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REVIEW ARTICLE

WT1 cancer vaccine for the treatment and prevention

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ABSTRACT

Wilms' tumor gene 1 (WT1) overexpresses in almost all kinds of hematological malignancies and solid tumors (hereinafter referred to as cancer). Therefore, WT1 protein is a ubiquitous tumor-associated antigen (TAA). Many clinical studies of WT1-targeted cancer immunotherapies (hereinafter referred to as WT1 cancer vaccine), including WT1 peptide vaccine, WT1 peptide-pulsed dendritic cell (WT1-DC) vaccine, and WT1 mRNA-electroporated dendritic cell (WT1 mRNA-DC) vaccine had been conducted for the treatment of almost all kinds of cancer. The clinical effect was promising, whereas the major side effects were the temporary fever and skin reaction on the vaccine injection sites and not significant. The appropriate combination therapy of WT1 cancer vaccine and chemotherapy enhanced WT1 immune response against cancer. Gemcitabine (GEM), for example, increased WT1 immune response through the promotion of the expression of WT1 antigen protein and HLA class I/II molecules in cancer. Furthermore, WT1 cancer vaccine immediately after hematopoietic stem cell transplantation (HSCT) induced sufficient WT1 immune response regardless of severe immunocompromised conditions and exerted sufficient clinical effect, suggesting that the immune condition immediately after HSCT should be suitable for the priming of WT1 immune response. Moreover, the combination therapy of WT1 cancer vaccine and immune checkpoint inhibitors (ICIs) was promising. Compared to the other TAAs, WT1 is especially unique in that it expresses not only in cancer cells but also in their stem cells at the quiescent state of cell-cycle, which are resistant to chemo- and radio-therapies. This uniqueness of WT1 largely contributes to cure cancer through the complete eradication of WT1-expressing cancer stem cells by WT1 immune response against them. Since the complete eradication of cancer stem cells is essential to cure cancer, and since only immune cells against cancer are considered to be able to kill the cancer stem cells at the quiescent state of cell-cycle, the introduction of immunotherapy, especially of WT1 cancer vaccine with sufficient safety is essential in the cure-oriented treatments of cancer. Accumulated clinical results suggest that WT1 cancer vaccine should be useful for cancer prevention, and the development of WT1 cancer prevention vaccine is awaited.

1. Introduction

WT1 is a gene responsible for childhood renal tumor, Wilms' tumor, and encodes a transcription factor that regulates many genes.[1, 2] The WT1 gene is originally defined as a tumor suppressor gene, but it seems to be rather an oncogene. The WT1 gene plays an important role in cell proliferation and differentiation, embryogenesis, angiogenesis, leukemogenesis and tumorigenesis.[3, 4, 5, 6] The WT1 gene overexpresses in acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) [7, 8] and the suppression of WT1 overexpression by WT1-specific anti-sense oligomers stopes the leukemic cell growth.[9, 10] The WT1 gene also overexpresses in many kinds of solid tumors, [11, 12] and higher WT1 expression in cancer is correlated to worse prognosis.[13] These results strongly indicate that the WT1 gene is an oncogene.

The accumulated data show that WT1 is a universal marker for the detection of minimal residual leukemic (MRL) cells, which remain in leukemia patients after treatment and cause the relapse. WT1 mRNA quantitation assay made it possible to detect MRL cells in acute AML [14, 15, 16] ALL, [14, 15, 16, 17] and myelodysplastic syndrome (MDS) [18, 19, 20] WT1 mRNA quantitation assay for the detection of the MRL cells of AML, ALL, and MDS is available as a clinical test, WT1 mRNA Assay Kit II "Otsuka", which is covered by national health insurance in Japan. This test is also available as WT1 mRNA OneStep Assay Otsuka in Europe and Wilms' tumor-1 gene (WT1) mRNA RT-PCR Assay Kit Otsuka in China.

WT1 protein overexpression in not only hematological malignancies but also almost all kinds of solid tumors indicated that WT1 protein should be a ubiquitous TAA that was useful for cancer immunotherapy. To confirm this, pre-clinical studies were conducted. Mouse MHC class I-restricted WT1 peptides induced WT1-specific cytotoxic lymphocytes (WT1-CTLs) and the WT1-CTLs killed WT1-expressing leukemia cells in vitro and in vivo with an HLA class I restriction manner. [21, 22] Furthermore, human HLA class I-restricted WT1 (WT1-I) peptides in vitro induced WT1-CTLs that were able to kill human WT1-expressing leukemia cells. [23, 24, 25]

Based on these findings, National Cancer Institute, USA evaluated 75 popular TAAs for the clinical utility and ranked the WT1 antigen as the top among them (Figure 1). [26]

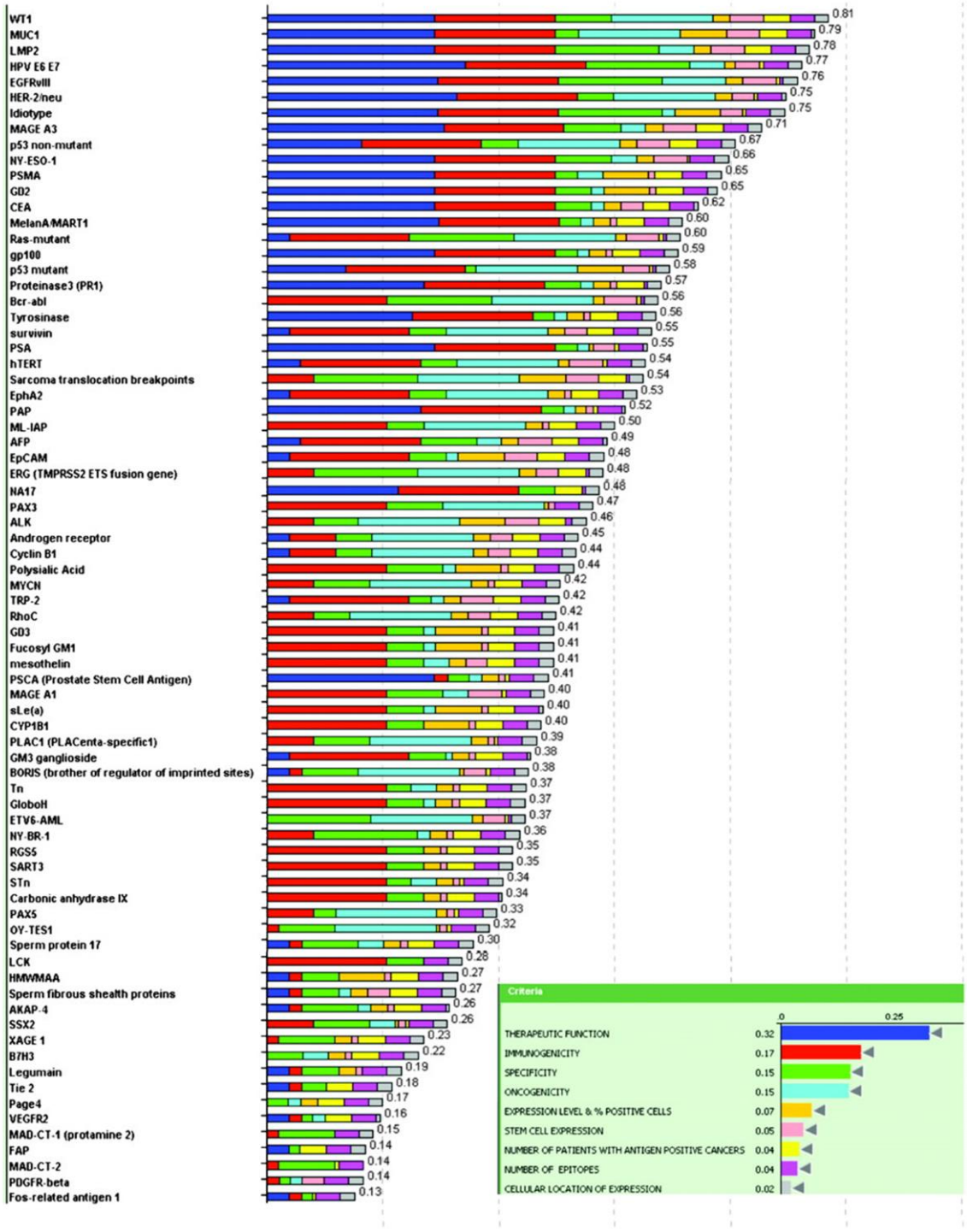
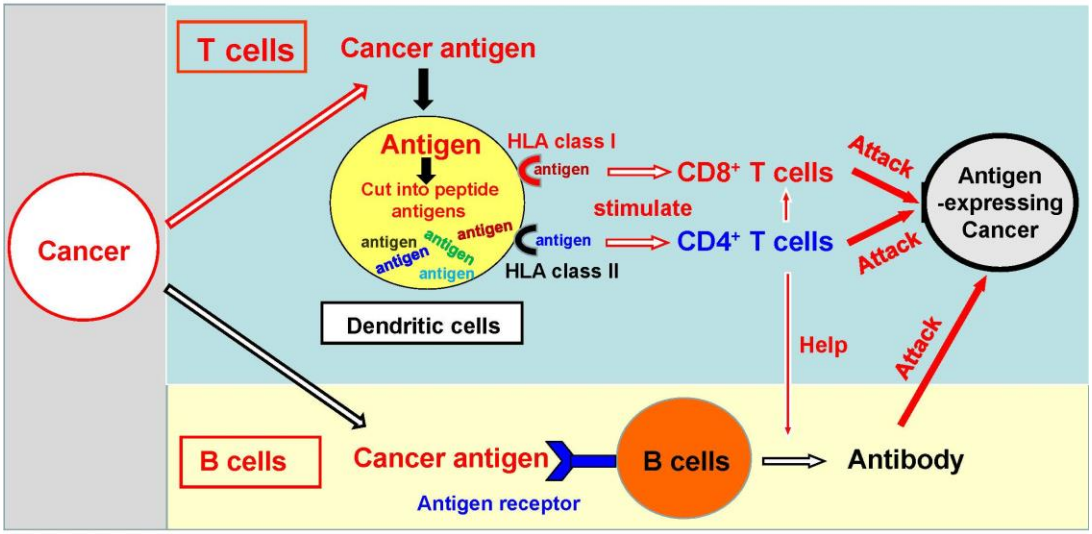


