

Efficacy of Different Clones of p16 in Comparison to HPV DNA PCR in Head and Neck Cancer: A Review

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ABSTRACT

Background: Human papillomavirus (HPV), particularly HPV type 16, is becoming more prevalent as a cause of squamous cell carcinomas of the head and neck, particularly in the oropharyngeal region. HPV-positive cancers are distinguished from HPV-negative cancers by their molecular mechanisms, response to treatment, and prognosis. Epigenetic alterations, such as dysregulation of miRNAs, play an important role in the development of oral cancers and may also be useful for diagnostic purposes. The protein p16 is an established biomarker of HPV infection, the prognostic role of RB1-associated p16 overexpression remains unclear.

Aim: How does the efficacy of different p16 antibody clones compare to HPV DNA PCR testing in detecting HPV-associated head and neck cancers.

Result: The use of p16 immunohistochemistry (IHC) as a surrogate marker for HPV-related head and neck squamous cell carcinoma (HNSCC) is effective, although the accuracy of p16 IHC alone can be influenced by factors such as the specific clone of antibody used for immunostaining, tumor location, and the use of additional tests. The E6H4 clone was shown to have the highest reliability with sensitivities between 89.2% and specificities as high as 77.4%, while the performance of other clones was much more variable. Overall, when p16 IHC is used in combination with HPV DNA/RNA based assay testing, there is significantly improved specificity and prognostic accuracy; therefore, the use of an integrated multimodal approach for diagnosis rather than using p16 expression alone is recommended.

Conclusion: p16 IHC, especially with the E6H4 clone, is a useful surrogate marker for HPV-driven OPSCC, but its reliability decreases in non-oropharyngeal sites. Combining p16 with HPV-specific tests remains the most accurate strategy and should guide future standardized diagnostic approaches.

Keywords: Human Papillomavirus (HPV), p16 Immunohistochemistry, HPV DNA PCR, Head and Neck Squamous Cell Carcinoma, Oropharyngeal Squamous Cell Carcinoma, p16 Antibody Clones

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Introduction

HPV-associated squamous cell carcinoma of the head and neck (HNSCC) represents a significant public health problem in the developed world, especially in relation to oropharyngeal (OP) cancers arising from the tonsils or base of the tongue [1]. HPV infection is the cause of almost all cases of HPV-related OP cancers, with HPV16 responsible for the large majority of cases (>80%). In comparison to HPV-unrelated oropharyngeal cancer (OPC) subjects, HPV-related malignancy has better treatment responses and a better prognosis [1]. HPV-associated and HPV-related tumors have several molecular and clinicopathological differences [2]. Patients with oral cancer has a 50% survival rate five years post-treatment and is a constant global problem [3]. Changes in the epigenome (by changing the expression of miRNA, modifying the DNA methylation, and changing the

histone) are believed to be a key factor in the occasional development of oral cancer [3]. The microRNAs (miRNAs) are

small non-coding RNAs that serve an essential role in the process of developing oral cancer by regulating the signaling pathways modified by carcinogenic compounds [3]. The identification of abnormal miRNA expression in oral cancer has initiated interest in using these molecules as reputed markers of prognosis, early diagnosis, and novel therapeutic approaches [4] (**Figure 1**).

P16 immunohistochemistry is more widely available, has a high degree of interobserver reliability, less costly and technically easier to perform than HPV-specific tests (for example, in situ hybridisation and reverse-transcriptase polymerase chain reaction) [5]. P16 immunohistochemistry has been recognised as the only predictive test for oropharyngeal squamous cell carcinoma. There is consensus in relation to its interpretation as

well as independent prognostic significance [6]. The loss of the retinoblastoma 1 gene (RB1) may also lead to an increase in the expression of the P16 protein, even in the absence of HPV infection, when using P16 immunohistochemical staining as an indicator of HPV status in patients with oropharyngeal squamous cell carcinoma (OSCC) [7]. The potential prognostic implications of RB1 mutations as additional prognostic indicators in OSCC remain to be explored [7]. 20–40% of oropharyngeal cancers (OPC) are thought to be caused by HPV infection, with HPV16 accounting for more than 80% of these cases [8, 1]. Compared to OPC without HPV infection, patients with HPV-related cancers respond better to treatment and have a greater overall survival rate [9–14]. HPV infection is more common in SCC than in healthy skin, particularly in head and neck squamous cell carcinoma (HNSCC), despite the fact that HNSCC has not gotten as much academic attention as oropharyngeal squamous cell carcinoma (OPSCC) [15].

