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Overcoming Challenges in Process Development of Cellular Therapies

Steven L. Highfill[1] · David F. Stroncek[1]

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Abstract

Purpose of the Review Cellular therapy using chimeric antigen receptor (CAR) T cells as a treatment option for patients with lymphoma and leukemia has proven to be remarkably efficacious. This success has sparked the development of new cellular therapy products for numerous indications. Similar to pharmaceutical products, challenges exist at nearly every stage of process development; however, the unique nature of a cellular therapy product can present exceptional challenges that are just beginning to emerge. The purpose of this review is to explore some of the most common challenges experienced during the early phases of development of CAR T cell products and to provide suggestions for navigating these challenges.

Recent Findings Recent articles focused on CAR T cells are highlighted with special attention on aspects that relate to CAR T cell process development and clinical manufacturing. We examine the various stages of process development for CAR T cells and outline some of the obstacles that must be overcome in order to move from pre-clinical development into clinical manufacturing.

Summary As the field of CAR T cell therapy continues to grow, it is important to quickly move new CAR T cell products into and through early phase clinical trials and to ensure that the result of these trials can be adequately compared. Having laboratory and clinical investigators and GMP manufacturing facilities aligned on the numerous aspects of new product development will facilitate this process.

Keywords CAR T cells · GMP manufacturing · Cellular therapy · Process development

Introduction

T cells genetically engineered to express chimeric antigen receptors (CAR) have become important therapies for B cell malignancies. Typically, CAR T cells are manufactured from cells collected from the patient to be treated (autologous therapy). CAR T cells are manufactured over 7 to 11 days and the process involves several distinct steps or unit operations; T cell enrichment, stimulation, transduction, and expansion. Manufacturing CAR T cells is relatively simple, but consistently producing high quality CAR T cells is difficult. The methods used to manufacture CAR T cells are reviewed with a special emphasis on the most challenging aspects.

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✉ Steven L. Highfill
steven.highfill@nih.gov

David F. Stroncek
dstroncek@cc.nih.gov

[1] Center for Cellular Engineering, Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD, USA

T cell Collection and Enrichment

Cell Source

The first step in manufacturing CAR T cells is the collection of T cells. Currently, most CAR T cells are produced from autologous T cells. In many respects, it would be easier and less expensive to manufacture CAR T cells from healthy subjects (allogeneic therapy) in lots or batches that were large enough to treat scores of patients, similar to the production and use of mesenchymal stromal cells [1]. However, if there is an HLA mismatch between patient and donor, the CAR T cells could cause graft-versus-host disease (GVDH) or they could be rejected in host-versus-graft due to the development an immune response directed toward the CAR T cells. Consequently, most CAR T cells are currently produced from autologous cells. However, several groups are engineering CAR T cells in order to produce "universal" CAR T cells from healthy donors that could be used for all patients.

Most efforts involve the use of gene editing technology to knock out the T cell receptor (TCR) or to target insertion into the TCR to stop GVHD [2–5]. Some protocols involve knocking out both the TCR to prevent GVHD and beta$_2$-microglobulin to prevent the expression of HLA class I molecules and, therefore, prevent immune rejection [3].

The production of universal donor or "off-the-shelf" CAR T cells has several advantages over autologous CAR T cells. The production of large lots of universal donor CAR T cells would allow the costs associated with manufacturing, testing, and quality review to be spread over many CAR T cell doses which would reduce the per patient cost. In addition, there would be little or no delay in providing CAR T cell therapy. The CAR T cells could be given immediately or within a few days of identifying a patient in need of the therapy. Allogeneic universal CAR T cells are being tested in early phase clinical trials [6•, 7].

