

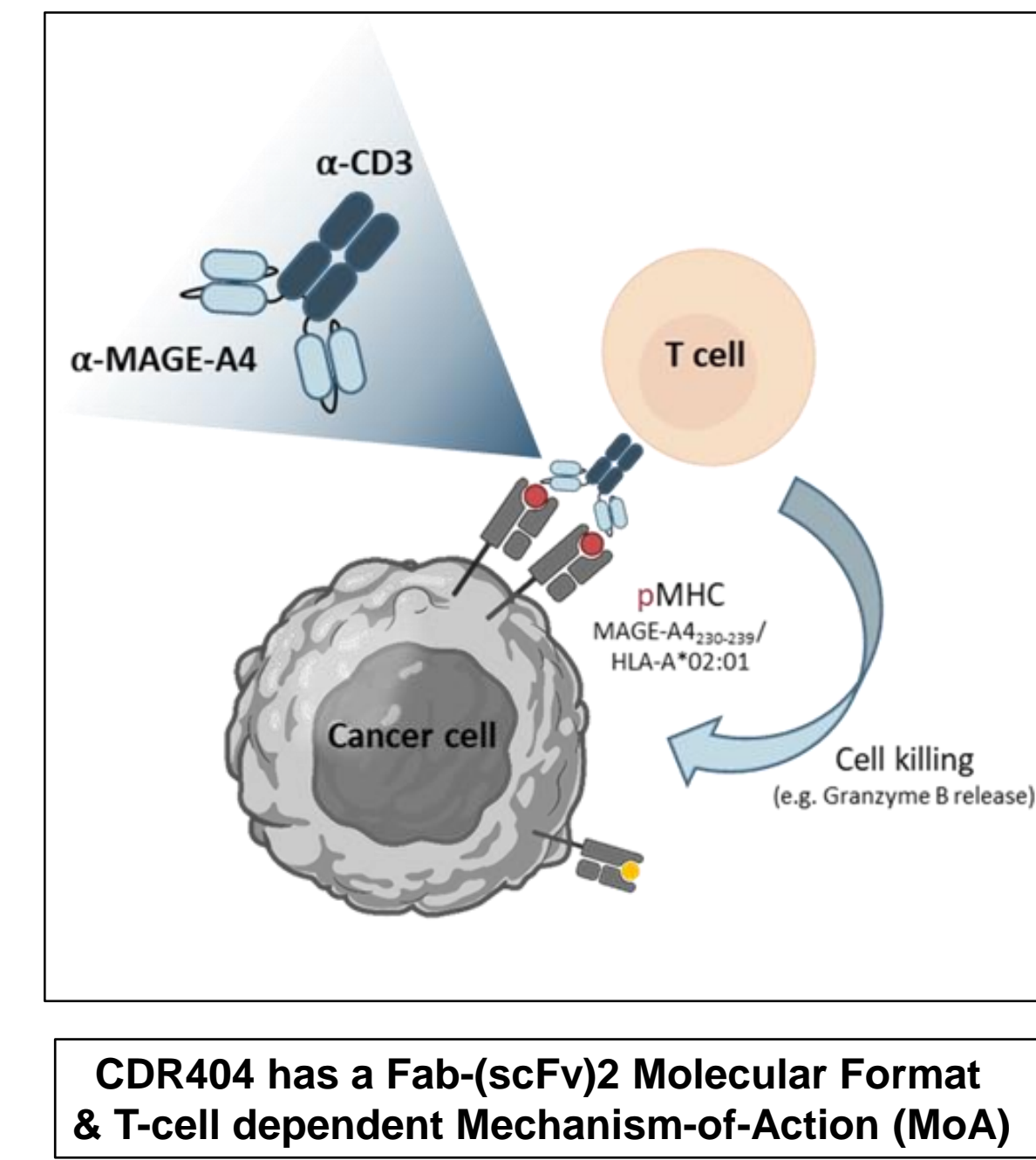
200P Precise Tumor & Patient Selection for CDR404: A Bispecific & Bivalent MAGE-A4 T-Cell Engager

