Clinical significance of TP53 mutations in adult T-cell leukemia/lymphoma

Yuma Sakamoto,1 Takashi Ishida,2 Ayako Masaki,1 Takayuki Murase,1 Morishige Takeshita,3 Reiji Muto,3 Hiromi Iwasaki,4 Asahi Ito,5 Shigeru Kusumoto,5 Nobuaki Nakano,6 Masahito Tokunaga,6 Kentaro Yonekura,7 Yukie Tashiro,8 Shinsuke Iida,5 Atae Utsunomiya,6 Ryuzo Ueda9,10 and Hiroshi Inagaki1

1Department of Pathology and Molecular Diagnostics, Graduate School of Medical Sciences, Nagoya City University, Nagoya, 2Department of Immunology, Nagoya University Graduate School of Medicine, Nagoya, 3Department of Pathology, Faculty of Medicine, Fukuoka University, 4Department of Hematology, National Hospital Organization Kyushu Medical Center, Fukuoka, 5Department of Hematology and Oncology, Graduate School of Medical Sciences, Nagoya City University, Nagoya, 6Department of Hematology, Imamura General Hospital, Kagoshima, and 7Department of Tumor Immunology, School of Medicine, Aichi Medical University, Nagakute, Japan

Received 22 April 2021; accepted for publication 20 July 2021

Correspondence: Takashi Ishida, Department of Immunology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showaku, Nagoya, Aichi 466-8560, Japan. E-mail: itakashi@med.nagoya-u.ac.jp

Hiroshi Inagaki, Department of Pathology and Molecular Diagnostics, Graduate School of Medical Sciences, Nagoya City University, 1-Kawasumi, Mizuho-ku, Nagoya, 467-8601, Japan. E-mail: hinagaki@med.nagoya-cu.ac.jp

Summary

Adult T-cell leukaemia/lymphoma (ATL) patients have a poor prognosis. Here, we investigated the impact of TP53 gene mutations on prognosis of ATL treated in different ways. Among 177 patients, we identified 47 single nucleotide variants or insertion-deletions (SNVs/indels) of the TP53 gene in 37 individuals. TP53 copy number variations (CNVs) were observed in 38 patients. Altogether, 67 of 177 patients harboured TP53 SNVs/indels or TP53 CNVs, and were categorized as having TP53 mutations. In the entire cohort, median survival of patients with and without TP53 mutations was 1-0 and 6-7 years respectively (P < 0.001). After allogeneic haematopoietic stem cell transplantation (HSCT), median survival of patients with (n = 16) and without (n = 29) TP53 mutations was 0-4 years and not reached respectively (P = 0.001). For patients receiving mogamulizumab without allogeneic HSCT, the median survival from the first dose of antibody in patients with TP53 mutations (n = 27) was only 0-9 years, but 5-1 years in those without (n = 42; P < 0.001). Thus, TP53 mutations are associated with unfavourable prognosis of ATL, regardless of treatment strategy. The establishment of alternative modalities to overcome the adverse impact of TP53 mutations in patients with ATL is required.

Keywords: adult T-cell leukaemia, Lymphoma, TP53, Mutation, allogeneic haematopoietic stem cell transplantation.
Introduction

Adult T-cell leukaemia/lymphoma (ATL) is a peripheral T-cell neoplasm caused by human T-cell lymphotropic virus type-1 (HTLV-1), and has a poor prognosis.1–3 The entire landscape of genetic aberrations in ATL has been delineated.4 In that report, TP53, which is the most commonly mutated gene in human cancers,5–7 was also frequently mutated in ATL.4 In general, TP53 mutations are associated with adverse prognosis in many sporadic cancers,8 but TP53 single nucleotide variants (SNVs)/insertion-deletions (indels) or copy number variations (CNVs) were not shown to be prognostic factors for ATL patients in an earlier study.9 On the other hand, another earlier study reported that ATL patients with TP53 mutations (n = 10) had a significantly shorter survival than those without such mutations (n = 46).10 Other investigators suggested that, during multistep oncogenesis, TP53 mutations played a role in later stages of ATL development.11 Additionally, several studies indicated a close association between HTLV-1 and TP53 for tumourigenesis.12–14 Thus, based on these earlier studies, the aim of the present study was to determine the clinical significance of TP53 mutations in ATL according to the treatment strategies which the patients received.

Methods

ATL patients

The present study included 177 ATL patients. Details are available in Data S1.2

Nucleic acid extraction

Details are available in Data S1.

Detection of TP53 SNVs/indels by targeted next-generation sequencing

Details are available in Data S1.

Detection of TP53 copy number variations

Details are available in Data S1.15

Detection of CCR4 and CD28 gene mutations

Details are available in Data S1.16,17

Statistical analysis

The start date for assessing overall survival (OS) was defined as the day when the tumour sample was obtained. Details are available in Data S1.

