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Phase Ib study on the humanized anti-CCR4 antibody, KW-0761, in advanced solid tumors

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ABSTRACT

Tregs infiltrate tumors and inhibit antitumor immunity. KW-0761 (Mogamulizumab) is a humanized anti-CCR4 monoclonal antibody that could eliminate activated Tregs with high immunosuppressive activity that express CCR4. In this phase Ib trial, KW-0761 was used as a cancer immunotherapeutic reagent to deplete Tregs in patients with advanced or recurrent solid CCR4-negative tumors. Thirty-nine patients with solid cancer were treated with KW-0761 at a dose of 0.1 or 1.0 mg/kg. The safety, clinical responses, and effects of Treg depletion were analyzed. Any grade and grade 3–4 treatment-related adverse events (AEs) were observed in 36 (92%) and 14 (36%) out of 39 patients, respectively. All treatment-related AEs were manageable. One and 5 patients achieved a partial response and stable disease, respectively, during treatment and were long survivors. The efficient depletion of Treg in peripheral blood was confirmed in both cohorts. Therefore, the administration of KW-0761 was safe, resulting in the depletion of Tregs in peripheral blood and potential immune responses in patients with solid cancer. The combined use of KW-

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0761 to deplete Tregs and other immunotherapies is a promising approach to augment immune responses.

Keywords: solid cancer patients, mogamulizumab, regulatory T cells, clinical trial, CCR4

Abbreviations:
Tregs: regulatory T cells
AEs: adverse events
TME: tumor microenvironment
mAbs: antibodies
PBMCs: blood mononuclear cells

INTRODUCTION

Immunotherapy, primarily with immune checkpoint inhibitors (ICIs) such as anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and anti-programmed cell death protein 1 (PD-1) monoclonal antibodies (mAbs), has markedly improved the survival rate of patients with cancer, even in the advanced stage[1,2,3]; however, the efficacy of ICIs is unsatisfactory in the majority of patients and, thus, other therapies, including immunotherapy, are urgently needed. In the escape phase of the concept of cancer immunoediting, tumor cells have developed strategies to establish an immunosuppressive state within the tumor microenvironment (TME) by producing immunosuppressive cytokines, such as vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), and indoleamine 2,3-dioxygenase (IDO), and recruiting regulatory immune cells, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs).[4] Therefore, the manipulation of immunosuppressive cells or TME is an anticancer therapeutic strategy.

Tregs constitute 5–10% of $CD4^+$ T cells in the periphery and play an important role in maintaining immune tolerance.[4,5] Tregs inhibit the development of antitumor immunity, thereby hindering the immune surveillance of cancer and preventing effective antitumor immune responses in tumor-bearing hosts. The presence of a large number of Tregs and a low ratio of $CD8^+$ T cells to Tregs in the TME correlate with a poor prognosis in various cancer types.[6,7] Tregs promote tumor progression by suppressing antitumor immunity,[8,9] manipulating Tregs, or targeting the immunosuppressive factors produced by these cells, and, thus, have potential as an anticancer treatment strategy. Despite promising pre-clinical studies, Treg cell-targeted therapy has not yet been successfully applied to clinical settings.

KW-0761 (mogamulizumab) is a humanized anti-CCR4 immunoglobulin G1 (IgG1) mAb with enhanced antibody-dependent cellular cytotoxicity.[10,11] It was developed as an orphan drug for adult T-cell leukemia-lymphoma (ATLL), which expresses CCR4 on cell surfaces, and has been approved for T-cell lymphomas, including ATLL and other peripheral T-cell lymphomas, by the Pharmaceuticals and Medical Devices Agency, Food and Drug Administration, and European Medicines Agency.[12,13,14] Evidence is emerging to show that KW-0761 suppresses the function of effector Tregs (eTregs), which is a subpopulation of Tregs with high expression levels of FoxP3 and CCR4, and exhibits strong immunosuppressive activity against tumors, leading to robust $CD8^+$ T-cell proliferation in ATLL.[15,16] Therefore, we conducted a clinical trial to examine the treatment effects of KW-0761 on solid cancers based on a novel concept, namely, Treg depletion. In a previous phase Ia clinical trial, KW-0761 was administered to 7 patients with lung cancer and 3 with esophageal cancer with a dose escalation design of 0.1, 0.5, and 1.0 mg/kg.[17] All doses were confirmed to be safe and well tolerated. Four out of the 10 patients achieved stable disease (SD) during the treatment and were long survivors. The monitoring of $FoxP3^+$ Tregs in

