

Inflammatory Myofibroblastic Tumors of the Female Genital Tract Are Under-recognized

A Low Threshold for ALK Immunohistochemistry Is Required

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Abstract: Inflammatory myofibroblastic tumor (IMT) of the female genital tract is under-recognized. We investigated the prevalence of ALK-positive IMT in lesions previously diagnosed as gynecologic smooth muscle tumors. Immunohistochemistry (IHC) for ALK was performed on tissue microarrays of unselected tumors resected from 2009 to 2013. Three of 1176 (0.26%) “leiomyomas” and 1 of 44 (2.3%) “leiomyosarcomas” were ALK IHC positive, confirmed translocated by fluorescence in situ hybridization (FISH) and therefore more appropriately classified as IMT. On review significant areas of all 4 tumors closely mimicked smooth muscle tumors morphologically, but all showed at least subtle/focal features suggesting IMT. Recognizing that the distinction between IMT and leiomyoma/leiomyosarcoma

can be subtle, we then reviewed 1 hematoxylin and eosin slide from each patient undergoing surgery for “leiomyoma” from 2014 to 2017 and selected cases for ALK IHC with a low threshold. Of these, 30 of 571 (5.3%) underwent IHC. Two were confirmed to be IHC positive and FISH rearranged. Of the 6 IMTs, only 1 tumor with a previous diagnosis of leiomyosarcoma, an infiltrative margin and equivocal necrosis, metastasized. Of note it demonstrated a less aggressive clinical course compared with most metastatic leiomyosarcomas (alive with disease at 6 y). The patient was subsequently offered crizotinib to which she responded rapidly. In conclusion, IMTs may closely mimic gynecologic smooth muscle tumors. IMTs account for at least 5 of 1747 (0.3%) tumors previously diagnosed as leiomyoma and 1 of 44 (2.3%) as leiomyosarcoma. These tumors may be recognized prospectively with awareness of subtle/focal histologic clues, coupled with a low threshold for ALK IHC.

Key Words: inflammatory myofibroblastic tumor, ALK, leiomyoma, leiomyosarcoma, uterus

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Inflammatory myofibroblastic tumor (IMT) is a unique and increasingly well characterized mesenchymal neoplasm. Given its significant potential for local recurrence (25% of extrapulmonary cases) but low risk of distant metastasis (< 2%), IMT is classified as a tumor of intermediate biological potential under the World Health Organization classification of tumors of soft tissue and bone.^{1–3}

Approximately half of all IMTs can be proven to harbor gene rearrangements involving the *anaplastic lymphoma kinase (ALK)* gene at 2p23.⁴ Although multiple different *ALK* fusion partners have been recorded, all reported fusions result in the 3′ kinase-containing portion of *ALK* being joined to the 5′ portion of a constitutively expressed gene.^{5–11} The resulting fusion oncogene drives neoplasia. Importantly, it may also be targeted by already

available rationally designed tyrosine kinase inhibitors such as crizotinib or ceritinib which have resulted in dramatic and durable responses in some patients with metastatic disease.^{12–16} Rearrangements of the genes encoding other tyrosine kinases including *ROS1*, *NTRK3*, *PDGFR β* , and *RET*, which may also be therapeutically targetable, have been reported in many of the remaining extrauterine IMTs not associated with *ALK* rearrangement but, to date, never in cases arising in the uterus.^{3,5–7,11,17}

Immunohistochemistry (IHC) for *ALK* serves as a highly sensitive and specific marker for pathogenic *ALK* gene rearrangements in IMT and other tumors.^{2,4,6,18} Because of the difficulty in identifying certain subtle rearrangements, particularly intrachromosomal inversions, correctly optimized *ALK* IHC may in fact be more sensitive than fluorescence in situ hybridization (FISH) studies which are often considered the gold standard in clinical practice.^{6,18}

Although IMTs may present across a broad range of anatomic sites and at any age, historically they have been considered to most commonly arise in the lung, retroperitoneum, and abdominal cavity of children and young adults.^{1–3} IMT of the uterus is considered very rare. Indeed, at the time of writing we are aware of only 42 reported cases.^{6,19–27} Furthermore all large series are multi-institutional and show a very strong bias toward consultation and referral cases. For example, Haines et al's⁶ recent series of 11 IMTs included consultation cases from at least 4 renowned consultation pathologists and in Parra-Herran et al's²³ series of 10 cases, 9 (90%) were external consultation cases. Although morphologic clues to the diagnosis of IMT including myxoid change and a lymphoplasmacytic infiltrate have been described, uterine IMTs may have fascicular areas which closely mimic uterine smooth muscle tumors.²³ In this context it is particularly interesting to note that of the 42 reported cases of uterine IMTs, 6 (14.3%) were initially categorized as leiomyosarcoma ($n = 4$),²⁷ smooth muscle tumor of uncertain malignant potential (STUMP) ($n = 1$)²⁶ or leiomyoma ($n = 1$)⁶ before *ALK* IHC, FISH, or molecular testing was performed either on archived material solely for the studies^{26,27} or as part of a comprehensive molecular panel performed as part of a personalized medicine approach for metastatic disease.²⁶

Given that so many cases were recognized only on review, we suspected that gynecologic IMTs may be significantly more common than previously appreciated. We therefore sought to systematically investigate the incidence and clinicopathologic features of IMTs in a large and unselected population of tumors initially classified as smooth muscle in origin.

METHODS

We first performed *ALK* IHC in large and unselected gynecologic leiomyoma and leiomyosarcoma tissue microarray (TMA) cohorts which we had previously used to investigate fumarate hydratase deficiency.²⁸ For the purpose of this study we term these

initial TMA cohorts the “calibration cohorts.” The leiomyoma calibration cohort comprised two 1 mm cores from all patients undergoing resection of uterine/gynecologic leiomyoma at our institution during calendar years 2009 to 2013. The leiomyosarcoma calibration cohort included two 1 mm cores from all patients undergoing surgery for gynecologic leiomyosarcoma from June 1998 to December 31 2013. In patients with multiple tumors, the largest tumor was selected for annotation and TMA construction.

IHC for *ALK* was performed using previously described methods which we have validated as highly sensitive and specific for *ALK* gene rearrangements in lung carcinoma in our laboratory.¹⁸ We used a mouse monoclonal antibody at a high concentration (dilution 1:10, clone 5A4; Novocastra, Leica Biosystems, United Kingdom). An automated staining platform was used—the Leica Bond III autostainer (Leica Biosystems, Mount Waverley, Vic., Australia) with heat-induced epitope retrieval in the manufacturer's alkaline retrieval solution ER2 (VBS part no; AR9460). All cases were interpreted by a single observer who was blinded to all clinical and pathologic data (A.J.G.) and any degree of cytoplasmic or nuclear staining for *ALK* was interpreted as positive. Cases which demonstrated any positive staining on the calibration TMA sections then underwent *ALK* IHC on whole sections and, if interpreted as positive using the same criteria, proceeded to *ALK* FISH.

