Serous Effusions
Part II

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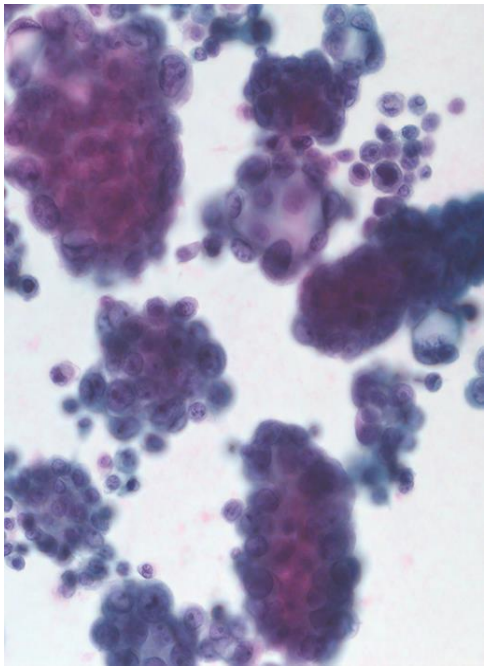
University Hospital
Basel

Outline

- Pre-analytics
- Non-neoplastic effusions
- Malignant effusions
  - Morphological features of the most common malignancies
  - Predictive marker testing
- Ancillary techniques
  - Immunocytochemistry
  - FISH

Malignant mesothelioma



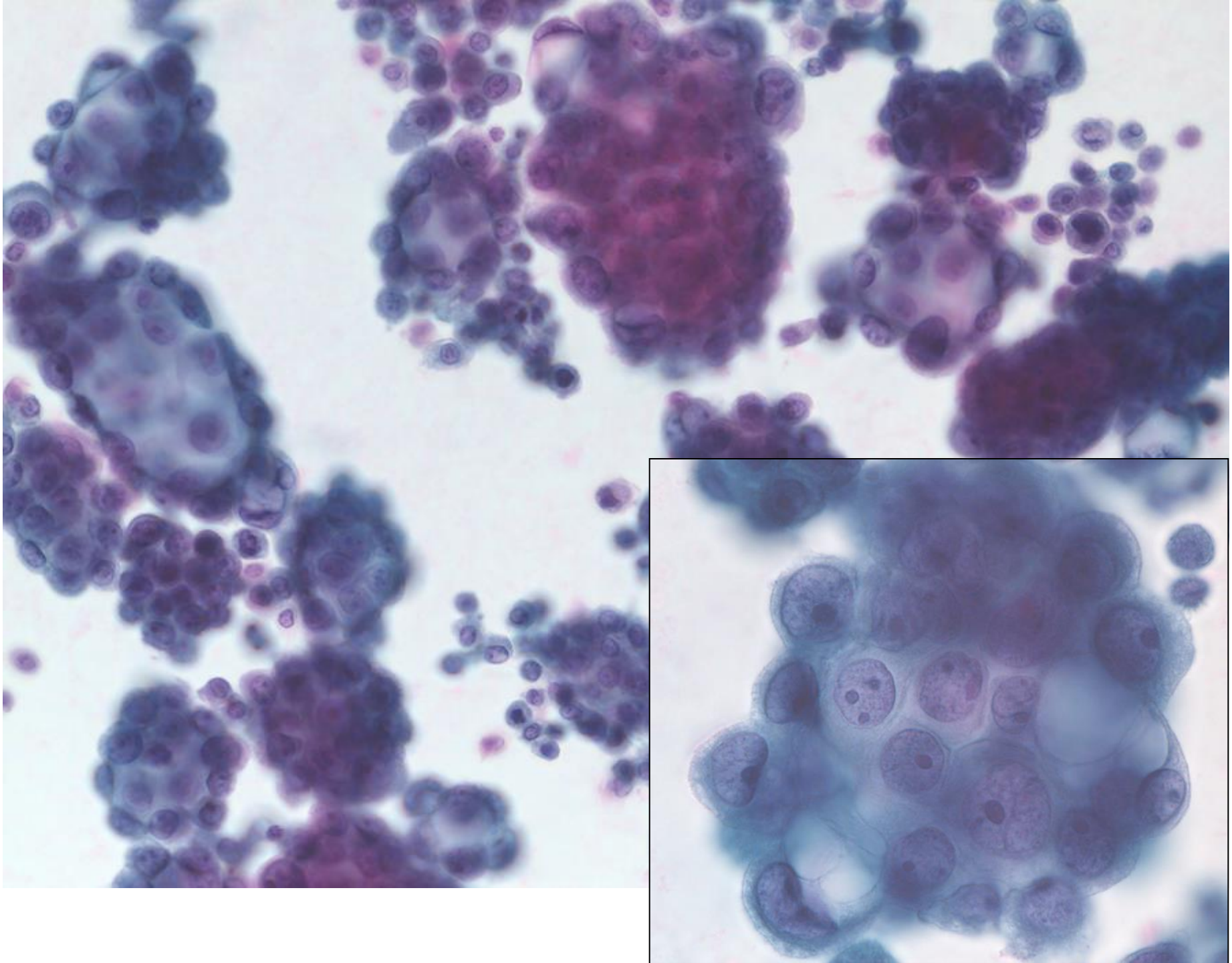
Sensitivity 32-76%:

DD reactive vs. MM

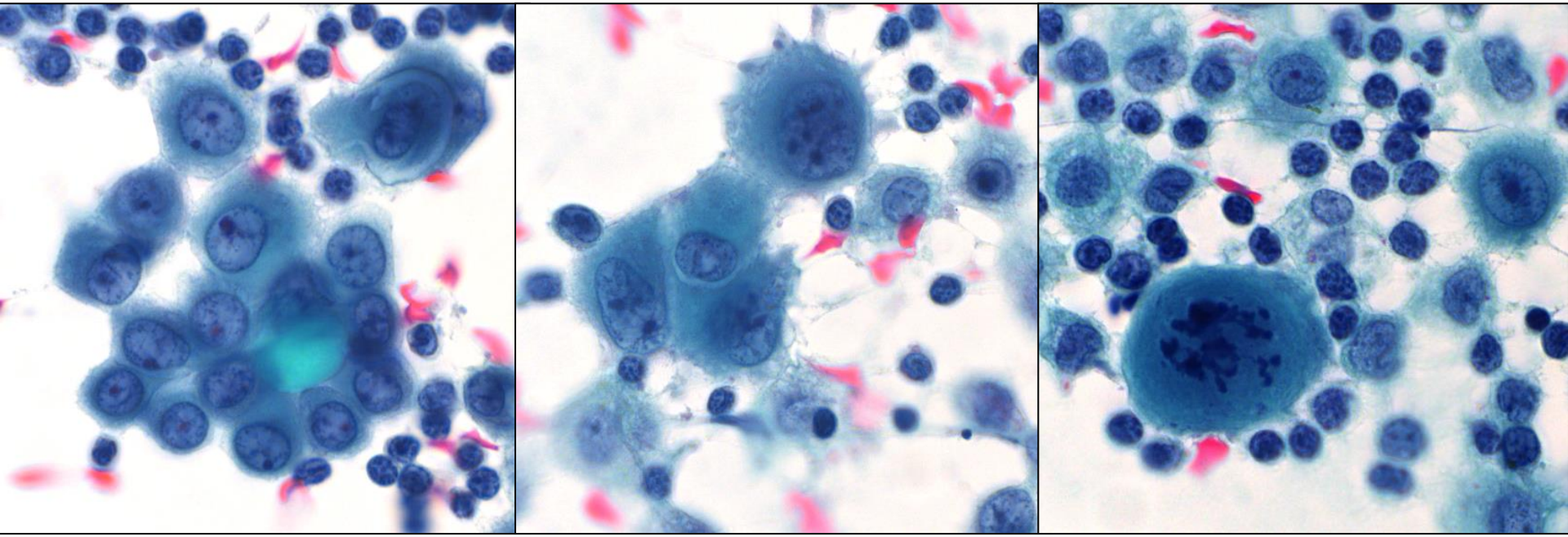
Features of epithelioid MM

- Numerous, large (>50 cells) papilliform cell groups
- Macronucleoli
- Nuclear atypia

Whitaker D Cytopathology 2000



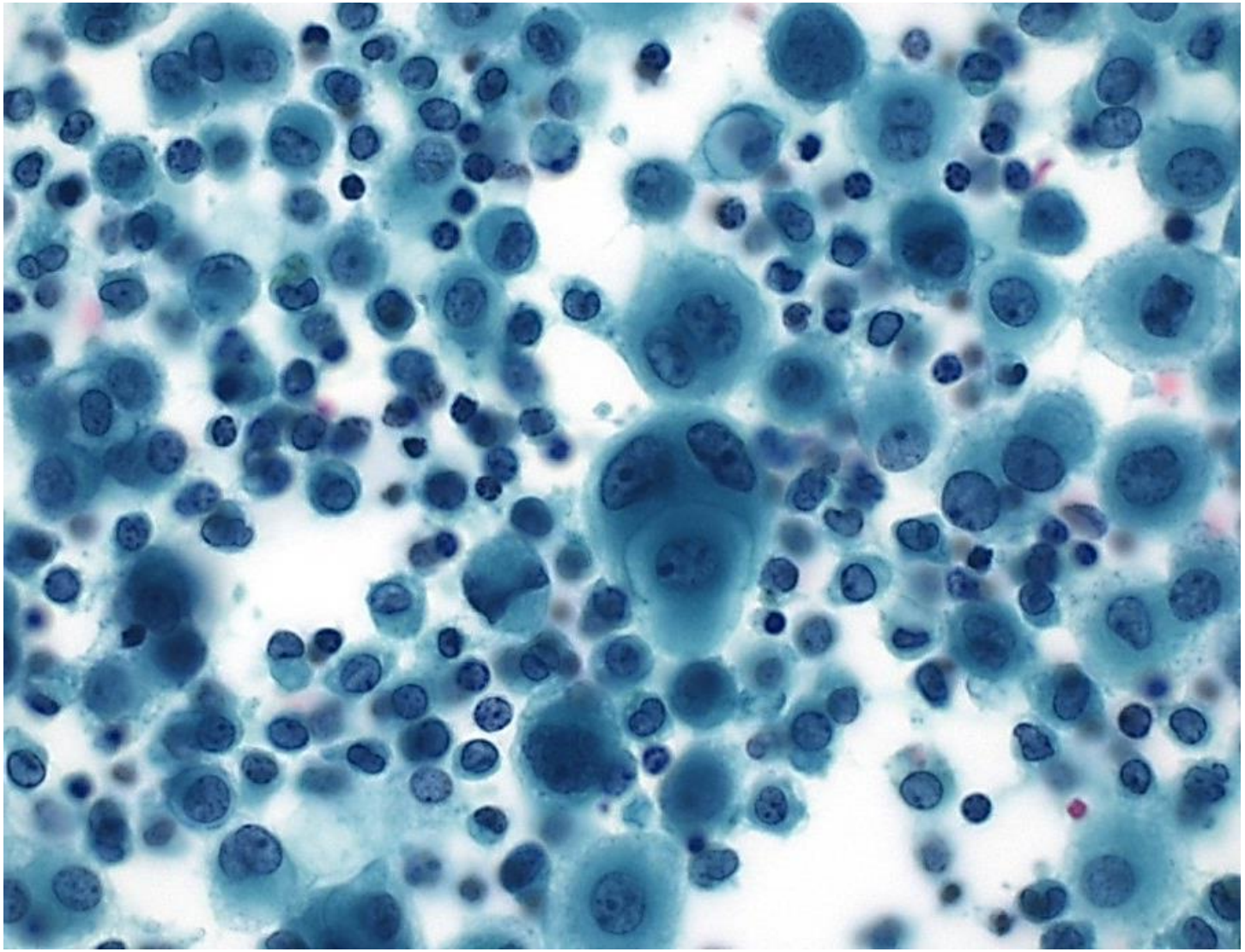
Reactive mesothelial cells



mimics of adenocarinoma and malignant mesothelioma

pericard - radiation/chemotherapy - cirrhosis

54y F, recurrent pleural effusion



Immunocytochemistry

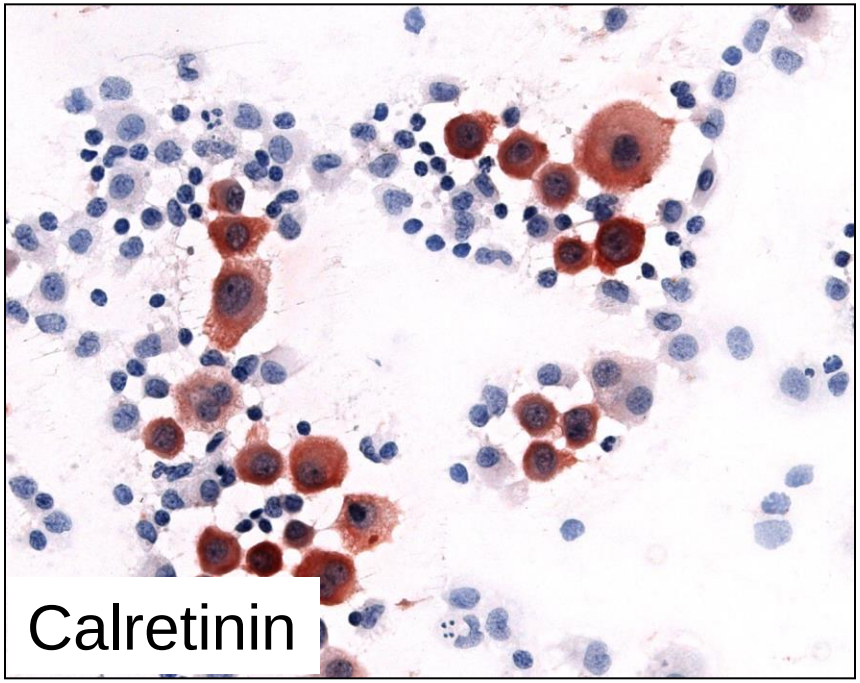
Mesothelial	**Epithelial**
Calretinin	BerEP4
D2-40	CD15
WT-1	TTF1, PAX8,…

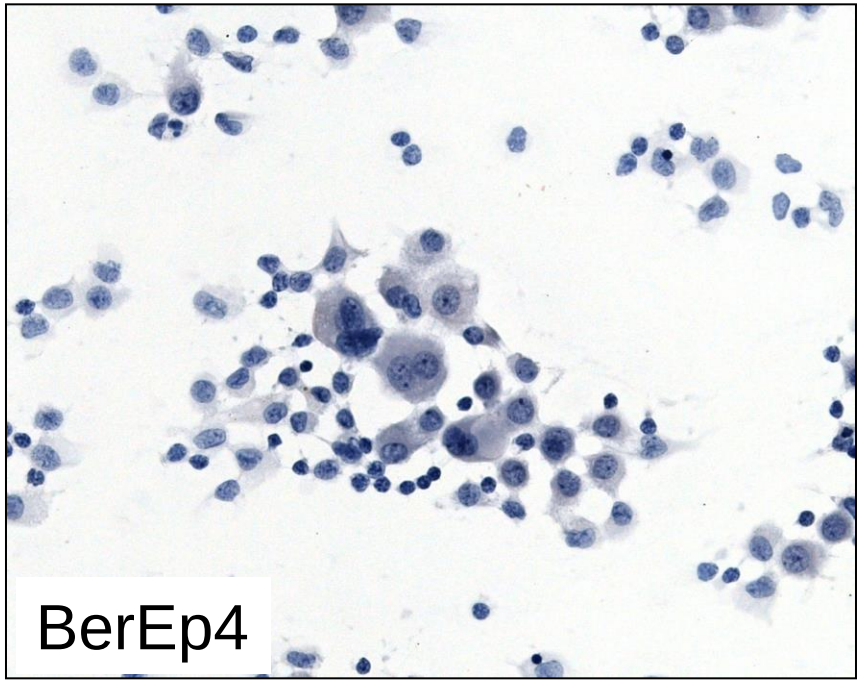
Panel depends on:

- Location (pleural vs peritoneal)
- Type of tumor in the DD

Husain AN et al. Arch Pathol Lab Med 2013

ICC





Cells of mesothelial origin

Reactive mesothelial cells vs MM

Antibody	Reactive Mesothelium, No. (% Positive)	Mesothelioma, No. (% Positive)
Desmin	34/40 (85)	6/60 (10)
EMA	8/40 (20)	48/60 (80)
p53	0/40 (0)	27/60 (45)
GLUT-1	5/150 (3)	103/153 (67)
IMP3	0/64 (0)	33/45 (73)

