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Cancer Vaccine

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1. Introduction

According to the GLOBOCAN 2008 estimates, about 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008; of these, 56% of the cases and 64% of the deaths occurred in the economically developing world (Jemal et al., 2011).

It is now 51 years since Macfarlane Burnet and Peter Medawar won the Nobel Prize in Physiology or Medicine for the discovery of acquired immunological tolerance, and Burnet's 'hypothesis that called for experiment' has driven an enormous amount of progress. A recent advance in anti-cancer therapies has been the use of cancer antigen to develop vaccines. However, immunization with cancer cell-based vaccines has not resulted in significant long-term therapeutic benefits. The search for human tumor antigens as potential targets for cancer immunotherapy has led to the discovery of several molecules expressed mainly or selectively on cancer cells.

Vaccination is an effective medical procedure of clinical oncology setting based on the induction of a long-lasting immunologic memory characterized by mechanisms endowed with high destructive potential and specificity. In the last few decades, identification of tumor-associated antigens (TAA) has prompted the development of different strategies for antitumor vaccination, aimed at inducing specific recognition of TAA in order to elicit a persistent immune memory that may eliminate residual tumor cells and protect recipients from relapses. Current data from trials with cancer vaccine for patients with advanced cancer are however not uniform. Because enormous problems arise from the variability of protocols in the preparation of vaccine, such as dendritic cell-based or peptide vaccine, and the vaccination itself.

Widely occurring, over-expressed TAAs have been detected in different types of tumors as well as in many normal tissues, and their over-expression in tumor cells can reach the threshold for T cell recognition, breaking the immunological tolerance and triggering an anticancer response. Many antigens have been identified and studied as potential targets for vaccine therapy, and several vaccine methods have been investigated to target them. The most well-studied and promising vaccines for the treatment of cancer can be subdivided into three main groups: antigen-specific vaccines, tumor cell vaccines, and dendritic cell vaccines.

Active immunotherapy is aimed either at eliciting a specific host immune response against selected cancer antigens by employing cancer vaccines or at amplifying the existing antitumor immune response by administering nonspecific proinflammatory molecules or adjuvants. Dendritic cells (DCs) are the most potent antigen-presenting cells in vitro and in

