Myeloid/lymphoid neoplasms associated with eosinophilia and rearrangements of *PCM1::JAK2* with erythroblastic sarcoma: a case report and literature review

Translocations and rearrangements involving *JAK2* are less common. Among them, the *PCM1::JAK2* fusion gene derived from t(8;9)(p22;p24) is the most frequent. In the 2016 World Health Organization (WHO) classification, myeloid/lymphoid neoplasms with eosinophilia (MLN-Eo) and *PCM1::JAK2* fusions were defined as new provisional entities, although eosinophilia may be absent in a subset of cases. These neoplasms share common features, such as eosinophilia; large immature erythroid islands, with some lymphoid aggregates; myelofibrosis, usually in combination with splenomegaly; and predominance in males.[1] Myeloid sarcoma (MS) is a rare solid tumor formed by primitive or naive myeloid cells in extramedullary sites, mostly secondary to hematologic malignancies. Erythroblastic sarcoma (EBS) is an erythroblastic form of MS, with only a handful of case reports published to date.[2] Here, we report an MLN-Eo case with *PCM1::JAK2* rearrangements and EBS that was challenging to diagnose and treat.

Ethical review and approval were not required for this study on human participants by the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the patient.

A 72-year-old male presented with fatigue in April 2021. A complete blood count analysis revealed a white blood cell count of 10.8×10^9/L, hemoglobin (HGB) level of 90 g/L, platelet (PLT) count of 70×10^9/L, and eosinophil level of 2.12×10^9/L. However, he did not consult a hematologist. In January 2022, his fatigue worsened, with a HGB level of 75 g/L. The patient underwent a positron emission tomography/computed tomography (PET/CT) scan, which showed moderate splenomegaly with a mild increase in diffuse fluorodeoxyglucose metabolism. He experienced the first marrow aspiration but could not receive a definite diagnosis. Iron supplementation was given but was ineffective. The HGB level decreased to 59 g/L, and the PLT count decreased to 34×10^9/L. The patient was referred to our institution. Repeated aspiration and biopsy of the bone marrow indicated myelofibrosis with eosinophilia and left-shifted erythroid predominance (Figure 1A-D).

Chromosome analysis revealed a 46,XY,t(8;9)(p22;p24)[16]/46,XY[4] karyotype (Figure 2A). Reverse transcription polymerase chain reaction (RT-PCR) (Figure 2B) and fluorescence *in situ* hybridization (FISH) assay (Figure 2C, D) showed *PCM1::JAK2* gene fusions in the marrow. A 248 gene DNA-based myeloid-targeted next-generation sequencing (NGS) panel was performed on the marrow with a sequencing depth of 4,544X. Runt-related transcription factor 1 (*RUNX1)* mutations were found with a variant allele frequency of 8%. The *BCR::ABL, MPLW515L/K, CALR, PDGFRA, PDGFRB*, and *FGFR1* genes were analyzed by RT-PCR, and all results were negative.

A biopsy of an enlarged right posterior cervical lymph node was performed. The results revealed neoplastic cells that were positive for erythroid-specific markers such as the transferrin receptor (CD71) and glycophorin 235a (Figure 1E, F) but negative for conventional pan myeloid markers including CD34 and MPO.

Therefore, all findings were diagnostic of EBS arising from an MLN-Eo with *PCM1::JAK2* rearrangements. The patient declined chemotherapy, involved-field radiotherapy, and *JAK2* inhibitors due to the low PLT level. Considering a normal erythropoietin concentration of 7.33 mIU/mL (normal range: 2.59-18.5 mIU/mL) at diagnosis, erythropoietin (at a dose of 10,000 units) was initially administered three times per week. The HGB level gradually increased to 105 g/L on day 165 (day 0 corresponds to erythropoietin initiation) without transfusions. However, the HGB level gradually decreased to 76 g/L on day 312 for unknown reasons. Shortly after, prednisone (30 mg daily) was added in addition to the original erythropoietin treatment and tapered to a maintenance dose of 10 mg daily on day 326. At the last follow-up (day 370), the HGB level increased to 119 g/L. The PLT and eosinophil counts were maintained at 36×10^9/L and 2.3×10^9/L, respectively. The patient is currently under close follow-up and enjoying an excellent performance status. The treatment of erythropoietin and prednisone continues.