Consumption of Alcohol, smoking, betel, tobacco & pan masala chewing have historically been linked to head and neck squamous cell carcinoma (HNSCC), a diverse illness that includes malignancies of the larynx, pharynx, and oral cavity [16] (**Figure 2**). Patients are often deemed disease-free after five years, with the majority of recurrences or deaths from disease-specific causes occurring during the first two to three years. Human papillomavirus (HPV), specifically oropharyngeal squamous cell carcinoma (OPSCC), has been linked to the development of HNSCC within the past 20 years. In HPV-negative cancers prognosis differ with HPV-associated malignancies by Their histological appearance and risk factor [17]. HPV modifies the expression of genes and promotes epigenetic alterations, which alter cellular immune surveillance and cellular homeostasis, similar to many other oncogenic viruses. The first effect is increased cellular proliferation that contributes to viral persistence and replication [18] (**Figure 1**).

p16 IHC is the key surrogate marker for HPV-associated oncogenesis and its diagnostic reliability must be evaluated based on the differing reports of variability related to the use of different clones of p16 antibodies. Similarly, while HPV DNA PCR testing is the gold standard for detecting HPV directly, it has challenges in terms of cost, availability, and the technical expertise required for use. In this context, the present study aimed to explore how does the efficacy of different p16 antibody clones compares to HPV DNA PCR testing in detecting HPV-associated head and neck cancers.

HPV DNA PCR: The Gold Standard

In order to identify HPV-positive cases, the most common method relies on PCR amplification of viral DNA. Several independent studies have shown that the specificity of methods based on PCR amplification of HPV DNA identification is insufficient to establish viral causation [19–23]. High sensitivity and the ability to detect even a small number of DNA copies in a sample, many PCR-based methods typically yield false-positive results, which only reflect transient infections [24–26]. Other indicators, such as the identification of E6/E7 mRNA transcripts and p16 (INK4a) expression, offer a more precise categorisation of HPV-mediated HNC (head and neck cancer) in the context of HPV-driven transformation [22, 27–31]. HPV E6/E7 oncogene transcripts were established as the standard of care, and it is currently crucial to detect the carcinogenic effect of HPV E6 and E7 oncoproteins for HPV-related malignancies [32].

Using sequence-specific primers, HPV DNA PCR entails amplifying particular HPV genomic areas, usually the highly conserved L1, E6, or E7 sections [33] (**Figure 3**). High sensitivity in recognize even a trace amounts of viral DNA in tissue samples is guaranteed by this amplification. In addition to qualitative detection (if HPV DNA is present or absent) and quantitative evaluation (viral load measurement), PCR-based techniques are very flexible and can be used for genotyping to distinguish between HPV subtypes, including HPV16 and HPV18, which are most closely linked to HNSCC [34].

Diagnostic Utility of differentiating HPV-driven HNSCC from non-HPV-associated patients, HPV DNA PCR is a crucial tool for proper prognosis and treatment classification. Since HPV-positive HNSCC is linked to improved overall and disease-specific survival, treatment planning must take HPV status into account [35,36]. In case of epidemiological HPV DNA PCR has been greatly for the improvement to understanding the HPV-driven HNSCC and also the rising incidence in the younger as well as non-smoking populations through the demonstration of the high-risk in HPV strains [37]. In therapeutic implication the Clinical decision-making was aided by the accuracy in HPV detection while the using DNA PCR, especially in the de-escalation trials for the HPV-positive HNSCC, which occurs the lower treatment intensity is the intended to avoid long-term their toxicities while this preserving high cure rates [37, 38].

Sensitivity and Specificity of HPV DNA PCR can detect even minute quantities of viral DNA; it is a very sensitive diagnostic technique. Even formalin-fixed paraffin-embedded (FFPE) tissue samples, which are used in nearly every histopathological examination relying on genotype identification, may harbour HPV DNA [39]. One of the distinctive aspects of HPV DNA PCR is the ability to differentiate between high-risk and low-risk HPV strains, which provides informative data regarding the infection's ability to develop into cancer. High-Grade Squamous Cell Carcinoma (HNSCC) is associated with the most aggressive type of Human Papillomavirus (HPV) infections. Specifically, HPV-16 is frequently found at the site of HNSCC and is thought to be a major cause of the disease. Quantitative PCR (qPCR) may be used to evaluate the viral load to provide more insight into the severity of the infection and its clinical significance [40].

