Human papillomavirus-independent cervical cancer

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ABSTRACT

Cervical cancer is the fourth most frequent cancer in women worldwide, representing nearly 8% of all female cancer deaths every year. The majority of cases of cervical cancer are caused by human papillomavirus (HPV); however, up to 5% of tumors are not associated with HPV-persistent infection and, moreover, the new WHO Female Genital Tumors classification subdivided cervical squamous and adenocarcinomas into HPV-associated and HPV-independent tumors. Based on this new information, the aim of this review is to provide an overview of HPV-independent cervical cancer, evaluating diagnostic techniques, molecular profiles, and clinical outcomes. The HPV-independent tumors are characterized by a differentiated molecular profile with lower proliferative activity, a p53 immunostaining, a decreased expression of cyclin-dependent kinase inhibitor proteins, such as p16, p14, and p27, and alterations in *PTEN, p53, KRAS, CTNNB1, ARID1A,* and *ARID5B*. HPV-independent tumors are associated with both adenocarcinomas and squamous histologic subtypes, with lymph node involvement in the early stages, more distant metastasis, and generally worse oncological outcomes. Thus far, no specific therapeutic strategies have been developed based on HPV status; however, with advancing knowledge of differences in the molecular profiles and possible targetable alterations, novel approaches may offer potential options in the near future. Investigators should report on clinical outcomes, evaluating the overall response rates to specific treatments, and consider new biomarkers to establish more accurate prognostics factors.

INTRODUCTION

Cervical cancer is the fourth most frequent cancer in women, with 604 127 new cases in 2020 and more than 341 831 deaths, representing nearly 8% of all female cancer deaths every year.[1] Of the estimated incidence and mortality from cervical cancer, approximately 84% of all cases and 88% of all deaths occurred in low- and middle-income countries.[2] Human papillomavirus (HPV) is a sexually transmitted virus that, if it establishes a persistent infection with high-risk genotypes, such as HPV 16 and 18, there is high association with cervical cancer.[3] Both of the HPV sub-types jointly cause 70–75% of all cervical cancers and 40–60% of its precursor lesions.[2]

Epidemiological studies report that almost all cases of cervical cancer are caused by HPV[3]; however, approximately 5% of tumors are not associated with HPV-persistent infection.[4] In 2009, zur Hausen stated that although more than 95% of cervical cancer biopsies contain high-risk HPV genomes, this does not necessarily imply that all of these tumors are caused by the infection.[5] A meta-analysis involving 40 679 women with cervical cancer from 229 studies, that used broad-spectrum consensus polymerase chain reaction (PCR) assays based on the primers MY09/11, PGMY09/11, GP5+/6+, SPF10, SPF1/GP6+, or L1C1/L1C2, reported that 10.6% (8.4–13.9%) of cases were HPV-negative and this percentage varied with geographic location.[6]

In 2020, the WHO updated the Female Genital Tumors classification (5th edition) and recognized that a proportion of cervical cancers are not associated with HPV infection, especially adenocarcinomas.[7] Based on this statement the Tumor Editorial Board subdivided the cervical squamous lesions into HPV-associated and HPV-independent tumors, and adenocarcinomas into HPV-associated, including (1) usual type: villoglandular variant; (2) mucinous type: mucinous not otherwise specified (NOS) adenocarcinoma, intestinal adenocarcinoma, signet-ring cell adenocarcinoma, and stratified mucin-producing adenocarcinoma, and HPV-independent tumors, including (1) gastric type adenocarcinoma; (2) clear cell adenocarcinoma; (3) mesonephric adenocarcinoma; and (4) endometrioid adenocarcinoma.[7]

The aim of this review is to provide an overview of HPV-independent cervical cancer, evaluating diagnostic techniques, molecular profiles, and clinical outcomes.

