Antitumor Activity of T Cells Expressing a Novel FOLR1 Costimulatory Antigen Receptor (CoStAR) in a Human Xenograft Murine Solid Tumor Model and Implications for In-Human Studies

Poster 2535

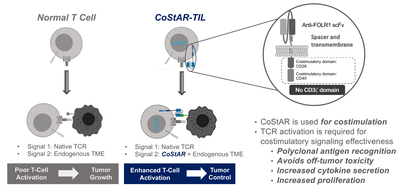
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BACKGROUND

- Tumor-infiltrating lymphocytes (TILs) are a polyclonal cell product that encompasses broad diversity of antigen recognition with an unrestricted T-cell receptor (TCR) repertoire, thereby offering the broadest diversity of antitumor reactivity[1,2]
- TIL activity in certain tumors is limited by poor costimulation in the tumor microenvironment (TME)[3-5]
- To improve T-cell functional activity, a novel platform was engineered to leverage the diverse TCR repertoire of TILs while amplifying their antitumor reactivity via a synthetic costimulatory antigen receptor (CoStAR; **Figure 1**)[6]

Figure 1. CoStAR Platform Overview



CoStAR, costimulatory antigen receptor; FOLR1, folate receptor alpha; scFv, single-chain variable fragment; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment.

- ITIL-306 is an autologous TIL cell therapy that supplements native TCR-specific antigen recognition with robust costimulation via the novel CoStAR transgene upon engagement with folate receptor alpha (FOLR1)
- Immunodeficient murine models in efficacy studies of novel adoptive cell therapies are well established and may help provide the rationale for clinical studies[7-9]

OBJECTIVES

- To assess long-term interleukin (IL)-2–independent effector function by anti-FOLR1 CoStAR T cells in vitro
- To validate in vitro IL-2 serial stimulation findings of anti-FOLR1 CoStAR T cells in vivo
- To evaluate the impact of CoStAR modification on in vivo antitumor efficacy, pharmacokinetics, and survival of T cells in a murine solid tumor model

METHODS

IN VITRO

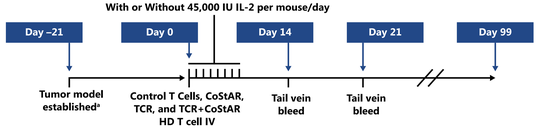
- Healthy donor T cells were manufactured to express anti-FOLR1 CoStAR (hereafter CoStAR) or left nontransduced (control T cells)
- Product cells were stimulated with a single (Day 0; ±IL-2) or serial (Day 0, 7, 14, 21; no IL-2) addition of target cells expressing membrane-anchored OKT3 (muromonab-CD3; to allow for TCR/CD3 complex crosslinking) and FOLR1 (to provide signaling through anti-FOLR1 CoStAR)
- T-cell activation and proliferation were measured

METHODS *(Continued)*

IN VIVO

- For in vivo studies, a human xenograft murine solid tumor model was established by engraftment of human carcinoembryonic antigen (CEA)-positive H508 cells engineered to express FOLR1 and injected into 6 mice/group on Day –21 (**Figure 2**)
- Control T cells or healthy donor T cells expressing anti-CEA TCR only (TCR), CoStAR only, or dual TCR and CoStAR (TCR+CoStAR) were given intravenously on Day 0 and compared with phosphate buffered saline control (no T cells)
 - For the TCR conditions, a transgenic, HLA-A*02–restricted, high-affinity CEA peptide-reactive TCR was introduced as a surrogate for polyclonal TCRs[10]
- Tumor growth, survival, and T-cell expansion and persistence in the periphery were assessed with 2 donors up to Day 99
- Studies were performed with and without exogenous IL-2 support on Days 0-7

Figure 2. Study Design for In Vivo Study

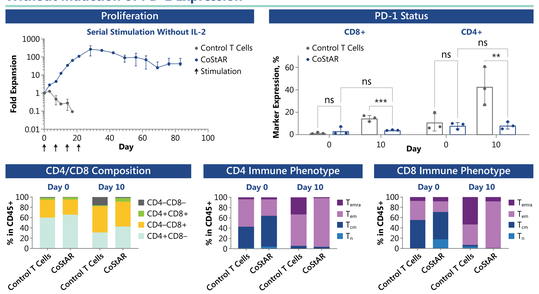


[a]Human carcinoembryonic antigen-positive H508 cells engineered to express FOLR1 were injected into 6 mice/group subcutaneously.
CoStAR, costimulatory antigen receptor; HD, healthy donor; IL, interleukin; IV, intravenous; TCR, T-cell receptor.

