Summer 2007
ISSN 1538-8786

BioProcessing JOURNAL

Trends and Developments in BioProcess Technology

Vol. 6/No. 2

www.bioprocessingjournal.com

Personalized Cell-Based Medicine: *Activated and Expanded T Cells for Adoptive Immunotherapy*

By BRUCE L. LEVINE

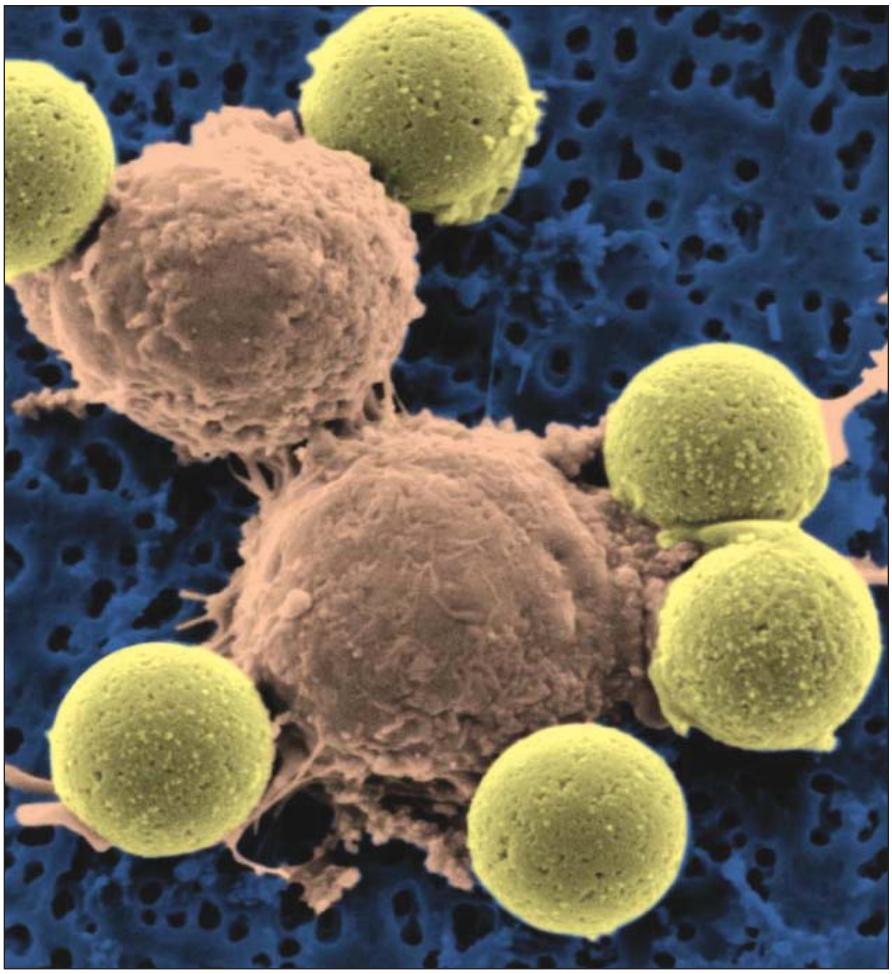

Figure 1. First generation artificial antigen-presenting cells. Magnetic beads, 4.5 µM in diameter are coupled with monoclonal antibodies directed against CD3 and CD28 on T lymphocytes. Beads are added to T lymphocytes at a 3:1 ratio during *ex vivo* activation and expansion.

The aim of personalized medicine is to provide the customized treatment likely to work best for each individual. A narrow interpretation of the definition attributes the appropriate treatment to be based on the patient's molecular phenotype. A broader interpretation includes cell-based therapies that are derived from a patient's own cells, or cells from a related or tissue-matched donor. Basic research findings contributing to the knowledge of the molecular and cellular basis of immune-mediated control of cancer and infectious diseases have created opportunities to develop new forms of cell-based vaccination for cancer and chronic infections like HIV.