vivo. DCs have a central function in the activation of specific effector T cells. On this basis, vaccination strategies with DC were regarded as a promising therapeutic approach even in advanced tumor diseases. DC have always been described as having two distinct functional stages: 1) immature, with high antigen uptake and processing ability, and poor T-cell stimulatory function; 2) mature, with high stimulatory function and poor antigen uptake and processing ability. DC internalize cancer antigens and process their proteins then display them as short peptides on the extracellular surface, in conjunction with major histocompatibility complex (MHC) class I and II molecules. DC then migrates into the corresponding lymph nodes, where it matures and present antigen to naïve T lymphocytes. Helper T cells ($CD4^+$) recognize their cognate antigens (MHC class II molecules) located on DCs, whereas $CD8^+$ cytotoxic T lymphocytes (CTLs) recognize affected foreign or cancer cells which display the complementary peptide-MHC class I molecule on their cell surfaces. Targeted cell death occurs by perforin/granzyme-induced apoptosis or FAS-L/Fas interaction. Activation of $CD4^+$ T cells leads to the secretion of cytokines such as IFN-γ and IL-12, which in turn augment the stimulation of active $CD8^+$ T cells. Cancer vaccine aimed at inducing specific recognition of TAA as well as eliciting persistent immune memory T lymphocytes. Programmed death-1 (PD-1) and anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) are induced on T cells after a TCR signal, and result in cell cycle arrest and termination of T-cell activation. Blocking by either CTLA-4 or PD-1 monoclonal antibodies can sustain the activation and proliferation of tumor-specific T cells (Hirano et al., 2005; Hodi et al., 2008). Although, to date, no autologous cellular immunotherapy has gained wide use in clinical practice, the first such therapy to show clinical efficacy in a phase 3 study recently gained U.S. Food and Drug Administration (FDA) approval for the treatment of prostate cancer. Sipuleucel-T consists of autologous PBMCs loaded with recombinant human prostatic acid phosphatase (PAP) linked to granulocyte-macrophage colony stimulating factor (PAP-GM-CSF), which has proven to be effective in phase III clinical trials. DC based Vaccine are typically prepared by harvesting large numbers of autologous peripheral blood mononuclear cells (PBMCs) by leukapheresis, then culturing these cells and loading them with antigens ex vivo and injecting them back into the patient. Three general methods have been described concerning DC based vaccine: (1)differentiating DCs from non-proliferating monocyte precursors (so-called "monocyte-derived DCs"; (2)differentiating DCs from proliferating $CD34^+$ hematopoietic progenitor cells; or (3)directly isolating DCs or mixed APCs from periphereal blood. Autologous DC can be loaded with a wide assortment of antigen types, including whole tumor cells or cell lysates, or TAA in the form of synthetic peptides, purified or recombinant proteins, RNA, plasmid DNA or non-replicating recombinant viral vectors (Mayordomo et al., 1995; Thurner et al., 1999). Immunogenicity may be enhanced by using antigens combined or fused with other more immunogenic molecules, including xenogeneic proteins such as Keyhole Limped Hemocyanin (KLH) or IL-2, TNF-α, IFN-γ or Toll-like receptor agonist. Adapting single peptide for vaccine is not preferable, because after complete objective response to NY-ESO-1 peptide vaccine, but later recurred with a NY-ESO-1–negative tumor, proving that single-target immunization can result in immune escape tumor variants after initial response (Odunsi et al., 2007). A desirable alternative to vaccines are multiepitope or whole tumor antigen vaccines created using autologous tumor lysate or tumor-derived RNA, which may have universal applicability (Chianese-Bullock et al., 2008; Tsuda et al., 2007).
However, the immune responses are often weak, and data on clinical efficacy are limited, as most of these have been small, single arm studies designed only to evaluate safety and immunogenicity. An enormous problem arises from the variability of protocols in the

preparation of DC and in the vaccination itself. A meta-analysis of 56 published peer-reviewed immunotherapy trials of melanoma that used either molecular defined synthetic antigens or whole tumor antigen (4,375 patients) found that only 25.3% of patients vaccinated had objective clinical control (Chi & Dudek , 2011).

A number of studies have found that development of tumor and an unfavorable prognosis for cancer patients were accompanied by accumulation of natural $CD4^{+}CD25^{+}Foxp3^{+}$ T regulatory cells (Tregs) in peripheral blood, as well as of peripherally induced Tregs in the tumor itself (Wilczynski et al., 2008). Furthermore, depletion of Treg is a critical maneuver to enhance vaccine therapy. Different therapeutic immune strategies have been tested preclinically and are currently in evaluation in early phase I and II trials. DC based vaccine is usually given to peripheral site, whereas Natural Killer (NK)-T cell and LAK are either delivered systematically or into the tumor site. Results from these trials vary, but the overall increased survival and/or clinical efficient benefit obtained so far has been limited. In addition, MHC expression level vary cancer type and stage, it seems difficult to eradicate cancer just administrating vaccine. Because CTL induced by vaccine targets MHC expressed cancer cell, whereas NK cell attacks MHC non-expressed cancer cell.

