CoStAR (Costimulatory Antigen Receptor) Enhancement of Tumour Infiltrating Lymphocyte Therapy

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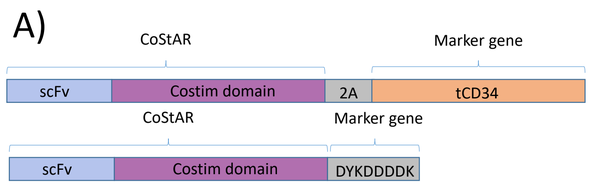
Introduction

- Adoptive T-cell therapies are proving to be hugely successful for ameliorating various chemo-refractory cancers, with CD19-CAR for leukaemia and tumour infiltrating lymphocyte (TIL) therapy for melanoma in particular showing success.
- Full and sustained T-cell activation requires synergistic signalling through the TCR (signal 1) and co-stimulatory molecules (signal 2). Tumour cells rarely express ligands for costimulatory receptors leading to a reduced T-cell response.
- The introduction of a chimeric costimulatory antigen receptor (CoStAR), which provides signal 2 upon tumour antigen encounter would provide an artificial signal 2 to T-cells leading to enhanced effector function
- This approach is particularly attractive to TIL therapy where the T-cells harbour natural tumour reactivity through the endogenous TCR.

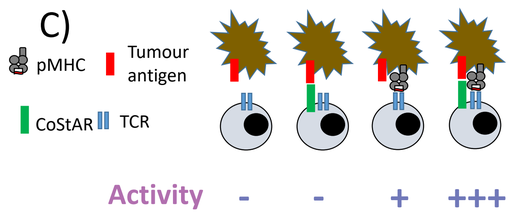
Development of CoStAR model system

- A CoStAR consisting of a tumour specific scFv fused to a costimulatory domain with either a CD34 or DYKDDDDK marker gene was generated (A).
- CoStARs targeting a number of tumour associated antigens were designed (B).
- Transduced T-cells were incubated with antigen +ve cell lines engineered to express a membrane-bound OKT3 (anti-CD3) molecule with a GFP tag.
- It was hypothesised that the presence of CoStAR would enhance functional activity in response to the OKT3 mediated signal 1 (C).
- Cells were enriched for marker gene expression, or intracellular flow staining performed and transduced cells gated using the relevant marker gene.

A)



C)

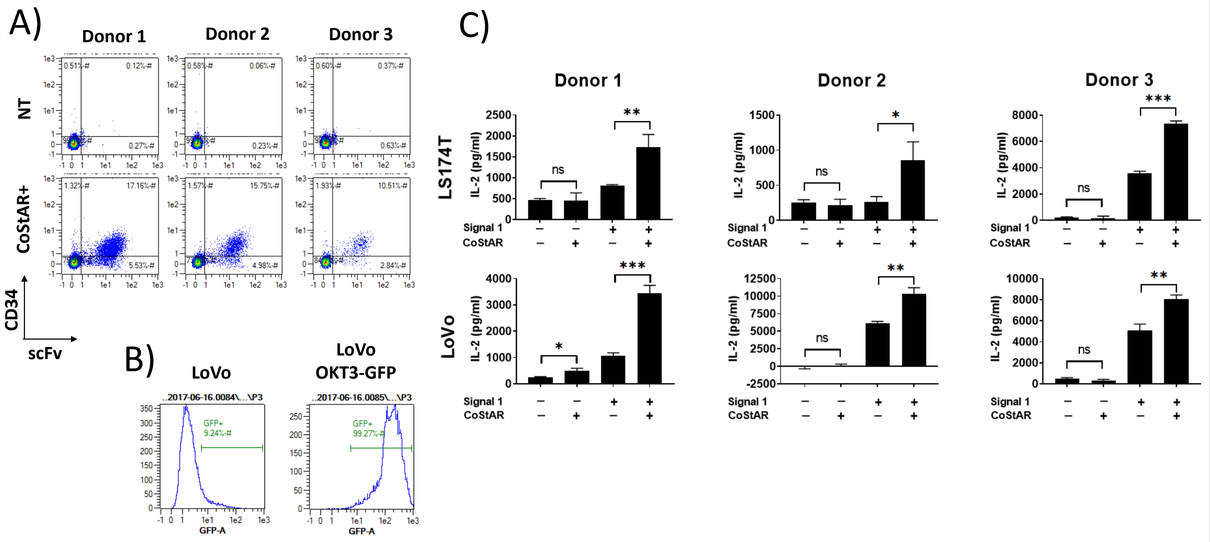


B)