peripheral blood mononuclear cells (PBMCs) during the treatment indicated efficient depletion even at the lowest dose of 0.1 mg/kg.

To investigate the safety and efficacy of eTreg depletion and clinical responses in patients with solid cancer in more detail, we conducted a phase Ib clinical trial on KW-0761 in 39 patients with CCR4-negative cancers.

PATIENTS AND METHODS

Patients

Eligible patients had CCR4-negative treatment-refractory or advanced cancer with target lesions. Archival or newly obtained tumor samples from patients were screened for the expression of CCR4 by immunohistochemistry (IHC) as previously described.[18] CCR4 expression was confirmed by the review committee with a central evaluation. Inclusion criteria were described in the phase Ia study.[17]

Study Design

This study was designed as a multi-institutional, open-label, two-arm, investigator-initiated phase Ib clinical trial on KW-0761. The investigational drug KW-0761was provided by Kyowa Hakko Kirin. The study was registered with ClinicalTrials.gov as NCT01929486 and was approved by local Institutional Review Boards. All participating patients provided written informed consent before enrolment, in accordance with the Declaration of Helsinki. The primary objectives were to characterize the safety and efficacy of eTreg depletion in PBMCs by KW-0761 for patients with advanced or recurrent solid cancer. The secondary objectives were to assess clinical responses, including the overall response rate, progression-free survival (PFS), and overall survival (OS), and select the recommended dose for a phase II trial. Twenty and nineteen patients were randomly enrolled into cohorts with doses of 0.1 and 1.0 mg/kg, respectively, and received 8 intravenous infusions of KW-0761 weekly followed by monthly infusions until disease progression. These two doses of KW-0761 were established in the phase Ia study as the maximum-tolerated and minimal doses, respectively. Oral antihistamines and acetaminophen were administered prior to each KW-0761 treatment, and hydrocortisone was simultaneously infused intravenously with the first KW-0761 treatment to prevent infusion reactions.

Toxicity Evaluation

Toxicity was evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. Patients were assessed for toxicity weekly during the first 8 treatments and every 4 weeks thereafter until 24 weeks after the last treatment. The independent data monitoring committee evaluated safety data for each dose level.

Clinical Response Evaluation

Responses were evaluated 12 weeks after the first KW-0761 treatment or at the study discontinuation based on computed tomography (CT) scans according to RECIST (ver. 1.1),[19] and/or immune-related (ir) RECIST. OS was defined as the duration from the first day of KW-0761 treatment until the day of death from any cause. PFS was defined as the duration from the first day of KW-0761 treatment until either the day of progressive disease (PD) detection or death from any cause. Tumor responses, OS, and PFS were confirmed by a central evaluation for each patient.

