Anti-folate receptor alpha (FRα) CoStimulatory Antigen Receptor (CoStAR™) drives distinct cytokine-mediated proliferation responses in CD4+ and CD8+ T cells.

Martina Sykorova[1], Robert E. Hawkins[1], Mark E. Dudley[1], Gray Kueberuwa[1], and John S. Bridgeman
Instil Bio. Inc. Dallas, Texas, USA

INTRODUCTION

- There are several limiting factors for the development of cell therapy modalities in solid tumors, including tumour cell clonal heterogeneity, high expression of inhibitory receptors and poor endogenous costimulation (1-5).
- Tumor-infiltrating lymphocytes (TILs) contain a hugely diverse T-cell receptor (TCR) repertoire (6), thereby offering the broadest diversity of anti-tumor reactivity.
- TIL therapy has a proven track record of effective clinical efficacy (7-9).
- We have developed the CoStimulatory Antigen Receptor (CoStAR) platform to overcome the suppressive TME.
- The CoStAR described here consists of a FRα-specific single chain antibody fragment (scFv) fused to the signaling domains from CD28 and CD40.
- We have previously shown that engagement of CoStAR with concomitant TCR signalling leads to enhanced T cell activity (10,11).
- Here we explored the cytokine-mediated proliferation responses of COStAR+ T cells.

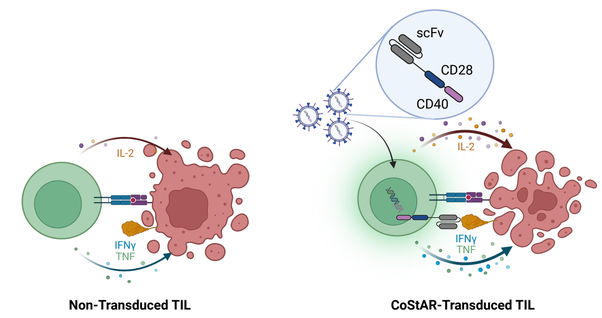

Figure 1: Wild-type non-transduced TIL (left) mediate tumour recognition via TCR binding of pMHC and can result in ineffective anti-tumour activity. CoStAR is introduced to TIL via lentiviral gene transfer (right). CoStAR recognition of tumour antigen (e.g. FRα) amplifies TCR signalling leading to enhanced effector function.

METHODS

- CoStAR consists of a FRα-specific single chain antibody fragment (scFv), fused to the signalling domain of CD28, CD28.CD137 or CD28.CD40. Additional control receptors consisting of anti-FRα or FMC63 (anti-CD19) fused to the HLA-A*02 transmembrane domain were also included.
- Healthy donor T cells were engineered with either FRα specific anti-FRα.CD28, CD28.CD137, CD28.CD40, or non-signaling anti-FRα.HLA-A2 CoStAR. Additional cells were engineered with a CD19 specific FMC63.HLA-A2 CoStAR (Fig. 2).
- Cells were normalised for each donor to the lowest expressing construct using Non-Td T cells (OvCAR-3 model), or enriched for CoStAR expression and sorted for CD4+ or CD8+ cells by negative selection (BA/F3 model).
- T cells were stimulated with FRα+ OvCAR3 or BA/F3 cells expressing a membrane anchored OKT3 molecule (OvCAR.OKT3 or BA/F3.OKT3.FRα) at an 8:1 E:T ratio, every seven days in the presence or absence of exogenous cytokine or conditioned media as indicated.
- T cell counts were made at seven-day intervals and CoStAR expression measured to assess expansion and enrichment.

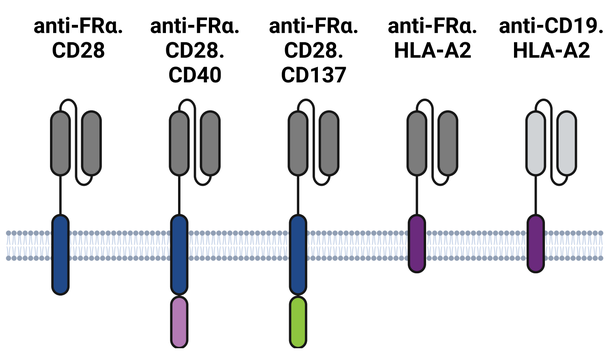

Figure 2: Schematic representation of CoStAR constructs used in this study.

