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Morphologic overlap between inflammatory myofibroblastic tumor and IgG4-related disease: lessons from next-generation sequencing

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Abstract

Introduction: Inflammatory myofibroblastic tumor (IMT), a locally aggressive neoplasm capable of metastasis, may show an IgG4-rich lymphoplasmacytic infiltrate. Prior reports suggest that storiform-fibrosis and obliterative phlebitis aid in the distinction of IMT from IgG4-related diseases. Herein, we highlight the morphologic overlap between the two diseases, and emphasize the importance of a multiplex fusion assay in the distinction of IgG4-RD from IMT.

Methods: We identified 7 IMTs with morphologic and immunohistochemical features of IgG4-RD; 3 patients were originally diagnosed with IgG4-RD. Demographic, clinical and morphologic data was recorded. We also re-evaluated 56 patients with IgG4-RD. We performed immunohistochemistry for IgG4, IgG, ALK and ROS1. In situ hybridization for IgG4 and IgG was performed in selected cases. A multiplex next-generation sequencing (NGS) based RNA assay for gene fusions was performed to detect all known IMT-related gene fusions.

Results: All 7 IMTs showed a dense lymphoplasmacytic infiltrate and storiform-type fibrosis, with obliterative phlebitis noted in 3 cases. The neoplastic stromal cells constituted <5% of overall cellularity and stromal atypia was either absent or focal and mild. Elevated numbers of IgG4 positive cells and increased IgG4 to IgG ratio was identified in all cases. Four cases showed *ALK* related abnormalities; while two patients showed *ROS1* and *NTRK3* fusions. One tumor was negative for known IMT-related gene fusions. All 56 IgG4-RD cases were negative for ALK and ROS1 on immunohistochemistry; 6 cases were negative on the fusion assay.

Conclusion: Highly-inflamed IMTs are indistinguishable from IgG4-RD both histologically and on immunohistochemistry for IgG4. We advocate scrutinizing patients with presumptive single organ IgG4-RD for IMT and the diagnostic algorithm should include ALK and ROS1 immunohistochemistry and, in selected cases, a NGS-based fusion assay that covers known IMT-associated gene fusions.

Keywords

Inflammatory myofibroblastic tumor; IgG4-related disease; ALK; ROS1

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Introduction:

Inflammatory myofibroblastic tumor (IMT), a mesenchymal neoplasm of intermediate biological potential characterized by a prominent inflammatory infiltrate, is increasingly defined by recurring genomic alterations.(1–4) Among these, fusions involving *ALK* and *ROS1* have emerged as common genetic abnormalities, although recent efforts have uncovered a range of other alterations.(1–4) The morphologic heterogeneity is widely recognized in IMTs, ranging from low-grade spindle cell neoplasms to high-grade sarcomas and tumors with an epithelioid phenotype (5–7); the wide morphologic spectrum increases reliance on immunohistochemical and/or genetic testing. The success of kinase inhibitors such as Crizotinib have greatly increased the importance of a precise diagnosis of IMT.(8)

IgG4-related disease (IgG4-RD), a multi-system IgG4-rich fibroinflammatory disease, predominantly affects elderly patients and responds, generally swiftly, to immunosuppressive therapy. IMT, a close mimic of IgG4-RD (9, 10), may also show elevated numbers of IgG4 positive cells (11–14). However, storiform-type fibrosis and obliterative phlebitis, believed to represent characteristic histologic features of IgG4-RD, are generally not observed in IMT.(11–14)

The study was prompted by the identification of IMT-associated genetic abnormalities in two patients initially diagnosed as IgG4-RD. Herein, we illustrate a series of IMTs with histologic features indistinguishable from IgG4-RD and evaluate the value of next-generation sequencing (NGS) in this scenario. The study also surveys a cohort of IgG4-RD patients evaluated at this institution to identify undiagnosed IMTs.

