Urinary Cytology

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Outline

- Pre-analytics
- The Paris System (TPS):
  - Background
  - Diagnostic categories
  - Morphologic criteria for each category
- Ancillary FISH testing
- Examples

Urinary Cytology: Ancillary FISH Testing

Ancillary FISH Testing

- Why?
- When?
- How?

Urinary Tumor Markers

- DNA-based
  - UroVysion® FISH Test
  - Microsatellite/LOH detection
- RNA- and protein-based
  - Cxbladder (uRNA-2)
  - uCyt+/ ImmunoCyt
  - BTA stat/ TRAK
  - NMP22
- Epigene-based
  - DNA methylathion

Add significant benefit?
Cost-effectiveness?
Standardization?
Validation?

Sapre N et al. Urol Oncol 2014
Ashish MK e al. Eur Urol 2012

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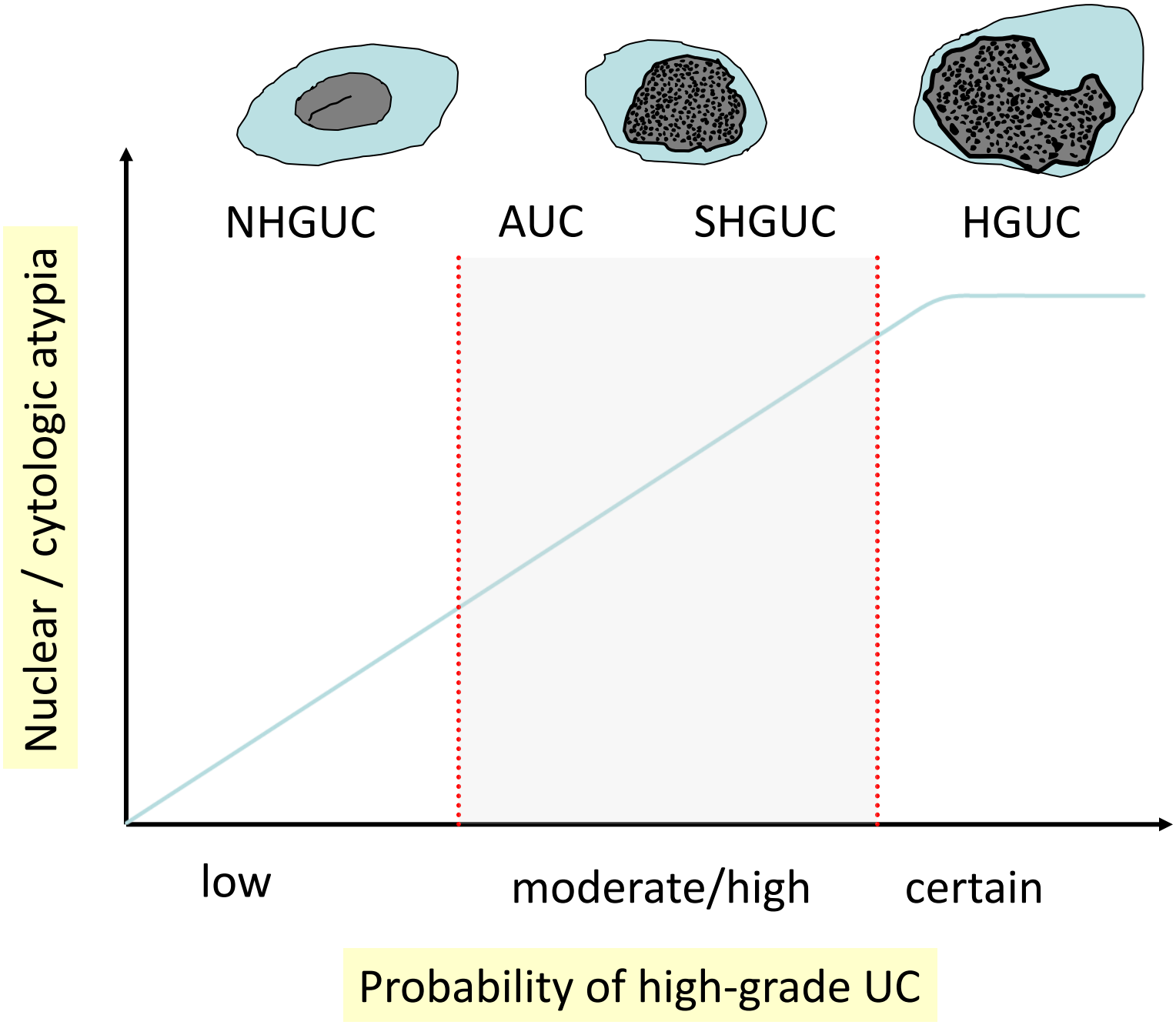

NHGUC
AUC
SHGUC
HGUC
Nuclear / cytologic atypia
low
moderate/high
certain
Probability of high-grade UC

Atypical

High grade UC

Spec./PPV >90%

Malignant

↓

Diagn. work-up
Treatment

Atypical

High grade UC

8-31%

Risk of malignancy

21-71%

Ubago JM et al. Cancer Cytopathol. 2013
Rosenthal DL et al. Cancer Cytopathol. 2013

Atypical

High grade UC

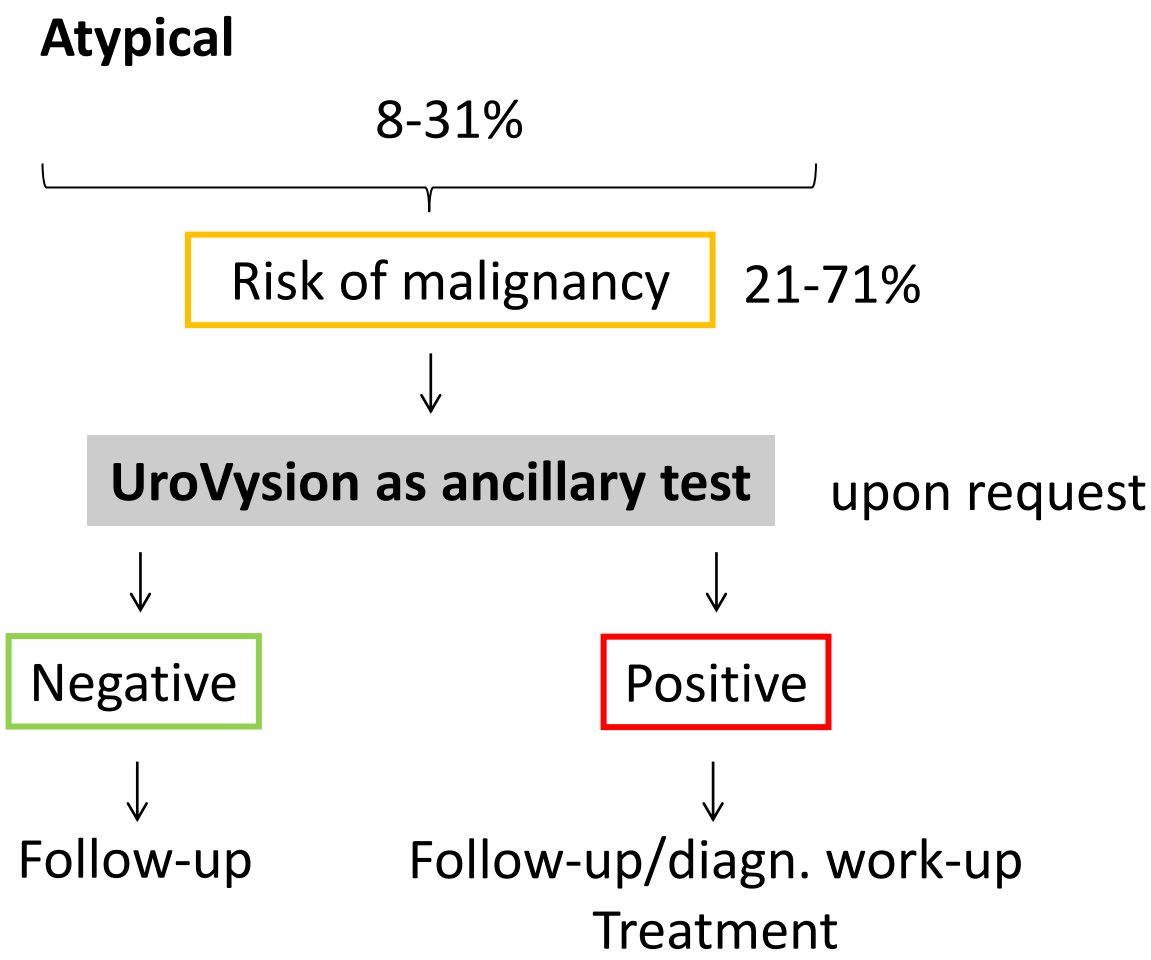
8-31%

Risk of malignancy 21-71%

negative or equivocal cystoscopy

↓

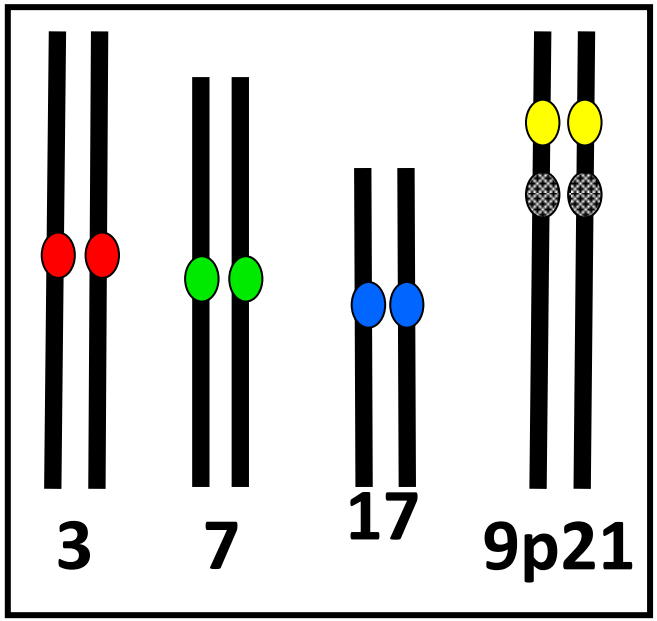
?


Atypical
High grade UC
8-31%
Risk of malignancy
21-71%
UroVysion as ancillary test
upon request
Negative
Positive
Follow-up
Follow-up/diagn. work-up
Treatment

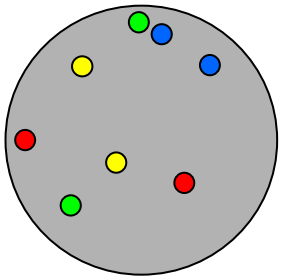
UroVysion FISH Test

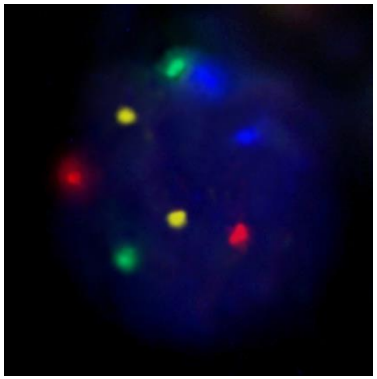
- High-grade UC
  - no additional benefit compared to cytology (sens. & spec. 70-100%)
- Low-grade UC
  - can increase sens. of cytology from 25% to 60-75%
  - usually visible by cystoscopy
- Ancillary test for clarification of atypical urothelial cells
  - detection of non-visible high-grade UC

UroVysion®
(Vysis / Abbott Mol.)

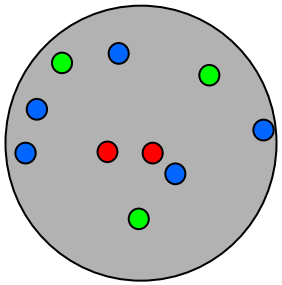


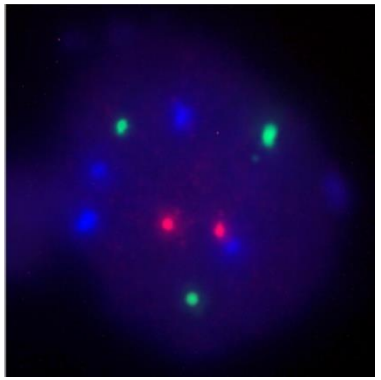
FISH **positive** result:
≥4/25 cells with gains in ≥2 chr. or
≥12/25 cells with homocyg. del 9p21





negative





positive

Sokolova IA et al. J Mol Diagn 2000

UroVysion FISH Test

- Sensitivity: range 8-100%
- Specificity: range 29-100%

→ lack of standardization

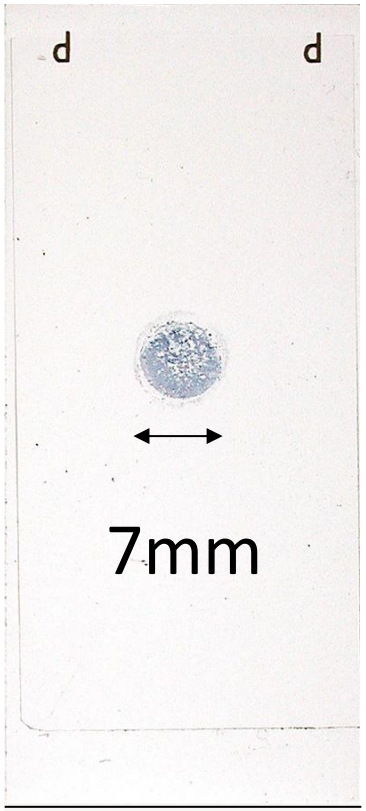
Dimashkieh H et al. Cancer Cytopathol 2013

UroVysion FISH Test

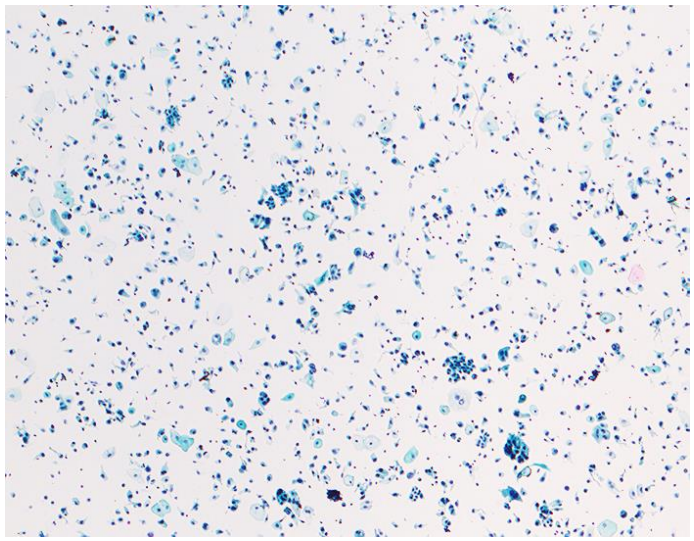
Performance depends on:

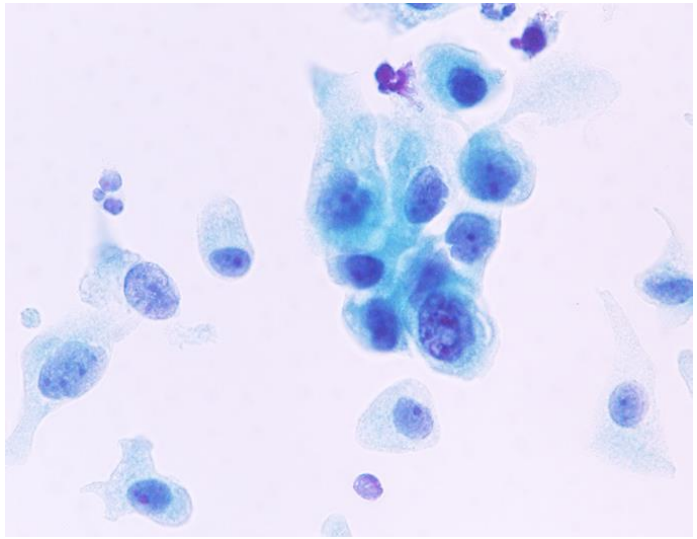
- UC: Low-grade – high-grade
- Urinary specimen (cellularity)
- Technical procedures
  - FISH on PAP stained slide – on remaining cellular material
- Test evaluation
  - automated relocation
  - definition of a positive result
  - pitfalls (false positivity): Tetraploidy, radiation

