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# Flow cytometric assessment of T-cell receptor β-chain constant region 1/2 skewing as a surrogate marker for clonality in human T-cell leukaemia virus type-1–infected cells

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## Summary

Estimating T-cell clonality within human T-cell leukaemia virus type 1 (HTLV-1)–infected cell populations may aid risk assessment and diagnosis of adult T-cell leukaemia/lymphoma (ATL). However, direct assessment of HTLV-1 integration-site clonality requires genetic analyses that are costly and labour-intensive. We developed a simple flow cytometric surrogate assay by combining the HTLV-1 analysis system by flow cytometry (HAS-Flow) method, which efficiently identifies HTLV-1–infected cells, with T-cell receptor β-chain constant region (TRBC1/2) staining. We analysed 53 HTLV-1–infected individuals, including asymptomatic carriers ($n=25$), patients with HTLV-1–associated myelopathy ($n=4$) and patients with ATL ($n=24$). TRBC1/2 skewing was quantified using the dominant TRBC ratio. The dominant TRBC ratio was significantly higher in ATL than in non-ATL cases and effectively discriminated ATL from non-ATL cases (Area under the receiver operating characteristic curve [AUC], 0.913; 95% confidence interval, 0.803–1.000; $p<0.0001$). It correlated with established markers of disease progression, including abnormal lymphocyte burden, proviral load and soluble interleukin-2 receptor levels, as well as clonality assessed by two independent HTLV-1 integration-site–based methods (viral integration site and RAISING-CLOVA). These findings indicate that assessment of TRBC1/2 skewing in HTLV-1–infected cells (HAS-TRBC method) is a simple, cost-effective surrogate for estimating T-cell clonality within HTLV-1–infected cell populations, with potential clinical utility.

**KEYWORDS**
adult T-cell leukaemia/lymphoma, clonality, flow cytometry, human T-cell leukaemia virus type 1, T-cell receptor β-chain constant region

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# INTRODUCTION

Most individuals infected with human T-cell leukaemia virus type 1 (HTLV-1) remain asymptomatic throughout life and are considered asymptomatic carriers.[1–4] Approximately 5% of carriers eventually develop adult T-cell leukaemia/lymphoma (ATL) after a long latent period.[1–4] Individuals with a family history of ATL, a high proviral load, an increased number of HTLV-1–infected cells, clonal proliferation of infected cells and the accumulation of genetic mutations are at high risk of ATL.[5–15]

Among these, clonality assessment is the most direct indicator of neoplastic transformation.[8–12] However, currently available methods have several limitations. Classical Southern blotting and inverse polymerase chain reactions (PCR) make quantitative evaluations difficult.[16,17] Methods based on HTLV-1 integration-site analysis are labour-intensive and expensive.[8–11] Therefore, simpler methods are required to estimate clonality.

In other T-cell malignancies, T-cell receptor (TCR)-based clonality assessment is routinely used.[18–20] Recently, a flow cytometric method for the simple estimation of clonality based on evaluation of the two mutually exclusive TCR β-chain constant regions (TRBC1 and TRBC2) expressed by all αβ T cells has been developed.[21,22] However, unlike other T-cell malignancies, the diagnosis of ATL requires the identification of clonality specifically within HTLV-1–infected cells. Therefore, a clonality assessment based solely on the TCR is insufficient. HTLV-1 analysis system by flow cytometry (HAS-Flow) accurately identifies HTLV-1–infected cells based on cell adhesion molecule 1 (CADM1) and cluster of differentiation (CD) 7 expression in $CD4^+$ T cells.[7,23–25] We hypothesised that combining HAS-Flow with TRBC1/2 dual staining would enable simple estimation of T-cell clonality within HTLV-1–infected cell populations. In this study, we aimed to evaluate the utility of this approach in HTLV-1–infected individuals.

# METHODS

Detailed methods for patient enrolment, flow cytometry, genetic analyses and statistical analyses are provided in the Supplementary Methods.

## Patients

This study enrolled patients who were treated at four institutions: three in Japan and one in the United Kingdom. Samples were collected between May 2021 and January 2026. All participants provided written informed consent, in accordance with the Declaration of Helsinki.

## Flow cytometric analysis

HTLV-1–infected and uninfected cells were identified based on CD3, CD4, CD8, CADM1 and CD7 expression, according to previously reported methods,[7,23–25] and the TRBC distribution was evaluated using TRBC1/2 staining.[21,22] The antibodies used in this study are summarized in Table S1. TRBC1/2 skewing was quantified as the dominant TRBC ratio, defined as follows:

Dominant TRBC ratio = Higher TRBC fraction (%)/Lower TRBC fraction (%).

## Proviral load

Proviral load was measured by real-time PCR as previously described.[6,26–28]

## VIS and genomic mutation analysis

Unique viral integration sites (VISs) and genomic mutation analyses were performed using capture-based targeted sequencing of sorted $CD4^+CADM1^+$ cells, as previously described.[13]

## RAISING-CLOVA analysis

Clonality was also assessed using the Rapid Amplification of Integration Sites without Interference by Genomic Deoxyribonucleic Acid (DNA) contamination (RAISING)-Clonality Value (CLOVA) method, as previously described.[10,11]

# RESULTS

## TRBC1/2 skewing as an indicator of disease progression in HTLV-1–infected individuals

We analysed 53 individuals from three institutions in Japan and one in the United Kingdom. The cohort included 25 HTLV-1 asymptomatic carriers, four patients with HTLV-1–associated myelopathy (HAM) and 24 patients with ATL. Among the ATL cases, nine were smouldering, eight were chronic and seven were acute. The participant characteristics are summarized in Table S2.

