JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2010;40(5)377–387
doi:10.1093/jjco/hyp194
Advance Access Publication 15 April 2010

Review Article

WT1 (Wilms' Tumor Gene 1): Biology and Cancer Immunotherapy

Haruo Sugiyama*

Osaka University Graduate School of Medicine, Osaka, Japan

*For reprints and all correspondence: Haruo Sugiyama, Osaka University Graduate School of Medicine, Department of Functional Diagnostic Science, Osaka 565-0871, Japan. E-mail: sugiyama@sahs.med.osaka-u.ac.jb

Received September 25, 2009; accepted December 18, 2009

Wilms' tumor gene WT1 encodes a transcription factor and plays an important role in cell growth and differentiation. The WT1 gene is highly expressed in leukemia and various types of solid tumors, whereas WT1 is a tumor marker convenient for the detection of minimal residual disease of leukemia. The WT1 gene was originally defined as a tumor suppressor gene, but we proposed that it was, on the contrary, an oncogene. Furthermore, the WT1 protein has proven to be a promising tumor-associated antigen, in which many human leukocyte antigen class I- or II-restricted WT1 epitopes have been identified. Clinical trials of WT1-targeted immunotherapy have confirmed its safety and clinical efficacy. WT1-specific cytotoxic T lymphocytes and WT1 antibodies are spontaneously induced in tumor-bearing patients, probably because of high immunogenicity of the WT1 protein. WT1-specific cytotoxic T lymphocytes make a major contribution to the graft-versus-leukemia effect after allogenic stem cell transplantation. When 75 cancer antigens including WT1 were prioritized according to several criteria such as therapeutic function and immunogenicity, WT1 was ranked as the top antigen. These findings suggest that a new era of WT1 immunotherapy is imminent.

Key words: WT1 – immunotherapy – Wilms' tumor

WILMS' TUMOR GENE (WT1)

The WT1 gene was isolated as the gene responsible for a childhood renal neoplasm, Wilms' tumor, which was thought to arise as a result of inactivation of both alleles of the WT1 gene located at chromosome 11p13 (1,2). This gene encodes a zinc finger transcription factor that plays an important role in cell growth and differentiation (3) and its expression is restricted to a limited set of tissues, including the gonad, uterus, kidney and mesothelium, and to progenitor cells in various types of tissues (4–6). WT1 knock-out mice were found to have defects in the urogenital system and died on ED 13.5, probably due to heart failure (7).

HIGH EXPRESSION OF THE WT1 GENE IN LEUKEMIAS AND SOLID TUMORS

The WT1 gene is highly expressed in the majority of acute myeloid leukemias (AML) and acute lymphoid leukemias (ALL) (Fig. 1) (8–18). This makes WT1mRNA a tumor marker for leukemic blast cells and one leukemic cell in 100 000 normal peripheral blood mononuclear cells (PBMCs) can be detected by quantitation of WT1mRNA (WT1 assay) (10). In chronic myelogenous leukemia (CML) (10) and myelodysplastic syndrome (MDS) (19), WT1mRNA expression levels were seen to increase along with disease progression. The WT1 assay is currently considered to be an essential test managing acute leukemia and MDS by means of detection of minimal residual disease (MRD) of leukemia. The WT1 gene is also expressed at high levels in almost all types of solid tumors (20–32), with its expression level serving as a significant prognostic factor.

THE WT1 GENE PERFORMS AN ONCOGENIC RATHER THAN TUMOR SUPPRESSOR ONE FUNCTION

The WT1 gene was originally defined as a tumor suppressor gene (33–38), but we proposed, on the basis of the accumulating evidence, that the WT1 gene plays an oncogenic function in leukemogenesis and tumorigenesis (39). For example, growth of WT1-expressing leukemic and solid cancer cells

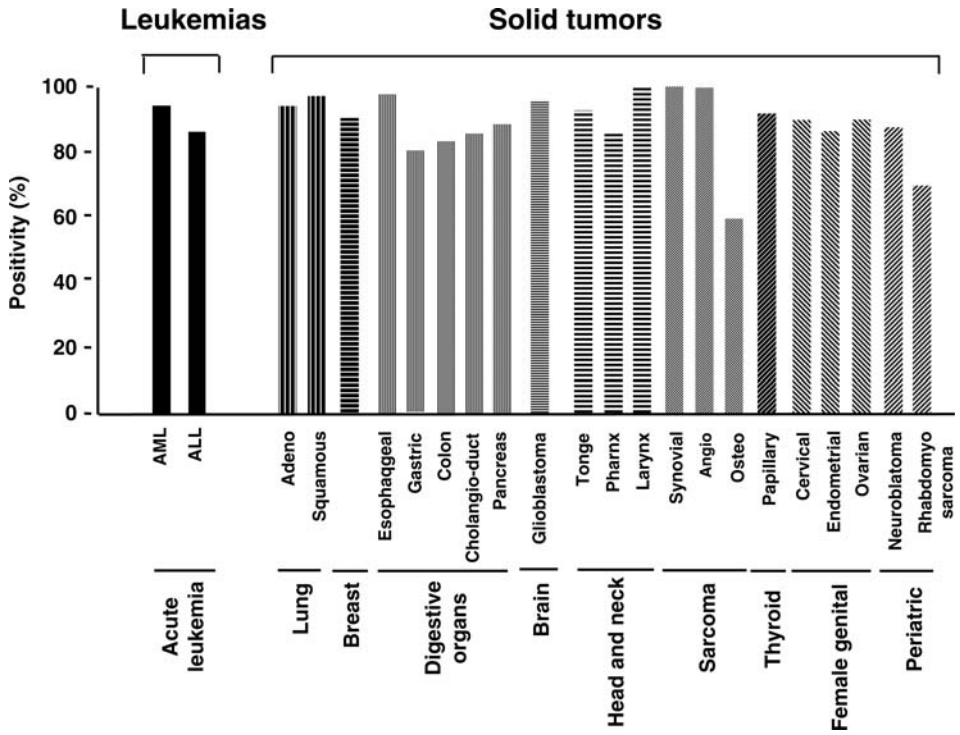

Figure 1. Positivity of WT1 overexpression in leukemia and various types of solid cancers. WT1 overexpression was determined by immunostaining with anti-WT1 antibodies and/or RT–PCR. RT–PCR, reverse transcriptase–polymerase chain reaction; AML, acute myeloid leukemias; ALL, acute lymphoid leukemias.

was inhibited by treatment with WT1 antisense oligomers (40,41) and WT1-specific siRNA (42). Conversely, forced expression of the WT1 gene promoted cell growth (43–45) and motility (46), suppressed apoptosis (47) and induced leukemia in WT1-transgenic mice (48). WT1mRNA expression levels in human leukemic cells and human normal $CD34^+$ hematopoietic progenitor cells (HPCs) were comparatively determined at single-cell level by using single-cell reverse transcriptase–polymerase chain reaction methods (49). Surprisingly, ~1.2% of the $CD34^+$ HPCs expressed WT1mRNA at levels similar to those in leukemic cells. These results indicated that WT1-expressing $CD34^+$ HPCs are the normal counterparts of leukemic cells and that leukemic cells are mainly generated as a result of leukemic transformation of the WT1-expressing $CD34^+$ HPCs. Since it is known that progenitor cells of various types of tissues express WT1 (50), we hypothesized that WT1-expressing progenitor cells can differentiate into tissue-specific cells by down-regulation of WT1 expression, but that if this down-regulation is impaired, WT1-expressing progenitor cells continue to proliferate and transform as a result of occurrence of secondary, tertiary or further genetic events (Fig. 2).

WT1 PROTEIN AS A TUMOR REJECTION ANTIGEN

High expression of the WT1 gene in leukemias and solid tumors indicated that the WT1 protein might be a promising tumor-associated antigen (TAA). Therefore, the murine WT1 protein-derived, MHC class I-restricted, 9-mer WT1 peptides Db126 (aa 126–134), Db227 (aa 227–235) and Db 235 (aa 235–243) were tested for their ability to induce WT1-specific cytotoxic T lymphocytes (CTLs) in a mouse model (51,52). The immunized mice rejected the challenges with WT1-expressing leukemic cells and survived, but showed no histopathological damage of organs that physiologically expressed WT1. Gaiger et al. (53) reported that immunization with the murine MHC class I-binding WT1 peptides p136–144, p235–243 and p117–139 induced WT1-specific CTLs in mice and lysed WT1-expressing tumor cells, while no evidence of autoimmune toxicity was observed. Finally, Gao et al. (54) showed that CML $CD34^+$ cells exposed to WT1-specific CTLs failed to develop leukemia in the recipient severe combined immunodeficiency mice, but CTL treatment did not inhibit engraftment of normal $CD34^+$ HPCs. These findings indicated that the WT1 protein could well be an attractive tumor rejection antigen.

