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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

Cell therapy manufacturing and bioprocessing

In partnership with

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CELL & GENE THERAPY INSIGHTS

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Cell therapy manufacturing and bioprocessing

Why choose a modular, automated approach to autologous CAR T manufacturing?

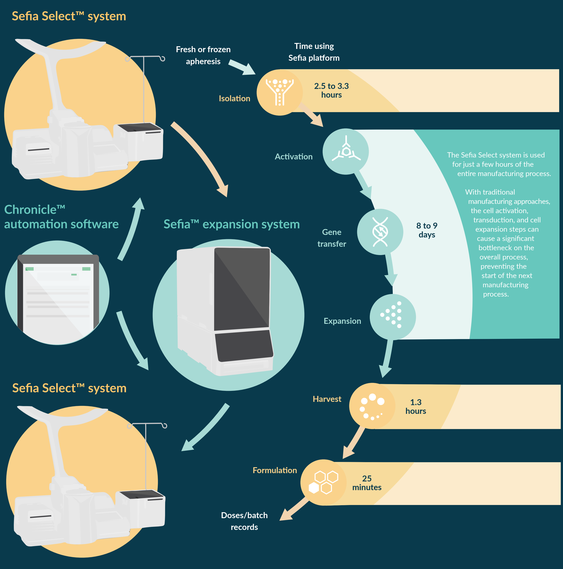
With over 10 commercially available therapies and more than 25,000 patients now treated, autologous CAR-T therapies have proven themselves in the clinic. However, significant challenges still face manufacturers, including high costs, frequent batch failures and delays, and traditional technologies that do not integrate well into advanced manufacturing workflows.

This infographic provides insights and case study data demonstrating the benefits of a modular manufacturing approach, which helps to increase productivity by optimizing equipment usage.

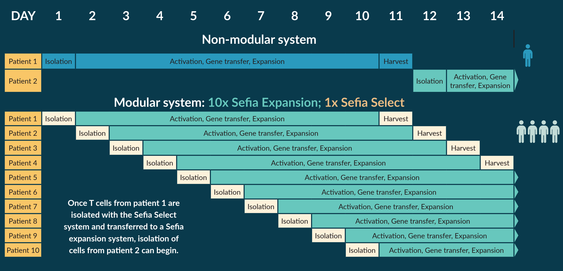
A modular system can mitigate risk, optimize available resources, and increase productivity

Modular approaches to manufacturing can help to address multiple issues facing the cell therapy space by helping to mitigate risk, optimize available resources, and significantly increase productivity.

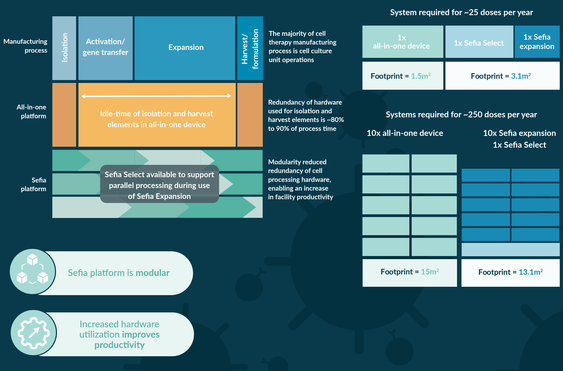
The Sefia™ cell therapy manufacturing platform is a modular, digitally integrated platform which combines two functionally closed systems to cover the entire cell therapy manufacturing workflow.



A modular system reduces redundancy by enabling parallel cell processing



Optimizing facility utilization



Sefia platform is modular

Increased hardware utilization improves productivity

Additional advantages of a modular approach

Optimized implementation and upgrade time

New technologies or improvements can be integrated into the workflow by upgrading specific systems without overhauling the entire platform.

Improved maintainance

Each system in the modular platform can be maintained or replaced independently, reducing downtime and maintenance costs.

Risk mitigation

Problems in one system do not necessarily affect the entire platform, allowing minimization of impact on overall operations.

Reduced labor

Modular approaches supported by digitally integrated systems create simple, streamlined processes that require less manual labor.

Speedy turnaround

Achieve improved turnaround times and facility productivity due to reduced equipment redundancy

10 Flexible manufacturing scales

Add Sefia expansion systems to increase production as needed. One Sefia Select system can support up to 10 Sefia expansion systems (assuming a 10-day process).

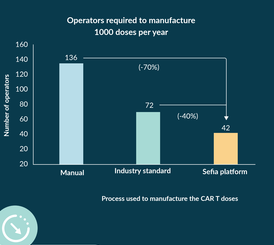
Importantly, modular approaches are most effective when using a digitally integrated system, creating a simple, streamlined approach that will reduce labor requirements

Case study

Modular manufacturing systems and automation of key steps reduce operator handling time and increase productivity

Operators required to manufacture X doses per year	25	100	250	1000	2500
Manual	4	14	34	136	338
Industry standard*	2	8	18	72	178
Sefia platform	2	6	12	42	102
Batches per operator to manufacture X doses per year	**25**	**100**	**250**	**1000**	**2500**
Manual	6	7	7	7	7
Industry standard*	13	13	14	14	14
Sefia platform	13	17	21	24	25

*Semi-automated process using several systems to cover the full manufacturing workflow.



Automating steps produces more batches per operator

Less handling time reduces operators required per dose

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CELL THERAPY MANUFACTURING

SPOTLIGHT

EXPERT INSIGHT

Full speed ahead: how rapid CAR-T manufacturing can shape the cell therapy landscape

Mackenzie M Lieberman and Kathryn A Henckels

Autologous CAR-T cell therapy has revolutionized treatment options and improved therapeutic outcomes for B cell leukemia, lymphoma, and multiple myeloma patients worldwide. However, patient access, cost and time to receive their personalized medicine remain a hurdle for many patients waiting to receive a cell therapy. Reducing CAR-T cell manufacturing time is one strategy to shorten the vein-to-vein time, bringing life-saving therapies to patients faster. Beyond expedited access to treatment, an accelerated manufacturing process may also enhance CAR-T cell durability, leading to improved clinical responses. This review highlights current advancements in the rapid cell therapy manufacturing space, while also discussing the challenges to widespread adoption of a rapid process including meeting required clinical doses, the need for development of expedited release assays and quality control procedures. Despite the challenges, adoption of a rapid process holds promise to increase manufacturing capacity and reduce costs to help further improve patient access.

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INTRODUCTION

Almost a decade ago, the first CAR-T cell therapy was granted US FDA approval for the treatment of pediatric B cell acute lymphoblastic leukemia (ALL) [1]. Since then, immuno-oncology and more specifically, cell therapy has emerged as a leading modality in the treatment and eradication of various hematologic malignancies including several lymphoma subtypes and multiple myeloma. Autologous CAR-T cell therapies, to date, are the most developed of all genetically engineered lymphocyte therapies, with several others on the horizon. Today, there are a total of seven commercial CAR-T cell products that have made astonishing impacts on patients' lives worldwide (Table 1) [2–10]. However, the rise of CAR-T therapies has also presented new challenges, spanning from concerns for neurotoxicity and the onset of secondary T cell malignancies to scaling manufacturing and increasing the

TABLE 1

Vein-to-vein time for commercial CAR-T cell therapies.

Product name	Commercial name	Indication	Vein-to-vein time
Tisagenlecleucel	Kymriah®	FL, DLBCL, ALL	3–4 weeks
Axicabtagene ciloleucel	Yescarta®	FL, DLBCL	3.5 weeks
Brexucabtagene autocel	Tecartus®	MCL, ALL	2–3 weeks
Lisocabtagene maraleucel	Breyanzi®	FL, LBCL, MCL, CLL, SLL	3–4 weeks
Obecabtagene autoleucel	Aucatzyl®	ALL	3 weeks
Idecabtagene vicleucel	Abecma®	MM	4 weeks
Ciltacabtagene autoleucel	Carvykti®	MM	4–5 weeks

ALL: acute lymphoblastic leukemia. CLL: chronic lymphocytic leukemia. DLBCL: diffuse B cell lymphoma. FL: follicular lymphoma. LBCL: large B cell lymphoma. MCL: mantle cell lymphoma. MM: multiple myeloma. SLL: small lymphocytic lymphoma.

accessibility and affordability of these life-saving therapies [11–16].

The goal of this review is to shed light on potential solutions to some of the largest bottlenecks in CAR-T cell therapy–patient access and vein-to-vein (V2V) time. Currently, the time between patient apheresis and drug product (DP) infusion, which will be subsequently referred to as V2V time, can range from 3–5 weeks, with a median time of 31 days (Table 1) [17]. Despite improvements in process engineering designed to increase scalability of CAR-T manufacturing, including increased reliance on automation and construction of large capacity manufacturing suites, time to administration remains a significant barrier for many patients with aggressive disease. Eligible patients for CAR-T cell therapy are generally heavily pretreated and have exhausted at least 1–3 lines of prior therapy before meeting eligibility criteria for CAR-T cell therapy. Specifically, B cell lymphoma patients are at an increased risk of disease progression making faster access critical in these situations. Put into perspective, it is estimated that nearly 30% of patients who are initially prescribed a CAR-T therapy never undergo leukapheresis, and 20% of patients who do undergo leukapheresis do not proceed to infusion of the therapy [18]. Furthermore, rapid disease progression, clinical ineligibility and declining clinical status have been identified as the leading factors for patient drop-off [18]. In additional support of these findings, mathematical simulations have illustrated that the V2V time has significant implications on patient outcomes including mortality rates, and life expectancy post CAR-T cell infusion [19,20].

The V2V time encompasses a wide range of activities including shipping and receiving of the apheresis starting material, manufacturing the drug product, release testing, and finally transportation to the infusion site. Clinical care considerations for the patient as well as clinician availability and scheduling also factor into the V2V time. This insight will focus exclusively on one aspect of the V2V time—the CAR-T cell manufacturing process; however, we acknowledge that reducing time to infusion will require a combined effort on multiple fronts. While the duration of the manufacturing process is only one determining factor of the V2V time, shortening this time will not only expedite time to patient infusion, but may also result in a more durable, higher quality drug product (DP) capable of inducing even deeper clinical remissions.

When considering all commercial autologous CAR-T cell products, conventional manufacturing processes can require

7–14 days of cell manipulation to generate the clinical dose and require many distinct process steps to produce the drug product [21,22]. Briefly, the autologous CAR-T process begins upon receipt of patient apheresis at the manufacturing site. Bulk apheresis is composed of a heterogeneous population of peripheral blood mononuclear cells (PBMCs), which may be further enriched for $CD3^+$ T cells through a selection or sorting process. Alternatively, $CD4^+$ and $CD8^+$ cells may be isolated together or separately to achieve a desired CD4:CD8 ratio, requiring a more labor-intensive manufacturing process. Furthermore, there have been additional efforts to isolate specific T cell subsets to serve as the starting material in the manufacturing process. T cell differentiation status has been shown to correlate with anti-tumor efficacy and *in vivo* persistence which has inspired an effort to isolate naïve and memory T cell subsets defined by a panel of surface markers including CD62L, CCR7, CD127, CD45RA, and CD45RO [23–25].

Upon enrichment of the intended T cell population, the cells are typically stimulated through activating antibodies to the CD3ζ chain of the T cell receptor (TCR) as well as the co-stimulatory molecule, CD28 [26]. Additionally, cytokine signals such as IL-2, IL-7, and/or IL-15 are included to support T cell expansion and CAR-Transgene delivery and favor preservation of less differentiated cell populations [27–29]. Currently, all commercial CAR-T products rely on lenti- or retro-viral transduction to drive stable CAR-Transgene expression. Once genetically modified, CAR-T cells are permitted to expand in culture for several days to ensure the intended clinical dose is met. The expansion process is performed under culture conditions that are favorable to T cell cultivation which can assist in the elimination of contaminating cell populations and additional impurities such as residual lentiviral plasmid DNA. Finally, the cells are harvested and formulated at the intended dose in cryoprotectant to preserve cell viability during the freezing and thawing process [26]. This marks the conclusion of the manufacturing process, at which point the material is tested for release. CAR-T cells are evaluated for several safety, characteristic and functional critical quality attributes including purity, sterility, transgene copy number, viability, and potency. The final dose that will be administered to the patient is a reflection of the percentage of surface CAR expressing T cells as a proportion of total T cells present in the DP. Once the DP passes all release testing, it is shipped back to the infusion center and the patient is prepared for treatment, which commonly includes a lymphodepletion regimen to generate space for T cell engraftment.

The subsequent sections will delve deeper into each phase of the manufacturing process, highlighting differences between conventional and rapid manufacturing and the accompanying considerations surrounding the adoption and integration of a rapid process.

CURRENT LANDSCAPE OF RAPID MANUFACTURING

Reducing the current autologous CAR-T manufacturing process from several days to 24–72 h is a paradigm shift when considering the widespread use of first-generation CAR-T cell manufacturing processes for several clinical and commercial CAR-T products (Table 2). Here, we will review various stages of the manufacturing process and how current efforts toward achieving a rapid process must address these critical process steps to generate a safe and efficacious DP.

Patient apheresis or starting material

Most pharmaceutical companies have adopted a centralized manufacturing

TABLE 2

Manufacturing overview and development state of rapid (<72 h) and expedited (4–6 days) CAR-T programs.

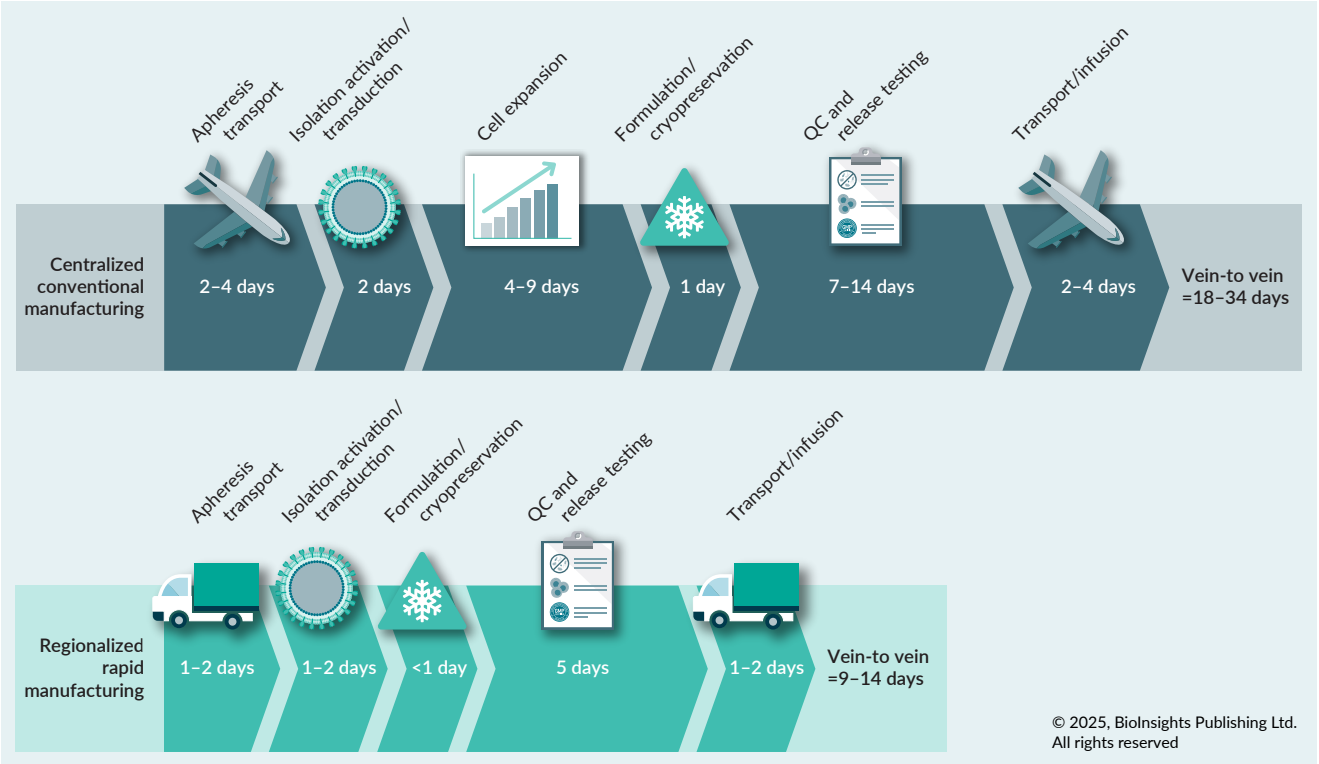
Rapid CAR T (target)	Activation	Gene editing strategy	Manufacturing time	QC/release	Development stage
GC012F (CD19/BCMA) FasTCAR Platform	CD3/CD28	Lentivirus	1 day	8 days	Phase 1b
BMS986354 (BCMA) NexT Platform	n.a.	Lentivirus	5–6 days	n.a.	Phase 1
YTB323 (CD19) T-Charge Platform	CD3/CD28	Lentivirus	<2 days	6 days	Phase 2
Dash CAR T (CD19)	CD3/CD28	Retrovirus	2–3 days	n.a.	Preclinical
KITE-753 (CD19/CD20)	n.a.	Lentivirus	5 days	<9 days	Phase 1
PRGN-3007 (ROR1), PRGN-3005 (MUC16), PRGN-3006 (CD33), UltraCAR-T Platform	None	Electroporation	1 day	n.a.	Phase 1
Ingenui-T (CD19)	n.a.	Lentivirus	<3 days	n.a.	Preclinical

n.a.: not accessible.

design, wherein patient apheresis is shipped from a clinical collection site to a central manufacturing facility within a predetermined time window. To meet global patient demand, there may be several manufacturing sites distributed throughout North America, Europe, and Asia to support late-stage clinical and commercial manufacturing. If apheresis receipt cannot occur within the pre-determined time window due to transportation constraints, the apheresis may be cryopreserved prior to shipment to the manufacturing site to preserve cell quality (Figure 1). Given the uncertainties that may cause delays in shipment, cryopreserving apheresis near the collection site can be used to reduce the risk of manufacturing failures due to apheresis quality and simplifies manufacturing logistics. However, there is also a benefit to employing fresh apheresis, especially when considering moving toward a rapid process. The cryopreservation process imposes an extrinsic stress on PBMCs which can negatively impact the quality and viability of cells that are recovered after thaw [30]. These effects could be exacerbated when considering patient material that may be more fragile in nature. On average, the recovery rate for cryopreserved PBMCs is 80%, therefore utilizing fresh material will permit access to greater numbers of T cells, which could be critical in meeting clinical dose in the context of a shortened process [31]. Additionally, cryopreserved material is likely to experience greater cell death over the first 48 h of culture, which may significantly impact cell yield from a rapid process [32]. However, the potential rewards of fresh apheresis are not without risk–pursuing a path to fresh material in a centralized manufacturing model will require extensive effort in the transport logistics to ensure that the material can be sent from the apheresis collection center to the manufacturing site on an expedited timeline (typically within 24–48 h) to maintain high cell viability and functionality. When considering the implementation of a rapid process, the starting material can have potentially large implications on critical quality attributes of the DP including cell viability. Fresh apheresis generally provides a benefit to T cell health due to the elimination of the

FIGURE 1

Overview of process development stages and accompanying timeframes in conventional (top) and rapid (bottom) manufacturing settings.



cryopreservation process, which can help improve cell performance in the manufacturing process.

T cell enrichment

CAR-T cell manufacturing begins upon receipt of the apheresis at the manufacturing site. Typically, the apheresis is washed prior to T cell enrichment or selection. Despite some conventional manufacturing processes beginning with unselected PBMCs, the shortened duration of a rapid process poses increased purity challenges of the resulting DP. The culture conditions and cytokine supplements used in conventional manufacturing processes favor the expansion of $CD3^+$ T cells and ultimately deter the growth of contaminating tumor cells, lymphocyte, and monocyte populations. A shortened process increases the risk of infiltrating non-T cell types in the final DP and therefore an enrichment process is generally preferred to control for DP purity. Rapid processes are currently relying on either density gradient centrifugation approaches (Novartis's T-Charge™ platform), CD4/CD8 positive selection microbeads, or CD3/CD28 Dynabeads™ (AstraZeneca/Gracell's FasTCAR-T and Hrain Biotechnology's Dash CAR-T platforms), which can both purify and activate T cells [33–43]. While the enrichment times are comparable across these platforms, the use of a multipurpose bead-bound activation reagent (i.e., CD3/CD28 Dynabeads) requires de-beading prior to DP formulation and can result in significant cell loss because of the affinity of the surface receptors to the crosslinking antibodies.

While some current commercial products may begin the manufacturing process

with bulk apheresis or leukapheresis material, adoption of this trend in an abbreviated process raises concerns regarding purity of the DP. Therefore, the field has largely agreed that an enrichment step is necessary to remove any unwanted or contaminating cell populations prior to T cell activation and transduction.

T cell activation

Nearly all conventional CAR-T cell manufacturing processes require naïve T cell activation to facilitate viral transduction. The cell therapy field has largely conformed to activating T cells through the CD3/CD28 mediated pathway. The use of crosslinking antibodies to these receptors has been widely adopted in commercial manufacturing which simulates the interaction between a T cell and an antigen presenting cell (APC), and ultimately invites a T cell-mediated immune response accompanied by T cell differentiation and clonal expansion. The agonistic antibodies to CD3 and CD28 can be bead bound (Dynabeads), soluble or plate immobilized, or conjugated to polymeric nanomatrices (TransAct™). T cell activation triggers a series of transcriptional and metabolic changes within the cell, and ultimately induces expression of surface proteins that are critical to viral infection. Viral fusion to the cell membrane and subsequent endocytosis is dependent upon the binding of the viral glycoprotein G from the vesicular stomatitis virus (VSV-G) to the low-density lipoprotein receptor (LDL-R) on the T cell surface. It has been previously demonstrated that LDL-R expression is upregulated upon T cell activation, specifically peaking around 24 h post activation [44,45]. These findings have provided justification for T cell activation prior to lentivirus introduction in virally engineered CAR-T cell therapies. Unsurprisingly, quiescent, non-dividing lymphocytes, such as naïve T cells, are historically less susceptible to viral infection. They have demonstrated accompanying low rates of reverse transcription of viral RNA and generally have reduced levels of transgene expression which poses a significant challenge when attempting to reach a specific clinical dose of CAR expressing cells [41].

However, recent findings in both preclinical and clinical stage research have demonstrated that potent CAR-T cells can be generated without an activation stimulus, enabling the retention of naïve or progenitor T cell subsets which demonstrate enhanced *in vivo* persistence and superior metabolic fitness [46,47]. Ghassemi *et al.* illustrated a lentiviral based approach to 24-h CAR-T generation without T cell activation [41]. Utilizing a multifaceted approach including the addition of cytokine signals, IL-7 and IL-15, deoxynucleosides, short periods of serum starvation, as well as re-design of the culture vessel to increase the colocalization of viral particles with T cells, the authors demonstrated that surface CAR protein could be detected as early as 12 h after lentivirus addition. Although the translation of this approach into clinical scale manufacturing has yet to be demonstrated, the potential therapeutic benefits of a naïve T cell-derived DP should inspire additional efforts in this space [48–51].

Similarly, Precigen, a clinical stage biopharmaceutical company, has developed an approach that does not utilize *ex vivo* activation. The UltraCAR-T® platform is an overnight manufacturing process that uses a semi-closed electroporation system, UltraPorator™, for gene transfer [52,53]. Non-viral genetic engineering strategies are more susceptible to gene transfer without an activation signal than lentiviral based approaches, however, they can pose additional toxicity concerns, as later described.

While there are advantages to infusing a naïve T cell population including retention of a quiescent cell population, conventional manufacturing processes rely on the

use of an activation stimuli to facilitate lentiviral integration and ultimately drive CAR expression which has resulted in potent, efficacious DP. When considering a rapid process, it is possible that alternative activation strategies may be preferred to facilitate earlier CAR-Transgene integration and reduce the impacts of activation induced cell death or terminal differentiation programs. Finally, it is well understood that T cell activation drives clonal expansion, which encompasses several days in a conventional manufacturing setting. However, in the context of a rapid manufacturing process, the cell product is harvested prior to the logarithmic expansion phase with the expectation that expansion will commence upon *in vivo* infusion. It remains unclear if *in vivo* expansion kinetics of unactivated and previously activated CAR-T cell products are comparable and may be an important area of investigation when developing a rapid process.

Vector delivery

As discussed previously, current clinical CAR-T cell therapies rely on viral vectors to deliver the CAR-Transgene and drive stable surface CAR expression. However, additional non-viral genetic engineering strategies are under development, some of which have been employed in rapid manufacturing settings (Table 2) [37,53]. Briefly, non-viral gene editing and delivery strategies including transposons, designer nucleases, electroporation and nanoparticles have been reviewed in detail elsewhere [54]. While many of these strategies are accompanied by their own unique sets of advantages, inefficient plasmid delivery, impacts to cell viability, safety concerns for off-target delivery and scalability remain significant hurdles that hinder adoption into clinical manufacturing.

Currently, the majority of rapid manufacturing efforts including those by Novartis (T-Charge), and AstraZeneca/Gracell (FasTCAR) (manufacturing time <72 h) as well as expedited manufacturing efforts by Kite (huCART19-IL-18) and Bristol Myers Squibb (NexT; manufacturing time 4–6 days), use viral-based delivery strategies (Table 2) [33,36,40,42,55]. While the timing of the transduction can span from the time of activation to ~24 h post-activation, mechanistically much has to happen inside of the cell to achieve stable CAR surface expression. First, the viral envelope protein VSVG must bind to low-density lipoprotein (LDL) receptors on the T cell surface to enter the cell. Viral RNA must then be unpackaged, reverse transcribed and subsequently integrated into the host cell genome. Complete integration of the CAR encoding DNA and subsequent transcription, translation and trafficking to the cell surface can require 72–96 h. The desire to shorten the entire CAR-T manufacturing process to less than 72 h therein poses a challenge, since accurate detection of surface CAR protein through conventional flow cytometric approaches is required to formulate the final dose.

Although viral vectors are the most established of the genetic engineering strategies, there are additional challenges that could impede their widespread use in rapid processes. Rapid processes employing 'no-expansion' protocols need to begin with cell numbers that far exceed the number of cells needed to meet the clinical dose. Not only must the DP bag be produced, but material needed for release testing, regulatory retains and potentially a back-up DP dose must also be generated while accounting for some cell loss during the harvest process. Given that there will likely be cell loss within the first 24 h due to activation-induced cell death or transduction-related toxicity, this could mean starting the process with greater than 1×10^9 $CD3^+$ T cells. Depending on the multiplicity of infection (MOI) used to transduce the material, this places a substantially increased demand on viral vector supply and will subsequently

increase the cost of raw materials required to generate DP. As previously indicated, the cost of cell therapies is one aspect with direct implications on patient access. Therefore, it may be worthwhile to allocate resources for the exploration of more economical strategies of gene transfer.

Aside from the use of lenti- or retro-virus, electroporation of T cells with the Sleeping Beauty transposon system to simultaneously drive expression of an anti-MUC16 CAR and safety kill switch in a 1-day manufacturing process is currently being investigated in a Phase 1/1b trial for recurrent platinum resistant ovarian cancer (Table 2) [38,53]. This process is achieved using Precigen's proprietary UltraPorator electroporation system and is capable of meeting large scale manufacturing requirements as evidenced by the infusion of clinical doses over 300×10^6 UltraCAR-T cells. This platform has demonstrated, preliminarily, that electroporation can be implemented into an expedited process while circumventing the risk of impurities associated with viral transduction. However, it should be noted that while this process removes the bottleneck associated with generating large volumes of high titer viral material, electroporation of purified T cells at this scale (starting material $>1 \times 10^9$ cells to generate a clinical dose of 300×10^6) will require large amounts of GMP grade plasmids which can be a significant expense in raw materials. Additionally, there are ongoing safety concerns from regulatory agencies surrounding the use of transposons in CAR-T manufacturing due to the concern for potential off-target integration, insertional mutagenesis and oncogenic risk to the patient requiring close long-term monitoring of adverse events in the treated population [56].

The use of both viral and non-viral approaches in rapid CAR-T cell manufacturing have been employed, however both strategies are subject to high raw material costs required to generate clinical doses upwards of 100×10^6 CAR^+ cells. Looking forward to the technological advancements in CAR design including advanced armoring strategies and secretion of tumor microenvironment (TME) modulating agents, it is possible that future generations of CAR-T cell products may demonstrate clinical efficacy at reduced clinical doses which would make a rapid process more achievable from a process and manufacturing capacity perspective [57].

Elimination of expansion phase

The end-to-end time for many of the rapid processes in development and under clinical investigation are within 72 h. This process duration aligns with the lag observed in T cell expansion kinetics after activation. Prior to clonal expansion, T cells must undergo several cellular processes including upregulation of specific genes, expansion in cell size and entrance into an active cell cycle from a quiescent state. The lag in T cell proliferation can span from 24–72 h and therefore minimal cell expansion happens in the context of a rapid protocol (Figure 1).

Without a period of T cell expansion, one of the greatest challenges for process development is the ability to meet the clinical dose. However, current data indicate that non-expanded cells have a superior phenotype and higher *in vivo* proliferative capacity, therefore it is possible that an effective dose may be significantly lower than conventional T cell therapies. Consider an example in B cell lymphoma—in the standard manufacturing Phase 1 trials, a dose range of $25–200 \times 10^6$ was evaluated. In the rapid manufacturing trials for YTB323, which expresses the same validated CD19-targeting CAR as tisgenlecleucel, and huCART19-IL-18 the dose ranges were significantly lower ranging from $3–70 \times 10^6$ cells per patient [34,55]. It is encouraging that the overall response rates (ORR) and complete response rates

(CRR) were comparable across both manufacturing platforms [34,55]. On the other hand, when considering the multiple myeloma indication where doses in the rapid process were decreased ~90% from 100–300 × 10^6 to only 10–20 × 10^6, the CRR achieved by infusion with the rapidly manufactured DP was 60% lower than those observed in the CARTITUDE-1 and IMMagine-1 trials [33,58,59]. These findings suggest that dose escalation trials will need to be performed with the rapidly generated CAR-T cells, as efficacious doses may depend on the disease indication or tumor associated environments. Although it is likely not feasible to perform a head-to-head comparison of CAR-T cells generated in a rapid process and conventional process due to additional confounding variables, this information would be beneficial to the ongoing development efforts in this space.

Regarding the solid tumor landscape, clinical efficacy with conventional CAR-T cells remains a challenge, with no current commercial products on the market. Due to the inefficiencies of CAR-T cell trafficking from the blood to the solid tumor site, it is possible that solid tumor indications could mandate higher infusion doses than what is currently required for hematologic indications, which could be difficult to achieve in a rapid process. However, the superior T cell phenotype resulting from a rapid or expedited process could be a benefit in enhancing CAR-T durability, which may be ultimately more advantageous in treating solid tumor indications. It remains to be explored if therapeutic combinations or novel TME-directed approaches could enable the successful entrance of rapid CAR-T cell products into the solid tumor space.

