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Introduction

- Squamous non-small cell lung cancer (SQ-NSCLC) is the 2nd most common type of lung cancer
- There is a high unmet need in SQ-NSCLC to develop effective 2nd-line immunotherapies for patients with disease progression after immune checkpoint blockade
- Due to prevalence and expression level (based on mRNA expression), MAGE-A4 is a promising target in squamous cell lung carcinoma (ESMO 2023, Poster 200P)
- CDR404 is a highly specific and potent T-Cell engager which targets MAGE-A4/HLA-A2 on cancer cells and CD3 on T-cells
- The objectives of these studies were:
 - To confirm by IHC the high prevalence and expression observed for MAGE-A4 mRNA in SQ-NSCLC and
 - Demonstrate anti-cancer activity of CDR404 in *in vitro* and *in vivo* models of squamous cell lung carcinoma

Methods

MAGE-A4 mRNA analysis using The Cancer Genome Atlas (TCGA):

MAGE-A4 prevalence and expression in lung squamous cell carcinoma (LUSC) was analyzed using the TCGA database (<https://www.cancer.gov/tcga>).

In vitro cytotoxicity assay using a SQ-NSCLC cell line:

CDR404 target cell killing in the presence of human PBMCs was assessed using the human SQ-NSCLC cell line NCI-H1703. HLA-A*02:01 positive/MAGE-A4 negative cancer cells were used as controls. Cell killing was analyzed with the live cell imaging platform Incucyte S3, a fluorescence microscopy system that records in real-time the disappearance of fluorescent target cells.

In vivo efficacy

In vivo activity of CDR404 in SQ-NSCLC was evaluated with an NCI-H1703 xenograft model in NSG mice.

MAGE-A4 IHC in human SQ-NSCLC samples:

MAGE-A4 protein expression was confirmed using immunohisto-chemistry (IHC) in fifty human SQ-NSCLC samples (clone E701U).

1 MAGE-A4 mRNA distribution profile in lung squamous cell carcinoma (LUSC)

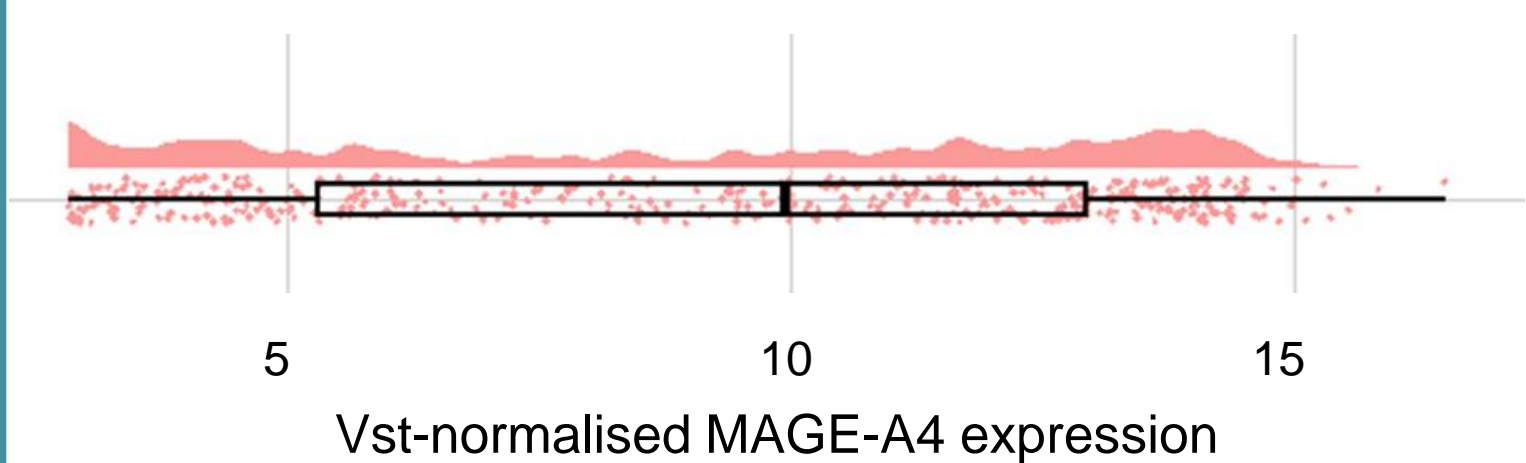


Figure 1. RainCloud distribution plot for MAGE-A4 mRNA expression in LUSC

High number of high MAGE-A4 expressing tumors within LUSC make it a highly attractive target for CDR404 immunotherapy