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Introduction

- CDR404 is a first-of-its-kind Fab-(scFv)₂ bispecific antibody-based T-cell engager that binds bivalently to a MAGE-A4₂₃₀₋₂₃₉ peptide displayed on HLA-A*02:01 on cancer cells and monovalently to CD3 on T cells
- MAGE-A4 is an attractive cancer target since it is transcriptionally silenced in normal somatic tissues, but expressed in certain tumours
- We evaluated the prevalence and mRNA expression levels of MAGE-A4 mRNA in 32 solid cancers in The Cancer Genome Atlas (TCGA), and its relationships with tumour mutational burden (TMB) & oncogenic pathways
- For prospective selection of patients with MAGE-A4+ tumours into the phase 1 trial using immunohistochemistry (IHC), we compared the specificity of two anti-MAGE-A4 monoclonal antibodies for the development of an investigational use only (IUO) assay



2 MAGE-A4 mRNA expression in 8 epithelial cancers with high unmet medical need

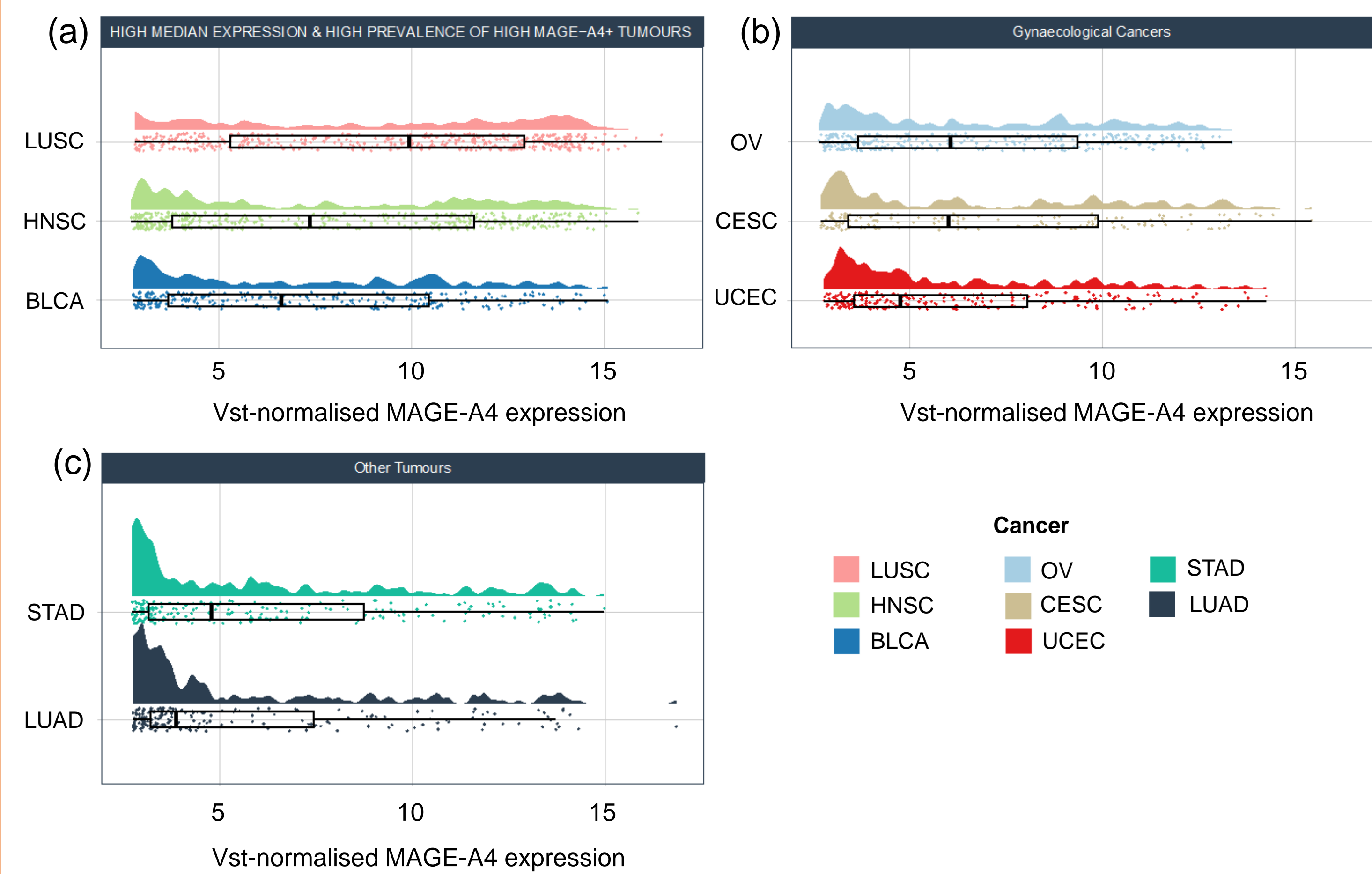


Figure 2. RainCloud distribution Plots for MAGE-A4 mRNA expression in 8 epithelial TCGA cancers

