Abbreviations

CxCa: Cervix Cancer; CIN: Cervical Intraepithelial Neoplasia; HWE: Hardy-Weinberg Equilibrium; HNSCC: Head and Neck Squamous Cell Carcinoma; HPV: Human Papillomavirus; OPSCC: Oropharyngeal Squamous Cell Carcinoma; SNP: Single Nucleotide Polymorphism

Introduction

Cancer is a complex, multistep process involving environmental, lifestyle, and genetic predisposing factors in addition to possible infectious agents that promote carcinogenic transformation [1]. Approximately, 18% of cancers worldwide are related to infectious diseases [1,2]. This proportion varies in different regions of the world from high of 25% in Africa to less than 10% in the developed world [1]. In addition to viruses, certain bacteria and parasites are also suspected to have a carcinogenic effect. It has been suggested that viruses are one of the most important risks factor for cancer development in humans, second only to tobacco use [3]. Although many specific viruses were associated with particular tumors, Human Papillomavirus (HPV) stands out as being implicated in several types of human carcinomas including not only cervix uterine and other anogenital cancers but also subgroup of head and neck cancers as roughly 96%, 60% and 30% of these malignancies are positive for HPV [4-6].

HPV are groups of DNA-viruses that affect skin and moist membranes in the body. Currently there are about 120 genotypes that infect human. Infection is common and the virus is primarily transferred by direct contact between infected skin to skin or to mucous membranes. Around 40 types of HPV viruses affect the genital area and risk of contracting infection increases during the active sexual life. High risk HPV genotypes are the primary
cause of cervical cancer (CxCa) and the incidence of this tumor is regarded as a surrogate marker for HPV infection particularly in countries lacking epidemiological studies [7].

The turning points in prevention to reduce the associated cancer risk are cytological screening for precancerous lesions, molecular screening for high-risk HPV infection and the availability of vaccines against the most common HPV genotypes currently in use in many developed countries [8]. In contrast, basic health care is often lacking in disadvantaged populations such as in resource-limited countries, marginalized and immigrants due to shortage of point-of-care access and the complexities of social and economic situations [9]. As a result, there is a tremendous disparity in health consequences for HPV-infected people, as compared to advantaged populations [10]. Therefore, initiatives were proposed to highlight these differences so that health authorities can be more aware of this global cancer disparities.

It was intriguing to note that, although there has been significant reduction of the incidence of Head & Neck cancers in US and North America as a result of the antismoking campaigns since the late eighties, there was a significant increase in the incidence of oropharyngeal squamous cell carcinomas in young (40-55 years) specifically in the tonsils and tongue base where most of these patients are not alcohol or tobacco consumers [11,12]. About 60% of these tumors were found positive for HPV16, the same type that leads to HPV-associated anogenital cancers. In a systematic meta-analysis, Kreimer et al., have reviewed 60 eligible studies that included 5046 cases of squamous cell carcinomas of the head and neck [13]. HPV was prevalent in 35.6% of or pharyngeal, in 23.5% of oral and in 24% of laryngeal cancers. HPV16 was by far the commonest subtype in all HPV positive cancers (87% of oropharyngeal, 60% of oral and 69% of laryngeal cancers). HPV18 was the next most common subtype. Such a wider implication of HPV involvement beyond a genital cancers is important for health authorities even in countries where CxCa is not a major concern [7].

HPV has more recently been implicated in subgroups of colorectal and breast malignancies, as roughly 42% and 23% of these cancers are positive for HPV, respectively [14-16]. The extrapolation of these rough estimated percentages of HPV-positive Head & Neck, colorectal and breast cancers in countries with low incidence of CxCa [7], such as Saudi Arabia is given in Figure 1. In total, potential HPV-related cancers would represent about 10% of all cancers in both genders. We have reviewed the evidence for HPV involvement in breast and colorectal cancers from related publications and results are summarized in Table 1. Although some studies were negative, the average percentage of HPV-positive samples were 26% and 33% for colorectal and breast cancers, respectively.

