Retrospective analyses of other iatrogenic immunodeficiency-associated lymphoproliferative disorders in patients with rheumatic diseases

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Summary
Other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs) occur in patients receiving immunosuppressive drugs for autoimmune diseases; however, their clinicopathological and genetic features remain unknown. In the present study, we analysed 67 patients with OIIA-LPDs, including 36 with diffuse large B-cell lymphoma (DLBCL)-type and 19 with Hodgkin lymphoma (HL)-type. After discontinuation of immunosuppressive drugs, regression without relapse was achieved in 22 of 58 patients. Spontaneous regression was associated with Epstein–Barr virus positivity in DLBCL-type (P = 0.013). The 2-year overall survival and progression-free survival (PFS) at a median follow-up of 32.4 months were 92.7% and 72.1% respectively. Furthermore, a significant difference in the 2-year PFS was seen between patients with DLBCL-type and HL-type OIIA-LPDs (81.0% vs. 40.9% respectively, P = 0.021). In targeted sequencing of 47 genes in tumour-derived DNA from 20 DLBCL-type OIIA-LPD samples, histone-lysine N-methyltransferase 2D (KMT2D; eight, 40%) and tumour necrosis factor receptor superfamily member 14 (TNFRSF14; six, 30%) were the most frequently mutated genes. TNF alpha-induced protein 3 (TNFAIP3) mutations were present in four patients (20%) with DLBCL-type OIIA-LPD. Cases with DLBCL-type OIIA-LPD harbouring TNFAIP3 mutations had shorter PFS and required early initiation of first chemotherapy. There were no significant factors for spontaneous regression or response rates according to the presence of mutations. Overall, OIIA-LPDs, especially DLBCL-types, showed favourable prognoses.

Keywords: immunosuppressive drug, lymphoproliferative disorders, autoimmune disease, methotrexate, tumour necrosis factor alpha-induced protein 3.
Introduction

Patients with rheumatoid arthritis receiving methotrexate (MTX) therapy are likely to be afflicted with lymphoproliferative disorders (LPDs). Epidemiologically, patients treated with MTX, for autoimmune diseases tend to suffer from LPDs or lymphoma, which are defined as other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs), according to the World Health Organization (WHO) classification. Among patients treated with MTX, the reported cases of OIIA-LPDs are most often of diffuse large B-cell lymphoma (DLBCL)-type (35–60%) and Hodgkin lymphoma (HL)-type (12–25%).

Patients and methods

This study was approved by the appropriate institutional review boards of the University of Tsukuba Hospital (H24-075), Toranomon Hospital (1710-H), and Toranomon Hospital Kajigaya (1710-B). In accordance with the Declaration of Helsinki, informed consent was obtained from participants whose genomic DNA was available from their frozen tissue samples or formalin-fixed and paraffin-embedded (FFPE) tissues.

Patient selection

In this study, 67 cases of OIIA-LPDs diagnosed between 2009 and 2018 at the University of Tsukuba Hospital, Toranomon Hospital and Toranomon Hospital Kajigaya were selected, and their clinico-pathological features were retrospectively analysed. Cases of the reactive lymphadenitis subtype were excluded from this study. The diagnosis was made by the pathologists at the participating centres. Furthermore, all cases were pathologically reviewed by an expert haematopathologist. In this study, tumour cells consisted of morphologically large B cells in DLBCL-type OIIA-LPDs. According to the WHO classification, DLBCL, not otherwise specified (DLBCL-NOS) and Epstein–Barr virus (EBV)-positive DLBCL-NOS were included in this study. A diagnosis of the EBV status was made based on the results of EBV-encoded small RNA (EBER) in situ hybridisation. We found that EBER-positive cells accounted for >50% of all EBV-positive tumour cells.

Tumour samples

A total of 15 fresh frozen tissue samples and five FFPE tissue samples available at the participating centres were selected. Genomic DNA was extracted from fresh frozen tissue samples or FFPE tissues using the QiAamp DNA Mini Kit or QiAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). DNA integrity numbers were calculated to evaluate the quality of FFPE tissues using an Agilent FFPE QC kit (Agilent Technologies, Santa Clara, CA, USA). FFPE tissue-derived DNA samples were used for library preparation only if the normalised integrity score (ΔΔCq values) was <1.5.

