Finding Consistency and Scalability in Allogeneic CAR-T Production

Lorraine Borland with Julia Hengst and Qasim Rafiq

In recent years, cell therapies have advanced in both safety and efficacy, transitioning from a fifth- to second-line treatment option. A notable example came with the US Food and Drug Administration (FDA) approval of Johnson & Johnson's Carvykti (ciltacabtagene autoleucel) immunotherapy for multiple myeloma on 5 April 2024 (**1**). That milestone reflects the evolving landscape of cell therapies, which can be characterized by three trends: enhanced effectiveness in blood-cancer treatment, treatment of solid-tumor progression, and groundbreaking advancements in treating autoimmune disease (**2**).

Autologous therapies have remained the dominant approach for immune cells from 2021 to 2024, accounting for ~65% of treatments, whereas allogeneic therapies represent around 35% (**2**). During that period, products based on chimeric antigen receptor (CAR) T cells have surged to the forefront, now making up 49% of all cell therapies. Oncology is the primary target area and accounts for 74% of all treatments. However, as the scope of treatable indications expands, demand for allogeneic therapies is increasing.

Allogeneic workflows are gaining traction and might eventually become more widespread than autologous therapies. Scientists can use allogeneic stem, CAR T, and natural killer (NK) cells to treat a broad spectrum of autoimmune diseases, including lupus nephritis, type-1 diabetes, multiple sclerosis, and myasthenia gravis. Multiple candidates based on such cells are undergoing phase 1 and 2 clinical trials, with potential to change how patients manage autoimmune disease. For example, a team from University Hospital Erlangen in Germany published a study showing that CD19-targeted CAR-T therapy resulted in drug-free remission for patients with severe lupus erythematosus (**3**).

Such reports demonstrate the transformative potential of cell therapies for autoimmune disease but also reveal challenges that cell-therapy manufacturers must face to ensure performance and scalability while adhering to stringent regulatory guidelines. Cell-therapy workflows are intricate, comprising several unit operations from donor-cell sourcing to selection, activation, modification, and expansion (Figure 1). The expansion process is particularly complex for CAR-T therapies. Scientists perform expansion in both static conditions and agitated bioreactors until reaching the requisite number of cells for clinical dose requirements. Both expansion phases can occur within closed manufacturing systems.

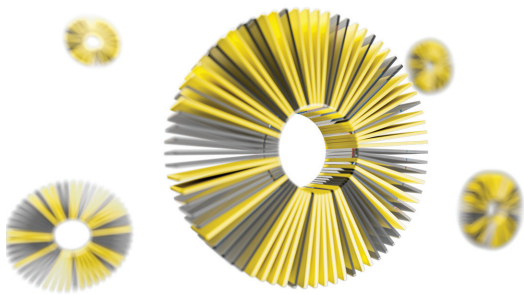

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Figure 1: A process-mapping summary for immune-cell workflows

Known for their rapid growth, CAR T cells can require daily perfusion to sustain proliferation. As the industry transitions from small-batch autologous processes to large-batch allogeneic ones, manufacturers need scalable systems that provide consistent performance to meet rigorous regulatory standards. Below, I speak with Julia Hengst, who joined Sartorius in 2022 as an external collaborations manager for advanced-therapy applications. In that role, Hengst is leading a partnership with Qasim Rafiq, a professor of cell- and gene-therapy bioprocessing at University College London (UCL) in the United Kingdom. Rafiq's research focuses on developing, optimizing, and intensifying CAR-T processes in stirred-tank bioreactors (STRs), with the goal of scaling up production.

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What difficulties do therapy developers and manufacturers face in engineered-cell manufacturing, particularly for CAR-T therapies? What new technologies could address those issues? Manufacturers today face challenges such as high cost of goods (CoG), high production failure rates, long vein-to-vein process times, and lengthy quality-control and product-release testing. The complexities of supply-chain management and the logistics of individualized products create additional obstacles.

New trends are emerging in the CAR-T market to overcome such challenges. Specifically, manufacturers are using rapid manufacturing strategies, in vivo CAR-T therapy, and next-generation CAR constructs to improve the potency of allogeneic products.

Intensifying CAR-T manufacturing processes (e.g., using STRs) would enable off-the-shelf allogeneic cell-therapy development at a relatively low cost. To adopt that strategy, manufacturers need bioreactor platforms with small-scale, automated models that support screening and process development to enable seamless transitions to robust, large-scale manufacturing platforms.

