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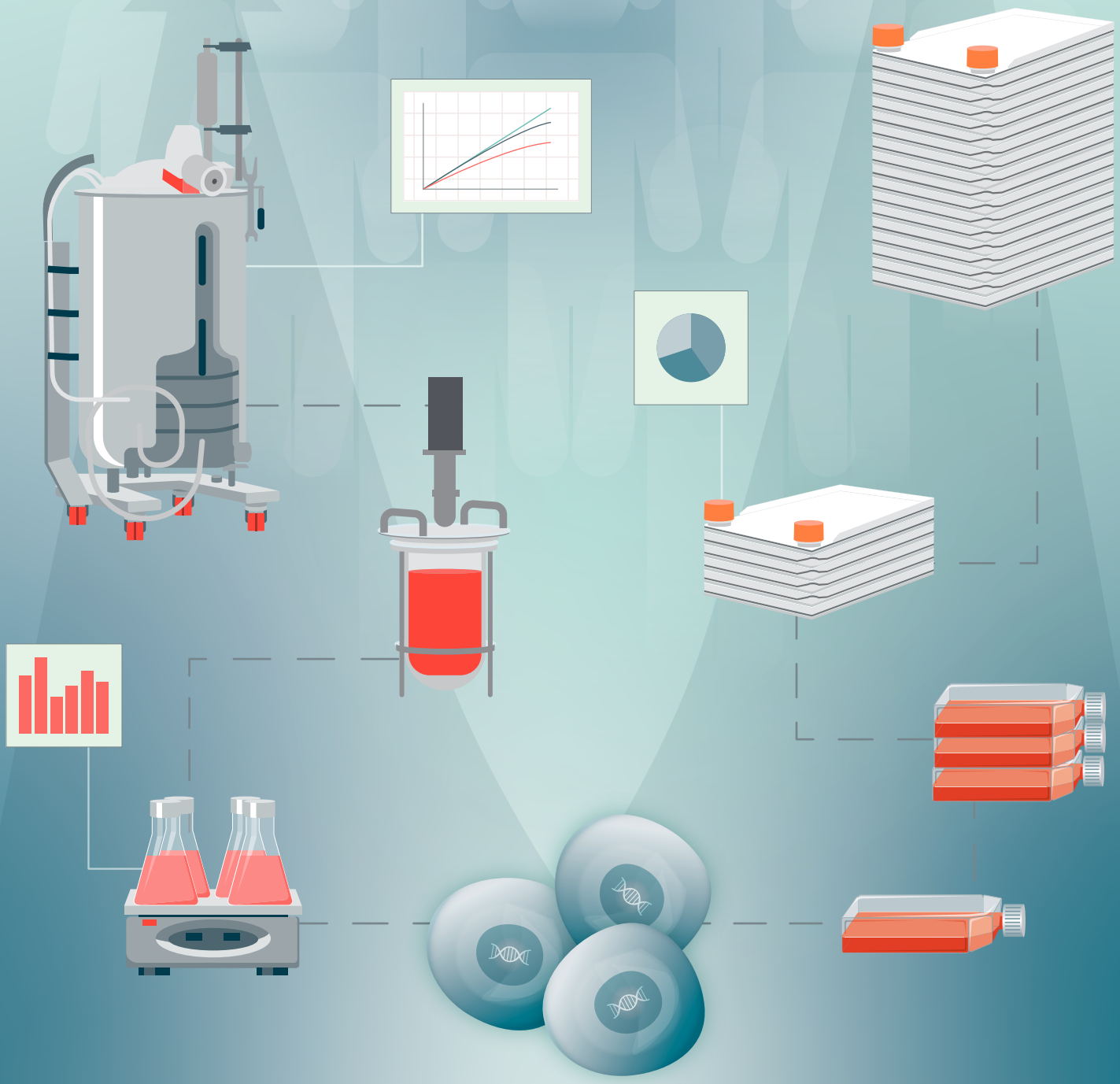
CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON
Scale-up/scale-out of cell and gene therapy manufacturing



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The cell therapy industrial revolution: current successes, existing challenges, and expansion into new domains

Joel Gaston and Matthew Li

Cell therapies continue to demonstrate their clinical success in the oncologic realm. The field has developed significantly from the initial hematopoietic stem cell transplants over a half century ago through the curative potential of single treatment CAR-T therapies, to the most recent regulatory approvals for the first CRISPR gene-edited hematopoietic stem cell (Casgevy®) and first tumor infiltrating lymphocyte therapy for solid tumors (Amtagvi™). The expedited success of these relatively new modalities validates that industrialization is still lagging. Widespread platform production methods are challenging due to the differing biological, scale, and platform requirements that are highly dependent on cell type and disease indication. Addressing these challenges will enable developers to better focus on reaching patients, rather than continually re-establishing new manufacturing paradigms. Continued forward progress will ensure that the field will enter an even more productive phase within oncology and beyond to areas such as autoimmune disease once the current field and market challenges abate.

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PROMISE OF CELL THERAPY

Cell therapies utilize autologous or allogeneic cells as either topical or injectable treatments to address patient populations underserved by current modalities. These therapies include those designed to eliminate pathogenic cells (i.e., CAR-Ts) while others aim to replace cells that have been lost due to innate pathology and/or as collateral to toxic therapeutic

agents (i.e., hematopoietic stem cell [HSC] transplants or dopaminergic neurons). To date, the US FDA has approved 22 cell therapy products (Table 1) [1]. Recent successes of CAR-Ts and HSCs in the treatment of various hematologic cancers have been hard-won, yet hundreds of cell therapy clinical trials have yielded lackluster results, and patient response to proven therapies has not been as robust as expected [2].

Cell therapies offer several advantages over traditional small molecules and biologics:

1. A dynamic drug that can sense, respond to, and influence their surrounding environment;
2. The ability to localize to diseased tissues and minimize off-tissue/target toxicity [3,4];
3. Self-renewing cells that can sustain a durable response [5];
4. The ability for single to low doses needed which can support needs of the patients, insurers, and medical centers.

However, as we delve into the following sections, numerous challenges have been encountered and identified that need to be addressed prior to cell therapies cementing themselves as a true cornerstone of modern medicine.

AUTOLOGOUS VERSUS ALLOGENEIC

Autologous cell therapy is defined by removing cells from the patient, potentially performing alterations such as genetic manipulation to endow disease-specific enhancements, a possible expansion culture phase to reach requisite cell numbers, formulation/finish, and then reintroduction to the patient. The primary benefit of autologous cell therapies is the lack of immune rejection and minimal graft versus host disease (GvHD) risk. Autologous therapies currently have the best track record of success, with 14 FDA approved therapies currently on the market. The key challenge in widespread adoption of currently approved therapies and expansion into other indications is cost; the high expense and complexity of scale-out manufacturing is a major hurdle for expanding autologous therapies into other treatment areas.

Allogeneic, off-the-shelf cell therapies employ a cell source derived from a separate donor with clear advantages, including healthy starting material, potentially more scalable production, reduced lot variability, improved manufacturability, improved economies of scale, and decreased time to treatment. However, allogeneic therapies come with inherent rejection risks, due to the immune mismatch between donor and recipient (which can be potentially ameliorated through donor matching). Furthermore, interactions between the donor cells and host immune system frequently result in reduced potency of the cell therapy compared to potential autologous equivalents [6]. To address this challenge, many allogeneic therapies currently under development include gene edits to evade the immune system [7–9].

CAR-T CELLS

The foundational autologous CAR-T therapies targeting oncologic B-cell malignancies continue to demonstrate efficacy and promise through durable complete response (defined as curative in some cases), ever increasing designations for lines of therapy, and indication expansion into autoimmunity [10]. However, with extended time in clinic, several biological, safety, and production hindrances have come more into focus that may impede widespread adoption.

Cytokine release syndrome and neurotoxicity due to excessive CAR-T cell activation is well known to both developers and regulatory agencies alike [11]. The relatively high incidence of cytokine release syndrome is a key impediment to more widespread adoption of CAR-T therapies, and new CAR

TABLE 1

Current list of FDA approved cell therapies, encompassing both autologous and allogeneic products. Therapeutic areas range from oncology to regenerative medicine.

Product	Manufacturer	Indication	Therapeutic area	Cell type	Autologous or allogeneic	Use of genetic engineering technology
Abecma®	Celgene	Multiple myeloma	Oncology	CAR-T	Autologous	Yes
Amtagvi™	Iovance Biotherapeutics	Metastatic melanoma	Oncology	Tumor infiltrating leukocyte	Autologous	Yes
Breyanzi®	Juno Therapeutics	B-cell lymphoma	Oncology	CAR-T	Autologous	Yes
Carvykti®	Janssen Biotech	Multiple myeloma	Oncology	CAR-T	Autologous	Yes
Casgevy®	Vertex	Sickle cell disease	Regen med	HSC	Autologous	Yes
Gintuit™	Organogenesis Inc	Mucogingival	Skin graft	Keratinocytes and fibroblasts	Allogeneic	No
Kymriah®	Novartis	B-cell lymphoma	Oncology	CAR-T	Autologous	Yes
Lantidra™	CellTrans	Type 1 diabetes	Regen med	Pancreatic cells	Allogeneic	No
Laviv®	Fibrocell	Nasolabial fold wrinkles	Regen med	Fibroblasts	Autologous	No
Lenmeldy®	Orchard Therapeutics	Early onset metachromatic leukodystrophy	Regen med	HSC	Autologous	Yes
Maci®	Vericel	Full thickness cartilage defects	Regen med	Chondrocytes	Autologous	No
Omisirge®	Gamida Cell Ltd	Hematologic malignancies	Oncology	HSC	Allogeneic	No
Provenge®	Dendreon Corp	Prostate cancer	Oncology	Antigen presenting cells	Autologous	No
Rethymic®	Enzyvant Therapeutics GmbH	Congenital athymia	Regen med	Thymic tissue	Allogeneic	No
Skyson™	Bluebird Bio Inc	cerebral adrenoleukodystrophy	Oncology	HSC	Autologous	Yes
Stratagraft®	Stratatech Corp	Thermal burns	Regen med	Keratinocytes and dermal fibroblasts	Allogeneic	No
Tecartus®	Kite Pharmaceuticals	Mantle cell lymphoma and B-cell lymphoma	Oncology	CAR-T	Autologous	Yes
Yescarta®	Kite Pharmaceuticals	B-cell lymphoma and refractory follicular lymphoma	Oncology	CAR-T	Autologous	Yes
Zynteglo™	Bluebird Bio Inc	ß-thalassemia	Regen med	HSC	Autologous	Yes
Allocord	Glennon Children's Medical Center	Unrelated donor hematopoietic progenitor cell transplantation	Oncology	HSC	Allogeneic	No
Clevecord™	Cleveland Cord Blood Center	Unrelated donor hematopoietic progenitor cell transplantation	Oncology	HSC	Allogeneic	No
Ducord®	Duke University	Unrelated donor hematopoietic progenitor cell transplantation	Oncology	HSC	Allogeneic	No
Hemacord®	NY Blood Center	Unrelated donor hematopoietic progenitor cell transplantation	Oncology	HSC	Allogeneic	No
HPC, Cord blood	Clinimmune Labs	Unrelated donor hematopoietic progenitor cell transplantation	Oncology	HSC	Allogeneic	No
HPC, Cord blood	MD Anderson	Unrelated donor hematopoietic progenitor cell transplantation	Oncology	HSC	Allogeneic	No
HPC, Cord blood	LifeSouth Community Blood Centers	Unrelated donor hematopoietic progenitor cell transplantation	Oncology	HSC	Allogeneic	No
HPC, Cord blood	Bloodworks	Unrelated donor hematopoietic progenitor cell transplantation	Oncology	HSC	Allogeneic	No

HSC: Hematopoietic stem cells.

designs and additional gene edits are being considered to limit these effects. Additionally, the risk of secondary cancers has arisen, causing the application of a black box warning on all CAR-T therapies. It is currently unclear if this risk is due to the CAR-T cells themselves or the viral vector through which the CAR is inserted [12]. While concerning, developers and clinicians still support these therapies, pointing to the outsized benefit versus risk to patients in need, which the agency also acknowledges [13]. Nevertheless, the outcome of these recent findings may have significant effects on the adoption of CAR-T based therapies outside the realm of oncology.

Manufacture of CAR-T therapies is difficult due to the differences between individual patients, process variability, and the technology available for the GMP process. The starting material quality and quantity of heavily pre-treated patients in oncologic indications affects the transduction efficiency, cell expansion rate, and overall cell fitness [14–16]. The variable expansion rates between patients and processes directly impacts the final drug product, as is evidenced by the effective dose of Kymriah® varying between 0.6 to 6.0×10^8 CAR-positive viable T cells [17]. It is also well known that scale-out manufacturing of autologous therapies is challenging [18]. Not only are the logistics challenging for these personalized medicines, but the economies of scale are directly tied to the ability of an organization to efficiently operationalize their scale-out approach.

To address technical challenges surrounding scale-out manufacturing, the field is undergoing a boom in automation technologies. Flavors of systems include: GMP-in-a-box (Miltenyi Biotech Prodigy, Lonza Cocoon, Adva X3), modularized systems (Ori Biotech Iro, Cellular Origins Constellation, Multiply Labs), through to device platform and service offerings (Cellares). The inherent goal of these technologies is to minimize the human workload that can be overtly onerous in these complicated manufacturing processes. However, the strategies differ greatly, and it remains to be seen how the market will respond to and potentially adopt these technologies. Each strategy also has its own set of drawbacks. GMP-in-a-box systems require end users to adapt their cell type into a relatively locked device ecosystem and lack true unit-scale models to enable high-powered DOE studies. Modular systems continue to place the burden of development on the end user and currently still have limited systems in which modularity is possible. Full-service system and service offerings assume that a one stop shop will meet the needs of therapy developers from a cost, time, and technical requirements perspective. These platforms are also predominantly built off an autologous CAR-T paradigm and may not be readily adaptable as differing specifications arise from advancement of other cell types.

It is unlikely that there is sufficient market space for all these technologies to coexist. While both late and early-stage companies appear to be assessing these technologies, the current evolving nature of the field makes it difficult to predict what a true enabling platform technology will look like. The majority of these technologies are best suited for autologous scale-out therapies, yet with the heavy interest in allogeneic, off-the-shelf products, the field may see a dichotomy in manufacturing platforms to support scale-out versus scale-up therapeutics.

HEMATOPOIETIC STEM CELLS

HSCs are a cornerstone of modern medicine for both autologous and allogeneic cell therapies for oncologic indications, with the first transplantations occurring in the 1960s [19]. Recently, HSCs were one of the first cell types identified as a candidate for genetic modification (vector- and CRISPR-based) due to their capacity for self-renewal, multipotency, and established clinical record of transplantation [20,21]. Most recently, Casgevy® was approved for the treatment of sickle cell disease and β-thalassemia, both strictly for autologous

use only. Although currently approved exclusively for autologous use, ongoing clinical trials are investigating the possibility of using genetically modified HSCs for HLA donor matched allogeneic treatment [22,23]. Notably, gene editing cannot be used to solve the problem of HLA matching in HSCs; the engrafted donor HSCs would recapitulate all aspects of the blood organ system and would almost certainly be targeted by the host immune system, leading to engraftment failure or graft versus host disease [24,25]. A tremendous leap in biology and additional further development will be needed to achieve an off-the-shelf allogeneic therapy without HLA matching.

Autologous and allogeneic HSC treatments both suffer from the same industrialization hurdles that surround scale-out approaches for autologous CAR-Ts, and similarly, processes using automation are becoming more common place. The larger challenges that surround HSC manufacturing lie in their innate biology. The therapeutic fraction of HSCs in the body, canonically labeled long-term HSCs, reside in the bone marrow and do not undergo significant proliferation which clearly hinders an industrialized, off-the-shelf approach [26]. Cytokine-driven methods for inducing proliferation *ex vivo* often drive HSCs down the path of myeloid or lymphoid maturation, limiting their capacity to successfully engraft and repopulate the bone marrow in the recipient [27]. While acutely therapeutic, the loss of long-term stemness leads to a loss in the durability of the treatment [26]. Recent progress has been made using small molecules such as UM171, which allows for significant expansion of HSCs *ex vivo* without loss of stemness [28,29]. It should be noted that UM171 is fully proprietary to ExCellThera and is currently in clinical trials for allogeneic use. If clinically successful, it would spur continued innovation from competitors, leading to further advances and a better understanding of HSC expansion pathways, and bring about an evolution to the next stage beyond donor derived HSC transplants. As of today, scale-up to address a large patient population and maintain durable potency remains challenging. The creation of an HSC by means of induced pluripotent stem cells (iPSCs) could conceivably be one method through which scale-up could be achieved.

INDUCED PLURIPOTENT STEM CELLS

iPSC based therapies hold the possibility of an entirely different treatment paradigm: true off-the-shelf allogeneic treatment. At first glance, iPSCs are extremely attractive for two primary reasons: a nearly unlimited capacity for self-renewal, and the theoretical ability to differentiate into any cell type in the body. Combined, these characteristics appear to fill current holes in allogeneic therapies, capable of generating large cell banks and creating cell types that recapitulate functions that have been difficult for traditional therapies to treat. This has been capitalized by several companies already and clinical trials are recruiting for a wide variety of iPSC-derived cell types, including but not limited to NK cells, cardiomyocytes, retinal pigment epithelium, dopaminergic neurons, T cells, and mesenchymal stromal cells (Table 2).

iPSC-based therapies are highly amenable to the creation of GMP master and working cell banks. Gene edits, including insertions, deletions, or a combination thereof, can be performed on a bulk culture. Subsequently, a single cell with all desired edits can be picked from which to generate a cell bank [30]. By cryopreserving millions to billions of cells, a stable master cell bank (MCB) can be generated, which can be used for the creation of working cell banks and drug product throughout the product life cycle, while reducing the risk of genetic drift. Creation of cell therapies from a qualified manufacturing process using cell banks results in not only a more reproducible product, but also greatly eases process development due to a robust and homogenous starting material.

Despite these advantages, iPSC-based therapies have significant drawbacks due to both biology and current manufacturing paradigms. Much like CAR-T and HSC therapies, the starting cell source has a significant downstream impact on the eventual drug product [31]. The iPSC donor, specific clone, reprogramming method used, and even the original tissue that the cell was derived from prior to reprogramming must be considered, even prior to gene editing [32,33]. The high proliferation rate and pluripotent state of iPSCs also means that mutations are rapidly acquired during cell division and subsequent culture, which can impact genomic stability and the safety profile of the final product. This is further compounded by gene editing, which can introduce a significant number of off-target effects.

iPSC maintenance and clone selection require considerable manual labor. This is especially evident during clone selection where thousands of cultures must be grown and monitored prior to final clone selection. Several cell culture automation systems, such as the Freedom EVO 200 (Tecan) or the CellExpress AI (Molecular Devices) have been tested with iPSCs, but to date, no system has been built specifically for GMP iPSC culture. Furthermore, this cell culture is performed in an open system using standard tissue culture flasks, elevating the costly risk of contamination during all parts of handling. A commercially available, GMP compatible closed platform specifically built for iPSC culture and clonal selection would be a significant advantage for the development of iPSC-based therapies. To the potential benefit of developers, regulatory guidelines at present appear to suggest that starting material for MCBs (i.e., gene-modified iPSCs clones) may have the ability to be produced under good laboratory practice and good documentation practice (GLP/GDP), under the assumption that the appropriate safety testing along the production path is performed. Therapy developers should perform appropriate risk-assessments and internal resourcing exercises to better understand if taking on clonal selection is a savvy decision.

After generation of the gene edited iPSC MCB, the challenges shift to differentiation and manufacturing scale. First and foremost, the recapitulation of eons of evolutionary development and condensing human development from months to weeks in a "dish" is a tremendous and astounding feat. Development of differentiation processes is difficult to scale; the fluid dynamics and mass transport of large-scale bioreactors is significantly different from small culture plates in which differentiation protocols are typically developed. Achieving the 'right' cell type is a herculean effort between R&D and CMC to ascertain key critical attributes of the cell product and the desired impact on a specific disease indication. Depending on the stimuli needed, multiple different types of bioreactors and culture media may be needed at various phases of the overall process, further increasing closed system complexity and process failure points. The cost and complexity of chemically defined, GMP compatible cell culture media is also a significant consideration, especially considering that most differentiation processes take weeks to months to complete. Fortunately, the use of representative material and even the MCB itself allow the process to be developed and iterated dozens to hundreds of times to develop the drug product.

Despite the considerable promise of iPSC-based therapies and ongoing clinical trials, none are currently approved for market use by regulatory agencies. The next few years will be crucial for determining if iPSC-based therapies are ready for the spotlight, or if further development is needed. Navigating the IP landscape is also becoming increasingly complex as more patents are filed relating to iPSC technologies. This is not confined to just disease- or gene editing-specific applications; recent years have seen patent applications purely for differentiating iPSCs into a specific cell type. As iPSC-based therapies progress through clinical trials and possible

TABLE 2

Current list of ongoing and completed clinical trials using iPSC-derived cell therapies.

Sponsor	Associated company	iPSC derived differentiated cell type	Indication	Autologous or allogeneic	Status	Clinicaltrials.gov ID
HeartWorks	HeartWorks	Heart cells	Congenital heart disease	Autologous	Recruiting	NCT05647213
Beijing Tongren Hospital	N/A	Retinal pigment epithelium	Macular degeneration	Autologous	Recruiting	NCT05445063
University of Alabama at Birmingham	N/A	Mesoderm cells and $CD34^{+}CD45^{+}$ cells	Diabetic retinopathy	Autologous	Recruiting	NCT03403699
Zhejiang University	Qihan Biotech	CLL1 or CD33 targeted CAR-NK cells	AML	Allogeneic	Recruiting	NCT06367673
Century Therapeutics	Century Therapeutics	CD19 targeted CAR-NK cells (CNTY-101)	CD19-positive B-cell malignancies	Allogeneic	Recruiting	NCT05336409
Century Therapeutics	Century Therapeutics	CD19 targeted CAR-NK cells (CNTY-101)	Refractory B cell-mediated autoimmune diseases	Allogeneic	Recruiting	NCT06255028
Shanghai East Hospital	N/A	Dopaminergic neural precursor	Parkinson's disease	Autologous	Not yet recruiting	NCT06145711
National Eye Institute	N/A	Retinal pigment epithelium	Age-related macular degeneration	Autologous	Recruiting	NCT04339764
Institute of Hematology & Blood Diseases Hospital, China	Qihan Biotech	CLL1 or CD33 targeted CAR-NK cells	AML	Allogeneic	Not yet recruiting	NCT05987696
National Institute of Neurological Disorders and Stroke	N/A	Dopaminergic neuron	Parkinson's disease	Autologous	Enrolling by invitation	NCT06422208
Zhejiang University	Qihan Biotech	CLL1 or CD33 targeted CAR-NK cells	AML	Allogeneic	Recruiting	NCT06367673
Zhejiang University	Qihan Biotech	CLL1 targeted CAR-NK cells	AML	Allogeneic	Recruiting	NCT06027853
Help Therapeutics	Help Therapeutics	Cardiomyocytes	Ischemic heart failure	Allogeneic	Recruiting	NCT05566600
Cynata Therapeutics	Cynata Therapeutics	Mesenchymal stromal cell (CYP-001)	Respiratory failure	Allogeneic	Completed	NCT04537351
Masonic Cancer Center	Fate Therapeutics	Non-cleavable CD16 NK cells (FT536)	Recurrent ovarian, fallopian tube, and primary peritoneal cancer	Allogeneic	Recruiting	NCT06342986
Allife Medical Science and Technology Co	Allife Medical Science and Technology Co	Endothelial progenitor cells	Critical limb ischemia	Allogeneic	Recruiting	NCT06359912
Fate Therapeutics	Fate Therapeutics	NK cells	Advanced solid tumors	Allogeneic	Completed	NCT03841110
Fate Therapeutics	Fate Therapeutics	BCMA targeted NK cells (FT576)	Multiple myeloma	Allogeneic	Active not recruiting	NCT05182073
Fate Therapeutics	Fate Therapeutics	Non-cleavable CD16 NK cells (FT516)	Ovarian cancer	Allogeneic	Completed	NCT04630769
University of California, San Diego	Sumitomo Pharma America	Dopaminergic progenitor (CT1-DAP001)	Parkinson's disease	Allogeneic	Recruiting	NCT06482268
Heartseed	Heartseed	Cardiomyocyte spheroids (HS-001)	Heart failure	Allogeneic	Recruiting	NCT04945018
Anhui Provincial Hospital		Mesenchymal stromal cell	Steroid refractory graft versus host disease	Allogeneic	Recruiting	NCT06321198
Aspen Neuroscience	Aspen Neuroscience	Dopaminergic neuron (ANPD001)	Parkinson's disease	Autologous	Enrolling by invitation	NCT06344026
Cynata Therapeutics	Cynata Therapeutics	Mesenchymal stromal cell (CYP-001)	Steroid refractory graft versus host disease	Allogeneic	Completed	NCT02923375
Eyestem Research Pvt. Ltd	Eyestem Research Pvt. Ltd	Retinal pigment epithelium (Eyecyte-RPE)	Age related macular degeneration	Allogeneic	Recruiting	NCT06394232

AML: Acute myeloid leukemia, iPSC: Induced pluripotent stem cells, NK: Natural killer.

FDA approval, extended freedom to operate investigations will need to be carried out for the editing performed, the disease being investigated, and the differentiation pathways used in the manufacturing process.

Manufacturing considerations

A key consideration is the internalization or externalization of manufacturing. With a current emphasis on the 'process is the product', guarding of trade secret production methods is a point for consideration. The complexity of manufacturing methods and specific needs for varying cell types means that a unified set of process specifications that span the industry does not exist. This complicates tech transfer and manufacturing campaigns due to constant training of new methods and is compounded by the relatively high turnover rate of manufacturing operators. Thus, much of the intent of internalized cGMP manufacturing at earlier phases is due to the increased control of the sponsor on the final production and testing of the drug product. In the relatively recent cash-flush years of cell and gene therapy, numerous companies built internal facilities with demand models that justified their cost effectiveness.

Yet, the variable and fixed costs to design, build, commission, qualify, potentially validate, and fully operationalize a cGMP facility cannot be underestimated. During this current industry consolidation phase, it will be interesting to compare the predicted demand and success that companies forecast in comparison to the realities currently being faced. In the foreseeable future market, it would be highly prudent for sponsors to perform their cost-benefit and break-even calculations with an overly conservative lens to ensure that resources are deployed as efficiently as possible.

TRANSLATION INSIGHT: ARE WE THERE YET?

Although we are in the midst of a tumultuous period of market consolidation, cell therapies at large currently reside in a predictable phase within the Gartner® Hype Cycle (Figure 1) [33]. This cycle is a graphical depiction of the maturity, adoption, and application of a given technology.

While the majority of the current crop of therapies generally target cancer or autoimmunity, progression through this lifecycle will begin to unlock the next phases of cell replacement therapy, regenerative medicine, and potentially even tissue engineering. All of these therapeutic verticals fundamentally rely on the ability to be able to manufacture cells in a consistent, robust manner, the foundations of which are being built today.