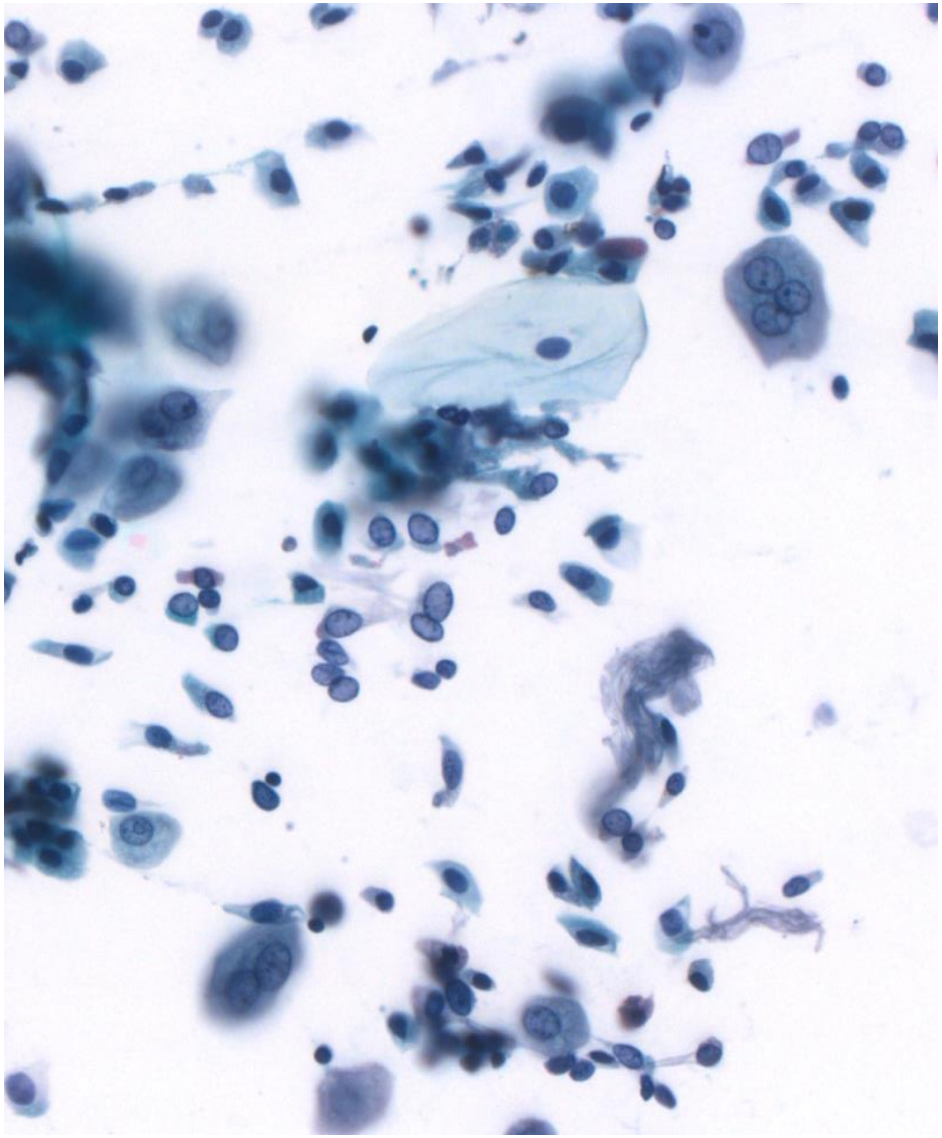
- Fresh
- 50% ethanol 1:1

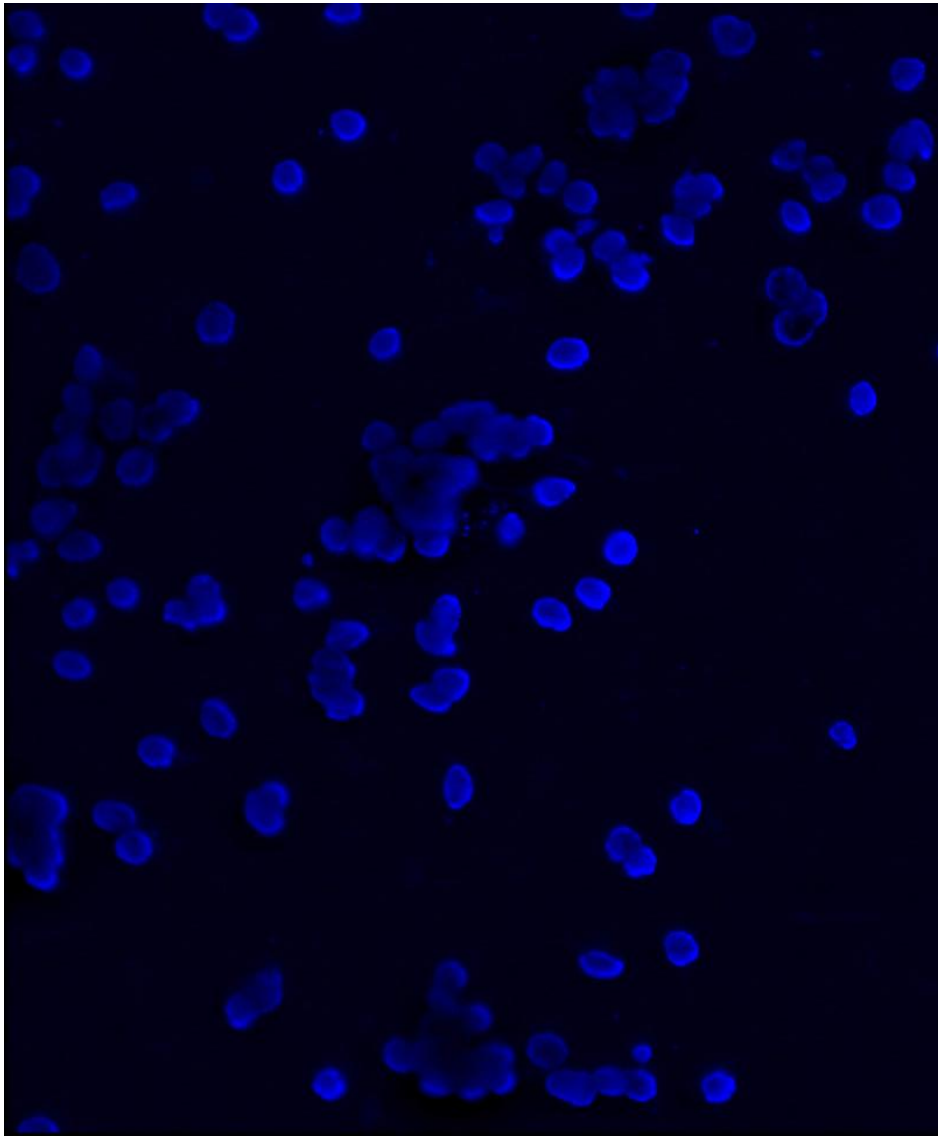


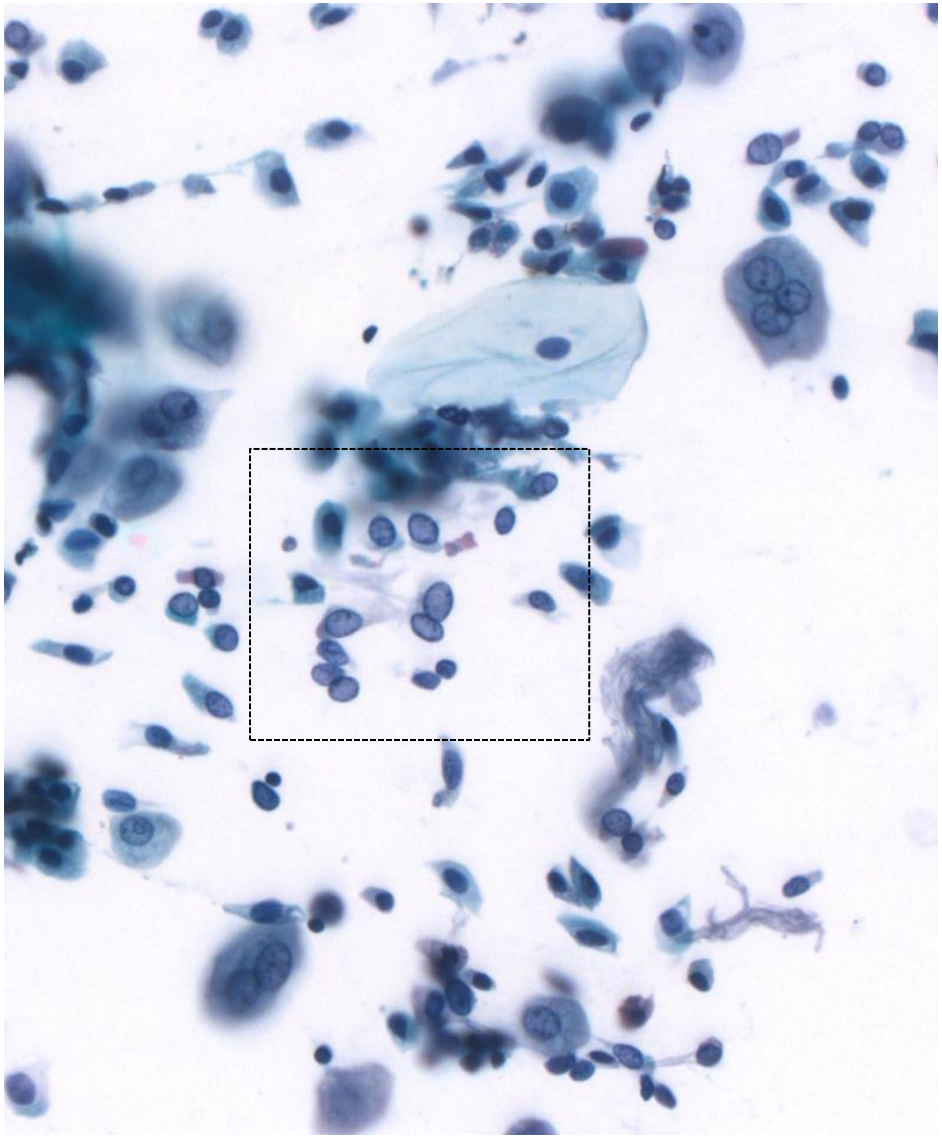
coated glass slide

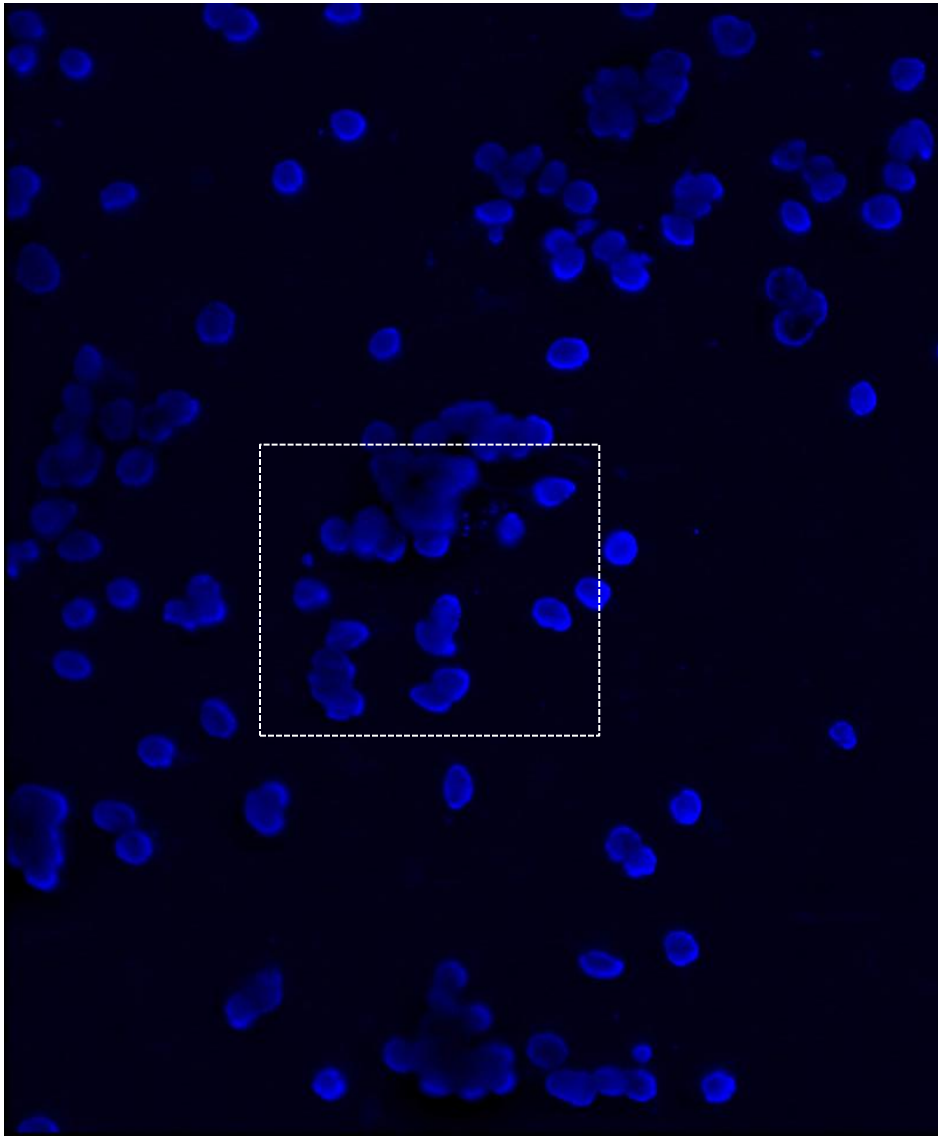


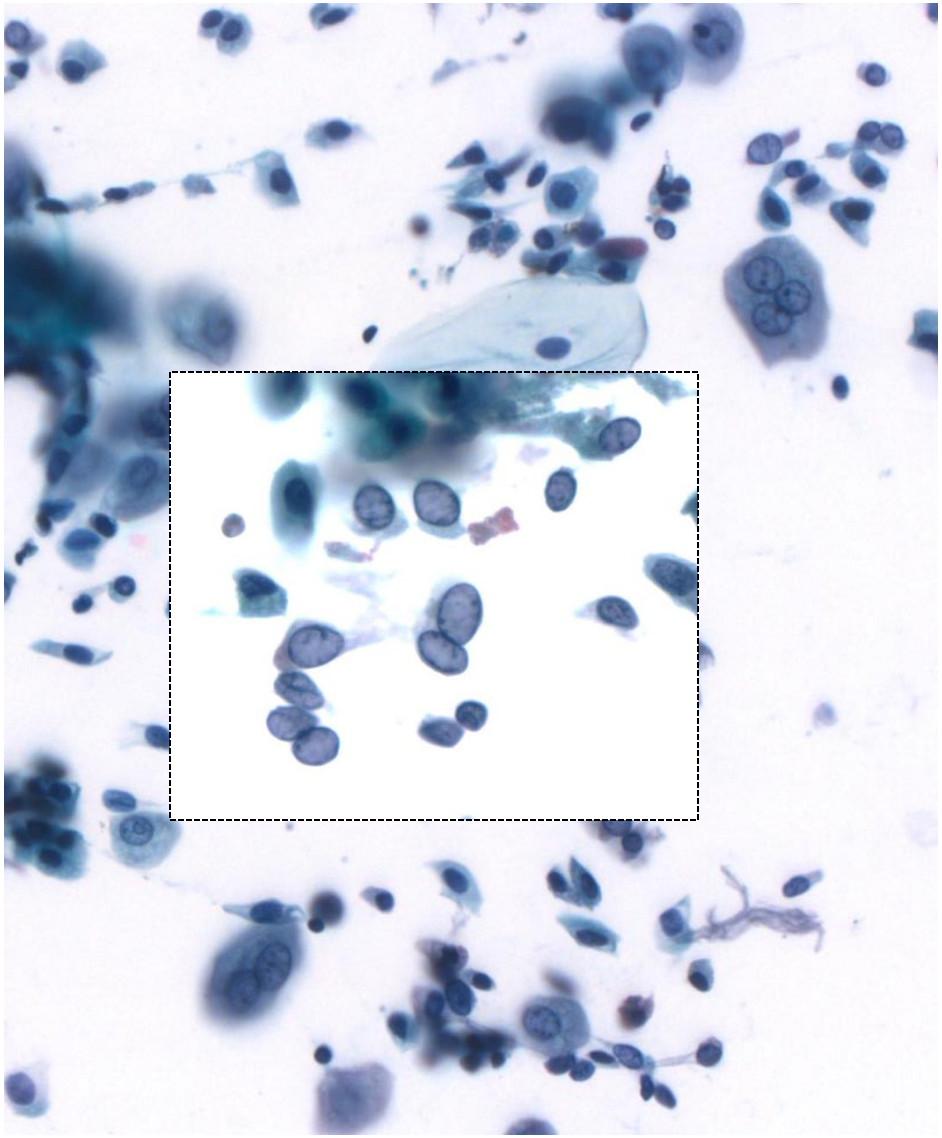


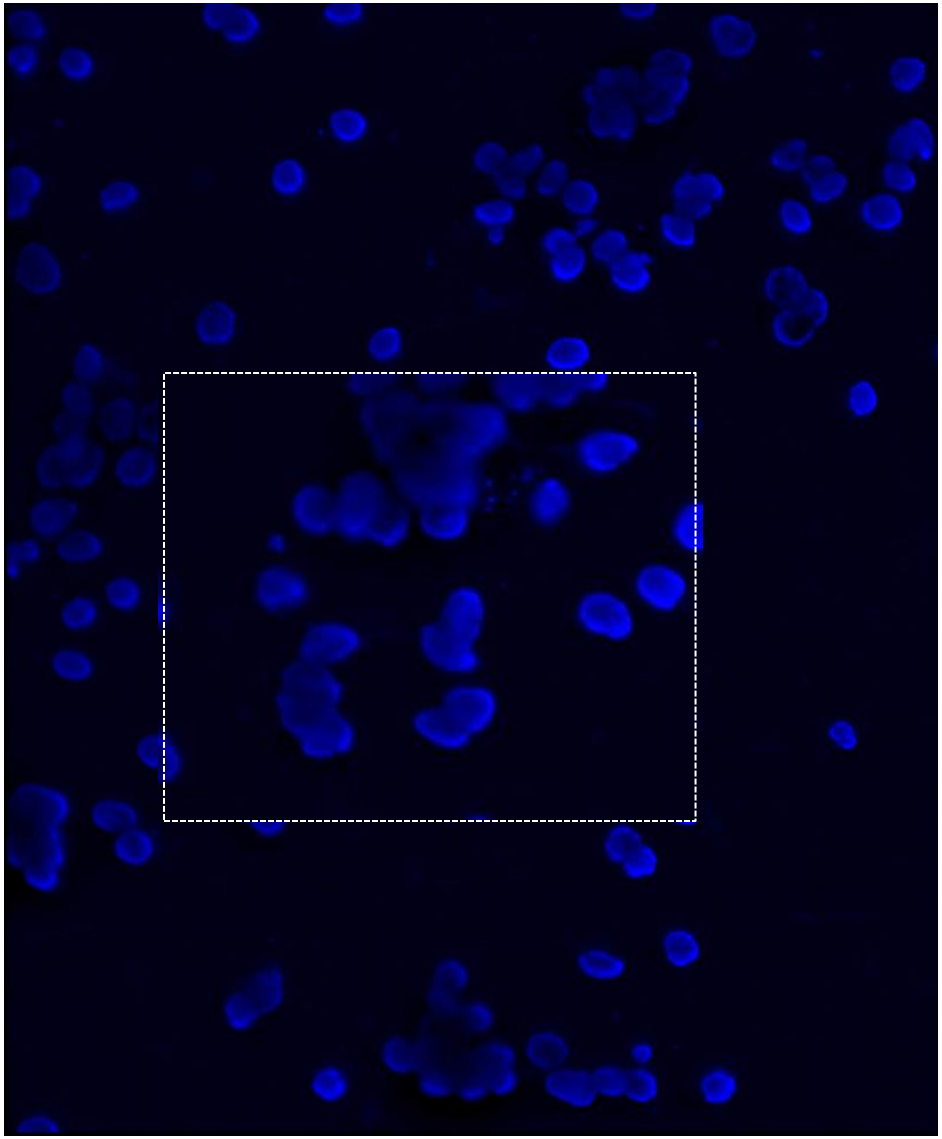




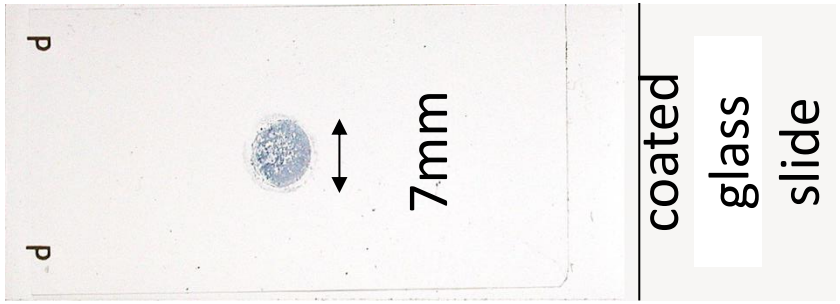


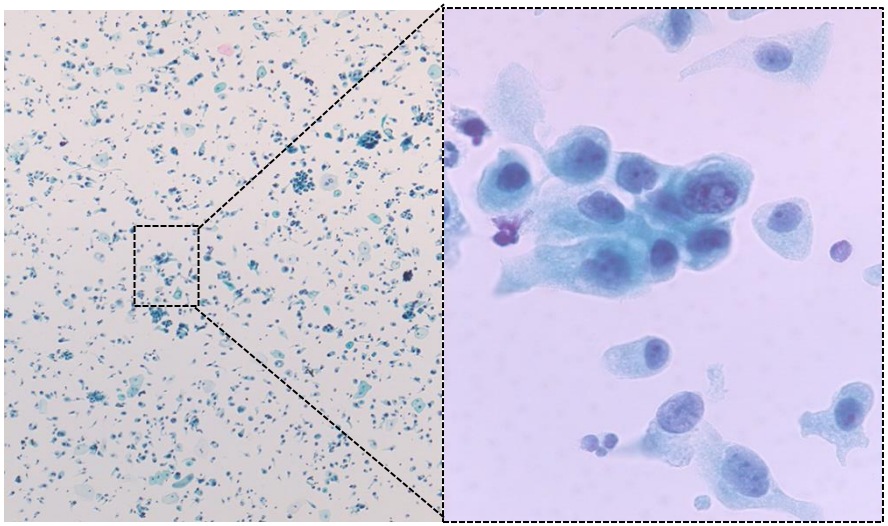






UroVysion FISH on cytological specimens

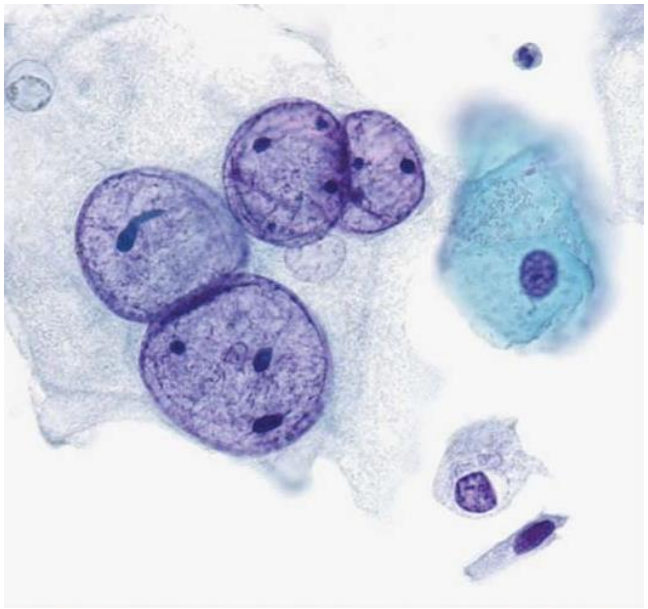




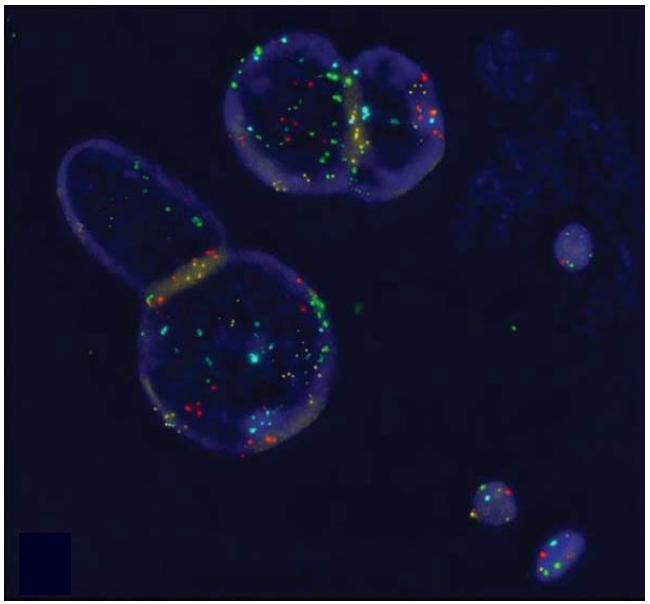
Automated cross table with relocation

Chromosomal aberrations in benign urinary cytology: False positive FISH

Reactive umbrella cells



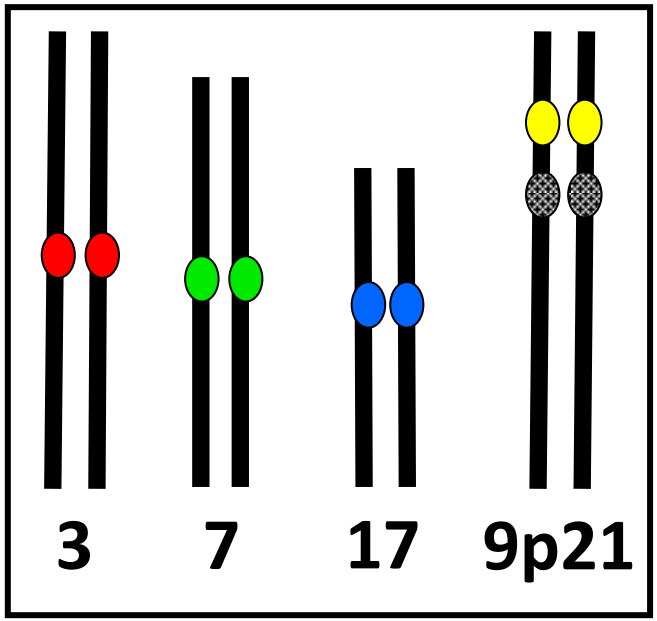
Polyploidy

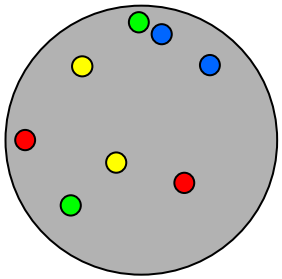


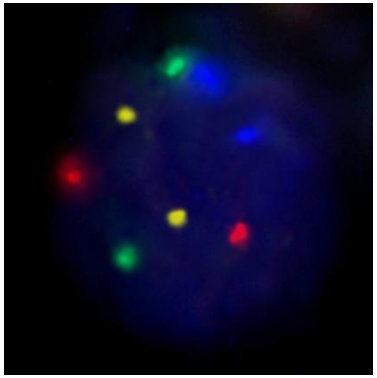
- Tetraploidy common in benign urothelial cells
- Radiation induced chromosomal aberrations
- No 9p21 deletions

Tapia C et al. Cancer Cytopathol 2011
Wojcik EM et al. Annal Quant Cytol Histol 2000

UroVysion®
(Vysis / Abbott Mol.)

