

Abstract

New York esophageal squamous cell carcinoma 1 (NY-ESO-1) is a member of the cancer testis antigen family and is an effective target for cancer immunotherapy. NY-ESO-1 is expressed in various types of tumors but not in healthy normal tissue outside of testis and ovary. The NY-ESO-1 cancer testis antigen has been identified as a biomarker in oral squamous cell carcinoma (OSCC) patients and can induce both specific NY-ESO-1 antibody and T cell responses. Its high tumor-specificity and immunogenicity make NY-ESO-1 an attractive target for specific immunotherapy of OSCC. To evaluate its potential as a diagnostic marker and therapeutic target in oral cancer development, we determined NY-ESO-1 expression across the range of pathologically distinct, progressive stages of oral cancer. Preserved samples from patients with oral dysplasia or cancer (N=262) were histologically examined and categorized into normal, non-dysplastic, mild, moderate, or severe dysplasia and carcinoma. Standard Immunohistochemical procedures were carried out followed by expression analysis using the H-scoring method. Statistical analysis of these new data will identify differential NY-ESO-1 expression during oral carcinogenesis and progression. The patient cohorts have been classified into low-risk and high-risk groups based on pathology which were analyzed for association with NY-ESO-1 expression. The high-risk group, severe dysplasia & carcinoma, showed increased NY-ESO-1 expression. To determine whether NY-ESO-1 expression is associated with patient outcomes, survival analysis will be stratified by NY-ESO-1 level. These same patient sample cohorts have been characterized for T cell infiltration (CD4, CD25, FoxP3 and CD8) and immune checkpoint expression (PD-1 and PD-L1). We will add NY-ESO-1 to our analyses to identify correlations between its level and tumor immune infiltration and activation status. These analyses will enhance our understanding of the predictive value of NY-ESO-1 expression in the oral cavity tumor microenvironment. Extension of this study to patient samples pre and post immune checkpoint blockade may address the native role of NY-ESO-1 in the immune response to oral cavity cancer. This study provides new data to support exploration of combination therapy using NY-ESO-1 specific immunotherapeutic interventions with checkpoint inhibition and other candidates to unleash the immune response against OSCC.

