

CHALLENGES OF
COMMERCIAL-SCALE
AUTOLOGOUS
CAR-T CELL MANUFACTURING

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INTRODUCTION PAGE 4

HISTORICAL BACKGROUND PAGE 7

MANUFACTURING TECHNOLOGIES FOR CELL THERAPIES PAGE 11

**DESIGN OF LATE-STAGE AND COMMERCIAL
PROCESSES AND MANUFACTURING SUITES** PAGE 16

MANY PATHS, ONE DESTINATION PAGE 21

REFERENCES PAGE 23

ABOUT THE AUTHORS PAGE 28



The field of cell therapy is relatively young and advancing quickly. Both product and process innovations are needed for bringing new cell therapies to market. Success requires a deep understanding of cell biology to modify cells of interest and expand cultures to sufficient numbers for treatment. The authors discuss the current state of product and process development in autologous cell therapy as well as what is needed to meet the demand for these life-saving treatments. Automation is the main focus along with other technologies used to produce the high numbers of doses required to treat large populations.

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The clinical successes of chimeric antigen receptor (CAR) T lymphocytes for the treatment of CD-19-expressing B cell malignancies paved the way for the US Food and Drug Administration (FDA) approval of Yescarta and Kymriah therapies in 2017. Both engineered T-cell products displayed potent activity against hematological malignancies in early phase clinical trials more than a decade ago (1). With multicenter trials reporting complete response rates of 40–74%, both products evolved from last-resort options to fully curative treatments (2, 3). With remarkable efficacy and durability, a total of six autologous CAR-T products are now approved by the FDA for the treatment of aggressive B-cell lymphomas, mantle-cell lymphomas, and B-cell acute lymphoblastic leukemia (Table 1). More recently, CAR-T cells have been directed toward B-cell maturation antigen (BCMA) to treat multiple myeloma. The documented potential patient populations for B-cell lymphoma (544,352), B-cell leukemia (64,190) and multiple myeloma (176,404) totals over three quarters of a million patients (Table 1), with the actual numbers likely to be much higher.

Today, there are more than 2000 active clinical trials for CAR-T cell therapies worldwide, with more than 200 ongoing phase 3 clinical trials (4). Clinical CAR-T manufacturing accounted for 78% of the cell-therapy market in 2022, and commercial autologous production is predicted to take over as soon as 2025. Such successes also provide justification for CAR-T cell therapies to be prescribed earlier in treatment. The FDA approved Yescarta and Breyanzi therapies as second-line treatments in 2022, increasing the available patient pool for the former by more than 11,000 in the United States alone (5). This trend is likely to continue, with more indications being treated now at earlier stages in the standard of care than ever before. For example the FDA recently approved Carvykti (ciltacabtagene autoleucel) and Abecama (idecabtagene vicleucel) as second- or third-line treatments for refractory myeloma

Table 1: Overview of licensed chimeric antigen receptor (CAR) T-cell therapy indications and annual incidences

Product	Company	Year Licensed	Target Antigen	Indications	Potential Patients
Kymriah (tisagenlecleucel)	Novartis	2017	CD19	B-cell acute lymphoblastic leukemia (ALL) ² B-cell non-Hodgkin lymphoma (NHL) ¹	544,352 ¹ 64,190 ²
Yescarta (axicabtagene ciloleucel)	Kite/Gilead	2017	CD19	B-cell non-Hodgkin lymphoma (NHL) ¹ Follicular lymphoma	
Tecartus (brexucabtagene autoleucel)	Kite/Gilead	2020	CD19	Mantle cell lymphoma (MCL) B-cell acute lymphoblastic leukemia (ALL) ²	
Breyanzi (lisocabtagene maraleucel)	Juno/BMS	2021	CD19	B-cell non-Hodgkin lymphoma (NHL) ¹	
Abecma (idecabtagene vicleucel)	Celgene/ BMS	2021	BCMA	Multiple myelomac	176,404 ³
Carvykti (ciltacabtagene autoleucel)	Legend/ Janssen	2022	BCMA	Multiple myelomac	

¹ Mafra A, et al. Global Patterns of Non-Hodgkin Lymphoma in 2020. *Int. J. Cancer* 151(9) 2022: 1474–1481; <https://doi.org/10.1002/ijc.34163>.

² Yi M, et al. Global Burden and Trend of Acute Lymphoblastic Leukemia from 1990 to 2017. *Aging* 12(22) 2020: 22869–22891; <https://doi.org/10.18632/aging.103982>.

³ Huang J, et al. The Epidemiological Landscape of Multiple Myeloma: A Global Cancer Registry Estimate of Disease Burden, Risk Factors, and Temporal Trends. *Lancet Haematol.* 9(9) 2022: e670–e677; [https://doi.org/10.1016/s2352-3026\(22\)00165-x](https://doi.org/10.1016/s2352-3026(22)00165-x).

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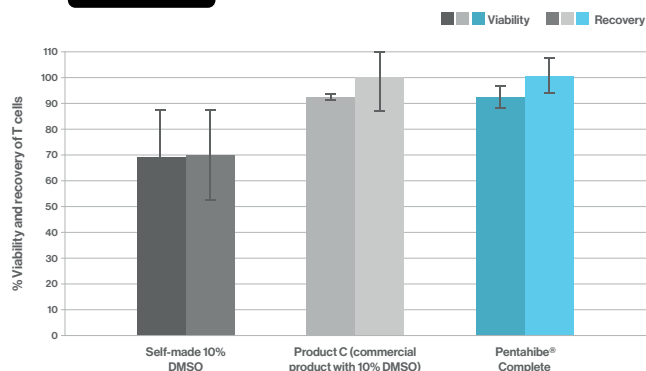
2%
DMSO



DMSO
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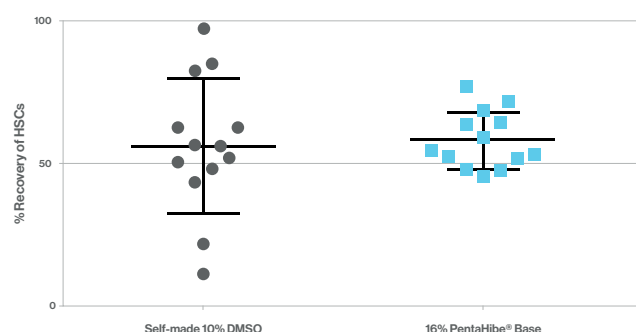
T cells¹



T-cells purified from buffy coats from three healthy donors, cryopreserved and analysed for post-thaw viability and recovery.

1. Hastrup EK et al. Transplantation and Apheresis Science, 2021; Vol 60(4): 103138

HSCs²



Recovery of hematopoietic stem cells from apheresis samples after thawing, expressed as the percentage of the number of HSCs before cryopreservation. Each data point represents duplicate measurements for each donor.

2. Svalgaard JD et al. Transplantation and Cellular Engineering, 2016 May; Vol 56(5): 1088-1095

respectively. Analysts predict that the eligible patient population for CAR T-cell therapies will exceed two million patients by 2029 (6).

However, access to these life-saving therapies is hampered by current cell-therapy manufacturing capacity, which is unable to meet the projected demand. During a 2022 clinical trial of heavily pretreated multiple-myeloma patients, 40% of participants waited a year to receive the Abecma CAR T-cell therapy — and about 25% of patients died waiting for treatment (7). Manufacturing of all six FDA-approved therapies provided enough material to treat an estimated 8,750 patients in 2023 (Figure 1), based on a calculation of product revenues and the cost of treatment (8, 9). That number contrasts with the thousands of patients who are diagnosed with lymphoma and leukemia worldwide — and multiple myeloma exceeded 750,000 patients (Table 1).

To understand the discrepancy between the numbers of potentially eligible patients and those actually receiving treatment, we need to acknowledge that the numbers being treated while drugs move through clinical development increase only slightly compared with demand that will manifest once commercial approval comes. Treating 100 patients can be considered the norm for clinical supplies of small-molecule drugs or protein biologics; the numbers of patients treated with autologous cell therapies is much lower than that. In Phase 3 trials, Yescarta and Kymriah subjects numbered 108 and 63, respectively (13, 14). As of December 2023, the total number of patients treated with those therapies was only 24,737 (<50-fold growth and ~14× the number of patients treated in 2019) (Figure 1).

Most CAR-T cell therapies originate from academic laboratories. Almost by definition, these benchtop solutions start small and will need to be scaled up or out to meet the demand for clinical and then commercial production. Transitioning to commercial scale from research and development (R&D) brings new variables to the forefront. Most developers focus on maintaining the highest quality of cells to treat patients, the speed of development to market, and process economics.

Both product and process innovation are needed for bringing a new cell therapy to market. Such treatments require not only novel applications of how to use cells to treat disease, but also a deep understanding of cell biology to modify cells of interest and expand cultures to sufficient numbers for treatment. The field of cell therapy is relatively young, with high rates of product and process innovation (Figure 2). Using living cells as drugs requires

Figure 1: Number of patients treated (derived from yearly revenue figures for each drug and the expense of each treatment)

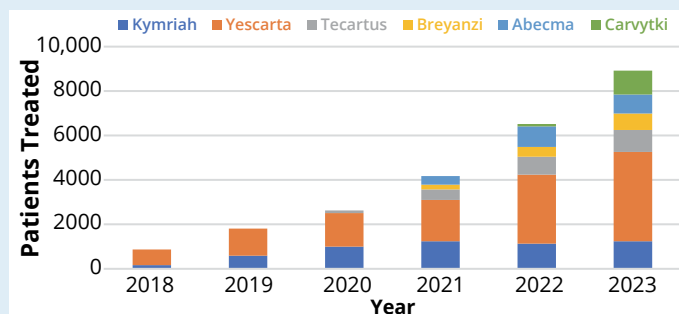
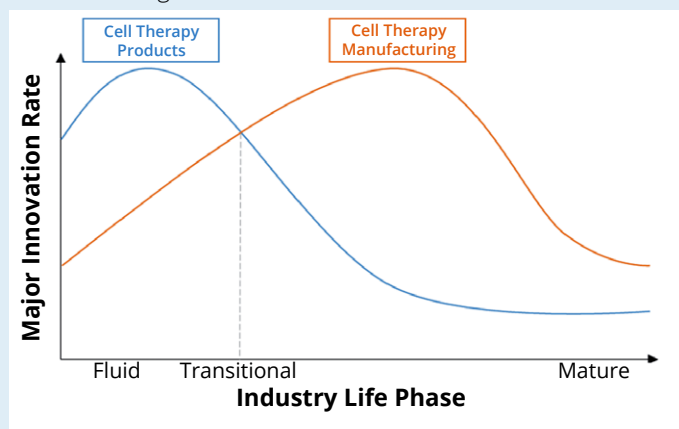


Figure 2: Product and process innovation — cell biology allows for innovation of products, and automation drives manufacturing innovation.



different products and processes compared with those of protein biologics and small-molecule drugs (15, 16). Herein, we discuss the current state of product and process development in autologous cell therapy, as well as what is needed to meet the demand for these life-saving therapeutics. We focus on automation and other technologies used to produce increasing numbers of doses required to treat patients.

