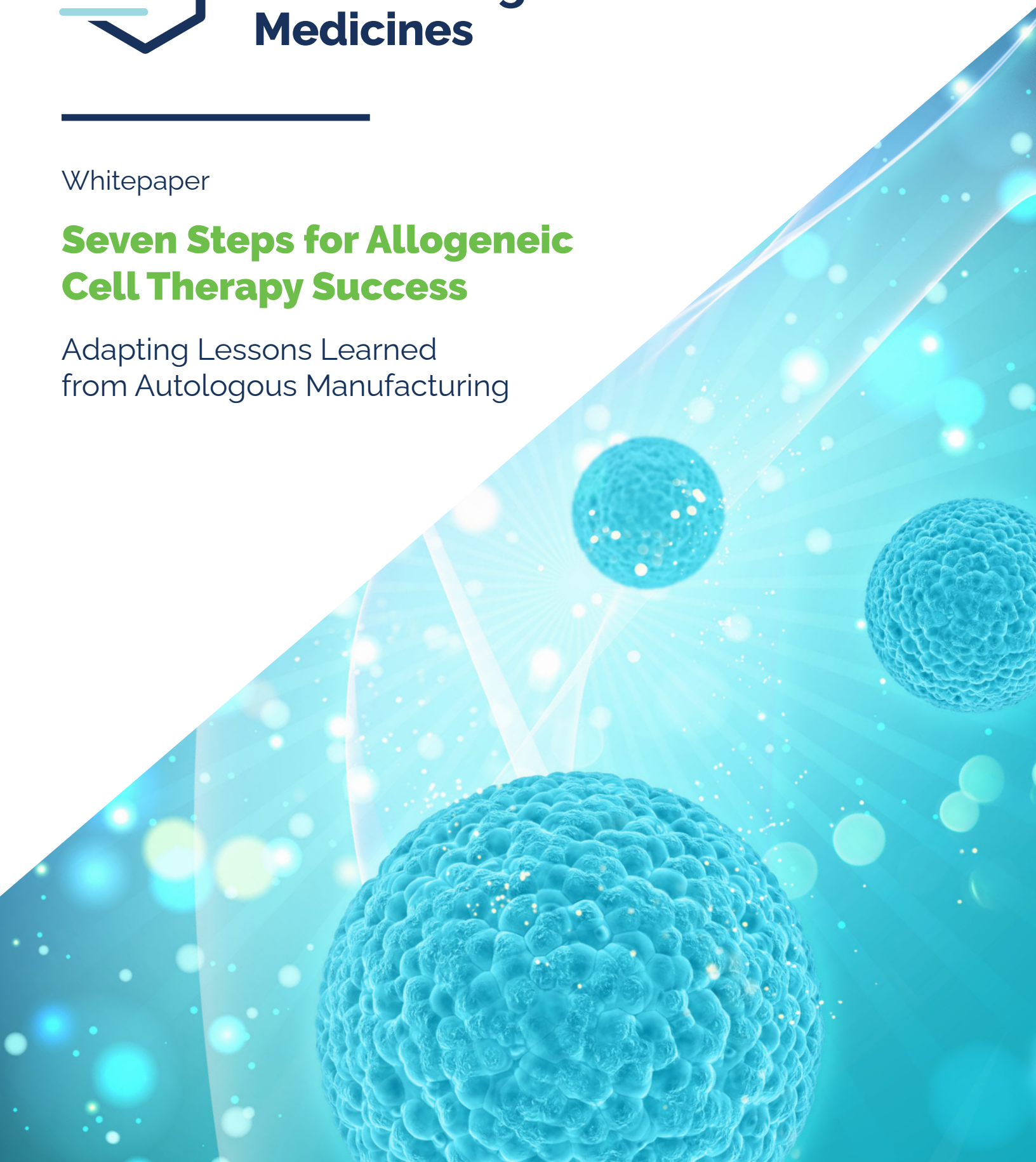
Center for Breakthrough Medicines

Whitepaper

Seven Steps for Allogeneic Cell Therapy Success

Adapting Lessons Learned from Autologous Manufacturing



PREPARING FOR SUCCESSFUL COMMERCIALIZATION OF ALLOGENEIC CELL THERAPIES

Allogeneic, or "donor-derived", CAR T cells have the potential to broaden access to treatment for patients. To prepare for commercial scale, companies must think through a number of considerations around scale-up, aseptic processing, aseptic processing, single-use systems, facility design, starting material, supply chain, and logistics.

