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REVIEW ARTICLE

# New insights into human papillomavirus-associated head and neck squamous cell carcinoma

## *Aggiornamento sul carcinoma squamoso testa-collo associato al papillomavirus umano*

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### SUMMARY

Human papillomavirus (HPV)-associated head and neck squamous cell carcinoma (HNSCC) is an entity with peculiar clinical and molecular characteristics, which mainly arises from the reticulated epithelium lining the crypts of the palatine tonsils and the base of the tongue. The only head and neck site with a definite etiological association between persistent high-risk (HR) HPV infection and development of SCC is the oropharynx. HPV-positive malignancies represent 5-20% of all HNSCCs and 40-90% of those arising from the oropharynx, with widely variable rates depending on the geographic area, population, relative prevalence of environment-related SCC and detection assay. HPV-16 is by far the most common HR HPV genotype detected in oropharyngeal SCC (OPSCC), and the only definitely carcinogenic genotype for the head and neck region. Patients with HPV-induced OPSCC are more likely to be middle-aged white men, non-smokers, non-drinkers or mild to moderate drinkers, with higher socioeconomic status and better performance status than subjects with HPV-unrelated SCC. HPV-induced HNSCCs are often described as non-keratinizing, poorly differentiated or basaloid carcinomas, and are diagnosed in earlier T-category with a trend for a more advanced N-category, with cystic degeneration, than the HPV-unrelated carcinomas. HPV positivity is associated with better response to treatment and modality-independent survival benefit. Treatment selection in HPV-related oropharyngeal carcinoma is becoming a critical issue, and although there is no evidence from randomized, controlled trials to support a treatment de-escalation in HPV-positive SCC, some investigators argue that intensive combined modality strategies may represent an overtreatment.

KEY WORDS: Human papillomavirus • Head and neck cancer • Squamous cell carcinoma • Oropharynx • Treatment de-escalation

### RIASSUNTO

*Il carcinoma squamoso testa-collo associato al papillomavirus umano (HPV) è una patologia con peculiari caratteristiche cliniche e molecolari che origina principalmente dall'epitelio criptico delle tonsille palatine e linguale. L'orofaringe rappresenta, infatti, l'unica sede per la quale a tutt'oggi sussista un sicuro nesso eziologico tra infezione da HPV e insorgenza di un carcinoma squamoso. I tumori maligni HPV-positivi rappresentano il 5-20% di tutti i carcinomi testa-collo e il 40-90% di quelli che originano dall'orofaringe, con tassi variabili di prevalenza in funzione dell'area geografica, del peso relativo degli altri fattori di rischio, della popolazione considerata e dei metodi di rilevamento del virus. Il paziente con tumore HPV-associato è più spesso un maschio di razza bianca, di mezza età, non-fumatore, non-bevitore o bevitore occasionale, presenta uno status socio-economico più elevato e un performance status migliore rispetto al soggetto con carcinoma HPV-negativo. Dal punto di vista istologico i tumori HPV-associati sono più spesso descritti come carcinomi non cheratinizzanti, scarsamente differenziati, con aspetti basaloidi e si presentano con una categoria T meno avanzata e una categoria N più avanzata, con aspetti di degenerazione cistica, rispetto ai tumori HPV-negativi. I carcinomi HPV-associati presentano una miglior risposta al trattamento e hanno una sopravvivenza migliore indipendentemente dal tipo di trattamento. La selezione del trattamento dei carcinomi orofaringei HPV-correlati sta diventando un punto critico poiché, nonostante non vi siano evidenze derivate da trials randomizzati controllati per giustificare una de-intensificazione del trattamento nei carcinomi squamosi HPV-positivi, alcuni ricercatori sostengono che una strategia di trattamento multimodale intensificata possa costituire in tali casi un over-treatment.*

PAROLE CHIAVE: Papillomavirus umano • Cancro testa-collo • Carcinoma a cellule squamose • Orofaringe • De-intensificazione del trattamento

## Introduction

In 2012, the International Agency for Research on Cancer stated that human papillomavirus (HPV) type 16 causes cancer of the oropharynx<sup>1</sup>. HPV-driven oropharyngeal squamous cell carcinoma (OPSCC) is a rising sexually transmitted entity with peculiar clinical and molecular characteristics. Interestingly, compared with environmental-related head and neck squamous cell carcinomas (HNSCC), patients with HPV-related malignancies display a better response to treatment and a lower risk of death and progression<sup>2</sup>.

