Counteracting TCR-T cell dysfunction in solid tumors through combination of FAS-based switch receptors and CD8-coreceptor

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Background

The immunosuppressive tumor microenvironment (TME) inhibits the effectiveness of T cell receptor-engineered T cell therapy (TCR-T) for solid tumors. Tumor-resident cells express multiple inhibitory ligands, including FAS ligand (FASL), a transmembrane protein that binds to the FAS receptor on T cells, triggering apoptosis[1]. Moreover, cancer cells lack costimulatory ligands mediating optimal T cell activation upon T cell receptor (TCR) engagement, which is critical for T cell engraftment and persistence.

We hypothesized that expression of switch receptors (SwR) composed of the extracellular FAS-domain and intracellular costimulatory domains will overcome the inhibitory effects of FASL and provide co-stimulation to improve T cell functionality and persistence (Fig. 1).

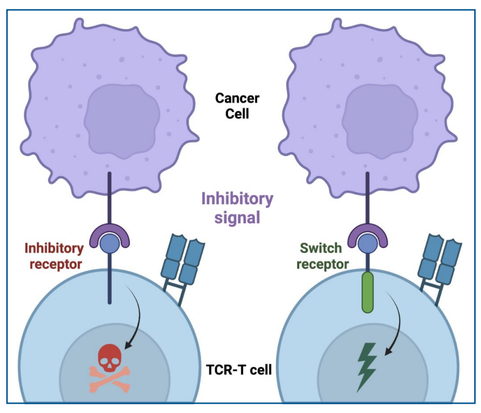

Fig 1. Switch receptors exploit negative signaling to trigger pro-survival, proliferation and activation circuits

Furthermore, incorporation of a CD8 coreceptor, resulting in CD4+ T cell engagement and subsequent coordinated action of CD4+ and CD8+ TCR-T cells[2], has the potential to broaden and deepen clinical responses. This raises payload and expression challenges in TCR-T construct design.

Here, we screened a library of FAS-based SwRs in combination with an HLA-A*02:01-restricted MAGE-A1 TCR and either a wild type or enhanced single chain CD8 co-receptor (Fig.2; for details on CD8 co-receptor design and constructs please visit Poster #375) and determined lead SwR candidates that enhanced TCR-T cell function while providing efficient resistance to FASL-expressing milieus.

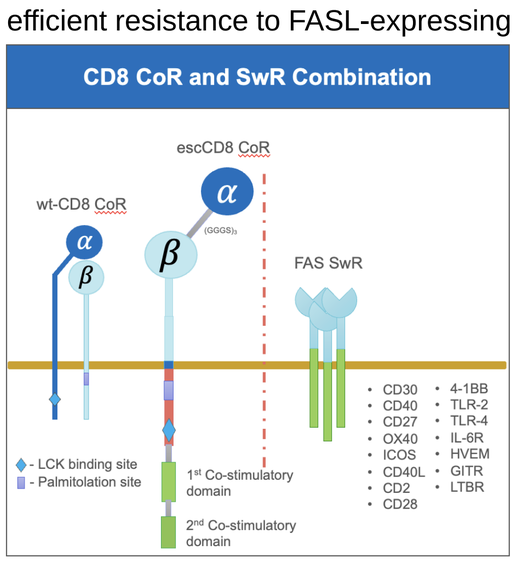

Fig 2. Combination of wild-type (wt-CD8 CoR) or enhanced single chain CD8 co-receptor (escCD8 CoR) with a library of FAS-based switch receptors with different intracellular (IC) signaling domains.

Methods

Nineteen different SwR constructs in combination with a MAGE-A1-targeting TCR, currently under clinical development (NCT05430555), were retrovirally delivered to CD8+ T cells. Functional activity against FASL-expressing cancer cell lines was evaluated for the SwR candidates that were efficiently expressed, as determined by flow cytometry.

Three SwR lead candidates, in combination with MAGE-A1 TCR and escCD8 CoR, were further analyzed in CD4+ and CD8+ T cells by assessing the T cell phenotype and functional activity against FASL-expressing cancer cell lines under sustained or repeated antigen stimulation.

Cytotoxicity was assessed by live cell imaging using the IncuCyte Zoom S3. Cytokine secretion was determined by LEGENDplex ™ (BioLegend).