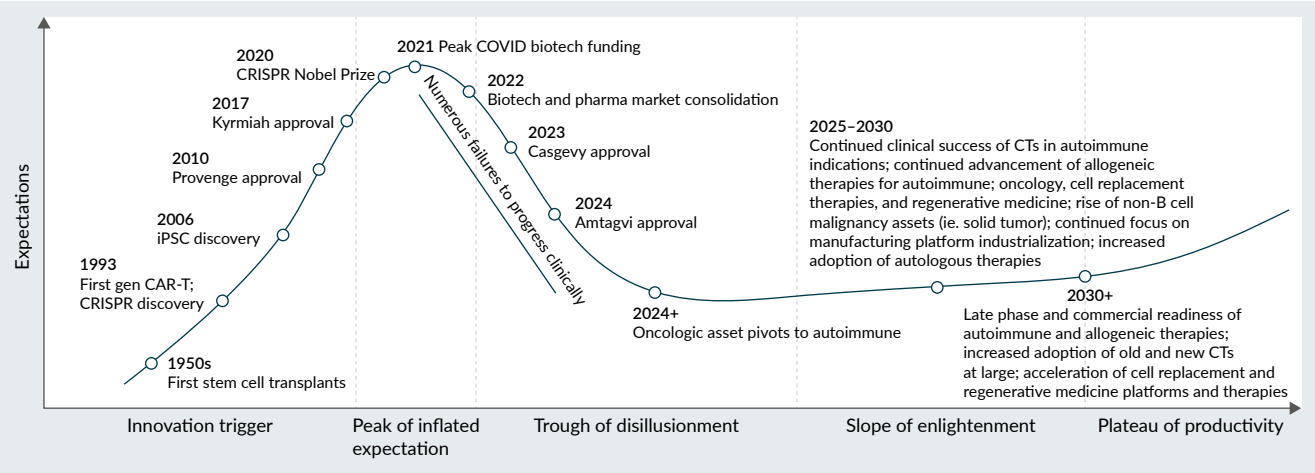
The field is currently working through the 'Trough of Disillusionment': funding conservatism, shifts in marketability of early-stage high risk technologies, focus on clinical assets, frequent company closures, and near daily reports of layoffs. Whether or not we have reached the nadir of the phase is still to be seen. This phase, while unfortunate for many living through it, is important for the field to progress. Through this period, therapy developers, tool developers, service providers, investors, and partners are forced to prioritize on the promises that have been touted. Curative and biological potential has been demonstrated, but without a reality in which accessibility exists, these therapies will be unable to reach their full potential to the detriment of patients in need. At the moment, the question is not 'are we there yet?' (which we clearly are not) but rather is 'what will it take?' For the remainder of this article, we highlight several considerations that may lead to the next phase: the 'Slope of Enlightenment'.

Competing modalities

As repeatedly stated, the efficacy of cell therapies is beyond doubt. While the field is still uncovering biological aspects that continue to make them successful, the multi-modal ability of a 'living therapy' likely contributes to their success and their complexity as a treatment. We have herein expounded allogeneic

FIGURE 1

Several key cell therapy inflection points are presented along a Gartner hype cycle. Historical, contemporaneous, and future predictions/opinions on what the next may entail along the next leg of the cell therapy journey.



therapies and the potential of this modality to bring treatment to the masses. However, until this is realized, more traditional modalities may still provide an advantage from a product concept and commercialization angle. The most direct competitor to cell therapies are their *in vivo* gene therapy counterparts, which aim to deliver the engineering payload within the body rather than outside [34], reducing the complexity of the manufacturing process. Progress from *in vivo* CAR-T companies such as Umoja, Capstan, Kelonia, and Orna will be closely watched. Parallel path modalities such as antibody drug conjugates, which bring cytotoxic payloads to their targets, or cell engagers, which attempt to bring target and activated effector cells together to facilitate cell-mediated killing, are showing significant impact and are expected to have a USD$27BN market share by 2033 [35,36]. The commonality of these non-cell therapies lies in the ability, maturity, and/or relative simplicity of manufacturing a drug product at relatively sizeable commercial scale (biologics, nucleic acids, etc.). A major potential benefit of cell therapies is the possibility for a durable, one-time therapy requiring no redosing as compared to other modalities. While all these modalities can coexist, the existence of cheaper and more scalable methods could impinge on the reach of cell therapies in the current and/or future markets when nearing commercial scale (assuming comparable potency).

Expanding market potential

The potential to effectively address a large patient population is attractive to larger pharma companies due to the high profitability of these products. At the moment, the oncology markets, namely B-cell malignancies, are showing upside with a target market size of ranging from $7–55BN by 2030 [37,38]. By comparison, recent wins in the GLP-1 space have expectations of over $130–170BN by 2030 [39,40]. With the recent push of CAR therapies into the CD19 and BCMA-driven autoimmune disease space and the associated necessity to treat more patients than the currently oncologic indications by orders of magnitude [41,42], the path to commercial-scale levels of quality and productivity become more paramount and challenging. This shines an even more critical spotlight on enabling technologies for both scale-out and scale-up. Both autologous and allogeneic modalities for autoimmune diseases are still being proven; forthcoming clinical trial data in the next couple years will be closely watched by companies and investors alike.

With the mechanism of action being repeatedly demonstrated, late-stage development and commercial market drivers may act as the catalyst to usher cell therapies into their industrialization revolution era.

Product maturation and market development

How would one expect these catalysts to manifest during the 'Slope of Enlightenment' period? Simply put, continued therapeutic success in more and increasingly larger markets, and subsequently, understanding how to manufacture these products at scale. In an ideal scenario, platform production methods also arise with application to future cell types. This applies to the current commercial therapies as they begin to move beyond 'last line of defense' and increase patient access, the emerging potential of cell therapies to treat autoimmune diseases, and applications and developments of autologous and allogeneic modalities.

While a world will likely exist in which autologous and off-the-shelf allogeneic therapies coexist, off-the-shelf treatments are the only hope in which a patient could receive a diagnosis and even conceivably receive treatment the literal or figurative 'same day'. However, this type of therapeutic availability is still years away, requiring advances in nearly every aspect of cell therapy design and logistics. Some of the developments on this front will undoubtedly be prioritized as allogeneic cell therapies reach the 'Plateau of Productivity', while others will simply become necessary over time. Interestingly, the COVID-19 pandemic led to considerable improvements for cold-chain logistics, storage, and distribution; an essential aspect of making cell therapies widely available [43,44].

Cost

The current cost of cell therapies is eye-watering, as is demonstrated by the representative long and expensive road of CAR-Ts. Insurance coverage for expensive gene and cell therapies is expected to vary, with many current health plans not covering existing cell and gene therapies [45]. The insurance approval process can take several weeks, not including additional contracting time between the medical center and the patients insurer [46]. Following that, if approved, the insurance provider will provide a care package including T cell collection, CAR-T manufacturing, and inpatient and outpatient care. The costs associated with cell isolation, gene editing, and expansion, along with specialized facilities, equipment, and trained personnel, are reflected in the overall price with most CAR-T therapies priced between US$350,000–$500,000. The overall cost-effectiveness of current CAR-T therapies may be palatable for their current indications as a life-saving measure or second-line treatment for oncology; it remains to be seen if the pricing will be acceptable for current clinical trial indications such as lupus and other autoimmune disorders.

Although reduction in manufacturing costs through more efficient processes, decentralized manufacturing, or decreased cost of goods have the ability to drive lower pricing of CAR-T and adjacent therapies, patents, legal disputes, and R&D costs associated with reaching a successful therapy can be large market forces behind the high costs associated with these products [47]. Allogeneic iPSC-based therapies may provide some relief on pricing structure as edited master cell banks could theoretically be used indefinitely for a variety of indications, somewhat defraying the costs associated with lengthy R&D processes. Nevertheless, there is a distinct lack of evidence that competition for small molecule drugs actually reduces price; it remains to be seen if cell-based therapies will also follow this trend [48].

Current pricing models of cell and gene therapies are value-based, ostensibly setting the price of the drug at a value that the patient is willing to pay as opposed to the costs purely associated with development, manufacturing, and baseline profit. The cost

of these therapies has been priced through a combination of manufacturing cost, market size, patent longevity, and cost that the patient would be paying over their lifetime with existing treatment options. The bottom line is that the single treatment, potentially curative nature of cell and gene therapies does not fit within the current framework of the healthcare model practiced in most of the developed world, which is sustained by repeated administration of therapies. Undoubtedly, the cost of developing a new cell therapy, currently estimated to be roughly US$1.94BN, must be recouped, with an additional profit margin [49]. However, with current pricing models, the overall adoption and market penetration of cell therapies remains to be seen.

Costs are also related to the reach of these therapies. The field is exploring methods of disseminating accessible CAR-T therapies to relatively under-resourced areas of the world. Initiatives such as those from Caring Cross and the Brazilian Ministry of Health exemplify needed efforts to democratize lifesaving, yet highly complex therapies [50]. While every nation will have their own pricing and reimbursement structure, learnings and innovations should be taken into account across all global products and markets. Spain provides an interesting case study of a hospital-brewed CAR-T that has received regulatory approval and supportive reimbursement by their national healthcare system at slightly less than US$100,000—roughly a quarter of commercially-priced competitors [51,52]. Innovative and collaborative efforts such as these that synergize developers, clinical centers, payers, and regulators have the ability to make a tremendous impact for patients in need globally.

SUMMARY

We are living in a challenging yet exciting time. Cell therapies have progressed a tremendous amount from the early days of stem cell transplants and yet regulatory approval is not necessarily an indicator of strong commercial success, which is ultimately driven by patient supply and reach. Furthermore, the current market consolidation and scrutiny on resourcing necessitate focus on delivering beyond the science and making a product. Industrialization will be the key to the next era of cell therapies ranging from increased automation of autologous treatments, the continued rise in allogeneic off-the-shelf therapies (healthy donor- and stem cell-derived), and unleashing the promise of iPSCs, as well as further increasing alignment of automated and scalable manufacturing akin to more traditional cell-line based biologics manufacturing. The field at large wishes to pass through this consolidation phase quickly, but the reassessment of priorities and efforts is necessary to reach the field's full potential.

REFERENCES

1. Research C for BE and approved cellular and gene therapy products. *FDA* 2024. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products.

2. Wang V, Gauthier M, Decot V, Reppel L, Bensoussan D. Systematic review on CAR-T cell clinical trials up to 2022: academic center input. *Cancers* 2023; 15(4), 1003.

3. Schenkel JM, Pauken KE. Localization, tissue biology and T cell state—implications for cancer immunotherapy. *Nat. Rev. Immunol.* 2023; 23(12), 807–823.

4. Anselmo AC, Gokarn Y, Mitragotri S. Non-invasive delivery strategies for biologics. *Nat. Rev. Drug Discov.* 2019; 18(1), 19–40.

5. Haddad A, Gazitt T, Feldhamer I, Feld J, Cohen AD, Lavi I, *et al.* Treatment persistence of biologics among patients with psoriatic arthritis. *Arthritis Res. Ther.* 2021; 23(1), 44.

6. Fürst D, Neuchel C, Tsamadou C, Schrezenmeier H, Mytilineos J. HLA Matching in Unrelated Stem Cell Transplantation up to Date. In: *Transfusion Medicine and Hemotherapy.* 2019; Karger Publishers, 326–336.

7. Zhang X, Jin X, Sun R, Zhang M, Lu W, Zhao M. Gene knockout in cellular immunotherapy: application and limitations. *Cancer Lett.* 2022; 540, 215736.

8. Vivier E, Rebuffet L, Narni-Mancinelli E, Cornen S, Igarashi RY, Fantin VR. Natural killer cell therapies. *Nature* 2024; 626(8000), 727–736.

9. Li Y-R, Halladay T, Yang L. Immune evasion in cell-based immunotherapy: unraveling challenges and novel strategies. *J. Biomed.* Sci. 2024; 31(1), 5.

10. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat. Rev. Clin. Oncol.* 2023; 20(6), 359–371.

11. Juluri KR, Wu QV, Voutsinas J, Hou J, Hirayama AV, Mullane E, *et al.* Severe cytokine release syndrome is associated with hematologic toxicity following CD19 CAR T-cell therapy. *Blood Adv.* 2022; 6(7), 2055–2068.

12. Research C for BE and approved cellular and gene therapy products. FDA requires boxed warning for T cell malignancies following treatment with BCMA-directed or CD19-directed autologous chimeric antigen receptor (CAR) T cell immunotherapies. *FDA* 2024. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/fda-requires-boxed-warning-t-cell-malignancies-following-treatment-bcma-directed-or-cd19-directed.

13. Bilodeau K. CAR-T developers respond to FDA black box warning. *PharmaVoice* 2024. https://www.pharmavoice.com/news/car-t-cell-therapy-cancer-bristol-myers-jnj-gilead-kite-novartis/705927/ (accessed Jul 19, 2024).

14. Fesnak AD. The challenge of variability in chimeric antigen receptor T cell manufacturing. *Regen. Eng. Transl. Med.* 2020; 6(3), 322–329.

15. Dulobdas V, Kirkwood AA, Serpenti F, *et al.* Risk factors for CD19-targeting CAR T manufacturing failure and patient outcomes: a report from the UK national CAR T panel. *Blood* 2023; 142(S1), 495.

16. Vormittag P, Gunn R, Ghorashian S, Veraitch FS. A guide to manufacturing CAR T cell therapies. *Curr. Opin. Biotechnol.* 2018; 53, 164–181.

17. Research C for BE and KYMRIAH. *FDA* 2024. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/kymriah.

18. Nam S, Smith J, Yang G. Driving the next wave of innovation in CAR T-cell therapies. *McKinsey* Dec 13, 2019. https://www.mckinsey.com/industries/life-sciences/our-insights/driving-the-next-wave-of-innovation-in-car-t-cell-therapies (accessed Jul 19, 2024).

19. Henig I, Zuckerman T. Hematopoietic stem cell transplantation—50 Years of evolution and future perspectives. Rambam Maimonides *Med. J.* 2014; 5(4), e0028.

20. Bhalla N, Bhargav A, Yadav SK, Singh AK. Allogeneic hematopoietic stem cell transplantation to cure sickle cell disease: a review. *Front. Med.* 2023; 10.

21. Tsakiris DA, Gavriilaki E, Chanou I, Meyer SC. Hemostasis and complement in allogeneic hematopoietic stem cell transplantation: clinical significance of two interactive systems. *Bone Marrow Transplant.* 2024, 1–11.

22. Study details: a long-term follow-up study of patients who received VOR33. *ClinicalTrials.gov* Jan 26, 2024. https://clinicaltrials.gov/study/NCT05309733?term=HSC%20allogeneic%20edited&aggFilters=status:rec&viewType=Card&rank=2 (accessed Sep 9, 2024).

23. Study details: allogeneic engineered hematopoietic stem cell transplant (HCT) lacking the CD33 protein, and post-HCT treatment with Mylotarg, for patients with $CD33^+$ AML or MDS. *ClinicalTrials.gov* Aug 29, 2024. https://clinicaltrials.gov/study/NCT04849910?term=HSC%20allogeneic%20edited&aggFilters=status:rec&viewType=Card&rank=1 (accessed Sep 9, 2024).

24. Horan J, Wang T, Haagenson M, *et al.* Evaluation of HLA matching in unrelated hematopoietic stem cell transplantation for nonmalignant disorders. *Blood* 2012; 120(14), 2918–2924.

25. Nowak J. Role of HLA in hematopoietic SCT. *Bone Marrow Transplant.* 2008; 42(2), S71–S76.

26. Kumar S, Geiger H. HSC niche biology and HSC expansion *ex vivo*. *Trends Mol. Med.* 2017; 23(9), 799–819.

27. Wilkinson AC, Igarashi KJ, Nakauchi H. Haematopoietic stem cell self-renewal *in vivo* and *ex vivo*. *Nat. Rev. Genet.* 2020; 21(9), 541–554.

28. Chagraoui J, Lehnertz B, Girard S, *et al.* UM171 induces a homeostatic inflammatory-detoxification response supporting human HSC self-renewal. *PLOS ONE* 2019; 14(11), e0224900.

29. Liu B, Klatt D, Zhou Y, Manis J, Sauvageau G, Brendel C, *et al.* UM171 Enhances fitness and engraftment of gene modified hematopoietic stem cells from sickle cells disease patients. *Blood* 2023; 142, 18.

30. Dashnau JL, Xue Q, Nelson M, Law E, Cao L, Hei D. A risk-based approach for cell line development, manufacturing and characterization of genetically engineered, induced pluripotent stem cell–derived allogeneic cell therapies. *Cytotherapy* 2023; 25(1), 1–13.

31. Sanchez-Freire V, Lee AS, Hu S, *et al.* Effect of human donor cell source on differentiation and function of cardiac induced pluripotent stem cells. *J. Am. Coll. Cardiol.* 2014; 64(5), 436–448.

32. Scesa G, Adami R, Bottai D. iPSC preparation and epigenetic memory: does the tissue origin matter? *Cells* 2021; 10(6), 1470.

33. Gartner hype cycle research methodology. *Gartner*. https://www.gartner.com/en/research/methodologies/gartner-hype-cycle (accessed Jul 19, 2024).

34. Short L, Holt RA, Cullis PR, Evgin L. Direct *in vivo* CAR T cell engineering. *Trends Pharmacol. Sci.* 2024; 45(5), 406–418.

35. Rubahamya B, Dong S, Thurber GM. Clinical translation of antibody drug conjugate dosing in solid tumors from preclinical mouse data. *Sci. Adv.* 2024; 10(22), eadk1894.

36. Antibody drug conjugate market to capture USD 27.37 billion by 2033 (size, trends, shares and analysis report). *BioSpace* Jul 18, 2024. https://www.biospace.com/antibody-drug-conjugate-market-to-capture-usd-27-37-billion-by-2033-size-trends-shares-and-analysis-report (accessed Jul 19, 2024).

37. CAR-T cell therapies market size, share. CAGR of 13.8%. *Market.US* Feb 2023. https://market.us/report/car-t-cell-therapies-market/ (accessed Jul 19, 2024).

38. Cell therapy market size to hit USD 97 billion by 2033. *Precedence Research* Apr 2024. https://www.precedenceresearch.com/cell-therapy-market (accessed Jul 19, 2024).

39. Lloyd E. Global GLP-1 agonist market trends. *HMP Global Learning Network* Apr 3, 2024. https://www.hmpgloballearningnetwork.com/site/pln/news/global-glp-1-agonist-market-trends (accessed Jul 19, 2024).

40. GLP-1 receptor agonist market size & share report, 2030. *Grand Review Research* 2024. https://www.grandviewresearch.com/industry-analysis/glp-1-receptor-agonist-market (accessed July 19, 2024).

41. Schett G, Mackensen A, Mougiakakos D. CAR T-cell therapy in autoimmune diseases. *Lancet* 2023; 402(10416), 2034–2044.

42. Moore B. Guest blog: a major health crisis: the alarming rise of autoimmune disease. *National Health Council* Mar 28, 2024. https://nationalhealthcouncil.org/blog/a-major-health-crisis-the-alarming-rise-of-autoimmune-disease (accessed Jul 19, 2024).

43. Andoh EA, Yu H. A two-stage decision-support approach for improving sustainable last-mile cold chain logistics operations of COVID-19 vaccines. *Ann. Oper. Res.* 2023; 328(1), 75–105.

44. Fahrni ML, Ismail IA-N, Refi DM, Almeman A, Yaakob NC, Saman KM, *et al*. Management of COVID-19 vaccines cold chain logistics: a scoping review. *J. Pharm. Policy Pract.* 2022; 15(1), 16.

45. Wong CH, Li D, Wang N, Gruber J, Lo AW, Conti RM. The estimated annual financial impact of gene therapy in the United States. *Gene Ther.* 2023; 30(10), 761–773.

46. Cliff ERS, Kelkar AH, Russler-Germain DA, *et al*. High cost of chimeric antigen receptor T-cells: challenges and solutions. *Am. Soc. Clin. Oncol. Educ. Book* 2023; 43, e397912.

47. Fiorenza S, Ritchie DS, Ramsey SD, Turtle CJ, Roth JA. Value and affordability of CAR T-cell therapy in the United States. *Bone Marrow Transplant.* 2020; 55(9), 1706–1715.

48. Sarpatwari A, DiBello J, Zakarian M, Najafzadeh M, Kesselheim AS. Competition and price among brand-name drugs in the same class: a systematic review of the evidence. *PLOS Med.* 2019; 16(7), e1002872.

49. Sabatini MT, Chalmers M. The cost of biotech innovation: exploring research and development costs of cell and gene therapies. *Pharm. Med.* 2023; 37(5), 365–375.

50. Silverman E. Nonprofit seeks to widen access to CAR-T cell therapy in Brazil, India. *STAT* May 8, 2024. https://www.statnews.com/pharmalot/2024/05/08/cell-gene-therapies-brazil-india-caringcross-medicines-cancer-hiv (accessed July 19, 2024).

51. Joseph A. In the U.S., scientists see barriers to the development of CAR-T cell therapies. In Spain, a hospital brews its own. *STAT* Dec 19, 2023. https://www.statnews.com/2023/12/19/car-t-therapy-spain-hospital (accessed Sep 13, 2024).

52. Ortíz-Maldonado V, Rives S, Castellà M, *et al*. CART19-BE-01: a multicenter trial of ARI-0001 cell therapy in patients with CD19$^+$ relapsed/refractory malignancies. *Mol. Ther.* 2021; 29(2), 636–644.

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CELL & GENE THERAPY INSIGHTS

SCALE-UP/SCALE-OUT OF CELL AND GENE THERAPY MANUFACTURING

SPOTLIGHT

INNOVATOR INSIGHT

Navigating cold chain complexity to enable clinical-to-commercial transition of advanced therapies

Susan Li

Cell and gene therapy (CGT) products often require ultracold temperatures for both manufacturing and distribution, which poses significant challenges, especially when transitioning from clinical to commercial stages. To maintain product integrity and manage complex global logistics, efficient supply chains, integrated end-to-end services, and compliance with evolving regulatory requirements are crucial. In this article, the benefits of leveraging a global network and innovative cold chain solutions to address these hurdles and ensure successful commercialization and global distribution of CGT products will be discussed. Specific aspects ranging from the implementation of precise temperature-controlled packaging to late-stage customization will be examined. Finally, an illustrative case study of the transition of a first-in-class allogeneic cell therapy from clinical to commercial stages will be shared.

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NAVIGATING SUPPLY CHAIN CHALLENGES IN THE EVOLVING CELL AND GENE THERAPY MARKET

Medicinal products that are dependent on cold chain maintenance, especially cell and gene therapies (CGT), have experienced double-digit CAGR in recent years, with this trend of strong growth expected to continue as more and more products receive marketing approval. However, significant challenges arise with the rapid increase in the number of products with ultra-cold chain (UCC) requirements that are transitioning from clinical to commercial stages (Figure 1).

ADDRESSING GLOBAL CHALLENGES IN CGT CLINICAL TRIALS

Maintaining the integrity of UCC CGT products demands close attention, especially regarding the infrastructure and logistics required for global distribution.

When considering commercialization, it is crucial to ensure there is proper infrastructure and support for global operations, whether a centralized or a decentralized manufacturing model is selected.

Aside from the manufacturing itself, efficient and secure logistics are vital to the success of geographically complex clinical trials. When setting up a global supply chain network, it is important to select an appropriate service provider that supports production and patient distribution both globally and regionally. For example, manufacturers of CGT products often require regional infrastructure that allows for the maintenance of −80 °C and cryogenic cold chains all the way to the point of care.

The solution to this challenge is selecting a service provider that offers the global infrastructure to support ultracold products in clinical trials and their distribution around the world. This involves access to a global network of CGMP facilities equipped with −80 °C and cryogenic storage, regional distribution centers with the same cold storage capabilities, and a global network of qualified couriers that can ensure rapid delivery and reduce the risks from complex multinational shipping regulations.

For example, Thermo Fisher Scientific is part of the Patheon global network, which can support UCC clinical and commercial products worldwide through a network of GMP-compliant facilities for storage, distribution, manufacturing, packaging, and labelling.

OVERCOMING LATE-STAGE CUSTOMIZATION HURDLES

Late-stage customization of CGT products presents another challenge to cold chain management. Autologous cell therapies, allogeneic cell therapies, and AAV-based *in vivo* gene therapies each have distinct supply chain models designed to minimize both the number of steps required and the associated risks.

For autologous cell products, packaging, labelling, and distribution typically occur at the manufacturing site to shorten the vein-to-vein timeline. In contrast, off the shelf allogeneic cell and gene therapy products often require late-stage customization such as patient-specific dosing, human leukocyte antigen (HLA) typing, and packaging and labelling while the products are still in a cryogenic state to maintain their stability.

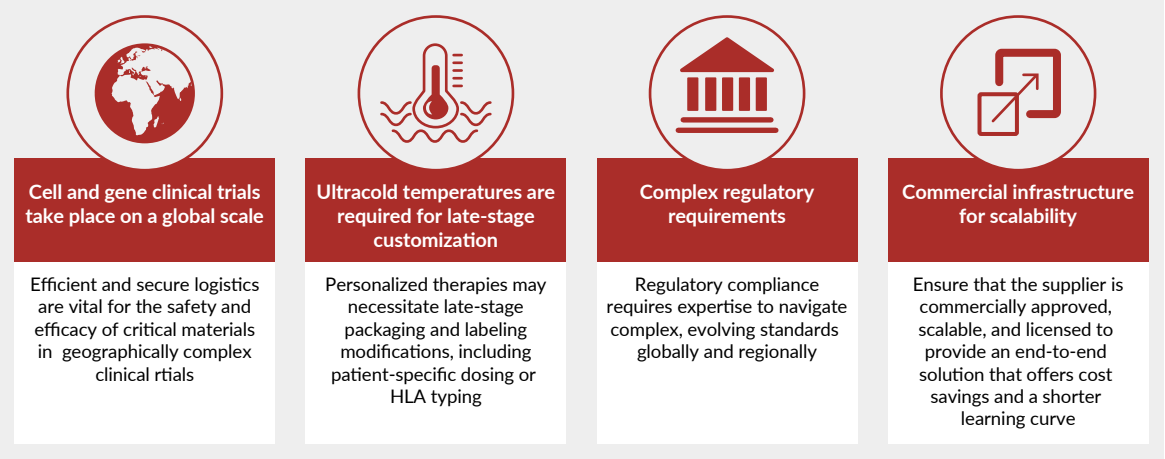
Precise, temperature-controlled packaging and labelling for clinical and commercial products, including custom and patient-specific labels, are a prerequisite for off the shelf CGTs. For example, when a patient is identified and a specific dosage needs to be prepared at late notice, it is crucial to have procedures in place to manage the short timeframe while minimizing temperature excursions in order to maintain product integrity and viability.

NAVIGATING COMPLEX REGULATORY REQUIREMENTS

As a relatively young industry, the CGT field faces evolving regulations that differ across

FIGURE 1

Considerations for temperature-sensitive CGT products transitioning from clinical to commercial stages.



countries and regions, making compliance something of a moving target. Regulatory requirements involve many complexities, depending on the market where the product is manufactured and sold. For example, UCC CGT products have unique regulatory requirements for packaging, distribution, import, export, customs clearance, duties, and taxes, all of which may vary from one country or region to another.

To overcome these challenges, it is crucial to involve a partner with expertise in implementing comprehensive Standard Operating Procedures (SOPs) based on both regional regulations and international best practices for robust Quality Management Systems (QMS) and the management of cold and UCC materials. For example, there are specific requirements for UCC Advanced Therapy Medicinal Products (ATMPs) in the EU market, including documentation to support the Qualified Person (QP) declaration.

ADDRESSING COMMERCIAL INFRASTRUCTURE CHALLENGES FOR SCALABILITY

The infrastructure required for the commercialization and distribution of UCC CGT products is not yet fully developed in terms of the scalability needed by the rapidly growing industry. While there are many service providers operating in the CGT commercialization and storage spaces, traditional supply chain and distribution networks are often inadequate for the specific needs of these products.

The key to overcoming this challenge is to start early in the clinical stage by engaging with a service provider that has a proven network and infrastructure for bringing UCC products to the market. Additionally, given the low volume and high value of CGT products, it is critical that they have experience in scaling up or down as needed. This flexibility is crucial both for adapting to fluctuating market demand and ensuring efficient operations.

An additional important aspect to consider is the implementation of integrated solutions to ensure efficient operations. Product handoffs can be minimized with an end-to-end solution. Having a one-stop shop for product receiving, importation, storage, late-stage customization, and distribution is beneficial in reducing unnecessary handoffs and associated risks.

