heat-killed mycobacterium tuberculosis H37RA (Difco). Mice also received 200 ng of pertussis toxin (List Biological Laboratories) i.p. in 0.2 ml of PBS on the same day and 2 days later. Clinical signs of EAE were assessed daily with a 0–6 scoring system (0, no signs; 1, tail limpness; 2, impaired righting reflex; 3, hind limb weakness; 4, hind limb paralysis; 5, hind limb paralysis with fore limb weakness; 6, death).

Isolation of Mouse B Cells and Adoptive Transfer

For B cell isolation, splenic B cells were purified by negative selection of $CD43^+$ cells with anti-CD43 magnetic beads (Miltenyi Biotec). The enriched B cell population was >95% positive for B220 staining. The B cells (2×10^7 cells) from spleen of $Mb1^{Cre/+}$ and $Prdm1^{f/f}Mb1^{Cre/+}$ mice 28 days after MOG_{35-55} immunization or wild-type and $Sell^{-/-}$ mice were transferred intravenously into μMT mice 24 hr before EAE induction.

Isolation and Stimulation of Human B Cells from Healthy Blood Donors

Mononuclear cells were isolated from peripheral blood of healthy donors by centrifugation over Ficoll-Paque PLUS (GE Healthcare). B cells were enriched by positive selection of $CD19^+$ cells with anti-human CD19 magnetic beads (Miltenyi Biotec) and were routinely >95% positive for CD19 staining. The purified B cells (5×10^5 cells/ml) were cultured for 96 hr with IL-2 (10 ng/ml; R&D), IL-6 (10 ng/ml; R&D), and CpG ODN 2006 (CpG; 1 μg/ml; InvivoGen) in the presence of IFN-α (1,000 U/ml; PBL Biomedical Laboratories) or IFN-β (1,000 U/ml; PBL Biomedical Laboratories). This study was approved by the ethics committees of Research Institute for Microbial Diseases, Osaka University. Healthy volunteers were recruited after obtaining informed consent.

ELISA and ELISPOT

MOG-specific IgG in serum was measured by ELISA with a plate coated with 10 μg/ml MOG_{35-55} and then detected with goat anti-mouse IgG and HRP-conjugated anti-goat IgG Abs (SouthernBiotech). For measurement of cytokine release by autoantigen-reactive lymphocytes, single-cell suspensions of the dLNs prepared from mice 14 days after EAE induction were cultured with a range of MOG_{35-55} doses for 48 hr. For measurement of IL-10 production by mouse B cells, purified B cells (1×10^6 cells/ml) were cultured for 48 hr with 10 μg/ml of LPS (Sigma-Aldrich) and then stimulated with 10 μg/ml of anti-mouse IgM $F(ab)'_2$ (Jackson Immunoresearch). In some experiments, $CD19^+CD138^-$ and $CD138^+CD44^{hi}$ cells harvested from dLNs of wild-type mice 14 days after EAE induction were stimulated with 100 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) plus 1 μM ionomycin (Sigma-Aldrich) for 5 or 24 hr. For measurement of IL-10 and IgM production by human peripheral blood B cells, purified B cells (5×10^5 cells/ml) were cultured for 96 hr with IL-2, IL-6, IFN-α, and CpG. IFN-γ, IgM, IL-4, IL-6, IL-10, IL-13, IL-17a, IL-27, IL-35, and TGF-β1 in the culture medium were detected by ELISA and Bio-Plex suspension assay according to the manufacturer's protocol (Biolegend, BIO-RAD, Bethyl Laboratories, or R&D). IgM secretion by human B cells was detected by ELISPOT according to the manufacturer's protocol (R&D).

Statistical Analysis

We performed statistical evaluation with Prism software (GraphPad). A two-tailed, unpaired Student's t test was applied for statistical comparison of two groups. In case of unequal variance, t test with Welch's correction was used. Comparisons of two nonparametric data sets were done by the Mann-Whitney U test. A p value of less than 0.05 was considered statistically significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures, one table, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2014.10.016.

AUTHOR CONTRIBUTIONS

M.M. planned and performed most of the experiments, analyzed data, and prepared the manuscript; A.B. assisted with experiments; T.Y. contributed to morphological characterization of cultured cells; Y.O. provided technical contributions to ChIP assays; H.K. and K.T. provided $Il10^{Venus/+}$ reporter mice; H.N. and S.S. collected human blood and provided reagents; A.K. and S.L.N. provided $Prdm1^{gfp/+}$ mice and edited the manuscript; Y.B. and T.K. supervised the project; T.K. contributed to manuscript writing; and Y.B. designed the study, performed some experiments, interpreted data, and wrote the manuscript.

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ORIGINAL ARTICLE

Reactivation of hepatitis B virus (HBV) infection in adult T-cell leukemia–lymphoma patients with resolved HBV infection following systemic chemotherapy

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Abstract Reactivation of hepatitis B virus (HBV) infection may occur in adult T-cell leukemia–lymphoma (ATL) patients with resolved HBV infection who receive monotherapy with the anti-CC chemokine receptor 4 monoclonal antibody, mogamulizumab. However, there is little evidence regarding the incidence and characteristics of HBV reactivation in ATL patients receiving systemic chemotherapy, including the use of this antibody. We conducted a retrospective study for 24 ATL patients with resolved HBV infection underwent regular HBV DNA monitoring to assess HBV reactivation in Nagoya City University Hospital between January 2005 and June 2013. With median HBV DNA follow-up of 238 days (range 57–1420), HBV reactivation (defined as the detection of HBV DNA) was observed in three (12.5 %) of 24 patients with resolved HBV infection. No hepatitis due to HBV reactivation occurred in those patients who were diagnosed with HBV DNA levels below 2.1 log copies/mL and who received antiviral drugs. Mogamulizumab was administered prior to HBV reactivation in two of three HBV-reactivated patients. In the mogamulizumab era, further well-designed prospective studies are warranted to estimate the incidence of HBV reactivation and to establish regular HBV DNA monitoring-guided preemptive antiviral therapy for such patients.

Keywords Reactivation · HBV · CCR4 · Mogamulizumab · ATL

Abbreviations

HBV	Hepatitis B virus
ATL	Adult T-cell leukemia–lymphoma
HBsAg	Hepatitis B surface antigen
Anti-HBc	Antibodies against hepatitis B core antigen
Anti-HBs	Antibodies against hepatitis B surface antigen
CCR4	CC chemokine receptor 4

Introduction

Reactivation of hepatitis B virus (HBV) infection has been reported as a potentially fatal complication of systemic chemotherapy [1–6]. HBV reactivation may occur not only in hepatitis B surface antigen (HBsAg)-positive patients, but also in patients with resolved HBV infection who are seronegative for HBsAg but seropositive for antibodies

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against hepatitis B core antigen (anti-HBc) and/or antibodies against HBsAg (anti-HBs).

Chemotherapy containing the anti-CD20 monoclonal antibody, rituximab plus steroids has been shown to be an important risk factor for HBV reactivation in B-cell lymphoma patients with resolved HBV infection [2, 3]. Recently, the anti-CC chemokine receptor 4 (CCR4) monoclonal antibody, mogamulizumab, was developed and introduced into the management of adult T-cell leukemia–lymphoma (ATL) [7–12]. A dose-finding study showed that mogamulizumab monotherapy could induce HBV reactivation-related hepatitis in an ATL patient with resolved HBV infection [9, 13].

However, there is little evidence regarding the incidence and characteristics of HBV reactivation in ATL patients with resolved HBV infection who were receiving systemic chemotherapy including this antibody. We conducted here a retrospective study in a single institution to evaluate the risk of HBV reactivation in these patients who underwent regular monitoring of HBV DNA levels during and after chemotherapy.

Patients and methods

Between January 2005 and June 2013, 66 patients were diagnosed with ATL in Nagoya City University Hospital. Baseline serological markers for HBsAg, anti-HBc, and anti-HBs were measured to evaluate their viral status before systemic chemotherapy. Antiviral prophylaxis was provided to the HBsAg-positive patients before the initiation of systemic chemotherapy. HBV DNA levels were assessed in HBsAg-negative patients who were seropositive for anti-HBc and/or anti-HBs. Patients seronegative for HBsAg but with detectable of HBV DNA were considered to have occult HBV infection, and antiviral prophylaxis was provided to those patients. HBsAg-negative patients seropositive for anti-HBc and/or anti-HBs but without detectable of HBV DNA were considered to have resolved HBV infection and their HBV DNA levels were monitored regularly (monthly in principle) for HBV DNA levels during chemotherapy and at least 1 year after chemotherapy; HBV reactivation was defined as the detection of HBV DNA. If HBV reactivation was confirmed, antiviral drugs were given immediately (preemptive antiviral therapy).

All baseline serological markers of HBsAg, anti-HBc and anti-HBs were measured by the laboratory in this hospital, using the following methods and cut-off values: CLEIA with cut-off values for HBsAg, anti-HBc and anti-HBs were 1.0 C.O.I, 1.0 INH % and 10.0 mIU/mL, respectively, from January 2005 to December 2010, CLEIA with cut-off values for HBsAg, anti-HBc and ant-HBs were 0.03 mIU/mL, 1.0 C.O.I, and 10.0 mIU/mL, respectively, from January 2011.