Fig. 1 Cancer antigen pilot prioritization: representation of ranking based on predefined and preweighted criteria and subcriteria. Inset, the color used to designate each creterion and its relative weight. Number at the end of each bar relative rank of that antigen.

After that, human HLA class II-restricted WT1 (WT1-II) peptides were identified and applied to WT1 cancer vaccine. [27, 28] The combinational use of WT1-I and WT1-II peptides largely increased the clinical effect, compared to the use of WT1-I peptides alone. Cellular and humoral immune responses against cancer were schematically described (Fig.2)

Fig.2 Cellular and humoral immune response against cancer



A First-in-Human clinical study of WT1 peptide vaccine using the WT1-I peptides alone started in 2001. [29, 30] Afterwards, many clinical studies of WT1 cancer vaccine, including WT1 peptide vaccine,[31, 32, 33, 34] WT1-DC vaccine,[35, 36] and WT1 mRNA-DC vaccine,[37] are being conducted for hematological malignancies and various kinds of solid tumors.

The aim of this review article is to summarize many clinical studies of WT1 cancer vaccine and to show the advantages of the combination of WT1 cancer vaccine and the other treatments and the importance of WT1 cancer vaccine in the cure-oriented cancer treatments. Application of WT1 cancer vaccine to cancer prevention are also discussed.

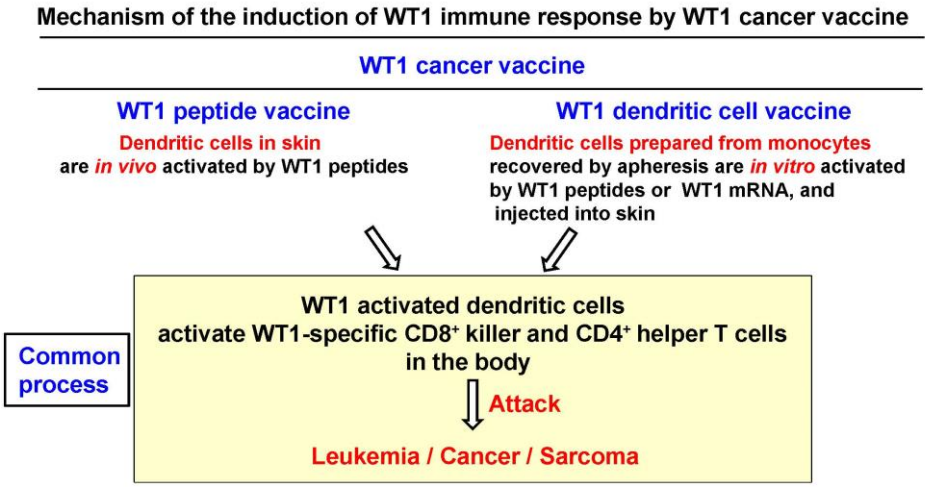
2. Clinical study of WT1 cancer vaccine

2.1 Procedure

In WT1 peptide vaccine, WT1-I (killer) peptides alone, or the cocktail of the WT1-I (killer) and WT1-II (helper) peptides was cutaneously injected in the emulsion with adjuvants. On the other hand, in WT1-DC and WT1 mRNA-DC vaccines, monocytes were recovered by

apheresis from patients and matured into DCs. The matured DCs were pulsed with WT1 peptides (WT1-I peptides alone, or the cocktail of the WT1-I and WT1-II peptides), or electroporated with WT1 mRNA, and cutaneously injected (see the references for the details of WT1 peptides, adjuvants, methods of WT1 DC maturation and WT1 mRNA electroporation, and the others). (Fig.3)

Fig.3



Recently, to promote the endocytosis of WT1 antigen into DCs, antibody fusion proteins consisting of WT1 protein fragments and an antibody specific for endocytic receptor DEC205 on human DCs had been developed.[38]

2.2 Clinical study

2.2.1 Acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL)

Oka et al provided the first report of WT1 peptide vaccine for AML.[30] Twelve patients were treated with WT1 peptide vaccine containing either natural or mutated WT1 peptide with the dose escalation.[39] The decrease in leukemic blast cells or WT1 mRNA levels was observed in 5 patients within 3 WT1 vaccinations. Four of 5 patients survived for unexpected long time. Three patients who had molecular relapse before WT1 vaccination are alive over 17 years. Tsuboi et al reported in detail these 3 long-term survivors.[40] These 3 patients achieved complete remission (CR) by the repeated WT1 peptide vaccination and survive over 17 years until now without significant adverse effects, indicating the cure of AML. Mailander et al treated a patient in relapse with 30% leukemic blasts with WT1 peptide vaccine alone, and a complete hematological remission was confirmed 16

weeks after the vaccination.[41, 42] Yasukawa et al treated a patient in relapse with WT1 peptide vaccine and reported that this patient achieved CR after the vaccination and survived during the follow-up time of 3 years without an increase in leukemic blasts.[33] Uttenthal et al reported that in 2 of the 5 evaluable patients who were treated with WT1 peptide vaccine, WT1-CTLs were induced, and WT1 mRNA levels, which reflected amount of leukemic cells, decreased to normal levels.[43] Brayer et al treated 16 patients with AML in first or second CR with WT1 peptide vaccine.[44] Two patients in CR2 at the time of vaccination demonstrated relapse-free survival (RFS) >1 year with duration of their remission exceeding duration of their first remission. There was a comparative and statistically significant improvement in overall survival (OS) in the WT1-vaccinated patients compared to the historical cohort (495 days versus 165 days, p=0.0175) although RFS was not statistically significant between the two (319 days versus 131 days, p=0.19). These results showed the clinical effect of WT1 peptide vaccine for relapse-high risk AML patients. Shah et al treated one AML and 3 ALL patients who had relapse after HSCT with WT1-DC vaccine with donor lymphocyte infusion.[45] Although obvious clinical effect was not observed, WT1-specific immune response was detected in all of the 3 ALL patients. Nakata et al treated 20 patients in CR but at high risk for relapse with WT1 peptide vaccine.[46, 47] Two-year disease-free survival (DFS) rate was 25%, and 2-year OS rate was 40%. Maslak et al treated 22 patients with WT1 peptide vaccine (galinpepimut-S), which consisted of multiple WT1 peptides. Three-year OS rate was 47%.[34, 48] Furthermore, Anguille et al treated 30 patients with WT1 mRNA-DC vaccine.[49, 50] Five-year OS rate was 40%. Importantly, these 3 independent clinical studies demonstrated a similar clinical efficacy of WT1 cancer vaccine, indicating high reliability of these 3 clinical studies. Since these OS rates were equivalent to those of HSCT, HSCT might be in part replaced with WT1 cancer vaccine in the future. On the other hand, a clinical study of WT1 peptide vaccine containing WT1-II (helper) peptide alone (OCV-501) was done. Kobayashi et al and Kiguchi et al conducted a multicenter, randomized, double-blind, placebo-controlled phase II study to evaluate the efficacy and safety of OCV-501.[51, 52] Elderly AML patients who achieved first CR were randomly allocated to receive either OCV-501 (N=69) or placebo (N=65). Median DFS in OCV-501 and placebo groups was 12.1 and 8.4 months, respectively, and the difference was not significant. However, importantly, OCV-501 responders with the WT1 immune responses such as the production of IgG antibodies against vaccinated WT1 helper peptide and induction of WT-specific $CD4^+$ T cells had significantly longer OS compared to placebo arm ($p<0.0001$), indicating the potential clinical effect of OCV-501 for AML.

