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Ⅲ.　研究成果の刊行物・別刷

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Clinical Cancer Research

Prognostic Significance of Tryptophan Catabolism in Adult T-cell Leukemia/Lymphoma

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Abstract

Purpose: Indoleamine 2,3-dioxygenase 1 (IDO1: IDO), an enzyme catabolizing tryptophan (Trp) into the kynurenine (Kyn) pathway, is increasingly being recognized as an important microenvironmental factor suppressing antitumor immune responses. The purpose of the present study was to determine the prognostic significance of Trp catabolism in adult T-cell leukemia/lymphoma (ATL).

Experimental Design: We quantified serum Trp and Kyn in 96 ATL patients, 38 human T-cell lymphotropic virus type-1 asymptomatic carriers (HTLV-1 ACs), and 40 healthy adult volunteer controls. The relationships between various clinical parameters including overall survival were analyzed. IDO expression was evaluated in the affected lymph nodes of ATL patients.

Results: Serum Kyn concentrations and Kyn/Trp ratios were significantly higher in HTLV-1 ACs than healthy controls. Both increased significantly with progression from HTLV-1 AC to ATL. However, there were no significant differences in the serum Trp concentrations between ATL patients, HTLV-1 ACs, and controls. IDO was possibly produced by ATL and/or cells of the microenvironment. Multivariate analyses demonstrated that a high serum Kyn/Trp ratio and high Kyn level, but not a high Trp level, were significantly independent detrimental prognostic factors in ATL, as well as in that subset of patients with aggressive variant ATL.

Conclusions: Quantification of serum Kyn and Trp is useful for predicting prognosis of an individual ATL patient. Furthermore, ATL, especially in patients with a high serum Kyn/Trp ratio, is an appropriate disease for testing novel cancer immunotherapies targeting IDO. *Clin Cancer Res; 1–10. ©2015 AACR.*

Introduction

Adult T-cell leukemia/lymphoma (ATL), a peripheral T-cell neoplasm, is caused by human T-cell lymphotropic virus type-1 (HTLV-1; refs. 1–3). ATL patients are severely immunocompromised, and have a very unfavorable prognosis (4–7). There have been several studies suggesting a high degree of immunogenicity of ATL cells, caused by HTLV-1–associated antigens such as Tax (8–10) or tumor-specific antigens such as NY-ESO-1 (11). In addition, the possible existence of graft-versus-HTLV-1 and/or graft-versus-ATL effects after allogeneic hematopoietic stem cell transplantation also supports strong immunogenicity of ATL cells (12–14). On the basis of this scenario, not only the established ATL cells, but also HTLV-1–infected cells in HTLV-1 asymptomatic carriers (AC), would need to have immunosuppressive function in order to evade the host immune response despite their immunogenicity. The possible mechanisms responsible for the immunologic escape of HTLV-1–infected cells, especially established ATL cells, can be partially explained by findings that ATL cells from a subset of patients function as regulatory T (Treg) cells (15, 16), and/or that they produce immunosuppressive cytokines such as IL10, TGFβ, or IL5 (17–19). Here, we have focused on Indoleamine 2,3-dioxygenase 1 (IDO1: IDO), an enzyme catabolizing tryptophan (Trp) into the kynurenine (Kyn) pathway, because Trp catabolism in malignant tumors is increasingly being recognized as an important microenvironmental factor that suppresses antitumor immune responses, and creates a favorable environment for tumor cells to escape from host immunity (20–23). The clinical significance of IDO expression has been investigated in many types of cancer. These studies suggest that IDO negatively regulates the recruitment of antitumor immune cells, and increases the proportion of Treg cells in the tumor-infiltrating lymphocytes, thus contributing to an unfavorable prognosis. Hoshi and colleagues reported that IDO was expressed in ATL cells, and that the serum Kyn, a Trp catabolite, level was decreased by anti-ATL treatment (24). However, details of Trp catabolism in

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Indoleamine 2,3-dioxygenase 1 (IDO1: IDO), an enzyme catabolizing tryptophan (Trp) into the kynurenine (Kyn) pathway, is an important microenvironmental factor suppressing antitumor immune responses. The present study demonstrated that adult T-cell leukemia/lymphoma (ATL) cells and/or cells of the tumor microenvironment possibly produce IDO, which would lead to a high Kyn/Trp ratio and a high Kyn level not only in the tumor microenvironment, but also in the blood. It was found that a high serum Kyn/Trp ratio and a high serum Kyn level were both independent significant detrimental prognostic factors in ATL patients. Thus, measurement of serum Kyn and Trp concentrations is useful for predicting prognosis of an individual ATL patient. Furthermore, IDO has now become a very attractive target for developing novel anticancer agents. ATL, especially in patients with a high serum Kyn/Trp ratio, is an appropriate disease for testing novel cancer immunotherapies targeting IDO.

ATL have not been fully explored yet. Therefore, the aim of the present study was to demonstrate the prognostic significance of Trp catabolism in ATL patients.

