Genomic determinants impacting the clinical outcome of mogamulizumab treatment for adult T-cell leukemia/lymphoma

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Abstract

In order to identify genomic biomarkers for the outcome of mogamulizumab-containing treatment, an integrated molecular analysis of adult T-cell leukemia/lymphoma (ATL) was conducted on 64 mogamulizumab-naïve patients. Among driver genes, *CCR4* and *CCR7* alterations were observed in 22% and 11% of the patients, respectively, both consisting of single nucleotide variants (SNV)/insertion-deletions (indels) in the C-terminus. Patients with *CCR4* alterations or without *CCR7* alterations exhibited a more favorable clinical response (complete response [CR] rate 93%, 13/14; *P*=0.024, and CR rate 71%, 40/56; *P*=0.036, respectively). Additionally, *TP53*, *CD28*, and *CD274* alterations were identified in 35%, 16%, and 10% of the patients, respectively. *TP53* alterations included SNV/indels or copy number variations (CNV) such as homozygous deletion; *CD28* alterations included SNV, CNV such as amplification, or fusion; *CD274* alterations included CNV such as amplification, or structural variants. Univariate analysis revealed that *TP53*, *CD28* or *CD274* alterations were associated with worse overall survival (OS) (hazard ratio [HR]: 2.330, 95% confidence interval [CI]: 1.183-4.589; HR: 3.191, 95% CI: 1.287-7.911; HR: 3.301, 95% CI: 1.130-9.641, respectively) but that *CCR4* alterations were associated with better OS (HR: 0.286, 95% CI: 0.087-0.933). Multivariate analysis indicated that in addition to performance status, *TP53*, *CCR4* or *CD274* alterations (HR: 2.467, 95% CI: 1.197-5.085; HR: 0.155, 95% CI: 0.031-0.778; HR: 14.393, 95% CI: 2.437-85.005, respectively) were independently and significantly associated with OS. The present study contributes to the establishment of precision medicine using mogamulizumab in ATL patients.

Introduction

Adult T-cell leukemia/lymphoma (ATL) is a peripheral T-cell neoplasm caused by human T-cell lymphotropic virus type-1, and has a very poor prognosis.[1,2] It is generally accepted that allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment for ATL. Hence, it is recommended that younger patients (≤ nearly

70 years of age) and those with relatively well-controlled ATL receive this treatment, aiming for long-term survival.[3,4] However, treatment-related adverse events associated with allogeneic HSCT are generally severe compared to other treatments. In addition, many Japanese ATL patients are indeed older (median age at diagnosis, 68 years).[5] Accordingly, ATL patients who are candidates for allogeneic HSCT by virtue of younger age are decreasing year by year. CC chemokine receptor 4 (CCR4) is expressed on the tumor cells of most patients with ATL, and it was therefore proposed as a molecular target for immunotherapy. The humanized anti-CCR4 monoclonal antibody mogamulizumab was developed for this purpose.[6] This antibody has a defucosylated Fc region, which enhances antibody-dependent cellular cytotoxicity (ADCC).[7,8] It is approved in Japan both for patients with newly diagnosed or relapsed/refractory ATL.[9,10] Currently, most patients deemed unsuitable for allogeneic HSCT receive mogamulizumab-containing treatment as first-line therapy.[10,11] Moreover, most relapsed or refractory ATL patients receive mogamulizumab.[9,12] However, some patients are initially refractory to mogamulizumab, or acquire resistance after treatment. The mechanisms responsible for this have not yet been determined.

An entire landscape of genetic aberrations in ATL has been identified, and it is clear that diverse multistep oncogenic events, from infant to elderly adult, are involved in the development of this disease.[13] The next step would be individualized treatments for ATL based on each patient's genomic biomarkers, to tailor therapy to the specific disease entity. To this end, we performed an integrated molecular analysis for mogamulizumab-naïve ATL patients, in order to identify genomic biomarkers influencing the clinical outcome of mogamulizumab-containing treatment. Our ultimate goal is to establish precision medicine for patients with ATL based on their genomic profiles.

Methods

Adult T-cell leukemia/lymphoma patients and samples

Study subjects were mogamulizumab-naïve ATL patients without prior allogeneic HSCT, who received mogamulizumab-containing treatment. ATL diagnosis and clinical subtype assignment were according to the Japan Lymphoma Study Group recommendations.[2] Therapeutic efficacy of mogamulizumab treatment was assessed according to the international consensus response criteria and classified as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD).[14] The current genomic study was approved by the Institutional Review Boards at all participating sites, and all patients provided written informed consent before blood or tissue sampling. Exome sequencing was performed using paired tumor–derived and normal tissue DNA from the same patient, the latter almost always being peripheral blood mononuclear cells (PBMC) from the patient in hematological remission after treatment. Details are available in the *Online Supplementary Appendix*.

DNA/RNA preparation for genomic and epigenetic analysis

Details are available in the *Online Supplementary Appendix*.

Exome library preparation and sequencing

Details are available in the *Online Supplementary Appendix*.

RNA sequencing

Details are available in the *Online Supplementary Appendix*.[15]

Somatic variant call

Details are available in the *Online Supplementary Appendix*.[16-21]

Fusion gene detection with RNA sequencing

Details are available in the *Online Supplementary Appendix*.[22,23]

Detection of structural variants in the 3' UTR of the *CD274* gene

Details are available in the *Online Supplementary Appendix*.[24]

Adult T-cell leukemia/lymphoma driver genes

A total of 81 genes was defined as ATL driver genes (*Online Supplementary Table S1*).[13] Details are available in the *Online Supplementary Appendix*.

Human leukocyte antigen genotyping and alteration call

Details are available in *Online Supplementary Appendix*.[25-28]

Statistical analyses

Progression-free survival (PFS) was defined as the time from the first dose of mogamulizumab to progression, relapse, or death resulting from any cause, whichever occurred first. Overall survival (OS) was measured from the day of the first dose to death resulting from any cause. The survival estimate was calculated with all transplanted patients (n=9) censoring at the day of allogeneic HSCT. Details are available in *Online Supplementary Appendix*.[29,30]

Results

Patients' characteristics

Sixty-four mogamulizumab-naïve ATL patients comprising 33 men and 31 women were included. Their median age was 68 years (mean, 66; range, 36-86), and disease clinical subtypes included 46 acute, seven lymphoma, and 11 chronic ATL. The 11 chronic patients all had an unfavorable subtype.[3,14] Twenty-six patients had not been previously treated, but the remaining 38 had previously received systemic chemotherapy (*Online Supplementary Table S2*). Forty-five patients received mogamulizumab-containing combination therapies, such as a mogamulizumab plus VCAP-AMP-VECP (vincristine, cyclophosphamide, doxorubicin, prednisone; doxorubicin, ranimustine, prednisone; vindesine, etoposide, carboplatin, prednisone)-like regimen, or CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone)-like regimens, whereas 19 received mogamulizumab monotherapy.[31] Patients received a median of seven mogamulizumab infusions at 1 mg/kg, with a mean of eight (range, 1-42). Nine patients received allogeneic HSCT after mogamulizumab-containing treatment.