Cell therapy laboratories have developed from their roots in bone marrow transplantation and blood banking into what can now be described as cellular engineering laboratories where cells can be isolated, enriched, transduced, activated, expanded and otherwise manipulated in ways to change or enhance the function of *in vivo*-derived cells for eventual reinfusion.

Accordingly, in the past two decades there has been a dramatic increase in cell therapy clinical trials around the world. The remarkable potential of cell-based vaccines has also built upon previous work in the area of blood and bone marrow transplantation in recent years. Since the first administration of gene-modified cells to two patients with congenital immune deficiency in 1990, there has been a remarkable increase in the number of cell and gene therapy Investigational New Drug applications (INDs) and amendments submitted to the FDA's Center for Biologics Evaluation and Research. As a surrogate marker of innovation, INDs submitted to FDA can show where the future of medicine is heading. *Thus, there has been a paradigm shift from the use of cells*

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and tissues for homologous function to the engineering of cells for non-homologous or improved function.

An ideal cell therapy would have the following properties: 1) demonstrated potency against tumor or infectious organism; 2) efficient engraftment enabling a high effector-to-target ratio; 3) long term persistence and memory; and 4) be easily obtained and efficiently manufactured. Among the cell therapies currently under investigation, T lymphocytes meet the first three criteria.

An inherent barrier to widespread clinical application remains the manufacturing difficulties, and the access to robust and efficient methods for the expansion of input T lymphocytes. Resolving this particular issue up front, at the early clinical development phase, is one of the cornerstones toward eventual commercialization and marketing of this promising new form of personalized therapy for cancer and infectious diseases. As described below, our laboratory has developed methods for the efficient activation, expansion, and gene transduction of T lymphocytes to meet the fourth property noted above.

T Cell Therapy and *Ex Vivo* Culture Methods

Although the clinical application of T cell-based therapeutics has gained extensive momentum within the past 20 years, the concept that immune responses can be induced to generate anti-tumor and anti-infective immunity is not a new one. In fact, the first "effective" immunotherapeutic intervention for cancer occurred in the 1890s and stemmed from Dr. William Coley's observation of a cancer patient having a complete remission following two attacks of erysipelas caused by acute infection with the bacteria *Streptococcus pyogenes*. Dr. Coley subsequently went on to develop an extract of these "toxins" and vaccinated over 800 patients; a significant portion of whom exhibited tumor regression.[1]

For the next century, a debate ensued on whether the immune system could recognize and mount an effective response to malignant tumors. If true, this offered the possibility of manipulating the immune system for therapeutic benefit.

A number of critical discoveries were made in the late 1980s to support the realization of T cell-based therapy's effective response to malignant tumors. These discoveries included the identification of the first T cell antigens that were later tested as the first cancer vaccines.[2] During this time, the first clinical trials administering the cytokine IL-2 directly into patients were being conducted. Cytokines are chemical messengers produced by cells of the immune system; many of which activate T cells and stimulate T cell responses. One limitation of IL-2 and other cytokines used as immunotherapeutic agents is that they can cause life-threatening or fatal side effects when directly administered to patients.[3]

Dendritic cells (DC) present foreign antigen to T cells and are critical to the initiation of the adaptive immune response.[4,5] There have been a large number of studies suggesting that DCs, when appropriately activated and induced to present tumor-associated antigens, can elicit tumor-specific T cell immunity. This dendritic cell therapeutic approach is currently being pursued by several biotechnology companies, but has limitations in that the ability to generate dendritic cells varies from patient-to-patient, and this variability may result in short-term or insufficient T cell activation to generate an effective immune response.