Furthermore, such an integrated solution means that a service provider not only offers end-to-end services, but also leverages the entire supply chain. This may include the supply chain management of critical raw materials, consumables, and equipment. Such an integrated network provides better

visibility and planning, and again, reduces risks associated with extra handoffs.

KEY STEPS IN PARTNERING WITH A GLOBAL UCC PACKAGING AND LABELLING SERVICES PROVIDER

Firstly, the complexities of transitioning to the commercial stage mean that it is optimal to select a global UCC packaging and labelling partner as early as Phase 2 clinical development (especially if the product is on a fast-track regulatory pathway) and certainly no later than the commencement of pivotal clinical trials. It is advisable to then stay with the same service provider from clinical to commercial stages in order to reduce the learning curve and minimize handoffs.

Working with a service provider in this space generally begins with the project setup. During this phase, order requirements are gathered, including packaging materials, shipping lanes, and shippers (Figure 2).

Next, during the tech transfer stage, the batch record, master batch record, specific packaging, and SOPs are developed. Validation of necessary documentation for regulatory submissions is carried out, whether it is for an US FDA Biologics License Application (BLA) or other specific regulatory agency requirements. This process typically takes 6–9 months, depending on the complexity of the supply chain process. Another crucial element to consider is serialization: while serialization may not be the main focus during preclinical or early clinical development work, certain gene therapies and allogeneic cell therapies require serialization during tech transfer unless a waiver is obtained.

Next, once the Process Performance Qualification (PPQ) runs have been carried out and the documentation and process validation are finalized, the preparation for site inspection readiness occurs.

Once the site is inspection-ready and all regulatory documentation has been submitted, it is time to seek final approval from a regulatory body, which may inspect either the entire supply chain or select service providers within it. Once regulatory approval is granted, commercial execution commences.

INNOVATIONS IN COLD CHAIN PACKAGING AND LABELLING

To address the specific manufacturing and supply chain needs for UCC CGT products, unique packaging and labelling solutions are required.

For example, gene therapy products face challenges in the requisite minimization of exposure to CO_2 (particularly dry ice). This issue may arise during the packaging, labelling, and distribution processes. To address this issue, Thermo Fisher Scientific has developed CO_2-free labelling and packaging at −80 °C without the use of dry ice.

Another example involves the choice of packaging materials for UCC CGT products. Labels, cartons, and other packaging materials such as foam inserts must withstand UCC temperatures for extended durations in order to qualify for commercial application submissions. Thermo Fisher Scientific has developed proprietary validated packaging materials designed to ensure product integrity and performance at UCC temperatures.

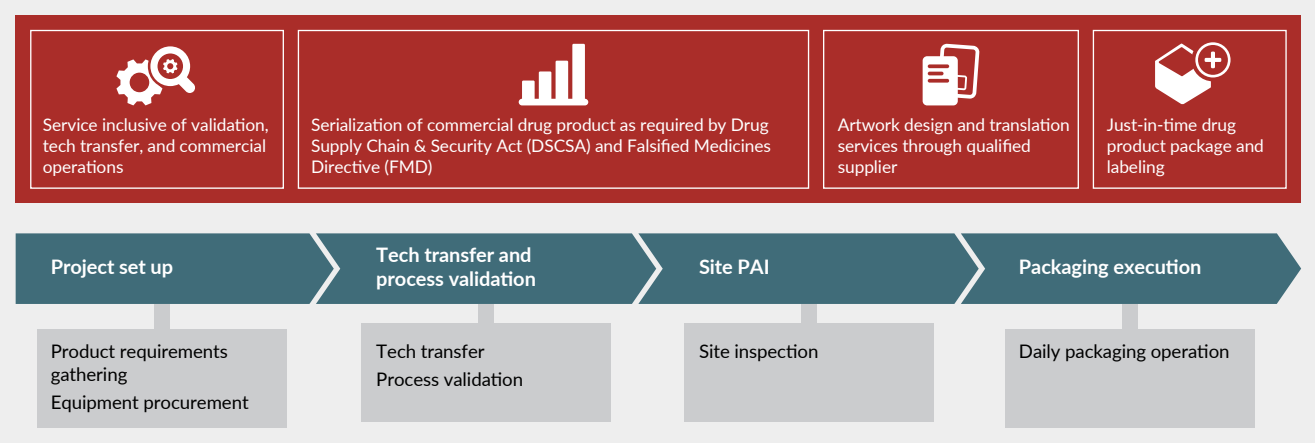
Lastly, as previously mentioned, one of the most challenging aspects of the delivery of off the shelf CGT products to patients is the requirement for late-stage customization. In order to apply or update a label for a product that is already in a frozen state without compromising temperature integrity, Thermo Fisher Scientific has developed UCC product labelling to eliminate temperature excursions.

CASE STUDY: A FIRST-IN-CLASS ALLOGENEIC CELL THERAPY APPROVED IN THE EU

Thermo Fisher Scientific supported the transition of an allogeneic cell therapy product

FIGURE 2

Timeline of commercial packaging development for UCC CGT products to support clients in the USA and EU.



from clinical to commercial stages and provided clinical support for the developer's global trials. This process involved supporting the developer's Phase 3 open-label studies, which required just-in-time (JIT) packaging and distribution to clinical sites in both the USA and the EU. More specifically, the requirements included JIT packaging in a 3-day turnaround time, variable information on secondary package labels, multiple doses and shipments per patient, and packaging in cryotemperatures such as −196 °C.

In order to meet these requirements, Thermo Fisher Scientific applied the innovations and solutions mentioned above, completing JIT late-stage packaging, labelling, and distribution to support the client's global clinical trials in the USA, EU, and other regions.

Beyond clinical support, Thermo Fisher Scientific collaborated on the tech transfer process, identified critical steps, supported SOP development, designed processes, and conducted validation during PPQ runs to ensure site readiness. Ultimately, Thermo Fisher Scientific successfully supported the product's commercial launch in the EU, beginning patient shipments in early 2024.

In summary, the cold chain innovations developed by Thermo Fisher Scientific ensured on-time delivery for every shipment with zero time spent outside of the required product temperature range, thus enabling the commercial success of an allogeneic cell therapy product.

Q&A

Susan Li

Q Do you provide secondary packaging and labelling for products at dry ice or liquid nitrogen (LN_2) temperatures?

SL: At Thermo Fisher Scientific, we provide secondary packaging and labelling services for clinical and commercial CGT products at −80 °C and cryogenic temperatures to reduce temperature excursions. We also provide labelling services for primary vials, if needed.

Q Can you provide real-time data visibility for the product and its transportation?

SL: When dealing with patient-specific materials that are precious or irreplaceable, it is crucial to have real-time visibility throughout the transportation and distribution process. We have developed a proprietary platform that provides in-house tracking for all global shipments. Regardless of which service provider you use, our network includes over 40 providers and integrators.

All shipments have visibility through our real-time track-and-trace platform, including GPS tracking and temperature sensors. This ensures we know the exact location of the product at all times, allowing us to make quick decisions to deliver the products to patients in the most efficient way.

Q What services do you offer for commercialization and in which markets?

SL: Currently, we provide packaging and labelling services for commercial products in the USA and EU markets, but these locations can also support other market needs following

detailed discussions. Our commercial services include primary and secondary packaging, labelling, storage, distribution, and QP services.

All of our sites have distribution and third-party logistics provider licenses, enabling direct shipment from the manufacturing site to the patient location. Additionally, we offer transportation services.

Q What regulatory services do you provide for ATMPs in the EU?

SL: We offer ATMP QP services, including the appropriate licenses and permits for importation and conducting relevant activities onsite. We also support important export duties, tax management, and provide regulatory consulting services. Additionally, our tech transfer process ensures the required documentation for regulatory submissions.

Finally, we partner with experts for artwork design and management, and handle serialization requirements to support a successful CGT product transition from clinical to commercial stages.

BIOGRAPHY

SUSAN LI joined Thermo Fisher BioServices and Specialty Logistics, Rockville, MD, USA in November 2020 as Director of Customer Solutions and SME for Cell and Gene Therapy. She and her team develop innovative and customized solutions to support many clients to conduct global clinical trials and commercial distribution. Susan was promoted to the Senior Director of Client Services and Solution Service in May 2023. In her new role, Susan will increase collaboration across the group to provide best-in-class support of customer experience and onboarding. Susan bought in-depth knowledge in cell therapy supply chain solutions from her role at Celgene (now Bristol Myers Squibb) as Director of Cell Therapy Logistics. She developed clinical and commercial supply chain strategy to support the launch of autologous CAR-T cell therapy products. Susan has extensive experience as senior healthcare cold chain logistics strategist at United Parcel Service. Susan also has marketing commercial operation experience from Abbott Laboratories and drug discovery research knowledge from AstraZeneca as a molecular biologist.

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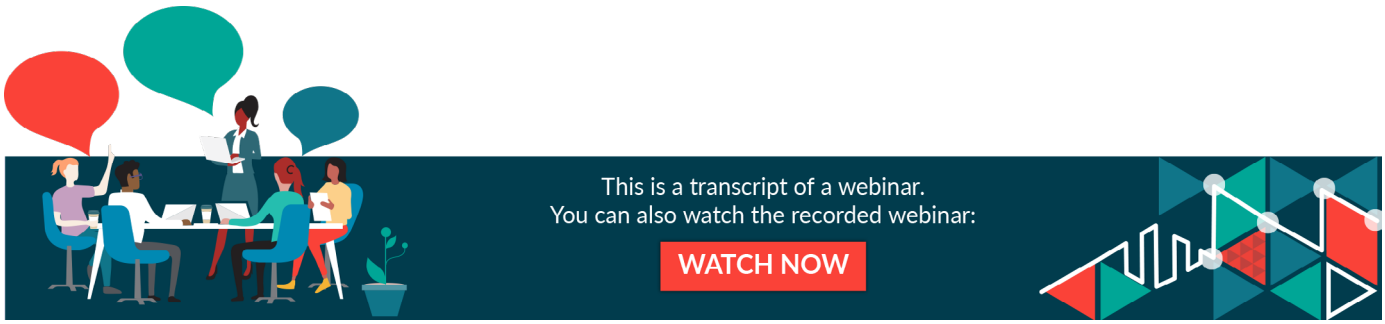

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- Secondary cold chain packaging and labeling
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CELL & GENE THERAPY INSIGHTS

SCALE-UP/SCALE-OUT OF CELL & GENE THERAPY MANUFACTURING

SPOTLIGHT

Decentralizing gene and cell therapy manufacturing: changing tides or uphill battle

Magdi Elsallab
Massachusetts General Hospital and Harvard Medical School

"A concerted effort must be made to decentralize manufacturing, particularly for autologous products, and to adapt the regulatory framework to reflect the unique nature of [cell and gene] therapies."

VIEWPOINT

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INTRODUCTION

The pharmaceutical landscape is rapidly evolving with the advent of advanced therapies, including gene and cell therapies. These therapies, heralded as game-changers, are transforming the treatment of a wide range of diseases, from hematologic malignancies and monogenic disorders to autoimmune diseases. The promise of

personalized, highly targeted treatment options has garnered substantial interest from researchers, clinicians, and patients alike. As of today, the US FDA has approved over 30 advanced therapies, with expectations that this number will continue to rise in the coming years [1].

However, as this field continues to mature, especially in the context of autologous therapies, a crucial question remains: How can we ensure the timely and consistent supply of these life-saving therapies to those who need them? To address this challenge, many stakeholders have begun advocating for the decentralization of manufacturing, particularly for autologous products. The ability to decentralize, coupled with regulatory flexibility, may be key to unlocking the full potential of gene and cell therapies.

CHALLENGES IN THE CURRENT MANUFACTURING LANDSCAPE

Gene and cell therapies, particularly those based on autologous starting material, present a unique set of challenges that traditional pharmaceutical supply chains did not evolve to handle. Unlike small molecules and biologics such as antibodies and recombinant proteins, which are produced in large batches for multiple patients, autologous products require individualized manufacturing for each patient. This process is time-sensitive, expensive, and logistically complex, often resulting in delays that could critically affect patient outcomes [2].

A significant example of the impact of these challenges is the current state of BCMA CAR T-cell therapies. Studies have shown that only 40% of patients scheduled for commercial BCMA CAR T products receive their therapy within one year, and tragically, 25% of patients die before receiving the product [3]. These stark figures underscore the urgent need for faster access to life-saving products and build the case for alternative models that can fulfill the growing demand.

THE CASE FOR DECENTRALIZATION

Decentralized manufacturing models offer a potential solution to some of these challenges. By bringing the manufacturing process closer to the patient or at the point-of-care, this could reduce logistical burdens and improve the timeliness of therapy delivery, with potential for cost savings. The question then becomes, how can a product commercialized under a single trade name be deemed of comparable quality across a decentralized network of manufacturing sites?

Establishing minimum quality criteria focused mainly on an equivalent safety profile for the products could be a reasonable ask. On the other hand, setting parameters for product identity, viability, and biological activity could be more challenging. Validation of the comparability of the process instead of the products could be an approach to qualify the sites, proving that all the products are produced using the same process. The current generation of automated manufacturing platforms might struggle with these requirements, as there is still a chance of operator variability and human error when interacting with the machine. This will become much easier with the implementation of several novel technologies that incorporate robotic arm approaches into the manufacturing process of these therapies. Cellares' Cell Shuttle is an example of a robotic arm unit that can be deployed in a decentralized model, eliminating operator variability from the production process [4]. Multiply Labs' robotic arm system could also be an answer for implementing these units within hospitals without the need for a clean environment.

THE US REGULATORY ECOSYSTEM: UNIQUE CHALLENGES

The reimbursement system in the US differs significantly from that of other countries due to its reliance on a combination of private insurers and government programs, rather

than a national health insurance model. This multi-payer structure and open-market dynamics introduce complexities in implementing decentralized manufacturing, creating both logistical challenges and financial barriers. While the FDA has taken steps to adapt its regulatory framework for advanced therapies, there is room for advancement in decentralized manufacturing adoption.

One of the primary obstacles is that gene and cell therapies are still viewed through the lens of traditional biologics manufacturing. While gene and cell therapies share similarities with biologics, they also incorporate key elements from transfusion medicine and transplantation. To truly unlock the potential of gene and cell therapies, the pharmaceutical industry, regulatory bodies, and payers must recognize the fundamental differences between these therapies and traditional biologics.

THE EVOLVING LANDSCAPE OF DECENTRALIZED MANUFACTURING WORLDWIDE

To ensure the success of decentralized manufacturing, national support and large-scale initiatives are essential. Several countries are already moving toward a national implementation of decentralized models. The UK's Medicines and Healthcare products Regulatory Agency (MHRA) has adopted a policy to implement point-of-care manufacturing [5]. In Canada, a coalition has been formed under the Canadian-led Immunotherapies in Cancer (CLIC) to produce locally manufactured CAR-T products [6]. A program was launched in the European Union to expand academically developed decentralized manufacturing to other EU countries. The CAR-T cell product (ARI-0001), which was successfully developed at the University of Barcelona and is currently manufactured in a network of hospitals across Spain under the hospital exemption, became one of the drugs participating in this EU pilot program [7]. Additionally, Caring Cross has partnered with the Fundação Oswaldo Cruz (Fiocruz) Foundation in Brazil and ImmuneAct in India to deliver locally manufactured, affordable CAR-T cell therapies.

INDUSTRY-DRIVEN DECENTRALIZATION OF MANUFACTURING

A growing question is whether there is a commercial case for decentralized manufacturing. Can the pharmaceutical industry evolve this model to improve access and maintain the commercial viability of products? A few companies are beginning to test this model, including early movers like Galapagos, which is working on implementing decentralized manufacturing approaches on a commercial scale. The company has been running several clinical studies across Europe and the United States. Early results indicate a significant reduction in turnaround time, achieving a seven-day vein-to-vein turnaround time [8]. However, the question remains as to how well such an approach can be implemented on a wider scale, given the requirement of setting up a dedicated space within a healthcare facility or a local manufacturer to manage the operations and implement the quality management system.

CONCLUSION: A CALL FOR CHANGE

Gene and cell therapies represent one of the most promising areas of modern medicine, with the potential to treat and even cure previously untreatable diseases. However, the current centralized manufacturing model, coupled with the regulatory environment, might limit their accessibility and scalability. A concerted effort must be made to decentralize manufacturing, particularly for autologous products, and to adapt the regulatory framework to reflect the unique nature of these therapies.

The international successes in point-of-care manufacturing demonstrate that this approach is both feasible and beneficial. Now,

it is up to regulators, pharmaceutical companies, and healthcare providers to embrace this model and work collaboratively to bring these life-saving therapies to more patients, faster. As gene and cell therapy continues to evolve, so must the systems that support it. Only then can we ensure that these groundbreaking treatments are accessible to all who need them, regardless of geographic or financial barriers.

REFERENCES

1. FDA. Approved cellular and gene therapy products. US Food & Drug Administration. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products (accessed Oct 1, 2024).

2. Elsallab M, Maus MV. Expanding access to CAR T cell therapies through local manufacturing. *Nat. Biotechnol.* 2023; 41(12), 1698–1708.

3. Al Hadidi S, Szabo A, Esselmann J, *et al.* Clinical outcome of patients with relapsed refractory multiple myeloma listed for BCMA directed commercial CAR-T therapy. *Bone Marrow Transplant.* 2022; 58(4), 443–445.

4. Abou-El-Enein M, Elsallab M, Feldman SA, *et al.* Scalable manufacturing of CAR T cells for cancer immunotherapy. *Blood Cancer Discov.* 2021; 2(5), 408–422.

5. MHRA. *Consultation on Point of Care Manufacturing.* Aug 12, 2021; Medicines & Healthcare Products Regulatory Agency. https://www.gov.uk/government/consultations/point-of-care-consultation/consultation-on-point-of-care-manufacturing.

6. Kekre N, Hay KA, Webb JR, *et al.* CLIC-01: manufacture and distribution of non-cryopreserved CAR-T cells for patients with CD19 positive hematologic malignancies. *Front Immunol.* 2022; 13, 1074740.

7. Juan M, Delgado J, Calvo G, Trias E, Urbano-Ispizua Á. Is hospital exemption an alternative or a bridge to european medicines agency for developing academic chimeric antigen receptor T-Cell in Europe? Our experience with ARI-0001. *Hum. Gene Ther.* 2021; 32(19–20), 1004–1007.

8. Kersten MJ, Saevels K, Beguin Y, *et al.* Seven-Day Vein-to-Vein Point-of-Care Manufactured CD19 CAR T Cells (GLPG5101) in Relapsed/Refractory NHL: Results from the Phase 1 Atalanta-1 Trial. Poster presented at: American Society of Hematology, Washington, DC, USA. Dec 9, 2023.

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CELL & GENE THERAPY INSIGHTS

SCALE-UP/SCALE-OUT OF CELL & GENE THERAPY MANUFACTURING

SPOTLIGHT

INNOVATOR INSIGHT

Mastering AAV production: best practices for small to large-scale manufacturing success and reduced development times

Emily Jackson-Holmes

The article discusses the optimization and scalability of AAV production, highlighting the primary challenges in small-scale AAV production, such as long lead times, low titers, and high costs. The article further discusses the scalability of AAV production to commercial scales while maintaining performance and quality, and it presents best practices for achieving high AAV titers with a particular focus on the transfection step and optimizing plasmid ratios.

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ADDRESSING THE CHALLENGES IN SMALL-SCALE UPSTREAM AAV PRODUCTION

AAV vectors are widely used in *in vivo* gene therapies due to their efficient gene delivery and scalable manufacturing, which enhances accessibility for patients. However, specific considerations related to the manufacturing process must be addressed in order to optimize AAV production.

The first key challenge in small-scale AAV production is the long lead times required to establish the AAV production process.

This challenge can be mitigated through fit-for-purpose solutions specifically designed for AAV manufacturing, together with pre-optimized and streamlined protocols for rapid implementation

The second common challenge is obtaining low titers and a low percentage of full particles from the upstream process. To address this issue, it is necessary to implement a complete production system with integrated components that work together to deliver maximal AAV titers.

Thirdly and finally, cost remains a significant concern. Manufacturing costs can be reduced by increasing productivity and lowering the amount of DNA used during transient transfection.

The Gibco™ AAV-MAX Helper-Free AAV Production System (AAV-MAX system) is a complete system for scalable, high-titer AAV production. It contains critical components required for AAV production through suspension-based transient transfection in mammalian cells. Additionally, all of these components are fit-for-purpose for gene therapy applications.

SHORTENING DEVELOPMENT TIMELINES AND OPTIMIZING COST–EFFECTIVENESS IN SMALL-SCALE AAV PRODUCTION

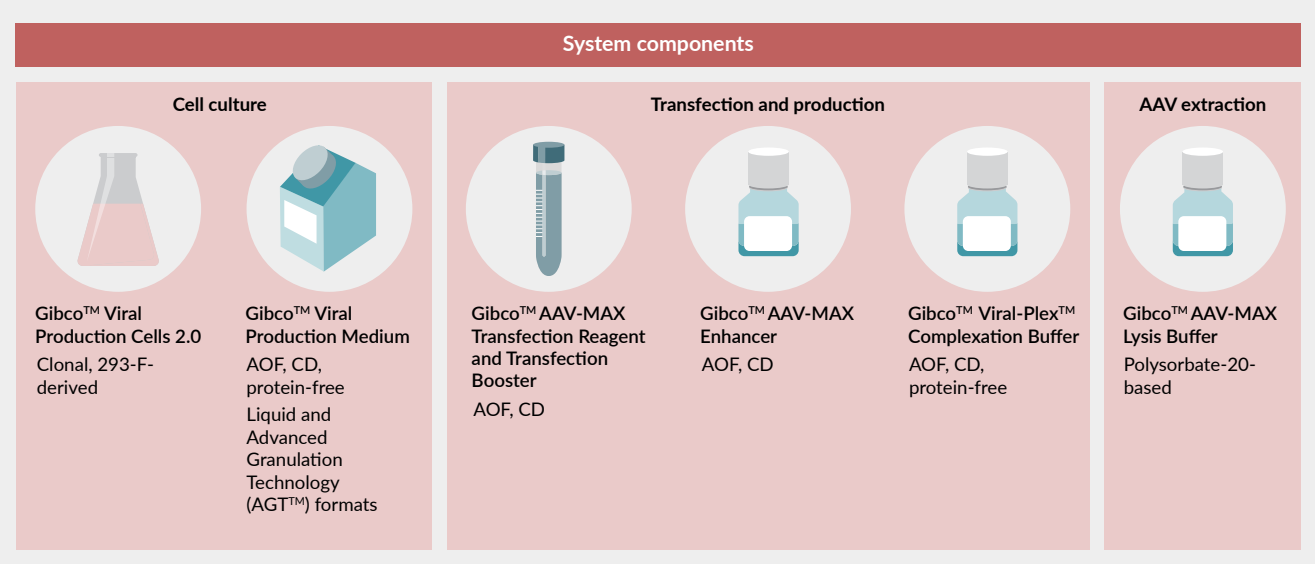
Establishing a new AAV upstream process can be both time-consuming and expensive due to the necessity of identifying, testing, and optimizing each component to achieve high vector quality while minimizing cost. The AAV-MAX system addresses these challenges by ensuring all system components are optimized to work together seamlessly, with streamlined protocols that are easy to implement.

Figure 1 illustrates the components of the AAV-MAX system. The first component is the Gibco™ Viral Production Cells 2.0, a clonal 293F-derived suspension cell line. These cells are cultivated during cell expansion and AAV production in the Gibco™ Viral Production Medium. This medium is animal-origin-free, chemically defined, and protein-free, available in liquid and Advanced Granulation Technology™ (AGT) options.

The AAV-MAX system utilizes several key components for the plasmid DNA

FIGURE 1

Components of the AAV-MAX system.



AOF: animal origin-free, CD: chemically defined.

transfection step. The primary components include the Gibco™ AAV-MAX Transfection Reagent, which is a cationic lipid-based reagent. Alongside this, the Gibco™ AAV-MAX Transfection Booster works with the transfection reagent to enhance the entry of the plasmid DNA into the cells. During this step in the protocol, the Gibco™ ViralPlex Complexation Buffer is employed for the dilution of the plasmid DNA. This buffer is chemically defined and animal-origin-free. Additionally, the Gibco™ AAV-MAX Enhancer is introduced into the cultures at the time of transfection to further amplify the production of AAV.

At the end of a production run, the Gibco™ AAV-MAX Lysis Buffer is used to extract the AAV from the cells. This Polysorbate 20-based buffer is also animal-origin-free and chemically defined.

Increasing AAV productivity in the upstream process remains of critical importance due to the high dose requirements for *in vivo*-based gene therapies, especially for systemically administered therapies and those targeting large patient populations. The AAV-MAX system was specifically developed to meet the productivity needs of gene therapy developers. The graph in Figure 2 depicts AAV titers measured in crude samples via droplet digital (dd)PCR across five AAV serotypes. The titers are in the 10^{11} vg/mL range for all five serotypes.

High titers were consistently achieved across various bench-scale production volumes, alleviating concerns about titer loss during scale-up. However, it is not just titer that matters: the key quality attributes of the particles are also crucial, including the percentage of full particles.

The data set in Figure 3 provides an in-depth analysis of titer and percentage full capsid across four AAV serotypes, comparing the performance of the AAV-MAX Transfection Kit to other available AAV transfection reagents.

The left column of graphs shows titers from crude samples measured using ddPCR. The right column of graphs shows the percentage of full particles measured by mass photometry. All studies were conducted in 24 deep-well plates in triplicate. In each graph, the AAV-MAX condition is the bar to the far left. For this condition, the complete AAV-MAX system was used per the recommended protocols. For each of the three other conditions (FectoVIR-AAV, PEIpro, and TransIT-VirusGEN), the AAV-MAX Transfection Kit was replaced with the respective reagent, following the vendor's recommendations.

Additionally, a Design of Experiments (DOE)-based approach was used to identify the optimal plasmid ratio for each transfection reagent. The data presented in Figure 3 reflects the optimized plasmid ratio for each reagent for each stereotype. It can be further seen that the full AAV-MAX system achieved the highest titers across all four serotypes compared to the other transfection reagents. In terms of percentage full particles, the AAV-MAX system achieved approximately 20–50% full particles in crude samples, depending on the serotype. These values were equivalent to or higher than those obtained with other transfection reagents. This study demonstrates that the AAV-MAX system

FIGURE 2

AAV titers measured in the crude samples of five serotypes in the 1×10^{11} vg/ml range.

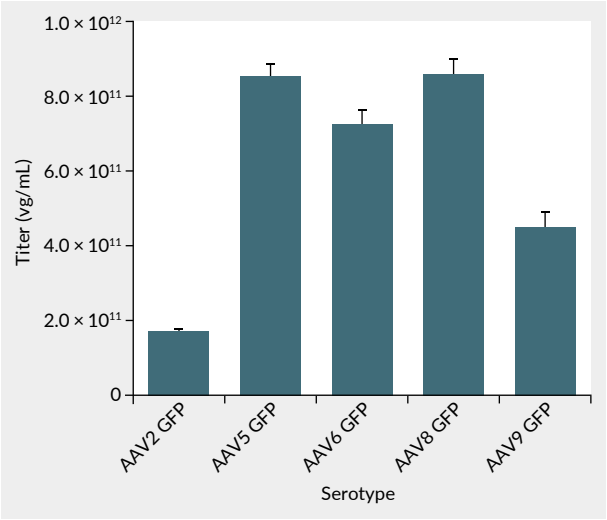


FIGURE 3

Bar graph analysis of AAV titers (determined by ddPCR) and percent full particles (determined by mass photometry) across four AAV serotypes.