Clinical responses to mogamulizumab, and progression-free survival and overall survival according to clinical parameters

Objective responses to mogamulizumab-containing treatment were noted in 54 of the 64 patients, including 43 CR. Three patients had SD and seven PD. Median PFS and OS were 1.0 year (95% CI: 0.5-1.5) (*Online Supplementary Figure S1A*) and 1.6 years (95% CI: 1.1-2.2) (*Online Supplementary Figure S2A*), respectively. There were no significant differences in PFS and OS according to age (> or <70 years) (*Online Supplementary Figures S1B* and *S2B*, respectively). PFS and OS of those patients with a performance status (PS) of 2-4 was significantly worse than of those with a PS of 0 or 1 (median PFS, 0.7 *vs*. 1.3 years, P=0.015, *Online Supplementary Figure S1C*, and median OS, 1.1 *vs*. 2.7 years, *P*=0.008, *Online Supplementary Figure S2C*, respectively). PFS and OS of patients with a higher serum LDH level (> upper limit of normal [ULN]) was significantly worse than in those with a lower level (< ULN) (median PFS, 0.6 *vs*. 1.4 years, *P*=0.009, *Online Supplementary Figure S1D*, and median OS, 1.3 *vs*. 5.1 years, *P*=0.020, *Online Supplementary Figure S2D*, respectively). There were no significant differences in PFS or OS according to sex (data not shown), between previously untreated or treated patients (*Online Supplementary Figures S1E* and *S2E*, respectively), or between patients treated with mogamulizumab monotherapy or combination therapy (*Online Supplementary Figures S1F* and *S2F*, respectively). There were also no significant differences in PFS or OS among the patients with different clinical subtypes (acute, lymphoma, or chronic) (*Online Supplementary Figures S1G* or *S2G*, respectively).

PFS of patients with CR (median, 1.4 years) was significantly better than of those with PR (median, 0.7 years, *P*=0.039), SD (median, 0.2 years, *P*<0.001), or PD (median, 0.1 years, *P*<0.001). Also, PFS of patients with PR was significantly better than of those with SD (*P*=0.008) or PD (*P*<0.001). There were no significant differences of PFS between patients with SD and PD (*Online Supplementary Figure S1H*). OS of patients with CR (median, 4.6 years) was significantly better than of those with PR (median, 1.5 years, *P*=0.016), SD (median, 0.5 years, *P*<0.001), or PD (median, 0.5 years, P<0.001). Finally, the OS of patients with PR was significantly better than of those with SD (*P*=0.016), or PD (*P*=0.001). There were no significant differences of OS between patients with SD and PD (*Online Supplementary Figure S2H*).

Driver gene alterations associated with clinical response to mogamulizumab

Sixty-three patients whose samples passed the quality assessments during exome analyses (n=64) and RNA sequencing (n=63) were evaluable for driver gene alterations. Frequencies, distributions and types of alterations in driver genes are shown in Figure 1A and B. The former included hot spot truncations in the C-terminal position of *CCR4*, *CCR7* and *NOTCH1* genes, and hot spot missense SNV in *PLCG1*, *PRKCB*, *VAV1*, *STAT3* and *CARD11*. The latter included homozygous deletion of 9p21.3 (*CDKN2A*), amplification of 2q33.2 (*CD28*) and 9p24.1 (*CD274* [*PD-L1*]), 18q21.33, 6p25.3 (*IRF4*) and 14q32.2 (*BCL11B*) (the gene symbol in parenthesis indicates the presumed driver gene on the segment).[32] In addition, a total of eight fusion genes was detected in six cases (1 each of *ATXN1-GMPR*, 1 *CBLB-GJC1*, 1 *CD58-SLC16A1*, 3 *ICOS-CD28*, 1 *CTLA4-CD28*, and 1 *SLC38A1-ARID2*). Three structural variants were identified in the *CD274* gene, including two deletions and one translocation of the 3' UTR. Analysis of combinations of driver SNV/indels, CNV, fusion, and structural variants revealed a comprehensive landscape of ATL driver alterations. In the current cohort, of the 81 driver genes (*Online Supplementary Table S1*), alterations were detected in 66, at an average of 5.5 alterations per patient (Figure 2; *Online Supplementary Table S3*).

Next, we analyzed associations between clinical responses to mogamulizumab (CR *vs*. non-CR) and 30 driver gene alterations (presence or absence). The presence of *CCR4* alterations or the absence of *CCR7* alterations was associated with CR (CR 93%, 13/14; *P*=0.024; CR 71%, 41/56; *P*=0.036, respectively) (*Online Supplementary Table S4*). There were no patients with both *CCR4* and *CCR7* alterations (Figure 1A). No other driver gene alterations associated with clinical response to mogamulizumab were identified, according to these criteria (i.e., *P*<0.050,

