

LETTER TO THE EDITOR

Comprehensive genomic profiling identifies a novel *TNKS2–PDGFRA* fusion that defines a myeloid neoplasm with eosinophilia that responded dramatically to imatinib therapy

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Myeloid and lymphoid neoplasms with *PDGFRA* gene rearrangements are a category of rare diseases that typically manifest with peripheral blood eosinophilia accompanied by eosinophilic tissue infiltrates, and may be associated with typical or atypical mast cell proliferations. These tumors can present as myeloproliferative neoplasms, acute myeloid leukemias or lymphoblastic leukemias/lymphomas and are, consequently, classified within the larger category of 'Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*' in the 2008 World Health Organization (WHO) classification. For neoplasms harboring *PDGFRA* rearrangements, *FIP1L1–PDGFRA* is by far the most common fusion detected, reflecting an 800-kb intrachromosomal deletion [del(4)(q12q12)].¹

Identification of *PDGFRA* rearrangement is both an important diagnostic and predictive marker, as these neoplasms are typically responsive to treatment with imatinib.^{2,3} The atypical mast cell proliferations that accompany these tumors may result in a mistaken diagnosis of systemic mastocytosis (SM),^{4,5} which is associated with an activating D816V alteration in *KIT*.⁶ The discrimination of these entities is important, as *PDGFRA*-rearranged tumors with eosinophilia, unlike SM harboring *KIT* D816V, are typically imatinib responsive.⁷

We describe a novel *TNKS2–PDGFRA* fusion in a myeloid neoplasm with eosinophilia, detected via comprehensive genomic profiling with a next-generation sequencing-based assay (FoundationOne), the presence of which correlated with the results from multiprobe fluorescent *in situ* hybridization (FISH) testing. Initially this patient was diagnosed with an aggressive SM (aSM); however, detection of this novel fusion, a translocation between chromosomes 4(q12) and 10(q23.3), resulted in diagnostic reclassification and led to targeted drug therapy with a resulting dramatic clinical response.

A 58-year-old Caucasian woman presented with left upper quadrant pain and hepatosplenomegaly with the splenic tip palpable to 15 cm below the left costal margin and liver palpable 5 cm below the right costal margin, with 6 years of chronic, untreated hepatitis C and hepatic cirrhosis diagnosed 2 years prior. An abdominal magnetic resonance imaging scan confirmed splenomegaly with the evidence of splenic infarct and a nodular liver, consistent with cirrhosis. A small amount of ascites was present. Her medical history was significant for mild thrombocytopenia of several years duration with no evidence of bleeding.

A bone marrow core biopsy was performed and was hypercellular (100% cellularity) with dense infiltrates and aggregates of >15 spindle mast cells as well as increased eosinophils. Mast cells were positive for CD2 and CD25 expression by immunohistochemistry. Targeted genomic analysis for mutations in *KIT* was ordered, but ultimately failed due to insufficient sample material. Cytogenetic analysis was ordered but the results were not immediately available, given the inherent time needed for processing. On the basis of these findings, the patient was initially

diagnosed with aSM. A peripheral blood count performed a month after initial diagnosis revealed leukoerythroblastic features with a marked absolute eosinophilia (white blood cells of $20.6 \times 10^9/l$, absolute eosinophil count of 3708/ μl) and no circulating blasts. An expanded blast population was not seen either in the marrow aspirate smear or using CD34 immunohistochemistry on the core biopsy. The patient's clinical status deteriorated rapidly with worsening thrombocytopenia and progressive anasarca, necessitating hospitalization. Diuretics were of little benefit.

Subsequent FISH testing was reported positive for a *PDGFRA* gene rearrangement and was attributed to presence of the canonical *FIP1L1–PDGFRA* fusion. However, karyotyping identified a translocation involving chromosomes 4 and 10. Comprehensive genomic profiling by FoundationOne performed on a bone marrow aspirate fully characterized the presence of a t(4:10) abnormality, identified as a novel fusion of *TNKS2* and *PDGFRA* (Figure 1). Further, a *KRAS* G12D point mutation was detected. These findings resulted in reclassification of this disease from aSM to a myeloid neoplasm with eosinophilia harboring a *PDGFRA* rearrangement.

On the basis of this diagnosis, the patient was started on a course of imatinib (400 mg, daily) and the clinical benefit was dramatic with immediate onset of diuresis, resolution of the anasarca, reduction in splenomegaly and steady improvement in the platelet count.

At the time of publication, the patient remains on a maintenance dose of imatinib (100 mg, daily) and a subsequent bone marrow biopsy 4 months after initiation of therapy revealed a dramatic normalization in marrow cellularity without eosinophilia or mast cell proliferation. Follow-up multiprobe FISH for *PDGFRA* abnormality at this time was negative.

A bone marrow aspirate was collected via needle biopsy and comprehensive genomic profiling (FoundationOne) was performed in a Clinical Laboratory Improvement Amendments-certified laboratory, College of American Pathologists and New York State accredited (Foundation Medicine, Cambridge, MA, USA). Methods of the clinical cancer gene assay used to analyze this patient have been previously published and the assay performance has been validated rigorously.⁹ Here we provide a brief summary. DNA is extracted from formalin-fixed, paraffin-embedded tissue ($\geq 1 \text{ mm}^3$) containing no less than 20% tumor nuclei by enzymatic digestion and subsequent purification. DNA is fragmented by sonication to 200 bp segments. Indexed sequencing adapters are ligated to the DNA fragments and PCR amplified to yield 500 ng of sequencing library. Hybridization selection is performed using individually synthesized baits targeting 3679 exons of 236 cancer-related genes and 47 introns of 19 genes frequently rearranged in cancer. The Illumina HiSeq 2500 (Illumina Inc, San Diego, CA, USA) platform is used in 49×49 paired-end sequencing. Sequence data are mapped to the human genome (hg19) using BWA aligner v0.5.9.¹⁰ Sequence data are analyzed through a computational analysis pipeline to call variants present in the sample, including substitutions, short insertions and deletions, rearrangements and copy-number variants.

