Clinical significance of soluble CADM1 as a novel marker for adult T-cell leukemia/lymphoma

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**ABSTRACT**

Adult T-cell leukemia/leukemia (ATLL) is an aggressive peripheral T-cell malignancy, caused by infection with the human T-cell leukemia virus type 1 (HTLV-I). We recently showed that the cell adhesion molecule 1 (CADM1), a member of the immunoglobulin superfamily, is specifically and consistently overexpressed in ATLL cells, and functions as a novel cell surface marker. In this study, we first show that a soluble form of CADM1 (sCADM1) is secreted from ATLL cells by mainly alternative splicing. After developing the Alpha linked immunosorbent assay (AlphaLISA) for sCADM1, we show that plasma sCADM1 concentrations gradually increased during disease progression from indolent to aggressive ATLL. Although other known biomarkers of tumor burden such as soluble interleukin-2 receptor α (sIL-2Rα) also increased with sCADM1 during ATLL progression, multivariate statistical analysis of biomarkers revealed that only plasma sCADM1 was selected as a specific biomarker for aggressive ATLL, suggesting that plasma sCADM1 may be a potential risk factor for aggressive ATLL. In addition, plasma sCADM1 is a useful marker for monitoring response to chemotherapy as well as for predicting relapse of ATLL. Furthermore, the change in sCADM1 concentration between indolent and aggressive type ATLL was more prominent than the change in the percentage of CD4+CADM1+ ATLL cells. As plasma sCADM1 values fell within normal ranges in HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients with higher levels of serum sIL-2Rα, the measurement of sCADM1 may become a useful tool to discriminate between ATLL and other inflammatory diseases, including HAM/TSP.
Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a refractory CD4+ T-cell malignancy associated with human T-cell leukemia virus type 1 (HTLV-1).\(^1,3\) It is estimated that 15-20 million people are currently infected with HTLV-1 worldwide, and a high prevalence of HTLV-1 infection can be found in many parts of the world, including southwestern Japan, Melanesia, South America, sub-Saharan Africa, the Caribbean, Romania, central parts of Australia, and the Middle East. ATLL is classified into four subtypes – acute, lymphoma, chronic, and smoldering type. Patients with indolent ATLL (chronic or smoldering) have a better prognosis and watchful waiting or combined zidovudine (AZT) and interferon-α (IFN-α) therapy is standard treatment for indolent disease. Patients with aggressive forms (acute and lymphoma) have a very poor prognosis due to the intrinsic chemotherapy resistance of malignant cells. Allogeneic hematopoietic stem-cell transplantation (allo-HSCT), mogamulizumab, an anti-CC chemokine receptor 4 (CCR4) monoclonal antibody, or AZT/IFN therapy are important for the treatment of ATLL; however, the prognosis is unfavorable in many cases.\(^4,7\)

Cell lines

The HTLV-1-negative human T-cell acute lymphoblastic leukemia (T-ALL) cell line MOLT4, the cutaneous T-cell lymphoma (CTCL) cell line HUT78, the HTLV-1-infected T-cell line MT2, and ATLL-derived cell lines (S1T and ST1) were maintained in RPMI 1640 medium (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS) and 50 µg/mL of penicillin/streptomycin in a 5% CO\(_2\) chamber at 37°C. The IL-2-dependent ATLL-derived cell lines KK1 and KOB were maintained in complete RPMI 1640 medium supplemented with 0.75 g/mL of recombinant human IL-2 (Peprotech, Rocky Hill, NJ, USA). The human osteosarcoma cell line Saos-2 was cultured in Dulbecco’s modified Eagle’s medium (DMEM; Wako) supplemented with 10% FBS and 50 µg/mL of penicillin/streptomycin. MOLT4 was obtained from the Fusaki Cell Center, Hayashibara Biochemical Laboratory (Okayama, Japan). MT2 was kindly provided by Dr. H. Iha (Oita University, Japan). ST1, KOB, and KK1 were kindly provided by Dr. H. Iha (Oita University, Japan). Saos-2 was obtained from the RIKEN Bioresource Center (Tsukuba, Japan).

