KSQ-004: UNBIASED PAIR-WISE DISCOVERY OF SOCS1 AND REGNASE-1 AS THE TOP CRISPR/CAS9 DUAL-EDIT COMBINATION ENHANCING IN VIVO TIL POTENCY AGAINST SOLID TUMORS

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<http://dx.doi.org/10.1136/jitc-2021-SITC2021.204>

Background The application of CRISPR/Cas9-gene-editing to enhance the anti-tumor activity of T cell Adoptive Cell Therapies (ACT) is a promising approach in the treatment of patients with solid tumors. We developed an in vivo CRISPR² screening approach and interrogated the top dual-edit combinations enhancing T cell anti-tumor function. We discovered that across all possible dual-edit combinations of T cell targets, inactivation of Regnase-1 and SOCS1 led to the greatest enhancement in anti-tumor T cell potency in vivo. We applied these findings to discover KSQ-004, a Regnase-1/SOCS1 dual-edited human CRISPR/Cas9-engineered TIL (eTIL) therapy currently under development for therapeutic use.

Methods We generated randomly paired CRISPR guide libraries (CRISPR2) targeting top hits from previous single-gene Immune CRISPRomics[®] screens. CRISPR² libraries with over 1200 gene pairs were screened in primary mouse OT1 and PMEL-TCR-Tg-T cells in the relevant syngeneic tumor models. Top dual-edit combinations were then evaluated in the immunotherapy-refractory B16F10 metastatic lung tumor model. The efficacy of the top combo was further evaluated in a mouse TIL model wherein TIL from B16-Ova tumors were expanded and engineered ex vivo and adoptively transferred into tumor bearing hosts for efficacy assessment.

Results Of the 1200+ combinations tested in the CRISPR² screens, the Regnase-1/SOCS1 combination ranked amongst the top dual-edits, with this combination enhancing T cell infiltration into tumors >3500-fold in comparison to controls. Studies conducted in the checkpoint therapy refractory B16F10 lung metastasis model revealed that Regnase-1/SOCS1 dual-edited PMEL-TCR-Tg-T cells conferred remarkable survival benefit relative to controls, significantly extending median survival of animals from 21 days to 53 days. Furthermore, Regnase-1+SOCS1-edited mouse TIL isolated and expanded from B16-Ova tumors exerted complete control of tumors upon re-infusion into hosts, suggesting rejuvenation of tumor-experienced TILs by this edit combination. To apply these insights for therapeutic use, we discovered KSQ-004, a human Regnase-1/SOCS1 dual-edited CRISPR/Cas9-engineered TIL (eTIL). Methods were developed to manufacture KSQ-004 from melanoma and NSCLC tumor samples, with eTIL demonstrating robust expansion and viability comparable to unedited control TIL with over 90% knockout of both targets. Importantly, KSQ-004 produced elevated IFN γ upon autologous tumor stimulation and exerted greater control of tumor spheroids in vitro.

Conclusions We used a novel CRISPR² screen approach to identify Regnase-1/SOCS1 as the top dual edit combination enhancing T cell function in the tumor microenvironment. We translated these findings to therapeutic use with the discovery of KSQ-004, a dual-edited eTIL therapy engineered that possesses enhanced anti-tumor potency and persistence against solid tumors.