Quality control and release criteria

Despite the headway that has been achieved in shortening the autologous manufacturing process, bringing us closer to being able to treat patients faster with a higher quality DP, product release testing and compliance with global regulatory bodies encompasses an additional challenge to overcome. The implementation of a shorter manufacturing timeframe often eliminates the in-process culture washing and media exchanges which help to remove or dilute impurities from the DP, including residual viral vector or non-T cell populations. In order to meet defined product specifications with regard to product identify, safety and purity it will be critical to demonstrate that removal of any process related impurities can be achieved within a shortened manufacturing timeframe [26,60]. Logistically, this will require minimizing the concentration of raw materials used within the process and implementing additional wash steps during the harvest procedure.

Perhaps the largest concern for product release testing with an accelerated process is validation of product identity and potency. Currently, under standard manufacturing conditions, evaluation of CAR-Transgene expression is performed using a flow cytometric approach of surface protein expression or a PCR-based viral copy number assay to quantify the number of transcripts integrated into the host cell genome. These methodologies can reliably measure stable CAR expression which plateaus in a conventional 5–10 day process. However, the ability to reliably measure CAR expression 48–72 h post-transduction has proven to be challenging using existing, qualified flow cytometry methods. It has been previously reported that integration of lentivirally-delivered genetic material is accompanied by a delay of 2–3 days prior to detection which can be explained by integration of the transgene, translation of the mRNA transcript and trafficking of the CAR molecule to the T cell surface [61]. Additionally, previous reports of pseudotransduction and unstable CAR expression at these early time points have been identified as an area of concern, as these

measurements may yield unreliable results for formulation and dosing [41]. With the advent of rapid processes, it is possible that additional orthogonal assays that are not reliant on surface CAR expression may need to be developed to facilitate product release under accelerated manufacturing timelines.

Additional safety release testing encompasses mycoplasma, endotoxin, and sterility testing to ensure the DP is free from harmful contaminants. Conventional sterility testing can require up to 14 days. There has been promising development surrounding rapid sterility testing using sensitive cytometry systems capable of detecting fluorescently labeled microorganisms or non-growth based 16S and 18S amplicon sequencing modalities which can significantly reduce this 14 day timeframe to several hours to days [62,63]. An additional solution encompasses an all-in-one approach to release testing in which multiple assays are performed in parallel using automated technology. An in-process QC technology platform, coined CellQ®, is currently deployed by Cellares to resolve manual QC processing bottlenecks associated with release testing and could be integrated into a rapid manufacturing setting. Additionally, this automated, high throughput platform can generate results using minimal sample volumes and reduce the need for any re-testing due to operator error. While these types of all-in-one QC platforms are enticing, they still rely heavily on the development of technologies that can reliably assess the critical parameters in a premature cell population, most challengingly CAR-T cell identity and potency.

Potency assays assessing CAR-T cell functionality inherently face the same challenges as CAR detection/identity assays given they rely on CAR-mediated recognition of tumor antigen which drives a cytotoxic T cell response. Currently, regulatory agencies recommend the development and utilization of orthogonal assays to measure potency including direct tumor cell killing and cytokine secretion prior to product release. Whether or not CAR-T cell cytotoxic performance or interferon-γ (IFN-γ) production at rapid harvest timepoints are representative of the DP attributes upon stable transgene integration remain an open question and active area of investigation.

In attempt to circumvent the challenges surrounding CAR detection and potency at early time points, an alternative strategy is to retain a subculture of the DP for a specified time period until flow cytometric approaches can be reliably used to estimate the dose before release of the DP. This approach does however add complexity to DP formulation and filling, QC release testing and pharmacy manual instructions. Additionally, with the recent development of rapid sterility methods, subculture techniques to confirm CAR identity could impose another bottleneck that delays DP release. Therefore, the development and validation of molecular-based analytical methods that can be reliably used at earlier stages in the manufacturing process may offer a preferable solution to detect accurate CAR^+ cell numbers from a rapid process. In summary, acceleration of DP release requires innovative solutions that minimize sample volumes, employ automation, and implement novel methods to accurately identify CAR^+ cells and report sterility with rapid turnaround time.

Decentralized manufacturing facilities

As previously mentioned, most pharmaceutical companies that have commercial CAR-T products have adopted a centralized manufacturing model in which the patient's apheresis is shipped to a dedicated production facility to manufacture the DP. This model is preferred from a quality control and standardization perspective with less opportunity for production or method protocol variation. Additionally,

these large-scale facilities are equipped to accommodate large numbers of manufacturing starts each day making them financially attractive options. However, centralized manufacturing designs can extend the V2V time given additional transportation times on both the front and back end of the process, while also mandating cryopreservation of the DP prior to re-infusion. Adoption of a regionalized production scheme in which a patient's drug is manufactured relatively close to the infusion center would simplify the transport logistics and could potentially enable delivery of a fresh DP to the patient (Figure 1). Prior reports have demonstrated potential benefits of a fresh DP over its frozen counterpart including improved *in vivo* functionality and improved product quality [64,65]. Additionally, a previous clinical study has demonstrated production feasibility in a decentralized model with a low frequency of manufacturing failures [64]. While both scientific and clinical evidence support moving toward a more dispersed manufacturing paradigm, this shift is challenging for large cell therapy manufacturers to implement.

Unfortunately, regionalized, or decentralized models are subject to their own set of challenges that must be overcome to reap the benefits of this manufacturing model. As alluded to previously, this would require the infrastructure to support multiple manufacturing facilities strategically placed across the USA, Europe, and Asia to limit barriers to patient access. Moreover, it is critical that each independent site is aligned not only on the manufacturing process and corresponding batch records, but also the execution of release methods to ensure consistency in the drug product [66]. These efforts will require significant oversight and coordination, posing a significant CMC and Quality challenge. Specifically, instituting a quality organization to manage and resolve any deviations or OOSs that may arise across multiple sites represents a significant barrier to integration of this model. Additionally, despite the continued push for end-to-end process automation, at this time, semi-automated processes do still require hands-on operator time to oversee the process and perform necessary manipulations of the material [67–70]. In a regionalized or de-centralized model, the manufacturing sites may be located in less metropolitan locations which could pose significant staffing challenges or mandate rigorous training programs to ensure operators are qualified to lead the manufacturing efforts.

Considering the future of cell therapy, adoption of a decentralized or point-of-care manufacturing setting would revolutionize patient access. In an idealized scenario, immediately after leukapheresis, a patient's material would enter manufacturing on site eliminating the need for formulation and cryopreservation. If a rapid manufacturing process (<72 h) is employed in a near patient setting, the patient DP can be harvested and prepared for infusion within 4 days, plus the time required for the completion of rapid release testing. Adoption of this process can reduce the V2V time by at least 7 days—the time associated with transport plus the difference between conventional (6–12 day) and rapid (3 day) manufacturing (Figure 1) [71]. A reduction in V2V time has been demonstrated to have significant clinical implications on patient outcomes and predicted life expectancy gains following CAR-T treatment [20,60]. Finally shortening of this timeframe could not only help limit disease progression and potentially mitigate the need for poorly tolerated bridging therapy, but may also help ensure that more patients become eligible for these life-saving treatments.

DRUG PRODUCT ATTRIBUTES AND THERAPEUTIC BENEFIT

Increased patient access is the primary clinical driver of the development of an

accelerated manufacturing process, but the higher quality of the DP that results from a shorter *ex vivo* culture time may improve patient responses. Early preclinical and clinical data have shown that a shortened *ex vivo* culture time reduces terminal T cell differentiation and subsequent acquisition of a transcriptionally exhausted program [35,41,72]. Additionally, cells harvested under restricted cultivation conditions demonstrate improved anti-tumor functionality, increased proliferative potential, and expanded *in vivo* persistence, all of which are indicative of attributes of a naïve/central memory T cell.

Previous preclinical investigations using patient material have demonstrated that CD19-targeting CAR-T cells harvested at earlier time points (3 or 5 days) have increased anti-leukemic activity in a murine xenograft model of ALL as compared to those harvested at later time points (9 days) [72]. Effectively, sub-therapeutic doses of earlier harvest CAR-T cells were able to induce disease remission in this model. Phenotypically, longer culture periods also enriched for effector $CD8^+$ T cells as defined by the phenotypic signature $CD45RO^+$, $CCR7^-$ whereas naïve-like cells were more prevalent after shorter *ex vivo* culture durations. Additionally, CAR-T cells harvested after only 3 days in culture showed increased fold expansion upon tumor antigen exposure *in vitro*, as well as long term persistence *in vivo* [72].

In the clinical space, Bristol Myers Squibb initiated a Phase 1 study investigating their accelerated NEX-T 5–6 day CAR-T manufacturing process. The next-generation BCMA asset, BMS-986354, illustrates a significant enrichment in central memory ($CD45RA^-$, $CCR7^+$) cells in the DP as compared to the control CAR-T, orcabtagene autoleucel (orva cel) as well as increased production of pro-inflammatory cytokines, IFN-γ, IL-2, and TNF-α [43]. Patients enrolled in the dose escalation trial received $20–80 \times 10^6$ CAR^+ T cells, a 2.5–7.5-fold reduction in dose, relative to the orva cel trials. Despite receiving a fraction of the standard manufacturing process dose, both products demonstrate comparable post-infusion expansion, suggesting that the NEX-T products have increased proliferative potential and persistence. Finally, from a safety and efficacy perspective, BMS-986354 illustrates an overall response rate of 95% with an accompanying profile of low-grade CRS and neurotoxicity [43]. The reduction in therapeutic dose may explain the low-grade adverse events and further reinforce the clinical advantages to adopting a rapid process.

Novartis has also developed a rapid (<2-day) manufacturing process coined, T-Charge, which has been implemented in DLBCL, CLL, ALL, and MM indications [33,34]. Initial clinical evaluation of safety and preliminary efficacy of the CD19-targeted CAR, YTB323, are extremely promising. Relative to the traditionally manufactured product, tisagenlecleucel, YTB323 retained a higher frequency of naïve and stem cell memory T cell subsets which was further reinforced by stem-like gene signatures present in bulk and scRNA-seq analyses. Functionally, rapidly manufactured cells demonstrate better killing capability upon repetitive stimulation suggesting these cells have increased survival capacity and resistance to acquisition of an exhausted transcriptome. In line with BMS-986354, the pharmacokinetics of YTB323 also illustrate enhanced proliferative potential *in vivo* as compared to tisgenlecleucel despite being administered at a 25-fold lower dose, as well as a desirable safety profile [34]. Analogously, BCMA-targeted CAR-T cells manufactured with the T-Charge platform termed, PHE885, illustrate strong clinical efficacy, self-renewal capacity and long-term persistence at 6 months of follow-up [33]. Furthermore, given the reduced V2V time, less than 30% of patients required bridging therapy prior to CAR-T infusion [73]. Collectively, the

early Phase 1 findings discussed in this section support the continued investment in developing rapid processes across hematologic disease indications.

Several other companies have made significant headway in this space including Kite/Tmunity, AstraZeneca/Gracell, Precigen, and Hrain Biotechnology, all leading Phase 1 trials utilizing accelerated manufacturing (Table 2). Many of the DP attributes echo the previous reports highlighting the robustness of rapid manufacturing across varying processes, tumor targets, and disease indications. Despite most investigations remaining largely focused on hematologic malignancies, there has been an expansion of cell therapies into solid tumors (ovarian, triple negative breast) and autoimmune indications (systemic lupus erythematosus) [38,53,74]. Solid tumors have remained a challenge for CAR-T cell therapy for nearly a decade, and it remains unclear if heightened cellular potency will be sufficient to overcome hurdles including T cell trafficking and suppressive tumor microenvironments. However, if combined with an optimized CAR design and appropriate manufacturing strategies, the field is hopeful that this approach could improve clinical responses and bolster cell therapy expansion into solid tumor malignancies.

TRANSLATION INSIGHTS

Rapid manufacturing processes are one approach to reduce patient V2V time and increase the accessibility of cell therapies. In addition to the clinical benefits including reduced opportunity for disease progression and generation of a higher quality DP, there are additional benefits of a rapid process from a manufacturing perspective. Reducing the time required to generate a clinical dose will increase manufacturing capacity enabling a scale up of the number of patients who can be manufactured per unit time. For example, cutting the manufacturing time in half will double the number of patients who can be manufactured in any given facility. This not only offsets facility costs on a per patient basis but will also reduce labor costs of operators required to perform critical manufacturing steps and provide oversight to the process. The extensive list of incentives for pursuing a rapid process have inspired key players in the cell therapy landscape to continue tackling the challenges preventing widespread implementation of a rapid process. These challenges include controlling for DP purity, achieving an efficacious clinical dose without a cell expansion phase and rapid, reliable release methods. Accurate characterization of the DP at an early timepoint is difficult with the current set of acceptable release assays, however, developing and qualifying new technologies capable of predicting stable CAR expression from a transient readout may be able to address these challenges. Alternatively, redesigning the dosing strategy could also alleviate these issues. Purity of the DP without additional washes during the process raises concerns for heightened lentiviral, non-T cell populations, and other process related impurities. However, rigorous washing during the formulation process as well as the development of increased sensitivity assays to detect these contaminants may help alleviate surrounding concerns. Finally, regarding dosing guidelines, it is likely that advancements in CAR design and armoring strategies will enable lower efficacious clinical doses that can be readily achieved with a rapid process, making treating solid tumors indications more feasible.

CONCLUSION

CAR-T cell therapy has made a profound impact on patients' lives in the last decade, however, there remains an unmet clinical need–to reduce the time to administration and increase the number of patients who can successfully receive this form of therapy. One component of the V2V time

is the time required to generate the DP *ex vivo*. Recent advances in cell manufacturing have demonstrated small scale feasibility and increased clinical efficacy of CAR-T cells generated within 72 h. However, widespread adoption of a rapid process will require innovative process and analytical development efforts, regionalized manufacturing models that enable the use of fresh apheresis, as well as early and more frequent interaction with global regulatory agencies. In addition, the early preclinical and clinical data suggesting that rapidly manufactured cells are inherently more potent may warrant further clinical investigation into defining an efficacious dose with a heightened awareness of potential toxicities that may arise. Despite the current financial and logistical challenges, rapid processes can significantly increase manufacturing capacity while the adoption of automated platforms can further increase scalability and reduce labor costs to justify the upfront investment.

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MANUFACTURING AND BIOPROCESSING

SPOTLIGHT

INNOVATOR INSIGHT

Ensuring compliance through collaboration: managing raw material changes in cell and gene therapy regulatory filings

Kasey Kime and Xiao Peng

The manufacturing of cell and gene therapy products relies on a complex network of specialized raw materials, each playing a vital role in ensuring product consistency, efficacy, and safety. From cell selection reagents and gene-editing enzymes used in upstream processes to process buffers, excipients, and cryoprotectants in downstream production, these materials directly impact the quality and performance of advanced therapies. However, despite their critical role, raw materials are often overlooked in regulatory filing change management strategies.

Global supply chain disruptions, intensified by the COVID-19 pandemic, have highlighted the vulnerabilities associated with reliance on single-source suppliers and rigid material registration practices. To mitigate these risks, regulators and industry stakeholders are shifting toward flexible, science-based approaches that integrate QbD principles into the regulatory filing and management of raw materials.

Ensuring compliance with global regulatory filing expectations requires a proactive approach to raw material change management encompassing shared responsibility between drug developers and suppliers. This article examines the challenges of raw material changes across clinical development and commercial manufacturing, the regulatory considerations involved, and best practices for mitigating risks associated with post-approval modifications. It highlights the importance of collaboration between suppliers and end users to minimize regulatory risks. By exploring case studies, this article highlights the role of both suppliers and drug developers in ensuring raw material consistency, quality, and regulatory compliance.

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DEFINITIONS OF RAW MATERIALS

Regulatory agencies and international guidelines use varying terminologies to define raw materials in cell and gene therapy manufacturing. While the core concept remains similar—referring to materials used in production but not intended to be part of the final product—there are some regional differences in terminology. Table 1 below outlines these variations [1–7].

REGULATORY FILING EXPECTATIONS FOR DRUG DEVELOPERS

Raw materials used in the manufacturing process of drug substances should be listed in CTD section 3.2.S.2.3. Control of Materials [8]. Each material's name, its use in the process, and details on its quality and control should be provided. While the supplier's name is not always required, some health authorities may request it for critical materials. Compendial or multi-compendial grades should be indicated where applicable, and specifications should be included for non-compendial materials. Evidence must be provided to demonstrate that the quality of raw materials meets the standards appropriate for their intended use. For instance, biologically sourced raw materials may need thorough evaluation to determine the presence or absence of harmful endogenous or adventitious agents.

According to ICH Q11 guidelines, any potential for raw material attributes to impact drug substance (DS) critical quality attributes should be identified [9]. ICH Q11 states: *"Raw materials used near the end of the manufacturing process pose a higher risk of introducing impurities into the DS compared to those used upstream; therefore, tighter quality controls should be considered"*. Risk assessment strategies to define raw material control can include evaluating the manufacturing process capability, detectability of attributes, and severity of impact. For example, the process's ability to eliminate an impurity or limitations in detecting issues (e.g., viral safety) should be factored in. Typically, risks related to impurities are managed through raw material specifications or robust purification steps during manufacturing.

During the investigational phases, any change to a raw material that could affect drug product quality must be submitted to the US FDA as an information amendment to the IND, in accordance with 21 CFR 312.31(a)(1) [10]. This submission should occur before the modified material is used in clinical investigations. For complex changes, it is possible to discuss the matter with the FDA; however, no specific timeframes are provided for such discussions. Therefore, it is advisable to request FDA feedback well in advance of implementing the change [11]. Unlike post-approval change guidance, there is limited detailed guidance available for examples of changes during the investigational phases. However, if a raw material change has the potential to impact drug product quality, a comparability study is required to demonstrate that the drug product maintains consistent quality when manufactured with the new material.

In the post-approval phases, when a drug manufacturer plans a change, assessing the potential impact on the process and product quality is essential and requires classification of the change according to regional regulatory guidelines. Changes are typically classified as major, moderate, or minor based on their nature and potential impact. A major change requires submission and approval by a health authority before distributing post-change material. A moderate change typically requires submission but may not need approval before distribution. A minor change only requires reporting to the health authority after implementation and does not need prior submission. These classifications

TABLE 1

Comparison of raw material terminology related to cell and gene therapies.

Region	Terminology used	References	Comments
USA	Ancillary materials used in manufacturing but not intended to be part of the final product; examples include fetal bovine serum, enzymes, growth factors, cytokines, antibiotics, media, detergents	USP <1043> Ancillary materials for cell, gene, and tissue-engineered products [1]; FDA Guidance for Industry: CMC information for human gene therapy IND applications (Jan 2020) [2]	Emphasize risk assessment for potential introduction of adventitious agents or impurities
EU	Raw materials are the reagents that are used during the manufacturing process but are not part of the final product; examples include fetal bovine serum, trypsin, digestion enzymes (e.g., collagenase, DNase), growth factors, cytokines, monoclonal antibodies, antibiotics, resins, cell-separation devices, and media and media components	EMA Guideline on Quality, non-clinical and clinical requirements for investigational advanced therapy products in clinical trials (Jan 2025) [3]; Ph Eur. 5.2.12 Raw materials of biological origin for the production of cell-based and gene therapy medicinal products [4]	Qualification and testing follows European Pharmacopoeia 5.2.12 guideline
International	Ancillary material is one that comes into contact with cellular therapeutic products during cell processing but is not intended to be part of the final formulation	ISO 20399 Ancillary materials present during the production of cellular therapeutic products and gene therapy products [5]	Definition aligns with USA and EU, focusing on process contact rather than final product inclusion
	Raw materials are starting materials, reagents, and solvents intended for use in the production of intermediates or APIs; critical raw materials are defined as all materials affecting the quality of the API; critical material sttribute (CMA) is a physical, chemical, biological, or microbiological property or characteristic of an input material that should be within an appropriate limit, range, or distribution to ensure the desired quality of output material	ICH Q7 Good manufacturing practices guide for active pharmaceutical ingredients [6]	Broad definition but not specific for cell and gene therapies; note that critical raw materials have critical material attributes (CMAs); while CMA a commonly recognized term, it is not defined within ICH guidance
	Raw material is a general term used to denote reagents, solvents, and excipients intended for use in the production of cell or gene therapy products	IPRP Reflection Paper General considerations for raw materials used in the manufacture of human cell and gene therapy products, Feb 2023 [7]	Broader definition that aligns to ICH Q7

determine the necessary data to demonstrate comparability between pre- and post-change and helps ensure no negative impact on product quality.

An applicant is typically required to include the following information for any major or moderate quality changes [12]:

- A detailed description of, including a rationale for, the change
- The product(s) involved
- The manufacturing site(s) or area(s) affected
- A description of the method(s) used, and studies performed to evaluate the effects of the change on the product quality, and data derived from these studies
- Relevant validation protocols and data
- A reference list of relevant standard operating procedures (SOPs)
- A comparability protocol before distribution of a product made using the change outlined in the protocol

Global health authorities may differ in their classification of changes, assessing the associated risk to product quality and specifying documentation or data requirements. Table 2 outlines classifications

TABLE 2

Reporting classifications for post-approval raw material changes for biologics across regions.

Health authorities	Guidelines	Reporting category	Raw material examples included?	Is supporting data for an RM change clearly outlined?
Health Canada	Post-Notice of Compliance (NOC) Changes: Quality Document [13]	Major: level 1; Moderate: level II; Minor: level III and IV	Yes, highly detailed	Yes
US FDA	CMC changes to an approved application: certain biological products [12]; Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products [14]; CMC post-approval manufacturing changes for specified biological products to be documented in annual reports [15]; § 314.70 Supplements and other changes to an approved NDA [16]; Comparability Protocols for Post Approval Changes to the Chemistry, Manufacturing, and Controls Information in an NDA, ANDA, or BLA [17]	Major: PAS; Moderate: CBE30/CBE supplement; Minor: AR	Yes	No
EMA	Commission regulation (EC) No 1234/2008 [18]; Commission guidelines on the details of the various categories of variations, on the operation of the procedures laid down in Chapters II, IIa, III, and IV of Commission Regulation (EC) No 1234/2008 and on the documentation to be submitted pursuant to those procedures [19]; EMA post-authorization procedural advice for users of the centralized procedure [20]; EMA Questions and answers on comparability considerations for advanced therapy medicinal products (ATMP) [21]	Major: type II; Moderate: type IB; Minor: type IA and IA_{IN}	Yes, highly detailed	Yes
WHO	Guidelines on procedures and data requirements for changes to approved biotherapeutics products, Annex 3, TRS No 1011. 2018 [22]	Major: major quality changes; Moderate: moderate quality changes; Minor: minor quality changes and quality changes with no impact	Yes	Yes
PMDA	Guideline for Descriptions on Application Forms for Marketing Approval of Drugs, etc. under the Revised Pharmaceutical Affairs Law [23]	Major: partial change approval application; Moderate: minor change notification; Minor: non-approved matters	Yes	No
NMPA	Technical Guideline for CMC Changes to Approved Biological Products [24]; Technical Guideline for CMC Changes to Approved Vaccines [25]	Major: major; Moderate: moderate; Minor: minor	Yes, highly detailed	Yes

AR: Annual report. CBE: Changes being effected. CBE30: Changed being effected in 30 days. PAS: Prior approval supplements. RM: Raw material.

assigned (based on published guidance) for raw material changes for biologics across different regions [12–25]. Not all health authorities include all types of changes in their post-approval guidance, and some have more detailed expectations than others for raw material changes. If there is any uncertainty, it is generally advisable to consult regulators directly to clarify change classifications.

Many post-approval changes cannot be implemented until they have been reviewed and approved by health authorities, a process that can take significant time. During the technical review, additional time and resources may be needed

to respond to information requests from these agencies. Furthermore, the lack of global harmonization across regions makes it difficult to predict how long each health authority will take to grant approval. This can result in several years passing before a change is able to be fully implemented worldwide. Although modern registration strategies, which are based on a scientific understanding of raw material attributes, exist for managing raw material changes, these strategies have not become mainstream due to perceived barriers in regulatory acceptance [26].

REGULATORY EXPECTATIONS FOR CRITICAL RAW MATERIAL SUPPLIERS

While raw material suppliers are not directly regulated in the same way as drug manufacturers, their change control practices, quality management systems, and regulatory knowledge can significantly impact drug developers' ability to maintain regulatory filing compliance.

There are established guidelines [1,5,27–29], certification schemes [30], and voluntary master files in certain regions [31–33] that guide the manufacturing and quality standards for raw materials. However, despite these guidelines, supplier practices can vary widely, leading to differences in raw material change management and change communication.

Basic practices for suppliers include providing timely change notifications, adequate supporting documentation for the end user to assess changes and understanding how their internal change classifications align with those of their end users. However, inconsistencies often arise when a supplier's internal change classification does not match the regulatory requirements of the drug manufacturers who use these materials. Suppliers should aim to be aware of common guidelines used by industry to classify changes and help ensure access to proper documentation and risk assessments (Table 3) [12,13,23,27,28,31–37]. For example, how a change in the cell culture media manufacturing process, such as moving manufacturing to a new site may impact the raw material trace element impurity profile. For this reason, USP <1023> recommends that change control notifications from suppliers include trace element testing data from a minimum of three lots [27].

Suppliers should also have knowledge of industry guidelines such as BioPhorum's Registration of Innovative Raw Material Using Quality by Design (QbD) Principles [26] and ICH Q12's [38] lifecycle management approach when assessing the potential impact of raw material changes on regulated end users. Suppliers who integrate these strategies into their quality management systems help facilitate smoother regulatory processes and improve overall compliance for drug developers.

For drug developers, key supplier management best practices may include:

- Establishing Quality Agreements: defining change notification timelines, documentation requirements, and regulatory expectations for raw material modifications
- Supplier Qualification and Audits: conducting regular audits and risk assessments to verify compliance with industry standards such as, ISO 20399 and USP <1043>
- Change Control Coordination: ensuring that supplier-initiated changes are assessed against regulatory expectations and, where necessary, included in regulatory submissions or comparability assessments. Changes to non-compendial raw materials, such as cell culture media or gene editing reagents, typically have a potentially higher impact than simple chemically defined raw materials

TABLE 3

Common supplier guidelines used to classify raw material changes.

Source	Document title
ASME BPE	ASME BPE—Bioprocessing Equipment, 2024 Edition [34]
BPSA	An Industry Proposal for Change Notification Practices for Single Use Biomanufacturing Systems, 2017 [35]
Health Canada	Guidance on Procedures and Administrative Requirements for Master Files: Overview, Jan 2024 [31]
Health Canada	Post-Notice of Compliance (NOC) Changes: Quality Document [13]
EDQM	Guideline on Requirements for Revision/Renewal of Certificates of Suitability to the European Pharmacopoeia Monographs [36]
Japan PMDA	Guideline on Utilization of Master File for drug Substances, etc. 2005 [32]
Japan PMDA	PFSB/ELD Notification 0210001: Guideline for Descriptions on Application Forms for Marketing Approval of Drugs, etc. Under the Revised Pharmaceutical Affairs Law [23]
US FDA	FDA Draft Guidance for Industry: Drug Master Files, 2019 [33]
US FDA	CMC Changes to an Approved Application: Certain Biological Products, 2021 [12]
US FDA	Guidance for Industry: Deciding When to Submit a 510(k) for a Change to an Existing Device, 2017 [37]
United States Pharmacopeia	*USP* <1195> Significant Change Guide for Bulk Pharmaceutical Excipients [28]
United States Pharmacopeia	*USP* <1023> Evaluation Strategy for Trace Elements in Cell Culture Media Used in the Manufacture of Recombinant Therapeutic Proteins [27]

BEST PRACTICES FOR THE REGISTRATION AND CHANGE MANAGEMENT OF RAW MATERIALS

Traditional methods of describing non-compendial materials in filings such as relying on brand names, suppliers, or part numbers—can restrict supply flexibility, as substitutions require regulatory approval, delaying timelines and disrupting continuity. A modern, QbD approach offers a solution by shifting the focus to the material's role and critical quality attributes [26,39].

This attribute-driven strategy includes defining a target material profile (TMP), assessing material attributes, reviewing the overall product control strategy, and identifying critical material attributes (CMAs) to help ensure quality and safety. By fostering science-based raw material specifications and integrating them into the broader product control framework, this approach enhances supply flexibility, enables consistent product quality, and simplifies regulatory lifecycle management in alignment with ICH Q12. With detailed, attribute-driven descriptions in regulatory submissions, companies can achieve efficient supply chain management without compromising compliance.

To drive industry-wide adoption of this modern, QbD-based approach, collaboration and shared guidance are essential. Organizations like BioPhorum have taken a leadership role by creating detailed frameworks to guide the registration of innovative raw materials, helping companies navigate complexities and adopt best practices [26]. These guidelines offer practical tools to implement science-based material specifications and integrate them into regulatory submissions, ultimately fostering a more resilient and flexible supply chain. By providing clear pathways for industry to follow, such initiatives can help ensure that innovation and quality

remain at the forefront of raw material management.

The recent EMA guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials states: *"For all raw materials of biological origin, the information on the supplier or the criteria for material selection should be provided and the potential impact of using several sources or suppliers on the quality of active substance needs to be addressed"* [3]. This requirement allows flexibility for raw material registration based on the understanding of critical material attributes and is aligned with ICH Q12.

Despite these improvements in raw material registrations, the global regulatory landscape for managing post-approval changes remains fragmented, posing significant challenges for sponsors with global submissions. Recognizing this, the World Health Organization (WHO) has emphasized the need for regulatory reliance and collaboration among National Regulatory Authorities (NRAs) to harmonize post-approval CMC change processes and reduce delays [40]. However, achieving regulatory review efficiency requires a secure, unified global platform. In response, the International Council for Harmonisation (ICH) is working toward the creation of such a system, which would enable regulators to jointly review post-approval changes in chemistry, manufacturing, and controls [41]. By streamlining regulatory processes and fostering global alignment, this platform has the potential to transform how raw material changes are managed, helping ensure that supply chains remain robust and responsive in an increasingly complex world.

The following case studies illustrate the practical implementation of these best practices, showcasing collaborative efforts, regulatory engagement, and tailored strategies to help ensure quality, compliance, and supply chain resilience across diverse global markets.

CASE STUDY 1

This section covers a supplier-initiated change in the manufacturing facility for a non-compendial, critical raw material and highlights how collaboration among the supplier, end users, and regulatory authorities facilitated a smooth transition. It underscores the importance of well-characterized raw materials and understanding end-user expectations for comparability data.

A supplier of cell culture media, used in several FDA-approved cell therapy products, needed to increase manufacturing capacity by expanding to a sister facility in Europe. While both sites operated under harmonized quality management systems, there were concerns regarding the comparability of the media produced at the new facility.

Given the proprietary nature of the media formulation, a Type II US Master File had been established with the FDA. This existing master file enabled the supplier to submit the comparability plan as a quality amendment, requesting FDA feedback. The plan, aligned with ICH Q5E, emphasized an analytical comparability approach for the manufacturing transfer. It included detailed information on the target material profile, critical material attributes, and potential risks to product quality, as outlined in ICH Q9 (Table 4).

