Cancer Science
Japanese Cancer Association JCA
Open Access

Phase 1/2 study of the WT1 peptide cancer vaccine WT4869 in patients with myelodysplastic syndrome

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Key words

Acute myeloid leukemia, azacitidine-refractory patients, myelodysplastic syndrome, synthetic peptide vaccine, Wilms' tumor 1 protein

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Funding Information

This work was supported by Sumitomo Dainippon Pharma Co., Ltd.

Received June 8, 2017; Revised September 14, 2017; Accepted September 21, 2017

Cancer Sci **108** (2017) 2445–2453

doi: 10.1111/cas.13409

WT4869 is a synthetic peptide vaccine derived from the Wilms' tumor gene 1 (WT1) protein. This phase 1/2 open-label study evaluated the safety and efficacy of WT4869, and biomarkers for response, in patients with myelodysplastic syndrome. WT4869 (5–1200 μg/dose) was administered intradermally every 2 weeks, according to a 3 + 3 dose-escalation method in higher-risk (International Prognostic Scoring System score ≥1.5) or lower-risk (score <1.5) red blood cell transfusion-dependent patients with myelodysplastic syndrome. Twenty-six patients were enrolled and treated (median age, 75 years; range, 32 to 89). The most common adverse event was injection site reaction (61.5%). Main grade 3 or 4 adverse events were neutropenia (30.8%), febrile neutropenia, pneumonia, elevated blood creatine phosphokinase levels and hypoalbuminemia (all 7.7%). Dose-limiting toxicities occurred in 1 patient in the 50 μg/dose cohort (pyrexia, muscle hemorrhage and hypoalbuminemia) and 1 patient in the 400 μg/dose cohort (pneumonitis); however, the maximum tolerated dose could not be determined from this trial. The overall response rate was 18.2%, the disease control rate was 59.1% and median overall survival was 64.71 weeks (95% confidence interval: 50.29, 142.86) as assessed by the Kaplan–Meier method. Subgroup analysis of azacitidine-refractory patients with higher-risk myelodysplastic syndrome (11 patients) showed median overall survival of 55.71 weeks (approximately 13 months). WT1-specific cytotoxic T lymphocyte induction was observed in 11 of 25 evaluable patients. WT4869 was well tolerated in patients with myelodysplastic syndrome and preliminary data suggest that WT4869 is efficacious. This trial was registered at www.clinicaltrials.jp as JapicCTI-101374.

Myelodysplastic syndrome (MDS) covers a group of refractory clonal disorders characterized by ineffective hematopoiesis, peripheral cytopenia and increased risk of progression to acute myeloid leukemia (AML). The prognosis for MDS is very poor for some patients, with a reported median overall survival of 3.5 to 5.7 years for untreated lower-risk (low and intermediate-1) MDS, and 0.4 to 1.2 years for untreated higher-risk (high and intermediate-2) MDS, according to the International Prognostic Scoring System (IPSS).[1]

Although progress has recently been made for MDS patients through improvements in treatments and determining genetics, the need for additional treatment options remains. MDS presents as a variety of pathologic states and treatment strategies are determined based on risk, as assessed by IPSS or revised IPSS scores.[2–5] In higher-risk patients, the aim of treatment is to prevent transformation to leukemia. The only available curative treatment is allogeneic hematopoietic stem cell transplantation (HSCT), which is performed only in higher-risk patients owing to the poor prospects for long-term survival; however, allogeneic HSCT is associated with potentially fatal consequences, particularly in older patients, and not all patients are eligible for this approach. Azacitidine is the current standard of care for higher-risk patients, but many of these patients experience treatment failure, with a median overall survival of less than 6 months.[6]

Although immunosuppressive or erythropoiesis-stimulating agents are used in lower-risk patients, no curative treatments are available and no standard of care has been determined for patients who are not eligible for such treatments. Thus, therapeutic options for MDS are limited and the development of new treatments is warranted.

WT4869 is a novel peptide derived from the tumor-associated antigen Wilms' tumor gene 1 (WT1), which is commonly overexpressed in leukemias, MDS[7] and a variety of solid tumors.[8] WT1 is ranked the most useful among 75 cancer antigens by the National Institutes of Health, and is attracting attention as a peptide suited for use as a cancer vaccine.[9]

The $WT1_{235–243}$ peptide derived from the WT1 gene product is restricted to the human leukocyte antigen (HLA)-A*24:02

that is present in approximately 60% of the Japanese population. In this population, $WT1_{235–243}$ can induce WT1-specific cytotoxic T lymphocytes (CTL).[10,11] $WT1_{235–243,\ 2M→Y}$ is $WT1_{235–243}$ with 1 amino acid substitution. Compared to $WT1_{235–243}$, $WT1_{235–243,\ 2M→Y}$ has a higher binding affinity for HLA-A*24:02 and induces CTL more effectively.[12–14] WT4869 is a further modified version of $WT1_{235–243,\ 2M→Y}$. The WT1-specific CTL are expected to show a therapeutic effect by damaging malignant myeloid stem cells or leukemic bone marrow blasts.[12,13] Expected adverse events (AE) of WT1 vaccine use include injection-site reactions and pancytopenia.[13,15]

Based on the above, we conducted a phase 1/2 clinical study of the safety and efficacy of WT4869, and biomarkers for response, in HLA-A*24:02-positive patients with MDS.

Material and Methods

Trial design. This was an open-label, uncontrolled, multicenter study, with data collected from 10 medical facilities. The objective of phase 1 was to assess the safety of WT4869 in patients with MDS, and to determine a maximum tolerated dose (MTD) and the recommended dose, using a 3 + 3 dose-escalation method. The objective of phase 2 was to evaluate the safety and efficacy of the recommended dose determined in phase 1.

