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TOOLS & TECHNIQUES

ZIOPHARM BETS ON THE HLA-A ODDS

By Michael Leviten, Senior Writer

Ziopharm Oncology Inc. is adding gene editing to its repertoire with a strategy to increase the odds of finding a match for hematopoietic stem cell transplants. While the system is still short of an allogeneic supply, the company believes it can go beyond the one donor-one recipient paradigm and employ a single donor's cells to benefit multiple patients.

In a study published last month in *Scientific Reports*, the company presented its case that by eliminating the HLA-A gene from donor stem cells it could increase patients' chances of finding a match, and showed that engineered stem cells lacking HLA-A could engraft and differentiate into mature blood cells following transplantation in mice.

CEO Laurence Cooper told BioCentury one of the big goals in transplantation is to make off-the-shelf cells that "are made in advance of their need and then deployed on demand." But those cells are often genetically dissimilar between donor and patient, so it becomes a "question of will those cells survive for long enough before they'll be rejected."

In earlier work performed while a professor at the University of Texas MD Anderson Cancer Center, Cooper partnered with Sangamo BioSciences Inc. to use its zinc finger nuclease (ZFN) platform to eliminate expression of TCR and HLA-A genes in T cells. The collaborators applied the technology to chimeric antigen receptor (CAR)-expressing T cells specific for CD19, and showed the cells retained antitumor activity *in vitro*. Cooper joined Ziopharm last May.

The next step, he said, was to show the cells can work *in vivo*. "In the off-the-shelf technology version 2.0, which is where we are now, the question is can you not only knock out the TCR, but can you make the cells engraft for the long term without having to immunocompromise the patients?"

The key factor determining whether the transplant will engraft is the match between patient and donor histocompatibility markers, which are cell surface proteins encoded by HLA genes and represent the main way immune cells distinguish self from foreign cells.

The human genome has six primary HLA loci, in which MHCI genes include HLA-A, HLA-B and HLA-C, and MHCII genes include HLA-DR. The most prevalent form of HLA-DR is HLA-DRB1, and the gold standard for compatibility between donor and host cells is to obtain a match for seven out of eight alleles of HLA-A, HLA-B, HLA-C and HLA-DRB1.

Cooper told BioCentury that as head of bone marrow transplants at MD Anderson he became familiar with the complexity of HLA genetics, and worked with the National Marrow Donor Program (NMDP) in the U.S.

Through discussions with the program he realized that by using the database to look at the statistics, he could increase the odds of matching recipients to donors if he could manipulate which HLA genes were expressed.

That analysis showed that not only could elimination of HLA-A remove a significant percentage of mismatches, but that it could in particular benefit some racial minorities, for whom the odds of finding a match are very low.

For example, in sickle cell disease which largely affects patients of African descent, eliminating HLA-A could increase the chances of finding a donor from 17% to 73%.

According to Sangamo VP of Research Michael Holmes, increasing those chances is a key benefit.

"I think the big thing that's really new is the idea of specifically targeting the HLA-A locus as a way to improve the ability to match donors to recipients where their HLA repertoire isn't

well covered within the database," he said. "I think that is the real novelty."

THE A-GAME

In its analysis of the NMDP database, Ziopharm looked at about nine million donor entries. For European Caucasians, the probability of finding a match for eight out of eight alleles was above 70%, whereas for other races it ranged from about 20% to 50%.

The team calculated the probability of finding a match for six out of six alleles after removal of HLA-A, HLA-B, HLA-C or HLA-DRB1. Removing HLA-A had the biggest impact, increasing the probability to a range of about 50% to 95% and producing a major increase for most racial minorities investigated.

Next, the researchers used an artificial pair of ZFNs from Sangamo that bind and cleave within the third exon of HLA-A, producing a mutant gene that is not expressed, and eliminated HLA-A in a $CD34^+$ subpopulation of stem cells from fresh human umbilical cord blood (UCB).

Using *in vitro* colony-forming assays, the team showed the HLA-A-modified stem cells could generate many of the normal hematopoietic lineages.

Finally, the team injected the edited cells into irradiated mice, and showed after 16 weeks they had engrafted in peripheral blood, bone marrow and spleen with the same efficiency as standard hematopoietic stem cells. Using lineage-specific markers, the researchers showed the engrafted cells differentiated *in vivo* to produce human myelocytes, monocytes and T cells.

