Targeting cold tumors using iPSC-derived CAR T Cells directed to the immune checkpoint molecule and tumor-associated antigen B7-H3

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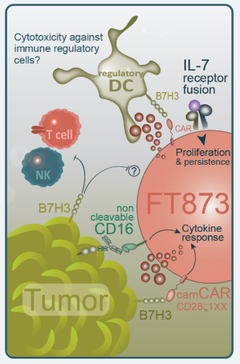
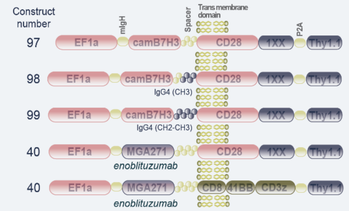
INTRODUCTION

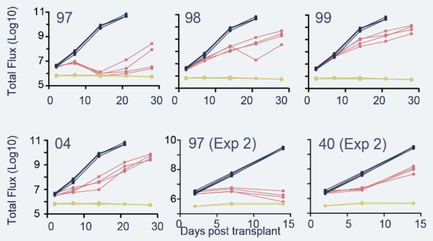
B7 homolog 3 protein (B7-H3) is a cell-surface protein that is broadly expressed on tumors as well as tumor-associated stromal cells, where it provides inhibitory signals to T and NK cells. Here, we present FT873, an iPSC-derived B7-H3 CAR T cell product, engineered with a single tricistronic expression cassette into the T-cell receptor α constant locus, for the treatment of solid tumors. This novel donor cassette encodes a VHH camelid antibody against B7-H3 fused to a CD28-CD3z-1XX signaling domain followed by an IL-7 receptor fusion protein and a high affinity non-cleavable version of the CD16 Fc receptor, providing the T cells with improved potency and fitness and, unlike naturally occurring T cells, the ability to mediate antibody-dependent cell-mediated cytotoxicity (ADCC).

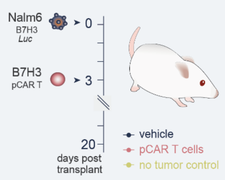


Camelid binder domains provide enhanced cytotoxicity

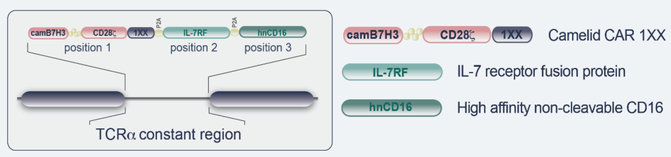


Binder optimization Primary T cells were transduced with various CAR constructs (left figure) and evaluated in vivo for their ability to suppress tumor growth (bottom figure). Primary CAR T cells expressing camelid binding domains fused via a short linker to CD28-CD3z-1XX signaling domains provided superior tumor control compared to all other constructs tested.

