

Anti-folate Receptor Alpha (FRα) CoStimulatory Antigen Receptor (CoStAR™) Improves T-cell Function Across Physiologically Relevant Ranges of FRα Expression and T-cell Receptor Affinities

Martina Sykova¹, Michelle Mojadidi¹, Leyuan Bao¹, Eric Gschweng¹, Milena Kalaitidou¹, Gray Kueberuwa¹, Xingliang (Tim) Zhou¹, Rubén Alvarez-Rodríguez¹, and John S. Bridgeman¹

¹Instil Bio, Inc., Dallas, TX, USA

Poster 282

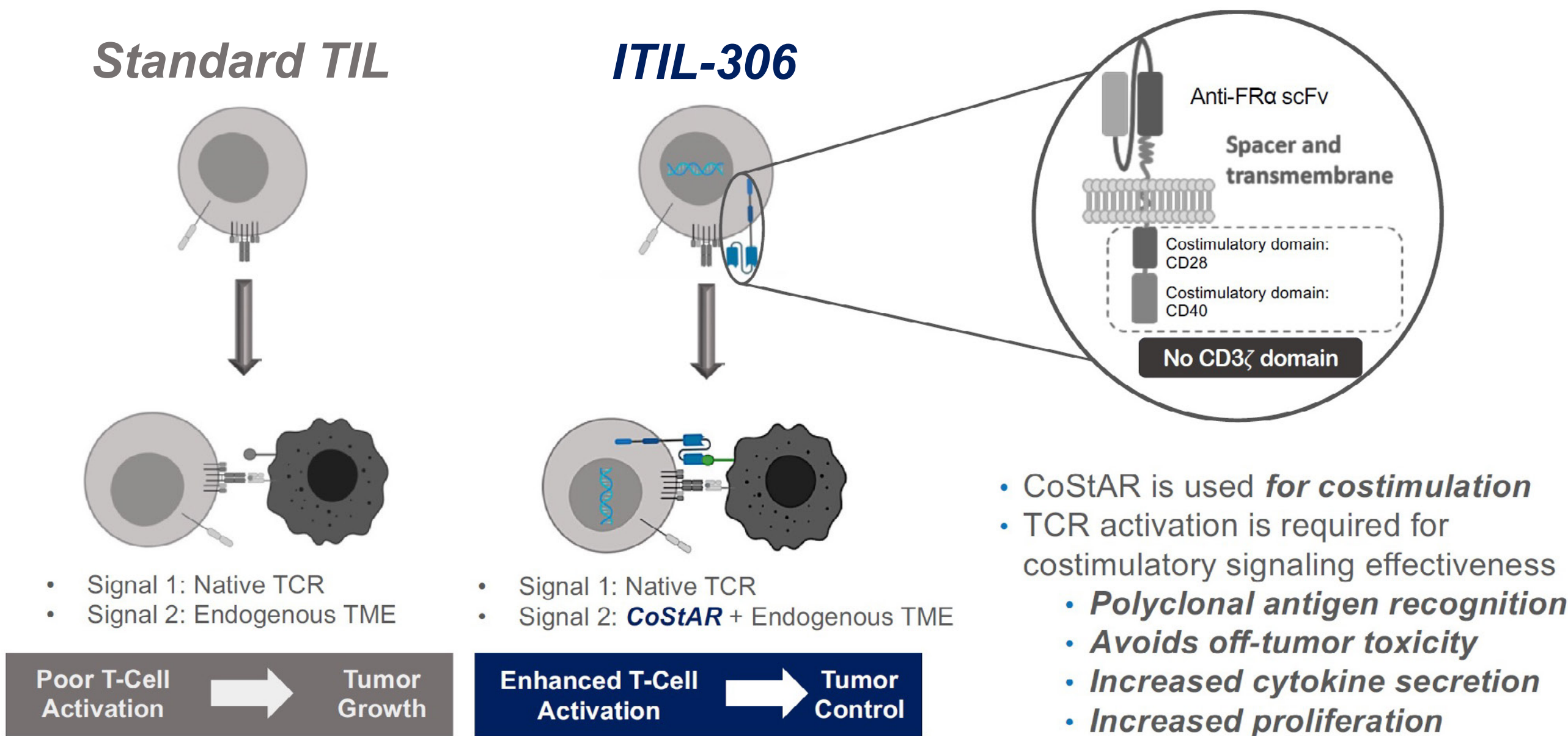
INTRODUCTION

- Major limiting factors for development of cell therapy modalities in solid tumors include clonal heterogeneity and the related lack of universally expressed tumor-specific antigens (1-3).
- As the only truly polyclonal cell product in advanced development, tumor-infiltrating lymphocytes (TILs) encompass the maximum possible diversity available with an unrestricted T-cell receptor (TCR) repertoire, thereby offering the broadest diversity of anti-tumor reactivity and demonstrate effective clinical efficacy (4,5,6).
- The suppressive tumor microenvironment is characterized by high expression of coinhibitory receptors and suboptimal costimulation (7,8).
