TO THE EDITOR:

CCR4 mutations associated with superior outcome of adult T-cell leukemia/lymphoma under mogamulizumab treatment

Yuma Sakamoto,[1,*] Takashi Ishida,[2,3,*] Ayako Masaki,[1] Takayuki Murase,[1] Kentaro Yonekura,[4] Yukie Tashiro,[5] Masahito Tokunaga,[6] Atae Utsunomiya,[6] Asahi Ito,[3] Shigeru Kusumoto,[3] Shinsuke Iida,[3] Ryuzo Ueda,[7] and Hiroshi Inagaki[1]

[1]Department of Pathology and Molecular Diagnostics, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan; [2]Division of Hematology and Oncology, Department of Internal Medicine, School of Medicine, Iwate Medical University, Morioka, Japan; [3]Department of Hematology and Oncology, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan; [4]Department of Dermatology, [5]Department of Pathology, and [6]Department of Hematology, Imamura General Hospital, Kagoshima, Japan; and [7]Department of Tumor Immunology, School of Medicine, Aichi Medical University, Nagakute, Japan

Adult T-cell leukemia/lymphoma (ATL) is caused by human T-cell lymphotropic virus type 1.[1-3] Patients, especially those with an aggressive variant (acute, lymphoma, and unfavorable chronic subtypes),[4,5] have an extremely poor prognosis.[1-7] Because CCR4 is expressed on tumor cells from most patients with ATL,[8-10] a therapeutic anti-CCR4 monoclonal antibody, mogamulizumab, has been developed.[11-13] Gain-of-function mutations of *CCR4* have been reported in ATL, associated with alterations at the carboxyl terminus[14-16]; therefore, the aim of the present study was to determine their significance for responsiveness of such patients in the mogamulizumab era.

We analyzed tumor samples from 116 patients with ATL. Diagnosis and assignment of clinical subtypes of ATL were conducted according to Japan Lymphoma Study Group recommendations.[17] Tumor samples were obtained at the time of initial presentation at the hospital, and we used the clinical characteristics recorded at that time as baseline data. All donors provided written informed consent before tumor 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 and Imamura General Hospital.

Using the QIAamp DNA FFPE Tissue Kit (56404; QIAGEN Inc., Germantown, MD), genomic DNA was extracted from formalin-fixed, paraffin-embedded biopsy tissues in the 82 patients diagnosed with ATL by histopathology. Genomic DNA was extracted from peripheral blood mononuclear cells of 34 patients with ATL using the AllPrep DNA/RNA Mini Kit (80204; QIAGEN Inc.). Only peripheral blood mononuclear cells containing >30% abnormal lymphocytes were included in the present study.

DNA fragments encompassing codons 322 to 348 of *CCR4* were amplified from genomic DNA using the primers shown in supplemental Table 1, available on the *Blood* Web site. Polymerase chain reaction products were purified and directly sequenced using the BigDye Terminator 3.1 System (Applied Biosystems, Foster City, CA) and analyzed on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). The mutations were validated using both sense and antisense sequence primers (supplemental Table 1), and all samples were tested in at least 2 independent experiments.

DNA fragments containing codons 322 to 348 of *CCR4* were amplified in the same manner (supplemental Table 1), and *CCR4* nonsense mutations C329*, Q330*, Y331*, Q336*, and Y347*, which have been previously reported,[14-16] were detected using the SNaPshot Multiplex Kit (Applied Biosystems). The fluorescence type and size of the extended products were determined by capillary electrophoresis on an ABI PRISM 3130 Genetic Analyzer with the aid of GeneMapper v4.0 software (Applied Biosystems). The mutations were validated using both sense and antisense probe primers (supplemental Table 1), and all samples were tested in at least 2 independent experiments.

Differences between the 2 groups were examined with the Fisher's exact test or Mann-Whitney *U* test. Survival was estimated using the Kaplan-Meier method and compared using the log-rank test. The start date for assessing progression-free survival (PFS) and overall survival (OS) was the day when the tumor sample was obtained. PFS was defined as time to progression, relapse, or death resulting from any cause, whichever occurred first. All analyses were carried out with the SPSS Statistics 17.0 package (IBM, Armonk, NY), and $P < .05$ (2 sided) was considered significant.

The patients with ATL enrolled in this study included 54 men and 62 women (age range, 41-90 years; median, 64 years). They included 73 patients with acute, 20 with lymphoma, 7 with unfavorable chronic, 4 with favorable chronic, and 12 with smoldering subtypes. *CCR4* gene alterations leading to amino acid changes were observed in 38 patients (32.8%). They included 11 C329*, 4 Q330*, 9 Y331*, 5 Q336*, 2 R323fs, 1 F326fs, 1 C329fs, 2 Y331fs, and 3 S345fs *CCR4* mutations (supplemental Figures 1 and 2).