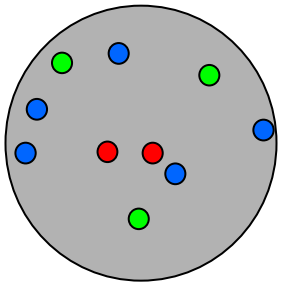


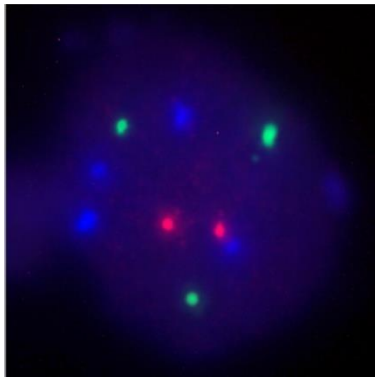




negative

FISH **positive** result:
≥4/25 cells with gains in ≥2 chr. or
≥12/25 cells with
homocyg. **or heteroc. del 9p21**
Tetraploidy >10/25 cells

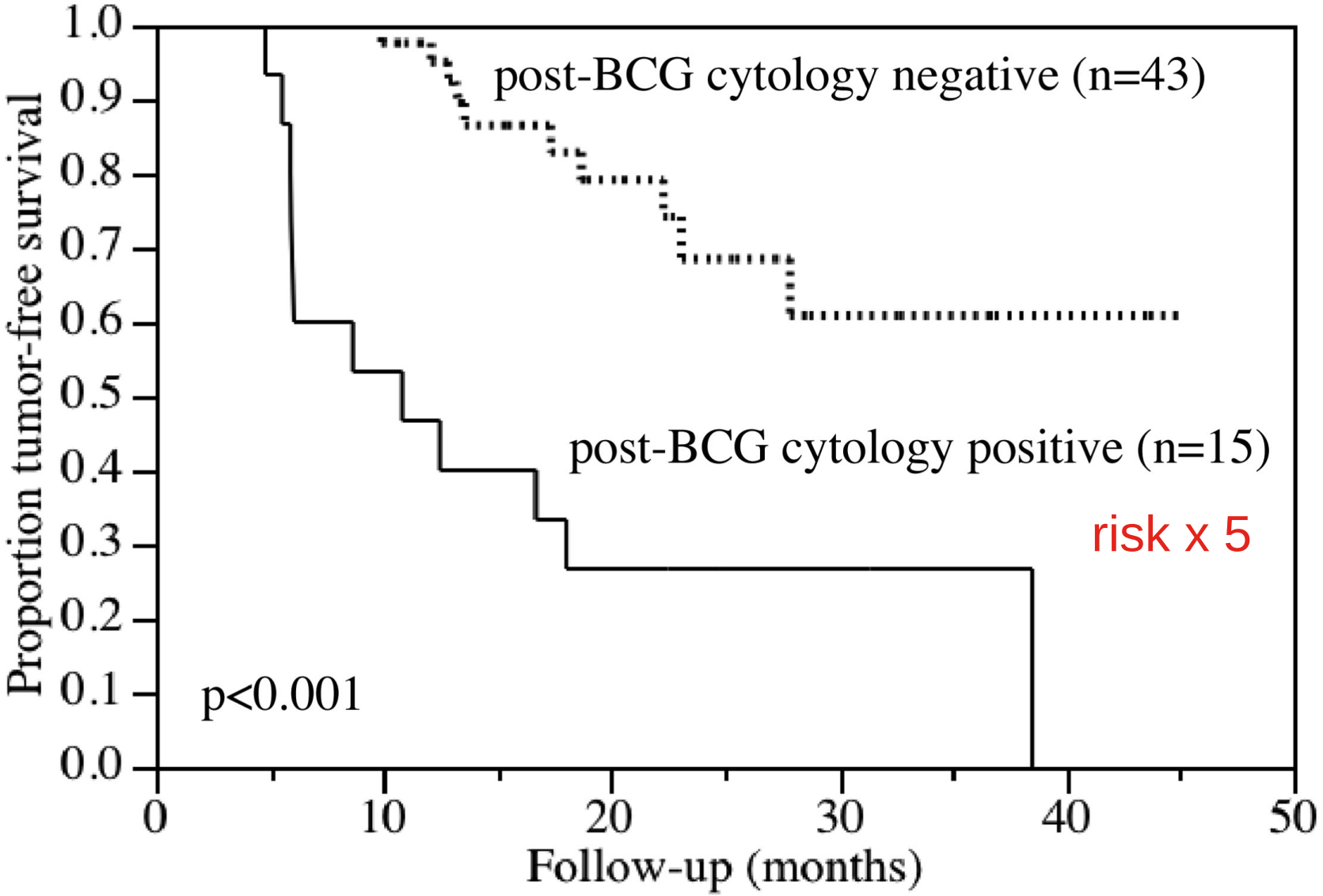




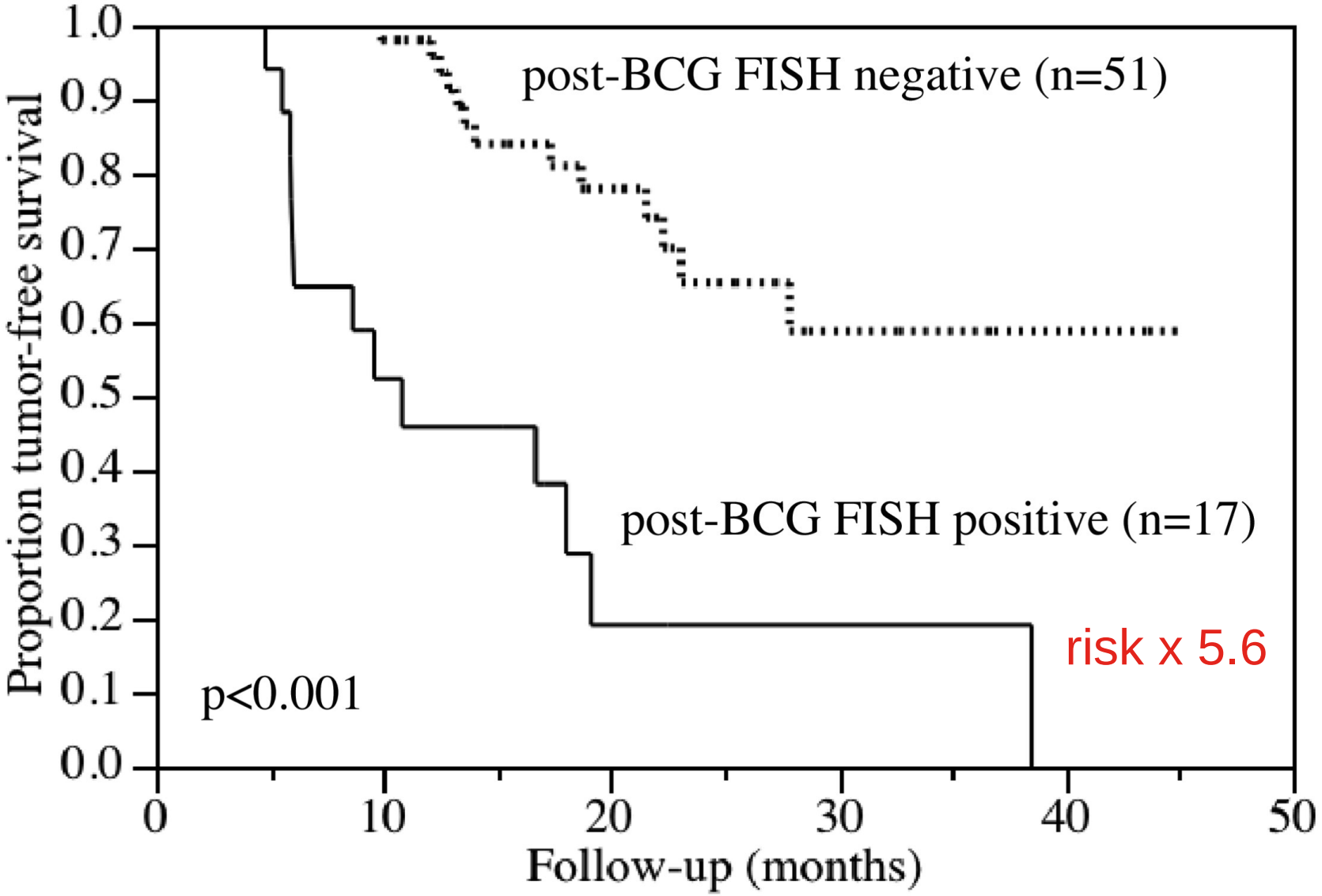
positive

Savic S et al. Int J Cancer 2009
Zellweger T et al. Int J Cancer 2006

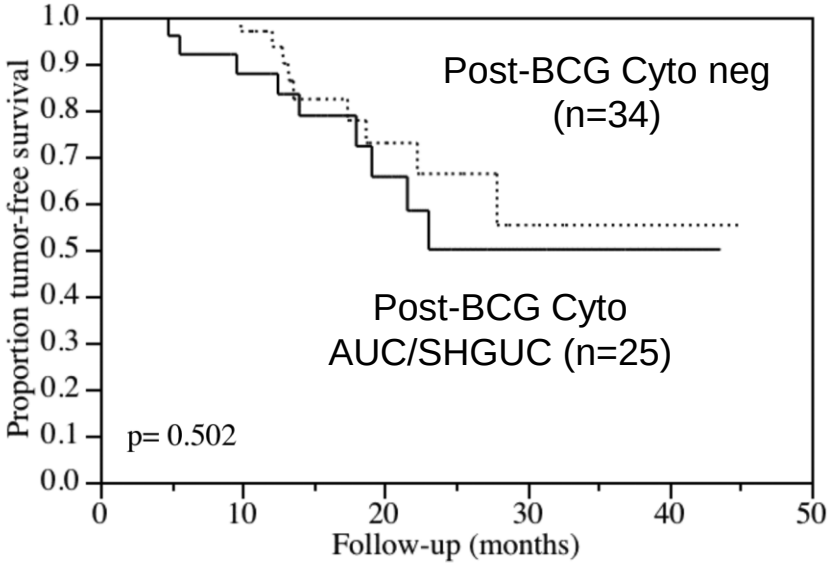
Cytology after BCG

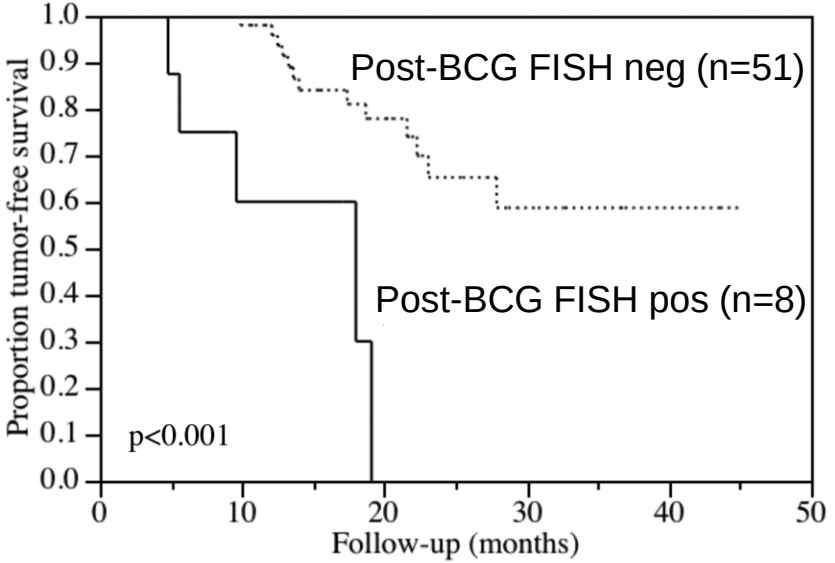


FISH after BCG

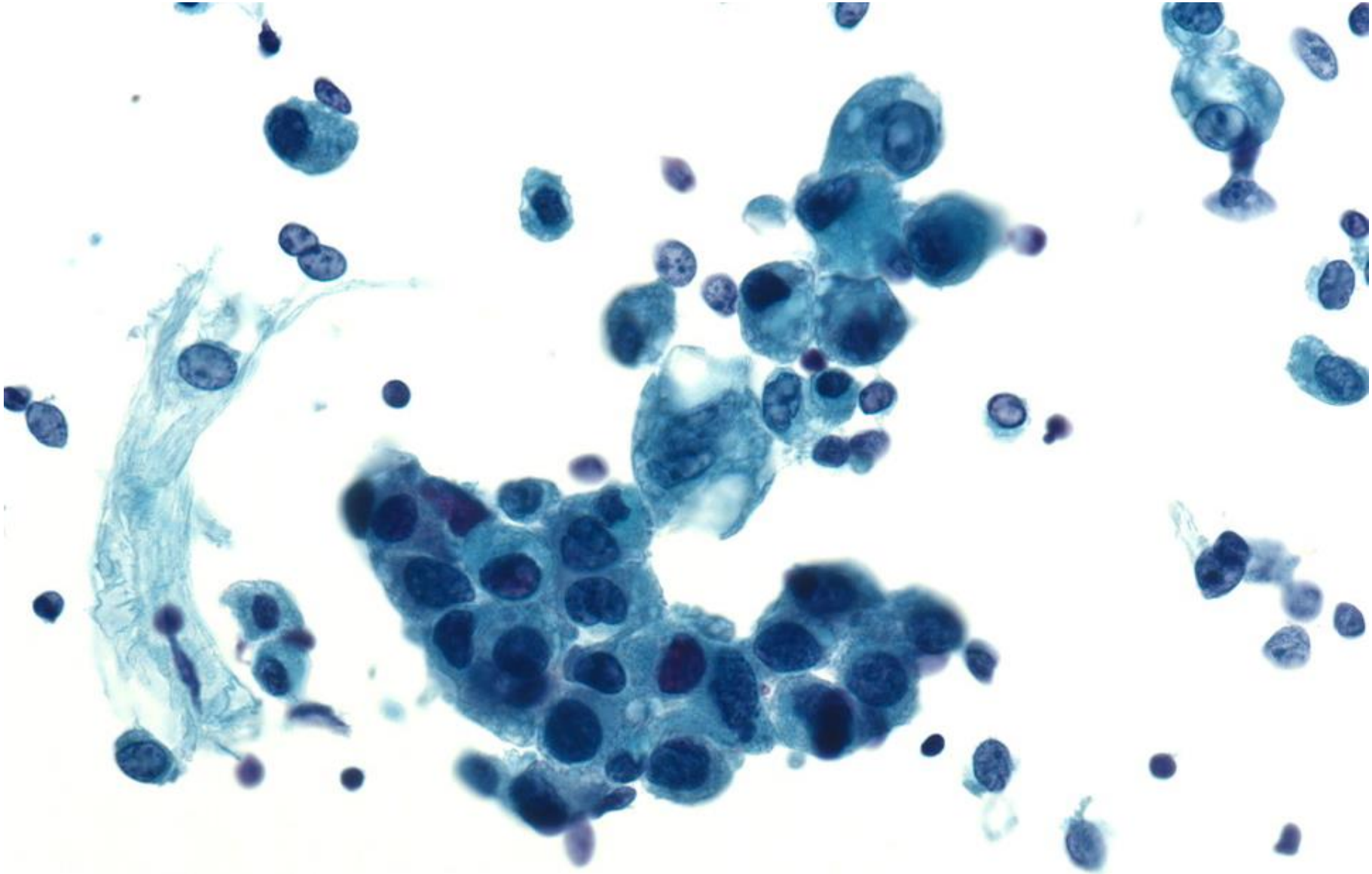


FISH after BCG in non-conclusive Cytology
(AUC, SHGUC)

