Interest of Tumor-Specific CD4 T Helper 1 Cells for Therapeutic Anticancer Vaccine

Jeanne Galaine 1,2,3, Christophe Borg 1,2,3,4, Yann Godet 1,2,3 and Olivier Adotévi 1,2,3,4,*

1 INSERM UMR1098, Besançon cedex F25020, France; E-Mails: jeanne.galaine@edu.univ-fcomte.fr (J.G.); christophe.borg@efs.sante.fr (C.B.); yann.godet@univ-fcomte.fr (Y.G.)
2 Université de Franche-Comté, Besançon cedex F25020, France
3 EFS Bourgogne Franche-Comté, Besançon cedex F25020, France
4 Department of Medical Oncology, University Hospital of Besançon, Besançon cedex F25020, France
* Author to whom correspondence should be addressed; E-Mail: olivier.adotevi@univ-fcomte.fr; Tel.: +33-3-8166-9351; Fax: +33-3-8166-8708.

Academic Editor: Mary Lenora (Nora) Disis

Received: 18 May 2015 / Accepted: 24 June 2015 / Published: 30 June 2015

Abstract: Nowadays, immunotherapy represents one promising approach for cancer treatment. Recently, spectacular results of cancer immunotherapy clinical trials have confirmed the crucial role of immune system in cancer regression. Therapeutic cancer vaccine represents one widely used immunotherapy strategy to stimulate tumor specific T cell responses but clinical impact remains disappointing in targeting CD8 T cells. Although CD8 T cells have been initially considered to be the main protagonists, it is now clear that CD4 T cells also play a critical role in antitumor response. In this article, we discuss the role of tumor antigen-specific CD4 T cell responses and how we can target these cells to improve cancer vaccines.

Keywords: CD4 T cell; tumor antigen; helper peptide; cancer vaccine

1. Introduction

The proof of concept of cancer immunotherapy efficacy has reached an important inflection point in the history of cancer therapy. Encouraging responses are consistently being reported for a broad range of human cancers with immunotherapies agents, notably so-called immune checkpoint inhibitors
(CTLA-4, PD-1 and PDL-1) [1]. The impressive response obtained with immune checkpoint inhibitors indicated that the presence of preexisting antitumor immune response is required for their efficacy. These emerging clinical data highlight the critical role of adaptive T cell immunity and suggest that cancer immunotherapy is likely to become a key part of the clinical management of cancer [2,3].

Therapeutic cancer vaccine is an active immunotherapy whose primary aim is to induce or enhance an adaptive antitumor immunity, notably T cells against tumor cells. Many vaccine approaches such as peptides, proteins, viruses, DNA, dendritic cells, and tumor cell lysates have been used to sustain T cell responses \textit{in vivo} [4]. Although therapeutic cancer vaccines have very good safety and tolerability in clinical settings, their efficacy was limited on several accounts, notably by immune suppression microenvironment within the tumor [5]. The challenge now is to develop new and optimized therapeutic vaccines for harnessing tumor specific T cell responses. Although cytotoxic CD8 T cells have been considered to be the main protagonists in the production of anti-tumor therapeutic effects resulting from vaccines, increasingly, several aspects of CD4 T-cell biology suggest that this T-cell population can effectively be used for cancer immunotherapy. Hence, previous studies demonstrated that CD4 T cells could be involved in the efficacy of many cancer immunotherapies [6–10]. Several approaches have been developed to stimulate antitumor CD4 T cell immunity, during therapeutic cancer vaccines. The most common strategy relies on the use of a helper peptide derived either from non-tumor antigens or tumor antigens, and more recently from neoantigens [11].

2. Critical Roles of CD4 Helper T Lymphocytes in Antitumor Immunity

CD4 T cells orchestrate a broad range of immune responses and are equipped to differentiate into multiple sublineages, which can induce and maintain immune responses against tumor antigens. Although originally defined as Th1 and Th2 subsets, new Th CD4 T cells subsets emerged the last decades such as suppressive regulatory T cells (Tregs) and pro-inflammatory Th17, and more recently for Th9, Th22 and follicular helper T cells [12–17]. Although Th1 and Th2 subsets are initially considered as the most stable and mutually exclusive lineages, it now appears that they depend on the differentiation state [18]. Likewise, Th17 and Treg subsets do not represent stable differentiation processes and retain plasticity allowing them to adapt to different environments [18,19].

Among these various Th subpopulations, the Th1 subset that produces interferon-gamma (IFN-\(\gamma\)), tumor necrosis factor-alpha (TNF-\(\alpha\)) and interleukine-2 (IL-2), plays a clear antitumor role by orchestrating cell-mediated immunity against cancer cells [20,21]. Earlier studies in mouse model showed that successful generation of antitumor CD8 T cell responses depend on the presence of CD4 T cells [20,22]. The induction of DC activation represents one major helper mechanism used by Th1 cells to sustain antigen presentation and to provide costimulatory signals such as CD40-CD40L to effector CD8 T cells [23–25]. Importantly, it has also been shown that Th1 cells enhance the CD8 T cells infiltration into the tumor [26–29]. This effect was mediated by IFN-\(\gamma\) dependent production of chemokines such as CXCL9 and CXCL10 [27,30]. However, CD4 Th1 cells also exhibit CD8 T cells’ independent antitumor activity. The IFN-\(\gamma\) secreted by Th1 cells exerts anti-proliferative, pro-apoptotic actions and inhibit angiogenesis in tumor cells [31]. Furthermore, Th1 cells also recruit and activate inflammatory cells (macrophages, granulocytes, eosinophils and NK cells) in around the tumor [20,21]. Furthermore, IFN-\(\gamma\) induces the upregulation of major histocompatibility complex (MHC) molecules on tumor cells.
leading to enhanced effector T cells recognition [31]. This mechanism enables MHC Class II restricted killing independently of B, NK or other T cells [32,33]. Indeed, some CD4 Th1 cells have also direct tumor-recognizing ability [34]. They are able to kill MHC-II* tumors through perforin and granzyme, TNF-related apoptosis inducing ligand (TRAIL) receptor and Fas/Fas ligand (FasL) pathways [20,21]. Finally, CD4 T cells can also provide help to themselves. Th-Th interaction enables the activation of CD4 T cells specific for a poorly immunogenic epitope [11].