- LUSC had the highest no. tumours of high MAGE-A4+ followed by HNSC and BLCA. LUAD had the longest distribution tail, but the lowest no. of high MAGE-A4+ tumours (Figure 2a)
- In TCGA gynaecological cancers, median mRNA expression levels were similar between OV and CESC, and UCEC had the lowest median. OV had the highest no. of high MAGE-A4+ tumours followed by UCEC and CESC (Figure 2b)
- Although 100% of OV samples in the TCGA had detectable MAGE-A4 mRNA, the low objective responses rates (ORR) reported in a prior soluble TCR T-cell engager phase 1 trial (NCT03973333), where prospective selection for tumour MAGE-A4 was absent in ovarian cancer patients (Sweis et al, 2022), is potentially explained by the low median MAGE-A4 expression and relatively low no. high MAGE-A4+ tumours in OV compared to LUSC

3 Differences in median TMB between high vs. low/intermediate expressing MAGE-A4+ tumours in 8 TCGA cancers

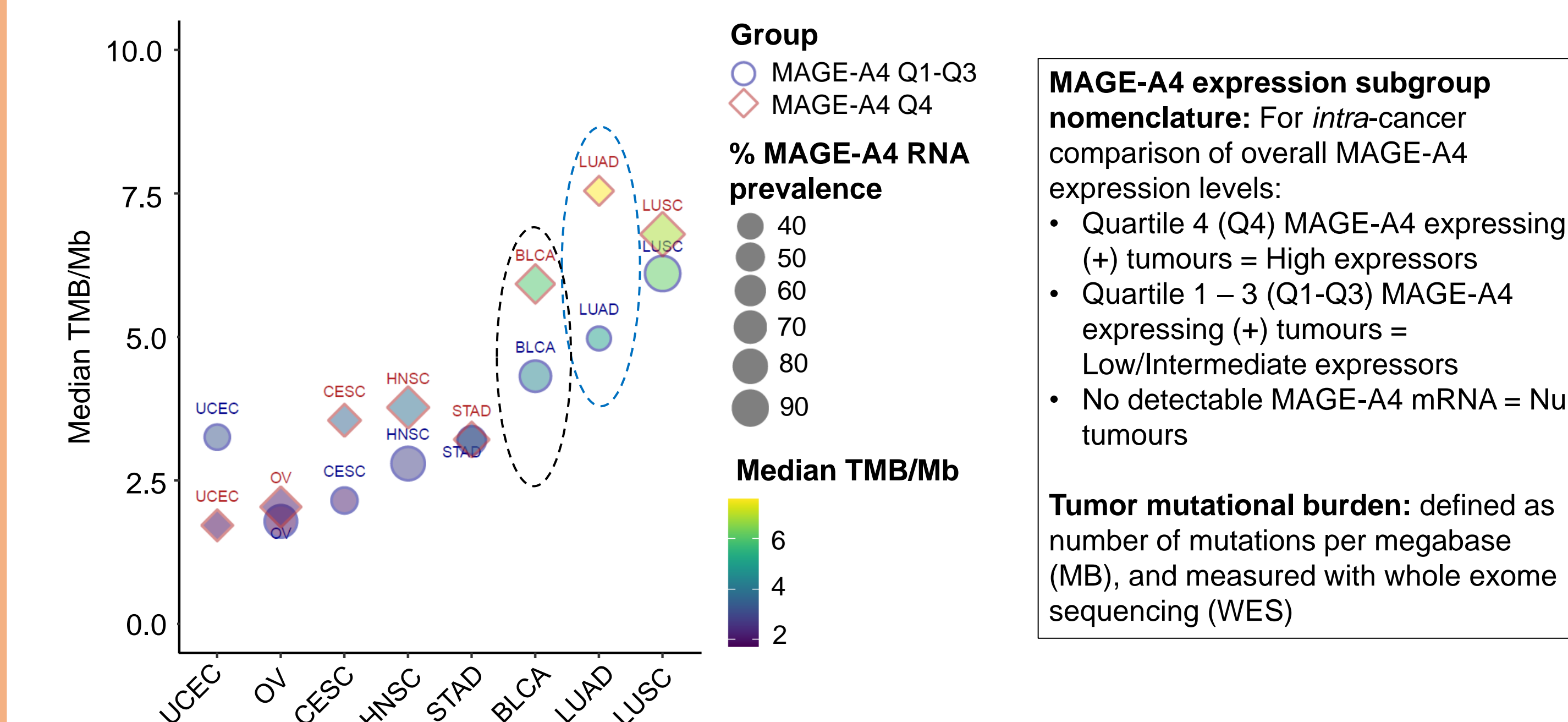


Figure 3. Median TMB between high vs. low/intermediate expressing MAGE-A4+ in 8 epithelial cancers with high unmet medical need

- As previously published, with exception of cutaneous melanoma, LUSC, LUAD, HNSC & BLCA had the highest median TMBs compared to other cancer types in the TCGA (Weinstein et al, 2013).
- In LUAD, high MAGE-A4+ expressing tumours had a higher median TMB compared to low/intermediate MAGE-A4+ tumours ($p < 0.002$; dashed blue circle).
- A similar, but lower significant difference, was seen in BLCA ($p < 0.004$; dashed black circle). Given these associations with TMB, further analysis is required to ascertain whether patient age is a confounding factor for tumour MAGE-A4 expression