Materials and Methods

ATL patients, HTLV-1 ACs, and control subjects

This study included 96 ATL patients and 38 HTLV-1 ACs. Forty healthy volunteers participated as control subjects, and their samples were anonymized and not traceable. All donors provided written informed consent before blood sampling according to the Declaration of Helsinki, and the present study was approved by the Institutional Ethics Committees of Nagoya City University Graduate School of Medical Sciences (Nagoya, Japan) and Imamura Bun-in Hospital (Kagoshima, Japan). Diagnoses and assignment of clinical subtypes of ATL were made according to the criteria proposed by the Japan Lymphoma Study Group (4). ATL patients with the acute and lymphoma subtypes have an aggressive clinical course, whereas ATL patients with the chronic and smoldering subtypes have longer survival (4, 5). Thus, the acute and lymphoma subtypes of ATL were designated aggressive variant and the chronic and smoldering subtypes as indolent variant in the present study. The clinical characteristics of the ATL patients analyzed in this study comprised age, sex, clinical subtype, Eastern Cooperative Oncology Group performance status (ECOG PS), white blood cell count (WBC), hemoglobin level (Hb), and platelet count (Plt) in the blood, serum calcium (Ca), serum albumin (Alb), serum lactate dehydrogenase (LDH), and serum soluble interleukin-2 receptor (sIL-2R) levels. When the serum Alb level was less than 4.0 g/dL, serum Ca was adjusted by the concentration of serum Alb as follows: adjusted Ca level (mg/dL) = measured Ca level (mg/dL) + [4.0 − Alb level (g/dL)]. Blood samples of all ATL patients were obtained at the time of initial presentation at the hospital, and we used the clinical characteristics that were recorded at that time. Although the treatments administered to the ATL patients enrolled in this study varied, a modified LSG15 protocol (25, 26), or cyclophosphamide-doxorubicin-vincristine-prednisolone (CHOP-like regimens) were initially administered to many with aggressive variant. Patients with indolent variant were mostly carefully managed on a watch-and-wait basis until disease progression. Some refractory or relapsed patients received mogamulizumab (27, 28).

Measurement of serum Trp and Kyn

L-Tryptophan (L-Trp) and L-kynurenine (L-Kyn) used to construct standard curves, were purchased from Sigma-Aldrich. L-Tryptophan-d_5 (L-Trp-d_5), used as an internal standard, was purchased from Cambridge Isotope Laboratories, Inc.. Trp and Kyn were measured using an ultra-high-performance liquid chromatography (UPLC) - tandem mass spectrometry (MS-MS, Quattro Premier XE mass spectrometer) system (Waters Corporation) as described previously (29). A 10-μL sample solution that was pretreated by a solid phase extraction (SPE) method using an Oasis MCX 30 mg/1 cc SPE cartridge (Waters Corporation) was injected into an Acquity UPLC BEH C18 column (2 × 100 mm; Waters Corporation) at room temperature. Chromatography was performed at a flow rate of 0.3 mL/minute using a step gradient alternating between methanol and 0.08% aqueous ion pair reagent (IPCC-MS3, GL Sciences). Trp and Kyn were analyzed by multiple reaction monitoring mode of MS-MS in positive ion mode. The cone voltage was 12–15 V, collision energy was 9–10 eV, and transitions were m/z 205.0 → 188.0 for L-Trp, m/z 209.0 → 192.0 for L-Kyn, and m/z 261.9 → 84.8 for L-Trp-d_5.

Histologic and immunofluorescence staining analyses

Hematoxylin and eosin (H&E) staining, immunostaining, and immunofluorescence analyses were performed on formalin-fixed, paraffin-embedded sections of the affected tissues of ATL patients. The patients provided written informed consent in accordance with the Declaration of Helsinki, and the present study was approved by the Institutional Ethics Committee of Nagoya City University Graduate School of Medical Sciences, and Imamura Bun-in Hospital. The 28 affected tissues biopsied at the time of blood sampling for serum Trp and Kyn measurement were used for immunostaining of IDO and tryptophan-2,3-dioxygenase (TDO). This was performed using rat anti-human and -mouse IDO mAb (sc-53978; Santa Cruz Biotechnology), and mouse anti-human TDO/TDO2 mAb (2A4; LifeSpan BioSciences, Inc.). IDO and TDO expression levels were classified semiquantitatively based on the percentage of ATL tumor cells with IDO or TDO staining, as in an earlier study (30). Positivity was scored as zero if <5% of ATL cells were stained, 1 if 5% to 30% were stained, 2 if 30% to 70%, and 3 if >70% were stained (Supplementary Fig. S1). Immunofluorescence analyses were performed using mouse anti-human CC chemokine receptor 4 (CCR4) mAb (1G1; BD Bioscience), and rat anti-human and -mouse IDO mAb (sc-53978) as primary antibodies, and Alexa Fluor 555 goat anti-mouse IgG (H + L; Invitrogen, Ltd.) and Alexa Fluor 488 goat anti-rat IgG (H + L; Invitrogen Ltd.) as secondary antibodies, respectively. CCR4 was used as an ATL cell membrane marker because it was expressed on the tumor cells of most patients with ATL (31). Nuclei were stained by VECTASHIELD mounting medium with DAPI (Vector Laboratories, Inc.). Slides were viewed using a fluorescence microscope (OLYMPUS BX53, Olympus Corporation), and images were obtained using CellSens Standard software (Olympus Corporation).

Statistical analysis

Correlations between two variables were assessed using the Spearman rank correlation coefficient (r_s). The differences between two groups were examined with the Mann–Whitney U test or

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Fisher exact test. The probability of survival was estimated by the Kaplan–Meier method, and survival times were compared using the log-rank test. The starting date of survival analysis was the day when serum was obtained. The clinically meaningful cut-off values for serum concentrations of Kyn, Trp, and Kyn/Trp ratios in ATL patients have not been determined. Thus, we attempted to divide ATL patients into two groups according to their serum levels of Kyn, Trp, and the Kyn/Trp ratio. The cut-off values for each in the ATL patients were tested at 11 points between median±SD. Univariate analysis for survival was performed by the Cox proportional hazards regression model for each parameter at each of the 11 cut-off points. In the present study, the cut-off point yielding the minimum P value was chosen as the most clinically meaningful cut-off value. Multivariate analysis by Cox proportional hazards regression models were used to evaluate variables potentially affecting overall survival (OS). All analyses were performed with SPSS Statistics 17.0 (SPSS). In this study, $P < 0.05$ (two-sided) was considered significant.

Results

Characteristics of the HTLV-1 ACs and ATL patients

The 96 ATL patients enrolled in this study comprised 51 males and 45 females (age range 40–91 years, median, 64 years). They included 60 acute-type, 19 lymphoma-type, 8 chronic-type, and 9 smoldering-subtype patients (Supplementary Table S1). The 38 HTLV-1 ACs enrolled in this study were 14 males and 24 females (age range 28–86 years, median, 49 years).

