

Baseline WT1-specific immunity and immune responses to WT1-targeted immunotherapy in pediatric patients with malignant solid tumors at high risk for relapse: results from a biomarker-driven multicenter translational phase II clinical trial

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Abstract

Background Biomarkers useful for identifying pediatric patients likely to achieve durable clinical outcomes from Wilms tumor gene 1 (WT1)-targeted cancer immunotherapy (WT1-targeted CIT) and for predicting such outcomes have not been identified.

Methods A phase II clinical trial of WT1-targeted CIT was conducted. Patients underwent repeated WT1₂₃₅ peptide vaccination. The primary endpoint was the 2-year progression-free survival (PFS) rate. Four immune signatures [WT1-specific cytotoxic T lymphocyte (CTL) frequency, CTL phenotype composition, serum anti-WT1₂₃₅ IgG and IgM antibody (Ab) titers, and delayed-type hypersensitivity (DTH) to WT1₂₃₅ peptide] were examined to identify candidate biomarkers.

Results Of 21 enrolled patients (median age, 6.5 years; range, 1.4–16.5; 10 rhabdomyosarcoma, 8 neuroblastoma, and 3 other bone/soft tissue tumors), 3 were unevaluable for immune response. The 2-year PFS rates—primary endpoint—for patients with a baseline CTL frequency of $\geq 0.1\%$ or $< 0.1\%$ were 90.9 % (50.8–98.7) and 30.0 % (7.1–57.8), respectively ($p < 0.001$). Immune responders were positive for DTH at week 11 or showed prespecified increases in WT1-specific CTL counts and/or frequencies. The 2-year PFS rate was significantly higher ($p = 0.003$) for 12 immune responders (91.7%; 53.9–98.8) than for 6 nonresponders (33.3%; 4.6–67.6). All 9 immune responders with a baseline CTL frequency of $\geq 0.1\%$ remained alive after 7 years of vaccination. Clinical responders were defined as patients without imaging progression during the first 12 months after vaccination. Immune responses were significantly associated with clinical responses ($p = 0.007$). At month 2 of vaccination, CTL frequency ($p = 0.007$) and the effector memory CTL proportion ($p = 0.047$) were significantly greater for clinical responders, defined as patients without imaging progression through 12 months, than for nonresponders. In clinical responders, the anti-WT1₂₃₅ IgG Ab titer at months 2 ($p = 0.007$) and 3 ($p = 0.004$) of vaccination increased significantly compared to baseline titers. Grade 3 injection site reaction and sinusitis occurred in two and one patients, respectively.

Conclusions Baseline WT1-specific CTL frequency may identify patients most likely to achieve durable clinical outcomes from WT1-targeted CIT. Immune signatures—CTL frequency, CTL phenotype composition, and anti-WT1 IgG Ab titer—were identified as candidate biomarkers for the early prediction of such durable clinical outcomes.

Trial registration UMIN000013251.

Introduction

Pediatric malignant solid tumors, including rhabdomyosarcoma (RMS) and neuroblastoma (NB), generally respond to multimodality therapy. However, patients with recurrent or progressive disease continue to carry a dismal prognosis [1, 2].

Wilms tumor gene 1 (*WT1*) antigen, which is ranked first in the cumulative score among 75 cancer antigens [3], is one of the most promising target antigens for cancer immunotherapy (CIT) [4]. *WT1* is overexpressed in many pediatric malignancies while showing only limited expression in normal tissues [5]. Pediatric soft tissue/bone tumors and NB are conventionally considered to be poorly responsive to immunotherapy due to their low immunogenicity caused by reduced major histocompatibility (MHC) class I expression [6] or by low tumor mutational burden [7]. However, MHC class I and II expression on these tumor cells is enhanced after chemotherapy [8] and radiotherapy [9, 10]. These findings suggest that conventional therapy might enhance antigen presentation, providing a rationale for the development of *WT1*-targeted cancer immunotherapy (*WT1*-targeted CIT).

Despite a great variety of therapeutic efforts made to date, no standard therapy has been established for recurrent or relapsed pediatric malignant solid tumors. Furthermore, survivors of these malignancies are at risk of treatment-related late complications (e.g., secondary cancer and organ dysfunction) when treated by conventional therapies (surgery, radiation, and chemotherapy including high-dose chemotherapy) [11]. Therefore, the development of an effective therapeutic modality with fewer treatment-related late complications is required.

Despite *WT1*-targeted CIT can induce antigen-specific immune responses, biomarkers capable of identifying patients likely to achieve durable clinical outcomes and predicting such outcomes remain undefined. Therefore, we integrated comprehensive immunomonitoring into a multicenter phase II clinical trial to determine whether baseline and vaccine-induced *WT1*-specific immune signatures could serve as clinically useful biomarkers.

The objective of the present trial was to identify clinically useful baseline and early on-treatment immune biomarkers associated with durable clinical outcomes in pediatric patients undergoing *WT1*-targeted CIT.

Methods

Patients

The present phase II, multicenter, open-label, uncontrolled clinical trial of this vaccine (UMIN000013251) enrolled patients—1) who had either a bone/soft tissue malignant tumor or NB and 2) who were assessed to present partial response (PR)/complete response (CR) and at high risk for recurrence/progression after standard multimodality therapy. Patients were recruited by referral at two medical institutions from February 15, 2011 to July 31, 2018.

Eligible patients met all of the following inclusion criteria: below 20 years of age at the time of enrollment; 0–2 in performance status (PS) according to Eastern Cooperative Oncology Group PS scores [12] for patients aged ≥ 5 years or $\geq 50\%$ in the Lansky Play-Performance scale for patients aged < 5 years [13]; evaluable lesions, without regard to the presence or absence of measurable lesions that are defined in the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [14] and that are assessed by

radiologists at each medical institution; the expression of WT1 protein in tumor tissue at the onset, recurrence, or progression of the tumor by immunostaining conducted at the central laboratory at Osaka University; a genotype [human leukocyte antigen (HLA)*24:02] in the allele group—however, both the recipient and donor should have the genotype when allogeneic hematopoietic stem cell transplantation was conducted. Details of the eligibility criteria for RMS, osteosarcoma and soft tissue sarcomas other than RMS, and for NB are provided in Supplemental Material that provides the ethical principles, as well as countermeasures for referral and selection biases. Clinical responders were defined as patients without imaging progression during the first 12 months after vaccination.

The present study was conducted after approval from the ethics committee at Osaka University Hospital (approval number: 8091) and by the ethics committee at St. Luke's International Hospital (approval number: 10–127) and was conducted in accordance with the Declaration of Helsinki and the committee-approved protocol.

WT1 peptide vaccine

Peptide Institute (Osaka, Japan) prepared Good Manufacturing Practice-grade, HLA-A*24:02-restricted, 9-mer-modified WT1 peptide (mp235-243; CYTWNQMNL) stimulating cytotoxic T lymphocytes (CTLs) that induce stronger cytotoxicity than its natural form [15]. The pharmaceutical details of the vaccine were published previously [4]. The vaccine, which had previously been emulsified with an adjuvant—Montanide™ ISA 51VG; Seppic, France [16]—at a weight ratio of 1:1, was injected intradermally to patients.

Primary endpoints, biomarkers, and secondary endpoints

The primary endpoint was the 2-year progression-free survival (PFS) rate. WT1-specific immune signatures (CTL frequency, CTL phenotype composition, serum anti-WT1₂₃₅ peptide antibody (Ab) titer, and delayed-type hypersensitivity to WT1₂₃₅ peptide) were analyzed to identify candidate biomarkers for the prediction of durable clinical outcomes. The secondary endpoints were the categories of efficacy and safety: regarding efficacy, 4-year PFS and OS rates for all patients; 2- and 4-year PFS and OS for RMS, and NB; regarding safety, treatment-emergent adverse events (TEAEs) graded according to the Common Terminology Criteria for Adverse Events version 4.0 for all patients.

Procedures

A water-in-oil emulsion, composed of a WT1 peptide solution and the abovementioned adjuvant, was used as the WT1 peptide vaccine. The investigators at two medical institutions intradermally injected the vaccine at the following doses: 0.5, 1, 2, and 3 mg for body weights of < 10, 10 –<20 kg, 20 –<40 kg, and ≥40 kg, respectively. Vaccinations were conducted according to the following proposed schedule: the

first 12 vaccinations weekly; 1 vaccination every 2 weeks for the next 3 months; 1 vaccination every 2–3 weeks for the next 6 months; and 1 vaccination every month thereafter.

At year 3 of vaccination, medical institutions determined vaccination interval, doses, and continuation/discontinuation of vaccination. Investigators were authorized to extend the vaccination interval until week 8 when disease was well controlled or to conduct vaccination weekly when necessary. The injection sites are shown in Supplemental Material.

Immunomonitoring

Immune responses in PB to WT1-targeted CIT were examined by determining 1) CTL frequency, 2) CTL phenotype composition, 3) anti-WT1₂₃₅ peptide immunoglobulin M (IgM) and G (IgG) Ab titers measured in the form of absorbance, and 4) delayed-type hypersensitivity (DTH) to WT1 peptide at baseline (allowance: 1 week before the first day of vaccination) and at the timepoints specified by the protocol.

