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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON: Industrializing immunooncology manufacture & supply chain

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Volume 8, Issue 5

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Preparing next-gen cellular immunotherapies for commercialization

David McCall, Editor, *Cell & Gene Therapy Insights*, talks to **Peter Olagunju**, Chief Technology Officer, TCR² Therapeutics

PETER OLAGUNJU is a Cell & Gene Therapy Executive, who has successfully worked to drive four C&GT products through clinical development to commercialization: PROVENGE®, ZYNTEGLO®, ABECMA®, and SKYSONA®. Mr Olagunju joined TCR² in 2021 as Chief Technology Officer. He brings over 20 years of experience in cell and gene therapy, clinical development, program management, manufacturing and technical operations. Prior to joining the company, he was Senior Vice President of Technical Operations at FerGene Inc., where he led the technical operations function for the commercialization of a gene therapy for bladder cancer. Before that, Mr Olagunju was Vice President of Global Patient Operations at bluebird bio, Inc., where he held several roles of increasing responsibility and was the program lead and functional head of manufacturing supporting the European approval for ZYNTEGLO®, a transformational gene therapy for Transfusion dependent Thalassemia. Earlier in his career, he held senior positions in Commercial Technical Operations and served as the Head of Quality at Dendreon Corp. and ZymoGenetics, Inc. Mr Olagunju holds an MBA from the University of Washington and a BS in Biology from the University of Illinois at Urbana–Champaign.

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Q What are you working on right now?

PO: I am enthused to be working on a unique T Cell Receptor (TCR) platform that is being used in solid tumors. We are moving into an area where several other cell therapies have not been successful.

It is a unique format that leverages the full TCR complex independent of human leukocyte antigen (HLA). Key differentiators include our clinical data that suggests the full TCR is required for solid tumor efficacy. HLA independence allows us to broadly enroll and treat patients, which is an important factor for commercial reasons.

Q What are the key specific considerations for the manufacture of TCR^2's TRuC-T cells, and how are these reflected in the company's ongoing manufacturing model/strategy?

PO: The first thing to mention is it is an autologous cell therapy – each batch that is manufactured is linked to one specific patient. With autologous cell therapy comes operational complexity in the logistics of a one-for-one manufacturing paradigm. The starting material comes from a patient, is cryopreserved, and then becomes the starting material for the unique drug product manufacturing process for that patient.

A further consideration is scaling up. As we look to treat more patients in clinical studies and potentially commercially, we need to look at scaling those processes, given some of the unique considerations of an autologous cell therapy.

In terms of how that influences our manufacturing strategy and network, we utilize multiple strategies for manufacturing. We work with a couple of contract development and manufacturing company (CDMO) partners to leverage external manufacturing. Typically, the outsourcing component is one that start-up cell therapy companies utilize until they reach the key inflection point where they can invest and internalize the manufacturing.

Additionally, a year ago we made an investment in a facility in Rockville, Maryland, which we intend to be our manufacturing center of excellence for both clinical and commercial production. Supporting such a large scale is typically difficult for operationally complex products such as autologous cell therapies. The commercial manufacturing center we are building will be able

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to support the scaling we need through late-stage clinical trials and our early commercialization phase.

Q While TCR² Therapeutics has led with an autologous approach, it is bringing allogeneic cell therapy into the earlier stage R&D pipeline. What adaptations are you making to your manufacturing operations and strategic approach to cater for this?

PO: From a baseline perspective, many folks are interested in allogeneic as a modality as an alternative to autologous. The body and quality of clinical data we have today points to autologous as being an important piece of the cell and gene therapy puzzle as we move forward, but when thinking about that operational complexity, the idea of having allogeneic therapies with potentially one starting material that are widely applicable for many patients is very appealing. This will remove some of the complexities in terms of logistics, whilst also leading to a cost of goods reduction.

That being said, there are some real biological hurdles from an allogeneic perspective, specifically when considering graft versus host disease (GvHD) and the corresponding need for gene editing. This means either knocking in or knocking out certain components to get a material that is broadly applicable to many patients and overcomes the limitations of GvHD.

Another aspect of a traditional transition from autologous to allogeneic could be using different cell types and cell sources. Many companies are looking at NK cells, gamma-delta T cells, and other cell types, and sources such as cord blood as a potential allogeneic play, which could also broaden applicability.

From a manufacturing perspective, it is still early days for us. We have some exciting preclinical data that suggests compelling aspects of our allogeneic cell therapy program. We are in the early stages of designing how the allogeneic manufacturing structure will differ to the autologous structure – the needs for allogeneic manufacturing are markedly different. We are looking to scale-up what we have seen on the preclinical side and build out a differentiated and dedicated allogeneic approach.

Q Reducing vein-to-vein time continues to be an important goal for companies in this space – what can you share about TCR² Therapeutics' approach to achieving it?

PO: Vein-to-vein time is important, especially in a therapeutic indication area like blood cancer, where patients are often progressing in a matter of days and weeks.

However, the term 'vein to vein' is somewhat problematic. The term my team is using now is 'turnaround time', because there are certain operational elements that take place in that vein-to-vein window that we have no control over – for example, once a product is released, patient availability, physician choice, and patient choice are all factors that affect the

vein-to-vein time. We can focus within our scope of responsibility up to and including product release, optimizing processes and systems to reduce that timeframe.

From an assay perspective, we are looking at rapid sterility and safety tests to reduce the long lead times typically associated with cellular immunotherapy product testing. This will ensure that once the product is manufactured, we are minimizing the amount of time it takes to release that product and make it available to the patient.

"There are some exciting innovations at the forefront. One particular therapy that comes to mind is cilta-cel (Carvykti), a Legend Biotech/Janssen product, which ... has an astounding profile when it comes to outcomes."

Q Where have advances been made in the field in terms of improving cost effectiveness with market and patient access to these therapies in mind?

PO: The industry has a responsibility to patients to broaden access to these therapies that have shown profound clinical data. To broaden access, the areas of focus are cost reduction and improving the efficiency and scalability of processes.

There are some exciting innovations at the forefront. One particular therapy that comes to mind is cilta-cel (Carvykti), a Legend Biotech/Janssen product, which is showing overall response rates in the high 90% range, and complete response rates near 80%. It has an astounding profile when it comes to outcomes.

A group at the University of Pennsylvania, alongside Novartis, has published data on an expedited *ex vivo* manufacturing process. Novartis recently presented the T-Charge platform, which implements a 48 h *ex vivo* process versus a typical 7–10+ days of *ex vivo* manufacturing. The data from the University of Pennsylvania actually showed a 24 h *ex vivo* manufacturing process, where much of the cell expansion would occur *in vivo* – the process was optimized, including the reagents and the cytokines, to enable most of the expansion to take place inside the patient. This is an exciting development which could be a game-changer.

Enabling technologies and particularly automation have been another key focus area, including Miltenyi Biotec's Progidy and work from Ori Biotech on a closed-system, benchtop manufacturing platform. Adva Biotechnology is coming out with some intriguing technology relating to closing the manufacturing process with an efficient footprint, so that you can scale in a limited amount of space. Another company, Cellares, has the Cell Shuttle – a dedicated, closed manufacturing area with several workstations to allow the addition of reagents and solutions within an enclosed space. This translates into the ability to design a manufacturing facility that is more efficient, as the enclosed system takes away the need to have a dedicated, classified space that requires increased monitoring and personnel counting. This is where a lot of the cost resides in the current manufacturing paradigm.

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Q You have been involved in the pivotal-phase and commercial manufacture of a number of advanced therapy products with the likes of Dendreon, bluebird bio, and FerGene – can you distil any key learnings you bring forward to TCR^2 Therapeutics from those experiences?

PO: The first learning is the importance of early characterization to understand both your process and your product. This means identifying and testing from early on to generate a body of characterization data. As you optimize and make changes, if you do not have that data from a characterization perspective, it can be tough to identify the impacts of those changes. Related to that is planning the right assays starting early on, before development, qualification, and validation of your late-stage studies. In particular, having a suitable plan for potency has been a stumbling block for several companies of late.

My second insight relates to something that my esteemed colleague Jason Carstens described as the "chemistry, manufacturing and controls (CMC) mousetrap". Often with a cell or gene therapy, we are fortunate in that we see an efficacy signal early in a Phase 1 clinical study. Phase 1 studies are primarily designed for safety, of course, but when we see an early efficacy signal, it opens the door for potential expedited clinical development. There are certain early designations (eg. RMAT) that allow for expedited development. This is great for the patient and the companies developing the products. However, it does create a tension point on the CMC side – regulators' expectations from a characterization and process understanding perspective do not change just because you are on an expedited development pathway.

What happens is folks get excited about the early clinical data, and they start to develop timelines based on the potential of an expedited pathway. They perhaps do not give enough weight to the importance of CMC – of needing to go through the requisite steps of characterizing the process, understanding the assays, and qualifying and validating. It is critical to drive internal alignment early on and to see how this is interconnected on the CMC side, in order to come up with an appropriate timeline for all parties and functions.

Q What will the cellular immunotherapy products we are manufacturing in 5–10 years' time look like – and what does this means for today's facility design?

PO: It is truly unknown what those products will look like. However, gene editing is growing in its prevalence and how it

"What happens is folks get excited about the early clinical data, and they start to develop timelines based on the potential of an expedited pathway. They perhaps do not give enough weight to the importance of CMC..."

is being incorporated into advanced therapy development. RNA is another developing area. These breakthroughs promote the idea that *in vivo* manipulation or adjustment, versus all the *ex vivo* manufacturing happening today, can potentially leapfrog what the allogeneic approach allows you to accomplish.

These further developments in gene editing, RNA, and *in vivo* processes will require companies to develop manufacturing facilities that are nimble – to look at modular designs and ensure the ability to pivot and switch should technologies change. Any time I see a facility that is completely built out, I wonder if it allows for the ability to pivot as needed. Being flexible enough – having latitude in the design space and being able to change as the technologies change – will be a requirement moving forward.

Q Finally, what are some key goals and priorities, both for yourself in your own role and for TCR² Therapeutics as a whole, over the coming 12–24 months?

PO: Our lead product, gavocabtagene autoleucel (gavo-cel), is being investigated in a number of solid tumors, including ovarian cancer, mesothelioma, cholangiocarcinoma, and non-small cell lung cancer. We recently initiated a Phase 2 expansion cohort for gavo-cel, in which we will treat a large number of patients over the next 12–18 months. This trial has multiple cohorts, one of which has three arms, including an arm with gavo-cel as a mono-agent, and the two other arms with gavo-cel in combination with checkpoint inhibitors, Opdivo and Yervoy, which our clinical trial collaborator Bristol Myers Squibb is providing. Some important data will be generated from these studies.

In addition, we have a new clinical program called our TC-510 program, which is a version of gavo-cel with a built-in enhancement – a PD-1:CD28 switch. The idea is to take a negative signal and turn it into a positive, enhancing the signal over time, which would potentially lead to greater persistence of the product in the tumor microenvironment.

From a manufacturing perspective, we are continuing to make investments in our manufacturing network to prepare for commercialization. With late-stage clinical development in mind, process characterization, qualification and validation of assays, and commissioning of our intended commercial facility are all on the radar.

The facility is a significant build, considering the scaling and the number of patients we are looking to treat versus the number we have treated in the past. The organizational design, investments in building out teams, leveraging appropriate IT solutions, process analytical development, and manufacturing and supply chain quality are all key considerations. It is going to be a big next couple of years from that perspective, and we are excited by the opportunity to broaden access for this product to more patients.

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Key considerations for cytokine supplier selection for cell therapies

Timothy Manning, Senior Product Manager, Protein Business Unit, Bio-Techne

In the cell therapy space, GMP ancillary materials include cytokines and growth factors used as culture supplements to make cell-based medicines. The success of a cell therapy can depend on the quality and consistency of supply of these ancillary materials, making the choice of supplier a crucial decision to get right.

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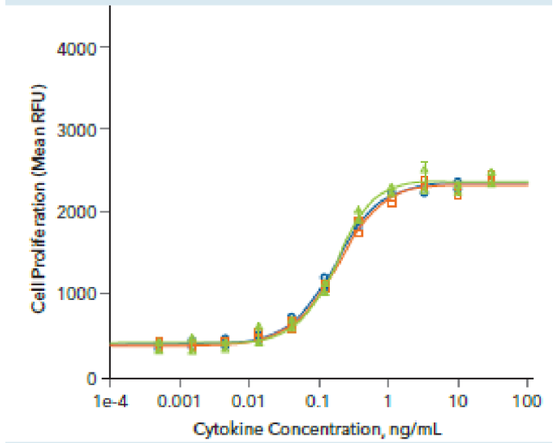
Effective partnering is central to ensuring end-users' primary considerations of patient safety, lot-to-lot consistency, and supply chain reliability are addressed. Relationships with suppliers must be more than transactional.

A smooth transition into clinical production can be facilitated by integrating RUO-grade cytokines with equivalent GMP-grade options early in discovery **(Figure 1)**.

Figure 1. Manual steps required to perform Ella and ELISA immunoassays.



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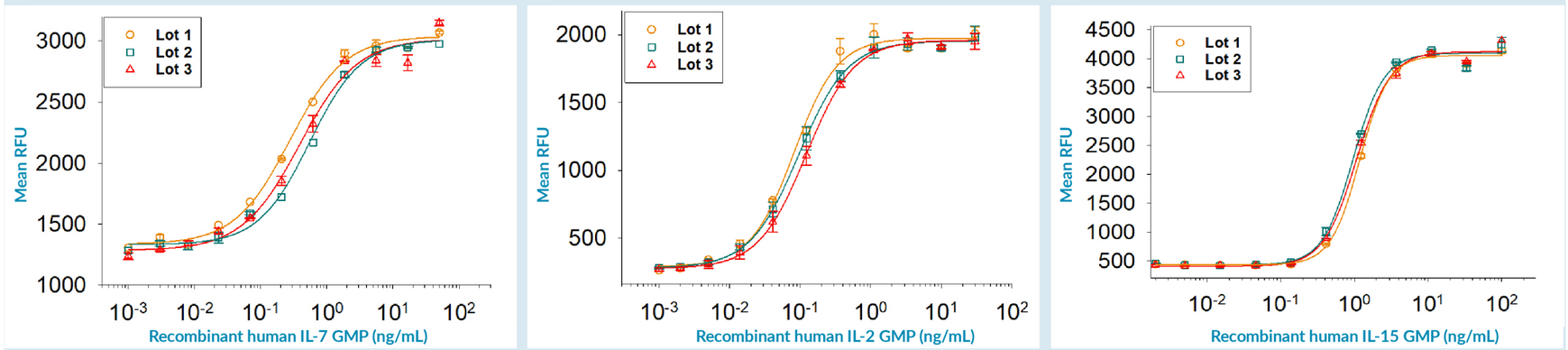

CONSISTENCY

Cytokines and growth factors are inherently prone to variability, due to their origins in biological systems. Partnering with the right suppliers will help to maintain production consistency throughout the lifecycle of a product. Spanning lots is almost inevitable, so to ensure consistency in cell culture, data from at least three past lots should be observed to confirm lot-to-lot consistency. If possible, testing material from three separate lots to examine the consistency in data **(Figure 2)** should be performed.

Controlling for assay variability can be managed using master control lots. These master control lots are tested with each new lot and should have identical activity every time. Variation in the activity of the known control is indicative of variability in the assay itself.

SUPPLY CHAIN

Planning early and anticipating late-stage requirements will help to avoid disruptive changes later. Ensuring that a supplier can meet your needs through later stage trials and commercialization requires a few considerations. First, lot size, including history of lot size, lot-to-lot consistency and past stability data should be queried. A master supply agreement can give the supplier visibility of the client's needs, whilst also giving the client confidence that material will be available when required. Considering a secondary supplier will also decrease risk. These considerations should be made early on to avoid the necessity of significant changes to critical raw materials, like cytokines and growth factors, mid-stream.

CELL & GENE THERAPY INSIGHTS

INDUSTRIALIZING IMMUNO-ONCOLOGY MANUFACTURE & SUPPLY CHAIN

SPOTLIGHT

COMMENTARY

Why we need a revolution for personalized cell therapies

Simone Steiner, Benjamin Gilgen, Eileen Pernot, Florence Salmon & Dorothea Ledergerber

The dawn of cell and gene therapies has revolutionized the treatment of several debilitating or deadly diseases. However, the current paradigm in the development of cell therapies needs a profound remodeling as the ecosystem that supports new innovative treatments is not fit for the future in terms of patient access, costs, speed, and ecological footprint. In addition, the gap between academia and industry is widely recognized by all but not addressed. Ignoring this elephant in the room is no longer an option as the industry strives to maintain a steady flow of life-saving innovative medicine reaching patients in need. One major improvement would be the use of closed manufacturing platforms, in a distributed setup but supported by standardized control, that could accelerate the translation of innovative treatments from bench to bedside. Reshaping the cell and gene therapy landscape needs a joint commitment of all stakeholders to ultimately offer the best possible personalized care to patients.

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WITH THE CELL & GENE THERAPY FIELD COMING OUT OF INFANCY, THE HEALTHCARE SYSTEM WILL BE OVERSTRAINED, & THE PATIENTS NOT WELL SERVED

The dawn of cell and gene therapies has revolutionized the treatment of several debilitating or deadly diseases. While CAR-Ts have shown transformative efficacy in hematological cancers, solid tumors remain a large

challenge. Innovation in other cell therapies has bloomed, building on the development of dendritic cell and T-cell culture breakthroughs during the last decade. With the rise of personalized immunocellular therapies, such as tumor-infiltrating lymphocytes directed at tumor-specific antigens and genetically modified T cell therapies, the potential to achieve the eradication of solid cancers is on the horizon.