In cancer patients, spontaneous CD4 T cell responses against tumor antigens have been detected in several studies [28,35–43]. Accordingly, a high density of tumor-infiltrating Th1 cells has been identified as a good prognostic marker in several human cancers [44,45]. On the other hand, subsets such as Th2, Tregs, or, under some circumstances, Th17 cells, may have tumor-promoting activity, which may need to be curtailed to obtain optimal antitumor responses [46]. Finally, only Th1 immune response has been shown to mediate bona fide anticancer effects in cancer patients, providing a strong rationale to develop antitumor Th1 immunity-stimulating immunotherapy, which is supported by many clinical trials.

3. Interest to Stimulate CD4 T Helper 1 Response for Therapeutic Cancer Vaccine

The role of CD4 Th cells in cancer vaccine is supported by early studies in animals showing that the depletion of CD4 T cells inhibited vaccine-induced protective immunity [47–49]. Based on the critical role of CD4 Th1 cells in antitumor immunity, we believe that effectiveness of cancer vaccine could be greatly enhanced by stimulating suitable tumor-reactive Th1 immunity, making a “good” tumor microenvironment for the immune effector cells action (CD8 T cells, NK cells and M1 macrophages) and a “bad” one for the immune suppressive cells (Tregs, MDSC, etc.). Hence, a reasonable alternative to boost helper response is to focus on MHC class II-binding peptides from tumor antigens.

To do this, we propose that four major criteria have to be considered to select tumor-specific Th1-inducer helper peptides for cancer vaccine: (i) the helper peptide must derive from a shared overexpressed tumor antigen; (ii) the tumor antigen should have crucial role in the oncogenesis to avoid immune escape; (iii) the tumor-reactive helper peptide should be highly promiscuous to immunize a large segment of the human population; and (iv) the helper peptides should preferentially stimulate Th1 immunity to prevent the induction of detrimental Th responses [50]. Several antigens with some of these properties have already been described such as human telomerase reverse transcriptase (hTERT) [51], HER2 [52], survivin [53], NY-ESO-1 [54], and MUC1 [55]. In order to stimulate CD4 Th1 responses in cancer patients, increasing attention has been focused on identifying MHC class II-restricted epitopes from relevant human tumor antigens to actively target these cells for cancer vaccine [56,57]. However, only a few tumor-reactive helper peptides are evaluated in clinical settings.

One common approach to stimulate CD4 T cell helper response is the use of xenogenic or non-tumor antigens that recall immune memory or provide a non-specific help. The synthetic helper peptide PADRE derived from keyhole limpet hemocyanin (KLH) and the tetanus toxoid-derived helper peptide are commonly used in anticancer vaccines [58,59]. In recent randomized vaccine trials, melanoma patients were vaccinated with multi melanoma-derived cytotoxic T lymphocytes (CTL) peptides vaccine either in the presence of a tetanus-derived helper peptide or with melanoma-derived helper peptides [8,60]. In contrast to the group that received CTL peptides and tetanus helper peptide, a high clinical objective response rate (ORR) was observed in patients treated with Melanoma-derived helper peptides [8,9].
Interestingly, significant ORR was also observed when melanoma-helper peptides were used alone as compared to CTL-peptides vaccination. One possible explanation for these observations is that helper peptides unrelated to tumor antigens may be ineffective in guiding effector CD8 T cells within the tumor [61].

These observations are in line with previous reports by using tumor-derived helper peptides such as HER2-neu and hTERT [62,63]. Hence, a hTERT-derived helper peptide vaccine called GV1001 has been evaluated in many cancers [64]. In lung cancer patients GV100 vaccination induced a durable T-cell memory response and increased survival in immune responders [63]. However GV1001 vaccination fails to reach the main end point overall survival in pancreatic adenocarcinoma [65]. Recently, our group described novel antitumor Th1-inducer peptides derived from hTERT also called Universal Cancer Peptides (UCP) [28,37]. We found that the immunoprevalence and magnitude of UCP-specific Th1 responses are higher than GV1001 in several cancers (manuscript submitted). Thus, we believe that UCPs are attractive helper peptides for therapeutic cancer vaccine and a clinical trial with these peptides is planned to be conducted in lung cancer.

Instead of vaccinating with a mix of Th and CTL epitopes, it was proposed later, to conjugate the two epitopes to form a single longer linear hybrid peptide named synthetic long peptide (SLP) [66–68]. This strategy allowed the improvement of the immunogenicity by increasing the duration of in vivo epitope presentation [69] and by inducing a broader immune response with the stimulation of both CD8 and CD4 T cells [49,70,71]. The efficacy of SLP-based vaccination has been shown in pre-clinical models [72,73] and in patients with cervical cancer [74,75].

Recently, Aarntzen et al. reported that the use of tumor-derived helper peptides greatly improved the efficacy responses of DC-based vaccination [76]. Furthermore, there exists clear evidence for epitope spreading, following immunization of cancer patients with tumor-reactive helper peptides [77–79]. Collectively, these results highlighted the necessity of tumor-specific CD4 T cell stimulation for vaccine success [49,80–82].

4. Emerging Personalized Vaccine Using CD4 Helper Peptides from Neoantigens

Conventional cancer vaccines have targeted shared self-antigens with the advantage of being universally available for patients. However central and peripheral tolerance could deplete the most reactive specific T cells. In this regard, it seems interesting to design cancer vaccines targeting non-self-tumor-specific antigens like those emerging from mutations, namely cancer neoantigens.

