Study offers a potential strategy to improve T cell therapy in solid tumors

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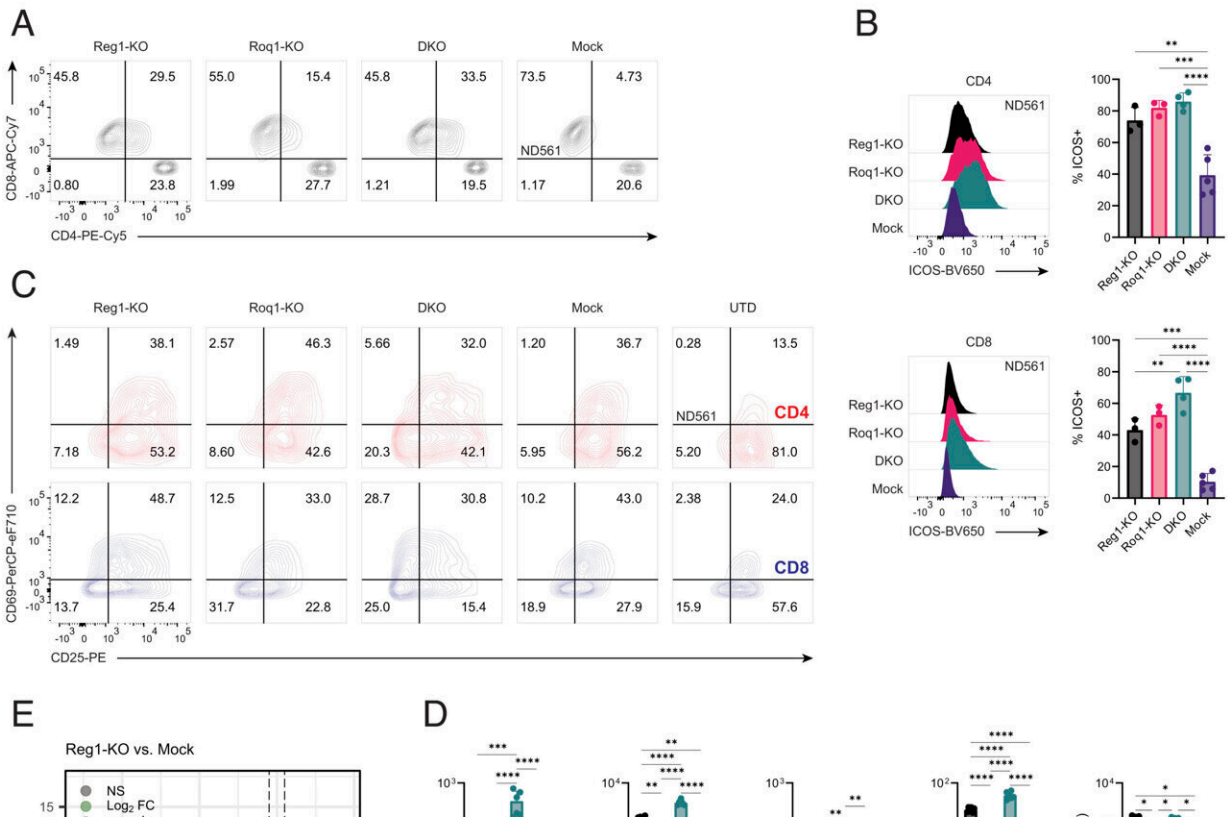

Regnase-1 and Roquin-1 double knockout alters the activation profile of resting engineered T cells. (A) Healthy human donor T cells were subject to CAR-T cell expansion and cryopreserved. Thawed CAR-T cells were rested overnight in media supplemented with cytokines before flow cytometry analysis of CD4 and CD8. Knockout of Regnase-1 and/or Roquin-1 increased expression of CD4, an activation marker, on CD8 T cells. Plots shown are representative of three independent donors. (B) Expression of ICOS, a costimulatory receptor, from CAR-T cells rested overnight with cytokines. Left flow plots shown are