ALK FISH studies were performed on whole sections using a commercial probe and interpreted using standard criteria.¹⁸ We employed the Vysis LSI *ALK* Dual Color, Break Apart Rearrangement Probe (Abbott Molecular). Fifty representative nuclei were assessed by an observer who was not blinded to the results of *ALK* IHC (A.C.), and cases were reported as positive for *ALK* rearrangement when 2 signal diameters were seen between the red and green signals in >15% of tumor cells.

Having reviewed the morphology of *ALK* translocated cases from the calibration cohort and with a better understanding of the morphologic similarities and differences between usual smooth muscle tumors and IMTs, we then investigated *ALK* gene rearrangements in other cohorts which for the purposes of this study we called the “test cohorts.” For the leiomyoma test cohort we selected a single hematoxylin and eosin (H&E)-stained slide from all patients who underwent surgery for (presumed) benign leiomyoma at our institution from 1 January 2014 until 31 January 2017 for morphologic review. Again when multiple tumors were present, the largest tumor was selected and annotated. This H&E-stained slide was reviewed by a pathologist who was blinded to all clinical and pathologic features (A.J.G.). Cases from the test cohort were then selected for *ALK* IHC on whole sections with a low threshold. That is *ALK* IHC was performed if the diagnosis of IMT was considered possible based on the presence of any feature (eg, myxoid change, inflammatory infiltrate, or tapered nuclei) even if these changes were subtle or focal. If cases were positive by IHC, confirmatory FISH studies were undertaken.

TABLE 1. Clinical and Pathologic Details of IMTs

Case	Age (y)	Resection Type	Site	Original Diagnosis	Duration of Follow-up (mo)	Status at Follow-up
1	47	Hysterectomy	Uterus submucosal	Leiomyoma	74	ANED
2	32	Enucleation (piecemeal)	Broad ligament	Leiomyoma	69	ANED
3	39	Myomectomy*	Uterus submucosal pedunculated	Leiomyoma	—	—
4	37	Myomectomy*	Uterus submucosal	Leiomyoma	30	ANED
5	45	Curettage (piecemeal)	Uterus submucosal	Leiomyoma or IMT	4†	Residual IMT 13 mm resected
6	43	Enucleation (piecemeal)	Uterus	Leiomyosarcoma (low grade)	66	AWD

*Pregnant, resected at time of lower segment Cesarean section.

†Computed tomographic scan and ultrasound at 2 months demonstrated bulky uterus and 2 lesions, 20 and 13 mm, with imaging characteristics of leiomyoma. The 13 mm submucosal lesion found to be residual IMT at hysterectomy at 4 months. The 20 mm lesion was a usual (ALK negative) leiomyoma.

‡Necrosis considered equivocal after expert review: 2 experts interpreted it as coagulative necrosis, and 1 expert thought it was hemorrhagic/apoplectic change.

ANED indicates alive with no evidence of disease; AWD, alive with disease (metastatic).

In view of our experience in this study and previous reports of IMT occurring in pregnancy,^{6,23} we sought to specifically study the relationship between pregnancy and IMT by investigating an additional test cohort which included all patients from June 1998 to January 2017 who were known to be pregnant at the time of leiomyoma surgery. We also postulated that due to unusual morphologic features or myxoid change some IMTs may have been initially classified as STUMP. We therefore also created a test cohort of all patients who underwent surgery for tumors classified as STUMP from June 1998 to January 2017. These 2 test cohorts underwent ALK IHC on whole sections with the intention to perform FISH if IHC was thought to be positive. This study was approved by the Northern Sydney Local Health District Human research ethics committee.

RESULTS

A total of 1176 patients underwent surgery for tumors classified as benign leiomyoma from 2009 to 2013 and had assessable material in the TMA calibration cohort. Three (0.26%) demonstrated positive staining for ALK on the TMA sections, and were subsequently confirmed to be positive on whole sections and *ALK* gene rearranged with FISH studies. In all cases, all areas of the tumor demonstrated diffuse strong expression of ALK, even in regions where the morphology exactly mimicked leiomyoma. The details of the clinical, pathologic, and immunohistochemical features are presented in Tables 1 and 2.

Patient 1 was 47 years old and underwent simple hysterectomy for menorrhagia. The uterus was in secretory phase and lacked hyperplasia. Three “leiomyomas” were identified macroscopically and 2 sampled. The smaller was 12 mm, had the typical appearance of leiomyoma and was ALK negative. The larger was 17 mm, submucosal and confirmed ALK IHC positive and *ALK* gene rearranged. The morphology is presented in Figure 1. The tumor did not particularly stand out against the benign myometrium and there were large areas with a fascicular architecture closely mimicking leiomyoma. There was no myxoid change but in areas the

architecture was less compact. There was a mild predominantly perivascular lymphocytic infiltrate which was more conspicuous than usually seen in leiomyoma, but otherwise the tumor very closely mimicked leiomyoma. This patient was alive and disease free 6 years and 2 months after surgery.

Patient 2 was 32 years old and underwent laparoscopy with biopsies of multiple sites for investigation and subsequent confirmation of endometriosis. At the time an incidental presumed leiomyoma of the left broad ligament was resected piecemeal with the combined fragments measuring 80 × 80 × 20 mm and weighing 42 g. A diagnosis of leiomyoma with focal myxoid and degenerative change was issued. On review the individual fragments were well circumscribed and large areas demonstrated typical morphology for leiomyoma (Fig. 2). Interestingly some areas demonstrated nuclear palisading. Areas with myxoid change accounted for < 10% of the lesion and in these areas the loose myxoid stroma, “tissue culture-like” appearance, scattered lymphocytes and myofibroblastic morphology typical of IMT could be appreciated. Five years and 9 months later the patient underwent a second laparoscopy for treatment of endometriosis. Although not specifically sought, no tumors were identified. Endometriosis was confirmed on biopsies of the right pararectal space, and right and left pelvic sidewall. No IMT was evident on review of these biopsies.

Patient 3 underwent myomectomy for a 35 mm presumed leiomyoma encountered at the time of lower segment Cesarean section at 39 years of age. Macroscopically the tumor was described as a pedunculated polyp. At the time mild inflammation and stromal edema attributed to pregnancy effect were reported. On review there were large areas with morphology in keeping with leiomyoma and smaller areas in which the myxoid change, less fascicular architecture, “tissue culture-like” stroma and subtle inflammatory infiltrate typical of IMT were appreciated (Fig. 3). No further follow-up is available for this patient, but she is not known to have presented to our service for investigations of any other neoplasms 5 years after resection.

