Review

Wilms' Tumor 1 (WT1): The Vaccine for Cancer

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ABSTRACT

Vaccines have been used to fight and protect against infectious diseases for centuries. With the emergence of immunotherapy in cancer treatment, researchers began investigating vaccines that could be used against cancer, especially against tumors that are resistant to conservative chemotherapy, surgery, and radiotherapy. The Wilms' tumor 1 (WT1) protein is immunogenic, has been detected in almost all types of malignancies, and has played a significant role in prognosis and disease monitoring. In this article, we review recent developments in the treatment of various types of cancers with the WT1 cancer vaccine; we also discuss theoretic considerations of various therapeutic approaches, which were based on preclinical and clinical data.

Keywords: peptide-based vaccine, immunotherapy, *WT1* gene, leukemia, solid cancer, toxicity, preclinical trails, clinical trials, cancer medicine, cancer vaccine

INTRODUCTION

Immunotherapy, or stimulating the body's immune system to fight disease, has been used in the fight against cancer since the late 18th century.[1] Immunotherapy has an *active form* consisting of therapeutic cancer vaccines, immunostimulatory cytokines, and checkpoint inhibitors, and a *passive form* that includes tumor-targeting monoclonal antibodies, immunomodulatory monoclonal antibodies, oncolytic viruses, and adoptively transferred T cells.[2–6] Tumor response to immunotherapy can take more time than tumor response to chemotherapy, and for most patients who receive immunotherapy, the tumor progresses before it regresses; therefore, treatment outcome in immunotherapy cannot be evaluated by the same criteria used to evaluate response to chemotherapy.[7]

In 2009, the National Cancer Institute, selected for study 75 cancer antigens on the basis of (1) therapeutic function, (2) immunogenicity, (3) specificity, (4) oncogenicity, (5) cells' positive rates for antigens, (6) stem cell expression, (7) number of patients who were positive for the antigen, (8) number of epitopes, and (9) cellular location of expression.[8] According to these well-vetted criteria, generated by expert panels, Wilms' tumor 1 (WT1) ranked as the most promising among the 75 cancer antigens.[8]

RELATIONSHIP BETWEEN WT1 PROTEIN AND NEOPLASMS

The *WT1* gene, located on human chromosome 11 (band p13), is important in transcriptional regulation, which consists of a proline and glutamine-rich region and 4 zinc finger domains.[9] Homogenous deletion of both alleles is required for the development of Wilms' tumor, a childhood kidney tumor, in which *WT1* was identified as a tumor suppressor gene.[10]

Oji et al[11] found that *WT1* plays an important role as both a tumor suppressor gene and oncogene. Cells from the 32D clone cell line that were infected with wild-type *WT1* proliferated without differentiation, whereas normal control cells and mutant *WT1*-infected 32D clone cells differentiated into mature cells after granulocyte colony-stimulating factor stimulation.[12] Miwa et al[13] concluded that the *WT1* gene plays a crucial role in the early stage of hematologic differentiation.

Higher levels of both WT1 mRNA and protein have been seen in prostate carcinoma than in benign prostate tumors.[14] Miyagi et al[15] also reported that *WT1* gene expression could be detected in several types of immature lymphoid or myeloid leukemia cells without gene mutation. WT1 protein is detected immunohistochemically in biopsy specimens of most types of cancer.[16] Although very little or no *WT1* gene expression was

noted with immunostaining for the *WT1* gene, more *WT1* gene expression was observed with reverse transcriptase–polymerase chain reaction, and Oji et al[17] determined that this discrepancy was primarily due to tests with various levels of sensitivity.[18]

The *WT1* gene is important not only for prognosis but also for diagnosis in hematologic malignancies. Increased levels of *WT1* gene expression had a significant role in predicting disease relapse in leukemic patients with complete remission.[19] As in Wilms' tumor, mutation of the *WT1* gene was reported in acute myeloid leukemia (AML), but it was required primarily for disease progression rather than for disease initiation.[20] Simultaneous production of IgG and IgM antibodies was found not only in patients with acute lymphoblastic leukemia, but also in patients with hematologic malignancies, such as AML, chronic lymphoblastic leukemia, and myelodysplastic syndrome, owing to repeated and continuous activation of WT1 antigens from leukemic cells.[21]

Study of the *WT1* gene significantly improved our understanding of solid tumor malignancies. The *WT1* gene is expressed not only in all colorectal carcinomas but also in most normal-appearing colorectal mucosal tissues, and expression of this gene has been reported to be greatly varied.[22] This kind of variation in gene expression was also noted in almost all types of thyroid cancers, in thyroid adenoma, and in normal-appearing thyroid tissue, but was present in only 30% to 70% of tumor cells in adenomas that were weakly stained immunohistochemically for WT1 protein.[23] Expression without mutation was observed in bone and soft-tissue sarcoma.[24] Malignant mesothelioma can be differentiated from other types of cancers, such as adenocarcinoma and squamous cell carcinoma, by WT1 immunostaining.[25]

TYPES OF WT1 VACCINE

The *WT1* gene acts as an oncogene that initiates the proliferation of malignant cells.[26] Loss or mutation of the gene, which can lead to loss of immune vigilance, is not common with WT1 antigen[27] and immune response against WT1 is illustrated in Figure 1.[28] Clinical trials for the WT1 vaccine are summarized in Table 1.

