Importance of Vaccination in Uterine Cancer Prevention in Adolescence

Ioannis Drikos[1,2]*, Alexandros Sachinidis[3], Ioanna Vassi[4] and Argyrios Ioannidis[5]

[1]Pediatric Clinic, General Hospital of Elefsina, Thiassio, Greece – Hellas, Gennimatas Avenue Magoula, 19600, Greece

[2]Department of Neonatology, National and Kapodistrian University of Athens, Greece – Hellas, Greece

[3]Second Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, Hippocration Hospital, Thessaloniki, Greece – Hellas, Greece

[4]National Kapodistrian University of Athens, Department of Philoshophy, Neurolinguistics in Education, Greece

[5]Sismanoglion General Hospital, Department of Surgery, Athens, Greece – Hellas, Greece

*Corresponding author: Dr. Ioannis Drikos, Pediatric Clinic, General Hospital of Elefsina, Thiassio, Greece - Hellas, Gennimatas Avenue Magoula, 19600, Greece, Tel: 2105563292; E-mail: johndrikos@yahoo.com

Abstract

Traditional therapeutic approaches in uterine cancer treatment may improve survival of patients with cancer. The specificity of uterus cancer treatment could improve the specific target therapy and understanding of mechanisms of cytotoxicity in normal cells. Developing cancer vaccines seems to achieve proper creation of specialized immune memory in order to prevent the tumor progression. Preventive prophylactic vaccination of women and men seems to be promising in disease progression considering the number of HPV strains involved and available vaccines being specific for 2 strains only to help eradication of cervical cancer.

Keywords: Cancer; Cervical cancer; HPV; HPV E6; HPV E7; Therapeutic vaccines; DNA vaccines

Introduction

The advances of cancer biology research allowed the use of targeted therapies against specific molecular targets. The improvement in aggression of a tumor can be exerted by active immunization with a non-toxic therapeutic agent potentially capable of eliciting for immune responses against tumor in patients with primary and metastatic cancer [1,2].

A significant number of antigens have been already recognized in tumor cells by specialized T lymphocytes. Most of the cancer proteins are self-antigens and according this the creation of cancer vaccines are based to the disruption of immunological tolerance without causing significant autoimmune reactions [2,3].

Vaccination in cancer patients should be able to trigger the activation of CD8+ cytotoxic T lymphocytes (CTLs) in order to discard cancer cells as identifying tumor-associated antigen on the surface of cancer cells [3].

The cervical cancer is the third most common cancer type in United States. Due to cervical cancer screening test annual disease rates have been reduced last decades [4,5]. About 80% of the total 500,000 annual cases of cervical cancer occur in developing countries. In US, the annual incidence is about 11,000 cases with more than 60% of cases occurring in populations of under-served women [4,5].

Literature Review

Human Papilloma Virus (HPV) associated with uterus cancer

According to literature HPV - 15 are associated with the occurrence of cancer in almost all cases of uterus cancer and appear to be important cause of cervical cancer [6].

The carcinogenesis of uterus may appear as progression from low differentiation to high quality morphological changes could be summarized in four distinct stages:

- HPV infection.
- HPV persistent infection.
- Progression of persistent cervical infection as pre-cancer stage.
- Progression in carcinoma status [6,7].

HPV is the most common sexually transmitted disease. HPV infections, including HPV carcinogenic genotypes, are usually transient within 6-12 months causing mild morphological changes. HPV cancer genotypes are the essential and common cause of cervical cancer.

Women with persistent HPV carcinogenic genotypes reveal high risk of developing precancerous alterations [6,7]. If precancerous stages are not diagnosed a significant proportion of infections may develop into carcinogenic [7,8]. Based on

genome analysis HPV is a DNA virus contains 8,000 bp encoding two classes of proteins, early and late proteins [9]. L1 and L2 proteins are structural components of the viral capsid, while the proteins E1, E2, E4, E5, E6 and E7 are vital as life cycle regulators.

E1 and E2 regulate the replication of DNA while E2 controls the RNA transcription. On the other hand, E4 controls the reorganization of the cytoskeleton, while E5, E6 and E7 mediate cellular transformation. In cervical cancer, viral integration into the host genome often leads to deletion of various gene-proteins (E2, E4, E5) leading to an increased expression of oncogenes E6 and E7. These oncogenes E6 and E7 inactivate the p53 and Rb tumor suppressor genes preventing cellular apoptosis and promoting cell growth [9,10].

