Engineered T cells behind enemy lines in solid tumors

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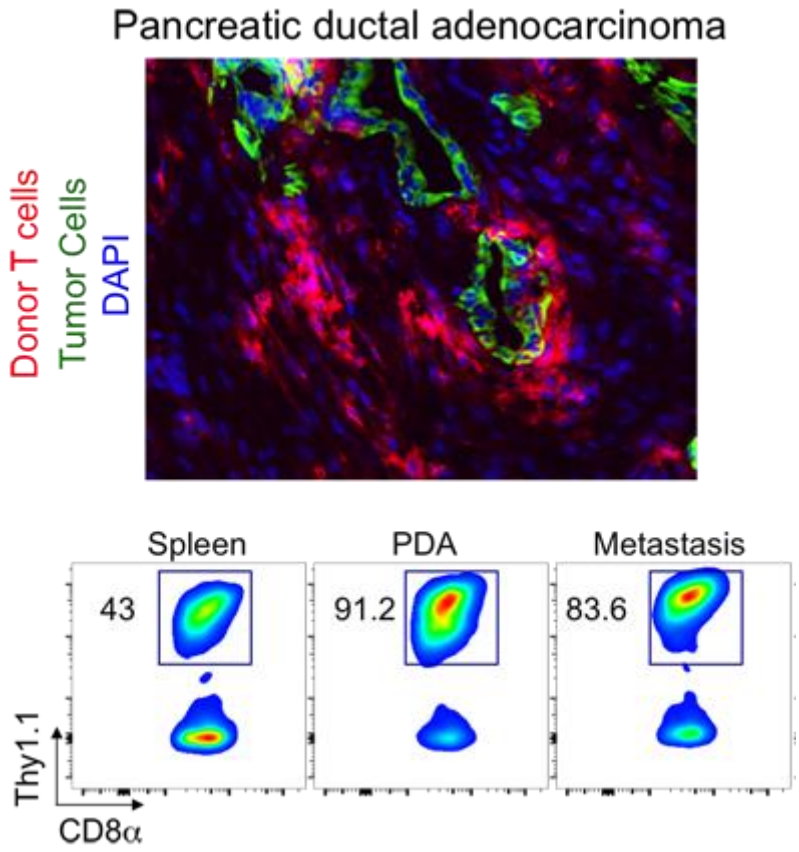

Immunofluorescent microscopy demonstrates that T cells engineered to target MSLN infiltrate solid PDA tumors. Engineered donor T cells shown in red are in the immediate vicinity of MSLN expressing tumor cells shown in green. Engineered T cells can alternatively be detected using flow cytometry in PDA primary tumors as well as in metastatic lesions.

Image provided by Dr. Ingunn Stromnes (Clinical Research Division).

The human immune system recognizes non-native antigens and targets them for destruction. This mechanism recognizes viruses, bacteria, fungi, transplanted tissue, and even cancers. In cancer, transformed cells must escape detection by the immune system and continue proliferating to become clinically relevant tumors. Different cancers exploit unique mechanisms to prevent detection including: low mutation rates, physical barriers, expression of immunosuppressive proteins, prevention of antigen processing/presentation, and growth in immune privileged sites. These adaptations must be overcome when developing immunotherapies. Some of the first successful T cell based therapies surmounted the issues of physical barriers and immune privilege growth sites by targeting B cell malignancies, which do not form solid tumors and circulate in the same environment as T cells. On the heels of these successes in leukemia, scientists in the Greenberg

and Hingorani Laboratories in the Clinical Research Division have demonstrated that engineered T cells are also effective against solid tumors. In a recent publication in *Cancer Cell*, these researchers engineered T cells to target pancreatic ductal adenocarcinoma (PDA) in a genetically engineered mouse model. Targeted immunotherapy nearly doubled the survival time in these preclinical studies.

PDA is an incredibly difficult target for immunotherapy: it is genetically stable with few mutations or chromosomal rearrangements, builds stromal barriers that limit perfusion, and often expresses inhibitory T cell ligands. One of the keys to achieving success in this study was choosing the correct T cell antigen. Previous studies found mesothelin (MSLN), a cell adhesion protein that promotes invasiveness, to be an attractive target in PDA, and explored immunization against it. In this study, they verified in their mouse model of PDA that MSLN is rarely expressed in adult tissues and is consistently expressed in all stages of PDA. To isolate the highest affinity T cell receptor (TCR) against this native protein, mice were immunized with eight different peptides and T cells were isolated. After identifying the T cells with highest affinity for MSLN and elevated cytokine production, researchers sequenced the TCR expressed in these cells.

With this high affinity TCR in hand, they found it functioned independent of the CD8 co-receptor, suggesting either CD8 or CD4 T cells could be engineered, however, only CD8 T cells were used in this study. Engineered T cells were injected intravenously into mice bearing spontaneous PDA tumors and were found to concentrate in the tumor compared to spleen. Once in the tumor region, the cells induced tumor apoptosis and loss of extracellular matrix – one of the barriers to immune access to tumors. These results were observed eight days after infusion, yet after 28 days, while engineered T cells concentrated in the tumor, the total number of T cells had significantly decreased. Phenotypic and functional characterization of T cells 28 days after infusion demonstrated signs of immune dysfunction. Some level of repression is expected in cells with chronic TCR signaling, however, non-activated engineered T cells also experienced immunosuppression. This suggests the tumor microenvironment was contributing in part to these observations.

The simple solution for the failure of engineered T cells to proliferate is to administer more cells, yet it is possible the first exposure may activate immune regulatory pathways in the tumor, rendering the second exposure useless. Surprisingly, when mice were dosed at 0 and 20 days, then analyzed 28 days after initial injection the engineered T cell population in tumors was 10 fold higher than with the single dose. Moreover, the second dose of engineered T cells demonstrated minimal signs of immune supression compared to cells persisting from the first dose. Having learned from these studies, researchers enrolled PDA bearing mice in a preclinical trial administering engineered T cell infusions every two weeks. Under this regimen 62.5% of mice experienced a decrease in tumor

volume, and overall survival time was nearly doubled. Moreover, there was a 20-30% decrease in metastatic disease and, as Dr. Stromnes, a research associate in the Greenberg Lab said, "We did not observe detectable clinical symptoms or histopathological evidence for toxicity with this approach."

In order to move forward into human studies the team also identified a high-affinity TCR targeting the human sequence of MSLN. However, they are not stopping there. According to Dr. Stromnes, "Obviously, it is wonderful to be part of a team that will translate this work to patients as soon as possible. However, I am certain we can do better. We are currently testing ways to further engineer the T cells for sustained benefit, including co-opting factors in the immunosuppressive environment to paradoxically sustain T cell survival, persistence and function rather than interfere with it." Another possibility suggested by Dr. Stromnes is targeting multiple antigens, "The Greenberg lab already has T cells in clinical trials targeting Wilms' tumor antigen (WT1), and WT1 is also expressed in PDA." Likewise, work done with CAR T cells in the Riddell lab may also benefit affinity enhanced TCR therapy, "Modulating the T cell subsets that are engineered for therapy, including provision of CD4 helper T cells, may also greatly improve upon efficacy of this approach." said Dr. Stromnes.

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Stromnes IM, Schmitt TM, Hulbert A, Brockenbrough JS, Nguyen HN, Cuevas C, Dotson AM, Tan X, Hotes JL, Greenberg PD, Hingorani SR. (2015). T Cells Engineered against a native antigen can surmount immunologic and physical barriers to treat pancreatic ductal adenocarcinoma. *Cancer Cell*. 28(5):638-52.