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Mitigation of Tumor Microenvironment-Mediated Immunosuppression Using a PD1-41BB Switch Protein with Optimal Affinity TCRs for First-In-Class, 3rd Generation TCR-T Therapies

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Background

- Despite showing promising early efficacy, TCR-T therapies face several challenges to optimising outcomes in the clinic, one of which is to sustain anti-tumor cell function in solid tumor microenvironments (TME) (Fig. 1).
- Tumor antigen levels and PD-L1 expression strongly influence T cell function. PD-L1 signalling to T cells via the PD-1 receptor limits proliferation, cytokine secretion and cytotoxic response, while exhaustion is induced by repetitive TCR signalling in the absence of T cell co-stimulation.
- Both impairments in TCR-T cells can be mitigated by co-expression of an optimal affinity TCR combined with a PD1-41BB costimulatory switch protein (CSP), comprised of the extracellular domain of PD-1 with the intracellular signalling domain of 4-1BB (CD137) (Fig. 2).
- Here we present data from two first-in-class, 3rd generation TCR-T therapies, MDG1015 and MDG2011, which were developed to express optimal affinity TCRs specific for a cancer-testis antigen (CTA), NY-ESO-1/LAGE-1a, and a neoantigen, mutant Kirsten rat sarcoma virus (mKRAS) G12V, respectively, with MDG1015 being the most advanced program.

Figure 1: Solutions to address unmet needs for TCR-T therapy of solid cancer to improve clinical responses without impacting safety

