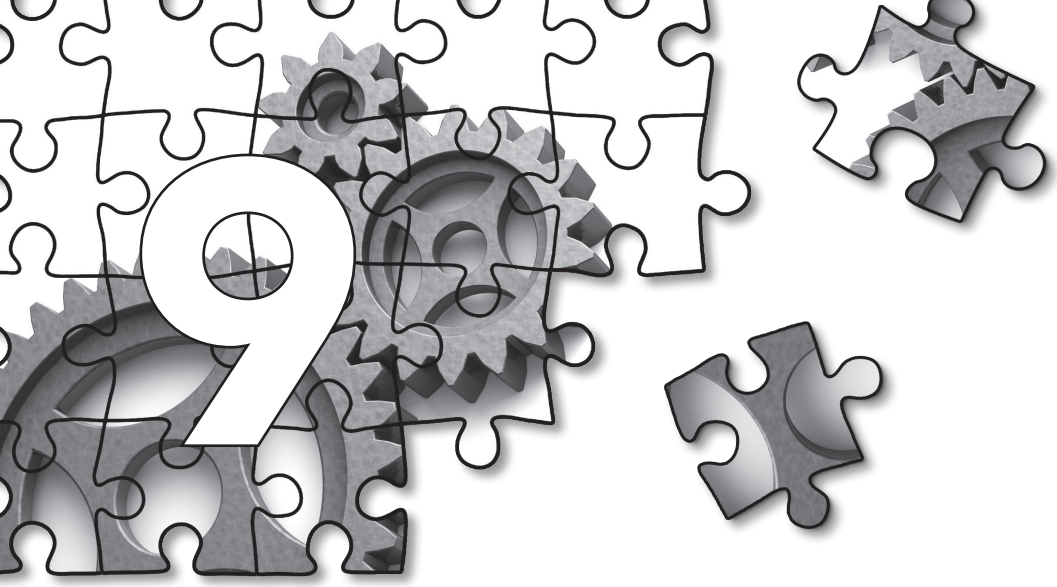

NEW APPROACHES IN VACCINE-BASED IMMUNOTHERAPY FOR HUMAN PAPILLOMAVIRUS-INDUCED CANCER

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ABSTRACT

The identification of Human Papillomavirus as the etiological factor for cervical cancer provides an opportunity to treat these malignancies by vaccination. Although therapeutic vaccination against viral oncogenes regularly induces a specific T cell response, clinical effectivity remains low. Three factors are particularly important for clinical outcome: the balance between cytotoxic T cells and regulatory immune subsets, the balance between cytotoxic T cells and tumor cells and finally the killing efficiency of cytotoxic T cells within the tumor. To improve these three factors, therapeutic vaccination is combined with other treatments. Here, we review those studies that are based on understanding the inhibitory mechanisms that prevent unleashing the full power of therapeutic vaccine-induced T cells and utilize combinatorial interventions based on these insights.

HIGHLIGHTS

Understanding of escape from immunotherapy allows targeting of the mechanism(s)

Combination treatment must increase the level of intra-tumoral cytotoxic T cells

Conventional treatment synergizes with therapeutic cancer vaccination

INTRODUCTION

The goal of many immunotherapeutic interventions for cancer is to induce $CD8^+$ cytotoxic T lymphocytes (CTLs) that can recognize and kill cancer cells expressing defined tumor-specific antigens (TSAs). Four major classes of TSAs have been characterized; differentiation antigens, overexpressed antigens, mutated antigens (neo-antigens) and viral antigens ((1), Melief, *et al.* submitted). Since central tolerance can be induced to self-antigens, therapeutic vaccinations preferably target mutated and viral antigens. Since the diversity in mutated antigens is large, therapeutic vaccination for these antigens is possible (2-4) but remains challenging (1). Viral antigens however are shared between patients and are often indispensable for the transformed state of malignant cells. Of the known human cancer viruses, most immunotherapeutic interventions, apart from adoptive T cell transfer of malignancies induced by EBV, have been conducted in premalignant disorders and cancer caused by high risk HPV. Therefore this review will focus on therapeutic interventions for treatment of high risk HPV-induced cancers.

