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WT1 peptide vaccine for the treatment of cancer

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Wilms' tumor gene *WT1* is expressed in various kinds of cancers. Human WT1-specific cytotoxic T lymphocytes (CTLs) were generated, and mice immunized with WT1 peptide rejected challenges by *WT1*-expressing cancer cells without auto-aggression to normal organs. Furthermore, WT1 antibodies and WT1-specific CTLs were detected in cancer patients, indicating that WT1 protein was immunogenic. These findings provided us with the rationale for cancer immunotherapy targeting WT1. Clinical trials of WT1 peptide vaccination for cancer patients were started, and WT1 vaccination-related immunological responses and clinical responses, including reduction of leukemic cells, reduction of M-protein amount in myeloma, and shrinkage of solid cancer, were observed. Valuable information about immune responses against tumor antigens can be obtained by the analysis of samples from the vaccinated patients, which should lead to further improvement of cancer vaccine.

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Introduction

Wilms' tumor gene *WT1* was isolated as a gene responsible for a childhood renal neoplasm, Wilms' tumor [1,2]. This gene encodes a zinc finger transcription factor and plays important roles in cell growth and differentiation [3,4]. Although *WT1* gene was categorized at first as a tumor-suppressor gene, it was recently demonstrated that the wild-type *WT1* gene performed an oncogenic rather than a tumor-suppressor function in many kinds of malignancies. It is highly expressed in malignancies, including hematological malignancies such as acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML) and myelodysplastic syndromes (MDS), and solid cancers [3–12] (all the literature were not cited because of limited space) (Table 1). *WT1* mRNA level in peripheral blood (PB) or bone marrow (BM) is now being used as a marker of minimal residual disease (MRD) of leukemia [3,5,7].

$CD8^+$ cytotoxic T lymphocytes (CTLs) are the most important effectors for antitumor immune responses, and they recognize tumor-associated antigen (TAA)-derived peptides that are 'processed' and presented on cancer cell surface in association with major histocompatibility complex (MHC) class I molecules, leading to killing of the cancer cells [13,14]. Clinical evidence for effectiveness of antitumor immune responses was obtained in several clinical settings including graft-versus-leukemia (GVL) effect after allogeneic hematopoietic stem cell transplantation (HSCT) [15].

These findings strongly suggested that WT1 protein might be a promising target antigen for cancer immunotherapy [4,16–18]. Tumor escape from immune surveillance as a result of down-regulation of *WT1* expression is unlikely to occur, because expression of *WT1* seems to have an essential role in leukemogenesis or tumorigenesis, and to be required to maintain the transformed phenotype/function [8,11,12]. This is a theoretical advantage for using WT1 protein as a target antigen for cancer immunotherapy.

Identification of WT1 protein-derived CTL epitopes and *in vitro* generation of WT1-specific CTLs

For the development of WT1 peptide cancer vaccine, the identification of HLA class I-restricted CTL epitopes derived from WT1 protein is essential [4]. Several groups succeeded in the identification of the CTL epitopes with the restriction of HLA-A*0201 or HLA-A*2402, which was a frequent HLA class I type in Caucasian or Japanese, respectively [4,16,18–24] (Table 2). These WT1 peptide-induced CTLs killed endogenously *WT1*-expressing cancer cells [18–26], indicating that the epitope peptides were 'processed' from WT1 protein in cancer cells, followed by presentation on the cell surface in association with HLA class I molecules to be recognized by WT1-specific CTLs.

A modified HLA-A*2402-restricted WT1 peptide, in which a single amino-acid substitution was introduced

Table 1

Malignant diseases that express WT1

Hematopoietic malignancies	Solid cancers
Acute myeloid leukemia (AML)[a]	Lung cancer[a]
Acute lymphocytic leukemia (ALL)	Breast cancer[a]
Chronic myelogenous leukemia (CML)	Head and neck squamous cell carcinoma
Myelodysplastic syndromes (MDS)[a]	Thyroid cancer
Multiple myeloma (MM)[a,b]	Esophageal cancer
Non-Hodgkin lymphoma (NHL)	Gastric cancer
	Colorectal adenocarcinoma
	Biliary duct cancer
	Pancreatic ductal adenocarcinoma
	Renal cancer[a]
	Prostate cancer
	Ovarian cancer
	Uterus cancer
	Primary astrocytic cancer[a]
	Bone and soft-tissue sarcoma
	Malignant melanoma
	Malignant mesothelioma
	Testicular germ cell tumor