Although HPV DNA PCR allows the classification of both latent and active infections, it also possesses many limitations. It detects the presence of viral DNA; however, it is unable to distinguish between transcriptionally active and transcriptionally latent HPV. Transcriptionally inactive HPV is unlikely to cause skin cancer, and therefore this limitation of the HPV DNA PCR can lead to false-positive results. False-negative results due to poor sample quality may occur in degraded samples of HPV DNA, e.g., those that have been fixed for too long or not properly extracted; [41]. Furthermore, HPV DNA PCR is more expensive than alternatives like p16 immunohistochemistry (IHC) and is not as widely adopted in routine clinical practice, particularly for large-scale screening [42].

Immunohistochemistry (IHC) p16^{INK4a}

p16 immunohistochemistry (IHC) is one of the most used surrogate markers for detecting HPV-associated carcinogenesis, especially those of head and neck squamous cell carcinoma (HNSCC) [43]. The cellular protein p16, which is encoded by the CDKN2A locus, controls the cell cycle as a cyclin-dependent kinase inhibitor (CDKI) to stop the cell cycle in the G1 stage [44, 45]. In HPV-driven oropharyngeal squamous cell carcinoma (OPSCC), HPV E7 inactivates Rb, leading to an increase in p16, a known biomarker of transcriptionally active HPV infection (**Figure 4**). Crucially, p16 positive, which is highly clinically predictive in OPSCC, is included in the AJCC TNM staging scheme [46]. The prognostic value of p16 for survival is exactly the same as the prognostic value of HPV DNA detection, making it a valuable predictor of HPV status [44].

Overview of p16 IHC Clones

A popular surrogate marker for identifying HPV-associated carcinogenesis, especially in head and neck squamous cell carcinoma (HNSCC), is p16 immunohistochemistry (IHC). The diagnostic accuracy and clinical usefulness of different clones of the p16 antibody can be variable dependent on their specific individualities. Comparatively assessing the various p16 clones enables the establishment of the comparable differences in staining characteristics, specificities, and sensitivity that each has referred [47].

The evaluation of diverse p16 antibody clones across multiple diagnostic pathology studies has indicated differences in their ability to provide an accurate measure of p16 expression for diagnosis (i.e., sensitivity, specificity and staining characteristics). Using the following commonly used p16 antibody clones: JC8 from Santa Cruz Biotechnology, E6H4 from Roche/Ventana, and G175-405 from BD Biosciences, differences in diagnostic performance are seen between them when evaluating their ability to detect p16 expression in patients with head and neck squamous cell carcinoma (HNSCC) [48]. Large-scale comparison studies (approximately 500 HNSCC cases) evaluating the performance of the various p16 antibody clones (i.e., E6H4, JC8, and G175-405) identified the E6H4 clone as having the highest level of diagnostic accuracy (sensitivity — 98%; specificity — 96%) and as having the lowest levels of inter-observer variability among pathologists when used for routine clinical practice [48]. In contrast, the JC8 and G175-405 clones demonstrated much lower levels of sensitivity (82%; 74%, respectively). Additionally, the JC8 and G175-405 clones had much lower degrees of specificity (88%; 90%, respectively) than the E6H4 clone. Overall, the data demonstrated that E6H4 would be the best of the available p16 antibodies to use for routine clinical practice [48] (**Table 1**).

Most guidelines recommend that p16 IHC be the primary screening test for the detection of HPV related oropharyngeal squamous cell carcinoma (OPSCC). This is due to its reasonable practicality and high specificity. Confirmation of diagnosis should be obtained by performing tests for either HPV DNA and/or E6/E7 mRNA using PCR or ISH [49]. According to the 2018 College of American Pathologists (CAP) and American Society of Clinical Oncology (ASCO) guidelines, the routine use of p16 IHC is limited to oropharyngeal cancer and to cases of cervical lymph node metastasis where there is an unknown primary site, because the HPV prevalence and predictive value are much lower in the other subsites of the head and neck. Proper interpretation also requires that the interpreting pathologist have a large amount of experience with p16 IHC, as equivocal staining intensity or focal staining patterns can lead to incorrect interpretations [50].

p16 IHC has high success rates for the diagnosis of HPV-positive HNSCC. P16 positive tumors have high sensitivity (~90%) and moderate to high specificity (>80%) for HPV positivity in accordance with prior studies [51-53]. The College of American Pathologists' guidelines state that a p16 positive tumor with strong diffuse nuclear and cytoplasmic staining in $\geq 70\%$ of tumor cells can be used as a reliable surrogate marker for HPV-positive OPSCC. [54].

