

研究成果の刊行に関する一覧表

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雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ishida T, Joh T, Uike N, Yamamoto K, Utsunomiya A, Yoshida S, Sasaki Y, Miyamoto T, Takemoto S, Suzushima H, Tsukasaki K, Nosaka K, Fujiwara H, Ishitsuka K, Inagaki H, Ogura M, Akinaga S, Tomonaga M, Tobinai K, Ueda R.	Defucosylated Anti-CCR4 Monoclonal Antibody (KW-0761) for Relapsed Adult T-Cell Leukemia-Lymphoma: A Multicenter Phase II Study.	J Clin Oncol	30	837-42	2012

Defucosylated Anti-CCR4 Monoclonal Antibody (KW-0761) for Relapsed Adult T-Cell Leukemia-Lymphoma: A Multicenter Phase II Study

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Submitted June 3, 2011; accepted December 5, 2011; published online ahead of print at www.jco.org on February 6, 2012.

Supported by Kyowa Hakko Kirin (Tokyo, Japan).

Presented in part at the 52nd Annual Meeting of the American Society of Hematology, December 4-7, 2010, Orlando, FL.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

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0732-183X/12/3099-1/\$20.00

DOI: 10.1200/JCO.2011.37.3472

ABSTRACT

Purpose

Adult T-cell leukemia-lymphoma (ATL) is usually resistant to conventional chemotherapies, and there are few other treatment options. Because CC chemokine receptor 4 (CCR4) is expressed on tumor cells from most patients with ATL, KW-0761, a humanized anti-CCR4 monoclonal antibody, which markedly enhances antibody-dependent cellular cytotoxicity, was evaluated in the treatment of patients with relapsed ATL.

Patients and Methods

A multicenter phase II study of KW-0761 for patients with relapsed, aggressive CCR4-positive ATL was conducted to evaluate efficacy, pharmacokinetic profile, and safety. The primary end point was overall response rate, and secondary end points included progression-free and overall survival from the first dose of KW-0761. Patients received intravenous infusions of KW-0761 once per week for 8 weeks at a dose of 1.0 mg/kg.

Results

Of 28 patients enrolled onto the study, 27 received at least one infusion of KW-0761. Objective responses were noted in 13 of 26 evaluable patients, including eight complete responses, with an overall response rate of 50% (95% CI, 30% to 70%). Median progression-free and overall survival were 5.2 and 13.7 months, respectively. The mean half-life period after the eighth infusion was 422 ± 147 hours (\pm standard deviation). The most common adverse events were infusion reactions (89%) and skin rashes (63%), which were manageable and reversible in all cases.

Conclusion

KW-0761 demonstrated clinically meaningful antitumor activity in patients with relapsed ATL, with an acceptable toxicity profile. Further investigation of KW-0761 for treatment of ATL and other T-cell neoplasms is warranted.

J Clin Oncol 30. © 2012 by American Society of Clinical Oncology

INTRODUCTION

Adult T-cell leukemia-lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by human T-cell lymphotropic virus type I. The disease is resistant to conventional chemotherapeutic agents, and there currently exist limited treatment options; thus, it has a poor prognosis.¹⁻⁴ A recent phase III trial for previously untreated patients with aggressive ATL (acute, lymphoma, or unfavorable chronic type) age 33 to 69 years demonstrated that a dose-intensified multidrug regimen, VCAP-AMP-VECP (vincristine, cyclophosphamide, doxorubicin, and prednisone; doxorubicin, ranimustine, and prednisone; and vindesine, eto-

poside, carboplatin, and prednisone), resulted in median progression-free (PFS) and overall survival (OS) of 7.0 and 12.7 months, respectively.⁵ This remains unsatisfactory compared with responses in other hematologic malignancies. Allogeneic hematopoietic stem-cell transplantation has evolved into a potential approach to treating patients with ATL over the last decade. However, only a small fraction of patients with ATL have the opportunity to benefit from transplantation, such as those who are younger, have achieved sufficient disease control, and have an appropriate stem-cell source.^{6,7} Therefore, the development of alternative treatment strategies for patients with ATL is an urgent issue.