$CD4^+$ T cells were subdivided into P, D, N and $CADM1^+$ fractions according to CADM1 and CD7 expression (Figure S1). The P fraction mainly represents HTLV-1–uninfected cells, whereas the $CADM1^+$ fraction is enriched for HTLV-1–infected cells.[7,23–25] Each fraction was further analysed for TRBC1 and TRBC2 expression and classified into three subpopulations: TRBC1-positive, TRBC2-positive and TRBC1/2 double-negative (Figure 1A). The dominant TRBC ratio was defined as the ratio of the higher to the lower proportion of $TRBC1^+$ and $TRBC2^+$ cells (Figure 1A). Cases in which the double-negative population exceeded both single-positive populations were classified as TRBC1/2 double-negative and excluded from TRBC ratio analyses.

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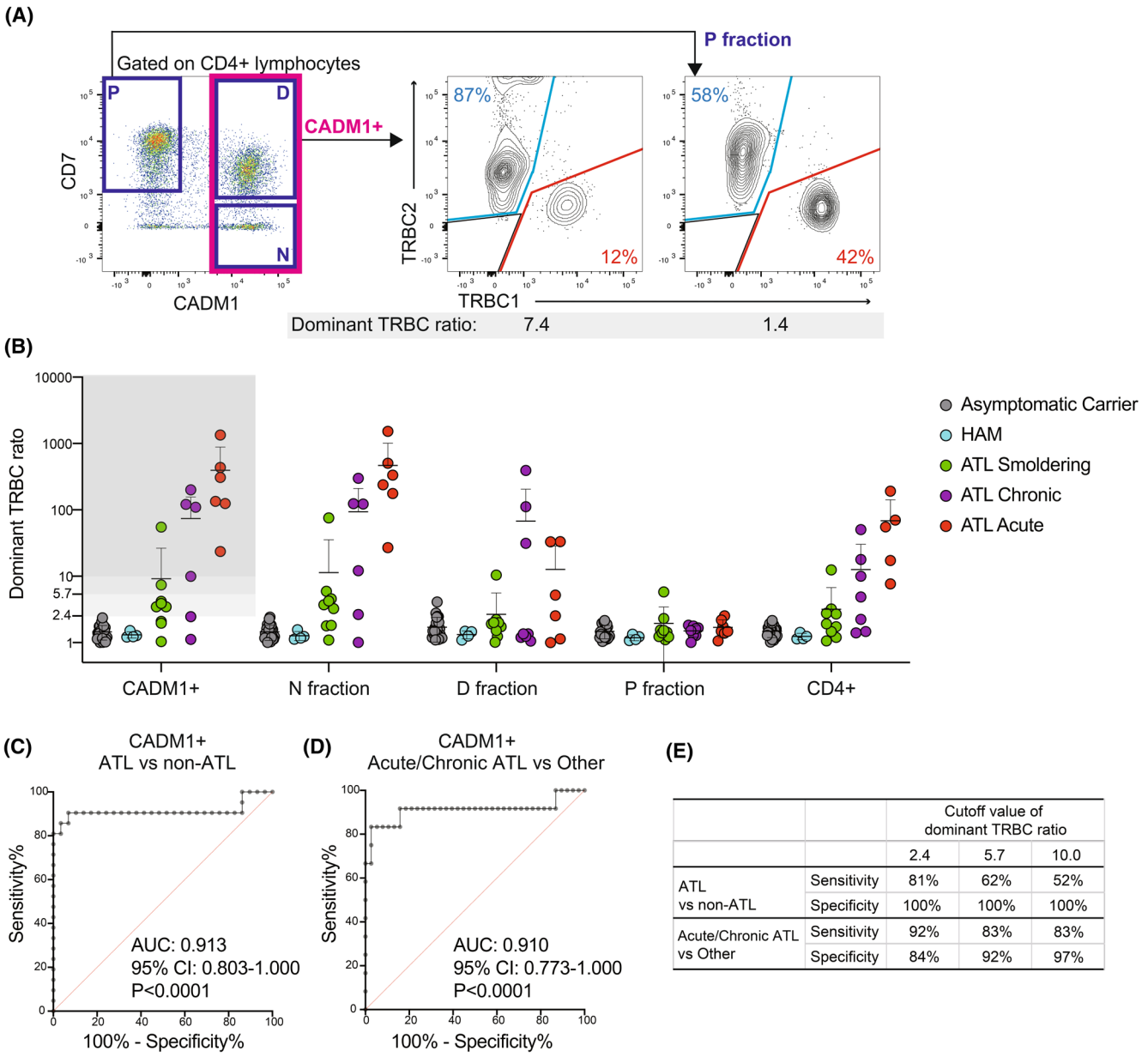


| | | Cutoff value of dominant TRBC ratio | | |
|---|---|---|---|---|
| | | 2.4 | 5.7 | 10.0 |
| ATL vs non-ATL | Sensitivity | 81% | 62% | 52% |
| | Specificity | 100% | 100% | 100% |
| Acute/Chronic ATL vs Other | Sensitivity | 92% | 83% | 83% |
| | Specificity | 84% | 92% | 97% |

**FIGURE 1** TRBC1/2 skewing according to disease stage and subtype in HTLV-1–infected individuals (A) Representative flow cytometry plots demonstrating TRBC1/2 skewing in this study. (B) The dominant TRBC ratio in each fraction ($CD4^+CADM1^+$ fraction, $CD4^+$ N fraction, $CD4^+$ D fraction, $CD4^+$ P fraction and total $CD4^+$ fraction) according to disease status. Data are presented as mean ± standard deviation. (C, D) Receiver operating characteristic (ROC) curves for discriminating ATL from non-ATL cases (C) and acute/chronic ATL from other cases (D) based on the dominant TRBC ratio in the $CD4^+CADM1^+$ fraction. Non-ATL cases included asymptomatic carriers and patients with HTLV-1–associated myelopathy (HAM), whereas other cases included asymptomatic carriers, patients with HAM, and patients with smouldering ATL. (E) Sensitivity and specificity of three cut-off values (2.4, 5.7 and 10.0) for the dominant TRBC ratio in the $CD4^+CADM1^+$ fraction for discriminating ATL from non-ATL cases and acute/chronic ATL from other cases.