Conclusions

- We successfully expressed a TCR, a switch receptor and a CD8 co-receptor on primary T cells using an all-in-one vector approach.
- Armoring TCR-T cells with FAS-CD40 or FAS-CD27 switch receptors and an enhanced single-chain CD8 co-receptor (escCD8 CoR) enabled generation of functional CD4/CD8 TCR-T product candidates and mediated strong cytotoxic activity upon sustained or repeated antigen stimulation at low E:T ratios.
- Our data support this dual approach as a promising strategy to overcome tumor resistance mechanisms coming from the immunosuppressive TME and the lack of co-stimulation by cancer cells, thus providing the potential for improved clinical outcomes.

References

1.Yamamoto T. N. et al., J Clin Invest, 2019, doi: 10.1172/JCI121491
2. Bajwa, G., et al. (2021). *Journal for ImmunoTherapy of Cancer* I (Suppl 2), A173-A173

Results

Ten switch receptor candidates were successfully expressed on primary T cells in combination with MAGE-A1-targeting TCR

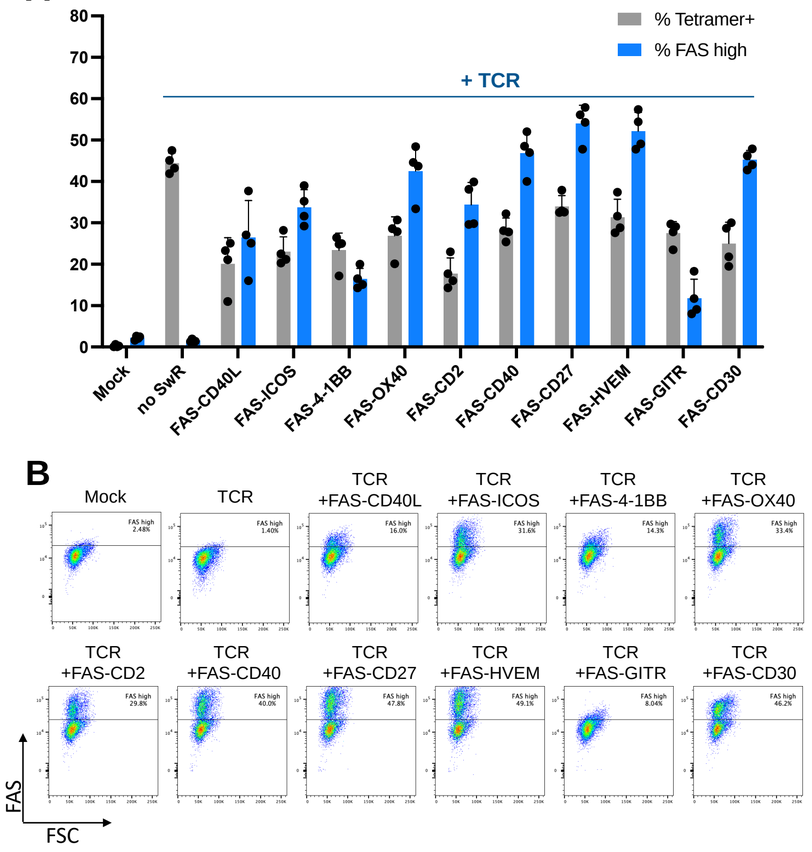

Fig 3. TCR and switch receptor expression on CD8+ T cells transduced with MAGE-A1 TCR and different switch receptor candidates.
A) Out of 19 FAS-based switch receptor candidates with different intracellular domains, ten show high levels of FAS expression at low (GITR, 4-1BB), medium (CD40L) or high (ICOS, OX40, CD2, CD40, CD27, HVEM, CD30) percentage. FAS is absent from TCR and Mock control, as expected. Data are shown as mean ± SD of 4 donors **B**) Representative flow cytometry plots of data presented in panel A.