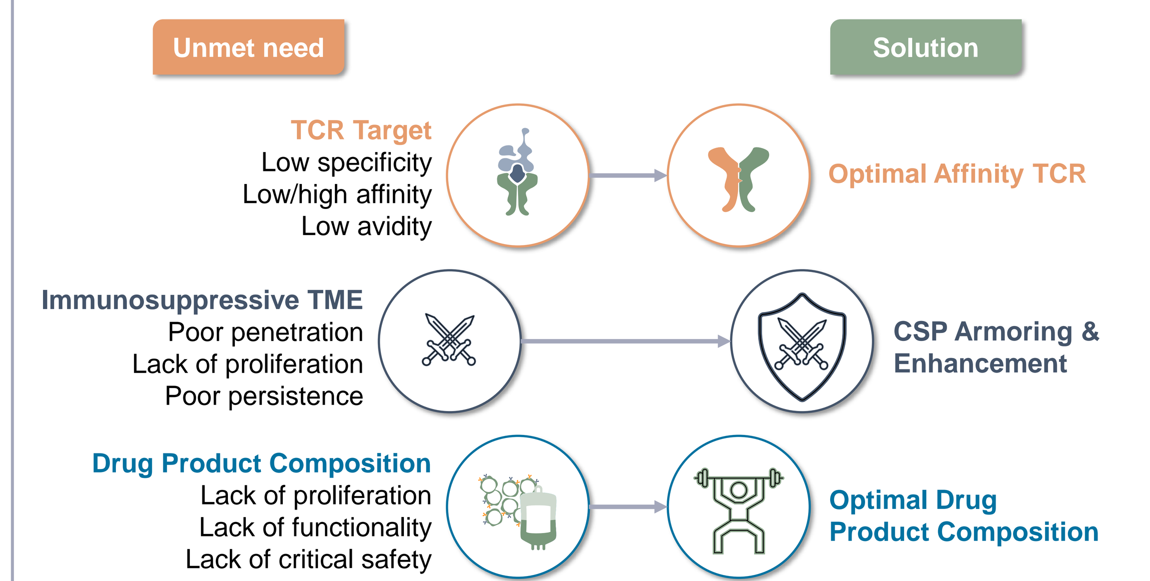
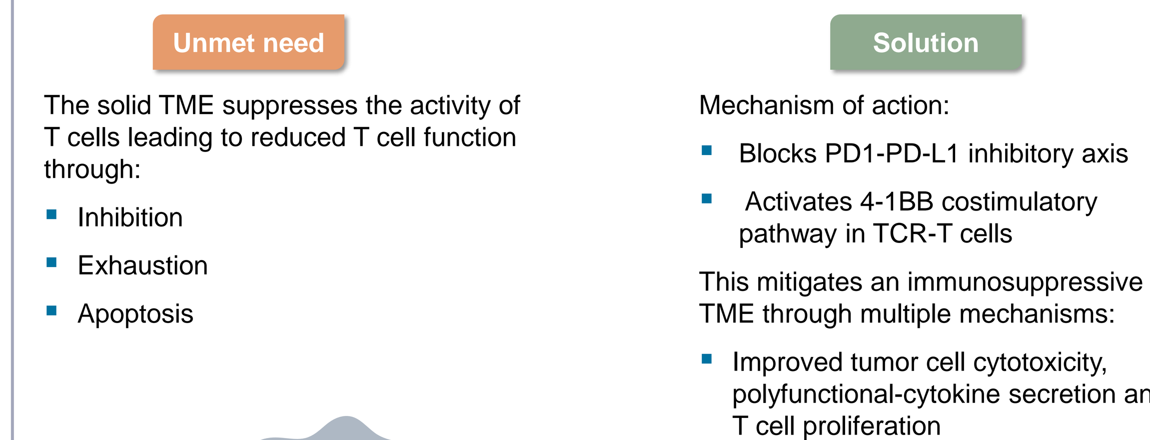
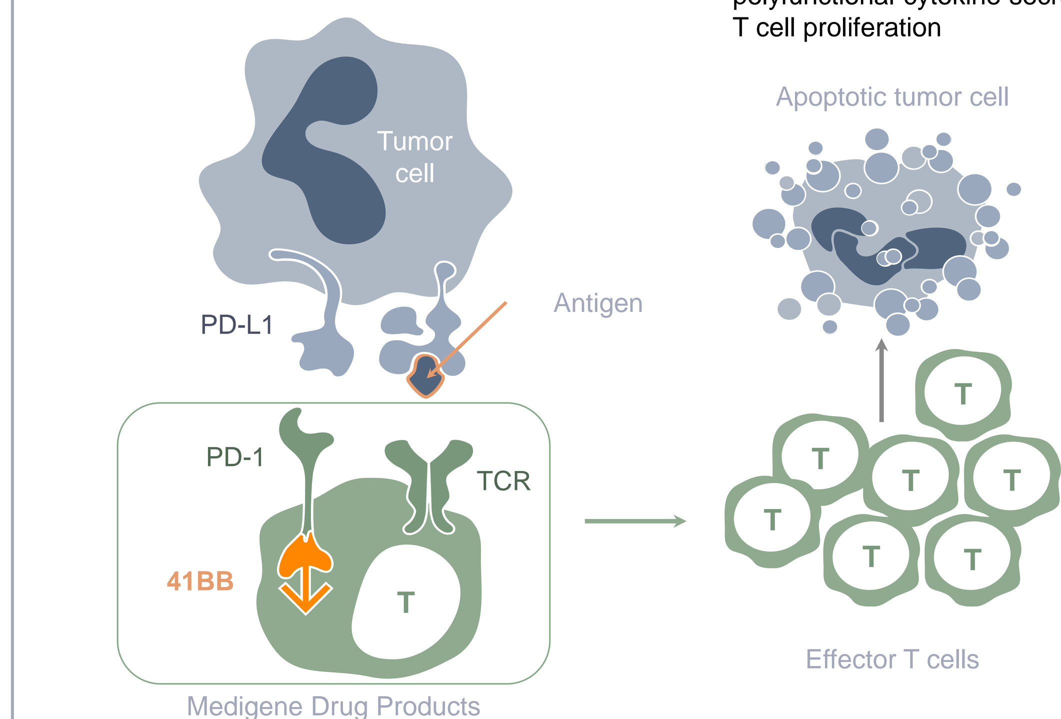


Figure 2: PD1-41BB, a CSP that armors & enhances TCR-T therapies to overcome the solid tumor TME