The FDA responded promptly to the quality amendment, enabling the transfer of product manufacturing to proceed and enhancing supply resilience for commercial cell therapy products in Europe. The comparability plan also served as a template for future cell culture media transfers, establishing a regulatory-accepted strategy for the company and pharmaceutical customers.

The documentation package provided to end users contained a summary of the comparability results, an overview of the harmonized quality management systems,

TABLE 4

Example cell culture media target material profile.

Description	Cell culture media consisting of a mix of amino acids, vitamins, recombinant proteins, and human serum albumin needed for cell growth and function
Intended function	Cell culture media shall provide the necessary nutrients, growth factors, minerals, and hormones for cell growth and function, as well as regulating the pH and osmotic pressure of the culture
Required characteristics to perform function	Quality criteria: Media pH and osmolality is suitable for its intended use; Media can support the intended cell growth; Media can support the intended cell function; Media CoA and COO are available Safety criteria: Sterile. Sterility testing complies with USP <71> and/or Ph. Eur. 2.6.1; Non-pyrogenic. Endotoxin testing complies with USP <85> and/or Ph. Eur. 2.6.14; Free of mycoplasma. Mycoplasma testing complies with USP <63> and/or Ph. Eur. 2.6.7; Low/No adventitious agent risk. Sufficient safeguards are in place to minimize or eliminate the risk of transmitting adventitious agents when using human- or animal-derived components; Absence of visible particulates or obvious precipitations; Non-toxic. Media elemental impurities, residual solvents and trace elements level should not affect cell performance Manufacturability: Media container and size is compatible with the existing manufacturing process and equipment

Material attribute	Target	Justification/control
Appearance	Clear golden liquid and free of visible particulates	A visual inspection to check for any signs of contamination or abnormalities, such as cloudiness, discoloration, precipitates, or visible debris
Sterility	No growth	General safety attribute
Endotoxin	≤1.0 EU/mL	General safety attribute
Performance	≥X × 10^6 viable cells/ml and ≥XX% viability	Changes in performance can reflect raw material inconsistency therefore it is important to test the performance of the media for its intended use
pH	≥6.8 to ≤7.4	This attribute confirms the right environmental conditions are present for cell growth
Mycoplasma	Negative	As these products contain animal origin components mycoplasma testing is performed to help ensure its absence as its presence can inhibit cell growth

and a statement confirming that the change had been discussed with the FDA and that the master file had been updated to include the new manufacturing site.

CASE STUDY 2

A large USA-based drug developer approached a supplier's regulatory team with their plans for global product registration of a new therapy using a chemically defined proprietary media. As raw master files are not available outside of the USA, Canada, and Japan, the supplier's regulatory team had to work with the drug developer on a post-submission plan for requests for information and change management regarding the raw materials. A list of countries and submission target dates was drafted along with a raw material registration strategy for each market. The supplier's regulatory team was able to populate the plan with the regulatory pathway for raw material disclosures in each market, recommend a change notification strategy to meet local requirements and share experiences on managing changes to raw materials across the target markets (Table 5).

The drug developer enrolled in the supplier's change notification program and formalized a commercial supply agreement with the manufacturing site. Additionally, a dedicated regulatory representative was appointed to support Requests for Information (RFIs) from health authorities concerning the raw materials. These measures help ensure that all regulatory requirements for post-approval changes

TABLE 5

Example of regional differences in raw material registration strategies.

Target market	Raw material filing strategy	Additional supplier aspects to consider
USA	Master File	Compliance with DMF Holder regulatory obligations; Supplier's Change Notification, and Supply Agreements
Canada	Master File	
Japan	Master File	
EU	No master file option, disclosure of qualitative media composition to the Applicant	Supplier's Change Notification and Supply Agreements, supplier regulatory lead for enquiries
China	No master file option, disclosure of qualitative media composition to the Applicant	Supplier's Change Notification and Supply Agreement, supplier regulatory lead for enquiries
Australia	With TGA approval, the supplier may submit a master file directly to the TGA to support the Applicant's filing; alternatively, a disclosure of qualitative media composition to the Applicant may be possible	Supplier's Change Notification and Supply Agreement, supplier regulatory lead for enquiries

related to critical raw materials are fully managed and addressed.

CONCLUSION

Managing raw material changes in cell and gene therapy regulatory filings is a complex process that requires close coordination between drug developers, suppliers, and regulatory authorities. These changes—whether prompted by supplier modifications, material shortages, cost considerations, or evolving industry standards—can pose significant risks to manufacturing consistency, product quality, and regulatory compliance.

To mitigate these risks, a proactive and collaborative approach is essential. Suppliers who develop a strong understanding of how raw material changes are potentially classified within pharmaceutical regulations help the industry by providing adequate documentation to support regulatory filings. By ensuring that drug developers have the necessary data and documentation to assess and justify raw material changes, suppliers can play a critical role in maintaining compliance and enabling efficient regulatory submissions in the advanced therapy landscape.

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SPOTLIGHT

REVIEW

Technical and regulatory opportunities and challenges for cell and gene therapies in low earth orbit: a status report

Gary C du Moulin, Ian Sands, Mari Anne Snow, and Yupeng Chen

The unique advantages exhibited by microgravity in enhancing the biological and chemical interactions for cells and tissues have come into greater focus following a quarter century of biological investigation aboard the International Space Station (ISS) in low earth orbit (LEO). One of its primary biomedical research purposes has been to investigate and mitigate the health risks faced by astronauts during prolonged spaceflight. However, as this status report describes, the hundreds of experiments aboard the ISS have also produced a vast quantity of knowledge opening up new possibilities for improving therapeutic modalities for the unmet medical needs of patients on earth. Among its many functions and capabilities, the ISS has been a preeminent biomedical research laboratory for biotechnology and drug development. Public–private partnerships have created the necessary collaborations and supplied the resources to conduct sophisticated biomedical experiments which have led to the improvement in the applications of stem cell biology, gene therapies, tissue engineering, and regenerative medicine. Technological advancements have also resulted in 3D bio printing of soft tissues such as blood vessels and micro physiological systems (Tissue on a Chip) using cells organized in a predetermined architecture. Nanomaterials assembled in microgravity formed with increased homogeneity and bioactivity can function as delivery platforms for cancer therapeutics or may be shaped into extracellular matrix supporting tissue regeneration therapies. Given these exciting innovations, and with the expectation that a robust regulatory framework will emerge, sustainable biomanufacturing in LEO is poised to unlock a transformative economic potential and accelerate the development of advanced next-generation therapeutics.

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FIGURE 1

The ISS has been circling in LEO since 2000.

Photo courtesy of NASA.

Low earth orbit (LEO) is a zone which extends from an altitude as low as 100 miles (160 km) to an altitude of 1,200 miles (2,000 km) above the surface of the earth. Within LEO, at an altitude of 250 miles, the 356 ft. International Space Station (ISS) has been in continuous operation since 2000, circling the earth every 93 minutes at 17,500 miles per hour (Figure 1) [1].

Aboard the ISS astronaut-scientists observe 16 sunrises and sunsets every 24 hours. As the ISS nears its 2030 retirement and potential deorbiting, over 4,000 research investigations have been undertaken by numerous scientific organizations from 108 countries [2]. Expeditions carrying experiments and scientists continue to be transported to the ISS sponsored by governmental, academic, medical, and commercial organizations all of whom utilize this celestial laboratory to better understand the effects of microgravity, radiation and a near continuous vacuum on human physiology, biology, material, and the physical sciences [3]. While microgravity may be simulated on earth, long term environmental conditions of microgravity cannot be duplicated. The vast knowledge gained aboard the ISS over the past quarter century have shown that the properties exhibited in microgravity can have a dramatic effect upon spaceflight mediated risks of radiation, isolation, and confinement, distance from earth, gravity effects, and the hostile and closed environments that affect the physical, chemical, and biological systems of astronauts (Box 1) [4–9].

Mitigation of these risks will lead to plans for long-term residency of humans on the moon and preparations for long duration space travel to the planet Mars [10]. Planning for the maintenance of crew health and provision of medical care when emergency evacuation is not possible is an essential part of human led explorations to the moon and beyond. These goals will require system development and biomedical technological innovations to ensure

the health of astronauts in the years ahead [11]. The ISS is entering its third decade of use as the world's preeminent orbital microgravity innovation laboratory. The efforts of the scientists working in areas of biology, physics, biomedicine, materials, earth, and space science have produced innumerable research discoveries and demonstrations of advanced technology, all of which will continue to return significant benefits to humanity on earth. This status report is intended to provide an overview of the current state of knowledge specific to the cell and gene therapy sector of LEO and to summarize those areas under active investigation in which the microgravity environment has been shown to exert an impact on the function of cells and tissues.

Much of the knowledge gained from research conducted in space over the past quarter century will contribute to improvements for life on earth. As a result, the creation of new and rapidly evolving initiatives is underway whereby the manufacturing of advanced materials and pharmaceutical products can take advantage of the unique environmental properties of LEO [12–16]. In anticipation of these new initiatives and possibilities, National Aeronautics and Space Agency (NASA) and the International Space Station National Laboratory (ISSNL) have partnered with a number of commercial organizations to facilitate the installation of research facilities aboard the ISS in support of business models promoting the creation of manufacturing platforms in LEO. Malshe describes the key drivers for a commercial space infrastructure for the servicing and assembly of orbital manufacturing facilities [17]. These include:

- Resource consumption limits due to population growth
- Human exploration advancements
- Declining launch costs

BOX 1

Space-flight mediated health hazards.

Molecular and cellular features

- Oxidative stress
- DNA damage
- Mitochondrial dysregulation
- Epigenetic and gene regulation changes
- Telomere length dynamics
- Passive osteogenic differentiation
- Microbiome shifts
- Hemoglobin degradation
- Shortened cell cycles

Systemic and physiological health risks

- Cardiovascular deconditioning and dysregulation
- CNS impairments
- Increased blood–brain barrier permeability
- Increased cancer risk
- Muscle degeneration
- Osteoarthritis and bone loss
- Cartilage degradation
- Defects in wound and bone fracture healing
- Immune dysfunction
- Increased liver disease and lipid dysregulation
- Circadian rhythm dysregulation
- Space associated neuro-ocular syndrome
- Altered mechanics of blood flow

Spaceflight mediated health hazards collectively affect multiple biological systems spanning space radiation, microgravity, confinement/isolation, a hostile/close environment, and distance from earth. These areas require scientific advancement to enable deep space exploration [5–9].

- Evolving in-space policies
- Geopolitics
- Advanced spacecraft accessibility
- Demand for space technology platforms [17]

In fact, a number of companies are vying for the opportunity to replace the ISS so as to continue research objectives under conditions of microgravity. Among those

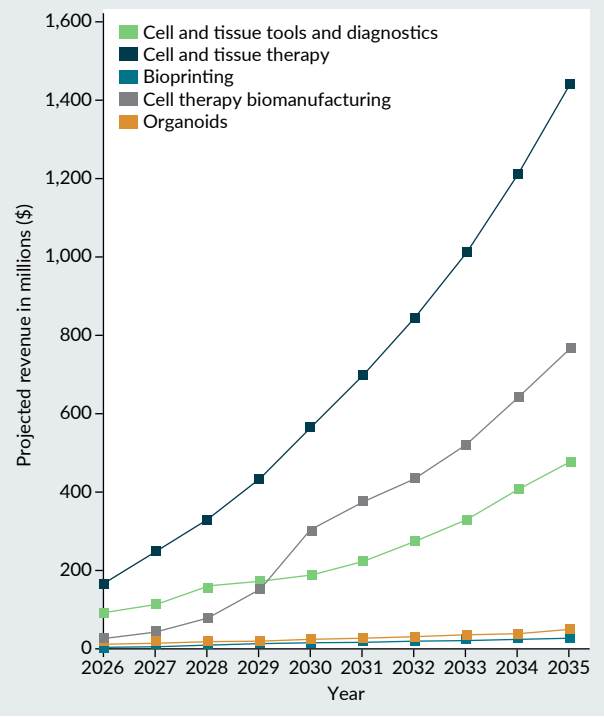
companies that have evaluated the impact, value creation, and feasibility, and are anxious to design, build, and launch their facilities into LEO are Vast Space, Blue Origin, StarLab Space, Sierra Space, SpaceX, and Axiom Space.

BIOTECHNOLOGY AND DRUG DEVELOPMENT IN SPACE

Presently, over 300 commercial entities have been established focused on the development and support of space manufacturing efforts in areas including advanced materials, biofabrication, and biotechnology [18]. The number of patents using the terminology 'microgravity' in the title or abstract rose from 21 in 2000 to 155 in 2020 [19]. Of these, a number are committed to the areas of biotechnology, biological research, and drug development of therapeutic modalities including cell and gene therapy, nanomaterial therapeutics, biologics, and medical devices [15,20,21]. To date, over 900 research articles have been published on biological and biotechnological adaptations to microgravity [22]. Opportunities for expanding business through space manufacturing of biopharmaceuticals including cell and gene therapies have been deemed feasible as revenue projections for making certain products in orbit exceed the operational costs of space manufacturing [23]. For example, McKinsey estimates that in orbit production of pharmaceutical products at maturity could reach an annual revenue at maturity of between 2.8 and 4.2 billion dollars [19]. With respect to biomanufacturing, Sharma determined revenues projected through 2035 and broke down the biomanufacturing market into five subsegments including the manufacturing of cell and tissue therapies (Figure 2) [24]. One company, Varda Space, has recently raised $90 million toward this effort in developing spacecraft that can autonomously manufacture active pharmaceutical ingredients with an ability to deliver these materials back to earth [25]. Deloitte consultants, who have studied the potential for commercialization and industrialization of space predicted that by the year 2035 there will be a vibrant economy situated in LEO resulting in an annual market of 312 billion dollars, an 8-fold increase in today's economic value of LEO [16].

FIGURE 2

Biomanufacturing in LEO market sub-segmentation revenue projection.



The LEO biomanufacturing market for regenerative medicine is broken down into five primary sub-segments: cell and tissue tools and diagnostics; cell and tissue therapy; bioprinting; cell therapy biomanufacturing; and organoids. Projections are for the next 15 years. Redrawn with permission from Sharma *et al.* [24].

CHARACTERISTICS OF A MICROGRAVITY ENVIRONMENT

The force of gravity exists in space. In fact, the gravitational field where most human spaceflight occurs is significant. At 250 miles above the earth's surface the gravitational field is 88.8% of 'normal gravity' (1 g) at sea level. Spacecraft that orbit

the earth maintain their position in orbit through the force of gravity and the speed with which the craft is traveling. That speed, 17,500 miles per hour, sufficient to maintain a state of continuous free-fall is considered to be a 'state of orbit' [26]. The two forces, centripetal force from the circular motion of spacecraft orbiting around the earth at 17,500 miles per hour and the gravitational force pulling the spacecraft toward earth are equal and balanced. While the ISS can be considered in a state of free fall its high horizontal velocity ensures that the station's flight trajectory will never touch the earth's surface. The ISS provides a microgravity value around 10^{-5} g, periodically increasing to 10^{-4} g during a re-boost phase that occurs approximately once per month when the ISS adjusts its orbit, an event that lasts about 30 seconds. Objects inside the ISS appear to be floating or 'weightless' due to this state of 'microgravity' [27].

This phenomenon has been studied extensively within aircraft that fly parabolic arcs to create brief periods of microgravity or in zero gravity research facilities containing drop towers in which test packages can be dropped into a vacuum to create brief moments of weightlessness [28]. Life appeared on earth 4 billion years ago adapting to the planet's gravitational pull. However, in an environment of microgravity where there is a lack of an up or down, significant alterations in the biochemistry of living things, including man become evident [15].

A quarter of a century of research within the ISS has provided an extensive record in understanding the effects microgravity has on the myriad of physical, chemical, and biological properties of life. The characteristics of microgravity are summarized in *NASA's Microgravity Science on the ISS: A Primer for new Researchers* (Box 2) [26].

The biological laboratory facilities installed aboard the ISS provide access to a microgravity environment and are made available to allow for biological experimentation. Among its many scientific functions, the ISS is well equipped as a biological and biomedical research laboratory (Figure 3) [29,30].

Each space agency including NASA, European Space Agency (ESA), Japan Aerospace Exploration Agency (JAXA), Russian Federal Space Agency (Roscosmos) and the Canadian Space Agency (CSA) has

BOX 2

Characteristics of microgravity.

- Absence of convection: there is no convection due to differences in relative densities
- Absence of sedimentation: In microgravity substances of different relative densities, such as water and oil, will disperse evenly
- Absence of buoyancy: buoyancy becomes insignificant in the space environment, light and heavy materials can be mixed uniformly
- Absence of hydrostatic pressure: almost no hydrostatic pressure exists in microgravity
- Containerless float: In microgravity liquids can float in the air without a container
- Dominance of diffusive properties: In microgravity diffusion is the dominant process, a gentler mixing that enables more perfect, uniform, and precise structures at the level of individual molecules and groups of atoms
- Dominance of materials surface tension: Microgravity allows surface tension features to dominate for more precise adhesion, contact, and interactions between layers of similar and dissimilar constituents

The ISS as a Biomedical Research Laboratory for Cell and Gene Therapy.

Reproduced from [26].

FIGURE 3

A 3D floating laboratory in space.

NASA astronaut, Sunita Williams works on StemCellEX-H1, a technology for in-space production of human stem cells that are used as therapies for certain blood diseases and cancers. Photo taken on Oct 2, 2024, courtesy of NASA.

contributed to the construction and operation of the ISS and has included in its instrument designs, laboratory research facilities to conduct biological and biotechnological experiments supporting cell and gene therapy applications. Flexible modular racks and lockers are dedicated to biological research and come with sophisticated instrumentation to support the experiment packages routinely transported to the ISS by institutions from around the world. A partial list of the basic equipment available for biological experimentation of cells and tissues aboard the ISS include:

- Life Sciences Glovebox (LSG; biosafety cabinet)
- Refrigerators/freezers
- Animal housing facilities for small mammals
- Centrifuges
- Incubators
- Growth chambers
- Greenhouses
- Microscope
- Aquariums
- UV spectrophotometers

Specialized equipment developed by commercial partners and NASA's Ames Research Center and provided to academic research groups have been designed to facilitate biological research in microgravity environments. These devices include the Bioculture System, Space Automated Bioproduct Laboratory (SABL), BioCell, Microscope Platform, PCR

FIGURE 4

The BioCulture System and one of the ten hollow fiber bioreactor cassettes designed for cell culture studies within the Bioculture System.

Each cassette provides the structural support, power, data, gas supply, incubator and refrigerator compartments. Photo courtesy of NASA.

analysis equipment (Wetlab-2), and DNA sequencer.

BioCulture System

The BioCulture system was developed by NASA's Ames Research Center and Tissue Genesis, Inc. to replace the Cell Culture Module that was originally designed for use in the Space Shuttle Program. The flight proven design and lessons learned were incorporated into a design that would be conducive to bioresearch studies in microgravity involving both stem cell and specialized cell lines. The system consists of a docking station, command module, gas supply assembly that houses 10 individually controlled experiment cassettes (Figure 4). Each cassette is a hollow fiber bioreactor and provides structural support, power, data, gas supply, incubator, and refrigerator compartments. The BioCulture system has since been used to support a wide diversity of tissue, cell, and microbiological cultures and experimental methods [6].

Academic and commercial researchers use the Bioculture System to study a wide range of biological processes in microgravity that are relevant to human health. These experiments have delivered a greater understanding about how gravity affects the physiology, biochemistry, genetics and gene expression of living cells, tissues, and microbes for purposes of drug discovery, countermeasure analyses, or to study infectious disease processes [30]. Studies of tissue engineering, regeneration, and wound healing are also possible applications of this system (Figure 5).

Space Automation Bioproduct Laboratory (SABL)

The Space Automation Bioproduct Laboratory designed and built by Bioserve Space Technologies, Inc. is a dual function

FIGURE 5

An astronaut-scientist conducting cell culture studies by manipulating the hollow fiber bioreactor within the Life Sciences Glovebox (LSG).

Photo courtesy of NASA.

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incubator/freezer that supports space life science experiments on the ISS for conducting cell culture and other biological experiments. The instrument provides advanced incubator technology, and active CO_2 in support of mammalian cell culture. Three units have been installed aboard the ISS.

BioCell

Bioserve Space Technologies, Inc. also designed cell culture hardware to function aboard the ISS in place of cell culture flasks or multiwell culture plates. The BioCell meets the strict NASA guidelines for safety and biological containment. The devices are compatible with the microscopy platform and the plate reader components. The Biocell supports fluid injections, media exchanges, fixation, and culture preservation.

BioServe Microscope Platform (Figure 6) [31]

A Nikon Eclipse TS100 professional inverted microscope allows for bright field and phase-contrast microscopy and is capable of providing full high-definition Imaging. The microscope is equipped with high quality objectives ranging in power from 2× to 40×. Additional objectives and other add-ons can be flown to the ISS on a per-experiment basis.

Polymerase Chain Reaction (PCR) analysis (Wetlab-2)

Wetlab-2 is a research platform for conducting real-time quantitative gene expression analysis aboard the ISS. This facility enables spaceflight genomic studies involving a wide variety of biospecimen types in the unique microgravity environment of space. WetLab-2 was developed at NASA's Ames Research Center and enables the traditional use of quantitative PCR, such as measuring gene transcription or rapid detection of gene targets that indicate infectious disease, cell stress, changes in cell cycle, growth and development, and/or genetic abnormality. The Wetlab-2 facility includes a commercial PCR instrument

FIGURE 6

NASA astronaut K Rubins at the microscope platform aboard the ISS.

She is studying the effects of spaceflight on human induced pluripotent stem cell-derived cardiomyocyte structure and function [31]. Photo courtesy of NASA.

(Cepheid SmartCycler®) that can perform up to 16 PCR reactions in parallel, a sample transfer tool for retrieving samples from culturing hardware and a set of fluidic modules to enable sample preparation work. Researchers working in a weightless environment can use the full facility to produce quantitative PCR information or extract RNA from their samples for analysis on the ground or by other facilities available on the ISS.

DNA sequencer (MinION)

Sequencing is a technology that addresses several critical spaceflight needs: infectious disease diagnosis, population metagenomics, gene expression changes, and accumulation of genetic mutations. Based on size, power, and ease of use considerations, the MinION™ DNA sequencer (Oxford Nanopore Technologies, Oxford, UK) was the most spaceflight-ready of commercially available sequencers. This device sequences DNA and RNA by measuring current changes caused by nucleic acid molecules passing through protein nanopores embedded in membranes; the change in current is diagnostic of the sequence of the DNA or RNA occupying the pore at a given time [32].

PUBLIC–PRIVATE PARTNERSHIPS IN FOSTERING OUTER SPACE INNOVATIONS IN BIOTECHNOLOGY AND BIOPRODUCTION

The recognition that space is a catalyst for economic growth and that commercialization will populate LEO and beyond with human activity, a number of public-private research and development partners have been established focused on broadening our biological knowledge of cells and tissues in space environment [24,33]. These partnerships serve to stimulate open-source research improving intellectual activity and productivity through input sharing, labor pooling and cross fertilization of ideas and knowledge in a concept known as 'agglomeration externality' [33]. Collaborations in the area of biotechnology and bioproduction have investigated how sustained microgravity influences cellular behavior including pluripotency, multipotency, cell division, cytokine and growth factor secretion, differentiation, cell to cell interactions, tissue development and regeneration, aggregate interactions in the context of the whole organism, and changes to stem cell proliferation rates [34]. These collaborations have produced an enormous amount of information leading to a greater understanding of stem cell properties and cell behavior in microgravity. Major medical centers have participated in public–private partnerships that are focused on space-based programs for stem cell science [24,31,35–40]. Among the institutions participating in these partnerships includes such prestigious medical centers as: Cedars-Sinai Regenerative Medicine Institute, Stanford University Consortium for Regenerative Medicine, the University of California at San Diego, Center for Regenerative Biotherapeutics and Department of Laboratory Medicine and Pathology, Mayo Clinic, Loma Linda University School of Medicine, New York Stem Cell Foundation Research Institute, Department of Molecular Medicine Scripps Research Institute and Emory University School of Medicine and the Children's Healthcare of Atlanta and Department of Biomedical Engineering, Georgia Institute of Technology. The investigations into human stem cell science in space undertaken by these organizations are relevant to the study and treatment of human disease on earth.

A number of large pharmaceutical manufacturers have also been committed to space-based research programs, many conducting research on board the ISS. Pharmaceutical developers currently

investing in space research include the following:

- AstraZeneca: nanoparticle drug delivery systems for therapeutic cancer vaccines
- Bristol Myers Squibb: protein crystallization
- Merck and Co: monoclonal antibodies (pembrolizumab, Keytruda®) as crystalline suspensions to enhance drug delivery. Protein crystal growth is smaller, much purer and consistent leading to lower viscosity with better injectability
- Gilead: increase COVID-19 therapeutic remdesivir to improve drug efficiency and reduce risk profile
- Amgen: preclinical trial of two osteoporosis drugs, Evenity® and Prolia®, on mice in microgravity
- Eli Lilly and Company: dosed mice with a muscle boosting antibody before their trip to the ISS and found that the treatment pre-empted the atrophying effect of microgravity on muscles
- Schering-Plough Research Institute: microgravity experiments on alpha interferon, Intron A®, produced large quantities of high-quality crystals

To assist these organizations in deploying their experimental packages for execution in the ISS, a number of commercial companies have been focused on developing or modifying the scientific tools in accordance with NASA's design and safety specifications to ensure compatibility with the systems onboard the ISS. These organizations include Space Tango, Axiom Space, Sierra Space, BioServe Space Technologies, and Redwire Space. Examples of those commercial entities with significant commitments to the development of therapeutic modalities and the biological and pharmaceutical development of manufacturing platforms in LEO include:

- Varda Space Industries: collaborating with pharmaceutical companies to improve their drugs and develop therapies by taking advantage of the unique properties of space and then returning those materials to earth
- Axiom Space: as an example of a commercial entity having sustained presence in space. Exclusive access to a module of the ISS was awarded by NASA. Axiom Space is creating an innovation platform for the in-space production of advanced materials and biomedical products that support the development of a robust commercial economy in LEO and beyond
- BioServe Space Technologies: has been designing and developing space flight certified equipment for over 34 years. Affiliated with the University of Colorado. BioServe has expertise in cultivation of mammalian cell and tissue culture, tissue engineering, organoids, bioreactors, and organ-on-a-chip technologies. Most recently, developing the 'BioServe In-space Cell Expansion Platform' or BICEP which is currently under evaluation aboard the ISS
- Space Tango: is focused on the design, certification, and operation of systems across space platforms, automated data collection and space manufacturing. Projects ongoing within the ISS have included layer-by-layer deposition, development of stem cells, tissue chips, organoid manufacturing, and 3D bio-printing platforms. Their activity is focused on agility, automation and reusability and minimal reliance by crew for all space platforms using their standardized CubeLab hardware

- Eascra Biotech: in-space fabrication of DNA-inspired Janus base nanomaterials (JBNs) for RNA therapeutics and cartilage tissue repair achieving the product development of a Technology Readiness Level (TRL) 7 and a Market Readiness Level (MRL) 5. Successful completion of these initial studies will provide the foundation for continued development of JBN technology development that has the potential to provide significant benefit to the industry and patients across a wide variety of therapeutic applications

- MicroQuin: crystallization of transmembrane proteins which regulate a cell's internal environment and its eventual death. Awarded ISSNL and Boeing funded MassChallenge accelerator program to crystallize transmembrane proteins

- LamdaVision: fabricated artificial retinas intended to restore vision in people who are blind takes advantage of microgravity to deposit atoms-thick protein films on a polymer membrane

- Angiex, Inc.: treatment targeting the blood supply of tumor cells, which kills cancer cells by depriving them of oxygen and nutrients. The company's Angiex cancer therapy investigation takes advantage of the space station's microgravity environment to culture endothelial cells, which line the walls of blood vessels, to see whether they might provide a valid model to help develop safer and more cost-effective cancer treatments

- Neuronix, sponsored by the ISSNL, demonstrates the formation of 3D neuron cell cultures in microgravity and tests a neuron-specific gene therapy. Gene therapy shows promise as a potential treatment for people with paralysis and neurological diseases such as Alzheimer's and Parkinson's, but the 3D models needed to test these therapies do not form in Earth's gravity. Creating 3D cell cultures in microgravity could provide a platform for drug discovery and gene therapy testing

Government organizations involved in space based biological research to support the long-term goals of space exploration and commercial development of space include:

- NASA—In Space Production Applications (InSPA) portfolio (advanced materials, tissue engineering, and biomanufacturing):

  - Technology Readiness Levels (TRL): a type of measurement system used to assess the maturity level of a particular technology. Each technology project is evaluated against the parameters for each technology level and is then assigned a TRL rating based on the project's progress. There are nine technology readiness levels. TRL 1 is the lowest and TRL 9 is the highest

  - Marketing Readiness Levels (MRL): Market Readiness Level refers to how ready the product or service is to be taken to market as a commercial offering for a group of customers. MRL frameworks tend to include an idea about a perceived need in the market-to-market leader. MRL 1 is the lowest level and MRL 9 is the highest level

- Space Biosciences Division, NASA Ames Research Center, Moffett field, CA Studies of Somatic/Embryonic Stem cells for long duration Space flight

- ISSNL (Center for Advancement of Science in Space (CASIS) (2024 fiscal year

budget—$5 million for NASA to pursue cancer related research on the ISS) The Center for the Advancement of Science in Space™ (CASIS) is the non-profit organization that manages the ISSNL receiving at least 50%of the US research allocation on the ISS to facilitate research that benefits humanity. NASA manages the other 50% and focuses on research for space exploration purposes

One public–private partnership recently established has been the launch of the Astrobiotechnology Hub, a consortium consisting of academic, industry and government participants [41]. Under the aegis of the Sanford Stem Cell Institute of the University of California, San Diego, this group is focused on translating the basic research findings of stem cell biology in space. By taking advantage of the properties presented in LEO, this consortium coordinates clinical trials and develops commercial products through biomanufacturing of novel drugs, biofilms, and stem cell therapies.

BIOMEDICAL APPLICATIONS AND RESEARCH OBJECTIVES UNDER STUDY AT THE ISS

Long-term space missions will expose crew members, their cells, as well as their microbiomes to prolonged periods of microgravity, ionizing radiation, and environmental stressors for which almost no earth-based organism have evolved to survive [7]. Applications for stem cell research, tissue engineering and regenerative medicine are being advanced within the environment of microgravity [6,42]. The objective is to create new business models that would attract capital investment for a robust commercial use of LEO as proposed by the ISSNL with the following objectives:

- Exploit the benefits of stem cell research in the microgravity environment for therapeutic applications on Earth
- Demonstrate an organoid or multicellular culture system to model human diseases that can be used for testing therapeutics
- Develop or leverage existing systems on the space station for the production of tissues or other biocompatible materials for regenerative medicine

Requests for investigative programs have been issued to focus on stem cell properties, tissue chips, organoids, and 3D bio fabrication [42,43].

