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

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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.

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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 prednisone)-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 C329*, 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

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

Characteristics	*TP53* mutations		*P* value
	Absent	Present	
Number (%)	110 (62)	67 (38)	
Sex			0·167
Female	52 (47)	39 (58)	
Male	58 (53)	28 (42)	
Clinical subtype			0·108
Chronic, smouldering	18 (16)	5 (7)	
Acute, lymphoma	92 (84)	62 (93)	
ECOG PS*			0·001
0, 1	91 (83)	40 (60)	
2, 3, 4	18 (17)	27 (40)	
Serum sIL-2R (U/ml)**			0·005
≤20 000	73 (70)	30 (47)	
>20 000	32 (30)	34 (53)	
Serum Ca (mg/dl)***[$]			0·029
≤11·0	99 (93)	51 (81)	
>11·0	8 (7)	12 (19)	
Serum albumin (g/dl)****			0·062
≥3·5	79 (74)	38 (66)	
<3·5	28 (26)	26 (33)	
Age (years)			0·050
Mean	65	68	
Median	64	67	
Range	41–90	41–85	
WBC (/μl)*****			0·680
Mean	15 907	13 098	
Median	8 120	8 560	
Range	2 900–232 100	2 500–51 100	
Hb (g/l)*****			0·075
Mean	130	124	
Median	134	125	
Range	60–171	61–163	
Plt ($x10^3$/μl)*****			0·754
Mean	224	228	
Median	217	204	
Range	4–622	56–602	
CCR4 gene mutation			0·251
Absent	71 (65)	49 (73)	
Present	39 (35)	18 (27)	
CD28 gene mutation			0·057
Absent	75 (68)	36 (54)	
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; Plt, 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.

types of *CD28*-related fusions, two had a *CD28*-related fusion and CNVs, and one had a *CD28*-related fusion and SNVs (data not shown).

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

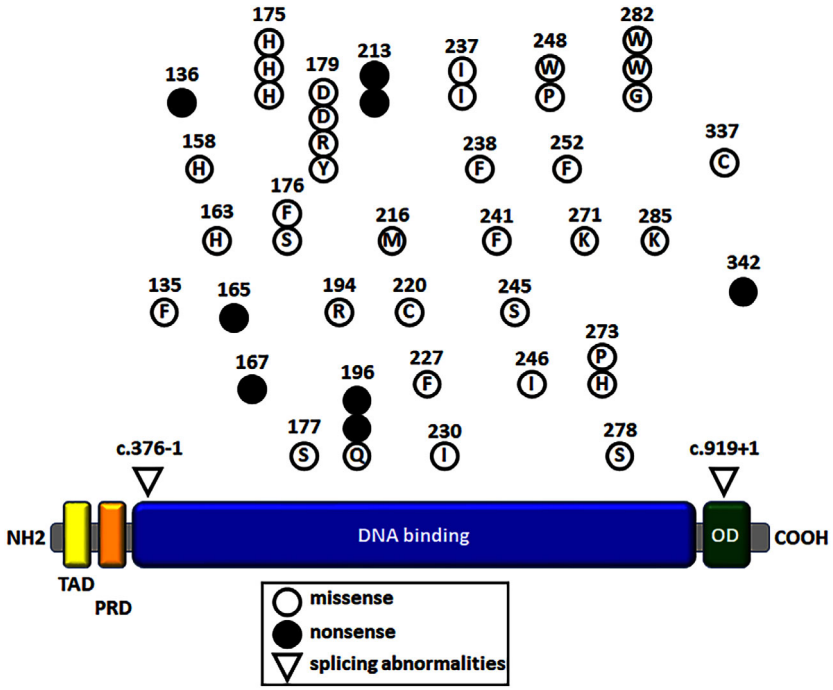


Fig 1. Schematic representation of *TP53* and the mutations detected. Each circle represents one patient with open symbols representing missense and closed symbols representing nonsense mutations. The number beside each circle indicates amino acid position, and the mutated amino acid is indicated in 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]

