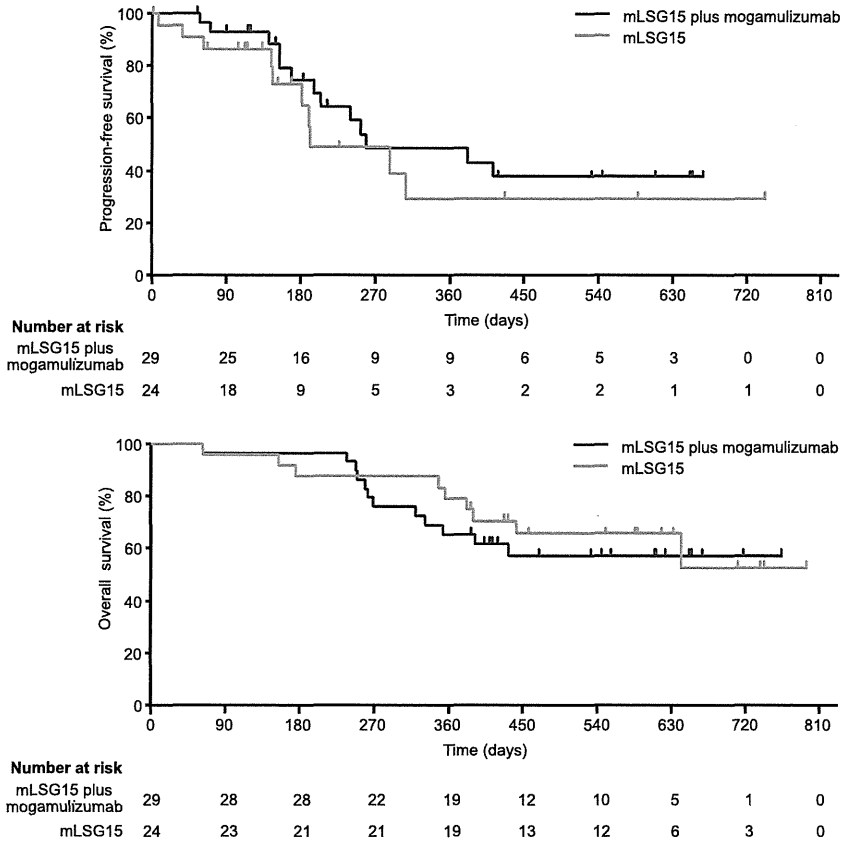

Fig 3. Progression-free survival and overall survival. (A) Kaplan–Meier curve of estimated progression-free survival (median, 8·5 months and 6·3 months in the mLSG15-plus-mogamulizumab and mLSG15 arms, respectively). (B) Kaplan–Meier curve of estimated overall survival (median, not achieved in either arm). The median follow-up periods in the mLSG15-plus-mogamulizumab and mLSG15 arms were 413 days (range, 63–764 days) and 502 days (range, 62–794 days), respectively.

than those in the mLSG15 arm ($0{\cdot}1478 \times 10^9$/l; $0{\cdot}133 \times 10^9$/l; $0{\cdot}059$–$0{\cdot}368 \times 10^9$/l) ($P < 0{\cdot}001$) (Fig 4A). Similarly, the numbers of CD4/CD25/FOXP3-positive cells in the blood immediately before VCAP therapy (Cycle 3) in the mLSG15-plus-mogamulizumab arm ($0{\cdot}0085 \times 10^9$/l; $0{\cdot}004 \times 10^9$/l; 0–$0{\cdot}048 \times 10^9$/l) were significantly lower than those in the mLSG15 arm ($0{\cdot}2432 \times 10^9$/l; $0{\cdot}074 \times 10^9$/l; $0{\cdot}018$–$2{\cdot}77 \times 10^9$/l) ($P < 0{\cdot}001$), and the numbers of these cells 28 days after VECP therapy (Cycle 4) in the mLSG15-plus-mogamulizumab arm ($0{\cdot}0054 \times 10^9$/l; $0{\cdot}003 \times 10^9$/l; 0–$0{\cdot}037 \times 10^9$/l) were significantly lower than those in the mLSG15 arm ($0{\cdot}0684 \times 10^9$/l; $0{\cdot}0435 \times 10^9$/l; $0{\cdot}016$–$0{\cdot}25 \times 10^9$/l) ($P < 0{\cdot}001$, Fig 4B).