A growing number of research papers about HPV-driven carcinogenesis in HNSCC have been published in recent years. The present review highlights the controversies and advances in HPV-related HNSCC to provide the otolaryngologists with a summary of the findings of selected research contributions mainly published in the last years.

## Epidemiology of HPV-induced HNSCC

More than 50,000 cases of head and neck (HN) cancers, mostly SCC, are estimated to have occurred in the United States in 2012, with about 13,000 deaths<sup>3</sup>. HN cancers represents about 3.5% of all malignant tumours in the United States<sup>3</sup> and Europe<sup>4,5</sup>, but in many other parts of the world, such as India, Southeast Asia and Brazil, they are much more prevalent, being altogether the 5<sup>th</sup>/6<sup>th</sup> most common malignancy worldwide<sup>3,6,7</sup>.

Despite histological homogeneity, HNSCCs are an extremely heterogeneous group of tumours both from molecular<sup>8-11</sup> and clinical points of view<sup>12</sup>. The main clinical heterogeneity factor is the site of origin, which correlates with specific risk factors, symptoms, stage at diagnosis, tendency to local and distant metastasis, chemo- and radiosensitivity and prognosis<sup>12,13</sup>. The best-established risk factors for HNSCC are tobacco and alcohol abuse<sup>12,13</sup>. High-risk (HR) HPV infection, whose role in carcinogenesis of the uterine cervix has been extensively studied<sup>13</sup>, is now a well-recognized<sup>14-17</sup> and emerging risk factor for HNSCC that probably underlies the marked increase in the incidence of OPSCC<sup>18,19</sup>, especially in the young<sup>20</sup>. Indeed, while the overall incidence of HNSCC has fallen in the last three decades, an increase in the incidence of OPSCC, mainly tonsil and tongue base cancers, has been seen both in USA and in Europe<sup>18,19,20-23</sup>. OPSCC now represents a significantly higher proportion of HNSCCs. This rise in incidence is mostly occurring in individuals aged 40–55 years, without environmental risk factors, and is associated with persistent infection with HR HPV<sub>s</sub><sup>18,20</sup>. Although the rate of HPV-positive OPSCCs varies widely depending on accuracy in the distinction of anatomical borders of the oropharynx, the competing effect of environmental risk factors, quality of tissue biopsies and HPV-testing accuracy<sup>1</sup>, HPV-related OPSCCs account for 40–

80% of OPSCCs diagnosed in the United States<sup>24</sup> and a growing proportion of these neoplasms in Europe<sup>21,22</sup> ranging from 18% to 90%<sup>25,26</sup>.

The above data prompted some authors to speak about an HPV epidemic, leading to a significant rise of OPSCC incidence worldwide, which led to interests of HPV-vaccine producing companies about head and neck oncology. Furthermore, the increasing epidemiologic role of HPV and its value as a prognostic marker in head and neck oncology has stimulated a growing number of studies in the last decade.

Nevertheless some critical issues, such as the actual incidence of HR HPV infection in sites outside the oropharynx, and the best detection method to diagnose the infection itself in HNSCC, have not yet been definitely clarified. A recent rigorous and influential study describes an overall HR HPV infection incidence of 14% in an HNSCC North American population, with a 53% positivity rate in oropharyngeal cases and a markedly lower incidence in the other sites<sup>10</sup>.

This and other recent studies<sup>27,28</sup> seem to suggest that the so-called “HPV epidemic” may have an impact mainly in the oropharynx, and that the prevalence of HPV infection among SCCs of other head and neck sites has actually been overestimated by several authors. The above-cited epidemiological data indirectly confirm the latter assumption, since if other head and neck subsites were significantly affected by HPV-related carcinogenesis, an increase in their incidence as in the oropharynx, which instead has been the only site with a rising frequency of SCC in the last 30 years, should have been observed<sup>23</sup>.

Nevertheless, among the other extra-oropharyngeal subsites, HPV may have a role in the supraglottic larynx<sup>28</sup>, whose marginal region is contiguous with the oropharynx, and it may account for the HR HPV infection rate among laryngeal SCCs reported in some studies<sup>29,30</sup>. As for the oral cavity SCCs, many authors reported frequent HR HPV involvement by considering p16 overexpression equivalent to HPV infection<sup>31,32</sup>; nevertheless, recent data suggest that p16 overexpression in oral cancers is due to different mechanisms and that HR HPV infection is very rarely detectable in oral SCCs<sup>27,28</sup>.

In the first paper by Gillison<sup>15</sup>, demonstrating the carcinogenic role of HPV in the oropharynx, 50 of 55 HPV-positive cancers harboured HPV type 16, and at present HPV16 remains the only HPV genotype that is classified as cancer-causing in the head and neck<sup>10,17,28</sup>. The role of the other HR genotypes, if any, is undoubtedly less relevant.

