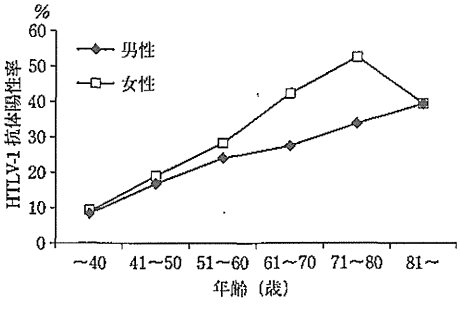

図 1 HTLV-1 高浸淫地域における年齢男女別抗体陽性率

偶者間の新規感染のため一定の年齢以降の感染率が高くなるというものである。

1. 母子感染

HTLV-1 の母子感染については，疫学的，実験的な結果から主な経路は母乳を介したものであることが判明した。ATL は母子間感染により乳児期に感染したキャリアから発症すると考えられているため，母乳を介した母子感染を遮断し，ATL を主とする将来の HTLV-1 関連疾患の発症を予防しようとする試みが行われてきた。すなわち抗体が陽性と判明した妊婦のなかで，母乳を与えないことに同意したキャリア母親には出産後母乳分泌抑制剤が投与され児の人工栄養が行われた。この結果，キャリア母親から出生した児の感染率は，6ヵ月以上の母乳栄養で20.5%であるのに対して，人工栄養では2.4%と有意に低下することが示された[4)5)]。

2. 性行為に伴う感染

HTLV-1 の配偶者間感染については，配偶者間での HTLV-1 陽性の一致率が高く，とくに年齢の高い夫婦ほど配偶者両者とも HTLV-1 が陽性となる率が高いことが判明している。また HTLV-1 キャリアの陰性の配偶者（たとえば夫が陽性で妻陰性）を長期にフォローすると，陰性であった配偶者の抗体陽転化が一定の割合で生じる（妻が陽性になる）[6)7)]。さらにこのようなケースにおける両者のウイルスの遺伝子配列を比較すると同一であることから，配偶者間感染が証明されている[8)]。感染は女性から男性においても成立しうるが，男性から女性において頻度が高い。このことは疫学的に，HTLV-1 抗体陽性率が中年以降において女性のほうが男性よりも高いという事実と合致している。配偶者間感染が性行為に伴って起こることについての直接的な証拠はない。しかし，HTLV-1 陽性男性の精液に感染性が証明されていることや[9)]，このウイルスの感染には細胞間接触が必要なことを合わせて考えると，性行為に伴っての感染が最も考えやすい。成人における配偶者感染によって ATL が発症したという明らかな報告はないが，完全に否定されたわけではない。また成人における感染においても HAM や HU 等の HTLV-1 関連疾患の発症は認められている。母子感染においては母乳哺育遮断という感染防止の方法論が確立されているが，性行為感染においてはこのような感染防止法として確立したものはないのが現状である。

V. HTLV-1 感染症への対策

前述したように，母子感染については母乳を介した感染の遮断により感染率を大幅に減少させることに成功している。しかしながら，母乳感染遮断の介入を行った場合にも2～3%程度の児に感染が成立することが判明しており，母乳遮断のみによる母子感染防止には課題が残る。今後の可能性のひとつとしてワクチンによる感染予防法の開発がある。これが実現すれば，現在全く対策のない配偶者間感染や医療機関における針刺し事故などの対策においても福音となると思われる。HTLV-1 感染に対するワクチン開発も基礎研究レベルでは開始されており，今後さらなる発展が期待される。また新たな感染を予防することに加えて，現在国内に108万人いると考えられている HTLV-1 キャリアについては HTLV-1 関連疾患発症予防法や治療法の開発が必要である。これまでの研究から，感染細胞数の多いキャリアから HTLV-1 関連疾患が発症することが判明しており[10)]，今後キャリアのウイルス量を減らす方法が開発されれば，HTLV-1 関連疾患の発症防止に大きく貢献できる可能性がある。

2010年に国において「HTLV-1 総合対策」が取りまとめられ，HTLV-1 感染予防，相談支援，医療体制の整備，啓発・情報提供，研究開発の推進が重点施策として挙げられており，今後の発展が期待されている。

ま と め

本ウイルスが発見されて30年が経過し，ウイ

ルスに関する基礎的な研究の発展はめざましいものがある。また，母子感染予防法の開発は大きな貢献といえる。しかし本邦においてはいまだ100万人以上のキャリアが存在しており，キャリアからウイルスを駆逐する方法や関連疾患を予防する方法は発見されていない。また今回の特集の主題「ストップザ性感染症」の観点からは男女間のHTLV-1の新規感染予防法については手つかずの状態であり，残された課題は大きい。我が国はいわゆる先進工業国の中では唯一のHTLV-1高浸淫国であり，これらの課題解決に今後も取り組んでいくことは世界的に対する貢献としてもぜひ必要であると思われる。

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LYMPHOMAS (J ARMITAGE AND P MCLAUGHLIN, SECTION EDITORS)

Targeting Chemokine Receptor CCR4 in Adult T-Cell Leukemia-Lymphoma and Other T-Cell Lymphomas

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Abstract Peripheral T-cell lymphoma (PTCL) is a group of lymphoid malignancy that remains difficult to treat, as most PTCL becomes refractory or relapses, and thus there is an unmet medical need for novel treatment modalities. CC chemokine receptor 4 (CCR4) is expressed in various types of PTCL including adult T-cell leukemia-lymphoma (ATL), which has the worst prognosis among them. A phase II study of a defucosylated, humanized anti-CCR4 monoclonal antibody, mogamulizumab (KW-0761), yielded an overall response rate of 50 % (13/26) and a median progression-free survival of 5.2 months in relapsed patients with CCR4-positive ATL who had been previously treated with chemotherapy. Mogamulizumab also showed potential efficacy for cutaneous T-cell lymphoma in a US phase I/II study. Further preclinical and clinical investigations are needed to examine whether concomitant use of this novel agent with other agents with different mechanisms of action would be more effective for ATL and other PTCLs.

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Keywords Chemokine receptor · CCR4 · Adult T-cell leukemia-lymphoma · ATL · Peripheral T-cell lymphoma · PTCL · Monoclonal antibody · Mogamulizumab · KW-0761

Introduction

Peripheral T-cell lymphoma (PTCL) represents a small, heterogeneous group of non-Hodgkin lymphoma (NHL) which is derived from more mature T-cells and natural killer (NK) cells, and accounts for approximately 10 % of NHL cases in Western countries [1, 2] and for approximately 20 %–25 % of those in Japan [3, 4]. PTCL, a collective entity of nearly 20 different subtypes defined according to morphology, immunophenotype, genotype, and clinical features [5], can be largely classified into the following two groups according to clinical features including the sites of lesions: (1) cutaneous T-cell lymphoma (CTCL), which is the general term for diseases that initially or mainly occur in the skin, and (2) PTCL other than CTCL. Treatment strategies have been separately developed for these two groups [6].