[a] Diseases for which WT1 peptide vaccination-induced clinical responses were shown in the literature.
[b] MM cells are susceptible to WT1-specific CTLs in spite of rather low expression of *WT1* mRNA in MM cells (see text for details).

at an anchor residue of a natural peptide, was reported [27]. Binding affinity of the modified peptide to HLA-A*2402 molecule was much increased, and the peptide elicited WT1-specific CTLs more efficiently than the natural peptide. Thus, this modified peptide was considered to be very useful for vaccination of HLA-A*2402$^+$ cancer patients. Another modified peptide with the restriction of HLA-A*0201 was also reported recently [28].

Table 2

WT1 protein-derived CTL epitopes that elicit WT1-specific CTLs

HLA-A*0201 restriction (the 2nd and 9th amino acids are anchor positions)	
RMFPNAPYL[a]	[126]
SLGEQQYSV	[187]
CMTWNQMNL[b]	[235]
YMFPNAPYL[c]	[126] (modified at the 1st position of peptide-a)
HLA-A*2402 restriction (the 2nd and 9th amino acids are anchor positions)	
CMTWNQMNL[b]	[235]
CYTWNQMNL[d]	[235] (modified at the 2nd position of peptide-b)
RWPSCQKKF	[417]

[b]This peptide elicits WT1-specific CTLs with the restriction of both HLA-A*0201 and HLA-A*2402. Peptide-c is not a natural WT1 peptide, but was modified from peptide-a. Peptide-d is not a natural WT1 peptide, but was modified from peptide-b. Numbers in brackets represent the first amino acid positions among the whole amino acid sequences of human WT1 protein. These peptides shown here are candidates for WT1 peptide vaccine.

Since several kinds of normal cells, including hematopoietic progenitor cells, physiologically express *WT1*, it is critical to know whether WT1-specific CTLs cause damage to normal tissues, if we apply WT1-directed immunotherapy to the clinical setting. It was demonstrated that WT1-specific CTLs, which killed *WT1*-expressing leukemia cells, did not inhibit colony-formation by BM cells, indicating that the CTLs did not attack *WT1*-expressing normal hematopoietic progenitor cells [18–20]. The selective CTL killing of leukemia cells but not normal hematopoietic progenitor cells, both of which express *WT1*, may be explained by the difference in *WT1* expression level between malignant and normal hematopoietic cells [19]. Further studies are needed to address this issue.

Human WT1-specific CTLs and the restricting HLA allele-matched *WT1*-expressing cancer cells were transplanted in immunodeficient mice to investigate the CTLs' killing activity *in vivo* [21,29,30]. In these experiments, inhibition of cancer cell growth because of attack by the CTLs and preferential accumulation of the CTLs to tumor site was observed. It was also shown that the CTLs did not inhibit engraftment of normal CD34$^+$ hematopoietic stem cells [30]. These results strongly suggested that WT1-specific CTLs attacked cancer cells but not normal cells *in vivo* as well as *in vitro*.

Spontaneous immune responses against WT1 protein in cancer patients

Recent investigations demonstrated that immune responses against WT1 protein, both humoral and cellular, were naturally elicited in cancer patients, indicating that WT1 protein is immunogenic [31–36,37•]. These findings provided us with a rationale for developing WT1-targeting cancer immunotherapy.

In a report, it was demonstrated that many patients with hematological malignancies such as AML, CML, and MDS responded to leukemia cell-derived WT1 protein and produced IgM-type and IgG-type WT1 antibody [33], indicating that not only WT1-responding B cells but also T cells needed to induce class-switch of WT1 antibody were activated in these patients. Analysis of MDS patients revealed that class-switch of WT1 antibody from IgM to IgG occurred along with the disease progression from refractory anemia (RA) to refractory anemia with excess of blast (RAEB), and further to RAEB in transformation (RAEB-t), that is with an increase in amount of tumor that stimulates patients' immune system. Furthermore, in AML patients, WT1 antibody disappeared after the achievement of complete remission, suggesting that decrease in stimulation of the immune system by leukemia cell-derived WT1 protein gave rise to discontinuation of WT1 antibody production.