Results

Clinical characteristics of the ATL patients enrolled in the present study

The ATL patients enrolled in this study included 86 men and 91 women (age range 41–90 years; median 64 years; Table I). Tumour samples were obtained from each patient at the time of initial presentation at the participating hospital, and we used the clinical characteristics including clinical subtypes recorded at that time. Treatments administered to the ATL patients enrolled in the present study varied, as they were determined at each investigator’s clinical discretion. A VCAP-AMP-VECP ( vincristine, cyclophosphamide, doxorubicin, and prednisone; doxorubicin, ranimustine, and prednisone; and vindesine, etoposide, carboplatin, and prednisolone)-like, or CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone)-like regimen with or without mogamulizumab was initially administered to many patients with acute or lymphoma subtypes.18–21 Relatively younger patients (≤70 years) were planned to receive allogeneic haematopoietic stem cell transplantation (HSCT) while in remission after chemotherapy without mogamulizumab,22–25 because pre-HSCT mogamulizumab can result in increased severity of graft-versus-host disease.26–28 Some patients received lenalidomide.29 Patients with chronic or smouldering subtypes were mostly managed on a careful watch-and-wait basis until disease progression to acute or lymphoma subtypes. The treatments administered to the patients, right after their tumour sampling, are shown in Table SI.

TP53 gene mutations in ATL patients

Forty-seven non-synonymous SNVs/indels of the TP53 gene were identified in 37 ATL patients (20-9%), and five patients were found to harbour more than one of these (Fig 1). TP53 CNVs, such as homozygous and heterozygous deletions detected by fluorescence in-situ hybridization (FISH), were observed in 10 and 28 patients (5.6% and 15.8%) respectively. To illustrate the FISH analysis, TP53:SE17 signal numbers of 2:2, 0:2 (homozygous deletion), and 1:2 (heterozygous deletion) are shown in Fig 2A–C respectively.

CCR4 and CD28 mutations in ATL patients

CCR4 mutations were detected in 57 patients (32-2%), including two R323fs, two F326fs, 15 C329fs, one C329fs, one I337fs, four Q330*, one Q330fs, 17 Y331*, four Y331fs, seven Q336*, and three S345fs CCR4 SNVs (data not shown). CD28 mutations were found in 66 patients (37-3%), including 24 CD28-related fusions (four CTLA4-CD28 and 20 ICOS-CD28), three activating SNVs (F51I, D124V, and D124E), and 44 CNVs (27 gains and 17 amplifications). Thus, two patients simultaneously harboured two different
Clinical characteristics of ATL patients stratified by TP53 mutations

ATL patients harbouring any of the identified TP53 SNVs/indels or CNVs were categorized as having TP53 mutations. In the present study, 67 of 177 patients (37.9%) had TP53 mutations according to this definition. Patients with TP53 mutations had a significantly worse Eastern Cooperative Oncology Group (ECOG) performance status (PS), a higher serum-soluble interleukin-2 receptor (sIL-2R) level, and a higher serum-adjusted calcium (Ca) level, relative to those without mutations. Patients with TP53 mutations tended to be older. There were no significant differences in the presence or absence of CCR4 mutations between patients with or without TP53 mutations, but there was a trend for patients with TP53 mutations to be more likely to harbour CD28 mutations (Table I).

OS of ATL patients stratified by TP53 mutations

The median OS of all patients enrolled in the present study was 1.8 years (Fig 3A). OS of patients with a higher serum sIL-2R level was significantly shorter than of those with a lower level (Fig 3B), and patients with a worse ECOG PS had a significantly shorter OS than those with a better PS (Fig 3C). Also, the OS of patients with acute or lymphoma subtypes was significantly shorter than of those with chronic or smouldering subtypes (Fig 3D).

The median OS of all the patients with and without TP53 mutations was 1.0 and 6.7 years respectively ($P = 0.001$; Fig 3E). Those with TP53 mutations were divided into two groups as follows: those having TP53 SNVs/indels with or without TP53 CNVs ($n = 37$), and those having TP53 CNVs without TP53 SNVs/indels ($n = 30$). Accordingly, the former group was designated “TP53 SNVs/indels ± CNVs”, and the latter “TP53 CNVs”. The median OS of patients with TP53 SNVs/indels ± CNVs was only 1.0 year, but this was significantly better than the OS of those with TP53 CNVs, which was 0.8 years ($P = 0.025$; Fig 3F). Regarding other gene mutations, there were no significant differences in OS between patients with or without CCR4 mutations (median OS, 1.9 vs. 1.4 years respectively; $P = 0.992$; Fig 3G). In contrast, the median OS of patients with or without CD28 mutations was significantly different at 1.0 and 2.6 years respectively ($P = 0.010$; Fig 3H).