Treatment options are substantially different for B-cell and T-cell lymphomas. Rituximab, an anti-CD20 monoclonal antibody, was developed for the treatment of B-cell lymphomas. The introduction of this agent into clinical practice has greatly improved the prognosis of patients with B-cell lymphoma [7]. Recently, bendamustine, which has little cross resistance with other chemotherapeutic agents presumably associated with its unique chemical structure of an alkylating agent and a nucleoside analog, has been developed as effective treatment of relapsed or refractory B-cell lymphoma, considering its lack of cross resistance with other chemotherapeutic agents [8]. However, PTCL remains extremely difficult to treat, because most PTCL subtypes become refractory to even aggressive chemotherapy

regimens or relapse, with the exception of anaplastic lymphoma kinase-positive anaplastic large cell lymphoma (ALK^+ ALCL), which responds well to the cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) regimen [9]. Among the various entities of PTCLs, adult T-cell leukemia-lymphoma (ATL) harbors the worst prognosis [10]. Here, we will discuss novel agents that have been developed for the treatment of ATL and other PTCLs, mainly focusing on mogamulizumab/KW-0761, which is a humanized monoclonal antibody targeting CC chemokine receptor 4 (CCR4) that has been actively developed for clinical use in Japan and the United States.

PTCL and Novel Agents

PTCL-not otherwise specified (PTCL-NOS) and angioimmunoblastic T-cell lymphoma (AITL), which are the most common subtypes of PTCL (PTCL-NOS, 26 %; AITL, 19 %), show a poor prognosis with 5-year overall survival (OS) and failure-free survival (FFS) of about 30 % and 20 %, respectively [10]. Several new agents have recently been developed for the treatment of PTCL, mainly in patients with relapsed or refractory disease. Such agents have various mechanisms of action, including an immunomodulator (lenalidomide), a proteasome inhibitor (bortezomib), histone deacetylase inhibitors (vorinostat, romidepsin, panobinostat), antifolate (pralatrexate), and biologics including antibodies and antibody-toxin/drug conjugates (alemtuzumab, siplizumab, denileukin diftitox, and brentuximab vedotin) as well as nucleoside analogs such as fludarabine, gemcitabine, nelarabine, and forodesine [11]. Of these agents, pralatrexate and romidepsin have been recently approved by the U.S. Food and Drug Administration (FDA) and are now being used in the U.S. for the treatment of relapsed or refractory PTCL. In 2011, brentuximab vedotin (formerly known as SGN-35) was also approved for the treatment of relapsed or refractory ALCL and Hodgkin lymphoma.

ATL has the worst prognosis among PTCL, with 5-year OS and FFS of 14 % and 12 %, respectively [10]. ATL is a peripheral T-cell malignancy associated with human T-cell lymphotropic virus type I (HTLV-1), and is relatively frequent in southwestern Japan, West Africa, the Caribbean islands, and Brazil, which are HTLV-1 endemic areas [12]. It is estimated that there are about 1.2 million HTLV-1 carriers in Japan, of whom a few percent develop ATL [13], and approximately 700 to 1000 people die of this disease per year [14]. ATL is classified into four disease subtypes (acute, lymphoma, chronic, and smoldering), based on clinical features including leukemic changes, high lactate dehydrogenase, hypercalcemia and organ infiltration, and the median survival time varies according to the disease type: acute type, 6 months; lymphoma type, 10 months; chronic type, 24 months; and smoldering type, 3 years or more [15]. It is recommended that treatment strategies should be selected according to the disease subtype [15]. In Japan, the acute type, lymphoma type, and chronic type with unfavorable prognostic factors have been regarded as aggressive ATL subtypes requiring immediate treatment, and intensive combination chemotherapy or allogeneic hematopoietic stem-cell transplantation are generally recommended therapeutic options [16].

The Japan Clinical Oncology Group-Lymphoma Study Group (JCOG-LSG) has been investigating the efficacy of combination chemotherapy for aggressive lymphomas including ATL or for ATL alone since the early 1980s. At the start of the investigation, CHOP-like regimens were evaluated because ATL was considered to be a type of NHL, but the outcome was poor [17]. Then, the LSG15 regimen consisting of the drugs used in the CHOP regimen plus four other drugs (ranimustine, vindesine, etoposide, and carboplatin) with the prophylactic use of granulocyte colony-stimulating factor (G-CSF) was evaluated. In a phase III trial, JCOG9801, this dose-intensified multiagent chemotherapy regimen was shown to be more effective than CHOP-14 regimen, with a complete response rate of 40 %, 3-year OS of 24 %, and median survival time of 12.7 months [18, 19]. However, since the outcome of this dose-intensified regimen was still inferior to that in other PTCLs and B-cell lymphomas, further improvement is necessary. In Western countries, combination therapy with interferon-α and zidovudine has been widely used for all disease subtypes of ATL. A recently published meta-analysis suggested the effectiveness of this combination therapy for ATL, especially leukemic forms such as acute and chronic types [20].

Several new antibodies are currently under development for the treatment of T-cell lymphoma. They are based on the unique immunophenotypic features of ATL cells, which express mature T-cell antigens such as CD2, CD25 (interleukin [IL]-2 receptor), and CD52. Because of the unique intense expression of CD25 compared to that in other PTCL, monoclonal antibodies targeting the IL-2 receptor (anti-Tac), either radiolabeled or unlabelled (daclizumab), have been tested in patients with relapsed or refractory ATL. However, the clinical efficacy appears to be limited [21]. An anti-CD2 monoclonal antibody (siplizumab) [22], anti-CD52 antibody (alemtuzumab) [23, 24], and anti-transferrin receptor antibody (A24) [25] are also under development, but data are currently limited.

Currently Available Therapeutic Agents for ATL

Pentostatin and sobuzoxane are chemotherapeutic agents that were previously approved for the treatment of ATL in Japan. Pentostatin, a purine nucleoside analog that inhibits adenosine deaminase, has been reported to be effective for

T-cell malignancies, including T-cell prolymphocytic leukemia, CTCL, and PTCL [26]. The clinical efficacy of pentostatin was evaluated in patients with ATL from the 1980s to 1990s, and a phase II study of pentostatin revealed a response rate of 32 % (10 of 31) in patients with relapsed or refractory ATL [27]. Other drugs that are often used in patients with relapsed or refractory ATL are some combination chemotherapy regimens, including EPOCH (etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin) and ESHAP (etoposide, methylprednisolone, high-dose cytarabine and cisplatin); however, there is no apparent evidence of an advantage of these combination chemotherapies over other therapeutic options. In the U.S., pralatrexate and romidepsin have been approved for the treatment of PTCL and can also be used for ATL. The efficacy of these drugs for ATL is not clear because they have been evaluated only in a very limited number of patients (the efficacy of pralatrexate was evaluated in a clinical study in one patient) [28].