Materials and Methods

We identified 7 IMTs histologically and immunohistochemically resembling IgG4-RD; 3 received in consultation by one of the authors (VD) and the other 4 from our institutional files. Three cases were initially diagnosed as IgG4-RD. To uncover other IMTs that may have been misinterpreted as IgG4-RD, we assembled a cohort of 56 patients diagnosed and treated as IgG4-RD at this institution. We also evaluated 4 additional pulmonary IMTs and 5 additional patients with IgG4-related pulmonary disease. The hematoxylin and eosin stained slides were reviewed for histological features of IgG4-RD; specifically, storiform type fibrosis and obliterative phlebitis. Elastic stains were performed on selected cases.

Immunohistochemistry

Immunohistochemistry for IgG4 and IgG was performed using antibodies to IgG4 (1:200 dilution, Zymed) and IgG (1:3000 dilution, Dako).(15) Antigen retrieval was conducted after protease digestion, and antigen detection was achieved using UltraView diaminobenzidine chromogen (Ventana Medical Systems; Tucson, AZ). Three high power fields (HPF) with the highest number of IgG4-positive cells were identified and the mean counts in these fields were recorded. The number of IgG-positive plasma cells within these 3 fields was also recorded, enabling derivation of IgG4 to IgG ratio. Immunohistochemistry for ALK (clone

5A4, 1:50 dilution, Novacastra) and ROS1 (clone D4D6, 1:200, Cell Signaling Technology) was performed.

An in-situ hybridization approach was used in selected cases in which either the IgG4 or IgG preparation showed marked non-specific background reactivity. Briefly, RNA in-situ hybridization probes (Affymetrix, Santa Clara, CA) were designed against the IgG4 and IgG transcripts as identified in the NCBI nucleotide database. The IgG4 probe is isotype-specific, while the IgG probe targets RNA sequences to all subclasses of IgG. Additional details regarding this in-situ hybridization platform are available in a prior publication.(16)

Fluorescence in-situ hybridization (FISH)

ALK FISH was conducted on formalin-fixed and paraffin-embedded tissue using a dual-color break-apart probe specific to the *ALK* locus (Vysis LSI *ALK* Dual Color, Break Apart Rearrangement Probe; Abbott Molecular). To detect *ROS1* rearrangement we used a break-apart FISH approach using BAC clones corresponding to the 5' (RP11-835I21) and 3' (RP11-1036C2) sequences flanking the *ROS1* gene labeled by nick translation in green and red. Samples were considered positive if more than 15% of lesional cells showed split signals.

Fusion assay

We used a clinically validated laboratory-developed assay based on Anchored Multiplex PCR (AMP) for targeted fusion transcript detection involving 51 genes using NGS.(17) cDNA was obtained off total nucleic acid extracted from formalin fixed paraffin embedded tumor tissue. ArcherDx FusionPlex Solid Tumor Kit primers were used in 2 hemi-nested PCR reactions and the library so prepared was sequenced on an Illumina NextSeq (2 × 150 base paired-end sequencing). A laboratory-developed algorithm was used for fusion transcript detection and annotation (version 2.1.0). The assay is designed to detect fusions involving the following genes: *ALK* (19-22, intron 19), *BRAF* (7-12, 15), *EGFR* (2-7 exon skipping/vIII variant, 7-9, 16, 20, 24, 25), *EWSR1* (4-14), *FGFR2* (2, 8-10, 17), *MAML2* (2,3), *MET* (exon 14 skipping), *NRG1* (1-3, 6), *NUTM1* (3), *RET* (8-13), *ROS1* (31-37), *AKT3* (1-3), *ARHGAP26* (2, 10-12), *AXL* (19,20), *BRAF* (7-12, 15), *BRD3* (9-12), *BRD4* (10, 11), *ERG* (2-11), *ESR1* (3-6), *ETV1* (3-13), *ETV4* (2, 4-10), *ETV5* (2, 3, 7-9), *ETV6* (1-7), *FGFR1* (2, 8-10, 17), *FGFR3* (8-10, 17, intron 17), *FGR* (2), *INSR* (12-22), *JAZF1* (2-4), *MAML2* (2,3), *MAST1* (7-9, 18-21), *MAST2* (2, 3, 5, 6), *MET* (13, 15), *MSMB* (2-4), *MUSK* (7-9, 11-14), *MYB* (7-9, 11-16), *NOTCH1* (2, 4, 26-31, internal exon 3-27 deletion), *NOTCH2* (5-7, 26-28), *NRG1* (1-3, 6), *NTRK1* (8,10-13), *NTRK2* (11-17), *NTRK3* (13-16), *NUMBL* (3), *PDGFRA* (7, exon 8 deletion, 10-14), *PDGFRB* (8-14), *PIK3CA* (2), *PKN1* (10-13), *PPARG* (1-3), *PRKCA* (4-6), *PRKCB* (3), *RAFI* (4-7, 9-12), *RELA* (3, 4), *RSPO2* (1, 2), *RSPO3* (2), *TERT* (2), *TFE3* (2-8), *TFEB* (1,2), *THADA* (28), and *TMPRSS2* (1-6).