HPV infections are associated with several malignancies, such as ano-genital cancers and a subset of head and neck cancers. Of these malignancies cervical cancer is the most prevalent with the highest morbidity rate (5). Although many subtypes of HPV have been identified, HPV16 is responsible for approximately 50% of all cervical cancers (6). The second most prevalent subtype is HPV-18, present in an additional 10-15% of all cervical malignancies (6). The two well-known HPV oncogenes E6 and E7 are constitutively expressed by HPV-associated tumors, and are critical for the induction and maintenance of cellular transformation (7), creating ideal targets for therapeutic vaccination (8, 9). Current approaches for therapeutic vaccinations include live-vector-based, nucleic acid-based, cell-based, and peptide- and protein- based vaccination (9). Recent reviews have focused on the latest clinical progress in therapeutic interventions for HPV induced lesions (9-11). From these reviews it is clear that a variety of therapeutics can induce an HPV-specific CD4 and/or CD8 T cell response but that clinical responses are still rare, except in patients with pre-malignant lesions (12, 13), and are scarcely observed in patients with advanced gynecological lesions. For instance, we have shown that synthetic long overlapping peptides of the oncogenes E6 and E7 of HPV16 (HPV16-SLP) induce clinical responses in mice and in patients with premalignant lesions (12, 14). Patients with a complete response had a stronger and broader HPV-specific T cell response ((12, 15), van Poelgeest, Welters, *et al.,* submitted). When this vaccine is applied in patients with advanced or recurrent HPV16-induced gynecological carcinoma there is no apparent effect on survival (16, 17), indicating that a more potent therapeutic modality is needed to treat this group of patients (18). To become clinically successful, combination of vaccination with one or more treatment modalities that can boost the vaccine-induced T cell response and down- modulate the regulatory mechanisms in the tumor micro-environment is needed (18).

PRECLINICAL MODELS AS A GUIDE FOR NEW COMBINATORIAL THERAPIES

Preclinical models have provided key mechanistic insights into anti-tumor responses and immune-escape mechanisms utilized by tumors, that have been vital for the development of FDA

approved immunotherapies (19). In case of HPV-induced cervical cancer, several preclinical models have proved their usefulness for the development of HPV targeting vaccination and continue to be useful to study local immune regulation, vaccination efficacy and treatment combinations. Because these preclinical studies are too numerous to discuss, we focus on those that are based on understanding of the inhibitory mechanisms that prevent unleashing of the full power of therapeutic vaccine-induced T cells and utilization of combinatorial interventions based on these insights.

BREAKING LOCAL IMMUNE REGULATION

The ratio between CD8 T cells and Foxp3$^+$ regulatory CD4 T cells (Tregs) is an independent prognostic factor in cervical cancer (20, 21) (figure 1). Enhancing the clinical effect of vaccination often focuses on the induction of CD8 T cells, resulting in an improved CD8 T cell/Treg ratio. Preclinical mouse models offer the possibility to evaluate intratumoral T cell responses in detail shortly after vaccination. As in patients, the percentage of systemic antigen-specific CD8 T cells is usually low, but after immunotherapy up to 40% of all tumor infiltrating CD8 T cells can be vaccine specific (22, 23), resulting in a favorable CD8 T cell/Treg ratio. Additionally, depletion of Tregs by an anti-CD25 antibody prior to E7/Hsp70 DNA vaccination in tumor bearing mice enhances the number of vaccine-induced CD8 T cells and results in enhanced survival (24).

