Development of KSQ-001, an engineered TIL (eTIL™) therapy for solid tumors through CRISPR/Cas9-mediated inactivation of SOCS1

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Abstract

Background: Adoptive cell therapy with ex vivo expanded tumor infiltrating lymphocytes (TIL) offers a potentially curative treatment for cancer. However, the immunosuppressive tumor microenvironment limits the effectiveness of TIL therapy. To address this medical need, we used our Immune-CRISPRomics® Platform to perform a series of genome-wide CRISPR/Cas9 screens to identify targets enhancing the ability of T cells to infiltrate and kill solid tumors in an in vivo setting. These screens identified SOCS1 as a top target that restrains T cell anti-tumor immunity. Based on these findings, we developed KSQ-001, an engineered TIL (eTIL) therapy created via CRISPR/Cas9-mediated editing of SOCS1 for the treatment of solid tumors.

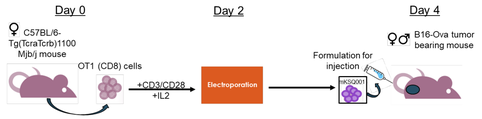
Methods: Genome-wide CRISPR/Cas9 screens were conducted in in vitro primary human T cells and TIL cultures and in in vivo primary mouse OT1 and PMEL TCR-Tg T cells in syngeneic tumor models. The efficacy of surrogate murine KSQ-001 (mKSQ-001), in which the SOCS1 gene is inactivated by CRISPR/Cas9 in OT1 or PMEL-TCR-Tg T cells, was evaluated in both the B16-Ova and CRC-gp100 syngeneic tumor models, with memory formation and efficacy evaluated both in the presence and absence of cyclophosphamide-mediated lymphodepletion. KSQ-001 was manufactured from human TIL using SOCS1-targeting sgRNAs selected for therapeutic use based on potency and selectivity, with KSQ-001 characterized for in vitro function and in vivo anti-tumor efficacy.

Results: Upon adoptive transfer of a single dose into solid tumor-bearing hosts, mKSQ-001 was found to robustly enhance anti-tumor efficacy and eradicate tumors in 7/10 mice in the PD1-sensitive OT1/B16-Ova model and to drive responses in the PD-1 refractory PMEL/CRC-gp100 syngeneic tumor model. mKSQ-001 also showed a ten-fold increase in anti-tumor potency in vivo compared to unengineered T-cell product and established durable anti-tumor memory by persisting in the form of T central memory cells detectable at high frequency in the peripheral blood of complete responder mice. In the setting of lymphodepletion, mKSQ-001 also displayed heightened anti-tumor potency, accumulation, and memory formation in comparison to inactivation of PD-1. Importantly, human KSQ-001 displayed a transcriptional signature indicative of increased anti-tumor function, produced increased amounts of pro-inflammatory cytokines, exhibited a hypersensitivity to IL-12 signaling, and demonstrated increased anti-tumor function both *in vitro* and *in vivo* solid tumor models.

Conclusion: Based on insights from our Immune-CRISPRomics platform and demonstrated efficacy across multiple preclinical tumor models, we have developed KSQ-001, a novel eTIL therapy. These preclinical data support clinical testing of KSQ-001 in a variety of solid tumor indications.

Methods

In Vivo Studies: Generation of mouse KSQ-001 cells (mKSQ-001)



- To serve as a negative control, T cells are edited with a sgRNA targeting OLF1, an olfactory receptor with no function in T cells

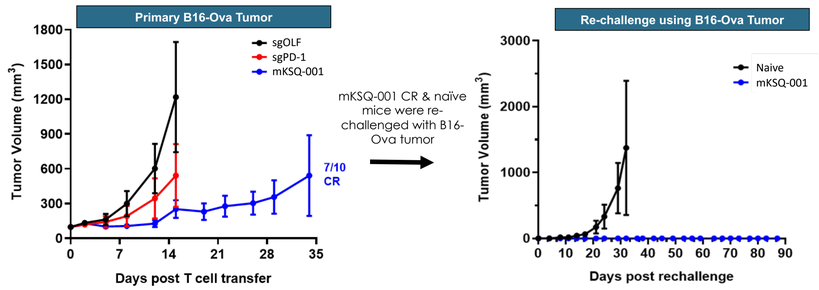
Human KSQ-001:

- Human KSQ-001 from melanoma and NSCLC donors were generated using a baseline eTIL Process
- Unedited TILs ('No EP' TIL) do not undergo the gene editing step and serve as controls
- Both No EP and KSQ-001 are cryopreserved at the end of the process with characterization assays performed following thaw

Figure 1: CRISPR/Cas9 inactivation of SOCS1 Enhances Murine KSQ-001 anti-Tumor Function and Persistence

1a)

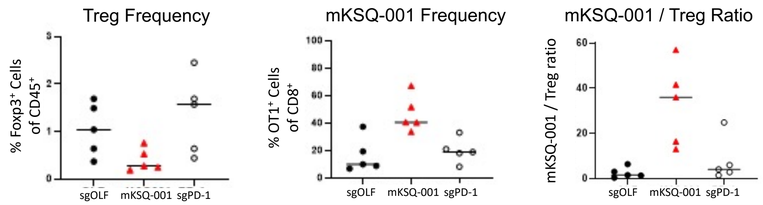
Robust mKSQ-001 efficacy & functional memory formation



1b)

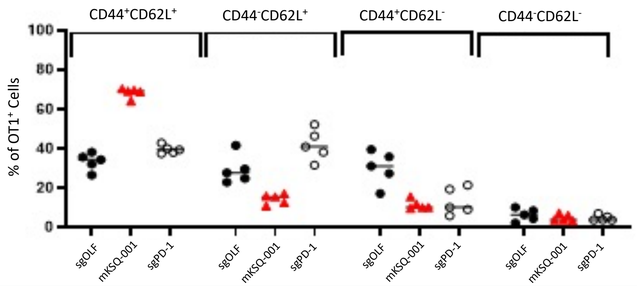
Enhanced accumulation of mKSQ-001 in tumor & depletion of Tregs

B16-Ova tumors show reduced levels of Tregs and enrichment of mKSQ-001 versus sgPD-1-edited OT1s 7 days following transfer

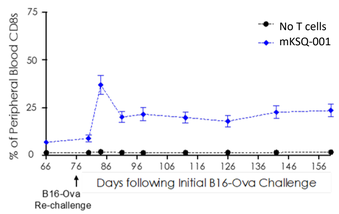


1c) **Accumulation of mKSQ-001 as Tcm cells in the tumor draining lymph nodes**

- Tumor draining lymph nodes were assessed 7 days following transfer, with the mKSQ-001 treated group showing enhanced enrichment of $CD44^+CD62L^+$ Tcm cells vs. sgPD-1-edited
- Enhanced accumulation of mKSQ-001 as Tcm cells was also observed in spleen



1d) **Long-term persistence of mKSQ-001 as Tcm cells following tumor re-challenge**



- mKSQ-001 displays a $CD44^{hi}CD62^{hi}$ Tcm phenotype prior to tumor re-challenge
- mKSQ-001 expands and differentiates into $CD44^{hi}CD62L^{lo}$ Tem cells following tumor re-challenge, followed by contraction and re-gain of a Tcm state
- mKSQ-001 is detectable >150 days following transfer in blood as Tcm cells

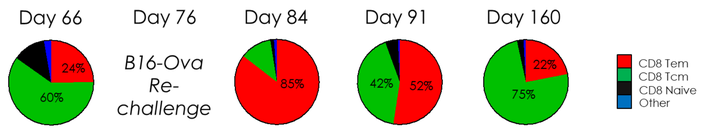
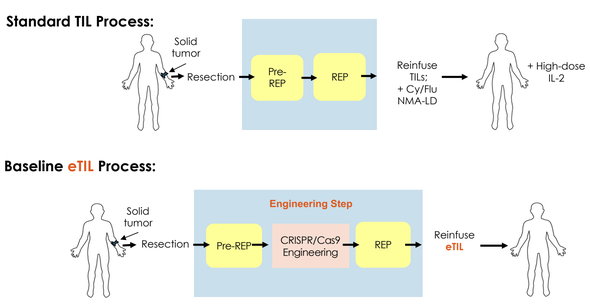