2 *In vitro*, the dual pMHC CDR404 T-cell engager elicits highly efficient killing against MAGE-A4+ cancer cells

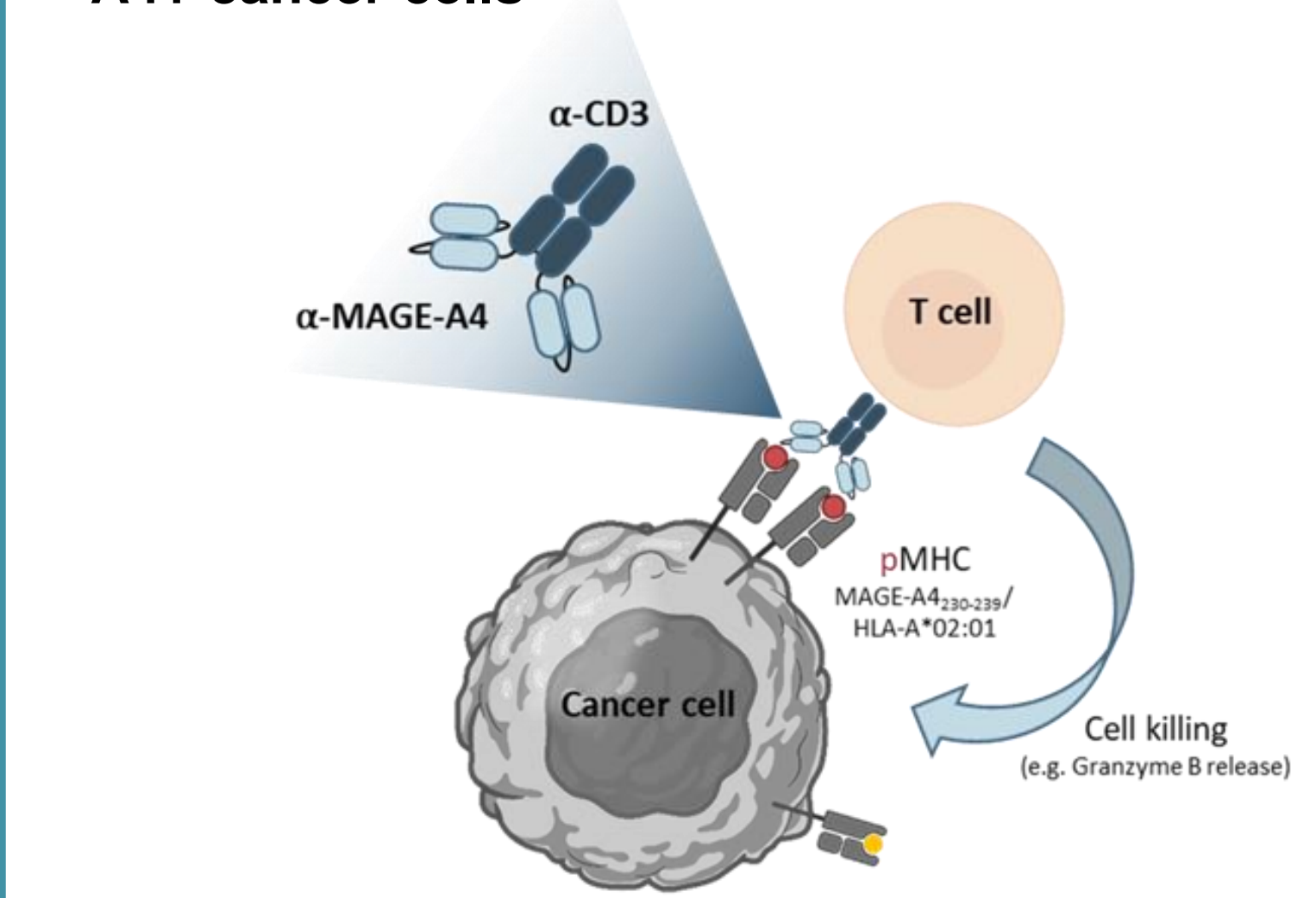


Figure 2. Mechanism of action of CDR404

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.