In addition to a favorable CD8 T cell/Treg ration, macrophage infiltration is related to disease progression in cervical intraepithelial neoplasia (25), and infiltration of mature M1 macrophages is an independent prognostic factor for survival in cervical cancer (20), suggesting that re-education of macrophages rather than depleting them by therapeutic intervention would be an attractive immunotherapeutic option. *In vivo* studies indicate that HPV transformed TC-1 tumors are highly infiltrated with Tumor Associated Macrophages (TAM). Macrophage depletion reduces tumor growth, correlates with enhanced tumor infiltration of tumor specific CD8 T cells (26), and improves responses to chemotherapy (27), together confirming the immunosuppressive function of these cells. However, stimulation with IFN-γ and CD40L can reverse a M2 phenotype to a M1, pro-inflammatory macrophage phenotype (28). Interestingly, anthracyclin-type chemotherapeutics and low-dose local radiotherapy were recently shown to reprogram macrophage differentiation to support intra-tumoral recruitment of CD8 T cells (29, 30). Additionally, our own data indicate that E7 long peptide vaccination induces tumor infiltrating cytokine producing CD8 T cells that modify intra-tumoral macrophage subsets required for tumor regressions (31). Together, these studies argue for a M1 macrophage-skewing, rather than a macrophage-depleting strategy in clinical practice.

UNLEASHING THE FULL POTENTIAL OF CYTOTOXIC T CELLS

T cell responses in patients with advanced gynecological lesions are often weak and therefore not sufficient to induce a robust anti-tumor response. To enhance the magnitude and functionality of HPV specific immune responses, vaccination is frequently combined with danger signals and immune stimulating antibodies.