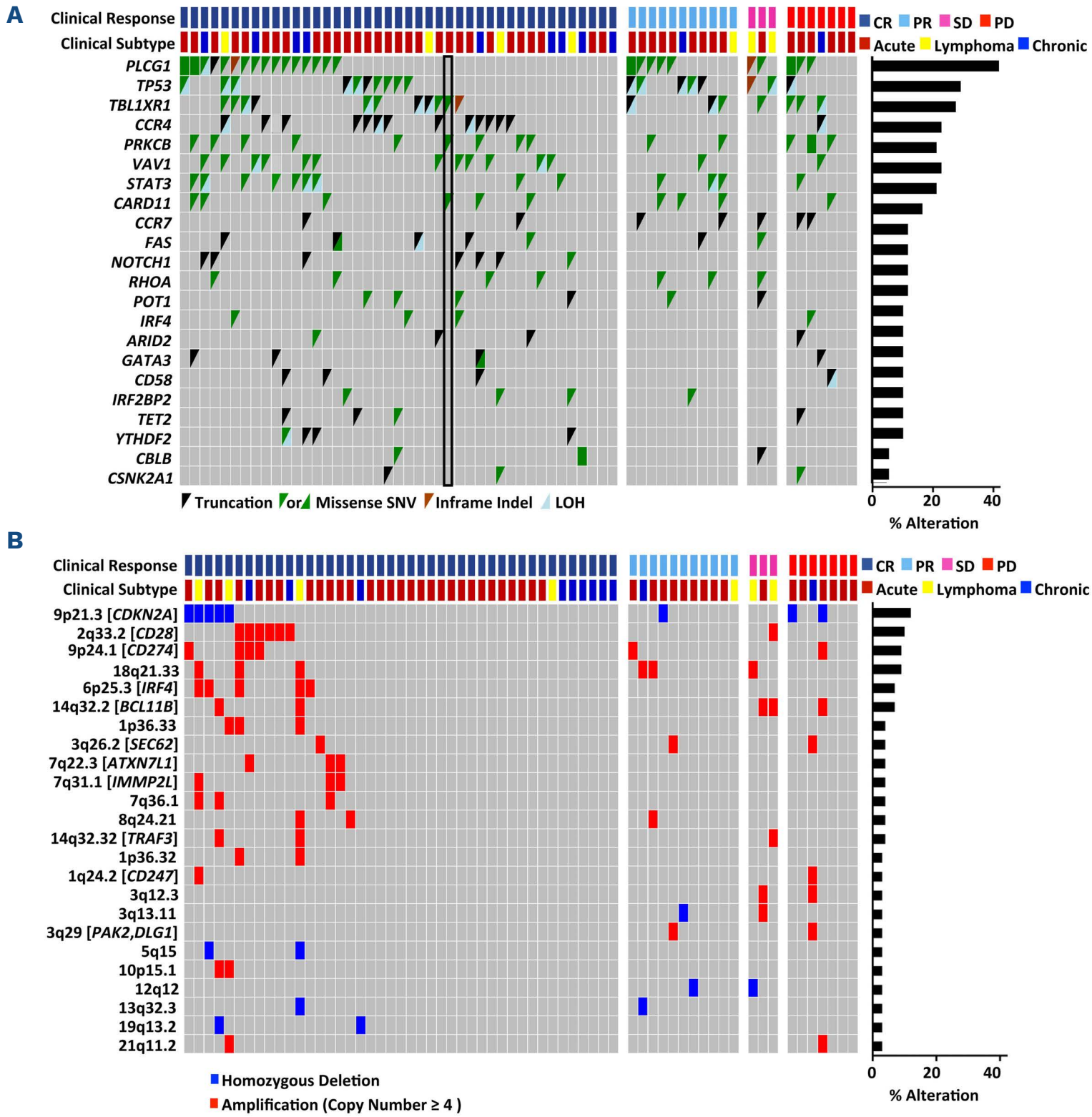

Figure 1. Driver gene alterations in adult T-cell leukemia/lymphoma cells. (A) Truncating mutations, missense single nucleotide variant (SNV), in-frame insertion-deletions (indels), loss of heterozygosity (LOH) in wild-type allele by copy number (CN) loss, in the 64 adult T-cell leukemia/lymphoma (ATL) patients by exome sequencing are presented according to clinical response to mogamulizumab using Oncoprint. Color code is as follows: truncating mutation, black; missense SNV, green; in-frame indels, brown; and LOH, light blue triangles. The altered gene name is indicated on the left, and their frequencies are indicated on the right. The case outlined by the black square lacked RNA-sequencing data, and thus SNV or indels were not evaluable. (B) CN variations such as homozygous deletions and amplifications of the gene segment, in the 64 ATL patients are presented according to clinical response to mogamulizumab using Oncoprint. Color code is as follows: homozygous deletions, blue; and amplifications, red squares. CN gain (CN=3) or loss (CN=1) are not presented. The gene segments are shown in descending order of their altered frequency from top to bottom. The locations of the altered gene segments and the gene name in the segments are indicated on the left, and their frequencies are indicated on the right.

number of cases with altered genes >3 in the cohort, and confirmation of altered allele expression by RNA sequencing).

Univariate analyses of progression-free and overall survival according to driver gene alterations

Univariate analysis of PFS was performed for the 48 driver gene alterations (presence or absence) occurring in more than one patient. Of these *TP53* and *CD274* alterations were associated with worse PFS (HR: 2.001, 95% CI: 1.058-3.784; HR: 2.924, 95% CI: 1.117-7.651, respectively). On the other hand, alterations in *CCR4* were associated with better PFS (HR: 0.363, 95% CI: 0.142-0.928). *DLG1* and *PAK2* alterations were associated with worse PFS (HR: 5.427,

95% CI: 1.250 to 23.566; n=2 for both), but did not meet the present prognostic criteria (number of cases with altered genes >3 in the cohort) (Figure 3).

Univariate analysis of OS was also performed. It was found that patients with *TP53*, *CD28* or *CD274* alterations had poorer OS (HR: 2.330, 95% CI: 1.183-4.589; HR: 3.191, 95% CI: 1.287-7.911; HR: 3.301, 95% CI: 1.130-9.641, respectively). As with PFS, *CCR4* alterations were associated with better OS (HR: 0.286, 95% CI. 0.087-0.933) (Figure 4).

Multivariate analyses of progression-free and overall survival according to driver gene alterations

Multivariate analysis of PFS of the 63 ATL patients receiving mogamulizumab was performed using the following five variables: *TP53*, *CCR4* and *CD274* alterations, Eastern Cooperative Oncology Group (ECOG) PS, and serum lactate dehydrogenase (LDH). Of these, four variables were significantly associated with PFS, namely, a worse PS (HR: 2.092, 95% CI: 1.024-4.277) and the presence of *TP53*, *CCR4* or *CD274* alterations (HR: 2.074, 95% CI: 1.069-4.026; HR: 0.355, 95% CI: 0.127-0.991; HR: 5.846, 95% CI: 1.890-18.081, respectively) (Table 1). The PFS of patients with or without *TP53*, *CCR4* or *CD274* alterations is depicted in Figure 5A to C, respectively.

Multivariate analysis of the OS of these 63 patients included the six variables *TP53*, *CCR4*, *CD28* and *CD274* alterations, ECOG PS, and serum LDH. Of these, four were significantly associated with OS, namely, a worse PS (HR: 2.362, 95% CI: 1.078-5.175) and the presence of *TP53*,