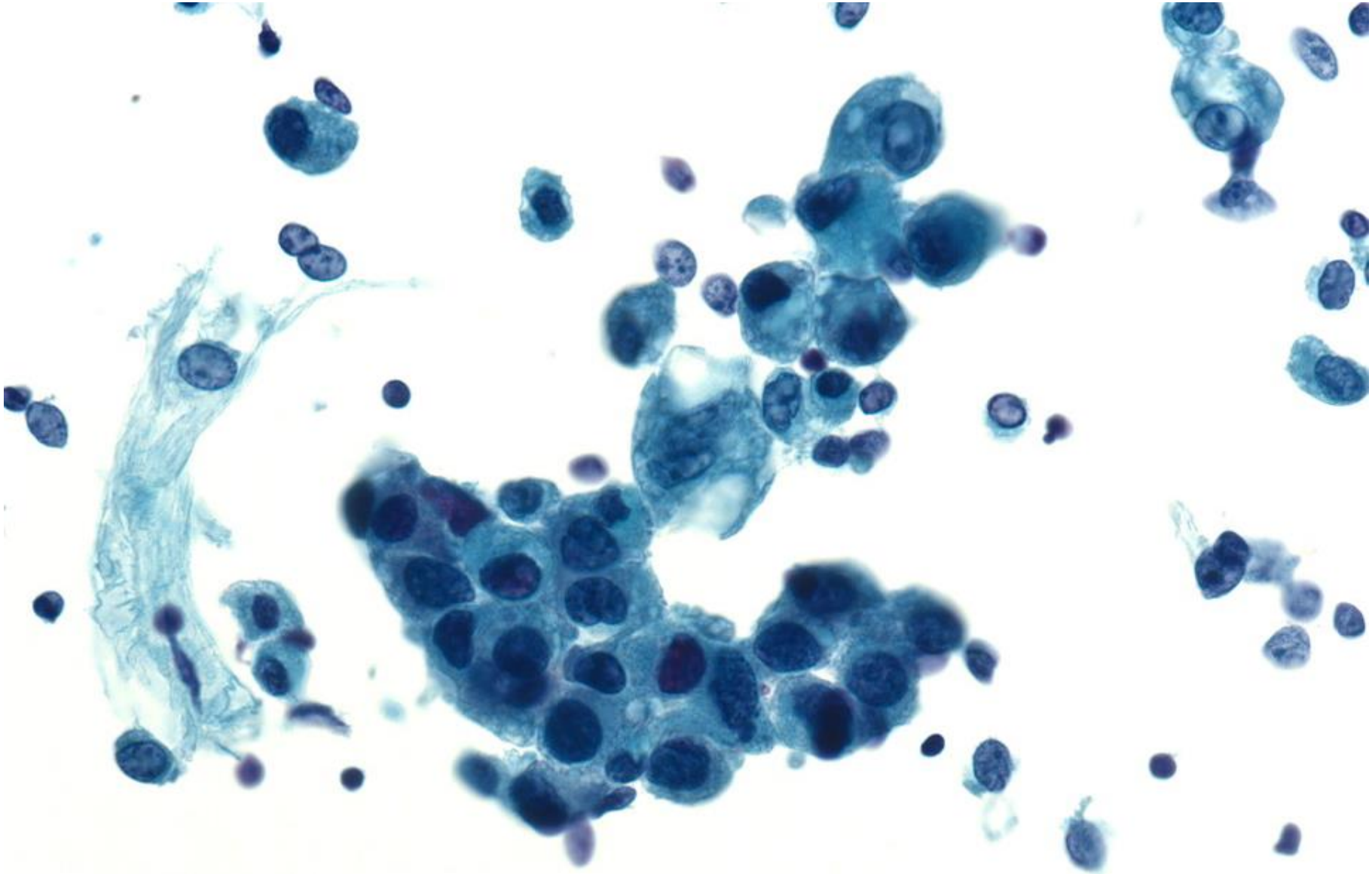




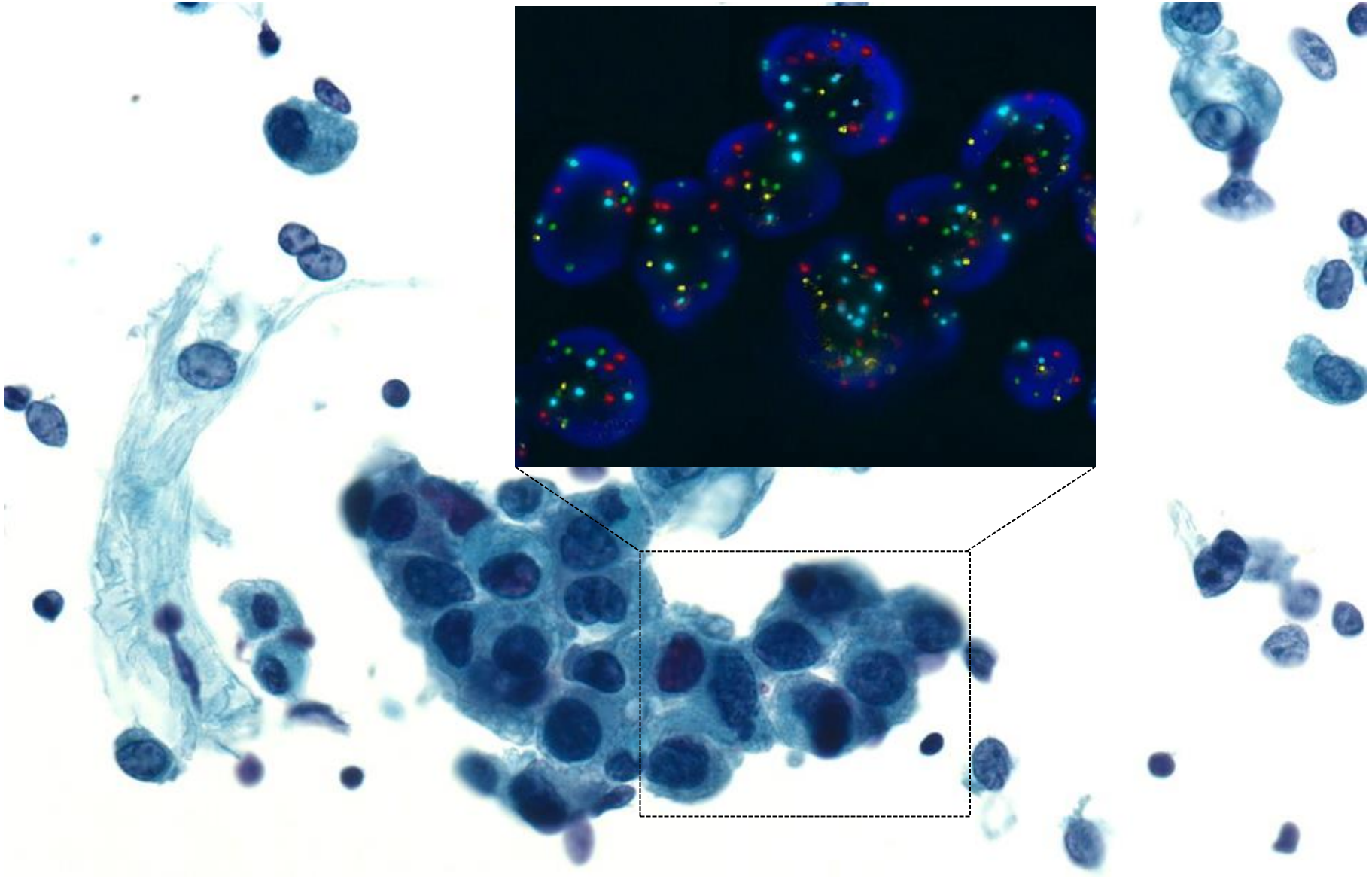
79y, renal pelvic washing, history of pTaG2 high grade of the bladder



