

Case Study

# Human herpesvirus 8-unrelated primary effusion lymphoma-like lymphoma following tyrosine kinase inhibitor treatment for chronic myelogenous leukemia

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A 69-year-old man was diagnosed with chronic myelogenous leukemia (CML) and treated with dasatinib. After two years on dasatinib, the patient achieved complete molecular response, but dasatinib treatment was discontinued due to exacerbation of pleural effusion. Nilotinib and imatinib were started but stopped due to an increase in pleural effusion. Thoracentesis was performed and he was diagnosed with human herpesvirus 8-unrelated primary effusion lymphoma (PEL)-like lymphoma. Complex chromosomal abnormality, including *BCL6* rearrangement, was found on chromosome analysis. To the best of our knowledge, this is the first report of PEL-like lymphoma following tyrosine kinase inhibitor treatment for CML.

**Keywords:** primary effusion lymphoma-like lymphoma, chronic myelogenous leukemia, tyrosine kinase inhibitor, *BCR-ABL*, *BCL6*

## INTRODUCTION

Chronic myelogenous leukemia (CML) is a chronic myeloproliferative neoplasm that originates from multipotent hematopoietic stem cells and is associated with the *BCR-ABL* fusion gene located on the Philadelphia (Ph) chromosome.<sup>1</sup> Based on historical data prior to any effective therapy, median survival times for CML ranged between 2 and 3 years.<sup>2</sup> However, in the current era of tyrosine kinase inhibitor (TKI) therapy using imatinib, dasatinib, nilotinib, and bosutinib, the prognosis has been markedly improved.<sup>3</sup> Currently, an important issue is the management of adverse events. Pleural effusion is one of the major problems. The TKIs have different patterns of side effects, and pleural effusion is more frequently observed with dasatinib compared with imatinib, nilotinib, and bosutinib.<sup>4-7</sup>

Primary effusion lymphoma (PEL) is a rare type of non-Hodgkin lymphoma (NHL) confined to the body cavities, usually without a tumor mass.<sup>1</sup> It is associated with infection of human herpesvirus 8 (HHV-8), and Epstein-Barr virus (EBV) coinfection is common, especially with a background of human immunodeficiency virus type-1 (HIV) infection.<sup>8</sup> However, some cases of HHV-8-negative primary lymphomatous effusion have been reported and termed HHV-8-unrelated PEL-like lymphoma.<sup>9,10</sup>

In this report, we present a rare case of PEL-like lymphoma that was found during pleural effusion treatment with TKIs for CML.

## CASE REPORT

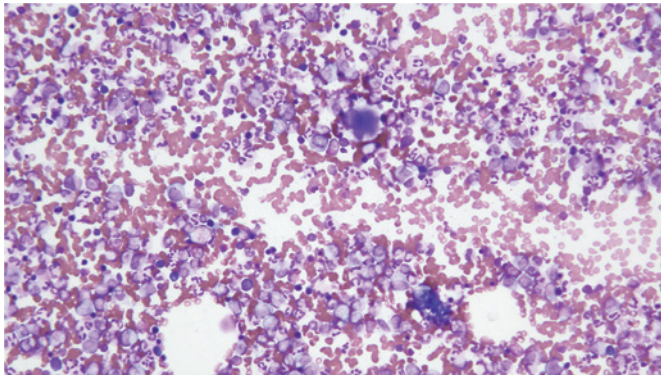
A 69-year-old Japanese male was hospitalized for cerebral infarction. Peripheral blood evaluation revealed a hemoglobin level of 12.2 g/dl, a platelet count of  $98.6 \times 10^4 / \mu\text{l}$ , a white blood cell count of  $26,800 / \mu\text{l}$  with 86% neutrophils, 6% lymphocytes, 1% monocytes, 1% eosinophils, 2% basophils, 2% myelocytes, and 2% metamyelocytes. Hepatitis B, Hepatitis C, HIV, and HTLV-1 were negative. Hypercellular bone marrow with an increase of megakaryocytes was observed on the bone marrow test, and the myeloblast count was less than 5% (Figure 1). Chromosomal analysis was abnormal with 46, XY, t(9;22)(q34;q11.2) [20/20]. The patient was diagnosed with CML in the chronic phase. Dasatinib was started at 100 mg once daily. After a year of dasatinib treatment, the patient achieved major molecular response (MMR). However, he developed dyspnea and bilateral pleural effusion was observed on chest X-ray; therefore, diuretics were started. After two years on dasatinib, the patient achieved complete molecular response (CMR), but dasatinib treatment was discontinued due to exacerbation of