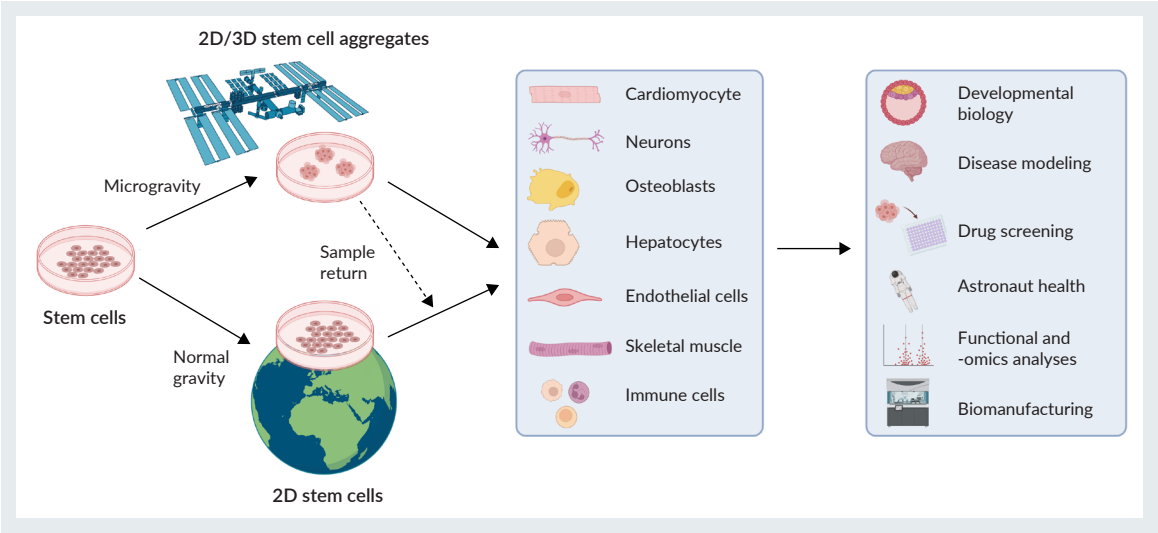
Physiological effects of microgravity on stem cell biology

Pluripotency is the ability to transform induced pluripotent stem cells (iPSC) into tissue cells. Earth's gravity causes challenges of maintaining pluripotency of iPSC during their production including expansion and growth of cell populations [6,39]. On earth, 2D cultures conditions do not entirely recapitulate the native environment of the human body. However, in microgravity it appears that 3D cell growth more closely resembles how cells grow within the human body [6,39,43–45].

Exposure to microgravity also causes significant mechanical unloading of mammalian tissues, resulting in rapid alterations of their physiology, which poses a significant risk for long-duration manned spaceflight [6]. The immediate degenerative effects of spaceflight understood best are those studied during short-term LEO experiments, and include rapid microgravity adaptive bone and muscle loss, loss of cardiovascular capacity, defects in wound and bone fracture healing, and impaired immune function. Over the long-term, exposure to microgravity may cause severe deficits in mammalian stem cell-based tissue regenerative health, including osteogenesis, hematopoiesis, and lymphopoiesis, as well as significant stem cell-based tissue

FIGURE 7

Testing the effects of microgravity on stem cell culture and downstream applications.



A better understanding of human disease and improvements in clinical therapies and biomanufacturing are emerging from parallel cell culture studies conducted in microgravity and on earth. Reproduced with permission from Arzt *et al.* [35].

degeneration in amphibian tail and lens regeneration [6]. In 2013, the ISSNL issued a Request for Information for partners interested in conducting stem cell research in a microgravity environment. The goal of the request was to leverage a LEO-based platform to gain insights into the control and optimization of stem cell pluripotency and multipotency, proliferation and expansion, genomic and epigenomic integrity, differentiation, and maturation [6,24].

Studies would be performed to enhance the growth of large amounts of safe and high-quality clinical grade stem cells with minimal cell differentiation and to evaluate the feasibility of successful harvest and transport of the space expanded stem cells back to earth (Figure 7) [6,39,43–45]. Experiments would be conducted and protocols standardized in accordance with the International Society for Stem Cell Research (ISSCR) to encourage the growth of stem cells in space for patient use on earth and to ensure the safety and efficacy of space produced stem cell therapeutic modalities [34]. Goals for this research would be focused on improving astronaut health for long duration space travel but also to gain insights into developmental biology of stem cells and their potential use in disease modeling or drug screening. The research would also be geared to developing approaches for high-throughput biomanufacturing of stem cell therapies, capability of working autonomously and remotely, employing miniaturization, microfluidics, robotics, machine learning, and artificial intelligence [2].

The types of stem cells that have been studied in space have included mesenchymal stem cells (MSC), hematopoietic stem cells (HSC), cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CM), cardiovascular progenitor cells (CPC), and neural stem cells (NSC) [37]. However, over the past quarter century many more types of stem cells and specialized cells have been the focus of innumerable experiments by academic centers (Box 3) [24,31,34–40,43]. Categories of stem cell research and disease entities conducted in LEO are shown in Box 4.

Recently, to address the on-earth limitations and challenges of expanding umbilical cord blood derived stem cells, in-space

expansion of hematopoietic stem cells is being evaluated for its technical, economic, and commercial capabilities [46]. Specialized bioreactor technology designed and deployed to the ISS is being evaluated for its ability to expand hematopoietic stem cells collected on Earth from umbilical cord blood or adult mobilized peripheral blood hematopoietic stem cell populations, cryopreserved and transported to future commercial space platforms. Once expanded in microgravity stem cell products can be cryopreserved and returned to earth for clinical use. The novel spaceflight culture system designed by BioServe Space Technologies is termed the 'BioServe In-Space Cell Expansion Platform' (BICEP). The hope is that hematopoietic stem cells expanded in space can improve upon the quantity, quality, cell type distribution, genetic stability, function, and clinical safety of earth produced stem cell products.

The BICEP technology has the capability of multiple bioprocessing functions including thawing of cryopreserved cells, seeding into specialized cells to initiate cellular expansion with media supply and control fluids. Cells are incubated at 37 °C in an atmosphere of 5% CO_2 after which the expanded cells are harvested after 10 days, cryopreserved and returned to earth [46].

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Tissue engineering efforts in space has long been a major goal. Before the advent of the ISS, 3D constructs of chondrocytes on scaffolds comprised of polyglycolic acid were grown in bioreactors aboard the Russian Mir space station [47]. The environment in space yielded cartilage constructs composed of viable cartilage cells expressing proteoglycans and type II collagen, markers for hyaline cartilage [47]. The shape, structure, composition, and function of these cartilage constructs produced under conditions of microgravity were consistent with those grown on earth. The utilization of space and the absence of gravity facilitates the rapid maturation and acceleration of cell growth mimicking the aging process allowing investigators to study changes in cells due to aging.

BOX 3

Studies of stem cells and specialized cells conducted in LEO [24,31,35-40,43].

Stem cell type

- Mesenchymal stem cells (MSC)
- Hematopoietic stem cells (HSCs)
  - Cardiomyocytes derived from induced pluripotent stem cells
  - Cardiac progenitor spheres derived from induced pluripotent stem cells
- Cardiovascular progenitor cells (CPCs)
  - Neural stem cells (NSCs)
  - Embryonic stem cells
  - Pig fetal liver stem cell line (PICM-19)
  - Cancer stem cells ($CD133^+$)
  - Oligodendrocyte progenitors

Specialized cells

- Retinal pigmented epithelia (ARPE-19 cells)
- Human aortic smooth muscle cells (HASMCs)
- Human cardiomyocyte line (AC16)
- Endothelial cells (EA.hy926)
- Human dermal microvascular endothelial cells (HMEC-dBL)
- Human microvascular endothelial cells (HMEC-1))
- Peripheral blood mononuclear cells (PBMCs)
- T cells (CD8 and CD4)
- Lymphocytes
- Neutrophils
- Monocytes
- Dendritic cells (DC)
- B cells (CD19 and lymphocyte depleted (LD)
- M1 and M2 Macrophages
- Primary T cells
- Primary macrophages
- Human chondrocytes
- Meniscus fibrochondrocytes
- Thyroid cells (FRTL5)
- Primary human dermal fibroblasts
- Primary skin tissue from C57BL/6J *Mus musculus* mic

BOX 4

Categories of stem cell research and disease entities conducted in LEO [42].

Mesenchymal stem cells (MSCs)

- MSCs grown in space maintain their morphology, phenotype, and proliferation capabilities
- Enhanced immunosuppressive properties were observed
- Microgravity may inhibit differentiation, preserving the stemness of MSCs, which is beneficial for clinical applications
- Space-grown MSCs could be used to treat central nervous system diseases, such as spinal cord injuries, due to increased neural development markers in MSCs grown in microgravity

Hematopoietic stem cells (HSCs)

- Microgravity affects HSC proliferation and differentiation, with an observed preservation of stemness
- Space-grown HSCs showed suppressed erythropoiesis and increased macrophage differentiation
- Applications include potential therapies for anemia and other blood-related disorders

Cardiomyocytes derived from induced pluripotent stem cells (iPSCs)

- iPSC-derived cardiomyocytes grown in space demonstrated structural and functional integrity
- Microgravity enhanced gene pathways related to mitochondrial function and calcium signaling
- Applications include advanced cardiac repair therapies and models for studying spaceflight-induced cardiac remodeling

3D models of the human brain derived from iPSCs

- Complex human models containing iPSC-derived neurons, astrocytes, oligodendrocytes, and microglia can be used for disease modeling and drug discovery
- Applications focus on regenerative therapies for neurodegenerative diseases like Alzheimer's and Parkinson's

Cardiovascular progenitor cells (CPCs)

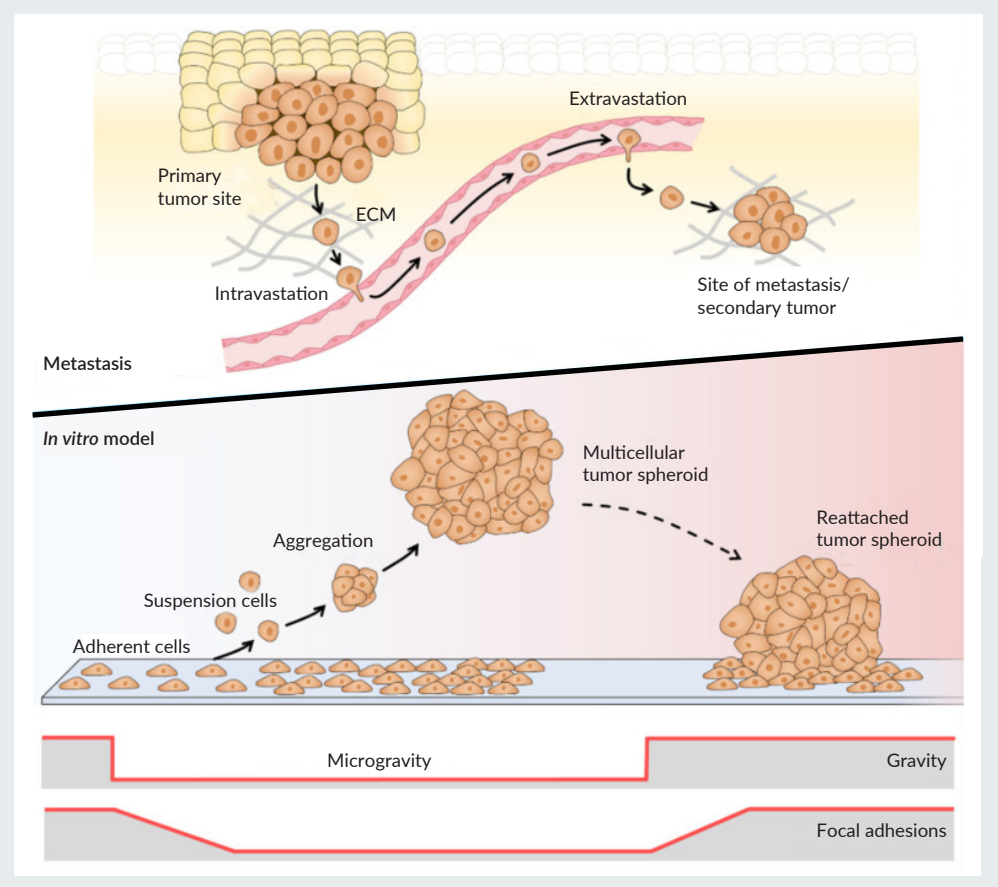
- CPCs in space displayed increased DNA repair capabilities and enhanced differentiation into cardiac tissues
- Applications focus on cardiac regeneration and repair through enriched and functional cardiomyocytes

Tissue engineering in microgravity has been given special emphasis [11,48,49]. This unique environment is seen to allow for the production of delicate tissue constructs through bio fabrication. The environment of space can facilitate the maturation and strengthening of growing 3D tissues without collapsing into less useful 2D forms as gravity would produce on earth. These characteristics have been exploited by a number of organizations who wish to improve patient care and to better understand cell behavior in order to advance regenerative medicine [11,49].

Terrestrial-based cell culture techniques face limitations in the complexity and consistency of cell systems that can be developed. Without the shearing and sedimentation forces present on earth, microgravity allows creation of larger, complex, and more delicate tissues, such as blood vessels, which can enable the formation of sophisticated organoid systems [11,48]. While tissue engineering has many potential applications, efforts often face limitations in culturing tissues resembling those in the body, advancing the research into organ growth, or attaining higher accuracy to validate personalized drug testing.

FIGURE 8

The dynamics of cell formation into spheroids in an *in vitro* model of tumor metastasis under conditions of microgravity are presented in this schematic.



When gravity is restored, spheroids will reattach to a substrate. Reproduced with permission from Grimm [52].

Tissues grown on earth are constrained by gravity which results in flattening and deformation of 3D constructs. However, those grown in an environment of microgravity offer specific advantages. For example, larger tissue constructs are allowed to form without special restriction into 3D structures termed spheroids. Moreover, evidence suggests that tissues grown in space can elicit a response similar to the aging process. This characteristic can accelerate drug development and disease modeling of cellular function [50].

Organoids are self-organizing 3D aggregates of cells differentiated from stem cells and whose spherical shape and cellular structure may resemble full organs [50,51]. These organoids or spheroids in a tissue culture environment can serve as simplified organ systems which can be used for accurate and scalable disease modeling and drug testing investigations (Figure 8) [52]. Organoids can also be used as tissue batches for regenerative medicine applications. The integrated biological function in organoids serves as a powerful model of human disease states, and applications of this kind of these advanced *in vitro* systems could enable a wide variety of experiments conducted in microgravity. For example, experiments have been conducted with stem cell derived brain and neural organoid models by the National Stem Cell Foundation to better understand

mechanisms behind neurodegenerative diseases such as Parkinson's and primary progressive multiple sclerosis [38]. There may also be applications for use in personalized medicine and potentially address the shortage of organs for transplantation [50].

Culturing stem cells and progenitor cells in microgravity stimulates proliferation as well as preserving 'stemness', helping to maintain population numbers in culture [48]. In addition, microgravity forces cells to interact and anchor to each other promoting the development of tridimensional cultures, producing larger and wider cell clusters with higher order structures (Figure 8) [51,52]. Microgravity therefore could be a platform to optimize conditions for large-scale production of organoids and spheroids for research, regenerative medicine applications, and pre-clinical testing of drug candidates [50].

3D bio-printing

Tissues expressing specific architecture such as muscles, vasculature, nerve tissues and heart valves can be created through 3D bio-printing [53]. The 3D Biofabrication Facility (BFF) and Advanced Space Experimental Processor (ADSEP) developed by Redwire Space and installed aboard the ISS worked on creating knee meniscus, muscle, vasculature, and nerve tissues [54,55]. Heart valves can be created as microgravity allows tissue to retain its shape [54,55]. In fact, a bio printer developed in Finland by Brinter AM Technologies is currently under modification by Redwire Space to meet the stringent requirements of compatibility with systems aboard the ISS. In support of future long duration deep space missions these devices would have the capability of producing replacement damaged tissues when access to earth bound medical facilities would be impossible. These devices would also enhance our knowledge of the biological mechanisms of tissue regeneration and aging.

On earth, gravity constrains engineered tissue by deforming and flattening 3D constructs while in microgravity cells are able to form complex 3D structures without the need for structural support. These structures are similar to tissues naturally found in the human body and facilitates the study of accelerated disease modeling, cell behavior, especially the effects of aging, and may have a role in advancing regenerative medicine and testing the effects of new drugs. Without the need for scaffold matrixes a variety of mechanisms can be applied in space to produce soft human tissues, such as blood vessels [54,55]. Larger tissues may be constructed by utilizing biofabrication capabilities that enable the production of 3D structures [54,55]. These technologies can pave the way for the development of therapies for repair or replacement of damaged tissues and organs [53]. Long-term success of biofabrication may enable potential medical breakthroughs, including the creation of patient-specific replacement tissues or patches and could ultimately help reduce the current shortage of donor organs.

More recently, the Wake Forest University School of Medicine's Institute for Regenerative Medicine (WFIRM) made significant advancements in the innovations for constructing biological models containing vascular tissues. Using 3D bioprinting technology these investigations were able to mimic vascularized liver tissue constructs [56,57]. NASA has selected Wake Forest Institute for Regenerative Medicine (WFIRM), of Winston-Salem, North Carolina, for a Phase 1 program In Space Production Applications (InSPA) award that takes advantage of the microgravity environment of space to develop and validate a platform and strategy for manufacturing vascularized and perfused liver tissue [56]. The proposed work will leverage microgravity for manufacturing clinical scale liver tissue constructs with intrinsic vascular networks that allow perfusion and integration into the recipient's

peripheral circulation for the treatment of liver disease [57].

3D tissue chips

Tissue chips are small devices, similar in size to a USB drive, engineered to grow human cells on an artificial scaffold to model the structure and function of human tissues and organs [9,58]. Also termed 'microphysiological systems' (MPS), tissue chips are constructed using human cells organized in a predetermined architecture and are designed to replicate facets of the physical environment cells experience inside the body, providing higher accuracy models that can lead to advancements in predictive medicine and personalized healthcare. By better replicating the complexity of the tissue architecture, tissue chips are also leveraged for therapeutic screening for multiple disease indications [9,59]. In microgravity, tissue chips have the potential to capture accelerated disease conditions on a complex tissue level to advance our understanding of the mechanisms behind disease progression, thereby increasing opportunities for drug development. Tissue chip devices provide a more financially accessible alternative to convention *in vivo* models with precise control over microenvironmental cues (e.g., shear stress, oxygen gradients, biomolecule delivery). This democratizes access for advanced screening models with even higher specificity and the opportunity for integrated monitoring systems for complex analysis. Tissue chip applications have been utilized by a number of investigational groups collaborating with the ISSNL to advance the study the effects of microgravity upon the human body including blood–brain barrier function mechanisms, accelerated deterioration of muscle, bone loss representative of osteoporosis, decreased cardiopulmonary function, and immune deficiency, all of which have been observed and documented in space [59–62].

Use of tissue chip applications for these studies can accelerate the understanding of ageing while revealing targets that potentially can reverse these processes. Three examples developed and placed on test within the ISS have been 'heart on a chip', 'tumor on a chip', and 'cartilage on a chip'.

Heart on a chip

Cedars-Sinai investigations have pioneered new technology to test chemotherapies and other cancer drugs for heart toxicity. Cardiomyocytes and vascular endothelial cells derived from induced pluripotent stem cells can screen for drug-induced alterations in cardiovascular cell function and survival. Specialized 3D chips containing these cell types are enclosed in separate chambers but are connected with channels that allow the introduction of fluids and facilitate the interaction of the cells. These unique test systems allow the formation of mature heart muscle cells and vascular cells which together form a test platform for precise drug toxicity studies [63].

Tumor on a chip

Another NASA funded organization, Encapsulate, Inc. developed an automated 'tumor on a chip' which allows cancer cells to be evaluated for their response to chemotherapeutic agents prior to their administration to patients [51]. In this way the most effective chemotherapeutic agent can be selected for a patient's specific cancer. Again, challenging these systems in microgravity facilitates the study of cancer cells since cells form 3D structures which more closely resemble the growth and behavior of cells within the human body [51]. The biomimetic microenvironment of the tumor within the chip in an automated system can control cell growth, maintenance, and be accurately monitored simulating the environment of the tumor within the human body [51].

Cartilage on a chip

A physiologically relevant joint model was successfully reproduced and tested in a microgravity environment. Viable and reproducible human cartilage, bone, and synovium cultures were generated [58,62,64]. This resulted in a reproducible baseline for one orthopedic condition, post traumatic osteoarthritis. With this 'cartilage on a chip' innovation treatment effects of drugs used to treat inflammation, and pain can more accurately be assessed. Cartilage repair strategies can also be assessed in space. This technology can be utilized on earth for treatment of post-traumatic osteoarthritis (PTOA) in athletes, a common medical problem especially in female athletes.

In an effort to extend the longevity of MPSs to a minimum of 6 months NASA is collaborating with the US FDA, National Institutes of Health (NIH), and the Biomedical Advanced Research and Development authority (BARDA). This extended lifespan would allow researchers to investigate the effects of acute and chronic stressors in a spaceflight environment and allow for longer duration studies to better assess:

- Disease models
- Drug development
- Clinical trial designs
- Chemical and environmental exposures and countermeasures
- Physiological changes due to the spaceflight environment [65]

Gene therapy

The ISSNL has sponsored opportunities to utilize the microgravity environment as a platform for the development of gene therapies [66,67]. The first gene therapy and investigational ophthalmic therapy has been pioneered by Oculogenex, Inc. in the testing of a novel gene therapy to prevent and possibly even reverse vision loss from age related macular degeneration (AMD), a leading cause of blindness in older adults [66,68]. This gene therapy technology addresses the root cause of dry macular degeneration by targeting the epigenetic switch which plays a fundamental role in retinal homeostasis on the mitochondrial enhancement of a cellular response to oxidative stress [66,69]. This approach restores the functionality of damaged cells and prevents senescence and death of retinal cells. Partially funded by NASA because astronauts can be afflicted with spaceflight associated neuro-ocular syndrome, forty female mice treated with the gene therapy were sent to the ISS with an equal number treated and remaining on earth. Exposure to microgravity by the gene therapy treated mice will accelerate the oxidative stresses that encourage the onset of AMD.

Axonis Therapeutics is developing a neuroregeneration gene therapy designed to silence the expression of PTEN, an inhibitory protein that suppresses the ability of axons to regrow after injury. The gene therapy was targeted to central nervous system (CNS) neurons only since the gene also plays a role in the suppression of growth in other non-neural cells [70]. Using an AAV as a viral vector and tailoring the vector's gene promoter only CNS neurons would be targeted. By deleting the expression of PTEN, the CNS neurons are reprogrammed back into a state of growth to allow regrowth of damaged axons. The model was successfully tested in rodent models. Aboard the ISS, experiments were designed to exploit microgravity and create a 3D model of the human brain by co-culturing iPSC derived mature neurons and astrocytes and forming brain organoids [67]. This achievement would result in a CNS model in order to test the gene therapy. Without an artificial

matrix substrate and other growth factors creating similar models on earth would be difficult.

When vials of the mature neurons, astrocytes and AAV vectors sent to the ISS were combined along with a fluorescent protein gene for microscopic visualization the astronauts would be able to evaluate the rate of self-assembly of the brain organoids culture. After 72 hours the neurons and astrocytes were seen to self-assemble into 3D organoids. Importantly, the functions of the neuron specific AAV gene therapy vector in suppressing the PTEN protein could also be visualized. The successful demonstration of the gene therapy would now be used to justify the chemistry, manufacturing and control elements needed to plan and execute clinical investigations [66].

NANOMATERIALS

Nanomaterials hold a strong potential for a number of therapeutic applications [71–74]. The FDA has approved a number of nanomedicines to include therapeutics for cancer, skin conditions, and regenerative medicine [75]. Janus Base Nanomaterials (JBNs) are noncentrosymmetric monomers that possess the Watson-Crick hydrogen bond 'donor-acceptor' motifs present in DNA. Mimicking DNA base pairs, a family of JBN monomers achieve controlled self-assembly at ambient temperatures to form a collection of 2D nano-rosettes which further assemble into 3D nanotubes. Each JBN monomer is 400 Da and the self-assembly process is achieved by suspending thousands of monomers in aqueous solution without the need for catalysts or cross linkers. The final resulting Janus Base Nanotube (JBNt) maintains supramolecular helicity and stability through inter-rosette hydrogen and π-π bonds. When self-assembled in the presence of biomolecules such as proteins, the resulting structure includes a biocompatible and mechanically robust nano matrix (JBNm) that mimics natural extracellular matrix [76]. Because of the characteristic of self-assembly Janus base nanomaterials are seen as ideal for in-space manufacturing.

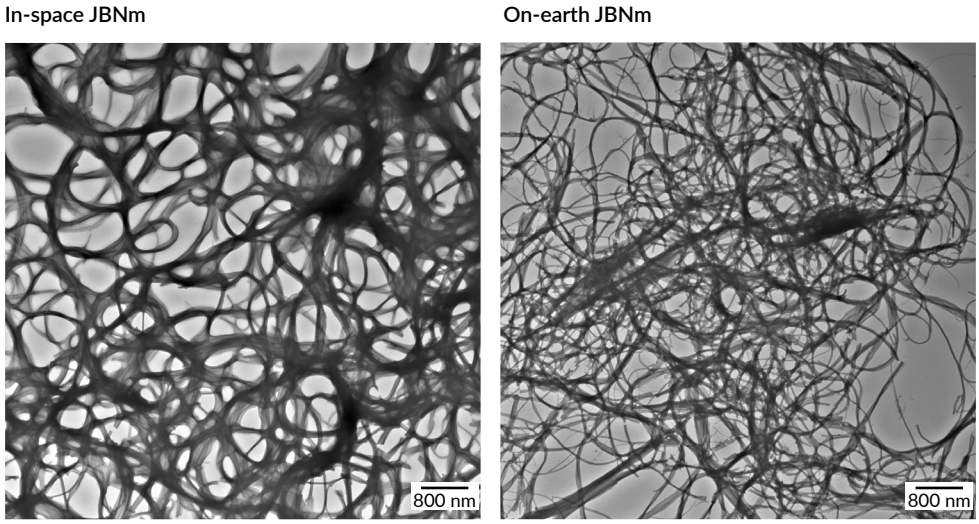
As an adjunct to tissue regeneration and cartilage repair the in-space fabrication of JBNms was recently demonstrated [77,78]. When creating JBNm on Earth, gravity-driven sedimentation can limit the assembly process. However, when manufacturing JBNm in microgravity, the lack of these forces allows for increased homogeneity and bioactivity (Figure 9). Scientists at the University of Connecticut's Nanomedicine Laboratory and Eascra Biotech have embarked upon studies aboard the ISS to evaluate this therapeutic nanomaterial; their goal is to overcome the decay of cartilage caused by the effects of microgravity and lack of mechanical loading that affect astronauts living and working in space especially on long duration missions [76–78].

Cartilage damage, whether through trauma or arthritis continues to be an unmet medical need on earth as well as in space [79–81]. Articular cartilage defects are seen in 60–66% of knees undergoing arthroscopy and osteoarthritis is a chronic and debilitating joint disease affecting over 600 million individuals worldwide over the age of 40 [82]. Natural hyaline cartilage, when damaged, has a limited ability for self-repair due to the absence of pluripotent cells, a sparse distribution of chondrocytes, no lymphatic drainage or nerve distribution and the lack of vasculature [79–81].

JBNms manufactured in space which utilize microgravity to minimize the effects of sedimentation were produced with improved homogeneity, low toxicity, and high cell biocompatibility [78]. This process can provide a better understanding of the disease mechanisms that promote cartilage degeneration in space and demonstrated a novel approach for improving tissue engineering of cartilage repair on earth.

FIGURE 9

Transmission electron microscope images of JBNm strands manufactured in space and on earth.



The width of the JBNm bundles manufactured in space were significantly larger than those manufactured on Earth. In space JBNs demonstrated improved homogeneity and scaffold assembly, increasing cell bioactivity indicating low toxicity with high biocompatibility. The mission supported methodology for manufacturing nanomaterials in space and successfully demonstrated the promise of utilizing microgravity for improved JBN assembly and bioactivity [77].

FDA REGULATION OF IN SPACE BIOMANUFACTURING FACILITIES

In 1984, writing in the *Food, Drug, Cosmetic Law Journal*, Robert Altman reiterated that, biological products would be the "most promising drugs for production in space" [83]. To exploit the benefits of a microgravity environment and facilitate the separation of impurities in the drug production process, a goal of the space program was to place in LEO a fully functioning automated pharmaceutical manufacturing laboratory. To achieve this goal, Altman discusses the problems confronting industry and the FDA and its role in supporting space technology. At that time, in order to attract investment from the private sector legislation had been introduced into the US Congress to relax existing FDA regulations. However, these legislative actions were unsuccessful. Only two companies, McDonnell Douglas Astronautics Co and Johnson and Johnson expressed interest in commercializing LEO for drug production in space [83].

FDA had not previously considered its role in space technology and the impact new technology would have in its regulatory scheme. FDA had to consider if its regulatory mandate in protecting the public health would discourage private investment in space programs or if the challenges emanating from the research, development, manufacture, and processing of pharmaceutical products in outer space would impede the entrance of private industry into the space market.

Thirty-four years later those concerns continue to confront industry in its plans to commercialize outer space. In 2018, the FDA and NASA issued a Memorandum of Understanding (MOU 225-18-027) that establishes formal communication between the two agencies to "discuss providing technical expertise for planning, performance, or review in areas of mutual interest". Under this MOU, the two agencies "seek opportunities to participate in

collaborative efforts, in furtherance of their respective objectives and as permitted under appropriate statutory authority and applicable law, as resources permit to:

- Look for potential collaborative studies on the utilization of already funded FDA projects that would enhance NASAs medical risk reduction exploration research
- Encourage space related health research through the exchange of expertise, scientific and technical information, date, and publications
- Discuss providing technical expertise for planning performance, or review in areas of mutual interest, subject to program priorities and availability of fund and personnel
- Facilitate and enhance research and development activities by either agency, including distributing information on research opportunities such as NASA Research announcements
- Coordinate publicity of mutually reinforcing activities, publications, and research results
- Include representatives from FDA and NASA in workshops, including NASA's Human research Program Investigator's Workshop, working groups, seminars, and other related activities" [84]

In 2021, this collaboration intensified when NASA solicited science investigations from multiple government agencies, including FDA, to extend the longevity of 3D tissue chips and microphysiological systems for modeling acute and chronic stressors in astronauts during long duration spaceflight. FDA's chief scientist, Rear Admiral Denise Hinton commented that:"FDA remains deeply engaged in identifying and fostering strategies that can bring alternative testing methods such as microphysiological systems to FDA for integration into the review process, collaboration with our partners in the public and private sectors has been critical to advancing our efforts in this area, particularly with respect to medical countermeasures." [61]. In the view of FDA these technical innovations could also advance the way drugs can be investigated and reviewed by regulators. Captain Tracy MacGill, Director of Medical Countermeasures (MCM) Regulatory Sciences noted that, "We expect that extending the lifespan of the microphysiological systems will provide more relevant and predictive models, for example, this will enable us to look at the effects of drugs or other FDA regulated products over a longer duration in both normal cells and those with acute and chronic diseases, the research had the potential to provide a wider window into safety and efficacy of a variety of medical products." [61]. The purpose of this NASA–FDA collaboration is to study a wide variety of biological changes including neurotoxic stressors, radiation exposure, and acute and chronic exposures to drugs that could result in unanticipated discoveries to improve the operational capabilities and medical status of astronauts as benefiting patients back on earth.