Results:

IMTs mimicking IgG4-RD (n=7)

This cohort showed a male: female ratio of 1:1.33 with a mean age of 38 years (range: 10-75 years) (table 1). There were 5 pulmonary tumors, 2 of which predominantly involved the trachea. One tumor each involved the pharynx and stomach. Three tumors were initially diagnosed as IgG4-RD (cases #1, 2 and 6). Multicentric involvement was not noted, and serum IgG4 was normal (n=3).

Morphological and immunophenotypic overlap:

All 7 tumors showed a dense lymphoplasmacytic infiltrate with inconspicuous stromal cells. All tumors showed storiform fibrosis (Figures 1C; 2A; 3B,C), while obliterative phlebitis (Figure 1E, F) was noted in 3 cases. The atypia in stromal cells varied from absent (n=2) (Figure 1B) to mild (n=5) (Figure 2B, 3C). Even with cases showing mild atypia, the overwhelming majority of stromal cells lacked atypia. The stromal cells were virtually indistinguishable from similar cells seen in IgG4-RD. Notably, lymphocytes, plasma cells and occasional eosinophils constituted the most prevalent cells, outnumbering stromal cells, often by a ratio of >20:1 (Figure 1B, 2A, 3C).

All cases showed increased numbers of IgG4 positive plasma cells as well as an IgG4 to IgG ratio that exceeded 40% (table 1) (Figure 1D; 2C, D; 3D). Four tumors (cases #4-7) were positive for ALK (Figure 2B). All tumors were negative for ROS1.

Genetic alterations:

The genetic alterations included three *ALK* related fusions, and one patient each with *ROS1* and *NTRK3* fusions (Figure 5). Although showing morphologic overlap with IgG4-RD, the stromal cells in three cases with *ALK* alterations showed strong ALK reactivity on immunohistochemistry, distinguishing this disease from IgG4-RD. NGS-based assay identified 3 unique fusion transcripts: *TFG-ALK*, *THBS1-ALK* and *DCTN1-ALK* (Figure 5B). One notable tumor (case 6), although positive for ALK immunohistochemistry, lacked genetic alteration on FISH as well as NGS, raising the possibility of an alternative mechanism of ALK activation (see discussion). Finally, case #3, classified as an IMT, lacked a detectable genetic alteration, but showed stromal atypia beyond that typically seen in IgG4-RD (Figure 4F).

Both patients with non-*ALK* rearrangements were originally diagnosed as IgG4-RD. Case 1 showed histological (dense lymphoplasmacytic infiltrate, storiform fibrosis and obliterative phlebitis) and immunohistochemical (elevated numbers of IgG4 plasma cells and elevated IgG4 to IgG ratio) features of IgG4-RD. The diagnoses was revised following the identification of *ETV6-NTRK3* and *TFG-ROS1* fusion.

Case #2, a 27-year old female, presented with difficulty in swallowing. A PET-CT showed a lobulated mass in the left lower lung measuring 9.3×5.7 cm. A **needle biopsy** revealed a dense lymphoplasmacytic infiltrate with storiform-type fibrosis and increased IgG4 positive cells, prompting a diagnosis of IgG4-RD (Figure 1A, B). She received 60 mg per day of

prednisone. The lack of radiographic response prompted weekly rituximab for 8 weeks. Post-rituximab CT showed no improvement. Her serum IgG4 was not elevated [70.6 mg/dL (normal=3.9-86.4mg/dL)]. Areas indistinguishable from IgG4-RD were identified on **thoracoscopic biopsy** (dense lymphoplasmacytic infiltrate, increased IgG4 positive plasma cells and elevated IgG4 to IgG ratio). Foci with prominent stromal cells were also detected, a minority showed mild cellular atypia (Figure 1C,D,F). Next-generation sequencing identified a *TFG-ROS1* transcript, a finding supported by FISH for *ROS1* (Figure 5B). She was treated with oral Crizotinib for 3 months with marked symptomatic improvement and underwent esophagus sparing resection. Histologically, the mass, replaced by large zones of fibrosis and calcification, lacked viable tumor cells.