Although no safety concerns were identified, enrollment of new patients was discontinued during phase 1. This was done to prioritize the development of a novel WT1 peptide vaccine instead of evaluating the efficacy and safety of WT4869 in the planned phase 2 part of this study. The novel WT1 vaccine induces CTL, which recognize WT1 antigens, and includes a WT1-derived helper peptide. Patients wishing to continue administration of WT4869 were transitioned to a separately planned extended administration study. This trial was registered at www.clinicaltrials.jp as JapicCTI-101374.

Participants. The main eligibility criteria for phase 1 were as follows: diagnosis of MDS based on *World Health Organization Classification* (4th edition);[16] an IPSS score of ≥1.5 or <1.5 with dependency on red blood cell transfusions; age ≥18 years; being HLA-A*24:02 positive; inability to undergo allogeneic HSCT; inability to receive or being unresponsive to other therapies; performance status of 0 to 2 (Eastern Cooperative Oncology Group); expected survival of at least 3 months; ≥5% myeloblasts in bone marrow or <5% myeloblasts in bone marrow with ≥1 of the following: hemoglobin <11 g/dL, neutrophil count <1000/μL and platelets $<10 \times 10^4$/μL; serum creatinine ≤1.5 times the facility upper limit of normal; total bilirubin ≤1.5 times the facility upper limit of normal; and aspartate aminotransferase and alanine aminotransferase ≤3 times the facility upper limit of normal. Patients were not eligible if they had received chemotherapy or a molecularly targeted drug within the past 28 days, or if they had previously received allogeneic HSCT.

This study was conducted according to Good Clinical Practice and ethical principles based on the Declaration of Helsinki. Approval was obtained from the clinical trial ethical review board of each medical facility prior to conducting the study. Written consent for study participation was given voluntarily and obtained from all patients.

Interventions. Administration of WT4869 was planned at 5, 15, 50, 100, 200, 400, 600, 1200 and 1800 μg/dose according to a 3 + 3 design. If a dose-limiting toxicity (DLT) developed in 1 of 3 patients evaluated in a particular dose cohort, 3 more patients were added to the cohort and 6 patients were evaluated. If a DLT developed in 1 of these 6 patients, the dose was escalated. If no patient developed a DLT at the 5 μg/dose, the dose was escalated to 50 μg/dose (skipping the 15 μg/dose), and if no patient developed a DLT at 50 μg/dose, the dose was escalated to 200 μg/dose (skipping the 100 μg/dose).

A WT4869 peptide suspension was prepared for injection by adding 0.5 mL of water to either 0.5 mg of WT4869 or 5 mg of WT4869. The WT4869 peptide suspension was then added to the originally formulated water/oil pre-emulsion WT4869 at a ratio of 3:7. Administration of 100 μL of dosing emulsion was performed intradermally at two locations (5–600 μg/dose) or four locations (1200 μg/dose) every 2 weeks, as shown in Figure 1. Treatment was continued until the discontinuation criteria (unacceptable AE or disease progression) were met.

Safety. The primary end point of phase 1 was safety. AE were observed from the initial dose of the study drug to 28 days after the final treatment. AE severity was determined according to the maximum applicable grade from the *Common Terminology Criteria for Adverse Events* version 4.0 JCOG Japanese edition. The seriousness of the AE was determined according to criteria specified in the protocol (e.g. death, persistent disability or incapacity). AE for which a causal

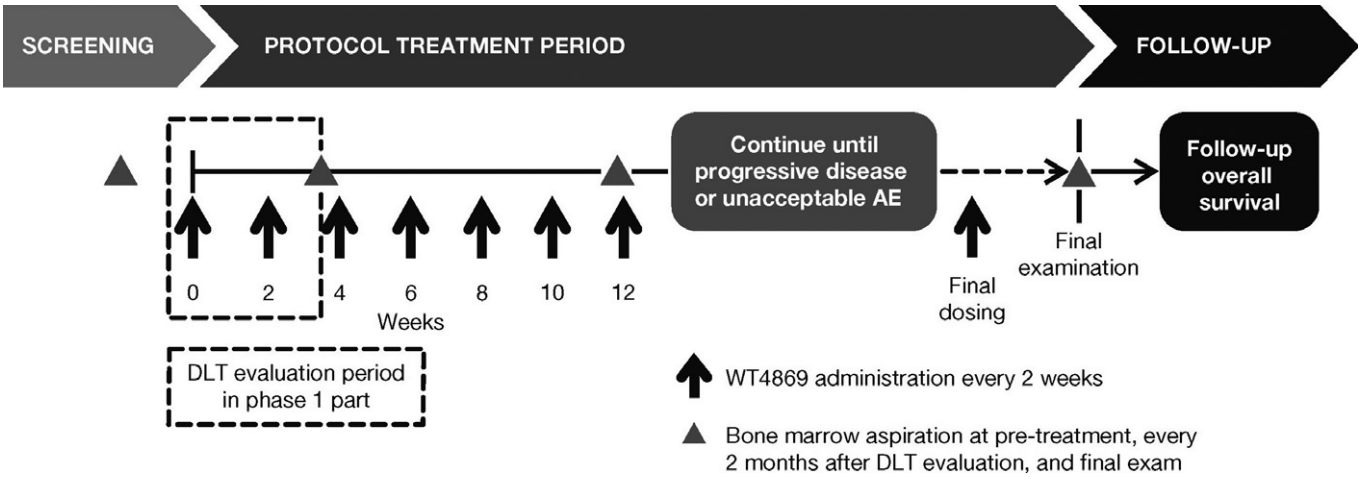

Fig. 1. Trial design. AE, adverse event; DLT, dose-limiting toxicity.

relationship to the study drug could not be ruled out were defined in the protocol as adverse drug reactions.

