Chapter 8

Dendritic Cell-Based Cancer Immunotherapy Targeting Wilms' Tumor 1 for Pediatric Cancer

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Abstract

The treatment of advanced pediatric cancers that have metastasized to distant organs remains difficult. Investigations evaluating the potential treatment of these cancers using therapeutic vaccination with an active dendritic cell (DC)-based immunotherapy are also being conducted. This method induces an efficient immune response by the acquired immune system against tumor-associated antigens. Cancer vaccination therapies have been prepared using autologous monocyte-derived mature DCs exposed to granulocyte–macrophage colony-stimulating factor

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and interleukin-4, which are the molecules principally attributed to the presence of tumor-associated antigens. Wilms' tumor 1 (WT1), an attractive target antigen that has been widely detected in cancers including sarcoma and leukemia, has been shown to be the most potent tumor-associated antigen. DC-based immunotherapy targeting WT1 may have a potentially strong therapeutic activity against cancers. DC vaccines primed with human leukocyte antigen (HLA) class I-/II-restricted WT1 peptides (WT1-DC) are a feasible option. A 6-year-old girl with neuroblastoma and a 14-year-old girl with WT received autologous DC vaccination pulsed with a modified WT1 peptide compatible with HLA-A*24:02. The patients received 20 and 25 vaccines, respectively, and experienced no adverse effects aside from a grade 2 skin reaction at the injection site and a fever with tolerable elevation. WT1tetramer analysis after vaccination detected WT1-specific immune responses. This treatment strategy may be safe, tolerable, and even feasible for all patients who are refractory to treatment and for pediatric patients who have relapsed with neoplasms.

Key words: Cancer vaccination; Dendritic cells; Pediatric neoplasm; Tetramer analysis

Introduction

Despite significant advances in cancer therapeutics, including the introduction of immune checkpoint inhibitors (1–6), it remains extremely difficult to treat advanced cancers affecting multiple organs and involving distant metastases. Ralph Steinman, the Nobel Prize-winning scientist who discovered dendritic cells (DCs) in 1973 (7), experimentally immunized himself with DC vaccination therapy against his pancreatic cancer and survived for 4.5 years. The manufacturing technology used in the production of antigen-presenting cell (APC)-based immunotherapies involving active DCs, the immune system's most potent APCs, is currently under development as a means of therapeutic vaccination against cancer (8). DC-based immunotherapy not only appears to be associated with few adverse reactions but also has limited clinical effectiveness when assessed using conventional evaluation methods such as response evaluation criteria in solid tumors (9, 10). Due to a slow clinical response, a low response rate, and few differences in patient median survival time (MST), long-term cancer immunity results in a delayed separation of treated and untreated patient survival curves, with an eventual treatment advantage in prolonged overall survival (OS) (11, 12).

An *ex vivo* technique is being developed for DC-based cancer vaccination to promote strong induction of T cells against tumor antigens. Oil adjuvants for peptide vaccines act by locally accelerating the activation of lymphocytes (13). However, DCs have the potential antigen bioactivity and may be used as a suitable adjuvant (14–16). Human leukocyte antigen (HLA) molecules harbor cancer antigen peptides that promote DCs binding with receptors on $CD8^+$ killer and $CD4^+$ helper T cells, leading to an immune response against cancers (Figure 1). In contrast,