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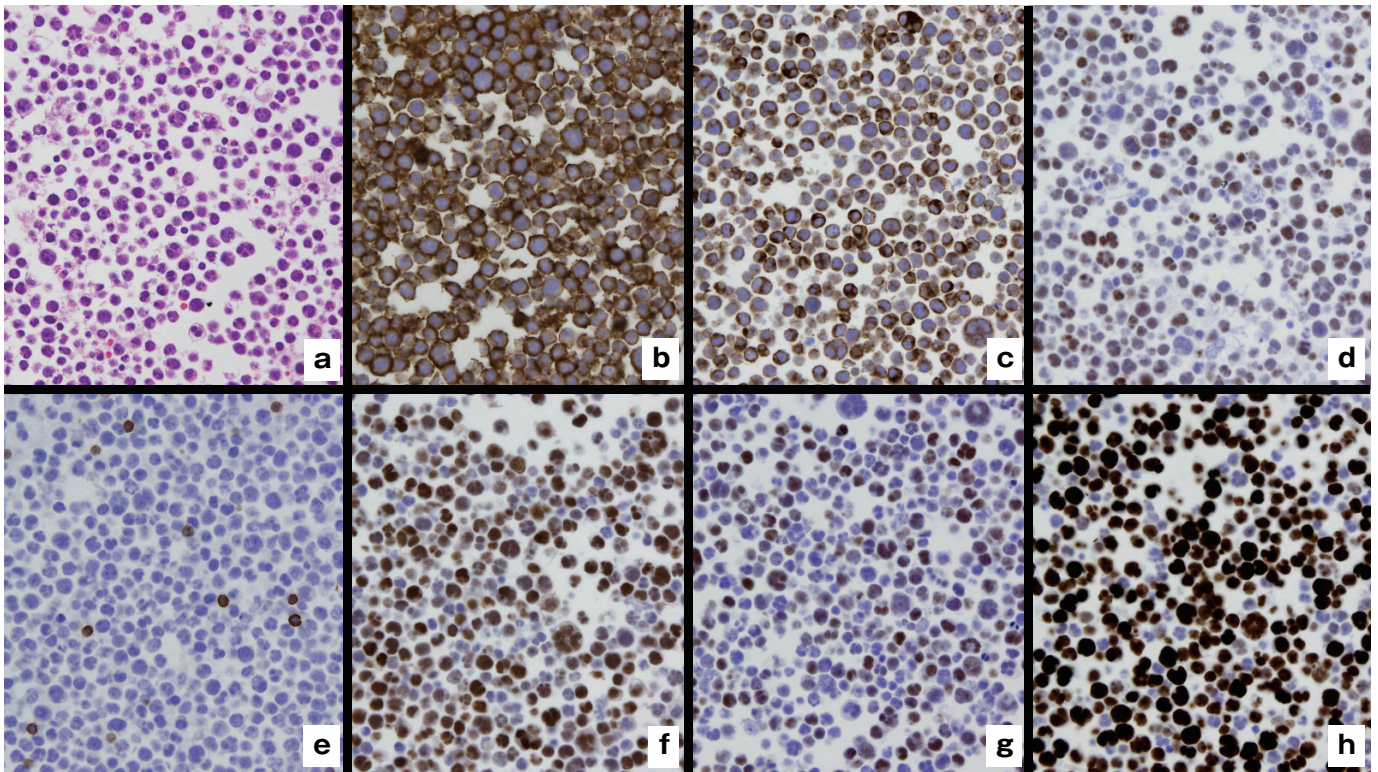
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pleural effusion resistant to diuretics and steroids. Nilotinib at 150 mg twice daily was selected because the pleural effusion had decreased, but the pleural effusion increased again, and nilotinib was discontinued after two weeks. Imatinib at 400 mg once daily was started after discontinuation of nilotinib, but was stopped after only ten days because pleural effusion increased again. Even after TKIs were stopped, the pleural effusion did not decrease and his dyspnea remained. Thus, thoracentesis was performed. IgM, bcl-2, cytoplasmic CD79a, and  $\kappa$ -chain were positive on flow cytometry, and molecular testing on the pleural effusion sample indicated a monoclonal immunoglobulin heavy chain gene rearrangement. Microscopically, in the cytocentrifuge preparation of the pleural effusion, cells composed of mid to large-sized pleomorphic nuclei with abundant cytoplasm, and