HPV Tests: Screening and Genotyping

HPV-independent cervical cancers are clinically relevant due to their biological behavior and possible worst prognosis. HPV-negative status may be associated with different potential scenarios: (1) HPV-independent (true negative) cancers, such as some subtypes of adenocarcinomas and a few cases of squamous carcinoma; (2) loss of the HPV genome during the integration process; (3) presence of viral genotypes not included in the molecular tests; (4) failure in detection of the diagnostic method employed; or (5) misclassification of cancers as primary cervical (metastases or primary uterine corpus neoplasms).[4 8 9]

Table 1 HPV test FDA approved for cervical cancer screening[16 17 24]

Commercial name	Assay type	Genotypes	Target genes	Analytical sensitivity	Sensitivity % (95% CI)	Specificity % (95% CI)
Hybrid Capture System 2*[1]	Signal amplification	13 HR-HPV 5 LR-HPV	Whole viral genome	1000–5000 copies/reaction	92.7 (85.6 to 97.0)	39.4 (33.1 to 46.0)
Cervista-HR*[2]		14 HR-HPV	*L1/E6/E7*	1250–7500 copies/reaction	92.8 (83.9 to 97.6)	46,0 (44.2 to 47.0)
Cervista 16/18[3]		16, 18	*L1/E6/E7*	625–1200 copies/reaction	77.3 (56.6 to 89.9)	67.3 (63.9 to 70.6)
Cobas HPV Test[4]	DNA amplification	16, 18 + 12 HR-HPV	*L1*	150–1200 copies/mL	93.5 (82.5 to 97.8)	69.3 (66.9 to 71.5)
BD Onclarity HPV assay[5]		16, 18, 45 + 11 HR-HPV	*E6/E7*	251–2367 copies/mL	91.4 (77.6 to 97.0)	62.0 (59.6 to 64.4)
APTIMA HPV assay[6]	mRNA amplification	16, 18, 45 + 11 HR-HPV	*E6/E7*	19–239 copies/reaction	97.7 (92.0 to 99.4)	52.9 (49.1 to 56.6)

*Does not determine specific type. 1: Qiagen, USA; 2: Hologic Inc, USA; 3: Hologic Inc, USA; 4: Roche Molecular Systems Inc, USA; 5: Becton Dickinson, USA; 6: Hologic Inc, USA.
FDA, US Food and Drug Administration; HPV, human papillomavirus; HR-HPV, high risk HPV; LR, low risk HPV.

The WHO initiative on preventive strategies for eradication of cervical cancer include HPV vaccination in combination with the implementation of effective screening programs with HPV-based testing for risk estimation of CIN3 +, and the proper management of pre-invasive lesions and cervical cancer.[10 11] Therefore, it is important to select an appropriate and validated test in terms of clinical accuracy, reproducibility, and cost-effectiveness before screening implementation.[12–14] In general, molecular tests are widely used in epidemiological studies, during HPV surveillance, and in monitoring the impact of HPV vaccination.[15]

HPV testing is a highly sensitive technique with high negative predictive value (97.9–99.3%)[12 16]; however, the optimal performance of an HPV test depends on a large number of factors such as sample collection, nucleic acid extraction methodology, primers, and use of internal controls.[17] The most commonly used methods to detect the HPV genome are based on PCR and the use of hybridization probes targeting the *L1* gene, as this is the most conserved gene in the HPV genotypes.[18] These tests are highly sensitive and specific (Table 1); however, they may not be capable of detecting HPV genomes that do not specifically bind to the designed primers and probes, and therefore a viral genotype that diverges in genomic sequence from the designed primer/probe sequences may escape amplification and/or hybridization and remain undetected.[19–21]

There are currently commercial tests approved by the US Food and Drug Administration for cervical cancer screening based on viral DNA amplification and mRNA amplification. Another group include signal amplification systems (Table 1). Signal amplification methods have a lower sensitivity than DNA amplification methods and may cause false negatives, especially in cases where the viral load is low. In addition, the absence of an internal control increases the proportion of false negatives, likely due to degradation of the viral genome.[22 23] Most PCR-based tests only amplify the *L1* region of the virus. Therefore, PCR false negatives may be associated with the loss of this region during the viral integration process[24]; whereas the *E6/E7* mRNA expression evaluation could be associated with the presence of a high-grade lesion or cervical cancer, since it is known that the *E6/E7* mRNA proportion increases after integration of the viral genome into host cells.[24]