IDENTIFICATION OF HUMAN WT1 PROTEIN-DERIVED CTL (HLA CLASS I) AND HELPER (CLASS II) EPITOPES

When PBMCs from HLA-A*0201-positive healthy donor were stimulated with T2 cells (HLA-A*0201-positive antigen-presenting cell line) pulsed with either HLA-A*0201-restrited 9-mer WT1 peptide Db126 (aa 126–134) or WH186

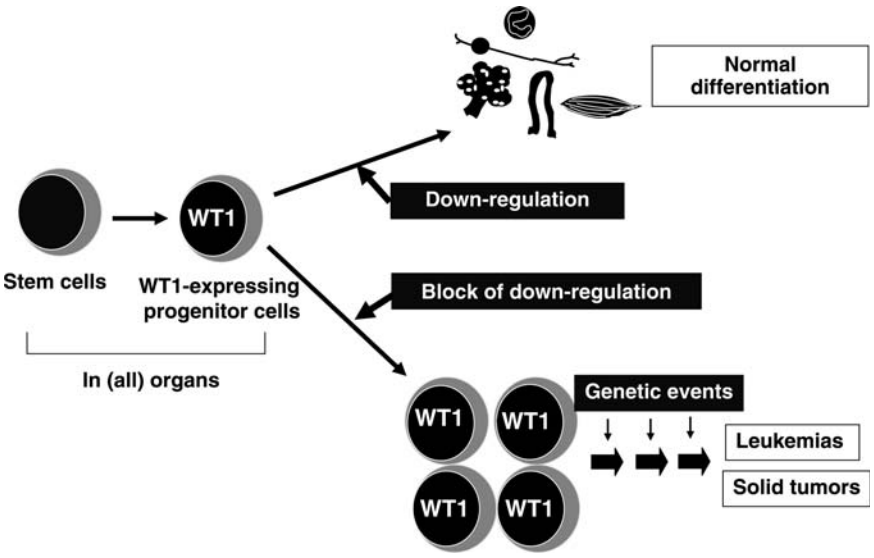

Figure 2. Hypothesis of WT1-involved leukemogenesis and tumorigenesis. Progenitor cells with high WT1 expression exist in almost all organs and differentiate into organ-specific cells along with down-regulation of WT1 expression. When this down-regulation is impaired by undetermined causes, the WT-expressing progenitor cells continue to proliferate and eventually transform into leukemic and solid tumor cells as a result of accumulation of secondary, tertiary or further genetic events. Some specific cells such as podocytes and methothelium physiologically express WT1 at high levels.

(aa 187–195), which were selected to have anchor motifs for the binding to HLA-A*0201 molecules, WT1 peptide-specific CTLs were induced and killed WT1-expressing leukemic cells, but not WT1-non-expressing cells, in an HLA-A*0201-restricted manner (55). Gao et al. (56) showed that P126 WT1 peptide (the same as Db126 in our study)-specific CTLs were elicited by the stimulation of PBMCs from HLA-A*0201-positive healthy donors with HLA-A*0201 stimulator cells that presented P126 WT1 peptide and that the induced CTLs killed endogenously WT1-expressing leukemic cells also in an HLA-A*0201-restiricted manner. Ohminami et al. (57) reported that the TAK-1, WT1-specific $CD8^+$ CTL clone, which was established by repeated stimulation of $CD8^+$ T cells from HLA-$A2402^+$ healthy donor with an HLA-A*2402-restricted 9-mer WT1 peptide (aa 235–243, CMTWNQMNL), recognized naturally processed WT1 peptide and killed endogenously WT1-expressing leukemic cells in a WT1-specific HLA-A*2402-restricted manner. On the other hand, TAK-1 did not inhibit the colony formation of normal bone marrow (BM) cells from HLA-A*2402-positive healthy donors. Tsuboi et al. (58) reported that a modified WT1 peptide, which had a single amino-acid substitution (M → Y) at position 2 of the natural WT1 peptide (aa 235–243) and increased binding affinity to HLA-A*0201 molecules, elicited WT1-specific CTLs more effectively than did the natural WT1 peptide from PBMCs of HLA-A*2402-positive healthy donors. The CTLs induced by the modified WT1 peptide killed endogenously WT1-expressing leukemic cells in an HLA-A*2402-restricted manner. This modified WT1 peptide is currently being used for clinical studies of WT1 peptide-based cancer immunotherapy for HLA-A*2402-positive patients. Azuma et al. (59) identified a novel WT1 peptide (aa 417–425) that elicited WT1-specific, HLA-*A2402-restiricted CTLs, which was followed by identification of many kinds of HLA-A*0201- or -A*2402-restricted WT1 CTL epitopes (60–65).

As for WT1 helper (HLA class II) epitopes, Knights et al. (66) identified an HLA-DRB1*0401-restricted WT1 helper peptide (aa 124–138) that activated dendritic cells, resulting in the sensitization of T cells and secretion of IFN-γ from T cells. Muller et al. (67) demonstrated two HLA-DRB1*0401-restricted WT1 peptides, WT12e and WT331. Fujiki et al. (68,69) also identified a WT1 332 helper peptide (aa 332–347), which could bind to at least four HLA class II molecules (HLA-DRB1*0405, -DRB1*1501, -DRB1*1502 and -DPB1*0901) because of its promiscuous-binding nature. Others have also identified HLA class II-restricted WT1 peptides (70,71).

CLINICAL STUDY OF WT1-TARGETED CANCER IMMUNOTHERAPY

Phase I Clinical Study

A Phase I clinical study of cancer immunotherapy targeting WT1 protein was initiated in 2001. Patients with AML, MDS, lung or breast cancer were intradermally injected with an HLA-A*2402-restricted natural (CMTWNQMNL) or modified (-Y-------) 9-mer WT1 peptide emulsified with Montanide ISA51 adjuvant at 0.3, 1.0 or 3.0 mg/body at 2-week intervals, after which toxicity and clinical and immunological responses were assessed (Table 1) (72–74). The WT1 vaccination was administered to 26 patients. In patients with breast or lung cancer or AML with adequate normal hematopoiesis, toxicity was detected only as local

Table 1. Summary of Phase I clinical study

Patient no.	Age/sex	WT1 peptide (mg/body)	Disease	Stage	Adverse effect (local/systemic)	Clinical responses
1	46/F	(Natural) 0.3	LC (Ad)	IV	+/−	+ (CEA↓)
2	52/M	0.3	LC (Ad)	IIIB	+/−	SD
3	54/F	0.3	LC (Ad)	IV	+/−	Unevaluable
4	70/M	0.3	LC (Sq-ad)	IV	+/−	+ (SLX↓)
5	47/F	Modified (M) 0.3	LC (Ad)	IV	+/−	Unevaluable
6	67/F	M 0.3	MDS		+/leukopenia	+ (leukemic cells↓)
7	56/M	M 0.3	MDS		+/leukopenia	+ (leukemic cells↓)
8	56/M	M 0.3	LC (Ad)	IV	+/−	PD
9	34/M	M 0.3	LC (Ad)	IV	+/−	PD
10	58/M	M 0.3	LC (Ad)	IV	+/−	PD
11	68/M	M 0.3	LC (Ad)	IV	+/−	PD
12	56/F	M 0.3	BC (T-P)	IIIA	+/−	+ (PR)
13	54/F	1.0	AML (M1)	CR	+/−	Unevaluable
14	63/M	1.0	AML (M3)	CR	+/−	PD
15	54/M	1.0	AML (M3)	CR	+/−	Unevaluable
16	46/F	1.0	BC (T-P)	IV	+/−	+ (PR)
17	50/M	M 1.0	LC (Sq)	IIIA	+/−	+ (SCC↓)
18	56/M	M 1.0	AML (M3)	CR	+/−	Unevaluable
19	45/M	M 1.0	AML (M3)	CR	+/−	Unevaluable
20	42/F	3.0	AML (M4)	CR	+/−	+ (WT1↓)
21	32/M	3.0	AML (M4E)	CR	+/−	+ (WT1↓)
22	40/F	3.0	AML (M2)	CR	+/−	PD
23	49/F	M 3.0	AML (M1)	CR	+/−	+ (WT1↓)
24	60/F	M 3.0	AML (M3)	CR	+/−	+ (WT1↓)
25	26/M	M 3.0	AML (M5)	CR	+/−	PD
26	56/F	M 3.0	AML (M3)	CR	+/−	+ (WT1↓)