Discussion

This study showed that the %CR in patients who received mLSG15 plus mogamulizumab was higher than that obtained in those treated with mLSG15 alone (52% vs. 33%; difference, 18·4%). The increase in the %CR with the addition of mogamulizumab observed in this study surpassed the predicted, targeted, clinically significant 15% increase in patients with ATL. Importantly, the %CR in patients with lesions in the blood compartment was higher in the combination arm, leading to the increase in overall %CR. This finding was consistent with that observed in previous studies, in which ATL lesions in the blood were found to be more sensitive to mogamulizumab monotherapy than ATL lesions at other disease sites (Yamamoto *et al*, 2010; Ishida *et al*, 2012b).

Infections were more frequent in the combination arm. In particular, cytomegalovirus infection was observed in 14% of patients in the combination arm, whereas it was not observed in the chemotherapy alone arm. Furthermore, cytomegalovirus-related SAEs occurred in three patients in the combination arm. Cytomegalovirus reactivation is observed in approximately 60% of patients with ATL during systemic chemotherapy (Ogata *et al*, 2011). Our study suggests that the addition of mogamulizumab to systemic chemotherapy might further increase the incidence of cytomegalovirus infection; therefore, careful monitoring for cytomegalovirus infection and appropriate use of antiviral therapy are recommended when systemic chemotherapy in combination with mogamulizumab is administered to patients with ATL.

In our previous study of mogamulizumab monotherapy for patients with relapsed ATL, skin rashes, including Stevens–Johnson syndrome, were the most frequently observed AEs (63%) (Ishida *et al*, 2012b, 2013). In the present study, as expected, AEs involving skin and subcutaneous tissue disorders were more frequent in the combination arm than in the chemotherapy alone arm. Even though no severe skin-related AEs, such as Stevens–Johnson syndrome or toxic epidermal necrolysis, occurred in the present study, special attention should be paid to these skin-related AEs

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Table III. Treatment-emergent adverse events in the mLSG15-plus-mogamulizumab (n = 29) and mLSG15 (n = 24) arms.