Immunohistochemistry (IHC) has several benefits for identifying head and neck squamous cell carcinoma (HNSCC), making it a useful tool in finding tumors associated with HPV (human papillomavirus). Although the use of IHC-p16 for diagnosis has not been thoroughly examined across all HNSCC patients, it is still a viable tool for identifying HPV-driven changes in patients due to its ease of use, short turnaround time, and cost-effectiveness compared with molecular techniques. [50].

One major advantage of p16 expression when using IHC to determine HPV infection is its establishment as a surrogate marker for HPV infection and the use of p16 overexpression as an indicator of HPV-associated tumors. Although there is limited evidence about the role of HPV in oral cavity cancers, p16 expression remains an important marker to utilize when trying to determine the presence of HPV contributions in these cancers [55]. A recent cross-sectional study showed the incidence of p16 positivity was approximately 60% for head and neck SCC cases, and had a significant association to site of the tumor and lymph node status [56].

In addition, the use of p16 IHC could help to identify patients with field cancerisation at surgical margins as it can detect increasingly early molecular changes in histopathologically normal tissue which would indicate that they are at higher risk for recurrence even if their hematoxylin & eosin (H&E) examination is negative [57].

P16 IHC provides high sensitivity and specificity for diagnosing HNSCC (head and neck squamous cell carcinoma). Pooled data from recent meta-analyses of all head and neck cancers have an estimated pooled sensitivity of 94% and specificity of 90% for identifying HPV-associated carcinomas via p16 IHC [50]. A systematic review of oropharyngeal tumors indicated that using p16 as a single diagnostic marker had a sensitivity of approximately 94% and specificity of approximately 83%; the sensitivity increased to nearly 96% when combined with HPV DNA by PCR [58].

In addition, the expression of p16 was found to correlate with a number of clinicopathological factors including patient's age (younger), gender (male), the involvement of particular subsites of the oral cavity (e.g., tongue, buccal mucosa), and lymph nodal metastasis. The identification of p16 as a potential prognostic marker will help improve risk stratification for patients with head and neck cancer [57].

There are numerous limitations associated with the use of p16 immunohistochemistry (IHC) as a diagnostic and prognostic tool for oral cavity squamous cell carcinoma (OSCC). Unlike oropharyngeal carcinoma, where p16 is viewed as a reliable surrogate marker for identifying human papillomavirus (HPV), it is much less reliable when applied to OSCC specimens that have been taken from the oral cavity. Many of the cases of OSCC that are expressing p16 do not actually contain HPV-related disease but instead are the result of other molecular processes, such as disruption of the pRb pathway, which leads to decreased specificity of p16. For example, in one study investigating p16-positive OSCC cases, only 1-10% of cases had evidence of HPV using the gold standard technique of in situ hybridisation (ISH). Thus, the positive predictive value (PPV) for p16 expression when compared to detection of HPV using E6/E7 RNA detection is approximately 40%. In addition, many studies have revealed poor concordance between p16 positivity and HPV detection using either polymerase chain reaction (PCR) or ISH methods in OSCC specimens, with a reported correlation coefficient of approximately -0.01. Therefore, this indicates that there are a substantial number of false-positive p16 results. [34]

The interpretation of p16 staining can also be complex because of the different ways that tissue samples exhibit different amounts of expression, as well as a lack of universally agreed upon cut-off points for OSCC. The most common cut-off points for p16 staining ($\geq 70\%$ diffuse strong nuclear and cytoplasmic staining) was originally developed for oropharyngeal SCC, and may not apply to oral cavity tumours since many cases have intermediate staining ($< 70\%$), partial staining, and basal-predominant patterns, which complicate interpretation [59]. Even though intermediate patterns of p16 staining exist, they are rarely reported and have not yet been described in detail with respect to their clinical importance or meaning in OSCC [60].

Additionally, while p16 positivity in oropharyngeal SCC is related to prolongation of overall survival, the prognostic role of p16 positivity in OSCC has shown inconsistency among several studies, including several that demonstrated no difference in disease-specific or disease-free survival with respect to p16 status [60].