Measurement of sCADM1 concentrations in the blood by AlphaLISA

The anti-CADM1 antibody (103-109) was generated by phage-display technology.\(^6\) AlphaLISA was performed in 96-well microtiter plates containing 5 µL of plasma sample, 10 µL of biotinylated anti-CADM1 antibody (SE1, MBL, Nagoya, Japan) (0.1 nM),\(^{15}\) 10 L of 10 µg/mL anti-CADM1 antibody (103-109)-conjugated AlphaLISA acceptor beads (10 µg/mL), and 25 µL of streptavidin-coated AlphaLISA donor beads (40 µg/mL) in AlphaLISA
form of that sCADM1 transcript. Semiquantitative RT-PCR showed located in exon 6 and intron 7 of CD4+CADM1+ leukemic cell populations from various lines (expressed in all of the HTLV-1-positive ATLL-related cell mRNA skipped both exons 9 and 10 (PCR analysis revealed that the majority of the cing between exons 7 and 11 (Figure 1A) and the inclusion of exon 9 confers shedding susceptibility to CADM1.31 RT-

The soluble form of CADM1 mRNA is efficiently expressed in ATLL

As sCADM1 can be generated through alternative splicing by an intron retention event27,28 or ectodomain shedding of CADM1 protein,29,30 the mRNA expression of a soluble splice variant of CADM1 and the level of CADM1 shedding were initially determined in ATLL-related cell lines and leukemia cells from patients with acute-type ATLL by RT-PCR and western blotting, respectively. Because the sCADM1 transcript contains the 118-bp sequence of intron 7 (Figure 1A),27 we used PCR primers located in exon 6 and intron 7 of CADM1 to amplify the sCADM1 transcript. Semiquantitative RT-PCR showed that sCADM1 mRNA, along with the membrane-bound form of CADM1 (mbCADM1) mRNA, was highly expressed in all of the HTLV-1-positive ATLL-related cell lines (Online Supplementary Figure S1A). We then performed quantitative RT-PCR analysis with purified CD4+CADM1+ leukemic cell populations from various types of ATLL patients.27 A significantly higher abundance of sCADM1 mRNA was observed in all ATLL samples tested, including smoldering, chronic, and acute-type ATLL, compared with CD4+ T cells from healthy volunteers (Figure 1B). Similarly, the levels of mbCADM1 or total CADM1 (tCADM1) were significantly higher in all subtypes of ATLL than controls (Figure 1B). In order to quantify CADM1 shedding in ATLL, we analyzed the levels of two membrane-associated C-terminal fragments (αCTF, 18 kDa and CTF, 35 kDa), which are generated by the proteolytic cleavage of CADM1 by ADAM and unidentified proteases, respectively.29,31 Although the CTF fragment of CADM1 was detected in ATLL-related cell lines, very weak or no signal was found in leukemia cell samples from ATLL patients (Online Supplementary Figure S2A-B). Additionally, CADM1 undergoes alternative splicing between exons 7 and 11 (Figure 1A) and the inclusion of exon 9 confers shedding susceptibility to CADM1.31 RT-PCR analysis revealed that the majority of the mbCADM1 mRNA skipped both exons 9 and 10 (Online Supplementary Figure S1A-B). Therefore, these results suggest that upregulated expression of sCADM1 mRNA, which is generated by retention of intron 7, might be the main cause of the increased level of sCADM1 protein in ATLL.