3 CDR404 induces killing of a MAGE-A4+/HLA-A*02:01+ SQ-NSCLC cancer cell line including at low E:T ratios

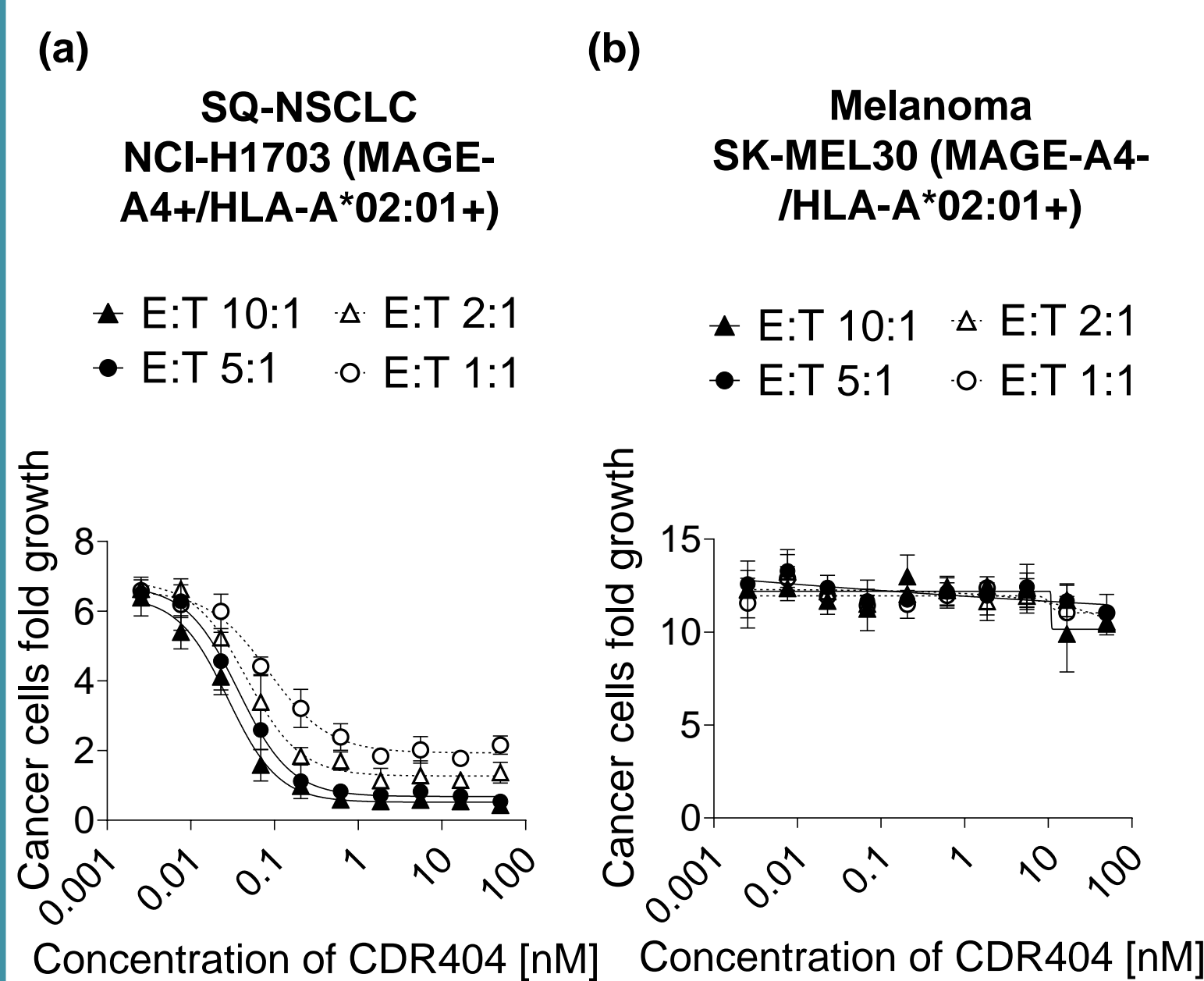


Figure 3. *In vitro* potency of CDR404. MAGE-A4+/ HLA-A*02:01+ NCI-H1703 (SQ-NSCLC) were co-cultured with PBMCs at different effector to target (E:T) ratios and a dose titration of CDR404 was applied. MAGE-A4-/ HLA-A*02:01+ SK-MEL-30 cancer cells were used as control. Cytotoxicity was measured up to 72h using live cell imaging of fluorescently labeled cancer cells by Incucyte.

CDR404 promotes efficient killing of NCI-H1703 SQ-NSCLC cancer cells down to E:T ratios of 1:1 (a), while no killing of MAGE-A4-/ HLA-A*02:01+ was observed for any of the E:T ratios tested (b).

