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Considerations in T Cell Therapy Product Development for B cell leukemia and lymphoma immunotherapy

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Abstract

Based on laboratory and clinical research findings and investments in immunotherapy by many institutions in academia, government funded laboratories, and industry, there is tremendous and deserved excitement in the field of cell and gene therapy. In particular, understanding of immune mediated control of cancer has created opportunities to develop new forms of therapies based on engineered T cells. Unlike conventional drugs or biologics, the source material for these new therapies is collected from the patient or donor. The next step is commonly either enrichment to deplete unwanted cells, or methods to positively select T cells prior to polyclonal expansion or antigen specific expansion. As the first generation of engineered T cell therapies have demonstrated proof of concept, the next stages of development will require the integration of automated technologies to enable more consistent manufacturing and the ability to produce therapies for more patients.

Keywords

cellular therapy; immunotherapy; gene therapy; T-lymphocytes; leukemia; lymphoma

Introduction

Engineered T cell therapies are entering a new stage of therapeutic development. From the early beginnings in the 1980's and early 1990's, the adoptive transfer of viral specific T cells was investigated in clinical trials (1–4). These manufacturing strategies, while novel, were laborious and time consuming, often taking as long as 6 months to manufacture each cell product (2). These cell products also involved the use of antigen presenting cells such as fibroblasts which had to be infected by live CMV, and then used to present antigen to the T cells. Nearly twenty-five years later, cellular therapy has emerged as one of the most

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promising treatments of cancer and other disorders. Manufacturing strategies and technologies have also advanced. Not only can virus-specific T cells be manufactured in 10 days and targeting 6 viruses, but T cells can now be manufactured from cord blood (5–7), from naïve T cells (8) to target leukemia (9, 10) and other cancers as well – and with apparent clinical benefit (9, 11). The description of chimeric antigen receptors to redirect T cell specificity (12, 13), led to early clinical trials in HIV and cancer that demonstrated the feasibility of this approach, but showed limited or no demonstration of clinical activity (14–16). These early trials relied on previous generations of manufacturing processes and adaptation of legacy equipment. Promising clinical trials and easily modifiable platforms have sparked an enormous interest and investment in the field (17–19) resulting in a massive increase in the number of clinical trials of engineered T cells around the world. Logistic and regulatory challenges accompany the move into later phase multi-center and global clinical trials (20). Simultaneously, a deepening understanding of T cell biology and enhanced methods of genetic manipulation have brought within sight the vision of engineered T cells as therapeutic autonomous vehicles. The manufacturing process thus continues to evolve incorporating new science and new technologies (Figure 1). The next generation cell therapy manufacturing will enjoy the benefits of instruments designed with cell therapy applications in mind- offering improved standardization, automation and closed systems for each unit of processing in the manufacturing cycle. These advances are critical to providing wider access to patients as cell therapies move forward on the path to commercialization.

Collection

Apheresis mononuclear cell (MNC) collection is a common source of cellular material for cellular immunotherapy manufacturing. During MNC collection, the patient's whole blood enters the apheresis instrument, is anticoagulated and then is separated by centrifugal force. The MNC layer, which includes T lymphocytes as well as B lymphocytes and monocytes, is identified and removed as the remaining material is simultaneously returned to the patient in real time. As extracorporeal blood volumes are small, large blood volumes may be processed and billions of leukocytes may be obtained. Several instruments are available for this purpose including the COBE Spectra and Spectra Optia (TerumoBCT) as well as the Amicus (Fenwal). While each instrument offers a unique set of advantages and disadvantages, comparison of the COBE Spectra and Spectra Optia illustrates the general direction in which the field of cell therapy manufacturing is moving. Compared to the COBE Spectra, the Spectra Optia incorporates a system to automatically detect the cell interface and adjust flow rates accordingly. The Spectra Optia also includes a feature to allow collection of a subset of the buffy coat layer. Both features highlight the goal of cell therapy equipment manufacturers to provide automated instruments that produce high purity MNC products.