..., they should not be solely relied on for MM diagnosis in individual cases

Husain AN et al. Arch Pathol Lab Med 2013

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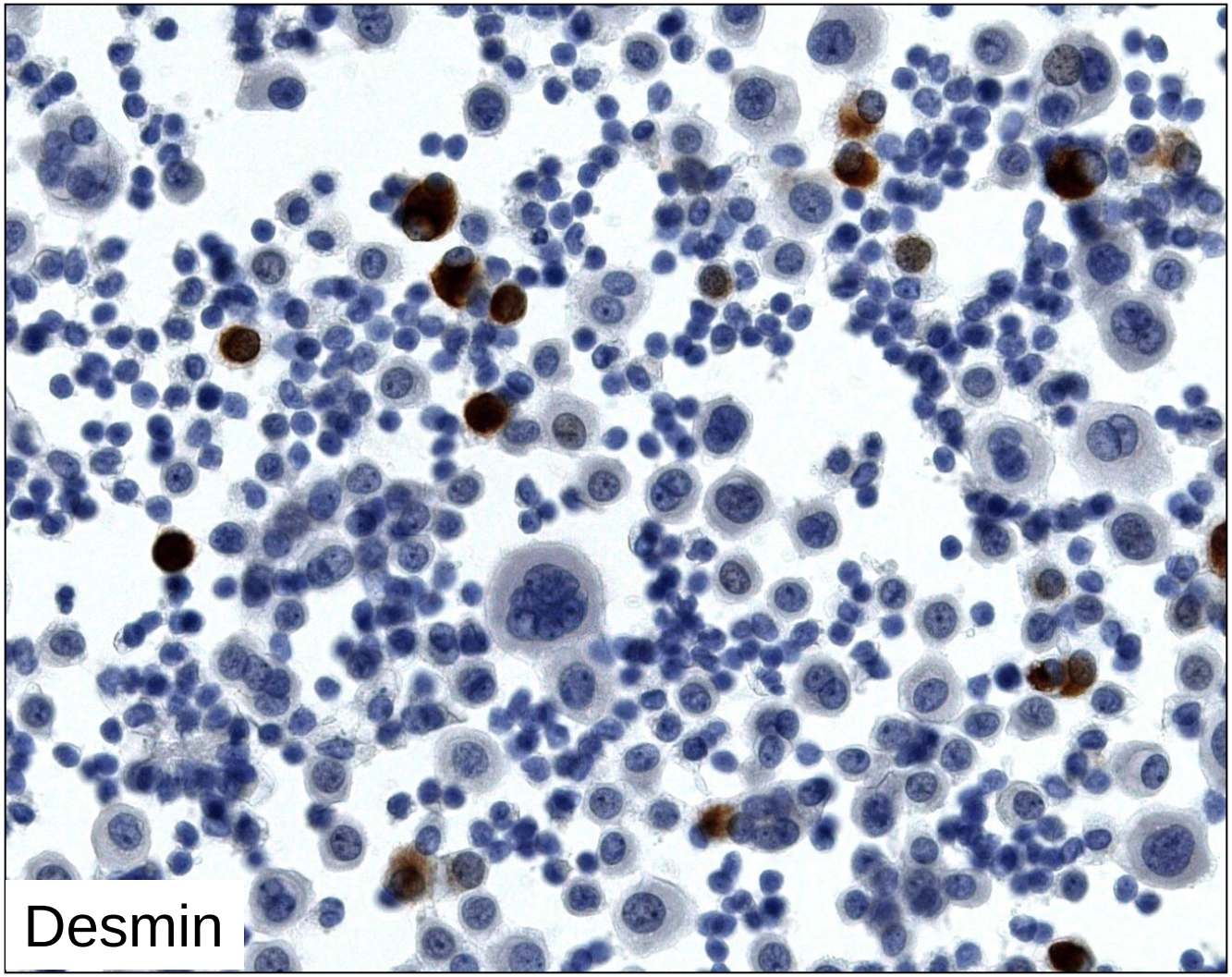
..., they should not be solely relied on for MM diagnosis in individual cases

BAP-1: loss of expression in 50% of MM

Husain AN et al. Arch Pathol Lab Med 2013



Loss of desmin expression:
Cells of malignant mesothelioma?

UroVysion FISH

	FISH		p-Value	Total
	Negative (%)	Positive(%)		n (%)
Mesothelioma	11 (21)	41 (79)	<0.01	52 (65)
BMC	28 (100)	0		28 (35)

Sensitivity= 78.8%, Specificity= 100%, PPV= 100%, NPP= 71.8%

Savic S et al, Chest 2010

UroVysion FISH

	FISH		p-Value	Total
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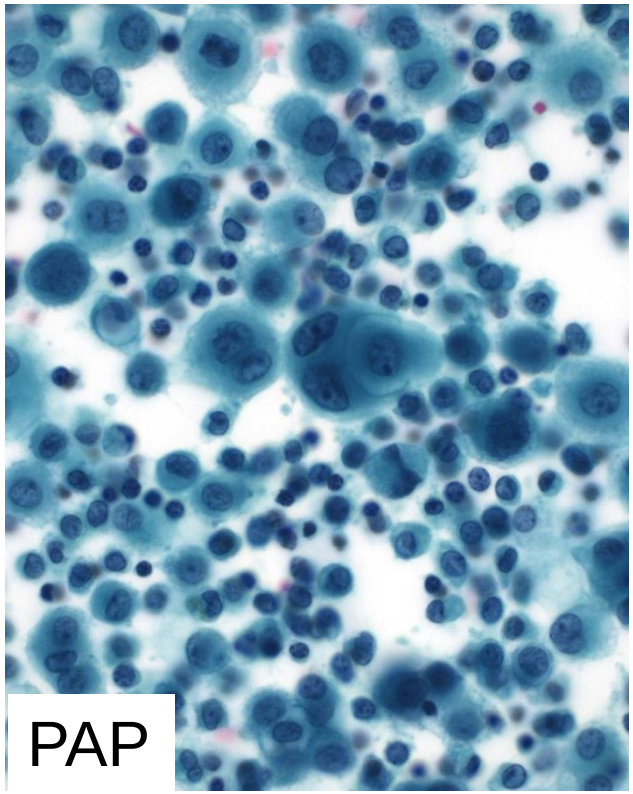
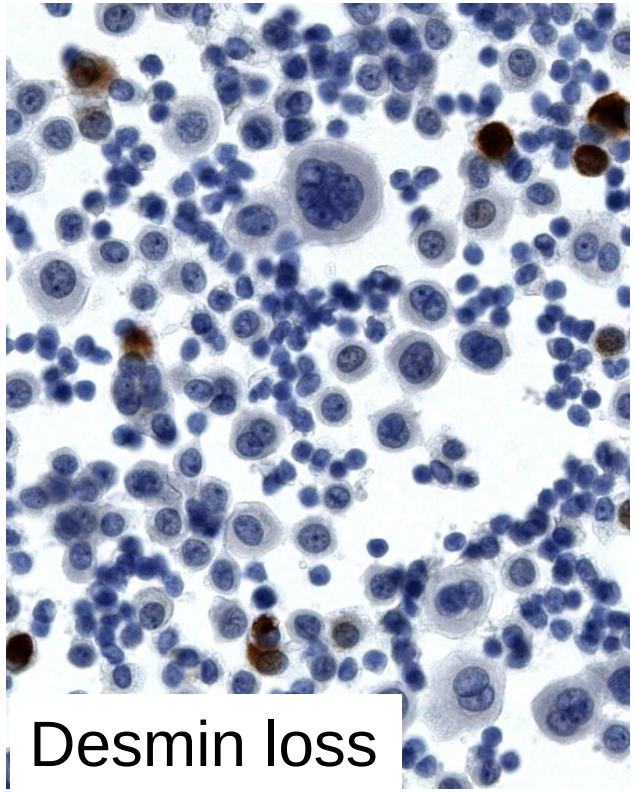
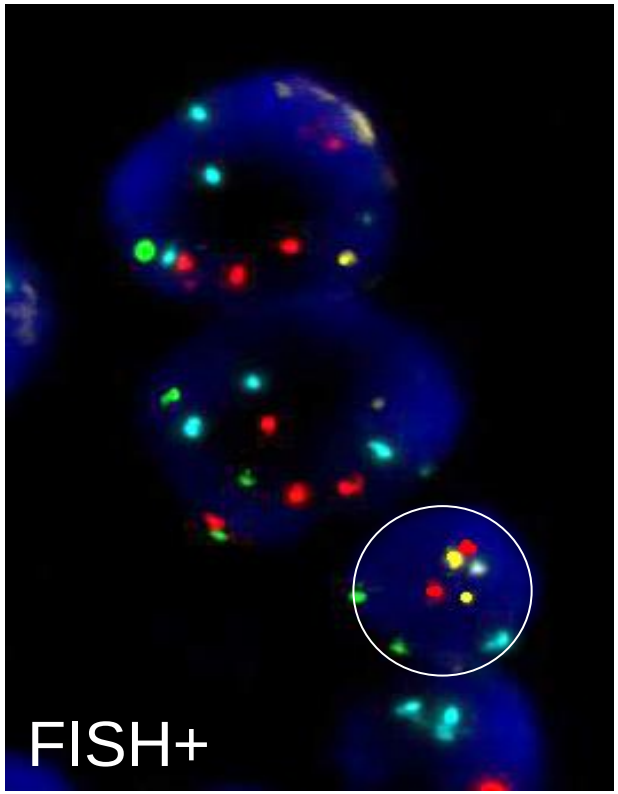
BMC	28 (100)	0		28 (35)

Sensitivity= 78.8%, Specificity= 100%, PPV= 100%, NPP= 71.8%

- positive FISH exculdes benign mesoth. cells
- negative FISH does not exclude MM
  - 20-30% of pleural epith. MM FISH-
  - >50 % of peritoneal epith. MM FISH-

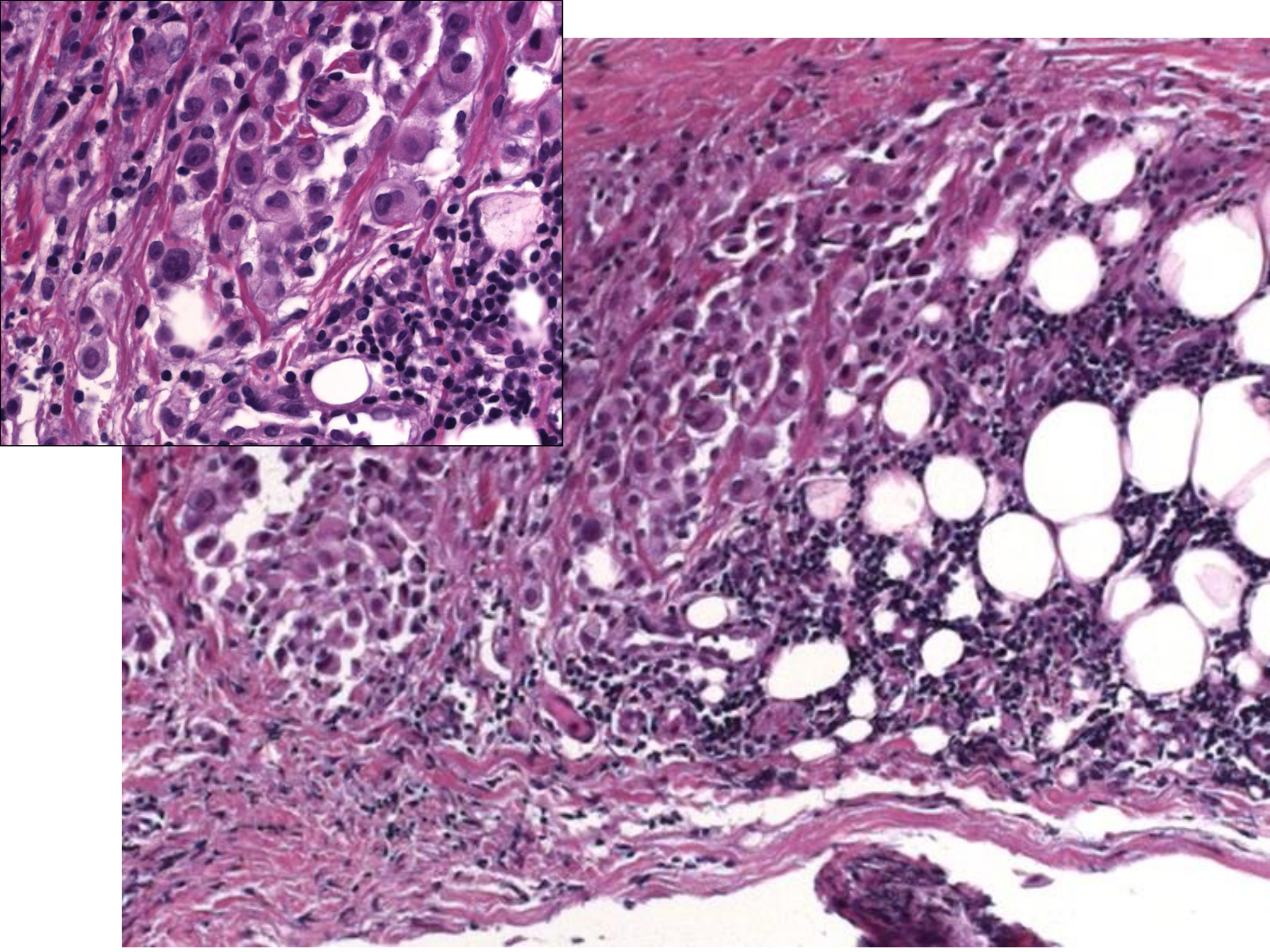






Malignant mesothelioma

In the setting of appropriate radiologic findings



Guidelines for Pathologic Diagnosis of Malignant Mesothelioma

2012 Update of the Consensus Statement from the International Mesothelioma Interest Group

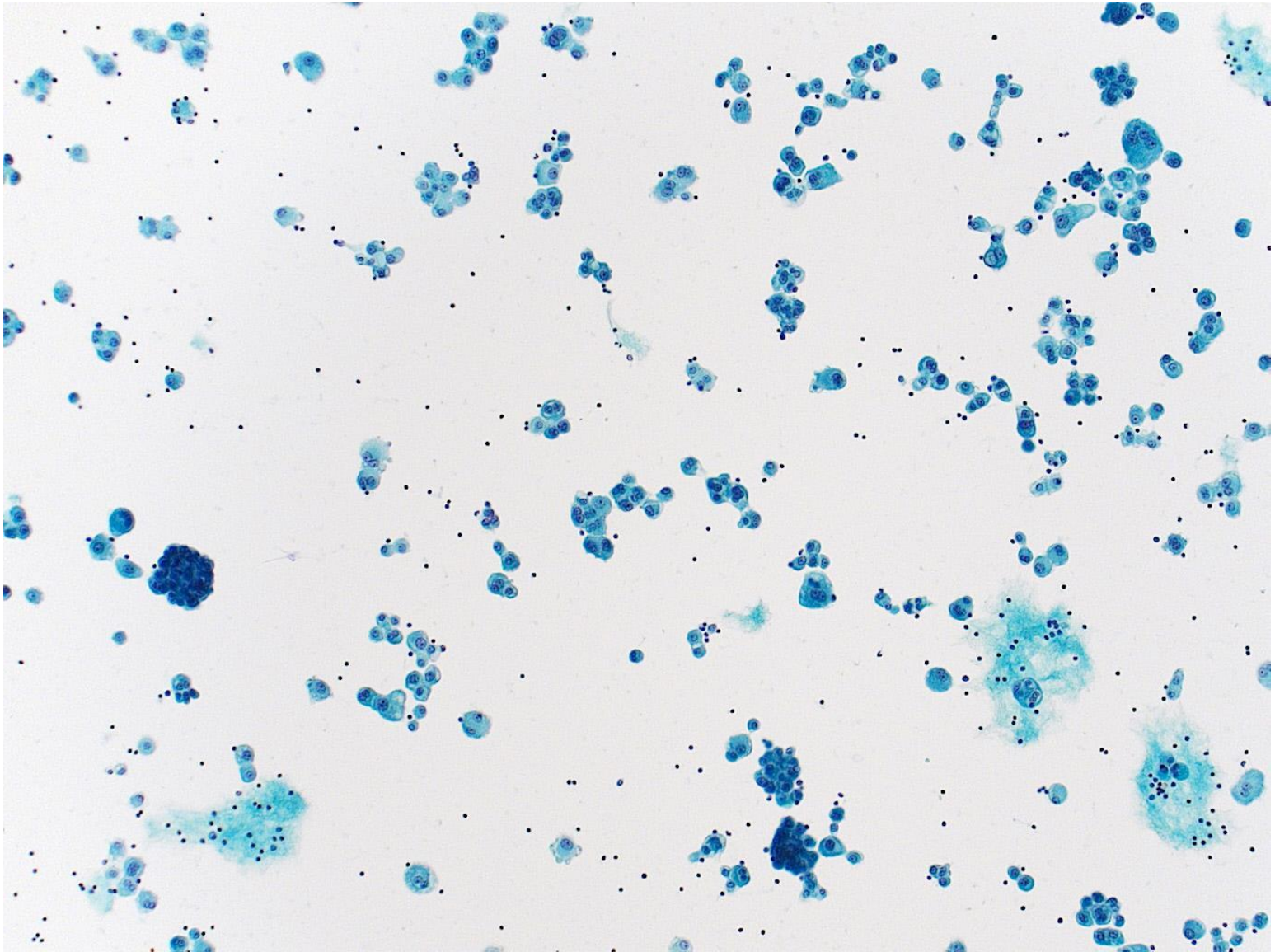
Aliya N. Husain, MD; Thomas Colby, MD; Nelson Ordonez, MD; Thomas Krausz, MD; Richard Attanoos, MB, BS; Mary Beth Beasley, MD; Alain C. Borczuk, MD; Kelly Butnor, MD; Philip T. Cagle, MD; Lucian R. Chirieac, MD; Andrew Churg, MD; Sanja Dacic, MD, PhD; Armando Fraire, MD; Francoise Galateau-Salle, MD; Allen Gibbs, MD; Allen Gown, MD; Samuel Hammar, MD; Leslie Litzky, MD; Alberto M. Marchevsky, MD; Andrew G. Nicholson, DM; Victor Roggli, MD; William D. Travis, MD; Mark Wick, MD