WT1-specific CTLs among CD8⁺CD3⁺ T cells in PB were stained with the WT1₂₃₅ modified tetramer (MBL, Tokyo, Japan). Staining procedures were described previously [4]. The CTL phenotype composition analysis was also conducted based on the CCR7 and CD45RA expressions for WT1-specific CTLs among CD8⁺ T cells in PB to examine the percentages of naïve (N) T cells (CCR7⁺, CD45RA⁺), central memory (CM) T cells (CCR7⁺, CD45RA⁻), effector memory (EM) T cells (CCR7⁻, CD45RA⁻), and effector (E) T cells (CCR7⁻, CD45RA⁺) [17, 18]. Flow cytometry used the following monoclonal antibodies: the anti-CD3-AmCyan Ab (BD Biosciences, San Jose, CA), anti-CD8-APC-Cy7 antibody (BD Biosciences), anti-CD45 RA-FITC Ab (BD Biosciences), and anti-CCR7 Pacific Blue Ab (BioLegend, San Diego, CA). FACSCanto™ II (BD Biosciences, Piscataway, NJ) was then used to conduct the flow cytometry of 7-AAD (eBioscience, San Diego, CA)-negative cells. Subsequently, the data were analyzed with the FlowJo software (Tree Star, Ashland, OR). Murao et al. found that the WT1-specific CTL frequency in healthy individual PB did not exceed 0.1% [19], which drove us to establish a baseline cutoff value of 0.1%.

DTH to WT1 HLA class I peptide was assessed by intradermally injecting 0.05 mL of a saline solution or modified WT1 peptide (mp235-243; CYTWNQMNL) (10 µg). At 48 hours after injection, DTH was considered positive when an eruption of ≥2 mm in diameter developed. Anti-WT1₂₃₅ IgG and IgM Ab titers in serum were determined at the onset, 2 weeks, as well as 1, 2, 3, and 6 months as described previously.[20, 21] WT1 aa 235–245 (CMTWNQMNLPPK) peptide was used as the capture antigen for the anti-WT1₂₃₅ IgM or IgG Ab. Absorbance was measured at a wavelength of 450 nm by using a MTP-310 Lab microplate reader (Hitachi High-Tech Science Corporation, Tokyo, Japan). The Ab titer in each serum sample was determined as the average absorbance of the duplicate wells after subtracting the absorbance of the negative control well. The cutoff levels for positivity for the anti-WT1₂₃₅ IgM and IgG Abs were set at 0.10 and 0.15, respectively.

Efficacy assessments

Because previously reported 2-year PFS were below 50% [22–24], the expected 2-year PFS rate was set at 50% as the threshold. The verification day of CR/PR on imaging studies within 21 days after the completion of prior conventional treatment was defined as the start to examine the vaccination effects as assessed by the primary and secondary endpoints (Supplemental Material). The antitumor effect of the WT1 peptide vaccine was assessed according to the RECIST version 1.1 [14]—CR, PR, progressive disease (PD), stable disease, and not evaluable (NE). Pediatric patients were followed up to examine their long-term prognosis through immunomonitoring and imaging studies. Immune responders were defined as patients who were positive for DTH to WT1 peptide at week 11 or who showed the following increases in WT1-specific CTL counts and/or frequencies in 3 mL of peripheral blood (PB) by week 11 from baseline: 0 CTL to ≥ 10 CTLs; 1–9 CTLs to ≥ 10 CTLs and $\geq$ twofold the frequency; as well as ≥ 10 CTLs to ≥ 10 CTLs and $\geq$ twofold the frequency. The baseline was defined as the start of vaccination with a preceding allowance of 1 week. Clinical responders were defined operationally as patients without disease progression on imaging studies for 12 months after the start of vaccination.

Immunohistochemistry

WT1 positivity was determined according to the following judgment criteria: stronger staining than internal control cells (lymphocytes and fibroblasts) in the same histological sample; and 2) $\geq 10\%$ in WT1-positive cell proportion among all tumor cells. WT1 immunostained slides were centrally reviewed by two experienced pathologists. Discordant assessments were resolved by consensus. The procedures of immunohistochemistry are as described by Nakatsuka et al. [25].

Statistical analyses

According to the 2002–2011 medical records at Osaka University Hospital, 21 patients with intractable tumors (11 NB and 10 RMS) had undergone standard therapy after the day of diagnosis, and the 2-year PFS rates for all patients, NB, and RMS were 33.3%, 36.4%, and 30.0%, respectively. Sample size to detect a significant difference in the 2-year PFS rate compared with the threshold PFS rate was calculated to be 23 according to the exponential distribution parameter estimation. The threshold 2-year PFS rate was set at 33.3%, and the expected 2-year PFS rate was set at 50%.

Survival probability was estimated according to the Kaplan-Meier method at baseline to investigate the impacts of baseline WT1-specific CTL frequency of $\geq 0.1\%$ or $< 0.1\%$ (i.e., preexisting immune status). Furthermore, survival probability was also estimated according to the Kaplan-Meier method including the landmark analysis at week 11 after the start of vaccination to examine the presence or absence of vaccination-induced immune responses as manifested by an increase in WT1-specific CTL frequency and by the emergence of DTH on the subsequent patients' prognosis. The landmark analysis was made to avoid "guarantee-time" bias [26] in patients who died or relapsed by week 11 after the start of vaccination. Between-group differences were analyzed using the log-rank test, Wilcoxon rank sum exact test, Wilcoxon signed-rank test, and Fisher's exact test. Kendall's rank correlation coefficient was used to

test correlations. The association of factors with time to events was analyzed using the Cox proportional hazards model. Age was expressed as median, and the 95% confidence interval (CI) was calculated for survival rates. Three-group comparisons were made using one-way analysis of variance, with Bonferroni correction to correct groups of p-values for “family-wide significance.” All statistical analyses were made with a statistical software “EZR” [27]. A value of $p < 0.05$ was considered statistically significant.

Results

Patient characteristics at baseline and patient disposition

The present trial (February 15, 2011 to July 31, 2018) recruited 23 patients at 2 medical institutions in Japan. Two patients were excluded after the acquisition of informed consent because of ineligibility—PD. Consequently, 21 patients at 2 medical institutions were enrolled and analyzed for the 2-year PFS rate, the 4-year PFS rate, the 4-year OS rate, and safety. Three of 21 patients did not receive the first 12 vaccinations because of the following reasons: acute pneumonia in 1 patient with NB who had shown CR; and PD in 2 patients (Fig. 1).

PD, progressive disease.

Table 1 summarizes the baseline characteristics of 11 boys and 10 girls (median age, 6.5 years; range, 1.4–16.5). RMS was predominant in enrolled patients (10 patients, 47.6%)—6 patients presented group III embryonal rhabdomyosarcoma ($n = 4$) and group III alveolar rhabdomyosarcoma (ARMS, $n = 2$); and 4 patients presented group IV RMS of alveolar type, 3 of whom were found to have the *PAX3-FOXO1* gene. Three of 8 patients (37.5%) with stage IV NB presented *MYCN* gene amplification. Each of 3 patients (14.3%) with other malignant soft tissue tumors had osteosarcoma, desmoplastic small round cell tumor, and extrarenal rhabdoid tumor. At baseline, there were 8, 10, and 3 patients in CR1, PR1, and PR2/CR2, respectively; however, 2 of 7 patients in CR1 developed a tumor that was confirmed to remain pathologically after surgical resection. No statistically significant difference was found with respect to all characteristics examined between immune responders and nonresponders (Table 1–2).

Table 1
1 Patient characteristics at baseline

UPN*	Sex/age, y	Primary disease	Primary site	Group/stage, histologic subtype, or disease stage	Molecular genetics	Disease status [†] on the initial day
1	M/1	RMS	Bladder	Group III [‡] , ERMS [§] , invasion to the prostate		PR
2	M/3	RMS	Middle ear	Group III [‡] , ERMS [§]		PR
4	F/4	NB	Left adrenal gland	Stage 4 , unfavorable histology [¶] , Stage M ^{**}	<i>MYCN</i> ⁻⁺⁺ , diploidy ^{‡‡}	PR
5	M/4	NB	Left neck sympathetic ganglion	Stage 4 , unfavorable histology [¶] , Stage M ^{**}	<i>MYCN</i> ⁻⁺⁺	1st CR
6	M/3	RMS	Retroperitoneum	Group III [‡] , ERMS [§]		PR
8	M/6	RMS	Middle ear	Group III [‡] , ERMS [§]		PR
9	F/1	NB	Right adrenal gland	Stage 4 , unfavorable histology [¶] , Stage M ^{**}	<i>MYCN</i> ⁺⁺ , diploidy ^{‡‡}	1st CR
10	F/11	NB	Mediastinum	Stage 4 , stage M ^{**}	<i>MYCN</i> ⁻⁺⁺ , diploidy ^{‡‡}	2nd CR
11	F/9	Osteosarcoma	Left humerus	Conventional central		1st CR ^{§§}
12	F/8	Extrarenal rhabdoid tumor	Left humerus	Group I [‡]		1st CR ^{§§}

UPN*	Sex/age, y	Primary disease	Primary site	Group/stage, histologic subtype, or disease stage	Molecular genetics	Disease status [†] on the initial day
13	F/15	RMS	Perineal perineum	Group III [‡] , ARMS [§]	<i>PAX3- FOXO1</i>	PR
14	F/4	RMS	Neck	Group III [‡] , ARMS [§]	Li- Fraumeni	2nd CR
15	F/3	NB	Right adrenal gland	Stage 4 , Stage M ^{**}	<i>MYCN</i> ⁻⁺⁺	PR
16	M/8	RMS	Left forearm	Group IV [‡] , ARMS [§]	<i>PAX3- FOXO1</i>	1st CR
17	F/3	NB	Right adrenal gland	Stage 4 , unfavorable histology [¶] , Stage M ^{**}	<i>MYCN</i> ⁺⁺	1st CR
18	F/12	RMS	Right thigh	Group IV [‡] (BM), ARMS [§]	<i>PAX3- FOXO1</i>	PR
19	M/12	RMS	Left thigh	Group IV [‡] (LN), ARMS [§]	<i>PAX3- FOXO1</i>	2nd PR
20	M/3	NB	Right adrenal gland	Stage 4 , unfavorable histology [¶] , Stage M ^{**}	<i>MYCN</i> ⁺⁺	1st CR
21	M/16	RMS	Left palm	Group IV [‡] , ARMS [§]		1st CR
22	M/16	DSRCT	Rectum	Stage 4	<i>EWS-WT1</i>	PR
23	M/9	NB	Right adrenal gland	Stage 4 , unfavorable histology [¶] , Stage M ^{**}	<i>MYCN</i> ⁻⁺⁺	PR

* 2 patients (UPNs 3 and 7) were excluded before enrollment due to progressed primary disease.