Therefore, HPV-associated OPSCC is a growing distinct clinical and molecular entity with a less strong association with tobacco and alcohol. On the other hand, the scientific evidence that links HR-HPV to SCC from other head and neck sites is substantially weaker<sup>1</sup>.

## HPVs and molecular mechanisms of HPV-induced carcinogenesis

Human papillomaviruses (HPVs) are a heterogeneous group of small non-enveloped epitheliotropic DNA viruses targeting the basal cells of stratified epithelia at either mucosal or cutaneous sites, and constitute the Papillomaviridae family. More than 90 HPV types have been fully sequenced, and independent studies indicate that many additional types exist<sup>33</sup>. The IARC Working Group has classified HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 as carcinogenic and type 68 as probably carcinogenic to humans<sup>17</sup>; these are responsible for virtually all carcinomas of the cervix and different proportions of carcinomas of the anus, vagina, penis, vulva and oropharynx<sup>34</sup>.

Globally, HPVs contribute significantly to viral associated neoplasms, accounting for approximately 600,000 cases (5%) of cancers worldwide annually<sup>35</sup>. In particular, HPV 16 accounts for approximately 50% of cervical carcinomas and more than 90% of HPV-positive carcinomas of the oropharynx (and the other ano-genital sites). Other HPV types have a low prevalence in cervical cancers and are classified as possibly carcinogenic (HPV 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85) or non-carcinogenic (low risk).

HPV infections are mainly sexually transmitted through direct skin or mucosa contact and represent the most common sexually transmitted infection worldwide; the probability of transmission is very high, with an estimated life-time risk of cervical HPV infection in sexually active women of up to 80%. Exposure to HPV is determined by well known risk factors for most sexually transmitted infections, while determinants of susceptibility and infectivity are much less established<sup>36</sup>.

Knowledge on the natural history of HPV infection derives from studies on cervical infection. The large majority of infections clear spontaneously in approximately 12-24 months (time depends on HPV type and host factors, such as immune status); the virus can either be completely cleared or remain in a latent form (i.e. undetectable by diagnostic tests) that can be reactivated later in life. Only a small fraction of infections cause clinical lesions; spontaneous regression occurs in most low grade lesions and in a fraction of high grade ones, while progression to invasive cancer is a very rare event and is preventable by surgical treatment of high grade lesions<sup>37</sup>.

The HPV genome is organized into three regions: a non-coding "long control region" (LCR) regulating gene expression and replication, and two protein-coding regions, the early (E) region coding proteins required for gene expression, replication and survival, and the late (L) region coding capsid proteins. Three early genes encode three viral oncoproteins: E5 and the best known E6 and E7. Studies on mucosal HR HPVs have demonstrated that E6

and E7 play a key role in both benign proliferation and malignant transformation.

E6 of HPV 16 (and of the other HR types) is able to induce degradation of the tumour suppressor protein p53 via the ubiquitin pathway<sup>38</sup>. P53 is a transcription factor that induces cell cycle arrest or apoptosis in response to cellular stress or DNA damage<sup>39</sup>. E6 binds to the cellular protein E6AP, and the E6/E6AP complex is responsible for ubiquitination and subsequent proteasome degradation of p53 protein. Since p53 has a crucial role in safeguarding the integrity of the genome by inducing growth arrest or apoptosis, its malfunction is a common feature of many human malignancies; while in most cancers this is determined by p53 mutations, in HPV-associated carcinomas wild-type functional p53 is degraded by E6 oncoprotein. Moreover, cells expressing HPV type 16 E6 show chromosomal instability, an important step towards malignant transformation<sup>33</sup>.

E7 of HPV 16 (and of the other HR types) inactivates pRb, which is in control of the G1-S phase transition by binding the transcription factor E2F. As a consequence, E2F is released (under physiological conditions this is determined by cyclin-dependent kinase (CDK)-mediated phosphorylation of pRb), with consequent promotion of cell G1-S phase transition<sup>40</sup>, and transcription of genes, such as cyclin E and cyclin A, which are required for cell cycle progression. Inactivation of pRB by E7 induces overexpression of p16<sup>INK4A</sup>; this can be visualized immunohistochemically and is generally considered as a marker of active HPV infection. E7 proteins from low-risk mucosal HPV genotypes (e.g. HPV types 6 and 11) bind pRb with a weak affinity compared with HPV type 16 E7<sup>33</sup>.