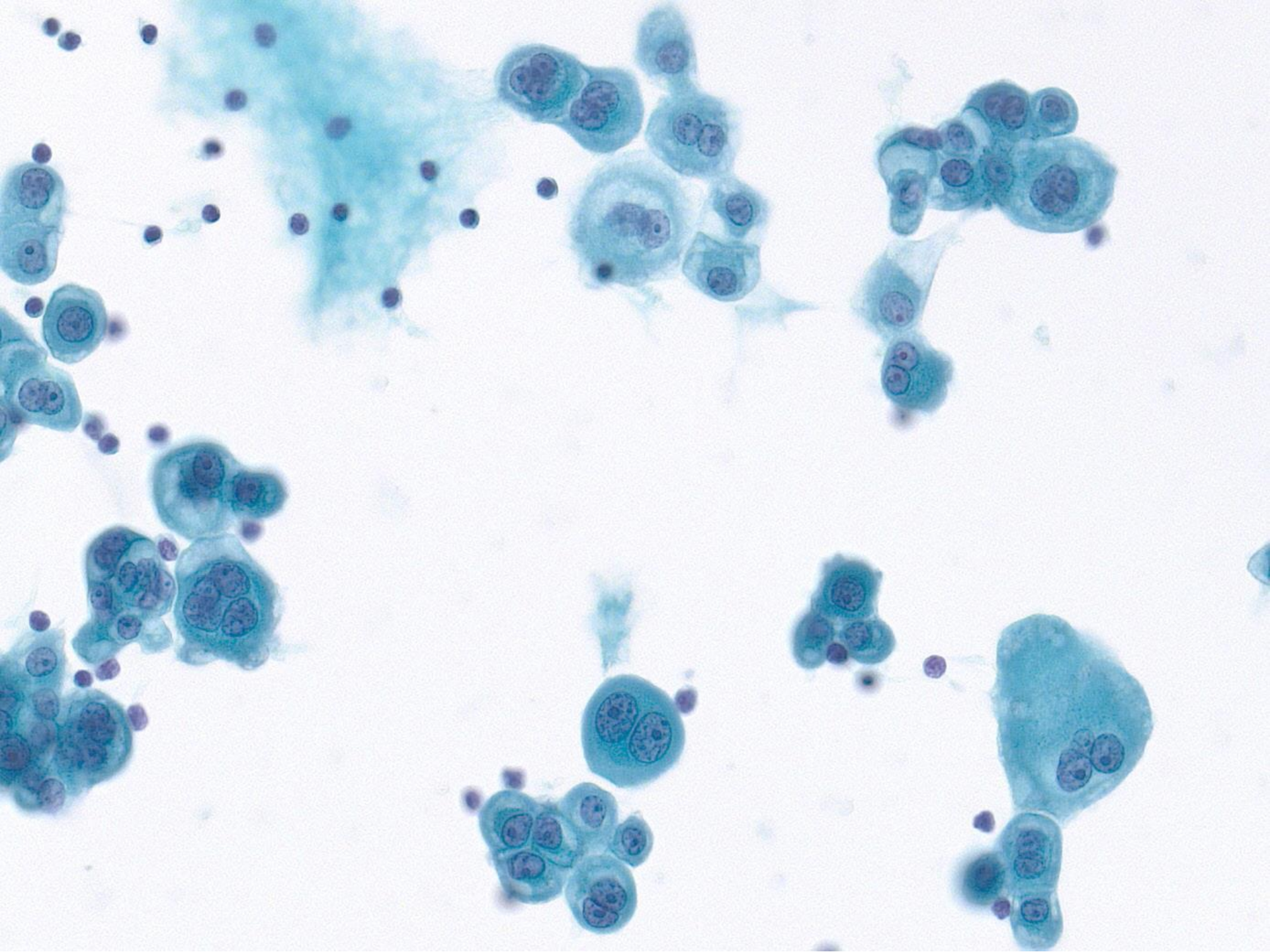
***Arch Pathol Lab Med* 2013**

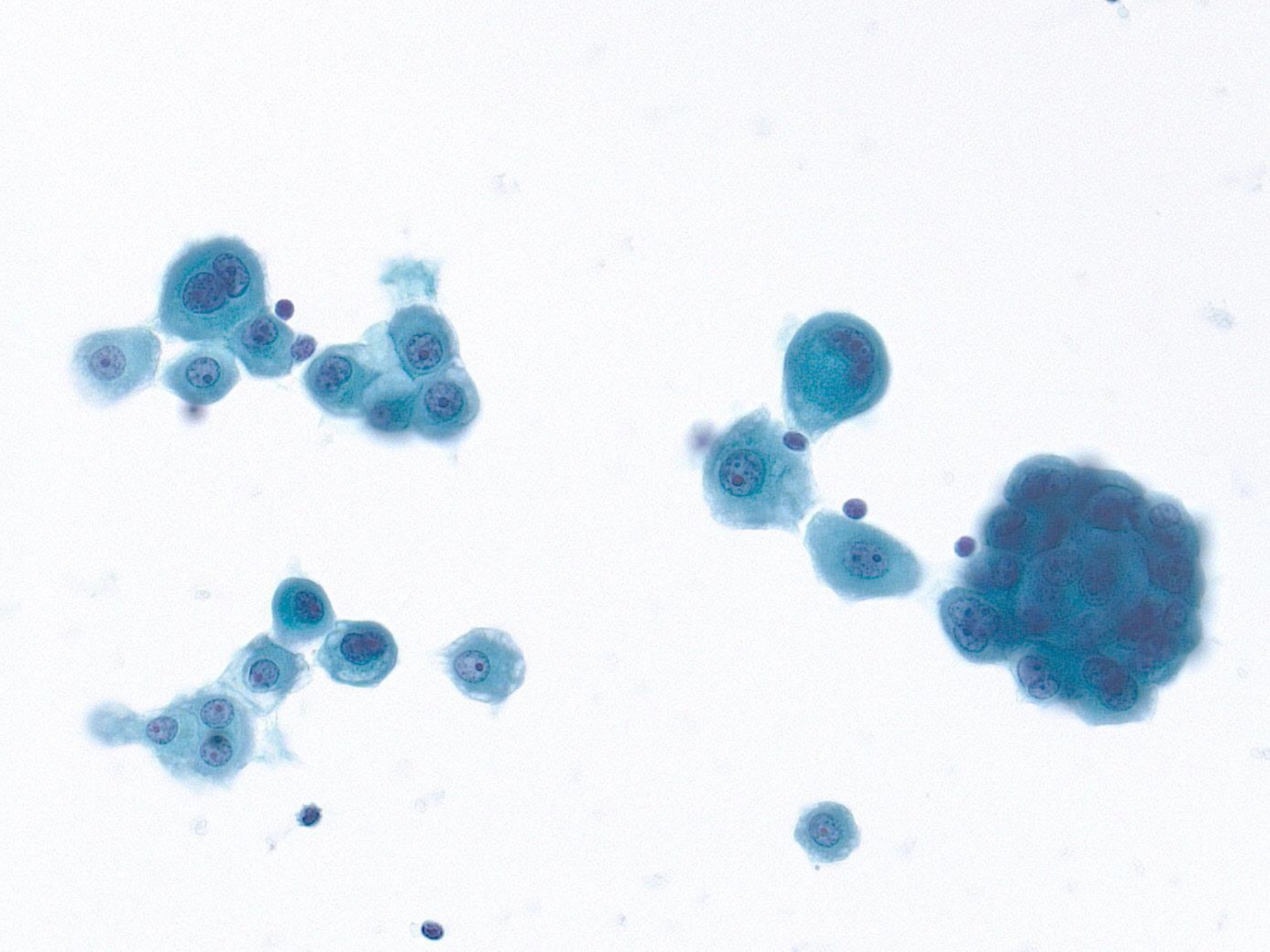
Diagnosis of MM should always be based on a biopsy (less commonly cytology) in the context of appropriate clinical and radiological findings

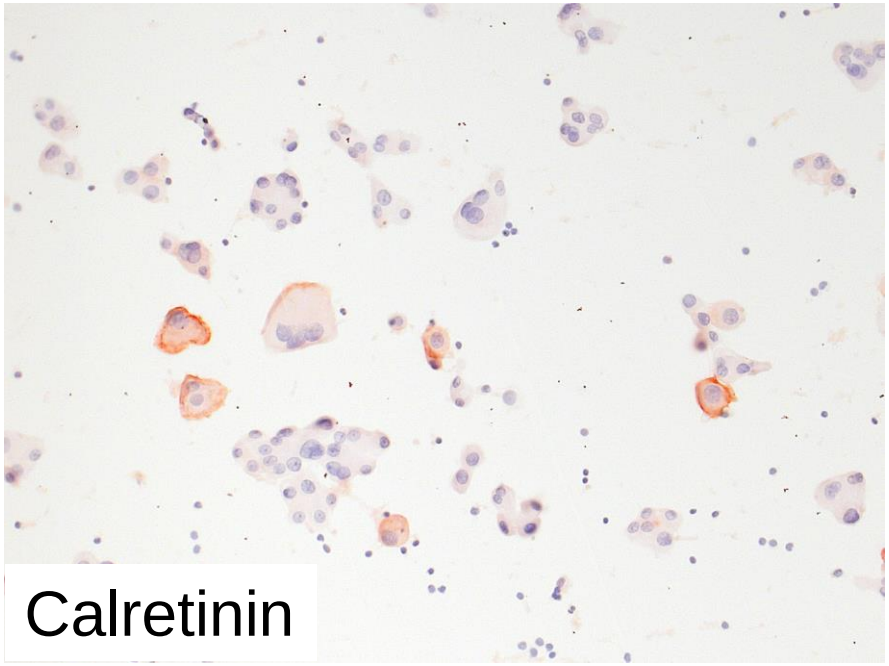
Molecular testing more widely available and helpful in selected cases

