

# Human Papillomavirus: Mini-review and Collateral Expected Benefits of the Vaccine

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Cervical neoplasia is the second leading cause of neoplastic death in Latin America. It is generally accepted that all cervical carcinomas have at least one high risk Human Papillomavirus (HPV). Due to the causal relationship of specific HPV types and cervical cancer and its role as precursor of skin lesions it is important to identify the involved genotype. HPV, as other tumor-viruses, induces oncogenesis by manipulating an array of different cellular pathways, which leads to immortalization and proliferation of the infected cells by disrupting the mitotic checkpoint upon infection of the host cell. Actually the role of the immune response in the development of cervical cancer is unknown as is the relationship between the type and level of expression of messenger RNA (mRNA) of interferon gamma (IFN- $\gamma$ ), transforming growth factor beta 1 (TGF- $\beta$ 1) and interleukin (IL)-4 in the cervical microenvironment within each of the stages of carcinogenesis with the HPV genotype causing the infection. An average annual cost to treat cervical cancer is U.S. \$ 10,283 per patient. Taking into account the World Population Prospects: The 2010 Revision, in ten years the accumulated cases of cervical cancer might be 3,286,534, thus making a total budget of U.S. \$ 33,795.4 million to treat all women. Universal vaccination against HPV might result in extended benefits as the decrease in mouth and oropharynx cancers as well as the reduction in health cost for the attendance of several neoplasias.

**Keywords:** human papillomavirus, immunology, treatment, vaccine.

## INTRODUCTION

Human Papillomavirus (HPV) infection is high in several countries, being described until now more than 100 genotypes, classified into species from genus  $\alpha$  or  $\beta$ ; most of them causing clinically imperceptible infections and if perceptible, most will appear as skin papilloma or mucosal papilloma (warts).<sup>1,2</sup> Besides these lesions HPV infection is associated with several types of anogenital tumors, particularly cervical carcinoma. Fortunately, less than 5% of women infected with HPV will develop cervico-uterine cancer.<sup>3</sup>

In Mexico, cervical carcinoma is the second leading cause of mortality among women older than 25 years. The Mexican National Health System offers medical attention to about 9,000 cases of cervicouterine cancer and 4,000

deaths are registered annually.<sup>4</sup> In women examined with the Papanicolaou cervical test in the city of Durango, Mexico, a 48% prevalence of HPV infection was found, being genotype 16 the most common in that survey.<sup>5</sup> In men, HPV prevalence in the genital tract tends to be similar to that in women with a prevalence in external genitalia of 46.4%, 20.8% in the urethra and 12.1% in the meatus.<sup>6</sup>

It is generally accepted that all cervical carcinomas have at least one high risk HPV.<sup>7</sup> Moreover, genotypes 16 and 18 are found in approximately 70% of the cases.<sup>8,9</sup> From all currently identified genotypes, more than 40 infect the genital tract, from which 15-20 (genotypes 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) have been associated with cervical cancer or high-grade of cervical intraepithelial neoplasia (CIN) and for this reason are classified as high risk types.<sup>5,7</sup> On the contrary, some

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HPV genotypes (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81) are classified as low risk types.<sup>9</sup> Due to the causal relationship of specific HPV types and cervical cancer and its role as precursor of skin lesions it is important to identify the involved genotype.<sup>8</sup>

#### HPV types and associated diseases

HPV is a non enveloped double stranded DNA virus, with around 8000 base pairs divided into early region (E), late region (L) and control region. HPV has 8 open reading frames (ORFs) in one chain. The initial ORF encodes structural proteins. There is a central region (1 kb in length) called upstream regulatory region (toward the 5' end) between the initial and final ORF that may determine the HPV specificity for a given tissue. There are also internal transcription signals, both, activators and inhibitors, which can act as cis elements of action by binding cellular and viral proteins that transregulate viral genome function.