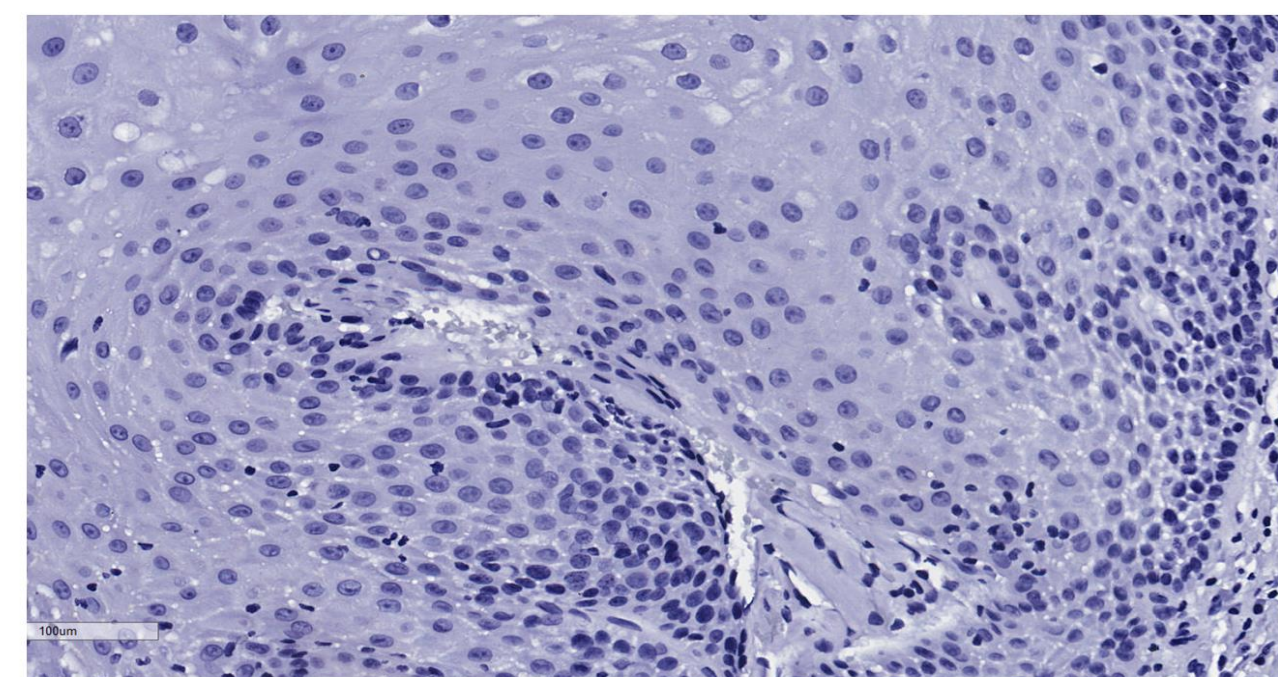
Introduction

New York esophageal squamous cell carcinoma 1 (NY-ESO-1) is a member of the cancer testis antigen family and is an effective target for cancer immunotherapy. NY-ESO-1 is expressed in various types of tumors but not in healthy normal tissue outside of testis and ovary. The NY-ESO-1 cancer testis antigen has been identified as a biomarker in oral squamous cell carcinoma (OSCC) patients and can induce both specific NY-ESO-1 antibody and T cell responses. Its high tumor-specificity and immunogenicity make NY-ESO-1 an attractive target for specific immunotherapy of OSCC. To evaluate its potential as a diagnostic marker and therapeutic target in oral cancer development, we determined NY-ESO-1 expression across the range of pathologically distinct, progressive stages of oral cancer.

