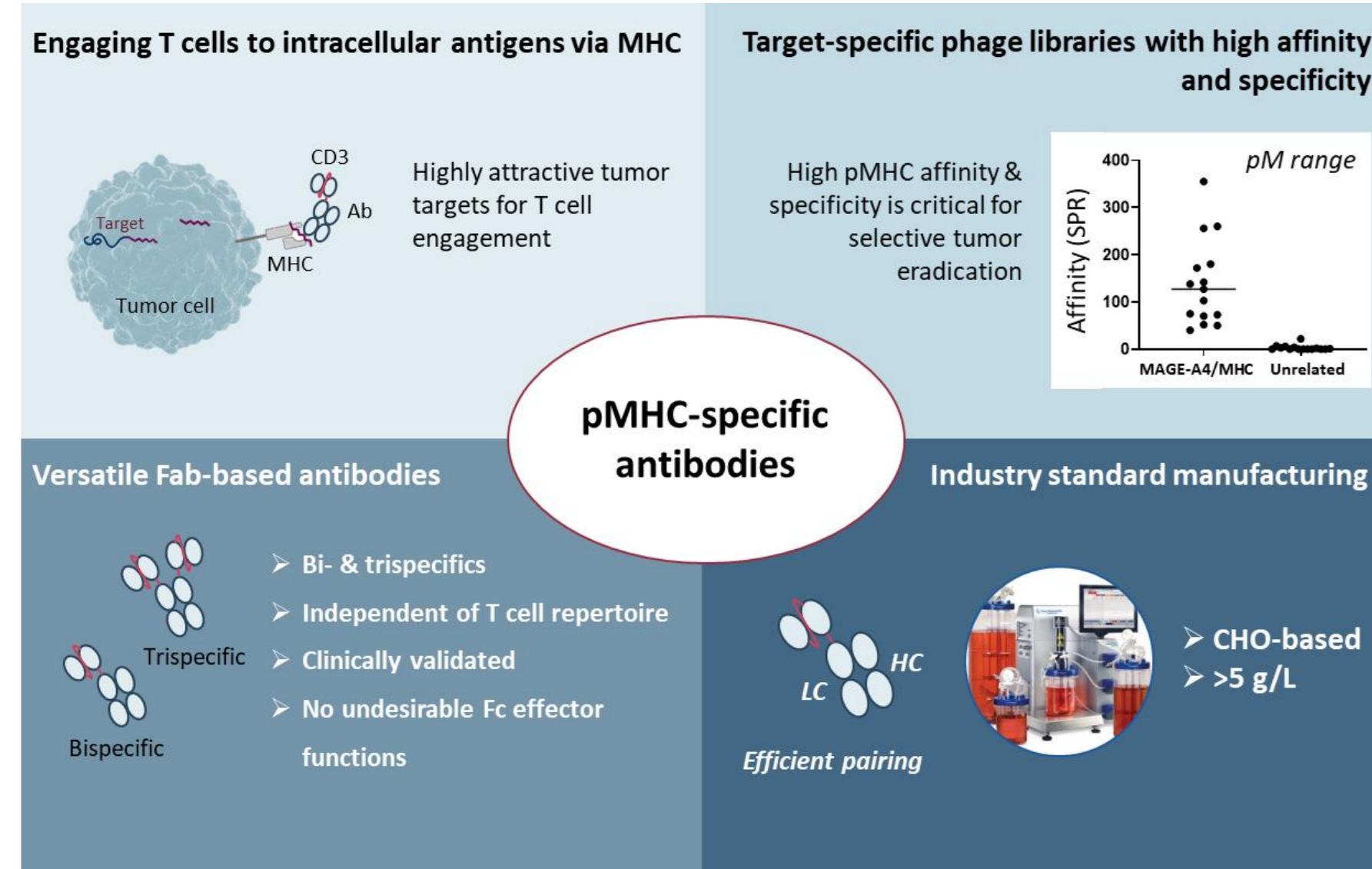


## Background

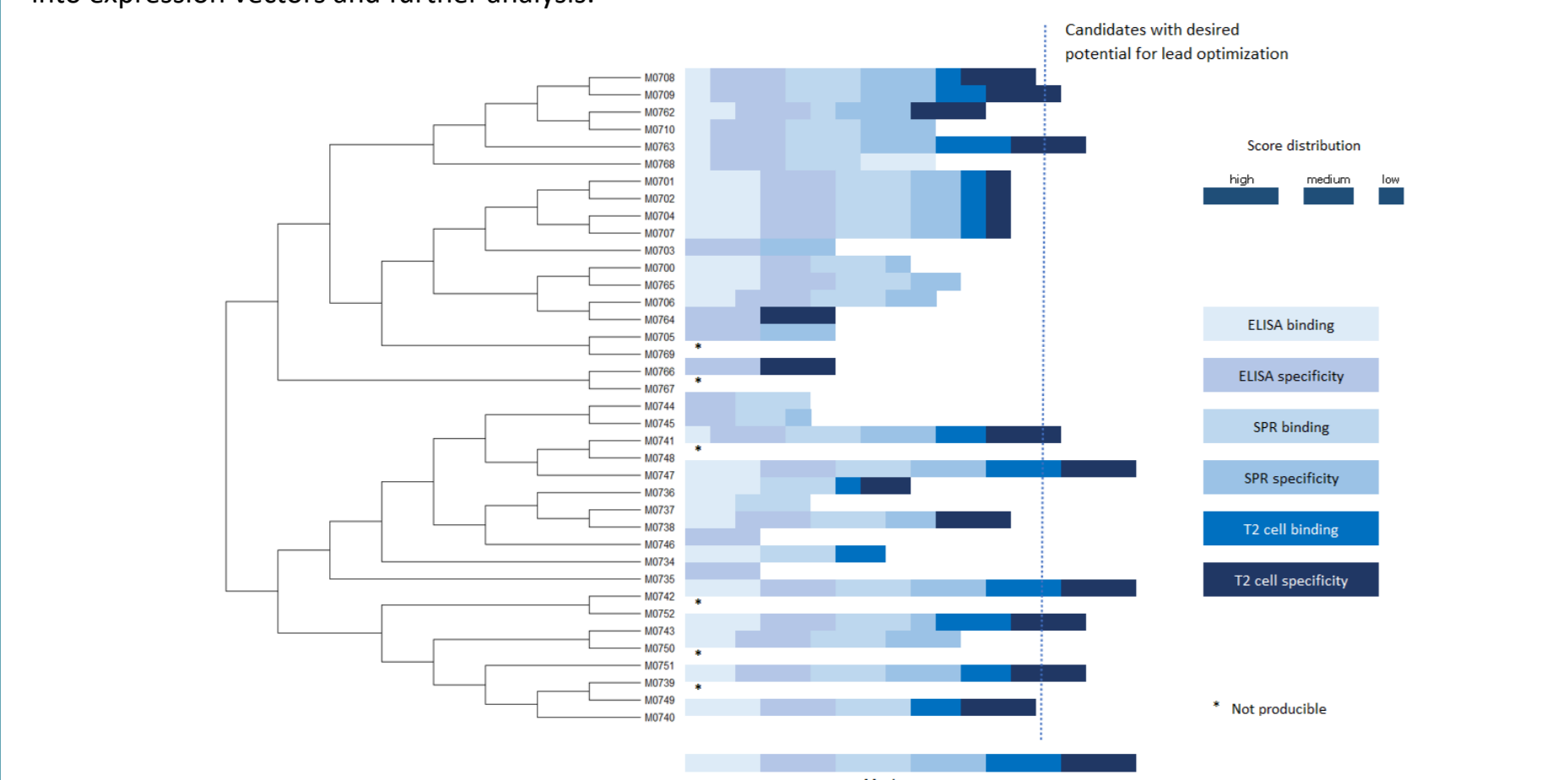
- Current immunotherapies are limited to cancer cell surface antigens that are rarely unique to tumor cells.
- Targeting peptides presented in major histocompatibility complexes (pMHCs) accesses the intracellular reservoir of cancer-specific antigens.
- The use of soluble T-cell receptors (TCRs) to target pMHC tumor-specific antigens is hampered by their intrinsic low affinity and challenges related to production.
- Here, we present a platform for the discovery and development of TCR-like antibodies with high specificity and affinity to pMHC antigens and using this platform, we selected a panel of binders against HLA-A\*02:01 (HLA-A2) in complex with GYVDGREHTV peptide of MAGE-A4.
- MAGE-A4 x CD3 bispecific T cell engagers demonstrate potent *in vitro* killing of multiple MAGE-A4 expressing cancer cell lines and completely eradicate a lung cancer xenograft *in vivo*.
- The high specificity, i.e. no binding and no killing of multiple HLA-A2+ cell lines that do not express MAGE-A4, make these antibodies particularly attractive as off-the-shelf TCR-like therapies.

