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Case report

Burkitt leukemia with precursor B-cell features that developed after ruxolitinib treatment in a patient with hydroxyurea-refractory *JAK2*^{V617F}-myeloproliferative neoplasm

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A 62-year-old woman, who had a 16-year history of *JAK2*^{V617F}-mutated myeloproliferative neoplasm (MPN), developed Burkitt leukemia (BL) 16 months after treatment with ruxolitinib to control hydroxyurea-refractory conditions. BL cells were CD10^{+}, CD19^{+}, CD20^{-}, CD34^{-}, cytoplasmic CD79a^{+}, and TdT^{+}, and lacked surface immunoglobulins but expressed the cytoplasmic μ heavy chain. In the bone marrow, nuclear MYC^{+} BL cells displaced the MPN tissues. t(8;14)(q24;q32) occurred at a CG dinucleotide within *MYC* exon 1 and at the IGHJ3 segment, and an N-like segment was inserted at the junction. The V-D-J sequence of the non-translocated IGH allele had the unmutated configuration. DNA from peripheral blood at a time of the course of MPN exhibited homozygous *JAK2*^{V617F} mutation, while that at BL development included both *JAK2*^{V617F} and wild-type DNAs. Although the association between JAK1/2 inhibitor therapy for MPN and secondary development of aggressive B-cell neoplasm remains controversial, this report suggests that, in selected patients, close monitoring of clonal B-cells in the BM is required before and during treatment with JAK1/2 inhibitors.

Keywords: myeloproliferative neoplasm, *JAK2*^{V617F} mutation, ruxolitinib, precursor B-cell Burkitt leukemia, t(8;14)(q24;q32)/*MYC*-IGH

INTRODUCTION

Burkitt lymphoma is a rapidly growing B-cell tumor characterized by chromosomal translocations involving the *MYC* gene on chromosomal band 8q24 and either one of the three immunoglobulin (IG) loci.[1] The disease initially presents not only in extranodal sites but also as an acute leukemia with extensive peripheral blood (PB) and bone marrow (BM) involvement, i.e., Burkitt leukemia (BL) or the L3 type of acute lymphoblastic leukemia in the French-American-British (FAB) group scheme.[1] Although leukemia cells of BL typically express cell surface IG μ heavy chain (IgM) with a restrictive light chain, B-cell antigens, and germinal center (GC) markers,[1] small fractions of both pediatric and adult cases have a precursor B-cell phenotype, i.e., expression of TdT and sometimes CD34, and absence of CD20 and surface IG expression (Supplementary Table S1).[1-9]

Whether there is an increased risk of developing lymphoproliferative disorders in patients with myeloproliferative neoplasm (MPN) remains controversial.[10-14] Reported lymphoid neoplasms include both T-cell and B-cell tumors and a wide range of histopathological subtypes.[11,15,16] Currently, it remains to be determined whether this association indicates the presence of common pathogenetic pathways between the myeloid and lymphoid tumors or is limited to patients who have received JAK1/2 inhibitor therapy.[10] In this report, we describe a case of BL that developed in a patient with MPN who was treated with a JAK1/2 inhibitor, ruxolitinib. Although leukemia cells exhibited a cytomorphology typical of BL and carried a t(8;14)(q24;q32) translocation, the cells showed precursor B-cell features.

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CASE PRESENTATION

A 47-year-old woman was referred to our department due to thrombocytosis. Her hemoglobin level was 14.6 g/dL, white cell count was 12.5 × 10^3/μL, and platelet count was 1,736 × 10^3/μL. The BM showed marked hypercellularity with the proliferation of large to giant megakaryocytes. Eight years after the initial presentation, hydroxyurea was initiated to control progressive leukocytosis and splenomegaly. Examination of the BM at the age of 60 years again showed marked hematopoietic hypercellularity with an increase of megakaryocytes (Figure 1A). No cytogenetic abnormality was found. Fourteen years after the initial presentation, she developed hemorrhage from gastric varices along the greater curvature of the stomach, which was successfully obliterated by cyanoacrylate injection. To reduce the collateral blood flow from the spleen, we initiated ruxolitinib in combination of hydroxyurea, leading to a reduction in the size of the spleen and collateral vessels.