Cell Collection

Autologous T cells used to manufacture CAR T cells are obtained from the peripheral blood. Relatively few (hundreds of millions) of T cells are needed to manufacture CAR T cells. While several hundred mL of blood from most healthy subjects contain a sufficient number of T cells for CAR T cell manufacturing, the concentration of T cells in the blood of patients needing these therapies is highly variable and is often low due to their underlying disease and prior therapies [8]. In order to obtain a sufficient quantity of cells for all patients, T cells are collected by apheresis using a blood cell separator. Blood cell separators isolate cells based on their density and the T cell rich peripheral blood mononuclear cell (PBMC) concentrate collected by apheresis is also rich in B cells and monocytes. The apheresis product can contain "contaminating" cell populations, including, but not limited to lymphoblasts (in patient samples), NK, red blood cells (RBCs), platelets, and granulocytes [9]. These cells can act to hinder downstream processes such as activation and/or transduction through mechanisms described below.

In order to ensure sufficient quantities of T cells are collected, it is helpful to measure the concentration of T cells in the patient's blood shortly before the apheresis collection procedure. Since the rate of collection of T cells is dependent on the concentration of T cells, the duration of the apheresis procedure can be adjusted based on the T cell count to ensure enough cells are collected [8].

When developing new CAR T cell manufacturing methods, it is important to test the method using PBMC concentrates with a wide variety of cell types and cell concentrations. It is relatively easy to obtain PBMC concentrates collected by apheresis from healthy subjects and most manufacturing protocols are developed using healthy donor cells. However, the composition of cells collected from patients may vary considerably for those collected from healthy subjects and manufacturing methods developed using only healthy donor cells may not be as effective with patient cells. Therefore, if possible, patient cells collected by apheresis should be incorporated into the product development process.

T cell Enrichment

Since the PBMC concentrates collected by apheresis contain many types of cells in addition to T cells [10], T cell enrichment is necessary before manufacturing process begins because the contaminating cells (including the leukemic blast cells) may reduce vector number copy in the T cells or interfere with the cell's expansion. Several methods have been used to enrich the mononuclear cell concentrate for T cells; including density gradient separation [11], counter-flow elutriation [12], and antibody selection [13–15]. All of these methods can be performed using automated instruments and closed systems, but the cost and effectiveness of the methods varies.

Density gradient separation removes granulocytes and RBC from mononuclear cells collected by apheresis, but monocytes, B cells and NK cells will not be separated from T cells. Counter-flow elutriation separates monocytes and granulocytes from T cells, but the T cell fraction will also contain B cells and RBCs [12].

Some CAR T cell manufacturing methods stimulate T cell expansion using anti-CD3 and anti-CD28 conjugated to beads, which function as artificial antigen presenting cells. These anti-CD3/CD28 beads (Dynabeads, Thermo Fisher) can be used to isolate T cells by incubating the mononuclear cell concentrate with the anti-CD3/CD28 beads for 2–4 h and then using a magnet to remove the beads and the bound CD3+ cells [13]. While this method is effective, the CD3+ cell recoveries can be relatively low, due in part to cell loss from many subsequent large volume washes. In addition, if the mononuclear cell concentrate contains large quantities of monocytes, these monocytes can phagocytosis the beads making the beads unavailable to the stimulate T cell expansion [16].

One, very effective method for T cell isolation involves selection with a combination of anti-CD4 microbeads and anti-CD8 microbeads. Closed systems are available that have been specifically designed for the clinical scale isolation of T cells using anti-CD4/CD8 microbeads and yields T cells with few other contaminating cells [14•]. Anti-CD4/CD8 microbead systems are able to isolate T cell populations of relatively high purity even when the PBMC concentrate collected by apheresis contains a very small proportion of T cells. While anti-CD4/CD8 microbead antibody selection yields a much more efficient separation, the cost is much greater.