This case is a unique presentation of an EBS, an exceptionally rare entity. To the best of our knowledge, only a handful of EBS cases have been reported in the English literature so far.[2-5] EBS in pediatric patients was manifested as either isolated sarcomas without systemic involvement or with associated erythroid leukemia, while those developed in adults present with myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CMML), primary myelofibrosis (MF), MLN with *PDGFRA* or *JAK2* rearrangement, myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN), and erythroid leukemia. Due to the small number of cases, it is difficult to predict survival and optimal treatment. Generally, EBS has short-term survival and poor prognosis; in seven of the previously reported cases, the patients died within 6 months of diagnosis. In terms of MLN-Eo with

t(8;9)(p22;p24)/*PCM1::JAK2* rearrangement and synchronous MS, only three cases have been reported to date (Table 1).[5-7] The treatment was unknown in one case and the other two cases were treated with local radiotherapy and chemotherapy. One of them then received allogenetic stem cell transplantation (ASCT), and the other was awaiting transplantation. There is currently no consensus on the management of sarcoma with MLN. Treatment can be aggressive or expectant based on patients' characteristics, preferences, and experience of the treating physician. If aggressive treatment is not initiated upfront, then close follow-up is needed.

This case also presented uncommon *PCM1::JAK2* rearrangement. *PCM1::JAK2* rearrangement affects a wide variety of hematopoietic cell lines and, therefore, can cause both myeloid and lymphoid malignancies, while MPN is the most common among the diseases. These patients had spanned the entire gamut of ages with a median age of 47 years. Nearly 80% of reported cases were male. The reason for the male preponderance is not clear.[8] Lierman *et al.*,

