Inflammatory Myofibroblastic Tumors harbor multiple potentially actionable kinase fusions

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Running Title: Multiple actionable kinase fusions in IMT

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Abbreviations list: Inflammatory Myofibroblastic Tumor (IMT), Anaplastic Lymphoma Kinase (ALK), immunohistochemistry (IHC), Next-Generation Sequencing (NGS), Food and Drug Administration (FDA), fluorescence in situ hybridization (FISH).

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ABSTRACT
Inflammatory myofibroblastic tumor (IMT) is a neoplasm which typically occurs in children. The genetic landscape of this tumor is incompletely understood and therapeutic options are limited. While 50% of IMTs harbor ALK rearrangements, no therapeutic targets have been identified in ALK negative tumors. We report for the first time that IMTs harbor other actionable targets, including ROS1 and PDGFRβ fusions. We detail the case of an 8 year old boy with treatment-refractory ALK negative IMT. Molecular tumor profiling revealed a ROS1 fusion, and he had a dramatic response to the ROS1 inhibitor, crizotinib. This case prompted assessment of a larger series of IMTs. Next generation sequencing revealed that 85% of cases evaluated harbored kinase fusions, involving ALK, ROS1, or PDGFRβ. Our study represents the most comprehensive genetic analysis of IMTs to date and also provides rationale for routine molecular profiling of these tumors to detect therapeutically actionable kinase fusions.

STATEMENT OF SIGNIFICANCE
Our study describes the most comprehensive genomics based evaluation of inflammatory myofibroblastic tumor (IMT) to date. Since there is no ‘standard of care’ therapy for IMT, the identification of actionable genomic alterations, in addition to ALK, is expected to redefine management strategies for patients with this disease.
INTRODUCTION

Inflammatory Myofibroblastic Tumor (IMT) is a rare mesenchymal tumor which can occur at any age, but has a predilection for children, adolescents, and young adults (1). An estimated 150-200 new cases are diagnosed annually in the United States (2). These soft tissue tumors can occur at multiple anatomic sites, but most commonly involve lung, abdomen/pelvis, and retroperitoneum. The mainstay of treatment for IMT is surgical resection; however, treatment options are limited for patients with unresectable and/or advanced disease.

IMTs are diagnosed pathologically using criteria established by the World Health Organization (WHO) (3). These tumors are characterized histologically by a spindle myoepithelial cell proliferation with a lymphoplasmacytic inflammatory infiltrate (4). Approximately 50% of IMTs are positive for anaplastic lymphoma kinase (ALK) expression by immunohistochemistry (IHC). The most common mechanism of ALK expression and activation involves structural rearrangements in the \textit{ALK} gene, leading to the formation of a chimeric fusion protein. Several \textit{ALK} fusion partners have been identified retrospectively (5), as tumor sequencing is not yet the standard of care for IMTs. ALK fusions have been validated as a therapeutic target. A patient with a \textit{RANBP2-ALK} positive IMT had a partial response to the ALK tyrosine kinase inhibitor (TKI), crizotinib, while a patient whose IMT lacked an ALK fusion did not respond to this agent (6).

In contrast, actionable genomic alterations have not yet been described in the 50% of IMT samples which are negative for ALK by IHC. ALK negative IMTs may be more aggressive with a higher frequency of metastasis compared to ALK positive IMT (7). Little is known on the genomic level regarding potential oncogenic drivers in this subset of IMTs and, as such, there are no targeted therapies available for these patients.

Here we describe the case of an 8 year old boy with treatment-refractory ALK negative IMT. Targeted next generation sequencing (NGS) based genomic profiling identified the presence of a ROS1 kinase fusion within his tumor. Based on this finding, he was treated with...
the ROS1/ALK/MET TKI, crizotinib, with rapid symptomatic improvement and significant
decrease in his tumor burden. This case prompted us to perform genomic analysis on a larger
series of this rare tumor. Our data show for the first time that kinase fusions are found in the
majority of IMTs. These data not only offer insight into this disease but also provide rationale for
routine molecular profiling to detect therapeutically actionable kinase fusions and thereby offer
patients rational therapeutic strategies with existing TKIs based on the genomic profile of the
tumor.