More than 1300 cell therapy trials were active in April 2021, representing an increase of 43% compared to 2020 [1]. While already an annual cost rise in the health care systems of 2–3% leads to fierce social and political debates, a global annual growth of 20–30% in approved cell and gene therapies will likely drive us on the road of a system collapse or glaring inequalities in patient access. It is also clear that the costs of research and development together with the manufacturing costs are the frontline factors driving the high prices that will overstrain the healthcare system. Moreover, these prices lead to many therapies only being available in a small number of countries, thus limiting access of patients to the best possible personalized care.

The current paradigm in the development of cell therapies needs a profound remodeling as the ecosystem that supports new innovative treatments is not fit for the future in terms of patient access, costs, speed, and ecological footprint as discussed below (Figure 1).

A fundamental shift in the way the main stakeholders in this ecosystem interact is required to accelerate the transformation of the cell therapy industry and guarantee fair access of patients to novel treatments (Figure 2). Such change can only arise through close collaboration between academia and industry, but also between payers and hospitals, without forgetting the patient's voice.

The dialogue between academia, clinicians and industry should occur early when generating novel therapeutic ideas to ensure an effortless continuum during clinical development, especially at the manufacturing level. One major improvement would be the use of dedicated manufacturing platforms that could facilitate the transition from a creativity-driven approach to an industrialized production. In this context, a patient-centric production model that allows for on-time production guided by the treating physician, with the necessary quality controls, would complement and benefit the entire cell therapy ecosystem.

TAMING THE ELEPHANT IN THE ROOM: CATALYZING THE COLLABORATION BETWEEN ACADEMIA & INDUSTRY

The gap between academia, clinicians and industry is widely recognized by all but not

FIGURE 1

The current growth pharma model is not fit for purpose for the growth in approved cell and gene therapies.

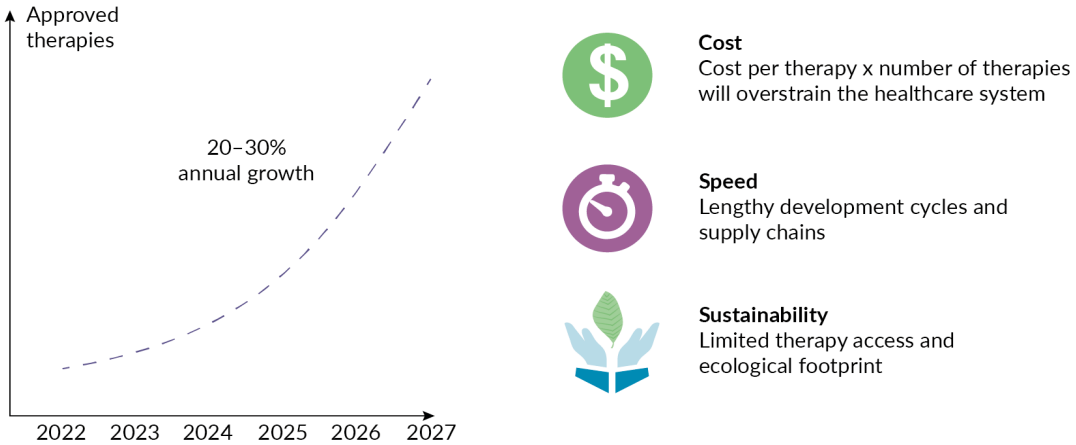

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FIGURE 2

Key drivers for an accelerated industry transformation are people, technology, data and regulations.

Early collaboration
Mutual understanding and co-development

Closed platforms
Integrated data, process development and new technologies

Patient centricity
On-time, guided by physician, standardized quality control

addressed properly. Ignoring this elephant in the room is no longer an option as the industry strives to maintain a steady flow of life-saving innovative medicine reaching patients in need.

Cell therapy treatments are usually developed in sequence by both the academia and the industry, and approved products are distributed worldwide by the industry [1]. Hospitals and academic institutions are paving the way for scientific innovation through proof-of-concept studies and early-stage clinical trials aimed at evaluating the safety and early clinical activity of new cell therapies. These studies are of utmost importance in the discovery of innovative treatments for patients, where many would otherwise remain without any available therapeutic option. Many leading academic hospitals are routinely manufacturing cellular therapies, mainly for autologous and allogenic stem cell transplantation, or for CAR-T therapies in the frame of clinical trials or single-patient use. The complexity of the production and associated costs of cell therapies in academic settings has been described elsewhere [2–6]. Late-phase clinical studies are usually carried out by the industry due to the costs, required infrastructure, operational complexity, and personnel needed to conduct large international studies.

The highly manual and adaptable manufacturing processes originated in academia are well suited for the early phase of cell therapy development because they provide enough freedom to explore different modalities via in-house manufacturing in a single center. However, albeit compliant with Good Manufacturing Practice, they represent a challenge for later stages of clinical development and post-approval, as highly standardized and consistent systems are required to ensure product quality in commercial production. The transition from an academic to an industrial manufacturing process requires substantial process optimization steps that usually need to be validated by additional clinical studies. As a consequence, the development and time to market availability of life-saving cell therapies can be delayed by two to three years. Such delays increase the risk for biotech and pharmaceutical companies, raises costs significantly, and affects economic benefits if not compensated by high list prices. For instance, prior to a new cell therapy Phase 2–3 clinical trial, at least 10–15 million USD need to be allocated only for process development and technology transfer to large facilities to increase manufacturing capacity. Ensuring a smooth transition of manufacturing processes between academia and industry could not only halve the manufacturing costs and subsequently decrease the overall therapy cost by 25%, but also would allow for a rapid diffusion of new science to physicians and their patients across the world.

In a word, throwing innovative therapies over the fence that separates academia from industry and hope for an optimized and sustainable collaboration has not proven favorable so far. Finding the sweet spot between exploratory freedom and standardized scale-out is crucial to increase the speed with which

cell therapies reach the right patients (Figure 3). Certainly, this is an area where regulators need to be involved in order to assess risk factors.

Supporting academia with experienced teams for setting up a scalable system at the early stage of the development would enable academic institutions to translate effortlessly their creative proof-of-concept into viable cell therapies without the burden of additional process development.

STANDARDIZED MANUFACTURING PLATFORMS REPRESENT THE ONLY SUSTAINABLE OPTION FOR TIMELY AND AFFORDABLE ACCESS TO NOVEL THERAPIES

One of the biggest challenges perceived by academic manufacturing facilities in building up their capacity for cell therapy production is the limited understanding of logistics and regulatory requirements. The definition and implementation of suitable quality policies according to the phase of the product development and their enforcement was reported to be another challenge; additionally, quality systems staffing was considered under-resourced [4].

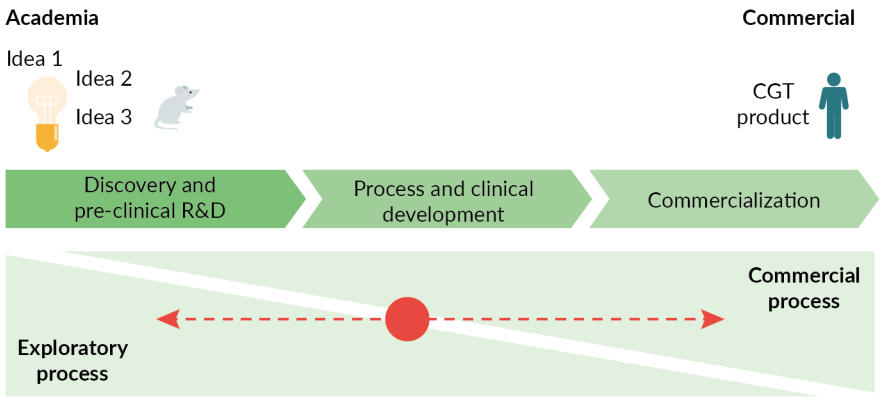
A versatile platform composed of a set of qualified equipment in an automated, fully aseptically closed setting, backed by a robust technical process, would have the potential to reshape the cell therapy landscape. It will allow the manufacturing of new modalities in a system supporting late-stage clinical studies with minimal process development. Additionally, by closing and automating the process, it will become possible to carry out manufacturing of T-cell-based therapies in D-level clean rooms instead of expensive B-level rooms. These features could dramatically increase the global manufacturing capacity and radically improve a broad patient access to such therapies. Standardized equipment that could be adapted to fit different manufacturing platform designs could provide enough flexibility throughout all stages of clinical development.

DISTRIBUTED, CLOSE-TO-BEDSIDE MANUFACTURING IS A MUCH-NEEDED ALTERNATIVE TO CENTRAL MANUFACTURING

Currently, the late-stage and commercial manufacturing mainly follows the traditional standards of the pharmaceutical industry that were built on large-volume products: the production is centralized or limited to a few production sites scattered across the globe to supply certain regions. Despite numerous efforts to build new production facilities, the currently available global manufacturing

FIGURE 3

Finding the right balance between exploratory freedom and standardized scale out is crucial for collaboration.



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capacity does not allow for the timely production of the right products to treat cancer patients in urgent need. Time is crucial for the survival of the patients as several cancers show aggressive and fast progression. Cell logistics require a very precise timing complicated by cross-border transportation. The waiting time from diagnosis to treatment is often too long, and the slightest disturbance (e.g., a patient leukapheresis appointment delayed) can break the entire flow.

There is an increasing demand of society towards the pharmaceutical industry to purposefully adhere to their pledge of sustainability. The environmental footprint of central manufacturing sites is substantial: patient's cells are shipped deep frozen with special courier from hospitals all around the world to a central manufacturing site and sent back again to the patient, leaving a major CO_2 footprint. For instance, to treat 100,000 patients worldwide, 200,000 van journeys and airfreight shipments (with at least half of them in liquid nitrogen tanks) are needed.

The availability of personalized cell-based treatments manufactured closer to the patient's bedside, in a distributed setup and cancer centers, would reduce the turnaround time significantly. In such a setting, a standardized quality management system and real-time quality control is imperative to ensure highest quality standards and compliance to current Good Manufacturing Practice requirements. The combination of the closed, automated platform described above, and a standardized quality system could make cell therapies also available in underserved regions of the world – more specifically, in regions where the logistics and cold chain may be problematic, and in regions where no B-level clean rooms and highly trained workforce are available.

New technologies, AI, machine learning, fast internet connections, full data integration, and equipment will be of paramount importance to ensure a distributed manufacturing compliant with Good Manufacturing Practice. A standardized quality management system is mandatory to support understaffed academic teams to meet regulatory requirements and ensure the safety of patients.

Finally, the price of cell therapies also circumscribes the availability of this type of treatment to the richest countries. To date, no considerable progress has brought the manufacturing capacity to bearable costs for society in developing countries. A fully closed and serviced equipment platform set up close to the patient's bedside in a distributed setup could contribute to broaden the reach of cell therapy to developing countries, by limiting the costs of logistics, qualified personnel, and cleanroom maintenance.

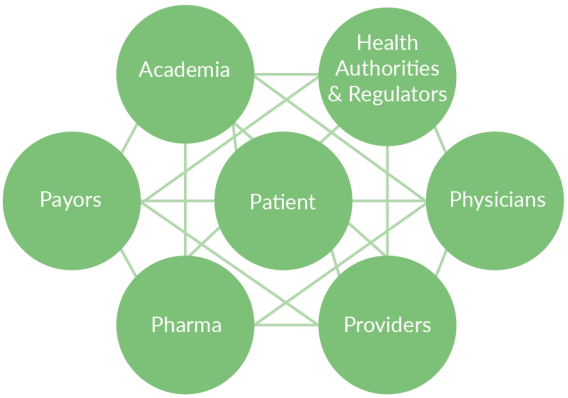
A REVOLUTION IN THE WAY THE ACTORS OF THE CELL & GENE THERAPY ECOSYSTEM INTERACT IS WARRANTED

A distributed setup for cell therapy manufacturing supported by expert service has the potential to accelerate the translation of innovative treatments from bench to bedside. However, this transformation needs a deep commitment by all stakeholders to shape the cell and gene therapy landscape together (Figure 4):

- Insurance companies, payors, and policy makers: these major players need to be involved in the development process to substantiate their assumptions and calculations with hands-on data and insights;
- Regulatory authorities: regulators must be consulted prior to starting and throughout product development to discuss plans, quality systems, potential findings, and to assess risk evaluation criteria;
- Patients: the distributed manufacturing facilities will offer new therapeutic options to patients in need but may require them to get treatment in regional specialized centers;

FIGURE 4

The stakeholders in the cell and gene ecosystem adapt at different pace – with individual drivers for change.



- Academia: new collaborative approaches to integrate experiences and analogies from commercial partners will have the potential to boost the development pipeline;
- Pharmaceutical companies: a mind shift from a blockbuster approach to a more agile therapy portfolio approach will be required from pharmaceutical companies. Their strength in market access and distribution will be a key driver for distributed manufacturing. The industrial partners will also need to actively support academia in their strive for creativity and innovation;
- Physician and health care providers: the platform approach will enable specialized hospitals to get back into the driver seat of cell therapy use. It will allow for an accelerated access to patients, including in rare indications and underserved geographical regions. Furthermore, it will support personalized care by allowing the right therapy to be tailored specifically for each patient at the right time.

All these actions would result in a situation that all stakeholders have been aspiring to for years: putting the patient at the center and give them the best possible personalized care.

TRANSLATION INSIGHT

A distributed setup for cell therapy manufacturing supported by standardized control has the potential to accelerate the translation of innovative treatments from bench to patient's bedside and reshape the cell therapy landscape.

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CELL & GENE THERAPY INSIGHTS

INDUSTRIALIZING IMMUNOONCOLOGY MANUFACTURE & SUPPLY CHAIN

SPOTLIGHT

INTERVIEW

Blazing a trail for commercial TCR T cell therapy

David McCall, Editor, *Cell & Gene Therapy Insights*, **talks to Helen Tayton-Martin**, Chief Business Officer, Adaptimmune

DR HELEN TAYTON-MARTIN has served as our Chief Business Officer since March 2017 and is a member of our Executive Team. She formerly served as our Chief Operating Officer since 2008, a role in which she oversaw the transition of all operations in the company from five to 300 staff, through transatlantic growth, multiple clinical, academic and commercial collaborations and private and public financing through to its Nasdaq IPO. As our CBO, Dr Tayton-Martin is responsible for optimizing the strategic and commercial opportunity for Adaptimmune's assets, leading on business development and alliance management. Her role encompasses all aspects of pipeline and technology assessment, strategic portfolio analysis and partnerships, including the company's strategic partnerships with GlaxoSmithKline (LSE/NYSE: GSK), Astellas and Genentech, a member of the Roche Group (SIX: RO, ROG; OTCQX: RHHBY). Dr Tayton Martin has over 26 years of experience working within the pharma, biotech and consulting environment in disciplines across preclinical and clinical development, outsourcing, strategic planning, due diligence and business development. She co founded Adaptimmune from the former company, Avidex Limited, where she had been responsible for business development of the soluble TCR program in cancer and HIV from 2005 to 2008. Dr Tayton Martin previously served as a non executive director of Trillium Therapeutics Inc. (Nasdaq and TSX: TRIL) from October 2017 through the sale of the company in November 2021to Pfizer Inc. She holds a PhD in molecular immunology from the University of Bristol, UK and an MBA from London Business School.

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Q Since the successful launch of the first CAR-T products, what have been the key learnings in the field that lead you to believe that patients with solid tumor indications will benefit from cell therapies?

HT-M: There has been a great deal of focus on trying to treat patients with solid tumors with cell therapies, hoping to emulate what has been seen with the CD19 and BCMA CAR-Ts in the hematological malignancy space. The first companies to commercialize in this space have faced significant challenges, but those products are now growing and becoming successful.

There are reasons to believe we can get to something similar in solid tumors. For example, Adaptimmune's data has established the basis to soon file a BLA submission in synovial sarcoma. Beyond that, we have seen responses in five different solid tumor types. We have moved into a Phase 2 setting in esophageal and esophagogastric junction cancers and are planning the same in ovarian cancer. We are also anticipating multi-indication strategies for rapid development.

The responses are there in various indications, some of which have been durable. The cells are persisting, and late-stage patients are benefiting from these therapies. And if we can move away from human leukocyte antigen (HLA) restriction, with our HLA-independent platform, we will be increasing the patient population who can potentially benefit.

Getting to more patients and getting to them earlier are both really important. Part of that requires educating solid tumor cancer centers, so they become more familiar with cell therapy as a modality – for example, they understand what screening for HLA looks like, and how and when to book apheresis slots.

We've built that knowledge from the ground up for these centers and have sought to create a mindset around cell therapy being part of the cancer treatment armamentarium. Initially, cell therapy can be seen as complex and challenging by the centers but as they see the benefits being delivered to patients, they become more and more interested.

Our SPEARHEAD-1 trial in synovial sarcoma was enrolled in record time - just over 12 months from start to finish in the first cohort. Producing good data gains buy-in and brings still more patients. It is a long and complex journey, but we have established cell therapy on the map in solid tumor settings.

Q What are the current obstacles to cell therapies being optimally and cost-effectively delivered?

HT-M: The obstacles include creating the infrastructure and the operational basis to provide these therapies, with a focused effort on key centers in a clinical trial setting. Another is breaking down the barriers to get to patients earlier.

Getting to patients earlier is going to be critical, and a key element of that is diagnosing earlier. This means educating on the importance of biomarker and HLA testing. If clinicians are thinking about whether their patients could be eligible for these therapies earlier, then

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there is potential for earlier treatment. We also need to think through the development pathways and combinations. We are bringing that into our SURPASS trial, with an additional arm, because of the importance of being able to test the therapy in earlier lines, including combinations with checkpoint inhibitors.

One of the challenges is that by the time a late-stage patient becomes eligible, many things need to occur, including screening for the antigens and HLA, getting the apheresis booked, and cell therapy manufacture. Late-stage solid tumor patients can be very unwell, so earlier treatment will be highly beneficial to them.

We also want to make the therapies more available and cost effective. For us, one of the biggest costs is the infrastructure surrounding manufacture, so improving capacity utilization is important. This will lead to more quickly delivered and more affordable therapies for patients.