CCR4 as a Novel Therapeutic Target

Chemokines act as signaling molecules in the migration and tissue homing of various leukocytes. Among them, thymus and activation-regulated chemokine (TARC) and monocyte-derived chemokine (MDC) induce the selective recruitment of distinct subsets of T-cells through triggering of a chemokine receptor, CCR4. CCR4 is a seven-transmembrane G-protein coupled receptor and selectively expressed on Th2 cells and regulatory T cells [29, 30]. The expression on normal cells such as Th2 cells can be partly regulated by the ligand, especially MDC [31], while the regulation by the ligands on cancer cells are not yet understood. Ishida et al. analyzed 103 patients with ATL, and found that tumor cells from about 90 % of patients showed CCR4 expression [32]. They also found that patients with CCR4-positive ATL were more likely to have skin infiltration and had a worse outcome than those with CCR4-negative ATL, indicating that CCR4 played an important pathogenetic role in ATL [32]. In addition, Yoshie et al. found that the expression of CCR4 was increased in association of HTLV-1 and showed a relationship to Fra-2/Jun D which induces downstream genes such as c-Myb and SOX4, and MDM2 which promotes growth and inhibits apoptosis [33]. CCR4 is also expressed on other types of PTCL (29 % of total cases; PTCL-NOS, 38 %; AITL, 35 %; ALK^- ALCL, 67 %; mycosis fungoides [MF], 41 %) [34]. Jones et al. independently reported that some types of PTCL expressed CCR4, as well [35]. In addition, analysis of 50 patients with PTCL-NOS revealed that CCR4-positive patients had significantly shorter survival than CCR4-negative patients [34]. Nakagawa et al. analyzed 51 patients with PTCL-NOS using the array comparative genomic hybridization technique, and found that patients with PTCL-NOS with genomic aberrations had a significantly higher frequency of CCR4 positivity and a worse outcome than those with PTCL-NOS without genomic aberrations [36]. These findings resemble those observed in patients with ATL. Although the role of CCR4 in the tumorigenesis and progression of PTCL-NOS has not been fully elucidated, CCR4 seems to be a promising target molecule in the treatment of PTCL as well as in ATL.

Clinical Trials of Mogamulizumab

Mogamulizumab/KW-0761 is a humanized monoclonal antibody that recognizes the N-terminal region of human CCR4 [37–39]. It is a therapeutic antibody produced using a novel glycoengineering technology that enhances antibody-dependent cellular cytotoxic (ADCC) activity [40]. Mogamulizumab and its human-mouse chimeric version, KM2760, showed potent antitumor activity mediated by enhanced ADCC against ATL cell lines and primary ATL cells in vitro and in vivo [39, 41, 42].

A phase I clinical study (0761–0501 Study: ClinicalTrials.gov Identifier NCT00355472) has been conducted in patients with CCR4-positive relapsed PTCL, including ATL [43•]. The primary objectives of the study were to assess the safety of mogamulizumab, and analyze its maximum tolerated dose (MTD) and pharmacokinetics. The secondary objectives were to determine the best overall response rate (ORR) and progression-free survival (PFS). Mogamulizumab was intravenously administered once a week for 4 weeks at four dose levels (0.01, 0.1, 0.5, and 1.0 mg/kg) according to the conventional 3+3 design. Enrolled in the study were 16 patients, of whom 13 had ATL (11 acute type, 2 lymphoma type), 1 had tumor-stage MF, and 2 had PTCL-NOS. All 16 patients receiving mogamulizumab were included in the safety and efficacy analyses. No dose-limiting toxicity (DLT) was observed in any of the 13 patients who received mogamulizumab at a dose of 0.01–1.0 mg/kg, and thus MTD was not reached. Then, three additional patients were enrolled to receive 1.0 mg/kg, the highest dose. One patient showed grade 4 neutropenia, grade 3 febrile neutropenia, and grade 3 skin eruption, but these adverse events occurred in only 1 of the 6 patients who received a dose of 1.0 mg/kg, indicating that this drug would be tolerated at least up to 1.0 mg/kg. The best ORR in the total 16 patients was 31 % (of those, 2 had complete response [CR] and 3 had partial response [PR]), and the best ORR was also 31 % in patients with ATL (of those, 2 had CR and another 2 had PR). Pharmacokinetic analysis revealed a plasma mogamulizumab trough concentration of 7.5–19.6 μg/mL after the 1st to 4th administration of mogamulizumab at a dose of 1.0 mg/kg. These concentrations were sufficient to kill

primary ATL cells by ADCC activity in vitro (10 μg/mL). After the 4th administration of mogamulizumab at a dose of 1.0 mg/kg, its plasma half-life was approximately 18 days, which is comparable to the half-life (14 to 21 days) of endogenous human IgG. Lastly, although MTD was not reached, a tendency toward an increased incidence of grade 3 or higher toxicity was observed at 1.0 mg/kg. Therefore, it was concluded that a dose of 1.0 mg/kg should be recommended for a subsequent phase II trial of this novel agent [44].

A subsequent phase II study of mogamulizumab (0761–002 Study: ClinicalTrials.gov Identifier NCT 00920790) was conducted in patients with CCR4-positive relapsed ATL [45•]. The primary endpoint was the best ORR, and the secondary endpoints included the best response of each disease site such as peripheral blood ATL cells, skin and nodal/extranodal lesions as well as PFS and OS. It was planned for 25 patients to be enrolled for efficacy analysis, assuming the expected ORR of 30 % with a 5 % threshold response rate. Mogamulizumab was intravenously administered once a week for 8 weeks at a dose of 1.0 mg/kg. In this study, 28 patients in total were enrolled. Of these, 27 patients who received mogamulizumab were included in the safety analysis, and 26 patients, excluding 1 patient who was judged ineligible for enrollment after starting mogamulizumab administration, were evaluated in the efficacy analysis. Of the 27 patients who received mogamulizumab, 14 had acute type, 6 lymphoma type, and 7 chronic type with unfavorable prognostic factors. The best ORR was 50 % (13/26) including 8 CR. With the lower limit of the 95 % confidence interval (30 % to 70 %) exceeding the threshold response rate of 5 %, the clinical efficacy of mogamulizumab was confirmed. Responses according to disease sites were 100 % (of 13 patients, all CR) for peripheral blood, 63 % (of 8 patients, 3 CR and 2 PR) for skin, and 25 % (of 12 patients, 3 CR/CRu) for nodal and extranodal lesions. Median PFS and OS were 5.2 and 13.7 months, respectively. The best ORR was also calculated for each disease subtype, giving 43 % in patients with acute type (of 14 patients, 5 CR and 1 PR), 33 % in patients with lymphoma type (of 6 patients, 1 CR and 1 PR), and 83 % in patients with unfavorable chronic type (of 6 patients, 2 CR and 3 PR). Thus, it was demonstrated that mogamulizumab induced favorable responses in patients with any disease subtype of ATL. In addition, for each age group, the best ORR was 39 % (of 13 patients, 3 CR and 2 PR) in patients younger than 65 years, and 62 % (of 13 patients, 5 CR and 3 PR) in patients 65 years or older. The most common adverse events observed during the study were lymphopenia (96 %), neutropenia (52 %), and thrombocytopenia (52 %) as hematologic toxicity, and acute infusion reaction (89 %) and skin eruption (63 %) as non-hematologic toxicity. There was no death related to mogamulizumab in either the phase I or phase II study. Of 8 serious adverse events with a relationship to mogamulizumab in the phase I and II studies, 5 events including 4 cases of skin eruption and 1 case of Stevens-Johnson syndrome occurred during the phase II study. However, these reactions were manageable with supportive measures including corticosteroid or other drugs in all patients. Considering the seriousness of the disease, even skin eruption might be considered acceptable for the treatment of ATL by treating physicians, while close and appropriate follow-up of the event is necessary. Elucidation of the mechanism of skin eruption and preventive measures against it are awaited.

