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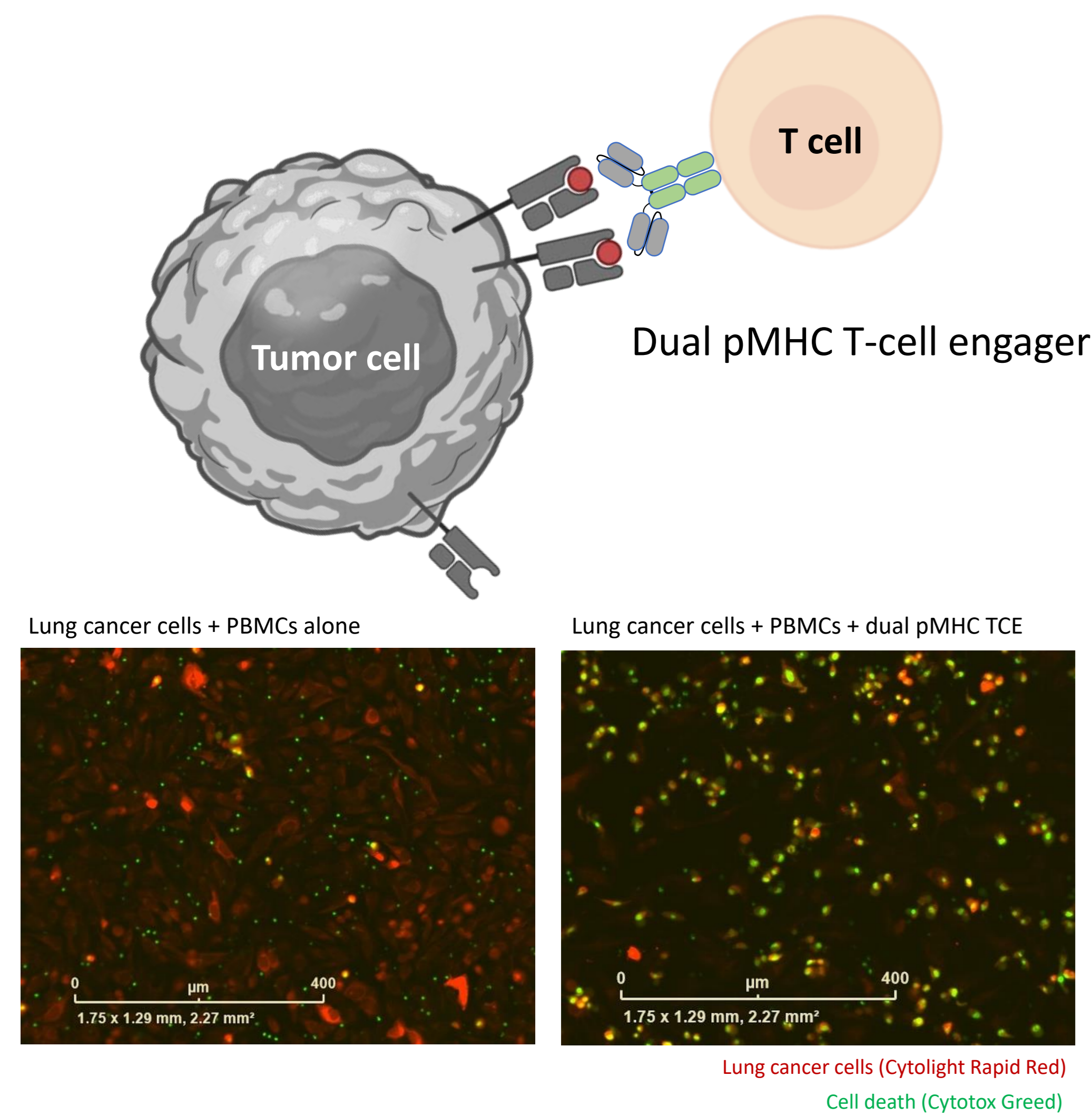
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Background