Concentrations and correlations of serum Kyn, Trp, and the Kyn/Trp ratio in healthy volunteers, HTLV-1 ACs, and ATL patients

The concentration of serum Kyn in the healthy volunteers was 7.7×10^{-1}, 7.3×10^{-1}, 4.4×10^{-1} to 12.0×10^{-1} μmol/L (mean, median, range). The corresponding values in the HTLV-1 ACs and ATL patients were 1.2, 1.1, 0.6 to 3.5 μmol/L, and 2.1, 1.6, 0.5 to 10.9 μmol/L, respectively. The serum Kyn concentration was significantly higher in the HTLV-1 ACs than in the healthy volunteers ($P < 0.001$), and in the ATL patients relative to the HTLV-1 ACs ($P = 0.001$; Fig. 1A–C). The concentration of serum Trp in the healthy volunteers was 130.1, 129.0, 87.9 to 175.9 μmol/L (mean, median, range). The corresponding values in the HTLV-1 ACs and ATL patients were 137.0, 119.1, 68.7 to 286.6, and 128.3, 118.0, 31.4 to 322.5 μmol/L, respectively. There were no significant differences in the serum Trp concentrations between any two groups among healthy volunteers, HTLV-1 ACs and ATL patients (Fig. 1A–C). The serum Kyn/Trp ratio [Kyn (μmol/L)/Trp (μmol/L) $\times 10^3$] in the healthy volunteers was 6.0, 5.7, 3.6 to 9.7 (mean, median, range). The corresponding values in the HTLV-1 ACs and ATL patients were 9.3, 8.4, 4.5 to 18.7, and 17.8, 12.7, 3.7 to 75.5, respectively. The serum Kyn/Trp ratio was significantly higher in the HTLV-1 ACs than in the healthy volunteers ($P < 0.001$), and in the ATL patients versus the HTLV-1 ACs ($P < 0.001$; Fig. 1D). There was a significant positive correlation between the concentrations of serum Kyn and Trp in the healthy volunteers ($r_s = 0.510$, $P = 0.001$; Fig. 1A), HTLV-1 ACs ($r_s = 0.505$, $P = 0.001$; Fig. 1B), and ATL patients ($r_s = 0.372$, $P < 0.001$; Fig. 1C).

Clinical characteristics of ATL patients according to serum Kyn/Trp ratio, Kyn, and Trp levels

In the present study, the cut-off value for the serum Kyn/Trp ratio was set at 15.3 (Supplementary Table S2). A high serum

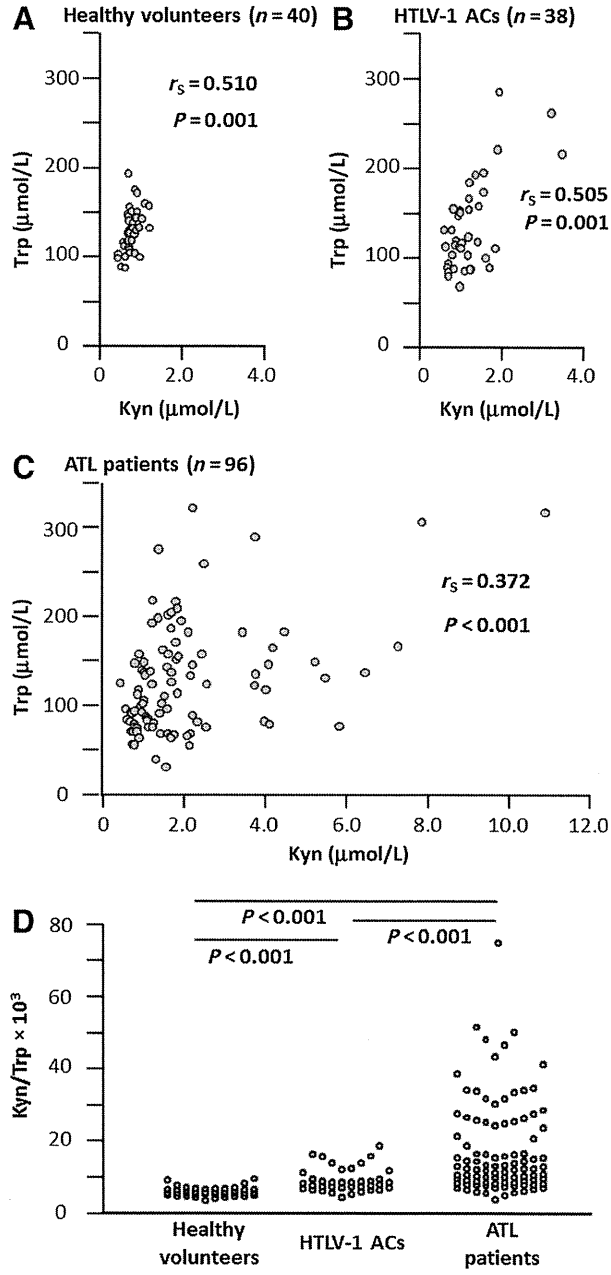

Figure 1.
Correlations of serum Kyn and Trp levels, and Kyn/Trp ratios in healthy volunteers, HTLV-1 ACs, and ATL patients. Serum Kyn and Trp concentrations are plotted on the x-axis and y-axis, respectively. Each dot plot in A, B, and C represents a different healthy volunteer, HTLV-1 asymptomatic carrier, and ATL patient, respectively. The Spearman rank correlation coefficient (r_s) between serum Kyn and Trp concentrations, and the P values are indicated in each panel. The significance of the differences of Kyn and Trp concentrations between healthy volunteers, HTLV-1 ACs and ATL patients were established by the Mann–Whitney U test. D, the Kyn/Trp ratios [Kyn (μmol/L)/Trp (μmol/L) $\times 10^3$] in serum samples obtained from healthy volunteers, HTLV-1 ACs and ATL patients are shown. Each dot plot in the panel indicates a different individual. The significance of the differences of the Kyn/Trp ratios were assessed by the Mann–Whitney U test, and P values are indicated in the panel. n, number.