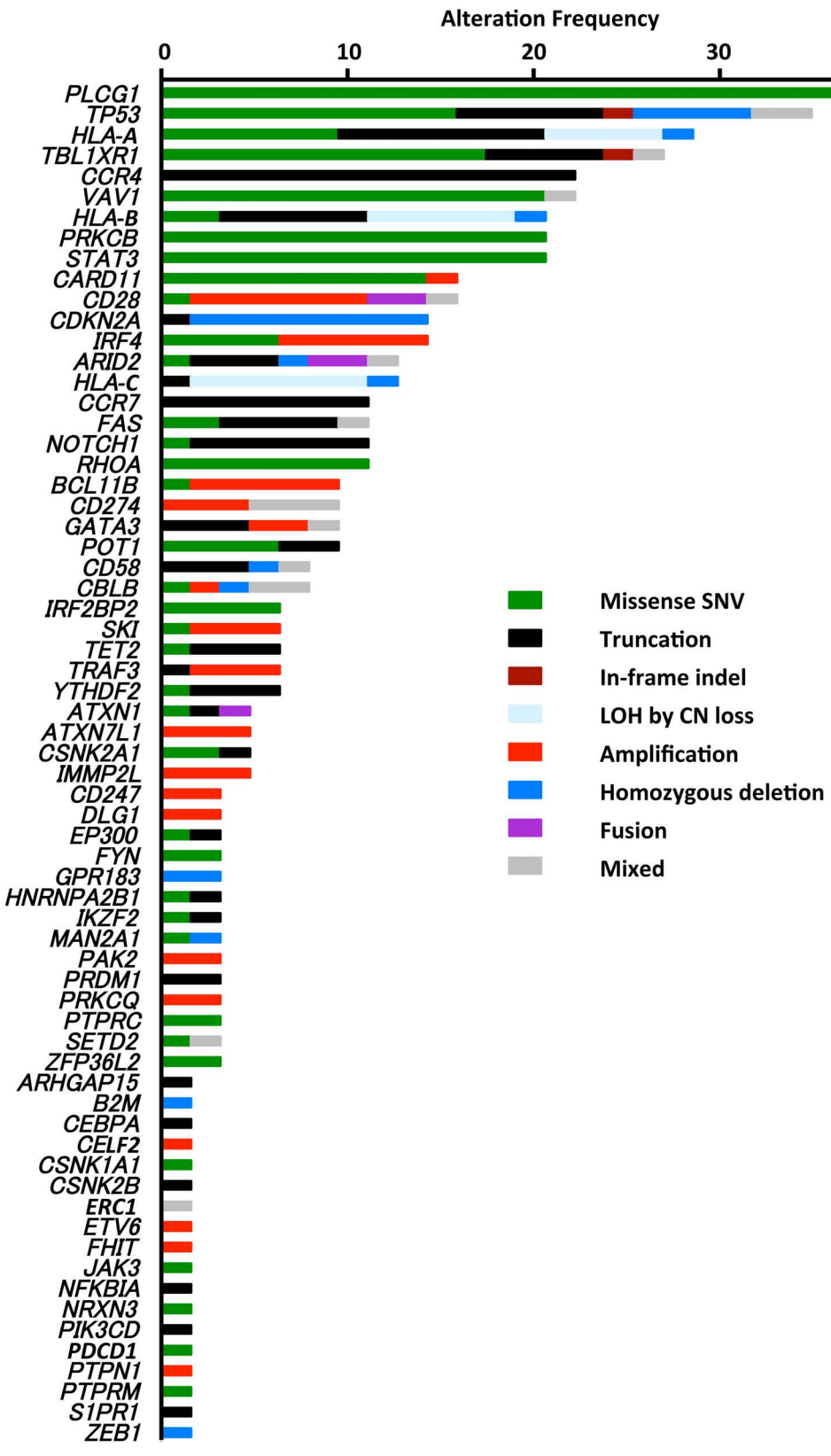

Figure 2. Combinations of driver gene alterations in adult T-cell leukemia/lymphoma cells. Combination of missense single nucleotide variant (SNV), truncation, in-frame insertion-deletions (indels), loss of heterozygosity (LOH) by copy number (CN) loss, amplification, homozygous deletion, gene fusion, and mixed, of the driver genes in the 64 adult T-cell leukemia/lymphoma (ATL) patients. “Mixed” indicates overlapped detection of more than two types of alterations in a gene. Color code is as follows: missense SNV, green; truncation, black; in-frame indels, brown; LOH, light blue; amplification, red; homozygous deletion, blue; fusion, purple; and mixed, gray. The altered gene name is indicated on the left, and they are shown in descending order of their combined altered frequency from top to bottom. X axis indicates the frequency of combined alterations.

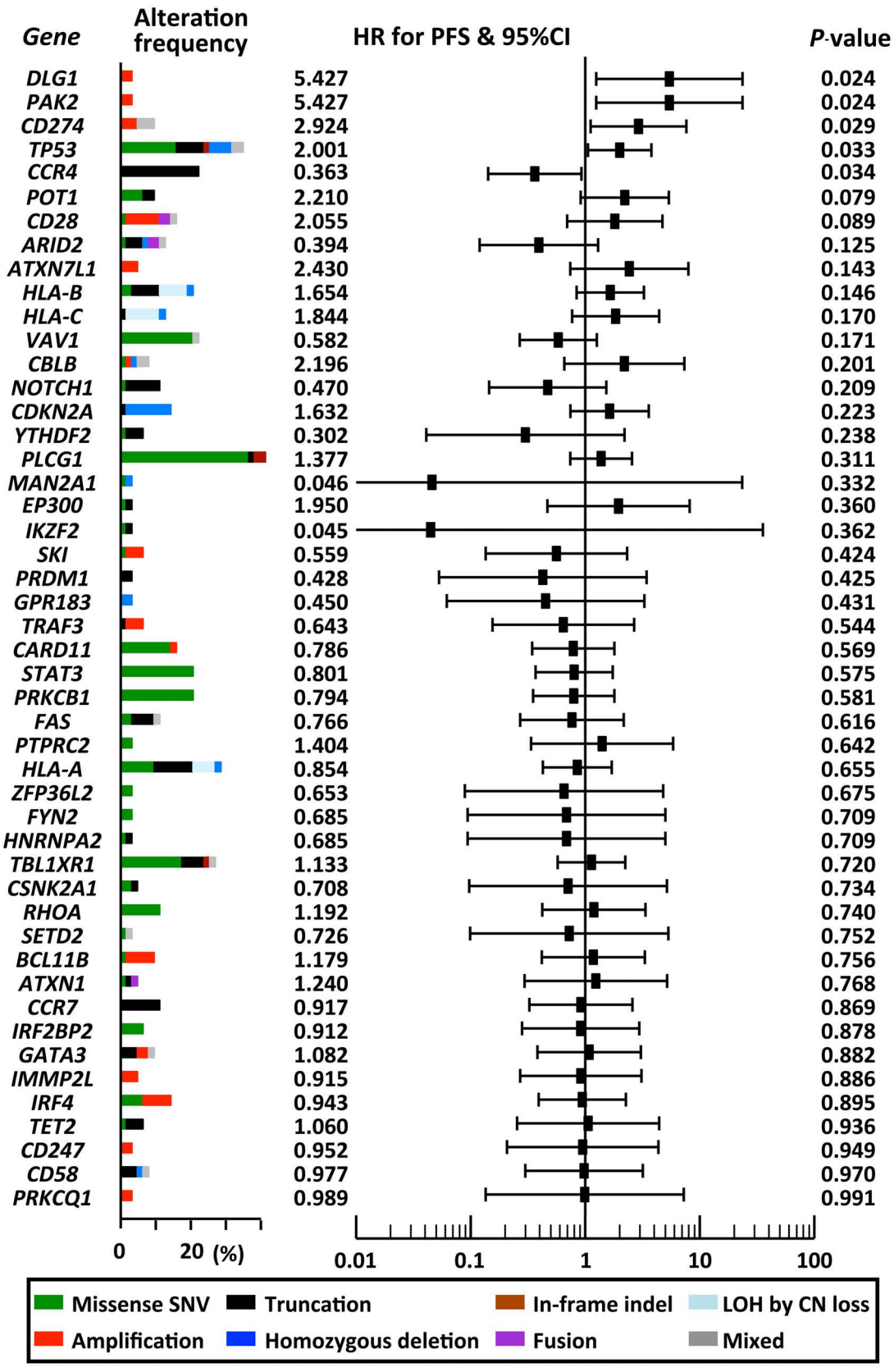

Figure 3. Univariate analyses of progression-free survival according to driver gene alterations. Forest plot of hazard ratio (HR), and 95% confidence interval (CI) for progression-free survival (PFS), and *P*-value for patients with each driver gene alteration, obtained from univariate analysis for PFS by the Cox proportional hazards regression model. The altered gene is indicated on the left, and they are shown in ascending order of the *P*-value from top to bottom.

CCR4 or *CD274* alterations (HR: 2.467, 95% CI: 1.197-5.085; HR: 0.155, 95% CI: 0.031-0.778; HR: 14.393, 95% CI: 2.437-85.005, respectively) (Table 2). The OS of patients with or without *TP53*, *CCR4*, *CD274* or *CD28* alterations is depicted in Figure 5D to G, respectively.

KIR3DL1 allelic polymorphism and *HLA-B* epitopes

We classified patients into three groups, namely strong, weak, or non-interactors based on the extent of natural killer (NK) cell inhibition, according to killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1) and HLA-B subtyping.[33] However, we found no significant differences in PFS or OS of patients in the strong (n=14), weak (n= 12), or non-interactor (n=38) groups (data not shown).

FCGR genotyping

Patients were stratified into three groups, according to *FCGR3A* genotype. However, also for this factor, there were no significant differences in OS between patients carrying *FCGR3A* 158V/V (n=5) *versus* *FCGR3A* 158V/F (n=21) or 158F/F (n=38), although the former (V/V) tended to have better PFS compared to the latter (V/F or F/F) (median PFS, 4.6 *vs*. 0.8 years, *P*=0.090) (data not shown).