RESULTS

IN VITRO

Figure 3. CoStAR Supports Prolonged/Sustained IL-2–Independent Proliferation Without Induction of PD-1 Expression



Data from 1 donor; results are representative of 3 donors.
Two-way ANOVA (Šidák's multiple comparisons test) was performed to assess statistical significance for PD-1 status; **P<.01, ***P<.001.
CoStAR, costimulatory antigen receptor; IL, interleukin; ns, nonsignificant; PD-1, programmed cell death protein 1; T_{cm}, central memory T cell (CD62L+CD45RO+); T_{em}, effector memory T cells (CD62L–CD45RO+); T_{emra}, terminally differentiated effector memory T cells (CD62L–CD45RO–); T_n, naive T cell (CD62L+CD45RO–).

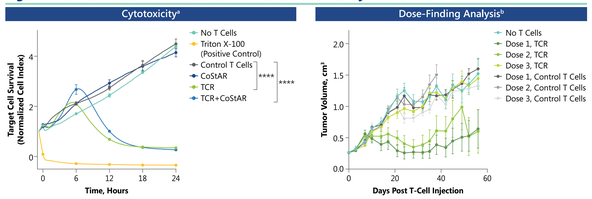
- CoStAR T cells showed sustained proliferation and persistence when stimulated multiple times (**Figure 3**)
- CoStAR T-cell proliferation in response to stimulation did not require exogenous IL-2

RESULTS *(Continued)*

- On Day 10, the expression of PD-1 (programmed cell death protein 1) was significantly increased in control T cells compared with CoStAR transduced CD4+ and CD8+ T cells; expression of PD-1 on CoStAR CD4+ and CD8+ T cells was similar at Day 0 and Day 10 (**Figure 3**)
- The fraction of CD4+ and CD8+ T cells was not impacted by CoStAR
- CoStAR transduction was associated with a lower fraction of terminally differentiated effector memory T cells (T_{emra} cells)
- CoStAR can maintain a "younger" T-cell phenotype, as demonstrated by lower PD-1 expression, lower fraction of T_{emra}, and high proliferation potential

CYTOTOXICITY AND DOSE-FINDING ANALYSIS

Figure 4. In Vitro and In Vivo Control of Tumor Growth by TCR T Cells

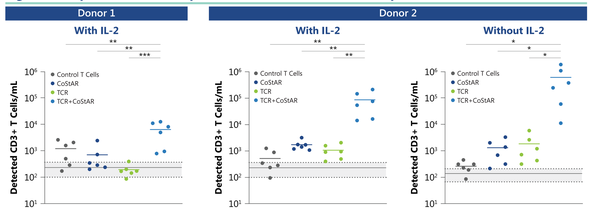


[a]Data are representative of Donor 1. T-cell groups were assessed against H508.Luc.Puro.FOLR1 target cells at a 10:1 T cell to target ratio over 24 hours. The normalized cell index, which is a measure of H508.Luc.Puro.FOLR1 cell growth, is shown over time following the addition of treatment groups. N=3 technical replicates; statistical significance was assessed by two-way analysis of variance with the Tukeys test for multiple comparisons; ****P<.0001.
[b]Data are shown up to Day 56. The drop in the green curve at Day 52 reflects the average tumor growth after the removal of a group member due to reaching maximal tumor volume. Mean ± standard error of the mean values are plotted for each condition (6 mice per group).
CoStAR, costimulatory antigen receptor; TCR, T-cell receptor.

- In vitro cytotoxicity activity was observed in TCR and dual TCR+CoStAR T cells compared with control T cells (**Figure 4**)
- CoStAR T cells did not show any cytotoxicity in vitro
- After a dose finding analysis, Dose 2 was selected as the optimal TCR T-cell dose to be administered based on tumor volume
- Infusion of TCR T cells at the two higher doses resulted in modest tumor control, indicating that TCR functions alone in vivo to control tumor growth
 - A cell dose with limited activity was selected to better measure the effects of CoStAR

IN VIVO

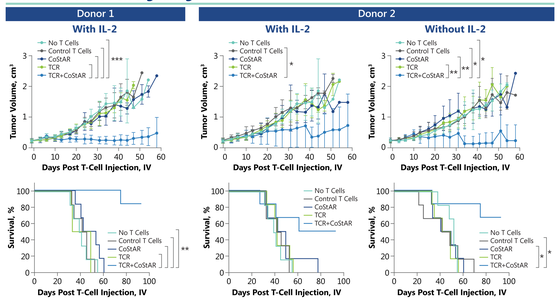
Figure 5. Improved In Vivo Expansion of CoStAR+ T Cells Independent of IL-2



Detection of T cells in mice that received no T cells was used to define the detection limit of the assay as shown by the solid and dotted lines; solid line, mean; dotted line, standard deviation. Mean and individual data points are depicted. One-way analysis of variance with the Tukeys test for multiple comparisons was performed to assess statistical significance; *P<.05, **P<.01, and ***P<.001.
CoStAR, costimulatory antigen receptor; IL, interleukin; TCR, T-cell receptor.