The importance of having a purified population of T cells as a starting material for CAR T ell manufacturing has recently taken center stage. In 2018, it was reported that a 20-year-old patient with ALL had relapsed with CD19 CAR transduced B cell leukemia that originated from a single leukemic cell

during manufacturing [17••]. In other studies, investigators have gone to such lengths as to not only purifying T cells, but also setting a defined composition of T cells for treatment. It was shown that a CAR T cell product set at a 1:1 ratio of CD4 and CD8 T cells led to a 93% rate bone marrow remission from 29 B-ALL patients [18]. While many methods are being used for the production of CAR T cells, the optimal separation method used is dependent on the population of patients that is to be treated. All methods are effective for patients with relatively normal white blood cell and differential counts. However, for patients whose blood contains a large proportion of white blood cells that are blasts or NK cells, manufacturing results may be more consistent if T cells are isolated using anti-CD4/CD8 selection.

Cell Stimulation

Antibodies and Antigen Presenting Cells

Polyclonal (antigen independent) T cell stimulation is used extensively to facilitate T cell expansion to reach appropriate numbers to treat patients. Careful considerations must be paid to the type of stimulation used, as this can have a dramatic impact on the phenotype and function of the expanded cells. Perhaps the simplest and most common method of stimulating human T cells is to use soluble anti-CD3 antibody (OKT3 clone), which is a high affinity antibody for CD3 antigen. Our group has shown that stimulation of PBMC's using OKT3 at 50 ng/mL plus IL-2 at 300 IU/mL results in moderate expansion of T cells with a mean fold expansion of 4.2 ± 2.0 after 7 days of culture [9]. These cells were highly functional after infusion and were capable of inducing clinical responses in patients with multiple myeloma (MM) when expressing a chimeric antigen receptor (CAR) specific for B cell maturation antigen (BCMA) [19, 20].

TCR stimulation using OKT3 (signal 1) in the absence of co-stimulation (signal 2) results in premature T cell apoptosis or anergy [21]. When using PBMC's, the signal 2 requirement is achieved by the antigen presenting cells (APC) within the PBMC population, such as the monocytes, macrophages, and dendritic cells. Although the use of PBMC's as starting material for CAR T cell manufacturing will be adequate for most patient samples, there are instances where the disease burden can be high and there is an increased frequency of B cell blasts or monocytes within the buffy coat. These cells can act to suppress T cell activation through the secretion of anti-inflammatory cytokines or the expression of negative regulatory molecules such as PD1 [22, 23]. As stated in the previous section, under these circumstances, it is recommended that the T cells are purified prior to stimulation. When using a purified population of T cells; however, an alternative method must be utilized to achieve co-stimulation in order to retain healthy expanded T cells. Another common method employed is the use of magnetic beads coated with anti-CD3/anti-CD28 antibodies (Dynabeads, Thermo Fisher) plus IL-2. If using this approach, methods must be developed to ensure T cells are 'bead-free' at the culture harvest and prior to infusion in to the patient. CAR T cells manufactured for clinical protocols at the NIH using this approach have T cell fold expansions that are, in general, much higher than for OKT3 plus IL-2 (approximately 20–30 fold expansion for CD22 CAR T cells; unpublished results). This higher fold expansion may come at a price, and it has been reported bead stimulated cells not only bias cell expansion toward CD4 T cells, but also result in a CD8 T cell population that has a phenotype more characteristic of terminally differentiated cells, while OKT3 stimulated cells have a higher frequency of cells that appear to have a less differentiated phenotype (CCR7+, CD45RA+, CD27+, CD28+) [24]. Studies using TIL have shown that adoptive therapy using cells with a more naïve phenotype results in enhanced in vivo persistence and a more robust anti-tumor response [25, 26]. Other published reports directly link the persistence of disialoganglioside (GD2) CAR T cells in patients with neuroblastoma to the number of CD4 T cells infused and the number of central memory cells (CD45RO+, CD62L+) in the infused product [27]. Despite these results, CD19 and CD22 CAR T cells manufactured for clinical protocols at the NIH using anti-CD3/anti-CD28 beads have resulted in dramatic clinical responses, and it remains to be seen whether or not differences will be observed in long-term outcomes between protocols using coated beads versus OKT3 [28, 29]. Another reagent that is becoming more common is TransAct (Miltenyi), which is a soluble, colloidal reagent covalently attached to anti-CD3/anti-CD28 antibodies. TransAct is easily removed from the culture with a simple wash which eliminates the need for bead removal, saving time and enhancing cell recovery. A comparison of traditional methods of activation and culture was recently performed using Dynabead stimulation plus X-Vivo10 medium (Lonza) vs. TransAct stimulation plus TexMACS medium (Miltenyi). The results showed that expansion of Dynabead/X-Vivo10 activated cells was significantly greater, but transduction efficiency was significantly higher for TransAct activated cells, making the overall number of transduced cells approximately equal [30].