Only three randomized phase 3 clinical trials of DC/APC vaccines for the treatment of cancer have been published. The first study compared subcutaneously administered cytokine-matured, Mo-DCs loaded with a mixture of MHC class II and II-restricted peptide antigens to conventional chemotherapy in patients with stage IV melanoma. Designed to compare clinical response rates as measured by tumor regression, the study showed no statistically significant difference in clinical outcomes between the two treatments. With the FDA-approval of sipuleucel-T, cancer vaccine has become an accepted approach for the treatment of cancer. However, it is not known if the use of dendritic cells or mixed APCs for the active immunotherapy of cancer has an advantage over more conventional vaccine approaches, which are simpler and much less expensive. We usually propose WT1, MUC1, CEA, CA125, HER-2/neu, and PSA as cancer antigens for DC based therapy according to the patient's primary lesion and elevated tumor marker (Sugiyama, 2005; Mukherjee et al., 2000; Nair et al., 1999; Larbcurrentet et al., 2007). It has been reported that WT1 and MUC1 is antigens with high immunogenicity and their-targeted immunotherapy have confirmed its safety and clinical efficacy, although there is few description concerning cancer vaccine adapting WT1 and MUC1 simultaneously to cancer antigen (Ramanathan et al., 2005). Dr Okamoto and his colleagues have already reported that OK-432 generates mature DCs via Toll-like receptor 4 signaling and that OK-432-activated DCs stimulates $CD8^{+}$ T cells to induce antigen-specific CTLs (Ahmed et al., 2004, Itoh et al., 2003; Nakahara et al., 2003; Okamoto et al., 2003, 2004, 2006; Oshikawa et al., 2006). In this analysis efficacy of cancer vaccine, different potential means of DC based vaccination in experimental settings and preliminary data from clinical trial have been examined.

2. Material and method

2.1 Patients, treatment and sampling

This retrospective study was carried out in accordance with the standards of our Institutional Committee for the Protection of Human Subjects. Eligible patients must be those who have failed standard treatment. Informed written consent according to the Declaration of Helsinki was obtained from all patients before giving this therapy, and the collection of the samples was approved by the Institutional Review Board. From 2007 to 2010, 127 patients with advanced cancer refractory to standard treatment were treated with DC-based immunotherapy (DC vaccine alone or DC vaccine plus NK-T cell therapy) at Kudan Clinic Immune Cell Therapy Center.

Initial patient evaluations included a medical history and physical examination; measurement of performance status, hemoglobin, WBC count, platelet count, blood urea nitrogen, creatinine, alkaline phosphatase, lactate dehydrogenase, AST, ALT, bilirubin, and tumor marker levels; HbA1c; Computed Tomography (CT) scans or Magnetic Resonance Imaging (MRI) of whole body. Patients with evidence of operable tumor were ineligible. To be eligible, patients were required to have an ECOG performance status of less than 3.

Eligible Adequate hematologic, hepatic, and renal function, within the following parameters: WBC count of 2,500/μl or greater; platelet count of 100,000/μl or greater; hemoglobin value of 10 g/dl or greater; blood urea nitrogen value less than 50 mg/dl; serum bilirubin level less than 5.0 mg/dl; AST level lower than 500 IU.

Autologous DCs (1×10^7 cells) were administered intradermally at 14-day intervals. Tolerable 1 to 5 KE of OK-432 (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), a streptococcal immunological adjuvant, was administered together with DC vaccine. NK-T cells were simultaneously injected in as many patients at 14-day intervals.

The clinical response was evaluated on the basis of the Response Evaluation Criteria in Solid Tumors (RECIST) Ver1.0 as follows: complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD). Adverse events were evaluated by grading the toxicity according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.