Transcriptome subtypes of adult T-cell leukemia/lymphoma and clinical outcomes

Consensus clustering analysis using variably expressed genes across the samples (gene number =1,966 with variance >0.0865) revealed four transcriptome subtypes (TS)

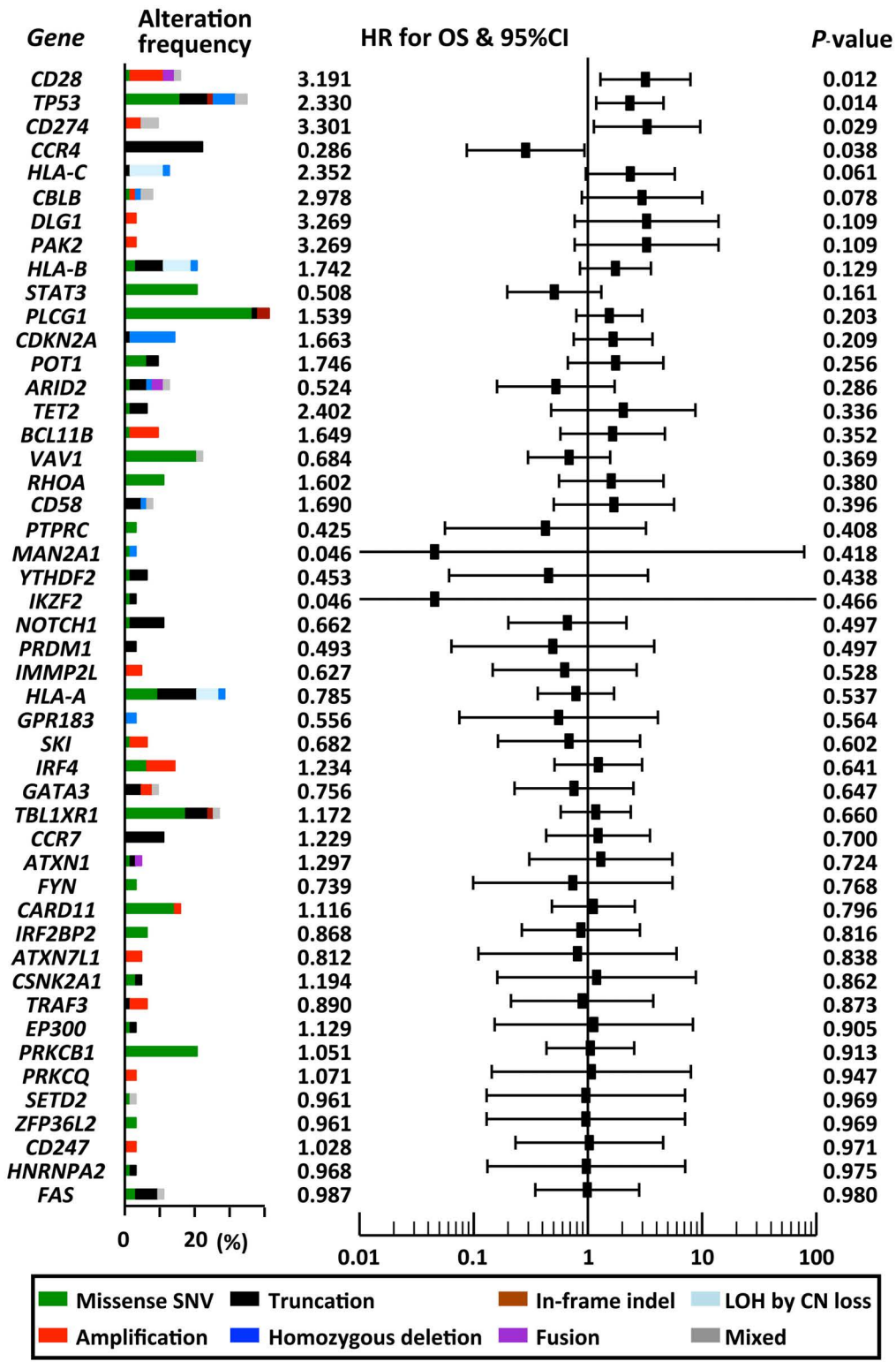

Figure 4. Univariate analyses of overall survival according to driver gene alterations. Forest plot of hazard ratio (HR) and 95% confidence interval (CI) for overall survival (OS) of patients with each driver gene alteration obtained from univariate analysis for OS by the Cox proportional hazards regression model. The altered gene is indicated on the left, and they are shown in ascending order of the *P*-value from top to bottom.

labeled A, B, C and D, which were distinguished by five gene modules (Figure 6A). TS-A was characterized by low tumor cell content and by the expression of "dendritic cell maturation", "Triggering Receptor Expressed on Myeloid cells 1 (TREM1) signaling" and "neuroinflammation signaling pathway" members. The genes of the "Th1 and Th2 activation pathway" and "T-cell receptor signaling" were highly expressed in TS-B. TS-C was a cluster derived from lymph node samples, which prominently expressed "hepatic fibrosis/hepatic stellate cell activation" genes. Expression of "IL-23 signaling pathway" and "*TP53* signaling" genes were enriched in TS-D samples.

Although clinical responses to mogamulizumab were not associated with these TS (data not shown), OS of patients with TS-D was significantly worse than of those with TS-A (median OS, 0.9 years *vs*. 5.1 years, *P*=0.040) (Figure 6B), or the pooled TS-A, -B, and -C groups (median OS, 2.1 years) (Figure 6C). We analyzed the association between TS and 30 driver gene alterations (presence or absence) found in more than three patients. Of these, *TBL1XR1* alterations were not associated with TS-A, *STAT3* alterations were associated with TS-D, and *SKI* and *TRAF3* alterations were associated with TS-C (*Online Supplementary Table S5*). The other 26 gene alterations showed no positive or negative associations with TS (*P*≥0.050).

HLA genotypes, somatic alterations, and clinical outcomes

HLA-A, *-B*, *-C*, *-DPB1*, *-DQB1* and *-DRB1* genotypes were analyzed if carried by more than three patients, but no associations were found between any of the *HLA* genotypes and clinical responses to mogamulizumab (CR *vs*. non-CR). Nonetheless, of nine *HLA-A* genotypes, patients with *HLA-A**26:03 had a worse OS (HR: 2.926, 95% CI: 1.001-8.552)

Table 1. Multivariate analysis including the gene alterations for progression-free survival in patients with adult T-cell leukemia/lymphoma.

Variables	N of patients	HR	95% CI	*P*-value
TP53 alterations				
absence	41	1,000		Reference
presence	22	2,074	1.069-4.026	0,031
CCR4 alterations				
absence	49	1,000		Reference
presence	14	0,355	0.127-0.991	0,048
CD274 alterations				
absence	57	1,000		Reference
presence	6	5,846	1.890-18.081	0,002
ECOG PS				
0, 1	47	1,000		Reference
2, 3, 4	16	2,092	1.024-4.277	0,043
LDH				
< ULN	21	1,000		Reference
> ULN	42	1,773	0.842-3.733	0,131

CI: confidence interval; HR: hazard ratio; ECOG PS: Eastern Cooperative oncology Group performance status; LDH: lactate dehydrogenase; ULN: upper limit of normal.

Table 2. Multivariate analysis including the gene alterations for overall survival in patients with adult T-cell leukemia/lymphoma.