2.2.2 Chronic myeloid leukemia (CML)

Oji et al and Narita et al conducted a phase I/II clinical study of WT1 peptide vaccine for patients with CML who were treated with

imatinib, but became resistant to it.[53, 54, 55] After WT1 peptide vaccination, WT1-CTLs were induced, and then bcr/abl mRNA levels decreased to and stayed at, undetectable ones. Hughes et al also showed that WT1-CTLs became detectable in major molecular response and molecular response after the tyrosine kinase inhibitor (TKI) therapy although they was not detected at diagnosis.[56] These results indicated that spontaneous WT1-CTL induction should contribute at least in part to the clinical effect of TKI therapy. Interestingly, Zhi-Dong et al showed that the appearance of graft-versus-leukemia (GVL) effect by HSCT for CML was associated with the induction of WT1-CTLs, which resulted from the spontaneous WT1 immune response against WT1-expressing CML cells.[57] These results indicated that the GVL effect was at least in part due to the WT1 immune response against WT1-expressing CML cells.

2.2.3 Myelodysplastic syndrome (MDS)

Oka et al treated 2 MDS patients with WT1 peptide vaccine, which was conducted as a First-in-Human clinical study.[29] Unexpectedly, white blood cells (WBC) promptly decreased in the 2 patients after the vaccination, and sepsis occurred in the 2 patients. It was thought that these adverse effects resulted from the prompt induction of WT1-CTLs, followed by the killing of MDS leukemic stem/progenitor cells by the CTLs. Since MDS is a stem cell disease, the majority of patients' WBC is derived from WT1-expressing leukemic stem cells. Therefore, it was reasonable to think that the prompt killing of the WT1-expressing leukemic stem cells by WT1-CTLs gave rise to the prompt reduction in WBC located downstream of the stem cells because WBC was continuously being producing from the stem cells. Therefore, WT1 peptide vaccine was very effective. Yasukawa et al treated a patient with frequent red blood cell (RBC) transfusion with WT1 peptide vaccine.[33] After the repeated vaccination, RBC counts were maintained at more than 7.0 g/dL without the transfusion during 3 years of follow-up time. Brayer et al treated 2 high-risk patients with WT1 peptide vaccine, and one of 2 experienced a prolonged decrease in transfusion dependence, and the bone marrow biopsy collected after the WT1 peptide vaccination showed a 35-40% reduction in myeloblast frequency.[44] These 4 clinical studies showed that WT1 peptide vaccine was effective. On the basis of these clinical results, company (Sumitomo Pharma, SP) -led clinical studies of WT1 peptide vaccine were conducted. Ueda et al treated 26 patients with WT4869 WT1 peptide vaccine, which was consisted of HLA-A*24:02-restricted WT1-I (killer) peptide and adjuvant.[58] One and 12 of 22 patients evaluable for hematological response were marrow CR (4.5%) and SD (54.5%), respectively. The overall response rate was 18.2%; the disease control rate was 51.9%; and median OS was 64.7 weeks. Median OS of 13.0 months in WT4869 arm for azacitidine-refractory patients was longer than median OS of 5.6 months in a historical control. Since these clinical results encouraged the further clinical study, a company (SP)-led phase I/II clinical

study of DSP-7888, WT1 peptide vaccine consisting of a cocktail of WT1-I (killer) and WT1-II (helper) peptides was conducted for azacitidine-resistant, relapse-high risk MDS. Miyakoshi et al and Usuki et al reported that median OS of the patients who were treated with DSP-7888 was 8.5 months while median OS of historical control was 5.6 months.[59, 60, 61] Importantly, median OS was 10.25 months and 4.94 months in WT1-specific DTH (delayed type hypersensitivity to WT1 peptide)-positive and -negative patients, respectively, and this difference in median OS was statistically significant (p=0.0045), indicating that the clinical effect of DSP-7888 was produced by the WT1 immune response.

2.2.4 Malignant lymphoma

Ogasawara et al treated 5 patients (2 diffuse large B cell lymphoma, one follicular lymphoma, one mantle cell lymphoma, and one peripheral T cell lymphoma) with WT-DC vaccine.[62] One follicular lymphoma was CR; one mantle cell lymphoma was partial remission (PR); one diffuse large B cell lymphoma and one peripheral T cell lymphoma were stable disease (SD); and one diffuse large B cell lymphoma was progressive disease (PD). A 60-year-old male with follicular lymphoma who had residual disease after the several courses of R-CHOP chemotherapy achieved CR after WT1-DC vaccine, and survived over 48.7 months. A 70-year-old male with mantle cell lymphoma who relapsed after intensive chemotherapy of R-CHOP and R-MEOP achieved PR after the vaccination, and survived for 37.9 months. A 51-year-old male with diffuse large B cell lymphoma who relapsed regardless of intensive chemo-radio therapies achieved SD after the vaccination and survived for 39.7 months. A 90-year-old female with peripheral T cell lymphoma who relapsed after THP-COP chemotherapy achieved SD by the vaccination, and survived for 44.0 months. It was noteworthy that there was a clear correlation between WT1 immune response and a prolongation of disease stabilization and survival time, showing that WT1-DC vaccine was effective. Shah et al treated one patient with relapsed Hodgkin lymphoma with WT-DC therapy. [45] However, obvious clinical effect was not observed. Israyelyan et al discovered that a large percentage of non-Hodgkin lymphoma (NHL) of all grades maintained WT1-CTLs.[63] They examined 63 patients on the frequencies of functional WT-CTLs by the ex vivo CD137 assay. The frequencies of patients with the WT1-CTLs was 55.8% in high/intermediate grade NHL patients and 20.0% in low grade NHL ones, and the difference was statistically significant (p=0.011). These results indicated the involvement of WT1-CTLs in spontaneous anti-cancer immunity against NHL

2.2.5. Multiple myeloma (MM)

Tsuboi et al showed that 3 of 4 patients with multidrug-resistant MM who were treated with WT1 peptide vaccine had clinical effects.[64] In one Bence-Jones type patient, the frequency of myeloma cells in bone marrow decreased from 85 % to 25 % during three month (a total of 12

injections) of WT1 peptide vaccination, and multiple bone lesions were improved. Tyler et al examined the significance of WT1-CTL response in patients with relapsed MM and high-risk cytogenetics who were undergoing allogeneic T cell-depleted HSCT followed by donor lymphocyte infusion (DLI).[65] All of 24 patients subsequently developed increments of WT1-CTL frequencies that were associated with reduction in specific myeloma markers in the absence of graft-versus-host disease (GVHD). These results showed an association between the emergence of WT1-CTLs and graft-versus-myeloma effect, indicating that WT1-CTLs induced spontaneously after HSCT contributed to graft-versus-myeloma effect. Taken together, these results indicated that WT1 peptide vaccine was effective for MM.

2.2.6 Brain tumor

Izumoto et al treated 21 patients with recurrent glioblastoma multiforme (GBM) with WT1 peptide vaccine.[66] Two, 10 and 9 of them were PR (9.5%), SD (47.6%), and PD (42.9%), respectively, at the time points of 3 months after the WT1 vaccination. Disease control rate was 57.1%, and median PFS was 20 weeks. Therefore, these clinical results were promising. Two SD patients became CR by the repeated WT1 peptide vaccination thereafter. Some patients were miss-judged as PD because of the pseudo-progression of tumor although the WT1 peptide vaccine was effective for them. Therefore, actual clinical effects were considered to be more. Furthermore, Hashimoto et al examined the effect of WT1 peptide vaccine on relapse prevention for 7 GBM patients who received the complete surgical resection of tumor.[67] OS of the patients who received WT1 peptide vaccine was much longer, compared to OS of historical control. In the above 2 clinical studies, only WT1-I (killer) peptides were used. On the other hand, Tsuboi et al treated 14 patients with recurrent malignant glioma with the combination of WT-I (killer) and WT1-II (helper) peptides.[68] Eleven of 14 patients completed 6 WT1 vaccinations, while 3 patients dropped put due to early disease progression. Six of the 11 evaluable patients were SD (54.5%), and the remaining 5 patients were PD (45.5%). Median OS and one-year OS rate were 24.7 weeks and 36 %, respectively. Although a 58-year-old male with GBM received partial resection of the tumor, followed by the combination therapy of radiation and temozolomide, the GBM was progressive. Therefore, this patient was treated with a total of 49 WT1 vaccinations over one year and 9 months. The tumor growth became stable, and the reduction in Gadolium-enhanced lesion and improvement of the midline shift on MRI were observed. Frequencies of WT1-CTLs notably increased from 0.017 to 2.50% 4 weeks after WT1 peptide vaccination. A 31-year-old male with relapsed anaplastic astrocytoma who was treated with WT1 peptide vaccine and radio-chemotherapies survived in progression-free over 320.1 weeks. Chiba et al examined the relationship between the clinical effect and either WT1 protein or MIB-1 (a marker of cell proliferation) expression levels in GBM, and provided valuable findings.[69] The both median

