

Abstract

Background: Leukoplakia is an oral disorder present in 1% of the population that holds malignant potential. Two distinct clinical subtypes exist: localized leukoplakia (LL) and proliferative leukoplakia (PL). LL is normally at a single site and is typically found in men, smokers, and has a low malignant potential (15%). Proliferative leukoplakia is a more aggressive form characterized by large or multifocal lesions, typically found in non-smokers, women, with a high malignant transformation rate (with 90% of patients developing cancer in 10 years). Unlike LL, which is addressed with ablation or excision, PL is difficult to treat because of its multifocality. Treatment options for PL can include CO2 lasering, scalpel surgery, photodynamic therapy, and various topical agents; but none have proven efficacious and recurrence rates remain quite high. Immune checkpoint inhibitors targeting PD-1 are now FDA approved for the treatment of advanced oral squamous cell carcinoma, yielding both durable disease control and improved survival in a subset of patients. Of interest, recent data suggests that nearly 50% of high-grade oral leukoplakia samples are PD-L1 (the ligand of PD-1) positive. Thus, immune checkpoint inhibitors may serve as a useful strategy for treating high-risk oral precancerous lesions.

Clinical findings: We aim to determine expression patterns of PD-1 and PD-L1 in LL, PL, and oral squamous carcinoma (OSCC) which may help predict PD-1 inhibitor efficacy earlier in the natural history of oral malignant transformation. Chen XJ et al. has shown that PD-L1 protein is detectable in 97.6% of OSCC, 61.9% of LL, and often absent in normal oral mucosa; and that there was no significant difference in expression between lesions with and without epithelial dysplasia. Another study by Kouketsu et al. similarly determined that PD-1 and PD-L1 expression was greater in OSCC compared to precursor lesions, with little difference between lesions with varying degrees (high, medium, and low) of dysplasia. To our best of knowledge, PD-1 and PD-L1 inhibitors are not yet being utilized as a treatment for high-risk oral dysplasia such as PL. By quantifying PD-1 and PD-L1 expression in various oral pre-malignant lesions, we can better understand the role of immune checkpoint receptor:ligand interactions in this disease.

Conclusion: PD-1 and PD-L1 expression is increased in OSCC lesions and in oral precancerous lesions, thus we anticipate that immune checkpoint blockade with PD-1 and PD-L1 inhibitors may hold therapeutic potential earlier in the natural history of oral carcinoma. Further research is needed to determine if precancerous lesions such as LL and high-risk PL would benefit from immunotherapy to mitigate progression to invasive OSCC.

(**Abbreviations:** HD, severe dysplasia or carcinoma in situ; HK, hyperkeratosis or hyperplasia; LD, mild to moderate dysplasia)

Immune Checkpoint Inhibitors (ICIs)

- Immune checkpoints are naturally occurring processes in our bodies that promote self-tolerance in order to prevent the body's immune system from attacking itself.
- Inhibitory pathways such as the CTLA-4 : B7 and PD-1 : PD-L1 interactions prevent immune cells from destroying themselves.
- Certain cancers have the propensity to upregulate PD-L1 in order to prevent an immune response from being initiated, thus are able to survive and multiply.
- FDA approved ICIs

- Ipilimumab** targets cytotoxic T-lymphocyte antigen-4 (CTLA-4),
- Pembrolizumab** and **nivolumab** target programmed death-1 (PD-1)

- Anti- PD-L1, anti-PD-1, and anti-CTLA-4 monoclonal antibodies have been FDA approved for treatment of certain cancers such as breast, bladder, cervical, colon, head and neck, Hodgkin's lymphoma, liver, lung, renal, melanoma, stomach, and rectal cancers.
- Challenges of ICI's: Assessing which patients are going to benefit from this therapy. Only a fraction of patients benefit because of the complex tumor microenvironment.
 - There are 3 major subtypes based on the infiltration of immune cells.
 - Immune desert: little to no T cells suitable for priming or activation
 - Immune excluded: has several chemokines, vascular factors, and stromal inhibition, but immune cells cannot infiltrate the environment
 - Immune inflamed: presence of multiple immune cells
 - From a few clinical findings, it has been seen that PD-L1 and PD-1 expression is increased in precancerous lesions as well as oral squamous cell carcinoma
 - Next steps:
 - Quantifying the amount of PD-L1 and PD-1 in oral precancerous lesions of LL and PL.

