OncoImmunology

ISSN: (Print) 2162-402X (Online) Journal homepage: informahealthcare.com/journals/koni20

Immunological monitoring of anticancer vaccines in clinical trials

Chizuru Ogi & Atsushi Aruga

To cite this article: Chizuru Ogi & Atsushi Aruga (2013) Immunological monitoring of anticancer vaccines in clinical trials, OncoImmunology, 2:8, e26012, DOI: 10.4161/onci.26012

To link to this article: https://doi.org/10.4161/onci.26012

OncoImmunology 2:8, e26012; August 2013; © 2013 Landes Bioscience

Immunological monitoring of anticancer vaccines in clinical trials

Chizuru Ogi and Atsushi Aruga*

Cooperative Major in Advanced Biomedical Sciences; Joint Graduate School of Tokyo Women's Medical University and Waseda University; Tokyo, Japan

Keywords: cancer, clinical trial, immunological analysis, immunotherapy, regulatory science

Therapeutic anticancer vaccines operate by eliciting or enhancing an immune response that specifically targets tumor-associated antigens. Although intense efforts have been made for developing clinically useful anticancer vaccines, only a few Phase III clinical trials testing this immunotherapeutic strategy have achieved their primary endpoint. Here, we report the results of a retrospective research aimed at clarifying the design of previously completed Phase II/III clinical trials testing therapeutic anticancer vaccines and at assessing the value of immunological monitoring in this setting. We identified 17 anticancer vaccines that have been investigated in the context of a completed Phase II/III clinical trial. The immune response of patients receiving anticancer vaccination was assessed for only 8 of these products (in 15 distinct studies) in the attempt to identify a correlation with clinical outcome. Of these studies, 13 were supported by a statistical correlation study (Log-rank test), and no less than 12 identified a positive correlation between vaccine-elicited immune responses and disease outcome. Six trials also performed a Cox proportional hazards analysis, invariably demonstrating that vaccine-elicited immune responses have a positive prognostic value. However, despite these positive results in the course of early clinical development, most therapeutic vaccines tested so far failed to provide any clinical benefit to cancer patients in Phase II/III studies. Our research indicates that evaluating the immunological profile of patients at enrollment might constitute a key approach often neglected in these studies. Such an immunological monitoring should be based not only on peripheral blood samples but also on bioptic specimens, whenever possible. The evaluation of the immunological profile of cancer patients enrolled in early clinical trials will allow for the identification of individuals who have the highest chances to benefit from anticancer vaccination, thus favoring the rational design of Phase II and Phase III studies. This approach will undoubtedly accelerate the clinical development of therapeutic anticancer vaccines.

*Correspondence to: Atsushi Aruga; Email: aruga@abmes.twmu.ac.jp
Submitted: 07/12/2013; Accepted: 08/02/2013
Citation: Ogi C, Aruga A. Immunological monitoring of anticancer vaccines in clinical trials. OncoImmunology 2013; 2:e26012; http://dx.doi.org/10.4161/onci.26012

Introduction

The tumor microenvironment is structured by a cellular compartment (including fibroblasts, immune cells and endothelial cells), biologically active agents such as cytokines (including various interleukins [ILs] and transforming growth factor β [TGFβ]), and numerous components of the extracellular matrix (compassing collagen and fibronectin). Such constituents of the tumor microenvironment interact with cancer cells and are intimately involved in oncogenesis and tumor progression. The physical interactions between malignant cells and tumor-infiltrating lymphocytes (TILs) are critical for the elicitation of anticancer immune responses, be them cellular, such as those triggered by therapeutic anticancer vaccines, or humoral.

Lymphocytes

T lymphocytes can be classified in multiple subsets based on their phenotype. Among various activities, $CD8^+$ cytotoxic T cells stimulate the immune system to produce cytokines such as tumor necrosis factor α (TNF α) and promote the expression of the death receptor CD95 (also known as FAS) on the surface of cancer cells, hence favoring their apoptotic demise. $CD4^+$ helper T cells often undergo one of two distinct functional programs that are generally referred to as T_H1 and T_H2 polarization. Thus, while T_H1 cells robustly stimulate cellular immune responses, their T_H2 counterparts promote humoral immunity. In particular, T_H2 cells play a major role in the differentiation of B lymphocytes, hence promoting the development of antibody-producing plasma cells. Of note, both cytotoxic T cells and helper T cells express a monospecific T-cell receptor (TCR) on their surface as well as the co-receptorial complex CD3. A particular subset of T lymphocytes is represented by regulatory T cells, which express CD4, CD25 and forkhead box P3 (FOXP3). These cells produce high levels of immunosuppressive cytokines (including TGFβ and IL-10), hence potently inhibiting the activity of conventional $CD8^+$ and $CD4^+$ T lymphocytes.