[†] Assessed according to RECIST.

[‡] Assessed according to the Children's Oncology Group: Surgical-Pathological Group System.

[§] Assessed according to the JRSG.

^{||} Assessed according to the INSS.

[¶] Assessed according to the INPC.

^{**} Assessed according to the INRGSS.

^{††} 10 copies of the gene are called *MYCN* gene amplification.

^{‡‡} Two sets of chromosomes are defined diploidy.

^{§§} Tumor remaining pathologically after secondary tumor resection

UPN, unique patient number; RECIST, Response Evaluation Criteria in Solid Tumors; M, male; RMS, rhabdomyosarcoma;

ERMS, embryonal rhabdomyosarcoma; F, female; JRSG, Japan Rhabdomyosarcoma Study Group; INSS, International Neuroblastoma Staging System; INPC, International Neuroblastoma Pathology Classification; INRGSS, International Neuroblastoma Risk Group

Staging System; NB, neuroblastoma; CR, complete response; ARMS, alveolar rhabdomyosarcoma; BM, bone marrow; LN, lymph node; DSRCT, desmoplastic small round cell tumor; PR, partial response.

Table 1
2 Base characteristics by immune response to WT1-targeted cancer immunotherapy

Characteristic	Responders (n = 12)	Nonresponders (n = 6)	Total (N = 18)	p-value
Sex				
Male, n (%)	6 (50.0)	2 (33.3)	8 (44.4)	0.638
Female, n (%)	6 (50.0)	4 (66.7)	10 (55.6)	
Age at vaccination, years, median [range]	5.0 [1–16]	4.0 [1–12]	4.0 [1–16]	0.670
Histologic type				
RMS, n (%)	5 (41.7)	4 (66.7)	9 (50.0)	0.529
Neuroblastoma, n (%)	5 (41.7)	2 (33.3)	7 (38.9)	
Others, [†] n (%)	2 (16.7)	0 (0.0)	2 (11.1)	
Disease status at vaccination				
CR1/PR1, n (%)	12 (100.0)	5 (83.3)	17 (94.4)	0.333
CR2/PR2, n (%)	0 (0.0)	1 (16.7)	1 (5.6)	
Prior chemotherapy courses, median [range]	11.0 [4–24]	7.5 [5–14]	9.5 [4–24]	0.346
Prior radiotherapy, n (%)	10 (83.3)	6 (100.0)	16 (88.9)	0.529
Prior HDT/HSCT, n (%)	6 (50.0)	2 (33.3)	8 (44.4)	0.638
Days from last day of prior treatment to the start day of vaccination, median [range]	63.5 [32–254]	39.5 [33–120]	47.0 [32–254]	0.261

P-values were calculated using Fisher's exact test for categorical variables and Mann–Whitney U test for continuous variables.

[†] Include osteosarcoma (n = 1) and extrarenal rhabdoid tumor (n = 1).

Note

3 patients with unevaluable immune responses (UPN 10, 19, 22) are excluded from this analysis.

RMS, rhabdomyosarcoma; NB, neuroblastoma; CR, complete response; PR, partial response; HDT, high-dose therapy; HSCT, hematopoietic stem cell transplantation

Furthermore, the distributions of prior conventional treatment, including chemotherapy, radiotherapy, surgery, and high-dose therapy/HSCT, were comparable between immune responders and nonresponders, indicating no apparent treatment-related imbalance favoring immune responders (Fig. 2).

The mark “X” indicates relapse.

The filled circle indicates the initiation of vaccination.

UPN, unique patient number; NA, not applicable.

Biomarkers and efficacy

PFS was significantly higher for 11 patients with a high baseline CTL frequency of $\geq 0.1\%$ than for their 10 counterparts ($< 0.1\%$) (HR, 0.05; 0.007–0.43; $p < 0.001$) (Fig. 3A). The 2-year PFS rate was 90.9% (95% CI, 50.8–98.7%) for patients with a high baseline CTL frequency, whereas the rate was 30.0% (95% CI, 7.1–57.8%) in patients with a low baseline CTL frequency. OS was significantly higher for 11 patients with a high baseline CTL frequency of $\geq 0.1\%$ than for their 10 counterparts ($< 0.1\%$) (HR, 0.087; 0.011–0.711; $p = 0.004$) (Fig. 3B). The 4-year OS rate for patients with a high baseline CTL frequency of 90.9% (50.8–98.7%), with a low their counterparts 40.0% (12.3–67.0%).

Notably, all 9 patients—who were categorized to WT1-specific immune responders defined in Methods and who also showed a high baseline CTL frequency of $\geq 0.1\%$)—remained alive after 7 years of vaccination without developing recurrence/progression (Figs. 3C, D). The numbers of clinical responders and nonresponders, defined in METHODS, were 14 and 7, respectively. The 2- and 4-year PFS rates for clinical responders ($n = 14$) were 92.9% (59.1–99.0) and 85.7% (53.9–96.2%), respectively.

*: Drawn according to the Kaplan-Meier method including the landmark analysis at week

11 after the start of vaccination

The numbers of WT1-specific immune responders and nonresponders were 12 and 6, respectively. The 2- and 4-year PFS rates for WT1-specific immune responders were identical being 91.7% (95% CI: 53.9–98.8), while the 2- and 4-year PFS rates for WT1-specific immune nonresponders were 33.3% (95% CI: 4.6–67.6) and 16.7% (95% CI: 0.8 to 51.7), respectively (Fig. 4A). The 4-year OS rates were 91.7% (53.9–98.8) and 50.0% (11.1–80.4) for WT1-specific immune responders and nonresponders, respectively (Fig. 4B). To minimize confounding by disease status at vaccination, an exploratory landmark analysis was conducted restrictedly to patients in CR1/PR1 for all patients [Figs. 4C (PFS), D (OS)]. Even within this clinically more homogeneous subgroup, WT1-specific immune responders showed significantly favorable PFS compared with nonresponders ($p = 0.006$; Fig. 4E) and favorable OS compared with nonresponders ($p = 0.040$; Fig. 4F), supporting an association between vaccine-induced immune response and long-term outcome.

*: Drawn according to the Kaplan-Meier method including the landmark analysis at week 11 after the start of vaccination

The 4-year OS rates after the start of vaccination were 92.9% (59.1–99.0%) and 14.3% (0.7–48.6%) for clinical responders (n = 14) and nonresponders (n = 7), respectively (Fig. 5).

* Includes 1 patient with NB who presented recurrence after the nineteenth vaccination, underwent chemotherapy with irinotecan and temozolomide, high-dose chemotherapy rescued with autologous peripheral blood stem cells, KIR ligand-mismatched cord blood stem cell transplantation, radiation for primary tumor, and immunotherapy with dinutuximab.

PFS, progression-free survival; WT1, Wilms tumor gene 1; OS, overall survival; HR, hazard ratio; CI, confidence interval.

Namely, clinical responders showed long-lasting, favorable clinical outcomes. The maximum frequency of WT1-specific CTLs by week 11 was significantly higher ($p = 0.007$) in clinical responders than in nonresponders (Fig. 6A-1). Typical time-course changes in WT1-specific CTL frequency in clinical responders and nonresponders are shown (Suppl. Figures S1A and B, respectively). The sustained WT1-specific CTL-producing ability as expressed by the area under the curve (AUC) of WT1-specific CTL frequency by week 11 (Suppl. Figure S1A) was significantly greater ($p = 0.004$) in clinical responders than in nonresponders (Fig. 6A-2). WT1-specific CTL frequency was higher in clinical responders than in nonresponders and persisted until month 6 (Fig. 6B).

At month 2 after the start of vaccination, the proportion of WT1-specific effector memory (EM) CTLs was significantly greater ($p = 0.047$) in clinical responders than in nonresponders, while the proportion of WT1-specific effector (E) CTLs at month 2 tended to be greater in clinical nonresponders than in responders (Fig. 6C). At month 6, the proportion of N CTLs, which are functionally inactive, tended to be greater in clinical nonresponders than in responders; the proportion of WT1-specific EM CTLs, albeit not significantly different, exceeded 10% in 6 of 9 clinical responders in sharp contrast to the low proportion ($\leq 10\%$) of EM WT1-specific CTLs in all of 4 clinical nonresponders (Fig. 6D).

A significant association was noted between a high baseline CTL frequency of $\geq 0.1\%$ and clinical response ($p = 0.016$). On the one hand, a high baseline CTL frequency of $\geq 0.1\%$ tended to be associated with the induction of immune response ($p = 0.097$, a value not statistically significant, presumably due to a small sample size).

DTH-positive patients, who were negative for DTH at baseline but turned positive at week 11, seemed to show a favorable clinical outcome compared with DTH-negative patients. All DTH-positive patients survived at least for 7 years (Suppl. Figures S2A, B).