Because CC chemokine receptor 4 (CCR4) is expressed on tumor cells from most patients with ATL,^{8,9} we postulated that it might represent a novel molecular target for immunotherapy. Accordingly, KW-0761, a next-generation humanized anti-CCR4 immunoglobulin G1 (IgG1) monoclonal antibody (mAb) with a defucosylated Fc region, which markedly enhances antibody-dependent cellular cytotoxicity (ADCC), was developed.^{10,11} We demonstrated that robust ADCC by the defucosylated anti-CCR4 mAb against primary tumor cells from patients with ATL mediated by autologous effector cells was triggered both in vitro and in a humanized mouse model in vivo.¹¹⁻¹³ These promising preclinical results prompted us to conduct a phase I clinical trial of KW-0761 for patients with relapsed CCR4-positive peripheral T-cell lymphoma (PTCL), including ATL. This study demonstrated good tolerability, predictable pharmacokinetics, and preliminary evidence of potent antitumor activity and resulted in a recommended dose of 1.0 mg/kg for subsequent clinical trials.¹⁴ Herein, we report the results of a multicenter phase II study designed to assess the efficacy, pharmacokinetic profile, and safety of KW-0761 monotherapy in patients with relapsed CCR4-positive aggressive ATL.

PATIENTS AND METHODS

Patients

Patients 20 years of age or older with CCR4-positive aggressive ATL (acute, lymphoma, or unfavorable chronic type)¹⁴ who had relapsed after at least one prior chemotherapy regimen were eligible. The unfavorable chronic type of ATL was defined by the presence of at least one of the following three factors: low serum albumin, high lactate dehydrogenase, or high blood urea nitrogen concentration.⁵ CCR4 expression was determined by immunohistochemistry or flow cytometry using a mouse anti-CCR4 mAb (KM2160)^{8,14} and confirmed by a central review committee. All patients were required to have an Eastern Cooperative Oncology Group performance status of 0 to 2. Eligibility criteria also included the following laboratory values: absolute neutrophil count $\geq 1500/\mu\text{L}$, platelet count $\geq 50,000/\mu\text{L}$, hemoglobin ≥ 8.0 g/dL, AST $\leq 2.5 \times$ the upper limit of the normal range (UNL), ALT [Iteuq] $2.5 \times$ UNL, total bilirubin $\leq 1.5 \times$ UNL, serum creatinine $\leq 1.5 \times$ UNL, corrected serum calcium ≤ 11.0 mg/dL, and arterial partial oxygen pressure ≥ 65 mmHg or arterial blood oxygen saturation $\geq 93\%$. Patients were excluded if they had an active infection, a history of organ transplantation, active concurrent cancers, CNS involvement, a bulky mass requiring emergent radiotherapy, or seropositivity for hepatitis B virus antigen, hepatitis C virus antibody, or HIV antibody.

Study Design

This study was a multicenter, single-arm, phase II trial. Objectives of the study were to evaluate the efficacy, pharmacokinetic profile, and safety of KW-0761 monotherapy. Patients received intravenous infusions of KW-0761 once per week for 8 weeks at a dose of 1.0 mg/kg.¹⁴ Oral antihistamine and acetaminophen were administered before each KW-0761 infusion to prevent infusion reactions. The primary end point was overall response rate (ORR), and secondary end points included the best response by disease site, PFS, and OS. Objective responses were assessed after the fourth and eighth infusions of KW-0761 by an independent efficacy assessment committee according to the modified response criteria for ATL.⁴ It was estimated that 25 patients would be required to detect a lower limit of the 95% CI exceeding the 5% threshold of ORR based on the assumptions that the minimum required ORR for a new drug for relapsed, aggressive ATL is 5%,¹⁵ with an expected ORR for KW-0761 of 30%¹⁴ with 90% power. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for AEs, version 3.0. The presence of human anti-KW-0761 antibodies in the patients' plasma was examined using enzyme-linked immunosorbent assay. Blood samples col-

lected at times strictly in accordance with the protocol were employed for the pharmacokinetic analysis. Samples were obtained from patients who had received at least one dose of KW-0761 up to all eight doses. When any event resulted in an alteration in the infusion protocol, only those samples taken before the alteration were used for the analysis. The following parameters were calculated for plasma KW-0761: maximum drug concentration and trough drug concentration of each KW-0761 administration, area under the blood concentration time curve from 0 to 7 days after the first and eighth doses, and half-life period ($t_{1/2}$) after the eighth dose. As an additional research parameter, we investigated blood T-cell subset distribution during and after KW-0761 treatment and compared these values with those of 10 healthy donors as controls (five men, five women; median age, 45 years; range, 41 to 57 years).

Statistical Analysis

Survival estimates were calculated using the Kaplan-Meier method. PFS was defined as the time from the first dose of KW-0761 to progression, relapse, or death resulting from any cause, whichever occurred first. OS was measured from the day of the first dose to death resulting from any cause. Regarding T-cell subset analysis, differences between the patients' values before KW-0761 treatment and those of the controls were examined using the Mann-Whitney U-test. Differences between KW-0761 pretreatment values and those at each time point after KW-0761 treatment were examined using the Wilcoxon signed-rank test. All analyses were performed with SPSS Statistics 17.0 (SPSS, Chicago, IL). In this study, $P < .05$ was considered significant.