In the U.S., a phase I/II study (ClinicalTrials.gov Identifier: NCT00888927) in patients with relapsed or refractory PTCL including CTCL has been conducted [46]. Mogamulizumab was well tolerated at doses of 0.1–1.0 mg/kg in 42 patients including 1 with PTCL-NOS. MTD was not reached and thus 1.0 mg/kg was chosen for subsequent studies. A promising ORR of 42 % (of 38 evaluable patients with CTCL, 3 CR and 13 PR) was achieved, although expression of CCR4 on lymphoma cells was not mandatory for patient enrolment in this particular phase I/II study. Regarding subtypes of CTCL, ORR in Sezary syndrome (SS) patients was 50 % and in MF patients was 36 %. Eighty-seven percent of SS patients had a response in peripheral blood, with 50 % CR. Further study of mogamulizumab is warranted in patients with nodal PTCL as well as CTCL.

Conclusions

It is evident that there are limitations to improvement of the treatment outcome of PTCL, especially ATL, with the currently available chemotherapeutic agents alone. Mogamulizumab has a less severe toxicity profile and induces a high response rate in patients with ATL, even in elderly patients.

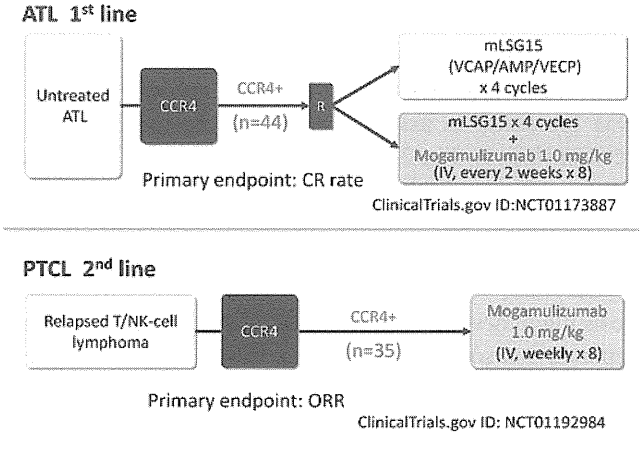

Fig. 1 Ongoing clinical studies of mogamulizumab/KW-0761 for peripheral T-cell lymphomas in Japan

Therefore, it may provide an effective treatment option for the disease, especially for elderly patients who are not eligible for intensive chemotherapy or hematopoietic stem-cell transplantation.

In Japan, based on the results of the clinical studies mentioned above, a single-arm phase II study of mogamulizumab monotherapy in patients with CTCL and PTCL (ClinicalTrials.gov Identifier: NCT01192984) and a randomized phase II study of dose-intensified combination chemotherapy (mLSG15 regimen) with or without mogamulizumab in untreated patients with ATL (ClinicalTrials.gov Identifier: NCT01173887) are being conducted as shown in Fig. 1. Patient enrollment has already been completed in these phase II studies. In the U.S., based on the aforementioned phase I/II study in patients with relapsed or refractory CTCL, a pivotal phase III study in patients with relapsed or refractory CTCL is being planned. In conclusion, mogamulizumab is expected to provide new, promising treatment options in patients with ATL and other T-cell lymphomas.

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ORIGINAL REPORT

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

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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 (± 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.

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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, etoposide, 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)[1,4] 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/μL, platelet count $\geq$ 50,000/μL, hemoglobin $\geq$ 8.0 g/dL, AST $\leq$ 2.5 $\times$ the upper limit of the normal range (UNL), ALT [lteuq] 2.5 $\times$ UNL, total bilirubin $\leq$ 1.5 $\times$ UNL, serum creatinine $\leq$ 1.5 $\times$ UNL, corrected serum calcium $\leq$ 11.0 mg/dL, and arterial partial oxygen pressure $\geq$ 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 collected 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.

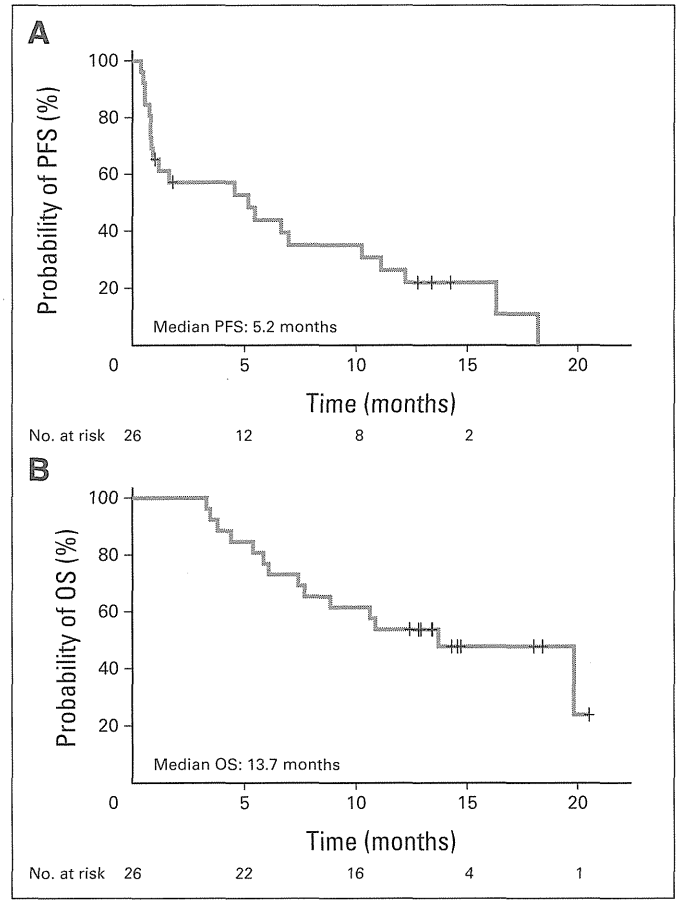

Fig 1. Kaplan-Meier curves of estimated (A) progression-free survival (PFS; median, 5.2 months) and (B) overall survival (OS; median, 13.7 months).