OS of ATL patients stratified by TP53 mutations, after censoring transplanted patients on the day of allogeneic HSCT

The median HSCT-censored OS of all patients enrolled in the present study was 1.9 years (Fig 4A), whereas this was 1.3 years

Table I. Characteristics of ATL patients according to TP53 mutations.

| Characteristics            | TP53 mutations |   | P value |
|----------------------------|----------------|---|---------|
|                            | Absent         | Present |         |
| Number (%)                 | 110 (62)       | 67 (38) | 0.167   |
| Sex                        |                |         |         |
| Female                     | 52 (47)        | 39 (58) |         |
| Male                       | 58 (53)        | 28 (42) |         |
| Clinical subtype           |                |         |         |
| Chronic, smouldering       | 18 (16)        | 5 (7)   | 0.108   |
| Acute, lymphoma            | 92 (84)        | 62 (93) |         |
| ECOG PS*                   | 0, 1           | 18 (17) | 0.001   |
| 2, 3, 4                    | 91 (83)        | 40 (60) |         |
| Serum sIL-2R (U/ml)**      | 73 (70)        | 30 (47) | 0.005   |
| ≤20 000                    | 32 (30)        | 34 (53) |         |
| >20 000                    | 99 (93)        | 51 (81) |         |
| Serum Ca (mg/dl)**         | 8 (7)          | 12 (19) | 0.029   |
| ≤11-0                      | 28 (26)        | 26 (33) |         |
| >11-0                      | 79 (74)        | 38 (66) |         |
| Serum albumin (g/dl)***** | ≥3-5           | 68      | 0.050   |
|                            | <3-5           | 67      |         |
| Age (years)                | 65             | 68      |         |
| Mean                       | 64             | 67      |         |
| Median                     | 41–90          | 41–85   |         |
| WBC (/μl)*****             | 15 907         | 13 098  | 0.680   |
| Mean                       | 8 120          | 8 560   |         |
| Median                     | 2 900–232 100 | 2 500–51 100 |         |
| Hb (g/l)******             | 130            | 124     | 0.075   |
| Mean                       | 134            | 125     |         |
| Median                     | 60–171         | 61–163  |         |
| Pt (x10⁹/μl)******         | 224            | 228     | 0.754   |
| Mean                       | 217            | 204     |         |
| Median                     | 4–622          | 56–602  |         |
| CCR4 gene mutation         |                |         |         |
| Absent                     | 71 (65)        | 49 (73) | 0.251   |
| Present                    | 39 (35)        | 18 (27) |         |
| CD28 gene mutation         |                |         |         |
| Absent                     | 75 (68)        | 36 (54) | 0.057   |
| Present                    | 35 (32)        | 31 (46) |         |

Alb, albumin; ATL, adult T-cell leukaemia/lymphoma; Ca, calcium; CCR4, CC chemokine receptor 4; ECOG, Eastern Cooperative Oncology Group; Hb, haemoglobin; Pt, platelet count; PS, performance status; sIL-2R, soluble interleukin-2 receptor; WBC, white blood cell count.

*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)].

*A patient’s data were unknown.

**Eight patients’ data were unknown.

***Seven patients’ data were unknown.

****Six patients’ data were unknown.

*****Five patients’ data were unknown.

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Fig 1. Schematic representation of TP53 and the mutations detected. Each circle represents one patient with open symbols representing missense and closed symbols representing nonsense mutations. The number beside each circle indicates amino acid position, and the mutated amino acid is indicated in each circle. Positions of nucleotide substitutions associated with splicing abnormalities in the intron are indicated by inverted triangles. TAD, Transactivation domain, PRD, Proline rich domain, OD, Oligomerization domain. [Colour figure can be viewed at wileyonlinelibrary.com]

Fig 2. TP53 copy number variations (CNVs) in adult T-cell leukaemia/lymphoma (ATL) by fluorescence in-situ hybridization (FISH). FISH analyses on FFPE sections from three individual ATL patients. TP53 signals on chromosome 17p13 are red, and centromeric signals of chromosome 17 are green. TP53 signal number:centromeric signal number ratios were 2:2 (A), 0:2 (B), and 1:2 (C). [Colour figure can be viewed at wileyonlinelibrary.com]

Fig 3. Overall survival (OS) of all adult T-cell leukaemia/lymphoma (ATL) patients enrolled in the study, stratified according to TP53 gene mutations. (A) OS of all ATL patients enrolled in the study (n = 177). The median OS was 1.8 years [95% confidence interval (CI), 1.3–2.3 years]. (B) OS according to serum-soluble interleukin-2 (sIL-2R) level, showing a significant association with OS [>20 000 U/ml compared with ≤20 000; hazard ratio (HR), 2.676; 95% CI, 1.730–4.138]. (C) OS according to Eastern Cooperative Oncology Group (ECOG) performance status (PS), which was significantly associated with OS (2–4 compared with 0 or 1; HR, 3.342; 95% CI, 2.127–5.252). (D) OS according to ATL clinical subtype, significantly associated with OS (acute or lymphoma subtypes compared with chronic or smouldering subtypes; HR, 4.286; 95% CI, 1.571–11.691). (E) OS according to TP53 mutations, showing significant associations with OS [TP53 mutations (+) compared with (−); HR, 3.003; 95% CI, 1.963–4.592]. (F) OS according to the types of TP53 mutations significantly associated with OS [TP53 single nucleotide variants (SNVs) or insertion-deletions with or without copy number variations [TP53 SNVs/indels ± copy number variations (CNVs)] compared with TP53 mutations (−); HR, 2.368; 95% CI, 1.436–3.906]. TP53 copy number variations without single nucleotide variations or insertion-deletions [TP53 CNVs] compared with TP53 mutations (−); HR, 4.238; 95% CI, 2.470–7.271]. TP53 CNVs compared with TP53 (SNVs/indels ± CNVs; HR, 1.981; 95% CI, 1.080–3.627]. (G) OS according to CCR4 mutations [CCR4 mutations (+) compared with (−); HR, 0.998, 95% CI, 0.638–1.561, not significant, n.s.]. (H) OS according to CD28 mutations, which are significantly associated with OS [CD28 mutations (+) compared with (−); HR, 1.718; 95% CI, 1.131–2.610].
TP53 mutations in adult T-cell leukemia/lymphoma

