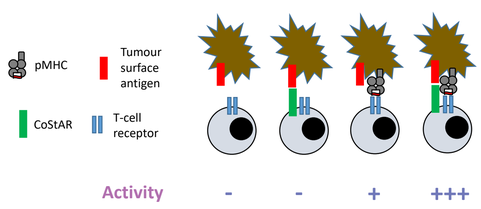
In vitro analysis of Tumour Infiltrating Lymphocytes engineered with costimulatory antigen receptors delivering targeted costimulation

John S. Bridgeman[1]; Michelle L. Le Brocq[1]; Aysha Patel[1]; Martina Sykorova[1]; Tania Katopodi[1]; Joanne McCaffrey[1]; Ryan D. Guest[1]; Robert E. Hawkins[1]

[1] Immetacyte Ltd. UMIC Bio-Incubator, 48 Grafton Street, Manchester, M13 9XX, UK;

Introduction

Tumour infiltrating lymphocytes (TIL) can be isolated from tumour biopsies and expanded to huge numbers ex vivo before reinfusion into lymphodepleted cancer patients. This form of treatment has proved remarkably successful in numerous clinical trials, particularly for the treatment of melanoma, and could be used in multiple other cancer indications such as ovarian cancer. In an effort to enhance Full and sustained T-cell activation requires synergistic signalling through the TCR (signal 1) and costimulatory molecules (signal 2). Tumour cells rarely express ligands for costimulatory receptors leading to a reduced T-cell response. We hypothesised that introduction of a chimeric costimulatory antigen receptor (CoStAR), which provides signal 2 upon tumour antigen encounter, would provide an artificial signal 2 to TIL leading to enhanced effector function, particularly IL-2, a classical signal 2 response.



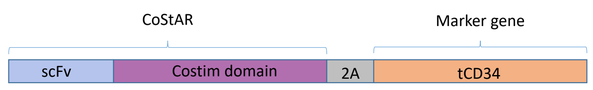


Figure 1: CoStAR Schematic. CoStAR enhances T-cell activity upon tumour antigen binding by synergising with pMHC/TCR signals (A). The prototype receptor consists of a tumour antigen specific scFv fused to a costimulatory receptor signalling domain split from a truncated CD34 marker gene by a 2A cleavage sequence (B).

Validation of CA125 and CEA specific CoStAR

Our basic prototype CoStAR consisting of a single chain antibody fragment (scFv) fused to a costimulatory receptor was expressed along with a truncated (t) CD34 marker gene in JRT3-T3.5 cells. Following marker gene mediated enrichment we validated expression of the scFv via staining with His-tagged Protein L, and binding of the scFv to its cognate ligand using His- or hFc-Tagged soluble CA125 or CEA respectively, both followed by fluorochrome conjugated anti-His/hFc antibodies (Figure 2)

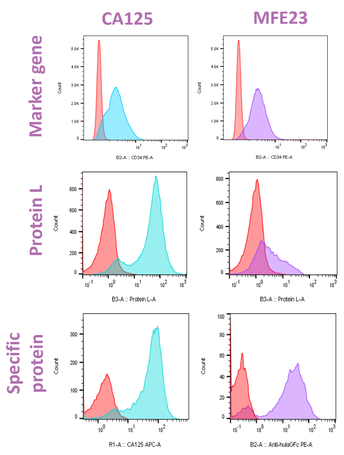


Figure 2: Flow cytometric analysis of CoStAR expression in JRT3-T3.5 cells

CoStAR enhances IL-2 production in T-cells

Primary human T-cells from three normal donors were transduced with CoStAR lentivirus. Figure 3 Shows the expression profile of the three donors using anti-CD34 and CEA.hFc. Cells were enriched to >90% using paramagnetic beads. Cells were cocultured at varying E:T ratios with CEA+ LoVo/LS174T or LoVo/LS174T-OKT3 (LoVo or LS174T engineered with membrane bound OKT3 – see Figure 4) for 24 h before analysis of supernatant by ELISA (Figure 5).

