

Case report

# T-cell large granular lymphocytic (LGL) leukemia consists of CD4<sup>+</sup>/CD8<sup>dim</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> LGL populations in association with immune thrombocytopenia, autoimmune neutropenia, and monoclonal B-cell lymphocytosis

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CD3<sup>+</sup>/CD57<sup>+</sup> T-cell large granular lymphocyte leukemia (T-LGLL) is an indolent neoplasm, exhibiting mostly CD8<sup>+</sup>, less frequently CD4<sup>+</sup> phenotypes, and T-LGLL consisting of 2 populations with CD8<sup>+</sup> and CD4<sup>+</sup> phenotypes is markedly rare. An 87-year-old female was admitted under a diagnosis of immune thrombocytopenia (ITP) with a platelet count of 5.0×10<sup>9</sup>/L and increased number of LGL with unknown etiology. Her neutrophil count also decreased to 0.27×10<sup>9</sup>/L and she was positive for antineutrophil antibody. The WBC count was 2.7×10<sup>9</sup>/L with 34.7% LGL and flow cytometry (FCM) analysis revealed 16% CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>dim</sup>/CD57<sup>+</sup> and 20.9% CD3<sup>+</sup>/CD8<sup>+</sup>/CD57<sup>+</sup> populations. These populations also expressed granzyme B and perforin. Circulating mononuclear cells were found to be clonal by PCR analysis of T-cell receptor β-chain gene. Serum immunofixation and bone marrow FCM analyses demonstrated 2 clonal B-cells producing IgG-λ and IgA-λ. Deep amplicon sequencing of *STAT3* and *STAT5B* genes revealed a *STAT3* R302G mutation with an allele burden of 2.6%. The thrombocytopenia and neutropenia were successfully treated by prednisolone and romiplostim with negative conversion of antineutrophil antibody. This is the first reported case of T-LGLL with dual components of CD4<sup>+</sup>/CD8<sup>dim</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> populations in terms of multiple comorbidities related to the respective CD8<sup>+</sup> and CD4<sup>+</sup> T-LGLLs.

**Keywords:** T-cell large granular lymphocytic leukemia, CD4, CD8, immune thrombocytopenia, autoimmune neutropenia, clonal B-lymphocytosis

## INTRODUCTION

T-cell large granular lymphocytic leukemia (T-LGLL) is an indolent neoplasm, accounting for 3% of mature lymphoid leukemias.<sup>1-3</sup> T-LGLL typically exhibits a CD3<sup>+</sup>, TCRαβ<sup>+</sup>, CD4<sup>-</sup>, CD5<sup>dim</sup>, CD8<sup>+</sup>, CD27<sup>-</sup>, CD28<sup>-</sup>, CD45RO<sup>-</sup>, CD45RA<sup>+</sup>, and CD57<sup>+</sup> phenotype.<sup>2,3</sup> CD3<sup>+</sup>/CD56<sup>+</sup> T-LGL leukemia is associated with *STAT5b* mutation and exhibits a more aggressive clinical course than CD3<sup>+</sup>/CD57<sup>+</sup> T-LGLL.<sup>4,5</sup> CD3<sup>+</sup>/CD57<sup>+</sup> T-LGLL mostly and rarely exhibits CD8<sup>+</sup> and CD4<sup>+</sup> phenotypes, respectively,<sup>2,6-8</sup> and only 3 cases of that consisting of 2 populations with CD8<sup>+</sup> and CD4<sup>+</sup> phenotypes have


been described.<sup>9,10</sup> CD3<sup>+</sup>/CD8<sup>+</sup>/CD57<sup>+</sup> and CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>dim</sup>/CD57<sup>+</sup> T-LGLLs have characteristic genetic backgrounds of *STAT3*<sup>11,12</sup> and *STAT5b*<sup>10</sup> mutations, respectively. CD3<sup>+</sup>/CD8<sup>+</sup>/CD57<sup>+</sup> T-LGLL is frequently associated with large granular lymphocytosis, autoimmune disease, such as rheumatoid arthritis, autoimmune cytopenia, such as pure red cell aplasia, immune thrombocytopenia (ITP), and autoimmune hemolytic anemia.<sup>12-15</sup> However, CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>dim</sup>/CD57<sup>+</sup> T-LGLL is not associated with autoimmune disease/cytopenia, but with B-cell neoplasia such as B-cell chronic lymphocytic leukemia and monoclonal gammopathy with undetermined significance/monoclonal B-cell lymphocytosis.<sup>6,16</sup>