There were no significant differences in age, sex, clinical variant, Eastern Cooperative Oncology Group performance status, serum soluble interleukin-2 receptor level, serum-adjusted calcium, Ann Arbor stage, white blood cell counts, hemoglobin, or platelet counts between patients with or without *CCR4* mutations (Table 1). There were also no significant differences in mogamulizumab treatment strategies between patients with or without *CCR4* mutations (supplemental Table 2).[4,5] Therefore, no obvious clinical features distinguish patients with ATL with *CCR4* mutations from those without.

Five-year PFS and OS in the present study were 27.5% (supplemental Figure 3A) and 46.2% (Figure 1A), respectively. Neither PFS (supplemental Figure 3B) nor OS (Figure 1B) was significantly different on stratification according to *CCR4* mutations. Five-year PFS and OS of patients with an indolent variant were 74.0% and 93.8%, but only 19.0% and 38.0% for those with an aggressive variant, respectively ($P = .001$ and $P = .002$, respectively; supplemental Figure 3C; Figure 1C). In

Table 1. Characteristics of patients with ATL according to CCR4 mutations

Characteristic	Total N (%) of patients		P
	CCR4 mutations		
	Absence (n = 78)	Presence (n = 38)	
Age, years			.243
≤70	57 (73)	32 (84)	
>70	21 (27)	6 (16)	
Sex			.693
Female	43 (55)	19 (50)	
Male	35 (45)	19 (50)	
Clinical variant			.258
Indolent	13 (17)	3 (8)	
Aggressive	65 (83)	35 (92)	
ECOG PS			.393
0-1	53 (68)	29 (76)	
2-4	25 (32)	9 (24)	
sIL2R, U/mL			.403
≤20 000	44 (61)	26 (70)	
>20 000	28 (39)	11 (30)	
Serum Ca*, mg/dL			1.000
≤11	62 (85)	31 (84)	
>11	11 (15)	6 (16)	
Serum Alb, g/dL			.664
≥3.5	50 (68)	27 (73)	
<3.5	23 (32)	10 (27)	
Stage			.270
I, II	7 (9)	1 (3)	
III, IV	71 (91)	37 (97)	
White blood cell counts, ×10⁹/L			.418
Mean	16.4	19.1	
Median	9.6	7.7	
Range	2.5-115.9	2.5-232.1	
Hemoglobin, g/dL			.586
Mean	12.6	12.8	
Median	12.8	13.0	
Range	7.9-16.0	8.8-16.3	
Platelet counts, ×10⁹/L			.083
Mean	200	239	
Median	200	236	
Range	28-443	120-602	

Alb, albumin; Ca, calcium; ECOG PS, Eastern Cooperative Oncology Group performance status; sIL2R, soluble interleukin-2 receptor.

*When serum Alb level was <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)].

the latter, there were also no significant differences in PFS or OS according to *CCR4* mutations (supplemental Figure 3D; Figure 1D). In patients not receiving mogamulizumab-containing treatment or allogeneic HSCT, *CCR4* mutation status had no significant impact on PFS (supplemental Figure 3E) or OS (Figure 1E). The same was true for patients who did receive allogeneic HSCT (supplemental Figure 3F; Figure 1F). Together, these indicate that *CCR4* gain-of-function mutations do not have any prognostic impact in patients with ATL receiving different therapies not including mogamulizumab.

In contrast, for patients who did receive mogamulizumab-containing treatment (but no allogeneic HSCT), 5-year PFS and survival from the day of the first dose of antibody were 63.6% and 72.2% in those with *CCR4* mutations, but not reached and only 26.2% in those without, respectively ($P = .023$ and $P = .027$, respectively; supplemental Figure 3G; Figure 1G). Subdividing the cohort into patients with or without an aggressive variant revealed that those receiving mogamulizumab-containing treatment (but no allogeneic HSCT) had 5-year PFS and OS from the day of the first antibody dose of 70.0% and 80.0% in the group with *CCR4* mutations, but only not reached and 24.7% in the group without, respectively ($P = .007$ and $P = .006$, respectively; supplemental Figure 3H; Figure 1H). These findings are likely due to *CCR4* gain-of-function mutations leading to impaired CCR4 internalization upon ligand binding, resulting in increased CCR4 expression even in the presence of the ligand.[14,15] In any event, these findings in patients with ATL with *CCR4* mutations who received mogamulizumab-containing treatment, as observed in the present study, do seem surprising and suggest that this group responds especially well to this approach. Thus far, it has been generally accepted that allogeneic HSCT is the only curative treatment for ATL,[2,18] but our present study indicates that a subset of patients with gain-of-function *CCR4* mutations actually experienced long-term survival with mogamulizumab-containing treatment without allogeneic HSCT. Mogamulizumab is currently approved for the treatment of CCR4$^+$ peripheral T-cell lymphomas other than ATL in Japan[19] and will be approved for the treatment of cutaneous T-cell lymphoma globally.[20,21] The *CCR4* gain-of-function mutations represent excellent biomarkers for mogamulizumab treatment outcome not only in ATL, but likely also in other types of T-cell lymphoma including cutaneous T-cell lymphoma.