The infiltration of neoplastic lesions by specific subsets of lymphocytes has been attributed a clinical prognostic value in multiple independent studies. For example, by means of a specific meta-analysis, Gooden et al. not only showed that increased amounts of $CD3^+$ or $CD8^+$ T cells within neoplastic lesions are associated with a positive effect on patient survival (with an hazard ratio [HR] of 0,58 and 0,71, respectively), but also suggested that the ratio between specific TIL subsets may be even more

informative than their absolute intratumoral level.[1] Along similar lines, Fridman and colleagues demonstrated that high densities of intratumoral $CD3^+$, $CD8^+$ and $CD45RO^+$ T cells are associated with increased patient survival.[2] Further extending this concept, Galon et al. showed that an immunological score taking into account the density of $CD8^+$ and $CD45RO^+$ cells in the center as well as at the invasive margins of primary tumors has robust prognostic and predictive value.[3]

Circulating lymphocytes may also be indicative of ongoing anticancer immune responses and hence provide information on disease outcome. Some reports suggested indeed that – upon therapy – the activation status of circulating lymphocytes would be higher in patients with pre-existing antitumor immunity than in patients without. In particular, Reynolds et al. reported that the administration of an anticancer vaccine was much more likely to increase the circulating levels of $CD8^+$ T cells specific for a tumor-associated antigen (TAA)—namely, melanoma antigen, family A, 3 (MAGEA3)—in melanoma patients exhibiting pre-vaccination immune responses ($p = 0.0007$).[4] Along similar lines, Speiser et al. reported that $CD8^+$ T-cell responses to melanoma-targeting peptide vaccines occurred primarily in patients with T cells that were pre-activated by endogenous TAAs. In this setting, patients who eventually responded to immunotherapy had a significantly higher percentage of immune cells activated prior to vaccination than patients who failed to respond ($p < 0.01$).[5]

Malignant Cells

An elevated tumor burden is generally associated with poor clinical outcomes in response to therapeutic anticancer vaccines. Indeed, advanced tumors are often robustly infiltrated by regulatory T cells and myeloid-derived suppressor cells (MDSCs), which exert intense immunosuppressive effects. Thus, Kobayashi et al. reported that prevalence of FOXP3+ regulatory T cells increased in a stepwise manner during the progression of hepatocarcinogenesis.[6] Along similar lines, Diaz-Montero and colleagues showed that the amounts of circulating MDSCs correlate with the stage of solid tumors as determined by the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) TNM classification.[7]

We have previously performed a retrospective survey showing that therapeutic vaccines failed to provide actual clinical benefits to cancer patients in 74% of completed or terminated Phase III clinical trials testing this immunotherapeutic intervention.[8] In addition, 69% of such failed studies did not meet their primary endpoint, even in spite of considerable efforts to reduce tumor burden by surgery or neo-adjuvant chemotherapy before vaccination. Thus, we believe that not only tumor burden but also immunological parameters should be taken into careful consideration to determine which patients might truly benefit from the administration of therapeutic anticancer vaccines.

Here, we conducted a retrospective study to clarify the design of previously completed Phase II/III clinical trials testing the efficacy of therapeutic vaccination in cancer patients, and to assess the value of immunological monitoring in the clinical development of these immunotherapeutic agents. Our analysis might provide useful hints for the development of successful anticancer vaccines.

Inclusion Criteria for Completed Phase II/III Trials

When we performed our survey, 23 Phase II/III clinical trials testing 17 distinct therapeutic anticancer vaccines appeared as completed. Eighteen of these studies had failed to achieve their primary objectives, while 4 had succeeded. The remaining trial was a confirmatory study for immunopharmacological analysis of patients affected by Stage III melanoma. Of the 18 failed trials, 11 (77%) had employed tumor stage to select or stratify patients prior to initiation of the study. Of the 4 successful trials, 2 (50%) had defined tumor stage as part of the criteria of patient inclusion in the study. In total, 14 of 23 (61%) completed Phase II/III trials had used tumor stage to select patients at enrollment (**Table 1**). Conversely, none of the 25 completed Phase II/III trials investigating the efficacy of therapeutic vaccination in cancer patients had included any immunological parameter among inclusion criteria.

Timing of Anticancer Immune Responses and Disease Outcome

Among the 23 Phase II/III clinical trials mentioned above, 15 studies (corresponding to 8 distinct anticancer vaccines) investigated the correlation between immune responses to vaccination and clinical outcome. Of note, such a correlation was most often evaluated in Phase II trials (7 studies). Only 4 studies assessed the correlation between vaccine-elicited immune responses and disease outcome in a Phase III setting. Finally, 4 reports did not explicitly mention the phase of clinical development at which this relationship was evaluated, but contained reliable indications in this respect (**Table 2**).

Methods to Evaluate Immune Responses and Disease Outcome

Among the 15 Phase II/III trials investigating the correlation between immune responses and clinical outcome, 14 used either overall survival (OS) or a combination of disease-free survival (DFS) and event-free survival (EFS) as indicators of clinical outcome, while 1 employed objective responses only. Thirteen out of 15 trials (87%) analyzed humoral immune responses by quantified the titer or circulating TAA-specific antibodies by ELISA. Ten trials (67%) analyzed cellular immune responses, which were detected by enzyme-linked immunospot (ELISPOT) assays, T-cell proliferation tests, intracellular cytokine staining coupled to flow cytometry, or delayed-type hypersensitivity (DTH) tests. All these assays were performed to analyze immune responses as induced of boosted by therapeutic anticancer vaccines. Of note, in 1 trial investigating the therapeutic value of Provenge®, both ELISPOT and T-cell proliferation assays were performed to assess cellular immune responses. Along similar lines, in 1 trial testing Canvaxin®, both DTH tests and the immunohistochemical quantification of TILs were employed for immunomonitoring (**Table 2**). Such an immunohistochemical approach for the

Table 1. Completed Phase III trials for therapeutic cancer vaccines and their inclusion criteria by tumor stage

