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Review

Overcoming the hurdles of randomised clinical trials of therapeutic cancer vaccines

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ARTICLE INFO

Article history:
Received 7 February 2010
Received in revised form 12 March 2010
Accepted 16 March 2010
Available online 21 April 2010

Keywords:
Cancer vaccine
Randomised clinical trial
Immune response
Personalisation

ABSTRACT

Most of the recent randomised clinical trials of therapeutic cancer vaccines have failed to demonstrate a meaningful therapeutic benefit to patients over existing treatments. Furthermore, some clinical trials have demonstrated a detrimental effect on patients, resulting in poorer outcomes. These unexpected results have shed light on several important issues to be solved for further development of cancer vaccines. As has been discussed with respect to the use of granulocyte–macrophage colony-stimulating factor (GM-SCF) as an adjuvant, the failures of clinical trials may be explained, in part, by a vaccine-specific adverse event, i.e. the induction of an 'inconvenient immune response' that inhibits pre-existing host immunity. This hypothesis may be supported by the fact that randomised trials of personalised peptide vaccines that were selected in consideration of pre-existing host immunities in individual patients resulted in clear benefit to patients. The development of reliable biomarkers for the selection of appropriate patients and vaccine antigens would thus be pivotal to prevent such vaccine-specific adverse events. This article discusses possible ways to overcome the hurdles of randomised clinical trials of therapeutic cancer vaccines based on a review of recently conducted clinical trials.

1. Introduction

The field of cancer vaccines has moved forward dramatically since 1991, when Boon and his colleagues reported their discovery of the first tumour-associated antigen.[1] Numerous tumour-associated antigens have been identified since that time, and some of them have been clinically tested with encouraging results in immunotherapy against patients with various types of cancer.[2–5] To date, however, no therapeutic cancer vaccines have been generally approved as a standard treatment for any type of cancer. Despite optimism and enthusiasm for cancer vaccine development, most of the randomised clinical trials designed to gain approval for clinical use have failed to demonstrate a meaningful therapeutic benefit to patients over existing treatments.[6,7] This situation has been further complicated by recent reports of several large

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2010.03.013

clinical trials in which cancer vaccines sometimes had a detrimental effect on patients, resulting in poorer outcomes.[6–8] Such unexpected results have shed light on several important issues that must be resolved for future development of cancer vaccines. Although the FDA has recently published a guidance for industry to facilitate the marketing approval of these vaccines,[9] it has not fully addressed the issues raised by the failure of recent clinical trials, leading us to re-consider possible ways to overcome the hurdles of randomised clinical trials of therapeutic cancer vaccines.

2. Current status of cancer vaccine development

In the field of cancer vaccines, one of the most notable advances has been the recent development of two prophylactic vaccines against human papillomavirus (HPV) infection, Gardasil® (Merck & Co.) and Cervarix® (GlaxoSmithKline Biologicals), which contain L1 virus-like particles of high risk types of HPV.[10] In contrast to the great success of these preventive vaccines, there are still multiple hurdles to overcome for therapeutic cancer vaccines. Namely, most of the follow-up late-phase trials have failed to achieve their main endpoints (Table 1). For example, despite much hope and promising preliminary data, a large phase III clinical trial of GVAX immunotherapy for symptomatic metastatic prostate cancer (VITAL-2, Cell Genesys Inc.), which is comprised of two prostate tumour cell lines secreting granulocyte–macrophage colony-stimulating factor (GM-SCF), were prematurely terminated due to an imbalance in deaths between the treatment and control arms of the study [hazard ratio (HR) = 1.4; $P = 0.02$; median overall survival, 12.4 months (treatment) versus 14.8 months (control)].[11] Another phase III randomised trial with GVAX immunotherapy for asymptomatic metastatic prostate cancer (VITAL-1, Cell Genesys Inc.) was also terminated based on the result of a futility analysis conducted at the company's request.[11] In addition, two randomised clinical trials of soluble protein idiotypic vaccination for follicular lymphoma have recently failed to achieve clinical benefits, although these 'personalised' vaccines got much attention. Of note, one of these studies showed a statistically significant difference in the time to progression (TTP) in favour of the patients treated with the control product [HR = 1.384; $P = 0.019$; TTP, 9.0 months (treatment) versus 12.6 months (control)].[12] Although a detailed analysis of these negative results has been awaited to help identify factors that are related to clinical benefit of idiotypic vaccination, the failure of these clinical trials is suggested to be due to the defect in clinical trial designs rather than due to the properties of vaccines themselves.[12]