The main question is: how do these therapies fit within the existing standard of care and existing infrastructure, as well as the understanding of both payers and clinicians?

We must build the evidence and push through the barriers to undertake the trials and provide data that will show the benefits in patients to clinicians. We also want to be able to document those benefits for a larger audience. Building the evidence is important to build credibility for clinical execution, so that a broader subset of clinicians will buy into the therapy.

Q As you prepare to launch products at Adaptimmune, what are you learning about the differences and similarities regarding manufacturing and delivering cell therapies in the clinical versus the commercial setting?

HT-M: From a clinical perspective, I have highlighted that the education and effort required for solid tumor centers in order to be able to run the trials, and of course to be able to prescribe in a commercial setting. As we build out the educational pathways into broader referral networks, those centers that refer into the treatment centers themselves also become critical. Patient advocates can be strong partners, particularly for a rare disease indication, which is where we are starting out with synovial sarcoma. There are patient advocacy organizations that are excited about something that could be a game-changer for patients. They act as a point of reference, so we need to ensure the educational information is ready for them, too.

From a commercial perspective, we need this information to be broadly available

"For us, one of the biggest costs is the infrastructure surrounding manufacture, so improving capacity utilization is important. This will lead to more quickly delivered and more affordable therapies for patients."

within an interface. We will be launching Adaptimmune Assist, for example - an interface which will enable patient and clinician interactions with the infrastructure and capabilities needed to help patients access the therapy and understand reimbursement. We must also focus on the actual treatment operation for delivery, especially on the manufacturing side, where we need understanding of the likely demand to ensure availability.

We are scaling up our manufacturing capabilities, using fallow space set aside in our Navy Yard facility. We are building beyond the capabilities needed for clinical execution into commercial and will go through FDA inspections to ensure they are compliant. Of course, we must ensure that both the vector we use and the cells we manufacture are going to be compliant from a commercial product perspective. This involves planning both, in terms of the quality systems and quality approach, as well as infrastructure and capacity.

"One of the other ultimate long-term aims for the field is moving towards an off-the-shelf product. The dream is to have a consistently characterized product from a potency, safety, and efficacy perspective, sitting on the shelf."

Q Personalized medicine is exciting, but the cost of making such therapies is high and finding the right patients with requirements to be treated can be difficult. Do you see potential for cell therapy to become easier to administer and reimburse?

HT-M: I have mentioned some of the near-term aspects around tackling earlier lines of therapy, awareness, and importance of screening patients early. This all feeds into a faster and more efficient autologous cell product delivery process.

One of the other ultimate long-term aims for the field is moving towards an off-the-shelf product. The dream is to have a consistently characterized product from a potency, safety, and efficacy perspective, sitting on the shelf. This means that when an eligible patient is screened, you do not have to wait for apheresis, manufacture, release, and shipping. With an off-the-shelf product, you only need to ship, thaw, and treat. We are fortunate to have an autologous near-term pipeline of products, which we think will be commercially viable soon. This gives us an idea of what those cells need to look like for an allogeneic version, as a replica of the optimal characteristics of the autologous products in an off-the-shelf setting. This will ultimately make it much easier and more cost-effective to administer cell therapy products in a similar way to today's established biopharma products, but hopefully involving just one dose.

The high degree of variability in making a cellular product means that however hard we lock down the characterization of the process, we are dependent on the starting apheresis material, which will always be affected by an individual patient's disease and prior courses of

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therapy. We can standardize the cells in an off-the-shelf format, taking a lot of that variability out of the equation. I think this will have the biggest impact down the line. However, allogeneic cell therapy products will still have to be safe and efficacious, and establishing this in the autologous setting is the first step.

Q In addition to education and partnering with patient advocates, what are some important steps towards ensuring such novel and personalized medicines find their patients and the physicians who prescribe them?

HT-M: Building evidence that can be published and presented at key meetings is important. Influential meetings, such as those from the American Society of Clinical Oncology (ASCO), where prescribing physicians go to get their latest information, will help to build awareness more broadly.

Ultimately, payers are of the upmost importance. What prescriber clinicians want to do can be much more impactful with payers than anything pharmaceutical companies can do.

Q Big pharma has been getting most of its recent innovation from biotech through collaboration or acquisition. Do you see this trend as a risk or an opportunity?

HT-M: Always an opportunity!

I have been accountable for business development transactions for Adaptimmune from the beginning. I see collaboration as a potential win for both parties. From Adaptimmune's perspective, mainstream pharmaceutical collaborations have been critical to both our survival and our success as an independent company.

In the early days, GSK took an option on our NY-ESO TCR program and largely financed its development whilst we built the capabilities we needed. We shared learnings along the way. We built both the Adaptimmune organization and our credibility through that initial deal.

We also saw the importance of allogeneic cell therapy development from an early stage. Our initial collaboration with Universal Cells, which was ultimately acquired by Astellas Pharma, helped us to build a relationship with them to ultimately partner and co-develop products. The deal enabled us to continue to develop a platform with a company that has built an oncology portfolio around transformative new modalities.

Last year, we made a deal with Genentech around a tremendous vision for where off-the-shelf T cell therapies could go in the future. Ultimately, the goal is to allow any patient to have their own T cell receptors in their own T cells given back to them. There is a lot of biology still to work out, but both companies could jointly see a personalized, off-the-shelf approach to T cell therapies was potentially possible with our current platform and Genentech's vision.

At every point in Adaptimmune's development, there was more opportunity in collaborating than risk. In the current market, there is still much opportunity for companies to do deals with pharmaceutical companies, based on the tremendous innovation that has occurred in the last five years. Pharma companies are increasingly seeing cell therapy as part of the future, both with the cutting-edge innovation in the field and products now showing demonstrable commercial value. As long as there is an aligned interest for the biotech and pharma companies, and both companies are contributing in some way, strong partnerships will exist.

Such deals also create non-dilutive capital for a company. Particularly in immuno-oncology, gaining access to non-dilutive capital and access to research and development capabilities can be synergistic and value-creating.

There are always challenges with bigger organizations coming together with smaller ones. In our experience, the more you can try to navigate that ahead of time in the collaboration agreement, the better the basis for a transparent collaboration. The more transparent you can be in a collaboration, the better the flow of ideas and the better the development decisions.

I see huge potential for more pharmaceutical and biotech transactions. It may be the key thing that emerges from the current state of the financial market. Pharmaceutical investment in R&D and product development is long-term, and usually goes way beyond financial cycles. The fundamentals that drive deals are still the same today as they have always been, but I feel there is possibly more opportunity around today, partly because of the amount of innovation out there, but also because there are a lot of companies looking for collaboration right now.

Q Specifically in oncology, will we see more biotech companies emerging that have the potential to become a Genentech?

HT-M: It depends on where a company is now in development and what assets they have. If there are companies with innovative therapies and the ability to develop them in innovative ways, then yes, they do have that potential. The global pharma and government investment in mRNA vaccines during the COVID-19 pandemic is a good example of what biotech companies can achieve.

Companies often talk about doing things "on COVID time" now in terms of drug development. Those with the capability of operating on COVID time are the consolidators of that innovation, as are the companies that think outside the box in terms of development pathways and opportunities. Genentech is unique, but there is always room for innovative companies with transformative commercial products.

Seeing the opportunity and acting on it is the basic premise. The question is whether

"I see huge potential for more pharmaceutical and biotech transactions. It may be the key thing that emerges from the current state of the financial market."

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a biotech company can do that through partnering and investment in a timeframe that is going to allow them to mature. The sector is possibly more intense now than it was when Genentech first flourished, with more risk, but there has been a lot of investment and so, there is potential.

Q Are healthcare systems ready to deliver the benefits that cell therapy can offer to patients, in terms of budgets, structure, capacity, and experience?

HT-M: Healthcare systems are moving towards being ready for cell therapy. The current CAR Ts on the market have demonstrated that cell therapies can be real products. A different paradigm is needed in order to assess their benefit over the longer term. Companies trying to develop and commercialize these products must build the evidence to enable payers to see those benefits, and to see that the innovation is worth rewarding.

With the initial CAR Ts there were many issues, certainly in the US, with out-of-pocket expenses, such as the additional costs that in-patient hospital treatment requires, and the new treatment add-on payments. Europe is different, as the various jurisdictions all have a slightly different approach to providing therapies on approval versus reimbursing them. Building the evidence is going to take time and the various systems around the world will continue to adjust as this occurs.

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CELL & GENE THERAPY INSIGHTS

INDUSTRIALIZING IMMUNOONCOLOGY MANUFACTURE & SUPPLY CHAIN

SPOTLIGHT

INNOVATOR INSIGHT

Scaling non-viral cell therapy approaches for solid tumor treatments

Evan Zynda, Nektaria Andronikou & Kyle Jacoby

The need for standardization and high manufacturing success rates are critical drivers of innovation in cell therapy. Thermo Fisher Scientific has built a fit-for-purpose portfolio of modular instrumentation platforms designed to support closed, large-scale cell manufacturing, enabling automation of the end-to-end manufacturing workflow. Automation of the isolation / activation and bead removal steps ensure that the manufacturing workflow can be closed at the outset of the process. The transformation of immune cells into a functional therapeutic can involve a genetic modification step. There has been a renewed interest in non-viral gene modification approaches as an alternative to viral vectors due to the increased focus on personalized therapies in solid tumor indications. Non-viral electroporation is emerging as the method of choice, especially given demonstrated efficacy, safety benefits, and flexibility allowing the utilization of CRISPR-Cas9 gene editing. This article explores the emergence of modular closed, automated technologies and non-viral gene engineering tools that are fit-for-purpose for large-scale cell therapy manufacturing and discusses their application for a non-viral engineered cell therapy process.

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SECTION 1: Evan Zynda & Nektaria Andronikou

Gibco™ CTS™ DynaCellect™ Magnetic Separation System

The Gibco CTS DynaCellect Magnetic Separation System is a closed and automated system for consistent cell isolation/activation and bead removal. DynaCellect is designed with an automated magnet-rocker and fluidics panel for target cell separation and subsequent bead removal. This is controlled by an intuitive touchscreen interface, designed for both process development and clinical and commercial use. The liquid handling and fluidics panel houses a peristaltic pump, bubble sensors, pressure sensor, and pinch valves. Processes carried out on this instrument can be scaled to address current and future autologous and allogeneic workflows.

The Gibco™ CTS™ DynaCellect™ Cell Isolation Kit is specifically designed for the isolation and activation of immune cells. The design consists of a 1 L isolation chamber that fits in the rocker nest and 8 PVC tubes for sterile welding. The protocols are validated for 10 mL to 1 L, with 10 million cells/mL optimal density per reaction. It can process 100 million to 10 billion target cells, thus accommodating multiple scales and workflows. The isolation process time is a little over an hour, whereas current automated offerings consistently take up to 4 hours. With Gibco™ CTS™ Dynabeads™ CD3/CD28, we have achieved 90% target cell recovery, a purity of 92%, and a 3% coefficient of variation.

The Gibco CTS™ DynaCellect™ Cell Bead Removal Kit is a single-use kit designed for bead removal. The configuration consists of a bag chamber designed to allow bead-cell solutions to flow over the entire surface of the magnet for continuous flow. The consumable has 4 PVC tubes for sterile welding, filters, a fluidics cassette, and a tube organizer. This process has been validated up to 10 L.

Customers who tested the instrument found it easy to use, precise, and fast. In one test, DynaCellect was compared to an existing process. The total bead removal processing time on the DynaCellect was 25 minutes compared to two hours with the existing process. Cell recovery and viability were similar in both methods. The residual bead removal was significantly greater in the DynaCellect with near-zero beads left, which was a significant improvement over the existing method. Furthermore, customers found using the DynaCellect and the intuitive touch screen, in particular, to be a substantial improvement over current instruments.

For scaling up for clinical and commercial manufacturing, the CTS DynaCellect Magnetic Separation System is a key piece in establishing a standardized closed, automated, end-to-end manufacturing workflow.

Gibco™ CTS™ Xenon™ Electroporation System

After isolation and bead removal using the DynaCellect, the next step in the process is cell engineering. While existing methodologies utilized in the autologous workflow tend to leverage viral-based systems, there are limitations such as safety concerns, cost of production, payload size, and increased testing burden. The industry is shifting to address these limitations by leveraging non-viral technologies.

Leveraging electroporation for cell therapy and *ex vivo* genetic modification of cells has shown promise. However, the systems available do not fit the safety and performance profile required at scale. In response, the small-scale, research use only Invitrogen™ Neon™ transfection system was leveraged to develop the Xenon, a large-scale electroporation platform. The Xenon is designed to mimic the pulse profile of the Neon transfection system, and the Xenon protocols were designed to scale directly from the Neon transfection system.

The Xenon electroporation system is an open platform which leverages the ability to manipulate the voltage, pulse width, and pulse number to find the optimal conditions

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for a wide variety of cell types and payloads. It has an additional variable of pulse interval, which can allow a reduction in processing time, and a modular design that can be leveraged upstream and downstream of other unit operations, providing consistent performance across various scales. The Xenon can also be connected into an existing platform (such as the DeltaV automation system) with the built-in OPC-UA, and it also enables compliance with the 21 CFR part 11 regulation.

Xenon consumables

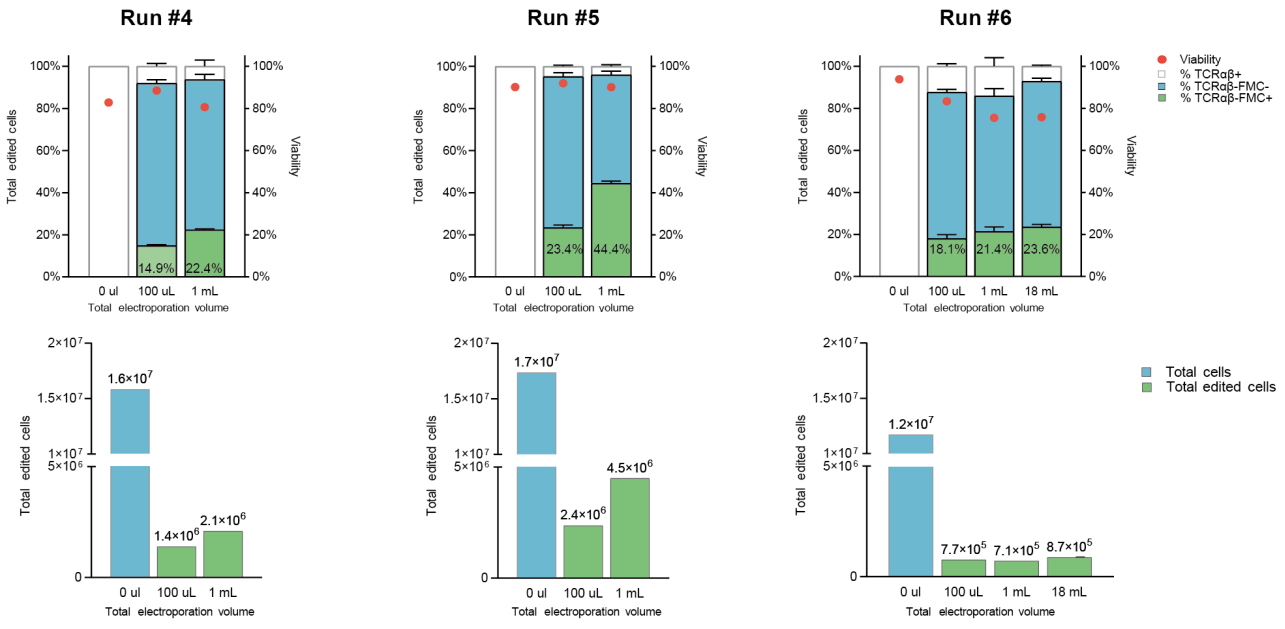
The Xenon electroporation system works with two plastic consumables, including a 1 ml SingleShot chamber that can be used for process development optimization. It can process 20–100 million cells in a single electroporation run of 1 ml volume. The MultiShot cartridge is used to process volumes from 5–25 mL, and cell numbers from 100 million to 2.5 billion. Electroporation buffer is provided in bag format to allow for automated, closed cell modification. There is also a newly developed genome editing buffer which enables genome editing-based applications and improves performance. An embedded touchscreen user interface allows for direct onscreen programing with walkthroughs for loading the consumables.

Both the instrument and the consumables are manufactured in a GMP facility under ISO 13485 and 9001 certifications. The chambers themselves also undergo extensive testing for sterility, biocompatibility, extractables, and particulate testing to ensure safety of the materials that will eventually be introduced into patients. The buffers, which come into direct contact with the cells, meet all relevant ancillary/raw material requirements.

The MultiShot cartridge, loaded onto the Xenon system, allows for closed system processing with connectible input and output lines. It has a hybrid PVC C-Flex tube that allows for sterile welding, as well as tube connectors. It has been designed with pre-routed tubes and a user-friendly interface to allow error-free loading. It has also been designed to minimize cell loss and preserve volume.

FIGURE 1

Total edited cells and variability.



Experimental data

In order to demonstrate the performance of the system, a standard workflow was used in which cells were isolated from a fresh apheresis product using the Rotea system, before activation with Dynabeads. After three days, the cells were de-beaded and prepared for electroporation on the Xenon system. Density at time of electroporation was 50 million cells/mL. The cells were then returned to culture in Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free (SFM) Medium, and analyzed for expression of the gene of interest (GOI).

Figure 1 shows data from three runs. Runs 4 and 5 demonstrates the differences in performance between the Neon transfection system 100 µL tip and the Xenon 1 mL single shot. Superior performance with the Xenon system was observed across the scales tested with no significant impact from electroporation itself on the viability of the cells. The 'total edited cells' are the cells that were seeded post-electroporation at equivalent seeding densities.