Targeted sequencing and mutational analysis

Targeted sequencing of DLBCL-type OIIA-LPDs was performed using our custom Haloplex® panel designed for 47 lymphoid cancer-related genes (Table SI). Libraries were prepared from 50 ng of tumour-derived DNA using the Haloplex® kit (Agilent Technologies) according to the manufacturer’s instructions. Libraries were then sequenced on a HiSeq 4000 instrument (Illumina, San Diego, CA, USA). Sequencing data were aligned to the hg19 human reference genome. Two different bioinformatics pipelines [SureCall tool (version 4.0) and Genomon2 pipeline (https://github.com/Genomon-Project)] were used for variant calling. All variants were confirmed by visual inspection using the Integrative Genomics Viewer (version 2.4.10; https://software.broadinstitute.org/software/igv).

Statistical methods

The OS was estimated from the date of diagnosis to that of death or last follow-up. Progression-free survival (PFS) was calculated from the date of diagnosis until that of progression after first chemotherapy, relapse, or death. In addition to OS and PFS, freedom from treatment (FFT) was analysed. FFT was defined as the time from the date of diagnosis to that of commencing the first chemotherapy, death, or the last follow-up. OS, PFS, and FFT were measured using the Kaplan–Meier method. The Cox proportional hazards model was used for multivariate analysis. Factors were analysed in univariate analysis, and all factors with a P < 0.05 were used for the multivariate model. All P values were two-sided and a P < 0.05 was considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R.
Results

Patient characteristics

A total of 67 cases of OIIA-LPDs were identified, of which 30 were diagnosed at the University of Tsukuba Hospital, 27 at Toranomon Hospital and 10 at Toranomon Hospital Kajigaya. The histological subtypes of OIIA-LPDs are summarised in Table I. The most common OIIA-LPDs were DLBCL-type (n = 36) and HL-type (n = 19), accounting for 80% of OIIA-LPDs. Therefore, DLBCL-type and HL-type were compared in subsequent analyses. Patient characteristics for all OIIA-LPDs and the most predominant subtypes (DLBCL-type and HL-type) are shown in Table II. In addition, patient characteristics for OIIA-LPDs with the subtypes other than DLBCL-type and HL-type are listed in Table SII. Among all patients with OIIA-LPDs, the male-to-female ratio was 1:3:2, and the median (range) age at diagnosis was 69 (30–85) years. Of the 67 patients with OIIA-LPDs, 62 had rheumatoid arthritis. In the remaining five patients, the autoimmune diseases were systemic lupus erythematosus (SLE) (one), myasthenia gravis (one), polymyalgia rheumatica (one), polymyositis (one) and ulcerative colitis (one). Of the two patients with SLE, one had both rheumatoid arthritis and SLE and the other only had SLE. MTX had not been used in seven patients from the time of diagnosis of autoimmune diseases to the diagnosis of OIIA-LPD. In these seven patients, the underlying disease was rheumatoid arthritis in three, SLE in one, myasthenia gravis in one, polymyalgia rheumatica in one and ulcerative colitis in one.

Table I. Subtypes of other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs).

| Subtype                                    | Number of high-quality DNA obtained from FF or FFPE |
|--------------------------------------------|-----------------------------------------------------|
| DLBCL                                      | 35 (52)                                              |
| DLBCL and follicular lymphoma              | 1 (1)                                                |
| Hodgkin lymphoma                           | 19 (28)                                              |
| B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma | 1 (1)                                                |
| MALT lymphoma                              | 2 (3)                                                |
| PTCL-NOS                                    | 3 (4)                                                |
| Angioimmunoblastic T-cell lymphoma          | 1 (1)                                                |
| Extramedial NK/T-cell lymphoma              | 1 (1)                                                |
| Burkitt lymphoma                            | 1 (1)                                                |
| Polymorphic infiltrates                     | 3 (4)                                                |

DLBCL, diffuse large B-cell lymphoma; FF, fresh frozen; FFPE, formalin-fixed paraffin-embedded; MALT, mucosa-associated lymphoid tissue; NK, natural killer; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified.

