Off-the-Shelf iPSC-derived CAR-T cells Targeting KLK2 Demonstrate Prolonged Tumor Control and Survival in Xenograft Models of Prostate Cancer

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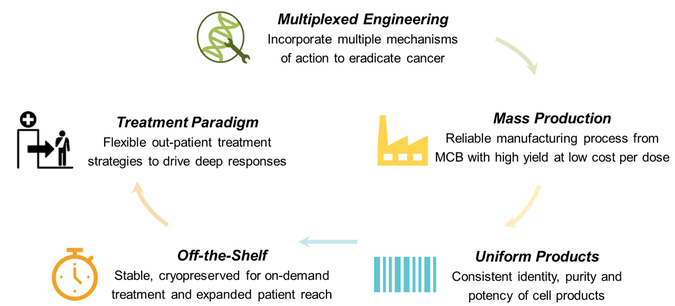
Executive Summary

Human kallikrein-related peptidase 2 (KLK2) is an antigen with prostate-restricted expression which is maintained during prostate cancer progression - making it an attractive therapeutic target for chimeric antigen receptor (CAR) T cells. While CAR-T cell therapies have shown remarkable success in hematologic malignancies, application to solid tumors has been broadly unsuccessful. Cost of treatment, manufacturing consistency, and scalability remain significant hurdles to broader patient access. To overcome these challenges, we are developing a KLK2 targeted off-the-shelf CAR-T cell product using our induced pluripotent stem cell (iPSC)-derived immunotherapy platform.

iPSC-derived CAR-KLK2 iT cells expressed a cell-surface profile consistent with a pure population of T lymphocytes; no TCRαβ cell-surface expression was detected, cells showed homogenous expression of CD45/CD7 (>99%), and uniform CAR expression driven by TRAC (>99%). Notably, the complete loss of T-cell receptor expression by genetic knock-out eliminates the potential of graft-versus-host disease in an allogeneic setting. Preclinical *in vitro* analyses of these CAR-KLK2 iT cells demonstrated potent and specific cytotoxicity against multiple prostate cancer cell lines, including VCaP cells which naturally express KLK2, PC3 cells engineered to express KLK2, and DU-145 cells engineered to express KLK2. *In vivo,* a multi-dose regimen of CAR-KLK2 iT cells controlled established (>100 mm3) VCaP and PC3-KLK2 subcutaneous xenograft models with greater than 90% tumor growth inhibition and associated increased survival. Follow-up dose titration studies demonstrated that a single dose of CAR-KLK2 iT cells was sufficient to mediate approximately 70% tumor growth inhibition.

Graphical Representation



Conclusion

Here we describe a novel multiplexed-engineered, off-the-shelf CAR T-cell therapy targeting KLK2 that is derived from a clonal master iPSC line for the treatment of prostate cancer. We show that the one-time engineered master iPSC line can serve as renewable starting material for the mass production of KLK2 CAR T cells that are uniform in CAR composition and completely absent of TCR expression, eliminating the risk for graft-versus-host disease in an allogeneic setting.

The presented preclinical *in vitro* and *in vivo* data suggest that KLK2 TRAC-CAR iT cells have the potential to effectively eliminate prostate cancer cells in a highly selective manner. The preclinical data also suggests that the anti-tumor performance can be enhanced with further modifications associated with T cell signaling. Since the behavior of engineered iT cells and additional edits in this novel platform are both currently not predictable, significant additional work is ongoing to generate an appropriate clinical candidate.

Results

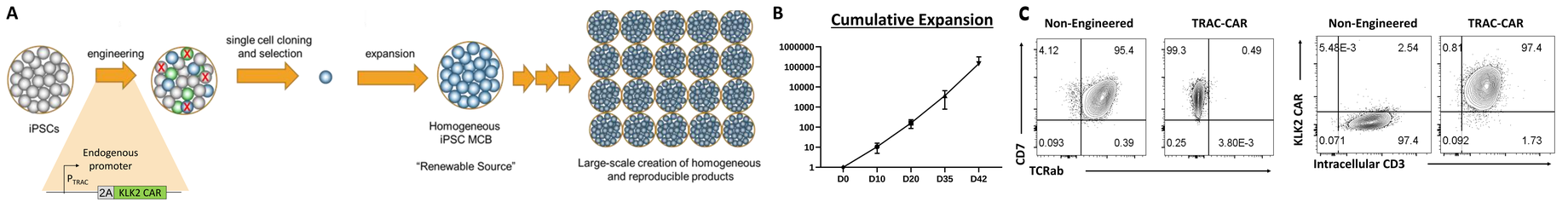


Figure 1. Precision engineered, iPSC-derived T cells facilitate the scalable production a homogenous population of KLK2 CAR iT cells. **(A)** Schematic illustration of Fate Therapeutic's iPSC cell therapy platform. iPSCs are generated from donor T cells and are engineered to express a novel anti-KLK2 CAR under control of the endogenous TRAC promotor. These TRAC-KLK2 CAR iPSC undergo selection and single cell cloning prior to expansion to establish a master cell bank. Master cell bank generation occurs one time in the lifecycle of the product and can support the generation of thousands of doses of iT cell product. **(B)** Cellular expansion was quantified over a 42-day differentiation, maturation, and expansion process from iPSC to mature CAR iT cell, resulting in greater than 1E5 iT cells generated for each starting iPSC. **(C)** Phenotypic profiling of mature iT cells demonstrates a uniform population of T cells expressing the lymphoid marker CD7, intracellular CD3 and lacking cell-surface TCRαβ, and expressing anti-KLK2 CAR.