Functional killing was observed by the CAR T cells generated with the Xenon system following cryopreservation on day 9. The cells were thawed and placed in culture for three days before performing the killing assay shown in Figure 2. As seen by the graph on the left, the CAR expression is sustained after a freeze-thaw cycle, achieving >50% expression of the CAR. When the cells are added to a co-culture, effective killing is observed over a range of ratios of effector to target cells.

Overall, the Xenon system has proven to be a robust system for the cell therapy workflow, and can be directly scaled from the Neon transfection system into process development and clinical manufacturing.

SECTION 2: Kyle Jacoby discusses: PACT^NV™ non-viral precision genome engineering at scale PACT Pharma: personalized adoptive cell therapy for the treatment of solid tumors

PACT Pharma has developed a robust single-step, targeted, non-viral method for the

FIGURE 2

Cytotoxicity killing assay.

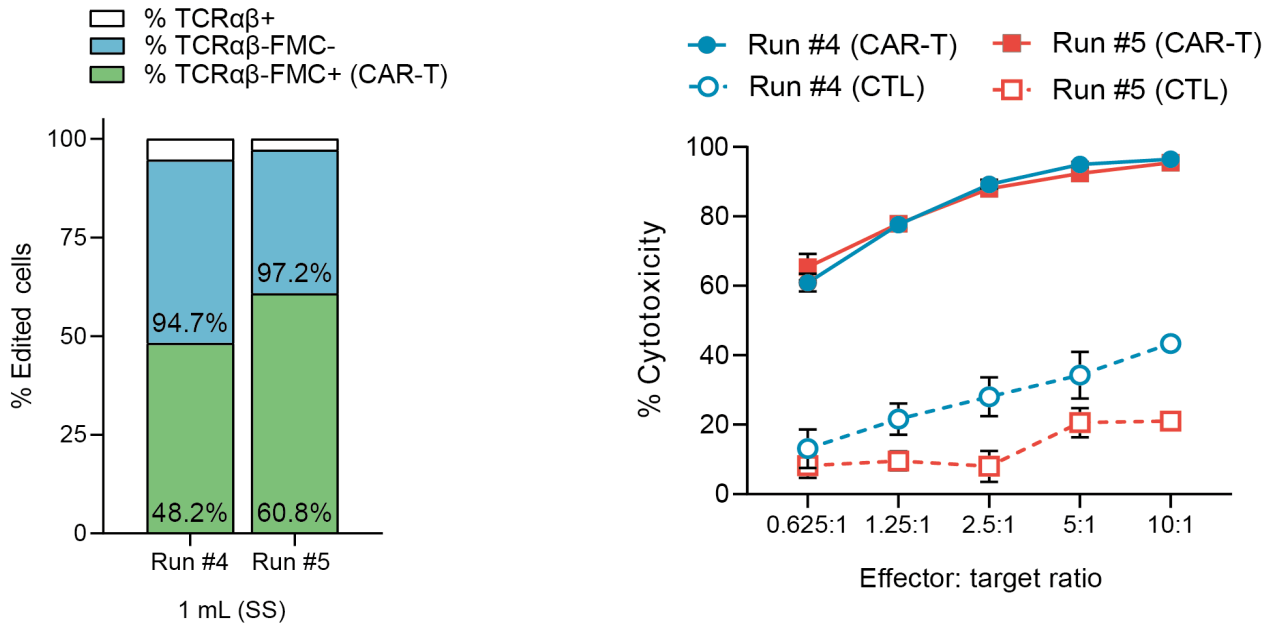


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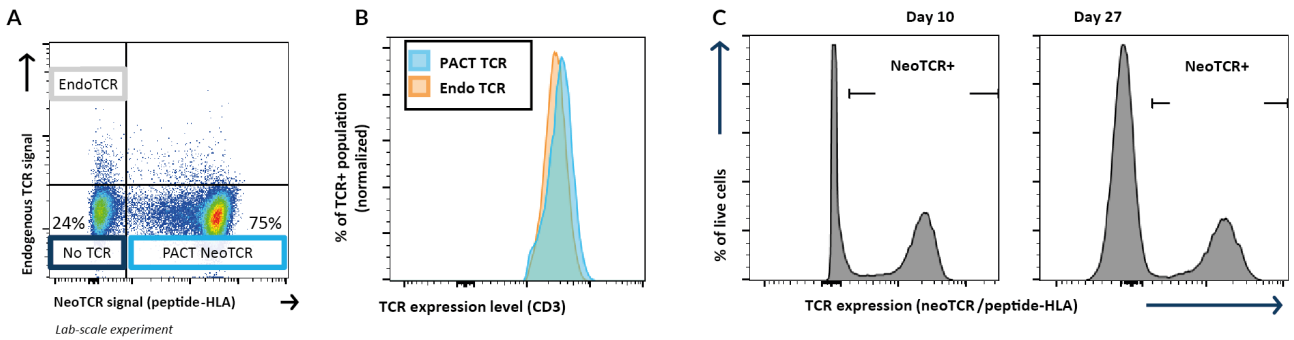
FIGURE 3

PACT^NV: physiological and sustained target gene expression for naturally regulated function.



(A) Single-step knock-in of neoTCR and knock-out of the endogenous TCR is highly efficient. **(B)** Site-specific integration of target gene into the endogenous locus results in consistent, physiological levels of target protein expression **(C)** Use of endogenous promoters results in sustained expression of target protein
(No downregulation of artificial promoters).

manufacturing of personalized adoptive cells therapies for the treatment of solid cancers (NCT03970382). In this process, a neoepitope-specific receptor (neoTCR) is precisely inserted into the endogenous locus while simultaneously eliminating the expression of the endogenous TCR. This results in neoTCR-specific T cells in which neoTCR expression is naturally regulated and not impeded by competition for CD3 by the endogenous TCR. Issues associated with retroviral manufacturing are avoided, including cost and dysregulation from random integration. PACT's single-step non-viral precision genome engineering technology can knock-out, knock-down, knock-in, and precisely regulate additional genes in a single step. These modifications have the potential to expand the applicability of drug products and are broadly applicable to a variety of other cellular therapies and research models.

PACT is developing an adoptive therapy that aims to create curative therapies tailored to each individual patient. Every patient's cancer is personal and unique, with highly specific mutations. Neoepitopes (neoE) are peptides originating from tumor-specific mutations that bind to the patient's human leukocyte antigen (HLA). These neoE-HLAs are highly specific targets for CD8 T cells. However, less than 1% of neoE-HLA targets are shared among individuals with solid tumors.

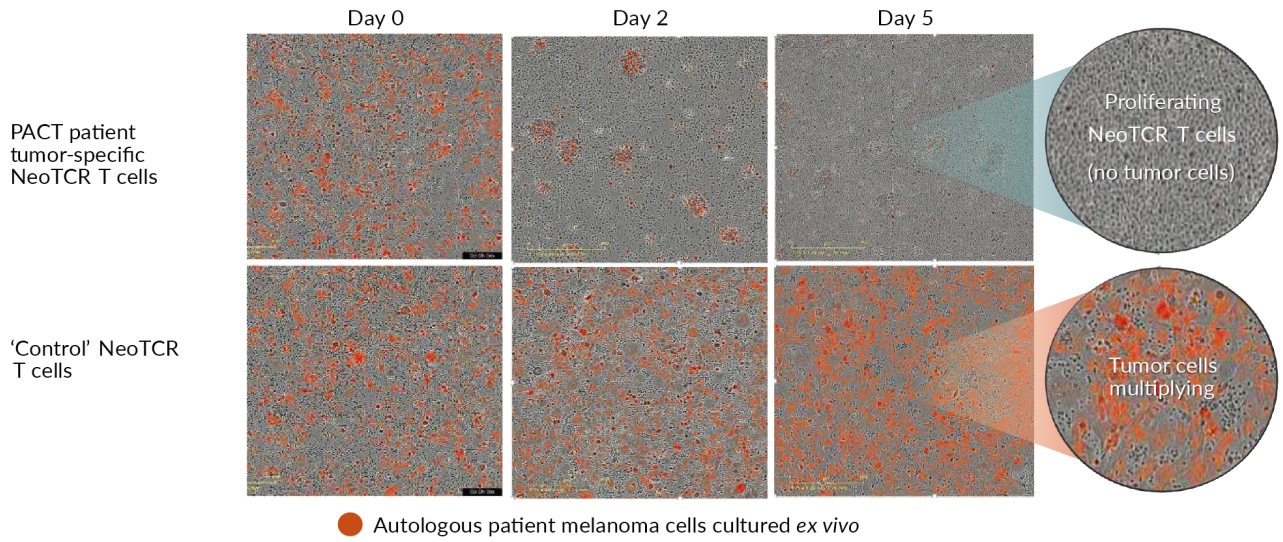
Developing personalized therapies allows the treatment of all patients with cancer. It is a multi-step process, which begins with the determination of patient private mutations. From this information, patient-specific peptide-HLA targets can be predicted. Subsequently, these predictions are prioritized and a library of several hundred peptide-HLAs are expressed for each patient. Each peptide-HLA reagent is then barcoded, fluorescently labeled, and multimerized to be used to capture T cells with receptors (TCRs) that specifically recognize and bind a cancer-specific neoE-HLA. For validation and characterization of the isolated TCRs, fresh T cells derived from a healthy donor are then engineered to express the isolated TCRs and functionally characterized. Validated TCRs can then be used to create either 'off-the-shelf' or patient-specific products.

PACT^NV™, the PACT non-viral precision genome engineering platform

The PACT^NV platform enables single step, targeted and site-specific, non-viral precision

FIGURE 4

PACT^NV engineered NeoTCR-T cells kill patient autologous tumor cells.



Tumor cells that we derived from a patient with melanoma are labelled in red and were cocultured with two separate T cell products. On the top, T cells engineered with a neoTCR specifically selected from this patient for this patient were add. On the bottom, T cells expressing a neoTCR that was isolated for a different patient were added as a control.

genome engineering that creates long-lasting and permanent modifications. This platform uses plasmid DNA-based, non-viral payload delivery, making it less expensive and quicker than a using a viral-based vector. Site-specific nucleases are delivered together with the neoTCR-coding plasmid for knock-in into the to the endogenous TCR-α locus and knock-out of the TCR-β genes. This process is highly reproducible and safe due to the low off-target profile of the site-specific nucleases. PACT's gene delivery system is currently the only clinically validated *ex vivo* non-viral gene delivery platform for GMP manufacture of cell therapy products.

Non-viral gene editing has enabled PACT to have the first and only in-human personalized neoTCR adoptive cell therapy in the clinic. In the small-scale data presented in Figure 3A, very efficient rates of gene editing were observed, with ~90% of cells exhibiting knock-out of the endogenous TCR and 75% of cells expressing the neoTCR. Expression of the neoTCR from the endogenous TCR promoter results in natural TCR regulation and TCR expression levels, as shown in Figure 3B. Knock-in at the TRAC locus has shown beneficial effects on the T cells, both in terms of reduction of variability of expression and resultant increased performance [1].

Importantly NeoTCR T cells are highly active when they engage the cognate tumor target, resulting in cytokine production, proliferation and antigen-specific killing of patient's autologous tumor cells as measured by *ex vivo* assays (Figure 4). As shown in the top panels, by day 5 all of the patient's tumor cells have been completely eradicated while the same tumor cells, when incubated with the neoTCR T cells targeting a neoE from a different patient were not. Important for *in vivo* persistence, the patient's T cells also proliferate (more black cells) as a result of encountering the cognate antigen.

PACT manufacturing & next-generation products

To rapidly generate the reagents required for manufacturing while adhering to a patient-focused timeline, PACT's personalized products are manufactured in-house. These reagents include the GMP plasmid DNA

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which serves as the homology-directed repair (HDR) template and codes for the patient-derived neoTCR. This non-viral process allows PACT Pharma to generate new plasmid vectors specific for each patient's tumor in a time- and cost-effective manner. The HDR template is used in two places: for the functional characterization of the isolated TCRs before product selection and the GMP generated HDR template is used later for the manufacturing of clinical product. Furthermore, PACT's clinical manufacturing process is focused on the generation of a final product in which the majority of the cells are of central memory or memory-stem cell phenotype.

An immunosuppressive tumor microenvironment may require a T cell drug product with added functionalities. The PACT^NV platform is capable of edits in addition to the native-TCR knock-out and neoTCR knock-in and can be used to generate T cells that are resistant to immunosuppression or have additional functions. For example, this system has been used to express short hairpin RNAs (shRNAs), knock-out additional genes, or knock-in additional genes and regulatory elements. The flexibility of this system can also be applied to other cell types such as natural killer (NK) cells and hematopoietic stem cells (HSCs).

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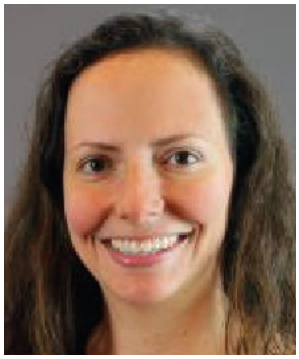
Ask the experts

Evan Zynda, **Nektaria Andronikou** & **Kyle Jacoby** answer your questions.



EVAN ZYNDA

Q Is the DynaCellect equipped to function as a standalone instrument, and is it cleanroom certified?

EZ: DynaCellect is designed to be standalone or integrated into end-to-end workflows with other devices. It is flexible and cleanroom B and C certified.

Q Do the DynaCellect kits come pre-welded to bags with buffers?

EZ: The kits are not pre-welded to bags. Our reason for leaving the tubes open ended is to allow flexibility in the design of the workflow, and to not pigeonhole our customers into pre-set workflows with bags pre-filled with specific liquids at certain locations. This gives customers more flexibility in designing their own processes, if they want.

Q For the DynaCellect, what cell separation protocols are available, or are they user-customizable?

EZ: Currently there is a default isolation protocol that is completely customizable and a bead removal protocol that is customizable as well. The device is designed to have default protocols for process development, which can be locked in later for clinical use.

NEKTARIA ANDRONIKOU

Q What are the major challenges when scaling up the electroporation process? Are there different optimization parameters that should be considered?

NA: When we were looking to scale the Neon technology to develop the Xenon, our engineers identified quickly that the open Neon tip and its submersion in buffer posed challenges to exactly replicate the pulse profile and equivalent performance in the closed chamber of the Xenon. There were pressure and temperature differences in the Xenon consumable when compared to the Neon tip.

The engineering team performed extensive temperature and pressure characterization studies to understand if this would impact functional performance. Keeping those factors in a balance to ensure equivalent performance was one of the biggest challenges during development of the Xenon.

From a biological perspective, optimization is ideally performed with your desired cell type and payload, and critical to identification of optimal performing conditions. From an internal development perspective, during the Xenon development, we discovered that the critical variables were how healthy cells were going into the system and the pulse voltage from the electroporation profile. However, there are many variables that can be optimized, such as the cell and payload type or concentration, the culture media system utilized pre- and post-electroporation, and the post-electroporation cell-seeding density.

Q What are the key variables from an engineering perspective that affect electroporation performance?

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NA: The other factor was to ensure consistent voltage being delivered to the sample during electroporation. Specifically, when scaling up using the MultiShot, we had to ensure there was equivalent performance from each individual shot to be able to have equivalent or superior performance to the SingleShot.

This meant equivalent filling of the MultiShot chamber during electroporation, as well as maintaining pressure inside the chamber to eliminate the risk of arcing. These were critical factors that the engineering team focused on during development.

Q How does the multi-shot cartridge process 150 mL if it can only accommodate 5–25 mL?

NA: To process 150 mL at this time, you would have to use multiple MultiShot cartridges. The upper limit is 25 mL due to the gold electroplating on the consumables. We have plated it to a set thickness to allow for electroporation of 25 mL, and to ensure that the base metals are not exposed.

If you have an upstream cell processing system that is doing the buffer exchange and sample preparation, you can have that sample ready to go and unload the used cartridge and load a new cartridge. We do have efforts in development now that we have launched the Xenon system focused on addressing larger volumes needed for electroporation in some cell therapy applications.

Q Besides CRISPR/Cas9, what other molecules can be used for the Xenon system?

NA: The Xenon system can be utilized with transcription activator-like effector nucleases (TALEN), mRNA, or DNA constructs. mRNA is a very easy payload to get into the cytoplasm itself, and we have even seen high efficiency in difficult-to-transfect cells, like naïve T cells. Fairly small DNA molecules, around 5–8 kbs, work well, but any larger can get tough, especially when you get up to 15 kb.

KYLE JACOBY

Q The comparison of the TRAC targeting approach to the traditional lentiviral approach to generate CAR T cells showed a wider range of expression, but also higher expression. Does this expression profile negatively impact function and is that applicable to TCR therapies?

KJ: We were focused on trying to get natural levels of TCR expression as opposed to the highest levels possible. It is important in the case of CAR T cells, where tonic signaling can negatively impact cells phenotype and lead to cell exhaustion.

However, for TCR T cells, we have seen good cell killing with our product. There was a paper from the Mueller group in *Nature* in 2021 [1] that highlighted specifically that for TCR T cells, the more consistent level of expression translates to improved cell killing both *in vitro* and *in vivo*. It works well for us using this system.

Q Targeted knock-in efficiencies seem to be around 75% in the small-scale data presented, and around 40–50% for manufacturing. Can you speak to this discrepancy, and whether there are limitations to scaling this technology?

KJ: 75% is on the high end of what we typically observe. However, we have also seen these numbers using large-scale systems. The technology is not intrinsically limited when attempting to scale.

Beyond that, there are also trade-offs between efficiency and cell death or cell yield when working with electroporation. As indicated, we are averaging about 40–50%. There are probably additional optimizations that could be done, as the technology is still relatively young.

Q On what day of process are you seeing 75% knock-in rates?

KJ: We start to see TCR expression within about 48 hours after electroporation. 75% is persistent across the process, remaining the same a week or two after transfection. We do not have a competitive growth advantage or disadvantage with the modified T cells.

Q What is the viability after electroporation?