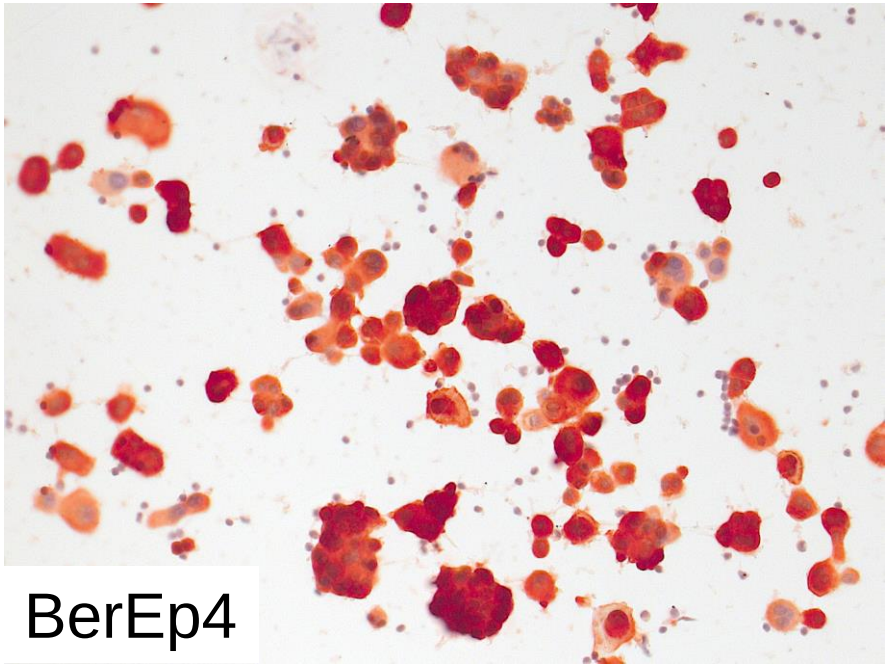
70y M, unilateral pleural effusion

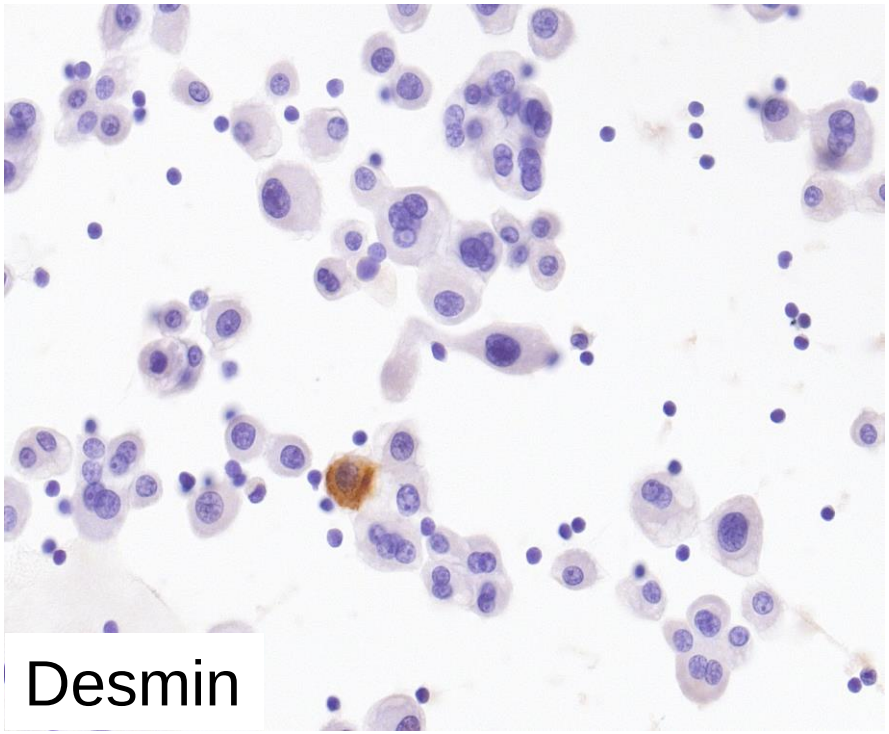




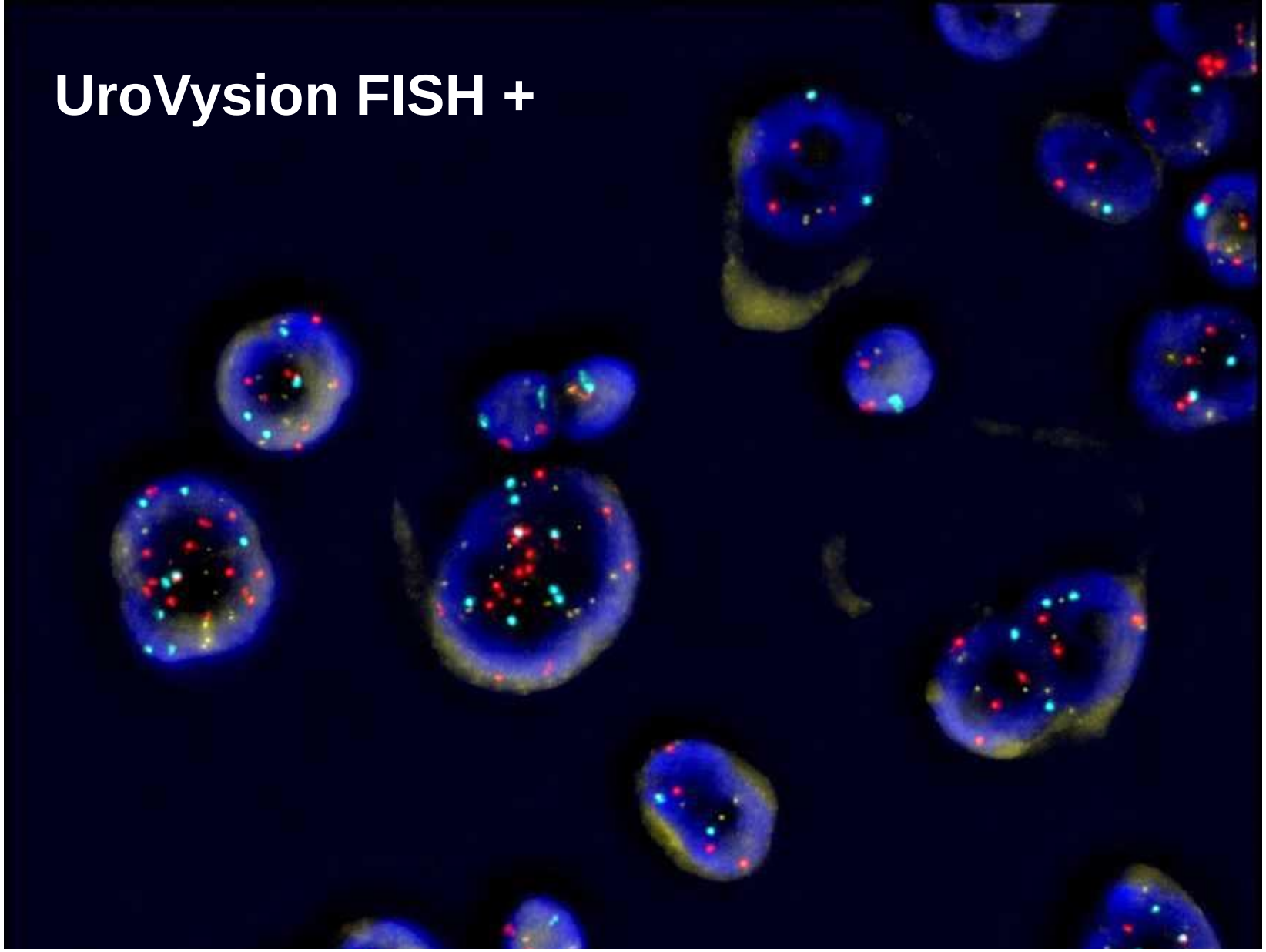



Calretinin


BerEp4


Desmin

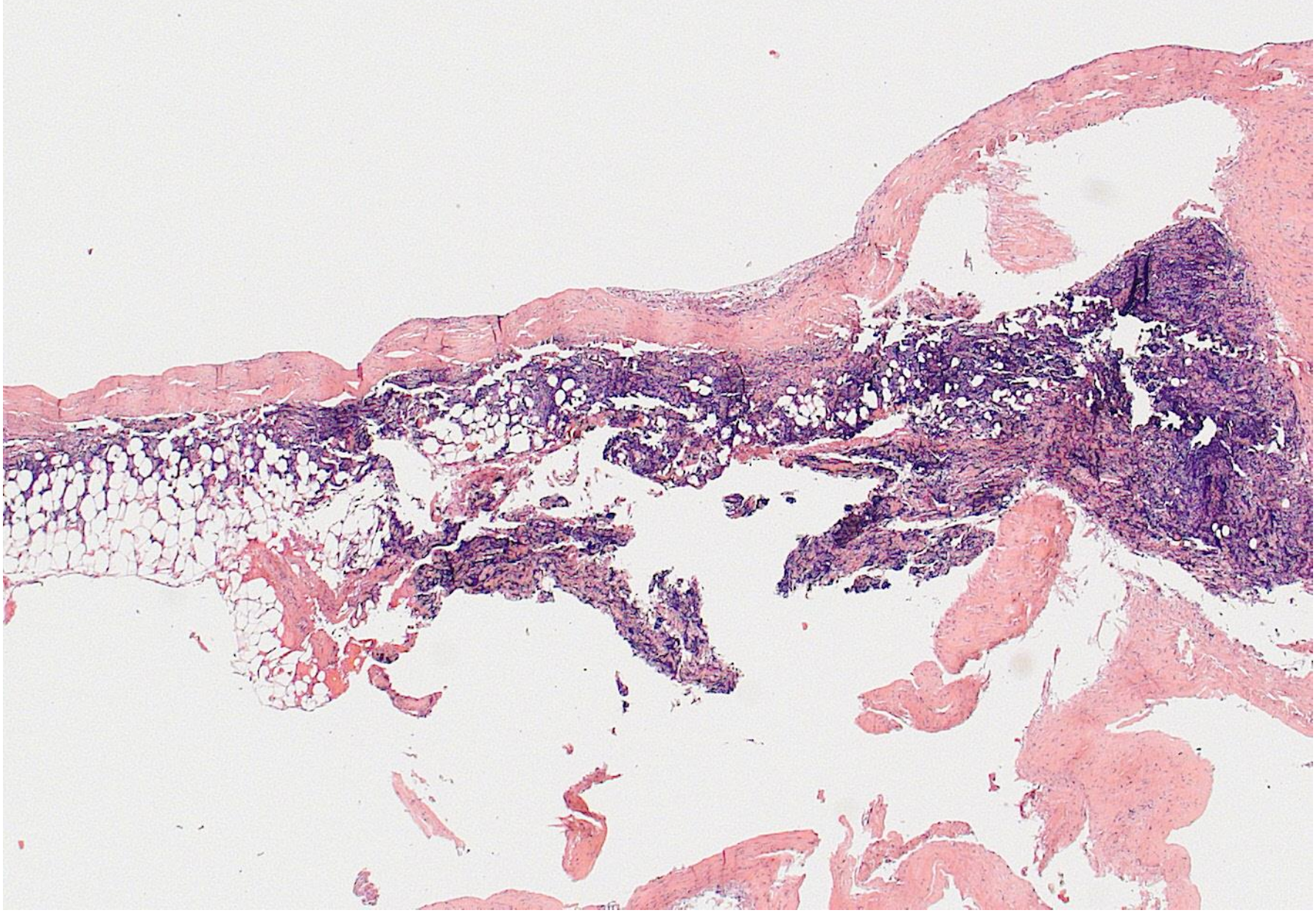
UroVysion FISH +

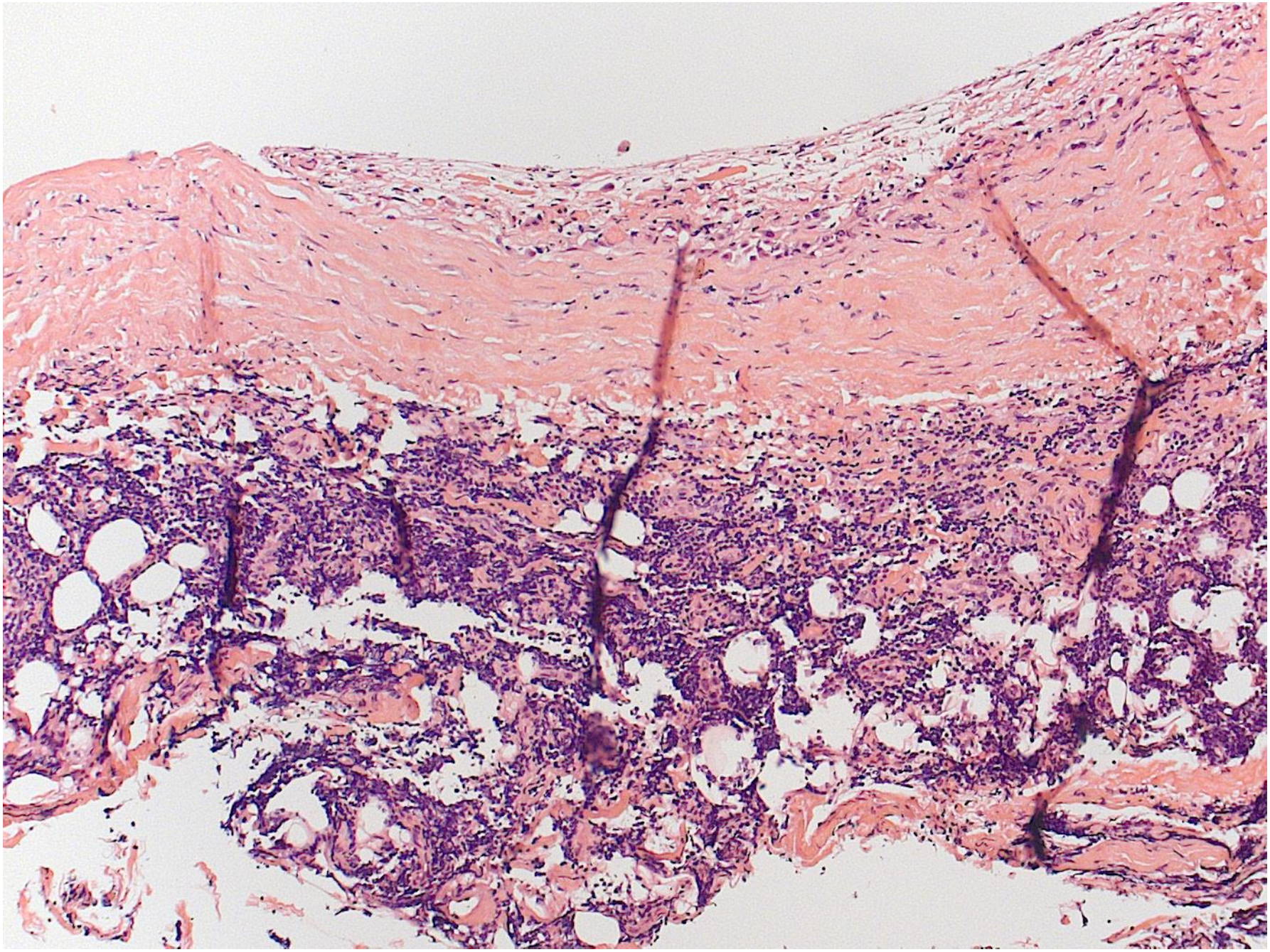


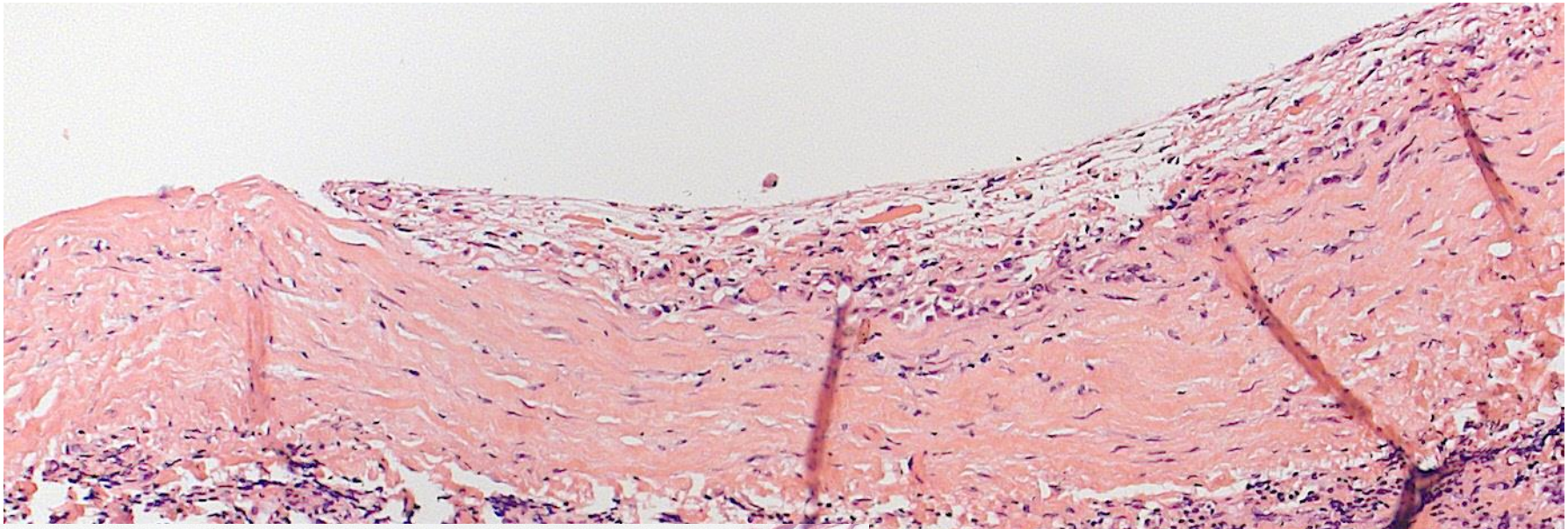
Malignant mesothelioma

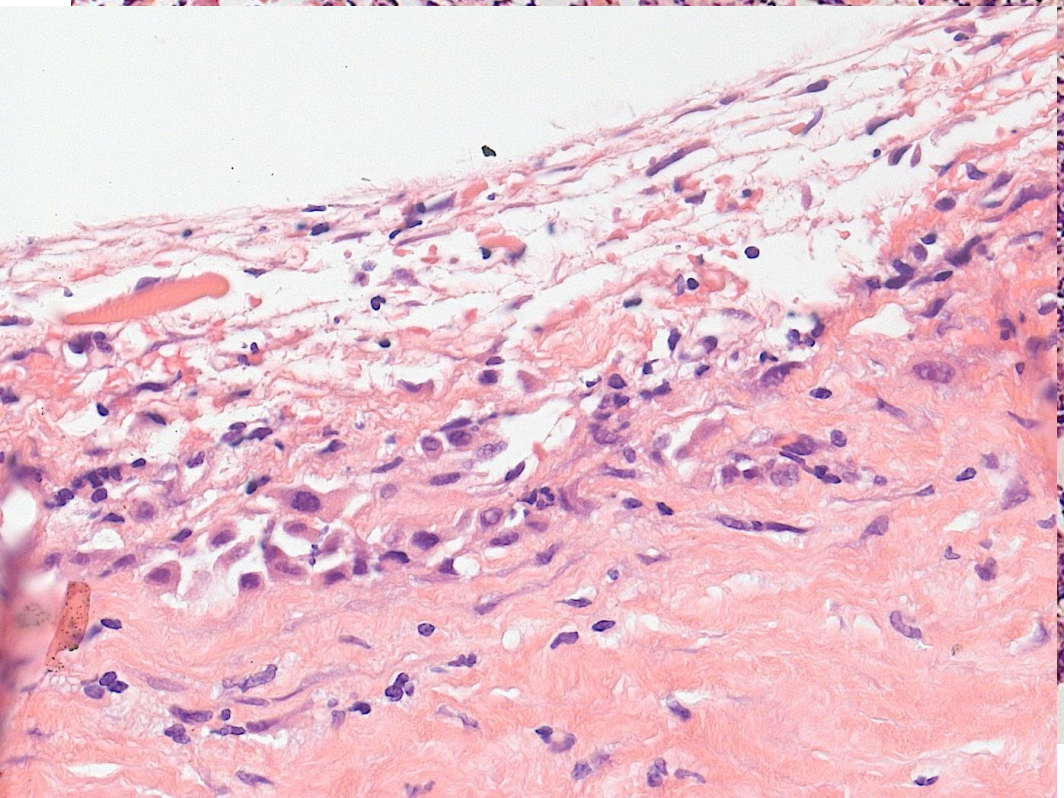
In the setting of appropriate radiologic findings

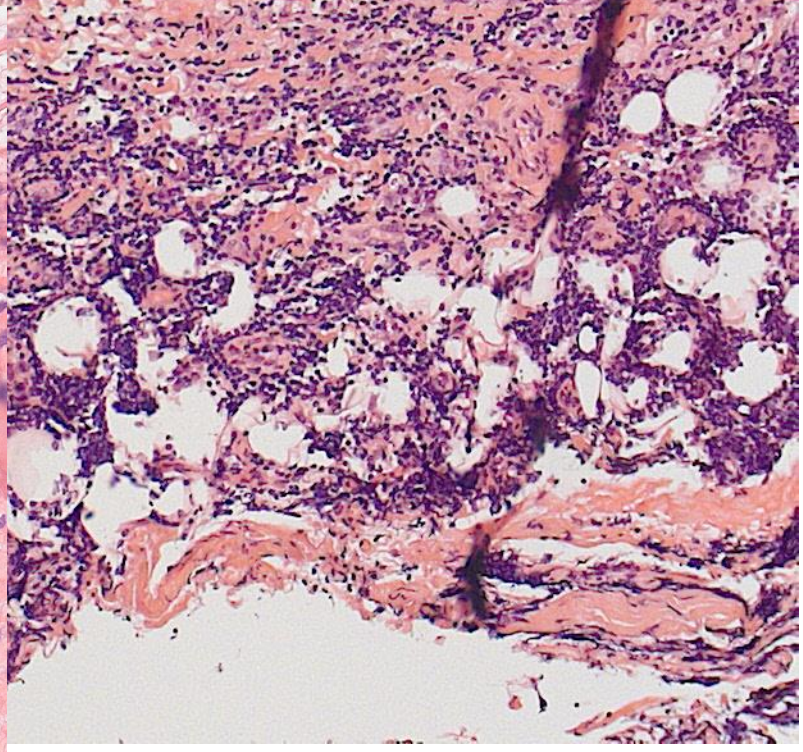
Radiologic findings not suggestive of MM

