EXCEPTIONAL CASE REPORT

blood advances

Lymphoid blast transformation in an MPN with *BCR-JAK2* treated with ruxolitinib: putative mechanisms of resistance

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Key Points

- Lymphoid blast transformation of an MPN with *BCR-JAK2* was associated with detection of an *IKZF1* deletion and upregulation of *IL7R* and *CRLF2.*
- Phenotypic shift from cytokine receptor dependence to B-cell receptor–like signaling dependence may represent a mechanism of resistance to ruxolitinib.

The basis for acquired resistance to JAK inhibition in patients with *JAK2*-driven hematologic malignancies is not well understood. We report a patient with a myeloproliferative neoplasm (MPN) with a BCR activator of RhoGEF and GTPase (*BCR*)–*JAK2* fusion with initial hematologic response to ruxolitinib who rapidly developed B-lymphoid blast transformation. We analyzed pre-ruxolitinib and blast transformation samples using genome sequencing, DNA mate-pair sequencing (MPseq), RNA sequencing (RNA-seq), and chromosomal microarray to characterize possible mechanisms of resistance. No resistance mutations in the *BCR-JAK2* fusion gene or transcript were identified, and fusion transcript expression levels remained stable. However, at the time of blast transformation, MPseq detected a new *IKZF1* copy-number loss, which is predicted to result in loss of normal IKZF1 protein translation. RNA-seq revealed significant upregulation of genes negatively regulated by IKZF1, including *IL7R* and *CRLF2*. Disease progression was also characterized by adaptation to an activated B-cell receptor (BCR)–like signaling phenotype, with marked upregulation of genes such as *CD79A*, *CD79B*, *IGLL1*, *VPREB1*, *BLNK*, *ZAP70*, *RAG1*, and *RAG2*. In summary, *IKZF1* deletion and a switch from cytokine dependence to activated BCR-like signaling phenotype represent putative mechanisms of ruxolitinib resistance in this case, recapitulating preclinical data on resistance to JAK inhibition in *CRLF2*-rearranged Philadelphia chromosome-like acute lymphoblastic leukemia.

Introduction

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic neoplasms characterized by expansion of 1 or more myeloid lineages.[1] Most MPNs are associated with somatic activating fusions or mutations in tyrosine kinase genes, including the *BCR-ABL1* gene fusion in chronic myeloid leukemia (CML) and *JAK2* V617F in polycythemia vera, primary myelofibrosis, and essential thrombocythemia.

Submitted 24 January 2021; accepted 18 June 2021; final version published online 10 September 2021. DOI 10.1182/bloodadvances.2020004174.

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The full-text version of this article contains a data supplement.

Fusion of *JAK2* with various gene partners, including *PCM1*, *ETV6*, and *BCR* has been reported in multiple hematologic malignancies.[1-3] *BCR-JAK2* fusions have been reported across a spectrum of hematolymphoid malignancies, including MPNs or myelodysplastic/myeloproliferative neoplasms, chronic eosinophilic leukemia, and B-cell acute lymphoblastic leukemia (B-ALL).[2,4-10] These neoplasms would be best classified in the World Health Organization category of "Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1* or with *PCM1-JAK2*." Ruxolitinib, a JAK1/JAK2 kinase inhibitor, has been shown to induce complete clinical or cytogenetic remissions in neoplasms with *JAK2* fusion genes; however, responses tend not to be durable.[8,11-13]

We report a patient with a CML-like MPN harboring a *BCR-JAK2* fusion who initially responded to ruxolitinib, but his disease quickly transformed to lymphoid blast phase. Blast transformation was associated with the detection of an *IKZF1* deletion, upregulation of *IL7R* and *CRLF2* RNA expression, and adaptation to an activated B-cell receptor (BCR)-like signaling phenotype, highlighting potential mechanisms of acquired ruxolitinib resistance.