The regulatory implications of bio manufacturing in LEO are challenging, with fundamental regulatory and legal questions that will require answers and policy decisions in the not-too-distant future. In a March 13, 2024 article entitled, *'Are FDA astronauts coming soon? Implication of the revolution in space based drug manufacturing'* the author poses the following questions:

- Will FDA need to recruit astronauts to inspect space based manufacturing facilities?

- What are the intellectual property opportunities and risk for space made drugs?
- Would a generic or biosimilar version of a space made drug also be required to be manufactured in space [85]?

The advantages and scientific rationale of space-based manufacturing have been identified through years of investigations by major pharma organizations conducted aboard the ISS over the last quarter century. For example, the quality and consistency of drug substances comprised of protein crystals have been shown to be of higher quality and consistency when formed in microgravity [86]. Removal of impurities is facilitated by manufacturing that can overcome the earth-bound effects of convection and sedimentation. Affordable access to space and the need for new and improved existing drugs have justified the current investment climate.

REMOTE REGULATORY ASSESSMENT (RRA) OF BIOMANUFACTURING FACILITIES IN LOW EARTH ORBIT

As a result of the COVID epidemic regulatory agencies have adopted criteria and procedures for remote regulatory assessments [87]. In recent publications FDA, through the Office of Study Integrity and Surveillance (OSIS), have developed a variety of surveillance tools and new oversight approaches formalized criteria for such assessments, although not considered on site GMP compliance inspections, but would rather support FDA's review of marketing applications. The Remote Regulatory Assessment criteria are comparable to the format of an inspection and could be adapted for facilities operating in LEO [88]. These adaptations could include visits to on earth manufacturing facilities where inspectors could become familiar themselves with the on orbit equipment and operations. FDA inspectors could then request specific documentation and review the following:

- Records of specific lots or batches and product specific information, such as product quality reports
- Summaries of batches manufactured in LEO and their disposition
- Visualize electronic systems with Read-only access to electronic databases
- Standard operating procedures and records on quality systems
- Interview relevant staff

These reviews could take place at the company's on earth location or remotely facilitated by interactive technological advancements on autonomous manufacturing, telemetry, internet connectivity, video conferencing platforms, screen sharing, remote livestreaming, or pre-recorded video of on orbit operations. Additional discussion of FDA personnel with pharmaceutical developers can improve upon design of the criteria by which FDA could gain access to on orbit manufacturing operations in order to assess regulatory compliance. Following such an assessment the FDA would not issue an FDA 483 (Report of Observations), but RRA observations would be shared in writing and discussed at a close-out meeting.

Despite the location of manufacture for drug substances, FDA will expect and rely upon its core principles of CMC (Chemistry, Manufacturing and Controls) [89]. Information will be reviewed at the Pre IND/IND stage to assess identity, strength, safety, quality and purity of the drug substance and drug product [89]. Sponsors would need to supply the preliminary critical quality attributes for the drug substance, along with the primary structure, control of

starting materials, preliminary manufacturing process and controls for DS/DP, physical characterization data, assay/impurities test methods such as sterility testing and endotoxin methodologies, preliminary DP formulation, etc. A change to the drug development process that now includes a manufacturing facility site change to a low orbit location would require a comprehensive risk assessment with data that demonstrates comparability and process optimization resulting from an environment of microgravity [90].

Appropriate clinical trial designs may be modified if they occur in deep space locations including personalized clinical trials used to assess the safety and efficacy of ultra-rare diseases or Phase 1 trials which entail micro-dosing of a medication over a short time period. The appropriate clinical design that would be executed in space would have to be discussed at a pre-IND meeting [91].

TRANSLATION INSIGHT: THE ECONOMIC POTENTIAL OF BIOMANUFACTURING IN LEO

Economic potential forecasting of biomanufacturing in LEO in terms of revenue and growth have been assessed by a number of expert analysts [12,15,16,19,92–96]. One analyst predicts that by the year 2040 over $1 trillion of the global economy will move into space [20]. CNBC reported that in 2023 $12.5 billion was raised with 39 merger and acquisition deals made across the sector [97].

The cost to place a satellite into LEO aboard a Delta E rocket in the 1960s was $168,000/kg [98]. Today the cost to launch a satellite into LEO from a SpaceX Falcon Heavy rocket is approximately $1500/kg, about 30X less than the launch cost of a NASA Space Shuttle [99]. It is now estimated that the cost to launch SpaceX's next vehicle, the Starship Rocket, can place a satellite into orbit for a cost per kilogram of $100 [94].

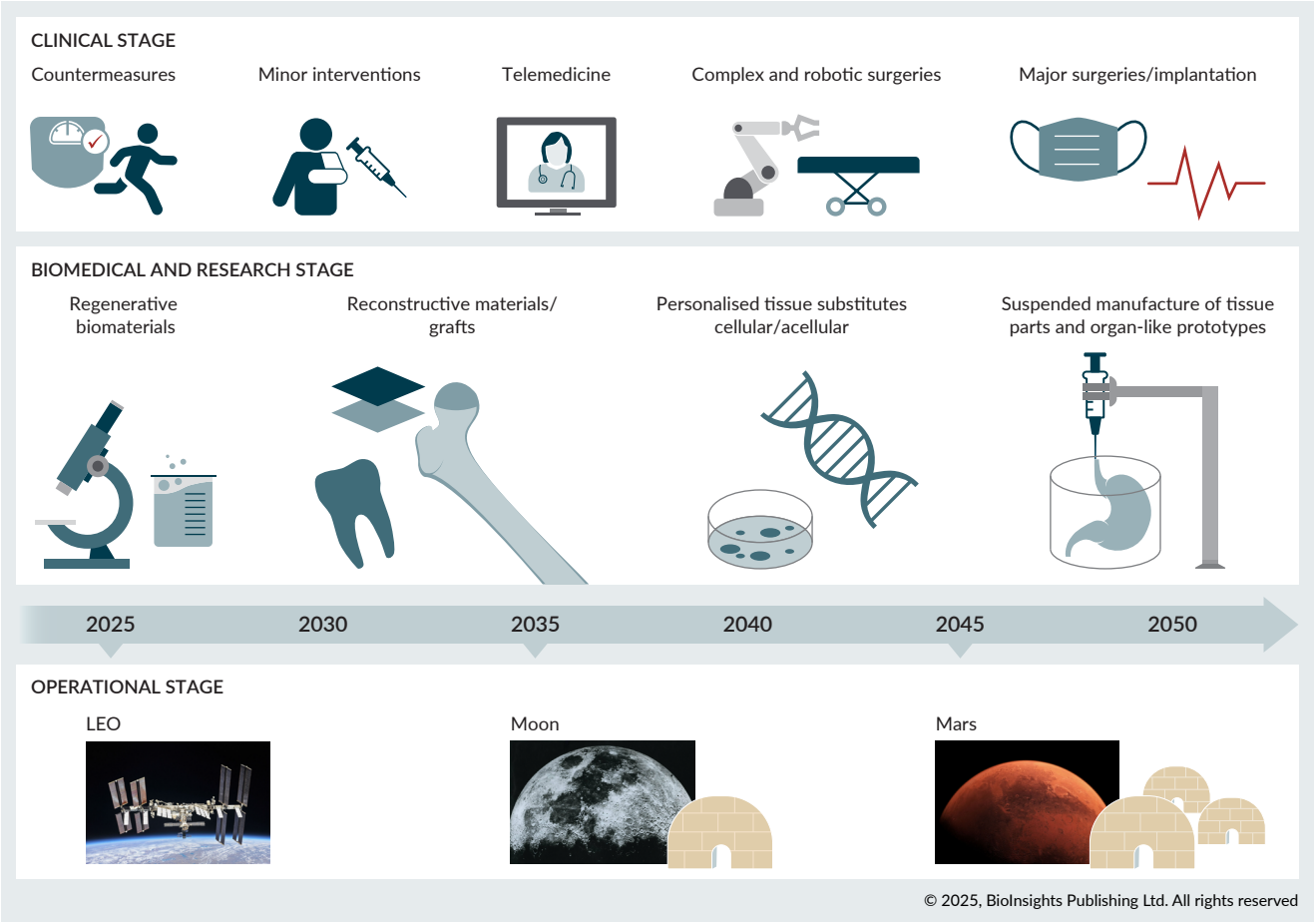
Space has now become economically feasible and a source of value and a return on investment for a number of business sectors including pharmaceutical development and biomanufacturing [11]. *Harvard Business Reviews* reported that in 2019, 95% of the estimated $366 billion in revenue earned in the space sector was for the 'Space for earth' economy—goods or services produced in space for use on earth [93]. Venture capital is flowing into the commercialization of space, $15 billion in 2021 according to the space consultancy BryceTech [95]. The retirement and deorbiting of the ISS will be replaced by an ever complex technical infrastructure being assembled in orbit by a number of commercial entities including fully autonomous orbiting drug manufacturing vehicles. In 2016, Axiom Space, Inc., was awarded a contract for exclusive access to a module of the ISS. This has allowed Axiom to build its own module for commercial activity on the ISS with plans to have it independently operated when the ISS is retired in 2030 [100].

Deloitte analysts predict that the commercial potential of LEO will require the following incentives to:

- Deliver lower cost, higher cadence human-related access to space
- Significantly increase down-mass (mass of materials returned from space) capacity and industrialization of on-orbit manufacturing operations
- Establish multiple on-orbit destinations for human-rated depot-centric, and other mission specific activities
- Better align the resources and complementary technical capabilities of public and private sector players
- Enable access to LEO and execute missions and activities at the speed of business [101]

FIGURE 10

A timeline for the biomedical and medical infrastructure of a life support system over the next decades of space exploration is proposed in this schematic.



Over the next decade, dominated by the construction of planetary habitats on the moon and, later on, Mars, it very likely that innovations in regenerative biomaterials, patches for skin damage repair, injectable systems and acellular therapies would eventually lead to the manufacture of tissue parts and functional organ replacements. Telemedical, robotic, and remote medical assistance capability will be developed and perfected over the next decades. Reproduced with permission from Lordachescu [11].

For a quarter century, cutting edge research and development has been conducted in space aboard the ISS since its initial launch and construction in the early 2000s. Companies like Bristol Myers Squibb and Merck have used the environmental qualities of microgravity to optimize drug efficacy by improving crystal formulation [102]. While much of the medical advances being made in space have an initial focus on lunar colony residency or the 2–3 year commitment for a round trip journey to Mars, the innovations and discoveries will also have a direct effect on the improvement of healthcare on earth (Figure 10) [10,11].

Numerous public-private partnerships have completed proof of principle studies for the 3D bio printing of tissues and organs, and manufacture of 'organs on a chip', stem cell cultivation, and creation of metabolically functional and vascularized heart tissue among others [9,34]. Knowledge of the blood brain barrier, immunoscenescence, pulmonary infection, cardiac dysfunction, post-traumatic osteoarthritis, proteinuria, kidney stones, and inflammation of the intestine and a host of other pathological

conditions that might affect astronauts on long duration space flights are also important health problems for large segments of the human population.

Tissue substitutes, nanomaterials for tissue scaffolds and efficient drug delivery, hemostatic agents or biomaterials such as dental fillers will all be available for astronaut crews to manage tissue damage and medical emergencies in deep space. These innovations will also find uses on earth for military medical professionals, or emergency medical first responders in the nation's emergency rooms [11].

New therapeutic applications and modalities for stem cell derived products that might include scaffolds and matrices, cell–cell and cell–matrix interactions, stem cell and tissue engineering and reprogramming, cellular immunotherapies, organoid development, cellular biomanufacturing, or system integration between biological components will require protocols and procedures to ensure safety and efficacy [39]. Regulatory review and approvals will have to be adapted to include the therapeutic translation using space as a manufacturing platform. Remote regulatory assessments to ensure appropriate chemistry, manufacturing, and quality control elements in accordance with regulatory compliance requirements must be present and verifiable, especially for their suitability in clinical trials as gene or cell therapies [34]. These new protocols and procedures will also be germane to the production and regulation of artificial tissues and organs in space [11].

Development of patient specific gene therapy will benefit from the knowledge that many cell types including induced pluripotent stem cells grow faster in space and that microgravity fosters natural 3D stem cell growth, mimicking the human body's environment more effectively than earth-based 2D cultures. Space-based research also enhances our ability to understand differentiation, proliferation, and tissue regeneration. Biomedical applications will range from drug discovery to regenerative medicine, disease modeling, and biomanufacturing in space for clinical use on earth [2,3,103,104].

Continued research and validation will be needed to fully understand the complex effects of microgravity on cellular function [103]. The future decades of biomedical commercialization of space will provide the opportunity for the synergies of cell and gene therapy, tissue engineering and microgravity to offer the types of innovations that will transform treatment strategies for human healthcare and medicine on earth [103,104].

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MANUFACTURING

SPOTLIGHT

INNOVATOR INSIGHT

Next-gen cell therapy manufacturing: leveraging flexibility and automation for success

David Shaw, Fred Parietti, and Carl Dargitz

Despite several cell therapy approvals, existing manufacturing capacity is inadequate to meet high patient demand. Automation is poised to play a crucial role in enhancing global patient access to these treatments. This article delves into the advanced technologies and automation strategies that are essential for navigating the evolving cell and gene therapy landscape, featuring insights from both the biopharma and tool provider sectors.

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THE ROCHE/GENENTECH CELL AND GENE THERAPY PORTFOLIO

David Shaw

Roche/Genentech has partnered with pioneering, innovative companies in order to build a diverse cell and gene therapy portfolio. One such collaboration is with Sarepta Therapeutics, which has resulted in the development of Elevidys for the treatment of Duchenne muscular dystrophy in patients aged 4 years and above, regardless of ambulatory status. Similarly, partnering with Spark Therapeutics to develop Luxturna® for the treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy led to Spark becoming the first company to receive US FDA approval for a gene therapy for a genetic disease.

Roche/Genentech also have an exclusive worldwide collaboration with Lineage Cell Therapeutics for the development and commercialization of an allogeneic stem cell-derived regenerative medicine, OpRegen, for the treatment of ocular disorders, including advanced dry age-related macular degeneration with geographic atrophy. In September 2024, OpRegen received Regenerative Medicine Advanced Therapy (RMAT) designation from the FDA for the treatment of geographic atrophy secondary to dry age-related macular degeneration. Other regenerative stem cell therapies are also in development.

Additionally, Roche/Genentech is developing donor-derived allogeneic T cell

therapies through a collaboration with Poseida Therapeutics. These include multiple CAR-T cell therapy programs for the treatment of cancer and other diseases, including P-BCMA-ALLO1 for the treatment of relapsed refractory multiple myeloma, which has also received RMAT designation.

A further venture in partnership with BioNTech is the development of an mRNA-based individualized neoantigen specific immunotherapy (iNeST) candidate, autogene cevumeran, for resected pancreatic ductal adenocarcinoma (PDAC). Autogene cevumeran is currently undergoing an open-label multicenter, randomized Phase 2 trial.

Roche/Genentech: purpose and vision

Roche/Genentech's purpose and vision for the cell and gene therapy field incorporates the following beliefs:

- Cell and gene therapies have the potential to cure serious diseases, whether used independently or alongside traditional treatment;
- Manufacturing is the key to success;
- In order to achieve speed to market with cell and gene therapies, it is crucial to adopt a lean manufacturing organization. This lean organization is based on the rationale that biotechs and academic labs dominate the early development of cell and gene therapies due to their ability to make rapid progress;
- Patients should have access to cell and gene therapies at a sustainable cost to society.

How to achieve worldwide patient access?

Achieving global patient access hinges on sustainable, cost-effective manufacturing. While the cell and gene therapy industry still needs time to reach this goal due to immature supporting technology, rapid advancements are being made. However, introducing automated manufacturing presents several challenges:

- The critical need for speed to clinical outcomes results in rapidly developed and often poorly understood processes;
- Small clinical trial patient populations make early-phase automation investment hard to justify;
- Due to funding constraints and high development costs, there may be insufficient resources for extensive comparability studies between manual and automated processes;
- Rapid transitions to pivotal trials leave little time to introduce and establish automation;
- The GMP manufacturing workforce skilled in manual production of cell and gene therapies differs significantly from one trained to manage automated workflows, necessitating a specialized workforce for automation.

ROBOTIC MANUFACTURING OF CELL THERAPIES

Fred Parietti

The chief goal of Multiply Labs is to leverage the latest robotic technology to achieve the industrial scale manufacture of next-generation advanced therapies, including gene-modified cell therapies. Owing to the complexity of the processes used to manufacture these lifesaving therapies, the application of robotics is fundamental to achieving both industrial scale and efficiency.

In the USA in 2023, some 250,000 patients were diagnosed with hematological malignancies, yet only approximately

8,000 patients were treated with CAR-T cell therapy. Over this same period, 100,000 deaths from hematological malignancies were recorded. Furthermore, the majority of patients who received CAR-T cell therapies were in the USA, meaning patients located elsewhere around the globe could not receive these advanced treatments.

The main manufacturing bottlenecks that are causing this supply chain issue are:

- Lack of efficiency in terms of space (inefficient large facilities are being used for manufacturing);
- Low utilization of equipment (particularly where manual operations are conducted in a large facility. This is largely unavoidable as most of the currently approved CAR-T products originated from fully manual processes);
- All of the approved therapies were discovered and developed in academic labs, which means the original processes cannot be repeated at commercial manufacturing scale.

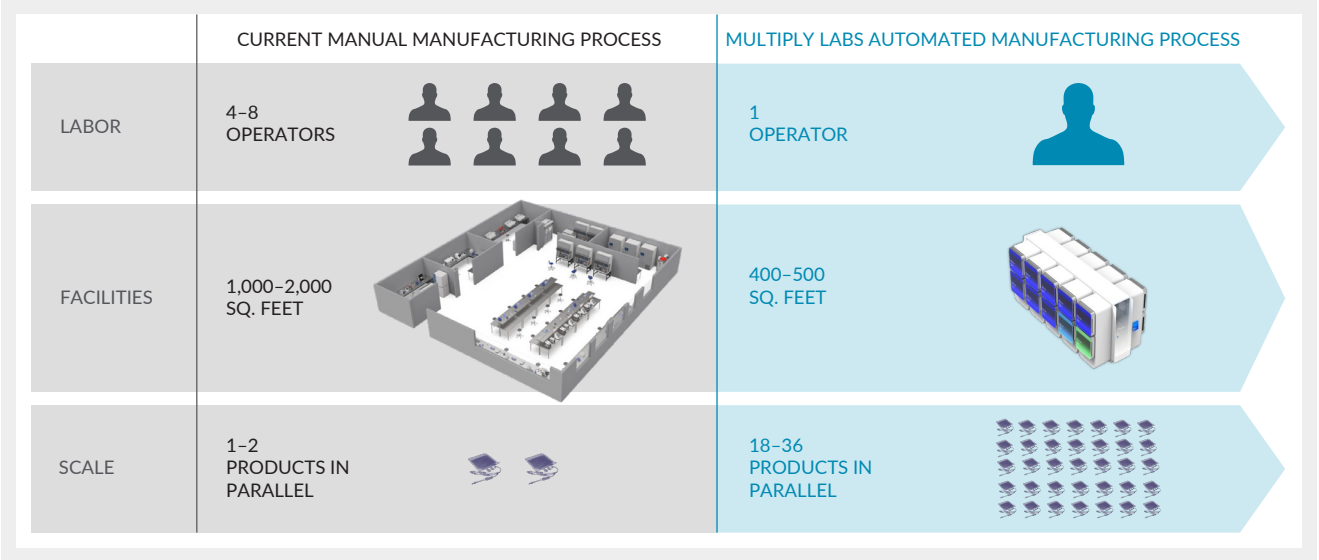
Automation is the key to meeting growing patient demand

Multiply Labs believes that automation is key to enabling efficiency in advanced therapy manufacturing (Figure 1). Typically, a manufacturing suite requires between 4 and 8 operators to support the manufacture of cell therapies. However, with robotic systems, only one operator is needed. In addition, this operator' need not be onsite as the robots could be monitored remotely through cameras that can be accessed via a secure portal. Loading and unloading the robots will require a person to be onsite, but otherwise the operation of the system is entirely autonomous.

There is also a major difference in space requirements. A typical cell therapy manufacturing suite requires 1,000–2,000 ft² (93–183 m²). In contrast, a robotic system would only need 400–500 ft² (37–46 m²)—a quarter of the footprint. Robotic arms are able to operate side-by-side and therefore do not require as much space. It is also possible to stack robots. Consequently, the same or even increased throughput may be achieved with a smaller footprint. A further advantage is that robotic systems can be housed

FIGURE 1

Comparison between manual and automated processes.



in lower grade cleanrooms. With human operators, there is a need for biosafety cabinets and a high degree of air filtration, both of which add to the cost of manufacturing.

Another significant advantage is the ability to scale up effectively. Manual processes are typically limited in the number of products that can be manufactured simultaneously to prevent cross-contamination. However, robotic systems greatly reduce these constraints. Currently, approximately 18 separate products can be accommodated within a single Thermo Heracell™ VIOS CO_2 incubator, and Multiply Labs has utilized up to 10 incubators in parallel within their robotic system (Figure 2).

A crucial aspect of Multiply Labs' technology is maintaining the original manual manufacturing process unchanged. Altering the process can result in comparability issues, leading to regulatory challenges, and can also affect cell growth, as cells may respond differently to changes in instruments or reagents.

Instead, Multiply Labs works with leaders in the space such as Thermo Fisher to place each instrument used in the original process—for example, the Gibco™ CTS™ Rotea™ Counterflow Centrifugation System—into its own closed module. Within these modules, the robots operate the instruments automatically. Multiply Labs refers to this as an 'additive approach', where automation is integrated into the existing process without altering it. It is essential that automation does not detract from the original process in any manner.

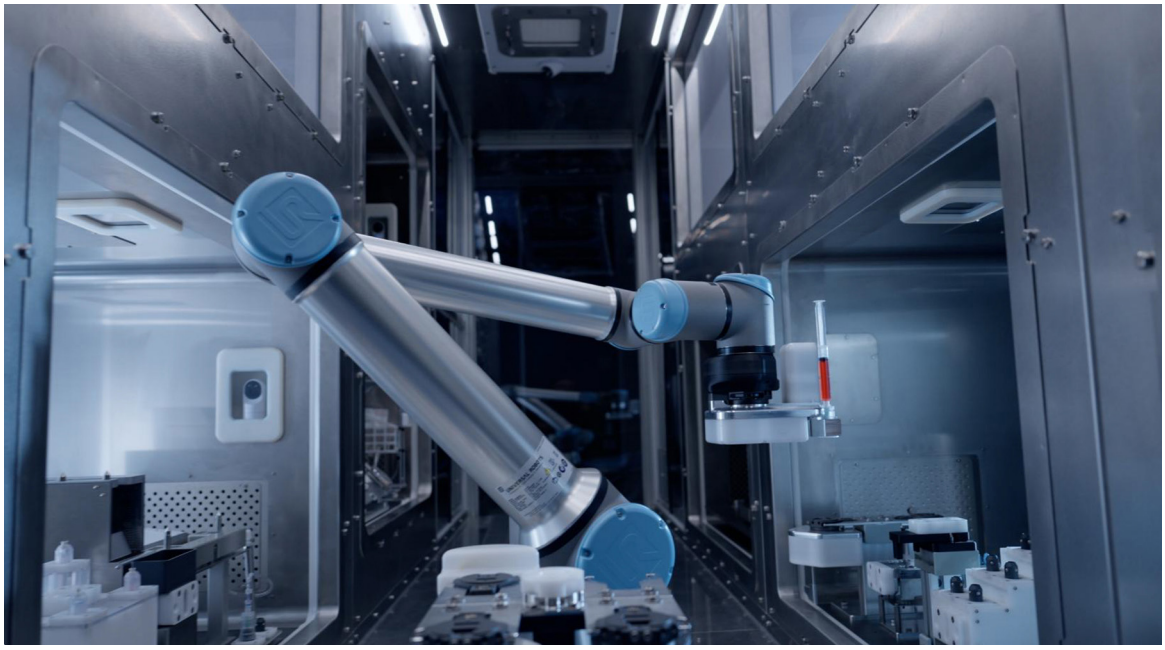
Collaboration with industry leaders

Multiply Labs has collaborated with Thermo Fisher from the initial stages of the development of this technology. The two key modules announced so far as part of this collaboration are the CTS Rotea system module and the Heracell VIOS CO_2 incubator module. The incubator module is of fundamental importance to achieving high throughput due to the opportunities it presents for parallel product manufacture described previously.

Each cell type utilized in cell therapy requires a different cell culture. From the

FIGURE 2

Robotic arms within one of Multiply Lab's automated units.



point of view of automation, these different cell cultures are all operated by the same robotic system inside the same incubator, demonstrating the flexibility of this approach.

The CTS Rotea system also lends itself well to automation owing to its design, with valves, sensors, and rotating chamber located on the same slanted surface. This makes it easy for the robotic system to insert and remove the consumables. This is important as it enables the robot to operate and set up and then clean or clear every instrument autonomously, which is more efficient than having the process conducted manually. It is therefore essential that these instruments are automation friendly. The main goal of the incubator is to hold as many products as possible and in this regard, both the CTS Rotea System and Heracell VIOS CO_2 incubator are conducive to an automated process.

Modules increase flexibility

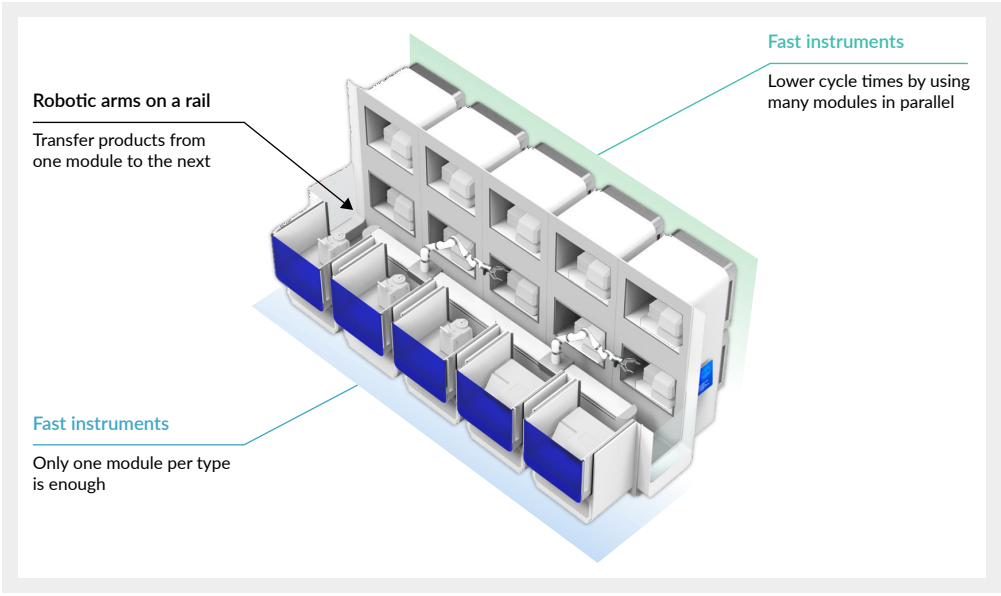
Once instruments are automated within modules, these modules can be combined in the same robotic system. Cell therapy developers can choose, add, or remove modules as needed. Updated or different instruments can be swapped into a module without affecting the rest of the system, similar to a standard manufacturing suite. Each module is standardized, but their combination is flexible. Multiply Labs offers various robotic modules, including incubation, refrigeration, centrifugation, bioreactor, isolation, and fill-and-finish modules, enabling any biomanufacturing process through a flexible, 'building block' approach.

High throughput with multiple parallel modules

This flexibility can also be harnessed to overcome manufacturing bottlenecks by using multiple modules of the same type operating in parallel in order to achieve high throughput (Figure 3). This method is commonly used with incubators, where a fully automated incubator can hold up to 18 1 L bioreactors, meaning 18 different therapies can be held in parallel. It is important to firstly optimize the number of units, doses, and products that can fit into a single incubator before then multiplying the units.

FIGURE 3

Multiple parallel modules working simultaneously can achieve high throughput.



However, there are some limits. It is wise not to utilize a system that is not significantly larger than 400–500 ft² (37–46 m²) as this causes difficulties with assembly and transport/mobility.

The future: AI imitation learning for robots

It is essential for robots to learn to replicate manufacturing tasks precisely. As such, Multiply Labs is currently developing an AI imitation learning stack whereby the robot can replicate the data and video simulation, which is then applied to a real robotic system. One example of this would be resuspension of a bioreactor task where the robot is designed to shake the flask, imitating the manual operation.

NEXT-GEN CELL THERAPY MANUFACTURING: LEVERAGING FLEXIBILITY AND AUTOMATION FOR SUCCESS

Carl Dargitz

Bridging the gap to optimal cell therapy workflows

To bridge the gap between the current state of cell and gene therapy development and our future goals, we must address three main challenges. The foremost issue is patient safety; ensuring that therapies are safe and providing tools to develop these safe therapies is of utmost importance. The second major concern is the high production cost. Thirdly, we need to enhance overall consistency across different processes. Central to overcoming these challenges are improvements in automation, flexibility (whether it pertains to the entire workflow, a specific process, or the ability to handle different patient types, indications, or cell yields), and scalability.

Defining automation in cell therapy

There are three main areas of automation within cell and gene therapy development:

- Unit operations, which relates to the automation of manual processes. Unit operations in cell therapy workflow refers to the distinct, sequential steps involved in the manufacturing process of cell-based therapies. Automating these individual workflow steps with specialized instruments ensures precise control and validation, thereby ensuring the production of high-quality cell therapy products;
- Digital data automation, which describes the creation of digital modules that allow for control from a central terminal and are also able to capture data for compliance with regulatory requirements for electronic batch records. Digital automation also refers to the interconnectivity between different instruments in the workflow;
- Process automation, which describes automation between unit functions (i.e., tools and systems exist for automating a unit function—however, the drug product ultimately needs to be transferred between each unit function).

Therefore, there is a need to automate the entire cell therapy workflow.