Effects after withdrawal of MTX or tacrolimus

Clinical outcomes after withdrawal of MTX or tacrolimus at the onset of OIIA-LPDs are summarised in Table III and Table SIII. At diagnosis of OIIA-LPDs, 59 patients (88%) were treated with MTX or tacrolimus; 50 (85%) were treated with MTX alone and four (7%) were treated with tacrolimus alone. Five patients were treated with a combination of MTX and tacrolimus. For 55 patients using MTX at the onset of OIIA-LPDs, MTX was discontinued in 54 (98%). For all nine patients using tacrolimus at the onset of OIIA-LPDs, tacrolimus was discontinued. In the remaining five cases, MTX had already been withdrawn before the onset of OIIA-LPDs. In these five patients, including two with the DLBCL-type and three with the HL-type, OIIA-LPDs occurred at a median (range) duration of 6.8 (2.8–10.9) years from the time of MTX cessation. For the three patients with the polymorphic subtype, regression without relapse after withdrawal was observed. For the DLBCL-type, regression without relapse was observed in 15 (47%) of 32 patients after MTX or tacrolimus withdrawal. Among these 15 patients with DLBCL-type, EBV was detected in 10. Regression was achieved at a significantly higher rate in the EBV-positive patients than in the EBV-negative patients (67% vs. 33%; P = 0.013) (Table SIV) while comparing DLBCL-type patients with or without regression. In contrast, EBV positivity did not affect the rate of spontaneous regression in patients with HL-type (67% vs. 33%; P = 1).

Response to chemotherapy

After the onset of OIIA-LPDs, 21 patients with the DLBCL-type and 15 with the HL-type OIIA-LPD received chemotherapy. Among the 21 patients with DLBCL-type OIIA-LPD, a patient was transferred to another hospital within a month after the initiation of first-line chemotherapy. For the remaining 20 patients with DLBCL-type and 15 with HL-type OIIA-LPD, the overall response rates (ORRs) were 90% and 63%, respectively, with 65% and 53% of patients achieving complete response (Table IV).

Survival outcomes and prognostic factors

At a median (range) follow-up of 32.4 (0.9–139.5) months, the 2-year OS, PFS, and FFT rates for all patients with OIIA-LPDs were 92.7% [95% confidence interval (CI) 81.7–97.2], 72.1% (95% CI 58.4–82.0) and 35.9% (95% CI 23.9–48.1) respectively (Fig 1). When analysed by subtype, the 2-year OS rates of patients with DLBCL-type and HL-type OIIA-LPDs were 93.4% (95% CI 75.8–98.3) and 86.7% (95% CI 56.4–96.5) respectively (P = 0.257). The 2-year PFS rates for DLBCL-type and HL-type OIIA-LPDs were
Table II. Clinical features of other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs).

