Cytogenetically cryptic *ZMYM2-FLT3* and *DIAPH1-PDGFRB* gene fusions in myeloid neoplasms with eosinophilia

More than 70 tyrosine kinase (TK) fusion genes have been identified in myeloid neoplasms as a consequence of reciprocal translocations or other genomic rearrangements. These TK fusions are generally primary drivers of myeloproliferation and important therapeutic targets, as well as being major criteria for the diagnosis of specific disorders. For example, chronic myeloid leukemia is defined by the presence of *BCR-ABL1*, and myeloid/lymphoid neoplasms with eosinophilia are defined by fusions involving *PDGFRA*, *PDGFRB*, *FGFR1* or *PCM1-JAK2*.[1] Other TK fusions have been described in patients with various subtypes of myeloproliferative neoplasms (MPN) or myelodysplastic/myeloproliferative neoplasms (MDS/MPN). Most of these individuals have pronounced eosinophilia,[2] but occasional cases have other phenotypes such as polycythemia vera (PV) or systemic mastocytosis.[2, 3] Apart from *FIP1L1-PDGFRA,* which is formed by a small deletion at 4q12,[4] TK fusions are almost always associated with visible karyotypic abnormalities. Despite their apparent prominence in the literature, TK fusions are in fact uncommon and the pathogenesis of the majority of MPN with eosinophilia (MPN-eo) remains unexplained. Some TK-fusion negative cases test positive for *KIT* D816V or *JAK2* V617F, whereas others are positive for mutations in a range of genes associated with myeloid disorders such as *TET2*, *ASXL1*, *EZH2* and *SETBP1*.[5,6,7] We hypothesized that hitherto undetected cryptic TK fusion genes may drive MPN-eo as well as other disorders such as *JAK2*-unmutated PV.

We used RNAseq to search for TK fusion genes in cases with MPN-eo or hypereosinophilia of unknown significance (HE_{US}) with a normal karyotype (n=14), PV with low or normal erythropoietin levels that tested negative for MPN phenotype driver mutations (n=6) and cell lines that were derived from MPN or MDS patients that had transformed to acute myeloid leukemia (F-36P, ELF-153, FKH-1, GDM-1, SKK-1, SKM-1). RNA extraction, polyA+ RNA-Seq library preparation, stranded RNAseq protocol and 100bp paired-end sequencing was performed with multiplexing for a minimum of 75 million reads/sample using an Illumina HiSeq 2000. Bowtie and TopHat-Fusion were used to align reads, resolve splice junctions, identify and filter potential TK fusions as previously described.[8] Confirmation and

screening of fusions was performed by RT-PCR and Sanger sequencing (Supplementary Table 1).

Of the 20 patient samples, two novel TK fusions were identified. In frame *DIAPH1-PDGFRB* and *ZMYM2-FLT3* fusion mRNAs (Figure 1; Supplementary Figures 1 and 2) were found in single patients with MPN-eo. None of the cases were positive for *TNIP1-PDGFRB*, a recently described cryptic fusion in MPN-eo.[9] Unusually, the fusion breakpoints in our cases fell within exons of both the partner and TK genes. No TK fusions were detected in the PV cases, but the FKH-1 and SKK-1 cell lines were positive for *ETV6-ABL1* and *ETV6-NTRK3*, respectively (Supplementary Figure 3). Although these fusions have been described previously, neither line was known to be positive and the presence of these fusions was not suspected on the basis of the karyotype. [10, 11]

DIAPH1 and *PDGFRB* are located 8.5Mb apart at 5q31.3 and 5q32, respectively. They are both oriented from telomere to centromere and thus the fusion presumably arose as a consequence of a tandem duplication or a translocation t(5;5)(q31.3;q32), both of which would be difficult to detect by routine cytogenetics. The affected patient, a 37-year-old male, was diagnosed with an MPN-eo and contemporaneous T-cell lymphoblastic lymphoma, most likely representing extramedullary lymphoid blast phase [12]. The karyotype was normal. The patient received intensive chemotherapy and achieved complete hematological remission (CHR) with disappearance of the lymphadenopathy. Two weeks later he developed leukocytosis ($119x10^9$/L) with significant eosinophilia ($21x10^9$/L), hepatosplenomegaly but with no recurrence of lymphadenopathy. Consolidation intensive chemotherapy treatment was started without response. Molecular analyses revealed overexpression of *PDGFRB* [13] and the *DIAPH1-PDGFRB* fusion was subsequently identified by RNAseq analysis. He received imatinib 100 mg/day and achieved CHR within 4 weeks but died due to a rapidly progressive neurodegenerative disorder at month 27 whilst still in complete remission. To test if *DIAPH1-PDGFRB* is a recurrent abnormality, we screened 50 additional cases with MPN-eo by RT-PCR but did not identify any further positive cases.