	All grades		≥Grade3	
	mLSG15 + mogamulizumab n = 29	mLSG15 n = 24	mLSG15 + mogamulizumab n = 29	mLSG15 n = 24
Blood and lymphatic system disorders	29 (100%)	22 (92%)	29 (100%)	22 (92%)
Anaemia	28 (97%)	22 (92%)	28 (97%)	19 (79%)
Febrile neutropenia	26 (90%)	21 (88%)	26 (90%)	21 (88%)
Gastrointestinal disorders	29 (100%)	23 (96%)	7 (24%)	7 (29%)
Stomatitis	16 (55%)	13 (54%)	4 (14%)	4 (17%)
General disorders and administration site conditions	29 (100%)	21 (88%)	6 (21%)	0 (0%)
Pyrexia	24 (83%)	15 (63%)	4 (14%)	0 (0%)
Infections and infestations	19 (66%)	16 (67%)	10 (34%)	7 (29%)
Bacteraemia	4 (14%)	3 (13%)	3 (10%)	3 (13%)
Pneumonia	4 (14%)	2 (8%)	3 (10%)	1 (4%)
Cytomegalovirus infection	4 (14%)	0 (0%)	2 (7%)	0 (0%)
Cytomegaloviral pneumonia	2 (7%)	0 (0%)	2 (7%)	0 (0%)
Investigations	29 (100%)	24 (100%)	29 (100%)	24 (100%)
Neutropenia	29 (100%)	23 (96%)	29 (100%)	22 (92%)
Thrombocytopenia	29 (100%)	23 (96%)	26 (90%)	17 (71%)
Lymphopenia	28 (97%)	23 (96%)	28 (97%)	18 (75%)
Leucopenia	29 (100%)	22 (92%)	29 (100%)	21 (88%)
Albuminaemia	12 (41%)	11 (46%)	2 (7%)	1 (4%)
Alanine transaminase increased	12 (41%)	10 (42%)	2 (7%)	2 (8%)
Aspartate transaminase increased	9 (31%)	8 (33%)	2 (7%)	1 (4%)
Potassium decreased	9 (31%)	6 (25%)	3 (10%)	1 (4%)
Sodium decreased	8 (28%)	7 (29%)	4 (14%)	2 (8%)
Phosphorus decreased	8 (28%)	3 (13%)	3 (10%)	1 (4%)
Blood pressure increased	7 (24%)	2 (8%)	5 (17%)	2 (8%)
Oxygen saturation decreased	4 (14%)	1 (4%)	2 (7%)	0 (0%)
Metabolism and nutrition disorders	27 (93%)	19 (79%)	14 (48%)	6 (25%)
Decreased appetite	23 (79%)	15 (63%)	8 (28%)	3 (13%)
Hyperglycaemia	13 (45%)	7 (29%)	4 (14%)	0 (0%)
Hyponatraemia	4 (14%)	3 (13%)	2 (7%)	2 (8%)
Hypophosphataemia	4 (14%)	3 (13%)	4 (14%)	2 (8%)
Hypokalaemia	5 (17%)	1 (4%)	2 (7%)	1 (4%)
Respiratory, thoracic and mediastinal disorders	21 (72%)	9 (38%)	4 (14%)	1 (4%)
Interstitial lung disease	3 (10%)	0 (0%)	3 (10%)	0 (0%)
Skin and subcutaneous tissue disorders	29 (100%)	20 (83%)	15 (52%)	1 (4%)
Papular rash	12 (41%)	0 (0%)	6 (21%)	0 (0%)
Erythematous rash	8 (28%)	0 (0%)	2 (7%)	0 (0%)

when mogamulizumab is administered to patients with ATL.

Adult T-cell leukaemia-lymphoma cells constitutively express CD25 (Waldmann *et al*, 1984), and the present study had an eligibility criterion of CCR4 positivity. Hence, most of the CD4/CD25/CCR4-positive cells were considered ATL cells. Compared to the chemotherapy alone arm, the combination arm showed a significant reduction in the number of CD4/CD25/CCR4-positive cells. This finding is consistent with the proposed antitumour mechanism of mogamulizumab, in that mogamulizumab kills CCR4-expressing ATL cells by increasing antibody-dependent cellular cytotoxicity (Shinkawa *et al*, 2003; Ishii *et al*, 2010; Yamamoto *et al*, 2010). In humans, CCR4 is expressed on CD45RO-positive, CD45RA-negative, FOXP3-positive activated regulatory T (Treg) cells (Miyara *et al*,

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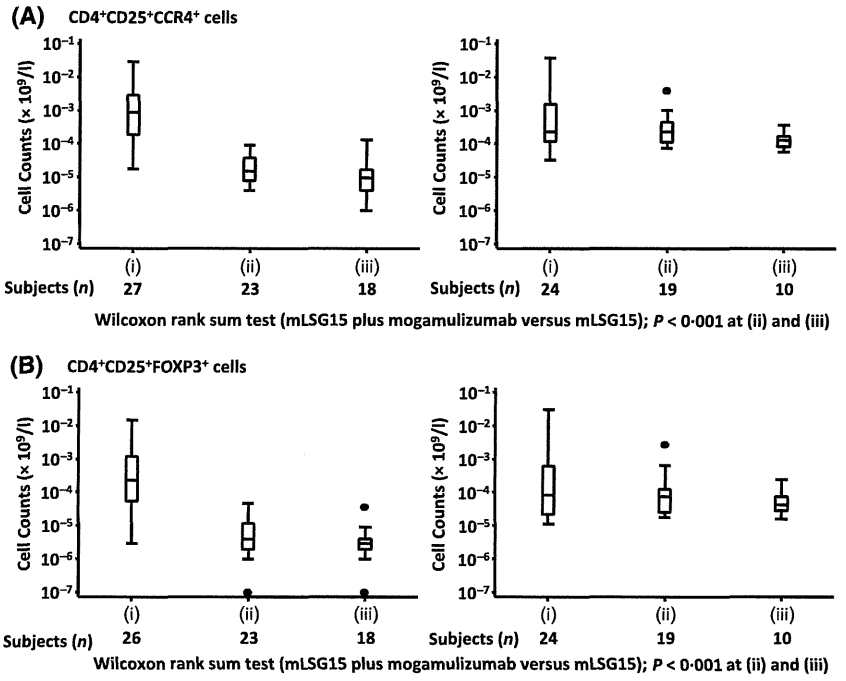

