

Athanasia Dasargyri¹, Stephanie Jungmichel¹, Nagjie Alijaj¹, Anna Sobieraj¹, Fabian Scheifele¹, Philip Knobel¹, Philipp Richte¹, Hannes Merten¹, Romina Doerig¹, Anna-Maria Evangelopoulou¹, Martina Priola¹, Blaz Pavlovic¹, Ariadna Vilarrasa¹, Thomas Schleier¹, Laure-Anne Bickel¹, Gonzalo Acuña¹, Swethajit Biswas¹, Tim Fugmann², Christian Leisner¹, Leonardo Borrás¹

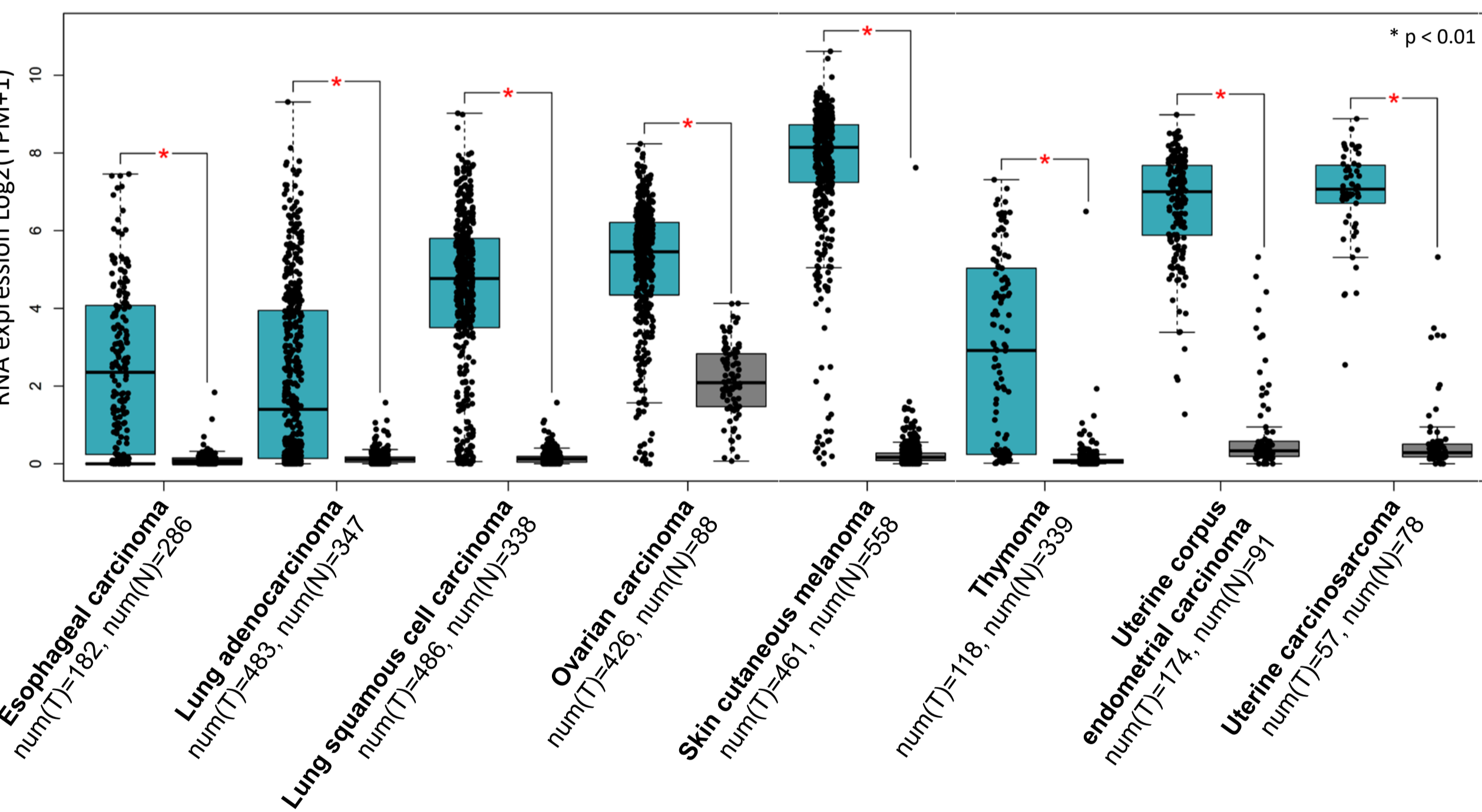
¹CDR-Life, Horgen, Switzerland (www.cdr-life.com), ²Alithea Biotechnology, Freiburg, Germany. Corresponding author: athanasia.dasargyri@cdr-life.com

Background

- Preferentially Expressed Antigen in Melanoma (PRAME) is a cancer/testis antigen expressed in a broad range of solid and hematological cancers. PRAME₄₂₅₋₄₃₃ peptide SLLQHLIGL presented on HLA-A*02:01 is a promising tumor target as its presentation is mainly restricted to cancer.
- CDR813 is an antibody fragment-based TCE which bivalently binds to the HLA-A*02:01-restricted PRAME₄₂₅₋₄₃₃ peptide on tumors. The target affinity of CDR813 was optimized to achieve high potency and target specificity.
- CDR813 was compared in potency, cytokine release, safety and developability properties against two comparators identical to clinical-stage compounds, which were produced in-house.