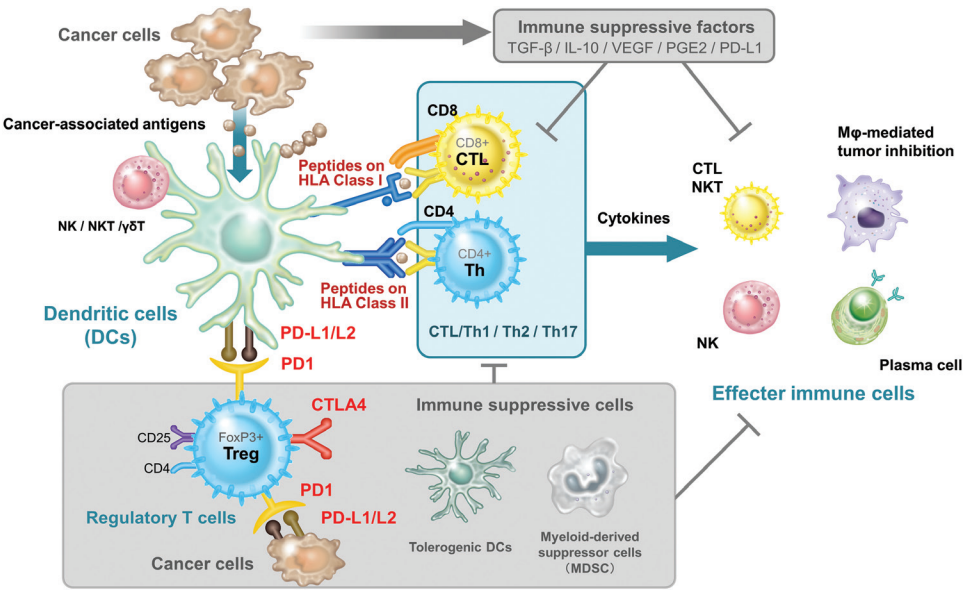

Figure 1. Dendritic cells and other immune cells in the cancer environment. Human leukocyte antigen (HLA) molecules harbor cancer antigen peptides, which induce DC binding with receptors on $CD8^+$ killer and $CD4^+$ helper T cells, leading to anticancer immune responses. In contrast, immune suppressor cells, such as regulatory T cells, tolerogenic DCs, and myeloid-derived suppressor cells, suppress autoreactive and cancer-derived mechanisms. (Original figure by Shimodaira S.)

immune suppressor cells, such as regulatory T cells, tolerogenic DCs, and myeloid-derived suppressor cells, suppress autoreactive and cancer-derived mechanisms (17–21). Immune suppressive factors are also stimulated by the presence of cancer cells. These factors are shown in Figure 1 and include transforming growth factor-β, interleukin (IL)-10, vascular endothelial growth factor, prostaglandin E2 (PGE2), and programmed death-ligand 1 (PD-L1) (22). The efficacy of DC vaccination can likely be attributed to the inhibition of these immune suppressors.

DCs are generated from peripheral monocytes following exposure to granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-4. DCs expressing tumor-specific antigens have been used in active cancer immunotherapies (23, 24). The most common approach to DC vaccination is the preparation of autologous, mature, monocyte-derived DCs *ex vivo* with consequent, homogeneous, and functional DC generation. Cancer vaccination therapies are principally attributed to the presence of tumor-associated antigens using peptide, protein, tumor lysate, and RNA (25–29). Sipuleucel-T (Provenge®) is a US Food and Drug Administration-approved autologous DC-based immunotherapy for men with metastatic hormone-refractory prostate cancer, which provides a new treatment option for patients with this type

of cancer. Sipuleucel-T is manufactured by exposing an individual patient's affected blood cells to a recombinant fusion protein composed of a prostatic acid phosphatase fused to GM-CSF, enhancing immune cell activity against this type of cancer. The patient's own DC product is administered intravenously as part of a three-dose schedule, with approximately 2-week intervals between each dose. This regimen yields a survival benefit of 4.1 months in patients with hormone-resistant prostate cancer (30). According to the requirement for antigens, such as Wilms' tumor 1 (WT1), mucin 1, cell surface associated, human epidermal growth factor receptor 2, carcinoembryonic antigen, survivin, and prostate-specific antigen, WT1 was identified as the most potent cancer-associated antigen. WT1 has confirmed immunological and clinical effectiveness with respect to therapeutic functions, immunogenicity, specificity, and oncogenicity (31). HLA-restricted WT1 peptides were identified as being compatible with HLA-A*02:01- or HLA-A*02:06-restricted (126–134: RMFPNAPYL) and class II compatible with HLA-DRB1*04:05 (332–347: KRYFKLSHLQMHSRKH). The WT1 peptide was restricted to HLA-A*24:02 and modified $WT1_{235–243}$ peptide (CYTWNQMNL). Methionine (M), the second amino acid, was replaced with tyrosine (Y), which can induce cytotoxic T cells (CTLs) to be more effective than the wild-type peptide (32–35). The percentage results for HLA genotyping were as follows: genotypes of HLA-A*24:02 (60%), A*02:01 (20%), and A*02:06 (15%) and HLA class II genotypes of HLA-DRB1*04:05, DRB1*08:03, DRB1*15:01, DRB1*15:02, DPB1*05:01, or DPB1*09:01 (90%). Phase I clinical trials have been conducted with this regimen for various types of solid tumors and hematological malignancies (36–38). DC vaccines primed with HLA class I-/II-restricted WT1 peptides (WT1-DC) have been determined to be safe and feasible, with few adverse reactions reported by patients with advanced cancers, including lung, breast, stomach, biliary tract, pancreas, ovary, and even high-grade glioma (39–47). Clinical studies have indicated that the efficacy of DC vaccination may be enhanced by the off-target effects of chemotherapeutic drugs (39–44, 48) and chemoradiotherapy (47, 49, 50), suggesting a survival benefit in some patients. Different combinations with adjuvant chemotherapy and/or radiotherapy have been investigated, along with the periods required for adaptation. The development of combination therapy regimens, which could potentially include immune checkpoint inhibitors, should improve the outcomes of personalized therapy for patients with cancer (51). However, DC vaccination has been only rarely utilized to treat pediatric patients. There are a few reports describing its use in acute leukemia after allogeneic hematopoietic stem transplantation (52, 53). This article focuses on a pilot study evaluating autologous DC vaccination targeting WT1 in pediatric patients with neuroblastoma or WT.