Variables	N of patients	HR	95% CI	*P*-value
TP53 alterations				
absence	41	1,000		Reference
presence	22	2,467	1.197-5.085	0,014
CCR4 alterations				
absence	49	1,000		Reference
presence	14	0,155	0.031-0.778	0,024
CD28 alterations				
absence	53	1,000		Reference
presence	10	1,941	0.648-5.813	0,236
CD274 alterations				
absence	57	1,000		Reference
presence	6	14,393	2.437-85.005	0,003
ECOG PS				
0, 1	47	1,000		Reference
2, 3, 4	16	2,362	1.078-5.175	0,032
LDH				
< ULN	21	1,000		Reference
> ULN	42	1,440	0.600-3.456	0,415

CI: confidence interval; HR: hazard ratio; ECOG PS: Eastern Cooperative Oncology Group performance status; LDH: lactate dehydrogenase; ULN: upper limit of normal.

and of eight *HLA-B* genotypes, those with *HLA-B**40:02 had a worse OS (HR: 2.582, 95% CI: 1.246-5.348). Of eight *HLA-C* genotypes, a worse OS was associated with *HLA-C**03:04 (HR: 2.381, 95% CI: 1.222-4.638) and of seven *HLA-DPB1* genotypes, patients with *HLA-DPB1**05:01 had a better OS (HR: 0.458, 95% CI: 0.226-0.927). All the other *HLA-A*, *-B*, *-C*, and *-DPB1* genotypes, as well as the nine HLA-*DQB1* and 13 HLA-*DRB1* genotypes, were not associated with OS (*P* >0.050) (data not shown). *HLA-A*, *-B*, *-C* somatic alterations did not have any significant impact on PFS (Figure 3) or OS (Figure 4). A *B2M* gene somatic alteration was observed in only one patient (Figure 2).

Next, we performed multivariate analysis of factors influencing OS in the 64 ATL patients using the following six variables: *HLA-A**26:03 (+ or -), *HLA-B**40:02 (+ or -), *HLA-C**03:04 (+ or -), *HLA-DPB1**05:01 (+ or -), ECOG PS (0, 1 *vs*. 2-4) and serum LDH (> ULN *vs*. < ULN). Of these, two variables significantly affected OS, namely, the presence of *HLA-DPB1**05:01 (HR: 0.409, 95% CI: 0.182-0.921), and a worse PS (HR: 2.454, 95% CI: 1.044-5.769) (*Online Supplementary Table S6*).

Discussion

This is the first integrated molecular analysis including exome sequencing, copy number variation assessment and RNA sequencing to evaluate genomic influences on clinical outcomes of mogamulizumab-naïve ATL patients receiving mogamulizumab-containing treatment. The critical inclusion criteria of the study were that patients had to be mogamulizumab-naïve, with no history of allogeneic HSCT. Although the present study included both previously-treated and untreated patients, there were no significant differences of PFS or OS between these two populations. In addition, the present study included patients who received mogamulizumab monotherapy or combination therapy, but there were also no significant differences of PFS or OS between these. These data are consistent with findings from our prospective clinical study of mogamulizumab-naïve ATL patients (the MIMOGA study, clinicaltrials go. Identifier: UMIN000008696).[12] Accordingly, because the heterogeneity of these patients did not directly associate with PFS or OS, the cohort is considered appropriate for achieving the aim of the present study. The observed PFS or OS according to clinical response to mogamulizumab-containing treatment (CR, PR, SD, or PD) thus indicates that this response directly influenced survival outcomes for these patients.

The frequency and types of alterations of the driver genes in ATL cells in the current cohort are almost identical to those in an earlier study.[13] For example, *PLCG1* (41%), *TP53*

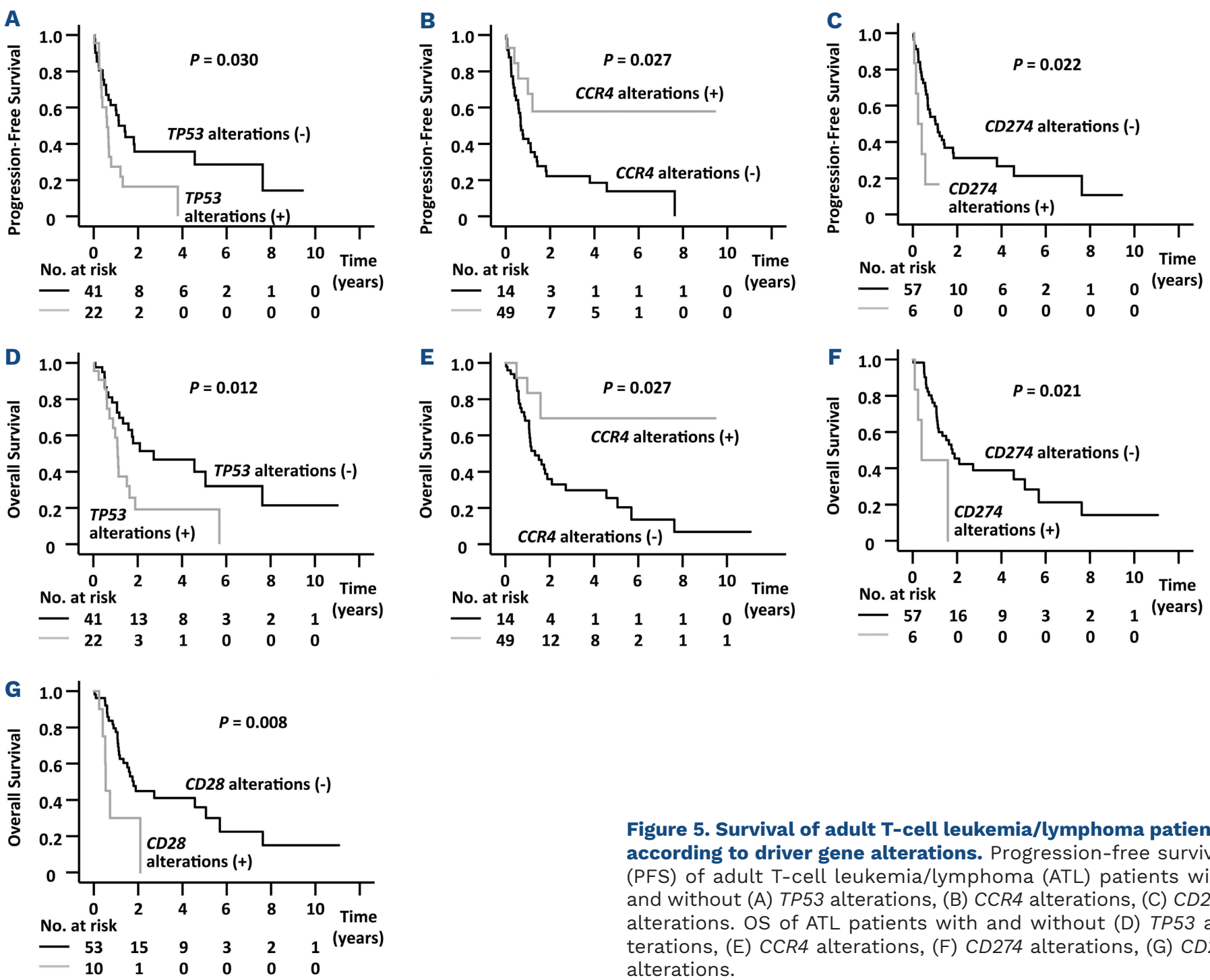

Figure 5. Survival of adult T-cell leukemia/lymphoma patients according to driver gene alterations. Progression-free survival (PFS) of adult T-cell leukemia/lymphoma (ATL) patients with and without (A) *TP53* alterations, (B) *CCR4* alterations, (C) *CD274* alterations. OS of ATL patients with and without (D) *TP53* alterations, (E) *CCR4* alterations, (F) *CD274* alterations, (G) *CD28* alterations.

