Unlocking precision: a next-generation multi-specific CD3 Switch-DARPin with enhanced function to tackle current limitations of T cell engagers in ovarian cancer

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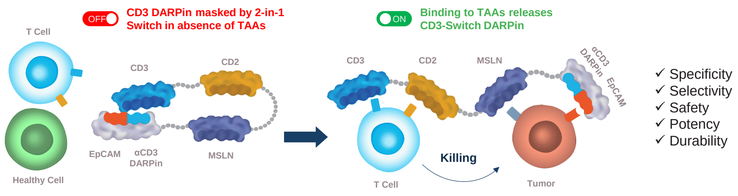
Introduction

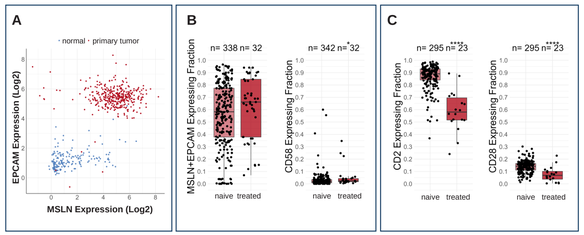
Ovarian cancer (OC) is characterized by a lack of durable response and the absence of specific, targeted therapy leads to resistance. Immunotherapy has had limited success, attributed to the absence of clean tumor-associated antigens (TAAs), presence of dysfunctional T cells, and an immunosuppressive tumor microenvironment. To address all these challenges in one molecule, we boosted our mesothelin (MSLN) x CD3 DARPin T cell engager with a CD2-engaging domain for co-stimulation and engineered a second "switchable" TAA-targeting DARPin (EpCAM) with a CD3-mask to ensure activation ONLY in the presence of these TAAs – further referred to as CD2/CD3 Switch.



- ✓ Specificity
- ✓ Selectivity
- ✓ Safety
- ✓ Potency
- ✓ Durability

EpCAM & MSLN are highly co-expressed in OC tumors

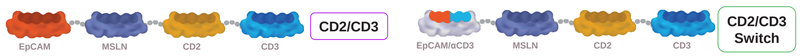
RNAseq data from human tissue biopsies, cancer cells and T cells



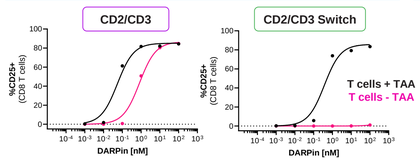
(A) Tissue sample biopsies from TCGA and GTEx were analyzed for MSLN and EpCAM expression (RNAseq, bulk). (B,C) Public single-cell RNAseq data were curated and re-analyzed in-house. The fraction of cells expressing the gene of interest was determined. (n= number of samples; unpaired Wilcoxon test). Panel A: tissue biopsies; Panel B: cancer cells; Panel C: T cells.

In-house curated data show that ~60% of untreated primary tumours co-express MSLN and EpCAM, while this is rare in healthy tissue. CD58, the ligand for CD2, is often down-regulated in OC, whilst >60% of infiltrating T cells express CD2, compared to <15% for CD28.

CD3 Mask prevents T cell activation in the absence of TAAs

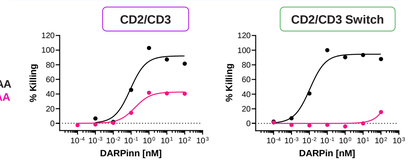


T cell Activation



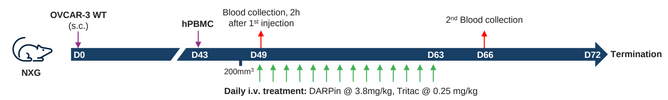
Pan T cells co-cultured with or without target cells (OVCAR-3 WT, 5:1). Activation (CD25) of CD8 T cells measured by flow cytometry after 48h.

T cell Cytotoxicity

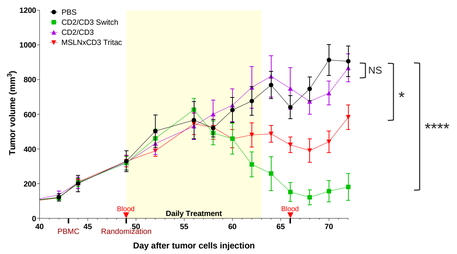


Pan T cells co-cultured with CHO WT or CHO-EpCAMxMSLN (1:1). LDH is measured in the supernatant after 48h and normalized to 100% killing.

CD2/CD3 Switch induces tumor regression in humanized mice

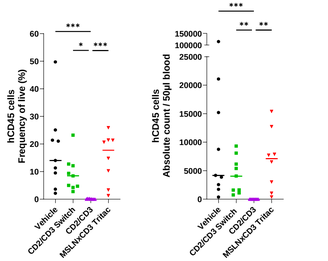


Tumor growth curves of OVCAR-3 s.c. model



10 mice/group, 2 PBMC donors engrafted at 100mm³, average +/- SEM, two-way ANOVA

Human CD45+ in blood on Day 66

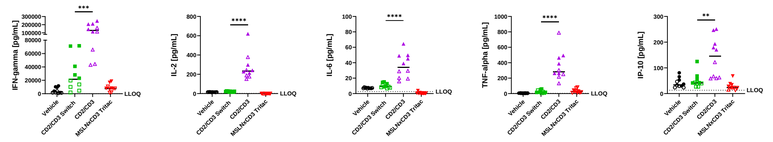


Kruskal-Wallis test for all mice (median)*

CD2/CD3 Switch induces significant tumor regression. Masking of CD3 ensures activation specifically in the tumor, whereas unmasked CD2/CD3 is not efficacious, likely due to loss of CD45+ T cells as a result of non-specific T cell activation in the periphery.