To understand cell-based product innovation cycles, it helps to examine first the development of monoclonal antibodies (mAbs) and therapeutic proteins. Today's cell and gene therapies (CGTs) have come about after years of innovation for related products, processes, and delivery methods.

HISTORICAL BACKGROUND

The birth of modern-day biopharmaceuticals coincides with the introduction of mAbs by Köhler and Milstein in 1975 (17). Their approach was to generate a hybridoma by fusing cells from a myeloma cell line with immortalized spleen cells from a mouse. That resulted in an immortal cell line that produced a single specific antibody. Several mammalian expression systems since have been developed for mAbs, including NSO murine myeloma cells, PER.C6 human cells, and Chinese hamster ovary (CHO) cells. Today, the main focus is on production of recombinant mAbs through in vitro expression of genes using the CHO-cell platform, which once was considered difficult to work with. After two decades of optimization of cell-line, media, and bioreactor conditions, current mAb yields are >10 g/L and cell densities of >20 million cells/mL in fed-batch processes (18).

Standard practice in mAb development includes clonal selection through high-throughput screening and cloning of the highest-producing construct into a commercially available cell-expression platform. That typically includes a finely tuned expression vector along with optimized growth media and supplements. Cells often are expanded in a “seed train” based on single-use bioreactor systems to develop an inoculum for introduction to a production bioreactor of up to 12,000 L in volume. Advances in on-line monitoring and analytical methods have helped the industry implement robust and reproducible upstream and downstream processes and validation approaches for production-scale good manufacturing practice (GMP) operations.

History of Gene Therapy: The gene-therapy approach had a promising start in 1990 with the cure of severe combined immunodeficiency (SCID) by viral delivery of a missing gene to patients (19). Within a decade, however, gene-therapy development halted in its tracks after the death of a clinical trial subject who had a severe immune response to the vector preparation — as well as the triggering of leukemia in several children who'd been treated with another viral vector (20, 21). Further development of safer vector systems and improved clinical trial support, however, brought a resurgence for gene therapy in the early 2010s. Adenoassociated virus

BACK TO CONTENTS



Autologous cell-therapy protocols historically involve many hands-on steps and manual processes, as in this image from Lonza Group.

([HTTPS://WWW.LONZA.COM/CELL-AND-GENE](https://www.lonza.com/cell-and-gene))

(AAV) vectors have proven to be effective in delivering therapeutic genes to patients who suffer from rare, monogenic diseases. At the same time, AAV and lentivirus vectors (LVVs) also have been demonstrated to deliver gene-modifying payloads effectively to T lymphocytes for the production of CAR-T cell therapies. Such treatments have had great success against blood cancers.

Viral-vector supply has become an expensive bottleneck, whether for ex vivo treatments of genetic diseases or genetic modification of immune cells in cancer treatments. Only recently has the manufacture of viral vectors begun to close the gap between supply and demand. Most vectors currently are cultured using mammalian cells in stirred-tank bioreactors. LVVs expressed transiently by host cells after a plasmid-based transduction step can help to fill supply needs for smaller-scale programs, such as autologous blood-cancer treatments using CAR-T cells (22). Increasing production scale by switching to suspension-based insect-cell systems could alleviate some strain on manufacturing capacity (23). Although scaled-up processes can deliver high levels of fully infectious particles, the FDA has set a high bar for batch characterization. Developers must establish and validate methods for monitoring impurities and empty capsids produced by such protocols.

History of Cell Therapy: The first **hematopoietic stem-cell** (HSC) transplant took place between twin sisters in 1959. Discovery of the human leukocyte antigen (HLA) in 1969 made typing transplants between nonidentical siblings possible (24). Today, HSCs are used to treat a variety of blood cancers, genetic diseases, and immunodeficiency syndromes. The cells are derived from three sources: bone marrow, peripheral blood, and umbilical cord blood.

Bone-marrow aspirate is collected from the iliac crest (pelvis) of a patient under general anesthetic. HSCs are present as a small fraction of the CD34+ white blood cells found in peripheral blood. Typically, HSC numbers are enhanced by treatment (mobilization) of the patient's blood with a cytokine, granulocyte colony stimulating factor (G-CSF). For HSC transplants from cord-blood sources, patients must undergo slightly different preparative treatments to ensure cell engraftment.

Postoperative treatments differ depending on the relationship between donors and recipients. HSC transplants have become common medical practice, providing life-saving treatments and increased quality of life to thousands of patients every year (25).

Embryonic Stem Cells (ESCs) and Induced Pluripotent Stem Cells (iPSCs): To many, the modern era of regenerative medicine began in 1998 with the derivation of stem cells from a human embryo (26). With the capacity to differentiate into any cell type of the human body, ESCs were thought to be a source of “spare parts” for treating many degenerative conditions as well as tools for drug screening and development. Researchers used specialized culture conditions for expansion and controlled methods for directed differentiation to produce a number of specialized cell types, including neurons, cardiomyocytes, and

Although scaled-up processes can deliver high levels of fully infectious virus particles, the FDA has set a **HIGH BAR** for batch characterization. Developers must establish and validate methods for monitoring impurities and empty capsids produced by such protocols.

pancreatic beta cells. In 2007, the Yamanaka laboratory generated induced pluripotent human stem cells (hiPSCs) through forced reprogramming of adult somatic cells using four transcription factors, eliminating the need for human embryos as a source (27). Now, iPSCs can be derived from patients carrying disease-causing mutations and differentiated into relevant cell types that can be used to model disease states and screen new therapeutic compounds. The cells have been studied clinically since 2014.

Several challenges have emerged in the generation of clinical-grade ESCs and iPSCs. Because stem cells are adherent, most if not all cultures have grown in dishes and flasks. Even multilayer cell stacks have made production difficult because of the need to visually inspect and physically pick colonies or partial colonies when passaging the cells. Cells had to be scraped from cultures and broken into clumps by titration, not plated as single cells until the addition of rho-kinase (ROCK) inhibitors could improve their survival (28). Recognizing the correct size of the cell clumps was part of the “art.”

Expansion and differentiation of ESCs and iPSCs are manual processes for a number of reasons. Culture conditions have expanded beyond using layers of murine feeder cells and serum-containing media. A growing understanding of stem-cell biology, including self-renewal and differentiation, has improved growth surfaces, media, and handling techniques. Feeder cells were replaced first with the Matrigel matrix (Corning Life Sciences), which is produced from mouse sarcoma-cell-secreted proteins, and later with more defined coatings based on extracellular matrix proteins such as vitronectin, laminin, and fibronectin (29). Serum-free media for ESCs and iPSCs have evolved; however, some current iterations still contain basic fibroblast growth factor (bFGF) and transforming growth factor β 1 (TGF- β 1).

The expansion of undifferentiated human ESCs and iPSCs remains a demanding process, and the cells are prone to spontaneous differentiation. Cultures can take several months to complete, and stem cells are not karyotypically stable if automated processes are used to passage them. Many manufacturers believe that such cells need special handling through isolation, expansion, and differentiation steps to ensure that nothing goes wrong. Once the cells have been differentiated, they can be grown and scaled using bioreactors.

Many iPSC-derived cell therapeutics are in development. Cynata Therapeutics is the most advanced company in this area, with its iPSC-derived mesenchymal stem cell (MSC) product for the treatment of osteoarthritis (OA) in phase 3 clinical trials (30). Other organizations developing iPSC-derived therapeutics include

- the US National Institutes of Health (NIH), with an iPSC for retinal disease (31)
- Fate Therapeutics in collaboration with University of Minnesota, with iPSCs alongside natural killer (NK) and CAR-T cell candidates (32)

Expansion of undifferentiated **STEM CELLS** remains a demanding process, and the cells are prone to spontaneous differentiation.

- Japanese Healios KK and Sumitomo Dainippon Pharma, with retinal cell candidates for the treatment of macular degeneration, all in early phases (33).

MSCs and Exosomes: MSCs are progenitor cells capable of forming osteoblasts, chondrocytes, myoblasts, and adipocytes. They have been studied since the 1960s, when they were first isolated from bone marrow (34). Arnold Caplan was the first scientist to grow MSCs from human sources, identifying them by their ability to adhere to plastic tissue-culture dishes (35). It was not until 2006 that the International Society for Cell and Gene Therapy (ISCT) defined minimum criteria for research purposes. According to the ISCT definition, MSCs must

- adhere to plastic
- express CD105, CD90, and CD73 cell-surface antigens
- not express CD45, CD19, CD14, CD11b, CD34, CD79a and HLA-Dr antigens
- have the ability to differentiate into osteoblasts, chondrocytes, and adipocytes (36).

MSCs have been used to modulate immune responses, prevent inflammation, and promote healing, and they have been in clinical trials for over two decades. Recently, these cells have been shown to release growth factors and cytokines as well as extracellular vesicles. Despite more than 10,000 patients treated in nearly 1,000 clinical trials so far (37), only in March 2024 did the FDA give the first approval to apply for a biologics license application (BLA) to Mesoblast Limited for remestemcel-L in pediatric patients with steroid-refractory acute graft versus host disease (SR-aGVHD) (38).

