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Commentary

# Moving forward with human papillomavirus immunotherapies

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## ABSTRACT

Persistent human papillomavirus (HPV) is the primary etiologic agent of cervical cancer and causes a significant number of vulvar, penile, anal and oropharyngeal cancers. The development of highly effective HPV therapeutic vaccines is a reasonable goal given the recent advances in basic and applied immunology. A number of vaccine strategies designed to induce systemic T cell responses have been tested in clinical trials against high grade cervical or vulvar high grade neoplasia and cancers, but with limited success. In line with the emerging trend to focus more on the epithelial context of HPV infection and premalignant disease, it might be advantageous to develop vaccination strategies that promote trafficking of HPV-specific T cells into lesions and overcome the local immunosuppressive environment. The development of more biologically relevant animal models would improve the preclinical evaluation of therapeutic vaccine candidates. Finally, persistent infection and low grade lesions may prove to be easier targets for therapeutic vaccines, and these vaccines would likely be commercially viable in high income countries and valuable components in screen and treat programs in low resource settings.

KEYWORDS: Human papillomavirus | cancer | immunotherapy | therapeutic vaccines | mucosal immunity

Human papillomavirus is a large family of epitheliotropic non-enveloped circular DNA genome viruses. Most HPV infections are cleared, however persistent infection with high-risk HPV, especially types 16 and 18, can lead to the development of intraepithelial neoplasia and carcinoma in the cervical, vulvar/vaginal, penile and anal and oropharyngeal mucosa.[1] Systematic screening programs to detect high grade cervical intraepithelial neoplasia and ablative treatment are effective to reduce the incidence of cervical cancer.[2] Because early detection programs for non-cervical sites are not yet available, malignant lesions or carcinoma are often diagnosed at an advanced stage and often require debilitating treatments. Prophylactic vaccines against high-risk HPV infections are attractive interventions to reduce the burden of HPV-induced neoplasia in the long term. However, in the absence of full vaccine coverage and the presence of large number of women with prevalent infections, the incidence of HPV-induced neoplasia will remain a global health concern for many years. Persistent infection and maintained expression of the HPV oncoproteins E6 and E7 are critical factors in the development of intraepithelial neoplasia and cancers, and therefore constitute promising targets for therapeutic vaccines.[3] The evolution of persistent HPV infection to cancer is a slow process that generally takes several decades upon first exposure, which gives a broad window of opportunity to intervene.

## Clinical evaluation of immunotherapeutic interventions against HPV-associated neoplasia

Most clinical trials of therapeutic vaccines and immunotherapies against HPV induced diseases have targeted high grade precancers and cancers of the cervico-vaginal and vulvar mucosa. Numerous available vaccine platforms have been tested (see [4,5] for comprehensive reviews). Most of these vaccines were given systemically or remotely from the site of the lesions. The vaccines platforms evaluated include naked DNA,[6-8] viral vectors such as vaccinia and MVA,[9-12] bacteria such as oral Lactobacillus,[13] proteins [14-16] and short and long peptides.[17-19] In spite of evidence for the induction of cellular immune responses against E6 and/or E7, these clinical trials showed regression in a subset of subjects, at best. Most trials did not have a randomized placebo control group to establish the rate of spontaneous regression in the trial subjects and so the efficacies of the vaccines were often difficult to estimate. The few randomized

controlled trials have demonstrated modestly higher regression rates in the vaccine recipients. To the best of our knowledge, therapeutic HPV vaccines have not been formally evaluated in a randomized phase III trial and consequently none has received regulatory approval.

Perhaps, the most notable response rates were observed in a large trial involving multiple rounds of intracervical or intralesional injection at other genital sites with an MVA vector expressing bovine papillomavirus E2.[20,21] Most subjects experienced clinical and virological cures. However, it is unclear whether induction of an adaptive immune response to E2 or the viral antigens in the lesions was involved in the mechanism of regression, and the need for multiple intralesional injections may prove to be a impediment to wide spread implementation.

In addition to traditional vaccines, adoptive T cell transfer therapy has been recently evaluated in a small number patient with metastatic cervical cancer and showed a correlation between clinical response (3 out of 9 subjects) and persistence of HPV-specific transferred T cells.[22] The study provides important proof of concept that cervical cancers can be susceptible to T cell-mediated effector mechanisms.