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 (± standard deviation) of the eighth infusion were 42.9 ± 14.2 μg/mL and 33.6 ± 10.6 μg/mL, respectively. Mean area under the blood concentration time curve from 0 to 7 days after the eighth infusion was 6,297 ± 1,812 μg × 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 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

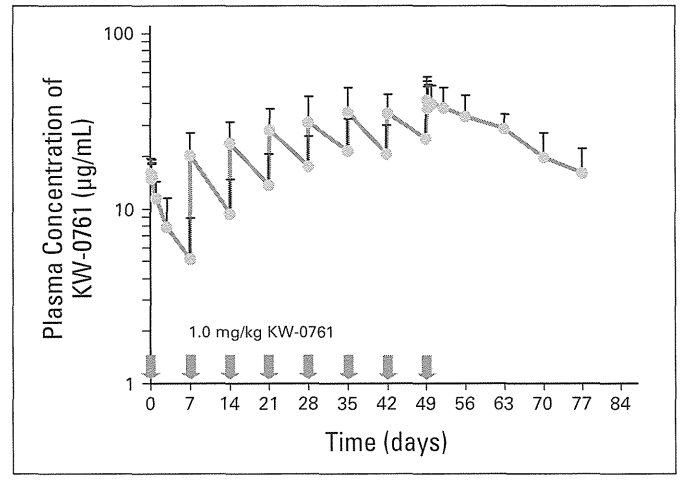

Fig 2. Pharmacokinetics of KW-0761. Mean KW-0761 plasma concentrations during and after 1.0 mg/kg KW-0761 infusions once per week for 8 weeks. Bar indicates upper limit of standard deviation.

Table 2. Adverse Events (n = 27)*

Adverse Event	Grade (No. of patients) 1	2	3	4	All Grades No. of Patients	%	Infusion Reaction Related (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 hypoproteinemia, BUN elevation, CRP, glycosuria, hypochloremia, and hyperammoniemia.

§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 al[20] 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

Fcγ 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 $v \geq$ five) indicated a significant association between clinical response and skin rashes (no or grade 1 v 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 al[37] 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.

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Reply to H. Charalambous et al

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We agree with Charalambous and Silbermann[1] that action needs to be taken to improve the skills of oncologists to manage chronic cancer pain. Their suggestion for clinical training programs at first seems logical; they cite findings that classroom training did not improve residents' knowledge,[2] a finding consistent with ours, that is, that continuing medical education in cancer pain management seemed to be ineffective.[3] They also cite a study showing that clinically based training in palliative care is effective.[4] In that study, however, there was only a 10% improvement, with statistically significant improvement in only six of 25 questions. In addition, the program was optional, which might suggest that those who took it were more motivated than most, making the generalizability of these findings questionable. Thus, although we agree that change is critically needed, the way to accomplish that change remains elusive. We continue to study this issue and hope that a more complete characterization of this problem will inform the development of more effective programs to support best practices in pain management and palliative care for the broad oncology community.

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Concurrent Chemoradiotherapy for Localized Nasal Natural Killer/T-Cell Lymphoma: An Updated Analysis of the Japan Clinical Oncology Group Study JCOG0211

To the Editor: Extranodal natural killer (NK)/T-cell lymphoma (NKTCL), nasal type,[1,2] is a predominantly extranodal lymphoma associated with Epstein-Barr virus. Before the early 2000s, no prospective clinical trials had been conducted for localized nasal NKTCL. In the November 20, 2009, issue of *Journal of Clinical Oncology*, we reported the results of our first analysis of a phase I/II study of concurrent chemoradiotherapy for newly diagnosed localized nasal NKTCL (Japan Clinical Oncology Group study JCOG0211).[3] Our first analysis demonstrated improved overall survival (OS) and progression-free survival (PFS) at 2 years with a median follow-up of 32 months (range, 24 to 62 months) compared with a historical control of radiotherapy (RT) alone.[3,4] Soon after the publication of our study, a Korean group reported promising results from a phase II study of concurrent chemoradiotherapy.[5] Since then, concurrent chemoradiotherapy has been regarded as one of the reasonable treatment options for newly diagnosed localized nasal NKTCL.[6] However, to our knowledge, no long-term follow-up studies on survival or complications of concurrent chemoradiotherapy have been published. We report the results of a long-term follow-up of the JCOG0211 study.

A total of 33 patients were enrolled and received concurrent chemoradiotherapy that consisted of 50 Gy of RT and three cycles of dexamethasone, etoposide, ifosfamide, and carboplatin (DeVIC). Two doses, which consisted of a two-thirds dose of DeVIC (2/3DeVIC) and a full dose of DeVIC (100%DeVIC), were evaluated in the phase I portion, and 2/3DeVIC was selected for the phase II portion.[3] In total, 27 patients were treated with RT and 2/3DeVIC (RT-2/3DeVIC), and six patients were treated with RT and 100%DeVIC (RT-100%DeVIC). Clinical parameters of all 33 patients were comparable with those of the 27 patients treated with RT-2/3DeVIC.

The data used for this analysis were updated as of December 2011. No patients received prophylactic therapy for CNS relapse. Moreover, no patient with an objective response underwent consolidative hematopoietic stem-cell transplantation. The median follow-up time for the 27 patients who were treated with RT-2/3DeVIC was 67 months (range, 61 to 94 months). The OS at 5 years was 70% (90% CI, 53% to 82%; 95% CI, 49% to 84%; Fig 1A), which was superior to the historical control of RT alone (40%)[4] that we used in the previous analysis. The PFS at 5 years was 63% (90% CI, 46% to 76%; 95% CI, 42% to 78%; Fig 1B). No disease progression was observed after the first analysis. These results demonstrate that RT-2/3DeVIC provides reasonably long response durability for newly diagnosed localized nasal NKTCL. The median follow-up time for all 33 patients was 68 months (range, 61 to 94 months). The OS at 5 years was 73% (90% CI, 57% to 83%; 95% CI, 54% to 85%), and the PFS at 5 years was 67% (90% CI, 51% to 78%; 95% CI, 48% to 80%; Fig 2). Recurrence within the RT field was observed in only two patients. Thus, the planning target-volume control rate at 5 years was 94% (31 of 33 patients).

The late toxicities were acceptable and manageable (Table 1). One patient treated with RT-2/3DeVIC experienced perforation of the nasal skin and received plastic surgery 18 months after RT. This event was scored as a grade 4 late RT adverse event (AE), although the patient had massive involvement of the nasal skin and subcutaneous tissue before the protocol treatment. One patient treated with RT-100%DeVIC experienced grade 3 irregular menstruation. No other grade 3 or higher late AEs were observed. Eleven patients (33%) experienced grade 1 or 2 late RT AEs of the eye, but none of these patients required ophthalmologic surgery as a result of late RT AEs other than cataracts. However, five of the 11 patients had not recovered from the late RT AEs of the eye at the last follow-up.

