**Background** Adoptive cell therapy with ex vivo expanded tumor infiltrating lymphocytes (TIL) offers a potentially curative treatment for cancer. To improve the clinical durability of TIL, we have developed KSQ-001, a CRISPR/Cas9 engineered TIL (eTIL) product with inactivation of SOCS1 gene. SOCS1 is a negative regulator of cytokine signaling in T cells that we previously identified as a top target restraining T cell in vivo anti-tumor function and long-term persistence in genome-wide in vivo CRISPR screens. The clinical manufacture of TIL has historically required a complex multi-step baseline process involving a pre-Rapid Expansion Protocol (pre-REP) followed by a Rapid Expansion Protocol (REP) with feeder cells. To simplify and shorten the manufacture of KSQ-001, we developed a next-generation ExPRESS manufacturing process involving fewer steps and eliminating the use of feeder cells. We demonstrated that ExPRESS can robustly manufacture KSQ-001 with high functional potency at clinical scale from tumor starting material in 21 days or less across multiple solid tumor types.

**Methods** Viably-cryopreserved-tumor-fragments (VCTF) from different solid tumor indications were used as starting materials for eTIL manufacture. T cells in the tumor fragments are activated to propagate for 7-11 days, after which TIL were electroporated with ribonucleoprotein (RNP) complexes containing the SOCS1-targeting guide RNA (gRNA). Following electroporation, TIL were further expanded for an additional 7-11 days prior to cryopreservation. The editing level, phenotypic characteristics, and functionality of KSQ-001 was assessed by NGS, flow cytometry, and in vitro functional assays.

**Results** KSQ-001 was successfully manufactured from 10/11 VCTF starting materials from melanoma and NSCLC samples to clinically relevant doses, demonstrating robust viability at the time of cryopreservation and following thaw. KSQ-001 manufactured using ExPRESS showed >90% editing of the SOCS1 gene as well as complete knockdown of SOCS1 protein in all donors tested. Importantly, KSQ-001 manufactured using ExPRESS exhibited heightened anti-tumor function when compared to un-engineered TIL, including increased production of IFNγ and anti-tumor potency in an in vitro TIL/tumor co-culture system. KSQ-001 also retained high diversity of the TCR repertoire and specificity for autologous tumor. Lastly, adoptively transferred eTIL showed enhanced persistence and anti-tumor efficacy in immunodeficient mouse tumor models.

**Conclusions** We describe a shortened and streamlined process for the manufacture of CRISPR/Cas9-edited eTIL. KSQ-001 can be manufactured at clinically relevant doses in 21 days or less and displays enhanced functional potency. These data support evaluating KSQ-001 manufactured by the ExPRESS process in the treatment of patients with metastatic treatment-refractory solid tumors.