No significant difference was found in time-course changes in the anti-WT1₂₃₅ IgM Ab titers between clinical responders and nonresponders, and the titers remained very low until month 6 (Fig. 6E). In contrast to the anti-WT1₂₃₅ IgM Ab titer, the anti-WT1₂₃₅ IgG Ab titer significantly increased compared to the baseline titers in clinical responders at months 2 ($p = 0.007$) and 3 ($p = 0.004$) (Fig. 6F). Anti-WT1₂₃₅ IgG Ab titer-positive patients, who showed a detection level-reaching increase in the IgG Ab titer after the start of vaccination, tended to show the greater 2-year PFS rate compared with their negative counterparts (Fig. 6G; $p = 0.142$); of 7 patients who turned positive for the anti-WT1₂₃₅ IgG Ab by month 6 after baseline, 5 showed at least 7.3 years of PFS after the start of vaccination except for the following 2: one patient with Li-Fraumeni syndrome who developed osteosarcoma as a secondary cancer at year 3.5 after the start of vaccination and one who had osteosarcoma recurrence at month 6.3 and survived 8.1 years after the start of vaccination. The 4-year OS rate tended to be higher ($p = 0.102$) for anti-WT1₂₃₅ IgG Ab-positive patients than for their negative counterparts (Fig. 6H); notably, a correlation was noted between the WT1₂₃₅ IgG Ab-positive and -negative groups (Kendall rank correlation coefficient, $p = 0.025$), although the log-rank test did not reveal a statistically significant difference between them. The typical time-course changes in anti-WT1₂₃₅ IgM and G Ab titers are shown (Suppl. Figures S3A, B). WT1-specific CTL frequency at baseline and at week 11 CTL phenotype composition, and serum anti-WT1₂₃₅ IgG Ab titer were identified as biomarkers associated with favorable clinical outcomes.

Note) Patients became positive for the anti-WT1₂₃₅ IgG Ab when exhibiting Ab positivity at least once by month 6 of vaccination. The impact of the presence or absence of the anti-WT1-specific IgG Ab on the patient's prognosis was examined.

Ab, antibody; Ig, immunoglobulin. Horizontal bar: median; box plot: upper and lower quartiles. WT1, Wilms tumor gene 1; CTL, cytotoxic T lymphocyte; CM, central memory; EM, effector memory; E, effector.

The immunomonitoring and imaging data of UPN13, who exhibited immune responses (positive DTH and an increase in WT1-specific CTL frequency involving the sustainment of a considerable proportion of the EM subset) and the conspicuous clinical response, are shown (Figs. 7A-E).

Arrows indicate the location of the tumor.

UPN, unique patient number; DTH, delayed-type hypersensitivity; CTL, cytotoxic T lymphocyte; WT1, Wilms tumor gene 1.

The 2- and 4-year PFS (Fig. 8A) and 4-year OS (Fig. 8B) rates for all patients after the start of vaccination were 61.9% (95% CI: 38.1–78.8), 57.1% (33.8–74.9), and 66.7% (42.5–82.5), respectively.

Treatment outcomes by histologic type are shown in Table 2 and Suppl. Figures S4A–D. Ten patients with RMS and eight patients with NB were evaluable for immune response at week 11 of vaccination. Four of 5 WT1-specific immune responders with RMS survived 7 years (Suppl. Figures S4E, F) and 5 of 5 WT1-specific immune responders with NB survived 7 years (Suppl. Figures S4G, H).

Table 2 Treatment outcomes by histologic type

	Histologic types			Total (N=21)
	RMS (n=10)	NB (n=8)	Others* (n=3)	
Number of vaccinations				
Median [minimum–maximum]	43 [5–60]	54.5 [8–101]	28 [9–39]	43 [5–101]
Completion of scheduled 12 vaccinations, [†] n (%)	9 (90.0)	7 (87.5)	2 (66.7)	18 (85.7)
Reasons for discontinuation at month 36, n				
Disease progression	4	3	1	7
Treatment-emergent adverse events			0	2
Acute pancreatitis [‡]	1	1		
Acute pneumonia [§]				
Response to treatment, n (%)				
CR/PR/SD	6 (60)	6 (75)	2 (66.7)	14 (66.7)
PD	4 (40)	2 (25)	1 (33.3)	7 (33.3)

*: 1 case each of osteosarcoma, desmoplastic small round cell tumor, and extrarenal rhabdoid tumor

[†]: Did not show delayed vaccination.

[‡]: Developed after the sixth vaccination.

[§]: Developed after the eight vaccination.

RMS, rhabdomyosarcoma; NB, neuroblastoma; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease

Regarding disease status on the start of vaccination, the HR was significantly higher for CR2/PR2 than for CR1 (HR, 7.4, 95% CI 1.18–46.4, $p=0.033$), and PFS was significantly higher for CR1 than for CR2/PR2 (Suppl. Figure S5A); the HR was significantly higher for CR2/PR2 than for PR1 (HR=2.264, 95% CI=1.004–5.107, $p=0.049$) and CR1 (HR=10.01, 95%CI 1.67–61.0, $p=0.013$), and the OS was significantly higher for PR1 and CR1 than for CR2/PR2 (Suppl. Figure S5B).

Safety

Table 3 summarizes the common TEAEs (incidence: ³10%) that occurred during the present study. The overall incidence of TEAEs was 100.0%, in which the incidence of grade 1/2 was 90.5%. TEAEs could be managed without requiring the discontinuation of WT1-targeted CIT in all patients except 2 patients (Table 3). Serious TEAEs (STEAEs)—grade 3/4 and grade 5—were 14.3% and 9.6%, respectively. The most common grade 3 STEAE was injection site reaction (9.5%). Two grade 5 STEAEs were acute pneumonia and secondary cancer. The independent Data and Safety Monitoring Committee adjudicated both events as unrelated to WT1-targeted CIT; vaccination for these 2 patients was discontinued.

Table 3 Most common treatment-emergent adverse events

			Grades*				
			1	2	3	4	5
TEAEs, n (%)							
Injection site reaction	21 (100.0)	12 (57.1)	7 (33.3)	2 (9.5)	0	0	
Nasopharyngitis	6 (28.6)	0	6 (28.6)	0	0	0	
Sinusitis	4 (19.0)	1 (4.8)	2 (9.5)	1 (4.8)	0	0	
ALT increased	3 (14.3)	1 (4.8)	2 (9.5)	0	0	0	
STEAEs, n (%)							
Injection site reaction	2 (9.5)	0	0	2 (9.5)	0	0	
Sinusitis	4 (19.0)	0	0	1 (4.8)	0	0	
Acute pneumonia	1 (4.8)	0	0	0	0	1 (4.8)	
Secondary cancer	1 (4.8)	0	0	0	0	1 (4.8)	

*: Assessed according to Common Terminology Criteria for Adverse Events version 4.0

TEAEs, treatment-emergent adverse events (incidence: ³10%); ALT, alanine transaminase; STEAEs, serious treatment-emergent adverse events.

Discussion

Two major findings emerged from the present study. First, baseline WT1-specific immunity identified candidate biomarkers for selecting patients most likely to achieve durable clinical outcomes from WT1-targeted CIT; this finding was supported by the observation that patients with a baseline WT1-specific CTL frequency of $\geq 0.1\%$ achieved significantly better PFS and OS than those with a lower CTL frequency. Second, vaccine-induced immune signatures enabled the early prediction of such durable clinical outcomes; this finding was supported by the fact that three immune signatures—CTL frequency, CTL phenotype composition, and anti-WT1 IgG antibody titer—were identified as candidate biomarkers for the prediction of durable clinical outcomes as early as month 2 after the start of vaccination. These

favorable outcomes were likely attributable to the efficient induction of WT1-specific CTLs by WT1 peptide vaccination, which is consistent with the finding that PFS and OS were significantly longer for immune responders than for immune nonresponders. The proportion of immune responders was as high as 66.7%, reflecting the appreciable immunogenicity of WT1 protein since WT1 is a stem cell antigen [28].

The release of cancer antigens (step 1) from dying cancer cells after chemotherapy and/or radiotherapy in the cancer-immunity cycle [29] promotes WT1 epitope presentation by antigen-presenting cells (e.g., DCs), followed by the induction of epitope-targeting CTLs. WT1-targeted CIT may have promoted the sustained activation of the cycle—the fundamental immunologic mechanism for preventing the recurrence/progression of malignant tumors in patients with good WT1-specific immune signatures at baseline and in the early period of vaccination, thereby contributing to durable tumor control (≥ 7 years).

Patients whose WT1-specific CTL frequency at baseline was $\geq 0.1\%$ showed the significantly higher 2-year PFS and 4-year OS rates compared with their counterparts with a baseline CTL frequency of $< 0.1\%$; this observation is congruent with the study of Nakata et al. [30] who reported on an association between the high prevaccination frequency ($\geq 0.03\%$ of WT1-specific CTLs in $CD8^+$ T cells) and favorable clinical outcomes. Hence, we consider that baseline CTL frequency of $\geq 0.1\%$ was useful for identifying pediatric patients undergoing WT1-targeted CIT who were likely to achieve durable clinical outcomes.

Prior clinical studies have linked effector/memory T-cell phenotypes with reduced metastasis, recurrence prevention, and favorable survival [31–33], supporting the biological plausibility that CTL phenotype composition may contribute to long-term prognosis after WT1-targeted CIT. $CD8^+$ T cells with effector function—EM and E CTLs—are the main effector cells in generating antitumor immune responses; furthermore, memory $CD8^+$ T cells can durably remain functional [34]. Early signs of activated or proliferating lymphocytes are associated with an improved likelihood of clinical response [35]. In the present study, the proportion of WT1-specific EM CTLs which have both effector and memory functions was significantly greater in clinical responders than in nonresponders at as early as month 2 after the start of vaccination (Fig. 6C). At month 6, 6 of 9 clinical responders, but none of clinical nonresponders, had a high proportion ($\geq 10\%$) of WT1-specific EM CTLs; the proportion of WT1-specific E CTLs—which are devoid of memory function—tended to be greater in clinical nonresponders than in responders (Fig. 6C). The findings of the CTL phenotype composition analysis strongly suggested that EM CTLs likely played a critical role in inducing good clinical responses, while E CTLs may represent terminally differentiated cells with limited functional capacity. In addition, the greater proportion of N CTLs in clinical nonresponders seems to reflect the failure of efficient WT1-specific CTL priming (Fig. 6D).