Target	Cell lines	Expression
CEA	LoVo, LS174T, H508	Colorectal, Gastric, Lung, Pancreatic
CA-125 (MUC-16)	OVCAR3	Ovarian, Endometrial
Folate receptor (FOLR1)	LoVo, OVCAR3	Ovarian, Renal

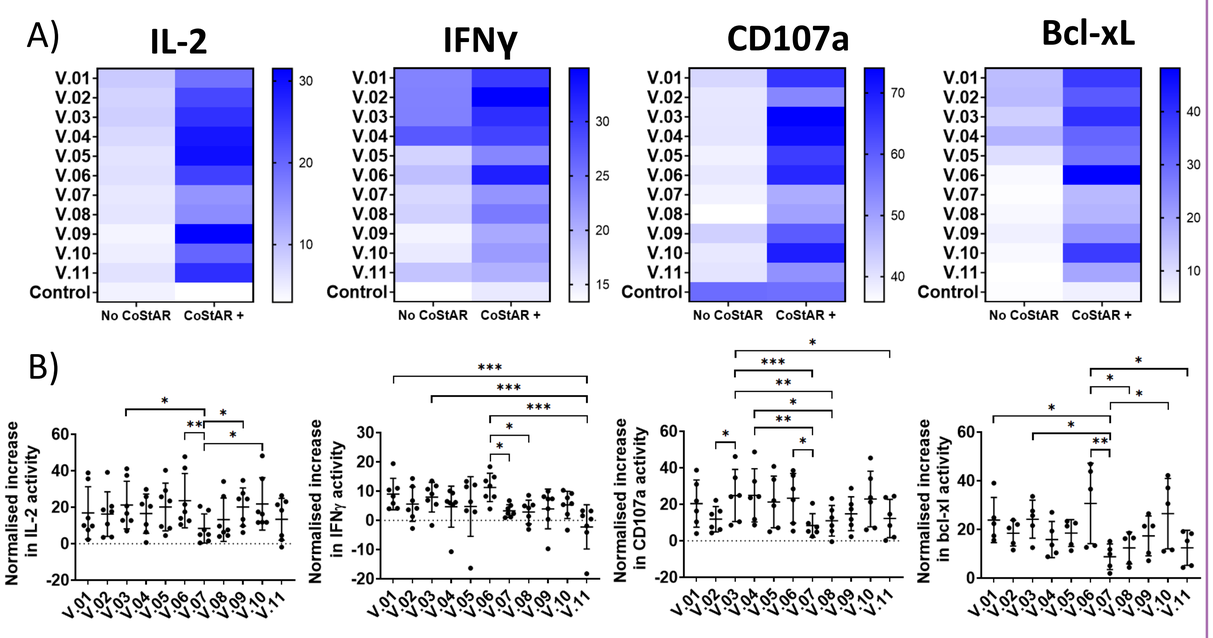
CoStAR enhances IL-2 production in T-cells

- Primary human T-cells from three healthy donors were transduced with CoStAR lentivirus.
- Transduction efficiency was evaluated using anti-CD34 antibodies (marker gene) and CEA.hFc (scFv detection) (A) before bead enrichment of transduced cells.
- Cells were co-cultured with CEA+ LoVo/LS174T or LoVo/LS174T-OKT3 for 24 h (B) before analysis of supernatant by ELISA for IL-2 production (C).
- CoStAR increases IL-2 production compared to TCR induced stimulation alone.