1. Implementing Scale-Up vs. Scale-Out

In autologous products where one batch supports one patient, processes must be scaled out to enable multiple products to be produced simultaneously (see **Figure 1**). Scale up of allogeneic therapies presents a different set of challenges. While the upstream process for allogeneic therapies is similar to that for autologous therapies, it is performed in much larger vessels (up to 2,000 L). Cell therapies typically comprise approximately 10^9 cells/dose.[1] To produce tens-to-hundreds of thousands of doses, a typical harvest goal per batch of an allogeneic therapy must be in the range of 10^{11} to 10^{14} cells – between a hundred-fold to million-fold increase over an autologous batch.

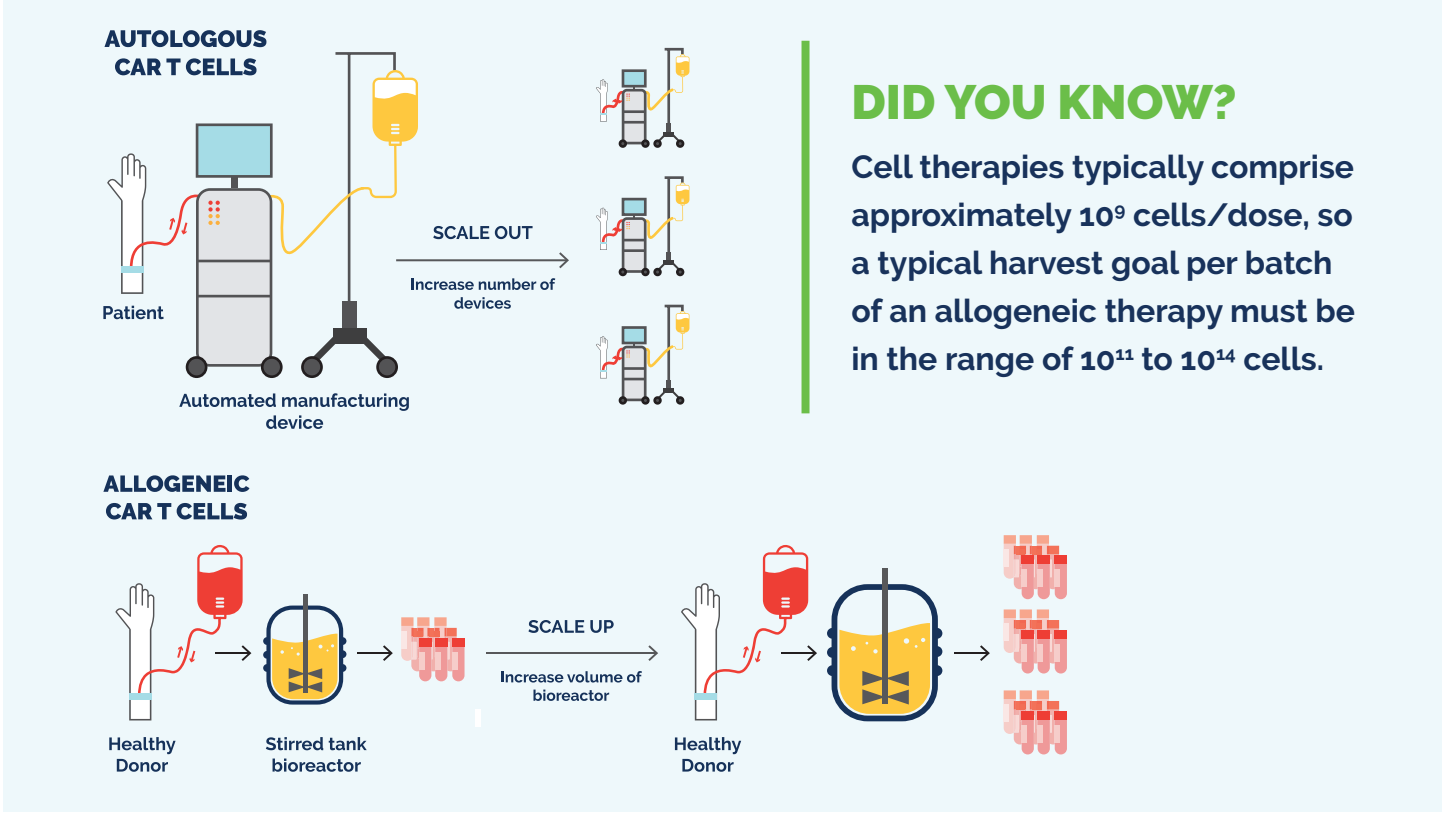


Figure 1: Scale up vs scale out

Each batch, whether produced via suspension (including microcarrier-based) cell culture in stirred-tank reactors or adherent cell culture in fixed-bed bioreactors, must afford cellular products with consistent properties and quality. The necessary equipment is not always available. For instance, fit-for-purpose mixers that accommodate microcarrier-based cell culture solutions and avoid applying undue shear stress to the cellular product are still under development.

In addition, achieving an appropriate number of cells while maintaining cellular integrity and functionality can be challenging.[2] As cell density increases, these properties tend to decrease. Furthermore, greater heterogeneity occurs, which can impact cellular performance and thus, quality and efficacy. Furthermore, the extensive level of expansion introduces the potential to generate cells with more differentiated, less efficacious phenotypes than cell populations that have been less expanded.

Downstream processing, meanwhile, presents logistical operational concerns when volumes reach 2000 L.[1,2] The quality and characteristics of the cell therapy product filled into the last vial must be the same as the product filled into the first vial. Typically, small-scale purification solutions are not practically implemented at large scale, requiring the development of processes using different equipment, such as continuous centrifugation and tangential-flow filtration (see **Figure 2**). Filling machines that operate at larger scale were not designed for cell therapies, however, and allogeneic therapy developers must contend with this issue before purpose-built fillers for cell therapies are commercially available.