It was also demonstrated that Th1-type, but not Th2-type, WT1 antibody significantly increased in PB of patients with leukemia or MDS, compared to healthy volunteers [34], indicating that Th1-biased WT1-specific immune responses, which were essentially needed for cancer immunotherapy targeting WT1, might be elicited in these patients.

The question of whether WT1-specific $CD8^+$ T cell responses, the most important responses in cancer immunotherapy targeting WT1, spontaneously occurred in leukemia patients was investigated [35,36]. T cells recognizing HLA-A*0201/WT1 peptide complex could be detected at a relatively high rate by ELISPOT or intracellular IFN-γ detection assay in PBMCs of HLA-$A*0201^+$ AML patients [35], which provided us direct evidence for spontaneous CTL responses against WT1 protein in leukemia patients. It is interesting that the responses to leukemia-related antigens, including WT1, were higher in CML or ALL patients after HSCT than those before HSCT [36,37•]. The increased responses to WT1 in these patients after HSCT may be one of the possible explanations for GVL effect of allogeneic HSCT. Using quantitative reverse transcription-PCR to measure IFN-γmRNA production by $CD8^+$ T cells, T cell responses directed against HLA-A*0201-restricted WT1 epitopes in leukemia patients and healthy donors were also detected [38].

As for solid cancer, functional HLA-A*0201/WT1 tetramer-binding T cells were expanded from tumor-draining lymph nodes in patients with early stage breast cancer [39], which suggested that WT1 protein-responding CTLs were enriched or activated in the tumor-draining lymph nodes. One report strongly suggested that WT1-specific CTLs were involved in graft-versus-tumor (GVT) effect in HSCT for solid cancer [40].

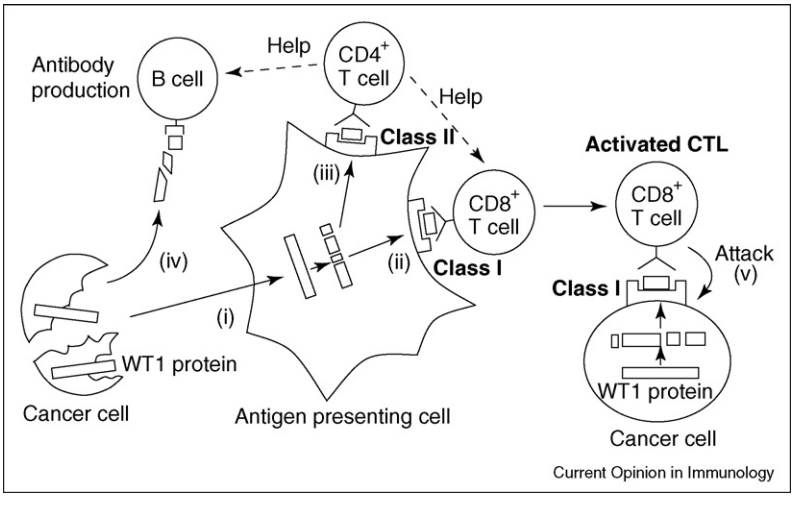
In cancer patients with HLA-A*2402, as well as those with HLA-A*0201, it was also shown that HLA-A*2402/WT1 tetramer-binding $CD8^+$ T cells were detected in PB of patients with leukemia or solid cancer at higher frequencies than that of healthy donors [41••].

Mouse *in vivo* models for WT1 peptide cancer vaccine

Mouse models are very useful to see whether WT1 protein can serve as a tumor rejection antigen *in vivo*.