Due to variations in the methodological approaches used to detect HPV, different primers, and diverse sensitivities and specificities, Petry et al[25] recommend the use of an additional PCR-based test as a part of the differential diagnosis of possible HPV-negative cervical cancer. However, when HPV detection fails by the conventional methodologies, other molecular techniques such as high-throughput sequencing can be used to identify the specific genotype in case of HPV infection. Likewise, if the cDNA is sequenced, the data can show whether there is transcriptional activity of the virus, which is fundamental in both the initiation and maintenance of the malignant phenotype.[26 27] The evidence shows that in cases of re-testing of suspected HPV-independent tumors, especially those performed with deep sequencing, between 48–57% of cervical cancer samples with a negative result by PCR remain truly negative[28] both in cases of adenocarcinomas and squamous cell carcinomas.

Molecular Profile of HPV-Independent Tumors

The HPV carcinogenesis associated with the development of cervical cancer is well described[3]; however, the mechanism associated with HPV-independent cancers is unclear.[29] Several studies have evaluated the differential gene expression between the HPV-associated and HPV-independent cervical cancers.[4 30 31] There are differences in the expression of markers between HPV-positive and HPV-independent tumors, evaluating cell proliferation markers such as PCNA[32] and Ki67[33]; tumor suppressor proteins such as p53,[33–37] p16,[35–38] p14, p21, and p27[36]; and proto-oncogenes such as epidermal growth factor receptor (EGFR),[33] c-myc,[34] and c-Erb-2.[33]

The HPV-independent tumors have a lower proliferative activity, suggesting that the viral infection induces an increased cellular proliferation.[32] Additionally, HPV-independent tumors show p53 nuclear immunostaining, and thus a useful marker in the differentiation of the viral independent tumors.[33–36] Nicolás et al[37] reported that tumors with an HPV-negative result showed a high rate of p53 abnormal (p53abn) immunostaining pattern, suggesting a mutational phenotype associated with the capacity of tumor deregulation, with increased growth potential and metastasis. Finally, HPV-positive tumors show increased expression of cyclin-dependent kinase (CDK) inhibitor proteins, such as p16, p14, and p27,[36] as a surrogate marker of HPV infection.[38]

With the development and implementation of novel molecular techniques, the comparison of genetic profiles between HPV-associated and HPV-independent tumors has been possible.[4 30 31 39 40] *WIG-1* is a p53-regulated gene that encodes a transcription factor. *WIG-1* can interact with heterogeneous nuclear ribonucleoprotein (hnRNP A2/B1), RNA helicase A, and double strand RNA (dsRNA), which plays an important role in RNA and protein stabilization.[41] *WIG-1* is frequently amplified in tumors, including cervical cancer.[39] *WIG-1* mRNA expression was higher in the HPV-independent cervical cancer cell lines (C33-A and HT-3) than in the HPV-positive cell lines, suggesting a possible role of *WIG-1* in HPV-negative cervical carcinogenesis. The authors reported statistically significant higher *WIG-1* protein staining intensity in HPV-independent cervical cancer tumors compared with HPV-associated tumors, both in squamous (p=0.002) and in adenocarcinomas (p=0.049).[39]

Differences in expression levels of miRNAs—a class of small non-coding RNA molecules that regulate key cellular processes—between high risk-HPV E6/E7 mRNA positive and high risk-HPV E6/E7 mRNA negative cervical cancer tissue samples have been evaluated. While miR-9 was downregulated,[40] miR-21 and miR-155[31] were upregulated in high risk-HPV E6/E7 mRNA negative cancer tissue samples. The miRNA regulation mechanism involves high risk-HPV E6/E7 proteins; therefore, the absence of these proteins could be deregulating the expression of miR9, miR21, and miR155, impacting regulation of metastasis, cell proliferation, inflammation-associated carcinogenesis, and tumor metabolism.[31 40]

The Cancer Genome Atlas (TCGA) Research Network[4] reports that HPV-independent cervical cancer encompassed a distinct subgroup within the CpG island hypermethylated (CIMP)-low cluster, with a lower mean promoter methylation, typically observed on healthy epithelial tissue. Functional epigenetic analysis showed differential subnetworks for HPV-associated and HPV-independent tumors, with one common subnetwork centered around *Forkhead Box A2* (*FOXA2*) gene (high DNA-methylation and low gene expression in HPV-positive cases). HPV-independent tumors also have a lower activation of NF-κB, p53, and MAPK signaling, a significantly higher epithelial-mesenchymal transition (EMT) mRNA score, and a lower frequency of APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) mutagenesis signature, and are characterized by mutations in *KRAS, ARID1A,* and *PTEN*.

