A Novel *PCM1-PDGFRB* Fusion in a Patient with a Chronic Myeloproliferative Neoplasm and an *ins(8;5)*

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A 54-year-old male patient presented in December 2008 with raised eosinophils on a routine full blood count. The white cell count was $10.3 \times 10^9/L$ with hemoglobin 13.6 g/dL, platelets $154 \times 10^9/L$, and eosinophils $1.4 \times 10^9/L$. He was otherwise asymptomatic. *BCR-ABL1* and *FIPIL1-PDGFRB* were both negative and his cytogenetics was reported as *del(5)(q22q31)* in 10 of 30 metaphases. Based on the above findings, he was formally diagnosed with myeloproliferative neoplasms, unclassified (MPN-U) with chromosome 5 abnormalities and eosinophilia. He was initially treated with low-dose dasatinib (the physician's choice), with a good hematological response and rapid resolution of the peripheral blood eosinophilia, but the treatment was discontinued after 10 months due to recurrent, symptomatic pleural effusions.

In 2013, nearly 5 years after the initial diagnosis, the patient continued to have persistent but asymptomatic mild eosinophilia off-therapy with otherwise normal blood counts. Cytogenetics was repeated and showed a *46,XY,ins(8;5)(p23;q33q35)* in all 30 metaphases analyzed. Retrospective analysis of the original diagnostic sample revealed the same abnormality, which had originally been misinterpreted due to poor-quality metaphases. Fluorescence in situ hybridization (FISH) analysis using *PDGFRB* breakapart probes (Cytocell, Cambridge,

UK) confirmed disruption of *PDGFRB* in both the original and follow-up samples. Based on this new finding and the reported efficacy of imatinib in *PDGFRB*-rearranged MPN [1, 2], the patient was treated with low-dose imatinib (200 mg daily) in July 2013. This was well tolerated and within 3 months of therapy, a repeat bone marrow assessment showed complete morphological and cytogenetic remission including FISH negativity for the *PDGFRB* rearrangement. Ongoing assessments indicated continuing morphological and cytogenetic remission whilst being maintained on low-dose imatinib, with FISH negativity in January 2015 and July 2016.

In order to have a marker for more sensitive minimal residual disease analysis, we retrospectively sought to identify the putative *PDGFRB* fusion partner using archived frozen cells taken at the time of initial diagnosis. Despite only being able to extract poor-quality RNA from the sample (RNA integrity number <5), we performed RNA-seq analysis. PolyA+ RNA extraction, library preparation, and 100-bp paired-end sequencing was performed with multiplexing for a minimum of 75 million reads per sample using an Illumina HiSeq 2000. Bowtie, TopHat, and TopHat-Fusion were used to align reads, resolve splice junctions, and identify and filter potential tyrosine kinase fusions, as previously described [3]. On

Fig. 1. *PCM1-PDGFRB* fusion. **a** Fusion junction of the chimeric *PCM1-PDGFRB* gene. **b** RT-PCR analysis: *PCM1-PDGFRB* was amplified from patient cDNA (E3419) but not negative control cases (E1180, E1181) using the primers PCM-AF (5'-ACACAGACTACTTGAGACAGAGGGC-3') in *PCM1* exon 25 and PDGFRB-AR (5'-GCTCCGACATAAGGGCTTGC-TTC-3') in *PDGFRB* exon 14. **a, c** MRD analysis. *PCM1-PDGFRB* cDNA was amplified by nested PCR for 30 cycles, each round using the primers PCM-AF plus PDGFRB-AR in the first step, followed by reamplification with the primers PCM-DF (5'-TGCTGATAATGCTAGTGTCCTGTCT) in *PCM1* exon 27 and PDGFRB-DR (5'-TGATAAGGGAGATGATGGTGAGCAC-3') in *PDGFRB* exon 11 for the second step. **c** The resulting product of 134 bp was seen in dilutions of the ins(8;5) patient's presentation cDNA down to 10^{-3} , but not in control samples or in blood or bone marrow samples taken 3 years after starting imatinib. **b** Confirmation of sample quality by amplification of normal *ABL1* cDNA using primers ABL 123 F (5'-TGGAGATAACACCTTAAGCATTA-ACTAAAGGT-3') and ABL R (5'-GATG-TAGTTGGTTGGGACCCA-3').

Fig. 2. Predicted structure of the *PCM1-PDGFRB* fusion protein. TM, transmembrane domain; TK, tyrosine kinase.

initial analysis no *PDGFRB* abnormalities were identified, but on relaxation of the filtering parameters, two breakpoint-spanning reads were identified with *PCM1* fused to *PDGFRB*. Although this fusion has not been reported before, *PCM1* was a plausible candidate since it is located at 8p22 and fuses to the tyrosine kinase *JAK2* in MPN with eosinophilia as a consequence of a recurrent t(8;9)(p22;p24) [4]. The *PCM1-PDGFRB* fusion was confirmed by RT-PCR and Sanger sequencing analysis, with *PCM1* exon 27 joined to *PDGFRB* exon 11, and thus preserving the reading frame (Fig. 1a, b). The fusion protein is predicted to retain the bulk of *PCM1*, including several coiled-coil domains and the entire transmembrane and cytoplasmic domains of *PDGFRβ* (Fig. 2).

Having identified and confirmed a *PCM1-PDGFRB* fusion in this case, we designed nested PCR primers for sensitive detection of minimal residual disease after therapy. Using serial dilutions of the (poor-quality presentation) cDNA in water, we found that the nested PCR was able to amplify the fusion down to a 1:1,000 dilution. We then tested blood and bone marrow samples taken from the patient 3 years after starting imatinib; residual *PCM1-PDGFRB* mRNA was not detected in either sample (Fig. 1c).

It is known that signaling through *PDGFRB* plays an important role in mitogenesis, cytoskeletal rearrangements, and chemotaxis [5, 6]. Disruption of *PDGFRB*, normally located on chromosome 5q33, was first described as the consequence of the t(5;12), in which the 5'

end of *ETV6* is juxtaposed to the 3' end of *PDGFRB* [7]. Subsequently, many more translocations involving 5q31–33 have been identified, resulting in the identification of >30 distinct *PDGFRB* fusion genes [8].

Pericentriolar material 1 (*PCM1*) encodes a component of centriolar satellites and is essential for the correct localization of several centrosomal proteins, and for anchoring microtubules to the centrosomes [9]. *PCM1-PDGFRB* and *PCM1-JAK2* are thus further examples of fusions involving centrosome components, with potentially important functional consequences [10].

Many patients with *PDGFRB* fusions achieve long-term molecular remission on imatinib therapy [2], and it appears that our case is no exception. Whether, as in chronic myeloid leukemia, some of these patients may be functionally cured and can safely stop treatment [11] remains to be seen. In the meantime, *PCM1-PDGFRB* should be added to the list of abnormalities associated with the WHO-defined subtype “myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*, or with *PCM1-JAK2*” [12].

Disclosure Statement

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