Development status	Product	Cancer	Tumor stage	Completed Phase III	Result	Reference
Approved (US)	Provenge®	Prostate cancer	No	III D9901	F (Efficacy)	9
			No	III D9902A	F (Efficacy)	9
			No	III D9902B, IMPACT	S	10
Approved (Russia)	Oncophage®	Renal cell carcinoma	Stage I, II, III, IV	III C-100–12	F (Efficacy)	11,12
Approved (Switzerland)	M-Vax™	Melanoma	Stage IIIb, IIIc	III	NA	13
Discontinued	Canvaxin®	Melanoma	Stage III	III MMAIT-III	F (Efficacy)	14
			Stage IV	III MMAIT-IV	F (Efficacy)	14
Discontinued	PANVAC™-VF	Pancreatic cancer	Stage IV	III	F (Efficacy)	15
Discontinued	Theratope®	Breast cancer	No	III	F (Efficacy)	16
Discontinued	L-BLP25	Breast cancer	No	III STRIDE	F (Safety)	17
Discontinued	Specifid™	Non-Hodgkin's lymphoma	Grade 1, 2, 3 (WHO)	III	F (Efficacy)	18
Discontinued	MyVax®	Non-Hodgkin's lymphoma	Stage III, IV	III	F (Efficacy)	19
Discontinued	GM2-KLH vaccine	Melanoma	Stage IIb, III, IV	III	F (Efficacy)	20
Discontinued	BEC2	Small cell lung cancer	No	III SILVA	F (Efficacy)	21
Unknown	Insegia™	Pancreatic cancer	No	III (single agent)	S	22
			Stage II, III, IV	III (combination)	F (Efficacy)	22
Unknown	OTS-102	Pancreatic cancer	No	II/III PEGASUS-PC	F (Efficacy)	23
Unknown	Oncophage®	Melanoma	Stage IV	III C-100–21	F (Efficacy)	24
Ongoing	OncoVAX®	Colorectal cancer	Stage II, III	IIIa 8701	S	25
Ongoing	Allovectin-7®	Melanoma	Stage III, IV	III (low-dose)	F (Efficacy)	26
Ongoing	GV1001	Pancreatic cancer	No	III PriomoVax	F (Efficacy)	27
Ongoing	L-BLP25	Non-small cell lung cancer	Stage IIIa, IIIb	III START	F (Efficacy)	28
Ongoing	BiovaxID®	Non-Hodgkin's lymphoma	Grade 1, 2, 3a (WHO) / Stage III, IV	III	S	42-44

quantification of TILs was undertaken only in this study, while the levels of peripheral blood lymphocytes (PBLs) were frequently employed to monitor immune responses.

Canvaxin®

Canvaxin® is a polyvalent whole-cell vaccine against melanoma. Canvaxin® was originally developed for commercialization by CancerVax Corp. (which merged with Micromet Inc. to become Amgen Inc.). However, because of the poor efficacy demonstrated in Phase III trials, the development of Canvaxin® was officially discontinued in 2005. Morton et al. have examined (by univariate and multivariate analyses) the prognostic significance of immunological parameters for melanoma patients receiving Canvaxin®, using a historical database.[31] In this setting, the histopathology of bioptic specimens was analyzed, revealing that patients who had Canvaxin® showed increased levels of TILs. Further phenotypic studies revealed a reduction in tumor-infiltrating $CD8^+$ cells coupled to an increase in the $CD4^+/CD8^+$ T-cell ratio ($p = 0.10$) as well as in the levels of intratumoral $CD25^+$ ($p < 0.05$) and $CD56^+$ ($p < 0.04$) cells. However, this study did not investigate the association between the amounts of pre-existing TILs and clinical outcome. Accordingly, subsequent clinical studies testing Canvaxin® did not stratify or select patients based on their immunological profile.

Theratope®

Theratope® is generated by conjugating sialyl-Tn (STn), a TAA linked to poor prognosis in patients affected by several cancers, with keyhole limpet hemocyanin (KLH). A Phase II trial testing the therapeutic potential of Theratope® has been conducted in patients with histologically proven, recurrent metastatic ovarian, breast or colorectal carcinoma. In this setting, humoral responses were analyzed by the ELISA-assisted quantification of circulating STn-specific antibodies, while pre- and post-vaccination cellular responses were monitored by the cytofluorometric quantification of $CD69^+$ and $CD4^+CD69^+$ PBLs. According to Reddish et al., the Cox proportional hazards analysis demonstrated a significant association between low amounts of $CD69^+$ PBLs before vaccination and increased survival ($p = 0.023$) or delayed disease progression ($p = 0.0016$) upon treatment.[38] Along similar lines, low levels of $CD4^+CD69^+$ PBLs before immunotherapy were associated with increased survival following vaccination ($p = 0.004$).