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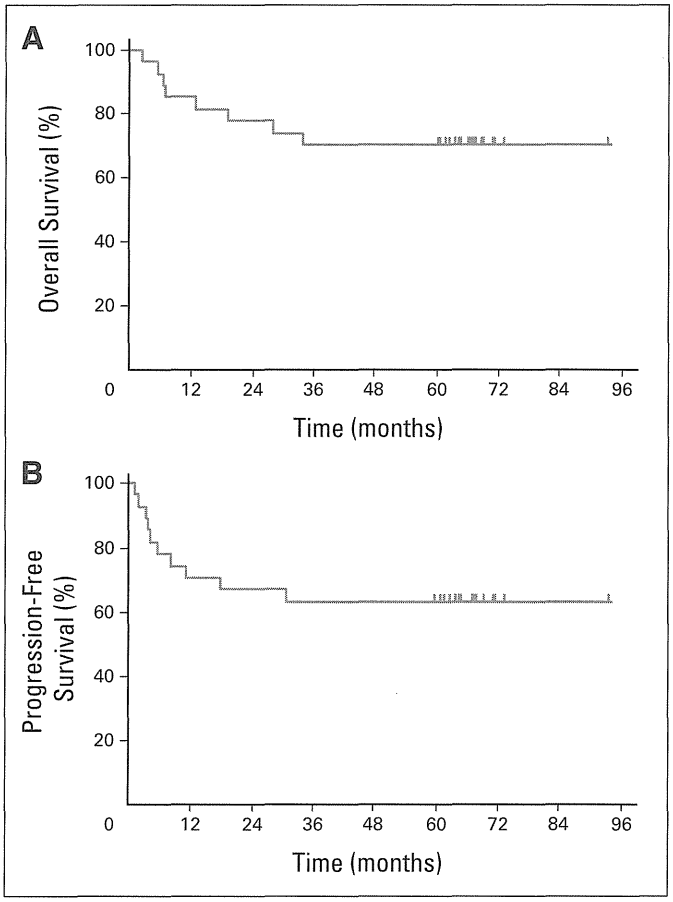

Fig 1. (A) Overall survival and (B) progression-free survival of 27 patients treated with radiotherapy and a two-thirds dose of dexamethasone, etoposide, ifosfamide, and carboplatin.

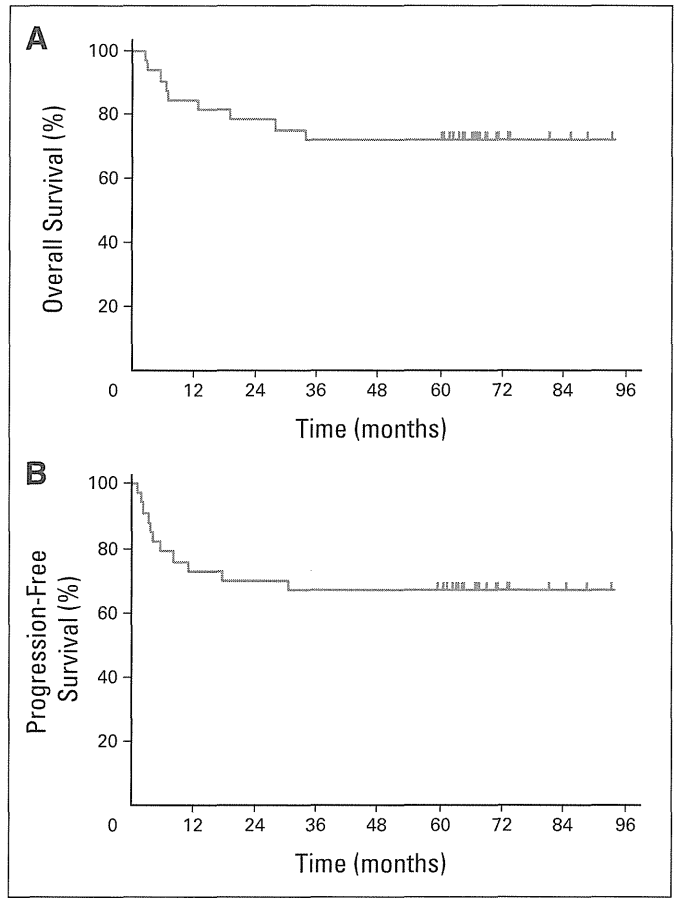

Fig 2. (A) Overall survival and (B) progression-free survival of 33 patients treated with radiotherapy and dexamethasone, etoposide, ifosfamide, and carboplatin.

Of note, four of the five patients had been treated with RT-100%DeVIC. With consideration of these results, and because the patient who experienced grade 3 amenorrhea had been treated with RT-100%DeVIC, it is unlikely that the full dose of DeVIC is appropriate for concurrent chemoradiotherapy because of the excessive acute and late toxicities, although the number of evaluated patients was small.

Our updated analysis confirmed that both the survival benefit and disease control provided by concurrent chemoradiotherapy with RT and DeVIC were maintained for more than 5 years, and to our knowledge, this analysis is the first to reveal the profile of late AEs of concurrent chemotherapy for this disease. We conclude that RT-2/3DeVIC is one of the most recommendable options as a first-line treatment for localized nasal NKTCL.

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Table 1. Incidence and Maximum Severity of Late Adverse Events During Follow-Up (N = 33)

Adverse Event	Grade 1		Grade 2		Grade 3		Grade 4	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Late RT adverse event, RTOG/EORTC Late Radiation Morbidity Scoring Scheme								
Mucous membrane, head and neck	11	33	3	9	0	0	0	0
Salivary glands	3	9	5	15	0	0	0	0
Skin, head and neck	7	21	0	0	0	0	1*	3
Subcutaneous tissue, head and neck	2	6	0	0	0	0	1*	3
Spinal cord	0	0	0	0	0	0	0	0
Brain	1	3	0	0	0	0	0	0
Eye	7	21	4	12	0	0	0	0
Other late adverse event, NCI-CTC 2.0								
Irregular menses	0	0	0	0	1†	3	0	0
Secondary malignancy							0	0

Abbreviations: EORTC, European Organisation for Research and Treatment of Cancer; NCI-CTC, National Cancer Institute Common Toxicity Criteria; RT, radiotherapy; RTOG, Radiation Therapy Oncology Group.
*The same patient underwent plastic surgery.
†This 30-year-old patient had been treated with RT and full-dose dexamethasone, etoposide, ifosfamide, and carboplatin and recovered from this adverse event after 3 years.