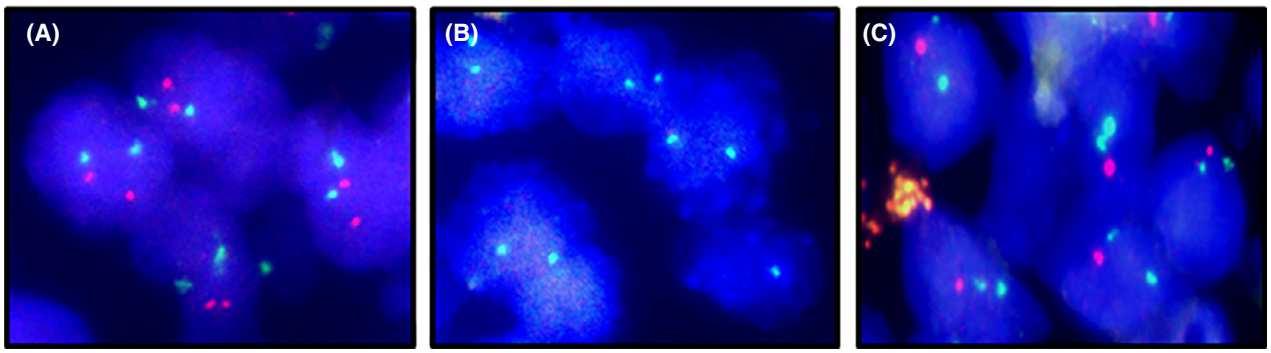
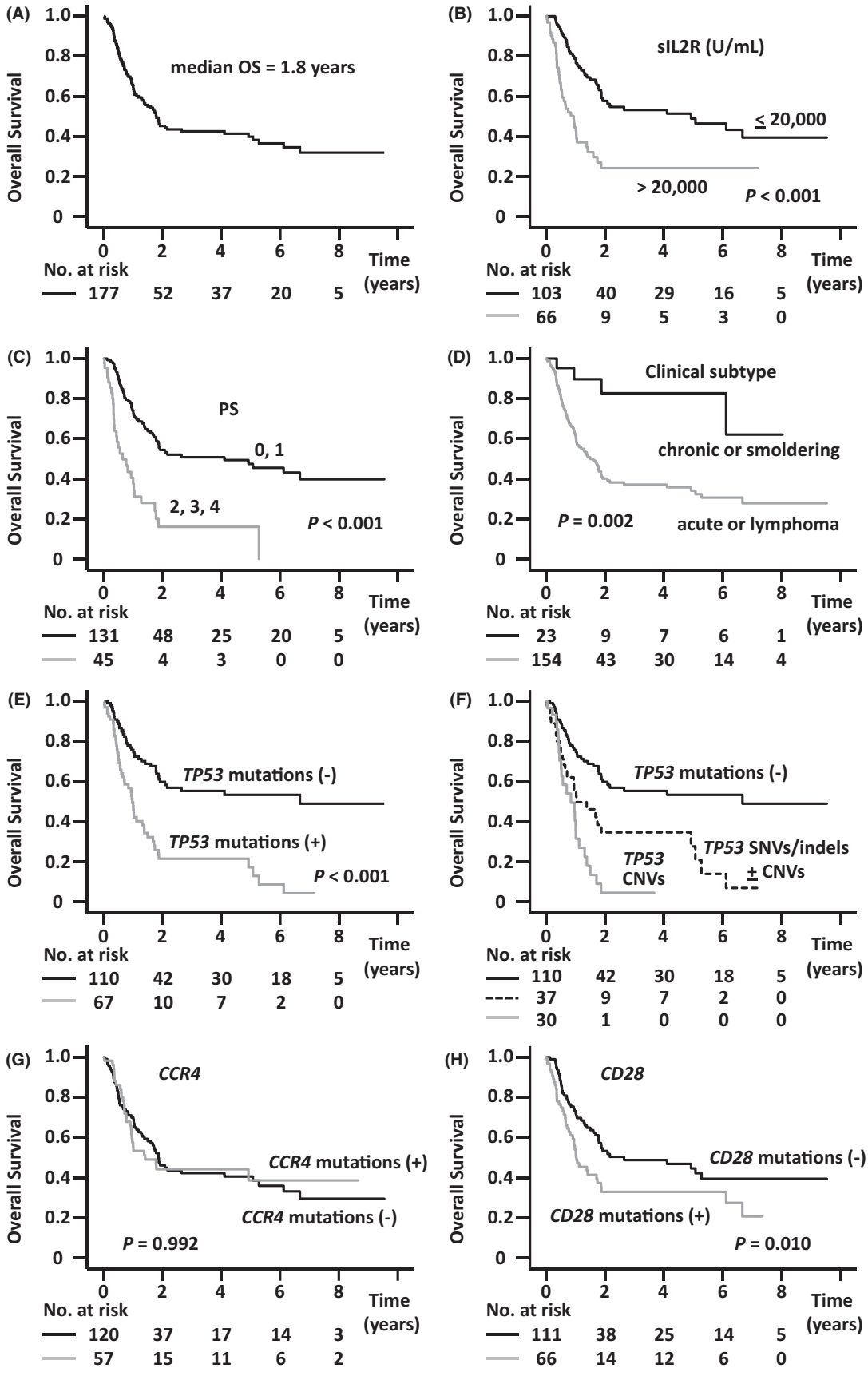