Lead switch receptor candidates were selected based on long-term cytotoxic activity at low E:T ratios against FASL-overexpressing cancer cells

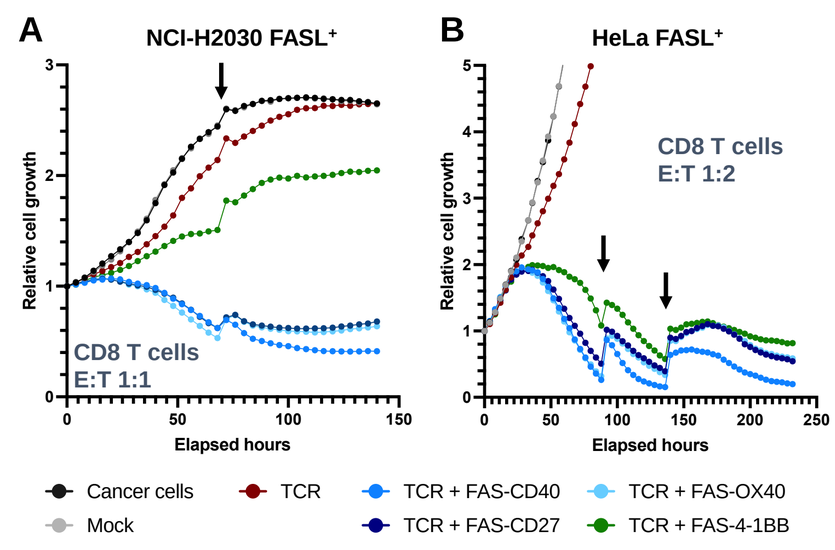

Fig 4. Addition of switch receptors to TCR-transduced CD8+ T cells enables effective elimination of FASL-expressing cancer cells. While FAS-OX40, FAS-CD40 and FAS-CD27 switch receptors mediated efficient cytotoxicity in all experimental settings, FAS-4-1BB only partially protected T cells from FASL-mediated cell death.
T cells were co-cultured with MAGE-A1 expressing NCI-H2030 cells transduced with HLA-A2, GFP and FASL (**A**), or HeLa cells transduced with MAGE-A1, HLA-A2, Nuclight Red and FASL (**B**). Cancer cells cultured without T cells or with Mock T cells served as controls. Arrows indicate addition of tumor cells. Data are shown as mean of 3 (A) and 2 (B) replicates, with 4 image areas per replicate. Graphs are representatives of 2 donors.

Switch receptors enable sustained cytotoxicity in conditions reflecting the immune suppressive tumor microenvironment

Co-culture of TCR-T cells with a mix of different ratios of FASL+ and FASL- cancer cells

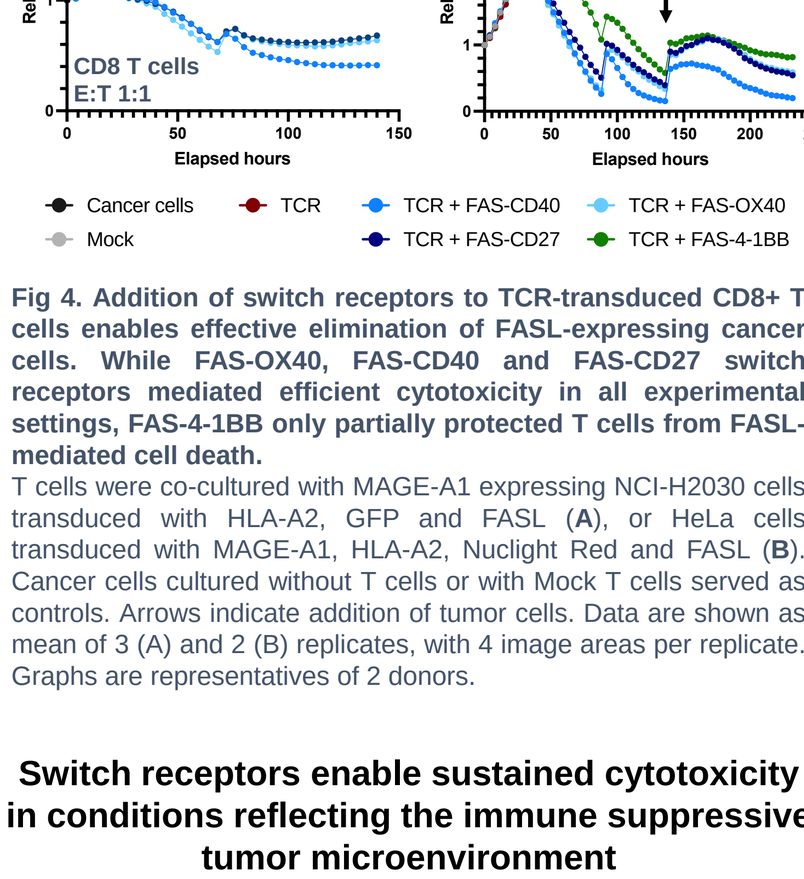

Fig 5. CD8+ T cells not expressing a switch receptor lose cytotoxic capacity proportionally to the FASL+ cancer cell content. Incorporation of switch receptors enabled sustained cytotoxicity irrespective of FASL-expression on cancer cells.
NCI-H2030 cells transduced with HLA-A2, GFP, with or without FASL were mixed at the indicated ratios. Data are shown as mean of 3 replicates, with 4 image areas per replicate.