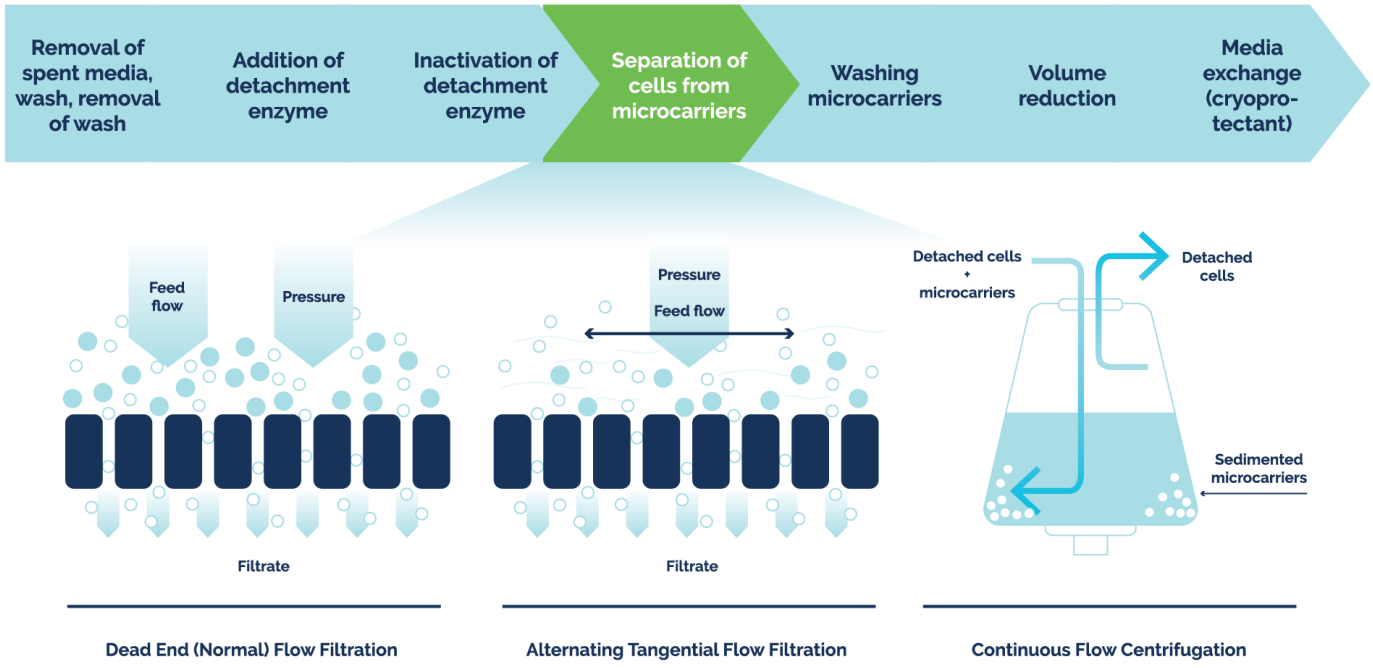


Figure 2: Downstream processing operations for cell therapies produced in suspension using microcarrier supports

2. Maintaining Aseptic Processing

While small-scale manufacturing for autologous therapies can be performed using open processes in biosafety cabinets, such approaches are not suitable for allogeneic processing, as the risk of contamination from humans and the environment is too great. Use of automated processes performed in closed systems such as isolators eliminates these issues and also decreases the risk of cross-contamination.[3] If manual operations are used, it is imperative that employees be consistent in good laboratory practices, remain up to date on training, and stay focused while processing to minimize variation between operators.

For closed systems, proof of closure is necessary and should include consideration of all parts of the system that play a role in achieving and maintaining closure (acceptable bioburden, cleanliness, and integrity). Methods must also be developed to ensure equipment integrity and prevent uncontrolled material exchange with the surroundings. Correct material addition and removal must also be verified.

Risk assessment and risk management methodology should be leveraged to target aspects of the process that present the greatest risk. Wherever possible, manufacturers should minimize tubing manipulations and simplify processes to reduce risk. Aseptic filling is also a high-risk process that for cell therapies often must be performed quickly due to limited product stability. Real-time monitoring systems are essential, and use of automated, robotic filling systems is highly recommended.[5]

Cell therapy production involves multiple manipulations, creating opportunities for the disruption of aseptic conditions.

3. Adopting Single Use Systems for Scale

Unlike stainless-steel equipment that requires cleaning between batches, single use (SU) systems come pre-sterilized. This elimination of cleaning and cleaning validation steps saves on labor, reduces setup and changeover times and further reduces the risk of cross-contamination.

SU systems are also available at commercial scale - bioreactors and other SU technologies developed for mAb and viral-vector manufacturing - can be leveraged for allogeneic cell therapy. These technologies will need to be optimized for cell therapy – for instance, shear force during mixing in a stirred-tank bioreactor may impact cell health.