Immune modulators based on TLR7/8 agonists have been approved for the treatment of genital warts and basal cell carcinomas.[23,24] The use of Imiquimod for the treatment of CIN2/3 or VIN2/3 has shown limited clinical benefits and topical application was more or less well tolerated depending on the study.[25-27]

## Why therapeutic vaccines have shown limited efficacy against HPV associated neoplasia

Multiple reasons could explain the limited success of therapeutic vaccines against HPV-induced neoplasia to date. Numerous clinical trials have shown induction of T cell responses against E6 and E7, but the magnitude of the response induced against HPV antigens might have been insufficient to clear HPV infected or transformed cells. The balance of T cell responses between $CD8^+$ cytotoxic T cells (CTL), $CD4^+$ Th1, Th2, Th9, Th17 or Treg induced against HPV antigens included in the vaccine might have been unfit to trigger a clinical response.[28,29] In addition, induction of the recently described genital tissue resident memory T cells (Trm) may be critical for highly effective vaccines against HPV infections and their associated cancers.[30]

The modulation of the local immune environment by HPV infected or transformed cells might have prevented T cells to effectively clear lesions. Some of the mechanisms described to participate in immune escape of HPV infected cells to CTL induction and/or killing are MHC dowregulation, defects in class I antigen processing,[31] disruption of the inflammatory cytokine response by keratinocytes,[32] upregulation of inhibitory molecules such as PD-L1 or IL-10,[33,34] evolution of a Th2 skewed helper response during neoplastic progression,[35] and induction of regulatory T cells.[36] Deregulation of local homeostasis such as disruption of the chemokine gradient in situ has been shown to prevent infiltration of lesions by T cells.[37] Loss of Langerhans cells, the primary antigen presenting cells in stratified epithelium, is a common feature in lesions induced by high risk mucosal HPV types.[38] In addition, the microbiota might also play a role in the development of HPV-induced neoplasia and shaping an immune suppressive environment, but its role remains unclear.[39,40]

Finally, most HPV therapeutic vaccines have been given systemically or in a remote mucosal site, which might induce a bona fide response but with limited trafficking to the lesions. Failure of T cells to infiltrate infected epithelium appears to be a major mechanism of immune escape in cervicovaginal HPV infection.[37] With few exceptions, the induction of tissue resident memory $CD8^+$ T cells in the genital epithelium after systemic immunization has not been yet addressed in clinical trials and, optimal regimens and vaccine platforms able to induce genital Trm remain to be defined. Importantly, the exclusion of Trm from the circulation implies that classical blood-based immunomonitoring might not predict their induction.

## Improving the efficacy of therapeutic vaccines against HPV

Combination of vaccines with topically administered immune modulators, such as TLR agonists, have been evaluated in preclinical models.[41,42] Such approaches induce local inflammation that can recruit circulating T cells to the lesions and therefore increase the ratio of effector to target cells. In addition, this “prime-pull” approach to enhance the recruitment of T cells to the application site might also render infected and transformed cells more susceptible to killing by CTL and mitigate repressive immune modulation. It is encouraging that clinical trials of systemic immunization followed by local stimulation with a TLR agonist are underway (e.g. NCT00788164).

Combination of vaccines with systemic administration of an immune checkpoint inhibitor or stimulatory antibodies is an alternative method to overcome direct

inhibition of T cells by ligands expressed by tumor cells or APC in the tumor environment. Immune checkpoint inhibitors have been recently approved to treat lung cancer and melanoma and are being evaluated against many other cancer types.[43] Expression of PD-L1 by HPV-infected and transformed epithelial cells has been documented,[33] suggesting that it could prevent infiltration and lysis of infected cells by HPV-specific T cells. Antibodies targeting PD-L1 or its receptor PD-1 on HPV-specific T cells might unleash a T cell response at the lesions. Although current approaches are promising for advanced stage carcinomas, they might not be amenable for treatment of premaligant disease because of the substantial adverse events that are frequently seen with systemic application of immune checkpoint inhibitors.[44] The topical application of immune checkpoint inhibitors on the lesions might mitigate the systemic toxicity of immune checkpoint inhibitor while retaining efficacy. IgG is transported bidirectionally across the cervicovaginal epithelium via the neonatal Fc receptor.[45] Therefore, monoclonal antibodies that inhibit immune check points might reach T cells and other immune cells in the genital submucosa in locally active concentrations after intravaginal application.

In preclinical models, intravaginal vaccination induces large numbers of local antigen-specific intraepithelial T cells, whereas systemic vaccination does not. Viral vector-mediated vaccination at distant mucosal sites, e.g., intranasal, was much less efficient at inducing intraepithelial T cells in the genital tract than intravaginal vaccination.[46,47] A recent pilot clinical trial of CIN2/3 patients compared intramuscular, intradermal and direct intralesional injection of a naked E6/E7-expressing plasmid DNA vaccine. Consistent with the preclinical studies, intralesional $CD8^+$ T cell infiltrates increased after intralesional, but not after intramuscular or intradermal injection.[48] However, there was no difference in the limited clinical and virological responses observed with the 3 routes of delivery. An interesting question is whether it is preferable to introduce a vaccine directly into the HPV neoplastic lesions or elsewhere in the cervicovaginal tract. Intralesional vaccination might maximize the focus of the induced immune cells to the infected cells. However, a local suppressive environment might inhibit induction of an effective immune response, particularly during priming. Since intraepithelial $CD8^+$ T cells are highly mobile within the lower epithelial layers,[49] T cells induced or amplified at adjacent sites should be able to reach the infected cells. In our opinion, alternative intravaginal vaccination strategies deserve future clinical evaluation.