- To overcome poor endogenous costimulation, we have developed the CoStimulatory Antigen Receptor (CoStAR) platform which provides costimulation via a chimeric CD28.CD40 module upon engagement with tumor associated antigens such as human folate receptor 1 (FRα), which is overexpressed in a number of malignancies including lung, ovarian and renal cancer.
- Here we explored the parameters which may affect CoStAR activity in response to the heterogeneity of the tumor microenvironment, including varying levels of FRα, the effect of serial provision of signal 1/signal 2, and the effect pMHC affinity and concentration (9).

METHODS

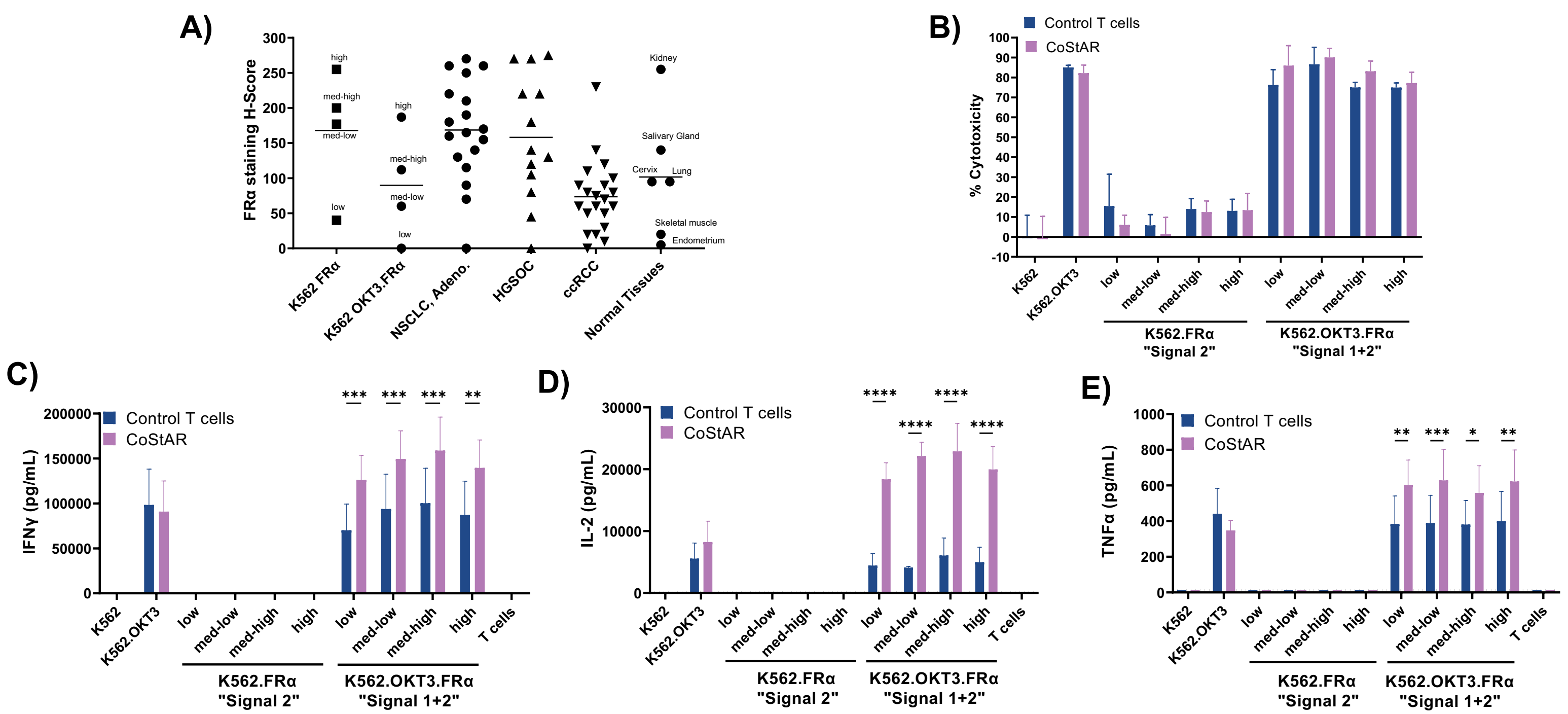
- Tumor from several indications, healthy tissue, and engineered K562 lines were analyzed for FRα expression.
- Healthy donor T cells were engineered with CoStAR and cocultured with the target lines before assessment of IL-2, TNFα and IFNγ in the supernatant and counts of remaining target cells.
- A serial stimulation assay was established using Ba/F3 cells engineered with either OKT3 (Signal 1) and/or FRα (Signal 2), to recapitulate scenarios in which CoStAR cells may encounter tumor and normal tissue in sequence.
- Healthy donor T cells engineered with CoStAR were cocultured with the indicated Ba/F3 cells presenting either signal 1 alone, signal 2 alone, both or neither. After 7 days, the T cells were restimulated with additional Ba/F3 cells before analysis of cytokines.
- Healthy donor T cells were singly or co-transduced with an HLA-A*02 Melan-A/MART-1 specific TCR and FRα specific CoStAR.
- Non-transduced or FRα transduced HLA-A*02+ T2 cells were pulsed with Melan-A/MART-1 heteroclitic (ELAGIGILTV 17 μM) or altered peptide ligands of varying antigenicity FATGIGITV (3 μM), ELTGIGILTV (82 μM) and ALGIGILTV (undefined low affinity) (10,11) and cytokine secretion measured after 20 h coculture.
- For cytokine analysis 2-way ANOVA with Sidak's or Tukey's multiple comparisons was used.
- For EC50 values nonlinear regression curves were fitted with a log (agonist) versus response (3 parameters) model to calculate LogEC50 fits. Comparisons of best fit LogEC50 values were calculated. Log EC50 values from 3 donors were analyzed by Friedman statistical test with Dunn's multiple comparisons.