Despite the failure of most of the recent randomised trials, there have been some encouraging advances. The phase III trial of dendritic cell (DC)-based vaccine (Provenge®, Dendrion Corporation) loaded with a recombinant fusion protein containing prostatic acid phosphatase (PAP) and GM-CSF has recently demonstrated a significantly longer overall survival in asymptomatic metastatic prostate cancer patients.[7] This result has been submitted to the FDA for approval of this product as the first therapeutic cancer vaccine in the United States. However, before this treatment modality can be made clinically available worldwide, multiple hurdles must be overcome, including the complicated protocols and the extremely high money, labour and time costs involved in the preparation of standardised vaccines. In addition, in April 2008, an autologous, tumour-derived heat-shock protein (glycoprotein 96)-peptide complex (vitespen; Oncophage®, Antigenics Inc.) became the first cancer vaccine approved in Russia for use as an adjuvant treatment for renal cell carcinoma patients with intermediate risk of disease recurrence.[7] However, post-marketing studies will still be needed to confirm its consistent clinical benefits, because this product showed no advantage in patient survival in the randomised phase III trials of renal cell carcinoma and melanoma.[7]

Recent early-phase clinical trials have also demonstrated significant advances in therapeutic peptide vaccines.[4,5,13–17] For example, therapeutic HPV vaccines have been reported to be effective for people with high risk of developing HPV-related cancers. Melief and his colleagues showed that a vaccine composed of a synthetic long peptide pool derived from HPV-16 E6/E7 oncoproteins successfully induced HPV-specific immune responses and caused measurable regression of HPV-infected precancerous genital lesions in a majority (79%) of patients.[13] In addition to these antigens derived from oncogenic infectious agents that are recognised as foreign by the host immune system, vaccination with 'self'-antigen peptides also has shown substantial progress. In

Table 1 – Randomised clinical trials of cancer vaccines with negative results.

Product	Immunogen	Target cancer	Disease status	Company (organisation)
Melacine	Allogeneic cell lysate	Melanoma	Adjuvant	Corixa
Canvaxin	Allogeneic cells	Melanoma	Metastasis, adjuvant	CancerVax
PANVAC-VF	CEA, MUC-1	Pancreas cancer	Metastasis	Therion Biologics
Oncophage	Vitespen, heat-shock protein	Renal cell cancer	Metastasis, adjuvant	Antigenics
Oncophage	Vitespen, heat-shock protein	Melanoma	Metastasis	Antigenics
GM2-KLH21	GM2-KLH21	Melanoma	Adjuvant	EORTC[a]
TroVax	MVA-5T4	Renal cell cancer	Adjuvant	Oxford BioMedica
MyVax	Id-KLH + GM-CSF	Non-Hodgkin's lymphoma	Adjuvant	Genitope
FavId	Id-KLH + GM-CSF	Non-Hodgkin's lymphoma	Adjuvant	Favrille
GVAX	GM-CSF producing cells	Prostate cancer	Metastasis, refractory	Cell Genesys
Theratope	sTn-KLH	Beast cancer	Metastasis	Oncothyreon

[a] EORTC: European Organisation for Research and Treatment of Cancer.

particular, improved strategies for vaccination, such as pre-screening of antigens for 'personalisation', combination of multiple peptides and combined therapy with other treatment modalities have increased clinical benefits.[13–17]