What interests you about applying STRs for CAR-T cell culture? Commercially approved CAR-T products are autologous. As I mentioned, that approach entails high CoG and lengthy timelines for processing, shipment, quality and release testing, and ultimately, administration. For allogeneic therapies, scientists can use STRs to intensify manufacturing processes and produce off-the-shelf cell products at much lower costs.

STRs are scalable, making them popular options for large-scale production of vaccines and protein-/antibody-based therapeutics. During CAR T-cell expansion in an STR, scientists can perform

on-line monitoring of specific parameters such as pH, dissolved-oxygen levels, and agitation rates. Manufacturers can also scale up STRs seamlessly from small-scale platforms for screening and process development to robust, large-scale manufacturing systems.

How will the Rafiq laboratory's work support the advancement of allogeneic CAR-T therapies? Because of the potential benefits of STRs, Rafiq and his group at UCL are investigating whether such technologies can be used to manufacture CAR T cells. Allogeneic CAR-T products require manufacture of multiple doses at a scale that is beyond current manufacturing-technology capacity, making STRs critical to investigate for large-scale production of allogeneic CAR-T formats.

Sartorius is collaborating with Rafiq and his group with the goal of advancing allogeneic CAR-T therapy development. Together, we are trying to leverage process optimization and intensification to produce more doses per manufacturing run. In time, such work can significantly reduce CoG for these transformative therapies while increasing patient accessibility.

What makes that application of STRs unique? Rafiq's team has pioneered studies of CAR T-cell production in STRs since 2017. Based on a design-of-experiments (DoE) approach, the team intensified a perfusion process in an Ambr 250 high-throughput bioreactor. After optimization, culture settings were scaled up for a 2-L Univessel single-use bioreactor system.

That approach is distinctive because most scientists do not apply perfusion or perform CAR T-cell expansions in STRs with on-line process monitoring. In addition, the intensified process produced the initial dose twice as fast and increased total cell yield by almost fivefold compared with what can be achieved in a fed-batch culture.

Sartorius and UCL also integrated a Ksep 400 single-use centrifugation system to enable closed, automated, and continuous concentration and harvest of final product. That integration enables a scalable CAR-T manufacturing workflow across upstream and downstream processing. Postharvest results suggest that the intensified perfusion process and Ksep centrifugation step had no significant effect on CAR T-cell phenotype, viability, or functionality. That outcome highlights the importance of establishing an integrated approach to CAR-T manufacturing, including process optimization, process intensification, and connectivity of closed upstream and downstream operations.

How did the team intensify CAR T-cell expansion? We compared a fed-batch and perfusion process using the Ambr 250 high-throughput perfusion bioreactor. We applied a DoE approach to examine multiple process parameters in the bioreactor system and to understand their impacts on CAR T-cell expansion, phenotype, and function. The DoE methodology provides a systematic approach to studying each process parameter and its concomitant effect on other investigated parameters, enabling us to investigate a larger design space than would be possible using

CAR-T Resources from Scientists at Sartorius and University College London

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approaches. Consequently, we can identify a more representative optimum compared with single-factor studies.

To identify critical process parameters for optimized CAR T-cell growth, we established an experimental space based on 32 expansion runs in an Ambr 250 high-throughput perfusion bioreactor. We tested two cell-culture media — RPMI media supplemented with 10% serum and 4Cell Nutri-T serum-free, xeno-free media — and two perfusion parameters. Because donor-to-donor variability is a challenge for CAR T-cell production, we tested the robustness of our identified process parameters by including T cells from three healthy donors.

Taking the two tested perfusion parameters and the CAR T-cell fold expansion into account, we used MODDE software to establish the DoE studies, analyze the data, and identify the best operating window for each medium. Our analysis showed that some heterogeneity manifested among the donors and that the best operating window for the process differed between the two media. Ultimately, the software identified optimized perfusion parameter settings that we could determine systematically for each media.

References

1 *Carvykti Is the First and Only BCMA-Targeted Treatment Approved by the US FDA for Patients with Relapsed or Refractory Multiple Myeloma Who Have Received at Least One Prior Line of Therapy* (press release). Johnson & Johnson: Horsham, PA, 6 April 2024; https://www.jnj.com/media-center/press-releases/carvykti-is-the-first-and-only-bcma-targeted-treatment-approved-by-the-u-s-fda-for-patients-with-relapsed-or-refractory-multiple-myeloma-who-have-received-at-least-one-prior-line-of-therapy.

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