MSCs have been grown in polystyrene dishes, flasks, and stacks. For allogeneic products and/or large batch sizes, MSCs can be cultured in hollow-fiber bioreactors, packed-bed bioreactors, and on microcarriers in stirred-tank bioreactors. Culture conditions have moved away from serum-containing media to serum-free media, although not without difficulty.

MSCs have demonstrated broad potential for therapeutic use. The unique ability to target damaged tissues and inhibiting immune and inflammatory responses to facilitate repair demonstrates MSCs' potential as model drugs (39). However, to be clinically successful, MSCs must be defined strictly. Standards for isolation and characterization of MSCs could eliminate the biological diversity of the starting materials. Off-the-shelf allogeneic MSC products to treat GVHD and acute respiratory distress syndrome (ARDS) have demonstrated safety in phase 1 and 2 clinical trials and received approval for markets outside the United States (39, 40).

The term *exosome* first was used to describe small extracellular vesicles that are secreted by all cell types for transfer of proteins, metabolites, and nucleic acids to recipient cells (41). We group exosomes with MSCs here because they have been hypothesized to be responsible for many of the functional attributes of those cells.

We group **EXOSOMES** with mesenchymal stem cells here because they have been hypothesized to be responsible for many of the functional attributes of those cells.

Collecting exosomes from bone-marrow–derived MSCs is relatively simple: Supernatant from adherently grown MSCs yields a rich collection of them. As a disease treatment, exosomes have the same effects as whole MSCs, are well tolerated, they have low immunogenicity (42). As with the source cells, problems arise in scale-up, isolation, characterization, and preservation. Nevertheless, exosomes are the subject of much research, including over 300 clinical trials at the time of this writing (43).

Immunotherapy and CAR-T Cells: Harnessing the immune system to attack cancer has been researched for over 100 years. In the 1950s, researchers sought to combat blood cancers through bone-marrow transplants. Although T lymphocytes in bone marrow can kill cancer cells, they required help in finding their targets (44). For that, mAbs can provide precise recognition and targeting capabilities. Finally, T lymphocytes were genetically engineered with CAR-T cells, bringing together the antibody guide with internal activation signals to help T cells locate and kill cancer cells (45). In the late 1990s, methods were developed to expand and activate engineered T cells in a laboratory setting. Around 2011, the first chronic lymphocytic leukemia and B-cell acute lymphoblastic leukemia patients were treated with CAR-T cell therapies; about 90% of those patients had a complete response to the treatment (46). In August 2017, the FDA approved its first CAR-T cell immunotherapy: the Kymriah treatment from Novartis.

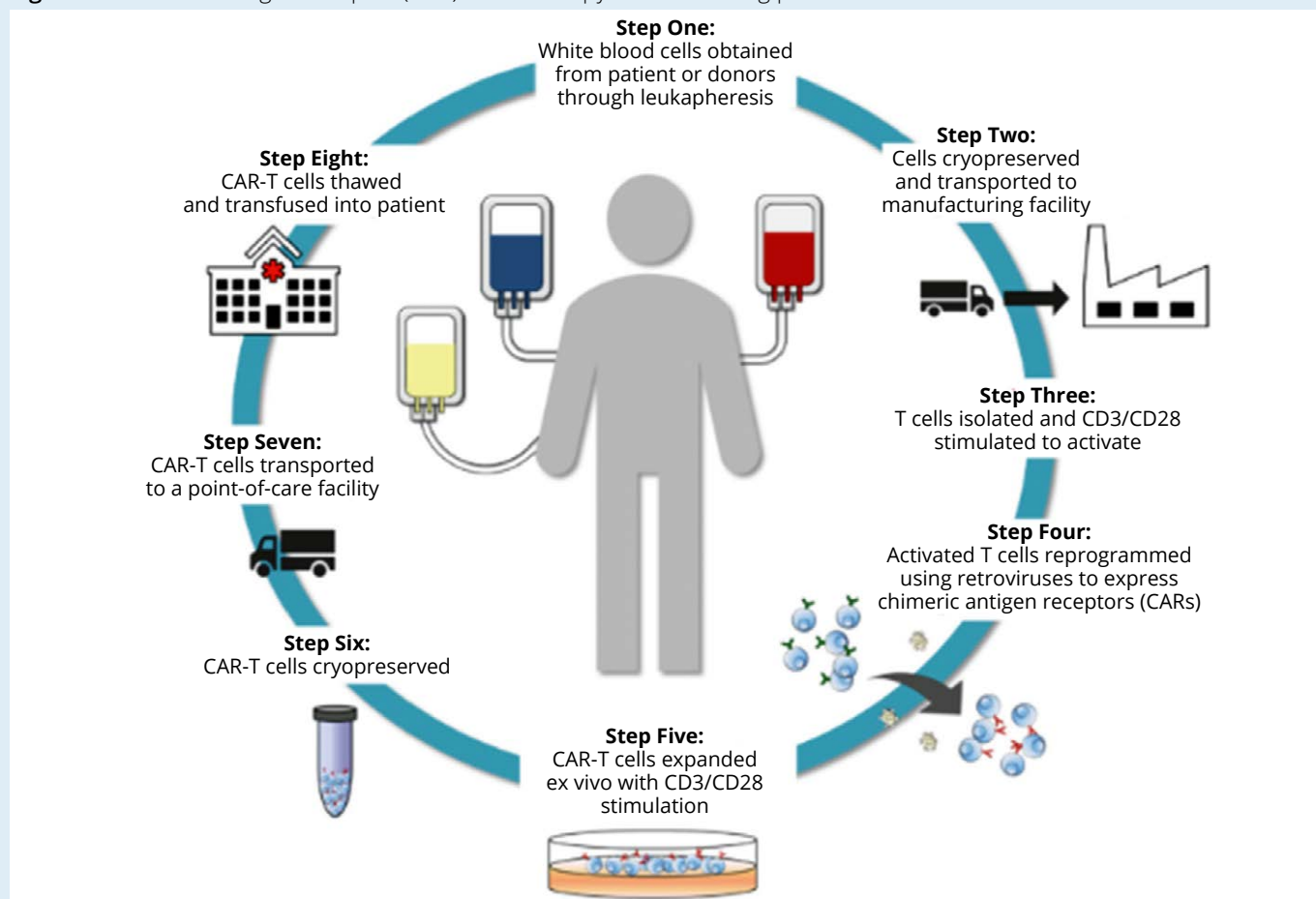
Evolution of Manufacturing Technology for Cell Therapy: The manufacture of gene-modified cell therapies is a complex, multistep process in which mononuclear cells are isolated from a patient, and then T lymphocytes are activated and genetically modified for a many-fold expansion of the cells. The expanded cells are harvested, washed, and prepared for cryopreservation before being reinfused to the same patient (47). That path from laboratory to clinic, then to commercial cell-therapy manufacturing and back, can take many different routes. Below, we describe a few possible paths starting with an R&D laboratory and moving into early phase clinical processes and late-stage clinical and commercial processes (including suite design). We also forecast some potential future process options.

MANUFACTURING TECHNOLOGIES FOR CELL THERAPIES

Introductory Bench-Scale Production: Discovery is the focus of academic research. Process optimization is time consuming and doesn't often get published in high-impact journals. In laboratories relying on research grants to operate, cost becomes a critical consideration. Depending on the cell type being studied, most academic laboratories culture cells in well plates, Petri dishes, T flasks, shake flasks, and/or spinner flasks. When large numbers of cells are required, scientists use cell stacks and gas-permeable bags. Working with traditional plastic allows maximum flexibility at low cost, with all the trade-offs of open

[BACK TO CONTENTS](#)

Figure 2: Chimeric antigen receptor (CAR) T-cell therapy manufacturing process flow



Source: Bhattacharya B, Telang A. Manufacturing and Qualification Challenges for Cell and Gene Therapy Products and Best Practices for Success. *Cell Culture Dish* 6 March 2023; <https://cellculturedish.com/manufacturing-and-qualification-challenges-for-cell-and-gene-therapy-products-and-best-practices-for-success>.

systems. And when developing new platforms for which the goals are speed to clinic or acquisition by a large company, many researchers use data from phase 1 and 2 trials with a few patients.

Producing gene-modified cell therapies, especially those in later-phase trials and commercial manufacturing, requires the use of automated systems (48). Many laboratories, especially those of well-healed organizations, use all-in-one turn-key solutions. Such systems decrease the number of open steps, reduce human errors, increase reproducibility and scalability, generate run reports, and are compatible with many process operations.

All-in-One Systems: All-in-one systems represent convenient solutions for closed, automated production of cell therapies. Such systems are capable of many, if not all, of the unit operations required to select T-cell populations from apheresis bags, activate and genetically modify cells by transduction or transfection, expand cells to desired lot sizes, and perform final formulation of products in compliance with current GMPs (CGMPs) using single bench-top devices. Because such devices provide functionally closed environments, processes can be moved out of biosafety cabinets and into less stringent cleanroom spaces (49).

Popular options include the CliniMACS Prodigy (Miltenyi Biotec) and the Cocoon (Lonza, Octane) platforms. Both systems represent remarkable achievements and act as turn-key processes for modern immunotherapies. However, they are expensive devices involving complex, costly single-use disposables. If parallel processing is needed for simultaneous production of multiple autologous samples, then the price of devices, disposables, and associated reagents can limit the scale of production.

Cell yields from CliniMACS Prodigy systems are in the range of 2×10^9 T cells from 3×10^7 to 5×10^7 selected T cells that were expanded for up to 13 days (47). Such yields can be increased if expansion phases are facilitated with a G-Rex device (Wilson Wolf) (50). Operational flexibility of the CliniMACS Prodigy system is limited by the fact that the software does not allow users to make on-site programming changes. A process may be changed only by Miltenyi's in-house scientists, and that can slow down operations. Additional caveats include the fact that newly developed processes will be owned by Miltenyi and not the company manufacturing a given therapy. Thus, it is not possible to test your own in-house process on a Prodigy system before purchasing the device. Finally, CliniMACS Prodigy users are required to use Miltenyi reagents and media, which may render cost of goods (CoG) unoptimizable while also limiting the flexibility of material sourcing.