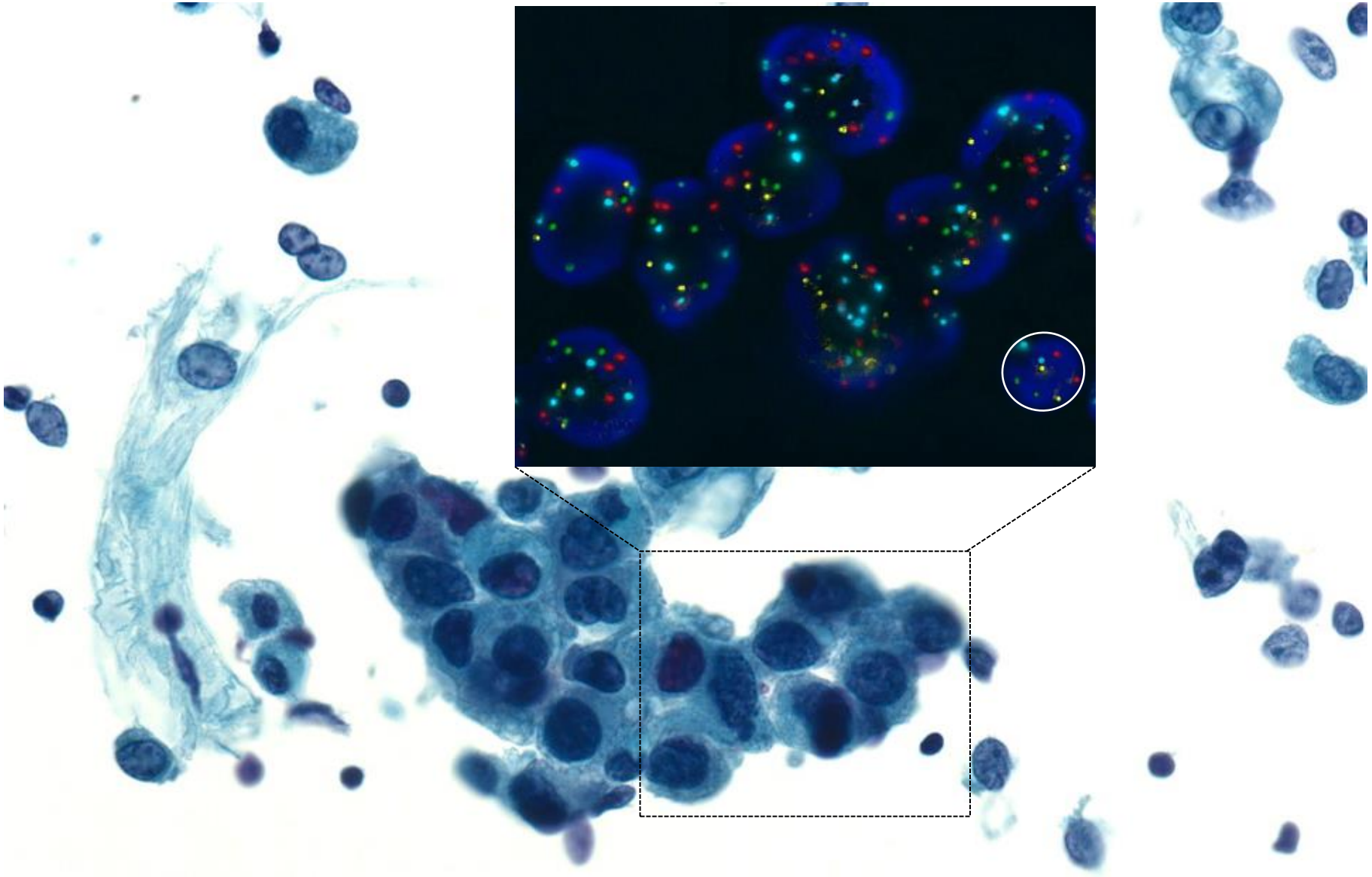
AUC



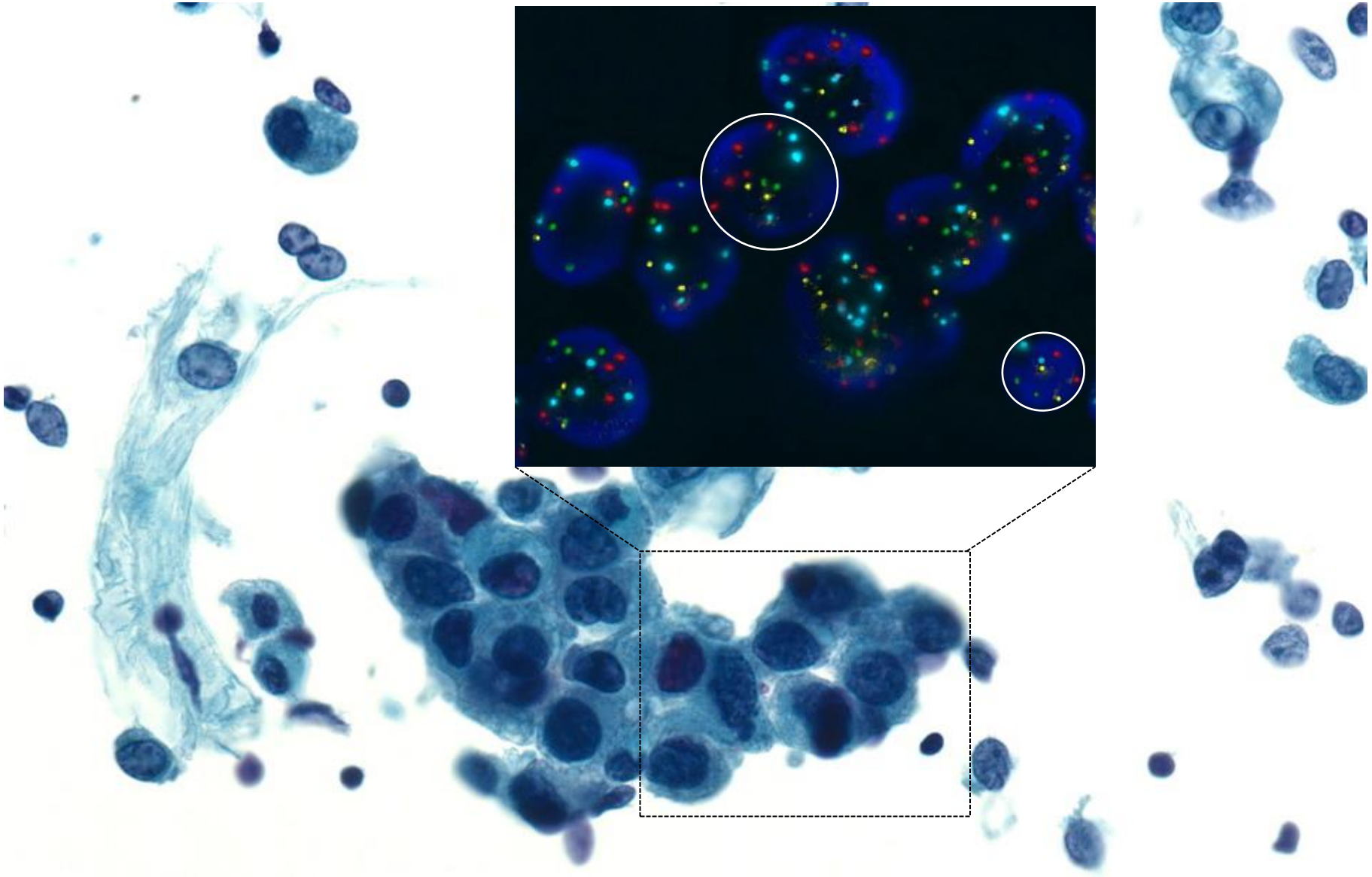
AUC



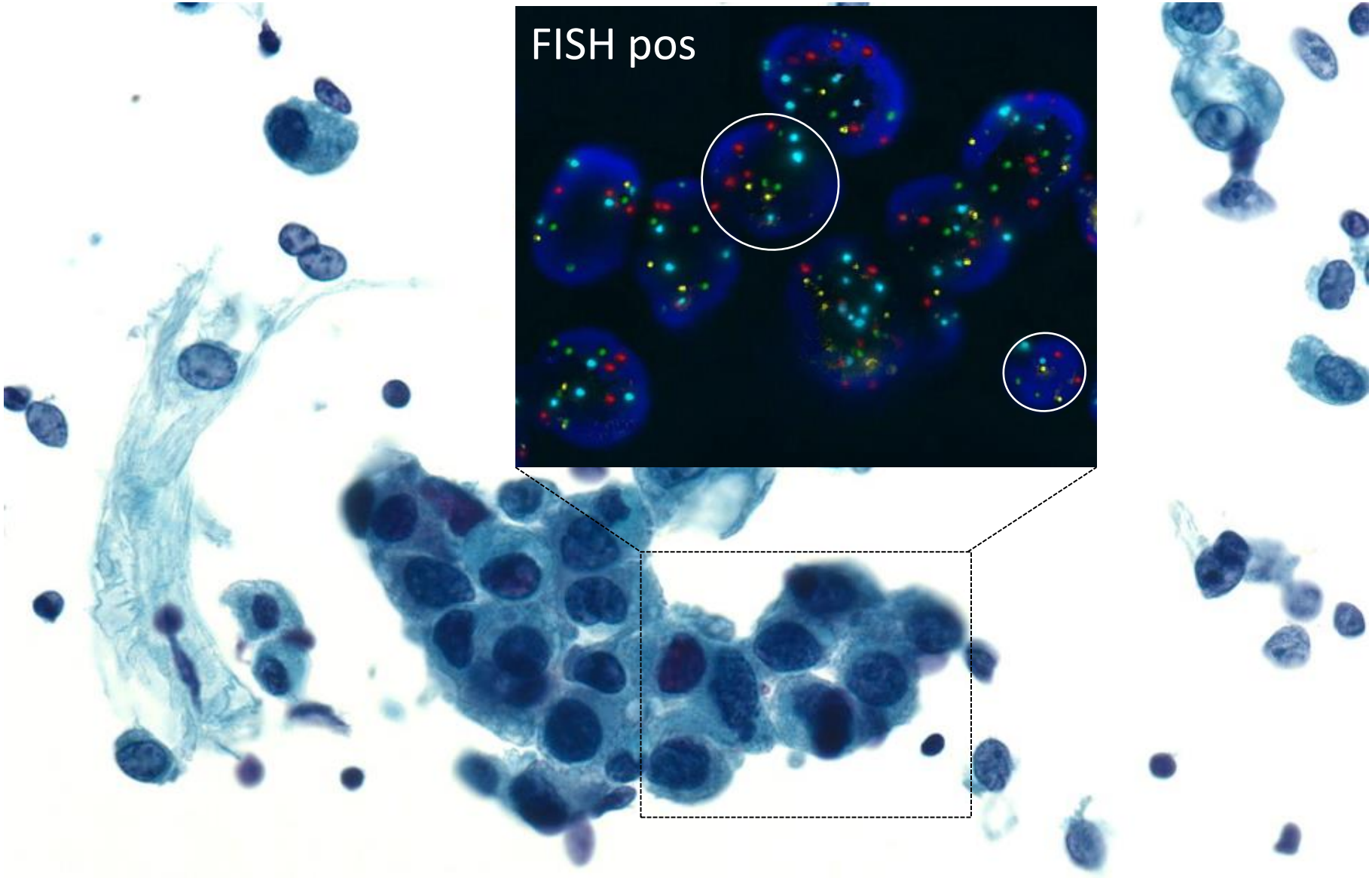
AUC



AUC



AUC:
Urothelial neoplasm, cannot exclude HGUC



UroVysion FISH Test

Why?

- to clarify atypical urothelial cells/SHGUC
  - non-visible high-grade UC
  - avoid unnecessary invasive procedures

→ Communication with urologist

UroVysion FISH Test

When?

- Atypical urothelial cells:
  - after intravesical therapy for high-grade UC (BCG)
  - in upper urinary tract cytology
    - Cytology with low sensitivity
    - 60% invasive (urinary bladder: 15%)

- Not in “benign” and “malignant” cytology

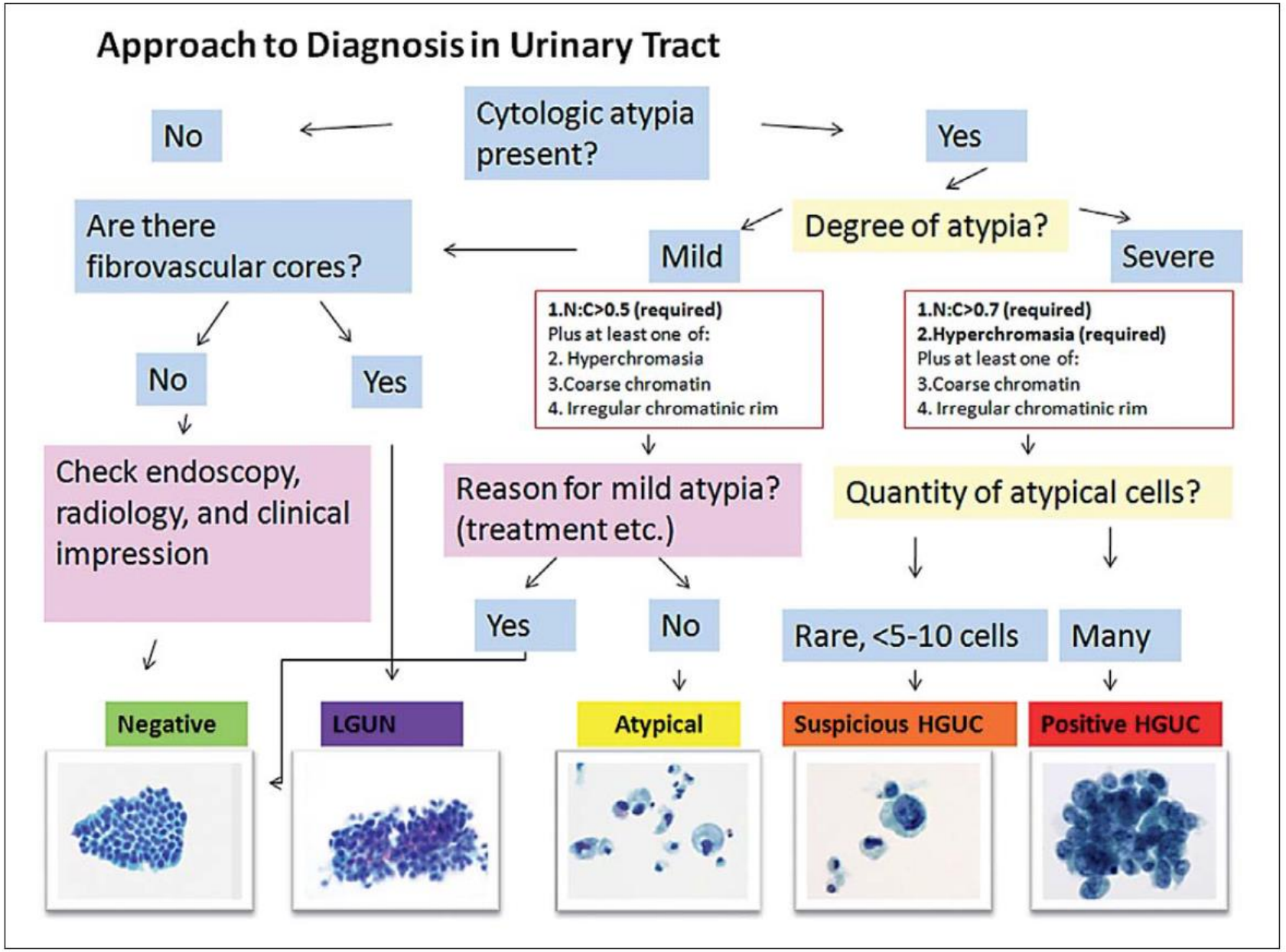
UroVysion FISH Test

How?

- On diagnostic PAP-stained slides
- With automated relocation of target cells
- By cytomorphologists trained in FISH analysis