progression-free survival (PFS) and median OS were significantly longer in the WT1 protein high expression group compared to WT1 protein low expression group (p=0.022 for median PFS, p=0.035 for median OS) whereas they were not related to MIB-1 expression levels. These results indicated that WT1 immune response against WT1 protein in GBM led to the clinical effect, and that higher WT1 protein expression in GBM induced stronger WT1 immune response against GBM, resulting in the occurrence of the better clinical effect. Sakai et al treated 7 relapsed patients (3 GBM, 2 anaplastic astrocytoma, 1 anaplastic oligoastrocytoma, and 1 anaplastic oligodendroglioma) with the combination of WT1-DC vaccine and chemotherapy.[70] All patients excerpt for 2 GBM patients with PD achieved SD. One GBM patient were still alive 46 months after the initial diagnosis. These results showed that WT1-DC vaccine was effective. Sakai et al also reported two patients who were treated with WT1-DC vaccine in the progressive diseases after the failure in the initial standard treatment.[71] WT1-DC vaccine led to a marked decrease in tumor size and an improvement of performance status and neurological disorders in association with an increase in WT1-CTLs. Hashii et al reported a case of a 13-year-old boy with relapsed diffuse midline glioma (grade IV).[72] The patient who presented vertigo, diplopia, and right hemiplegia received radiotherapy and chemotherapy with temozolomide. However, since the tumor regrew, this patient was treated with WT1 peptide vaccine. After the WT peptide vaccination, tumor size was obviously reduced with the induction of WT-CTLs, and his quality of life drastically improved so much as to the extent that this patient was able to live his daily life without difficulty. Best response was SD, and OS was 6.5 months from the start of the vaccination and 8.3 months from the relapse, which was markedly longer than 3.2 months of the median OS in conventional therapies. Yokota et al treated 105 patients with malignant glioma with WT1 peptide vaccine after surgical resection, completed 12 or more vaccinations for 54 patients, and provided important findings regarding the rationale of WT1 cancer vaccine.[73] Of the 54 patients, 27 PD patients received second tumor resection. Therefore, the paired tumor specimens before and after the vaccination were able to be obtained from 20 of the 27 PD patients and pathologically examined for the expression of WT1 protein and HLA class I molecules. Interestingly, the both of WT1 protein and HLA class I molecule expression significantly decreased in the tumors resected after the vaccination compared to those resected before the vaccination (p=0.012 for WT1 protein, p=0.008 for HLA class I molecule). Importantly, higher WT1 protein expression in the tumors resected before the vaccination was significantly associated with a longer OS (p<0.05). Furthermore, higher WT1 protein expression in the tumors resected after the vaccination was also significantly associated with a longer PFS (p=0.012) and OS (p<0.01). These results indicated that at least one cause by which anti-tumor effect of WT1 peptide vaccine was diminished was the decrease in WT1/HLA

complexes on the tumor cell surface, which played an essential role in the recognition and killing of tumor by WT1-CTLs. These results also strongly indicated that anti-tumor effect by WT1 peptide vaccine was produced by WT1 immune response against WT1-expressing GBM tumor cells. These results clearly demonstrated that the loss or decrease in WT1 and/or HLA class I molecule expression was a mechanism of escape of tumor from WT1 immune response. On the basis of these results, a company (SP)-led phase I clinical study of WT2725, WT1 peptide vaccine consisting of one HLA-A*02:01/-A*02:06-restricted WT1 peptide and adjuvant was conducted for advanced GBM. Fu et al reported that 7 of 21 patients survived over one year, and 3 of the 7 patients survived over 18 months.[74] Two of the 7 patients achieved CR after pseudo-progression and survived over 2 years. Based on these promising clinical results, 2 clinical studies, a phase I/II and II clinical studies of DSP-7888 were conducted for pediatric malignant glioma and adult GBM, respectively. Fujisaki et al reported that one and 3 of 11 patients with diffuse intrinsic pontine glioma (DIPG) were PR (9.1%) and SD (27.3%), respectively, and that 2 of 5 patients with GBM and one of 2 patients with high grade glioma were SD (40.0% for GBM, 50.0% for high grade glioma).[75] Median OS of DIPG was 5.4 months and better than 2.7 months of the historical control. One DIPD patient survived over 20 months. Global clinical study for adult GBM (WIZARD 201G), where bevacizumab and bevacizumab + DSP-7888 arms were randomized at a ratio of 1:1, had been conducted.

2.2.7 Head and neck cancer

Ogasawara et al treated 11 patients (2 oral cavity, 2 gingiva, 2 nasopharynx, 2 larynx, one hypopharynx, and 2 oropharynx) with WT1-DC vaccine combined with conventional chemotherapy.[76] Five and 6 of 11 patients were SD (45.5%) and PD (54.5%), respectively. Median PFS was 13.0 and 2.8 months in SD and PD patients, respectively, and the difference was statistically significant (p=0.0014). Median OS was 30.3 and 8.1 months in SD and PD patients, respectively, and the difference was also statistically significant (p=0.0126). Importantly, median PFS was 13.0 and 2.8 months in WT1 immune response-positive and-negative patients, respectively, and the difference was statistically significant (p=0.0025). Similarly, median OS was 30.3 and 7.6 months in WT1 immune response-positive and-negative patients, respectively, and the difference was statistically significant (p=0.0025). These results indicated that WT1-DC vaccine was effective, and that the patients who were able to induce WT1 immune response were able to induce clinical effect.

2.2.8 Lung cancer

Oka et al and Tsuboi et al reported that 4 of 8 evaluable patients who were treated with WT1 peptide vaccine at the advanced stage had clinical effects.[30, 77] In a 76-year-old male at the terminal stage, tumor decreased, and tumor markers became normal, and cancerous

lymphangiopathy improved after 2 WT1 peptide vaccinations. This patient survived for 2 years and one month. To evaluate the clinical effect of WT1 peptide vaccine on the prevention of relapse after the complete resection of non-small cell lung cancer at clinical stages ⅠB and ⅡA, 12 patients were treated with WT1 peptide vaccine a total of 19 times (5 times weekly, 4 times biweekly, and then 10 times monthly) for one year. Then WT1 peptide vaccination was discontinued and the patients were observed without any additional treatments thereafter. In the phase I part, 11 of the 12 patients who were treated with WT1 peptide vaccine survives over 7 years and 6 months until today without significant adverse effect. OS of WT1-vaccinated patients was much longer compared to the OS of the historical control patients who were treated with operation alone or operation + chemotherapy (manuscript in preparation). These results showed the strong preventative effect of WT1 peptide vaccine on the relapse of non-small cell lung cancer. Krug et al treated 3 patients with WT1 peptide vaccine, and in 2 evaluable patients, WT1-specific $CD4^+$ and $CD8^+$ T cell responses were detected although clinical effect was not able to be evaluated because of a small number of patients.[78] Takahashi et al treated 62 patients with inoperable or recurrent lung cancer with WT1 and/or muc-1 peptide-pulsed DC vaccine.[79] One, 4, 26, and 31 of the 62 patients were CR (1.6%), PR (6.5%), SD (41.9%), and PD (50.0%), respectively. Median OS was 27 months from diagnosis and 12 months from the start of the DC vaccine, showing the longer OS compared to that of historical control. Importantly, two items of the use of WT1 peptide in the DC vaccine and Hb concentration were prognostic factors, but not the use of muc-1. These results indicated that the clinical effect resulted from the WT1 immune response against tumor.

2.2.9 Malignant mesothelioma

Malignant mesothelioma is a typical tumor that expresses WT1 at high levels. May et al identified the WT1 peptide epitopes specific for $CD4^+$ and $CD8^+$ T cells that recognized and killed malignant mesothelioma cells.[80] As a clinical application of these WT1 peptides, Krug et al vaccinated 9 patients with these WT1 peptides.[78] Eight patients were PD, but one relapsed patient remained without progression 36 months after the start of the study, suggesting the clinical effect. In the following clinical study, Zauderer et al randomized 41 patients to galinpepimut-S, which contained 4 WT1 peptides that stimulated both $CD4^+$ and $CD8^+$ T cells, with GM-CSF + Montanide arm, or GM-CSF + Montanide arm, after surgery and another treatment modality.[81] One-year PFS rate was 33% and 45% in the control and vaccine arms, respectively. Median PFS was 7.4 months and 10.1 months and median OS was 18.3 months and 22.8 months in the control and vaccine arms, respectively. Therefore, clinical effect was observed although it was underpowered because of a small number of patients.