Fig 4. T-cell subset analysis. Blood samples were taken (i) immediately before the initiation of treatment, (ii) immediately before VCAP therapy for cycle three, and (iii) 28 days after VECP therapy for cycle four. The numbers of CD4/CD25/CC chemokine receptor 4 (CCR4)-positive cells (A) and CD4/CD25/FOXP3-positive cells (B) are shown as box and whisker plots indicating the minimum, lower, median, upper quartile, and maximum values. The number of samples used for analysis at each point is indicated below the graph. The differences of each point [(ii) & (iii)] between the mLSG15-plus-mogamulizumab and mLSG15 arms are indicated as p-values (Wilcoxon signed-rank test) below the graphs. CCR4 was detected by using a monoclonal antibody (clone 1G1), with its binding to CCR4 being unaffected by the presence of mogamulizumab. VCAP: vincristine, cyclophosphamide, doxorubicin, and prednisolone; VECP: vindesine, etoposide, carboplatin, and prednisolone.

2009; Ishida & Ueda, 2011; Sugiyama *et al*, 2013). In addition, ATL cells from a subset of patients express FOXP3 and function as Treg cells (Yano *et al*, 2007). Thus, the CD4/CD25/FOXP3-positive cells included not only endogenous activated Treg cells, but also ATL cells, in some patients. Our study indicated that compared to the chemotherapy alone arm, the combination arm showed a significant reduction in the number of CD4/CD25/FOXP3-positive cells, which is consistent with the findings from our previous study of mogamulizumab monotherapy. In general, decreasing the number of Treg cells is considered a promising strategy for boosting antitumour immunity in patients with cancer, because the numbers of these cells increase in the tumour microenvironment, and they may play an important role in the ability of the tumour to escape host immunity in several different types of cancer (Ishida & Ueda, 2011; Jacobs *et al*, 2012). On the other hand, because alterations in Treg cell frequencies and/or function may contribute to various autoimmune diseases (Michels-van Amelsfort *et al*, 2011), immune-related AEs, such as skin disorders, which were also observed in our study, should be carefully monitored.

The present study was conducted according to the premise that mLSG15 is the most recommended chemotherapeutic regimen for patients with newly diagnosed aggressive ATL. We found higher rates of treatment-related toxicities with mLSG15 compared to what has been reported for CHOP-14 (Tsukasaki *et al*, 2007). In the context of this scenario, this study suggests that a younger patient population, particularly those aged <56 years, will benefit from VCAP-AMP-VECP, while an older population consisting of those aged 56–69 years will not; there are no data regarding mLSG15 therapy for patients with ATL aged >69 years (Tsukasaki *et al*, 2007). In the present study, the median ages in the mLSG15-plus-mogamulizumab and mLSG15 arms were 61 years and 64 years, respectively; patients potentially benefiting from mLSG15 (<56 years) accounted for only 38% of the patients in the mLSG15-plus-mogamulizumab arm and 25% of those in the mLSG15 arm. Adult T-cell leukaemia-lymphoma generally occurs in older individuals, with a median age at diagnosis of approximately 66 years (Iwanaga *et al*, 2012); therefore, further investigations are needed to determine whether mLSG15 is indeed the most suitable systemic chemotherapeutic regimen when combined with mogamulizumab.