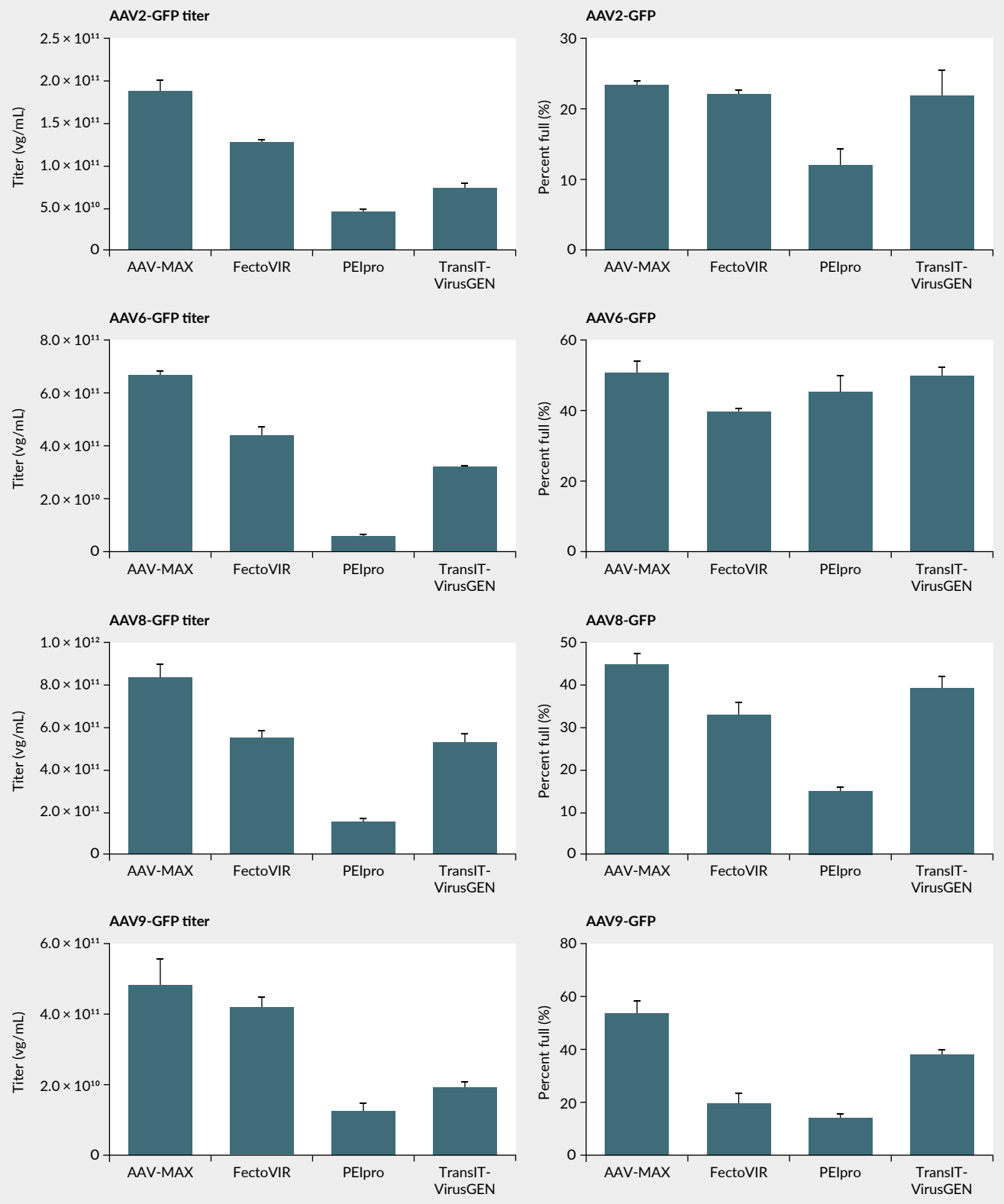
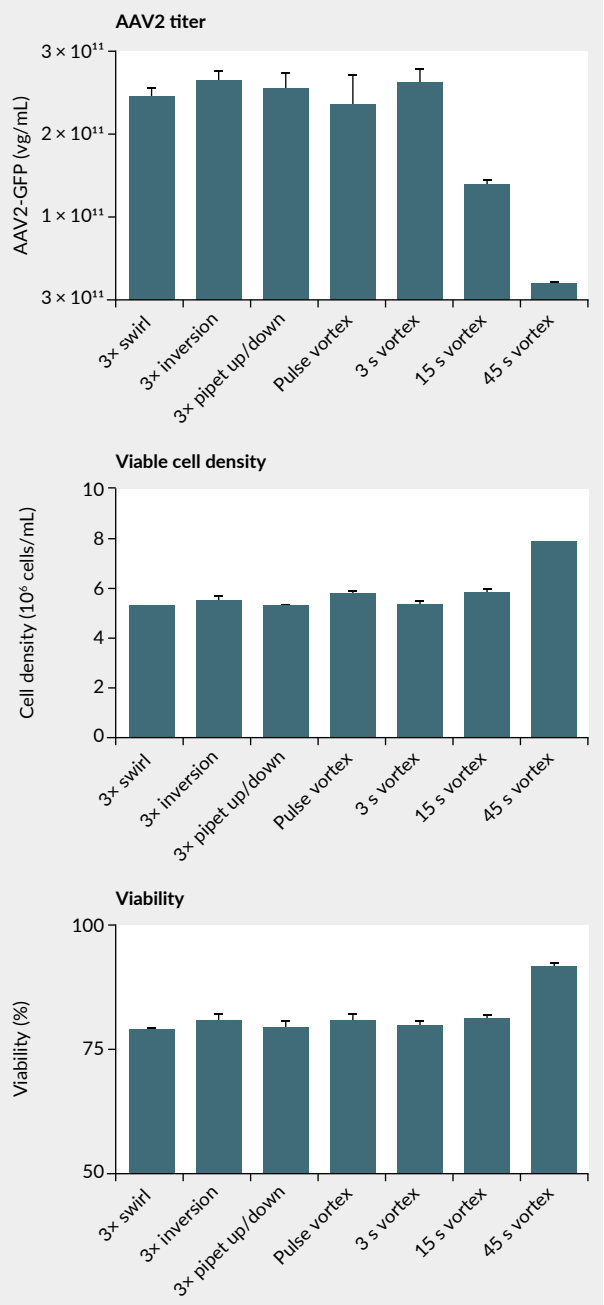


FIGURE 4

Impact of various mixing techniques on AAV2 titer, viable cell density, and viability.



Parameter	Value
Format	125 mL shake flasks
Culture volume	30 mL
DNA	1.5 µg/mL
GOI:RC2:Help	1:4:4 mass ratio
Complexation time	20 min

improves both productivity and percentage full particles compared to other available transfection reagents.

Further, the impact on manufacturing and development costs, which are crucial considerations for AAV researchers and gene therapy developers alike, can be considered. The aforementioned increases in productivity and quality result in cost savings in two key areas: firstly, the cost per amount of AAV produced in the upstream process is reduced; secondly, downstream costs associated with purifying out empty particles are also lowered. Together, these factors lead to a reduction in the cost per AAV therapeutic dose.

Additional cost savings can be achieved as the AAV-MAX Transfection Reagent uses up to 25% less plasmid DNA than other transfection reagents. Lastly, using a pre-optimized system reduces the lead time required to establish a new AAV process, further lowering development costs.

OPTIMAL PRACTICES FOR HIGH AAV TITERS: TRANSFECTION

The transfection step is critical in the upstream workflow and significantly impacts overall process performance. For the complexation of the transfection reagent with plasmid DNA, key parameters include the method of mixing the transfection reagent, the temperature of the reagents during this step, and the timing of the incubation of the reagent and DNA before adding the complex to the cell culture.

For small-scale AAV production, such as in multi-well plates and shake flasks, the transfection step should be performed at room temperature with the AAV-MAX Transfection Kit. Gentle mixing of the AAV-MAX Transfection Reagent with the DNA is recommended, with a 20–30-minute incubation time. Figure 4 shows data demonstrating relative performance of various methods for gentle mixing of the AAV-MAX Transfection Reagent and AAV-MAX Transfection Booster with the DNA, including swirling, inversion, and gentle pipetting. The titer data

indicated that all gentle mixing techniques were effective except for longer periods of vortexing, which can disrupt the transfection reagent/plasmid DNA complex.

Another critical aspect of transfection that significantly impacts both titers and percentage full particles is the ratio of plasmids used in the process. For triple transfection, optimizing the ratio of plasmids specific to the application is crucial.

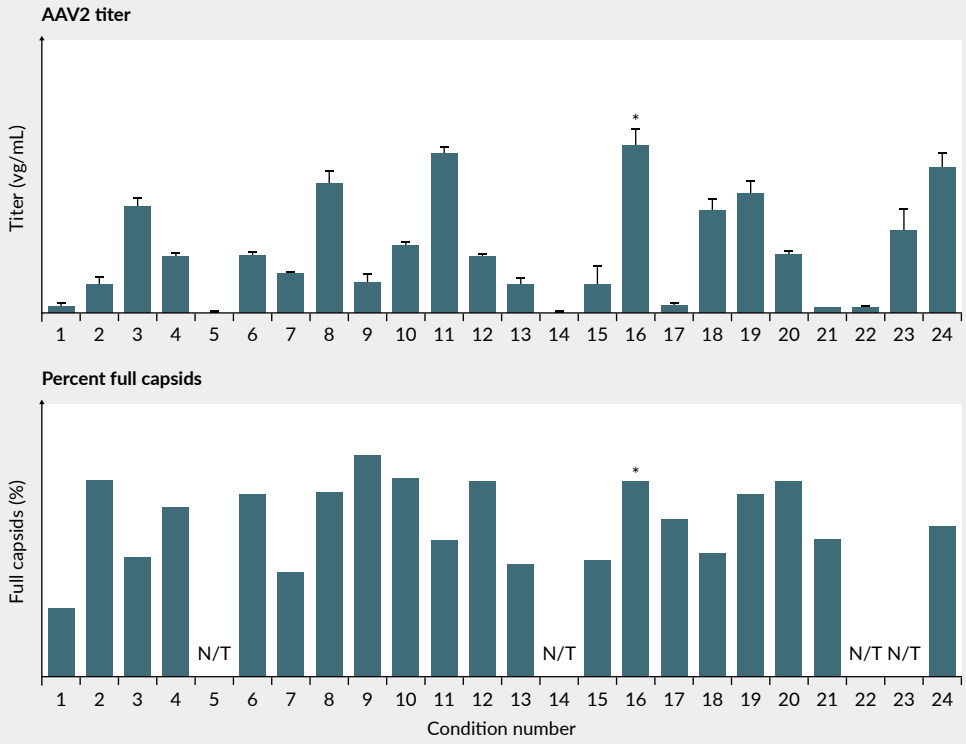
Figure 5 depicts the impact of plasmid ratio on the AAV2-GFP production using the full AAV-MAX system in 24 deep-well plates. A DOE study was performed with 24 conditions, evaluating titers via ddPCR and percentage full capsids via mass photometry, respectively. This data is shown in the bar graphs. Subsequently, statistical modeling software was utilized to analyze the experimental results and determine the plasmid ratio that optimized both titer and percentage full capsids. The outcomes of that modeling are presented in the table at the bottom of Figure 5. The plasmid ratio identified by the model closely resembled condition 16, which exhibited the highest titer and a favorable percentage full capsids.

ADDRESSING LARGE-SCALE AAV PRODUCTION NEEDS

Scaling-up an upstream process whilst maintaining performance and reproducibility remains challenging. As a result, Thermo Fisher Scientific has made significant investments in addressing

FIGURE 5

Effects of varying plasmid molar ratio of AAV2 titer and percentage full capsids, and the subsequent optimal ratios.

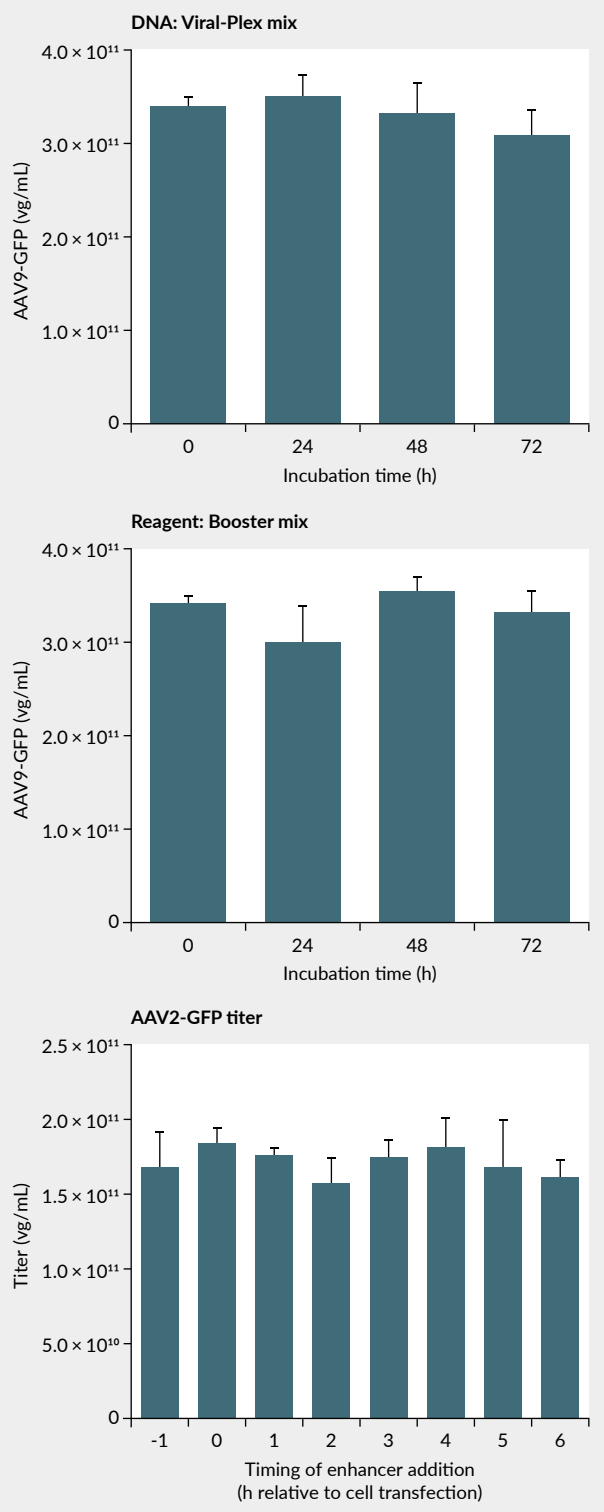


Plasmid	Optimal molar ratio	Optimal mass ratio
pITR-GFP	1	1
pRepCap2	4.4	5.6
pHelper	1.4	3.4

scalability challenges through product design, recommended protocols, and making comprehensive technical support resources available.

FIGURE 6

Flexibility without compromise of robustness in premixed transfection kits and the timing of enhancer addition.



From the outset, the AAV-MAX system was designed with an inherently scalable, suspension-based upstream process in mind. The system features a clonal 293F-derived cell line that is known for robust growth and scalability. Additionally, consumable components are available in configurations suitable for large-scale production. This includes off-the-shelf formats capable of supporting production up to 100 L, as well as larger-scale formats with further customization options. Furthermore, the media are made available in both liquid and proprietary Advanced Granulation Technology (AGT™) medium format, specifically designed to support large-scale manufacturing needs.

Scalability in a GMP manufacturing environment requires protocols that offer flexibility without compromising robustness. Two examples of this flexibility can be seen in Figure 6. Firstly, with the AAV-MAX Transfection Kit, studies were conducted demonstrating that certain steps can be prepared in advance, such as diluting plasmid DNA and mixing the AAV-MAX Transfection Reagent and Booster. The bottom graph of Figure 6 illustrates that titers of AAV9-GFP remain consistent, whether these steps are performed at the time of transfection or up to 72 hours beforehand.

Secondly, flexibility was demonstrated in the timing of AAV-MAX Enhancer addition. This enhancer, added at the transfection stage to enhance titers, can be introduced one hour before transfection or up to six hours after, as depicted in the top two graphs of Figure 6. This flexibility maintains consistent titers across different timing scenarios.

OPTIMIZATION OF A SCALABLE COMPLEXATION PROCESS: CASE STUDY 1

Another critical aspect to be addressed is the transfection step, which, while pivotal to achieving high titers, can be challenging to scale effectively. A series of studies was conducted to explore the optimization of

large-scale transfection using the AAV-MAX Transfection Kit with the broader AAV-MAX system.

The initial focus was on optimizing the mixing of the AAV-MAX Transfection Reagent and Transfection Booster with plasmid DNA. Various approaches were studied, including mixing in bioprocessing bags on a rocker platform and mixing in stirred-tank bioreactors. Mock transfections were first conducted using colorimetric reagents to identify strategies that achieved homogenous mixing. Subsequently, real transfections were performed under optimized conditions to validate the findings.

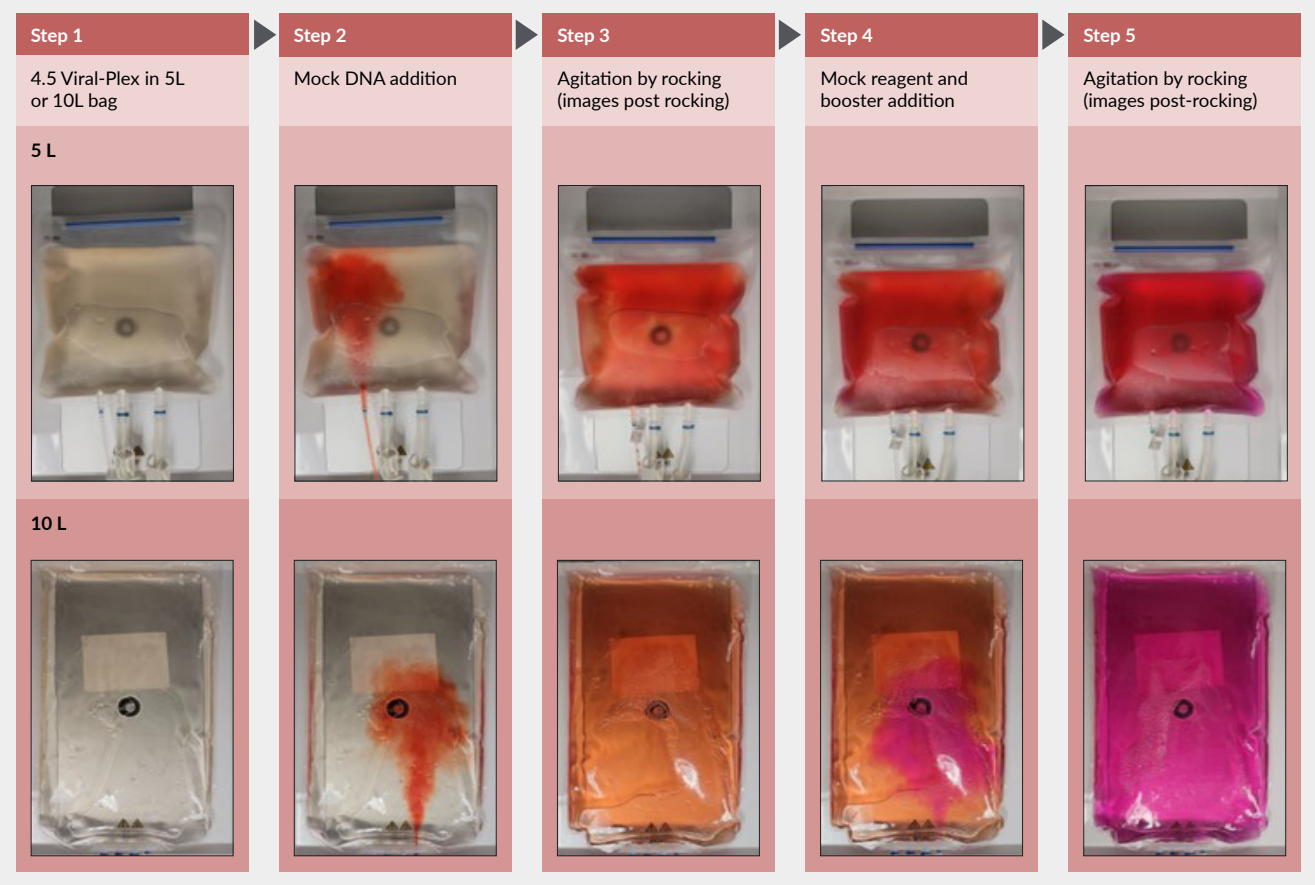
Throughout these studies, key parameters such as agitation conditions, container headspace, reagent temperature, and incubation timing, were carefully considered and adjusted as necessary.

In the first series of studies, transfection reagent-plasmid DNA mixing was conducted in bioprocessing bags on a rocker platform. In these studies, 5 L complexation reactions were carried out, which corresponds to the volume required for a 50 L AAV production run. Figure 7 depicts two mock complexations with colorimetric reagents, which were instrumental in determining the best strategy to achieve homogenous mixing.

The images on the top row of Figure 7 follow a 5 L mock complexation that was performed in 5 L bags. Firstly, 4.5 L of Viral-Plex Complexation Buffer was added to a 5 L bioprocessing bag (step 1). Next, a solution of phenol red was used to simulate the

FIGURE 7

Mock transfections using colorimetric reagents in 5 L (top) and 10 L (bottom) bioprocessing bags for a 50 L AAV production scale run.



dilution of DNA in a Viral-Plex Complexation Buffer. The DNA was added at a flow rate of 400 mL/minute (step 2). Following DNA addition, the platform was turned on to mix at 12 °C and 20 RPM for a minute (step 3). The mock DNA was not adequately mixed immediately after pumping, and even by the end of the rocking period, the mock DNA remained visibly unevenly distributed, as evidenced by the non-uniform red color in the image.

Next, a solution of 1N NaOH was pumped in to mimic the AAV-MAX Transfection Reagent and Booster (step 4). This was done under the same settings followed by rocking for one minute. If homogenous mixing has occurred, the color should have transitioned from red to magenta. However, after rocking was complete, the solution mainly remained red with only some magenta visible at the corners of the bag (step 5). Overall, this mock complexation study revealed that performing a 5 L complexation in a 5 L bag under these conditions did not achieve optimal mixing.

In the bottom row set of images in Figure 7, a mock complexation of the same 5 L volume was performed; however, this time in a 10 L bioprocessing bag. Firstly, 4.5 L of Viral-Plex Complexation Buffer was added to a 10 L bioprocessing bag (step 1). The second and third images show the addition of mock DNA to the buffer (steps 2–3). The third and fourth images depict the subsequent addition of the mock transfection reagent and booster to the diluted mock DNA (steps 3 and 4). In both sets of steps, the initial pumping step achieved better distribution of the reagents compared to the 5 L bag study. Once rocking began, performed 5–10 times, a homogenous mixture was fully achieved as indicated by the color changes. After the addition of the mock DNA solution, the solution appears homogeneously red. Following the addition of the mock reagent booster, the color shift from red to magenta indicated complete mixing.

Overall, through this mock complexation study, optimal mixing conditions using a bioprocessing bag on a rocking platform were identified. The increased headspace provided by the 10 L bag enabled superior mixing results.

OPTIMIZATION OF A SCALABLE COMPLEXATION PROCESS: CASE STUDY 2

In a second set of studies, the use of a stirred-tank bioreactor for mixing the transfection reagent and booster with the plasmid DNA was investigated. Similar to the previous studies, this was firstly performed with mock transfections using colorimetric reagents to optimize mixing conditions before proceeding to real transfections.

In this experiment, a 2 L complexation reaction was performed in a 3 L stirred-tank bioreactor, a reaction volume suitable for a 20 L AAV production run. Firstly, a mixture of plasma DNA and Viral-Plex Complexation Buffer was added to the reactor. The Viral-Plex was added first before the drive shaft was turned on and mock DNA was pumped into the reactor via the bottom siphon with a 12-second transfer time.

Subsequently, the AAV-MAX Transfection Reagent and Booster mixture were introduced through the bottom port. Throughout this step, the drive shaft remained on. As the mixture was added, cloudiness began to form, indicating the formation of the transfection reagent and DNA complex. After one minute of AAV-MAX Transfection Reagent and Booster mixture addition, the mixture underwent an additional minute of mixing. Following this, the drive shaft was turned off to allow static incubation.

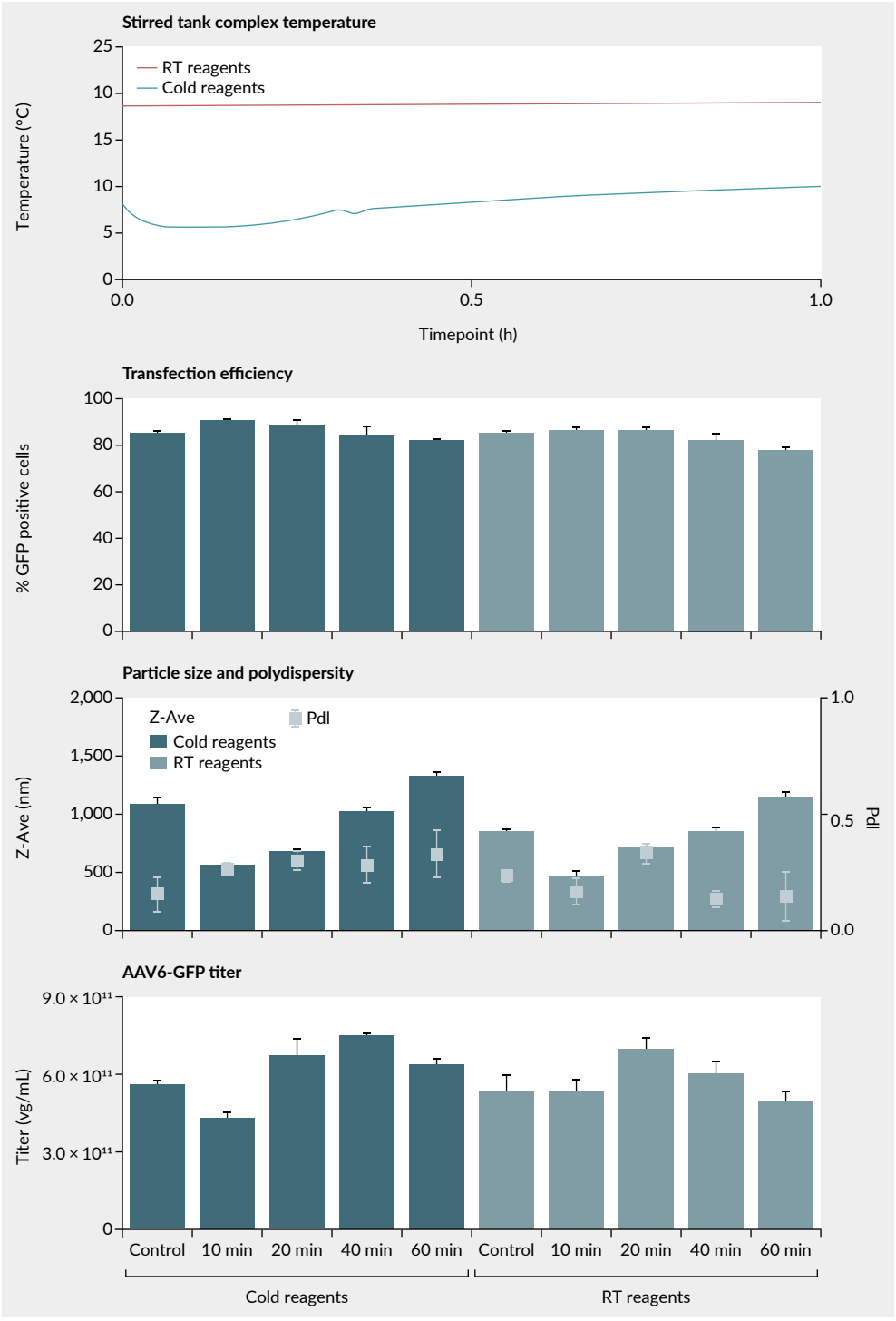
To validate the successful mixing strategy demonstrated in stirred-tank bioreactors, a time course study was performed. Two complexation mixing experiments were performed using 2 L and 3 L reactors with reagents maintained either at cold (4 °C) or room temperatures. Cold reagents were brought to room temperature immediately before use. The addition of the AAV-MAX Transfection Reagent and Booster and plasmid DNA, followed by mixing, was executed

exactly as in the previous study. As a control, complexation was also conducted in 50 mL conical tubes.