Liu et al[30] identified 17 differentially expressed genes between HPV-positive and HPV-negative tumors. Following mRNA and protein level determinations, the authors reported seven genes with significantly higher expression in HPV-negative cervical cancer cells and tissues than in HPV-positive cervical cancer and normal cells or tissues. Particularly, *MEX3A,* an RNA binding gene, and *TTYH3,* a chloride-channel-responsive gene, correlated with shorter overall survival of patients with HPV-independent cervical cancer, representing a possible new therapeutic target.

Based on the expression of HPV *E6/E7* oncogenes, Banister et al[42] classified cervical tumors into HPV-active and HPV-inactive, based on the transcriptional state of the mRNA. The HPV-inactive group is associated with lower DNA methylation levels and therefore overexpression of several genes. According to the non-synonymous and synonymous mutation profile, the cancer driver genes *PTEN, p53, CTNNB1, AKT, ARID1A,* and *ARID5B* tend to be mutated, independently of the APOBEC pathway, suggesting that HPV-inactive tumors use alternative pathways to sustain tumor growth; additionally, the expression of inflammatory associated genes is decreased.

Clinical Outcomes of HPV-Independent Tumors

Currently, the proposed first-line treatment for early stages of cervical cancer (stage IA1 with lymph vascular space invasion to IB2 and IIA1 International Federation of Gynecology and Obstetrics (FIGO) 2018) is an open radical hysterectomy with pelvic lymph node assessment.[43 44] Adjuvant treatment with chemoradiotherapy may be necessary based on pathologic findings. For advanced stages (stage IB3, IIA2 to IVA FIGO 2018) the standard treatment is concomitant platinum-based chemoradiotherapy, and for metastatic disease (IVB FIGO 2018) platinum-based therapy with bevacizumab.[43–45]

Primary treatment of cervical cancer is based on clinical, imaging, and pathological results. However, there is no specific treatment based on histological type, genomic alteration or HPV status defined in the current guidelines. Several studies have reported that patients with HPV-independent tumors could have a worse prognosis than HPV-associated tumors; however, the clinical impact of HPV detection to determine treatment is still not clear.[46–49] There is no prospective evidence evaluating the outcomes of patients with HPV-independent cervical cancer.

A retrospective cohort study of 136 patients[50] with cervical cancer, including squamous cell carcinoma and adenocarcinoma, showed that of 14 initially HPV-independent tumors, determined by the Hybrid Capture system (Qiagen, USA), only eight were confirmed by PCR. These patients had a worse disease-free survival (51.9 vs 109.9 months; p=0.010) and this was considered a prognostic factor even after multivariate analysis. The authors found that despite being more common in adenocarcinomas, these poor outcomes were also demonstrated in non-keratinizing squamous histological types.[51]

Some additional retrospective studies analyzed the association between HPV negativity and oncological outcomes. In a study including 248 patients—108 patients who underwent surgery and 140 patients treated with chemoradiation—Chong et al[52] reported that 18.5% of cervical cancers were HPV-independent and those tumors were associated in a multivariate analysis with poorer disease-free survival when compared with HPV-associated tumors (HR 3.97, 95% CI 1.84 to 8.58; p=0.0005). Several reports have demonstrated a similar pattern in patients with HPV-associated head and neck squamous cell carcinoma, showing greater radiosensitivity and better prognosis, and this is strongly related to the molecular differences between HPV-associated and HPV-independent tumors.[53] Another retrospective analysis included 214 tumors,[37] classified as squamous cell carcinoma, adenocarcinoma, adenosquamous, or neuroendocrine. Using reverse hybridization for HPV genotyping and p16 immunostaining, the authors found a 10% rate of HPV-independent tumors. Patients with HPV-independent tumors had higher rates of lymph node invasion (67% vs 36%, p<0.01) and worse disease-free survival (59.8 vs 132.2 months, p<0.01) and overall survival (77.0 vs 153.8 months, p=0.01) compared with women with HPV-associated tumors. However, only advanced FIGO stage and lymph node metastases after multivariate analysis were associated with a poor prognosis.