CCR4 is expressed on the surface of tumour cells of patients from a subgroup of PTCL other than ATL, which also has an unfavourable prognosis (Ishida *et al*, 2004; Nakagawa *et al*, 2009). We have already completed a multicentre phase II study of mogamulizumab monotherapy for patients with relapsed CCR4-positive PTCL in Japan (Clinicaltrials.gov: NCT01192984) (Ogura *et al*, 2014). Furthermore, other clinical trials of mogamulizumab for PTCL (Clinicaltrials.gov: NCT01611142) or cutaneous T-cell lymphoma (Clinicaltrials.gov: NCT01728805) are currently underway worldwide. Further studies are expected to allow the determination of the efficacy of combining mogamulizumab with chemotherapy or other novel molecular target therapies for PTCL subtypes other than ATL.

Although this study offers a novel treatment option for newly diagnosed aggressive ATL, some limitations should be discussed. First, this study was designed to set the %CR as a primary endpoint; as a result, this study does not have enough power or a long enough follow-up period to detect

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PFS and OS differences between the two arms. Thus, although a tendency towards prolongation of PFS in the combination arm was observed in the present study, this was not confirmed. Second, the treatment after the study protocol, including allo-HCT and mogamulizumab, varied among the patients. Because the use of mogamulizumab for relapsed/refractory ATL was approved in Japan during the study period, the patients, including those in the chemotherapy alone arm, may have a chance to receive this drug. Both of these factors may affect the OS (Chihara *et al*, 2013).

In conclusion, although mLSG15 plus mogamulizumab was found to be associated with a potentially less favourable safety profile, particularly for infectious and skin-related events, the majority of the AEs were manageable. The %CR was higher with combination therapy. Accordingly, this combination treatment appears to be a better option for managing patients with newly diagnosed aggressive ATL. Further clinical studies are necessary to evaluate the survival parameters in patients treated with chemotherapy plus mogamulizumab and to determine a more suitable combination regimen.

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Author contributions

T.I., K.U., K.Y., N.U., A.U., K.T., S.A. and R.U. contributed to the conception and design of the study; T.I., T.J., S.T., H.S., K.U., K.Y., N.U., Y.S., K.N., A.U., K.T., H.F., K. Ishitsuka, S.Y., N.T., Y.M., K. Imada and T.M. contributed to the acquisition of data; T.I., K.T., S.A., M.T. and R.U. analysed and interpreted the data; all authors drafted and reviewed the manuscript and approved the final version for submission.

Conflicts of interest

T.I. has received honoraria and travel grants from Kyowa Hakko Kirin, and research funding from Kyowa Hakko Kirin, Chugai, Bayer and Celgene, and has served on the speakers bureau for Kyowa Hakko Kirin. T.J. has received honoraria and travel grants from Kyowa Hakko Kirin. S.T. has received travel grants and research funding from Kyowa Hakko Kirin. H.S. has served on the speakers bureau for Kyowa Hakko Kirin. K.Y. has a consultancy/advisory role with Kyowa Hakko Kirin and Novartis, has received honoraria from Kyowa Hakko Kirin, Novartis, Takeda and Janssen, and has received research funding from Kyowa Hakko Kirin, Novartis, Pfizer and ARIAD. Y.S. has received honoraria and research funding from Kyowa Hakko Kirin. K.N. has received honoraria from Kyowa Hakko Kirin. A.U. has received honoraria and research funding from Kyowa Hakko Kirin. K.T. has received honoraria from Kyowa Hakko Kirin and research funding from Kyowa Hakko Kirin, Celgene, Eisai, Solasia Pharma and Mundipharma. S.Y. has received honoraria and research funding from Kyowa Hakko Kirin. Y.M. has received honoraria, travel grants and research funding from Kyowa Hakko Kirin. K. Imada has received research funding from Kyowa Hakko Kirin. S.A. is employed by Kyowa Hakko Kirin, and is a stock owner. M.T. has a consultancy/advisory role with Kyowa Hakko Kirin, and has received honoraria from Kyowa Hakko Kirin. R.U. has a consultancy/advisory role with Mundipharma, and has received honoraria, travel grants and research funding from Kyowa Hakko Kirin and Chugai, and has served on the speakers bureau for Kyowa Hakko Kirin. The remaining authors declare no competing financial interests.