One year and 4 months after the initiation of ruxolitinib, she, at the age of 62, presented with severe anemia and multiple skin petechiae on the body and extremities. The spleen was enlarged below the level of the umbilicus. Her hemoglobin was 7.0 g/dL, white cell count was 12.50 × 10^3/μL including 36.0% BL cells, and platelet count was 2 × 10^3/μL. The level of lactate dehydrogenase was 18,248 U/L and uric acid was 20.0 mg/dL. She developed pulmonary hemorrhage and died shortly after. Her entire course is illustrated in Supplementary Figure S1.

BL cells in PB were medium in size, having deeply basophilic cytoplasm with vacuoles. Flow cytometry (FCM) revealed that these cells were $CD10^+$, $CD19^+$, $CD20^-$, $CD21^-$, $CD22^{+/-}$, $CD23^-$, $CD24^+$, $CD34^-$, $CD38^+$, HLA-DR^+, cytoplasmic $CD79a^+$, and TdT^+. The cells lacked surface IGs but expressed the IgM in the cytoplasm (Figure 1B). BM was 'dry tap' and the biopsy demonstrated replacement by BL cells associated with starry sky macrophages (Figure 1C). The cells were positive for nuclear MYC expression and Ki-67 positivity was >95%. $CD10^+$, $CD20^-$, $CD79a^+$, $CD34^-$, and TdT^+ were confirmed by immunohistochemistry (Figure 1C). BCL2, BCL6, and Epstein-Barr virus–encoded small RNA were negative. The FAB-L3 morphology could be more favorably viewed in the touch preparation of the biopsy specimen (Figure 1C).

G-banding of the metaphase spreads obtained from PB demonstrated t(8;14)(q24;q32), and FISH using the *MYC*-IG heavy locus (IGH) dual fusion probe revealed the generation of *MYC*-IGH fusion signals at der(8) and der(14) chromosomes (Supplementary Figure S2). FISH on interphase nuclei confirmed the rearrangement of *MYC*, while no rearrangements of *BCL2* or *BCL6* were detected. To characterize t(8;14)(q24;q32)/*MYC*-IGH, we performed long-distance (LD-) polymerase chain reaction (PCR) using primers designed for *MYC* exon 2 and for the enhancer (En) and IGHM, IGHG, and IGHA constant genes of IGH.[17] The results showed that the *MYC*-En and *MYC*-IGHM primer combination generated approximately 6-kb and >10-kb LD-PCR products, respectively (Figures 2A and B). Nucleotide sequencing of the former products demonstrated that breakage and reunion occurred at a CG dinucleotide located 479-bp upstream of the noncanonical start site of *MYC* within exon 1 and at 5-bp 3′ of the 5′ end of the IGHJ3 segment, and 5 N-nucleotides were inserted at the junction (Figure 2C).

Multiplex PCR for the antigen receptor gene rearrangements generated a single species of PCR products of the IGH. Using the consensus IGHJ (reverse) and custom IGHV (forward) primers, we obtained the entire length of the IGH V-D-J sequence in frame that encoded a functional IG molecule. An IgBLAST search revealed that sequences of each segment matched those of IGHV3-30*18/IGHV3-30-5*01, IGHD3-10*01, and IGHJ6*02 with 100% identity. The length of CDR3 in codons was 25 (Supplementary Figure S3). We confirmed that the V-D-J sequences obtained represented the IGH gene that was not involved in t(8;14)(q24;q32) by LD inverse PCR (data not shown).[18]

To investigate the status of *JAK2*^{V617F} mutation, we prepared DNA from PB obtained at a time of the course of MPN, in addition to that at the development of BL, in which both myeloid cells and BL cells were present, and subjected them to melting curve assay.[19] As shown in Figure 2D, the melting curve of DNA at MPN course overlapped that of *JAK2*^{V617F} reference DNA, while DNA at BL development exhibited a minor melting peak at the position of *JAK2* wild-type (wt) DNA. We then amplified a fragment encompassing the site of mutation and subjected the PCR products to Sanger sequencing. The resulting electropherograms demonstrated a single thymidine (T) peak at nucleotide position 1,849 (c.G1849T; NCBI reference sequence, NP_001309123.1) in DNA at MPN course, while a heterozygous guanine (G) peak appeared at this position in DNA at BL development, confirming the presence of both *JAK2*^{V617F} mutant and *JAK2*^{wt} DNAs in the latter material.