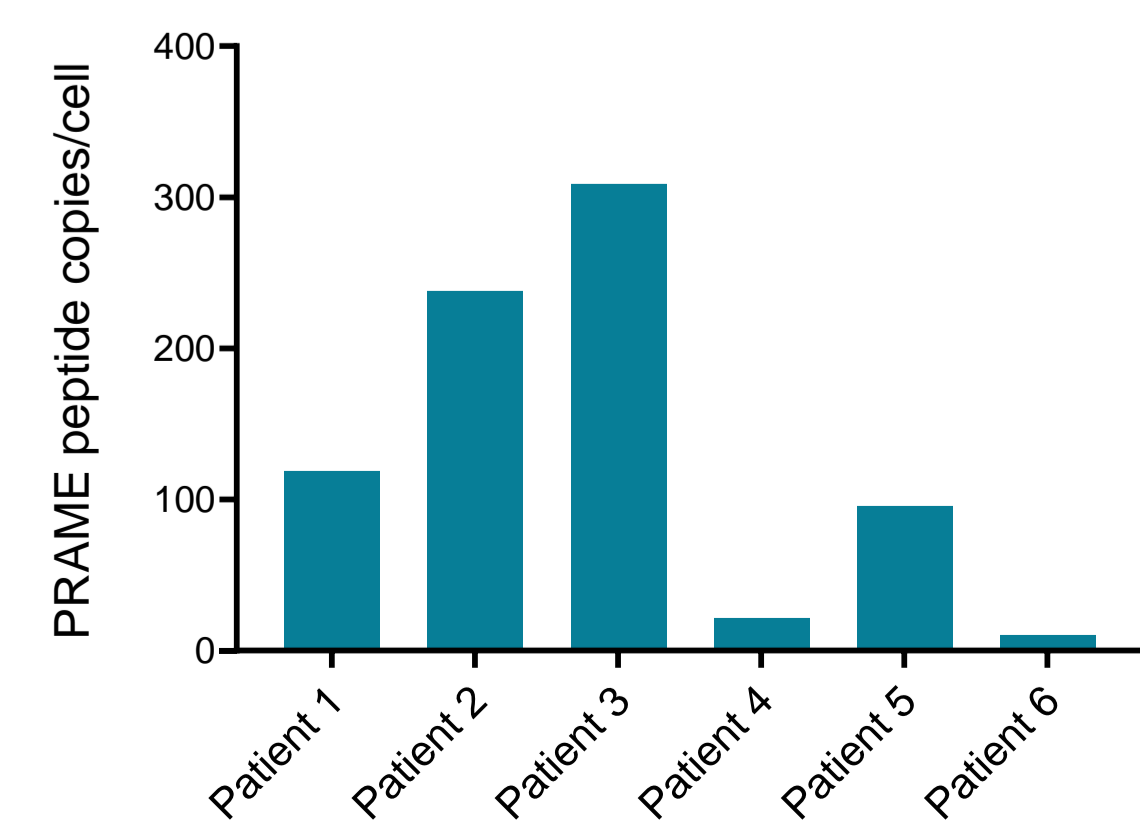
1 PRAME is a highly specific and prevalent target in multiple solid cancers with high unmet need

PRAME RNA expression in normal (N, grey) and tumor (T, blue) tissues was obtained from the TCGA and GTEx gene expression data using the GEPIA server (Tang et al. 2017, gepia.cancer-pku.cn).