Types are identified when they show more than a 10% difference between their E6, E7 y L1 genetic sequences. A subtype or variant reaches only 2-5% of genetic differences.<sup>10</sup> HPV 16 is the most frequent genotype in the world, except in Indonesia and Algeria where genotype 18 is more frequent. Africa and Latin America have a high prevalence of the oncogenic types 16, 18, 31, 33, 35, 45, 51, 52, 58, 59. In Western Africa, HPV 45 is present with high frequency. In Central America and South America types 33, 39 and 59 are highly frequent.<sup>10</sup>

There are 30-40 genital HPV types that cause venereal infections that are highly and commonly contagious, showing 26.8% prevalence in USA women ages 24 to 59 years old. Women in Mexico demonstrate around a 14% prevalence rate. Most of these infections are not clinically manifested, others are detected as vulvar, vaginal, cervical or penile papilloma, and some others appear as warts. Cluster forms are common for HPV types 6 and 11 and less common for HPV 16 types. Intraepithelial lesion is more commonly present on 16, 18, 31, 6 and 11 types. HPV 16 is observed in oral warts. 10 Types 16 and 18 cause 70% of cervical neoplasia worldwide.<sup>11</sup>

Types 16 and 18 had been detected in vulvar, vaginal, penile, perianal and anal region neoplasias. They are also present in superior respiratory tract and esophagus neoplasias. HPV 33 is present in 44% of the cases of mammary ductal invasive cancer. Urethral and prostatic cancer show types 16 and 18 being involved.<sup>11</sup>

#### Transmission

Transmission is by direct contact with infected skin, mucosal or sexual fluids, mainly by sexual act with penetration. This is the most frequent sexually transmitted disease, in fact, 50% of sexually active individuals are considered infected at some time in their lives.<sup>10</sup> The risk of vertical transmission of HPV genotypes is relatively low

although, children of mothers' who are HPV-positive at the post-partum visit are about 5 times more likely to test HPV-positive than children of corresponding HPV-negative mothers.<sup>12</sup>

Prevalence of HPV infection rises annually in women 14 to 24 years old and gradually declines at 59 years old.<sup>11,13</sup> Even though prevalence is higher in men, HPV causes more damage in women.<sup>14</sup> Prevalence is higher in prostitutes.<sup>15</sup> It rises with a higher number of sexual partners and with early start of sexual activity.

The infection can be of only one type, or may involve many types. Likewise, it may pass several years before developing cancer or pre-malignant lesions, however, a minor part of detected precancer lesions evolve to cancer.<sup>16</sup>

#### Pathogenicity

HPV gene expression is polycistronic initiating from multiple promoters. Gene regulation occurs at transcriptional, but particularly post-transcriptional levels, including RNA processing, nuclear export, mRNA stability and translation. A close association between the virus replication cycle and epithelial differentiation adds a further layer of complexity.

HPV infected cells can be cleared by apoptotic pathways or they may persist in a chronic infection state that can develop neoplasia. As with other oncovirus, HPV develops oncogenesis by manipulation in arrangement on different cellular pathways that lead to immortalization and proliferation of infected cells by altering mitosis checkpoints often complemented by functional inhibition or proteasome degradation of many tumor suppressor proteins by products of viral genetic code.<sup>17</sup> Genetic regulations take place at the transcriptional level and with particularity on a post-transcriptional level that includes RNA process, nuclear exportation, mRNA stability and translation. It follows a complex level of association between viral replication and epithelial differentiation.<sup>18</sup>

HPV oncogenic high risk types succeed in integrating to the host cells the viral genome.<sup>10</sup> Thirty percent of type 16 cervical cancer uses an episomal way ends up on: production and activation of E6 and E7 proteins. Another significant difference between low and high oncogenic type risk is the affinity of E6 and E7 to the p53 and retinoblastoma (pRB) proteins.<sup>10</sup> Additionally, oncogenic HPV types code for an E5 protein involved in carcinogenic transformation and immune evasion, while many non oncogenic types lack an ORF for E5 or lacks a start codon for it.<sup>2</sup>

HPV as other tumor-viruses induce oncogenesis through manipulating an array of different cellular pathways, which lead to immortalization and proliferation of the infected cells by disrupting the mitotic checkpoint upon infection of the host cell. This is often accomplished by functional inhibition or proteasomal degradation of many tumor

suppressor proteins by virally encoded gene products. The virally infected cells can either be eliminated via cell-mediated apoptosis or persist in a state of chronic infection. Importantly, the chronic persistence of infection by tumor viruses can lead to oncogenesis.<sup>17</sup> In this regard; the most important involved proteins are E1, E2, E6, E7 and E1-E4.<sup>18-25</sup>