4 Oncogenic signaling pathways mutated in LUAD stratified by MAGE-A4+ expression subgroups

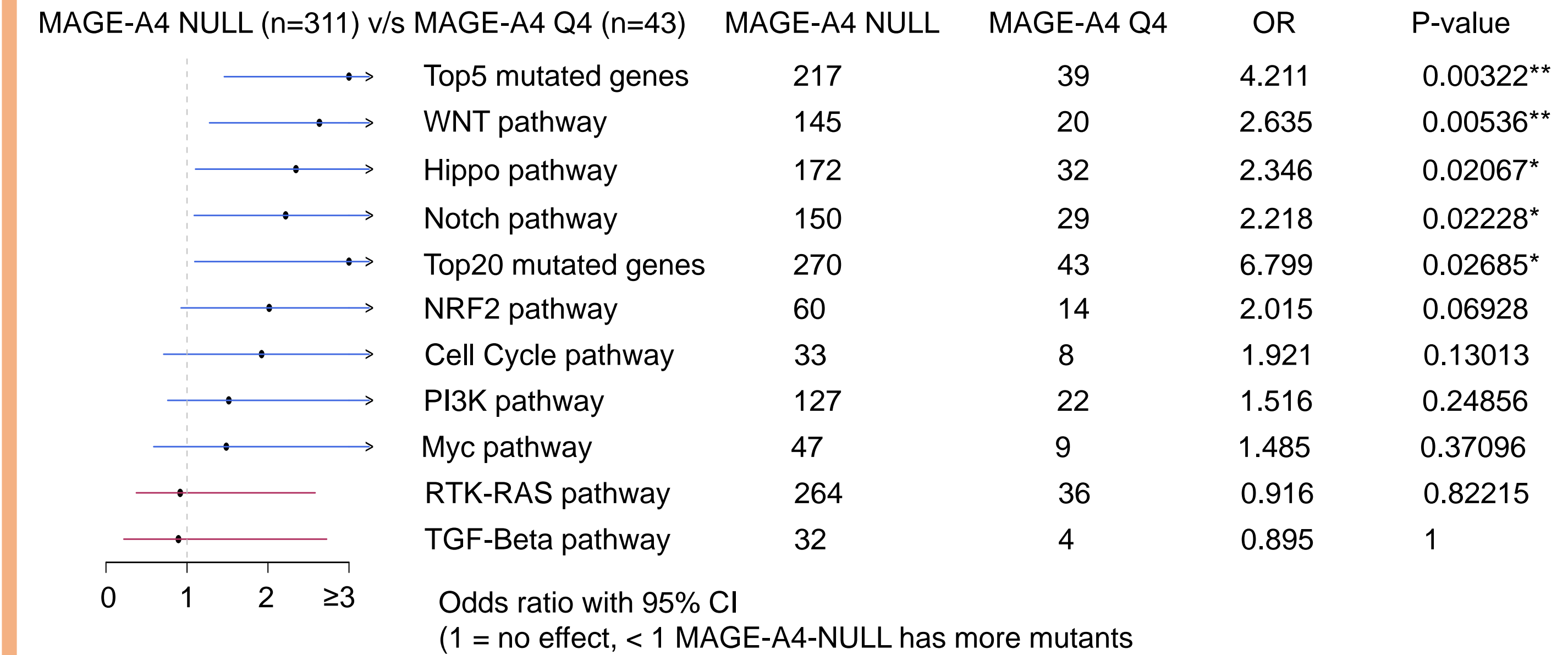


Figure 4. Genomic evaluations in LUAD to include an understanding of the associations between MAGE-A4 expression and oncogenic signalling pathways.

A higher frequency of genetic mutations in the top 5 mutated genes in LUAD (including *TP53*), as well as in stemness/de-differentiation genes