The WT1 vaccine can be categorized into the following 4 groups, depending on the use of the WT1 antigens: (1) human leukocyte antigen (HLA)-restricted peptide vaccines, (2) non–HLA-restricted long peptides vaccines, (3) dendritic cell (DC) vaccines loaded with HLA-restricted peptide, and (4) DC vaccines loaded with mRNA encoding full-length WT1.[28] Among the various types of WT1 vaccines, HLA-restricted WT1 peptides had been used in most of the trials and were extensively investigated.[29] Although HLA-restricted WT1 peptides have the advantage of being simple and effective, Van Driessche et al[29] concluded that they were restricted to individual patients' HLA haplotype and could activate only cytotoxic $CD8^+$ T cells.

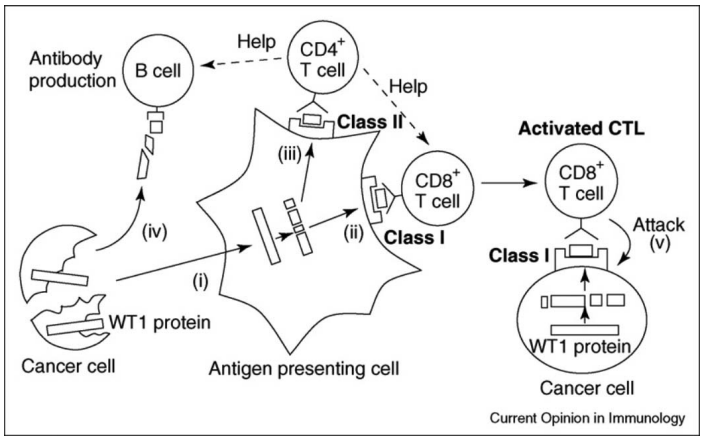

Figure 1.—Elicitation of immune responses against Wilms' tumor 1 (WT1) protein in cancer patients. Cancer cell-derived WT1 protein is ingested by antigen-presenting cells (APCs), such as dendritic cells (DCs) (i), and is processed in them, followed by presentation of WT1 peptides in association with human leukocyte antigen (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") cytotoxic T lymphocytes (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). Reprinted from *Current Opinion in Immunology*, Vol 20, Oka Y, Tsuboi A, Oji Y, et al, WT1 peptide vaccine for the treatment of cancer, 211-220, Copyright 2008, with permission from Elsevier.

When the heteroclite WT1-A1 peptide's sequence was inserted into the longer WT1-122A1–long peptide, which can activate both $CD4^+$ and $CD8^+$ cells, the peptide vaccine was enhanced to be better recognized by T-cell receptors and to have increased immunogenicity over the various HLA subtypes.[30] When WT1 peptide and keyhole limpet–hemocyanin-pulsed donor-derived DC vaccine were given to a patient with relapsed AML after allogeneic hematopoietic stem cell transplantation, no graft-versus-host disease or other serious adverse events were noticed, and only local erythema with grade 2 itching at the injection site was reported.[31]

Although a strong immune response was detected, no clinical response was observed, and Kitawaki et al[31] concluded that sufficient potency of WT1-specific responses for the growing leukemic cells might be needed. Kitawaki et al[31] also recommended treating patients with detectable WT1-specific memory $CD8^+$ T cells before immunization and only those patients with less invasive tumors, such as patients with minimal residual disease or those whose disease is in remission.