HBV DNA levels were measured by an outside laboratory (SRL, Inc.; Tokyo, Japan) or by the laboratory in this hospital, using the following methods and cut-off values: transcription-mediated amplification test with a cut-off value of 3.7 LGE/mL from January 2005 to April 2006, Amplicor HBV monitor test with a cut-off value of 2.6 log copies/mL from April 2006 to May 2008, COBAS AmpliPrep/COBAS TaqMan HBV test (v1.0) with a cut-off value of 1.8 log copies/mL from May 2008 to July 2009, and COBAS AmpliPrep/COBAS TaqMan HBV test (v2.0) with a cut-off value of 2.1 log copies/mL from July 2009.

For the analysis of HBV sequences, nucleic acids were extracted from the preserved serum specimens (200 μL) and subjected to PCR to amplify HBV genomes within the short S region [nucleotides (nt) 427–607] and the basal core promoter (BCP)/precore (PC) regions [nt 1628–2047] followed by direct sequencing using the ABI Prism Big Dye ver. 3.1 kit in an ABI 3100 DNA automated sequencer (Applied Biosystems, Foster City, CA). HBV genotypes were determined by molecular evolutionary analysis [14].

To compare the baseline characteristics and ATL treatment of the patients with and without HBV reactivation, we used the Chi-square test and two-sided Fisher's exact test for categorical data, and the Mann–Whitney U test for continuous variables. A two-tailed p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS (version 22.0) statistical software for Windows, using data fixed on August 31, 2013. This study was approved by the Institutional Review Board of Nagoya City University. All patients gave written informed consent.

Results

The status of HBV infection at baseline was as follows (Fig. 1): HBsAg-positive (n = 2, 3.0 %), HBsAg-negative (n = 63, 95.5 %), and no serological HBV assessment (n = 1, 1.5 %). Of the 63 HBsAg-negative patients, 31 (49.2 %) were anti-HBc positive and/or anti-HBs positive. Of the remaining 32 patients, 31 were anti-HBc negative and anti-HBs negative, and one had no data for anti-HBc and anti-HBs. Because HBV DNA below 1.8 log copies/mL was detected at baseline in one patient who was anti-HBc positive and anti-HBs positive at baseline (and who was therefore judged to have occult HBV infection), antiviral drugs were administered before initiating systemic chemotherapy. Finally, 24 of 31 patients with resolved HBV infection underwent regular HBV DNA monitoring (Fig. 1). For these 24 ATL patients, initial systemic chemotherapy included the following regimens: CHOP (n = 7, 29.2 %), VCAP-AMP-VECP (n = 13, 54.2 %) and others (n = 4, 16.6 %) (Table 1). Systemic chemotherapy was started in 6

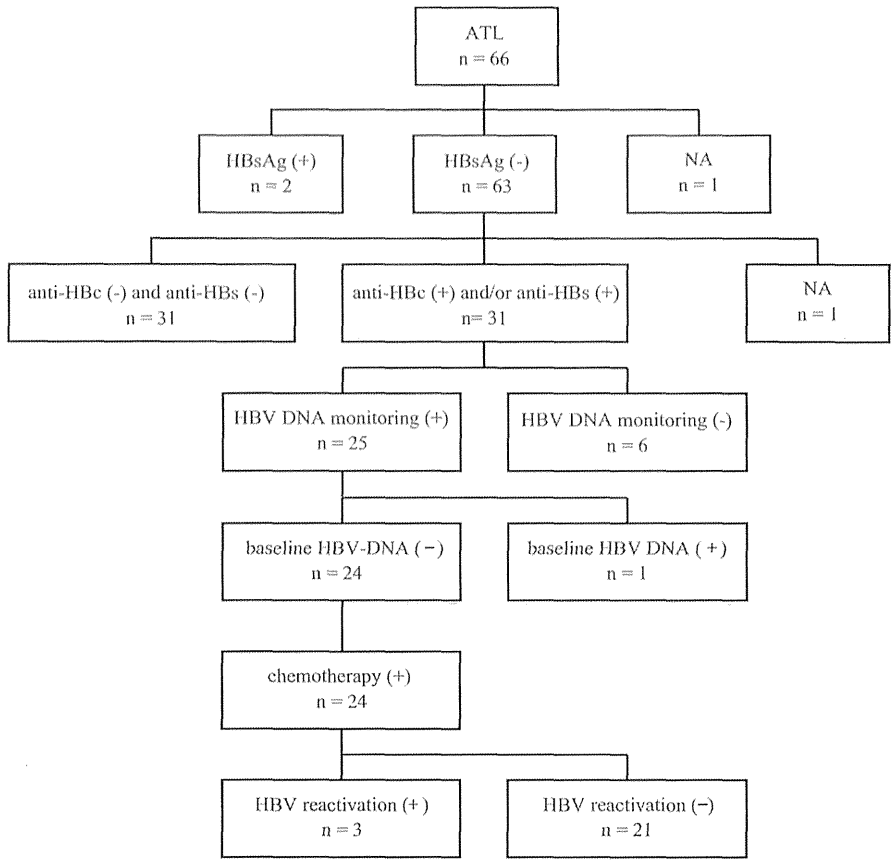

Fig. 1 Baseline serological markers of HBV infection in the 66 ATL patients. Two patients were HBsAg-positive, 63 were HBsAg-negative, the last was not available for serological HBV assessment. Of the 63 HBsAg-negative patients, 31 were anti-HBc-positive and/or anti-HBs-positive. One patient had detectable HBV DNA at baseline, and was judged as having occult HBV infection. Regular HBV DNA monitoring was performed in 24 of 31 patients with resolved HBV infection and 3 patients suffered HBV reactivation. *HBV* hepatitis B virus, *ATL* adult T-cell leukemia–lymphoma, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibodies against hepatitis B core antigen, *anti-HBs* antibodies against hepatitis B surface antigen, *NA* not available

patients before HBV DNA monitoring. For the 24 patients with resolved HBV infection during and after systemic chemotherapy, regular monitoring of HBV DNA was conducted with a median interval of 30 days (range 2–703).

HBV reactivation was observed in 3 (12.5 %) of 24 patients with resolved HBV infection, with a median HBV DNA follow-up of 238 days (range 57–1420). No hepatitis due to HBV reactivation occurred in those patients who were diagnosed with HBV DNA levels below 2.1 log copies/mL and who received antiviral drugs (entecavir, 0.5 mg/day), resulting in no detectable HBV DNA levels during antiviral treatment.

There was no statistically significant difference in baseline characteristics and ATL treatment between patients with and without reactivation in this retrospective analysis (Table 1). The characteristics of 3 patients with HBV reactivation are shown in Table 2; all were male, and seropositive for anti-HBc and anti-HBs at baseline, and received the VCAP-AMP-VECP regimen as initial treatment. Mogamulizumab was administered prior to HBV reactivation in 2 of 3 HBV-reactivated patients. The anti-HBs titers of 3 patients decreased at reactivation compared to baseline titers in 3 patients. Their HBV genotypes were determined as C. HBV mutations were not found in the precore region or basal core promoter. One patient died due to ATL progression.

The clinical course of case 1 is shown in Fig. 2. HBV reactivation was confirmed with HBV DNA levels below 2.1 log copies/mL, 3 months after initiating mogamulizumab-containing chemotherapy as initial treatment for ATL. The patient presented with elevation of transaminase levels after detection of HBV DNA, it considered not viral hepatitis, but drug-induced liver damage because of transient and slight increase of HBV DNA levels. Reemergence of HBV was observed repeatedly after withdrawal of antiviral drugs following the development of drug-induced allergic rash or interstitial pneumonia. The patient maintains complete remission of ATL with undetectable of HBV DNA after withdrawal of antiviral drugs over 3 years after mogamulizumab-containing chemotherapy.