Appendix I

List of the review committees and medical experts who participated in this trial:

1 Junji Suzumiya, Shimane University Hospital
2 Takashi Terauchi, Research Centre for Cancer Prevention and Screening National Cancer Centre
3 Ukihide Tateishi, Yokohama City University Graduate School of Medicine
4 Junichi Tsukada, University of Occupational and Environmental Health
5 Koichi Nakata, University of Occupational and Environmental Health
6 Shigeo Nakamura, Nagoya University Graduate School of Medicine
7 Hiroshi Inagaki, Nagoya City University Graduate School of Medical Sciences
8 Koichi Ohshima, Kurume University School of Medicine
9 Michinori Ogura, Nagoya Daini Red Cross Hospital
10 Tetsuo Nagatani, Hachioji Medical Centre of Tokyo Medical University
11 Akimichi Morita, Nagoya City University Graduate School of Medical Sciences
12 Kazunari Yamaguchi, Institute of Molecular Embryology and Genetics, Kumamoto University
13 Yasuaki Yamada, Nagasaki University Graduate School of Biomedical Sciences
14 Shuichi Hanada, National Hospital Organization Kagoshima Medical Centre.

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Original Article

Mutation Analysis for *TP53* in Chronic-Type Adult T-Cell Leukemia/Lymphoma

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Adult T-cell leukemia/lymphoma (ATLL) is a T-cell neoplasm caused by human T-cell leukemia virus type I (HTLV-I). ATLL is classified into four clinical subtypes based on the clinical manifestation: acute, lymphoma, chronic and smoldering. Approximately half of chronic type ATLL cases progressed to the acute type. We previously demonstrated that genomic alterations related to the cell cycle de-regulation such as *CDKN2A* and immune escape such as *CD58* alteration can serve as predictive biomarkers for acute transformation of the chronic type. Although alteration of *TP53*, which is known to be a major regulator of cell cycle, has been identified in several types of cancers including acute type ATLL, no copy number alteration of *TP53* was found in the chronic type by array comparative genomic hybridization. In the present study, mutation of *TP53* was further analyzed by sequencing for these cases as well as HTLV-I carriers with oligo-clonality. However, no *TP53* mutation was identified. These results suggested that *TP53* mutation plays a role for the later stage of ATLL development. [*J Clin Exp Hematop 55(1) : 13-16, 2015*]

Keywords: adult T-cell leukemia/lymphoma, acute transformation, *TP53* mutation

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATLL) is a T-cell neoplasm caused by human T-cell lymphotropic virus type I (HTLV-I).[1-3] Currently, approximately 10 to 20 million people are estimated to be infected by HTLV-I in the world, and high prevalence of the virus are recognized in south-western Japan, Caribbean islands and South America.[4,5] Over 90% of HTLV-I infected peoples remain asymptomatic carriers during one's life time, but approximately 2 to 5% of the carriers develop ATLL.[6]

ATLL has been classified into four types based on the clinical manifestation: smoldering, chronic, lymphoma and acute.[7] Among these subtypes, chronic and smoldering types are regarded as indolent ATLL because patients with these types have better prognosis than those with lymphoma and acute types. However, about half of patients with chronic type ATLL are reported to progress to the acute type and subsequently die.[8] Although combination therapy of interferon α and zidovudine are recommended for indolent ATLL patients who are symptomatic, chronic type ATLL patients with poor prognostic factors such as high level of lactate dehydrogenase are needed to be treated by intensive chemotherapy including stem-cell transplantation.[9]

