Genetic modifications improve the therapeutic efficacy of iPSC-derived CAR-T cells against solid tumors

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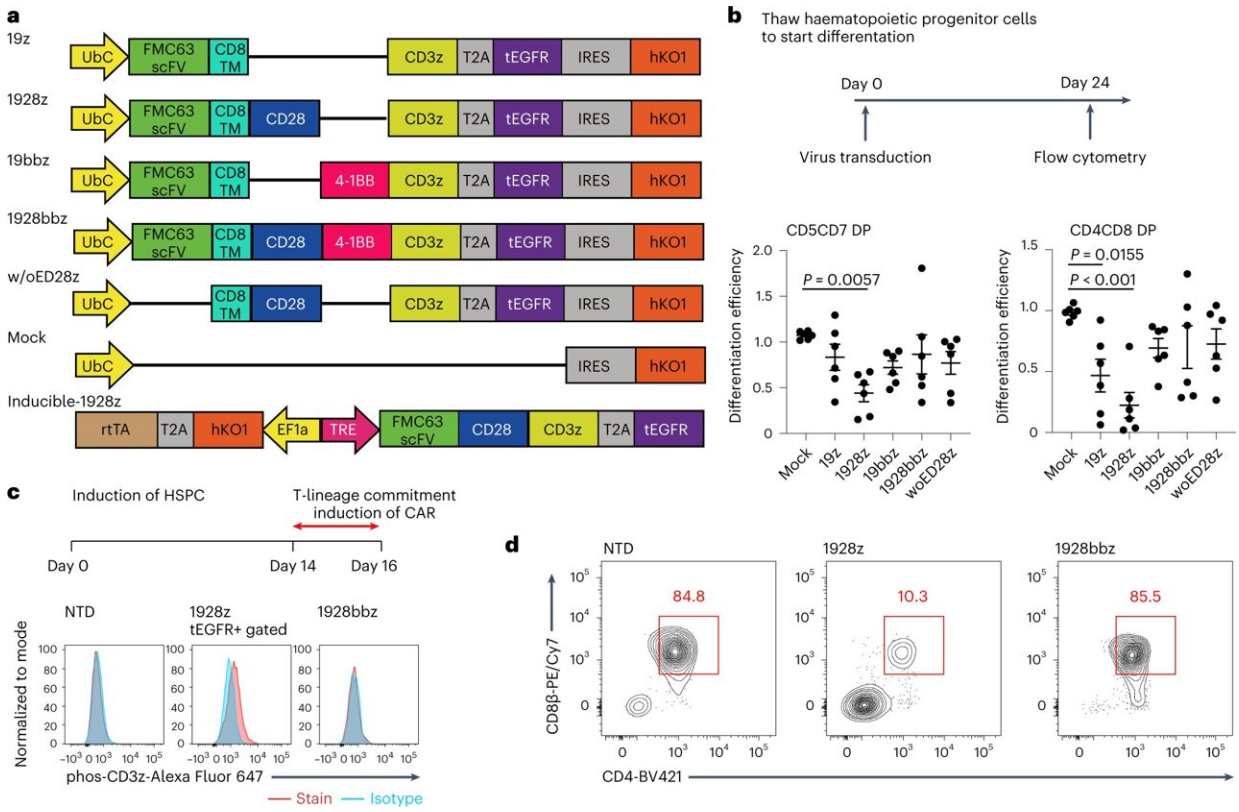

The CAR construct impacts the differentiation of CAR-iPSCs into T-cell lineages. a, Schematic presentation of the CAR constructs 19z, 1928z, 19bbz, 1928bbz and w/oED28z CAR (w/oED28z lacks the extracellular scFv domain of 1928z CAR). Each construct was inserted at the indicated internal promotor(s) of the lentiviral vector pCS. b, Comparison of surface antigen profiles of immature iCAR-T cells from various kinds of iCARs. Haematopoietic progenitor cells derived from iPSCs were transduced with CARs (see a) and

differentiated into T-cell lineages. To compensate for well-to-well variation, we used hKO1-negative cells in the same well as internal controls. Differentiation efficiencies were calculated as follows: CD5CD7 DP = (percentage of CD5CD7 DP cells in hKO1-positive cells)/(percentage of CD5CD7 DP cells in hKO1-negative cells); CD4CD8 DP = (percentage of CD4CD8 DP cells in hKO1-positive cells)/(percentage of CD4CD8 DP cells in hKO1-negative cells). Each dot represents biological replicates (n = 6). One-way ANOVA with Dunnett's multiple comparisons test. c, Haematopoietic progenitor cells derived from inducible 1928z or 1928bbz CAR-transduced T-iPSCs were divided into two groups and subsequently differentiated on FcDLL4 for 2 days in the presence (2 μg ml^{-1}) or absence of doxycycline. Representative FCM data representing the phosphorylation of CD3ζ in the two groups are shown. d, Representative FCM data comparing surface antigen expression of CD4 and CD8β on immature iCAR-T cells from 1928z-transduced and 1928bbz-transduced iPSCs. NTD, not transduced. Credit: *Nature Biomedical Engineering* (2022). DOI: 10.1038/s41551-022-00969-0

The Shin Kaneko laboratory at CiRA has developed a method to generate CAR-T cells from iPS cells (iCAR-T cells) that possess vastly improved anti-tumor activity in a mouse model of solid tumors.

In order to use T cells expressing chimeric antigen receptor (CAR) effectively in immunotherapy against solid tumors, it is critical for the intravenously administered CAR-T cells to accumulate and proliferate at the tumor site, where they will work continuously to target and eliminate the deleterious cancer cells.

The research group led by Professor Kaneko and Dr. Tatsuki Ueda, a former CiRA researcher now at the University of Chicago, began by examining whether different CAR constructs affected the differentiation of iPS cell-derived hematopoietic progenitor cells into T cells and ultimately selected one that did not have any negative impact on

differentiation efficiency.

Next, the team successfully showed in a solid tumor mouse model that two additional genetic manipulations of these iCAR-T cells—designed to augment T cell activation—enhanced their proliferation at the tumor site and extended their survival longer than ever shown before. Strikingly, the modified iCAR-T cells suppressed tumor growth and prolonged the recipient animals' survival even when only 1/20th of the original dose tested for unmodified iCAR-T cells was administered.

These modifications designed by Kaneko's team are expected to make the long sought-after practical use of iPSC-derived CAR-T cells for solid tumor treatment one step closer to reality.

The results of this study were published online in *Nature Biomedical Engineering* on December 12, 2022.

More information: Tatsuki Ueda et al, Optimization of the proliferation and persistency of CAR T cells derived from human induced pluripotent stem cells, *Nature Biomedical Engineering* (2022). DOI: 10.1038/s41551-022-00969-0

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