Patients who received two doses of the study drug or who developed a DLT after the first dose were included for DLT evaluation. DLT were defined as any of the following events developed during the DLT evaluation period (defined as 28 days following administration of the first dose of the study drug) for which a causal relationship to the study drug could not be ruled out. Hematologic toxicities considered DLT were grade 4 febrile neutropenia or grade 3 febrile neutropenia that persisted for ≥7 days. Non-hematologic toxicities considered DLT were: (i) grade 4 electrolyte abnormalities or grade 3 electrolyte abnormalities that persisted for ≥7 days; (ii) grade 4 injection site reactions or grade 3 uncontrolled injection site reactions; (iii) grade 4 infections or grade 3 infections that persisted for ≥7 days; and (iv) grade 3 or higher non-hematologic toxicities (excluding electrolyte abnormalities, injection site reactions and infections).

Efficacy. *Determination of clinical response.* Hematologic response, hematologic improvement and cytogenetic response were evaluated using the International Working Group 2006 response criteria.[17] Hematologic response was defined as complete, partial or marrow complete response (mCR), or stable disease. Patients who achieved a complete response, partial response or mCR were defined as responders. Responders or those who had stable disease were defined as disease controlled. Hematologic improvement was evaluated based on erythroid, platelet and neutrophil response criteria. Cytogenetic response was evaluated as either complete or partial. Clinical response was defined as the best response that persisted for the duration of the study as stipulated by the International Working Group 2006 response criteria from responses observed at each analytical time point.

Time to transformation to acute myeloid leukemia or death, and overall survival. Time to transformation to AML or death was the duration of time from the first WT4869 treatment day to the day of a definite diagnosis of AML or the day of death from any cause (whichever occurred first). For patients without AML transformation or death at the time of analysis, the censor was the last day of the visit being free from events, such as AML transformation or death. Starting from the first WT4869 treatment day, overall survival was considered the period until day of death from any cause. For patients alive at time of analysis, the censor was the furthest day from the first treatment day on which survival was confirmed.

Biomarkers. Biomarkers (excluding a delayed-type hypersensitivity [DTH] response to WT1 peptides) were each measured centrally at the biomarker measurement facility before first WT4869 treatment (within 4 weeks), at prescribed timings, and at the end of treatment.

Delayed-type hypersensitivity response to Wilms' tumor gene 1 peptides. Delayed-type hypersensitivity response is investigated in a variety of clinical studies[18,19] to confirm induction of cellular immunity. A WT4869 suspension and a negative control of diluting solution for WT4869 injection was injected intradermally into the same forearm. The diameter of redness was measured 2 days after intradermal injection.

Wilms' tumor gene 1 peptide-specific cytotoxic T lymphocyte induction activity. This biomarker was chosen to confirm the mechanism of action of WT4869 and examine the relationship between this biomarker and efficacy. The percentage of induced CTL in $CD8^+$ lymphocytes was measured in peripheral blood by an HLA tetramer assay.

Other biomarkers. The level of WT1 mRNA expression in bone marrow and peripheral blood was measured using the WT1 mRNA Assay Kit "Otsuka" (Otsuka, Tokyo, Japan). A relationship between efficacy and serum titer of antibodies to the WT1 protein and peptide has been suggested.[20] The serum titer of antibodies to the truncated WT1 protein corresponding to amino acids 181 to 324 (Fragment A) or 294 to 449 (Fragment B) was measured using an enzyme-linked immunosorbent assay method.

Expression levels of HLA-A*24 in blast cells and ratio of regulatory T cells in peripheral blood were measured using a flow cytometry method. Lymphocyte fractions were extracted by isolating cells corresponding to the size and internal structure of lymphocytes. A $CD4^+$ lymphocyte fraction was resolved from this fraction by extracting anti-CD4 antibody-positive cells. Following this, the $CD25^+/Foxp3^+$ fraction of these lymphocytes was evaluated.

HLA-A*24 expression levels in peripheral blood blast cells were determined by a dot-plot method using anti-CD45

Table 1. Patient characteristics

Characteristics	*n* = 26 (%)
Sex, *n* (%)	
Male	16 (61.5)
Female	10 (38.5)
Median age [range], years	75 [32–89]
Performance status, *n* (%)	
0	14 (53.8)
1	9 (34.6)
2	3 (11.5)
IPSS, *n* (%)	
Intermediate-1/Low	9 (34.6)
Intermediate-2	10 (38.5)
High	7 (26.9)
World Health Organization classification, *n* (%)	
RCMD	8 (30.8)
RAEB-1	2 (7.7)
RAEB-2	13 (50.0)
Others	3 (11.5)
Prior azacitidine treatment, *n* (%)	
All	15 (57.7)
Higher risk	11 (42.3)
Platelet count, ($\times 10^3/\mu L$)	
Mean (SD)	57.0 (66.49)
Median	25.5
White blood cell count, $\times 10^3/uL$	
Mean (SD)	2.17 (1.243)
Median	1.90
Bone-marrow blasts, %	
Mean (SD)	1.62 (3.741)
Median	0.00
Neutrophil, %	
Mean (SD)	39.50 (18.637)
Median	40.00
Neutrophil count, $\times 10^9/L$	
Mean (SD)	0.8604 (0.63641)
Median	0.6985
Minimum, maximum	0.152, 2.365

IPSS, International Prognostic Scoring System; RCMD, refractory cytopenia with multilineage dysplasia; RAEB, refractory anemia with excess blasts; others, MDS-U (2 patients) and 5q-syndrome (1 patient); higher risk, IPSS score ≥1.5 and azacitidine non-responder.