Case description

A previously healthy 41-year-old male was found to have leukocytosis and thrombocytopenia on routine complete blood count (white blood cell count [WBC], 27.9×10^9/L, hemoglobin 13.9 g/dL, platelet count, 121×10^9/L) (Figure 1). Approximately 1 month later, his leukocytosis progressed with a left shift without eosinophilia (WBC, 46.6×10^9/L; hemoglobin, 14.4 g/dL; platelet count, 110×10^9/L; granulocytes, 92.7%). Molecular studies were negative for *BCR-ABL1* gene fusion, *JAK2* V617F, and other *JAK2* mutations in exons 12 to 14. Chromosome analysis demonstrated 46,XY,t(9;22)(p24;q11.2)[15]/46,XY[5](Figure 2A), which was further evaluated by metaphase fluorescence in situ hybridization (supplemental Figure 1). A local bone marrow was read as atypical CML, *BCR-ABL1*-negative. Mast cells were not increased, and lymphoblasts were not present in the marrow. He was started on hydroxyurea and referred to Stanford Hematology for further evaluation.

After receiving hydroxyurea for 2.5 months, his WBC decreased to 18.2×10^9/L. Genome sequencing of DNA isolated from peripheral blood (time point 1) identified a translocation and inversion event with 3 intronic fusions: *BCR-JAK2*, *JAK2-PPM1F*, and *PRAMENP-BCR* (*PRAMENP* is a putative pseudogene) (Figure 2B-D; supplemental Table 1). Reverse transcription polymerase chain reaction (RT-PCR) demonstrated an in-frame fusion between exon 1 of *BCR* and exon 19 of *JAK2*, which is identical to the fusion transcript previously described[14] (supplemental Figure 2; supplemental Table 1). RT-PCR also identified a fusion between exon 16 of *JAK2* and exon 2 of *PPM1F*; however, this results in the introduction of a stop codon at the first complete *PPM1F* codon. A *PRAMENP-BCR* fusion transcript was not detected.

Hydroxyurea was discontinued because of disease progression after 14 months (progressive leukocytosis and myeloid immaturity without eosinophilia). Similar to his initial presentation, neither palpable lymphadenopathy or splenomegaly were detected. He provided informed consent for an institutional review board–approved single-patient compassionate use protocol with ruxolitinib, initiated at a dose of 15 mg twice per day in 28-day cycles. Peripheral blood specimens were collected under protocols approved and overseen by the Stanford University Administrative Panel for the Protection of Human Subjects and the Stanford Cancer Institute Scientific Review Committee. The participant was counseled and gave consent for genomic sequencing studies. Specimens were collected before the start of treatment with ruxolitinib (time point 2). At this time, peripheral blood demonstrated left-shift neutrophilia and leukocytosis with myeloid predominance without an increase in blasts. Bone marrow aspirate and core biopsy demonstrated hypercellular marrow without an increase in blasts (supplemental Figure 3A-D).

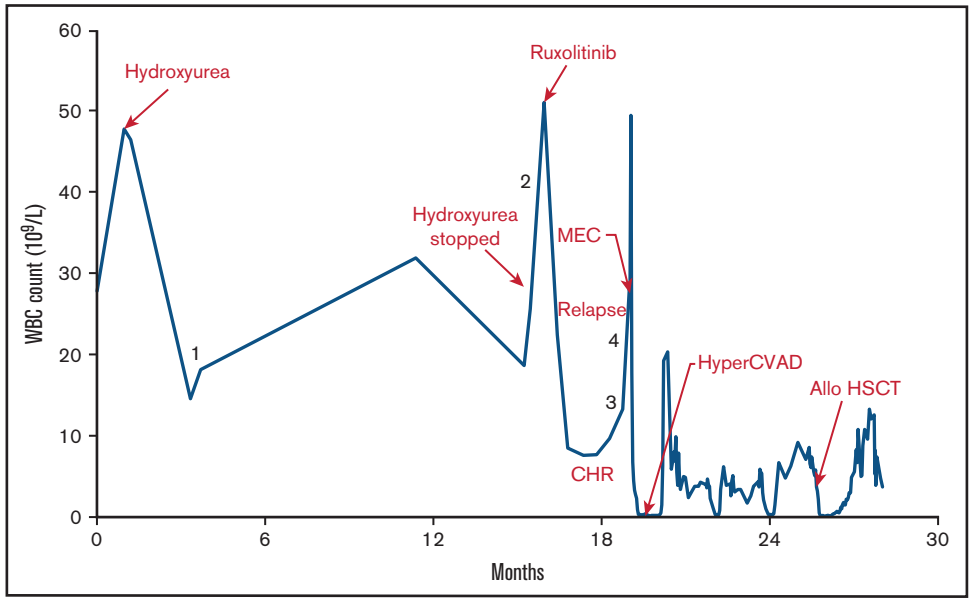

Figure 1. WBC count vs time (in months) with major events labeled. The numerals 1 to 4 indicate corresponding time points of correlative analyses. Allo HSCT, allogeneic hematopoietic stem cell transplantation; CHR, complete hematologic remission; hyperCVAD, cytarabine, vincristine, doxorubicin, dexamethasone; MEC, mitoxantrone, etoposide, cytarabine.