Another limitation exists due to inconsistent expression patterns of p16 in oral cavity dysplasia and premalignant lesions; this means that some of these lesions show either overexpression or a decrease/absence of staining depending on the grade of dysplasia, the antibody clone used and the criteria utilized to score lesions which has resulted in contradictory findings among studies [61]. Additionally, in some patient populations that were studied, there were completely negative p16 staining results found in both OSCC and the control oral tissues. However, positive p16 staining was observed in 26.8% of oral lichen planus cases and therefore does not establish p16 as a reliable early malignancy transformation biomarker [62].

The variability of p16 detection methods can also be influenced by the technical aspects of the antibodies (for example, antibody clone). In general, antibody clones E6H4 demonstrate a greater degree of sensitivity, specificity and inter-observer agreement

than other clones (for example, antibody clone G175-405, ABS377 or JC8); however, the E6H4 clone tends to be most expensive even though they produce better reproducible staining results than other antibodies [63].

Result

Immunohistochemistry (IHC) p16 is frequently used as an alternative marker in the diagnosis of HPV-related head and neck squamous cell carcinoma (HNSCC). Although IHC is a standard test, it has variable diagnostic quality. Diagnostic performance may differ between antibody clones or based on tumour subtype; the use of molecular or other types of testing may also have an effect on diagnostic reliability depending on antibody clone and tumour site. Among the clones evaluated for diagnostic reliability in HNSCC, E6H4 was consistently ranked as having the highest diagnostic reliability with sensitivity ranging from 89% and specificity 77.4 %, thereby being established as the reference standard. Other antibody clones (MXB and JC8) had acceptable but lower sensitivity relative to E6H4 while G175405 had moderate to significantly variable diagnostic precision (**Table 1**).

In multiple studies examining different diagnostic modalities for disease diagnoses, p16 expression was found to be highly associated with tumours caused by HPV, especially OPSCC; however, when examining only cancers of the oral cavity, there was little prognostic or diagnostic utility from p16 expression alone. Many studies have suggested that p16 positivity should be validated using either HPV DNA or RNA methods to provide greater specificity through combined testing methods, and also allow for differentiation between transcriptionally active HPV infections vs incidental viral presence. Advanced molecular approaches, including RNA in-situ hybridization and multiplex mRNA detection, further enhanced diagnostic precision and prognostic stratification. Overall, the evidence indicates that while p16 IHC is a practical first-line biomarker, optimal diagnostic accuracy in HNSCC is achieved through integrated multimodal testing rather than reliance on p16 expression alone.

Discussion

This review summarises currently available research on how well different HPV DNA PCR-associated p16 IHC clones can predict and diagnose HNSCC, specifically in OPSCC, where HPV causes the large majority of cases. Accurate identification of HPV-positive tumors is essential for developing prognostic characteristics and individualised treatment methods for patients with HPV-related OPSCC [1, 9]. The p16 IHC marker is now widely used and cost-effective because of its strong association with transcriptionally active HPV infection and prognosis in OPSCC. HPV DNA PCR remains the gold standard for direct viral detection based on higher sensitivity and genotyping potential [5, 6, 50].

Our analysis highlights that not all p16 antibody clones perform equally. The clone E6H4 consistently demonstrates superior diagnostic performance, with reported sensitivity and specificity often exceeding 89.2% and 77.4%, respectively, when compared to HPV DNA PCR or RNA ISH in OPSCC [48,50] (**Table 1**). Large-scale comparative studies confirm that E6H4 offers the highest interobserver reliability and is recommended for routine clinical use [47]. In the contrast, clones like as JC8 & G175-405 those like show the variable and generally lower sensitivity and its specificity, which may occur to diagnostic the inaccuracy, if it's not properly validated [17, 48, 63] (**Table 1**). This was the variability underscores that their importance of the standardizing the antibody clone which used for diagnostic pathways to ensure the consistent and the reproducible results for across laboratories. the recent studies finding the significant discrepancy in the performance of the p16 IHC across that the different anatomical subsites. In the OPSCC, p16 overexpression occurs robust indicator of HPV-driven carcinogenesis, with the strong prognostic value [47, 51]. However, in the oral cavity SCC (OSCC), p16 was positivity is a poor surrogate for the HPV

infection due to their frequent Rb pathway to alterations unrelated to the HPV, leading to low specificity and the positive predictive value (PPV) [34, 59]. As the example, according to their several reports in our assessment included, only the small minority of the p16-positive OSCC were the HPV-positive by PCR or ISH [8, 34]. This was shown that the p16 IHC should not be used the standalone test for non-oropharyngeal anatomic sites.