Nos. 21, 23 and 24 (in molecular relapse) have been WT1-vaccinating for more than 6 years and 6 months until now and are in continuous complete remission. WT1↓ represents reduction in WT1mRNA, a leukemic blast marker. Disease: LC, lung cancer; MDS, myelodysplastic syndrome; BC, breast cancer; AML, acute myeloid leukemia; CR, complete response; CEA, chorio-embryonic antigen; SD, stable disease; PD, progressive disease; PR, partial response; SCC, squamous cell carcinoma.

erythema at the WT1 vaccine injection sites (72), whereas severe leukocytopenia occurred as the result of only a single dose of 0.3 mg of modified WT1 peptide in both MDS patients (Fig. 3) (73). This severe leukocytopenia was thought to have resulted from an attack by WT1-specific CTLs against WT1-expressing transformed HPCs, from which almost all leukocytes were derived. As expected, *de novo* AML in complete remission with sufficient normal hematopoiesis remaining, no leukocytopenia occurred because normal HPCs were not attacked by WT1-specific CTLs. Both breast cancer patients and three of eight lung cancer patients who were WT1-vaccinated showed clinical effects such as regression of tumor masses and a decrease in tumor markers. These early clinical studies confirmed both the safety and clinical effectiveness of WT1 peptide immunotherapy for patients with adequate normal hematopoiesis and indicated the advisability of reducing the dose of WT1 peptide vaccine for patients with stem cell leukemia such as MDS and CML. In these patients, the majority of the hematopoietic cells were derived from WT1-expressing transformed HPCs and normal hematopoiesis was very poor (Fig. 4). Mailander et al. (75) vaccinated HLA-A*-0201-positive AML patients with 30% blasts in BM with WT1 peptide (aa 126−134) at a dose of 0.2 mg admixed with 1.0 mg of keyhole limpet hemocyanin, and injected granulocyte macrophage colony-stimulating factor at the same sites as those of the WT1 vaccine. Ten weeks after the vaccination, BM analysis showed that blasts accounted for less than 5% of cells and complete hematological remission was confirmed 16 weeks after the WT1 vaccination.

Further Phase II Clinical Studies

A Phase I/ II clinical study of WT1 peptide vaccination was initiated for patients with solid tumors or hematopoietic malignancies other than MDS and CML. WT1 vaccine containing 3.0 mg of modified WT1 peptide was intradermally injected at weekly intervals for 3 months (a total of 12 injections), after which adverse effects and clinical response were assessed. Since the Phase I study showed that skin erythema at the WT1 vaccine injection sites was the only adverse effect and thus confirmed the safety of this WT1 vaccination protocol (76), Phase II clinical studies of WT1 peptide vaccination are currently being carried out for patients with various types of solid tumors or hematopoietic malignancies other than MDS and CML.

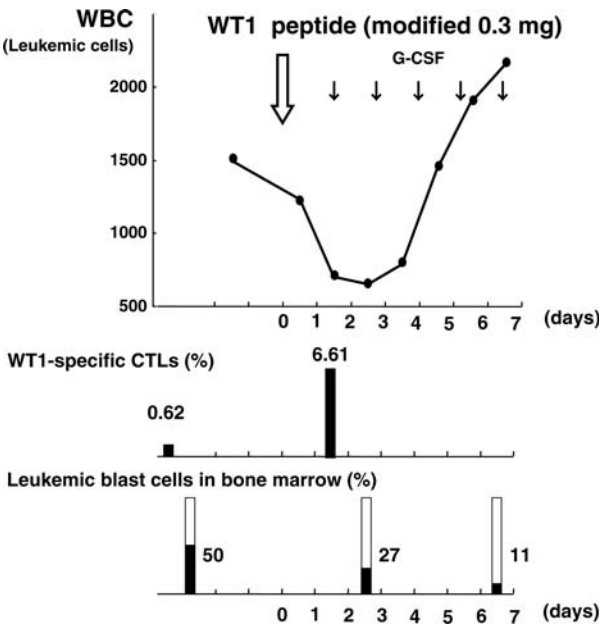

Figure 3. Clinical course of a patient (No. 7 in Table 1) with MDS. A single dose of WT1 vaccine rapidly induced WT1-specific CTLs (0.62% → 6.61%) and nearly eradicated leukemic blast cells (50% → 11%). Severe leukocytopenia, followed by sepsis overcome with administration of G-CSF and steroids. MDS, myelodysplastic syndrome; CTLs, cytotoxic T lymphocytes; G-CSF, granulocyte colony-stimulating factor; WBC, white blood cells.

Glioblastoma Multiforme

WT1 vaccine was administered to 23 HLA-A*2404-positive patients with recurrent glioblastoma multiforme that was resistant to standard therapies (77). The only adverse effect was again local erythema at the WT1 vaccine injection sites. Clinical response according to RECIST criteria was partial response in 2 patients (Fig. 5), stable disease (SD) in 10 patients and progressive disease in 9 patients. Overall response and disease control rates were 9.5% and 57.1%, respectively. Median progression-free survival (PFS) time and 6-month PFS rate were 20.0 weeks and 33.3%, respectively. For two SD patients, long-term SD of more than 236 and 184 weeks, respectively, with only skin erythema at the WT1 vaccine injection sites has continued until the time of writing.

Renal Cell Carcinoma

In two of three HLA-A*2402-positive patients with advanced renal cell carcinoma (RCC) who were WT1-vaccinated, tumor growth was suppressed, so that clinical response was evaluated as SD (78). Especially noteworthy is that development of new metastases was stopped for a prolonged period.

Multiple Myeloma

Myeloma cells are highly sensitive to the granule exocytosis pathway mediated by WT1-specific CTLs (79). A HLA-A*2402-positive patient with advanced therapy-resistant multiple myeloma was treated with WT1 vaccine (Figs 6 and 7) (80). Myeloma cells in BM decreased from 85% to 25%, and

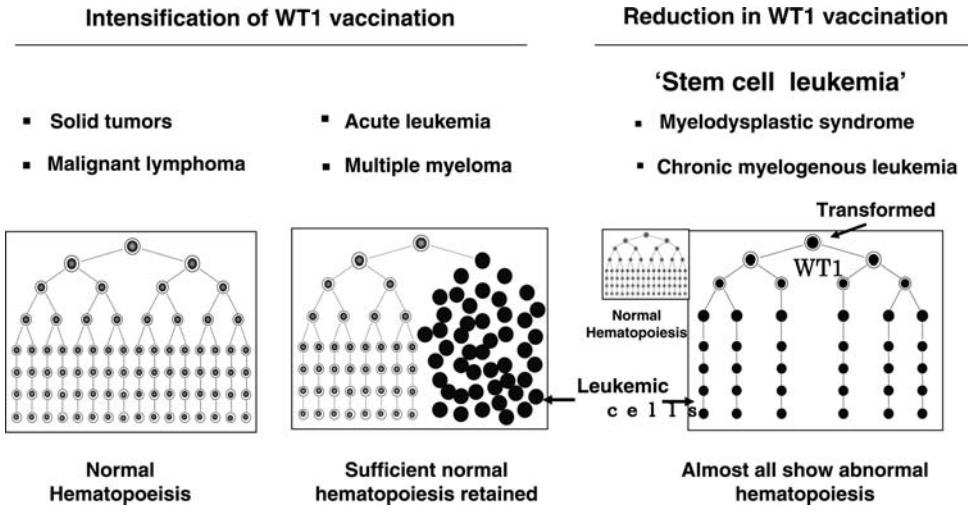

Figure 4. Strategy of new clinical studies.