## Need for more accurate preclinical models of HPV persistent infection and low-grade lesions

Numerous preclinical animal models have been used to evaluate therapies against HPV-associated malignancies. Preclinical therapeutic efficacy of vaccines has usually been tested in subcutaneous or orthotopic mouse models in which high numbers of in vitro passaged syngeneic tumor cells are transplanted.[50,51] In spite of the impressive protection displayed by some vaccine candidates in these models, the preclinical findings have not generally translated into clinical efficacy. This discrepancy might be due to differential immunogenicity of the vaccine platforms in mice and humans. In particular, adjusting the dosage of vaccine antigens between mice and humans is difficult to achieve and might lead to over estimating human immunogenicity using mouse models. Also, due to the rapid growth of the transplanted tumor cells and time needed to induce a cellular response, therapeutic vaccine must be given at relatively early stages when the tumors are still small and presumably before immune escape mechanisms are fully developed. In addition, although the in vivo biology of the tumors and the surrounding microenvironment are not well documented, they probably do not closely match those of slowly arising intraepithelial neoplasia in humans.

Current models using E6 and E7 transgenic mice permit the study of the cellular and molecular mechanisms involved in the development of premalignant diseases and immunosuppression at early stages.[52-54] However, the tissue wide expression of E6 and E7 does not reflect their focal expression in human neoplasia, and tissue wide expression of the oncogenes does not permit selective T cell targeting of the transformed cells in the lesions. Genetic models allowing for focal expression of E6 and E7 oncoproteins that drive the in situ development of intraepithelial neoplasia in cervicovaginal, anal or oropharyngeal epithelium would likely be more predictive preclinical models to evaluate immunotherapies and vaccines. Development of this type of models should be a high priority.

## Handicapping targets for HPV therapeutic vaccines

Effective therapeutic vaccines would be useful across the entire spectrum of HPV induced neoplasia, from persistent infection and its histological manifestation, grade 1 intraepithelial neoplasia, to malignant cervicovaginal, anal, and oral cancers. Effective therapeutic vaccines for cancer are the most pressing need. Unfortunately, they will almost certainly be the most difficult to develop, due to the many well documented ways that the genetically unstable tumors can evolve to escape immune surveillance. Most clinical trials of therapeutic HPV vaccines have targeted CIN3 (or CIN2/3), in large part because these lesions (especially CIN3) infrequently regress and, when identified

in screening programs, require treatment, currently with ablative therapies.[55] While generally effective, these destructive treatments are associated with a significant number of failures and adverse events. Nevertheless, the general effectiveness and tolerability of current CIN3 therapies places a rather high bar for immunotherapeutic approaches.

In contrast to CIN3, ablative therapy of anal intraepithelial neoplasia (AIN3) is more often associated with substantial morbidity and a more benign immunotherapy would address a large unmet need.[56] Important questions that deserves further inquiry are what proportion of CIN3s and AIN3s are genetically unstable and have already undergone immune selection and whether these features vary by HPV type. The possibility remains that they may be closer to cancers than asymptomatically infected tissues and low grade lesions with regard to susceptibility to immune interventions.

CIN2 is a rather difficult to define intermediate class of lesions that might be an attractive target for immunotherapy because of the current inability to estimate the likelihood that a specific lesion will regress or progress to CIN3.[57] Thus there is an unmet need for a more benign treatment of these lesions.

CIN1/persistent cervical infection has received relatively little interest as a target for HPV immunotherapy. In part, this may be because it is not considered a treatable disease with current therapies. Also, therapeutic efficacy trials would have to be relatively large because of the high rate of spontaneous regression. However, with the increasing adoption of virological-based primary screening for cervical cancer risk in high income countries, there will be increasingly large numbers of women who will be informed that they have a potentially oncogenic sexually transmitted infection, but there is no treatment option for them unless it progresses to a high grade precancer. These women would likely provide a large and motivated market for a therapeutic vaccine, provided it was benign enough for use against infections that have a 90% spontaneous clearance rate. More importantly from an international public health point of view, a safe immunotherapeutic vaccine against persistent infection might greatly facilitate cervical cancer prevention programs in low resource settings that could involve point of care HPV detection followed immediately by therapeutic (and perhaps also prophylactic) vaccination of women who test positive for oncogenic HPV types.