Table 2. Methods of immune response and clinical outcome evaluation for therapeutic cancer vaccines

	Response		Humoral immune response	Cellular immune response								
	Sample			Peripheral blood lymphocytes				Skin	Tumor lesion	Clinical outcome	Ref.
Product	Cancer	Phase	ELISA	ELISPOT	T cell proliferation assay	Intracellular cytokine staining	Flow cytometry	DTH testing	Pathologic assessment		
Provenge®	Prostate cancer	P I/II	Y	Y	Y					TTP	29
		P III (IMPACT)	Y		Y					OS	10
Canvaxin®	Melanoma (Stage IV)	P II	Y					Y		OS	30
	Melanoma (Stage II)	P II	Y							DFS	30
	Melanoma (Stage IIIa, IV)	After P II	Y					Y	Y	OS	31
Specifid™	Non-Hodgkin's lymphoma	P II	Y			Y				OR	32
		P II (after rituximab)	Y			Y				OR, EFS	33
BEC2	Small cell lung cancer	P III	Y							OS	21
		During P III	Y							OS, RFS	34
Insegia™	Pancreatic cancer	P II	Y							OS	35
		P III (single agent)	Y							OS	22
M-Vax™	Melanoma (Stage III)	Before P III						Y		OS	36
		P III						Y		OS	13
MyVax®	Non-Hodgkin's lymphoma	Before P III	Y		Y					PFS	37
Theratope®	Breast cancer	P II	Y				Y			OS	38
Total trials			13	1	3	2	1	4	1		

Abbreviations: OS, overall survival; TTP, time to progression; DFS, disease free survival; EFS, event free survival; RFS, recurrence free survival; PFS, progression free survival; OR, objective response

Finally, there was a significant association between elevated pre-vaccination levels of mucin 1 (MUC1) in the serum and decreased survival following immunotherapy in breast (p = 0.0153) and ovarian (p = 0.0105) cancer patients. Theratope® has also been tested in a Phase III clinical trial enrolling patients affected by metastatic breast carcinoma. Thus, Miles et al. reported that Theratope® did not improve time to progression (TTP) or OS, although patients receiving the vaccine developed high titers of IgM and IgG antibodies to ovine submaxillary mucin.[39] In this setting, the median TTP of patients treated with Theratope® and KLH only was 3.4 and 3.0 mo, respectively (Cox proportional hazards model, p = 0.353; Log-rank test, p = 0.305), while the median OS in the Theratope® and KLH groups was 23.1 and 22.3 mo, respectively (Cox proportional hazards model, p = 0.916).

Evaluation of Immune Responses and Clinical Outcome

Thirteen trials performed statistical analyses to determine the correlation between immune responses and disease outcome, whereas two trials gathered case reports but did not perform statistical tests. The statistical approaches included the Log-rank

test for univariate analysis (based on the Kaplan-Meier model), and the Cox proportional hazards model for multivariate analysis. They were used in 13 (100%) and 6 (46%) clinical trials, respectively.

Of the 13 clinical trials that conducted Log-rank tests, 12 (92%)—corresponding to 7 distinct anticancer vaccines—revealed a positive correlation between immune responses and disease outcome upon treatment. Nonetheless, only 2 of these vaccines exerted significant effects in terms of primary endpoint in Phase III trials (**Table 3**). All the 6 clinical trials that employed a Cox proportional hazards model—corresponding to 3 anticancer vaccines—concluded that immune responses to vaccination constitute a prognostic factor. However, also these vaccines did not exert significant efficacy in terms of primary endpoint in Phase III studies (**Table 4**).

Evaluation of Pre-Existing Immune Responses and Clinical Outcome

Only in a Phase II clinical trial (testing Theratope® in patients with histologically proven, recurrent metastatic ovarian, breast or colorectal carcinoma), the association between pre-existing

Table 3. Evaluation of immune response and clinical outcome after therapeutic cancer vaccines by log-rank test using the Kaplan-Meier model

Product	Cancer	Phase	Evaluation results	Positive Correlation	Reference
Provenge®	Prostate cancer	P I/II	TTP correlated with development of an immune response to prostatic acid phosphatase (PAP) and with the dose of dendritic cells received.	Y	29
		P III (IMPACT)	An antibody titer of more than 400 against PA2024 or PAP after baseline lived longer than did those who had an antibody titer of 400 or less ($p < 0.001$ and $p = 0.08$, respectively). No survival difference could be detected between patients in the sipuleucel-T group who had T-cell proliferation response to PA2024 or PAP and those who did not.	Y	10
Canvaxin®	Melanoma (Stage IV)	P II	5-y OS rate was 75% for patients who had an elevated level of anti-TA90 IgM and a strong DTH response, 36% for patients who had either an elevated IgM response or a strong DTH response, and only 8% if neither response was strong ($p < 0.001$)	Y	30
	Melanoma (Stage II)	P II	Anti-TA90 IgM levels $\geqq$ 1:800 were significantly correlated with improved 5-y DFS and improved 5-y OS.	Y	30
	Melanoma (Stage IIIa and IV)	After P II	Survival correlated significantly with delayed cutaneous hypersensitiity ($p = 0.0066$) and antibody response ($p = 0.0117$).	Y	31
Specifid™	Non-Hodgkin's lymphoma	P II (after rituximab)	There was no correlation observed between the development of anti-Id immune response and the achievement of an objective response or duration of EFS.	N	33
BEC2	Small cell lung cancer	P III	The survival of responders was better than that of non-responders, although this did not reach statistical significance (median survival, 19.2 v 13.9 mo for responders v non-responders; $p = 0.0851$).	Y	21
Insegia™	Pancreatic cancer	P II	Median survival was 217 d for the antibody responders and 121 d for the antibody non-responders. The difference in survival between the antibody responders and non-responders was significant ($p = 0.0023$).	Y	35
		P III (single agent)	Patients developing anti-G17DT responses (73.8%) survived longer than non-responders or those on placebo (median survival, 176 v 63 v 83 d; log-rank test, $p = 0.003$).	Y	40
M-Vax™	Melanoma (Stage III)	Before P III	The development of a positive DTH response to unmodified autologous melanoma cells was associated with significantly longer 5-y survival (71% v 49%; $p = 0.031$).	Y	36
		P III	OS after relapse was significantly longer in patients who developed positive DTH to unmodified tumor cells (25.2% v 12.3%; $p < 0.001$).	Y	13
MyVax®	Non-Hodgkin's lymphoma	Before P III	Patients who mounted humoral immune responses had a longer PFS than those who did not (8.21 v 3.38 y; $p = 0.018$).	Y	37
Theratope®	Breast cancer	P II	51 patients who generated titers higher than median value for anti-STn+ mucin IgG survived longer than 46 patients who generated lower titers below the median.	Y	38

