ALK IHC and molecular analysis in the Diagnosis of uterine Inflammatory Myofibroblastic Tumor

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USCAP 109TH ANNUAL MEETING
2020 EYES ON YOU

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Carlos Parra-Herran reported no relevant financial relationships

PLEASE TURN OFF YOUR CELL PHONES

INFLAMMATORY MYOFIBROBLASTIC TUMOR

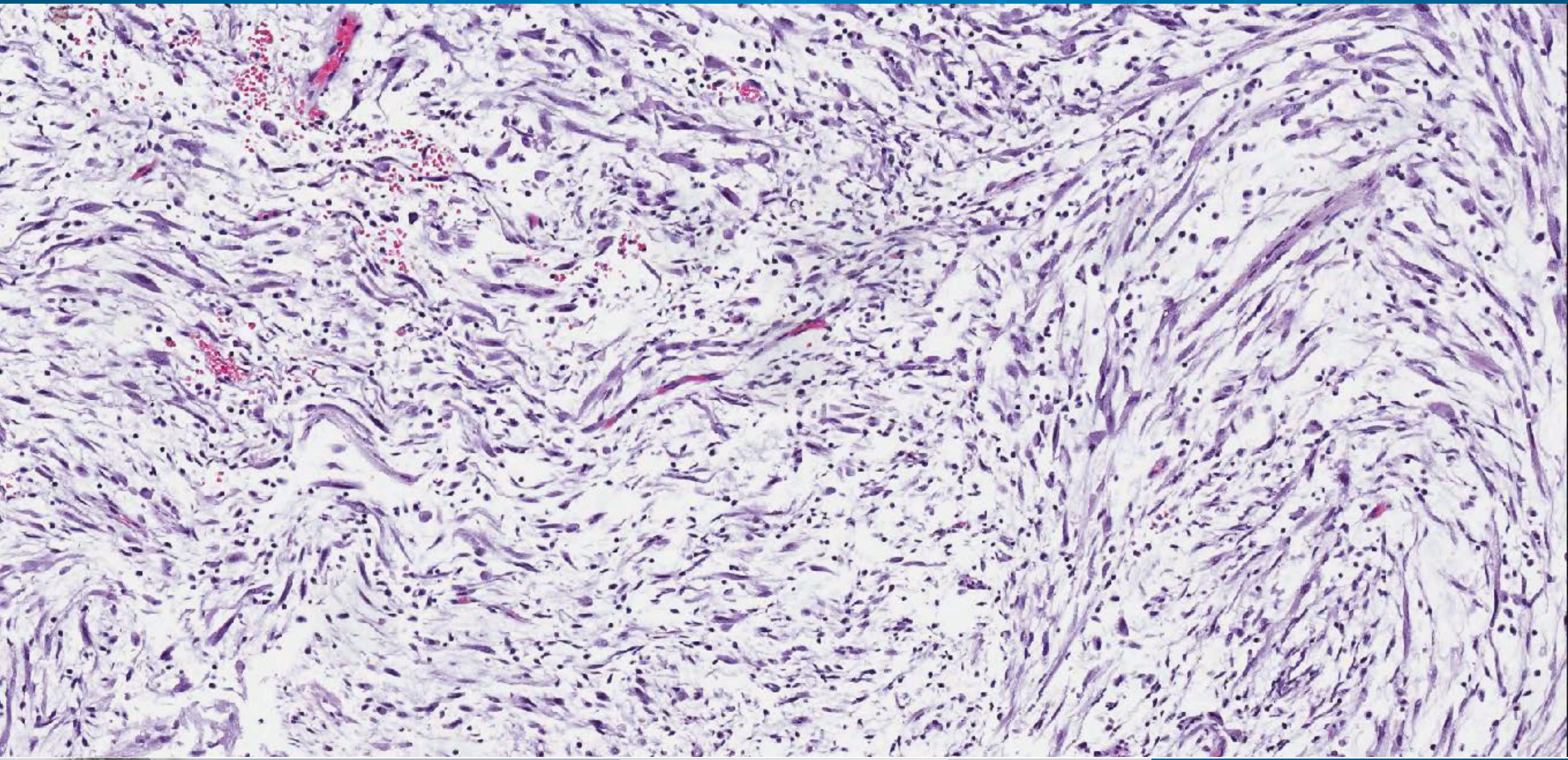
- Rare in the gynecologic tract
  - Most cases occur in the uterus; rarely cervix, ovary
- Young adults
- Association with pregnancy
- Benign clinical course in most cases
- Aggressive behavior has been documented
  - Metastases, local recurrence, death of disease

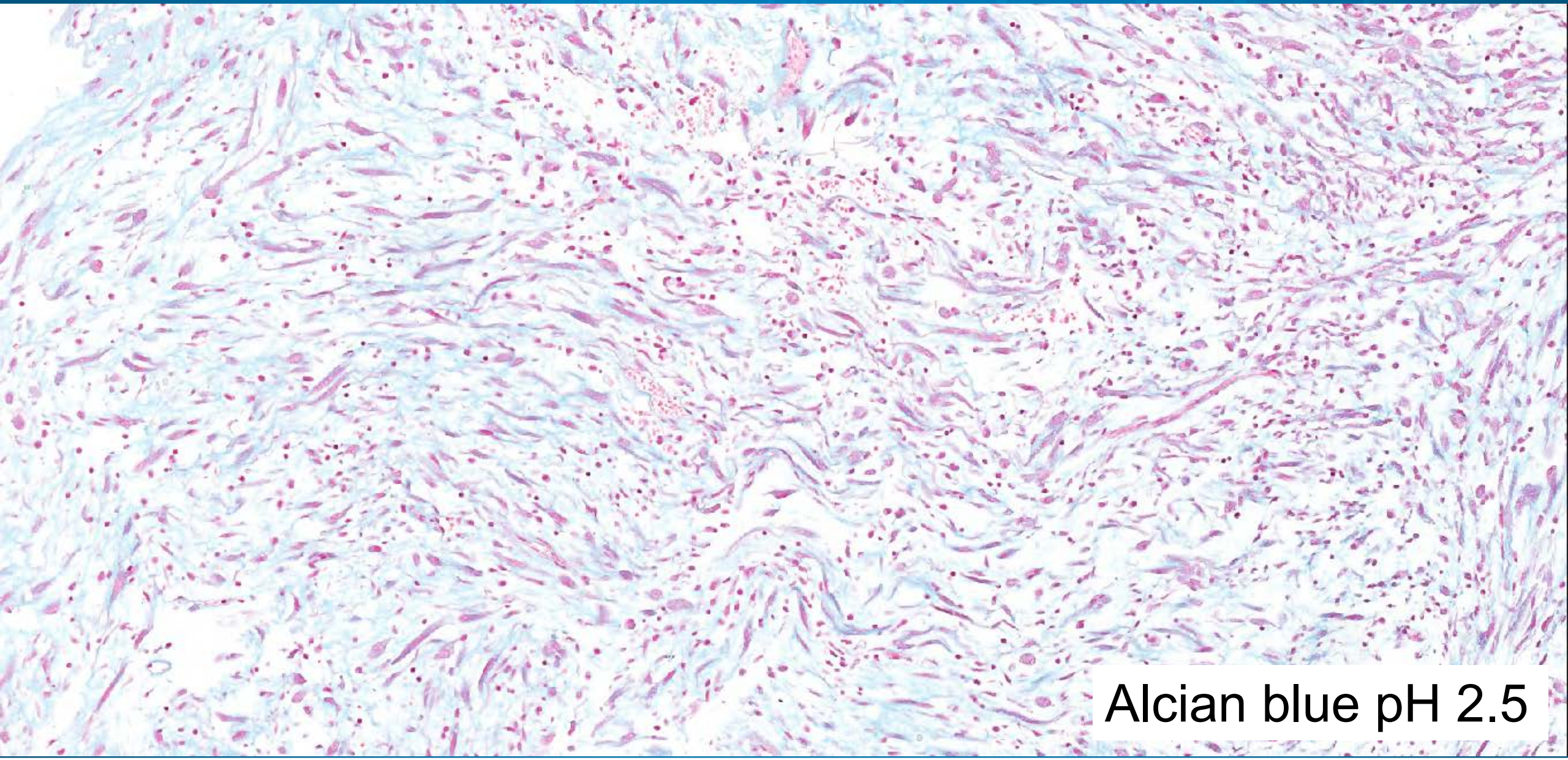
Am J Surg Pathol 2005;29:1348-55
Am J Surg Pathol 2015 39:157-68
Am J Surg Pathol 2017;41:1433-42

INFLAMMATORY MYOFIBROBLASTIC TUMOR - ARCHITECTURE

- Myxoid
  - Spindle cells individually dispersed in an abundant myxoid matrix (tissue culture / fasciitis – like)
- Fascicular / compact
  - Smooth muscle-like appearance
- Hyalinized collagenous pattern