Kyn/Trp ratio was significantly associated with aggressive variant ATL ($P = 0.002$), worse PS from 2 to 4 ($P = 0.014$), a high serum sIL-2R level (>20,000 U/mL; $P = 0.001$), a high serum

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Table 1. Characteristics of ATL patients according to serum Kyn/Trp ratio, Kyn, and Trp

	Serum Kyn/Trp × 10^3			Serum Kyn, μmol/L			Serum Trp, μmol/L		
Characteristics	**≤15.3**	**>15.3**		**≤2.0**	**>2.0**		**>180.0**	**≤180.0**	
Total patients, number (%)	**58**	**38**	***P***	**66**	**30**	***P***	**18**	**78**	***P***
Age, y			0.073			1.000			0.065
≤70	53 (91)	29 (76)		56 (85)	26 (87)		18 (100)	64 (82)	
>71	5 (9)	9 (24)		10 (15)	4 (13)		0 (0)	14 (18)	
Sex			0.213			0.122			1.000
Female	24 (41)	21 (55)		27 (41)	18 (60)		8 (44)	37 (47)	
Male	34 (59)	17 (45)		39 (59)	12 (40)		10 (56)	41 (53)	
Clinical variant			0.002			0.253			0.016
Indolent	16 (28)	1 (3)		14 (21)	3 (10)		7 (39)	10 (13)	
Aggressive	42 (72)	37 (97)		52 (79)	27 (90)		11 (61)	68 (87)	
ECOG PS			0.014			0.347			0.048
0, 1	45 (78)	20 (53)		47 (71)	18 (60)		16 (89)	49 (63)	
2, 3, 4	13 (22)	18 (47)		19 (29)	12 (40)		2 (11)	29 (37)	
Serum sIL-2R, U/mL			0.001			0.003			0.032
≤20,000	43 (74)	14 (37)		46 (70)	11 (37)		15 (83)	42 (54)	
>20,000	15 (26)	24 (63)		20 (30)	19 (63)		3 (17)	36 (46)	
Serum LDH[a]			0.032			0.366			0.008
≤2N	41 (71)	18 (47)		43 (65)	16 (53)		16 (89)	43 (55)	
>2N	17 (29)	20 (53)		23 (35)	14 (47)		2 (11)	35 (45)	
Serum Ca[b], mg/dL			0.023			0.736			0.118
≤11.0	55 (95)	30 (79)		59 (89)	26 (87)		18 (100)	67 (86)	
>11.0	3 (5)	8 (21)		7 (11)	4 (13)		0 (0)	11 (14)	
Serum Alb, g/dL			<0.001			0.013			0.383
≥3.5	50 (86)	19 (50)		53 (80)	16 (53)		15 (83)	54 (69)	
<3.5	8 (14)	19 (50)		13 (20)	14 (47)		3 (17)	24 (31)	
Eosinophil count, /μL			0.002			0.025			1.000
≤500	57 (98)	30 (79)		63 (95)	24 (80)		17 (94)	70 (90)	
>500	1 (2)	8 (21)		3 (5)	6 (20)		1 (6)	8 (10)	
WBC, /μL			<0.001			0.094			0.725
Mean	10,179	30,547		17,106	20,739		20,311	17,764	
Median	6,200	139,00		6,700	9,750		6,700	7,600	
Range	1,300–81,700	1,600–208,600		1,300–208,600	1,600–115,900		3,200–115,900	1,300–208,600	
Hb, g/dL			0.123			0.041			0.142
Mean	12.5	11.7		12.5	11.5		12.8	12.0	
Median	13.0	11.8		13.0	11.9		13.3	12.7	
Range	7.3–16.7	8.2–16.7		7.3–16.7	8.2–16.7		8.4–15.3	7.3–16.7	
Plt, ×10^3/μL			0.200			0.758			0.428
Mean	197	186		191	194		199	191	
Median	189	159		182	179		184	181	
Range	35–443	20–546		35–495	20–546		38–322	20–546	

[a]LDH was expressed as a ratio in which the LDH value in the patient was divided by the upper limit of normal for LDH at the laboratory at the respective hospital. [b]When serum Alb level was less than 4.0 g/dL, serum Ca was adjusted by the concentration of serum Alb as follows: adjusted Ca level (mg/dL) = measured Ca level (mg/dL) + [4 − Alb level (g/dL)].

LDH level (greater than twice the upper limit of normal; $P = 0.032$), hypercalcemia (adjusted Ca level >11.0 mg/dL; $P = 0.023$), low serum albumin (<3.5 g/dL; $P < 0.001$), and blood eosinophilia (>500/μL; $P = 0.002$). WBCs were significantly higher in ATL patients with a high serum Kyn/Trp ratio ($P < 0.001$; Table 1).

The cut-off value for serum Kyn was set at 2.0 μmol/L (Supplementary Table S2). A high serum Kyn level was significantly associated with a high serum sIL-2R level ($P = 0.003$), low serum albumin ($P = 0.013$), and blood eosinophilia ($P = 0.025$). Hb values were significantly lower in ATL patients with a high serum Kyn level ($P = 0.041$; Table 1).

Finally, the cut-off value for serum Trp was set at 180.0 μmol/L (Supplementary Table S2). Here, a low serum level was significantly associated with aggressive variant ATL ($P = 0.016$), worse PS ($P = 0.048$), a high serum sIL-2R level ($P = 0.032$), and a high serum LDH level ($P = 0.008$; Table 1).