TABLE 1. (continued)

Size (mm)	Margin	Predominant Architecture	Necrosis	Cytologic Atypia	Mitotic Count Per 10 hpf	Morphologic Features
17	Circumscribed	Fascicular	Absent	Nil	1	Remarkably similar appearance to adjacent myometrium. No myxoid change. Mild infiltrate of lymphocytes and histiocytes
80	Circumscribed	Fascicular	Absent	Nil	1	Focal myxoid change comprising < 10% of area. Mild infiltrate of lymphocytes. Nuclear palisading
35	Circumscribed	Fascicular	Absent	Nil	6	Myxoid change comprising approximately 30% of area. Moderate infiltrate of lymphocytes and histiocytes
40	Circumscribed	Fascicular	Absent	Nil	4	Myxoid change comprising 50% of area but scattered in discrete foci. Mild chronic inflammatory infiltrate of lymphocytes and histiocytes
40	Unable to ascertain due to nature of specimen	Fascicular	Absent	Mild	2	Mild cytologic atypia with prominent nucleoli and mild nuclear pleomorphism. Myxoid change present in which cytologic atypia is focally more pronounced. Mild infiltrate of lymphocytes
190	Infiltrative	Fascicular	Equivocal‡	Mild	1 in most areas (focally 7)	Focal mild cytologic atypia. Focal myxoid change present (30% of lesion). Metastasis demonstrated same morphology

Having identified these cases in the calibration cohort and with greater awareness that the morphologic features of IMT can be very subtle and focal in tumors which would otherwise be classified as leiomyoma we reviewed the morphology of consecutively resected “leiomyomas” from January 2014 to January 2017 in the test cohort. A single H&E-stained slide from 571 cases was reviewed and 30 cases were selected for ALK IHC on the basis of focal myxoid change, tapered nuclei, less fascicular architecture or other unusual features. Two cases (patients 4 and 5) demonstrated diffuse strong positive staining and were confirmed to be *ALK* translocated by FISH. Four other cases showed very weak nonspecific staining which could be attributed to background uptake in myxoid stroma but not the neoplastic cells themselves. These cases lacked gene rearrangements on FISH studies and therefore the diagnosis of IMT was considered excluded.

Patient 4 had a 40 mm tumor resected by myomectomy at the time of lower segment Cesarean section at 37 years of age. At the time of initial reporting unusual morphologic features were appreciated including myxoid change and a mitotic rate of 4 per 10 hpf and the tumor was referred to 2 nationally recognized experts in gynecologic pathology who rendered a diagnosis of leiomyoma with pregnancy-related changes. On review the most notable feature was that although much of the tumor demonstrated leiomyoma-like features, there were multiple discrete foci of myxoid change where the high power appearances were more in keeping with IMT. As a result of this study, this patient was recalled 2½ years after initial resection and offered a dedicated computed tomographic scan and pelvic ultrasound both of which demonstrated no residual disease. The patient was counselled about the

uncertainties of the natural history of this lesion and is currently being followed.

Patient 5 had uterine curettage at 45 years of age for menorrhagia and a presumed submucosal leiomyoma was resected in multiple fragments 40 × 40 × 5 mm in aggregate. Prominent, albeit focal, myxoid change was noted and there was mild cytologic atypia. There were some areas in which typical features of IMT were identified. This case was encountered during preparation of the manuscript and the possibility of IMT was considered by 1 pathologist involved in reporting this case but not by another. On subsequent follow-up computed tomographic scan and ultrasound at 2 months the patient had a residual bulky uterus with 20 and 13 mm lesions which demonstrated the imaging characteristics of leiomyoma. She was counselled about the uncertainties of the natural history of this lesion and underwent simple hysterectomy. The 13 mm submucosal lesion was found to be residual IMT while the 20 mm lesion was a usual (ALK negative) leiomyoma.

The clinical and pathologic associations of the entire cohort of ALK-positive IMTs (n = 5) from both the calibration and test cohorts compared with the remaining leiomyomas (n = 1742) are presented in Table 3. The IMTs and leiomyomas were similar size (mean, 33 vs. 50 mm; *P* = 0.44). However the IMTs demonstrated a strong tendency to occur at a significantly younger age—mean 40 years (range, 32 to 48 y) compared with mean 49 years (range, 24 to 94 y; *P* = 0.07). The 0.26% incidence in the 2009 to 2013 cohort screened by IHC was very similar to the 0.35% incidence in the cohort screened first with morphology (*P* = 0.664).

There was a strong association between IMTs and pregnancy with IMTs being found in 2 of 19 (10.53%) of

TABLE 2. Immunohistochemical Features of IMTs

Patient	ALK	Estrogen Receptor	Progesterone Receptor	SMA	Desmin	Calponin	P53*	Ki67	CD10	S100	SOX-10	P16*
								Proliferative Index (%)				
1	Cytoplasmic	2+	3+	3+	3+	3+	Normal	2	Negative	Negative	Negative	Abnormal (positive)
2	Cytoplasmic (PNA)	1+	2+	3+	3+	3+	Normal	< 1	Negative	Negative	Negative	Abnormal (positive)
3	Cytoplasmic (PNA)	Neg	3+	3+	2+ focal	2+ focal	Normal	2	3+	Negative	Negative	Normal
4	Cytoplasmic (PNA)	Neg	2+	3+	2+ focal	2+ focal	Normal	2	3+	Negative	Negative	Normal
5	Cytoplasmic (PNA)	3+	3+	3+	3+ focal	3+	Normal	1	Negative	Negative	Negative	Abnormal (positive)
6	Cytoplasmic	1+ focal	3+	3+	2+ focal	3+	Normal	4	Negative	Negative	Negative	Normal

*P53 and P16 expression was interpreted as normal if patchy or heterogeneous staining and abnormal if either strong and diffuse or completely absent.²⁹ PNA indicates paranuclear accentuation of cytoplasmic ALK staining.

the pregnant patients from the calibration and test cohorts (2009 to 2017), compared with 3 of 1728 (0.17%) of the nonpregnant patients ($P = 0.001$; odds ratio, 66.2; 95% confidence interval, 7.475-471.7). We therefore sought to investigate the incidence of IMTs in an expanded cohort of pregnant patients. We searched our database for all “leiomyomas” resected during pregnancy and performed ALK IHC on whole sections. A further 44 patients were investigated with ALK staining on whole sections, comprising 17 of the previously screened cases from the 2009 to 2017 cohorts and another 27 from 1998 to 2008. All these additional cases were all ALK IHC negative on whole sections. We further investigated ALK staining on whole sections from 16 STUMP from 1998 to 2017 and found no positive cases.