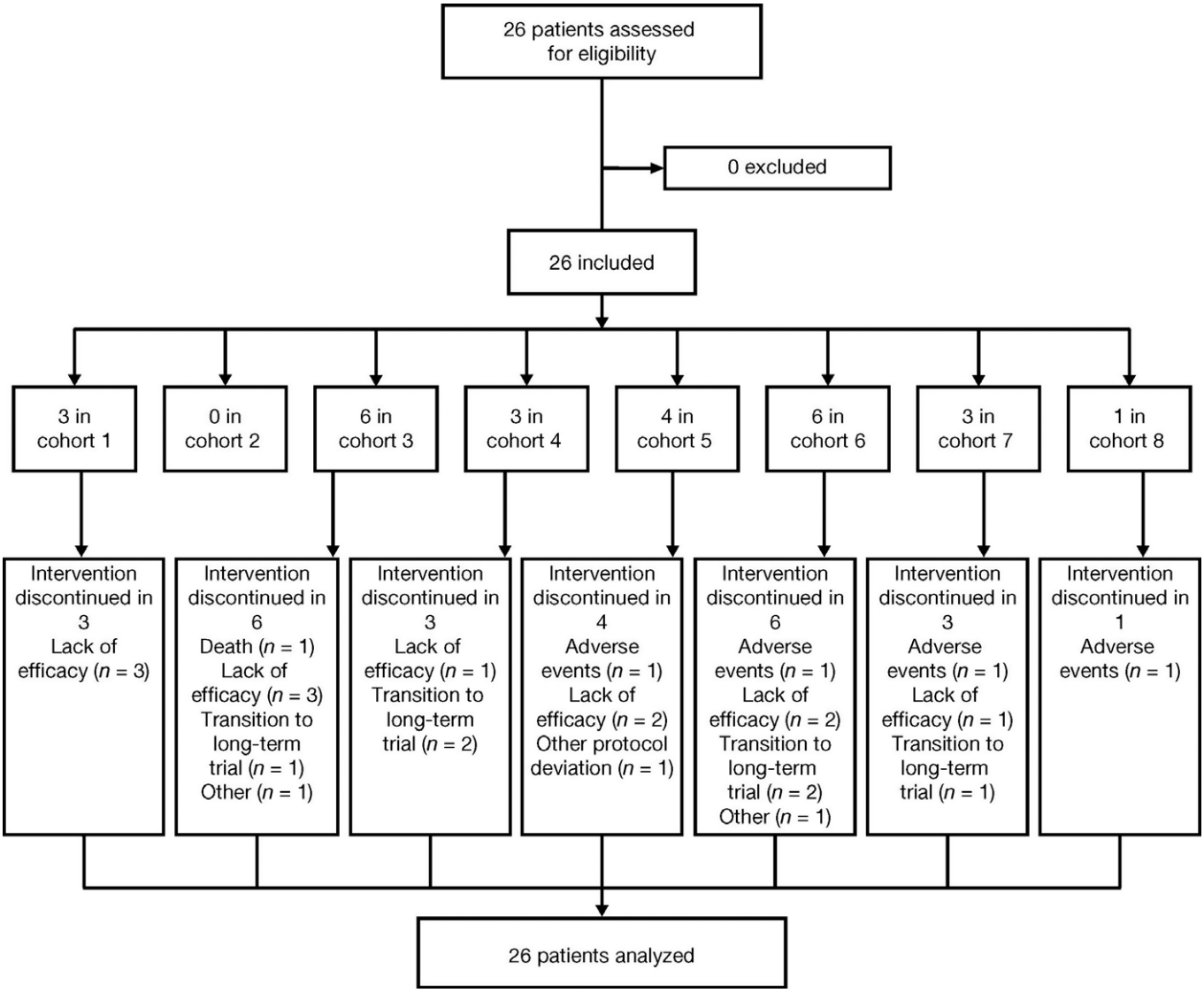

Fig. 2. Patient flow diagram. Cohort 1, 5 μg/dose; cohort 2, 15 μg/dose; cohort 3, 50 μg/dose; cohort 4, 100 μg/dose; cohort 5, 200 μg/dose; cohort 6, 400 μg/dose; cohort 7, 600 μg/dose; cohort 8, 1200 μg/dose.

Table 2. Adverse events of any cause with frequency of 15% or higher

	Total (n = 26)
System organ class	
Preferred term, n (%)	
All events	26 (100)
Neutropenia	8 (30.8)
Stomatitis	4 (15.4)
Injection site reaction	16 (61.5)
Pyrexia	8 (30.8)
Fall	6 (23.1)
Contusion	4 (15.4)

Events were aggregated after combining dose cohort groups.
Source: *Medical Dictionary for Regulatory Activities* version 16.1 and *Common Terminology Criteria for Adverse Events* version 4.0 (11 September 2010 JCOG Japanese edition).

antibodies and cell internal structures. The cell population that weakly bound anti-CD45 antibodies (blast cells) was evaluated and presented as a histogram of anti-HLA-A*24 antibodies, where the percentage of anti-HLA-A*24 antibody-positive blast cells and fluorescent intensity was evaluated.

Statistical methods. The sample size during phase 1 was calculated as a maximum of 54 patients based on the 3 + 3 design with 3 to 6 patients in each dose cohort (maximum of 9 dose cohorts). AE were converted to the *Medical Dictionary for Regulatory Activities* version 16.1 terminology. The clinical response rate was calculated as the proportion of responders among patients in the analysis set. The Kaplan–Meier method was used to calculate median survival with a 95% confidence interval (CI). Subgroup analysis of efficacy was performed in higher-risk azacitidine non-responders as a post hoc analysis. All statistical analyses were performed using SAS software version 9.2 (SAS Institute, Cary, NC, USA).

