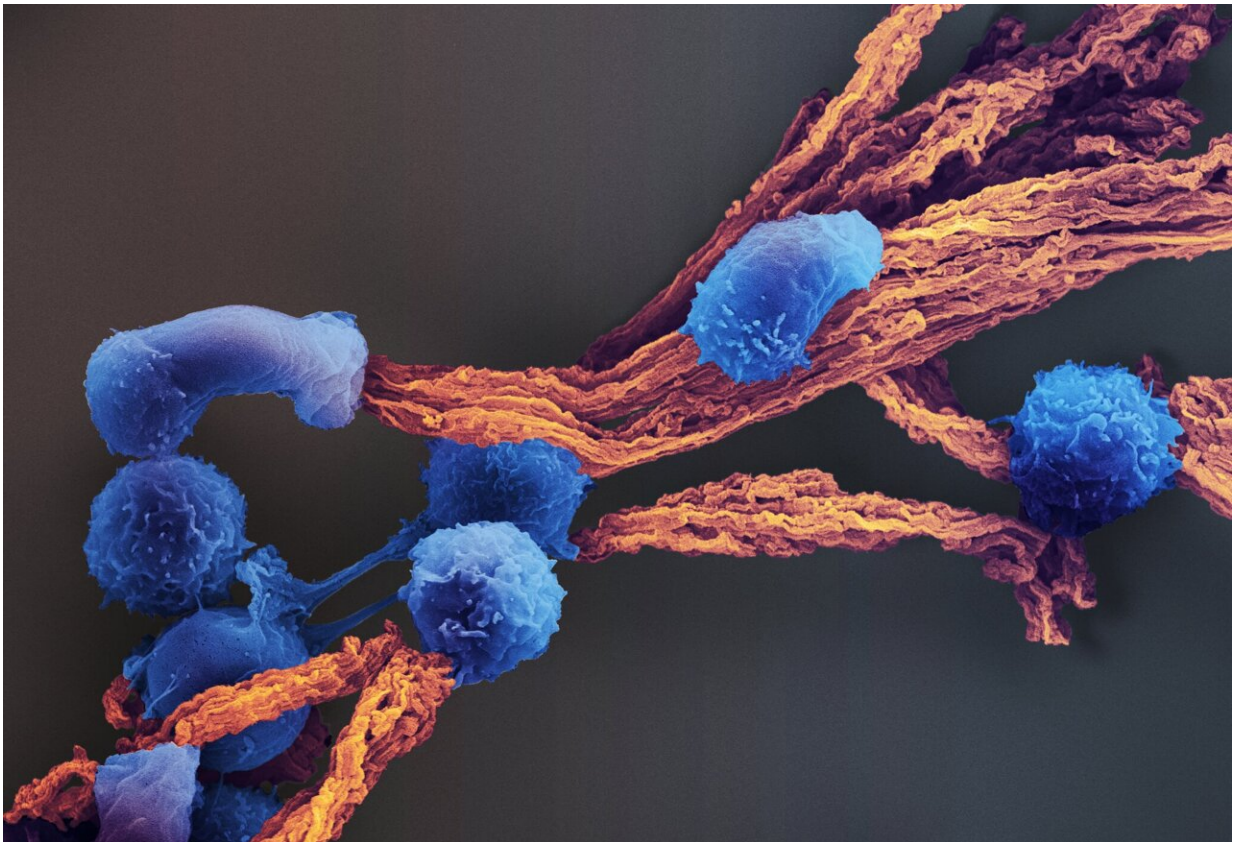# CAR-T cell cancer immunotherapy gets personal

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A new study performed in collaboration by David Mooney's team at the Wyss Institute and Harvard SEAS and Catherine Wu's team at the DFCI used these artificial antigen-presenting cell-mimicking scaffolds (APC-ms) to create personalized CAR-T cell products from patients' T cells. Credit: Wyss institute at Harvard University

New adoptive T cell therapies—in which T cells, the immune system's natural hunters patrolling the body for foreign adversaries, are retrieved from cancer-riddled patients, super-charged and amplified outside the body, and then infused back into the same patient—are changing the prospects of cancer patients.

Since 2017, when CAR ([chimeric antigen receptor](#))-T cells were green-lighted as the first modified therapeutic cells by the Federal Drug Administration (FDA) to treat leukemia, five similar products have since been approved and more than 20,000 people have been treated with this game-changing immunotherapy.

CAR-T cells are engineered to carry synthetic membrane-spanning receptor molecules that use their outside-facing portion to bind to antigens on cancer cells, which their inside-facing portion responds to by switching on a powerful tumor cell-destroying program. However, not all patients respond equally well to CAR-T cell therapies, and cancer immunologists have been trying to figure out what makes them work well or fail.

Despite a budding understanding of differences between cancer patients' T cells and healthy individuals' T cells, these insights have not been taken into account in CAR-T cell manufacturing processes. All processes use a similar type of stimulation with T-cell specific agonists and general immune-stimulating cytokines to create infusible CAR-T cell products, irrespective of variations in the original T cells' phenotype.