Discussion

This study showed that the incidence of HBV reactivation among ATL patients with resolved HBV infection who received systemic chemotherapy was 12.5 %. Preemptive antiviral therapy, guided by regular HBV DNA monitoring,

Table 1 Baseline characteristics and treatment of 24 ATL patients with resolved HBV infection who underwent HBV DNA monitoring following systemic chemotherapy

	HBV reactivation (+) $n = 3$	HBV reactivation (−) $n = 21$	p value
Median age (range)	59 (58–65)	64 (41–77)	0.822
Sex			0.217
Male	3	9	
Female	0	12	
ATL type of disease			0.090
Acute	1	17	
Lymphoma	2	1	
Chronic	0	2	
Smoldering	0	1	
ECOG performance status			0.530
0 or 1	3	14	
2 or more	0	7	
Baseline HBV status			1.00
Anti-HBc positive and anti-HBs positive	3	18	
Anti-HBc positive and anti-HBs negative	0	3	
Anti-HBc negative and anti-HBs positive	0	0	
Baseline anti-HBs titers (mIU/mL)			0.728
<10	0	3	
≥10, <100	2	8	
≥100	1	10	
Initial chemotherapy regimen[a]			0.396
CHOP	0	7	
VCAP-AMP-VECP	3	10	
Others	0	4	
Mogamulizumab administration[b]			0.576
(+)	2	9	
(−)	1	12	
Allogeneic HSCT[c]			1.00
(+)	1	5	
(−)	2	16	
Year enrolled for HBV DNA monitoring			–
2005–2006	0	0	
2006–2008	0	4	
2008–2009	0	3	
2009–2013	3	14	
Median HBV DNA follow-up time (range)[d]	640 (637–1030)	227 (57–1420)	–

HBV hepatitis B virus, *ATL* adult T-cell leukemia–lymphoma, *ECOG* Eastern Cooperative Oncology Group, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibodies against hepatitis B core antigen, *anti-HBs* antibodies against hepatitis B surface antigen, *CHOP* cyclophosphamide, doxorubicin, vincristine, prednisolone, *VCAP-AMP-VECP* VCAP (vincristine, cyclophosphamide, doxorubicin, prednisolone)-AMP (doxorubicin, ranimustine, prednisolone)-VECP (vindesine, etoposide, carboplatin, prednisolone), *HSCT* hematopoietic stem cell transplantation

[a] Initial chemotherapy regimen for adult T-cell leukemia–lymphoma was given during HBV DNA monitoring

[b] In 2 of 3 HBV-reactivated cases, mogamulizumab was given prior to HBV reactivation

[c] One patient received allogeneic hematopoietic stem transplantation after HBV reactivation

[d] HBV DNA follow-up time indicates the time from the date of baseline HBV DNA measurement until the date of the last HBV DNA measurement

was effective in preventing hepatitis due to HBV reactivation in all three patients. Most of HBV reactivation has been reported to occur in B-cell lymphoma, especially in those who received rituximab-containing chemotherapy [2–4, 6]. This is the first report regarding the risk of HBV reactivation focused on ATL patients with resolved HBV infection, which suggesting that the risk of HBV reactivation in ATL patients may be similar to that in B-cell lymphoma patients [15, 16].

ATL is a mature T-cell lymphoma and human T-cell leukemia virus type-1 plays a role in its pathogenesis. Aggressive ATL has been reported to have a poor prognosis with a median overall survival of approximately 1 year, regardless of intensive chemotherapy [17]. The anti-CCR4 monoclonal antibody, mogamulizumab has been shown recently to be effective and safe for aggressive ATL patients in the setting of monotherapy or combined with conventional chemotherapy [9, 11, 18]. It is expected that mogamulizumab will enable long-term disease control, so more HBV reactivation events may be predicted because CCR4 is a chemokine receptor expressed on T-helper type 2 and regulatory T cells [7, 19], and is thought to have an important

Table 2 Characteristics of 3 patients with HBV reactivation

	Case 1	Case 2	Case 3
Age	65	59	58
Sex	Male	Male	Male
Type of ATL	Lymphoma	Lymphoma	Acute
ECOG performance status	1	1	0
Baseline HBV status			
HBsAg	(−)	(−)	(−)
Anti-HBc titers	98.1 %	3.6 C.O.I	1.5 C.O.I
Anti-HBs titers	20.0 mIU/mL	24.0 mIU/mL	>1000.0 mIU/mL
HBV DNA levels	Not detectable	Not detectable	Not detectable
Chemotherapy regimens before HBV reactivation			
	VCAP-AMP-VECP plus mogamulizumab	VCAP-AMP-VECP	VCAP-AMP-VECP Mogamulizumab CHOP DeVIC etc.
Number of regimens	1	1	7
Allogeneic HSCT[a]	No	Yes	No
After HBV reactivation			
Time to reactivation (day)[b]	90	71	541
HBV DNA levels at reactivation (log copies/mL)	<2.1	<2.1	<2.1
Peak HBV DNA levels (log copies/mL)	2.3	<2.1	<2.1
Anti-HBs titers	17.6 mIU/mL	22.0 mIU/mL	566.5 mIU/mL
HBV genotype	C	C	C
HBV mutation of precore region or basal core promoter	Wild	Wild	NA
Antiviral drugs	Entecavir, lamivudine	Entecavir	Entecavir
Hepatitis due to HBV reactivation	No	No	No
HBV DNA follow-up time (day)[c]	1030	640	637
Outcome	Alive (CR1)	Alive (CR1)	Death due to ATL progression

HBV hepatitis B virus, *ATL* adult T-cell leukemia–lymphoma, *ECOG* Eastern Cooperative Oncology Group, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibodies against hepatitis B core antigen, *anti-HBs* antibodies against hepatitis B surface antigen, *VCAP-AMP-VECP* VCAP (vincristine, cyclophosphamide, doxorubicin, prednisolone)-AMP (doxorubicin, ranimustine, prednisolone)-VECP (vindesine, etoposide, carboplatin, prednisolone), *CHOP* cyclophosphamide, doxorubicin, vincristine, prednisolone, *DeVIC* dexamethasone, etoposide, ifosfamide, carboplatin, *CR1* first complete response

[a] One patient (case 2) received allogeneic hematopoietic stem transplantation after HBV reactivation

[b] Time to reactivation indicates the time from the date of baseline HBV DNA measurement until the date of the confirmation of HBV reactivation

[c] HBV DNA follow-up time indicates the time from the date of baseline HBV DNA measurement until the date of the last HBV DNA measurement

role in maintaining the balance of the human immune system. The mechanism whereby mogamulizumab causes HBV reactivation is not fully understood; a reduction of numbers of CCR4-expressing cells following this antibody treatment might be associated with an imbalance of antiviral immunity, resulting in the development of HBV reactivation [9, 13]. Although HBV reactivation was confirmed in 2 of 11 patients who received mogamulizumab, this study did not prove that HBV reactivation is associated with mogamulizumab therapy, partly because of the small sample size.

This study has the following limitations: a retrospective study in a single institution with a small sample size, and the diagnosis of HBV reactivation at early stage when only when HBV DNA became detectable (below 2.1 log copies/mL) by PCR. Because antiviral treatments after the onset of hepatitis are often insufficient to control HBV reactivation, preemptive antiviral therapy guided by regular HBV DNA monitoring, whereby the antiviral drug is given immediately when HBV DNA becomes detectable, is recommended by some guidelines to prevent hepatitis due to HBV reactivation [20, 21]. However, the definition of HBV reactivation and cut-off values of HBV DNA levels, along with the timing of initiation of antiviral treatment in patients with resolved HBV infection, have not been fully investigated yet.