(35%), *HLA-A* (29%), *TBL1XR1* (27%), *CCR4* (22%), *VAV1* (22%), *HLA-B* (21%), *PRKCB* (21%), *STAT3* (21%), *CARD11* (16%), and *CD28* (16%) were the 11 most frequently altered genes in the present study. Of these, eight genes other than *HLA-A*, *HLA-B*, and *CD28* comprised the top eight most frequent genes with SNV/indels in the earlier study.[13] It should also be noted that these alterations in ATL are similar to those in Sezary Syndrome, which is another mature CD4 T-cell neoplasm. It was reported that the six most frequently altered genes with SNV/indels in Sezary Syndrome are *TP53* (24%), *PLCG1* (18%), *CARD11* (15%), *ARID1A* (10%), *FAS* (10%), and *CCR4* (7%).[34] Thus, four of these six (but not *ARID1A* and *FAS*) are included in the top 11 genes in the present ATL study. Together with the fact that the Food and Drug Administration and European Medicines Agenecy approved mogamulizumab for the treatment of patients with Sézary Syndrome in 2018, these genomic similarities are important for the establishment of appropriate therapeutic strategies for these difficult-to-treat diseases.

In the present study, *TP53*, *CCR4*, and *CD274* alterations in addition to worse PS were independent and significant prognostic factors not only for PFS, but also for OS. Among them, worse PS is a generally accepted unfavorable prognostic factor already established by retrospective studies of ATL patients who did not receive mogamulizumab-containing treatment.[35] In addition, our prospective study of mogamulizumab therapy also supports this finding.[12] With respect to *TP53*, the most commonly altered gene in human cancer, an earlier study of ATL reported that SNV/indels and CNV of this gene were detected in 18% and 23% of patients, respectively.[13] Although the earlier study reported that neither SNV/indels nor CNV of the *TP53* gene had prognostic impact on the OS of ATL patients, in that study, the majority of patients did not receive mogamulizumab-containing treatment.[36] Therefore, this difference of the impact of *TP53* alterations between the two studies may be due to differences in the treatment the patients received. On the other hand, the current adverse impact of *TP53* alterations is consistent with our previous report.[37] The establishment of alternative treatment strategies other than or in addition to mogamulizumab, which can overcome the refractoriness caused by *TP53* alterations in ATL, is warranted.

With respect to *CCR4*, alterations in 25-30% of patients with ATL were reported previously.[13,38,39] Importantly, their association with superior outcomes of mogamulizumab-containing treatment was also previously reported, and the present study is consistent with those findings.[39] In addition, a higher CR rate in patients with *CCR4* alterations was also observed in the present study. These findings are likely due to the fact that mutations in the C-terminus lead to impaired CCR4 internalization upon ligand binding, resulting in its increased surface expression even in the presence of the ligand, and hence to an increased availability of target molecules for mogamulizumab.[13,38] Thus far, a number of successful therapies targeting products of altered critical genes in cancer have been developed, such as those targeting *bcr-abl* fusion products in chronic myelogenous leukemia, mutated *epidermal growth factor receptor* in non-small-cell lung cancer (NSCLC), *EML4-ALK* fusion in NSCLC, or *BRAF* mutations in melanoma.[40] In this context, based on the results presented here, mogamulizumab for patients with *CCR4*-altered ATL could represent a new addition to this group of successful targeted treatments. In addition, CCR7 is a seven-transmembrane G-protein-coupled chemokine receptor, alterations of which were associated with fewer CR, the opposite of *CCR4* alterations. *CCR7* and *CCR4* alterations seem to be mutually exclusive in the present ATL cohort. The reasons for these paradoxical findings are unclear, and further investigations are warranted.

With respect to *CD274*, it was also reported that the amplification of this gene was an unfavorable prognostic factor in ATL, although the treatments received by patients in that study were different from the present study.[36] The mechanism responsible for this may be that higher expression of CD274 by ATL cells results in the suppression of anti-tumor cytotoxic T lymphocytes (CTL) via enhanced CD274/PD-1 signaling.[41] Thus, in general, higher expression of CD274 by gene amplification or structural variants seems to lead to ATL cells' escape from host immune attack by CTL, and thus to their survival advantage.[24] These observations indicate the importance of the immune system for clinical outcome in ATL, as previously reported.[12,42,43] However, we must take special note of the fact that rapid progression of ATL after CD274/PD-1 blockade has also been seen.[44] In this context, a close relationship between regulatory T (Treg) cells and ATL cells has been reported, and also that blockade of CD274/PD-1 signaling leads to the activation and proliferation of PD-1-positive Treg cells.[45-48] Collectively, these data indicate that higher expression of *CD274* in ATL cells might theoretically result in suppression of the activation or proliferation of PD-1-positive ATL cells themselves via enhanced CD274/PD-1 signaling between them. However, in fact, CD274 alterations leading to higher expression of the CD274 protein were significantly associated with worse PFS and OS in patients receiving mogamulizumab in the present study. These findings indicate that CD274/PD-1 signaling in ATL cells is very important, and we are therefore currently conducting further detailed investigations.

With respect to the patients´ *HLA* genotypes or genetic polymorphisms, those with *HLA-DPB1**05:01 had better OS in the present study. The biological mechanisms responsible for this are unknown. Mogamulizumab mediates ADCC, but not complement-dependent cytotoxicity or direct antitumor activities.[7,8] In this context, NK cells are