In DC-based vaccine, vaccine antigen needs to be in contact with DCs to generate an immune response.[32] The extent of antigen needed for the DCs was unknown.[33] Zityogel et al[34] found that these antigens could be used in immunotherapy for cancer in clinical trials. For example, expression of WT1 mRNA can be detected in bone marrow and peripheral blood in patients with AML.[35–40] Protein vaccination can acti-

Table 1.—Clinical trials for WT1 peptide-based vaccine in solid and hematological malignancies

NCT Number	Reference	Types of Neoplasm	Intervention	Sample Size	End Point	Outcomes	Adverse Effects
NR	53	AML	WT1 vaccine in combination with zoledronate	3	WT1 immune response was noted in patients with transient decline in leukemic cells and stable disease	SD – 1 PD – 1	No serious adverse effect
00398138	54	AML	WT1 peptide vaccine	9	Median overall survival – 35+ mo Mean time for follow-up from diagnosis – 30 mo	CCR – 5 RD – 4	No serious adverse effect except in 1 patient who developed generalized urticaria and laryngeal spasm as a delayed grade 2 reaction to vaccine
NR	55	AML	WT1 peptide vaccine	10	Immune responses that are closely related to clinical response	Molecular remission – 5	No serious adverse effect except in 1 patient with pain in axillary LN along drainage and another patient who exhibited transient decline in platelet count and mild flare-up of preexisting Achilles and foot tendonitis
00313638	56	Myeloid malignancies (AML, MDS, CML)	WT1 and PR1 peptide vaccines	8	Median follow-up of 252 d (105–523 d)	SD – 2 CR – 3 RD – 2 Mol R – 1	No serious adverse effect except in 1 patient who developed chest pain that resolved without intervention, probably owing to GM-CSF
00665002	57	Myeloid neoplasm	WT1 peptide vaccine	13 (MDS – 2, AML – 11)	Patients tolerated the vaccine	Transfusion dependence was noted to be temporally less in a patient with MDS, and relapse-free survival was reported to be longer than 1 year in 2 AML patients	No serious adverse effect
00923910	58	AML, ALL, HL	WT1 peptide vaccine with DLI	5 (ALL – 3, AML – 1, Hodgkin – 1)	WT 1 vaccine is safe and tolerable after allogenic HCT	PD – 5	No serious adverse effect except in 1 patient with grade 1 skin GVHD resolved without systemic treatment
NR	59	Head and neck cancer	WT1 peptide-pulsed dendritic cell vaccination combined with conventional chemotherapy	11	PFS – 6.4 mo OS – 12.1 mo	SD – 5 patients PD – 6 patients	No serious adverse effect
NR	60	Advanced biliary tract cancer	WT1 and/or MUC1 peptide-pulsed dendritic cell vaccination	65	MST from diagnosis – 18.5 mo, MST from the first vaccination – 7.2 mo (WT1 alone – 5.1 mo, MUC1 alone – 6.6 mo, WT1+MUC1 – 8.2 mo)	PR – 4 SD – 15 PD – 44 UE – 2	No serious adverse effect
NR	61	Advanced pancreatic or biliary tract cancer	Combination therapy of WT1 vaccine and GEM	18	MST – 278 d (biliary tract cancer – 288 d, gallbladder cancer – 153 d, intrahepatic bile duct cancer – 384 d, extrahepatic bile duct cancer – 301 d, pancreatic cancer – 259 d)	DCR pancreatic cancer – 89% gallbladder cancer – 25% intrahepatic bile duct cancer – 100% extrahepatic bile duct cancer – 50%	Cytopenia owing to GEM was reported in all patients, grade 3–4 neutropenia in 11 patients and grade 3 anemia in 3 patients
NR	62	Advanced lung cancer	WT1 peptide vaccine	2	Detection of a higher level of WT1-specific CTLs precursors in the pleural fluid than in PBMC	Decline in tumor markers, such as CEA and SLX, was seen after serial WT1 vaccinations in lung cancer patients	No serious adverse effect

Table 1.—Continued.

NCT Number	Reference	Types of Neoplasm	Intervention	Sample Size	End Point	Outcomes	Adverse Effects
NR	63	Breast or lung cancer, MDS, or AML	WT1 peptide vaccine	26	WT1 immune response was noted in 8/11 patients with clinical response (1 patient could not evaluate the immune response because of inadequate PBMC due to pancytopenia)	Clinical response – 12 SD – 2 UE – 6 PD – 6	No serious adverse effect
NR	64	Breast cancer, glioblastoma, malignant fibrous histiocytoma, primary neuroectodermal tumor, and rectal cancer	WT1 peptide vaccine	10	Toxicity for the weekly vaccination was acceptable and antitumor effect was noted	PR – 1 SD – 5 PD – 4	No serious adverse effect
NR	65	Gynecologic malignancies (ovarian cancer, cervical cancer, uterine carcinoma and, corpus cancer)	WT1 peptide vaccine	12	DIC – 25%	SD – 3 PD – 9	No serious adverse effect
00398138	66	Mesothelioma and non–small cell lung cancer	WT1 peptide vaccine	11	MST from initial vaccination – 14 mo	PD – 8 SD – 1 Relapse – 2	No serious adverse effect
NR	67	Pancreatic cancer	WT1 peptide vaccine combined with multimodal therapy	6	Median OS – 1796.5 d Median PFS – 607 d	SD – 3 PD – 3	No adverse effect is mentioned
NR	68	Pediatric cancer	WT1 peptide vaccine	5	WT1 vaccine has potential therapeutic outcomes	Clinical response – 1 SD – 1 PD – 3	No serious adverse effect