2 PRAME₄₂₅₋₄₃₃ peptide is presented by HLA-A*02:01 in lung cancer patient samples

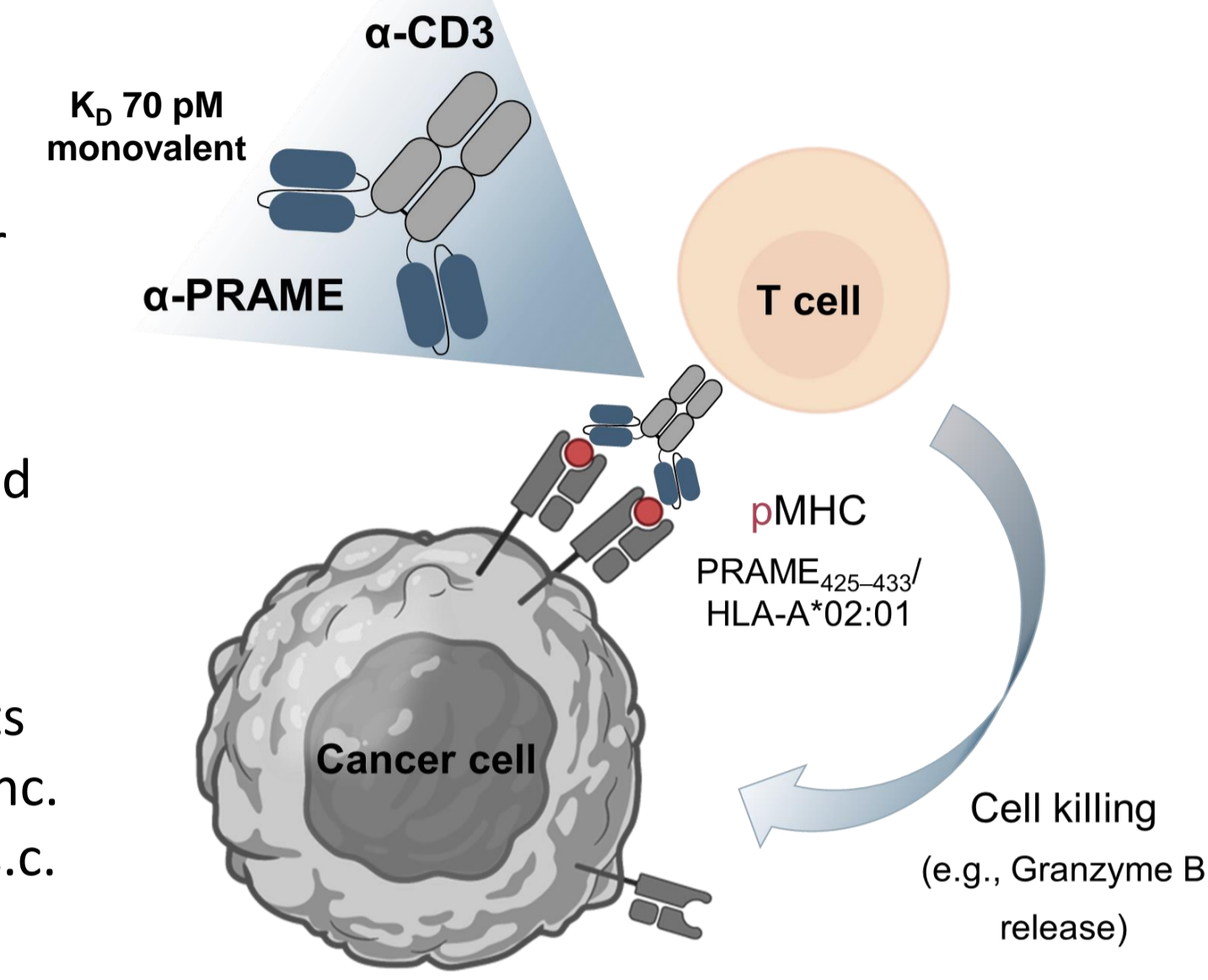
HLA-A*02:01 positive tumor samples from lung adenocarcinoma patients were used for immunoprecipitation followed by mass spectrometry analysis. The PRAME₄₂₅₋₄₃₃ peptide was identified in 6 of 12 samples. Approximate copy number per cancer cell was extrapolated from the analyzed tumor samples.



3 M-gager CDR813 targeting PRAME

M-gager features:

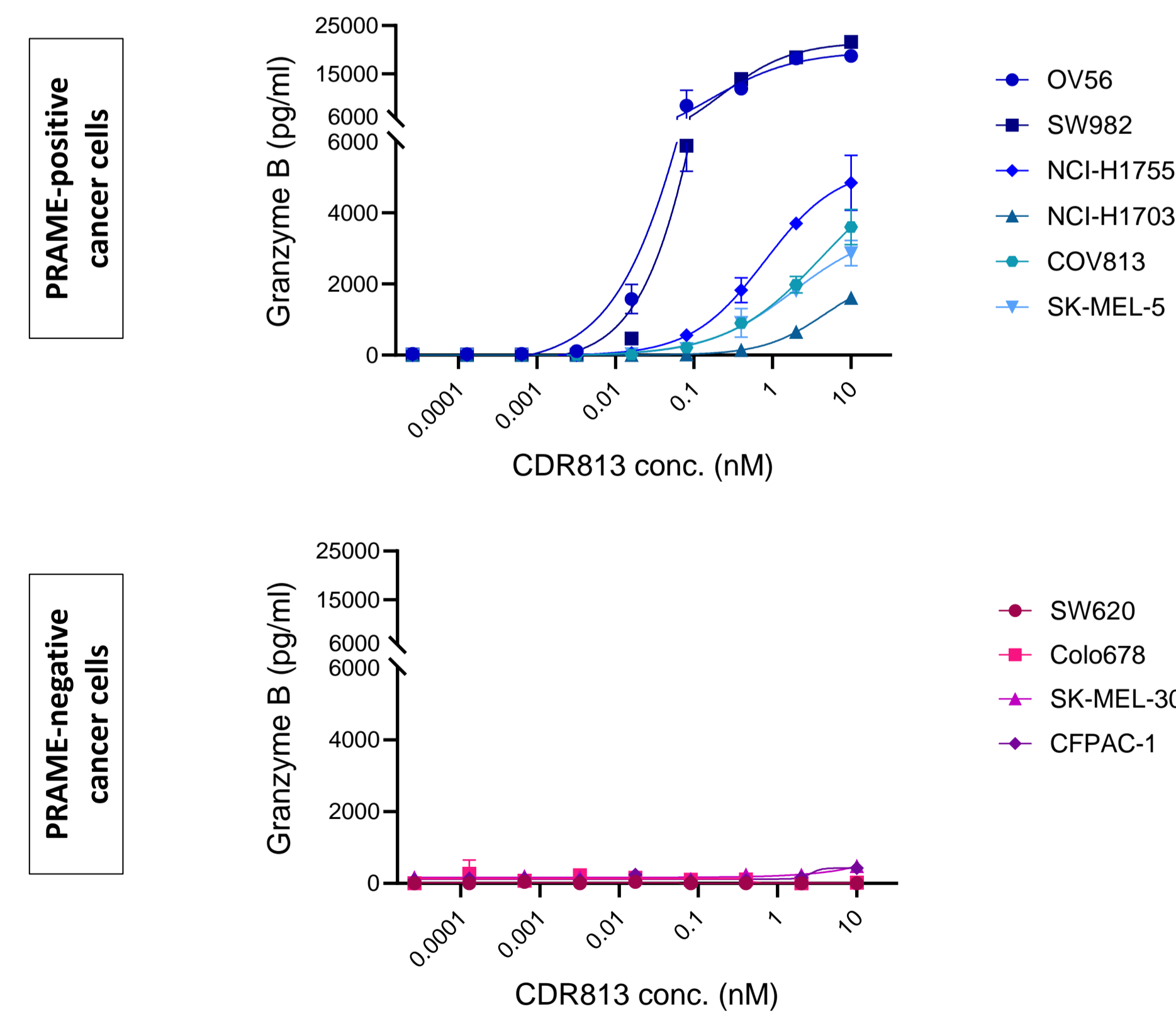
- Bivalent binding of pMHC for increased potency
- Efficient cancer cell killing and minimal cytokine release
- Drug Product profile supports liquid formulation in high conc. (> 100 mg/mL), suitable for s.c.
- Excellent manufacturability properties in CHO (mAb-like titers)



| Comparators | Description |
|-------------|--|
| sTCRxCD3 | Recombinant soluble TCR fused to an anti-CD3 scFv |
| TCR-TCE Fc | Half-life extended TCR and low-affinity T cell recruiter |

