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 adenoscarcinomas 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 adenoscarcinomas 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.4 Epidemiological studies report that almost all cases and 80% 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. The HPV sub-types jointly cause 70–75% of all cervical cancers and 40–60% of its precursor lesions. Epidemiological studies report 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 adenoscarcinomas.7 Based on this statement the Tumor Editorial Board subdivided the cervical squamous lesions into HPV-associated and HPV-independent tumors, and adenoscarcinomas into HPV-associated and HPV-independent tumors, including (1) usual type: villoglandular variant; (2) mucinous type: mucinous not otherwise specified (NOS) adenoscarcinoma, intestinal adenocarcinoma, signet-ring cell adenoscarcinoma, and stratified mucin-producing adenoscarcinoma; and (3) mesonephric adenocarcinoma; (4) endometrioid adenoscarcinoma.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 adenoscarcinomas 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).8


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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. Therefore, it is important to select an appropriate and validated test in terms of clinical accuracy, reproducibility, and cost-effectiveness before screening implementation. In general, molecular tests are widely used in epidemiological studies, during HPV surveillance, and in monitoring the impact of HPV vaccination.

HPV testing is a highly sensitive technique with high negative predictive value (97.9–99.3%) 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. 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. 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.

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. 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; 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.

Due to variations in the methodological approaches used to detect HPV, different primers, and diverse sensitivities and specificities, Petry et al 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. 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 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; however, the mechanism associated with HPV-independent cancers is unclear. Several studies have evaluated the differential gene expression between the HPV-associated and HPV-independent cervical cancers. There are differences in the expression of markers between HPV-positive and HPV-independent tumors, evaluating cell proliferation markers such as PCNA and Ki67; tumor suppressor proteins such as p53 and p16, p21, and p27; and proto-oncogenes such as epidermal growth factor receptor (EGFR), c-myc, and c-ErbB-2.

The HPV-independent tumors have a lower proliferative activity, suggesting that the viral infection induces an increased cellular proliferation. Additionally, HPV-independent tumors show p53 nuclear immunostaining, and thus a useful marker in the differentiation of the viral independent tumors. Nicolás et al 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, as a surrogate marker of HPV infection.
With the development and implementation of novel molecular techni
tiques, the comparison of genetic profiles between HPV-associated
and HPV-independent tumors has been possible.4 36 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 processesa—
between high risk-HPV E6/E7 mRNA positive and high risk-HPV E6/
E7 mRNA negative cervical cancer tissue samples have been evalu-
ated. While miR-9 was downregulated,40 miR-21 and miR-15541
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.51 40

The Cancer Genome Atlas (TCGA) Research Network4 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 differen-
tial 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 higher
activation of NF-kB, 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 characte-
erized by mutations in KRAS, ARID1A, and PTEN.

Liu et al50 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, repre-
senting a possible new therapeutic target. Based on the expression of HPV
E6/E7 oncogenes, Banister et al42 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.33 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 meta-
static disease (IVB FIGO 2018) platinum-based therapy with beva-
cizumab.53 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 prog-
nosis than HPV-associated tumors; however, the clinical impact of
HPV detection to determine treatment is still not clear.46–48 There is
no prospective evidence evaluating the outcomes of patients with
HPV-independent cervical cancer.

A retrospective cohort study of 136 patients39 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 prog-
nostic 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 associa-
tion 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 al43 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 radio-
sensitivity 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 prog-
nostic biomarkers analyzed 464 cases with IB endocervical
tumors and demonstrated a significant difference in the outcomes
between HPV-negative and HPV-positive tumors.52 Several studies
have shown that the use of HPV testing is associated with improved
outcomes in patients with cervical cancer.53–55 This is consistent with
the findings of the current study, which showed that HPV-negative
tumors had a worse prognosis compared with HPV-positive tumors.

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 study55 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 retrospective 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 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

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**Figure 1** Schematic representation of the hypothetical hit and run mechanism. Created with BioRender.com. Adapted from Ferreira et al59 and Niller et al.60
cervical lesions showed diffuse p16INK4A 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 or the CDKN2A pattern shown in HPV-negative cases. Furthermore, the presence of tumors with a different biological behavior, mediated by alterations in signaling pathways independent of viral infection, such as programmed death protein 1/programmed cell death ligand-1 (PD-1/PD-L1) inhibitors, suggests 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 and CHECKMATE-358 trial 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 expression. However, Chen et al. 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 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. With these data, further investigations based on the evaluation of these proteins as tumor markers in cases of HPV-negative tumors, PI3K/mTOR inhibitors and tyrosine kinases inhibitors (dasatinib) may improve the response rate in these patients. The lower expression of inflammatory associated genes 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 and CHECKMATE-358 trial 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 expression. However, Chen et al. reported no significant difference in PD-L1 expression among different histologic types of endocervical adenocarcinomas.
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 prognostic factors.

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REFERENCES
16 Chrysoffomou AK, Kostrikis LG. Methodologies of primary HPV testing currently applied for cervical cancer screening. Life 2020;10:290.

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