Figure 2. TRAC CAR-KLK2 iT cells have specific cytotoxic function against PC3 prostate cancer cells engineered to express KLK2. **(A)** KLK2-CAR iT cells eliminate PC3-KLK2 prostate cancer cells (solid red circles,) while sparing KLK2 negative PC3 parental cells (solid blue circles). Backbone iT cells show minimal cytotoxicity against both KLK2 expressing and parental PC3 cells (open red and open blue circles, respectively). **(B)** TNFα and IFNγ production by KLK2-CAR iT cells co-cultured in the presence of KLK2 expressing and parental PC3 prostate cancer cells is robust and specific to KLK2 expressing target cells (purple bars).

Figure 3. KLK2-CAR iT cells demonstrate specific and robust cytotoxic activity against KLK2 positive tumor spheroids. KLK2-CAR iT cells target tumor spheroids from PC3 and DU145 prostate cancer cells engineered to express KLK2 or an endogenously expressing KLK2 positive prostate cancer cell line VCaP (red curves, upper panels). KLK2-CAR iT cells demonstrate more efficient cytotoxicity against 2 of 3 target cell lines compared to primary CAR-T cells (red vs orange curves). 3D tumor spheroids images demonstrate specific activity against KLK2 expressing targets and not their wild-type counterparts (lower panels).

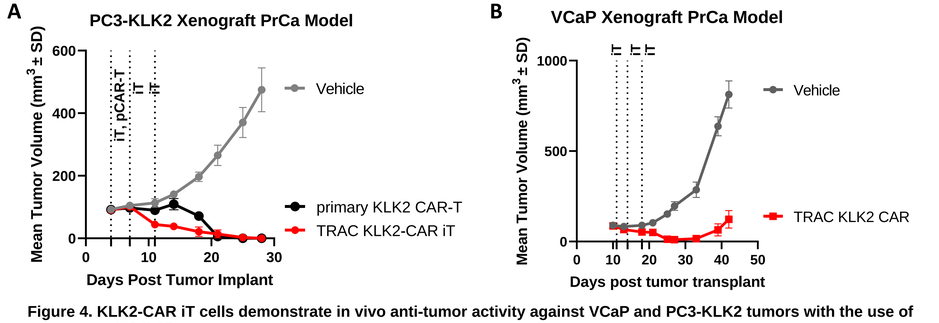


Figure 4. KLK2-CAR iT cells demonstrate in vivo anti-tumor activity against VCaP and PC3-KLK2 tumors with the use of exogenous IL2/IL15 cytokines. **(A)** KLK2-CAR iT cells demonstrate robust and durable tumor growth control of VCaP prostate cancer cells. Growth control is steep and durable to 30+ days post tumor implant. **(B)** KLK2-CAR iT cells demonstrate potent and long-lasting tumor growth control of PC3-KLK2 prostate cancer tumors in the presence of exogenous cytokine support. KLK2-CAR iT cells control tumor growth on par with primary CAR T cells and achieve near complete elimination of tumors.

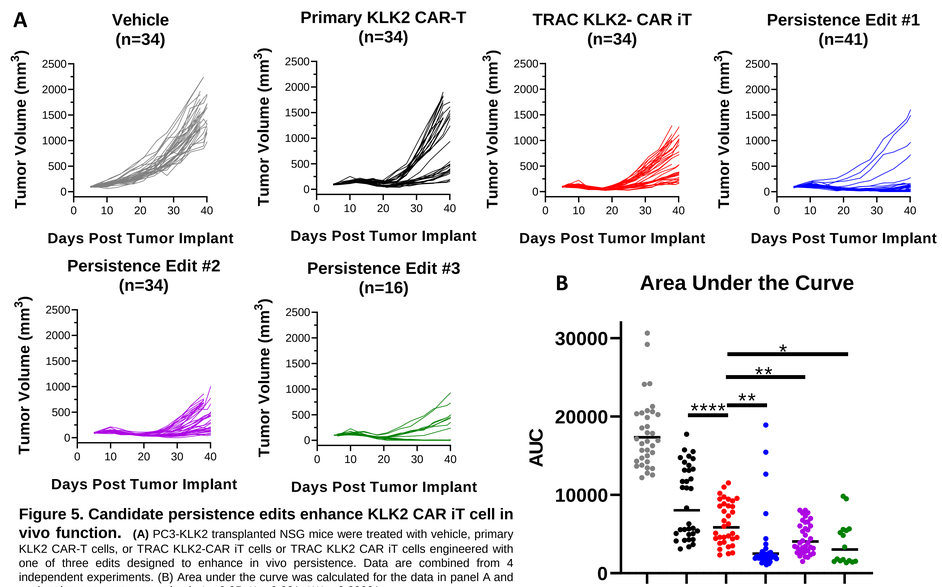


Figure 5. Candidate persistence edits enhance KLK2 CAR iT cell in vivo function. **(A)** PC3-KLK2 transplanted NSG mice were treated with vehicle, primary KLK2 CAR-T cells, or TRAC KLK2-CAR iT cells or TRAC KLK2 CAR iT cells engineered with one of three edits designed to enhance in vivo persistence. Data are combined from 4 independent experiments. (B) Area under the curve was calculated for the data in panel A and each point represents one animal. *p<0.05; **p<0.001, ****p<0.00001.