Study Oversight

The study was sponsored by Kyowa Hakko Kirin Company (Tokyo, Japan). The academic investigators and the sponsor were jointly responsible for the study design. The protocol was approved by the institutional review board at each participating site, and all patients and controls provided written informed consent before enrollment according to the Declaration of Helsinki.

RESULTS

Patients

Of the 28 patients enrolled onto the study, 27 (12 men, 15 women) received at least one infusion of KW-0761. One patient was withdrawn for aggravation of the general condition before the administration of KW-0761. Demographics and clinical characteristics of the 27 patients are summarized in Table 1. Median age was 64 years (range, 49 to 83). The disease subtypes included 14 acute, six lymphoma, and seven unfavorable chronic type ATL. Of these 27 patients, 14 (52%) completed the schedule of eight planned infusions. Of the remaining 13 patients, 11 (41%) discontinued treatment because of disease progression, one (4%) because of skin rash, and another (4%) because of concurrent colon cancer, for which this patient was excluded from the efficacy evaluation.

Efficacy of KW-0761

Of 26 patients evaluable for efficacy, objective responses were noted in 13 patients (ORR, 50%; 95% CI, 30% to 70%), including eight complete responses (CRs). Responses according to disease site were 100% (13 of 13; all CRs) for blood, 63% (five of eight) for skin, and 25% (three of 12) for nodal and extranodal lesions. Responses according to disease subtype were 43% (six of 14) for acute, 33% (two of six) for lymphoma, and 83% (five of six) for unfavorable chronic type ATL. Responses according to number of prior chemotherapy regimens were 48% (10 of 21) in those who had one prior regimen and 60% (three of five) for those who had two or three prior regimens. Median PFS and OS were 5.2 and 13.7 months, respectively (Figs 1A, 1B).

Table 1. Patient Demographics and Clinical Characteristics (n = 27)*

Characteristic	No.	%
Age, years		
Median	64	
Range	49-83	
≥ 65	13	48
Sex		
Male	12	44
Female	15	56
ECOG performance status†		
0	15	56
1	7	26
2	5	19
Disease subtype		
Acute	14	52
Lymphoma	6	22
Chronic	7	26
Prior chemotherapy regimens, No.		
1	22	82
2	3	11
3	2	7

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
 *Of 28 patients enrolled, 27 received at least one infusion of KW-0761.
 †ECOG performance status scores range from 0 (normal activity) to 5 (death), with higher scores indicating more severe disability.

Pharmacokinetics

KW-0761 plasma concentrations over eight infusions, once per week, at 1.0 mg/kg are shown in Figure 2. Mean maximum drug concentration and trough drug concentration (\pm standard deviation) of the eighth infusion were $42.9 \pm 14.2 \mu\text{g/mL}$ and $33.6 \pm 10.6 \mu\text{g/mL}$, respectively. Mean area under the blood concentration time curve from 0 to 7 days after the eighth infusion was $6,297 \pm 1,812 \mu\text{g} \times \text{hours/mL}$. The mean $t_{1/2}$ after the eighth infusion was 422 ± 147 hours.

AEs

Table 2 lists AEs that occurred in at least 15% of patients or at grades 3 to 4, which were determined as possibly, probably, or definitely KW-0761 related. The most common nonhematologic AE was an infusion reaction (89%). In addition, 80% or more of the following recorded AEs occurred along with an infusion reaction: fever, chills, tachycardia, hypertension, nausea, and hypoxemia (Table 2). These events occurred primarily at the first infusion, becoming less frequent with subsequent treatments. The infusion reactions and component events were transient, and all patients recovered, although some needed systemic steroids. Skin rashes were observed as another frequent nonhematologic AE (63%), mostly occurring after the fourth or subsequent infusions. Of the 14 patients who developed grade 2 or higher skin rashes, objective responses were noted in 13 patients (93%), including eight CRs. On the other hand, of the 12 patients who developed no or grade 1 skin rashes, no objective responses were observed. A typical clinical course of the rash is depicted in Appendix Figures A1A and A1B (online only). The skin rash observed in this patient appeared after the seventh infusion, and the corresponding skin biopsy revealed mild perivascular CD8-positive cells dominating an inflammatory reaction, with an absence of ATL cells. The skin rash recovered on application of topical steroid. Of the 17 patients who