Figure 2: Human KSQ-001 Manufactured at Clinical Scale Demonstrates Enhanced anti-Tumor Activity and Hypersensitivity to Cytokine Signals

KSQ-001: An autologous, single-gene edited tumor-infiltrating lymphocyte (TIL) cell product manufactured using the baseline eTIL process, which includes an engineering step wherein Cas9/sgRNAs targeting the SOCS1 gene are electroporated (EP) into TIL

2a)

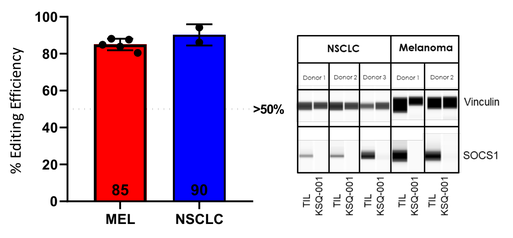
Manufacture of KSQ-001 at Clinical Scale



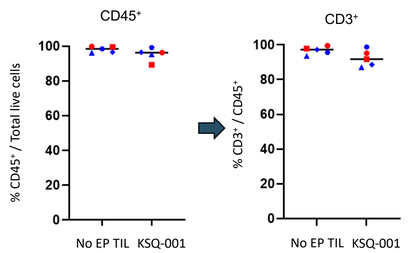
2b)

Characterization of Human KSQ-001: Robust Editing of the SOCS1 gene

>80% editing of SOCS1 at the DNA level and complete knockdown of SOCS1 protein using therapeutic sgRNAs using a clinical-scale engineering step



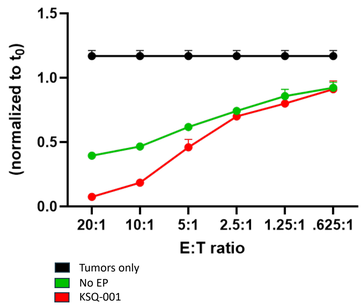
KSQ-001 is Composed of $CD45^+CD3^+$ T cells



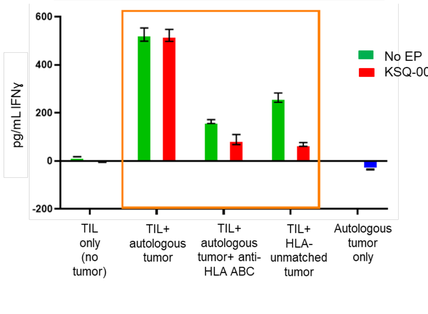
2c)

Heightened in vitro anti-tumor activity of KSQ-001 with hypersensitivity to cytokine signals

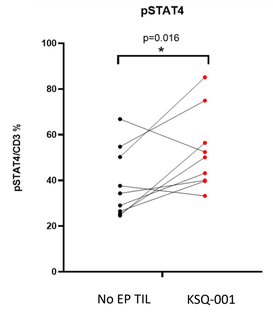
KSQ-001 showed superior control of tumor growth than 'No EP' TIL in vitro



KSQ-001 retains MHC class I-restricted reactivity for autologous tumor



Hypersensitivity of KSQ-001 to IL-12 signals through pSTAT4



Conclusions

- SOCS1 identified from KSQ's Immune CRISPRomics Platform as a top target enhancing anti-tumor activity and memory formation of T cells in vivo
- KSQ-001 is a single-edit eTIL product with inactivation of the SOCS1 gene, and can be manufactured at clinical scale
- KSQ-001 displays enhanced anti-tumor function in comparison to TIL, including hypersensitivity to cytokines