ZMYM2 and *FLT3* are both located at 13q12 and are in opposite orientations. *ZMYM2-FLT3* is thus predicted to arise as a consequence of an 8Mb inversion (Supplementary Figure 3).

ZMYM2 is the fourth gene reported to fuse to *FLT3* in myeloid neoplasms[2] but the first *FLT3* fusion that is cytogenetically cryptic. We screened 105 additional cases with MPN-eo, HE_{US} or other atypical MPN by RT-PCR. One additional positive case was detected, with similar but not identical breakpoints to the initial case (Figure 1). PCR analysis of genomic DNA for the second case (DNA was not available from Case 1) revealed that the cDNA and genomic breakpoints were identical, indicating the formation of a fusion exon by the inversion. We note that a third case with *ZMYM2-FLT3* has been reported recently in a patient with *BCR-ABL1*-like acute lymphoblastic leukemia.[14]

Both cases with *ZMYM2-FLT3* had MPN-eo. Case 1, a 48 year old female, presented with leukocytosis (30 x 10^9/L), eosinophilia (2 x 10^9/L, elevated serum tryptase (37µg/L), splenomegaly and a hypercellular bone marrow (BM) with increased numbers of loosely scattered mast cells. Cytogenetics was normal, *FIP1L1-PDGFRA*, *KIT* D816V and *JAK2* V617F were all negative and no relevant mutations were identified by myeloid panel analysis (28 genes). After 10 months, she progressed to myeloid blast phase. Because the disease was resistant to AML-induction chemotherapy, an allogeneic peripheral blood stem cell transplant was performed from an unrelated donor 13 months after diagnosis. She died 6 months later from chronic graft versus host disease and septic shock; the *ZMYM2-FLT3* fusion was identified post mortem.

Case 2, a 47 year old male, presented with eosinophilia (4.7 x 10^9/L), elevated serum tryptase (42µg/L) and a hypercellular BM. Cytogenetics was normal and *FIP1L1-PDGFRA*, *KIT* D816V and *JAK2* V617F were all negative. There was no response to steroids or hydroxyurea. Following the finding of *ZMYM2-FLT3* positivity, treatment with sunitinib off-label at 50mg/day was commenced. Blood counts started to improve from day 4 and normalized after 3 weeks. During a pause of 3 weeks due to pulmonary infection, leukocytes/eosinophils rapidly increased, but normalized again within weeks after restart of sunitinib, initially at a dose of 25mg/day and then subsequently 35mg/day. The patient has been maintained on sunitinib for 10 months (since re-start) and remains in CHR (Figure 2).

In conclusion, we have found that *ZMYM2-FLT3* and *DIAPH1-PDGFRB* fusion genes are novel, cytogenetically cryptic and therapeutically targetable abnormalities in MPN-eo, and are thus

reminiscent of *FIP1L1-PDGFRA* positive myeloid neoplasms. Due to their extensive diversity and clinical importance, we believe that genome wide or targeted RNAseq is rapidly becoming the method of choice to detect rare TK fusions.

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Conflicts of interest

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Figure legends

Figure 1. Fusion junctions for *DIAPH1-PDGFRB* and *ZMYM2-FLT3* identified by RNAseq analysis (panels A and B), plus the additional *ZMYM2-FLT3* positive case detected by RT-PCR screening.

Figure 2. *ZMYM2-FLT3* fusion (case 2): longitudinal measurements of absolute leucocytes and eosinophil values during treatment with prednisolone (PRD in mg/day), hydroxyurea (HU in mg/day), and sunitinib (in mg/day).