Closed, scalable and automated instruments for cell therapy

Thermo Fisher has an array of different unit function solutions that can help in automating a workflow (Figure 4). For processes involving cell isolation, activation, and bead removal, Thermo Fisher relies on the Giboc™ CTS™ DynaBead™ technology, which is paired with the Giboc™ CTS™ DynaCellect™ Magnetic Separation System. This allows these processes to be conducted in a single step whilst also cutting down on donor-to-donor variability. The CTS Rotea Counterflow Centrifugation System can aid cell processing (e.g., washing, concentration,

FIGURE 4

Closed, scalable, and automated instruments for cell therapy development.

buffer exchange), which can take place at many different points withing the workflow, including before a gene-editing step or a cryopreservation step. For gene delivery and gene-editing applications, Thermo Fisher provides both viral and non-viral production tools. Non-viral tools include the Gibco™ CTS™ Xenon™ Electroporation System, while for viral vector production, they offer solutions for both lentivirus (LV) and adeno-associated virus (AAV). The Gibco™ LV-MAX™ Lentiviral Production System and Gibco™ AAV-MAX™ Helper-Free AAV Production System are designed to maximize yield and efficiency, providing scalable options for both research and clinical applications. All viral and non-viral tools utilize the Gibco™ CTS™ Cellmation™ Software for DeltaV™ System. This is key for reducing the total number of manual touch points while capturing data. Another critical step is wash/formulation/fill and finish, which can be achieved using Thermo Fisher's forthcoming Gibco™ CTS™ Compleo™ Fill Finish System. In terms of analytics and characterization, Thermo Fisher's Attune™ NxT Flow Cytometer can accomplish multiple assays relating to genomics, identity, and purity.

The following standalone devices can be digitally and physically integrated to enhance automation levels. These instruments are intended for both manufacturing and process development. They are designed for speed and precision, featuring compact footprints, and they facilitate data collection for regulatory documentation.

Q&A



David Shaw (left), **Fred Parietti** (centre), **Carl Dargitz** (right)

Q What are some key considerations for selecting automation tools that can adapt to evolving cell therapy processes?

DS We have found that automation tools that provide transparency in how they execute their unit operation allow us to troubleshoot more effectively. We have encountered tools that are proprietary, and their operation occurs in a way that leads to uncertainty. Consequently, the cell therapy processes in which they are applied are not well understood. Our goal is to choose tools that help us to learn more about our cell therapy processes so that we have the best possible understanding of what is going on, and thus, allow us to better control the manufacturing process and successfully deliver our medicines to our patients.

It has taken decades of industrial manufacturing expertise and experience to make today's antibody biologics, and we should learn from that rich area of technology in order to pick out what works well for cell and gene therapy.

Q What future advancements in automation do you foresee having the most significant impact on cell therapy development and production?

FP We anticipate advancements in two key areas. The first is the automation of an increasing number of unit operations, driven primarily by demand from cell therapy developers. We regularly survey our partners, who inform us about the specific instruments they are using or the capabilities they need to add to automate the end-to-end cell therapy manufacturing process. This feedback directly influences our development efforts.

New instruments often require the development of new robotic tools to ensure the robotic system can operate the instrument and handle the reagents. For instance, many reagents are delivered in vials, sometimes lyophilized, requiring resuspension, mixing, extraction, and addition to the product. Robots need to be capable of performing these tasks, which drives innovation.

The second area involves robots manufacturing multiple lots and doses simultaneously. This has made loading and unloading robots a significant bottleneck. When processes are automated, the manual steps performed before or after the automated process can become bottlenecks. Currently, we load and unload the robots manually, but we have several new developments in progress to address this issue.

Cell & Gene Therapy Insights 2025; 11(4), 439–451 · DOI: 10.18609/cgti.2025.053

Q What are the primary benefits of incorporating automation into cell therapy manufacturing processes?

CD **There are numerous benefits, but most importantly, it's about enabling the flexibility to produce therapies for diverse patients with varying health statuses, indications, and variable cell numbers.** The goal is to apply these solutions broadly. We aim to provide flexible and modular instruments that allow different cell therapy developers to use the same equipment and processes, regardless of the specific indication or patient status.

We are all striving towards the same objective, using different tools and approaches to achieve it. For example, we consistently heard from customers that the fill-finish step was a missing component in unit function automation. This feedback led us to develop the Compleo system to address this need.

Q How important is flexibility in cell therapy manufacturing, and how can automation systems be designed to accommodate this need?

CD **Flexibility is crucial.** One of the central goals of our product development is to ensure our systems are adaptable. We need to provide solutions that cater to various indications and patient statuses whilst also being scalable. For example, the Rotea system can handle starting cell volumes of less than 1 L and scale up to 20 L.

Switching systems entirely disrupts consistency and results, especially with significant increases in scale. Flexibility in our systems enables easier scaling, optimization, and adjustment to necessary parameters. This adaptability is critical and a core focus of our efforts to advance the field.

Q Can you discuss any specific challenges you have encountered when integrating automation into cell therapy workflows?

DS **Currently, there are very few automation platforms for cell and gene therapy, and a major challenge is the lack of standardization, which limits our ability to adopt specific technologies.** Our diverse interests in cell therapy require different workflows and automation for each cell type. It may be unrealistic to expect autologous or allogeneic T cell therapies to align seamlessly with stem cell-derived therapies, but this is a goal we need to work towards.

This diversity poses a challenge when adopting platform technologies, or what we hope will become platform technologies. Additionally, demonstrating comparability is costly and complex, especially when transitioning to automated workflows. As the industry matures, we may find a path to lifecycle changes after licensure, but the current lack of understanding of these cell therapy processes makes demonstrating comparability challenging.

Q Can you share your experience with integrating robotics into current instrumentation for cell therapy manufacturing workflows?

FP It is very important to work closely with the team that developed the original product and process. We have many case studies relating to this and it's very striking how these cell therapy manufacturing processes tend to be so manual and can require so much attention from highly experienced scientists. For example, it's very common for us to review batch records or otherwise, very detailed descriptions of the processes. Then, when it is time to implement these on the robotic system, we do a side-by-side comparison of the robotic system versus the manual process.

That is what we are doing with our robots in Palo Alto, as part of our current collaboration with Stanford University. When scientists arrive there to run these experiments, we observe how they do the manual process. There are always a few details that they are adding that were not captured in their description in the batch record or in their process. For example, they may shake a container in a different way, or shake it more times, etc. Their manual methods have not been precisely defined, and it is striking how much key information is not captured in written records. Standardization is very important. It is shocking sometimes how manual these processes are, but after a few initial days of adjustment, it will be possible to have a fully replicated process that is robotic.

Q How does automation impact the scalability and quality of cell therapy production?

FP In terms of quality, we observe a significant improvement right from the initial runs when comparing manual and robotic processes. Standard deviations across key process parameters tend to decrease because the robotic system consistently performs tasks with precision. We have digital records that verify the robots' actions, ensuring they move in the exact same way, adhere to time limits between operations, and maintain cells within the incubator for the specified duration. This consistency leads to higher predictability and consequently, higher quality.

From an efficiency standpoint, once we demonstrate that robots can replicate a process with statistical equivalence to the manual method, it becomes a matter of determining the number of modules and robotic arms needed to meet production demands. After establishing the initial unit process, scaling up is straightforward, as the robots consistently perform the same motions and enforce the same constraints, allowing for rapid scalability.

The initial optimization phase involves working on a limited number of processes simultaneously. Once this is fine-tuned, efficiency increases exponentially. The primary constraint is the number of incubators integrated into the robotic system, which determines the maximum production capacity.

Cell & Gene Therapy Insights 2025; 11(4), 439–451 · DOI: 10.18609/cgti.2025.053

Q Can you share with us some examples of how automation has improved efficiency or reduced costs in cell therapy manufacturing?

DS Some of the automation systems we have evaluated offer increased walk-away time and even remote monitoring, which is highly beneficial for reducing time spent in a GMP suite. One of the significant improvements, in my opinion, is the more streamlined data acquisition. Data analysis and data mining will be crucial for the rapid advancement of cell and gene therapy manufacturing. By leveraging these tools, what took decades for antibody biologics to achieve in terms of successful manufacturing could be accomplished in just a few years for cell and gene therapies. This increase in efficiency is expected to ultimately help reduce costs.

Q What key challenges have you encountered when automating critical unit operations within cell therapy manufacturing workflows?

CD From a development standpoint, the primary challenge for instrumentation lies in balancing versatility with the ability to deliver precise results. An instrument that claims to perform all tasks may not excel in any specific function, making it less suitable for cell therapy manufacturing. It is essential to thoroughly understand the needs of all cell therapy developers to ensure the equipment meets their specific requirements effectively.

Balancing instruments capabilities with manufacturing process needs (e.g., process volumes ranging from 1 mL to 1,000 L on the same instrument) likely means compromising on performance. Even though we have adaptable tools like the Rotea system, there are inherent limitations. The main challenge has been finding the right balance between flexibility and the specific requirements of cell therapy manufacturing. This challenge can only be addressed by collaborating closely with customers and partners to understand where flexibility is crucial and where a more fixed approach is necessary for the manufacturing process.

BIOGRAPHIES

David Shaw is currently Head of Cell Therapy Engineering and Process Development at Genentech, Inc. The group is responsible for developing cell therapy processes to support clinical manufacturing of T cell therapies and stem cell therapies. The group collaborates with external partners, CDMO partners and internal Research groups to enable the cell therapy portfolio. David has 24 years of service at Genentech/Roche with 7 years in Cell Therapy Process Development, 8 years in CHO Cell Line Technology Development and 9 years as Head of Molecular Biology and Protein Sciences at Roche Palo Alto. Prior to joining Genentech/Roche, David was a Research Professor at the South Carolina Cancer Center researching retroviral gene therapy to alleviate the myelosuppressive side effects of chemotherapy. David has a PhD in Biochemistry and Molecular Biology from the University of South Carolina, Columbia, SC, USA and a BSc in Biochemistry from the University of British Columbia, Vancouver, BC, USA.

David Shaw PhD, Senior Director, Head of Cell Therapy Engineering and Process Development, Pharma Technical Cell and Gene Therapy, Genentech, Inc., San Francisco, CA, USA

Fred Parietti holds a PhD in robotics from MIT, Cambridge, MA, USA and has extensive experience in the design of autonomous robotic systems (awarded with the MIT Mechanical Engineering De Florez Design Award). Before MIT, Fred worked on advanced robotics at Carnegie Mellon University, the Polytechnic University of Milan and ETH Zurich. Fred has authored or co-authored 16 peer-reviewed scientific publications, which have been cited more than 1,500 times. He is also a co-inventor in 10 patents and patent applications.

Fred Parietti, Co-Founder and CEO, Multiply Labs, San Francisco, CA, USA

Carl Dargitz is a Senior Manager in the Cell and Gene Therapy Platforms R&D group and has been developing cell therapy instrumentation at Thermo Fisher Scientific for the past 5 years. Previously, he developed reprogramming and characterization assays for PSCs and immune cells at Thermo Fisher. Before joining Thermo Fisher, he worked at the Salk Institute for Biological Studies at the stem cell core facility managing the core's reprogramming and characterization services. He received his MS from California Polytechnic State University, San Luis Obispo, CA, USA in Biomedical Engineering with a specialization in stem cell research.

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CELL THERAPY MANUFACTURING

SPOTLIGHT

The path to PAT: the role of process analytical technologies in advancing CAR-T therapy production

This article is part of our 'Rising Stars' series, giving a platform to the emerging leaders of the sector. In this series, we share the perspectives of fledgling thought-leaders, chosen by our Editorial Advisory Board members as future stars in their field.

John Moscariello, Chief Technical Officer at Neuvogen Therapeutics, had this to say about his Rising Star nominee:

> "Almost immediately upon the approval of the first gene-modified cell therapy products, process developers asked how we can make the product cheaper. While this emphasis has led to significant advances in automation, many people have missed what I think is a more impactful question—how can we make products with better clinical outcomes? Sarah Rajani is the industry-leader in developing adaptive cell therapy processes in which real-time measurements of cell attributes can be used to change process conditions. Published literature has shown that clinical outcomes can correlate to specific ranges of critical quality attributes and Sarah's work has the potential to change the manufacturing process to ensure those critical quality attributes are within those ranges. Sarah brings innovation, inclusion, and passion to this multifunctional challenge that can make a significant impact on patients using gene-modified cellular therapies."

RISING STARS

INTERVIEW

"PAT should be a key pillar in a manufacturing process and can become just as critical as your electroporator or expansion bioreactor."

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, speaks to **Sarah Rajani**, Senior Scientist, Process Analytical Technologies (PAT), Bristol Myers Squibb, about the role of PAT in assisting with the analytical monitoring of CAR-T cell therapies in manufacturing, the limitations of current PAT, and the potential improvements that can be made to enhance CAR-T therapy production. They also discuss the emergence of holographic imaging and ensuring PAT can meet the diverse needs of cross-functional teams.

Cell & Gene Therapy Insights 2025; 11(4), 433–438 · DOI: 10.18609/cgti.2025.052

Q What are the challenges/bottlenecks you see in the analytical monitoring of CAR-T cell therapies in manufacturing?

SR From a technical perspective, there are two main considerations: one is the analytical approach—how do we collect the data to begin with? In CAR-T cell therapy, particularly autologous cell therapies, the products are quite complex, meaning that the type of information we are interested in is also complex. Therefore, novel analytical approaches are needed to be able to obtain this information, whether it is CAR-T cell identity, activation state, or differentiation state. Another layer of difficulty lies in the material availability. All in-process material is precious; every cell contributes to the final dose that could save someone's life. Process analytical platforms require sampling methods that minimize or bypass the destruction of any of our in-process material. Sampling, specifically how much you sample to monitor drug product quality attributes, is a hot topic and an interesting challenge for both technology and therapeutics developers. A third challenge lies in developing for material heterogeneity. For autologous CAR-T cell therapies, there is variability between patients and over time as immune cells respond to the process that we are putting them through to create the CAR-T cell therapy product. Measurement accuracy may be impacted by changes in cell size, cell functionality, and donor-to-donor variability, so monitoring platforms must be robust across a wide range of possibilities.

The second consideration is how we harness that data. When we talk about personalized CAR-T cell manufacturing or adaptive manufacturing, process control hinges on automated, operator-free, unsupervised analysis, and reporting capabilities for these tools. To pair with this analytical method, we also need to develop a robust data analysis algorithm that is accurate across a wide range of measurement values. This data must be in a format that can be digested and reported/communicated to our process equipment via digital interfaces.

Q How are in-process analytical technologies poised to assist in these challenges? How do these technologies enable the identification of critical process parameters (CPPs) and critical quality attributes (CQAs)?

SR There has been growing momentum in the development of these systems, and it is an exciting time to be talking about developing and implementing

PAT in a way that can be integrated into our cell therapy process. There are several tools available that have been adapted to support continuous and non-destructive monitoring of traditional process parameters that are indicative of general cell culture performance. We can learn from our small molecule and biologics predecessors, and we know factors such as pH, glucose, and viable cell concentration matter. There are tools that have emerged that can be seamlessly implemented in our manufacturing processes.

There is also the growing sophistication and miniaturization of both novel and traditional analytical methods. We have seen more innovative methods leveraging lasers and label-free imaging. There is also miniaturization of traditional techniques such as using mass spectrometry to interrogate cell therapy attributes in a way that allows us to take a sample, get new information, and incorporate this into our process development sphere.

There is a lot of value in implementing the tools that exist in process development. Your ability to learn increases exponentially with the amount of information that you can collect. That end-to-end data allows us to understand the process from starting material all the way through the evolution and probe different processing set points. This makes it possible to identify relationships from the beginning, middle, and end and determine your design space to help pursue a targeted development strategy. The final piece of the puzzle is finding technologies that can be integrated all the way through from development to commercial manufacturing so you can keep learning, iterating, and harnessing that data.

Q What are the key limitations of current PAT technologies, and what innovations would you like to see in the coming years to enhance CAR-T therapy production?

SR If I had one wish, it would be to harness a non-invasive monitoring technology or a non-destructive sampling approach. There is such power in the analytical information that these tools have been developed for. The need for large sampling volumes or extensive sample preparation introduces limitations; we are limited by how often we can collect this information and in which batches. We learn the most by collecting information and identifying process parameters or quality attributes that are predictive of batch failure from patient lots that are struggling. However, those are the lots that we cannot sample from, which can be very constricting. If there is a way to adapt the technologies on the market to support a non-destructive or a non-invasive approach, we can accelerate this adaptive manufacturing vision that we all share.

Q Holographic imaging has emerged as a promising PAT tool—how do you see its application in cell therapy process monitoring, and what advantages does it offer over traditional imaging techniques?

SR In general, imaging has become a spotlight technology once again, especially with the emergence of machine learning and AI, which allows us to grasp new details from images. We can extract even more information on the shape or internal texture of cells which can inform their functionality and their phenotypic state.

"Looking ahead, we can start to establish clinical outcomes as we begin to manufacture CAR-T therapies for patient treatment."

Holographic imaging is a unique approach to support product attribute monitoring and address some of the challenges we described earlier. This contrasts with fluorescence imaging that requires specific labeling and sample preparation. It also allows us to obtain more insight than from traditional bright field imaging methods through improved capture of intracellular content. With phase imaging, you are able to harness the shift (i.e., the refractive index) over the course of the cellular thickness. This allows us to compute how the shift occurs at different points within the cell. You can then start to clearly quantify the heterogeneity in the cells and how they are metabolizing and responding to your process, through their morphology.

As holographic imaging is label-free, we can develop non-destructive sampling approaches and send cells back to the culture once imaged. Label-free holographic imaging enabled by non-destructive sampling allows us to quantify cellular response in real time and continuously, obtaining powerful insights without impact.

Q How do you collaborate with cross-functional teams (e.g., R&D, clinical, regulatory) to ensure that PAT tools meet both scientific and operational needs in CAR-T manufacturing?

SR Collaboration is what is most exciting to me. I started in the industry specifically doing PAT, and what attracted me the most to this role was being able to learn from these different schools of thought, all driving towards an ultimate goal. From an R&D perspective, you are able to learn about any early insights. What process parameters are coming up as important when developing gene transfer methods? What cellular attributes are important to track in these indications/diseases? As we work towards commercialization, how do we address variability to create a robust process and product? It is certainly key to be able to learn and then provide those tools. PAT stands to transform the way we do development by harnessing the information that we collect, whether process parameters or product quality attributes, and developing a mechanistic understanding. This means we can define the causality of a cellular response to a process and see how it starts to relate to our drug product quality attributes.

Looking ahead, we can start to establish clinical outcomes as we begin to manufacture CAR-T therapies for patient treatment. We can harness in-process phenotypic state in addition to starting material characteristics to establish relationships between manufacturing performance and clinical efficacy. We can then use this understanding to develop process control strategies that allow us to meet a target product profile, therefore helping us create consistent high-quality products.

At the moment, advanced technologies are in the spotlight, with PAT and adaptive manufacturing playing their own parts. The interfacing of what we measure, how it allows us to improve our process, and how we get it to patients and demonstrate robustness means it is important that we have cross-functional collaboration to achieve this. It is the best part of my job.

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Q How do you foresee the role of PAT evolving in the scalability of CAR-T cell therapies, particularly as the demand for personalized treatments increases and the need for widespread access to cell analysis becomes more important?

SR Cell therapy manufacturing is rapidly evolving to meet growing demand; we are creating closure in our manufacturing process, introducing complete automation, and introducing control mechanisms that can be implemented without a human to address process variability.

Process variability is only one side of the coin; biological variability is the other. Without PAT that allows you to monitor and modulate biological variability, the potential of true automation will not be realized. The need to develop control to the caliber described in these concepts hinges on the quality and quantity of the information collected while manufacturing these batches. These measurements must be biologically relevant and taken frequently to enable us to establish trends and act upon them. This level of sophistication will be critical.

Guidance for industry from the US FDA on the need for PAT was introduced as early as 2004. As cell therapy addresses gaps in process automation, analyzing and control critical in-process attributes and parameters will likely shift from a 'nice to have,' to 'why aren't you doing this?' You are leaving chips on the table when you are automating your entire process but not identifying tools that can be integrated to monitor and address biological variability though automation, increasing manufacturing success and clinical outcomes through adaptive control, while still reducing costs. That scalability is dependent on our ability to address both sources of variability.

Q What are your key goals for yourself and your team over the next 1–2 years?

SR Two things come to mind: one is to learn; learn from failures, learn from technology performance, and be unafraid to iterate. There is great value in experience; if we can develop technologies faster and integrate them earlier, we can use these tools to monitor and collect information to accelerate process development and PAT monitoring capabilities. As we transition from development on healthy donor to patient material being able to learn and to iterate is going to be a key component for the next year or two.

We can also learn from the field; we have so many brilliant innovators that are all keen to share their knowledge. I was so inspired by some of the conferences I attended last year. There is a drive across the field to better serve our patients with faster, more robust, more accessible products for cell therapy. We would be doing a disservice by not opening ourselves up to the rest of the industry.

The second goal is to think outside the box and to be bold enough to do so. In order to fully realize our vision of real-time product monitoring and adaptive CAR-T manufacturing, there is a need to develop PAT solutions for a wide variety of indications and cell therapy processes, whether this be a gene-edited process or an allogeneic one. PAT should be a key pillar in a manufacturing process and can become just as critical as your electroporator or expansion bioreactor. Thinking outside of the box and being creative by looking at existing tools in a new light so that we can reach our goals is paramount to success.

BIOGRAPHY

Sarah Rajani is a Senior Scientist who has been leading Process Analytical Technology Development efforts for cell therapies at Bristol Myers Squibb for the last 5 years. During this time, she has led a team of enthusiastic scientists towards evaluation, development and integration of novel monitoring technologies into process that enable the production of more robust cell therapies through adaptive CAR-T manufacturing. Sarah is a strong advocate for PAT integration, speaking previously on the promise of Quality-By-Design and adaptive manufacturing approaches provide for improved CAR-T drug product quality.

Sarah Rajani, Senior Scientist, Bristol Myers Squibb

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MANUFACTURING AND BIOPROCESSING

SPOTLIGHT

Driving innovation to advance allogeneic cell therapies through manufacturing platform development

INTERVIEW

"Our objective is to develop an end-to-end solution that enhances automation, ensures process closure, intensifies production where necessary, and ultimately simplifies the workflow."

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, speaks to Jason Dowd, Global Lead Cell Therapy Platform Process, Bayer, to explore the latest advancements in allogeneic cell therapy platform development, automation strategies, and hurdles that industry must address to ensure broader patient access.

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Q Can you tell me about the allogeneic cell therapy platform development that you are involved in?

JD My role focuses on development planning for the 6 months to 3 years. Currently, we are looking to identify technologies that will drive both scale-up

and scale-down improvements, particularly in terms of cost efficiency. We do this by taking a platform-based approach and evaluate the entire process to determine whether modular components can be implemented in either a 2D or 3D format. Even within these frameworks, there may be variations that need consideration. Our objective is to develop an end-to-end solution that enhances automation, ensures process closure, intensifies production where necessary, and ultimately simplifies the workflow.

The overarching goal is to increase patient access to these therapies at the lowest possible cost. As cost remains a critical factor, it directly impacts reimbursement discussions. Achieving a price point of USD$1,000 per dose significantly enhances the feasibility of widespread adoption. In regenerative medicine, we have a unique opportunity to make this vision a reality.

Q What upstream and downstream processing technology innovations are required to reach wider patient populations in terms of delivering the cost and time savings and improvements in quality and consistency?

JD The process begins with the cells, which are required to undergo gene edits to enhance their ability to reach larger patient populations—particularly by making them immune-evasive. Various strategies exist to achieve this; however, this remains a critical area of advancement.

Next, optimizing cell banking is essential. We aim to enhance scaling-up working cell banks to the largest possible size while also maximizing the per-vial or per-starting-point capacity. A larger initial starting volume shortens the overall manufacturing timeline by reducing need for prolonged expansion at small scales. In terms of expansion, we are focused on developing more cost-effective approaches. Media costs alone can account for 50 to 70% of overall production expenses. Reducing waste in this area is crucial, particularly given high cost of cytokines, which contribute significantly to these expenses.

Similarly, differentiation remains a time-intensive step. Engineering solutions for media exchanges are essential to optimizing this phase. In many cases, the process requires cycling between 0 and 100% concentrations of certain compounds. While this may seem like an advanced plumbing challenge, it must be executed in a GMP-compliant environment—something that has not been extensively developed before. We are actively collaborating with companies on harvesting technologies and media exchange systems. While these aspects may not seem particularly innovative, they are fundamental to establishing an end-to-end process that effectively reduces the CoG.

Finally, in drug product manufacturing, we are focused on designing highly automated equipment capable of performing visual inspections, in-process analytics, and integrating certain release criteria directly into the process. This level of automation is key to achieving efficiency, consistency, and scalability in cell therapy production.

"One of the most significant challenges remains ensuring the genetic stability of cells. Demonstrating an absence of rare genetic events is inherently difficult, as it is impossible to prove a negative."

Q How can the cell therapy field continue migrating away from traditional multi-step processes toward automated production on a single platform? How can upstream and downstream processes be seamlessly integrated to ensure streamlined manufacturing?

JD Our goal is to develop modular, plug-and-play systems that seamlessly integrate each stage of the process. Depending on the specific biological requirements, each module should feed directly into the next in a fully closed, automated manner—eliminating traditional separation between upstream and downstream processing. Historically, these functions were managed by distinct teams, but we are now driving a shift toward a more unified approach, ensuring cross-training across both disciplines.

This cross-training is often underappreciated but is essential for fostering a workforce capable of seamlessly managing an entire process. Encouraging team members to alternate between upstream and downstream responsibilities enhances flexibility and operational efficiency. Additionally, there is growing interest among academic institutions in developing talent for these evolving roles. However, hands-on experience remains crucial for achieving true proficiency in managing integrated manufacturing platforms.

Seamless integration hinges on process closure and automation. When both upstream and downstream operations are incorporated into a single system, such as integration expansion, differentiation, and downstream processing, validation efforts become more streamlined. Instead of qualifying multiple individual unit operations, a single, fully integrated system can be validated, ensuring consistency, efficiency, and scalability. Each stage functions as a puzzle piece within a cohesive framework, enabling more effective and cost-efficient cell therapy production.

Q Looking at bag filling technologies, how is the field working to establish automation in this area?

JD Several promising technologies have been introduced in this space, and some have already been implemented. However, for larger allogeneic therapies, there is a recognized need to develop custom solutions while leveraging existing advancements. The key to progress lies in collaboration with equipment providers rather than solely relying on them to develop solutions independently or purchasing equipment outright. As a therapeutics company rather than an equipment manufacturer, the priority is to engage in strategic partnerships that drive innovation.

By fostering collaboration, organizations can ensure that equipment providers incorporate process-specific requirements into their designs. In many cases, sharing intellectual property in exchange for co-development opportunities allows for the refinement of

manufacturing processes while also enabling equipment manufacturers to bring commercially viable solutions to market. Through beta prototypes and joint prototyping efforts, companies can gain early insights into how their processes integrate with emerging technologies. Additionally, licensing or sharing intellectual property with industry partners can facilitate creation of valuable equipment that can be showcased at industry events and adopted more widely.

Looking ahead, it is expected that within 3 years, many equipment manufacturers will have commercialized solutions that incorporate these collaborative advancements. Ultimately, establishing mutually beneficial partnerships will accelerate automation in backfilling technologies while improving the scalability and efficiency of cell therapy manufacturing.

Q What are your key goals and priorities for the next 1 to 2 years?

JD The primary goal is to enhance the robustness of our Phase III manufacturing process to the highest possible level, targeting a success rate of over 95%. While achieving this benchmark presents a challenge, it is a necessary objective for delivering high-quality treatments to patients.

To ensure consistent production of a reliable therapeutic product, the manufacturing process must be exceptionally robust, minimizing the risk of failure. In reality, the ultimate target extends over 95%, aiming for a success rate of 98 to 99%. Reaching this level of consistency would represent a significant accomplishment and a major step forward in advancing scalability and reliability of cell therapy manufacturing.

Q Finally, what do you see as the biggest hurdle for the cell therapy field as we move into 2025?

JD One of the most significant challenges remains ensuring the genetic stability of cells. Demonstrating an absence of rare genetic events is inherently difficult, as it is impossible to prove a negative. The ability to confirm that no unforeseen genetic anomalies have occurred is a critical concern, particularly for therapies intended to provide durable, long-term treatment—potentially lasting up to 30 years in a patient's body. Moving forward, the field must continue to refine and validate methodologies that provide confidence in long-term genetic integrity of cell-based therapies.

BIOGRAPHY

Jason Dowd received his PhD from the UBC Michael Smith Biotechnology Laboratories, VA, Canada in Biological & Chemical Engineering and has recently completed his MBA, specializing in Technology Management. Dowd has directly mentored and grown progressively larger development and manufacturing teams with over 20 years' experience in diverse companies, launching new services, R&D and commercialized therapeutic products with multi-hundred million USD per year revenues. Dowd is enabling development, platform and GMP teams to

reach their potential and goals. Dedicated to innovation and 'Let's make it happen', Dowd joined Bayer in August, 2023.

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MANUFACTURING

SPOTLIGHT

Exploring strategies for scaling up allogeneic natural killer cell therapy

INTERVIEW

"...designing a scalable, closed-system manufacturing process from the outset ensures a seamless transition from clinical to commercial scale."

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, speaks to **Will Rosellini**, President, CytoImmune Therapeutics, about the advancements in natural killer (NK) cell therapies for cancer and autoimmune diseases. They also discuss the role of decentralized manufacturing in improving therapy accessibility.

Cell & Gene Therapy Insights 2025; 11(4), 459–463 · DOI: 10.18609/cgti.2025.055

Q What are you working on right now at CytoImmune?

WR CytoImmune is currently focused on advancing allogeneic NK cell therapy programs, particularly in oncology and autoimmune diseases. Our lead program targets solid tumors, and we are also exploring novel strategies for autoimmune disorders, such as rheumatoid arthritis, by leveraging NK cells' ability to selectively eliminate autoreactive immune cells. Additionally, we are strengthening our manufacturing capabilities through partnerships with companies such as Hemostemix, hoping to launch a commercial stem cell product under GMP later this year.

Q Can you tell us more about your in-house manufacturing facility in Puerto Rico, and what advantages does having a dedicated facility bring?

WR Our in-house GMP manufacturing facility in Puerto Rico is a strategic investment that provides us with greater control over production, quality, and cost. The decision to keep manufacturing in-house was driven by several key factors.

Firstly, Puerto Rico offers significant financial incentives, including Act 60 tax benefits and R&D credits, which help optimize operational costs by approximately 50%. Secondly, by owning and operating our own facility, we can maintain rigorous quality standards and directly manage compliance with the US FDA and global regulatory agencies. Thirdly, having this facility allows us to rapidly scale production as we progress through clinical trials and into commercialization without being dependent on third-party contract manufacturers. Lastly, with in-house production, we reduce bottlenecks in supply chain logistics and manufacturing lead times, enabling us to accelerate clinical trial execution. All things considered, this approach ensures that we can produce high-quality, scalable allogeneic NK cell therapies efficiently and cost effectively.

Q What are the major hurdles in manufacturing allogeneic cell therapies at scale, and how does CytoImmune overcome them?