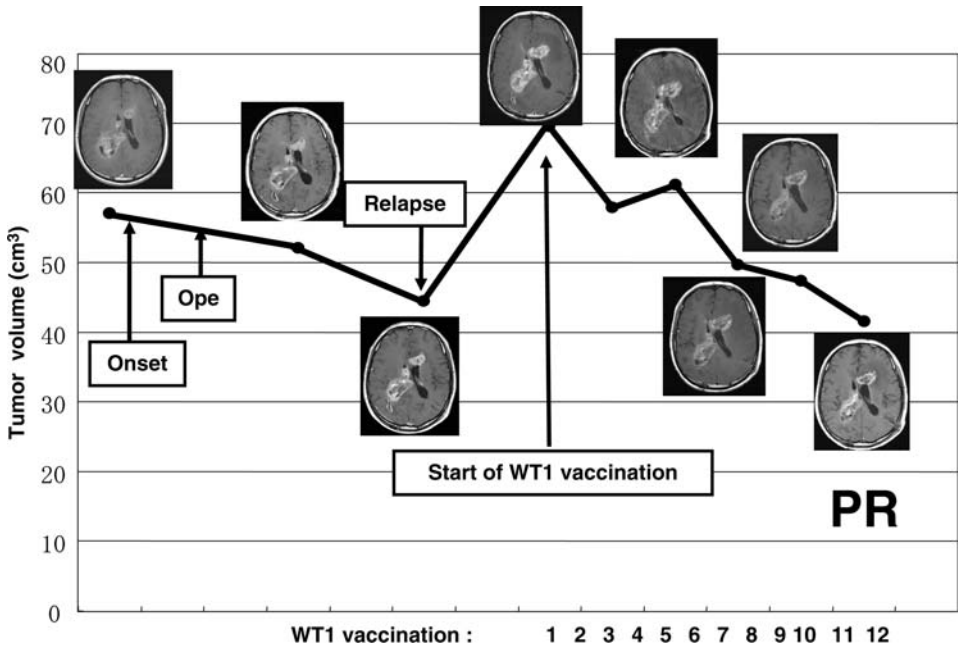

Figure 5. Clinical course of a patient with recurrent glioblastoma multiforme. Patient (33/M) with glioblastoma multiforme was vaccinated with WT1 at relapse (modified WT1 peptide, 3.0 mg/body, once a week).

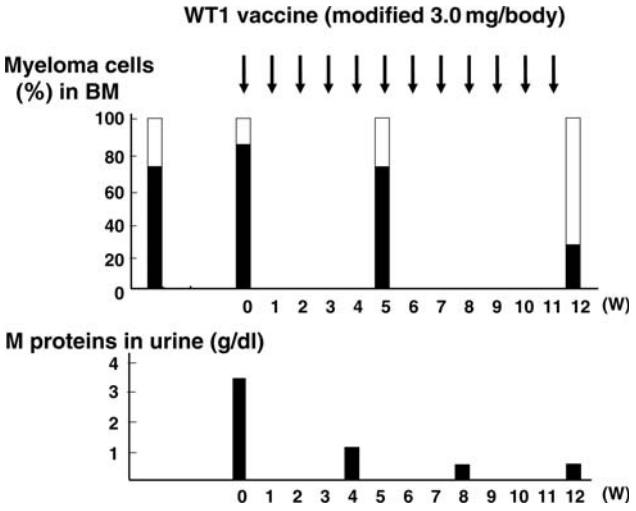

Figure 6. Clinical course of a patient with multiple myeloma. Myeloma cells in BM and M protein in urine in this patient (57/F) with Bence–Jones type multiple myeloma gradually decreased as a result of repeated WT1 vaccination. BM, bone marrow.

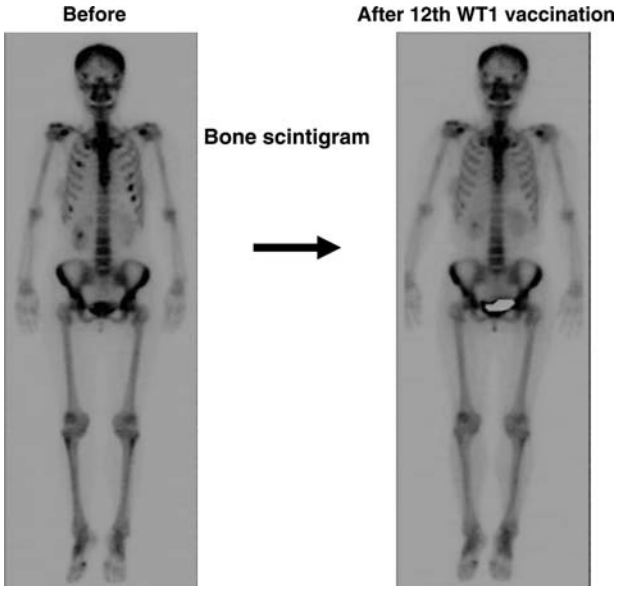

Figure 7. Improvement of bone lesions. Bone scintigram of the same patient with multiple myeloma showed that WT1 vaccination improved bone lesions especially in ribs.

the amount of M protein in the urine decreased from 3.6 to 0.6 g/day after the WT1 vaccination. A bone centigram showed improvement of bone lesions, especially of the ribs, so that the clinical response was assessed as minimal (EGBMT criteria).

Acute myeloid leukemias and myelodysplastic syndrome

Rezvani et al. (81) performed a Phase I clinical study of WT1 peptide vaccine combined with proteinase 3-derived PR1 peptide vaccine for HLA-A*0201-positive patients with AML or MDS and reported a decrease in MRD. Keilholz et al. (82) treated 17 AML patients and 2 patients who had refractory anemia with excess blasts together with HLA-A*0201-restricted, WT1 126–134 peptide and 1 mg keyhole limpet hemocyanin. Objective response in the AML patients was 10 SD. An additional four patients showed clinical benefits after initial progression, i.e. one complete remission and three SD. Yasukawa et al. (83) recently reported one HLA-A*2402-positive AML patient and one

MDS patient who were vaccinated with 1.0 mg body of natural WT1 235–243 peptide emulsified with Montanide adjuvant at 2-week intervals. In one AML patient in the second relapse stage, myeloblasts decreased from 7% to 3% after the fifth vaccination and the patient has survived without an increase in myeloblasts for more than 3 years.

Lung Cancer

Tsuboi et al. (74) reported that two patients with advanced lung cancer were WT1-vaccinated with 0.3 mg of an HLA-A*2402-restricted WT1 peptide at 2-week intervals, resulting in a reduction in tumor markers such as chorio-embryonic antigen and tumor size.

Breast Cancer

Gillmore et al. (84) analyzed paired tumor-draining lymph nodes and peripheral blood samples from five HLA-A2-positive patients with Stage I/II breast cancer to quantify WT1-specific CTLs. WT1 tetramer-binding T cells expanded from all lymph node samples but from none of the corresponding peripheral blood samples. This finding demonstrated that WT1-specific CTLs could expand from the tumor-draining lymph nodes of breast cancer patients and perform a peptide-specific effector function. Oka et al. (72) reported that two patients with advanced breast cancer were treated with 0.3 mg of modified WT1 peptide or 1.0 mg of natural WT1 peptide, respectively, and that both patients showed the regression of metastatic tumors. The latter patient's disease was progressive with ileus due to metastatic tumors in colon before WT1 vaccination. After the second WT1 vaccination, a computed tomographic scan showed that thickening of the walls of the colon tract had significantly improved, accompanied by improved appetite, and the patient survived for 3 years and 1 month after WT1 vaccination.

Infantile Cancer

A 6-year-old girl with metastatic alveolar rhabdomyosarcoma persisting after conventional therapies, including operation, intensive chemo- and radiotherapies, was treated with an HLA-A*2402-restricted modified WT1 peptide (85). After 3 months of WT1 vaccination, her bone metastasis became undetectable in association with an increase in WT1-specific CTLs. WT1 vaccination is being continued and she has survived in complete remission for more than 3 years and 5 months after the start of WT1 vaccination.

A NEW PHASE I CLINICAL STUDY FOR STEM CELL LEUKEMIA, MDS AND CML

The Phase I study demonstrated that MDS, which is a stem cell leukemia, was very sensitive to WT1 peptide vaccination, so that vaccination with the usual dose of WT1 peptide proved to be dangerous because of the rapid reduction in leukocytes derived from WT1-expressing transformed leukemic hematopoietic cells (72). Therefore, a new Phase I study, in which the WT1 peptide dose started from 5 μg/body and reached a maximum of 50 through 15 μg/body, was started in 2005. A 57-year-old male patient with chronic myelomonocytic leukemia was vaccinated biweekly with a very low dose of 5 μg/body of modified WT1 peptide (Fig. 8) (86). After the WT1 peptide vaccination, leukocyte and monocyte counts (13 780 and 1930/μL, respectively) gradually decreased to normal range in association with an increase in WT1-specific CTLs. This case demonstrated for the first time that vaccination with as little as 5 μg/body of WT1 peptide could induce WT1-specific immune response and the resultant clinical effect.