4 CDR404 induces GrzB and IFNγ release on a MAGE-A4+/ HLA-A*02:01+ SQ-NSCLC cancer cell line including at low E:T ratios

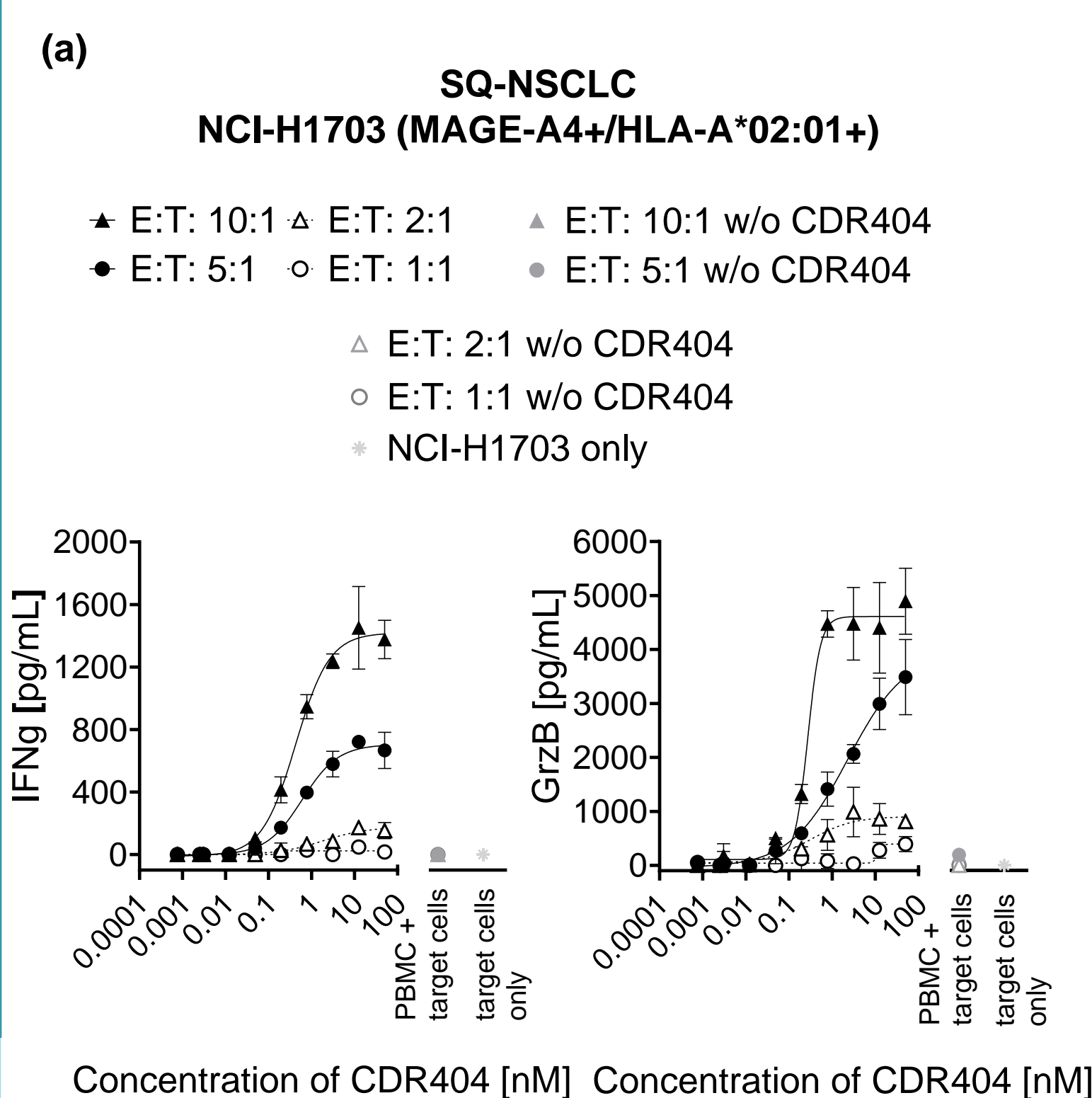


Figure 4. IFNγ and Granzyme B release induced by CDR404. MAGE-A4+/ HLA-A*02:01+ NCI-H1703 (SQ-NSCLC) were co-cultured with PBMCs at different E:T ratios and a dose titration of CDR404 was applied. MAGE-A4-/ HLA-A*02:01+ SK-MEL-30 cancer cells were used as control (data not shown). IFNγ and Granzyme B release were measured by ELISA at 24h.

CDR404 induces efficient release of the cytolytic molecule GrzB and the cytokine IFNγ when incubated with PBMCs and target positive cancer cell lines.