The Cocoon system is a relatively new addition to the cell-therapy manufacturing space (51). This platform focuses on cell activation, transduction, and expansion, offering capabilities in other unit operations such as automated T-cell purification and magnetic debinding (52). As with the Miltenyi technology, cell-expansion volume is fixed at ~210 mL. A recent publication compared the Cocoon system with gas-permeable bags, concluding that it yielded results that were equal to or better than theirs while meeting all critical quality attributes (CQAs) for clinical autologous CAR T-cell products (53). After two separate 10-day processes using OKT3 or CD3/CD28 Dynabeads separators (Thermo Fisher Scientific) for T-cell activation, viable cell yields were $>2.1 \times 10^9$, which is comparable to Prodigy yields and greater than those from static cell-culture bags ($>1.5 \times 10^9$). A key distinction of the Cocoon system is that it allows users to perform process development and optimization through a standalone protocol builder. One caveat of this system is that Lonza owns the intellectual property of "nonserializable" protocols once they've been developed (54). The system was designed specifically for autologous cell-therapy manufacturing, with hardware and software that runs many Cocoon units simultaneously when arranged in a series of vertical "trees."

Early Phase Clinical Process: As cell-therapy manufacturers move from bench to preclinical and early phase clinical manufacturing schemes, some processes simply are not amenable to the software/hardware limitations of all-in-one devices. To serve a relatively small number of patients in compliance with CGMPs and to maintain flexibility, many developers want a

As cell-therapy manufacturers move from bench to preclinical and early phase clinical manufacturing schemes, some processes simply **ARE NOT AMENABLE** to the software/hardware limitations of all-in-one devices.

semiautomated solution. Developing a modular process with separate devices for individual manufacturing steps (Tables 2 and 3) provides that flexibility but can increase complexity and integration challenges while presenting unforeseen failure modes involving just one part of a process. At minimum, five steps must be automated: cell selection and enrichment, cell activation (with or without beads), transduction or transfection, expansion, and formulation and fill–finish of the final product.

Automated cell expansion has been the most investigated step of the process. The Automation Partnership (later TAP Biosystems, now part of Sartorius) pioneered the robotic handling of cells grown in flasks inside an aseptic cabinet for use in high-throughput screening (HTS). That technology has been a staple of the pharmaceutical industry for over 20 years.

Among other cell-expansion systems is the mostly manual G-Rex device from Wilson Wolf. Cells are cultured on a gas-permeable membrane at the bottom of a culture vessel, which offers two advantages: Cells resting on the membrane are not diffusion-limited for gas exchange (as is the case in a traditional flask), and access to O₂ and CO₂ allows for relatively large volumes of culture media, which can provide for several weeks of undisturbed cell expansion. This device comes in several sizes up to 5 L, and it can expand cultures to achieve a dose of 100 billion cells from multiple 5-L chambers over a total culture time of 21 days (52).

The Xuri cell-expansion system (Cytiva) is an outgrowth of the WAVE bioreactor (Cytiva) concept, in which a culture bag rests on a rocking platform. Xuri systems are automated with temperature and gas controls as well as a pumping system that can supply media in either fed-batch or perfusion feeding schemes (55). Culture bags for this system come in sizes up to 20 L, although 1-L to 2-L bags probably are most suitable for autologous CAR-T manufacturers. After optimizing feed rates, cell seeding, rocking dynamics, and pH and dissolved oxygen (DO) rates, users can obtain 20 billion cells from a 2-L culture bag (52). A rocking platform was used in many early CAR-T cell-manufacturing protocols (56).

The Quantum Flex hollow-fiber bioreactor (Terumo BCT) is a fully automated cell-expansion system. Hollow-fiber bioreactors were designed originally for kidney dialysis and have been used for over 20 years to produce mAbs and other biologics. These bioreactors separate their culture space into two compartments with a semipermeable membrane. Cells grow on the inside of the hollow fibers, and perfusion across the membrane provides for precise regulation of metabolites, gases, and nutrients. The molecular weight cut-off (MWCO) of the membrane is ~17 kDa, which allows some cytokines to diffuse away from the cells. A Quantum device can support both adherent- and suspension-cultured cells and can feed them continuously while simultaneously removing waste products. Precise management of the cell microenvironment has produced yields of >20 billion cells

SCALE-UP of anchorage-dependent human cells cultured as aggregates or grown on microcarriers to commercial-manufacturing scales presents a unique set of challenges.

(57, 58). This system also has proven to be a viable option for LVV production (59). Scaling out to larger batch sizes for clinical applications has been demonstrated by simultaneous use of multiple Quantum bioreactors and pooling harvested cells to produce GMP-grade clinical lots of >10 billion neural stem cells in nine days (60). The system could be useful for early stage trials and vector-development laboratories.

Scale-up of anchorage-dependent human cells cultured as aggregates or grown on microcarriers to commercial-manufacturing scales presents a unique set of challenges. Cell therapies derived from PSCs typically grow on two-dimensional planar cultures as monolayers. However, when culturing them as single cells or small aggregates in suspension cultures, you must take special care to maintain cell cluster morphology. With human ESCs and iPSCs expanded as undifferentiated cells before differentiation, the size and shape of those clusters can compromise such processes. Hydrodynamic environments inside traditional stirred-tank bioreactors using horizontal blade impellers can present cells with dead zones or high-velocity “tornadoes,” both of which create different-sized aggregates. One solution to the problem is a PBS vertical-wheel bioreactor (PBS Biotech), which maintains pluripotency during expansion and scale-up (61, 62). Vertical-wheel bioreactors come in several volumes from 0.1 L to 15 L and provide uniform hydrodynamic environments. However, many other parameters must be optimized for large-scale use, including timely gas and media exchange and cell-harvesting methods.

Another scale-up solution on the path toward a 50-L stirred-tank bioreactor is the Mobius CellReady 3-L single-use bioreactor (MilliporeSigma), a presterilized, rigid-walled system that is compatible with most desktop controllers and provides all the functionality of a conventional glass bioreactor. Expansion and activation of CAR-T cells in stirred-tank bioreactors have been demonstrated. Increased agitation of T-cell cultures led to higher cell densities than are achieved typically in static T-flasks (63). Although not commonly used for CAR-T cell expansion, stirred-tank bioreactors could become common as allogeneic cell therapies are developed. The Mobius bioreactor is intended for process development, in which its performance is predictive of both 50-L and 200-L results.

When developing a cell-therapy manufacturing process using multiple devices with diverse functions, nearly every step will require cell separation, selection, wash, and concentration before the next unit operation. Three devices that supply that essential functionality for a GMP environment are the Gibco CTS Rotea counterflow-centrifugation system (Thermo Fisher Scientific), the Lovo cell-processing system (Scale Ready), and the Cue cell-processing system (Scale Ready). The Lovo and Cue systems both use spinning membranes for buffer exchange and cell washing without centrifugation or cell resuspension. The Lovo system can retain total nucleated cells while washing out platelets from an

When developing a cell-therapy manufacturing process using multiple devices with diverse functions, nearly **EVERY STEP** will require cell separation, selection, wash, and concentration before the next unit operation.

Table 2: Equipment applicability per unit operations; IV = intravenous, NA = not applicable, WC = wash and concentrate

Device Name	Suspension or Adherent Cells, Fresh or Frozen Leukopaks?	Preloaded Media and Reagents?	Magnetic Cell Selection?	Activation?	Transduction, Transfection?	Expansion?	Final Formulation?
Cocoon	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Prodigy	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Quantum Flex	Yes	Yes		Yes	Yes	Yes	No
Xuri	Yes	IV pole	Yes	Yes	Yes	Yes	Yes
G-Rex	Suspension only	NA	Yes	Yes	Transduction only	Yes	No ¹
Mobius 3L bioreactor	Yes	No	No	Yes	Yes	Yes	Yes
PBS vertical wheel	Yes	NA	No	No	Neither	Yes	No ²
ROTEA	Yes ³		Yes ⁴	WC	WC	WC	WC

¹ Pairs with Cue system from Fresenius Labs³ Separation of peripheral blood mononuclear cells from monocytes² Can suspend cells uniformly for dispensing⁴ Including live/dead (elutriation)

apheresis product in <11 minutes. Similarly, the Cue system is an effective alternative to centrifuge-based separations for processing the final harvest of ascitic tumor cells (ATCs), tumor-infiltrating lymphocytes (TILs), and MSCs (64).

The Rotea system uses centrifugation to separate cells based on both density and size. Counterflow is a gentler process than traditional centrifugation, providing for increased cell viability of ~10% (65). Spinning-chamber size determines device capacity, with the Rotea system made for autologous and early phase allogeneic scale processes. It provides a closed system for cell washing and buffer exchange, cell concentration, peripheral blood mononuclear cell (PBMC) preparation in <30 minutes, dead-cell removal, and formulation for cryopreservation. Other systems that use spinning chambers include elutriators from Beckman Coulter and the Ksep scalable single-use centrifugation system (Sartorius), which have larger capacities (66).

Cell-processing technology from Curate Biosciences is designed to replace Ficoll 400 gradient centrifugation (MilliporeSigma), elutriation, and magnetic-bead separations for isolation of PBMCs from leukopaks. Using a microfluidic device, the Curate system's gentle processes for size-based separation and washing preserves more naive T cells while providing the requisite 3-log cell wash to remove soluble factors from a closed system. It also has been shown to remove platelets and platelet aggregates, which can release proinflammatory cytokines that drive regulatory T-cell (Treg) differentiation (67).