WR Manufacturing allogeneic cell therapies at scale presents several key challenges. To begin with, there are hurdles associated with cell expansion and yield. Maintaining robust NK cell expansion while preserving potency and functionality is critical. To address this complexity, we optimize our media formulations and bioreactor conditions. Furthermore, there are challenges associated with batch-to-batch consistency. To mitigate this hurdle, it is crucial to implement stringent quality control measures and in-line analytics to monitor cell characteristics throughout the process. Additionally, there are supply chain complexities, and the availability of critical raw materials, such as high-quality cytokines and growth factors, can impact production timelines. We mitigate these risks through strategic sourcing and redundancy planning. Finally, the evolving regulatory landscape for cell therapy demands strict adherence to GMP guidelines. Our in-house facility allows us to align our processes with the FDA expectations early in development.

Overall, by leveraging automation, real-time analytics, and process optimization, CytoImmune proactively addresses these challenges to ensure scalable and reproducible NK cell manufacturing.

Q What are your key strategies in formulating and executing a successful CMC compliance strategy, particularly with the ever-increasing complexity of engineered cell therapy products?

WR A strong CMC compliance strategy requires an integrated approach that involves early process characterization, automated process control,

Cell & Gene Therapy Insights 2025; 11(4), 459–463 · DOI: 10.18609/cgti.2025.055

regulatory alignment, and scalability considerations. Firstly, defining critical quality attributes and process parameters early ensures robustness and regulatory alignment. Secondly, real-time monitoring and analytics help maintain consistency and mitigate variability in manufacturing. Thirdly, engaging with the FDA and global regulatory bodies early helps streamline approval pathways and prevent compliance setbacks. Finally, designing a scalable, closed-system manufacturing process from the outset ensures a seamless transition from clinical to commercial scale.

Q Scaling up cell therapy manufacturing while ensuring cost efficiency is a significant challenge. How do you navigate this issue, and what role do you see automation playing in the future of large-scale cell therapy manufacturing?

WR Scaling up cell therapy manufacturing while maintaining cost efficiency requires process automation, optimized supply chain, modular manufacturing systems, and cost-effective cryopreservation.

First and foremost, automation will play an increasingly vital role in reducing costs, increasing batch-to-batch reproducibility, and improving overall efficiency in cell therapy manufacturing. We are integrating automation into our workflow, including closed-system bioreactors, robotic handling, and AI-driven analytics to reduce manual labor and variability. Additionally, securing reliable sources for key raw materials and streamlining logistics helps prevent bottlenecks and cost overruns, whilst designing flexible, modular systems enables rapid scale-up without requiring extensive facility redesigns. Finally, developing optimized cryopreservation and logistics solutions ensures the efficient distribution of cell therapies globally.

Q How do you see decentralized or distributed manufacturing models contributing to the scalability and accessibility of engineered, allogeneic NK cell therapies, particularly for cancer patients?

WR Decentralized and distributed manufacturing models offer significant advantages for making cell therapies more accessible. Localized manufacturing hubs minimize the risk of delays due to shipping or regulatory barriers in different regions, ultimately reducing supply chain constraints. These models also allow patients to receive fresh, potent cell therapies closer to their treatment centers, reducing logistical complexities. Furthermore, decentralized models enable real-time process adjustments based on patient needs and regulatory requirements in different regions.

Overall, we see decentralized manufacturing playing a key role in the future, particularly for allogeneic NK cell therapies, where consistent, large-scale production is essential.

> "...we see decentralized manufacturing playing a key role in the future, particularly for allogeneic NK cell therapies..."

"...CytoImmune aims to drive the next wave of innovation in engineered, allogeneic natural killer cell therapies..."

This model aligns with our vision of bringing innovative cancer immunotherapies to a broader patient population efficiently.

Q What are your key goals and priorities in your work over the next 1–2 years?

WR There are several key priorities at CytoImmune for the next few years. Firstly, we will focus on advancing clinical trials and expanding our allogeneic NK cell therapy programs into later-stage trials, with a focus on both solid tumors and autoimmune diseases. We will also focus on scaling manufacturing by enhancing our in-house capabilities to meet the increasing clinical demand while preparing for commercialization. Thirdly, CytoImmune will be working closely with the FDA and international regulatory agencies to accelerate approval pathways, as well as collaborating with biopharma and academic institutions to explore combination therapies and novel indications. Lastly, we will be further optimizing our NK cell platforms to enhance persistence, efficacy, and targeting capabilities.

By focusing on these areas, CytoImmune aims to drive the next wave of innovation in engineered, allogeneic NK cell therapies, improving patient outcomes while ensuring scalable, cost-effective manufacturing.

BIOGRAPHY

Will Rosellini is the President of CytoImmune Therapeutics, Inc., a clinical stage biotechnology company. Previously, Rosellini was the CEO of Perimeter Medical, Inc. (TSX:V 'PINK') where he oversaw two 510K clearances, an RTO and $30 million in capital raised. Prior to that he was the CEO Nexeon Medsystems, Inc., ('OTC:QB, NXNN') a medical device manufacturing company that went public in 2017. Before that he founded, raised $16 million across A/B rounds and led Lexington Technology Group, LLC, a database company commercializing an electronic health record database solution to an exit ('DSS' NYSE). Before that he founded Sarif Biomedical LLC, a stereotactic cancer microsurgery with IP spun-out of Medtronic and led company to an exit with Marathon Patent Group, Inc. ('MARA' NSDQ). He subsequently served on the Marathon board of directors and chaired the Audit committee. Rosellini completed two acquisitions to form Telemend Medical, Inc., a clinical engineering services company, and led that company to an exit in 2016. Rosellini was also CEO at Microtransponder, an implantable neurostimulation developer with solutions for stroke rehabilitation. He is a former minor league pitcher with the Diamondbacks of the Arizona League, holds a JD, MBA, MS of Accounting, MS of Computational Biology, MS of Neuroscience and MS of Regulatory Science.

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MANUFACTURING

SPOTLIGHT

Digitalization and data analytics: shaping the future of cell bioprocessing

INTERVIEW

"...we must think about what tools can help us the most with digitalization and be bold."

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, speaks to Mohamed Noor, Digitalization Manager, National Institute for Bioprocessing Research (NIBRT), who shares his perspectives on how digitalization and data analytics will help to catalyze the transformation of biopharma and cell therapy bioprocessing, with the goal of producing reliable and scalable therapies for widespread use.

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Q What are you working on right now?

MN I work at the National Institute for Bioprocessing Research and Training (NIBRT). My main mandate is education surrounding automation, digitalization, and data analytics, because often, those involved in drug development struggle to make sense of their bioprocesses and truly harness the value that can be drawn from data analytics.

"Digitalization can help by flagging when something deviates from the norm, allowing humans to intervene only when necessary."

Developers often have the expectation that digital tools are highly complicated and require dedicated IT teams—but this is not necessary. There are solutions out there, including lightweight solutions such as manufacturing execution systems (MES), that are hosted in the cloud and do not require any dedicated staff to run. One must figure out where they are in terms of clinical or commercial development, and from there, identify the data analytics and digital platforms that are best suited to their needs.

Q What is the significance of digitalization for the biopharma industry? How do you think it will transform cell therapy?

MN At the moment, digitalization is viewed as if it is an optional element in bioprocessing, but regulators during inspections are starting to ask: 'What is your data strategy? What is your strategy for replacing paper records with digital systems?'. In 2 or 3 years' time, this will likely be the norm.

For example, during the COVID-19 pandemic, organizations realized that paper records must be taken from the manufacturing environment for data analytics purpose, where the teams are working from home, so paper can really be in one place at a time. What happens if a piece of paper is lost from a bundle of pages?

We heard from a manufacturer that they could not find the records of a batch, and the product then had to be placed in the quarantine zone in the warehouse. That is a real case of lost opportunity.

Q How has digitization transformed cell therapy supply chains and manufacturing specifically?

MN The impact of digitization on supply chains depends on the manufacturing model. In decentralized manufacturing, where therapies are produced at the point of care, logistics become crucial. Managing patient samples, ensuring paperwork is streamlined, and avoiding the need for Qualified Persons (QPs) to check every data point manually is where digital tools can help.

For example, QPs can be limited in terms of the number of products that they are qualified to sign off on. You don't want one QP to oversee 10 or 20 products; they must understand the process in detail so that the review is meaningful from a regulatory perspective. Digitalization can help by flagging when something deviates from the norm, allowing humans to intervene only when necessary. If you design a process with digitalization in mind, then this is easier to do.

An analogy can be drawn to driving a car: if you exceed the speed limit, your dashboard alerts you, helping you stay within safe parameters. Similarly, digital tools help manufacturers stay ahead of potential issues, rather than reacting to problems on the shop floor.

Q Can you explain what is meant by 'Cell Therapy 4.0'? And what are the key components that constitute Cell Therapy 4.0?

MN The term 'Cell Therapy 4.0', (sometimes referred to as 5.0) embodies a mindset where digitalization and automation are central. In early-stage biotech companies or academic settings, resources are limited, but they must still think about how to streamline processes as they move toward GMP manufacturing.

How do you compress the timeline for your processes, for example? It's not about using any specific technology like an MES system, it is about creating a comprehensive framework. Cell Therapy 4.0 is about how you manage data—such as storing quality control (QC) analytics data, managing standard operating procedures (SOPs), and handling deviation records.

You have to always be prepared for regulatory inspections, too. Ultimately, it's about thinking backwards from patient quality and meeting the expectations of regulators and investors, ensuring that everything is in place from the start.

Q How can novel algorithms be harnessed to enable the adaptation of autologous process to meet the needs of patients at scale?

MN The genetic background of the patient is a key factor in adapting autologous processes. For instance, population-specific variations, such as those seen in different ethnic groups, reveal that common health metrics like body mass index (BMI) may not be applicable across all populations. When developing cell therapies, we must ask, 'Does this treatment work for all patients, or just a specific group?'.

For clinical trials, a controlled environment is necessary, and this includes considering a specific patient's background. Inclusion criteria must be well-defined, but the goal is to ensure treatments are broadly applicable, rather than being limited to a small segment of the population. This is especially important when thinking about national regulatory or healthcare payer systems. There is also an ethical dimension to this—for example how do we balance the benefits of a treatment for older patients versus younger patients? This requires a thorough understanding of both the biological and ethical implications of treatment options, as well as how these treatments will be reimbursed in healthcare systems.

As another example, sometimes particular drugs work brilliantly in only one specific patient population. We often see this within cancer treatment, with drugs only working if a patient is expressing a certain level of a specific marker. What about someone who has a slightly different biomarker expression? Will they be included in a clinical trial?

There are so many questions that must be considered surrounding collecting data from the patient and the patient's background. The bioinformatics and manufacturing processes need to be capable of addressing that, which can only be achieved by having a robust data engineering and analytics platform.

Q What challenges does the rapid digitalization of biotechnology pose to the wider field of cell therapy?

MN **The biggest challenge is that while the technology is available, many people in the industry are not yet ready to embrace it.** One of the issues is workforce readiness—undergraduate students are familiar with digital tools in their personal lives, but when they enter the manufacturing environment, they are often confronted with paper-based systems. This creates a disconnect and can be frustrating for new employees.

Furthermore, while many undergraduate biochemistry programs provide only a limited exposure to statistics, much of the biopharma industry relies heavily on data analysis. There is a gap in education and training for the workforce, especially when it comes to preparing students for digital tools and data-centric processes in the field and having a quality-first mindset.

Q How can the cell therapy field combat the skilled workforce shortages in this period of digitalization transformation?

MN **Workforce training is essential.** At NIBRT, we focus on providing training that is aligned with the current needs of biopharma. We regularly engage with vendors, manufacturers, and regulators to understand what is expected from developers and ensure our training programs meet those needs.

Ultimately, every single product is different when it comes to cell and gene therapy. This is vastly different from, say, protein-based therapeutics, where the bioprocess steps are almost always the same. Cell therapy processing requires an understanding of the needs from a regulatory perspective.

A locked-down GXP environment will not provide an adequate opportunity to train staff in all the intricacies of clean room experiments. We train in GMP-like environments to create opportunities for trainees to experience troubleshooting first-hand—this helps build confidence and practical knowledge. It's about preparing the next generation to adapt to digital tools and fostering a mindset where they can take ownership of the processes they are learning while being mindful of regulatory expectations.

Q What are you most excited about for 2025 and why?

MN **I am excited about the potential for cell and gene therapy to replace traditional biologics in the coming years.** Rather than taking medications for life, we are moving toward cures. The progress made in therapies that can treat conditions like deafness, for example, is remarkable.

"Cell therapy processing requires an understanding of the needs from a regulatory perspective."

We must move fast, because there are so many patients waiting. At the same time, we must think about what tools can help us the most with digitalization and be bold. As we embrace digitalization, we must also ensure that we can demonstrate to regulators that our products are manufactured in a robust, reproducible way. The ultimate goal is to produce therapies that are not only groundbreaking but also reliable, cost-effective and scalable for widespread use.

BIOGRAPHY

Mohamed Noor is the Digitalization Manager at the National Institute of Bioprocessing Research and Training (NIBRT), where he leads the efforts to help clients to address their needs for digitalization in bioprocessing. He has more than 10 years of experience, including prior roles in Janssen Sciences and Regeneron Ireland focusing on process sciences for large-scale biologics manufacturing. As the Digitalization Manager, Mohamed also works with vendors, non-profit (ISPE Ireland) and consulting companies to create partnerships to leverage their knowledge for industry development. Mohamed is dual-qualified with a PhD in Biochemistry and two degrees in Computer Science/Data Science.

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CELL & GENE THERAPY INSIGHTS

UPSTREAM PROCESSING

Exploring innovations in AAV upstream processing to advance gene therapy development

INTERVIEW

"...my goal is to introduce innovative designs and new concepts to the AAV manufacturing field."

Jokūbas Leikauskas, Editor, *Cell & Gene Therapy Insights*, speaks to **Hao Liu**, Viral Vector Core Research Associate III, Horae Gene Therapy Center, UMass Chan Medical School, about addressing the challenges in viral vector upstream processing with novel AAV manufacturing platforms to boost vector purity, reduce costs, and support the broader mission of advancing gene therapies from research to clinical application.

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Q What are you working on right now? What are the key areas of focus at Horae Gene Therapy Center, UMass Chan Medical School?

HL My primary focus is on producing high-purity, high-titer AAV vectors at a reduced cost. My first work of low-cis triple transfection was published in *Molecular Therapy Methods & Clinical Development* last year [1]. This new method significantly improves vector purity with much reduced plasmid demand. In addition, I also work on AAV capsid engineering and recently published a co-first-authored paper in *Molecular*

Therapy Methods & Clinical Development on AAV2 capsid engineering for improved retinal delivery [2].

Regarding the Horae Gene Therapy Center, it covers everything from A to Z in human gene therapy research and clinical trials. This includes AAV manufacturing, disease modelling, preclinical research, and clinical trials.

Q What are the critical key challenges in the upstream processing of viral vectors, especially AAV?

HL **One of the key challenges is achieving high vector purity.** For example, the purity of AAV produced through triple transfection is suboptimal, often containing various impurities, including residual plasmid DNA (pDNA), host cell genome fragments, and helper plasmids, among others. Additionally, the empty-full capsid ratio at harvest is low, which creates a major challenge for downstream purification and enrichment. As the gene therapy field advances and matures, it is almost inevitable that the US FDA will impose increasingly stringent purity standards for AAV products. When more gene therapy drugs enter the commercial stage and patients with rare diseases have options to choose from, I believe their top priority will be safety. Since purity is directly linked to the safety profile, it will become even more crucial. Beyond purity, there are also significant hurdles associated with scalability, cost, and yield.

Q What approaches are you taking to address the existing challenges in viral vector upstream processing? Are there any technologies that you find particularly promising?

HL **My main goal is to design advanced viral vector manufacturing platforms that introduce novel mechanisms or concepts into AAV production.** For example, AAVPure^{Mfg}, which I presented at the American Society of Gene & Cell Therapy (ASGCT) annual meeting last year, is one example. Furthermore, I will be presenting AAVPCR at the ASGCT annual meeting this year, which represents a next-generation AAV manufacturing platform.

Over the past 30 years, plasmid-based AAV manufacturing systems have required massive amounts of pDNA to produce sufficient materials for AAV production. For instance, reducing the pRep/Cap plasmid amount by tenfold typically results in a tenfold decrease in rAAV titer. AAVPCR platform challenges this concept by introducing an *in cellulo* replication process to selectively amplify the targeted pDNA, such as the Rep/Cap genome. Our current data suggest that the AAVPCR system can amplify the Rep/Cap genome by roughly 200 times, which allows for a 30–100-fold reduction in pRep/Cap and pGOI plasmid usage while still achieving rAAV titers comparable to those of the triple transfection method.

This substantial reduction in plasmid usage significantly facilitates the efficiency of AAV production. Additionally, since AAVPCR alters the production dynamics and underlying mechanism, it significantly increases AAV product purity compared to triple transfection. For example, we found that the empty-full capsid ratio at harvest is improved by

"...introducing this in cellulo plasmid DNA replication process into AAV manufacturing will fundamentally revolutionize viral vector production."

2–10-fold, while plasmid backbone contamination is reduced by 8–20-fold. These improvements, particularly the enhanced full capsid percentage, will greatly facilitate downstream processes such as affinity and anion exchange chromatography, ultimately saving both time and money.

Another key advantage of AAVPCR is its ability to package non-permissive transgenes, which remains a challenge in the current triple transfection system. I believe introducing this *in cellulo* plasmid DNA replication process into AAV manufacturing will fundamentally revolutionize viral vector production.

Q You were recently awarded the Excellence in Research Award by the ASGCT in 2024. Can you tell us more about the AAVPure^{Mfg} method you developed to produce high quantities of rAAV vectors?

HL Over the past decades, researchers have introduced iterations and modifications to the triple transfection method. For example, some have combined pHelper and pRep/Cap to create a two-plasmid system, while others have merged pRep/Cap and pGOI into a single plasmid to develop another dual-plasmid transfection system.

The design of the AAVPure^{Mfg} system is different. It was developed by inserting an inverted terminal repeat (ITR)-flanked transgene cassette into 3′ of the Rep gene. I found that the Rep C-terminus is highly flexible and amenable to peptide insertion. Through a Bxb1-mediated recombination process, we can excise the attP- and attB-flanked transgene cassette, generating a minicircle DNA that contains only the ITR-flanked transgene without any bacterial sequences and a pRep-attR/Cap for initiating AAV production.

With this new design, the plasmid backbone ratio is dramatically decreased by 20–50-fold, setting the lowest level ever recorded in plasmid-based AAV manufacturing. Additionally, before recombination, the transgene cassette stops Cap expression, thus reducing empty capsid formation and increasing the empty-full capsid ratio at harvest by 1.5–3-fold. Through these improvements, AAVPure^{Mfg} overcomes some inherent limitations of triple transfection, setting a new standard for high-purity AAV manufacturing.

Ultimately, my goal is to introduce innovative designs and new concepts to the AAV manufacturing field. I hope that my efforts will enhance purity, improve safety in clinical trials, and reduce costs, making gene therapies more accessible to patients.

Q What are the advantages of transient transfection versus stable producer cell lines for AAV manufacturing?

HL The first major advantage of transient transfection is flexibility and faster turnaround. In contrast, developing a stable producer cell line typically takes several months, given everything goes smoothly. That is a long time, especially for preclinical or Phase 1 studies, which is why researchers often rely on plasmid-based

"The performance of AAVPCR is already very promising but I am aiming to enhance the in cellulo amplification efficiency to further increase rAAV titer and purity and reduce plasmid input."

transient transfection systems at the early stages because they are much faster and more flexible.

Another advantage is that the vector titer achieved through transient transfection is still higher in many cases. Based on current publications, producer cell lines have not yet achieved titers comparable to transient transfection systems—they can be lower by as much as 3–10-fold. Yet at last year's ASGCT meeting, some companies showcased producer cell lines with extremely high titers, although it is unclear how these cell lines were generated or how stable they are.

Q What are your goals and priorities over the next 1–2 years, both for yourself and for Horae Gene Therapy Center as a whole?

HL My main priority is publishing AAVPure^{Mfg} and AAVPCR. The performance of AAVPCR is already very promising, but I am aiming to enhance the *in cellulo* amplification efficiency to further increase rAAV titer and purity and reduce plasmid input. Currently, we can achieve a 100-fold reduction in plasmid usage by AAVPCR. In the future, I think we can potentially achieve several hundred-fold reductions. In theory, as long as a single copy of the plasmid enters the nucleus, it can be amplified to produce a robust AAV titer with high purity. However, achieving this will require further genetic engineering.

Another goal is to develop packaging cell lines or stable producer cell lines that incorporate the AAVPCR genetic components. This would further simplify the system and significantly reduce plasmid usage.

Beyond AAV manufacturing, I am also involved in directed molecular evolution, hoping to develop new capsids or viral vectors that could open new possibilities in human gene therapy.

As for the Horae Gene Therapy Center, the top priorities remain focusing on conducting comprehensive A-to-Z studies, from preclinical research to clinical trials and from AAV manufacturing to disease modelling and animal research.

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BIOGRAPHY

Hao Liu is a molecular virologist specializing in adeno-associated virus (AAV) and adenovirus research. He holds a PhD in Cell and Developmental Biology from the University of Michigan, Ann Arbor, MI, USA, and currently serves as a Viral Vector Core Research Associate III at the UMass Gene Therapy Center. His research is focused on developing innovative AAV manufacturing methods that enhance viral vector purity, titer with reduced costs and engineering novel AAV capsids with specific tissue tropism. Dr Liu is passionate about leveraging technical innovations to make gene therapy medicines more accessible and effective for patients.

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CELL & GENE THERAPY INSIGHTS

UPSTREAM PROCESSING

Key considerations for scalable retroviral vector production—with a focus on lentivirus

INTERVIEW

"Evaluation should always start with estimating your commercial requirements in terms of viral vector quantities."

Viral vectors based on viruses from the retrovirus family are a common choice for gene therapy applications due to their ability to transduce dividing and non-dividing cells. They are usually produced in mammalian cells via transient transfection or stable expression and naturally 'bud from cells, offering opportunities for perfusion harvest and continuous manufacturing.

In this interview, **Alex Chatel**, Senior Product Manager, Donaldson Life Sciences, discusses the benefits and drawbacks of different approaches when producing vectors for cell therapy applications, and outlines important considerations for developers when selecting a suitable production process.

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Q What are you currently working on?

A I'm currently focused on advancing both process and product development within Donaldson's Life Sciences businesses, particularly around our advanced manufacturing technologies. A key part of my work involves supporting

emerging therapeutic modalities like viral vectors and nucleic acids. I also oversee the development and use of advanced cost of goods modeling capacities for bioprocessing. My background is in chemical and biochemical engineering, and I've held a mix of technical and commercial roles across academia and industry, which gives me a broad perspective on how to bring innovative technologies to market.

Q What unique benefits—and drawbacks—do retroviruses offer for cell and gene therapy applications?

AC Two members of the retrovirus family are of particular interest for advanced therapies: lentiviruses (LVs) and to a lesser extent, gamma retroviruses (gRVs). Retroviruses are mainly known and used for their ability to transduce dividing and non-dividing cells, which is particularly useful in genetically-modified cell therapies, such as chimeric-antigen receptor T cell therapies (CAR-T), sometimes referred to as *ex vivo* gene therapies (GTs).

As of today, ten market-approved *ex vivo* gene therapies use retroviruses—eight of which use lentiviruses, and two use gamma-retroviruses. They are also currently investigated in hundreds of clinical trials. It's important to note that although the market-approved retrovirus therapies are all *ex vivo* therapies, there's a growing interest in using retroviruses, mainly LV in this case, for *in vivo* therapeutics. One recent example is the platform developed by EsoBiotec, a Belgium-based company developing a therapy using lentiviruses for *in vivo* use, that was recently acquired by AstraZeneca.

When it comes to drawbacks, LVs are originally derived from HIV, so there is a level of biosafety quality control that needs to be ensured. The LV used in therapies is replication-deficient, but this needs to be tested with every batch and justifies extensive quality control. On the other hand, gRVs pose an inherent higher risk of insertional mutagenesis, which has been linked to an increased chance of leukemia in clinical trials in the early 2000s. For this reason, LVs are now generally favored.

Beyond safety, a key challenge with these viruses is simply that they are hard to manufacture, especially in a cost-effective manner. This means gene therapy drug products are typically associated with high price tags, which impact patient accessibility and in some cases even the commercial viability of these life-saving medicines.

Q Could you frame for us the current challenges in the production of LV and gRV vectors for applications in cell therapy, as you see them?

AC It's important to remember that in almost all cases LVs and gRVs are produced in mammalian cells via either transient transfection, or sometimes stable production in more modern processes. One of the challenges of producing these vectors in a cost-effective manner is that depending on the target indication, the quantity of viral vector required can vary up to a million times from relatively low-dose *ex vivo* gene therapies to high dose *in vivo* therapies, with an additional impact of disease prevalence and patient population size.

"Two members of the retrovirus family are of particular interest for advanced therapies: lentiviruses (LVs) and to a lesser extent, gamma retroviruses (gRVs)."

This breadth makes it challenging to produce retroviruses cost-effectively with standard manufacturing technologies. It means that there is not simply a one-size-fits-all system for manufacturing, and flexibility is needed. For instance, an *ex vivo* GT or an *in vivo* GT for ultra-rare disease may be cost-effectively produced with a bench-top production system, while on the other end of the spectrum, a highly automated, industrialized-size manufacturing platform will be needed for large indication *in vivo* gene therapies.

In a typical process, the cells need to be grown to a target density upon which production will start. If using transfection, the most common method today, this will be initiated with the addition of plasmid DNA and a transfection agent. This is a delicate step as the transfection mix is both time and shear-sensitive, and can lead to cell toxicity. Stable producer cell lines don't require this step because they already possess the inherent genetic information to produce the virus, but it takes time to develop a robust cell line. As of today the titers obtained from producer cells are still lower than in transfection, which means that this option hasn't yet been widely adopted—although I anticipate it will be in the future as technology improves.

A key consideration for both LV and gRV is the use of serum as a cell culture supplement. By definition, serum is chemically undefined. It's an animal-sourced reagent, and requires complex procurement and quality control to use. When the vectors are used as an ingredient to cell transduction, such as for CAR-T production, the purity requirements aren't as stringent as for direct injection. However, as therapies evolve and move into more *in vivo* uses, there will be increased emphasis and scrutiny on process-related impurities. Cost and procurement considerations aside, this is likely to keep pushing developers away from using serum, and the use of suspension allows this in some instances.

Finally, another important challenge is identifying the appropriate manufacturing platform. Developers will usually start with developing processes at the lab scale using adherent cells because this process is simpler to adopt at the small scale. Upon successful transition to the next stage, manufacturing will need to be considered with commercial needs in mind. Typically, the decision developers will need to make is whether to stay with an adherent system or to move to suspension if the target production scale justifies it.

Q Can you expand on the relative pros and cons of adherent versus suspension cell culture approaches?

AC For suspension-adapted cells, stirred-tank bioreactors (STRs) have long been the gold standard. They have been used for decades and have a proven ability to meet the high-demand requirements for many biologics—starting with antibodies and now moving into the field of viral vector production. They scale well and they offer flexible capacity for many applications. They also provide the ability to produce in serum-free conditions, but there are cases for which their basic design can be a hurdle.

For example, for high cell density perfusion cultures, which is a growing trend in viral vector production, the set up can be complex to run and also requires a cell retention device, which is a somewhat difficult process to both develop and operate. Another hurdle is the

simple fact that at large scale, you will be producing a very large volume of harvest material of relatively dilute product, which is also highly heterogeneous in nature and will contain cells, often a lot of cell debris, host cell impurities such as DNA and proteins, and the product. These components need to be removed, which is both time-consuming and costly, and can lead to reduced downstream processing efficiency and yield. Finally, STRs run by agitating cells in liquid medium and as such can generate high hydrodynamic shear, especially at the impeller tip. Cells and product are constantly exposed to this because they are freely suspended and in contact with these impellers. This might not always be an issue depending on application, but LVs and gRVs are notoriously shear-sensitive, and some cell types are too, so this can result in product loss. Bubble damage linked to aggressive oxygen sparging can also be a recurring challenge.

As for adherent cell lines, there are two broad categories of manufacturing technologies. We have traditional flatware, such as multi-layer culture dishes or single-layer culture dishes at the lab scale, or we have what we call fixed-bed reactors. Traditional flatware is perfectly suitable for R&D and is widely used across labs and sometimes also for very small-scale production. However, it doesn't offer the same level of process control as a bioreactor would. Additionally, flatware approaches can scale out, but not up, which leads to unsustainable costs for commercial production.

Fixed-bed bioreactors on the other hand, provide a controlled environment in the same way that a STR does, with pH, temperature, and DO control, as well as GMP-compatible data recording and access control to monitor the batch. They also can be used all the way from lab to commercial scale.

Q How can structured fixed-bed bioreactors help to address these issues?

AC First generation fixed-bed bioreactors based on randomly packed matrix have helped address some limitations of adherent cell-based production, but face scalability and reproducibility issues due to variable compaction levels and lack of intermediate scale options. Next-gen fixed-bed reactors, such as the scale-X™ platform from Univercells Technologies (a Donaldson Life Sciences business), have been developed as a result of these limitations.

The scale-X bioreactor can be more accurately described as a structured fixed-bed. Indeed, the surface provided for cell growth is homogeneous throughout the reactor, and the packing density of the material that is designed for cell anchorage is the same throughout the whole vessel. This provides predictable and consistent cell growth, and therefore production of the viral vector.

A structured fixed-bed reactor is characterized by its surface area for cell growth, the same way flatware would be. This can also be equated to the volume of an STR. The smallest scale-X bioreactor, the scale-X nexo, is 0.5 m^2 which is roughly equivalent to a 1 L STR. For mid-scale production the scale-X carbo comes both in 10 and 30 m^2 and can produce the equivalent of up to a 200 L STR. At the top end of the scale we have the scale-X nitro, which is either 200 or 600 m^2 and can yield throughput equivalent to 2,000 L or more.

There are a number of advantages to choosing a structured fixed-bed platform. Firstly, the cells are retained within the bed itself. This means that processes which could benefit from perfusion or intermittent harvest—such as for retroviruses which have a biological

"The LV used in therapies is replication-deficient, but this needs to be tested with every batch and justifies extensive quality control."

capacity to 'bud' from cells—don't require an external cell retention device, unlike with an STR. You can perform intermittent harvest, which opens process design possibilities such as the ability to collect fractions of the harvest at fixed time points along the process. You also have opportunities to collect them in a way that protects the virus, such as to cool them right after harvest, for instance, to maintain product integrity.

Secondly, as the cells are protected within the fixed-bed, they are also protected from shear linked to impeller and sparging, which helps support high viabilities, and, typically, increased specific viral productivities compared to alternative technologies.

Finally, thanks to the structured fixed-bed design, reproducible cell entrapment, growth and productivities can be achieved linearly throughout scale-up, from 0.5 m^2 to 600 m^2. This is a major advantage for process development and scale-up, especially in a time- and cost-constrained setting.