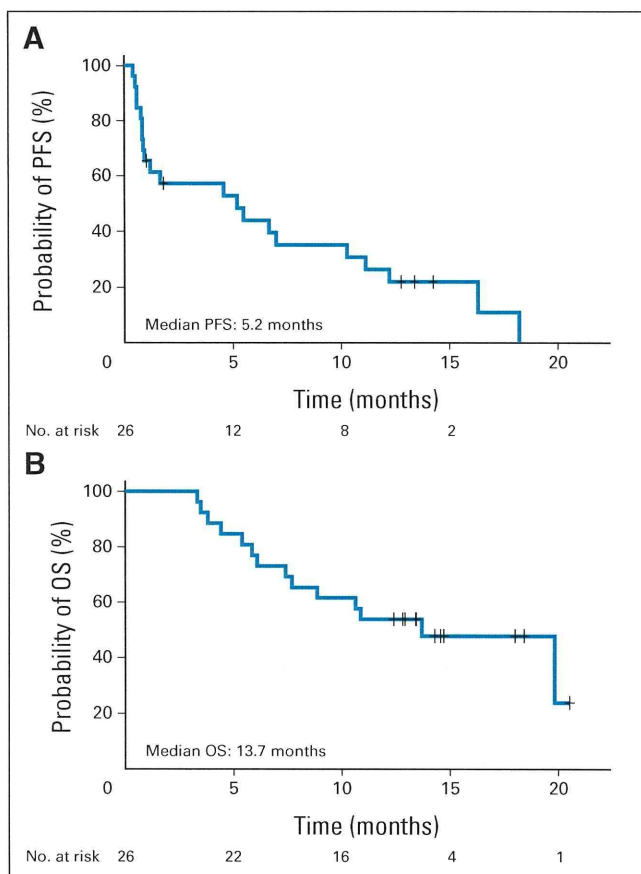


Fig 1. Kaplan-Meier curves of estimated (A) progression-free survival (PFS; median, 5.2 months) and (B) overall survival (OS; median, 13.7 months).

developed skin rashes, one developed Stevens-Johnson syndrome, which was determined as possibly KW-0761 related, although that patient also received trimethoprim/sulfamethoxazole, fluconazole, and acyclovir for prevention of infection according to the protocol. This patient stopped those preventive agents and was treated with

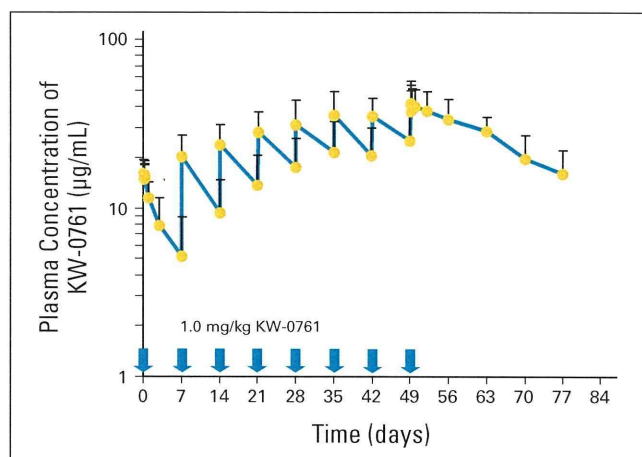


Fig 2. Pharmacokinetics of KW-0761. Mean KW-0761 plasma concentrations during and after 1.0 mg/kg KW-0761 infusions once per week for 8 weeks. Bar indicates upper limit of standard deviation.

Table 2. Adverse Events (n = 27)*

Adverse Event	Grade (No. of patients)				All Grades		Infusion Reaction Related (No. of patients)	
	1	2	3	4	No. of Patients	%	All Grades	≥ Grade 2
	Nonhematologic							
Infusion reaction	1	22	1	0	24	89		
Fever	20	2	0	0	22	82	18	2
Rash	3	9	5	0	17	63	1	0
Chills	14	2	0	0	16	59	16	2
ALT	5	4	2	0	11	41		
AST	3	5	2	0	10	37		
Tachycardia	9	0	0	0	9	33	9	0
Hypertension	6	2	0	0	8	30	8	1
Albuminemia	7	1	0	0	8	30		
ALP	4	2	0	0	6	22		
Weight gain	5	0	0	0	5	19		
Nausea	4	1	0	0	5	19	5	1
Hyponatremia	5	0	0	0	5	19		
Hypoxemia	0	2	3	0	5	19	4	4
Hypotension	2	2	0	0	4	15	3	1
Pruritus	0	3	1	0	4	15		
γ-GTP	0	1	3	0	4	15		
Hypophosphatemia	0	4	0	0	4	15		
Hyperuricemia	4	0	0	0	4	15		
Hypercalcemia	1	1	0	1	3	11		
Hypokalemia	1	0	2	0	3	11		
Erythema multiforme†	0	0	1	0	1	4		
Hyperglycemia	0	0	1	0	1	4		
Tumor lysis syndrome	0	0	1	0	1	4		
Metabolic/laboratory, other‡	4	7	3	0	14	52		
Hematologic								
Lymphopenia§	0	6	9	11	26	96		
Leukocytopenia	3	7	8	0	18	67		
Thrombocytopenia	7	2	3	2	14	52		
Neutropenia	5	4	5	0	14	52		
Hemoglobin	4	3	1	0	8	30		