| Clinical characteristics | All patients (n = 67) | DLBCL-type (n = 36) | HL-type (n = 19) |
|--------------------------|----------------------|---------------------|-----------------|
| Age, years, median (range) | 69 (30–85) | 71 (30–85) | 67 (52–79) |
| Sex (male/female), n | 16/51 | 5/31 | 6/13 |
| ECOG PS score 0–1/2–4, n | 50/15 | 24/12 | 16/2 |
| Stage I/II/III/IV, n | 15/11/15/12 | 11/9/3/13 | 3/2/6/8 |
| Underlying disease, n/N (%) | | | |
| Rheumatoid arthritis | 62/67 (93) | 33/36 (92) | 18/19 (95) |
| SLE | 3/67 (4) | 1/36 (3) | 2/19 (11) |
| Myasthenia gravis | 1/67 (1) | 0/36 (0) | 0/19 (0) |
| Polymyalgia rheumatica | 1/67 (1) | 0/36 (0) | 1/19 (5) |
| Polymyositis | 1/67 (1) | 1/36 (3) | 0/19 (0) |
| Immunosuppressant, n/N (%) | | | |
| MTX | 60/67 (90) | 32/36 (89) | 17/19 (90) |
| MTX and biological agents | 19/67 (28) | 10/36 (28) | 6/19 (32) |
| Biological agents without MTX | 2/67 (3) | 1/36 (3) | 1/19 (5) |
| B symptoms, n/N (%) | 19/65 (29) | 6/36 (17) | 9/18 (50) |
| IPI score 3–5, n/N (%) | 29/63 (46) | 17/36 (47) | 9/18 (50) |
| HB level ≤100 g/l, n/N (%) | 12/65 (18) | 5/36 (14) | 5/19 (26) |
| Elevated serum LDH level, n/N (%) | 37/65 (57) | 21/36 (58) | 13/19 (68) |
| Serum CRP level >50 mg/dl, n/N (%) | 15/65 (23) | 8/36 (22) | 6/19 (32) |
| Serum albumin level ≤3 g/dl, n/N (%) | 14/65 (22) | 8/36 (22) | 4/19 (21) |
| Lymphocyte count ≤800/µl, n/N (%) | 19/64 (30) | 10/35 (29) | 5/18 (28) |
| Duration of MTX administration, years, median (range) | 7.4 (0–2–24) | 8.4 (0–5–22) | 8.2 (0–2–24) |
| (n = 58) | (n = 32) | (n = 15) |
| Extranodal disease, n/N (%) | 32/63 (51) | 20/36 (56) | 6/18 (33) |
| EBER positive, n/N (%) | 39/65 (60) | 15/36 (42) | 15/18 (83) |
| Died, n/N (%) | 8/67 (12) | 4/36 (11) | 4/19 (21) |

CRP, C-reactive protein; DLBCL, diffuse large B-cell lymphoma; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EBER, Epstein–Barr virus-encoded small RNA; Hb, haemoglobin; HL, Hodgkin lymphoma; IPI, International Prognostic Index; LDH, lactate dehydrogenase; MTX, methotrexate; SLE, systemic lupus erythematosus. [Correction added on 24 December 2021, after first online publication: The data in the third and fourth columns of the second row were corrected in this version.]

Table III. Clinical outcome following withdrawal of MTX or tacrolimus in the onset of other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs).

| MTX or tacrolimus use in the onset | All patients (n = 59) | DLBCL-type (n = 32) | HL-type (n = 15) |
|-----------------------------------|----------------------|---------------------|-----------------|
| Only MTX use in the onset, n (%) | 50 (85) | 27 (84) | 13 (87) |
| Only tacrolimus use in the onset, n (%) | 4 (7) | 2 (6) | 1 (7) |
| MTX and tacrolimus use in the onset, n (%) | 5 (8) | 3 (9) | 1 (7) |
| MTX or tacrolimus cessation, n | 58 | 32 | 15 |
| Regression and no relapse, n (%) | 22 (38) | 15 (47) | 3 (20) |
| Relapse after regression, n (%) | 12 (21) | 4 (13) | 5 (33) |
| Persistent, n (%) | 12 (21) | 5 (16) | 4 (27) |
| Initiation of immediate chemotherapy, n (%) | 11 (19) | 8 (25) | 2 (13) |
| Lost follow-up, n (%) | 1 (1) | 0 | 1 (7) |
| MTX continuation, n | 1 | 0 | 0 |

DLBCL, diffuse large B-cell lymphoma; HL, Hodgkin lymphoma; MTX, methotrexate.

Table IV. Initial chemotherapy for other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs).