**HPV risk factor and mechanisms of tumor genesis**

HPV is the most prevalent viral infection among both men and women worldwide. The current data base contains 120 different HPV types that do not share the same properties [17]. It is estimated that 80% of sexually active adults have been infected with at least one HPV type [18]. Its association with the cancer of uterine cervix has long been recognized by the medical community [17-19]. The HPV are grouped according to their association with CxCa and their genomic sequence into: oncogenic high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), probable oncogenic high-risk types (HPV 26, 53 and 66), and non-oncogenic low-risk types (HPV 6, 11, 42, 43, 44, 54, 61, 70, 72, 81 and 89). Low-risk HPVs are primarily found in genital warts, while high-risk HPVs are found in CxCa and precancerous lesions [20]. *In vitro* experimental studies have shown that high-risk HPV can transform human cells in tissue culture [21,22].

Oncoproteins E6 and E7 of high-risk HPV have a high affinity for binding and degrading p53 and pRb, resulting in essentially immortal cells. In contrast, the E6 and E7 proteins of low-risk HPVs do not bind p53 or pRb at detectable levels and have no effect on p53 or pRb stability *in vitro* [23]. During carcinogenesis the oncoproteins E6 and E7 of high-risk HPVs interact with host cellular proteins through different pathways, particularly the p16INK4A-cyclin D1-CDK4/6-pRb-E2F and p14ARF-MDM2-p53 pathway [24,25]. This interaction subverts cell cycle checkpoints, resulting in cell over-proliferation, genome instability, and carcinogenesis [26]. p14ARF and p16INK4A arise from the same gene and are strongly associated with HPV-positive CxCa [27]. High-risk HPV E6 binds to p53 and degrades it through the ubiquity pathway. P14ARF act as a cell cycle regulator, inhibiting MDM2 proteins and blocking the formation of MDM2-p53. This prevents the degradation of p53 by MDM2 [28]. High-risk HPV E7 binds to and degrades pRb, and releases the cellular transcription factor E2F, leading to increased expression of cyclin A, cyclin E and p16INK4A [29]. Recently, over expression of p16 is considered sufficient for HPV diagnostic and suggested to be used as prognostic factor for treatment outcome in head and neck squamous cell carcinoma (HNSCC) according to the recently published Head and Neck Cancer international guidelines [30-31].

HPVs have a specific tropism for squamous and glandular epithelial cells, and the stage of productive infection is related to the stages of the cell differentiation. To establish an infection, viruses have to infect basal stem cells of mucosal epithelium. In
Table 1: Reviewed publications on HPV detection in breast and colorectal cancers.