Q ...and what about the impact on downstream processing?

AC The ability to easily produce in a perfusion setup offers advance for further processing, as the product can be directly stored in more appropriate conditions—for example at lower temperature. This helps improve titers and product quality, facilitating downstream operations.

Interestingly, it has also been shown that a fixed-bed approach reduces contaminants such as debris, proteins and host cell DNA in the harvest. This is the result of a simple physical effect wherein the fixed bed acts a bit like a filter. Cells will anchor and attach as they grow, but even as they lyse a large proportion of the contaminants remain trapped within the fixed bed. The harvest collected will therefore be cleaner when compared to an STR, where all of the debris and impurities are resuspended along with the product. This results in a significant improvement in the downstream processability of the broth.

The filter area needed to clarify the broth prior to the capture step will be smaller, and also the efficiency of the following chromatographic steps, if used, will be enhanced thanks to the lower contaminant burden.

Q What advice would you give to developers looking to evaluate which production process will be most suitable for their own application?

AC Evaluation should always start with estimating your commercial requirements in terms of viral vector quantities. This will define the scale at which production will need to take place once the process is developed and safety and efficacy has been demonstrated at the clinical stage. Next, there are a number of strategic considerations to carefully evaluate, including in-house production vs outsourcing to a CMDO, the type of expression system (transient transfection versus stable producer), the size and breadth of the drug pipeline; and linked to that the eventual choice to go for a platform approach.

Manufacturing considerations, including scale-up strategy, should be assessed as early as possible, as this will have a significant impact on development speed and on the final manufacturing cost of goods. In turn this will impact the final return on investment and margin of producing the drug product. A poorly designed process can significantly impact production costs and could even delay market entry.

The flexibility of your manufacturing solution should also be considered. For example, the scale-X carbo enables the production of manufacturing batches releasable under GMP guidelines using a compact benchtop system. This is cost-effective as it contributes to reducing large capital and operating expenses or commitments during the clinical phase, and can delay them to a stage where the risk of failure is lower. Avoiding spending too much upfront, and instead only doing so when your chances of success have increased, is an efficient use of your capital.

Particularly for retrovirus products, the ability to increase product yields by implementing semi or continuous product harvest strategies by design, without a cell retention device, is highly cost-effective. Even above the cost of plasmid DNA or transfection reagents, the most effective way to reduce the cost of goods per dose is to increase the total amount of product that can be produced from the reactor. The higher your productivity, the more significant the impact on reducing cost.

To conclude, it all comes down to scalability, speed, and cost. Choosing a manufacturing platform that supports low-footprint, low-cost production at high yields while supporting rapid scale-up can be instrumental in achieving commercial success and getting these life-saving therapies to those in need. At Donaldson Life Sciences, our Univercells Technologies business is committed to making this a reality.

BIOGRAPHY

Alex Chatel is a Senior Product Manager at Donaldson Life Sciences, based in Lyon, France, where he leads product initiatives in viral vectors, nucleic acids, and vaccines. He also supports Univercells Technologies, a Donaldson Life Sciences business located in Brussels, Belgium, where Alex developed and launched the scale-X technology prior to his current role He has also held positions as an Enterprise Fellow in technology transfer at University College London, London, UK and as a Research Engineer at GlaxoSmithKline in Stevenage, UK. Alex holds an EngD in Biochemical Engineering from University College London and an MEng in Chemical Engineering from The University of Manchester, Manchester, UK.

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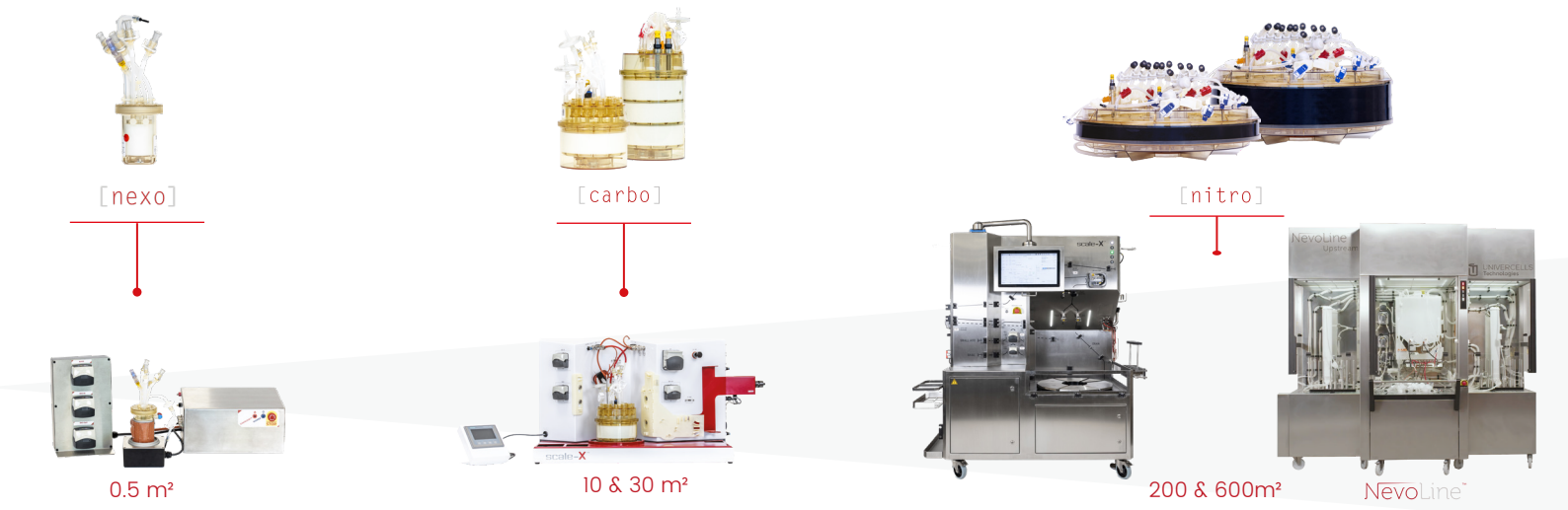

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CELL & GENE THERAPY INSIGHTS

UPSTREAM PROCESSING

Optimizing upstream processing to improve consistency and scalability in viral vector production

INTERVIEW

"It is exciting to build something from an early stage...and getting to enjoy the results of that work."

Jokūbas Leikauskas, Editor, BioInsights, speaks to **Katerina Rigaki**, Upstream Manager PD Vector, Autolus Therapeutics, about overcoming the technical and logistical challenges in cell and gene therapy (CGT) manufacturing, particularly around transient transfection, raw material variability, and scalability in adherent and suspension systems.

Cell & Gene Therapy Insights 2025; 11(4), 505–510 · DOI: 10.18609/cgti.2025.060

Q What are you working on right now, and what are the key focus areas at Autolus Therapeutics?

KR Currently, the main priority of the Vector Process Development team at Autolus Therapeutics is strengthening our upstream capabilities and gaining a better understanding of the critical process parameters. The goal is to improve the reproducibility of our development runs as the process scales, in order to evaluate the impact of factors such as biological starting material variability and to efficiently perform process optimization.

We emphasize the acquisition and analysis of high-quality data, as they form the basis of improving our process capabilities. They guide both immediate actions and long-term strategic planning for our research and product pipeline.

Q In the context of CGT manufacturing, what are the key challenges associated with upstream viral vector production?

KR The most challenging part is the narrow optimization window for transient transfection. Most viral vector-producing companies use transient transfection of the host cell line with a set of plasmids and a transfection reagent. Typically, producer cell lines are not used due to the longer development timeline. Transient transfection is linked to incomplete viral packaging, which also impacts downstream processing and necessitates further analytical development. The narrow optimization window becomes even more challenging during the scaling up of the process. For instance, the complexation kinetics at larger scales are different, and the mixing and interaction of complexes with cells can be affected by a multitude of physical and biological parameters in different bioreactor systems. These lead to suboptimal transfection efficiency and productivity compared to the development scale.

Other key challenges include raw material variability and cost, especially because most developers do not use producer cell lines, meaning they need large quantities of plasmids and transfection reagents, which contribute the most to the Cost of Goods per batch.

In the case of adherent cell lines, serum is often utilized, which introduces a lot of variability and can, in turn, lead to inconsistencies and even false positives during development. It becomes tricky when developers must make decisions based on fine-tuning or small input differences. For instance, chemically defined media is an option mainly for suspension cell culture, but less so for adherent platforms. However, designing or optimizing new media requires time to integrate into upstream development timelines, and not everyone has the luxury to do that. While some suppliers provide off-the-shelf or customized media options, serum is typically added separately, and that adds to the complexity.

Another difficulty with adherent cell lines is the lack of reliable, scale-down models. Most adherent cell lines are initially developed in 2D flatware and then either scaled out (by increasing the number of flasks) or scaled up (using a fixed-bed bioreactor with a completely different hydrodynamic environment). This means parameters optimized at laboratory scale might need to be revalidated or re-optimized once you move to a fixed-bed bioreactor. Scaled-down models for fixed-bed bioreactors, especially for adherent cells, are quite limited.

Another major hurdle is the absence of real-time or continuous process analytical technology tools. While tools exist to monitor cell growth and culture health, such as biomass sensors or Raman probes, they do not give real-time insights into productivity. Analytics are difficult because the upstream harvest materials are notoriously low in purity. Tools such as qPCR, ELISA, or infectious titer testing are more reliable after clarification and do not always allow for quick turnaround of results. However, this delay means developers do not immediately see the impact of upstream changes or understand how they interact with other process variables throughout the run.

Lastly, there are other challenges that depend on whether the system is adherent or suspension-based, and whether the product is extracellular or intracellular. These differences

"Generating a fully characterized stable producer cell line is both resource-intensive and time-consuming..."

also bring varying levels of impurities, which must be addressed alongside downstream processing and analytics. It requires a coordinated effort across different departments.

Q How can these hurdles be addressed? What strategies or innovations could help streamline upstream processing?

KR Instead of relying on transient transfection that requires a lot of optimization, there have been efforts around developing integrated cell lines that stably produce the genes of interest along with the necessary viral and accessory proteins. This concept is largely inspired by traditional biologics such as monoclonal antibodies, where stable producer cell lines are the standard approach.

However, in early development, when flexibility and quick turnaround are essential, you cannot afford to dedicate three to twelve months to generating a stable cell line while still optimizing other aspects of the process, such as plasmid constructs or transfection reagents. Generating a fully characterized stable producer cell line is both resource-intensive and time-consuming, taking at least six months in most cases. The only practical alternative at this stage is to invest time in thoroughly characterizing the kinetics of any host cell line, assessing the transfection complex formation step and uptake by the cell culture step.

In order to better manage the limitations of transient transfection, improved analytics are key. Specifically, assessing the molecular size of complexes formed through the mixing of DNA and transfection reagents could help, but more research is needed. There is some support from suppliers of transfection reagents that offer general guidelines, but much still depends on internal research within each developer group. Techniques such as dynamic light scattering and other technologies that can characterize these complexes at the molecular level are important for optimizing transfection outcomes.

Q What are the key bottlenecks with starting and raw materials, and how can they be addressed?

KR The experience of working in the tech transfer function within CDMOs since the early days of my career gave me a solid understanding of the gaps developers often encounter and how important it is to maintain consistency in materials from process development through to manufacturing. Without that consistency, any performance differences can be attributed to material variability. Of course, maintaining that consistency is not always possible due to tight lead times and the pressure to initiate development projects quickly in one's product lifecycle. Ideally, all critical materials, such as the host cell line, plasmids, and culture media, should come from the same supplier, produced at the same manufacturing site using the same raw materials.

"...having a strong supply chain, planning ahead, and having a plan B for every critical material in the process is fundamental."

Regarding host cell lines, most companies rely on off-the-shelf options, which often come with licensing fees. In many cases, they still need to be adapted to internal conditions and have their growth kinetics studied, which is a time-consuming process. Developers might end up using a non-optimized or fully characterized host cell line for a highly sensitive process, just to get results faster and support speed to market.

Furthermore, there is the issue of lot-to-lot variability in plasmids. While high-quality GMP-grade plasmids are available, they typically have long lead times of around six months. One alternative is shifting from biologic to synthetic plasmids, which offer shorter lead times. However, they come with their own set of impurities, which then need to be incorporated into the purification strategy and evaluated for further impact.

There is also variability in culture media, particularly due to supply disruptions. In the UK specifically, Brexit has played a role here, and it has become clear that we now need to plan much further in advance than previously. Delays, especially for materials coming from Europe, have become more common. Therefore, having a strong supply chain, planning ahead, and having a plan B for every critical material in the process is fundamental.

Q How does the use of suspension versus adherent cell cultures impact upstream processes for viral vector production?

KR **There are commercially viable strategies for both adherent-based processes and suspension-based ones.** The main difference comes down to infrastructure and scalability. Adherent processes require a scale-out approach, meaning you end up needing significantly more space for incubators, biological safety cabinets, and a different kind of working environment. In contrast, suspension systems operate in stirred-tank bioreactors, which are closed systems, so you do not necessarily require a Grade A cleanroom classification; a Grade B or C environment is often sufficient.

Another key aspect is that most traditional biologics companies that have transitioned into vector production already have in-house expertise in mass transfer and scale-up parameters relevant to suspension systems. On the other hand, with adherent cell lines, there is much less historical reference and established know-how, particularly in the context of viral vectors, especially when moving to fixed-bed bioreactors instead of scaling out using flasks. While suppliers do offer some support, it often means starting from scratch to understand your process, which takes more time.

Flexibility is another factor. Adherent systems are generally more labor-intensive. Although they can offer higher efficiencies early on, since the cells grow in a monolayer with direct contact with the media and typically achieve higher cell densities than suspension systems, they also require more manual handling. The use of serum provides a rich environment, which supports cell growth, but that does not always translate to higher productivity at later stages. When scaling out, these systems demand a lot more people to operate the process.

Suspension systems, on the other hand, offer greater consistency and scalability. There are also more commercially available automation systems for suspension culture. However,

one advantage of adherent cultures is that they yield cleaner harvests. Because the cells are attached to surfaces, especially in fixed-bed bioreactors, where you have a packing material, the outlet stream is a lot cleaner. This results in lighter clarification needs and can reduce the number and complexity of downstream unit operations, ultimately affecting the footprint needed in a cleanroom.

Overall, the differences impact facility design, labor requirements, and how you operate the process. There are unique challenges with each approach, particularly around scalability and workflow planning. Lastly, transfection efficiency remains an issue in both suspension and adherent systems.

Q What are your goals and priorities over the next 1–2 years, both for yourself and for Autolus Therapeutics as a whole?

KR I want to keep growing professionally by deepening my knowledge and experience, particularly in lentiviral vector production. It is exciting to build something from an early stage, understanding the challenges that come with it, and getting to enjoy the results of that work.

One of the things I appreciate about working at Autolus is the opportunity to develop that understanding while also seeing the direct impact our work has on patients' lives. It is meaningful and fulfilling to work on something that truly makes a difference.

With CAR-T cell therapy programs like Obe-cel, Autolus aims to strengthen upstream capabilities and take a holistic approach to what we supply. Building a strong vector team is part of that vision, and I am glad to be part of it.

BIOGRAPHY

Katerina Rigaki is Vector Process Development Manager at Autolus, where she leads the design and scale-up of upstream processes for viral vector manufacturing. With over a decade of experience in bioprocessing and MSAT across companies including Lonza, MeiraGTx, and Ascend Advanced Therapies, she specialises in technology transfer, GMP readiness, and data-driven process optimisation for advanced therapy medicinal products.

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Essential manufacturing scale-up considerations for cell and gene therapies

Demand for cell and gene therapy treatments continues to outstrip supply, with only a small portion of eligible patients receiving cell and gene therapeutic treatment, such as CAR-T cell cancer therapy — in part, due to limited manufacturing capacity and high costs. Scaling up both manufacturing programs and processes of gene and gene-modified cell therapies in preparation for IND filing presents significant challenges, from transitioning through discovery and clinical phases to achieving GMP-compliant commercialization. Open systems, manual processes, and contamination risks often complicate the process, making early consideration of scalability essential to avoiding delays and costly adjustments. This infographic provides expert tips and key considerations for developing a scale-up or scale-out strategy in cell and gene therapy manufacturing, to bring these transformative therapies to more patients in need.

KEY CONSIDERATIONS WHEN PLANNING YOUR PROGRAM AND PROCESSES

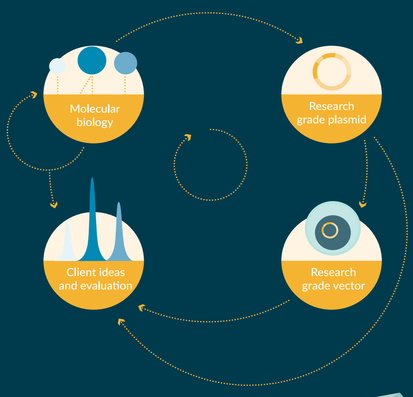
1 Set out your target product profile

Start with the product, not the process. Know your target population, intended use, and critical safety and efficacy characteristics.

Candidate selection

- Identify your lead candidate early, but know that development work can happen at any stage—it's not as simple as "consolidate it as soon as possible."
- Research-grade feedback loops can be used to narrow down the candidate selection.

Research-grade feedback loops



Source: Charles River

2 Plan ahead, stay flexible but prepare to fail

Keep the end product in mind, but know that program milestones can ebb and flow

Process development

Allow for unknowns and have contingency plans in place for unexpected events.

Plan ahead for scale-up based on existing platforms and data, but keep in mind that individual products can behave differently, even within standardized platforms, so plans may need to evolve.

Balance long-term and short-term objectives but don't fall into the trap of characterizing endlessly.

"Anything that can go wrong, will go wrong."

Edward Murphy Jr.

3 Optimize genetic engineering

There are a number of pitfalls and potentials to consider when selecting a genetic engineering platform. Process optimization is key.

Newer technologies, such as CRISPR, have the potential to improve gene delivery and reduce manufacturing costs.

4 Regulatory considerations

Product characterization

With specific regulatory guidance for the cell and gene therapy field still evolving, the twin imperatives to 'start early' in development on product characterization and embrace novel, fit-for-purpose analytical technologies continue to grow in importance.

Starting materials

Leveraging research-grade materials focused on safety, efficacy and ease-of-use from the early stages is also critical to ensuring efficient progress through development, and alleviating CMC-related issues at the approval and commercialization stages.

Manufacturing services

Charles River offers cell therapy manufacturing services (as well as AAV, Adenovirus, Lentivirus and Retrovirus gene therapy manufacturing) for various autologous and allogeneic cell types and starting materials, including marrow-infiltrating lymphocytes (MILs), dendritic cells (DCs), natural killer (NK) cells, T cells, CAR-T, bone marrow-derived mesenchymal stem cells (BMSCs), mesenchymal stem cells (MSCs), whole blood, apheresis, leukapheresis, tumor isolates, and stem cells.

5 Choose your system and processes wisely

Look for utility and overall benefit, rather than technical specifications, and consider the entire process rather than focusing on a single step.

A question of scale

A larger-scale 3L system can generate material for evaluation in downstream processing, or be used for assay development. Smaller-scale systems may only provide enough material for upstream evaluation.

Be sure to choose a trusted CDMO partner that can work at the most effective scale for you.

Advantages of platform production

6

Streamline development

Expedite timelines

Standardize processes

Reduce space and labor requirements

Reduce cost

Platform products

Charles River offers a range of platform products throughout the development and manufacturing timeline offering:

Expedited development and 100% in-house analytics.

- Partnering with a trusted CDMO with fully integrated testing.
- Cost-effective, reliable path to GMP and clinic.

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nAAVigation®

AAV production.

Cell Therapy Flex Platform

7 Robust QC and CMC

When it comes to selecting the right analytical tools with which to characterize your product, key considerations include cost, time to result, sensitivity, flexibility, and the acceptability of the assay by regulatory authorities.

The following are questions to ask of any candidate analytical tool under evaluation:

"Work the problem."

Gene Kranz

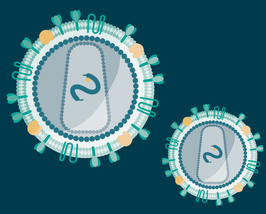
? Has the assay been used with clinical-stage or approved advanced therapies before?

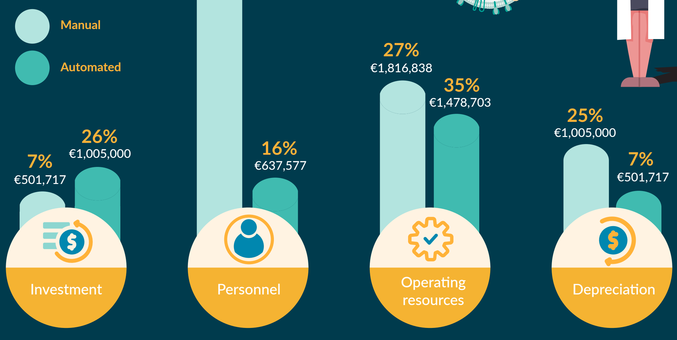
? How easy will it be to validate against compendial methods?

? Is it 'QC-friendly' in terms of the likely future quality control requirements of your particular cell or gene therapy product at clinical and commercial scales?

8 Automate for scale out and scale up

Process automation for scale-up and scale-out reduces costs, improves robustness of the program and enables the power of in-line analytics and process decisions in real time.





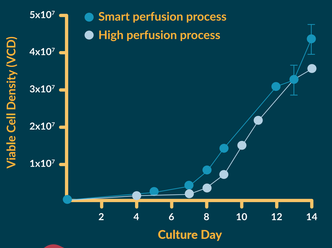
Source: Nießing B, Kiessel R, Herbst L, Schmitt RH. Source: Techno-economic analysis of automated iPSC production. *Processes* 2021, 9(2); 240. DOI: 10.3390/pr9020240.

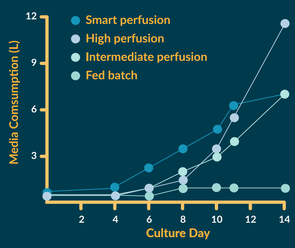
9 Harness AI

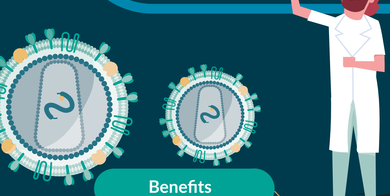
AI and machine learning continue to push the boundaries of process automation, especially for research and discovery data.

Smart perfusion

"Smart perfusion" uses machine learning to determine the right amount of media for T cell density, with a 50% reduction in media consumption.







10 Don't neglect fill/finish

Fill/finish is too often considered a side note and not addressed early in the process.

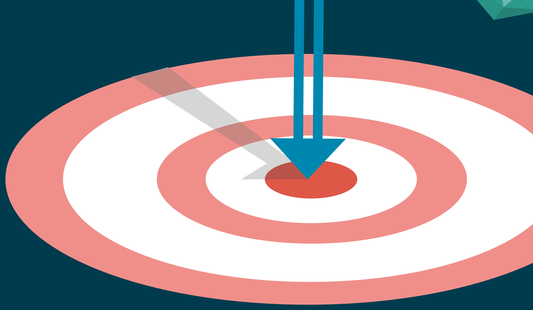
Benefits

- Closing downstream processes for large and small scale can reduce labor, space, and COGs.
- Laboratory information management systems (LIMS) can be used for high-throughput labeling and tracking.

Closed manufacturing processes

Limitations

- Open formulation.
- Fill/finish steps.



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Quickly and accurately measure key analytical attributes of large vectors with the KaritroMP

Maria Jacintha Victoria, Market Development Manager, CGT, Refeyn Ltd.

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Traditional methods for the characterization of large viral vectors often require substantial time and specialized scientific expertise to produce consistent results. This poster introduces a novel instrument designed to address these challenges—offering rapid, cost-effective, and user-friendly measurement of critical quality attributes in large vectors, such as adenovirus, using minimal sample volumes.

HOW DOES MACRO MASS PHOTOMETRY WORK?

Mass photometry (MP) measures the mass of individual molecules by detecting light scattering as they land on a glass surface, enabling label-free, real-time analysis in solution. In comparison to MP, macro mass photometry (MMP) introduces a vertical sweep, which is used to determine particle size, in addition to the mass measurement seen with traditional MP (Figure 1). MMP characterizes samples by measuring both contrast (a proxy for particle mass) and size (particle diameter).

ESTIMATING AdV EMPTY/FULL

The KaritroMP enables characterization of diverse adenovirus (AdV) populations including empty, full, and fragmented capsids and protein aggregates. Empty and full capsids are the same size, but are resolvable based on mass (two contrast peaks).

To assess the accuracy of KaritroMP in estimating AdV empty/full ratio across the range, pure empty and full AdV samples were mixed in different ratios, from 0% to 100% full, and measured with the KaritroMP alongside other orthogonal methods. The results of the method accuracies are presented in Table 1. All KaritroMP datapoints were within 10% of the theoretical value and the best correlation between the % full theoretical and measured was observed when using MMP.

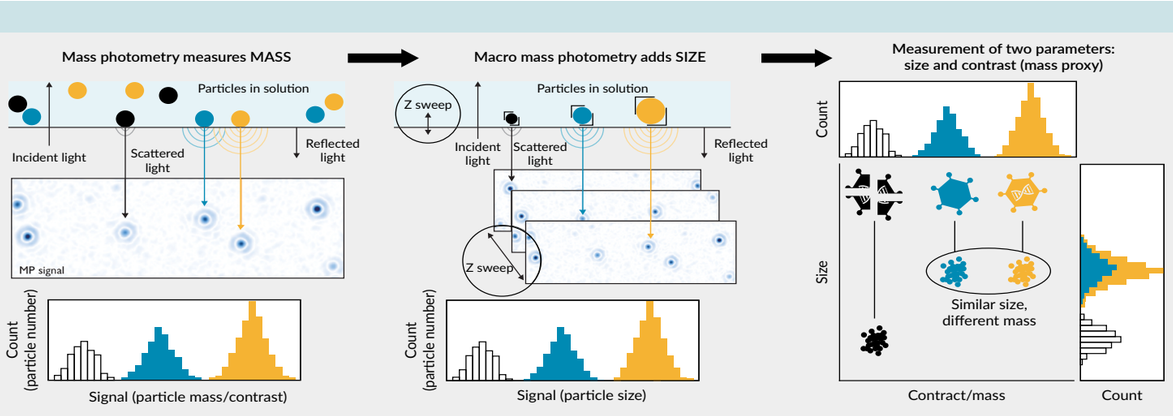
PROCESS MONITORING

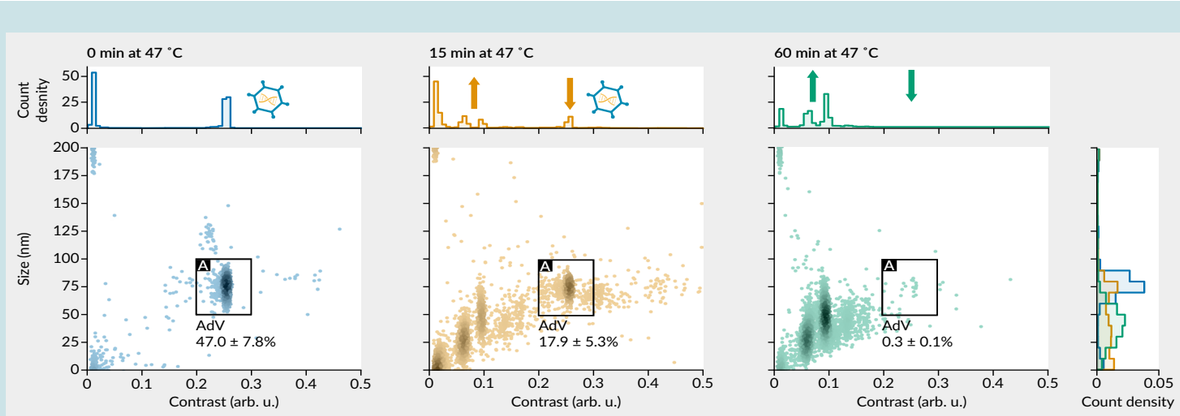
The KaritroMP was tested for its suitability as a process analytical tool for in-process monitoring. Using minimal sample and with high population resolution, KaritroMP was shown to be able to monitor enrichment of the desired population (full AdV) throughout production processes (Figure 2).

STABILITY TESTING

The KaritroMP was also identified as a suitable tool for determining AdV stability. In the stability assessment shown in Figure 3, samples were heated to 47°C in a thermal degradation study. As the intact population dropped from 47% (0 min) to 18% (15 min) and 0% (60 min), populations of fragments grew. The KaritroMP was able to monitor population changes, reliably differentiating intact from broken viral particles in a single measurement.

The KaritroMP is also easy to use and learn, with an intuitive workflow allowing automated data acquisition for up to 14 samples, and new technician proficiency in less than half a day.





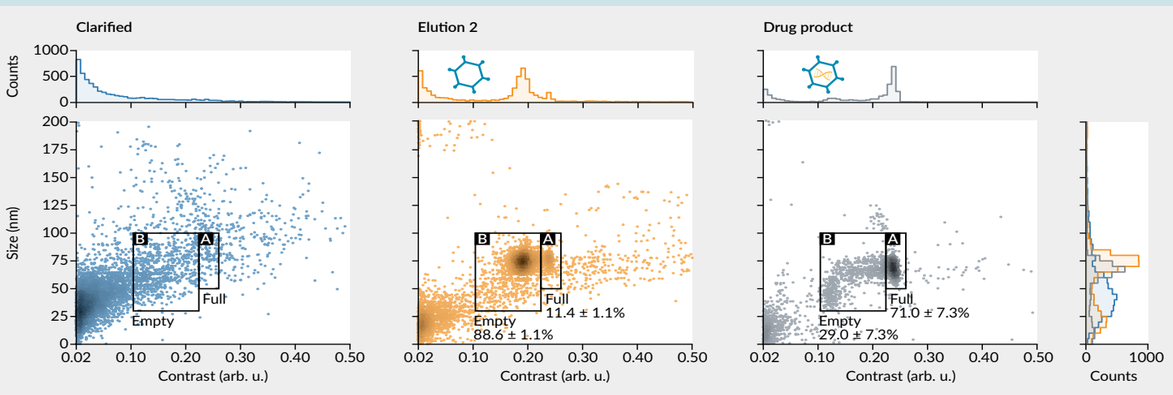


Table 1. Correlation between the % full theoretical and measured values for AdV empty/full ratio.

Method	R^2 value
MMP	0.9985
AUC	0.9861
nsEM	0.9277
A260:A280	0.8373
AEX-HPLC	0.8663

AEX-HPLC: anion exchange high-performance liquid chromatography, AUC: analytical ultracentrifugation, MMP: macro mass photometry, nsEM: negative-staining electron microscopy.

Figure 1. Top left. Measurement functionality of MP versus MMP. MP: mass photometry, MMP: macro mass photometry.

Figure 2. Bottom left. The KaritroMP can monitor purity across AdV production. AdV: adenovirus.

Figure 3. Top right. Stability determination of AdV on the KaritroMP. AdV: adenovirus.