RESULTS

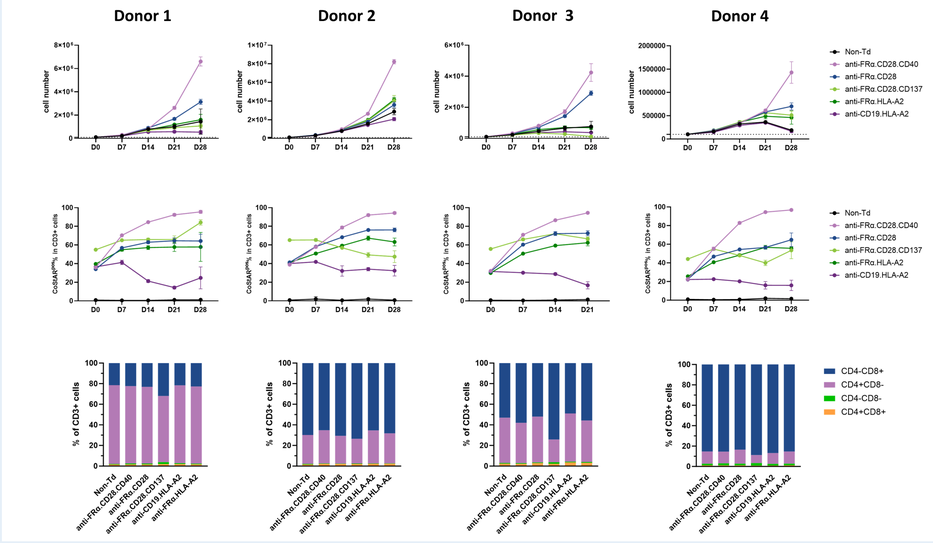

Figure 3: CoStAR-mediated proliferation and enrichment responses in four donors, in the presence of exogenous IL-2, and associated CD4:CD8 ratios.

- CoStAR-mediated proliferation was seen in all four donors in the presence of exogenous IL-2, with anti-FRα.CD28.CD40 mediating the greatest enhancement in proliferation (Fig. 3).
- In the absence of IL-2 there was more donor variability, with anti-FRα.CD28.CD40 T cells from one donor expanding well, displaying a slower rate of attrition in two donors and showing no benefit in a fourth. Flow cytometric analysis seemed to suggest a concordance between T cell proliferation and the CD4:CD8 ratio, with better CoStAR expansion in CD4+ enriched T cells in the absence of IL-2 (Fig. 4).
- Despite this, anti-FRα.CD28.CD40 engineered T cells demonstrated optimal enrichment in all donors tested suggesting that there was a still a survival benefit over time even in the absence of cytokine.

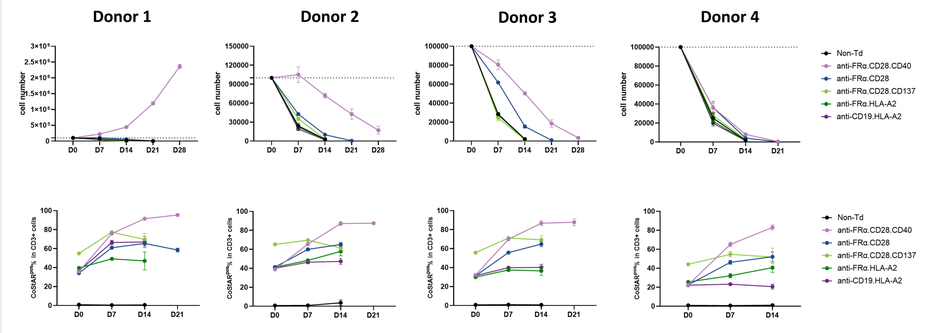

Figure 4: CoStAR-mediated proliferation and enrichment responses in four donors, in the absence of exogenous IL-2.

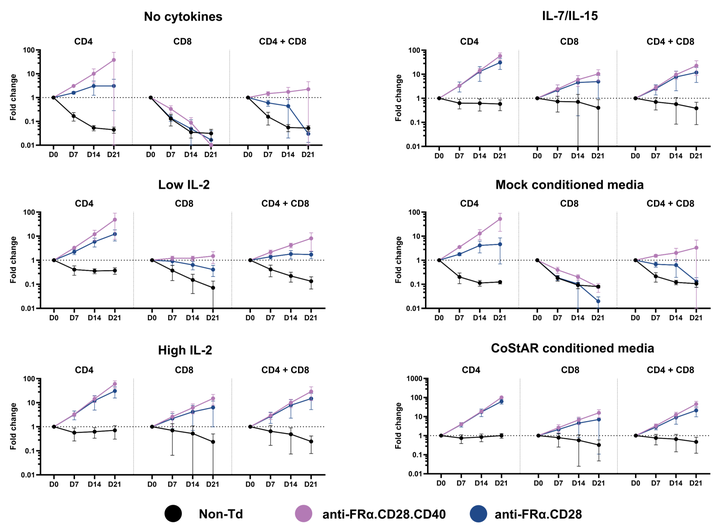

Figure 5: CoStAR-mediated proliferation responses from three donors in the presence and absence of different cytokines or conditioned media.