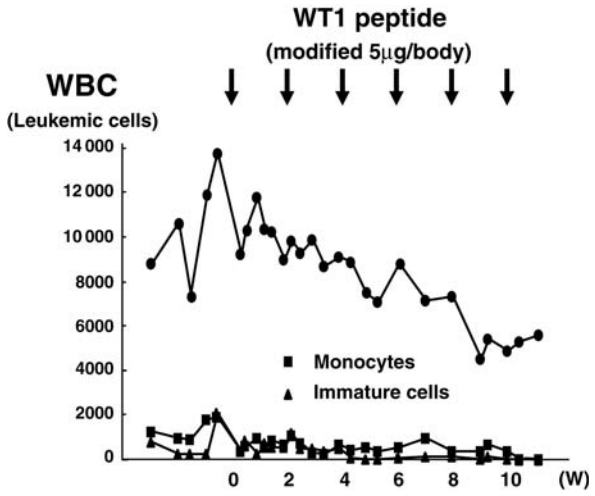

Figure 8. Clinical course of a patient with MDS. Patient (62/M) with MDS (chronic myelomonocytic leukemia) was vaccinated with a very low dose of WT1 peptide (5 μg/body) at biweekly intervals.

HUMORAL AND CELLULAR IMMUNE RESPONSES IN TUMOR-BEARING PATIENTS

Gaiger et al. (87) reported that 16 of 63 patients (25%) with AML possessed serum antibodies reactive with WT1 full-length protein. Similarly, antibodies reactive to WT1 full-length protein were detectable in the serum of 15 of 81 CML patients (19%). In contrast to the results for leukemia patients, antibodies reactive with WT1 full-length protein were detected in only 2 of 96 normal individuals. Elisseeva et al. reported that in 73 patients with hematopoietic malignancies (16 AML, 11 ALL, 13 CML and 33 MDS), IgM, IgG and IgM + IgG WT1 antibodies were detected in 40 (54.8%), 40 (54.8%) and 24 (32.8%), respectively (88). On the other hand, the corresponding values for 43 healthy volunteers were 7 (16.2%), 2 (4.7%) and none (0%). Furthermore, immunoglobulin isotype class switching of WT1 antibodies from IgM to IgG occurred along with disease progression of MDS, suggesting that WT1-specific helper T cells had been induced. A study to determine

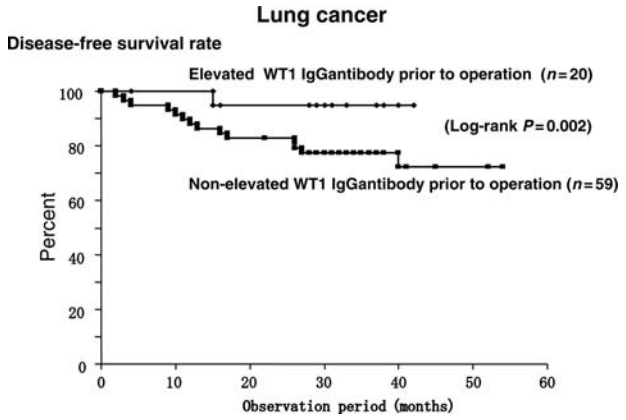

Figure 9. Clear correlation between WT1 antibody titers and disease-free survival rate in patients with lung cancer. WT1 IgG antibody titers were measured before operation for lung cancer.

whether IgG humoral immune response against WT1 protein were Th1- or Th2-type showed that Th1-biased humoral immune responses against WT1 protein were generated in leukemia and MDS (89). Oji et al. (90) analyzed WT1 IgG antibodies in patients with non-small cell lung cancer (NSCLC) and reported that pre-operative elevation of WT1 antibodies was significantly associated with longer disease-free survival in NSCLC patients with Stages I–III, which suggests that WT1-specific immune response has an important role in anticancer immunity (Fig. 9). Rezvani et al. (91) reported that WT1-specific $CD8^+$ T cell responses were detected in 5 of 8 CML patients, 4 of 6 AML patients and 7 of 12 healthy donors. The magnitude and extent of these $CD8^+$ T cell responses were greater in patients with myeloid leukemia than in healthy donors. They confirmed the presence of high-avidity T cell clones with a WT1-specific repertoire. Scheibenbogen et al. (92) found that T cells recognizing HLA-A2.1-binding epitopes from WT1 or proteinase 3 could be detected *ex vivo* in 5 of 15 HLA-A2-positive AML patients, thus providing the first evidence of spontaneous T cell response against defined antigens in AML patients. Finally, Gannagé et al. (93) reported that WT1, hTert, PR1 and bcr74 tetramer$^+$ $CD8^+$ T cells were detected in 85%, 82%, 67% and 61% of patients.

ASSOCIATION OF WT1-SPECIFIC $CD8^+$ T CELLS WITH GRAFT-VERSUS-LEUKEMIA EFFECT AFTER ALLOGENEIC HEMATOPOETIC STEM CELL TRANSPLANTATION

Donor-derived CTLs that respond to tumor antigens emerge after hematopoietic stem cell transplantation (HSCT). Rezvani et al. (94) reported that responses to WT1, PR1 or BCR-ABL were observed in 9 of 14 patients with CML before HSCT and in 5 of 6 after HSCT, and that the responses were higher in patients with CML than in healthy donors and highest after HSCT. Morita et al. (95) used HLA-A*0201 tetramers to analyzed the frequency of CTLs against PR1, PRAME and WT1 after HSCT and found that 1 (1.0%), 10 (10.3%) and 39 (40.2%) of 97 samples from 35 HLA-A*02-positive patients (9 AML, 11 MDS, 2 CML, 4 ALL, 7 lymphomas and 2 RCC) were positive for PR1, PRAME and WT1, respectively. Serial analysis of WT1-specific CTLs during the clinical course after HSCT was performed for two patients with RCC and the results showed that higher positive rates of WT1-specific CTLs correlated with good clinical response. Rezvani et al. (96) analyzed absolute numbers of WT1-specific $CD8^+$ T cell as well as WT1mRNA levels that reflected MRD of leukemia in PB during the early phase of immune recovery after HSCT in 10 HLA-A*0201-positive ALL patients and demonstrated that the emergence of WT1-specific $CD8^+$ T cells was associated with a decrease in WT1mRNA levels, suggesting the presence of a WT1-driven graft-versus-leukemia (GVL) effect. Loss of WT1-specific $CD8^+$ T cell responses was associated with reappearance of WT1mRNA, which is consistent with a molecular relapse ($P < 0.001$). These results support the notion of immunogenicity of WT1 after HSCT for ALL and a very high potential for WT1 vaccines to boost GVL after HSCT for ALL. Kapp et al. (97) reported that 28 patients, screened after allo-HSCT for T cell responses toward proteinase-3, WT1 and MUC1, showed a significant relationship between relapse and the absence of a TAA-specific T cell response, since only 2 of 13 (15%) patients with TAA-specific CTL relapsed, in contrast to relapse in 9 of 15 (60%) patients without TAA-specific CTL responses ($P < 0.005$).

DISCUSSION

The main focus of this review was on three aspects of the WT1 gene. First, the WT1 gene is highly expressed in leukemia and various types of solid tumors, thus making WT1mRNA as a useful tumor marker. For leukemia, the clinical usefulness of the WT1mRNA assay to detect MRD has now been established, and the assay is covered by public health insurance in Japan.

Second, the WT1 gene was originally defined as a tumor suppressor gene on the basis of the following findings: intragenic deletions or mutations in Wilms' tumor, germline mutation in patients with leukemic predisposition syndromes, and WT1-mediated growth suppression of Wilms' tumor cells expressing a WT1 splicing variant. On the other hand, we proposed that the WT1 had an oncogenic function rather than that of a tumor suppressor gene. The question of whether the WT1 gene is a tumor suppressor gene or an oncogene, or has a biphasic function combining the two characteristics remains an important and interesting issue to be resolved.

Third, a discussion about the potential of the WT1 protein as a cancer antigen should be 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 done only by clinical studies that are very laborious, and conversely, only clinical studies can determine their potential as cancer antigens. It is therefore laborious and time-consuming work to determine and confirm the clinical usefulness of a given cancer antigen.

Very recently, 75 representative cancer antigens including WT1 were prioritized (98). 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 percent of antigen-positive cells, (vi) stem cell expression, (vii) number of patients with antigen-positive cancers, (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 result can be expected to promote the development of WT1-targeted cancer immunotherapy.

Cancer immunotherapy is considered to be the fourth cancer therapy after the three major cancer therapies, surgery, chemotherapy and radiotherapy. It is thought that complete eradication of cancer stem cells is essential for cure of cancer and that only immunotherapy is capable of killing non-dividing, quiescent cancer stem cells. I would therefore like to put forward the strategy described in Table 2. For maximal efficacy, 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. I therefore believe that a new era of immunotherapy is imminent.