(A) Overall Survival

median OS = 1.8 years

No. at risk

0 Time

years

(B) sIL2R (U/mL)

≤ 20,000

> 20,000

P < 0.001

No. at risk

0 Time

years

(C) Overall Survival

PS

0, 1

2, 3, 4

P < 0.001

No. at risk

0 Time

years

(D) Overall Survival

Clinical subtype

chronic or smoldering

P = 0.002

acute or lymphoma

No. at risk

0 Time

years

(E) Overall Survival

TP53 mutations (-)

TP53 mutations (+)

P < 0.001

No. at risk

0 Time

years

(F) Overall Survival

TP53 mutations (-)

TP53 mutations (+)

TP53 SNVs/indels

TP53 CNVs

P = 0.992

No. at risk

0 Time

years

(G) Overall Survival

CCR4

CCR4 mutations (+)

CCR4 mutations (-)

P = 0.992

No. at risk

0 Time

years

(H) Overall Survival

CD28

CD28 mutations (-)

CD28 mutations (+)

P = 0.010

No. at risk

0 Time

years

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Fig 4. Overall survival (OS) of adult T-cell leukaemia/lymphoma (ATL) patients stratified by TP53 mutations after censoring transplanted patients on the day of allogeneic haematopoietic stem cell transplantation (HSCT). (A) HSCT-censored OS of all ATL patients enrolled in the study (n = 177) showing a median OS of 1.9 years [95% confidence interval (CI), 0.0–2.3 years]. (B) HSCT-censored OS according to TP53 mutations [TP53 mutations (+) compared with (−)]; hazard ratio (HR), 2.639; 95% CI, 1.612–4.319]. (C) HSCT-censored OS according to the type of TP53 mutation [TP53 single nucleotide variants or insertion-deletions (SNVs)/indels ± copy number variations (CNVs) compared with TP53 mutations (−)]; HR, 2.163; 95% CI, 1.231–3.802. TP53 CNVs compared with TP53 mutations (−)); HR, 4.231; 95% CI, 2.134–8.390. TP53 CNVs compared with TP53 SNVs/indels ± CNVs; HR, 2.082; 95% CI, 0.963–4.499]. (D) HSCT-censored OS according to CCR4 mutations [CCR4 mutations (+) compared with (−)]; HR, 0.907, 95% CI, 0.527–1.563, n.s.]. (E) HSCT-censored OS according to CD28 mutations [CD28 mutations (+) compared with (−)]; HR, 2.129; 95% CI, 1.298–3.493].
and not reached (NR; \( P < 0.001 \)) for patients with and without \( TP53 \) mutations respectively (Fig 4B). The median HSCT-censored OS of patients with \( TP53 \) SNVs/indels ± CNVs was only 1·7 years, but this trended to be better than patients with \( TP53 \) CNVs (1·0 year; \( P = 0·057 \); Fig 4C). Regarding other gene mutations, there were no significant differences in the HSCT-censored OS between patients with or without \( CCR4 \) mutations (1·8 vs. 1·9 years respectively; \( P = 0·726 \); Fig 4D). In contrast, the median HSCT-censored OS of patients with or without \( CD28 \) mutations was significantly different at 1·1 and 4·9 years respectively (\( P = 0·002 \); Fig 4E).

**Survival of ATL patients receiving allogeneic HSCT stratified by \( TP53 \) mutations**

The median survival from the day of allogeneic HSCT in all 45 transplanted patients was 1·4 years (Fig 5A). There was no difference in survival between patients with higher or lower serum sIL-2R levels (Fig 5B) or between those with a better or worse ECOG PS (Fig 5C). There was also no difference in survival between patients with acute or lymphoma, and chronic or smouldering subtypes (Fig 5D).

The median survival of patients with and without \( TP53 \) mutations was 0·4 years and NR respectively (\( P = 0·001 \); Fig 5E). On the other hand, there was no significant difference in survival from the day of allogeneic HSCT between patients with \( TP53 \) SNVs/indels ± CNVs and \( TP53 \) CNVs (median survival 0·3 vs 0·4 years respectively; \( P = 0·790 \); Fig 5F). Regarding other gene mutations, there were also no significant differences in survival between patients with or without \( CCR4 \) mutations (median survival 0·6 vs. 1·5 years respectively; \( P = 0·938 \); Fig 5G), or those with or without \( CD28 \) mutations (median survival 0·6 vs. 1·4 years respectively; \( P = 0·968 \); Fig 5H).