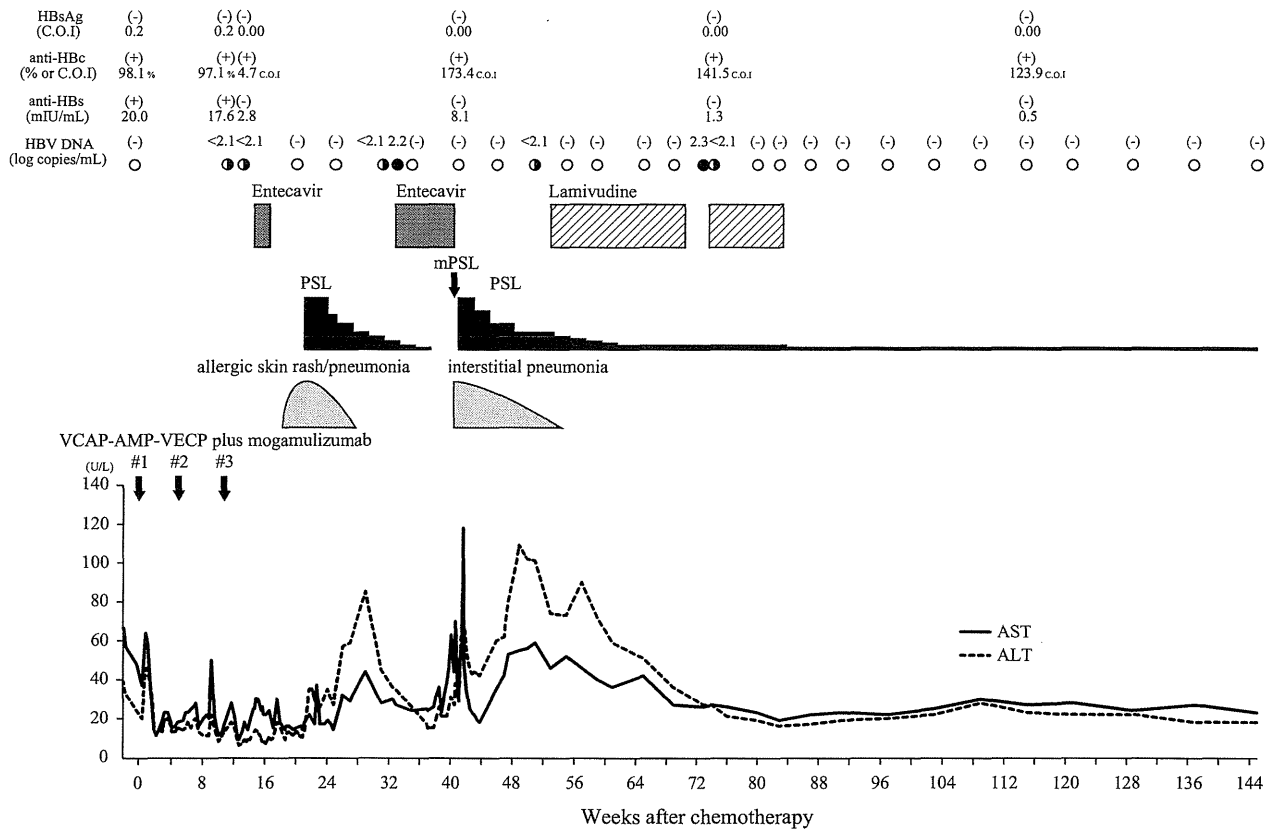

Fig. 2 Clinical course of case 1. A 65-year-old male was diagnosed as having adult T-cell leukemia–lymphoma of lymphoma type and received VCAP-AMP-VECP plus mogamulizumab combined chemotherapy. At 3 months after systemic chemotherapy, HBV reactivation was confirmed with HBV DNA levels below 2.1 log copies/mL and antiviral therapy (entecavir, 0.5 mg/day) was given immediately with no HBV-related hepatitis. He presented with elevation of transaminase levels after detection of HBV DNA, it considered not viral hepatitis but drug-induced liver damage because of transient and slight increase of HBV DNA levels. Because he suffered from an allergic rash and interstitial pneumonia (IP), entecavir could not be continued. Consequently, reemergence of HBV (HBV DNA levels of 2.2 log copies/mL) was observed at 3 months after the first detection of HBV reactivation. However, he discontinued entecavir because of the occurrence of IP, and HBV reactivation was again observed. Lamivudine was given for HBV reactivation, but was discontinued due to mild renal dysfunction, which resulted again in replication of HBV (HBV DNA levels of 2.3 log copies/mL) at 18 months after initiating mogamulizumab-containing chemotherapy. The *open circles* show undetectable PCR signals during HBV DNA monitoring; the *half-filled circles* show PCR signals indicating HBV DNA levels below 2.1 log copies/mL; and the *filled circles* show detectable PCR signals indicating HBV DNA levels of 2.1 log copies/mL or more. *HBV* hepatitis B virus, *PSL* prednisolone, *mPSL* methylprednisolone, *AST* aspartate transaminase, *ALT* alanine aminotransferase, *VCAP-AMP-VECP* VCAP (vincristine, cyclophosphamide, doxorubicin, prednisolone)-AMP (doxorubicin, ranimustine, prednisolone)-VECP (vindesine, etoposide, carboplatin, prednisolone)

In conclusion, the incidence of HBV reactivation was 12.5 % in ATL patients with resolved HBV infection following systemic chemotherapy. In mogamulizumab era, further well-designed prospective studies are warranted to estimate the incidence of HBV reactivation and to establish regular HBV DNA monitoring-guided preemptive antiviral therapy for these patients.

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bjh research paper

Dose-intensified chemotherapy alone or in combination with mogamulizumab in newly diagnosed aggressive adult T-cell leukaemia-lymphoma: a randomized phase II study

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Summary

This multicentre, randomized, phase II study was conducted to examine whether the addition of mogamulizumab, a humanized anti-CC chemokine receptor 4 antibody, to mLSG15, a dose-intensified chemotherapy, further increases efficacy without compromising safety of patients with newly diagnosed aggressive adult T-cell leukaemia-lymphoma (ATL). Patients were assigned 1:1 to receive mLSG15 plus mogamulizumab or mLSG15 alone. The primary endpoint was the complete response rate (%CR); secondary endpoints included the overall response rate (ORR) and safety. The %CR and ORR in the mLSG15-plus-mogamulizumab arm ($n = 29$) were 52% [95% confidence interval (CI), 33–71%] and 86%, respectively; the corresponding values in the mLSG15 arm ($n = 24$) were 33% (95% CI, 16–55%) and 75%, respectively. Grade ≥ 3 treatment-emergent adverse events, including anaemia, thrombocytopenia, lymphopenia, leucopenia and decreased appetite, were observed more frequently (≥10% difference) in the mLSG15-plus-mogamulizumab arm. Several adverse events, including skin disorders, cytomegalovirus infection, pyrexia, hyperglycaemia and interstitial lung disease, were observed only in the mLSG15-plus-mogamulizumab arm. Although the combination strategy showed a potentially less favourable safety profile, a higher %CR was achieved, providing the basis for further investigation of this novel treatment for newly diagnosed aggressive ATL. This study was registered at ClinicalTrials.gov, identifier: NCT01173887.

Keywords: adult T-cell leukaemia-lymphoma, CCR4, mogamulizumab, randomized phase II study, antibody therapy.

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Adult T-cell leukaemia-lymphoma (ATL) is an aggressive, peripheral T-cell neoplasm caused by human T-cell lymphotropic virus type I (Uchiyama *et al*, 1977; Matsuoka & Jeang, 2007), and is classified into four clinical subtypes: smouldering, chronic, lymphoma and acute (Shimoyama, 1991). Intensive chemotherapy has been recommended for patients with newly diagnosed acute lymphoma or with unfavourable chronic subtypes of ATL (i.e. aggressive ATL) (Tsukasaki *et al*, 2009). A phase III trial was performed in previously untreated patients with aggressive ATL to compare the effects of a dose-intensified multidrug regimen, namely the modified LSG15 (mLSG15) regimen (VCAP-AMP-VECP: vincristine, cyclophosphamide, doxorubicin and prednisolone; doxorubicin, ranimustine and prednisolone; vindesine, etoposide, carboplatin and prednisolone) (Yamada *et al*, 2001) with the effects of CHOP-14 (cyclophosphamide, doxorubicin, vincristine and prednisolone). The complete response rate (% CR) was higher in the mLSG15 arm (40%) than in the CHOP-14 arm (25%; $P = 0{\cdot}020$). The overall survival (OS) rates at 3 years were 24% and 13% in the mLSG15 and CHOP-14 arms, respectively, with a significant difference ($P = 0{\cdot}028$) observed between the two arms after adjustment for imbalances in baseline prognostic factors (Tsukasaki *et al*, 2007). However, the median survival time of 12·7 months in the mLSG15 arm (CHOP-14 arm, 10·9 months) was lower than that observed for other haematological malignancies. Moreover, allogeneic haematopoietic cell transplantation (allo-HCT) has been explored as a promising treatment for ATL, and it has been reported that allo-HCT can potentially provide cures for 30–40% of transplant recipients. However, only few ATL patients benefit from transplantation, such as those who are younger, achieve sufficient disease control and have an appropriate stem cell source (Hishizawa *et al*, 2010; Ishida *et al*, 2012a).

Because CC chemokine receptor 4 (CCR4) is expressed on the surface of the tumour cells of most patients with ATL (Yoshie *et al*, 2002; Ishida *et al*, 2003), it has been postulated to represent a novel molecular target for immunotherapy for ATL. Therefore, a humanized anti-CCR4 monoclonal antibody with a defucosylated Fc region, mogamulizumab (KW-0761) was developed, and has been shown to markedly enhance antibody-dependent cellular cytotoxicity (Shinkawa *et al*, 2003; Ishii *et al*, 2010). A phase I clinical study of mogamulizumab was performed in patients with relapsed CCR4-positive peripheral T-cell lymphoma (PTCL), including ATL (Yamamoto *et al*, 2010). This study showed good tolerability, predictable pharmacokinetics and preliminary evidence of the antitumour activity of mogamulizumab, and the recommended dose was determined to be 1·0 mg/kg (Yamamoto *et al*, 2010). In the subsequent phase II study, mogamulizumab monotherapy showed an overall response rate (ORR) of 50% in patients with relapsed ATL, with an acceptable toxicity profile (Ishida *et al*, 2012b). Accordingly, mogamulizumab was approved in Japan in 2012 for patients with CCR4-positive relapsed/refractory ATL.

Herein, we report the results of a multicentre, randomized phase II study, the aim of which was to evaluate whether or not the addition of mogamulizumab to mLSG15 increases efficacy without compromising safety for patients with newly diagnosed aggressive ATL.