### Immunology

HPV is considered an important factor in the origin and evolution of pre-malignant lesions of cervicouterine cancer, however, exposure to the virus is necessary but not sufficient to cause CIN.<sup>26</sup> Fortunately, in 90% of the cases HPV can be cleared by the immune system in about two years.<sup>11,13-15,27</sup>

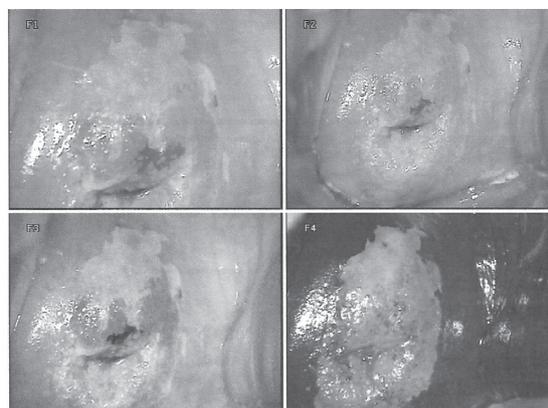
Scott et al. found that the pattern of Th1 immune response through the expression of interferon gamma (IFN- $\gamma$ ) and interleukin (IL)-2 is associated with the presence of HPV in cervical tissue.<sup>28</sup> Al-Saleh et al. showed that CIN progression to cancer was associated to an immunoregulatory Th2 response (IL-4 and 6).<sup>29</sup> Wu et al. showed that tumor necrosis factor alpha (TNF- $\alpha$ ) in addition to IL-2 and IFN- $\gamma$  play a critical role in regulating the susceptibility of cervical keratinocytes to HPV infection in patients with CIN 2 and 3.<sup>30</sup> Th1 immune response is predominant in premalignant lesions, with or without HPV, being the IFN- $\gamma$  expression related to the severity of the premalignant lesions.

Viral infections are eliminated by the cellular immune response (CIR) that consists of the recognition and lysis of infected cells by T lymphocytes (LT) CD8+, an event promoted by IFN- $\gamma$  secreted by the cells, also by the Natural killer cells (NK) and Th1 subpopulation of LT CD4+.<sup>31</sup> There are however two known subpopulations named Th2 and Th3 that secrete IL-4 and transforming growth factor beta 1 (TGF- $\beta$ 1), respectively.<sup>32</sup> Among the main functions of IL-4 and TGF- $\beta$  is the CIR inhibition and another function of IL-4 is to activate the humoral immune response (HIR), however, activation of the HIR may allow progression of tumors caused by virus, therefore, the type and level of cytokine expression determines the course of the immune response and the outcome of viral infection and carcinogenesis.

In low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL) the CIR alteration is a favorable factor for progression to cervical cancer as has been demonstrated in HIV-coinfected patients.<sup>33</sup> Actually the role of the immune response in the cervical cancer development and the relationship between the type and level of expression of messenger RNA (mRNA) of IFN- $\gamma$ , TGF- $\beta$  1 and IL-4 in the cervical microenvironment within the stages of carcinogenesis or with the HPV genotype causing the infection is unknown.

### Diagnosis, prevention and treatment

Diagnosis requires gynecological medical examination that includes Papanicolaou, colposcopy (Figure 1) and histopathological techniques. Even though expensive, genetic molecular techniques like polymerase chain reaction (PCR) and hybridization have been added to identify the virus.



**Figure 1. Low grade HPV genital infection (Colposcopic image). F1) eutrophic cervix, positive for acetone, easy bleeding and epithelial erosion with bacterial vaginosis, F2) exocervical visualization with acetic acid 3%, F3) visualization of increased vascularization, F4) Schiller test.**

Since 2006 there have been vaccines approved by the Food and Drug Administration (FDA), the Advisory Committee on Immunization Practices (ACIP), and the Center for Disease Control and Prevention (CDC) and by the World Health Organization (WHO).

