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Myeloid neoplasm with histiocytosis and spleen tyrosine kinase fusion responds to fostamatinib

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Short Running Title: Fostamatinib in myeloid neoplasm with spleen tyrosine kinase fusion

Data Sharing Statement: For de-identified patient data and sequencing data please contact the corresponding author Sameek Roychowdhury (sameek.roychowdhury@osumc.edu). Academic researchers can gain access to Foundation Medicine data in this study by contacting the corresponding author and filling out a study review committee form. You and your institution will be required to execute a data transfer agreement. For further questions please reach out to Foundation Medicine, Cambridge, MA's compliance department (compliance@foundationmedicine.com).

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Conflict of Interest

Lee Albacker and Julia Hopkins were employees of Foundation Medicine, Inc. and shareholders of Roche Holdings AG during the execution of this study. Sameek Roychowdhury is a collaborator and consultant with Incyte pharmaceuticals, Merck and Co., and QED therapeutics. Zachary Risch is a shareholder of <5% in the following companies: Alnylam Pharmaceuticals, Geron Corporation, Lineage Cell Therapeutic, Oncolytics Biotech, Pfizer Inc., Repligen Corporation, Sangamo Therapeutics, and Viatris Inc. Sarah Wall has served on the educational bureau of CTI BioPharma and served as a one-time advisory board for Bristol Myers Squibb in calendar year 2022 and a one-time consultation for AbbVie Inc. Ben Kaffenberger is a collaborator with the following companies: Biogen, Merck, BMS, InflaRx, Onquality, and NIAMS and consultant for the following companies: ADC therapeutics, Novartis, Biogen, and OnQuality. Otherwise, to the best of our knowledge, the named authors have no major conflict of interest, financial, or otherwise.

Author Contribution Statement

ZR, BK, JB, Lynee A., SR, KW - Writing manuscript, Revisions/editing manuscript, Data collection, generation, or analysis

CC, ES, EH, TD, AS, SW, JR, MW, JH, Lee A, CE, AF - Revisions/editing manuscript, Data collection, generation, or analysis

Spleen tyrosine kinase (SYK) is an intracellular kinase involved in immune cell signaling that has emerged as a therapeutic target for autoimmune diseases and hematologic cancers. SYK inhibitors are currently FDA-approved for immune thrombocytopenia purpura and have been evaluated in clinical trials for autoimmune diseases and hematologic cancers^{1,2}.

Genomic alterations have been reported in SYK including point mutations and gene rearrangements. SYK fusions were first described in 2000 in a patient with myelodysplastic syndrome harboring a translocation between chromosomes 9 and 12 resulting in an *ETV6-SYK* fusion protein with constitutive kinase activation driving disease pathogenesis³. Additional cases of myeloid and lymphoid neoplasms harboring SYK fusions have been reported⁴⁻⁸. *ITK-SYK* fusions are known oncogenic fusions in T-cell lymphomas⁴. Additionally, a case series described 3 young children, between 1 to 15 months of age, with histiocytic neoplasms harboring *CLTC-SYK* fusions. These children presented with diverse clinical manifestations including a large chest wall mass, a soft tissue mass of the right arm resulting in fracture, and multiple café au lait without fulfilling clinical criteria for neurofibromatosis 1 syndrome⁹. *In vitro* experiments have shown that SYK inhibition results in decreased SYK phosphorylation and decreased tumor growth⁸. Additionally, several germline point mutations, described in autoimmune and inflammatory disorders, result in constitutive SYK kinase activation and a gain-of-function phenotype¹⁰⁻¹².

Here we present a patient with a myeloproliferative neoplasm with non-Langerhans cell histiocytosis and an *ETV6-SYK* rearrangement that responded to treatment with a SYK kinase inhibitor. This is the first patient with cancer harboring an activating genomic alteration in SYK treated with a SYK kinase inhibitor. Further, we describe the genomic landscape of SYK alterations across over 300,000 hematologic and solid malignancies.

