Background Immune-checkpoint inhibition (ICI) only benefits a subgroup of patients with head and neck squamous cell carcinoma (HNSCC). Several molecular and cellular components of the tumor microenvironment (TME) have been hypothesized to drive either response or resistance. Here, spatially defined whole transcriptome data were analysed in search of associations of compartment-specific gene-signatures with HNSCC immunotherapy outcomes.

Methods Pre-treatment biopsy samples from 50 immunotherapy-treated recurrent or metastatic HNSCC patients as well as 12 matched post-treatment biopsies obtained after 4 weeks of treatment, constructed in tissue microarray format (YTMA496), were included in the study. The GeoMx Human Whole Transcriptome Atlas (NanoString Technologies) assay was performed on samples to allow RNA quantification of 18,677 protein encoding genes, using in situ hybridization, in three molecularly defined tissue compartments; tumor (CK), leukocyte (CD45), macrophage (CD68). Differentially expressed genes (DEGs) (P< 0.05) between pre- and post-treatment biopsies in each of the tissue compartments were identified. Next, these DEGs were used as a ‘biological’ filter for the initial 18,677 gene set and analysed using LASSO logistic regression models with the aim to obtain pre-treatment gene expression signatures for best overall response (RECIST 1.1.). The performance of each compartment signature was evaluated using the receiver operating characteristic (ROC) curve and AUCs were calculated for Best Overall Response (BOR) to ICI. Genes comprising the highest performing signature were investigated for correlations with immune cell genes extracted from the ‘Single Cell RNA-Seq HNSCC’ (CIBERSORTx) gene matrix.

Results A six-gene signature (DDX4, COL17A1, HBA1, MMP1, GPNMB, TTN) in the CD45 compartment presented the highest AUC (0.83), followed by signatures in the CK and CD68 compartments (AUC: 0.72 and 0.68, respectively). Cross-testing of the CD45 signature in the other two compartments, as well as in a third, artificially generated ‘pseudo-bulk’ compartment (all compartments combined), showed poor performance, indicating spatial specificity. Interestingly, the CD45 signature included three extracellular-matrix protein-encoding genes (MMP1, COL17A1, TTN), all associated with resistance (negative coefficients in the BOR model) to ICI. Fibroblast and dendritic cell populations, characterized using the CIBERSORTx gene matrix, were the immune phenotypes most closely associated with the CD45 signature.

Conclusions Our results indicate that CD45 molecular tissue compartment gene expression demonstrates increased association with ICI resistance in HNSCC. Extracellular matrix genes rather than immune-cell related genes dominated the CD45 compartment signature, highlighting the importance of non-immune stromal components within the TME and the importance of the use of spatial information in the understanding of ICI resistance.

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Ethics Approval This study was approved by Yale Human Investigation IRB protocol ID 9505008219.