KJ: Viability quickly recovers after electroporation - within a couple of days, and typically within the 90+% range – and that is maintained through culture. Normal cell viability numbers recover quite rapidly.

Q What stem cell-like memory T (TSCM) cell markers do you use to characterize the stem cell population?

KJ: The primary markers we are using are CD45RA and CD62L. We also use CD27 and CD95 for our flow cytometry.

Beyond flow cytometry, we have also characterized our cells by using single-cell RNA sequencing and gene expression sets. Those results also align well with what we see by flow cytometry. Flow markers are not perfect, but they are the tool we have.

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Q Do you need to expand your edited TCR T cells after engineering, or do you directly put the engineered TCR T cells into the patients?

KJ: We do expand the cells after we engineer them, but only for a short period of time. Our total cell engineering process is under two weeks. While we do expand the total cell numbers, we do not select for specific cell populations.

Q When looking at the transfected cells (i.e., the circulating edited cells post-dosing) that have been removed from *the in vivo env*ironment, do they maintain their targeting potential?

KJ: We do analyze T cells recovered from our patients that have been dosed with neoTCR T cells. In fact, we have developed specific probes that allow us to distinguish neoTCR-edited cells from endogenous WT cells with the same TCR. This way, we do NOT have to use additional cell markers like EGFRT. We ensure the cells we are manufacturing only express the neoTCR. We know they bind their epitopes as this is how we identify them initially. Importantly, we found neoTCR T cells in post-infusion tumor biopsies whenever biopsies were available.

BIOGRAPHIES

EVAN ZYNDA has been with Thermo Fisher Scientific for almost 5 years. He serves as a Senior Scientist in R&D for the department of Cell Culture and Cellular Medicine and has been focused on Cell Therapy process development and product development. He first began studying T cell biology in 2005 at Roswell Park Cancer Institute, where he received a PhD in Molecular and Cellular Biophysics and Biochemistry. During his academic years, he elucidated mechanisms by which tumor cells evade the immune system and went on to apply this knowledge in drug development and cell therapy manufacturing.

NEKTARIA ANDRONIKOU is a Senior R&D Manager for Thermo Fisher Scientific's Biosciences Division focused on the development and improvement of mechanical based delivery products for use within cell and gene therapy workflows. She currently leads a small team of innovative scientists working with various primary cell models and payload/cargo types (CRISPR, mRNA, DNA, etc.) to improve non-viral based transfection and delivery. Throughout her time at Thermo Fisher Scientific, Nektaria was an integral member of the cross-functional teams that developed the Lipofectamine 3000, MessengerMAX and Invivofectamine 3.0 transfection reagents. She began her professional career at Ionis Pharmaceuticals (formerly ISIS Pharmaceuticals), as a research associate for the Cardiovascular Drug Discovery program. She received a Bachelor of Science in Biochemistry with a minor in Cellular and Molecular Biology from UCSD. She has been with Thermo Fisher for eleven years and has been dedicated to the understanding and commercialization of improving delivery technologies for clinical, translational and research markets.

KYLE JACOBY is currently the Senior Director of Gene Editing for PACT Pharma, a personalized adoptive T cell therapy company. The Gene Editing group has developed and characterized a non-viral precision genome engineering method to produce PACT's clinical cell products. Prior to PACT Pharma, Dr. Jacoby led the technology development group at Seattle Children's Program for Cell and Gene Therapy. This group was responsible for creating molecular tools and assays for engineered T cell and HSC therapies. His PhD was granted from the University of Washington's Molecular and Cell Biology program for work on the discovery and characterization of nucleases for genome engineering.

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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.*

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FASTFACTS

Driving CAR-T from early-stage development to clinical filing and lot release

Ulrike Herbrand, Julia Schüler & Sophie Vermond, Charles River Laboratories

***Ex vivo* gene therapies such as Chimeric Antigen Receptor T cells (CAR-T) and T Cell Receptor T cells (TCR-T) have emerged as promising cancer therapies and more continue to move into the clinic. While T cell therapies are novel and, in some cases, curative, they can have formidable limitations leading to a complex development process. For CAR-T cell therapy products, for example, this spans all stages of the pipeline, from CAR design to clinical trial filing.**

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BACKGROUND

To date, most success with CAR-T cells has been booked in liquid tumors. More challenges arise for CAR-T cell therapies for solid tumors, such as CAR-T cells penetrating and surviving in the tumor microenvironment or losing target antigen expression. In addition, even though CAR-T products can have high specificity and sensitivity, solid tumor target antigens are often expressed at low levels in healthy tissue as well, which can have a serious impact on the safety of the product due to on-target, off-tumor activity.

Therefore, in preclinical development of engineered T cell therapies, it is critical to establish and use high-quality, well-characterized materials, to develop and conduct *in vitro* efficacy and safety studies complementary to *in vivo* research for facilitating clinical approval. As the number of cellular immunotherapy products in clinical development increases, so does the need for an effective preclinical process to evaluate them, and a single integrated contract partner to streamline supplier management and more importantly help developers efficiently transition from scientific discovery to market.

Figure 1. CAR-T cell therapy case study overview.

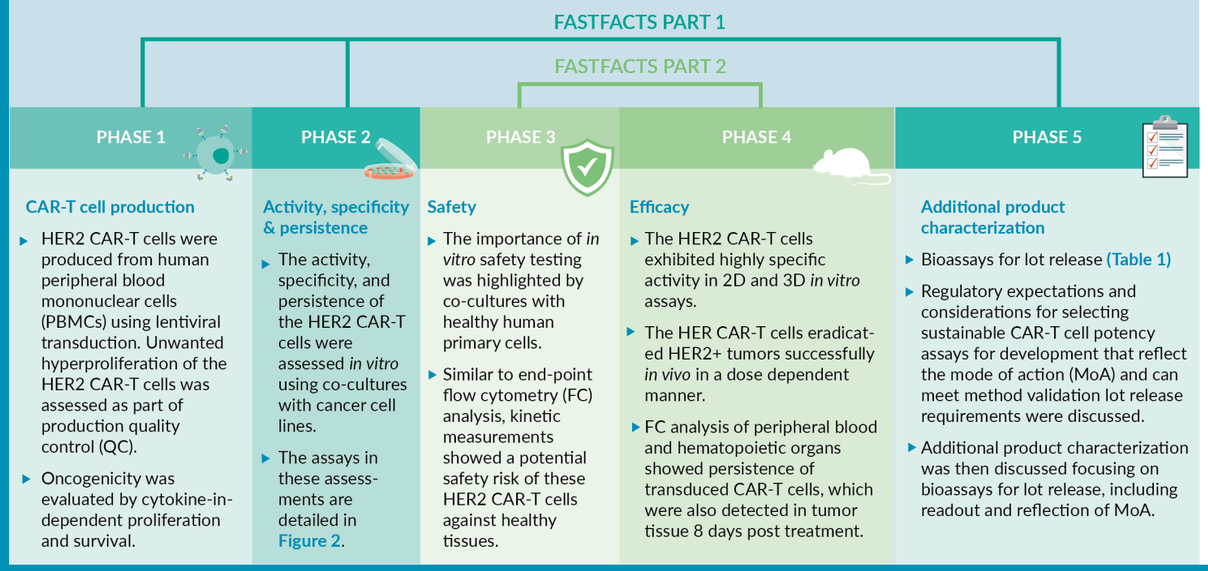
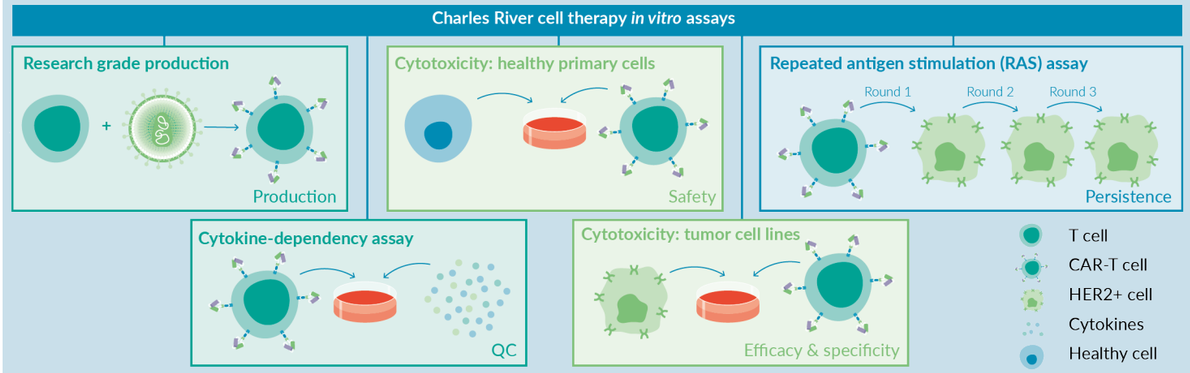


Figure 2. *In vitro* assays for activity, specificity, and safety.



CELL THERAPY CASE STUDY OVERVIEW

This two-part FASTFACTS series uses HER2-targeting CAR-T cells as a model system for targeting solid tumors (Figure 1). This model was developed and used by Charles River and is broadly applicable for cell therapy development with the aims to guide cell therapy developers on their path from early-stage development to clinical filing and lot release. This series also highlights how an integrated cell and gene therapy outsourcing partner, like Charles River, addresses needs across the different cell therapy development stages: from consistent, high-quality starting material supply, to lead optimization to cell engineering, manufacturing, and characterization, followed by *in vitro* and *in vivo* efficacy and safety assessments, and lastly, with lot release testing.

Table 1. Biological activity testing for CAR-T product lot release.

Assays	considerations
Cell surface marker expression	T-cell activity reflection but not truly MoA-reflective
Cytokine release	
Cell death	Highly MOA reflective

BENEFITS OF AN INTEGRATED OUTSOURCING PARTNER

In addition to sharing this model to help the market progress, Charles River is a comprehensive development and manufacturing outsourcing partner offering an integrated cell and gene therapy solutions platform that can support the entire development lifecycle, from high-quality material supply, including product characterization, preclinical evaluation through to human clinical trial enabling studies assessing potential toxicity risks, and manufacture and release for transition to the clinic.

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CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES:

CELL & GENE THERAPY INSIGHTS

INDUSTRIALIZING IMMUNOONCOLOGY MANUFACTURE & SUPPLY CHAIN

SPOTLIGHT

EXPERT INSIGHT

A new era of *in vivo* gene therapy: the applicability of a differentiated HSV-1 based vector platform for redosable medicines

Trevor Parry, Domenick A Prosdocimo & Suma Krishnan

Viral vector-based gene replacement approaches have traditionally focused on the use of adenoviruses, adeno-associated viruses, and lentiviruses for functional gene transmission. Innovation in payload delivery is critical for advancing the boundaries of genetic medicine. While underappreciated, herpes simplex virus type 1 (HSV-1) possesses a number of natural traits that make it an attractive alternative for gene therapy approaches, including episomal delivery, large payload capacity, a broad tissue tropism, and the ability to resist immune clearance via inhibition of innate and adaptive anti-viral immunity. Krystal Biotech has created a proprietary HSV-1-based gene delivery platform leveraging many of the natural properties innate to HSV-1, while engineering it to be replication-incompetent to reduce cytotoxicity. This platform has been validated clinically in dermatology, and its utility is being extended into programs across additional tissue types and organ systems, including initiation of a genetic pulmonary program in cystic fibrosis. This differentiated vector platform provides a broadly applicable, highly versatile gene delivery system for the development of direct and redosable genetically-coded medicines.

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The United States Food and Drug Administration (FDA) defines gene therapy as a means to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use [1]. An effective gene therapy approach requires efficient delivery of genetic material to target tissues or cells through the use of viral or non-viral vectors [2]. Central to the realization of gene therapy's potential is the advancement of vector technology to overcome the physical and immunological barriers to repeated gene delivery. While non-viral vectors may be an attractive future approach to gene therapy given their low cytotoxicity, reduced immunogenicity, and minimal risk of mutagenesis, the broad use of non-viral vectors has yet to be realized given the ongoing challenges related to gene transfer efficiency, gene expression duration, and safety [3]. In contrast, the use of viral vectors, which are the focus of this article, are the more commonly utilized tools for gene therapy given their evolutionarily-derived attributes of high efficiency gene transfer and specificity to target cells [4]. Viral vector-based gene replacement approaches have traditionally focused on the use of adenoviruses (Ad), adeno-associated viruses (AAV), and lentiviruses (LV) [5]. These viral vectors consist of a protein capsid and/or envelope that encapsulates the genetic payload, the transgene of interest, and regulatory elements that control stable or transient expression of the transgene as an episome or integrated into the host chromosome [2].

Adenovirus

Adenoviruses are non-enveloped viruses with double-stranded DNA that naturally cause infection of the upper respiratory tract. Their use as a vector in gene therapy was initially thought to be attractive given their payload capacity (4.5–6.5 kilobase (kb) transgene cassettes), high transduction efficiency (both in quiescent and dividing cells), epichromosomal persistence in the host cells, and broad tropism for various tissue targets [2, 6]. However, recent evidence suggest Ad vectors may have the potential to integrate into the host DNA through random (nonhomologous) recombination, thus raising safety concerns of their use in gene replacement approaches [7]. Further, the early generation Ad vectors proved to be highly immunogenic due to the innate immune response initiated by exposure of the host to the virus capsid protein resulting in severe cytokine storm [8–9]. This was evidenced in a 1999 clinical trial where a trial participant died from complications of Ad vector administration resulting in systemic inflammation and multiorgan failure [10–11]. While recent generation Ad vector-based vaccines and oncolytic therapies benefit from this intrinsic immunogenicity and cellular toxicity, these properties continue to limit their use as a treatment modality for genetic disease [2].

Adeno-associated virus

Adeno-associated viruses are generally recognized as versatile vectors for gene therapy given their wide-ranging tropism profiles. Indeed, a significant majority of gene therapy development today is based on AAV vectors [2, 12]. AAVs lack the essential genes needed for replication, and they undergo circularization via inverted terminal repeat (ITR) recombination to form stable and persistent episomal configurations [2]. While there have been over 200 clinical trials based on AAVs worldwide, their limited transgene payload capacity (<5 kb transgene cassettes) and inherent immunogenicity are standing challenges in the field [2]. Regarding the latter, host adaptive immunity to the capsid results in reduced AAV efficacy [13–14]. Moreover, while early studies suggested AAVs do not integrate into the host genome, additional evidence has suggested AAVs (*e.g.,* AAV2) may cause insertional mutagenesis in humans [15–17]. While AAVs have been shown to be relatively safe in humans, resulting in the U.S. FDA approval of two AAV-based gene therapies (*i.e.,* Luxturna® (voretigene

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neparvovec-rzyl) and Zolgensma® (onasemnogene abeparvovec-xioi), additional genetic engineering of AAV vectors is needed to begin to address the issues associated with AAV integration, as well as pre-treatment immunity due to the presence of neutralizing antibodies against serotypes commonly found circulating in the population [18–19].

Lentivirus

Lentiviruses (LVs) are part of the retroviridae family of single-stranded RNA viruses [20]. LVs possess a payload capacity of up to 9 kb and integrate into the host genome in both dividing and non-dividing cells, with a preference for transcriptionally active sites, allowing for long-term transgene expression [21–22]. With over a dozen completed clinical trials, the use of LV vectors appears well-tolerated [2]. However, LV-based approaches to gene therapy are primarily limited to *ex vivo* delivery due to the potential for insertional mutagenesis and the associated risk of cancer development inherent to their use [2, 23]. Moreover, recent reports suggest LV vector integration can activate neighboring genes, promote chimeric gene fusions, and may cause aberrant splicing of cellular transcripts, raising additional concerns about the oncogenic impact of such integration [24–26]. The FDA has also recently placed a clinical hold on a LV-vector based gene therapy approach for the treatment of cerebral adrenoleukodystrophy, as one participant reportedly developed myelodysplastic syndrome likely associated with LV treatment, highlighting the potential risks associated with uncontrolled LV integration into the host genome [27]. However, groups are exploring the use of non-integrating lentiviruses in the preclinical setting with the intent to circumvent the risk associated with integrating LVs currently used clinically [28].

There remains an unmet need for a gene therapy platform that addresses a number of the challenges faced by Ad-, AAV-, and LV-based genetic medicines, such as vector integration into the host genome, pre- and post-treatment neutralizing immunity, and limited payload capacity.

HERPES SIMPLEX VIRUS AS THE BASIS FOR KRYSTAL BIOTECH'S GENE THERAPY PLATFORM

Herpes simplex virus type 1 (HSV-1) belongs to the human herpes virus (HHV) family of double-stranded DNA viruses. Of the known HHVs, HSV-1 is the best characterized given that it is highly prevalent in the human population, with estimates suggesting that more than two-thirds of those ≥12 years of age in the US have been exposed to the virus [29]. The HSV-1 virion is ~220 nm in diameter with a linear, double-stranded DNA genome that circularizes upon cellular infection. Importantly, the HSV-1 genome remains fully episomal and does not integrate into, or otherwise disrupt, the host genome [30–31].

Upon cellular infection, the cascade of HSV-1 gene expression that ultimately leads to replication, which is necessary for lytic disease and secondary neuronal infection/spread, is a tightly controlled temporal process. This begins with expression of the five immediate early (IE) genes (Table 1), which are a focus in vector development given their essential role in both replication and immune evasion. Subsequent expression of early and late genes, and consequent assembly of fully infectious virions, is entirely dependent upon the expression of the IE genes. In humans, HSV-1 efficiently resists immune clearance, partially explained by the innate immune-evasive properties of HSV tegument proteins and the observations that HSV-1 has evolved a number of genes devoted, at least in part, to inhibiting both innate and adaptive anti-viral immunity [32–33].