Other vaccine strategies, including recombinant DNA and proteins, have also shown some promising results in clinical trials. For example, PROSTVAC-VF immunotherapy that comprises two recombinant viral (vaccinia- and fowlpox-based) vectors encoding transgenes for prostate-specific antigen (PSA) and three immune costimulatory molecules (B7.1, ICAM-1, and LFA-3) has recently demonstrated a 44% reduction in the death rate and an 8.5-month improvement in median overall survival in men with minimally symptomatic castration-resistant metastatic prostate cancer in a randomised, controlled and blinded phase II study.[18] Much attention has also been paid to the randomised clinical trial of vaccination with recombinant MAGE-A3 protein, which is now being evaluated by a big pharmaceutical company, GlaxoSmithKline Biologicals, as an adjuvant treatment for non-small cell lung cancer in a large-scale, international phase III trial.[7]

3. Critical issues for cancer vaccine development

3.1. *Selection of ideal tumour-associated antigens and broad availability of immune modulators to augment vaccine efficacy*

Over the past 20 years, a large number of tumour-associated antigens have been identified by several different approaches, including cDNA expression cloning, SEREX and reverse immunology. Although the number of potential cancer antigens is becoming almost limitless, individual antigens have not been fully characterised. Recently, the NCI pilot project has developed a prioritised list of cancer vaccine antigens, which will help select the most promising cancer antigens for further development and testing in clinical trials.[3] However, in view of the heterogeneity of tumours and complexity and diversity of immune responses, it may be a somewhat difficult task to select 'universal' antigens through the prioritisation of vaccine candidates. Instead, the selection of 'personalised' vaccine antigens ideal for individual patients through pre-screening of vaccine candidates would be a more rational approach.[15,16] Indeed, our recently conducted randomised trials of personalised peptide vaccinations in consideration of the pre-existing host immunity in each patient resulted in clear benefit for the patients.[17]

The immune system possesses exceedingly strict biological restrictions, which limit the efficacy of cancer vaccines. To overcome these restrictions, many new biological agents that potentially augment immune cell responses have been discovered.[19] However, the major problem is a lack of broad availability of the agents with already proven immunological functions. For example, few adjuvants have been made commonly available for broad testing in therapeutic cancer vaccines. GM-CSF, which was approved by the FDA as a haematopoietic growth factor, has been frequently used in academic cancer vaccine trials, in part because it is readily available. However, GM-CSF has not always shown promising results as an adjuvant, possibly because of its 'double-edged sword' effects, in which this factor augments or impairs immune responses depending on situations.[20] To enhance the clinical benefits of cancer vaccines, broad testing and approval of new immune modulators will be crucial.

3.2. *Standardisation of immune monitoring assays to identify reliable biomarkers*

Immune monitoring assays that accurately portray the characteristics of anti-tumour immune responses would increase our understanding of tumour immunity and guide further vaccine development and the next generation of immunotherapeutic strategies. In cancer vaccine trials, it will be crucial to characterise the specificity and breadth of T and B cell responses that occur following vaccination, which may be correlated with *in vivo* anti-tumour activity. Due to the complexity and diversity of immune recognition and responses, various T cell assays have been extensively studied and widely used. These include ELISPOT, ELISA, intracellular cytokine staining, cytotoxicity and proliferation assays and peptide-MHC multimer staining. Increasing numbers of studies have reported significant correlations between clinical benefits and immunological responses, but the problem is that no universal standards have been established in the currently available assays.[21] The high degree in variability of results makes the comparison between different facilities difficult and complicated. Optimisation and standardisation of assay protocols, including analysis, interpretation and reporting of data, may thus be crucial to facilitate cancer vaccine development. Currently, these issues are being addressed through large-scale collective initiatives with the goal of achieving assay standardisation, validation and harmonisation.[22] In addition, a reporting framework for T cell monitoring assays is being developed by a project, minimal information about T cell assays (MIATA), which recommends the minimum specific information required for a more objective assessment of both the quality of the laboratory infrastructure and the T cell assays themselves.[23]