- The effect of cytokine milieu on CD4+ and CD8+ cells was further explored with CD4/CD8 enriched cells, or CD4/CD8+ cells mixed at a 1:1 ratio (Fig. 5).
- In the absence of exogenous cytokines both anti-FRα.CD28 and anti-FRα.CD28.CD40 CoStARs mediated survival of CD4+ T cells with CD28.CD40 outperforming the CD28 alone variant. In contrast Non-Td T cells failed to proliferate.
- Anti-FRα.CD28.CD40 enhanced survival of mixed CD4+/CD8+ T cells but not CD8+ T cells in the absence of cytokine. Addition of low (20 IU/mL) or high (200 IU/mL) IL-2, or IL-7+IL-15 was able to recover the activity of CoStAR in CD8+ T cells in a dose dependent manner.
- The effect of CoStAR is partly mediated by a contact independent mechanism, as conditioned media from CoStAR-Td, but not Non-Td T cells was able to recapitulate the effect of exogenous cytokine.

RESULTS

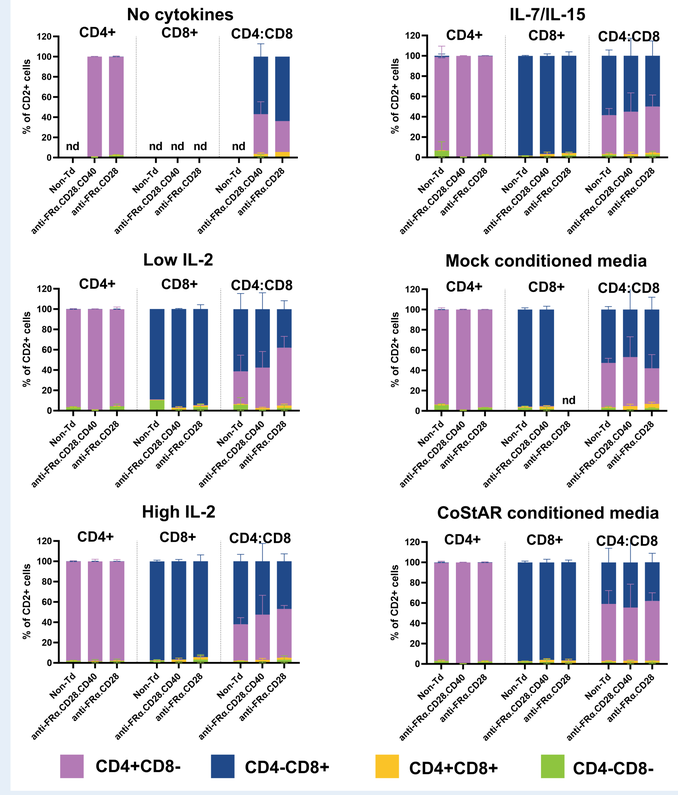

Figure 6: CD4:CD8 ratios of Non-Td and CoStAR-Td T cells following three rounds of tumour restimulation under different cytokine conditions (nd = no data, insufficient cells to analyse).

- CD4 and CD8 was assessed following three rounds of tumour stimulation (Fig. 6).
- Lack of bars indicates insufficient data for analysis.
- As expected, CD4 and CD8 was still expressed highly in previously enriched populations.
- Surprisingly, even in the absence of IL-2 the proportion of CD4 and CD8 cells in 1:1 CD4:CD8 cultures did not change dramatically over the course of the experiment, even in conditions which favour CD4 outgrowth (e.g. no cytokine).
- This observation further supports the observation that CoStAR-Td CD4+ T cells are capable of supporting CoStAR-Td CD8+ T cells.

CONCLUSION

- CoStAR provides proliferative benefit to both CD4+ and CD8+ T cells, and the provision of cytokine signals contributes to the overall magnitude of response.
- CD28.CD40 provides a substantially more robust signal for cytokine independent expansion of T cells compared to CD28-alone CoStAR .
- CoStAR engineered CD4+ T cells support CoStAR engineered CD8+ T cells through secreted factors.
- These data support the ability of CoStAR-transduced T cells to proliferate independent of cytokine support, while implying that potential clinical manipulations known to increase the levels of circulating cytokines, such as increasing intensity of lymphodepleting chemotherapy and addition of exogenous IL-2 infusions, could further augment the activity of CoStAR-TILs *in vivo*.

REFERENCES

1. Marofi F, et al. Stem Cell Res Ther. 2021;12:81.
2. Albelda SM. Cancer Immunol Res. 2020;8:2.
3. Schnell A et al. Cell Research 2020;30:285
4. Truxova I, et al. J Immunother Cancer. 2018;6:139.
5. Bandola-Simon J, Roche PA. Mol Immunol. 2019;113:31-37.
6. Spindler MJ, et al. Nat Biotechnol. 2020;38:609-619.
7. Rohaan MW, et al. J Immunother Cancer. 2018;6:102.
8. Betof Warner A, et al. Clin. Cancer. Res. 2023;29:1835.
9. Pillai M, et al. Am. J. Cancer. Res. 2022;12:3967
10. Sukumaran S, et al. JITC 2021; 2021-SITC 2021.198
11. Sykorova M, et al. JITC2022; SITC-2022.0282

All authors are current or former employees of Instil Bio and may have stock and/or stock options