In principle, standardization would enable the use of SU components from different suppliers, eliminating the reliance on single suppliers, which would lead to lower costs while minimizing supply chain disruptions. Disposable solutions designed specifically for cell-therapy manufacturing, particularly for downstream processing, would also be beneficial, particularly with respect to unit operations that can cause cell damage due to shear exposure. Shear forces present during transfer through tubing are of concern, and such transfers should be minimized during process development.

4. Future-proofing Facility Designs

Given that allogeneic cell therapy is still early in development, standardization of processes and equipment has yet to occur. Therefore, facility designs must be sufficiently flexible to accommodate ongoing changes as the sector matures.

Factors to consider for any cell therapy manufacturing plant include the locations for reagent delivery and processing, QC labs, and warehousing; the headcount needed to efficiently operate the facility; material and personnel flows and the number and types of cleanroom suites required and the necessary utilities.[5]

For allogeneic therapies, decisions include whether to build one large, multi-product facility or adopt a campus-style approach with different cell therapies produced in different smaller buildings and whether to start from an existing structure or construct a greenfield facility. Regardless, it is essential to design cell therapy facilities with the future in mind with respect to the potential for implementation of new technologies and/or equipment, the need to comply with new regulations and the introduction of new products.

That means redundant utilities should be isolated from the manufacturing floor so they can be upgraded without needing to halt manufacturing operations, and additional floor space should be included for increased manufacturing capacity, whether for current or additional products. Ballroom style production areas with closed equipment are attractive for both autologous and allogeneic therapies as a means for downgrading the production area environment and adding processing flexibility.[5]

5. Ensuring Starting Material Quality

The consistency of raw materials is a key criterion for successful biologics manufacturing. That becomes a huge hurdle for autologous cell therapies given that the key raw material is leukapheresis from sick patients, which by default leads to significant heterogeneity in the quality of cells.