2.2.10 Thymoma and Thymic cancer

Oji et al treated 4 patients with invasive thymoma and 8 patients with thymic cancer with WT1 peptide vaccine.[82, 83] Three and one of the 4 thymoma patients were SD (75.0%) and PD (25.0%), respectively. In a 53-year-old male with a pleural disseminated thymoma resistant to conventional therapies, the tumor size decreased after WT1 peptide vaccination and then stayed at SD for as long as one year and 7 months. Of 8 patients with thymic cancer, 6 and 2 were SD (75.0%) and PD (25.0%), respectively. In a 53-year-old male who had recurrent tumor, multiple lung and bone metastasis, and kidney metastasis that were resistant to intensive chemotherapy after the surgery followed by radiation, the tumor growth was stabilized for 4.5 months after WT1 peptide vaccination in the association with the WT1-CTL induction and WT1-specific DTH-positivity. These results showed that WT1 peptide vaccine was effective for thymoma and thymic cancer.

2.2.11 Breast cancer

Oka et al reported that in 2 patients with recurrent, metastatic breast cancer, the metastatic tumor decreased by WT1 peptide vaccine.[30] In one patient who was resistant to chemotherapy and had ileus due to involvement of metastatic tumor into colon, the ileus disappeared after 2 WT1 peptide vaccinations, and the patient survived over 3 years and one month, showing the clinical effect of the vaccine. Higgins et al treated the patients with stage II/III breast cancer with the vaccine (WT1-immunotherapeutic) combining the WT1 recombinant antigen with the AS15 immunostimulant, or placebo in 4 cohorts according to neoadjuvant treatment (aromatase inhibitors, chemotherapy, trastuzumab + chemotherapy, or HR^+ $HER2^-$ chemotherapy).[84] Clinical effect was not able to be evaluated because of patients' complex background and a small number of patients. However, importantly, in the cohort where aromatase inhibitors + WT1-immunotherapeutic were administered without chemotherapy and routine corticosteroids, all patients developed WT1-specific antibodies as a humoral immune response against WT1 antigen. On the other hand, none of the patients who were treated with chemotherapy + WT1-immunotherapeutic developed the WT1 antibodies. These results showed that chemotherapy suppressed the WT1 immune response but not aromatase inhibitors. Gillmore et al detected WT-CTLs in tumor-draining lymph node in patients with stage I/II breast cancer and demonstrated the endogenous and spontaneous immune response against WT1 antigen in breast cancer patients, showing that WT1 protein was really immunogenic in breast cancer.[85]

2.2.12 Salivary gland cancer

Sasabe et al treated with WT1 peptide vaccine a 66-year-old female with growing pulmonary metastasis regardless of chemo-radiotherapy, followed by the administration of lymphokine-activated killer cells + IL-2 + IFN-γ.[86] The tumor

growth stopped during one year of WT1 peptide vaccination, but the tumor regrew after the discontinuation of WT1 peptide vaccination. Shirakata et al reported that in a 56-year-old male with recurrence who was treated with WT1 peptide vaccine, rapid tumor growth was suppressed, and then the tumor growth decreased and stayed at SD for further 7 weeks until the end of the clinical study for 12 weeks. [87] Necrotic lesion in the tumor was found by CT examination at the end of WT1 peptide vaccination. WT1-CTLs increased after the WT1 peptide vaccination, and WT1-specific DTH became positive. These results showed that WT1 peptide vaccine was effective.

2.2.13 Esophageal cancer

Matsuda et al reported that they treated 10 patients with the advanced cancer with WT1-DC vaccine combined with docetaxel, and that one and 9 patients were SD (10%) and PD (90%), respectively.[88] Importantly, the frequencies of WT1-CTLs significantly increased after the vaccination compared to those before the vaccination (p=0.040). However, it seemed that the anti-tumor WT1 immune response was not strong enough to suppress tumor growth because of severe immunosuppressive conditions induced by intensive chemotherapy. Ogasawara et al reported that 15 patients on the recurrent or refractory status regardless of surgery, chemotherapy and/or radiotherapy were treated with WT1-DC therapy, and that one, 2, 3, and 9 of the 15 patients were CR (6.7%), PR (13.3%), SD (20.0%), and PD (60.0%), respectively.[89] Median PFS and OS were 4.1 and 7.0 months from the vaccination, respectively. In a 65-year-old female who had many metastatic lesions in para-aorta lymph nodes and abdomen cavity, these lesions gradually regressed and disappeared after the 14th WT1-DC + S-1 therapy, and CR was achieved. The CR was maintained by the repeated WT1-DC + S-1 therapy for as long as 14 months. Median OS was 18.3 and 5.8 months in the responding patients (CR, PR, and SD) and non-responding ones (PD), respectively, and it was significantly longer in the responding patients than the non-responding ones (p=0.0049). WT1-specific CTLs significantly increased in the responding patients after the DC vaccination (p=0.043), but not in the non-responding ones. Furthermore, the frequency of the WT1-specific CTLs after the DC vaccination was significantly higher in the responding patients than the non-responding ones (p=0.043). These results showed that WT1-DC was effective for recurrent or refractory esophageal cancer.

2.2.14 Gastric cancer

Kobayashi et al directly injected WT1 and muc-1 peptide-pulsed DCs into the tumor in an 80-year-old patient with recurrent gastric cancer. Surprisingly, the tumor completely disappeared, and CR was achieved.[90] Importantly, WT1-CTLs were induced at high frequency, indicating that the clinical effect was due to the WT1 immune responses against the gastric cancer. Lu et al treated 10 patients at stages III/IV with WT1-DC vaccine.[91] Six and 4 of the 10 patients were

SD (60%) and PD (40%), respectively. Two of the 4 PD patients was evaluated to be clinically effective although the 2 patients finally became PD. OS was significantly longer in SD patients compared to PD patients ($p<0.05$). These results indicated that WT1-DC vaccine was effective for gastric cancer.

2.2.15 Pancreatic cancer

Kaida et al conducted an open-labeled, dose-escalation phase I clinical study of WT1 peptide vaccine, and treated 9 patients with advanced cancer with the combination of GEM and WT1 peptide vaccine.[92] Eight and one of the 9 patients were SD (88.9%) and PD (11.1%), respectively, and disease control rate at 2 months was 88.9%. One patient showed a reduction in the tumor size at 3 months. Since WT1 immune responses were not examined in detail, the contribution of WT1 peptide vaccine to clinical outcome was not able to be evaluated. Nishida et al treated 32 patients with advanced, inoperable pancreatic cancer with the combination of GEM + WT1 peptide vaccine (GEM + WT1).[93] Six and 16 of 30 patients who were eligible for evaluation of clinical effect were PR (20.0%) and SD (53.3%), respectively. Median PFS and OS were 4.2 and 8.1 months, respectively. Six-month and one-year OS rates were 71.0% and 29.0%, respectively. These clinical results suggested that GEM + WT1 had better clinical effect than GEM alone. On the basis of these promising results, Nishida et al conducted the randomized clinical study of GEM alone versus GEM + WT1.[94] Patients with advanced pancreatic cancer were randomized to GEM arm, or GEM + WT1 arm at a ratio of 1 to1. Six- month PFS rates in the stage IV were 3.9 % and 37.8 % for GEM and GEM + WT1 arms, respectively, and the difference was statistically significant ($p=0.017$). The patients in the GEM + WT1 arm were divided into WT1-specific DHT-positive and-negative ones, and median PFS and 6-month PFS rates were analyzed. Median PFS was 6.5 and 2.5 months in DTH-positive and-negative patients, respectively, and 6-month PFS rate was 60.0 and 17.8 % in DTH-positive and-negative patients, respectively. Importantly, PFS in DTH-positive patients was significantly prolonged compared to that in DTH-negative patients ($p=0.001$), whereas PFS in DTH-negative patients was similar to that in the patients treated with GEM alone. These results strongly indicated that the clinical effect of GEM + WT1 resulted from WT1 immune response. This should be the first demonstration of the statistically significant clinical effect of TAA peptide-based cancer vaccine in randomized control trial. Kimura et al treated 36 patients at advanced stages with the combination of GEM/S-1 and DCs pulsed with WT1 peptide and 0 - 3 peptides of Muc-1, CEA, CA-125, and Her 2 peptides. Since clinical effect was 2 CR (6.7%), 4 PR (13.3%), 9 SD (30.0%), and 15 PD (50.0%) among 30 evaluable patients, it was sufficient for the advanced cancer.[95] However, the contribution of WT1-DC vaccine to clinical effect remained unclear since the relationship between the clinical effect and WT1 immune responses