2.2 Preparation of DCs and NK-T cells

PBMCs-rich fraction was obtained from leukapheresis (400 ml x 13 cycles) using COM.TEC (Fresenius Kabi, Homburg, Germany). The PBMCs were isolated from the heparinized leukapheresis products by Ficoll-Hypaque gradient density centrifugation (Böyum, 1967). These PBMCs were placed into 100 mm plastic tissue-culture plates (Becton Dickinson Labware, Franklin Lakes, NJ) in AIM-V medium (Gibco, Gaithersburg, Md). After 30 min of incubation at 37°C, nonadherent cells were removed, and the adherent cells were cultured in AIM-V containing granulocyte-macrophage colony stimulating factor (GM-CSF, 500 ng/ml; Primmune Inc., Kobe, Japan), and IL-4 (250 ng/ml; R&D Systems Inc., Minneapolis, MN) to generate immature DCs (Okamoto et al., 2004). The population of the adherent cells remaining in the wells was composed of 95.6 ± 3.3% $CD14^+$. After 5 days of the cultivation, the immature DCs were stimulated to be matured with OK-432 (10 μg/ml) and Prostaglandin E2 (50 ng/ml; Daiichi Fine Chemical Co. LTD., Toyama, Japan) for 24 hrs. It has been reported that Prostaglandin E2 acquires the ability to migrate to the lymph node to DCs (Sato et al., 2003). Peptides (20 μg/ml) for WT1, Her2 and CEA were pulsed into the DCs at 24 hrs after the treatment with OK-432 and with Prostaglandin E2, while MUC1 long peptide (30 mer) (20 μg /ml), CA125 protein (500 U/ml) and autologous tumor lysates (50 μg/ml) were added into the DC culture media at the same time as adding OK-432 and Prostaglandin E2, then incubated for 24 hrs (Kontani et al., 2002; Cannon et al., 2004). To prepare the autologous tumor lysates, tumor masses were obtained by surgical resection exclusion, and were then homogenized. Aliquots of the isolated tumor cells were then lysed by putting them through 10 freeze (in liquid nitrogen) and thaw (in a 37 °C water bath)

cycles. The lysed cells were centrifuged at 14000 g for 5 min, and the supernatants were passed through a 0.22 μm filter (Millipore Corporation, Bedford, MA). The protein contents of the resultant cell-free lysates were determined using DC protein assay kits (Bio-Rad Laboratories, Hercules Aliquots (500 μg/tube) were then cryopreserved at -135 ^{o}C until use (Nagayama et al., 2003). Surface molecules expressed in the DCs were determined using flow cytometry. The cells defined as the mature DCs were $CD14^{-}$, HLA-DR^{+}, HLA-ABC^{+}, $CD80^{+}$, $CD83^{+}$, $CD86^{+}$, $CD40^{+}$, and $CCR7^{+}$.
For preparation of NK-T cells, PBMCs were cultured with an immobilized monoclonal anti-CD3 antibody (5 μg/mL OKT3; Jansen Pharmaceutical K.K., Tokyo, Japan) in the presence of recombinant human IL-2 (175 U/mL; CHIRON, Benelux B.V., Amsterdam, Netherlands) and autologous plasma for 14 days. Fresh NK-T cells were prepared every injection as described above; it was composed of more than 85% of aβ-T cells and about 10% of NK/NKT cells.

2.3 Vaccine quality control
All vaccines were subjected to a quality-control evaluation, which were assessed the total number of live dendritic cell, monocyte-derived dendritic cell characteristics, and percentage of viable cells. For a vaccine to be deemed "adequate," there must have been 4 x 10^{7} viable dendritic cells.

2.4 FACS analysis
The frozen cells were allowed to thaw in a 37 ^{o}C water bath quickly and retrieved from the cryopreservation tubes by rinsing with 0.02% albumin containing Cell Wash™ (eBioscience, San Jose, CA) (FACS buffer). The FACS analysis was performed for cell surface antigen staining. FITC-labeled anti-human CD14, CD40, CD80, HLA-A, B, C, PE-labeled anti-human CD11c, CD83, CD197 ($CCR7^{+}$), HLA-DR and FACS Calibur Flowcytometer were purchased from BD Bioscience, and used for the FACS analysis.

3. Results

3.1 Clinical outcome of patients with DC-based vaccine
Computed tomography scans or MRI was done before and at the end of dendritic cell therapy. Of 127 patients who received DC vaccine, complete responses (n = 4; 3.1%), partial responses (n = 26; 20.5%), or stable disease were observed in 34 (26.8%) (Table 1.).
Although the study was not designed or powered to detect differences between other vaccine treatment groups, it was of interest to compare the response and survival of patients treated with other vaccines.
Most patients received NK-T therapy in combination with the DC vaccination as to induce Th (helper T cell) 1-dominant state for improved CTL response and to attack non-MHC expressed carcinoma cells. The NK-T cells generated according to the methods described in the "Materials and Methods" section secrete IFN-γ and IL-2, and induce helper T cell (Th) 1-dominant state in the cytokine balance of the patients (Chong et al., 1994).