Compared with HTLV-1 asymptomatic carriers, ATL cases showed increased TRBC1/2 skewing, especially in the $CADM1^+$ and N fractions (Figure 1B). In contrast, no significant skewing was observed in the P fraction regardless of the disease subtype. Receiver operating characteristic (ROC) analysis demonstrated that the dominant TRBC ratio in the $CADM1^+$ fraction provided the best discriminatory performance among the fractions evaluated, both for ATL versus non-ATL cases (area under the ROC curve [AUC], 0.913; 95% confidence interval [CI], 0.803–1.000; $p < 0.0001$) and for acute/chronic ATL versus other cases (AUC, 0.910; 95% CI, 0.773–1.000; $p < 0.0001$) (Figure 1C,D; Figure S2). Based on the ROC analyses, cut-off values of 2.4 and 10.0 were identified to maximize the sum of sensitivity and specificity for discriminating ATL from non-ATL cases and acute/chronic ATL from other cases respectively. We also evaluated a cut-off of 5.7, corresponding to the previously reported

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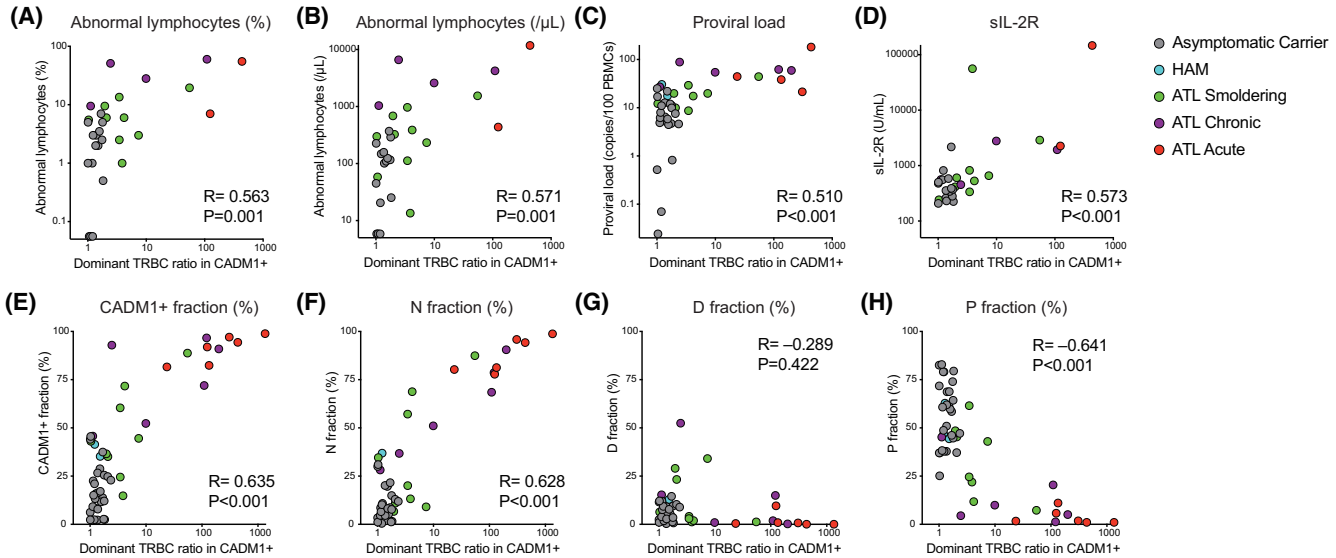


**FIGURE 2** Correlations between the dominant TRBC ratio in the $CD4^+CADM1^+$ fraction and several parameters. (A–D) Correlations between the dominant TRBC ratio in the $CD4^+CADM1^+$ fraction and the proportion of abnormal lymphocytes (A), absolute count of abnormal lymphocytes (B), proviral load (C) and soluble interleukin-2 receptor (sIL-2R) (D). (E–H) Correlations between the dominant TRBC ratio in the $CD4^+CADM1^+$ fraction and the proportions of the $CD4^+CADM1^+$ fraction (E), $CD4^+$ N fraction (F), $CD4^+$ D fraction (G) and $CD4^+$ P fraction (H) in $CD4^+$ T cells. Spearman's rank correlation coefficient was used for all analyses.

criterion of TRBC1 positivity of <15% or >85% for clonality assessment.[21,29] The sensitivity and specificity of these three cut-off values for discriminating ATL from non-ATL cases and acute/chronic ATL from other cases are shown in Figure 1E.

The dominant TRBC ratios in the $CADM1^+$ and N fractions were strongly correlated, whereas weaker correlations were observed for the D and P fractions (Figure S3). Together with the ROC analysis, these findings support the dominant TRBC ratio in the $CADM1^+$ fraction as a useful marker of disease progression in HTLV-1–infected individuals.

## Correlation between the TRBC ratio and markers of ATL progression

We examined the relationship between the dominant TRBC ratio in the $CADM1^+$ fraction and established indicators of ATL progression. The dominant TRBC ratio showed significant positive correlations with the proportion and absolute number of abnormal lymphocytes, proviral load and soluble interleukin-2 receptor (sIL-2R) levels (Figure 2A–D). Using a dominant TRBC ratio cut-off of 2.4 in the $CADM1^+$ fraction, all these parameters were significantly higher in the high group than in the low group (Figure S4). We then assessed the association between the proportion of each HAS-Flow fraction in $CD4^+$ T cells and the dominant TRBC ratio in the $CADM1^+$ fraction. The dominant TRBC ratio showed a significant positive correlation with the $CADM1^+$ and N fractions, no significant correlation with the D fraction, and a significant negative correlation with the P fraction (Figure 2E–H).