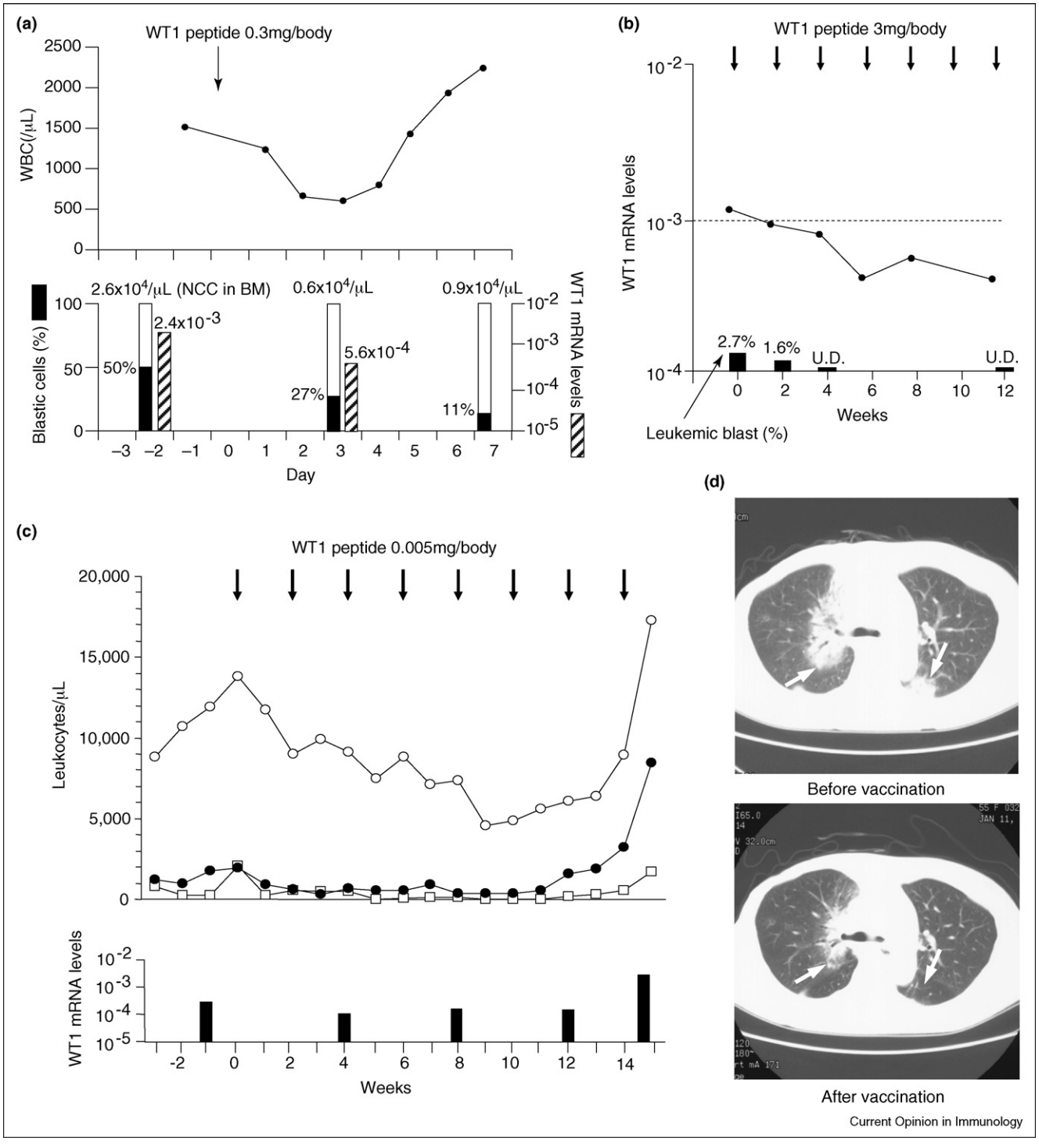
When C57BL/6 mice were immunized with activated APCs pulsed with WT1 peptide (Db126: a.a.126–134, RMFPNAPYL: underlined letters represent anchor motifs for $H\text{-}2D^b$), which had relatively high-binding affinity for $H\text{-}2D^b$ molecules, WT1-specific CTLs were generated from the spleen cells [17]. Furthermore, the immunized mice rejected challenges by *WT1*-expressing cancer cells more efficiently than non-immunized mice, while the vaccination-induced CTLs did not give damage to normal tissues that physiologically expressed *WT1* [17]. In mice injected with plasmid DNA encoding mouse full-length WT1 protein, the similar results were obtained [42].