DISCUSSION

Here, we describe the course of a patient who had a 16-year history of *JAK2*^{V617F}-mutated MPN. After 16 months of ruxolitinib therapy to control hydroxyurea-refractory conditions, she developed BL with t(8;14)(q24;q32), and nuclear MYC^+ BL cells displaced MPN tissue in BM. BL cells exhibited a precursor B-cell immunophenotype and carried unmutated IGH V-D-J sequences, most likely encoding the cytoplasmic IgM. Although precursor B-cell BL has been described primarily in the pediatric literature,[6,7] this report confirmed that this rare type of BL can occur in the adult population (Supplementary Table S1).[1-5,8,9]

As t(8;14)(q24;q32)/*MYC*-IGH breakpoints in sporadic cases of BL occur nearby or within *MYC* exon 1, which is a target of somatic hypermutation (SHM),[20] and within the switch regions of IGH, the translocation has been considered to be mediated by the SHM/class switch recombination (CSR) mechanism in the GC.[1,2] However, as BL cells of this case exhibited a precursor B-cell immunophenotype and

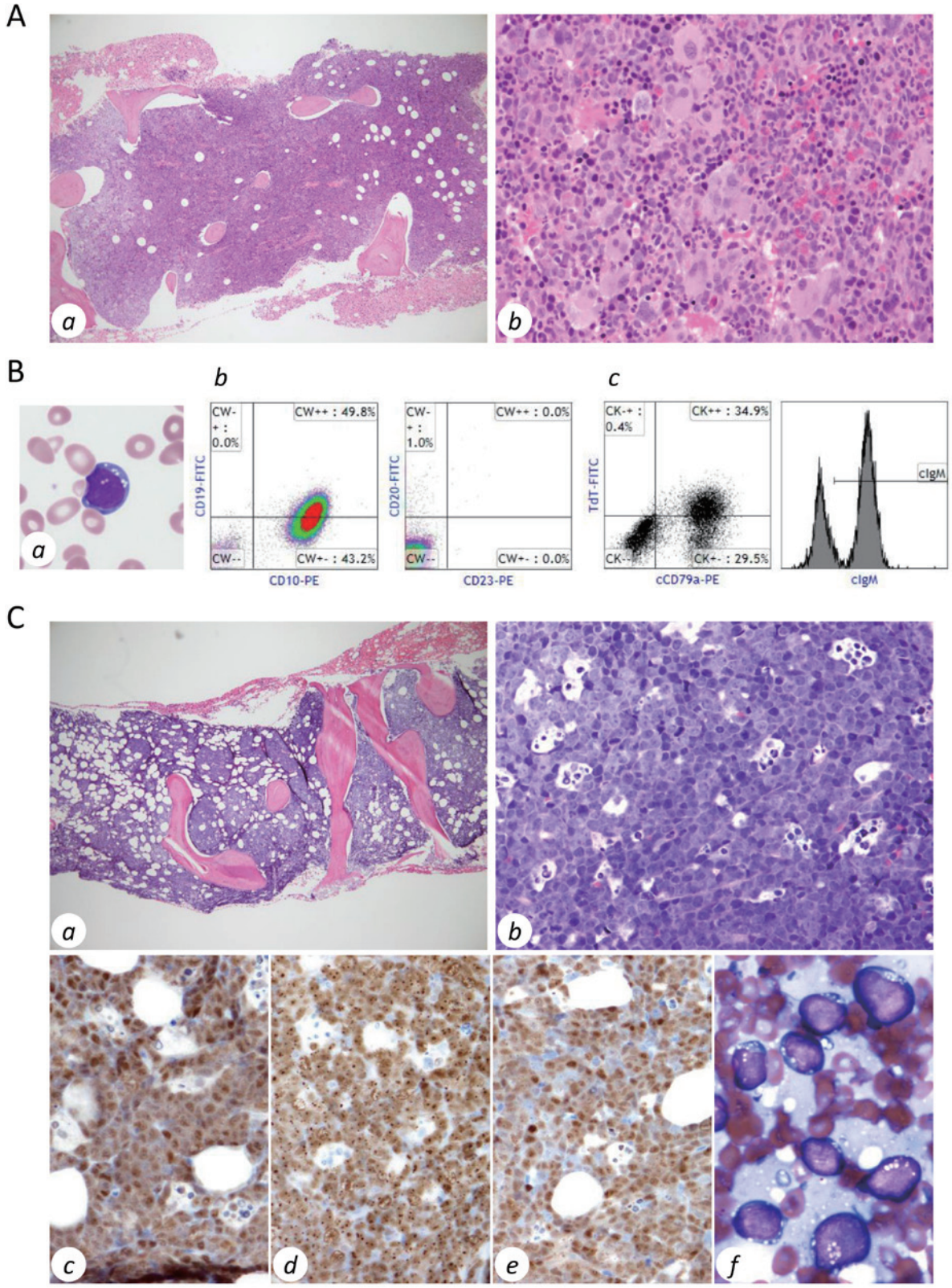

Fig. 1. (***A***) Hematoxylin & eosin (H&E)-stained BM biopsy specimen obtained at a time of the course of MPN (13 years after initial presentation). Original magnification of objective lens: ***a***, ×4; ***b***, ×40. (***B***) Morphology and FCM of BL cells in PB. ***a***, a Wright-stained circulating BL cell (×100); ***b***, FCM of cell surface antigens; and ***c***, FCM of Dako's IntraStain-treated cells detecting intracellular antigens. "c" denotes cytoplasmic. (***C***) Appearance of the BM biopsy at the development of BL. ***a*** (×4) and ***b*** (×40), H&E staining; ***c***, anti-MYC immunostaining (×40); ***d***, anti-Ki-67 (×40); ***e***, anti-TdT (×40); and ***f***, Wright-stained touch preparation of the biopsy (×100).