Within the apheresis process as determined by factors such as staff training, collection efficiency and the limiting of contamination by unwanted cell types can also have a direct impact on autologous cell therapy manufacturing outcomes.[6] In fact, it has been reported that apheresis product variability (See **Figure 3**) is a major cause of failed cell therapy manufacturing runs, largely due to a failure to meet target therapeutics cell yields. [7]

As previously mentioned, autologous cell therapy is hindered by the fact that individual patients have received many prior treatments, which not only contributes to greater heterogeneity, but also means cells can be compromised or non-viable as starting material. Making efficacious cell therapy products from shoddy starting materials is difficult to do.

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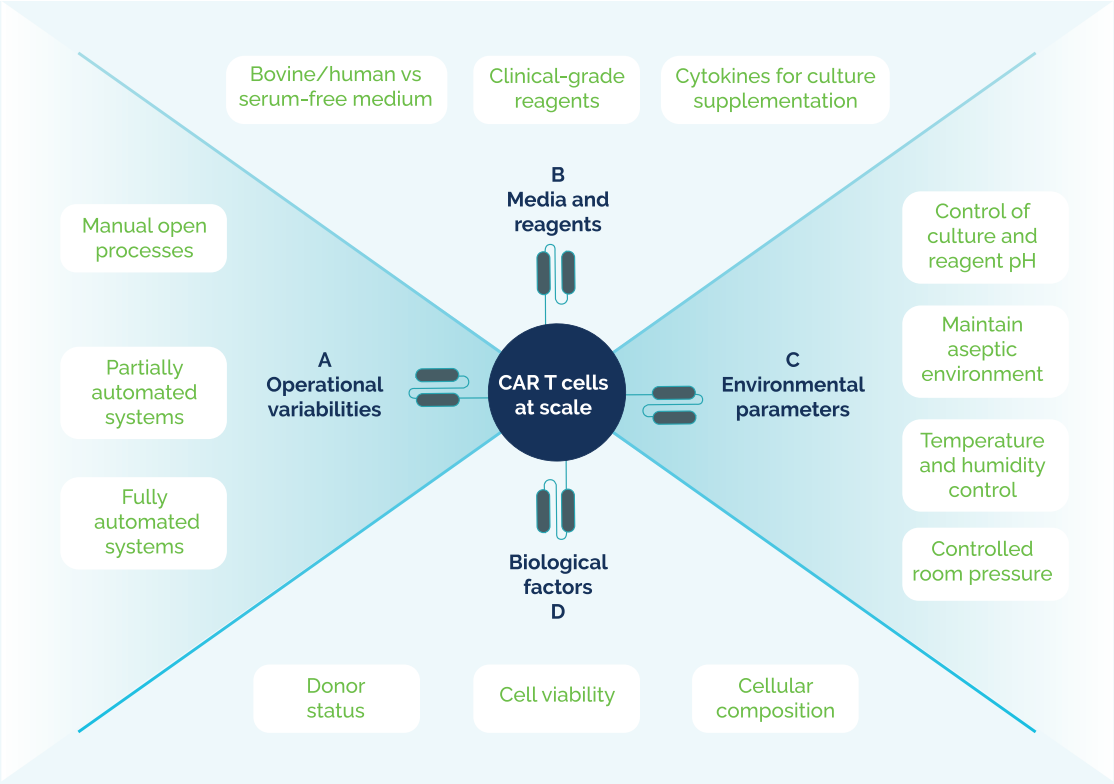


Figure 3: Manufacturing variables in CAR T cell production at scale

Allogeneic therapies benefit from the ability of manufacturers to carefully select the donor cells used to manufacture their products. However high-quality cell therapies cannot be produced without access to a reliable supply of high-quality donor cells. Partnering with recognized suppliers that have demonstrated experience is an optimal solution to this challenge. These suppliers should in turn have access to valued allogeneic donors who are dependably available for repeat apheresis collections.[6] Here too, standardization of apheresis center training, equipment and procedures would be highly beneficial.