OS of the whole ATL cohort according to their Kyn/Trp ratios, Kyn, and Trp levels

The OS of the whole cohort is shown in Fig. 2A. The median OS was 15.6 months [95% confidence intervals (CI), 10.1–21.2 months]. This OS was significantly shorter in ATL patients with a high relative to low serum Kyn/Trp ratio (median OS, 7.3 vs. 24.8 months, $P < 0.001$; Fig. 2B). It was also shorter in patients with a high serum Kyn level (median OS, 9.5 vs. 22.0 months, $P = 0.007$; Fig. 2C), and in those with a low serum Trp level (median OS, 13.2 vs. 37.1 months, $P = 0.015$; Fig. 2D). OS was significantly shorter in patients with an aggressive variant than in those with an indolent variant, as expected (median OS, 11.7 vs. 48.5 months, $P < 0.001$; Fig. 2E).

OS of aggressive variant ATL patients according to their serum Kyn/Trp ratios, Kyn, and Trp levels

Among the ATL patients with an aggressive variant, a high serum Kyn/Trp ratio and a high serum Kyn level were each

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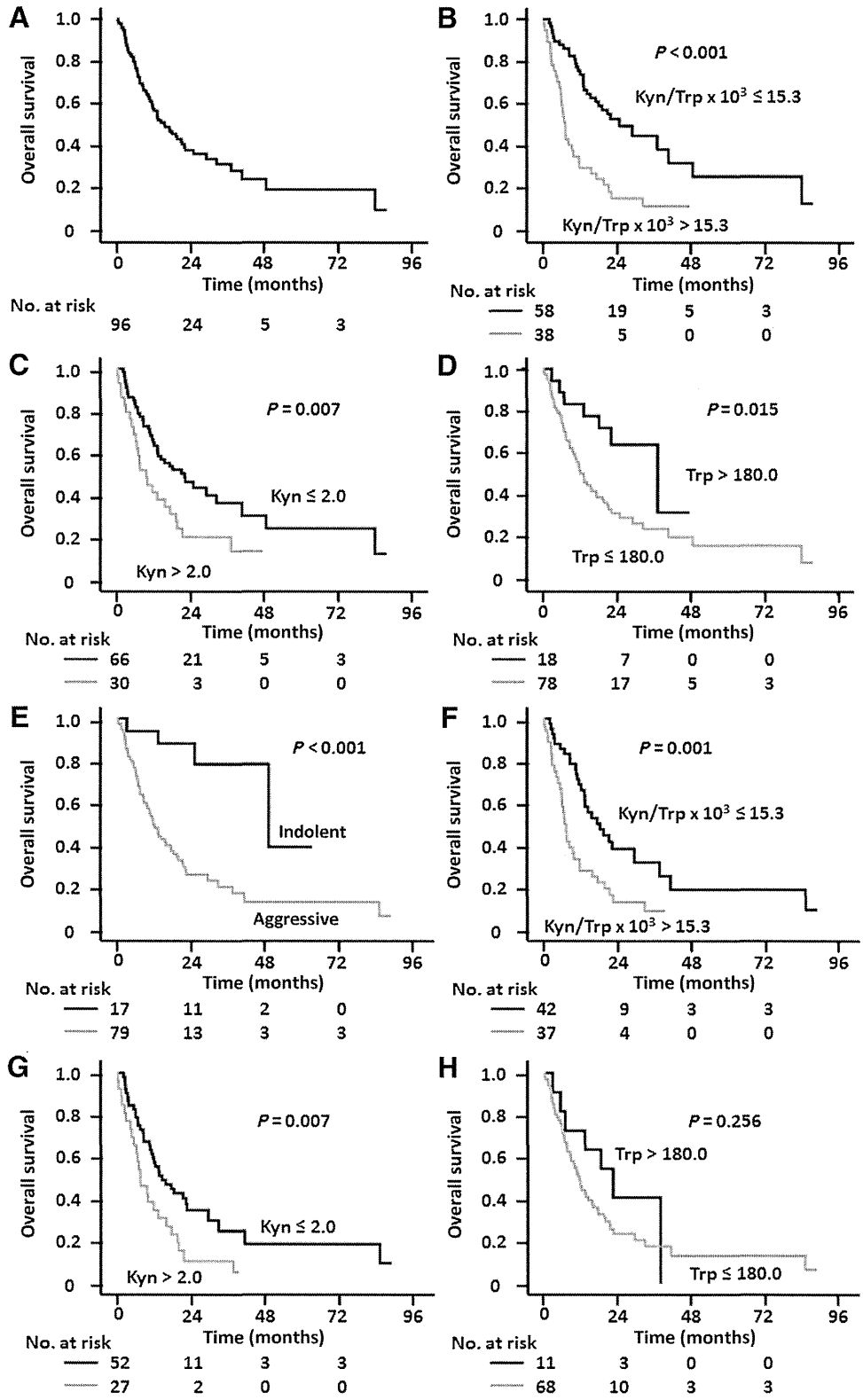

Figure 2.
OS of the ATL patients. A, OS curve of all ATL patients enrolled in the study ($n = 96$). B, OS curves of the ATL patients according to serum Kyn/Trp ratio. C, OS curves of the ATL patients according to serum Kyn level. D, OS curves of the ATL patients according to serum Trp level. E, OS curves of the 79 ATL patients with an aggressive variant and the 17 ATL patients with an indolent variant. F, OS curves of the ATL patients with aggressive variant according to serum Kyn/Trp ratio. G, OS curves of the ATL patients with aggressive variant according to serum Kyn level. H, OS curves of the ATL patients with aggressive variant according to serum Trp level. There was no significant difference in the OS between the patients with low and high serum Trp levels. The survival curves were compared using the log-rank test, and the *P* value is indicated in each panel. *n*, number.

significantly associated with shorter survival (median OS, 7.3 vs. 18.1 months, $P = 0.001$, and 7.4 vs. 14.2 months, $P = 0.007$, respectively; Fig. 2F and G). However, there was no significant difference in the OS between patients with an aggressive variant having a low versus high Trp level (median OS, 11.3 vs. 22.0 months; Fig. 2H).