Manufacture of a DC vaccine

Mature DCs (mDCs) were generated under Good Gene, Cell and Tissue Manufacturing Practice, conditions according to the "The Act on the Safety of Regenerative Medicine"

introduced in Japan on November 25, 2014 (54). Mononuclear cell-rich fractions (165 ml) were isolated from 4,000 ml of the patient's blood through apheresis using a COM.TEC® cell separator (Fresenius Kabi Japan K.K., Tokyo, Japan). Immature DCs were generated by culturing adherent cells in AIM-V® medium (Gibco, Gaithersburg, MD) containing GM-CSF (50 ng/ml; Gentaur, Brussels, Belgium) and IL-4 (50 ng/ml; R&D Systems Inc., Minneapolis, MN) in a CO_2 incubator equipped with a Cell Processing Isolator (H_2O_2-sterilizing system, Panasonic Corporation, Osaka, Japan) at the Shinshu University Hospital Cell Processing Center. After 5 days of culture, immature DCs were differentiated into mDCs by stimulation with OK-432 (10 μg/ml of streptococcal preparation; Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) and PGE2 (50 ng/ml; Daiichi Fine Chemical Co. Ltd., Toyama, Japan) for 24 h (55). The resulting mDCs were cryopreserved at −152°C or in the gas layer within a liquid nitrogen tank until the day of administration. Cell culture supernatants were collected for sterility testing at the time of mDC freezing. For each vaccination, an aliquot of frozen mDCs was thawed immediately prior to clinical use and primed with 100 μg/ml of good manufacturing practice-grade WT1 peptide (NeoMPS Inc., San Diego, CA) containing 1–2 KE of OK-432. WT1 peptides contained HLA-A*02:01- or A*02:06-restricted peptides (126–134: RMFPNAPYL), HLA-A*24:02-restricted modified WT1 peptides (CYTWNQML, residue 235–243), and/or class II peptide (332–347: KRYFKLSHLQMHSRKH) compatible with DRB1*04:05, DRB1*08:03, DRB1*15:01, DRB1*15:02, DPB1*05:01, or DPB1*09:01 (35, 43). One course of seven biweekly sessions was performed with 1–3 × 10^7 DCs with 1–2 KE of OK-432 intradermally injected at bilateral axillar and inguinal areas per session. For pediatric cases, the dose of adjuvant OK-432 was modified as 0.25–1.0 KE, and intradermal injection sits were selected at two points in either bilateral axillar or inguinal areas per session.

DC vaccine release criteria

The antigenic profiles of mDCs were determined using flow cytometry. mDCs were defined as $CD11c^+$, $CD14^-$, HLA^-DR^+, HLA^-ABC^+, $CD80^+$, $CD83^+$, $CD86^+$, $CD40^+$, and $CCR7^+$ cells (55). The criteria for DC vaccine administration were as follows: purity defined as >90% proportion of $CD11c^+$ $CD14^-$ $CD86^+$ HLA^-DR^+ >90% cells, >80% viability, mDC phenotype, negative for bacterial and fungal infection after 14 days, presence of endotoxin ≤0.05 EU/ml, and negative for mycoplasma (55).