A recent retrospective multicenter study evaluating prognostic biomarkers analyzed 464 cases with IB endocervical

adenocarcinomas using the International Endocervical Adenocarcinoma Criteria and Classification system and no molecular tests. They identified on multivariate analysis that the HPV-independent status was associated with worse recurrence-free survival (HR 2.31, CI 95% 1.02 to 5.46; p=0.05). The other associated factors for this cohort were the lymph vascular invasion and the presence of lymph node metastasis.[54]

Finally, in a re-testing study[55] including FIGO stage I–IV of 37 initially HPV-negative samples (corresponding to 14% of all the analyzed tumors), including squamous cell carcinoma and adenocarcinomas, only half were confirmed as HPV-independent. These tumors had a worse cancer-specific survival at 5 years (27% vs 69%, p=0.009) and a lower recurrence rate, although this was non-significant (27% vs 50%, p=0.061). A systematic review and meta-analysis was recently published exploring the value of HPV status in patients with cervical cancer.[56] The analysis of 17 retrospectives studies including 2838 patients showed that the oncological outcomes of patients with HPV-associated cancers were different. The overall survival was higher in this population (HR 0.610, 95% CI 0.457 to 0.814; p=0.001), as was the disease-free survival (HR 0.362, 95% CI 0.252 to 0.519; p<0.001), compared with HPV-independent cancer patients. This review has some limitations, given the lack of a registered protocol, the absence of a methods section, and the performance of meta-analysis even when high heterogeneity was present. It also has to be mentioned that the methods for HPV detection and the source of tissue varied through the different primary studies.

The American Joint Committee on Cancer (AJCC)[57] in its 9th edition, within the key modifications for cervical cancer, suggested defining HPV status as associated or independent, considering the evidence describing worse oncologic outcomes in the HPV-independent tumors. Despite the modest evidence that defines pathological and clinical characteristics of these tumors, the determination of HPV status prior to the start of treatment could be a useful tool for discussion of disease prognosis and potentially for establishing closer surveillance in these patients. It also encourages further research with the aim of determining carcinogenesis and biological behavior that might lead to personalized treatment and improved oncological outcomes.

Etiology of HPV-Independent Tumors

Thus far, it is difficult to explain the development of HPV-independent tumors, but the 'hit and run viral theory' could explain the absence of the viral genome in these cases. Viruses associated with human cancers promote an inflammatory process, change the microenvironment and cellular metabolism, and are associated with genomic instability. The 'hit and run theory' proposes that once a viral infection has caused sufficient cellular alteration, expression of viral proteins or viral infection is no longer required for tumor maintenance, and, consequently, the virus may be lost during cancer progression (Figure 1).[58 59]

It has been proposed that the *E6/E7* oncogenes start the process of carcinogenesis, but as the mutations accumulate over time, transcription of the viral genes is no longer necessary and therefore they are lost.[59] Additionally, it has been proposed that the 'hit and run' theory of oncogenesis may also leave permanent traces through epigenetic dysregulation. Chromatin remodeling may expose hotspots for viruses to impair transcriptional regulation, DNA repair, and permanent epigenetic alterations in the infected cell, as E7 HR-HPV oncoprotein that stimulates DNA methyltransferase 1 (Dnmt1) activity.[60] During the 'hit and run' process, the transient but regular presence of viral genomes or parts thereof in a pre-invasive stage of the respective tumor would be considered; thus the initial persistent infection with HPV in pre-invasive lesions could be the necessary hit for the development of HPV-independent cervical cancer after the viral run.[60] The existence of HPV-independent pre-invasive lesions has not been established thus far. However, it was recently reported that two of three HPV-independent pre-invasive