Importantly, persistent infection/CIN1/AIN1 are likely to be more susceptible to immunotherapies than advanced lesions since they are less likely to be genetically

unstable or to have undergone immune selection. Productive infections also provide more viral antigen targets, including E2, which appears to a major T cell target in natural infection.[58] In our opinion, it might be preferable to focus more attention on developing vaccines to treat productive infections, since they are more likely to be successful, and subsequently apply the lessons learned in their development to the presumably more difficult case of more advanced lesions.

## Conclusions

There are reasons to be optimistic that development of highly effective HPV therapeutic vaccines can be achieved, including the many recent advances in basic and applied immunonology and the current level of public sector and industry support. However, a relatively large number of reasonable vaccine strategies focused on induction and measurement of systemic immune responses have been tried, but with limited success. It is our opinion that the emerging trend to focus more on the epithelial context of HPV infection and premalignant disease should be strongly encouraged. Efficient trafficking of viral-antigen specific T cells to lesions and overcoming the local immunosuppressive environment once they arrive may be critical components to a successful vaccine. Development of more biologically relevant animal models would increase our ability to rank the potential of various candidates for clinical trials. Finally, we believe that HPV therapeutic vaccine development should move up the disease severity continuum rather that down it, as has been the case historically. In this regard, it seems important for the research community to clearly establish an investment case for vaccines targeting persistent infections, including detailed modeling of efficacy trials, in order obtain sufficient public sector and industry support for these endeavors.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

# References

1. Zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology 2009; 384(2):260-5; PMID:19135222; http://dx.doi.org/10.1016/j.virol.2008.11.046 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

2. Bosch FX, Broker TR, Forman D, Moscicki AB, GillisonML, Doorbar J, Stern PL, Stanley M, Arbyn M, Poljak M, et al. Comprehensive control of human papillomavirus infections and related diseases. Vaccine 2013; 31(Suppl 7):H1-31; http://dx.doi.org/10.1016/j.vaccine.2013.10.003 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

3. Stanley MA. Epithelial cell responses to infection with human papillomavirus. Clin Microbiol Rev 2012; 25(2):215-22; PMID:22491770; http://dx.doi.org/10.1128/CMR.05028-11 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

4. Skeate JG, Woodham AW, Einstein MH, Da Silva DM, Kast WM. Current therapeutic vaccination and immunotherapy strategies for HPV-related diseases. Hum Vaccin Immunother 2016; 12(6):1418-29: 1–12; PMID:26835746; http://dx.doi.org/10.1080/21645515.2015.1136039 [Taylor & Francis Online] [PubMed] [Web of Science ®], [Google Scholar]

5. Tran NP, Hung CF, Roden R, Wu TC. Control of HPV infection and related cancer through vaccination. Recent Results Cancer Res 2014; 193:149-71; PMID:24008298; http://dx.doi.org/10.1007/978-3-642-38965-8_9 [Crossref] [PubMed], [Google Scholar]

6. Bagarazzi ML, Yan J, Morrow MP, Shen X, Parker RL, Lee JC, Giffear M, Pankhong P, Khan AS, Broderick KE, et al. Immunotherapy against HPV16/18 generates potent TH1 and cytotoxic cellular immune responses. Sci Transl Med 2012; 4(155):155ra38; http://dx.doi.org/10.1126/scitranslmed.3004414 [Crossref] [Web of Science ®], [Google Scholar]

7. Trimble CL, Morrow MP, Kraynyak KA, Shen X, DallasM, Yan J, Edwards L, Parker RL, Denny L, Giffear M, et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and

E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. Lancet 2015; 86(10008):2078-88; http://dx.doi.org/10.1016/S0140-6736(15)00239-1 [Crossref] [Web of Science ®], [Google Scholar]

8. Kim TJ, Jin HT, Hur SY, Yang HG, Seo YB, Hong SR, Lee CW, Kim S, Woo JW, Park KS, et al. Clearance of persistent HPV infection and cervical lesion by therapeutic DNA vaccine in CIN3 patients. Nat Commun 2014; 5:5317; PMID:25354725; http://dx.doi.org/10.1038/ncomms6317 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

9. Maldonado L, Teague JE, Morrow MP, Jotova I, Wu TC, Wang C, Desmarais C, Boyer JD, Tycko B, Robins HS, et al. Intramuscular therapeutic vaccination targeting HPV16 induces T cell responses that localize in mucosal lesions. Sci Transl Med 2014; 6(221):221ra13; PMID:24477000; http://dx.doi.org/10.1126/scitranslmed.3007323 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

10. Baldwin PJ, van der Burg SH, Boswell CM, Offringa R, Hickling JK, Dobson J, Roberts JS, Latimer JA, Moseley RP, Coleman N, et al. Vaccinia-expressed human papillomavirus 16 and 18 e6 and e7 as a therapeutic vaccination for vulval and vaginal intraepithelial neoplasia. Clin Cancer Res 2003; 9(14):5205-13; PMID:14614000 [PubMed] [Web of Science ®], [Google Scholar]