DESIGN OF LATE-STAGE AND COMMERCIAL PROCESSES AND MANUFACTURING SUITES

For the scale-up or scale-out of a CGMP manufacturing process, three quarters of all developers turn to contract manufacturing organizations (CMOs) or contract development and manufacturing

[BACK TO CONTENTS](#)

Table 3: Closed-system platform comparison — engineered capabilities; DO = dissolved oxygen, NA = not applicable, ? = no information available

Device Name	Automated?	Functionally Closed, Single-Use?	Scalable?	Monitor/Control Temperature and Gases?	Integral Pumps, Sensors?	DO/CO ₂ Real-Time Feedback Process Control?	Bar-Coded Scanner?	Software for Users To Design/Control Flow Pathways?
Cocoon	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Prodigy	Yes	Yes	Yes	One zone	Yes	No	Yes	Preprogrammed by supplier and verified (closed)
Quantum Flex	Yes	Yes	Yes	One zone	Pump only	No	Yes	Yes
Xuri	Yes	Yes	Yes	One zone	Yes	Yes	Chronicle software	Yes
G-Rex	No	?	Yes	No	No	No	No	NA
Mobius 3L bioreactor	No	Yes	Yes	Heating blankets	Yes	Yes	No	No
PBS vertical wheel	Yes ²	Yes	Yes	Yes	Yes ¹	Yes ³	No	Yes ⁴
Rotea	Yes	Yes	Yes	No	Yes	Yes ⁵	Yes	Yes

¹ Three binary pumps, one bidirectional sampling pump

³ Positive regulation of CO₂, N₂, O₂, air, NaOH

² Agitation, temperature, pH, CO₂, DO, additions

⁴ Recipe capable

⁵ Camera-based

organizations (CDMOs). Many product sponsors lack GMP manufacturing capacity in their own facilities, and some have no manufacturing capacity whatsoever (68). The manufacture of a commercially viable cell therapy must be scaled beyond the bench, where open, manual processes dominate. Automated closed-cell processes can accommodate several different solutions for CDMO staffing and suite design. Quality control (QC) is a major component of autologous manufacturing processes, second only to scaled-out processes. For scaled-up allogeneic processes, expansion results in fewer quality tests but requires larger bioreactors. Viral-vector manufacturing requires full segregation of manufacturing systems for viral particles (69). To compete in the industry, a CDMO must be flexible in its offerings. The most straightforward option is a dedicated suite that is fully segregated, suitable for both open and closed processes, and can contain up to two closed-system bioreactors to support an entire process. Dedicated suites are small (200–400 ft²) and supply the needs of single customers in early stage clinical trials, while requiring relatively high International Organization of Standardization (ISO) ratings, making them expensive to operate.

In 2013, the International Society of Pharmaceutical Engineers (ISPE) defined the *ballroom manufacturing concept* as “a large manufacturing area that has no fixed equipment and minimal segregation due to the use of functionally closed systems” (70). The use of automated, single-use, closed systems enables relatively low cleanroom requirements. Equipment not required for a specific manufacturing process can be staged in a separate clean storage area. Equipment move-in and move-out are facilitated by a plug-and-play approach delivering different utilities and distributed

control systems, depending on the stage of a process. With closed systems, >10 bioreactors can be grouped in a single large room (500–1000 ft²). Nevertheless, it is hard to imagine how to meet the demands of the CGT market by serving 50 patients at a time.

To make the most of existing facilities, the “dance floor” concept has been introduced. Processes flow from upstream steps, including cell selection and activation to cell expansion, are housed in a separate suite. The expansion bioreactor typically causes a bottleneck, so several of them could be housed in a dedicated suite serving a separate downstream processing suite for formulation and fill–finish in adjoining rooms with sterile pass-throughs. Process closure is thus maintained for sample movement (69). Again, closed systems would require only the final process steps to have higher ISO ratings.

Manufacturing in a Box: End-to-end automation is the ultimate goal for cell-therapy manufacturing. Once patient material arrives at a manufacturing site, then a true end-to-end autologous process would include the following:

- cell thaw and wash, with defined constraints on the number of cells and final volume
- cell selection to ensure consistent starting materials
- cell activation or engineering, in which additional reagents could include antibody-coated magnetic beads or modifications to introduce genetic material to cell nuclei
- cell expansion to produce required doses
- cell harvest with bead removal, wash, and concentration steps
- product formulation and freezing for return to patients.

Cellares’ Cell Shuttle platform can provide end-to-end automation in a closed system, reducing cleanroom and workforce requirements and producing savings up to 70%. Capable of running 10 processes simultaneously with flexible hardware and software, this platform should reduce operator error and processing times. At the heart of the system is a GMP-in-a-box cartridge that performs all process steps and delivers cell-therapy products ready for infusion. Cellares recently raised US\$255 million to build a smart factory for producing more than 40,000 batches a year in 50 Cell Shuttle systems. The technology could reduce process-failure rates by 75% and manufacturing costs by 50% (71).

Multiply Labs offers another approach to automation: robotic systems for cell-therapy manufacturing. Using the unit operations approach described above, this company is producing modules such as bioreactors and incubators that incorporate each process step — including QC testing — and can be adapted to recreate any process. Each module contains a single instrument; parallel processing can reduce bottlenecks by incorporating multiple bioreactor modules or different wash and concentrate devices when needed. A robotic arm moves the process along by transferring

POINT-OF-CARE

manufacturing would eliminate the movement of patient samples from collection sites to manufacturing sites and the need to return completed products to patients.

materials to the next unit operation. The hard-walled system behaves like a conventional cleanroom. Modules are compatible with market-leading GMP instruments. However, the industry has yet to standardize tubing sizes, connectors, or materials in manufacturing. Such a lack of standardization can cause problems when, for example, a bag from one manufacturer's device must be moved to another manufacturer's device.

Point-of-Care (PoC) Production: One potential solution to a centralized, single-site global manufacturing model and its inherent costs and time-consuming journey is the move to PoC manufacturing. That would eliminate the movement of patient samples from collection sites to manufacturing sites and the need to return completed products to patients. Maintaining strict time and temperature requirements during transport is expensive and unpredictable. In addition, large geographic areas create complexities in scheduling.

Two models exist for PoC manufacturing: the hub model and the fully distributed model. The *hub model* collocates manufacturing with major medical centers through partnerships with existing CDMOs or investing in automation solutions such as those described above. A *fully distributed PoC solution* would require local hospitals to invest in automated manufacturing infrastructure that can deliver standardized products to patients. Such a model is the most common PoC option being pursued in the United States and Europe. Fully distributed PoC models must wait for the industry to improve its understanding of autologous cell-therapy manufacturing enough to allow for the required process standardization (72).

PoC manufacturing of autologous cell therapies could shorten manufacturing times, enhance product safety, and improve patient access, but challenges remain. Maintaining process control and product quality is difficult with a centralized facility, and doing so may be nearly impossible across multiple sites in a hub model. For example, finding skilled labor to oversee such operations is essential, but the difficulty of finding suitable employees increases proportionately with distance from academic and research centers (73). Such difficulty makes the use of automated processes critical. Regulatory approval and compliance across multiple sites accomplished by conventional methods will be costly and time-consuming, if not impossible. New modes of digital data collection and analytics will be required (72). Autologous cell-therapy manufacturing is still in its early stages, as are the digital technologies that will be needed to establish PoC manufacturing sites and make them cost-effective.

Accelerated Manufacturing Strategies: A major hurdle to the manufacture of autologous CAR-T cell therapies is the length of the process itself. A waiting time of up to three weeks has consequences for both patients and their cells. At a 2021 American Society of Hematology (ASH) meeting, Novartis launched the T-Charge platform for next-generation CAR-T production. When the

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cell expansion step of the process moves to *inside* a patient's body, the lengthy (ex vivo) culture time is eliminated and proliferation potential is preserved while producing fewer exhausted T cells. Novartis hopes that an optimized T-Charge process will take under seven days and remove the need to switch to allo-based technologies (74).

Another attempt to shorten production times and improve patient outcomes is the anti-CD19 cell therapy process called FastCAR (75). Fresh T-cells are isolated from PBMCs, then concurrently activated and transduced in a single step. On day 1 of the 72-hour process, ~200 million T cells are selected and seeded. They are transduced using LVVs and then collected, purified, and cryopreserved the next day. After about seven days of release testing, FastCAR cells have been infused into 25 pediatric and adult patients with B-cell acute lymphoblastic leukemia. Results have been promising from phase 1b and 2 investigational new drug (IND) studies using a B-cell maturation antigen (BCMA)/CD19 dual CAR-T therapy for patients with relapsed/refractory multiple myeloma (76) and for rapid CAR-T cells using the Cocoon platform (77). Although FastCAR therapy is still in early stages — with animal testing still in progress — the cells that have been produced so far appear to have memory phenotypes and display antitumor cytotoxicity in cell-based assays.

In another version of “patient as bioreactor,” an alginate scaffold was used to support in vivo manufacturing of CAR-T cells in a single day (78). Patient-derived PBMCs were activated by seeding them onto a scaffold with immobilized CD3/CD28 antibodies and encapsulated interleukin 2 (IL-2). Gamma-retroviral particles containing a CD-19-specific CAR also were immobilized on the scaffold. After subcutaneous implantation of the fully loaded scaffold in mice, CAR expression was detected for >32 days. The subcutaneously implanted scaffolds generated and released fully functional CAR-T cells in a xenograft model of lymphoma, increasing tumor-free survival when compared with conventionally produced CAR-T cells (78). This multifunctional, all-in-one scaffold platform reduced CAR-T manufacturing to a single day.

Different approaches to generate CAR-T cells transiently in vivo have used lipid nanoparticles (LNPs). LNPs displaying anti-CD5 T-cell targeting antibodies were used to deliver therapeutic messenger RNA (mRNA) and generate functional CAR T-cells in vivo (79). Direct infusion of replication-incompetent virus glycoprotein (VSV-G) LVVs carrying a CAR gene also were shown to produce persistent CAR T-cells in mouse models, eliminating the need for external manufacturing (80).

Clustered, regularly interspaced, short palindromic repeats (CRISPR) and the associated guide RNA to form programmable DNA nucleases (CRISPR-Cas9), have become major players in genome editing. Researchers have used such technology to modify T cells ex vivo for immunotherapies and HSCs that treat sickle-cell disease and β -thalassemia. The FDA also approved Casgevy

The same techniques required in **AUTOLOGOUS** CAR-T cell manufacturing also are required for **ALLOGENEIC** T-cell selection, activation, and genetic engineering. However, the resulting products can be scaled up during expansion to achieve large cell numbers, thereby allowing many patients to be dosed from a single collection of reprogrammed cells.