Persistent HR HPV infection is a prerequisite for the development of cervical precancerous lesions and invasive cancer. In fact, in order to induce stable malignant transformation of the host cells, E6 and E7 of HR HPVs need to cooperate with activated cellular proto-oncogenes. Proliferation-associated mutagenesis induced by persistent expression of viral oncogenes E6 and E7 may finally lead to activation of proto-oncogenes resulting in the fully malignant phenotype<sup>33</sup>.

Carcinogenic mechanisms in HPV-associated oropharyngeal cancers (OPSCC) may be similar to what described for cervical cancers, but since the oral cavity/oropharynx are exposed to higher levels of chemical carcinogens in comparison to the genital tract, it is likely that different mechanisms are implicated in cervical and oropharyngeal carcinogenesis. Moreover, in several studies HPV DNA-positive OPSCCs were heterogeneous in both biological and clinical behaviour, possibly due to differences in viral load and/or viral oncogene expression<sup>41</sup>. Low levels of HPV DNA and absence of viral transcriptional activity are likely to have no or limited biologic significance, and could indicate that HPV does not play a pathogenetic role in these malignancies<sup>42</sup>.

## Diagnostic challenges of HPV detection in HNSCC

Although the management of OPSCC does not require evaluation of HPV status, HPV-testing in subjects with OPSCC is becoming the standard of care in many institutions. HPV-induced OPSCC constitutes a new tumour entity with distinct clinical and histopathological features, improved performance status and better prognosis. Nevertheless, heterogeneity in both biological and clinical behaviour among HPV DNA-positive cases has been observed in several studies<sup>43-46</sup>. This may be due to differences in viral load and/or viral gene expression<sup>46</sup>, and highlights the need to assess the presence of HPV in the tumour using an algorithm that allows detection of biologically active virus only, and identifies cases with improved clinical outcome.

Detection of HPV-DNA is the most widely used method to diagnose HPV infection in clinical samples. The available diagnostic assays show variable sensitivity and specificity estimates, and no standardization on sample processing and testing has been defined. Moreover, as PCR-based assays are highly sensitive, cross contamination may be an issue. General consensus primers amplify fragments of the widely conserved HPV L1 gene, and HPV types are then identified by direct sequencing, or hybridization or restriction fragment length polymorphism analysis of the amplified products. Real-time (RT)-PCR assays permit to quantitatively detect and genotype HPV-DNA. By measuring viral load, RT-PCR may discriminate between HPV-related (high levels of viral DNA) and HPV-unrelated cancers (low levels of viral DNA possibly due to contamination)<sup>47</sup>.

In situ hybridization using HPV-type specific probes allows direct visualization of HPV in tissue samples and may discriminate between integrated and episomal infections and between oncologically relevant (clonal pattern) and non-oncologically relevant infections (low viral copies in few cells)<sup>24,48</sup>.

PCR-based detection of HPV E6 mRNA in frozen specimens is considered the gold standard for the diagnosis of oncologically-relevant HPV infection. Unfortunately, such a method is unlikely to be feasible in a routine pathology laboratory and at present does not allow its wide application in retrospective molecular studies on archival histologic material<sup>24</sup> because it usually requires good quality mRNA which is generally extracted from fresh materials stored at -80°C and/or in a medium with RNase inhibitors, although improved detection methods have most recently been developed also in formalin fixed paraffin embedded (FFPE) samples<sup>49</sup>.

Different biomarkers for the detection of biologically active HPV infections (i.e. translationally active infection accompanied by specific biological behaviour of tumours) in head and neck SCC have been evaluated and compared<sup>24,28,50,51</sup>.

Among these, the most popular is overexpression of p16<sup>INK4A</sup> protein (assessed by immunohistochemistry; IHC), which has been indicated as a suitable surrogate biomarker, and proposed as the first “screening” assessment<sup>16</sup>. p16<sup>INK4A</sup> is a CDK inhibitor, encoded by the CDKN2A gene, which arrests cells in G1<sup>52</sup>. The inactivation of pRb determined by HPV E7 is associated with up-regulation of CDKN2A and consequent protein overexpression. Conversely, in HPV-unrelated, environment-related HNSCC perturbation of pRb-pathway is uncommon and expression of CDKN2A is, in most cases although not always, downregulated<sup>53</sup>. Therefore, p16<sup>INK4A</sup> immunostaining in conjunction with HPV-DNA detection may be a useful tool to establish a diagnosis of HPV-related OPSCC cancer<sup>54</sup>; a guide for its interpretative relevance and consistency has recently been proposed<sup>55</sup>. Of note, results of recent studies have highlighted that p16<sup>INK4A</sup> overexpression is not a reliable surrogate marker of HPV presence in non-oropharyngeal head and neck sites<sup>28</sup>. Moreover, its use in OPSCC should be prudently advised to validate a positive result at HPV DNA evaluation, but should not be used alone as a diagnostic tool.