was not be able to be evaluated. Mayanagi et al evaluated the feasibility of, and immune response to WT1-DC vaccine combined with GEM as a first-line therapy in 10 patients with advanced pancreatic cancer.[96] The frequencies of WT1-CTLs significantly increased after the combination therapy (p=0.036). Six and 4 of the 10 patients were SD (60%) and PD (40%), respectively. OS were significantly longer in SD patients than PD patients (p=0.016). This combination therapy controlled the cancer progression in all patients with locally advanced disease without liver metastasis, but not in patients with liver metastasis. Hanada et al evaluated 6 patients who were treated with the combination of WT1-DC vaccine with chemotherapy, radiotherapy, and/or surgery.[97] In 2 of 4 WT1-specific DTH-positive patients, survival time was long (more than 2,747 and 1,134 days). However, clinical effect was not able to be evaluated because of a small number of patients. Nagai et al treated 10 patients with the combination of WT1/Muc-1-DC vaccine and chemotherapy after tumor resection.[98] The induction of WT1-CTLs was related to longer survival, although it was not statistically significant, suggesting the anti-tumor effect of WT1 immune response. Ota et al treated 48 patients with metastatic and unresectable or relapsed cancer with WT1/Muc-1 DC vaccine in combination of chemotherapy.[99] Seven, 20, and 21 of the 48 patients were PR (14.6%), SD (41.7%), and PD (43.8%). Median OS and PFS were 8.1 and 15.1 months from the start of the DC vaccine, respectively. Importantly, median OS and PFS were significantly longer in patients with the positive immune response to WT1 or Muc-1 compared to those without the positive immune response (p<0.001). In these 6 clinical studies of WT1-DC vaccine described above, only WT1-I (killer) peptides were used. On the other hand, Koido et al conducted a novel clinical study of WT1-DC vaccine where one arm used one each of WT1-I (killer) and WT1-II (helper) peptide while another used the both WT1-I and WT1-II peptides, and compared the clinical effect.[100, 101] The both of PFS and OS were significantly better in WT1-I and -II peptides-double pulsed DC arm than WT1-I or WT1-II peptide-pulsed DC arm (p=0.001 for PFS, and p=0.036 for OS). As expected, the frequencies of WT1-CTLs increased in the both arms after the vaccination, but the increase was significant in only WT1-I and WT1-II peptides-double pulsed DC arm (p=0.003). Furthermore, the frequencies of WT1-CTLs after the vaccination were higher in WT1-I and WT1-II peptides-double pulsed DC arm than WT1-I or WT1-II peptide-pulsed DC arm, and the frequencies were higher in the patients with OS≧1 year than those with OS＜1 year. Moreover, in the WT1-I and WT1-II peptides-double pulsed DC arm, the both of PFS and OS were significantly longer in WT1-specific DTH-positive patients than the DTH-negative patients (p=0.018 for PFS, and p=0.021 for OS). Importantly, a significant increase in WT1-specific $CD4^+$ ($WT1\text{-}CD4^+$) helper T cells was found in WT1-I and -II peptides-double pulsed DC arm (p<0.0001).

These results confirmed in the clinical practice a concept that the use of the both WT1-I (killer) and WT1-II (helper) peptides induced WT1-CTLs stronger than the use of one each of WT1-I and WT1-II peptides, followed by the better clinical effect. On the basis of these results, the following WT1-DC vaccine routinely used both WT1-I (killer) and WT1-II (helper) peptides. Yanagisawa et al treated 8 patients with WT1-I and WT1-II peptides-double pulsed DC vaccine with chemotherapy after tumor resection.[102] WT1-CTLs became detectable in 7 of the 8 patients after the vaccination although they were within the background levels before the vaccination. Two-year OS rate after surgery was significantly better in WT1 immune response-positive patients compared to the negative patients (71.4% vs. 0.0%, p=0.008). Katsuda et al conducted a double-blind randomized comparative clinical study of WT1-DC vaccine (TLP0-001) in combination with S-1 for advanced pancreatic cancer refractory to standard chemotherapy.[103] A total of 185 patients with inoperable or metastatic pancreatic cancer who were refractory or intolerant to standard primary chemotherapy with GEM + nab-paclitaxel were allocated to secondary treatment with either placebo in combination with S-1 (the control group) or TLP0-001 in combination with S-1 (the investigational product group). The primary objective of this trial was to evaluate the safety and efficacy (as measured by overall survival) of the investigational product by comparing the two groups. The clinical results are awaited.

2.2.16 Gallbladder and bile duct cancer

Kaida et al treated 8 patients with advanced gallbladder cancer and 8 patients with advanced bile duct cancer with the combination of WT1 peptide vaccine and GEM.[92] Objective clinical efficacy was not apparent although elongation of OS was suggested. However, WT1 immune response was observed as followed. A 59-year-old female with bile duct cancer had the increase in the frequencies of WT1-CTLs from undetectable levels before the vaccination to 14.9% 2 months after the vaccination, achieved SD, and survived over 686 days.

2.2.17 Colorectal cancer

Koesters et al showed that WT1-CTLs lysed WT1-expressing colon tumor cells, and identified WT1 protein as an attractive target for the development of antigen-specific immunotherapy in colon cancer.[104] Shimoraida et al treated 3 patients at advanced stages with WT1-DC vaccine after operation + chemotherapy.[105] DFS of 2 patients was 34.0 and 24.5 months, respectively, and OS of them was 43.6 and 32.4 months, respectively. Since PFS was long regardless of advanced stages of the diseases, WT1-DC vaccine seemed to be effective for colon cancer.

2.2.18 Renal cancer

Iiyama et al treated 3 patients with recurrent, multiple metastatic tumor with WT1 peptide vaccine.[106] In the two patients who completed 3-month vaccination, the tumor decrease and

became SD, and the appearance of new metastatic lesions was suppressed for a long time. Morita et al reported that in the 2 patients who received HSCT after chemotherapy, there was a clear relationship between the appearance of GVHD, better clinical effect, and high frequency of WT1-CTLs. [107] Therefore, these results indicated that WT1-CTLs that were spontaneously induced as the result of WT1-specific immune response against WT1-expressing renal tumor played at least a part of the graft versus tumor (GVT) effect by HSCT, aim of which was to induce GVT effect for the eradication of tumor. Ogasawara et al treated 5 patients at advanced stages with WT-DC vaccine, and reported that 4 of the 5 patients were SD (80%), 2 of which were a durable SD (11.7 months+, 19.3 months+), that WT-CTLs significantly increased in the SD patients after the vaccination ($p<0.01$) but not in the PD patient, and that the frequencies of WT-CTLs after the vaccination were significantly higher in the SD patients compared to the PD patient ($p<0.05$).[108] These results indicated that WT1 cancer vaccine was effective for renal cancer.

2.2.19 Bladder cancer

Ogasawara et al treated 5 patients with WT1-DC vaccine, and reported that 3 and 2 of 5 patients were SD (60%) and PD (40%), respectively.[108] Two patients without muscle invasion but at relapse regardless of transurethral resection (TUR) of tumor were treated with WT1-DC vaccine, and achieved SD. These 2 patients survived without relapse over 33.4 and 84.8 months, respectively, with the repeated WT1-DC vaccine. Another patient without muscle invasion who repeatedly relapsed regardless of TUR achieved SD by WT1-DC vaccine. However, relapse occurred 26.8 months after the vaccination, and total cystectomy was performed. This patient survived over 69.1 months. WT-CTLs significantly increased in the SD patients after the vaccination ($p<0.01$) but not in the PD patient, and were significantly higher in the SD patients compared to the PD patient ($p<0.05$). These results indicated that WT1-DC vaccine was effective for bladder cancer.