The quadrivalent vaccine, Gardasil® (HPV4), protects against infection with HPV types 6, 11, 16 and 18. This vaccine has demonstrated its effectiveness in preventing cervical, vaginal, vulvar and anal cancers as well as genital warts in both genders. The second HPV vaccine, Cervarix® (HPV2), is a bivalent vaccine that provides protection against HPV types -16 and -18. It is recommended in girls younger than 13 years.

Actually there is no official treatment for HPV infection. Papilloma lesions can be remissible by the individual's immune system and defence mechanisms. Many therapies are available for the treatment of HPV-associated disease, particularly external genital warts. However, at present, these therapies are for removing the lesion rather than specifically target HPV infection.<sup>25,34</sup> Understanding mRNA and its regulation on HPV infection related diseases will give clues to develop diagnosis techniques and antiviral therapy.<sup>18</sup> Future direct or indirect therapies will include the following: antiviral drugs against HPV functional proteins; increase immune system ability to solve the infection, apoptosis induction on HPV infected cells;<sup>34</sup> or small interference RNA (sRNAi) to target and inhibit viral

oncogenes in tumoral cells.<sup>35</sup>

Some advances on treatment research for HPV infection are based on: 1) curcumin treatment that inhibits HPV 16 transcription of E6/E7 and restores expression of the tumor suppressor proteins p53, retinoblastoma and PTPN<sup>13,36</sup> 2) proteasome inhibitors on HPV infected cervical cancer cells,<sup>37,38</sup> 3) development of a biphasic vesicles delivery system that injects IFN- $\alpha$ .<sup>39</sup> Future therapies will be directly or indirectly antiviral, targeting HPV protein functions or enhancing the ability of the immune system to resolve infection or inducing apoptosis indirectly in HPV-infected cells.<sup>34</sup>

Cervical cancer treatment depends on the stage and dissemination at the moment of diagnosis; options are surgery (hysterectomy), radiotherapy and chemotherapy.<sup>25</sup>

#### Collateral benefits of HPV vaccine

Cancer of the cervix is one of the most important neoplasias, with an estimated worldwide incidence of about half a million cases per year and 260,000 deaths around the world in 2005, of which 80% occurred in developing countries.<sup>40</sup> In Brazil, the annual cost per patient with cervical cancer is, approximately, U.S. \$4,970.00,<sup>41</sup> while in Tunisia it is U.S. \$ 3,180.00.<sup>42,43</sup> In some countries, the direct cost per case of cervical cancer reaches higher values, as in Belgium U.S. \$ 12,434.00, USA U.S. \$ 18,799.00, the United Kingdom U.S. \$ 8,810.00 and France U.S. \$ 13,505.00.<sup>44-47</sup> An average annual cost with the previous information is U.S. \$ 10,283 per patient. Taking into account the World Population Prospects: The 2010 Revision,<sup>48</sup> in ten years, the accumulated cases of cervical cancer might be 3,286,534, thus making a total budget of U.S. \$ 33,795.4 million<sup>13</sup> to treat all women. Of course this will not occur and the real expenditure will be much lower.

For the WHO, a 80% coverage of Pap screening among women 25 to 59 years would be enough to impact the morbidity and mortality indicators, which can be observed after four years of implementation of early detection actions.<sup>49</sup>

HPV also causes an estimated ~30,000 oropharyngeal cancers, worldwide.<sup>50</sup> In fact, HPV is now the major cause of oropharyngeal cancer in developed countries, detected in 45–90% of cases.<sup>51</sup> HPV has also been detected in a smaller subset of laryngeal (24%) and oral cavity (23%) cancers.<sup>50</sup>

Recent data demonstrate that HPV also plays a role in head and neck cancers, and non-cancerous conditions such as recurrent respiratory papillomatosis. As more and more information about the role of infection in non-cervical diseases is amassed, additional questions about whether prophylactic HPV vaccines will effectively prevent these conditions are raised.

