



Comprehensive immune profiling in oral cancers

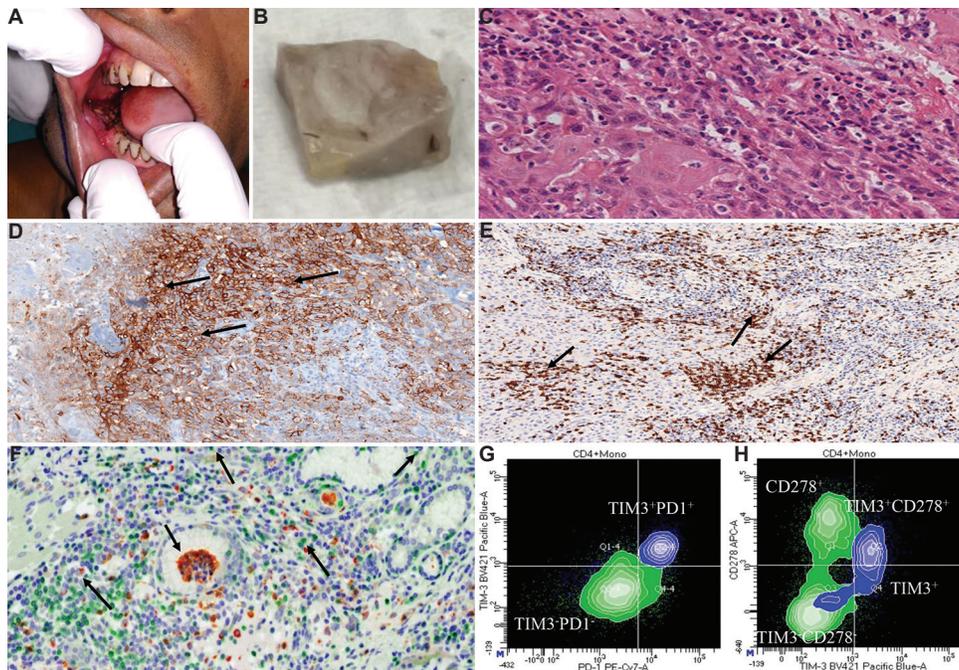


Figure. Immunohistochemistry and flowcytometry images of oral cancer. (A) Irregular growth in lower Gingivobuccal region. (B) Excised tumour from oral cancer patient. (C) Haematoxylin and eosin stain ($\times 40$) showing nucleoli islands, each with moderate to abundant cytoplasm, and hyperchromatic nuclei. (D) Scattered brown DAB staining indicates $CD3^+$ lymphocytes ($\times 20$). (E) Brown $PDL1^+$ ($\times 20$) expressing cells with marginal infiltration. (F) Bright field view ($\times 40$) composite $CD3$, $CD15$, arginase, granzyme, $CD68$, nuclei in green, yellow, orange, magenta, red, blue, respectively. (G) $TIM3^+ PD1^+$ co-expression in blue monocytes and $TIM3^+ PD1^-$ expressed by green lymphocytes. (H) $CD278^+$ in green lymphocytes & $TIM3^+ CD278^+$ coexpression in the monocytic population.

This work was performed at the Laboratory Haematology & Histopathology department, Tata Medical Center, Kolkata, India, from May 2017 to October 2019. Recent advancement in the immunotherapy include characterization of immune infiltrates (such as T cells, Tregs, B cells, NK cells, macrophages and dendritic cells) in tumour microenvironment (TME). In this study, immune cell subsets and checkpoint markers in the TME of oral-gingivobuccal squamous cell carcinoma have been

analyzed by flow cytometry immunophenotyping (FCI) as well as immunohistochemistry (IHC). The uniqueness of this study is to identify, quantify and correlate immune cells ($CD3$, $CD4$ and $CD8$); inducible costimulatory molecule ($CD278$); cytolytic (granzyme) and checkpoint ($TIM-3$ and $PD-1$) molecules with the clinicopathological status of the disease and genomics using the same set of tumour samples. The novelty of this study is biomarker identification by FCI and detection of

Informed consent from patients with oral gingivobuccal squamous cell carcinoma procured for sample collection.

immune prognostication scores by IHC to address novel immunotherapeutic targets. The IHC images in Figure A-D depict a representative case of oral gingivobuccal tumour with increased lymphoid infiltrate. These cells show high programmed death ligand-1 (PDL1) expression (Figure E). The FCI image (Figure G and H) shows increased CD3+ lymphocytes with expression of T cell immunoglobulin and mucin domain-containing-3 (TIM-3), PD-1 and CD278. Multiplex image (Figure F) also shows high expression of CD3, CD68, CD15 and granzyme. Evaluating TME will enhance our knowledge of oral cancer in management of patients for a better disease-free survival.

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Conflicts of Interest: None.

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