KSQ-001: A CRISPR/Cas9-Engineered Tumor Infiltrating Lymphocyte (eTIL™) Therapy for Solid Tumors

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Abstract

Adoptive cell therapy (ACT) with ex vivo expanded tumor infiltrating lymphocytes (TIL) offers a potentially transformative therapy for treatment refractory solid tumors. However, the immunosuppressive tumor microenvironment (TME) limits the effectiveness of TIL therapy. To systematically identify targets that have the potential to improve T-cell function in the TME, we conducted two CRISPR/Cas9 functional genomic screens of immune cells using our proprietary CRISPRomics® platform. The first screen employed primary mouse T cells and assessed in vivo T cell infiltration into tumors by measuring sgRNA guide enrichment. Notably, this genome-wide screen identified clinically active targets, such as PD-1, and identified multiple targets, including Cell Therapy-1 (CT-1). The potential of inactivating 'CT-1' to enable ACT in the clinic was demonstrated by our finding that CT-1 edited TCR-Tg mouse T cells are 10x more potent then control-edited T cells in an in vivo syngeneic solid tumor model. A second CRISPR/Cas9 functional genomic screen employed human TIL and assessed the impact of gene inactivation on human TIL expansion under standard manufacturing conditions. This screen also identified CT-1 as a top target. We therefore prioritized the development of KSQ-001, an engineered TIL (eTIL™) therapy, created via CRISPR/Cas9-mediated editing of CT-1. Potent and selective sgRNAs targeting CT-1 were identified and characterized, and manufacturing methods to engineer human TIL with high efficiency have been developed. KSQ-001 manufactured from human melanoma TIL possess an in vitro profile consistent with increased anti-tumor potency in comparison to TIL, including heightened production of IFNγ and TNFα. Together, these data demonstrate that our CRISPRomics® platform enables comprehensive target identification and validation of compelling new targets for the development of robust eTIL™ therapies and support the clinical evaluation of KSQ-001 as a next generation-adoptive cell therapy in treatment-refractory solid tumors.

Methods

In vivo T-cell screen : CD8 T cells were isolated from OT1 x Cas9 Transgenic mice, transduced with an sgRNA library and adoptively transferred into B16-OVA tumor bearing mice. After in vivo expansion, tumors were collected, gDNA was isolated and sgRNA accumulation was evaluated by NGS.

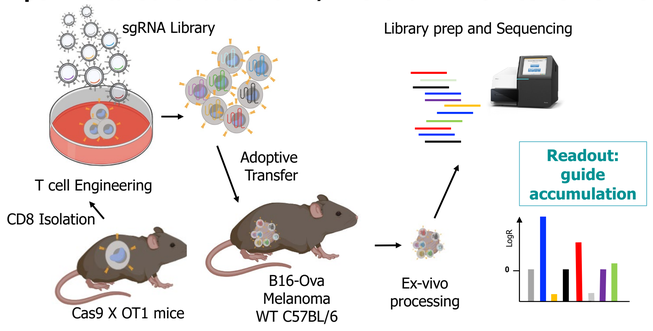
In vitro TIL screen : Human melanoma TILs were expanded by culture with human recombinant IL-2. After expansion, TIL were transduced with an sgRNA library and subsequently transfected with mRNA encoding Cas9. Engineered TILs were expanded in a standard Rapid Expansion Phase (REP) containing irradiated PBMCs, anti-CD3, and IL-2 for 14 days. After expansion gDNA was isolated and sgRNA accumulation was determined by NGS.

In vivo Target validation : B16-Ova melanoma cancer cells were injected s.c. into C57BL/6J mice. Mice were randomized for tumor volume (approx. mean 100mm^3) and either control or CT-1 edited OT1 T cells were injected i.v. Tumor volume and body weights were measured twice a week. Relative potency of mKSQ-001 vs control edited T cells was evaluated in the B16-Ova model at cell doses ranging from 0.41 to 41x10^6 cells. Day 21 post cell transfer, blood was collected from the highest dose groups and OT-1 T cells measured for expansion (Fig 2).

