A Cryptic *BCR-PDGFRB* Fusion Resulting in a Chronic Myeloid Neoplasm With Monocytosis and Eosinophilia: A Novel Finding With Treatment Implications

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ABSTRACT

RNA-seq was used to identify the partner gene and confirm the presence of a *BCR-PDGFRB* fusion. Identification of this fusion product resulted in successful treatment and long-term remission of this myeloid neoplasm. Based on our results, we suggest that despite current WHO recommendations, screening for *PDGFRB* rearrangement in cases of leukocytosis with eosinophilia and no other etiologic explanation is necessary, even if the karyotype is normal.

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Background

Myeloid and lymphoid neoplasms with eosinophilia and gene rearrangement constitute a distinct group of hematologic neoplasms in the current WHO classification for hematopoietic neoplasms.¹ Included in this category are neoplasms harboring abnormal gene fusions involving *PDGFRA*, *PDGFRB*, and *FGFR1* or *PCM1-JAK2*. These fusions result in constitutive activation of respective tyrosine kinases that can be targeted using specific kinase inhibitors. There are multiple recognized partner genes for *PDGFRA*, *PDGFRB*, *FGFR1*, and *JAK2*. *PDGFRB* located at chromosome 5q32 has >25 known fusion partners, with *ETV6* the most common.^{2–8} Although *PDGFRA* rearrangements often can be cryptic and not readily observed on routine karyotyping studies,^{9,10} *PDGFRB* rearrangements are believed to be nearly always visible on routine karyotype, and the WHO textbook specifically states that “molecular analysis for *PDGFRB* is not necessary for myeloid neoplasms with eosinophilia if routine karyotypic studies do not reveal a 5q abnormality.”¹¹

This report describes a myeloid neoplasm presenting with morphologic features of chronic myelomonocytic leukemia with eosinophilia harboring a cryptic and novel *BCR-PDGFRB* fusion. Discovery of this fusion led to successful treatment with imatinib, a tyrosine kinase inhibitor. To our knowledge, this is the first report of a *BCR-PDGFRB* fusion in a myeloid neoplasm associated with eosinophilia.

Methods

Next-generation sequencing (NGS) was performed on DNA obtained from the bone marrow (BM) aspirate using a whole exome panel of 28 genes commonly associated with myeloid neoplasms, as described previously.¹¹

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Conventional chromosomal analysis was performed on G-banded metaphases prepared from unstimulated 48-hour BM aspirate cultures using standard techniques; 20 metaphases were analyzed.

Fluorescence in situ hybridization (FISH) was performed using a *PDGFRB* dual-color breakapart probe set (Cytocell Ltd).

RNA-seq analysis was performed using 700 ng of total RNA and a TruSeq RNA Sample Preparation Kit v2 (Illumina). The library was enriched selectively by 12 cycles of PCR followed by size selection per the manufacturer's protocol. The resulting size-fractionated library was sequenced using a 75-bp short-read paired-end reads protocol on an Illumina HiSeq 2000 Sequencing System. The resultant BCL files were demultiplexed and converted to fastq files using Illumina's Consensus Assessment of Sequence and Variation (CASAVA) tool. After standard quality control using RNA-SeQC, fastq files were processed using an integrative analysis of multiple fusion detection methods with hg19 as the reference genome. To increase specificity, 3 fusion detection tools—TopHat-Fusion, MapSplice, and FusionMap—were

applied; only fusions detected by at least 2 tools were considered positive.^{12–14}

Results

The patient was a 26-year-old man who presented with a 2-week history of fatigue, sore throat, night sweats, muscle aches, and weight loss. A diagnostic workup was initiated with a clinical consideration of acute leukemia in view of the short symptomatic history and pathology report from another institution indicating a “packed” bone marrow. Physical examination of the patient showed splenomegaly. Renal function, liver function, and other biochemical parameters, including serum lactate dehydrogenase levels, were within normal limits. A CBC count revealed anemia (hemoglobin level, 11.8 g/dL; reference, 13.5–17.5 g/dL), leukocytosis (WBC count, $37.6 \times 10^9/L$; reference, $4–11 \times 10^9/L$), and a normal platelet count ($152 \times 10^9/L$; reference, $150–450 \times 10^9/L$). The differential leukocyte count showed monocytosis (47%) and eosinophilia (15%). BM examination showed hypercellular (~95%) BM with myelomonocytic hyperplasia and eosinophilia including increased eosinophilic