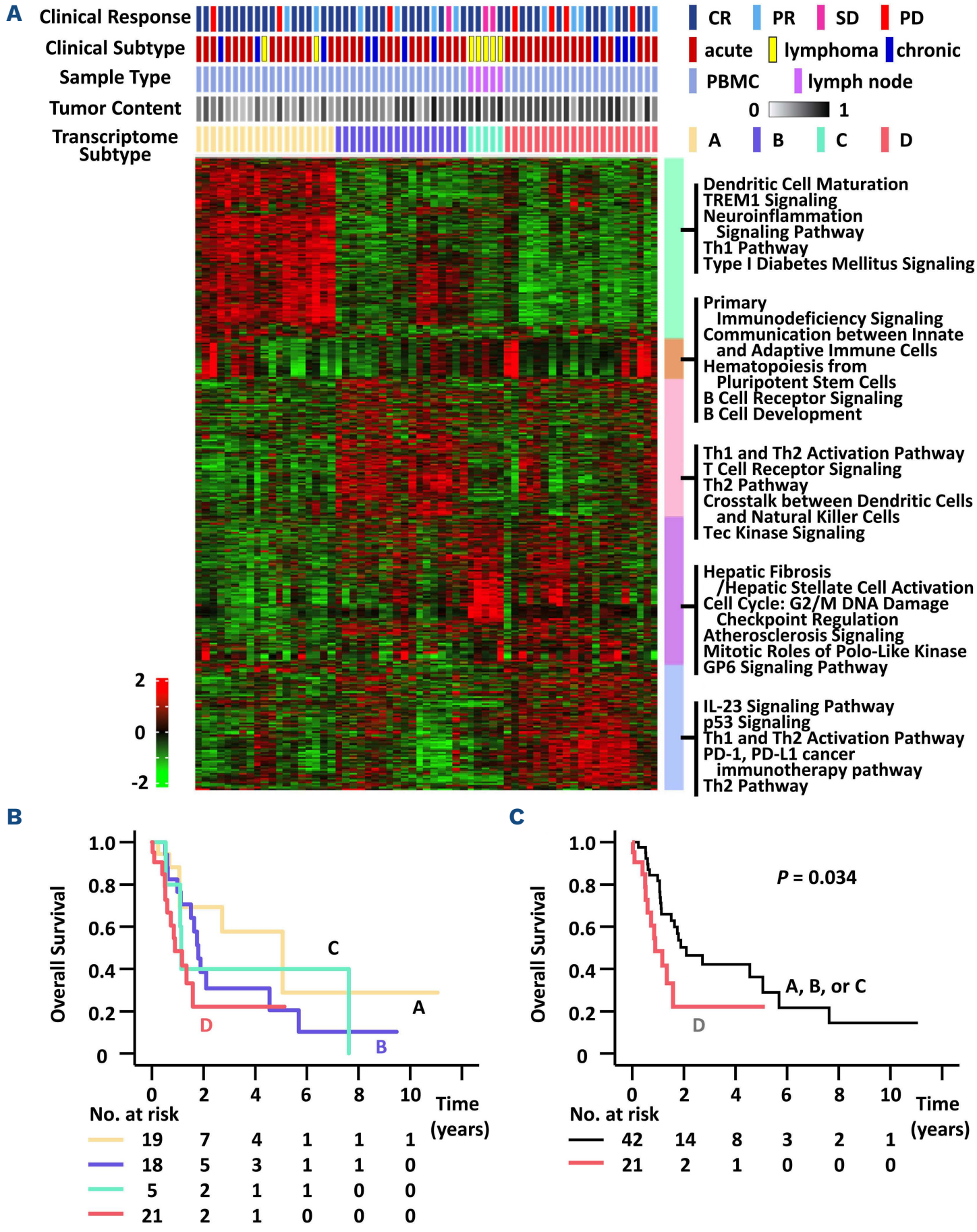

Figure 6. Transcriptome subtypes and overall survival. (A) Heatmap of gene expression used for clustering analysis is shown with color code for clinical information (clinical response to mogamulizumab, clinical subtype, sample type) and heatmap for tumor contents in the sample. Consensus clustering identified four transcriptional subtypes (A, B, C and D) in the cohort. Representative gene ontology annotations, identified by Ingenuity Pathway Analysis, for gene modules are shown on the right. (B) Overall survival (OS) of all adult T-cell leukemia/lymphoma (ATL) patients enrolled in the study, stratified according to transcriptional subgroups. (C) OS of all ATL patients in transcriptional subgroup D compared to pooled TS-A, -B, and -C groups.

considered to be the main effector cells of mogamulizumab-induced ADCC. However, *KIR3DL1* allelic polymorphism and *HLA-B* epitopes, which are associated with NK cell responses, were not associated with PFS or OS in the present study.[33] In addition, because the *FCGR3A* genotype influences the binding affinity of the FcγRIIIa on NK cells to the Fc portion of antibody, it is associated with the magnitude of ADCC.[49] Nonetheless, the *FCGR3A* genotype was not found to be associated with PFS or OS in the present study. This finding might be consistent with an

earlier report that defucosylated antibodies mediate strong ADCC regardless of *FCGR3A* genotype.[50] In any case, more detailed investigations of larger patient cohorts are needed to identify the germline characteristics determining the clinical outcome of mogamulizumab treatment.

In the present study, ATL were divided into four transcriptome subtypes. Patients with TS-D had a significantly worse prognosis than those in the other groups. Together with the present observations on somatic alterations, this is consistent with the findings that "TP53 signaling" and the "CD274/PD-1 cancer immunotherapy pathway" were enriched in TS-D. However, significant associations between *TP53* alterations and TS-D or *CD274* alterations and TS-D were not observed here. Again, more extensive investigations in larger patient cohorts are warranted in order to understand the clinical significance of TS in ATL. Although the present investigation offers significant observations regarding genomic biomarkers predicting clinical outcomes of ATL patients on mogamulizumab-containing treatment, some limitations of the study should be recognized. First, the number of patients enrolled was relatively low. Second, the study included both previously untreated and treated patients, and after enrollment, some patients received mogamulizumab monotherapy, whereas others received different combination therapies. Finally, the present exome-sequencing strategy required the patients' own enriched non-tumor cells, which were in many cases their PBMC after treatment, as the reference. Thus, patients whose clinical responses to mogamulizumab-containing treatment were good, might be more likely to be enrolled in the study, even though the sensitivity of ATL disease to mogamulizumab was extremely good for ATL cells in the blood, compared to other disease sites.[9,10] All of these considerations could affect the conclusions of the present study.

In conclusion, the present integrated genomic analyses identified somatic alterations in ATL cells which influence the clinical outcome of patients treated with mogamulizumab. *TP53* and *CD274* alterations were independently and significantly associated with worse OS, and *CCR4* alterations with better OS. The present study contributes to the establishment of precision medicine for patients with ATL. On the basis of these results, further genomic analyses in much larger cohorts are warranted.

Disclosures

SK received research funding from Chugai, Daiichi-Sankyo, and honoraria from Chugai, Kyowa Kirin. NN received honoraria from Novartis, Takeda, Chugai, Celgene, Otsuka, Nippon Shinyaku, Kyowa Kirin, Asahi-Kasei, and consulting fee from JIMRO. YS received research funding from Chugai, Novartis, Bayer, Eisai, Ono, Otsuka, Pfizer, Amgen, Daiichi Sankyo, Celgene, and Incyte. MH received honoraria from Chugai, and consulting fee from Symbio. KN received research funding from Chugai, Kyowa Kirin, and honoraria from Meiji Seika, Celgene, Eisai, Novartis and Consultant or advisory role from Kyowa Kirin.MY received honoraria from Novartis, Takeda, and consulting fee from Takeda. YI received honoraria from Bristol-Myers, Celgene, CHUGAI, Eisai, Kyowa Kirin, Meiji Seika, Nippon Shinyaku, Sanofi, SymBio, Sumitomo Dainippon. SI received honoraria and reserch grants from Janssen, Sanofi, Takeda, Ono, Celgene and Daiichi-Sankyo, also received research grants from Bristol-Myers, Abbvie, Glaxo-Smithklein, Chugai and Kyowa Kirin. AU has received honoraria from Kyowa Kirin, Daiichi-Sankyo, Bristol-Myers, Celgene, and has received consulting fees from HUYA Japan, JIMRO, Meiji Seika and Otsuka Medical Devices. HN received research funding and honoraria from Ono, Bristol-Myers, MSD, Chugai, and research funding from Taiho, Daiichi-Sankyo, Kyowa Kirin, Zenyaku, Oncolys BioPharma, Debiopharma, Asahi-Kasei, Sysmex, Fujifilm, SRL, Astellas, Sumitomo Dainippon and BD Japan outside of this study. RU received research funding from Kyowa Kirin, Chugai Pharmaceutical, and Ono. All other authors have no conflicts of interest to disclose.

Contributions

SM, TN, HN, RU and TI developed the concept and design of the research; NT, SM, KK, YO, OG, SK, NN, YS, AI, IC, OM, MH, KN, MY, YI, SI, AU and TI acquired and analyzed data; NT, SM, KK, YO and TI interpreted data. All authors wrote and approved the final version of the manuscript.

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Data-sharing statement

Dr. Takashi Ishida takes responsibility for the exome and RNA-sequencing data in the above manuscript, the data will be available on reasonable request by contacting him by email.

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