Case Report
Transformation of an Unclassified Myeloproliferative Neoplasm with a Rare BCR-JAK2 Fusion Transcript Resulting from the Translocation (9;22) (p24;q11)

A. N. Chamseddine, 1,2 P. Etancelin, 3 D. Penther, 3,4 F. Parmentier, 3,4 C. Kuadjovi, 3 V. Camus, 1,2 N. Contentin, 1,2 P. Lenain, 1,2 C. Bastard, 3,4 H. Tilly, 1,2,4 and F. Jardin 1,2,4

1 Department of Clinical Hematology, Henri Becquerel Cancer Center, 1 rue d'Amiens, 76038 Rouen, France
2 Blood and Marrow Transplant Unit, Henri Becquerel Cancer Center, 1 rue d'Amiens, 76038 Rouen, France
3 Molecular and Genetic Laboratory Department, Henri Becquerel Cancer Center, 1 rue d'Amiens, 76038 Rouen, France
4 INSERM U918 Unit, Henri Becquerel Cancer Center, 1 rue d'Amiens, 76038 Rouen, France

Correspondence should be addressed to A. N. Chamseddine; alichamseddine@hotmail.com and P. Etancelin; pascaline.etancelin@chb.unicancer.fr

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1. Introduction

Some myeloproliferative neoplasms (MPNs) are Philadelphia- (Ph-) negative, lacking the reciprocal t(9;22)(q34;q11) and its resultant BCR-ABL1 fusion gene. Currently, the most frequent genomic abnormality observed in Ph-negative MPN is a dominant gain-of-function V617F mutation in the JH2 kinase-like domain of JAK2 [1]. However, there are rare additional mechanisms described in Ph-negative MPNs that activate JAK2, such as chromosomal translocations that cause constitutive dimerization through the replacement of amino terminal sequences with a fusion partner [2, 3]. Indeed six different fusion partners have been associated with JAK2 (RPN1, SSBP2, PAX5, PCMI, BCR, and ETV6).

Here, we report a rare case of unclassified MPN (MPN-U) with a t(9;22)(p24;q11) leading to a 5′BCR/3′JAK2 fusion gene producing a fusion transcript that juxtaposed BCR exon 13 and JAK2 exon 17 and subsequently rapidly transformed into a myeloid granulocytic sarcoma. We also describe, 35 months after diagnosis and ∼24 months after ASCT, a prolonged and sustained complete clinical, hematologic, and cytogenetic remission after undergoing allogeneic stem cell transplantation (ASCT).

2. Case Presentation

We report a case of a 49-year-old man with no significant medical history. The patient was referred to our center in
October 2011. The blood count was abnormal with anemia (Hb 11.2 g/dL) and a platelet count of 78000/mm$^3$. The white blood cell count was 11500/mm$^3$ with 30% lymphocytes, 2% monocytes, 2% eosinophils, 0% basophils, 29% neutrophils, and 37% promyelocytes, myelocytes, and metamyelocytes. Clinical examination was unremarkable. The bone marrow aspiration and biopsy associated with initial molecular blood and medullary analyses led to diagnose an MPN-U. It did not reveal any BCR-ABL1 rearrangement neither V617F JAK2 mutation. In February 2012, the patient presented to the emergency room with a sudden onset of pyramidal tract deficiency syndrome and with an increase of leukocytosis and blood myeloid precursors. The MRI scan revealed a thoracic spinal epidural compression extending from T4 to T10. Emergent laminectomy was done. Histological analysis was performed on the laminectomy specimen and demonstrated the presence of a granulocytic (myeloid) sarcoma. Radiation therapy was then performed. Cytogenetic examination of the bone marrow aspiration of the patient was performed on two unstimulated short-term cultures (24 hrs and 48 hrs). The karyotype was obtained by conventional R-banding analysis [4]. Chromosome analysis (Figure 1) showed t(9;22)(p24;q11) as the sole abnormality in 60% of the analyzed metaphases (12/20). In 10% of the analyzed metaphases (2/20), it showed the latter translocation in addition to der(22) t(9;22)(p24;q11). The last 30% of the analyzed metaphases (6/20) were normal. Mutations of exons 12, 13, and 14 and, in particular, the V617F JAK2 gene mutation were not found. Considering the t(9;22)(p24;q11), that the exons 12, 13, and 14 and the V617F JAK2 mutations were absent, and that JAK2 had previously been shown to fuse with BCR in MPN-like patients, the best fusion gene candidates were JAK2 in 9p24 and BCR in 22q11.