Figure 1. Bone marrow aspirate smear showing (A) increased monocytes with atypical morphology (black arrows), eosinophilic precursors (orange arrows), and (B) occasional morphologically unremarkable mast cells (red circle) (Wright-Giemsa, original magnification $\times 1,000$). (C) Bone marrow trephine biopsy specimen showing hypercellular bone marrow with marked myelomonocytic hyperplasia with increased eosinophils and increased atypical megakaryocytes (some with separated nuclear lobes), the latter showing emperipolesis (hematoxylin-eosin, original magnification $\times 400$). (D) A mast cell tryptase stain shows increased mast cells; however, no abnormal aggregates were identified (immunohistochemistry with hematoxylin counterstain, original magnification $\times 400$).

myelocytes and metamyelocytes, erythroid hypoplasia, and no significant increase in blasts. Megakaryocytes were mildly increased but did not form distinct clusters; they had predominantly unremarkable morphology and showed frequent emperipolesis (Figure 1A–C). Mast cells were increased but did not form abnormal aggregates (Figure 1D). Ring sideroblasts were not identified. Flow cytometric immunophenotypic analysis showed a small (0.2%) population of myeloid blasts without immunophenotypic aberrancies. Monocytes were increased (26%) and showed aberrant CD56 expression. A small population of mast cells with aberrant expression of CD25 and CD30 was also present. Conventional cytogenetics showed a diploid male karyotype, 46,XY[20] (Figure 2A). FISH was negative for *BCR-ABL1*. Microarray-based comparative genomic hybridization (array CGH) revealed no chromosomal copy number alterations. NGS did not reveal any mutations. Because of the presence of eosinophilia,

additional FISH testing for *PDGFRA*, *PDGFRB*, and *FGFR1* was performed, revealing a *PDGFRB* rearrangement in 55% of the interphase nuclei analyzed (Figure 2B); *PDGFRA* and *FGFR1* were intact. A mapback FISH analysis on a G-banded metaphase showed that 3' *PDGFRB* (red) had translocated to a morphologically normal chromosome 22q and that 5' *PDGFRB* (green) had remained on chromosome 5q (Figure 2C). The RNA-seq FusionMap caller identified 2 unique fusion reads that defined an in-frame fusion joining exon 13 of *PDGFRB* on chromosome 5 (149504394; hg19) to exon 1 of *BCR* on chromosome 22 (23524426; hg19). The MapSplice caller further confirmed the same *BCR-PDGFRB* with similar breakpoints, with 3 unique read counts and 1 paired-read. The resultant fusion product was formed by the first 426 residues of *BCR* fused to the last 505 residues of *PDGFRB* containing the oligomerization domain and serine threonine kinase domain of *BCR* and the kinase domain of *PDGFRB* (Figure 2D). In light of these findings, a

Figure 2. (A) Chromosomal analysis showed a diploid male karyotype 46,XY. (B) FISH analysis using a CytoCell dual-color breakapart *PDGFRB* probe showed split signals, indicating *PDGFRB* rearrangement. Normal pattern: 2 fusion (2F) yellow signals; abnormal pattern: 1 red, 1 green, 1 fusion (1R1G1F) signals. (C) Mapback FISH analysis on a G-banded metaphase with a CytoCell dual-color breakapart *PDGFRB* probe revealed 3' *PDGFRB* (red) on chromosome 22 and 5' *PDGFRB* (green) on chromosome 5. (D) Schematic representation of the *BCR-PDGFRB* fusion product with donor and acceptor sequences.

Abbreviations: ch, chromosome; FISH, fluorescence in situ hybridization; Ig, immunoglobulin; S/T, serine/threonine; TK, tyrosine kinase; TM, transmembrane.

retrospective review of the routine karyotype was performed that confirmed the alteration to be cryptic.