Am J Surg Pathol 2005;29:1348-55
Am J Surg Pathol 2015 39:157-68

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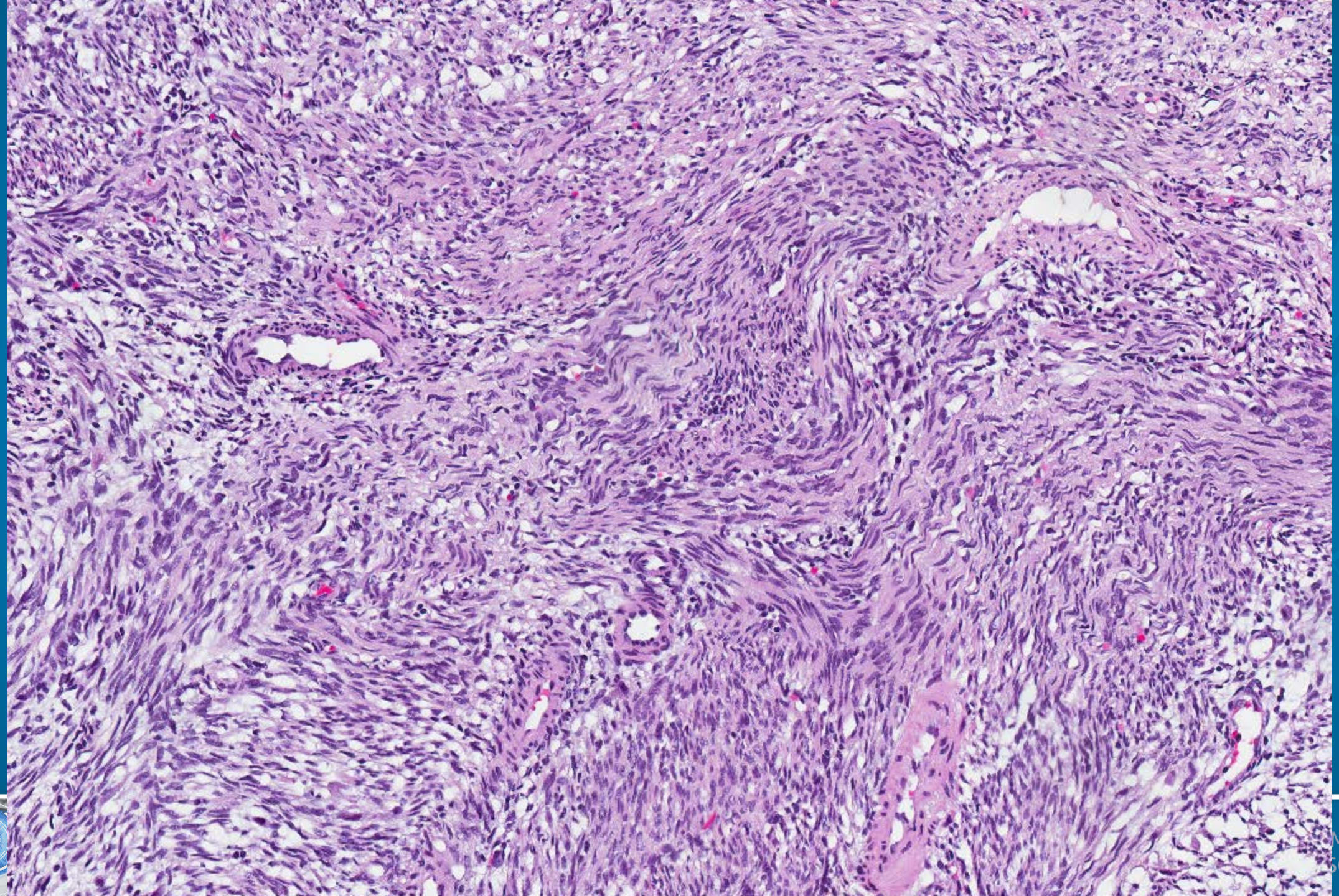
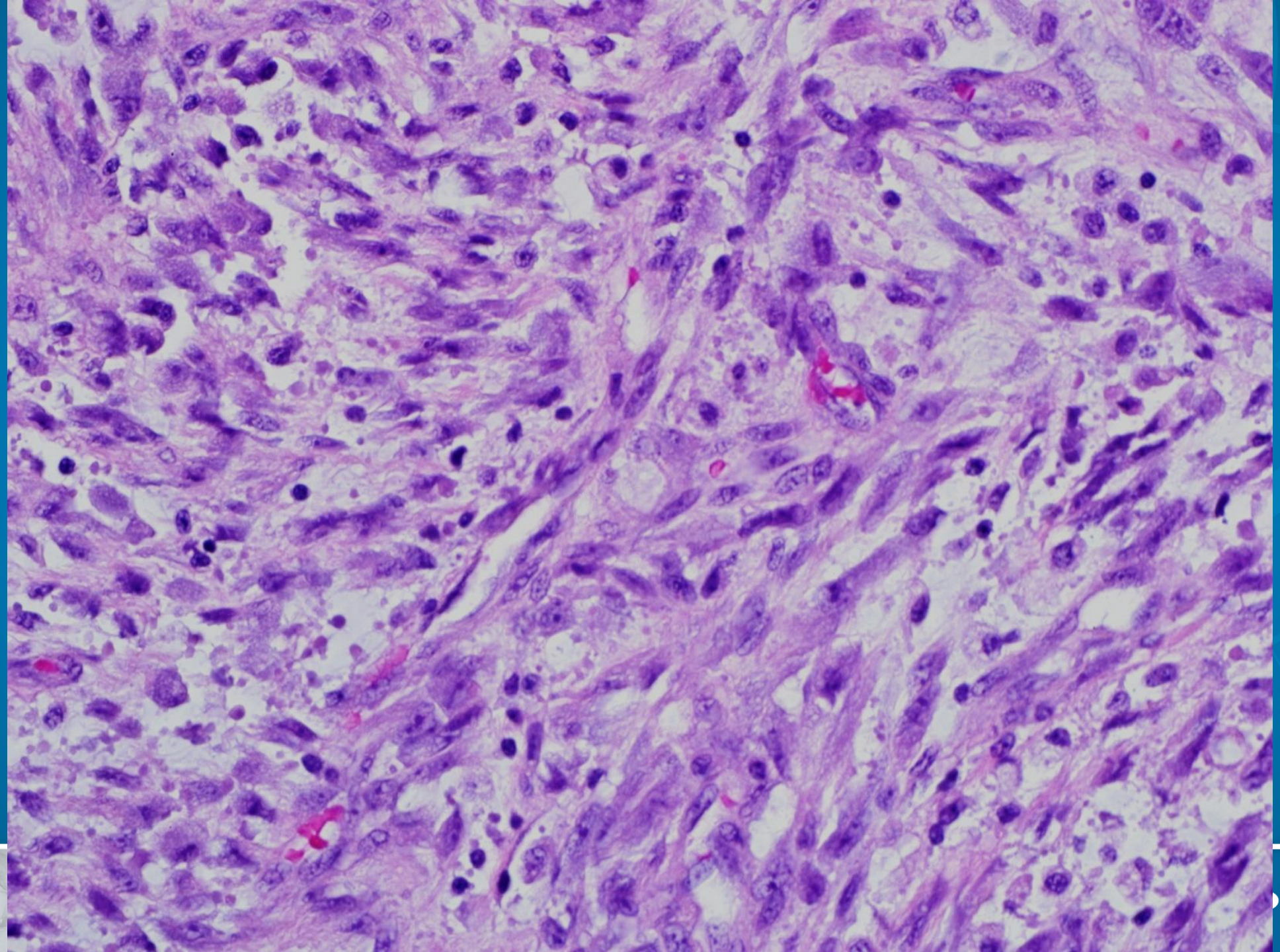
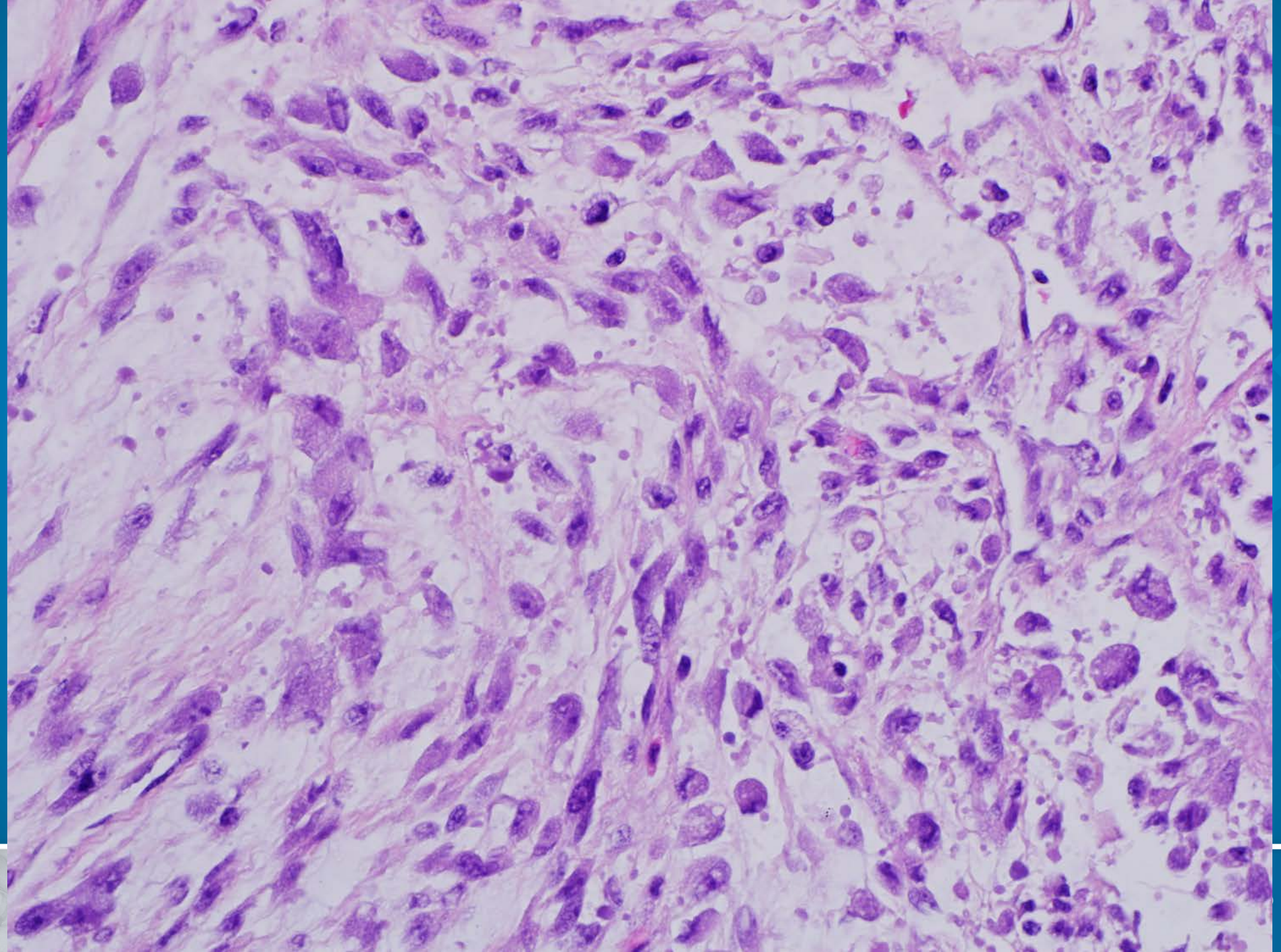