Variant CoStARs demonstrate enhanced activity

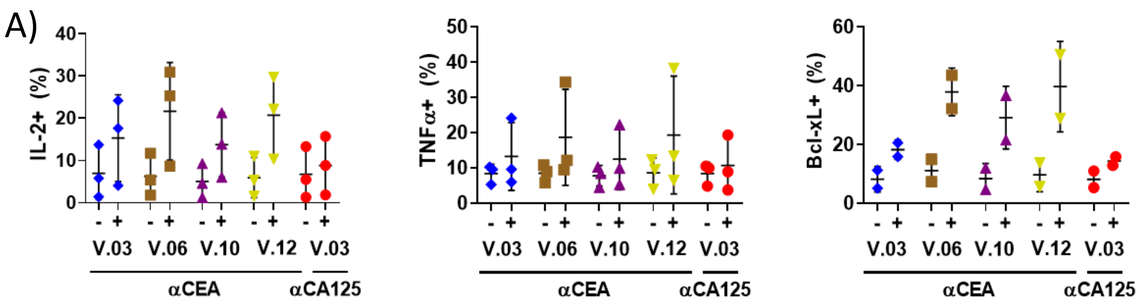
- A panel of CoStAR receptors to compare with the prototype (V.03) was designed
- Expression of the effector molecules IL-2, IFNγ and CD107a, as well as the anti-apoptotic protein Bcl-xL were assessed by flow cytometry.
- All receptors tested enhanced activity of all readouts except the control target mis-matched CoStAR (A).
- Comparisons of normalised increase in CoStAR-driven effector activity shortlisted V.06 and V.10 for further analysis (B).



Colorectal and ovarian cancer specific CoStARs demonstrate functional activity

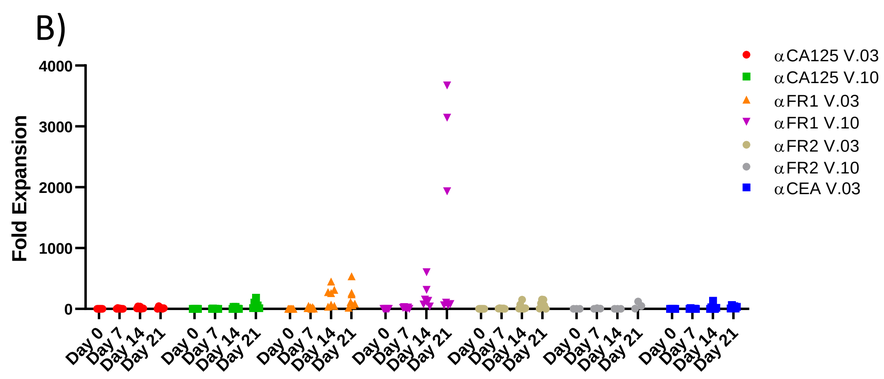
- A second round of CoStAR validation in LoVo-OKT3 v T-cell co-cultures was conducted with a panel of DYKDDDDK tagged CoStARs.
- V.03, V.06 and V.10, and also V.12, a hybrid of V.06 and V.10, were compared to each other and an antigen mismatched V.03 CoStAR as a negative control.
- All the CEA targeting receptors performed well across various effector functions (A).
- Expression of V.06 and V.12 was poor and as such were excluded from further analysis.

A)