While underappreciated as a gene delivery platform, HSV-1 addresses a number of challenges faced by other vector technologies currently utilized in gene therapy. HSV-1 is known to resist immune clearance and does not induce broadly neutralizing antibody

TABLE 1

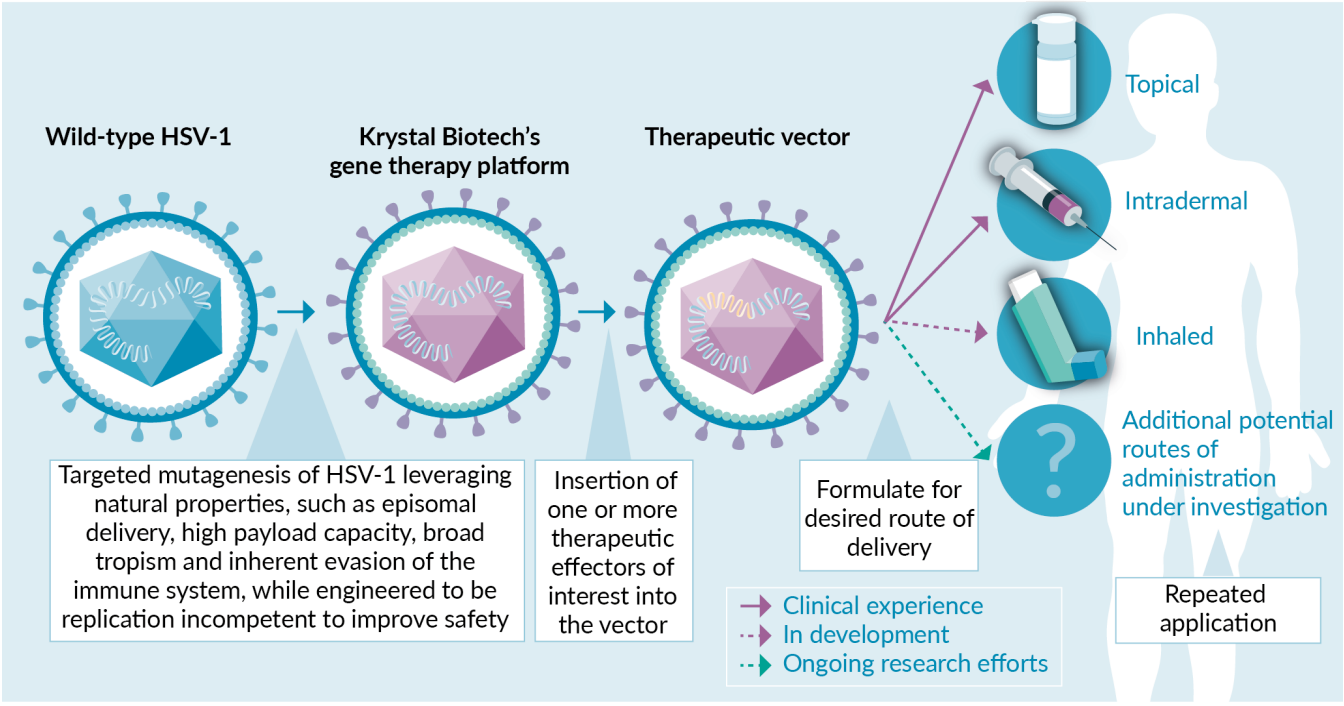
HSV-1 IE gene products and their functions

HSV-1 IE genes	Function
Infected Cell Protein (ICP) 0	Activates viral promoters to support downstream viral replication, plays a role in evasion of the innate immune response via inhibition of multiple pathways, and promotes cell cycle arrest and apoptosis [34–36]
ICP4	Obligate transactivator of downstream viral gene expression and HSV-1's lytic cycle [37]
ICP22	Plays several roles in viral gene expression, cell cycle control, viral assembly, and nuclear egress [38]
ICP27	Regulates viral gene expression through multiple mechanisms including splicing regulation, processing, and mRNA export, and plays a role in evasion of the innate immune response [39]
ICP47	Not regulatory but instead prevents immune recognition and destruction of HSV-1 by binding to transport associated protein, which prevents antigen loading of MHC class I molecules [40]

responses [32, 41]. It has a genome size that easily accommodates large or multiple genes, and it can transduce both dividing and non-dividing cells without integrating into, or otherwise disrupting, host cell DNA [31]. In addition to its native sites of infection, the skin and mucosa, the ubiquity of HSV-1 entry receptors on myriad human cell types raises the possibility of efficient delivery to multiple tissues and organ systems upon targeted administration [42]. Krystal Biotech has combined many of the beneficial properties inherent to HSV-1 with a modification strategy of targeted IE gene deletions in ICP4 and ICP22 to render the vector replication-incompetent and less cytotoxic resulting in the development of an *in vivo*, non-invasive, and redosable vector platform suitable for localized gene delivery (Figure 1). Also, Krystal Biotech's products are manufactured using fully characterized virus and cell banks, and thus, do not suffer from the same inefficiencies inherent to multiple plasmid-based transfection methodologies utilized in the production of

FIGURE 1

An overview of Krystal Biotech's gene therapy platform approach.



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AAVs or LVs. Further, because of the targeted IE gene deletion in ICP4, the HSV-1 vector does not grow in non-complementing cells lacking exogenous ICP4. As an additional precaution, Krystal Biotech's vector engineering strategy was specifically designed to maintain sensitivity to commonly prescribed antiviral medications (*e.g.,* acyclovir and valacyclovir) targeting herpes viruses, to address the extremely unlikely event that herpetic lesions or other viral-associated disease manifestations were to appear in a patient exposed to the modified virus.

CLINICAL VALIDATION OF KRYSTAL BIOTECH'S GENE THERAPY PLATFORM IN RARE SKIN DISEASE

Dystrophic epidermolysis bullosa (DEB) is a serious, ultra-rare genetic blistering disease caused by mutations in the *COL7A1* gene, encoding type VII collagen (COL7) [43–44]. Pathogenic mutations to *COL7A1* result in absent or dysfunctional anchoring fibrils and loss of adhesion of the epidermis to the dermis [45–46]. DEB is characterized by skin fragility that leads to widespread, painful, and lifelong recurrent blistering [45–47]. Patients with DEB are at increased risk for serious complications, including aggressive squamous cell carcinoma [44, 48–53]. A significant unmet need exists for therapies to molecularly correct the underlying cause of DEB.

Beremagene geperpavec (B-VEC) is an investigational topical, redosable gene therapy based on Krystal Biotech's vector platform that is designed to restore functional COL7 via delivery of full-length *COL7A1* genes [41, 54]. Preclinical data demonstrated that B-VEC efficiently restored COL7 expression in recessive DEB (RDEB) primary skin cells, as well as in a diseased animal model, demonstrating its capability for therapeutic gene delivery [41]. In an open-label, placebo-controlled Phase 1/2 clinical study (GEM-1; NCT03536143), repeated application of B-VEC resulted in full-length COL7 protein expression and anchoring fibril formation in nine patients with RDEB. Notably, B-VEC was well tolerated, and wounds treated with the vector demonstrated improvement in closure compared to placebo at 3 months [41]. A Phase 3, double-blind, placebo-controlled, intra-patient-randomized study (GEM-3; NCT04491604) evaluating the efficacy and safety of B-VEC in patients with DEB has been completed [54].

Autosomal recessive congenital ichthyosis (ARCI) is a life-long, severe genetic skin disease resulting from germline mutations in the *TGM1* gene encoding tranglutaminase-1, a protein essential for proper formation of the skin barrier [55]. Patients with *TGM1*-associated ARCI are typically born in a collodion membrane and develop plate-like scales on the skin following the shedding of the collodion membrane, [56]. Disease complications include pronounced dehydration, increased risk of infection, and a significantly decreased quality of life [56].

KB105 is a second investigational, topical, redosable gene therapy candidate developed using the modified HSV-1 vector platform via insertion of the functional form of *TGM1*. Preclinical data demonstrated that KB105 efficiently transduced ARCI patient keratinocytes *ex vivo* and barrier-impaired mouse skin *in vivo*, resulting in human *TGM1* expression [57]. A Phase 1 exploratory, open-label, placebo-controlled, intra-patient study (NCT04047732) evaluated three adult patients with a genetic diagnosis of *TGM1*-deficient ARCI to understand the safety and preliminary efficacy (molecular correction, phenotypic improvement) of KB105 [56]. Repeat dosing was well-tolerated with no drug-related adverse events or immune response to HSV-1 or TGM1. Treatment with KB105 restored functional TGM1 protein, which was correctly localized in the epidermis. Areas treated with KB105 showed reduced reversion to the ichthyotic scaling phenotype; however, phenotypic evaluation was limited by small treatment areas and these observations need to be confirmed in larger

studies. The Phase 2 portion of this study is ongoing.

DERMATOLOGY BEYOND RARE SKIN DISEASE

Given clinical substantiation of the underlying vector technology, there existed a unique opportunity to differentiate Krystal Biotech's platform beyond the traditional confines of the therapeutic setting.

Dermal collagen represents >90% of human skin and is composed primarily of type I collagen (COL1) and type III collagen (COL3) fibrils which provide strength to the skin and are critical for the maintenance of skin tissue architecture. COL3 appears early during collagen fibrillogenesis, and its subsequent replacement by COL1 is a critical step for collagen fibril maturation and extracellular matrix reorganization [58]. Due to the essential role collagen plays in the process of skin biorejuvenation, and the diminution of dermal collagen being the primary contributor to the aged phenotype, direct and indirect collagen stimulation/supplementation/replacement has been the focus of aesthetic product development for much of the last four decades. However, directed supplementation of functional full-length human COL3, produced by and secreted from the patient's own dermal cells, has not been explored clinically to treat superficial skin depressions.

Jeune Aesthetics, Inc., a wholly-owned subsidiary of Krystal Biotech, is evaluating KB301, an investigational aesthetic product based on Krystal Biotech's differentiated HSV-1 platform, encoding the *COL3A1* gene. A Phase 1 study with two cohorts evaluating the safety, tolerability, and preliminary efficacy in adults (PEARL-1; NCT04540900) has been conducted. In cohort 1, repeated intradermal injection of three different doses of KB301 were evaluated in seven healthy subjects, demonstrating tolerability with no clinically significant immunogenicity findings [59]. Cohort 2 was a randomized, double-blind, placebo-controlled assessment of the safety and preliminary efficacy of KB301 for the improvement of fine lines and skin texture in the lower and upper cheek, and for the improvement in skin thickness on the knee [60]. Twenty-seven adult patients were enrolled with the treatment side randomized 2:1 to receive KB301 or placebo as multiple micro depot injections. Low dose KB301 was evaluated in the knee. Low dose or high dose KB301 was evaluated in the lower cheek and low dose KB301 was evaluated in the upper cheek. Repeat administration of KB301 was well-tolerated with minimal injection site reactions, all resolving within 3–5 days. Treatment with KB301 also demonstrated clinical benefit versus placebo, including improved Subject Satisfaction Scores across all three treatment areas. Safety and efficacy of KB301 will be further evaluated in a Phase 2 study.

Following clinical proof-of-concept in skin to address both monogenic disease and aesthetic protein supplementation, Krystal Biotech's platform is now being investigated more broadly, including for its ability to deliver non-traditional effectors for genetic medicine. Given skin being the initial focus as a target tissue, the first departure from traditional gene replacement was an attempt to treat chronic, complex skin indications with vectors encoding synthetic constructs. Preliminary efforts in this regard focused on recombinant HSV-1 vectors to deliver and locally express therapeutic antibodies. A library of such vectors was engineered, including candidates encoding single-chain antibodies targeting TNFα, IL4-Rα, and IL-17, and preliminary *in vitro* and *in vivo* efficacy was demonstrated, including in multiple murine atopic dermatitis models [61]. The use of Krystal Biotech's vector platform, which enables effector expression at the site of application without systemic vector exposure and the ability to re-dose over time, could be particularly attractive for these alternative payloads. Discovery-phase exploration of vectors designed to deliver RNAi and gene editing machinery are under investigation.

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ADDITIONAL PLATFORM APPLICATIONS OUTSIDE OF THE SKIN

Cystic fibrosis (CF), a disease characterized by chronic pulmonary infections, increased airway secretions, and eventually respiratory failure, is the most common inherited genetic disorder in the United States [62]. Targeted delivery of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene to address the diseased phenotype, employing both viral and non-viral gene therapy approaches, have been explored extensively but have suffered from some combination of limited capacity to encode a large effector like *CFTR*, toxicity to the administered epithelium, robust immune system activation upon single or repeated exposure, and/or inefficient gene transfer to the apical (air-exposed) membrane of polarized airway cells.

Krystal Biotech is investigating the use of its gene delivery platform for the treatment of CF with KB407, an investigational therapeutic encoding two copies of full-length human *CFTR* [63]. Preclinical pharmacology of KB407 indicated that the vector capably transduces relevant primary CF patient airway epithelial cells in 2D culture, efficiently produces functional human CFTR protein, molecularly corrects multiple CFTR defects without significant toxicity in a clinically relevant 3D organotypic system, and effectively directs localized expression of human CFTR in mice and non-human primate lung epithelium. Krystal Biotech believes that direct supplementation or replacement of full-length human CFTR upon nebulization of KB407 presents a unique opportunity for safe, non-invasive, and mutation-agnostic molecular correction of CF. A Phase 1 study is planned to commence in 2022.

CONCLUSIONS

Much progress has been made to advance gene therapy over the years; however, it has also shed light on limitations of commonly used *ex vivo* and *in vivo* viral vectors. Krystal Biotech recognized the attributes of HSV-1 as the basis for a gene delivery system, established a differentiated vector platform through targeted modification to render the virus replication incompetent and less cytotoxic, and has clinically validated it in multiple dermatologic conditions, including a Phase 3 study of B-VEC in DEB. During the course of developing B-VEC from concept to clinic, there was a growing recognition of the potential applicability of the underlying vector technology in other skin conditions as well as additional organ systems. Evidence to date suggest this proprietary platform holds promise in the development of broadly applicable, redosable gene therapies that can be delivered via minimal- to non- invasive routes of administration.

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INNOVATOR INSIGHT

Removing technological barriers to efficient large-scale LV vector production

Charlotte Barker speaks to Scott Jeffers, Emily Jackson-Holmes, Rakel Lopez de Maturana, Steve Milian & Margherita Neri

SCOTT JEFFERS is the Chief Technology Officer at Gensight Biologics. He has worked with numerous biotech and pharma companies to bring novel gene therapies to the clinic both at clinical and commercial stages, for rare monogenetic diseases. Scott leads teams that utilize novel techniques and approaches including single-use suspension and adherent cell bioreactors, and adherent cell culture in Hyperstacks to produce viral vectors, such as AAV, adenovirus, lentivirus, retrovirus, and herpes virus.

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MARGHERITA NERI is the Vector Process Development Manager at AGC Biologics. She has considerable knowledge and experience in the design of vector production processes and at AGC she is involved in the process development and industrialization of large-scale production of lentiviral and AAV vectors in compliance with GMP requirements.

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As demand for lentiviral (LV) vectors for both *ex vivo* cell-based and *in vivo* gene therapy applications grows, the question of how to make LV vector processing faster, more productive, and more cost-effective becomes increasingly pressing. In this panel discussion, LV processing and quality experts from across the biotech, CDMO, and solution provider sectors will discuss how recent technological innovations in specific upstream and downstream LV process steps compare in terms of their impact on titer, process speed, and cost.

Q What can you share about your own experiences of seeking higher LV titers and improved process speed and cost through your choice of bioreactor and upstream production platform?

EJ: When developing products for upstream LV production, we use suspension-based systems, because they are advantageous in terms of scale-up, in addition to reducing variability and cost. To increase titer within the suspension system, we have used design of experiment (DoE) to optimize concentrations and timings of each component in the process, including the mammalian cell transfection process, cell line, transfection reagent, plasmid DNA, and any enhancers or supplements. This has resulted in a successful, optimized system that significantly increases titer and reduces cost.

MN: At AGC, the priority for our platform is to have good scalability between the full-scale and the small-scale processes. We chose Bioreactors for scale-up that have a fully representative scale-down model system. We have experience in scaling up to 200 L, with any major challenges being resolved at the small scale by applying DoE for all critical steps.

RL: From my experience, there are critical process parameters and key steps in production that we can optimize. For example, we can increase titers simply by changing the producer saline.

Q What recent technological innovations are having the greatest impact on downstream LV processing?

SJ: It is important to understand the full process and process steps to best gain efficiency. If you have a large-scale production in your suspension system, you have a large volume on the first-capture step and the filtration steps, and every step in between. Using DoE to understand your steps, inputs, and outputs is important. Analytics are also highly important in having tight control over your outputs.

MN: The downstream side for LV is challenging, particularly because of the ~0.1µ dimensions of the LV. The most critical step is the final sterile filtration, where a large part of vector preparation is often lost. Clients frequently ask for more concentrated vectors. From a CDMO perspective, we must balance the concentration with the yield of the final sterile filtration. The more concentrated the vector, the more aggregation in the vector preparation, and the greater difficulty in balancing sterile filtration. Improvement in the analytical possibility to evaluate the vector aggregation will be important to solve the downstream challenge. Recently, many new membranes and resins for purification have become available on the market, and we are testing these to improve LV purification.

RL: There have also been advances in the fields of affinity, size-exclusion, and ion-exchange chromatography. Quick analytics that enable definition of critical process controls and use small-sample volumes are key. Automation in fill-and-finish and other processes allows better stability.

Q What scales have you reached for suspension? Either for transfection or stable cell line?

EJ: For transient transfection suspension, we have reached 50 L in-house, but we do have customers reaching as high as 200 L with our products. When dealing with transfection at that scale, we have optimized different aspects, including the timing of the complexation, keeping the reagent cold, and the mechanics of adding the complex to the bioreactor.

Q What analytical tools are helping you to improve the identification and measurement of critical quality attributes (CQAs)?