Results

Baseline data. Between July 2011 and November 2014, 26 patients with a median age of 75 years (range 32 to 89) were enrolled and treated in this study. Patient characteristics and baseline data are shown in Table 1. Fourteen patients had a

Table 3. Adverse events of any cause

	Total $n = 26$		
System organ class Preferred term	Grade 3 *n* (%)	Grade 4 [Grade 5] *n* (%)	All grade *n* (%)
All events	10 (38.5)	8 (30.8) [1 (3.8)]	26 (100)
Blood and lymphatic system disorders			
Neutropenia	1 (3.8)	7 (26.9)	8 (30.8)
Thrombocytopenia	0 (0.0)	1 (3.8)	3 (11.5)
Febrile neutropenia	2 (7.7)	0 (0.0)	2 (7.7)
Anemia	1 (3.8)	0 (0.0)	1 (3.8)
Leukopenia	1 (3.8)	0 (0.0)	1 (3.8)
Cardiac disorders			
Arrhythmia	0 (0.0)	0 (0.0) [1 (3.8)]	1 (3.8)
Gastrointestinal disorders			
Gastrointestinal hemorrhage	1 (3.8)	0 (0.0)	1 (3.8)
General disorders and administration site conditions			
Pyrexia	1 (3.8)	0 (0.0)	8 (30.8)
Infections and infestations			
Pneumonia	2 (7.7)	0 (0.0)	2 (7.7)
Sepsis	0 (0.0)	1 (3.8)	1 (3.8)
Lung infection	1 (3.8)	0 (0.0)	1 (3.8)
Soft tissue infection	1 (3.8)	0 (0.0)	1 (3.8)
Injury, poisoning and procedural complications			
Spinal compression fracture	1 (3.8)	0 (0.0)	1 (3.8)
Investigations			
Blood creatine phosphokinase increased	2 (7.7)	0 (0.0)	2 (7.7)
Metabolism and nutrition disorders			
Hypoalbuminemia	2 (7.7)	0 (0.0)	2 (7.7)
Hypokalemia	1 (3.8)	0 (0.0)	1 (3.8)
Musculoskeletal and connective tissue disorders			
Muscle hemorrhage	1 (3.8)	0 (0.0)	1 (3.8)
Neoplasms benign, malignant and unspecified (including cysts and polyps)			
Bladder cancer	1 (3.8)	0 (0.0)	1 (3.8)
Pancreatic carcinoma	1 (3.8)	0 (0.0)	1 (3.8)
Respiratory, thoracic and mediastinal disorders			
Pneumonitis	1 (3.8)	0 (0.0)	1 (3.8)
Vascular disorders			
Hypertension	1 (3.8)	0 (0.0)	1 (3.8)

AE data have been aggregated after combining dose cohort groups. "All" refers to the total number of patients with a given AE of grade 1 to 5. Grade 3 or higher AE occurred in 19 of 26 patients (73.1%) (including grade 5 arrhythmia). AE, adverse event.

performance status of 0 and 17 patients were higher-risk (7 = high risk; 10 = intermediate-2 risk).

WT4869 treatment. Patient flow is presented in Figure 2. WT4869 (5–1200 μg/dose) was administered to all 26 enrolled patients. No patient received the 1800 μg/dose treatment. According to criteria stipulated in the protocol, the 15-μg/dose treatment was skipped because no DLT occurred in the 5-μg/dose cohort, but the 100-μg/dose treatment was administered because DLT occurred in the 50-μg/dose cohort.

The mean number of WT4869 treatments in each group ranged from 6.0 to 32.3. The mean overall treatment duration in each group was 11.14 to 65.24 weeks, and the mean total administered dose was 78.3 to 7200.0 μg.

Safety. The most common AE are shown in Table 2. AE were observed in all 26 patients. The most common AE was injection site reaction in 16 patients (61.5%), followed by neutropenia in 8 patients (30.8%), pyrexia in 8 patients (30.8%), fall in 6 patients (23.1%), stomatitis in 4 patients (15.4%) and contusion in 4 patients (15.4%). Non-hematologic AE were mostly grade 1 or 2 events.

Adverse events are shown grouped by grade in Table 3 and Table S1. Grade 3 or higher AE occurred in 19 of 26 patients (73.1%), of which neutropenia was the most common, occurring in 8 patients (30.8%), followed by febrile neutropenia, pneumonia, blood creatine phosphokinase increase, and hypoalbuminemia in 2 patients each (7.7%). A grade 5 AE of arrhythmia occurred in 1 patient in the 50-μg/dose cohort, but a causal relationship to the study drug was ruled out.

Among 25 patients (1 patient who did not finish DLT evaluation was excluded), DLT of pyrexia, muscle hemorrhage and hypoalbuminemia occurred in 1 patient in the 50-μg/dose cohort, and pneumonitis occurred in 1 patient in the 400-μg/dose cohort. All these events resolved or improved. No DLT were observed in any other treatment group (up to 1200-μg/dose cohort). The MTD was not determined from this trial.

Adverse drug reactions occurred in 22 patients (84.6%) and 6 patients (23.1%) discontinued the study treatment owing to AE.

Efficacy. *Determination of clinical response.* Clinical responses are shown in Table 4. Of 26 patients administered the study drug, efficacy was evaluated in all patients after excluding ineligible or unevaluable patients. The overall response rate (mCR + hematologic improvement) was 18.2% and the disease control rate (mCR + hematologic improvement + stable disease) was 59.1%. Among the 22 patients evaluable for efficacy, a hematologic response of mCR was observed in 1 patient and stable disease in 12 patients. A hematologic improvement was observed in 1 patient with mCR and in 3 patients with stable disease. A neutrophil response was observed in 2 patients, 1 with an erythroid/neutrophil response and the other with a platelet/erythroid/neutrophil response. Among 18 patients whose cytogenetic responses were evaluated, a complete cytogenetic response was observed in 1 patient and a partial cytogenetic response was observed in 1 patient.

Time to transformation to acute myeloid leukemia or death and overall survival. Transformation to AML or death occurred in 16 of 26 patients by the end of the study (median time, 60.43 weeks [95% CI: 18.71, 103.29]). Death occurred in 13 of 26 patients by the end of the study. Median overall survival was 64.71 weeks (95% CI: 50.29, 142.86).

Subgroup analysis. Subgroup analysis of higher-risk azacitidine-refractory patients (11 patients) showed a median time to transformation to AML or death of 31.14 weeks (95% CI: 5.43, 60.43). A Kaplan–Meier curve of overall survival is shown in Figure 3. Median overall survival was 55.71 weeks (approximately 13 months; 95% CI: 31.14, not applicable).