It should be noted, however, that the assays for detecting and monitoring antigen-specific T cells have their inherent limitations. Because the frequencies of antigen-specific T cells are usually quite low even after vaccination,[24] we should not expect a dramatic improvement in the performance characteristics of T cell assays. Although antigen-specific T cells can be expanded by *in vitro* culture with the specific antigens, *in vitro* expanded cells do not necessarily give a better picture of the T cell activity present *in vivo*. In contrast, B cell assays would have more potential for immune monitoring. Indeed, we have recently published several papers describing clear correlations between clinical outcomes and B cell responses measured by IgG antibody titers specific to vaccine antigens with a tiny amount of patient plasma after peptide vaccination.[15–17,25,26] The multiplex bead-based LUMINEX technology that we specially developed for monitoring B cell responses allows high throughput screening of IgG responses specific to large numbers of peptide antigens with high accuracy.[25] Compared to the 'classical' T cell assays, which have been continuously modified by introducing additional innovative parameters or techniques, B cell assays are simple, quick to optimise and highly reproducible. In support of our observa-

tions, vaccination with tumour antigen recombinant proteins has also been reported to elicit increased levels of antibodies specific to vaccinated antigens, which may efficiently prime naïve antigen-specific T cells through cross-priming.[27] Although T cell assays have been more widely used than B cell assays for immune monitoring in vaccinated patients, it will be important to address whether monitoring of antigen-specific B cell responses as biomarkers would yield beneficial information for predicting clinical outcomes in future randomised vaccine trials.

3.3. *Increased focus on 'inconvenient immune responses' as a vaccine-related adverse event*

Cancer vaccines have been generally believed to have fewer adverse events than other cancer therapies, but the induction of 'inconvenient immune responses' might be one of the most serious adverse events specifically associated with this treatment. This adverse event is usually difficult to detect or diagnose by the standard toxicity grading systems, such as CTCAE v3.0, but may occur more frequently than expected. Based on the current paradigm that the size and composition of the adaptive immune system is invariable and individual cells are constantly competing for limited space, inadequate vaccine antigens that introduce over-abundant quantities of non-specific memory cells may have catastrophic consequences for the host by displacing pre-existing beneficial memory cells.

Vaccine-induced inconvenient immune responses that are either non-specific to tumour cells or ineffective for tumour cell killing may cause suppression of pre-existing immunity, which results in acceleration of cancer progression or early death in vaccinated patients. Indeed, our previous clinical trials with non-personalised peptide vaccine regimens demonstrated a shorter survival in some advanced cancer patients, possibly due to the suppression of pre-existing host immunity.[28] After this painful experience, we have been conducting clinical trials of 'personalised' peptide vaccinations, in which up to four promising peptides are selected by excluding inadequate ones, based on screening of cellular and humoral responses to a set of vaccine candidates.[5,15–17,26] For example, our recent randomised trials of personalised peptide vaccines that were selected in consideration of the pre-existing host immunities in individual patients demonstrated clear benefit to the patients.[17] In contrast, the failure of some recent clinical trials may be explained, at least in part, by the induction of inconvenient immune responses. In particular, this may be the case with whole tumour vaccine strategies, such as GVAX immunotherapy. Especially if allogeneic tumour cells are used as an antigen source, it is highly possible that T cells against irrelevant antigens that are expressed only on the allogeneic tumour cells, but not on the host tumour cells, might dominate the adequate responses to real antigens expressed on the host tumour cells. Similarly, allogeneic T cell responses, or responses to minor histocompatibility antigens, might also dominate.[29]

3.4. *More flexible guidelines focused on cancer vaccine development*

Therapeutic cancer vaccines represent more complicated biological and/or clinical characteristics than traditional cytotoxic agents. However, the current guidelines used for investigational studies of cancer vaccines are generally based on criteria originally developed for cytotoxic agents.[2,9,30] Nevertheless, cancer vaccines are generally much safer than anticancer drugs, and no linear associations are observed between doses and clinical effects, possibly due to complicated, multi-step processes of vaccine-induced immune responses. Unlike cytotoxic drugs, the maximum tolerated dose (MTD) or pharmacokinetics is difficult to be determined in cancer vaccine trials. Early-phase trials should thus focus on the establishment of an active dose providing the proof-of principle to permit the rational design of late-phase randomised trials.[9,30]

In addition, cancer vaccines often need more time to elicit beneficial immune responses that demonstrate biological activities. Due to the delayed vaccine effects, patients often experience disease progression prior to the onset of biological activities or effects of vaccines. Therefore, conventional short-term tumour responses evaluated by shrinkage of established tumour masses are not always appropriate endpoints in cancer vaccine clinical trials.[2,9,30] Considering these unique and complicated characteristics of cancer vaccines, more flexible and focused guidelines that are adjusted for their biological features should be developed to facilitate clinical development.