It is possible that with the massive vaccination against HPV, other pathologies besides cervical cancer will be diminished in incidence. For example, according to the WHO, within all its members, the approximate number of deaths by mouth and oropharynx cancers were of 281,500 (April 2011).<sup>52</sup> Taking into account a constant ratio for mouth and oropharynx cancers of 3.9% of the worldwide population, with 45% of these malignancies associated to HPV and thinking positive with a hypothetical universal vaccination program against HPV, beginning with a coverage of 30%, a decrease of 253,063 cases would be accumulated in ten years.

#### CONCLUSIONS

The role of the immune response in the cervical cancer development has not been explained successfully until now. As the knowledge of this system grows, new therapeutical options are designed. Universal vaccination against HPV might result in extended benefits as the decrease in mouth and oropharynx cancers as well as the reduction in health cost for the attendance of several neoplasias.

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#### REFERENCES

1. HPV Study group in men from Brazil, USA and Mexico. Human papillomavirus infection in men residing in Brazil, Mexico, and the USA. *Salud Publica Mex.* 2008;50(5):408-18.
2. Castle PE, Jerónimo J, Schiffman M, Herrero R, Rodríguez AC, Bratti MC, et al. Age-related changes of the cervix influence human papillomavirus type distribution. *Cancer Res.* 2006;66(2):1218-24.
3. Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, Palan P, et al. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J Natl Cancer Inst.* 1995;87(18):1365-71.
4. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189(1):12-9.
5. Sánchez-Anguiano LF, Alvarado-Esquivel C, Reyes-Romero MA, Carrera-Rodríguez M. Human papillomavirus infections in women seeking cervical Papanicolaou cytology of Durango, Mexico: prevalence and genotypes. *BMC Infect Dis.* 2006;6:27.
6. Aguilar LV, Lazcano-Ponce E, Vaccarella S, Cruz A, Hernández P, Smith JS, et al. Human papillomavirus in men: comparison of different genital sites. *Sex Transm Infect.* 2006;82(1):31-3.