In conclusion, based on available knowledge the presence of HPV DNA in tissue biopsies is not always sufficient to attribute a cancer of the oropharynx to HPV, depending on the different sensitivity of the various assays relying on DNA detection, particularly in patients who also report tobacco/alcohol exposure, and appropriate algorithms could be used to define a tumour as HPV-induced. Assessment of HPV status is indicated in patients with oropharyngeal carcinomas, particularly when no environmental risk factors are present, and in patients with neck metastasis and carcinoma of unknown primary as HPV detection in metastatic lymph node samples is strongly indicative of a primary in the tonsilla or in the base of the tongue<sup>56</sup>. Unfortunately, there is no current standard for testing or interpretation of HPV detection assays, and each assay has technical limitations. In general, since tumours with low HPV DNA viral load are often negative for E6/E7 expression, use of assays with appropriate sensitivity for DNA detection (i.e. not too high)<sup>28</sup> and/or a combination of two different tests (i.e. HPV DNA detection validated by p16<sup>INK4A</sup> IHC in DNA positive cases) is considered as clinically adequate<sup>54,57</sup> when only FFPE samples are available, while mRNA detection remains the gold standard for fresh samples<sup>28</sup>.

## Oral HPV infection and detection of oral HPV in patients with HPV-induced OPSCC

Data on prevalence, incidence and natural history of oral HPV infection may be very important to understand the mechanisms of development of oropharyngeal cancers, identify populations at higher risk of tumour development and possibly define preventive strategies.

In a cross-sectional study recently conducted in the United States among more than 5,000 men and women aged 14 to 69 years, the overall prevalence of oral HPV infection was about 7%, with higher figures among men than women<sup>58</sup>. Higher prevalence rates (in the range of 20-40%) are consistently found among men who have sex with men (MSM) and among HIV-infected individuals, with the highest values for HIV-positive MSMs<sup>59,60</sup>.

Only a few studies have been published on the incidence and natural history of oral HPV infection; persistence of infection after 6-12 months has been recorded in about half of cases. As observed for HPV genital infection, immunosuppression may contribute to increased persistence or progression of oral HPV infection.

HPV sequences can be detected in oral exfoliated cells of patients with HPV-induced HNSCC; it has been shown that detection during follow-up is associated with disease progression<sup>61</sup>. Knowing the causal agent of a tumour provides the opportunity to prevent its development. Prophylactic vaccines targeting HPV 16 (plus type 18, or also 6 and 11) have been developed and are commercially available. High safety and efficacy in preventing the corresponding ano-genital infections and high-grade lesions have been demonstrated in phase III trials, and confirmed by post-licensure use. No specific data on efficacy in preventing oral infection and oropharyngeal cancers are available, but if proven efficacious, prophylactic vaccines hold great promise for primary prevention of HPV-associated oropharyngeal cancers, also because there are no screening strategies approved<sup>62</sup>. Indeed, while precancerous HPV-associated lesions that precede cervical carcinoma are well defined and widely employed in screening programmes, precancerous lesions in the oropharynx are poorly understood and not consistently classified, and their existence and scope for early diagnosis are controversial.

### Clinical features of HPV-induced carcinomas

As mentioned earlier, subjects with HPV-induced OPSCC are more likely to be middle-aged white men, non-smokers, non-drinkers or mild to moderate drinkers, and have a higher socioeconomic status and better performance status than subjects with HPV-unrelated OPSCC<sup>24,63</sup>. On the other hand, patients with HPV-induced OPSCC have a higher number of sexual partners and more oral sex partners<sup>24</sup>. Open-mouthed kissing was found to be associated with the development of oral HPV infection<sup>64</sup>. Nevertheless, HPV-induced oropharyngeal carcinoma occurs both among exposed and non-exposed to tobacco/alcohol, with cigarette smoking being a consistently associated risk factor for oral HPV infection and a suspected modifier of the natural history of HPV-induced HNSCC<sup>16,58,65,66</sup>.

HPV-induced SCC mainly develops from oropharynx with the palatine tonsils and base of the tongue being more frequently involved than other oropharyngeal sub-sites<sup>15,28,67,68</sup>.