4. Tips for overcoming the hurdles of randomised clinical trials

4.1. *Specific criteria for selection of patients and vaccine antigens*

The field of cancer vaccines does not have its own specific criteria for patient selection. In clinical trials of cancer vaccines, therefore, patients are usually selected based on the criteria originally developed for cytotoxic drugs, which pay no particular attention to the immunological status of patients.[2,9,30] In view of the diversity and complexity of immune responses, it will be essential to determine the immune responses to vaccine antigens before starting vaccination. Patients without immunological memory against vaccine antigens would take more time for development of anti-tumour immune responses. Furthermore, vaccines may inhibit beneficial anti-tumour immunity through induction and/or accumulation of antigen-specific immunosuppressive cells such as $CD4^+CD25^+$ regulatory T cells. Antigens that cannot induce appropriate immune responses would thus provide no clinical benefits, especially in advanced patients, who show a relatively quick disease progression. Therefore, development of novel criteria to select appropriate patients or adequate 'personalised' vaccine antigens based on pre-existing immune responses to a set of vaccine antigens would be beneficial. Our early-phase clinical trials strongly suggest the importance of personalisation of cancer vaccines.[5,15–17,26] For example, we previously reported that personalised peptide vaccination quickly induced infiltration of $CD45RO^+$ memory T cells, rather than naïve T cells or B cells, into cancer tissues.[31]

Recently, it has been well recognised that the detection of pre-existing immune cells infiltrating tumours, such as memory $CD8^+$ T cells and/or $CD4^+CD25^+$ regulatory T cells, is well

correlated with prognosis in cancer patients.[32,33] In addition, tumour microenvironment that is regulated by the interaction between tumour cells, stromal cells and host immune system has been reported to play important roles in tumour development and progression.[34] Indeed, it has been suggested that the phenotype and/or activity of immune cells infiltrating tumours may correlate with clinical responses after cancer vaccination in some clinical trials.[35] Because tumour tissues may contain immune cells specific for tumour antigens, they would yield more beneficial information for selecting adequate patients and/or vaccine antigens, although they are usually difficult to obtain for serial comparison before and during vaccinations.

4.2. *Detection of inconvenient immune responses*

Because both the immune cell repertoires of the host and immunological characteristics of tumours are diverse and heterogeneous, non-personalised antigens do not always elicit beneficial immune responses specific to tumours. Rather, if inadequate antigens are vaccinated without considering these unique characteristics of tumour immunity, they may induce harmful immune responses that prevent a pre-existing functional immunity in patients. Therefore, the development of novel biomarkers and criteria to predict and/or detect inconvenient immune responses will be important.

In view of the limited sensitivity and reproducibility of T cell assays, B cell assays that allow high throughput measurement of IgG responses specific to large numbers of antigens may be more reliable and promising. Since we have already confirmed the clinical significance of antigen-specific IgG antibody titers in selecting adequate peptides for personalised vaccination and monitoring vaccine-induced immune responses,[15–17,25,26] this technique may also be useful for predicting and monitoring inconvenient immune responses to a panel of antigens. Indeed, our previous clinical trials with non-personalised peptide vaccine regimens demonstrated a decrease of IgG levels reactive to non-vaccinated peptides in several advanced cancer patients who showed a shorter survival.[28]