Finally we investigated ALK status in 44 tumors which had received a diagnosis of uterine leiomyosarcoma. One case was IHC positive on TMA and confirmed positive and gene rearranged by FISH on whole sections

and represents patient 6. Patient 6 initially presented in 2011 when a 190 mm myometrial lesion was resected with morcellation. Much of the tumor showed features indistinguishable from leiomyoma, but approximately 30% of the tumor showed myxoid change (Fig. 4). In areas the contour was mildly infiltrative. It had a low mitotic rate of < 1 per 10 hpf in most areas but in very small areas up to 7 per 10 hpf. Most of the tumor was cytologically bland but very focally there were areas of mild cytologic atypia. There was some interobserver disagreement about whether there was coagulative necrosis or hemorrhagic/apoptotic change. In view of diagnostic uncertainty, this case had been referred to 2 national experts who made a diagnosis of low-grade leiomyosarcoma. Of note, the patient requested a further opinion and the slides were reviewed by an internationally renowned gynecologic pathologist who thought the tumor lacked coagulative necrosis, preferred a diagnosis of “atypical leiomyoma” and thought that recurrence was unlikely. She was found

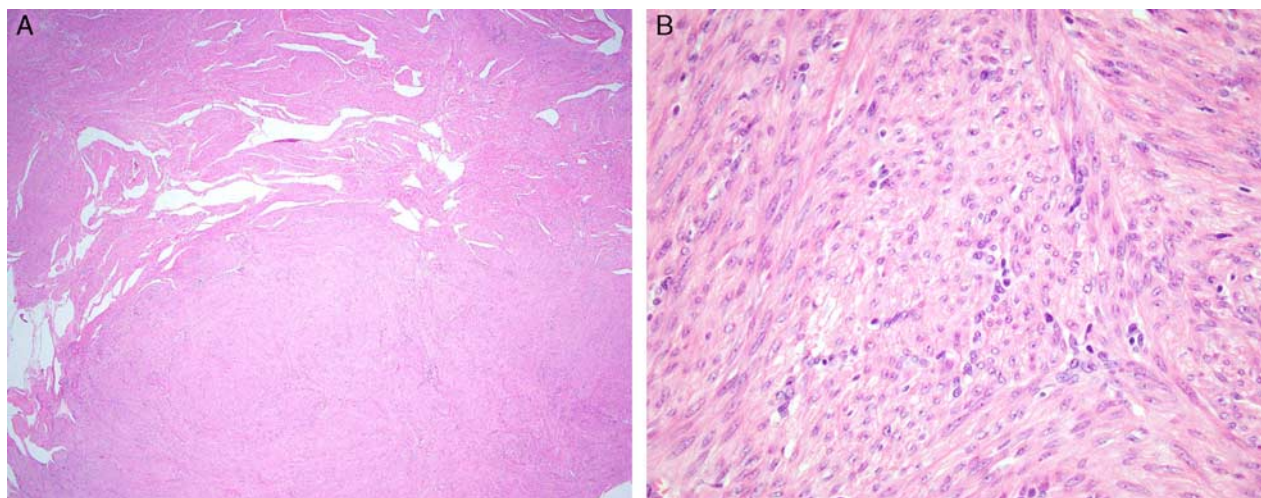


FIGURE 1. In patient 1 the IMT was somewhat ill-defined compared with the adjacent myometrium (A) but was clearly and crisply highlighted by ALK immunohistochemistry. At higher power a striking fascicular architecture and cytology indistinguishable from leiomyoma was noted (B).

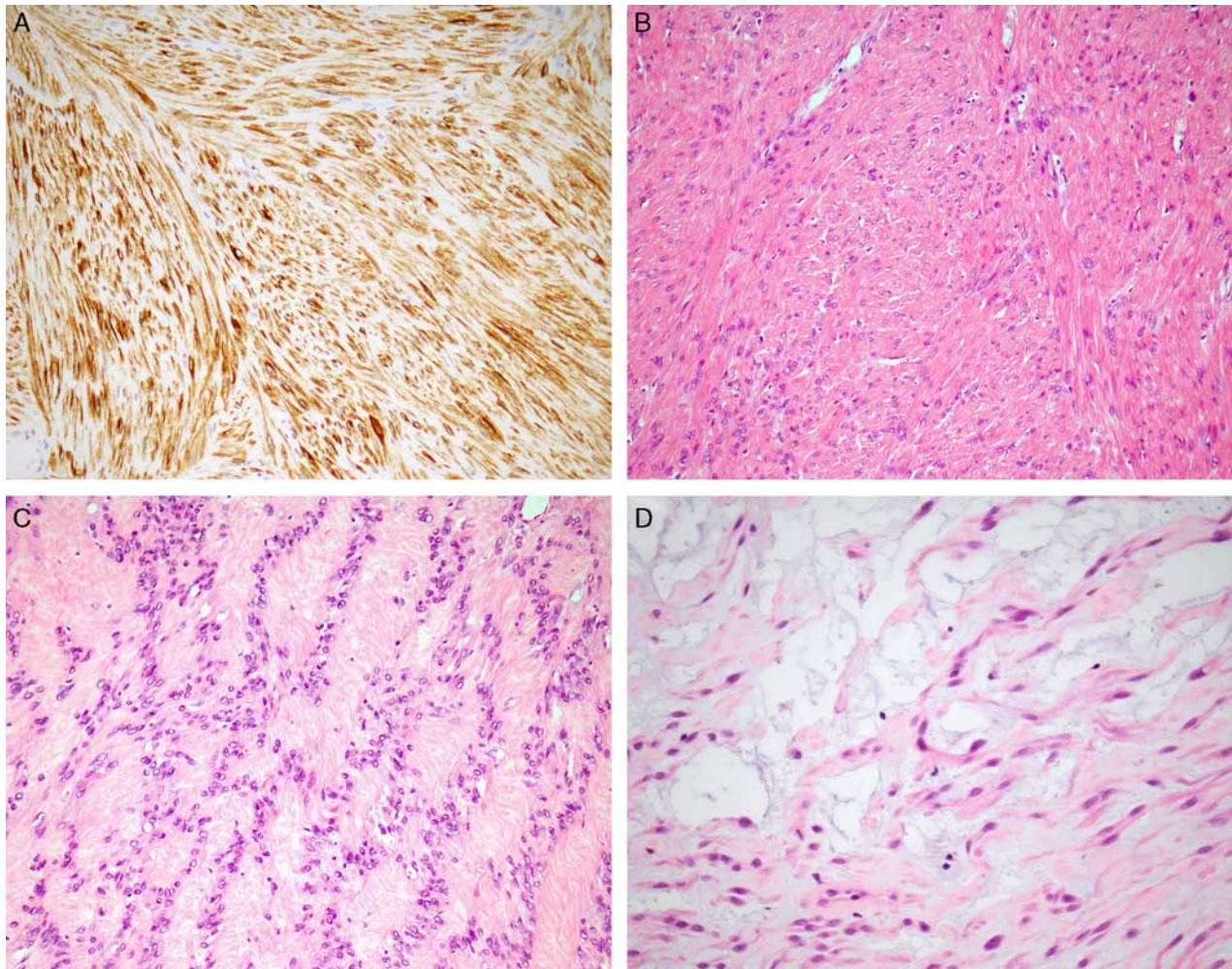


FIGURE 2. Like the other cases, the tumor from patient 2 demonstrated diffuse strong staining for ALK (A), in this instance with some paranuclear accentuation. Again there were areas with a well-developed fascicular architecture mimicking leiomyoma (B). Areas of nuclear palisading were noted (C). In small areas, <10% of the tumor, more typical features of IMT were present (D) including myxoid change, a loose “tissue culture-like” architecture and a subtle inflammatory infiltrate.