The reticulated epithelium covering the tonsillar crypts is in intimate contact with various cells of the immune system and may be more prone to HPV infection and subsequent malignant transformation<sup>69</sup>. Furthermore, the typical epithelial disruptions of the reticulated epithelium leave the basement membrane unprotected against viral deposition without the need for concomitant mechanical abrasion of the mucosa as in the cervix<sup>70</sup>. Thus, HPV-driven SCCs mainly arise from the tonsillar crypts, while environment-related SCCs arise from the superficial epithelium<sup>71,72</sup>.

Although there are no specific histologic features that can discriminate HPV-induced from HPV-un-induced SCCs, several authors have identified some morphologic characteristics associated with HPV-driven carcinogenesis. While the prototypic HNSCC is moderately differentiated, HPV-induced SCCs are predominantly non-keratinizing SCC often described as poorly differentiated carcinomas or basaloid carcinomas based on the lobular growth of cells with hyperchromatic nuclei, scanty cytoplasm and marked mitotic activity<sup>70,71,73-75</sup>.

SCCs with basaloid appearance are usually associated with poor patient outcomes<sup>76,77</sup>. However, basaloid SCCs are now recognized as a heterogeneous group of HPV-16 positive and HPV-16 negative tumours: the presence of HPV-16 has a dramatic impact on the prognosis denoting a subset of basaloid SCCs with a more indolent behaviour<sup>73,78</sup>.

HPV-induced HNSCCs have a peculiar clinical presentation with regard to both tumour and neck stage and characteristics. Compared to HPV-unrelated tumours, HPV-induced carcinomas are generally diagnosed in an earlier T-category with a trend for a more advanced N-category<sup>79</sup>. In this sense, the extreme clinical manifestation of HPV-related HNSCC is a neck metastasis from an occult primary tumour<sup>71,80</sup>. About a quarter of neck metastases from unknown primary cancers are HPV-positive suggesting the possibility that the occult primary lesion originates in the oropharynx. The microanatomy of the crypt characterized by a porous basal membrane may promote early invasion and prompt metastasis of clinically-occult carcinomas<sup>81</sup>. Therefore, in cases of cervical metastasis from occult primary carcinomas, additional immunohistological investigations (p16<sup>ink4a</sup>) or HPV-DNA analysis should be performed on the neoplastic tissue to assess HPV status<sup>71</sup>. Some authors have demonstrated the utility of identifying HPV-induced carcinomas by ISH in fine-needle aspiration biopsies of metastatic cervical lymph nodes in the prediction of an occult oropharyngeal primary tumour<sup>82</sup>.

Previous studies have demonstrated that about two-thirds of lymph node metastases from SCC of Waldeyer's ring

display cystic degeneration<sup>83</sup>. More recently, several authors reported that HPV-related lymph-nodes metastases are often cystic at radiological imaging and histological examination<sup>84-86</sup>. This feature is now recognized as an HPV-associated phenomenon<sup>84</sup>. The presence of cystic degeneration in the metastatic nodes from HPV-driven OPSCC is a common finding that appears to simulate the growth and pattern behaviour of the parent cell derived from the reticulated epithelium covering the tonsillar crypts<sup>70</sup>.

It is not excluded that a fraction of branchiogenic carcinomas may be revised as cystic neck metastases from an occult HPV-induced carcinoma of the tonsillar crypts<sup>87-88</sup>. Moreover, HPV-related lymph node metastases often undergo sudden changes in volume. Particularly, a spontaneous shrinkage before and an enlargement during radiation treatment have been observed in patients with HPV-related neck metastases<sup>89</sup>. These oscillations have implications both in the consistency of pre-treatment clinical staging and in the risk of treatment complications since the decreasing volume of the target may leave a higher amount of normal tissue exposed to radiation dose.

### Prognosis of HPV-induced carcinomas

The first line of evidence of the impact of HPV in prognosis comes from numerous small mono-institutional retrospective case series published in the last dozen years, which have shown that patients with HPV-positive HNSCC, particularly those with oropharyngeal primary, treated by radiotherapy, chemoradiotherapy, surgery, or combined modality therapy have better outcome than those with HPV-un-induced cancer<sup>15 44 90-95</sup>. In these original reports, patients with HPV-positive SCC were estimated to have up to an 80% reduction in risk of disease-failure compared to HPV-negative patients.