The combination of the p16 IHC with the HPV-specific testing (e.g., DNA PCR or E6/E7 mRNA ISH) that improves the diagnostic accuracy [2]. Studies by the Simoens et al. (2022) and the others have been shown that the while p16 IHC alone is the highly sensitive, it is specificity increases significantly when it's combined with the HPV DNA or RNA testing, it reducing the false positives from the non-functional HPV presence [2, 7] (**Table 2**). This is the particularly relevant in the regions with the low HPV prevalence, where the positive was predictive value of the p16 IHC is inherently lower [1, 34]. Moreover, the emerging multiplex assays, such as the QuantiGene-MPH assay, which was the simultaneously quantify HPV E7 and p16 mRNA, offer the promising approach to the distinguish truly HPV-driven cancers from the incidental infections [35].

From the clinical perspective, choice between p16 IHC and HPV DNA PCR involves while the trade-off between cost, as well as accessibility, and diagnostic precision. p16 IHC is the less expensive, faster, and widely available in making it their suitable for initial screening in the OPSCC. However, in the cases of equivocal p16 staining or in the non-oropharyngeal sites, confirmatory testing with the HPV DNA PCR or E6/E7 mRNA ISH occurs the essential to avoid misclassification [49,54]. Furthermore, the prognostic significance of the p16 is well-established in OPSCC but the remains controversial in OSCC, where it was does not consistently correlate with the improved survival [34,60].

Limitation of study

This review has several limitations that need to be consider when interpreting the findings. One major limitation is the lack of a sufficient number of published studies comparing the different p16 antibody clones in oral and head and neck cancers, limiting the ability to make a full comparison of the diagnostic performance of these clones. Another limitation is the number of relevant studies examining the combined use of p16 immunohistochemistry with HPV 16 and 18 detection methods is limited, therefore very little can be concluded regarding the effectiveness of this combined diagnostic method. Finally, some potentially relevant articles were excluded from this review, because they were behind a paywall or had high access costs, making their full contents and data unavailable.

Conclusion

p16 immunohistochemistry (IHC) remains an important and practical surrogate biomarker for detecting HPV-associated head and neck squamous cell carcinoma, particularly in oropharyngeal squamous cell carcinoma. However, its diagnostic reliability varies depending on the antibody clone, tumor subsite, and the use of complementary molecular tests. Among the evaluated clones, E6H4 shows the most consistent diagnostic performance and is considered the preferred clone for routine clinical practice. Although p16 overexpression strongly correlates with transcriptionally active HPV in OPSCC, it is not sufficiently reliable as a standalone marker in non-oropharyngeal tumors. Therefore, integrating p16 IHC with HPV-specific molecular assays such as HPV DNA PCR or E6/E7 mRNA testing improves diagnostic accuracy and helps ensure appropriate patient stratification and management.

Abbreviations

HPV – Human Papillomavirus, **OP** – Oropharyngeal, **DNA** – Deoxyribonucleic Acid, **miRNAs** – Micro Ribonucleic Acid, **PCR** – Polymerase Chain Reaction, **IHC** – Immunohistochemistry, **OSCC** – Oral Squamous Cell

Carcinoma, **RB1** – Retinoblastoma **1**, **OPC** – Oropharyngeal Cancer, **HNSCC** – Head and Neck Squamous Cell Carcinoma, **OPSCC** – Oropharyngeal Squamous Cell Carcinoma, **SCC** – Squamous Cell Carcinoma, **HNC** – Head and Neck Cancer, **HPV DNA PCR** – Human Papillomavirus Deoxyribonucleic Acid Polymerase Chain Reaction, **FFPE** – **Formalin-Fixed Paraffin-Embedded**, **qPCR** – Quantitative Polymerase Chain Reaction, **ISH** – In Situ Hybridization, **CAP** – College of American Pathologists, **ASCO** – American Society of Clinical Oncology, **H&E** – Hematoxylin and Eosin Stain.

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Declarations

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Figure 1: The connection between miRNAs and the main risk factors in oral cancer.

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Figure 2: Risk Factor of Oral Cancer

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Figure 3: Mechanism of oncoprotein in carcinogenesis

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Figure 4: Mechanism of cell cycle deregulation by Human Papillomavirus (HPV)**Table legends**

Table 1: Compare the efficacy of different IHC p16 antibody clones

Table 2: Comparing the Diagnostic Accuracy of Different Techniques for Head and Neck Cancer