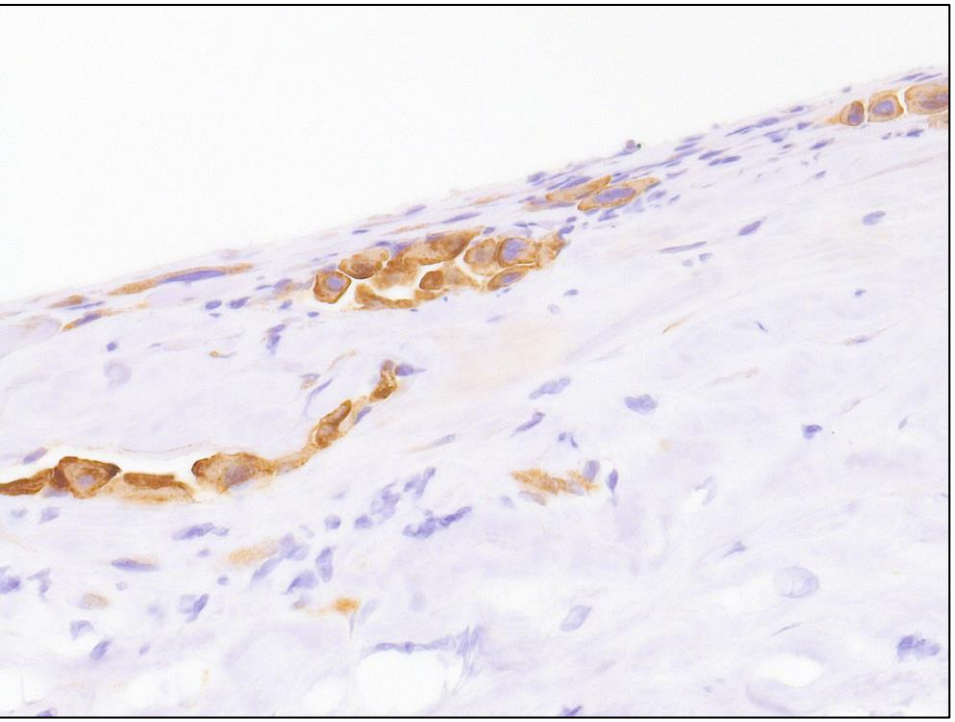




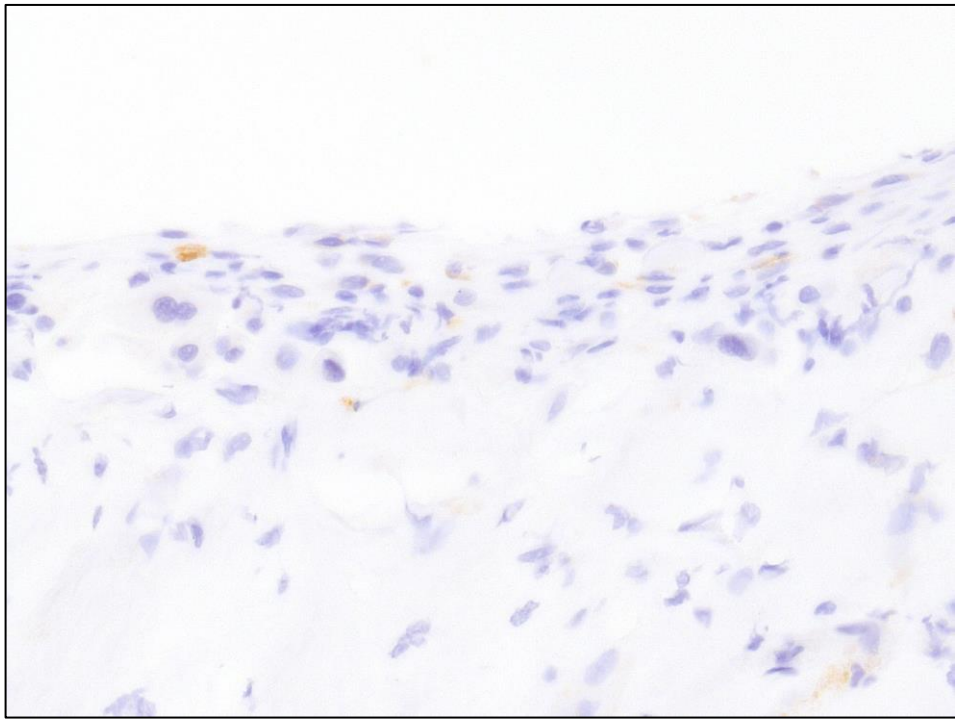




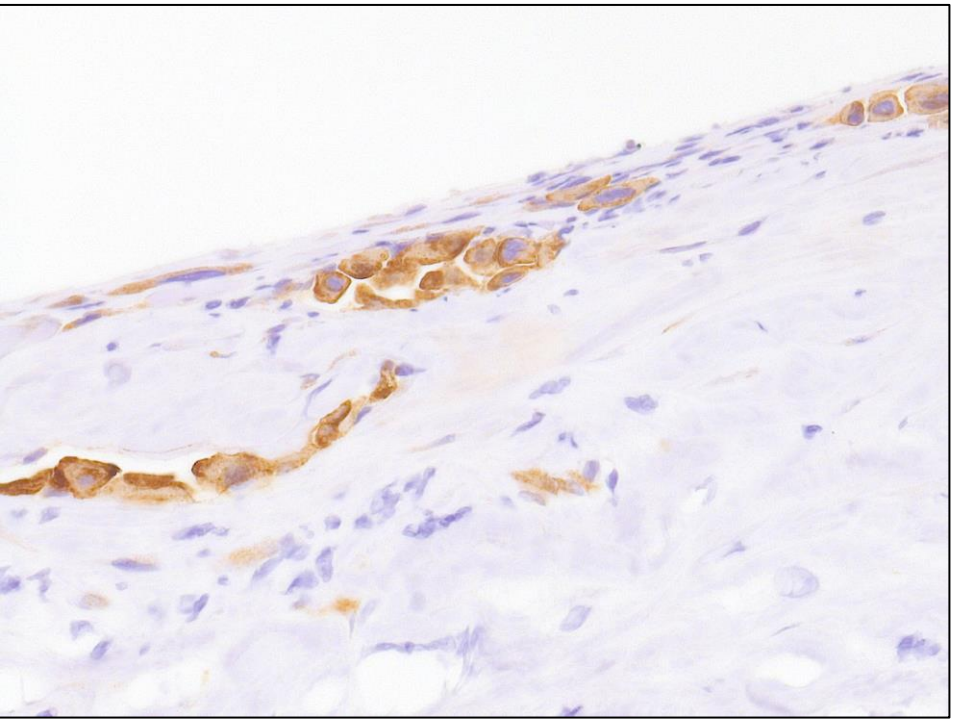




Calretinin



Desmin



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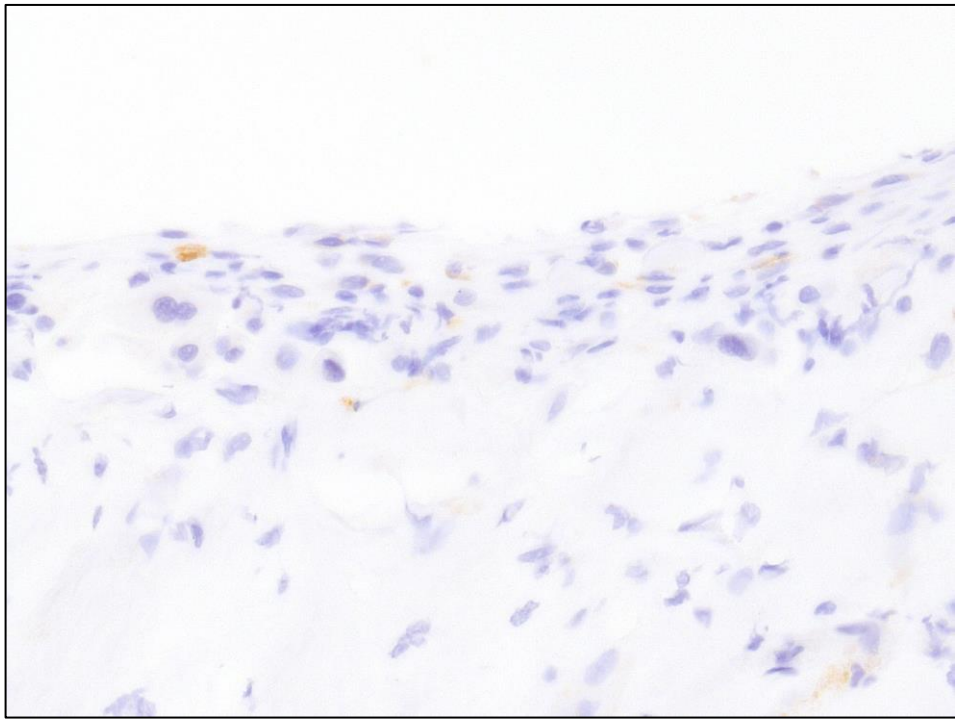
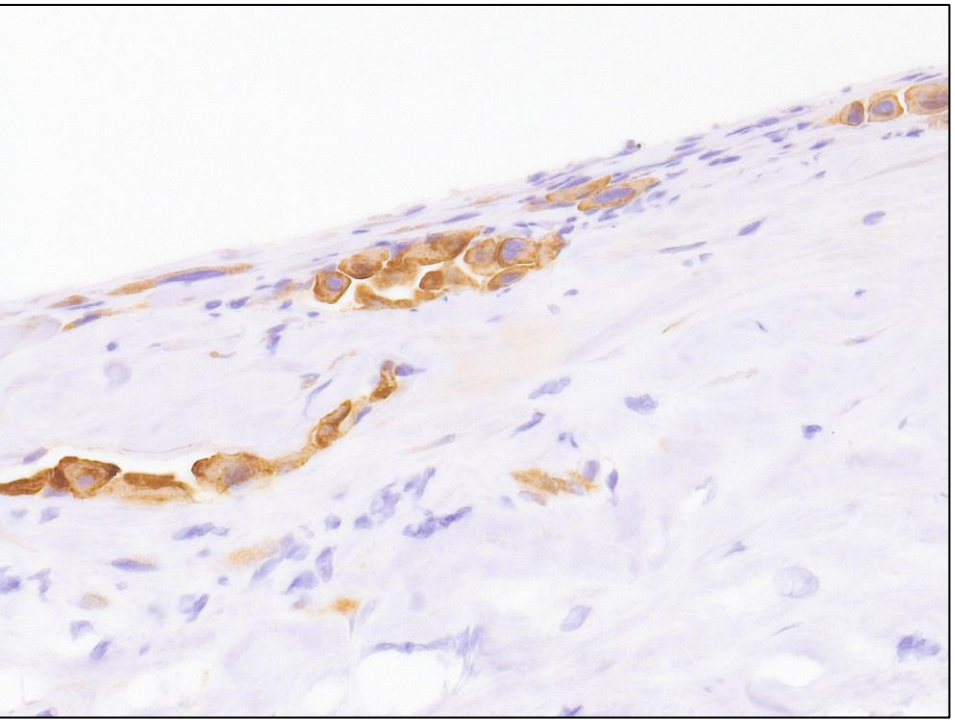


Desmin

Chronic, lymphocytic pleuritis.
Few mesothelial cells with loss of desmin.

Comment: VATS re-biopsy recommended



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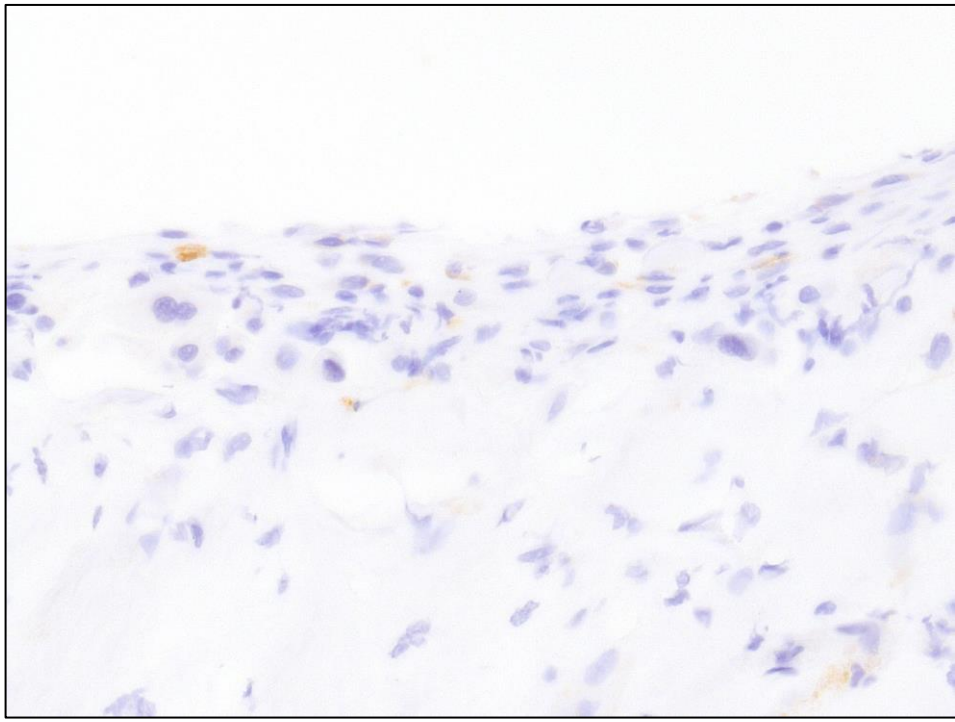
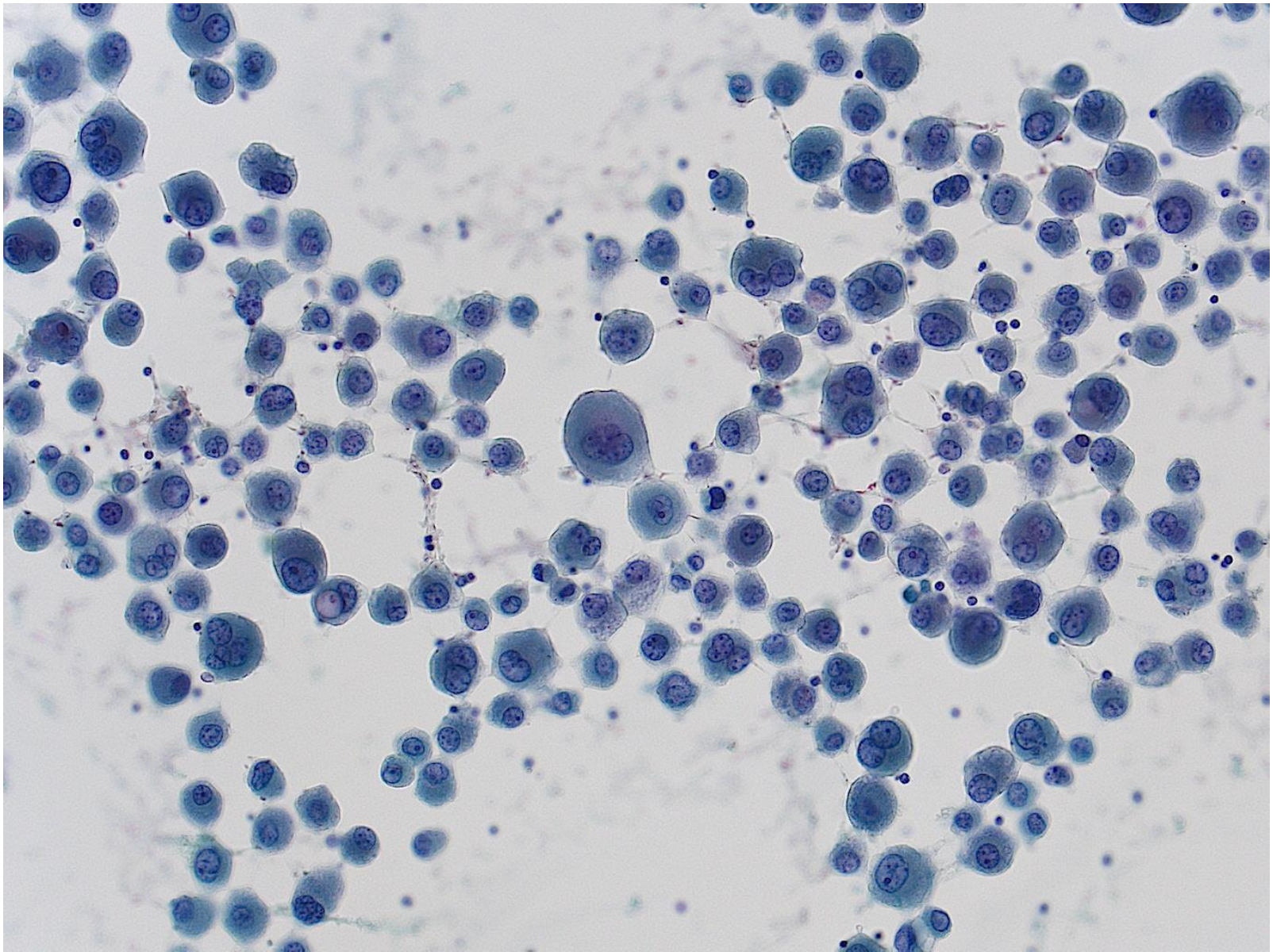


Desmin

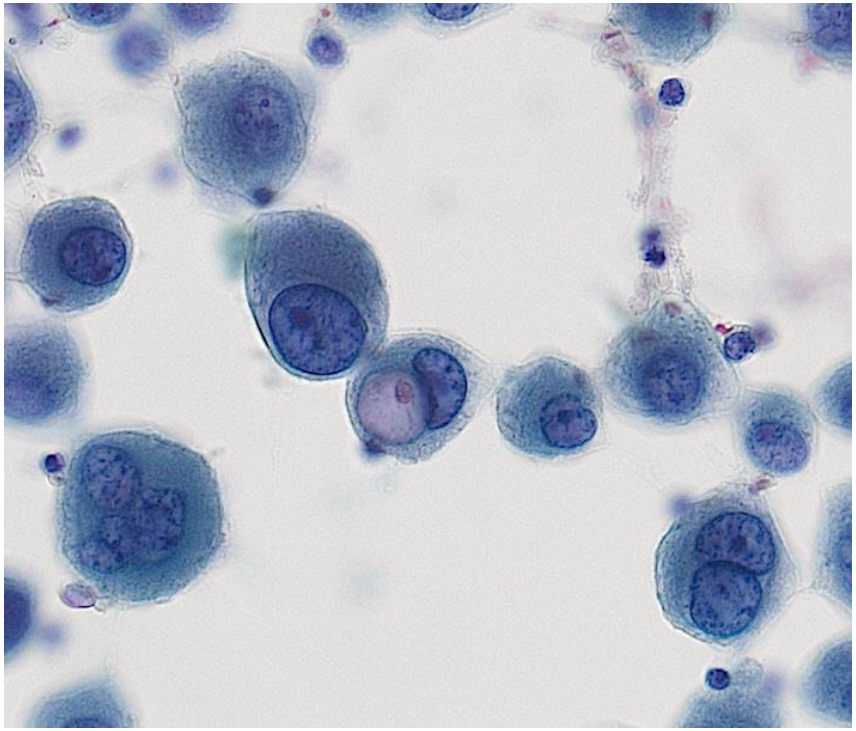
CT follow-up every 3 month:

- At 9 month follow-up: **Malignant mesothelioma**

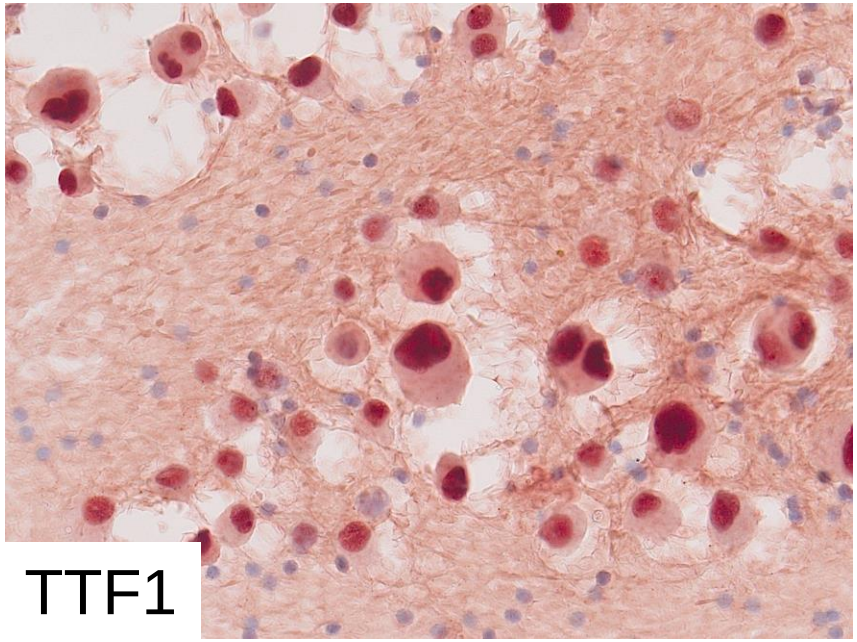
65y M, unilateral pleural effusion



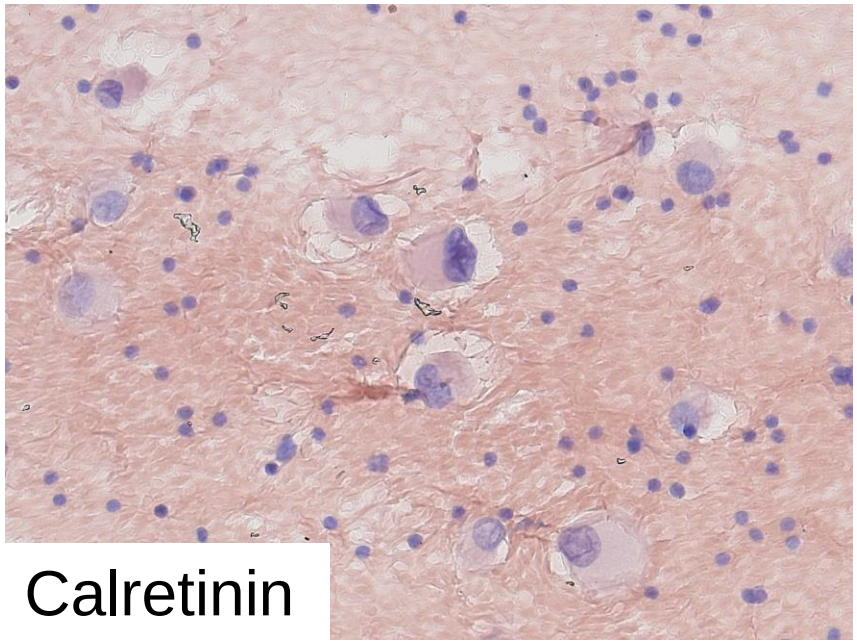
Adenocarinoma



Mucin

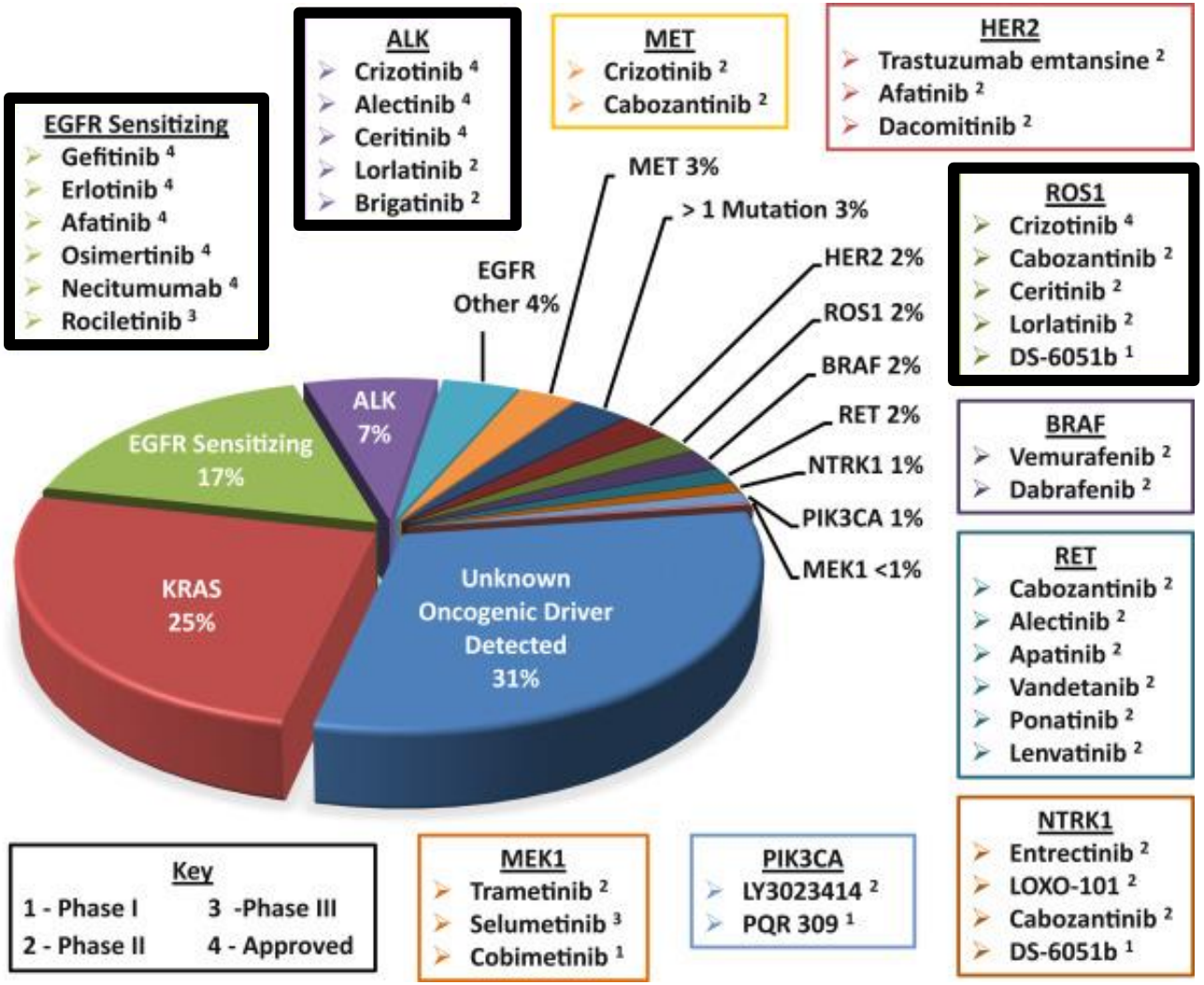


TTF1



Calretinin

ADC: Molecular Subtypes



EU:
Aug 2016
Crizotinib
approved

Tsao AS et al., JTO 2016

Stage IV
«Non-squamous» NSCLC

⇩

Predictive molecular testing:
EGFR, ALK, ROS1

Gene sequencing by NGS

DNA content for
NGS: ≈ 5 ng

ICC ALK & ROS1

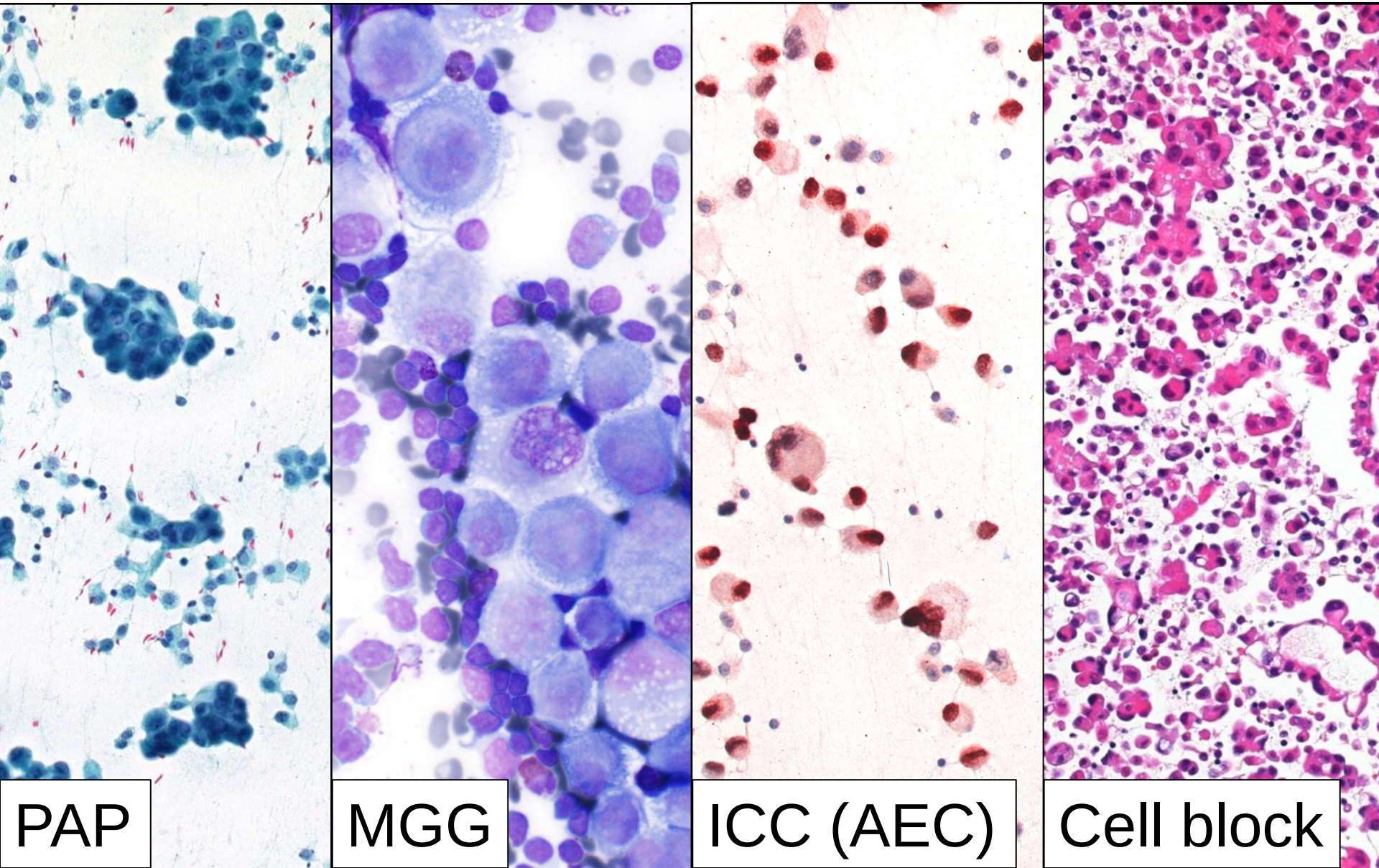
+ ↓

↓ - KRAS, EGFR WT

Confirmation by FISH

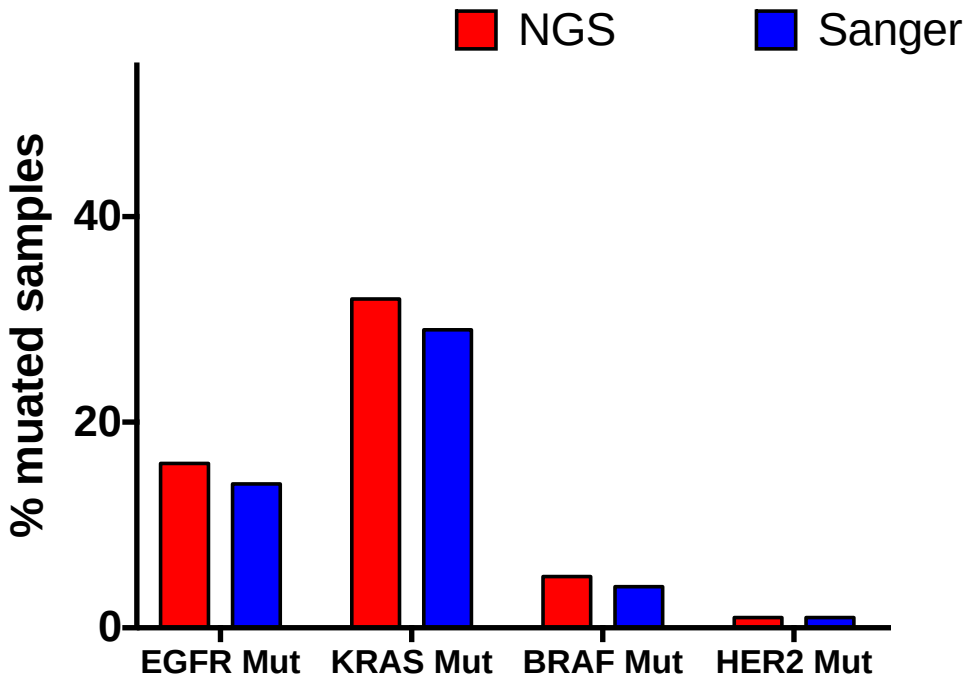
FISH: MET, RET on clinical request

DNA based analyses on cytological specimens

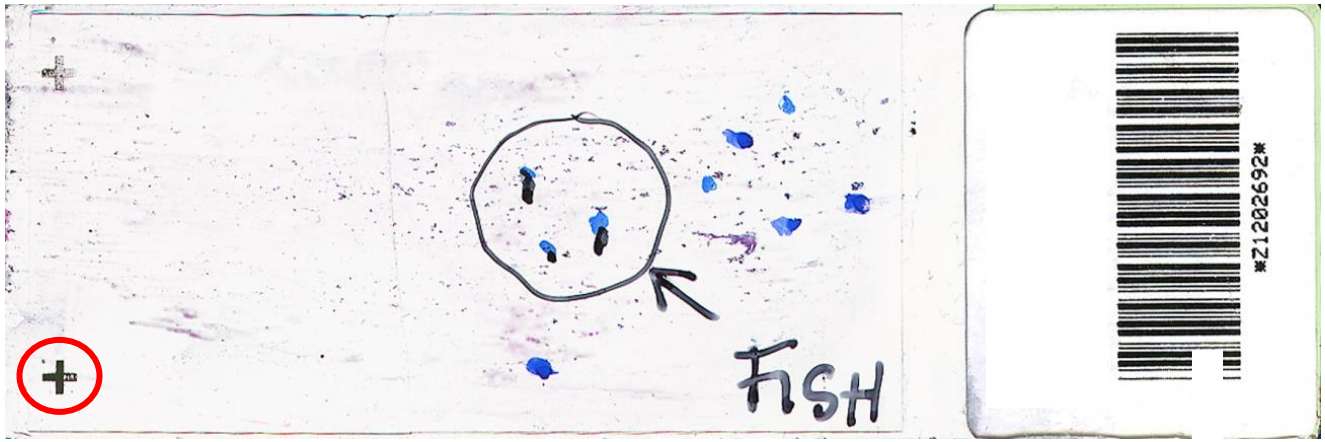


Molecular Pathology USB: From Sanger to NGS

- **April 2015 - April 2016:**
  - 1400 NGS, Ion Torrent (900 diagnostic, 500 research)
  - 65% from outside hospitals
  - 50% from cytology specimens: Adequacy rate 90%



How many cancer cells do we need?