ALL, acute lymphoblastic leukemia; APCs, antigen presenting cells; CCR, continuous complete remission; CD, cluster of differentiation; CEA, carcinoembryonic antigen; CLL, chronic lymphoblastic leukemia; CR, complete remission; GEM, gemcitabine; GVHD, graft versus host disease; HCT, hematopoietic cell transplantation; HL, Hodgkin lymphoma; LCLs, lymphoblastoid cell lines; MDS, myelodysplastic syndrome; Mol R, molecular response; MST, median survival time; MUC1, mucin 1 cell surface associated; NCT, national clinical trial; NR, no register; OS, overall survival; PBMC, peripheral blood mononuclear cell; PD, progressive disease; PR, partial response; RD, relapse disease; SD, stable disease; SLX: Sialyl Lewis (x); UE, unevaluable; WT, Wilms' tumor 1

vate humoral immune responses but is rarely used for immunization in clinical trials due to the lack of $CD8^+$ cytotoxic T-cell induction.[29] Full-length WT1 mRNA can be used to transfect DCs to activate not only humoral immunity but also cellular immunity to eradicate cancer cells.[29] Owing to the advantages of a better clinical safety profile and reproducibility, mRNA was used to transfect DCs for WT1 vaccination.[41–44]

After assessing the differences in immunogenicity between WT1 HLA class I peptide ($WT1_{235}$) and WT1 HLA class II peptide ($WT1_{332}$), Tsuboi et al[45] observed more promising immune responses in most of the patients by a surge of ($WT1_{235}$)-specific interferon (IFN)-γ–producing $CD8^+$ T cells and ($WT1_{332}$)-specific tumor necrosis factor-α–producing $CD4^+$ T cells after vaccination with the cocktail vaccine of WT1 HLA class I and II peptides. $CD4^+$helper T cells are important for both priming and effector phases for cytotoxic T lymphocytes (CTLs) via direct helper signals, such as cytokine or cell contact–mediated stimulation.[46] Results suggested that $CD4^+$ HTLs potentiated the immunotherapeutic effect of CTLs by promoting the functional activity of CTLs via an increase in IFN-γ–producing cell frequencies, and that this effect was stronger than that produced by increasing the tetramer of cell frequencies.[46] Because immature antigen-presenting cells cannot prime CTLs, $CD4^+$ HTLs are needed for differentiation of these immature cells.[47–49]

PRECLINICAL TRIALS

When mice were immunized with a WT1 peptide vaccine, we observed not only WT1-specific CTLs but also rejection across tumor cells, which expressed WT1 without autoimmunity, although podocytes of the kidney glomeruli and bone marrow $CD34^+$ cells expressed WT1.[50] Inhibition of cell growth was seen only in the WT1-expressing leukemic cell line when both the leukemic cell line, which expressed *WT1*, and a normal cell line, which did not express *WT1*, were tested with WT1 antisense oligomers; Yamagami et al[51] concluded that WT1 is an essential oncogene in leukemogenesis.

The cytotoxic ability of CTLs was seen in lung cancer cells, which expressed the *WT1* gene, but the antitumor effect of CTLs was inhibited by anti-HLA class I mAb.[52] Decreased cytolytic activity of WT-specific lung cancer CTLs was noted with the WT1-WT2–loaded autologous lymphoblastoid cell line, and the absence of cytotoxicity was observed when WT1-WT2–loaded HLA-A24–negative lymphoblastoid cell lines were tested with CTLs.[52] The growth of lung cancer cells in nude mice decreased when mice were treated with WT1-specific CTLs.[52]

CONCLUSIONS

Immunotherapy, reported to be the best option for eradicating residual malignant cells, has been found to be effective and to have with minimal toxicity in cancer patients. Having a better understanding of the WT1 protein can enhance the scope of cancer medicine, not only for diagnostic purposes but also for treatment protocols for malignancy, especially for patients with advanced or aggressive cancers. Because the outcomes of patients who underwent adjuvant treatments were more promising than were those of patients who did not undergo these treatments, the WT1 vaccine can be one of the reliable standard multimodal therapies in the near future. Although excellent overall survival and PFS rates were reported with WT1 vaccination, clinical trials with better protocol guidelines and larger sample sizes are still needed for data on the safety of the WT1 vaccine.

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