IgG4-RD cohort (n=56)

We also re-evaluated surgical resections from a cohort of 56 patients with established IgG4-RD. The mean age was 56 years and the male: female ratio was 2.2:1. The organs examined included the pancreas (22), lung, airways and pleura (10), salivary gland (7), lymph node (6), gallbladder (3), liver (2), retroperitoneum (2), bile duct (1), ampulla (1), appendix (1) and breast (1). Forty-seven (84%) cases showed storiform fibrosis, while obliterative phlebitis was identified in 41 (73%) (table 2). As with IMTs, inflammatory cells dominated, outnumbering stromal cells by a ratio of >20:1. The mean IgG4 count was 143 per HPF (range 22-550); the mean IgG4 to IgG ratio was 60.7% (range 21-99%). The patients responded favorably to steroids and/or Rituximab. The cases were also critically evaluated for atypia in the stromal compartment; atypia ranged from absent to mild (Figure 6B). Immunohistochemical stains for ALK and ROS1 were negative in all cases. NGS-based RNA assay for gene fusions was performed on a subset of cases (n=6) with mild nuclear atypia, similar to that observed in the IMT cohort; the 6 cases were negative for fusions involving *ALK*, *ROS1*, *NTRK3*, *RET* and *PDGFRB*.

Comparison of Pulmonary IMT cases with Pulmonary IgG4-RD

We compared the 6 pulmonary IMTs (2 from this series and 4 non-IgG4-RD mimics) with seven patients with IgG4-related pulmonary disease (2 from this series and 5 independent cases). The most distinctive histologic difference between IgG4-related pulmonary disease and IMT lay in the characteristic pattern of involvement of the adjacent lung; patients with IgG4-RD showed extension of the cellular fibroinflammatory infiltrate (5 of 7 cases) along the airways, interlobular septa and accompanying pulmonary veins, a finding not observed in IMTs. It should be noted that perilesional acellular fibrous septa were seen in both IMTs and IgG4-RD.

Discussion

IgG4-RD and IMT can involve virtually every organ, with both diseases characterized by a rich inflammatory infiltrate. Prior studies have alluded to the immunohistochemical overlap between IgG4-RD and IMT; a subset of IMTs may show an IgG4-rich inflammatory infiltrate. (11, 12, 14) Nevertheless, there has been a tacit understanding that the two diseases could be distinguished on routine histologic evaluation: 1) IgG4-RD is characterized by storiform-type fibrosis, obliterative phlebitis and an increase in IgG4

positive cells, 2) in contrast, IMTs lack these features and instead show a prominent population of spindle/epithelioid cells with nuclear atypia.(11, 12, 13), The current study underscores the inadequacy of some of these assumptions: highly-inflamed IMTs may be morphologically indistinguishable from IgG4-RD, particularly on needle biopsies. Both diseases may show storiform-type fibrosis, obliterative phlebitis, IgG4-rich plasma cell infiltrates and mild atypia in the stromal compartment. In these circumstances, the distinction is reliant on additional ancillary testing, including immunohistochemistry and/or FISH for *ALK* and *ROS1*. Our current generation NGS-based RNA assay for gene fusions detects all known IMT-related fusions: *ALK*, *ROS1*, *NTRK3*, *RET*, and *PDGFRA*. Of note, the assay is agnostic to the fusion partner, for example, the test can identify the more than a dozen fusions involving *ALK*.

