Supersizing adoptive T cell therapies

By Tracey Baas, Senior Editor

Despite the striking efficacy of chimeric antigen receptor–based T cell therapies in small clinical trials in patients with leukemia, the ability to rapidly provide T cells to a large number of recipients is limited by the lack of readily available tumor antigen–associated human T lymphocytes. To tackle this problem, a **Memorial Sloan-Kettering Cancer Center** team has incorporated patient-derived induced pluripotent stem cells into an immunotherapy protocol to provide large-scale production of T cells endowed with enhanced antitumor properties.[1]

The team was led by Michel Sadelain, director of MSKCC's Center for Cell Engineering. He also led the teams that produced second-generation CD19-specific chimeric antigen receptor (CAR)-expressing T cells that efficiently induced complete remission in five of five patients with chemotherapy-refractory acute lymphoblastic leukemia (ALL).[2]

In those studies, T cells were isolated from a patient, transduced with a specific tumor-associated antigen, expanded *ex vivo* and reinfused into the same patient.

The problem is that a personalized immunotherapy–based protocol may not be possible when collection or expansion of T cells is problematic, such as in individuals with small numbers of T cells because of immunosuppression or previous cancer treatment.

Thus, Sadelain's team wanted to design a protocol conducive to producing large quantities of either autologous or allogeneic T cells. Large batches of autologous T cells would allow multiple dosings to one patient. Large batches of allogeneic T cells generated from one donor would allow multiple recipients to be dosed. Both approaches would be more practical than current CAR protocols (*see* **Figure 1, "CAR-expressing T cell production to develop immunotherapeutics"**).

The solution, the group hypothesized, was induced pluripotent stem (iPS) cells. The goal would be to engineer patient-derived iPS cells to produce antigen-specific CAR–based T cells capable of large-scale expansion.

First, the researchers obtained peripheral blood T lymphocytes from a healthy volunteer and transduced the cells with two retroviral vectors that encoded the reprogramming factors *Klf4*, *Sox2*, *Oct4* and *c-Myc* (*MYC*). Resulting pluripotent cells then were transduced with a lentiviral vector encoding MSKCC's second-generation CD19-specific CAR.

Using a 3-step, 30-day protocol, the team differentiated the CAR-expressing iPS cells into CAR-expressing T cells by taking them through

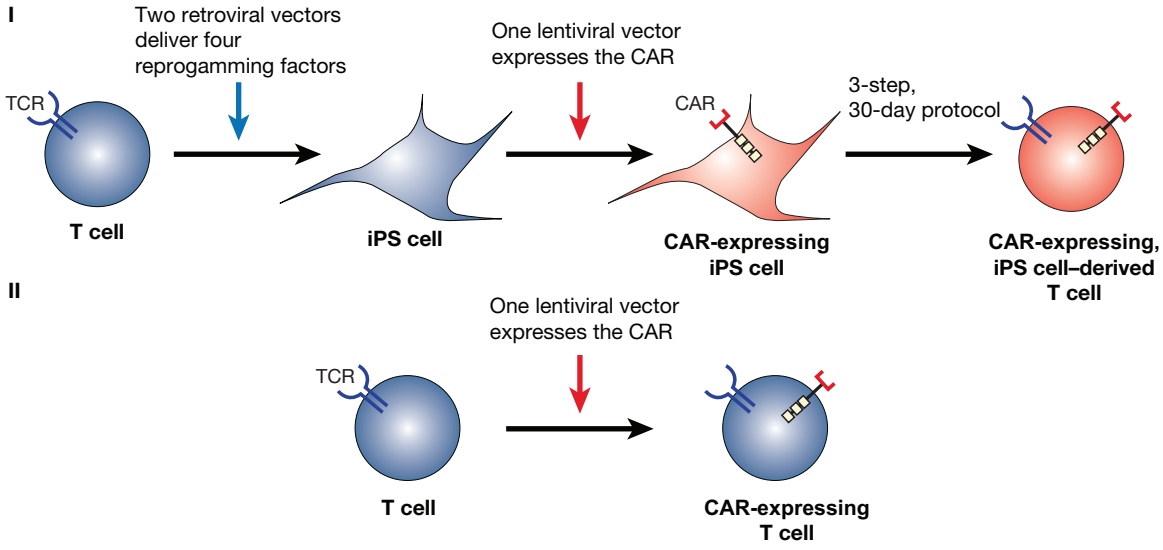

Figure 1. CAR-expressing T cell production to develop immunotherapeutics. (I) Themeli *et al.* transduced a healthy volunteer's T cells with two retroviral vectors encoding four transcription factors that reprogram the T cells into induced pluripotent stem (iPS) cells. The iPS cells then were transduced with a lentiviral vector encoding a chimeric antigen receptor (CAR) to provide CAR-expressing iPS cells. The team next differentiated the CAR-expressing iPS cells into T cells using a 3-step, 30-day protocol that takes the cells through phases of embryoid body formation, hematopoietic precursor specification and finally T cell commitment.

The resulting T cells expressed both the CAR and an endogenous T cell receptor (TCR) that matched the original TCR of the patient-obtained T cell. Methods to expand the CAR-expressing, iPS cell–derived T cells resulted in a 1,000-fold increase in numbers.

(II) CAR-expressing T cells also can be directly engineered from patient-obtained T cells using the lentiviral vector encoding CAR. Methods to expand the CAR-expressing T cells are not as proficient as those to expand CAR-expressing, iPS cell–derived T cells.

Copyright © 2013 Nature Publishing Group

phases of embryoid body formation, hematopoietic precursor specification and finally T lymphoid commitment.