Evaluation of eTreg Depletion on PBMCs

Blood samples were obtained at baseline, 4 and 8 weeks after the first KW-0761 treatment, and every 4 weeks during the continuous treatment until study-off. PBMCs were isolated from heparinized blood by density gradient centrifugation using Ficoll-Paque Plus (GE Healthcare, Fairfield, CT). Cells were stored in liquid N_2 until used. Treg depletion was evaluated by flow cytometry. After thawing, PBMCs were incubated with mAbs at 4°C for 20 minutes. Cells were stained with anti-CD4-PerCP (clone SK3; BD Biosciences, San Jose, CA), anti-CD25-APC (clone 2A3; BD Biosciences), and anti-CD45RA-FITC (clone ALB11; Beckman Coulter, Brea, CA) mAbs. The intracellular staining of FOXP3 was performed with anti-FoxP3-PE (clone PCH101; eBioscience) mAb and a FoxP3/Transcription Factor Staining Buffer Set (eBioscience, San Diego, CA) according to the manufacturer's instructions. After the incubation, cells were washed and analyzed by FACSCalibur (BD Biosciences). $CD45RA^+$ FoxP3 lo resting/naïve Tregs, $CD45RA^-$ FoxP3 hi activated/effector Tregs (eTregs), and $CD45RA^-$ FoxP3 lo, non-Tregs were analyzed as previously described.[15]

RESULTS

Patient Characteristics

In this phase Ib study, 39 patients with advanced CCR4-negative solid cancer were randomly assigned to receive a treatment with KW-0761 at 0.1 mg/kg (n=20) or 1.0 mg/kg (n=19) between October 2013 and April 2016 (Table 1). The median age of patients was 65 years. The cohort included 11 patients with esophageal cancer, 9 with lung cancer, 6 with malignant melanoma, 5 with gastric cancer, 5 with ovarian cancer, and 3 with mesothelioma with 2 to 23 infusions of KW-0761. Eleven patients dropped out before the completion of the first cycle with 8 infusions of KW-0761 due to the withdrawal of consent in 1 patient, disease progression in 4, and a decision by the principal investigators in 6. The median number of infusions was 8 (2–14) in the 0.1 mg/kg cohort and 8 (2–23) in the 1.0 mg/kg cohort. The median follow-up was 94 (21–498) days in the 0.1 mg/kg cohort and 99 (25–511) in the 1.0 mg/kg cohort. Seven (35%) patients in the 0.1 mg/kg cohort and 7 (36%) in the 1.0 mg/kg cohort died, and the cause of all deaths was disease progression.

Table 1 Patient characteristics

Dose (mg/kg)	ID	Age	Sex	Tumor Type	No. of Infusions	Best overall response	Time to progression (days)	OS (days)	Treatment-related AEs (All)	Treatment-related AEs (Grade 3–4)	Treg depletion
0.1	B-01	66	M	Esophageal cancer (SCC)	6	PD	42	96	+	+	+
0.1	B-03	70	F	NSCLC	8	PD	63	422	+	–	+
0.1	B-05	67	M	Melanoma	5	PD	28	73	+	–	+
0.1	B-07	68	F	Melanoma	8	PD	77	77	+	–	+
0.1	B-10	64	F	Esophageal cancer (SCC)	8	PD	63	249	+	+	+
0.1	B-11	70	M	Esophageal cancer (Other)	4	PD	25	391	+	+	NA
0.1	B-12	66	M	Melanoma	8	PD	76	150	+	–	+
0.1	B-15	57	M	NSCLC	8	PD	67	77	+	–	+
0.1	B-19	76	M	NSCLC	8	SD	63	92	+	+	+
0.1	B-21	67	M	Melanoma	8	PD	77	91	+	+	+
0.1	B-22	53	F	Ovarian cancer	8	PD	67	224	+	+	+
0.1	B-23	61	M	NSCLC	8	PD	63	498	+	+	+
0.1	B-24	70	M	Gastric cancer	8	PD	70	271	+	–	+
0.1	B-26	85	M	Gastric cancer	2	PD	21	21	+	–	+
0.1	B-28	71	M	Mesothelioma	4	SD	40	40	–	–	+
0.1	B-32	65	F	NSCLC	8	PD	63	395	+	–	+
0.1	B-33	63	F	Ovarian cancer	8	PD	69	77	+	–	+
0.1	B-35	47	F	Esophageal cancer (Other)	4	PD	29	31	+	–	+
0.1	B-37	65	M	Esophageal cancer (SCC)	9	SD	96	284	+	–	+
0.1	B-39	59	F	Esophageal cancer (SCC)	14	PD	70	251	+	+	+