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We report a rare case of T-LGLL consisting of 2 populations with CD3<sup>+</sup>/CD8<sup>+</sup>/CD57<sup>+</sup> and CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>dim</sup>/CD57<sup>+</sup> phenotypes, being associated with ITP, autoimmune neutropenia, and monoclonal B-cell lymphocytosis. This is the first reported case of T-LGLL with CD4<sup>+</sup>/CD8<sup>+</sup>-dual components and multiple comorbidities related to the respective CD8<sup>+</sup> and CD4<sup>+</sup> T-LGLLs.

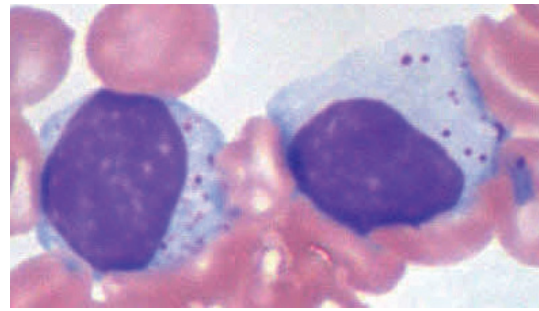
## MATERIALS AND METHODS

*STAT3* and *STAT5b* gene analyses were performed as previously described<sup>17</sup> using preserved DNA from white blood cells (WBC) after the T-cell receptor (TCR) gene clonal analysis. This study was approved by the ethics committees of Shinko Hospital and Shinshu University School of Medicine, and written informed consent was received from the patient's son.

## CASE REPORT

An 87-year-old female was admitted in November 2016 because of thrombocytopenia of  $2.5 \times 10^9/L$ , which was revealed to be ITP. At this time, an increased number of LGL ( $2.4 \times 10^9/L$ ) and neutropenia ( $0.29 \times 10^9/L$ ) were observed with an unknown etiology. As her medical history, she had been diagnosed with rectal cancer, stomach malignant lymphoma (diffuse large B-cell lymphoma: DLBCL), and thyroid cancer at the ages of 74, 75, and 76, respectively. For DLBCL, she received 6 courses of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) followed by 2 courses of rituximab alone at a previous hospital in 2005. The karyotype and immunohistopathological findings regarding the DLBCL were not available. For thyroid cancer, she underwent left lobe resection of the thyroid without following chemotherapy at another hospital in 2006. Chemotherapy was not performed after surgery for the rectal cancer in 2004. ITP was successfully treated by prednisolone (PSL) and romiplostim, and she was discharged. These agents were tapered and discontinued 10 months later.

In July 2017, she was readmitted because of the recurrence of ITP. Physically, several and many petechiae were observed in the oral cavity and on the bilateral forearms, respectively. Neither superficial lymph node swelling nor hepatosplenomegaly was noted. Laboratory examination demonstrated a WBC count of  $2.7 \times 10^9/L$ , with 12.9% neutrophils, 0.9% eosinophils, 1.3% basophils, 20.4% monocytes, 29.8% lymphocytes, and 34.7% LGL (Figure 1), a hemoglobin concentration of 11.3 g/dL, and a platelet count of  $5.0 \times 10^9/L$ . Regarding ITP and neutropenia, platelet-associated IgG was markedly increased to  $4,930 \text{ ng}/10^7 \text{ cells}$  (normally below  $46 \text{ ng}/10^7 \text{ cells}$ ) and anti-neutrophil antibody was positive. Other serological examinations including anti-nuclear antibody, complements (C3 and C4), rheumatoid factor, and immunoglobulin amounts, were non-specific. Serological tests for Epstein-Barr virus (EBV) revealed a pattern of previous infection, but EBV-DNA was not detected in