When the differentiated T cells were added to cultured CD19$^+$ cells, they expressed the T cell activation markers *IL-2 receptor α-chain* (*CD25*) and *CD69*, secreted type I cytokines and ultimately eliminated the CD19$^+$ cells. Those results suggested the CD19-specfic, iPS cell–derived T cells were functional.

This same process did not happen when the differentiated T cells were added to cultured CD19$^-$ cells, thus demonstrating the antigenic specificity of the engineered T cells.

With proof of concept established, the next step was to modify the protocol to enable the production of larger batches.

Coculturing with CD19$^+$, artificial antigen–presenting cells, IL-7 and IL-15 stimulated and expanded CD19-specific iPS cell–derived T cells. After three weekly stimulations, the CD19-specific iPS cell–derived T cell population increased by up to 1,000-fold.

In mice with CD19$^+$ lymphoma cells, the expanded T cells delayed tumor progression and initiated tumor regression, and they increased survival compared with no treatment. The results for the expanded cells were similar to those for endogenous T cells that were isolated from the same healthy volunteer and engineered to express MSKCC's second-generation CD19-specfic CAR.

The findings suggest large-batch engineering of CAR-based T cells using iPS cells can be performed without sacrificing functionality.

Results were published in *Nature Biotechnology*.

The safety scale

Jianxun Song, assistant professor of microbiology and immunology at **Pennsylvania State University Hershey College of Medicine**, said that key next steps would be to show safety and simplify the approach, regardless of whether iPS cells are used to generate allogeneic or autologous T cells.

"Adoptive transfer of iPS cell–derived T cells has the potential to trigger cross-reactivity or development of autoimmunity once the T cells are fully activated. Incorporating a suicide gene would be a good option," he said. "This allows the removal of the transferred T cells by the injection of a drug to induce the suicide gene and shut off the system."

In 2011, Song's group used antigen-specific T cell receptor (TCR)-expressing iPS cells in xenograft mice to show that the iPS cells differentiated into antigen-specific cytotoxic T cells and prevented tumor growth.[3]

Aya Jakobovits, president and CEO of cancer immunotherapy company **Kite Pharma Inc.**, said that the best use of the method could be instances in which very low levels of T cells are a problem.

"As more data become available, especially from multicenter trials using CAR- or TCR-engineered T cells, banking T cells upon diagnosis—before therapies begin—could become an attractive and affordable option," she said. "If at the time of diagnosis the patient already presents with an extremely low T cell number, then the iPS T cell approach can be beneficial."

However, Song said that the MSKCC group's 30-day protocol may not be worth the time. "iPS cell–derived T cells take a substantial time to generate, grow to large numbers, transduce with CARs or TCRs and expand to large numbers," he noted. This strategy might be worthwhile for creating T cells that can be banked for future use, but patients who need to start treatment may not be able to wait that long, he said.

Sadelain countered that the approach "does incorporate more time up front but opens up opportunities to stockpile large numbers of antigen-specific T cells, even before they are needed. This ultimately saves time when patients need to start treatment."

"Our approach also makes the first step toward off-the-shelf immunotherapeutics that can be used for allogeneic transfer," he continued. "At this stage, cell banks could be established where large numbers of antigen-specific T cells are stored and categorized using common donor HLA [human leukocyte antigen] haplotypes, and recipients for the cells can be selected using minimal mismatch criteria to ensure histocompatibility."

"At this stage, cell banks could be established where large numbers of antigen-specific T cells are stored and categorized using common donor HLA [human leukocyte antigen] haplotypes, and recipients for the cells can be selected using minimal mismatch criteria to ensure histocompatibility."

—Michel Sadelain, Memorial Sloan-Kettering Cancer Center

The MSKCC team now is taking a two-pronged approach to make its CARs more applicable to the allogeneic setting. The first, said Sadelain, is "disrupting endogenous TCRs using zinc finger nucleases or selecting for endogenous, virus-specific TCRs, which are less likely to cause graft-versus-host disease because the TCRs target virus proteins rather than host proteins."

T cells with virus-specific TCRs exist in any individual who has had a viral infection. These T cells are programmed to attack virus, not host proteins, so graft-versus-host disease would not be induced through the TCR.

The second approach would involve repressing HLA expression through additional genetic modifications to ensure histocompatibility.

Before incorporating further modifications into the protocol, both Song and Jakobovits wanted to see studies showing that the engineered T cells are not tumorigenic.

"Any leftover iPS cells that do not differentiate could lead to teratoma formation," acknowledged Sadelain. "Fortunately, immunologists are experts at sorting and isolating different subpopulations of immune cells, so if residual iPS cells remain, these cells could be removed."

MSKCC has filed a patent application for the method, and the IP is available for licensing.

Baas, T. *SciBX* 6(36); doi:10.1038/scibx.2013.983
Published online Sept. 19, 2013

REFERENCES

1. Themeli, M. *et al. Nat. Biotechnol.*; published online Aug.11, 2013; doi:10.1038/nbt.2678
   Contact: Michel Sadelain, Memorial Sloan-Kettering Cancer Center, New York, N.Y.
   e-mail: sadelaim@mskcc.org
2. Brentjens, R.J. *et al. Sci. Transl. Med.* **5**, 177ra138 (2013)
3. Lei, F. *et al. Cancer Res.* **71**, 4742–4747 (2011)

COMPANIES AND INSTITUTIONS MENTIONED

Kite Pharma Inc., Los Angeles, Calif.
Memorial Sloan-Kettering Cancer Center, New York, N.Y.
Pennsylvania State University Hershey College of Medicine, Hershey, Pa.

Copyright © 2013 Nature Publishing Group