Choosing the right donor cells is crucial. Donor cells clearly must be free of genetic abnormalities that underlie disease. Thus, genomic sequencing is essential. Currently there is no generally available assay that can confirm chromosomal aberrations are absent in the hundreds of millions of cells that make up a cell therapy. Work needs to be done in this area.

The HLA background is also an obvious characteristic that must be understood for matching purposes. Beyond these features, companies typically look at the cellular age, with younger cells thought to be more efficacious. Understanding of the phenotype for T cell therapy can also be important, such as stem cell memory and other departmental subsets, with CD4 seeming to be relevant for sustained response. Expansion capacity and cytotoxic profile are two other important features, with the latter related to how well the cell can maintain its level of cytotoxicity.

Because allogeneic cell therapies will be produced from donor cells obtained from multiple donors, specifications for starting T cell populations are needed so that testing of cells from different donors can be conducted to ensure consistency. This need is urgent, as even when donor cells meet key minimum criteria, differences in key characteristics may still exist. In one case, MSCs that met ISCT proposed criteria still differed with respect to cell growth potential and IL-6 production, properties that can directly impact manufacturing.[8] Separately, genetic variability was shown to have a greater impact on induced pluripotent stem cell differentiation potential than parental cell type.[9]

In addition to the need for consistent, robust, high-quality donor cells, some level of gene editing is required for these cells in order to avoid GvHD and other immunogenicity-related issues. The need for gene editing raises questions about safety and scalability. The safety of various genome editing techniques should be confirmed with assays that confirm high-fidelity editing.[10] The genetic stability of the construct during cell manipulation must also be demonstrated via in-process and release testing to ensure the integrity of the resulting cellular population so that patients are not subjected to additional risk through genetic abnormalities. Next-generation genomic sequencing performed post-genetic manipulation is essential for getting precise results.

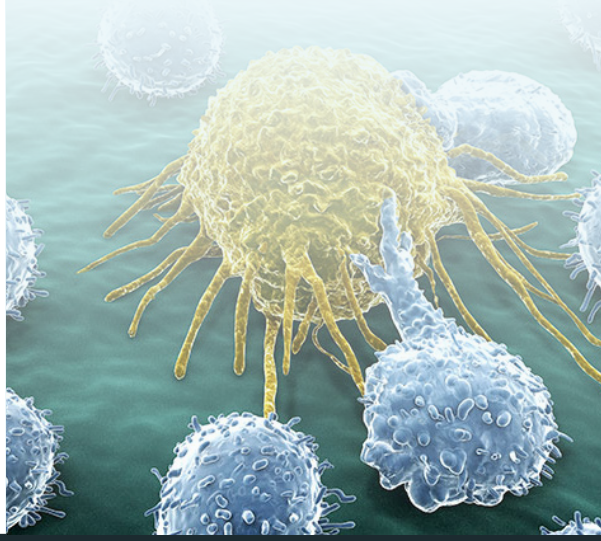
Knocking out the TCR gene to eliminate GvHD also creates issues with expansion following gene editing, as the majority of T-cell therapies are expanded to scale through the same TCR. Therefore, other methods of expansion must be used that can potentially be less effective. Furthermore, as cell populations are expanded to 10^{11} or 10^{12}, the risk of functional exhaustion increases, and cell therapy developers must navigate how to achieve high expansion levels without compromising cellular functionality.

Raw materials other than the donor cells can impact cell therapy manufacturing processes as well. The variability of human serum is an important issue. Access to fit-for-purpose media is another. To ensure that serum and media are consistent from batch, it is necessary to rely on strategic suppliers, which adds supply-chain risk and often cost. The goal is to develop processes that do not require the use of proprietary materials including human serum and specialized media formulations. Media can be produced in-house, sometimes even at reduced costs.

PROFILE: LOOKING BEYOND SIMPLE T CELLS

T cells are powerful agents of the immune system, but there are some challenges and limitations to using conventional -TCR (T-cell receptor) T cells as cellular therapies, particularly when genetically modified. It should be no surprise, then, that researchers are exploring the potential of many different types of T cells and other immune cells for future CAR therapies.

