KSQ-004EX: A SOCS1/Regnase-1 Dual-Edited eTIL® Therapy Demonstrating Enhanced Anti-Tumor Activity

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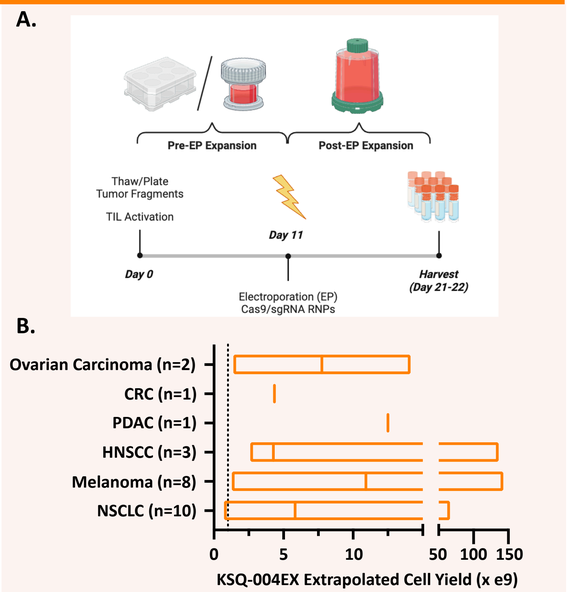
BACKGROUND

KSQ-004EX: A CRISPR/Cas9-engineered Tumor Infiltrating Lymphocyte (eTIL®) therapy with inactivation of the genes encoding SOCS1 & Regnase-1



(A) Schematics of KSQ-004EX generation. The process consist of TIL activation and extravasation of the processed tumor for 11 days, followed by CRISPR engineering by electroporation (EP) and post-electroporation expansion of 10 to 12 days. TIL expansion through out the process is supported by IL-2. Created with BioRender.com. **(B)** Full scale and extrapolated (scaled up or down) post-EP yield of freshly harvested end product, prior to cell washing, concentration, and cryopreservation. The dotted lines delineate the lower (1 x10^9) infusion dose. The line in each bar shows the mean cell yield from the indication. The number of donors per indication is listed. CRC, colorectal cancer; PDAC, pancreatic adenocarcinoma; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer. **(C)** Editing efficiency at *SOCS1* and *ZC3H12A*, which codes for Regnase-1, loci determined by NGS. n=17 independent donors. **(D)** Expression of SOCS1, Regnase-1 and Vinculin by WES capillary system. Four representative donors shown.

Figure 1. KSQ-004EX is Comprised Primarily of CD8+ Effector Memory T cells

Figure 1. **(A)** Frequency of CD3+ T cells of total CD45+ cells. **(B-F)** Frequency of CD8+ cells **(B)**, CD4+ cells **(C)**, γδ+ cells **(D)**, CD45RO+CCR7- cells **(E)** and CD25+ cells **(F)** of total live CD45+CD3+ cells. N= 26 matching No EP and KSQ-004EX donors. Statistical analysis was done using paired Students' *t* test. ** p<0.01.