DC vaccine study

Application and conditions for DC vaccine therapy

1. Adjuvant therapy after surgical resection or high risk of disease relapse
2. *De novo* cancer at an advanced stage or recurrent cancer after standard therapies

Indication for DC vaccine therapy and eligibility

1. Performance status: 0/1
2. No organ function abnormalities, no infectious diseases, no blood abnormalities, no bleeding tendency
3. Neither cardiovascular diseases nor respiratory disorders that would prevent blood apheresis
4. Tolerable to chemotherapy and radiotherapy as standard cancer treatments
5. Within 6 months of cancer diagnosis or recurrence, with cancer sensitivity to chemotherapy

Exclusion criteria

1. Requiring platelet or red blood cell transfusion or albumin infusion
2. Disseminated intravascular coagulation syndrome and deep vein thrombosis
3. An infectious disease such as viral hepatitis (following the standard of the Japanese Red Cross Blood Center)
4. Allergy to penicillin or OK-432
5. Steroid hormone therapy continuously administered for diseases other than the prevention of temporal chemotherapeutic drug allergy
6. Difficulty in arm vessel blood access for apheresis
7. A presumed length of survival period that would prevent seven sessions of one course at the outpatient clinic
8. No informed consent due to cancer
9. Inability to understand the risk and benefit of the DC vaccine therapy
10. Opposition to DC vaccine therapy
11. Pregnant or nursing women
12. Physician judgment that a patient is inappropriate for treatment

Evaluation of safety and effectiveness

1. In terms of safety evaluation, we evaluated (i) any allergic reaction after the intradermal injection of the DC vaccine (presence of reduced blood pressure, tachycardia, breathing difficulties, or rash) and (ii) local reactions, fever onset, nausea, vomiting, diarrhea, loss of appetite, ulcer of the mucosa, central nervous system damage, anemia, reduced white blood cells, reduced platelets, abnormal kidney function, and abnormal liver function either during or after the completion of treatment.
2. We assessed the cancerous lesions during the treatment course using various imaging techniques, such as computerized tomography, magnetic resonance imaging, and positron emission tomography, approximately 4 weeks after the completion of DC

vaccination. The DC vaccination study was conducted at Shinshu University Hospital and was approved by the Ethics Committee of Shinshu University School of Medicine (Approval Number 1199, December 2, 2008; Approval Number 2704, April 8, 2014).

Case report

Case 1: Neuroblastoma

A 6-year-old girl presented with adrenal gland neuroblastoma in December 2008 at the age of 4. Bone metastasis and bone marrow involvement were detected, resulting in a diagnosis of stage IV disease according to International Neuroblastoma Staging System (56). The patient underwent systemic chemotherapy according to the protocol of the Japanese Neuroblastoma Study Group, followed by surgical resection of the primary adrenal gland neuroblastoma. After intensive chemotherapy was administered in combination with thiotepa and melphalan, the patient subsequently underwent autologous hematopoietic stem cell transplantation (HSCT). The patient received 20 Gy of radiation therapy to the primary right adrenal gland area after recovery from myeloablation and achieved complete disease remission in December 2009. However, at the age of 6, she developed bone marrow relapse in June 2010 and was admitted for DC vaccination in combination with etoposide chemotherapy. Her HLA genotype was confirmed as HLA-A*24:02 compatible with modified WT1-235 peptide. One course (seven sessions, once every 3 weeks) of DC vaccination containing modified WT1-235 peptide (a total of 7.22×10^7 DCs; mean, 1.03×10^7 DCs per session) was administered from March to July 2011. DC vaccine-related toxicities were tolerable and included grade 2 skin reactions and pain at the injection sites along with grade 1 low-grade fever within 48 h of treatment. There were no ≥grade 3 adverse effects due to DC vaccination based on Common Terminology Criteria for Adverse Events (CTCAE), version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf). The tumor markers (neuron-specific enolase, urinary vanillylmandelic acid, and homovanillic acid) were normalized in August 2011, and the magnetic resonance imaging indicated the lesion significantly reduced.

However, the increase of the recurrent tumor with multiple metastasis of bone marrow was detected by MRI and metaiodobenzylguanidine scintigraphy in November 2011. Temozolomide was added, and the DC vaccination was also continued after one course for an additional 15 sessions until October 2012. The patient died due to disease progression in August 2013. Progression-free survival and OS from diagnosis were 5 months after DC vaccination and 4 years and 8 months, respectively.