At four intervals spanning one hour in total, complexes were removed from the reactor and analyzed using Dynamic Light

FIGURE 8

Complexation kinetics in a 3 L stirred-tank bioreactor.



Scattering (DLS) to assess particle size and polydispersity. In parallel, complexes from the same time intervals were used to transfect 125 mL cultures for evaluation of both transfection efficiency and AAV6-GFP titers.

The 3rd graph in Figure 8 illustrates particle size and polydispersity for the two complexation conditions. Particle size increased over time (represented by the dark blue bars), while polydispersity indices (shown as light blue dots) remained below 0.5 for both cold and room temperature reagents.

Transfection efficiency and crude titers are depicted in the 2nd and 4th graphs of Figure 8. Complexes collected at the 20- and 40-minute intervals produced the highest titers and had particle sizes ranging from 700–1,000 nm. A decrease in transfection efficiency and AAV titers was noted at the 60-minute time point, correlating with the highest complex particle size diameter.

This study confirmed the optimal mixing conditions for complexation in stirred-tank bioreactors. Furthermore, it demonstrated that both cold and room temperature reagents can be effectively used, with a 20–40-minute incubation period following mixing yielding the highest titers.

IMPACT OF PUMPING ON PARTICLE SIZE AND AAV PRODUCTION

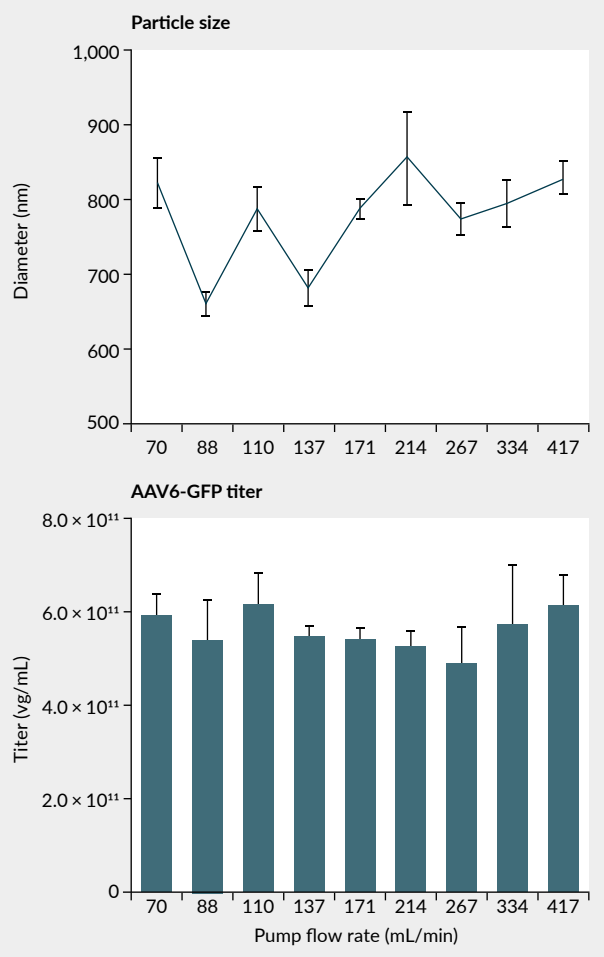
One final aspect of large-scale transfection to consider is the strategy for pumping the transfection reagent-DNA complex into reactors. As excessive fluid shear stress during pumping could potentially disrupt the complexes and negatively impact transfection efficiency and titers, this was investigated.

Both peristaltic pumps and low-shear pumps could be used for pumping. However, peristaltic pumping was chosen as a worst-case scenario in terms of fluid shear stress. In this study, transfection complexes were generated in 50 mL conical tubes and samples were taken. These samples were then pumped at various flow rates using a MasterFlex L/S digital drive peristaltic pump, a MasterFlex EasyLoad II pump head, and MasterFlex C-Flex Ultra size 16 tubing (1/8'' ID).

Subsequently, particle size was examined via DLS, and AAV production was performed by transfecting shake flask cultures in parallel. The graph on the top in Figure 9 shows particle sizes observed at different peristaltic pumping flow rates. Although the data shows some variability inherent in such experiments, the particle sizes generally ranged between 700 nm and 900 nm across the tested flow rates. The graph on the bottom of Figure 9 illustrates consistent crude titers observed across the tested flow rates. Collectively, these data demonstrate that within the range of flow rates tested,

FIGURE 9

Impact of pumping on particle size and AAV production.



peristaltic pumping did not negatively impact the transfection complex.

SCALING AAV PRODUCTION TO COMMERCIAL SCALE: CASE STUDY

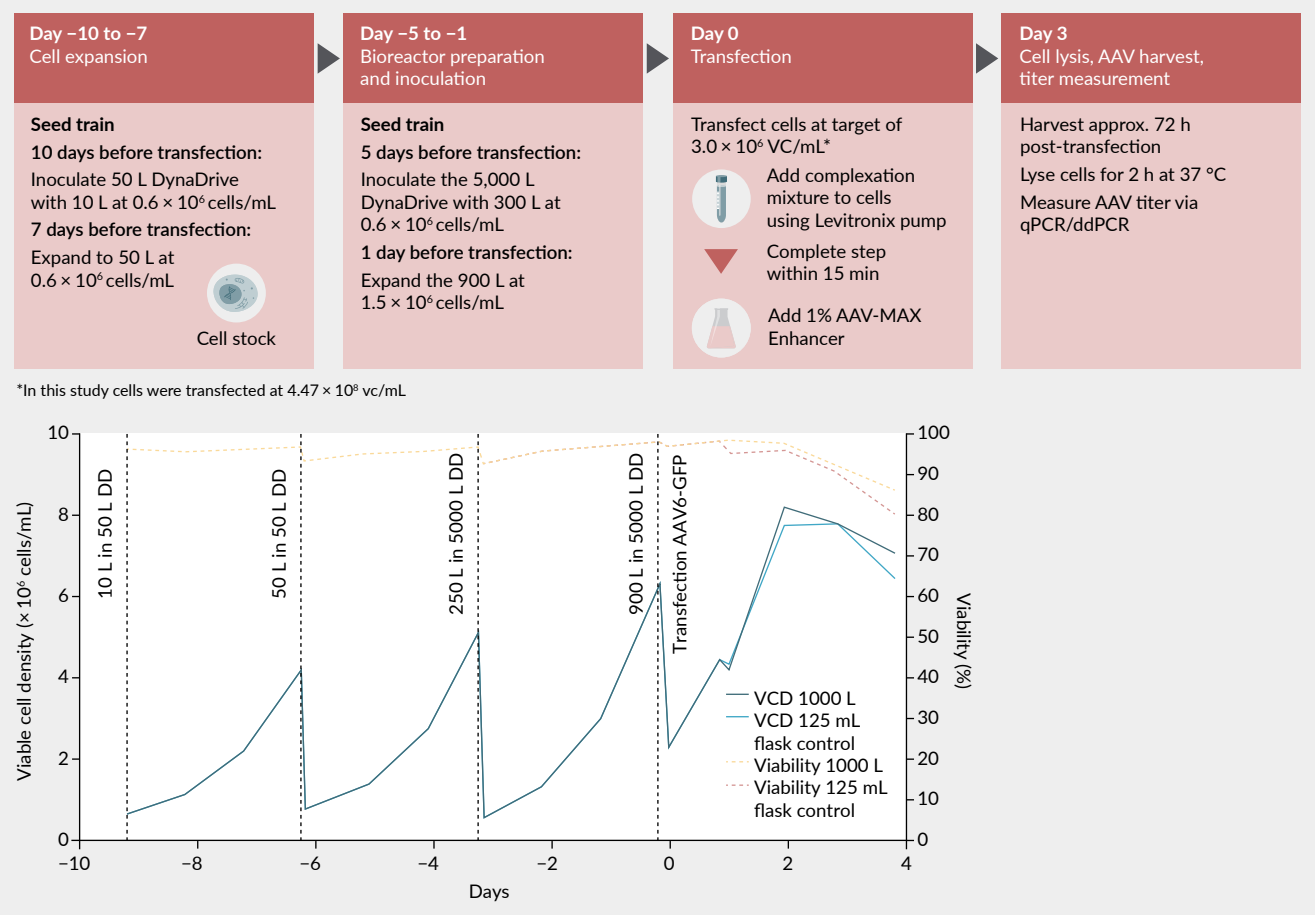
Following large-scale complexation characterization and optimization studies, the next step involved leveraging these protocols for large-scale AAV production runs. For these large-scale runs, AAV6-GFP was produced at both 500 L and 1,000 L scales using the Thermo Scientific™ DynaDrive Single-Use Bioreactor (SUB). The reagent volumes were scaled up proportionately to accommodate these larger production scales. The procedure focused on optimizing the seed train strategy, bioreactor conditions, and the transfection procedure. Key outputs included assessment of cell growth, metabolite profiles, AAV titers, and the percentage of full vs empty capsids.

Figure 10 depicts the procedure used for the 1,000 L production-scale run, with accompanying data on viable cell densities and viability. In this study, cell expansion began 10 days prior to transfection in a 50 L SUB, seeded at 0.6 million cells/mL. The first expansion step to 50 L was conducted seven days pre-transfection.

At 5 days pre-transfection, a 5,000 L DynaDrive bioreactor was inoculated with 300 L at a cell density of 0.6 million cells/mL. A final expansion step was performed 1 day pre-transfection, increasing the culture volume to 900 L. On the day of transfection, cells

FIGURE 10

Procedural schematic used for the 1,000 L production scale run (top) and accompanying data on viable cell densities and viability (graph, bottom).



were targeted at a density of 3 million cells/mL, although in this specific study, they were actually transfected at slightly more than 4 million cells/mL.

The transfection reagent-DNA complexation mixture was prepared in bioprocessing containers, manually mixed, and added to the bioreactor using a low-shear pump. Following complex addition, AAV-MAX Enhancer was introduced. Cultures were harvested at the 72-hour time point before undergoing lysis, with samples taken for subsequent processing and analytics.

The graph in Figure 10 illustrates the trends in viable cell density and viability percentage throughout the production run for both the large-scale run and controls in the 125 mL shake flasks. The data indicates robust scalability of cell growth in bioreactors, with viable cell density closely aligned with that for the shake flask controls. Additionally, following transfection on day 0, the observed drops in viability and viable cell density are indicative of a successful transfection process.

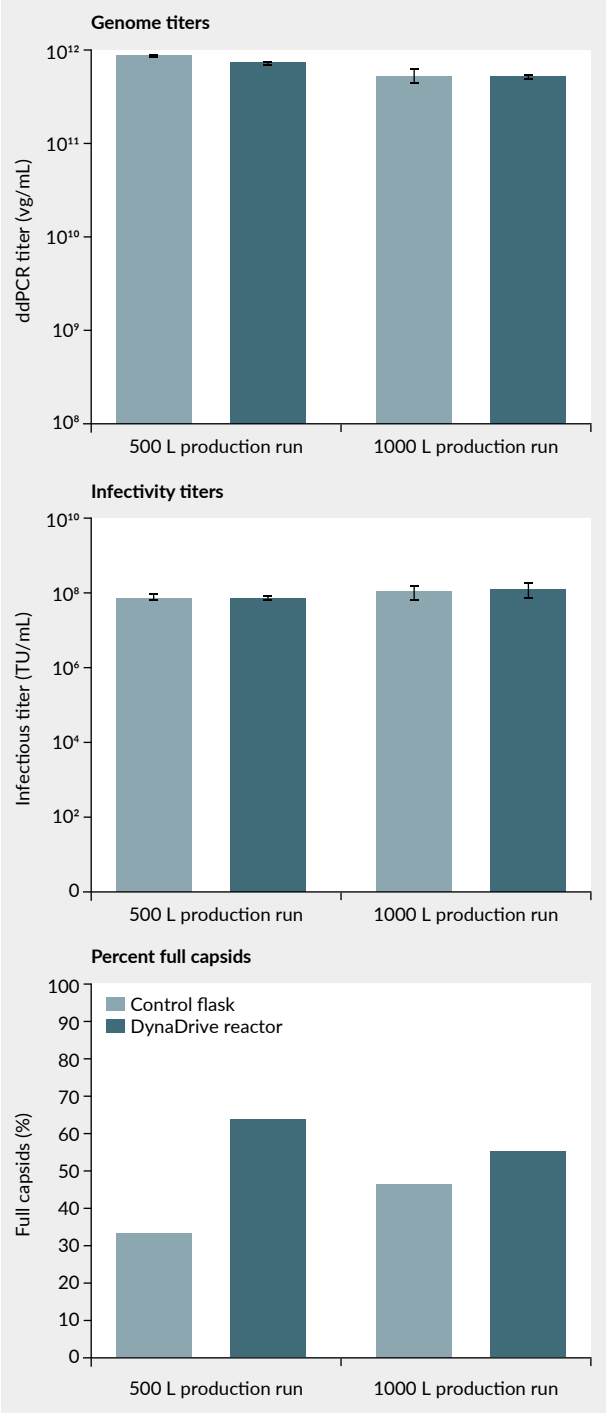
Samples collected from the 1,000 L production run were analyzed for genome titers, infectivity, and percentage full capsids, alongside data from a similarly performed 500 L run. Genome titers were quantified using ddPCR, infectivity was assessed with HT1080 cells, and percentage of full capsids was measured in crude samples via mass photometry.

The graphs presented in Figure 11 show data from the large-scale bioreactors in light blue, while corresponding shake flask controls are depicted in dark blue. For both the 500 L and 1,000 L runs, crude titers approached 10^{12} vg/ml and were comparable to those obtained in shake flasks. Infectivity showed similar levels across both large-scale runs and their corresponding shake flask controls. Notably, the percentage full capsids ranged from 50–60% for both 500 L and 1,000 L runs, with bioreactors showing a slight trend towards higher percentage full capsids compared to shake flask controls.

Overall, these findings demonstrate the robust scalability of the AAV-MAX-based upstream process to commercially relevant

FIGURE 11

AAV6-GFP productivity (genome titers), infectivity, and percentage full capsids in a 500 L and 1,000 L run of shake flask controls versus large-scale bioreactors (DynaDrive).



scales whilst maintaining both high productivity and quality.

CLINICAL AND COMMERCIAL SCALE AAV MANUFACTURING: SUPPLY CHAIN AND REGULATORY SUPPORT

A final critical aspect for consideration in AAV manufacturing pertains to the key requirements associated with the clinical and commercial stages of gene therapy manufacturing, and how the field is equipped to address those needs. Two crucial areas of this are supply chain and regulatory support.

The importance of establishing and maintaining a robust supply chain for the raw materials used in a manufacturing process is widely understood. This need can be addressed in multiple ways with one solution being CGMP-compliant products that are made available both as off-the-shelf catalog items and also as customizable products. This flexibility allows for tailored options such as side samples and customized packaging to align with specific commercial manufacturing needs. Additionally, leveraging extensive global reach and an established GMP-compliant supply chain, such as that of Thermo Fisher Scientific, can facilitate streamlined sourcing to ensure products reach clinical settings and beyond successfully.

On the regulatory front, established specialized expertise enables product design and accompanying documentation to meet the rigorous demands of gene therapy applications. Selecting products under the Thermo Fisher Scientific Cell Therapy Systems (CTS) brand ensures they are equipped with the necessary features to support gene therapy manufacturing seamlessly through clinical and commercial stages.

SUMMARY

In summary, challenges in small-scale AAV production, including the extended time required to establish production processes, low titers, and high costs, can be mitigated by using specialized, pre-optimized products and streamlined protocols.

The AAV-MAX system is a comprehensive solution for scalable, high-titer AAV production due to the inclusion of pre-optimized components for suspension-based transient transfection. Furthermore, the challenges of maintaining performance and reproducibility in large-scale AAV production can be addressed through optimized mixing and transfection techniques to achieve consistently high titers and quality across different production scales. The presented case studies showcase the AAV-MAX system's flexibility, allowing for various preparation and timing adjustments without compromising robustness. Additionally, the impact of different transfection methods and plasmid ratios on AAV production efficiency and the optimization of these parameters to maximize titers and percentage full capsids were demonstrated.

Overall, the AAV-MAX system meets the key needs of AAV gene therapy developers by addressing challenges in productivity, product quality, scalability, and cost.

Q&A

Emily Jackson-Holmes

Q Have you looked at the residuals such as host cell DNA and host cell protein?

EJH: The data for this was previously presented in a webinar and at a conference. In general, we see that residual host cell DNA and host cell protein levels are at or below industry standard levels. The same applies for residual plasmid DNA.

In one data set that we have shown previously, we assessed AAV6-αCD19 CAR constructs at a 50 L scale. We performed purification and examined the residuals both post-affinity and post-anion exchange. For those samples, regarding host DNA, we observed less than 10 ng per 10^{12} viral genomes. As for host cell protein, the levels were below the lower limit of quantification, thus the results showed good levels.

Q Could you provide more information on how the AAV-MAX Transfection Booster and Enhancers supplement the AAV-MAX Transfection reagent?

EJH: The AAV-MAX Transfection Booster is a reagent that forms a complex with the Transfection Reagent and the plasmid DNA. Its primary function is to facilitate the entry of DNA into the cells. We observe at least a two-fold increase in productivity when using the booster in conjunction with the reagent.

The AAV-MAX Enhancer is a reagent added to the cells at the time of transfection. As shown in the data, there is some flexibility in the timing of its addition, but whether you add it an hour before transfection or a couple of hours after, for example, the enhancer increases titers on average two-fold compared to not using it. Together, these three components—the booster, the enhancer, and the transfection reagent—work synergistically at the time of transfection. We have optimized them to achieve the best possible performance in terms of productivity.

Q Can you assess whether the AAV vectors are loaded with the correct DNA?

EJH: Mass photometry will only distinguish between AAV vectors that are loaded versus those that are not, but will not distinguish whether the AAV is loaded with the target DNA or an incorrect fragment or fragments. The data presented in the article was obtained using mass photometry to assess the percentage full capsids. However, we have used other methods in the past. With mass photometry specifically, we know the theoretical mass of an intact particle with the correct genome. This method provides a high confidence measure, although it is not direct evidence of having a full-length genome. To confirm the presence of the correct DNA, you would need to perform next-generation sequencing (NGS) or multi-primer PCR targeting different points along the genome.

In our experience, mass photometry offers a good trade-off, allowing us to process many samples efficiently. When conducting studies with numerous samples, we have found it to be a very effective method for saving time while measuring the percentage full capsids.

Q How did the optimal plasmid ratios vary from AAV serotype to AAV serotype?

EJH: In the DOE study presented in this article, AAV2 was used as an example—specifically, AAV2-GFP. We have also looked at other serotypes, such as AAV6, 8, and 9. We found that the optimal plasmid ratios for these other serotypes were very similar, if not identical. This similarity is due to the rep/cap plasmids being very similar in size.

However, if you are swapping out the serotype but not the gene of interest (GOI), it is still recommended to perform a DOE study to determine the best process output. Additionally, for different GOIs, we recommend conducting DOE studies to optimize the process, as GOIs can vary in size and may behave differently.

Q Can the CTS Viral Production Cells 2.0 be licensed?

EJH: Yes, we have both RUO and GMP banks of these cells. The RUO cells can be purchased off-the-shelf and used for internal research activities without a license, and they accompanied by a limited-use label license that allows for this. Licensing for RUO cells typically becomes relevant for service providers who wish to use the cells for their clients. In such cases, we offer multiple flexible licensing options.

For the CTS Viral Production Cells 2.0, a license is required to purchase them. We provide multiple flexible licensing options for these cells to ensure that our clients can access them and start working with them quickly. This license includes access to the cell line documentation package—a regulatory support package containing all requisite details of the qualification testing and traceability of the cells.

Q What kind of customizations are available, either for product or packaging?

EJH: For customizations, we offer a wide range of options through our custom media channel. In our catalog, we have products available in bottles, bioprocessing bags, and different pack sizes up to 100 L. Through the custom channel, clients can customize volumes (including larger volumes), formats, containers, and tubing connections. Additionally, you can modify the QC testing and add side samples.

BIOGRAPHY

EMILY JACKSON-HOLMES is a Product Manager at Thermo Fisher Scientific, Carlsbad, CA, USA. She supports products for upstream manufacturing of viral vectors, including the AAV-MAX system, as well as leading new product development. Emily has over a decade of experience in life sciences tools development, with expertise including viral vector production and microfluidic cell culture and analysis systems. Emily holds a PhD in Chemical and Biomolecular Engineering from the Georgia Institute of Technology, Atlanta, GA, USA and a BS in Chemical-Biological Engineering from the Massachusetts Institute of Technology, Cambridge, MA, USA.

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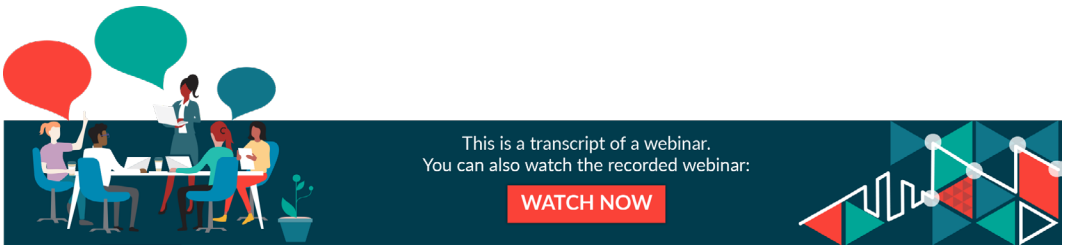

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Shifting to non-viral *in vivo* therapies to enhance global patient access to genetic medicines

David McCall, Senior Editor of BioInsights, talks to **Hari Pujar**, Chief Operating Officer, Tessera Therapeutics and Operating Partner, Flagship Pioneering, about current trends in the cell and gene therapy field aimed at addressing long-standing issues of manufacturing scalability, complexity, and high cost of goods.

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Q What are you working on right now?

HP: I have spent much of my more than two decades in industry in the vaccines space, including recent work on mRNA vaccines. However, in the past few years, I have shifted my focus more towards gene therapy and gene editing. Additionally, I have lately been working on developing nucleic acid delivery technologies to enable genetic medicines.

"By transitioning from *ex vivo* to *in vivo* methods, and from viral vectors to non-viral delivery vehicles, there can be a significant reduction in the cost of medicines."

Q What for you are the key current trends in the cell and gene therapy space, and what do they say about the general direction in which the field is heading?

HP: The cell and gene therapy field has been advancing in line with the central dogma of molecular biology, which is that DNA within the cell is transcribed into RNA, and RNA is then translated into proteins. The industry initially focused on small molecule drugs that act on proteins before the concept of using proteins as drugs emerged around four decades ago, establishing a significant and valuable industry.

Following this, the development of RNA medicines began, leading to the development of more than half a dozen RNA-based therapies. Subsequently, the industry ventured into DNA medicines, although we are still in the early stages. The first wave of DNA medicines, collectively termed gene therapy and gene editing, utilized viral vectors. These methods have been effective in treating various diseases, as evidenced by the success of CAR-T cell therapy for hematological malignancies and the initial accomplishments in AAV gene therapy. However, the use of viral vectors and the complex *ex vivo* cell therapy manufacturing model impose severe limitations on the full potential of gene therapy and genetic medicine.

So, the industry is now transitioning from viral vectors to non-viral delivery methods, and from *ex vivo* to *in vivo* therapies. Additionally, there is a focus on expanding the range of tissues that can be targeted with these medicines. Another significant technological trend is the shift towards highly specific genomic modifications, such as targeted edits of a mutation or precise insertion of nucleic acids into the genome. These developments represent the future direction of genetic medicine.

Q Let's dive into a couple of specific areas of importance to the investor community and the sector as a whole. Firstly, where are you seeing progress in addressing longstanding issues of high cost of goods in cell and gene therapy manufacturing?

HP: The same trends previously mentioned apply here. *Ex vivo* manufacturing and the use of viral vectors result in high production costs. By transitioning from *ex vivo* to *in vivo* methods, and from viral vectors to non-viral delivery vehicles, there can be a significant reduction

in the cost of medicines. This shift would also enable broader distribution, making these treatments accessible to a larger population, beyond the few individuals who can currently afford them or access them in specialized centers in developed countries.

Q And how do you see insufficient capacity and scalability issues being resolved?

HP: There are currently some highly effective medicines that, unfortunately, are not widely accessible due to limited capacity. A prime example is Carvykti® from Janssen, which has shown significant clinical benefit in relapsed and refractory multiple myeloma. However, patients are on waiting lists to receive it as there is insufficient capacity to treat everyone. This limitation arises largely from using viral vectors in *ex vivo* settings.

If these two factors can be addressed, the capacity would be significantly increased. We know that over a billion individuals worldwide were vaccinated with mRNA vaccines during the COVID-19 pandemic. By leveraging similar manufacturing capabilities for genetic medicines, we would be able to address such capacity limitations.

Similarly, once the field has moved away from *ex vivo* and viral vectors, scalability becomes much easier. This is not a criticism of *ex vivo* techniques or viral vectors, as they have successfully demonstrated the effectiveness of their mechanisms of action. However, we are now learning from these successes and are developing improved methods to achieve the same goals more efficiently.

Currently, there are at least two companies, Intellia and Verve Therapeutics, that have human clinical data demonstrating the use of non-viral delivery methods to edit the genome *in vivo*. Intellia Therapeutics has achieved protein knockout in the liver for two diseases, while Verve Therapeutics has utilized base editing of a gene in the liver. These advancements are gaining a lot of traction and are now extending beyond liver applications.

Notably, Tessera Therapeutics has recently announced promising non-viral delivery data that could enable *in vivo* gene editing for sickle cell disease. Tessera's proprietary LNPs were shown to efficiently deliver a reporter gene *in vivo* in non-human primates to an average of ~95% of long-term hematopoietic stem cells in bone marrow. Although human clinical trials are still forthcoming, this represents a groundbreaking development. Currently, the treatment for sickle cell disease involves a stem cell transplant, which requires mobilizing stem cells, manipulating them *ex vivo*, and then reintroducing them to the patient after ablating the existing stem cells with toxic chemotherapy. This complex process could potentially be circumvented with a simple IV injection of a lipid nanoparticle that targets hematopoietic stem cells.

The progress in non-viral delivery is substantial, and *in vivo* treatment becomes feasible with these advancements. Some companies are also exploring *in vivo* viral delivery, although non-viral approaches appear to have a number of significant manufacturing advantages.

Q The 'build vs buy' debate around manufacturing capabilities has swung back and forth over recent years for biotechs in the cell and gene therapy field. Where do venture organizations like Flagship Pioneering currently stand on this question, and how do you see the picture continuing to evolve moving forward?

HP: The 'build versus buy' question is approached as follows: if a platform company has the potential to develop multiple medicines from their platform, and if that manufacturing platform is novel with limited maturity in the external market, then building is the preferable option. Newer platforms are presumably simpler to manufacture and thus require minimal capital investment to establish manufacturing capabilities. Therefore, if a platform company anticipates multiple products and does not require a lot of capital investment, building is the recommended approach. Tessera Therapeutics exemplifies this strategy.

At Tessera, a platform company focused on Gene Writing™, we anticipated the development of multiple product candidates with the platform, which has proven to be true. The required capital investment was relatively modest, so we proceeded with building our manufacturing capabilities. This decision has been highly beneficial, as it allows us to control our manufacturing process both technologically and in terms of scheduling.