Discussion

Myeloid neoplasms with eosinophilia and *PDGFRB* rearrangement have been described more commonly in men (2:1 ratio) over a wide age range (8–72 years), with median age in the late 40s. The hematologic presentation in cases of myeloid neoplasms with *PDGFRB* rearrangement is most often that of chronic myelomonocytic leukemia, usually with eosinophilia, as was seen in this case.^{6,15} The *BCR-PDGFRB* we describe is a novel fusion, not yet documented in the published literature. The fusion retained the oligomerization and serine threonine kinase domains of *BCR*, supporting constitutive activation of the retained tyrosine kinase domain of *PDGFRB*. This hypothesis is supported by the prompt, effective, and long-lasting control of leukocytosis and eosinophilia through treatment with imatinib in this patient. The patient was treated with imatinib, 400 mg daily, which resulted in complete response with normalization of blood counts and disappearance of eosinophilia within 3 months. A follow-up BM sample performed 3 months after initial diagnosis was morphologically unremarkable and negative for *PDGFRB* rearrangement by FISH. At the time of writing, the patient is undergoing regular follow-up and continues to be experiencing disease remission after 3 years of therapy.

In addition to showing a novel fusion product, this case shows a clinically critical point: that screening for *PDGFRB* rearrangement in cases of leukocytosis with eosinophilia and no other etiologic explanation may be helpful and potentially lifesaving, even if the routine karyotype is diploid.

Due to the large number of potential fusion partner genes, molecular analysis, namely RNA-seq, can be helpful in identifying a partner gene once rearrangement is detected by FISH. In addition to confirming the diagnosis, molecular characterization of the fusion at diagnosis using RT-PCR or RNA sequencing will provide a potential target for monitoring treatment response and measuring residual disease. The presence of immunophenotypically abnormal mast cells as seen in this case is also a helpful clue to assess for *PDGFRB* alterations, because these cases frequently harbor abnormal mast cell populations, despite not meeting diagnostic criteria for systemic mastocytosis.^{1,16}

It is useful to perform immunohistochemistry for mast cells tryptase and/or CD117 to exclude systemic mastocytosis. Unlike systemic mastocytosis, the mast cells described in this case do not form dense aggregates and are not morphologically abnormal (spindled);

furthermore, a *KIT* D816V mutation was not detected. This case also highlights the importance of a systematic workup for potential recurrent gene rearrangements associated with myeloid neoplasms with eosinophilia. Early diagnosis of the underlying molecular aberration in myeloid neoplasms with eosinophilia will help in the timely assignment of appropriate targeted therapy and reducing the probability of potential tissue damage associated with eosinophilia. In some patients, timely identification might help induce a remission that may permit allogeneic stem cell transplant therapy.¹⁰ This case also highlights the utility of a stepwise approach in using *PDGFRB* FISH for accurate diagnosis of myeloid neoplasms with eosinophilia that do not have evidence of 5q31–33 alterations on routine karyotypic analysis, in which the presence of eosinophilia cannot be attributed to other molecular or cytogenetic alterations, such as *BCR-ABL1* translocation or alterations of *PDGFRA*.¹⁷ Currently FISH is considered to be more practical and readily available in most commercial and academic settings. Although more advanced technologies such as RNA-seq are useful in identifying unknown gene partners in these neoplasms, as shown in our case, they are not readily accessible outside of the research setting, and therefore we do not currently recommend their routine use in the workup of such cases.

Although limited by its observational nature, this case does highlight a potential pitfall in the diagnosis of myeloid neoplasms with eosinophilia, and supports the algorithmic approach recommended by Fang et al¹⁷ in their recent publication describing the systematic use of *PDGFRB* FISH in such neoplasms.

Conclusions

This report presents the case of a myeloid neoplasm with monocytosis and eosinophilia harboring a novel and cryptic *PDGFRB* rearrangement leading to a *BCR-PDGFRB* fusion. Identification of this fusion protein resulted in successful treatment and long-term sustained remission of this myeloid neoplasm.

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