Case 2: Wilms' tumor

A 14-year-old girl presented with WT derived from her left kidney in January 2002 at the age of 4. Tumor cells were involved from inferior vena cava to the right atrium, with metastases to the

liver, lung, and iliac bone. She was diagnosed with stage IV disease according to the National Wilms' Tumor Study criteria, and pathological findings as favorable histology. She underwent systemic chemotherapy with the protocol of SIOP 93-01, followed by surgical resection of the primary left renal WT. After intensive chemotherapy according to the JWiTs DD-4A protocol of the Japan WT Study group was performed, the patient subsequently underwent radiation therapy targeting the primary left renal area and achieved complete disease remission in December 2002. However, she developed inferior vena cava relapse in November 2006. Although chemotherapy was started, effectiveness was few, and thereafter, localized radiotherapy was performed. She was admitted for DC vaccination at the age of 14. The HLA genotype was confirmed as HLA-A*24:02, which was compatible with the modified WT1-235 peptide. Two courses (seven sessions, once every 3 weeks) of DC vaccination containing modified WT1-235 peptide together with tumor lysate (a total of 31.46×10^7 DCs; mean, 2.25×10^7 DCs per session) were administered from November 2011 to August 2012. During DC vaccination, residual tumor cells extending from the inferior vena cava to the right atrium were surgically resected in March 2012. Despite the surgery, new tumor lesions were detected at the hepatic portal area in March 2013. DC vaccination at 1- to 3-month intervals was continued for a total of 11 additional sessions by November 2014. The patient died due to disease progression in May 2015. DC vaccination-related toxicities were tolerable and included grade 2 skin reactions and pain at the injection sites, along with grade 2 low-grade fever within 48 h of treatment. There were no ≥grade 3 adverse effects due to DC vaccination based on CTCAE ver.4.0. Disease-free survival during DC vaccination was achieved for 12 months, and OS since the time of initial diagnosis was 13 years and 4 months.

Immune monitoring with tetramer analysis

Freshly isolated peripheral blood mononuclear cells were stained with phycoerythrin (PE)-conjugated human immunodeficiency virus/HLA-A*24:02 tetramer as a negative control or with PE-conjugated WT1-modified peptide/HLA-A*24:02 tetramer (MBL; Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Other stains included allophycocyanin-conjugated anti-CD3 mAb and fluorescein isothiocyanate-conjugated anti-CD8 mAb prior to the analysis by flow cytometry (BD FACSCalibur™ and BD FACSCanto™ II) in Figure 2A. The presence of WT1 antigen-specific CTLs (WT1-CTLs) was defined according to the following criteria: (i) greater than 0.02% WT1-positive cells of all CD8+ T cells analyzing 50,000–10,000 lymphocytes with no evidence of false-positive cells and (ii) WT1-positive population clustered and not diffused as described (57). WT1-CTLs were determined either by WT1-peptide/HLA-A*24:02 tetramer analysis or by interferon (IFN)-γ-producing clones used in enzyme-linked immunosorbent spot (ELISPOT) assays after DC vaccination as a proof-of-concept analysis. Before DC vaccination in both cases, WT1-CTLs were detectable at levels above 0.02% as previously defined (57). After one course of DC vaccination, the immune monitoring assay demonstrated that WT1-CTLs consisted of 0.05% and 2.05% of the $CD8^+$ T-cell population in cases 1 and 2, respectively (Figure 2B