Case: A 51-year-old woman presented with painful skin plaques involving 60% of her surface area, requiring opioid therapy, and over 40 pounds of weight loss (**Fig. 1A-B**). Laboratory

evaluation identified a leukocytosis (20.4 K/ μ l) with 79% neutrophils, mild absolute eosinophilia (range from 0.2 to 1.4 K/ μ l), and monocytosis (range from 0.4 to 1.2 K/ μ l). The remaining cells on the differential were within normal range. A skin biopsy from the right leg showed diffuse histiocytic proliferation (immunohistochemistry strongly positive for CD68 and CD163, weakly and focally positive for factor XIIIa, and negative for CD1a and S100) consistent with non-Langerhans cell histiocytosis (**Fig. 1C-D**). A bone marrow aspirate and biopsy demonstrated myeloid predominate hypercellular bone marrow (80%) with moderate eosinophilia (15%) without dysplasia. There was no evidence of dysgranulopoiesis, monocytosis, or basophilia. Flow cytometry on the bone marrow aspirate showed no increased blasts or abnormal lymphoid population. Brain MRI, transthoracic echocardiogram, nuclear whole-body bone scan, and computed tomography scan of the chest, abdomen, and pelvis were negative for additional extramedullary disease other than the described cutaneous lesions. After informed consent, the patient underwent genomic testing via OSU-13053, Precision Cancer Medicine for Advanced Cancer Through High-throughput Sequencing (NCT02090530). This IRB-approved study supports the collection of tumor specimens for CLIA-certified genomic testing, the return of clinically significant sequence results to patients, and routine clinical follow-up. Targeted RNA sequencing was performed on the previously collected right leg skin shave biopsy as previously described (OSU-SpARKFuse)¹³. RNA sequencing analysis identified a rearrangement involving *ETV6* (exons 1-5) and *SYK* that preserved the tyrosine kinase domain of *SYK* (exons 6-14) with 80 normalized fusion spanning reads (NFSR) (**Fig. 1E**)¹³. Targeted DNA sequencing using a panel of 279 genes from the skin biopsy did not identify any alterations associated with histiocytic disorders including in *BRAF*, *KRAS*, *JAK2*, *MAP2K1*, and *CSF1R*. Conventional cytogenetic analysis performed on bone marrow demonstrated aberrations involving chromosomes 9, 12, and 16. Next-generation sequencing using a 50 gene panel from the bone marrow aspirate did not identify any pathogenic genomic alterations including in *BCR*, *FLT3*, *JAK2*, *CEBPA*, *KRAS*, *MAP2K1*, and *BRAF*. Fluorescence *in situ* hybridization (FISH) analysis

of the bone marrow showed co-localization of *ETV6* and *SYK* on chromosome 9 in 78.9% of the cells analyzed (**Fig. 1F-G**) (locus-specific probes to *SYK* (9q22.2) and *ETV6* (12p13.2)). The presence of the same *ETV6-SYK* rearrangement in the histiocytic skin infiltrate and myeloproliferative neoplasm along with no other identifiable driver mutations in the bone marrow or skin led to the hypothesis that this rearrangement was driving both disease processes and could potentially respond to treatment with a SYK inhibitor.

The patient was started on the SYK inhibitor, fostamatinib, at 100 mg twice daily based on the dosing recommendations for treatment of immune thrombocytopenia purpura¹. Fostamatinib was obtained through an emergency IND and a single patient IRB protocol developed with Rigel. There was immediate improvement in the skin lesions, markedly reduced pain with discontinuation of opioid therapy, and weight gain of over 20 pounds (**Fig. 1H-I**). The patient experienced side effects of diarrhea and hypertension, which were managed with supportive care, anti-hypertensive medications, and a brief one-day dose interruption. Approximately six months after starting fostamatinib, the skin lesions worsened. RNA sequencing from a new skin biopsy showed the *ETV6-SYK* fusion with 78 NFSR. The fostamatinib dose was then increased to 150 mg twice daily. Three months later, repeat skin and bone marrow biopsies showed a reduction in histiocytes in the skin (**Fig. 1J-K**), and near normal bone marrow cellularity. There was also a reduction in the percentage of nuclei examined positive for the *ETV6* rearrangement by FISH from 78% at diagnosis to 58% after seven total months of fostamatinib (**Fig. 1L**). Additionally, the *ETV6-SYK* fusion was undetectable from a repeat skin biopsy using RNA sequencing. After 18 months of therapy, the patient experienced clinical progression and fostamatinib was discontinued. Repeat skin and bone marrow biopsies revealed an increase in histiocytic infiltrate and *ETV6* rearrangement to 80% by FISH, respectively (**Fig. 1L**). Repeat RNA sequencing from a skin biopsy obtained post-progression showed a rise in the *ETV6-SYK* fusion NFSR to 60 and did not identify any new mutations in the *SYK* gene. The patient then

underwent a peripheral blood allogeneic stem cell transplant with remission. Approximately 5 months post-transplant, the patient died from complications of graft-versus-host disease and infection.