Plasma sCADM1 levels increase with disease progression in ATLL

In order to investigate the clinical utility of sCADM1 in the diagnosis of ATLL patients, we developed a highly sensitive method for the measurement of plasma sCADM1 with the use of the AlphaLISA technology, which is based on energy transfer from a streptavidin donor bead to an AlphaLISA acceptor bead in close proximity.27,28 The donor and the acceptor beads were conjugated with biotinylated anti-CADM1 (3E1) and anti-CADM1 (103-189) antibodies, respectively (see Methods). Using recombinant human CADM1 protein, the assay system was capable of detecting sCADM1 in the plasma at concentrations as low as ~0.2 ng/mL (Online Supplementary Figure S3). First, we determined the sCADM1 levels in the peripheral blood of healthy volunteers, HTLV-1 carriers, and patients with HAM/TSP and various types of ATLL who had not been previously treated. The median values for plasma and serum sCADM1 from healthy volunteers were 181.3 ng/mL and 173.5 ng/mL with 5th-95th percentile ranges of 142.7-234.0 ng/mL and 151.7-215.5 ng/mL, respectively (Online Supplementary Figure S4A). There were neither a significant differences between the sCADM1 levels of males and females, nor amongst the age groups (Online Supplementary Figure S4B-C). In addition, the majority of HTLV-1 carriers and HAM/TSP patients had sCADM1 levels within the standard reference range (Figure 2). The median plasma sCADM1 concentration was slightly higher in smoldering-type ATLL patients (210.2 ng/mL) than in healthy volunteers (181.3 ng/mL), and it was elevated in chronic-type ATLL patients, with a median peak value of 267.1 ng/mL (Figure 2). The median plasma sCADM1 level was 1055.0 ng/mL (range: 173.6-6931.0 ng/mL) in acute-type ATLL patients, more than 5-fold higher than in the control group (Figure 2 and Online Supplementary Figure S5A). In addition, 2 of 10 patients with the unfavorable chronic-type ATLL had high levels of plasma sCADM1 (Online Supplementary Figure S6).

Next, we determined the association between plasma sCADM1 concentrations and other clinical and/or biochemical parameters in ATLL patients (Online Supplementary Figure S5A-E). Among these subjects, serum concentrations of sIL2R were higher in patients with all subtypes of ATLL compared to healthy volunteers, and concentrations increased with disease progression to acute-type or lymphoma-type ATLL (Online Supplementary Figure S5B). Importantly, while the patients with HAM/TSP showed significantly higher sIL2R levels compared with healthy volunteers, as reported previously,4 plasma sCADM1 levels were in the healthy range (Online Supplementary Figure S5A-B), confirming that sCADM1 is a specific marker for ATLL. Most patients with acute-type or lymphoma-type ATLL had elevated serum lactate dehydrogenase (LDH) levels (Online Supplementary Figure S5C), HTLV-1 proviral load (PVL) in the peripheral blood increased in smoldering-type ATLL patients and was elevated in chronic-type and acute-type ATLL, and was similar to white blood cell (WBC) concentrations (Online Supplementary Figure S5D-E). Serum sIL2R levels strongly correlated with plasma sCADM1 levels (r=0.66, P<0.001) (Figure 3A), and moderate correlations were found between plasma sCADM1 and LDH levels (r=0.52, P<0.001) (Figure 3B) as well as plasma sCADM1 and PVL levels in the peripheral blood (r=0.47, P<0.001) (Figure 3C). The correlation between plasma sCADM1 and PVL levels was higher than that between plasma sCADM1 and the levels of oligoclonality of HTLV-1-infected clones (Online Supplementary Figure S7A-B). There was a weak correlation between WBC counts and plasma sCADM1 levels (r=0.57, P<0.001) (Figure 3D). Additionally, the increase in plasma concentrations of sCADM1 was much higher than the

Results

The soluble form of CADM1 mRNA is efficiently expressed in ATLL

All measurements were done in duplicate. 

Chemiluminescence emission was read on an EnSpire Alpha microplate reader (PerkinElmer Waltham, MA). The concentrations of sCADM1 were obtained from a standard curve generated by recombinant CADM1 protein (Sino Biological, Beijing, China) using four-parameter logistic curve fitting, and multiplied by the dilution factor. All measurements were done in duplicate. Additional methods are provided in the Online Supplementary Appendix.