<table>
<thead>
<tr>
<th>AUTHOR, YEAR</th>
<th>POPULATION</th>
<th>NO. OF CASES</th>
<th>HPV +</th>
<th>% HPV +</th>
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<tbody>
<tr>
<td><strong>HPV IN BREAST CANCER</strong></td>
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<tr>
<td>de Leon <em>et al.</em>, 2009 [108]</td>
<td>N/A</td>
<td>51</td>
<td>15</td>
<td>29.4</td>
</tr>
<tr>
<td>Aceto <em>et al.</em>, 2010 [62]</td>
<td>Italy</td>
<td>5</td>
<td>3</td>
<td>60.0</td>
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<tr>
<td>Ong <em>et al.</em>, 2009 [109]</td>
<td>Singapore</td>
<td>92</td>
<td>32</td>
<td>35</td>
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<tr>
<td>Cazzaniga <em>et al.</em>, 2009 [110]</td>
<td>Italy</td>
<td>70</td>
<td>11</td>
<td>15.71</td>
</tr>
<tr>
<td>Mendizabal-Ruiz <em>et al.</em>, 2009 [111]</td>
<td>Mexico</td>
<td>67</td>
<td>3</td>
<td>4.4</td>
</tr>
<tr>
<td>Khan <em>et al.</em>, 2008 [112]</td>
<td>Japan</td>
<td>124</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>AKil <em>et al.</em>, 2008 [113]</td>
<td>Syria</td>
<td>113</td>
<td>69</td>
<td>61.06</td>
</tr>
<tr>
<td>de Cremoux <em>et al.</em>, 2008 [114]</td>
<td>France</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Choi <em>et al.</em>, 2007 [115]</td>
<td>Korea</td>
<td>123</td>
<td>8</td>
<td>6.5</td>
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<tr>
<td>Lindel <em>et al.</em>, 2007 [116]</td>
<td>Germany</td>
<td>81</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Gumus <em>et al.</em>, 2006 [117]</td>
<td>Turkey</td>
<td>50</td>
<td>37</td>
<td>74</td>
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<tr>
<td>Kroupis <em>et al.</em>, 2006 [118]</td>
<td>Greece</td>
<td>107</td>
<td>17</td>
<td>15.9</td>
</tr>
<tr>
<td>Wrede <em>et al.</em>, 1992 [119]</td>
<td>London, UK</td>
<td>80</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Kan <em>et al.</em>, 2005 [120]</td>
<td>Australia</td>
<td>50</td>
<td>24</td>
<td>48</td>
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<tr>
<td>de Villiers <em>et al.</em>, 2005 [121]</td>
<td>Germany</td>
<td>29 (BC), 29 (Mamilla)</td>
<td>25 &amp; 20</td>
<td>86.21 &amp; 69</td>
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<tr>
<td>Tsai <em>et al.</em>, 2005 [122]</td>
<td>Taiwan</td>
<td>62</td>
<td>8</td>
<td>12.9</td>
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<tr>
<td>(Damin <em>et al.</em>, 2004 [123]</td>
<td>Brazil</td>
<td>101</td>
<td>25</td>
<td>24.75</td>
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<tr>
<td>Liu <em>et al.</em>, 2001 [124]</td>
<td>USA</td>
<td>17</td>
<td>6</td>
<td>35</td>
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<tr>
<td>Yu, 2000 [125]</td>
<td>China</td>
<td>32</td>
<td>14</td>
<td>43.8</td>
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<tr>
<td>Hennig <em>et al.</em>, 1999 [126]</td>
<td>Norway</td>
<td>41 &amp; 38 (CIN III)</td>
<td>19 &amp; 32</td>
<td>46 &amp; 84</td>
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<tr>
<td>Yu <em>et al.</em>, 1999 [127]</td>
<td>China, Japan</td>
<td>72</td>
<td>N/A</td>
<td>41.7 &amp; 11.1</td>
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<tr>
<td>Czerwenka <em>et al.</em>, 1996 [128]</td>
<td>Austria</td>
<td>20</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Gopalkrishna <em>et al.</em>, 1996 [129]</td>
<td>India</td>
<td>26</td>
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<tr>
<td>Brathauer <em>et al.</em>, 1992 [130]</td>
<td>USA</td>
<td>28</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>HPV IN COLORECTAL CANCER</strong></td>
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<tr>
<td>Bodagi <em>et al.</em>, 2005 [131]</td>
<td>N/A</td>
<td>55 &amp; 107</td>
<td>28 &amp; 38</td>
<td>51 &amp; 36</td>
</tr>
<tr>
<td>Liu <em>et al.</em>, 2011 [132]</td>
<td>N/A</td>
<td>96</td>
<td>32</td>
<td>33.30</td>
</tr>
<tr>
<td>Gornick <em>et al.</em>, 2010 [133]</td>
<td>USA, Israel, Spain</td>
<td>279</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Yavuzer <em>et al.</em>, 2011 [134]</td>
<td>N/A</td>
<td>168</td>
<td>0</td>
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<tr>
<td>Perez <em>et al.</em>, 2010 [135]</td>
<td>Argentina</td>
<td>75</td>
<td>33</td>
<td>44</td>
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<tr>
<td>Deschoolmeester <em>et al.</em>, 2010 [136]</td>
<td>Belgium</td>
<td>N/A</td>
<td>N/A</td>
<td>14.2</td>
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<tr>
<td>Salepci <em>et al.</em>, 2009 [137]</td>
<td>Turkey</td>
<td>56</td>
<td>N/A</td>
<td>82.14</td>
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<tr>
<td>Giuliani <em>et al.</em>, 2008 [138]</td>
<td>Italy</td>
<td>66</td>
<td>22</td>
<td>33.33</td>
</tr>
<tr>
<td>Damin <em>et al.</em>, 2007 [139]</td>
<td>Brazil</td>
<td>72</td>
<td>60</td>
<td>83.3</td>
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<tr>
<td>Buyru <em>et al.</em>, 2006 [140]</td>
<td>Turkey</td>
<td>43</td>
<td>31</td>
<td>72.09</td>
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<tr>
<td>Yu <em>et al.</em>, 2002 [141]</td>
<td>China</td>
<td>32</td>
<td></td>
<td>21.9</td>
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<tr>
<td>Lee <em>et al.</em>, 2001 [142]</td>
<td>Taiwan</td>
<td>19</td>
<td>16</td>
<td>84</td>
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</tbody>
</table>

Breast Cancer: Percentage of HPV+ (average): 32.7%. Lowest percentage of HPV+ 4.47%. Highest percentage of HPV+: 86.21%.