Abbreviations: ALP, alkaline phosphatase; BUN, blood urea nitrogen; CRP, C-reactive protein; GTP, glutamyl transpeptidase.
*Of 28 patients enrolled, 27 received at least one infusion of KW-0761. Listed are adverse events determined as possibly, probably, or definitely KW-0761 related that occurred in at least 15% of patients or were of grade 3 to 4 severity.
†One patient diagnosed as having Stevens-Johnson syndrome.
‡Other metabolic and laboratory test abnormalities included hypoproteinaemia, BUN elevation, CRP, glycosuria, hypochloremia, and hyperammonemia.
§Lymphopenia included decrease of abnormal lymphocytes.

systemic steroids, but improvement required the passage of 4 months. Lymphopenia, including a decrease in the number of ATL cells, occurred in 26 (96%) of the 27 patients. Grades 3 to 4 thrombocytopenia was observed in five patients (19%) but was not associated with bleeding, and grade 3 neutropenia also occurred in five patients but did not lead to a febrile episode. The latter two hematologic AEs improved in all patients. None of the patients developed detectable anti-KW-0761 antibody.

T-Cell Subset Analysis

The numbers of circulating blood CD4+ CCR4+, CD4+ CD25+ FOXP3+, CD4+ CCR4-, and CD4- CD8+ cells from

KW-0761-treated patients and those from the 10 controls are presented as box and whisker plots in each graph (Appendix Figs A2A to A2D, online only). The numbers of CD4+ CCR4+ and CD4+ CD25+ FOXP3+ cells in patients with ATL before treatment were significantly higher than those in the controls but were significantly reduced after the first KW-0761 infusion. The reduction lasted for at least 4 months after the eighth infusion (Appendix Figs A2A, A2B; online only). The numbers of CD4+ CCR4-, and CD4- CD8+ cells in patients with untreated ATL were significantly lower than those in the controls. KW-0761 treatment led to a transient further reduction of those cells; however, recovery took place by the fifth infusion (Appendix Figs A2C, A2D; online only).

DISCUSSION

In the present multicenter phase II study, KW-0761 monotherapy demonstrated significant responses in patients with relapsed ATL with an acceptable toxicity profile. An ORR of 50% and median PFS and OS values of 5.2 and 13.7 months, respectively, were observed. Because the lower limit for an ORR with a 95% CI was 30%, this study met the primary end point. These results suggest an improvement over what has been achieved with other agents in relapsed ATL.¹⁵ Cladribine was associated with an ORR of 7% (one of 15 patients),¹⁶ and irinotecan hydrochloride treatment had an ORR of 38% (five of 13 patients) with a median duration of response of 31 days.¹⁷ Antiviral therapy consisting of a combination of zidovudine and interferon, which has been proposed as a standard first-line therapy in leukemic subtypes of ATL,¹⁸ was initially reported as having a median OS of 3.0 months in 19 patients with acute or lymphoma type ATL.¹⁹ In addition, White et al²⁰ reported three objective responses lasting longer than 1 month with zidovudine plus interferon in 18 patients with ATL, of whom 15 had received prior therapy. Those observations collectively suggest that KW-0761 may offer an advantage over or provide an additional therapeutic option to the currently available therapy for relapsed ATL, although there were no direct comparisons.

On examining the results of ATL treatment according to disease site, disease in blood seemed to be more sensitive to KW-0761 than at other disease sites. Currently, we are unable to fully explain this difference; however, factors such as the KW-0761 delivery or the amount of ADCC effector cells such as natural killer (NK) cells and monocytes/macrophages in each disease site may be important.

Pharmacokinetic analyses demonstrated that the $t_{1/2}$ after the eighth administration of KW-0761 was nearly the same as that of circulating endogenous human IgG1, indicating good stability of this antibody in vivo. In addition, no anti-KW-0761 antibody was detected, suggesting that the antigenicity of this novel defucosylated mAb is not likely to be a problem clinically, consistent with findings in our preceding phase I study.¹⁴

The infusion reactions observed in the present study may also provide novel insights into problems associated with antibody therapy. It is generally recognized that complement plays a major role in infusion reactions,²¹ but this mechanism cannot apply to KW-0761, because the agent is unable to mediate complement-dependent cytotoxicity.¹¹ Therefore, the infusion reactions observed here may have a different mechanism compared with those of other antibody therapies, such as rituximab. KW-0761 has a defucosylated Fc region, which markedly enhances ADCC because of increased binding affinity to the

Fc γ receptor on effector cells. Defucosylated IgG1 is a more potent activator of NK cells than nondefucosylated IgG1 during ADCC.²² We surmise that the infusion reactions to KW-0761 were mainly induced by cytokines and related cytotoxic molecules released from highly activated NK cells.