Biomarkers. *Delayed-type hypersensitivity response to Wilms' tumor gene 1 peptides.* Delayed-type hypersensitivity response was evaluated using the diameter of redness at the WT4869 injection site. The diameter of redness at the WT4869 injection site was ≥2 mm larger than at the negative control injection site in 5 patients.

Wilms' tumor gene 1 peptide-specific cytotoxic T lymphocyte induction activity. The percentage of induced CTL was evaluated among lymphocytes and $CD8^+$ lymphocytes (CTL cell

Table 4. Clinical response

All patients (n = 26)	Patient	Age/sex	WHO	IPSS	Previous azacitidine	Number of vaccinations	Response	Survival, weeks	Maximum CTL† (%)
5 μg/dose	10401	77/M	RAEB-1	Int-2	No	21	SD (HI-E)	142.9	0.0087
	10601	82/F	RAEB-1	Int-2	No	21	mCR (HI-E)	123.9+	0.1213
	10602	69/M	RCMD	Int-1	Yes	5	PD	101.7	0.0027
50 μg/dose	30101	32/M	RCMD	Int-1	No	53+	SD	112.9+	0.0133
	30201	79/M	RAEB-2	Int-2	No	5	NE	8.6	0.0008
	30202	58/M	RAEB-2	Int-2	Yes	31	SD	90.9+	0.0055
	30301	75/M	RAEB-2	High	No	28	PD (cytogenetic PR)	113.1+	0.0121
	30501‡	53/M	RAEB-2	Int-2	Yes	2	NE	60.4	0.0016
	30603	43/F	RCMD	Int-1	No	36	SD (cytogenetic CR)	94.0+	0.0925
100 μg/dose	40302	61/M	RAEB-2	Int-2	Yes	44+	SD	95.7+	0.0103
	40701	80/F	MDS-U	Int-2	Yes	8	PD	50.3	0.0025
	40801§	75/M	RCMD	Int-1	Yes	45+	SD (TI-P)	95.0+	0.0093
200 μg/dose	50604	72/F	RAEB-2	Int-2	Yes	5	PD	40.0	0.0060
	50702	76/M	RCMD	Int-2	Yes	25	SD (HI-E,N)	55.7	0.0043
	50703	71/M	RAEB-2	Int-2	Yes	4	PD	64.7	0.0628
	50802	89/F	RCMD	Int-1	No	1	NE	16.7	—¶
400 μg/dose	60203	73/M	RAEB-2	High	Yes	4	PD	40.1+	0.0035
	60402††	68/M	RAEB-2	High	Yes	1	NE	31.1	0.0068
	60704	81/F	MDS-U	Int-1	Yes	2	PD	50.6	0.0154
	60705	76/F	RCMD	Int-1	Yes	7	SD	51.0+	0.0013
	60901	76/F	5q-syndrome	Int-1	No (lenalidomide)	22+	SD (HI-P,E,N)	48.1+	0.0091
	60902	88/F	RAEB-2	High	No	19+	SD	47.1+	0.0235
600 μg/dose	70102	65/M	RAEB-2	High	Yes	3	PD	39.4+	0.0135
	71001	75/M	RAEB-2	High	Yes	3	PD	7.1	0.0033
	71002	76/M	RCMD	Low	No	15+	SD	30.3+	0.3385
1200 μg/dose	80903	84/F	RAEB-2	High	No	6	SD	25.0	0.0198
Summary of clinical response: Evaluable patients (n = 22) ‡‡									
Clinical response, n (%)									
ORR (mCR + HI)							4 (18.2)		
mCR with HI							1 (4.5)		
SD with HI							3 (13.6)		
SD without HI							9 (40.9)		
DCR (mCR + HI + SD)							13 (59.1)		
PD							9 (40.9)		

†After administration of WT4869. ‡Experienced DLTs (pyrexia, muscle hemorrhage, and hypo-albuminemia). §Reached and maintained TI-P for 7 months during treatment period. ¶No CTL value recorded after administration of WT4869. ††Experienced DLT (pneumonitis). ‡‡Four patients were excluded from the efficacy analysis due to being non-evaluable. +Still being treated or surviving on the cut-off day. CR, complete response; DCR, disease control rate; F, female; HI, hematologic improvement; HI-E, hematologic improvement - erythroid response; HI-E,N, hematologic improvement - erythroid response, neutrophil response; HI-P,E,N, hematologic improvement - platelet response, erythroid response, neutrophil response; Int, intermediate; IPSS, International Prognostic Scoring System; M, male; mCR, marrow complete response; MDS-U, myelodysplastic syndrome - unclassifiable; NE, not evaluable; ORR, overall response rate; PD, progressive disease; PR, partial response; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; SD, stable disease; TI-P, transfusion-independency platelet; WHO, World Health Organization.

group). CTL induction measured at baseline and after administration of at least 1 study drug was evaluable in 25 patients; 11 patients reported an increased percentage of CTL induction. Measurement of the change in CTL in each patient evaluable for efficacy (n = 22) over time is shown in Figure 4a and b. Comparing induced CTL percentages (after administration) grouped by hematologic responses, percentages of induced CTL in the any response + stable disease group (13 patients) were higher than in the progressive disease group (9 patients). No statistical significant difference was found on the maximum value of CTL from each patient between the any response + stable disease group and the progressive disease group, based on the Wilcoxon two-sample test (P = 0.2349).

Other biomarkers. Increases and decreases of WT1 mRNA expression levels in bone marrow and peripheral blood were observed in some patients, but no clear trend was reported.

In measuring the serum titer of antibodies to WT1 protein and peptides, there was no clear increase in the titer of antibodies to fragment A after initiation of the study drug; however, a decrease was observed in several patients. Overall, no clear tendency was observed in the titer of antibodies to fragment B before and after initiation of the study drug.

No clear change in HLA-A*24 expression levels in blast cells and ratio of regulatory T cells in peripheral blood was observed before and after initiation of the study drug in almost any patient.

