MANUFACTURE OF KSQ-001, A CRISPR/CAS9-ENGINEERED TIL (ETIL) THERAPY, FOR THE TREATMENT OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Background Head and neck squamous cell carcinoma (HNSCC) is characterized by frequent immune infiltration and an immunosuppressive tumor microenvironment. In recent years, adoptive cell therapy with ex vivo expanded tumor infiltrating lymphocytes (TIL) has shown clinical efficacy among HNSCC patients (pts), although the durability of responses to TIL therapy remains limited. To address this issue, we developed KSQ-001, a novel engineered TIL (eTIL) product with CRISPR/Cas9 mediated inactivation of SOCS1 gene. This gene was identified as a top target for enhancing T cell anti-tumor potency and persistence in vivo from our genome-wide CRISPRomics screens.

Methods Viably cryopreserved primary tumors and/or metastatic lymph nodes (mLN) from HNSCC pts were processed into small fragments and cultured in the presence of IL-2 through the pre-Rapid Expansion Protocol (pre-REP). TILs were then electroporated with ribonucleoprotein (RNP) complex containing the SOCS1-targeting guide RNA (gRNA). Following electroporation, engineered TIL (KSQ-001), and an unengineered control TIL were expanded under the REP. Both KSQ-001 and control TIL were harvested on day 27 and cryopreserved. SOCS1 editing in KSQ-001 was assessed by Next Generation Sequencing (NGS). The phenotypic characteristics and functionality of thawed KSQ-001 drug product from HNSCC tumors were assessed by flow cytometry, TCR sequencing, in vitro functional assays, and in vivo models.

Results KSQ-001 was successfully manufactured from 7/8 (88%) of HNSCC tumors with an average of 2000-fold post-electroporation expansion at the REP phase and an average of 95.2% editing at the SOCS1 locus as assessed by NGS. KSQ-001 from all donors showed levels of TCR diversity similar to those of the matched unedited TIL, and displayed hypersensitivity to IL-12 with elevated pSTAT4 levels upon stimulation. In comparison to donor-matched TIL, KSQ-001 showed greater cytotoxicity and enhanced IFNγ production against 3D tumor spheroids in vitro. Further, after infusion into immuno-compromised mice, KSQ-001 was detected in circulation at a significantly higher level than control TIL at 7-days post infusion, suggesting hyperresponsiveness to IL-2 and greater persistence in vivo.

Conclusions We demonstrated feasibility and robust manufacture of KSQ-001, a SOCS1-edited eTIL product, from HNSCC tumors at clinical scale. KSQ-001 from HNSCC tumors consistently displayed enhanced functional potency while retaining a highly diverse TCR repertoire. These data provide a compelling rationale for evaluating KSQ-001 as a therapeutic strategy for patients with HNSCC.