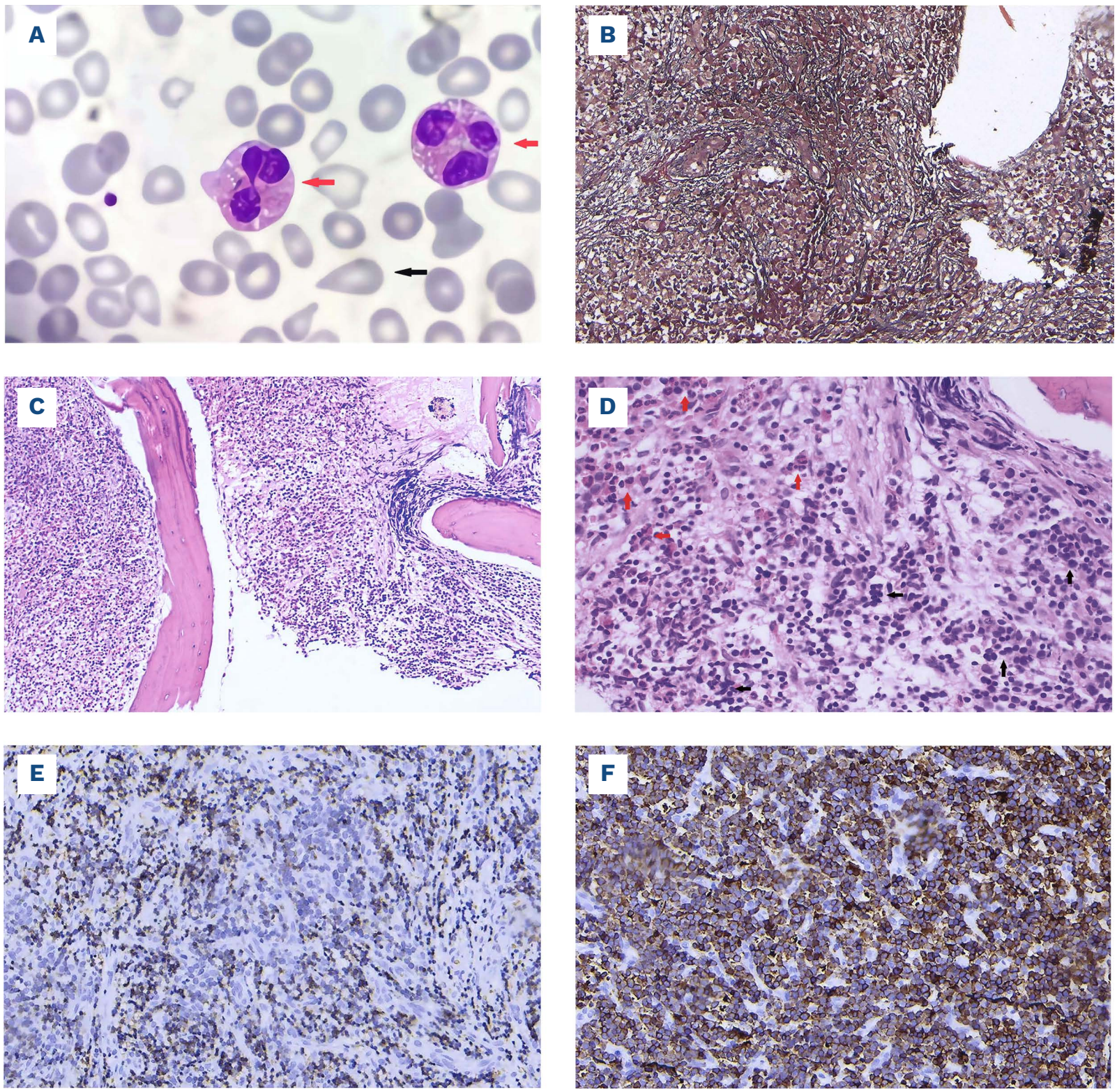

Figure 1. Erythroblastic sarcoma arising in a myeloid neoplasm with *PCM1::JAK2* rearrangement: morphology and immunohistochemistry at diagnosis (A) Eosinophils (red arrow) and teardrop erythrocytes (black arrow) were easily seen on the peripheral blood smear (×1,000 magnification). (B) Bone marrow biopsy showed a marked increase in argentophilic reticulin via silver impregnation staining and indicated marked reticulin fibrosis (×400 magnification). (C, D) Marrow biopsy showed numerous clusters of erythroid precursors (black arrow) and predominantly eosinophils (red arrow) via hematoxylin and eosin (H&E) staining (C, ×100 magnification; D, × 400 magnification; D is the magnified image of C). (E, F) Biopsy of the patient's right posterior cervical lymph node via immunohistochemistry showed sheets of neoplastic cells highlighted by CD235a (E, ×400 magnification) and CD71 (F, ×400 magnification) immunostaining.

first reported the use of ruxolitinib, a potent *JAK1/2* inhibitor, for chronic eosinophilic leukemia with *PCM1::JAK2* rearrangement at an initial dose of 10 mg twice daily, followed by a cautious increase to 15-20 mg twice daily. A complete cytogenetic response was observed after 15 months without recurrence for at least 36 months.[9] Since then, there have been several positive responses to ruxolitinib treatment against such diseases, but the small