Despite the impressive work accomplished to monitor T-cell reactivity against large collections of shared epitopes, the cumulative fraction of tumor-infiltrating T cells that were reactive with these epitopes showed to be low [83,84]. One possible explanation for this observation could be that neoantigens specific T cells form a significant component of tumor specific T cell responses and that these studies were focused on CD8 T cells. Prior works have clearly established that neoantigens can be recognized in human cancers, and that reactivity against patient-specific antigens can be stronger than the one against shared antigens [7,85]. Neoantigens can arise from mutation or cancer specific splice isoforms. Recurrent alternative splicing events have been identified [86] and some of them are immunogenic [38,87]. However, until now, the major known somatic alterations in the cancer genome leading to neoantigens include nucleotide substitution mutations, small insertion/deletions (indels) and
chromosomal rearrangements. Missense mutations are the most frequent DNA modifications observed [88] and most recent works focused on these mutations within exonic region [10,89,90]. However, insertions, deletions and chromosomal translocations are believed to be more immunogenic than missense as they could induce frameshifts and the immunogenicity of splice site mutations is poorly studied. Despite these limits, several epitopes derived from neoantigens are described [10,89–91] and neoepitopes derived from these mutations preferentially stimulate CD4 T cell responses [10]. In addition, the efficacy of checkpoint inhibitors seems to rely on mutation load [92,93]. These recent observations pointed out once more, the role of CD4 T cells in antitumoral immune response and the interest in targeting them to increase clinical benefit of cancer vaccines.

Neoantigens appear advantageous for antitumor vaccine because CD4 T cell precursors exist in a nontolerized form within the repertoire of the nonimmunized individual. With exon and RNA sequencing, algorithms already exist to rapidly match neoepitopes with MHC alleles, thereby facilitating the identification of peptides for personalized cancer medicine [94].

However, neoepitopes targeting strategies meet several hurdles. Firstly, data from genome sequencing and single cell sequencing analysis have revealed a surprising tumor genetic heterogeneity [95,96]. Thus, targeting a unique neoantigen would probably lead to selection of antigen non-expressing tumor cells. Secondly, assessing the immunogenicity of each neoepitope is not reasonably applicable on a large scale. To bypass these hurdles, Kreiter et al. have proposed to select strong HLA binders that are highly expressed [10]. However, the number of neoepitopes that are needed is not determined in humans. Thirdly, vaccination with neoepitopes could lead to cross-reactions, due to peptide mimicry, inducing autoimmunity as it has been observed for MAGE-3 [97]. Nonetheless, clinical trials targeting neoepitopes are ongoing (NCT00683670, NCT02287428, NCT01970358, NCT02129075, NCT02035956) and results will help us to establish the relevance of this vaccine strategy.

5. Conclusions and Perspectives

Owing to the central role of CD4 T cells in antitumor immunity, targeting MHC-II restricted antigens will doubtless improve cancer vaccine efficacy in the future. Beyond the effector immune cells, the choice of antigens remains crucial, and each of them presents advantages as well as drawbacks. Shared antigens are broadly relevant in a non-personalized vaccination strategy while neoantigens are patient and tumor-specific. However, neoantigens-specific T cells are not selected in the thymus.

The success story of checkpoint inhibitors, anti-cytotoxic T lymphocytes antigen 4 (CTLA-4) (ipilimumab) and anti-programmed death 1 PD-1 (nivolumab, pembrolizumab), reinforces the notion that it exists a preexisting antitumor immune response but it fails to eradicate all tumor cells because of immune suppressive establishment in tumor microenvironment [1,3,98]. Thus, immunotherapy strategy, particularly vaccine strategy, could be more effective when combined with molecules, which break cancer immunosuppression such as chemotherapy depleting Tregs and myeloid-derived suppressor cells (MDSC) [99,100], tyrosine kinase inhibitor [101] or antibody directed against checkpoint receptors [102,103].
Acknowledgments

Authors are supported by grants from the Ligue contre le cancer, the ICB network of the University of Franche-Comté, the Conseil Régional de Franche-Comté, the Agence Nationale de la Recherche (Labex LipSTIC, ANR-11-LABX-0021). The authors would like to thank Guadalupe Tizon for English writing assistance.

Author Contributions

Jeanne Galaine, Christophe Borg, Yann Godet and Olivier Adotévi contributed to the writing and revision of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Sharma, P.; Allison, J.P. Immune checkpoint targeting in cancer therapy: Toward combination strategies with curative potential. Cell 2015, 161, 205–214.
2. Pardoll, D.M. The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer 2012, 12, 252–264.
3. Chen, D.S.; Mellman, I. Oncology meets immunology: The cancer-immunity cycle. Immunity 2013, 39, 1–10.
4. Butterfield, L.H. Cancer vaccines. Br. Med. J. 2015, doi:10.1136/bmj.h988.
5. Ajewski, T.F.; Meng, Y.; Harlin, H. Immune suppression in the tumor microenvironment. J. Immunother. 2006, 29, 233–240.
6. Hunder, N.N.; Wallen, H.; Cao, J.; Hendricks, D.W.; Reilly, J.Z.; Rodmyre, R.; Jungbluth, A.; Gnjatic, S.; Thompson, J.A.; Yee, C. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N. Engl. J. Med. 2008, 358, 2698–2703.
7. Tran, E.; Turcotte, S.; Gros, A.; Robbins, P.F.; Lu, Y.C.; Dudley, M.E.; Wunderlich, J.R.; Somerville, R.P.; Hogan, K.; Hinrichs, C.S.; et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 2014, 344, 641–645.
8. Slingluff, C.L.; Lee, S.; Zhao, F.; Chianese-Bullock, K.A.; Olson, W.C.; Butterfield, L.H.; Whiteside, T.L.; Leming, P.D.; Kirkwood, J.M. A randomized phase II trial of multiepitope vaccination with melanoma peptides for cytotoxic T cells and helper T cells for patients with metastatic melanoma (E1602). Clin. Cancer Res. 2013, 19, 4228–4238.
9. Reed, C.M.; Cresce, N.D.; Mauldin, I.S.; Slingluff, C.L.; Olson, W.C. Vaccination with melanoma helper peptides induces antibody responses associated with improved overall survival. Clin. Cancer Res. 2015, doi:10.1158/1078-0432.CCR-15-0233.
10. Kreiter, S.; Vormehr, M.; van de Roemer, N.; Diken, M.; Löwer, M.; Diekmann, J.; Boegel, S.; Schrörs, B.; Vascotto, F.; Castle, J.C.; et al. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. Nature 2015, 520, 692–696.
11. Zanetti, M. Tapping CD4 T Cells for Cancer Immunotherapy: The Choice of Personalized Genomics. *J. Immunol.* 2015, 194, 2049–2056.