Colorectal Cancer: Percentage of HPV+ (average): 25.6%. Lowest percentage of HPV+ 14.2%. Highest percentage of HPV+: 84%.

Most infections the virus stays in an episomal state. In invasive cervical tumors, the viral DNA is integrated into host genome while in benign tumors the integration is a rare event, and HPV16 integration frequency has been reported to increase in parallel with the severity of cervical lesions [32]. Since integration often disrupts the E2 gene, current assays are based on quantification with real-time PCR of E6 relative to E2 DNA [33]. During integration part of HPV E2 inhibitory gene is deleted leading to increased expression of E6 and E7 oncoproteins [34]. High-risk HPV E6 and E7 impairs p53 and pRb functions and causes the cell to escape cell cycle checkpoint surveillance, subsequently leading to genome instability and cell immortalization [35]. HPV variants.
 (>98 % similarity) may have different biological and biochemical properties important to cancer risk [23]. These variants often appear to have disparate geographical and ethnic origins. Most studies of HPV variants focus on high-risk HPV 16. HPV 16 has 5 known variants: European (E), Asian (As), Asian-American (AA), African-1 (Af1) and African-2 (Af2). Asian-American variants have apparently higher oncogenic potential than European variants [36]. Viral integration in the host genome has been reported to be associated with carcinogenesis and could be the cause of persisting HPV infection [32]. Multiple HPV co-infections (mostly double-co-infections) have been reported in the literature [37-41].

Genetic risk cofactor

Magnusson et al., reported that biological daughters of women with CxCa had an increased risk of the disease as compared to adopted daughters, with an approximately 50% reduced risk for half-sisters [42]. These findings suggested that genetic predisposition may contrive to the risk of cancer. Gene polymorphisms in tumor suppressor genes or genetic variations in immune-and other genes might be related to HPV persistence and progression to cancer [43]. In particular, a central node along an oncogenic pathway that is composed of the two major proteins, the tumor suppressor p53 (encoded by TP53 gene) and its negative regulator the proto-oncoprotein MDM2 (encoded by HDM2 gene). MDM2 is an ubiquitin ligase that plays a critical role in regulating levels of and activity of p53 protein, which is a central tumor suppressor [44,45] (Table 2). TP53 and HDM2 are examples of candidate genes that have been suggested to affect the oncogenic potential of the HPV.

The TP53 gene contains a common polymorphism that is a G-to-C transversion at codon 72 (rs1042522) located in exon 4. This non-synonymous SNP results in Arg to Pro substitution in the amino acid sequence of the p53 protein. Storey et al., found an association between the arginine form of p53 and CxCa development and suggested that the corresponding G genotype is more susceptible to HPV E6 mediated degradation (Figure 2) [19]. Since then, there have been many reports on this TP53 polymorphism and risk for various cancers [46]. The codon 72 is located within the proline-rich region and amino acid substitution variant may affect the structure of the putative SH3-binding domain. It has been shown that TP53 Arg72 induces apoptosis markedly better than does the Pro72 variant [47]. This may suggest suboptimum Pro72 protein function culminating in less “apoptotic” cell death associated with genetic instability and cancer pre-disposition. In fact, most previous studies related to TP53 codon 72 SNP and also in its downstream effector CDKN1A codon 31 SNP, were concerned with their potential association with cancer [48].

Similar to SNPs affecting the function of TP53, a functional polymorphic variant in the HDM2 promoter at position 309 (rs2279744) have been found to affect cellular response. This SNP309, resulting in either a T or G allele (T/G substitution), was shown to affect the transcriptional activator SP1 binding thereby modulating HDM2 transcription, and hence, its levels. The G variant has been shown to increase the affinity for Sp1, resulting in higher levels of HDM2 mRNA and protein and the subsequent attenuation of the p53 pathway [49]. In humans, SNP309 is shown to associate with accelerated tumor formation in both hereditary and sporadic cancers, whereby a model was proposed suggesting that HDM2 SNP309 serves as a rate-limiting event in carcinogenesis [49]. This SNP could increases susceptibility to cancer and decreases the response of cancer cells to certain forms of treatment, such as radiation therapy and DNA-damaging drugs [50].