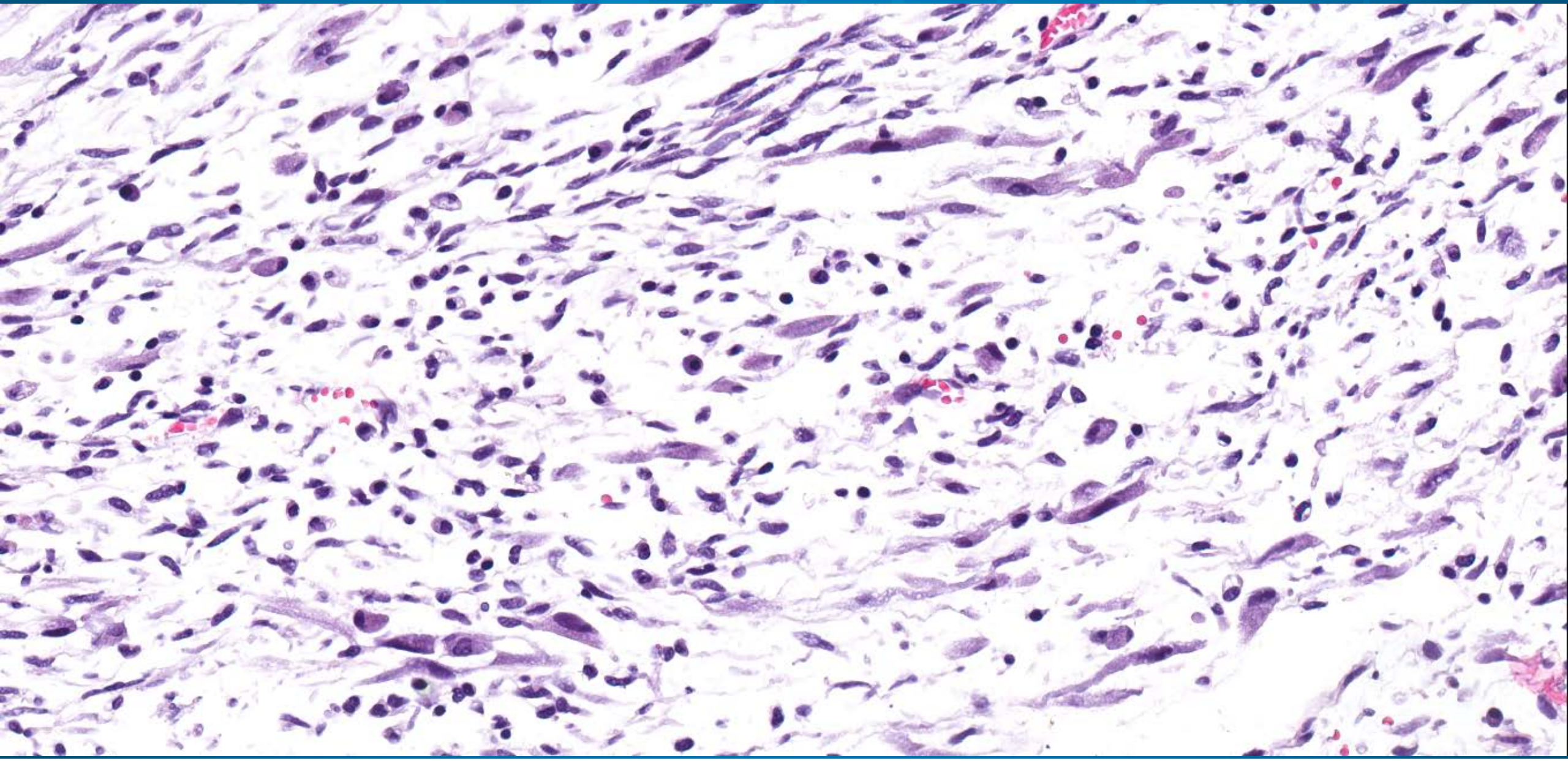
INFLAMMATORY MYOFIBROBLASTIC TUMOR - CYTOMORPHOLOGY

- Myofibroblastic cell population
  - Spindle with wispy cytoplasm and elongated nuclei
  - Vesicular, evenly dispersed chromatin
  - Ganglion-like, epithelioid, giant cell components can occur
- Inflammatory cell component
  - Typically lympho-plasmacytic
  - Eosinophils, neutrophils, mast cells less often

Am J Surg Pathol 2005;29:1348-55
Am J Surg Pathol 2015 39:157-68







DIFFERENTIAL DIAGNOSIS

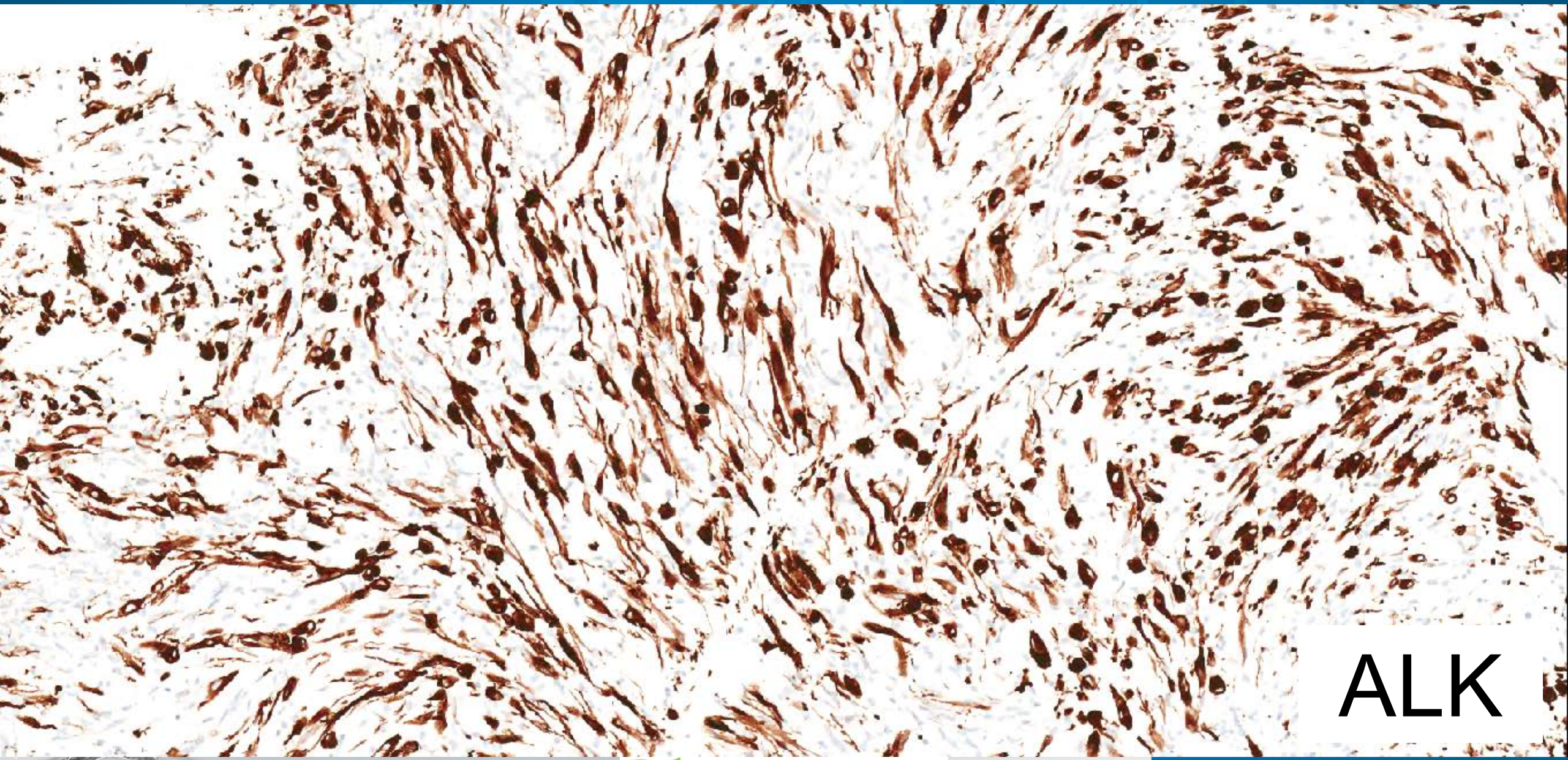
- **Smooth muscle neoplasia**
  - Leiomyoma (if predominantly fascicular)
  - Myxoid leiomyosarcoma (if predominantly myxoid)
  - Smooth muscle tumor of unknown malignant potential (STUMP)
    - IMTs mimicking STUMP usually has myxoid features
- **Endometrial stromal neoplasia**
  - Fibromyxoid low-grade endometrial stromal tumors (ESN, LG-ESS)
  - BCOR-altered high-grade uterine sarcomas