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Table 2. Multivariate analysis for OS in ATL patients

Variables	Number	HR (95% CI)	P
ECOG PS			
0, 1	65	1.000	Reference
2, 3, 4	31	1.840 (1.038–3.263)	0.037
Age, y			
≤70	82	1.000	Reference
>70	14	2.285 (1.174–4.448)	0.015
Serum Alb, g/dL			
≥3.5	69	1.000	Reference
<3.5	27	1.017 (0.553–1.870)	0.956
Serum sIL-2R, U/mL			
≤20,000	57	1.000	Reference
>20,000	39	1.606 (0.935–2.758)	0.086
Clinical variant			
Indolent	17	1.000	Reference
Aggressive	79	2.761 (0.926–8.233)	0.068
Serum Kyn/Trp $\times 10^3$			
≤15.3	58	1.000	Reference
>15.3	38	1.905 (1.082–3.352)	0.025

Prognostic significance of serum Kyn/Trp ratios, Kyn, and Trp levels in ATL patients

Multivariate analysis for OS in the 96 ATL patients was performed using the following six variables: PS (0–1 or 2–4), age (70 or >70 years), serum Alb (3.5 or <3.5 g/dL), serum sIL-2R (20,000 or >20,000 U/mL), ATL clinical variant (indolent or aggressive), and serum Kyn/Trp ratio. Of these six variables, three significantly affected OS; these were worse PS (HR, 1.840; 95% CI, 1.038–3.263), older age (HR, 2.285; 95% CI, 1.174–4.448), and a high serum Kyn/Trp ratio (HR, 1.905; 95% CI, 1.082–3.352; Table 2).

Multivariate analysis in these 96 patients was also performed using the six variables PS, age, serum Alb, serum sIL-2R, ATL clinical variant, and serum Kyn level. Of these, four variables significantly affected OS, as follows: worse PS (HR, 1.972; 95% CI, 1.127–3.449), older age (HR, 2.803; 95% CI, 1.414–5.559), aggressive variant (HR, 3.097; 95% CI, 1.040–9.224), and a high serum Kyn level (HR, 1.756; 95% CI, 1.004–3.072; Supplementary Table S3).

Finally, multivariate analysis was also performed using PS, age, serum Alb, serum sIL-2R, ATL clinical variant, and serum Trp level. Of these, only one variable, older age, significantly affected OS (HR, 2.319; 95% CI, 1.185–4.540). In this analysis, HR and 95% CI of a low serum Trp level were 1.326 and 0.573–3.069, respectively (Supplementary Table S4).

Prognostic significance of serum Kyn/Trp ratios, Kyn, and Trp levels in ATL patients with an aggressive variant

Multivariate analysis for OS in the 79 patients with aggressive ATL was performed using the following five variables: PS, age, serum Alb, serum sIL-2R, and serum Kyn/Trp ratio. Of these, two variables significantly affected OS, namely older age (HR, 2.257; 95% CI, 1.162–4.384) and a high serum Kyn/Trp ratio (HR, 2.010; 95% CI, 1.127–3.582; Table 3).

Multivariate analysis in these same 79 ATL patients was also performed using the following five variables: PS, age, serum Alb, serum sIL-2R, and serum Kyn level. Of these, three variables significantly affected OS; they were worse PS (HR, 1.898; 95% CI, 1.085–3.321), older age (HR, 2.825; 95% CI, 1.422–5.611), and a high serum Kyn level (HR, 1.908; 95% CI, 1.074–3.392; Supplementary Table S5).

Finally, multivariate analysis of the aggressive variant patients was also performed using PS, age, serum Alb, serum sIL-2R, and serum Trp level. Again, only older age significantly affected OS (HR, 2.361; 95% CI, 1.202–4.639) in this case. In this analysis, HR (95% CI) for a low serum Trp level was 1.037 (0.446–2.408; Supplementary Table S6).

Immunostaining analyses in the affected tissues of ATL patients

Immunostaining for IDO in the affected tissues of 28 individual ATL patients yielded nine cases scored as having no expression, four cases scored as 1, seven cases as 2, and eight scored as 3. Collectively, ATL cells produced IDO as identified by histology in 68% (19/28) of patients. The concentration of serum Kyn in the ATL patients whose IDO expression level scored 0 was 1.9, 1.1, 0.7 to 7.9 μmol/L (mean, median, range). The corresponding values in the ATL patients scored 1, 2, and 3 for IDO were 4.2, 2.4, 1.0 to 0.9 μmol/L, 3.5, 2.6, 0.8 to 7.3 μmol/L, and 2.1, 1.7, 0.9 to 4.2 μmol/L, respectively. There were no significant differences in the serum Kyn concentrations between any two groups among the ATL patients scored 0 to 3. The concentration of serum Trp in patients with zero IDO expression was 124.7, 87.4, 55.4 to 307.0 μmol/L (mean, median, range). The corresponding values in patients scored 1, 2, and 3 were 168.1, 136.0, 83.0to 317.2 μmol/L, 128.6, 138.6, 76.1 to 166.9 μmol/L, and 124.0, 136.7, 39.2 to 186.8 μmol/L, respectively. There were also no significant differences in serum Trp concentrations between any two groups among these. Finally, the serum Kyn/Trp ratio in the ATL patients with zero IDO expression was 15.1, 12.4, 6.4 to 38.8 (mean, median, range) and the corresponding values for those scored 1, 2, and 3 were 21.3, 21.9, 7.0 to 34.3, 27.6, 33.8, 5.5 to 43.6 and 19.1, 19.5, 5.8 to 34.2, respectively. Again, there were no significant differences in the serum Kyn/Trp ratio between any two groups. Some of the cells in the ATL microenvironment, including monocytes/macrophages and small lymphocytes, were positive for IDO. The IDO expression levels of these cells varied among the cases, regardless of level of IDO expression in the ATL cells themselves. Immunostaining for TDO in the affected tissues from the 28 individual ATL patients was negative in all cases.