The impact of this genetic variation on HDM2 levels have as now-balling effect on p53 amounts in the cell, and consistently, the G allele which leads to higher HDM2 transcription was shown to attenuate the TP53 response. Furthermore, since the SP1-binding site is found in the estrogen receptor (ER)-binding motif on the HDM2 promoter, it was shown that estrogen preferentially stimulates the transcription of HDM2 from SNP309 G allele and

<table>
<thead>
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<th>Table 2: TP53 and HDM2 in summary.</th>
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<tbody>
<tr>
<td><strong>Gene</strong></td>
</tr>
<tr>
<td>Name(s):</td>
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<tr>
<td>Gene/Prot.:</td>
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<tr>
<td>Mediator:</td>
</tr>
<tr>
<td>Function:</td>
</tr>
<tr>
<td>Result:</td>
</tr>
<tr>
<td>Outcome:</td>
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<tr>
<td>Oncogenic:</td>
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</tbody>
</table>

Figure 2 Burden of potentially HPV-Mediated cancers in Saudi Arabia having low incidence of cervical cancer (CxCa).
increased the levels of \textit{HDM2} protein in estrogen-responsive cells homozygous for SNP309 (G/G) [51]. These results suggested the possibility that SNP309 G allele would be more sensitive to estrogen response compared with the T allele and may contribute to gender-specific tumorigenesis through further elevating the \textit{HDM2} levels and disrupting the TP53-HDM2 oscillation [52]. Consistently, this provided explanation for the original results described by Bond \textit{et al.}, who have found a correlation between the G allele and earlier onset of breast carcinoma in younger females who would generally have higher estrogen levels than their older postmenopausal counterparts [53]. The direct relationship, independent from p53, between Mdm2 and HPV is still elusive.

There have been many reports on the \textit{TP53} codon 72 polymorphism and risk for cervical and the results are inconsistent [46]. The differences between the studies could both be due to methodological aspects contributing to Hardy-Weinberg disequilibrium and study design properties resulting in less than ideal circumstances for evaluating this polymorphism [54]. Still, the lack of confirmation between studies can also reflect locus heterogeneity between populations [55-59]. Perez \textit{et al.}, have found significant association indicating that \textit{TP53} cod on 72 arginine homozygous genotype may represent a genetic predisposing factor for colon cancer development [60]. Wang \textit{et al.}, have compared in case-case comparison study, the HPV16 status in tumor specimens and \textit{TP53} cod on 72 and also p73 G4C14-to-A4T14 polymorphisms in 309 oropharyngeal cancer patients [61]. Results showed both p53 variant genotypes (Arg/Pro+Pro/Pro) and p73 variant genotypes (GC/AT+AT/AT) were significantly associated with HPV16-positive tumors that had greater association for the combined variant genotypes (p53 Pro carriers and p73 AT carriers) compared with combined wild-type genotypes (p53 Arg/Arg and p73 GC/GC), suggesting that variant genotypes of \textit{TP53} and p73 genes may be individually, or more likely jointly, associated with tumor HPV16-positive oropharyngeal cancer patients, particularly in never smokers.

Identification of such susceptible biomarkers would greatly help individualize cancer prevention and treatment to improve prognosis. In a study involving 5 juvenile breast cancers, Aceto \textit{et al.}, has reported tumor-associated HPV 16/18 E6 sequences in 3 patients [62]. All the HPV-positive cases were homozygous for arginine at \textit{TP53} cod on 72, a genotype associated with HPV-related cancer risk, and the tumors showed p16 (INK4A) immunostaining, a marker of HPV-associated cancers.

The role of Mdm2 SNP in HPV-positive patients has less been investigated. Nunobiki \textit{et al.}, found significant association between MDM2-SNP309 and high-risk HPV infection in CxCa in a Japanese population [63]. In a case-control study involving oropharyngeal cancer, Chen \textit{et al.}, have found significant association between HM2D2 309 SNP and HPV16 L1 seropositivity and that was particularly pronounced in never smokers/drinkers [64].