Early methods of T cell culture demonstrated that it was feasible to generate Epstein-Barr virus (EBV)-specific[6] or cytomegalovirus (CMV)-specific[7] T cells that could be infused. Limitations in lymphocyte numbers occasioned by shortcomings in tissue culture technology have prevented the routine application of adoptive immunotherapy. There is now a greater understanding of the receptor signaling pathways for T cell activation. In particular, it has been recognized that both a primary specificity signal via the T cell receptor (TCR) (Signal 1) and a co-stimulatory/regulatory signal via the CD28 receptor (Signal 2) are simultaneously required for the generation of full T cell effector function and a long-lasting immune response.[8]

The CTLA4 gene is a co-stimulatory receptor that can deliver a negative signal to T cells. In fact, there is a family of co-stimulatory receptors that can deliver either a positive or a negative signal to T cells.[9]

Magnetic Bead-Based Artificial Antigen-Presenting Cells

With this knowledge, we have developed efficient and reproducible methods of mimicking the signal provided to T cells by dendritic cells, but without delivering a negative co-stimulatory signal. With artificial antigen-presenting cells (aAPC), appropriate signals can reproducibly be delivered to T cells to improve on the function, activation/expansion and length of T cell survival *in vivo*. These aAPC methods allow for T cells to be grown rapidly *ex vivo* to clinical scale for therapeutic applications. The technology enables direct T cell activation instead of indirect activation via vaccines which can be modulated by the nature of cell dose as necessary to achieve a clinical response.[10,11]

We developed the first generation of off-the-shelf aAPC by covalently linking clinical grade anti-human CD3 and anti-CD28 monoclonal antibodies to magnetic Dynal beads (Invitrogen Corp., Carlsbad, CA, USA), which serves to crosslink the endogenous CD3 and CD28 receptors on the T cell (Figure 1). This bead-based aAPC enables the most efficient reported growth of human polyclonal naïve and memory CD4+ T cells.[11] The peripheral T cell pool appears to be the source of the expanded CD4+ cells. In terms of cell function, the expanded cells retain a highly diverse TCR repertoire, and by varying the culture conditions, can be induced to secrete cytokines characteristic of T helper 1 (Th1) or T helper 2 (Th2) cells.[12]

One important advantage of this bead-based system is that it does not cross-react with CTLA4 and therefore provides unopposed CD28 stimulation for more efficient expansion of T cells. Another unanticipated discovery was

that the cross-linking of CD3 and CD28 with bead-immobilized antibody renders CD4+ T lymphocytes highly resistant to HIV infection. This is due to the downregulation of CCR5, a necessary co-receptor for the internalization of HIV, and the induction of high levels of β-chemokines, the natural ligands for CCR5,[13-15] and allows for the efficient culture of CD4+ T cells from HIV-infected study subjects. *Ex vivo* expansion may also indirectly enhance T cell activity by removing T cells from a tumor-induced immunosuppressive milieu.[16-19] Other key features are that exogenous growth factors (or accessory cells) are not needed to enable the T cell stimulation and expansion, as with previous methods.