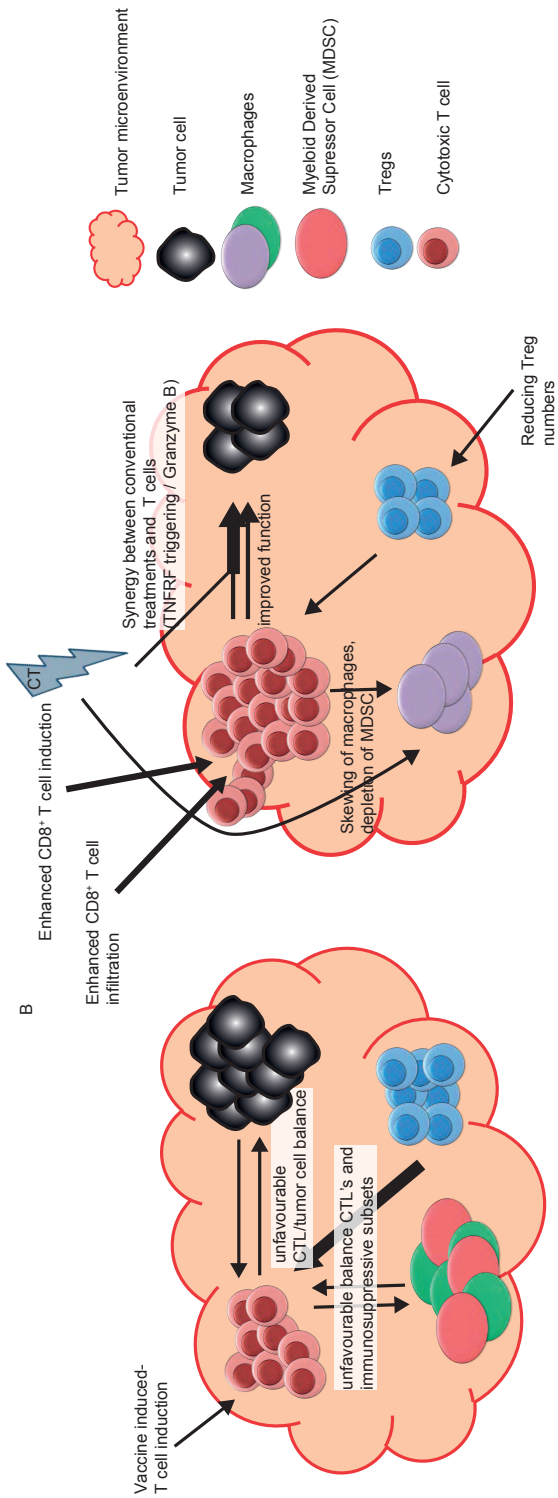

Figure 1. Improving therapeutic vaccination by combination therapies. A) Although vaccination can enhance the number of CD8 T cells, combination with treatment modalities that can boost the vaccine-induced T cell response or modulate intratumoral regulatory mechanisms is needed (18). **B)** Improving the balance betweencytotoxic T cells and immunosuppressive subsets may be achieved via enhancement of the cytotoxic T cell response, depletion of Tregs or Myeloid Derived Suppressor Cells and skewing or depletion of macrophages (24, 26, 29-33). Improved killing of tumor cells by cytotoxic T cells may be achieved through enhanced T cell function or the synergistic action of conventional debulking treatments and T cells (23, 44, 50-56).

Boosting T cell responses

The addition of TLR ligands to long peptide vaccination resulted in significantly enhanced anti-tumor responses ((32) and reviewed by (33)) while in an orthotopic murine model for cervical cancer the intravaginal (IVAG) application of TLR3 and 9 agonists promoted attraction of E7-specific CD8 T cells to the tumor site (34). IVAG application of the TLR9 ligand CpG enhanced CCR5 and CXCR3 levels on CD8 T cells, suggesting that the expression of these receptors allowed an improved attraction of CD8 T cells into the tumor (34). IVAG application of the TLR7 agonist Imiquimod induced IFN-**γ mediated,** local induction of CXCL9 and CXCL10, which enhanced infiltration of $CXCR3^+$ vaccine-induced CD8 T cells. Imiquimod was shown to be effective in the treatment of VIN (35) and is currently combined with vaccination (36).

Checkpoint blockade

Immune checkpoint blockade has emerged as a promising strategy to attack tumors (37). By releasing the brakes on T cells, immune checkpoint blockers enhance the tumor-specific T cell responses. Since a combination of blocking the immune checkpoint molecule CTL-associated antigen 4 (CTLA-4) and vaccination with GM-CSF — transduced tumor cells can alter the balance between regulatory T cells and effector T cells (38, 39), the combination of an HPV targeting vaccine with CTLA-4 blocking seems an attractive approach in the therapeutic treatment of HPV induced malignancies but preclinical and clinical data for HPV induced malignancies using this approach are limited. Due to serious side effects (40), systemic treatment with CTLA-4 blocking antibodies is not preferred. However, similar to local anti-CD40 agonist antibody (41), the controlled local delivery of anti-CTLA4 can be equally effective as systemic treatment (42). In fact, local treatment with CTLA-4 antibodies via secretion by the tumor results in an enhanced CD8/Treg ratio in HPV transduced tumors (43), however we have not found any study that combines HPV vaccination with CTLA-4 targeting.

The role of another immune checkpoint protein, programmed cell death protein 1 (PD-1) and its ligand PD-L1, is studied more extensively in HPV-induced cancer. In HPV-associated head and neck cancer, PD-1-expressing tumor-infiltrating T cells are a favorable prognostic biomarker. *In vitro* assays for a small group of patients suggest that PD-1 blockade enhances the IFN-γ production by CD8 T cells (44), while *in vivo* data indicate that E7 polypeptide vaccination combined with PD-L1 blockade significantly enhanced survival when compared to untreated or single treated tumor-bearing mice (44). Furthermore, PD-1 blocking synergizes with cyclophosphamide to enhance the anti-tumor capacity of E7-specific vaccination with short peptide vaccination combined with agonistic CD40 antibodies and GM-CSF in Incomplete Freund's Adjuvant (45). The effectiveness in patients, however, remains to be tested.