**Fig. 1.** Bone marrow test showed hypercellular bone marrow with an increase of megakaryocytes and a myeloblast count less than 5%.

perinuclear halo consistent with plasmacytoid differentiation were occasionally seen. Immunohistochemical staining for CD20, CD79a, CD99, BCL2, BCL6 and MUM-1 was positive, but MPO, CD3, CD5, CD7, CD10, CD34, CD56 and TdT were negative, and EBER RNA in situ hybridization was negative. The MIB-1 labeling index indicated more than 90% tumor cells (Figure 2). HHV-8 immunohistochemistry was negative and HHV-8 DNA quantification in the pleural effusion was less than 100 copy/mL. Chromosome analysis based on a G-banding analysis demonstrated the karyotype  $82<3n>,XXY,+Y,+2,+der(3)?t(3;14)(q27;q32)x2,?t(3;9;14)(q27;p13;q32),-6,-7,add(7)(p22),+der(9)?t(3;9)(q27;p13),+13,+add(14)(q32),+18,+19,+21,+5mar$ . FISH analysis was negative for *BCR-ABL* and positive for *BCL6* (Figure 3). The translocation of 9p13 may have caused an abnormality in *PAX-5*, but FISH analysis was not performed. Based on these findings, a diagnosis of PEL-like lymphoma was made. Mild hypocellular marrow without lymphoma cells and dysplasia was found on bone marrow biopsy, and the marrow karyotype was normal. The patient was initially treated for de novo PEL-like lymphoma with the R-CHOP (rituximab, cyclophosphamide, vincristine, doxorubicin, and predonisolone) regimen. After two cycles of R-CHOP, doxorubicin was stopped due to possible anthracycline cardiomyopathy even though the patient had no risk of cardiac disease before R-CHOP. After 3 additional cycles of R-CVP treatment, we stopped the chemotherapy because of systemic edema due to heart failure. Computed tomography (CT) revealed remnant bilateral pleural effusion, but FDG accumulation was not observed with Positron Emission Tomography



**Fig. 2.** Histopathological and immunohistochemical appearance of PEL-like lymphoma. (a) Hematoxylin-eosin staining, (b) CD20, (c) BCL2, (d) BCL6, (e) CD3, (f) CMYC, (g) MUM1 and (h) MIB1