Figure 1. MOA



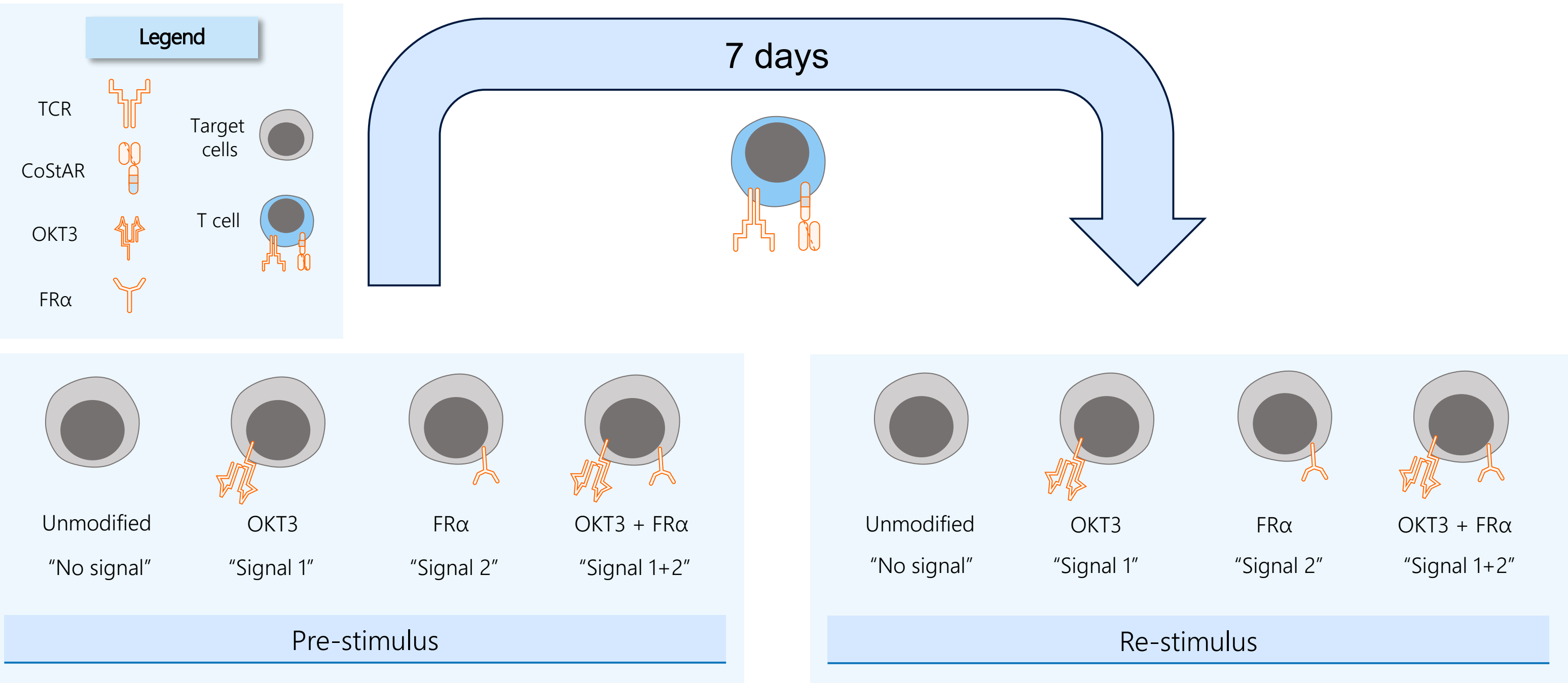
RESULTS

Figure 2 – CoStAR synergizes with signal 1 agonists even at low levels of FRα



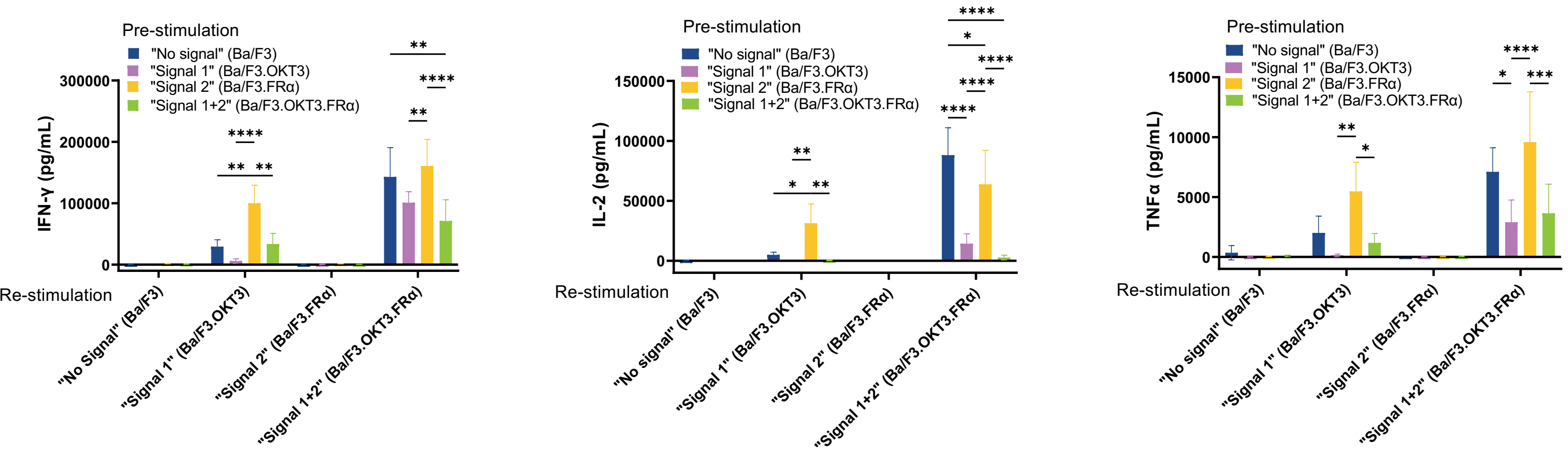
- K562 clonal lines were established expressing FRα levels within the range seen in normal and tumor tissues (A).
- Enhanced lysis of K562 target lines (B) and cytokine release was not observed (C-E) in the absence of signal 1, regardless of the level of FRα present, indicating that FRα alone is insufficient to elicit effector function.
- In conditions with OKT3 expressing lines, CoStAR demonstrated FRα dependent enhancement in activity, with all levels of FRα significantly enhancing IL-2, IFNγ and TNFα release (C-E).
- Enhancements in cytokine production at low FRα levels was observed, an expression level equivalent to that observed at the lower end of the range seen in lung, ovarian and renal cancer.