(Continued)

1.0	B-02	65	F	Ovarian cancer	8	PD	66	413	+	–	+
1.0	B-04	68	F	Melanoma	8	PD	79	142	–	–	+
1.0	B-06	49	M	Melanoma	8	PD	56	233	+	–	+
1.0	B-08	51	F	Esophageal cancer (SCC)	2	PD	27	93	+	–	+
1.0	B-09	64	M	Esophageal cancer (SCC)	23	PR	491	511	+	+	+
1.0	B-13	54	F	Ovarian cancer	8	PD	65	318	+	–	+
1.0	B-14	68	F	Gastric cancer	4	PD	35	35	–	–	+
1.0	B-16	57	M	NSCLC	3	PD	25	25	+	+	NA
1.0	B-17	67	M	Gastric cancer	5	PD	47	47	+	–	+
1.0	B-18	68	M	NSCLC	8	PD	62	65	+	+	+
1.0	B-20	54	M	SCLC	8	PD	56	58	+	–	+
1.0	B-25	61	M	NSCLC	8	PD	63	497	+	–	+
1.0	B-27	64	M	Esophageal cancer (SCC)	5	PD	36	99	+	+	+
1.0	B-29	45	M	Gastric cancer	8	PD	70	272	+	–	+
1.0	B-30	77	M	Esophageal cancer (SCC)	8	PD	63	68	+	–	+
1.0	B-31	68	M	Mesothelioma	10	SD	124	124	+	+	+
1.0	B-34	65	M	Esophageal cancer (Other)	8	PD	81	88	+	+	+
1.0	B-36	80	M	Mesothelioma	10	SD	160	168	+	–	+
1.0	B-38	73	F	Ovarian cancer	8	PD	76	84	+	–	+

NSCLC: non-small cell lung cancer
SCLC: small cell lung cancer
SCC: squamous cell carcinoma
PD: progressive disease
SD: stable disease
PR: partial response
AEs: adverse events
NA: not assessed

Adverse events (AEs)

Any grade treatment-related AEs occurred in 19 (95%) out of 20 patients in the 0.1 mg/kg cohort and in 17 (89%) out of 19 in the 1.0 mg/kg cohort; grade 3–4 treatment-related AEs occurred in 7 (35%) patients in the 0.1 mg/kg cohort and in 6 (32%) in the 1.0 mg/kg cohort (Table 1). Table 2 lists all treatment-related AEs, with 65 and 49 AEs being observed in the 0.1 and 1.0 mg/kg cohorts, respectively. The most frequently observed categories of treatment-related AEs were skin disorders and lymphopenia. Grade 3–4 treatment-related AEs were lymphopenia (4 [20%] in the 0.1 mg/kg cohort and 4 [21%] in the 1.0 mg/kg cohort), rash, increased alanine aminotransferase, increased aspartate aminotransferase, hypophosphatemia, increased gamma-glutamyltransferase, and appetite loss. All treatment-related AEs were manageable or recovered without any treatment, and no drug-related deaths were observed.

Table 2 Treatment-related adverse events

Grade	0.1 mg/kg (N=20)				1.0 mg/kg (N=19)			
	1	2	3	4	1	2	3	4
Cases	16	12	6	1	10	14	4	2
Total events	34	23	7	1	23	20	4	2
Non-hematological								
General								
Fever	1				1	2		
Fatigue	1				2			
Appetite loss	1						1	
Edema	1							
Weight loss		1						
Skin and subcutaneous tissue								
Rash	8	5	1		5	5		
Pruritus	1							
Drug eruption						1		
Gastrointestinal								
Diarrhea					1	1		
Nausea					1			
Vomiting					1			
Ear and labyrinth								
Vertigo positional		1						
Ear discomfort		1						
Cardiac disorders								
Arrhythmia	1				2			
Electrocardiogram T wave inversion					1			
Hypertension						1		