SM: Some of the common tools that are currently deployed include digital PCR (dPCR), quantitative PCR (qPCR), P24 assays which are typically ELISA-based, and flow cytometry. Without a doubt, dPCR and qPCR are the bread and butter for LV analytics, giving several CQAs such as genome titer, infectious titer, and residual DNA. These methods can also provide us some insight into the average number of genes of interest (GOIs) that have integrated into a cell line, which is an important CQA.

Genome titering has been challenging in the past, mostly because of residual levels of the genome of interest (GOI) that can lead to an overestimation. Strategies to overcome this include optimizing the endonuclease step to remove residual DNA. This is useful for quick turnaround results, compared to the cell-based infectious unit (IU) titer methods.

There are several commercially available kits for P24. They bring some challenges, such as a lack of differentiation between free P24 and particle-associated P24. This is an important consideration, as certain processing steps can damage the virus and give erroneous titers. Commercial vendors are working on this, but there is certainly some room for improvement.

Lastly, flow cytometry is another important method, mostly used in potency assays. Some of the challenges in flow cytometry revolve around data analysis and ensuring proper gating parameters.

MN: Regarding analytics, in our CDMO we have a strong interaction between process and analytical development. Together, we define the main parameters that we want to monitor in our process. According to these, we decide the best method for each step. Each step is always monitored, with orthogonal assays for each CQA. For LV vectors, the main test for potency evaluation is infectious viral titer. The analytical method for this assay must be robust, reliable, and reproducible across labs, in order to control the consistency of the vector production processes. If the vector is applied for *ex vivo* gene therapy, we need to use a cell line for infectious viral titer evaluation that is transducible with the same efficiency of the target cells (e.g., HSC or T cells). To have a robust method, it is important to have good reliable cell line stock, and a positive control. To have a reliable test, we need to control all these parameters, starting from the beginning of the development of the process, to ensure the same robust method is used throughout.

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EJ: From our perspective, we use analytics primarily when developing new cell lines and reagents to monitor and validate the changes and improvements we make. We look at typical things such as particle titer, genome titer, and infectious titer, with challenges including variability of assays.

RL: We use conventional methods, such as PCR and ELISA by default. There are new tools now, such as equipment based on microfluidics or optics, which are beginning to be used more frequently, together with conventional methods. Automation of both new and traditional assays is helping us to use a smaller sample volume and get quicker analytics.

Q What is your opinion on full versus empty analysis in LV vector manufacture?

SM: It has not been a priority as it has been in AAV, primarily because, up to now, LV has been mostly *ex vivo*. Considerations about the load of empty particles into a patient are not as high, but there are tools out there, including high-performance liquid chromatography (HPLC)-based methods, being used for this. As we do start to move more into *in vivo* therapies for LV, this will become an important CQA.

Q For analytical requirements, is total particle count important?

SM: It is certainly an attribute that we measure using P24 ELISA or other methods. Even though we are mostly focused on infectious titers, understanding the particle-to--activity ratio goes back to the question of empty versus full.

Q What are the considerations and best practices to ensure robustness relating to assay selection and evaluation?

MN: When we select an assay, even if it is based on a commercial kit, for example the P24, we need to exercise qualification to reduce further variability of the analytical methods. It is important to take into consideration interference studies in the process, as in each step, the vector is in a different media or buffer. These buffers could affect the results, so we must evaluate this interference to ensure that the analytical methods are fully reliable and reproducible.

SJ: The analytics with LV must be robust, quantifiable, qualifiable, and eventually validatable in commercial productions. Knowing your assays, how they work, and what the pitfalls are is paramount.

SM: The big question is, 'do we have the assay under control'? We want low variance and high repeatability. One of the most important attributes of the assay is the ability to have different people do it – on different days, using different instruments – and still get the same answer. When assays are performed incorrectly, we should be investigating the impact of those changes on the assay itself to build a better understanding of how robust the methods are. If we notice that small changes are dramatically impacting the assays, it hints that the assay is not robust. We need to start building a library of investigations, to determine what are the critical parts of an assay and how they can be negatively impacted.

RL: In terms of analytics and how they contribute to process development, infectivity and viral titer is the key parameter. It is key to control the limits of the assay, and then identify the factors that affect the assay.

Q What will be the key next steps for bioprocess and analytical innovation in the LV field to drive further scalability and quality/consistency improvements?

SM: The need for rapid in-process analytics for viral vectors still remains. This is a particular concern due to the fragility of the LV particles themselves. We want to minimize hold and processing times as much as possible and to do so we need rapid, reliable analytics. Focusing on rapid particle titering or GOI titering is going to be a key driver for scalability, product quality, and consistency.

The introduction of commercially available 'off-the-shelf' solutions is also going to be an important part of developing consistent manufacturing processes, helping to standardize the analytics across the industry. Right now, the use of different methods makes it difficult for us to make comparisons and causes challenges for regulators in setting industry guidance.

RL: There are three key components for bioprocessing. One is the development of more producer cell lines to increase productivity. Second, automation is key, both in process and analytics. The third key point is the development of new serotyping strategies that better target the cell to be transfused, and the development of transduction enhancers. This is key to developing more cost-effective processes so that more patients can be treated.

MN: Another step, from a process point of view, is the reduction of the dead volume in downstream instruments. The systems that are now on the market for concentration have been created for large-scale processes and the dead volume in the instruments is limiting the vector concentration. Suppliers should help us with having more flexibility in size and measure for the single-use instruments.

EJ: One key aspect is ensuring early setup with products and tools that have a clear path to commercial launch. Historically, a challenge in this space has been the lack of fit-for-purpose reagents. Starting early in development with serum or animal-containing components makes things more complicated from a downstream processing or regulatory point

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of view. Therefore, the components in a process that are intended to be used in cell and gene therapy applications must be identified and come with appropriate product documentation to maintain a streamlined path to the clinic.

Q Which parameters do you need to optimize for scaling up your process?

EJ: Optimizing the density the cell line is grown at and transfected at are both key. In our experience, there are many aspects to optimize at the transfection step, such as plasmid DNA, ratios of plasmids, the timing of complexations depending on transfection agent, and harvest time. We see various impacts from optimizing each of those steps, including benefits in terms of the titer.

Q How might the evolving regulatory landscape impact the picture?

RL: In my experience as QP, as viral production has developed and knowledge has increased, the number of applications for gene therapy has also increased. Regulatory bodies are increasing their demands for process control and products of a higher quality. This directly impacts the standards for LV manufacture and prioritization. We need to control more CQAs to tighter certifications. We are looking for products of better quality, with fewer contaminants.

SM: Regulators want us to demonstrate our processes are controlled, both in the consistency of processing and in product safety and quality. We must consider and monitor the residuals that end up in the product due to the process that we have. Regulators require safety first and foremost, which should be our major focus.

SJ: The landscape has changed tremendously over the last 5 years. Regulators have placed great importance on quality and ensuring that we are monitoring our processes. Even from the early stages of process development, it is recommended to think about the final stages of commercialization. Understanding your process, with quality in mind from the beginning, is important to ensure you can get through the regulatory pathways. This ensures patients are safe, which is of primary importance.

Q What are the key challenges to overcome to enable greater automation – for example, in leveraging in-process analytics?

MN: There has been good innovation within in-line process controls, including the recent Raman spectroscopy technologies. This kind of technology, despite its promise, is very demanding in terms of resources to be invested. It needs a dedicated team to interpret the data and evaluate the metabolites that best correlate with the CQAs. We are now

scouting and evaluating these new technologies because they offer an interesting opportunity to expedite process development.

Q What is your experience in trying to implement greater automation levels in the manufacturing process?

SJ: To make the best possible analytical technology from beginning to end, thinking about the future is important. Understanding the process and having a way to look at every parameter, from the bioreactor to the chromatography, is ideal. We want to move towards full automation of processes.

SM: One of the largest barriers to entry is cost. Instruments are expensive and one key challenge we have right now is being able to deploy these instruments. The second thing is matrix interference as a key barrier to ensuring that we have reliable process analytics, especially in upstream bioprocessing. This can be overcome, but there is potential for miscalibration or poor data due to matrix issues.

RL: Automation is expensive, but if it is correctly implemented, it is cost-effective. It should be seen as an investment decision by the company. It must be qualifiable, validated, and on time.

Q In downstream processing of LV vectors, is tangential flow filtration (TFF) done before chromatography, and what kind of chromatography do you use to purify and concentrate your vector?

MN: Regarding chromatography for LV vectors, we now use anion exchange chromatography. To ensure GMP, we use ready-to-use columns. Regarding TFF, our approach has been to develop the adherent process with a hollow fiber step after chromatography as an additional vector concentration step. For the suspension system, we are evaluating the addition of a first fiber concentration step at the beginning of the downstream in order to reduce the volume of the bulk vector prior to proceeding with purification. This step could be essential when the USP scale will increase to 1000-2000 L or more.

Q When it comes to chromatography, which specifications are important, and where do you see gaps in the currently used media?

MN: For the development of the chromatography step, we consider the quality of the vector that we obtain. The suspension after clarification is still rich in proteins and host cell DNA, so there is a need for a stronger endonuclease step. We are working on the chromatography, fine-tuning the amount of resin needed for the vector. At the end of the chromatography step, we are looking at yield, in terms of physical particles, and the infectivity

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and stability of the vector. Moreover, we evaluate the presence of impurities, mainly host cell proteins, total DNA, and other product and process-related residuals.

Q How do innovations in LV vector processing and analytical toolkits impact decisions on whether to outsource LV process development and manufacture or keep them in-house?

SJ: From a small biotech perspective, we need CDMOs to produce these viral vectors. A virtual company that may have an office space in a large lab may not even have the facilities to do process development. They need to rely on partners and collaboration between teams to get this work done. At CDMOs, there are subject matter experts (SMEs), who spend time ensuring that we are successful.

EJ: We are always going to see the need for CDMOs and CMOs. Greater standardization in analytical assays and bioprocessing solutions is going to make transfer easier, whether you start early development in-house and then outsource, or vice versa.

RL: LV manufacturing is such a complex service, and needs such a degree of optimization, that outsourcing can be much more reasonable. From an economic point of view, the costs associated with a GMP facility with trained personnel are so high that outsourcing is often a good idea.

MN: The cost and the setting of the quality systems for GMP manufacturing are so well-established in CDMOs, that small-to-medium companies need to rely on that experience for vector manufacture, particularly in a clinical setting.

SM: CDMOs are key to ensuring that we get these drugs to patients as soon as possible. The complexity and the amount of investment in infrastructure and equipment are very high that it is a huge barrier to execution. Turning to the analytical side, as more kits are commercially available, it changes the dynamic, making it easier for people to in-source some of these assays that previously had to be outsourced. Even with that, there is certainly going to be space for CDMOs to help deliver those products to patients.

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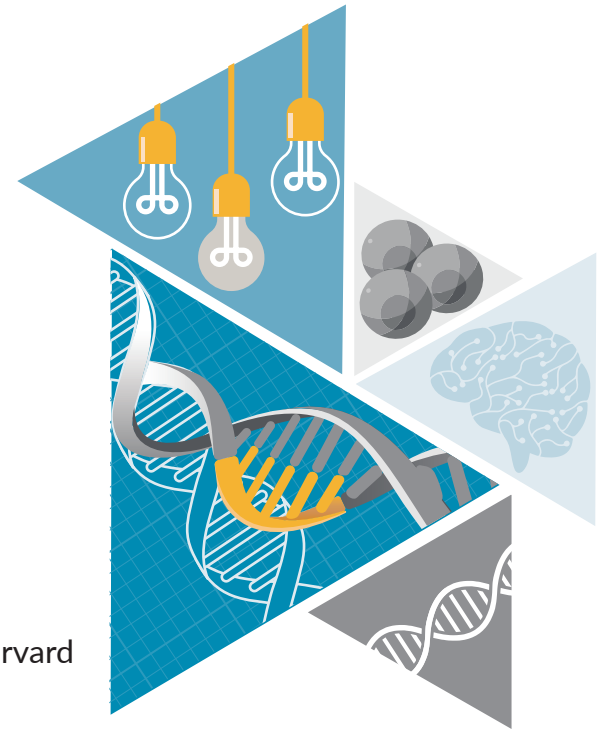
CELL & GENE THERAPY INSIGHTS



Innovation Insights

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INTERVIEW

David McCall, Editor, *Cell and Gene Therapy Insights* speaks to **Brock Reeve,** Executive Director of the Harvard Stem Cell Institute

Driving stem cell innovation

BROCK REEVE has been the Executive Director of the Harvard Stem Cell Institute since 2006. In partnership with the Faculty Directors, he has overall responsibility for the operations and strategy of the Institute which is comprised of the schools of Harvard University and its affiliated hospitals and research institutions. Under the leadership of its Executive Committee, HSCI invests in scientific research and its faculty has grown to include over 370 Principal and Affiliated members. The Institute is engaged with several leading pharmaceutical companies and foundations in joint research projects and its faculty have founded over 40 stem cell-related startup companies and serve on leading Scientific Advisory Boards. Brock came to HSCI from the commercial sector with extensive experience in both management consulting and operations for technology-based companies, with a focus on life sciences. Prior to Harvard, Brock was COO and Managing Director of Life Science Insights a consulting and market research firm specializing in information technology in life sciences. As a consultant, Brock has additional experience in the healthcare/life sciences market with IBM, Viant Corp. and SRI Consulting, where his clients included some of the leading pharmaceutical, biotechnology and medical device companies. Brock received a BA and MPhil from Yale University and an MBA from Harvard Business School.

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Q Tell us about the Harvard Stem Cell Institute

BR: Harvard Stem Cell Institute (HSCI) works in both early-stage science and clinical applications across eight different disease programs, including cancer and diabetes. We are doing different things in different disease areas, depending on the particular issues in each disease area.

Q How has the stem cell research field evolved over the lifetime of the Institute?

BR: HSCI started officially in 2004, back when there was a lot of political debate around use of embryonic stem cells in the US. On the science side, there was also much discussion about how certain tissues repaired themselves, and the existence of tissue specific stem cells. This was before Shinya Yamanaka's discovery of reprogramming, and the gene editing tools that existed were primitive compared to CRISPR today. In short, it was a different world 15 years ago, and we were asking different questions. However, the mission and vision of understanding stem cell behavior and development as a way of ultimately treating disease was there from the beginning.

We have made advances, such as the ability to make induced pluripotent stem cells (iPSCs), and our understanding of cellular turnover in organs like the pancreas or the heart, compared to the blood, skin, or intestine. For example, we now know that adult motor neurons can regrow if you give them the right environment and right stimulus.

We have learned a lot about how different cell populations behave, and we are learning even more about how we can instruct them. This could be through reprogramming, gene editing, or a combination of the two. We are getting more specific about effects of the environment or niche that a particular cell inhabits, including their substrates and who their neighbors are. There is lots of work in single-cell analysis, both *in vitro* and *in vivo*, which will open the next level of potential therapies.

Q How have the organization of HSCI and its collaborative activities evolved over this period, particularly in creating environments that are conducive to R&D innovation?

BR: Our mantra from the beginning was 'it takes a village' – we tried to create a village across Harvard and its multiple hospitals. We are governed by an executive committee and not by an individual. Two faculty directors, myself, and an executive committee of a dozen senior scientists from across Harvard, all decide together where we put our money. We also partner with disease foundations and companies in some of our research. Originally, we had 30 or 40 faculty and we have expanded to having over 375 faculty now affiliated with the Institute across Harvard and the eight Harvard hospitals.

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Despite this growth, our focus on collaboration has not changed. In the past, many people in scientific careers (particularly in an environment like Harvard) thought only about the work in their own lab, and were less used to collaboration across disciplines, domain areas, or organ systems. We deliberately set up vehicles to do just that, such as our junior faculty programs where at least three or four faculty had to come from at least two different institutions to work on a common problem. We tried to bake collaboration into how we conduct science. So, we have certain collaborative grants, but also reward individual ideas by having, for example, an annual innovation award for a 'big picture' concept.

"...something that will progress quickly is the increasing specificity of gene therapy."

We also work with foundations and companies to bring different labs together to tackle problems in processes that cut across organ systems. For example, we have a couple of collaborations with large pharma companies where we have people studying the fibrotic response in different tissue types, including the lungs, heart, liver, and kidneys. In these collaborative projects we can compare notes, understand common pathways, and learn from one another. The faculty and the scientists enjoy this collaboration because it makes life more interesting and their work more fruitful.

Q Can you expand on the key current trends within the stem cell research field, as you see them?

BR: Gene editing tools have advanced to the point where some of them are becoming clinically tractable. In other words, delivering a certain gene to a certain cell type – for example, gene correction for hematopoietic stem cells in sickle cell anemia – is in the clinic now. Another example is cell therapy in diabetes or Parkinson's disease, where cell replacement strategies are also in the clinic. This is starting to happen in a meaningful way.

Many things are still in the early stage of research. However, something that will progress quickly is the increasing specificity of gene therapy. Some of the gene therapy failures in the clinic have been because certain gene therapies cannot be redelivered or end up accumulating in the liver and creating toxicity. Now, there are new tools and methods for delivering a gene product to a particular tissue of interest preferentially so that it avoids, for example, the liver.

There is a project working towards the clinic at Harvard and the Broad Institute in which a gene fragment is being delivered to skeletal muscle preferentially for treating Duchenne muscular dystrophy. There have also been recently published papers investigating ways to turn on certain transcription factors in specific cell types. The tools to deliver something systemically but only have it turn 'on' in certain cells are starting to become available. That will increase the specificity with which we can think about cell and gene therapy, and how we can influence cell behavior within certain tissues in the body.

Q What do these advances mean for likely future directions in the field?

BR: One big question is: how do you get organ level repair for organs that cannot repair themselves?

Blood can repair itself, and the liver can do a pretty good job. But the kidney, the heart, and the lung cannot. Repairing an organ that has a complex architecture without a native stem cell population is complicated and requires thought about the different tools we could use.