3.2 Adverse events
Therapy was well-tolerated during the treatment and 3 months after last administration. None of the patients experienced adverse events of grade 3 or higher during the treatment period, grade 1 to 2 fevers, grade 1 injected-site reaction consisting of erythema, induration

Cancer type	patient No.	CR (%)	PR (%)	SD (%)	PD (%)	total (%)
esophagus	10	0	20.0	10.0	70.0	100
gastric	24	0	16.7	20.8	62.5	100
colorectal	9	0	22.2	44.4	33.3	100
hapetocellular	8	12.5	25.0	12.5	50.0	100
pancreas	18	5.6	27.8	38.9	27.8	100
lung	14	7.1	21.4	42.9	28.6	100
breast	21	0	9.6	33.3	57.1	100
gynecological	3	0	0	33.3	66.6	100
malignant lymphoma	3	33.3	0	0	66.6	100
prostate	10	0	60.0	20.0	20.0	100
thyroid	3	0	0	0	100	100
malignant melanoma	4	0	0	0	100	100
total (No.)	127	4	26	34	63	100
total (%)		3.1	20.5	26.8	49.6	100

Table 1. Clinical Outcome of Patients treated with DC based vaccine

and tenderness, lasting 24 to 48 hours after injection in 8 patients and resulted in no dose modifications or delays. No signs or symptoms of auto-immune phenomena (eg, arthritis, colitis, inflammation of skin) were observed either during or after therapy.

4. Discussion

Interest in antitumor vaccination arose around 1900 when a series of microbial vaccines by Dr William B. Coley proved to be effective. Boon T and others provided an unambiguous definition of TAA, an important finding the genetic and molecular identification of a large series of TAA (Coulie, 1997; Robbins & Kawakami, 1996).

In many cases, TAA are peptides presented by class I and class II glycoproteins of the MHC. A similar picture is emerging from phase I studies on vaccination of cancer patients. However, clinical responses to the immunotherapy with DC vaccination have only been observed in a minority of patients with solid cancer. Initiation of immune responses requires that professional APC deliver a first signal to T-lymphocytes through the binding of the T-cell receptor by the peptide enclosed in the HLA molecule, that is responsible for the specificity of the immune response, and a second or co-stimulatory signal that is not antigen-specific but it is required for T-cell activation mainly through CD80 (B7-1) and CD86 (B7-2) binding to CD28 receptor, or the CD40:CD40L pathway (Janeway & Bottomly, 1994). Moreover, the capacity of DC to activate NK cells by ligation of the CD40 molecule with its counter-receptor has recently been demonstrated (Cayeux et al., 1999; Kitamura et al., 1999).

Therefore, given the complex network of regulatory signals by professional APC and naïve and memory T lymphocytes occurring in antigen-specific immune responses, it is not surprising that tumor cells may fail to induce efficient immune reactions even when a well known TAA is present. From among the professional APC, DC are the most potent stimulators of T cell responses and play a crucial role in the initiation of primary immune responses (Banchereau & Steinman, 1998). DC have always been described as having two distinct functional stages: 1) immature, with high antigen uptake and processing ability, and poor T-cell stimulatory function; 2) mature, with high stimulatory function and poor antigen uptake and processing ability. Despite several immunotherapeutic approaches having been tested for colon cancer patients, only one study has reported clinical results in a prospective randomized study (Vermorken et al., 1999). Experimental data and clinical evidence suggest that antitumor vaccines will be a new form of tumor treatment that will be able to be adopted for the management of defined stages of carcinoma, in sequential association with conventional treatments (Sadanaga et al., 2001). Prediction of when the efficacy of antitumor vaccination will be assessed and will become a routine procedure is beyond a simple scientific evaluation. While pre-clinical research has identified several possible targets and strategies for tumor vaccination, the clinical scenario is far more complex. The main cell populations taking part in immunoregulation of tumor growth are presented in Fig. 1.