## Validation of the dominant TRBC ratio as an indicator of clonal expansion

To validate the dominant TRBC ratio as an indicator of clonal expansion, we compared it with clone-size estimates that were derived from the genomic analysis of HTLV-1 integration sites (VIS analysis).[13] Cases with a polyclonal VIS pattern showed dominant TRBC ratios close to 1.0, whereas the two cases with a monoclonal VIS pattern showed markedly higher ratios of 55.1 and 439.9 respectively (Figure 3A). The acute ATL case showed apparent oligoclonality despite marked TRBC skewing (Figure 3A). Furthermore, in cases with confirmed clonal expansion, somatic mutations that are frequently observed in ATL were detected at high variant allele frequencies (Table S3).

We next compared the dominant TRBC ratio with clonality values obtained by RAISING-CLOVA.[10,11] A significant positive correlation was observed between the clonality value and the dominant TRBC ratio (Figure 3B). Using the previously reported clonality cut-off of 0.5,[10] the dominant TRBC ratio was significantly higher in the high-clonality group than in the low-clonality group (Figure 3C).

These findings support the dominant TRBC ratio in the $CADM1^+$ fraction as a simple surrogate marker of ATL clonal expansion.

## TRBC1/2 double negativity in progressive ATL

Among the 53 cases, three (6%) exhibited TRBC1/2 double negativity within the $CADM1^+$ fraction. All were progressive ATL cases (two chronic and one acute ATL), whereas no double-negative cases were observed among asymptomatic

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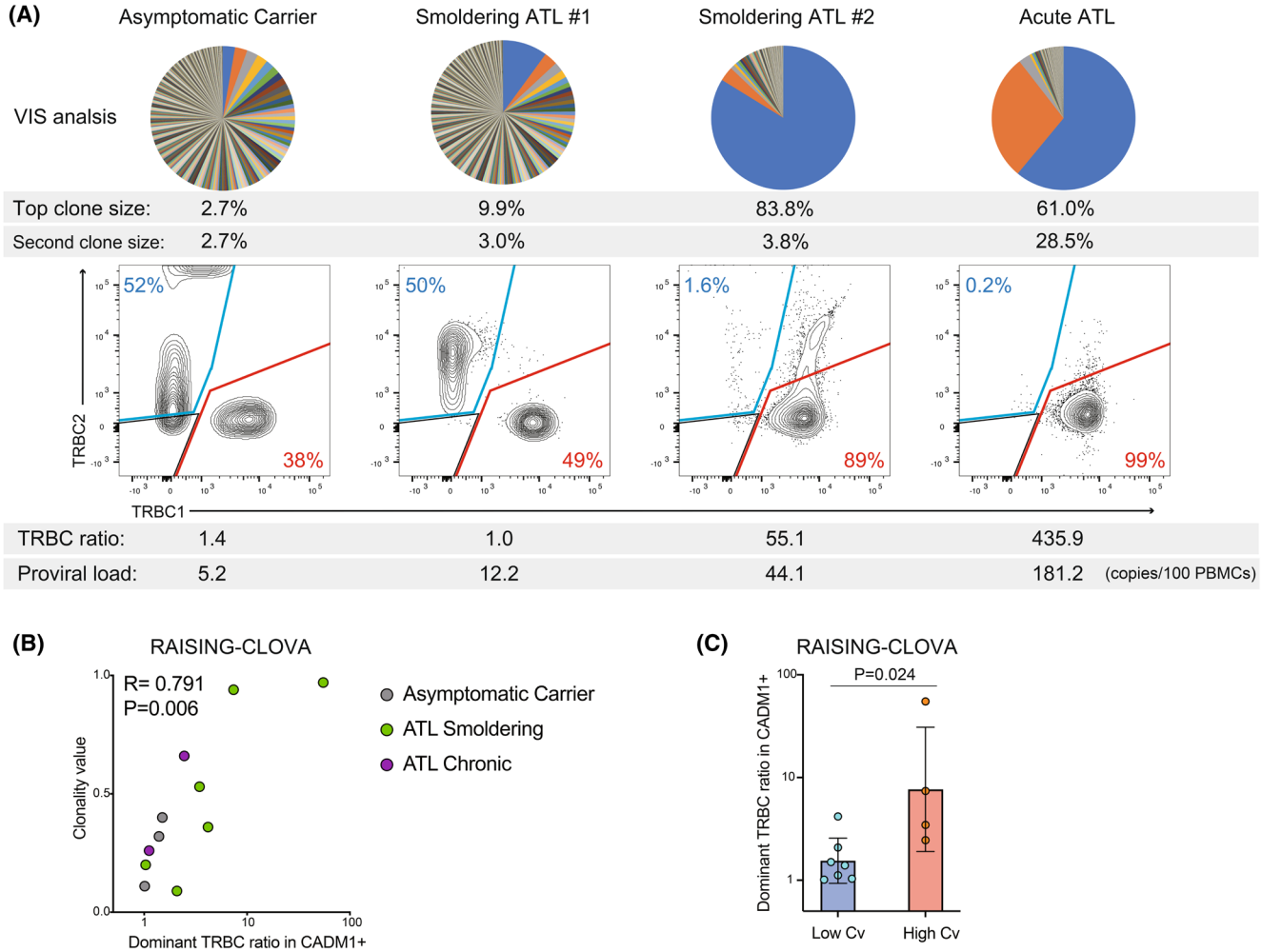