Conversely, Generate Biomedicines' modality-agnostic platform, which designs proteins using generative AI, poses a different manufacturing context. This leads to a preference for outsourcing in the early years. The 'buy versus build' strategy will evolve as Generate's pipeline matures. Ultimately, the decision depends on the specific circumstances, of course.

Capital efficiency is crucial in either scenario. The examples of Tessera Therapeutics and Generate Biomedicines illustrate different approaches to achieving capital efficiency. However, capital efficiency does not always equate to buying, as outsourcing can lead to manufacturing errors or delays, which can undermine the anticipated efficiency. Thus, the decision must be carefully evaluated based on the specific situation at hand.

Q Tell us more about Tessera Therapeutics and how the trends we have discussed so far are reflected in your strategy

HP: Tessera Therapeutics aims to address various genomic changes needed to treat or cure diseases through Gene Writing. Our Gene Writing platform is designed to introduce therapeutic messages into the genome by efficiently changing single or multiple base pairs, thereby precisely correcting or rewriting the genome, or adding longer exon-length sequences or even whole genes.

Equally as important as the Gene Writing platform is Tessera's proprietary delivery platform. This is being developed to facilitate *in vivo* delivery of the Gene Writing cargo to different cell

"We are still in the early stages of delivering nucleic acid medicines, reaching only a limited number of target areas within the body—much of the body remains untapped."

types within the body. We believe that the combination of these two platforms will enable the creation of transformative genetic medicines for a wide range of diseases.

At two different recent conferences—ASGCT and FASEB—we presented a significant amount of transformative data on therapeutic candidates that we believe can alter the course of many diseases. For instance, we shared data in α-1 antitrypsin deficiency demonstrating an estimated 56% rewriting efficiency in hepatocytes in the liver in non-human primates, effectively reaching potentially curative editing levels with a single dose of a lipid nanoparticle. Similarly, for sickle cell disease, a single delivery of a lipid nanoparticle achieved therapeutically relevant levels of rewriting in the *HBB* gene responsible for sickle cell disease. Additionally, we described a T cell delivery vehicle combined with our Gene Writers that has the potential to enable *in vivo* CAR-T cell therapy in the future.

Tessera is actively implementing these concepts and demonstrating proof of concept in non-human primates. It is well known that successfully demonstrating proof of concept in non-human primates in genetic medicine significantly increases the probability of success in clinical trials. Unlike small molecule drugs, where Phase 2 clinical success does not guarantee further success in Phase 3, positive data for genetic medicines in non-human primates often translates well to humans.

Q Lastly, please can you sum up one or two key goals for both Tessera Therapeutics and Flagship Pioneering over the foreseeable future?

HP: Tessera's foremost priority is to advance these innovative medicines into clinical trials. Additionally, a significant focus of mine, in collaboration with Tessera, is the continued expansion of non-viral delivery methods beyond our current capabilities. We are still in the early stages of delivering nucleic acid medicines, reaching only a limited number of target areas within the body—much of the body remains untapped. Within Flagship, I have been heavily involved in nurturing an effort to enhance the delivery of nucleic acid medicines to other parts of the body and improve the efficiency of delivery to areas we have already targeted. I am excited about creating this capability and building out the organization to support this endeavor.

BIOGRAPHY

HARI PUJAR is Chief Operating Officer of Tessera Therapeutics, Cambridge, MA, USA as well as Operating Partner at Flagship Pioneering, Cambridge, MA, USA. At Tessera, his responsibilities span across research, manufacturing, program strategy and management, and IP. Hari is a global biopharmaceutical executive with over 20 years of value creation in the biologics and vaccine industry. Before Flagship, Hari served as Chief Technology Officer of Spark Therapeutics, leading the technical operations, process and technology development and quality assurance organizations. At Spark, Hari was responsible for growing and scaling the company's cutting-edge technology capabilities for an expanding development pipeline. Prior to Spark, Hari was Head of Technical Development and Manufacturing at Moderna Therapeutics. At Moderna, he built and led the technology and early manufacturing organizations that delivered on supply for more than a dozen clinical programs in a brand-new technology area. Previously, Hari held a variety of scientific and cross-functional leadership positions during over 18 years at Merck & Co in the company's commercial, R&D and manufacturing divisions, culminating in the franchise leadership of pediatric and adult vaccines representing over $2 billion in revenue. Hari has a PhD in Chemical Engineering from the University of Delaware, Newark, DE, USA and an MBA from the Wharton School at the University of Pennsylvania, Philadelphia, PA, USA. He is a Fellow of the American Chemical Society and the American Institute of Medical and Biological Engineers.

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Leveraging the baculovirus-insect cell expression system for optimal AAV scale-up

David McCall, Senior Editor, BioInsights, speaks to VectorY Therapeutics' **Barbara Sanders**, Co-founder and CTO, and **Femke Hoeksema**, Director of Process Development, about their innovative work on gene therapies for neurodegenerative diseases, including vectorized antibody treatments for amyotrophic lateral sclerosis. They also discuss the considerations and advantages in utilizing a baculovirus-based AAV production system versus other upstream viral vector production platforms.

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Q What are you working on right now?

BS: At VectorY, we focus on developing gene therapies for neurodegenerative diseases. More specifically, we are working on vectorized antibody treatments for amyotrophic

lateral sclerosis (ALS) by utilizing viral vectors to express intracellular antibodies in diseased cells that can bind to disease targets for therapeutic effect. For ALS, this means targeting and clearing misfolded, toxic TDP-43 aggregates. By binding and removing TDP-43 toxic species, we aim to restore diseased motor neurons to health, providing a therapeutic approach with disease-modifying potential for patients with ALS.

We are highly experienced in combining antibodies and vectors, which makes VectorY unique. Our team includes scientists with expertise in antibody development, protein degradation, while Femke and I develop strategies for the large-scale production of gene therapy vectors for patient populations worldwide.

FH: We also focus on developing our platform process to secure in-house production of AAVs, and a robust platform for further scale-up and clinical manufacturing in order to support all our pipeline programs, especially our lead program in ALS.

Q Tell us more about VectorY Therapeutics' platform and R&D pipeline—what differentiates it?

BS: Firstly, not many researchers in the field are developing antibodies that are vectorized, especially intracellular antibodies. Additionally, the focus on targeting proteinopathies in CNS diseases with intracellular antibodies is a major differentiating factor for VectorY. While ALS is our lead indication, many neurodegenerative diseases can be addressed with our approach such as Huntington's disease, Parkinson's disease, and Alzheimer's disease.

Neurodegenerative diseases are very complex and often multifactorial—and the CNS is very challenging to reach, of course. AAV vectors are the most established delivery method for gene therapies, and we use them to develop vectorized antibodies for one-time CNS delivery. However, to add to the complexity and the challenge, in order to target CNS diseases, we need to be able to manufacture large volumes of high-quality, highly concentrated AAV vectors.

As I alluded to earlier, the blend of experts from various fields, including protein degradation, antibodies, and vector production, is a further differentiating factor. We also continuously make strategic investments in process development, meaning we invest in a scalable process from molecular design through all unit operations in order to deliver our platform. As previously mentioned, our ultimate goal is to treat large patient populations, so we must scale up production to 1,000 L and beyond. Unlike the smaller indications common to the AAV gene therapy space, which might only need a 50-liter bioreactor to cater for a clinical trial, we must scale-up to a considerably larger degree in order to deliver treatments to a much greater number of patients in need.

Additionally, we invest in technology, including manufacturing and analytical technology, and different capsid technologies to improve vector delivery. We also invest in technologies to improve our binders' ability to target multiple cells, and enhance their efficacy in degrading toxic protein species.

FH: We recently launched an observational trial for ALS in collaboration with the University of Utrecht in the Netherlands. This trial, led by one of the University's Professors, will help us validate biomarkers and enable efficacy readout strategies, which is important as we advance our lead program to clinical development.

"Our team includes scientists with expertise in antibody development, protein degradation, while Femke and I develop strategies for the large-scale production of gene therapy vectors for patient populations worldwide."

Q Talk us through the pros and cons in utilizing a baculovirus platform for viral vector production—for instance, versus HEK293?

FH: The transfection-based HEK293 system is currently the most widely used method for AAV production because it is fast and straightforward. With the right transfection reagents and plasmids, it enables the speedy generation of AAV materials at small scales. While it can be a good platform of choice for young companies in the discovery phase that are testing various AAV vectors, it might not be sufficient if high quantities of AAV vector are required. This is where the enhanced scalability of the baculovirus-insect cell expression system is advantageous. For example, gene therapy products for some retinal diseases typically require relatively low doses of approximately 1 to 1.5×10^{11} vg, whereas ALS and other CNS indications often need much higher doses, especially for treatments involving systemic delivery, where dosages can be 1,000-fold higher.

As companies transition from the clinical phases to commercialization, it is important to use easily scalable production systems that allow for high yields while maintaining product quality. To accommodate these needs, some gene therapy companies switch from HEK-based platforms to different systems that are capable of commercial manufacturing capabilities. However, this transition can be challenging as it requires proving comparability between the two systems, which can lead to development delays. At VectorY, we made a deliberate choice to use the baculovirus-insect cell platform because our founders, including Barbara and Sander van Deventer (CEO), have extensive experience with it. From the very start of the company, we decided to invest in this scalable platform alongside our discovery activities. This was with the end-goal in mind of developing a high-yielding and robust platform that can streamline CMC activities in our product pipeline and ensuring product availability for larger patient populations.

The baculovirus-insect cell platform is quite straightforward to scale up. Furthermore, having a scalable stirred-tank bioreactor allows one to optimize cell growth conditions and scale to 2,000 L and beyond. Then, after a few days' growth in the bioreactor, a simple infection step is needed, which is also relatively easy to achieve.

We already have substantial data showing that scaling up with the baculovirus-insect cell platform is linear due to fully characterized seed stocks. In contrast, scaling up the HEK-based system is more challenging, as it requires optimizing mixing conditions to ensure consistent quality and yield at large scales, which can make it more difficult to achieve high consistency. Additionally, the HEK-based system has higher costs due to the need for expensive GMP-compliant plasmids and transfection reagents.

Apart from scalability, another benefit of the baculovirus-insect cell system is consistency, which again is thanks to the availability of fully characterized seed stocks. Utilizing the same seed stock as the biological starting material for multiple productions ensures high consistency in the upstream vector product. This in turn leads to high reproducibility in the downstream process, resulting in consistent yield and product quality.

"From the very start of the company, we decided to invest in this scalable platform alongside our discovery activities. This was with the end-goal in mind of developing a high-yielding and robust platform that can streamline CMC activities in our product pipeline and ensuring product availability for larger patient populations."

According to our internal data, the baculovirus-insect cell platform shows very consistent upstream yields of more than 10^{15} vg/L, with <1% residual host cell DNA present. Following downstream purification, the large majority of the purified AAVs are filled with full-length transgene products, showing very high product integrity.

In comparison, HEK-based production systems typically deliver AAV vectors with only approximately ≤15% full particles, resulting in a higher level of process-related impurities such as empty capsids. In gene therapy, the dose is determined by the number of full viral genomes that the patient receives. Therefore, if a manufacturing batch contains a large percentage of capsids filled with truncations or contaminants such as the helper plasmid, the patient would need to receive far more viral particles in their dosing regimen, which may compromise the treatment's safety and efficacy. In essence, utilizing a baculovirus-based system has allowed us to keep these impurities as low as possible throughout our development trajectory and scale-ups, significantly improving the quality and safety of the final gene therapy products, and providing promise for eventual commercial manufacturing success.

Finally, our investments in high-quality baculovirus starting materials also support the viability and scale-up of the product. At VectorY, we demonstrated that these materials are very stable over multiple passages.

BS: The baculovirus-insect cell system offers an additional significant advantage from a safety perspective. Because we use insect cells and baculovirus, any residual DNA is inert in humans, meaning that even the very low residual levels are completely non-functional in human cells. Furthermore, insect cells do not propagate human viruses, adding another layer of safety. In contrast, the HEK-based system theoretically has more residual DNA, including human DNA and helper plasmids with antibiotic-resistance genes, which could negatively impact the product's safety.

Q How is the baculovirus-insect cell system continuing to evolve? How is it being improved and optimized?

BS: The baculovirus-insect cell system has come a long way since the initial designs such as the triple baculovirus system with a split Rep gene, developed by Urabe, *et al.*, in 2002, which was extremely genetically unstable upon passaging [1]. Furthermore, using three seeds introduced significant variability in the process. AAV is a mammalian virus, not an insect virus, meaning the molecular splicing that occurs in HEK cells differs from that in insect cells. Therefore, molecular adjustments to the insect cells are necessary to produce the same particles as with the HEK system. Unfortunately, there are only a few effective options for molecular modifications, including artificial introns and attenuated Kozak sequences.

However, transitioning from a triple system to a dual baculovirus system has been a massive improvement. At VectorY, we have also eliminated the pre-production steps by utilizing highly characterized frozen seed stocks. As a result, the production process is more robust and consistent.

Q What are the remaining critical issues with, and needs for, virus-based production systems at large-scale?

BS: As we have already mentioned, our biggest challenge is the need to produce high yields for genuinely large-scale AAV production. AAV is one of the hardest viruses to produce because it does not replicate on its own, requiring helper viruses or helper genes for efficient production. Then, once the AAV vector has been produced, there are downstream processing challenges, such as distinguishing empty capsids from full capsids, which makes it even more complex and challenging to move towards high-volume commercial production. Unlike the production of antibodies or viruses such as Polio that replicate easily, allowing a single bioreactor to produce millions of doses, an AAV bioreactor might only provide enough doses for 18 to 30 patients.

However, these challenges could be solved by developing a scalable and high-yielding process. Furthermore, reducing COGs to make large-scale treatments more affordable is also an important goal for us and for the gene therapy field at large. Gene therapy companies and payers face complex processes and significant investments to produce viral vectors, which impacts pricing. For example, one of the latest US FDA-approved gene therapy products, Hemgenix, costs USD$3.5 million per dose to reimburse. Our objective is to lower COGs significantly by achieving higher yields and increasing recovery during downstream processing, thereby alleviating the need for such high price points.

Q What are some key goals and priorities, both for yourselves in your own roles and for VectorY as a whole, over the foreseeable future?

BS: VectorY's primary focus is getting our first product to the clinic. Simultaneously, we are developing multiple research programs, such as working on new binders for other CNS diseases. We plan to leverage the platform we have built over the years to help speed up the development of these programs as soon as they are validated in research.

FH: My ultimate goal is to be an internal facilitator to ensure our product pipeline progresses as efficiently as possible. In the meantime, Barbara and I will also focus on maintaining and further optimizing our platform with second-generation process optimizations and technology development. For example, more efficient capsids can lead to lower doses and reduced COGs, as we have previously discussed.

REFERENCE

1. Urabe M, Ding C, Kotin RM. Insect cells as a factory to produce adeno-associated virus type 2 vectors. *Hum. Gene Ther.* 2002; 13(16), 1935–1943.

BIOGRAPHIES

BARBARA SANDERS is CTO and co-founder of VectorY, Amsterdam, Netherlands and has served as Vice President Vector Development since the company's inception in 2020. She has over 15 years of experience in viral vector development within large pharmaceutical and biotechnology companies where she led multiple Discovery and CMC teams. Barbara completed a PhD in Molecular Virology at the University of Amsterdam, Amsterdam, Netherlands and is an inventor of eight patents.

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EXPERT INSIGHT

Analytics for cell and gene therapy products in early development: points to consider before preparing an IND for a first-in-human clinical trial

William E Janssen and Scott R Burger

In the transition from research lab to formal pre-clinical studies and initial clinical trial, manufacturing and testing of novel cell and gene therapy products must undergo significant development. Often, however, more effort is devoted to scaling, closing, and automating production methodology than to development of supporting analytical methods. Yet well-controlled, consistent testing is crucial to ensure product quality. Here, we address the feasibility and benefit of early development of analytical methods, and discuss the product quality attributes that must be addressed by process control and release analytics, the quality attributes of assays, and critical analytical considerations such as sample size and management, and sampling method.

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Analytical characterization is fundamental to the development and production of all cell and gene therapy (CGT) products [1]. The analytical methods available for CGT applications grow ever-more sophisticated and powerful. However, the capabilities of

any analytical method can be undercut by inadequate control or poor application, hampering CGT product development. In our experience, characterization testing problems are especially evident when transferring CGT products from R&D laboratory settings, in industry as well as academia, and reflect limited understanding of proper analytical development and application, and the misperception that analytical rigor is less necessary before the transition to GMP manufacturing for Phase 1. Here, we discuss considerations for characterization testing development and control as an integral part of the earliest proof-of-concept development and testing. We submit that not only will this enhance the scientific integrity of the early pre-clinical studies, but, crucially, will facilitate the conduct of later-stage development and efforts to secure regulatory approval. In fact, some aspects of analytical method development necessarily must be addressed in the course of clinical development, but much of the analytical foundation can be built during preclinical R&D. In the following sections, we will address the critical elements of analytical methods, noting those that can feasibly be developed at the earliest phases of the product lifecycle (Table 1).

Novel concepts for CGT products have their roots in R&D laboratories. Thus, initial characterization of these products, and the underlying assays from which the characterization is derived, have been developed in the service of research aims. While these early assays, which we may refer to as 'pre-qualification assays', are the starting point for product characterization, process control, and product release analytics, there are multiple layers of control that must be developed, as well as possible additional assays, before a novel product may be considered for initial clinical development. Prior to initiation of clinical trial, the analytics must become 'qualified assays'. Many aspects of product analytics that will be of significant consequence through the development lifecycle [2] may feasibly be more fully developed and transitioned from pre-qualification to qualified during preclinical R&D.

Throughout this discussion, we will use cell counting as an example to illustrate key analytical concepts. Cell counting is perhaps the most commonly performed test of CGT intermediates and products, and whether the intended product is a cell-based therapy or viral vector, accurate and precise measurement of cell concentration is essential [3].

ASSAY METHODOLOGIC CONSIDERATIONS: PROCEDURE

Within the framework of CGT, procedures for the conduct of analytics are driven substantially by the procedures employed in the manufacturing of a CGT product, and the intermediate products within that manufacturing that may be sampled for process control or product characterization purposes. For example, prior to an extended incubation for cell expansion, the number of cells present is small, and removal of any poses a risk of compromising the final product. Similarly, the media in which an intermediate product is suspended may produce matrix effects within an assay, requiring that assays be developed using that same media matrix.

In laboratories where novel CGT products are developed, cell counting is among the most routine analytical procedures. Often, this is done manually, with little control other than performing the count twice and checking to see whether a similar result is obtained both times. This is, obviously, an inadequate level of control.

The cell counting standards published by the International Standards Organization (ISO) provide a useful framework for establishing well-controlled methods for determining cell concentration [3,4]. FDA and ISO standards require a written procedure for performing cell counts and that every person who performs cell counts must be trained on and follow that procedure [2–4]. The procedure must identify the specific steps that must be performed for the counting procedure, the

TABLE 1

CGT product development lifecycle.

Development stage	Typical features of manufacturing	Analytics development recommendations
Conception: laboratory observation/experimental result	Specific product not identified; process described in lab notebooks but may be one-off	Lab developed assays
Early gestation: functional demonstration that a selected, cultured, or gene-modified collection of cells or gene/vector pairing can be produced that has potential to treat a pathologic condition	Continued use of methods from basic research; non-qualified or minimally-qualified materials; procedures written down, but may still be altered on the fly	For most commonly used assays begin quality attribute measurements (specificity, linearity, accuracy, precision); create written procedures
Late gestation: proof of principle studies in animal model(s)	Continued use of existing methods; written procedures may be maintained in a methods notebook	Begin development of analytics for SISPQ elements not addressed with existing assays
Pre-clinical development: preparation for production for early phase clinical trial	SOPs created, batch record forms; larger product vessels and reagent volumes	Ensure that SOPs are complete and quality attributes have been determined for all assays; stage-appropriate sampling specified; preparation for method qualification
Phase 1 clinical trial: production of small lots for administration to very small number of trial participants	Formal SOPs and batch record forms used; manufacturing performed in areas with environmental monitoring and control, closed manufacturing systems where practical/possible; CDMO may be employed	Qualification of analytical methods prior to manufacturing clinical trial material; evaluate candidate potency assays; ensure that all analytics are included in tech transfer to manufacturer
Phase 2 clinical trial: production of small lots for administration to a much larger (10-fold) number of trial participants	More extensive automation, more extensive use of closed-system processing	Refine and develop analytic methods as necessary to accommodate evolving manufacturing methods and lot sizes
Phase 3 and clinical trial	Need to increase production to support commercial scale	If any analytics employed are compendial, verification studies must be performed; for all assays, compendial and non-compendial, expand quality attribute testing for full validation, including measures of robustness
Licensure and commercialization	Locking in of CMC manufacturing methods	Locking in of CMC analytics methods

details of the sample to be tested, materials and equipment to be used, and possible sources of error and how to mitigate them. If more than one counting method is available in a facility, there must also be a procedure for determining which counting method is correct for the cells being counted.

In this example, if a manual counting method is employed, the type of counting chamber, stain, microscope, and power of both eyepiece and objective must be specified. The written procedure must describe how the sample is prepared, such as dilution(s), staining method, and maximum time post-staining, as well as how the microscope is prepared for cell counting, including cleaning, and adjustment of the microscope's illumination system, substage diaphragm, and determination of correct focus. Criteria for valid test results should be specified as well. For example, how many times should the count be repeated, and what is the acceptable range of results?

Similarly, if an automated counter is employed, the manufacturer and model must be identified, along with how, and how often, it is calibrated, and specifications for acceptable results. The description of calibration must include the source of controls. Consumable reagents required for the counting instrument must be identified, along with how they are qualified for use, and the

instrument itself must have been qualified, and the qualification along with all routine maintenance, cleaning and calibration that the manufacturer specifies must be documented so that personnel using it can check to see that it is in compliance prior to each use.

As with the above cell counting example, these basic details of procedure, consumable materials, and required instrumentation must be identified for any analytic procedure, whether employed for process control, for final product release, or both. And, as with the cell counting example, these details can be defined at the same time as early proof-of-principle experiments are being conducted with the associated intended therapeutic product [5]. Having a detailed, clearly written procedure for any analytic method, with this information defined and adhered to each time that analytic test is performed, strengthens the quality of the proof of principle experiments and data generated. Moreover, the documented presence of such procedural integrity and discipline strengthens ensuing regulatory submissions, as qualification of both manufacturing and associated analytic methods must be performed prior to initiating GMP manufacturing for Phase 1 clinical trial.

ASSAY METHODOLOGIC CONSIDERATIONS: SAMPLING

It can be argued that sampling receives insufficient attention, given its potential to affect analytical results. Not all aspects of sampling methodology lend themselves to being defined early in product development, as some changes in product manufacturing—such as transitioning from an open to a closed production system—will necessitate adjustments in sampling. There are, however, methodological details of sampling that can and must be determined early in development [2].

The first of these is sample size. This can be particularly challenging when sampling cellular starting material, as any material removed for testing reduces the amount of material available for product manufacturing, and there are limits to the amount of cellular material that can be safely taken from a single donor. For this reason, sample size typically is determined as a volume small enough to avoid excess loss of product. Although this is an important consideration, it is not the only factor to bear in mind and can risk compromising the test for which the sample is being obtained. Continuing with the cell counting example, consider a counting method that has a lower limit of detection of 1,000 cells/mL. To minimize sample volume, a 0.25 mL sample is withdrawn and then diluted up to a full 1 mL for the count, If the cell concentration of the originally sampled cell suspension is less than 4,000 cells/mL, then the final diluted sample will have less than 1,000 cells/mL, and may not show a count at all.

Compounding the problem, in this example, is that while the limit of detection may be 1000 cells/mL, the lower limit for accurate quantitation may be 10,000 cells/mL, and so even if a non-zero count is obtained, it is likely to be inaccurate. It is imperative that the specified sample size ensures that sufficient analyte is obtained, from concentrations likely to be encountered, to allow the test to produce an accurate result.

Sample size determination must be based on considerations of the intermediate or final product being sampled and the assay quality characteristics discussed below. Another critical element of sampling methodology is the need for consistency. The procedure for performing the test, or the manufacturing procedure, or both, must describe the sampling device, how to use it, and how to manipulate the intermediate or product prior to sample collection. The sampling methodology must be described in the procedure(s) in sufficient detail that every operator can understand exactly how to take the sample correctly, enabling consistent sampling and analytical precision.

Because terminal sterilization is not possible for CGT products, sampling methodology

must be in keeping with the overall requirement for aseptic methods throughout manufacturing of the intended product. Generally, this requires consideration of the environment within which the sample is obtained, whether closed or open system manufacturing is employed, and how much sample is actually required.

Finally, sample management must be defined. This requires well-defined and clearly readable sample labeling. It also includes the container and closure system that will contain the sample between removal from the product and initiation of the analytic procedure, the maximum time interval between sampling and test initiation, and range of temperatures within which the sample should be maintained. If the test is to be performed by a contract laboratory, the external packaging for shipping, carrier(s) that may be used for sample transit, and how temperature monitoring during shipment will be conducted and documented must be defined. If the sample is in a liquid form, primary and secondary containers as well as absorbent material in case of leakage must be defined. Finally, if the sample is being transported through any publicly utilized passageway, including public hallways in a hospital or other multi-occupant building, public sidewalks, roadways, and utilizing couriers, trucking, rail or air carriers, then necessary biohazard labeling must be defined.

ASSAY QUALITY CHARACTERISTICS

Analytical methods have essentially three main applications in CGT development and manufacturing. The first is characterization of the product, i.e., establishing an analytical understanding of the product's composition and function. Product characterization is critical early in development, though understanding of the product continues to be refined. Product characterization serves as the benchmark for the other two applications—process control and product acceptance for release and monitoring of stability. In process control testing, test(s) are used to measure critical process parameters, determine whether specific process intermediates meet pre-defined criteria, and support process validation.

In product acceptability determination, testing serves to establish whether the final product is suitable for release, distribution, and administration. Product acceptability is also applied when monitoring product stability during post-release storage. The qualities of the product that are required to be measured for these purposes are safety, identity, strength, and purity [6], identified here by the acronym SISPQ [7]. Some of the analytical methods used may evaluate more than one of the SISPQ qualities, but all will address at least one.

There are in turn critical qualities of each analytical method that must be determined to ensure that the method performs correctly. These qualities are specificity, linearity, accuracy, precision, range, quantitation limit, and detection limit [2,8]. It is imperative that assessment of all of these measures be performed using samples derived from the manufacturing of the specific product that the analytic methods are designed to assess, and that testing is performed using the same sample matrix as would be in place during manufacturing runs for products for clinical use. Detailed methods for measuring each of these quality attributes are beyond the scope of this paper, but are readily available from regulatory, compendial and scientific literature sources, including ICH Q2(R2) [8–10].

Specificity

Specificity is the measure of how well the analytic method measures only the intended analyte and not other components of the sample being tested. Using our example of cell counting, the specificity of a counting method would be defined as the number of true cells counted divided by the count obtained, where the count obtained might include pieces of debris, leftover cell

separation beads, or other impurities that are not actually cells. Thus, if a sample contains 90,000 cells/mL, and our counting method yields a result of 100,000 cells/mL, the specificity of that method would be determined to be 90%. This simplified description of what is implied by specificity is in no way intended to a prescription for measuring specificity. In the likely situation that a sample with a precisely known analyte concentration is not available, there are multiple approaches to determining specificity of an analytic method. Inasmuch as assay results support furtherance of a product concept into continued development, the specificity of analytic methods can and should be determined early in the product development lifecycle.