- Intracellular tumor antigens presented as peptides on MHC (pMHC) class I molecules are attractive targets for more tumor-selective immunotherapeutic approaches in solid tumors.
- A series of monovalent and bivalent antibody constructs composed of anti-MAGE-A4 binding arms, ranging in affinities from ~30 nM to 100 pM, were fused to an anti-CD3 Fab fragment with lower affinity than commonly seen for TCR-fusions and evaluated for selective killing of double positive MAGE-A4/HLA-A*02:01 cancer cells versus a panel of different MAGE-A4 negative/HLA-A*02:01 positive cell lines.
- The dual pMHC bispecific T-cell engager (TCE) was optimized for CD3 affinity and MAGE-A4/HLA-A*02:01 target affinity to achieve high potency while maintaining specificity by minimizing binding to similar and physiologically relevant non-MAGE-A4 peptides (S1, S16).
- The dual pMHC TCE was compared in potency, cytokine release, and specificity against a comparator molecule (recombinant soluble TCR fused to an anti-CD3 scFv), a construct that is currently in clinical development.

1 Dual pMHC T-cell engager elicits highly efficient anti-tumor responses

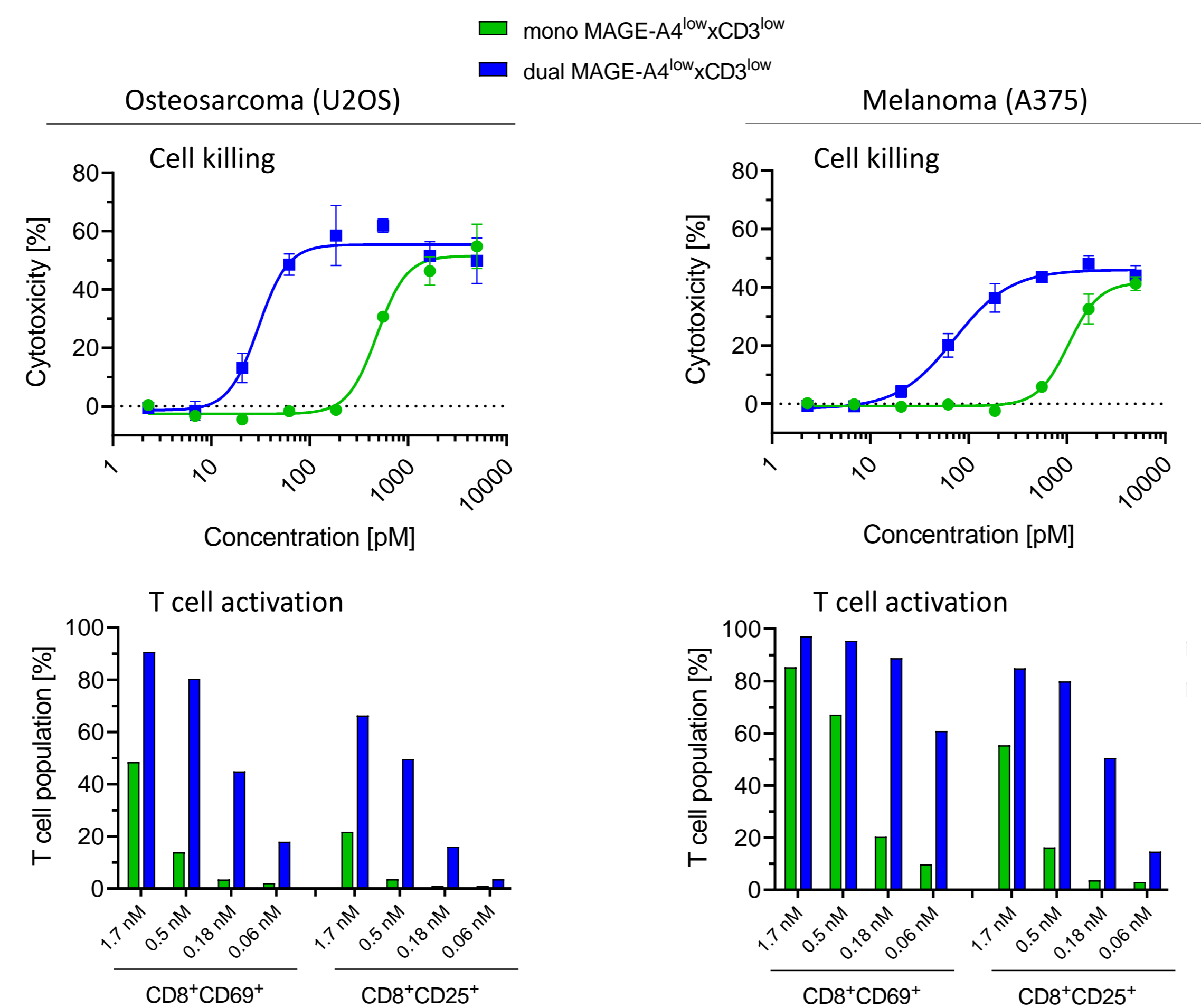
Live cell imaging of MAGE-A4 positive NCI-H1703 lung squamous carcinoma cells co-cultured with human PBMCs in presence of a dual pMHC TCE with specificity for MAGE-A4/HLA-A*02:01.