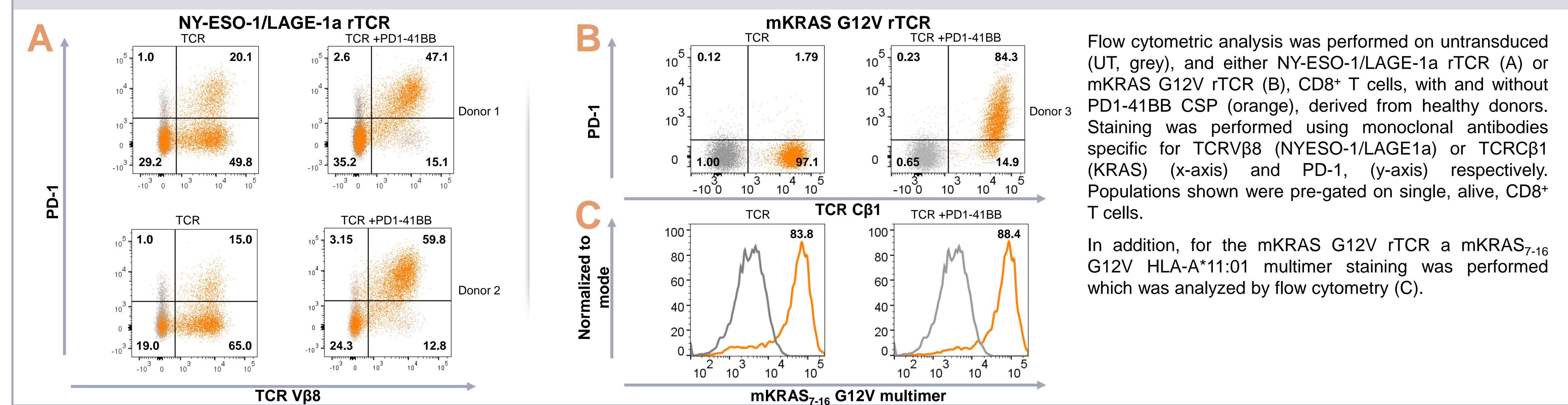


Results

PD1-41BB CSP improves CD8⁺ TCR-T cell polyfunctionality by increasing production of effector, stimulatory and chemoattractive cytokines

- >75% of NY-ESO-1/LAGE-1a and >85% of mKRAS G12V recombinant TCR (rTCR)-transduced CD8⁺ T cells co-express PD1-41BB CSP (Fig. 3)
- T cells producing multiple cytokines, so-called "polyfunctional" T cells, are known to provide more effective immune responses
- CD8⁺ rTCR-T cells co-expressing PD1-41BB CSP display a 4-to-6-fold increase in polyfunctionality (Fig. 4a) and a 6-to-12-fold higher Polyfunctional Strength Index (PSI) (Fig. 4b)
- Superior polyfunctionality is mainly related to proteins associated with effector, stimulatory and chemoattractive properties (Fig. 4c)

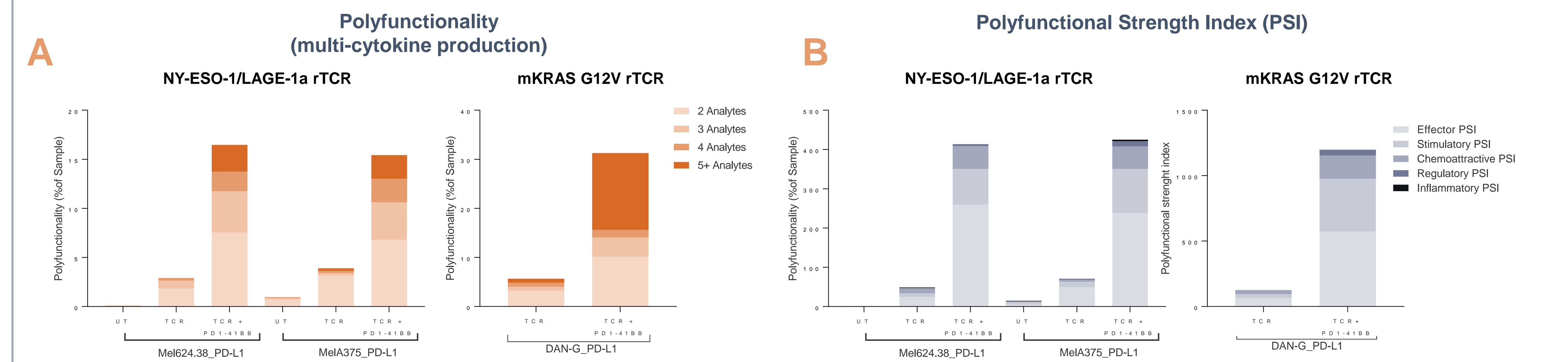
Figure 3: Both NY-ESO-1/LAGE-1a and mKRAS G12V CD8⁺ rTCR-T prominently co-express the rTCR and PD1-41BB CSP at the cell surface