Fig 2. *TP53* copy number variations (CNVs) in adult T-cell leukaemia/lymphoma (ATL) by fluorescence *in-situ* hybridization (FISH). FISH analyses on FFPE sections from three individual ATL patients. *TP53* signals on chromosome 17p13 are red, and centromeric signals of chromosome 17 are green. *TP53* 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].



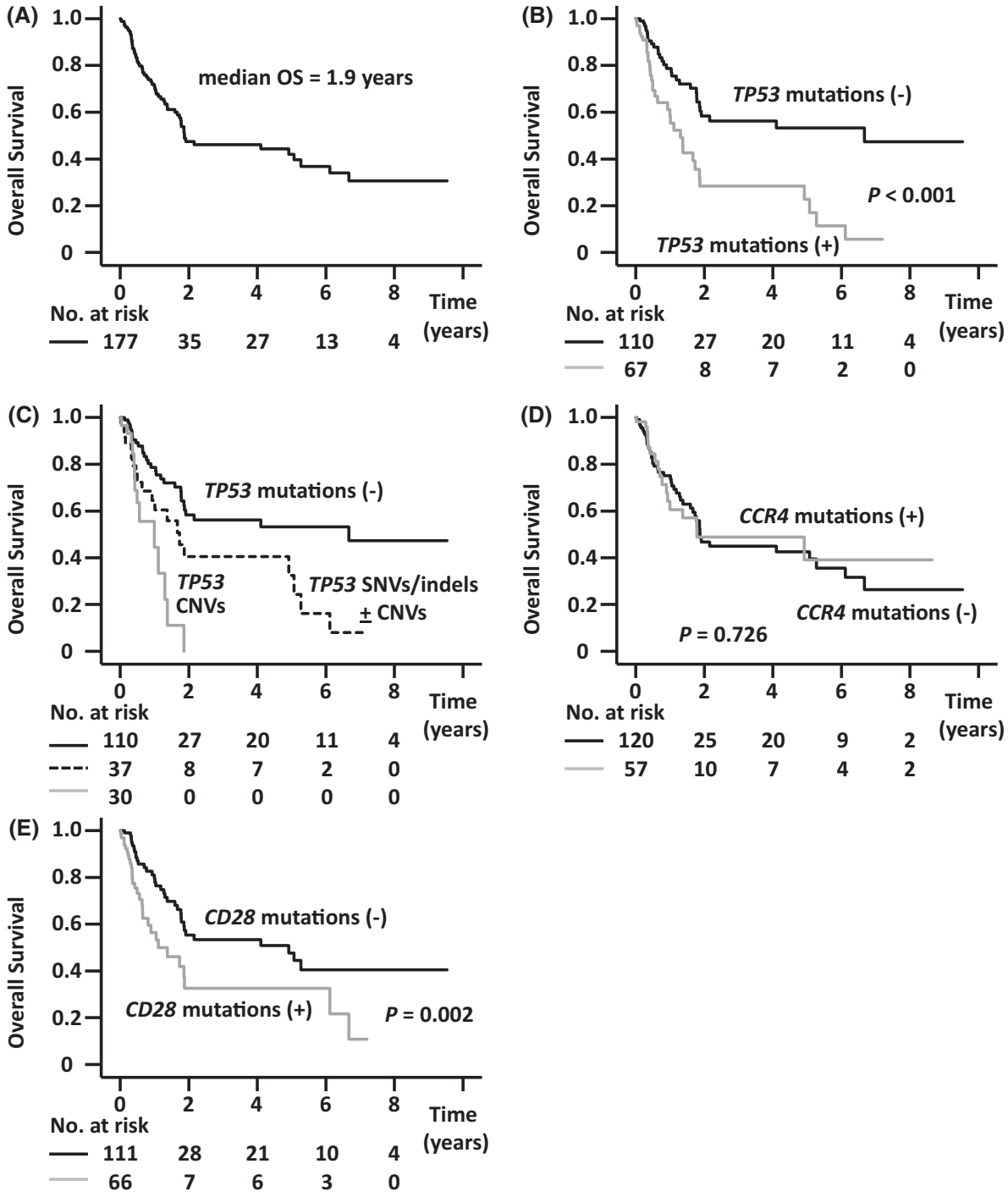


Fig 4. Overall survival (OS) of adult T-cell leukaemia/lymphoma (ATL) patients stratified by *TP53* mutations after censoring transplanted patients on the day of allogeneic haematopoietic stem cell transplantation (HSCT). (A) HSCT-censored OS of all ATL patients enrolled in the study (n = 177) showing 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; $P < 0{\cdot}001$) 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 tended to be better than patients with *TP53* CNVs (1·0 year; $P = 0{\cdot}057$; 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; $P = 0{\cdot}726$; 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 ($P = 0{\cdot}002$; 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 ($P = 0{\cdot}001$; 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; $P = 0{\cdot}790$; 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; $P = 0{\cdot}938$; Fig 5G), or those with or without *CD28* mutations (median survival 0·6 vs. 1·4 years respectively; $P = 0{\cdot}968$; 