Enhanced cancer immunotherapies through cytokine-labeled T cells

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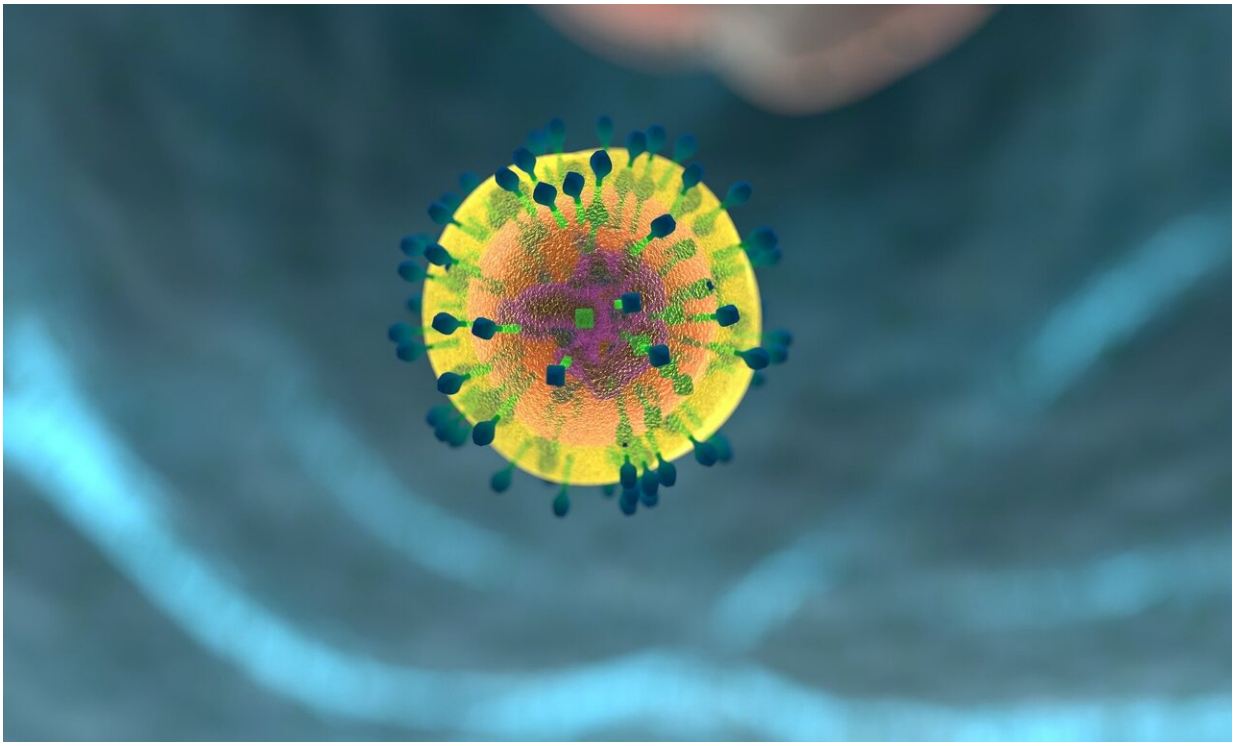

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The recent years have seen a wave of adoptive cell therapies (ACTs), a type of immunotherapy in which T cells (T cell transfer therapy) and other immune cells are obtained from patients, activated and multiplied outside the body, and infused in larger numbers back into the blood circulation to help fight cancers.

In the successful version of ACT known as CAR-T cell therapy, immune oncologists, in addition, genetically engineer a chimeric antigen receptor (CAR) into T cells that with one of its parts binds a specific tumor cell type and with another helps unleash the T cell's tumor cell-destroying activity.

CAR-T cell therapies have advanced into clinical practice to treat tumors of the immune system, such as leukemias and lymphomas, and more recently multiple myeloma, which affects white blood cells in the bone marrow. However, T cell transfer therapies have not yet been successfully applied to solid tumors because T cells do not easily penetrate solid tumor masses and persist long enough in them, and because their activity is muted by an immune-suppressive tumor microenvironment.

One way to overcome these limitations could be to pair T cell transfer therapies with cytokine therapy. Cytokines are small proteins that are secreted by certain immune cells and can enhance tumor-destroying activities of other immune cells, including transferred T cells. A serious downside of this approach, however, are significant side-effects resulting from cytokines freely circulating in the body, leading to toxicity and potentially lethal inflammatory syndromes. In addition, despite the risks cytokines pose when given systemically, they often are cleared too fast to produce the desired cancer therapeutic effects.

Now, a research collaboration at the Wyss Institute for Biologically Inspired Engineering at Harvard University, Harvard John A. Paulson School of Engineering and Applied Sciences (SEAS), and Dana-Farber Cancer Institute (DFCI) has developed a nanotechnology-driven solution to these problems. The method utilizes an unnatural sugar that is taken up and integrated into the T cells' external coating, which can then be used to anchor cytokines.