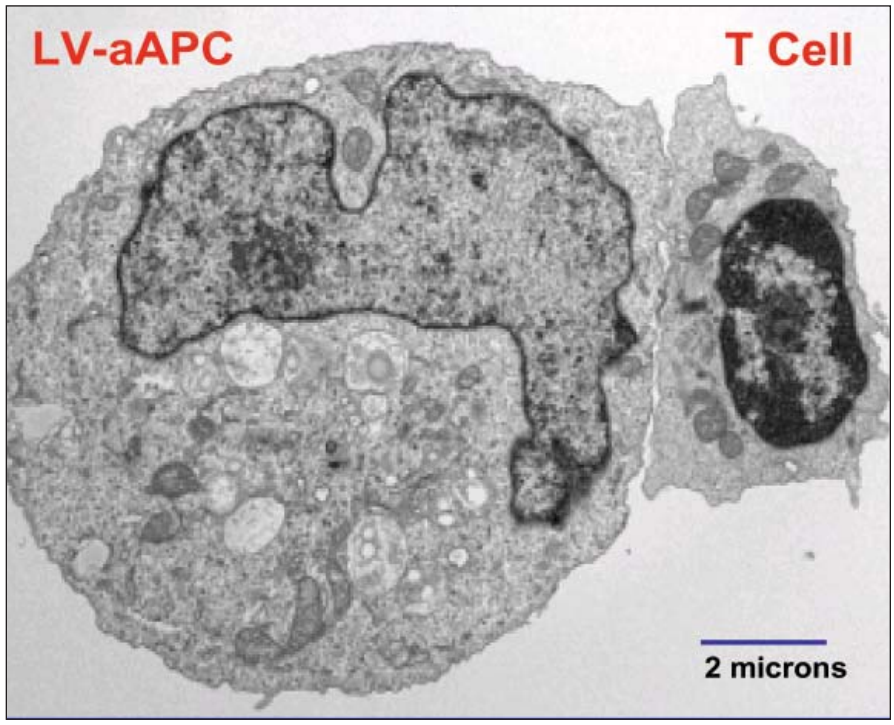

Figure 2. Second generation artificial antigen-presenting cells optimized for CD8 T lymphocytes and antigen-specific T lymphocyte activation. K562 cell lentivirally transduced to express CD64 and CD137L, and loaded with anti-CD3 and anti-CD28 Abs stimulates a CD8+ T lymphocyte.

Cell-Based Artificial Antigen-Presenting Cells

Dr. Carl June and Dr. James Riley have recently developed aAPC lines derived from the chronic myelogenous leukemia line K562.[20-22] K562 cells do not express major histocompatibility complex (MHC) or T co-stimulatory ligands, and these cells may represent a DC precursor that retains many other attributes that make DCs such effective aAPCs, such as cytokine production, adhesion molecule expression, and macropinocytosis. These cells have been transduced with a library of lentiviral vectors that allows for the customized expression of stimulatory and co-stimulatory molecules that can be used to activate and expand different subsets of T cells and be further modified to amplify antigen specific T cells in culture. These aAPCs offer the advantage of expression of molecules in addition to CD3 and CD28 on their surface.

The K562 aAPCs have been transduced with vector to express the antibody fragment crystallizable (Fc)-binding receptor and the co-stimulatory molecule 4-1BB. The expression of CD64, the high affinity Fc receptor, on K562 aAPCs allows the flexibility of loading antibodies directed against T cell surface receptors. CD3 and CD28 antibodies are added to the cells and are bound by the Fc receptor to yield a cell that expresses anti-CD3, anti-CD28 and 4-1BB. These cell-based aAPCs have proven to be more efficient at activating and expanding T cells, especially CD8+ and antigen-specific T cells (Figure 2), than the magnetic bead-based aAPC. In addition, the cells are capable of stimulating CD4 cells efficiently.

Thus, K562 cells may represent ideal scaffolds to which the desired MHC molecules, co-stimulatory ligands, and cytokines can be introduced in order to establish a DC-like aAPC that has the following advantages of natural DCs: a) high levels of MHC expression; b) a wide array of co-stimulatory ligands; and c) the ability to engage in cytokine crosstalk with the T cell. The following disadvantages of natural DCs are averted: a) the need to derive natural DCs from either granulocyte colony-stimulating factor (G-CSF) mobilized CD34+ cells or monocytes using cytokines that are not currently available as GMP reagents; b) patient-specific expansion; c) limited life span; and d) limited replicative capacity.

Moreover, these cells have been injected into humans as part of a tumor vaccine,[23] signifying that these cells can be used in a GMP manner. Additionally, our lab and our collaborators have now developed either bead or cell-based aAPCs optimized for Th2 cells,[12,24] and for T regulatory cells.[25]

Manufacturing Process

Independent of which of the above aAPCs is used, the manufacturing procedure remains similar, starting with an apheresis product (Figure 3). Alternatively, T cells can be derived from a blood draw, bone marrow, ascites, or tumor infiltrating lymphocytes. The pheresis product may be washed out of collection buffer in a COBE 2991 cell washer, a Baxter CytoMate, or directly loaded in the Gambro Elutra cell separation system for depletion of monocytes and isolation of lymphocytes.