More recently, retrospective analyses of archival tumour specimens from patients enrolled in phase II and III trials, which received more specific treatment regimens<sup>16 96-99</sup>, and three meta-analyses<sup>2 100 101</sup>, confirmed that patients with HPV-induced HNSCC have significantly better prognosis than patients with HPV-unrelated tumours. In these studies, the survival benefit was most predominant or restricted in patients with an oropharyngeal primary tumour. In a very recent extensive and exhaustive meta-analysis, a 72% reduction in both HNSCC and tonsillar SCC specific mortality was reported. Progression in patients with HPV-positive HNSCC and OPSCC was also significantly lower by 60% and 52% respectively. HPV-positive HNSCC and OPSCC patients were, respectively, 59% and 63% less likely to experience cancer recurrence than HPV-negative patients. Furthermore, compared with HPV-negative patients, those with HPV-positive HNSCCs, OPSCCs and tonsillar SCCs showed a 54%, 53% and 50% reduction in overall mortality, respectively<sup>2</sup>.

The reason why patients with HPV-induced HNSCC have better prognosis than those with HPV-unrelated cancer remains elusive. In particular, the better overall survival of HPV-positive patients may depend on their younger age at diagnosis, superior performance status, lower smoking and alcohol related morbidity, distinct biology of the cancer, reduced risk of second primary tumours or a more aggressive treatment strategy. The favourable outcome of HPV-induced SCC may be attributable to enhanced sensitivity to treatment due to a wild-type TP53, allowing an apoptotic response of cancer cells to radiation and chemotherapy<sup>102</sup>.

Robust data indicate that cigarette smoking may modify the clinical behaviour of HPV-positive SCC, adversely affecting the prognosis of these neoplasms<sup>16 103</sup>. Recently, a recursive partitioning analysis showed that the combination of tumour HPV status, smoking and TN category segregates patients with stage III and IV OPSCCs into 3 groups with different prognoses: patients with HPV-induced SCCs were considered to be at low risk, with the exception of smokers with advanced nodal category, who were considered to be at intermediate risk; patients with HPV-negative SCCs were considered to be at high risk, with the exception of non-smokers with tumours of stage T2 or T3, who were considered to be at intermediate risk<sup>16</sup>. These results were recently validated by an Italian single-institutional retrospective database<sup>103</sup>.

Some authors have argued that HPV status may reduce the overall prognostic significance of nodal category<sup>104</sup>. Recently, extra-capsular spread was shown to not be predictive of poor prognosis in surgically treated patients with p16<sup>INK4A</sup>-positive SCC of the oropharynx<sup>105</sup>.

Other biological markers have been recognized as prognostic factors in HNSCC. The HR HPV E6 and E7 oncoproteins target tumour suppressor signalling pathways. A major transforming property of HPV16 E6 is its ability to induce degradation of the tumour suppressor protein p53 via the ubiquitin pathway<sup>38</sup>. Furthermore, in transcriptionally active HPV infections, HPV16 E7 inactivates pRb. This event is associated with up-regulation of CDKN2A, which codes for p16<sup>INK4A</sup><sup>53</sup>. The absence of TP53 gene mutations is significantly associated with better overall survival and p16<sup>INK4A</sup> positivity, irrespective of HPV status, and is also associated with better outcomes. As a consequence, the survival benefit observed in HPV-induced HNSCC may not be the result of HPV positivity per se, but rather the result of the absence of TP53 gene mutations or CDKN2A deletion in HPV positive tumours, which are responsible for poor prognosis in HPV-negative patients<sup>11</sup>.

Another unclear aspect is the predictive nature of HPV positivity: is HPV status a prognostic marker, a predictive marker for response to a specific treatment or both? The available data support the hypothesis that HPV positivity results in a treatment-independent survival benefit<sup>106</sup>.

However, large randomized clinical trials including the stratification of patients according to HPV status are needed to provide a conclusive answer.

### Management of HPV-induced HNSCCs

Treatment of HPV-induced SCC is a pressing issue, as although there is no evidence from randomized, controlled trials to support a de-escalation of treatment intensity in HPV-positive oropharyngeal carcinomas, some investigators have argued that intensive concomitant chemoradiation regimens may represent overtreatment<sup>107 108</sup>. Actually, an aggressive multimodality strategy, which may result in high rates of acute and long-term severe toxicity, would be not appropriate for HPV-positive patients who are younger and have prolonged survival. In this context, most efforts are targeted toward de-escalation of treatment intensity in HPV-positive SCCs with the intent to reduce toxicity and thereby improve the long-term quality of life, while maintaining efficacy<sup>106</sup>.