5 Ensuring safety of SQ-NSCLC and other Phase 1 trial patients by using a matrix of non-clinical experiments

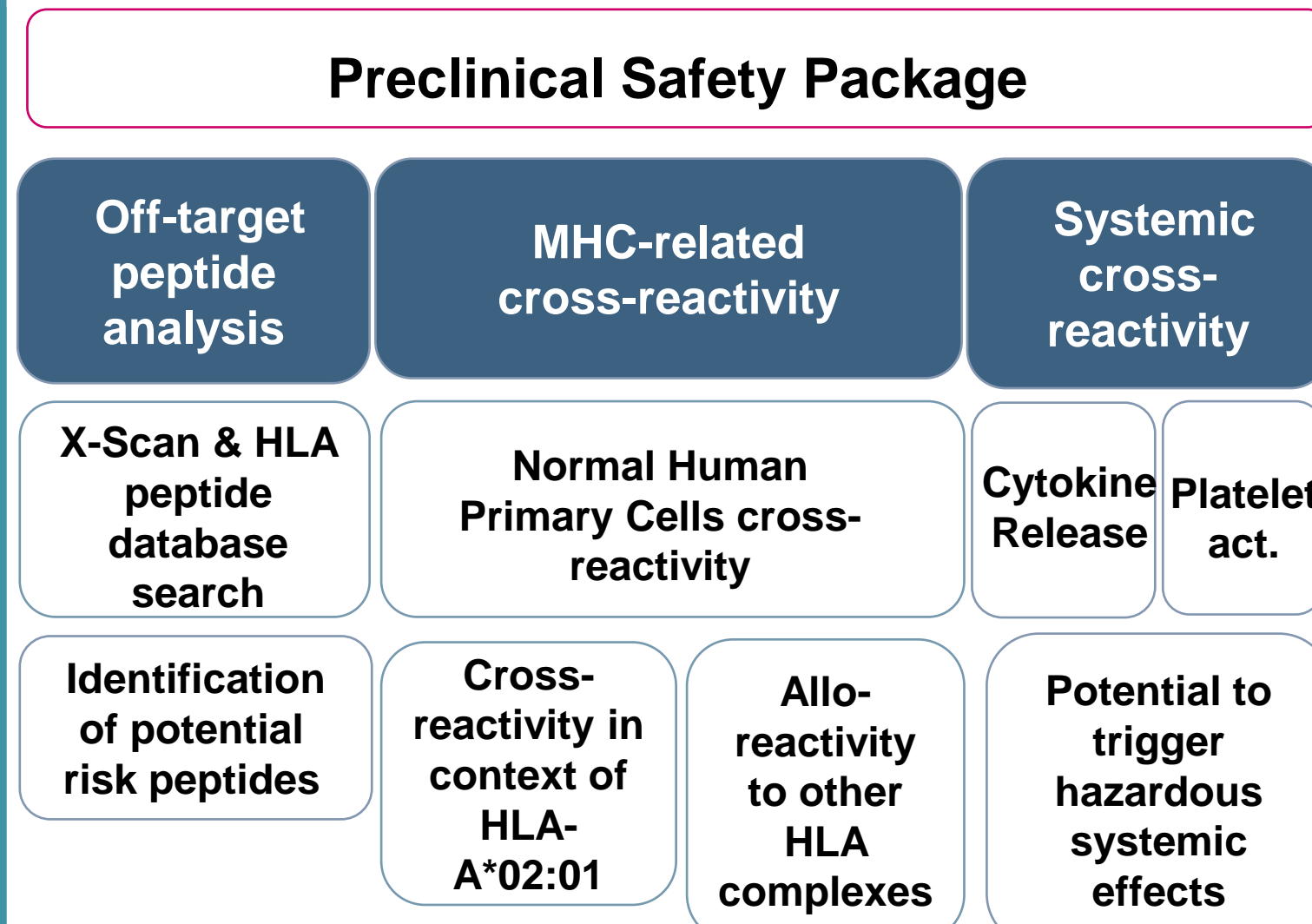


Figure 5. Overview of the preclinical safety package. Due to a lack of pharmacologically relevant animal models, it is not possible to assess the toxicity of CDR404 with *in vivo* studies. Alternative approach: *in vitro* and *in silico* based, preclinical safety strategy to demonstrate safety and target selectivity of CDR404, based on precedence set by other programs developing peptide/MHC-targeting molecules.

In the absence of pharmacology relevant species, the CDR404 preclinical safety assessment of selectivity and safety was conducted *in vitro*.