In the largest study to date, Antonescu and co-workers evaluated 62 IMTs including 25 children and 37 adults. (1) Among these, 35 patients (56%) showed *ALK* gene rearrangement, 6 (10%) showed *ROS1* gene rearrangement, with a single case of *RET* rearrangement. Other reported IMT-related kinase fusions include *NTRK3* and *PDGFRB*.(3, 4) Thirty-two percent of IMTs lacked detectable gene fusions with adults accounting for 90% of fusion-negative IMTs.(1) Notably, two cases in the current series lacked detectable genetic alterations. Patient #6, showed strong unequivocal reactivity with ALK on immunohistochemistry but lacked a genetic correlate. However, given the morphologic appearance, the specificity of the ALK antibody, and alternative means of activating ALK (including amplification, point mutation, alternate transcription initiation), all of which would be difficult to diagnose given the very low cellularity of the neoplastic cells(18), the lesion is best characterized as an IMT. Patient #3 highlights the current challenge associated with distinguishing IgG4-RD from fusion negative IMT. Although negative for known fusions, the morphologic appearance, particularly stromal atypia, and the knowledge that 49% of adults lacked known genetic alterations, would support the diagnosis of an IMT.(1)

Immunohistochemistry for ALK is a robust surrogate marker for the corresponding fusion. (2,19) The sensitivity and specificity of ROS1 immunohistochemistry is >90%, although only a limited number of IMTs have been examined.(20, 21) In one study all 3 *ROS1*-rearranged IMTs showed immunohistochemical reactivity for ROS1.(2) However, another study reported 1 ROS1 immunohistochemistry negative IMT among 4 *ROS*-rearranged IMTs while a second case showed weak reactivity.(1) Notably, case#2 in our series, a *ROS1*-rearranged IMT, was negative on immunohistochemistry and the patient was only identified after she failed to respond to treatment for IgG4-RD. The antibody clone used in this study was similar to that used in prior publications.(1, 2) Collectively, while ALK serves as a robust marker of *ALK*-rearranged IMTs, ROS1 immunohistochemistry may be a less reliable surrogate marker in this context. Pan-TRK immunohistochemistry has been reported as a sensitive method to detect *NTRK* fusions, (22) although we have not evaluated it in the context of a highly-inflamed IMT. There are no reliable immunohistochemical markers for other IMT-associated kinase fusions: *PDGFRB* and *RET*.

In addition to IMTs, *NTRK* fusions have been identified in high-grade uterine sarcomas (*NTRK1* and *NTRK3* fusion)(23), lipofibromatosis-like neural tumors (*NTRK1* fusion)(24), myopericytic neoplasms (*NTRK1* fusion)(25) and infantile fibrosarcoma (*NTRK3* fusion).

Given that recurrent *NTRK* fusions are identified in a variety of epithelial and mesenchymal neoplasms, the ultimate classification would necessarily rely on a careful morphologic evaluation. (26) Based on the diffuse lymphoplasmacytic infiltrate, the bland appearance of neoplastic cells and overlap with other highly-inflamed IMTs, case #1 is best classified as an IMT. Ultimately, given the remarkable success of tropomyosin receptor kinase (TRK) inhibitor that is agnostic to tumor type, the precise classification may prove a secondary consideration, with therapy driven by the fusion.(26)

Immunohistochemistry for ALK and ROS1 would nevertheless be the first line assay in biopsies showing a dense lymphoplasmacytic infiltrate with fibrosis/storiform fibrosis. NGS-based RNA assay for gene fusions, a powerful means of evaluating multiple genes, some with no immunohistochemical surrogates, may overcome false negative results of ROS1 immunohistochemistry. Although a second line test, NGS is nevertheless an essential component of the diagnostic algorithm. Finally, it must be emphasized that almost half of all adult IMTs currently lack a distinctive genetic signature. Morphology remains a critical element and any degree of stromal atypia should be viewed with suspicion, and a thorough work-up to exclude a diagnosis of IMT and identify other evidence of IgG4-RD would be warranted. An alternative approach would be to perform FISH for *ALK* and *ROS1*. A significant shortcoming of FISH testing is the low percentage of neoplastic cells in highly-inflamed IMTs; the neoplastic cells generally constitute <10% of total cellularity. In contrast, the sensitivity of the NGS-based RNA fusion is 5% or less. The evidence thus favors multiplex NGS-based assay over a FISH-based approach.