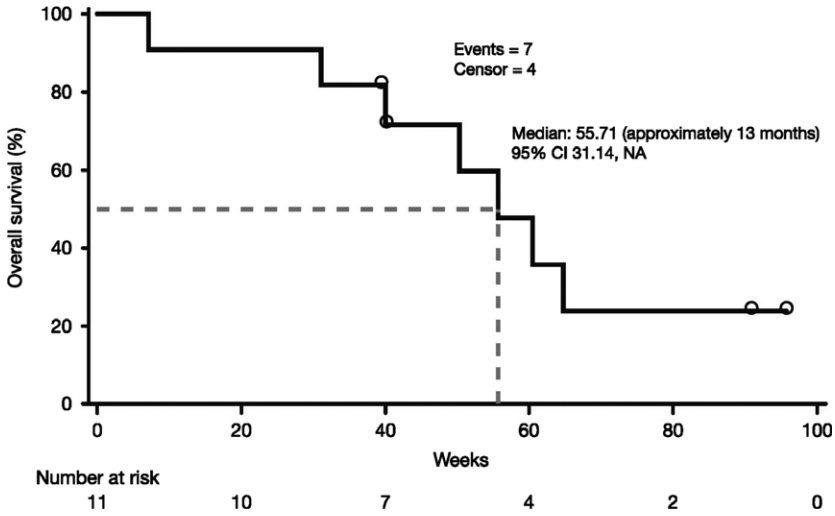

Fig. 3. Overall survival of higher-risk azacitidine-resistant patients (n = 11). CI, confidence interval; NA, not applicable.

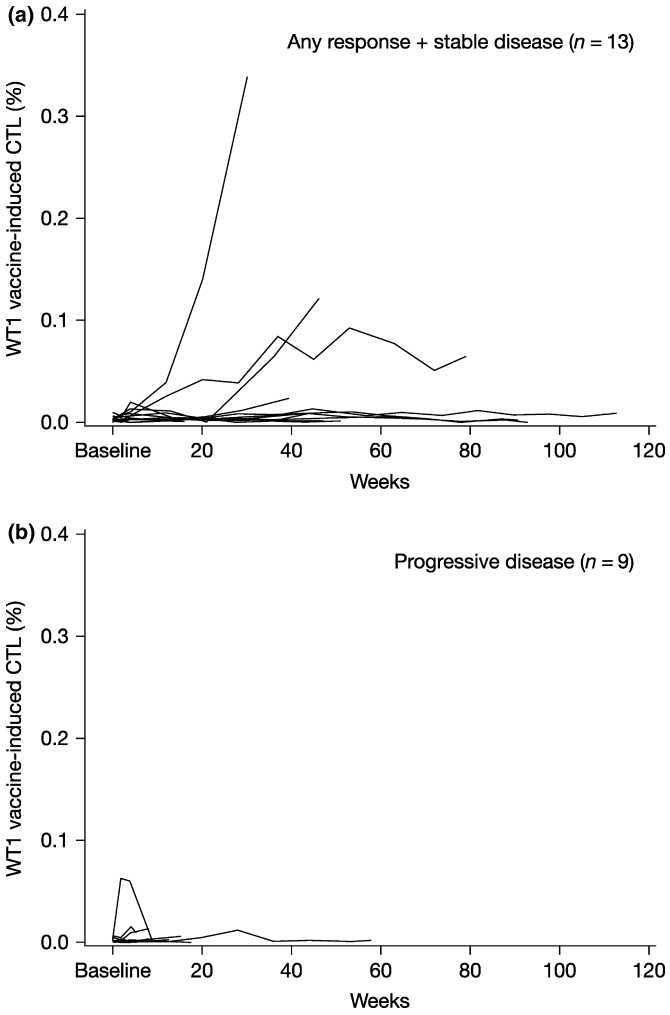

Fig. 4. Cytotoxic T lymphocytes (CTL) induction with Wilms' tumor gene 1 (WT1) vaccine: (a) Percentage of WT1 vaccine-induced CTL in patients with any response + stable disease (n = 13) and (b) percentage of WT1 vaccine-induced CTL in patients with progressive disease (n = 9). "Any response" includes marrow complete response, stable disease with hematologic improvement, and stable disease with cytogenetic complete response. CTL, cytotoxic T lymphocyte; WT1, Wilms' tumor gene 1.

Discussion

We conducted a phase 1/2 clinical study of WT4869 using 5 μg/dose up to 1200 μg/dose in patients with MDS. Among 26 patients, 6 discontinued treatment owing to AE in phase 1 of the study. No significant issues related to AE or DLT were reported. A grade 5 arrhythmia occurred in 1 patient in the 50-μg/dose cohort, which was deemed not study drug-related. An MTD and recommended dose could not be determined in this study; however, the MTD was confirmed to be ≥400 μg/dose based on DLT results. Preliminary efficacy results included an overall response rate of 18.2% and a disease control rate of 59.1%.

This study showed that WT4869 can be safely administered to patients with MDS up to 1200 μg/dose. The most common AE was injection-site reaction and the most common event of grade 3 or higher was neutropenia. DLT only occurred in 2 patients: pyrexia, muscle hemorrhage and hypoalbuminemia in 1 patient in the 50-μg/dose cohort (suggesting a possible immune response that improved upon steroid administration), and pneumonitis in 1 patient in the 400-μg/dose cohort. Most of the AE reported were mild and all DLT resolved without issues. A review of WT1 peptide vaccination clinical trials in patients with MDS and AML found no grade 3 or 4 AE in 8 of 9 clinical trials, and, in the remaining trial, grade 3 or 4 erythema, dyspnea and fever were observed.[21] In 1 case report, sepsis was reported in a patient during a phase 1 study due to leukopenia associated with the WT1 peptide vaccine.[14,15] Of hematopoietic organ tumors, our study targeted patients with MDS, in whom we observed AE of grade 3 or higher in 19 of 26 patients. The AE observed in our study may have differed from those observed in previous studies in which some patients had been in the remission phase of AML, unlike the patients in this study. Consequently, the results show that AE from WT4869 treatments in this study were manageable, and safety and tolerability were acceptable.