To identify additional cases of *SYK* rearrangements or activating mutations in cancer, we evaluated sequencing data from over 320,000 tumors in the Foundation Medicine, Inc. database (FoundationCORE®), 10,337 tumors from The Cancer Genome Atlas (TCGA), 24,289 tumors from The International Cancer Genome Consortium (ICGC), and 11,242 tumors from the Oncology Research Information Exchange Network (ORIEN) database. We identified 2 cases with *SYK* rearrangements that retained the kinase domain, a T cell lymphoma, and a myeloproliferative neoplasm. Both harbored *ITK-SYK* rearrangements, that included exons 8-14 and the tyrosine kinase domain of *SYK* (**Fig. 2A-B**). In addition, we identified 4,635 cases (1.5%) with somatic point mutations in *SYK*, of which 1,772 were unique. Of the cases with point mutations, 67 (1.4%) were identical to case reports of patients with germline *SYK* variants that resulted in gain-of-function and multi-organ inflammatory disease (**Fig. 2C**)¹⁰⁻¹². The 67 cancers with gain-of-function *SYK* mutations included 15 non-small cell lung cancers, 11 colorectal cancers, 4 breast cancers, 8 cancers of unknown primary, and 4 melanomas. (**Fig. 2D**).

Here we report the clinical, pathologic, and molecular features of a patient with a myeloproliferative neoplasm presenting with non-Langerhans cell histiocytosis and a *SYK* rearrangement. This patient experienced an initial response to treatment with the *SYK* inhibitor, fostamatinib, with clinical improvement in skin lesions and bone marrow involvement and improved quality of life, before succumbing to disease. The detection of identical *SYK* rearrangements along with clinical, pathologic, and molecular response to targeted kinase inhibition in the skin and bone marrow supports our initial hypothesis that these two disease processes are biologically associated and driven by the same genomic alteration. This phenomenon has been reported in other cases of concurrent non-Langerhans cell histiocytosis

and myeloid neoplasms, but this is the first case of a patient with a *SYK* rearrangement being treated with targeted therapy in cancer.

Patients with non-Langerhans cell histiocytosis have a high prevalence of concurrent myeloid neoplasms. Non-Langerhans cell histiocytosis are clonal disorders with a high prevalence of activating mutations in known oncogenic drivers including *BRAF*, *PIK3CA*, and *N/KRAS*. Approximately 10.1% of these cases have a concurrent myeloid neoplasm¹⁴. Several case reports have detected identical driver mutations in both disease processes and treatment with targeted kinase inhibition has resulted in clinical responses^{14 15}. We propose that patients presenting with myeloproliferative neoplasms or histiocytic disorders should undergo comprehensive genomic testing for potentially actionable alterations.

In this paper, we also describe the landscape of gain-of-function mutations in *SYK*. Additional characterization of *SYK* rearrangements, mutations, and signaling may help guide therapy opportunities for these patients. Currently, *SYK* inhibitors are FDA-approved for the treatment of immune-related thrombocytopenia (fostamatinib) and have been studied in various other autoimmune diseases and malignancies. There are no studies investigating gain-of-function genomic alterations in *SYK* despite the interest as a target in cancer. This paper illustrates how genomic alterations in *SYK* could guide therapy selection for some patients.