12. Mosmann, T.R.; Coffman, R.L. TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 1989, 7, 145–173.

13. Sakaguchi, S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat. Immunol.* 2005, 6, 345–352.

14. Korn, T.; Oukka, M.; Kuchroo, V.; Bettelli, E. Th17 cells: Effector T cells with inflammatory properties. *Semin. Immunol.* 2007, 19, 362–371.

15. Végran, F.; Apetoh, L.; Ghiringhelli, F. Th9 cells: A novel CD4 T-cell subset in the immune war against cancer. *Cancer Res.* 2015, 75, 475–479.

16. Trifari, S.; Kaplan, C.D.; Tran, E.H.; Crellin, N.K.; Spits, H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from TH-17, TH1 and TH2 cells. *Nat. Immunol.* 2009, 10, 864–871.

17. Crotty, S. Follicular helper CD4 T cells (TFH). *Annu. Rev. Immunol.* 2011, 29, 621–663.

18. Geginat, J.; Paroni, M.; Maglie, S.; Alfen, J.S.; Kastirr, I.; Gruarin, P.; de Simone, M.; Pagani, M.; Abrignani, S. Plasticity of human CD4 T cell subsets. *Front. Immunol.* 2014, doi:10.3389/fimmu.2014.00630.

19. Zhu, J.; Paul, W.E. CD4 T cells: Fates, functions, and faults. *Blood* 2008, 112, 1557–1569.

20. Kennedy, R.; Celis, E. Multiple roles for CD4+ T cells in anti-tumor immune responses. *Immuno. Rev.* 2008, 222, 129–144.

21. Kim, H.J.; Cantor, H. CD4 T-cell subsets and tumor immunity: The helpful and the not-so-helpful. *Cancer Immunol. Res.* 2014, 2, 91–98.

22. Pardoll, D.M.; Topalian, S.L. The role of CD4+ T cell responses in antitumor immunity. *Curr. Opin. Immunol.* 1998, 10, 588–594.

23. Ridge, J.P.; di Rosa, F.; Matzinger, P. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature* 1998, 393, 474–478.

24. Bennett, S.R.; Carbone, F.R.; Karamalis, F.; Flavell, R.A.; Miller, J.F.; Heath, W.R. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 1998, 393, 478–480.

25. Smith, C.M.; Wilson, N.S.; Waithman, J.; Villadangos, J.A.; Carbone, F.R.; Heath, W.R.; Belz, G.T. Cognate CD4+ T cell licensing of dendritic cells in CD8+ T cell immunity. *Nat. Immunol.* 2004, 5, 1143–1148.

26. Wong, S.B.J.; Bos, R.; Sherman, L.A. Tumor-specific CD4+ T cells render the tumor environment permissive for infiltration by low-avidity CD8+ T cells. *J. Immunol.* 2008, 180, 3122–3131.

27. Bos, R.; Sherman, L.A. CD4+ T-cell help in the tumor milieu is required for recruitment and cytolytic function of CD8+ T lymphocytes. *Cancer Res.* 2010, 70, 8368–8377.

28. Dosset, M.; Godet, Y.; Vauchy, C.; Beziaud, L.; Lone, Y.C.; Sedlik, C.; Liard, C.; Levionnois, E.; Clerc, B.; Sandoval, F.; *et al.* Universal cancer peptide-based therapeutic vaccine breaks tolerance against telomerase and eradicates established tumor. *Clin. Cancer Res.* 2012, 18, 6284–6295.

29. Dosset, M.; Vauchy, C.; Beziaud, L.; Adotevi, O.; Godet, Y. Universal tumor-reactive helper peptides from telomerase as new tools for anticancer vaccination. *Oncoimmunology* 2013, doi:10.4161/onci.23430.
30. Nakanishi, Y.; Lu, B.; Gerard, C.; Iwasaki, A. CD8+ T lymphocyte mobilization to virus-infected tissue requires CD4+ T-cell help. *Nature* 2009, 462, 510–513.

31. Ikeda, H.; Old, L.J.; Schreiber, R.D. The roles of IFNγ in protection against tumor development and cancer immunoediting. *Cytokine Growth Factor Rev.* 2002, 13, 95–109.

32. Xie, Y.; Akpinarli, A.; Maris, C.; Hipkiss, E.L.; Lane, M.; Kwon, E.K.M.; Muranski, P.; Restifo, N.P.; Antony, P.A. Naive tumor-specific CD4+ T cells differentiated *in vivo* eradicate established melanoma. *J. Exp. Med.* 2010, 207, 651–667.