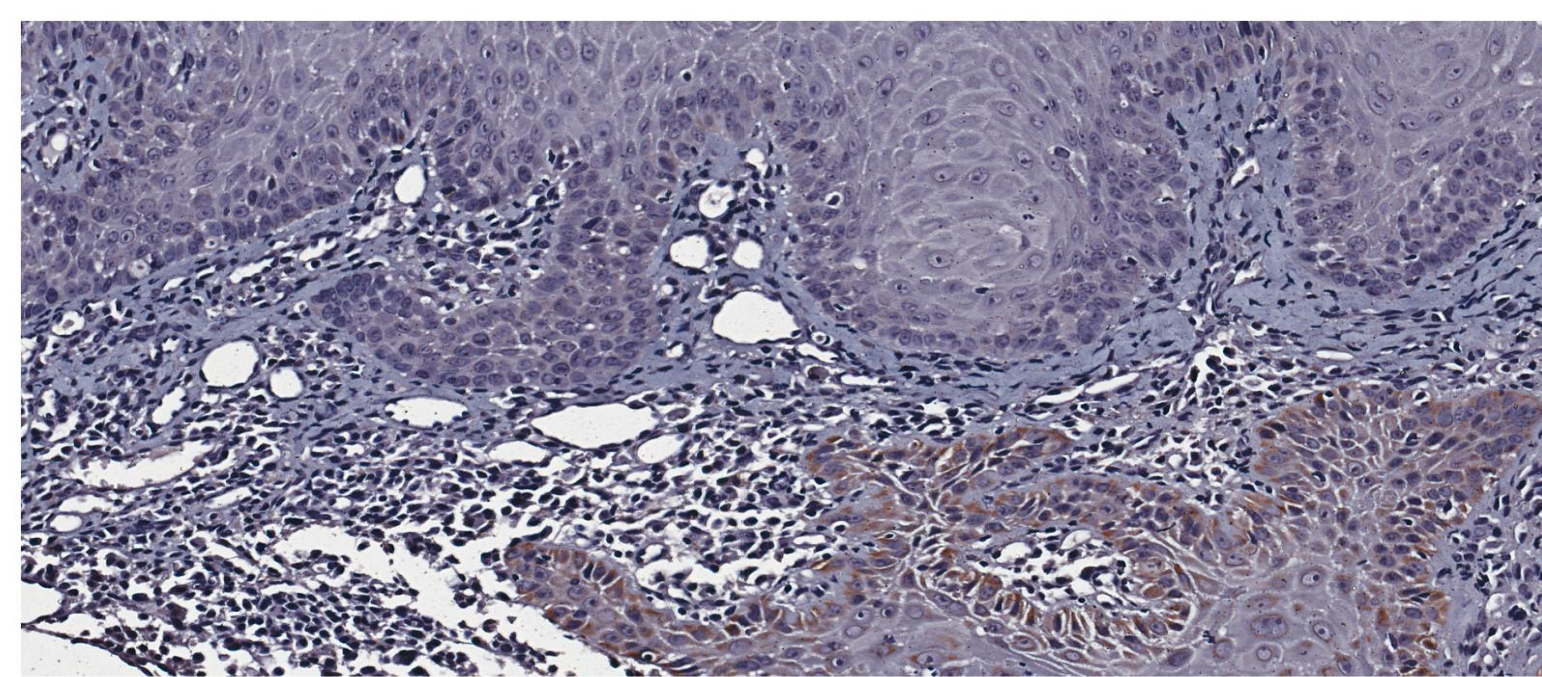
Methods

This study used preserved samples from patients who had been referred to the head and neck surgery department at Roswell Park Cancer Institute, Buffalo with Oral Potentially Malignant Disorders (OPMD) or cancer (N=287) and were histologically examined and categorized into normal, non-dysplastic, mild, moderate, severe dysplasia and carcinoma. Standard Immunohistochemical procedures were carried out to find out the expression of NYESO1. Briefly, slides were deparaffinized in xylene, rehydrated and incubated in 0.3% methanol/H₂O₂ at room temperature for 15 min to block the endogenous peroxidase activity. Antigen retrieval was performed by keeping 20 min on steamer in citrate antigen buffer, followed by the addition of the primary NY-ESO-1 (E978) mouse monoclonal antibody raised against human origin (Santa Cruz Biotechnology, 1:100) and overnight incubation in a moist chamber at 4 °C temperature. Next, slides were incubated with Mouse and Rabbit Specific HRP/DAB (ABC) Detection system, visualized with DAB Substrate Chromogen System (Abcam, USA) and counterstained with hematoxylin. Digital images of IHC stained sections were acquired using an Aperio ScanScope slide scanner (Leica Biosystems, USA) at an initial magnification of 20x. A digital image of each section is stored in the database for further evaluations. Two independent observers blind to the pathology of the samples evaluated these scanned images using Leica Imagescope software at a total magnification of 400x. H-score method performed for the NY-ESO-1 expression analysis where the H-score is determined by adding the results of multiplication of the percentage of cells with staining intensity ordinal value (scored from 0 for “no signal” to 3 for “strong signal”) with 300 possible values. In this system, <1% positive cells is considered to be a negative result. All statistical analyses were performed using the SPSS v25 statistical software. Statistical testing included the Spearman correlation and ANOVA. Statistical significance was set at p<0.05 for all tests.