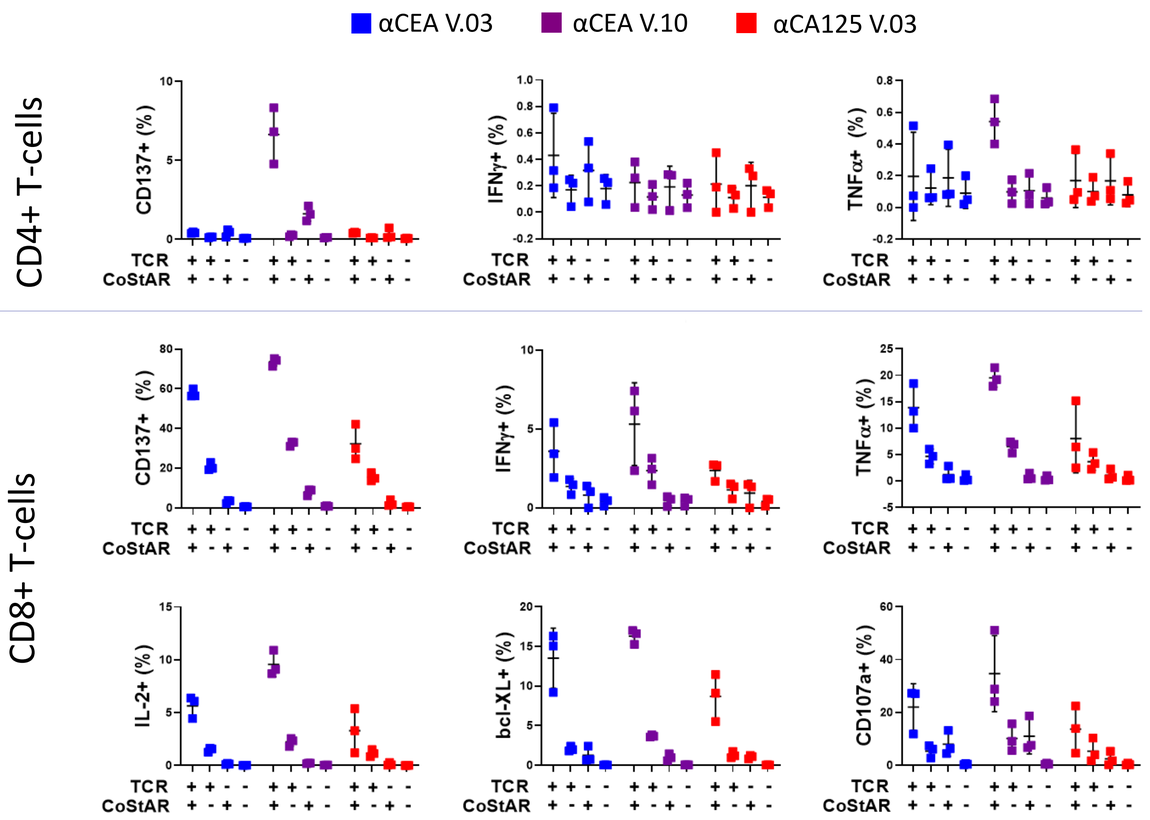
- V.03 and V.10 were cloned with antigen binding regions for CA125 or FOLR1.
- The CA125+/FOLR1+ cell line OVCAR3 was transduced with OKT3 and used in co-culture experiments with engineered T-cells.
- Both CA125 and FOLR1 targeting CoStARs enhanced IL-2 production by T-cells but only the FOLR1 targeting CoStAR enhanced specific proliferation of transduced cells, with V.10 performing best (B).

B)



CoStAR synergises with a recombinant monoclonal TCR

- A model of TCR and CoStAR dual transduction was developed taking advantage of a previously described HLA-A*02 restricted CEA TCR (Parkhurst et al. Clin. Cancer Res 2009)
- Effector activity of CEA targeting V.03 and V.10 receptors and a CA125 antigen mismatched V.03 receptor in both singly and TCR dual transduced CD4+ and CD8+ T-cells was assessed.
- The presence of CoStAR enhanced TCR mediated stimulation, with V.10 CoStAR enhancing a number of activities above and beyond the impact of V.03, in particular CD137 induction and IL-2 secretion.



Summary

- A chimeric costimulatory receptor (CoStAR) which provides T-cells with a costimulatory signal upon engagement with a defined tumour antigen has been optimised.
- CoStARs against CA125 and FOLR1 (ovarian cancer) and CEA (GI cancers) have been validated.
- Optimised CoStARs demonstrate enhanced activity and proliferative capacity in T-cells.
- Choice of CoStAR target can impact on the proliferative capacity of the engineered T-cells.
- CoStARs work synergistically with introduced monoclonal TCRs.

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