The prototypic patient with IgG4-RD is an elderly male with multiorgan involvement.(9, 27) However, a significant proportion of patients are younger than 50; although, pediatric disease remains uncommon. (9, 27) Thus, there is significant overlap in the demographic features of the two diseases. Additionally, one-third of patients show disease isolated to a single organ.(28) Furthermore, serum IgG4 is not elevated in about 50% of patients with IgG4-related disease; elevated serum IgG4 is more likely in patients with multi-organ disease.(28) In a study by Ebbo and co-workers, only 10% of patients with elevated serum IgG4 levels were diagnosed with IgG4-RD, highlighting the lack of specificity of this assay. (29) Given the significant clinical and histologic overlap, and the relatively large proportion of IMTs lacking detectable genetic alterations, the current study raises questions regarding our current diagnostic approach to IgG4-RD. While we did not identify additional misdiagnosed patients in our IgG4-RD cohort, it is conceivable that IMTs may have been mischaracterized as IgG4-RD. Our patients with IgG4-RD had long term follow-up and/or responded favorably to immunosuppression. We would therefore advocate that the possibility of an IMT should be strongly considered in young/middle aged patients and those with single organ disease, regardless of the histologic appearance. This distinction is important because IMT is a neoplasm with intermediate biological behavior with a potential for metastasis and surgical resection and/or targeted therapy is the mainstay of treatment. Conversely, IgG4-RD respond swiftly to steroids and surgical resection is unwarranted. Ironically, in the pre-IgG4-RD era, this disease was occasionally misdiagnosed as IMT.

The majority of our cases involved the respiratory system. The characteristic pattern of pulmonary involvement in IgG4-RD, extension of a cellular and inflamed component along

the airway and interlobular septa would support the diagnosis of this fibroinflammatory disease. (30) Conversely, IMTs do not spread along lymphatic planes. However, these features are appreciable only on a resection specimen.

In conclusion, highly-inflamed IMTs are histologically and immunohistochemically indistinguishable from IgG4-RD. In a young patient or a patient with single organ disease, the pathologic diagnostic algorithm for IgG4-RD necessitates the use of immunohistochemistry for ALK and ROS1. NGS-based RNA fusion assay, a powerful means of distinguishing IMT from its inflammatory mimics, overcomes many of the shortcomings of the immunohistochemical platform and FISH based assays, and could represent a first-line ancillary test, albeit in selected cases. These guidelines are particularly applicable to biopsies, given that the stromal atypia may not be captured on a needle biopsy. The availability of highly effective targeted therapy for actionable kinases underscores the importance of distinguishing IgG4-RD from IMT, as illustrated by the dramatic response to Crizotinib in case 2. Conversely, IgG4-RD responds swiftly to immunosuppressive agents. Patients diagnosed with IgG4-RD but failing to respond to treatment should also prompt diagnostic reconsideration.

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Figure 1:

Inflammatory myofibroblastic tumor with *TFG-ROS1* fusion (case 2). Biopsy on Panel A and B, thoracoscopic biopsy on Panel C, D, E and F. The needle biopsy (Panel A and B) showed a dense plasma cell infiltrate and elevated numbers of IgG4 positive cells (not illustrated). The thoracoscopic biopsy (panel C and inset) although less inflamed, shows prominent stromal cells with atypia. Diffuse increase in IgG4 positive plasma cells (immunohistochemical stain for IgG4) (Panel D). Panel E inset shows a focus of obliterative

phlebitis (* highlights the outlines of the obliterated vein). The obliterated vein is highlighted on an elastic stain (Panel F).

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Figure 2.

Inflammatory myofibroblastic tumor (case 5) with *THBS1-ALK* fusion. Storiform-type fibrosis (Panel A). * marks the center with fascicles of fibroinflammatory cells (arrow) emanating from this region. The neoplastic cells are diffusely positive for ALK (Panel B) Note the occasional stromal cells with vesicular nuclei (Panel B, inset). The plasma cells are diffusely positive for IgG4 in-situ hybridization (Panel C and inset) and IgG in-situ hybridization (Panel D and inset). The IgG4 to IgG ratio was > 90%.

Figure 3.