The present study demonstrated that compared with the levels in the controls, KW-0761 led to a significant and lasting decrease in the number of CD4+ CCR4+ but not CD4+ CCR4- or CD4- CD8+ cells in patients with ATL. Consistent with the fact that CCR4 is expressed not only on T-helper type 2 cells but also on regulatory T (Treg) cells,²³⁻²⁶ KW-0761 treatment also resulted in a significant and lasting decrease in CD4+ CD25+ FOXP3+ cells, including both ATL cells and endogenous non-ATL Treg cells.²⁷⁻²⁹ Reduction or suppression of Treg cells is expected to be a potentially promising strategy for boosting antitumor immunity in patients with cancer, as observed in studies with ipilimumab,³⁰⁻³³ although ipilimumab and KW-0761 have different targets; the former suppresses Treg cell function, and the latter decreases their number. Hence, KW-0761 could also lead to activation of antitumor immunity, which might also contribute to its potent anti-ATL response. Because ipilimumab causes immune-related AEs such as diarrhea and colitis, we were especially vigilant in monitoring for this type of AE. Because CCR4 contributes to lymphocyte skin-specific homing,³⁴ it was not surprising that skin rashes, which could be an immune-related AE, were frequently observed in the present KW-0761 study. Skin rashes, including the most severe case of Stevens-Johnson syndrome, the causal association of which with concomitant medications other than KW-0761 could not be excluded, proved to be manageable, and patients improved in all cases, although some needed systemic or topical steroid treatment. The observed better responses to KW-0761 in patients with grade 2 or higher skin rashes were highly impressive. However, the underlying mechanisms for this finding are not clear; thus, further detailed investigation is warranted. All of the 14 patients who developed grade 2 or higher skin rashes received five or more KW-0761 infusions according to the protocol, whereas only three of the 12 patients who developed no or grade 1 skin rashes received five or more KW-0761 infusions. This suggests the possibility that skin rashes were associated with the number of KW-0761 infusions. The Cochran-Mantel-Haenszel test stratified by the number of KW-0761 infusions (\leq four ν \geq five) indicated a significant association between clinical response and skin rashes (no or grade 1 ν grades 2 to 4; $P = .009$). However, the sample size is insufficient to draw such a conclusion.

Following on a phase III study (JCOG9801 [Japan Clinical Oncology Group 9801]) for untreated aggressive ATL,⁵ the present promising results for KW-0761 monotherapy prompted us to conduct a subsequent randomized trial of VCAP-AMP-VECP chemotherapy with or without KW-0761 for previously untreated ATL (Clinicaltrials.gov: NCT01173887). CCR4 is also expressed on tumor cells from a subgroup of PTCL other than ATL, which also has an unfavorable prognosis.^{2,35,36} Thus, we are currently conducting a phase II study of KW-0761 monotherapy for relapsed CCR4-positive PTCL (Clinicaltrials.gov:NCT01192984). In addition, Duvic et al³⁷ recently reported a phase I/II study of KW-0761 for refractory cutaneous T-cell lymphoma. They found that KW-0761 was well tolerated at doses of 0.1 to 1.0 mg/kg, and a promising ORR of 39% (15 of 38 patients) was achieved, although expression of CCR4 on lymphoma cells was not included as one of the eligibility criteria (Clinicaltrials.gov: NCT00888927). Furthermore, clinical trials of KW-0761 for

patients with Hodgkin's lymphoma may be worth trying, because it has been reported that Hodgkin's lymphoma tumor cells produce CCR4 ligand molecules, and migratory CCR4-expressing Treg cells prevent a host immune attack on tumor cells, thereby creating an immunologically favorable environment for the tumor cells.³⁸

Although this phase II study offers a novel promising treatment option (KW-0761) for patients with relapsed ATL, some limitations should be discussed. First, the present phase II study was relatively small, with consequent limitations on drawing definitive conclusions about the efficacy and safety profile of KW-0761. Second, patients received different prior systemic chemotherapy regimens, which could affect the results of the present study. Finally, the enrolled patients all had aggressive ATL, but three clinical subtypes (acute, lymphoma, and unfavorable chronic type) were included. Although there may be no significant differences in susceptibility to conventional chemotherapies between these subtypes, the heterogeneity of the enrolled patients might have affected the results.