to have a biopsy-confirmed pulmonary metastasis on initial staging investigations. She suffered a peritoneal recurrence including the port site 1 year later and had further debulking surgery. She was subsequently treated with antihormonal therapy (letrozole) and had a very good partial response in the lung which has been maintained. However, she had multiple intra-abdominal recurrences and has had a total of 5 resections comprising multiple abdominal, pelvic, and peritoneal deposits up to 85 mm resected at 9 months, residual 100 mm tumor in the uterus and peritoneal disease resected at 13 months, and further peritoneal and mesenteric disease at 23, 54, and 55 months. She was then diagnosed with progressive disease with a large retroperitoneal mass.

At 62 months the diagnosis was modified to IMT with *ALK* gene rearrangement as part of this study. The patient was therefore offered crizotinib therapy to which she had a dramatic response. Dose limiting side effects led to a change to the second generation tyrosine kinase

inhibitor ceritinib to which she has had an ongoing response now at 4 months after initial therapy commencement. Her pulmonary metastasis has not progressed and it is possible that tumor morcellation at the time of initial resection contributed to the multiple pelvic and abdominal recurrences.

DISCUSSION

Our study provides evidence that at least 5 of 1747 (0.3%) lesions otherwise thought to be uterine leiomyomas actually represent IMTs and that these tumors can be recognized prospectively by morphology in combination with a low threshold for ALK IHC. Given that leiomyomas are among the most commonly resected visceral neoplasms,³⁰ our findings suggest that the uterus may be the most common anatomic location for IMTs. Indeed in our department during the study period from January 2009 to the present we only encountered 5 other cases of

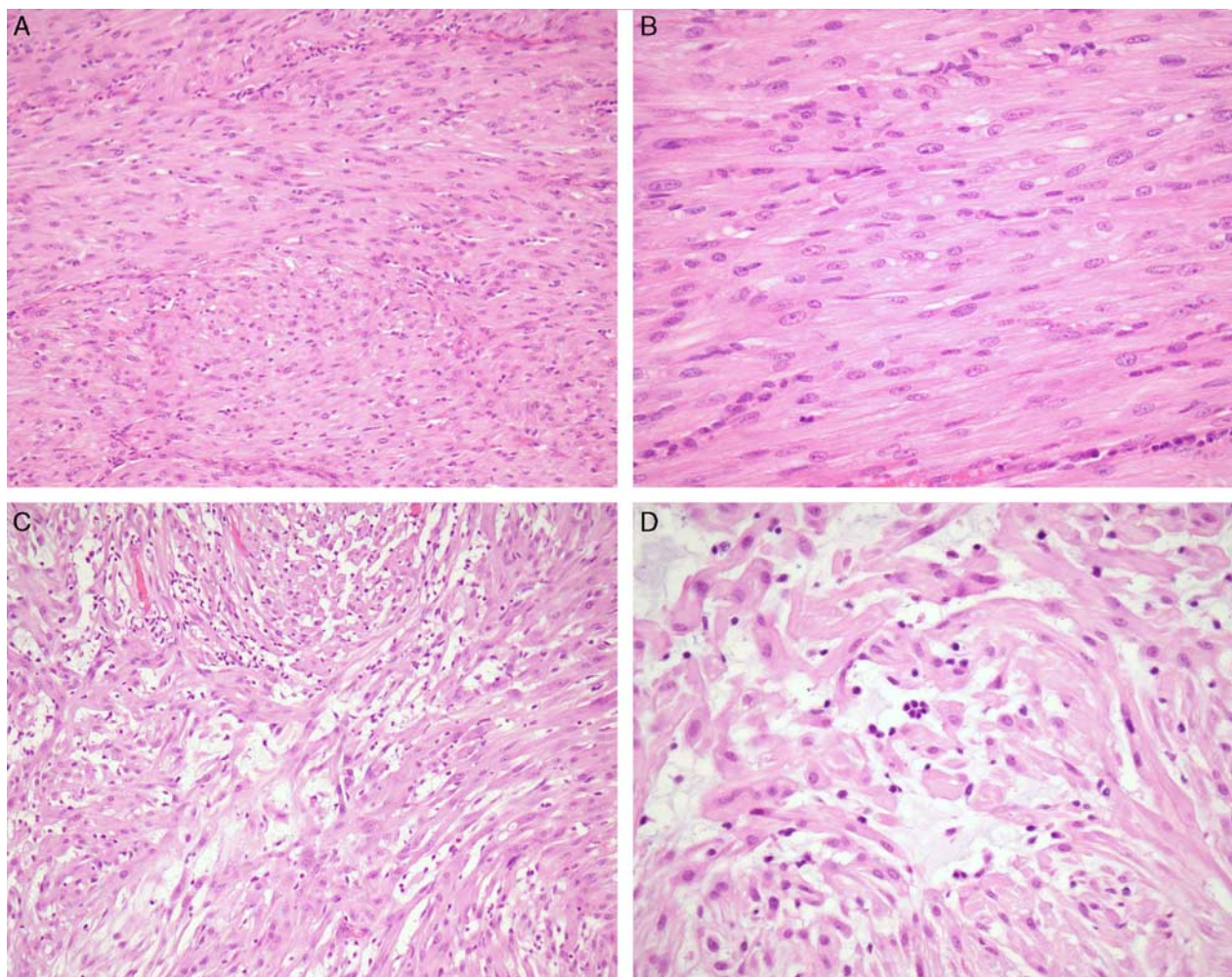


FIGURE 3. Most of the IMT from patient 3 demonstrated a well-developed fascicular architecture (A) and cytology closely mimicking leiomyoma (B) although a few inflammatory cells were noted. Focally the tumor demonstrated features more typical for IMT including myxoid change, tapered nuclei and a lymphocytic inflammatory infiltrate (C, D).