Inflammatory myofibroblastic tumor with *ETV6-NTRK3* fusion (case 1). The lung lesion is well circumscribed (Panel A). Note the storiform-type fibrosis (Panel B). On high-power the inflammatory cells dominate with only occasional stromal cells with vesicular nuclei (Panel C); storiform-type fibrosis is also seen. Immunohistochemical stain for IgG4 showing an IgG4-rich infiltrate (Panel D).

Figure 4:

Gastric inflammatory myofibroblastic tumor (case #6), Panel A-D. The tumor involved the submucosa and muscularis propria (Panel A) and showed a prominent storiform-type pattern (Panel B). Occasional larger cells with vesicular nuclei are present (Panel C). These cells are diffusely positive for ALK (Panel D, immunohistochemistry). Tracheal tumor, likely representing an inflammatory myofibroblastic tumor (case #3) (Panel E and F). The tumor shows a prominent storiform-type pattern (Panel E). Note the stromal cells, similar to those

depicted in figures 1–3 (Panel F) (arrow). However, the fusion panel was negative for IMT-related genetic alterations.

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Figure 5:

A Schematic of the human *ALK*, *ROS1*, and *NTRK3* loci. Exon numbers are shown below their respective boxes for RefSeq *ALK* transcript variant 1 (NM_004304.4), *ROS1* (NM_006180.4), and *NTRK3* transcript variant 1 (NM_001012338.2). Fusion breakpoints are shown as dotted lines for the indicated cases; all breakpoints are intronic. Note that the three schematics are not at the same scale (amino acid residue numbers are listed above first and last exons), exons are drawn at a larger scale than introns, and introns are not drawn to the same scale for each gene (*ALK* locus is ~739 kB, *ROS1* is ~137 kB, and *NTRK3* is ~384 kB). Domains LDLR: LDL Receptor (A/B); F3: Fibronectin type III; MAM: meprin, A-5, mu-receptor; TM: Transmembrane Helix). **B**: Schematic of predicted fusion protein products (see also Table 1). Triangles and Ex notation indicate the fusion breakpoints, preceding

partner gene exon, and subsequent kinase exon. Purple shaded domains are those predicted or shown to induce dimerization or trimerization in the fusion partner (Pointed: sterile alpha motif (SAM) / helix loop helix (HLH) oligomerization domain; PB1: Phox and Bem1p interaction domain; vWFC von Willebrand Factor C domain; CAP-Gly: cytoskeleton-associated protein glycine-rich domain); Green shaded domains are other annotated sequence features. Gray shaded TM: transmembrane domain. Proteins are drawn to scale (*DCTN1-ALK* fusion = 1383 AA).

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Figure 6:

IgG4-related retroperitoneal fibrosis. Low power view (Panel A). High-power view highlights the prominent population of stromal cells with mild nuclear atypia in the background of lymphocytes and plasma cells (Panel B). Obliterative phlebitis (Panel C) (arrow). Immunohistochemical stain for IgG4 showing increased numbers of IgG4 positive plasma cells (Panel D).

Clinical, histologic and molecular feature of inflammatory myofibroblastic sarcoma mimicking IgG4 related disease

Table 1:

case	Age	Gender	Tumor size	Location	Histology	IgG4 counts	IgG4/IgG (%)	IHC for ALK/ROS1	FISH/Translocation assay	Follow-up
1	49	F	1.5 cm	Lung	Storiform fibrosis, obliterative phlebitis, dense LP infiltrate	52	42%	Negative	ETV6-NTRK fusion; FISH for ALK/ROS1 negative	ANED 26 months
2	27	F	9.3	Lung	Storiform fibrosis, obliterative phlebitis, dense LP infiltrate	63	79%	Negative	TFG-ROS1 fusion ROS1 FISH positive	Recurrent tumor *
3	33	M	2.9	Trachea	Subtle storiform fibrosis, dense LP infiltrate	77	55%	Negative	FISH for ALK negative; Fusion assay negative	Tumor recurred, LTFU after resection of recurrence
4	44	M	2.0	Trachea	Subtle storiform fibrosis, dense LP infiltrate	82	61%	ALK positive, ROS1 negative	TFG-ALK fusion	ANED - LTFU after 12 months
5	10	M	2.8	Pharynx	Marked fibrosis with subtle storiform fibrosis, dense LP infiltrate	344	85%	ALK positive, ROS1 negative	THBS1-ALK fusion	ANED 38 months
6	75	F	2.5 cm	Stomach	Storiform fibrosis, obliterative phlebitis, dense LP infiltrate	99	45%	ALK positive, ROS1 negative	FISH for ALK negative; fusion assay negative	ANED 15 months
7.	27	F	3.6 cm	Lung	Dense LP, storiform fibrosis	86	45%	ALK positive, ROS1 negative	DC7N1-ALK fusion	ANED 24 months