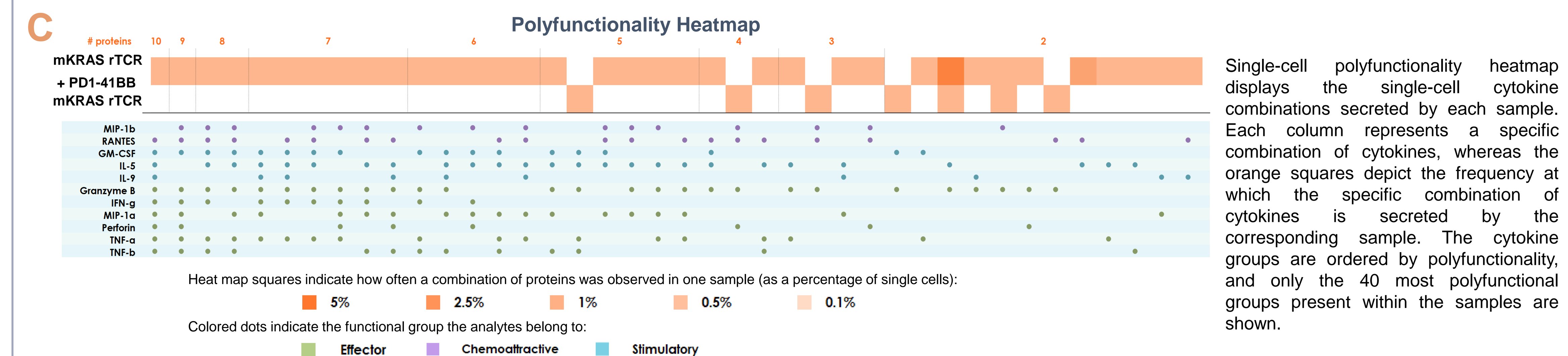
Flow cytometric analysis was performed on untransduced (UT, grey), and either NY-ESO-1/LAGE-1a rTCR (A) or mKRAS G12V rTCR (B), CD8⁺ T cells, with and without PD1-41BB CSP (orange), derived from healthy donors. Staining was performed using monoclonal antibodies specific for TCRVβ8 (NYESO-1/LAGE1a) or TCRCβ1 (KRAS) (x-axis) and PD-1, (y-axis) respectively. Populations shown were pre-gated on single, alive, CD8⁺ T cells.

In addition, for the mKRAS G12V rTCR a mKRAS$_{7-16}$ G12V HLA-A*11:01 multimer staining was performed which was analyzed by flow cytometry (C).

Figure 4: Enhanced polyfunctionality of both CD8⁺ rTCR-T cells specific for NY-ESO-1/LAGE-1a or mKRAS G12V through co-expression of rTCR and PD1-41BB CSP compared to CD8⁺ rTCR-T cells alone



NY-ESO-1/LAGE-1a or mKRAS G12V-specific CD8⁺ rTCR-T cells with (TCR+PD1-41BB) and without (TCR) co-expression of PD1-41BB CSP were analyzed after 24 hours of co-culture with tumor target cell lines Mel624.38_PD-L1 and MelA375_PD-L1 (for NY-ESO-1/LAGE-1a rTCR) and DAN-G_PD-L1 (for mKRAS G12V rTCR) using single cell proteomic analysis of a panel of 32 secreted cytokines, chemokines, and cytotoxic molecules (IsoLight® technology, PhenomeX). Co-culture with untransduced T cells (UT) of the same donor served as control. Cells secreting ≥2 cytokines are considered polyfunctional. A) Polyfunctionality of single TCR-T cells displayed as % of the sample and categorized in % of cells expressing 2, 3, 4 and 5+ analytes (shades of orange) at the same time. B) Polyfunctional Strength Index (PSI) of the displayed samples is defined as the number of T cells secreting ≥2 effector molecules per cell (polyfunctional T cells in A)), multiplied by mean fluorescence intensity (MFI) of the proteins secreted by the respective cells and categorized in proteins associated with effector, stimulatory, chemoattractive, regulatory and inflammatory properties (shades of grey).