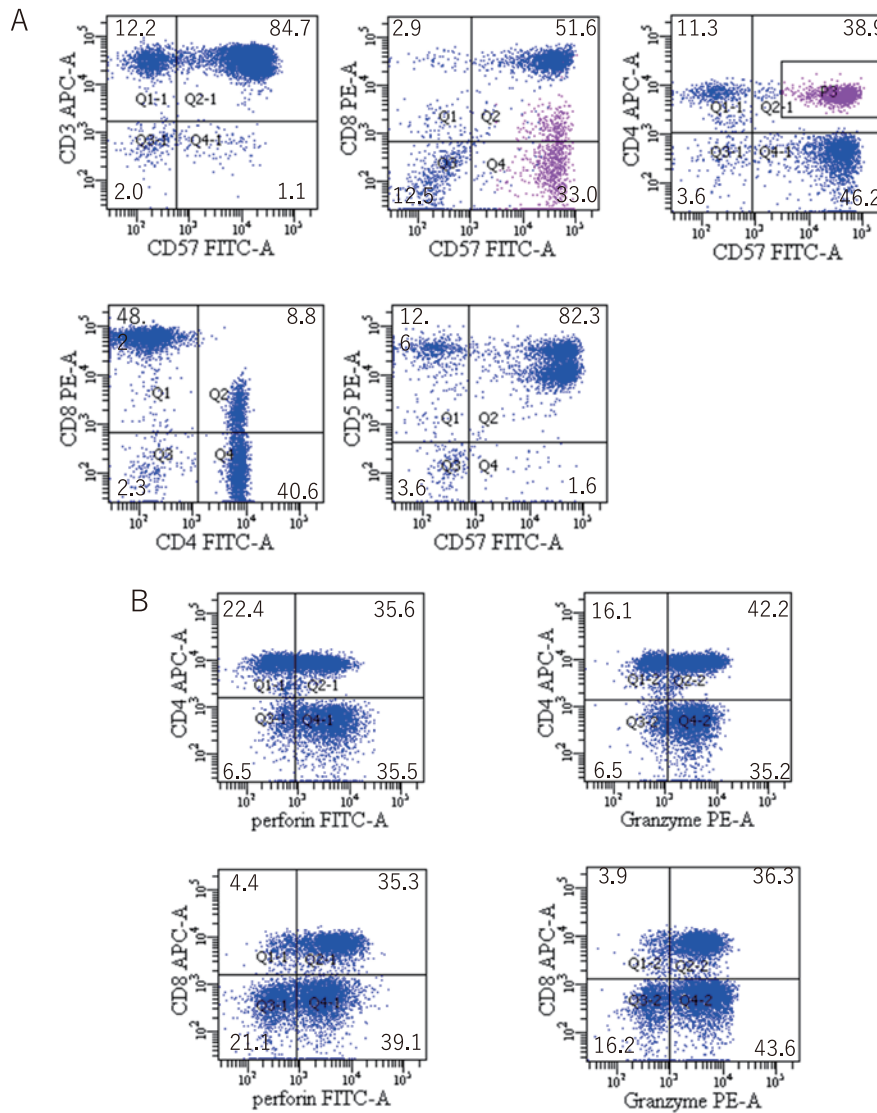
**Fig. 1.** Large granular lymphocytes in the peripheral blood in July 2017 (Wright-Giemsa staining,  $\times 1,000$ ).

the blood by multiplex PCR assay. The antibody for human immunodeficiency virus (HIV) was negative. Serological testing for human T-cell leukemia virus type 1 (HTLV-1) was not performed.