Figure 1



Elicitation of immune responses against WT1 protein in cancer patients. Cancer cell-derived WT1 protein is ingested by antigen-presenting cells (APCs) such as dendritic cells (DCs) **(i)**, and is processed in them, followed by presentation of WT1 peptides in association with HLA class I or II molecules on the surface of the APCs **(ii and iii)**, while the WT1 protein stimulates B lymphocytes to produce anti-WT1 antibody **(iv)**. WT1 peptide/HLA class I complexes stimulate $CD8^+$ T cells to make WT1-specific cytotoxic T lymphocytes (CTLs) (ii). WT1 peptide/HLA class II complexes stimulate $CD4^+$ T cells to make WT1-specific helper T cells (iii), which more activate ('help') CTLs and B cells, respectively. Activated B cells produce anti-WT1 antibody of class-switched IgG-type as well as IgM-type. Activated WT1-specific CTLs attack cancer cells that have WT1 peptide/HLA class I complexes on the cell surfaces **(v)**.

Figure 2



Representative cases that showed clinical responses to WT1 peptide vaccination. **(a)** An MDS-derived overt AML patient. After a single injection of WT1 peptide, leukemic blast cells and *WT1* mRNA levels in BM decreased (lower). At the same time, leukocytopenia appeared (upper) because hematopoiesis was mainly sustained by *WT1*-expressing malignant hematopoietic stem/progenitor cell-derived blood cells in MDS patients (see text for details). Modified from a figure in *Int J Hematol* 2003, **78**:56–61 (Copyright). **(b)** A *de novo* AML patient. During WT1 peptide vaccination, leukemic blast cell percentage and *WT1* mRNA level gradually decreased. In contrast to an MDS patient (a), leukocytopenia did not occur. Modified from a figure in *Proc Natl Acad Sci U S A* 2004, **101**:13885–13890 (Copyright). **(c)** A CMML patient treated with a very low dose of WT1 peptide. During WT1 peptide vaccination, counts of white blood cells (open circles), monocytes (closed circles), and myelocytes plus metamyelocytes (open squares) as well as *WT1* mRNA level 'gradually' decreased. This clinical course was in contrast to an MDS patient treated

The question of whether WT1 peptide vaccination had the potency to reject cancer cells in therapeutic settings was also addressed [43]. Intradermal injection of *Mycobacterium bovis* bacillus Calmette-Guerin cell wall skeleton (BCG-CWS) as an adjuvant to C57BL/6 mice, followed by intradermal injection of WT1 peptide (Db126) at the same site on the next day, generated WT1-specific CTLs and led to rejection of *WT1*-expressing cancer cells which had been implanted into the mice before the vaccinations. Mice treated with WT1 peptide and BCG-CWS survived significantly longer than those vaccinated with WT1 peptide alone, or injected with BCG-CWS alone. No damage in physiologically *WT1*-expressing normal tissues was observed in the treated mice.

Clinical trials of WT1 peptide cancer vaccine

On the basis of preclinical results, which strongly suggested occurrence of WT1-directed immune responses in patients (Figure 1), clinical trials of WT1 peptide vaccine were started (Table 2).

Clinical responses

Patients with myeloid malignancies, including AML and MDS

Oka *et al.* reported results about a phase I clinical trial of WT1 peptide vaccine for HLA-A*2402$^+$ cancer patients [41••,44,45]. 0.3, 1.0, or 3.0 mg of WT1 peptide was injected intradermally at two-week intervals with Montanide ISA51 adjuvant. Clinical responses in leukemia or MDS patients were reported [41••,44].

A patient with MDS-derived leukemia was injected with 0.3 mg of WT1 peptide emulsified with Montanide ISA51 (Figure 2a). The vaccination resulted in an increase in frequency of WT1-specific CTLs in PB, followed by a rapid reduction in leukemic blast cells and *WT1* level in BM, accompanied with severe leukocytopenia in PB. It was speculated that leukocytopenia was induced because most normal-appearing hematopoietic cells in MDS patients were derived from *WT1*-expressing malignant hematopoietic stem/progenitor cells, which were attacked by the vaccination-induced CTLs. It is probably that leukocytopenia was specific to MDS patients in whom hematopoiesis was mainly sustained by transformed hematopoietic cells. Therefore, the induced leukocytopenia was considered to be a clinical effect as well as an adverse effect. Another WT1 peptide-vaccinated MDS patient (collectively, two among two MDS patients) also showed the similar clinical course.