in the *WNT*, *NOTCH* and *HIPPO* signalling pathways were found in high MAGE-A4+ LUAD tumours compared to null tumours (Figure 4).

5 Evaluation of anti-MAGE-A4 monoclonal antibody specificity for use in a patient selection IHC IUO assay

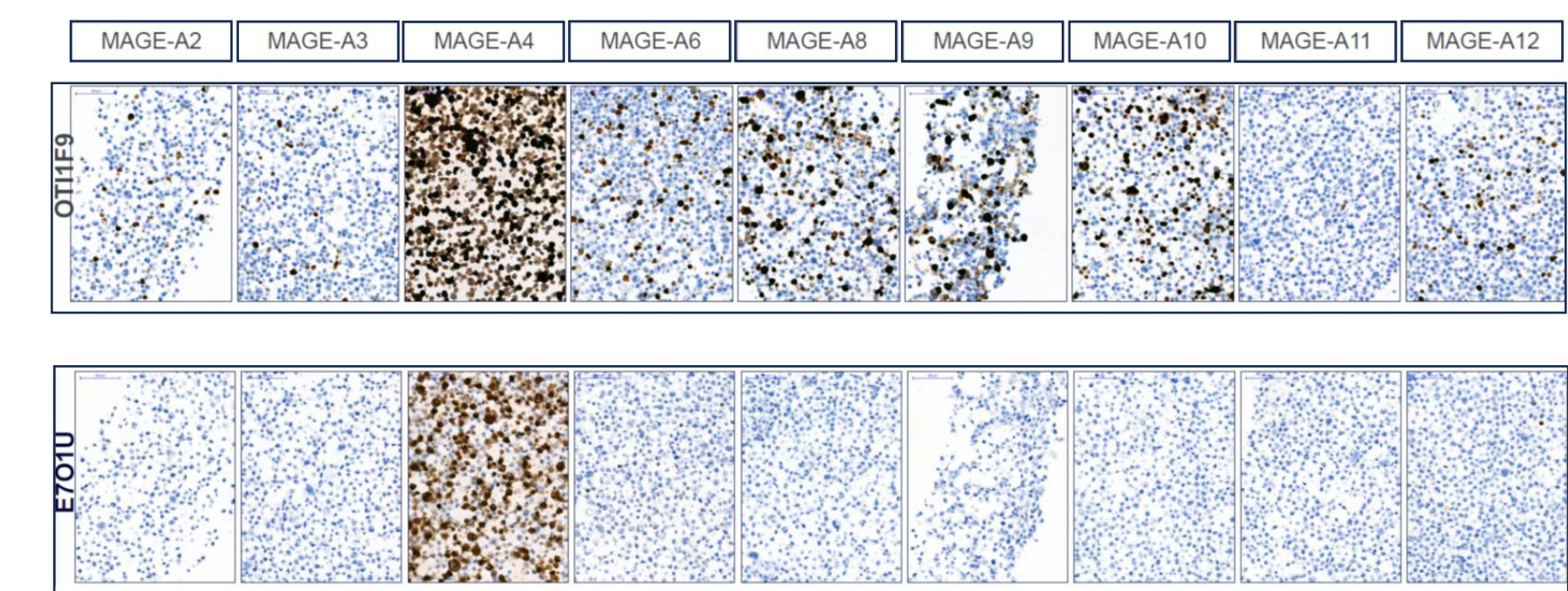


Figure 5. Anti-MAGE-A4 IHC monoclonal antibody cross-reactivity experiments using an anti-rabbit E7O1U clone and an anti-mouse OT1F9 clone.