None of 14 clinical responders died of primary disease and all survived for at least 7 years after the start of vaccination. This exceptionally durable survival suggests the sustained suppression of minimal residual disease (MRD). Regarding the prevention of late relapse, we speculate that WT1-targeted CIT successfully sustained the generation of CM and EM CTLs sufficient to provide the long-term suppression of tumor regrowth through $CD4^+$ T cell help that was presumably delivered by WT1-specific

helper T cells, the activation of which was suggested by the generation of the anti-WT1₂₃₅ IgG Ab and EM CTLs at month 2.

Immunomonitoring results afforded immunologic evidence to support the speculation that CD4⁺ T cells increase the magnitude and quality of CTL and B cell responses as described previously [36]. Follicular helper T cells provide B cells with CD4⁺ T cell help, resulting in the induction of IgM-to-IgG class switch [37]. CD4⁺ helper T cells promote the priming of CD8⁺ T cells capable of differentiating into EM CTLs, thus affording the effector and memory functions of CTLs [36]. The production of the anti-WT1₂₃₅ IgG Ab may reflect WT1-specific CD4⁺ T cell help [38] and was associated with a favorable prognosis. Despite the previous observation that an increase in serum IgM Ab titer precedes that in IgG Ab titer in repeated exposure to cancer antigens and nontumor antigens, even clinical responders did not exhibit marked preceding increases in anti-WT1₂₃₅ IgM Ab titer. The possibility that IgM-to-IgG class switching occurred before week 2—when the measurements of the anti-WT1₂₃₅ IgG and IgM Ab titers started—cannot be excluded. Another possibility is that the IgM-to-IgG class switch, which had already occurred prior to the start of vaccination, generated a very low titer of the anti-WT1₂₃₅ IgG Ab, whose titer increased to be detectable after the start of vaccination.

In 2004, Oka et al. [4] demonstrated in adult patients with leukemia, myelodysplastic syndromes, lung cancer, or breast cancer that WT1-targeted CIT induced the generation of WT1-specific CTLs and the resultant tumor regression. These findings prompted us to examine the immune response caused by this vaccine in 5 patients with acute lymphoblastic leukemia or sarcomas [39]. Consequently, we found that the greater potential of inducing tumor regression could be expected for patients with MRD compared with those with high tumor burden. Hence, we designed the present study in patients with solid malignancies in remission or who had PR in expectation of recurrence prevention for the former and progression prevention for the latter by the vaccine. Consistent with this concept, tumor shrinkage was also observed (Suppl. Figures S4 D, E).

Finally, this vaccine did not provoke any TEAEs of clinical concern so severe as to require the discontinuation of WT1 peptide vaccination during the study period, although the incidence of injection site reaction was 100%.

Collectively, these findings support a translational framework in which baseline WT1-specific immunity may serve as a clinically useful biomarker for identifying pediatric patients who may benefit from WT1-targeted CIT, whereas vaccine-induced immune signatures may serve as on-treatment biomarkers reflecting treatment-induced immune responses and informing future WT1-targeted immunotherapy studies.

The present trial has several limitations. First, the immune signatures identified in the present study represent exploratory candidate biomarkers and require prospective validation in independent patient cohorts before being translated into clinical practice. Second, the relatively small sample size and single-arm phase II clinical trial design limited the statistical power, precluded multivariable subgroup analyses,

and the generalizability of the findings. Therefore, the optimal cutoff value for baseline CTL frequency ($\geq 0.1\%$) and its predictive performance require further validation in larger prospective studies. Third, although the associations of CTL phenotype composition and anti-WT1 IgG antibody responses with favorable long-term prognosis were demonstrated, the underlying immunological mechanisms were not directly investigated. Functional analyses of WT1-specific CD4⁺ and CD8⁺ T cells, as well as the tumor microenvironment, are warranted to establish causal relationships. Fourth, because the present study included multiple pediatric solid tumor types, the predictive value of the identified immune signatures could not be evaluated separately for each tumor type. Therefore, tumor-specific validation will be necessary in future studies.

Conclusions

Baseline WT1-specific CTL frequency may identify patients most likely to achieve durable clinical outcomes from WT1-targeted CIT. Immune signatures—CTL frequency, CTL phenotype composition, and anti-WT1 IgG Ab titer—were identified as candidate biomarkers for the early prediction of such durable clinical outcomes. Collectively, these findings provide a rationale for biomarker-driven WT1-targeted CIT in pediatric solid tumors.

Abbreviations

Ab Antibody

ARMS Alveolar rhabdomyosarcoma

AUC Area under the curve

CIT Cancer immunotherapy

CTL Cytotoxic T lymphocyte

CI Confidence interval

CM Central memory

CR Complete response

DC Dendritic cell

DTH Delayed-type hypersensitivity

E Effector

HLA Human leukocyte antigen

HSCT Hematopoietic stem cell transplantation

Ig Immunoglobulin

MHC Major histocompatibility

N Naïve

NE Not evaluable

OS Overall survival

PD Progressive disease

PFS Progression-free survival

PR Partial response

PS Performance status

NB Neuroblastoma

RECIST Response evaluation criteria in solid tumors

RMS Rhabdomyosarcoma

STEAEs Serious treatment-emergent adverse events

TEAEs Treatment-emergent adverse events

UMIN University hospital medical information network

UPN Unique patient number

WT1 Wilms tumor gene 1

Declarations

Ethics approval and consent to participate

The present clinical trial was approved by the ethics committee at Osaka University Hospital (approval number: 8091) and by the Ethics Committee of St. Luke's International Hospital (approval number: 10–127) and was conducted in accordance with the Declaration of Helsinki. All procedures were approved by the ethics committee review board of each participating hospital. Pediatric patients (16–19 years) and their legal representatives provided written informed consent, and pediatric patients (aged < 16 years) endeavored to give written informed assent and their legal representatives provided written informed consent. Pediatric patients and their caregivers were instructed to visit the hospital regularly for clinical follow-up.

Competing interests

The authors declare no conflicts of interest.

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Author Contribution

YH and YoH contributed equally to this work. YH, YoH, HM, and YO designed the clinical trial and reviewed all data. YH, HM, and YoH recruited patients, collected clinical data, contributed to data analysis, and participated in manuscript preparation. YH and SH conducted the statistical analysis. All authors coordinated and supervised the trial at their respective institutions, interpreted the data, critically reviewed the manuscript, and approved the final version for submission.

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Data Availability

The datasets used and/or analyzed during the present trial are available from the corresponding author on a reasonable request.

Supplementary Information

The online version contains supplementary material available at <https://xxxxxxxxxxx>.

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Figures

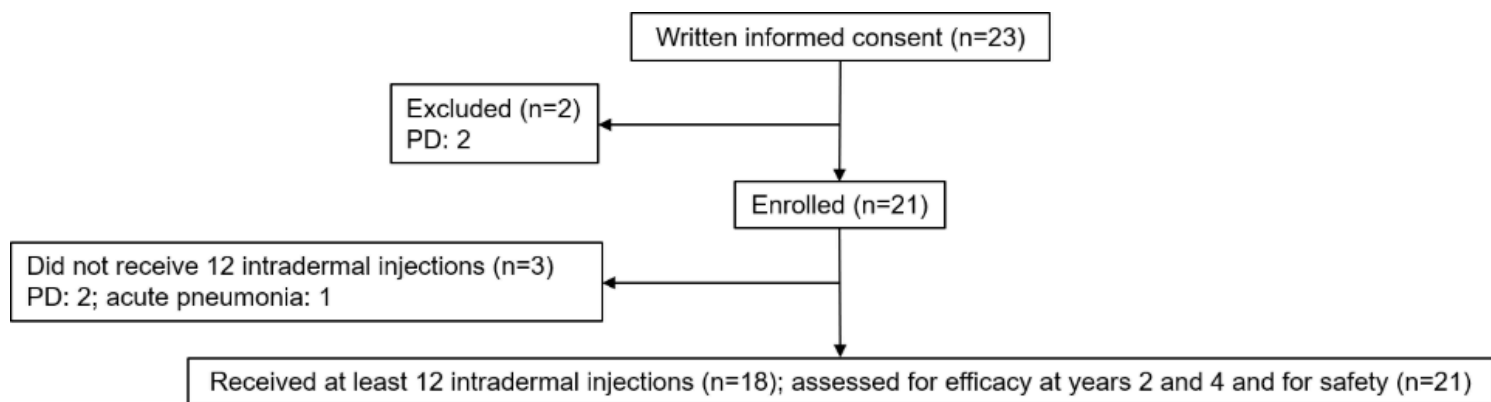


Figure 1

Patient disposition

PD, progressive disease.