Often, MNC product total cell yield and purity are competing interests. The MNC layer itself contains multiple cell types, some of which may not be desirable for a given application. Further, non-MNC contaminants can also inhibit downstream manufacturing. Collecting adequate lymphocyte yields and purities for cell manufacturing from patients with B cell malignancies can be challenging. These patients frequently have low circulating absolute lymphocyte counts. To improve yield, more total blood volume can be processed as the patient tolerates. However, if the MNC layer is small at the time of collection due to low

absolute lymphocyte count (ALC), simply increasing the volume processed may increase total cell yield, but produce a low purity product. Without compensatory adjustments, fluctuations in blood flow through the instrument change the location of the target cell layer and alter the contents collected. When dealing with a narrow MNC layer, this is particularly noticeable. Non-MNC cell types (red blood cells, granulocytes) are frequently collected during MNC collection from patients with low ALC counts due to difficulty obtaining or maintaining the proper interface during collection. Granulocytic cells may inhibit ex vivo T cell expansion by a number of mechanisms(21). Within the MNC layer itself, non-target lymphocytes (eg. B cells, circulating lymphoid tumor cells) may add to product impurity. Residual contaminant tumor cells inhibit T cell activation and expansion, and monocytes interfere with activation (22). Further complicating matters, required cell yield and purity depends on downstream manufacturing capabilities. For example, a protocol in which ex vivo expansion is robust may prioritize purity over yield, whereas a protocol that includes extensive purification may value yield over purity. Given this complexity, to ensure the best change of manufacturing success, a collection approach should be tailored to each protocol's needs and clearly communicated to the collection site.

While MNC collection is the primary source of starting material for cell therapies, it is not the sole source. Cell therapies have been generated from tumor infiltrating lymphocytes obtained by surgical resection, from mobilized hematopoietic stem cell products, bone marrow, cord blood, and even whole blood (23, 24). Each of these sources offers a unique cost/benefit profile with respect to yield and purity. It is important to note that many current challenges in MNC collection of B cell malignancy patients also limit use of alternative sources of cellular material. In addition, it has now been demonstrated that cytotoxic chemotherapy commonly used in B cell malignancies can impair the ability of benign T cells to expand ex vivo (25). This suggests treating a patient with conventional lymphotoxic therapies may limit the ability to successfully manufacture a cell therapy from that patient if cells are collected before T lymphocyte counts and function are reconstituted. Nevertheless, whole blood is useful in instances where an allogeneic donor is used, such as after hematopoietic stem cell transplantation, where tumor-associated antigen tumor antigen associated (TAA)-specific T cells in the case of leukemia,(9, 10) or Epstein-Barr Virus (EBV)-specific T cells in the case of EBV+ lymphoma,(26–28) can be expanded from the donor and transferred to the recipient. As more is learned about the long-term consequences of these treatments on benign immune cells, collection timing can be optimized and the likelihood of manufacturing success improved.

Enrichment

Post collection T cell enrichment can be achieved through several selection or depletion methods. Depending on the target cell population and the relative frequency of contaminant cell types, enrichment may be performed through density-based, size- and density-based, or immunophenotype-based techniques. Density gradients, such as Ficoll can further deplete granulocytes and red blood cells from lymphocytes and monocytes. While Ficoll has traditionally been performed in centrifuge tubes, an open and laborious method, a large scale, closed system Ficoll protocol has been reported making this approach more feasible in the Good Manufacturing Practices (GMP) setting (29, 30). A density gradient alone,

however, cannot distinguish lymphocyte and monocyte fractions. Alternative separation techniques that rely on both size and density, such as elutriation, do allow for isolation of a lymphocyte fraction from a monocyte-rich product (31, 32). Closed system elutriators are commercially available and widely used in cell therapy applications (Elutra, TerumoBCT; Elutriation System, Beckman Coulter). Elutriation, however, cannot separate subsets of lymphocytes such as tumor lymphocytes or T regulatory cells that decrease efficiency of downstream manufacturing.

Separation by cell surface immunophenotype offers the highest resolution of subset isolation. Monoclonal antibodies fused to magnetic particles or fluorophores may be used to distinguish between target and non-target cells. If marking target cells, the bead-coated cells can be retained in a magnetic field or sorted by flow cytometry while non-desirable and unmarked cell types are removed. Alternatively, antibody-bead/fluorophore coated non-target cells can be negatively selected. The closed and semi-automated CliniMACS system (Miltenyi Biotec) is a magnetic bead based separation platform in which a specific immunophenotypic subset can be positively or negatively selected. Flow cytometric cell sorters can be setup for clinical grade cell sorting (33, 34). While density gradients, elutriation and magnetic bead separation have been in use in the clinical setting for years, novel cell therapy applications have driven continued effort to improve the standardization, automation and closed nature of these systems.

