

Overcoming Obstacles to Develop a Cancer Immunotherapeutic

Clinicians developing cell- and gene-based therapeutics including gene therapy, RNAi-based therapeutics, vaccines and other immunotherapeutics face multiple challenges that can impede the initiation of clinical trials. Safety, scalability, efficiency and the need for regulatory acceptance are the primary concerns when developing a therapeutic that involves delivering genes or gene constructs into a cell. Dr. Malcolm Brenner, Professor of Medicine and Director of the Center for Cell and Gene Therapy at Baylor College of Medicine, describes the events leading up to his choice to use the approach developed by MaxCyte, Inc., a pioneer in the development of loading technologies involved in cell and gene therapies. Dr. Brenner and MaxCyte are currently engaged in a Phase III clinical trial to assess the safety of escalating doses of an immunotherapeutic using MaxCyte's cell loading system.



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A promising approach to cancer therapy involves modifying the patient's cancer cells in order to enhance the immune response against them. Such modifications may be accomplished by inserting appropriate genetic information into the cells. The safety and efficiency of such a procedure depends in large part upon the method chosen for gene delivery into the cancer cells. While the use of viruses as vectors for gene delivery has been studied extensively, many safety problems remain that have resulted in complex and cumbersome regulatory requirements. Chemical methods are also available, but these lack reproducibility and are difficult to scale up.

The shortcomings of available technologies to deliver genetic information to cancer cells prompted Dr. Brenner to seek a novel approach to develop a cellbased therapeutic for Chronic Lymphocytic Leukemia (CLL), the most common type of leukemia in the United States and Europe. Having attempted various methods, he turned to MaxCyte's gene delivery technology to expedite taking his potential therapeutic from the bench to the clinic.

CD40L and IL-2 Stimulate Significant Immune Responses in CLL

CLL is a disease in which mature lymphocytes become cancerous and gradually replace normal cells in lymph nodes. The number of cancerous mature lymphocytes first increases in the blood and lymph nodes, then spreads to the liver and spleen, both of which begin to enlarge. Most types of CLL progress slowly, but inexorably, to a fatal result. No available therapies cure the disease. Dr. Brenner initiated an investigation to alter CLL cells in order to improve their limited ability to produce an immune response to fight the disease. Leukemic cells from CLL patients do not stimulate a significant immune response, but if the cells express the protein CD40 ligand (CD40L), the immune response is greatly enhanced and leads to diminished tumor mass. *In vitro* animal and human studies have shown the combination of CD40L and interleukin-2 (IL-2) stimulates an even greater immune response.

The Shortcomings of Existing Delivery Technologies

Dr. Brenner's initial clinical results were encouraging, but the technology required to accomplish the cell modifications was insufficient. A study that utilized a gene that produced mouse CD40L demonstrated regression of cancer cells, but the use of murine reagents was undesirable. Lentiviral vectors were able to infect CLL cells, but safety concerns related to lentiviral vectors for clinic applications remains high.

Dr. Brenner and the Baylor group investigated use of an adenoviral vector to deliver genes for CD40L and IL-2 to CLL cells. Unfortunately, CLL cells were not efficiently transfected with adenoviruses, and gene expression, especially of CD40L, was difficult to achieve. Additionally, the adenoviruses used to load the cells resulted in considerable cell death, making it difficult to effectively load clinically relevant volumes.

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Dr. Brenner and his collaborators next used a co-culturing method, first transfecting an embryonic fibroblast cell line (MRC-5) with adenovirus that coded for CD40L. When CLL cells were cultured with the CD40L-expressing MRC-5 cells, they would express CD40L and could then be used to treat CLL patients. This approach prevented the cell death that was seen when adenovirus was used directly to load CLL cells.

"We used the MRC-5 cells as a feeder cell line, and getting B cells to express CD40L with this method was robust and reproducible enough to secure Food and Drug Administration (FDA) approval for our proposed Phase I clinical trial protocol," said Dr. Brenner. "However, the logistics of such a method were a huge drawback. To generate the MRC-5 cell line, transduce with adenovirus and grow B cells on the cell line would require one or two technicians a week or more of work just to produce one patient's treatment. Generating autologous vaccine to treat enough patients for a Phase I clinical trial was extremely expensive and logistically difficult."

MaxCyte's Cell-Loading Solution

MaxCyte offered an alternate approach that efficiently loaded CLL cells with both CD40L and IL-2, preserved acceptable cell viability, reduced regulatory issues and was logistically simpler.

"MaxCyte's approach allows the loading of CLL cells in a manner that is much more scalable than our MRC-5 method. One technician can load the cells from several patients in a single day, versus the week required to load cells for a single patient using our alternate method," Dr. Brenner described.

"After we had already secured FDA clearance for the MRC-5 method to load cells, we weren't anxious to have to go through resubmission of more cell loading protocols to the FDA," explained Dr. Brenner. "But MaxCyte's cell loading approach received extremely fast approval from both the Recombinant DNA Advisory Committee and the FDA. In fact, I've worked with many clinical trials before, and MaxCyte's cell loading approach has led to the fewest FDA concerns."

With its biologically neutral, FDA-accepted cell loading technology, MaxCyte overcomes regulatory and safety hurdles inherent in current *ex vivo* cell loading protocols by eliminating the use of viral vectors and chemical transfection reagents. The company's technology uses electrical pulses to briefly make cell membranes permeable and can be used with either primary cells or cell lines. MaxCyte's flow-based cell loading technology allows the continuously scalable loading of cell volumes from the sub-milliliter level up to liter quantities, in a closed, sterile unit. Its ability to increase throughput and improve cell viability allows the processing of volumes that are relevant to clinical applications. MaxCyte's technology also provides a tamper-proof record of cell loading parameters, which can support a cGMP manufacturing process and later become part of FDA submissions.

The company's non-viral approach has many applications in therapeutic areas involving *ex vivo* cell-based approaches. "I am hoping to use MaxCyte's approach for other immunotherapeutics that I'm developing," stated Dr. Brenner.