The concentrated cytokines locally enhance T cell functions without producing unwanted systemic side-effects. In mice with melanoma, an aggressive solid tumor type, the approach also stimulated the host immune system against tumor cells, which inhibited tumor growth. As an add-on to CAR-T cell therapy, it allowed complete regression of lymphoma tumors at otherwise non-curative cell doses. The results are published in the *Proceedings of the National Academy of Science*.

"The results that we see suggest a major step toward developing ACTs with efficacy against solid tumors and ACTs that work more consistently against a variety of blood cancers," said senior author David Mooney, Ph.D., a Founding Core Faculty member of the Wyss Institute and the Robert P. Pinkas Family Professor of Bioengineering at the Harvard John A. Paulson School of Engineering and Applied Sciences. "Our approach can be easily scaled and integrated with the processes currently used to manufacture therapeutic T cells, including CAR-T cells, and thus could have a relatively short path into clinical application."

Mooney's combined their bioengineering expertise with that of cancer immunologist Kai Wucherpfennig, M.D., Ph.D. Wucherpfennig is Director of DFCI's Center for Cancer Immunotherapy Research, Professor of Neurobiology at Brigham and Harvard Medical School, and an Associate Member of the Broad Institute of MIT and Harvard.

Sugar plus cytokine equals enhanced T cell therapy

To be able to track cancer reactive dendritic cells, which orchestrate a broader immune response in lymph nodes, Mooney's group had previously developed a biomaterials-based method that allowed them to attract the cells into a 3-D scaffold in living animals, where they took up a synthetic reactive sugar molecule and used it as a building unit for the complex sugar chains on the cell surface.

"In our new study we similarly harnessed the natural sugar metabolism of cells, but delivered the reactive azido sugar to T cells via nanoparticles in a culture dish. The cells' sugar metabolism utilizes the sugar and metabolically integrates it into the complex sugar chains on the cell surface," said first-author Yutong Liu, who is a graduate student working with Mooney.

"In a second step, using click-chemistry, we then exploited the sugar molecules' azido group to link specific cytokine molecules that are modified with a highly compatible chemical group [DBCO] to them. Only having to add sugar-containing nanoparticles and later the cytokines to the culture media makes the method extremely simple and fully compatible with the adoptive cell manufacturing pipeline."

After optimizing the conjugation process with a collection of cytokines in cultured T cells, and ensuring that the cells' viability and general functions were not affected, the team tested their approach in mice burdened by solid melanoma tumors. They found that melanoma-specific T cells carrying the anti-tumor cytokine interleukin-12 (IL-12) at non-curative cell doses significantly delayed the growth of the tumors, and prolonged the animals' life span by 50%. In comparison, the same number of melanoma-specific T cells adoptively transferred along with a systemic injection of IL-12 produced much weaker effects.

The adoptively transferred T cells also had an improved ability to survive and differentiate into tumor-destroying cells in the animals and involved other T cell types and immune cells with roles in a broader immune response against tumors. "We saw far greater increases in T helper and cytotoxic T cells both in dissected tumors and spleens of animals that received melanoma-specific metabolically labeled T cells conjugated with IL-12 than in our control conditions, and clear signs that these had increased tumor-specific activities," said Liu.

The researchers think that part of the explanation could be that dendritic cells (DCs), which are key orchestrators of the broader tumor-directed immune response, were more strongly stimulated by melanoma-specific T cells with conjugated IL-12 than T cells without IL-12.

"We think that our approach could enhance a tumor-specific immune cycle. First, the adoptively transferred IL-12-conjugated T cells differentiate and kill a subset of tumor cells, resulting in the release of different tumor-specific antigens that are taken up and processed by DCs, which present them to other tumor-specific T cells in nearby lymph nodes that then also invade the tumors and directly contribute to tumor cell killing and the spread of yet more antigens," hypothesized Liu.

The antigen-spreading effect observed by the team could be very relevant for the treatment of solid tumors which often have a highly heterogenous cell composition and thus are more difficult to attack by targeting just one antigen.

In the last piece of their study, the researchers took their T cell approach to CAR-T cell therapy in a mouse xenograft lymphoma model. Metabolically labeled CAR-T cells with conjugated IL-12 were able to control the development of tumors and prolong the survival of mice that before had been injected with lymphoma cells, and at doses at which CAR-T cells lacking IL-12 were not able to cure the animals.

"The straight-forward and elegant nature of new approach to cancer immunotherapy offers huge potential for cancer patients. We are excited to support this effort through the Wyss Institute's high-priority Validation Project program, which will hopefully expedite its advancement towards the clinic," said Wyss Founding Director Donald Ingber, M.D., Ph.D. who is also the Judah Folkman Professor of Vascular Biology at Harvard Medical School and Boston Children's

Hospital, and the Hansjörg Wyss Professor of Bioinspired Engineering at SEAS.

More information: Yutong Liu et al, Cytokine conjugation to enhance T cell therapy, *Proceedings of the National Academy of Sciences* (2022). DOI: 10.1073/pnas.2213222120

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