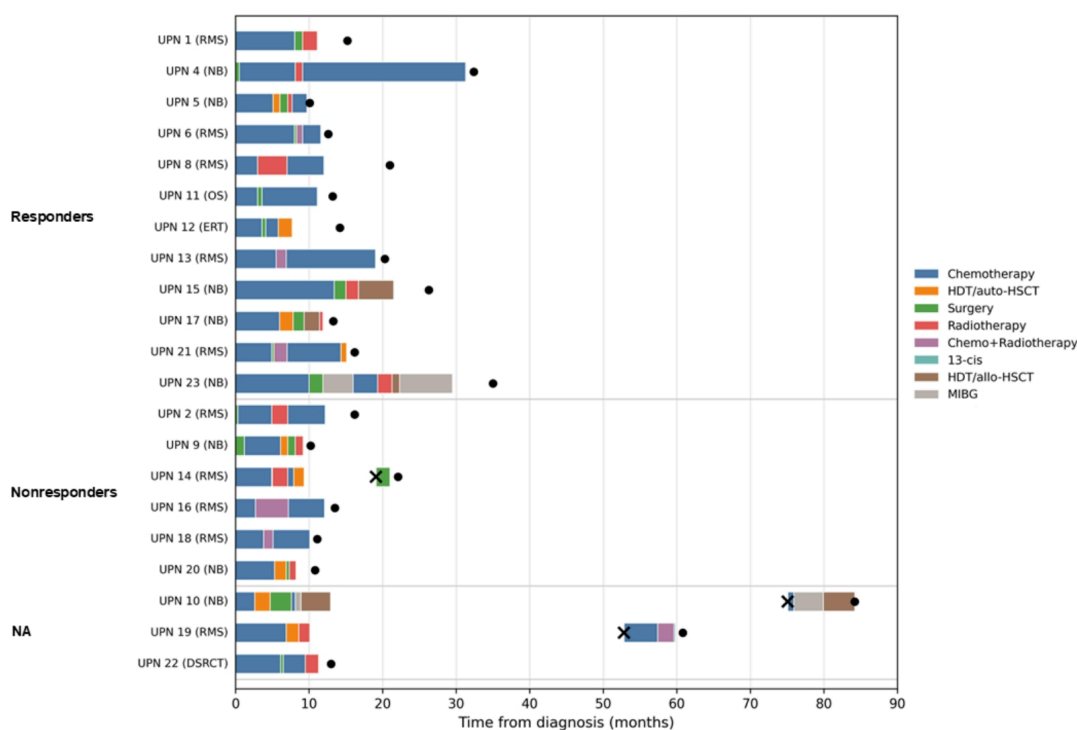


Figure 2

Swimmer plot of 12 immune responders, 6 immune nonresponders, and 3 not applicable patients.

The mark "X" indicates relapse.

The filled circle indicates the initiation of vaccination.

UPN, unique patient number; NA, not applicable.

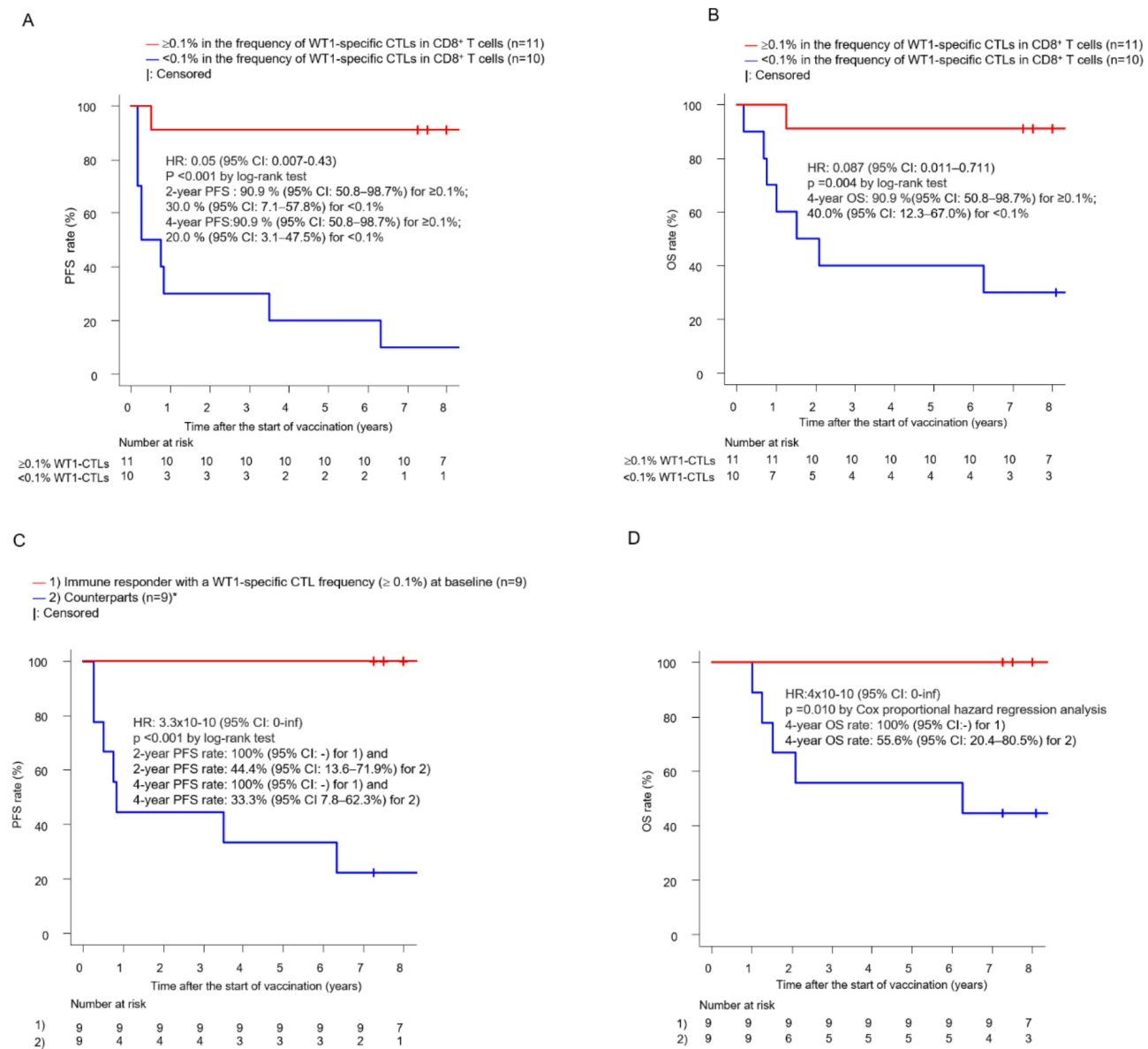


Figure 3

A. Kaplan-Meier curves of the PFS rates for patients whose frequency of WT1-specific CTLs in CD8⁺ T cells was $\geq 0.1\%$ or $< 0.1\%$. **B.** The Kaplan-Meier curves of the OS rates for patients whose frequency of WT1-specific CTLs in CD8⁺ T cells was $\geq 0.1\%$ or $< 0.1\%$. **C.** Kaplan-Meier curves* of the PFS rates for immune responders with a baseline WT1-specific CTL frequency of $\geq 0.1\%$ and for their counterparts. **D.** Kaplan-Meier curves* of the OS rates for immune responders with a baseline WT1-specific CTL frequency of $\geq 0.1\%$ and for their counterparts

*: Drawn according to the Kaplan-Meier method including the landmark analysis at week

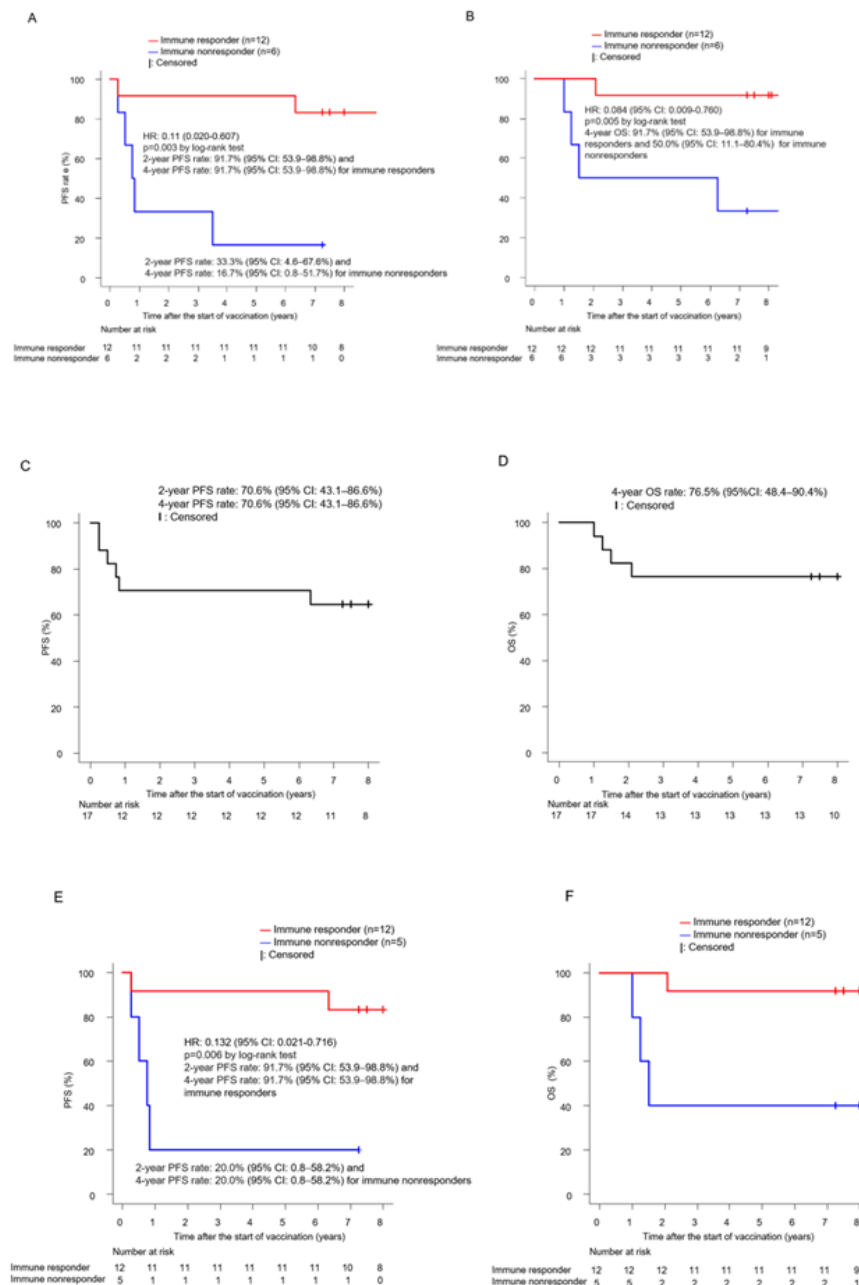


Figure 4

A. The Kaplan-Meier curves* of PFS for immune responders and nonresponders. **B.** The Kaplan-Meier curves* of OS for immune responders and nonresponders. **C.** Kaplan-Meier curve of the PFS rates for patients in CR1/PR1. **D.** Kaplan-Meier curve of the OS rates for patients in CR1/PR1. **E.** Kaplan-Meier

curves* of the PFS rates for immune responders and nonresponders among patients in CR1/PR1.

