Phase I Trial of Wilms’ Tumor 1 (WT1) Peptide Vaccine with GM-CSF or CpG in Patients with Solid Malignancy

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Abstract. Background: The aim of this study was to investigate the safety and efficacy of combinatorial use of granulocyte-macrophage colony-stimulating factor (GM-CSF) and CpG oligodeoxynucleotides (CpG-ODN) as immunoenhancement adjuvants in Wilms’ Tumor 1 (WT1) vaccine therapy for patients with solid malignancy. Patients and Methods: The patients were placed into treatment groups as follows: WT1 peptide alone, WT1 peptide with GM-CSF (100 μg) and WT1 peptide with CpG-ODN (100 μg). HLA-A*2402 or *0201/*0206-restricted, WT1 peptide emulsified with Montanide ISA51 was injected intradermally every week for eight weeks. Toxicities were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events ver. 3.0. Tumor size, which was measured by computed tomography, was determined every four weeks. The responses were analyzed according to Response Evaluation Criteria in Solid Tumors. Results: The protocol was well tolerated; only local erythema occurred at the WT1 vaccine injection site. The disease control rate of the groups treated with WT1 peptide alone (n=10), with combinatorial use of GM-CSF (n=8) and with combinatorial use of CpG-ODN (n=10), in the initial two months was 20%, 25% and 60%, respectively. Conclusion: Addition of GM-CSF or CpG-ODN to the WT1 peptide vaccine for patients with solid malignancy was safe and improved the effectiveness of clinical response.

Recent advances in tumor immunology have resulted in the identification of a large number of tumor-associated antigens (TAAs) that might be used for cancer immunotherapy, since their epitopes, associated with human leukocyte antigen (HLA) class I molecules, are recognized by cytotoxic T-lymphocytes. One such identified TAA is the product of the Wilms’ tumor gene, WT1 (1, 2).

We performed a phase I clinical trial to examine the safety of a WT1-based vaccine, as well as the clinical and immunological response of patients with a variety of cancer types, including leukemia, lung cancer and breast cancer (3). The WT1 peptide vaccine, emulsified with Montanide ISA51 adjuvant and administered at a dosage of 0.3, 1.0, or 3.0 mg at two-week intervals, was safe for patients, other than those with myelodysplastic syndromes. Furthermore, it has been confirmed that the potential toxicities of the weekly WT1 vaccination treatment schedule (3.0 mg dose) with the same adjuvant agent were also acceptable (4). In the past, clinical response to weekly WT1 peptide-based immunotherapy in phase II trials has been reported for renal cell carcinoma (5), multiple myeloma (6), glioblastoma multiforme (7) and gynecological malignancies (8). In these studies, the activity of WT1 peptide alone was examined and no specific adjuvant, that would activate immune reactions, was included. As a result, the peptide vaccine had limited effectiveness against malignant tumors.

In clinical studies, the identification of predictive factors of treatment is extremely important for the improvement of clinical response. The most representative factor that predicts the outcome of cancer peptide vaccine therapy is the expansion and/or induction of TAA-specific cytotoxic T-lymphocytes (CTLs). Klebanoff et al. reported that not only the induction of effector CTLs, but also the maintenance of memory CTLs, are required for ideal antitumor immune response in tumor-bearing patients (9). Moreover, Fujiki et al. confirmed that occurrence of an antigen-specific helper T-cell (Th) response predicted good clinical response of CTL epitope
vaccination (10). We have demonstrated that the percentage of dendritic cells (DCs) in peripheral blood may represent a new interesting biological marker predicting therapeutic response in patients treated with WT1 peptide vaccination (11). The main function of DCs is to process antigen material and present it on their surface of other cells (e.g. Th and CTLs) of the immune system. In accordance with these results, we focused on the adjuvant agent used to activate antigen-presenting cells (e.g. DCs and macrophages), in order to enhance the therapeutic efficacy of cancer peptide vaccination.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that functions as a white blood cell growth factor. GM-CSF stimulates stem cells to produce granulocytes and monocytes. The various cellular responses (i.e. division, maturation and activation) are induced through GM-CSF binding to specific receptors, expressed on the cell surface of target cells (12). GM-CSF increases the cytotoxicity of monocytes towards certain neoplastic cell lines (13).

CpG oligodeoxynucleotides (CpG-ODN) are short, single-stranded, synthetic DNA molecules that contain a cytosine "C" followed by a guanine "G". The "p" refers to the phosphodiester backbone of DNA, however some ODNs have a modified phosphorothioate backbone. When these CpG motifs are unmethylated, they act as immunostimulants (14). CpG motifs are considered pathogen-associated molecular patterns (PAMPs) due to their abundance in microbial genomes and their rarity in vertebrate genomes (15). The CpG-ODN PAMP is recognized by the pattern recognition receptor toll-like receptor 9 (TLR9).

