Review

Overcoming the hurdles of randomised clinical trials of therapeutic cancer vaccines

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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-CSF) 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.

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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
clinical trials in which cancer vaccines sometimes had a detrimental effect on patients, resulting in poorer outcomes.\textsuperscript{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,\textsuperscript{9} it has not fully addressed the issues raised by the failure of recent clinical trials, leading us to reconsider 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\textsuperscript{10} (Merck & Co.) and Cervarix\textsuperscript{11} (GlaxoSmithKline Biologicals), which contain L1 virus-like particles of high-risk types of HPV.\textsuperscript{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)].\textsuperscript{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.\textsuperscript{11} In addition, two randomised clinical trials with GVAX immunotherapy for asymptomatic metastatic prostate cancer patients.\textsuperscript{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\textsuperscript{12}, 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.\textsuperscript{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.\textsuperscript{7}

Recent early-phase clinical trials have also demonstrated significant advances in therapeutic peptide vaccines.\textsuperscript{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. Relief 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.\textsuperscript{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

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<tr>
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\textsuperscript{4} 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.5 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-
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.26 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

3.3. 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.

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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 antitumour 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. 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. 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. 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, 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.

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. 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. While cancer vaccination itself is a promising novel approach, its combination with additional therapies could produce much more synergistic effects.

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.

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