The optimal immunophenotype of an effective B cell directed cell therapy is under investigation. Because this remains largely unknown, the active ingredient in these therapies is somewhat ill-defined. For example, engineered T cell therapies are often defined as CD3+ cells that carry the transgenic modification. However, many infused products contain a variable number of CD4+, CD8+ and memory T cell subsets. Because the optimal immune subset for each potential therapeutic application is still under investigation, the best cell subset to initiate cultures and variables among patients is also unknown.

Manufacture and reinfusion of defined CD4 and CD8 subsets has been shown to generate potent anti-tumor responses (35), however it is not clear that a particular CD4 to CD8 ratio consistently enhances potency over bulk T cells. Memory T cell subsets appear to correlate with clinical response and can be promoted in culture (25, 36, 37). Better understanding of in vivo effector and memory responses of these cells will continue to drive manufacturing protocol development to skew cultures toward production of potent and persistent anti-tumor cells.

Expansion

To achieve a clinically relevant dose of cells, many cell therapy protocols require ex vivo expansion. Cell therapy culture expansion may occur in static culture or dynamic culture. Static culture may occur in flasks or gas permeable culture bags. These systems allow for culture of small and large volumes and are easily scalable. Many bag systems include docking for heat-sealed sterile tubing, which allows for the system to remain closed through sampling, media exchange or addition of reagents. Some bags are also qualified to be cryopreserved, simplifying materials needed for harvest and cryopreservation procedures.

For flasks and bags, gas exchange only occurs at the liquid-air interface. The G-Rex device, which allows gas transfer through a gas-permeable membrane on the bottom of the device, permits the addition of cultures with larger volumes since the gas diffusion does not occur through the top of the culture as in most flasks and tissue culture plates.(38) The use of the G-Rex device has allowed for more rapid expansion and increased cell yield in many processes that tend to be laborious.(7, 8) One disadvantage of the original G-Rex is that the device has a large, open top, which increases the potential for contamination. WilsonWolf, the manufacturer, has recently released a closed version of the device, which could help lead the translation of these devices into clinical manufacturing – particularly in later phase clinical studies.

Dynamic bioreactors address both of these potential limitations. Dynamic bioreactors such as the WAVE Bioreactor System (GE) gently agitate cells and media in a bag while simultaneously applying heat. Here too, the bioreactor systems frequently include sterile docking to maintain a closed system during media exchange or sampling. Newer systems record performance and quality data to streamline auditing. While bioreactors improve gas exchange and even distribution of culture components, such systems add capital cost and can be challenging to scale across a production line. Installation and ongoing maintenance can be expensive. Preclinical collaborators rarely have access to these instruments, placing additional demands on clinical manufacturing validation.

The Miltenyi Prodigy combines the cell selection capabilities of the CliniMACS with the dynamic bioreactor technology available in other bioreactor systems.(40) The closed nature of this device, along with its automation, make this instrument appealing to programs or companies which have limited space, staff, and experience and are also appealing to industry for many of the same reasons, along with their ability to standardize procedures. For example, because the Prodigy can select subpopulations of cells based on the expression of surface markers,(41) T cells recognizing EBV antigens could theoretically be selected based on secretion of interferon gamma and then used as a treatment for lymphoma such as post-transplant lymphoproliferative disease (PTLD).(42) CAR T cell manufacturing may also be adapted to the Prodigy (43). Significant capital cost outlay and ongoing maintenance are also considerations with this device that are balanced with savings in labor.

The use of dynamic bioreactors and selection of cell populations is feasible for chimeric antigen receptor (CAR) T cell therapies and for selecting and infusing specific cell populations. In contrast, the expansion of TAA-specific T cells such as those targeting the leukemia antigens Preferentially Expressed Antigen of Melanoma (PRAME), Wilm's Tumor Antigen (WT-1), and Survivin, involves the expansion of rare cell populations, likely derived from the naïve T cell population, and priming of T cells followed by repeated stimulations of expanded T cells with antigen-presenting cells such as dendritic cells. Because of the small cell volume and complexities associated with these expansions, the use of closed bioreactor systems has proven challenging, and a more rapid process will be required before it is widely implemented.