After cycle 2 of ruxolitinib, he achieved WBC normalization with minimal myeloid immaturity. After cycle 3, peripheral blood showed return of myeloid immaturity with increased blasts (time points 3 and 4, collected 1 week apart). A bone marrow biopsy showed lymphoid blast transformation. The blasts were positive for CD10 and CD79a and negative for myeloperoxidase by immunohistochemistry (supplemental Figure 3E-I). Flow cytometric analysis reiterated these findings, with the $CD34^+$ blast population expressing CD79a and terminal deoxynucleotidyltransferase. During the course of ruxolitinib therapy, no cytogenetic response was observed, and no additional karyotype abnormalities were noted at the time of lymphoid blast disease. We performed analysis of the genome, DNA mate-pair sequencing (MPseq), RNA sequencing (RNA-seq), and chromosomal microarray (CMA) data from the pre-ruxolitinib (time point 2) and blast transformation specimens (time points 3 and 4) to identify acquired genetic changes associated with disease progression. The *BCR-JAK2* fusion was persistent throughout the treatment course up to this point, detected at time points 1 to 4 (Figure 1).

The patient underwent induction chemotherapy with mitoxantrone, etoposide, and cytarabine followed by hyperfractionated cytarabine, vincristine, doxorubicin, and dexamethasone for residual disease. He achieved a complete hematologic and cytogenetic remission but suffered a cytogenetic relapse before proceeding to a matched unrelated donor allogeneic hematopoietic stem cell transplantation with busulfan-cyclophosphamide conditioning. His transplant course was complicated by infections and acute graft-versus-host disease of the skin, but he achieved a complete hematologic and cytogenetic remission. As of the writing of this article, he is still alive and without evidence of disease 7 years after hematopoietic stem cell transplantation.

Methods

To characterize potential molecular causes of acquired resistance, we performed genome sequencing, MPseq, RNA-seq, and CMA on the lymphoid blast transformation and pre-ruxolitinib samples as described in the supplemental Materials.

Results and discussion

MPseq demonstrated a subclonal 90-kb *IKZF1* deletion encompassing exons 2 to 7 (NM_006060.6; chr7:50 306 990-50 395 984; hg38) in the blast transformation sample (time point 3), with no evidence of the *IKZF1* deletion in the pre-ruxolitinib sample (time point 2) (data not shown). Because exon 2 contains the ATG translational start site, these deletions are predicted to result in loss of normal IKZF1 protein translation from these deletion alleles. We cannot distinguish whether the *IKZF1* deletion was acquired near the time of lymphoid blast transformation or represents the outgrowth of a minor preexisting clone. No other deletions were detected in other genes commonly altered in B-ALL with *IKZF1* deletion, including *CDKN2A*, *CDKN2B*, *EBF1*, and *PAX5* before or after blast transformation. Genome sequencing did not identify any new candidate coding