Patients and methods

Patients

Eligible patients included those newly diagnosed with CCR4-positive aggressive ATL who were aged ≥20 years. CCR4 expression was determined by using immunohistochemistry or flow cytometry with a mouse anti-CCR4 monoclonal antibody (KM2160) (Ishida *et al*, 2003; Yamamoto *et al*, 2010) and confirmed by a central review committee. All patients were required to have an Eastern Cooperative Oncology Group performance status of 0–2. Furthermore, the eligibility criteria included the following laboratory parameters: abso-

lute neutrophil count $\geq 1{\cdot}5 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l, haemoglobin level ≥80 g/l, aspartate aminotransferase level ≤2·5 × the upper limit of the normal range (ULN), alanine aminotransferase level ≤2·5 × ULN, total bilirubin level ≤2·0 mg/dl, serum creatinine level ≤ 1·3 mg/dl, and arterial partial oxygen pressure ≥65 mmHg or arterial blood oxygen saturation ≥93%. Patients were excluded if they had a severe infection, a history of organ transplantation, active concurrent cancer, central nervous system involvement, a bulky mass requiring emergent radiotherapy, or seropositivity for hepatitis B virus surface antigen, hepatitis C virus antibody or human immunodeficiency virus antibody.

Randomization and masking

Eligible patients were randomly assigned in a 1:1 ratio to the two treatment groups based on dynamic allocation and minimization (Pocock & Simon, 1975) by a central randomization centre (Bell Medical Solutions, Inc., Tokyo, Japan). For randomization, the first stratification factor was clinical subtype, and the second was age (<56 or ≥56 years). The study had an open-label design.

Procedures

This was a multicentre, randomized, phase II study to compare the efficacy and safety of mLSG15 plus mogamulizumab with that of mLSG15 alone. Subjects assigned to the mLSG15-plus-mogamulizumab arm received eight intravenous 1·0 mg/kg mogamulizumab infusions during four mLSG15 cycles. Typically, mogamulizumab was administered the day before VCAP and VECP administration except for the first VCAP administration (Fig 1). When VCAP or VECP administration was delayed for any reason, mogamulizumab administration was delayed accordingly.

The primary endpoint was %CR, and the secondary endpoints included ORR, %CR and response rate according to disease site; progression-free survival (PFS); OS and safety. We estimated that 22 patients per arm would be required to achieve an 80% probability of detecting a higher %CR in the mLSG15-plus-mogamulizumab arm than in the mLSG15 arm, based on the selection design (Simon *et al*, 1985). We assumed that an increased %CR of 15% achieved upon adding mogamulizumab would imply clinical significance. This 15% increase in the %CR corresponded to the difference observed between mLSG15 and CHOP-14, with a previous phase III study showing that the former treatment prolonged OS (Tsukasaki *et al*, 2007). Thus, if the true difference is 15%, there is an 80% chance of selecting the right treatment when one chooses the treatment with the higher CR rate. Objective responses were assessed after the second and fourth chemotherapy cycles in each arm by an independent efficacy assessment committee according to the modified response criteria for ATL (Tsukasaki *et al*, 2009). Adverse events (AEs) were graded according to the National Cancer Institute's Common Terminology Criteria for AEs version 4·0 (http://evs.nci.nih.gov/ftp1/CTCAE/Archive/CTCAE_4.02_2009-09-15_QuickReference_8.5x11.pdf), and were summarized according to the Medical Dictionary for Regulatory Activities System Organ Class and preferred terms. The presence of human anti-mogamulizumab antibodies in plasma was also determined. Blood samples were collected from

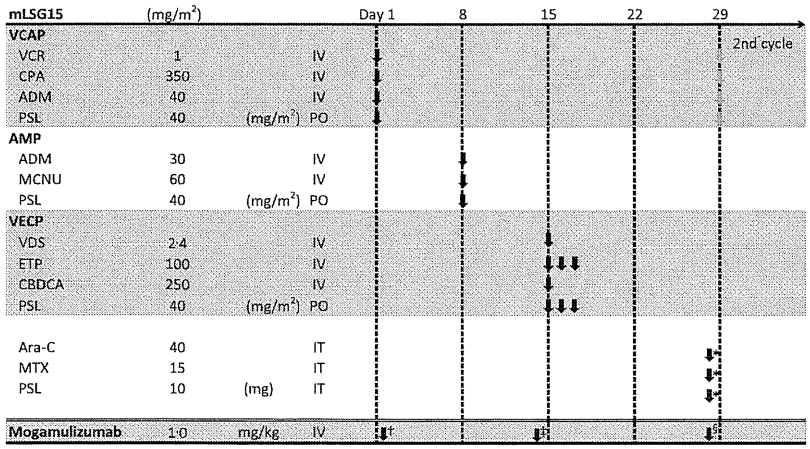

Fig 1. Treatment protocol. The mLSG15 protocol consists of three chemotherapeutic regimens, namely VCAP, AMP and VECP. Subjects assigned to the mLSG15-plus-mogamulizumab arm received up to eight infusions of mogamulizumab during four cycles of mLSG15. Cytarabine, methotrexate and prednisolone were intrathecally injected before initiation of VCAP administration in cycles 2 and 4. VCAP: vincristine, cyclophosphamide, doxorubicin, and prednisolone; AMP: doxorubicin, ranimustine, and prednisolone; VECP: vindesine, etoposide, carboplatin, and prednisolone; IV, intravenous; PO, per os (oral administration); IT, intrathecal; VCR, vincristine; CPA, cyclophosphamide; ADM, doxorubicin; PSL, prednisolone; MCNU, ranimustine; VDS, vindesine; ETP, etoposide; CBDCA, carboplatin; Ara-C, cytarabine; MTX, methotrexate. *Before cycles 2 and 4 (Days −2 to −1). †After VCAP in Cycle 1 (Days 2 to 5). ‡Preceding VECP in Cycles 1–4 (Days 12 to 14). §Preceding VCAP in Cycles 2–4 (Days −3 to −1).

patients who had received at least one dose of mogamulizumab at time points determined by the protocol for the pharmacokinetic analysis. The maximum drug concentration (C_{max}) and trough drug concentration (C_{trough}) for each mogamulizumab administration were calculated. We also investigated the distributions of blood T-cell subsets (CD4/CD25/CCR4-positive cells and CD4/CD25/FOXP3-positive cells) during and after treatment in each arm.

Statistical analysis

Survival estimates were calculated by using the Kaplan–Meier method. PFS was defined as the time from the day of starting the protocol treatment to progression, relapse, or death from any cause. OS was measured from the day of starting the protocol treatment to death from any cause. The numbers of T-cell subsets in the two arms were compared by employing the Wilcoxon signed-rank test for each sampling point at a significance level of 0·05.

Study oversight

The study was sponsored by Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan. The academic investigators and the sponsor were jointly responsible for the study design. The protocol was approved by the institutional review boards at each participating site and all patients provided written informed consent before enrolment, in accordance with the Declaration of Helsinki.

Results

Patients

Between August 2010 and September 2011, 54 patients with newly diagnosed aggressive ATL were enrolled at 18 institutions. Of these 54 patients, 29 in the mLSG15-plus-mogamulizumab arm and 24 in the mLSG15 arm received treatment according to our study protocol. One patient assigned to the mLSG15 arm was withdrawn from the study, owing to the patient's treatment having to be deferred due to abnormal laboratory values that met the protocol criteria, and the patient was unable to wait for the protocol treatment due to deterioration of his/her general condition. The demographics and characteristics of the remaining 53 patients are summarized in Table I. Fifteen patients in the mLSG15-plus-mogamulizumab arm did not complete the planned treatment; of these, seven dropped out because of AEs, including infectious diseases; four dropped out because of progressive disease (PD); and the remaining four dropped out for different reasons, including withdrawal of consent and start of an alternative treatment. Thirteen patients in the mLSG15 arm did not complete the planned treatment; among these, four had AEs, four had PD, and the remaining five dropped out for other reasons (Fig 2).

Table I. Demographics and clinical characteristics.

	mLSG15 + mogamulizumab (n = 29)	mLSG15 (n = 24)*
ATL subtype		
Acute	20 (69%)	17 (71%)
Lymphoma	6 (21%)	7 (29%)
Chronic†	3 (10%)	0 (0%)
Age, years		
Median	61	64
Range	49–81	37–74
<56	11 (38%)	6 (25%)
≥56	18 (62%)	18 (75%)
Sex		
Male	12 (41%)	16 (67%)
Female	17 (59%)	8 (33%)
ECOG PS		
0	16 (55%)	13 (54%)
1	10 (35%)	9 (38%)
2	3 (10%)	2 (8%)

ECOG, Eastern Cooperative Oncology Group; PS, performance status.
*25 patients were randomized; 24 were treated.
†Chronic type with poor prognostic factors.

Efficacy

Of the 29 and 24 patients evaluable for efficacy in the mLSG15-plus-mogamulizumab and the mLSG15 arms, 25 patients [ORR, 86%; 95% confidence interval (CI), 68–96%] and 18 patients (ORR, 75%; 95% CI, 53–90%), respectively, had objective responses. The %CR, including unconfirmed CR, was higher in the mLSG15-plus-mogamulizumab arm (52%; 95% CI, 33–71%) than in the mLSG15 arm (33%; 95% CI, 16–55%), with a between-group difference of 18·4% (95% CI, −8·9 to 43·8%; Table II). The %CR according to the disease site in the mLSG15-plus-mogamulizumab and mLSG15 arms were 100% (14/14) and 43% (3/7) for blood, 92% (24/26) and 73% (16/22) for nodal and extranodal lesions and 50% (4/8) and 60% (3/5) for skin lesions, respectively. The response rate according to the disease site in the mLSG15-plus-mogamulizumab and mLSG15 arms were 100% (14/14) and 100% (7/7) for blood, 92% (24/26) and 77% (17/22) for nodal and extranodal lesions and 75% (6/8) and 80% (4/5) for skin lesions, respectively. The median PFS in the mLSG15-plus-mogamulizumab and mLSG15 arms were 8·5 months and 6·3 months, respectively (Fig 3A). The median OS was not reached in either arm (Fig 3B).