7. Fontaine V, Mascaux C, Weyn C, Bernis A, Celio N, Lefèvre P, et al. Evaluation of combined general primer-mediated PCR sequencing and type-specific PCR strategies for determination of human papillomavirus genotypes in cervical cell specimens. *J Clin Microbiol.* 2007;45(3):928-34.
8. De Guglielmo Z, Rodríguez A. Methods used in the identification of human papillomavirus. *An Sist Sanit Navar.* 2010;33(1):71-7.
9. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. *Am J Epidemiol.* 2008;168(2):123-37.
10. Rivera ZR, Aguilera TJ, Larraín HA. Epidemiología del virus papiloma humano (HPV). *Rev Chil Obstet Ginecol.* 2002;67(6):501-6.
11. Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, et al. Prevalence of HPV infection among females in the United States. *JAMA.* 2007;297(8):813-9.
12. Castellsagué X, Drudis T, Cañadas MP, Goncé A, Ros R, Pérez JM, et al. Human Papillomavirus (HPV) infection in pregnant women and mother-to-child transmission of genital HPV genotypes: a prospective study in Spain. *BMC Infect Dis.* 2009;9:74.
13. Ferreccio C, Prado RB, Luzoro AV, Ampuero SL, Snijders PJ, Meijer CJ, et al. Population-based prevalence and age distribution of human papillomavirus among women in Santiago, Chile. *Cancer Epidemiol Biomarkers Prev.* 2004;13(12):2271-6.
14. Lazcano-Ponce E, Herrero R, Muñoz N, Hernández-Avila M, Salmerón J, Leyva A, et al. High prevalence of human papillomavirus infection in Mexican males: comparative study of penile-urethral swabs and urine samples. *Sex Transm Dis.* 2001;28(5):277-80.
15. Juárez-Figueroa LA, Wheeler CM, Uribe-Salas FJ, Conde-González CJ, Zampilpa-Mejía LG, García-Cisneros S, et al. Human papillomavirus: a highly prevalent sexually transmitted disease agent among female sex workers from Mexico City. *Sex Transm Dis.* 2001;28(3):125-30.
16. Castle PE, Rodríguez AC, Burk RD, Herrero R, Wacholder S, Hildesheim A, et al. Long-term persistence of prevalently detected human papillomavirus infections in the absence of detectable cervical precancer and cancer. *J Infect Dis.* 2011;203(6):814-22.
17. Saha A, Kaul R, Murakami M, Robertson ES. Tumor viruses and cancer biology: Modulating signaling pathways for therapeutic intervention. *Cancer Biol Ther.* 2010;10(10):961-78.
18. Graham SV. Human papillomavirus: gene expression, regulation and prospects for novel diagnostic methods and antiviral therapies. *Future Microbiol.* 2010;5(10):1493-506.
19. Rivera ZR, Delgado GJ, Painel VP, Barrero PR, Larrain HA. Mecanismo de infección y transformación neoplásica producido por virus papiloma humano en el epitelio cervical. *Rev Chil Obstet Ginecol.* 2006;71(2):135-40.
20. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med.* 2010;363(1):24-35.
21. Kong CS, Narasimhan B, Cao H, Kwok S, Erickson JP, Koong A, et al. The relationship between human papillomavirus status and other molecular prognostic markers in head and neck squamous cell carcinomas. *Int J Radiat Oncol Biol Phys.* 2009;74(2):553-61.
22. Nobre RJ, Herráez-Hernández E, Fei JW, Langbein L, Kaden S, Gröne HJ, et al. E7 oncoprotein of novel human papillomavirus type 108 lacking the E6 gene induces dysplasia in organotypic keratinocyte cultures. *J Virol.* 2009;83(7):2907-16.
23. Lizano-Soberón M, Carrillo-García A, Contreras-Paredes A. Infección por virus del Papiloma Humano: Epidemiología, Historia Natural y Carcinogénesis. *Cancerología.* 2009;4:205-16.
24. Thompson DA, Zacny V, Belinsky GS, Classon M, Jones DL, Schlegel R, et al. The HPV E7 oncoprotein inhibits tumor necrosis factor alpha-mediated apoptosis in normal human fibroblasts. *Oncogene.* 2001;20(28):3629-40.
25. Torpy JM, Burke AE, Glass RM. JAMA patient page. Human papillomavirus infection. *JAMA.* 2007;297(8):912.
26. Sedlacek TV. Advances in the diagnosis and treatment of human papillomavirus infections. *Clin Obstet Gynecol.* 1999;42(2):206-20.
27. Sánchez-Aleman MA, Uribe-Salas F, Conde-González CJ. Human papillomavirus infection, a possible biological marker of sexual behavior among university students. *Salud Publica Mex.* 2002;44(5):442-7.
28. Scott M, Stites DP, Moscicki AB. Th1 cytokine patterns in cervical human papillomavirus infection. *Clin Diagn Lab Immunol.* 1999;6(5):751-5.
29. Al-Saleh W, Giannini SL, Jacobs N. Correlation of T-helper secretory differentiation and types of antigen-presenting cells in squamous intraepithelial lesions of the uterine cervix. *J Pathol.* 1998;184(3):283-90.
30. Wu R, Coleman N, Stanley M. Different susceptibility of cervical keratinocytes containing human papillomavirus to cell-mediated cytotoxicity. *Chin Med J (Engl).* 1996;109(11):854-8.
31. Gey A, Kumari P, Sambandam A, Lecuru F, Cassard L, Badoual C, et al. Identification and characterisation of a group of cervical carcinoma patients with profound downregulation of intratumoral Type 1 (IFN $\gamma$ ) and Type 2 (IL-4) cytokine mRNA expression. *Eur J Cancer.* 2003;39(5):595-603.
32. Spellberg B, Edwards JE. Type 1/Type 2 immunity in infectious diseases. *Clin Infect Dis.* 2001;32(1):76-102.
33. Fernandes AP, Goncalves MA, Duarte G, Cunha FQ, Simoes RT, Donadi EA. HPV16, HPV18, and HIV infection may influence cervical cytokine intralesional levels. *Virology.* 2005;334(2):294-8.