RESULTS

Case Report

A 6 year old boy presented with a 1 year history of cough and fatigue. Imaging
demonstrated the presence of a large left sided chest mass. Biopsy of the mass revealed IMT,
negative for ALK expression by standard clinical immunohistochemistry and for ALK
rearrangement by break-apart fluorescence in situ hybridization (FISH). The tumor was deemed
unresectable due to its intimate association with the pulmonary vein, aorta, and esophagus. At
the time of diagnosis, his laboratory parameters were indicative of a microcytic anemia and an
inflammatory state. Several treatment regimens were administered including anti-inflammatory
agents (naproxen, corticosteroids, and indomethacin) as well as cytotoxic chemotherapy
(methotrexate/vinorelbine) over the course of 24 months (Figure S1), with no anti-tumor
response and minimal improvement of his anemia. While he was receiving methotrexate/
vinorelbine, we performed targeted NGS-based genomic profiling of his tumor using formalin-
fixed and paraffin embedded (FFPE) tissue and surprisingly detected a TFG-ROS1 fusion
(Figure 1A). ROS1 tyrosine kinase inhibitors, such as crizotinib, have proven to be an effective
therapeutic strategy in lung cancers harboring ROS1 kinase fusions (8, 9). Therefore, he was
treated with crizotinib 250 mg twice daily orally, obtained through a compassionate access
program. He experienced grade 1 diarrhea and visual disturbance, both of which resolved with no dose reduction. Within 3 cycles of crizotinib therapy, he symptomatically felt better with decreased cough and significantly increased energy. Imaging studies revealed, for the first time since diagnosis, a decrease in the size of his tumor mass (Figure 1B). Notably, his hemoglobin (HgB) and mean corpuscular volume (MCV) rapidly increased and his erythrocyte sedimentation rate (ESR) decreased (Figure 1C, Table S1). He has now been on crizotinib for 4 months with excellent tolerance, improved quality of life, and continued decrease in his tumor burden.

**Patient and tumor characteristics**

In an effort to further characterize cases of both ALK positive and ALK negative IMT, we obtained 37 samples from 33 patients with this rare disease (Table 1). Patients ranged in age from infancy (less than 1 year old) to age 41. As is typical for IMT, the tumors arose at multiple anatomic locations, including thorax, mesentery, peritoneum, and bladder. The pathological diagnosis was established based on criteria according to the WHO classification (3). ALK IHC was completed on each sample as part of the standard pathologic evaluation (Supplementary Methods). 11/37 (30%) of the cases were ALK IHC negative and 26/37 (70%) of the cases were ALK IHC positive.

**Targeted next-generation sequencing identified ALK, ROS1, and PDGFRβ tyrosine kinase fusions in a collection of IMT samples.**

We hypothesized that further insight into the biology of known fusions as well as discovery of novel kinase fusions would provide new therapeutic targets to treat patients with IMT. To address this hypothesis, we analyzed genomic DNA from all 37 IMT samples using a targeted NGS based assay (FoundationOne™) which assesses 3769 exons of 287 cancer genes and 47 introns of 19 commonly rearranged genes, including 8 tyrosine kinases (Table
This platform has been previously described and successfully employed in several large genomic studies of various tumor types (10-12). In each case, tumor DNA was isolated from FFPE tissue. Average coverage was 543x. Targeted NGS was successfully performed in 22/26 ALK positive and 11/11 ALK negative specimens (Table 1, Figure S2). In cases in which there was sufficient tumor material available, the kinase fusions were verified with RNA sequencing.

Among the 11 ALK IHC negative cases, kinase fusions were identified in 8/11 (73%) of the cases (Table 1, Figure 2A,B). 2 cases harbored ALK fusions (sample L5: EML4-ALK, sample L9: TPM3-ALK) which were missed by ALK IHC testing alone. Amongst the other 9 ALK negative samples, 4 contained distinct ROS1 fusions (sample L3/L4: YWHAE-ROS1, sample L6: TFG-ROS1), including the index patient (sample L37), and 2 contained a PDGFRβ fusion (samples L7/L10: NAB2-PDGFRβ). Notably, neither ROS1 nor PDGFRβ fusions have been described in IMT to date. The genomic coordinates for each fusion identified are summarized in Table S3. Importantly, all kinase fusions identified in this study (ALK/ROS1/PDGFRβ) are therapeutically targetable with existing FDA approved TKIs (8, 13-15). No other recurrent alterations were identified (Table S3). Further analysis of the 3/11 samples for which a kinase fusion was not detected in this targeted NGS assay is ongoing.