**Prognostic value of HPV infection**

Once cancer is diagnosed, clinical staging is important for treatment planning and estimation of prognosis. Besides a number of prognostic factors like depth of stromal invasion, tumor differentiation, or nodal involvement, the presence of HPV has been suggested as an important prognostic marker of disease severity in early-stage cervical and HN cancers, and could also be associated with vascular invasion, lymph node metastases and tumor size [65-68]. The prognostic role of HPV genotype in HPV-associated cancers has, however, been an issue of controversy. In CxCa, some earlier studies had found that HPV-negative tumors were more aggressive [69,70]. some found HPV-18 being associated with poorer prognosis as compared with HPV-16-positive tumors [71-74], one study found HPV-16 to be a predictor for a worse survival [75], whereas others did not find a prognostic value for HPV genotype [76,77]. In addition, some studies have revealed that the physical state of the virus (integrated or episomal) could also be an important prognostic marker [78]. Thus, the interpretation of these results remains uncertain.

Evidence exists in vitro and in vivo that viral proteins can alter cellular sensitivity to chemo-radiation [79,80]. The constitutive expression of the E6 protein promotes rapid tumor development and leads to radio resistance [81]. E6 protein abrogates the inhibition of DNA synthesis and suppresses p53 and p21 protein induction in the epidermis after treatment with ionizing radiation [82]. These results suggest a negative influence of HPV infection on clinical outcome. However, it has also been shown that degradation of p53 by E6 is not functionally equivalent to the loss of wild-type p53 function through somatic mutations of p53 gene [83]. Ishikawa \textit{et al.}, found that CxCa patients with wild-type p53 had a significantly better curative radiotherapy outcome [84]. In a similar study, Harima \textit{et al.}, found that HPV-negative patients had a highly significant shorter survival than the HPV-positive group [85]. Several studies reported a low rate of p53 mutations among HPV-positive tumors [86-88], and HPV-positive tumors appear to express significant amounts of functional p53, which may contribute to the cellular response.

Recent evidence, however, is mounting that HPV positivity improves prognosis in patients with OPSCC. This observation has led the International Collaboration on Oropharyngeal Cancer Network for Staging (ICON-S) to develop a staging system for HPV-related oropharyngeal cancer [89]. In addition; combining HPV status with other biomarkers was suggested to improve its prognostic power. In a recent meta-analysis looking at various biomarkers of overall survival (OS) in oropharyngeal squamous cell carcinoma (OPSCC) patients, including raised bcl2; amplification of 11q3 and loss of 16q genes; and low c-met, ki67, IMD, PLK, FHIT, nuclear surviving, or nuclear cycling D1, it was concluded that there was a significant OS benefit for patients with HPV positive and p16 positive tumors [90]. It has also been reported that HPV-positive OPSCCs are characterized by over expression of p16 and a significantly higher response rate to radiotherapy when compared to HPV-negative cancers [30]. Patients with HPV-positive OPSCC had a statistically significant higher overall survival, local control rate and distant metastases free survival than those with HPV-negative SCC, in several prospective clinical trials [30,91-93].

Recently, HPV and over expression of p16 were suggested to be used as prognostic factors for overall and progression free survival and might also be a predictive marker of response
to treatment in HNSCC [30]. Based on the evidential higher response rate of HPV-related HNSCC, currently there are at least three prospective studies evaluating treatment outcome with reduced doses of radiotherapy in locally advanced OPSCC (NCT01084083/ECOG-E1308, NCT01088802/J0988 and NCT01221753). RTOG1016 is another phase III running study evaluating radiotherapy plus cetuximab vs. chemotherapy in HPV-related OPSCC. Thus, it would be tempting to speculate that HPV positivity and p16-expression could also serve as prognostic markers not only in HNSCC, but also colorectal and breast cancer particularly if a significant portion of those tumors are mediated by HPV.

