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Centralized vs Decentralized Manufacturing of Personalized Cell Therapies: How to Implement Local Manufacturing of CAR T-Cells

Bryon D. Johnson, PhD

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Among the current immune therapies for cancer, chimeric antigen receptor (CAR)-engineered T-cell therapies have provided some of the most impressive clinical results to date [1-3]. While CAR T-cells have been most effective in patients with B-cell malignancies, there is hope that that these treatments will also soon demonstrate clinical efficacy in patients with solid tumors. Thus far, CAR T-cells have been predominantly manufactured from autologous, patient-derived T-cells, in a process that involves several steps. A centralized manufacturing model has been employed for FDA-approved CAR T-cell products, whereby peripheral blood collected from patients is shipped fresh or frozen to the offsite manufacturing facility, and the manufactured cells are shipped back to the site of origin in a cryopreserved form for administration. While centralized manufacturing reduces the chance of product variability that can occur when multiple manufacturing sites are involved in providing a given CAR T-cell product, this process can take time, and it is not ideal for patients with rapidly growing disease. Local CAR T-cell manufacturing is an alternative approach that can be used to ensure that products get to patients quicker, but there are several challenges to implementation of local manufacturing. This document briefly discusses the key steps and considerations when moving forward with local manufacturing of CAR T-cells.

Steps in the CAR T-Cell Manufacturing Process

Developing a robust manufacturing platform is one of the key steps in implementing local CAR T-cell manufacturing to support clinical trials. In the United States (US), the FDA mandates that these cells be manufactured using current good manufacturing practices (cGMP). Each manufacturing process is typically tested in at least three validation runs to demonstrate feasibility before being brought to the clinic. The main steps in CAR T-cell manufacturing are shown in Figure 1. First, peripheral blood mononuclear cells are collected from the patient as a source of T-cells. Typically, this is accomplished by apheresis due to the relatively high numbers of T-cells that can be obtained. Apheresis collections are usually done in a hospital or blood bank setting, and they require specialized equipment that costs $50,000 (USD) or more per instrument [4]. Each apheresis procedure usually costs $1,500-$3,000 for disposables, and the process needs to be conducted by highly trained staff [4]. Some have suggested that sufficient numbers of T-cells for CAR T-cell manufacturing can be obtained from whole blood, particularly for younger patients [5], but it is still unclear whether sufficient numbers of T cells can be routinely obtained from a whole blood collection.

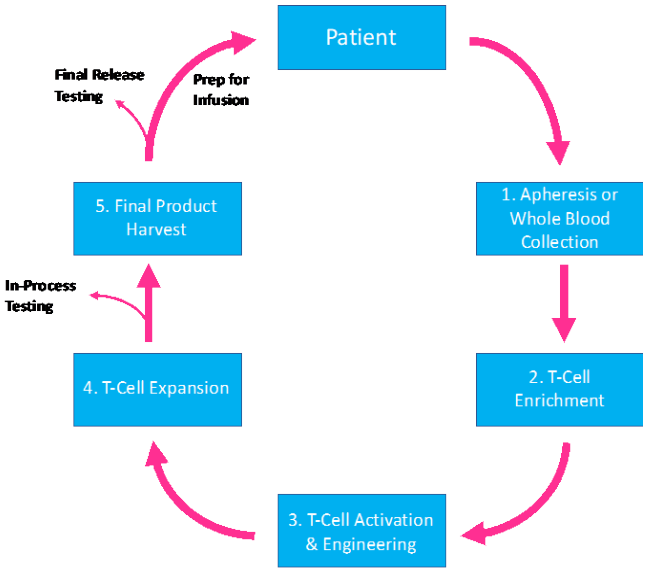

Figure 1. Steps in CAR T-cell manufacturing

As a second step, T-cell enrichment can be done to eliminate contaminating cells, such as monocytes and granulocytes that can inhibit T-cell expansion [6-8] or circulating tumor cells, if present in the patient. Starting the process with enriched T cells can also help standardize the manufacturing process. cGMP-compliant systems such as the CliniMACS Plus and CliniMACS Prodigy (Miltenyi Biotec) have been used to enrich total T-cells (CD4 plus CD8) or various T-cell subsets by immunomagnetic sorting.

