Adoptive cell therapy (ACT) with identification and validation of targets for the development of CRISPR/Cas9 engineered TIL immunosuppressive tumor microenvironment (TME) limits the effectiveness of TIL therapy. The first screen was genome-wide and employed primary T cells in vivo, assessing T cell infiltration into tumors (sgRNA guide enrichment). Notably, this screen identified clinically active molecules, such as PD-1, and identified multiple targets, including a target referred to as CT-1, that enhanced anti-tumor T-cell function superior to PD-1. The potential of CT-1 for ACT was validated in multiple syngeneic tumor models by adoptive transfer of CT-1-edited TCR-Tg CDB T cells into tumor-bearing mice, which showed robust efficacy in both PD-1 sensitive and insensitive models in the absence of any conditioning regimens. CT-1-edited TCR-Tg CDB T cells established durable anti-tumor memory, with edited CDB T cells characterized by enhanced accumulation and a T central memory phenotype.

The second screen employed human TIL and assessed the impact of gene inactivation on in vitro human TIL expansion under standard manufacturing conditions. This screen identified CT-1 as a top target, indicating that CT-1 inactivation enhances both anti-tumor activity and cell expansion. Potent and selective sgRNAs targeting CT-1 were identified, and the in vitro functional profile of CT-1 edited CRISPR/Cas9-engineered TIL (eTILs™) characterized, with increased production of pro-inflammatory cytokines observed. Together, these data demonstrate that the Immune-CRISPRomics® platform enables comprehensive target identification and validation of targets for the development of CRISPR/Cas9 engineered TIL (eTILs™) as a next generation adaptive cell therapy.

In vitro TIL screen: Human melanoma TILs were expanded by culture with human recombinant IL-2. After expansion, TIL were transduced with an sgRNA library and subsequently transfected with mRNA encoding Cas9. Engineered TILs were expanded in a standard REP containing transduced PRMKS, anti-CD3, and IL-2 for 14 days. After expansion, sgDNA was isolated and sgRNA accumulation was determined by NGS.

In vitro Target validation: B16-Ova melanoma or gp100 CRC cancer cells were injected s.c. into C57BL/6 mice. Mice were randomized for tumor volume (approx. mean 100mm³) and either control, PD-1, or CT-1 edited OT1 or PMEL T cells were injected i.v. Tumor volume and body weights were measured twice a week. For B16-Ova tumor re-challenge study, mice with complete tumor response 10 days following CT-1 treatment were re-injected with B16-Ova tumors and monitored for tumor regrowth. Peripheral blood draws were collected at baseline, and multiple timepoints post tumor re-challenge to assess the phenotype of adaptively transferred CT-1 edited OT1 cells (fig. 3). Relative potency of CT-1 vs control edited T cells was evaluated in the B16-Ova model at cell concentrations ranging from 0.41 to 41x10⁶ cells. Day 21 post cell transfer, blood was collected from the highest dose groups and OT1 T cells measured for expansion (fig. 4).

B16-Ova mice cured post transfer of CT-1 edited OT1 T cells do not re-establish tumors following re-challenge. CT-1 edited OT1 cells in peripheral blood display a high central memory phenotype prior to re-challenge, an effector phenotype at day 8 post tumor re-challenge, and progressive differentiation back to a Tcm state.

In vivo Target validation: B16-Ova melanoma or gp100 CRC cancer cells were injected s.c. into C57BL/6 mice. Mice were randomized for tumor volume (approx. mean 100mm³) and either control, PD-1, or CT-1 edited OT1 or PMEL T cells were injected i.v. Tumor volume and body weights were measured twice a week. For B16-Ova tumor re-challenge study, mice with complete tumor response 10 days following CT-1 treatment were re-injected with B16-Ova tumors and monitored for tumor regrowth. Peripheral blood draws were collected at baseline, and multiple timepoints post tumor re-challenge to assess the phenotype of adaptively transferred CT-1 edited OT1 cells (fig. 3). Relative potency of CT-1 vs control edited T cells was evaluated in the B16-Ova model at cell concentrations ranging from 0.41 to 41x10⁶ cells. Day 21 post cell transfer, blood was collected from the highest dose groups and OT1 T cells measured for expansion (fig. 4).

B16-Ova mice cured post transfer of CT-1 edited OT1 T cells do not re-establish tumors following re-challenge. CT-1 edited OT1 cells in peripheral blood display a high central memory phenotype prior to re-challenge, an effector phenotype at day 8 post tumor re-challenge, and progressive differentiation back to a Tcm state.

Methods

In vivo T cell screen: CDB T cells were isolated from OT1 x Cas9 Transgenic mice, transduced with an sgRNA library and adoptively transferred into B16-Ova tumor bearing mice. After in vivo expansion, tumors were collected, sgDNA was isolated and sgRNA accumulation was evaluated by NGS.

In vitro TIL screen: Human melanoma TILs were expanded by culture with human recombinant IL-2. After expansion, TIL were transduced with an sgRNA library and subsequently transfected with mRNA encoding Cas9. Engineered TILs were expanded in a standard REP containing transduced PRMKS, anti-CD3, and IL-2 for 14 days. After expansion, sgDNA was isolated and sgRNA accumulation was determined by NGS.

In vivo Target validation: B16-Ova melanoma or gp100 CRC cancer cells were injected s.c. into C57BL/6 mice. Mice were randomized for tumor volume (approx. mean 100mm³) and either control, PD-1, or CT-1 edited OT1 or PMEL T cells were injected i.v. Tumor volume and body weights were measured twice a week. For B16-Ova tumor re-challenge study, mice with complete tumor response 7 days following CT-1 treatment were re-injected with B16-Ova tumors and monitored for tumor regrowth. Peripheral blood draws were collected at baseline, and multiple timepoints post tumor re-challenge to assess the phenotype of adaptively transferred CT-1 edited OT1 cells (fig. 3). Relative potency of CT-1 vs control edited T cells was evaluated in the B16-Ova model at cell concentrations ranging from 0.41 to 41x10⁶ cells. Day 21 post cell transfer, blood was collected from the highest dose groups and OT1 T cells measured for expansion (fig. 4).

Conclusions

Murine in vivo T cell and human in vitro TIL CRISPR screening identified CT-1 as a top target with potential to increase efficacy of TIL adoptive cell therapy.

CT-1 edited T cells exhibit increased and durable anti-tumor potential associated with memory formation in murine syngeneic models.

CT-1 edited human eTIL demonstrate enhanced inflammatory properties in vitro.