**FIGURE 3** Correlation between TRBC skewing and clone-size estimation based on the HTLV-1 integration site analysis. (A) Results of unique viral integration site (VIS) analysis (pie charts), dominant TRBC ratios (flow cytometry plots), and proviral load in four cases (one asymptomatic carrier, two smouldering ATL cases, and an acute ATL case). Larger top-clone sizes indicate higher clonality. (B) Correlation between the dominant TRBC ratio in the $CD4^+CADM1^+$ fraction and clonality values obtained using the RAISING-CLOVA method ($n = 11$). Spearman's rank correlation coefficient was used for the analysis. (C) Comparison of dominant TRBC ratios between two groups stratified by a clonality value (CV) cut-off of 0.5. Data are presented as mean ± standard deviation. *p*-values were calculated using the Mann–Whitney *U* test.

carriers, HAM or smouldering ATL (Figure 4A,B; Table S2). When the analysis was restricted to 15 cases of chronic and acute ATL, 20% were classified as TRBC1/2 double-negative. Notably, intracellular TRBC1/2 staining revealed marked TRBC restriction in two of the three surface double-negative cases, whereas the remaining case remained TRBC1/2 double-negative (Figure 4B). In the P fraction, which is enriched for HTLV-1–uninfected cells, surface TRBC expression was preserved in all cases (Figure S5).

As TRBC forms a complex with CD3 and reduced TRBC expression is associated with decreased CD3 expression,[30,31] we hypothesised that CD3 expression is reduced in TRBC1/2 double-negative cases. Consistent with this hypothesis, all TRBC1/2 double-negative cases showed reduced CD3 expression in the $CADM1^+$ fraction compared with the P fraction (Figure 4C). CD3 expression in the $CADM1^+$ fraction was significantly lower in TRBC1/2 double-negative cases than in other chronic and acute ATL cases (Figure 4D). Intracellular staining did not result in a marked increase in CD3 detection in the TRBC1/2 double-negative cases (Figure 4E).

## Further potential applications of TRBC assessment

We evaluated the dominant TRBC ratio after treatment in patients with acute ATL. Cases in which abnormal lymphocytes decreased to ≤1% after treatment showed significantly lower dominant TRBC ratios than those with persistent peripheral blood involvement (Figure 5A), suggesting that TRBC skewing decreases with treatment response. We also assessed TRBC skewing in tissue specimens. Marked TRBC1/2 skewing was observed in specimens with ATL infiltration, whereas minimal skewing was observed in the specimen without ATL infiltration (Figure 5B; Table S4),

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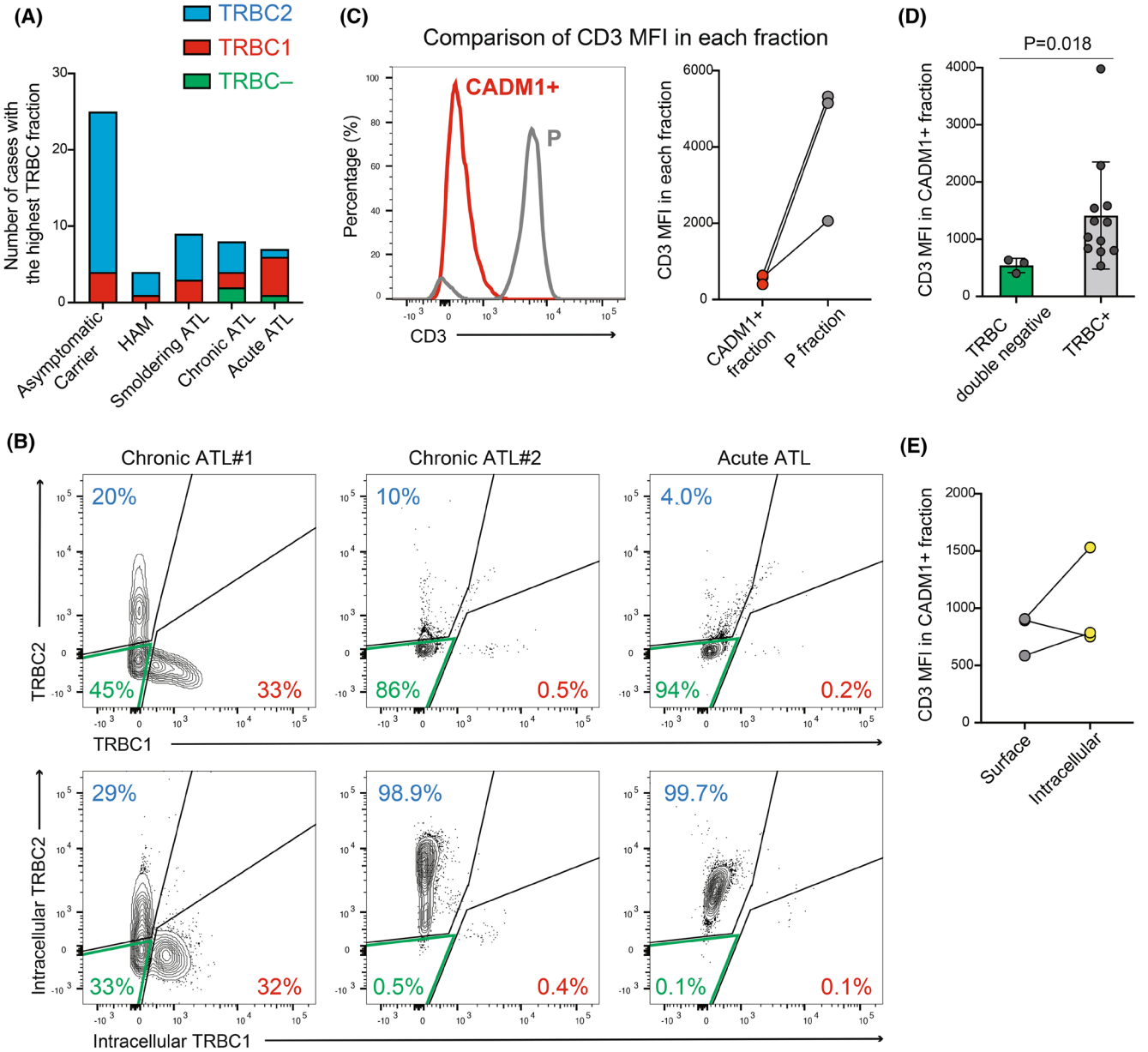