11. Borysiewicz LK, Fiander A, Nimako M, Man S, Wilkinson GW, Westmoreland D, Evans AS, Adams M, Stacey SN, Boursnell ME, et al. A recombinant vaccinia virus encoding human papillomavirus types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. Lancet 1996; 347(9014):1523-7; PMID:8684105; http://dx.doi.org/10.1016/S0140-6736(96)90674-1 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

12. Brun JL, Dalstein V, Leveque J, Mathevet P, Raulic P, Baldauf JJ, Scholl S, Huynh B, Douvier S, Riethmuller D, et al. Regression of high-grade cervical intraepithelial neoplasia with TG4001 targeted immunotherapy. Am J Obstet Gynecol 2011; 204(2):169 e1-8; PMID:21284968; http://dx.doi.org/10.1016/j.ajog.2010.09.020 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

13. Kawana K, Adachi K, Kojima S, Taguchi A, TomioK, Yamashita A, Nishida H, Nagasaka K, Arimoto T, Yokoyama T, et al. Oral vaccination against HPV E7 for treatment of cervical intraepithelial neoplasia grade 3 (CIN3) elicits E7-specific mucosal immunity in the cervix of CIN3 patients. Vaccine 2014; 32(47):6233-9; PMID:25258102; http://dx.doi.org/10.1016/j.vaccine.2014.09.020 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

14. Frazer IH, Quinn M, Nicklin JL, Tan J, Perrin LC, Ng P, O'Connor VM, White O, Wendt N, Martin J, et al. Phase 1 study of HPV16-specific immunotherapy with E6E7 fusion protein and ISCOMATRIX adjuvant in women with cervical intraepithelial neoplasia. Vaccine 2004; 23(2):172-81; PMID:15531034; http://dx.doi.org/10.1016/j.vaccine.2004.05.013 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

15. Davidson EJ, Faulkner RL, Sehr P, Pawlita M, Smyth LJ, Burt DJ, Tomlinson AE, Hickling J, Kitchener HC, Stern PL. Effect of TA-CIN (HPV 16 L2E6E7) booster immunisation in vulval intraepithelial neoplasia patients previously vaccinated with TA-HPV (vaccinia virus encoding HPV 16/18 E6E7). Vaccine 2004; 22(21–22):2722-9; PMID:15246603; http://dx.doi.org/10.1016/j.vaccine.2004.01.049 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

16. de Jong A, O'Neill T, Khan AY, Kwappenberg KM, Chisholm SE, Whittle NR, Dobson JA, Jack LC, St Clair Roberts JA, Offringa R, et al. Enhancement of human papillomavirus (HPV) type 16 E6 and E7-specific T-cell immunity in healthy volunteers through vaccination with TA-CIN, an HPV16 L2E7E6 fusion protein vaccine. Vaccine 2002; 20(29–30):3456-64.;PMID:12297390; http://dx.doi.org/10.1016/S0264-410X(02)00350-X [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

17. de Vos van Steenwijk PJ, Ramwadhdoebe TH, Lowik MJ, van der Minne CE, Berends-van der Meer DM, Fathers LM, Valentijn AR, Oostendorp J, Fleuren GJ, Hellebrekers BW, et al. A placebo-controlled randomized HPV16 synthetic long-peptide vaccination study in women with high-grade cervical squamous intraepithelial lesions. Cancer Immunol Immunother 2012; 61(9):1485-92.;PMID:22684521; http://dx.doi.org/10.1007/s00262-012-1292-7 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

18. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, Essahsah F, Fathers LM, Offringa R, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N Eng J Med 2009; 361(19):1838-47; http://dx.doi.org/10.1056/NEJMoa0810097 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

19. Coleman HN, Greenfield WW, Stratton SL, Vaughn R, Kieber A, Moerman-Herzog AM, Spencer HJ, Hitt WC, Quick CM, Hutchins LF, et al. Human papillomavirus type 16 viral load is decreased following a therapeutic vaccination. Cancer Immunol Immunother 2016; 65(5):563-73; PMID:26980480; http://dx.doi.org/10.1007/s00262-016-1821-x [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

20. Rosales R, Lopez-Contreras M, Rosales C, Magallanes-Molina JR, Gonzalez-Vergara R, Arroyo-Cazarez JM, Ricardez-Arenas A, Del Follo-Valencia A, Padilla-Arriaga S, Guerrero MV, et al. Regression of human papillomavirus intraepithelial lesions is induced by MVA E2 therapeutic vaccine. Hum Gene Ther 2014; 25(12):1035-49; PMID:25275724; http://dx.doi.org/10.1089/hum.2014.024 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