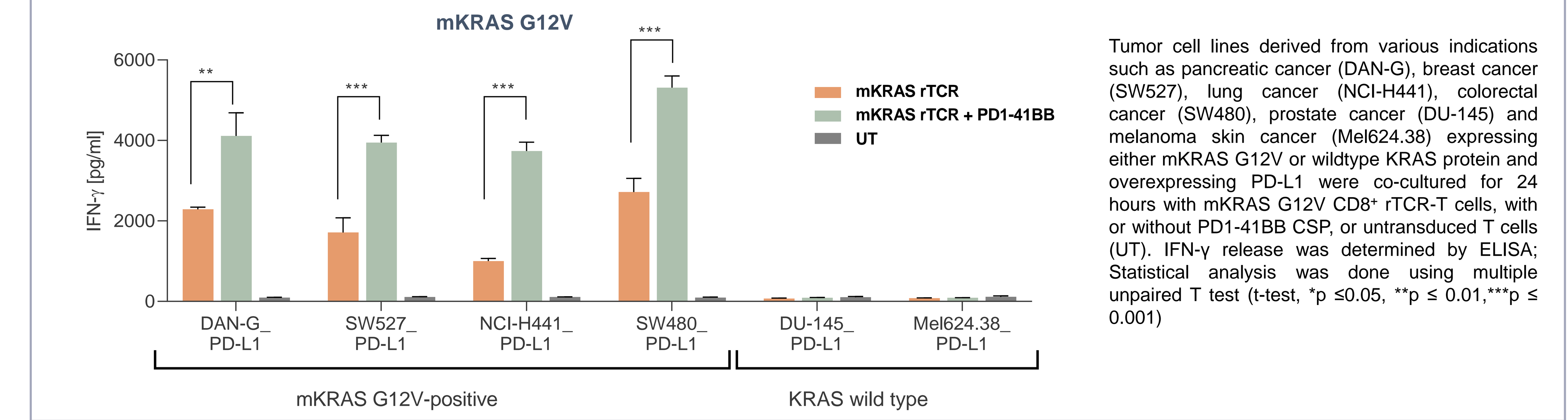


Single-cell polyfunctionality heatmap displays the single-cell cytokine combinations secreted by each sample. Each column represents a specific combination of cytokines, whereas the orange squares depict the frequency at which the specific combination of cytokines is secreted by the corresponding sample. The cytokine groups are ordered by polyfunctionality, and only the 40 most polyfunctional groups present within the samples are shown.

PD1-41BB enhances activation and prolongs cytotoxic activity of CD8⁺ rTCR-T cells upon repeated stimulation

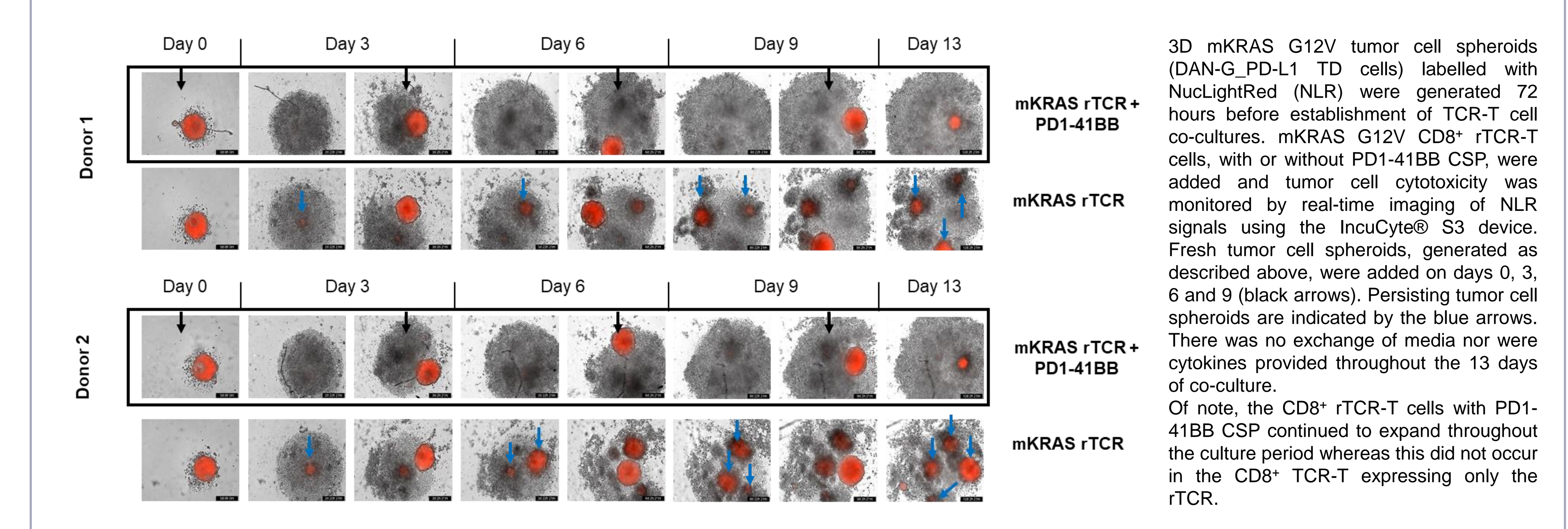
- No CD8⁺ rTCR-independent IFN-γ production is observed in response to target-negative but PD-L1-positive 3D tumor cell lines (Fig. 5)
- CD8⁺ rTCR-T cells co-expressing PD1-41BB CSP display a 2-3-fold increase of IFN-γ production upon co-culture with target-positive and PD-L1-positive tumor cell lines (Fig. 5)
- CD8⁺ rTCR-T cells co-expressing PD1-41BB CSP show prolonged and enhanced cytotoxic responses upon serial rechallenge with target- and PD-L1-positive 3D tumor spheroids (Fig. 6)