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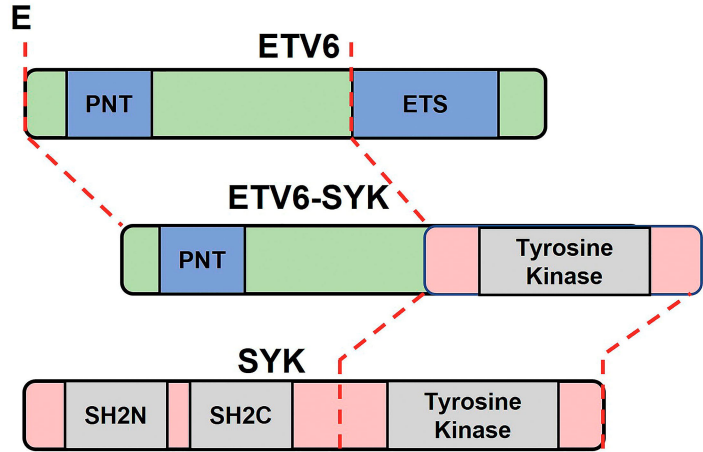
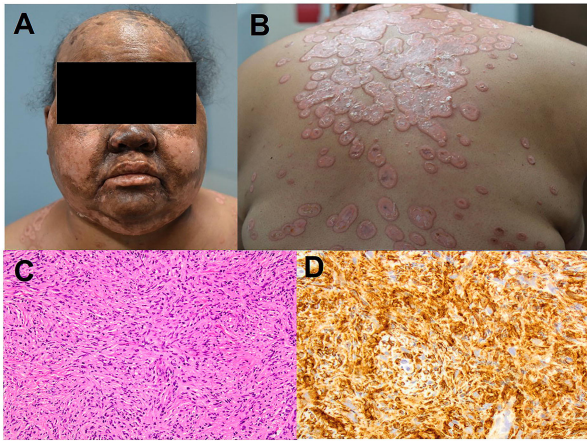
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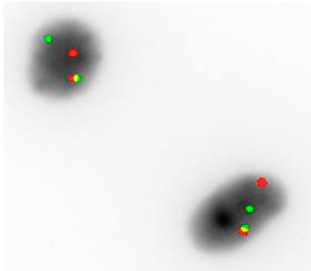
Figure 1. Myeloproliferative neoplasm with non-Langerhans Cell Histiocytosis and *ETV6-SYK* fusion responds to fostamatinib. (A-B) Clinical photographs taken approximately 6 months before treatment with fostamatinib. (C-D) Pre-treatment skin biopsy (H&E, 200X) (C) and CD163 IHC (200X) (D) showing histiocytic infiltrate. (E) Schematic of an *ETV6-SYK* rearrangement identified from skin biopsy preserving exons 6-14 and the tyrosine kinase domain of *SYK*. (F) Interphase FISH analysis from bone marrow aspirate showing *ETV6* rearrangement using an *ETV6* break-apart probe. When *ETV6* is intact, the red and green signals are adjacent and appear yellow; rearrangement splits the yellow signal into its red and green components. (G) Metaphase FISH analysis from bone marrow aspirate shows co-localization of the *ETV6* (green) and *SYK* (red) locus-specific probes on the abnormal der(9) chromosome. (H-I) Clinical photographs taken after 5 months of fostamatinib therapy show reduction in skin lesions. (J-K) Skin biopsy (H&E, 200X) (J) and CD163 IHC (200X) (K) taken after 9 months of fostamatinib treatment showing reduction in histiocytic infiltrate. (L) Pre-treatment interphase FISH analysis from bone marrow aspirate using *ETV6* break-apart probe showed 78.9% of the nuclei examined were positive for *ETV6* rearrangement. This improved to 58% after 7 months of therapy. At time of progression this increased to 80%.

Figure 2. Landscape of *SYK* gain of function genomic alterations in cancers. (A, B) Schematic of two cases of *ITK-SYK* rearrangements identified via review of FoundationCORE®, The Cancer Genome Atlas (TCGA), International Cancer Genome Consortium (ICGC), and Oncology Research Information Exchange Network (ORIEN). (A) A T cell neoplasm involving exons 1-13 of *ITK* and (B) a myeloproliferative neoplasm involving exons 1-5 of *ITK*. Both rearrangements retain the tyrosine kinase domain of *SYK*. (C) Lollipop diagram demonstrating unique *SYK* gain of function single nucleotide variants (SNV) in exons 8-14 identified through a review of clinical-genomic database from FoundationCORE®, ICGC, TCGA and ORIEN. (D) Frequency of gain-of-function SNVs (illustrated in Fig. 2C) by cancer type.

Pre-Treatment

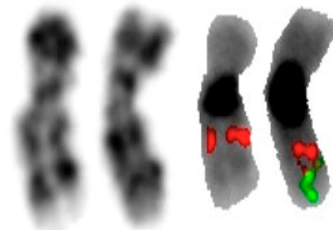


F Interphase FISH



ETV6 break apart probes
 Yellow - *ETV6* intact
 Red/Green - *ETV6* rearrangement

G Metaphase FISH



Locus specific probes
ETV6 - green
SYK - red

Post-Treatment