Dual (bivalent) pMHC targeting T-cell engager efficiently crosslinks T cells to tumor cells, leading to potent redirected T cell cytotoxicity of antigen positive cancer cells.

2 Bivalent targeting of antigen positive cancer cells greatly potentiates activity of the pMHC TCE

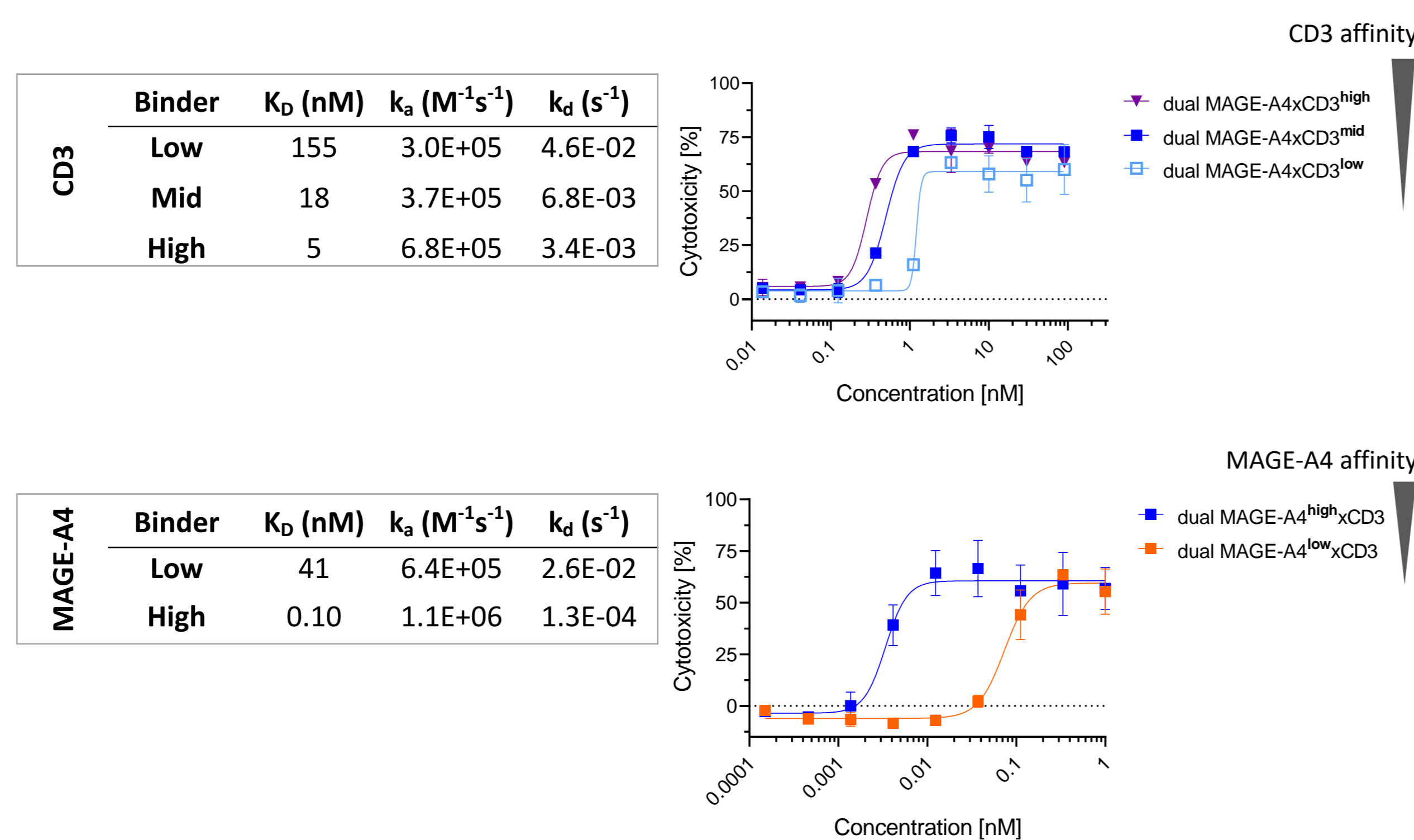
MAGE-A4 positive osteosarcoma (U2OS) or melanoma (A375) cells were co-incubated with PBMCs (E:T 10:1) and either monovalent or dual/bivalent pMHC TCE comprising the same MAGE-A4 and CD3-binding antibody fragments. T cell-mediated cytotoxicity was determined by measuring LDH release after 48h. T cell activation was determined by quantification of CD69 and CD25 markers on the CD8 T cell population after 24h using flow cytometry.