Am J Surg Pathol. 2019;43:64-74
Adv Anat Pathol 2017;24:354-61
J Hematol Oncol 2015;8:66
Virchows Arch 2018;473:583-90

INFLAMMATORY MYOFIBROBLASTIC TUMOR - IMMUNOHISTOCHEMISTRY

- Negative for S100, keratins, CD34, CD117, ROS
- Normal p53 and p16 expression
- Smooth muscle markers and CD10 are frequently expressed
  - Fascicular / compact areas +++
- ALK positivity in ~95% of uterine IMTs reported

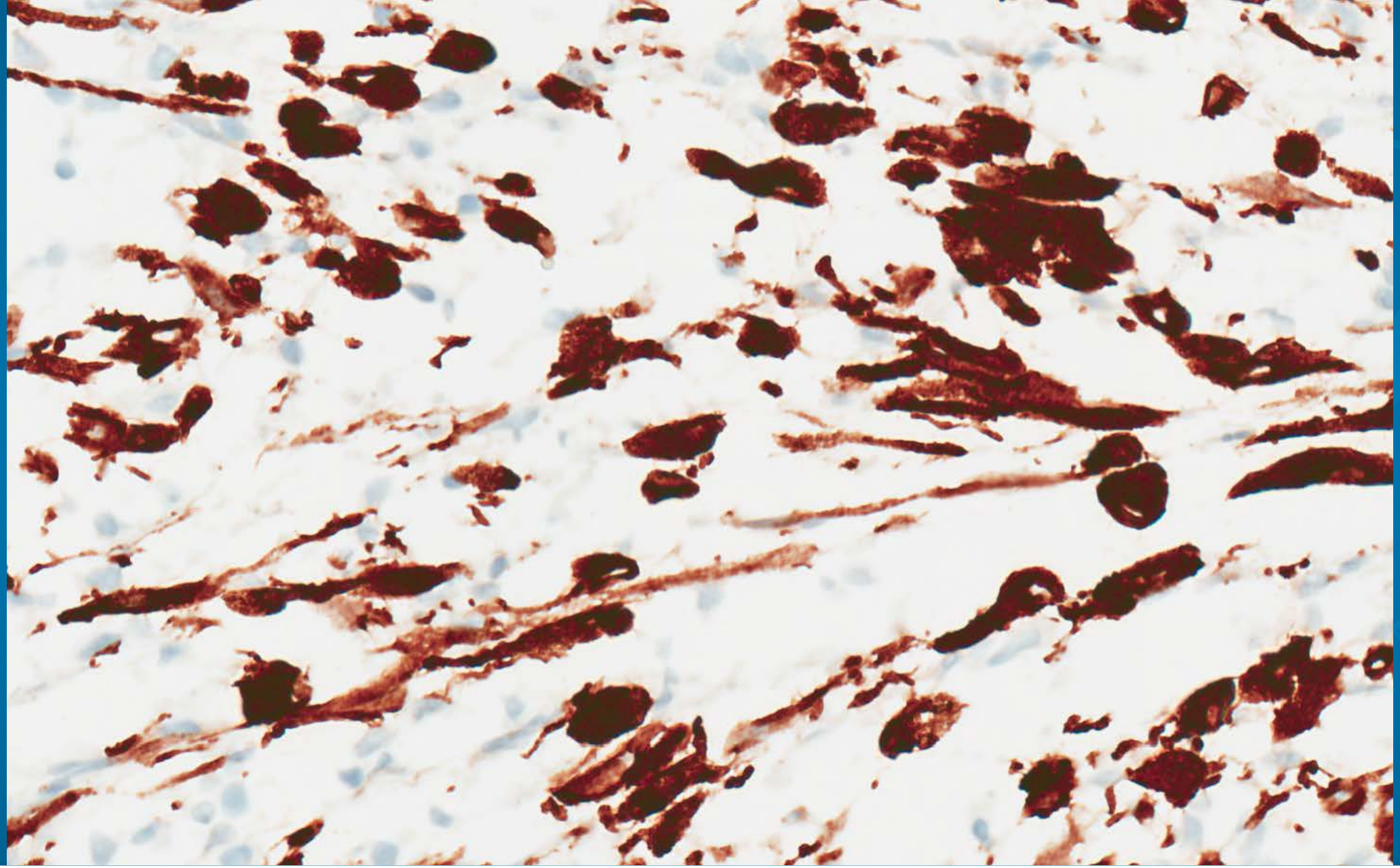
Mod Pathol. 2017;30:1489-1503
Am J Surg Pathol. 2015;39:157-68
Histopathol 2017;70:1138-46
Am J Surg Pathol 2017;41:773-80