33. Quezada, S.A.; Simpson, T.R.; Peggs, K.S.; Merghoub, T.; Vider, J.; Fan, X.; Blasberg, R.; Yagita, H.; Muranski, P.; Antony, P.A.; *et al.* Tumor-reactive CD4+ T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. *J. Exp. Med.* 2010, 207, 637–650.

34. Matsuzaki, J.; Tsuji, T.; Luescher, I.; Old, L.J.; Shrikant, P.; Gnjatic, S.; Odunsi, K. Nonclassical antigen-processing pathways are required for MHC class II-restricted direct tumor recognition by NY-ESO-1-specific CD4+ T Cells. *Cancer Immunol. Res.* 2014, 2, 341–350.

35. Ayyoub, M.; Pignon, P.; Classe, J.-M.; Odunsi, K.; Valmori, D. CD4+ T effectors specific for the tumor antigen NY-ESO-1 are highly enriched at ovarian cancer sites and coexist with, but are distinct from, tumor-associated Treg. *Cancer Immunol. Res.* 2013, 1, 303–308.

36. Campi, G.; Crosti, M.; Consogno, G.; Facchinetti, V.; Conti-Fine, B.M.; Longhi, R.; Casorati, G.; DellaBona, P.; Protti, M.P. CD4+ T cells from healthy subjects and colon cancer patients recognize a carcinoembryonic antigen-specific immunodominant epitope. *Cancer Res.* 2003, 63, 8481–8486.

37. Godet, Y.; Fabre, E.; Dosset, M.; Lamuraglia, M.; Levionnois, E.; Ravel, P.; Benhamouda, N.; Cazes, A.; le Pimpec-Barthes, F.; Gaugler, B.; *et al.* Analysis of spontaneous tumor-specific CD4 T-cell immunity in lung cancer using promiscuous HLA-DR telomerase-derived epitopes: Potential synergistic effect with chemotherapy response. *Clin. Cancer Res.* 2012, 18, 2943–2953.

38. Vauchy, C.; Gamonet, C.; Ferrand, C.; Daguindau, E.; Galaine, J.; Beziaud, L.; Chauchet, A.; Henry Dunand, C.J.; Deschamps, M.; Rohrlich, P.S.; *et al.* CD20 alternative splicing isoform generates immunogenic CD4 helper T epitopes. *Int. J. Cancer* 2015, 137, 116–126.

39. Wang, X.F.; Kerzerho, J.; Adotevi, O.; Nuyttens, H.; Badoual, C.; Munier, G.; Oudard, S.; Tu, S.; Tartour, E.; Maillère, B. Comprehensive analysis of HLA-DR- and HLA-DP4-restricted CD4+ T cell response specific for the tumor-shared antigen survivin in healthy donors and cancer patients. *J. Immunol.* 2008, 181, 431–439.

40. Kudela, P.; Sun, Z.; Fourcade, J.; Janjic, B.; Kirkwood, J.M.; Maillere, B.; Zarour, H.M. Epitope hierarchy of spontaneous CD4+ T cell responses to LAGE-1. *J. Immunol.* 2011, 186, 312–322.

41. Ohue, Y.; Eikawa, S.; Okazaki, N.; Mizote, Y.; Isobe, M.; Uenaka, A.; Fukuda, M.; Old, L.J.; Oka, M.; Nakayama, E. Spontaneous antibody, and CD4 and CD8 T-cell responses against XAGE-1b (GAGED2a) in non-small cell lung cancer patients. *Int. J. Cancer* 2012, 131, E649–E658.

42. Tsuji, T.; Matsuoka, J.; Ritter, E.; Miliotto, A.; Ritter, G.; Odunsi, K.; Old, L.J.; Gnjatic, S. Split T cell tolerance against a self/tumor antigen: Spontaneous CD4+ but not CD8+ T cell responses against p53 in cancer patients and healthy donors. *PLoS ONE* 2011, 6, e23651.
43. Yoshida, N.; Abe, H.; Ohkuri, T.; Wakita, D.; Sato, M.; Noguchi, D.; Miyamoto, M.; Morikawa, T.; Kondo, S.; Ikeda, H.; et al. Expression of the MAGE-A4 and NY-ESO-1 cancer-testis antigens and T cell infiltration in non-small cell lung carcinoma and their prognostic significance. *Int. J. Oncol.* **2006**, *28*, 1089–1098.

44. Zhang, Z.; Huang, J.; Zhang, C.; Yang, H.; Qiu, H.; Li, J.; Liu, Y.; Qin, L.; Wang, L.; Hao, S.; et al. Infiltration of dendritic cells and T lymphocytes predicts favorable outcome in epithelial ovarian cancer. *Cancer Gene Ther.* **2015**, *22*, 198–206.

45. Fridman, W.H.; Dieu-Nosjean, M.C.; Pagès, F.; Cremer, I.; Damotte, D.; Sautès-Fridman, C.; Galon, J. The immune microenvironment of human tumors: General significance and clinical impact. *Cancer Microenviron.* **2013**, *6*, 117–122.

46. Fridman, W.H.; Pagès, F.; Sautès-Fridman, C.; Galon, J. The immune contexture in human tumours: Impact on clinical outcome. *Nat. Rev. Cancer* **2012**, *12*, 298–306.

47. Fayolle, C.; Deriaud, E.; Leclerc, C. *In vivo* induction of cytotoxic T cell response by a free synthetic peptide requires CD4+ T cell help. *J. Immunol.* **1991**, *147*, 4069–4073.

48. Antony, P.A.; Piccirillo, C.A.; Akpinarli, A.; Finkelstein, S.E.; Speiss, P.J.; Surman, D.R.; Palmer, D.C.; Chan, C.C.; Klebanoff, C.A.; Overwijk, W.W.; et al. CD8+ T cell immunity against a tumor/self-antigen is augmented by CD4+ T helper cells and hindered by naturally occurring T regulatory cells. *J. Immunol.* **2005**, *174*, 2591–2601.