F.Kaplan-Meier curves* of the OS rates for immune responders and nonresponders among patients in CR1/PR1.

*: Drawn according to the Kaplan-Meier method including the landmark analysis at week 11 after the start of vaccination

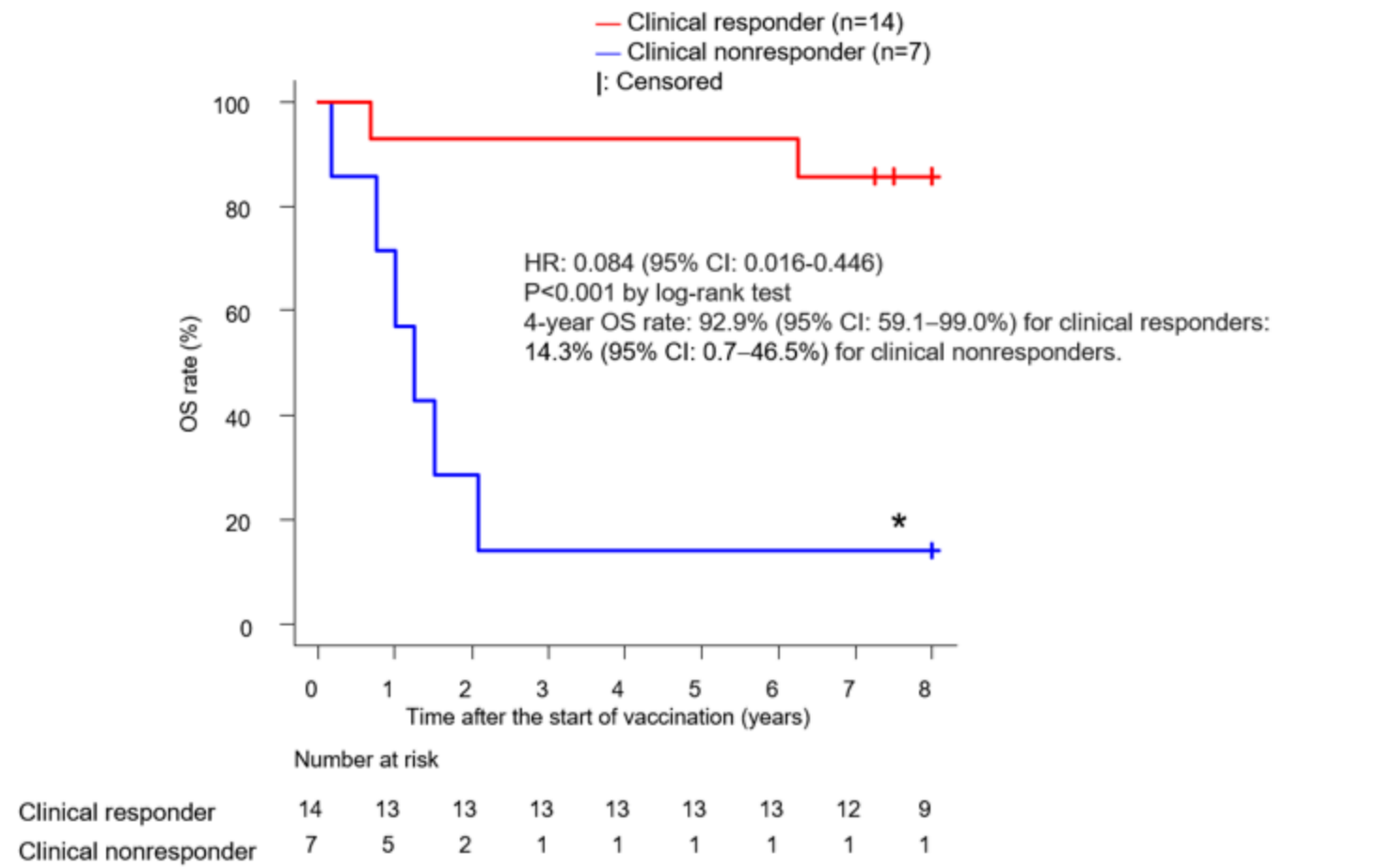


Figure 5

Kaplan-Meier curves of the OS rates for clinical responders and nonresponders.

* Includes 1 patient with NB who presented recurrence after the nineteenth vaccination, underwent chemotherapy with irinotecan and temozolomide, high-dose chemotherapy rescued with autologous peripheral blood stem cells, KIR ligand-mismatched cord blood stem cell transplantation, radiation for primary tumor, and immunotherapy with dinutuximab.

PFS, progression-free survival; WT1, Wilms tumor gene 1; OS, overall survival; HR, hazard ratio; CI, confidence interval.

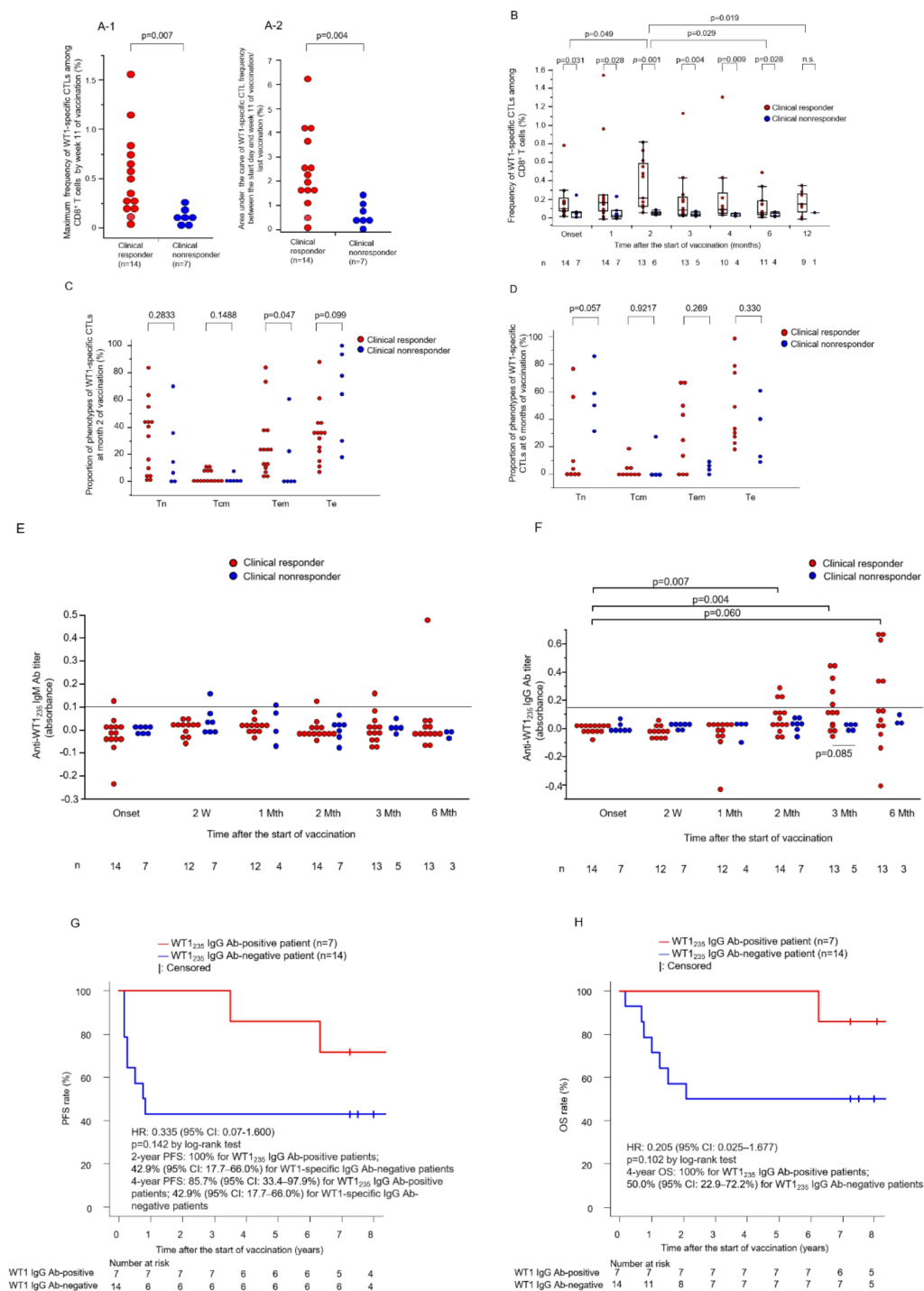


Figure 6

A-1. Maximum frequency of WT1-specific CTLs among CD8⁺ T cells (%) at week 11 of vaccination. **A-2.** Area under the curve of WT1-specific CTL frequency (percentage ´ week; a representative patient UPN4

shown in supplement Fig. 3) in clinical responders and nonresponders. **B.** Time-course changes in the frequency of WT1-specific CTLs between clinical responders and nonresponders. **C.** Proportion of WT1-specific CTL phenotypes at month 2 of vaccination. **D.** Proportion of WT1-specific CTL phenotypes at month 6 of vaccination. **E.** Time-course changes in anti-WT1₂₃₅ IgM Ab titer (absorbance) and **F.** IgG Ab titers (absorbance), both in clinical responders and nonresponders. **G.** The PFS rate for all of pediatric patients who were anti-WT1₂₃₅ IgG Ab positive or negative. **H.** The OS rate for all of pediatric patients who were anti-WT1₂₃₅ IgG Ab positive or negative.