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High expression of sCADM1 transcript in adult T-cell leukemia/lymphoma. (A) Schematic representation of the structure and domain organization of membrane-bound cell adhesion molecule 1 CADM1 (mbCADM1) and its soluble form (sCADM1). mbCADM1 contains an extracellular domain (exons 2-10) containing three immunoglobulin-like C2-type domains, a transmembrane domain (exon 11), and a cytoplasmic domain (exon 12). sCADM1 is generated by alternative splicing in which intron 7 is retained and an in-frame stop codon is found immediately downstream of the immunoglobulin-like domain. Arrows indicate the position and direction of primers used in RT-PCR reactions. The mbCADM1, sCADM1, and total CADM1 (tCADM1) transcripts were amplified using primers for exon 7 and exon 8, exon 6 and intron 7, and exon 4 and exon 5, respectively. Numbers indicate exon numbers of the CADM1 gene. (B) Quantitative RT-PCR analysis of tCADM1, mbCADM1, and sCADM1 expression in CD4+ T lymphocytes from healthy volunteers (n=5) and sorted CADM1+ cells from peripheral blood of patients with smoldering-type (n=6), chronic-type (n=6), and acute-type (n=7) ATLL. The data were normalized to β-actin gene expression and expressed relative to a healthy control sample. Mean ± standard deviation is shown, **P<0.01 (Mann-Whitney U test).
increases in the percentages of CD4+ CADM1+ T cells, WBC concentrations, or serum sIL2R levels in acute-type ATLL patients compared to chronic-type ATLL patients (Online Supplementary Figure S8A-D). Notably, a strong positive correlation was found between the absolute number of circulating CD4+ CADM1+ T cells and sCADM1 concentration in the peripheral blood of chronic-type and acute-type ATLL patients (Online Supplementary Figure S9A-B). Given that sCADM1 expression did not significantly differ between chronic-type and acute-type ATLL (Figure 1B), the drastic increase in sCADM1 levels in acute-type ATLL is possibly ascribed to an increase in numbers of circulating tumor cells. Additionally, univariate and multivariate logistic regression analyses were performed to assess factors associated with aggressive ATLL compared with indolent ATLL. In the univariate analysis, the risk factors associated with aggressive type ATLL were sCADM1, sIL2R, LDH, and WBC (Table 1). On the other hand, in multivariate analysis, sCADM1 was the only risk factor associated with aggressive ATLL (Table 1). Furthermore, the Kaplan-Meier analysis in aggressive type ATLL patients showed a trend towards worse overall survival for patients with high sCADM1 (Online Supplementary Figure S10A-D, Online Supplementary Table S2). Taken together, these results suggest that sCADM1 has potential as a novel diagnostic biomarker in ATLL, and that it may be useful for monitoring disease progression.

The plasma sCADM1 level is a useful clinical indicator of the leukemic cell burden in ATLL patients

In order to investigate the clinical value of plasma sCADM1 concentration, we evaluated the levels of plasma sCADM1 along with sIL2R, LDH, PVL, and WBC in the peripheral blood of patients with various types of ATLL.

Table 1. Univariate and multivariate analysis of factors predicting aggressiveness of adult T-cell leukemia/lymphoma.

| Variable  | OR      | 95% CI      | P         | OR     | 95% CI      | P         |
|-----------|---------|-------------|-----------|--------|-------------|-----------|
| sCADM1    | 5.46    | 3.13-9.54   | <0.0001   | 4.33   | 2.06-9.10   | <0.0001   |
| sIL2Rα    | 1.85    | 1.49-2.28   | <0.0001   | 1.22   | 0.885-1.67  | 0.205     |
| LDH       | 6.93    | 3.48-13.8   | <0.0001   | 2.22   | 0.944-5.21  | 0.068     |
| WBC       | 1.72    | 1.22-2.44   | 0.002     | 0.617  | 0.349-1.09  | 0.096     |
| PVL*      | 1.14    | 0.94-1.38   | 0.158     | 0.555  | 0.387-0.91  | 0.065     |
| Age       | 1.01    | 0.98-1.04   | 0.385     | 1      | 0.957-1.05  | 0.963     |