4 CDR813 shows specific killing of multiple HLA-A*02:01/PRAME (+) cancer cell lines

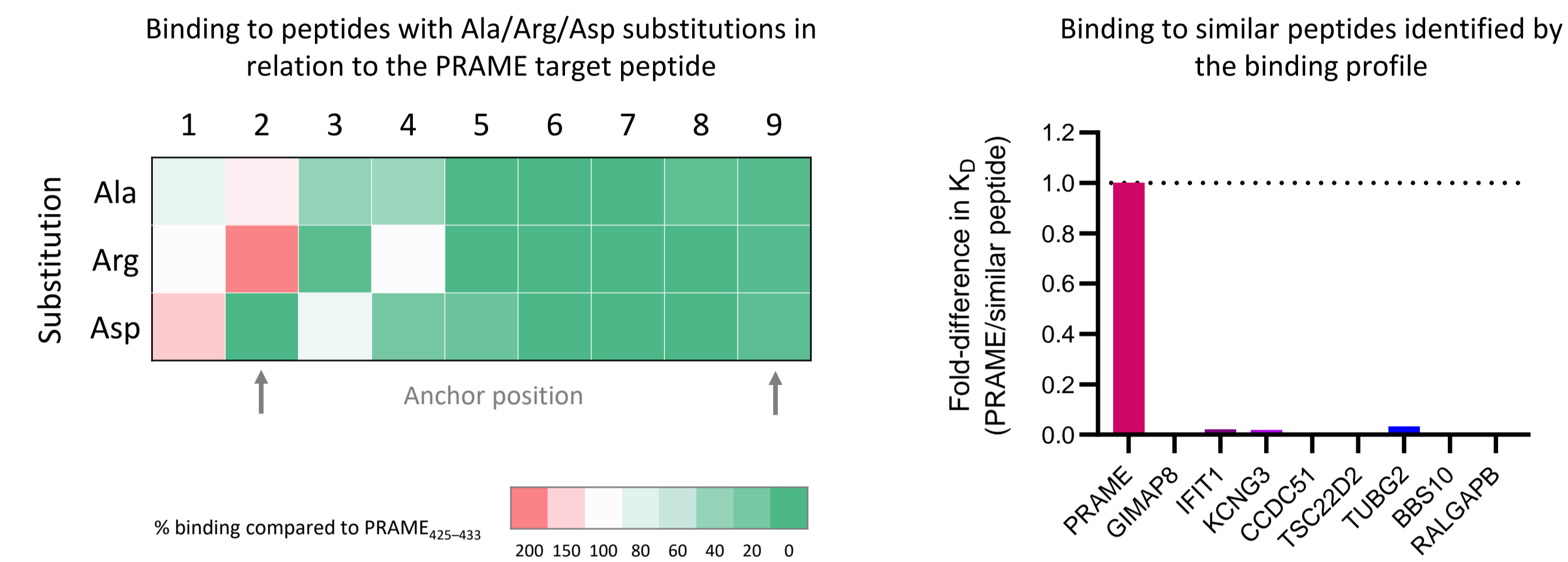
Cancer cell lines with varying HLA-A and PRAME RNA expression levels (Scholtalbers et al. 2015) or PRAME-negative cell lines were co-incubated with PBMCs and CDR813. Granzyme B release was determined at 24 h.



| Cell line | Disease | HLA-A allele 1 | HLA-A allele 2 | PRAME RPKM | HLA-A RPKM |
|-----------|----------------------------------|----------------|----------------|------------|------------|
| OV56 | Ovarian carcinoma | 02:01 | 02:01 | 148 | 1858 |
| SW982 | Synovial sarcoma | 02:01 | 24:02 | 99 | 3779 |
| NCI-H1755 | Lung adenocarcinoma | 02:01 | 02:01 | 278 | 1101 |
| NCI-H1703 | Lung squamous cell carcinoma | 01:01 | 02:01 | 118 | 1710 |
| SK-MEL-5 | Melanoma | 11:01 | 02:01 | 944 | 1506 |
| COV318 | Ovarian carcinoma | 03:01 | 02:01 | 191 | 950 |
| SW620 | Colorectal adenocarcinoma | 24:02 | 02:01 | 0.7 | 1407 |
| Colo-678 | Colon carcinoma | 02:01 | 02:01 | 1 | 451 |
| SK-MEL-30 | Melanoma | 01:01 | 02:01 | 0.5 | 2806 |
| CFPAC-1 | Pancreatic ductal adenocarcinoma | 03:01 | 02:01 | 4.1 | 3775 |