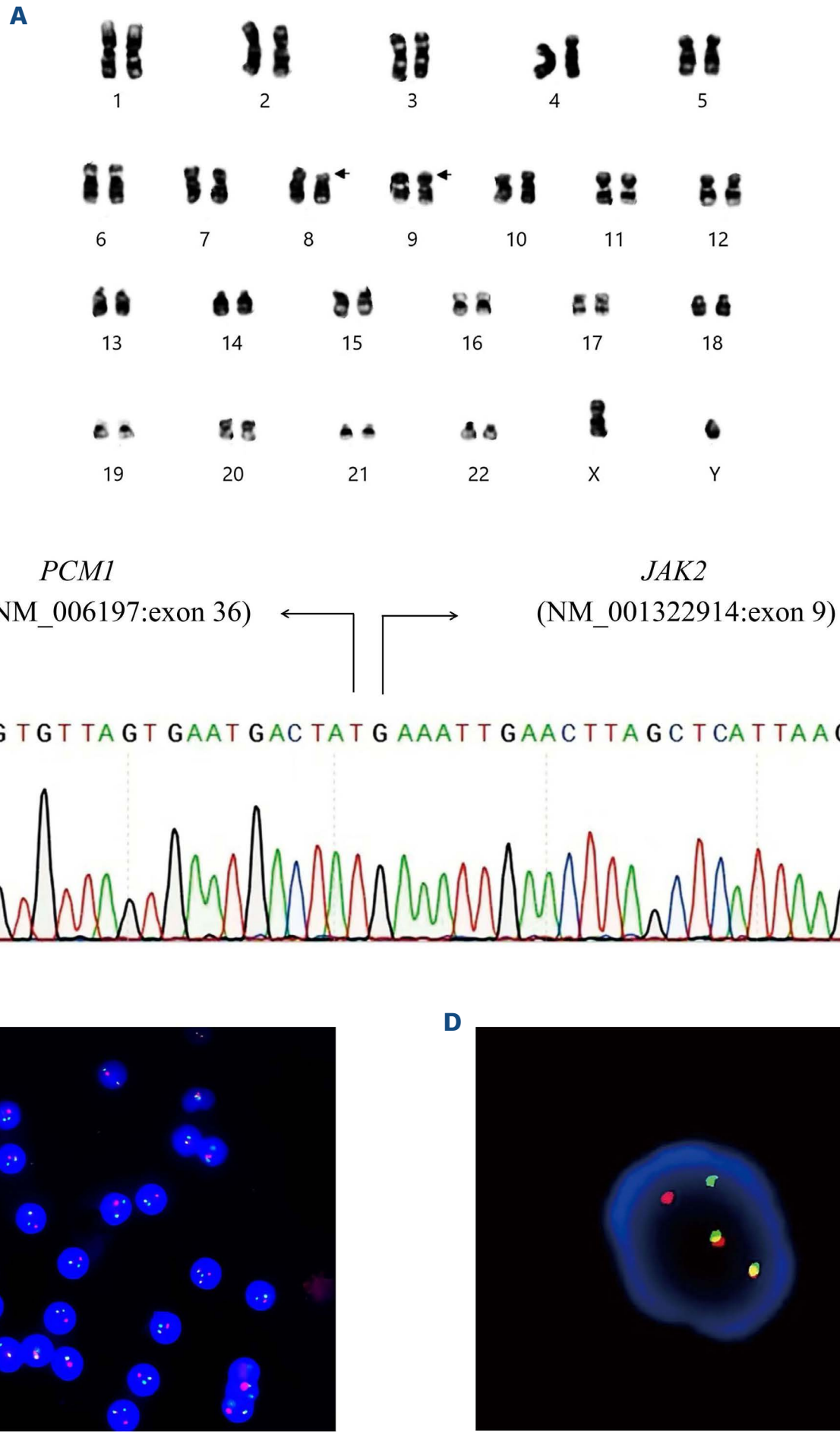

Figure 2. Erythroblastic sarcoma arising in a myeloid neoplasm with *PCM1::JAK2* rearrangement: genetic features at diagnosis
(A) Chromosome analysis of marrow revealed a 46,XY,t(8;9)(p22;p24) karyotype. (B) Reverse transcription polymerase chain reaction revealed the sequence of the chimeric *PCM1::JAK2* gene showing in-frame fusion between exon 36 of *PCM1* and exon 9 of *JAK2*. (C) Fluorescence *in situ* hybridization (FISH) analysis with a *JAK2* dual colour break-apart probe showed split signal patterns (1 red, 1 green, and 1 yellow) in 74% cells. (D) FISH analysis with a *PCM1* (green)/*JAK2* (red) dual-colour dual fusion probe showed a signal consistent with 2 fusions (2 yellow, 1 red, and 1 green) and *PCM1::JAK2* rearrangement.

number of patients reported precludes a definitive statement regarding its effect on survival. Further studies are needed to determine how best to utilize ruxolitinib. Therefore, currently, ASCT is still the only way to cure the disease, particularly in the absence of acute leukemia.[8] For those patients who are unsuitable for transplantation, the prospect seems very bleak. Some patients with MPN received hydroxyurea or interferon for initial treatment and then followed a strategy of watch and wait.[10]

Although effective standard treatment strategies have not yet been established for elderly patients who are not candidates for transplantation, here, we illustrate the value of symptom-directed conventional drug therapies in certain settings. The patient initially responded to erythropoietin, although he experienced progressive anemia after 4 months. Low levels of serum erythropoietin at the onset of therapy may be a predictor of the efficacy of erythropoietin. In primary MF patients, prednisone was an option for MF-associated anemia.[11] Low-dose prednisone was, thus given, resulting in rapid improvement in overall symptom burden and anemia. Further, enhanced CT examination of cervical lymph nodes didn't reveal enlargement of the originally involved lesion.