49. Knutson, K.L.; Disis, M.L. Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. *Cancer Immunol. Immunother.* **2005**, *54*, 721–728.

50. Cecil, D.L.; Holt, G.E.; Park, K.H.; Gad, E.; Rastetter, L.; Childs, J.; Higgins, D.; Disis, M.L. Elimination of IL-10-inducing T-helper epitopes from an IGFBP-2 vaccine ensures potent antitumor activity. *Cancer Res.* **2014**, *74*, 2710–2718.

51. Beatty, G.L.; Vonderheide, R.H. Telomerase as a universal tumor antigen for cancer vaccines. *Expert Rev. Vaccines* **2008**, *7*, 881–887.

52. Kobayashi, H.; Wood, M.; Song, Y.; Appella, E.; Celis, E. Defining promiscuous MHC class II helper T-cell epitopes for the HER2/neu tumor antigen. *Cancer Res.* **2000**, *60*, 5228–5236.

53. Piesche, M.; Hildebrandt, Y.; Zettl, F.; Chapuy, B.; Schmitz, M.; Wulf, G.; Trümper, L.; Schroers, R. Identification of a promiscuous HLA DR-restricted T-cell epitope derived from the inhibitor of apoptosis protein survivin. *Hum. Immunol.* **2007**, *68*, 572–576.

54. Jäger, E.; Jäger, D.; Karbach, J.; Chen, Y.T.; Ritter, G.; Nagata, Y.; Gnjatic, S.; Stockert, E.; Arand, M.; Old, L.J.; et al. Identification of NY-ESO-1 epitopes presented by human histocompatibility antigen (HLA)-DRB4 *0101-0103 and recognized by CD4+ T lymphocytes of patients with NY-ESO-1-expressing melanoma. *J. Exp. Med.* **2000**, *191*, 625–630.

55. Hiltbold, E.M.; Ciborowski, P.; Finn, O.J. Naturally processed class II epitope from the tumor antigen MUC1 primes human CD4+ T cells. *Cancer Res.* **1998**, *58*, 5066–5070.

56. Kobayashi, H.; Celis, E. Peptide epitope identification for tumor-reactive CD4 T cells. *Curr. Opin. Immunol.* **2008**, *20*, 221–227.

57. Adotévi, O.; Dosset, M.; Galaine, J.; Beziaud, L.; Godet, Y.; Borg, C. Targeting antitumor CD4 helper T cells with universal tumor-reactive helper peptides derived from telomerase for cancer vaccine. *Hum. Vaccines Immunother.* **2013**, *9*, 1073–1077.
58. Del Guercio, M.F.; Alexander, J.; Kubo, R.T.; Arrhenius, T.; Maewal, A.; Appella, E.; Hoffman, S.L.; Jones, T.; Valmori, D.; Sakaguchi, K.; et al. Potent immunogenic short linear peptide constructs composed of B cell epitopes and Pan DR T helper epitopes (PADRE) for antibody responses in vivo. Vaccine 1997, 15, 441–448.

59. Valmori, D.; Pessi, A.; Bianchi, E.; Corradin, G. Use of human universally antigenic tetanus toxin T cell epitopes as carriers for human vaccination. J. Immunol. 1992, 149, 717–721.

60. Slingluff, C.L.; Petroni, G.R.; Chianese-Bullock, K.A.; Smolkin, M.E.; Ross, M.I.; Haas, N.B.; von Mehren, M.; Grosh, W.W. Randomized multicenter trial of the effects of melanoma-associated helper peptides and cyclophosphamide on the immunogenicity of a multipeptide melanoma vaccine. J. Clin. Oncol. 2011, 29, 2924–2932.

61. Woods, K.; Cebon, J. Tumor-specific T-cell help is associated with improved survival in melanoma. Clin. Cancer Res. 2013, 19, 4021–4023.

62. Disis, M.L.; Wallace, D.R.; Gooley, T.A.; Dang, Y.; Slota, M.; Lu, H.; Coveler, A.L.; Childs, J.S.; Higgins, D.M.; Fintak, P.A.; et al. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. J. Clin. Oncol. 2009, 27, 4685–4692.

63. Brunsvig, P.F.; Kyte, J.A.; Kersten, C.; Sundström, S.; Möller, M.; Nyakas, M.; Hansen, G.L.; Gaudernack, G.; Aamdal, S. Telomerase peptide vaccination in NSCLC: A phase II trial in stage III patients vaccinated after chemoradiotherapy and an 8-year update on a phase I/II trial. Clin. Cancer Res. 2011, 17, 6847–6857.

64. Kyte, J.A. Cancer vaccination with telomerase peptide GV1001. Expert Opin. Investig. Drugs 2009, 18, 687–694.

65. Middleton, G.; Silcocks, P.; Cox, T.; Valle, J.; Wadsley, J.; Propper, D.; Coxon, F.; Ross, P.; Madhusudan, S.; Roques, T.; et al. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): An open-label, randomised, phase 3 trial. Lancet Oncol. 2014, 15, 829–840.

66. Widmann, C.; Romero, P.; Maryanski, J.L.; Corradin, G.; Valmori, D. T helper epitopes enhance the cytotoxic response of mice immunized with MHC class I-restricted malaria peptides. J. Immunol. Methods 1992, 155, 95–99.

67. Shirai, M.; Pendleton, C.D.; Ahlers, J.; Takeshita, T.; Newman, M.; Berzofsky, J.A. Helper-cytotoxic T lymphocyte (CTL) determinant linkage required for priming of anti-HIV CD8+ CTL in vivo with peptide vaccine constructs. J. Immunol. 1994, 152, 549–556.