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Cancer Rehabilitation Evaluation System (CARES) and CARES-SF Now Publicly Available

To the Editor: I was very pleased to read your special issue of *Journal of Clinical Oncology* (April 10, 2012) focused on "Caring for the Whole Patient: The Science of Psychosocial Care." The issue does an excellent job of amplifying the findings of the recent Institute of Medicine report[1] by including review articles that provide in-depth presentation of strategies that can be used to implement the recommendations of the committee report. One of the major failures of our current oncology practice is the lack of a systematic approach to evaluating the unmet needs of patients with cancer, and this is well described in the article by Carlson et al.[2]

Early in the 1980s, my colleagues Coscarelli (Schag) and Heinrich developed a needs assessment tool, initially called the Cancer Inventory of Problem Situations[3] and then later refined as the Cancer Rehabilitation Evaluation System (CARES)[4] and a short form called the CARES-SF.[5] I have used this tool for intervention research,[6] outcomes in clinical trials,[7] and clinical care. It is described among a variety of instruments in Table 2 of the article by Carlson et al[2] as a reliable and useful tool for assessing the unmet needs of patients with cancer. Unfortunately, the widespread use of the CARES and CARES-SF was limited by a copyright and user fee that the developers chose to impose. Fortunately, this is no longer the case. The CARES, CARES-SF, user manual and scoring sheets, along with a listing of many related publications are now publicly available at the Jonsson Comprehensive Cancer Center Web site.[8] I would encourage anyone interested in a comprehensive needs assessment tool to review the CARES and consider its use. It is well

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Paradoxical expression of *IL-28B* mRNA in peripheral blood in human T-cell leukemia virus Type-1 mono-infection and co-infection with hepatitis C Virus

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Abstract

Background: Human T-cell leukemia virus type-1 (HTLV-1) carriers co-infected with and hepatitis C virus (HCV) have been known to be at higher risk of their related diseases than mono-infected individuals. The recent studies clarified that IL-28B polymorphism rs8099917 is associated with not only the HCV therapeutic response by IFN, but also innate immunity and antiviral activity. The aim of our research was to clarify study whether IL-28B gene polymorphism (rs8099917) is associated with HTLV-1/HCV co-infection.

Results: The genotyping and viral-serological analysis for 340 individuals showed that IL-28B genotype distribution of rs8099917 SNP did not differ significantly by respective viral infection status. However, the IL-28B mRNA expression level was 3.8 fold higher in HTLV-1 mono-infection than HTLV-1/HCV co-infection. The high expression level was associated with TT (OR, 6.25), whiles the low expression was associated with co-infection of the two viruses (OR, 9.5). However, there was no association between down-regulation and ATL development (OR, 0.8).

Conclusion: HTLV-1 mono-infection up-regulates the expression of IL-28B transcripts in genotype-dependent manner, whiles HTLV-1/HCV co-infection down-regulates regardless of ATL development.

Keywords: IL-28B, IL-λ3, HTLV-1, HCV, SNP

Introduction

A retrovirus, human T-cell leukemia virus type-1 (HTLV-1), and a positive-strand RNA virus, hepatitis C virus (HCV), are completely different in terms of virologic characteristics. Nevertheless, they play a similar role in the pathogenesis of viral-induced malignant neoplasms, such as adult T-cell leukemia (ATL) in HTLV-1- infected individuals, and hepatocellular carcinoma (HCC) and B-cell lymphoma in HCV- infected individuals, during long-term chronic infections.

Furthermore, it is known that co-infection with HCV and HTLV-1 is frequently observed in an area endemic for HTLV-1. HCV/HTLV-1 co-infected individuals have been reported to be at higher risk for developing HCC than those infected with HCV alone [1-3]. Although the pathologic mechanism of the co-infection remains to be elucidated, it is thought that the impaired immunity due to HTLV-1 infection may contribute to HCV infection and HCV-related disorders, which is suggested by previous reports. Kohno et al. reported that the severe immunodeficiency and anergic state in patients with ATL may be associated with a functional property of leukemic cells originating from regulatory T-cells expressing CD4, CD25, CCR4, GITR and Foxp3 [4]. Kishihara et al. also reported that impairment of the immune response by HTLV-1 could explain the reduced effectiveness of interferon (IFN) treatment in patients co-infected with HTLV-1 and HCV [5].

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Recently, genome-wide association studies of patients with HCV have made great advances in viral clearance associated with *IL-28B* single nucleotide polymorphisms (SNP) [6,7]. IL-28B is a type III Lambda interferon (IFN-λ) and a cytokine similar to IL-10 with IFN-like activities [8]. This new IFN-λ family includes IFN-λ1 (IL-29), IFN-λ2 (IL-28A) and IFN-λ3 (IL-28B) [9]. Although the IFN-λ genomic structure resembles that of the IL-10 family [10], the amino acid and functional level of IFN-λs are more closely related to type I IFNs than IL-10. The IFN-λs are induced by stimulation with several single-strand RNAs (ssRNA) and several kinds of viruses. The IL-28B SNPs, such as rs8099917, rs12979860, and 12980275, have been reported to be associated with spontaneous clearance [10], innate HCV immunity [9], HCV-related disease chronicity, and therapeutic response to pegIFN-α and ribavirin (RBV) [6,7].

From these observations, we hypothesized that IFN-λ3 encoded from the *IL-28B* gene would be associated with HTLV-1 infection. The aim of the present study was to examine the mutual association between IL-28B polymorphism (rs8099917 SNP) and mono-infected-HTLV-1 and co-infected HTLV-1 with HCV subjects.

Materials and methods

Clinical subjects

All subjects were of Japanese origin living in Nagasaki City, an endemic area for HTLV-1 in Japan. For genomic specimens, 340 blood samples were randomly collected from patients who visited a liver clinic and liver transplantation center from April 2009 to March 2011 from the departments of Hepatology and a Hematology Clinic. One hundred and twenty-four of the 340 samples were also available for total RNA tests. Accordingly, most patients had either chronic liver disease (CLD) or adult T-cell leukemia (ATL). This study was done under informed consent after the approval of the Nagasaki University hospital IRB (IRB Approval No.10050). Since the samples used here were un-linked materials, patient information was restricted.

Cell lines

Eight HTLV-1-infected T-cell lines, Hut 102, MT-1, MT-2, ST1, KK1, KOB, OMT, and LMY-1, were used for IL-28B mRNA quantification. The first three were purchased and latter five were established in our laboratory [11].

Serological and genetic tests for HCV and HTLV-1

HCV and HTLV-1 infections were mainly serologically detected using commercially available kits, CLEIA-anti-HTLV-1, Lumipulse-II Ortho HCV (Fujirebio-INC, Tokyo, Japan). The confirming examination was genetically performed by the Cobas TaqMan HCV test (TaqMan HCV; Roche Tokyo INC, Tokyo, Japan) for HCV and in-house HTLV-1 proviral real-time RT quantifiable PCR [12]. Genomic DNA and total RNA were extracted from peripheral blood mononuclear cells (PBMC) using commercially available QuickGene DNA Whole blood kits (FUJIFILM Corp., Tokyo, Japan) and PureLink RNA Micro Kits (Invitrogen Corp., Carlsbad, Ca, USA). The extraction protocol was performed according to the manufacturer's instructions.