Linearity, range, limits of detection, and quantitation

Linearity measures the proportionality of assay results relative to the amount of analyte present in a sample. Returning to the cell counting example, if four samples contained 90,000, 180,000, 270,000, and 360,000 cells/mL, and our cell counting assay yields results of 100,000, 200,000, 300,000, and 400,000 cells/mL, respectively, then our assay is linear over the range of 90,000–360,000 cells/mL. In other words, the result of the assay over that range can be described as result=specificity × actual-concentration. Generally, analytic methods will have a range of analyte concentrations within which the assay is linear, but at very low or very high analyte concentrations, results will not be linear. This is illustrated in Figure 1. The range of analyte concentrations that produce linear results with the analytic method is referred to as the linear range, and generally can be thought of as the assay's quantifiable range. The quantifiable range of an analytic method refers to the interval between the lowest and the highest analyte concentrations in which the procedure has a suitable level of response, accuracy, and precision, to be discussed in more detail below. If the anticipated concentration of an analyte to be measured is likely to be outside of the assay range, then that analytic method cannot be considered suitable. Referring back to our cell counting example, if a particular cell counting instrument has a quantification range from 10,000 cells/mL up to 200,000 cells/mL, but cell concentrations are anticipated to routinely be 5,000 cells/mL or less, then that instrument cannot be considered satisfactory, even if the lower limit of detection is 1,000 cells/mL, since at that lower level, the counts are not sufficiently accurate. The quantitation limits for a given analytic method are, in fact, the lower and upper ends of the range. That is, the limits within which quantitation of an analyte has been documented to be accurate. The detection limits, usually applied to the lower detection limit, refer to the lowest concentration of the analyte that the method can consistently detect, albeit without consistent accuracy.

Accuracy

Accuracy is the measure of how close to the correct measure of an analyte results from the analytic method when it is applied to a sample with a known amount of the analyte, generally through use of known reference material or spiking of the sample matrix with a fixed amount of the analyte. Accuracy is defined to be the mean percent recovery of a known amount of analyte over multiple tests. The tests should include multiple concentrations of analyte, and multiple replicate tests at each concentration.

While accuracy is often described as a stand-alone measure, it must be pointed out that it is, in fact, closely interrelated with linearity and quantifiable range for an assay. Thus, by extension, these measures are also interrelated with specificity.

Precision

Precision is the ability of the analytic method to produce the same result from multiple tests of the same sample. Precision may be

reported as either the coefficient of variation (%CV) from multiple tests, that is, the standard deviation divided by the mean, or as the 95% confidence interval from the same repeated tests. Beyond simple measurements of precision, that is, same sample, same testing conditions, advanced precision testing should be performed. Intermediate precision, goes beyond simple precision in that additional factors including anticipated typical variations such as different personnel conducting the assay, different days of the week, and different items of small equipment (e.g., pipettors) employed will be introduced. Reproducibility of the assay goes to another level of precision through conduct of an interlaboratory trial to demonstrate that the assay is generalizable.

Robustness

All of the above measures of analytic quality characteristics can be measured and determined early in the CGT development lifecycle, as they will not change as long as the assay procedure, materials and instrumentation remain unchanged. Having these measures in hand will serve to enhance developer confidence in the product that is being developed, and will also strengthen regulatory submissions through all phases of the product development lifecycle.

Robustness of an analytic method, however, cannot be determined early in development. Over the course of product development, the robustness of analytic techniques should be assessed. That is, as different samples from different products, possibly produced in different facilities or by different personnel, or using different starting materials (as is the case with any type of cellular therapy), the performance of the analytic technique using the measures described above should be assessed and documented [8].

ADDITIONAL CONSIDERATIONS

In the development of analytics for CGT, a difficulty that is faced by all product developers is obtaining cellular material that is the same as will be used for the intended product. This is particularly problematic when developing products which will be for autologous use. Fortunately, there are now several commercial entities that can provide 'healthy donor' cells, including apheresis products and bone marrow aspirates. Material from patients who have a medical condition that is the intended indication for the product in development requires IRB approval, which may be difficult to obtain for the purpose of collecting cells without benefit to the subject patient. It is important, however, that effort be made to obtain such materials for analytics development.

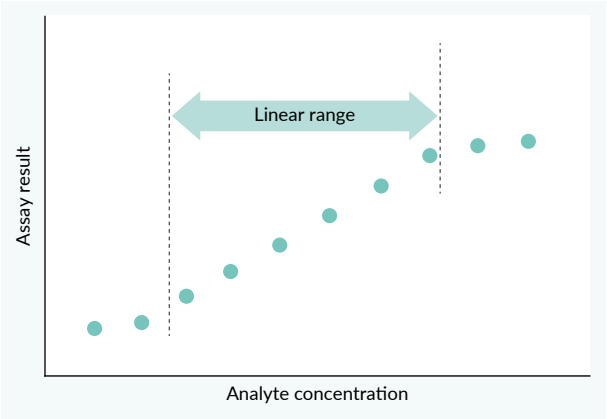
Once an analytic method has been qualified, it becomes imperative to continue to apply quality monitors to ensure that the assay is continuing to function. Specific controls must be defined, and the assay must be run with these controls on a routine basis. The results of these controlled runs must be followed and analyzed for outlier results and trends that may indicate problems with the assay.

TRANSLATIONAL INSIGHT

The development of robust analytic methods is crucial for the successful transition of cell and gene therapy (CGT) products from basic

FIGURE 1

Linear range of an assay.



research to clinical trials and eventual market approval. The authors, in their capacity as consultants assisting product developers with chemistry, manufacturing and control (CMC) development and with regulatory applications, have often seen problems arise in the course of product development that are linked to shortcomings in the analytic methods for characterization of manufacturing intermediates or final products. These analytical limitations, in turn, lead to regulatory problems and delays.

Early attention to the development of analytic methods can significantly enhance the quality and reliability of both process control and product release testing. By addressing key elements such as written procedures, sampling methodologies, and assay quality characteristics early in the development lifecycle, developers can ensure that their products meet regulatory requirements and are well-positioned for successful clinical and commercial outcomes. Effective, proactive development of analytics is feasible and desirable even at early stages of CGT product development, strengthening the scientific foundation of the product and facilitating smooth navigation of the regulatory pathway.

REFERENCES

1. FDA. *Q10 Pharmaceutical Quality System, Guidance for Industry*. Apr 2009; US Food & Drug Administration. https://fda.gov/media/71553/download.

2. FDA. *Analytical Procedures and Methods Validation for Drugs and Biologics, Guidance for Industry*. Jul 2015; US Food & Drug Administration. https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf.

3. ISO. *ISO 20391-1 Biotechnology—Cell Counting. Part 1: General Guidance on Cell Counting Methods.* 2018; International Standards Organization. https://iso.org/standard/68879.html.

4. ISO. *ISO 20391-2 Biotechnology—Cell Counting. Part 2: Experimental Design and Statistical Analysis to Quantify Counting Method Performance*. 2019; International Standards Organization. https://iso.org/standard/67892.html.

5. FDA. *Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs), Guidance for Industry*. Jan 2020; US Food & Drug Administration. https:// fda.gov/regulatory-information/search-fda-guidance-documents/chemistry-manufacturing-and-control-cmc-information-human-gene-therapy-investigational-new-drug.

6. US Code of Federal Regulations. *21CFR210 Good Manufacturing Practices.* 2020; US Government Printing Office.

7. National Institutes of Health Division of Technical Resources. Preparing for FDA pre-operational review of APF projects. *Design Requirements Manual News to Use* 2020; 2(40): 1.

8. FDA. *Q2(R2) Validation of Analytical Procedures, Guidance for Industry*. Mar 2024; US Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q2r2-validation-analytical-procedures.

9. Gupta PC. Method validation of analytical procedures. *PharmaTutor* 2015; 3(1), 32–39.

10. USP. *<1046> Cellular and Tissue Based Therapies.* 2011; United States Pharmacopeia.

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CELL & GENE THERAPY INSIGHTS

ANALYTICS CHANNEL

CHANNEL CONTENT

INTERVIEW

Advancing CAR-T therapies: navigating innovation, challenges, and the future of biopharma analytics

Abi Pinchbeck, Editor, *Cell and Gene Therapy Insights*, speaks to **Adam Fung**, a CMC and quality strategy and operations leader in cell and gene therapy with over 15 years of experience. The discussion emphasizes the importance of automation, innovation, and strategic planning in shaping the future of biopharma analytics, particularly in the development of CAR-T cell therapies.

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Q You have spent over 15 years in the biopharma analytics field. Can you take us on a journey through your time in the field and the changes you've witnessed, particularly in cell and gene therapies (CGT)? What have been the key inflection points for the area over this period?

AF: The commercial approvals for genetically modified CAR-T cell therapies over the last years, especially for B cell malignancies, clearly demonstrates the promise of these cell

therapies in oncology. Building on those CAR-T approvals serves as both a foundation and an inflection point. We are now in an era of diversity and differentiation. For instance, there is significantly more diversity beyond autologous and allogeneic CAR-T cell therapies, including the use of different cell types like natural killer (NK) cells, induced pluripotent stem cells (iPSCs), and tumor-infiltrating lymphocytes (TILs), which are advancing in clinical development and even receiving commercial approvals.

Therapies have expanded beyond treatment options to include products that can at least partially mitigate genetic irregularities, such as those for sickle cell disease and β-thalassemia, as well as therapies that are potentially curative. There has also been a growing diversity in how genetic material is transferred to cells, whether *in vivo* or *in vitro*, and an increase in non-viral delivery mechanisms and gene editing technologies. Additionally, there is substantial interest in using these therapies for diseases beyond oncology. This explosive growth highlights the differentiation happening in the field.

The approved CAR-T products are moving into earlier lines of therapy and broader indications, thereby increasing demand. However, manufacturing capacity and vein-to-vein turnaround times remain significant bottlenecks. Sponsors developing CGTs are now more cognizant of these common bottlenecks and are taking steps to secure manufacturing capacity and reduce costs. They are designing manufacturing processes to reduce or eliminate cell expansion steps or yield more potent drug products, introducing more automation for bioprocessing and analytical testing, and exploring ways to minimize safety risks and make autologous cell collections less invasive to improve the patient experience. These considerations will shape the future of the field because while being first to market is coveted, the ability to deliver products reliably, quickly, at lower cost, and with improved safety profiles can heavily influence the delivery of therapies to patients and shape market potential.

Finally, there are now numerous guidance documents published by health authorities specifically focused on CGTs. These guidance documents, while imperfect and more applicable to more mature CAR-T cell therapies, provide baseline roadmaps for managing product life cycles and health authority expectations. The availability of these guidance documents aligns with the rapid pace of CGT development, highlighting the growth of the field.

Q There's lots of excitement in the CGT space surrounding applications of cell therapies in autoimmune disease—can you elaborate on the promises seen here?

AF: There is significant excitement around the potential of cell therapies to treat diseases beyond oncology. There is substantial potential for cell therapies to treat autoimmune diseases, and this is reflected in the number of investigational therapies being developed for neurological, skin, muscular, and other autoimmune diseases associated with autoreactive B cells in both adults and pediatric patients.

"The more lifecycle management is incorporated early on, the simpler it can be during later stages of development."

Numerous publications and presentations at conferences have summarized ongoing clinical studies demonstrating that B cells can be successfully depleted with anti-CD19 CAR-T cell therapies, leading to remission in refractory diseases like lupus (systemic lupus erythematosus), myositis, and other autoimmune diseases. The promise of these therapies is their potential to be curative and penetrate tissues that monoclonal antibodies cannot. Following B cell depletion, healthy B cells are produced, potentially resetting the immune system. A range of autoimmune diseases, both rare and with large patient populations, could potentially be treated using these therapies. One key to success will be mitigating the CMC challenges to deliver products to patients, given that the patient populations for autoimmune diseases could far surpass those seeking treatment with cell therapies for oncology.

Q On the CMC and analytics side, what are the current main limitations and challenges being faced in the development of commercial CAR-T cell therapies?

AF: On the CMC side, having a solid baseline end-to-end roadmap to support the product development lifecycle is a key challenge. Product development is a process that spans numerous years and likely involves multiple changes. Being able to map out and align the CMC and clinical development plans with clear strategies for managing changes is essential for a smoother path from proof of concept to commercial readiness. The more lifecycle management is incorporated early on, the simpler it can be during later stages of development.

Moreover, it is incredibly important to define the quality target product profile (QTPP) as early as possible, using the QTPP to drive development efforts towards achieving a product quality profile that ensures patient safety and maximizes clinical benefits in accordance with the patient population. This requires early investment in developing a simple yet robust process that minimizes manual manipulations and open operations, alongside QC-friendly methods, and a clear plan for managing changes, as it is rare to employ a commercial-ready platform process during the early stages of development.

Change management is perhaps the Achilles' heel of product development. Changes are inevitable, but thoughtful implementation and ensuring lineage across the development lifecycle will reduce obstacles later on. Sometimes going slower early on helps accelerate progress later.

Finally, manufacturing capacity and alignment between securing the necessary capacity for clinical or commercial demand remains a real challenge. With complicated manufacturing

"The bottom line is to gain as much experience as possible in development labs using materials, consumables, and equipment similar to those in a GMP environment."

processes and laborious or highly variable analytical methods, technology transfer timelines are long, and defining success criteria for process and analytical transfers can be particularly challenging if process development and manufacturing experience is limited or if method performance is not appropriately monitored and controlled.

The bottom line is to gain as much experience as possible in development labs using materials, consumables, and equipment similar to those in a GMP environment. Employing best practices with analytical methods is key, for example, implementing appropriate assay controls, monitoring method performance, and having a robust sample retain program to enable future comparability studies and tech transfers.

Additionally, a robust manufacturing supply strategy is critical to success. With multiple examples where commercial launch or capacity expansion is occurring at contract manufacturers, partnering with organizations with late-stage and commercial experience, as well as companies offering fully automated and integrated solutions with flexible capacity, is key. An end-to-end roadmap, clear milestones, and readiness to implement at the right times are essential.

Q What emerging analytical assays, platforms, and workflows are emerging to help drive the requisite improvements in the quality and consistency of cell therapy manufacturing?

AF: Similarly to the introduction of automation within manufacturing processes, including end-to-end solutions, one of the biggest areas of growth for analytical methods is automation and rapid analysis methods. Instrument manufacturers are increasingly offering higher throughput or plate-based instruments to improve sample measurement workflows and now offer integrated solutions for automated sample preparation, particularly for flow cytometry and PCR-based methods. Robust robotic solutions have become more common and accessible, and deploying automated solutions improves real-time testing, efficiency, and consistency, allowing highly skilled operators and analysts to focus on sample management and data analysis rather than manual sample preparation. Instrument manufacturers are also building compliance with 21 CFR Part 11 requirements into their software to improve QC readiness, acknowledging user requirements for smoother implementation in cGMP environments, including equipment and electronic systems validation.

Another emerging area is methods capable of detecting multiple quality attributes. These approaches, including deep sequencing techniques, resemble how mass spectrometry

techniques became more widely used for monitoring and measuring multiple attributes in a single measurement for biological products. This approach expands the analytical characterization toolbox and may reduce the number of methods needed for release testing in the future.

Q Where are the most pressing requirements for future innovation in the cell therapy analytics toolkit? What would be at the top of your wishlist for innovation in this space?

AF: Innovations in automation, multiple attribute monitoring, and end-to-end analytical solutions have the potential to improve efficiency and reduce labor and turnaround times. We are just at the beginning of an evolutionary period where these automated analytical platforms are becoming more accessible, and more innovation is needed to bring these solutions to the forefront.

Integration of these tools at earlier stages of the product development lifecycle is tremendously important to support growing demand and reduce major bottlenecks associated with method changes later in the analytical lifecycle. Analytical bridging can be a huge challenge with long timelines of troubleshooting and further optimization needed, so developing automated and higher throughput options either from the beginning or in parallel with early analytical methods will enable deploying those solutions when the time is right. The analytical methods or tools used to monitor process performance, demonstrate safety, and ensure acceptable product quality, including patient dose, must focus on consistency and reproducibility.

Q Turning now to the regulatory space, where are the roadblocks, and what future developments are needed for regulatory guidance to keep up with rapid advancements in cell therapies?

AF: Diversity and differentiation are occurring within the field in concert with, and even driving, some of the rapid growth. This is excellent from scientific and therapeutic perspectives, but it makes the regulatory landscape quite challenging because each investigational product has unique considerations. With guidance documents aligning more closely with commercial products, given recent approvals, there is still room for interpretation and creativity at earlier stages of development and product-specific considerations.

A simple example is the recent draft guidance for manufacturing changes and comparability for human CGT products, which seems more tailored to autologous cell therapies like those recently approved that are in pivotal to commercial stages of development. The guidance is therefore limited when considering examples such as allogeneic CAR-T cell therapies and the unique challenges related to managing changes and comparability, given the relatively small number of batches manufactured for allogeneic therapies compared to autologous therapies.

Regardless, it is encouraging and tremendously helpful to have these guidance documents to use as a starting point. They represent a level of convergence that can help streamline development and set expectations for product development. A persistent bottleneck, though improving, is the growth within health authorities to support the sheer volume of regulatory submissions. Growth at the regulatory agencies is needed to help sponsors get the support needed to keep moving ahead. However, the predictability of growth rates and the ability to scale in concert with the growth of the field may still be difficult to manage.

This highlights the need for sponsors to have a good end-to-end product development roadmap to plan regulatory strategies, prepare for regulatory interactions, and get the most value from those interactions by obtaining feedback on current and prospective activities. Managing the product lifecycle over the entirety of the program and planning for success by implementing strategies at the right times is crucial.

BIOGRAPHY

ADAM FUNG has a PhD in Biochemistry with over 16 years of experience with functional and end-to-end product development of cell therapies, biologics, and small-molecules. He has led and supported functional and cross-functional product development teams as an Analytical Lead, Product Quality Lead, and CMC Strategy Lead for multiple early-stage, late-stage, and commercial programs. Particular areas of focus include process and analytical development, process and product control strategies, technology transfers, comparability studies, developing and managing end-to-end lifecycle management strategies, and operational leadership and oversight to support development of therapies intended to treat patients across a global regulatory landscape.

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CELL & GENE THERAPY INSIGHTS

ANALYTICS CHANNEL

COMMENTARY

Illuminating pathways and balancing precision: maximizing economic value in breast cancer gene therapy through integrated CMC, analytics, and collaboration

Arya Bhushan and Preeti Misra

Gene therapy has emerged as a promising approach for treating breast cancer, particularly in cases where traditional therapies have proven inadequate. The success of gene therapy in this context depends heavily on rigorous Chemistry, Manufacturing, and Controls (CMC) protocols and advanced analytics. This article discusses the crucial role of CMC and analytics in developing gene therapies for breast cancer, emphasizing their contributions to ensuring safety, efficacy, and scalability. By employing meticulous manufacturing processes and cutting-edge analytical techniques, researchers can address challenges, enhance therapeutic outcomes, and move closer to a future where breast cancer can be effectively targeted at the genetic level. As gene therapy for breast cancer continues to advance, innovation and collaboration in CMC and analytics will be key to unlocking its full potential and improving patient care.

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Breast cancer is a significant health concern globally, representing the most common invasive cancer in women [1]. The integration of cell and gene therapy (CGT) in its treatment holds promise but faces several critical challenges [2]. CGT stands at the forefront of revolutionary medical treatments, offering hope for countless individuals battling previously incurable genetic diseases [3]. While the spotlight often shines on groundbreaking research and clinical trials, behind-the-scenes efforts in Chemistry, Manufacturing, and Controls (CMC) and analytics are equally pivotal. These disciplines form the backbone of gene therapy development, ensuring safety, efficacy, and scalability. As the fight against breast cancer intensifies [4,5], gene therapy emerges as a beacon of hope, offering tailored interventions to combat this complex disease. In this Commentary, we delve into the significance of CMC and analytics in advancing gene therapy, exploring their contributions, challenges, and future prospects for breast cancer treatment.

CGT IN BREAST CANCER

Breast cancer is the most common invasive cancer in women worldwide, accounting for 30% of female cancer cases and the incidence is increasing by about 3% annually [6–8]. It is a heterogeneous disease characterized by uncontrolled cell growth in breast tissue, classified into subtypes such as hormone receptor-positive (HR+), HER2-positive, and triple-negative breast cancer (TNBC) [9]. HR+ breast cancer expresses estrogen and/or progesterone receptors and often responds well to hormonal therapies, while HER2-positive breast cancer overexpresses the HER2 protein and can be targeted with HER2 inhibitors. TNBC lacks estrogen, progesterone, and HER2 receptors, making it more aggressive with fewer targeted treatment options, often necessitating chemotherapy. Several clinical trials are currently exploring CGT approaches for breast cancer [10], including CAR-T cell therapies [11], oncolytic virotherapy [12], and gene therapies [13]. CAR-T cell therapies are being investigated for targeting HER2, with promising early results in tumor regression (NCT03696030) [14]. Several OVs have been studied for breast cancer treatment, including adenovirus, protoparvovirus, vaccinia virus, reovirus, and herpes simplex virus type I (HSV-1) [15,16]. Currently, there is no OV registered for breast cancer treatment, however, there are a number of ongoing preclinical trials focusing on a variety of viruses, such as those using talimogene laherparepvec (T-VEC), which aim to selectively infect and kill breast cancer cells (NCT02658812) [17]. Gene therapy trials are exploring CRISPR/Cas9 to correct genetic mutations driving breast cancer or to enhance the anti-tumor immune response (NCT04438083) [18].

Despite the potential of CGT in breast cancer, several challenges persist, including the tumor's heterogeneity, immunogenicity, safety, delivery efficiency, and cost. The genetic diversity of breast cancer complicates the development of universally effective CGT products, necessitating tailored therapies. Viral vectors [19] and CAR-T cells [20] can provoke immune responses, leading to side effects like cytokine release syndrome (CRS) or neurotoxicity. Efficiently delivering therapeutic genes to the tumor site is challenging due to barriers like the dense extracellular matrix and the immune-suppressive tumor microenvironment (TME). Additionally, CGT products are expensive to manufacture and scale, limiting patient accessibility. Streamlined production processes and cost-effective strategies are essential for making these therapies broadly available.

CGT, once a realm of speculation and promise, is now gaining traction as a viable approach in the fight against breast cancer [10]. They are different from other biologics and small molecules because of their inherent complexity and variability. This innovative strategy harnesses the power of genetic engineering to directly target the underlying molecular mechanisms driving cancer growth

and metastasis. Unlike traditional treatments that often cause collateral damage to healthy tissues, gene therapy holds the promise of precision medicine, delivering tailored interventions with minimal side effects. One of the most promising avenues of gene therapy research in breast cancer revolves around targeted gene delivery systems. By leveraging viral vectors or nanoparticle-based carriers, researchers aim to selectively deliver therapeutic genes to cancerous cells, effectively silencing oncogenes or enhancing tumor suppressor activity [19]. The selection of an appropriate vector for CGT products targeting breast cancer will determine the CMC strategy and significantly impact the economic assessment. Viral vectors like AAV and lentivirus offer high transduction efficiency and stable gene expression, crucial for therapies such as p53 gene delivery in breast cancer models [21]. However, they pose safety risks, including immunogenicity and insertional mutagenesis, although advancements like helper-dependent adenoviral vectors and self-inactivating lentiviral vectors mitigate these issues [22,23]. Non-viral vectors, including lipid nanoparticles (LNPs) and polymeric nanoparticles, offer safer alternatives with lower immunogenicity and better scalability. LNPs, for instance, have successfully delivered siRNA targeting HER2 in breast cancer [24]. Despite lower transduction efficiency, they can be engineered for enhanced targeting and penetration of the TME. The choice between viral and non-viral vectors should also consider the target cell type within the heterogeneous breast cancer population, the TME, and the intended therapeutic outcome. This choice profoundly influences the CMC strategy and economic viability, making it a critical decision in CGT product development for breast cancer. This targeted approach not only maximizes the therapeutic effect but also minimizes off-target effects, thereby improving the safety profile of treatment. However, this seemingly straightforward concept entails a complex journey from laboratory bench to patient bedside. To enhance the discussion on the development of CGT products for breast cancer, it is important to incorporate insights from various stages of the CMC process and their integration with analytics. These stages include upstream and downstream process development, analytical method validation, regulatory compliance, and economic assessments [25–28]. Additionally, integrating CMC technologies with analytics will provide a more detailed and cohesive discussion on the development pathway and practical implications of CGT products [29,30]. Expanding the review to include detailed descriptions of specific challenges in CGT production for breast cancer treatment can provide a more comprehensive understanding of the current landscape and future directions.

THE CRUCIAL ROLE OF CMC: CHALLENGES AND SOLUTIONS IN THE PRODUCTION OF CGT

While the promising potential of CGT is evident from clinical trials, companies encounter operational hurdles post-FDA approval, hindering efficient scaling and optimization of manufacturing processes [31]. Identifying these common roadblocks to commercializing CGTs is crucial. With over 1000 CGT assets in clinical trials, emerging companies must proactively evaluate risks and tackle operational and commercial challenges. Table 1 outlines the key challenges faced by emerging companies in the CGT industry, along with corresponding solutions to address these challenges effectively.

In the context of breast cancer gene therapy, precision manufacturing techniques not only enhance therapeutic efficacy but also drive economic value. CMC protocols encompass the meticulous orchestration in optimizing vector production, purification, and formulation processes ensuring consistent product quality and regulatory compliance. By implementing stringent manufacturing standards and robust QC measures, researchers can mitigate variability and ensure consistency in therapeutic outcomes. The potential

TABLE 1

Manufacturing challenges and solutions in the CGT industry.

Challenges	Solutions
Effective demand planning	Develop new capabilities for effective demand planning and manufacturing to accommodate patient-specific variability and time-dependent process steps
Management and storage of raw materials and consumables	Predictable availability of raw materials and consumables through key knowledge acquisition during the development phase
Incoming raw materials testing time constraints	Investment in improving efficiencies and capacity in QC processes during ramp-up
Process execution for treatment delivery	Establish cross-functionally aligned product supply and delivery processes that are both predictable and agile
Material sourcing and quality management	Invest in quality during procurement and sourcing of raw materials, and establish partnerships with CMOs
High demand/limited selection of CMOs in the CGT industry	Develop early manufacturing and sourcing strategies to ensure adequate capacity and resources based on long-term business plans
Quantity and quality of raw materials	Implement scalable QC and management systems for critical process components, such as viral vectors
High cost of goods sold (COGS)	Drive manufacturing innovation to reduce COGS, considering labor and direct materials costs. Develop growth and process improvement strategies before therapy approval to avoid complexities in post-submission changes

COGS: Cost of goods sold; CMOs: Contract manufacturing organizations; QC: Quality control.

economic impact of integrating critical stages of CMC and analytics in CGT products for breast cancer should be expanded upon. Efficient CMC strategies and robust analytics can significantly reduce production costs and accelerate time-to-market, as seen with CAR-T cell therapies for leukemia and lymphoma [32]. For example today the production costs for CAR-T therapies, such as Kymriah (tisagenlecleucel), can range from $200,000 to $500,000 per patient due to complex manufacturing processes and QC measures [33] which can demonstrate reduced costs through optimized manufacturing processes. These economic assessments provide a baseline for future breast cancer CGT products, emphasizing the need for scalable, cost-effective production methods and rigorous analytical standards to ensure product quality and efficacy, ultimately improving accessibility and affordability for patients. Moreover, CMC strategies facilitate scalability, enabling the translation of promising preclinical findings into clinically viable treatments for breast cancer patients. By optimizing manufacturing processes, minimizing waste, and maximizing resource utilization, CMC strategies contribute to cost-effective production of gene therapy products. Moreover, efficient CMC protocols accelerate the translation of promising preclinical findings into clinically viable treatments, reducing time-to-market and overall development costs.

Along with manufacturing techniques, analytical tools also play a crucial role in evaluating the efficacy, purity, and stability of gene therapy products for breast cancer. However, the CGT field itself faces various challenges in characterizing, monitoring, and ensuring product quality [34]. Some common analytical challenges are summarized in Table 2. Addressing these analytical challenges requires interdisciplinary collaboration, innovative assay development, and continuous adaptation to evolving regulatory requirements and technological advancements in the field of CGT.

