A novel *PCM1-PDGFRB* fusion in a patient with a chronic myeloproliferative neoplasm and an ins(8;5)

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 x 10^9/L with haemoglobin 13.6 g/dL, platelets 154 x 10^9/L and eosinophils 1.4 x 10^9/L. He was otherwise asymptomatic. *BCR-ABL1* and *FIPIL1-PDGFRA* 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 MPN-U with chromosome 5 abnormalities and eosinophilia. He was initially treated with low dose dasatinib (physician choice) with good haematological response and rapid resolution of peripheral blood eosinophilia but treatment was discontinued after 10 months due recurrent, symptomatic pleural effusions. In 2013, nearly 5 years after 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 analysed. Retrospective analysis of the original diagnostic sample revealed the same abnormality, which had been misinterpreted originally due to poor quality metaphases. Fluorescence in situ hybridisation (FISH) analysis using *PDGFRB* break apart 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 MPNs (1) (2), the patient was treated with low dose imatinib (200mg 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 100bp paired-end sequencing was performed with multiplexing for a minimum of 75 million reads/sample using an Illumina HiSeq 2000. Bowtie,

TopHat and TopHat-Fusion were used to align reads, resolve splice junctions, identify and filter potential TK fusions as previously described (3). On 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 myeloproliferative neoplasms (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 (Figure 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 (Figure 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 in 1000 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 (Figure 1c).

It is known that signalling 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 more than 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 World Health Organisation-defined subtype 'myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*, or with *PCM1-JAK2*' (12)

Muna Ghazzawi,[1,2] Varun Mehra,[3] Marcin Knut,[1] Lorretta Brown[3]; William Tapper,[1] Andrew Chase, [1,2] Hugues de Lavallade,[3] Nicholas C. P. Cross[1,2]