| Patients receiving initial chemotherapy, % (n/N) | 58 (21/36) | 75 (15/19) | 0.149 |
|-----------------------------------------------|-------------|-------------|--------|
| ORR, % (n/N) | 90 (18/20) | 63 (9/15) | 0.052 |
| CR, % (n/N) | 65 (13/20) | 53 (8/15) | 0.511 |
| The content of initial chemotherapy | RCHOP (n = 17) | RCHOP (n = 2) | |
| Rituximab (n = 3) | CHOP (n = 3) | | |
| RTHPCOP (n = 1) | ABVD (n = 2) | | |
| ABVD, doxorubicin (adriamycin), bleomycin, vinblastine, and dacarbazine; CR, complete response; CVP, cyclophosphamide, vincristine, and prednisone; DLBCL, diffuse large B-cell lymphoma; HL, Hodgkin lymphoma; ORR, overall response rate; RCHOP, rituximab and CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone); RTHPCOP, rituximab, and THPCOP (pirarubicin, cyclophosphamide, vincristine, and prednisone). |
81.0% (95% CI 62.5–91.0) and 40.9% (95% CI 16.4–64.3) respectively ($P = 0.021$). Additionally, the 2-year FFT rates were 44.7% (95% CI 27.5–60.5) and 10.2% (95% CI 8.3–33.5) respectively ($P = 0.088$) (Fig 2). As for PFS, the DLBCL-type showed a significantly better prognosis than the HL-type. Although there was no difference in the OS and PFS between EBV-positive DLBCL-type and EBV-negative DLBCL-type, the FFT for EBV-positive cases exhibited a significantly better prognosis than for EBV-negative cases among DLBCL-type patients (Fig S1). In total, eight patients included in the study died, with OIIA-LPDs as the cause of death in seven and interstitial pneumonia in one. Tables SV–SVII show the results of uni- and multivariate analyses of the risk factors for PFS in all patients, and in those with the DLBCL-type and HL-type OIIA-LPD respectively. In multivariate analysis, B symptoms were an independent risk factor for PFS.

**Mutational profile of DLBCL-type OIIA-LPDs**

Targeted sequencing of tumour-derived DNA was conducted on 20 DLBCL-type OIIA-LPD samples. The mean and median coverage in our panel were 458.7x (range 243–957) and 313x (range 210–691), respectively (Table SVIII). The gene mutations identified in DLBCL-type OIIA-LPD samples are listed in Table SIX. The median (range) number of mutations per patient was 4 (2–25). The mutational frequencies for DLBCL-type OIIA-LPDs are shown in Fig S2. The most frequently mutated genes were histone-lysine N-methyltransferase 2D ($KMT2D$; eight samples, 40%) and tumour necrosis factor receptor superfamily member 14 ($TNFRSF14$; six samples, 30%). Figure 3 shows a mutational heat map. Forest plots summarise the results of univariate analyses of gene alterations for FFT and PFS (Fig S3). Almost all mutations, except for TNF alpha-induced protein 3 ($TNFAIP3$) shown in Fig S3, were detected in one or two cases. Therefore, mutations in $TNFAIP3$, a negative regulator of the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) pathway, were focussed upon. $TNFAIP3$ mutations were significantly associated with shorter FFT and PFS than wild-type $TNFAIP3$ (Fig 4). Positions and types of somatic mutations encoded in $TNFAIP3$ were detected in four cases, including non-synonymous mutations in three cases and frame-shift deletion in one case. Figure 4 also shows $TNFAIP3$ mutations observed in 204 DLBCL-NOS cases, including missense mutations in 45 cases and truncating mutations in 159 cases described in previous studies. Table SX shows clinical features of DLBCL-type OIIA-LPDs with $TNFAIP3$ mutations. There were no specific features among DLBCL-type OIIA-LPDs with or without $TNFAIP3$ mutations. We analysed spontaneous regression rates, ORRs, and complete response rates (CRR) according to the presence of mutations in our cohort; however, there were no statistically significant differences.
Discussion

In the present study, we performed clinicopathological analyses of OIIA-LPDs, as well as mutational analyses of DLBCL-type OIIA-LPDs. As for PFS, DLBCL-type OIIA-LPDs showed significantly better outcomes than the HL-type OIIA-LPDs. EBV positivity was significantly associated with regression after withdrawal of MTX or tacrolimus. Based on the results of mutational analysis of OIIA-LPDs, TNFAIP3 mutations were found to be associated with a poor prognosis.

Contrary to our present results, the DLBCL-type was associated with poor OS in some previous reports. However, in those reports, reactive lymphadenitis was included in the non-DLBCL-type, and the authors did not compare the prognosis of the DLBCL-type with the HL-type. In another report, FFT differed significantly between the DLBCL-type and HL-type (12.1 vs. 6.4 months; \( P = 0.001 \)). The results of that previous report and those obtained in our present study imply that the prognosis of the DLBCL-type is favourable compared with that of the HL-type.