Table 2. Cancer treatment strategy

When a cancer is detected, it is important to immediately start immunotherapy and continue it until it is eradicated
Operation
Start immunotherapy before the operation and continue it even after the operation
Chemotherapy
Strong anticancer drugs should be used as short-term strategy
If the anticancer drug turn out to be ineffective, long-term usage should be avoided
Radiotherapy
Because irradiation lowers immunity, excessive exposure to radiation is not recommended, except for prospective cases
Immunotherapy + operation
Immunotherapy + chemotherapy
Immunotherapy + radiotherapy
It is important to make the most of the advantages of the four therapies: operation, chemotherapy, radiotherapy and immunotherapy

Funding

Grant-In-Aid for Cancer Research from the Ministry of Health, Labour and Welfare.

Conflict of interest statement

None declared.

References

1. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms tumor locus. *Cell* 1990;60:509–20.
2. Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GAP. Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. *Nature* 1990;343:774–8.
3. Sugiyama H. Wilms' tumor gene WT1: its oncogenic function and clinical application. *Int J Hematol* 2001;73:177–87.
4. Buckler AJ, Pelletier J, Haber DA, Glaser T, Housman DE. Isolation, characterization, and expression of the murine Wilms tumor gene (WT1) during kidney development. *Mol Cell Biol* 1991;11:1707–12.
5. Park S, Schalling M, Bernard A, Maheswaran S, Shipley GC, Roberts D, et al. The Wilms tumor gene WT1 is expressed in murine mesoderm-derived tissues and mutated in a human mesothelioma. *Nature Genet* 1993;4:415–20.
6. Davies R, Moore A, Schedl A, Bratt E, Miyahawa K, Ladomery M, et al. Multiple roles for the Wilms' tumor suppressor, WT1. *Cancer Res* 1999;59(Suppl 7):1747–1750s; discussion 1751s.
7. Moore AW, Mclnnes L, Kreidberg J, Hastie ND, Schedl A. YAC complementation shows a requirement for Wt1 in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development* 1999;126:1845–57.
8. Miwa H, Beran M, Aunders GF. Expression of the Wilms tumor gene (WT1) in human leukemias. *Leukemia* 1992;6:405–9.
9. Miyagi T, Ahuja H, Kubota T, Kubonishi I, Koeffler HP, Miyoshi I. Expression of the Candidate Wilms tumor gene, WT1, in human leukemia cells. *Leukemia* 1993;7:970–7.
10. Inoue K, Sugiyama H, Ogawa H, Nakagawa M, Yamagami T, Miwa H, et al. WT1 as new prognostic factor a new marker for the detection of minimal residual disease in acute leukemia. *Blood* 1994;84:3071–9.
11. Bergmann L, Miething C, Maurer U, Brieger J, Karakas T, Weidmann E, et al. High levels of Wilms' tumor gene (wt1) mRNA in acute myeloid leukemias are associated with a worse long-term outcome. *Blood* 1997;90:1217–25.
12. Brieger J, Weidmann E, Fenchel K, Mitrou PS, Hoelzer D, Bergmann L. The expression of the Wilms tumor gene in acute myelocytic leukemias as a possible marker for leukemic blast cells. *Leukemia* 1994;8:2138–43.
13. Menssen HD, Renkl H-J, Rodeck U, Maurer J, Notter M, Schwartz S, et al. Presence of Wilms tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. *Leukemia* 1995;9:1060–7.
14. Menssen HD, Renkl HJ, Rodeck U, Kari C, Schwartz S, Thiel E. Detection by monoclonal antibodies of the Wilms' tumor (WT1) nuclear protein in patients with acute leukemia. *Int J Cancer* 1997;70:518–23.
15. Cilloni D, Gottardi E, De Micheli D, Serra A, Volpe G, Messa F, et al. Quantitative assessment of WT1 expression by real time quantitative PCR may be a useful tool for monitoring minimal residual disease in acute leukemia patients. *Leukemia* 2002;16:2115–21.
16. Tamaki H, Mishima M, Kawakami M, Tsuboi A, Kim EH, Hosen N, et al. Monitoring minimal residual disease in leukemia using real-time quantitative polymerase chain reaction for Wilms tumor gene (WT1). *Int J Hematol* 2003;78:349–56.
17. Ogawa H, Tamaki H, Ikegame K, Soma T, Kawakami M, Tsuboi A, et al. The usefulness of monitoring WT1 gene transcripts for the prediction and management of relapse following allogeneic stem cell transplantation in acute type leukemia. *Blood* 2003;101:1698–704.