68. Hiranuma, K.; Tamaki, S.; Nishimura, Y.; Kusuki, S.; Isogawa, M.; Kim, G.; Kaito, M.; Kuribayashi, K.; Adachi, Y.; Yasutomi, Y. Helper T cell determinant peptide contributes to induction of cellular immune responses by peptide vaccines against hepatitis C virus. J. Gen. Virol. 1999, 80, 187–193.

69. Bijker, M.S.; van den Eeden, S.J.F.; Franken, K.L.; Melief, C.J.M.; van der Burg, S.H.; Offringa, R. Superior induction of anti-tumor CTL immunity by extended peptide vaccines involves prolonged, DC-focused antigen presentation. Eur. J. Immunol. 2008, 38, 1033–1042.

70. Zeng, G.; Li, Y.; El-Gamil, M.; Sidney, J.; Sette, A.; Wang, R.; Rosenberg, S.A.; Robbins, P.F. Generation of NY-ESO-1-specific CD4+ and CD8+ T cells by a single peptide with dual MHC class I and class II specificities: A new strategy for vaccine design. Cancer Res. 2002, 62, 3630–3635.
71. Van der Burg, S.H.; Melief, C.J. Therapeutic vaccination against human papilloma virus induced malignancies. *Curr. Opin. Immunol.* 2011, 23, 252–257.

72. Zwaveling, S.; Mota, S.C.F.; Nouta, J.; Johnson, M.; Lipford, G.B.; Offringa, R.; Burg, S.H. van der Melief, C.J.M. Established human papillomavirus type 16-expressing tumors are effectively eradicated following vaccination with long peptides. *J. Immunol.* 2002, 169, 350–358.

73. Vambutas, A.; DeVoti, J.; Nouri, M.; Drijfhout, J.W.; Lipford, G.B.; Bonagura, V.R.; van der Burg, S.H.; Melief, C.J.M. Therapeutic vaccination with papillomavirus E6 and E7 long peptides results in the control of both established virus-induced lesions and latently infected sites in a pre-clinical cottontail rabbit papillomavirus model. *Vaccine* 2005, 23, 5271–5280.

74. Kenter, G.G.; Welters, M.J.P.; Valentijn, A.R.P.M.; Lowik, M.J.G.; Berends-van der Meer, D.M.A.; Vloon, A.P.G.; Essah, F.; Fathers, L.M.; Offringa, R.; Drijfhout, J.W.; *et al.* Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N. Engl. J. Med.* 2009, 361, 1838–1847.

75. Disis, M.L.; Gooley, T.A.; Rinn, K.; Davis, D.; Piepkorn, M.; Cheever, M.A.; Knutson, K.L.; Schiffman, K. Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. *J. Clin. Oncol.* 2002, 20, 2624–2632.

76. Aarntzen, E.H.J.G.; de Vries, I.J.M.; Lesterhuis, W.J.; Schuurhuis, D.; Jacobs, J.F.M.; Bol, K.; Schreibelt, G.; Mus, R.; de Wilt, J.H.W.; Haanen, J.B.A.G.; *et al.* Targeting CD4+ T-helper cells improves the induction of antitumor responses in dendritic cell-based vaccination. *Cancer Res.* 2013, 73, 19–29.

77. Wierecky, J.; Müller, M.R.; Wirths, S.; Halder-Oehler, E.; Dörfel, D.; Schmidt, S.M.; Häntschel, M.; Brugger, W.; Schröder, S.; Horger, M.S.; *et al.* Immunologic and clinical responses after vaccinations with peptide-pulsed dendritic cells in metastatic renal cancer patients. *Cancer Res.* 2006, 66, 5910–5918.

78. Inderberg-Suso, E.M.; Trachsel, S.; Lisluer, K.; Rasmussen, A.M.; Gaudernack, G. Widespread CD4+ T-cell reactivity to novel hTERT epitopes following vaccination of cancer patients with a single hTERT peptide GV1001. *OncoImmunology* 2012, 1, 670–686.

79. Hu, Y.; Petroni, G.R.; Olson, W.C.; Czarkowski, A.; Smolkin, M.E.; Grosh, W.W.; Chianese-Bullock, K.A.; Slingluff, C.L. Immunologic hierarchy, class II MHC promiscuity, and epitope spreading of a melanoma helper peptide vaccine. *Cancer Immunol. Immunother.* 2014, 63, 779–786.

80. Protti, M.P.; Monte, L.D.; Lullo, G.D. Tumor antigen-specific CD4+ T cells in cancer immunity: From antigen identification to tumor prognosis and development of therapeutic strategies. *Tissue Antigens* 2014, 83, 237–246.

81. Simon, T.; Fonteneau, J.F.; Grégoire, M. Requirement of tumor-associated antigen-specific CD4+ T cells for an efficient dendritic cell vaccine in antitumor immunotherapy. *Immunotherapy* 2013, 5, 565–567.

82. Grabowska, A.K.; Kaufmann, A.M.; Riemer, A.B. Identification of promiscuous HPV16-derived T helper cell epitopes for therapeutic HPV vaccine design. *Int. J. Cancer* 2015, 136, 212–224.

83. Andersen, R.S.; Thue, C.A.; Junker, N.; Lyngaa, R.; Donia, M.; Ellebæk, E.; Svane, I.M.; Schumacher, T.N.; Thor Straten, P.; Hadrup, S.R. Dissection of T-cell antigen specificity in human melanoma. *Cancer Res.* 2012, 72, 1642–1650.
84. Benlalam, H.; Labarrière, N.; Linard, B.; Derré, L.; Diez, E.; Pandolfino, M.C.; Bonneville, M.; Jotereau, F. Comprehensive analysis of the frequency of recognition of melanoma-associated antigen (MAA) by CD8 melanoma infiltrating lymphocytes (TIL): Implications for immunotherapy. *Eur. J. Immunol.* 2001, 31, 2007–2015.