For logistic regression analysis, numerical values of soluble form of cell adhesion molecule 1 (sCADM1), serum interleukin-2 receptor α (sIL2Rα), lactate dehydrogenase (LDH), white blood cell (WBC), and proviral load (PVL) were converted to base 2 logarithm transformation. The data from 49 aggressive type (40 acute-type and nine lymphoma-type) and 83 indolent type adult T-cell leukemia/lymphoma (ATLL) cases (18 chronic-type and 65 smoldering-type) (Online Supplementary Figure S5) were used in the analysis. *Forty-two cases with missing values on PVL (eight acute-type, eight chronic-type, and 17 smoldering-type) and nine lymphoma type ATLL were excluded.

Figure 2. Plasma sCADM1 levels in adult T-cell leukemia/lymphoma patients examined by AlphaLISA. The plasma soluble form of cell adhesion molecule 1 (sCADM1) levels in 34 healthy volunteers, 78 human T-cell leukemia virus type 1 (HTLV-1) carriers, 12 HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients, 77 smoldering-type, 23 chronic-type, 13 lymphoma-type, and 43 acute-type ATLL patients who were previously untreated were measured by AlphaLISA using anti-CADM1 antibodies. The box and whisker plots show the 5th, 25th, 50th (median), 75th, and 95th percentile values, with outliers marked by solid dots. *P<0.05, **P<0.01, ***P<0.001 versus healthy volunteers or HTLV-1 carriers (Kruskal-Wallis test/Dunn’s multiple comparison test). Median and 5th and 95th percentile values are indicated at the top of each column. The dot line indicates the 95th percentile of plasma sCADM1 in healthy subjects.
before and after treatment with chemotherapy (Figure 4). Although the pre- and post-treatment samples were not derived from the same patients, median plasma sCADM1 levels were significantly decreased in the peripheral blood of patients with acute and lymphoma-type ATLL after chemotherapy compared to before treatment (Figure 4A). Additionally, the serum sIL2R levels in acute and lymphoma-type ATLL patients after chemotherapy were sig-

Table 2. Receiver operating characteristic (ROC) curve analysis of plasma sCADM1 concentrations.

| Marker                        | Cut-off Point | Sensitivity | Specificity | AUC   | P      |
|-------------------------------|---------------|-------------|-------------|-------|--------|
| ATLL (acute/lymphoma type)    | sCADM1 (ng/mL)| 232.4       | 0.873       | 0.971 | 0.937  | P<0.0001|
| versus healthy WBC (×100/μL) | 92.5          | 0.630       | 0.969       | 0.786 | P=0.0002|
| volunteers                    | sIL2Rα (U/mL) | 494.5       | 1.0         | 1.0   | 1.0    | P<0.0001|
| ATLL (chronic type)           | sCADM1 (ng/mL)| 233.4       | 0.689       | 0.971 | 0.739  | P=0.0024|
| versus healthy WBC (×100/μL) | 76.5          | 1.0         | 0.875       | 0.981 | P<0.0001|
| volunteers                    | sIL2Rα (U/mL) | 470.0       | 1.0         | 1.0   | 1.0    | P<0.0001|
| ATLL (smoldering type)        | sCADM1 (ng/mL)| 214.6       | 0.481       | 0.912 | 0.657  | P=0.0082|
| versus healthy WBC (×100/μL) | 62.5          | 0.627       | 0.719       | 0.692 | P=0.0020|
| volunteers                    | sIL2Rα (U/mL) | 436.5       | 0.955       | 1.0   | 0.986  | P<0.0001|

The Youden index was used to determine cut-off values. ATLL: adult T-cell leukemia/lymphoma; AUC: area under the ROC curve; SCADM1: soluble form of cell adhesion molecule 1; WBC: white blood cell; sIL2Rα: serum interleukin-2 receptor α.