With the burgeoning growth in the cell therapy field, especially for T cells, we are beginning to see a rapid development of GMP-grade reagents available to support early and late phase clinical trials. There are now a number of different types of stimulatory reagents (examples listed in Table 1), most of which hinge on CD3/CD28 stimulation, and more research is

Table 1 Examples of GMP-grade stimulatory reagents

Trade name	Manufacturer	Type
Anti-CD3 OKT3 antibody	Miltenyi Biotech, Takara, others	Murine IgG2a antibody recognizing human CD3
Humanized anti-CD3 OKT3	Sino Biological	Humanized antibody IgG4a recognizing human CD3
Soluble anti-CD28	Miltenyi Biotech, Sino Biological	Murine IgG1/2 antibody against human CD28
CTS™ (cell therapy systems) Dynabeads™ CD3/CD28	Thermo Fisher	Anti-CD3 and anti-CD28 antibodies coupled to a superparamagnetic polymer particle
MACS® GMP T cell TransAct™	Miltenyi Biotech	Colloidal polymeric nanomatrix covalently attached to humanized recombinant agonists against human CD3 and CD28
Cloudz™ T cell activation	R&D Systems	CD3/CD28 nonmagnetic, dissolvable microparticles
ImmunoCult™ Human CD3/CD28 T cell activator (GMP version expected 2020)	StemCell Technologies	Soluble tetrameric antibody complexes that results in cross linking of CD3 and CD28

needed to determine the nuances between them and the downstream effects on the final product cells.

Cytokines

Determining the optimal cytokine(s) for support of T cell growth is yet another critical factor to consider for CAR T cell manufacturing. As is with stimulation, there are a number cytokine cocktails available that can result in significant changes in cell differentiation status and function at the end of the culture [31]. In most ongoing clinical trials, IL-2 (PROLEUKIN® (aldeskleukin), is the principal growth factor supplemented into the medium to support CAR T cell expansion. However, in an effort to tackle the ongoing problem of limited in vivo persistence, the use of other yc chain cytokines such as IL-7, IL-15, and/or IL-21 to better preserve the more naïve cell to be used for treatment are being investigated. The use of IL-7 plus IL-15 in the setting of a second generation CD19 CAR T cell resulted in an increased frequency of $CD8^+CD45RA^+CCR7^+$ T-memory stem cells (Tscm) that had a significantly increased proliferative capacity, persistence and effector function in xenograft tumor models when compared to cells grown in IL-2 [32]. Similarly, using a GD2-specific CAR, Gargett et al. has shown IL-7 and IL-15 in combination with CD3/CD28 stimulation results in optimal expansion and effector function of "stem-like" T cells against GD2-expressing neuroblastoma and melanoma cell lines [33]. A phase I/II clinical trial is currently underway at the NIH in which streptamer-selected (IBA Lifesciences) naïve T cells are manufactured using CD3/CD28 bead stimulation plus IL-7 and IL-21 with the addition of the glycogen synthase kinase beta 3 (GSK3b) inhibitor TWS119 to more strongly promote the generation of cells with a Tscm phenotype. Here, the authors have shown this method to be capable of generating CD19 CAR^+ cells that had a very high frequency (52.2% ± 12.52%) of cells with a Tscm phenotype compared to cells grown under traditional conditions using IL-2 (median of 1.36%) [34]. Further, these cells displayed a much higher degree of functionality in vitro and in vivo when compared to traditional 19 CAR T cells. From a manufacturing perspective, one has to also consider the total number of cells required to reach the targeted dose level. If employing anything other than IL-2, it may be necessary to initiate the cell cultures with a larger number of cells given that cell expansion will likely be much more limited.