immune responses and disease outcome was analyzed. In this setting, pre-existing immune responses were indeed found to positively correlated with clinical outcome. Nonetheless, patients were not selected or stratified based on pre-vaccination immunological parameters in the context of the subsequent Phase III clinical trial testing Theratope® in metastatic breast carcinoma patients.

Discussion

Tumor stage is a well-established prognostic factor and is often used to select or stratify patients in clinical trials. The prognostic value of the immunological profile, as defined by a multiparametric immunoscore, has been first investigated in colorectal carcinoma patients by Mlecnik et al.[41] In this context, an elevated immunoscore was shown to positively correlate with DFS, disease-specific survival (DSS) and OS (HRs of 0.64, 0.60, and 0.70, respectively; $p < 0.005$). Moreover, multivariate Cox regression analyses including the AJCC/UICC TNM stage and the immunoscore revealed that only the latter was significantly associated with DFS, DSS, and OS. Thus, it appears that the immunological profile of cancer patients might be an important prognostic factor, even more than tumor stage, at least in some circumstances.

Thus, we are convinced that cancer patients should be selected or stratified for clinical trials based not only on tumor stage, but also on immunological profile. This might allow for the prospective identification of patients with an immunological status that allows them to optimally respond to therapeutic vaccination. Immune responses are often monitored in the context of Phase I clinical trials to identify the optimal dosage and administration route for therapeutic anticancer vaccines. In addition, the efficacy of these immunotherapeutic interventions is generally investigated in the exploratory trials using patients selected or stratified based on tumor stage, followed by the assessment of the correlation between immune responses and disease outcome. We believe that pre-vaccination immunological parameters associated with optimal vaccine-elicited immune responses should be identified in such early phase clinical studies.

In patients, immune responses are nowadays evaluated by quantifying the circulating titers of TAA-specific antibodies (as an indicator of humoral antitumor immunity) or the proliferative and functional profile of T cells (as an indicator of cellular antitumor immunity). In Phase I clinical trials, this is generally assessed both before and after treatment, so to discriminate between the elicitation and the enhancement of immune responses by therapeutic vaccination. As shown by our survey, the correlation between immune responses and disease outcome has been mainly evaluated in the context of Phase II or III studies. Most of these analyses were conducted starting from the characterization of PBLs or DTH (skin) assays. In the future, we would like to evaluate the association between the pre-vaccination immunological profile and vaccine-elicited immune responses in early phase studies, to identify patients who have the highest chances to respond to treatment. For example, circulating $CD4^+$ helper T cells and $CD4^+CD25^+FOXP3^+$ regulatory T cells may be characterized for their ability to secrete immunomodulatory cytokines and hence modulate humoral immune responses which only contribute to the secretion of antibodies by plasma cells. In addition, bioptic specimens may be employed to quantify intratumoral $CD8^+$ cytotoxic T cells and hence obtain insights into local cellular immune responses. In this context, a strong lymphocytic infiltration has been correlated with improved clinical outcomes in patients affected by different tumor types, and high intratumoral densities of $CD3^+$ T lymphocytes, $CD8^+$ cytotoxic T cells, and $CD45RO^+$ memory T cells has been associated with increased patient survival.[2] However, contradictory findings for particular types of cancer have also been reported.[2] Thus, both tumor type and the immunological profile of patients should be carefully considered for the evaluation of clinical trials testing therapeutic anticancer vaccines.

Most of the clinical trials included in our survey demonstrated a positive correlation between immune responses and disease outcome upon therapeutic anticancer vaccination. Nonetheless, the majority of Phase III clinical trials testing the same immunotherapeutic products failed to reveal a significant efficacy. As mentioned above, the pre-vaccination immunological profile of cancer patients is an important factor for predicting clinical outcomes. However, only 1 clinical trial included in our survey evaluated the relationship between pre-existing immunological conditions and disease outcome, and this finding was not used to select or stratify patients in a subsequent Phase III study.

If the pre-treatment immunological profile had been accepted as a prognostic factor, and hence patients had been stratified accordingly in the following Phase III study, different clinical outcomes might have been revealed in distinct patient subsets. Moreover, if only patients with an optimal immunological profile had been included in the study, the study might have revealed a statistically significant effect for vaccination, which was not the case with unselected patients. Therefore, to successfully develop therapeutic anticancer vaccines, clinical outcome should be evaluated at Phase II or III among a patient subset properly selected for immunological profile in previous exploratory studies.