## 1 Unique platform to access intracellular target reservoir



## 2 A broad collection of antibodies specific to HLA-A2/MAGE-A4

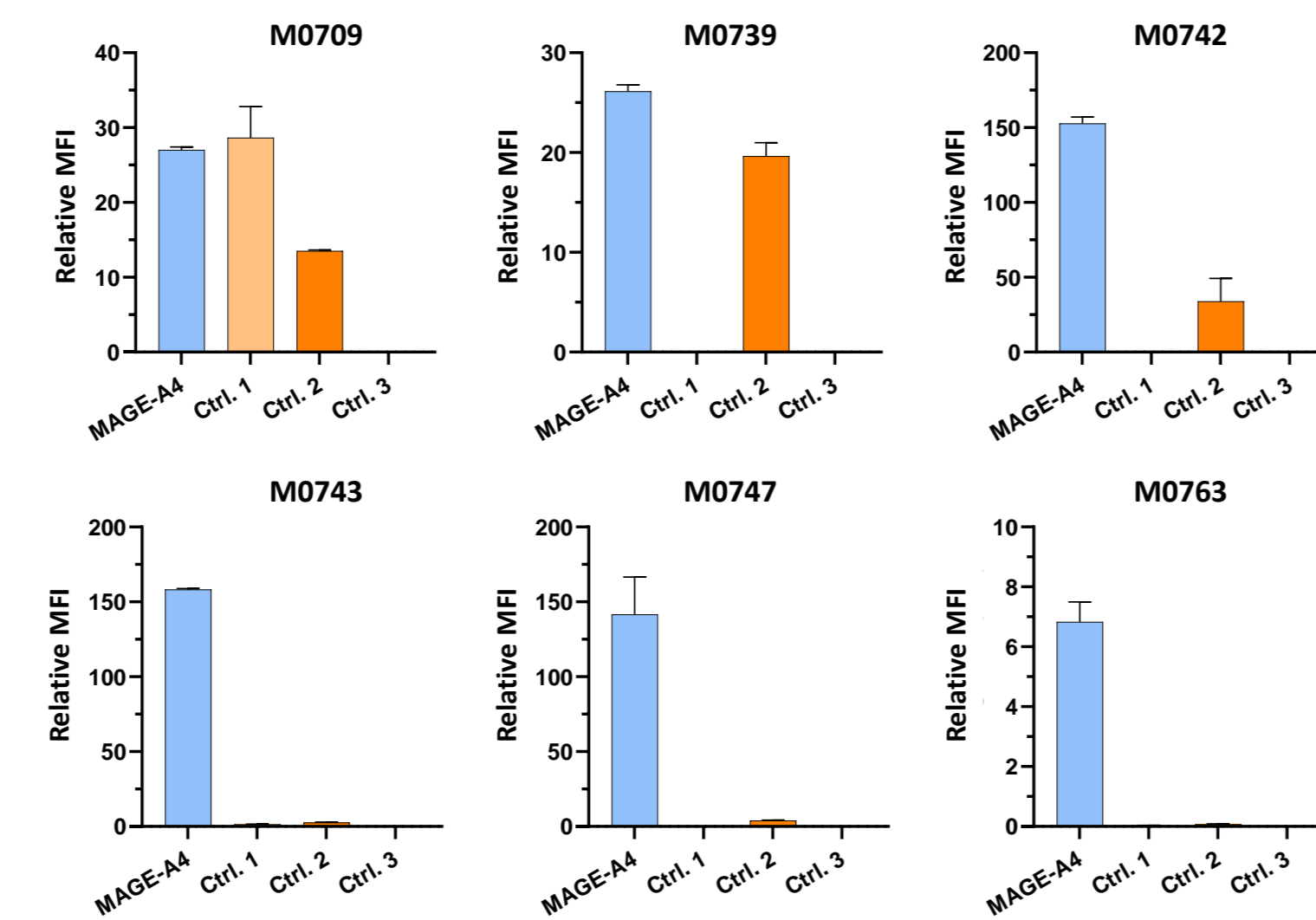
>500 monoclonal phages binding to the HLA-A2/MAGE-A4 but not to HLA-A2 complexed with unrelated control peptides were identified after three rounds of biopanning. Based on ELISA and DNA fingerprint 32 clones were selected for cloning into expression vectors and further analysis.



Six clones with best binding and specificity profile were considered to represent lineage-related antibody groups and selected for further characterization

## 3 Binding analysis reveals different specificity profiles of selected antibodies

TAP-deficient T2 cells were pulsed with HLA-A2-restricted peptides (MAGE-A4 or control peptides) and incubated with MAGE-A4 binders followed by fluorophore-labeled specific detection antibodies and analysis by flow cytometry. Peptide loading was confirmed with PE-labeled anti-HLA-A2 antibody BB7.2. Results of the ratio of binding efficiency over peptide loading capacity are shown as Relative Median Fluorescence Intensity (MFI).