Chemo-immunotherapy

Certain chemotherapeutics can enhance the anti-tumor effect of therapeutic vaccination. A number of trials reported that combining chemotherapy with immunotherapy improved clinical responses (46). A significant number of preclinical studies for HPV-induced malignancies indicate that especially cisplatin works well with T cell-inducing vaccinations. While others have recently reviewed immunogenic effects of cisplatin and the molecular pathways underlying

this immunogenicity (47, 48), we focus here on the coordinated collaboration between cisplatin and vaccination. Cisplatin-immunotherapy can induce a type 1 interferon and TLR4 dependent activation and migration of antigen-loaded dendritic cells into tumor-draining lymph nodes where they stimulate CD8 T cells (22). In fact, for a subset of therapeutic vaccines it has been described that cisplatin can enhance the systemic or local vaccine-induced T cell response (22, 49, 50), while for other vaccines it has no effect on the induction, activity, localization, or migrating capacity of vaccine-induced T cells (23, 51), but differences are primarily observed in the tumor microenvironment. Tumors of mice treated with cisplatin and vaccination generally have a decreased tumor cell density compared to single treated tumors (23, 51). This is explained by the reduced proliferative capacity of tumor cells (23) and the enhanced tumor cell death upon combined treatment (figure 1), (23, 50-52). This enhanced cell death is explained by the increased sensitivity of tumor cells for CTL-mediated killing as result of cisplatin-induced enhanced sensitivity of tumor cells for granzyme B mediated killing (51-53) and the enhanced sensitivity of tumor cells for cisplatin-induced apoptosis by T cell produced TNFα (23). Various preclinical *in vivo* and *in vitro* studies indicate synergistic mechanisms between specific chemotherapeutics and triggering of one of the members of the TNF receptor family (23, 54, 55). Accordingly, the combined treatment with an adenoviral vector expressing E7 with gemcitabine and cisplatin enhanced the intratumoral expression of a variety of proinflammatory chemokines, supported a favorable M1/M2 macrophage ratio and inhibited (treatment-induced) accumulation of systemic Tregs, B cells, and myeloid derived suppressor cells (MDSCs; indicated as Gr-1$^+$/CD11b$^+$) (49). Furthermore, also radiated tumor cells have an increased sensitivity to E7 vaccine-driven CTL-mediated killing (56), implying that similar mechanisms as those induced by cisplatin are involved in the enhanced anti-tumor responses.

Surprisingly, other chemotherapeutics such as oxaliplatin and doxorubicin did not enhance vaccine induced anti-tumor responses but did also not weaken the effect of HPV vaccination (22, 23). Together indicating that although chemotherapy does not impair vaccine induced anti-tumor immunity, combining vaccination with cisplatin would be the most favorable choice to test in patients.

NEW APPROACHES TRANSLATED INTO THE CLINIC

A huge body of preclinical and clinical studies indicates that therapeutic vaccination against HPV- induced malignancies is improved by combination therapy. Since the full power of a therapeutic T cell response will only be unleashed when immunosuppression by (tumor-induced) immune inhibitory cells is abolished, clinical efficacy of the combination therapies will be determined by the effect of the combined treatments on the tumor microenvironment and immune cell subsets. Understanding the role of individual components in these interactions allows targeting of the relevant mechanisms by therapy and will guide the road ahead to improved clinical responses and decreased toxicity in patients with advanced gynecological lesions.

ANNOTATED REFERENCES

References and recommended reading
• of special interest
•• of outstanding interest

• Ref 12
This study demonstrates for the first time that therapeutic vaccination with a highly immunogenic vaccine can result in complete and durable clinical responses in a significant number of patients with HPV16-induced premalignant lesions.

•• Ref 20
This study shows that in addition to a high CD8 T cell/Treg ratio, the presence of matured M1 macrophages improves patient survival, indicating that macrophage polarization may be an attractive component of immunotherapy for cervical cancer.

•• Ref 22
In this study the authors show that cisplatin-immunotherapy induces a type 1 interferon and TLR4 dependent activation of and migration of antigen-loaded dendritic cells into tumor-draining lymph nodes where they stimulate CD8 T cells. This study shows that chemotherapy allows an adjuvant-free priming of CD8 T cells by vaccination.

•• Ref 23
This study shows that vaccine-induced T cells migrate to the tumor and produce Tumor Necrosis Factor which strongly enhances the sensitivity of tumor cells for cisplatin, resulting in tumor cell death and enhanced survival of mice treated with timed vaccination and cisplatin treatment.