Selection of specific HPV antigens for therapeutic vaccination

The connection between HPV infection and cervical cancer lead to development of many HPV vaccines. HPV prophylactic vaccines are designed to prevent HPV infection by inducing specific neutralize antibodies. The understanding of the protective humoral immune responses against primary HPV infection has can be used to express targeting of L1 and L2 proteins of the viral capsid. Experiments of animal cell lines encoding the L1 protein of the capsid leads antibody production which may provide immunoprotection [11].

There are two main vaccines for HPV protection. Gardasil contain L1 VLP, which is derived from the HPV-6, 11, 16 and 18 viral genotypes being extremely successful in protection against infection of most important types of HPV, HPV-6, 11 and HPV-16, 18. Gardasil has been shown to significantly reduce the incidence of CIN that is associated with genotypes HPV-16 and 18 [12].

Another HPV prophylactic vaccine containing L1 VLP is derived from HPV-16 and 18. The L1 VLP vaccine is administered with AS04 adjuvants. Cervarix offers partial cross-protection against HPV types 31 and 45, which are not included in the first reported vaccine. Gardasil and Cervarix are highly immunogenic and are capable of producing high titters of L1 protein neutralizing antibodies from HPV types, often associated with cervical cancer [HPV-16 and 18] [12].

In order to develop an effective therapeutic vaccine, there must be suitable target antigens against which immune response can be activated. Preventive administration of HPV vaccines targets L1 and L2 capsid proteins produce neutralizing antibodies. E6 and E7 proteins are ideal targets for therapeutic vaccination against HPV infections such tumor specific antigens. E6 and E7 are essential molecules in cell transformation, with constant expression in cancer cells. Their decisive role in the pathogenesis of tumors regulates their functionality. E6 and E7 proteins are typically selected as target antigens in the development of therapeutic vaccines against HPV 15 [13].

HPV vaccines

Many different forms of vaccines have been used to develop the appropriate therapeutic agents. These methods include the generation of peptides or proteins with immunogenicity such as DNA or RNA vaccines. Each form has its own advantages and disadvantages. Vaccines containing a peptide segment are known to be well tolerated, stable and readily prepared on a large scale [12,13].

Attributable and immunogenicity of these vaccines is limited caused by histocompatibility antigens [MHC I] expressed in one individual. Other immunoregulatory agents, such as Toll-like receptors [TLR], cytokines and co-stimulatory molecules [13], are necessary for this type of vaccine.

Whole cell vaccines include combination of dendritic cells and cancer cells. This therapeutic approach requires the preparation of individual dendritic cells [AX] with E6/E7 peptides, DNA or RNA encoding these peptides or transfection with live vectors carrying E6/E7.

Cancer cells used to make vaccines require the systematic management of whole cancer cells to help identify HPV-related tumor-associated antigens from the immune system. The introduction of new cancer cells in patients raises concerns about their safety [14].

On the other hand DNA vaccines are being an attractive form of therapeutic vaccines against HPV. DNA vaccines have many advantages over other forms of HPV therapeutic vaccines. Compared to live vector bases are relatively safe and can be given repeatedly to the same person without losing their efficacy. DNA vaccines do not directly affect the immune response of the vaccinated patient and are appropriate in cases where functions are required to be achieved. DNA vaccines are also stable, easy to prepare, exported in high purity and relatively inexpensive [15].

The presence of full-length complementary DNA simultaneously provides many epitopes, thus overcoming the restriction of MHC, to peptide-based vaccines. Plasmid DNA itself contains unmethylated CpG motifs that can function as potent DNA vaccines are also able to provide stable release of antigenic proteins, thereby enhancing immune memory. They can be to express HPV peptides or proteins, allowing DNA vaccines to exhibit antigen-conferring property, enhancing the CD4+ and CD8+ T lymphocytes *in vivo* responses [15,16].

Although significant progress has been made in the development of HPV prophylactic vaccines with the commercialization of Gardasil and Cervarix, the increase in HPV population associated with abnormalities and precancerous lesions, underlines the need progress in the field of therapeutic vaccines for all genotypes of the HPV viruses.

The availability of different forms of HPV therapeutic vaccines creates opportunities for the development of co-administrative regimens in order to enhance the therapeutic activity of DNA vaccine [17,18]. It is also important to consider the use of immunomodulatory agents in conjunction with the therapeutic DNA vaccines may prevent the success of an effective immune therapy. The use of HPV therapeutic

vaccines can potentially be combined with other therapeutic methods, such as chemotherapy and radiotherapy in order to increase the therapeutic effects of the vaccine. Chemotherapeutic agents such as cisplatin and bortezomib have been shown to render E7-expressing cancer cells-important for the cytotoxic activity of T lymphocytes [19-21]. DNA vaccine against HPV regulates E7 tumor expression and significantly enhances the therapeutic option of HPV DNA vaccine [21].

