Background Immune Checkpoint Inhibitors (ICI) are approved for the treatment of recurrent or metastatic Head and Neck Squamous Cell Carcinomas (HNSCC), but are only effective in a minority of patients. To improve efficacy of ICIs, detailed insight into the HNSCC tumor immune microenvironment (TiME) is required. Previous research in our group showed that high tumor-infiltrating B-lymphocyte (TIL-B) frequencies significantly improved patient survival in oral SCC (OSCC) based on immunohistochemistry (IHC) staining for CD19. Besides, TIL-B infiltration seemed independent of CD8 T cell infiltration. The phenotypes of TIL-B and their function within the TiME of OSCC remain unclear and were the main topic of this research.

Methods NanoString gene expression analysis was performed on 10 TIL-B rich and 10 TIL-B low OSCC, using a panel of 627 immune related genes. Furthermore, multiplexed fluorescent-immunohistochemistry (mFHC) on matched OSCC FFPE material was used to spatially characterize the phenotypes of TIL-Bs and their interaction with other immune subsets in the TiME. Additionally, fresh HNSCC tumor biopsies were interrogated for B cell subpopulations using multi-parameter flow cytometry.

Results Confirming our initial IHC data, CD8 gene signatures were not different between TIL-B rich and TIL-B low samples. TIL-B rich tumors showed significant upregulation of IGHG3, IGHV4–59 and IGHAI RNA expression indicating active immunoglobulin production in the TiME of TIL-B rich OSCC. In addition, increased expression of IRF4 and XBP1 transcription factors was observed, also indicating enhanced plasma cell activation. Gene Set Enrichment Analysis showed upregulation of genes associated with B cell receptor signaling, MHC-II antigen presentation and the complement system, indicating B cell activation, antigen presentation and a potential role of the complement system in the humoral anti-tumor response in OSCC. Preliminary flow cytometry data support the presence of high plasma cell frequencies among the TIL-B population. Using our TIL-B mFHC panel, we spatially confirmed the presence of significantly higher plasma cell numbers in the tumor invasive margin of TIL-B rich OSCC compared to TIL-B low OSCC. Besides, we observed a significant increase in the naïve B-lymphocyte population which colocalized primarily with CD4 T helper cells.

Conclusions Based on the gene expression, flow cytometry and multiplex IHC data, we concluded that the TIL-B rich OSCC contain increased plasma cell populations and increased Immunoglobulin RNA expression. We will next study the functional implications of plasma cell presence and immunoglobulin production in OSCC.

Ethics Approval Written informed consent was obtained from all patients from whom fresh tumor biopsies were used for research, as part of the HNcol protocol at the Department of Otolaryngology | Head and Neck Surgery of Amsterdam UMC (location VUmc) as approved by the Institutional Review Board of the VU medical center (registered with the US Office for Human Research Protection as IRB00002991) (ID: 2008.071|A2016.035). Non-WMO approval was obtained from the Amsterdam UMC (VUmc) IRB for the immune profiling of retrospective HNSCC patient cohorts using FFPE or fresh frozen samples (2021.0511).