Among the 22 ALK positive cases analyzed, 20 harbored ALK fusions with various previously described 5’ gene fusion partners, including TPM3, TPM4, SEC31A, TFG, RANBP2, CLTC, and FN1 (Table 1, Figure 2A,B). Of note, the FN1-ALK fusion detected in samples L26 and L29 harbors an atypical breakpoint within intron 18 of ALK. This fusion was initially missed by genomic DNA analysis (which targeted only intron 19 of ALK), but later identified with RNA sequencing. Novel ALK fusions were also detected, including LMNA-ALK (sample L12) and PRKAR1A-ALK (sample L35). The remaining 2 ALK IHC positive cases were also negative for ALK kinase domain mutations and ALK amplification, suggesting a different mechanism of ALK expression in these tumor samples.
DISCUSSION

IMT is a rare tumor that can arise at multiple anatomic locations. There are limited systemic therapeutic options available for patients with surgically unresectable and/or metastatic disease. Previous data has demonstrated that approximately 50% of IMTs are positive for ALK expression based on results from immunohistochemistry (IHC). Responses to the tyrosine kinase inhibitor, crizotinib, have been documented in patients with ALK+ IMT, demonstrating the importance of identifying this target (6, 14).

In our study, we successfully performed targeted NGS in 20/22 ALK IHC positive IMT samples and identified several different ALK fusions, with various 5’ gene fusions partners. Several of these fusions partners have been previously described, including TPM-3/-4, ATIC, CLTC, CARS, and RANBP2 (5). However, we also identified novel ALK fusions, such as LMNA-ALK and PRKAR1A-ALK, the latter of which was detected in a congenital IMT. In addition, we identified ALK fusions with non-canonical fusion breakpoints. FN1-ALK, which has previously been described in ovarian cancer, has a breakpoint in intron 18 of the ALK gene, whereas most fusions have a breakpoint ALK intron 19 (16). Since patients with tumors harboring intron 1(exon 19) ALK fusions can derive clinical benefit from ALK inhibitor therapy (17), there is a need to incorporate these atypical but recurrent fusions into NGS based diagnostic platforms. Notably, we also detected ALK fusions in 2/11 IMT samples which tested negative for ALK expression by IHC. Therefore, the possibility of targeted therapy with an ALK inhibitor would have been missed for these patients with ALK testing by IHC alone.

In contrast, there are currently no data regarding potential oncogenic ‘drivers’ in the ALK negative subset of IMTs. We identified actionable kinase fusions in 8/11 ALK negative IMT tumors analyzed by targeted NGS, including ROS1 and PDGFRβ kinase fusions which have not yet been described in this disease. PDGFRβ fusions have been described in myeloproliferative disorders (18). ROS1 kinase fusions have been detected in a variety of malignancies, including lung cancer, glioblastoma, cholangiocarcinoma, and Spitz tumors [reviewed in reference (19)].
Crizotinib, which is FDA approved for the treatment of ALK fusion positive lung cancer, is also a potent ROS1 inhibitor. Preliminary results from the phase I clinical trial of crizotinib in ROS1 fusion positive lung cancer demonstrated an objective response rate of 56% (9). However, responses in other ROS1 fusion positive cancers have not yet been documented. Here, we report that a young boy with ROS1 fusion positive IMT responded to crizotinib. This was the first anti-tumor response this patient has experienced since his initial diagnosis more than 2 years prior to starting crizotinib; his tumor previously did not respond to four different lines of therapy including cytotoxic chemotherapy or anti-inflammatory agents. His tumor mass decreased in size, his paraneoplastic anemia improved, and symptomatically he felt better. This case clearly illustrates the need for improved diagnostic and therapeutic paradigms in this disease.