Gene Transfer

Viral Vectors

To obtain CAR T cells, one has to introduce the correct genetic material to the cell in such a way that the cell can process and express the CAR on the cell surface. There are a number of methods that can be used to achieve this goal; we will highlight a few of the most commonly used methods here.

Viral transduction is the most common method to introduce genetic material into the cells. Both y-retro and lentiviruses attach to the host cell in a receptor independent manner and retrotranscribe their RNA genome, which then becomes integrated into the host cell genome. The replication cycle of y-retroviruses differs from that of lentiviruses in that the former is not capable of passing through the intact nucleus and requires that the cell is undergoing division (mitosis) to facilitate integration [35]. Lentiviruses, however, can translocate across the nuclear pore of an intact nuclear envelope and are therefore capable of integration in both dividing and non-dividing cells [36]. This is an important distinguishing characteristic, because the timing at which optimal transduction occurs after T cell stimulation is more critical for y-retro than for lentiviruses.

Safety of viral vectors has been a top concern since it was discovered that patients treated for X-linked severe combined immunodeficiency (SCID-X1) with a viral vector transduction of bone marrow derived stem cells had developed T cell leukemia. In this unfortunate event, retroviral vector insertion into the LMO2 proto-oncogene promoter of CD34 bone marrow cells lead to increased transcription and expression of the LMO2 gene, causing premalignant cell proliferation [37]. In a series of subsequent experiments to determine genotoxic potential of viral vectors, it was discovered that lentiviral vectors appeared to have lower genotoxicity than y-retroviral vectors

[38–40]. Compared to y-retroviral vectors, lentiviral vectors do not favor integration into regulatory elements or transcriptional start sites and are associated with a lower frequency of tumor formation in vivo [40, 41]. It is important to note that there has never been an observed oncogenic event when T cells are transduced with either y-retro or lentiviral vectors, and it has been shown that T cells are much more resistant to transformation by y-retro virus than stem cells [42].

There are several things to consider when developing a clinical protocol which utilizes viral transduction to generate CAR T cells. First, most of the manufacturing for clinical grade cells is performed in closed system, gas permeable cell culture bags. These bags come in various sizes and offer needle free valves for sterile access to reduce the possibility of contamination. For some CAR T cell protocols at the NIH, cells are activated in Origen PermaLife bags, generally PL120-PL240 in size. After 2 days in culture, T cells are transduced with y-retro or lentiviral vector and expanded for an additional 7–10 days. The transduction process takes place in smaller PL30 sized bags with using 15×10^6 cells/bag. For each protocol tested, it is customary to perform several iterations of the transduction process to ensure optimal expression of the CAR in the target cells. In addition to testing the timing of transduction and whether or not one vs. two separate events are required, we also determine the impact of coating bags with RetroNectin or other transduction enhancers and bag spinoculation (spinfection). RetroNectin (Takara Bio) is a human recombinant fragment of fibronectin that contains a domain that binds to the surface of the target cell via VLA-4/5 and another that binds to the virus using heparin-binding domains and acts to co-localize viral particles and cells. CAR T cells manufactured at large scale and transduced under conditions employing RetroNectin have been shown to have high transduction efficiencies [13]. Other transduction enhancers, such as Vectofusion (Miltenyi) and Polybrene may likewise benefit gene expression and are also currently being used [43, 44].

Spinoculation is a process by which target T cells and viral particles are centrifuged together usually at 1200×*g* for 2 h at 25 °C. This technique, used commonly in preclinical laboratories performing viral transductions, has been shown to significantly enhance transduction efficiencies through a process that results in sedimentation of viral particles on the target cells and thereby increasing viral binding by as much as 40 fold [45]. In preclinical laboratories, this process is performed in 6-well plates, but in effort to make this process applicable to clinical manufacturing, we have developed a method where this can be accomplished using closed system cell culture bags as well (unpublished observations). It is interesting to note that we have found that the efficacy of transduction is not always improved by these methods, even when using viral vectors containing the same backbones, and each product should be tested independently to determine if there is benefit.