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 ($P < 0{\cdot}001$; 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; $P = 0{\cdot}041$; 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; $P = 0{\cdot}059$; 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; $P = 0{\cdot}013$; 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; $P = 0{\cdot}014$; 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 ($P < 0{\cdot}001$; 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; $P = 0{\cdot}106$; 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 ($P = 0{\cdot}033$; 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; $P = 0{\cdot}052$; 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 ($P < 0{\cdot}001$), and tended to be better than in patients without either *TP53* or *CCR4* mutations ($P = 0{\cdot}084$; 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; $P = 0{\cdot}008$; 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

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

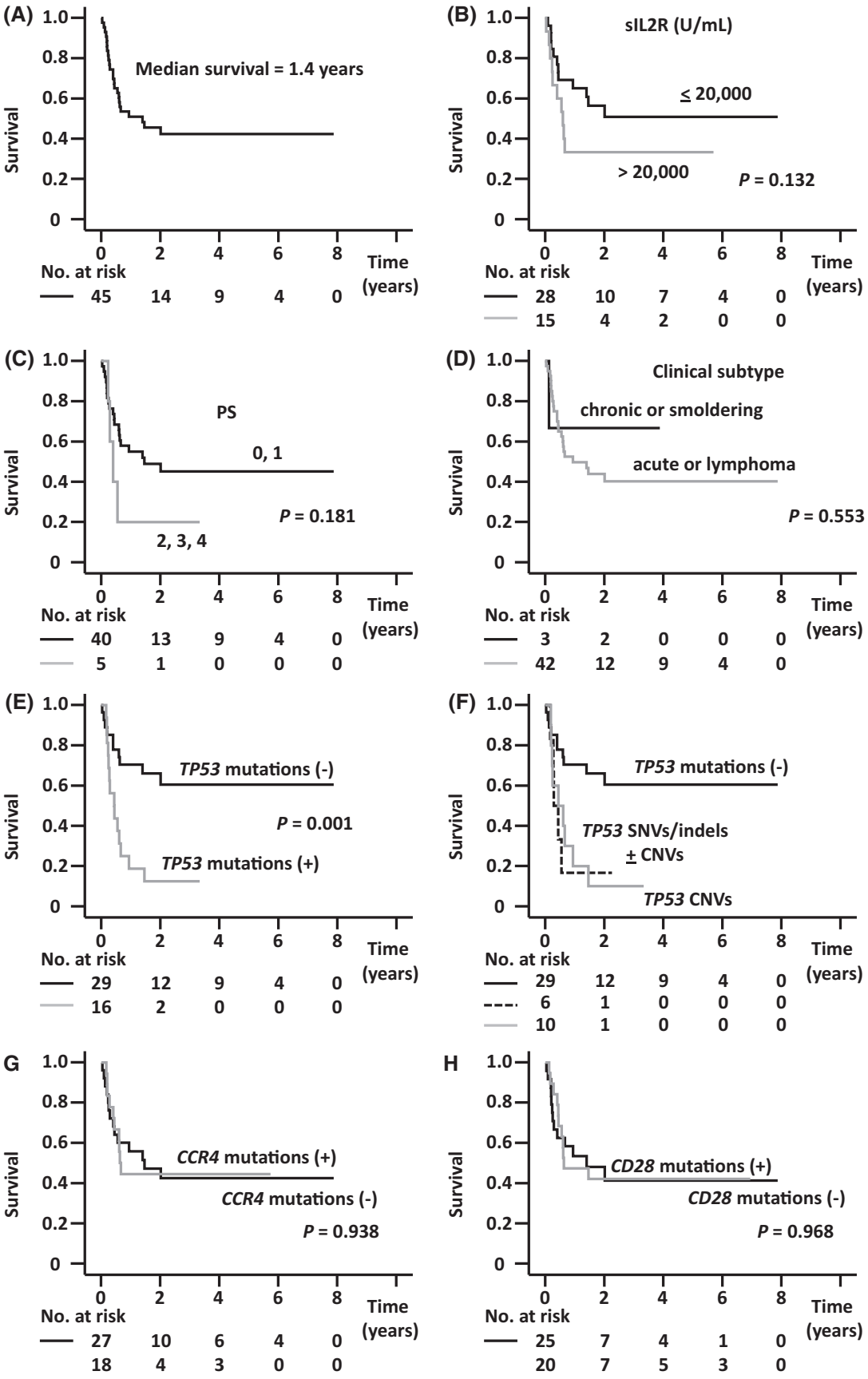
The present study documents that ATL patients with *TP53* mutations such as SNVs/indels or CNVs have a significant worse prognosis than patients without such mutations. To the best of our knowledge, this is the first report demonstrating the clinical significance of *TP53* mutations in a large cohort 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-