Identification of Completed Phase II/III Clinical Trials

Only completed Phase II/III trials testing therapeutic anticancer vaccines were included in this research. To identify these studies, all clinical trials registered on ClinicalTrials.gov as of June 25th, 2012 were screened based on the following terms: condition = "cancer," treatment = "vaccine therapy," and study type = "interventional." Completed Phase II/III clinical trials were selected from search results and manually reviewed. Additional Phase II/III studies were identified by screening the relevant scientific literature on PubMed as well as by checking the homepage of multiple companies currently developing therapeutic anticancer vaccines. The design of the studies, their results and additional information were obtained from the publicly available literature, and indications of products tested in completed Phase II/III trials were clarified.

Table 4. Evaluation of immune response and clinical outcome after therapeutic cancer vaccines by Cox proportional hazards model

Product	Cancer	Phase	Evaluation results	Positive association	Reference
Canvaxin®	Melanoma (Stage IV)	P II	Elevated anti-TA90 IgM and strong DTH to vaccine correlated with improved survival (p = 0.03 and 0.008, respectively).	Y	30
	Melanoma (Stage II)	P II	Anti-TA90 IgM was identified as an independent prognostic factor for OS and DFS.	Y	30
	Melanoma (Stage IIIa, IV)	After P II	It was revealed prognostic significance for site of metastases (p = 0.0001) and immunotherapy (p = 0.0001).	Y	31
M-Vax™	Melanoma (Stage III)	Before P III	The failure to develop DTH to unmodified autologous melanoma cells was associated with OS (HR = 2.54, p = 0.080). After adjustment for age only, the hazards ratios for RFS and OS increased and were statistically significant (p = 0.029 and 0.036, respectively).	Y	36
		P III	A positive DTH response to unmodified tumor cells remained statistically significant for both RFS and OS (p = 0.015 and 0.009, respectively).	Y	13
MyVax®	Non-Hodgkin's lymphoma	Before P III	Valine/valine genotype and humoral immune response were independent positive predictors for PFS (p = 0.0013 and 0.0015, respectively).	Y	37

Identification of Clinical Trials Evaluating Immunological Parameters, Immune Responses, and Disease Outcome

Methods to evaluate immune response and clinical outcome were surveyed for products identified as above, and categorized by evaluated sample and type of immune response. Their study phase and efficacy endpoint which investigated correlation between immune response and clinical outcome were also surveyed. Information on these trials was obtained by surveying the literature via PubMed or upon re-quotation of the paper published on the completed Phase II/III study.

Acknowledgments

This work was supported by the Global COE program, Multidisciplinary Education and Research Center for Regenerative Medicine (MERCREM), from the Ministry of Education, Culture, Sports Science, and Technology (MEXT), Japan.

Disclosure of Potential Conflicts of Interest

AA has no conflicts of interest to disclose. CO is an employee of Merck Serono Co., Ltd., but being part of the company has not influenced the results and discussion in this paper.

References

1. Gooden MJM, de Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. Br J Cancer 2011; 105:93-103; PMID:21629244; http://dx.doi.org/10.1038/bjc.2011.189
2. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012; 12:298-306; PMID:22419253; http://dx.doi.org/10.1038/nrc3245
3. Galon J, Pagès F, Marincola FM, Thurin M, Trinchieri G, Fox BA, Gajewski TF, Ascierto PA. The immune score as a new possible approach for the classification of cancer. J Transl Med 2012; 10:1; PMID:22214470; http://dx.doi.org/10.1186/1479-5876-10-1
4. Reynolds SR, Zeleniuch-Jacquotte A, Shapiro RL, Roses DF, Harris MN, Johnston D, Bystryn JC. Vaccine-induced CD8+ T-cell responses to MAGE-3 correlate with clinical outcome in patients with melanoma. Clin Cancer Res 2003; 9:657-62; PMID:12576432
5. Speiser DE, Rimoldi D, Batard P, Liénard D, Lejeune F, Cerottini JC, Romero P. Disease-driven T cell activation predicts immune responses to vaccination against melanoma. Cancer Immun 2003; 3:12; PMID:12962476
6. Kobayashi N, Hiraoka N, Yamagami W, Ojima H, Kanai Y, Kosuge T, Nakajima A, Hirohashi S. FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis. Clin Cancer Res 2007; 13:902-11; PMID:17289884; http://dx.doi.org/10.1158/1078-0432.CCR-06-2363
7. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother 2009; 58:49-59; PMID:18446337; http://dx.doi.org/10.1007/s00262-008-0523-4
8. Ogi C, Aruga A. Clinical evaluation of therapeutic cancer vaccines. Hum Vaccin Immunother 2013; 9:1049-57; PMID:23454867; http://dx.doi.org/10.4161/hv.23917
9. FDA's homepage Vaccines, Blood & Biologics, Cellular & Gene Therapy Products, Approved Products. Available from http://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/ucm210012.htm
10. Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, Provost N, Frohlich MW. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer 2009; 115:3670-9; PMID:19536890; http://dx.doi.org/10.1002/cncr.24429
11. Randazzo M, Terness P, Opelz G, Kleist C. Active-specific immunotherapy of human cancers with the heat shock protein Gp96-revisited. Int J Cancer 2012; 130:2219-31; PMID:22052568; http://dx.doi.org/10.1002/ijc.27332
12. Wood C, Srivastava P, Bukowski R, Lacombe L, Gorelov AI, Gorelov S, Mulders P, Zielinski H, Hoos A, Teofilovici F, et al.; C-100-12 RCC Study Group. An adjuvant autologous therapeutic vaccine (HSPPC-96; vitespen) versus observation alone for patients at high risk of recurrence after nephrectomy for renal cell carcinoma: a multicentre, open-label, randomised phase III trial. Lancet 2008; 372:145-54; PMID:18602688; http://dx.doi.org/10.1016/S0140-6736(08)60697-2
13. Berd D, Sato T, Maguire HC Jr., Kairys J, Mastrangelo MJ. Immunopharmacologic analysis of an autologous, hapten-modified human melanoma vaccine. J Clin Oncol 2004; 22:403-15; PMID:14691123; http://dx.doi.org/10.1200/JCO.2004.06.043