Figure 3. Association between plasma sCADM1 levels and various prognostic indicators in adult T-cell leukemia/lymphoma. (A) Scatter plot of plasma soluable form of cell adhesion molecule 1 (sCADM1) concentrations versus serum interleukin-2 receptor α (sIL2Rx) concentrations in 34 healthy volunteers, 78 human T-cell leukemia virus type 1 (HTLV-1) carriers, 77 smoldering-type, 55 chronic-type, 34 lymphoma-type, and 69 acute-type adult T-cell leukemia/lymphoma (ATLL) patients, including those who were previously untreated and treated with chemotherapy. Spearman correlation coefficient values (r) and P-values are shown on each of the graphs. (B) Scatter plot of plasma sCADM1 concentrations versus serum lactate dehydrogenase (LDH) concentrations in 45 HTLV-1 carriers, 69 smoldering-type, 38 chronic-type, 18 lymphoma-type, and 60 acute-type ATLL patients, including those who were previously untreated and treated with chemotherapy. Spearman correlation coefficient values (r) and P-values are shown on each of the graphs. (C) Scatter plot of plasma sCADM1 concentrations versus proviral load (PVL) in 66 HTLV-1 carriers, 56 smoldering-type, 56 chronic-type, and 61 acute-type ATLL patients, including those who were previously untreated and treated with chemotherapy. Spearman correlation coefficient values (r) and P-values are shown on each of the graphs. (D) Scatter plot of plasma sCADM1 concentrations versus white blood cell (WBC) counts in 32 healthy volunteers, 71 HTLV-1 carriers, 74 smoldering-type, 57 chronic-type, 33 lymphoma-type, and 69 acute-type ATLL patients, including those who were previously untreated and treated with chemotherapy. Spearman correlation coefficient values (r) and P-values are shown on each of the graphs.
Figure 4. Plasma sCADM1 levels are related to treatment efficacy in adult T-cell leukemia/lymphoma patients. (A) Soluble form of cell adhesion molecule 1 (sCADM1) concentrations in the plasma of 23 chronic-type, 13 lymphoma-type, and 43 acute-type adult T-cell leukemia/lymphoma (ATLL) patients who were previously untreated and of 39 chronic-type, 18 lymphoma-type, and 7 acute-type ATLL patients who were previously treated with chemotherapy were measured by AlphaLISA. *P<0.05, ***P<0.001 (Mann-Whitney U test). The dot line indicates the 95th percentile of plasma sCADM1 in healthy subjects. Note that the pre-treatment and post-treatment samples were derived from different individuals. (B) Serum interleukin-2 receptor α (sIL2Rα) concentrations in the serum of 19 chronic-type, 12 lymphoma-type, and 43 acute-type ATLL patients who were previously untreated and of 32 chronic-type, 16 lymphoma-type, and six acute-type ATLL patients who were previously treated with chemotherapy. *P<0.05, ***P<0.001 (Mann-Whitney U test). The dot line indicates the upper limit of normal serum sIL2Rα. The same samples as Figure 4A were used. Fifteen cases with missing values on sIL2Rα (one lymphoma-type and four chronic-type in the pre-treatment group and one acute-type, two lymphoma-type, and seven chronic-type in the post-treatment group) were excluded. (C) Lactate dehydrogenase (LDH) concentrations in the serum of 18 chronic-type, nine lymphoma-type, and 40 acute-type ATLL patients who were previously untreated and of 15 chronic-type, seven lymphoma-type, and five acute-type ATLL patients who were previously treated with chemotherapy. *P<0.05 (Mann-Whitney U test). The dot line indicates the upper limit of normal serum LDH. The same samples as Figure 4A were used. Forty-nine cases with missing values on LDH (three acute-type, four lymphoma-type, and five chronic-type in the pre-treatment group and two acute-type, 11 lymphoma-type, and 24 chronic-type in the post-treatment group) were excluded. (D) Human T-cell leukemia virus type 1 (HTLV-1) proviral load (PVL) in 15 chronic-type, 11 lymphoma-type, and 35 acute-type ATLL patients who were previously untreated and in 39 chronic-type, 18 lymphoma-type, and 7 acute-type ATLL patients who were previously treated with chemotherapy. **P<0.01 (Mann-Whitney U test). The same samples as Figure 4A were used. Eighteen cases with missing values on PVL (eight acute-type, two lymphoma-type, and eight chronic-type in the pre-treatment group) were excluded. (E) White blood cell counts (WBC) counts in 19 chronic-type, 11 lymphoma-type, and 43 acute-type ATLL patients who were previously untreated and in 33 chronic-type, 16 lymphoma-type, and 6 acute-type ATLL patients who were previously treated with chemotherapy. ***P<0.001 (Mann-Whitney U test). The dot line indicates the upper limit of normal for WBC counts. The same samples as Figure 4A were used. Thirteen cases with missing values on WBC counts (two lymphoma-type and four chronic-type in the pre-treatment group and one acute-type, two lymphoma-type, and four chronic-type in the post-treatment group) were excluded.
significantly lower than before treatment (Figure 4B). A significant reduction in serum LDH, PVL, and WBC after chemotherapy was also observed in acute-type ATLL patients (Figure 4C-E). Plasma sCADM1 levels were examined in three patients with acute-type ATLL who received allo-HSCT (Online Supplementary Table S3). Although all three patients exhibited elevated levels of serum sIL2Ra, 2 of the 3 patients showed normal (case 3) or slightly high levels (case 1) of plasma sCADM1. Notably, in one patient (case 2) with elevated levels of both sIL2Rα and sCADM1,