Host genetic polymorphic variations in candidate genes can also influence sensitivity and response to cancer treatment. The example of p53 Arg72Pro, discussed above under genetic predisposition, may also have implication on treatment outcome. In fact, we have demonstrated a statistically significant association between TP53 cod on 72 polymorphism, protein expression and cellular radio sensitivity in vitro [94]. We have also found significant association between HDM2 309 SNP and late normal tissue complications following radiotherapy in Head and Neck cancer patients [95]. Furthermore, we have very recently showed a borderline association between TP53 G72C and CxCa only in HPV-positive patients who were deviated from Hardy-Weinberg equilibrium (HWE) indicating co-selection, hence implicating the combination of HPV and SNPs in cancer predisposition [96]. Thus, SNPs could be more relevant biomarkers of susceptibility to cancer when associated with HPV infection. Therefore, the exact relationship between HPV genotype and polymorphism in candidate genes has to be investigated in our cancer patients as markers of susceptibility to HPV-infection, malignant transformation and predictive of treatment outcome, which if confirmed, will have significant translational implication allowing classifying patients according to their expected HPV-propensity and response to treatment.

**Vaccines and prevention of HPV-associated cancers**

Immunization is an effective measure in many infectious diseases [97,98]. Prevention by vaccinations has saved more lives than any medical treatment ever developed. Vaccines were developed against HPV infection to prevent CxCa and probably other HPV-related diseases [99]. Two types of prophylactic vaccines, a bivalent (Cervarix) that protect against HPV-16 and 18 and a quadrivalent (Gardasil) that is effective against HPV-6, 11, 16 and 18 are being widely introduced in Western countries to prevent HPV infection [100,101], and promising new broad-spectrum HPV vaccines are in development [102]. The short term results showed nearly complete efficacy against cervical cytological abnormalities, precancerous lesions, and even genital warts in the case of the quadrivalent vaccine [99,100].

Thus, even in countries with low incidence of CxCa, taking into consideration in the projection outlined in figure 1, in theory the vaccination is expected to protect about 10% of cancer patients which is, from an expenditure point of view, is cost-effective in view of the incidence of Head and Neck, colorectal, breast and anogenital cancers. In addition, many DNA vaccines are being developed for the treatment of HPV-16 and HPV-18-induced malignancies [103]. Most of these vaccines consist of a fusion of E6 or E7 with a “carrier-protein” to generate highly immunogenic E6 or E7 directed DNA vaccines. These vaccines can be used to treat HPV-positive cancers to improve outcome. Trimpe et al., have recently published the results of a randomized phase 2b trial [104]. The authors have assessed the safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic plasmid DNA vaccine in 167 patients with CIN2/3 from seven countries. At 36 weeks after the first dose of three intramuscular injections of the vaccine, 50% of plasmid recipients verses 31% of placebo recipients experienced histopathology regression to normal or CIN1 (P=0.03). The vaccine elicited humeral as well as cellular immune responses, both of which were greater among women who achieved histopathology regression and viral clearance [105]. This proof-of-principle trial represents a major breakthrough that therapeutic HPV vaccine is feasible. In addition, since p16 is consistently over expressed in all HPV-associated cancers, it can be a vaccine target antigen. A preliminary phase 1/2a study to test the safety and immunogenicity of a p16INK4a peptide (P16_37-63) vaccine in patients with advanced HPV-associated cancers, has showed induced cellular and humeral immune response [106]. These therapeutic vaccines could present a non-surgical option, reducing the destructive conventional treatment and changing the outlook for HPV-associated cancers.

**CONCLUSION**

Emerging evidences identify Human Papillomavirus (HPV) as causative agent not only for the well-known cervical and other anogenital cancers but also for Head and Neck squamous cell carcinomas (HNSCC), and more recently of colorectal and breast cancers [4-6,14,15]. Moreover, HPV-positive cancers appear to form separate subgroups among their counterparts with different epidemiology, histopathological characteristics, therapeutic response to chemo-radiation treatment and clinical outcome [31]. Furthermore, HPV-associated over expression of p16 protein is being considered diagnostic for HPV and prognostic for treatment outcome in HNSCC [30,31]. Thus, HPV detection and p16 expression are expected to gain importance in clinical settings for cancer patients. In fact, one of the most important developments in oncology of the past decade is the demonstration that patients with HPV-mediated oropharyngeal cancers have p16-expression along with significantly improved outcomes, compared to HPV-negative patients [5]. This has become the basis for clinical trials investigating the impact on “treatment deintensification” for patients with HPV-mediated cancers [31,91,107]. Preventive and therapeutic vaccines offer further options to improve the management of HPV-positive cancers. The significance of HPV in other head and neck and also colorectal and breast cancers is still to be clarified.

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