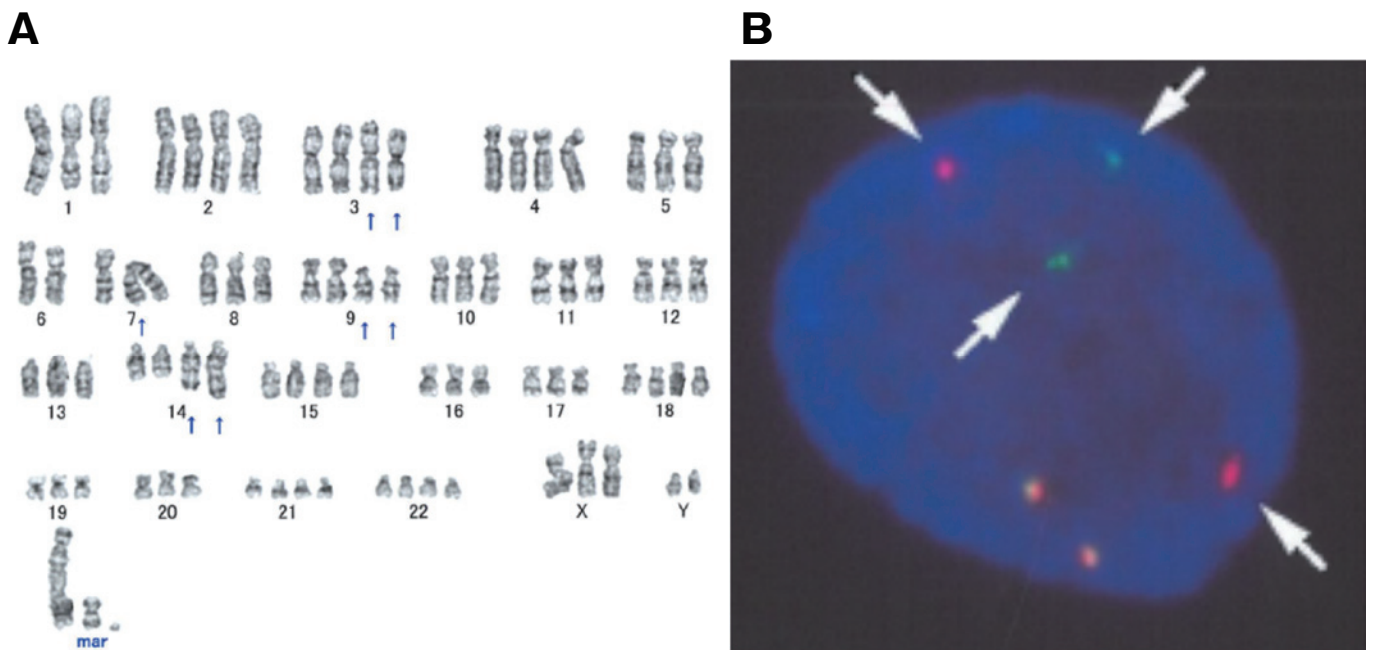
(PET)-CT. As lymphoma cells were not observed on thoracentesis, he was considered to have achieved complete remission. After chemotherapy for PEL-like lymphoma, *BCR-ABL* IS% increased (0.6795) and he was diagnosed with loss of MMR. The patient began bosutinib treatment, but discontinued due to diarrhea and anorexia. Nilotinib was re-started and the patient achieved MMR.

**DISCUSSION**

CML is thought to arise from a multi-potent hematopoietic stem cell that has undergone *BCR-ABL* translocation, resulting in a shortened chromosome 22, referred to as the Philadelphia (Ph) chromosome. Takahashi *et al.* reported that the Ph chromosome was found in pluripotent stem cells, but in a lower incidence among B lymphocytes than among neutrophils and monocytes.<sup>11</sup> Their hypothesis is that lymphocytes with the Ph chromosome fail to differentiate and the usual target of malignant transformation in CML is a more restricted stem cell committed to B-cell lineages. This is consistent with the B-cell population being chimeric with respect to *BCR-ABL*. In our case, *BCL6* was positive, but *BCR-ABL* was negative in FISH analysis; therefore, PEL like lymphoma originated from *BCL-ABL*-negative B lymphocytes. Non-Hodgkin lymphoma during CML treatment is rare. Ichinohasama R *et al.* reported two cases of Ph-negative non-Hodgkin lymphoma occurring in the chronic phase of Ph-positive CML, defined as a genetically different neoplasm from extramedullary localized blast crisis.<sup>12</sup> The author advocates there are three types of lymphoid neoplasms related with the Ph chromosome or status of CML as follows: Type A: Lymphoid blast crisis (both localized or not localized) consisting of Ph-positive cells in CML (same genetic

background for both lymphoid neoplasm and CML), Type B: Ph-negative lymphoid neoplasm occurring in CML (different genetic background between lymphoid neoplasm and CML), and Type C: Ph-positive lymphoid neoplasm in a patient without Ph-positive disorders. It is very important to distinguish the subtypes to determine the appropriate treatment. Our case corresponds with Type B, and he was in complete remission after rituximab-combined chemotherapy, which is the standard treatment for B-cell lymphoma, without needing a stronger regimen.