IMT at all other anatomic sites combined. Furthermore we note that some cases of IMT may not have been identified by this study and the incidence of IMT in the uterus may in fact be greater. For example we only screened the largest “leiomyoma” and may have missed IMTs masquerading as smaller leiomyomas. Although ALK expression was diffuse and strong in all IMTs we identified, in the calibration cohort we only screened cases

on TMA and may have missed cases with patchy or weak expression. For the test cohort we screened first by morphology on just 1 H&E-stained slide and may have missed subtle morphologies or focal change. Furthermore we only screened for ALK overexpressing and gene rearranged cases and we may have missed IMTs with non-*ALK* driver abnormalities such as *ROS1*, *NTRK3*, *PDGFRβ*, and *RET* gene rearrangements (although to date these have not been found in the uterus).^{5-7,11,17}

While it is impractical to test all leiomyomas, we demonstrate that with awareness of subtle and focal morphologies and a low threshold for ALK IHC, the prospective recognition of IMTs can be greatly increased. Indeed the 0.26% incidence in the 2009 to 2013 cohort screened by IHC did not differ significantly from the 0.35% incidence in the cohort screened first with morphology ($P = 0.664$). The morphologic clues to the diagnosis have been previously emphasized and include myxoid change often with a loose “tissue culture-like” appearance, interspersed inflammatory cells (in our

TABLE 3. Clinical and Pathologic Characteristics of 1747 Patients, Comparing True Leiomyomas (n = 1742) With ALK Rearranged IMTs (n = 5)

	ALK-Negative (Leiomyomas) (n = 1742) (99.71%)	ALK-Positive (IMTs) (n = 5) (0.29%)	P
Age (mean [range]) (y)	49 (24-94)	40 (32-48)	0.07
Size (mean [range]) (mm)	50 (2-300)	33 (17-40)	0.44

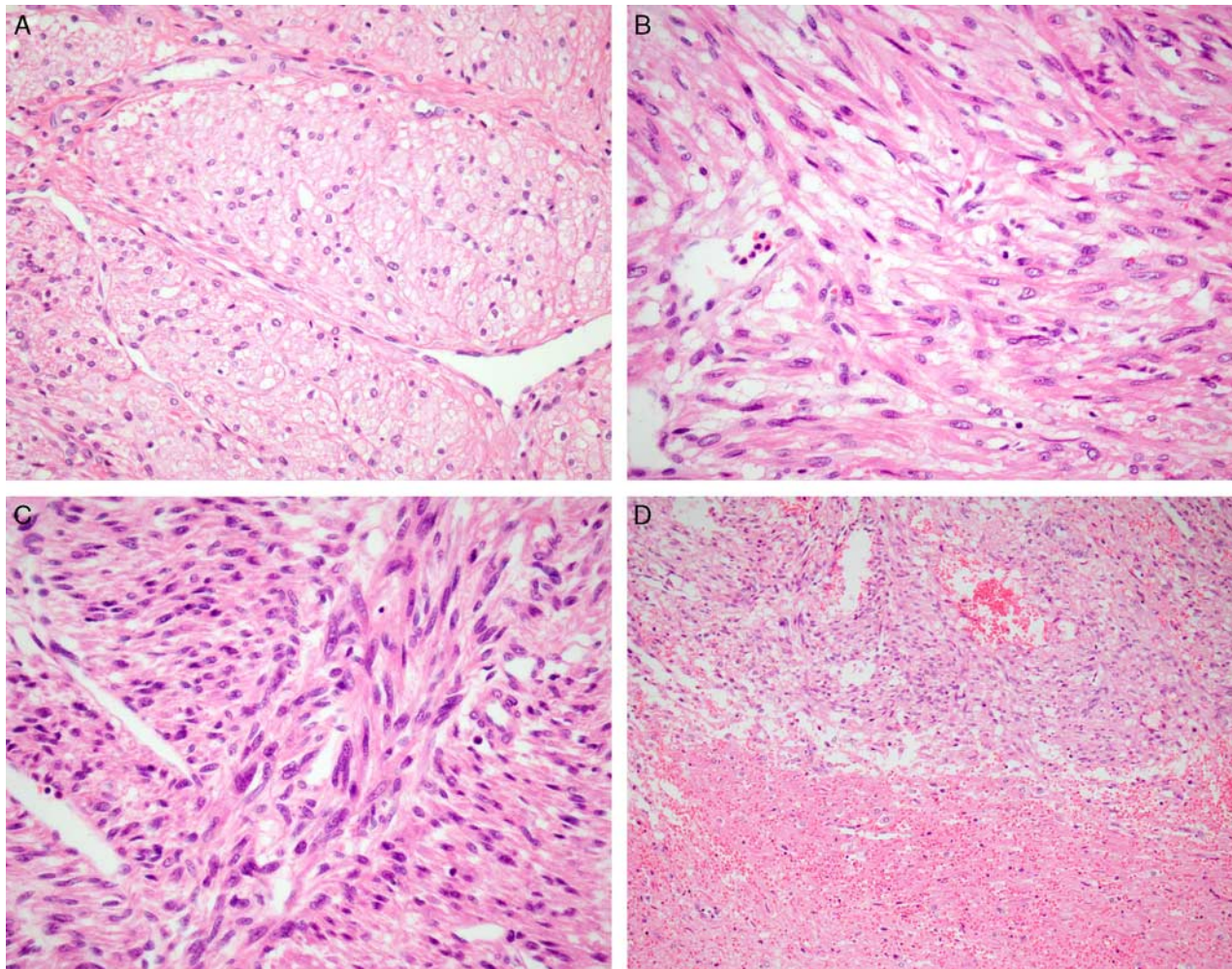


FIGURE 4. The IMT from patient 6 was the only case to demonstrate aggressive behavior during the follow-up period. Much of the tumor was morphologically indistinguishable from leiomyoma (A) but there were more discohesive areas associated with myxoid change more suggestive of IMT (B). Cytologic atypia was present but this was mild and focal (C). There were small areas of necrosis but there was disagreement amongst expert pathologists as to whether this represented true coagulative necrosis or hemorrhagic/apoplectic change (D).

experience particularly lymphocytes rather than plasma cells and sometimes predominantly perivascular), dispersed chromatin and genuinely fusiform, that is tapered rather than blunt-ended, nuclei.²³ However, we emphasize that all IMTs detected in this study demonstrated a predominantly fascicular histologic pattern with large areas closely resembling smooth muscle tumors both architecturally and cytologically and only relatively small areas demonstrating more typical IMT-like features. These leiomyoma-like areas all demonstrated diffuse strong ALK expression and harbored gene rearrangements on FISH studies confirming that they are part of the neoplasm.

We caution that in this study we performed screening IHC for ALK with a specific mouse monoclonal antibody (clone 5A4) at a high concentration (1:10) which has already been validated by us and others to be highly sensitive and specific for the presence of *ALK* gene rearrangements in lung carcinoma and other tumors.^{18,31,32} Others have

found similar high sensitivity and specificity with other clones including the rabbit monoclonal antibody D5F3.^{33,34} However not all ALK antibodies, dilutions and retrieval conditions are equally sensitive and specific³⁵ and we recommend ALK IHC be optimized and validated in individual laboratories before it is deployed clinically. Although rare and usually occurring when less specific antibodies are used, positive immunohistochemical staining for ALK has been reported in connective tissue neoplasms other than IMT.^{23,36,37} Therefore positive staining for ALK alone is not enough to make the diagnosis of IMT and the morphology should also be compatible.