85. Lennerz, V.; Fatho, M.; Gentili, C.; Frye, R.A.; Lifke, A.; Ferel, D.; Wölfel, C.; Huber, C.; Wölfel, T. The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. *Proc. Natl. Acad. Sci. USA* 2005, 102, 16013–16018.

86. Danan-Gotthold, M.; Golan-Gerstl, R.; Eisenberg, E.; Meir, K.; Karni, R.; Levanon, E.Y. Identification of recurrent regulated alternative splicing events across human solid tumors. *Nucleic Acids Res.* 2015, doi:10.1093/nar/gkv210.

87. Guilloux, Y.; Lucas, S.; Brichard, V.G.; Van Pel, A.; Viret, C.; de Plaen, E.; Brasseur, F.; Lethé, B.; Jotereau, F.; Boon, T. A peptide recognized by human cytolytic T lymphocytes on HLA-A2 melanomas is encoded by an intron sequence of the N-acetylglucosaminyl transferase V gene. *J. Exp. Med.* 1996, 183, 1173–1183.

88. Nehrt, N.L.; Peterson, T.A.; Park, D.; Kann, M.G. Domain landscapes of somatic mutations in cancer. *BMC Genomics* 2012, doi:10.1186/1471-2164-13-S4-S9.

89. Yadav, M.; Jhunjhunwala, S.; Phung, Q.T.; Lupardus, P.; Tanguay, J.; Bumbaca, S.; Franci, C.; Cheung, T.K.; Fritsche, J.; Weinschenk, T.; et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. *Nature* 2014, 515, 572–576.

90. Linnemann, C.; van Buuren, M.M.; Bies, L.; Verdegaal, E.M.E.; Schotte, R.; Calis, J.J.A.; Behjati, S.; Velds, A.; Hilkmann, H.; Atmioui, D.E.; et al. High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. *Nat. Med.* 2015, 21, 81–85.

91. Schumacher, T.N.; Schreiber, R.D. Neoantigens in cancer immunotherapy. *Science* 2015, 348, 69–74.

92. Snyder, A.; Makarov, V.; Merghoub, T.; Yuan, J.; Zaretsky, J.M.; Desrichard, A.; Walsh, L.A.; Postow, M.A.; Wong, P.; Ho, T.S.; et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* 2014, 371, 2189–2199.

93. Rizvi, N.A.; Hellmann, M.D.; Snyder, A.; Kvistborg, P.; Makarov, V.; Havel, J.J.; Lee, W.; Yuan, J.; Wong, P.; Ho, T.S.; et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015, 348, 124–128.

94. Paul, S.; Lindestam Arlehamn, C.S.; Scriba, T.J.; Dillon, M.B.C.; Oseroff, C.; Hinz, D.; McKinney, D.M.; Carrasco Pro, S.; Sidney, J.; Peters, B.; et al. Development and validation of a broad scheme for prediction of HLA class II restricted T cell epitopes. *J. Immunol. Methods* 2015, doi:10.1016/j.jim.2015.03.022.

95. Ding, L.; Ellis, M.J.; Li, S.; Larson, D.E.; Chen, K.; Wallis, J.W.; Harris, C.C.; McLellan, M.D.; Fulton, R.S.; Fulton, L.L.; et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. *Nature* 2010, 464, 999–1005.

96. Marusyk, A.; Almendro, V.; Polyak, K. Intra-tumour heterogeneity: A looking glass for cancer? *Nat. Rev. Cancer* 2012, 12, 323–334.

97. Linette, G.P.; Stadtmauer, E.A.; Maus, M.V.; Rapoport, A.P.; Levine, B.L.; Emery, L.; Litzky, L.; Bagg, A.; Carreno, B.M.; Cimino, P.J.; et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood* 2013, 122, 863–871.
98. Tumeh, P.C.; Harview, C.L.; Yearley, J.H.; Shintaku, I.P.; Taylor, E.J.M.; Robert, L.; Chmieleowski, B.; Spasic, M.; Henry, G.; Ciobanu, V.; et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014, 515, 568–571.

99. Walter, S.; Weinschenk, T.; Stenzl, A.; Zdrojowy, R.; Pluzanska, A.; Szczyllic, C.; Staehler, M.; Brugger, W.; Dietrich, P.Y.; Mendrzyk, R.; et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. *Nat. Med.* 2012, 18, 1254–1261.

100. Apetoh, L.; Ladoire, S.; Coukos, G.; Ghiringhelli, F. Combining immunotherapy and anticancer agents: The right path to achieve cancer cure? *Ann. Oncol.* 2015, doi:10.1093/annonc/mdv209.

101. Farsaci, B.; Donahue, R.N.; Coplin, M.A.; Grenga, I.; Lepone, L.M.; Molinolo, A.A.; Hodge, J.W. Immune consequences of decreasing tumor vasculature with antiangiogenic tyrosine kinase inhibitors in combination with therapeutic vaccines. *Cancer Immunol. Res.* 2014, 2, 1090–1102.

102. Gibney, G.T.; Kudchadkar, R.R.; DeConti, R.C.; Thebeau, M.S.; Czupryn, M.P.; Tetteh, L.; Eysmans, C.; Richards, A.; Schell, M.J.; Fisher, K.J.; et al. Safety, correlative markers, and clinical results of adjuvant nivolumab in combination with vaccine in resected high-risk metastatic melanoma. *Clin. Cancer Res.* 2015, 21, 712–720.

103. Sawada, Y.; Yoshikawa, T.; Shimomura, M.; Iwama, T.; Endo, I.; Nakatsura, T. Programmed death-1 blockade enhances the antitumor effects of peptide vaccine-induced peptide-specific cytotoxic T lymphocytes. *Int. J. Oncol.* 2015, 46, 28–36.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).