21. Garcia-Hernandez E, Gonzalez-Sanchez JL, Andrade-Manzano A, Contreras ML, Padilla S, Guzman CC, Jiménez R, Reyes L, Morosoli G, Verde ML, et al. Regression of papilloma high-grade lesions (CIN 2 and CIN 3) is stimulated by therapeutic vaccination with MVA E2 recombinant vaccine. Cancer Gene Ther 2006; 13(6):592-7; PMID:16456551; http://dx.doi.org/10.1038/sj.cgt.7700937 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

22. Stevanovic S, Draper LM, Langhan MM, Campbell TE, Kwong ML, Wunderlich JR, Dudley ME, Yang JC, Sherry RM, Kammula US, et al. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. J Clin Oncol 2015; 33(14):1543-50; PMID:25823737; http://dx.doi.org/10.1200/JCO.2014.58.9093 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

23. Beutner KR, Spruance SL, Hougham AJ, Fox TL, OwensML, Douglas JM, Jr. Treatment of genital warts with an immune-response modifier (imiquimod). J Am Acad Dermatol

1998; 38(2 Pt 1):230-9.;PMID:9486679; http://dx.doi.org/10.1016/S0190-9622(98)70243-9 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

24. Schulze HJ, Cribier B, Requena L, Reifenberger J, Ferrandiz C, Garcia Diez A, Tebbs V, McRae S. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from a randomized vehicle-controlled phase III study in Europe. Br J Dermatol 2005; 152(5):939-47; PMID:15888150; http://dx.doi.org/10.1111/j.1365-2133.2005.06486.x [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

25. Pachman DR, Barton DL, Clayton AC, McGovern RM, Jefferies JA, Novotny PJ, Sloan JA, Loprinzi CL, Gostout BS. Randomized clinical trial of imiquimod: an adjunct to treating cervical dysplasia. Am J Obstet Gynecol 2012; 206(1):42 e1-7; PMID:21907959; http://dx.doi.org/10.1016/j.ajog.2011.06.105 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

26. Lin CT, Qiu JT, Wang CJ, Chang SD, Tang YH, Wu PJ, Jung SM, Huang CC, Chou HH, Jao MS, et al. Topical imiquimod treatment for human papillomavirus infection in patients with and without cervical/vaginal intraepithelial neoplasia. Taiwan J Obstet Gynecol 2012; 51(4):533-8; PMID:23276555; http://dx.doi.org/10.1016/j.tjog.2012.09.006 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

27. Grimm C, Polterauer S, Natter C, Rahhal J, Hefler L, Tempfer CB, Heinze G, Stary G, Reinthaller A, Speiser P. Treatment of cervical intraepithelial neoplasia with topical imiquimod: a randomized controlled trial. Obstet Gynecol 2012; 120(1):152-9; PMID:22914404; http://dx.doi.org/10.1097/AOG.0b013e31825bc6e8 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

28. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nat Rev Immunol 2008; 8(4):247-58; PMID:18323851; http://dx.doi.org/10.1038/nri2274 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

29. Disis ML, Watt WC, Cecil DL. Th1 epitope selection for clinically effective cancer vaccines. Oncoimmunol 2014; 3(9):e954971; http://dx.doi.org/10.4161/21624011.2014.954971 [Taylor & Francis Online] [PubMed] [Web of Science ®], [Google Scholar]

30. Park CO, Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. Nat Med 2015; 21(7):688-97; PMID:26121195; http://dx.doi.org/10.1038/nm.3883 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

31. Sikorski M, Bobek M, Zrubek H, Marcinkiewicz J. Dynamics of selected MHC class I and II molecule expression in the course of HPV positive CIN treatment with the use of human recombinant IFN-gamma. Acta Obstet Gynecol Scand 2004; 83(3):299-307; PMID:14995928 [Taylor & Francis Online] [PubMed] [Web of Science ®], [Google Scholar]

32. Niebler M, Qian X, Hofler D, Kogosov V, Kaewprag J, Kaufmann AM, Ly R, Böhmer G, Zawatzky R, Rösl F, et al. Post-translational control of IL-1beta via the human papillomavirus type 16 E6 oncoprotein: a novel mechanism of innate immune escape mediated by the E3-ubiquitin ligase E6-AP and p53. PLoS Pathog 2013; 9(8):e1003536; PMID:23935506; http://dx.doi.org/10.1371/journal.ppat.1003536 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

33. Mezache L, Paniccia B, Nyinawabera A, Nuovo GJ. Enhanced expression of PD L1 in cervical intraepithelial neoplasia and cervical cancers. Mod Pathol 2015; 28(12):1594-602; PMID:26403783; http://dx.doi.org/10.1038/modpathol.2015.108 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