**Survival of ATL patients who received mogamulizumab, but did not receive allogeneic HSCT, stratified by \( TP53 \) mutations**

We evaluated the impact of \( TP53 \) mutations on survival of patients receiving mogamulizumab without allogeneic HSCT. The median survival from the first dose of mogamulizumab in 69 patients was 1·6 years (Fig 6A). There was a trend towards worse survival in patients with a higher versus a lower serum sIL-2R level (Fig 6B). Survival from the day of the first dose of antibody in patients with a poorer ECOG PS was significantly worse than in those with a better ECOG PS (Fig 6C). However, there was no significant difference in survival between patients with acute or lymphoma, and chronic or smouldering subtypes (Fig 6D).

The median survival from the first dose of antibody in patients with \( TP53 \) mutations was 0·9 compared to 5·1 years in those without \( TP53 \) mutations (\( P < 0·001 \); Fig 6E). For patients with \( TP53 \) SNVs/indels ± CNVs, median survival was 1·0 year, significantly better than for those with \( TP53 \) CNVs (0·3 years; \( P = 0·041 \); Fig 6F). Regarding other gene mutations, there was a trend towards better survival from the day of the first dose of antibody in patients with \( CCR4 \) mutations compared to those without such mutations (median survival NR vs. 1·6 years; \( P = 0·059 \); Fig 6G). Survival from the day of the first dose of antibody in patients with \( CD28 \) mutations was significantly worse than in those without \( CD28 \) mutations (median survival 0·7 vs. 1·5 years; \( P = 0·013 \); Fig 6H). Finally, survival from the day of the first dose of antibody in patients without \( TP53 \) but with \( CCR4 \) mutations \((n = 12)\) was significantly better compared to that in the other patients \((n = 57)\); median survival NR vs. 1·4 years; \( P = 0·014 \); data not shown).

**Survival of ATL patients receiving mogamulizumab stratified by \( TP53 \) mutations after censoring transplanted patients on the day of allogeneic HSCT**

The median HSCT-censored survival from the day of the first dose of mogamulizumab was 1·6 years for all patients (Fig 7A), but for those with \( TP53 \) mutations it was only 0·9 years, compared to 5·1 years for those without \( TP53 \) mutations (\( P < 0·001 \); Fig 7B). There was no significant difference in the HSCT-censored survival between patients with \( TP53 \) SNVs/indels ± CNVs or with \( TP53 \) CNVs (1·0 vs. 0·5 years; \( P = 0·106 \); Fig 7C). Regarding other gene mutations, the median HSCT-censored survival from the first dose of antibody in patients with \( CCR4 \) mutations was NR, compared to 1·6 years in those without (\( P = 0·033 \); Fig 7D). HSCT-censored survival in patients with \( CD28 \) mutations tended to be worse than in those without \( CD28 \) mutations (0·7 vs. 1·5 years; \( P = 0·052 \); Fig 7E). Finally, the HSCT-censored survival from the day of the first dose of antibody was analyzed in patients stratified by \( TP53 \) and \( CCR4 \) mutations. Median survival in patients without \( TP53 \) but with \( CCR4 \) mutations \((n = 15)\), without either \( TP53 \) or \( CCR4 \) mutations \((n = 31)\), with \( TP53 \) but without \( CCR4 \) mutations \((n = 23)\), and with both \( TP53 \) and \( CCR4 \) mutations \((n = 8)\), was NR, 1·8, 0·9, and 1·0 years respectively. Thus, HSCT-censored survival of patients without \( TP53 \) but with \( CCR4 \) mutations was significantly better than for those with \( TP53 \) but without \( CCR4 \) mutations (\( P < 0·001 \)), and tended to be better than in patients without either \( TP53 \) or \( CCR4 \) mutations (\( P = 0·084 \); Fig 7F). Accordingly, the median HSCT-censored survival in patients without \( TP53 \) but with \( CCR4 \) mutations \((n = 15)\) was significantly better compared to all other patients grouped together \((n = 62)\); NR vs. 1·6 years; \( P = 0·008 \); Fig 7G).

**Multivariate analysis of \( TP53 \), \( CCR4 \) and \( CD28 \) mutations influencing survival in ATL patients**

Multivariate analysis of survival from the day of allogeneic HSCT in 45 patients with ATL was performed using the

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following three variables: CCR4 mutations, CD28 mutations, and TP53 mutations. Of these, TP53 mutations were significantly associated with a worse survival (hazard ratio [HR], 3.987; 95% CI, 1.693–9.392; Table II). Multivariate analysis of survival from the day of the first dose of mogamulizumab in the 69 patients with ATL who did not receive allogeneic HSCT was performed using the same three variables; again, TP53 mutations were significantly associated with a worse survival (HR, 2.661; 95% CI, 1.303–5.434; Table III). Furthermore, multivariate analysis of HSCT-censored survival from the day of the first dose of antibody in 77 patients was performed using the same three variables, and again, TP53 mutations were significantly associated with a worse survival (HR, 2.733; 95% CI, 1.347–5.544; Table IV).