Now, a collaboration between bioengineers at the Wyss Institute for Biologically Inspired Engineering at Harvard University and Harvard John A. Paulson School of Engineering and Applied Sciences (SEAS) led by David Mooney, Ph.D. and cancer-immunologists at the Dana-Farber Cancer Institute (DFCI) led by Catherine Wu, M.D., Ph.D. has demonstrated that personalizing CAR-T cell stimulation during

manufacturing can significantly enhance the consistency and potency of the resulting CAR-T cell products.

By using artificial antigen-presenting cell mimicking scaffolds (APC-ms), the team was able to fine-tune the levels of T cell stimulation to match the phenotype of T cells obtained from leukemia patients, and significantly enhanced their ex vivo and in vivo tumor-clearing abilities. The findings are published in *Nature Communications*.

"We show that CAR-T cell products made from T cells derived from cancer patients are generally less functional than CAR-T cells products derived from healthy individuals," said Founding Wyss Core Faculty member Mooney.

"Matching the CAR-T cell antigen-stimulation dose to the phenotype of patients' T cells using a precisely controllable biomaterials approach that closely mimics natural antigen presentation can significantly improve their function. This approach could further personalize CAR-T cell therapy and remove an existing inadequacy from current T cell manufacturing."

Mooney also is Robert P. Pinkas Family Professor of Bioengineering at SEAS, and a lead of the Immunomaterials to Improve Immunotherapy (i3) Center coordinated at the Wyss Institute. This project was conceived at the Center, and Wu is one of its Principal Investigators.

Cutting the keys for personalized CAR-T therapies

The team investigated the phenotypes of T cells that they isolated from samples obtained from patients suffering from acute lymphoblastic leukemia (ALL) and chronic lymphoblastic leukemia (CLL), as well as from healthy donors. Next, they utilized APC-ms to provide the T cells with different doses of anti-CD3/anti-CD28 antigen stimulation and thus

created a CAR-T cell library.

All CAR-T cell products contained in the library were then probed again for functional differences, including their ability to kill [cancer cells](link) in vitro. The researchers directly compared their approach with one that is commonly used in CAR-T cell manufacturing, which presents the same antigens on rigid magnetic beads (Dynabeads) to T cells.

A key finding was that cancer patients' T cells were much more easily over-stimulated at antigen doses commonly used during CAR-T cell manufacturing than "healthy" T cells. This made them lose their functionality, or become more "exhausted" as immunologists say, and decreased their ability to proliferate. CAR-T cells not only need to be transformed into a functional state but also amplified by millions to be able to eliminate tumor cells and metastasis in the entire body.

"By exploring a precise, narrow range of stimulation doses made possible with APC-ms, we show that there is something like a personalized 'sweet spot' for patient-derived T cells that maximizes functionality and amplification, which is, on average, lower than the usual doses," said first-author David Zhang, who is a graduate student on Mooney's team.

"The APC-ms approach functions much more naturally than Dynabeads, because highly controllable levels of T-cell signals are embedded into a lipid bilayer, which allows the CAR-T cells to push and pull at them as just as T cells usually do across the 'immunological synapse' between them and antigen-presenting cells when T cell stimulation is at its best."

From in vitro studies to cell manufacturing

While the team did not observe any significant differences between CAR-T cells created from ALL and CLL patient samples, overall their

approach generated more cells with high cytotoxic potential toward tumor cells, a more balanced ratio between cytotoxic CD8^{+} T cells and CD4^{+} T cells that support their function, and more memory T cells that themselves are not cytotoxic but can be activated in later responses.

In a mouse in vivo study, infused CAR-T cell products created with different levels of stimulation also exhibited significantly different abilities to control CD19-expressing Burkitt's lymphoma, with cells again stimulated at lower than usual levels during manufacturing showing the strongest potential.

"We constructed a proof-of-concept model that is based on the quantifiable relationship between the phenotype of a T cell blood sample and its CAR-T cell products, and that outputs an optimal T cell stimulation dose for personalized CAR-T cell production," said Wu.

"Given that T cell samples are always fingerprinted for important markers at the beginning of the cell manufacturing process, similar strategies could be devised to further personalize the therapy using the APC-ms approach." Wu is the Lavine Family Chair, Preventative Cancer Therapies at DFCI, and Professor of Medicine at Harvard Medical School.

"Dave Mooney's team in the Wyss' Immunomaterials platform is pushing the envelope of CAR-T cell and other immunotherapies using entirely new engineering and materials-based approaches. Hopefully, this will eventually enable us to also mobilize the immune system against recalcitrant solid tumors for which no therapies exist yet. It's also a great example of where less is more," said Wyss Founding Director Donald Ingber.

  More information: David K. Y. Zhang et al, Enhancing CAR-T cell functionality in a patient-specific manner, *Nature Communications*

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