Patient T-cells are cultured with agonistic antibodies to CD3 and CD28 antigens to induce cell activation and proliferation. A variety of reagents are available to do this including Dynabeads (Invitrogen), TransAct (Miltenyi Biotec), Expamer (Juno Therapeutics) and ImmunoCult Human CD3/CD28 T Cell Activator (Stemcell Technologies). While Dynabeads need to physically be removed from the cultured cells at some point following activation, TransAct, Expamer and ImmunoCult reagents are either biodegradable or can be removed through washing steps. Shortly following T-cell activation, the T-cells are engineered to express the selected CAR gene(s). The most common methods for inducing CAR expression involve transduction of cells with lentiviral or γ-retroviral gene vectors, or using transposon/transposase technology [9,10]. Messenger RNA has also been used to induce expression of CARs in T-cells but may require multiple cell infusions since expression of the CARs is transient. Each one of these technologies comes with its own manufacturing challenges and the gene constructs require rigorous quality control. Due to the complexities of manufacturing and expensive safety testing required, the gene vector is usually one of the more costly components of the CAR T-cell manufacturing process.

It is becoming clear that cell culture conditions can have a major impact on the 'quality' of CAR T-cell products [11]. One factor is the length of manufacturing. While reducing the time of CAR T-cell culturing has been a recent focus of some researchers as an effort to obtain a 'less differentiated' T-cell product [12,13], conventional CAR T-cell manufacturing times have varied from 7-22 days in order to achieve the desired cell numbers [14]. There are a variety of cGMP-grade cell culture media available today, and while the move has been towards use of serum-free formulations, some CAR T-cell manufacturers still include human serum in their medium. If human serum is used, it is advisable to use a qualified source. Another important factor in the manufacturing process is the cytokine(s) used to drive T-cell expansion. Interleukin-2 (IL-2) or a combination of IL-7 & IL-15 are the most common cytokines used. IL-7/IL-15 are preferred by some labs as these cytokines have been shown to drive expansion of less differentiated T cell subsets [15,16].

There are many cell culture platforms that have been used for expansion of CAR T-cells. They include T-flasks, tissue culture plates, gas permeable bags, G-REX bottle bioreactors (Wilson Wolf Manufacturing), and rocking motion bioreactors such as the Biostat RM (Sartorius), Xuri Cell Expansion System (GE Healthcare Life Sciences) and SmartRocker (Finesse) [17]. Some of these platforms are partially automated, reducing the degree of labor involved in the manufacturing process, but each requires the need for well-trained staff. Fully automated, closed-system platforms are emerging (e.g., CliniMACS Prodigy [Miltenyi Biotec] & Octane Cocoon [Lonza]), which can significantly reduce labor costs, help minimize the risk of microbial contamination, and provide manufacturing consistency. Although these platforms are limited to one product at a time per device, each is well suited for local manufacturing.

A major advantage of local CAR T-cell manufacturing over centralized manufacturing is the ability to deliver fresh CAR T-cells to patients. For this to occur, the manufacturer must be able to ensure there are functional CAR T-cells present in the final products and that the cells are free of microbial

contaminants. Since not all release tests can be done on final CAR T-cell products in a timely manner for fresh infusion, rigorous in-process testing is critical for products that are to be infused fresh. In-process testing may include flow cytometry to demonstrate cell surface CAR expression, sterility assays (gram stain, mycoplasma, endotoxin & sterility cultures) and a functional assay such as intracellular cytokine flow cytometry (of CAR antigen-stimulated T-cells) or cell cytotoxicity (against antigen-(+)ve target cells). In patients that are unable to receive fresh CAR T-cells for medical reasons, the final products can be cryopreserved for later administration. Cryomedia should be carefully evaluated to achieve ideal and reproducible cell viabilities upon thaw. Formulations that are used to freeze hematopoietic progenitor cell products may not be ideal for CAR T-cell cryopreservation. Commercial GMP-grade cryomedia, such as CryoStor (formulations with 2, 5 or 10% DMSO), are available and have resulted in excellent post-thaw CAR T-cell viabilities. Use of controlled-rate freezing can also help achieve consistency in viable post-thaw cell recoveries.