**Discussion**

The present study documents that ATL patients with TP53 mutations such as SNVs/indels or CNVs have a significant worse prognosis than patients without such mutations. To the best of our knowledge, this is the first report demonstrating the clinical significance of TP53 mutations in a large cohort of ATL patients. The frequency of TP53 mutations in ATL was very similar to results in an earlier report.4

In the entire cohort examined here, ATL patients with TP53 mutations had unfavourable clinical parameters to a greater extent than those without such mutations, such as worse PS, a higher serum sIL-2R or adjusted Ca levels.30,31 However, we found that there were no significant correlations between TP53 mutations and clinical subtype (acute or lymphoma versus chronic or smouldering), unlike what was reported in an earlier study.11 Regarding clinical outcome, patients with TP53 mutations had a significantly worse OS, which was also found to be the case when HSCT-censored OS was examined. Among patients with TP53 mutations, those with TP53 CNVs had a significantly worse OS than those with TP53 SNVs/indels ± CNVs; this also remained similar when HSCT-censored OS data were analyzed. However, the mechanisms accounting for the observed differences between the types of TP53 mutations remain unclear at present.

In general, because prognostic factors vary according to the treatment strategy even in the same disease, we next investigated the prognostic significance of TP53 mutations in ATL patients stratified according to their treatment modality. In this respect, we found that in the cohort of ATL patients receiving allogeneic HSCT, clinical parameters such as serum sIL-2R level, ECOG PS, or clinical subtype were not significantly associated with prognosis. This was presumably because allogeneic HSCT results in replacement of the haematopoietic and immune systems by healthy donor-derived cells, when ATL disease is relatively well controlled.22-24 Thus, the impact of otherwise unfavourable clinical parameters at the time of tumour sampling seems not to be relevant. Similarly, there were no significant differences in survival measured from the day of HSCT regardless of the presence or absence of CCR4 or CD28 mutations. On the other hand, patients with TP53 mutations did have a significantly worse prognosis, compared to those without such mutations. It is generally accepted that allogeneic HSCT is the only curative treatment for ATL,22-25 but, accordingly, our present study indicates that even this approach can hardly overcome the refractoriness to treatment of TP53-mutated ATL.

Next, we evaluated the impact of TP53 mutations in patients receiving mogamulizumab, without allogeneic HSCT.20,21,32,33 Here, again, TP53 mutations were associated with a significantly worse outcome, also confirmed with HSCT-censored survival data. In addition, patients with TP53 CNVs had a significantly worse prognosis than those with TP53 SNVs/indels ± CNVs, but again, the reason for this difference remains unclear. Patients with CCR4 mutations had a clear trend towards a more favourable prognosis compared to those without. The significantly better survival of patients receiving mogamulizumab with CCR4 mutations was also confirmed by the analysis of HSCT-censored survival. These findings are consistent with our previous report,16 and likely due to the fact that CCR4 mutations in the C-terminus lead to impaired CCR4 internalization upon ligand binding.4,34 Importantly, patients with CCR4 mutations but without TP53 mutations had an extremely good prognosis, as also confirmed with HSCT-
TP53 mutations in adult T-cell leukemia/lymphoma

**A.** Median survival = 1.4 years

**B.** sIL2R (U/mL)

| Time (years) | No. at risk |
|--------------|-------------|
| 0            | 28          |
| 2            | 10          |
| 4            | 7           |
| 6            | 4           |
| 8            | 0           |

**C.** PS

| Time (years) | No. at risk |
|--------------|-------------|
| 0            | 3           |
| 2            | 2           |
| 4            | 0           |

**D.** Clinical subtype

| Time (years) | No. at risk |
|--------------|-------------|
| 0            | 42          |
| 2            | 12          |
| 4            | 9           |
| 6            | 4           |
| 8            | 0           |

**E.** TP53 mutations (-)

| Time (years) | No. at risk |
|--------------|-------------|
| 0            | 29          |
| 2            | 12          |
| 4            | 9           |
| 6            | 4           |

**F.** TP53 mutations (+)

| Time (years) | No. at risk |
|--------------|-------------|
| 0            | 16          |
| 2            | 2           |
| 4            | 0           |

**G.** CCR4 mutations (+)

| Time (years) | No. at risk |
|--------------|-------------|
| 0            | 27          |
| 2            | 10          |
| 4            | 6           |
| 6            | 4           |

**H.** CCR4 mutations (-)

| Time (years) | No. at risk |
|--------------|-------------|
| 0            | 18          |
| 2            | 4           |
| 4            | 3           |

**P** values:

- **A:** $P = 0.001$
- **B:** $P = 0.132$
- **C:** $P = 0.181$
- **D:** $P = 0.553$
- **E:** $P = 0.938$
- **F:** $P = 0.968$
- **G:** $P = 0.938$
- **H:** $P = 0.968$
censored survival data. Accordingly, such patients can be expected to achieve long-term survival under mogamulizumab-containing treatment without the necessity for allogeneic HSCT.