We previously analyzed genomic alterations of chronic and acute types by using high-resolution array comparative hybridization (aCGH) and compared the molecular characteristics of both types.[10] The study revealed that cell cycle de-regulation and immune escape mechanism play critical roles in acute transformation of the chronic type. However, a few of the chronic type cases progressed to acute type ATLL without these alterations. Although *TP53* alteration, which causes de-regulation of cell cycle, has been frequently identified in several types of cancers, no copy number alteration of *TP53* were observed in our analyzed chronic type cases.[10] Several previous studies identified genomic mutation of *TP53* in ATLL cases[11-13] and other several types of cancers. Missense mutations of *TP53* rather than the copy number losses are more frequently observed in various cancers.[14] Although Tawara *et al.* found that two cases of chronic type ATLL possessed a *TP53* mutation and that such patients had a poor clinical outcome,[13] long-term follow-up duration was not described. Therefore, it has remained fully elucidated whether *TP53* mutation can work as predictive markers for

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acute transformation in chronic type ATLL. It should be also noted that mutation analysis for *TP53* has not been performed in HTLV-1 carriers to date.

In order to precisely reveal genomic alterations which are related to cell cycle de-regulation, we analyzed *TP53* mutation in our chronic type ATLL cases, whose copy number alterations had been analyzed by array CGH. HTLV-1 carriers who showed oligo- clonality were also investigated.

MATERIALS AND METHODS

Samples

Eight chronic type ATLL cases and eleven HTLV-1 asymptomatic carriers, for which adequate genomic DNA was available, were analyzed (Table 1). Seven of the eight cases with chronic type ATLL progressed to the acute type. Each DNA was extracted from CD4-positive cells in peripheral blood as described in previous reports.[10,15] Inverse polymerase chain reaction (PCR) analysis for HTLV-1 integration site showed oligoclonal proliferation of CD4-positive cells in five of the HTLV-1 carrier samples which were used in this study.[15] aCGH analysis revealed that five out of eight chronic type ATLL cases used in this study had genomic alterations of the genes which were related to cell cycle (Table 1).[10] This study was approved by the Institute Review Board of the Kurume University School of Medicine (Kurume, Japan) and was conducted with the basis of Declaration of Helsinki.

Mutation analysis for TP53

Mutation analysis was performed as previously described.[16] In brief, exons 4-8 of *TP53* were amplified from genomic DNA using PCR. PCR primers used in this study were detailed in the previous study.[17] The sequence results were evaluated by using International Agency for Research on Cancer (IARC) TP53 database (http://p53.iarc.fr/).[18]

RESULTS

Results for *TP53* mutation were summarized in Table 2. One-nucleotide substitution registered as a single nucleotide polymorphism (SNP) in the NCBI database (http://www.ncbi.nlm.nih.gov/gene/) was found in 17 cases (c.215C > G, SNP rs1042522). Except for this SNP, no mutation was identified in analyzed 19 cases. Even in the three chronic type cases which do not possess the copy number alteration of cell cycle related genes, no mutation of *TP53* was observed.

DISCUSSION

Our previous study revealed that 17 of the 35 (49%) acute type ATLL cases had genomic loss of *CDKN2A* located in 9p21.3, which was also found in 5 of the 27 (19%) chronic type ATLL cases.[10] The previous study also found that 15 of the 27 chronic type cases (56%) had copy number alterations related to cell cycle-related genes including *CDKN2A*.

Table 1. Characteristics of the analyzed samples

Case No.*	Sample type	Proliferation**	Cell cycle alteration***	*CDKN2A* loss	Acute transformation
Carrier-4	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-6	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-7	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-8	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-10	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-11	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-12	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-13	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-14	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-15	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-16	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
C-1	Chronic type ATLL $CD4^+$	Monoclonal	+	-	+
C-3	Chronic type ATLL $CD4^+$	Monoclonal	+	-	+
C-6	Chronic type ATLL $CD4^+$	Monoclonal	+	+	+
C-10	Chronic type ATLL $CD4^+$	Monoclonal	+	+	+
C-12	Chronic type ATLL $CD4^+$	Monoclonal	+	-	+
C-21	Chronic type ATLL $CD4^+$	Monoclonal	-	-	-
C-23	Chronic type ATLL $CD4^+$	Monoclonal	-	-	+
C-24	Chronic type ATLL $CD4^+$	Monoclonal	-	-	+

NA, not available.