Single-chain CD8 CoR, but not the wt-CD8 CoR, can be co-expressed in combination with both a switch receptor and a MAGE-A1-targeting TCR

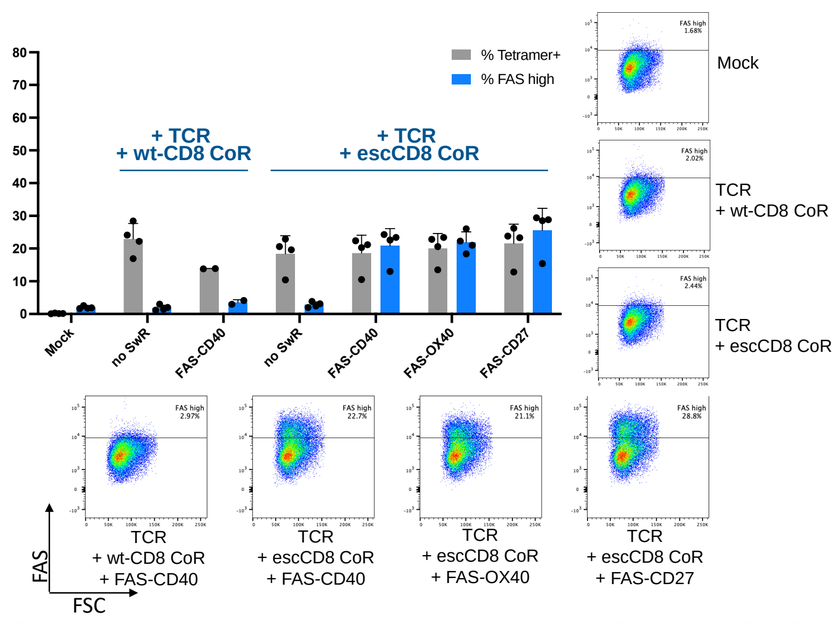

Fig 6. Switch receptor expression levels on CD4 and CD8 T cells transduced with MAGE-A1 TCR, different switch receptors, and either wt-CD8 CoR or escCD8 CoR.
A) Expression of switch receptor lead candidates was detected by flow cytometry using high FAS expression as readout. This was observed only in constructs incorporating escCD8 CoR, but not the wt-CD8 CoR. Data are shown as mean ± SD of 4 donors for escCD8 CoR and 2 donors for wt-CD8 CoR. **B**) Representative flow cytometry plots of data presented in panel A.

FAS-CD40 and FAS-CD27, in combination with escCD8 CoR, mediate long-term cytotoxicity at low E:T ratio against FASL-overexpressing cancer cells