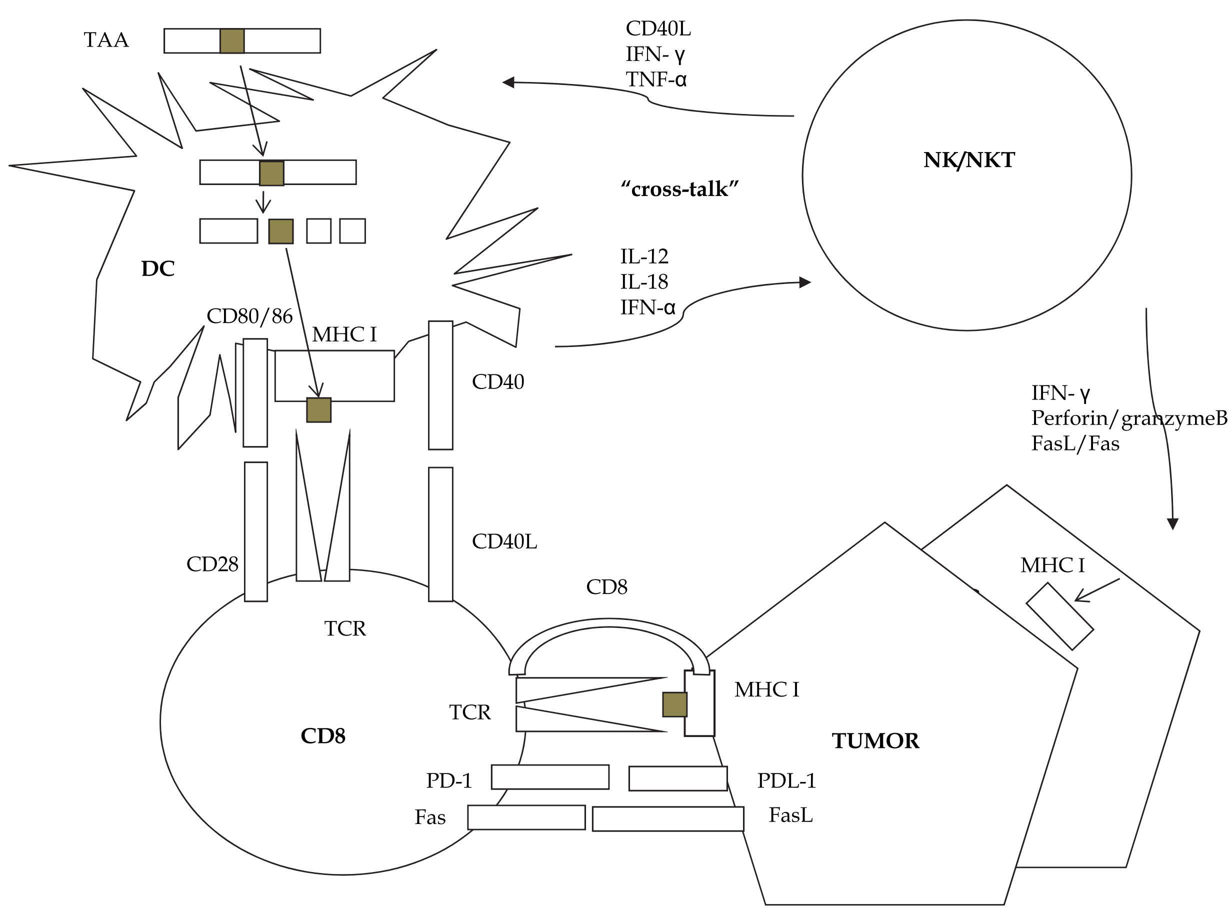

Fig. 1. The main anti-tumor immune cell responses by DC and NK/NKT cell

Most peptide-based vaccines have considered HLA class I restricted peptides only, whereas there is increasing evidence that tumor-specific $CD4^+$ T-cells may be important in inducing an effective antitumor immunity. The addition of peptides that bind class II HLA glycoproteins to peptide vaccines could lead to an amplification of the immune response as well as to better clinical effect. The possibility of effectively monitoring the immune response induced acquires critical importance since it may provide a much earlier surrogate end-point, predictive of the clinical outcome. An ideal TAA is a protein that is essential for sustaining the malignant phenotype, and that is not stripped or down modulated by the immune reaction. TAAs were sorted by their anti-tumor potential such as therapeutic function, immunogenicity, oncogenicity, specificity, expression level and % positive cells, and cancer stem cell expression. Among the 75 peptides evaluated by Dr Martin A. Cheever, WT1 was the 1st and MUC1 was the 2nd anti-tumor effect (Cheever et al., 2009). WT1 was originally identified as tumor suppressor gene for Wilm's tumor. WT1 over-expression has been detected in different malignant cell types including gastroenterological carcinoma, gynecological carcinoma, lung carcinoma, prostate, breast carcinoma and hematological malignancy. MUC1 is expressed at high levels over the entire surface of diverse types of carcinoma cells such as gastroenterological carcinoma, gynecological carcinoma, NSCLC, prostate and breast carcinoma. MUC1 transmembrane receptor has revealed a function for this subunit as an oncoprotein that is targeted to the nucleus and regulates gene expression. MUC1-C accelerates the malignant potential by regulating gene transcription, blocking stress-induced apoptosis and necrosis, and attenuating activation of death receptor pathways.

When compared with conventional cancer management, vaccination is a *soft*, non-invasive treatment free from particular distress and iatrogenic side effects. Antitumor vaccines can be expected to have a considerable social impact, but a few large clinical trials enrolling the appropriate patients are now necessary to assess their efficacy. In conclusion, even if cancer vaccines are an old dream, only recently has their design become a rational enterprise (Ehrlich, 1909). There are now many ways of constructing vaccines able to elicit a strong protective immunity. This progress is offering ground for optimism.