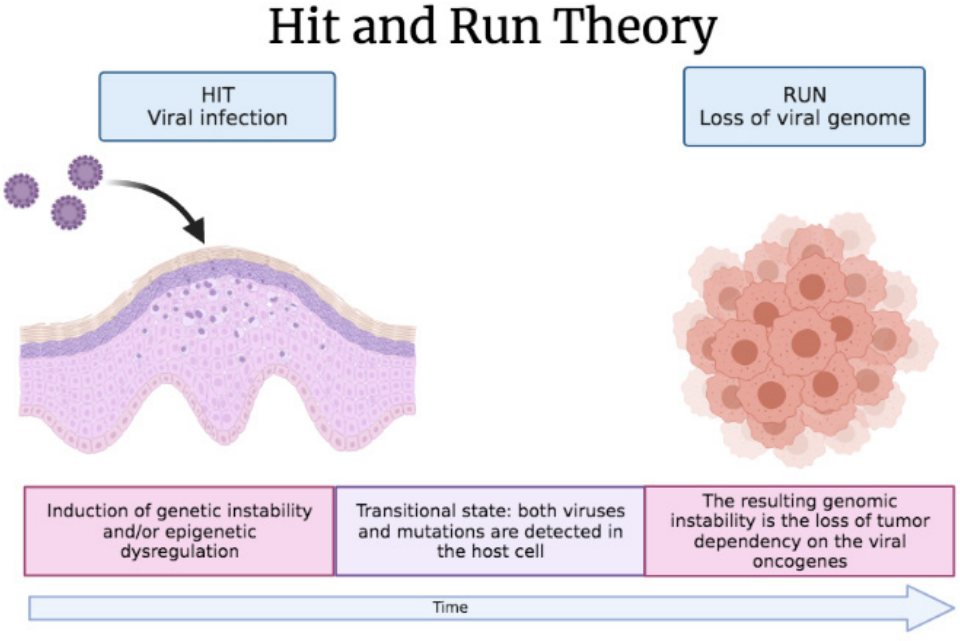

Figure 1 Schematic representation of the hypothetical hit and run mechanism. Created with BioRender.com. Adapted from Ferreira et al[59] and Niller et al.[60]

Table 2 HPV-independent cervical cancer reports

Author	Sample histology	Negative cases N (%)	Negative results according to sample histology	Technique
Riou et al[48]	89 SQ, 17 ADC	17/106 (16.03)	15 SQ, 2 ADC	Southern blot, PCR
Shikano et al[32]	39 ADC	14/39 (14.35)	14 ADC	CISH
Brewer et al[34]	30 LCNK, 23 LCK, 7 ADC, 5 ADS, 1 CC	5/66 (7.57)	Not reported	MY09/11 – *E6*
Lo et al[51]	107 SQ, 14 ADC	26/121 (21.48)	23 SQ, 3 ADC	MY09/11 - sequencing
Kedzia et al[33]	47 SQ	15/47 (31.91)	15 SQ	MY09/11 – *E6*
Park et al[35]	26 ADC unusuals	26/26 (100)	26 ADC unusuals	SPF10-LiPA system
Xu et al[39]	13 SQ, 25 ADC	17/38 (44.73)	4 SQ, 13 ADC	Multiplex PCR
Liu et al[40]	89 SQ, 10 ADC, 2 CC, 1 undifferentiated	14/101 (13.86)	Not reported	*E6*/*E7* mRNA
Rodríguez-Carunchio et al[50]	104 SQ, 32 ADC	8/136 (5.88)	3 SQ, 5 ADC	HC2, SPF10-LiPA system, GP5+/6+, *E7*-specific assay
Omori et al[36]	36 ADC usual, 8 ADC gastric	14/44 (31.81)	6 ADC usuals, 8 ADC gastrics	ISH, PCR
Park et al[31]	50 SQ, 2 ADC	15/52 (28.84)	Not reported	*E6*/*E7* mRNA
Burk et al[4]	144 SQ, 31 ADC, 3 ADS	9/178 (5.05)	4 SQ, 5 ADC	RNA seq
Banister et al[42]	212 SQ, 44 ADC, 5 ADS	20/261 (7.70)	10 SQ, 8 ADC, 2 ADS	RNA seq (*E6E7*/total genes)
Chong et al[52]	210 SQ, 38 ADC/ADS	46/248 (18.50)	33 SQ, 13 ADC/ADS	PAN Array HPV Genotyping Chip/Anyplex II HPV28
Nicolás et al[37]	168 SQ, 39 ADC, 4 ADS, 3 neuroendocrines	21/214 (9.81)	12 SQ, 6 ADC, 1 ADS, 2 neuroendocrines	SPF10-LiPA system
Kaliff et al[55]	169 SQ, 27 ADC, 4 ADS, 2 neuroendocrines	14/202 (6.9)	3 SQ, 9 ADC, 2 ADS	Anyplex II HPV28, RT-PCR *E6*/*E7*