3a – CoStAR engagement enhances subsequent stimulation to signal 1 agonism



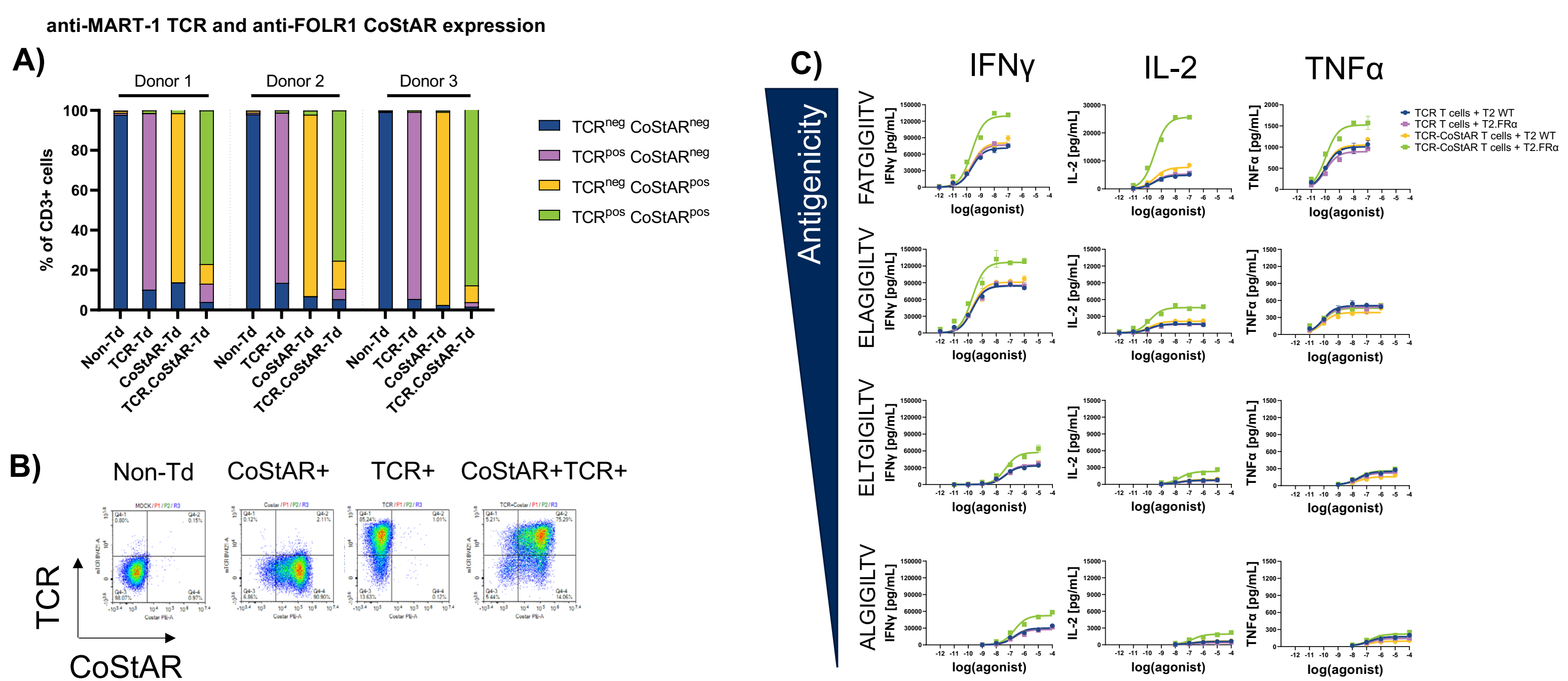
RESULTS (Continued)