In some *de novo* AML patients who were in hematological complete remission (CR) but had MRD at microscopic and/or molecular level, a decrease in leukemic cells and/or *WT1* mRNA level was observed (Figure 2b). In contrast to MDS patients, leukocytopenia was not observed, because normal hematopoiesis remained enough in *de novo* AML patients. In this phase I trial, of the 14 patients with leukemia, including *de novo* AML and MDS, 7 patients showed clinical responses such as a reduction of leukemic cells and/or *WT1* mRNA level.

Mailaender *et al.* reported a WT1 peptide-vaccinated HLA-A*0201$^+$ AML patient [16,46]. Injections of WT1 peptide with GM-CSF and KLH led the patient to CR, and the remission persisted for more than one year.

Very recently, Rezvani *et al.* reported results about a phase I trial in which WT1 peptide and proteinase 3-derived PR1 peptide in Montanide ISA51 were injected with GM-CSF to HLA-A*0201$^+$ patients with myeloid malignancies [47••]. Decrease in *WT1* expression level, a MRD marker, was observed after the combined vaccination in patients with AML or MDS.

Patients with multiple myeloma (MM)

WT1 mRNA level in myeloma cells was lower than that in acute leukemia cells, leading to a tentative conclusion that MM might not be a good target disease for WT1-directed cancer immunotherapy. However, a recent investigation showed that myeloma cells were lysed efficiently by WT1-specific CTLs in spite of rather low *WT1* mRNA level in the cells [48]. The high sensitivity of myeloma cells to CTL-mediated cytotoxicity appeared to be because of high susceptibility of the cell membrane to perforin. On the basis of these findings, a clinical trial of WT1 peptide vaccination for MM patients was started [49]. Clinical responses, including decrease in M-protein amount in urine, decrease in myeloma cell percentages in BM, and improvement in bone scintigram, were observed in a WT1 peptide-vaccinated MM patient.

Patients with MDS, vaccinated with 'very low dose' of WT1 peptide

As we mentioned before, injection of 0.3 mg WT1 peptide, a usual dose in peptide vaccination therapy, induced severe leukocytopenia as well as a decrease in leukemic blast cells and/or *WT1* mRNA level in MDS patients, which led us to construct new WT1 peptide treatment strategies for MDS patients with little normal hematopoiesis [41••,44,45]. Very low dose (0.005 mg/body) of WT1 peptide vaccine was intradermally injected at two-week intervals with Montanide ISA51 to a patient with chronic

with a usual dose of WT1 peptide (a). Modified from a figure in *Int J Hematol* 2007, **85**:426–429 (Copyright). **(d)** A breast cancer patient with lung metastasis. After the repeated WT1 peptide vaccination, tumor size of lung metastasis decreased. Modified from a figure in *Proc Natl Acad Sci U S A* 2004, **101**:13885–13890 (Copyright).

myelomonocytic leukemia (Figure 2c) [50]. The patient showed immunological responses such as an increase in WT1 tetramer$^+$ cell frequencies in PB, and the resultant clinical responses, including a 'gradual' decrease in leukocyte, monocyte, and immature cell count into a normal range, instead of 'rapid' leukocytopenia.

Patients with solid cancer

A phase I trial performed by Oka *et al.* also reported clinical responses in patients with lung or breast cancer [41••,51]. One clinical response-positive case is shown in Figure 2d, in which breast cancer metastasis in lung regressed after vaccination. One lung cancer patient and one breast cancer patient have been vaccinated for more than one year with maintained clinical benefit and good quality-of-life (QOL), which may be characteristic of immunotherapy. Among 12 patients with lung or breast cancer, 5 patients showed clinical responses such as a decrease in tumor size or tumor marker [41••].

A phase I/II trial of weekly injection of WT1 peptide vaccine for HLA-A*2402$^+$ patients with various kinds of solid cancers was started. The phase I part composed of the first 10 patients was finished [52]. The vaccination-related systemic toxicities were not observed. As disease-specific phase II part of this trial, a result about 21 recurrent glioblastoma patients was reported [10,53]. Two partial response (PR) and 10 stable disease (SD) in Response Evaluation Criteria in Solid Tumors (RECIST) were obtained. The median progression-free survival (PFS) period was 20.0 weeks, and PFS rate at 6 months was 33.3%. WT1 peptide vaccination was considered active for the treatment of recurrent glioblastoma, because a review of the literature suggested that an agent demonstrating a six-month PFS rate of 10% or greater would be considered active [53].