In the present study, we investigated the safety and efficacy of GM-CSF and CpG-ODN as immunoenhancement adjuvants in WT1 vaccine therapy for patients with solid malignancy.

**Patients and Methods**

**Trial protocol.** A phase I clinical trial of the WT1 with immunostimulatory adjuvants was designed to evaluate the safety and tumor response. Patients with histologically confirmed solid malignancies were eligible if they exhibited a performance status of the Eastern Cooperative Oncology Group of 0-2 and had measurable disease. Additional inclusion criteria were: (i) age ranging from 16 to 80 years; (ii) overexpression of the WT1 gene in the cancerous tissue as determined by immunohistochemistry; (iii) HLA-A*2402, or A*0201, or A*0206 positivity; (iv) disease refractory to conventional chemotherapy, radiotherapy, and/or hormonal therapy; (v) no history of antitumor therapy within 4 weeks prior to enrolment; (vi) in patients not having primary brain tumor, absence of brain metastases should be confirmed by computed tomography or magnetic resonance imaging; (vii) sufficient organ function and (viii) written informed consent.

Following written informed consent, the patients received injections of 3.0 mg of WT1 peptide emulsified with Montanide ISA51 adjuvant (SEPPIC S.A., Paris, France). The emulsion was injected intradermally into four different regions (bilateral axillary and inguinal region). The WT1 vaccinations were scheduled to be administered weekly, for eight consecutive weeks. The initial group of patients (cohort 1) received WT1 emulsion alone. The subsequent group of patients (cohort 2) received WT1 emulsion with GM-CSF (sargramostim) (Bayer Health Care Pharmaceuticals, LLC, Seattle, WA, USA). GM-CSF was administered subcutaneously as four separate injections of 100 μg in the same region as each vaccine dose. The final group of patients (cohort 3) received WT1 emulsion admixed with 100 μg CpG-ODN (5'-TCGTCGTTTTGTCGTTTTGTCGTT-3') (Hokkaido System Science Co., Ltd, Hokkaido, Japan).

The Independent Safety Monitoring Committee (ISMC) monitored and reviewed the protocol compliance, safety and on-schedule study progress. The protocol was approved by the Institutional Review Board and the Ethical Committee at Tokyo Women's Medical University. The study was registered in the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR) Clinical Trial (Unique trial number: UMIN 000002771) on November 11, 2009 (UMIN-CTR URL: http://www.umin.ac.jp/ctr/index.htm).

**WT1 peptide.** The WT1 peptide was manufactured by NeoMPS, Inc. (San Diego, CA, USA). For patients with HLA-A*2402, modified 9-mer WT1 peptide (amino acids 235-243 CYTWNQMNL) was synthesized, in which Y was substituted for M at amino acid position 2 (the anchor position) of the natural WT1 peptide. This variant induces stronger cytotoxic activity than the natural peptide (16). For patients with HLA-A*0201 or A*0206 an 9-mer WT1 peptide (amino acids 187-195 SLGEQQYSV), which is able to bind to both HLA-A*0201 and A*0206, was synthesized (17). Peptides were stored in dimethyl sulfoxide (DMSO) at –80°C and thawed on the day of injection. A water-in-oil emulsion vaccine was then prepared, consisting of the peptide (aqueous phase) and the adjuvant Montanide (oil phase), by combining equal volumes of the peptide and the adjuvant. All synthesis, production and formulation of the two different kinds of peptides were in accordance with applicable Good Manufacturing Practices and met the applicable criteria for use in humans.

**Immunohistochemical analysis.** Positive immunostaining of WT1 protein in the patient's tumor was a mandatory requirement for entry into the trial. A standardized staining protocol was adopted from a preceding trial (18). Briefly, formalin-fixed and paraffin-embedded tissue sections were first autoclaved in order to expose antigenic complex (ABC) kit (Vector Laboratories, Burlingame, CA, USA). Staining with a more specific monoclonal antibody, 6F-H2 (Dako, Glostrup, Denmark), was also performed and the results were consistent with those obtained with the polyclonal antibodies.

**Evaluation of toxicity.** Toxicities were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events ver. 3.0 (19). If an adverse event of grade 2 or 3 continued, further immunization was suspended until the problem was solved. An adverse event of more than grade 4 forced the immediate termination of the immunotherapy.