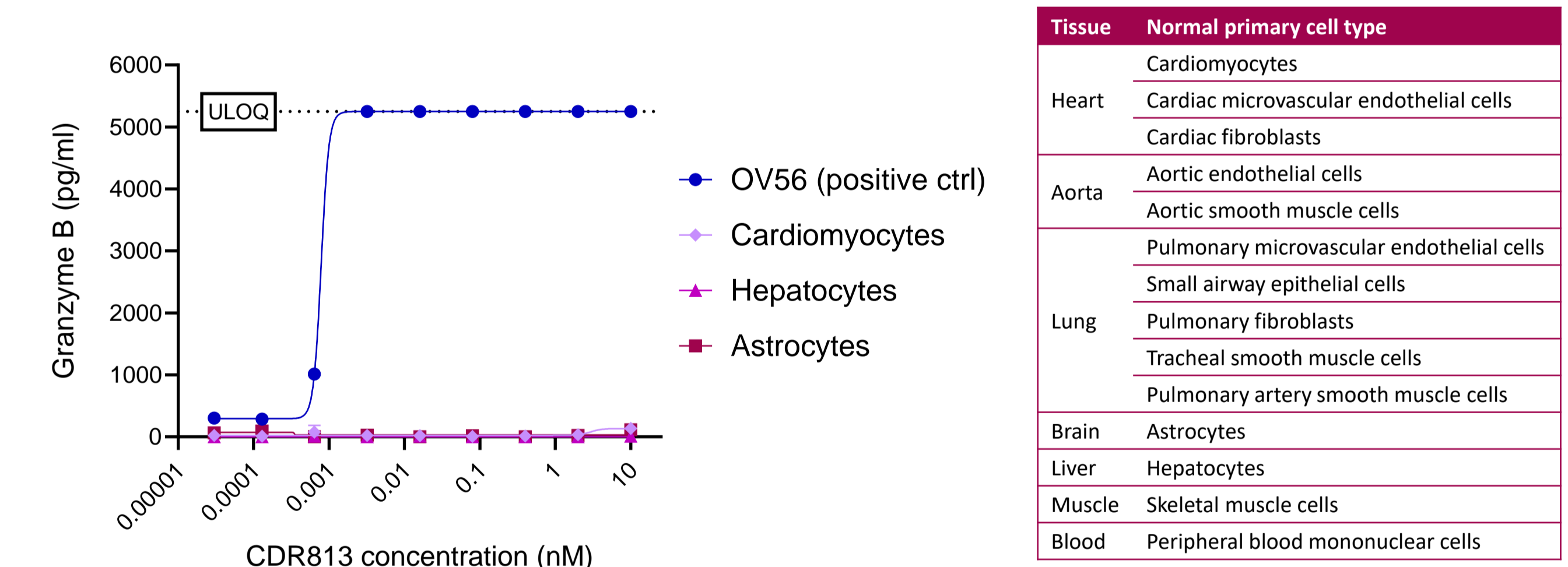
5 Binding studies suggest that CDR813 is highly specific

Binding of monovalent CDR813 was measured by SPR. Amino acids alanine, arginine or aspartic acid were substituted on the PRAME₄₂₅₋₄₃₃ peptide to establish which amino acids are critical for binding. Reduction in binding indicates that the substituted amino acid is important for binding. The binding profile enables to search for similar peptides in the human proteome. No relevant binding was observed to similar peptides presented in healthy tissues.



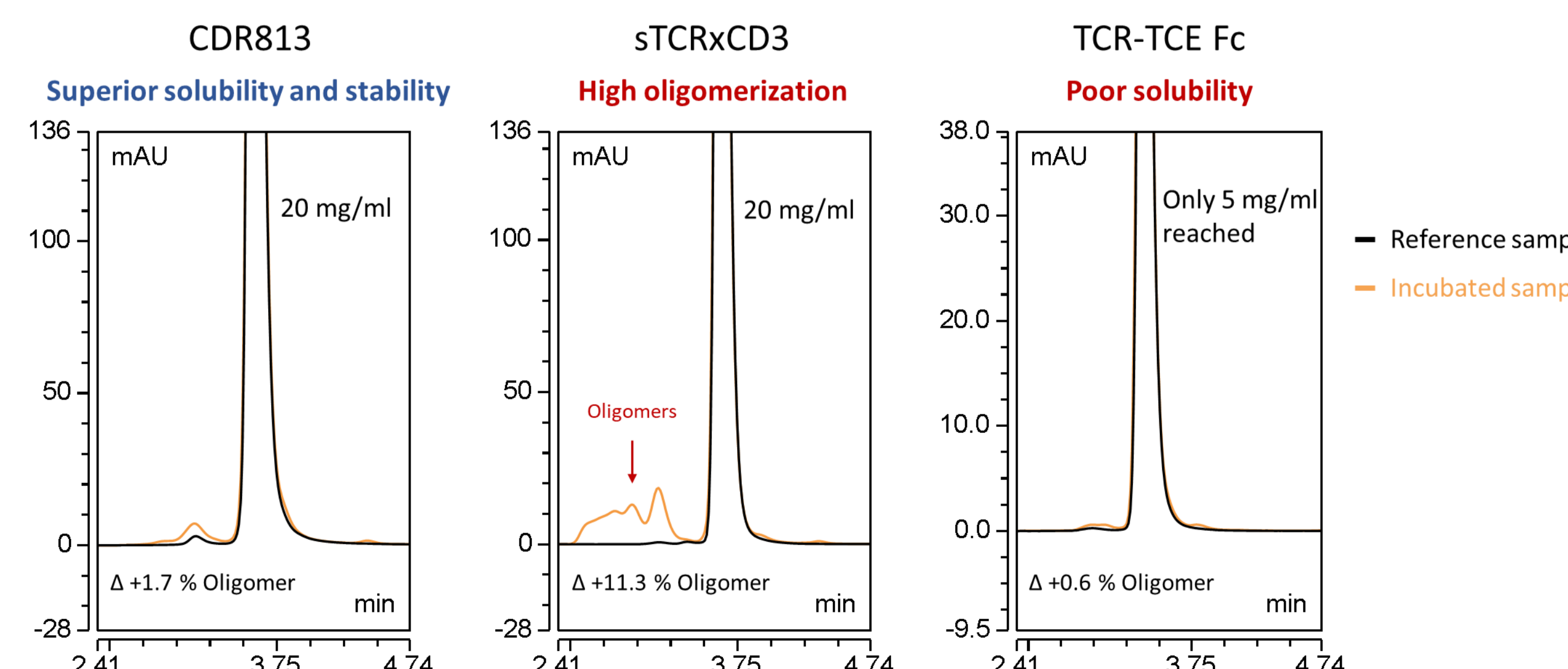
6 CDR813 exhibits a good safety profile in vitro