In previous studies, most spontaneous responses were reported to occur in EBV-positive OIIA-LPDs; we observed that spontaneous regression was associated with EBV positivity in the DLBCL-type. EBV positivity is associated with autoimmune diseases themselves or with immunosuppressors, such as MTX. Regarding DLBCL, EBV positivity is higher in immunodeficiency settings. The persistence and reactivation of EBV may cause the occurrence of EBV-positive DLBCL-type OIIA-LPDs. This finding implies that the recovery of the immune system after withdrawal of immunosuppressors could be achieved in EBV-positive DLBCL-type OIIA-LPDs, leading to spontaneous regression. In previous studies, lymphopenia was found to be associated with inferior OS, PFS, and regression after MTX withdrawal in patients with immunodeficiency-associated LPDs. However, in our present study, lymphopenia did not have a significant effect on OS, PFS, and FFT (Fig S4).

We observed that the 2-year OS and PFS for the HL-type OIIA-LPDs were 86.7% and 40.9% respectively. As the OS rate of classic HL (cHL) was 92% at 50 months, the prognosis of cHL is favourable, suggesting that the prognosis of HL-type OIIA-LPDs might be worse than that of cHL. However, the median (range) age at diagnosis of HL-type in OIIA-LPDs was 67 (52–79) years, which was higher than that of cHL in immunocompetent patients, and clinical outcome of patients aged >60 years with cHL treated in Switzerland showed that the 5-year OS among patients aged 60–70 years and 70–80 years was 78.2% (95% CI 70.8–86.9) and 52.2% (95% CI 42.3–64.3) respectively. Therefore, there may be no difference between the prognosis of HL-type OIIA-LPDs and cHL. However, the bias between cHL and HL-type OIIA-LPDs should be taken into account.

According to the current WHO classification, the mutational characteristics of OIIA-LPDs do not differ from those of lymphomas of similar histological types not associated
with immunosuppression. Although the genome-wide copy number and loss-of-heterozygosity profiles were analysed, the targeted sequencing of OIIA-LPDs has not been reported to date. Therefore, we performed targeted sequencing of DLBCL-type OIIA-LPDs, and TNFAIP3 mutations were found to be associated with poor outcomes.

DLBCL was classified into four prominent genetic subtypes, termed MCD [based on the co-occurrence of myeloid differentiation factor 88 (MYD88) L265P mutations and CD79B mutations], BN2 [based on B-cell lymphoma 6 (BCL6) fusions and Notch receptor 2 (NOTCH2) mutations], N1 (based on NOTCH1 mutations), and EZB (based on enhancer of zeste homolog 2 (EZH2) mutations and BCL2 translocations). As genetic aberrations targeting regulators of the NF-κB pathway are characteristics of BN2, TNFAIP3 mutations (negative regulators of NF-κB) were common in BN2. BN2 DLBCL showed favourable outcomes in a previous study. However, we show that the DLBCL-type with TNFAIP3 mutations was associated with a poor outcome, implying that the role of TNFAIP3 mutations might differ between DLBCL and DLBCL-type OIIA-LPDs; nevertheless, we must consider that the number of patients with TNFAIP3 mutations in the present study was limited.

In the present study, KMT2D (eight, 40%) and TNFRSF14 (six, 30%) were the most frequently mutated genes among DLBCL-type OIIA-LPDs. In previous reports, TNFRSF14 and TNFAIP3 were the candidate genes with single-nucleotide polymorphisms (SNPs) linked to rheumatoid arthritis. These rheumatoid arthritis risk genes are also related to the response of immune cells in the microenvironment. TNFAIP3 is one of the negative regulators of NF-κB signalling, and it has an important role in regulating the immune response. Kato et al. found loss-of-function mutations of TNFAIP3 (A20) in B-cell lymphomas and showed the tumour suppressor role of TNFAIP3 using functional assays. They revealed the pivotal role of TNFAIP3 as a tumour suppressor in the development of lymphoma. More recently, Carreras et al. performed genomic profiling of DLBCL-type MTX-LPD. They reported a loss in the copy number of TNFAIP3 and the copy neutral loss-of-heterozygosity of TNFRSF14. Thus, the results of these previous studies and those obtained in our present study support
the notion that TNFRSF14 and TNFAIP3 play crucial roles in the pathogenesis of DLBCL-type OIIA-LPDs.