Figure 5. Plasma sCADM1 is useful for monitoring disease progression in adult T-cell leukemia/lymphoma patients. Plasma soluble form of cell adhesion molecule 1 (sCADM1) concentrations along with various prognostic parameters including serum interleukin-2 receptor α (sIL2Rα), lactate dehydrogenase (LDH), and white blood count (WBC) count were monitored in six patients with adult T-cell leukemia/lymphoma (ATLL) before and after acute crisis (day 0). The drug regimen is indicated at the top of the figure: VP-16, etoposide; LSG15, VCAP-AMP-VECP (vincristine, cyclophosphamide, doxorubicin, and prednisone [VCAP], doxorubicin, ranimustine, and prednisone [AMP], and vindesine, etoposide, carboplatin, and prednisone [VECP]); PSL: prednisolone; KW-0761: mogamulizumab; THP-COP: pirarubicin, cyclophosphamide, vincristine, and prednisolone.
Discussion

In this study, we used AlphaLISA technology to measure sCADM1 concentrations in the peripheral blood. Although ELISA is a well-known technique to measure the plasma concentration of target proteins, we found that detection sensitivity of plasma sCADM1 in our AlphaLISA system was enhanced ~10-fold compared to a conventional ELISA method (data not shown). By using this system, we successfully determined the range of plasma sCADM1 concentrations in healthy subjects and cut-off points for plasma sCADM1 between healthy and ATLL patients.

The disease progression in ATLL patients may involve the accumulation of genetic alterations and clonal expansion of ATLL cells. Although recent studies have identified somatic mutations that act as drivers during ATLL progression, the application of this genetic information towards diagnosing patients is not easy, because the genomic abnormalities of ATLL cells are very complicated. In addition, clonality assessment is currently high-cost and time-consuming. Therefore, the development of effective methods for predicting disease progression is urgently needed.