(Continued)

Thoracic disorders								
Pleural effusion					1			
Nervous system disorders								
Dizziness	1							
Dysgeusia	1							
Headache					3			
Neuropathy peripheral					1			
Infections								
Oral candidiasis						1		
Upper respiratory tract Inflammation						1		
Cheilitis		1						
Endocrine								
Hypothyroidism	1				1			
Gynecomastia	1							
Hematological								
Leukopenia	2					1		
Lymphopenia	1	10	3	1		7	2	2
Thrombocytopenia					1			
Eosinophilia	1							
Hypokalemia	1	2						
Hyponatremia	1							
Hypophosphatemia	1							
Hypoalbuminemia	1						1	
GGT increased			1					
ALT increased	3		1					
AST increased	2		1					
Amylase increased		1						
LDH increased	1				1			
ALP increased	1	1						
TSH increased					1			
Glucose urine present	1							

ALT: alanine aminotransferase
AST: aspartate aminotransferase
LDH: lactate dehydrogenase
TSH: thyroid-stimulating hormone
GGT: gamma-glutamyltransferase
ALP: alkaline phosphatase

Clinical Responses

Confirmed objective responses with RECIST were SD in 3 patients with NSCLC, esophageal cancer, or mesothelioma in the 0.1 mg/kg cohort, and a partial response in 1 patient with esophageal cancer and SD in 2 patients with mesothelioma in the 1.0 mg/kg cohort (Table 1, Fig. 1). Median PFS was 67 and 65 days and OS was 271 and 272 days in the 0.1 and 1.0 mg/dl cohorts, respectively (Table 1). A durable response was observed in 2 patients (B-09, B-39) (Fig. 2).

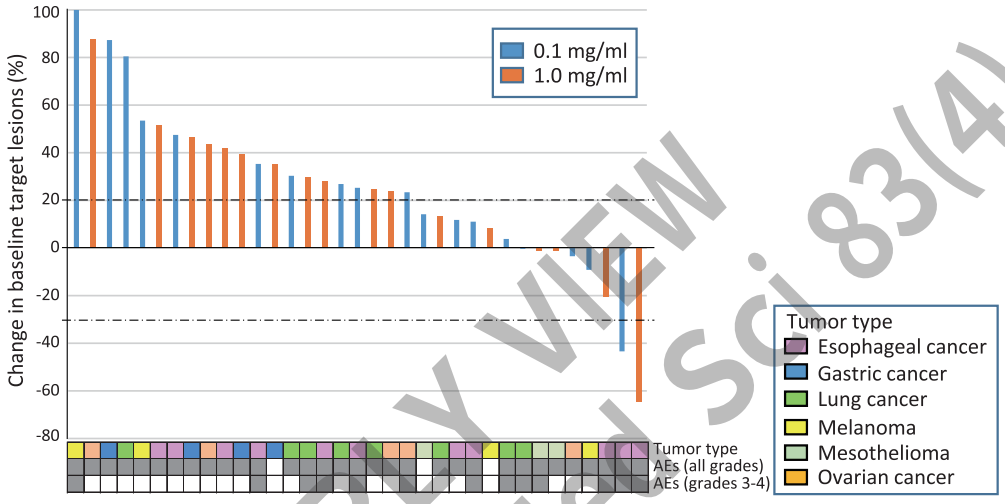

Fig. 1 Best percentage change in the target lesion tumor burden from baseline in patients with a CT assessment

Waterfall plot for the maximum percentage reduction in the target lesion tumor burden until disease progression (study-off) according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 progression. A positive change in the tumor burden indicates tumor growth; a negative change in the tumor burden indicates a tumor reduction. The horizontal dotted lines denote a 30% decrease and 20% increase, indicating an objective response and progressive disease, respectively, as per RECIST version 1.1. The tumor type and presence or absence of adverse events (AEs) are annotated for each patient. 0.1 mg/ml (n=18), blue bar; 1.0 mg/ml (n=17), orange bar.