Cooper told BioCentury that eliminating only HLA-A is safer than removing all the genes to make HLA-null cells because the cells would be attacked by natural killer (NK) cells.

"By being nuanced and just knocking out HLA-A and leaving behind B and C, not only do we reduce the number of donors that are needed to give maximum numbers of recipients for off-the-shelf cells, but we make a product that is both HLA-matched and resists killing by resident NK cells," he said.

GAME: SET AND MATCH

Emile Nuwaysir, president and COO of Cellular Dynamics International Inc., told BioCentury, "What this paper really does is it shows that you can expand the donor pool. You can edit them so that in theory you could take existing bone marrow samples and apply them to more patients that do not necessarily have a match within the registry."

He noted that successfully editing HLA is in itself "a pretty big step." In addition, he said it was a good choice to use zinc finger proteins because they have already been used in the clinic and have a GMP-compliant manufacturing method.

That should provide a clean and relatively straightforward regulatory path, he said.

Cellular Dynamics was acquired by Fujifilm Holdings Corp., and is using induced pluripotent stem (iPS) cells to build HLA-matched cell banks for autologous cell therapies.

However, Nuwaysir noted that while the technical steps are significant, the technology doesn't dramatically solve the supply problem because the technology hasn't yet been developed to expand the cells. "You are still faced with that basic roadblock, which is you can really only put one donor sample into one patient or a few patients," he said. "It's not a cell line; it's a population of cells you can engraft."

> "What this paper really does is it shows that you can expand the donor pool."
>
> Emile Nuwaysir, Cellular Dynamics

Donor cells can't be expanded because the process alters their biological properties, causing them to lose multipotency and become more lineage-directed, he said.

Cellular Dynamics is also playing the odds with HLA-typing to reduce the chances of a mismatch. The company has identified "super donors" with naturally low HLA diversity based on having the same HLA allele on each chromosome pair at many of the HLA loci.

Last year, the company told BioCentury its first two super donors collectively match about 19% of the U.S. population, and said iPS cells derived from them can be differentiated into at least 15 different cell types.

International Stem Cell Corp. is also making HLA-homozygous stem cells, but is using a parthenogenetic strategy that involves chemically stimulating an unfertilized oocyte to duplicate its 23 chromosomes, resulting in what the company terms "a variant of embryonic stem cells" containing a diploid set of 46 chromosomes in which all genes are homozygous.

Chris Parker, EVP and CBO of Cellular Dynamics, said the Ziopharm findings could benefit the iPS strategy as well. "This article demonstrates that you can apply genetic engineering concepts to an engraftable population of cells."

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>
> Emile Nuwaysir, Cellular Dynamics

That's important, he said, because it's not yet been possible to get iPS-manufactured hematopoietic stem cells to engraft. "When you compare a cell that's manufactured to one that's derived from the bone marrow, they're identical by marker expression. But when you put the stem cell-derived hematopoietic stem cells into an animal they don't engraft, whereas a derived bone marrow cell will."

Cooper said that the company is considering how far it can take the technology, and told BioCentury, for example, "a company like mine would be quite interested in off-the-shelf NK cells."

He said Ziopharm is not yet prepared to reveal its strategic plan, but that with its partner Intrexon Corp., is interested in creating off-the-shelf cells that are "universal and long-lived."

COMPANIES AND INSTITUTIONS MENTIONED

Fujifilm Holdings Corp. (Tokyo:4901), Tokyo, Japan
International Stem Cell Corp. (OTCQB:ISCO), Carlsbad, Calif.
Intrexon Corp. (NYSE:XON), Germantown, Md.
National Marrow Donor Program (NMDP), Minneapolis, Minn.
Sangamo BioSciences Inc. (NASDAQ:SGMO), Richmond, Calif.
University of Texas MD Anderson Cancer Center, Houston, Texas
Ziopharm Oncology Inc. (NASDAQ:ZIOP), Boston, Mass.

TARGETS AND COMPOUNDS

HLA - Human leukocyte antigen
HLA-A - Major histocompatibility complex class I A
HLA-B - Major histocompatibility complex class I B
HLA-C - Major histocompatibility complex class I C
HLA-DR - Major histocompatibility complex class II DR
HLA-DRB1 - Major histocompatibility complex class II DR β1
MHCI - Major histocompatibility complex class I
MHCII - Major histocompatibility complex class II
TCR - T cell receptor

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