

Title: Expression of PD-L1 in relation to human papillomavirus (HPV) and p16 $_{INK4a}$ protein in primary and metastatic squamous cell carcinomas of the head and neck (SCCHN).

Background : It is known that HPV plays an important role in the etiology of a subset of SCCHN. The degree of PD-L1 expression has been reported to be increased in those patients with HPV-positive disease. Recent clinical studies having shown efficacy of various therapies targeting the PD-1/PD-L1 pathway for patients diagnosed with SCCHN tumors. The determination of primary and/or metastatic tumors having HPV origins could be used in the identification of patients who may benefit from these more targeted treatment modalities.
Methods : Immunohistochemistry (IHC) and In situ Hybridization (ISH) assays on the VENTANA BenchMark ULTRA instrument were used to determine the HPV and P16 $_{INK4a}$ status as well as the PD-L1 expression level in 30 cases of SCCHN, with matching primary and metastatic resections. The VENTANA PD-L1 (SP263) Assay was used to determine tumor cell expression of PD-L1. HPV status of the tissues was determined using the INFORM HPV III Family 16 Probe (B) product while the detection of the p16 $_{INK4a}$ protein was completed using the CINtec p16 Histology assay.
Results: Evaluation of samples from the primary as well as the metastatic resections from each of the 30 cases (60 samples total) for percent expression of PD-L1 in tumor cells, HPV status and p16 $_{INK4a}$ status was completed. The PD-L1 expression level for this sample set ranged from 0% to 75%. Of the 30 cases represented 17 cases show indication of HPV infection. The resulting data were analyzed using a t-test of the log of the PD-L1 Expression Level (natural log scale) and was found to be marginally and positively associated with evidence of HPV infection (Diff. of means = 1.363: 95%Cl:[-0.176;2.902]; p-value=0.077). However, a larger sample set would be needed to validate these findings.
Conclusion: While the results from this small contingent are far from conclusive, the initial results show that a diagnostic approach including these three assays may lead to a better understanding of the tumor origin and thus aid in the determination of treatment options for SCCHN patients.

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