Although the present study is valuable for clinical hematologists, its limitations need to be borne in mind. The patient cohort was relatively small, with consequent limitations on our ability to draw definitive conclusions about the sensitivity of ATL to mogamulizumab treatment. Therefore, a confirmation study analyzing more tumor samples from patients enrolled in prospective trials such as the MIMOGA study (registered at http://www.umin.ac.jp/ctr/ as #UMIN000008696) is warranted.

In conclusion, *CCR4* gain-of-function mutations determine sensitivity to mogamulizumab treatment in ATL. Additional investigations to clarify the mechanisms responsible for the present observations are warranted.

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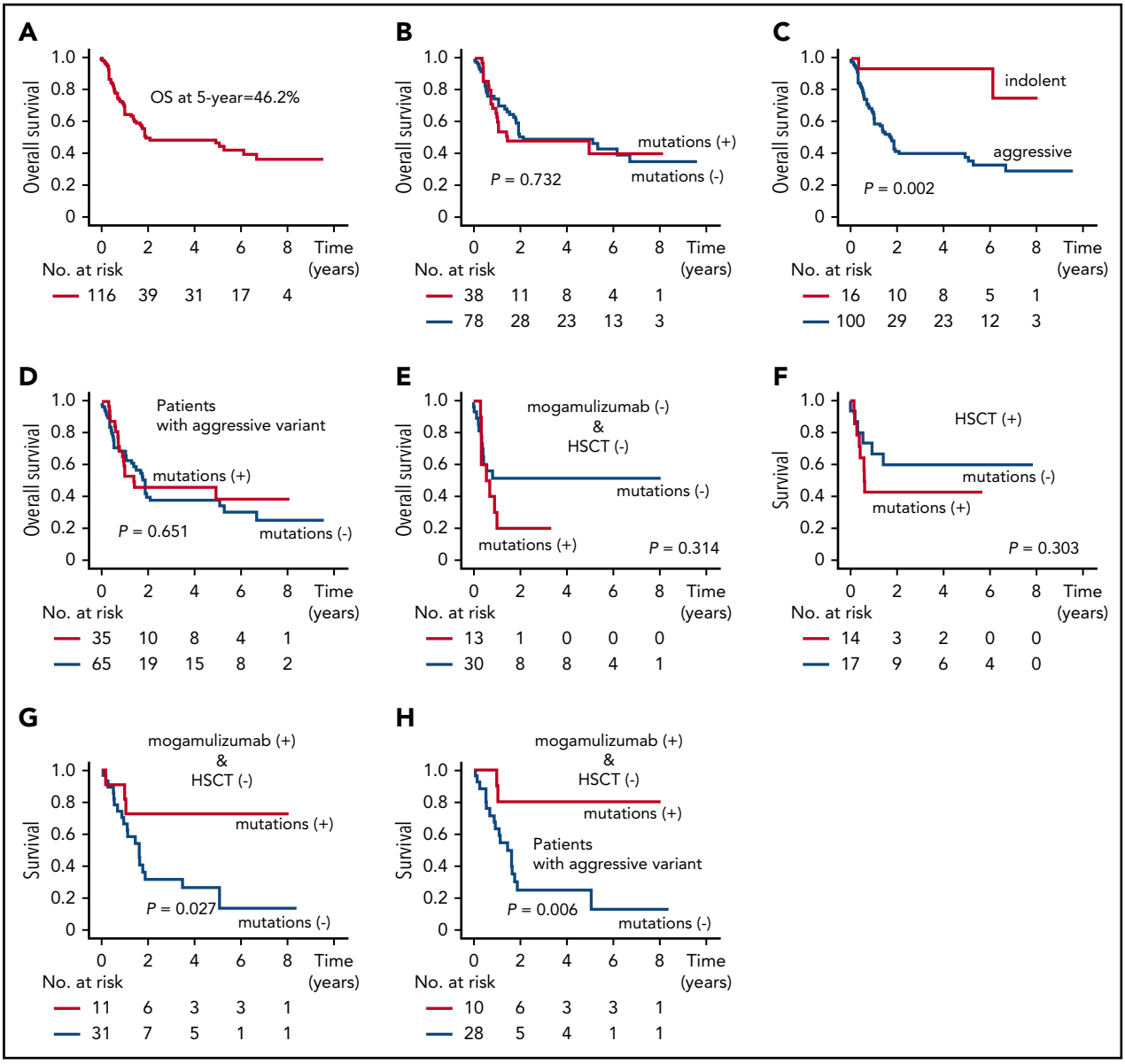