Whether culture of T cells occurs in dynamic bioreactors or static culture vessels, a critical determinant of cell number, efficiency of gene delivery, and potency is the culture media

formulation. Studies showing the importance of retaining a central memory or stem cell memory, rather than an effector memory, phenotype (44, 45) have provided rationale for the addition of cytokines such as IL-7 and IL-15 to culture media (46–48). Another approach to achieve the end goal of enhanced engraftment and potency has been to pharmacologically inhibit the kinase Akt (49, 50). Method of T cell stimulation can also enhance potency and expand a polyclonal, rather than oligoclonal population. This is particularly evident in chronic lymphoid leukemia (CLL), where there is an intrinsic defect in the immune synapse that results in impaired signaling and T cell function that can be reversed by stimulation with anti-CD3/anti-CD28 antibody coated beads or by lenolidomide (51, 52). Ibrutinib is a recently approved therapy for CLL that targets Bruton's Kinase (BTK). Although some patients progress on this therapy, the drug has a significant effect in restoring T cell function in patients with CLL and Mantle Cell Lymphoma (53, 54). This property of Ibrutinib is now being exploited in a clinical trial of CAR T cells (NCT02640209). Cell-based artificial antigen presenting cells can be tuned to express various combinations of stimulatory ligands that preferentially expand a selectable array of T cell subsets (55, 56). Serum free media and shorter culture times are additional enhancements to cell processing that have recently been reported (57). Ultimately, regardless of the technique, ex vivo expansion aims to increase the number of total cells for infusion. However, it is important to note that total cell number in a dose is not always a good predictor of potency and thus in vivo response. In vivo activation and proliferation vary from patient to patient due to T cell intrinsic and tumor intrinsic factors. Further, split dosing of more cells may improve safety while retaining efficacy (58). With better definition of the effective cell type, total doses will be reduced and robust ex vivo expansion will become less critical.

Genetic modification and editing

Efficient, GMP-compliant, ex vivo modification of T cells can be achieved by several mechanisms. Broadly speaking, T cells can be modified to express novel proteins or to ablate expression of endogenous proteins. In addition, such modifications can be transient or permanent. The appropriate strategy for introduction or ablation of targets is protocol dependent. Next generation T cell protocols are often multiplexed, introducing and/or ablating in a complementary fashion.

The most common T cell modification strategies introduce novel constructs into T cells by viral transduction or electroporation of DNA or RNA. Early work with retroviral transduction proved that efficient T transduction could be achieved; however, safety concerns were raised in light of oncogenesis observed in human retroviral based gene therapy trials (59). Lentiviral vectors have a potentially decreased risk of insertional oncogenesis compared to retroviral vectors, and do not require cell division for transduction. Sensitization of T cells to lentiviral transduction can be achieved by TCR-mediated activation or exposure to IL-2 and IL-7, among other methods (60, 61). Alternatively, DNA or RNA may be electroporated into cells. Electroporation transiently disrupts lipid bilayers, allowing passage of extracellular components into the cytosol. Electroporation of mRNA can lead to efficient, but transient expression of CARs (62). Electroporation of a DNA transposon with transposase can generate integration and stable expression of CARs (63).

Targeted gene disruption in T cells can be achieved through protein-endonuclease or RNA-endonuclease methods. Zinc Finger Nucleases (ZFN) and Transcription Activator-like Effector Nucleases (TALENs) share amino acid based targeting of specific DNA sequences. Amino acid arrays can be assembled to target sequences within the genomic DNA and when coupled with an endonuclease, double stranded breaks (DSB) can be generated at those loci. During DSB repair, bases may be lost or added, functionally disrupting the targeted locus. ZFNs and TALENs have been used to disrupt a number of endogenous proteins during ex vivo T cell modification (64, 65). ZFNs were the first gene editing method to be tested in humans (66). The RNA based Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 system can also be used to target disruption of genomic DNA. CRISPR-Cas9, unlike ZFN and TALEN systems, use RNA to confer specificity, however the ultimate goal of DNA disruption at the target locus is shared. CRISPR-Cas9 has the added advantage in that generation of GMP grade RNA is easier and more economical than creating customized, GMP-compliant protein or amino acid arrays. All gene disruption techniques can be used in combination with viral transduction to generate CAR T cells with additional modifications (67, 68).