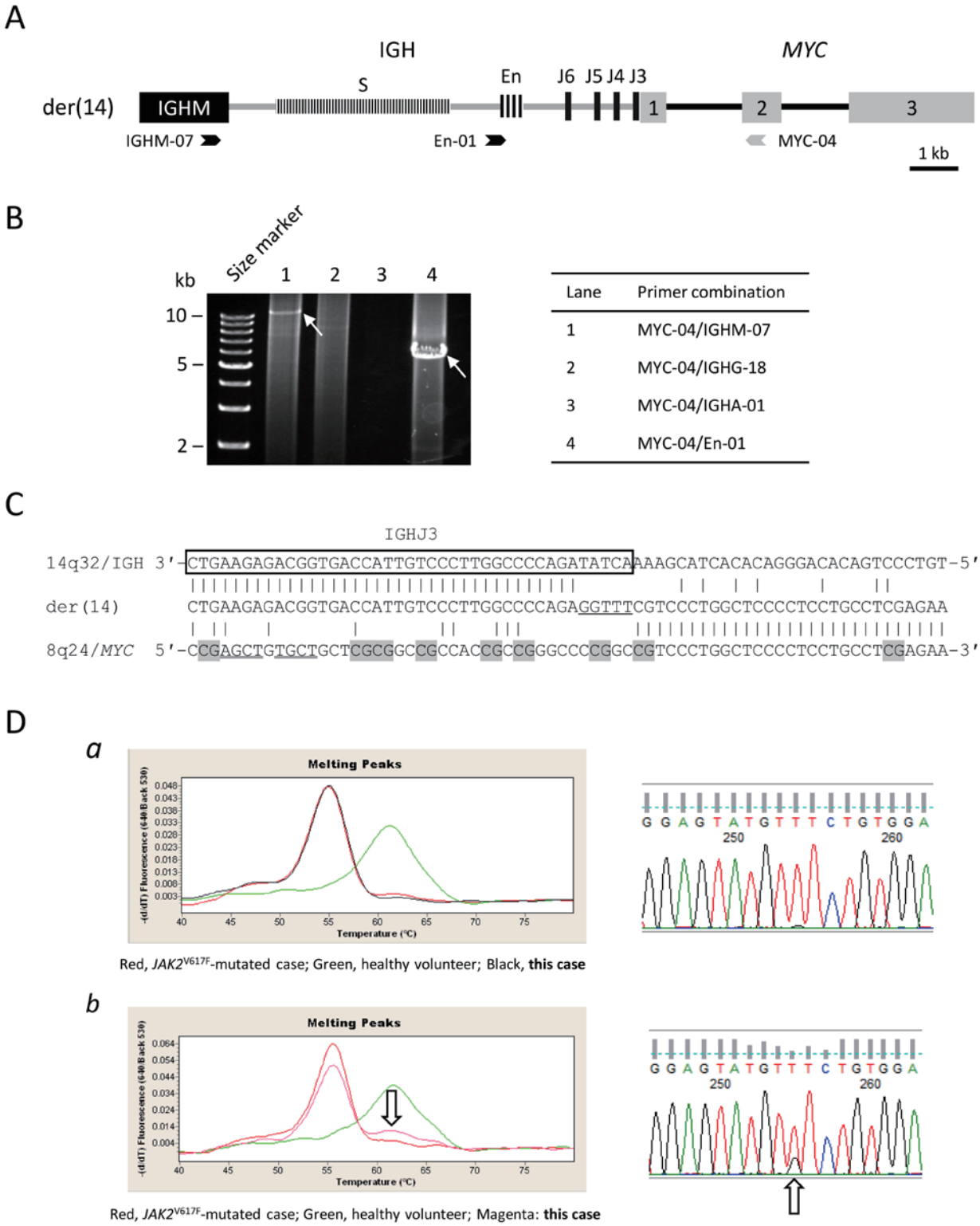

Fig. 2. (***A***) Diagram of the anatomy of t(8;14)(q24;q32)/*MYC*-IGH. Three exons of *MYC* as well as the enhancer (En), switch region (S), and IGHM constant gene are presented. (***B***) Ethidium bromide-stained gel electrophoresis of LD-PCR encompassing the t(8;14)(q24;q32) junction. Arrows indicate the products amplified by MYC-04/IGHM-07 and MYC-04/En-01 primer combinations. The positions of the primers are indicated in ***A***. (***C***) Nucleotide sequences of the t(8;14)(q24;q32)/*MYC*-IGH junction. Vertical lines indicate nucleotide identity. IGHJ3 segment at IGH, N-segment at the junction, and CG dinucleotides and WGCW motifs within the *MYC* sequence are boxed, underlined, and highlighted and double underlined, respectively. (***D***) Status of *JAK2*^{V617F} mutation determined by melting curve assay (*left*) and Sanger sequencing (*right*). DNA was extracted from PB at a time of MPN course (***a***, 8 years after initial presentation) and at the development of BL (***b***). Melting peak indicative of the presence of *JAK2*^{wt} DNA and heterozygous guanine/thymidine (G/T) peaks at position 1,849 in the latter material are indicated by open arrows. The former DNA was negative for rearrangement of IGH and IGK by multiplex PCR.

carried unmutated IGH V-D-J sequences, it is unlikely that the SHM/CSR machinery was active. In accordance with this, the breakpoint occurred at the IGHJ3 segment and an N-segment was inserted at the junction, indicating that aberrant V-D-J recombination activity within BM was responsible for the breakage and joining of the IGH side of the translocation; the same held true for IG-*MYC* breakpoints of precursor B-cell BL cases reported previously (Table 1).[2,4]