Figure 1. Survival of patients with ATL stratified according to CCR4 mutations. (A) Five-year OS of all 116 patients with ATL was 46.2% (95% confidence interval [CI], 35.8%-56.6%). (B) Five-year OS of the 38 patients with CCR4 mutations and 78 patients without mutations was 40.0% (95% CI, 20.0%-60.0%) and 48.9% (95% CI, 36.4%-61.4%), respectively. (C) Five-year OS of the 16 patients with indolent variant and 100 patients with aggressive variant was 93.8% (95% CI, 81.8%-100.0%) and 38.0% (95% CI, 26.8%-49.2%), respectively. The former was significantly higher than the latter. (D) Among the patients with ATL with aggressive variant (n = 100), 5-year OS of those with *CCR4* mutations (n = 35) or without (n = 65) was 38.5% (95% CI, 18.5%-58.5%) and 38.0% (95% CI, 24.5%-51.5%), respectively. (E) Among the 43 patients with ATL who did not receive any mogamulizumab-containing treatment or allogeneic hematopoietic stem-cell transplantation (HSCT), 5-year OS in the 13 with *CCR4* mutations and 30 without was not reached and 51.4% (95% CI, 31.2%-71.6%), respectively. (F) Among the 31 patients with ATL who did receive allogeneic HSCT, 5-year survival from the day of allogeneic HSCT in the 14 patients with *CCR4* mutations and 17 without was 42.9% (95% CI, 17.0%-68.8%) and 60.3% (95% CI, 35.6%-85.0%), respectively. (G) Among the 42 patients with ATL who received mogamulizumab-containing treatment but no allogeneic HSCT, 5-year survival from the day of the first dose of antibody in the 11 patients with *CCR4* mutations and 31 without was 72.7% (95% CI, 46.4%-98.7%) and 26.2% (95% CI, 8.0%-44.4%), respectively. This difference reached statistical significance. (H) Among the 38 patients with ATL with aggressive variant who received mogamulizumab-containing treatment but no allogeneic HSCT, 5-year survival from the day of the first dose of antibody in the 10 patients with *CCR4* mutations and 28 without was 80.0% (95% CI, 55.3%-100.0%) and 24.7% (95% CI, 6.3%-43.1%), respectively. This difference was also statistically significant. Survival curves were compared using the log-rank test, and the *P* value is indicated in each panel.

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Authorship

Contribution: Y.S., T.I., and H.I. designed the research; all authors performed the experiments; T.I., R.U., and H.I. analyzed and interpreted data; and all authors wrote and approved the manuscript.

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ORCID profiles: T.I., 0000-0002-1060-0777; H.I., 0000-0001-6157-636X.

Correspondence: Hiroshi Inagaki, Department of Pathology and Molecular Diagnostics, Graduate School of Medical Sciences, Nagoya City University, Nagoya 467-8601, Japan; e-mail: hinagaki@med.nagoya-cu.ac.jp.

Footnotes

*Y.S. and T.I. contributed equally to this study.

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TO THE EDITOR:

Treatment of IgM-associated immunoglobulin light-chain amyloidosis with rituximab-bendamustine

Richa Manwani, Sajitha Sachchithanantham, Shameem Mahmood, Darren Foard, Faye Sharpley, Tamer Rezk, Thirusha Lane, Cristina Quarta, Marianna Fontana, Helen J. Lachmann, Julian D. Gillmore, Carol Whelan, Philip N. Hawkins, and Ashutosh D. Wechalekar

National Amyloidosis Centre, University College London (Royal Free Campus), Rowland Hill Street, London, United Kingdom

Systemic amyloid light-chain amyloidosis (AL) is characterized by deposition of misfolded immunoglobulin light chains within organs. AL with an immunoglobulin M (IgM) monoclonal protein (IgM-AL) accounts for 5% to 7% of AL and exhibits more prevalent lymph node, neuropathic, and lung involvement, less prevalent cardiac involvement, and lower amyloidogenic light chains.[1]