MAGE-A4	G	V	Y	D	G	R	E	H	T	V
ctrl. peptide 1	G	L	A	D	G	R	T	H	T	V
ctrl. peptide 2	G	L	Y	D	G	P	V	H	E	V
ctrl. peptide 3	G	V	F	D	G	L		H	T	V

Alignment of MAGE-A4 peptide (aa230-239) and highly similar control peptides found to be expressed in normal human tissues. Asterisk indicates amino acids relevant for HLA-A\*02:01 anchoring.

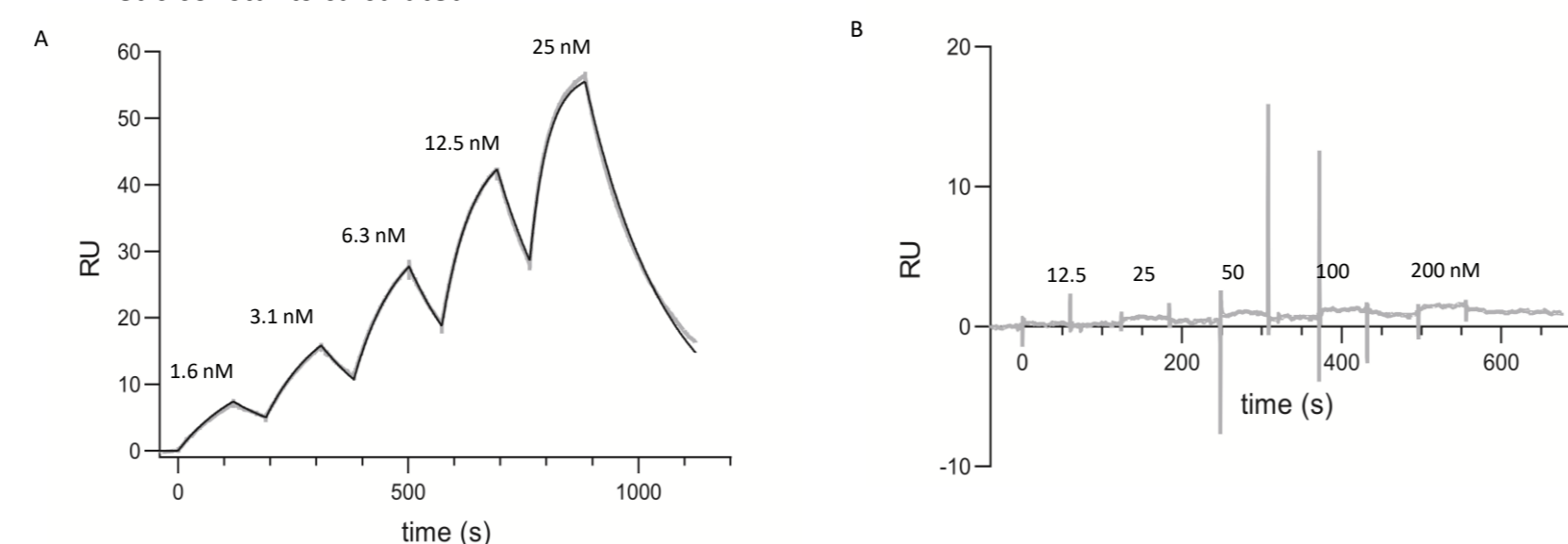
From cell binding assays using similar peptides being expressed in healthy tissues four MAGE-A4 binders with the highest specificity were selected for SPR affinity characterization.

## 4 SPR measurements confirm high specificity binding to MAGE-A4

Binder	K <sub>D</sub> ratio Ctrl. 1/MAGE-A4	K <sub>D</sub> ratio Ctrl. 2/MAGE-A4	K <sub>D</sub> ratio Ctrl. 3/MAGE-A4
M0742	80	6	Nb
M0743	126	81	Nb
M0747	229	43	Nb
M0763	> 330	> 240	Nb

K<sub>D</sub>-ratio (control peptide complex divided by MAGE-A4/HLA-A2) for exemplary binders. Nb: no binding for control peptide measurable