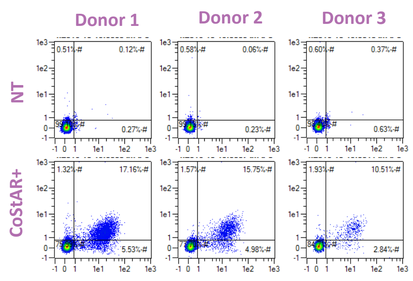


Figure 3: Flow cytometric analysis of CoStAR expression in x3 normal donor T-cells

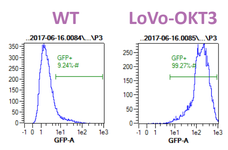


Figure 4: GFP expression in WT and OKT3-GFP engineered LoVo cells

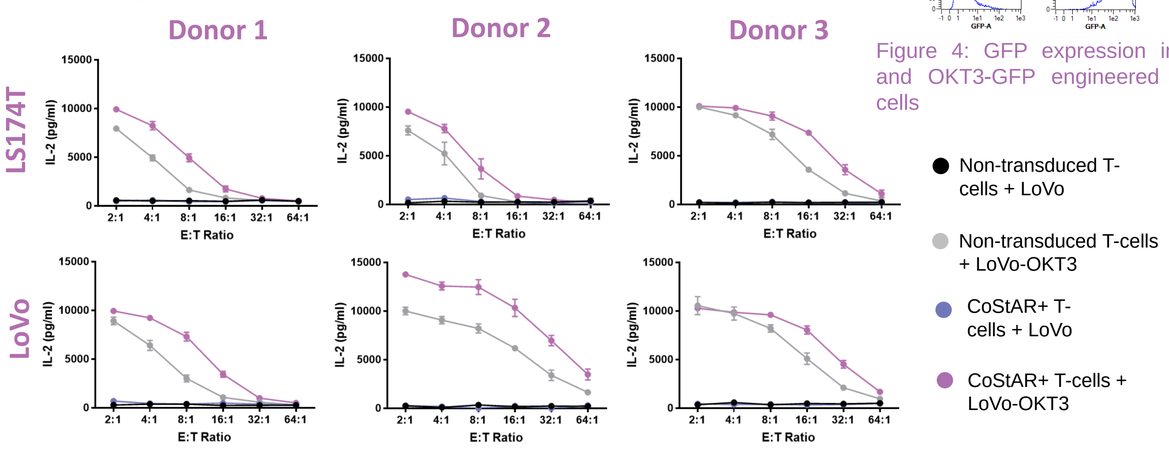


Figure 5: Analysis of IL-2 secretion from non-transduced and CoStAR+ T-cells in response to CEA+ LoVo or LS174T and OKT3 engineered LoVo or LS174T cells

CoStAR enhances IL-2 dependent and independent T-cell proliferation and survival

Proliferation dye loaded CoStAR engineered or non-engineered T-cells were mixed with LoVo or LoVo-OKT3 cells and the number of proliferation cycles the cells went through was then analysed at day 6 by flow cytometry. We found that a larger proportion of CoStAR engineered cells went though more divisions than non-engineered cells (Figure 6a). In another experiment CoStAR engineered or non-engineered cells were mixed with LoVo or LoVo-OKT3 cells in the absence of exogenous Il-2 and the absolute cell counts assessed at different time points. We found that CoStAR engineered cells had a proliferative and/or survival advantage over non-engineered cells in the presence of LoVo-OKT3 but not wild-type LoVo cells (Figure 6B)