*: Representations in previous Ohshima *et al.* and Yoshida *et al.* papers were used.

**: These results were extracted from previous Ohshima *et al.* and Yoshida *et al.* results.

***: These results were extracted from previous Yoshida *et al.* result. "+" means presense of the genomic alterations related to cell cycle pathway.

Table 2. Results of *TP53* mutation analyses

Case No.*	Exon4	Exon5	Exon6	Exon7	Exon8
Carrier-4	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-6	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-7	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
Carrier-8	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-10	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-11	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
Carrier-12	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-13	Wt	Wt	Wt	Wt	Wt
Carrier-14	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-15	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
Carrier-16	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
C-1	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
C-3	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
C-6	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
C-10	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
C-12	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
C-21	Wt	Wt	Wt	Wt	Wt
C-23	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
C-24	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt

Wt, wild type.
*: Representations in previous Ohshima *et al.* and Yoshida *et al.* papers were used.

Because the chronic type ATLL cases with the alterations of cell cycle-related genes progressed to the acute type, presence of these alterations can serve as biomarkers predicting acute transformation in chronic type ATLL. However, no copy number alterations of *TP53* were found in our previous study. It is therefore surmised that *TP53* mutation rather than copy number alteration exert a predictive biomarker in the chronic type. Mutation analysis for *TP53* was therefore performed in this study, but no case with *TP53* mutation was found.

It is speculated that *TP53* mutation could not be identified in HTLV-1 carriers because of few *TP53* mutated cells. Under the current method, ultra-deep-next-generation sequencing might detect such *TP53* mutated cells in HTLV-1 carriers because the sensitivity of this method is reported to be approximately 0.3%.[19] Additionally, flow cytometrical analysis by using CD3, CD7 and CADM1 antibodies can purify the HTLV-1 infected clones which can progress to ATLL even in the carrier samples.[20] Combination of these techniques would help us more precisely evaluate *TP53* mutation in the carriers, and this analysis could reveal the role of *TP53* mutation in the pathophysiology of ATLL.

Tawara *et al.* found that 2 of the 13 chronic type ATLL cases had a *TP53* mutation and that *TP53* mutation and *CDKN2A* loss were mutually exclusive events in ATLL.[13] Our current analysis was also applied for the five chronic type cases, who progressed to the acute type without *CDKN2A* loss, but no *TP53* mutation was identified in these cases. This finding suggests that other alterations contribute to acute transformation of the chronic type besides *TP53* mutation. A previous report showed that *TP53* mutation was observed in 43% of acute type ATLL, while only 7% of the chronic type had the mutation.[12] Our current study also suggested that *TP53* mutation is a rare event in the chronic type. From these results, it is speculated that *TP53* mutation plays an important role especially in pathophysiology of the acute type ATLL and that the mutation immediately induces the acute type.

Monti *et al.* reported that copy number alterations of cell cycle-related genes including *CDKN2A* loss predict more precisely poor prognosis in diffuse large B-cell lymphoma than international prognostic index (IPI).[21] Although several clinical factors such as serum calcium levels, serum soluble interleukin-2 receptor levels, and performance status are reported to serve as IPI in ATLL,[22,23] these prognostic indexes do not include molecular pathological factors. Molecular characteristics of ATLL have been elucidated by several groups including us.[10,24,25] Development of prognostic and predictive factors based on these molecular aspects might be valuable to stratify treatments for incurable ATLL cases.

In summary, our previous study revealed that genomic alterations of cell cycle-related genes including *CDKN2A* can serve as predictive markers for acute transformation of the chronic type. However, our current study focusing on *TP53* indicates that copy number alteration and nucleotide mutation of *TP53* are not useful for the prediction of acute transformation in the chronic type.

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