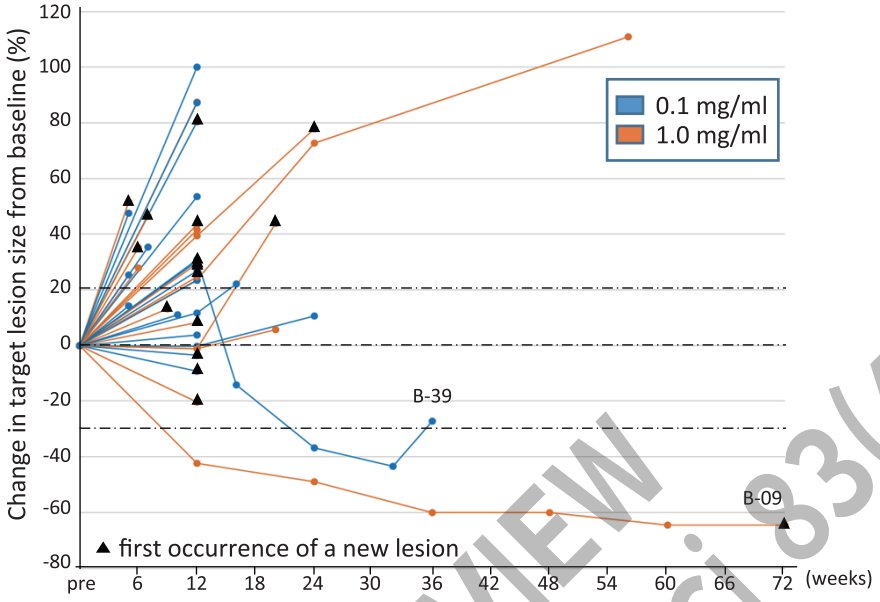

Fig. 2 Percentage change in the target lesion tumor burden from baseline over time
A spaghetti plot for changes from the baseline in the tumor burden, measured as the sum of the longest diameters of the target lesions by patients over time. The black triangle indicates the presence of a new lesion. Horizontal dotted lines denote a 30% decrease and 20% increase. 0.1 mg/ml (n=20), blue line; 1.0 mg/ml (n=19), orange line.

Efficacy of eTreg Depletion on PBMCs

eTreg depletion by KW-0761 was examined with PBMCs at baseline and during the KW-0761 treatment using flow cytometry. The percentage of eTregs in $CD4^+$ T cells markedly decreased in all patients, except for 2 for whom blood samples were not available after the start of the KW-0761 treatment (Table 1). The median percentages of eTregs in $CD4^+$ T cells in the 0.1 and 1.0 mg/kg cohorts were 2.1 and 2.1% at baseline and 0.22 and 0.21% after 4 infusions, respectively. The percentage of eTregs in blood remained low during the treatment with KW-0761.

DISCUSSION

The results of this phase Ib study confirmed the safety of KW-0761. Some treatment-related AEs were observed, including lymphopenia, which was the most frequent grade 3–4 AE; however, these AEs were all manageable and no drug-related deaths occurred. All AEs related to skin reactions in the present study were grades 1–2, except for one patient with a rash, which were less severe than those previously reported in ATLL patients.[11,13] No significant difference was noted in the incidence of AEs between the 0.1 and 1.0 mg/kg cohorts.