In summary, to the best of our knowledge, this is the first retrospective study to determine the mutational characteristics of OIIA-LPDs. In our present study, DLBCL-type OIIA-LPDs showed significantly better outcomes than HL-type OIIA-LPDs. Furthermore, TNFAIP3 mutations were found to be associated with shorter PFS and FFT in DLBCL-type OIIA-LPDs. As the number of samples in OIIA-LPDs was small, further studies are warranted to identify clinically relevant predictive markers. However, the number of samples in our present study was the largest owing to the rarity of OIIA-LPDs. Therefore, the results of the present study are the best available data on the clinical and mutational characteristics of OIIA-LPDs.

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Fig 4. Positions and types of somatic mutations encoded in tumour necrosis factor alpha-induced protein 3 (TNFAIP3). (A, B) Lollipop mutation plot of TNFAIP3. (A) four mutations detected in 20 patients with diffuse large B-cell lymphoma (DLBCL)-type other iatrogenic immunodeficiency-associated lymphoproliferative disorder (OIIA-LPD) in our cohort. (B) 204 mutations detected in previous studies, combining 2207 patients with de novo DLBCL. Green circles indicate missense mutations, whereas black circles indicate truncating mutations, including nonsense, frame-shift deletion, and frame-shift insertion. OTU, OTU-like cysteine protease domain; zf., A20-like zinc finger domain. (C) Freedom from treatment (FFT) of DLBCL-type OIIA-LPD with or without TNFAIP mutation. (D) Progression-free survival (PFS) of DLBCL-type OIIA-LPD with or without TNFAIP mutation.
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**Author contributions**

Daisuke Kaji, Manabu Kusakabe, Mamiko Sakata-Yanagimoto and Shigeru Chiba contributed to the study design; Daisuke Kaji and Manabu Kusakabe performed the experiments and analysed the extracted data. All authors critically reviewed the manuscript, approved the final version, and supported this publication.

**Conflicts of interest**

The authors declare no conflicts of interest in association with the present study.

**Supporting Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig S1.** Overall survival (OS), progression-free survival (PFS), and freedom from treatment (FFT) with Epstein–Barr virus (EBV)-positive DLBCL-type and EBV-negative DLBCL-type.

**Fig S2.** Frequently mutated genes in diffuse large B-cell lymphoma (DLBCL)-type.

**Fig S3.** (A) Forest plots of freedom from treatment (FFT) of gene alterations in DLBCL-type. (B) Forest plots of progression-free survival (PFS) of gene alterations in DLBCL-type.

**Fig S4.** Overall survival (OS), progression-free survival (PFS), and freedom from treatment (FFT) with other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs) with lymphocyte ≤800/µl and lymphocyte >800/µl.

**Table S1.** The lists of 47 lymphoid cancer-related genes.

**Table SII.** Clinical features of other iatrogenic immunodeficiency-associated lymphoproliferative disorders except for the subtype of diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL).

**Table SIII.** Clinical outcome following withdrawal of methotrexate (MTX) or tacrolimus in the onset of other iatrogenic immunodeficiency-associated lymphoproliferative disorders except for the subtype of diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL).

**Table SIV.** The effect of EBV positivity for spontaneous regression.

**Table SV.** Univariate and multivariate analysis of clinical characteristics for progression-free survival (PFS) in other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs).

**Table SVI.** Uni- and multivariate analysis of clinical characteristics for progression-free survival (PFS) in diffuse large B-cell lymphoma (DLBCL)-type.

**Table SVII.** Univariate and multivariate analysis of clinical characteristics for progression-free survival (PFS) in Hodgkin lymphoma (HL)-type.

**Table SVIII.** The mean and median coverage of diffuse large B-cell lymphoma (DLBCL)-type other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs).

**Table SIX.** Gene mutations identified with other iatrogenic immunodeficiency-associated lymphoproliferative disorder (OIIA-LPD) sample.

**Table SX.** Clinical features of diffuse large B-cell lymphoma (DLBCL)-type other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPDs) with tumour necrosis factor alpha-induced protein 3 (TNFAIP3) mutations.

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