Universal appeal for CARs

By Tracey Baas, Senior Editor

Chimeric antigen receptor-expressing T cells have shown dramatic efficacy in treating blood cancers, but so far only autologous, modified T cells can be used, making manufacturing labor intensive and expensive. Now, **The University of Texas MD Anderson Cancer Center** and **Sangamo BioSciences Inc.** have taken a step toward a universal immunotherapy by using gene-modifying tools to mask donor-derived T cells from the standard immune surveillance machinery in the recipient.¹ The MD Anderson team is already planning clinical trials with such cells targeting CD19.

Universal, chimeric antigen receptor (CAR)-expressing T cells would have two key traits—the cells would not attack the host via the graft-versus-host (GvH) response and the host would not attack the cells via the host-versus-graft (HvG) response.

GvH is mediated by T cell receptors (TCRs) on the infused allogeneic cells that are stimulated by mismatched human leukocyte antigens (HLAs) on the recipient's normal tissues. HvG is mediated by host-derived T cells that employ TCRs to recognize mismatched HLAs on infused allogeneic cells.

Mismatches occur because each individual presents a unique combination of HLA class I antigens and HLA class II antigens. *HLA* alleles are highly polymorphic, and finding an appropriately matched, unrelated, universal donor for multiple recipients would be unattainable.

With allogeneic bone marrow transplantation, optimal outcomes for unrelated donors occur when a minimum of class I antigens major histocompatibility complex class I A (HLA-A), HLA-B and HLA-C and class II antigen major histocompatibility complex class II DR (HLA-DR) have been matched between one donor and the intended recipient.²

What constitutes minimally matched T cells for cancer patient recipients is unknown. Therefore, genetic strategies have been developed to eliminate HLA expression on donor T cells.

In the new study, a team of researchers at MD Anderson co-led by Laurence Cooper and Hiroki Torikai collaborated with Sangamo to investigate whether an immune response to allogeneic, CAR-expressing T cells could be avoided by eliminating expression of one or more mismatched HLAs on donor-derived cells.

Cooper is a professor of pediatrics, section chief of cell therapy for the Children's Cancer Hospital and associate director of the Center

for Cancer Immunology Research at MD Anderson, and Torikai is a research scientist at MD Anderson.

The team had previously used zinc finger nucleases (ZFNs) to eliminate expression of TCRs on human CAR-expressing T cells specific for CD19. *In vitro*, the ZFN-edited, TCR⁻ cells retained their antitumor activity against malignant B cells and did not respond to TCR stimulation, which is the initiating factor for a GvH response.³

Based on the success using ZFNs to control the GvH response, the group hypothesized that it could also control HvG by eliminating HLAs to generate universal, CAR-expressing T cells.

As proof of concept, the team designed and used ZFNs to eliminate expression of *HLA-A2* and *HLA-A3* in an immortalized human cell line. Isolated cellular clones lacking the HLA-As were not recognized or lysed by HLA-A2- and HLA-A3-restricted cytotoxic T lymphocytes (CTLs).

The team then generated HLA-A⁻ primary T cells via a two-step procedure. First, the team used electroporation to deliver ZFN mRNA into T cells to eliminate *HLA-A2*, which is the most common *HLA-A* allele in Caucasians. The researchers then used antibody-coated paramagnetic beads to remove unedited HLA-A2⁺ T cells in which the ZFNs did not cleave *HLA-A2*. The result was a population of T cells in which more than 95% were HLA-A2⁻.

With proof of concept in hand for the procedure, the researchers turned their attention to modifying CD19-specific, CAR-expressing T cells. Using ZFN mRNA electroporation and paramagnetic bead selection, the team generated a population of HLA-A2⁻, CD19-specific, CAR⁺ T cells with around 99% enrichment.

Even after multiple days in culture, about 94% of the CD19-specific, CAR-expressing T cells remained HLA-A2⁻ and avoided attack by HLA-A2-restricted CTLs, suggesting the HvG response had been thwarted. Additionally, the modified T cells retained their ability to lyse CD19⁺ lymphoma cell lines as well as CD19⁺ primary lymphoma cells obtained from patients, indicating that the cells maintained their antitumor activity.

Next, the team simultaneously eliminated HLA-A2 and TCR in primary T cells by using two ZFN species. The result was elimination of both GvH and HvG.

Finally, the group attempted to protect the T cells from another type of HvG response that is initiated by NK cells. T cells lacking classical HLA class I molecules, such as HLA-A, are seen as nonself and are subject to elimination by NK cells.

The team simultaneously expressed nonclassical class I antigens HLA-E and HLA-G on HLA-A⁻ cells and showed that those expressing either one or both of the molecules were lysed significantly less often by NK cells than those lacking all class I molecules.

Results were published in *Blood*.

Stepwise into the clinic

The MD Anderson team is planning clinical trials of CD19-specific,

“What range of HLA class I or HLA class II molecules will need to be targeted, in conjunction with TCR editing, to provide the best therapeutic cells is hard to predict.”

—Carl June,
Perelman School of Medicine at the
University of Pennsylvania

CAR-expressing T cells using an escalating approach—first TCR⁺ cells and then TCR⁺ and HLA⁺ cells. Initially, TCR⁺ T cells will be tested in humans. An application has been submitted to the **NIH** Office of Biotechnology Activities seeking federal regulatory approval.