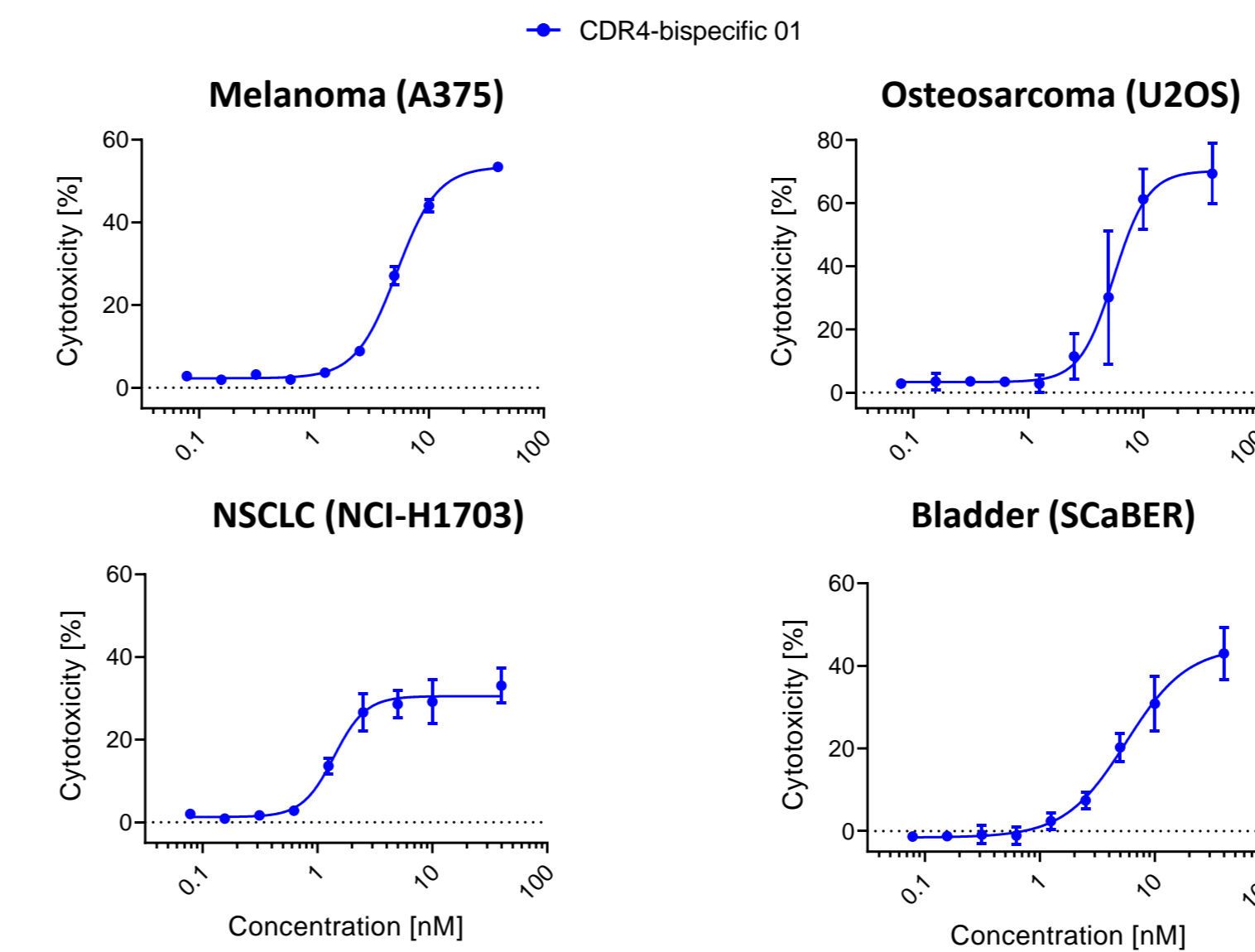
Exemplary sensograms of SPR-affinity determination of M0742 to A) HLA-A2-MAGEA4 and B) HLA-A2-Ctrl. 3. The HLA-A2/peptide complex was immobilized on a Streptavidin chip (SAHC30M, XanTec) and five consecutive binder injections were performed. The data (grey line) was fitted to a 1:1 Langmuir model (black line) and kinetic constants calculated.