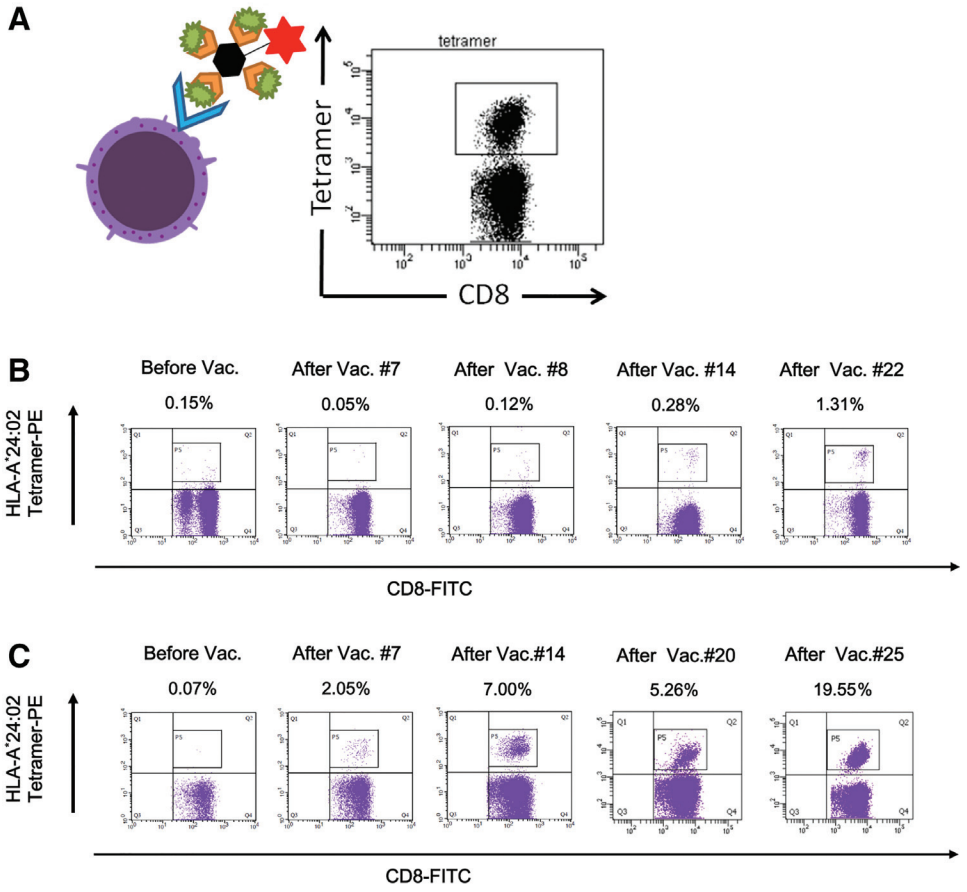

Figure 2. WT1 tetramer assay conducted during the course of DC vaccination. (A) PE-conjugated, WT1-modified peptide/HLA-A*24:02 tetramer was used to detect WT1-specific cytotoxic T cells. Before DC vaccination, WT1-CTLs were at detectable levels in both cases at a concentration of more than 0.02%. After one course of DC vaccination, the immune monitoring assay demonstrated that WT1-CTLs comprise 0.05% of $CD8^+$ T cells in case 1 (B) and 2.05% of $CD8^+$ T cells in case 2 (C). WT1-CTLs concentrations gradually increased after one course of DC vaccination. (Original figure by Shimodaira S.)

and C). WT1-specific T cells were markedly increased after one course during additional vaccination, contributing to the antitumor immune responses noted in our cases (Figure 2B and C).

DC vaccination technology for pediatric patients

Our preliminary study on pediatric patients has several limitations, such as the small sample size and a heterogeneous group of patients. However, DC vaccination targeting WT1 during a standard

therapy course may be both feasible and well tolerated for treating advanced neuroblastoma and WT. The findings also indicated that DC vaccination targeting WT1 generated immunogenicity. WT1-specific CTLs were detected at several levels at the time of initial vaccination, whereas they were distinctly increased after one course of additional vaccination and contributed to induction of the antitumor immune response in our cases. Spontaneous WT1-specific T-cell responses have been reported in acute myeloid leukemia patients (58). Therefore, one possible explanation for the immune response is that the WT1-specific T cells might have been spontaneously induced in these patients. WT1-specific T-cell responses were interestingly maximal at the last session of the course under their disease progression. It is possible that the response of WT1-specific CTLs might be merely boosted by tumor cell growth, although they were no longer able to control disease progression as described with a case of allogeneic DC vaccination targeting WT1 (53).

Case 1 with stage IV neuroblastoma, who relapsed 6 months, had the highest risk of death based on the time to first relapse (59). Our patient survived for 38 months after relapse under disease control with DC vaccination and low-dose etoposide, suggesting a survival benefit together with the maintenance of quality of life. WT1-DC vaccination would be helpful when selecting an optimal therapy for poor survival after neuroblastoma relapse. The WT in case 2 was classified as stage III very high risk for subsequent relapse among children with relapsed WTs (60). Despite the high-dose therapy, MST for very high-risk patients is less than 2 years. It is evident that an effect of the combined modality therapy including DC vaccination achieved the more than 8-year survival after the recurrence in this case, although the contribution of the WT1-DC vaccine to the patient's prolonged survival was unclear. As the number of WT1-CTLs was positively related to the WT1-specific IFN-γ production according to ELISPOT assays (57), the efficacy of DC vaccination would be presumed to be dependent on the number of WT-CTLs. However, the WT1-CTL response to neuroblastoma cells might be limited due to a lack of and downregulation of HLA-class I antigens in neuroblastoma and other renal cell cancers (61–63). Despite an increase in HLA-class I expression on neuroblastoma cells following exposure to IFN-γ (61), there is a concern regarding the attenuation of WT1 antigen in tumor cells during the course of WT1-DC vaccination.