This case also indicates that cytogenetic and molecular testing is a crucial part of the workup for hematopoietic neoplasms. In addition to conventional karyotype analysis, thorough evaluation should utilize a targeted sequencing panel to look for mutations that may have a significant impact on the patient's therapy and likely, prognosis. As hematological neoplasms with *PCM1::JAK2* rearrangement are rare, it is difficult to conduct large-scale clinical studies on the molecular characterization. NGS assays detected relatively small *RUNX1* mutation clones in the marrow of our case. Similar to *JAK2* and spliceosome genes, *RUNX1* showed the highest predictive value for myeloid neoplasms. Notably, mutations in *RUNX1* are usually not detected in the prediagnostic phase but are mostly identified in the context of a full-blown myelodysplastic syndrome or acute myelogenous leukemia (AML). For example, *RUNX1* is more frequently detected in secondary AML post-CMML than during CMML and is associated with disease progression and poor prognosis.[12] Unlike other MLN-Eo subgroups, such as *PDGFRA, PDGRFB*, and *PCM1::JAK2*, somatic mutations are common in the *FGFR1*-rearranged subgroup and frequently involve *RUNX1*.[13-14] A retrospective study involving 61 MLN-Eo cases found that 23% (14/61) of patients carried at least one mutation in addition to WHO-defined genetic changes. Eighty-three percent of cases (5/6) with *FGFR1* rearrangement were found with mutations, all of which involved *RUNX1*. However, mutations were detected in only 14% (1/7) of the *PCM1::JAK2* subgroup, which were *TET2*.[15] Of note, *TET2* is also mutated in older individuals without a hematologic malignancy, which was recently described as clonal hematopoiesis of indeterminate potential. Therefore, the role of *RUNX1* mutations in the occurrence, progression, and prognosis of our *PCM1::JAK2* fusion case remains uncertain.

In conclusion, we reported a novel case of *PCM1::JAK2* fusion mutation-related MLN-Eo with EBS. It is important that cytogenetics, sometimes FISH, PCR, and/or NGS, should be completed at the time of initial diagnosis. To date, ASCT is the only potentially curative treatment. Prospective studies are required to confirm whether ruxolitinib could be a first-line treatment option for this rare disease, especially for those who are not eligible for transplantations. Given the limitations of the data, at this point in time, supportive treatment and symptomatic care may be

Table 1. Clinical course of patients with t(8;9)(p22;p24)/*PCM1::JAK2* rearrangement accompanying myeloid sarcoma.

Reference	Age, yrs Sex	Type	Biopsy site	Initial diagnosis	Treatment	ASCT	Response	Survival (mths) (from diagnosis of MS to the last follow-up reported)
Rizzuto G, *et al.* 2022[6]	53 F	MS	Right ileo-psoas muscle	MF, B-ALL	Two cycles of FLAI regimen; a cycle of high-dose methotrexate and cytarabine, also for CNS prophylaxis; local radiotherapy; ASCT; ruxolitinib as post-transplant maintenance	ASCT after CR	CR	14
Luedke C, *et al.* 2020[5]	32 M	EBS	Axillary LN	Myeloid neoplasm	NA	NA	NA	NA
Song I, *et al.* 2016[7]	42 M	MS	Right axillary LN and an inguinal LN	MPN-U	Chemotherapy with arabinoside and erythromycin	Awaiting	Unknown	3

F: female; M: male; MS: myeloid sarcoma; EBS: erythroblastic sarcoma; LN: lymph node; MF: myelofibrosis; B-ALL: B-cell acute lymphoblastic leukemia; MPN-U: myeloproliferative neoplasm, unclassifiable; FLAI: Fludarabine + Cytarabine + Idarubicine; CNS: central nervous system; ASCT: allogeneic stem cell transplantation; NA: not available; CR: complete remission; yrs: years; mths: months.

considered for non-transplant candidates with relatively stable diseases.

Authors

Lina Zhang,[1] Xiaoqin Zhu,[2] Weiying Qu,[2] Yingjia Lu,[2] Zhou Feng[2] and Lin Zhao[2]

[1]Department of Biochemistry, Institute of Basic Medicine, Shanghai University of Traditional Chinese Medicine and [2]Department of Hematology, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China

Correspondence:
L. Z. ZHAO - sg1520@shutcm.edu.cn

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Contributions

LZ, LNZ, and XQZ gathered the clinical information and drafted the manuscript. LZ and LNZ revised the paper. LZ approved the final diagnosis and formulated the therapeutic strategies. WYQ, YJL, and ZF reviewed multiple drafts of the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Data-sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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