The manipulation of Tregs has attracted increasing attention as a novel therapeutic strategy for cancer. In this phase Ib study, eTregs were efficiently depleted on PBMCs after the KW-0761 treatment. Several potential strategies to target Tregs are currently being clinically and preclinically investigated alone or in combination, mostly with ICIs. One approach is the depletion of Tregs by targeting molecules specifically expressed on Tregs, such as CD25, CTLA-4, CCR4, OX40 (CD134), inducible T cell co-stimulator (ICOS), and glucocorticoid-induced TNFR-related

protein (GITR), or signals that are crucial for Treg cell survival and function, including T cell receptor (TCR) and IL-2 receptor signaling. Another approach for targeting Tregs is to control or modulate Treg cell function and infiltration, such as TGF-β, tyrosine kinase, phosphoinositide 3-kinase (PI3K), IDO, the CD39-CD73 axis, and vascular endothelial growth factor receptor (VEGFR) signaling. In these strategies, anti-CTLA-4 mAbs has attracted special attention because it yielded durable responses in a subset of cancer patients.[3,20] Recent findings from several preclinical studies indicated that Treg depletion is one of the mechanisms underlying the antitumor effects of anti-CTLA-4 mAbs used as a checkpoint inhibitor.[21,22] Anti-CTLA-4 mAb reportedly deplete Tregs from the TME, which may contribute to the clinical benefits of this agent.[20] However, direct Treg cell-targeted therapy has not yet been applied to clinical settings. Treg recruitment at local sites is driven by combinations of chemokines and their receptors, such as CCL22 to CCR4 and CCL1 to CCR8.[23,24] CCR4 is differentially expressed on the surface of various types of lymphocyte subpopulations. In a previous phase Ia study, we confirmed the high expression of CCR4 on eTregs and CCR4$^+$ eTreg enrichment in tumor specimens.[17,25] Moreover, an in vitro experiment on an anti-CCR4 mAb treatment with PBMCs showed the efficient induction of NY-ESO-specific CD4$^+$ and CD8$^+$ T cells due to the depletion of CCR4$^+$ eTregs. ATLL patients treated with KW-0761 showed the induction of NY-ESO-1-specific CD8$^+$ T cells, potentially contributing to the prolongation of survival. These findings suggest the contribution of KW-0761 to antitumor immune enhancements through Treg depletion.

In this clinical trial, while KW-0761 effectively depleted eTregs in PBMCs, most of the treated patients did not exhibit tumor regression, which is consistent with the findings of our previous phase Ia study; however, a durable clinical response was observed in 2 patients with esophageal cancer. A possible explanation for the low clinical efficacy of KW-0761 is that KW-0761 may deplete another type of CCR4$^+$ T-cell subset. The comprehensive assessment conducted in the previous phase Ia study showed the high expression of CCR4 on eTregs, Th2 CD4$^+$ T cells, and Th17 CD4$^+$ T cells and the low expression of CCR4 on Th1 CD4$^+$ T cells and CD8$^+$ T cells.[17] The efficacy of KW-0761 depends on the balance between the depletion of eTregs, which may improve antitumor immunity, and the depletion of other types of cells, including CD8$^+$ T cells or Th1 CD4$^+$ T cells, which may attenuate antitumor immunity. Another plausible explanation for impaired clinical responses by KW-0761 is that eTregs were not sufficiently depleted in the TME. It currently remains unclear whether these drugs selectively deplete Tregs in the TME. The density of Tregs in the TME is not always reflected in peripheral blood. In this clinical trial, we observed Treg reductions in the tumor of a patient with biopsy specimens that were collected at baseline and post-treatment (data not shown). Therefore, further basic and translational research is warranted to obtain a more detailed understanding of Treg cell functions, particularly in the TME.

In summary, KW-0761 was safely administered and efficiently depleted eTreg in PBMCs, with potential immune responses in solid CCR4-negative cancers. We are currently conducting a phase I clinical trial on the preoperative administration of KW-0761 combined with anti-PD-1 mAb to patients with advanced or recurrent solid cancer, with the expectation of the synergistic effects of these immunologically different functional treatments. Although further refinement of the regimen is required, the combined use of KW-0761 to deplete Tregs and other immunotherapies is a promising strategy to augment immune responses.

IN MEMORIAM

This study is dedicated to the memory of the late Dr. E. Nakayama.

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CONFLICTS OF INTEREST

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