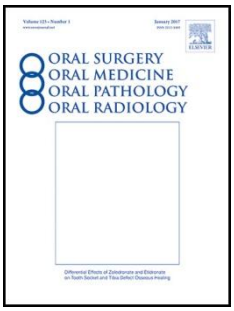
Accepted Manuscript



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PII: S2212-4403(17)31065-9
DOI: https://doi.org/doi:10.1016/j.oooo.2017.09.010
Reference: OOOO 1846

To appear in: *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*

Received date: 20-9-2017
Accepted date: 21-9-2017

Please cite this article as: R. Bryan Bell, Bernard A. Fox, Relationships matter in oral cancer: will single stain immunohistochemistry become irrelevant in the age of multispectral imaging?, *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology* (2017), https://doi.org/doi:10.1016/j.oooo.2017.09.010.

Relationships Matter in Oral Cancer: Will single stain immunohistochemistry become irrelevant in the age of multispectral imaging?

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The presence of low numbers of CD8+ T cells within malignant tumors has been shown in more than 100 peer-reviewed studies to be associated with poorer survival outcomes than in cancer patients who have high numbers of tumor infiltrating CD8+ T cells.[1,2] This is also true for oral squamous cell cancer (OSCC)[3-6] and suggests that patients with low numbers of CD8+ T cells lack an effective anti-cancer immune response.

Several years ago, Jerome Galon and his group began to delineate tumor-infiltrating immune cells using standard immunohistochemistry (IHC) coupled with digital imaging and computer algorithms. Termed "Immunoscore", this method, which qualifies and quantifies immune infiltrates, has proved to be a powerful prognostic biomarker, providing significantly ($p<0.001$) more prognostic power than histological AJCC/UICC TNM staging in patients with colon cancer.[7,8] The Immunoscore (I) quantifies CD8+ T cells and CD3+ T cells within the center of the tumor and the invasive margin of resected tumors to provide a score ranging from 0-4. An Immunoscore of 0 (I0) is found when low densities of both CD8+ and CD3+ T cell types are found in both regions, and an Immunoscore of 4 (I4) is scored when high densities are found in both regions. The "Immunoscore" was applied to 2 large independent cohorts, in which only 4.8% of patients with a high immunoscore (I4) relapsed after 5 years (86.2% 5-year survival) compared to the 72% of patients with low immunoscore (I0 and I1) that experienced tumor recurrence (27.5% 5-year survival).

A large international consortium (14 sites in 13 countries) validated these findings in 1336 colon cancer patients as part of a global effort to develop immunoscore as a prognostic biomarker.[9] The results, which were reported at the 2016 American Society of Clinical Oncology annual meeting, demonstrated that time to recurrence (TTR) was shorter among patients with stage I-III colon cancer who had low immunoscore compared to those with high immunoscore (Cohort 1: HR=0.35; $p<0.0001$; Cohort 2: HR=0.54; $p=0.006$), independent of age, sex, tumor stage or sidedness.[10] In fact, other investigations have shown that the immunoscore remained the only significant criteria over the classical AJCC/UICC TNM-classification for disease-free survival and overall survival, even out performing microsatellite instability.[11] It has thus been suggested that the prevalence of post-surgical immune infiltrates, and not tumor status, is the key indicator for recurrence, metastasis and therefore, clinical outcome.

Recently, our group collaborated with investigators from Halle, Germany to assess the immune infiltrates within a cohort of 119 patients with OSCC and showed that it is not only the number of CD8+ T cells in the tumor that defined immune response and clinical outcome, but *it was the location and relationship of the effector CD8+ T cells to suppressive elements (FoxP3+ or PD-L1+ cells) in tumors that most consistently predicted survival.*[6] We used sophisticated multiplex immunohistochemistry (mIHC) and multispectral imaging techniques to enumerate immune cell types and cartographic coordinates of each cell in formalin-fixed paraffin embedded (FFPE) tissue, to develop a highly significant prognostic biomarker for overall survival ($P<0.0005$) in patients with HPV-negative OSCC who were treated with conventional surgery plus risk adapted adjuvant radiation or chemoradiation.[6] This exploratory biomarker is called the "cumulative suppressive index" (CSI). The CSI scoring system combines the evaluation of FoxP3+ and PD-L1+ cells within 30 µm of CD8+ T cells at both the stromal and tumor side of the invasive margin. While stratifying based on CD8 T cell numbers provided a significant prognostic biomarker, enumeration of tumors for FoxP3+ or PD-L1+ cells (high or low) did not provide a prognostic significance. However, when geography was accounted for, we found a striking correlation between the number of FoxP3+ T cells within 30 microns of CD8 T cells and the number of PD-L1+ cells within 30 microns of CD8 T cells, and patient survival.