18. Greiner J, Ringhoffer M, Taniguchi M, Li L, Schmitt A, Shiku H, et al. mRNA expression of leukemia-associated antigens in patients with acute myeloid leukemia for the development of specific immunotherapies. *Int J Cancer* 2004;108:704–11.
19. Tamaki H, Ogawa H, Ohyashiki K, Ohyashiki J-H, Iwama H, Inoue K, et al. The Wilms' tumor gene WT1 is a good marker for diagnosis of disease progression of myelodysplastic syndromes. *Leukemia* 1999;13:393–9.
20. Oji Y, Ogawa H, Tamaki H, Oka Y, Tsuboi A, Kim EH, et al. Expression of the Wilms' tumor gene WT1 in solid tumors and its involvement in tumor cell growth. *Jpn J Cancer Res* 1999;90:194–204.
21. Oji Y, Suzuki T, Nakano Y, Maruno M, Nakatsuka S, Jomgeow T, et al. Overexpression of the Wilms' tumor gene WT1 in primary astrocytic tumors. *Cancer Sci* 2004;95:822–7.
22. Oji Y, Inohara H, Nakazawa M, Nakano Y, Akahani S, Nakatsuka S, et al. Overexpression of the Wilms' tumor gene WT1 in head and neck squamous cell carcinoma. *Cancer Sci* 2003;94:523–9.
23. Oji Y, Miyoshi Y, Koga S, Nakano Y, Ando A, Nakatuska S, et al. Overexpression of the Wilms' tumor gene WT1 in primary thyroid cancer. *Cancer Sci* 2003;94:606–11.
24. Oji Y, Yano M, Nakano Y, Abeno S, Nakatsuka S, Ikeba A, et al. Overexpression of the Wilms' tumor gene WT1 in esophageal cancer. *Anticancer Res* 2004;24:3103–8.
25. Oji Y, Miyoshi S, Maeda H, Hayashi S, Tamaki H, Nakatsuka S, et al. Overexpression of the Wilms' tumor gene WT1 in de novo lung cancers. *Int J Cancer* 2002;100:297–303.
26. Loeb DM, Evron E, Patel CB, Sharma PM, Niranjan B, Buluwela L, et al. Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. *Cancer Res* 2001;61:921–5.
27. Miyoshi Y, Ando A, Egawa C, Taguchi T, Tamaki Y, Tamaki H, et al. High expression of Wilms' tumor suppressor gene (WT1) predicts poor prognosis in breast cancer patients. *Clin Cancer Res* 2002;8:1167–71.
28. Oji Y, Nakamori S, Fujikawa M, Nakatsuka S, Yokota A, Tatsumi N, et al. Overexpression of the Wilms' tumor gene WT1 in pancreatic ductal adenocarcinoma. *Cancer Sci* 2004;95:583–7.
29. Oji Y, Yamamoto H, Nomura M, Nakano Y, Ikeba A, Nakatsuka S, et al. Overexpression of the Wilms' tumor gene WT1 in colorectal adenocarcinoma. *Cancer Sci* 2003;94:712–7.
30. Ueda T, Oji Y, Naka N, Nakano Y, Takahashi E, Koga S, et al. Overexpression of the Wilms' tumor gene WT1 in human bone soft-tissue sarcomas. *Cancer Sci* 2003;94:271–6.
31. Oji Y, Miyoshi Y, Kiyotoh E, Koga S, Nakano Y, Ando A, et al. Absence of mutations in the Wilms' tumor gene WT1 in primary breast cancer. *Jpn J Clin Oncol* 2004;34:74–7.
32. Oji Y, Miyoshi S, Takahashi E, Koga S, Nakano Y, Shintani Y, et al. Absence of mutations in the Wilms' tumor gene wt1 in de novo non-small cell lung cancers. *Neoplasma* 2004;51:17–20.
33. Coppes MJ, Campbell CE, Williams BRG. The role of WT1 in Wilms tumorigenesis. *FASEB J* 1993;7:886–95.
34. Rauscher FJI. The WT1 Wilms tumor gene product: a developmentally regulated transcription factor in the kidney that functions as a tumor suppressor. *FASEB J* 1993;7:896–903.
35. Haber DA, Park S, Maheswaran S, Englert C, Re GG, Hazen-Martin DJ, et al. WT1-mediated growth suppression of Wilms tumor cells expressing a WT1 splicing variant. *Science* 1993;262:2057–9.
36. Algar EM, Kenney MT, Simms LA, Smith SI, Kida Y, Smith PJ. Homozygous intragenic deletion in the WT1 gene in a sporadic Wilms' tumour associated with high levels of expression of a truncated transcript. *Hum Mutat* 1995;5:221–7.
37. Little M, Wells C. A clinical overview of WT1 gene mutations. *Hum Mutat* 1997;9:209–25.
38. Loeb DM, Sukumar S. The role of WT1 in oncogenesis: tumor suppressor or oncogene? *Int J Hematol* 2002;76:117–26.
39. Sugiyama H. Cancer immunotherapy targeting Wilms' tumor gene WT1 product. *Expert Rev Vaccines* 2005;4:503–12.
40. Yamagami T, Sugiyama H, Inoue K, Ogawa H, Tatekawa T, Hirata M, et al. Growth inhibition of human leukemic cells by WT1 (Wilms tumor gene) antisense oligodeoxynucleotides: Implications for the involvement of WT1 in leukemogenesis. *Blood* 1996;87:2878–84.
41. Algar EM, Khromykh T, Smith SI, Blackburn DM, Bryson GJ, Smith PJ. A WT1 antisense oligonucleotide inhibits proliferation and induces apoptosis in myeloid leukaemia cell lines. *Oncogene* 1996;12:1005–14.
42. Tatsumi N, Oji Y, Tsuji N, Tsuda A, Higashio M, Aoyagi S, et al. Wilms' tumor gene WT1-shRNA as a potent apoptosis-inducing agent for solid tumors. *Int J Oncol* 2008;32:701–11.
43. Inoue K, Tamaki H, Ogawa H, Oka Y, Soma T, Tatekawa T, et al. Wilms' tumor gene (WT1) competes with differentiation-inducing signal in hematopoietic progenitor cells. *Blood* 1998;91:2969–76.
44. Tsuboi A, Oka Y, Ogawa H, Elisseeva OA, Tamaki H, Oji Y, et al. Constitutive expression of the Wilms' tumor gene WT1 inhibits the differentiation of myeloid progenitor cells but promotes their proliferation in response to granulocyte-colony stimulating factor (G-CSF). *Leuk Res* 1999;23:499–505.
45. Li H, Oka Y, Tsuboi A, Yamagami T, Miyazaki T, Yusa S-I, et al. The lck promoter-driven expression of the Wilms' tumor gene WT1 blocks intrathymic differentiation of T-lineage cells. *Int J Hematol* 2003;77:463–70.
46. Jomgeow T, Oji Y, Tsuji N, Ikeda Y, Ito K, Tsuda A, et al. Wilm's tumor gene WT1 17AA(-)/KTS(-) isoform induces morphological changes and promotes cell migration and invasion in vitro. *Cancer Sci* 2006;97:259–70.
47. Ito K, Oji Y, Tatsumi N, Shimizu S, Kanai Y, Nakazawa T, et al. Anti-apoptotic function of 17AA(+)WT1 (Wilms' tumor gene) isoforms on the intrinsic apoptosis pathway. *Oncogene* 2006;25:4217–29.
48. Nishida S, Hosen N, Shirakata T, Kanato K, Yanagihara M, Nakatsuka S. AML1-ETO rapidly induces acute myeloblastic leukemia in cooperation with Wilms' tumor gene, WT1. *Blood* 2006;107:3303–12.
49. Hosen N, Sonoda Y, Oji Y, Kimura T, Minamiguchi H, Tamaki H, et al. Very low frequencies of human normal $CD34^+$ hematopoietic progenitor cells express the Wilms' tumor gene WT1 at the levels comparable to those in leukemia cells. *Br J Haematol* 2002;116:409–20.
50. Kanato K, Hosen N, Yanagihara M, Nakagata N, Shirakata T, Nakazawa T, et al. The Wilms' tumor gene WT1 is a common marker of progenitor cells in fetal liver. *Biochem Biophys Res Commun* 2005;326:836–43.
51. Oka Y, Udaka K, Tsuboi A, Elisseeva OA, Ogawa H, Aozasa K, et al. Cancer immunotherapy targeting Wilms tumor gene WT1 product. *J Immunol* 2000;164:1873–80.
52. Tsuboi A, Oka Y, Ogawa H, Elisseeva OA, Li H, Kawasaki K, et al. Cytotoxic T-lymphocyte responses elicited to Wilms' tumor gene WT1 product by DNA vaccination. *J Clin Immunol* 2000;20:195–202.
53. Gaiger A, Reese V, Disis ML, Cheever MA. Immunity to WT1 in the animal model and in patients with acute myeloid leukemia. *Blood* 2000;96:1480–9.
54. Gao L, Xue SA, Hasserjian R, Cotter F, Kaeda J, Goldman JM, et al. Human cytotoxic T lymphocytes specific for Wilms' tumor antigen-1 inhibit engraftment of leukemia-initiating stem cells in non-obese diabetic-severe combined immunodeficient recipients. *Transplantation* 2003;75:1429–36.
55. Oka Y, Elisseeva OA, Tsuboi A, Ogawa H, Tamaki H, Li H, et al. Human cytotoxic T lymphocyte responses specific for peptides of wild-type Wilms tumor gene WT1 product. *Immunogenetics* 2000;51:99–107.
56. Gao L, Bellantuono I, Elsasser A, Marley SB, Gordon MY, Goldman JM, et al. Selective elimination of leukemic CD34+ progenitor cells by cytotoxic T lymphocytes specific for WT1. *Blood* 2000;95:2198–203.
57. Ohminami H, Yasukawa M, Fujita S. HLA class I-restricted lysis of leukemia cells by a CD8+ cytotoxic T-lymphocyte clone specific for WT1 peptide. *Blood* 2000;95:286–93.
58. Tsuboi A, Oka Y, Udaka K, Murakami M, Masuda T, Nakano A, et al. Enhanced induction of human WT1-specific cytotoxic T lymphocytes with a 9-mer WT1 peptide modified at HLA-A*2402-binding residues. *Cancer Immunol Immunother* 2002;51:614–20.
59. Azuma T, Makita M, Ninomiya K, Fujita S, Harada M, Yasukawa M. Identification of a novel WT1-derived peptide which induces human leucocyte antigen-A24-restricted anti-leukaemia cytotoxic T lymphocytes. *Br J Haematol* 2002;116:601–3.
60. Bellantuono I, Gao L, Parry S, Marley S, Dazzi F, Apperley J, et al. Two distinct HLA-A0201-presented epitopes of the Wilms tumor antigen 1 can function as targets for leukemia-reactive CTL. *Blood* 2002;100:3835–7.
61. Li Z, Oka Y, Tsuboi A, Masuda T, Tatsumi N, Kawakami M, et al. WT1(235), a ninemer peptide derived from Wilms' tumor gene product,