Examples include virus-specific T cells (VSTs), invariant natural killer T (iNKT) cells, diverse natural killer T (NK) cells, iMR1-restricted T and mucosal-associated invariant T (MAIT) cells, TCR T cells and universal TCR cells, among others.[11,12] Engineered T cells with a bispecific T cell engager (BiTE) that down-regulate CD3/TCR have also been shown to produce much fewer cytokines yet still exhibit similar cancer-killing ability compared to CAR-T cells.[13]



6. Addressing Plasmid and Viral Vector Supply Chain Concerns

Gene-modified cell therapies, whether patient- or donor-derived, require access to some form of gene delivery vehicle. Lentiviral vectors are most widely used for the production of CAR T-cell therapies, although other types of viral vectors are being investigated.

The need for viral vectors is an issue in the current environment because demand for plasmid DNA (pDNA), the key starting material for vector production, currently exceeds supply. Despite the addition of pDNA capacity by many CDMOs, wait times for custom pDNA production can be as long as 18 months or more. A similar situation exists for viral-vector manufacturing. Many consider the supply chain for pDNA and viral vectors is the rate limiting step for the production of gene-modified cell therapies today.

Some cell therapy innovators, such as Kite Pharma, are responding by building in-house capability, but that can be challenging due to the lack of people with sufficient skills and experience in these areas. The benefit for allogeneic is that production can be aligned with pDNA and vector supply and the final product banked for use. Vector manufacturing failures, such as the one that affected commercial CAR T providers in Q2 2022, are less likely to impact immediate product delivery.[14]

7. Simplifying Scheduling, Logistics and Chain of Identity

The one-patient-one-dose paradigm of autologous cell therapies requires that patient pre-treatment and apheresis be scheduled in concert with logistics and manufacturing operations.[15] Hospitals and clinics must collaborate with manufacturing plants. Shipment of apheresis material and final products requires cold-chain solutions and must occur in minimal time. Chain of custody (CoC)/ chain of identity (CoI) for patient materials must be maintained so the right patient receives the right product. The vein-to-vein supply chain requires an audit trail for the specific CoI for each unique patient.

Allogeneic cell therapies do not face many of these challenges. Centralized manufacturing is expected as with conventional biologics, with large batches produced, filled into vials and stored for later distribution to multiple patients. Distribution strategies will need to be defined, however. They most likely will involve the use of regional hubs located near cancer treatment centers. Formulation and stability of allogeneic drug products will have a major impact, such as whether investment in cold-chain infrastructure and cryogenic storage facilities will be required.

Although there is no vein-to-vein audit trail required, the CoC of donor cells must still be tracked through manufacturing and distribution to the patients that receive the final cell therapy products. As such, there will still be significant need for collaboration amongst all players to ensure that supply of raw materials, the scheduling of manufacturing runs and the delivery cell therapy products is sufficiently organized and interconnected. The use of MES, process automation and digital technologies for data capture, analysis and sharing will therefore be as important for allogeneic therapies as they are for autologous products.

Center for Breakthrough Medicines

ABOUT THE CENTER FOR BREAKTHROUGH MEDICINES

CBM is a cell and gene therapy contract development and manufacturing organization (CDMO) based in the heart of Philadelphia's Cellicon Valley. CBM offers preclinical through commercial manufacturing capabilities including process development, plasmid DNA, viral vector manufacturing, cell banking, cell processing, and a full suite of complimentary testing and analytical capabilities. Through a single-source, end-to-end solution, CBM accelerates time to market without compromising quality.

Co-locating manufacturing, process development and analytical services prevents delays and handling errors. CBM's aim was to create one campus, one building, one manufacturing site. Our purpose built 700,000 sq. ft. manufacturing center is future-proofed in terms of infrastructure within and around the site. The current facility sits on over 1 million sq. ft. of space, allowing for future expansion to match the growing demand of the cell and gene therapy industry. Internally, the suites have been designed so that complementary services and labs are adjacent or nearby, to ensure we can accelerate time to market without compromising quality.

Scan or click the QR code to learn more about our cell therapy manufacturing

AUTHORS

John Lee,
VP, Head of Cell Therapy Manufacturing

Patricia Lindler,
Associate Director, Cell Therapy Manufacturing

David Aponte Jr.
Associate Director, Cell Therapy Manufacturing

Tatiana Nanda,
Head of Cell & Gene Therapies Drug Product Development,

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