A

DIAPH1 exon 11 — *DIAPH1* exon 11

AATGTGTTTGATGAACAAGGGGAAGAGGATTCCTAT

-N--V--F--D--E--Q--G--E--E--D--S--Y-

DIAPH1 breakpoint

PDGFRB exon 10 — *PDGFRB* exon 10

ACGCTGCTGGGGAACA***GTTCCGAAGAGGAGAGCCAG***

-T--L--L--G--N--S--S--E--E--E--S--Q-

PDGFRB breakpoint

DIAPH1 exon 11 — *PDGFRB* exon 10

AATGTGTTTGATGAACA***GTTCCGAAGAGGAGAGCCA***

-N--V--F--D--E--Q--F--R--R--G--E--P-

B

ZMYM2 exon 20 — *ZMYM2* exon 20

TACCAGTCTCATGATGATAGTTCTGACAATTCAGAA

-Y--Q--S--H--D--D--S--S--D--N--S--E-

ZMYM2 breakpoint

FLT3 exon 14 — *FLT3* exon 14

GAGTACTTCTACGTTGAT***TTCAGAGAATATGAATAT***

-E--Y--F--Y--V--D--F--R--E--Y--E--Y-

FLT3 breakpoint

ZMYM2 exon 20 — *FLT3* exon 14

TACCAGTCTCATGATGAT***TTCAGAGAATATGAATAT***

-Y--Q--S--H--D--D--F--R--E--Y--E--Y-

C

ZMYM2 exon 20 — *ZMYM2* exon 20

GGATACCAGTCTCATGATAGTTCTGACAATTCAGAA

-G--Y--Q--S--H--D--S--S--D--N--S--E-

ZMYM2 breakpoint

FLT3 exon 14 — *FLT3* exon 14

GTGACTGGCCCCCTGG***ATAATGAGTACTTCTACGTT***

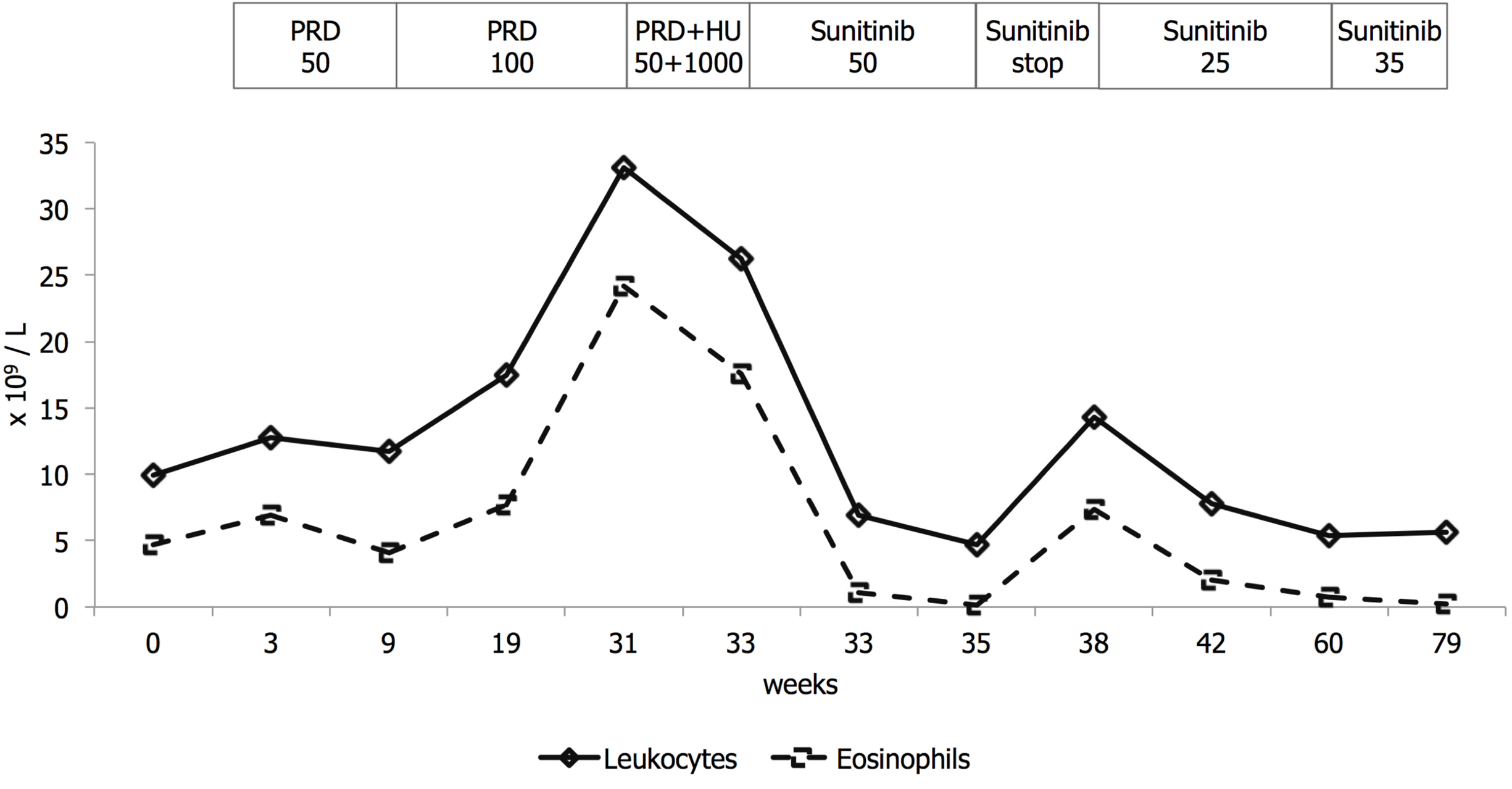
-V--T--G--P--L--D--N--E--Y--F--Y--V-

FLT3 breakpoint

ZMYM2 exon 20 — *FLT3* exon 14

GGATACCAGTCTCATG***ATGATGAGTACTTCTACGTT***

-G--Y--Q--S--H--D--D--E--Y--F--Y--V-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