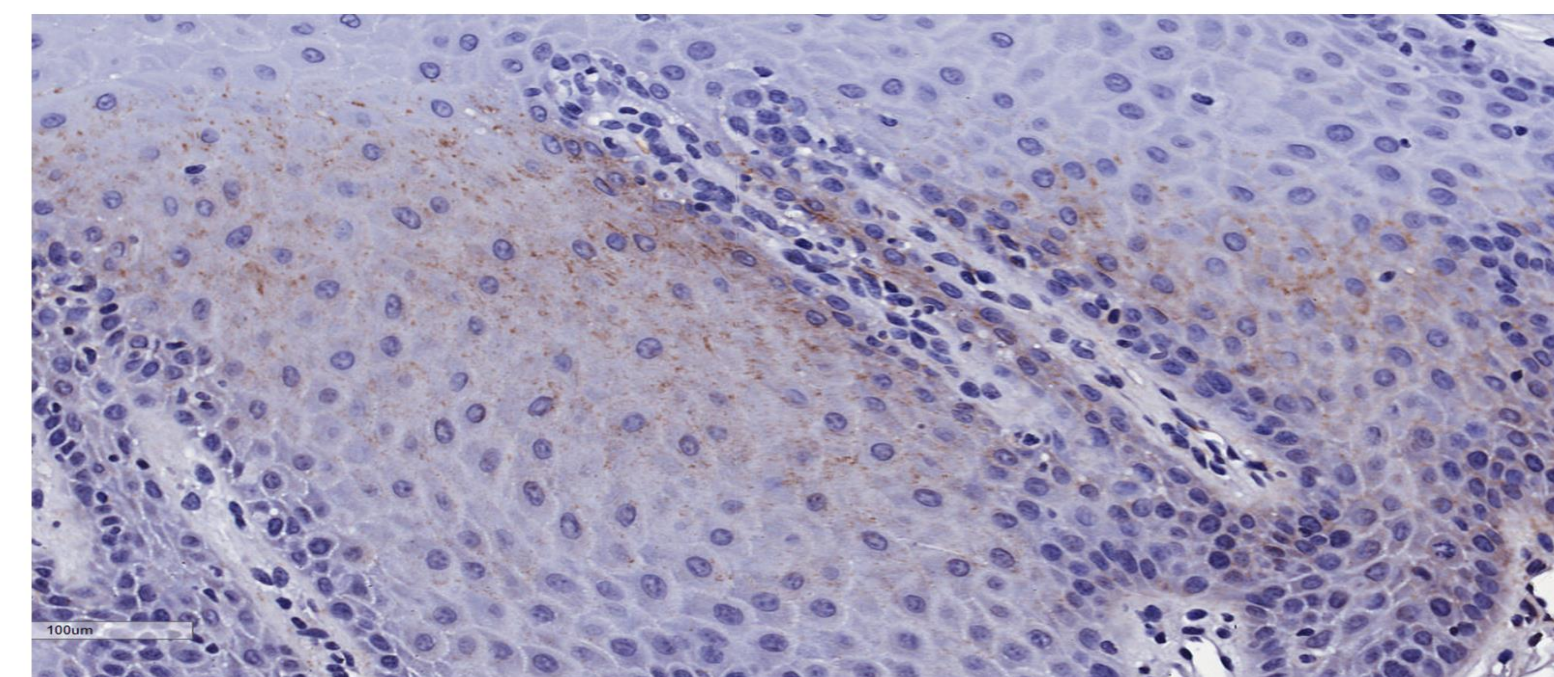
Results



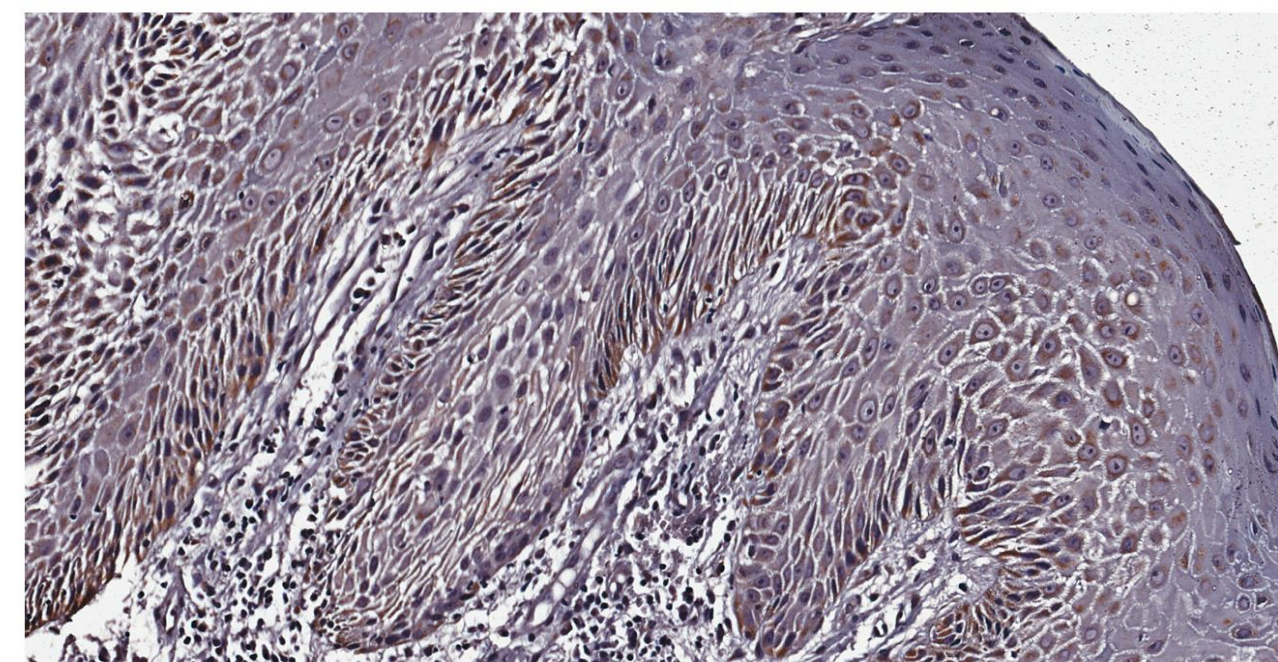
Normal



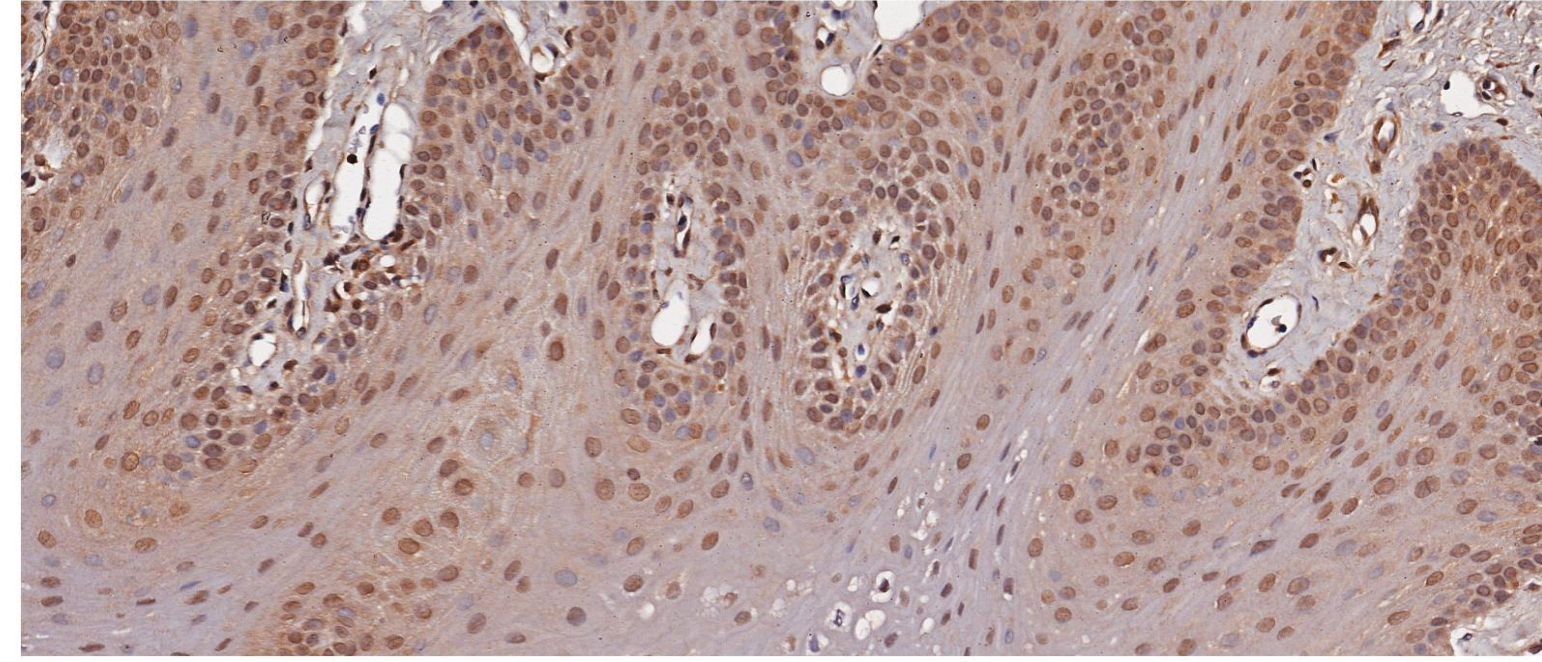
Non Dysplastic



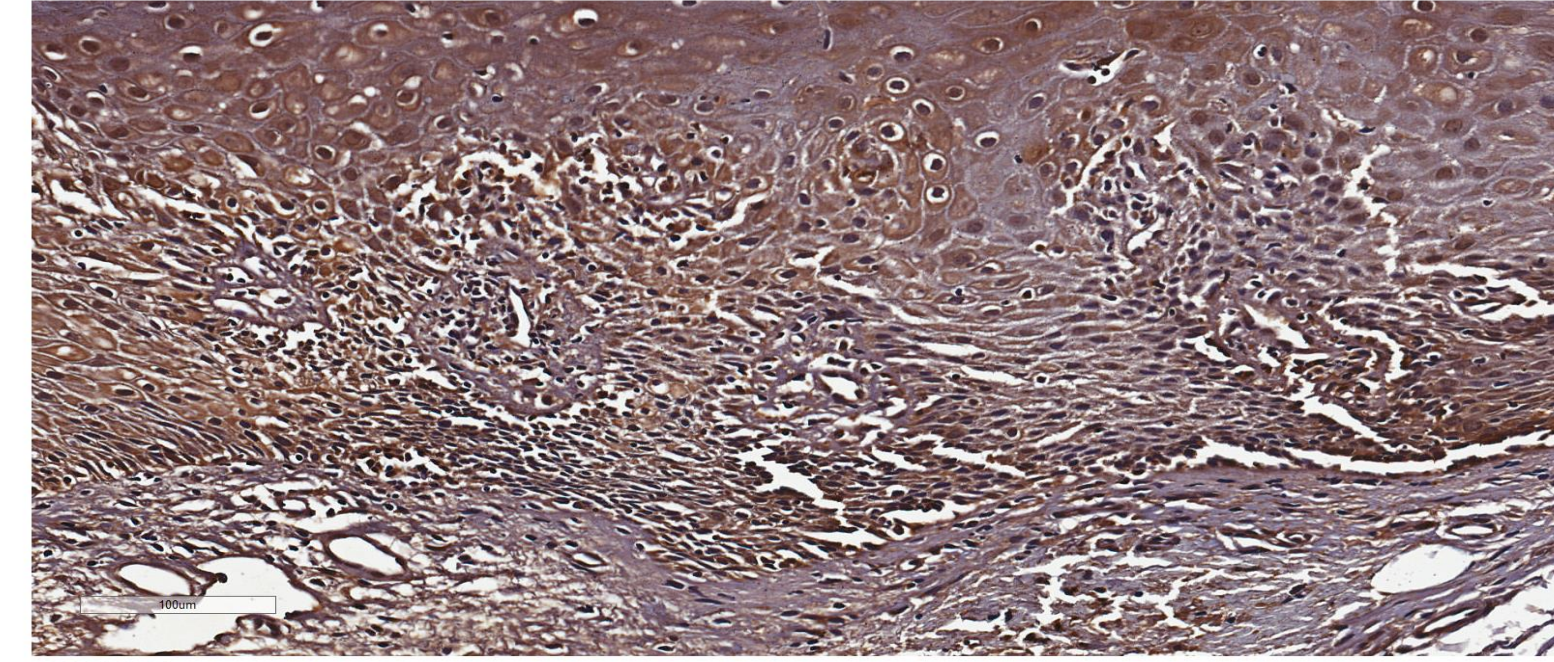
Mild Dysplastic



Moderate Dysplastic



Severe Dysplastic



OSCC

Figure 1: Distribution of NY-ESO-1 staining demonstrating the differential expression in various OPMD and Carcinoma.

- We investigated NY-ESO-1 expression in oral potentially malignant disorders (OPMD) and malignant sections (287 sections).
- Patient samples were categorized as normal, non-dysplastic, mild dysplastic, severe dysplastic and carcinoma based on the histopathology. **Staining for NY-ESO-1 increased as dysplasia increased (Figure 1).**
- **Along the dysplasia spectrum, NY-ESO-1 expression exhibited a positive correlation with OSCC progression (p<0.001).** The mean value of NY ESO1 expression in high-risk dysplasia (moderate and severe) and carcinoma were high (62.14±11.4, 63.17±10.47 and 52.32±12.36 respectively) compared to the normal, non-dysplastic and mild dysplastic patients whereas the expressions were 38±5.23, 25.6±6.56 and 35±6.5 respectively (**Figure 2**).