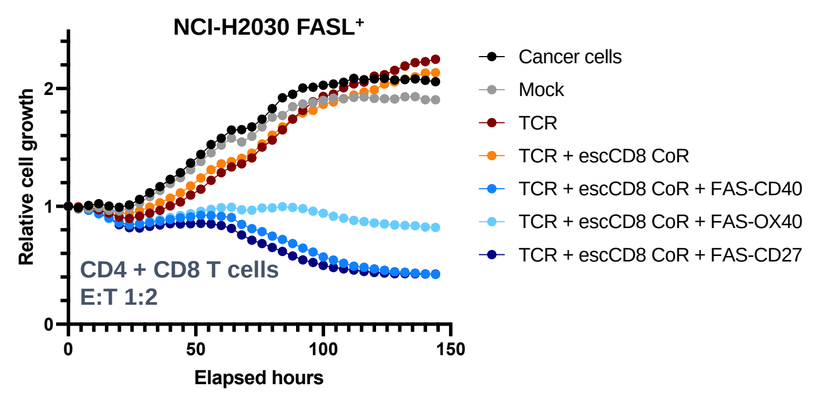

Fig 7. Addition of switch receptors enabled TCR-T product candidates comprising of both CD4 and CD8 T cells to effectively eliminate FASL- expressing cancer cells.
NCI-H2030 cells transduced with HLA-A2, GFP and FASL. T cells expressing TCR and escCD8 CoR together with FAS-CD40 or FAS-CD27 switch receptors efficiently mediated long-term cytotoxicity at very low E:T ratio, while FAS-OX40 only partially restricted growth of FASL-expressing cancer cells. TCR-T cells lacking switch receptors did not demonstrate cytotoxic activity against FASL-expressing cancer cells. Data are shown as mean of 3 replicates, with 4 image areas per replicate. Graph is representative of 4 donors.

T cells expressing FAS-CD40 or FAS-CD27 and escCD8 CoR maintain functional activity upon repeated stimulation with FASL-overexpressing cancer cells

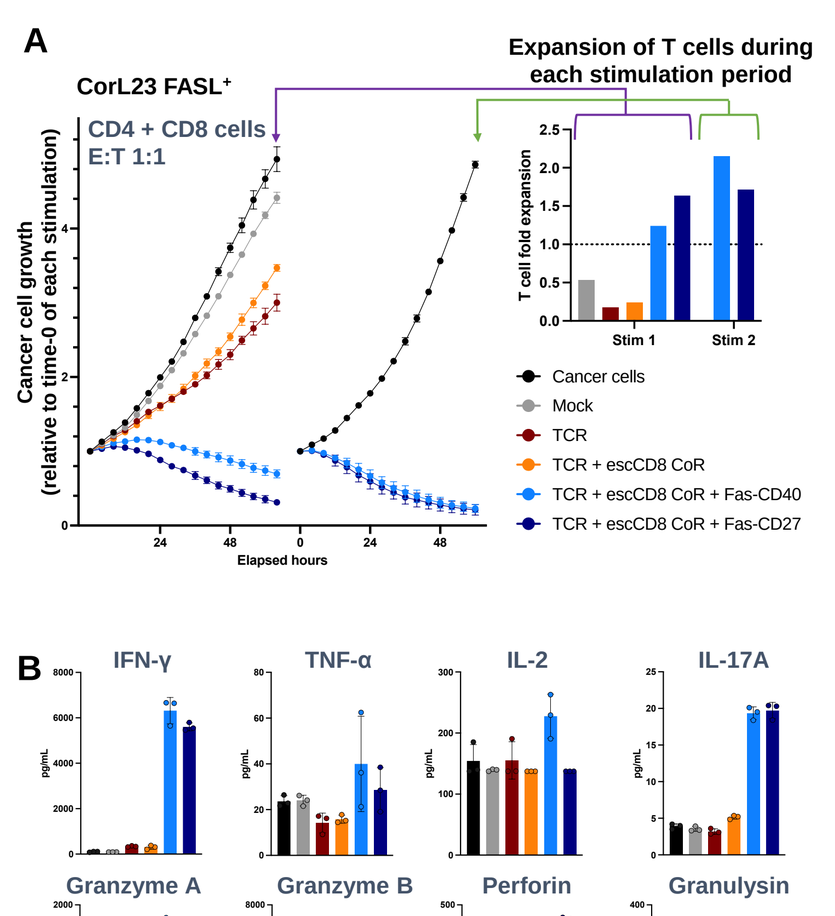

Fig 8. Armoring CD4/CD8 TCR-T products with FAS-CD40 or FAS-CD27 SwR and escCD8 CoR mediate stronger cytotoxic activity and cytokine secretion upon repeated antigen stimulation.
COR-L23 cells were transduced with HLA-A2, Nuclight Red and FASL. After each stimulation cycle, T cells were harvested, counted and replated with freshly pre-seeded cancer cells at an E:T ratio of 1:1. **A**) Simultaneous expression of TCR, escCD8 CoR and switch receptors enabled T cells to effectively eliminate FASL-expressing cancer cells for subsequent stimulation rounds. Data are shown as mean of 3 replicates, with 9 image areas per replicate. The bar graph on the right shows corresponding fold expansion of effector cells. **B)** Culture supernatants were collected after the first stimulation to assess cytokine production. Data are shown as mean ± SD of 3 replicates.