In the present multivariate analysis including TP53, CCR4, and CD28 mutations, TP53 mutations were identified as independent unfavourable prognostic factors in all patient cohorts tested (i.e. survival from allogeneic HSCT, survival from the first mogamulizumab injection without HSCT, and HSCT-censored survival from the first mogamulizumab injection). In the field of ATL treatment, several novel agents such as mogamulizumab and lenalidomide have now become available in the clinic.20,21,29,32 On the other hand, the present study suggests that the currently available therapies, including allogeneic HSCT, will hardly be able to overcome the treatment refractoriness of ATL with TP53 mutations.

Although the present investigation offers significant observations regarding TP53 mutations for clinical outcomes in ATL patients, some limitations should be recognized. First, the significance of each type of TP53 mutation, especially in cases stratified by treatment strategy, were not fully elucidated due to an insufficient number of patients in the cohort. Second, the clinical impact of relatively novel agents such as lenalidomide or brentuximab vedotin in patients with TP53 mutations were not fully examined in the present study.29,35 Thus, further detailed investigations in much larger cohorts are warranted.

In conclusion, the present study demonstrates that TP53 mutations are significantly associated with an unfavourable prognosis in ATL patients. This was the case not only for patients in the entire cohort, but separately for those receiving allogeneic HSCT, and for those receiving mogamulizumab without allogeneic HSCT. The establishment of alternative treatment strategies which overcome the adverse impact of TP53 mutations in patients with ATL is urgently required.

Funding information
This work was supported by grants-in-aid for Early-Career Scientists (20K16177 to YS), and the Nitto Foundation (YS), Grants-in-aid from the Japan Agency for Medical Research and Development (No. 20ae010104h0001 and 21ae010104h0001 to RU, and No. 20cm0106301h0005 and 21cm0106301h0005 to TI).

Author contributions
YS, TI and HI designed the research. YS, TI, AM, TM, MT, RM, HI, AI, SK, NN, MT, KY, YT, SI, AU, RU and HI performed the experiments. TI, RU and HI analyzed and interpreted data. All authors wrote and approved the manuscript.

Conflicts of interest
HI received research funding from Kyowa Kirin Co., Ltd. SK received research funding from Chugai Pharmaceutical Co., Ltd., and Daiichi Sankyo Co., Ltd., and received honoraria from Chugai Pharmaceutical Co., Ltd. and Kyowa Kirin Co., Ltd. NN received honoraria from Novartis, Takeda pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., Celgene, Otsuka Pharmaceutical Co., Ltd., Nippon Shinyaku Co., Ltd., Kyowa Kirin Co., Ltd., and Asahi Kasei Pharma Co., Ltd., and received consulting fee from JIMRO. KY received honoraria from AbbVie, Celgene, Daiichi Sankyo Co., Ltd., Eisai, Eli Lilly Japan, Janssen Pharmaceutica, Kaken Pharmaceutical, Kyowa Kirin Co., Ltd., Maruhoo, Minophagen Pharmaceutical, Novartis, Sanofi, Taiho Pharmaceutical, Torii Pharmaceutical, and UCB Japan. SI received honoraria from Janssen, Celgene, Ono, Takeda, Sanofi, and Daiichi Sankyo Co., Ltd., and received research funding from Sanofi, Chugai, Ono, Takeda, Kyowa Kirin Co., Ltd., Celgene, Janssen, Bristol-Myers Squibb, Abbvie, and GlaxoSmithKlein. AU received honoraria from Kyowa Kirin Co., Ltd, Daiichi Sankyo Co., Ltd., Bristol-Myers and Celgene, and received consulting fees from HUYA Japan, JIMRO, Meiji Seika Pharma Co., Ltd. and Otsuka Medical Devices Co., Ltd. RU received research funding from Kyowa Kirin Co., Ltd., Chugai Pharmaceutical Co., Ltd., and Ono Pharmaceutical Co., Ltd. The other authors have no conflicts of interest to disclose.

Fig 6. Survival of adult T-cell leukaemia/lymphoma (ATL) patients receiving mogamulizumab but not allogeneic haematopoietic stem cell transplantation (HSCT), stratified by TP53 mutations. (A) Survival of ATL patients from the first dose of mogamulizumab (n = 69). The median survival was 1-6 years [95% confidence interval (CI), 1.0–2.3 years]. (B) Survival according to serum-soluble sIL-2R level tended to be associated with survival ≥20 000 U/ml compared with ≤20 000; hazard ratio (HR), 1.95; 95% CI, 0.987–3.853). (C) Survival according to Eastern Cooperative Oncology Group (ECOG) PS, significantly associated with survival (2–4 compared with 0 or 1; HR, 2.916; 95% CI, 1.401–6.069). (D) Survival according to ATL clinical subtype (acute or lymphoma subtypes compared with chronic or smouldering subtypes; HR, 1.264; 95% CI, 0.386–4.136, n.s.). (E) Survival according to TP53 mutations, significantly associated with survival [TP3 mutations (+) compared with (−); HR, 3.166; 95% CI, 1.603–6.252]. (F) Survival according to the types of TP53 mutations, also significantly associated with survival [TP3 single nucleotide variants or insertion-deletions (SNVs)/indels ± copy number variations (CNVs) compared with TP3 mutations (−); HR, 2.745, 95% CI, 1.329–5.668. TP3 CNVs compared with TP3 mutations (−); HR, 5.679; 95% CI, 2.007–16.070. TP3 CNVs compared with TP3 SNVs/indels ± CNVs; HR, 2.947; 95% CI, 0.996–8.720). (G) Survival according to CCR4 mutations showing a trend towards an association with survival [CCR4 mutations (+) compared with (−); HR, 0.458, 95% CI, 0.200–1.051]. (H) Survival according to CD28 mutations, significantly associated with survival [CD28 mutations (+) compared with (−); HR, 2.243; 95% CI, 1.162–4.328].
TP53 mutations in adult T-cell leukemia/lymphoma