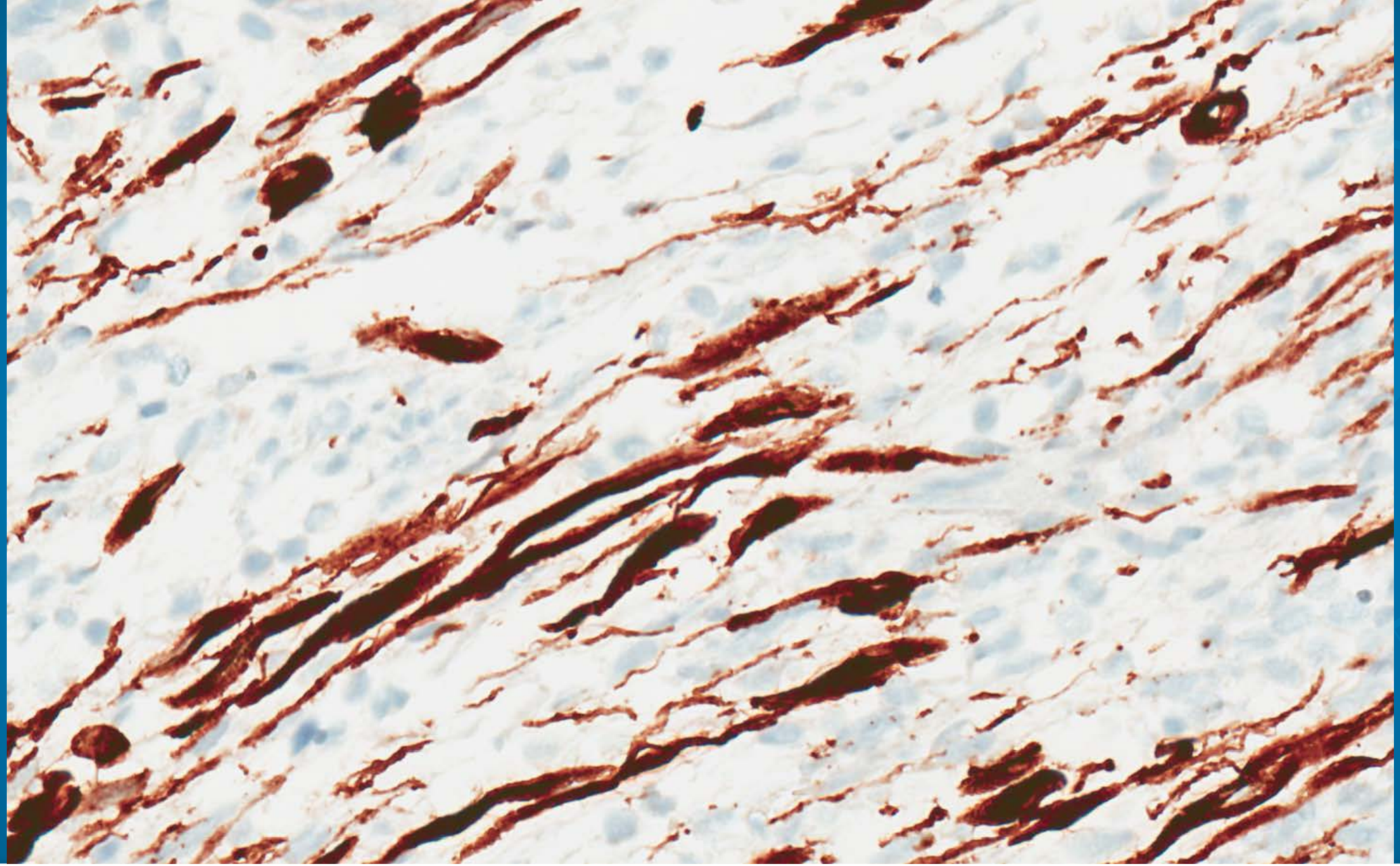

ALK

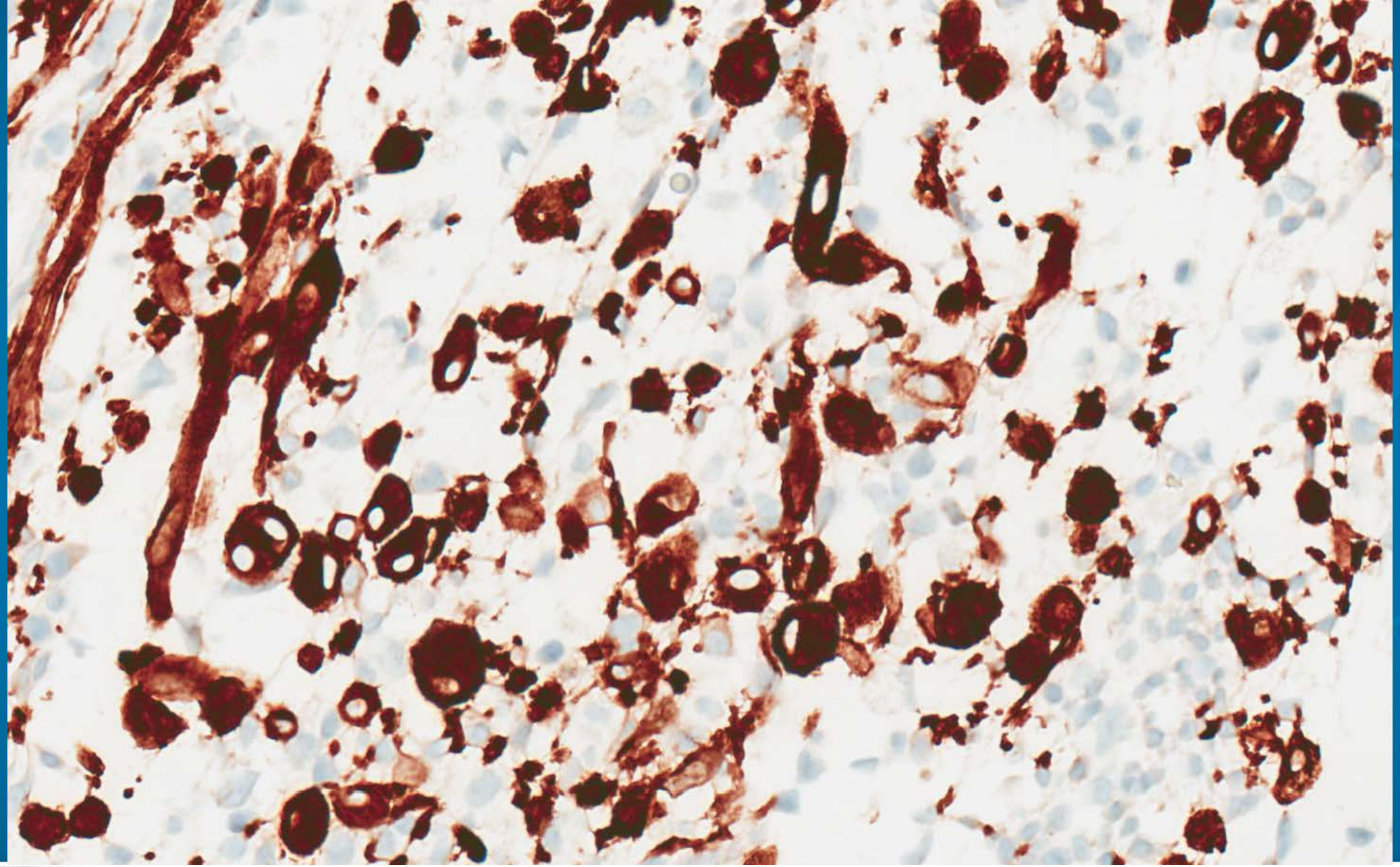
IMMUNOHISTOCHEMISTRY

- Cytoplasmic, membranous and/or nuclear staining
  - IMT Diffuse granular cytoplasmic is the most frequent pattern
    Perinuclear or membranous also observed
  - ALCL Strong nuclear and cytoplasmic
  - *EML-ALK* lung adenocarcinoma: At least 1+/2+ diffuse granular cytoplasmic
  - Merkel cell carcinoma: At least 1+/2+ diffuse granular cytoplasmic
- Reporting
  - Positive: any staining (1+, 2+ and/or 3+)
  - Negative: absent staining

https://www.nordiqc.org/epitope.php?id=14
NordiQC Assessment Run 57 (2019): ALK
Accessed January 9th, 2020







REFERENCE (PMID)	ALK Ab	DILUTION	ALK+ IMTs	ALK+ non-IMTs
Azuno (14526339)	**ALK1**	1:50	1/1 (100%)	NA
Rabban (16160478)	**ALK1**	1:100	5/5 (100%)	NA
Shintaku (16984620)	**ALK1**	1:50	1/1 (100%)	NA
Fuehrer (22646268)	**ALK1**	1:100	6/7 (86%)	NA
Kushnir (24371717)	**ALK1**	Not specified	0/1 (0%)	NA
Subbiah (26062823)	**ALK1**	Not specified	1/1 (100%)	NA
Mandato (29310405)	**ALK1**	Not specified	1/1 (100%)	NA
Haimes (28490045)	**ALK1**	1:50	11/11 (100%)	NA
Takahashi (29900760)	**ALK1**	Not specified	0/1 (0%)	NA
Mandato (29310405)	**ALK1**	Prediluted	1/1 (100%)	NA
Ptáková (30116888)	**ALK1**	Ready to use	1/1 (100%)*	22/22 (100%)*
	TOTAL		27/30 (91%)	22/22 (100%)

ALK1 clone (n=52)

Sensitivity = 91%

Specificity = 100%

REFERENCE (PMID)	ALK Ab	DILUTION	ALK+ IMTs	ALK- non-IMTs
Bennett (28664932)	**5A4**	Not specified	12/13 (92%)	NA
Pickett (28731868)	**5A4**	1:10	6/6 (100%)*	1304/1304 (100%)*
Mohammad (30015720)	**5A4**	1:50	3/3 (100%)*	260/260 (100%)*
Devereaux (29794871)	**5A4**	1:25	6/6 (100%)*	37/37 (100%)*
	TOTAL		27/28 (96%)	1601/1601 (100%)

REFERENCE (PMID)	ALK Ab	DILUTION	ALK+ IMTs	ALK+ non-IMTs
Parra-Herran (25321329)	**D5F3**	1:300	10/10 (100%)	NA
Parra-Herran (26866354)	**D5F3**	1:300	4/4 (100%)*	19/22 (89%)*
Zarei (30741845)	**D5F3**	Not specified	1/1 (100%)	NA
Ptáková (30116888)	**D5F3**	1:50	1/1 (100%)*	22/22 (100%)*
	TOTAL		16/16 (100%)	41/44 (93%)

5A4 clone (n=1629)

Sensitivity = 96%

Specificity = 100%

D5F3 clone (n=60)