•• Ref 47
This review extensively describes the literature on cisplatin-induced anti-tumor immunomodulation.

• Ref 48
This review extensively describes the literature on the molecular pathways involved in the immunogenic effects of platinum-based chemotherapeutics.

REFERENCE LIST

1. Coulie PG, Van den Eynde BJ, van der Bruggen P, Boon T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. Nat Rev Cancer 2014;14:135-46.
2. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014;515:577-81.
3. Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature 2014;515:572-6.
4. Castle JC, Kreiter S, Diekmann J, Lower M, van de Roemer N, de Graaf J, et al. Exploiting the mutanome for tumor vaccination. Cancer Res 2012;72:1081-91.
5. 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;189:12-9.
6. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121:621-32.
7. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87:796-802.
8. Melief CJ, Toes RE, Medema JP, van der Burg SH, Ossendorp F, Offringa R. Strategies for immunotherapy of cancer. Adv Immunol 2000;75:235-82.
9. Hung CF, Ma B, Monie A, Tsen SW, Wu TC. Therapeutic human papillomavirus vaccines: current clinical trials and future directions. Expert Opin Biol Ther 2008;8:421-39.
10. Eskander RN, Tewari KS. Immunotherapy: An Evolving Paradigm in the Treatment of Advanced Cervical Cancer. Clin Ther 2015;37:20-38.
11. Knoff J, Yang B, Hung CF, Wu TC. Cervical Cancer: Development of Targeted Therapies Beyond Molecular Pathogenesis. Curr Obstet Gynecol Rep 2014;3:18-32.
12. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N Engl J Med 2009;361:1838-47.
13. Roman LD, Wilczynski S, Muderspach LI, Burnett AF, O'Meara A, Brinkman JA, et al. A phase II study of Hsp-7 (SGN-00101) in women with high-grade cervical intraepithelial neoplasia. Gynecol Oncol 2007;106:558-66.
14. Zwaveling S, Ferreira Mota SC, Nouta J, Johnson M, Lipford GB, Offringa R, et al. Established human papillomavirus type 16-expressing tumors are effectively eradicated following vaccination with long peptides. J Immunol 2002;169:350-8.
15. Welters MJ, Kenter GG, de Vos van Steenwijk PJ, Lowik MJ, Berends-van der Meer DM, Essahsah F, et al. Success or failure of vaccination for HPV16-positive vulvar lesions correlates with kinetics and phenotype of induced T-cell responses. Proc Natl Acad Sci U S A 2010;107:11895-9.
16. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, et al. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. Clin Cancer Res 2008;14:169-77.
17. van Poelgeest MI, Welters MJ, van Esch EM, Stynenbosch LF, Kerpershoek G, van Persijn van Meerten EL, et al. HPV16 synthetic long peptide (HPV16-SLP) vaccination therapy of patients with advanced or recurrent HPV16-induced gynecological carcinoma, a phase II trial. J Transl Med 2013;11:88.
18. van der Burg SH, Melief CJ. Therapeutic vaccination against human papilloma virus induced malignancies. Curr Opin Immunol 2011;23:252-7.
19. Dranoff G. Experimental mouse tumour models: what can be learnt about human cancer immunology? Nat Rev Immunol 2012;12:61-6.
20. de Vos van Steenwijk PJ, Ramwadhdoebe TH, Goedemans R, Doorduijn EM, van Ham JJ,

Gorter A, et al. Tumor-infiltrating CD14-positive myeloid cells and CD8-positive T-cells prolong survival in patients with cervical carcinoma. Int J Cancer 2013;133:2884-94.

21. Jordanova ES, Gorter A, Ayachi O, Prins F, Durrant LG, Kenter GG, et al. Human leukocyte antigen class I, MHC class I chain-related molecule A, and CD8+/regulatory T-cell ratio: which variable determines survival of cervical cancer patients? Clin Cancer Res 2008;14:2028-35.

