To the Editor,

Rearrangements of PDGFRα, PDGFRβ, FGFR1 or JAK2 are established features of myeloid/lymphoid neoplasms with eosinophilia (MLN-Eo). The rearrangement of the fms-related tyrosine kinase 3 (FLT3) gene should also be associated with MLN-Eo, and ETV6, SPTBN1, GOLGB1 and TRIP11 have been identified as FLT3 rearrangement partner genes (Figure S1). Cases of MLN-Eo with FLT3 rearrangement are rare but have a poor outcome.

We encountered a patient who achieved a favourable long-term outcome by allogeneic haematopoietic stem cell transplantation (allo-HSCT) and without using tyrosine kinase inhibitors, despite being refractory to conventional chemotherapy. The coiled-coil domain containing an 88C (CCDC88C)-FLT3 translocation was identified in this patient who was diagnosed with myeloid neoplasm with T-cell lymphoblastic lymphoma (T-LBL). Chronic myelomonocytic leukaemia (CMML) was one of the differential diagnoses for the current patient; the criteria of chronic myelomonocytic leukaemia included not having the specific genes, such as PDGFRα, if eosinophilia was present. The current case showed a FLT3 rearrangement, and therefore we considered a diagnosis of MLN-Eo as reasonable. The CCDC88C-FLT3 translocation was identified in T-LBL, CD34-positive haematopoietic stem and multilineage cells.

1 | CASE

A 50-year-old woman was admitted to our hospital. Her bone marrow aspiration showed hypercellular marrow (>90% cellularity) with increased myeloid cell numbers and abundant eosinophils (10%–20% all nucleated bone marrow cells (Figure S2A)). In addition, T-LBL was detected in a tonsil biopsy. Tonsil biopsy showed areas with dysplastic lymphoid cells with a predominant T-cell phenotype (CD7, CD4, CD8, CD56, TdT, CD99 and bcl-2). The CD4/CD8 ratio revealed that abnormal lymphocytes were positive for CD3, CD5, CD7, CD4, CD8, CD56, TdT, CD99 and bcl-2. The CD4/CD8 ratio was high. Since eosinophils in tonsil specimen were not so condensed, we considered a diagnosis of MLN-Eo as reasonable. The presence of MLN-Eo cells were unclear.

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narrow areas on exon 13, 14 and 15 between the TK domain and juxtamembrane area (Figure S1). Therefore, we investigated the breakpoint using inverse RT-PCR (Table S1). We first identified the breakpoint using cDNA (Figure 1A, B) and then determined the precise breakpoint using DNA (Figure 1B). Sequencing of the junction using cDNA revealed that one nucleotide was deleted (Figure S1B) through splicing from RNA to cDNA. We specified the CCDC88C gene on chromosome 14 and the precise breakpoint. Band q12 on chromosome 13 was thus identified as corresponding to FLT3 and band q32 on chromosome 14 was identified as CCDC88C. While one study reported that CCDC88C was a fusion partner gene to PDGFRB, CCDC88C-FLT3 has only previously been reported in one case of juvenile myelomonocytic leukaemia in a 20-week-old boy. Therefore, this is the first case of MLN-Eo with CCDC88C-FLT3 translocation.

The CCDC88C breakpoint in the current case and previous report were located in the intron after exon 22 and intron after exon 23 respectively. The FLT3 breakpoint was located in exon 14. The FLT3 breakpoints in the current and previously reported translocations are all located near exon 14 (Figure S1).

We next examined if the identified translocation occurred only in MLN-Eo cells or if it also occurred in other lineage cells. Sufficient transcript levels of the CCDC88C-FLT3 fusion gene were present in sorted single cells to detect by electrophoresis when amplified by RT-PCR (Figure 1A-C and Table S1). The nested RT-PCR results for each single cell are shown in Figure 1C. The CCDC88C-FLT3 breakpoint was amplified in all lineages, indicating the presence of the translocation in both myeloid and lymphoid lineages. The FLT3 rearrangement thus occurred in CD34-positive haematopoietic stem cells that differentiated into multiple lineages. The possibility that nested PCR-negative T-LBL cells did not include the translocation was low, given that the previous study reported that a mouse model of MLN-Eo with FGFR1 rearrangement showed myeloid/lymphoid neoplasms. The function of CCDC88C is not completely known. Daple (encoded by CCDC88C) modulates Wnt signalling and leads to the activation of noncanonical Wnt signalling. Tyrosine kinases and Akt are also associated with the signalling. Bioinformatic analysis showed that the CCDC88C expression level enriched in Wnt signalling was positively correlated with CD4+ T cell activation. Our patient had the FLT3 rearrangement which involved tyrosine kinase domain and the T-LBL which was positive for CD4 strongly. Tyrosine kinase domain is the key factor for MLN-Eo. Wnt signalling may relate to the emergence of T-LBL.

In the current report, we identified CCDC88C as a novel fusion partner gene to FLT3, with the translocation occurring in CD34-positive haematopoietic stem cells that subsequently differentiate into multiple lineages. The patient achieved a favourable prognosis.

**FIGURE 1** CCDC88C-FLT3 breakpoints and occurrence in myeloid and lymphoid lineages. (A) Schema of single-cell sorting for nested PCR. (B) Electrophoresis of nested PCR products on a 2% agarose gel with bone marrow at diagnosis. RT-PCR primers were designed to detect 412-bp products of CCDC88C-FLT3 respectively. Lane A: CCDC88C-FLT3; lane C: ladder. Schematic of the position of primers for (C). Red arrows indicate forward and reverse primers for CCDC88C-FLT3. (C) The result of nested RT-PCR to each single cell. The positive wells are in which we could find 969-bp products of CCDC88C-FLT3. For example, we checked 50 wells after CD3+ single-cell sorting and found 17 positive wells respectively.
with allo-HSCT without TK inhibitor. Although CMML with eosinophilia was one of the differential diagnoses for the current patient, who had a high count of monocytes in the peripheral blood, the FLT3 rearrangement that indicates MLN-Eo does not lead to the diagnosis of CMML.1,2,10,11 MLN-Eo with FLT3 rearrangement should thus be included in the World Health Organization definition of MLN-Eo. Despite the rarity of MLN-Eo with FLT3 rearrangement, the poor outcome of these patients in the absence of allo-HSCT highlights the need to investigate the disease aetiology and to develop suitable treatments.

This study was performed in accordance with the Declaration of Helsinki, and informed consent was obtained from the patient for publication of this report.

CONFLICT OF INTEREST
The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS
Yuya Kurihara: Formal analysis (lead); Investigation (lead); Methodology (lead); Validation (lead); Writing – original draft (lead).
Hideaki Mizuno: Methodology (supporting); Project administration (supporting); Supervision (supporting); Writing – review & editing (lead).
Akira Honda: Conceptualization (supporting); Project administration (supporting); Writing – review & editing (supporting).
Arika Shimura: Investigation (supporting); Writing – review & editing (lead).
Yosei Fujioka: Supervision (supporting); Writing – review & editing (supporting).
Hiroaki Maki: Supervision (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Supervision (lead); Writing – review & editing (supporting).

DATA AVAILABILITY STATEMENT
The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

Correspondence
Mineo Kurokawa, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
Email: kurokawa@m.u-tokyo.ac.jp

ORCID
Mineo Kurokawa https://orcid.org/0000-0002-4034-2422