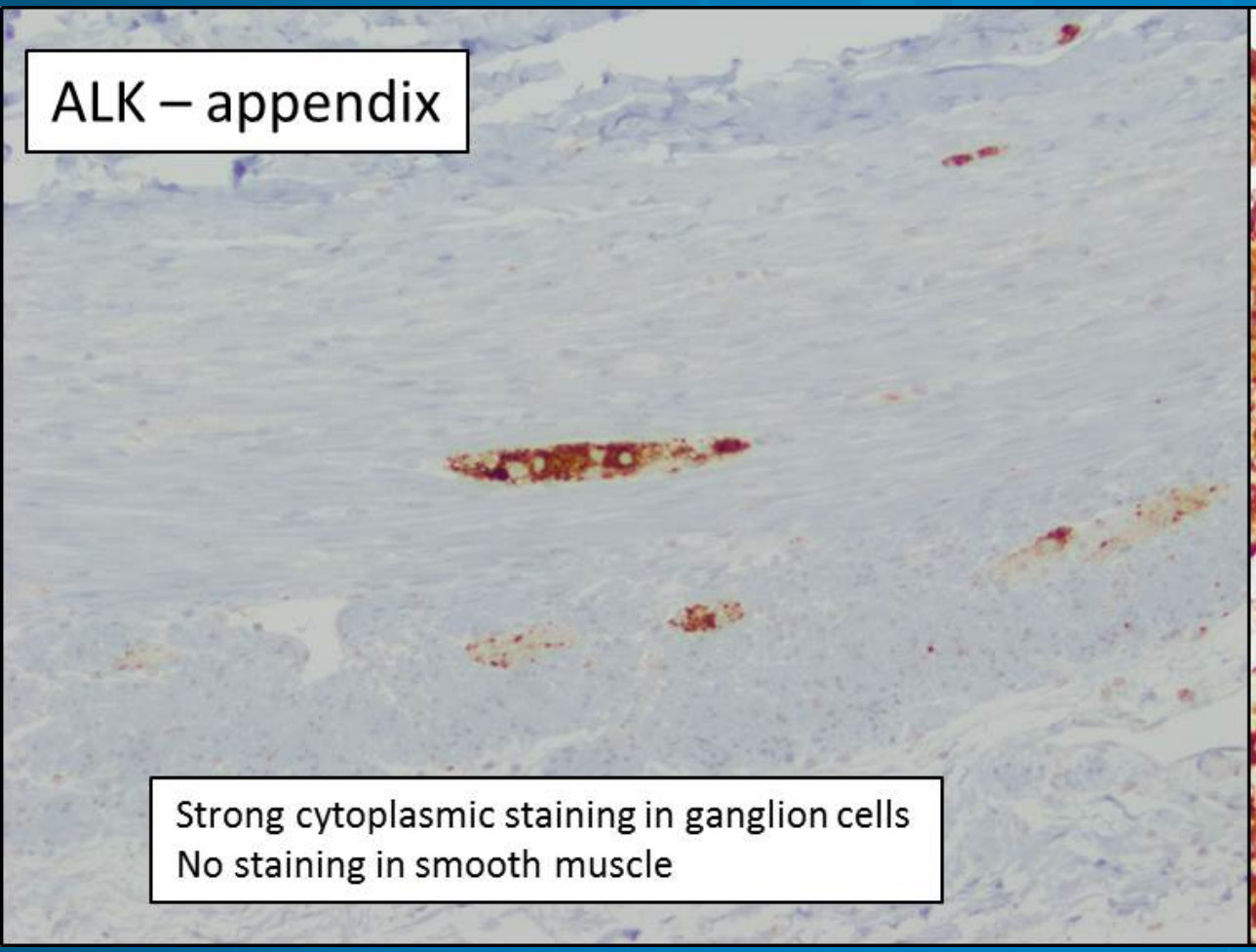
Sensitivity = 100%

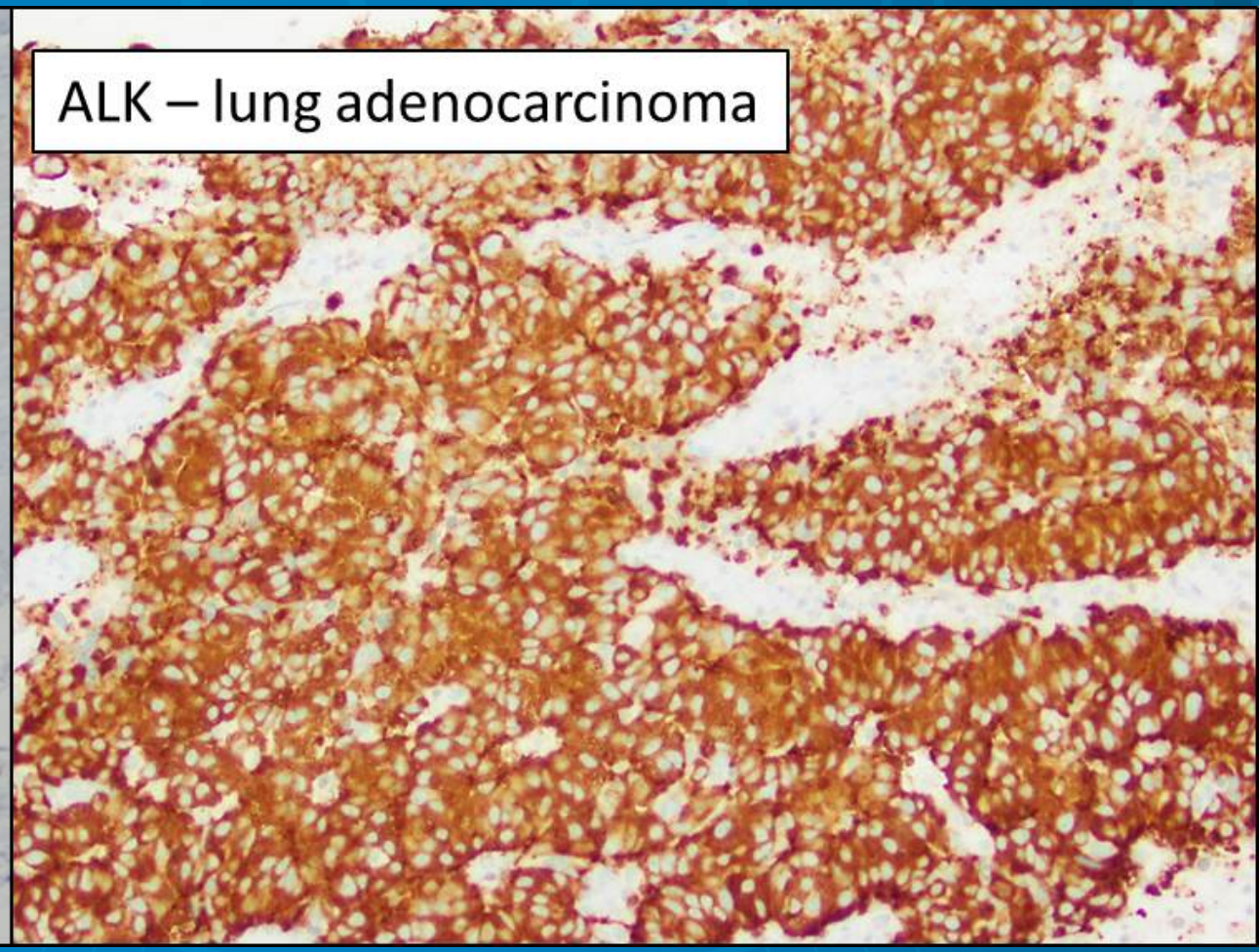
Specificity = 93%

ALK IHC TESTING

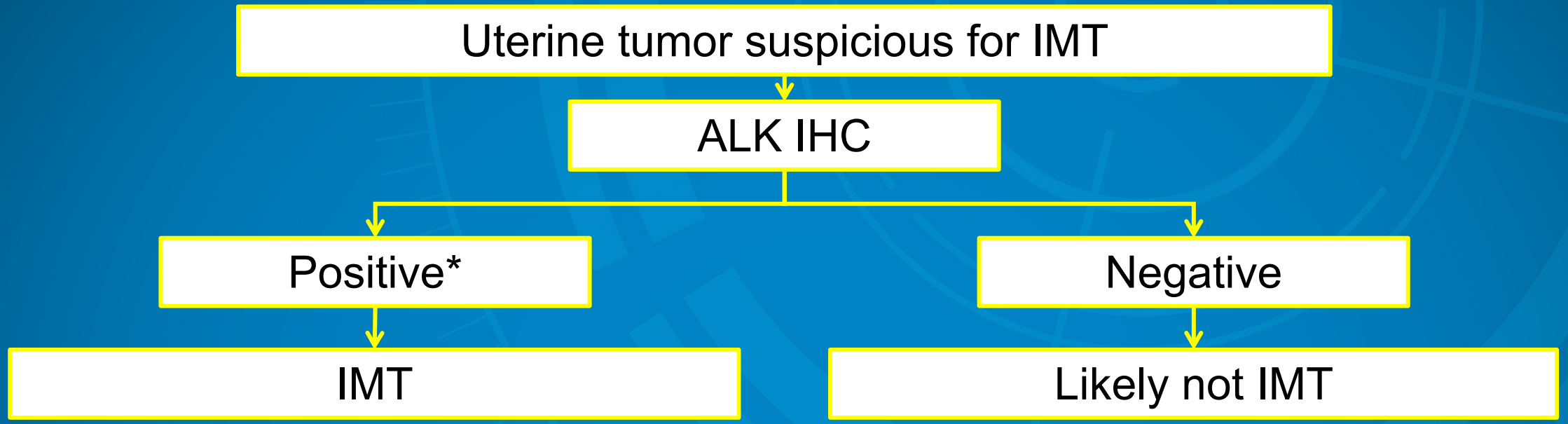
- Positive controls
  - Appendix (1+ to 2+ ganglion and myenteric plexus staining)
  - ALK-positive lung adenocarcinoma (confirmed by FISH)
- Negative control
  - Appendix (absent staining in epithelium and smooth muscle)
  - ALK-negative lung adenocarcinoma (confirmed by FISH)

https://www.nordiqc.org/epitope.php?id=14
NordiQC Assessment Run 57 (2019): ALK
Accessed January 9th, 2020





Courtesy of Dr. Elzbieta Slodkowska
(Sunnybrook Health Sciences Centre, Toronto ON Canada)