Here, we demonstrate that WT1 and/or MUC1 pulsed DC vaccination is feasible, safe, and sufficiently powerful to induce objective clinical and immune responses even in patients with significant tumor burden. Several studies of ex vivo, custom-manufactured cancer vaccines using patient-specific idiotype, idiotype-pulsed dendritic cells, and tumor lysate–pulsed dendritic cells have also demonstrated objective clinical responses and these prior results prompted our pursuit of the practicable in situ approach.

This approach-by its nature-must be studied in patients with clinically evident disease, in contrast to the described randomized studies (Flowers, 2007; Freedman et al., 2009). As compared with other comparably practical vaccines current vaccination has the potential advantage of using more potential TAA encompassing each individuals' relevant tumor antigens. In addition, several recent immune-response studies showing that vaccine-induced T cells peak at day 14 and decline sharply thereafter, have prompted earlier immune-response measurements in an ongoing follow-up study (Deng et al., 2004; Kim et al., 2007; Treanor et al., 2006). There is little or no controversy that patients with Treg-inducing tumors had poorer clinical outcomes after vaccination. This biomarker could be either a specific predictor of response to in situ vaccination or a general prognosticator of poor outcomes regardless of therapy. Interestingly, patients with highly Treg-infiltrated tumors have shown favorable clinical outcomes after standard therapy (Carreras et al., 2006; Tzankov et al., 2008). If Treg induction predicts good response to standard therapy, but a poor response to the in situ vaccine, then it would be a powerful clinical tool for selecting appropriate patients for vaccination. This interesting finding is still preliminary and is being evaluated prospectively in an ongoing follow study (ClinicalTrials.gov-ID: NCT00880581). That the vaccine preparation in current study was optimal was evident from quality-control assessments, because in the study presented here, all vaccines didn't fail to meet quality-control specifications. As prognosis of most advanced carcinoma who failed standard therapy is poor, establishment of effective therapeutic modality for advanced carcinoma is an urgent issue.