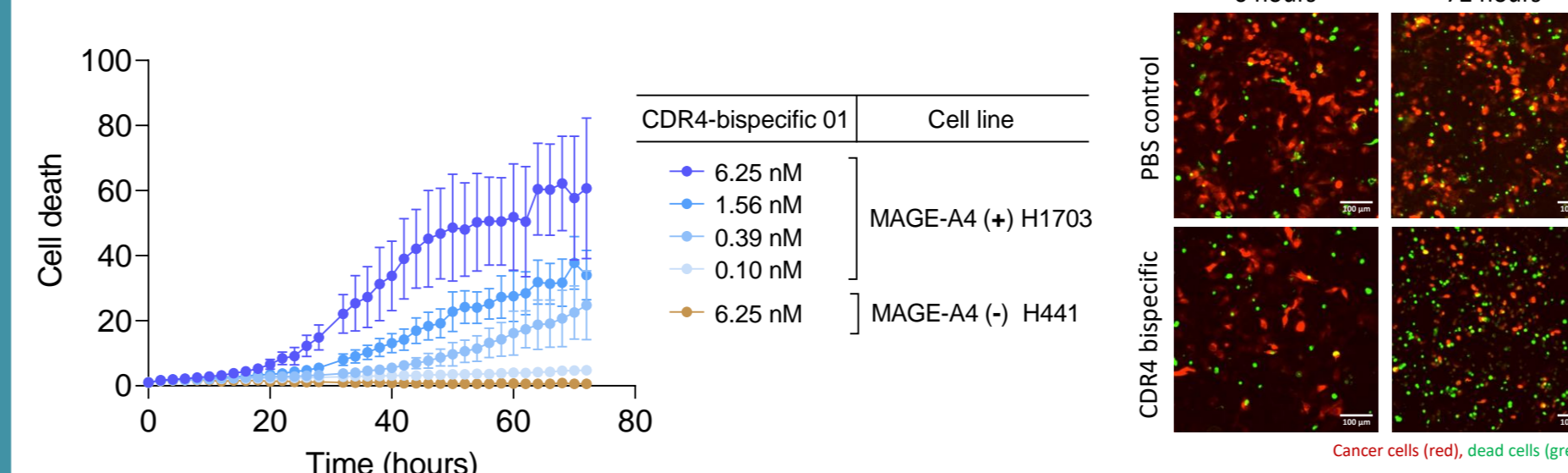
Affinity determination by SPR confirms high specificity binding of selected binders to MAGE-A4/HLA-A2 compared to physiologically relevant control peptide/HLA-A2 complexes

## 5 MAGE-A4 x CD3 bispecific potently kills multiple cancer cell types

*In vitro* T cell-mediated cytotoxicity: Cell killing was determined by measuring released LDH after 48h co-incubation of MAGE-A4 positive cell lines with PBMCs at E:T ratio 10:1 and indicated concentrations of CDR4-bispecific 01.



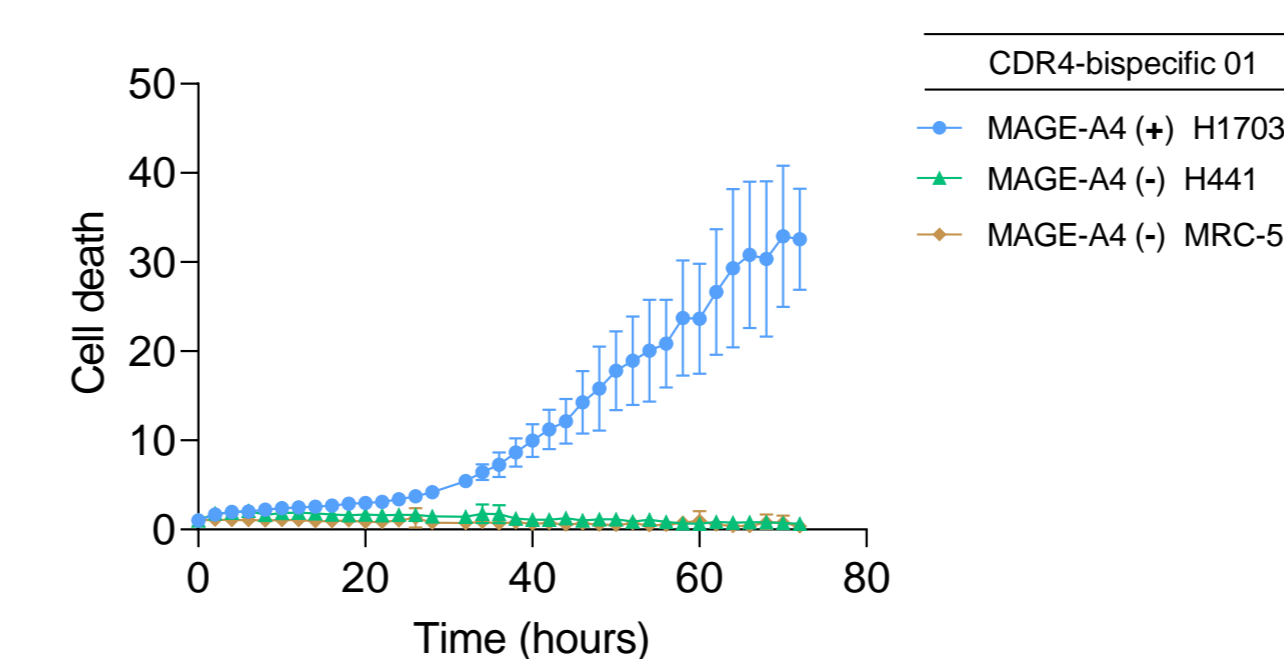
*Live cell imaging of in vitro* T cell-mediated cytotoxicity: MAGE-A4 positive NCI-H1703 cells were co-incubated with PBMCs (E:T 10:1) and CDR4-bispecific B01 at the indicated concentrations and images were recorded by the IncuCyte S3 system for up to 72h. Quantification of cytotoxicity is reported as ratio of green object count per image (dead cells, Cytotox Green Dye) to red area confluence (cell lines, Cytolight Rapid Red). MAGE-A4 negative/HLA-A2 positive H441 cells were used as control at the highest concentration (6.3 nM) of bispecific to demonstrate specific killing.