* Potential pitfall: Angiomatoid fibrous histiocytoma can be ALK positive

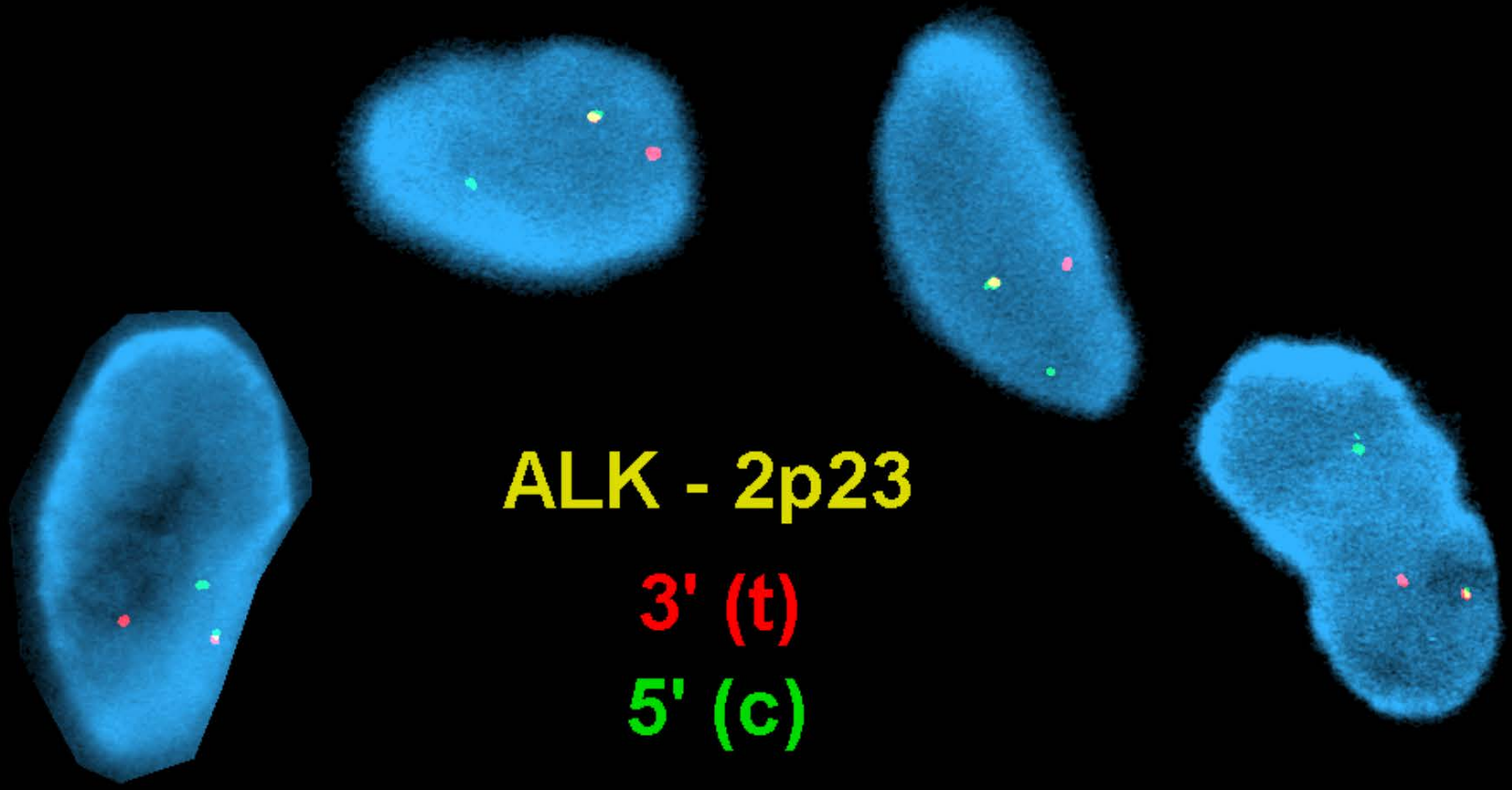
Am J Surg Pathol 2019;43:93-101

ALK REARRANGEMENTS

- *ALK* gene (2p23)
- Common *ALK* fusion partners in the uterus
  - *IGFBP5*, *THBS1, FN1, DES, TIMP3, DCTN1, SEC31, TPM3, PPP1CB*
- Fluorescence in situ hybridization (FISH)
  - Break apart probe
  - Rearrangement with split 3’ (telomeric) and 5’ (centromeric) signals
  - Rarely, isolated 3’ signals or 5’ (non-functional) signals

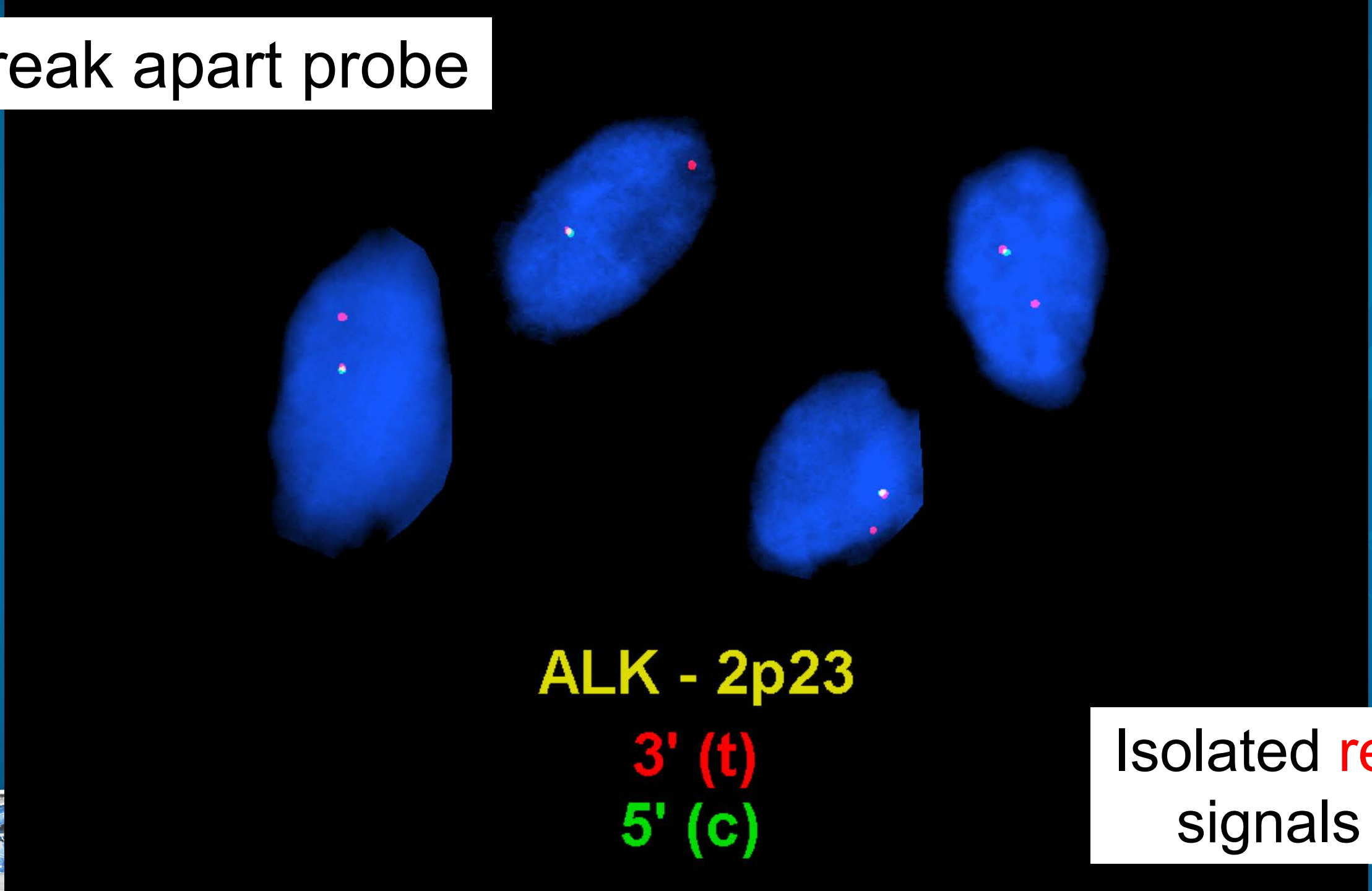
Arch Pathol Lab Med 2012;136:623-6
Am J Surg Pathol 2017;41:773-80
Am J Surg Pathol 2019;43:64-74
Int J Gynecol Pathol 2019 PMID 30741845

Break apart probe



Isolated red and green signals

Break apart probe



Isolated red signals

ALK REARRANGEMENTS

- FISH: ~75% sensitivity
- FISH-negative cases
  - Intrachromosomal inversions
  - *IGFBP5* and *FN1* are also located in chromosome 2 (2q35)
- Targeted RNA sequencing
  - Primers for specific *ALK* exons
  - Most fusions involve start of exons 17, 18 or 19
    - Kinase domain is encoded in exons 22-25
    - Transmembrane domain is encoded in exon 19

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Mod Pathol 2017;30:1489-1503
Am J Surg Pathol 2017;41:773-80

TABLE 2. Summary of RNA-Sequencing Analysis

Case	Diagnosis	Cq	Fusion Transcripts
1	IMT	27.8	*IGFBP5 (exon 1)—ALK (exon 19)*
2	IMT	21.9	*TIMP3 (exon 1)—ALK (exon 19)*/TIMP3 (exon1)—ALK (exon 20)*
3	IMT	24.3	*IGFBP5 (exon 1)—ALK (exon 19)*
4	IMT	32.4	*THBS1 (exon 4)—ALK (exon 19)*
5	IMT	23.4	*FN1 (exon/intron 15)—ALK (exon 18)*/FN1 (exon 15)—ALK(exon 19)*
6	IMT	23.7	*FN1 (exon 27)—ALK (exon 18)*/FN1 (exon 27)—ALK (exon 17)/FN1 (exon 27)—ALK (exon 19)*
7	IMT	23.7	*THBS1 (exon 4)—ALK (exon 19)*
8	IMT	25.4	*THBS1 (exon 4)—ALK (exon 19)*
9	IMT	24	*IGFBP5 (exon 1)—ALK (exon 19)*
10	IMT	29.8	None identified
11	IMT	25.8	None identified
12	IMT/m-LM†	24.5	*IGFBP5 (exon 1)—ALK (exon 19)*/IGFBP5 (exon 1)—ALK (exon 20)*

*Predominantly expressed isoform.
†IMT that was initially diagnosed as myxoid leiomyoma because of negative FISH finding.
m-LM indicates myxoid leiomyoma.

Am J Surg Pathol 2017;41:773-80

Table 4 Immunohistochemical and molecular findings of uterine inflammatory myofibroblastic tumors

Case	*ALK*	*CD10*	*Desmin*	*Caldesmon*	*FISH*	*Anchored multiplex assay*
1	3+	2+	3+	3+	Rearranged	IGFBP5-ALK
2	1+	3+	2+	2+	Rearranged	Negative (suboptimal)
3	3+	2+	2+	0	Failed	TIMP3-ALK
4	0	3+	0	0	Rearranged	DES-ALK (3 reads only)
5	3+	2+	3+	0	Rearranged	Negative
6	3+	3+	2+	1+	Abnormal 'Green only' signal	Negative
7	3+	2+	3+	0	Rearranged	IGFBP5-ALK
8	3+	3+	1+	1+	Not rearranged	SEC31-ALK
9	3+	0	2+	0	NP	TPM3-ALK
10	3+	NP	NP	NP	Rearranged	THBS1-ALK
11	3+	3+	NP	3+	Rearranged	THBS1-ALK
12	3+	1+	3+	0	NP	DES-ALK
13	2+	3+	3+	0	Rearranged	THBS1-ALK

Abbreviations: FISH, fluorescence *in situ* hybridization; NP, not performed; 3+, diffuse; 2+, multifocal; 1+, rare; 0, negative.