34. Syrjanen S, Naud P, Sarian L, Derchain S, Roteli-Martins C, Longatto-Filho A, Tatti S, Branca M, Erzen M, Hammes LS, et al. Immunosuppressive cytokine Interleukin-10 (IL-10) is up-regulated in high-grade CIN but not associated with high-risk human papillomavirus (HPV) at baseline, outcomes of HR-HPV infections or incident CIN in the LAMS cohort. Virchows Arch 2009; 455(6):505-15.;PMID:19908064; http://dx.doi.org/10.1007/s00428-009-0850-7 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

35. Bais AG, Beckmann I, Lindemans J, Ewing PC, MeijerCJ, Snijders PJ, Helmerhorst TJ. A shift to a peripheral Th2-type cytokine pattern during the carcinogenesis of cervical cancer becomes manifest in CIN III lesions. J Clin Pathol 2005; 58(10):1096-100; PMID:16189158; http://dx.doi.org/10.1136/jcp.2004.025072 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

36. Kojima S, Kawana K, Tomio K, Yamashita A, Taguchi A, Miura S, Adachi K, Nagamatsu T, Nagasaka K, Matsumoto Y, et al. The prevalence of cervical regulatory T cells in HPV-related cervical intraepithelial neoplasia (CIN) correlates inversely with spontaneous regression of CIN. Am J Reprod Immunol 2013; 69(2):134-41; PMID:23057776; http://dx.doi.org/10.1111/aji.12030 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

37. Trimble CL, Clark RA, Thoburn C, Hanson NC, Tassello J, Frosina D, Kos F, Teague J, Jiang Y, Barat NC, et al. Human papillomavirus 16-associated cervical intraepithelial neoplasia in humans excludes CD8 T cells from dysplastic epithelium. J Immunol 2010; 185(11):7107-14; PMID:21037100; http://dx.doi.org/10.4049/jimmunol.1002756 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

38. Leong CM, Doorbar J, Nindl I, Yoon HS, Hibma MH. Loss of epidermal Langerhans cells occurs in human papillomavirus α, gamma, and mu but not β genus infections. J Invest Dermatol 2010; 130(2):472-80.;PMID:19759549; http://dx.doi.org/10.1038/jid.2009.266 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

39. Piyathilake CJ, Ollberding NJ, Kumar R, Macaluso M, Alvarez RD, Morrow CD. Cervical microbiota associated with higher grade cervical intraepithelial neoplasia in women infected with High-Risk Human Papillomaviruses. Cancer Prev Res (Phila) 2016; 9(5):357-66; PMID:26935422; http://dx.doi.org/10.1158/1940-6207.CAPR-15-0350 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

40. Lee JE, Lee S, Lee H, Song YM, Lee K, Han MJ, Sung J, Ko G. Association of the vaginal microbiota with human papillomavirus infection in a Korean twin cohort. PloS One 2013; 8(5):e63514.;PMID:23717441; http://dx.doi.org/10.1371/journal.pone.0063514 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

41. Soong RS, Song L, Trieu J, Knoff J, He L, Tsai YC, Huh W, Chang YN, Cheng WF, Roden RB, et al. Toll-like receptor agonist imiquimod facilitates antigen-specific CD8+ T-cell accumulation in the genital tract leading to tumor control through IFNgamma. Clin Cancer Res 2014; 20(21):5456-67; PMID:24893628; http://dx.doi.org/10.1158/1078-0432.CCR-14-0344 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

42. Domingos-Pereira S, Decrausaz L, Derre L, Bobst M, Romero P, Schiller JT, Jichlinski P, Nardelli-Haefliger D. Intravaginal TLR agonists increase local vaccine-specific CD8 T cells and human papillomavirus-associated genital-tumor regression in mice. Mucosal Immunol 2013; 6(2):393-404; PMID:22968420; http://dx.doi.org/10.1038/mi.2012.83 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

43. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015; 348(6230):56-61.;PMID:25838373; http://dx.doi.org/10.1126/science.aaa8172 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

44. Boutros C, Tarhini A, Routier E, Lambotte O, Ladurie FL, Carbonnel F, Izzeddine H, Marabelle A, Champiat S, Berdelou A, et al. Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination. Nat Rev Clin Oncol 2016; PMID:27141885 [Epub ahead of print] [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

45. Li Z, Palaniyandi S, Zeng R, Tuo W, Roopenian DC, Zhu X. Transfer of IgG in the female genital tract by MHC class I-related neonatal Fc receptor (FcRn) confers protective immunity to vaginal infection. Proc Natl Acad Sci U S A 2011; 108(11):4388-93; PMID:21368166; http://dx.doi.org/10.1073/pnas.1012861108 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

46. Cuburu N, Wang K, Goodman KN, Pang YY, Thompson CD, Lowy DR, Cohen JI, Schiller JT. Topical herpes simplex virus 2 (HSV-2) vaccination with human papillomavirus vectors expressing gB/gD ectodomains induces genital-tissue-resident memory CD8+ T cells and reduces genital disease and viral shedding after HSV-2 challenge. J Virol 2015; 89(1):83-96; PMID:25320297; http://dx.doi.org/10.1128/JVI.02380-14 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