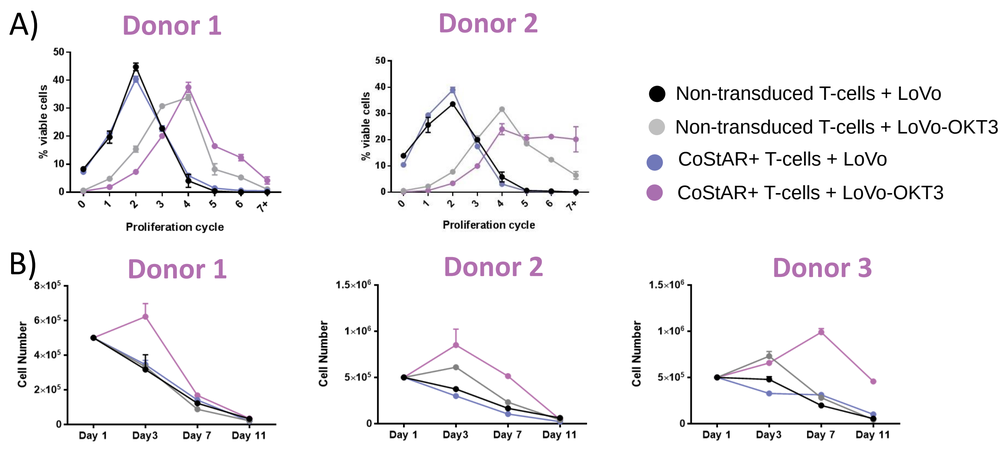


Figure 6: Proliferation analysis of CoStAR engineered cells. Proliferation at day 6 (A) and kinetics of survival/expansion (B) analysis of CoStAR+ and non-transduced cells in response to LoVo and LoVo-OKT3

Optimised expression and activity in TIL

We had initially found tCD34 to be difficult to express in TIL, we therefore compared this with two alternatives (Figure 7A). We found an alternative marker gene (herein labelled Marker gene A) which gives improved expression profiles in TIL. Ovarian TIL engineered with our prototype receptor show enhanced activity towards autologous tumour (Figure 7B)

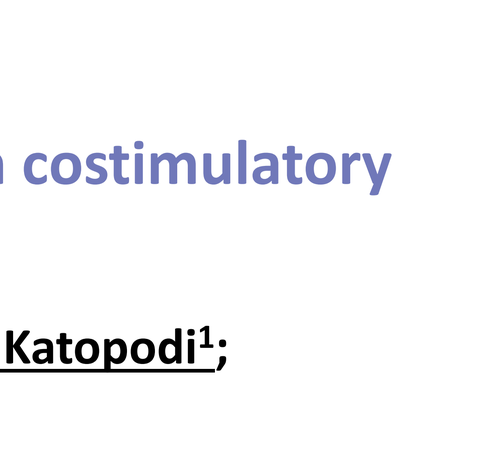


Figure 7: effect of marker gene on CoStAR expression and functional activity in Ovarian TIL of a CA125 targeting CoStAR

CoStAR enhances costimulation of CD8+ more than CD4+ T-cells

We assessed our prototype receptor (v.3) against two other receptor formats by intracellular cytokine staining. Transduced T-cells were mixed with LoVo or LoVo-OKT3 cells overnight before fixation and permeabilization, followed by staining with αIL-2 and αIFNγ antibodies. We observed IL-2 and IFNγ production by non-transduced cells in response to LoVo-OKT3 but not wt LoVo by CD4+ and CD8+ T-cells (Figure 8A). In the transduced population we observed an overall shift in the proportion of T-cells which were IL-2+ (Figure 8B).