Immunotherapy would be implied as one of the important therapeutic modalities against advanced carcinoma and even adjuvant settings because WT1 and MUC1, highly immunogenic target molecules for adaptive anti-tumor immune response, were frequently expressed in most carcinoma tissue (Cheever et al., 2009; Oka et al., 2006). DC-based vaccination has several advantageous aspects for induction and activation of tumor antigen-specific CTLs compared with CTL-epitope peptide-based vaccination (Melief & van der Burg, 2008). Patients with advanced carcinoma who failed standard therapy and met eligible criteria enrolled in current study. Response rate was 23.6%, whereas control ratio was 50.4%. It is a significant tumor control ratio compared with other historical modalities much less no severe adverse event. Is the strongest result from this trial the apparent increase in control ratio? It would be important to understand the mechanisms of immune system underlying the significant increase in cancer control ratio. These results indicate that WT1 and/or MUC1 pulsed DC-based vaccination can elicit significant clinical benefit even for the advanced cancer patients refractory to the standard therapies. Although there was a trend toward treatment being superior to standard treatment only, there was no statistical consideration. However, the study demonstrated that successful active specific immunotherapy with WT1 and/or MUC1 pulsed DC-based vaccine may be dependent on the quality of the vaccine as well as TAAs. These encouraging preliminary results suggest that WT1 and/or MUC1 pulsed DC-based vaccination warrants further study as a novel therapy for patients with advanced carcinoma. The combination of cytotoxic therapy and intratumoral immune stimulation has been studied preclinically for a variety of common tumor types and might also be directly translated to the clinic (Meng et al., 2005; Najar et al., 2008; VanOosten & Griffith, 2007). This trial clearly supports the idea that to be immunologically effective, control of the vaccine preparation and the quality assurance that the vaccine meets specifications are of the highest priority and must be considerations in any future tumor cell vaccine study. A key element in these novel strategies is the identification of suitable patients, the selection being based on detailed immunological and molecular characterization. The most promising finding that emerges from this study is that WT1 and/or MUC1 pulsed DC-based vaccine together with NK-T cell therapy elicit strong anti-tumor response. Progress in the formulation of cancer vaccines will be brought by a more precise knowledge of the requirements for the potent generation of efficient CTL induction and NK cell expansion as well as discovering potent TAA, together with the current ability to closely monitor molecular immune response prediction markers in, will likely provide powerful, individualized vaccines in the near future.

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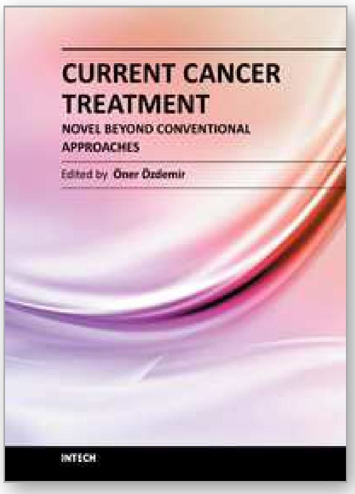

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Currently there have been many armamentaria to be used in cancer treatment. This indeed indicates that the final treatment has not yet been found. It seems this will take a long period of time to achieve. Thus, cancer treatment in general still seems to need new and more effective approaches. The book "Current Cancer Treatment - Novel Beyond Conventional Approaches", consisting of 33 chapters, will help get us physicians as well as patients enlightened with new research and developments in this area. This book is a valuable contribution to this area mentioning various modalities in cancer treatment such as some rare classic treatment approaches: treatment of metastatic liver disease of colorectal origin, radiation treatment of skull and spine chordoma, changing the face of adjuvant therapy for early breast cancer; new therapeutic approaches of old techniques: laser-driven radiation therapy, laser photo-chemotherapy, new approaches targeting androgen receptor and many more emerging techniques.

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