Long-term SD in renal cancer patients was also reported [54].

No damage to normal tissues by WT1 peptide vaccination

In phase I clinical trials for WT1 peptide vaccination, no damage to physiologically *WT1*-expressing normal tissues was reported [40,41••,46,47••,49], which was expected from preclinical data that was described before in this article [17–20]. This result demonstrated that WT1 vaccination-induced CTLs attacked *WT1*-expressing cancer cells, but not physiologically *WT1*-expressing normal cells. Even if WT1 peptide vaccination-related side effect might occur, the probability of occurrence of severe side effect should be very low.

Immunological responses and their correlation with clinical responses

In a phase I trial (AML, MDS, breast or lung cancer) reported by Oka *et al.*, a significant correlation between immunological (increase in frequencies of WT1 tetramer$^+$ CD8$^+$ T cells in PB) and clinical responses was observed in the 19 evaluable patients, indicating that WT1-specific CTLs induced by WT1 vaccination played important roles in the clinical responses [41••]. A case report (AML) by Mailaender *et al.* showed an increase in frequencies of WT1 tetramer$^+$ CD8$^+$ T cells in PB in correlation with a decrease in leukemic blast cells and *WT1* mRNA level [46]. In a phase I trial (AML, MDS, and CML) reported by Rezvani *et al.*, in which both WT1 and PR1 peptides were injected to patients, the emergence of WT1 or PR1 tetramer$^+$ CD8$^+$ T cells was associated with a decrease in *WT1* mRNA level, suggesting a vaccine-driven antileukemic effect [47••]. They also showed that the loss of WT1 or PR1 response was associated with reappearance of *WT1* transcripts, a MRD marker.

In a phase II trial targeting glioblastoma and a case report for an MM patient, WT1 vaccination-driven induction of clinical responses was not obviously associated with an increase in frequencies of WT1 tetramer$^+$ CD8$^+$ T cells in PB [49,53]. However, these patients had relatively high frequencies of the tetramer$^+$ cells already before the vaccination [49,53].

A case report for two renal cancer patients showed that delayed type hypersensitivity (DTH) response for WT1 peptide turned positive after the vaccination, associated with stabilization of disease [54]. In one of the two patients, an increase in frequencies of WT1 tetramer$^+$ CD8$^+$ T cells in PB was also observed after the vaccination.

It is difficult to directly compare immunogenecity of WT1 protein with that of other TAAs in the clinical setting, because each clinical trial did not use the same protocol. For example, the following might be different: first, were a single kind of peptide or multiple kinds of peptide administered?; second, if multiple kinds of peptide were administered, were pre-existing immune responses against target antigens examined before vaccination for 'selection' of the kinds of peptide to be administered? [55]; third, what are the patient characteristics? (early or advanced stages?; hematological malignancies or solid cancers?); and fourth, what kind of adjuvant or cytokine was used? According to accumulating evidences, however, it seems obvious that WT1 protein-derived CTL epitopes (peptides) identified so far are highly immunogenic in the clinical setting. It is notable that only a single kind of WT1 peptide, such as WT1-126 or WT1-235, could induce an increase in WT1 tetramer$^+$ CD8$^+$ T cell frequencies and/or make the peptide-specific DTH reaction positive after vaccination, leading to the emergence of clinical responses [41,44,46,50,54]. It was also shown that only a single injection of WT1 peptide could induce an increase in WT1 tetramer$^+$ CD8$^+$ T cells [44,47••].

Perspectives

New target diseases for WT1-directed cancer immunotherapy

Drakos *et al*. recently demonstrated that WT1 protein was frequently detected, primarily in the cytoplasm, of a subset of high-grade NHLs [56] (Table 1). Clinical trials of WT1 peptide vaccination for NHL patients should also be planned.

Enhancement of clinical efficacy and usefulness of WT1 peptide vaccine

Although it was revealed that WT1 peptide vaccination had clinical efficacy and usefulness at least for some patients [41••,44–46,47••,49–54], we need to further improve the vaccine. Strategies to immunologically enhance the power of the vaccine may include first, to find or develop more strong adjuvants; second, to use HLA class II-restricted helper epitopes which enhance induction/activation of CTLs and make 'memory' CTLs in combination with CTL epitopes [57–59,60•,61]; and third, to use multiple CTL epitopes [55,62••].