**FIGURE 4** TRBC1/2 double negativity and CD3 expression. (A) Bar chart showing the number of cases that were categorized according to the predominant subpopulation within the $CD4^+CADM1^+$ fraction among $TRBC1^+$, $TRBC2^+$ and TRBC1/2 double-negative (TRBC–) populations. (B) Representative flow cytometry plots showing surface (upper panels) and intracellular (lower panels) TRBC1/2 expression in the three cases classified as TRBC1/2 double-negative within the $CD4^+CADM1^+$ fraction. (C) Representative histograms of CD3 expression and comparison of CD3 mean fluorescence intensity (MFI) between the $CD4^+CADM1^+$ and $CD4^+$ P fractions in the three TRBC1/2 double-negative cases. (D) Comparison of CD3 MFI in the $CD4^+CADM1^+$ fraction between TRBC1/2 double-negative cases and TRBC-positive cases among patients with chronic or acute ATL. Data are presented as mean ± standard deviation. *p*-values were calculated using the Mann–Whitney *U* test. (E) Comparison of CD3 MFI in the $CD4^+CADM1^+$ fraction between surface and intracellular staining in the three TRBC1/2 double-negative cases.

suggesting that TRBC-based assessment of T-cell clonality may also be applicable to tissue specimens.

## DISCUSSION

In this multicentre study, we demonstrated the utility of a method that combines HAS-Flow and TRBC1/2 dual staining (hereafter referred to as the HAS-TRBC method) for HTLV-1–infected individuals.

The correlation between TRBC1/2 skewing in the $CADM1^+$ fraction and HTLV-1 integration-site clonality supports its use as a surrogate of clonal expansion. However, because TRBC1/2 provides a binary readout, accurate estimation of clone size may be limited in complex clonal states. Conversely, genetic clonality analysis alone may also be difficult to interpret in some cases. In the acute ATL case shown in Figure 3A, apparent oligoclonality by VIS despite marked TRBC skewing and a proviral load close to 200 copies/100 PBMCs suggested that two HTLV-1

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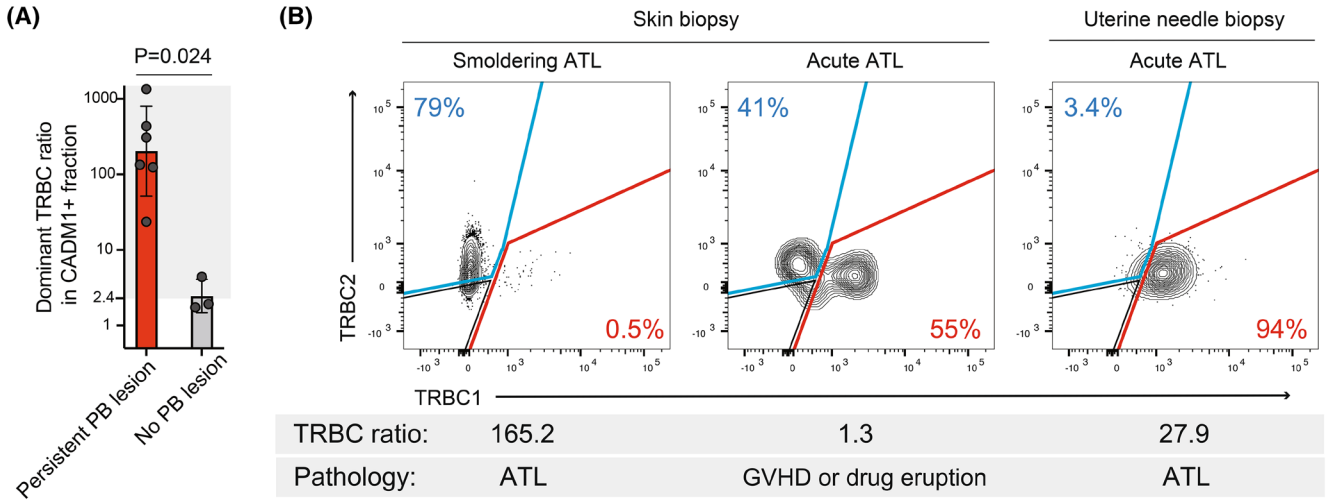


**FIGURE 5** Evaluation of TRBC skewing after treatment and in non–peripheral blood specimens (A) Comparison of dominant TRBC ratios in acute ATL cases between cases in which posttreatment peripheral blood abnormal lymphocytes decreased to ≤1% and those with persistent peripheral blood involvement. In two cases in the no PB lesion group, the dominant TRBC ratio was below the cut-off value of 2.4 used to distinguish ATL from non-ATL cases. Data are presented as mean ± standard deviation. *p*-values were calculated using the Mann–Whitney *U* test. (B) Flow cytometry plots and corresponding pathological findings from two skin biopsy specimens and one uterine needle biopsy specimen. GVHD, graft-versus-host disease.