3b – CoStAR engagement enhances subsequent stimulation to signal 1



- A model of serial tumor engagement was established to investigate the impact of combinatorial signal 1/ signal 2 provision to CoStAR T cells (Figure 3a).
- T cells pre-stimulated with FRα alone and then re-stimulated with OKT3 produced significantly more IL-2, IFNγ and TNFα than T-cells pre-stimulated on target cells without FRα. This suggests that FRα primes T cells to enhanced responsiveness to subsequent stimulation in the absence of CoStAR engagement.
- Restimulated T cells showed no response to non-transduced Ba/F3 cells or Ba/F3 cells expressing FRα alone, regardless of the pre-stimulation condition. This confirms a lack of off-target toxicity even after multiple stimulations.

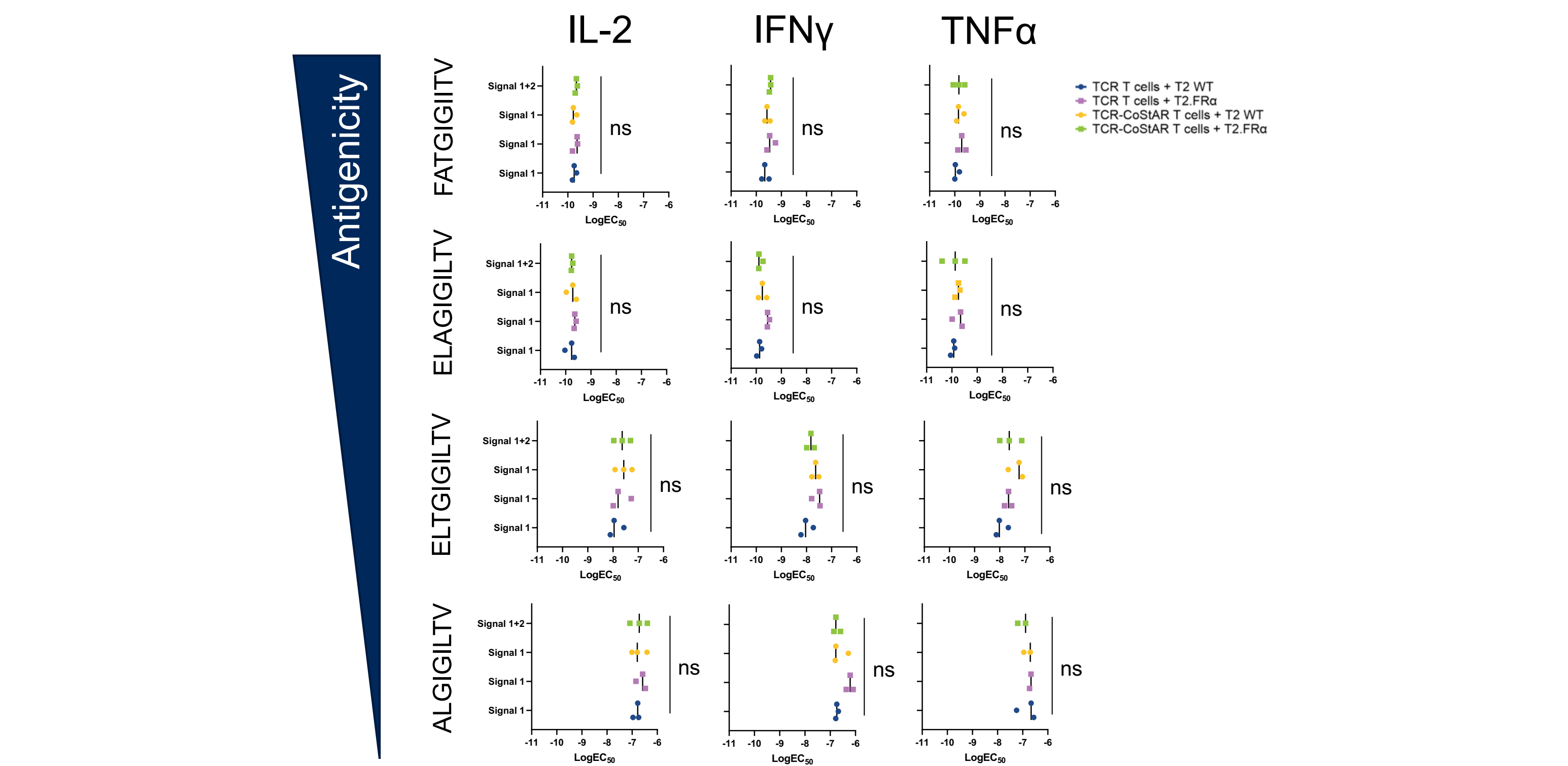
Figure 4 – CoStAR enhances the maximum cytokine responsiveness of T cells