The bivalent format of a MAGE-A4 targeting TCE shows superior cancer cell killing and T cell activation compared to its monovalent counterpart.

3 Potency of the dual pMHC TCE is influenced by valency and the intrinsic affinity of the binding arms

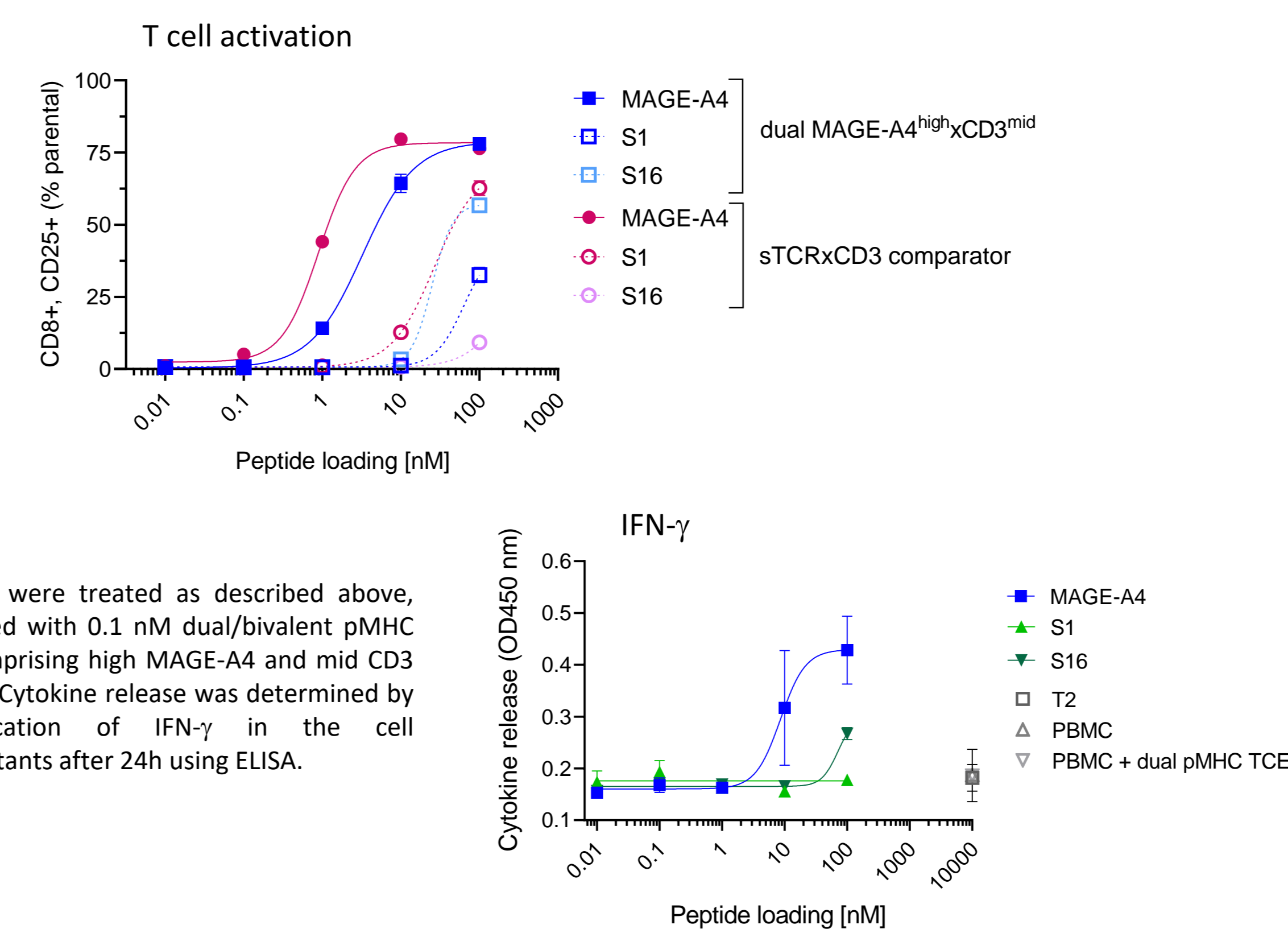
Apparent binding affinities for MAGE-A4/HLA-A*02:01 and CD3 antigens were determined in monovalent scFv format for CD3 and MAGE-A4 binders by surface plasmon resonance. Dual/bivalent pMHC TCEs comprising either the different CD3 or MAGE-A4 binders were evaluated for cell killing of MAGE-A4 positive U2OS cancer cells upon co-incubation with PBMCs (E:T 10:1). T cell-mediated cytotoxicity was determined by measuring LDH release after 48h.



