

STUDY OF THE TUMOR MICROENVIRONMENT OF ORAL SQUAMOUS CELL CARCINOMA USING MULTIPLEX IMMUNOFLOUORESCENCE AND IMAGE ANALYSIS APPROACHES

¹Frank Rojas*, ²Jebrane Bouaoud, ¹Edwin Parra, ²Pierre Saintigny, ¹Auriole Tamegnon, ¹Mei Jiang, ³Shanyu Zhang, ¹Pandurengan Renganayaki, ⁴Lucas Michon, ²Nicolas Gadot, ²Sylvie Lantuejoul, ⁴Philippe Zrounba, ²Jean-Philippe Foy, ²Karene Mahtouk, ²Chloe Bertolus. ¹MD Anderson Cancer Center, Houston, TX, USA; ²University Claude Bernard Lyon, Lyon, France; ³MD Anderson Cancer Center, Houston, TX, USA; ⁴Centre Leon Berard, Lyon, France

Background Head and Neck Squamous Cell Carcinoma is the 8th leading cancer worldwide and it is associated with significant morbidity and mortality.¹⁻² Tumor microenvironment (TME) is dynamic and it plays an important role in head and neck carcinogenesis.³⁻⁴ Cytotoxic T-cells, immune checkpoint molecules such as programmed cell death 1 (PD-1), its ligand (PD-L1), and other checkpoints molecules have been described in these tumors.¹⁻³ This study aimed to characterize the TME of oral squamous cell carcinoma (OSCC) and compare with their pathology features.

Methods Four microns thickness consecutive slides from representative OSCC (N=46) cases were stained and analyzed using 11 biomarkers (CK, CD3, CD8, CD68, PD1, PDL1, LAG3, TIM3, ICOS, VISTA, OX40) placed in two multiplex immunofluorescence panels to characterize the TME. For image analysis, the samples were divided in tumor, stroma and peritumoral compartment. Co-expression of markers (cell phenotypes) were analyzed as densities by mm² in each compartment. For PD-L1 expression by malignant cells (CK+PD-L1+) we set up a cutoff of positive case as \geq than 1%. Cell phenotypes were correlated with anatomopathological information retrieved from records such as tumor size, margin status, stage and perineural, lymphovascular, and bone invasion among others. Statistical analyses and plots were performed using SPSS and Graphpad prism8 software packages.

Results We found significant higher cell density for CK+PDL1+ (P= 0.038), CD3+PDL1+ (P= 0.027), CD3+CD8+PDL1+ (P=0.040) in female patients compared with the male population. Interestingly, smaller tumor size (\leq median, 25mm) showed higher densities of CD3+ (P= 0.006), CD3+CD8+ (P= 0.007), CD3+PDL1+ (P= 0.037), CD3+CD8+PDL1+ (P= 0.016), CD3+ICOS+ (P= 0.036), CD3+VISTA+ (P= 0.001), CD68+ (P= 0.001) and CD68+PD-L1+ (P= 0.008) than large tumors. Additionally, high cell density CD3+OX40+ (P= 0.011) was observed in tumors without margin invasion and high cell density for macrophages CD68+ (p= 0.005) in tumors without bone invasion. In ulcerative and infiltrative tumor pattern we observed higher cell density of CD3+PDL1+ (P= 0.020), CD3+CD8+PDL1+ (P=0.006) and CD3+OX40+ (P= 0.022) than non-ulcerate and no infiltrative pattern. Lastly, 58.7% of cases were PDL1+.

Conclusions Our findings of a diminished immune response in larger tumors might be correlated to their potential role in tumor aggressiveness and progression. Furthermore, high cell density of macrophages on tumor bone invasion may suggest an immune suppressive M2 response supported by the presence of PDL1+ expression. All these results can be the first approach for the development of a treatment based of immune interception.

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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.

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