There are several different approaches being actively pursued right now, developing learnings about biomaterials, gene editing, and developing cells to solve the problem at scale. For example, in the kidney and heart, mechanical devices have been around for a while but xenotransplants received a lot of publicity this year, as gene editing tools can now be used to make organs useful for transplant. People are also trying to de-cellularize organs to create the scaffold to then repopulate with cells of interest. Another approach is making biomaterials with hollow channels that can be loaded with cells of interest.

Another area is systemic interactions – how organs that we used to think about as being quite separate actually interact and influence one another. There has been a lot of discussion recently on the gut–brain axis. This explores how changes in the gut microbiome can impact the brain and the central nervous system. There was a study several years ago at Harvard and the Broad Institute that showed that changing the diet of certain mice changed their health outcome in terms of their mental capacity. There are also issues like exercise – what factors are released that have systemic benefit? Why does stimulating the vasculature lower the risk for Alzheimer's disease and enhance neural capacity? There is one theory that Parkinson's disease may actually start in the gut before it ultimately has its impact on the dopamine releasing neurons in the brain. People are also looking at the lung-brain axis right now, at how inflammation that happens in the lung can impact the brain. Understanding the connections between organs and systems in the body is a next-order question that people are starting to tease apart.

Stem cell-based tools allow this work to occur, because we can create *in vitro* mini-models of the brain, or, say, brain-vagus nerve-intestine organoids, to see how these tissues react and interact. We can start to explore those issues in a way we simply did not have the tools to do so a decade ago.

> "There are several different approaches being actively pursued right now, developing learnings about biomaterials, gene editing, and developing cells to solve the problem at scale."

Q Can you distill for us some key learnings relating to successful fostering of R&D innovation that you have derived during your career?

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BR: One of the key questions in deciding what to fund is whether to bet on a project or a person. The answer is usually a little bit of both. For example, back in 2009, we funded a small grant in Derrick Rossi's lab where he was proposing to use mRNA to reprogram cells. We took a chance on a bright guy with an interesting idea, and that work went on to become the basis of the intellectual property for Moderna. We have to be willing to take risks and learn from whatever happens. If it works, we understand what it can do. But if it does not work, what will we have learned from it?

It is also important to ensure that what you are doing is different and can make a difference if it works. Oftentimes, you must be willing to be open to interactions and learning from other people. If people hold things too tight to their chests, then collaborations do not work, and innovation gets stifled. Encouraging an atmosphere of openness whilst still giving people the credit they deserve is key. The whole is greater than the sum of the parts. This also adds to the excitement and team building and sense of camaraderie in that effort.

Q Finally, what are some key goals and priorities both for your own work and for the HSCI as a whole over the coming 12–24 months?

BR: Our priority is to keep funding exciting science that could potentially have an impact in the clinic and on disease. Our goal is to create the medicines of the future, whether the future is 2 or 10 years down the road. For example, we actively try and stimulate an entrepreneurial environment to encourage startup companies. This means increasing the number of startups when it makes sense to do so, licensing for a company, or bringing something into the clinic. Another goal is to keep having continued success in terms of fundraising, so that we can have enough money to create this flywheel of innovation.

One of the nice things about being in Boston is being surrounded by other world-class universities and hospitals, as well as the venture capital, biotech, and pharma communities. Boston is a real center of gravity as a life sciences ecosystem and making sure we are a vibrant part of that, interacting with the other entities so that we can accelerate progress, is increasingly important as the science advances and gets closer to being translatable.

We will be seeing more progress down the translational path, even though our mission is not to be a translational entity. Our mission is to do good science that can impact the world.

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Current technological trends & advancements in vector purification

Ying Cai, Nathalie Clément, Chantelle Gaskin, Matt Roach, Ashish Saksule

Given the current rapid growth in the gene therapy sector, it is imperative that downstream processing doesn't apply a handbrake to recent gains made in increasing AAV vector upstream process scale and titer. In a recent webinar, a panel of AAV vector processing experts discussed whether the potentially conflicting drivers of increasing the efficiency versus the sensitivity and robustness of downstream vector processing is reflected in today's purification toolkit, and asked 'what's next?' in terms of further development. Here, we sum up some of their key thoughts.

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WHAT ARE THE CURRENT TRENDS AND ADVANCEMENTS IN AAV VECTOR DOWNSTREAM PROCESSING?

"There are three key trends regarding AAV gene therapy. First, we want the enrichment for full AAV particles to be as high as possible. This is not only done by removing empty capsids, but also partially filled AAV, which is quite challenging. Secondly, there is a rising regulatory bar for the control of adventitious agents including viral clearance and inactivation. The third trend is manufacturing cost reduction from the clinical phase to commercial. Manufacturing cost consideration is becoming more important. We have seen high cost per dose, especially for AAV and cell therapies. Moving forward, we not only need to improve product quality, but we also need to reduce manufacturing cost per dose."

- Ying Cai, Senior Director of Process Development, Ultragenyx

"A newer trend I have seen is regarding novel variants and new serotypes. Generation and screening of libraries for AAV variants has emerged as a powerful method for identifying novel capsids. Novel capsids are emerging with numerous advancements in the construct design, and we have multiple synthetic capsid variants that can outperform their natural counterparts. These include new liver-tropic serotypes such as AAV-DJ or AAV-DJ/8, muscle-tropic AAV9MYO, or even the newer AAV7m8.

For downstream processing of this novel capsid, we are still using traditional methods, which were developed for the proteins and monoclonal antibody (mAB) space. There is a key technological need to focus on the newer novel serotypes."

- Ashish Saksule, Cell and Gene Therapy Process Development Lead, Takeda

"From the vendor side of things, I personally am looking at the column-free systems on the horizon. One example is essentially a liquid-liquid phase separation approach, based on a hydrophobic affinity reagent binding to the target molecule in the crude harvest phase. This is combined with tangential flow filtration to produce purified material. Another example is a single-use flow-path system using a chromatography resin in a recirculation flow path. The different process buffers are connected and are allowed to circulate in the flow-path along with the crude material. If they are applied at the correct time, then the purified material is eluted in a separate vessel."

- Chantelle Gaskin, Field Applications Scientist, Thermo Fisher Scientific

WHAT ARE THE CHIEF IMPLICATIONS OF RESIDUAL TESTING?

"Measuring residuals, whether DNA, protein, or product or process derived, has become a very hot topic over the past few years. The technology has advanced tremendously, so all the testing has become more sensitive and more accurate, for example ddPCR, next generation sequencing, and RNA sequencing. In parallel to the technology improvements in the assay itself, the clinical doses have dramatically increased, mostly because of the type of indication treated. With higher doses in the clinic, there comes a higher burden of residuals, and therefore a need to better determine the amount and the type of residuals. We have seen toxicity in humans during the course of several trials, furthering the importance of measuring residuals."

- Nathalie Clément, Unicorn Consultations (formerly Resilience)

HOW ARE CURRENT SOLUTIONS HELPING TO ADDRESS THE CHALLENGE OF EMPTY/FULL CAPSID SEPARATION?

"Companies are moving towards designing platforms for AAV. It has become more apparent just how different various AAV capsids are from each other. Additionally, you need to account for the differences in production systems, heterogeneity of viral proteins, and heterogeneity of packaging, which can be a challenge.

The good news is that many groups are tackling this. We have seen an increase in the number of resin and column manufacturers providing specific solutions to empty/full capsid separation."

- Matthew Roach, AAV Process Development Team Leader, Precision BioSciences

To find out more, read the full discussion or watch the video.

CELL & GENE THERAPY INSIGHTS

INTERVIEW

Evolution of analytical tools and techniques for hematopoietic cell therapy manufacture

David McCall, Editor, *Cell & Gene Therapy Insights,* **talks to**
Riccardo Biavasco, Senior Scientist at bluebird bio

RICCARDO BIAVASCO obtained his PhD in Cellular and Molecular Biology in 2018 working at the Telethon Institute for Gene Therapy, where he designed and generated a humanized mouse model for myeloid inflammatory neoplasms and characterized the effects of aberrant cellular senescence in hematopoietic stem and progenitor cells. In 2019 Riccardo joined bluebird bio analytical development group, where he coordinates the development of characterization assays for hematopoietic stem cell drug products and he is responsible for the interactions with regulatory agencies regarding drug product characterization assays.

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Q What are you working on right now?

RB: I am a senior scientist in the analytical development department of bluebird bio. We work on hematopoietic stem cell (HSC) gene therapies, mainly for three different diseases: a neurodegenerative disease, adrenoleukodystrophy (ALD), and two hemoglobinopathies, b-thalassemia and sickle cell disease, which are among the most frequently occurring

genetic disorders worldwide. My specific role as a group leader of HSC analytics is to characterize our drug products.

We have a panel of release assays that have been established over the years that we use to release our drug products. We must meet specific criteria for those assays to be able to use and infuse this drug product in the clinic. Then, we have a much larger panel of characterization assays that we use to get better insights into our drug products. The specific focus of my group is to perform these assays and to develop new ones to tackle the various questions that are coming from the clinic, and to find correlation between drug product attributes and clinical parameters.

I am also responsible for the interaction with regulatory agencies on this topic. I was the author for a Biologics License Application (BLA) section on characterization of drug products.

Q You learned your trade at the San Raffaele Telethon Institute for Gene Therapy (SR-Tiget). Can you share any insights from there that you carried forward to bluebird bio?

RB: The short answer is everything!

Studying for a PhD at SR-Tiget was a foundational experience for my career. Prior to that, I was studying in the San Raffaele University for both a bachelor's and master's degree. For my master's degree I was working on a rare inflammatory neoplasm.

We started our collaboration with some labs in SR-Tiget to take advantage of their extensive expertise in the manipulation of HSCs. I joined Dr Eugenio Montini's lab where I transduced human HSCs with a lentiviral vector expressing a mutated gene. Transduced cells were transplanted into a mouse to develop humanized model of inflammatory neoplasms, in order to find the molecular mechanisms behind this group of diseases and potential novel therapies.

For that project, I cloned and produced the lentiviral vectors, harvested cells from donor cord blood, enriched the stem cells, transduced them, and transplanted them into mice. So I got the whole gene therapy package within one project, including much HSC biology, flow cytometry, and characterization experience. I was very lucky to receive this exposure to gene therapy field.

Q What do you regard as the key technological advancements in the HSC space that have had a positive impact on your own work? Can you share any examples where you have seen novel analytical tools delivering benefits for cell therapy manufacture?

RB: I have a few examples in mind, including the colony-forming-cell assay for CD34+ cells, which has been used extensively for the past 20–30 years in academic literature. However, it is very operator-dependent - especially in the analysis portion, because you are looking, characterizing, and counting colonies under a microscope, and there are many

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variables that can affect your results. You end up with tens, or at best, a couple of hundred colonies per sample. These small numbers are highly susceptible to variability.

We have optimized the plating protocol. Automated imaging and analysis gave us the opportunity to move from characterization to a release assay. Today, we culture the cells in the incubator, put the plates under a proprietary cell counting machine, and if the numbers look good, we can use them straight away. In this way, we are removing the operator-to-operator variability on the analysis portion.

"In the future, the ability to automate cell culture would be beneficial because for HSC therapy drug products."

The other example is moving from standard quantitative PCR (qPCR) to digital droplet PCR (ddPCR) for assays such as vector copy number (VCN) or percent transduced cells. This provides a step up in the throughput of the samples that we can analyze. In the future, the ability to automate cell culture would be beneficial because for HSC therapy drug products, especially for those transduced with lentiviral vectors, you cannot assess the number of integrated copies immediately, straight from the drug product. The integration process takes time, and there are some copies that remain episomal, which are lost over time. You need at least seven days of culture after you have manufactured the drug product and cryopreserved the bag. This culture, depending on the assay used, can be more prone to operator-to-operator variability. The next technological advance we are looking for is machines that can culture these cells in incubators in a sterile way to remove that operator-to-operator variability, especially for the colonies where very few cells are plated from the start. We are getting there, but we are not there just yet.

If we want to truly scale-up the manufacturing of these drug products, each company or research institute cannot have sole responsibility for the assays – we will need to rely on contract testing organizations (CTOs) to perform the assays for us. Ideally, these organizations should be dispersed throughout the world to allow ease of access to the greatest number of patients possible, and we need for them all to perform the assays in exactly the same way. This is a very complex and daunting exercise, but it is one that we must undergo to have comparability across manufacturing and testing sites.

Compared to small molecules and even biologics like antibodies, HSC-based drug products are an order of magnitude more complex. They require assays that have much greater complexity, and with complexity comes variability. Automation and higher throughput technologies can help us improve standardization and reduce costs.

Q What learnings do you take from recent regulatory setbacks for sponsors relating to cell therapy CMC in general? How should developers respond to evolving regulator priorities and expectations?

RB: This is a very complicated question. I was at a conference recently where there were many different opinions. In the field there is a consensus about the necessity to start

open conversations with regulatory agencies, to align their expectations with the challenges that the industry is facing. It seems that regulators are currently very wary of the unknowns of gene therapy, such as the fact there are some adverse events for which we have no predictive markers, and that we can sometimes only tackle when it is unfortunately too late. However, these adverse events can be more or less tolerated depending on the disease. For example, there are neurodegenerative diseases that affect very small children, which are fatal within just a few years from the onset of symptoms. Some of the families of these children are willing to take risks that go beyond the feelings of the regulators.

In my opinion, we need to push companies to find the best possible treatments – preferably, curative ones, while holding them accountable for potential setbacks. Gene therapy companies need to work alongside regulators and not just lobby for a more relaxed view towards adverse events. As a collective of gene therapy companies, research institutes and regulators, we are still in the process of building the field of gene and cell therapy and at the moment we should push to understand what are the things we can control in our drug products, and what are the best standards we can achieve and develop.

Sometimes this is about adding more assays, and sometimes it is about removing them. There are some assays that in theory help us have a better understanding of the mechanism of action of our drug products, but they may also require a very complex culture system, which introduces artifacts and variability that reduce the relevance to the clinical efficacy and safety. Another assay might not add much to the characterization panel, but it will definitely add to the cost. Spending money and energy in developing assays that are not particularly informative means taking away resources that could be better invested in other areas.

I have been involved in the development and extensive analysis of many potency assays. One of the main takeaways of my presentation at a recent conference was that the closer we get to the mechanism of action, the more we lose predictivity of the clinical efficacy - in particular, in beta thalassemia and sickle cell disease.

If we use quantitative biomarkers like peripheral blood VCN or transgenic hemoglobin production in the blood, we see that the best predictors of the patient outcome are the easiest and less disease-specific characterization assays. If this will remain the case over the next years, removing some of the more complex assays from the characterization panels would allow to reduce manufacturing costs while maintaining the most valuable information.

For example, in beta thalassemia, cells lack b-globin and have an a-globin to b-globin chain imbalance, meaning the excess α-globins precipitate and block the maturation of the red blood cells. We can culture our drug product CD34 cells *in vitro*, and differentiate them to reticulocytes/erythrocytes, the final steps of maturation of erythroid cells. With this assay, we can measure how many cells fully differentiate and thus assess the degree of correction of the disease

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phenotype. However, this assay lasts for three-week and there is huge patient-to-patient variability in the starting material as well as in how cells respond to the differentiation culture. Therefore, this assay is not fully quantitative, rather we have established a threshold. Below this threshold, the drug product is not likely to be efficacious, and above a threshold it most likely will be. But we do not see a clear difference between something that barely makes it above the threshold and something going much higher. By contrast, if we can see that transduction efficiencies are doubled, we can be very confident that drug products are going to lead to much greater production of therapeutic hemoglobin.

In the last few years, we have been pushed by regulatory agencies to develop assays that are more specific for each disease. This is fundamental in such an early phase for cell therapies because we do not know which assays are going to be the most representative and informative and we do not have clear guidelines to follow. To move the field forward and quickly reach a consensus on these cell therapy characterization guidelines, I believe we need to have continued and frank conversations involving regulatory agencies, companies and research institutes to learn from each other's mistakes and find the optimal solutions for the outstanding issues and questions. bluebird bio could spend a lot of time developing characterization assays and from a competitive standpoint, we would want other companies to have to go through the same process. However, as people trying to treat patients and move forward cell therapies, we need to collaborate as a field and find a way to align the goals of all parties involved.

Q What are the roadblocks to further advances in the area of analytics development, and what are the next steps towards addressing them?

RB: On the one hand, I am very confident that in the next 10 years we will have instruments that will blow our minds, because we are in a very exciting period from a technology point of view – for example, in artificial intelligence.

However, we need to gain much more knowledge of the basic biology of cells. We use CD34+ cells and others use T cells, which are both harvested and purified from a bulk population. Not all CD34+ cells are stem cells, so not all will engraft into the patient. We do not fully know which ones are going to be effective in the long-term, how to recognize them, track them, and specifically manipulate them.

In hematology there is a great advantage as there is a good, reliable set of markers available to isolate stem cells. But when working with other organs, sometimes the stem cell can barely be identified, let alone potentially

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taken out of the body, edited, and then put it back in an efficient way, as is done for bone marrow transplantation, for example.

This is going to be the next quantum leap for these diseases. We get a lot of information by continuing to dig down into these cell-based drug products, continuing to analyze them with as many parameters as possible, and following patients for decades – and if we want to gather understanding long-term in the stem cell arena, we need to talk about decades, not years. Unfortunately, moving forward, mouse models are not that informative. We have gained a huge amount of information thanks to mouse models, but we are missing the details. Taking cells and putting them in an immuno-deficient mouse can answer some questions, but it cannot answer everything, especially in terms of safety and long-term efficacy.

The continued effort to analyze the incoming cells, the drug product cells, and how they behave upon infusion is going to be a huge priority for both academic and industry researchers. But it is also going to be a huge effort from the patient's point of view, because we will need their tissues and their bone marrow, not just their blood. Many of these patients are extremely brave and are more than willing to donate parts of their body to science, but we need to find the right balance. If we can do so, future gene therapies will be safe, effective, and will be available at a lower cost compared to now, making them more accessible to patients worldwide.

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