Figure 2. Enhanced Anti-Tumor Cytotoxicity and IFN-γ Production by KSQ-004EX

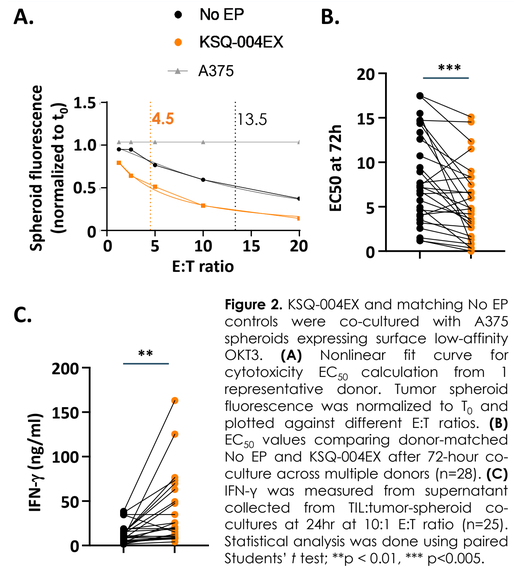


Figure 2. KSQ-004EX and matching No EP controls were co-cultured with A375 spheroids expressing surface low-affinity OKT3. **(A)** Nonlinear fit curve for cytotoxicity EC_{50} calculation from 1 representative donor. Tumor spheroid fluorescence was normalized to T_0 and plotted against different E:T ratios. **(B)** EC_{50} values comparing donor-matched No EP and KSQ-004EX after 72-hour co-culture across multiple donors (n=28). **(C)** IFN-γ was measured from supernatant collected from TIL:tumor-spheroid co-cultures at 24hr at 10:1 E:T ratio (n=25). Statistical analysis was done using paired Students' *t* test; **p < 0.01, *** p<0.005.

Figure 3. Enhanced Functionality by KSQ-004EX to Autologous Tumor from Checkpoint-refractory Melanoma Donor

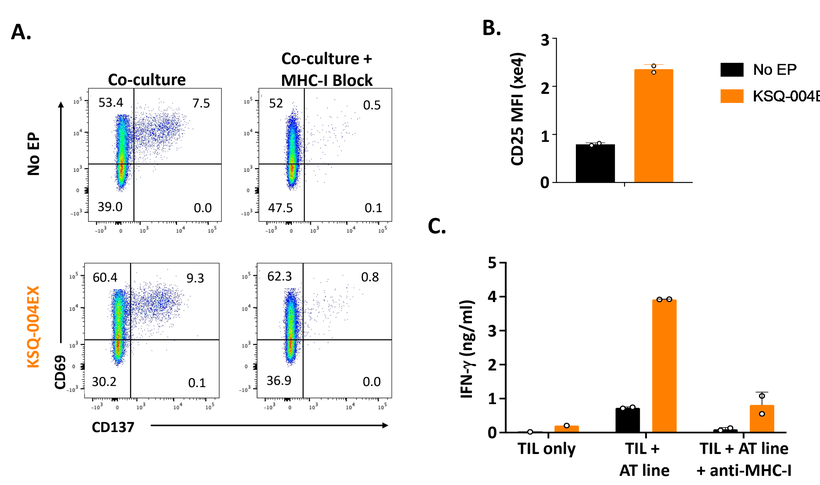


Figure 3. Paired KSQ-004EX, matching No EP control TIL and autologous tumor lines were generated from a checkpoint-refractory melanoma patient. TIL/autologous tumor pairs were cultured 1:1 for 24 hours. A representative donor is shown. **(A)** Frequency of CD137 and CD69 in $CD8^+$ T cells. **(B)** Expression of CD25 in $CD8^+CD137^+CD69^+$ cells. **(C)** Production of IFN-γ in culture supernatants measured by MSD. Duplicates were included in the assay.

Figure 4. KSQ-004EX Show Enrichment of Cytokine Signaling and Stemness Transcriptional Signatures

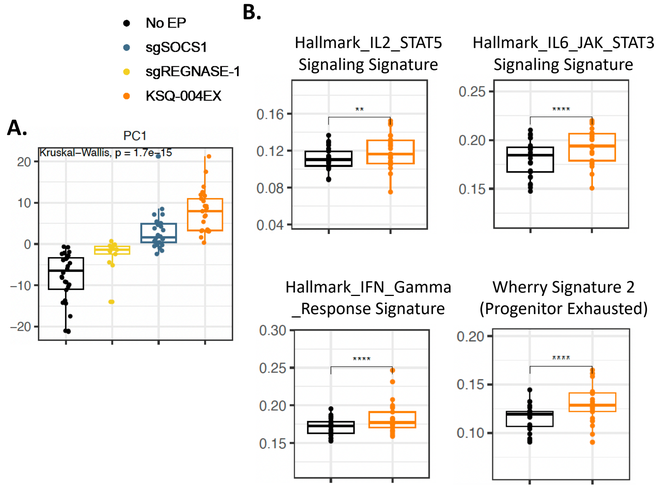


Figure 4. RNA-seq was performed in KSQ-004EX and matching No EP or single-edited controls upon harvest. **(A)** Enrichment of No EP, single-edited TIL or KSQ-004EX along Principal Components 1. Dots represent individual donors. **(B)** Enrichment of indicated pathways/gene sets. Statistical analysis was done using Wilcox test. ** p<0.01; **** p<0.001.