If a CD8+ or CD4+ T cell product is desired, the depletion of CD4+ or CD8+ T cells can be accomplished using a Miltenyi CliniMACS. This instrument is an electromechanical device intended to isolate certain cell subsets via large-scale magnetic cell selection in a closed and sterile system. Before selection, the washed cells from a pheresis product are magnetically labeled by using particles

conjugated with anti-CD4 or anti-CD8 MAb. A single-use tubing set, including separation columns, is then attached to the CliniMACS instrument and the cell preparation bag containing the labeled cells. After starting the selection program, the system automatically applies the cell sample to the separation column, performs a series of washing steps depending on the program chosen, and finally elutes the purified target cells.

The lymphocyte fraction from the Elutra cell separation system or enriched T cells are cultured in a nutrient media and stimulated to divide and grow via the addition of the antibody-coated magnetic beads or irradiated and antibody pre-loaded K562 aAPCs, each of which is described above. Utilizing either of these methods, gene transduction with retroviral or lentiviral vectors is very efficient.

The whole mixture of cells, growth media, vector and aAPC is added to a gas-permeable plastic bag (or alternative culture vessel) and then placed in a humidified 37°C, 5% CO_2 incubator. Tubing leads on the bags and a variety of connecting devices (via spike connectors and welds produced via Terumo's sterile connecting device) allow the cells to be grown in a closed system with minimal risk of contamination. The cultures are maintained for up to 12 days prior to harvesting and preparation for reinfusion or cryopreserved for later infusion. The activated cells are counted at least every other day and fresh medium is added to maintain the cells at an appropriate density (approximately 0.5–1.5x10^6 cells/ml) during the initial culture period. After gene vector washout (if needed) with the CytoMate and also during log phase cell growth, cultures are transferred to the Wave bioreactor where cell concentrations may reach 1.0x10^7 cells/ml or higher. We have optimized cell culture in both the Wave bioreactor 2/10 and 20/50 for our ongoing clinical trials, including gene therapy trials. The advantage of the Wave is that T cells can be grown at higher densities which saves labor during processing and cell harvest.

The next step prior to infusion (at approximately day 9–12) is to wash the cells out of the nutrient media and into an infusible solution. At times, the volume of the cell culture can be as much as 10 liters (2.5 gallons) or more. Washing and concentration is performed in a Baxter Fenwal Harvester, COBE 2991 cell processor, or equivalent device while maintaining a closed system. After washing three times, the cells are resuspended and cryopreserved in an infusible solution. Containers of cryopreserved cells are stored pending the results of quality control release testing which, for gene transfer protocols, usually takes several weeks. If the cells are to be infused fresh, in-process samples are taken for microbiological testing, viability, and cell phenotype by flow cytometry for the release. Testing is repeated on the final product, although results for some tests are not available until after the cells are infused. The physician would be notified in the event of a test excursion.

Clinical Trials of Engineered T Lymphocytes

To date, between our laboratory and those of our collaborators, several hundred infusions of bead-expanded T cells have been safely administered to treat hematologic cancers and HIV in clinical trials at several sites in the U.S. In hema-