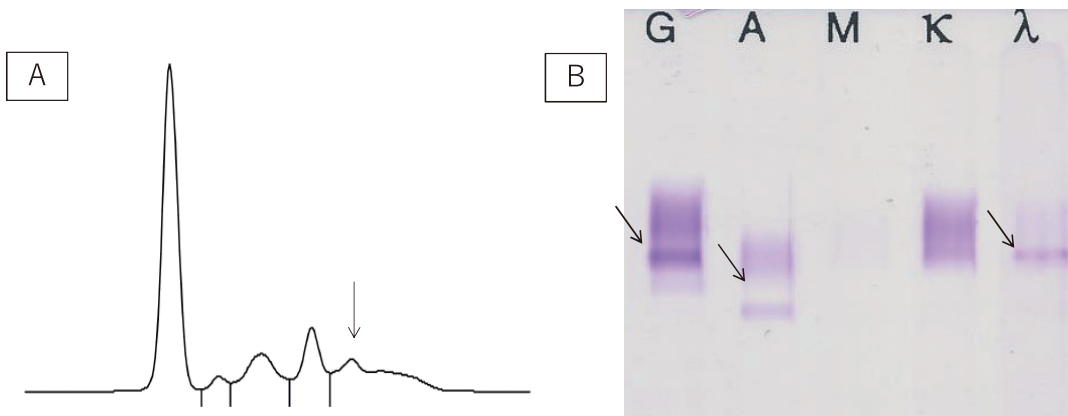
Flow cytometry (FCM) of peripheral blood demonstrated 16% CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>dim</sup>/CD57<sup>+</sup> and 20.9% CD3<sup>+</sup>/CD4<sup>-</sup>/CD8<sup>+</sup>/CD57<sup>+</sup> populations in nucleated cells with a CD4/CD8 ratio of 0.86 (Figure 2A). These cell populations also expressed granzyme B, perforin (Figure 2B), and TCR $\alpha\beta$  (data not shown). The summation of the percentages of CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>dim</sup>/CD57<sup>+</sup> and CD3<sup>+</sup>/CD4<sup>-</sup>/CD8<sup>+</sup>/CD57<sup>+</sup> populations by FCM was similar to that of the morphologically evaluated LGL (34.7%), suggesting that the LGL consisted of both CD4<sup>+</sup> and CD8<sup>+</sup> populations. CD16 of these mononuclear cells was negative on 2 incidences of FCM on the peripheral blood and bone marrow. Multiplex PCR analysis of WBC demonstrated monoclonal rearrangement of the TCR- $\beta$  chain gene but not  $\gamma$ -chain (data not shown). A monoclonally rearranged band of the TCR- $\beta$  gene was also observed on PCR analysis of bone marrow cells (data not shown).

An M-peak was observed on serum electrophoresis (Figure 3A), and immunofixation revealed monoclonal bands of IgG- $\lambda$  and faint IgA with an unknown light chain (Figure 3B). On FCM analysis of bone marrow cells, a B-cell population with a CD19<sup>+</sup>/CD38<sup>+</sup>/sm $\lambda$ <sup>+</sup>/sm $\kappa$ <sup>-</sup> phenotype comprised 3.8% of marrow nucleated cells (Figure 4). Of CD19<sup>+</sup> cells, cy $\alpha$ <sup>+</sup>/cy $\lambda$ <sup>+</sup>, cy $\lambda$ <sup>+</sup>, and cy $\kappa$ <sup>-</sup> cells comprised 1.2, 5.9, and 0.3% of marrow cells, respectively (Figure 4). Considering the results of serum immunofixation and marrow FCM analyses, there were 2 clonal B-cells producing IgG- $\lambda$  and IgA- $\lambda$  in the bone marrow. The bone marrow picture was non-specific except for LGL and lymphoplasmacytic cells (Figure 5), which comprised 5.0 and 5.6% of nucleated cells, respectively. We did not prepare marrow biopsy or clot preparations. Chromosomal examination of the marrow cells revealed an abnormal karyotype of 46,XX, ?t(3;9)(q27;q22) in 5 of the 20 dividing cells analyzed.

Based on these results, a final diagnosis of 2-population T-LGLL with CD4<sup>+</sup> and CD8<sup>+</sup> phenotypes, associated with ITP, autoimmune neutropenia, and clonal B-cell lymphocytosis consisting of 2 populations producing IgG- $\lambda$  and IgA- $\lambda$ , was made. ITP was successfully treated with PSL and romiplostim, with subsequent improvement of neutropenia