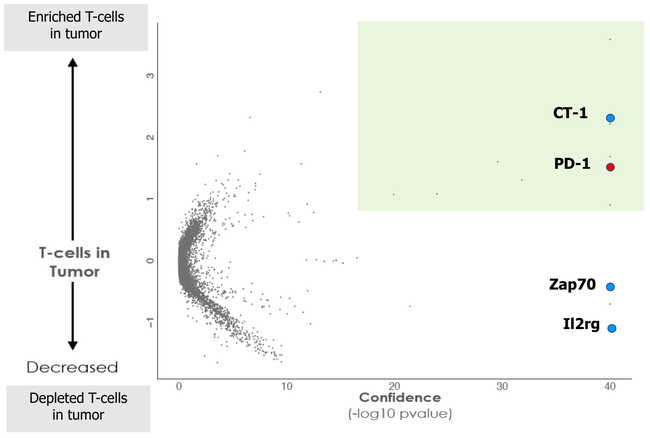
Manufacture & characterization of human KSQ-001 : For pre-REP, single cell tumor digests from Stage III treatment-naïve melanoma patients were cultured in 6000U IL-2 for 20-30 days, until ~50x10^6 TIL per donor were obtained. CRISPR/Cas9 RNPs targeting either the CT-1 gene or OR1A1 gene (sgControl) were electroporated into TIL, with edited TIL expanded in G-REX flasks with irradiated PBMCs, 30ng/ml OKT3 and 6000U IL-2 (REP). KSQ-001 and sgControl-edited TIL viability were assessed by AOPI stain from Nexcelom, with MART-1 tetramers from MBL.

Figure 1: A genome-wide CRISPR/Cas9 *in vivo* T- cell screen identifies CT-1 as a top target

Experimental schematic of OT1 / B16-Ova in vivo T cell CRISPR screen

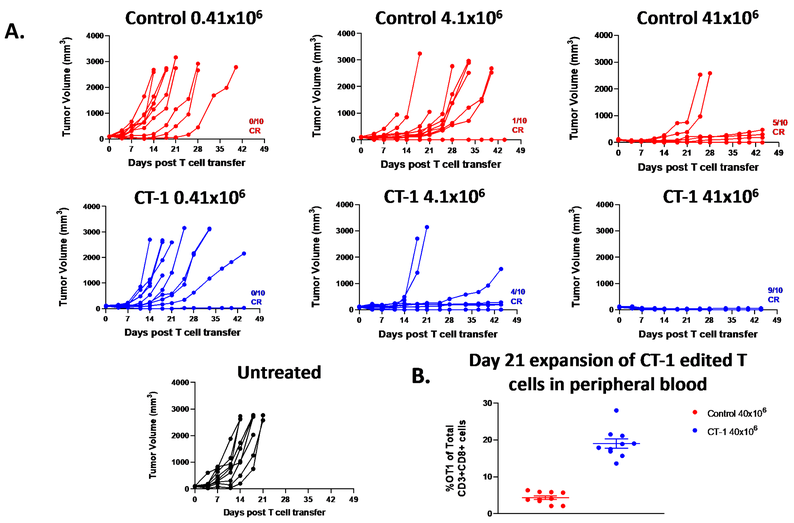


Genome-Scale in vivo Screen Identifies Novel T cell Targets



- Genome-wide *in vivo* screen IDs targets driving T cell enrichment in the tumor
- PD-1 serves as a positive control, the gene code-named Cell Therapy-1 (CT-1) demonstrates activity greater than PD-1
- Screen passes multiple QC checks, including recovery of >90% sgRNAs in library, depletion of T cell essential genes (Zap70, Il2rg), and expression of hits in T cells

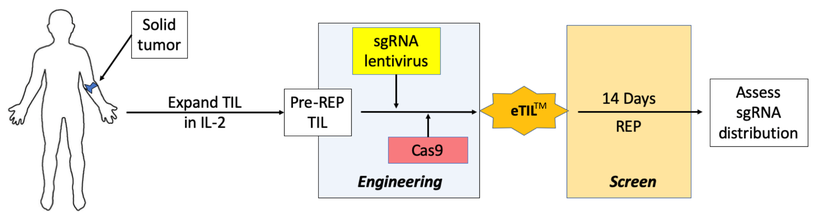
Figure 2: Murine KSQ-001 is 10x more potent and accumulates 5x greater than Control T cells