The IMTs did not differ significantly in size compared with the leiomyomas but they did show a strong trend toward occurring at a younger age: mean 40 years (range, 32 to 48 y) compared with mean 49 years (range, 24 to 94; $P = 0.07$). Strikingly there seemed to be a strong association with pregnancy with IMTs being found in 2 of

19 (10.53%) of the pregnant patients from the leiomyoma-like cohort (2009 to 2017), compared with 3 of 1728 (0.17%) of the non-pregnant patients ($P = 0.001$; odds ratio, 66.2; 95% confidence interval, 7.475-471.7). Although we did not identify additional IMTs in a further 27 patients with presumed leiomyoma resected in pregnancy from 1998 to 2008, it is worth noting that at least 3 of 21 patients from 2 recent series of IMTs were pregnant.^{6,23} We note that the majority of the IMTs expressed estrogen receptor and all strongly expressed progesterone receptors (Table 2) and that the 1 patient with metastatic disease partially responded to antihormonal therapy. Therefore it is possible that uterine IMTs may be particularly likely to grow under the hormonal influence of pregnancy. From a practical point of view we would recommend that the possibility of IMT be particularly considered when apparent leiomyomas are resected in pregnant patients.

Many of the previously reported uterine IMTs have been described as polypoid or submucosal lesions.^{6,20,22-25} Indeed in Rabban et al's¹⁹ report of 6 cases, 50% were polypoid masses resected by hysteroscopy. It is therefore worth noting that 4 of 6 IMTs in our series were submucosal including one case which presented as a pedunculated polyp. That is, a submucosal or polypoid growth pattern may be a clue to the diagnosis of IMT and should also precipitate a low threshold for ALK IHC.

In keeping with the experience at other sites, our findings indicate that the majority of gynecologic IMTs, even those treated by simple myomectomy or enucleation alone, do not recur. Furthermore if uterine IMTs are much more common than previously appreciated as our study indicates, the fact that metastatic IMT remains a very rare disease reinforces the indolent behavior of the great majority of IMTs.

However, as evidenced by patient 6 in this series and multiple previous reports, some gynecologic IMTs can behave aggressively with local recurrence or metastasis,^{12,23-27} and it can be extremely difficult to prospectively identify which tumors are at high risk of metastasis. In other sites molecular testing may provide a clue to the risk of aggressive disease. For example, in soft tissue certain ALK fusion partners such as *RANPB2* or ALK negative IMTs have a higher risk of aggressive disease.^{1,31} However the fusion partners commonly associated with ALK in IMTs may be different to those identified elsewhere and include *IGFBP5*, *THBS1*, *FN1*, and *TIMP3*,⁶ and there are currently no known phenotype-genotype correlations in the uterus.

Recently Parra-Herran et al²³ proposed morphologic criteria which may identify uterine IMTs at higher risk of metastasis including tumor size, coagulative necrosis, mitotic activity, a predominant myxoid pattern and infiltrative borders. The one lesion showing aggressive clinical behavior in our study was significantly larger than the others, and the only tumor to have an infiltrative margin and necrosis (albeit considered equivocal as one expert pathologist favored this as hemorrhagic/apoplectic change). Interestingly, despite its size, the great majority

of the tumor had a low mitotic rate and was indistinguishable from the other IMTs which did not progress.

The histologic descriptions of other lesions in the literature which have progressed beyond the uterus vary in their detail, but many seemed to show either classic histologic features of IMT (with myxoid stroma being frequently described) or to display sufficient atypia to warrant concern about a malignant process or an initial diagnosis of leiomyosarcoma as in this case.^{12,24,25,27} This raises the intriguing possibility that IMTs which closely mimic smooth muscle tumors and lack any conventionally atypical features have a particularly low malignant potential, although we would certainly exclude IMT before considering a diagnosis of "benign metastasizing leiomyoma," particularly if there is any suggestion of myxoid change. Pragmatically and having counselled patients with IMTs either removed by myomectomy alone or partially removed by curettage we can only advise that based on current knowledge the risk of progression is low but cannot be accurately predicted in individual cases.

Notwithstanding the difficulties in predicting outcome in individual cases, perhaps the most important reason to identify patients with IMT and differentiate them from smooth muscle tumors, particularly myxoid leiomyosarcoma, is the potential for targeted therapy with specific tyrosine kinase inhibitors including crizotinib and ceritinib.¹²⁻¹⁶ Our patient 6, who had undergone a total of 6 operations for multiple recurrences and metastatic disease over a 5-year period eventually demonstrated an excellent response to crizotinib and ceritinib when the diagnosis was changed to IMT as a result of this study and unequivocally illustrates the value in distinguishing IMTs from leiomyosarcoma.

In conclusion, IMTs arising in the uterus are much more common than previously appreciated and on review account for at least 5 of 1747 (0.3%) tumors previously diagnosed as leiomyoma and 1 of 44 (2.3%) as leiomyosarcoma. Many of these tumors can be recognized prospectively if ALK IHC is performed after appreciation of the subtle and often extremely focal histologic features, including tapered nuclei, a mild inflammatory cell infiltrate and myxoid change with a "tissue culture-like" appearance. We caution that pathologists need to be particularly aware that such changes can be focal in lesions which would otherwise have the appearance of leiomyoma or leiomyosarcoma. The diagnosis of IMTs should be particularly considered in pregnancy or if the tumor is submucosal/polypoid. It is difficult to predict the natural history of individual IMTs; however, it is known that only the minority will behave aggressively. If metastasis/recurrence occurs targeted therapy with tyrosine kinase inhibition is a valid and commonly successful therapeutic option but will only be considered if the correct diagnosis is made pathologically.

REFERENCES

1. Fletcher CDM, Bridge JA, Hogendoorn PCW, et al. *World Health Organization Classification of tumours of Soft Tissue and Bone*. Lyon France: International Agency for Research on Cancer; 2013.