2.2.20 Gynecologic cancer

Ohno et al treated 12 patients (6 ovarian, 4 cervical, one corpus cancer, and one uterine carcinosarcoma) who were resistant to standard therapies with WT1 peptide vaccine, and 9 patients were eligible for evaluation of clinical effect.[109] Two of 4 ovarian cancer patients were SD (50%), and all of 3 cervical cancer patients were SD (100%). One corpus cancer and one uterine carcinosarcoma patients were PD. In a 53-year-old patient with ovarian serous adenocarcinoma, CA125, a tumor marker returned to normal range, and SLD (sum of the longest diameters) became – 15.5% 3 months after the WT1 peptide vaccination, and pleural and cardiac fluid disappeared. In the following clinical study, Dohi et al reported that in 53-year-old patient with serous ovarian cancer who was treated with WT1 peptide vaccine, pelvic tumor mass reduced with the normalization of

CA125, and pleural and cardiac effusion disappeared.[110] Miyatake et al treated with WT1 peptide vaccine 40 patients with various kinds of tumors (24 ovarian, 11 cervical, and 5 uterine sarcoma) that were resistant to chemotherapy and radiotherapy.[111] Clinical effect was SD (40%) and PD (60%). Importantly, WT1-specific DTH positivity was significantly associated with OS as an independent prognostic factor (p=0.023). On the other hand, age, uterus or cervical cancer, and carcinoma or sarcoma were not prognostic factors. These results indicated WT1 immune responses led to clinical effect. Nishida et al treated 25 patients with recurrent or chemotherapy-resistant ovarian cancer with WT1 peptide vaccine.[112] Nineteen patients completed 3-month WT1 peptide vaccination, and 9 of 25 patients were SD (36.0%). In serous type, 7 and 10 of 17 patients were SD (41.2%) and PD (58.8%), respectively. In non-serous type, 2 and 6 of 8 patients were SD (25.0%) and PD (75.0%), respectively. WT1-CTLs significantly increased after WT1 peptide vaccination (p=0.0017), and WT1 peptide-specific antibodies against the vaccinated WT1 peptide were also produced after the vaccination. Therefore, the both of cellular and humoral immune responses against the WT1 peptide were induced. The frequencies of WT1-CTLs with higher avidity for WT1/HLA complexes were positively correlated with better PFS (p=0.0333), and higher titers of WT1 peptide-specific antibodies also predicted better PFS (p=0.0023). These results indicated that WT1 immune response induced the clinical effect in advanced ovarian cancer. On the other hand, WT1-DC vaccine was also conducted. Coosemans et al treated one ovarian carcinosarcoma (OCS) and one serous ovarian cancer (SOC) with WT1 mRNA-DC therapy, and reported the extended survival of 19 months for OCS and 12 months for SOC.[113] These results indicated the feasibility and T cell immunogenicity of WT1 mRNA-DC vaccine in ovarian cancer. Coosemans et al also treated 6 patients with uterine tumors (3 serous endometrial carcinoma, and 3 leiomyosarcoma) with WT1 mRNA-DC vaccine.[114, 115, 116] A 46-year-old patient with serous endometrial carcinoma at stage IV (Pat.1) showed a transient molecular response after the vaccination. The other 2 patients with leiomyosarcoma (Pats. 2 and 4) showed a radiological response, which was not maintained with booster vaccines. An oncological response was observed in 4 patients with the end-stage disease (Pats.1 and 2, and 2 patients with serous endometrial carcinoma) in association with immune response. Kobayashi et al evaluated the feasibility and clinical effect of WT1-DC vaccine for recurrent ovarian cancer.[117] Two, 14 and 32 of 56 patients were PR (3.6%), SD (25%), and PD (57%), respectively, and the remaining 8 patients were not able to be evaluated. In 17 patients who were examined for the frequencies of WT1-CTLs, they significantly increased after WT1-DC vaccine (p=0.004). These results showed that WT-DC vaccine was effective for ovarian cancer. On the basis of these clinical studies, a company (SP)-led phase I/II clinical study of the combination

therapy of DSP-7888 and ICIs for advanced ovarian cancer is being conducted.

2.2.21 Malignant melanoma

Nishida et al treated 7 patients with metastatic malignant melanoma with WT1 peptide vaccine, where BCG-CWS (Bacille-Calmette-Guerin cell wall skeleton) was used as an adjuvant instead of Montanide.[118] Three and 4 of 7 patients were SD (42.9%) and PD (57.1%), respectively. Of 3 SD patients, 2 with lung metastasis survived over one year after WT1 peptide vaccination. Nishioka et al reported that in a 64-year-old man at stage IV who was treated as described above, metastatic lesion in lung was growing before the vaccination, but the growth was stabilized (SD) and then suppressed after the vaccination. Since no new metastatic lesions appeared for 6 months, the lung lesion was surgically resected. WT1-specific DTH became positive after the vaccination.[119] Fukuda et al treated 9 patients on the stage Ⅳ with the combination of chemotherapy (carboplatin and paclitaxel) and DCs pulsed with WT1, gp100, tyrosinase peptides, and either MAGE-A3 (for HLA-A*24:02) or MAGE-A2 (for HLA-A*02:01) peptide.[120] Of 9 patients, one, 4 and 4 were PR (11.1%), SD (44.4%), and PD (44.4%), respectively, and disease control rate was 55%. Median PFS and OS were 2.3 months and 12.0 months, respectively. Immune response after the vaccination was evaluated by HLA tetramer assay. Positivity of the peptide-specific CTLs was 1/9 for gp100, 0/9 for tyrosinase, 0/9 for MAGE-A3/A2, and 4/9 for WT1. Therefore, positivity of the WT1-CTLs were highest among 4 common TAAs. Furthermore, OS rate was better in the patients with WT1 immune responses, compared to those without the responses (p=0.051), indicating that the clinical effect was at least in part induced by WT1 immune response. These results confirmed that WT1 peptide was the most potent TAA between WT1, gp100, tyrosinase, and MAGE-A3/MAGE-A2 peptides.

2.2.22 Soft tissue sarcoma

Hashii et al treated 4 patients at high risk for relapse with metastasis after intensive chemo-radiotherapy with WT1 peptide vaccine and chemotherapy. One rhabdomyosarcoma was CR; one osteosarcoma was PD; one liposarcoma was SD; and one synovial sarcoma was PD.[121] In a 12-year-old boy with osteosarcoma, a tumor marker, alkaline phosphatase was transiently decreased after the WT1 peptide vaccination although the final outcome was PD. In a 16-year-old boy with liposarcoma, the growth of ileocecal tumor was stabilized after the vaccination (SD). In a 19-year-old boy with synovial sarcoma, a transient SD was achieved after the vaccination although the disease finally became PD. These clinical results indicated that WT1 peptide vaccine was effective for soft tissue sarcoma. Ohta et al reported in detail the clinical course of the above rhabdomyosarcoma patient who achieved CR by WT peptide vaccine.[122] This 7-year-old girl had a mass on her lower left leg, a lymph node metastasis from the right axis to para-aortic legion, multiple bone

metastases in right parietal, right 4th rib and thoracic vertebrae, and bone marrow invasion. She received intensive chemotherapy, followed by operation, and then high-dose chemotherapy with autologous hematopoietic stem cell rescue, followed by radiotherapy on the metastatic lymph nodes. Although primary tumor and all metastatic lesions except for those on the lumbar vertebrae disappeared after these intensive therapies, the lesions on lumbar vertebrae remained and were positive for bone scintigraphy. However, bone scintigraphy on metastatic lesions on lumbar vertebrae became negative after 3 months (12 WT1 peptide vaccinations) from the start of the vaccination, and CR was achieved. She is alive without disease over 15 years and 10 months. Sawada et al treated one osteosarcoma, 2 rhabdomyosarcoma, and one Ewing sarcoma patients in CR but at high risk for relapse with WT1 peptide vaccine.[123] These 4 patients survived in continuous CR over 5 to 7 years, indicating that WT1 peptide vaccine was very effective. On the basis of these clinical results, Miyachi et al conducted a doctor-led phase II clinical study of DSP-7888 for patients with soft tissue sarcoma (19 rhabdomyosarcoma, one malignant rhabdoid tumor, one myoepithelial carcinoma, and one synovial sarcoma) in CR but at high risk for relapse after multi-modal treatment. The patients were randomized to DSP-7888 or control (no maintenance therapy) arm at a ratio of 1:1 after the front-line therapy.[124] One-year event-free survival (EFS) rate after randomization was 68% in DSP7888 arm and 34% in control arm. The hazard ratio for EFS of the DSP-7888 arm to the control arm was 0.29, and the difference was statistically significant (p=0.035). These results clearly demonstrated the usefulness of DSP7888 WT1 peptide vaccine for prevention of relapse of soft tissue sarcoma. To confirm it, a phase III clinical study is awaited.

2.2.23 Familial adenomatous polyposis

Familial adenomatous polyposis (FAP) is a dominant hereditary disease with one locus mutation of APC, a tumor-suppressor gene. In FAP, many colon adenomas appear in young age, and then transform into adenocarcinoma, followed by total colon resection. A doctor-led phase I/II clinical study of DSP-7888 was conducted for the evaluation of suppressive effect of WT1 peptide vaccine on the appearance and growth of adenoma and the transformation of the adenoma into adenocarcinoma. The clinical results are awaited.