CDR813 was tested in co-cultures of HLA-A*02:01+ normal human primary cells, or positive control cancer cells (OV56), with human PBMCs. Granzyme B release was quantified after 24 h. Fourteen cell types from seven different tissues were screened and showed no relevant reactivity with CDR813. Representative data from three normal cell types and OV56 cells are shown.



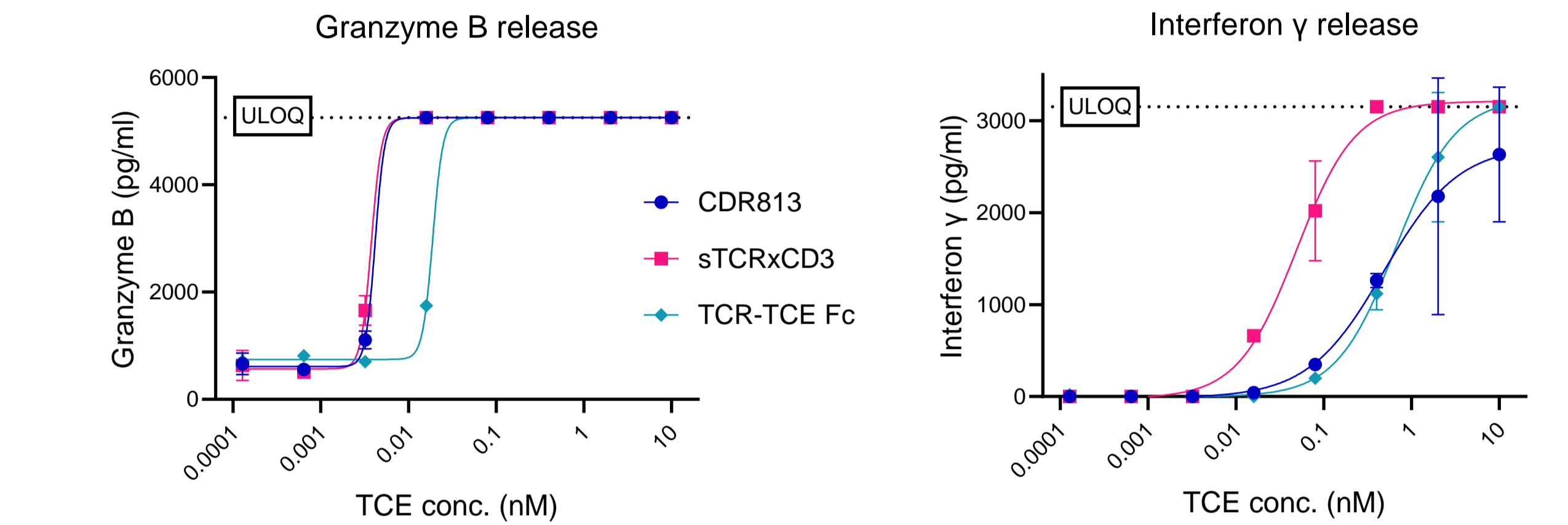
7 CDR813 demonstrates suitability for subcutaneous injection

CDR813 and two comparators were concentrated in artificial subcutaneous fluid buffer (Schuster et al. 2021) and incubated for one week at simulated physiological s.c. conditions (34°C, pH 7.4) at 20 mg/mL (or at 5 mg/mL for the TCR-TCE Fc). The incubated samples were analyzed by size exclusion chromatography. sTCRxCD3 showed a high tendency to form oligomers. TCR-TCE Fc was only soluble up to 5 mg/mL. CDR813 shows superior stability in simulated physiological s.c. conditions.