sCADM1 is transcriptionally upregulated in HTLV-1-infected T cells and ATLL cells in almost all cases, and in this study we found that the same CADM1 promoter appears to activate the expression of both sCADM1 and the membrane-bound form of CADM1 transcripts in ATLL. On the other hand, plasma sCADM1 levels were drastically increased in acute stage ATLL compared to chronic stage ATLL (median 1,066.7 ng/mL vs. 204.0 ng/mL, respectively), whereas the median percentages of CD4+CADM1+ T cells were 78.8% and 43.1% in acute and chronic-type ATLL, respectively (Online Supplementary Figure S8). Moreover, we observed increases in plasma sCADM1 levels during follow-ups with patients, which were accompanied by poor patient outcomes, whereas WBC counts were not changed (Figure 5). As multivariate analysis of several blood biomarkers for ATLL identified sCADM1 as the only independent serum marker for aggressive ATLL with significant differences (Table 1), plasma sCADM1 may be a potential risk factor of the aggressiveness of ATLL cells. In addition, while sIL2Rα is secreted not only by leukemia cells but also during various inflammatory responses, plasma sCADM1 was not increased in HAM/TSP patients or in a patient with GvHD after allo-HSCT for ATLL. Therefore, combined sCADM1 and sIL2Rα measurements may become a promising method for more accurately diagnosing ATLL development in HAM/TSP patients, or discriminating ATLL relapse and GvHD following allo-HSCT for ATLL. In the current clinical setting, assessment of treatment efficacy in ATLL is based on serum biochemical parameters such as sIL2Rα, LDH, and calcium levels, and clinical findings including computed tomography and positron emission tomography examination results, however, these tests are unable to determine the depth of response to treatment, which can be evaluated by detection of MRD. Given that sCADM1 seems to be a specific biomarker for ATLL, the measurement of plasma sCADM1 may be a useful in measuring the depth of response to therapy, and a change in the sCADM1 plasma level may become an important clinical criteria for not only determining the transplant adaptability, but also determining the choice of consolidation or maintenance treatments and their periods.

It has been reported that sCADM1 can bind to the extracellular domain of CADM1 through an interaction between their Ig domains. sCADM1 contains a PSD95/Dig/ZO-1 (PDZ) domain that interacts with T lymphoma invasion...
and metastasis 1 (TIAM1), and induces signaling to actin filaments, leading to cell structure remodeling.27 sCADM1 may activate this TIAM1-actin signaling to modulate cellular functions. It has also been reported that sCADM1 expression on tumor cells enhances the cytotoxic activities of cytotoxic T cells or natural killer cells via an interaction with Class I MHC-restricted T-cell-associated molecule (CRTAM).40,41 The CRTAM-CADM1 interaction acts as a costimulator of T-cell receptor (TCR) signaling that may help to eliminate tumor cells. Because sCADM1 has been reported to bind to the extracellular domain of CADM1 on the cell surface and inhibit CADM1 homophilic interactions,27 it is possible that sCADM1 may modulate immune responses under certain conditions. Future studies of the biological role of sCADM1 in anti-tumor immune responses will be important to obtain a better understanding of the molecular mechanisms of the immunological abnormalities in ATLL.44

To date, there has been no report identifying sCADM1 in cancers; however, it has been reported that CADM1 shedding was elevated in the lung epithelial cells of patients with idiopathic interstitial pneumonias,45 pulmonary emphysema,46 as well as in the pancreatic islets of patients with type 2 diabetes mellitus,47 which was accompanied by a decrease in full-length CADM1 levels and may be associated with the disruption of cell polarity and cell apoptosis.45,46 In ATLL, plasma sCADM1 levels drastically increased from chronic to acute-type ATLL with no apparent reduction in the expression of full-length CADM1, suggesting that sCADM1 may play a role in the malignant progression of ATLL.

In summary, sCADM1 is a promising biomarker not only for monitoring the leukemic burden of ATLL patients, but also for predicting disease status. Thus, sCADM1 measurement may be valuable for the diagnosis of ATLL.

Disclosures
No conflicts of interests to disclose.

Contributions
SN performed research, analyzed data and wrote the manuscript; CS, AN, KSa, MY, IN, and MS performed research and analyzed data; TK, KSh, YK, TH, AK, ST, NN, MI, YS, KMo, MA, KMa, ES, AO, KSh, and TW provided critical information and samples. YU, TM, and AI provided critical reagents; AU provided clinical information and samples, and critical evaluation of the manuscript; and KM conceived and designed the study, directed and supervised the research, and wrote the manuscript.

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