Dose dependent killing of MAGE-A4 expressing tumor cells with MAGE-A4 x CD3 bispecific

## 6 No killing of HLA-A2+ cell lines not expressing MAGE-A4

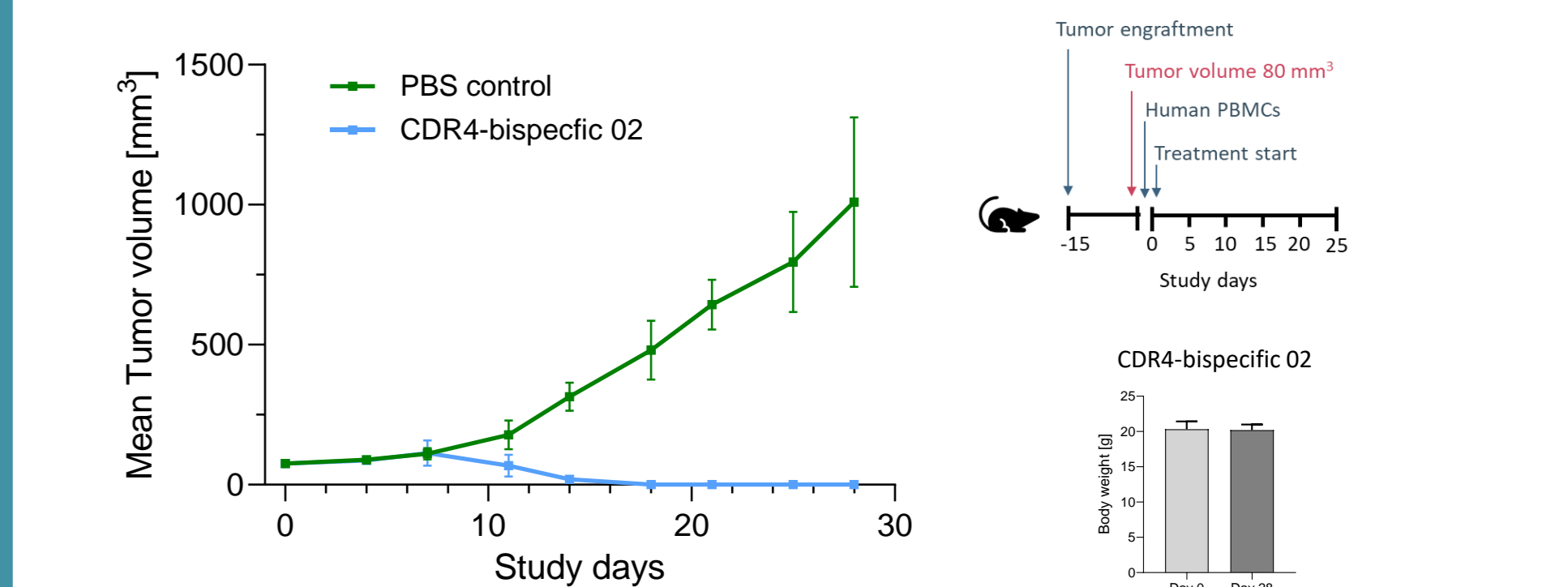
*Live cell imaging of in vitro* T cell-mediated cytotoxicity: MAGE-A4 positive/HLA-A2 positive NCI-H1703 cells or MAGE-A4 negative/HLA-A2 positive cells (H441 and MRC5) were co-incubated with PBMCs (E:T 10:1) and single concentrations of 0.8 nM CDR4-bispecific 01 and images were recorded with the IncuCyte S3 system for up to 72h as described above.