2.2.24 The other solid tumors

Sawada et al treated various kinds of solid tumors with WT1 peptide vaccine.[123] Of 3 patients with neuroblastoma who received autologous and allogeneic HSCT but were in overt diseases, one and 2 were SD (33.3%) and PD (66.6%), respectively. This SD patient survived for 7 months after WT1 peptide vaccination. They also treated 2 patients with hepatoblastoma with minimal residual diseases after HSCT. An 8-year-old boy had the stabilization of tumor growth by the WT1

peptide vaccination. After that surgery was performed, and tumor was completely resected. This patient survived in continuous CR over 7 years. A 14-year-old girl with neuroendocrine tumor with metastasis of liver, lung, and para-aortic lymph nodes had MR (minimal response). The metastases of para-aortic lymph nodes deceased, and those of liver were stabilized after the vaccination, although the lung metastases were progressive. Sugiyama et al reported a case of a 6-year-old boy with high-risk neuroblastoma with multiple bone metastases in skull bone, ribs, long bones of extremities, vertebral bodies, and pelvis, and bone marrow invasion.[125] This patient was treated with intensive chemo-radio therapies rescued by HSCT and ^{123}I-metaiodobenzylguanidine (3 times) in the combination with WT1 peptide vaccine (66 times), and achieved CR nine years after the diagnosis. It seemed that the clinical effect was ascribed to not only GVT effect but also WT1 immune responses against tumor. These clinical results indicated that WT1 peptide vaccine was effective for these solid tumors.

2.3 Adverse effects of WT1 cancer vaccine

Common adverse effects were fever, and redness, swelling and itching at the injection sites of WT1 peptide vaccine, WT1-DC vaccine, or WT1 mRNA-DC vaccine. Ulceration with pain at the injection sites was sometimes caused in the case of induction of strong WT1 immune response. In the case of the painful ulceration, the intervals of WT1 vaccination were extended, and/or steroids was applied to the skin ulcer. In any case, WT1 cancer vaccine was very safe and tolerable for almost all patients with advanced malignancies (see the references for the detailed adverse effects).

2.4 Importance of HLA class II-restricted WT1 helper peptides

It is well known that $CD4^+$ helper T cells plays an essential role in immune response against cancer as well as infection. In the vaccination with the combination of WT1-I (killer) and WT1-II (helper) peptides, WT1-specific $CD4^+$ (WT1-$CD4^+$) helper T cells are induced, and the induced WT1-$CD4^+$ helper T cells promote the induction and proliferation of WT1-CTLs (WT1-specific $CD8^+$ killer T cells). Furthermore, the WT1-$CD4^+$ helper T cells terminally differentiate into WT1-$CD4^+$ cytotoxic T cells, and the cytotoxic T cells attack and kill WT1-expressing tumor cells. Therefore, WT1-$CD4^+$ helper T cells initially help the induction and proliferation of WT1-CTLs and terminally differentiate into WT1-$CD4^+$ cytotoxic T cells. In a WT1-expressing tumor-bearing mouse model, the vaccination with the combination of WT1-I (killer) and WT1-II (helper) peptides induced higher frequencies of WT1-CTLs and maintained the WT1-CTLs at higher levels for a long time, compared to the vaccination with WT1-I (killer) peptide alone.[126] Furthermore, tumor-infiltrating WT1-CTLs significantly increased in the vaccination with the combination of WT1-I (killer) and WT1-II (helper) peptides, compared to that of WT1-I (killer) peptide alone ($p<0.05$). In

another mouse model, the vaccination with the combination of WT1-I (killer) and WT1-II (helper) peptides induced more frequently not only WT1-CTLs but also WT1- $CD4^+$ T cells in association with longer survival, compared to that with WT1-I (killer) peptide alone.[28] In fact, patients who had WT1-$CD4^+$ T cells that were endogenously induced before or 2 weeks after the vaccination with WT1-I (killer) peptide alone had better clinical effect of WT1-I (killer) peptide vaccine, compared to the patients without such WT1-$CD4^+$ T cells at the both time points.[127] Therefore, these results indicated that WT1- $CD4^+$ T cells played an essential role in the all aspects of the induction, proliferation, and tumor infiltration of WT1-CTLs, leading to better clinical effect. In the real world of clinical study of WT1 cancer vaccine, the combinational vaccination of WT1-I (killer) and WT1-II (helper) peptides largely improved the clinical effect, compared to the vaccination of WT1-I (killer) peptide alone, as described above. Therefore, in the recent clinical study of WT1 cancer vaccine, including DSP-788, the combinational vaccination of WT1-I (killer) and WT1-II (helper) peptides are being conducted.

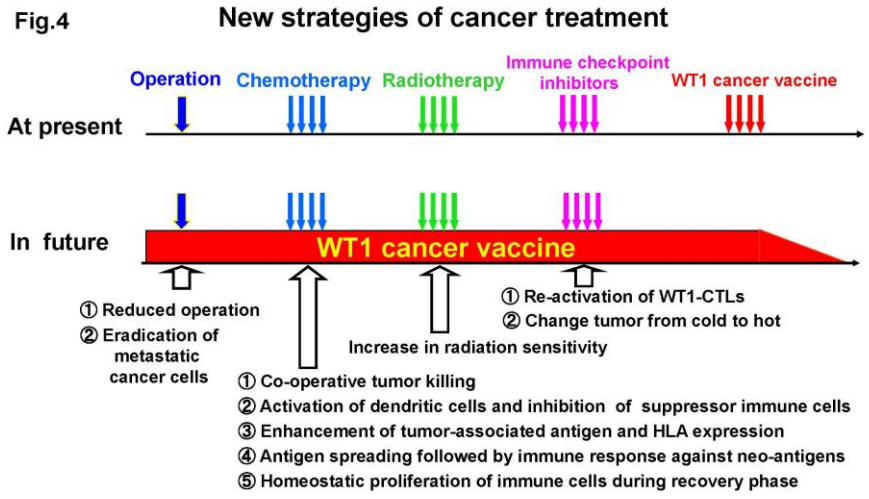
2.5 Combination therapy of WT1 cancer vaccine and the other treatments

Combination therapy of WT1 cancer vaccine and GEM was effective for the advanced pancreatic cancer. GEM treatment increased the WT1 protein, HLA class I and II molecule expression, and activated DCs, resulting in the improvement of the clinical effect of WT1 cancer vaccine.[128,129,130,131] The reader is referred to the other great reviews for general information on the immune effects of chemotherapeutics.[132, 133, 134] Furthermore, some clinical studies showed that combination therapy of WT1 cancer vaccine and HSCT should be a novel strategy for the treatments of intractable malignancies.[135,136,137,138] It is well known that immune checkpoint inhibitors (ICIs) are effective for only hot tumor containing many infiltrating immune cells, but not for cold tumor where the infiltration of immune cells is poor. On the other hand, WT1 cancer vaccine is able to change the cold tumor into the hot tumor containing many infiltrating immune cells. Therefore, the combination therapy of WT1 cancer vaccine and ICIs is surely promising.[139] A company (SP)-led phase I/II clinical study of the combination therapy of DSP-7888 and ICIs is being conducted for ovarian cancer.

3. Immunotherapy is essential for cure-oriented cancer treatments.

Cure-oriented treatments of cancer must have a potential of complete eradication of cancer stem cells that are resistant to chemo-and radio-therapies because they are in the quiescent state of cell-cycle. It is considered that the immune response against cancer plays an essential role in the eradication of the quiescent cancer stem cells. Therefore, immunotherapy should be essential for cure-oriented treatments.[140,141,142,143,144]. Thus, the TAAs targeted for the immunotherapy must be

expressed in the cancer stem cells. WT1 TAA is prominently unique in that it expresses not only in the growing cells of almost all kinds of cancer but also in their quiescent stem cells[145,146,147,148], implying that WT1 is one of the best targets for cure-oriented immunotherapy. As New strategies of cancer treatment, it will be recommended that WT1 cancer vaccine is begun immediately after the diagnosis of cancer, continued until the achievement of CR, and then tapered. Advantages of combinational use of WT1 cancer vaccine with operation, chemotherapy, radiotherapy, and immune checkpoint inhibitors were schematically summarized (Fig.4).



4. WT1 cancer prevention vaccine

WT1 cancer vaccine cured hematologic malignancies and many kinds of solid tumors, or suppressed their relapse for a long time as mentioned above. Therefore, the idea that WT1 cancer vaccine will be effective for cancer prevention is reasonable. FAP, hereditary breast and ovarian cancer syndrome (HBOC) with BRCA1/2 gene abnormality, intraductal papillary mucinous neoplasm (IPMN), heavy smokers with high risk of lung cancer, and senior people with high risk of cancer onset should be good targets for WT1 cancer prevention vaccine. A phase I/II clinical study of DSP-7888 WT1 peptide vaccine for FAP had been conducted as mentioned above.

COI

I am an inventor in a part of the patents needed for the commercial development of WT1 cancer vaccine.

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