Immunofluorescence analyses in the affected lymph node lesions from ATL patients

Immunofluorescence analysis for IDO (green signal) and CCR4 (red signal) in the affected lymph node lesions of 3 individual ATL patients is shown in Fig. 3. The percentage of IDO-positive ATL cells in the affected lymph nodes of patient 1 was 10% to 20% (scored as 1, top panels), whereas this was 30% to 40% in patient

Table 3. Multivariate analysis for OS in aggressive ATL patients

Variables	Number	HR (95% CI)	P
ECOG PS			
0, 1	49	1.000	Reference
2, 3, 4	30	1.760 (0.990–3.128)	0.054
Age, y			
≤70	65	1.000	Reference
>70	14	2.257 (1.162–4.384)	0.016
Serum Alb, g/dL			
≥3.5	54	1.000	Reference
<3.5	25	0.945 (0.507–1.763)	0.956
Serum sIL-2R, U/mL			
≤20,000	40	1.000	Reference
>20,000	39	1.644 (0.955–2.829)	0.086
Serum Kyn/Trp $\times 10^3$			
≤15.3	42	1.000	Reference
>15.3	37	2.010 (1.127–3.582)	0.018

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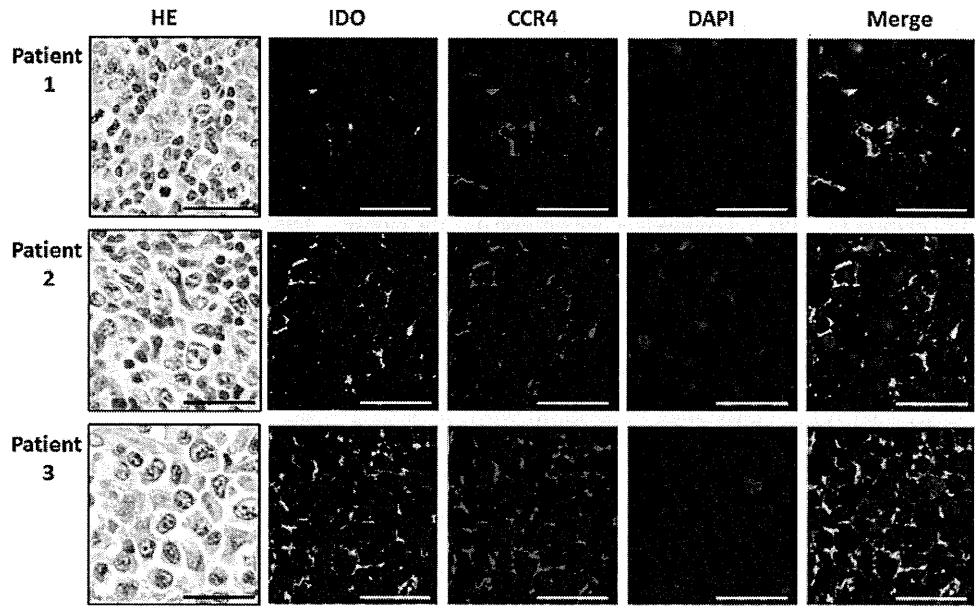

Figure 3.
IDO1: IDO expression in ATL cells. Immunofluorescence analyses in the affected lymph node lesions from 3 individual ATL patients. IDO was visualized by Alexa Fluor 488 (green), and CC chemokine receptor 4 (CCR4) by Alexa Fluor 555 (red). Nuclei are stained by DAPI (blue). The close proximity localization of IDO and CCR4 is discernible in the merged image (yellow). The scale bars in the pictures represent 50 μm.

2 (scored as 2, middle panels), and 80% to 90% in patient 3 (scored as 3, bottom panels). The merged images show yellow signals around the pleomorphic nuclei that are stained blue. This indicates that IDO was present around the ATL cell nuclei and also very close to the membrane as shown by the staining for CCR4. That is to say, the present immunofluorescence analyses demonstrated that IDO was certainly distributed throughout the cytoplasm of CCR4-positive ATL cells in all three cases.

Discussion

It has been reported that relative to healthy controls, serum Trp levels are significantly lower in several types of cancer, such as colorectal cancer (32) and ovarian carcinoma (33), in addition to ATL (24). This might be due to accelerated Trp catabolism mediated by the IDO produced by tumor cells and/or cells of the tumor microenvironment. However, in the present study, we found no significant differences in serum Trp concentration between healthy volunteers and ATL patients, even though ATL cells from some patients did produce IDO. In healthy people, systemic Trp levels are regulated within a certain range mainly by TDO, expressed at high levels in the liver, in cooperation with IDO and IDO2 (20–22, 34, 35). The present study suggested that, even in the presence of the HTLV-1 infection, the intrinsic TDO, IDO, and IDO2 system continued to regulate Trp levels within that certain range. We surmise that this regulation of Trp catabolism also leads to a significant positive correlation between the levels of serum Kyn and Trp, not only in healthy volunteers, but also in HTLV-1 ACs and ATL patients. On the other hand, serum Kyn concentrations and thus Kyn/Trp ratios were significantly elevated in both HTLV-1 ACs and ATL patients compared with controls. In addition, they both increased with progression from HTLV-1 AC to overt ATL. This suggests that IDO is produced not only by ATL cells themselves in some of the patients, which is confirmed in the present study, but also by nontransformed HTLV-1–infected cells in some of the HTLV-1 ACs, which would contribute to their survival in the face of the host immune response.

A high serum Kyn level did not seem merely to directly reflect the ATL tumor burden, because it was not significantly associated with either a high serum LDH level or aggressive clinical variant. It seems to rather reflect immune dysfunction because it was significantly associated with blood eosinophilia, which was possibly associated with a high IL5 level (19, 36). Unlike a high serum Kyn level, a low serum Trp level did rather seem to reflect the ATL tumor burden, because it was significantly associated with high serum sIL-2R and LDH levels, and aggressive clinical variant, but not with blood eosinophilia. With respect to serum Kyn/Trp ratios in ATL patients, these do seem to reflect both the ATL tumor burden and immune dysfunction, because they were significantly associated with high serum sIL-2R and LDH levels, aggressive clinical variant, higher WBC, and also blood eosinophilia. Furthermore, a high serum Kyn/Trp ratio in ATL patients also seems to reflect their poor general condition because of its significant association with worse PS and low serum albumin level.