integration sites were present within the same dominant clone. In contrast, the absence of TRBC skewing would be more consistent with true oligoclonality. Thus, HAS-TRBC may provide complementary information to genetic clonality analyses. Importantly, several limitations that have been associated with TRBC-based analyses of other T-cell malignancies are less relevant to ATL. Although TRBC skewing may occur in non-neoplastic conditions, such as infection,[32,33] HAS-TRBC restricts the analysis to the CADM1$^+$ fraction enriched for HTLV-1–infected cells, thereby reducing this concern. Moreover, because ATL typically expresses TCRαβ,[34,35] this method is well suited for ATL despite the limitation of TRBC staining in TCRγδ-positive tumours. Compared with TCR-Vβ–based approaches, which require a panel of 24 anti-TCR-Vβ antibodies,[12] HAS-TRBC requires only the addition of TRBC1 and TRBC2 antibodies to the standard HAS-Flow panel. Taken together, HAS-TRBC provides a simple approach for estimating T-cell clonality within HTLV-1–infected cell populations, analogous to the κ/λ ratio used to assess light-chain restriction in B-cell malignancies.[36]

Unlike conventional TRBC-based assessment of other T-cell neoplasms, in which TRBC restriction is typically evaluated within an immunophenotypically aberrant T-cell population,[21,37] the HAS-TRBC method assesses TRBC1/2 skewing within the CD4$^+$CADM1$^+$ fraction. Although this fraction is enriched for HTLV-1–infected cells, it is not defined by additional markers of malignant aberrancy and may therefore contain both clonally expanded and residual polyclonal infected cells. Consequently, the dominant TRBC ratio reflects not only clonality but also the relative abundance of the expanded clone within the HTLV-1-infected cell compartment. Thus, HAS-TRBC partially recapitulates conventional leukaemia/lymphoma immunophenotyping by restricting analysis to a biologically relevant population, but this distinction should be considered when interpreting modest TRBC skewing.

Several findings from this study indicate that the CD4$^+$CADM1$^+$ fraction is appropriate for assessing T-cell clonality within HTLV-1–infected cell populations. The dominant TRBC ratio in the CADM1$^+$ fraction showed superior discriminatory performance to that in total CD4$^+$ T cells and correlated positively with abnormal lymphocyte burden and the proportion of CADM1$^+$ cells. This superior performance likely reflects the enrichment of HTLV-1–infected cells within the CADM1$^+$ fraction, whereas TRBC skewing in total CD4$^+$ T cells may be diluted by residual uninfected and polyclonal T cells, particularly in cases with a lower abnormal lymphocyte burden or a smaller CADM1$^+$ fraction. Consistent with this interpretation, the dominant TRBC ratio in the CADM1$^+$ fraction was positively correlated with the proportion of the N fraction and negatively correlated with the P fraction. In line with previous reports,[23,24] these findings suggest that clonal expansion of HTLV-1–infected cells is accompanied by a relative decrease in uninfected cells and enrichment of clonally expanded cells within the N fraction. In contrast, no significant correlation was observed between the dominant TRBC ratio in the CADM1$^+$ fraction and the proportion of the D fraction, and TRBC skewing was not observed in some acute-type ATL cases (Figure 1B; Figure S3B). This may reflect progressive enrichment of clonally expanded cells in the N fraction, whereas the D fraction may retain a mixture of polyclonal infected cells. Although TRBC1/2 skewing in the N fraction showed discriminatory performance comparable to that in the CADM1$^+$ fraction, assessment of the entire CADM1$^+$ fraction may provide a more consistent measure of overall clonality across the disease spectrum from carriers to ATL. The dominant TRBC ratio also varied substantially among cases with high proviral

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load and among patients with smouldering or chronic ATL, populations that are heterogeneous in clinical course and clonality.[11,38] Because proviral load and infected-cell burden alone have limited predictive performance,[5–7] incorporating clonality assessment may improve evaluation of these heterogeneous populations.

An important consideration in interpreting TRBC1/2 skewing is the choice of cut-off value. The cut-off of 2.4 in the CADM1$^+$ fraction was derived from ROC analysis to distinguish ATL from non-ATL cases, providing 81% sensitivity and 100% specificity and should therefore be interpreted as a disease-discrimination threshold rather than a definitive threshold for clonality. This value was lower than the threshold previously used to define clonal T-cell populations based on TRBC1 restriction. TRBC1 positivity of <15% or >85%, which corresponds to a dominant TRBC ratio of approximately 5.7, has been used as a criterion for clonality assessment in other T-cell neoplasms.[21,29] In our cohort, a cut-off of 5.7 maintained 100% specificity for ATL but reduced sensitivity to 62%. In contrast, ROC analysis identified a higher cut-off of 10.0 for distinguishing acute/chronic ATL from other cases, with 83% sensitivity and 97% specificity. Although ratios around or above 10 have also been used as evidence of marked TRBC restriction in other T-cell neoplasms,[22] the cut-off of 10.0 in our study was independently derived for disease discrimination and was not intended as a universal clonality threshold. Thus, the optimal threshold may depend on the clinical purpose and T-cell population evaluated, and values near 2.4 should be interpreted cautiously rather than as evidence of clonality in isolation.