Examples

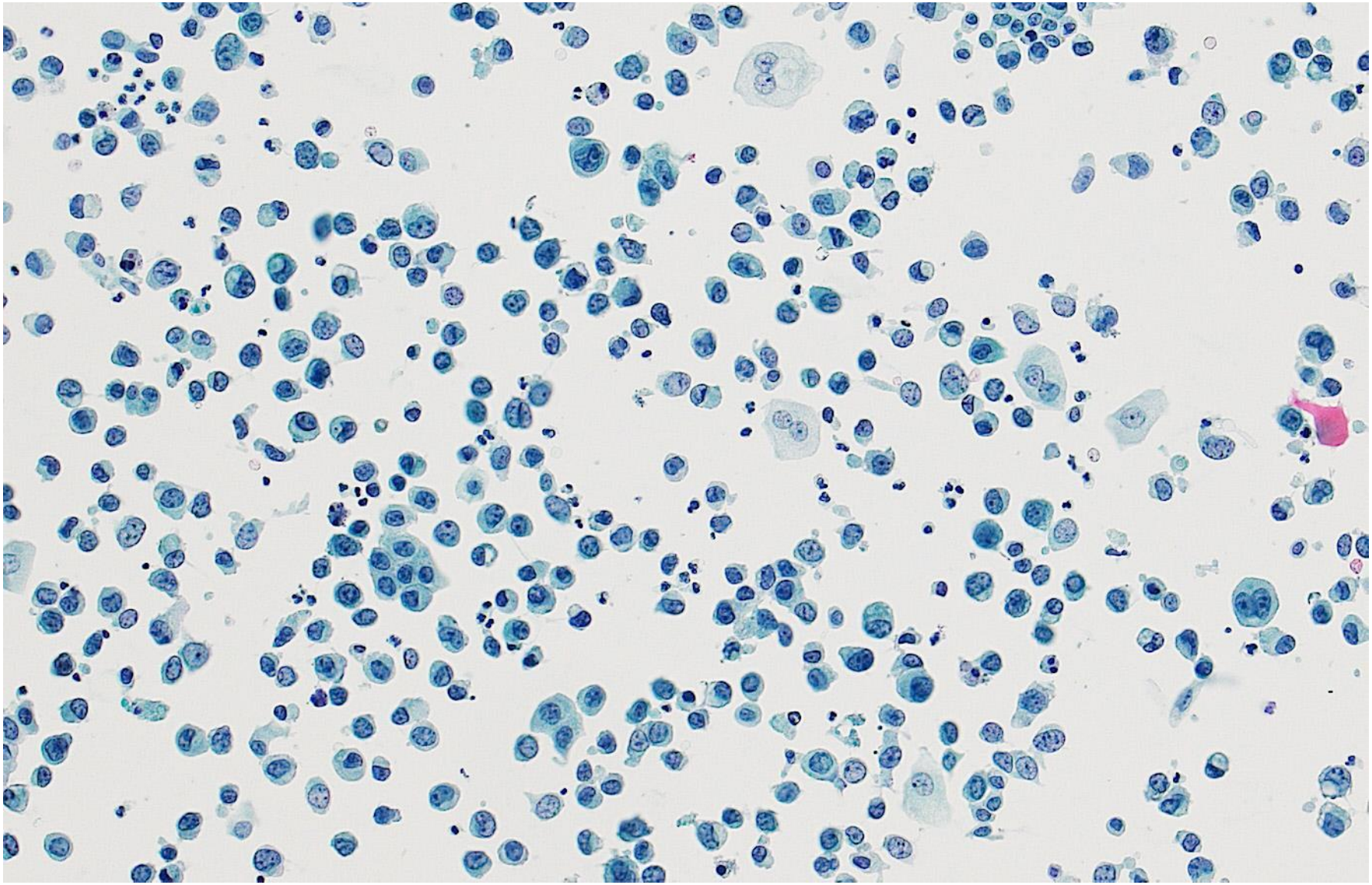
11 cases



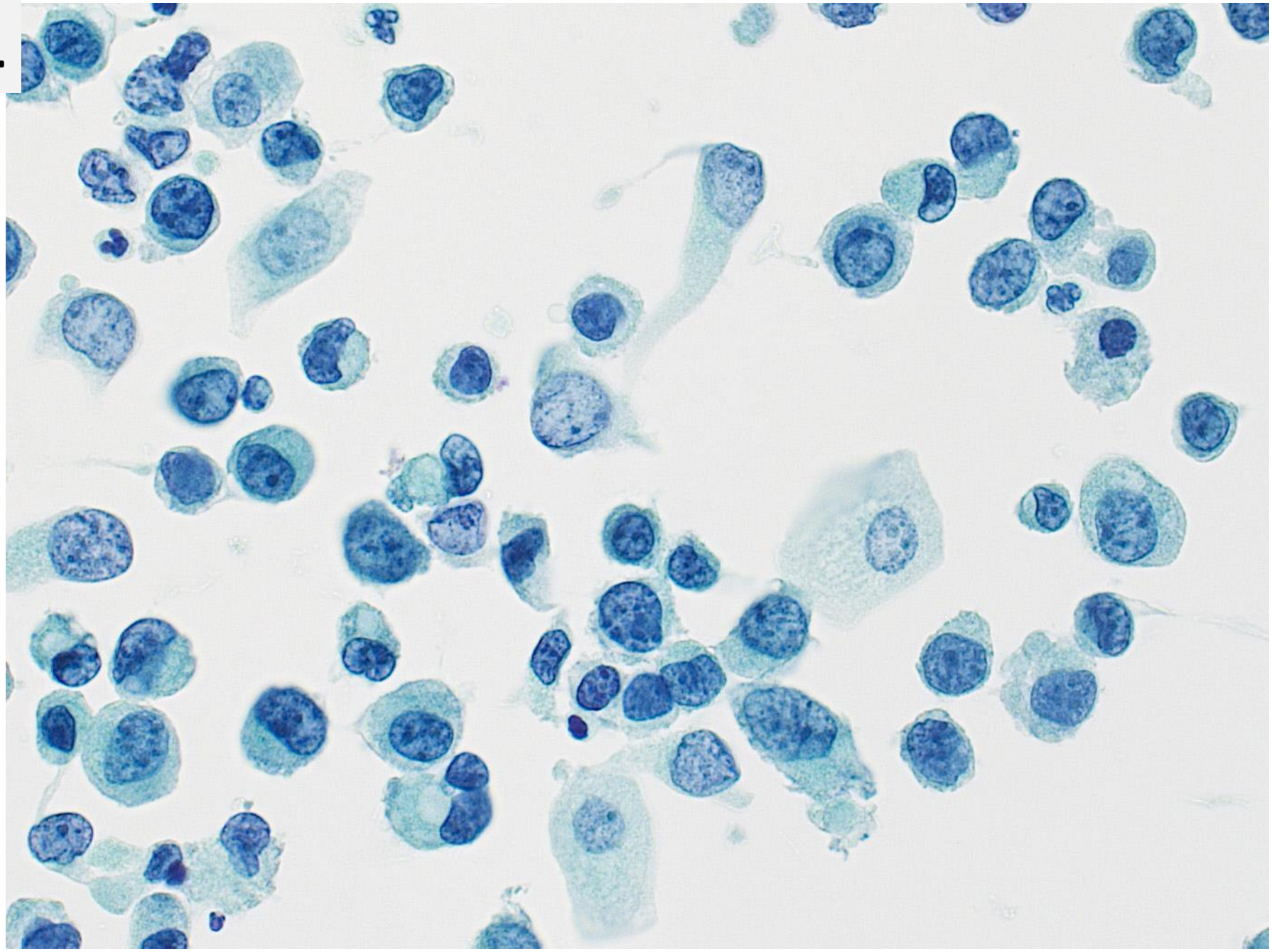
Barkan GA et al, Acta Cytol 2016; 60:185-197

1

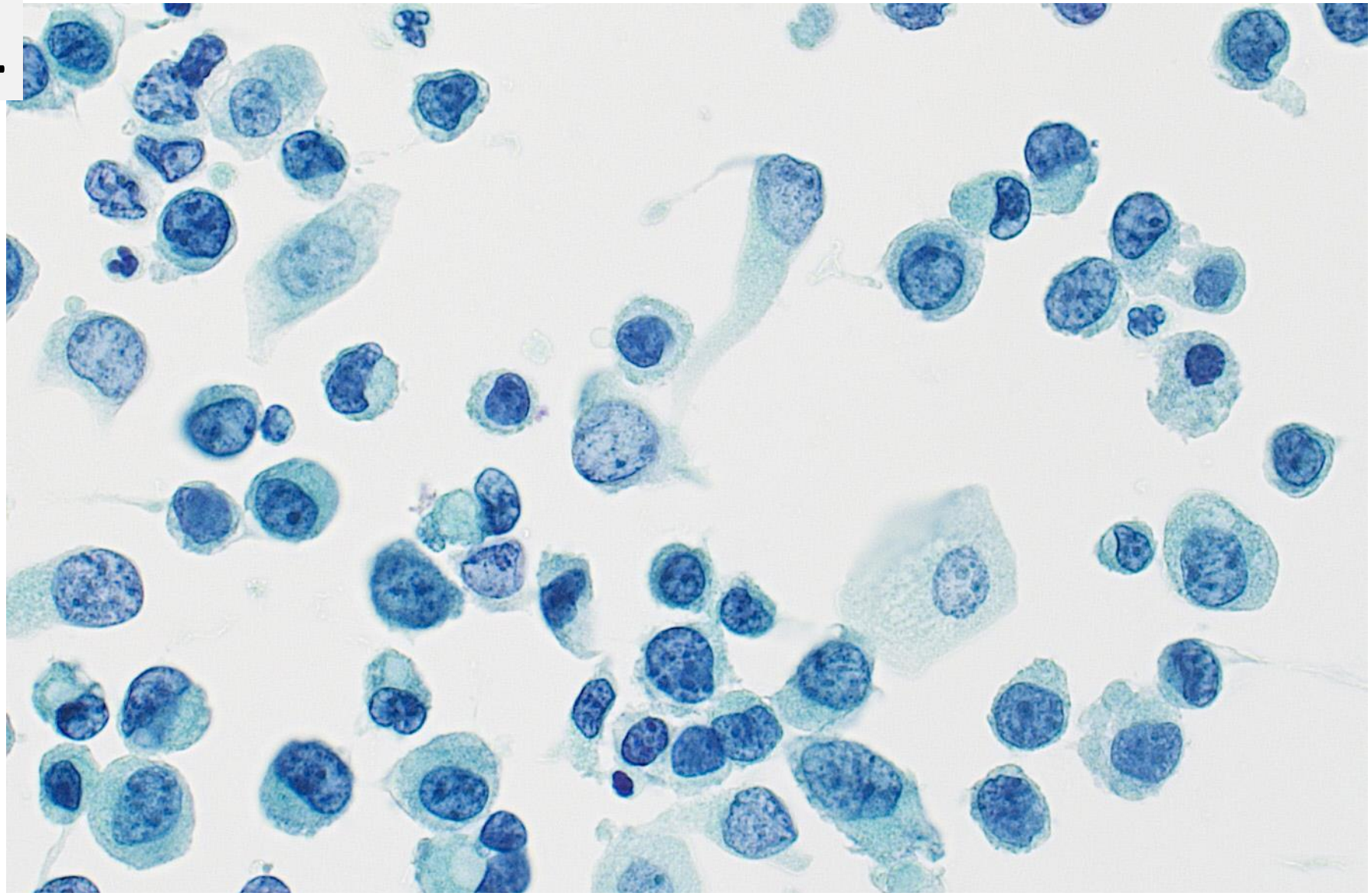
61y, history of pTaG2 LG, difficult cyst.