- T-cell persistence on Day 14 was increased in dual TCR+CoStAR versus TCR only mice (*P*<.01-.001; **Figure 5**)
- T-cell persistence on Day 14 was also increased in dual TCR+CoStAR versus all other treatment groups with and without exogenous IL-2 (*P*<.05-.001)

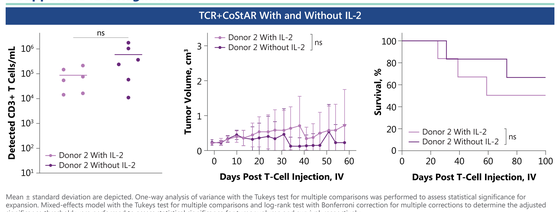
Figure 6. CoStAR Significantly Enhances Tumor Control and Survival Only When Combined With TCR Signaling



Mean ± standard deviation are depicted. One-way analysis of variance with the Tukeys test for multiple comparisons was performed to assess statistical significance for expansion. Mixed-effects model with the Tukeys test for multiple comparisons and log-rank test with Bonferroni correction for multiple corrections to determine the adjusted significance threshold were performed to assess statistical significance for tumor volume and survival, respectively; *P<.05, **P<.01, and ***P<.001.
CoStAR, costimulatory antigen receptor; IL, interleukin; IV, intravenous; ns, nonsignificant; TCR, T-cell receptor.

- In cells from Donor 1, dual TCR+CoStAR T cells enhanced control of tumor growth relative to control T cells, CoStAR, and TCR treatment groups (**Figure 6**)
- Similar control of tumor growth was observed in mice treated with dual TCR+CoStAR T cells from Donor 2 and occurred in the presence or absence of exogenous IL-2
- For both donors, only dual TCR+CoStAR mice had complete responses (undetectable tumors) at Day 58
- T cells expressing CoStAR alone did not control tumor growth, highlighting its dependence on TCR signaling to enhance tumor control and suggesting a favorable toxicity profile
- A significant improvement in survival was observed in dual TCR+CoStAR–treated mice compared with all other treatment groups, and this was independent of exogenous IL-2 (Donor 2)

Figure 7. Benefit From TCR+CoStAR Cells Was Observed Without the Administration of a Supportive IL-2 Regimen



Mean ± standard deviation are depicted. One-way analysis of variance with the Tukeys test for multiple comparisons was performed to assess statistical significance for expansion. Mixed-effects model with the Tukeys test for multiple comparisons and log-rank test with Bonferroni correction for multiple corrections to determine the adjusted significance threshold were performed to assess statistical significance for tumor volume and survival, respectively.
CoStAR, costimulatory antigen receptor; IL, interleukin; IV, intravenous; ns, nonsignificant; TCR, T-cell receptor.

- Enhanced in vivo expansion and persistence by TCR+CoStAR T cells did not require exogenous IL-2
- No significant differences in the control of tumor growth were observed in mice treated with dual TCR+CoStAR T cells across donors in the presence or absence of exogenous IL-2
- No significant differences in survival were observed in dual TCR+CoStAR–treated mice in the presence or absence of exogenous IL-2

CONCLUSIONS

- CoStAR significantly enhanced T-cell proliferation, persistence, and antitumor activity in vivo when combined with TCR signaling versus TCR alone, resulting in tumor control and prolonged survival
- Effects were not observed with CoStAR alone, underscoring that CoStAR only enhances T-cell effector function (including cytotoxicity) when combined with TCR signaling
- CoStAR can sustain proliferation of T cells without exogenous IL-2 in vitro and in vivo, supporting a clinical TIL regimen free of high-dose IL-2
- These results suggest that CoStAR may improve the clinical performance and benefit/risk profile of TILs by augmenting intrinsic TIL effector function while also eliminating the need for exogenous high-dose IL-2
- This will be explored in an upcoming first-in-human clinical study with ITIL-306

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