Genotyping for SNPs

SNP genotyping was performed using multiplex PCR amplification and Pyrosequencing technology. To amplify target regions, newly designed biotinylated-primers were employed: sense and anti-sense for rs8099917, 5'-TCCTCCTTTTGTTTTCCTTTCTG-3' and 5'-AAAAAGCCAGCTACCAAACTGT-3'. Then, the amplicon was sequenced according to the manufacturer's instructions based on Pyrosequence technology (Qiagen, Hilden, Germany). Biotin-labeled amplicons from the 1st PCR were captured by binding to streptavidin-coated Sepharose beads, and DNA was denatured to produce an ssDNA template for the Pyrosequencing assay. The ssDNA was released and combined with the sequencing primer, which was extended during the Pyrosequencing reaction to provide the sequence of the template DNA. Pyrosequencing data were produced in the form of Pyrograms, and genotypes were assigned by the peak pattern presented in the Pyrogram.

Real-time reverse transcription (RT) quantifiable PCR for IL-28B mRNA

mRNA for IL-28B transcribed into cDNA by a GoScript™ RT System (Promega, Madison, WI, USA) was quantified by a LightCycler System (Rosche, Mannheim, Germany) using newly designed sense and anti-sense primers, 5'-AAGGACTGCAAGTGCCGCT-3' and 5'-GCTGGTCCAAGACATCCC-3' (AY129149). A standard curve was generated using a tenfold dilution method with a reference material derived from pTAC-1.2735 inserted with 166 base fragments including the target. The amplicon was assayed by the Cyber green method. The raw data were normalized by *abl* mRNA density and evaluated as the relative value for *abl* gene expression calculated by *IL-28B* data/*abl* data × 10^4, modified from our previous mRNA real time RT qPCR method [12].

Statistical analysis

The minor-allele frequency (MiAF) was set as the less frequent allele in a population for SNPs analyzed. Viral infectious status was divided into 4 groups of HTLV-1 mono-infection, HCV mono-infection, HTLV-1/HCV-co-infection, and non-infection (double negative; DN).

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Differences in the genotype distribution of IL-28B SNPs among groups were compared using the Chi-square or Fisher exact test. The level of mRNA expression was compared using the Mann Whitney U test. Correlation analysis was performed by the Nonparametric Spearman's rank correlation method. The relationship between a factor and an outcome was estimated the magnitude of the association by the odds ratio with 95% confidence intervals (95%CI). Statistical analysis was performed using SAS 9.1. The statistical significance level was set at 0.05.

Results

IL-28B genotypes and the sero-status

Three hundred and forty samples were genotyped on IL-28B rs8099917 SNP and were serologically examined for viral infection of HTLV-1 and HCV. As summarized in Table 1. They consisted of 263 (77.4%) major TT homozygotes, 171 (20.9%) minor TG heterozygotes, and 6 (1.8%) minor GG homozygotes. The virus tests revealed that 59 were negative for both HTLV-1 and HCV, 73 were positive for HTLV-1 alone, 179 were positive for HCV alone and 29 were positive for both viruses. The genotypic distributions, as well as minor allele frequency (MAF) of the IL-28B gene, did not significantly differ among each viral infection status as a control of no-infection.

Since the HTLV-1 mono-infection group consisted of 47 ATL patients and 26 HTLV-1 carriers, we stratified the two groups of ATL patients and carriers and the minor allele frequencies of the two groups were compared; the difference between that of ATL and carriers was not statistically significant (p = 0.21). The prevalence of TT was not different statistically either (p = .495).

Next, the expression levels of IL-28B were quantified using 124 samples randomly collected during this study period.

Table 1 IL-28B genetic distribution and allele frequency in stratification based on the combination of HTLV-1 and HCV infection

		Genotype r(rs8099917)			Allele fequency		
	No	TT	TG	GG	T	G	
All cases	340	263 (77.4%)	71 (20.9%)	6 (1.8%)	0.86	0.14	
1) non-Infection	59	45(76.3)	10 (16.9)	4(6.8)	0.84	0.15	
2) HTLV-1 mono	73	55(75.3)	17 (23.3)	1(1.9)	0.87	0.13	P = 0.90
ATL patients	47	37(78.7)	10 (21.3)	0(0.0)	0.89	0.11	P = 0.11
carriers	26	18(69.2)	7(26.9)	1(3.8)	0.82	0.18	P = 0.46
3) HCV-mmono	179	141 (78.7)	37 (20.7)	1(1.0)	0.89	0.11	P = 0.68
4) co-infection	29	22(75.9)	7(24.1)	0(0.0)	0.88	0.14	P = 0.9

There was no significant difference in the genetic distribution and allele frequency among respective infectious states

P values were compared with non infection

IL-28B mRNA expression level and HCV/HTLV-1 co-infection

The expression levels of IL-28B mRNA were generally low in most cases with a median value of 5.2 in no-infection, 10.6 in HTLV-1 mono-infection, 3.9 in HCV mono-infection, and 2.8 in HTLV-1/HCV co-infection (Figure 1a). Notably, a small number of measurement values shown as open circles was high, and they were distributed only within the HTLV-1 mono-infected and HCV mono-infected groups. Moreover, all of those who had high values were exclusively TT homozygous, as shown in Figure 1b (samples marked by $^{A\text{-}E)}$ were the same in Figure 1(a) and Figure 1(b)). Surprisingly, the median value was the highest in HTLV-1 mono-infection and the lowest in the co-infection group (10.6 versus 2.8; p = 0.013). Therefore, to clarify whether ATL cells directly affect the expression of IL-28B mRNA, we compared the mRNA expression levels in mainly HTLV-1 carriers, ATL patients with ATL cells, and ATL cell lines. As shown in Figure 2, the median values were significantly higher in mono HTLV-1 carriers with TT (17.9 vs 5.6, $P < 0.05$) and ATL patients with TT having ATL cells than those of non-infected individuals (13.4 vs 5.6, $p < 0.05$). No high expression level was observed in two ATL or 16 carriers with HTLV-1/HCV co-infection. Surprisingly, these data were lower rather those from TG/GG. On the other hand, IL-28B mRNA expression in 8 HTLV-1-infected T-cell lines was undetectable in all but one (Hut 102). The genotype was TT in all cell lines.

In addition, there was no correlation between the IL-28B mRNA levels and HCV-RNA levels (non-parametric Spearman's rank correlation, R^2 = 0.0543, Figure 3).

Assessment by odds ratio analysis for an outcome if a risk factor is present

As shown in Figure 2, HTLV-1 was revealed to have an association similar to HCV and IL-28B mRNA. However, the up-regulated-action of HTLV-1 was nullified if the virus was co-infected with HCV. The prevalence of a major TT and minor TG/GG was similar among individuals infected with either HTLV-1 or HCV, as well as the allele frequency, indicating that there is no specific correlation between IL-28B and HTLV-1. Thus, to approach a causative clue, assessment by odds ratio (OR) analysis was performed (Table 2). Only the high mRNA level besides 3 states of HTLV-1 mono-infection,