Comparison of HPV E6,E7 mRNA and p16 Expression in Oral Cancer Samples Using Flow Cytometry and Immunohistochemistry

Yvette Carrasco1, Amanda Chargin1, Haitham Mirghani2, Bruce K.Patterson1
1IncellDx, Inc., Menlo Park, CA  2Institut Gustave Roussy, Villejuif, France
yvettec@incelldx.com

Although alcohol and tobacco have been considered the most prominent precursor to head and neck squamous cell carcinoma (HNSSC), there is an established link between HPV infection and a subset of patients with HNSSC. Better patient prognosis has been shown in patients with HPV associated HNSSC as well as response to PD-L1 therapy. We observed HPV E6,E7 mRNA overexpression by flow cytometry, to see if it correlated with p16 status. First, HPV infection was established by standard histology p16 test as a surrogate of E7 activity with positive p16 samples confirmed HPV positive by using INFORM HPV Probes ISH. Secondly, E6,E7 mRNA overexpression was quantified by flow cytometry as a marker of transcriptionally active and integrated virus. Additionally, we looked at the PD-L1 surface expression by fluorescent labeled antibody to PD-L1 in these tumors to see if it correlated with HPV mRNA overexpression.

RESULTS

30 patient samples* were analyzed by the E6,E7 mRNA/PD-L1 assay with flow cytometry as well as by p16 IHC/ HPV gDNA-ISH. Concordance between the two assays was 73.3% overall. All 8 non-concordant results were in HPV/pos calls by IHC/ISH.

PATIENT CHARACTERISTICS

Patient 43 - HPVpos : Positive by both p16 IHC/ISH and HPV mRNA Flow Cytometry

- Sex: Male
- Age: 71 yrs
- Smoker: Non-Smoker
- Treatment: Radiation Therapy
- Tumor Differentiation: Poor
- Location: Amygdale
- TMN Staging: T2N0

Patient 49 - HPVpos by p16/ISH : Positive by p16 IHC/ISH and Negative by HPV mRNA Flow Cytometry

- Sex: Male
- Age: 63 yrs
- Smoker: 45 packs annual
- Treatment: Chemoradiation Therapy
- Tumor Differentiation: Poor
- Location: Tonsil
- TMN Staging: T3N1

Patient 3 - HPVneg : Negative both p16 IHC/ISH and by HPV mRNA Flow Cytometry

- Sex: Female
- Age: 53 yrs
- Smoker: 40 packs annual
- Treatment: Chemoradiation Therapy
- Tumor Differentiation: Poor
- Location: Amygdale
- TMN Staging: T2N1

Flow Cytometry performed on Beckman Coulter CytoFLEX. The CytoFLEX is For Research Use Only. Not For Clinical Use.

CONCLUSION

This unique assay provides quantification of HPV E6, E7 mRNA and PD-L1 on a single cell level. This could have profound effect on the ability of clinicians to decide treatment options for patients regardless of disease progression. Clinicians could base decisions on objective, rather than subjective assay results whether at the screening or re-occurrence levels of disease.

Swabs taken from patients with lesions of the oral pharynx at the Institut Gustave Roussy were placed into a vial with a proprietary fixation solution and shipped overnight for processing. Upon receipt, samples were strained through a 35 μM filter. Collected cells underwent in situ hybridization with E6,E7 mRNA probe, staining with PD-L1 Ab (28-8), and a cell cycle dye used to verify single nucleated cells. Once processed, samples were analyzed by flow cytometry. Biopsy tissue was taken from the same swabbed region and processed into a FFPE block. P16 and ISH testing was performed on the FFPE slides at Institut Gustave Roussy.

30 patient samples* were analyzed by the E6,E7 mRNA/PD-L1 assay with flow cytometry as well as by p16 IHC/ HPV gDNA-ISH. Concordance between the two assays was 73.3% overall. All 8 non-concordant results were in HPV/pos calls by IHC/ISH.

PD-L1 expression was observed only in HPV negative samples by E6,E7 Flow Cytometry.

*Additional data added following abstract submission.