Figure 5: CD8⁺ T cells co-expressing the rTCR and PD1-41BB CSP showed enhanced IFN-γ secretion compared to CD8⁺ T cells expressing only the rTCR



Tumor cell lines derived from various indications such as pancreatic cancer (DAN-G), breast cancer (SW527), lung cancer (NCI-H441), colorectal cancer (SW480), prostate cancer (DU-145) and melanoma skin cancer (Mel624.38) expressing either mKRAS G12V or wildtype KRAS protein and overexpressing PD-L1 were co-cultured for 24 hours with mKRAS G12V CD8⁺ rTCR-T cells, with or without PD1-41BB CSP, or untransduced T cells (UT). IFN-γ release was determined by ELISA; Statistical analysis was done using multiple unpaired T test (t-test, *p ≤0.05, **p ≤ 0.01,***p ≤ 0.001)

Figure 6: Superior spheroid cytotoxicity by CD8⁺ T cells co-expressing the rTCR and PD1-41BB CSP compared to CD8⁺ T cells expressing only the rTCR observed upon serial tumor cells rechallenge



3D mKRAS G12V tumor cell spheroids (DAN-G_PD-L1 TD cells) labelled with NucLightRed (NLR) were generated 72 hours before establishment of TCR-T cell co-cultures. mKRAS G12V CD8⁺ rTCR-T cells, with or without PD1-41BB CSP, were added and tumor cell cytotoxicity was monitored by real-time imaging of NLR signals using the IncuCyte® S3 device. Fresh tumor cell spheroids, generated as described above, were added on days 0, 3, 6 and 9 (black arrows). Persisting tumor cell spheroids are indicated by the blue arrows. There was no exchange of media nor were cytokines provided throughout the 13 days of co-culture.
Of note, the CD8⁺ rTCR-T cells with PD1-41BB CSP continued to expand throughout the culture period whereas this did not occur in the CD8⁺ TCR-T expressing only the rTCR.

Conclusions

- The PD1-41BB CSP significantly increases the activity and proliferation of tumor antigen-specific TCR-T cells upon stimulation with tumor cells expressing both the target antigen NY-ESO-1/LAGE-1a or mKRAS G12V and PD-L1.
- The co-stimulatory effects of PD1-41BB are dependent on the rTCR-mediated recognition of the specific tumor-antigen and on the expression of the inhibitory ligand PD-L1 on tumor cells.
- Co-expression of the PD1-41BB CSP leads to a striking increase in polyfunctional T cells, driven by the production of effector, stimulatory and chemoattractive, rather than regulatory and inflammatory cytokines.
- PD1-41BB CSP drives a stronger but also prolonged T cell cytotoxic response as observed by serial rechallenge with 3D tumor spheroids.
- The combination of optimal affinity rTCRs combined with the PD1-41BB CSP provides strong protection of TCR-T cells against two variable mechanisms of TME immunosuppression based on the demonstration of CSP-enhanced poly-cytokine secretion, proliferation and mitigation against exhaustion *in vitro*.
- This observation warrants application in the clinic for this novel approach which has the potential to overcome major challenges in solid tumor TMEs leading to much needed improvements regarding depth and duration of responses seen with TCR-T therapies in solid tumor patients to date.

* Presenting author has no conflicts of interest to declare