Fig 5. Survival of adult T-cell leukaemia/lymphoma (ATL) patients receiving allogeneic haematopoietic stem cell transplantation (HSCT), stratified by *TP53* mutations. (A) Survival of ATL patients from the day of allogeneic HSCT (n = 45). The median survival was 1·4 years [95% confidence interval (CI), 0·0–3·0 years]. (B) survival according to serum-soluble sIL-2R level, showing lack of significant association with survival] >20 000 U/ml compared with ≤20 000; hazard ratio (HR), 1·891; 95% CI, 0·813–4·403, n.s.]. (C) Survival according to Eastern Cooperative Oncology Group (ECOG) PS (2–4 compared with 0 or 1; HR, 2·068; 95% CI, 0·697–6·141, n.s.). (D) Survival according to ATL clinical subtype (acute or lymphoma subtypes compared with chronic or smouldering subtypes; HR, 1·819; 95% CI, 0·245–13·525, n.s.). (E) Survival according to *TP53* mutations [*TP53* mutations (+) compared with (−); HR, 3·570; 95% CI, 1·182–10·784]. (F) Overall survival (OS) according to the types of *TP53* mutations [*TP53* single nucleotide variants or insertion-deletions (SNVs)/indels ± copy number variations (CNVs) compared with *TP53* mutations (−); HR, 3·570, 95% CI, 1·182–10·784. *TP53* CNVs compared with *TP53* mutations (−); HR, 3·563; 95% CI, 1·421–8·938. *TP53* CNVs compared with *TP53* SNVs/indels ± CNVs; HR, 0·860; 95% CI, 0·283–2·612]. (G) OS according to *CCR4* mutations [CCR4 mutations (+) compared with (−); HR, 1·033, 95% CI, 0·457–2·333, n.s.]. (H) OS according to *CD28* mutations [CD28 mutations (+) compared with (−); HR, 0·984; 95% CI, 0·440–2·200, n.s.].



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.