(exagamglogene autotemcel), a therapy from Vertex Pharmaceuticals and CRISPR Therapeutics, based on a CRISPR-Cas9 approach (81). In a separate phase 1 clinical trial, a specially designed LNP delivered CRISPR-Cas9 to patient hepatocytes in vivo, where the mutant gene that causes transthyretin amyloidosis is knocked out. The resulting therapy significantly reduced serum concentration of the misfolded protein (82). Similar base-editing strategies are planned for a wide array of diseases.

The Promise of “Off-the-Shelf” Allogeneic Cell

Therapies: Suspension-culture processes using allogeneic cells suggest an alternative to automated, closed-system processes for autologous cells. Suspension-culture processes could decrease manufacturing costs while producing larger batches than are possible with adherent cell cultures. Facilities using such processes require less QC testing space and can automate cultures earlier in development, reducing headcount requirements (83). Instead of starting with compromised cells from a sick patient, a uniform population of healthy donor cells can be used as starting materials. Donors might even become optional if developers rely on differentiated iPSCs (84).

The same techniques required in autologous CAR-T cell manufacturing also are required for allogeneic T-cell selection, activation, and genetic engineering. However, resulting products can be scaled up during expansion to achieve large cell numbers, thereby allowing many patients to be dosed from a single collection of reprogrammed cells (49). When surveyed, both large and small cell-therapy manufacturing companies were neutral on switching fully to allogeneic processes and thus plan to pursue both allogeneic and autologous processes (84). Only time will tell as both processes mature.

Several challenges remain for allogeneic processes to become mainstream, as well. One is the use of large stirred-tank bioreactors for expansion. Without thorough understanding of shear forces, impeller impact, gas exchange, and hydrostatic pressures, the nonphysiologic conditions of such reactors could derail allogeneic expansion process steps. And unlike biologic processes in which protein products are secreted into the culture medium, allogeneic cells are the products, themselves, and they could experience adverse effects during expansion (85).

One critical concern emerging from clinical trials of allogeneic cell therapies has been immunogenicity, including patient rejection of infused cells and GVHD development (86). Gene-editing techniques such as CRISPR-Cas9 can be used to disrupt T-cell receptors (TCRs) and major histocompatibility (MHC) loci, mitigating immunogenic recognition of allogeneic therapies and reducing or eliminating symptoms; however, improved understanding of such mechanisms is required (49).

In a comparison of allogeneic and autologous CAR-T cells, the autologous cells demonstrated greater performance in almost all aspects, especially persistence. Strategies for improving allogeneic

cell performance include using iPSCs as cell sources; maintaining constant, equal CD4+/CD8+ ratios in T-cell populations; using stem-cell memory and central memory T-cells; improving culture conditions for reduced cell doublings, improved efficacy, and lowered rates of cell exhaustion; and combining cell therapies with conventional treatments (87). Once the limitations above have been overcome, allogeneic cell therapies could become the model of choice for centralized CGT manufacturing facilities.

MANY PATHS, ONE DESTINATION

Cell-therapy manufacturing is still in its infancy. Just as the biologics industry progressed stepwise from hybridoma technologies to optimized cell-expression platforms, CGT manufacturing requires improved understanding of cell biology to achieve reproducible, scalable processes. However, understanding of pluripotent-cell biology and the influence of media, growth surfaces, cell-line development, and differentiation has been slow to advance.

Automation of undifferentiated ESC expansion and subsequent differentiation is difficult, and the process largely remains an art. Cell therapies based on iPSCs gained acceptance with their less-controversial sourcing and have progressed to late-phase clinical trials, but none have been approved for use in humans. Generally, differentiated iPSCs are considered to be a good source for rare cell types. Progenitor cells such as MSCs have potential for many cell therapies but suffer from a lack of standardization. Defining them merely by their ability to adhere to tissue-culture plastic leads to variability in starting materials and subsequent failure of many MSC clinical trials in the United States, although products have been approved for use in humans elsewhere. Similarly, exosomes have been difficult to characterize and purify. We have no doubt that pluripotent, multipotent, and progenitor cells will find a place in cell-therapy toolboxes as scientific understanding grows.

CAR-T cells are in the spotlight now. Multiple such therapies have been approved for use in humans, and hundreds more are in development. But the number of patients treated remains quite small compared to the overall patient population. Major challenges remain for CAR-T cell therapies, including adverse events following treatment such as cytokine release syndrome (CRS) and neurotoxicity. Overall tumor burden, the intensity of preceding lymphodepletion, and the size of therapeutic doses all could play a part in the severity of such adverse effects (88). Another major challenge is the high cost of treatment. Total treatment costs can exceed \$500,000, and with the current treatments taking up to 30 days, some patients will not survive to reap the benefits.

To date, CAR-T therapy has shown immense promise for treating hematological cancers but only limited success when treating solid tumors. Overcoming immunosuppressive tumor microenvironments will be the next chapter in T-cell engineering. Successes so far have led researchers to explore other immune cells such as NK cells, which are less immunogenic and could provide an “off-the-

BACK TO CONTENTS

Multiple **CAR-T THERAPIES** have been approved, and hundreds more are in development. But the number of patients treated remains quite small compared with the overall patient population. Major challenges for CAR-T cell therapies include adverse events following treatment.

shelf” therapy for certain applications. However, those bring another set of challenges: namely, limited life span and proliferation potential (88).

To meet ever-increasing CGT demands, automation must replace manual processes that use flasks and bags. A scalable, all-in-one system should be developed with increased process control and flexibility such that it could serve not just as an early-stage solution, but also as a stepping stone to PoC or dispersed-manufacturing solutions. Still, it is hard to imagine 50 units in a single ballroom as a viable long-term solution. Novel or complex processes will require step-by-step or unit-operations automation. Manufacturing-in-a box could contribute customizable automation solutions required for more sophisticated products. However, all-in-one devices still require highly skilled operators to be effective.

To meet the demand of autologous manufacturing, CDMOs must reduce human errors, costs, and processing times. New levels of standardization and process monitoring also will be required to make such manufacturing a viable option. Distributed manufacturing will require all automation and standardization described above while adding the challenge of finding local talent to operate PoC facilities. Several approaches for accelerating the manufacture of autologous T-cell therapies, including “patient as bioreactor,” could reduce greatly the number of manufacturing steps and time to treatment for patients. Finally, hearkening back to the early days of gene therapy, in vivo modification of T cells using CRISPR-Cas9 could give rise to a new class of drugs entirely.

We are in a phase of great invention as we work our way through the different means to engineer a patient’s own immune cells to fight cancer. Autologous cell therapies work and are worth pursuing. Meeting the demands of industrialization for these potential cures is a huge undertaking. Scale-out of such processes will require more than just a keen understanding of how stirred-tank bioreactor environments affect cells. One possible solution is the transition to “off-the-shelf” allogeneic cell therapies. Taming the immunogenicity of such cells and improving their persistence are just two of the challenges that must be overcome, however. Improvements in automation and understanding of cells’ biology as well as the environment in which they operate will facilitate the cost-effective treatment of hundreds of thousands of new patients.

REFERENCES

1 Kochenderfer JN, et al. Eradication of B-Lineage Cells and Regression of Lymphoma in a Patient Treated with Autologous T Cells Genetically Engineered To Recognize CD19. *Blood* 116(20) 2010: 4099–4102; <https://doi.org/10.1182/blood-2010-04-281931>.

2 Crump M, et al. Outcomes in Refractory Diffuse Large B-Cell Lymphoma: Results from the International SCHOLAR-1 Study. *Blood* 130(16) 2017: 1800–1808; <https://doi.org/10.1182/blood-2017-03-769620>.

3 Cappell KM, et al. Long-Term Follow-Up of Anti-CD19 Chimeric Antigen Receptor T-Cell Therapy. *J. Clin. Oncol.* 38(32) 2020: 3805–3815; <https://doi.org/10.1200/JCO.20.01467>.

BACK TO CONTENTS

- 4** Hunt T. *Cell and Gene State of the Industry Briefing*. Alliance for Regenerative Medicine: Washington, DC, 2023; <https://www.precisionmedicinegrp.com/wp-content/uploads/2023/06/WP-State-of-the-Industry-2023.pdf>.
- 5** Caffrey M. FDA Approves Axi-cel in Second Line for LBCL, Expands CAR-T Pool by 11,000 in US Alone. *AJMC* 1 April 2022; <https://www.ajmc.com/view/fda-approves-axi-cel-in-second-line-for-lbcl-expands-car-t-pool-by-11-000-in-us-alone>.
- 6** Mikulic M. Estimated Addressable Patient Population for CAR-T Cell Therapies Worldwide as of 2019. *Statista* 19 April 2022; <https://www.statista.com/statistics/1098463/global-addressable-patient-population-car-t-cell-therapies-estimate>.
- 7** Frellick M. Dying on the Wait List: Many with Multiple Myeloma Face CAR T Access Issues. *Oncology News Central* 13 December 2022; <https://www.oncologynewscentral.com/article/dying-on-the-wait-list-many-with-multiple-myeloma-face-car-t-access-issues>.
- 8** Keith R, Stabler C. Gene and Cell Therapies: Navigating the Challenges Around Rising Costs in Medical Insurance. *Guy Carpenter* 2023; https://www.guycarp.com/content/dam/guycarp-rebrand/insights-images/2023/09/Guy_Carpenter_GCT_Briefing%20_09_23.pdf.
- 9** Cliff ERS, et al. High Cost of Chimeric Antigen Receptor T-Cells: Challenges and Solutions. *ASCO Educat. Book* 2023; https://doi.org/10.1200/EDBK_397912.
- 10** Mafra A, et al. Global Patterns of Non-Hodgkin Lymphoma in 2020. *Int. J. Cancer* 151(9) 2022: 1474–1481; <https://doi.org/10.1002/ijc.34163>.
- 11** Yi M, et al. Global Burden and Trend of Acute Lymphoblastic Leukemia from 1990 to 2017. *Aging* 12(22) 2020: 22869–22891; <https://doi.org/10.18632/aging.103982>.
- 12** Huang J, et al. The Epidemiological Landscape of Multiple Myeloma: A Global Cancer Registry Estimate of Disease Burden, Risk Factors, and Temporal Trends. *Haematol.* 9(9) 2022: e670–e677; [https://doi.org/10.1016/S2352-3026\(22\)00165-X](https://doi.org/10.1016/S2352-3026(22)00165-X).
- 13** Kasamon Y, Bouchkouj N. *Yescarta (Axicabtagene ciloleucel) BLA Clinical Review*, STN: 125643. US Food and Drug Administration: Silver Spring, MD, 31 March 2017; <https://www.fda.gov/media/109149/download?attachment>.
- 14** O’Leary M. *Kymriah (Tisagenlecleucel): BLA Clinical Review*, STN: 125646. US Food and Drug Administration: Silver Spring, MD, 2 February 2017; <https://www.fda.gov/files/vaccines%2C%20blood%20%26%20biologics/published/Clinical-Review---KYMRIAH.pdf>.
- 15** Abernathy WJ, Utterback JM. Patterns of Industrial Innovation. *Technol. Rev.* 80(7) 1978: 41–47.
- 16** Rowley JA, Montgomery SA. The Need for Adherent Cell Manufacturing: Production Platform and Media Strategies Drive Cell Production Economics. *BioProcess Int.* 16(11–12) 2018: 34–49; <https://www.bioprocessintl.com/cell-therapies/the-need-for-adherent-cell-manufacturing-production-platform-and-media-strategies-drive-cell-production-economics>.
- 17** Köhler G, Milstein C. Continuous Cultures of Fused Cells Secreting Antibody of Predefined Specificity. *Nature* 256(5517) 1975: 495–497; <https://doi.org/10.1038/256495a0>.
- 18** Li F, et al. Cell Culture Processes for Monoclonal Antibody Production. *mAbs* 2(5) 2010: 466–479; <https://doi.org/10.4161/mabs.2.5.12720>.
- 19** Blaese RM, et al. T Lymphocyte-Directed Gene Therapy for ADA–SCID: Initial Trial Results After 4 Years. *Science* 270 (5235)1995: 475–480; <https://doi.org/10.1126/science.270.5235.475>.