Alternatives to Viral Vectors

With the interest now growing to develop CAR T cells for solid tumors, and shared antigen expression on critical healthy tissue being a major concern, safer alternatives to viral integration are being examined more rigorously. Several clinical trials have been conducted or are currently ongoing using mRNA electroporation into T cells, which result in transient expression of the gene of interest on the surface of the T cells (for detailed review see [46]). In vitro transcribed mRNA does not need to enter the nucleus to be translated into protein and, unlike viral vectors, they do not integrate into the genome and therefore do not pose a risk for insertional mutagenesis. There are considerable constraints to using mRNA-derived CAR T cells, however, such as the limited persistence of CAR expression on the cell surface, which only last days, and the apparent inability to eradicate tumor when given systemically [47]. This results in the requirement for each patient to have numerous treatments in order to have hope of achieving a durable response, which significantly increases the overall cost and labor of this type of approach. Ongoing studies utilizing new techniques to engineer and purify mRNA offer some solace for this methodology and have resulted in increased CAR expression on the surface and increased cytolytic activity [48].

There are important manufacturing challenges that need to be considered when developing this type of approach as well. First and foremost, there are only a small number of electroporation devices that offer large-scale, closed system GMP-compliant manufacturing solutions, and their availability for purchase at this time is very limited due to the rapid growth of the field. Once the device is received, it is sometimes critical that the user develops a strong relationship with the company's technical representative that the device was purchased from. The most widely used instruments are user friendly but there are a limited number of setting and adjustments. The downside of this simplicity of use is that the development of a new protocol can be difficult because trying to optimize parameters can be very restricted, due to the limited flexibility. The specific parameters of the process, the internal components of the instrument, and the material and reagents that are required for use are all proprietary. When developing a process for difficult-to-transfect cells, frequent interactions and cooperation of the instruments technical representative is required.

Cell Expansion

Culture in Bags and Specialized Flasks

CAR T cell manufacturing for patients at most academic institutions are currently utilizing gas-permeable cell culture bags that provide a sterile, closed system to support T cell

growth throughout the entire process. These bags are generally made of polyethylene, polyvinyl chloride, fluorinated ethylene propylene, or polyolefin and are offered in a wide variety of sizes and shapes, making their use highly flexible and adaptable to virtually any process. Processes developed using bags can be labor intensive and require staff that has a higher skill set, as fluid handling is performed manually through a variety of different types of ports and configurations. Other alternatives to bags include G-Rex cell culture devices (Wilson Wolf), which contain a rigid plastic outer shell and a silicone bottom membrane that offers the highest oxygen and carbon dioxide permeability coefficient [49]. We and others have shown that these devices are capable of supporting a large number of T cells ($\sim 20 \times 10^9$ per G-Rex500M flask) while additionally benefitting from its easy adaptation to automated harvests using GatheRex pumps, making this entire culture a closed process [50–52].

Bioreactors

There are a number of closed and semi-closed system bioreactors currently on the market that can be utilized for phase I/II trials and potentially beyond. Two of the most commonly encountered instruments used for CAR T cell production are the Xuri Cell Expansion System (GE Healthcare) and the Prodigy (Miltenyi) devices. The Xuri Cell Expansion System is a based on the more widely known WAVE bioreactor instrument and is a functionally closed, rocking instrument that can accommodate up to 25 L of product at a time. This bioreactor features a perfusion system that facilitates the removal of waste products and supplementation of glucose and glutamine and is capable of generating cells at a high density ($\sim 20 \times 10^6$cells/mL). Further, this offers a small instrument footprint that maximizes the use of limited manufacturing space. When manufactured on this instrument, TIL and TCR modified cells were comparable in terms of total cell number, overall fold expansion, and viability, but interestingly, the WAVE favored CD4 expansion over CD8 cells and differences in activation status were observed depending on whether the cells were TIL (lower activation status) or TCR modified cells (higher activation status) [53]. Manufacturing clinical grade CD19 CAR T cells on such a device has shown it to be capable of generating an expansion of 668-fold, yielding up to $\sim 2 \times 10^{10}$ total T cells over a period of 13 to 18 days [54]. Moreover, CD19 CAR T cells manufactured on the WAVE were shown to have an acceptable safety profile [55] and high functional activity in that they were able to induce minimal residual disease negative complete responses patients with relapsed and/or chemotherapy refractory B-ALL [56].