Affinity enhancement of the MAGE-A4 binding arms, but not of the CD3 binding arm, mediates greater degree of cancer killing.

4 Bivalent targeting of MAGE-A4 does not compromise selectivity of the bispecific molecule

TAP-deficient T2 cells were pulsed with HLA-A*02:01-restricted peptides (MAGE-A4 or similar control peptides S1 and S16) and co-incubated with PBMCs (E:T 5:1) and 0.1 nM of dual/bivalent pMHC TCE comprising the high MAGE-A4 and mid CD3 affinity or an in-house produced clinical stage comparator molecule. T cell activation was determined by quantification of CD25 markers on the CD8 T cell population after 24h using flow cytometry.

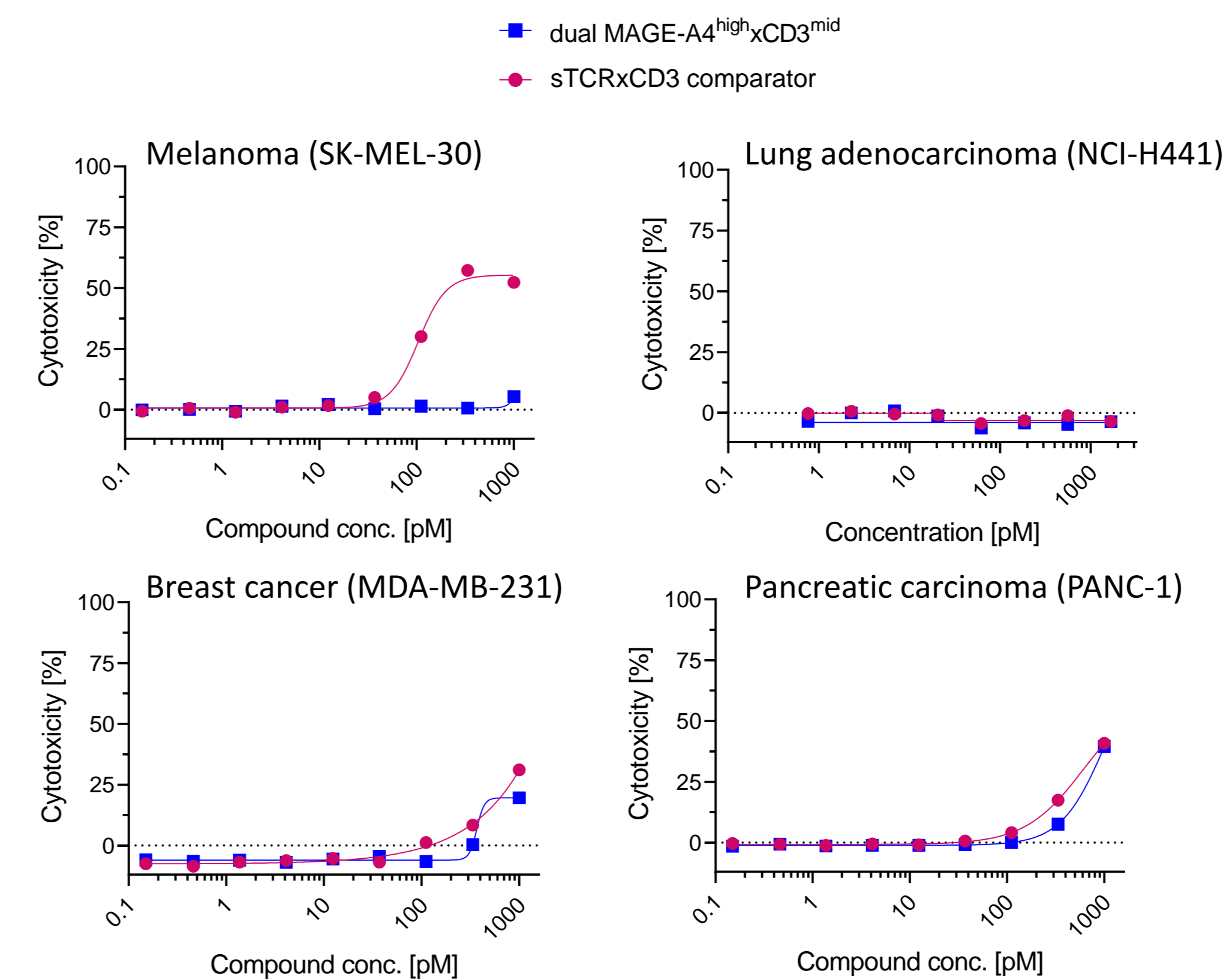


T2 cells were treated as described above, incubated with 0.1 nM dual/bivalent pMHC TCE comprising high MAGE-A4 and mid CD3 affinity. Cytokine release was determined by quantification of IFN-γ in the cell supernatants after 24h using ELISA.

Dual pMHC TCE (with picomolar affinity for MAGE-A4) elicits considerably lower T cell functional responses for the S1 and S16 off-target peptides than for the MAGE-A4 target peptide.