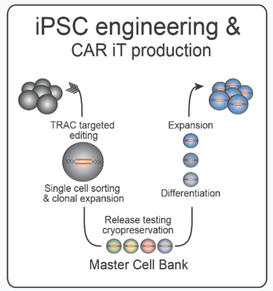


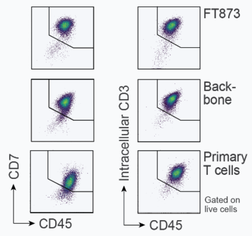


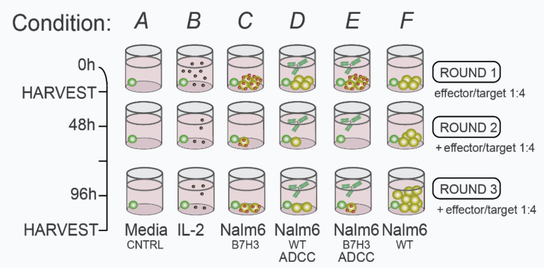
FT873 engineering strategy

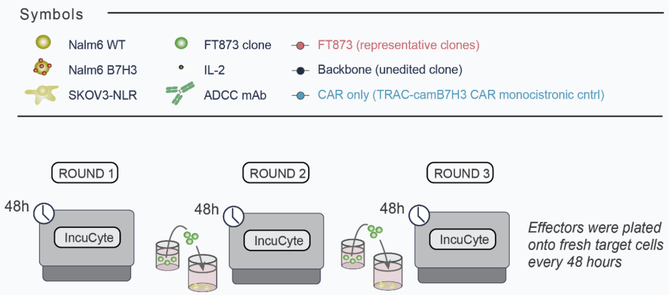


METHODS

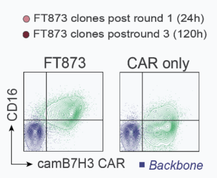


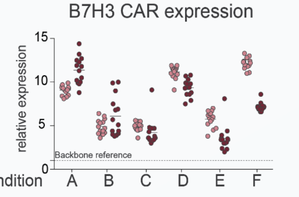


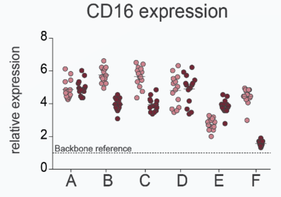




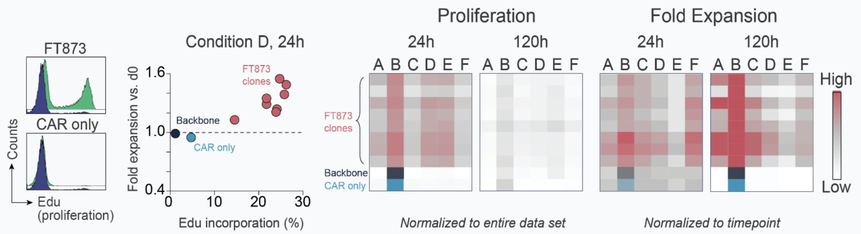
RESULTS



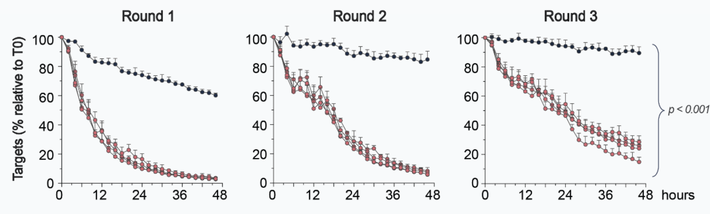


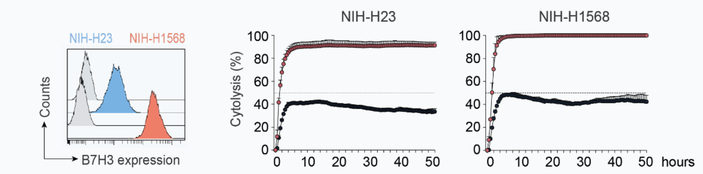


Left Function of FT873 clones was assessed in the presence or absence of tumor or cytokine support (see above). *Top*: CAR and CD16 expression relative to an unedited (backbone) clone. *Bottom*: Evaluation of IL-7RF function: FT873 clones showed improved proliferation and persistence relative to controls.



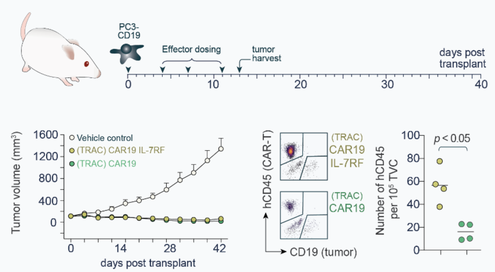
Right top Tumor killing under conditions with sustained high tumor burden. IncuCyte serial cytotoxicity assay with SKOV-3 ovarian tumors in the presence of herceptin for ADCC.





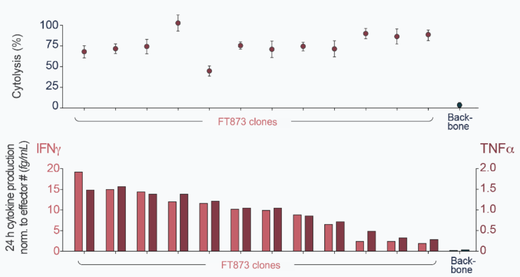
Low B7-H3 expressing tumors are controlled by FT873 xCELLigence cytotoxicity assays in the presence of cetuximab with two lines of NSCLC expressing different levels of endogenous B7-H3 antigen.

IL-7RF promotes CAR-iT cell tumor infiltration in vivo



Tumor infiltration In addition to demonstrating comparable tumor control, iPSC-derived T cells engineered with a bicistronic (CD19-CAR IL-7RF) TRAC-targeted cassette show increased abundance of tumor infiltrating effector cells relative to a monocistronic version without the IL-7RF insert.

Nalm6 cytolysis and cytokine response (condition E, 120h)



Antigen-induced cytokine response Percent cytolysis (top) and cytokine levels (bottom) after three restimulations. Bottom graph depicts cytokine production during the last 24h of culture normalized to the number of effector cells at the end of the round.

CONCLUSIONS

Multicistronic expression cassettes inserted into the TRAC locus provide an attractive approach for site-specific engineering of iPSC-derived CAR T cells, allowing timely and synchronized expression of transgenes during T cell differentiation and CAR function and, at the same time, eliminating the risk of graft-vs-host disease in patients. Here, we show that co-expression of IL-7RF promotes proliferation and persistence of B7-H3 CAR iT cells and improves the ability of the cells to home to and persist in the tumor microenvironment. Importantly, the co-expression of a high-affinity non-cleavable CD16 allows for co-engagement of CAR and ADCC pathways to maximize the cytotoxicity of CAR T cells leading to better target specificity and enhanced anti-tumor function, while inhibition of B7-H3, a potent immune checkpoint protein, has the potential to unleash dormant anti-tumor responses by endogenous NK and T cells. Ongoing preclinical activities are focused on final clone selection and the development of solid tumor models to demonstrate the effectiveness of multiplexed-engineered, iPSC-derived CAR-T cells targeting B7-H3, including in combination with therapeutic antibodies, for off-the-shelf treatment of solid tumors.