(A) median survival = 1.6 years

(B) siL2R (U/mL)

(C) PS

(D) Clinical subtype

(E) TP53 mutations

(F) TP53 mutations

(G) CCR4 mutations

(H) CD28 mutations

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**Figure 1.**

(A) Median survival = 1.6 years

(B) TP53 mutations (-) vs (+), *P < 0.001*

(C) TP53 mutations (-) vs (+) with TP53 SNVs/indels and CNVs

(D) CCR4 mutations (+) vs (-), *P = 0.033*

(E) CD28 mutations (-) vs (+), *P = 0.052*

(F) CCR4 (+) vs (-) & TP53 (-) vs (+)

(G) CCR4 mutations (+) & TP53 mutations (-), *P = 0.008*
Fig 7. Survival of ATL patients receiving mogamulizumab stratified by TP53 mutations, after censoring transplanted patients on the day of allogeneic HSCT. (A) Survival of ATL patients from the first dose of mogamulizumab (n = 77). The median survival was 1-6 years [95% confidence interval (CI), 1-2-20 years]. (B) Survival according to TP53 mutations showing significant associations with survival [TP53 mutations (+) compared with (−); hazard ratio (HR), 3-176; 95% CI, 1-605–6-282]. (C) Survival according to the types of TP53 mutations, significantly associated with OS [TP53 single nucleotide variants or insertion-deletions (SNVs)/indels ± copy number variations (CNVs) compared with TP53 mutations (−); HR, 2-830, 95% CI, 1-370–5-846. TP53 CNVs compared with TP53 mutations (−); HR, 4-651; 95% CI, 1-625–13-311. TP53 CNVs compared with TP53 SNVs/indels ± CNVs; HR, 2-404; 95% CI, 0-806–7-172]. (D) Survival according to CCR4 mutations significantly associated with OS [CCR4 mutations (+) compared with (−); HR, 0-416, 95% CI, 0-182–0-953]. (E) Survival according to CD28 mutations [CD28 mutations (+) compared with (−); HR, 1-903; 95% CI, 0-984–3-683, n.s.]. (F) Survival according to CCR4 and TP53 mutations, significantly associated with survival [CCR4 mutations (+) and TP53 mutations (−) compared with CCR4 mutations (+) and TP53 mutations (+); HR, 0-326; 95% CI, 0-072–1-466, compared with CCR4 mutations (−) and TP53 mutations (+); HR, 0-134; 95% CI, 0-037–0-482, and compared with CCR4 mutations (−) and TP53 mutations (−); HR, 0-346; 95% CI, 0-099–1-216]. (G) Survival from the first dose of mogamulizumab in the patients with CCR4 mutations (+) and TP53 mutations (−; n = 15) compared to survival of all other patients (n = 62; HR, 0-227; 95% CI, 0-069–0-748).

Table II. Multivariate analysis for survival from the day of allogeneic HSCT.

| Variables | Number | Hazard ratio | 95% CI | P value |
|-----------|--------|--------------|--------|---------|
| CCR4 mutations | | | | |
| Absent | 27 | 1-000 | | Reference |
| Present | 18 | 1-354 | (0-583–3-145) | 0-480 |
| CD28 mutations | | | | |
| Absent | 25 | 1-000 | | Reference |
| Present | 20 | 0-821 | (0-362–1-859) | 0-636 |
| TP53 mutations | | | | |
| Absent | 29 | 1-000 | | Reference |
| Present | 16 | 3-987 | (1-693–9-392) | 0-002 |

CI, confidence interval; HSCT, haematopoietic stem cell transplantation.

Table III. Multivariate analysis for survival from the first dose of mogamulizumab.

| Variables | Number | Hazard ratio | 95% CI | P value |
|-----------|--------|--------------|--------|---------|
| CCR4 mutations | | | | |
| Absent | 51 | 1-000 | | Reference |
| Present | 18 | 0-490 | (0-213–1-126) | 0-093 |
| CD28 mutations | | | | |
| Absent | 42 | 1-000 | | Reference |
| Present | 27 | 1-563 | (0-784–3-119) | 0-205 |
| TP53 mutations | | | | |
| Absent | 42 | 1-000 | | Reference |
| Present | 27 | 2-661 | (1-303–5-434) | 0-007 |

CI, confidence interval.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. The treatment approach administered to the patients right after their tumour sampling.

Data S1. Supplementary Methods.

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