Important Considerations for Implementing Local CAR T-Cell Manufacturing

While developing and validating a CAR T-cell manufacturing process is a key step in implementing local manufacturing, there are many important considerations before CAR T-cells products can ultimately be delivered to patients. The four following areas will be briefly covered:

- Facilities/Equipment/Resources
- Training
- Regulatory Compliance
- Quality Program

Facilities/Equipment/Resources: CAR T-cell manufacturing at academic centers needs to be done in laboratories that adhere to cGMP guidelines, and frequently these are labs that started with processing hematopoietic progenitor cell (HPC) products for transplant programs. Much of the basic equipment used for processing HPCs (biosafety cabinets, cell counters, centrifuges, flow cytometers, liquid nitrogen freezers, -80°C freezers, refrigeration, etc.) will be used for CAR T-cell manufacturing. However, depending on which CAR T-cell manufacturing platform is employed, a more stringent clean room environment may be required. If deemed necessary, this could involve renovations to current cell processing lab facilities or the construction of new space. This can obviously add a significant cost for starting local CAR T-cell manufacturing. Use of a fully closed CAR T-cell manufacturing platform, such as those mentioned earlier, could alleviate the need for expensive renovations or new construction. Depending on how much of the CAR T-cell product release testing is done in-house, other equipment that may be required includes a qPCR system to test for presence of replication competent virus and viral copy number integration (when viral vectors are used). At the Medical College of Wisconsin, we chose to purchase a droplet digital PCR (ddPCR) system, which provides a high degree of sensitivity, does not require generation of standard curves, and provides highly reproducible results between operators. However, the cost of ddPCR equipment is substantially higher than that required for conventional qPCR.

Training: CAR T-cell manufacturing requires highly trained staff. For those lab facilities involved with HPC processing, many of the basic training requirements will already exist (aseptic technique, tissue culture, automated cell counting, cryopreservation & thawing, cell product delivery, cleaning of equipment, etc.). Depending on whether the CAR T-cell manufacturing facility does its own flow cytometry, staff may need flow cytometry training. Training records need to thoroughly documented

and training updated as required. Training records are usually maintained as part of the Lab Quality Program. Finally, SOPs and associated work forms will need to be written to provide staff a step-by-step guide for the manufacturing process and prevent cross-contamination of CAR T-cell products.

Regulatory Support: Ensuring regulatory compliance with cGMP regulations for CAR T-cell manufacturing is not trivial. In the US, CAR T-cell products administered to human subjects must be approved by the FDA. This involves submission of an investigational new drug (IND) application. Details on the manufacturing are included in the chemistry, manufacturing, and controls (CMC) section of the IND application. Additional information provided in the CMC includes a description of the manufacturing facilities, a list of all components used in the manufacturing process, methods to determine identity, strength, quality and purity of the cell product, stability assessment, and product labeling information. For reagents that are not GMP-grade, certificates of analysis (COAs) need to be provided in the IND application. All assays used in the release of CAR T-cells for patient administration must be fully validated by qualified staff, and a COA should be provided with each final cell product to indicate that it has met all release criteria. The IND submission process can be laborious, so involving individuals familiar with the process can save a tremendous amount of time.

Quality Program: A robust Lab Quality Program should be in place when initiating local CAR T-cell manufacturing to ensure consistent manufacturing. The quality team should review all aspects of the CAR T-cell manufacturing process, ensure that training of the manufacturing staff is complete and current, approve all associated SOPs and work forms, and ensure that labeling of final CAR T-cell products is accurate. All CAR T-cell lot and infusion COAs should also get a final review by quality staff before the chart for each subject is finalized. The Quality Program team can also help identify alternative sources for critical materials should shortages occur.

Conclusions

As we move forward in these exciting times for immune cell therapies, it is likely that CAR T-cell manufacturing will continue to involve a mix of centralized and local manufacturing. Centralized manufacturing is well suited for larger scale production of FDA-approved CAR T-cell products to ensure product consistency. On the other hand, early testing of novel CAR T-cell products is feasible with local manufacturing, and local manufacturing can allow clinicians to get CAR T-cells into their patients more quickly, which is important for treatment of those with rapidly progressive disease. Implementing local CAR T-cell manufacture does have its challenges, but the advent of new fully automated manufacturing platforms helps to alleviate some of the challenges.

In a recent development, the Medicines and Healthcare products Regulatory Agency (MHRA) in the UK published a document online (posted August 26, 2021) that seeks consultation from industry, the medical community, patients and the public on a new proposed regulatory structure for point-of-care manufactured medicines (https://www.gov.uk/government/consultations/point-of-care-consultation/consultation-on-point-of-care-manufacturing). The goal of this guidance document is to formulate a modified regulatory structure to support increased point-of-care product manufacturing since such drugs, including cell therapeutics, often do not necessarily fit the “standard model” of regulation where manufacture is local and administration to the patient is immediate. Assuming MHRA is able to generate a final document that incorporates the proposed modified regulatory structure, this could have a positive impact on locally generated CAR T-cell products by more quickly facilitating their approval for use.

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