is a candidate peptide for the vaccination of HLA-A*0201-positive patients with hematopoietic malignancies. *Int J Hematol* 2005;82:458–9.
62. Pinilla-Ibarz J, May RJ, Korontsvit T, Gomez M, Kappel B, Zakhaleva V, et al. Improved human T-cell responses against synthetic HLA-0201 analog peptides derived from the WT1 oncoprotein. *Leukemia* 2006;20:2025–33.
63. Asemissen AM, Keilholz U, Tenzer S, Müller M, Walter S, Stevanovic S, et al. Identification of a highly immunogenic HLA-A*01-binding T cell epitope of WT1. *Clin Cancer Res* 2006;12:7476–82.
64. May RJ, Dao T, Pinilla-Ibarz J, Korontsvit T, Zakhaleva V, Zhang RH, et al. Peptide epitopes from the Wilms' tumor 1 oncoprotein stimulate CD4+ and CD8+ T cells that recognize and kill human malignant mesothelioma tumor cells. *Clin Cancer Res* 2007;13:4547–55.
65. Li Z, Oka Y, Tsuboi A, Fujiki F, Harada Y, Nakajima H, et al. Identification of a WT1 protein-derived peptide, WT1, as a HLA-A 0206-restricted, WT1-specific CTL epitope. *Microbiol Immunol* 2008;52:551–8.
66. Knights AJ, Zaniou A, Rees RC, Pawelec G, Muller L. Prediction of an HLA-DR-binding peptide derived from Wilms' tumour 1 protein and demonstration of in vitro immunogenicity of WT1(124–138)-pulsed dendritic cells generated according to an optimised protocol. *Cancer Immunol Immunother* 2002;51:271–81.
67. Muller L, Knights A, Pawelec G. Synthetic peptides derived from the Wilms' tumor 1 protein sensitize human T lymphocytes to recognize chronic myelogenous leukemia cells. *Hematol J* 2003;4:57–66.
68. Fujiki F, Oka Y, Tsuboi A, Kawakami M, Kawakatsu M, Nakajima H, et al. Identification and characterization of a WT1 (Wilms Tumor Gene) protein-derived HLA-DRB1*0405-restricted 16-mer helper peptide that promotes the induction and activation of WT1-specific cytotoxic T lymphocytes. *J Immunother* 2007;30:282–93.
69. Fujiki F, Oka Y, Kawakatsu M, Tsuboi A, Nakajima H, Elisseeva OA, et al. A WT1 protein-derived, naturally processed 16-mer peptide, WT1(332), is a promiscuous helper peptide for induction of WT1-specific Th1-type CD4(+) T cells. *Microbiol Immunol* 2008;52:591–600.
70. Guo Y, Niiya H, Azuma T, Uchida N, Yakushijin Y, Sakai I, et al. Direct recognition and lysis of leukemia cells by WT1-specific CD4+ T lymphocytes in an HLA class II-restricted manner. *Blood* 2005;106:1415–8.
71. Kobayashi H, Nagato T, Aoki N, Sato K, Kimura S, Tateno M, et al. Defining MHC class II T helper epitopes for WT1 tumor antigen. *Cancer Immunol Immunother* 2006;55:850–60.
72. Oka Y, Tsuboi A, Taguchi T, Osaki T, Kyo T, Nakajima H, et al. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proc Natl Acad Sci USA* 2004;101:13885–90.
73. Oka Y, Tsuboi A, Murakami M, Hirai M, Tominaga N, Nakajima H, et al. WT1 peptide-based immunotherapy for patients with overt leukemia from myelodysplastic syndrome (MDS) or MDS with myelofibrosis. *Int J Hematol* 2003;78:56–61.
74. Tsuboi A, Oka Y, Osaka T, Kumagai T, Tachibana I, Hayashi S, et al. WT1 peptide-based immunotherapy for patients with lung cancer. *Microbiol Immunol* 2004;48:175–84.
75. Mailander V, Scheibenbogen C, Thiel E, Letsch A, Blau IW, Keilholz U. Complete remission in a patient with recurrent acute myeloid leukemia induced by vaccination with WT1 peptide in the absence of hematological or renal toxicity. *Leukemia* 2004;18:165–6.
76. Morita S, Oka Y, Tsuboi A, Kawakami M, Maruno M, Izumoto S, et al. A phase I/II trial of a WT1 (Wilms' tumor gene) peptide vaccine in patients with solid malignancy: safety assessment based on the phase I data. *Jpn J Clin Oncol* 2006;36:231–6.
77. Izumoto S, Tsuboi A, Oka Y, Suzuki T, Hashiba T, Kagawa N, et al. Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme. *J Neurosurg* 2008;108:963–71.
78. Iiyama T, Udaka K, Takeda S, Takeuchi T, Adachi YC, Ohtsuki Y, Tsuboi A, et al. WT1 (Wilms' tumor 1) peptide immunotherapy for renal cell carcinoma. *Microbiol Immunol* 2007;51:519–30.
79. Azuma T, Otsuki T, Kuzushima K, Froelich CJ, Fujita S, Yasukawa M. Myeloma cells are highly sensitive to the granule exocytosis pathway mediated by WT1-specific cytotoxic T lymphocytes. *Clin Cancer Res* 2004;10:7402–12.
80. Tsuboi A, Oka Y, Nakajima H, Fukuda Y, Elisseeva OA, Yoshihara S, et al. Wilms tumor gene WT1 peptide-based immunotherapy induced a minimal response in a patient with advanced therapy-resistant multiple myeloma. *Int J Hematol* 2007;86:414–7.
81. Rezvani K, Yong AS, Mielke S, Savani BN, Musse L, Superata J, et al. Leukemia-associated antigen-specific T-cell responses following combined PR1 and WT1 peptide vaccination in patients with myeloid malignancies. *Blood* 2008;111:236–42.
82. Keilholz U, Letsch A, Busse A, Asemissen AM, Bauer S, Blau IW, et al. A clinical and immunologic phase 2 trial of Wilms tumor gene product 1 (WT1) peptide vaccination in patients with AML and MDS. *Blood* 2009;113:6541–8.
83. Yasukawa M, Fujiwara H, Ochi T, Suemori K, Narumi H, Azuma T, et al. Clinical efficacy of WT1 peptide vaccination in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Am J Hematol* 2009;84:314–5.
84. Gillmore R, Xue SA, Holler A, Kaeda J, Hadjiminas D, Healy V, et al. Detection of Wilms' tumor antigen–specific CTL in tumor-draining lymph nodes of patients with early breast cancer. *Clin Cancer Res* 2006;12:34–42.
85. Ohta H, Hashii Y, Yoneda A, Takizawa S, Kusuki S, Tokimasa S, et al. WT1 (Wilms tumor 1) peptide immunotherapy for childhood rhabdomyosarcoma: a case report. *Pediatr Hematol Oncol* 2009;26: 74–83.
86. Kawakami M, Oka Y, Tsuboi A, Harada Y, Elisseeva OA, Furukawa Y, et al. Clinical and immunologic responses to very low-dose vaccination with WT1 peptide (5 microg/body) in a patient with chronic myelomonocytic leukemia. *Int J Hematol* 2007;85:426–9.
87. Gaiger A, Carter L, Greinix H, Carter D, McNeill PD, Houghton RL, et al. WT1-specific serum antibodies in patients with leukemia. *Clin Cancer Res* 2001;7:761s–765s.
88. Elisseeva OA, Oka Y, Tsuboi A, Ogata K, Wu F, Kim EH, et al. Humoral immune responses against Wilms' tumor gene WT1 product in patients with hematopoietic malignancies. *Blood* 2002;99:3272–9.
89. Wu F, Oka Y, Tsuboi A, Elisseeva OA, Ogata K, Nakajima H, et al. Th1-biased humoral immune responses against Wilms tumor gene WT1 product in the patients with hematopoietic malignancies. *Leukemia* 2005;19:268–74.
90. Oji Y, Kitamura Y, Kamino E, Kitano A, Sawabata N, Inoue M, et al. WT1 IgG antibody for early detection of nonsmall cell lung cancer and as its prognostic factor. *Int J Cancer* 2009;125:381–7.
91. Rezvani K, Brenchley JM, Price DA, Kilical Y, Gostick E, Sewell AK, et al. T-cell responses directed against multiple HLA-A*0201-restricted epitopes derived from Wilms' tumor 1 protein in patients with leukemia and healthy donors: identification, quantification, and characterization. *Clin Cancer Res* 2005;11:8799–807.
92. Scheibenbogen C, Letsch A, Thiel E, Schmittel A, Mailaender V, Baerwolf S, et al. CD8 T-cell responses to Wilms tumor gene product WT1 and proteinase 3 in patients with acute myeloid leukemia. *Blood* 2002;100:2132–7.
93. Gannagé M, Abel M, Michallet AS, Delluc S, Lambert M, Giraudier S, et al. Ex vivo characterization of multiepitopic tumor-specific CD8 T cells in patients with chronic myeloid leukemia: implications for vaccine development and adoptive cellular immunotherapy. *J Immunol* 2005;174:8210–8.
94. Rezvani K, Grube M, Brenchley JM, Sconocchia G, Fujiwara H, Price DA, et al. Functional leukemia-associated antigen-specific memory CD8+ T cells exist in healthy individuals and in patients with chronic myelogenous leukemia before and after stem cell transplantation. *Blood* 2003;102:2892–900.
95. Morita Y, Heike Y, Kawakami M, Miura O, Nakatsuka S, Ebisawa M, et al. Monitoring of WT1-specific cytotoxic T lymphocytes after allogeneic hematopoietic stem cell transplantation. *Int J Cancer* 2006;119:1360–7.
96. Rezvani K, Yong AS, Savani BN, Mielke S, Keyvanfar K, Gostick E, et al. Graft-versus-leukemia effects associated with detectable Wilms tumor-1 specific T lymphocytes after allogeneic stem-cell transplantation for acute lymphoblastic leukemia. *Blood* 2007;110:1924–32.
97. Kapp M, Stevanović S, Fick K, Tan SM, Loeffler J, Opitz A, et al. CD8+ T-cell responses to tumor-associated antigens correlate with superior relapse-free survival after allo-SCT. *Bone Marrow Transplant* 2009;43:399–410.
98. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res* 2009;15:5323–37.