

IMMUNE LANDSCAPE AT THE INVASION FRONT OF SURGICALLY RESECTED ORAL SQUAMOUS CELL CARCINOMAS SHOWS SIGNIFICANT ASSOCIATIONS WITH DISEASE SPECIFIC SURVIVAL

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Background Oral squamous cell carcinomas (OSCC) prognosis remains poor. While AJCC TNM 8th edition has slightly improved patients' stratification with regard to prognostic, innovative approaches to are still needed. As in other tumor types, tumor immune microenvironment (TiME) might represent an opportunity to improve prognostic assessment.

Methods TiME landscape of 47 HPV-negative OSCC was analyzed using multiplex immunofluorescence (mIF). Markers for tumor cells (PanCK), tumor infiltrating lymphocytes (CD3, CD8), macrophages (CD68), inhibitory (PD-1, PD-L1, TIM3, LAG3, VISTA) or stimulatory (OX40, ICOS) immune checkpoints (ICP) were studied. Regions of interest (ROI), 5 in the tumor core and 5 at the invasion front, were subjected to cell markers identification and quantification (scoring) as well as tissue compartmentalization to divide them in tumor-epithelial and tumor-stroma compartments, respectively. A total of 20 cell phenotypes were defined based on previous work (CK+, CK+PD-L1+, CD3+, CD3+CD8+, CD3+PD-1+, CD3+CD8+PD-1+, CD3+PD-L1+, CD3+CD8+PD-L1+, CD3+PD-L1+PD-1+, CD3+CD8+PD-L1+PD-1+, CD68+, CD68+PD-L1+, CK+OX40+, CD3+VISTA+, CD3+ICOS+, CD3+LAG3+, CD3+OX-40+, CD3+TIM3+). Results were correlated with clinical features including disease-specific survival (DSS) using the Kaplan-Meier method and a multivariate Cox model. A multivariate general linear model (GLM) was built to test the specific association of each variable with a given cell density by correcting the possible confusion due to other variables.

Results Immune cells densities were significantly higher overall in the stroma. The intra-tumor stroma showed a significant enrichment of in CD3+PD-1+ T cells compared to peri-tumor stroma. None of the clinical or pathological (resection margin, tumor stage, lymph node invasion, perineural invasion) was significantly associated with DSS. In contrast, the following cell phenotypes in the tumor invasion front were strongly associated with a poor DSS, including CD3+PD-L1+ (P-value= 0.004), CD3+PD1+PD-L1+ (P-value= 0.02) and CD3+OX40+ (P-value= 0.02) T cells as well as CD3+CD8+PD-1+ (P-value= 0.048), CD3+CD8+PD-L1+ (P-value= 0.008) and CD3+CD8+PD1+PD-L1+ (P-value= 0.01) cytotoxic T cells. In the tumor core, CD68+PD-L1- macrophages (P-value= 0.06) were marginally associated with better DSS. Using a GLM, we found that tumor from smoker-drinker patients and/or with pN+, were significantly more infiltrated by PD-1- and/or PD-L1-positive immune cells. On the other hand, floor of mouth and gingiva-mandibular OSCC were significantly less infiltrated than others.

Conclusions The prognostic value of PD-1+ and/or PD-L1+ cells in the invasion front of resected OSCC was remarkable, underlying the importance of this area when studying the TiME. Incorporating TiME analysis in the invasion front may improve prognostic evaluation of patients treated for OSCC, especially in the context of immunotherapy.

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Ethics Approval The study was conducted in accordance with all applicable laws, rules, and requests of French and European government authorities. Written informed consent was obtained from all patients and the study was approved by the Centre Leon Bérard institutional review board (Lyon, France). Samples were obtained from the CRB Centre Léon Bérard (n°BB-0033-00050) which is quality certified according NFS96-900 French standard and ISO 9001 for clinical trials.

Consent Written informed consent was obtained from all patients and the study was approved by the Centre Leon Bérard institutional review board (Lyon, France)

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