MAGE-A4 x CD3 bispecific selectively kills MAGE-A4 expressing tumor cells but not normal lung fibroblast cells (MRC5) or tumor cells (H441) that do not express MAGE-A4

## 7 MAGE-A4 x CD3 bispecific eradicates NSCLC tumor *in vivo*

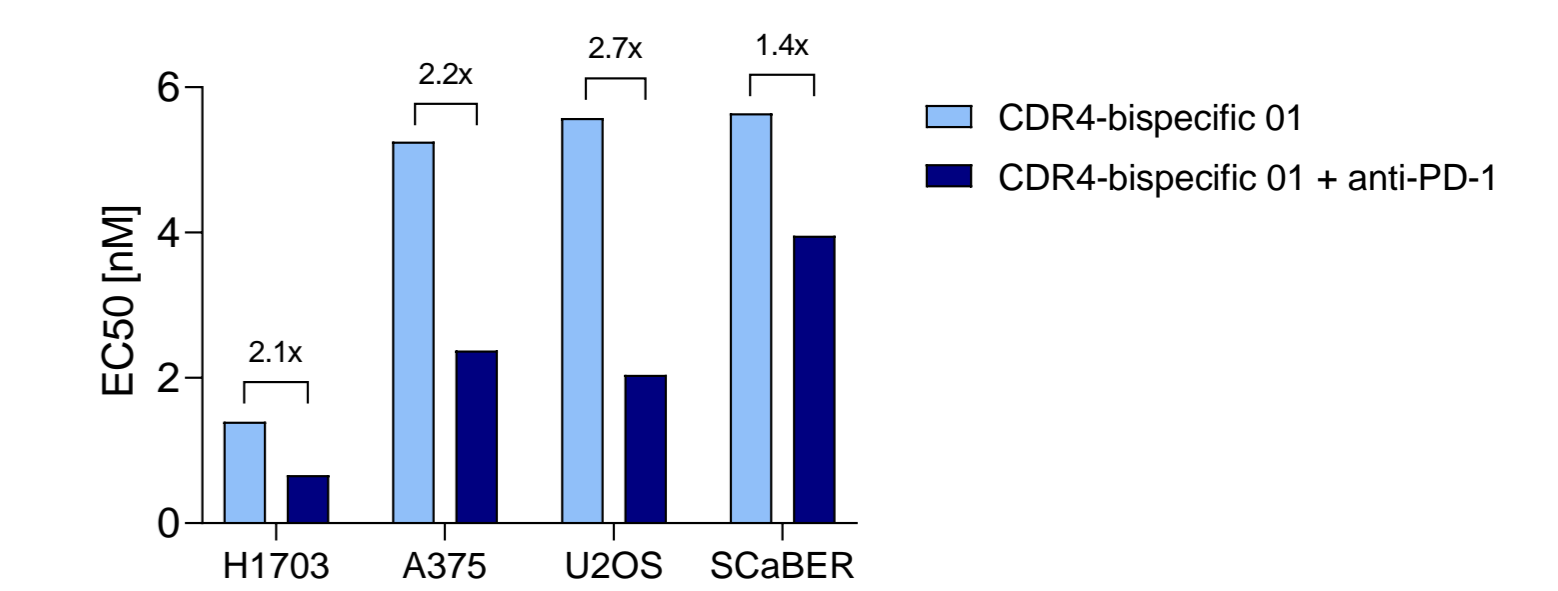
NSG mice were injected s.c. with 5x10<sup>6</sup> NCI-H1703 cells and received at an average tumor size of 80 mm<sup>3</sup> 5x10<sup>6</sup> PBMCs i.v. (2 donors, 4 mice/group). Mice were treated once daily with CDR4-bispecific 02 (2.5 mg/kg day 0-9, 5 mg/kg day 10-27) or a PBS control.



MAGE-A4 x CD3 bispecific led to complete regression of a lung cancer tumor xenograft and was safe and well tolerated in mice

## 8 Enhanced potency of MAGE-A4 x CD3 bispecific with immune checkpoint inhibitors

*In vitro* T cell-mediated cytotoxicity: EC50 for cell killing was determined by LDH release after 48h co-incubation of PBMCs and MAGE-A4 positive cell lines at E:T ratio 10:1 in presence of MAGE-A4 bispecific 01 (concentrations ranging from 0.078 to 40 nM) with or without 300 nM anti-PD-1 (Pembrolizumab).



Synergistic effects of MAGE-A4 x CD3 bispecific and immune checkpoint inhibition offer the opportunity to overcome immunosuppressive mechanisms.

## Conclusions

- CDR-Life's TCR-like antibody platform successfully delivered highly specific antibodies against a MAGE-A4 peptide presented on HLA-A\*02:01.
- The technology platform allowed straight forward isolation of high affinity and specificity MAGE-A4 binders that do not bind highly similar peptides expressed in healthy tissues.
- MAGE-A4 x CD3 bispecifics potently kill MAGE-A4 expressing cancer cells *in vitro* and *in vivo*.
- MAGE-A4 x CD3 bispecifics demonstrate safety *in vitro* and *in vivo*.
- Combining MAGE-A4 x CD3 bispecific and immune checkpoint inhibitor treatment potentiates cancer cell killing.
- Our T cell engaging TCR-like antibodies have great potential as off-the shelf therapies for a wide range of solid tumors.