6 CDR404 eradicates SQ-NSCLC in an *in vivo* model

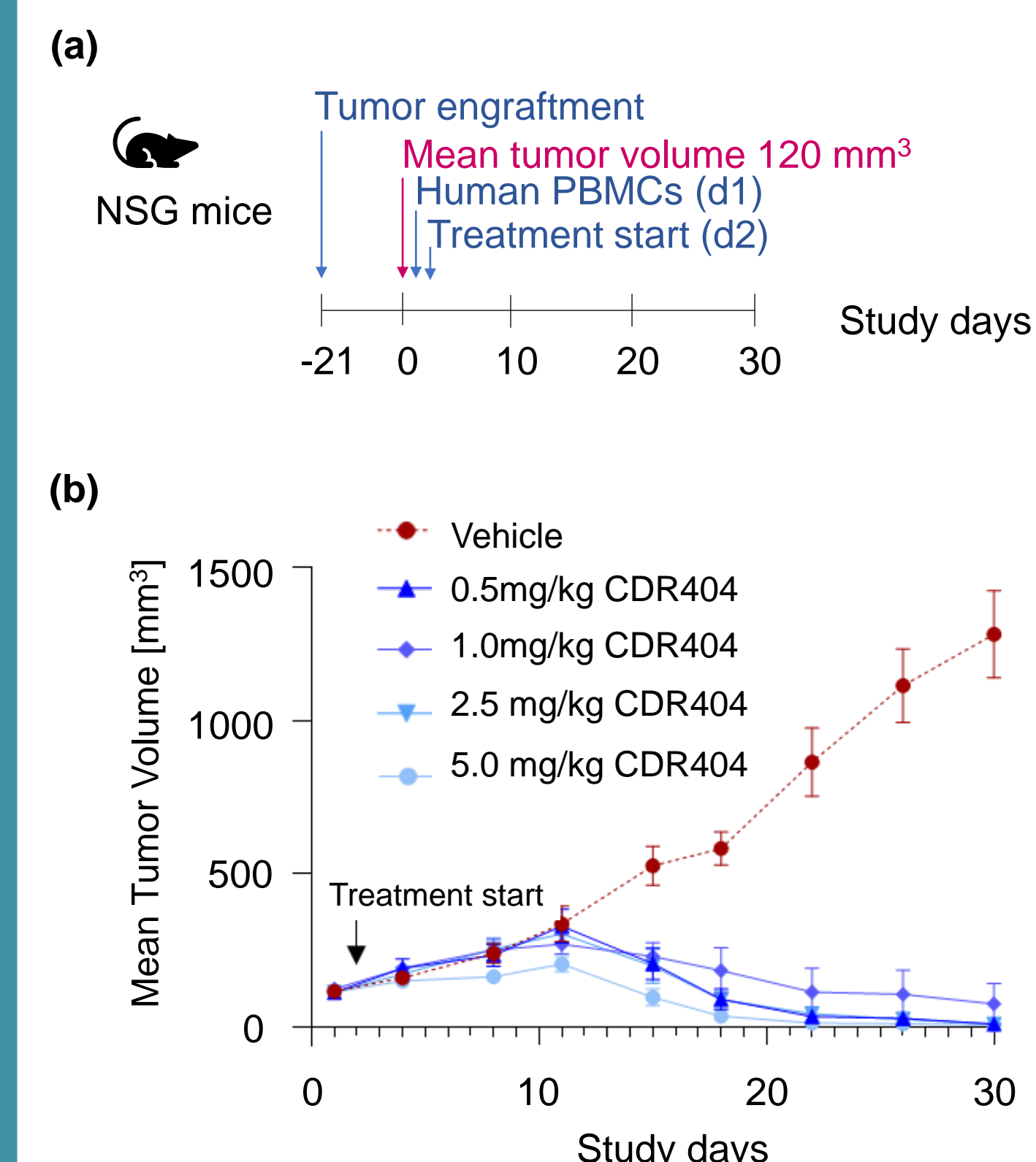


Figure 6. *In vivo* efficacy study of CDR404. (a) Experimental overview using NSG mice injected s.c. with 5×10^6 NCI-H1703 cells. Mice received at an average tumor size of 120 mm^3 5×10^6 PBMCs i.v. (2 donors, 3 mice/group). Mice were treated once daily with CDR404 (0.5 mg/kg; 1.0 mg/kg; 2.5 mg/kg; 5.0 mg/kg) or a vehicle control. (b) Tumor development was measured every 4th day.

CDR404 led to complete regression of squamous lung cancer tumors in a heterotopic mouse model across all doses tested.