22. Kang TH, Mao CP, Lee SY, Chen A, Lee JH, Kim TW, et al. Chemotherapy acts as an adjuvant to convert the tumor microenvironment into a highly permissive state for vaccination-induced antitumor immunity. Cancer Res 2013;73:2493-504.

23. van der Sluis TC, van DS, Huppelschoten S, Jordanova ES, Beyranvand NE, Sloots A, et al. Vaccine-induced tumor necrosis factor-producing T cells synergize with Cisplatin to promote tumor cell death. Clin Cancer Res 2015;21:781-94.

24. Chuang CM, Hoory T, Monie A, Wu A, Wang MC, Hung CF. Enhancing therapeutic HPV DNA vaccine potency through depletion of CD4+CD25+ T regulatory cells. Vaccine 2009;27:684-9.

25. Hammes LS, Tekmal RR, Naud P, Edelweiss MI, Kirma N, Valente PT, et al. Macrophages, inflammation and risk of cervical intraepithelial neoplasia (CIN) progression--clinicopathological correlation. Gynecol Oncol 2007;105:157-65.

26. Lepique AP, Daghastanli KR, Cuccovia IM, Villa LL. HPV16 tumor associated macrophages suppress antitumor T cell responses. Clin Cancer Res 2009;15:4391-400.

27. DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov 2011;1:54-67.

28. Heusinkveld M, de Vos van Steenwijk PJ, Goedemans R, Ramwadhdoebe TH, Gorter A, Welters MJ, et al. M2 macrophages induced by prostaglandin E2 and IL-6 from cervical carcinoma are switched to activated M1 macrophages by CD4+ Th1 cells. J Immunol 2011;187:1157-65.

29. Klug F, Prakash H, Huber PE, Seibel T, Bender N, Halama N, et al. Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell 2013;24:589-602.

30. Ma Y, Adjemian S, Mattarollo SR, Yamazaki T, Aymeric L, Yang H, et al. Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. Immunity 2013;38:729-41.

31. van der Sluis TC, Sluijter M, van Duikeren S., West BL, Melief CJ, Arens R, et al. Therapeutic peptide vaccine-induced CD8 T cells strongly modulate intratumoral macrophages required for tumor regression. Cancer Immunol Res 2015.

32. van Duikeren S, Fransen MF, Redeker A, Wieles B, Platenburg G, Krebber WJ, et al. Vaccine-induced effector-memory CD8+ T cell responses predict therapeutic efficacy against tumors. J Immunol 2012;189:3397-403.

33. Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nat Rev Cancer 2008;8:351-60.

34. Domingos-Pereira S, Decrausaz L, Derre L, Bobst M, Romero P, Schiller JT, et al. Intravaginal TLR agonists increase local vaccine-specific CD8 T cells and human papillomavirus-associated genital-tumor regression in mice. Mucosal Immunol 2013;6:393-404.

35. van SM, van BM, ten Kate FJ, Beckmann I, Ewing PC, Eijkemans MJ, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod. N Engl J Med 2008;358:1465-73.

36. Daayana S, Elkord E, Winters U, Pawlita M, Roden R, Stern PL, et al. Phase II trial of imiquimod and HPV therapeutic vaccination in patients with vulval intraepithelial neoplasia. Br J Cancer 2010;102:1129-36.

37. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.

38. Wada S, Jackson CM, Yoshimura K, Yen HR, Getnet D, Harris TJ, et al. Sequencing CTLA-4 blockade with cell-based immunotherapy for prostate cancer. J Transl Med 2013;11:89.

39. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells

co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med 2013;210:1695-710.

40. Attia P, Phan GQ, Maker AV, Robinson MR, Quezado MM, Yang JC, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 2005;23:6043-53.

41. Fransen MF, Sluijter M, Morreau H, Arens R, Melief CJ. Local activation of CD8 T cells and systemic tumor eradication without toxicity via slow release and local delivery of agonistic CD40 antibody. Clin Cancer Res 2011;17:2270-80.