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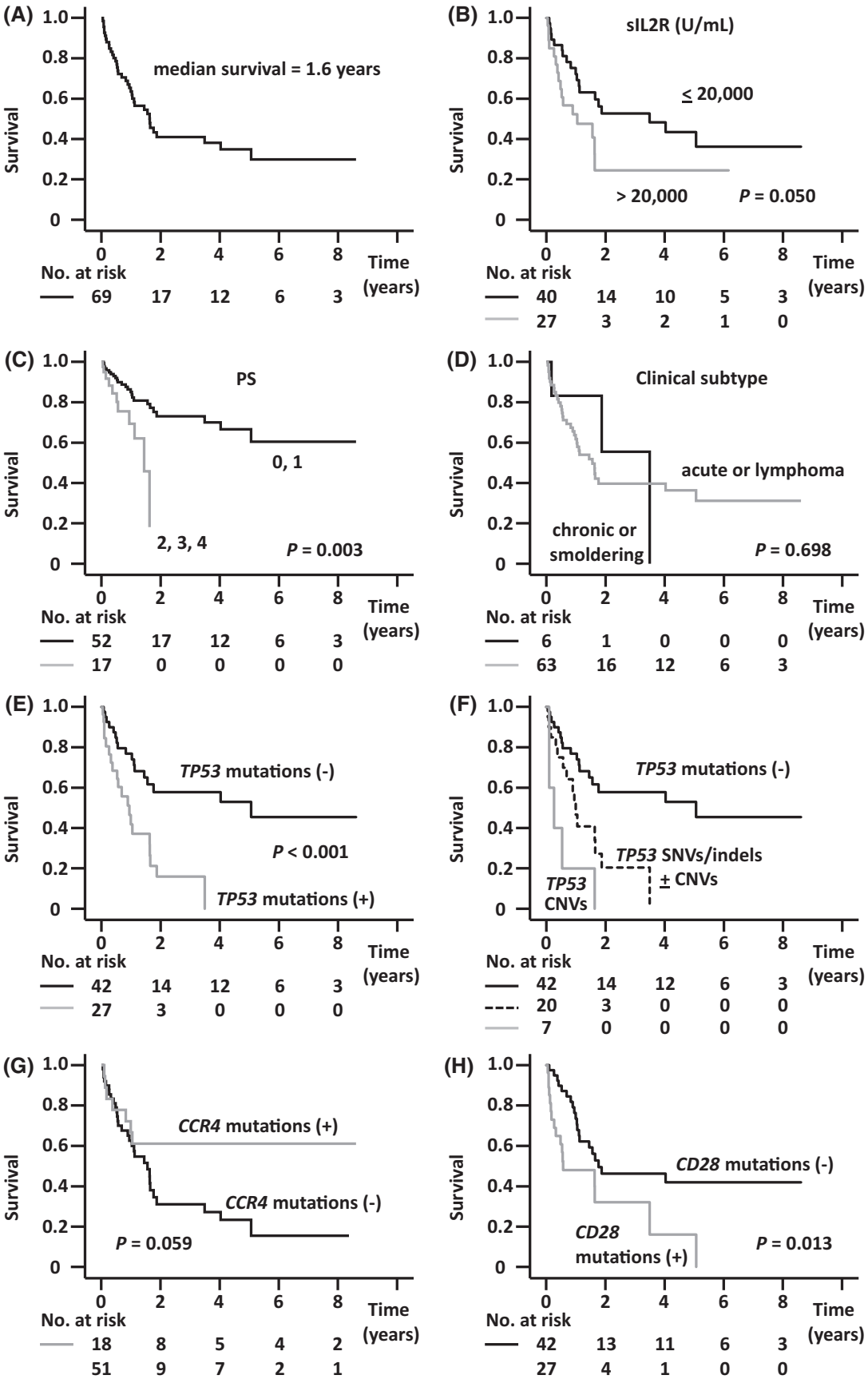
Author contributions

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 Pharmatceutical 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 Pharmaceuticals, Kaken Pharmaceutical, Kyowa Kirin Co., Ltd., Maruho, 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, Abbie, and Glaxo-Smith-Klein. 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), 1·950; 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 [*TP53* mutations (+) compared with (−); HR, 3166; 95% CI, 1·603–6·252]. (F) Survival according to the types of *TP53* mutations, also significantly associated with survival [*TP53* single nucleotide variants or insertion-deletions (SNVs)/indels ± copy number variations (CNVs) compared with *TP53* mutations (−); HR, 2·745, 95% CI, 1·329–5·668. *TP53* CNVs compared with *TP53* mutations (−); HR, 5·679; 95% CI, 2·007–16·070. *TP53* CNVs compared with *TP53* 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].