5 Dual pMHC TCE demonstrates limited cross-reactivity towards antigen-negative cells in vitro

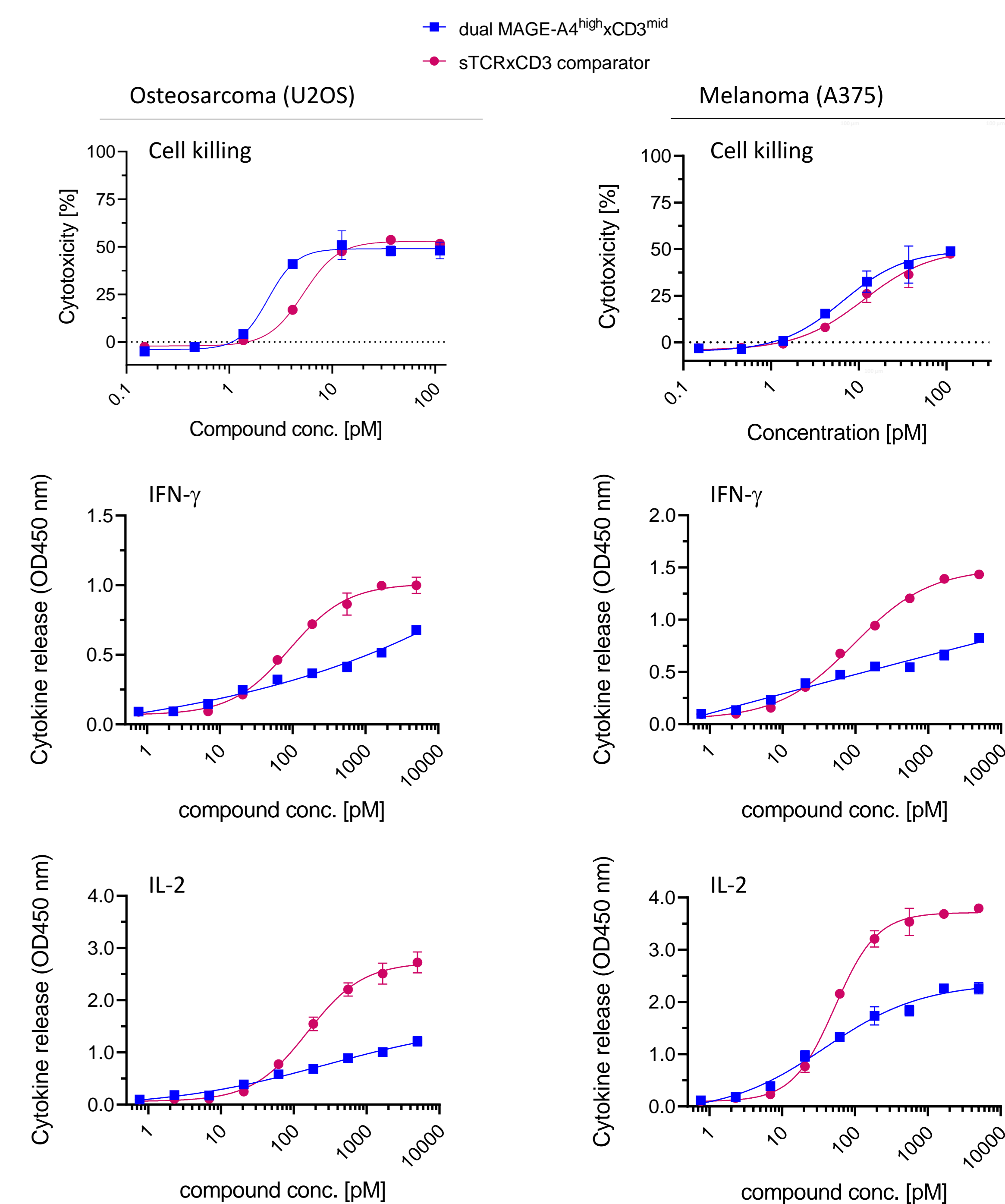
MAGE-A4 negative/HLA-A*02:01 positive cells (SK-MEL-30, NCI-H441, MDA-MB-231, PANC-1) were co-incubated with PBMCs (E:T 10:1) and either dual/bivalent pMHC TCE with the picomolar MAGE-A4 and mid CD3 affinity or an in-house produced clinical stage comparator molecule. T cell-mediated cytotoxicity was determined by measuring LDH release after 48h.



Dual pMHC TCE induces comparable or less cytotoxicity of MAGE-A4 negative/HLA-A*02:01 positive cells than sTCRxCD3 comparator (similar to clinical stage IMC-C103C compound)

6 High anti-tumor cytotoxicity profile with limited cytokine release by dual pMHC TCE

MAGE-A4 positive U2OS osteosarcoma U2OS (left panels) or A375 melanoma cells (right panels) were co-incubated with PBMCs (E:T 10:1) and either dual/bivalent pMHC TCE with high MAGE-A4 and mid CD3 affinity or an in-house produced clinical stage comparator molecule. T cell-mediated cytotoxicity was determined by measuring LDH release after 48h. Cytokine release was determined by quantification of IFN-γ and IL-2 in the cell supernatants after 20h using ELISA.



The dual pMHC TCE shows superior cell killing of MAGE-A4 positive cancer cells while eliciting significant lower cytokine release.

Conclusion

This novel dual pMHC TCE provides:

- Selective and efficient T cell-mediated target cell killing
- Effective activation of T-cells
- Lower cytokine release

Dual pMHC targeting with a TCE is highly potent while lower cytokine release may avoid T cell exhaustion, thus providing the promise of more effective and durable anticancer responses.