On the other hand, potential mechanisms involved in the breakpoints of the partner genes have been proposed.[21] The CpG-type breakage is observed in *BCL2* breakpoints of t(14;18)(q32;q21)/IGH-*BCL2*,[22] *MALT1* breakpoints of t(14;18)(q32;q21)/IGH-*MALT1*,[23] and a fraction of *CCND1* breakpoints of t(11;14)(q13;q32)/*CCND1*-IGH,[24] all of which are considered to develop in the pro-B/pre-B–cell stage of B-cell differentiation. In contrast, *MYC* breakpoints in t(8;14)(q24;q32) do not involve CpG, even though the *MYC* breakpoint region is rich in CpG, but instead prefer the WGCW motif (W = A or T).[24] These observations have led to the proposal of a model, in which CpGs are targeted in translocations occurring in pro-B/pre-B–cells when AID levels are low, whereas WGCW is more frequently targeted when AID levels are higher, as in GC B-cells.[21-24] In the current case, the *MYC* breakpoint occurred at a CG dinucleotide within exon 1 and was 29-bp apart from the closest WGCW motif (Figure 2C). Taken together with the IGHJ3 breakpoint on the IGH side, t(8;14)(q24;q32)/*MYC*-IGH in this case likely occurred at an early B-cell stage in the BM, in contrast to t(8;14)(q24;q32)/*MYC*-IGH in the majority of cases of sporadic BL that occurred in GC. Nevertheless, as reported *MYC* breakpoints were distributed over a large region encompassing the *MYC* coding exons (Table 1),[2,4] further studies are required to confirm the validity of the CpG breakage model in precursor B-cell BL.