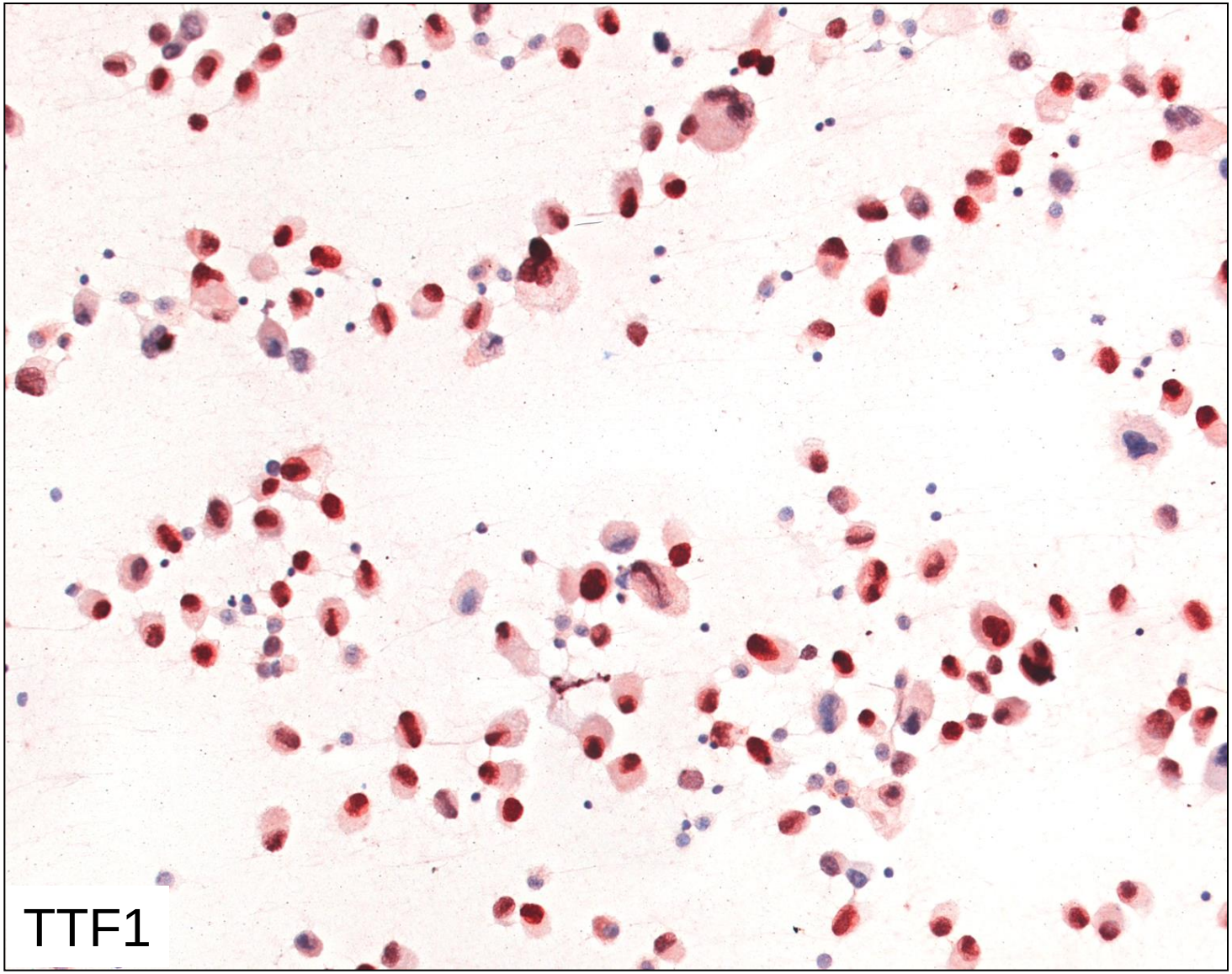


Gene Sequencing (DNA)

- NGS (IonTorrent):
  ≈ 200 (5ng), >20%
- Sanger:
  ≥ 50 (1-2ng), >30%

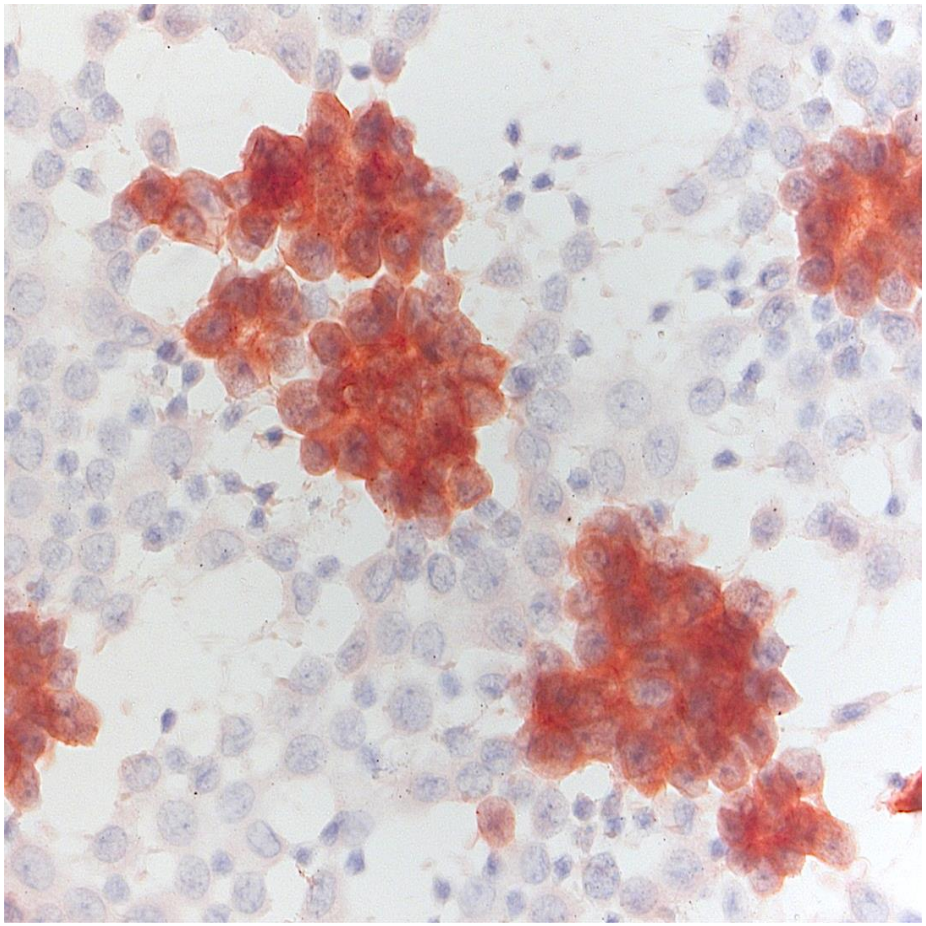
Gene Rearrangement

- FISH:
  50 - 100
- NGS (IonTorrent)(RNA):
  ≈ 500 – 1000, >20%

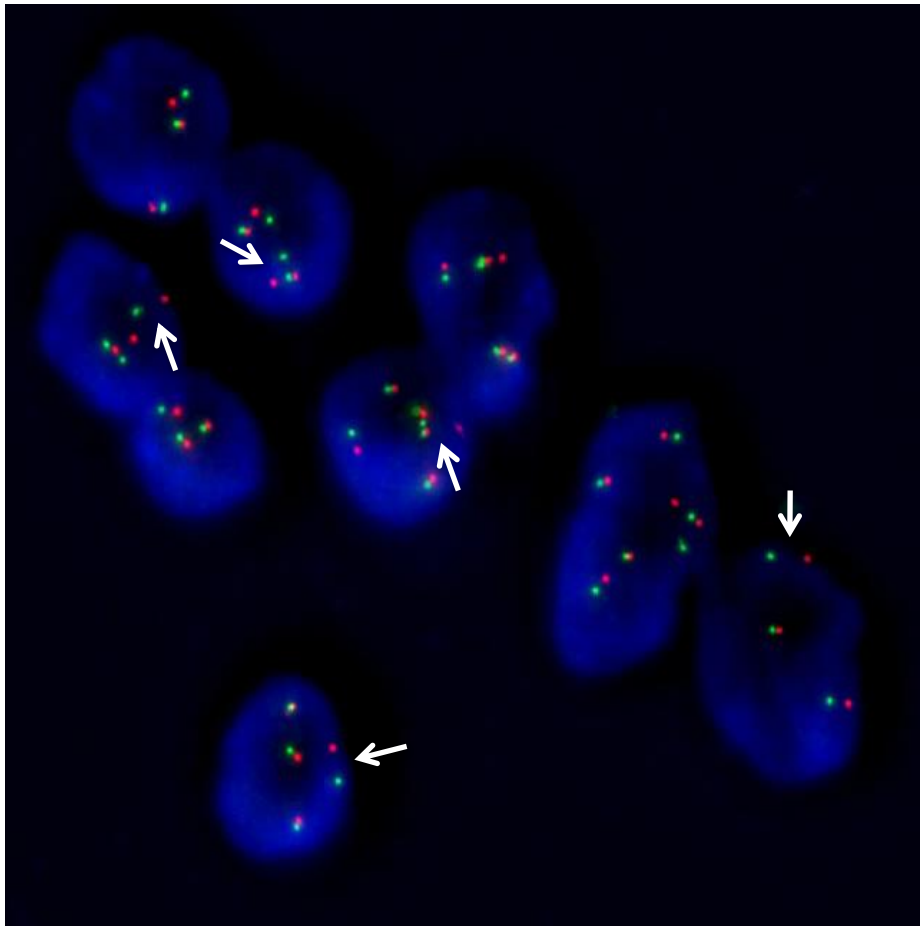


>30% cancer cells: Manual dissection

ALK Testing



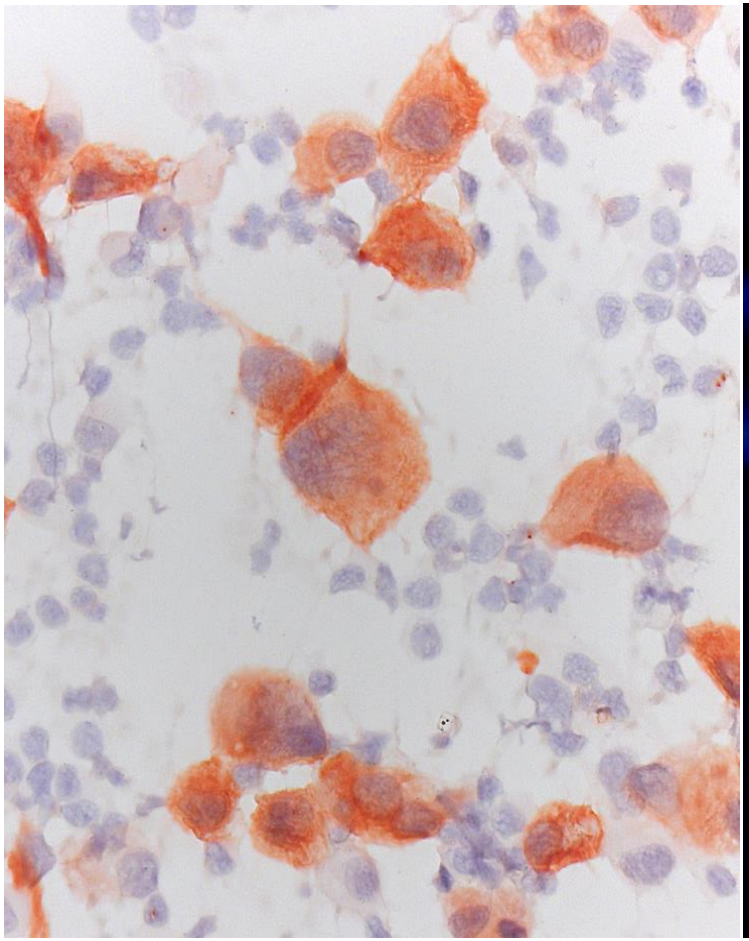
5A4 mAb
(BOND-MAX)



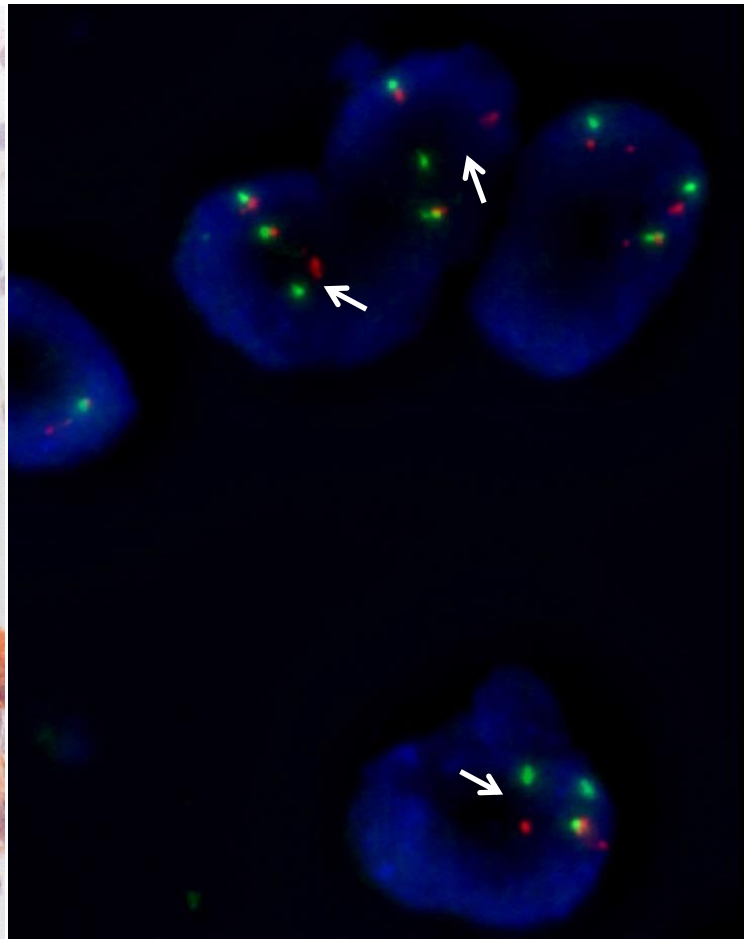
ALK FISH positive

Savic S et al, JTO 2013

ROS1 Testing



D4D6 mAb
(BOND-MAX)



ROS1 FISH positive

Bubendorf L et al, Virchows Arch 2016

Entnahme	23.08.2016
Eingang	23.08.2016
Ausgang	01.09.2016

Zytologische Diagnose

7510: Pleuraerguss, links:
Zahlreiche Zellen eines TTF-1 positiven Adenokarzinoms der Lunge.

Resultat der Gensequenzierungen des Oncomine™ Solid Tumor Panel (Tumorzellgehalt 70%):

Prädiktive Resultate:
EGFR: p.L747_P753delinsT im Exon 19 (24x%)
KRAS: keine Mutation
BRAF: keine Mutation
HER2: keine Mutation

Übrige Resultate:
TP53: p.R248W (27%), Bewertung: pathogen

Resultat der prädiktiven Immunzytochemie:
ALK (5A4 Antikörper, Leica): negativ
ROS1 (D4D6 Antikörper, Cell Signaling): negativ

Kommentar

Es zeigt sich eine bekannte, aktivierende und prädiktive EGFR Mutation. Auf die bestätigende ALK- und ROS1 FISH-Untersuchungen wird deshalb verzichtet.

Methodik

Link für die Mutationsreferenzen:
EGFR: p.L747_P753delinsT: http://www.ncbi.nlm.nih.gov/clinvar/variation/177822/
TP53: p.R248W: http://www.ncbi.nlm.nih.gov/clinvar/variation/12347/

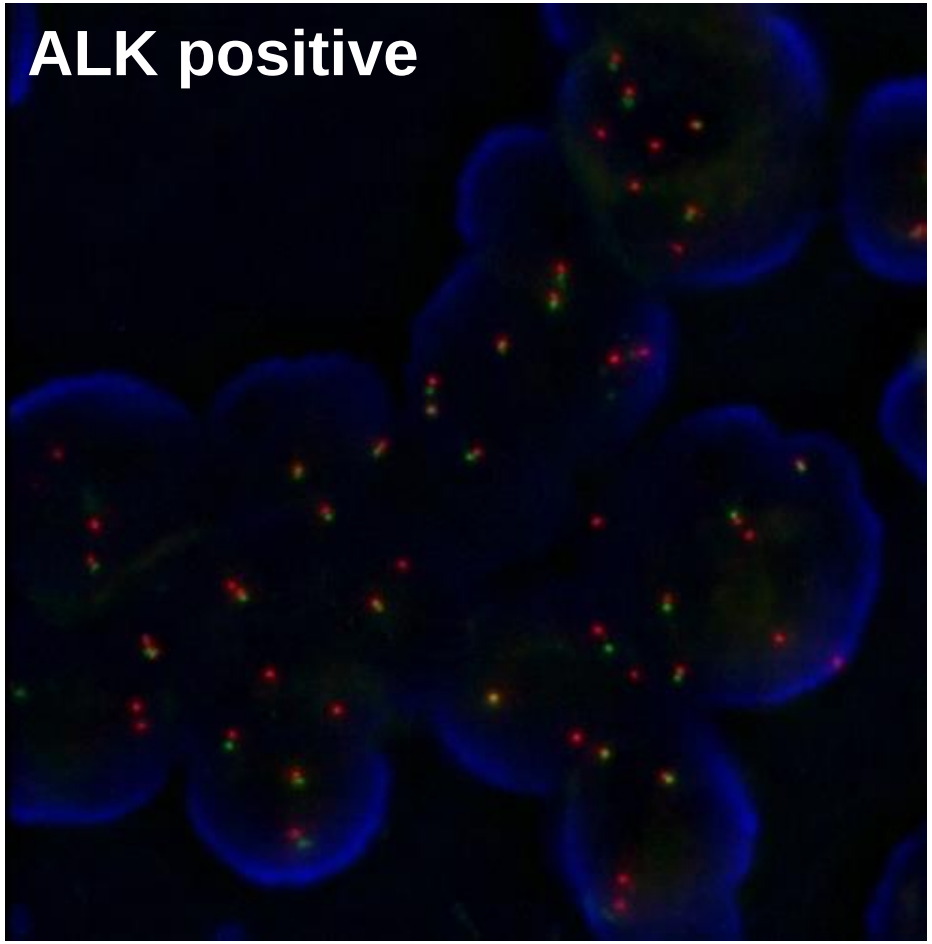
Der als Tumorzellgehalt angegebene Prozentsatz entspricht dem Verhältnis der Tumorzellen zur Gesamtanzahl der untersuchten Zellen. Anreicherung der Tumorzellen auf einen Anteil von mindestens 20% durch gezieltes mechanisches Ablösen von Objektträgern. Anschliessend folgen DNA-Extraktion aus Tumormaterial, Amplifikation der Zielsequenzen und Next-Generation-Sequencing (NGS) (Ion Torrent, Life Technologies).
Für weitere Informationen und Berichtsinterpretation siehe: http://pathologie.unispital-basel.ch (Informationen für Einsender / Molekulare Pathologie und Zytologie / NGS)

