Background Cutaneous squamous cell carcinoma (cSCC) is the second most common non-melanoma skin cancer and represents a major global health burden. Approximately 50% of cSCC patients develop primary resistance and 20% will develop secondary resistance to immune checkpoint inhibitors (ICI). There are few biomarkers that reflect the tumor dynamics predictive of response to ICI therapy and we thus need to better understand the cSCC tumor microenvironment (TME).

Methods Our retrospective study profiled tissues from 50 patients at the Princess Alexandra Hospital (Brisbane, AU). Study groups included patients with de novo local cSCC (n=10) and regional nodal metastasis (n=10) who have remained disease free for >2 years. Additional groups included patients with locoregional recurrent disease (n=10), recurrent and/or metastatic disease (n=10) treated with immunotherapy, and immunocompromised de novo or recurrent disease (n=10). In this exploratory study, we designed a high-dimensional >40-plex antibody panel identifying cell lineages, activation states, and checkpoints, as well as markers for cell metabolism, vasculature, and tissue structure. Whole-slide spatial phenotyping was conducted on the PhenoCycler®-Fusion spatial biology system and spatial features, including spatial phenotypic composition, cell-to-cell localization, and cellular neighborhoods were analyzed and compared against clinicopathological findings and response to ICI therapy.

Results We have profiled the TME of cSCC via ultrahigh-plex, single-cell spatial analyses of whole tissues. Our data have revealed unique cell phenotype compositions within the TME of different patient cohorts. Additionally, spatial phenotyping analysis revealed distinct cellular neighborhoods within the tissue cohorts. Taken together, this high-dimensional imaging approach of the cSCC TME has revealed new tissue insights.

Conclusions There is a need to understand the immune contexture of the cSCC immune microenvironment. Here we identify distinct cellular phenotypic compositions and cellular neighbourhoods in tissues from immune-competent and immunocompromised cSCC. We thereby confirm biological differences in tissue composition and cellular interactions between these tissues and provide putative new biomarkers for cSCC patient stratification.

Ethics Approval This study has Metro South Human Research Ethics Committee (HREC) Approval (LNR/2020/QMS/66612) and The University of Queensland ethics ratification.