- T cells from three healthy donors were engineered with a Melan-A/MART-1 specific TCR and CoStAR (A, B).
- TCR and TCR.CoStAR T cells responded in a dose dependent manner to peptide loaded T2 or T2.FRα cells via production of IFNγ, IL-2 and TNFα (representative example of response curves from one of the three donors).
- Differences were seen across the peptides tested, with the strongest HLA binder, FAT, eliciting a greater degree of activity followed in the order: ELA, ELT and ALG eliciting the lowest level of cytokine secretion.
- TCR engineered cells responded to peptide loaded T2 or T2.FRα cells and TCR.CoStAR cells responded to peptide loaded T2 cells with similar levels of activity.
- TCR.CoStAR cells responded more potently to peptide loaded T2.FRα cells against all peptides tested (C).

RESULTS (Continued)

Figure 5 – CoStAR does not affect the half-maximal effective antigen concentration (EC50) of T cells stimulated through pMHC



- EC50 values were calculated for each scenario (+/- CoStAR and +/- FRα) and plotted for each peptide and each effector function.
- CoStAR did not impact TCR mediated half maximal effective antigen concentration (EC50), which is consistent with CoStAR enhancing overall T cell activity without altering the trigger point for activation.

CONCLUSIONS

- Anti-FRα CoStAR enhances T-cell function in response to target antigen regardless of the degree of FRα expression, as long as FRα is expressed.
- CoStAR cells do not respond to FRα in the absence of TCR stimulation (cytokine production or cytotoxicity), even at physiologically high levels of FRα.
- Stimulation of CoStAR with FRα alone primes subsequent responses to TCR agonism, suggesting that priming of CoStAR with FRα may enhance subsequent activity towards any tumor targets lacking FRα expression.
- CoStAR enhances T-cell activity for pMHC ligands across a range of affinities, which should allow tumor reactive T cells harbouring CoStAR to respond more effectively to suboptimal conditions; however, CoStAR engagement does not change the EC50. This suggests that TCR functional avidity or promiscuity will not be affected by CoStAR engagement.
- These results support the clinical exploration of anti-FRα CoStAR in tumor indications expressing variable FRα levels.
- Phase 1a/1b, multicenter, clinical study (NCT05397093) evaluating the safety and feasibility of ITIL-306 in adult patients with advanced solid tumors whose disease has progressed after standard therapy is ongoing.

REFERENCES

1. Maroli F, et al. Stem Cell Res Ther. 2021;12:81.
2. Albelda SM. Cancer Immunol Res. 2020;8:2.
3. Shilyansky J, et al. Proc Natl Acad Sci U S A. 1994;91:2829-2833.
4. Spindler MJ, et al. Nat Biotechnol. 2020;38:609-619.
5. Rohaan MW, et al. J Immunother Cancer. 2018;6:102.
6. Pillai et al. Am J Cancer. Res 2022; 12: 3967-3984
7. Truxova L, et al. J Immunother Cancer. 2018;6:139.
8. Bandola-Simon J, Roche PA. Mol Immunol. 2019;113:31-37.
9. Bridgeman JS, et al. 2012;135(1):9-18.
10. Clement M, et al. J Immunol. 2011;187(2):654-63.
11. Ekeruche-Makinde J, et al. J Biol Chem. 2012;287(44):37269-81.

Copies of this presentation obtained through Quick Response (QR) Code are for personal use only and may not be reproduced without permission from the author of this poster.