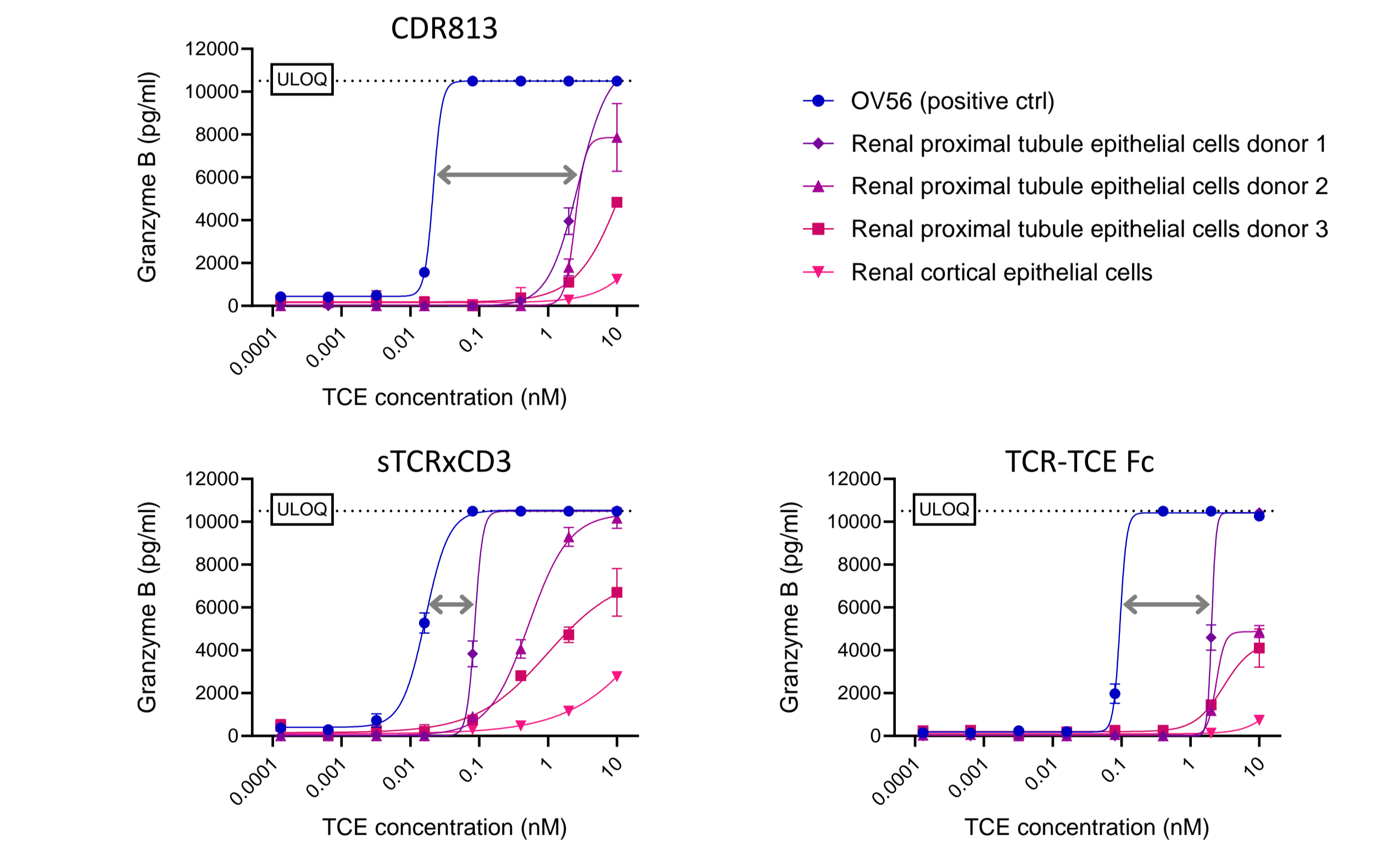
8 CDR813 induces potent cancer cell killing and low cytokine release

OV56 cancer cells were co-incubated with PBMCs and CDR813 or two comparators. Granzyme B and interferon γ release was determined at 24 h. CDR813 is more potent than TCR-TCE Fc and induces lower cytokine release than sTCRxCD3.



9 CDR813 shows the broadest window between on-target/on-tumor and on-target/off-tumor reactivity

CDR813 and the two comparators were tested in co-cultures of PBMCs with OV56 cells or with HLA-A*02:01+ normal human primary cells of renal origin expressing low levels of PRAME (data not shown). Granzyme B release was quantified after 24 h.



Conclusion

- PRAME is a highly prevalent target in multiple high need cancers.
- Clinical candidate CDR813 is a PRAME₄₂₅₋₄₃₃/HLA-A*02:01-targeting T cell engager with:
 - High potency on target-positive cancer cells with varying target expression levels
 - Highly specific target binding and minimal binding to similar peptides
 - A broad safety profile on healthy primary cells
 - Moderate cytokine release that could translate into lower CRS effect in the clinic
 - Good stability, favourable biophysical characteristics and excellent manufacturability properties
- CDR813 holds the potential to exhibit best-in-class characteristics in a clinical setting.