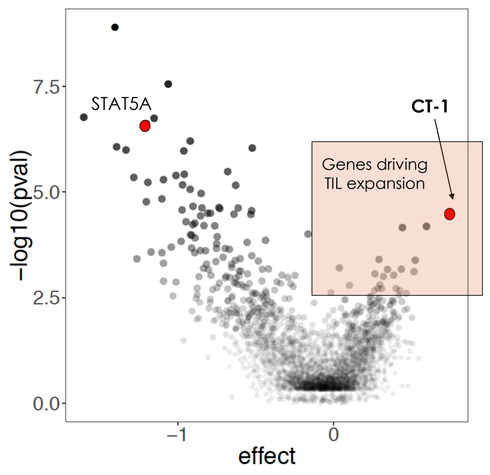


- CT-1 edited murine KSQ-001 (mKSQ-001) possess 10x potency increase in comparison to control edited T cells in the B16-Ova model
- At 21 days post adoptive transfer, mKSQ-001 are found at a 5x higher frequency in the peripheral blood compared to sgControl-edited T cells

Figure 3: A Human TIL Manufacturability CRISPR Screen Identifies CT-1 as a Top Target

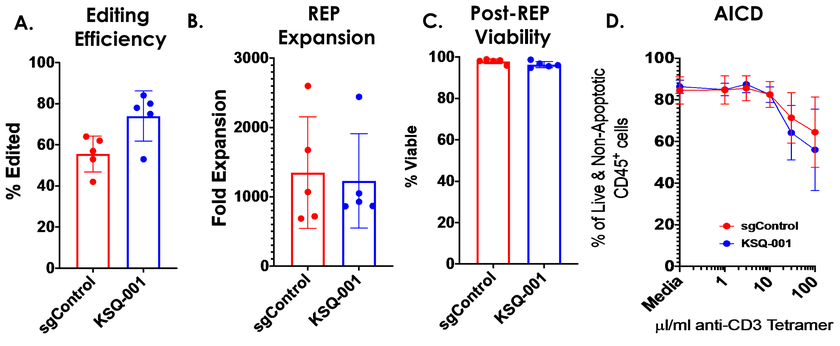
TIL CRISPR screen schematic:





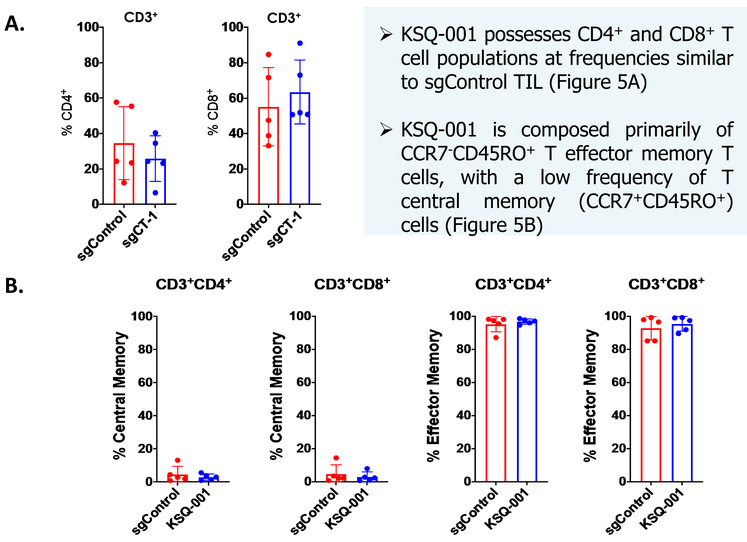
- A pooled CRISPR screen on the TIL manufacturing REP identified the CT-1 gene as the top target enhancing TIL expansion
- T cell relevant dropouts, including STAT5A, were observed in the screen, demonstrating screen quality