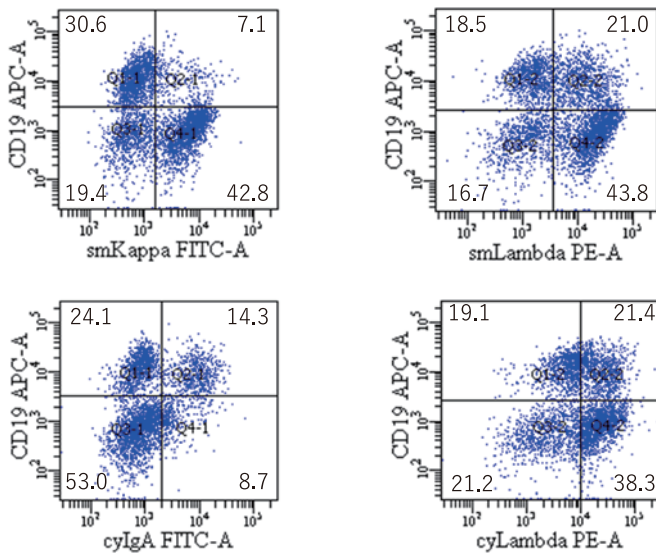


**Fig. 2.** Flow cytometric analysis of peripheral blood performed using strong CD45 gating. The value in each area in respective cytograms indicates % of cells among CD45-strongly positive mononuclear cells. Based on these data, the % of CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>dim</sup>/CD57<sup>+</sup> and CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>+</sup>/CD57<sup>+</sup> populations in WBC (nucleated cells) were 16.0% and 20.9%, respectively (Figure 2A). These CD4<sup>+</sup> and CD8<sup>+</sup> cell populations also expressed granzyme B and perforin (Figure 2B).

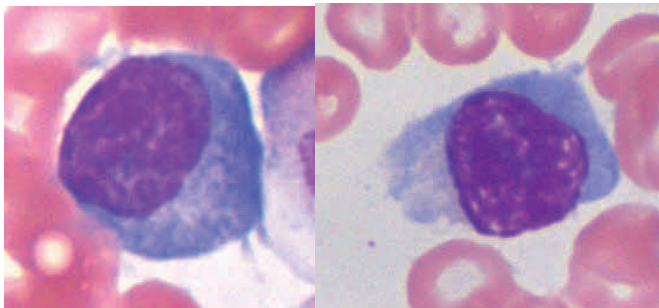


**Fig. 3.** An M-peak was observed on serum electrophoresis (arrow) (Figure 3A), and immunofixation showed monoclonal bands of IgG-λ and faint IgA with an unknown light chain (arrows) (Figure 3B).





**Fig. 4.** Flow cytometric analysis of bone marrow cells with strong CD45 gating. The value in each area in respective cytograms indicates the % of cells among CD45-strongly positive mononuclear cells. The analysis subsequently revealed a population with a CD19<sup>+</sup>/smλ<sup>+</sup>/smκ<sup>-</sup> phenotype comprising 3.8% of marrow nucleated cells. Cytoplasmic immunoglobulin analysis showed that cyα<sup>+</sup>/cyλ<sup>+</sup>, cyλ<sup>+</sup>, and cyκ<sup>-</sup> cells comprised 1.2, 5.9, and 0.3% of marrow nucleated cells, respectively. Surface and cytoplasmic IgG were not detected, possibly due to unknown problems associated with the use of monoclonal anti-IgG antibody in this analysis.



**Fig. 5.** Lymphoplasmacytic cells in the bone marrow comprised 5.6% of marrow nucleated cells (Wright-Giemsa staining, ×1,000).

and negative conversion of antineutrophil antibody, and the patient was discharged in August 2017. The T-LGLL was stable with platelet counts of around 55×10<sup>9</sup>/L. However, in November 2017, the patient died of DLBCL in the abdominal cavity, which may have been transformed from the above-mentioned marrow clonal B-cells because the pathological diagnosis of this lymphoma was DLBCL (CD20<sup>+</sup>/*Bcl-2*<sup>+</sup>/CD5<sup>-</sup>/CD10<sup>-</sup>/*c-myc*/*EBER*<sup>-</sup>) with plasmacytic differentiation, and FCM analysis confirmed 2 populations of abnormal B-cells with phenotypes of CD19<sup>+</sup>/CD20<sup>+</sup>/sm-cyκ<sup>+</sup>/cyIgM<sup>dim</sup>/CD5<sup>-</sup>/CD10<sup>-</sup>/smIg (A, D, M, G)/cyIg (A, D, G)/sm-cyλ<sup>-</sup> (33.4% of mononuclear cells) and CD45<sup>dim</sup>/CD19<sup>dim</sup>/CD10<sup>+</sup>/cyIgA<sup>+</sup>/CD5<sup>-</sup>/CD20<sup>-</sup>/smIg (A, D, M, G)/cyIg (M, D, G)/sm-cyκ<sup>-</sup> and λ<sup>-</sup> (6.0%). Chromosomal and fluorescence in situ hybridization (FISH) analyses were not performed on this lymphoma specimen.

Mutation analyses of *STAT3* and *STAT5* genes revealed a

*STAT3* R302G mutation with an allele burden of 2.6%.