Results

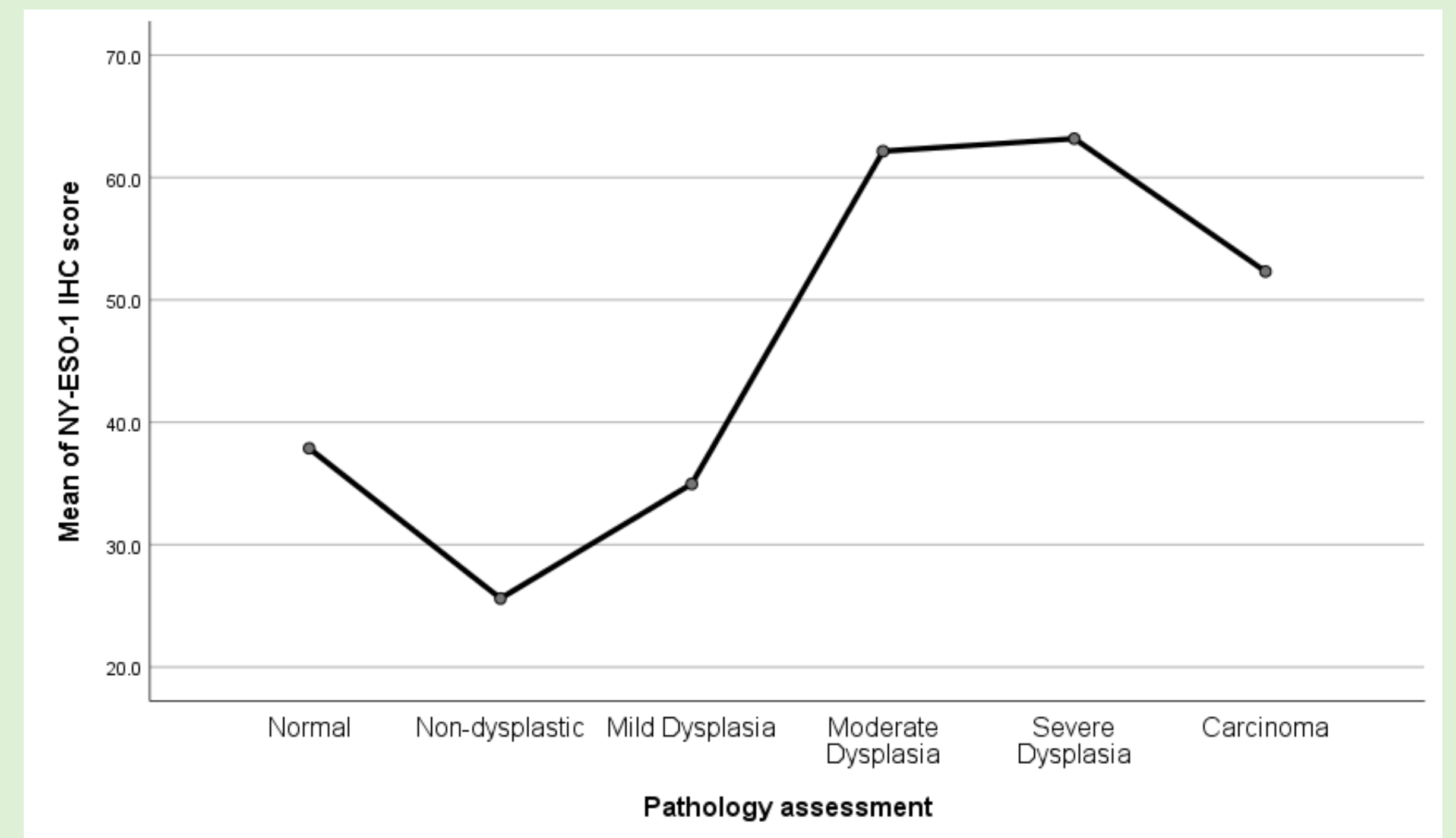


Figure 2: NY-ESO-1 mean IHC values in various types of OPMD and cancers.

Kruskal-Wallis testing indicated a strong association between NY-ESO-1 intensity and dysplastic progression (p<0.001).

Conclusion

- The complex, dynamic tumor microenvironment can thwart the effectiveness of immunotherapy in solid tumors; as a result, there are many efforts to identify tumor-specific antigens
- Several cancer testis antigens have been found to elicit a spontaneous immune response, the most immunogenic of them being NY-ESO-1
- In this study, we found that NY-ESO-1 expression was significantly related to histological grading in OPMD and OSCC, suggesting that it could serve as a prognostic factor as well as a target for advanced treatment therapies.
- We believe that this study is the first to test the differential expression of this specific antigen in OPMD samples and oral cancer samples
- As a result of their unique expression patterns, cancer testis antigens like NY-ESO-1 can serve as potentially useful markers for diagnosis and targets for immunotherapy