- 20** Sibbald B. Death but One Unintended Consequence of Gene-Therapy Trial. *CMAJ* 164(11) 2001: 1612; <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC81135>.
- 21** Cole A. Child in Gene Therapy Programme Develops Leukaemia. *BMJ* 336(7634) 2008: 13; <https://doi.org/10.1136/bmj.39436.582292.DB>.
- 22** Perry C, Rayat ACME. Lentiviral Vector Bioprocessing. *Viruses* 13(2) 2021: 268; <https://doi.org/10.3390/v13020268>.
- 23** van Oers MM, Pijlman GP, Vlak JM. Thirty Years of Baculovirus–Insect Cell Protein Expression: From Dark Horse to Mainstream Technology. *J. Gen. Virol.* 96(1) 2015: 6–23; <https://doi.org/10.1099/vir.0.067108-0>.
- 24** Juric MK, et al. Milestones of Hematopoietic Stem Cell Transplantation — From First Human Studies to Current Developments. *Front. Immunol.* 7, 2016: 470; <https://doi.org/10.3389/fimmu.2016.00470>.
- 25** Lopez, A. Hematopoietic Stem Cell Transplantation (HSCT). *Embryo Project Encyclopedia*. Arizona State University: Tempe, AZ, 2010; <https://hdl.handle.net/10776/2041>.
- 26** Thomson JA, et al. Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science* 282(5391) 1998: 1145–1147; <https://doi.org/10.1126/science.282.5391.1145>.
- 27** Takahashi K, et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* 131(5) 2007: 861–872; <https://doi.org/10.1016/j.cell.2007.11.019>.
- 28** Watanabe K, et al. A ROCK Inhibitor Permits Survival of Dissociated Human Embryonic Stem Cells. *Nat. Biotechnol.* 25(6) 2007: 681–686; <https://doi.org/10.1038/nbt1310>.
- 29** Melkounian Z, et al. Synthetic Peptide-Acrylate Surfaces for Long-Term Self-Renewal and Cardiomyocyte Differentiation of Human Embryonic Stem Cells. *Nat. Biotechnol.* 28(6) 2010: 606–610; <https://doi.org/10.1038/nbt.1629>.
- 30** Bloor AJC, et al. Production, Safety and Efficacy of iPSC-Derived Mesenchymal Stromal Cells in Acute Steroid-Resistant Graft Versus Host Disease: A Phase I, Multicenter, Open-Label, Dose-Escalation Study. *Nat. Med.* 26(11) 2020: 1720–1725; <https://doi.org/10.1038/s41591-020-1050-x>.
- 31** Bharti K, et al. Developing Cellular Therapies for Retinal Degenerative Diseases. *Invest. Ophthalmol. Vis. Sci.* 55(2) 2014: 1191–1202; <https://doi.org/10.1167/iovs.13-13481>.
- 32** Cichocki F, et al. iPSC-Derived NK Cells Maintain High Cytotoxicity and Enhance In Vivo Tumor Control in Concert with T Cells and Anti-PD-1 Therapy. *Sci. Transl. Med.* 12(568) 2020: eaaz5618; <https://doi.org/10.1126/scitranslmed.aaz5618>.
- 33** Kuroda T, et al. Robust Induction of Retinal Pigment Epithelium Cells from Human Induced Pluripotent Stem Cells by Inhibiting FGF/MAPK Signaling. *Stem Cell Res.* 39, 2019: 101514; <https://doi.org/10.1016/j.scr.2019.101514>.
- 34** Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in Transplants of Bone Marrow Cells. *J. Embryol. Exp. Morphol.* 16(3) 1966: 381–390.
- 35** Caplan AI. Mesenchymal Stem Cells. *J. Orthopaed. Res.* 9(5) 1991: 641–650; <https://doi.org/10.1002/jor.1100090504>.
- 36** Gomez-Salazar M, et al. Five Decades Later, Are Mesenchymal Stem Cells Still Relevant? *Front. Bioeng. Biotechnol.* 8, 2020: 148; <https://doi.org/10.3389/fbioe.2020.00148>.
- 37** Wright A, Arthaud-Day ML, Weiss ML. Therapeutic Use of Mesenchymal Stromal Cells: The Need for Inclusive Characterization Guidelines To

Accommodate All Tissue Sources and Species. *Front. Cell Dev. Biol.* 9, 2021: 632717; <https://doi.org/10.3389/fcell.2021.632717>.

38 Stansfield N. FDA Deems Mesoblast's Data on Remestemcel-L Cell Therapy Sufficient for BLA Submission in Pediatric SR-aGVHD. *CGT Live* 26 March 2024; <https://www.cgtlive.com/view/fda-deems-mesoblast-data-remestemcel-l-cell-therapy-sufficient-bla-submission-pediatric-sr-agvhd>.

39 Newman RE, et al. Treatment of Inflammatory Diseases with Mesenchymal Stem Cells. *Inflamm. Allergy Drug Targets* 8(2) 2009: 110–123; <https://doi.org/10.2174/187152809788462635>.

40 Bellingan G, et al. Safety and Efficacy of Multipotent Adult Progenitor Cells in Acute Respiratory Distress Syndrome (MUST-ARDS): A Multicentre, Randomised, Double-Blind, Placebo-Controlled Phase 1/2 Trial. *Intensive Care Med.* 48(1) 2022: 36–44; <https://doi.org/10.1007/s00134-021-06570-4>.

41 Couch Y, et al. A Brief History of Nearly EVerything — The Rise and Rise of Extracellular Vesicles. *J. Extracell. Vesicles* 10(14) 2021: e12144; <https://doi.org/10.1002/jev2.12144>.

42 Tang Y, Zhou Y, Li H-J. Advances in Mesenchymal Stem Cell Exosomes: A Review. *Stem Cell Res. Ther.* 12(1) 2021: 71; <https://doi.org/10.1186/s13287-021-02138-7>.

43 Rai A, et al. The Discovery of Extracellular Vesicles and Their Emergence As a Next-Generation Therapy. *Circ. Res.* 135(1) 2024: 1198–1221; <https://doi.org/10.1161/CIRCRESAHA.123.323054>.

44 Billingham RE, Brent L, Medawar PB. Quantitative Studies on Tissue Transplantation Immunity. II. The Origin, Strength and Duration of Actively and Adoptively Acquired Immunity. *Proc. Roy. Soc. Lond. B Biol. Sci.* 143(910) 1954: 58–80; <https://doi.org/10.1098/rspb.1954.0054>.

45 Fesnak AD, June CH, Levine BL. Engineered T Cells: The Promise and Challenges of Cancer Immunotherapy. *Nat. Rev. Cancer* 16(9) 2016: 566–581; <https://doi.org/10.1038/nrc.2016.97>.

46 Grupp SA, et al. Chimeric Antigen Receptor–Modified T Cells for Acute Lymphoid Leukemia. *N. Engl. J. Med.* 368(16) 2013: 1509–1518; <https://doi.org/10.1056/NEJMoa1215134>.

47 Alzubi J, et al. Automated Generation of Gene-Edited CAR T Cells at Clinical Scale. *Mol. Ther. Methods Clin. Dev.* 20, 2021: 379–388; <https://doi.org/10.1016/j.omtm.2020.12.008>.

48 Smith D, et al. Towards Automated Manufacturing for Cell Therapies. *Curr. Hematol. Malig. Rep.* 14(4) 2019: 278–285; <https://doi.org/10.1007/s11899-019-00522-y>.

49 Abou-el-Enein Mohamed, et al. Scalable Manufacturing of CAR T Cells for Cancer Immunotherapy. *Blood Cancer Disc.* 2(5) 2021: 408–422; <https://doi.org/10.1158/2643-3230.BCD-21-0084>.

50 Suarez L, et al. AIM Platform: A Novel Nano Artificial Antigen-Presenting Cell-Based Clinical System Designed To Consistently Produce Multi-Antigen-Specific T-Cell Products with Potent and Durable Anti-Tumor Properties. *Transfus. Med. Hemother.* 47(6) 2020: 464–471; <https://doi.org/10.1159/000512788>.

51 O'Connor J, et al. Manufacturing of Patient Specific Novel T Cell Therapies Using the Cocoon Platform Automated System. *Advancing Manufacture of Cell and Gene Therapies VII*. ECI Symposium Series: New York, NY, 2022; https://dc.engconfintl.org/cellgenetherapies_vii/12.