The Prodigy (Miltenyi) is an all-in-one functionally closed processing device. Not only is this instrument capable of supporting cell expansion, but it also has the benefit being able to perform automated cell selections, viral transductions, media exchanges/feeds and cell harvests. The Prodigy limits the need for user involvement in a number of steps, thereby reducing technician resource utilization and limiting the risk of cell contamination. This instrument has very flexible T cell Transduction (TCT) software suite that allows the user to build and define the timing and volume of processes within an "activity matrix." Initial publications using this instrument to manufacture CD19 CAR T cells have shown it to be capable of generating approximately 2×10^9 total lymphocytes with a transduction efficiency as high as 66% [30]. Since then, others have adapted the instrument for manufacturing CD20 CAR T cells using IL-7/IL-15 and even CD20/CD19 Bispecific CAR T cells, and modification of the procedure have increased the total cell numbers in the final harvest to approximately 4×10^9 after 13 days of culture [14, 15]. Interestingly, the majority of the cells express cell surface markers indicative of memory T cells rather than T effector cells, which is what is usually observed for T cells grown under a traditional approach using Dynabeads [31]. Others have shown that the greater the terminal differentiation of the T cells, the more likely it is to have an impaired anti-tumor efficacy [57]. One recent study has carefully examined the exhaustion phenotype of cells grown on the Prodigy under conditions of a much shorter 8-day culture process and has shown very low expression of common exhaustion markers found on T cells such as LAG-3 and PD-1 [58]. In 2018, we received approval of a CD19/CD22 bispecific CAR T cell protocol for treatment of pediatric patients with ALL using cells manufactured on this instrument (NCT03448393). Since then, we have averaged $2 \times 10^9 \pm 274 \times 10^6$ total T cells with transduction efficiencies of 78% ± 3% from only 7 days of culture and have witnessed favorable clinical outcomes (unpublished observations).

Drawbacks to this system include the inability to test multiple parameters at once for process development purposes, which translates into a significant amount of time that is required to develop complex processes. This is especially true for most academic development laboratories that only have access to one device. Also, an important consideration is cost, as the cost of reagents for a single manufacturing run can exceed $15 K if a CD4/CD8 selection is performed. On the positive side, the instrument is easily operated by a single user and having the centrifugation, selection process and incubator built-in significantly minimizes the GMP manufacturing space needed, which, in most cases, would offset the cost of the reagents.

Conclusions

Cellular immunotherapy using genetically modified CAR T cells has proven to be extremely successful for hematologic malignancies, and major efforts are currently underway to translate this success to patients with solid tumors. The rapid

growth in this field has spurred an equal amount of growth of regulatory oversight for these types of clinical trials. Clinical manufacturing methods that previously involved open culture plates, manual pipetting, and research grade reagents (such as y-retro or lentivirus) have almost completely evolved into closed system, automated, GMP-grade reagents. During this evolution, many new companies have begun to support clinical trials by supplying these types of systems and reagents. Development of processes that were once relatively simple and similar to preclinical production are now quite complex and can involve testing of material from numerous sources which adds cost and time. As the field continues to advance, solving the problem of complex CAR T cell manufacturing will be critical to the success of this type of therapy beyond phase III trials and result in patient access to more potent, better characterized cells at a cheaper cost.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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