The median time to transformation to AML or death was 60.43 weeks and the median overall survival was 64.71 weeks. In higher-risk azacitidine-refractory patients, the median overall survival was 55.71 weeks (approximately 13 months). The median overall survival in a historical cohort of higher-risk azacitidine-refractory patients (n = 435)[6] was 5.6 months. Previous studies of higher-risk patients with MDS, who were administered novel drugs after hypomethylating agent failure,

showed a median overall survival of 8.2 months with rigosertib (phase 3 study, $n = 299$),[22] 6.8 months with erlotinib (phase 2 study, $n = 35$)[23] and 7.6 months with dasatinib (phase 2 study, $n = 18$).[24]

Based on the mechanism of action of WT4869, we predicted an association between WT1 peptide-specific CTL induction activity and efficacy; however, no clear relationship between CTL induction and response was observed in this study. This could be due to the small number of patients in the study. There were also no clear changes in other biomarker levels associated with WT1 treatment, which is supported by previous studies reporting no correlation between immunologic response and clinical response.[25,26] In addition, the mechanism of action of WT4869 requires support from helper $CD4^+$ T cells to establish a memory response in CTL that can be expected to provide long-term efficacy. Future studies combining CTL activation-inducing cancer peptide vaccines with a therapy that simultaneously enhances helper T cell function are required to validate this hypothesis.

Cancer vaccines are typically considered more suited to chronic phase treatment than acute phase treatment because they require time to elicit an immunologic response; however, in this study 11 patients who were azacitidine non-responders survived for ≥6 months after termination of vaccination. Furthermore, only 2 of 26 patients developed febrile neutropenia (grade 3). The findings together suggest that WT1 peptide vaccine may improve outcomes compared to salvage treatments.[27,28] These results suggest that for patients who do not respond to azacitidine treatment, early treatment with WT1 peptide vaccine might lead to better treatment outcomes, a finding that warrants further investigation. WT1 peptide vaccines may also be suited to those with lower tumor burden, including decreased tumor volume following response to azacitidine.

This study was the first clinical trial in which WT4869 was administered to humans. A 3 + 3 design was used to determine the MTD starting from a low dose. As a result, limited toxicity and efficacy data were obtained because only 3 to 6 patients were included in each cohort. This study demonstrated a better trend than the typical prognosis for patients with MDS. The inclusion of patients with a performance status of 0 to 2 who were in relatively good physical condition might account for the favorable results observed in our study. Further study is warranted to confirm these results.

Wilms' tumor gene 1 peptide vaccine research has focused on patients with AML, with only 1 to 2 patients with MDS included in each previous study.[21] Our study had a larger sample size, including 26 patients with MDS. The knowledge obtained from this study is both important and encouraging in regards to the planning of future clinical trials of WT1 peptide vaccines for the treatment of patients with MDS.

This study demonstrated that WT4869 is safe and well tolerated in patients with MDS, and preliminary data suggest that it has promising efficacy. Vaccine therapy targeted WT-1 could potentially be used in patients who are not candidates for transplantation or whose prognosis has not improved with current treatments. Further investigation is warranted to develop therapies for elderly patients with MDS and AML.

Acknowledgments

The authors thank the following individuals and their teams at investigational sites for their contributions during the conduct of the trial: Keiya Ozawa, Division of Hematology, Department of Medicine, Jichi Medical University, Shimotsuke, Japan (affiliation during trial) and IMSUT Hospital, Division of Genetic Therapeutics, The Advanced Clinical Research Center The Institute of Medical Science, The University of Tokyo (current affiliation); Toshiki Uchida, Department of Hematology and Oncology, Nagoya Daini Red Cross Hospital, Nagoya, Japan; Tomoki Naoe, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan (affiliation during trial) and National Hospital Organization Nagoya Medical Center, Nagoya, Japan (current affiliation); Makoto Murata, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; Naokuni Uike, Department of Hematology, National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan (affiliation during trial) and Department of Palliative Care Medicine, Saga-ken Medical Center Koseikan, Saga, Japan (current affiliation); Yasunobu Abe, Department of Hematology, National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan; Michihiro Hidaka, Department of Hematology, National Hospital Organization Kumamoto Medical Center, Kumamoto, Japan; Masahiro Kizaki, Department of Hematology, Saitama Medical Center, Saitama Medical University, Kawagoe, Japan; Ryuzo Ohno, Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, Japan; and Masao Tomonaga, Japanese Red Cross Nagasaki Atomic Bomb Hospital (affiliation during trial) and Megumino-Oka Nagasaki Atomic Bomb Survivors Nursery Home Clinics (current affiliation). The authors also thank all the patients and their families who contributed to this study. This work was supported by Sumitomo Dainippon Pharma Co., Ltd. Medical writing assistance was provided by Michiko Kato from Statcom and Angelique Stalmach from Complete HealthVizion, which was contracted and compensated by Sumitomo Dainippon Pharma Co., Ltd.

Disclosure Statement

Y. U. received research funding from Sumitomo Dainippon Pharma Co., Ltd. M. O. received research funding from SymBio Pharmaceuticals and Celltrion. T. S. received honoraria from Kyowa Hakko Kirin and Nippon Shinyaku. K. O. received honoraria for lecture fees from Celgene and Novartis Pharma, and received research funding from Ono Pharmaceutical, Chugai Pharmaceutical, Bristol-Myers Squibb, Kyowa Hakko Kirin and Janssen Pharmaceutical. Y. M. received honoraria from Sumitomo Dainippon Pharma Co., Ltd. S. Tagashira and S. Tsuchiya are employees of Sumitomo Dainippon Pharma Co., Ltd. S. M. and Y. H. received no financial support for this manuscript.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Adverse events from any cause.