- 52** Song HW, et al. Scaling Up and Scaling Out: Advances and Challenges in Manufacturing Engineered T Cell Therapies. *Int. Rev. Immunol.* 41(6) 2022: 638–648; <https://doi.org/10.1080/08830185.2022.2067154>.
- 53** Trainor N, et al. Automated Production of Gene-Modified Chimeric Antigen Receptor T Cells Using the Cocoon Platform. *Cytotherapy* 25(12) 2023: 1349–1360; <https://doi.org/10.1016/j.jcyt.2023.07.012>.
- 54** Beltzer J. Bryan A (vice president and head of personalized medicine business, Lonza AG). Personal communication, October 2023.
- 55** Janas M, et al. Perfusion's Role in Maintenance of High-Density T-Cell Cultures. *BioProcess Int.* 13(1) 2015: 18–26; <https://www.bioprocessintl.com/bioreactors/perfusion-s-role-in-maintenance-of-high-density-t-cell-cultures>.
- 56** Porter DL, et al. Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia. *N. Engl. J. Med.* 365(8) 2011: 725–733; <https://doi.org/10.1056/NEJMoa1103849>.
- 57** Coeshott C, et al. Large-Scale Expansion and Characterization of CD3⁺ T-Cells in the Quantum Cell Expansion System. *J. Transl. Med.* 17, 2019: 258; <https://doi.org/10.1186/s12967-019-2001-5>.
- 58** Nankervis B, et al. Optimizing T Cell Expansion in a Hollow-Fiber Bioreactor. *Curr. Stem Cell Rep.* 4(1) 2018: 46–51; <https://doi.org/10.1007/s40778-018-0116-x>.
- 59** Sheu J, et al. Large-Scale Production of Lentiviral Vector in a Closed System Hollow Fiber Bioreactor. *Mol. Ther. Meth. Clin. Dev.* 2, 2015: 15020; <https://doi.org/10.1038/mtm.2015.20>.
- 60** Tirughana R, et al. GMP Production and Scale-Up of Adherent Neural Stem Cells with a Quantum Cell Expansion System. *Mol. Ther. Meth. Clin. Dev.* 10, 2018: 48–56; <https://doi.org/10.1016/j.omtm.2018.05.006>.
- 61** Lee B, et al. Cell Culture Process Scale-Up Challenges for Commercial-Scale Manufacturing of Allogeneic Pluripotent Stem Cell Products. *Bioengineering* 9(3) 2022: 92; <https://doi.org/10.3390/bioengineering9030092>.
- 62** Dang T, et al. Computational Fluid Dynamic Characterization of Vertical-Wheel Bioreactors Used for Effective Scale-Up of Human Induced Pluripotent Stem Cell Aggregate Culture. *Can. J. Chem. Eng.* 99(11) 2021: 2536–2553; <https://doi.org/10.1002/cjce.24253>.
- 63** Costariol E, et al. Demonstrating the Manufacture of Human CAR-T Cells in an Automated Stirred-Tank Bioreactor. *Biotechnol. J.* 15(9) 2020: 2000177; <https://doi.org/10.1002/biot.202000177>.
- 64** Ibenana L, et al. Assessment of the LOVO device for Final Harvest of Novel Cell Therapies: A Production Assistance for Cellular Therapies Multi-Center Study. *Cytotherapy* 24(7) 2022: 691–698; <https://doi.org/10.1016/j.jcyt.2022.01.010>.
- 65** Li A, et al. Improving Cell Viability Using Counterflow Centrifugal Elutriation. *Cytotherapy* 24(6) 2022: 650–658; <https://doi.org/10.1016/j.jcyt.2022.01.008>.
- 66** Li A, et al. Advances in Automated Cell Washing and Concentration. *Cytotherapy* 23(9) 2021: 774–786; <https://doi.org/10.1016/j.jcyt.2021.04.003>.
- 67** Backer D, Ward T. Modernizing Cell Therapy Manufacturing To Reduce Vein-to-Vein Times. *Cell Gene Ther. Ins.* 9(6) 2023: 843–850; <https://doi.org/10.18609/cgti.2023.104>.
- 68** Harris E. *A Summary of Cell & Gene Therapies Manufacturing Outlook Report: 2023 Edition*. Cell & Gene, 20 April 2023; <https://www.cellandgene.com/doc/a-summary-of-cell-gene-therapies-manufacturing-outlook-report-edition-0001>.

69 Rios M, Judd S. Multimodal Facility Design for Cell and Gene Therapies. *BioProcess Int.* 20(6) 2022: 20–28; <https://www.bioprocessintl.com/facility-design-engineering/multimodal-facility-design-for-cell-and-gene-therapies>.

70 *Baseline Guide: Volume 6 – Biopharmaceutical Manufacturing Facilities*. International Society of Pharmaceutical Engineers: North Bethesda, MD, November 2023; <https://ispe.org/publications/guidance-documents/baseline-guide-vol-6-biopharmaceutical-manufacturing-facilities-3rd-edition>.

71 Dunleavy K. Cellares Unveils \$255M Investment Round with Bristol Myers Among Those in the Fold. *Fierce Pharma*, 24 August 2023; <https://www.fiercepharma.com/manufacturing/cellares-unveils-255m-investment-round-bristol-myers-among-those-fold>.

72 Ozbilge K, Sullivan E, Gavin B. Will Point-of-Care Manufacturing Unlock the Value of Autologous Cell Therapies? *Cell & Gene*, 5 June 2023; <https://www.cellandgene.com/doc/will-point-of-care-manufacturing-unlock-the-value-of-autologous-cell-therapies-0001>.

73 Harrison RP, Medcalf N, Rafiq QA. Cell Therapy-Processing Economics: Small-Scale Microfactories as a Stepping Stone Toward Large-Scale Macrofactories. *Regen. Med.* 13(2) 2018: 159–173; <https://doi.org/10.2217/rme-2017-0103>.

74 Stanton D. Novartis at JPM: “7-Day CAR-T Process an Alternative to Allogeneic.” *BioProcess Insider*, 12 January 2023; <https://www.bioprocessintl.com/therapeutic-class/novartis-at-jpm-7-day-car-t-process-an-alternative-to-allogeneic>.

75 Yang J, et al. Next-Day Manufacture of a Novel Anti-CD19 CAR-T Therapy for B-Cell Acute Lymphoblastic Leukemia: First-in-Human Clinical Study. *Blood Cancer J.* 12(7) 2022: 104; <https://doi.org/10.1038/s41408-022-00694-6>.

76 Du J, et al. Updated Results of a Phase I, Open-Label Study of BCMA/CD19 Dual-Targeting FasTCAR-T GC012F for Patients with Relapsed/Refractory Multiple Myeloma (RRMM). *Hemasphere* 7, 2023: e84060bf; <https://doi.org/10.1097/01.HS9.0000970380.84060.bf>.

77 Laskowski T, Purpura K. Rapid CAR-T Cells: Accelerating Manufacturing To Enable Fast Transition of CAR-T Cell Therapies to the Clinic. Webinar. *Cell Gene Ther. Ins.* 7 December 2023; <https://www.insights.bio/cell-and-gene-therapy-insights/webinars/511/Rapid-CAR-T-cells-Accelerating-manufacturing-to-enable-fast-transition-of-CAR-T-cell-therapies-to-the-clinic>.

78 Agarwalla P, et al. Bioinstructive Implantable Scaffolds for Rapid In Vivo Manufacture and Release of CAR-T Cells. *Nat. Biotechnol.* 40(8) 2022: 1250–1258; <https://doi.org/10.1038/s41587-022-01245-x>.

79 Rurik JG, et al. CAR T Cells Produced In Vivo To Treat Cardiac Injury. *Science* 375(6576) 2022: 91–96; <https://doi.org/10.1126/science.abm0594>.

80 Rive CM, et al. Selective B Cell Depletion Upon Intravenous Infusion of Replication-Incompetent Anti-CD19 CAR Lentivirus. *Mol. Ther. Meth. Clin. Dev.* 26, 2022: 4–14; <https://doi.org/10.1016/j.omtm.2022.05.006>.

81 Sheridan C. The World’s First CRISPR Therapy Is Approved: Who Will Receive It? *Nat. Biotechnol.* 42(1) 2024: 3–4; <https://doi.org/10.1038/d41587-023-00016-6>.

82 Gillmore JD, et al. CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. *N. Engl. J. Med.* 385(6) 2021: 493–502; <https://doi.org/10.1056/NEJMoa2107454>.

83 Bream A, Salzmann B. The Difficulties of Manufacturing Cell and Gene Therapies at Scale. *BioProcess Int.* 19(4) 2021: 10–13; <https://www.bioprocessintl.com/cell-therapies/the-difficulties-of-manufacturing-cell-and-gene-therapies-at-scale>.

84 Walters P. Scalability in Cell and Gene Therapy Facilities: How Today's Developers Are Preparing for Tomorrow's Commercial Success. *BioProcess Int.* 20(4): 14–18; <https://www.bioprocessintl.com/facility-design-engineering/scalability-in-cell-and-gene-therapy-facilities-how-today-s-developers-are-preparing-for-tomorrow-s-commercial-success>.

85 Eaker S, et al. Bioreactors for Cell Therapies: Current Status and Future Advances. *Cytotherapy* 19(1) 2017: 9–18; <https://doi.org/10.1016/j.jcyt.2016.09.011>.

86 Wagner DL, et al. Immunogenicity of CAR T Cells in Cancer Therapy. *Nat. Rev. Clin. Oncol.* 18(6) 2021: 379–393; <https://doi.org/10.1038/s41571-021-00476-2>.

87 Liu Y, et al. Strategies To Enhance CAR-T Persistence. *Biomark. Res.* 10(1) 2022: 86; <https://doi.org/10.1186/s40364-022-00434-9>.

88 Mitra A, et al. From Bench to Bedside: The History and Progress of CAR T Cell Therapy. *Front. Immunol.* 14, 2023: 1188049; <https://doi.org/10.3389/fimmu.2023.1188049>.

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BACK TO CONTENTS