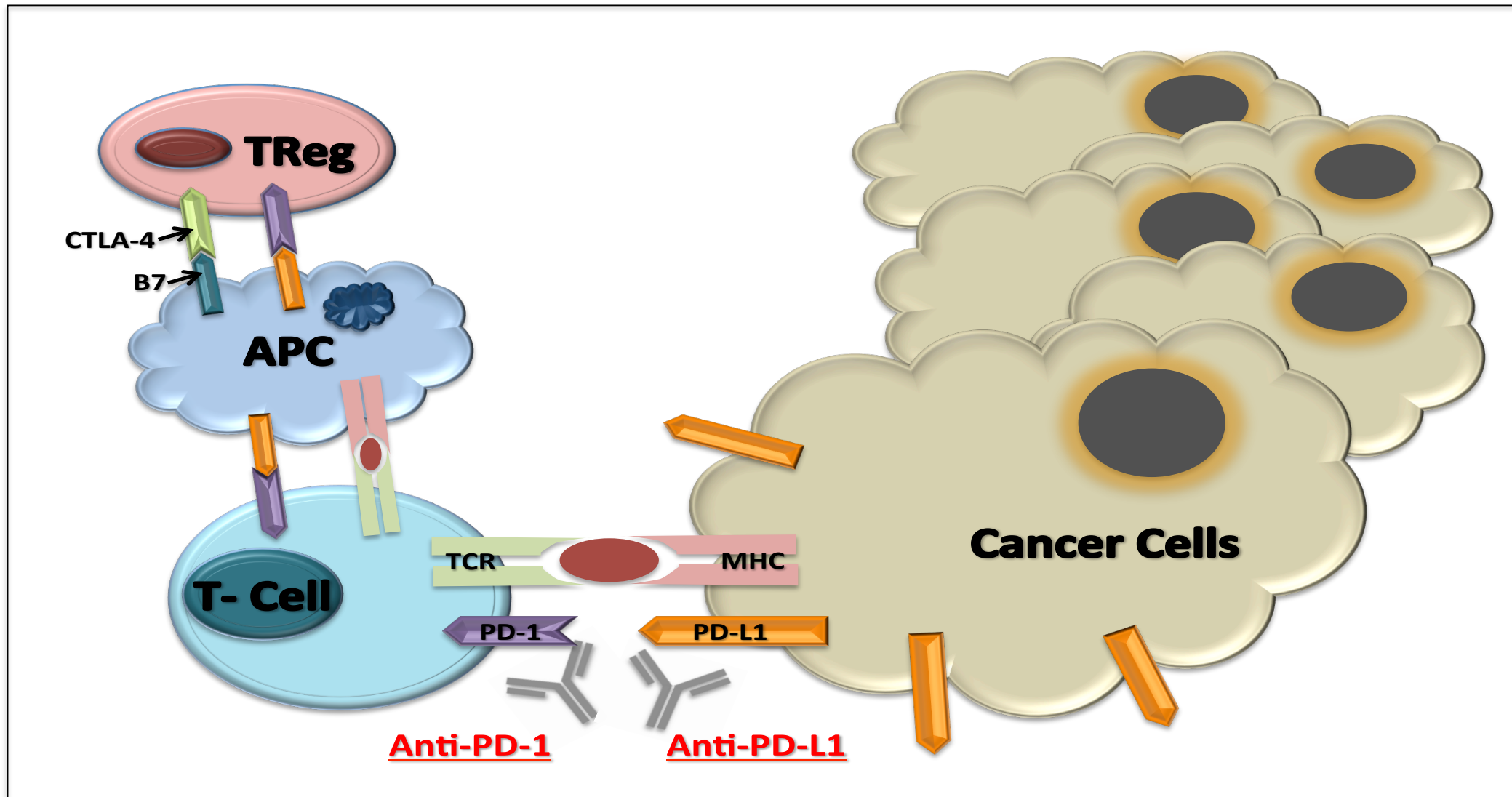


Figure 1): Cartoon representation of PD-1 and PD-L1 interaction.