The present multivariate analyses demonstrated that a high serum Kyn/Trp ratio and high Kyn level were independent significant unfavorable prognostic factors when considering the entire cohort of ATL patients. These analyses also indicated that the two factors both strongly influenced OS in ATL patients, because covariates such as older age, worse PS, high serum sIL-2R level, and a low serum Alb included in the present multivariate analyses have been identified as independent prognostic factors for acute and lymphoma-type ATL patients in a recent large nationwide retrospective study (37). The HR and significance for death conferred by a high serum Kyn/Trp ratio was higher than for a high serum Kyn level. Therefore, a high serum Kyn/Trp ratio seems to be a more robust unfavorable prognostic factor than merely a high serum Kyn level. This is presumably due to the Kyn/Trp ratio including both serum Kyn and Trp levels, despite the serum Trp level itself not representing a significant unfavorable factor in the present multivariate analysis. Here, we also demonstrated that a high serum Kyn/Trp ratio and Kyn level, but not a low serum Trp level, were independent unfavorable prognostic factors in that subset of ATL patients with an aggressive variant. It was also found that these two factors both strongly influenced OS in aggressive variant ATL. In addition, as seen in the whole ATL cohort, a high serum Kyn/Trp ratio seems to be a more important

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unfavorable prognostic factor than a high serum Kyn level also in ATL patients with aggressive variant.

There were no significant correlations between histologically defined ATL cell IDO expression levels and the serum Kyn/Trp ratio, or the serum Kyn or Trp levels, although this may have been because we were only able to examine 28 cases. ATL is a systemic disease, and it was reported that clonal heterogeneity is present in approximately 70% of cases (38, 39). Taken together, we surmise that the biopsy specimen, which is only a part of a systemic lesion, might not reflect the entire ATL disease condition, at least as far as Trp catabolism is concerned. It is also possible that IDO was produced by non-ATL cells including those in the tumor microenvironment.

How IDO exerts its immunomodulatory effects is not completely clear, but two main theories have been proposed, the Trp starvation theory and the Trp metabolite theory. In the former, Trp starvation induces cell-cycle arrest of host T lymphocytes and renders these cells more sensitive to apoptosis (40, 41). In the latter, Trp metabolites such as Kyn, 3-hydroxykynurenine and 3-hydroxyanthranilic acid, are toxic to host lymphocytes (42–44). In addition, the Trp metabolites directly contribute to tumor cell survival (45). Although these two theories are not mutually exclusive, the present study suggested that the latter was more relevant. That is to say, Trp metabolites such as Kyn compromise the host's immune system and thus contribute to tumor cell survival in the face of weakened immunity, despite maintained immunogenicity of the cancer (8–14). This is accompanied by a severely immunocompromised state of the host. In addition, the Trp metabolites could also directly promote ATL cell survival through the aryl hydrocarbon receptor expressed by these cells (45, 46). Together, these factors would contribute to the unfavorable prognosis of ATL patients with high IDO activity.

Most of the research in this area to date has focused on IDO as the central and immunobiologically relevant enzyme that catalyzes the conversion of Trp to Kyn. However, there are two other enzymes, TDO and IDO2 that also catalyze the same enzymatic step. In addition, this pathway is also responsive to nonspecific inflammation. Therefore, the Kyn/Trp ratio is merely one surrogate marker of IDO activity, and does not directly exclusively reflect IDO activity. Indeed, IDO2 was reported to be expressed in some cancers including pancreatic tumors (47, 48). Thus, although we confirmed IDO production by ATL cells in some patients, and lack of TDO production by ATL cells in all patients, further investigation of IDO2 expression in ATL cells is warranted.

In conclusion, ATL cells and/or cells of the tumor microenvironment are likely to produce IDO, which would lead to a high Kyn/Trp ratio and a high Kyn level not only in the tumor microenvironment, but also in the blood. A high serum Kyn/Trp ratio and a high serum Kyn level were both independent significant detrimental prognostic factors in ATL patients, as well as in that subset of patients with aggressive variant. These results provide novel insights for better understanding the immunopathogenesis of ATL. In addition, measurement of serum Kyn and Trp concentrations is useful for predicting prognosis of an individual ATL patient. Furthermore, IDO has now become a very attractive target for developing novel anticancer agents, and several IDO inhibitors are currently being investigated (20–23, 49, 50). ATL, especially in patients with a high serum Kyn/Trp ratio, is an appropriate disease for testing novel cancer immunotherapies targeting IDO.

Disclosure of Potential Conflicts of Interest

T. Ishida reports receiving commercial research grants from Bayer, Celgene, and Kyowa Hakko Kirin, Co., Ltd, and speakers bureau honoraria from Kyowa Hakko Kirin, Co., Ltd. R. Ueda reports receiving commercial research grants and speakers bureau honoraria from Chugai Pharmaceutical Co., and Kyowa Hakko Kirin Co., Ltd, and is a consultant/advisory board member for Mundipharma Co., Ltd. A. Utsunomiya reports receiving speakers bureau honoraria from Bristol-Myers Squibb Co., Chugai Pharmaceutical Co., Ltd., and Kyowa Hakko Kirin Co., Ltd. S. Iida reports receiving commercial research grants from Bristol-Myers Squibb Co., Celgene, Chugai Pharmaceutical Co., Ltd., Eli Lilly Japan K. K., Kyowa Hakko Kirin Co., Ltd, Nippon Kayaku Co., Ltd., and Taiho Pharmaceutical Co, Ltd.; speakers bureau honoraria from Celgene, and Janssen Pharmaceutical Company; and is a consultant/advisory board member for Ono Pharmaceutical Co., Ltd. No potential conflicts of interest were disclosed by the other authors.

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Clinical Cancer Research

Prognostic Significance of Tryptophan Catabolism in Adult T-cell Leukemia/Lymphoma

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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-