Masking CD3 may reduce risk of CRS providing a better safety profile

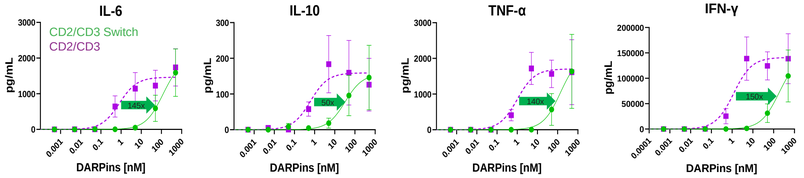
Cytokines measured in mice blood 2h after first dose



*Serum from blood taken at 2h post first injection was analyzed using MSD U-plex system. Significance shown with t-test **

CD2/CD3 Switch shows significantly lower cytokine levels shortly after first administration, indicating that masking CD3 could reduce the risk of cytokine release syndrome (CRS).

Cytokine release from a human whole blood assay

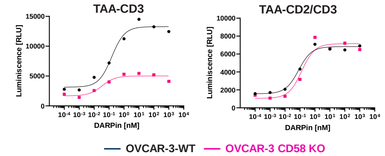


Whole blood from 3 donors incubated 24h with DARPin. Serum taken for cytokine analysis by MSD U-plex. Average +/- SEM.

CD2/CD3 DARPin induces non-TAA-specific cytokine release in human whole blood. CD2/CD3 Switch DARPin reduces non-specific cytokine release.

CD2 co-stimulation prevents loss of activity against CD58 KO cells

Reporter cell activation



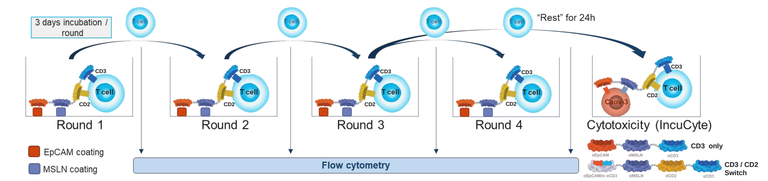
Absence of CD58 on target cells results in reduced efficacy of the CD3 DARPin whereas CD2/CD3 co-stimulation maintains efficacy, thus compensating for the lack of CD58.

Jurkat IL-2 cells cultured 1:1 with OVCAR-3 WT or CD58 KO cells over 24h. Activation measured by luminescence.

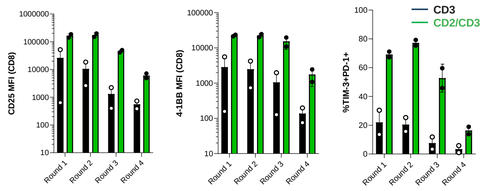
CD2 co-stimulation enables continued polyfunctionality of T cells

T cells repetitively stimulated with CD2/CD3 DARPins maintain cytotoxic capacity whereas T cells cultured in the presence of DARPins lacking CD2 show reduced capacity of target cell killing. T cells repetitively exposed to CD2/CD3 DARPin maintain a more activated phenotype than to CD3 alone.

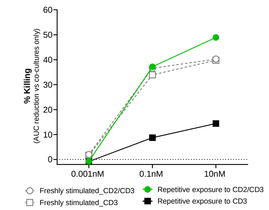
Repetitive stimulation scheme



Phenotype of T cells after repetitive stimulation



T cell cytotoxicity after 3 rounds of repetitive stimulation



T cells were repetitively exposed to DARPins captured by EpCAM and MSLN coated to the plates. After each round of stimulation, a portion of cells were taken for analysis by flow cytometry. After 3 rounds, T cells were co-cultured with NucLightRed (NLR) labeled Caov-3 cells and DARPins for 48h in an IncuCyte to measure the NLR signal over time.

Conclusions

We present a preclinical proof of concept for a multi-targeting DARPin T cell engager, with CD2 co-simulation and a TAA-based Switch to mask CD3 in the absence of TAAs. Our data show:

- ❖ Co-engagement of CD2 leads to sustained T cell activation and cytotoxic capacity, preventing dysfunction.
- ❖ Masking of CD3 allows higher specificity on the tumor, without compromising potency or needing to down-tune the CD3 affinity.
- ❖ CD2/CD3 Switch DARPin effectively induces potent tumor regression *in vivo,* whilst maintaining a good safety profile with reduced cytokine release compared to an unmasked CD3 with CD2 co-stimulation which ultimately leads to T cell depletion.

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