Overall, our data show for the first time that kinase fusions are found in the majority of IMTs (85% in our series). To our knowledge, this study represents the largest genomic analysis of this tumor to date, and our results redefine this heterogeneous disease as being largely a kinase fusion driven neoplasm. These data not only provide insight into this rare disease but also offer rational targeted therapeutic strategies with existing FDA approved TKIs based on the genomic profile of the tumor. Critical to successful deployment of this evolving therapeutic paradigm is incorporation of testing with highly sensitive NGS platforms capable of detecting both known and novel fusions in multiple oncogenes from a single tumor specimen.
METHODS

**Patients and Tumor Samples.** IMT samples and associated patient characteristics were analyzed with an Institutional Review Board (IRB) - approved protocol (#090572). All clinical data was obtained and maintained according to HIPAA standards. All unique identifiers have been removed prior to publication.

**Genomic DNA sequencing and analysis.** DNA was extracted from FFPE samples. Sequencing was performed for 3769 exons of 287 cancer genes and 47 introns of 19 commonly rearranged genes, including 8 tyrosine kinases (FoundationOne™ Panel, Table S2) as previously described(10). Tumor content was assessed by hematoxylin and eosin staining prior to analysis; no micro/macro dissection tissue enrichment was performed. Sequencing was performed on the HiSeq2000 instrument (Illumina) with 40 bp paired reads to an average depth of 543X. Resultant sequences were analyzed for base substitutions, insertions, deletions, copy number alterations, and select gene fusions (10). Additional information regarding the analytic validation of this assay as well as the sequencing of RNA is provided in the Supplementary Appendix.
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REFERENCES


Table 1: Summary of clinical characteristics and targeted next generation sequencing results for the study cohort. 37 formalin fixed paraffin embedded (FFPE) tumor samples from 33 different patients with IMT were included in the analysis. The following samples were obtained from the same patient at different times in his/her disease course: L3/L4, L7/L10, L31/L36, L32/L33/L34. There was 100% concordance in the kinase fusions detected across multiple samples from the same patient. *Sufficient material was available to verify these kinase fusions with RNA sequencing. #Initial results from the FoundationOne™ genomic DNA analysis were negative. The FN1-ALK fusion, which harbors an atypical breakpoint within intron 18 of ALK, was detected by RNAseq.
FIGURE LEGENDS

Figure 1: Response to crizotinib in an 8 year old boy with refractory IMT harboring a TFG-ROS1 fusion. (A) Schematic representation of the TFG-ROS1 fusion. ROS1 is located on chromosome 6q22 and TFG is located on 3q12. The break point occurs in-frame between exon 4 of TFG and exon 36 of ROS1. (B) CT scans prior to the initiation of crizotinib (left panel) and after 3 cycles of crizotinib (right panel) showing dramatic reduction in the tumor mass within the left lung. (C) Changes in hemoglobin (HgB), mean corpuscular volume (MCV), and erythrocyte sedimentation rate (ESR) over the course of the patient’s treatments. Arrows below the graphs indicate the initiation of the indicated therapies. The high (H) and low (L) limits of normal for each measured parameter are indicated on the blue graphs.

Figure 2: Kinase fusions identified in IMT by targeted sequencing. Starting with 37 formalin-fixed, paraffin-embedded (FFPE) IMT samples (26 ALK IHC positive and 11 ALK IHC negative), 33 tumors were evaluable with targeted next generation sequencing. (A) Genomic alterations identified in the 37 IMT tumor samples. The columns denote the samples, the rows denote the genes. Red bars represent ALK fusions, green bars represent PDGFRβ fusions, and blue bars represent ROS1 fusions. The identified gene fusions were mutually exclusive. No other recurrent genomic alterations were identified by targeted next generation sequencing in these samples. (B) Schematic representation of the distinct ALK, PDGFRβ, and ROS1 fusions identified. In each case, the exons encompassed within each gene fusion partner are indicated.
Pre-crizotinib
11/20/2013

Post 3 cycles of crizotinib
02/12/2014
(A) 

![Gene Expression Chart]

(B) 

**Targeted Therapy**

- **Crizotinib**
  - TFG
  - YWHAE
  - NAB2
  - EML4
  - TPM4
  - PRKAR1A
  - LMNA
  - TPM3
  - TFG
  - RANBP2
  - SEC31A
  - FN1
  - CLTC

- **Sorafenib**
- **Sunitinib**
- **Regorafenib**
- **Axitinib**

Lovly et al Fig. 2
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