At this point we may notice that E6 and E7 antigens need to be processed through the action of the proteasome. Activation of antigenic memory is related to the action of MHC and APC by activation of CD8 + T cells [22]. However, only some of these small peptides contain the sequence of antigenic fragments (epitopes) that can bind to MHC molecule with high affinity [23-25]. Most therapeutic vaccines are designed to elicit an immune response against the E7 antigen because it is better immunological characterized by the E6 antigen in preclinical models.

Current developments in vaccines include bacterial or viral vectors. These vectors replicate in the body and facilitate the spread of the antigen [26-28]. HPV therapeutic vaccines are highly immunogenic and can cause strong cellular and humoral immune responses [29]. Unfortunately vector-based live vaccines are a potential safety hazard, particularly in people with immunosuppression [29]. In addition, the effectiveness of the immune response after repeated immunization with the same vector is limited [27-29].

On the other hand, various bacterial vectors have been selected for the development of HPV therapeutic vaccines including *Listeria monocytogenes, Lactobacillus lactis*, *Lactobacillus plantarum* and *Lactobacillus casei* [30,31]. *Listeria* has been recognized as a promising vector due its ability to infect macrophages and secrete listeriolysin [30,31]. Also several viral vectors have been used to produce HPV antigens including adenovirus and lentivirus [30,31]. Also have been created by particles comprised of recombinant MVA including sequences encoding modified HPV16 E6/E7 and human IL-2 factor.

Another MVA-based vaccine, MVA E2 was created to transfer E2 proteins to vaccinated hosts and not to E6 and E7. The vaccine uses the E2 protein as an inhibitor for the expression of E6 and E7 oncoproteins. The host can suppress E6 and E7 activity in HPV-infected individuals and then reduce the transformation of infected cells and the survival capacity of cancer cells [32,33].

E2 protein has been shown to inhibit cell growth and induction of tumor cell apoptosis [33,34]. The MVA E2 vaccine may also result in the generation of CD8 + T cells targeting the E2 antigen. MVA E2 can produce therapeutic anti-cancer effects.

The main advantage of DNA vaccination is the production of non-living, non-reproducible, non-propagated antigens administered to APCs inducing CTL, Th1 and B-cell immunity. Additionally, DNA vaccination does not cause autoimmunity against the vector in patients, so multiple DNA administrations are possible without inducing an immune response against DNA plasmid [35]. This approach may therefore be particularly useful in the context of cancer therapeutic vaccination, where repeated vaccinations are often required to effectively increase T cell responses [35,36].

Several preclinical and clinical DNA vaccine studies have been conducted against HPV-induced malignancies. A plasmid DNA originally named ZYC101 and encoding the HPV16 HLA A2 epitope was developed to treat HPV16 infections in HLA-A2 positive individuals [36-38].

Another DNA vaccine targeting the oncoprotein E7, pNGVL4a-Sig/E7, was tested in phase I/II clinical trials for the treatment of patients with a positive history of HPV 16 and CIN 2/3 infection. This DNA plasmid encodes a mutant form of HPV16 E7. DNA vaccines produce moderate immunity in humans, E7 was fused to an accompanying protein, Hsp70 from Mycobacterium tuberculosis, to enhance presentation through APC and MHC class I.

The E7-Hsp70 antigen was further linked to a signal sequence, which resulted in the secretion of E7 and secreted antigen would be more likely to gain access to APC [39]. In addition, a sequential heterologous vaccine phase I test has already been performed using the same DNA plasmid pNGVL4a-Sig/E7 [detox] -Hsp70 with recombinant vaccine virus encoding the HPV16 and HPV18 E6/E7 fusion proteins. Metabolic immune changes included increased signaling of CD8 + in both stromal and epithelial sections [39,40].

HPV genotypes related to malignancies

Current vaccines are capable of eliciting immune responses against the two most common oncogenic types such as HPV-16 and HPV-18 but not against other high-risk HPV viruses [41,42]. It is obvious that multiple vaccines against a number of HPV viruses will have major impact and may develop a single L1 VLP combination vaccine. VLP vaccines are particularly effective against several types of viruses, mainly by targeting the L1 protein but their efficacy against other types of HPV is variable depending on their genomic integrity and similarity [43].