In vitro over-expression experiments for 9 MAGE-A family members using cell pellets. E7O1U was shown to only detect MAGE-A4 protein in these cell line models whereas OT1F9 widely cross-reacted with MAGE-A3, -A6, -A8, -A10, and -A12 proteins

Conclusions

- Multiple cancer indications are potentially eligible for the CDR404 phase 1 trial including 3 RNA^{HIGH} epithelial cancers with the strongest associations with a history of cigarette smoking, high TMB, and high global mortality: i.e., **LUSC, HPV-negative HNSC and BLCA**
- Sub-groups of high MAGE-A4+ expression are present across a wide range of solid cancers including OV>UCEC>STAD>LUAD, but since the sizes of high MAGE-A4 subgroups varies widely across TCGA tumour types evaluated in this study, a specific MAGE-A4 tumour selection assay will be essential for the identification of patients for the CDR404 phase 1 trial
- CDR404 might be a rational immunotherapeutic approach for LUAD patients with high MAGE-A4+ tumours, since both *WNT* pathway signalling and *TP53* mutations have been implicated in resistance to anti-PD-1/PD-L1 checkpoint blockade in the context of high TMB (Takeuchi et al, 2021; Zhu et al, 2023)
- The E7O1U rabbit monoclonal antibody clone (Cell Signalling Technology: MAGE-A4 XP® Rabbit, mAb #82491) demonstrated high specificity against MAGE-A4. Therefore, the E7O1U clone has been selected for development into an IUO IHC assay to precisely select patients for the CDR404 phase 1 trial
- IHC/mRNA concordance studies using E7O1U stained tumours could enable a future transition to an RNA-sequencing based patient selection assay. This could enable a more sensitive patient screening assay especially for cancers (e.g., LUAD & STAD) where MAGE-A4 expression is lower

Disclosures

This study was undertaken by CellCarta and Genevia sponsored by CDR-Life. The presenting author is employed by CellCarta. The authors would like to thank Kelly Gordon (Boudiccadx) for review.

Abbreviations for key solid cancers in TCGA: LUSC (squamous lung cancer); LUAD (adenocarcinoma lung cancer); BLCA (bladder cancer); HNSC (head & neck squamous carcinoma); UCS (uterine carcinosarcoma); TCGT (testicular germ cell tumour); CESC (cervical squamous carcinoma); ESCA (oesophageal squamous carcinoma); OV (high-grade serous ovarian carcinoma); STAD (stomach carcinoma); BRCA (breast cancer – all subtypes)

Methods

TCGA Bioinformatic Analysis

mRNA expression data from 32 cancers with solid tumours was obtained from TCGA and TCGA Pan-Cancer Atlas datasets.

Anti-MAGE-A4 IHC monoclonal antibody cross-reactivity experiments:

To assess protein expression, we compared specificity of two commercial MAGE-A4 antibodies: an anti-rabbit E7O1U clone and an anti-mouse OT1F9 clone. Cross-reactivity to MAGE-A family members was confirmed using IHC on cell pellets of individually transfected HEK293 cells.