Conclusion: Competing visions of next-gen cell therapy manufacturing

The field of cell therapy manufacturing for the treatment of B cell malignancies is aligned on many goals. It is universally recognized that standardized, automated and closed systems are needed to increase manufacturing capacity and scale out these treatments. Next generation equipment that meets these needs will find an enthusiastic and expanding market. While there is widespread agreement on the needs, the path to achieving these goals is more controversial.

In the future state, manufacturing may occur at centralized locations or be distributed to small scale laboratories, much like the model established by hematopoietic stem cell transplantation over the past 60 years. Central manufacturing supports greater standardization, quality oversight and cost savings through economy of scale and minimization of idle capacity. However, smaller manufacturing operations distributed closer to translational investigators, such as in university medical centers, support more collaborative efforts to accelerate new therapies to clinical manufacturing and, in some cases, alleviate the need to cryopreserve cells. These approaches are not mutually exclusive and could coexist with novel therapies developed in smaller academic GMP labs and more mature products being manufactured at scale in large centralized facilities.

Manufacturing protocols may employ modular or integrated systems. Modular systems allow the developer to optimize across several platforms and tailor a given manufacturing approach to the specific product specifications. While this requires a greater level of expertise during development, each individual component of the manufacturing cycle can be selected and optimized for a given process. Fully integrated protocols, however, allow for better automation, streamlining the process and standardization. Such standardization is critical to make these therapies commercially attractive and improve widespread availability. Manufacturing process design will also depend on the choice of manufacturing location. Centralized manufacturing makes control over and standardization of manufacturing easier.

On the other hand, logistics of material and product transport add costs and challenges. Ultimately, as some therapies move toward FDA approval, costs will be reconsidered in the setting of potential reimbursement. Though unknown, one hopes that compensation for these therapies will consider the potential systemic cost savings of potentially curative cell therapies.

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Figure 1.

Engineered T Cell Manufacturing Lifecycle - T cells can be obtained from a number of tissues depending on the application. While whole blood is most convenient, leukapheresis products contain much larger numbers of cells. Both cord blood and healthy donors may be a source for T cell product manufacture in the context of a hematopoietic stem cell transplant. Tumor infiltrating lymphocytes are already enriched for specificity. Examples of methods for isolation and enrichment, T cell activation, gene delivery, and culture provide flexibility dependent on the application. With variables at each processing step, the resulting final product derived from the same cell source will differ in cell number, phenotype, antigen specificity, gene expression level, and potency. The ultimate benefit to multiple available methods and technologies at each step is the opportunity for innovation and therapeutic development.

Table 1

Instrumentation used in the manufacture of cellular therapy products

Instrument	Process	Company	Advantages	Limitations
Optia	Apheresis Collection	Terumo BCT	Automated detection of interface	Limited in-process modification to collection
Spectra	Apheresis Collection	Terumo BCT	Vast prior experience	Soon to be no longer supported by manufacturer Yields/purities may be user dependent
Amicus	Apheresis Collection	Fenwal	Highly automated processing	Less frequent opportunity for in-process adjustments
Elutra	Enrichment	Terumo BCT	Closed system Distinguishes lymphocytes from monocytes	Cannot distinguish lymphocyte subsets or some tumor types
CliniMACS	Cell Selection	Miltenyi Biotec	Closed system Specific selection of cell populations *	Manual, lengthy procedure Expensive
SEPAX	Enrichment	GE Healthcare	Enrichment of mononuclear fractions Closed system	Limited processing volume
WAVE	Cell Expansion	GE Healthcare	Closed system Ability to control multiple variables	Footprint, Modified CD4+/CD8+ T cell ratios, Shear force damage(39)
Prodigy	Cell Expansion/Cell Selection	Miltenyi Biotec	Closed system Modular Contains centrifuge, Ability to enrich	Expensive, Not feasible for all processes
Quantum	Cell Expansion	Terumo BCT	Closed, automated	Limited reports on its use, Large, one size reactor
G-Rex	Cell Expansion	Wilson Wolf	Simple, Low cost design Effective	Potential for contamination, Limited sizes – small scale

* Food and Drug Administration (FDA) approval as a Humanitarian Use Device for specific indications