5. Limitation of cancer vaccines

Cancer cells escape from a variety of mechanisms that control their malignant behaviour. For example, cancer cells escape from the host immunological surveillance. Immunological pressure from the host often induces tumour cell variants that down-regulate antigen-presenting machineries and/or tumour antigens or express immune suppressive molecules. Therefore, even if a cancer vaccine alone is effective at the beginning of treatment, it often turns out to be ineffective after escaped cancer cells become dominant. In addition, cancer cells can also easily escape from other treatments, such as chemotherapy and hormone therapy. Of note, these escape mechanisms exist independently of each other and rarely exist at the same time in individual cancer cells.[36] Recently concomitant treatments, such as chemotherapy, radiotherapy and monoclonal antibodies, have been reported to enhance the therapeutic effects of cancer vaccines through multiple coordinated immune mechanisms, including activation of antigen-presenting cells or cytotoxic T cells and removal of suppressor cells.[37] While cancer vaccination itself is a promising novel approach, its combination with additional therapies could produce much more synergistic effects.[2,9,16,17,26]

6. Conclusions

To facilitate cancer vaccine development, it will be critical to recognise the reasons behind the failure of most of the recent randomised clinical trials and to fully address the raised issues in future trials. In particular, the vaccine-specific adverse event known as the inconvenient immune response may be at least one of the causes for the failure of randomised trials. The development of novel criteria and biomarkers to select appropriate patients and vaccine antigens would thus be a breakthrough in cancer vaccine development.

Conflict of interest statement

The authors indicated no potential conflict of interest except for the following: Akira Yamada and Kyogo Itoh received a research grant from the Green Peptide Co. Ltd.; Akira Yamada and Kyogo Itoh own stock in the Green Peptide Co.; Akira Yamada is a part-time executive of the Green Peptide Co.

Acknowledgements

This study was supported in part by Grants-in-Aid (KAKENHI) (no. 12213134 to KI), 'TOSHI aria jigyo to Kurume City' and 'High-Tech Research Center' Project for Private Universities: matching fund subsidy from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

REFERENCES

1. van der Bruggen P, Traversari C, Chomez P, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 1991;**254**(5038):1643–7.
2. Schlom J, Gulley JL, Arlen PM. Paradigm shifts in cancer vaccine therapy. *Exp Biol Med* 2008;**233**:522–34.
3. Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res* 2009;**15**:5323–37.
4. Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. *Nat Rev Drug Discov* 2007;**6**:404–14.
5. Itoh K, Yamada A, Mine T, Noguchi M. Recent advances in cancer vaccines: an overview. *Jpn J Clin Oncol* 2009;**39**:73–80.
6. Finke LH, Wentworth K, Blumenstein B, et al. Lessons from randomized phase III studies with active cancer immunotherapies – outcomes from the 2006 meeting of the cancer vaccine consortium (CVC). *Vaccine* 2007;**25**:B97–B109.
7. Goldman B, DeFrancesco L. The cancer vaccine roller coaster. *Nat Biotechnol* 2009;**27**:129–39.
8. Eggermont AMMM. Therapeutic vaccines in solid tumours: can they be harmful? *Eur J Cancer* 2009;**45**:2087–90.