Prevention of cancer disease is associated with additional oncogenic genotypes associated with HPV 16 and 18 (mainly HPV 31 and 45) can provide an important protection. The administration of a quadrivalent vaccine reveals a significant protective effect against HPV 31 (persistent infection in CIN2-3/AIS patients).

Bivalent vaccine may be useful after HPV 31 and 45 infections leading to development of CIN 2 [44,45]. In November 2009, Merck announced that Gardasil has 90% positive results in the prevention of genital lesions caused by genotypes 6, 11, 16 and 18 HPV in men aged between 16 to 26. Based on that Gardasil prevent the alteration of external genital organs in men adolescents [46,47].

According to National Cancer Institute more than 1,200 new cases of penile cancer are diagnosed each year. In 80% and 90% of cases the rectal cancer is the associated with HPV

infections usually HPV-16 [48,49]. Additionally, HPV is the most common sexually transmitted disease in the United States among men and women and 6.2 million new people being infected each year.

FDA approves vaccination in male and female. For women, Gardasil be started at 9-11 years of age with the vaccination period being recommended between the ages of 12 and 26 years [49].

Discussion

Pre-screening program is an important monitoring method of estimated the risk of cervical cancer. Despite the relative success of preventive testing in reducing cervical cancer, the disease has not been eliminated.

Also, available screening programs can reveal abnormal and precancerous cells in the cervix, but they do not prevent the cause of cervical abnormalities, ie persistent infection with oncogenic types of HPV.

From studies to date, it appears that L1 VLP vaccines are very effective in preventing new infections for the two most common oncogenic types of HPV, significantly reducing HPV rates

Associated cancers provided the vaccine is widely administered. To achieve these requirements, more studies need to be conducted to find a wide range of vaccines, possibly more economical. In addition, the above clinical benefits for the population under consideration will only arise in alignment with the applied [primary and secondary care] strategies of prevention and the provision of clear and complete community information over time.

In addition, the future goal of eliminating HPV genotypes associated with pathological conditions may be the local production of multiple-activity antigens among the various types of HPV.

Conclusion

In conclusion, it is obvious that as an effective cervical cancer screening program does not exist, vaccination of women and men may prove to be the best strategic choice, as it will not only make it possible in the long term to reduce the risk of developing the disease but will also reduce its cost to the country's health budget, since it will prevent cases that would otherwise require very costly treatment. On this basis, national health policy on the prevention of cervical cancer should focus on implementing a vaccination program targeting adolescents and adolescents for the ultimate reduction of HPV infections, which is a potentially carcinomatous condition.

Authors' Contributions

Ioannis Drikos, Alexandros Sachinidis, Ioanna Vassi, Argyrios Ioannidis participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

This article does not contain any studies with human participants performed by any of the authors.

References

1. Nencioni AFF, Patrone GF, Brossart P (2004) Anticancer vaccination strategies. Ann Oncol 15: 153–160.

2. Sung H, Simon R (2004) Candidate epitope identification using peptide property models: application to cancer immunotherapy. Methods 34: 460–467.

3. Huber CH, Wolfel T (2004) Immunotherapy of cancer: From vision to standard clinical practice. J Cancer Res Clin Oncol 130: 367–374.

4. Jemal A, Thun MJ, Ries LA, Howe HL, Weir HK, et al. (2008) Annual report to the nation on the status of cancer, 1975-2005, featuring trends in lung cancer, tobacco use, and tobacco control. J Natl Cancer Inst 100: 1672–1694.

5. Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. (2008) Cancer statistics, 2008. CA Cancer J Clin 58: 71–96.

6. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S (2007) Human papillomavirus and cervical cancer. Lancet 370: 890–907.

7. Wright TC Jr, Schiffman M (2003) Adding a test for human papillomavirus DNA to cervical-cancer screening. N Engl J Med 348: 489–490.

8. McCredie MR, Sharples KJ, Paul C, Baranyai J, Medley G, et al. (2008) Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: A retrospective cohort study. Lancet Oncol 9: 425–432.

9. Tindle RW (2002) Immune evasion in human papillomavirus-associated cervical cancer. Nat Rev Cancer 2: 59–65.

10. Zur Hausen H (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2: 342–350.

11. Villa LL, Perez G, Kjaer SK, Paavonen J, Muñoz N, et al. (2007) Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl JMed 356: 1915–1927.

12. Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, et al. (2006) Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomized control trial. Lancet 367: 1247–1255.

13. Jabbar IA, Fernando GJ, Saunders N, Aldovini A, Young R, et al. (2000) Immune responses induced by BCG recombinant for human papillomavirus L1 and E7 proteins. Vaccines 18: 2444–2453.

14. Hung CF, Ma B, Monie A, Tsen SW, Wu TC (2008) Therapeutic human papillomavirus vaccines: current clinical trials and future directions. Expert Opin Biol Ther 8: 421–439.

15. Donnelly JJ, Ulmer JB, Liu MA (1997) DNA vaccines. Life Sci 60: 163–172.

16. Gurunathan S, Klinman DM, Seder RA (2000) DNA vaccines: Immunology, application, and optimization. Annu Rev Immunol 18: 927–974.

17. Wlazlo AP, Deng H, Giles-Davis W, Ertl HC (2004) DNA vaccines against the human papillomavirus type 16 E6 or E7 oncoproteins. Cancer Gene Ther 11: 457–464.

18. Rittich S, Duskova M, Mackova J, Pokorna D, Jinoch P, et al. (2005) Combined immunization with DNA and transduced tumor cells expressing mouse GM-CSF or IL-2. Oncol Rep 13: 311–317.

19. Chuang CM, Hoory T, Monie A, Wu A, Wang MC, et al. (2009) Enhancing therapeutic HPV DNA vaccine potency through depletion of CD4+ CD25+ T regulatory cells. Vaccine 27: 684–689.

20. Tseng CW, Monie A, Wu CY, Huang B, Wang MC, et al. (2008) Treatment with proteasome inhibitor bortezomib enhances antigen-specific CD8+ T-cell-mediated antitumor immunity induced by DNA vaccination. J Mol Med 86: 899–908.

21. Chuang CM, Monie A, Wu A, Hung CF (2009) Combination of apigenin treatment with therapeutic HPV DNA vaccination generates enhanced therapeutic antitumor effects. J Biomed Sci 16: 49.

22. Yao Y, Huang W, Yang X, Sun W, Liu X, et al. (2013) HPV-16 E6 and E7 protein T cell epitopes prediction analysis based on distributions of HLA-A loci across populations: an in silico approach. Vaccines 31: 2289–2294.

23. Feltkamp MC, Smits HL, Vierboom MP, Minnaar RP, De Jongh BM, et al. (1993) Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. Eur J Immunol 23: 2242–2249.

24. Kumar A, Hussain S, Yadav IS, Gissmann L, Natarajan K, et al. (2015) Identification of human papillomavirus-16 E6 variation in cervical cancer and their impact on T and B cell epitopes. J Virol Methods 218: 51–58.

25. Riemer AB, Keskin DB, Zhang G, Handley M, Anderson KS, et al. (2010) A conserved E7-derived cytotoxic T lymphocyte epitope expressed on human papillomavirus 16-transformed HLA-A2+ epithelial cancers. J Biol Chem 285: 29608–29622.

26. Ma B, Maraj B, Tran NP, Knoff J, Chen A, et al. (2012) Alvarez RD, Hung CF, Wu TC. Emerging human papillomavirus vaccines. Expert Opin Emerg Drugs. 17: 469–492.

27. Ghittoni R, Accardi R, Chiocca S, Tommasino M (2015) Role of human papillomaviruses in carcinogenesis. E-Cancer Medical Science. 9: 526.

28. Doorbar J (2016) Model systems of human papillomavirus-associated disease. J Pathol 238:166–179.

29. Yang A, Jeang J, Cheng K, Cheng T, Yang B, et al. (2016) Current state in the development of candidate therapeutic HPV vaccines. Expert Rev Vaccines. 15: 989–1007.

30. Adachi K, Kawana K, Yokoyama T, Fujii T, Tomio A, et al. (2010) Oral immunization with a Lactobacillus casei vaccine expressing human papillomavirus (HPV) type 16 E7 is an effective strategy to induce mucosal cytotoxic lymphocytes against HPV16 E7. Vaccines 28: 2810–2817.

31. Bermudez-Humaran LG, Cortes-Perez NG, Le Loir Y, Alcocer-Gonzalez JM, Tamez-Guerra RS, et al. (2004) An inducible surface presentation system improves cellular immunity against human papillomavirus type 16 E7 antigen in mice after nasal administration with recombinant lactococci. J Med Microbiol 53: 427–433.