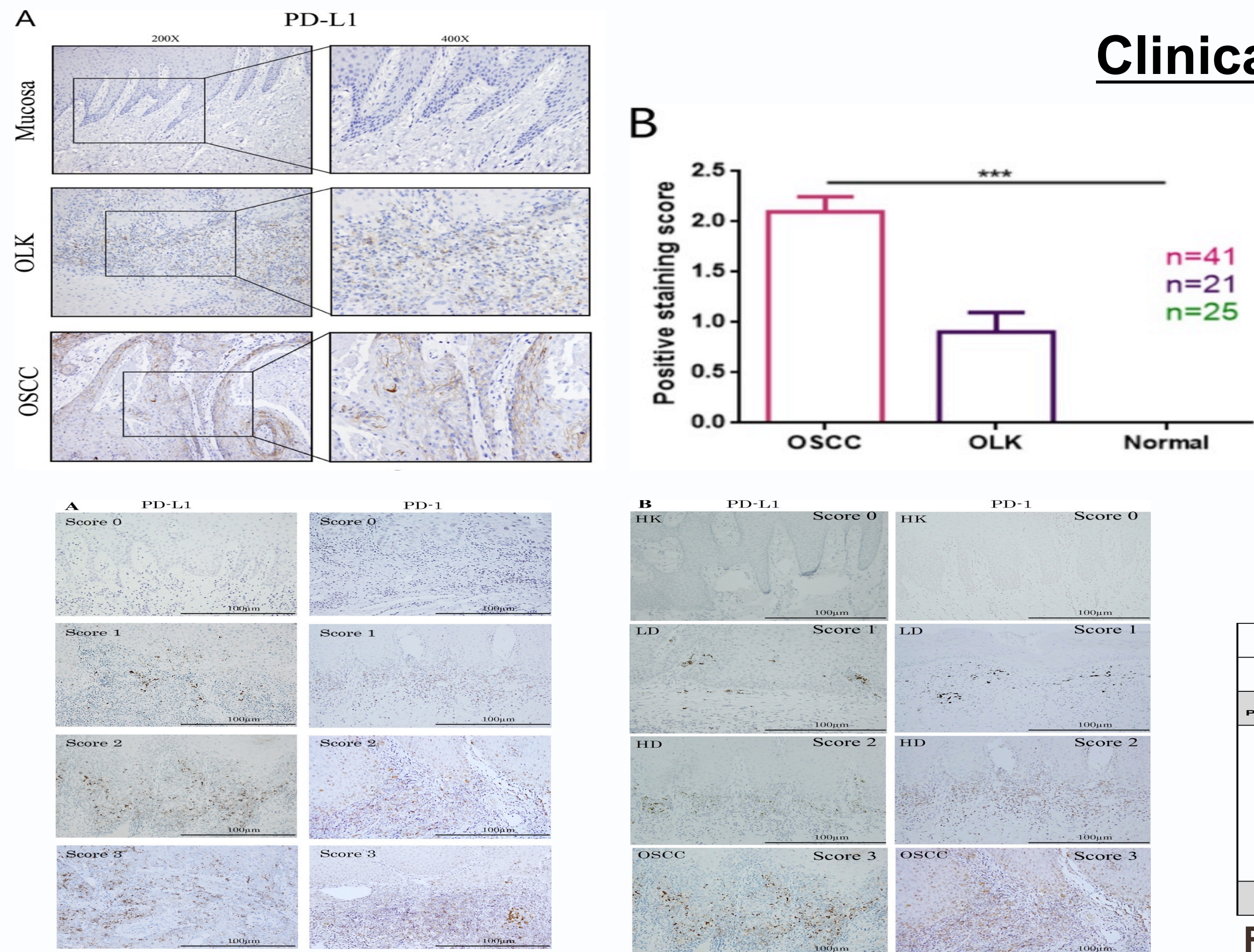


Figure 3): Kouketsu et al. **A)** Scoring of IHC reactivity for PD-L1 and PD-1.

B) Scored images of PD-L1 and PD-1 IHC in OEPL and OSCC.

Clinical Findings

Figure 2): Chen XJ et al. The expression of PD-L1 in OSCC, OLK patients and normal controls.

(A) The expression of PD-L1 in OSCC, OLK (oral leukoplakia) and normal tissue samples were detected by immunohistochemistry. Magnification: 200× (left), 400× (right).

(B) Semi-quantitative analysis of PD-L1 expression in OSCC, OLK patients and controls using positively stained score as follows: 0, no staining; 1, ≤30%; 2, 31–60%; and 3, 61–100%. The score was significantly increased in OSCC and OLK compared with control group (Mann-Whitney U test, P < 0.0001).

	Number of cases	PD-L1					PD-1				
		Score 0	Score 1	Score 2	Score 3	P value	Score 0	Score 1	Score 2	Score 3	P value
Oral Epithelial Precancerous Lesions	79	58 (73.4%)	4 (5.1%)	14 (17.7%)	3 (3.8%)	0.001	56 (70.8%)	11 (13.9%)	10 (12.7%)	2 (2.5%)	0.001
Leukoplakia without epithelial dysplasia (Hyperkeratosis or hyperplasia)	24	19 (79.2%)	2 (8.3%)	2 (8.3%)	1 (4.2%)	0.001	18 (75%)	2 (8.3%)	4 (16.7%)	0 (0%)	0.001
Low-grade epithelial dysplasia (Mild to moderate dysplasia)	31	21 (67.7%)	2 (6.5%)	6 (19.4%)	2 (6.5%)	0.001	20 (64.5%)	6 (19.4%)	3 (9.7%)	2 (6.5%)	0.001
High-grade epithelial dysplasia (Severe dysplasia or CIS)	24	18 (75.0%)	0 (0%)	6 (25.0%)	0 (0%)	0.001	19 (79.2%)	3 (12.5%)	3 (12.5%)	0 (0%)	0.001
Oral Squamous Cell Carcinoma	105	34 (32.4%)	11 (10.4%)	43 (40.5%)	18 (17.0%)	0.001	33 (31.1%)	9 (8.5%)	50 (47.2%)	14 (13.2%)	0.001

Figure 4) Kouketsu et al. Immunohistochemical reactivity for PD-L1 and PD-1 in OEPL and OSCC.

Clinical Photos



Figure 5) Localized Leukoplakia of right ventral tongue.



Figure 6) Proliferative Leukoplakia of left palate and bilateral dorsal tongue.



Figure 7) Proliferative Leukoplakia of right dorsal tongue and left dorsal tongue.

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Contact

If you have any questions please email me at:
nikhil_mistry@hsdm.harvard.edu