LP= lymphoplasmacytic

ANED = alive no evidence of disease

LTFU = lost to follow-up

* following resection and discontinuing Crizotinib the tumor recurred. The recurrent tumor responded to Crizotinib. Currently the patient has no evidence of tumor.

Table 2:

Histology and immunohistochemistry data on patients with an established diagnosis of IgG4 related disease.
All cases were negative for ALK and ROS1 immunohistochemistry

No.	Site	Storiform fibrosis	Obliterative phlebitis	IgG4	IgG	IgG4/IgG
1	Breast	Yes	Yes	47	187	25
2	Lung	Yes	Yes	121	458	27
3	Pancreas	Yes	Yes	121	140	87
4	Pancreas	Yes	Yes	93	210	44
5	Pancreas	Yes	Yes	37	50	75
6	Pancreas	Yes	Yes	187	294	63
7	Ampulla	Yes	Yes	160	336	48
8	Appendix	Yes	Yes	61	173	35
9	Lymph node	No	No	383	850	45
10	Gallbladder	Yes	No	51	60	86
11	Arytenoid	Yes	No	84	336	25
12	Pancreas	Yes	Yes	154	262	59
13	Lymph node	No	No	138	190	73
14	Gallbladder	Yes	Yes	32	55	58
15	Pancreas	Yes	Yes	187	196	95
16	Pancreas	Yes	Yes	126	175	72
17	Trachea	Yes	Yes	47	112	42
18	Lung	Yes	Yes	200	220	91
19	Salivary gland	Yes	Yes	112	178	63
20	Pancreas	Yes	Yes	290	304	95
21	Pancreas	Yes	Yes	257	336	76
22	Lymph node	No	No	147	200	74
23	Pancreas	Yes	Yes	136	290	47
24	Pancreas	Yes	Yes	220	458	48
25	Pancreas	Yes	Yes	154	187	83
26	Retroperitoneum	Yes	Yes	112	234	48
27	Salivary gland	Yes	Yes	70	173	41
28	Pancreas	Yes	Yes	364	369	99
29	Pancreas	Yes	Yes	28	37	75
30	Pancreas	Yes	No	35	74	47
31	Pancreas	Yes	No	178	238	75
32	Lymph node	No	No	176	224	79
33	Pancreas	Yes	Yes	138	148	93
34	Salivary gland	Yes	Yes	93	215	43
35	Salivary gland	Yes	Yes	290	322	90
36	Lymph node	No	No	134	353	38

No.	Site	Storiform fibrosis	Obliterative phlebitis	IgG4	IgG	IgG4/IgG
37	Pancreas	Yes	Yes	65	313	21
38	Pancreas	Yes	Yes	260	270	96
39	Pancreas	Yes	Yes	134	320	42
40	Bile duct	Yes	No	94	157	60
41	Gallbladder	Yes	Yes	41	75	55
42	Liver	Yes	No	94	114	82
43	Trachea	Yes	Yes	145	282	51
44	Salivary gland	Yes	Yes	160	190	84
45	Lung	Yes	Yes	164	210	78
46	Pancreas	Yes	Yes	41	67	61
47	Pancreas	Yes	Yes	110	240	46
48	Retroperitoneum	Yes	Yes	69	200	35
49	Pleura	No	No	105	200	53
50	Oropharynx	No	No	75	210	36
51	Lung	Yes	Yes	200	350	57
52	Salivary gland	Yes	Yes	126	177	71
53	Liver	No	No	82	150	55
54	Salivary gland	Yes	Yes	110	250	44
55	Lung	Yes	Yes	230	500	46
56	Lymph node	No	No	550	850	65