representative of three independent donors. Right graphs show percentage of ICOS+ T cells summarized among at least three independent donors. Top and Bottom figures refer to CD4 and CD8 T cells, respectively. Error bars represent SD. One-way ANOVA was used for analysis followed by Tukey's multiple comparisons test. (C) Expression of activation markers CD25 and CD69 on CD4 (red) or CD8 (blue) CAR-T cells and UTD controls after coculture with mesothelin-negative K562 cells overnight. Plots shown are representative of two independent experiments from two independent donors each performed in triplicate. (D) Secretion of Th1 and inflammatory cytokines (IL2, IFNg, TNFa, IL6, and GM-CSF) measured via Luminex assay. CAR-T cells and untransduced (UTD) T cell controls were cocultured with mesothelin-negative K562 cells overnight, and supernatants were collected for analysis. Data shown are pooled from two independent experiments from two independent donors each performed in triplicate. Error bars represent SD. One-way ANOVA was used for analysis followed by Tukey's multiple comparisons test. (E) Volcano plots showing differentially expressed genes between Regnase-1, Roquin-1, and double knockout versus mock engineered T cells (pooled n = 2 CAR-T and n = 1 TCR-T for total n = 3 independent donors) after thaw and overnight rest with cytokines. Genes that are statistically significant (adjusted $P \leq 0.005$) and have a Log2FC ≥ 1 are shown in red. Statistical significance was calculated using the Wald test with Benjamini–Hochberg multiple testing correction. (F) Expression levels of various inflammatory genes, including Th1 cytokines (IL2, IFNg, TNFa), activation markers (CD25, CD69, CD40LG, PD1, LAG3), and costimulatory receptors (CD28, ICOS). Values shown are log normalized transcript counts of bulk RNA sequencing data from three independent donors (n = 2 CAR-T and n = 1 TCR-T). Box plots show medians, interquartile ranges, minimum, and maximum values. Not shown = not significant, *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$. Credit: *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2218632120

A new approach that delivers a "one-two punch" to help T cells attack solid tumors is the focus of a preclinical study by researchers from the Perelman School of Medicine at the University of Pennsylvania. The findings, published in the *Proceedings of the National Academy of*

Sciences (PNAS), showed that targeting two regulators that control gene functions related to inflammation led to at least 10 times greater T cell expansion in models, resulting in increased antitumor immune activity and durability.

CAR T cell therapy was pioneered at Penn Medicine by Carl H. June, MD, the Richard W. Vague Professor in Immunotherapy at Penn and director of the Center for Cellular Immunotherapies (CCI) at Abramson Cancer Center, whose work led to the first approved CAR T cell therapy for B-cell acute lymphoblastic leukemia in 2017. Since then, personalized cellular therapies have revolutionized blood cancer treatment, but remained stubbornly ineffective against solid tumors, such as lung cancer and breast cancer.

"We want to unlock CAR T cell therapy for patients with solid tumors, which include the most commonly diagnosed cancer types," said June, the new study's senior author. "Our study shows that immune inflammatory regulator targeting is worth additional investigation to enhance T cell potency."

One of the challenges for CAR T cell therapy in solid tumors is a phenomenon known as T cell exhaustion, where the persistent antigen exposure from the solid mass of tumor cells wears out the T cells to the point that they aren't able to mount an antitumor response. Engineering already exhausted T cells from patients for CAR T cell therapy results in a less effective product because the T cells don't multiply enough or remember their task as well.

Previous observational studies hinted at the inflammatory regulator Regnase-1 as a potential target to indirectly overcome the effects of T cell exhaustion because it can cause hyperinflammation when disrupted in T cells—reviving them to produce an antitumor response. The research team, including lead author David Mai, a Bioengineering

graduate student in the School of Engineering and Applied Science, and co-corresponding author Neil Sheppard, DPhil, head of the CCI T Cell Engineering Lab, hypothesized that targeting the related but independent Roquin-1 regulator at the same time could boost responses further.

"Each of these two regulatory genes has been implicated in restricting T cell inflammatory responses, but we found that disrupting them together produced much greater anticancer effects than disrupting them individually," Mai said. "By building on previous research, we are starting to get closer to strategies that seem to be promising in the solid tumor context."

The team used CRISPR-Cas9 gene editing to knock out Regnase-1 and Roquin-1 individually and together in healthy donor T cells with two different immune receptors that are currently being investigated in Phase I clinical trials: the mesothelin-targeting M5 CAR (mesoCAR) and the NY-ESO-1-targeting 8F TCR (NYESO TCR). Neither engineered T cell product targets CD19, the antigen targeted by most approved CAR T cell therapies, as this antigen is not present in solid tumors.

After CRISPR editing, the T cells were expanded and infused in solid tumor mice models, where researchers observed the double knockout led to at least 10 times as many engineered T cells compared to disabling Regnase-1 alone, as well as increased antitumor immune activity and longevity of the engineered T cells. In some mice, it also led to overproduction of lymphocytes, causing toxicity.

"CRISPR is a useful tool for completely ablating the expression of target genes like Regnase and Roquin, resulting in a clear phenotype, however there are other strategies to consider for translating this work to the clinical setting, such as forms of conditional gene regulation," Sheppard said.

"We're certainly impressed by the antitumor potency that was unleased by knocking out these two non-redundant proteins in combination. In solid tumor studies, we often see limited expansion of CAR T cells, but if we're able to make each T cell more potent, and replicate them to greater quantities, we expect T cell therapies to have a better shot at attacking solid tumors."

Additional authors include Omar Johnson, Jordan Reff, Ting-Jia Fan, and John Scholler.

More information: David Mai et al, Combined disruption of T cell inflammatory regulators Regnase-1 and Roquin-1 enhances antitumor activity of engineered human T cells, *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2218632120

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