A mechanism that links JAK1/2 inhibitor-treated MPN and aggressive B-cell neoplasm was proposed by Porpaczy *et al*.[11] They first showed that JAK1/2 inhibitor therapy increased the risk of developing an aggressive B-cell lymphoma 16-fold in their MPN cohort. They then studied 4 patients who developed aggressive B-cell lymphoma after JAK1/2 inhibitor therapy for MPN, and showed that there was no evidence of mutations in MPN-driver genes in lymphoma cells, but instead the lymphoma clones pre-existed in BM during the MPN phase. The authors suggested that as JAK1/2 inhibitors act as immunosuppressive agents, JAK inhibition may cause progression from an indolent clonal B-cell proliferation to aggressive B-cell lymphoma.[11] In the current case, as the development of BL was associated with the appearance of *JAK2*^{wt} DNA in PB, BL cells likely lacked the *JAK2*^{V617F} mutation. In line with Porpaczy *et al*.,[11] it is possible that pre-existing clonal B-cells in BM at a precursor B-cell stage rapidly proliferated, when they acquired t(8;14)(q24;q32)/*MYC*-IGH, in the setting of ruxolitinib-induced immunosuppression. Of course, this hypothesis is very speculative; nevertheless, this report suggests that, in selected patients, close monitoring of clonal B-cells in the BM is required before and during treatment with JAK1/2 inhibitors.[10,25]

ETHICAL APPROVAL

All procedures performed in this study involving the patient were conducted in accordance with the 1964 Helsinki declaration.

INFORMED CONSENT

The patient consented to the use of her medical record and clinical materials for research purposes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1 Leoncini L, Campo E, Stein H, *et al*. Burkitt lymphoma. In: Swerdlow SH, Campo E, Harris NL, *et al*. (eds). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th edition, Lyon, IARC, 2017; pp. 330-334.
2 Wagener R, López C, Kleinheinz K, *et al*. IG-*MYC*$^{+}$ neoplasms with precursor B-cell phenotype are molecularly distinct from Burkitt lymphomas. Blood. 2018; 132 : 2280-2285.
3 Li Y, Gupta G, Molofsky A, *et al*. B Lymphoblastic leukemia/

Table 1. Breakpoints of IG-*MYC* rearrangements in precursor B-cell BL

Authors	Case no.	IG-*MYC*	Derivative chromosome	IG breakpoint	*MYC* breakpoint	N segment
Wagener *et al*.[2]	Case 1	IGH-*MYC*	der(8)t(8;14)(q24;q32)*	IGHV3-33	Intron 1	12 nucleotides
	Case 2	IGH-*MYC*	der(8)t(8;14)(q24;q32)*	IGHD2-15	~108 kb upstream	6 nucleotides
	Case 3	IGH-*MYC*	der(14)t(8;14)(q24;q32)	IGHJ4	1.7 kb upstream	6 nucleotides
	Case 4	IGK-*MYC*	der(2)t(2;8)(p11;q32)*	4 bp upstream of IGKJ4	279 kb downstream	5 nucleotides
Yoon *et al*.[4]	Case 2	IGH-*MYC*	der(14)t(8;14)(q24;q32)	IGHJ3	632 bp upstream	12 nucleotides
Fukutsuka *et al*.	This case	IGH-*MYC*	der(14)t(8;14)(q24;q32)	IGHJ3	Exon 1	5 nucleotides

*These sequences represent the reciprocal breakpoints of 5′ *MYC* and 5′ IGH sequences of t(8;14)(q24;q32)/*MYC*-IGH and those of 3′ *MYC* and 5′ IGK sequences of t(2;8)(p11;q24)/IGK-*MYC*.