In long-term follow-up of imatinib treatment for CML, the incidence of secondary neoplasm was 12/551 (2.2%) and that of hematological neoplasm was 5/551 (0.9%).<sup>13</sup> Thus, the incidence of secondary neoplasm was not increased by imatinib treatment for CML. Dasatinib treatment is also similar.<sup>14</sup> The onset mechanism of neoplasms by TKI is unclear. Kawamata *et al.* reported that imatinib reduces expression of AID by off-target effects, and it suppresses class switching and causes low gammaglobulinemia.<sup>15</sup> It is unknown whether dasatinib has the same effects, but if we apply this hypothesis to our case, lymphoma may have developed due to hypo-gammaglobulinemia. On the other hand, pleural effusion associated with TKIs is more frequently observed with dasatinib than with other TKIs.<sup>16</sup> The mechanism by which dasatinib induces pleural effusion is likely related to off-target kinase inhibition. In addition to Abl, dasatinib potently inhibits platelet-derived growth factor receptor beta (PDGFRB),<sup>17,18</sup> a receptor expressed in pericytes<sup>19</sup> that is involved in the regulation of angiogenesis.<sup>20</sup> PDGFRB-deficient mouse embryos lack microvascular pericytes, and develop defective blood vessels and edema.<sup>21</sup> Blockade of PDGFRB with CDP860, a humanized pegylated diFab in patients with advanced ovarian and colorectal



**Fig. 3.** (A) G-banded karyotype from the pleural effusion, revealing 82<3n>,XXY,+Y,+2,+der(3)?t(3;14)(q27;q32)x2,?t(3;9;14)(q27;p13;q32),-6,-7,add(7)(p22),+der(9)?t(3;9)(q27;p13),+13,+add(14)(q32),+18,+19,+21,+5mar. (B) Fluorescence in situ hybridization analysis for *BCL6* of pleural effusion. *BCL6* = yellow signal, 5' *BCL6* = red signal and 3' *BCL6* = green signal

carcinoma, resulted in early interruption of the study after three of the eight treated patients developed significant ascites and/or pleural effusion, likely resulting from a marked reduction in interstitial fluid pressure.<sup>22</sup> The negligible effects of imatinib on PDGFRB may explain the low incidence of this event with this agent.<sup>23-25</sup> Another possible explanation is that tyrosine kinases responsible for capillary integrity may be overexpressed in the pulmonary vasculature and/or pleural epithelium. Src regulates focal adhesion and *adherens* junctions, two subcellular matrix attachment structures key in regulating cell adhesion,<sup>26,27</sup> which may play a role in the stability of the pleural epithelium and pleural space homeostasis. The vascular permeability activity mediated by vascular endothelial growth factor is directly dependent on the Src-related kinases Yes and Src, which are widely expressed in lung tissue<sup>28,29</sup> and inhibited by dasatinib.<sup>17,18,30</sup> However, other dual Src and abl inhibitors, such as bosutinib, have not been associated with pleural effusion<sup>31</sup> and bosutinib does not inhibit PDGFRB.<sup>32</sup> Although there are some reports on the mechanism of TKI-related pleural effusion, PDGFRB rearrangement is usually associated with myeloid tumors, as typified by chronic eosinophilic leukemia, and the relationship with TKI-related pleural effusion and the onset mechanism of PEL-like lymphoma are unclear. Elucidation of their relevance is a future task.

To the best of our knowledge, this is the first report of treatment of PEL-like lymphoma following TKI treatment for CML. Accumulation of cases and further analysis are needed.

## CONFLICT OF INTEREST

The authors have no conflicts of interest or funding to disclose.

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