Figure 4: Robust Manufacture of KSQ-001 from Human Melanoma TIL



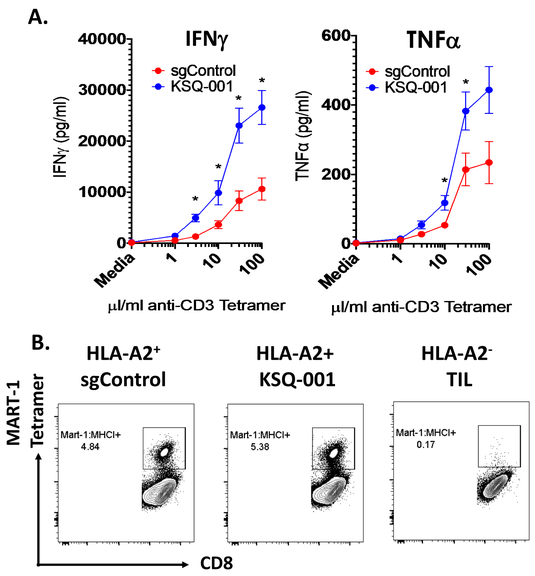
- KSQ-001 was manufactured from n=5 Stage III-IV human melanoma donors, with sgControl-edit TIL as comparators
- Robust editing of the CT-1 gene was observed across donors for KSQ-001 by amplicon sequencing (Figure 4A)
- KSQ-001 displays similar expansion, viability and anti-CD3 mediated AICD levels in comparison to sgControl-edited TIL following the REP (Figures 4B-D)

Figure 5: The Cellular Composition of KSQ-001 Matches Control TIL



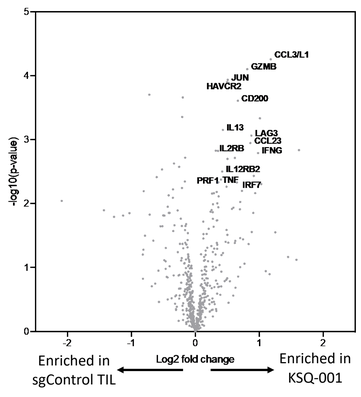
- KSQ-001 possesses CD4$^+$ and CD8$^+$ T cell populations at frequencies similar to sgControl TIL (Figure 5A)
- KSQ-001 is composed primarily of CCR7$^-$CD45RO$^+$ T effector memory T cells, with a low frequency of T central memory (CCR7$^+$CD45RO$^+$) cells (Figure 5B)

Figure 6: KSQ-001 Produces Increased Cytokines & Maintains Anti-Tumor Specificity



- KSQ-001 produced higher amounts of secreted IFNγ and TNFα following anti-CD3 stimulation in comparison to sgControl edited TIL (Figure 6A; combined data from 5 donors)
- An HLA-A2$^+$ donor contained specificity for the Mart-1 tumor antigen following pre-REP (Figure 6B). Following the REP, KSQ-001 from this donor retained Mart-1 specificity similar to sgControl-edited TIL. HLA-A1$^-$ donor shown as a control

Figure 7: KSQ-001 Displays Heightened Expression of Transcripts Associated with Anti-Tumor Function



- mRNA from n=5 KSQ-001 and sgControl samples were run on the Nanostring CAR-T Characterization Panel
- KSQ-001 exhibits increased expression of T cell activation markers (TIM3, LAG3, CD200); cytokines (CCL3, IFNg, TNFa, IL-13, CCL23) cytokine signaling (IL-2RB, IL12RB2, IRF7), cytolytic proteins (GrzB, Prf1) and T cell signaling pathway components (Jun) in comparison to sgControl TIL

Conclusions

- **CRISPR screens identified CT-1 as a top solid tumor ACT target**
- **Inactivation of CT-1 in mKSQ-001 increases anti-tumor potency by 10x versus unedited T cells**
- **Human KSQ-001 is an eTIL™ with comparable manufacturability, viability and cellularity profiles as unedited TIL**
- **Human KSQ-001 demonstrates an invitro functional profile consistent with enhanced anti-tumor properties**