34. Stanley M. Chapter 17: Genital human papillomavirus infections--current and prospective therapies. *J Natl Cancer Inst Monogr.* 2003;(31):117-24.
35. Chang JT, Kuo TF, Chen YJ. Highly potent and specific siRNAs against E6 or E7 genes of H. *Cancer Gene Ther.* 2010;17(12):827-36.
36. Maher DM, Bell MC, O'Donnell EA, Gupta BK, Jaggi M, Chauhan SC. Curcumin suppresses human papillomavirus oncoproteins, restores p53, Rb, and PTPN13 proteins and inhibits benzo[a]pyrene-induced upregulation of HPV E7. *Mol Carcinog.* 2011;50(1):47-57.
37. Lin Z, Bazzaro M, Wang MC, Chan KC, Peng S, Roden RB. Combination of proteasome and HDAC inhibitors for uterine cervical cancer treatment. *Clin Cancer Res.* 2009;15(2):570-7.
38. Phaeton R, Wang XG, Einstein MH, Goldberg GL, Casadevall A, Dadachova E. The influence of proteasome inhibitor MG132, external radiation, and unlabeled antibody on the tumor uptake and biodistribution of (188)re-labeled anti-E6 C1P5 antibody in cervical cancer in mice. *Cancer.* 2010;116(4 Suppl):1067-74.
39. Foldvari M, Badea I, Kumar P, Wettig S, Batta R, King MJ, et al. Biphasic vesicles for topical delivery of interferon alpha in human volunteers and treatment of patients with human papillomavirus infections. *Curr Drug Deliv.* 2011;8(3):307-19.
40. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol.* 2006;24(14):2137-50.
41. Da Fonseca AJ, Ferreira LP, Dalla-Benetta AC, Roldan CN, Ferreira ML. Epidemiology and economic impact of cervical cancer in Roraima, a Northern state of Brazil: the public health system perspective. *Rev Bras Ginecol Obstet.* 2010;32(8):386-92.
42. Goldie SJ, Kim JJ, Kobus K, Goldhaber-Fiebert JD, Salomon J, O'shea MK, et al. Cost-effectiveness of HPV 16, 18 vaccination in Brazil. *Vaccine.* 2007;25(33):6257-70.
43. Ben GH, Aounallah-Skhiri H, Oueslati F, Frikha H, Achour N, Hsairi M. Estimated cost of managing invasive cervical cancer in Tunisia. *Sante Publique.* 2009;21(6):561-9.
44. Annemans L, Rémy V, Lamure E, Spaepen E, Lamotte M, Muchada JP, et al. Economic burden associated with the management of cervical cancer, cervical dysplasia and genital warts in Belgium. *J Med Econ.* 2008;11(1):135-50.
45. Insinga RP, Ye X, Singhal PK, Carides GW. Healthcare resource use and costs associated with cervical, vaginal and vulvar cancers in a large U.S. health plan. *Gynecol Oncol.* 2008;111(2):188-96.
46. Brown RE, Breugelmans JG, Theodoratou D, Benard S. Costs of detection and treatment of cervical cancer, cervical dysplasia and genital warts in the UK. *Curr Med Res Opin.* 2006;22(4):663-70.
47. Arveux P, Bénard S, Bouée S, Lafuma A, Martin L, Cravello L, et al. Invasive cervical cancer treatment costs in France. *Bull Cancer.* 2007;94(2):219-24.
48. United Nations, Department of Economic and Social Affairs. [cited 2013 January 22]; <http://esa.un.org/unpd/wpp/Excel-Data/population.html>
49. World Health Organization. Manual on the prevention and control of common cancers. Geneva, Switzerland: WHO. Westerns Pacific Series, 20; 1998; National cancer control programmes: 8-31.
50. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev.* 2005;14(2):467-75.
51. D'Souza G, Dempsey A. The role of HPV in head and neck cancer and review of the HPV vaccine. *Prev Med.* 2011;53 Suppl 1:S5-11.
52. World Health Organization. Health statistics and health information systems. [cited 2013 January 22]; Available from: [http://www.who.int/healthinfo/global\\_burden\\_disease/estimates\\_country/en/index.html](http://www.who.int/healthinfo/global_burden_disease/estimates_country/en/index.html)