Mod Pathol 2017;30:1489-1503

ALK REARRANGEMENTS

- RNA-seq: 77-90% sensitivity
- RNA-seq false negative cases
  - Suboptimal tumor RNA quality
  - Primers not covered by assay
- *ROS1, PDGFRB* and *RET* fusions not yet reported in uterus
- One uterine IMT with *ETV6-NTRK3* fusion reported
  - Previously described in a subset of ALK-negative IMTs

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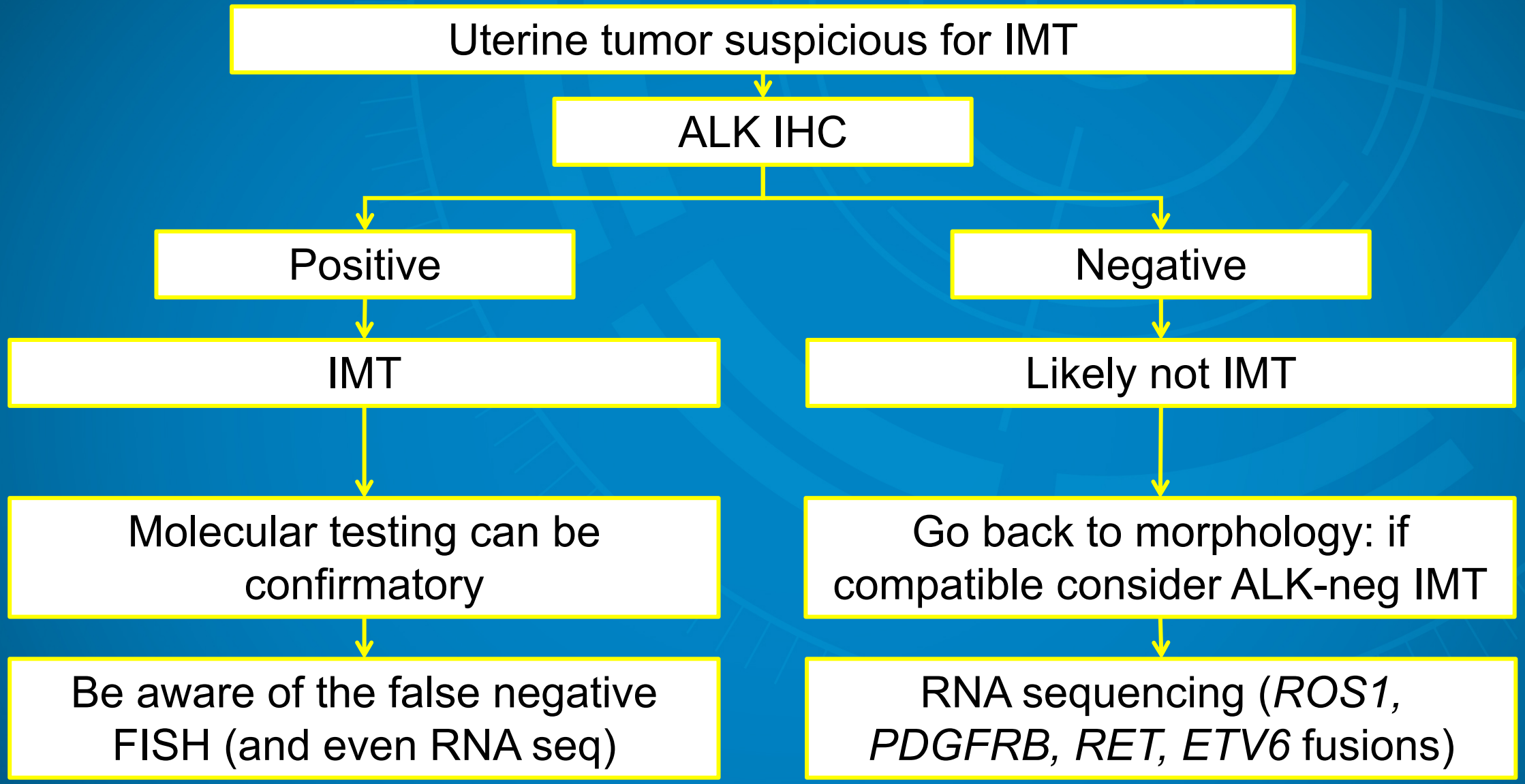
Mod Pathol 2017;30:1489-1503
Am J Surg Pathol 2017;41:773-80
J Int Med Res 2018;46:3498-3503
Am J Surg Pathol 2016;40:1051-61

ALK-NEGATIVE UTERINE IMT EXISTS

- It is very rare
- Likely to be misdiagnosed as leiomyoma or myxoid leiomyosarcoma – IHC overlap
- Molecular analysis more likely to help
  - Compatible morphology


Uterine tumor suspicious for IMT
ALK IHC
Positive
Negative
IMT
Likely not IMT
Molecular testing can be confirmatory
Go back to morphology: if compatible consider ALK-neg IMT
Be aware of the false negative FISH (and even RNA seq)
RNA sequencing (*ROS1, PDGFRB, RET, ETV6* fusions)

TARGETED THERAPIES FOR IMT

- Crizotinib
  - First-generation, ATP-competitive inhibitor of *ALK* and *MET* tyrosine kinases
  - Clinical activity in NSCLC with *EML4-ALK* rearrangements
  - Emerging experience with IMT

N Engl J Med 2010;363:1727
N Engl J Med 2010;363:1693
J Natl Compr Canc Netw 2018;16:115
J Oncol Pharm Pract 2019 PMID 31615346
J Clin Oncol 2017;35:3215

TARGETED THERAPIES FOR IMT

- Scant experience with gynecologic IMT
  - One patient with morcellated myometrial lesion
  - Pulmonary metastases found on staging work-up
  - Peritoneal recurrence 1 year later
  - Initial response with antihormonal Tx (letrazole)
  - Multiple abdominal and pelvic recurrences over a 5 year period
  - Dramatic partial response to Crizotinib and subsequent Ceritinib

Am J Surg Pathol 2017;41:1433-42

TARGETED THERAPIES FOR IMT

- Response in >50% of patients
  - Tumor regression seen as early as 1-2 months
  - Partial response or complete remission at follow-up (range 10-36 months)
  - Usually with combination of debulking, radiation and/or chemotherapy
  - Better response in abdominal vs thoracic IMTs
- Poor initial response -> adverse outcome
- Second-generation *ALK* inhibitors (ceritinib, alectinib)

N Engl J Med 2010;363:1727
J Natl Compr Canc Netw 2018;16:115
J Oncol Pharm Pract 2019 PMID 31615346
J Clin Oncol 2017;35:3215

Table 2. Results of phase studies with crizotinib in IMT.

	Phase trials	
	Phase 1b trial[28]	Non-randomized phase 2 trial[29]
Number of cases	9	20
Number of ALK translocated patients	9	12
Primary site at diagnosis (number of cases)		
Thorax	Not reported	2 (16%)
Abdominal		4 (34%)
Other or unknown		6 (50%)
ORR (%)[a]	66.7	50
CR (%)[a]	11.1 (one patients)	17 (two patients)
PR (%)[a]	55.6 (five patients)	33 (four patients)
Median DOR (weeks)[a]	74.1	36
PFS rate (%)[a]	In 2 years (66.7)	In 1 year (73)

[a]Only ALK-positive patients.
ORR: objective response rate; CR: complete response rate; PR: partial response rate; DOR: duration of response; PFS: progression-free survival.

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J Oncol Pharm Pract 2019 PMID 31615346
Am J Hematol 2018;93:607-614
Lancet Respir Med 2018;6:431-441

Outcome	IMT (n = 14)
Best overall response	
Complete response	5 (36)
Partial response	7 (50)
Stable disease	2 (14)
Progressive disease	0
Therapy duration, years, median (95% CI)	1.63 (0.55 to 2.30)
Time to first PR/CR, days, median (95% CI)	28.5 (27 to 134)

Unresectable IMT in pediatric patients
CR = complete response
PR = partial response
CI = confidence interval

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J Clin Oncol 2017;35:3215

SUMMARY

- **A low threshold** to consider ALK IHC is suggested
  - Any myxoid neoplasm of the uterus
  - Any smooth muscle-like neoplasm with unusual features (minor myxoid change, scattered inflammation, STUMP)
- In the uterus, **ALK IHC is highly specific**
  - Positive result is virtually confirmatory of IMT
  - High correlation with ALK rearrangements
  - Mimickers in the uterus are negative

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SUMMARY

- **ALK IHC sensitivity** is also good
  - A negative result is reassuring, but does not exclude IMT
  - Sensitivity of IHC, FISH and sequencing **≠ 100%**
  - ALK-negative IMT exists in the uterus
    - Case with *ETV6-NTRK3* fusion reported
- Available clones offer similar sensitivity and specificity
- Confirmation of IMT and ALK alterations has therapeutic potential

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THANK YOU!