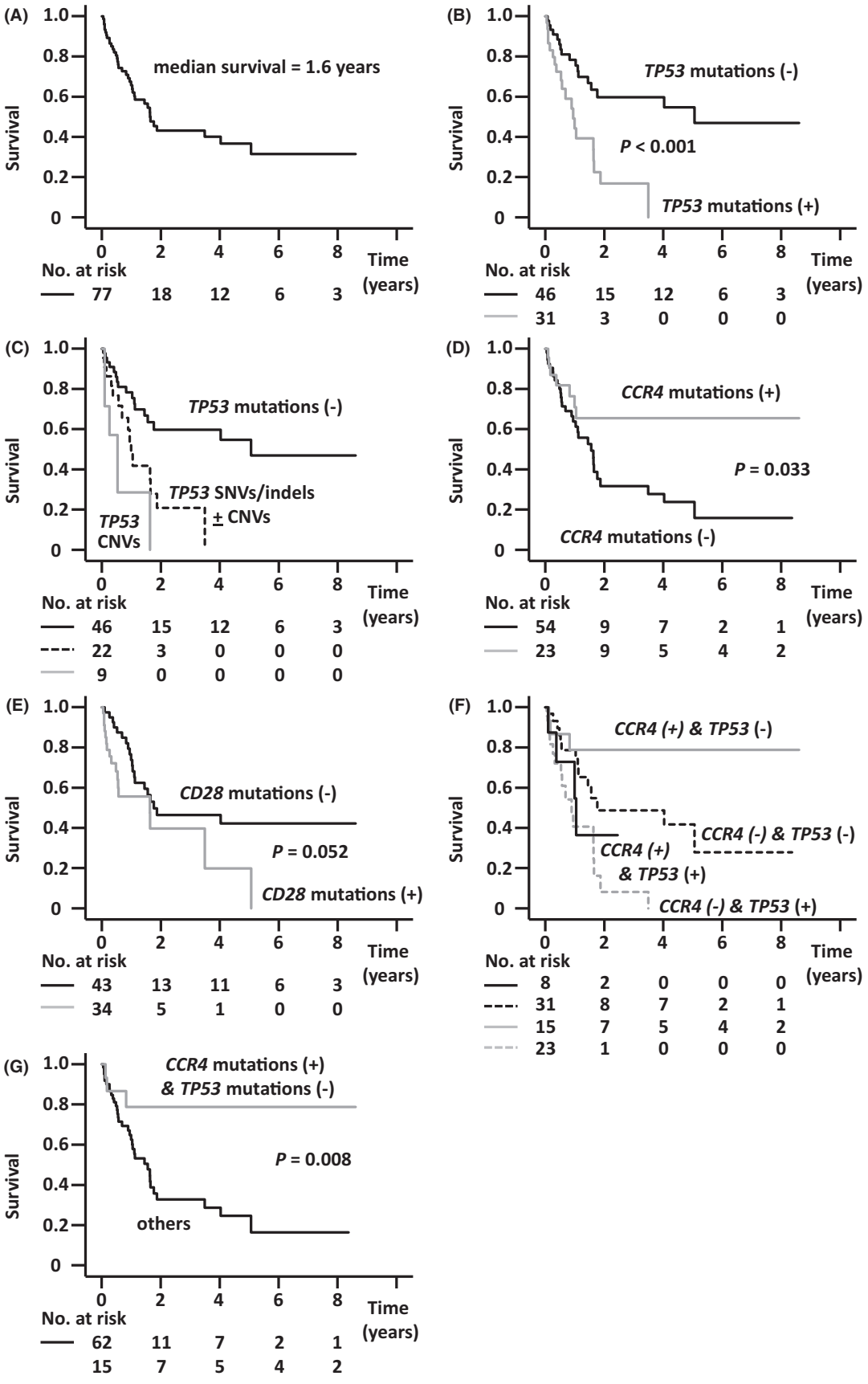


Fig 7. Survival of ATL patients receiving mogamulizumab stratified by *TP53* mutations, after censoring transplanted patients on the day of allogeneic HSCT. (A) Survival of ATL patients from the first dose of mogamulizumab (n = 77). The median survival was 1·6 years [95% confidence interval (CI), 1·2–2·0 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 SI. The treatment approach administered to the patients right after their tumour sampling.

Data S1. Supplementary Methods.

Table IV. Multivariate analysis for HSCT-censored survival from the first dose of mogamulizumab.

Variables	Number	Hazard ratio	95% CI	*P* value
CCR4 mutations				
Absent	54	1·000	–	Reference
Present	23	0·439	(0·191–1·008)	0·052
CD28 mutations				
Absent	43	1·000	–	Reference
Present	34	1·442	(0·728–2·855)	0·294
TP53 mutations				
Absent	46	1·000	–	Reference
Present	31	2·733	(1·347–5·544)	0·005

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

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