Figure 5. KSQ-004EX is Refractory to Exhaustion in a Chronic Re-Stimulation Assay

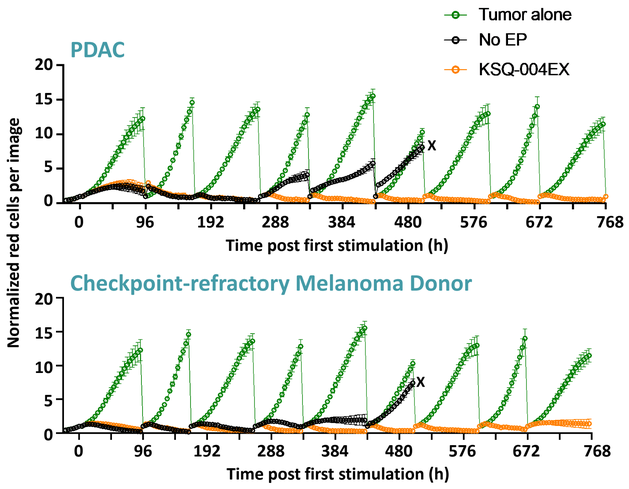


Figure 5. KSQ-004EX or No EP controls were added to RFP^+ A375 cells expressing high-affinity OKT3, with cytotoxicity assessed by Incucyte across 8 rounds of re-stimulation. A fraction of cells were seeded at each subsequent re-stimulation. Two representative donors are shown. Data shows Mean ± SD (n=10 replicates per condition). X indicates that No EP samples were removed due to lack of tumor control.

Figure 6. KSQ-004EX Demonstrates Enhanced Persistence in vivo

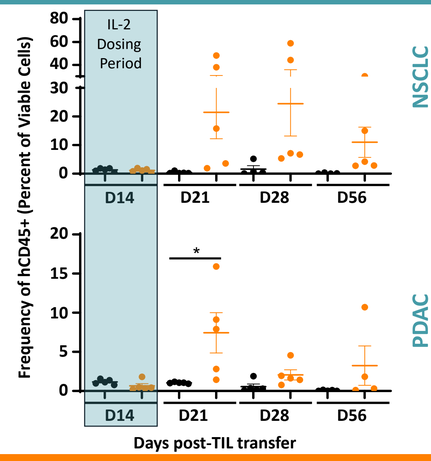


Figure 6. NSG mice injected with 20x10^6 No EP TIL or KSQ-004EX IV, with 45,000 IU IL-2 administered IP daily for 14 days. Spleens were harvested on samples analyzed by flow cytometry on the indicated timepoint. Statistical significance of all group comparisons was evaluated using a one-way ANOVA with Tukey's multiple comparisons test; *p < 0.05.

CONCLUSIONS

- ✓ KSQ-004EX, successfully generated from Melanoma, HNSCC, NSCLC, CRC, PDAC, and Ovarian Adenocarcinoma, is comprised primarily of $CD8^+$ T cells with an effector-memory population.
- ✓ KSQ-004EX show enhanced cytokine production and cytotoxicity upon co-culture with spheroids or autologous tumors.
- ✓ KSQ-004EX display increased serial killing and in vitro persistence relative to matching No EP controls, suggesting resistance to exhaustion.
- ✓ KSQ-004EX demonstrate enhanced persistence in vivo in NSG mice supplemented with IL-2 for 14d.
- ✓ KSQ-004EX show transcriptional characteristics of both single-edited cells.