**Evaluation of clinical response.** After the WT1 vaccine was administered eight times, the antitumor effect of the treatment was assessed by determining the response of the target lesions on
computed tomographic images. The tumor size was analyzed according to Response Evaluation Criteria in Solid Tumors (RECIST) (20), with results reported as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). The disease control rate was calculated as the percentage of the number of patients in which there was a CR, PR or SD divided by the total number of patients.

**Results**

**Patients’ characteristics.** Between January 2010 and November 2010, a total of 28 patients were enrolled in this study. Their clinical characteristics are summarized in Tables I-III. The mean age of the 28 enrolled patients was 55.3 (cohort 1: 59.4, cohort 2: 63.6, cohort 3: 54.3) years. All the patients had been treated with surgery as initial therapy. For recurrent diseases and disease progression after initial therapy, all patients received chemotherapy with or without radiotherapy.

**Administration protocol and toxicities.** The median number of vaccination was nine (cohort 1: 7.5, cohort 2: 9, cohort 3: 15.5), with a range from 2 to 47 (cohort 1: 4-25, cohort 2: 2-30, cohort 3: 6-47), with four patients still on treatment at the end of September 2011. Nine patients received fewer than nine vaccinations due to disease progression and poor general condition. The patients who had an effective response continued to receive weekly or biweekly vaccinations after the period of the clinical trial, until tumor progression was demonstrated.

All patients developed an injection-site reaction (grade 1 or 2), such as erythema, itching or swelling. Patient CpG 5 (Table III) had multiple colonic liver metastases with hepatic portal infiltration at the time of enrollment in the study. Eight weeks after the initial vaccination, bleeding from esophageal varices, which occurs as a result of portal-systemic shunting, was observed. Endoscopic variceal ligation was performed and hemostasis was promptly achieved. The ISMC review of this adverse event confirmed that the gastrointestinal bleeding was not related to WT1 treatment.

No other toxicities (grade 1-5) were observed. These results indicate that repeated WT1 vaccination with GM-CSF and CpG-ODN is sufficiently tolerable.
Clinical outcome. Clinical outcome data for all patients categorized by immunoenhancing adjuvants are summarized in Tables I-III. For primary analysis, clinical response was assessed according to the RECIST criteria. The disease control rate of cohort 1, 2 and 3 in the initial two months (the clinical trial period) was 20%, 25% and 60%, respectively.

Discussion

In this study, patients with HLA-A*2402, A*-0201 or A*-0206 were immunized by injecting the WT1 peptide, added with GM-CSF or CpG-ODN, intradermally once every week for eight weeks and evaluated the safety and efficacy. As vaccine-related adverse events, grade 1 and 2 injection-site reactions were observed within 24-72 h. The intensity of the skin reaction was augmented by repeated vaccinations, suggesting the reaction was a delayed-type hypersensitivity reaction towards WT1 peptide. It is reasonable to believe that the skin toxicity of vaccine therapy at the injection sites is due to the natural course of the immune activation. Therefore, the treatment was considered to be well-tolerated.

The potential of the WT1 protein as a cancer antigen is of considerable interest. Many cancer antigens are relatively easy to isolate because of advances in tumor and molecular immunology. Nevertheless, determination of the clinical efficacy of these cancer antigens can be achieved only by clinical studies that are very laborious, and moreover, only clinical studies can determine their potential as cancer antigens. It is therefore a laborious and time-consuming work to determine and confirm the clinical usefulness of a given cancer antigen. Recently, 75 representative cancer antigens including WT1 were prioritized (21). The selection and prioritization of these antigens were performed according to the following criteria: (i) therapeutic function, (ii) immunogenicity, (iii) role of the antigen in oncogenicity, (iv) specificity, (v) expression level and percentage of antigen-positive cells, (vi) stem cell expression, (vii) number of patients with antigen-positive cancer, (viii) number of antigenic epitopes, and (ix) cellular location of antigen expression. Although none of the 75 cancer antigens had all the characteristics of the ideal cancer antigen, WT1 was at the top of the ranking. This finding can be expected to promote the development of WT1-targeted cancer immunotherapy.

The cytokine GM-CSF is involved in the recruitment and maturation of antigen-presenting cells and has been incorporated into numerous clinical studies with cancer vaccines to enhance immune responses (22-24). Previous studies have revealed the safety of therapeutic application using WT1 peptides in Montanide adjuvant with GM-CSF in patients with myeloid malignancy (25-27) and mesothelioma (28). The present study also demonstrated that GM-CSF was safe as adjuvant in patients with various types of cancer. However, the disease control rate in the group of patients treated with the WT1 peptide vaccine with GM-CSF (cohort 2) (25%), was only slightly better than or comparable to that of the group treated with the WT1 peptide alone (cohort 1) (20%).