## DISCUSSION

In the present patient, an unusual lymphocyte population with a CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>dim</sup>/CD57<sup>+</sup> phenotype as T-LGLL was observed. The CD4<sup>+</sup> population, however, expressed CD57, granzyme B, and perforin, similar to the CD8<sup>+</sup> population. Although we did not perform clonal analyses, such as PCR or Southern blotting, on isolated CD4 and CD8 populations as Richards *et al.* did,<sup>9</sup> the expression of CD57 and cytotoxic molecules in these 2 T-cell populations supports that these 2 populations originated from the same clone. Therefore, this case was diagnosed as extremely rare 2-population T-LGLL with CD4<sup>+</sup> and CD8<sup>+</sup> phenotypes. Only 3 cases of this dual-population T-LGLL have been reported. However, comorbidities related to the respective CD8<sup>+</sup> and CD4<sup>+</sup> T-LGLL were not described.<sup>9,10</sup>

In normal T-cell differentiation from double-positive T-cells to single-positive cells, mutually exclusive transcription factors, *ThPOK* and *Runx3*, induce CD4<sup>+</sup>- and CD8<sup>+</sup> single-positive cells, respectively.<sup>18</sup> On the other hand, in a T-cell neoplasm, differentiation from double-positive T-cells to single-positive T-cells occurs with restriction of CD4<sup>+</sup>- or CD8<sup>+</sup> single-positive cells. In this situation, it is hypothesized that only one of the 2 transcription factors acts due to tumor-altered gene regulation. In the present case, both *ThPOK* and *Runx3* may have abnormally functioned at the stage of neoplastic double-positive T-cells, resulting in the generation of both CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>dim</sup>/CD57<sup>+</sup> and CD3<sup>+</sup>/CD4<sup>-</sup>/CD8<sup>+</sup>/CD57<sup>+</sup> populations. Of note, the former population also expressed cytotoxic molecules, granzyme B and perforin. *ThPOK* negatively regulates the expression of *Runx3* and CD8 lineage genes, resulting in the suppression of CD8, granzyme B, and perforin expression.<sup>19,20</sup> *ThPOK* in this patient, therefore, may have incompletely suppressed CD8 lineage molecules.

The CD4<sup>+</sup>/CD8<sup>+</sup>-dual populations demonstrated notable clinical pictures in this patient: 2 autoimmune phenomena of ITP and autoimmune neutropenia, which are frequently associated with CD8<sup>+</sup> T-LGLL,<sup>12-15</sup> and clonal B-cell lymphocytosis consisting of 2 B-cell populations, which is associated with CD4<sup>+</sup> T-LGLL.<sup>6,16</sup> The clinical picture of the present patient, therefore, is of interest and novel in terms of rare CD4<sup>+</sup>/CD8<sup>+</sup>-dual component T-LGL leukemia and multifarious comorbidities related to the respective CD8<sup>+</sup> and CD4<sup>+</sup> T-LGLLs.

The intra-abdominal DLBCL that developed in November 2017 may have originated from monoclonal marrow B cells because of plasmacytic differentiation of the DLBCL on pathological examination and the presence of cyIgA-positive abnormal B-cells, as previously detected in the bone marrow by FCM analyses. The abnormal karyotype of 46,XX, ?t(3;9)(q27;q22) observed in the bone marrow may also support this possibility because 3q27 involves *Bcl-6*, and abnormality of this gene is observed in many DLBCL. The relationship of stomach DLBCL at the age of

75 with the late-appearing DLBCL is unclear in this patient because immunohistochemical and chromosomal information of the stomach DLBCL were not available.

Mutation analyses of *STAT3* and *STAT5* genes revealed a *STAT3* R302G mutation. R302G mutation has not been reported in T-LGLL; however, R302W mutation was described in adenoma of the adrenal cortex in the Catalogue of Somatic Mutations in Cancer (COSMIC v88, March 2019), and classified as pathogenic. *STAT3* mutations are common in T-LGLL and considered to be useful for appropriately managing patients.<sup>2</sup>

In conclusion, we report the first case of T-LGLL with CD4<sup>+</sup>/CD8<sup>+</sup>-dual components and multiple comorbidities related to the respective CD8<sup>+</sup> and CD4<sup>+</sup> T-LGLs with *STAT3* R302G mutation.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this study.

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