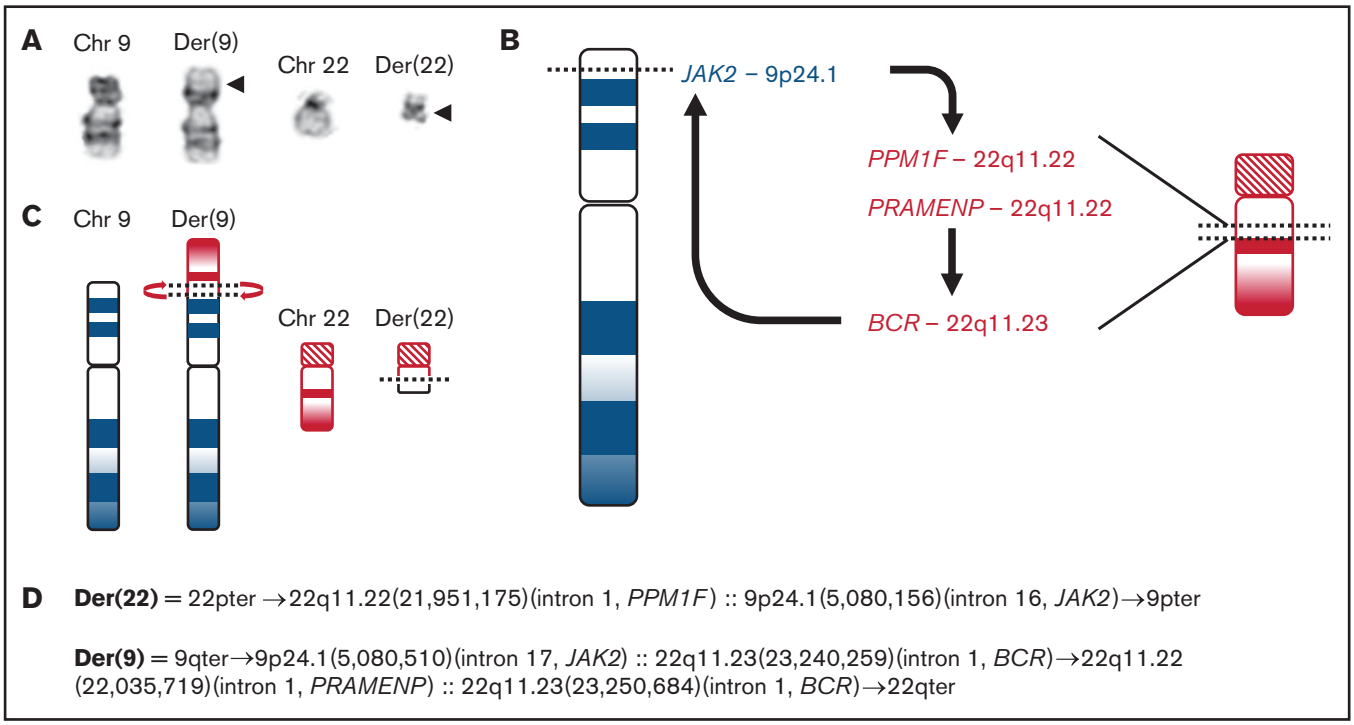

Figure 2. Complex genomic rearrangement resulting in *BCR-JAK2*. (A) A partial karyotype from a metaphase cell shows an apparently reciprocal translocation between the short (p) arm of chromosome 9 and the long (q) arm of chromosome 22 or a t(9;22)(p24;q11.2). Arrowheads mark the breakpoints on the derivative chromosomes 9 and 22. (B) Genome sequencing defined a more complex rearrangement involving a translocation between 9p24 and 22q11.2 and associated inversion involving 22q11.2. The arrows note the direction of the fusions moving from 5' to 3'. This rearrangement resulted in DNA fusions juxtaposing the intronic regions of *BCR-JAK2*, *JAK2-PPM1F*, and *PRAMENP-BCR*. (C) Idiograms and (D) inferred structure of the derivative chromosomes based on hg38 assembly, NM_014634.4 (*PPM1F*), NM_004972.3 (*JAK2*), NM_004327.4 (*BCR*), and NR_135291.1 (*PRAMENP*).

variants in the blast transformation sample when compared with the pre-ruxolitinib sample.