AEs

The treatment-emergent AEs (TEAEs) of ≥grade 3 that occurred in at least two patients are listed in Table III. The most common TEAEs of any grade in the mLSG15-plus-mogamulizumab arm were neutropenia (100%), thrombocytopenia (100%), leucopenia (100%), lymphopenia (97%),

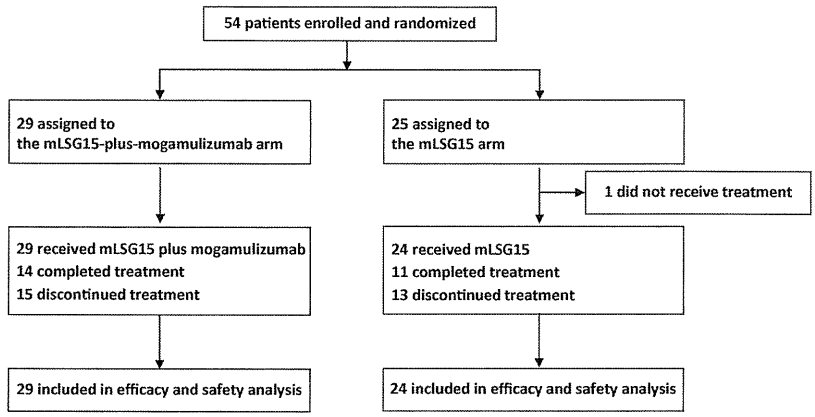

Fig 2. CONSORT diagram. Patients with newly diagnosed CC chemokine receptor 4 - positive aggressive adult T-cell leukaemia-lymphoma were assigned in a 1:1 ratio to receive treatment with mLSG15 plus mogamulizumab or mLSG15 alone. One patient assigned to the mLSG15 arm was withdrawn from the study, owing to the patient's treatment having to be deferred due to abnormal laboratory values that met the protocol criteria, and the patient was unable to wait for the protocol treatment due to deterioration of their general condition.

Table II. Response to treatment.

	mLSG15 + mogamulizumab (n = 29)	mLSG15 (n = 24)
CR	9	5
CRu	6	3
PR	10	10
CR + CRu	15	8
% CR (95% CI)	52% (33–71)	33% (16–55)
Between-group difference (95% CI)	18·4% (−8·9 to 43·8)	
CR + CRu + PR	25	18
ORR (95% CI)	86% (68–96)	75% (53–90)

CR, complete response; CRu, uncertified complete response; PR, partial response; %CR, complete response rate; CI, confidence interval; ORR, overall response rate.

anaemia (97%) and febrile neutropenia (90%). The corresponding percentages in the mLSG15 arm were 96%, 96%, 92%, 96%, 92% and 88%, respectively. The following TEAEs of grade ≥ 3 were more frequently observed (≥10% difference) in the mLSG15-plus-mogamulizumab arm than in the mLSG15 arm: anaemia (97% vs. 79%), thrombocytopenia (90% vs. 71%), lymphopenia (97% vs. 75%), leucopenia (100% vs. 88%) and decreased appetite (28% vs. 13%). Papular rash (21%), hyperglycaemia (14%), pyrexia (14%), interstitial lung disease (10%), erythematous rash (7%), cytomegalovirus infection (7%) cytomegaloviral pneumonia (7%) and oxygen saturation decreased (7%) occurred only in the mLSG15-plus-mogamulizumab arm.

Twenty serious AEs (SAEs) were reported in 12 patients in the mLSG15-plus-mogamulizumab arm. These included pneumonia in two patients, cytomegalovirus infection in two, interstitial lung disease in two, and the following events occurred in one patient each: febrile neutropenia, septic shock, cytomegaloviral pneumonia, pneumonitis, generalized erythema, viral encephalitis, oral disorder, bacteraemia, infection, exfoliative rash, ileus, cholecystitis, haemorrhagic cystitis and disease progression. The patient with septic shock did not recover and ultimately died. Another patient with haemorrhagic cystitis, which was suspected to be due to a viral infection, showed disease progression and died during the follow-up period due to the haemorrhagic cystitis as an SAE. The remaining 17 SAEs in the mLSG15-plus-mogamulizumab arm all improved or resolved.

Eleven SAEs were reported in nine patients in the mLSG15 arm. These included two patients with bacteraemia, and the following events in one patient each: infection, enterocolitis, pneumonia, soft tissue inflammation, myelodysplastic syndrome, ischaemic colitis, herpes zoster, neurogenic bladder and febrile neutropenia. The outcomes of all SAEs in the mLSG15 arm, with the exception of myelodysplastic syndrome, improved or resolved. There were no deaths during the treatment or follow-up period in the mLSG15 arm.

Pharmacokinetics and immunogenicity

Of the 29 patients enrolled in the mLSG15-plus-mogamulizumab arm, 16 (55%) completed the eight doses of mogamulizumab. The C_{max} (at the end of the eighth infusion) and C_{trough} (14 days after the eighth infusion) of mogamulizumab were 22·8 ± 4·6 and 94 ± 3·8 μg/ml (mean ± SD), respectively. None of the patients developed detectable levels of anti-mogamulizumab antibodies.

T-cell subset analysis

The numbers of circulating CD4/CD25/CCR4-positive cells in the blood immediately before VCAP therapy for cycle three in the mLSG15-plus-mogamulizumab arm (mean, 0·0246 × 10^9/l; median, 0·015 × 10^9/l; range, 0·004–0·094 × 10^9/l) were significantly lower than those in the mLSG15 arm (mean, 0·4693 × 10^9/l; median, 0·234 × 10^9/l; range, 0·077–3·991 × 10^9/l) (P < 0·001). The corresponding numbers of these cells 28 days after VECP therapy (Cycle 4) in the mLSG15-plus-mogamulizumab arm (0·0173 × 10^9/l; 0·0095 × 10^9/l; 0·001–0·133 × 10^9/l) were significantly lower

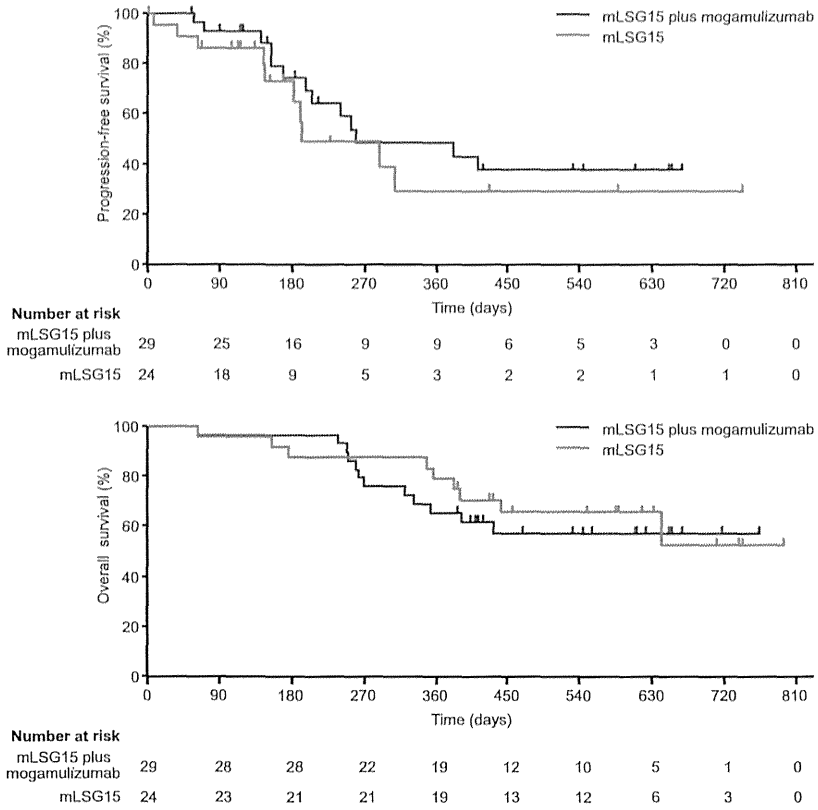

Fig 3. Progression-free survival and overall survival. (A) Kaplan–Meier curve of estimated progression-free survival (median, 8·5 months and 6·3 months in the mLSG15-plus-mogamulizumab and mLSG15 arms, respectively). (B) Kaplan–Meier curve of estimated overall survival (median, not achieved in either arm). The median follow-up periods in the mLSG15-plus-mogamulizumab and mLSG15 arms were 413 days (range, 63–764 days) and 502 days (range, 62–794 days), respectively.

than those in the mLSG15 arm (0·1478 × 10^9/l; 0·133 × 10^9/l; 0·059–0·368 × 10^9/l) ($P < 0·001$) (Fig 4A). Similarly, the numbers of CD4/CD25/FOXP3-positive cells in the blood immediately before VCAP therapy (Cycle 3) in the mLSG15-plus-mogamulizumab arm (0·0085 × 10^9/l; 0·004 × 10^9/l; 0–0·048 × 10^9/l) were significantly lower than those in the mLSG15 arm (0·2432 × 10^9/l; 0·074 × 10^9/l; 0·018–2·77 × 10^9/l) ($P < 0·001$), and the numbers of these cells 28 days after VECP therapy (Cycle 4) in the mLSG15-plus-mogamulizumab arm (0·0054 × 10^9/l; 0·003 × 10^9/l; 0–0·037 × 10^9/l) were significantly lower than those in the mLSG15 arm (0·0684 × 10^9/l; 0·0435 × 10^9/l; 0·016–0·25 × 10^9/l) ($P < 0·001$, Fig 4B).