In conclusion, this multicenter phase II study demonstrated that KW-0761 monotherapy showed clinically meaningful antitumor activity in patients with relapsed ATL, with an acceptable toxicity profile. Further investigation of KW-0761 for ATL and other T-cell neoplasms is warranted on the basis of the present results.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Shiro Akinaga, Kyowa Hakko Kirin (C) **Consultant or Advisory Role:** Michinori Ogura, Kyowa Hakko Kirin (C) **Stock Ownership:** None **Honoraria:** Takashi Ishida, Kyowa Hakko Kirin **Research Funding:** Takashi Ishida, Kyowa Hakko Kirin **Expert Testimony:** None **Other Remuneration:** None

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Acknowledgment

We thank all nurses and clinical research coordinators who were involved in this study for their patient care and schedule management. We are also grateful to Takeshi Takahashi, PhD, Masatoshi Sugiura, PhD, and Nobuhito Matsunaga, PhD (Kyowa Hakko Kirin) for their help in preparing this article.

Appendix

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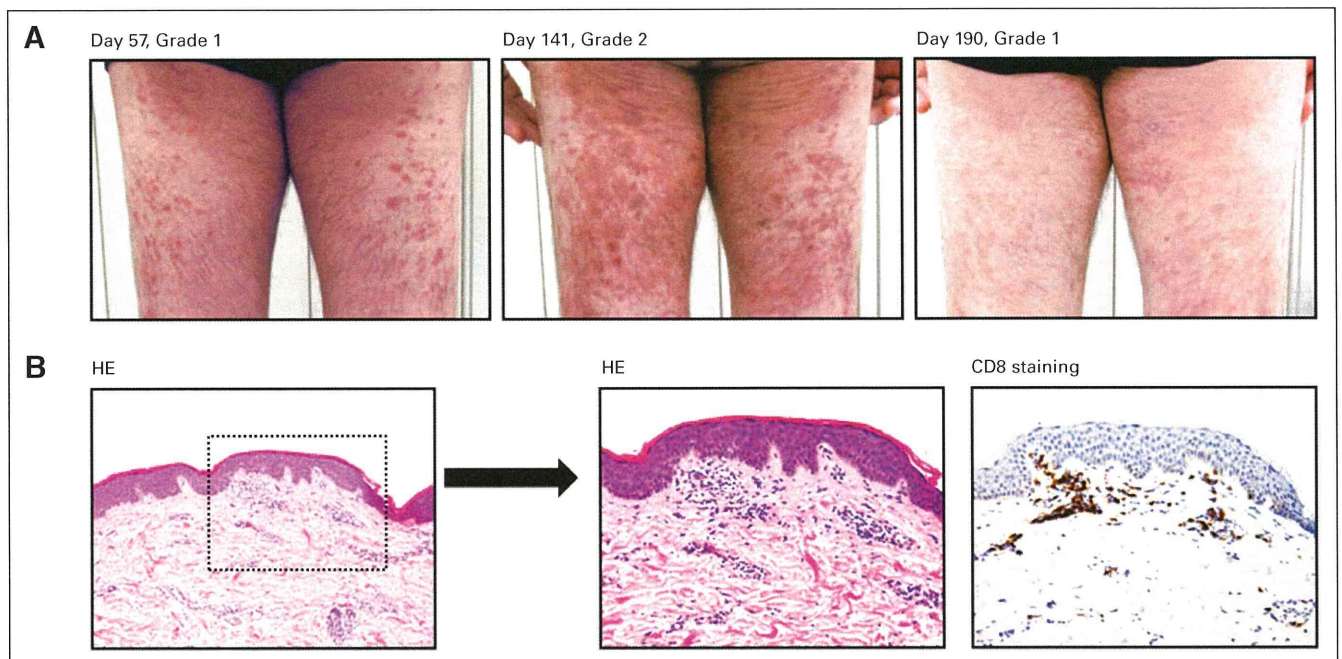


Fig A1. Example of typical clinical course of skin rash after KW-0761 treatment. (A) Macroscopic observations of patient whose skin eruptions appeared after the seventh infusion. (B) Corresponding skin biopsy at day 57 showing mild spongiosis and mild perivascular inflammation with dominant CD8-positive cells but absence of adult T-cell leukemia-lymphoma cells. The skin rash recovered on treatment with topical steroid. HE, hematoxylin and eosin.