It is noteworthy that surface TRBC assessment using the HAS-TRBC method was difficult in three cases (20%) of chronic and acute ATL with reduced CD3 expression. Given the known association between CD3 and TRBC expression[30,31] and previous reports of decreased CD3 expression with ATL progression,[39,40] reduced CD3 expression associated with disease progression may have contributed to impaired surface TRBC detection in these cases. The preservation of surface TRBC expression in all HTLV-1–uninfected fractions and in all HTLV-1–infected fractions from non-ATL cases further supports this interpretation. Importantly, intracellular TRBC1/2 staining revealed marked TRBC restriction in two of the three cases with predominant surface TRBC1/2 double negativity. These findings suggest that intracellular staining can overcome the limitations of surface TRBC assessment in some cases with reduced or absent surface CD3/TRBC expression, consistent with previous reports in other T-cell proliferations and neoplasms.[21,41–43] Intracellular staining did not substantially increase CD3 detection in these cases, suggesting that reduced CD3 expression may not be attributable solely to impaired surface expression. The remaining case remained TRBC1/2 double-negative even after intracellular staining, as has also been reported in other T-cell neoplasms.[21,44] In cases that remain TRBC1/2 double-negative even after intracellular staining, molecular or genetic approaches to clonality assessment may therefore be required.

Furthermore, TRBC1/2 assessment has demonstrated utility in several clinical scenarios, even after progression to aggressive ATL. In cases of acute ATL with decreased posttreatment peripheral blood involvement, a significant reduction in TRBC1/2 skewing was observed; however, the dominant TRBC ratio did not fall below the cut-off value of 2.4 in one case (Figure 5A). This finding suggests that, as peripheral blood involvement decreases, TRBC-defined clonal restriction may diminish in some cases while persisting in others. Thus, the HAS-TRBC method may be useful for assessing T-cell clonality in residual abnormal lymphocytes after treatment. In addition, this method may be applicable to tissue specimens. ATL frequently involves multiple tissues, and tissue biopsies are frequently required to assess the extent of the disease.[45,46] However, it is well recognized that a definitive diagnosis of ATL based solely on histopathological findings can be challenging.[34,47] Therefore, a simple method for estimating T-cell clonality within HTLV-1–infected cells may be useful to identify ATL cell infiltration. TRBC1 immunohistochemistry has also been reported to facilitate the identification of clonal T-cell populations in tissue specimens.[48] Unlike TRBC1 immunohistochemistry alone, however, the HAS-TRBC method enables TRBC1/2 skewing to be evaluated specifically within the CADM1$^+$ population enriched for HTLV-1–infected cells. Therefore, the HAS-TRBC method may serve as a valuable adjunct to histopathological diagnosis.

This study had some limitations. The primary limitation is the relatively small sample size. Second, standardized cut-off values for TRBC1/2 skewing have not been established, particularly for HTLV-1–infected CADM1$^+$ cells. Although our ROC analyses identified clinically informative thresholds, further validation in independent cohorts is required. Third, specific ATL subtypes with atypical immunophenotypes, such as CD8$^+$ ATL, were not evaluated. Finally, interinstitutional variability remains a potential limitation. Although the samples were analysed using standardized protocols, differences across institutions may have affected the reproducibility of the TRBC measurements. Further studies in larger independent cohorts are needed to validate the findings in this study.

In conclusion, this study demonstrated the utility of the HAS-TRBC method, a simple flow cytometry–based approach, for estimating T-cell clonality within HTLV-1–infected cells. This method is widely applicable, easy to perform and more cost-effective than genetic approaches for clonality assessment. Although further validation in larger cohorts is warranted, HAS-TRBC is a practical and clinically applicable tool for evaluating T-cell clonality within HTLV-1–infected cells.

## AUTHOR CONTRIBUTIONS

**Koji Jimbo:** Data curation; investigation; formal analysis; funding acquisition; visualization; resources; writing – original draft. **Aileen G. Rowan:** Conceptualization;

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methodology; investigation; supervision; funding acquisition; project administration; writing – review and editing. **Momoko Usui:** Methodology; investigation. **Kako Suzuki:** Investigation. **Makoto Yamagishi:** Investigation; writing – review and editing; funding acquisition. **Junya Makiyama:** Resources. **Lucy B. M. Cook:** Resources; writing – review and editing. **Yoshihisa Yamano:** Investigation. **Kaoru Uchimaru:** Writing – review and editing; project administration. **Yasuhito Nannya:** Supervision; funding acquisition; project administration; writing – review and editing. **Shigeo Fuji:** Conceptualization; methodology; supervision; funding acquisition; project administration; writing – review and editing; resources.

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## CONFLICT OF INTEREST STATEMENT

JM has received consulting fees from Daiichi Sankyo and Janssen Pharmaceutical; honoraria from AbbVie GK, AstraZeneca, Chugai Pharmaceutical, Daiichi Sankyo, Genmab, Janssen Pharmaceutical, Kyowa Kirin, Minophagen, Ono Pharmaceutical, Takeda Pharmaceutical, Meiji Seika Pharma, Nippon Shinyaku and Sanofi; and support for attending meetings and/or travel from Takeda Pharmaceutical.

## DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Sequencing data in FASTQ format have been deposited in the National Bioscience Database Center Human Database under accession number JGAS000553 (https://humandbs.dbcls.jp/en/hum0252-v3).

## ETHICS STATEMENT

This study was approved by the Institutional Review Boards of The Institute of Medical Science, The University of Tokyo, Osaka International Cancer Institute and Sasebo City General Hospital in Japan. Patients recruited in the United Kingdom donated blood for research after providing written informed consent. Research in the UK was conducted under the governance of the Communicable Diseases Research Group Tissue Bank, approved by the UK National Research Ethics Service (09/H0606/106, 15/SC/0089, 20/SC/0226, 25/SC/0209).

## PATIENT CONSENT STATEMENT

All participants provided written informed consent, in accordance with the Declaration of Helsinki.

## ARTIFICIAL INTELLIGENCE (AI) DISCLOSURE

The authors used ChatGPT (OpenAI) to assist with English-language editing. The authors reviewed and verified all AI-assisted edits and take full responsibility for the accuracy and integrity of the submitted work.

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## SUPPORTING INFORMATION

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