32. Arias-Pulido H, Peyton CL, Joste NE, Vargas H, Wheeler CM (2006) Human papillomavirus type 16 integration in cervical carcinoma in situ and in invasive cervical cancer. J Clin Microbiol 44:1755–1762.

33. Pett M, Coleman N (2007) Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? J Pathol 212: 356–367.

34. Gray E, Pett MR, Ward D, Winder DM, Stanley MA, et al. (2010) In vitro progression of human papillomavirus 16 episome associated cervical neoplasia displays fundamental similarities to integrant associated carcinogenesis. Cancer Res 70: 4081–4091.

35. Rosales R, Lopez-Contreras M, Rosales C, Magallanes-Molina JR, Gonzalez- Vergara R, et al. (2014) Regression of human papillomavirus intraepithelial lesions is induced by MVA E2 therapeutic vaccine. Hum Gene Ther 25: 1035–1049.

36. Hsu KF, Hung CF, Cheng WF, He L, Slater LA, et al. (2008) Enhancement of suicidal DNA vaccine potency by linking Mycobacterium tuberculosis heat shock protein 70 to an antigen. Gene Ther 8: 376–383.

37. Kim TW, Hung CF, Juang J, He L, Hardwick JM, et al. (2004) Enhancement of suicidal DNA vaccine potency by delaying suicidal DNA-induced cell death. Gene Ther 11: 336–342.

38. Varnavski AN, Young PR, Khromykh AA 2000) Stable high-level expression of heterologous genes in vitro and in vivo by non-cytopathic DNA-based Kunjin virus replicon vectors. J Virol 74: 4394–403.

39. Herd KA, Harvey T, Khromykh AA, Tindle RW (2004) Recombinant Kunjin virus replicon vaccines induce protective T-cell immunity against human papillomavirus 16 E7-expressing tumour. Virology 319: 237–248.

40. Sebastian M, Papachristofilou A, Weiss C, Fruh M, Cathomas R, et al. (2014) Phase IB study evaluating a self-adjuvanted mRNA cancer vaccine (RNActive(R)) combined with local radiation as consolidation and maintenance treatment for patients with stage IV non-small cell lung cancer. BMC Cancer. 14: 748.

41. Sheets EE, Urban RG, Crum CP, Hedley ML, Politch JA, et al. (2003) Immunotherapy of human cervical high-grade cervical intraepithelial neoplasia with microparticle-delivered human papillomavirus 16 E7 plasmid DNA. Am J Obstet Gynecol 188: 916–926.

42. Pokorna D, Rubio I, Muller M (2008) DNA-vaccination via tattooing induces stronger humoral and cellular immune responses than intramuscular delivery supported by molecular adjuvants. Genet Vaccines Ther 6:4.

43. Prausnitz MR, Mikszta JA, Cormier M, Andrianov AK (2009) Microneedle-based vaccines. Curr Top Microbiol Immunol 333: 369–393.

44. Voltan R, Castaldello A, Brocca-Cofano E, De Michele R, Triulzi C, et al. (2009) Priming with a very low dose of DNA complexed with cationic block copolymers followed by protein boost elicits broad and long-lasting antigen-specific humoral and cellular responses in mice. Vaccines 27: 4498–4507.

45. Jagu S, Karanam B, Gambhira R, Chivukula S, Chaganti R, et al. (2009) Concatenated multitype L2 fusion proteins as candidate prophylactic pan-human papillomavirus vaccines. J Natl Cancer Inst 101: 782-792.

46. Fraillery D, Baud D, Pang S, Schiller J, Bobst M, et al. (2007) Salmonella enterica serovar typhi Ty21a expressing human papillomavirus type 16 L1 as a potential live vaccine against cervical cancer and typhoid fever. Clin Vaccine Immunol 14: 1285-1295.

47. Varsani A, Williamson AL, Rose RC, Jaffer M, Rybicki EP (2003) Expression of human papillomavirus type 16 major capsid protein in transgenic Nicotiana tabacum cv. Xanthi. Arch Virol 148: 1771-1786.

48. Fernandez-San Millan A, Ortigosa SM, Hervás-Stubbs S, Corral-Martínez P, Seguí-Simarro JM, et al. (2008) Human papillomavirus L1 protein expressed in tobacco chloroplasts selfassembles into virus-like particles that are highly immunogenic. Plant Biotechnol J 6: 427-441.

49. Chitale R (2009) Merck hopes to extend gardasil vaccine to men. J Natl Cancer Inst 101: 222-225.