FISH analysis (Figure 2) using dual fusion probes for BCR (22q11.2) and ABL1 (9q34) regions (LSI BCR/ABL ES Dual Color Translocation Probe, Abbott Molecular; Vysis, Des Plaines, IL, USA) excluded the BCR-ABL1 fusion and showed an extra signal of the BCR probe on chromosome 9p. Moreover, a FISH assay was performed using JAK2 probes obtained from bacterial artificial chromosome (BAC) (RP11-39K24 AL161450, Sanger Institute, Cambridge, UK) labelled with spectrum red (Vysis, Downers Grove, IL, USA) and showed a split of the probe between 9p24 and 22q11. According to the cytogenetic examination and FISH results which showed a t(9;22)(p24;q11), a BCR-JAK2 fusion was suggested.

Blood from the patient was collected in EDTA, and RNA was isolated from 107 cells using the TRizol kit (Life Technologies, Carlsbad, CA, USA). cDNA synthesis was performed using Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen, Life Technologies, Carlsbad, CA, USA). The breakpoint Sanger sequencing using ABI 3130
inblue). Amino acids of the respective fusion gene BCR-JAK2 were carried out relative to the expression of the housekeeping gene ABL1. Molecular monitoring was able to detect low levels of disease. Hence, the assay was >4 logs more sensitive than conventional cytogenetic, detecting one copy of BCR-JAK2 to 10000 copies of ABL1 (0.0001%) and allowing us to follow up the effectiveness of treatment. The patient underwent acute myeloid leukemia-like chemotherapy induction and consolidation achieving a chronic phase in May 2012. An ASCT from a matched human leukocyte antigen- (HLA-) unrelated donor (MUD) was then undertaken in August 2012 with a TBI-Endoxan regimen conditioning and without any Graft-versus-host disease complications. The QPCR follow-up of BCR-JAK2 expression in both the bone marrow and peripheral blood mononuclear cells showed complete hematological and molecular (<0.0001%) remission 3 months later. With 35-month follow-up, the patient remains alive with undetectable BCR-JAK2 transcript levels in the blood and no transplant-related complications (Figure 4).

3. Discussion

We have described the presence of a BCR-JAK2 fusion gene in a patient with a rapid blast evolution. This fusion gene is the result of a reciprocal translocation between chr9 and chr22, implying the possible occurrence of a double break on chr9. A fragment of the 3′ end of exon 17 of the JAK2 on chr22 translocated to exon 13 of chr9 in the proper orientation to generate an in-frame fusion transcript with the 5′ end of the BCR gene. The resultant encoded 1330-amino-acid chimeric protein contained the N-terminal coiled-coil dimerization domain of BCR and the C-terminal tyrosine kinase domain of JAK2. The constitutive activation of this chimeric protein is mediated by oligomerization through the coiled-coil domain of BCR and by disruption of the autoinhibitory role of the inhibitory regions (IR) of the pseudo-kinase domain JH2 of JAK2. In fact, there are three inhibitory regions (IR1, -2, and -3) within JH2. IR3, at the C-terminus of JH2, directly inhibits JH1. IR2, in the C-terminal lobe of JH2, and IR1, extending from the N-terminal to the C-terminal lobe, enhance the IR3-mediated inhibition of JH1. Hence, the disruption of IR by mutation, deletion, or translocation increases basal JAK2 activity. Consequently, the BCR-JAK2 chimeric protein is entirely or partially deprived of IR1, which may result in the upregulation of JAK2 activity [5]. Preclinical studies implied a possible role of c-ABL1 in Jak2 activation in various Ph-negative myeloid malignancies [6] and demonstrated that the BCR-JAK2 fusion gene induces STAT5 activation and inhibits BCRxL gene expression, thereby promoting tumorigenic properties and increasing cell survival [7].

It was difficult to define the best therapy. JAK2 inhibitors alone or in combination with chemotherapy may not be effective against the BCR-JAK2 fusion gene malignancies [7, 8]. Compared with JAK2 mutations, JAK2 fusions are probably associated with more aggressive diseases such as...
Table 1: Characteristics of cases reported in the literature with BCR-JAK2 fusion gene.