2. Gleason BC, Hornick JL. Inflammatory myofibroblastic tumours: where are we now? *J Clin Pathol*. 2008;61:428–437.
3. Hornick JL, Scholl LM, Dal Cin P, et al. Expression of ROS1 predicts ROS1 gene rearrangement in inflammatory myofibroblastic tumors. *Mod Pathol*. 2015;28:732–739.
4. Coffin CM, Patel A, Perkins S, et al. ALK1 and p80 expression and chromosomal rearrangements involving 2p23 in inflammatory myofibroblastic tumor. *Mod Pathol*. 2001;14:569–576.
5. Antonescu CR, Suurmeijer AL, Zhang L, et al. Molecular characterization of inflammatory myofibroblastic tumors with frequent ALK and ROS1 gene fusions and rare novel RET rearrangement. *Am J Surg Pathol*. 2015;39:957–967.
6. Haimes JD, Stewart CJR, Kudlow BA, et al. Uterine inflammatory myofibroblastic tumors frequently harbour ALK fusions with IGFBP5 and THBS1. *Am J Surg Pathol*. 2017;41:773–780.
7. Alassiri AH, Ali RH, Shen Y, et al. ETV6-NTRK3 is expressed in a subset of ALK-negative inflammatory myofibroblastic tumors. *Am J Surg Pathol*. 2016;40:1051–1061.
8. Bridge JA, Kanamori M, Ma Z, et al. Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor. *Am J Pathol*. 2001;159:411–415.
9. Lawrence B, Perez-Atayde A, Hibbard MK, et al. TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. *Am J Pathol*. 2000;157:377–384.
10. Ma Z, Hill DA, Collins MH, et al. Fusion of ALK to the Ran-binding protein 2 (RANBP2) gene in inflammatory myofibroblastic tumor. *Genes Chromosomes Cancer*. 2003;37:98–105.
11. Lovly CM, Gupta A, Lipson D, et al. Inflammatory myofibroblastic tumors harbor multiple potentially actionable kinase fusions. *Cancer Discov*. 2014;4:889–895.
12. Ak G, Jaaback K, Yin H, et al. Malignant inflammatory myofibroblastic tumour of the uterus. *Pathology*. 2015;47:380–381.
13. Butrynski JE, D'Adamo DR, Hornick JL, et al. Crizotinib in ALK-rearranged inflammatory myofibroblastic tumor. *N Engl J Med*. 2010;363:1727–1733.
14. Mosse YP, Lim MS, Voss SD, et al. Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study. *Lancet Oncol*. 2013;14:472–480.
15. Mansfield AS, Murphy SJ, Harris FR, et al. Chromoplectic TPM3-ALK rearrangement in a patient with inflammatory myofibroblastic tumor who responded to ceritinib after progression on crizotinib. *Ann Oncol*. 2016;27:2111–2117.
16. Gaudichon J, Jeanne-Pasquier C, Deparis MJ, et al. Complete and repeated response of a metastatic ALK-rearranged inflammatory myofibroblastic tumor to crizotinib in a teenage girl. *Pediatr Hematol Oncol*. 2016;38:308–311.
17. Yamamoto H, Yoshida A, Taguchi K, et al. ALK, ROS1 and NTRK3 gene rearrangements in inflammatory myofibroblastic tumours. *Histopathology*. 2016;69:72–83.
18. Houang M, Toon CW, Clarkson A, et al. Reflex ALK immunohistochemistry is feasible and highly specific for ALK gene rearrangements in lung cancer. *Pathology*. 2014;46:383–388.
19. Rabban JT, Zaloudek CJ, Shekitka KM, et al. Inflammatory myofibroblastic tumor of the uterus: a clinicopathologic study of 6 cases emphasizing distinction from aggressive mesenchymal tumors. *Am J Surg Pathol*. 2005;29:1348–1355.
20. Shintaku M, Fukushima A. Inflammatory myofibroblastic tumor of the uterus with prominent myxoid change. *Pathol Int*. 2006;56:625–628.
21. Azuno Y, Yaga K, Suehiro Y, et al. Inflammatory myoblastic tumor of the uterus and interleukin-6. *Am J Obstet Gynecol*. 2003;189:890–891.
22. Olgan S, Saatli B, Okyay RE, et al. Hysteroscopic excision of inflammatory myofibroblastic tumor of the uterus: a case report and brief review. *Eur J Obstet Gynecol Reprod Biol*. 2011;157:234–236.
23. Parra-Herran C, Quick CM, Howitt BE, et al. Inflammatory myofibroblastic tumor of the uterus: clinical and pathologic review of 10 cases including a subset with aggressive clinical course. *Am J Surg Pathol*. 2015;39:157–168.
24. Kushnir CL, Gerardi M, Banet N, et al. Extrauterine inflammatory myofibroblastic tumor: a case report. *Gynecol Oncol Case Rep*. 2013;6:39–41.
25. Fraggetta F, Doglioni C, Scollo P, et al. Uterine inflammatory myofibroblastic tumor in a 10-year-old girl presenting as polypoid mass. *J Clin Oncol*. 2015;33:e7–e10.
26. Subbiah V, McMahon C, Patel S, et al. STUMP un“stumped”: anti-tumor response to anaplastic lymphoma kinase (ALK) inhibitor based targeted therapy in uterine inflammatory myofibroblastic tumor with myxoid features harboring DCTN1-ALK fusion. *J Hematol Oncol*. 2015;8:66.
27. Parra-Herran C, Schoolmeester JK, Yuan L, et al. Myxoid leiomyosarcoma of the uterus: a clinicopathologic analysis of 30 cases and review of the literature with reappraisal of its distinction from other uterine myxoid mesenchymal neoplasms. *Am J Surg Pathol*. 2016;40:285–301.
28. Harrison WJ, Andrici J, Maclean F, et al. Fumarate hydratase-deficient uterine leiomyomas occur in both the syndromic and sporadic settings. *Am J Surg Pathol*. 2016;40:599–607.
29. Schaefer I-M, Hornick JL, Sholl LM, et al. Abnormal p53 and p16 staining patterns distinguish uterine leiomyosarcoma from inflammatory myofibroblastic tumour. *Histopathology*. 2017;70:1138–1146.
30. Cramer SF, Patel A. The frequency of uterine leiomyomas. *Am J Clin Pathol*. 1990;94:435–438.
31. Paik JH, Choe G, Kim H, et al. Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. *J Thorac Oncol*. 2011;6:466–472.
32. Chou A, Fraser S, Toon CW, et al. A detailed clinicopathological study of alk translocated papillary thyroid carcinoma. *Am J Surg Pathol*. 2015;39:652–659.
33. Conklin CM, Craddock KJ, Have C, et al. Immunohistochemistry is a reliable screening tool for identification of ALK rearrangement in non-small-cell lung carcinoma and is antibody dependent. *J Thorac Oncol*. 2013;8:45–51.
34. Selinger CI, Rogers TM, Russell PA, et al. Testing for ALK rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. *Mod Pathol*. 2013;26:1545–1553.
35. Marino-Enriquez A, Wang WL, Roy A, et al. Epithelioid inflammatory myofibroblastic sarcoma: an aggressive intra-abdominal variant of inflammatory myofibroblastic tumor with nuclear membrane or perinuclear ALK. *Am J Surg Pathol*. 2011;35:135–144.
36. Cessna MH, Zhou H, Sanger WG, et al. Expression of ALK1 and p80 in inflammatory myofibroblastic tumor and its mesenchymal mimics: a study of 135 cases. *Mod Pathol*. 2002;15:931–938.
37. Li X-Q, Hisaoka M, Shi D-R, et al. Expression of anaplastic lymphoma kinase in soft tissue tumors: an immunohistochemical and molecular study of 249 cases. *Hum Pathol*. 2004;35:711–721.