42. Fransen MF, van der Sluis TC, Ossendorp F, Arens R, Melief CJ. Controlled local delivery of CTLA-4 blocking antibody induces CD8+ T-cell-dependent tumor eradication and decreases risk of toxic side effects. Clin Cancer Res 2013;19:5381-9.

43. Tuve S, Chen BM, Liu Y, Cheng TL, Toure P, Sow PS, et al. Combination of tumor site-located CTL-associated antigen-4 blockade and systemic regulatory T-cell depletion induces tumor-destructive immune responses. Cancer Res 2007;67:5929-39.

44. Badoual C, Hans S, Merillon N, Van Ryswick C., Ravel P, Benhamouda N, et al. PD-1-expressing tumor-infiltrating T cells are a favorable prognostic biomarker in HPV-associated head and neck cancer. Cancer Res 2013;73:128-38.

45. Mkrtichyan M, Najjar YG, Raulfs EC, Abdalla MY, Samara R, Rotem-Yehudar R, et al. Anti-PD-1 synergizes with cyclophosphamide to induce potent anti-tumor vaccine effects through novel mechanisms. Eur J Immunol 2011;41:2977-86.

46. Ramakrishnan R, Antonia S, Gabrilovich DI. Combined modality immunotherapy and chemotherapy: a new perspective. Cancer Immunol Immunother 2008;57:1523-9.

47. de Biasi AR, Villena-Vargas J, Adusumilli PS. Cisplatin-induced antitumor immunomodulation: a review of preclinical and clinical evidence. Clin Cancer Res 2014;20:5384-91.

48. Hato SV, Khong A, de Vries IJ, Lesterhuis WJ. Molecular pathways: the immunogenic effects of platinum-based chemotherapeutics. Clin Cancer Res 2014;20:2831-7.

49. Fridlender ZG, Sun J, Singhal S, Kapoor V, Cheng G, Suzuki E, et al. Chemotherapy delivered after viral immunogene therapy augments antitumor efficacy via multiple immune-mediated mechanisms. Mol Ther 2010;18:1947-59.

50. Tseng CW, Hung CF, Alvarez RD, Trimble C, Huh WK, Kim D, et al. Pretreatment with cisplatin enhances E7-specific CD8+ T-Cell-mediated antitumor immunity induced by DNA vaccination. Clin Cancer Res 2008;14:3185-92.

51. Bae SH, Park YJ, Park JB, Choi YS, Kim MS, Sin JI. Therapeutic synergy of human papillomavirus E7 subunit vaccines plus cisplatin in an animal tumor model: causal involvement of increased sensitivity of cisplatin-treated tumors to CTL-mediated killing in therapeutic synergy. Clin Cancer Res 2007;13:341-9.

52. Sin JI, Kim JM, Bae SH, Lee IH, Park JS, Ryoo HM. Adoptive transfer of human papillomavirus E7-specific CTL enhances tumor chemoresponse through the perforin/granzyme-mediated pathway. Mol Ther 2009;17:906-13.

53. Ramakrishnan R, Assudani D, Nagaraj S, Hunter T, Cho HI, Antonia S, et al. Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice. J Clin Invest 2010;120:1111-24.

54. Galligan L, Longley DB, McEwan M, Wilson TR, McLaughlin K, Johnston PG. Chemotherapy and TRAIL-mediated colon cancer cell death: the roles of p53, TRAIL receptors, and c-FLIP. Mol Cancer Ther 2005;4:2026-36.

55. van der Most RG, Currie AJ, Cleaver AL, Salmons J, Nowak AK, Mahendran S, et al. Cyclophosphamide chemotherapy sensitizes tumor cells to TRAIL-dependent CD8 T cell-mediated immune attack resulting in suppression of tumor growth. PLoS One 2009;4:e6982.

56. Ye GW, Park JB, Park YJ, Choi YS, Sin JI. Increased sensitivity of radiated murine cervical cancer tumors to E7 subunit vaccine-driven CTL-mediated killing induces synergistic anti-tumor activity. Mol Ther 2007;15:1564-70.