ADC, adenocarcinoma; ADS, adenosquamous; CC, clear cells; CISH, chromogenic in situ hybridization; HPV, human papillomavirus; ISH, in situ hybridization; LCK, large cell keratinizing; LCNK, large cell non-keratinizing; PCR, polymerase chain reaction; SQ, squamous.

cervical lesions showed diffuse p16^{ink4a} staining, similar to the pattern shown in HPV-associated lesions. The authors excluded somatic or germline mutations in the *RB* gene or the *CDKN2A* gene encoding the p16^{ink4a} protein,[61] which could suggest the action of the 'hit-and-run' mechanism.

NEW PERSPECTIVES

With the development of new technologies for HPV detection, the detection rate of HPV-negative cases has decreased.[9] However, several studies continue reporting HPV-independent cervical cancer through different methodologies, including deep sequencing, both in cases of adenocarcinomas and squamous cell carcinomas (Table 2). Additionally, the distinctive molecular profile of these HPV-independent tumors provides information regarding the presence of tumors with a different biological behavior, mediated by the alteration of signaling pathways independent of viral infection, and highlighting alterations in *PTEN, KRAS, p53, CTNNB1, ARID1A,* and *ARID5B*.[4 42] With these data, further investigations based on the evaluation of these proteins as tumor markers in cases of HPV-independent cervical cancer could have some implications for treatment.

A patient with cervical cancer with an HPV-negative test may constitute a biologically distinct subgroup, which may be associated with advanced FIGO stage and a poor prognosis, and may require a different therapeutic strategy.[24 42 62] It is well known that in HPV-independent oropharyngeal cancer, the response rate to chemotherapy and radiation treatment is lower than in HPV-associated cases.[53] Therefore, the lower progression-free survival and overall survival of HPV-independent cervical tumors may be associated with low responses to current standard treatment. However, to date there are no current data to support this hypothesis. Banister et al[42] proposed that, due to the somatic mutations shown by HPV-independent tumors, PI3K/mTOR inhibitors and tyrosine kinases inhibitors (dasatinib) may improve the response rate in these patients.

The lower expression of inflammatory associated genes[42] suggest that HPV-independent cervical cancers may have a worse response rate to checkpoint inhibitors-based immunotherapy, such as programmed death protein 1/programmed cell death ligand 1 (PD1/PD-L1) inhibitors. The KEYNOTE-028 trial[63] and CHECKMATE-358 trial[64] demonstrated that patients with HPV-associated cervical cancer (squamous cell type) had improved outcomes due to an elevated proportion of TCD8 + infiltrating lymphocytes (TILSs) and PD-L1.[65 66] However, Chen et al[67] reported no significant difference in PD-L1 expression among different histologic types of endocervical adenocarcinomas.

CONCLUSIONS

HPV-independent cervical cancer constitutes a unique biological entity with a different molecular profile when compared with HPV-associated tumors. The absence of p16 and the presence of founder mutations in genes such as *p53, KRAS, ARID1A,* and *PTEN*

characterize the HPV-independent tumors; thus the WHO recommends the use of HPV testing and p16 immunostaining for differentiation between HPV-associated and HPV-independent cervical cancer. HPV-independent tumors are associated with both adenocarcinomas and squamous histologic subtypes, with lymph node involvement in early stages, more distant metastasis, and generally worse oncological outcomes. However, there is no prospective information available that evaluates different interventions according to HPV status that will lead to changing clinical practice yet, and there is no specific treatment based on HPV status. There is need for future research, encouraging investigators to report on clinical outcomes, evaluating the overall response rates to specific treatments, and to consider new biomarkers to establish more accurate prognostics factors.

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