RNA-seq data were compared in the blast transformation specimen (time point 3) relative to the pre-ruxolitinib sample (time point 2). Overall, *JAK2* expression decreased by approximately twofold in the blast transformation specimen. RT-PCR followed by Sanger sequencing and RNA-seq failed to detect any newly acquired or potential resistance mutations in the *BCR-JAK2* fusion transcript, and the level of fusion transcript expression remained consistent between samples based on normalized junction-spanning read counts. Given the finding of an *IKZF1* deletion in the blast transformation specimen by MPseq, we also examined *IKZF1* splicing and expression. We did not detect evidence of aberrant *IKZF1* splicing. *IKZF1* expression demonstrated a minor but statistically significant decrease (1.2-fold; adjusted $P = 5.7 \times 10^{-8}$), and significant upregulation was observed for genes negatively regulated by *IKZF1*,[15,16] including *IL7R* (8.5-fold increase) and *CRLF2* (3.4-fold increase) (supplemental Table 2). Pre-BCR signaling genes, including *CD79A*, *CD79B*, *IGLL1*, *VPREB1*, *BLNK*, *ZAP70*, *RAG1*, and *RAG2* exhibited significant upregulation from 4.1-fold to 11.0-fold (supplemental Table 3) in a pattern similar to that reported in Philadelphia chromosome-like ALL (Ph-like ALL).[17] Upregulation of *PAX5* (4.5-fold) and *CD19* (4.7-fold) was also observed, further supporting the diagnosis of a lymphoid blast phenotype.

IKZF1 encodes for the protein Ikaros, a transcription factor integral in normal lymphopoiesis.[18] Acquired deletions of *IKZF1* have been associated with lymphoid blast transformation in CML and at least 1 case of a *BCR-JAK2* MPN.[5,18,19] In *BCR-ABL1*-positive ALL, *IKZF1* deletions have been hypothesized to contribute to tyrosine kinase inhibitor resistance by altering cell adhesion pathways, which results in leukemic cells being relocated to the bone marrow.[18] In Ph-like ALL, which is frequently associated with *CRLF2* rearrangement and concomitant activating *JAK2* mutations,[20,21] preclinical models demonstrate short-term response to ruxolitinib before leukemic cells overcome the effects of JAK1/JAK2 inhibition.[17] This resistance to JAK1/JAK2-inhibition in *CRLF2* rearranged Ph-like ALL is mediated by a genomic and phenotypic shift from cytokine receptor dependence to BCR-like signaling dependence.[17]

In summary, we report a rare case of a *BCR-JAK2* fusion MPN with initial near complete hematologic remission with ruxolitinib followed by rapid lymphoid blast transformation, a pattern suggestive of acquired resistance that has been observed in other *JAK2*-rearranged neoplasms.[5,8,13] The molecular basis for resistance to ruxolitinib in neoplasms with *JAK2* fusion genes has not been characterized, but *IKZF1* deletion and a switch from a cytokine dependence to an activated BCR-like signaling phenotype are strong candidates for contributors to the development of ruxolitinib resistance in this case. Although combinatorial approaches to treatment with agents such as ruxolitinib, dasatinib, idelalisib, and dexamethasone overcome the BCR-like signaling pattern of resistance in preclinical models,[17] this requires clinical evaluation in patients with myeloid or lymphoid neoplasms with *JAK2* fusion genes and Ph-like ALL.

Acknowledgment

J.G. thanks the Charles and Ann Johnson Foundation for supporting research on MPNs.

Authorship

Contribution: J.G. treated the patients; J.A.C., J.D.M., and J.G. designed the study; D.A.A., C.D.B., A.M.C., M.D.E., and R.S.O. performed histopathologic, cytogenetic, and fluorescence in situ hybridization analyses; Y.H., K.M.R., L.B.B., A.Z.F., L.F., H.M.K., S.B.M., K.E.P., B.A.P., and J.D.M. performed the genome sequencing, DNA MPseq, RNA-seq, and CMA experiments and analyzed the data; J.A.C., J.D.M., and J.G. wrote the initial manuscript; and all authors approved the final version except for A.M.C. who died before the manuscript was drafted.

Conflict-of-interest disclosure: J.A.C. received the AACR-AstraZeneca clinical immune-oncology research fellowship grant. D.A.A. has served on advisory boards and consulted for AbbVie, Amgen, Jazz Pharmaceuticals, Monsanto, and Roche. M.D.E. has served on advisory boards for Acceleron Pharma. S.B.M. is on the scientific advisory board for MyOme. J.D.M. has served on advisory boards and consulted for AbbVie, Bristol Myers Squibb, Illumina, and PierianDx and serves on the Board of Directors for the Association for Molecular Pathology. J.G. has served on advisory boards and received honoraria from Incyte (the manufacturer of ruxolitinib)and has received funding from Incyte for conducting clinical trials using ruxolitinib in MPNs. The remaining authors declare no competing financial interests.

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