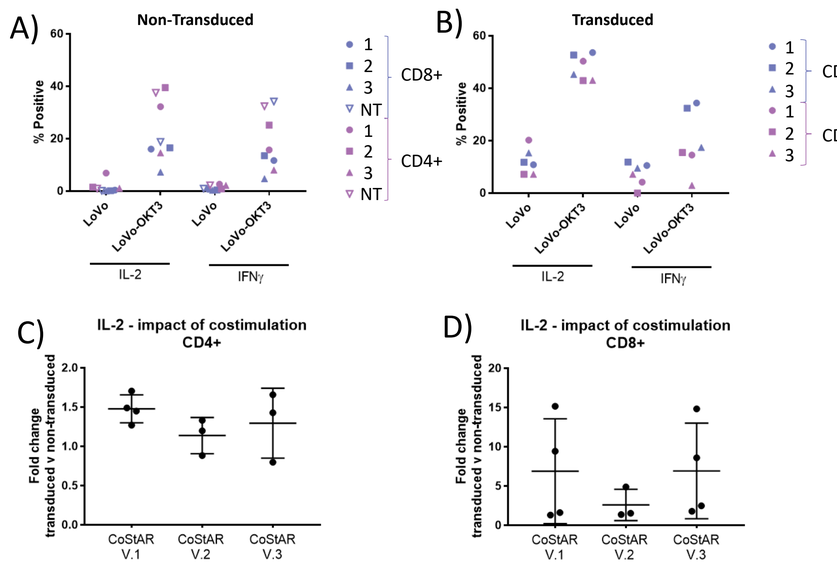


Figure 8: Analysis of IL-2 and IFNγ production from non-transduced and CoStAR+ T-cells in response to LoVo and OKT3 engineered LoVo cells by flow cytometry

To better understand this effect we looked at the fold change in the proportion of IL-2+ cells in the CD4+ and CD8+ populations over three donors. In CD4+ T-cells fold increase in IL-2 production by CoStAR (transduced v non-transduced cells) was 1.0-1.5-fold, whereas in CD8+ cells, with v.1 and v.3, we observed an average of over 5-fold increase in IL-2 production (Figure 8C & D).

Functional analysis of CoStAR variants reveals receptors with enhanced activity

Next we expanded on our previous analysis of three receptor variants by including a further eight. We conducted analysis solely in purified CD8+ T-cells as we expected to see the biggest difference in these cells. All variants showed functional activity by enhanced induction of IL-2 (Figure 9A & B). We analysed the fold induction of four effector functions (IL-2, bcl-xl, IFNγ & CD107a). Our initial analysis of four donors indicates that variants 5, 6 , 9 & 10 having potentially beneficial qualities (Figure 9C)

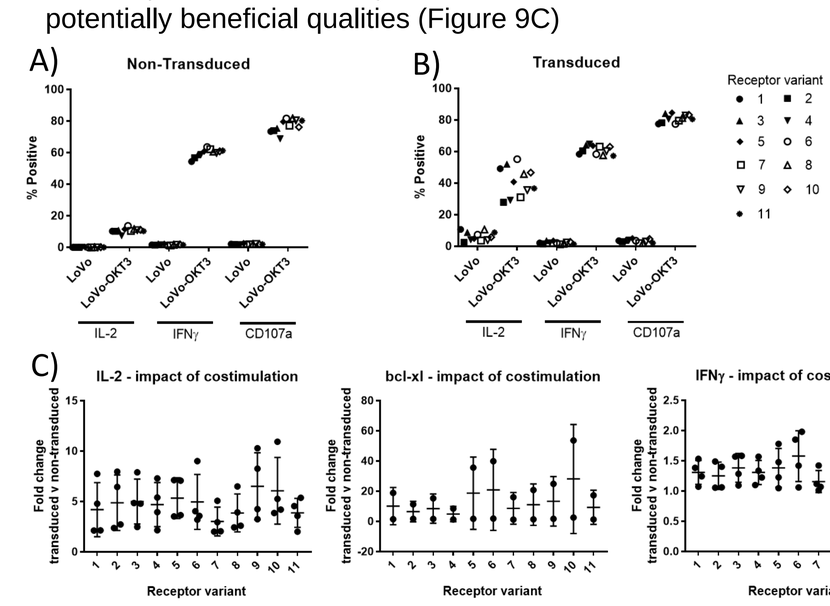


Figure 9: Analysis of IL-2 secretion from non-transduced and CoStAR+ T-cells in response to LoVo and OKT3 engineered LoVo cells

Summary

- We have developed a chimeric costimulatory receptor (CoStAR) which provides T-cells with a costimulatory signal upon engagement with a defined tumour antigen.
- We have validated CoStAR against CA125 (ovarian cancer) and CEA (GI cancers)
- We have identified an optimal marker gene for identification of CoStAR engineered TIL
- We have shown CoStAR enhances survival and proliferation and has enhanced activity in CD8+ T-cells
- We have identified several novel CoStARs which have enhanced functional activity

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