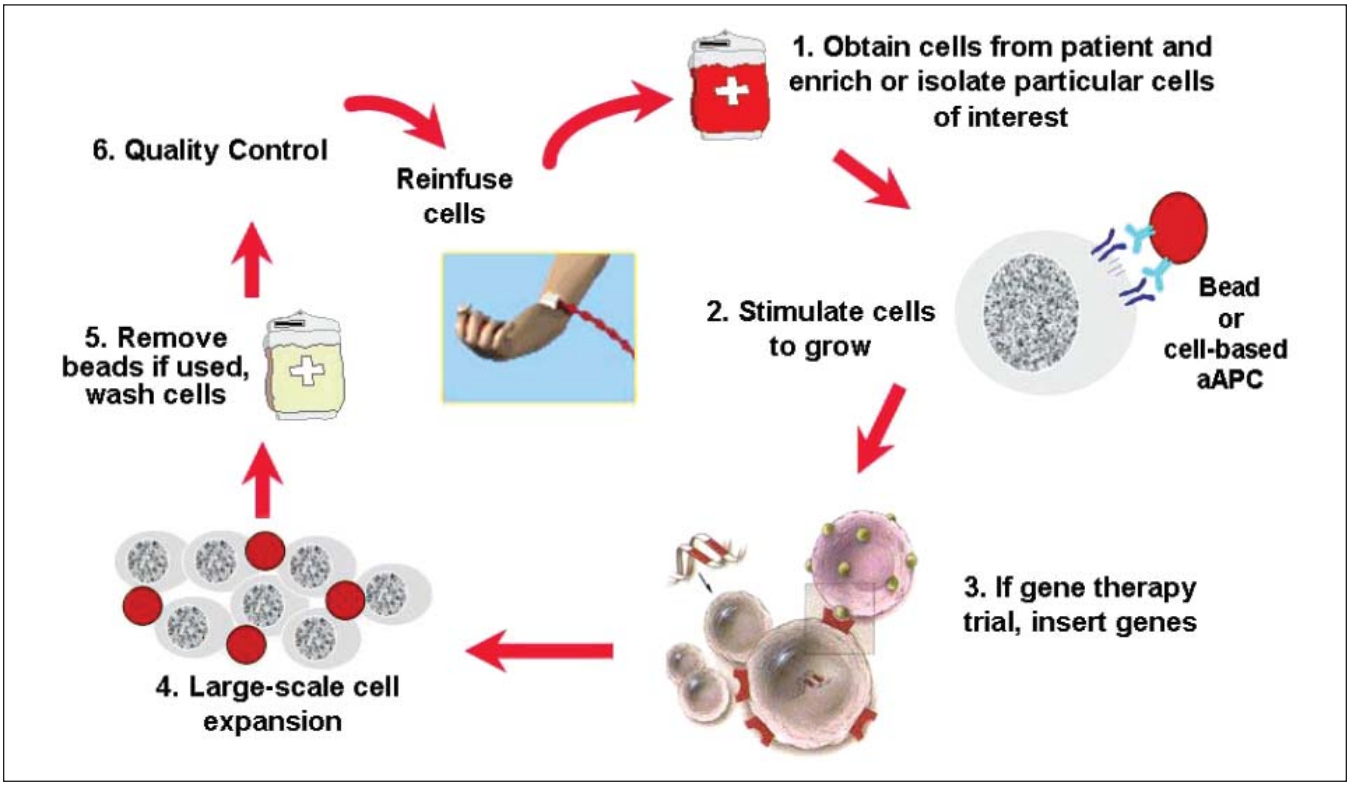

Figure 3. *Ex vivo* process for engineered T lymphocyte therapies.

tologic malignancies, we have completed four trials. During three of these trials, in lymphoma, chronic myelogenous leukemia (CML), and myeloma, patients were administered activated autologous T cells. In the first trial, patients with high-risk lymphoma were given one infusion of the cells on day 14 post-CD34 selected hematopoietic stem cell transplantation (HCT). Immune reconstitution was assessed following post-transplant T cell reconstitution. Five of 16 subjects had an unexpected lymphocytosis following T cell infusion and the frequency of interferon-secreting cells also increased markedly in some patients.