CpG-ODN can be synthesized for therapeutic use and has been evaluated as a vaccine adjuvant in several clinical studies. CpG-ODN acts as a very potent adjuvant in combination with Montanide, and has been shown to promote strong antigen-specific CD8+ T-cell responses in patients with melanoma (29, 30). In addition, intradermal injections of CpG-ODN around the excision site of melanoma activate the plasmacytoid DCs and myeloid DCs, and reduce the number of regulatory T-cells in sentinel lymph nodes (31, 32). Vaccination with NY-ESO-1 peptide in combination with CpG-ODN was reported to successfully induce NY-ESO-1-specific immune responses and revealed clinical benefit by extending survival in patients with NY-ESO-1-positive cancer (33). As established by the seminal
CD8+ CTLs, CD4+ Th1 cells and memory T-cells in mice immune responses, including idiotype- and myeloma-specific associated with an induction of strong humoral immune established myeloma. The therapeutic responses were not GM-CSF, not only efficiently protected mice from idiotypic vaccine combined with CpG-ODN or IFN-α, but killer (NK) cells were activated. These results suggest that not only tumor-specific acquired immunity, but also innate immunity were enhanced by this vaccination.

CpG-ODN can stimulate both innate immunity and adoptive immune responses through endosomal TLR9, which is expressed in plasmacytoid DCs in humans. Plasmacytoid DCs produce high levels of type I interferons, as well as a variety of other cytokines and chemokines to promote Th1-like immune responses involving other cell types, including additional DC subsets, monocytes, NK cells, and neutrophils (35-37). Therefore, CpG-ODN is considered to play important roles as an adjuvant for cancer vaccines using epitope peptides.

In our study, we have shown that the disease control rate in the group of patients treated with the WT1 peptide vaccine with CpG-ODN (cohort 3) (60%), was much higher than that of the other groups. Recently, Hong et al. (38) revealed that idiotypic vaccine combined with CpG-ODN or IFN-α, but not GM-CSF, not only efficiently protected mice from developing myeloma, but also eradicated the already established myeloma. The therapeutic responses were associated with an induction of strong humoral immune responses, including anti-idiotypic antibodies, and cellular immune responses, including idiotype- and myeloma-specific CD8+ CTLs, CD4+ Th1 cells and memory T-cells in mice receiving idiotypic vaccine combined with CpG or IFN-α. Furthermore, idiotypic vaccine, combined with CpG or IFN-α induced idiotype- and tumor-specific memory immune responses that protected surviving mice from tumor recrudescence. Thus, these results clearly show that CpG is a better immune adjuvant than GM-CSF. However, our study was still a phase I trial, and we will determine whether the immune response to WT1 can be induced by this vaccine protocol in the next phase II study.

For decades, investigators have relied on modified WHO criteria (39) or, more recently, RECIST (20) to assess the clinical activity of anticancer agents. These standard criteria were designed to capture effects of cytotoxic agents and depend on tumor shrinkage to demonstrate activity. However, the response patterns seen with immunotherapeutic agents extend beyond those of cytotoxic agents and can manifest, for example, after a period of stable disease in which there is no tumor shrinkage, or after initial tumor burden, an increase in, or the appearance of new lesions (e.g. tumor-infiltrating lymphocytes) (40-43). This potential delayed detection of clinical activity on radiographic assessment may reflect the dynamics of the immune system, the time required for T-cell expansion followed by infiltration of the tumor, and a subsequent measurable antitumor effect. For example, our previous trial (8, 44) and other studies (40-43) of clinical cancer vaccines demonstrated that patients with stable or progressive disease may have subsequent tumor regression, or initial mixed responses, with regression in some lesions, while other lesions remain stable or progress.

Such patterns have been noted by many investigators; however, they were inconsistently included in publications or were not systematically captured because of the absence of suitable response criteria, which, in turn, did not allow for their clinical significance to be adequately studied (45). It has become evident that RECIST and WHO criteria may not offer a complete description of the response to immunotherapeutic agents, and therefore either adjusted or new criteria are needed (45).

Cancer immunotherapy is considered to be the fourth cancer therapy after the three major cancer therapies of surgery, chemotherapy and radiotherapy. It is thought that complete eradication of cancer stem cells is essential for the cure of cancer and that only immunotherapy is capable of killing non-dividing, quiescent cancer stem cells. Therefore, ideal and future immunotherapy should be started as soon as possible after the diagnosis of cancer and continued as long as possible, so that surgery, chemotherapy and radiotherapy can be performed under conditions of enhanced cancer immunity.

In conclusion, the addition of GM-CSF or CpG-ODN to a WT1 peptide vaccine, for patients with solid malignancy, was safe and apparently improved the effectiveness of clinical response.

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References