47. Cuburu N, Graham BS, Buck CB, Kines RC, Pang YY, Day PM, Lowy DR, Schiller JT. Intravaginal immunization with HPV vectors induces tissue-resident CD8+ T cell responses. J Clin Invest 2012; 122(12):4606-20; PMID:23143305; http://dx.doi.org/10.1172/JCI63287 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

48. Alvarez RD, Huh WK, Bae S, Lamb LS, Jr, Conner MG, Boyer J, Wang C, Hung CF, Sauter E, Paradis M, et al. A pilot study of pNGVL4a-CRT/E7(detox) for the treatment

of patients with HPV16+ cervical intraepithelial neoplasia 2/3 (CIN2/3). Gynecol Oncol 2016; 140(2):245-52; PMID:26616223; http://dx.doi.org/10.1016/j.ygyno.2015.11.026 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

49. Ariotti S, Beltman JB, Chodaczek G, Hoekstra ME, van Beek AE, Gomez-Eerland R, Ritsma L, van Rheenen J, Marée AF, Zal T, et al. Tissue-resident memory CD8+ T cells continuously patrol skin epithelia to quickly recognize local antigen. Proc Natl Acad Sci U S A 2012; 109(48):19739-44; PMID:23150545; http://dx.doi.org/10.1073/pnas.1208927109 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

50. Feltkamp MC, Smits HL, Vierboom MP, Minnaar RP, de Jongh BM, Drijfhout JW, ter Schegget J, Melief CJ, Kast WM. Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. Eur J Immunol 1993; 23(9):2242-9; PMID:7690326; http://dx.doi.org/10.1002/eji.1830230929 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

51. Lin KY, Guarnieri FG, Staveley-O'Carroll KF, Levitsky HI, August JT, Pardoll DM, Wu TC. Treatment of established tumors with a novel vaccine that enhances major histocompatibility class II presentation of tumor antigen. Cancer Res 1996; 56(1):21-6; PMID:8548765 [PubMed] [Web of Science ®], [Google Scholar]

52. Schaper ID, Marcuzzi GP, Weissenborn SJ, Kasper HU, Dries V, Smyth N, Fuchs P, Pfister H. Development of skin tumors in mice transgenic for early genes of human papillomavirus type 8. Cancer Res 2005; 65(4):1394-400; PMID:15735026; http://dx.doi.org/10.1158/0008-5472.CAN-04-3263 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

53. Lambert PF, Pan H, Pitot HC, Liem A, Jackson M, Griep AE. Epidermal cancer associated with expression of human papillomavirus type 16 E6 and E7 oncogenes in the skin of transgenic mice. Proc Natl Acad Sci U S A 1993; 90(12):5583-7; PMID:8390671; http://dx.doi.org/10.1073/pnas.90.12.5583 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

54. Zhong R, Pytynia M, Pelizzari C, Spiotto M. Bioluminescent imaging of HPV-positive oral tumor growth and its response to image-guided radiotherapy. Cancer Res 2014; 74(7):2073-81; PMID:24525739; http://dx.doi.org/10.1158/0008-5472.CAN-13-2993 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

55. Stern PL, van der Burg SH, Hampson IN, Broker TR, Fiander A, Lacey CJ, Kitchener HC, Einstein MH. Therapy of human papillomavirus-related disease. Vaccine 2012; 30(Suppl 5):F71-82.;PMID:23199967; http://dx.doi.org/10.1016/j.vaccine.2012.05.091 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

56. Richel O, de Vries HJ, van Noesel CJ, Dijkgraaf MG, Prins JM. Comparison of imiquimod, topical fluorouracil, and electrocautery for the treatment of anal intraepithelial neoplasia in HIV-positive men who have sex with men: an open-label, randomised controlled trial. Lancet Oncol 2013; 14(4):346-53; PMID:23499546; http://dx.doi.org/10.1016/S1470-2045(13)70067-6 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]

57. Moscicki AB, Schiffman M, Kjaer S, Villa LL. Chapter 5: Updating the natural history of HPV and anogenital cancer. Vaccine 2006; 24(Suppl 3):S3/42-51; PMID:16950017 [PubMed], [Google Scholar]

58. Dillon S, Sasagawa T, Crawford A, Prestidge J, Inder MK, Jerram J, Mercer AA, Hibma M. Resolution of cervical dysplasia is associated with T-cell proliferative responses to human papillomavirus type 16 E2. J General Virol 2007; 88(Pt 3):803-13; http://dx.doi.org/10.1099/vir.0.82678-0 [Crossref] [PubMed] [Web of Science ®], [Google Scholar]