In addition to treatment using only cancer vaccine with appropriate adjuvant/cytokine, combined usage of cancer vaccine and other kinds of drugs, including molecular-target-based drugs such as imatinib [62••], or chemotherapy drugs [63–65], might be another strategy to develop a novel modality for anticancer action. It was recently reported that addition of a multiple peptide vaccine against BCR-ABL-derived fusion protein to imatinib treatment in CML patients induced improved cytogenetic responses [62••]. Blockade or removal of regulatory T cells by using approaches such as administration of cyclophosphamide or gemcitabine-containing chemotherapy might improve the efficacy of peptide vaccines [63–65]. Gemcitabine was also revealed in a mouse model to eliminate splenic Gr-1$^+$/CD11b$^+$ myeloid suppressor cells [65].

Optimization of WT1 peptide dose for the treatment of MDS patients is also a major subject. Although vaccination of a CMML patient with a very low dose of WT1 peptide safely reduced leukocyte, monocyte and immature cell count and *WT1* mRNA level, these parameters started to increase again three months after the start of the vaccination. The dose (0.005 mg/body) might be too low to elicit enough immune reaction.

Since human T cell receptors (TCRs) that recognized WT1peptide/HLA class I complex were cloned, WT1-specific T cell gene therapy is also expected [66–68].

Translational research and reverse-translational research

Translational research is composed of basic research and the subsequent clinical trials. The latter is based on 'scientific' data obtained from the former. Therefore, we can obtain 'science'-based valuable information about TAA-directed immune responses by analyzing samples from the vaccinated patients.

According to accumulating evidences, it seems convincing that WT1 vaccine has potential for anticancer action. As the next step, we must investigate what kind of immune responses are induced by the vaccination, and how the induced immune responses lead to clinical responses. Even after cancer cells are damaged by CTLs, immune reactions continue. For example, epitope (of WT1 protein)-spreading or antigen (other than WT1 protein)-spreading occurs, followed by the activation of various kinds of CTLs or helper T cells.

To clarify these immune reactions lead to construction of 'proof of concept' for WT1 peptide vaccination, and may lead to establishment of reliable immune reaction-monitoring methods to predict clinical responses, or improvement of cancer vaccine.

Evaluation of cancer vaccine-induced clinical responses

Cancer chemotherapy drugs directly attack cancer cells, while cancer vaccine does not. The latter indirectly give damage to cancer cells by the activation of immune system. Therefore, we may need cancer vaccine-specific response evaluation criteria [45,69,70].

It is probable that some of the cancer vaccine-treated patients survive long-term with good QOL even if tumor regression is not obvious [41••,53,54], or that the tumor growth may be stabilized after an initial increase in its size because immunotherapy is generally not as quick-acting as chemotherapy [45,69,70]. Therefore, when we evaluate vaccination-induced clinical responses with RECIST, which is a gold standard in the field of cancer chemotherapy, it may be recommended that SD is highly regarded in cancer immunotherapy, particularly when SD persists long-term. It may also be recommended that the stabilization of disease after initial progression of disease, which is categorized as progressive disease (PD) in RECIST, is also evaluated favorably, like 'SD after PD'.

Appropriate clinical settings for cancer vaccine

Once we accumulate evidences that show the potential of WT1 peptide vaccine to induce WT1-specific immunological responses that lead to clinical responses in early phase clinical trials, we should start clinical trials in 'adjuvant setting', in which disease is 'morphologically or radiologically undetectable but high risk of relapse' after operation and/or chemotherapy. In this setting, effecter/target ratio is high, and immuno-suppressive environment induced by high amount of cancer cells is improbable. Adjuvant setting should be the most appropriate setting in which cancer vaccine show its ability. Recurrence of disease may be reduced or postponed by the vaccination.

Conclusion

We summarized recent investigation about elicitation of cancer antigen WT1-directed immune responses, and its clinical application as WT1 peptide cancer vaccine. Our understanding of cancer antigen-directed immune responses at the cellular and molecular level continues to grow, which should lead to further development of cancer immunotherapy.

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- • of special interest
- •• of outstanding interest

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