Discussion

This study showed that the %CR in patients who received mLSG15 plus mogamulizumab was higher than that obtained in those treated with mLSG15 alone (52% vs. 33%; difference, 18·4%). The increase in the %CR with the addition of mogamulizumab observed in this study surpassed the predicted, targeted, clinically significant 15% increase in patients with ATL. Importantly, the %CR in patients with lesions in the blood compartment was higher in the combination arm, leading to the increase in overall %CR. This finding was consistent with that observed in previous studies, in which ATL lesions in the blood were found to be more sensitive to mogamulizumab monotherapy than ATL lesions at other disease sites (Yamamoto *et al*, 2010; Ishida *et al*, 2012b).

Infections were more frequent in the combination arm. In particular, cytomegalovirus infection was observed in 14% of patients in the combination arm, whereas it was not observed in the chemotherapy alone arm. Furthermore, cytomegalovirus-related SAEs occurred in three patients in the combination arm. Cytomegalovirus reactivation is observed in approximately 60% of patients with ATL during systemic chemotherapy (Ogata *et al*, 2011). Our study suggests that the addition of mogamulizumab to systemic chemotherapy might further increase the incidence of cytomegalovirus infection; therefore, careful monitoring for cytomegalovirus infection and appropriate use of antiviral therapy are recommended when systemic chemotherapy in combination with mogamulizumab is administered to patients with ATL.

In our previous study of mogamulizumab monotherapy for patients with relapsed ATL, skin rashes, including Stevens–Johnson syndrome, were the most frequently observed AEs (63%) (Ishida *et al*, 2012b, 2013). In the present study, as expected, AEs involving skin and subcutaneous tissue disorders were more frequent in the combination arm than in the chemotherapy alone arm. Even though no severe skin-related AEs, such as Stevens–Johnson syndrome or toxic epidermal necrolysis, occurred in the present study, special attention should be paid to these skin-related AEs

Table III. Treatment-emergent adverse events in the mLSG15-plus-mogamulizumab (n = 29) and mLSG15 (n = 24) arms.

	All grades		≥Grade3	
	mLSG15 + mogamulizumab n = 29	mLSG15 n = 24	mLSG15 + mogamulizumab n = 29	mLSG15 n = 24
Blood and lymphatic system disorders	29 (100%)	22 (92%)	29 (100%)	22 (92%)
Anaemia	28 (97%)	22 (92%)	28 (97%)	19 (79%)
Febrile neutropenia	26 (90%)	21 (88%)	26 (90%)	21 (88%)
Gastrointestinal disorders	29 (100%)	23 (96%)	7 (24%)	7 (29%)
Stomatitis	16 (55%)	13 (54%)	4 (14%)	4 (17%)
General disorders and administration site conditions	29 (100%)	21 (88%)	6 (21%)	0 (0%)
Pyrexia	24 (83%)	15 (63%)	4 (14%)	0 (0%)
Infections and infestations	19 (66%)	16 (67%)	10 (34%)	7 (29%)
Bacteraemia	4 (14%)	3 (13%)	3 (10%)	3 (13%)
Pneumonia	4 (14%)	2 (8%)	3 (10%)	1 (4%)
Cytomegalovirus infection	4 (14%)	0 (0%)	2 (7%)	0 (0%)
Cytomegaloviral pneumonia	2 (7%)	0 (0%)	2 (7%)	0 (0%)
Investigations	29 (100%)	24 (100%)	29 (100%)	24 (100%)
Neutropenia	29 (100%)	23 (96%)	29 (100%)	22 (92%)
Thrombocytopenia	29 (100%)	23 (96%)	26 (90%)	17 (71%)
Lymphopenia	28 (97%)	23 (96%)	28 (97%)	18 (75%)
Leucopenia	29 (100%)	22 (92%)	29 (100%)	21 (88%)
Albuminaemia	12 (41%)	11 (46%)	2 (7%)	1 (4%)
Alanine transaminase increased	12 (41%)	10 (42%)	2 (7%)	2 (8%)
Aspartate transaminase increased	9 (31%)	8 (33%)	2 (7%)	1 (4%)
Potassium decreased	9 (31%)	6 (25%)	3 (10%)	1 (4%)
Sodium decreased	8 (28%)	7 (29%)	4 (14%)	2 (8%)
Phosphorus decreased	8 (28%)	3 (13%)	3 (10%)	1 (4%)
Blood pressure increased	7 (24%)	2 (8%)	5 (17%)	2 (8%)
Oxygen saturation decreased	4 (14%)	1 (4%)	2 (7%)	0 (0%)
Metabolism and nutrition disorders	27 (93%)	19 (79%)	14 (48%)	6 (25%)
Decreased appetite	23 (79%)	15 (63%)	8 (28%)	3 (13%)
Hyperglycaemia	13 (45%)	7 (29%)	4 (14%)	0 (0%)
Hyponatraemia	4 (14%)	3 (13%)	2 (7%)	2 (8%)
Hypophosphataemia	4 (14%)	3 (13%)	4 (14%)	2 (8%)
Hypokalaemia	5 (17%)	1 (4%)	2 (7%)	1 (4%)
Respiratory, thoracic and mediastinal disorders	21 (72%)	9 (38%)	4 (14%)	1 (4%)
Interstitial lung disease	3 (10%)	0 (0%)	3 (10%)	0 (0%)
Skin and subcutaneous tissue disorders	29 (100%)	20 (83%)	15 (52%)	1 (4%)
Papular rash	12 (41%)	0 (0%)	6 (21%)	0 (0%)
Erythematous rash	8 (28%)	0 (0%)	2 (7%)	0 (0%)

when mogamulizumab is administered to patients with ATL.

Adult T-cell leukaemia-lymphoma cells constitutively express CD25 (Waldmann *et al*, 1984), and the present study had an eligibility criterion of CCR4 positivity. Hence, most of the CD4/CD25/CCR4-positive cells were considered ATL cells. Compared to the chemotherapy alone arm, the combination arm showed a significant reduction in the number of CD4/CD25/CCR4-positive cells. This finding is consistent with the proposed antitumour mechanism of mogamulizumab, in that mogamulizumab kills CCR4-expressing ATL cells by increasing antibody-dependent cellular cytotoxicity (Shinkawa *et al*, 2003; Ishii *et al*, 2010; Yamamoto *et al*, 2010). In humans, CCR4 is expressed on CD45RO-positive, CD45RA-negative, FOXP3-positive activated regulatory T (Treg) cells (Miyara *et al*,