Note) Patients became positive for the anti-WT1₂₃₅ IgG Ab when exhibiting Ab positivity at least once by month 6 of vaccination. The impact of the presence or absence of the anti-WT1-specific IgG Ab on the patient’s prognosis was examined.

Ab, antibody; Ig, immunoglobulin. Horizontal bar: median; box plot: upper and lower quartiles. WT1, Wilms tumor gene 1; CTL, cytotoxic T lymphocyte; CM, central memory; EM, effector memory; E, effector.

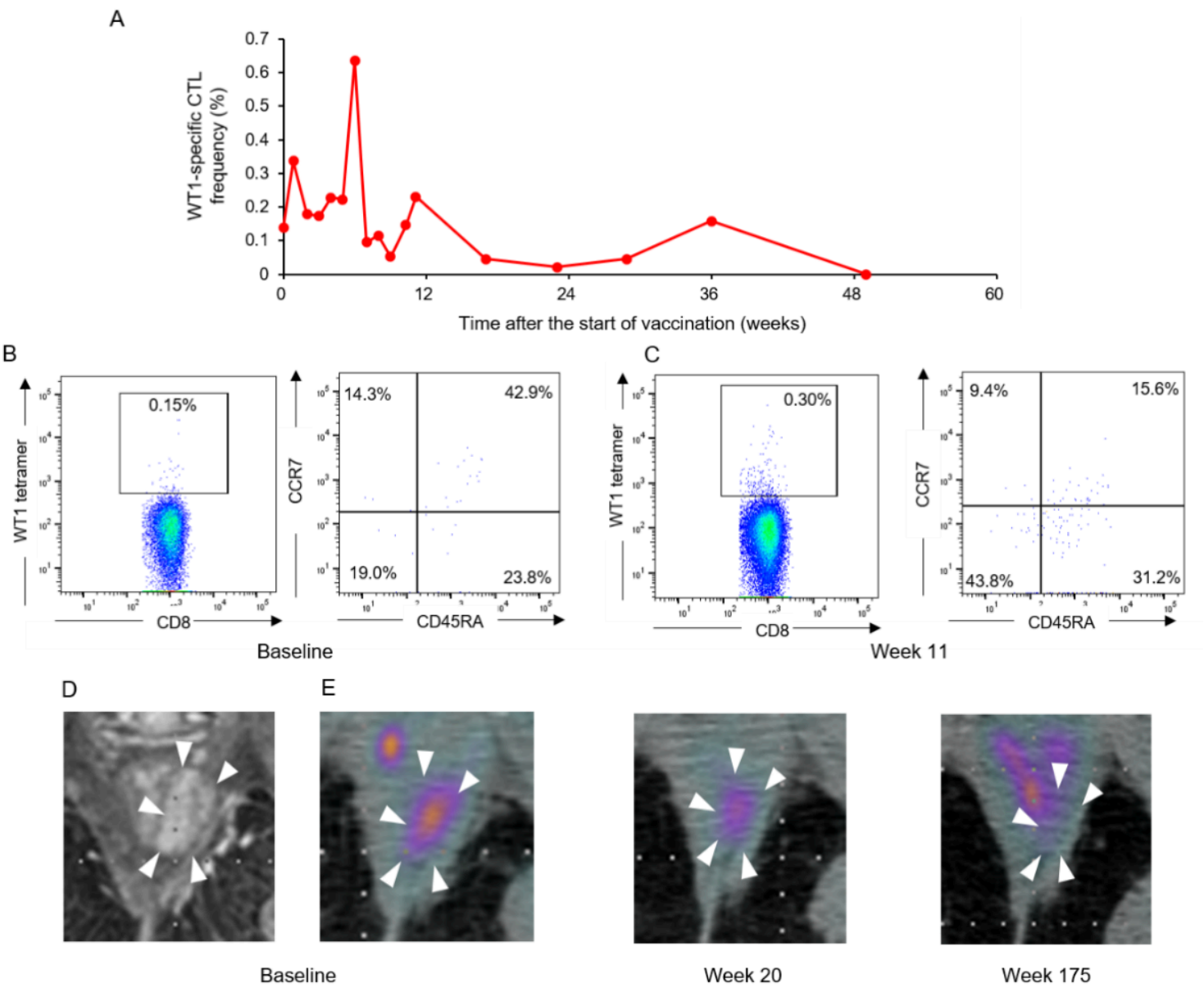


Figure 7

Immunomonitoring and imaging data of UPN13 with rectal rhabdomyosarcoma, who became positive for DTH to WT1 peptide at week 16 of vaccination, who showed an obvious association between immunologic and clinical responses, and who survived 9 years. **A.** Time-course changes in WT1-specific CTL frequency. **B.** Flow cytometry analysis of WT1-specific CTLs by using the WT1 tetramer and surface markers (N [CD45RA⁺ CCR7⁺], CM [CD45RA⁻ CCR7⁺], EM [CD45RA⁻ CCR7⁻], E [CD45RA⁺ CCR7⁻]) on the start of vaccination. **C.** Flow cytometry analysis of the CTLs at week 11 of vaccination. **D.** T2-weighted magnetic resonance image of the tumor. **E.** Fluorodeoxyglucose 18 positron emission tomograms of the tumor.

Arrows indicate the location of the tumor.

UPN, unique patient number; DTH, delayed-type hypersensitivity; CTL, cytotoxic T lymphocyte; WT1, Wilms tumor gene 1.

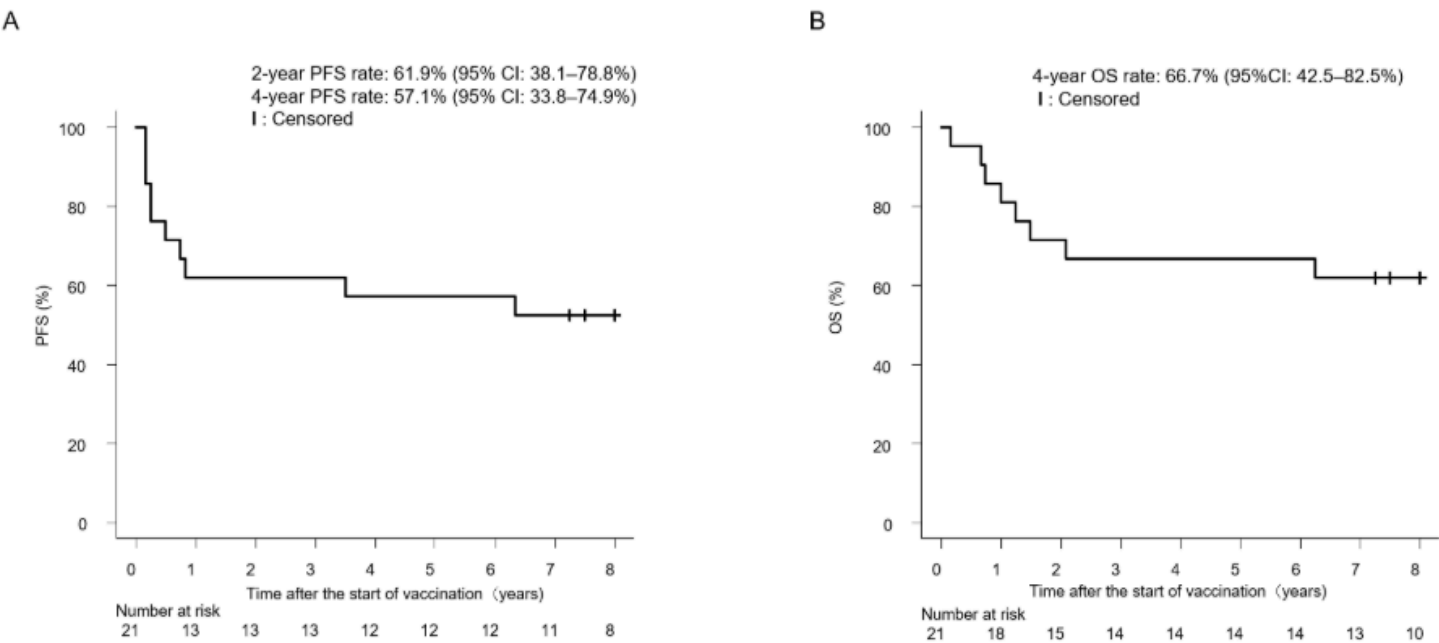


Figure 8

A. The PFS rates for all patients. **B.** The OS rates for all patients.

Supplementary Files

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- [SupplementalmaterialforJHO.docx](#)