A treatment de-escalation may be achieved by reducing the total dose of radiotherapy in a concurrent chemoradiotherapy setting by using radiotherapy and EGFR inhibitors instead of cis-platinum based chemoradiotherapy or radiotherapy alone instead of chemoradiotherapy, and primary surgery +/- de-intensified adjuvant treatment instead of up-front chemoradiotherapy. However, there are some challenges concerning a de-escalation strategy. A phase III non-inferiority trial for HPV-positive patients is considered difficult to conduct due to the large number of patients required<sup>109</sup>. Moreover, although HPV positivity results in a platform-independent survival benefit, the absolute superiority of any given platform is not yet known<sup>106</sup>. Currently, several randomized controlled clinical trials specifically designed to test the efficacy of a de-intensification strategy in HPV-positive patients are on-going (E1308, RTOG 1016, De-ESCALaTE HPV, NCT1088802/J0988, NCT01221753). These de-escalation protocols are mainly based on reduction in radiotherapy intensity (from 70 Gy down to 54 Gy) or on the substitution of cis-platinum with cetuximab in concurrent chemotherapy regimens. Treatment de-escalation strategies carry a risk of negatively impacting the overall favourable outcomes. Several investigators sustain that the more favourable prognosis in HPV-positive SCCs may be attributable to better compliance to chemoradiotherapy strategies. Furthermore, emerging data suggest that cetuximab-radiotherapy may not be the preferred therapy in patients with HPV-positive cancers<sup>110-113</sup>.

Trans-oral surgery is emerging as a feasible treatment option for early stage SCCs of the oropharynx. Minimally invasive trans-oral surgery can be performed by trans-oral laser microsurgery or trans-oral robotic surgery. The benefits of minimally invasive trans-oral surgery include low morbidity and mortality and excellent functional out-

comes. The FDA approved the use of trans-oral robotic surgery for T1 and T2 cancers of the oropharynx. Patients with HPV-induced SCC of the oropharynx may be the most ideal candidates for minimally invasive trans-oral surgery due to their younger age, early T category and long-term survival. Preliminary results have shown that trans-oral robotic surgery as a primary surgical modality, followed by adjuvant therapy as indicated, offers disease control in both HPV-negative and HPV-positive groups<sup>114</sup>. Very recently, a mono-institutional experience with definitive radiation alone for HPV-positive HNSCC confirmed the inherent radio-sensitivity of these tumours<sup>115</sup>.

Overall, there is insufficient evidence to treat HPV-positive SCCs with a de-intensified treatment strategy. This option should be restricted to controlled clinical trial settings with closely monitored safety assessments. Undoubtedly, it seems reasonable to exclude non-smoker patients with HPV-positive SCC from clinical trials using intensification of standard treatment. To date, the treatment of patients with HPV-positive OPSCC should not be different from standard treatment of patients with HPV-negative tumours. It should be based on stage of disease and the general conditions of the patient, maximizing the probability to treat early stage SCCs with a single modality therapy<sup>116</sup>.

### Conclusions

1. An increasing amount of OPSCCs, mostly arising from palatine and lingual tonsils, are caused by persistent HPV-16 infection.
2. Patients with HPV-driven OPSCCs are more likely to be middle-aged white men, non-smokers, non-alcohol abusers, and have a higher socioeconomic status and a better performance status than patients with HPV-unrelated OPSCCs.
3. Oral sex and open-mouthed kissing are associated with the development of oral HPV infection and OPSCC. In adult healthy people a 5-10% prevalence of oral HPV infection has been recorded; search for HPV sequences is not indicated in the absence of lesions.
4. HPV-induced SCCs are predominantly nonkeratinizing SCC often described as poorly differentiated carcinomas or basaloid carcinomas.
5. The typical clinical presentation of HPV-induced OPSCC is represented by an earlier T-category with a trend for a more advanced N-category than HPV-unrelated counterpart. Not uncommonly, HPV-induced SCC may clinically present as a neck metastasis from occult primary cancer; HPV-induced lymph-nodes metastases are predominantly cystic on imaging and often undergo sudden changes in volume.
6. HPV positivity results in a platform-independent survival benefit: overall, patients with HPV-induced SCC had a 54% better overall survival compared to HPV-negative patients.



7. Smoking may modify the clinical behavior of HPV-positive SCC, adversely affecting the prognosis of these neoplasms.
8. Although a de-escalation of treatment intensity is attractive in HPV-positive SCC, the current treatment of these patients should be not different from the standard treatment of patients with HPV-negative tumors and based on stage of disease and the general condition of the patient.
9. Standardization of diagnostic tests to correctly assign an oropharyngeal tumor to HPV is necessary, at present the gold standard in fresh samples is mRNA detection, in FFPE samples DNA detection with possibly a confirmatory IHC for p16 may be an appropriate diagnostic algorithm.

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