7 MAGE-A4 intensity scoring in human SQ-NSCLC tumors

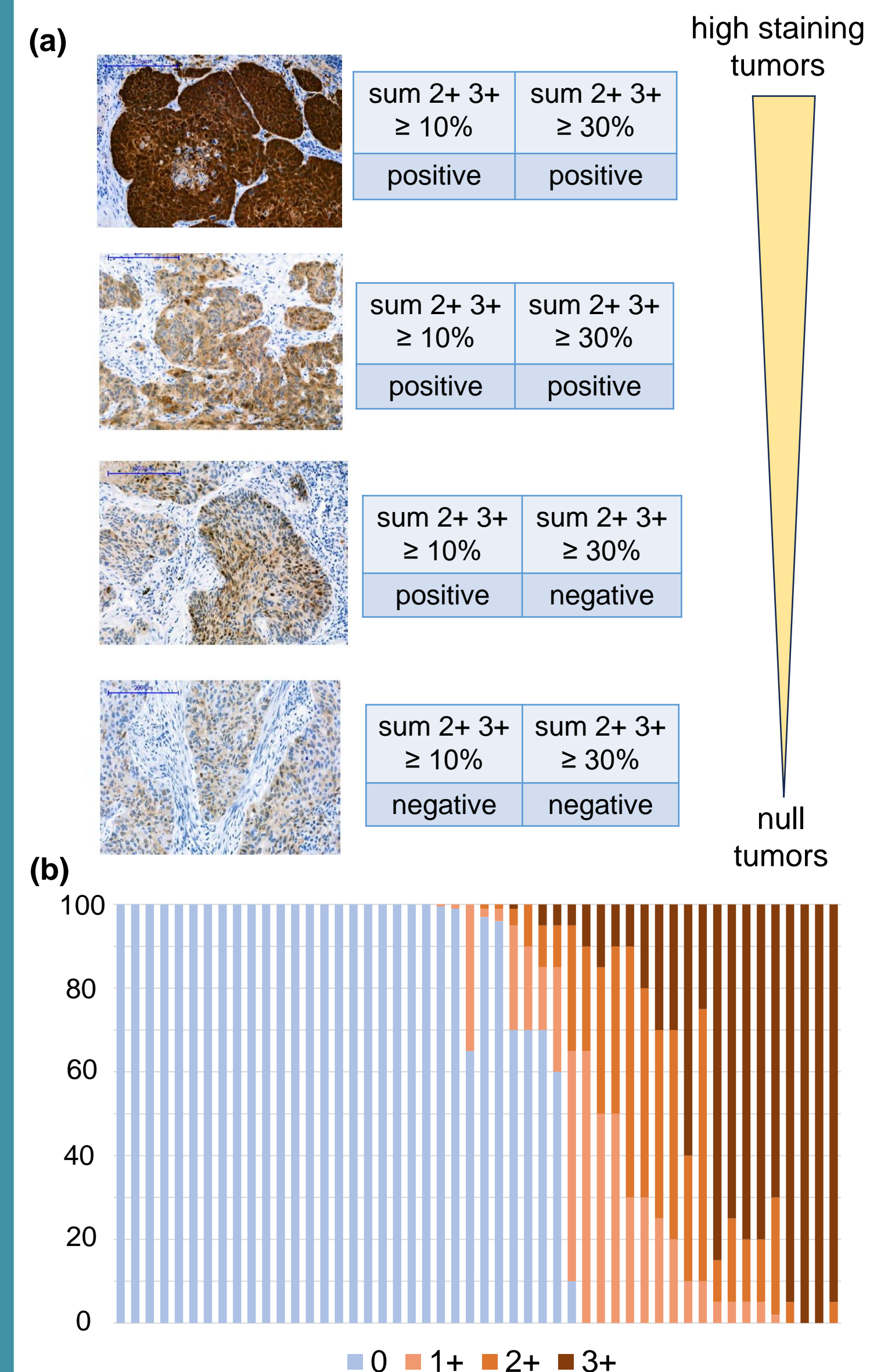


Figure 7. MAGE-A4 IHC Staining in Human SQ-NSCLC. (a) Photomicrograph panel of MAGE-A4 IHC Staining (High Staining = 100% cancer cells with +3 Intensity; Null Staining = 100% cancer cells with 0 Intensity) (b) MAGE-A4 IHC Intensity scoring profiles.

56% of the SQ-NSCLC samples had at least 0.5% at 1+ staining intensity (N= 28/50). 44% positive cases were detected with cut-off sum of 2+3+ ≥10% (N=22/50). 38% (N= 19/50) positive cases were detected with a cut-off sum 2+3+ ≥30%.

Conclusions

- High prevalence and expression of MAGE-A4 in squamous cell lung carcinoma tumors make it a highly attractive target for CDR404 immunotherapy.
- Specific IHC assay confirms MAGE-A4 expression in at least half of SQ-NSCLC tumor samples tested.
- Safety and specificity of CDR404 is best studied with *in vitro* and *in silico* model systems due to the human-specific nature of the tumor targeting arm.
- CDR404 promotes efficient killing and complete tumor eradication in relevant *in vitro* and *in vivo* models of squamous cell lung carcinoma