A breakthrough in DC-based vaccine technology is required to achieve further improvement in its cancer treatment efficacy. An allogeneic DC vaccination targeting WT1 may be another potential strategy for patients with relapsed leukemia after HSCT. This strategy may be safe, tolerable, and even feasible for pediatric donors and patients with relapsed leukemia after HSCT as described (52, 53). A 15-year-old girl with acute lymphoblastic leukemia received allogeneic DC vaccination pulsed with WT1 peptide after her third HSCT. The vaccines were generated from her third HSCT donor, the patient's younger 12-year-old sister, who matched with HLA-A*24:02. The patient received 14 vaccine doses with no occurrence of graft-versus-host disease and no systemic adverse effects apart from a grade 2 local skin reaction at the injection site.

WT1-specific immune responses were detected postvaccination by both WT1 tetramer analysis and ELISPOT assays. The patient experienced 44 months of remission after the third HSCT with DC vaccinations, whereas she had been in remission for less than 14 months between her second and third HSCT. This finding suggests that WT1-specific DC vaccination contributed to the extended period of remission following the patient's third HSCT (53). One potential approach to overcome the phenomenon of tumor cells escaping immune detection is the generation of IFN-DCs from monocytes using GM-CSF and IFN-α. Mature forms of IFN-DCs would induce CTLs together with their strong adaptive antitumor effects, with natural killer cell activity independent of HLA-class I antigen expression (64). Another approach is the administration of granulocyte colony-stimulating factor (G-CSF), resulting in the upregulation of monocyte adhesion molecules. An evaluation of the hypothesis that acceleration of acquired cancer immunity using a G-CSF-primed WT1-DC vaccine is related to the type of cancer is ongoing.

The efficacy of DC vaccination may be enhanced by the off-target effects of chemotherapeutic drugs such as gemcitabine (2′,2′-difluorodeoxycytidine, GEM) and a combination of tegafur, gimeracil, and oteracil (48). It has also been reported that WT1 antigen expression in pancreatic cancer cell lines is increased by GEM treatment (65). Initial radiotherapy with additional chemotherapeutic drugs acting through their off-target effects may have accelerated the development of acquired cancer immunity and induced antigen-specific CTLs in patients receiving WT1-targeted DC vaccinations (47). Therefore, DC vaccines in combination with chemotherapy and radiotherapy should promote treatment efficacy against advanced disease. It is necessary to determine the best combinations of the DC vaccine with chemotherapeutic drugs for treating WT and pediatric neoplasms. Immune checkpoint inhibitors are rapidly being developed as chemotherapeutic agents (66). Further studies are required to evaluate whether effector memory T-cell numbers prior to vaccination and the exhaustion of markers for PD1-positive CTLs after DC vaccination influence the efficacy of DC vaccination. Targeted clinical trials could reveal the effectiveness of DC vaccine in combination with immune checkpoint inhibitors as cancer treatments in the near future. Predictive biomarkers for use with DC vaccination targeting WT1 are highly relevant to the personalized cancer therapy.

Conclusion

Our preliminary study suggests that DC vaccination targeting WT1 administered during the course of standard cancer therapies may be both feasible for and well tolerated by patients with neuroblastoma and WT. The study findings indicate that induction of acquired immunity by targeting WT1 was detected by immune monitoring with tetramer analysis during the course of DC vaccination, confirming the positive results for this proof-of-concept investigation in pediatric patients. The results also suggest that WT1-DC vaccination may prolong the survival of pediatric patients with neoplasms. In contrast, it was not clearly

determined whether there was an improvement in patient prognosis following WT1-DC vaccination because both patients died due to disease progression. Therefore, the efficacy and safety of DC vaccination should be determined by phase I/II prospective trials enrolling larger numbers of patients with pediatric neoplasms.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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