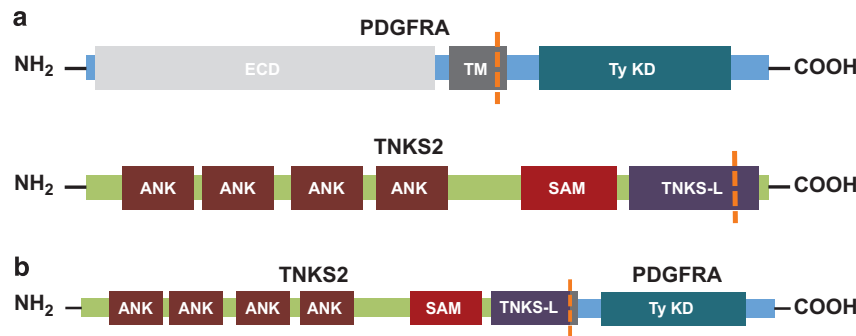


Figure 1. Intrachromosomal rearrangement of *TNKS2* and *PDGFRA* results in a likely fusion gene. ANK, ankyrin domains; ECD, extracellular domain; SAM, sterile alpha motif; TM, transmembrane; TNKS-L, tankyrase-like; TyKD, kinase domain.

FISH studies were performed with a standard commercially available multiprobe assay. Red and green probes hybridize to the 3' and 5' end of *FIP1L1*, respectively. An aqua probe hybridizes to *PDGFRA*. Loss of the 3' end of *FIP1L1*, as evidenced by an absent red signal, is considered a positive result for *PDGFRA-FIP1L1* fusion.

We present a patient initially diagnosed as harboring aSM and ultimately reclassified most appropriately as having a myeloid neoplasm with rearrangement of *PDGFRA* with a novel fusion partner, *TNKS2*, identified by comprehensive genomic profiling. Since the first detection of *FIP1L1-PDGFRA* fusions was in 2003,¹ many other fusion partners of *PDGFRA* have been discovered. These include translocations with chromosomes 4, 12 and 22, and partial insertions of chromosome 9.¹¹ We have observed a translocation of *TNKS2* and *PDGFRA* with breakpoint in intron 25 and exon 12, respectively. The discovery of a novel fusion of *TNKS2* with *PDGFRA* further demonstrates the diversity of alterations possible in these myeloid neoplasms with eosinophilia.

From our clinical observations, it is unknown whether *TNKS2* has a role identical to that of *FIP1L1* in the context of a fusion with *PDGFRA*. Further *in vitro* studies are needed to elucidate whether the identity of the fusion partner in a *PDGFRA* rearrangement has functional and clinical implications.

Unlike the near pathognomonic *KIT* D816V alteration in SM, *FIP1L1-PDGFRA* fusions are associated with a favorable response to imatinib therapy^{2,3} and mandate the diagnosis of a myeloid neoplasm with *PDGFRA* rearrangement. Other known fusion partners of *PDGFRA* described by Gotlib and Cools¹¹ have not yet been known to respond to imatinib.¹² The *TNKS2-PDGFRA* fusion described further extends the set of known targets for imatinib therapy. Interestingly, a concurrent *KRAS* G12D mutation was also detected in this patient. In some settings, this mutation has been associated with a poor response to tyrosine-kinase inhibition;⁸ yet the patient's response to imatinib was remarkable and has been durable to date. Others have described similar findings with crizotinib, demonstrating clinical efficacy despite the presence of concurrent *KRAS* and *MET* alterations in the same tumor.¹³

Unlike the well-characterized intrachromosomal *FIP1L1-PDGFRA* fusion of 4q12, the *TNKS2-PDGFRA* fusion observed in this patient is an interchromosomal translocation. In performing diagnostic assays, direct interrogation for the presence of *FIP1L1-PDGFRA* may not always be performed and patients are, instead, tested for deletion of *CHIC2*, a gene that lies in the region between *FIP1L1* and *PDGFRA*. This surrogate assay may be most effective for detecting recurrence of a typical *FIP1L1-PDGFRA*,⁵ but would not have resolved an interchromosomal event such as this one.

In summary, this report describes a novel *TNKS2-PDGFRA* fusion that diagnostically defines a distinct category of myeloid neoplasms with eosinophilia as defined within the WHO 2008 classification schema. Using FISH analyses, these neoplasms may be incorrectly interpreted as harboring a *FIP1L1-PDGFRA* rearrangement or may even be interpreted as fusion negative if the fusion is complex and FISH probes do not fully border the regions

of interest. Identification of a *PDGFRA* rearrangement is important for both diagnosis and therapy as these neoplasms are responsive to tyrosine kinase inhibitors. However, the implications for durability of therapeutic response and prognosis, particularly with respect to the specific *PDGFRA* fusion partner, are not yet known. Comprehensive genomic profiling offers an accurate and detailed analysis platform that reliably detects the complex interchromosomal events at gene-level resolution.

CONFLICT OF INTEREST

ZRC, SMA, GMF, JSR, RY, NAP, JAE, JC, PJS, VAM, RE and PVC are employed by and have equity interest in Foundation Medicine, Inc. The remaining authors declared no conflict of interest.

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