14. Sondak VK, Sabel MS, Mulé JJ. Allogeneic and autologous melanoma vaccines: where have we been and where are we going? Clin Cancer Res 2006; 12:2337s-41s; PMID:16609055; http://dx.doi.org/10.1158/1078-0432.CCR-05-2555
15. Therion Reports Results of Phase 3 PANVAC-VF Trial and Announces Plans for Company Sale. PR Newswire 28 June available from http://www.prnewswire.com/news-releases/therion-reports-results-of-phase-3-panvac-vf-trial-and-announces-plans-for-company-sale-56997582.html
16. Finke LH, Wentworth K, Blumenstein B, Rudolph NS, Levitsky H, Hoos A. Lessons from randomized phase III studies with active cancer immunotherapies--outcomes from the 2006 meeting of the Cancer Vaccine Consortium (CVC). Vaccine 2007; 25(Suppl 2):B97-109; PMID:17916465; http://dx.doi.org/10.1016/j.vaccine.2007.06.067
17. Oncothyreon Inc. Oncothyreon announces temporary suspension of Stimuvax clinical trials by Merck Serono. Press release; Mar 23, 2010.
18. Freedman A, Neelapu SS, Nichols C, Robertson MJ, Djulbegovic B, Winter JN, Bender JF, Gold DP, Ghalie RG, Stewart ME, et al. Placebo-controlled phase III trial of patient-specific immunotherapy with mitumprotimut-T and granulocyte-macrophage colony-stimulating factor after rituximab in patients with follicular lymphoma. J Clin Oncol 2009; 27:3036-43; PMID:19414675; http://dx.doi.org/10.1200/JCO.2008.19.8903
19. Schuster SJ, Neelapu SS, Gause BL, Janik JE, Muggia FM, Gockerman JP, Winter JN, Flowers CR, Nikcevich DA, Sotomayor EM, et al. Vaccination with patient-specific tumor-derived antigen in first remission improves disease-free survival in follicular lymphoma. J Clin Oncol 2011; 29:2787-94; PMID:21632504; http://dx.doi.org/10.1200/JCO.2010.33.3005
20. Fernandes LE, Gabri MR, Guthmann MD, Gomez RE, Gold S, Fainboim L, et al. NGcGM3 Ganglioside: a privileged target for cancer vaccines. Clinical and Development Immunology 2010; 2010: 814397; dx.doi.org/10.1155/2010/814397
21. Giaccone G, Debruyne C, Felip E, Chapman PB, Grant SC, Millward M, Thiberville L, D'addario G, Coens C, Rome LS, et al. Phase III study of adjuvant vaccination with Bec2/bacille Calmette-Guerin in responding patients with limited-disease small-cell lung cancer (European Organisation for Research and Treatment of Cancer 08971-08971B; Silva Study). J Clin Oncol 2005; 23:6854-64; PMID:16192577; http://dx.doi.org/10.1200/JCO.2005.17.186
22. Gilliam AD, Watson SA. G17DT: an antigastrin immunogen for the treatment of gastrointestinal malignancy. Expert Opin Biol Ther 2007; 7:397-404; PMID:17309331; http://dx.doi.org/10.1517/14712598.7.3.397
23. OncoTherapy Science, Inc. Press release. Feb 29, 2012.
24. Testori A, Richards J, Whitman E, Mann GB, Lutzky J, Camacho L, Parmiani G, Tosti G, Kirkwood JM, Hoos A, et al.; C-100-21 Study Group. Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100-21 Study Group. J Clin Oncol 2008; 26:955-62; PMID:18281670; http://dx.doi.org/10.1200/JCO.2007.11.9941
25. Uyl-de Groot CA, Vermorken JB, Hanna MG Jr., Verboom P, Groot MT, Bonsel GJ, Meijer CJ, Pinedo HM. Immunotherapy with autologous tumor cell-BCG vaccine in patients with colon cancer: a prospective study of medical and economic benefits. Vaccine 2005; 23:2379-87; PMID:15755632; http://dx.doi.org/10.1016/j.vaccine.2005.01.015
26. Bedikian AY, Del Vecchio M. Allovectin-7 therapy in metastatic melanoma. Expert Opin Biol Ther 2008; 8:839-44; PMID:18476795; http://dx.doi.org/10.1517/14712598.8.6.839
27. Pharmexa stops one of two Phase III trials. Drugs.com May 13, 2008 available from http://www.drugs.com/news/pharmexa-stops-one-two-phase-iii-trials-8180.html
28. Butts CA, Socinski MA, Mitchell P, Thatcher N, Havel L, Krzakowski MJ, et al. START: A Phase III study of L-BLP25 cancer immunotherapy for unresectable Stage III non-small cell lung cancer. J Clin Oncol 2013; 31:suppl; abstr 7500
29. Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV, Valone FH. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. J Clin Oncol 2000; 18:3894-903; PMID:11099318