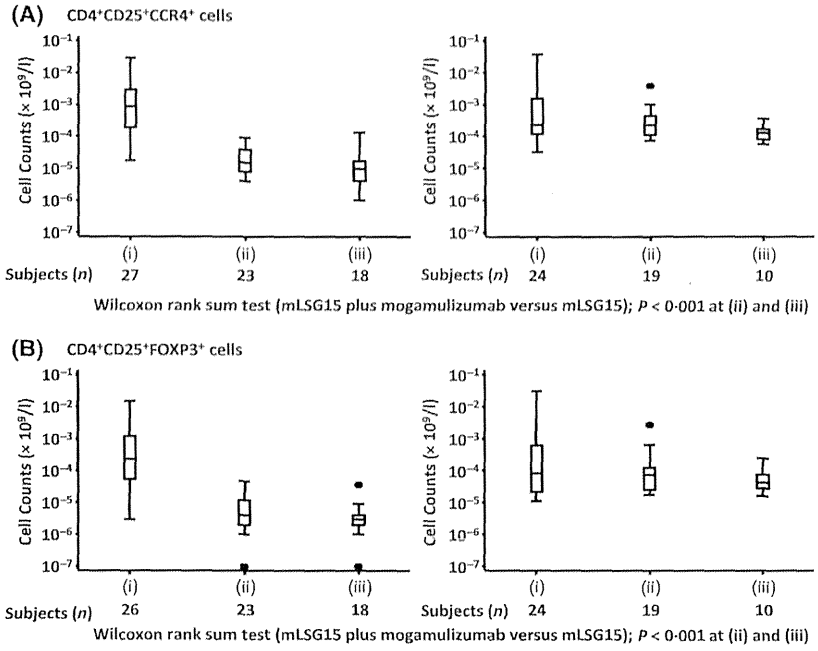

Fig 4. T-cell subset analysis. Blood samples were taken (i) immediately before the initiation of treatment, (ii) immediately before VCAP therapy for cycle three, and (iii) 28 days after VECP therapy for cycle four. The numbers of CD4/CD25/CC chemokine receptor 4 (CCR4)-positive cells (A) and CD4/CD25/FOXP3-positive cells (B) are shown as box and whisker plots indicating the minimum, lower, median, upper quartile, and maximum values. The number of samples used for analysis at each point is indicated below the graph. The differences of each point [(ii) & (iii)] between the mLSG15-plus-mogamulizumab and mLSG15 arms are indicated as p-values (Wilcoxon signed-rank test) below the graphs. CCR4 was detected by using a monoclonal antibody (clone 1G1), with its binding to CCR4 being unaffected by the presence of mogamulizumab. VCAP: vincristine, cyclophosphamide, doxorubicin, and prednisolone; VECP: vindesine, etoposide, carboplatin, and prednisolone.

2009; Ishida & Ueda, 2011; Sugiyama *et al*, 2013). In addition, ATL cells from a subset of patients express FOXP3 and function as Treg cells (Yano *et al*, 2007). Thus, the CD4/CD25/FOXP3-positive cells included not only endogenous activated Treg cells, but also ATL cells, in some patients. Our study indicated that compared to the chemotherapy alone arm, the combination arm showed a significant reduction in the number of CD4/CD25/FOXP3-positive cells, which is consistent with the findings from our previous study of mogamulizumab monotherapy. In general, decreasing the number of Treg cells is considered a promising strategy for boosting antitumour immunity in patients with cancer, because the numbers of these cells increase in the tumour microenvironment, and they may play an important role in the ability of the tumour to escape host immunity in several different types of cancer (Ishida & Ueda, 2011; Jacobs *et al*, 2012). On the other hand, because alterations in Treg cell frequencies and/or function may contribute to various autoimmune diseases (Michels-van Amelsfort *et al*, 2011), immune-related AEs, such as skin disorders, which were also observed in our study, should be carefully monitored.

The present study was conducted according to the premise that mLSG15 is the most recommended chemotherapeutic regimen for patients with newly diagnosed aggressive ATL. We found higher rates of treatment-related toxicities with mLSG15 compared to what has been reported for CHOP-14 (Tsukasaki *et al*, 2007). In the context of this scenario, this study suggests that a younger patient population, particularly those aged <56 years, will benefit from VCAP-AMP-VECP, while an older population consisting of those aged 56–69 years will not; there are no data regarding mLSG15 therapy for patients with ATL aged >69 years (Tsukasaki *et al*, 2007). In the present study, the median ages in the mLSG15-plus-mogamulizumab and mLSG15 arms were 61 years and 64 years, respectively; patients potentially benefiting from mLSG15 (<56 years) accounted for only 38% of the patients in the mLSG15-plus-mogamulizumab arm and 25% of those in the mLSG15 arm. Adult T-cell leukaemia-lymphoma generally occurs in older individuals, with a median age at diagnosis of approximately 66 years (Iwanaga *et al*, 2012); therefore, further investigations are needed to determine whether mLSG15 is indeed the most suitable systemic chemotherapeutic regimen when combined with mogamulizumab.

CCR4 is expressed on the surface of tumour cells of patients from a subgroup of PTCL other than ATL, which also has an unfavourable prognosis (Ishida *et al*, 2004; Nakagawa *et al*, 2009). We have already completed a multicentre phase II study of mogamulizumab monotherapy for patients with relapsed CCR4-positive PTCL in Japan (Clinicaltrials.gov: NCT01192984) (Ogura *et al*, 2014). Furthermore, other clinical trials of mogamulizumab for PTCL (Clinicaltrials.gov: NCT01611142) or cutaneous T-cell lymphoma (Clinicaltrials.gov: NCT01728805) are currently underway worldwide. Further studies are expected to allow the determination of the efficacy of combining mogamulizumab with chemotherapy or other novel molecular target therapies for PTCL subtypes other than ATL.

Although this study offers a novel treatment option for newly diagnosed aggressive ATL, some limitations should be discussed. First, this study was designed to set the %CR as a primary endpoint; as a result, this study does not have enough power or a long enough follow-up period to detect

PFS and OS differences between the two arms. Thus, although a tendency towards prolongation of PFS in the combination arm was observed in the present study, this was not confirmed. Second, the treatment after the study protocol, including allo-HCT and mogamulizumab, varied among the patients. Because the use of mogamulizumab for relapsed/refractory ATL was approved in Japan during the study period, the patients, including those in the chemotherapy alone arm, may have a chance to receive this drug. Both of these factors may affect the OS (Chihara *et al*, 2013).

In conclusion, although mLSG15 plus mogamulizumab was found to be associated with a potentially less favourable safety profile, particularly for infectious and skin-related events, the majority of the AEs were manageable. The %CR was higher with combination therapy. Accordingly, this combination treatment appears to be a better option for managing patients with newly diagnosed aggressive ATL. Further clinical studies are necessary to evaluate the survival parameters in patients treated with chemotherapy plus mogamulizumab and to determine a more suitable combination regimen.

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Author contributions

T.I., K.U., K.Y., N.U., A.U., K.T., S.A. and R.U. contributed to the conception and design of the study; T.I., T.J., S.T., H.S., K.U., K.Y., N.U., Y.S., K.N., A.U., K.T., H.F., K. Ishitsuka, S.Y., N.T., Y.M., K. Imada and T.M. contributed to the acquisition of data; T.I., K.T., S.A., M.T. and R.U. analysed and interpreted the data; all authors drafted and reviewed the manuscript and approved the final version for submission.

Conflicts of interest

T.I. has received honoraria and travel grants from Kyowa Hakko Kirin, and research funding from Kyowa Hakko Kirin, Chugai, Bayer and Celgene, and has served on the speakers bureau for Kyowa Hakko Kirin. T.J. has received honoraria and travel grants from Kyowa Hakko Kirin. S.T. has received travel grants and research funding from Kyowa Hakko Kirin. H.S. has served on the speakers bureau for Kyowa Hakko Kirin. K.Y. has a consultancy/advisory role with Kyowa Hakko Kirin and Novartis, has received honoraria from Kyowa Hakko Kirin, Novartis, Takeda and Janssen, and has received research funding from Kyowa Hakko Kirin, Novartis, Pfizer and ARIAD. Y.S. has received honoraria and research funding from Kyowa Hakko Kirin. K.N. has received honoraria from Kyowa Hakko Kirin. A.U. has received honoraria and research funding from Kyowa Hakko Kirin. K.T. has received honoraria from Kyowa Hakko Kirin and research funding from Kyowa Hakko Kirin, Celgene, Eisai, Solasia Pharma and Mundipharma. S.Y. has received honoraria and research funding from Kyowa Hakko Kirin. Y.M. has received honoraria, travel grants and research funding from Kyowa Hakko Kirin. K. Imada has received research funding from Kyowa Hakko Kirin. S.A. is employed by Kyowa Hakko Kirin, and is a stock owner. M.T. has a consultancy/advisory role with Kyowa Hakko Kirin, and has received honoraria from Kyowa Hakko Kirin. R.U. has a consultancy/advisory role with Mundipharma, and has received honoraria, travel grants and research funding from Kyowa Hakko Kirin and Chugai, and has served on the speakers bureau for Kyowa Hakko Kirin. The remaining authors declare no competing financial interests.

Appendix I

List of the review committees and medical experts who participated in this trial:

1 Junji Suzumiya, Shimane University Hospital
2 Takashi Terauchi, Research Centre for Cancer Prevention and Screening National Cancer Centre
3 Ukihide Tateishi, Yokohama City University Graduate School of Medicine
4 Junichi Tsukada, University of Occupational and Environmental Health
5 Koichi Nakata, University of Occupational and Environmental Health
6 Shigeo Nakamura, Nagoya University Graduate School of Medicine
7 Hiroshi Inagaki, Nagoya City University Graduate School of Medical Sciences
8 Koichi Ohshima, Kurume University School of Medicine
9 Michinori Ogura, Nagoya Daini Red Cross Hospital
10 Tetsuo Nagatani, Hachioji Medical Centre of Tokyo Medical University
11 Akimichi Morita, Nagoya City University Graduate School of Medical Sciences
12 Kazunari Yamaguchi, Institute of Molecular Embryology and Genetics, Kumamoto University
13 Yasuaki Yamada, Nagasaki University Graduate School of Biomedical Sciences
14 Shuichi Hanada, National Hospital Organization Kagoshima Medical Centre.

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