This trial suggested for the first time that it is possible to accelerate immune reconstitution in patients with advanced lymphoma who are given high-dose therapy and autologous stem cell transplantation. This discovery formed the basis for the myeloma trial described later.[26] In the CML trial, aside from feasibility, a secondary objective was to determine the frequency of hematologic, cytogenetic, and molecular remissions from the treatment approach of autologous transplants followed by T cell infusions. Of the four subjects who proceeded through the trial regimen, all had rapid recovery of lymphocyte counts following T cell infusion and had complete cytogenetic remissions early after transplantation. Three of the four also became PCR-negative for the bcr/abl fusion mRNA.[27]

The randomized Phase I/II study in subjects with advanced myeloma was designed to examine the relative benefits of pre- and post-transplant vaccine immunizations in combination with adoptive T cell transfer. Post-stem cell transplant lymphocyte reconstitution and Prevnar (pneumococcal) vaccine response were evaluated in 42 subjects. Similar to the lymphoma trial, the infusion of activated autologous T cells by day 14 post-transplant resulted in the induction of homeostatic T cell proliferation in the first few weeks following transplantation. This may prove to be a useful way to generate and/or enhance protective anti-tumor immunity.[28,29] In addition, only those subjects who received antigen-experienced T cells made appropriate antibody responses. A follow-on trial is now open in which the potency of a putative myeloma-specific vaccine is being tested to lead to a myeloma-directed T cell-mediated "graft vs. myeloma" effect. In the fourth completed trial, activated donor leukocyte infusions (aDLI) were administered to treat relapsed advanced hematologic malignancies after allogeneic bone marrow transplantation and standard DLI.[30] Of the 17 subjects evaluable for response, eight achieved a complete remission (CR) with six still alive in CR, a median of 17 months after aDLI.

This trial suggests that adoptive transfer of activated allogeneic T cells is feasible, and is associated with durable CR in a subset of subjects without excessive graft vs. host disease or other toxicity. In general, these trials demonstrate that activated and expanded T cells, in combination with other therapies such as stem cell transplantation, chemotherapy, and alkylating agent therapy (*i.e.,* melphalan-containing regimens) have been associated with complete and partial responses in the treated subjects.

With HIV, we have adoptively transferred activated autologous CD4+ T cells and observed a dose-dependent increase in CD4 counts and in the CD4/CD8 ratio following infusions. Sustained increases in CD4+ T cell numbers and decreases in the percentage of CD4+CCR5+ cells in patients were also found, suggesting augmentation of natural immunity to HIV infection.[31] More recently, we have shifted to the use of gene-modified T cells using vectors that express proteins or anti-sense that target specific HIV genes. We have assessed the safety and feasibility of this gene transduction and expansion method in the world's first lentiviral trial.[32,33]

In HIV+ study subjects that had failed at least two prior combination anti-viral drug regimens following T cell infusion, viral loads were stable or decreased in all five subjects. One subject has had a prolonged 2-log decrease in viral load for at least two years. CD4 counts remained stable in all patients, and circulating gene-modified cells were detected in all patients for at least six months. Sustained lentiviral gene transfer was demonstrated in all subjects, and has persisted for more than two years in three of the patients.

From these early trials, promising results in heavily pre-treated patients have led us to initiate a second series of randomized trials to begin addressing the efficacy of engineered T cell therapies. At the same time, we will begin our first trials with the second generation of aAPC, the modified K562 cell lines described above, for the expansion of tumor-specific T lymphocytes.

From Bench to Bedside to Market

To be commercially viable, adoptive T cell therapy has to be clinically effective, scalable, reproducibly manufactured, and appropriately priced and marketed. These are challenges beyond the proof of principle studies described above. Should cell therapies be viewed as a traditional vaccine manufactured in centralized plants or processing facilities, or more like surgery or stem cell transplantation? It is probable that most engineered T cell therapies will require stringent manufacturing controls that favor centralized manufacturing plants, whereas some forms of manufacturing for natural T cell therapies could be carried out at tertiary care medical centers. The major challenge facing the field at present is to conduct randomized clinical trials demonstrating sufficient clinical benefit to justify the logistics and expense of customized cellular therapies.

ACKNOWLEDGEMENTS

The author would like to acknowledge helpful discussions and assistance from Dr. Carl June, Dr. James Riley, Dr. Richard Carroll, Dr. Anne Chew, and Dr. Gwendolyn Binder.

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