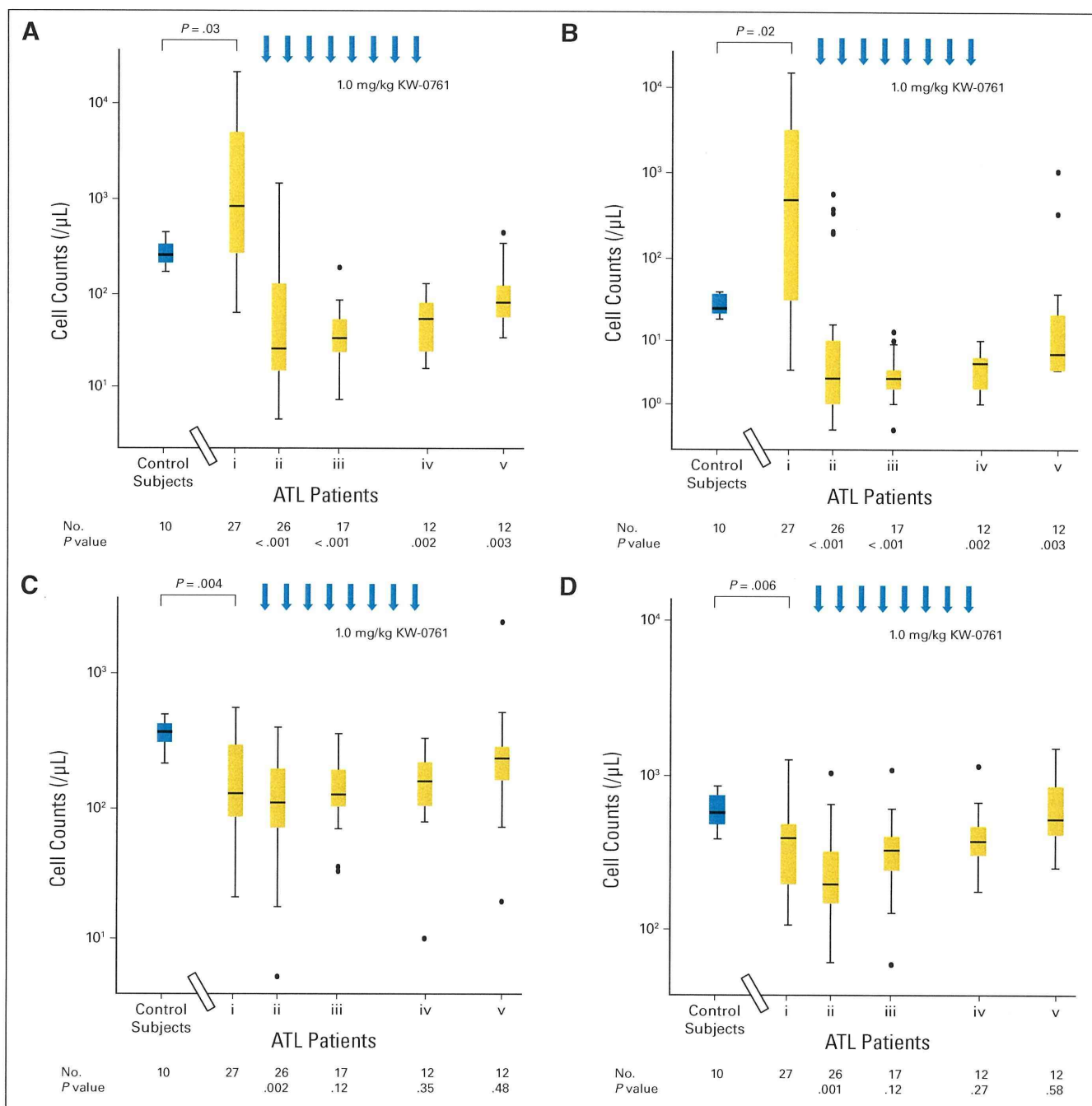


Fig A2. T-cell subset analysis. Blood samples collected at times in accordance with the protocol were employed. When any event resulted in a discontinuation of the infusion protocol, only those samples taken before this time were used for the T-cell subset analysis. (A) Numbers of CD4+ CCR4+ cells from KW-0761-treated patients with adult T-cell leukemia-lymphoma (ATL) in blood samples taken (i) just before the first KW-0761 infusion, (ii) just before the second infusion, (iii) just before the fifth infusion, (iv) 1 week after the eighth infusion, and (v) 4 months after the eighth infusion and those from 10 controls are shown as box and whisker plots indicating minimum, lower quartile, median, upper quartile, and maximum values. Differences between the patients' values before KW-0761 treatment and those of the controls are indicated as a P value (Mann-Whitney U-test) in the graph. The number of samples used for analysis at each point is indicated below the graph. The differences between before and each point after KW-0761 treatment [(i) v (ii), (iii), (iv), or (v)] are indicated as a P value (Wilcoxon signed-rank test) below the graph. CCR4 was detected by a monoclonal antibody (clone 1G1), where its binding to CCR4 was not affected by the presence of KW-0761. Numbers of (B) CD4+ CD25+ FOXP3+; (C) CD4+ CCR4-; and (D) CD4- CD8+ cells are presented in the same manner.