[1] Faculty of Medicine, University of Southampton, Southampton, UK

[2] Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury, UK

[3] Department Haematology, King's College Hospital, London, UK

FIGURE LEGENDS

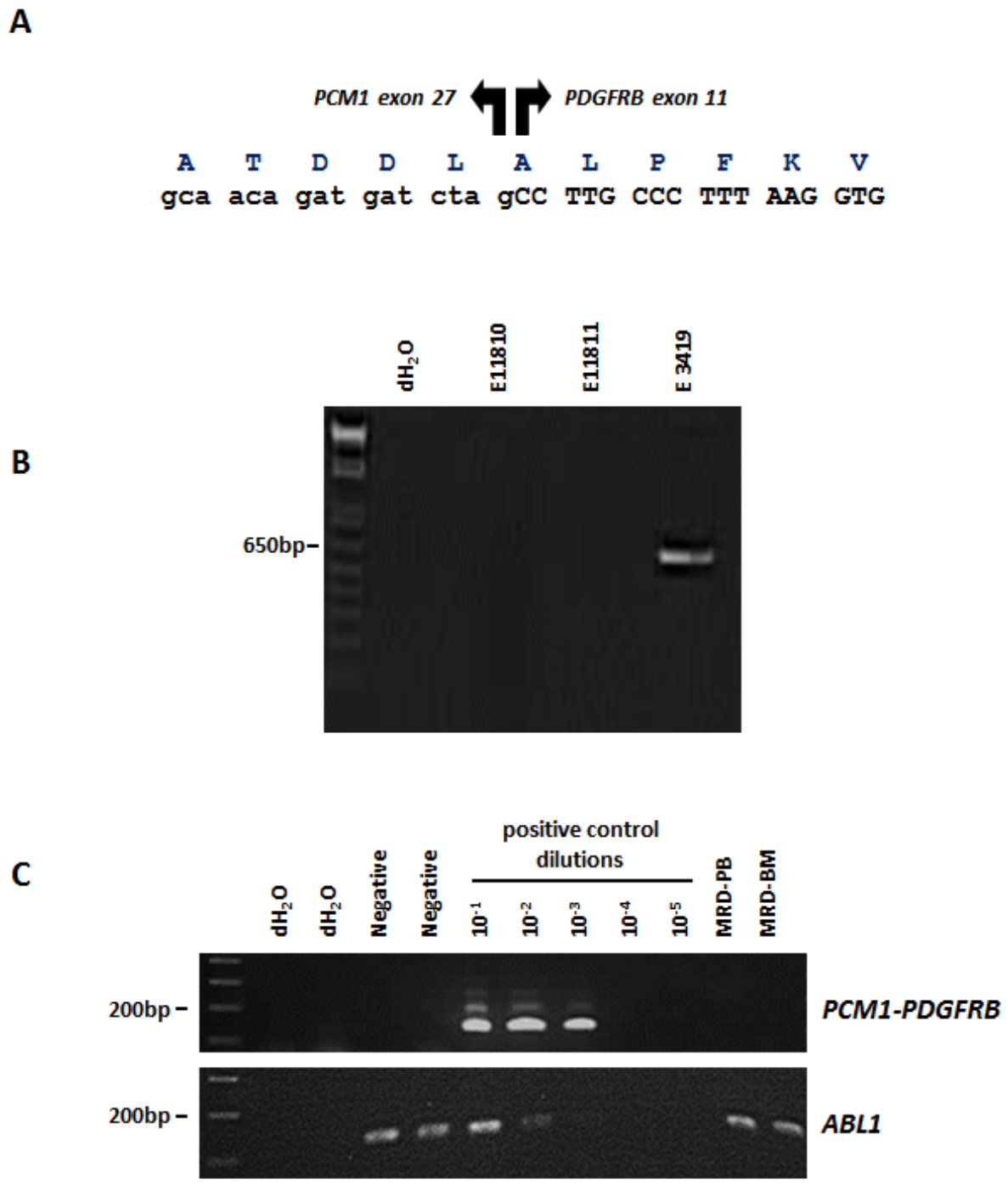

Figure 1: The *PCM1-PDGFRB* fusion. (A) Fusion junction of the chimeric *PCM1-PDGFRB* gene. (B) RT-PCR analysis: *PCM1-PDGFRB* was amplified from patient cDNA (E3419) but not negative control cases (E1180, E1181) using primers PCM-AF (5'-ACACAGACTACTTGAGACAGAGGGC-3') in *PCM1* exon 25 and PDGFRB-AR (5'-GCTCCGACATAAGGGCTTGCTTC-3') in *PDGFRB* exon 14. (C) (A) MRD analysis. *PCM1-PDGFRB*

cDNA was amplified by nested PCR for 30 cycles each round using primers PCM-AF plus PDGFRB-AR in the first step, followed by reamplification with primers PCM-DF (5'-TGCTGATAATGCTAGTGTCCTGTCT) in *PCM1* exon 27 and PDGFRB-DR (5'-TGATAAGGGAGATGATGGTGAGCAC-3') in *PDGFRB* exon 11 for the second step. The resulting product of 134bp 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'- TGGAGATAACACCTTAAGCATTAACTAAAGGT-3') and ABL R (5'-GATGTAGTTGGTTGGGACCCA-3').

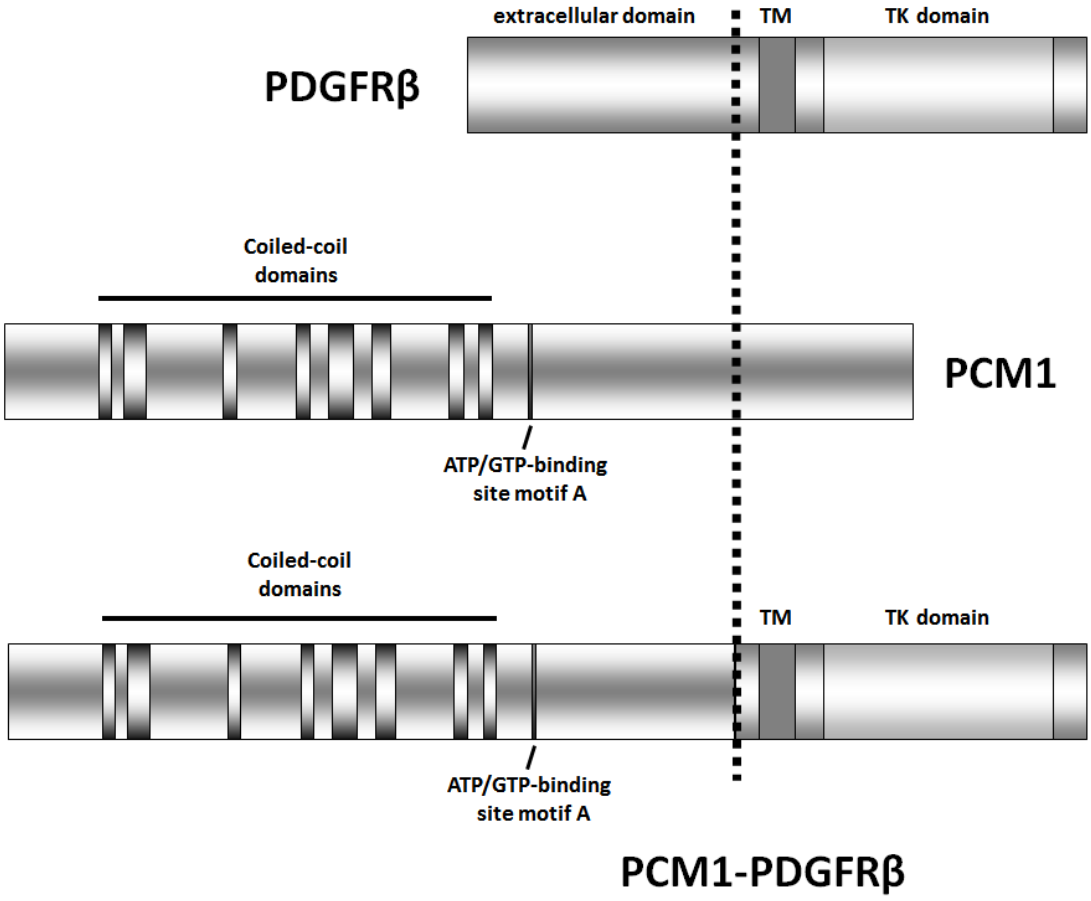

Figure 2. Predicted structure of the PCM1-PDGFRβ fusion protein. TM, transmembrane domain; TK, tyrosine kinase

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