Results

1 MAGE-A4 expression is enriched in cancers of squamous cell origin including HPV-negative HNSC

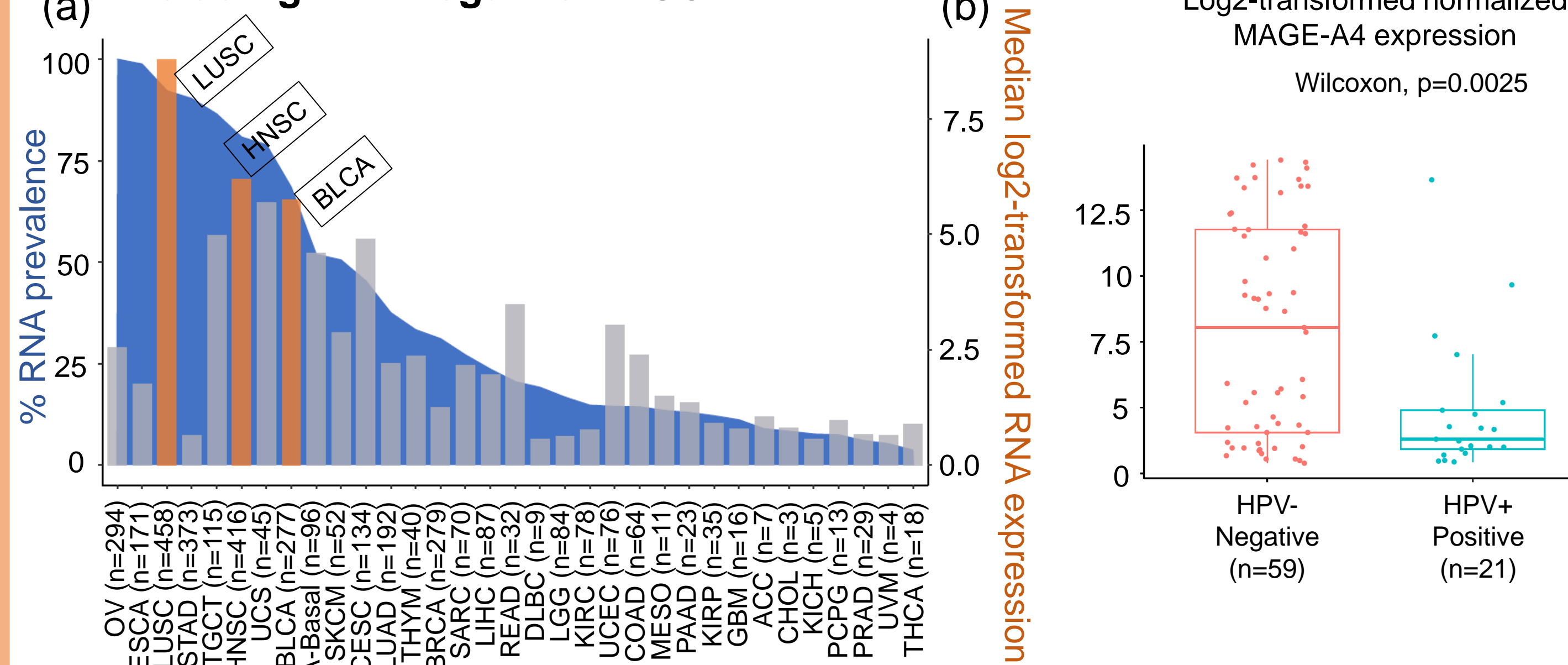


Figure 1. (a) MAGE-A4 mRNA prevalence (blue contour) & median expression levels (orange histograms: 3 epithelial tumours with the highest expression) in 32 TCGA cancers (x-axis: cancer type ranked left to right based on mRNA prevalence) (b) MAGE-A4 mRNA expression levels (y-axis) in HNSC stratified by HPV-negative vs. HPV-positive status (x-axis) in HNSC.

- MAGE-A4 mRNA prevalence (MAGE-A4 expression detected or not) and expression (normalized RNAseq) levels were overall positively correlated ($R^2=0.64$, $p=0.00012$). However, when ranked by median expression, the top three epithelial tumours with highest MAGE-A4 expression levels (RNA^{HIGH} epithelial group) were: **LUSC, HNSC, and BLCA** (Figure 1a, right y-axis). As a trend, MAGE-A4 expression was enriched in cancers of squamous cell origin: LUSC, HNSC & CESC
- Overall, this data suggests that MAGE-A4 expression is mainly, but not exclusively, associated with cigarette smoking associated cancers (i.e., LUSC, HNSC & BLCA). In HNSC, human papillomavirus status (negative vs. positive) evaluated by p16ARF expression was able to stratify tumour MAGE-A4 expression. MAGE-A4 mRNA levels were higher in HPV-negative (n=59) vs. HPV+positive (n=21) HNSC ($p=0.0025$; Wilcoxon text) (Figure 1b)