Advanced analytical techniques such as high-throughput sequencing, multi-parametric flow cytometry, mass spectrometry-based proteomics, real-time monitoring with process analytical technologies, and spectroscopic techniques enable precise characterization of vectors and cells, comprehensive assessment of impurities,real-time monitoring of critical process parameters, and non-destructive

TABLE 2

Analytical challenges in CGT.

Analytical challenges	Description
Vector characterization	Characterizing viral vectors for gene editing tools used in CGT, including assessing their purity, potency, stability, and integrity
Cell characterization	Evaluating the identity, potency, phenotype, viability, differentiation status, and immunogenicity of therapeutic cells used in CGT
QC testing	Conducting rigorous QC testing to ensure compliance with regulatory standards and product specifications, assessing identity, purity, sterility, and potency of CGT products
Stability testing	Assessing the stability of CGT products over time to determine shelf-life and storage conditions, detecting product degradation, impurities, and changes in potency
Biomarker identification	Identifying reliable biomarkers for predicting treatment responses and monitoring patient outcomes in CGT, including developing sensitive and specific assays for their detection
Process monitoring and optimization	Monitoring manufacturing processes, optimizing process parameters, and controlling critical process parameters to ensure consistent product quality and scalability in CGT
Data analysis and interpretation	Analyzing complex datasets generated from analytical testing in CGT, including data integration, interpretation, and visualization to derive meaningful insights and support decision-making

CGT: Cell and gene therapy; QC: Quality control.

evaluation of stability. These techniques pave the way for improved product quality, safety, and efficacy in CGT. By harnessing the power of analytical techniques, researchers can unravel the molecular mechanisms underlying breast cancer progression and treatment response, empowering clinicians to tailor treatment regimens based on individual patient profiles and maximize therapeutic efficacy while minimizing adverse effects.

The FDA plays a crucial role in guiding the development of CGT products through comprehensive guidelines for CMC. These guidelines are designed to ensure the safety, quality, and efficacy of CGT products by outlining requirements for manufacturing processes, characterizing starting materials, controlling critical quality attributes, and validating analytical methods [35]. Emphasizing the importance of robust manufacturing processes compliant with Good Manufacturing Practices (cGMP), the FDA guidelines also stress thorough product characterization, including assessments of identity, purity, potency, and stability. They cover expectations for both early and late-stage product development, facilitating accelerated pathways through FDA approval. To address challenges such as the variability in CMC frameworks, enhanced communication and knowledge-sharing among stakeholders is vital. It is essential to note that these guidelines not only assist sponsors of gene therapy Investigational New Drug applications but also provide valuable insights for developers seeking approval through other regulatory pathways. By adhering to these guidelines, CGT developers can effectively navigate regulatory pathways, mitigate risks, and advance promising therapies from research to clinical practice, ultimately benefiting patients and advancing the field of regenerative medicine.

NAVIGATING ECONOMIC REALITIES THROUGH ANALYTICS AND OPTIMIZING COLLABORATION FOR ECONOMIC SUSTAINABILITY

After addressing the challenges and solutions to enhance CMC for CGT in breast cancer treatment, attention now turns to their economic feasibility. This encompasses considerations of accessibility and affordability,

particularly for patients in developing countries [36]. Navigating economic realities through analytics in the CGT field involves leveraging data-driven insights to optimize resource allocation, mitigate financial risks, and maximize return on investment (ROI). Table 3 summarizes some insights on how analytics can be applied in the CGT industry.

Analytics are crucial for evaluating the economic viability and value proposition of breast cancer gene therapy, as they provide valuable insights and data-driven evidence on the effectiveness and cost–effectiveness of the treatment. Through cost–benefit analyses, health economic modeling, and market forecasting, analytics provide insights into the long-term economic impact of gene therapy adoption. By evaluating factors such as treatment efficacy, patient outcomes, and healthcare utilization, analytics help stakeholders make informed decisions regarding investment, reimbursement, and market access. Furthermore, real-time monitoring of treatment response and predictive analytics enable proactive management of healthcare resources, optimizing the allocation of funds and maximizing economic value.

Addressing economic challenges and charting a path forward in the context of breast cancer gene therapy requires a multifaceted approach that considers various factors influencing both the development and accessibility of these innovative treatments. Firstly, research and development (R&D) efforts must focus on optimizing gene therapy approaches specifically tailored to breast cancer subtypes, ensuring efficacy while minimizing potential side effects. Collaborative initiatives between academia, industry, and government entities are crucial for advancing preclinical and clinical research, as well as facilitating technology transfer and knowledge exchange. Moreover, cross-sector collaborations enable shared resources, expertise, and infrastructure, reducing development costs and accelerating the commercialization of CGT products. A study by Elorza (2021) underscores the economic advantages of collaborative research networks in CGT, demonstrating increased efficiency, productivity,

TABLE 3

Economic analysis of gene therapy for breast cancer.

Analytics	Example
Cost–benefit analysis	A pharmaceutical company conducts a cost–benefit analysis to assess the economic viability of developing a gene therapy for metastatic breast cancer. The analysis considers factors such as research and development costs, manufacturing expenses, clinical trial expenses, regulatory fees, and potential revenue from product sales
Market forecasting	Market research firms use data analytics to forecast the global demand for breast cancer therapies, including gene therapies. They consider factors such as breast cancer incidence rates, patient demographics, treatment trends, healthcare infrastructure, and regulatory landscape
Pricing optimization	A biotechnology company uses predictive analytics to optimize the pricing strategy for its gene therapy for HER2-positive breast cancer. By analyzing production costs, competitor pricing, reimbursement rates, and patient affordability, the company sets a pricing structure that maximizes revenue while ensuring access for patients
Supply chain management	Breast cancer therapy manufacturers implement advanced analytics tools to optimize their supply chain operations, including sourcing raw materials, managing production schedules, monitoring inventory levels, and optimizing distribution logistics to ensure timely delivery of therapies to patients
Risk management	A biopharmaceutical company uses predictive modeling and scenario analysis to assess the financial, regulatory, and clinical risks associated with developing a novel gene therapy for breast cancer. By identifying potential risks and developing mitigation strategies, the company minimizes the likelihood of project failure and protects its investment
Value-based healthcare	Healthcare payers and providers use analytics to evaluate the value proposition of gene therapies for breast cancer based on patient outcomes, cost–effectiveness, and overall impact on the healthcare system. By aligning reimbursement models with value-based principles, stakeholders ensure that therapies are reimbursed based on their demonstrated value to patients and society

and cost–effectiveness compared to isolated efforts.

Furthermore, initiatives such as the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) foster collaboration across academia, industry, and government to drive innovation, standardization, and workforce development in biopharmaceutical manufacturing, including CGT. Additionally, allocating resources towards enhancing infrastructure and expanding manufacturing capabilities for gene therapy production can effectively mitigate manufacturing costs and facilitate the scaling up of production to meet the growing demand.

To overcome economic barriers to access, innovative financing models and reimbursement strategies need to be explored. These may include value-based pricing arrangements that align reimbursement with treatment outcomes, as well as novel payment models that spread costs over time to alleviate financial burdens on patients and healthcare systems. Additionally, regulatory agencies play a pivotal role in fostering an enabling environment for breast cancer gene therapy by streamlining approval processes and providing clear guidance on clinical trial design and endpoints. Furthermore, patient advocacy groups and community organizations play a crucial role in raising awareness about breast cancer gene therapy, advocating for improved access to these treatments, and providing support to patients navigating the complexities of treatment decision-making.

Finally, continued investment in research and education aimed at understanding the long-term benefits and potential risks of gene therapy for breast cancer is essential for building confidence among patients, clinicians, and payers, ultimately paving the way for widespread adoption and integration of these transformative therapies into standard clinical practice.

CONCLUSION

In the quest to maximize value in breast cancer gene therapy, the integration of CMC, analytics, and economic considerations is paramount. By leveraging precision manufacturing techniques, advanced analytical tools, leveraging analytics to assess economic viability, and fostering collaboration among stakeholders, researchers can navigate the complexities of breast cancer biology and treatment response, paving the way for personalized and effective therapeutic interventions and ensure that gene therapy innovations translate into tangible economic benefits for patients, healthcare systems, and society as a whole. As we embark on this transformative path to navigate the complexities of economic realities, let us remain steadfast in our commitment to harnessing the power of science and innovation to combat breast cancer gene therapy in a manner that is both clinically effective and economically sustainable and improves the lives of patients worldwide.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 2018; 68(6), 394–424.

2. Lee SY, Kim S, Tyler PM, Park N, Zhou Y. Current status of gene therapy for breast cancer: progress and challenges. *J. Exp. Clin. Cancer Res.* 2020; 39(1), 1–20.

3. Elverum K, Whitman M. Delivering cellular and gene therapies to patients: solutions for realizing the potential of the next generation of medicine. *Gene Ther.* 2020; 27, 537–544.

4. Bhushan A, Gonsalves A, Menon JU. Current state of breast cancer diagnosis, treatment, and theranostics. *Pharmaceutics* 2021, 13, 723.

5. Grimm D. Recent advances in breast cancer research. *Int. J. Mol. Sci.* 2023; 24(15), 11990.

6. Loibl S, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast cancer. *Lancet* 2021; 397(10286), 1750–1769.

7. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J. Clin.* 2023; 73(1), 17–48.

8. Obeagu, EI, Obeagu GU. Breast cancer: a review of risk factors and diagnosis. *Medicine* 2024; 103(3), e36905.

9. Shaath H, Elango R, Alajez NM. Molecular classification of breast cancer utilizing long non-coding RNA (lncRNA) transcriptomes identifies novel diagnostic lncRNA panel for triple-negative breast cancer. *Cancers* 2021; 13(21), 5350.

10. Dastjerd NT, Valibeik A, Rahimi Monfared S, *et al.* Gene therapy: a promising approach for breast cancer treatment. *Cell Biochem. Funct.* 2022; 40(1), 28–48.

11. Niu Z, Wu J, Zhao Q, Zhang J, Zhang P, Yang Y. CAR-based immunotherapy for breast cancer: peculiarities, ongoing investigations, and future strategies. *Front. Immunol.* 2024; 15, 1385571.

12. Chowaniec H, Ślubowska A, Mroczek M, *et al.* New hopes for the breast cancer treatment: perspectives on the oncolytic virus therapy. *Front. Immunol.* 2024; 15, 1375433.

13. Mirza Z, Karim S. Nanoparticles-based drug delivery and gene therapy for breast cancer: recent advancements and future challenges. *Semin. Cancer Biol.* 2021; 69, 226–237.

14. ClinicalTrials.gov. A Study of CAR-T Cells Targeting HER2 in Patients With HER2-Positive Solid Tumors (NCT03696030). https://clinicaltrials.gov/study/NCT03696030.

15. Carter ME, Hartkopf AD, Wagner A, *et al.* A three-dimensional organoid model of primary breast cancer to investigate the effects of oncolytic virotherapy. *Front. Mol. Biosci.* 2022; 9, 826302.

16. Brito Baleeiro R, Liu P, Chard Dunmall LS, *et al.* Personalized neoantigen viro-immunotherapy platform for triple-negative breast cancer. *J. Immunother. Cancer* 2023; 11, e007336.

17. ClinicalTrials.gov. Oncolytic Virotherapy for Breast Cancer (NCT02658812). https://clinicaltrials.gov/study/NCT02658812.

18. ClinicalTrials.gov. Gene Therapy for Breast Cancer (NCT04438083). https://clinicaltrials.gov/study/NCT04438083.

19. Tseu GYW, Kamaruzaman KA. A review of different types of liposomes and their advancements as a form of gene therapy treatment for breast cancer. *Molecules* 2023; 28, 1498.

20. Chalermchai S, Nattaporn P, Kwanpirom S, *et al.* Cytotoxicity of fourth-generation anti-Trop2 CAR-T cells against breast cancer. *Int. Immunopharmacol.* 2024, 129, 111631.

21. Marvalim C, Datta A, Lee SC. Role of p53 in breast cancer progression: an insight into p53 targeted therapy. *Theranostics* 2023; 13(4), 1421–1442.

22. Xie H, Lim B, Lodish HF. MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. *Diabetes* 2017; 56(5), 1429–1436

23. Nayerossadat N, Maedeh T, Ali PA. Viral and nonviral delivery systems for gene delivery. *Adv. Biomed. Res.* 2012; 1, 27.

24. Wang H, Agarwal P, Zhao S, Xu RX, Yu J, Lu X. Hybrid nanoparticles of lipid-enveloped polymer for gene delivery to breast cancer cells. *J. Nanobiotechnol.* 2019; 17(1), 27.

25. Wurm FM. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat. Biotechnol.* 2004; 22(11), 1393–1398.

26. Shukla AA, Thömmes J. Recent advances in large-scale production of monoclonal antibodies and related proteins. *Trends Biotechnol.* 2010; 28(5), 253–261.

27. Jacobson PD, Milstein R. The 21st century health care workforce: how should we adapt to the age of the 'new medicine'? *Health Affairs* 2008; 27(1), w58–w68.

28. Faulkner E, Spinner DS, Ringo M, Carroll M. The growing value of gene therapy and the need for outcome-based pricing. *J. Market Acc. Health Pol.* 2019; 7(1), 1589136.

29. Rathore AS, Winkle H. Quality by design for biopharmaceuticals. *Nat. Biotechnol.* 2009; 27(1), 26–34.

30. Kulkarni RV, Roney CA. Integration of process analytical technology (PAT) in biopharmaceutical manufacturing: applications and case studies. *J. Pharm. Sci*. 2020; 109(2), 579–586.

31. Csaszar E, Mills S, Zandstra PW. Process evolution in cell and gene therapy from discovery to commercialization. *Canadian J. Chem. Eng.* 2021; 99(11), 2517–2524.

32. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. *EMBO Mol. Med.* 2017; 9(9), 1183-1197.

33. Scott AM, Wolchok JD, Old LJ. Antibody therapy for cancer. *Nat. Rev. Cancer* 2018; 12(4), 278–287.

34. Smith D, LeBlon C, Shapka S. Redefining contamination control for CGTs. *BioPharm Int.* 2021; 34(11), 32–35.

35. FDA. *Cellular and Gene Therapy Guidances*. 2024; US Food & Drug Administration. https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/cellular-gene-therapy-guidances.

36. Cornetta K, Bonamino M, Mahlangu J, Mingozzi F, Rangarajan S, Rao J. Gene therapy access: global challenges, opp ortunities, and views from Brazil, South Africa, and India. *Mol. Ther.* 2022; 30(6), 2122–2129.

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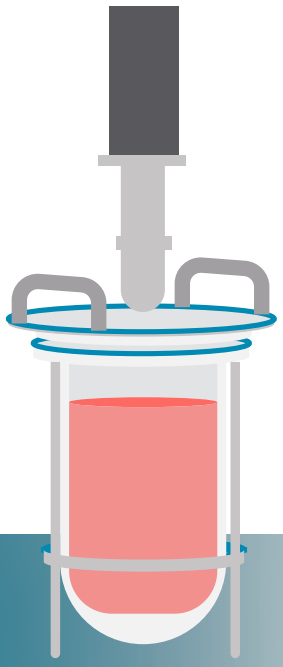


Demystifying AAV affinity capture: mapping AAV-affinity ligand footprints with cryo-electron microscopy

Nathaniel Clark, Scientist, Downstream Development, Repligen

AVIPure affinity resins provide efficient, cost-effective purification solutions for AAV vectors. These resins feature high-affinity ligands stable in hydroxide, making them robust through 20+ clean-in-place (CIP) cycles. This FastFacts poster explains how cryo-electron microscopy (EM) structures of AVIPure ligands in complex with AAV capsids reveals the binding regions, and aids in selecting capsids that pair with AVIPure resins.

AVIPURE SCAFFOLDS AND AAV AFFINITY RESIN

AVIPure technology is based on a diverse library of amino-acid-based scaffolds that deliver high-affinity ligands for biological epitopes. This technology rapidly identifies ligands without animal immunizations, and the ligands are inherently manufacturable, highly selective, and efficient at eliminating host cell proteins and DNA.

AVIPure affinity resins lower COG as they remain stable in NaOH for 20+ CIP cycles, making them highly reusable. **Figure 1** shows AVIPure AAV2 resin performance, consistently providing 4-log clearance of host cell proteins and 2.7-log clearance of DNA after 30 simulated CIP cycles (15 hours of 0.5M NaOH exposure), with no loss in capacity, peak broadening, or increase in back pressure.

Figure 1. AVIPure AAV2 performance data of host cell proteins and host cell DNA

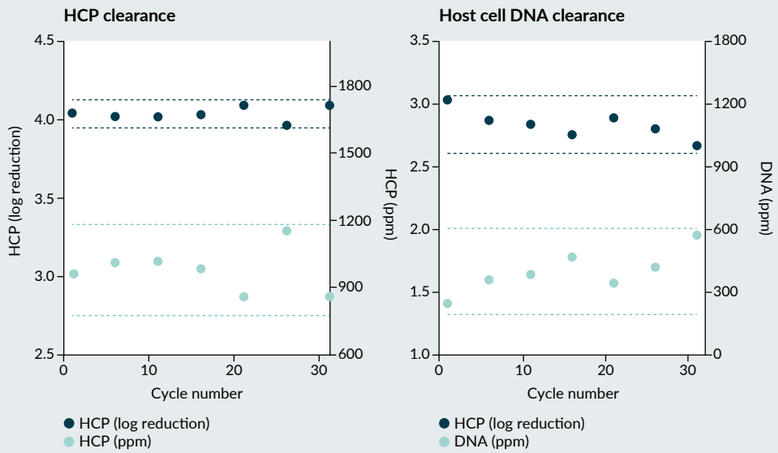
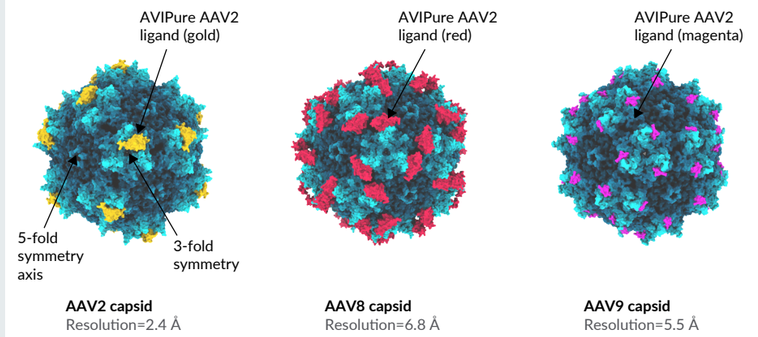


Figure 2. Cryo-EM structures of AVIPure AAV2, AAV8, and AAV9 bound to their respective capsids.



CRYO-EM STRUCTURES OF THE AVIPURE LIGAND: CAPSID COMPLEXES

Cryo-EM mapping identified which AAV capsid residues bind to AVIPure ligands. **Figure 2** displays cryo-EM structures of AAV2, AAV8, and AAV9 ligands bound to their respective capsids, with each ligand binding to distinct regions around the 3-fold symmetry axes.

The AAV2 ligand binds between the external lobes of the threefold axis and interacts with approximately 23 capsid residues, covering 1,060 Å2 of surface area. The AAV8 ligand also targets the threefold region but on its exterior, contacting 18 residues, and with a surface area of 778 Å2. Finally, the AAV9 ligand, a 1.5 kDa peptide with a 615 Å2 contact area, binds specifically to the galactose binding pocket of the AAV9 capsid and interacts with 10 residues. The AAV9 ligand makes very efficient use of its small size, delivering exquisite selectivity and purity of the vector product.

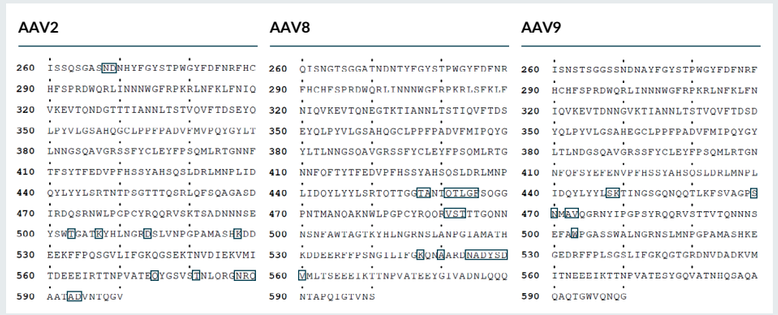
CAPSID RESIDUES AND SUCCESSFUL AVIPURE CAPTURE

The cryo-EM structures reveal specific residues recognized by AVIPure ligands. Engineered capsids that retain the boxed residues in **Figure 3** can be captured efficiently with AVIPure resins. This information can be valuable in the early capsid engineering and selection stages. If capsids that maintain the AVIPure epitopes progress to downstream development, an economical capture solution will already be in place.

SUMMARY

AVIPure technology delivers high-capacity, highly stable affinity resins capable of multiple CIP cycles in NaOH, offering superior process economics and environmental benefits. By leveraging cryo-EM analysis to map ligand-capsid interactions, this work enables a rational approach to pairing engineered capsids with appropriate AVIPure AAV affinity resins. This approach simplifies downstream process development, ensuring efficient purification and reducing production costs.

Figure 3. Sequence alignment of AVIPure resins where residues in each are noted in boxes.



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IN PARTNERSHIP WITH REPLIGEN

FASTFACTS

Automated cryopreservation process development for leukapheresis to support supply chains

Alexandre Michaux, Manager, Process Development, and MSAT, Cryoport Systems

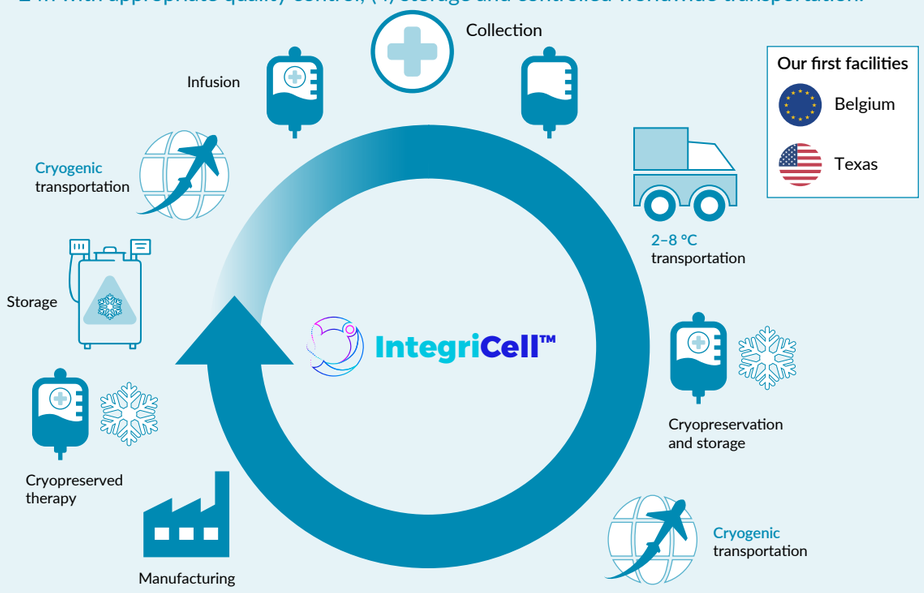
The supply chain of fresh leukapheresis starting materials faces challenges that can impact manufacturing quality and costs, primarily due to two reasons. First, the quality of starting material degrades over time, which can lead to low-quality drug products. Second, transporting fresh leukapheresis materials over long distances complicates manufacturing slot management and affects the quality of the final drug product. This FastFacts poster outlines the development of a robust cryo-process to support leukapheresis starting materials for cell therapy supply chains.

SOLUTIONS-DRIVEN INTEGRATED LEUKAPHERESIS SUPPLY CHAIN PLATFORM

Cryoport Systems supports the cell field with its IntegriCell™ platform, an integrated leukapheresis supply chain platform designed to ensure consistent, compliant, and high-quality starting materials for cell-based therapies. The platform optimizes manufacturing capacity planning, reducing risk and cost in drug production.

To further enhance cell therapy outcomes, Cryoport is establishing cryopreservation centers across the US and EU. This network ensures proximity to patients and standardizes leukapheresis cryopreservation within an integrated storage and distribution system, strengthening the global supply chain ensuring robust and reliable support for cell therapy manufacturing, as seen in **Figure 1**.

Figure 1. Leukapheresis supply chain platform collection support. (1) Leukapheresis pick up at collection site; (2) temperature-controlled transportation; (3) standardized cryopreservation <24h with appropriate quality control; (4) storage and controlled worldwide transportation.

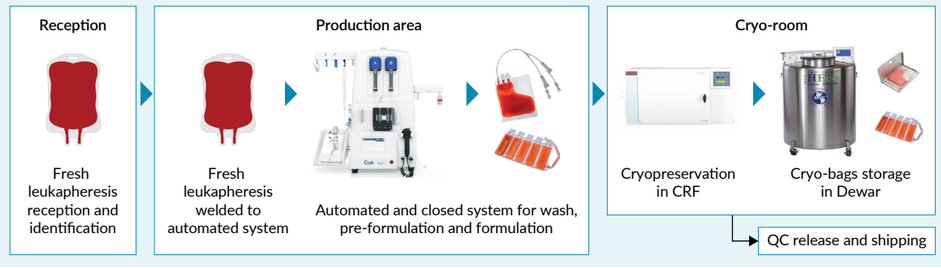


CLOSED CRYO-PROCESS FOR FRESH LEUKAPHERESIS

A DoE was conducted using the Cue® Cell Processing System from Fresenius Kabi to develop an automated closed process for leukapheresis, including buffer preparation, Cue system processing, post-processing, and freezing as illustrated in **Figure 2**. Cue system processing involved leukapheresis cell resuspension, membrane filtration washing, buffer exchange, and cryo-formulation. A QbD cryo-process was developed to automate and close the process, enhancing reproducibility.

Key process parameters, such as buffer content, cell concentration, critical process parameters (CPPs), and spinner wash flow rate were optimized. After CPP optimization, the automated cryo-process was then compared to a manual cryo-process. Cell recovery and viability of manual and automated cryopreserved products were measured using an automated cell counter. Both processes provide high viable nucleated cell (VNC) recovery and viability (90.2±8.7% and 91.8±6.6% recovery and 98.3±0.7% and 97.7±0.5% viability for manual and automated cryo-process respectively. Interestingly, manual cryo-process showed higher recovery variations with outliers potentially associated with operator variability while automated ensured the robust performance within the defined CQA, as illustrated in **Figure 3**. Additionally, the hematocrit/white blood cell ratio in the starting leukopaks shows no potential impact on the cell recovery, or viability during and after the automated cryo-processing.

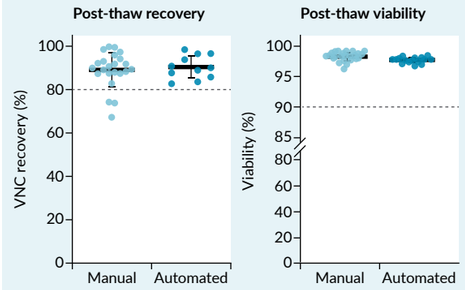
Figure 2. IntegriCell automated cryo-process for fresh leukapheresis.



SUMMARY

The DoE study demonstrated that the leukapheresis cryopreservation process using the IntegriCell™ platform enables automated, closed leukapheresis processing moving beyond a typical "one-size-fits-all" cryopreservation approach. The platform standardized the process, ensuring consistent cell viability and recovery while maintaining immune cell populations across multiple donor-derived leukapheresis. Cryopreservation, when performed appropriately, can provide a high-quality starting material for cell therapy manufacturing. The key goal of the IntegriCell™ platform is to enable increased and improved standardization for high-quality leukapheresis supply to support clinical and commercial partners globally.

Figure 3. Post-thaw recovery and post-thaw viability of GMP-compatible leukapheresis of both manual and automated cryo-processes.



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