| Reference       | Year | Age | Sex | Translocation                  | Isoform                               | Clinical presentation | Treatment                      | Follow-up (FU)                  |
|-----------------|------|-----|-----|---------------------------------|---------------------------------------|-----------------------|--------------------------------|--------------------------------|
| Griesinger et al. [11] | 2005 | 63  | F   | t(9;22) (p24;q11)               | BCR exon 1 fused to JAK2 exon 19     | aCML                  | Hy; Cy; Mit                    | Death from blast crisis        |
| Cirmena et al. [12]    | 2008 | 67  | F   | t(9;22) (p24;q11)               | BCR exon 14 fused to JAK2 exon 11    | AML                   | HD + ASCT(MSD)                 | Death from disease relapse     |
| Lane et al. [13]       | 2008 | 44  | M   | t(9;22) (p24;q11)               | BCR exon 1 fused to JAK2 exon 17     | aCML                  | ND                            | ND                            |
| Elnaggar et al. [14]   | 2012 | 84  | M   | t(9;22) (p24;q11)               | BCR exon 1 fused to JAK2 exon 19     | aCML                  | Hy; IM                         | ND                            |
| Tirado et al. [15]     | 2010 | 14  | M   | t(9;22) (p24;q11)               | ND                                   | ALL                   | Polychemotherapy, ASCT(MSD)    | CHR at 6-month FU              |
| Bellesso et al. [16]   | 2013 | 54  | M   | ins(22;9) (q11;p13;24)          | BCR exon 1 fused to JAK2 exon 19     | aCML                  | Hy + INF α                     | Death from aGVHD               |
| Xu et al. [17]         | 2013 | 28  | M   | t(9;18;22) (p11.3;q11.2)        | BCR exon 1 fused to JAK2 exon 15     | MPN-U                 | IM, DAS, INF α                  | CHR at 27-month FU             |
| Impera et al. [18]     | 2011 | 84  | M   | t(9;18;22) (p11.3;q11.2)        | BCR exon 1 fused to JAK2 exon 17     | aCML                  | Jak2 inh                       | CHR at 21-month FU             |
| Schwaabet al. [8]      | 2015 | ND  | M   | t(9;18) (p24;q12)*              | BCR exon 1 fused to JAK2 exon 15     | ALL                   | High-risk ALL protocol, ASCT, INF α | >6 years                      |
| Cuesta-Dominguez et al. [7] | 2012 | 58  | M   | 49, XY, +X, +2, +4, +9, +11, +19, +add(19) (q13), +20, +22, +mar +2, del(2) (p23), t(3;22;9) (p12;q11.2;p24) t(9;22) (p24;q11.2) | as part of complex karyotype | ALL                   |                                |                                |
| Roberts et al. [19]    | 2012 | 2.7 | M   | t(9;22) (p24;q11)               | BCR exon 1 fused to JAK2 exon 15     | ALL                   | ND                            | ND                            |
| Angelova et al. [20]   | 2011 | 53  | M   | t(9;22) (p24;q11)               | ND                                   | MPN-U                 | No treatment                    | Death from blast crisis        |
| Present case           | 2011 | 49  | M   | t(9;22) (p24;q11)               | BCR exon 13 fused to JAK2 exon 17    | MPN-U/GS              | 3 + 7, ASCT(MUD)               | CMR at 35-month FU             |

F: female; M: male; aCML: atypical chronic myeloid leukemia; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; MPN-U: unclassified myeloproliferative neoplasm; GS: granulocytic sarcoma; Hy: hydroxyurea; Cy: cytarabine; Mit: mitoxantrone; HD: high-dose chemotherapy; ASCT: allogeneic stem cell transplantation; ND: not described; MSD: matched sibling donor; MUD: matched unrelated donor; IM: imatinib; DAS: dasatinib, Jak2 inh: JAK2/JAK2 inhibitor ruxolitinib; aGVHD: acute graft-versus-host disease; CHR: complete hematological response; CMR: complete molecular remission. *The RNA sequencing indicated the presence of a BCR-JAK2 fusion gene. The BCR-JAK2 fusion was subsequently confirmed by RT-PCR and PCR from genomic DNA. BCR-JAK2 in this case is therefore likely to be the result of a small insertion of BCR into the JAK2 locus on the der(18).

acute leukemias (myeloid or lymphoblastic), atypical CML (aCML), and myelofibrosis [9]. In our review of literature (Table 1) seven patients presented with aCML/MPN-U, one patient presented with AML, and one patient with ALL. As it is usually observed in myeloid neoplasms, a predominance of the male gender is reported. Three of the reported patients were unsuccessfully treated with tyrosine kinase inhibitors (imatinib or ruxolitinib). In addition, it is important to note that the mortality rate was 50% in cases where the follow-up was described. In our opinion, ASCT is likely the only curative strategy in these MPN-Us. In fact, JAK2 translocations have been described in acute and chronic leukemias of myeloid and lymphoid phenotypes. Hence, we can hypothesize that MPN-U associated with rearrangements of JAK2 is a hematopoietic stem cell (HSC) disease that is only curable by HSC transplantation [10].

4. Conclusion
We described a rare Ph-negative case of MPN with a BCR-JAK2 transcript and a reciprocal t(9;22)(q34;q11) that was detected juxtaposing BCR exon 13 and JAK2 exon 17. This rare entity underlies often an aggressive clinical course with rapid progression to blast phase within the first 2 years after diagnosis (Table 1). To the best of our knowledge, this is the thirteenth case reported worldwide. Furthermore we report here the first described isoform fusion transcript juxtaposing BCR exon 13 and JAK2 exon 17. It revealed one of the longest sustained complete clinical, hematologic and cytogenetic remissions in a BCR-JAK2 fusion MPN-U. These rare BCR-JAK2 fusions suggest common pathways between JAK2 activation and the natural history of lympho/myeloproliferative hematologic malignancies. One should take into consideration JAK2 fusions when investigating Ph-negative MPN patients.

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.
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