Background Adenoid cystic carcinoma (ACC) is a rare and aggressive tumor commonly originating from the salivary glands, accounting for 1% of head and neck cancers. Usually slow growing, ACC spreads through perineural invasion and metastasizes through hematogenous dissemination. The MYB: NFIB gene fusion is the principal oncogenic driver and is found in more than 85% of cases. To date there are no effective systemic targeted therapies or immunotherapies, therefore a deeper understanding of the anti-tumor immune response is essential for finding new treatment options. Thus, we leveraged a novel automated multiplex immunofluorescence (mIF) platform to better characterize the tumor immune landscape.

Methods In a retrospective cohort study, we analyzed 20 ACC cases (16 salivary gland, 2 lung, 2 breast) and 4 squamous cell carcinomas. Multiplex immunofluorescence staining was performed with the COMET™ Platform (Lunaphore Technologies, Switzerland) using a 13-plex antibody panel including FoxP3, CD68, PD1, PDL1, PDL2, pan-cytokeratin, Ki67, R2M, CD45, CD3, CD4, CD8, and CD20. Regions of interest were annotated by a pathologist, and a DAPI nuclei segmentation mask was used to generate nuclei contours and surrounding cell border polygons. Each polygon was classified into either cancer cell, CD20+ B cell, CD3+/CD8+ T cell, CD3+/CD4+ T cells and CD68+ macrophages, prior to subsequent cell-subtype classification.

Results We discovered a complex immune landscape, with most tumors containing tertiary lymphoid structures (TLS), and variable proportions of immune cells relative to total cells (4–30%). All samples displayed a low percentage of cytotoxic T cells ranging between 0–4.5%, with very few tumor-infiltrating lymphocytes (TILs) within tumor glands (<0.1% of total immune cells in all cases). Interestingly, in 6 cases CD68+ macrophages constituted >50% of immune cells. None of the ACCs expressed significant levels of PDL1 or PDL2, and FoxP3 cells were sparse. In on-going analysis, these findings will be correlated with patient outcomes.

Conclusions With a cutting-edge mIF imaging platform and custom computational image analysis, we found ACCs to be ‘cold’ tumors with very few TILs. CD68+ macrophages were common in a subset of cases and may contribute to tumor growth and suppression of the anti-tumor immune response. A lack of PDL1 or PDL1 staining on tumors cells suggests therapeutic approaches other than checkpoint inhibition may be required. The role of TLSs needs further investigation, but it is complicated by the fact that salivary glands have prominent mucosa associated lymphoid tissue, which can be challenging to differentiate from an anti-tumor response.

Ethics Approval This study was approved by Mass General Brigham institution’s Ethics Board; approval number 2022P003132.