30. Hsueh EC, Morton DL. Antigen-based immunotherapy of melanoma: Canvaxin therapeutic polyvalent cancer vaccine. Semin Cancer Biol 2003; 13:401-7; PMID:15001158; http://dx.doi.org/10.1016/j.semcancer.2003.09.003
31. Morton DL, Foshag LJ, Hoon DSB, Nizze JA, Famatiga E, Wanek LA, Chang C, Davtyan DG, Gupta RK, Elashoff R, et al. Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine. Ann Surg 1992; 216:463-82; PMID:1417196; http://dx.doi.org/10.1097/00000658-199210000-00010
32. Redfern CH, Guthrie TH, Bessudo A, Densmore JJ, Holman PR, Janakiraman N, Leonard JP, Levy RL, Just RG, Smith MR, et al. Phase II trial of idiotype vaccination in previously treated patients with indolent non-Hodgkin's lymphoma resulting in durable clinical responses. J Clin Oncol 2006; 24:3107-12; PMID:16754937; http://dx.doi.org/10.1200/JCO.2005.04.4289
33. Koç ON, Redfern C, Wiernik PH, Rosenfelt F, Winter JN, Carter WD, Gold DP, Stewart ME, Ghalie RG, Bender JF. A phase 2 trial of immunotherapy with mitumprotimut-T (Id-KLH) and GM-CSF following rituximab in follicular B-cell lymphoma. J Immunother 2010; 33:178-84; PMID:20145546; http://dx.doi.org/10.1097/CJI.0b013e3181bfcea1
34. Grant SC, Kris MG, Houghton AN, Chapman PB. Long survival of patients with small cell lung cancer after adjuvant treatment with the anti-idiotypic antibody BEC2 plus Bacillus Calmette-Guérin. Clin Cancer Res 1999; 5:1319-23; PMID:10389914
35. Brett BT, Smith SC, Bouvier CV, Michaeli D, Hochhauser D, Davidson BR, Kurzawinski TR, Watkinson AF, Van Someren N, Pounder RE, et al. Phase II study of anti-gastrin-17 antibodies, raised to G17DT, in advanced pancreatic cancer. J Clin Oncol 2002; 20:4225-31; PMID:12377966; http://dx.doi.org/10.1200/JCO.2002.11.151
36. Berd D, Maguire HC Jr., Schuchter LM, Hamilton R, Hauck WW, Sato T, Mastrangelo MJ. Autologous hapten-modified melanoma vaccine as postsurgical adjuvant treatment after resection of nodal metastases. J Clin Oncol 1997; 15:2359-70; PMID:9196151
37. Weng WK, Czerwinski D, Timmerman J, Hsu FJ, Levy R. Clinical outcome of lymphoma patients after idiotype vaccination is correlated with humoral immune response and immunoglobulin G Fc receptor genotype. J Clin Oncol 2004; 22:4717-24; PMID:15483014; http://dx.doi.org/10.1200/JCO.2004.06.003
38. Reddish MA, MacLean GD, Poppema S, Berg A, Longenecker BM. Pre-immunotherapy serum CA27.29 (MUC-1) mucin level and CD69+ lymphocytes correlate with effects of Theratope sialyl-Tn-KLH cancer vaccine in active specific immunotherapy. Cancer Immunol Immunother 1996; 42:303-9; PMID:8706053; http://dx.doi.org/10.1007/s002620050287
39. Miles D, Roché H, Martin M, Perren TJ, Cameron DA, Glaspy J, Dodwell D, Parker J, Mayordomo J, Tres A, et al.; Theratope® Study Group. Phase III multicenter clinical trial of the sialyl-TN (STn)-keyhole limpet hemocyanin (KLH) vaccine for metastatic breast cancer. Oncologist 2011; 16:1092-100; PMID:21572124; http://dx.doi.org/10.1634/theoncologist.2010-0307
40. Gilliam AD, Broome P, Topuzov EG, Garin AM, Pulay I, Humphreys J, Whitehead A, Takhar A, Rowlands BJ, Beckingham IJ. An international multicenter randomized controlled trial of G17DT in patients with pancreatic cancer. Pancreas 2012; 41:374-9; PMID:22228104; http://dx.doi.org/10.1097/MPA.0b013e31822ade7e
41. Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindea G, Meatchi T, Bruneval P, Trajanoski Z, Fridman WH, Pagès F, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol 2011; 29:610-8; PMID:21245428; http://dx.doi.org/10.1200/JCO.2010.30.5425
42. Schuster SJ, Neelapu SS, Gause BL, Muggia FM, Gockerman JP, Sotomayor EM, et al. Idiotype vaccine therapy (BiovaxID) in follicular lymphoma in first complete remission: Phase III clinical trial results. J Clin Oncol 2009; 27:18s (suppl; abstr2)
43. Lee ST, Jiang YF, Park KU, Woo AF, Neelapu SS. BiovaxID: a personalized therapeutic cancer vaccine for non-Hodgkin's lymphoma. Expert Opin Biol Ther 2007; 7:113-22; PMID:17150023; http://dx.doi.org/10.1517/14712598.7.1.113
44. Shuster SJ, Neelapu SS, Gause BL, Muggia FM, Gockerman JP, Sotomayor EM, et al. Idiotype vaccine therapy (BiovaxID) in follicular lymphoma in first complete remission. ASCO2009 Annual Meeting.