30j. female, never smoker

- 10/14 Pleural effusion: TTF1+ ADC

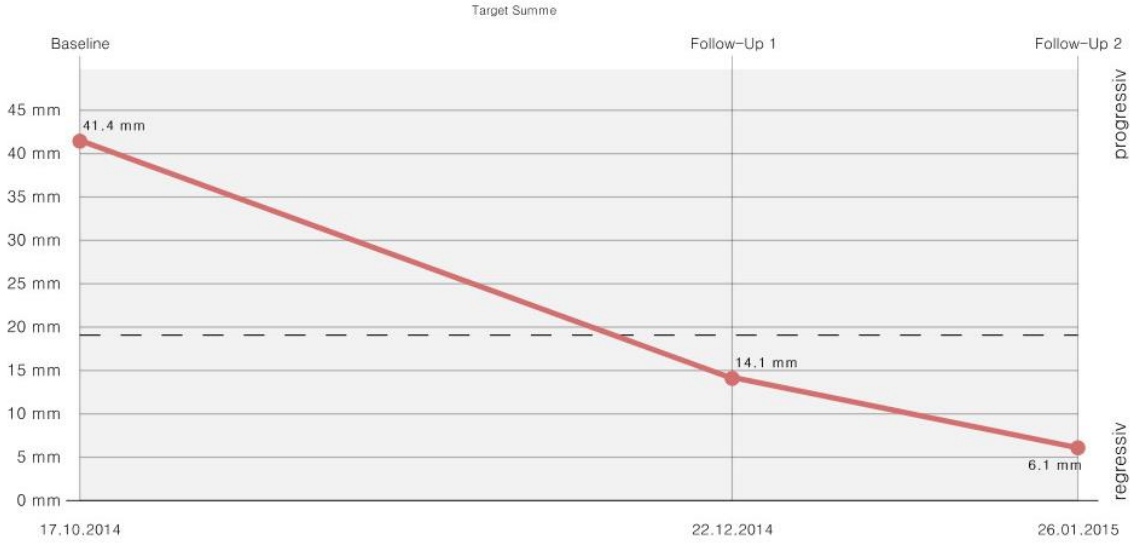
30j. female, never smoker

- 10/14 Pleural effusion: TTF1+ ADC



30j. female, never smoker

- 10/14 Pleural effusion: TTF1+ ADC, ALK+
- Treatment with Alectinib (ALEX clinical trial)



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- 10/14 Pleural effusion: TTF1+ ADC, ALK+
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- 03/15 Progressive disease: Pleural effusion

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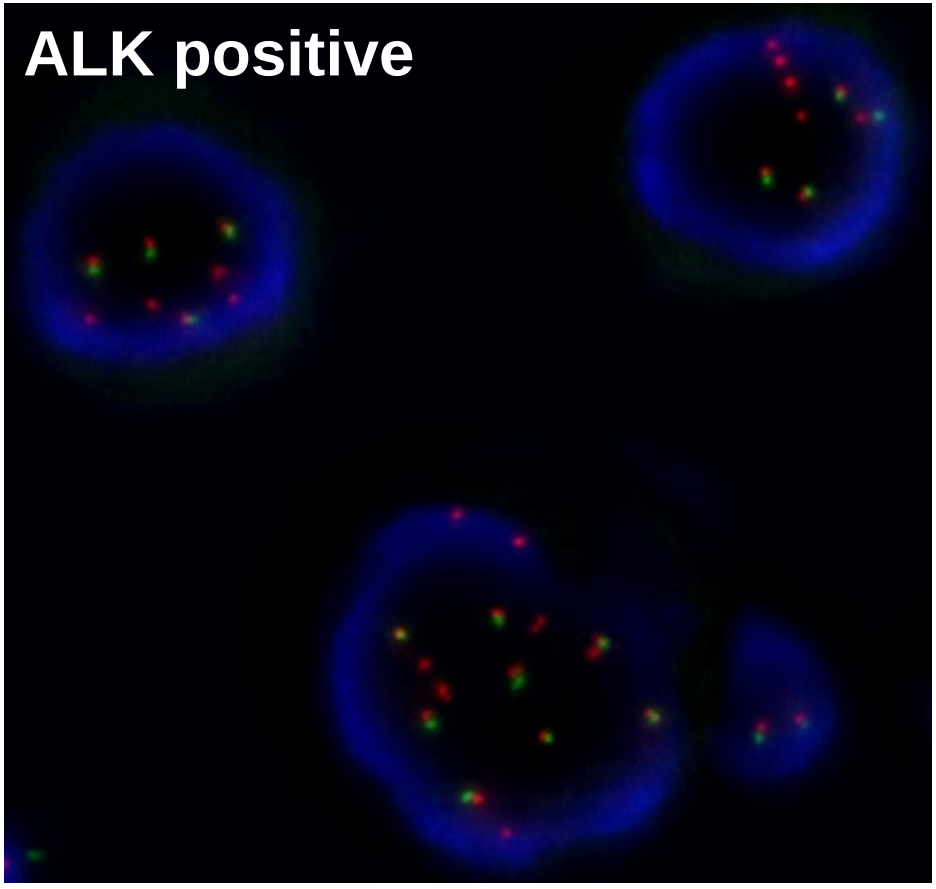
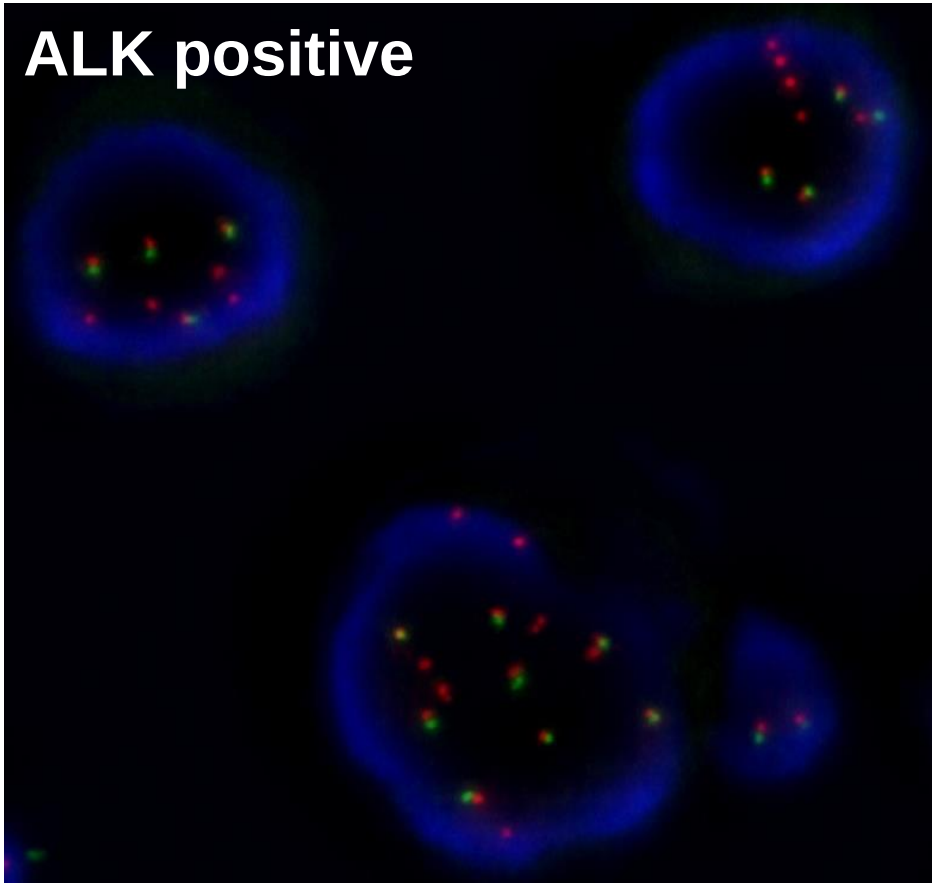
- 10/14 Pleural effusion: TTF1+ ADC, ALK+
- Treatment with Alectinib (ALEX clinical trial)
- 03/15 Progressive disease: Pleural effusion



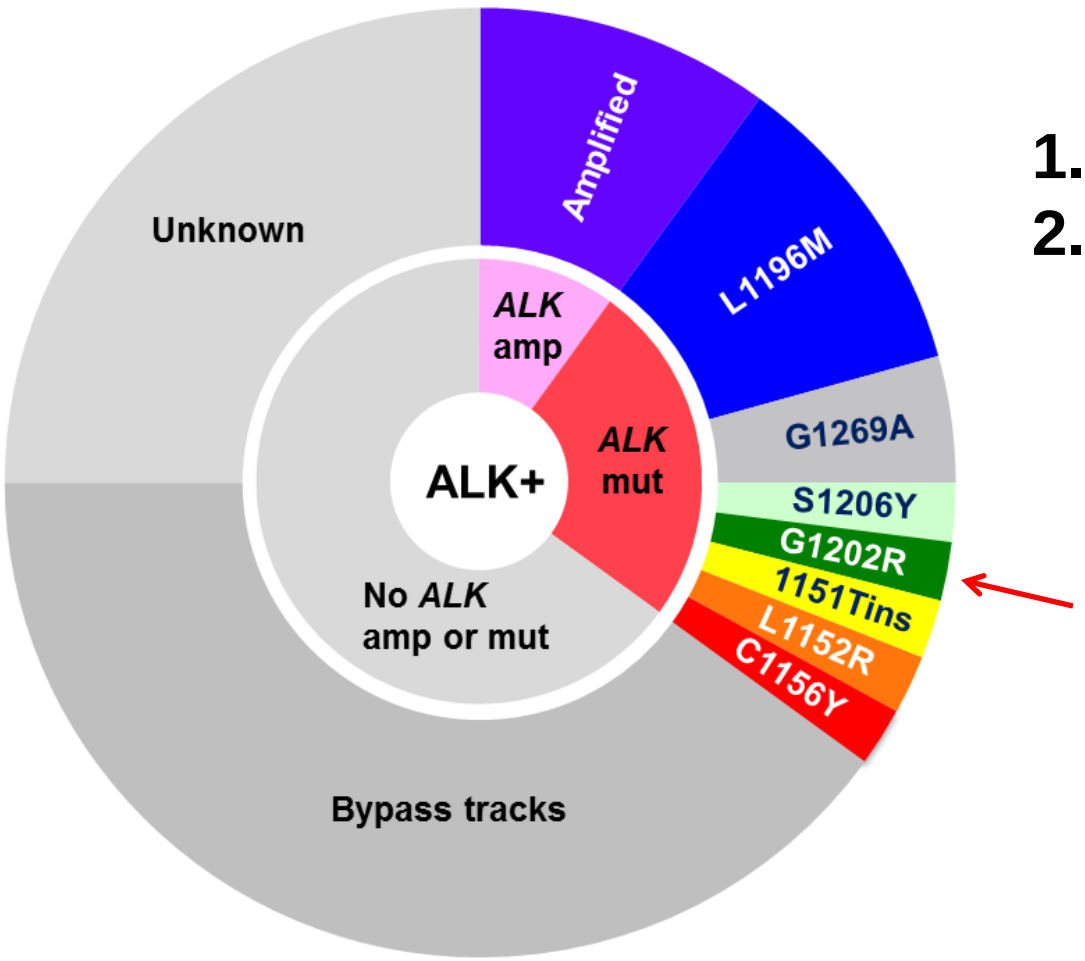
30j. female, never smoker

- 10/14 Pleural effusion: TTF1+ ADC, ALK+
- Treatment with Alectinib (ALEX clinical trial)
- 03/15 Progressive disease: Pleural effusion



NGS:
p.G1202R ALK mutation

Mechanisms of Resistance to Crizotinib



1. **Target modification**
2. **Bypass signalling**

Doebele RC et al, Clin Cancer

ALK resistance mutations and various ALK inhibitors

Cellular ALK Phosphorylation Mean IC50 (nM)

Mutation status	Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib
Parental Ba/F3	763.9	885.7	890.1	2774.0	11293.8
EML4-ALK V1	38.6	4.9	11.4	10.7	2.3
EML4-ALK C1156Y	61.9	5.3	11.6	4.5	4.6
EML4-ALK I1171N	130.1	8.2	397.7	26.1	49.0
EML4-ALK I1171S	94.1	3.8	177.0	17.8	30.4
EML4-ALK I1171T	51.4	1.7	33.6[a]	6.1	11.5
EML4-ALK F1174C	115.0	38.0[a]	27.0	18.0	8.0
EML4-ALK L1196M	339.0	9.3	117.6	26.5	34.0
EML4-ALK L1198F	0.4	196.2	42.3	13.9	14.8
EML4-ALK G1202R	381.6	124.4	706.6	129.5	49.9
EML4-ALK G1202del	58.4	50.1	58.8	95.8	5.2
EML4-ALK D1203N	116.3	35.3	27.9	34.6	11.1
EML4-ALK E1210K	42.8	5.8	31.6	24.0	1.7
EML4-ALK G1269A	117.0	0.4	25.0	ND	10.0
EML4-ALK D1203N+F1174C	338.8	237.8	75.1	123.4	69.8

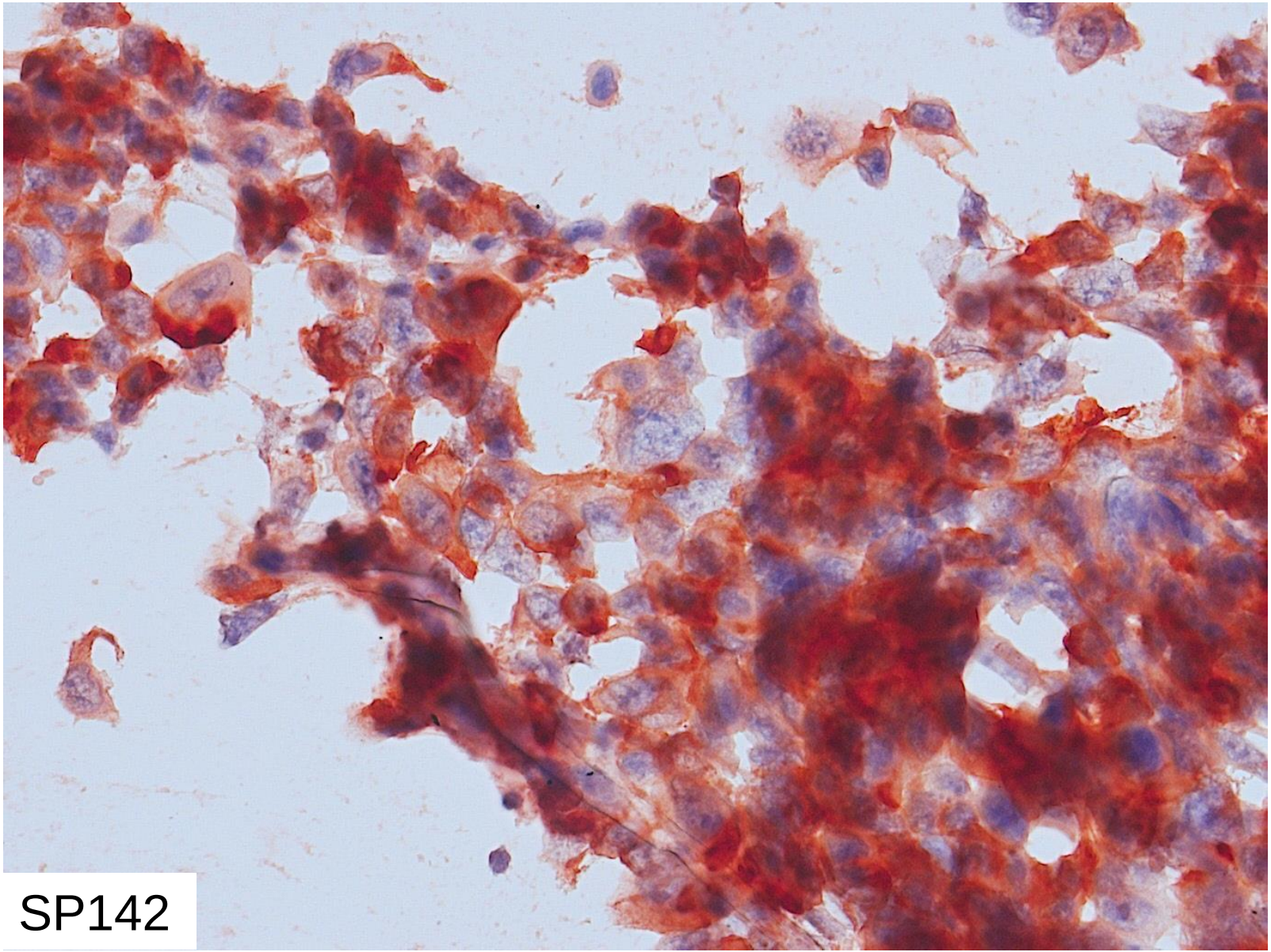
IC50 ≤ 50 nM

IC50 > 50 <200 nM

IC50 ≥ 200 nM

Gainor JF et al. et al., Cancer Discov 2016

PD-L1 ICC
(SP 142, 22C3, SP 163, E1L3N)



Summery

- Cave reactive atypia
- Foreign population in most carcinomas
- Don’t miss dissolute medium sized tumors
- Exclude MM in recurrent effusion
  - Even if there is no evidence of MM on imaging
  - Even if there is only mild cytological atypia

Summery

- ICC and molecular analyses common practice in pleural effusion cytology
  - Diagnostic and predictive ICC
  - FISH (9p21 del.) might allow for a def. diagnosis of MM
  - Reflex molecular predictive marker analyses in NSCLC

Thanks!

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Cytopathology team

Michel Bihl
Luca Quagliata