“Our first trial will infuse CD19-specific CAR T cells that have been genetically edited to eliminate the expression of endogenous TCR,” said Cooper. “These trials will then be modified to infuse CD19-specific CAR T cells that lack TCR as well as HLA-A to improve persistence and to avoid presentation of immunogenic genes.”

“To edit genes, we are working with Sangamo’s designer ZFNs. They have terrific experience supporting the human application of T cells edited with ZFNs. In the future, clinical-grade T cells, as well as other cells, will be genetically edited to eliminate undesired genes. These can be generated using a panel of artificial nucleases that is not limited to ZFNs but include CRISPR [clustered, regularly interspaced short palindromic repeats] or TALEN [transcription activator–like effector nuclease] gene editing systems,” added Cooper.

“Universal therapy using HLA-edited allogeneic T cells is absolutely desirable because banking HLA-matched cell lines would not cover all HLA matching options, and even more importantly, the outcome of the therapy would be very heterogeneous depending on the exact level of matching,” said Assaf Marcus, a postdoctoral researcher in the Department of Molecular and Cell Biology at the **University of California, Berkeley**.

Marcus and Zelig Eshhar, a professor of chemical and cellular immunology at the **Weizmann Institute of Science**, both think the CARs will require additional modifications before they can become off-the-shelf products.

“HLA-A⁺ cells are not universal cells; they are proof of concept that T cells can be made more stealth-like to CTLs,” said Eshhar. “For truly universal cells, all classical HLA I and HLA II molecules would need to be eliminated to prevent host-versus-graft reactions, while TCR would need to be eliminated to prevent graft-versus-host reactions.”

“And because NK cells can rapidly eliminate massive numbers of donor cells that lack HLA I expression, HLA-E and/or HLA-G expression would most likely be required to give donor cells a fighting chance to outmaneuver the recipient’s immune system and establish donor cell persistence,” said Eshhar.

“If one could ask to see the best next steps, that would be to generate allogeneic T cells lacking all classical HLA I and II molecules and TCR and expressing HLA-E and HLA-G,” proposed Bruce Levine, an associate professor in cancer gene therapy in the Department of Pathology and Laboratory Medicine at the **Abramson Cancer Center at the University of Pennsylvania**. “But just because you can edit any or all those proteins doesn’t make it necessary and doesn’t make it practical.”

Carl June, a professor in the Department of Pathology and Laboratory Medicine at the **Perelman School of Medicine at the University of**

“For truly universal cells, all classical HLA I and HLA II molecules would need to be eliminated to prevent host-versus-graft reactions, while TCR would need to be eliminated to prevent graft-versus-host reactions.”

**—Zelig Eshhar,
Weizmann Institute of Science**

Pennsylvania and director of the translational research program at Abramson, thought the MD Anderson and Sangamo concept is worth probing in the clinic.

June’s three-patient Phase I trial using CD19 CAR T cells in chronic lymphocytic leukemia (CLL) basically set the field ablaze^{4,5} and led to a deal in 2012 between the **University of Pennsylvania** and **Novartis AG** to develop and commercialize CAR immunotherapies for cancer.⁶

“What range of HLA class I or HLA class II molecules will need to be targeted, in conjunction with TCR editing, to provide the best therapeutic cells is hard to predict,” June said.

He said the planned escalation approach is a good idea. “Sequentially testing increasingly modified cells to determine safety and persistence” is a sound strategy, said June. “It is hard to overestimate the ability of the human immune system to detect and eliminate modified cells.”

“These types of studies will likely uncover modified allogeneic T cells that could be used as universal, CAR-based therapies for tumor knockdown or remission induction and therapies to prevent tumor relapse,” he continued. “However, I am doubtful that these universal cells will have long-term persistence and therefore may not be a substitute for autologous T cells.”

The University of Texas MD Anderson Cancer Center Office of Technology Commercialization and Sangamo have jointly filed for a patent covering universal CD19-specific, CAR-expressing T cells. The IP is available for licensing.

Baas, T. *SciBX* 6(28); doi:10.1038/scibx.2013.712
Published online July 25, 2013

REFERENCES

1. Torikai, H. *et al. Blood*; published online June 5, 2013; doi:10.1182/blood-2013-03-478255
Contact: Laurence J.N. Cooper, The University of Texas MD Anderson Cancer Center, Houston, Texas
e-mail: ljncooper@mdanderson.org
2. Flomenberg, N. *et al. Blood* **104**, 1923–1930 (2004)
3. Torikai, H. *et al. Blood* **119**, 5697–5705 (2012)
4. Porter, D.L. *et al. N. Engl. J. Med.* **365**, 725–733 (2011)
5. Kalos, M. *et al. Sci. Transl. Med.* **3**, 95ra73 (2011)
6. McCallister, E. *BioCentury* **20**(33), A7–A8; Aug. 13, 2012

COMPANIES AND INSTITUTIONS MENTIONED

Abramson Cancer Center at the University of Pennsylvania, Philadelphia, Pa.
National Institutes of Health, Bethesda, Md.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pa.
Sangamo BioSciences Inc. (NASDAQ:SGMO), Richmond, Calif.
University of California, Berkeley, Calif.
University of Pennsylvania, Philadelphia, Pa.
The University of Texas MD Anderson Cancer Center, Houston, Texas
Weizmann Institute of Science, Rehovot, Israel