9. US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for Industry; Clinical Considerations for Therapeutic Cancer Vaccines, draft guidance; 2009 [September].
10. Dunne EF, Datta SD, Markowitz LE. A review of prophylactic human papillomavirus vaccines: recommendations and monitoring in the US. *Cancer* 2008;**113**:2995–3003.
11. Lassi K, Dawson NA. Update on castrate-resistant prostate cancer: 2010. *Curr Opin Oncol*, in press.
12. Bendandi M. Idiotype vaccines for lymphoma: proof-of-principles and clinical trial failures. *Nat Rev Cancer* 2009;**9**:675–81.
13. Kenter GG, Welters MJP, Valentijn A, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N Engl J Med* 2009;**361**:1838–47.
14. Barve M, Bender J, Senzer N, et al. Induction of immune responses and clinical efficacy in a phase II trial of IDM-2101, a 10-epitope cytotoxic T-lymphocyte vaccine, in metastatic non-small-cell lung cancer. *J Clin Oncol* 2008;**26**:4418–25.
15. Yajima N, Yamanaka R, Mine T, et al. Immunologic evaluation of personalized peptide vaccination for patients with advanced malignant glioma. *Clin Cancer Res* 2005;**11**:5900–11.
16. Noguchi M, Mine T, Yamada A, et al. Combination therapy of personalized peptide vaccination and low-dose estramustine phosphate for metastatic hormone refractory prostate cancer patients: an analysis of prognostic factors in the treatment. *Oncol Res* 2007;**16**:341–9.
17. Noguchi M, Kakuma T, Uemura T, et al. A randomized phase II trial of personalized peptide vaccine plus low dose estramustine phosphate (EMP) versus standard dose EMP in patients with castration resistant prostate cancer. *Cancer Immunol Immunother*, in press.
18. Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J Clin Oncol* 2010;**28**:1099–105.
19. Cheever MA. Twelve immunotherapy drugs that could cure cancers. *Immunol Rev* 2008;**222**:357–68.
20. Eggermont AM. Immunostimulation versus immunosuppression after multiple vaccinations: the woes of therapeutic vaccine development. *Clin Cancer Res* 2009;**15**:6745–7.
21. Whiteside TL. Immune monitoring of clinical trials with biotherapies. *Adv Clin Chem* 2008;**45**:75–97.
22. Britten CM, Gouttefangeas C, Welters MJP, et al. The CIMT-monitoring panel: a two-step approach to harmonize the enumeration of antigen-specific CD8(+) T lymphocytes by structural and functional assays. *Cancer Immunol Immunother* 2008;**57**:289–302.
23. Janetzki S, Britten CM, Kalos M, et al. "MIATA" – minimal information about T cell assays. *Immunity* 2009;**31**:527–8.
24. Hida N, Maeda Y, Katagiri K, et al. A simple culture protocol to detect peptide-specific cytotoxic T lymphocyte precursors in the circulation. *Cancer Immunol Immunother* 2002;**51**:219–28.
25. Komatsu N, Shichijo S, Nakagawa M, Itoh K. New multiplexed flow cytometric assay to measure anti-peptide antibody: a novel tool for monitoring immune responses to peptides used for immunization. *Scand J Clin Lab Invest* 2004;**64**:535–45.
26. Hattori T, Mine T, Komatsu N, et al. Immunological evaluation of personalized peptide vaccination in combination with UFT and UZEL for metastatic colorectal carcinoma patients. *Cancer Immunol Immunother* 2009;**58**:1845–54.
27. Valmori D, Souleimanian NE, Tosello V, et al. Vaccination with NY-ESO-1 protein and CpG in Montanide induces integrated antibody/Th1 responses and CD8 T cells through cross-priming. *Proc Natl Acad Sci USA* 2007;**104**:8947–52.
28. Mochizuki K, Sato Y, Tsuda N, et al. Immunological evaluation of vaccination with pre-designated peptides frequently selected as vaccine candidates in an individualized peptide vaccination regimen. *Int J Oncol* 2004;**25**:121–31.
29. Chen W, McCluskey J. Immunodominance and immunodomination: critical factors in developing effective CD8(+) T-cell-based cancer vaccines. *Adv Cancer Res* 2006;**95**:203–47.
30. Hoos A, Parmiani G, Hege K, et al. A clinical development paradigm for cancer vaccines and related biologics. *J Immunother* 2007;**30**:1–15.
31. Noguchi M, Yao A, Harada M, et al. Immunological evaluation of neoadjuvant peptide vaccination before radical prostatectomy for patients with localized prostate cancer. *Prostate* 2007;**67**:933–42.
32. Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004;**10**:942–9.
33. Pages F, Berger A, Camus M, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. *N Engl J Med* 2005;**353**:2654–66.
34. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* 2008;**27**:5904–12.
35. Hodi FS, Butler M, Oble DA, et al. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. *Proc Natl Acad Sci USA* 2008;**105**:3005–10.
36. Homma S, Komohara Y, Harada M, et al. Differential levels of human leukocyte antigen-class I, multidrug-resistance 1 and androgen receptor expressions in untreated prostate cancer cells: the robustness of prostate cancer. *Oncol Rep* 2007;**18**:343–6.
37. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. *Nat Rev Immunol* 2008;**8**:59–73.