lymphoma with Burkitt-like morphology and IGH/*MYC* rearrangement: Report of 3 cases in adult patients. Am J Surg Pathol. 2018; 42 : 269-276.
4 Yoon J, Yun JW, Jung CW, *et al*. Molecular characteristics of terminal deoxynucleotidyl transferase negative precursor B-cell phenotype Burkitt leukemia with IGH-MYC rearrangement. Genes Chromosomes Cancer. 2020; 59 : 255-260.
5 Komrokji R, Lancet J, Felgar R, Wang N, Bennett JM. Burkitt's leukemia with precursor B-cell immunophenotype and atypical morphology (atypical Burkitt's leukemia/lymphoma): case report and review of literature. Leuk Res. 2003; 27 : 561-566.
6 Navid F, Mosijczuk AD, Head DR, *et al*. Acute lymphoblastic leukemia with the (8;14)(q24;q32) translocation and FAB L3 morphology associated with a B-precursor immunophenotype: the Pediatric Oncology Group experience. Leukemia. 1999; 13 : 135-141.
7 Sakaguchi K, Imamura T, Ishimaru S, *et al*. Nationwide study of pediatric B-cell precursor acute lymphoblastic leukemia with chromosome 8q24/ *MYC* rearrangement in Japan. Pediatr Blood Cancer. 2020; 67 : e28341.
8 Shiratori S, Kondo T, Fujisawa S, *et al*. c-myc rearrangement in B-cell lymphoblastic lymphoma with the involvement of multiple extranodal lesions. Leuk Lymphoma. 2011; 52 : 716-718.
9 Slavutsky I, Andreoli G, Gutiérrez M, *et al*. Variant (8;22) translocation in lymphoblastic lymphoma. Leuk Lymphoma. 1996; 21 : 169-172.
10 Rumi E, Baratè C, Benevolo G, *et al*. Myeloproliferative and lymphoproliferative disorders: State of the art. Hematol Oncol. 2020; 38 : 121-128.
11 Porpaczy E, Tripolt S, Hoelbl-Kovacic A, *et al*. Aggressive B-cell lymphomas in patients with myelofibrosis receiving JAK1/2 inhibitor therapy. Blood. 2018; 132 : 694-706.
12 Pemmaraju N, Kantarjian H, Nastoupil L, *et al*. Characteristics of patients with myeloproliferative neoplasms with lymphoma, with or without JAK inhibitor therapy. Blood. 2019; 133 : 2348-2351.
13 Maffioli M, Giorgino T, Mora B, *et al*. Second primary malignancies in ruxolitinib-treated myelofibrosis: real-world evidence from 219 consecutive patients. Blood Adv. 2019; 3 : 3196-3200.
14 Rumi E, Zibellini S, Boveri E, *et al*. Ruxolitinib treatment and risk of B-cell lymphomas in myeloproliferative neoplasms. Am J Hematol. 2019; 94 : E185-E188.
15 Rumi E, Passamonti F, Elena C, *et al*. Increased risk of lymphoid neoplasm in patients with myeloproliferative neoplasm: a study of 1,915 patients. Haematologica. 2011; 96 : 454-458.
16 Holst JM, Plesner TL, Pedersen MB, *et al*. Myeloproliferative and lymphoproliferative malignancies occurring in the same patient: a nationwide discovery cohort. Haematologica. 2020; 105 : 2432-2439.
17 Akasaka T, Akasaka H, Ohno H. Polymerase chain reaction amplification of long DNA targets: application to analysis of chromosomal translocations in human B-cell tumors (review). Int J Oncol. 1998; 12 : 113-121.
18 Akasaka T, Lee YF, Novak AJ, *et al*. Clinical, histopathological, and molecular features of mucosa-associated lymphoid tissue (MALT) lymphoma carrying the t(X;14) (p11;q32)/ *GPR34* -immunoglobulin heavy chain gene. Leuk Lymphoma. 2017; 58 : 2247-2250.
19 Lay M, Mariappan R, Gotlib J, *et al*. Detection of the *JAK2* V617F mutation by LightCycler PCR and probe dissociation analysis. J Mol Diagn. 2006; 8 : 330-334.
20 Pasqualucci L, Neumeister P, Goossens T, *et al*. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature. 2001; 412 : 341-346.
21 Lieber MR. Mechanisms of human lymphoid chromosomal translocations. Nat Rev Cancer. 2016; 16 : 387-398.
22 Tsai AG, Lu H, Raghavan SC, *et al*. Human chromosomal translocations at CpG sites and a theoretical basis for their lineage and stage specificity. Cell. 2008; 135 : 1130-1142.
23 Tsai AG, Lu Z, Lieber MR. The t(14;18)(q32;q21)/IGH-MALT1 translocation in MALT lymphomas is a CpG-type translocation, but the t(11;18)(q21;q21)/API2-MALT1 translocation in MALT lymphomas is not. Blood. 2010; 115 : 3640-3641.
24 Greisman HA, Lu Z, Tsai AG, *et al*. IgH partner breakpoint sequences provide evidence that AID initiates t(11;14) and t(8;14) chromosomal breaks in mantle cell and Burkitt lymphomas. Blood. 2012; 120 : 2864-2867.
25 Arcaini L, Cazzola M. Benefits and risks of JAK inhibition. Blood. 2018; 132 : 675-676.