1

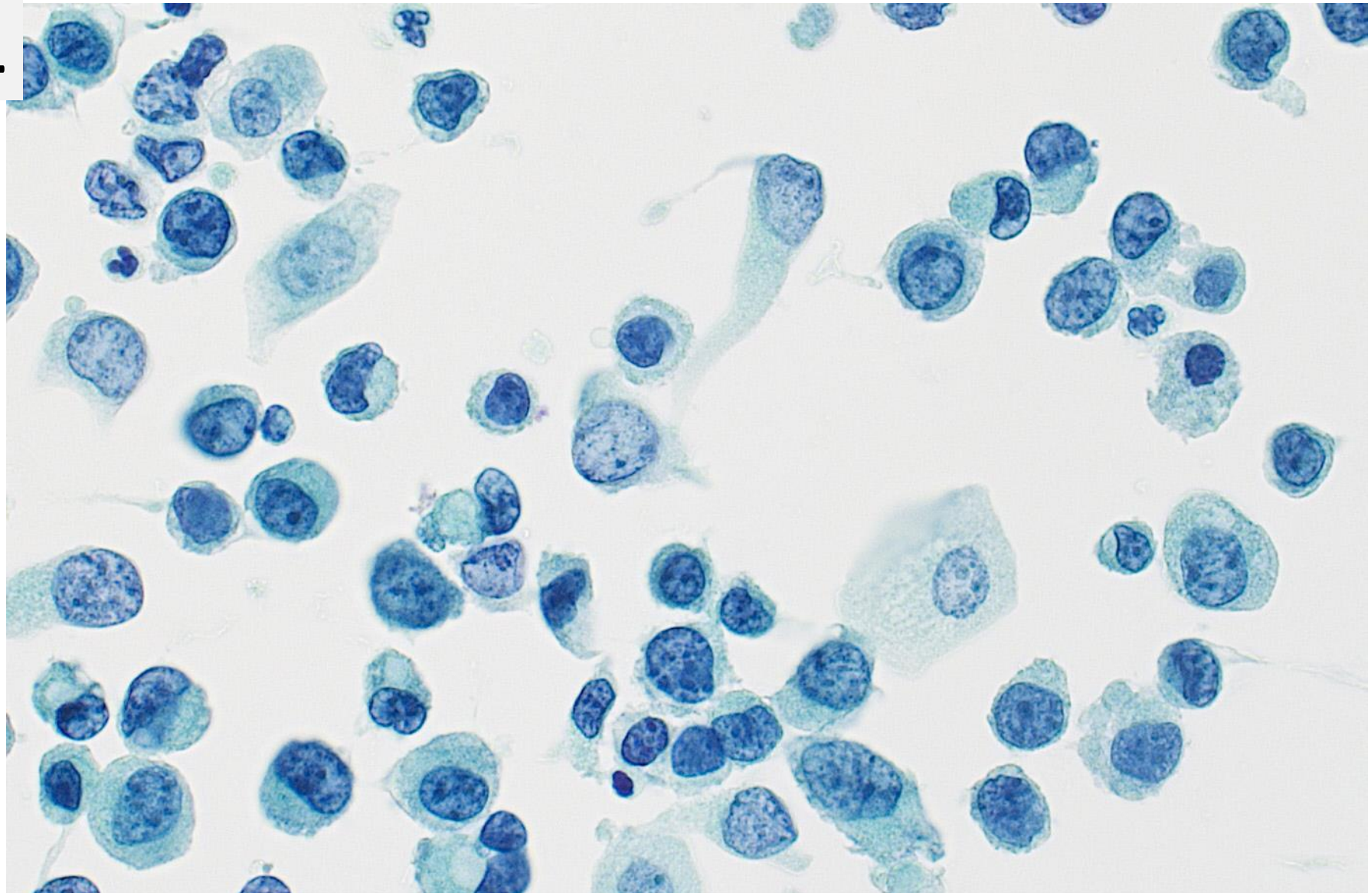


1



HGUC

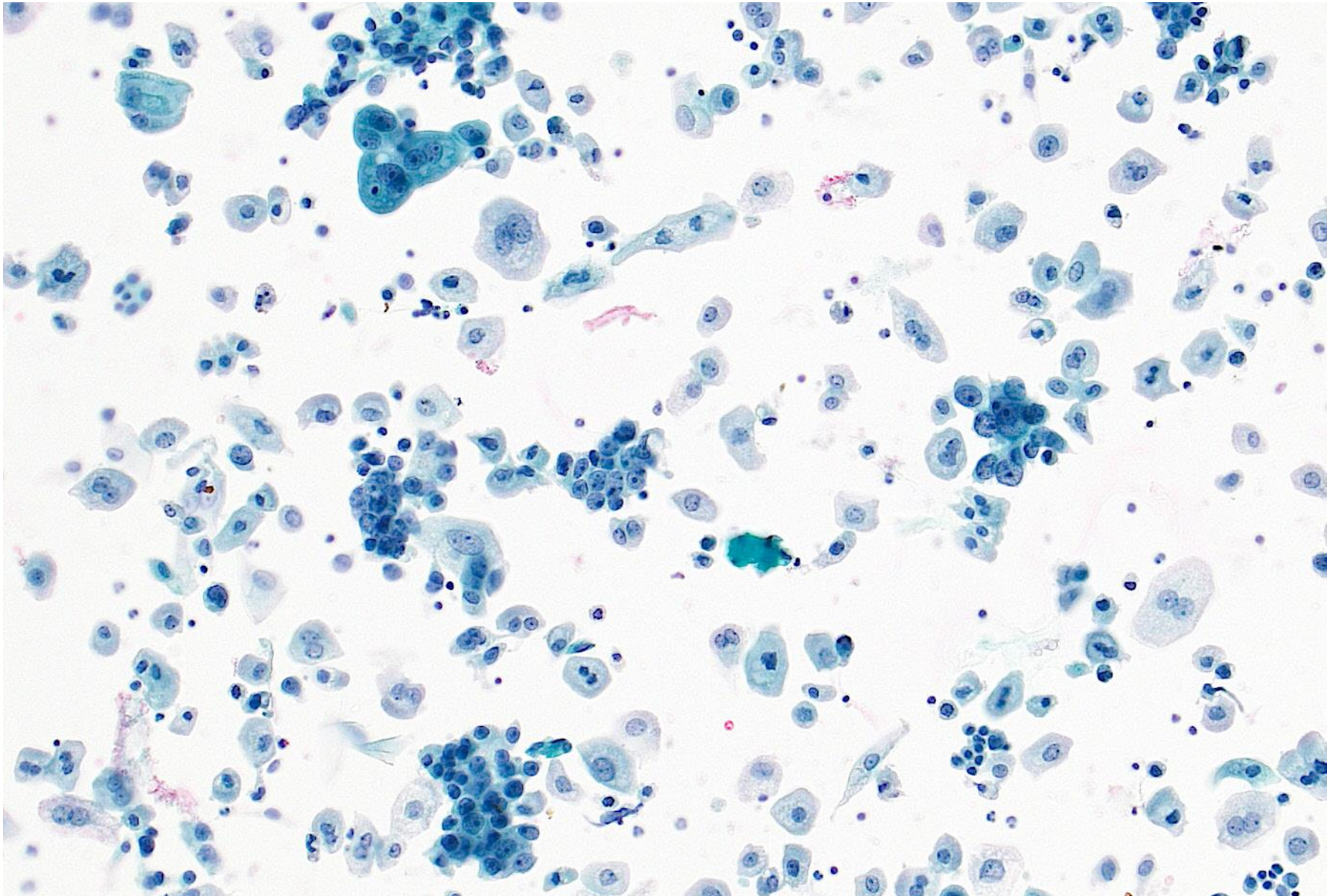
1



Biopsy: pT1, G2 high grade

2

72y, hematuria, cyst.: tumor



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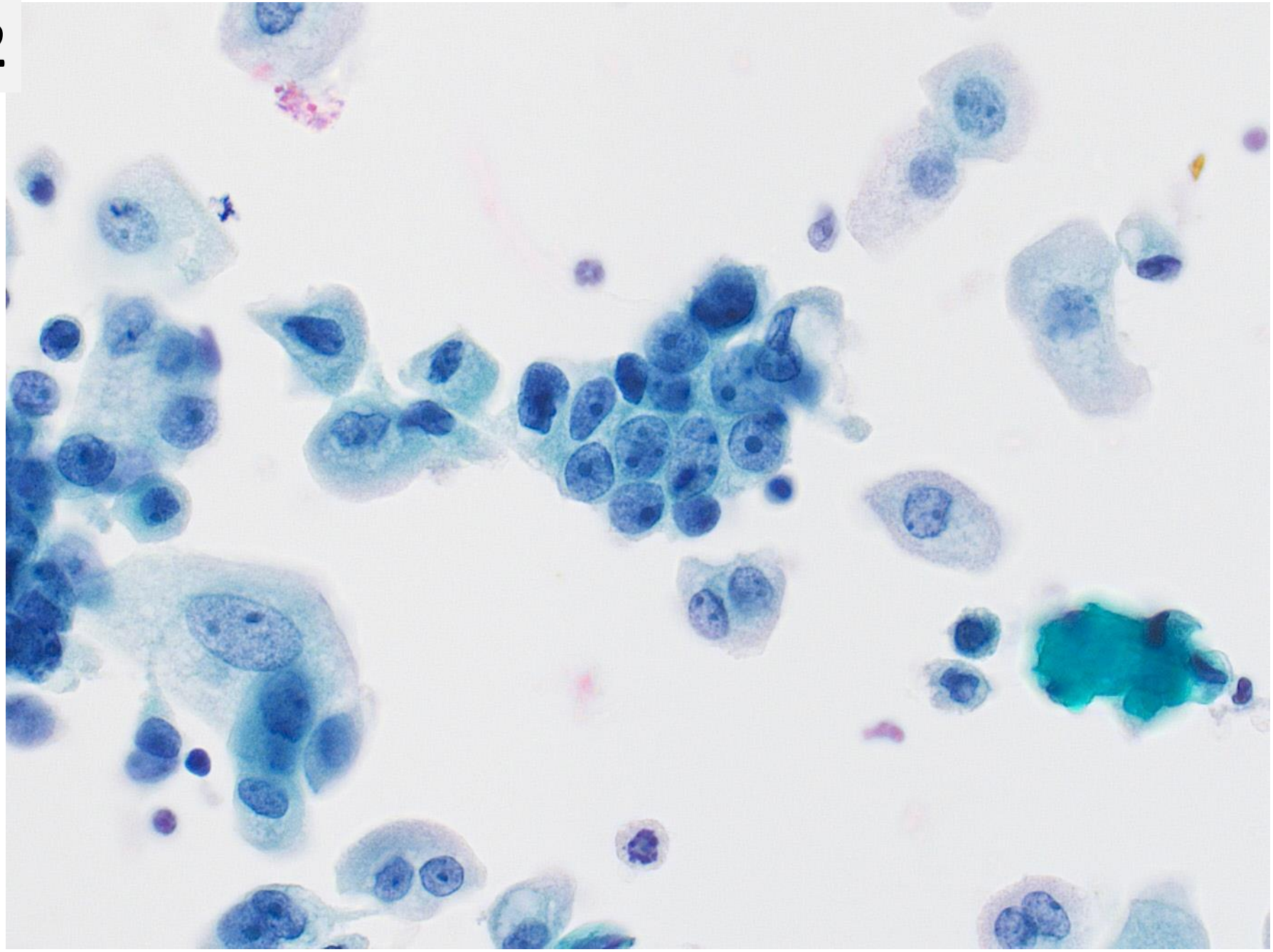

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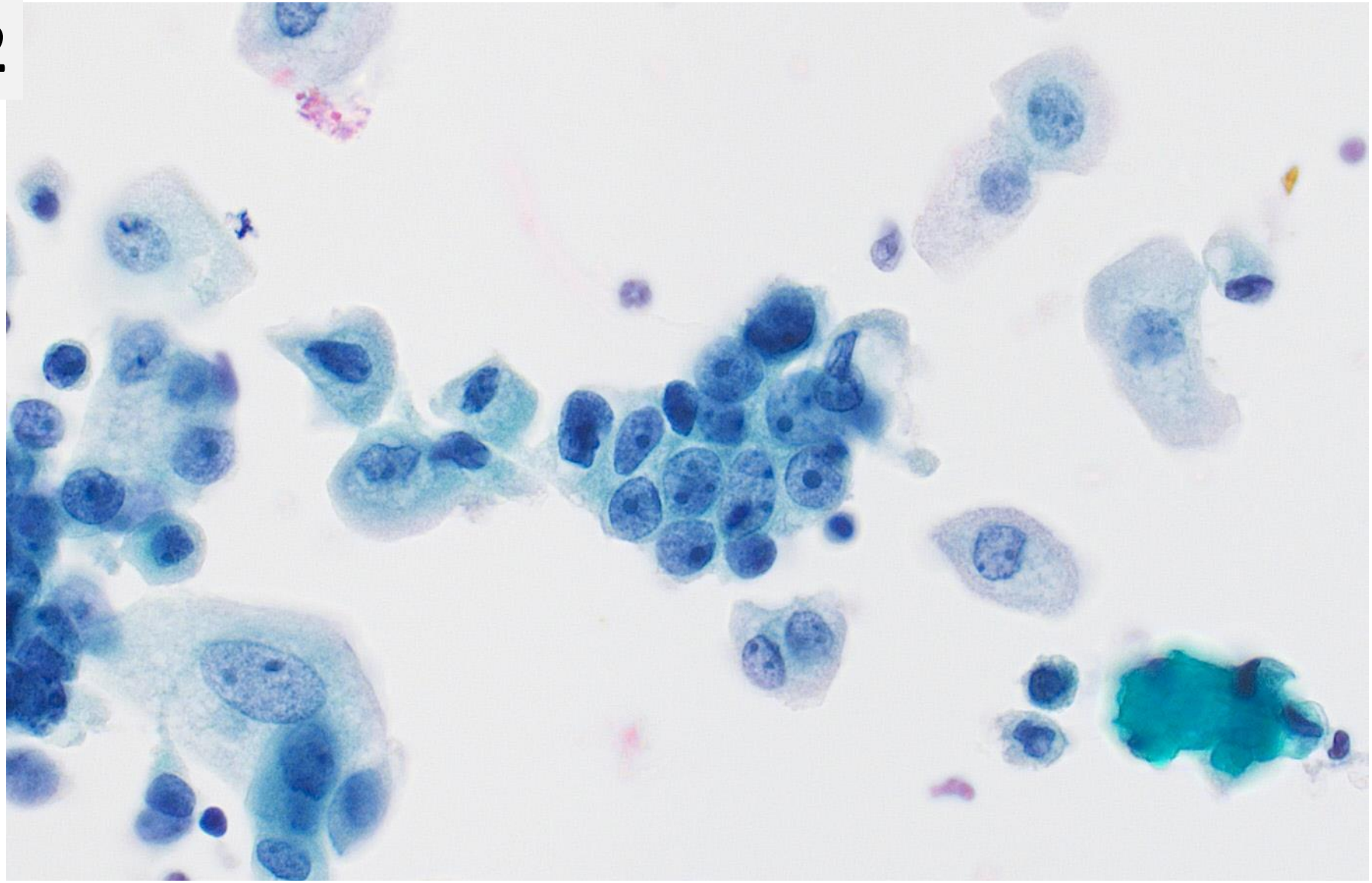
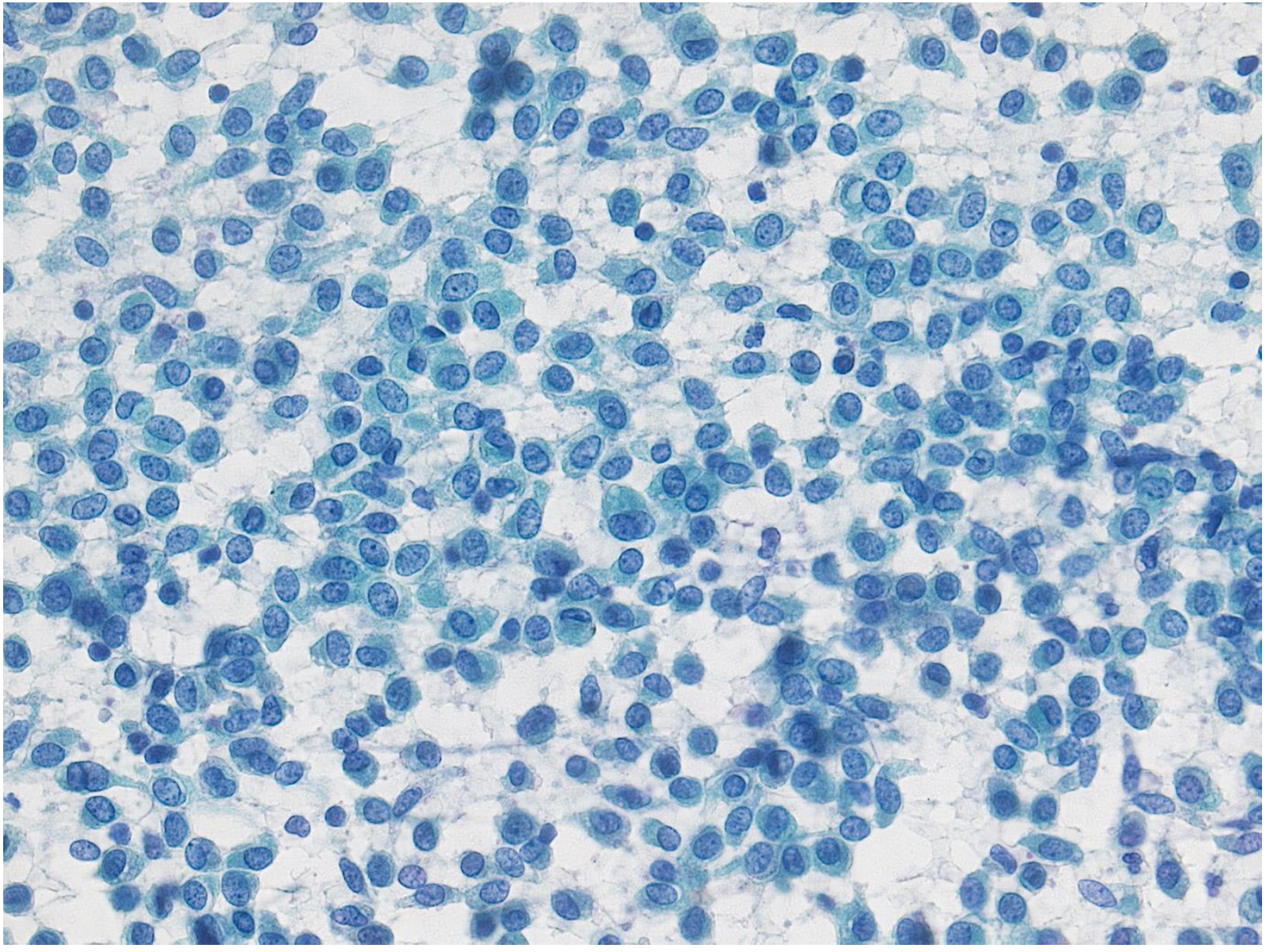


Prostatic adenocarcinoma

3 75y, history of pT1G3+CIS, cyst.: difficult



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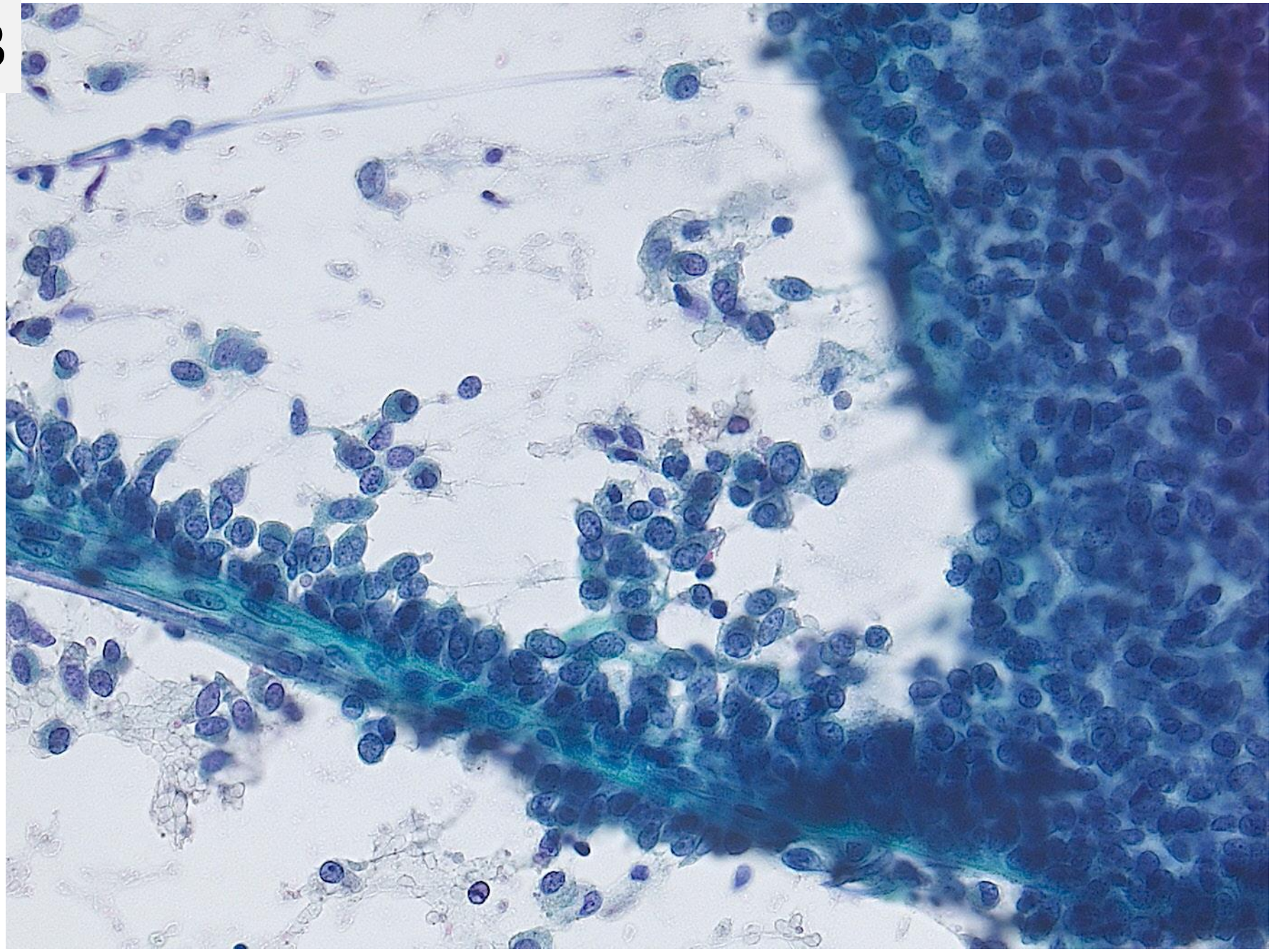

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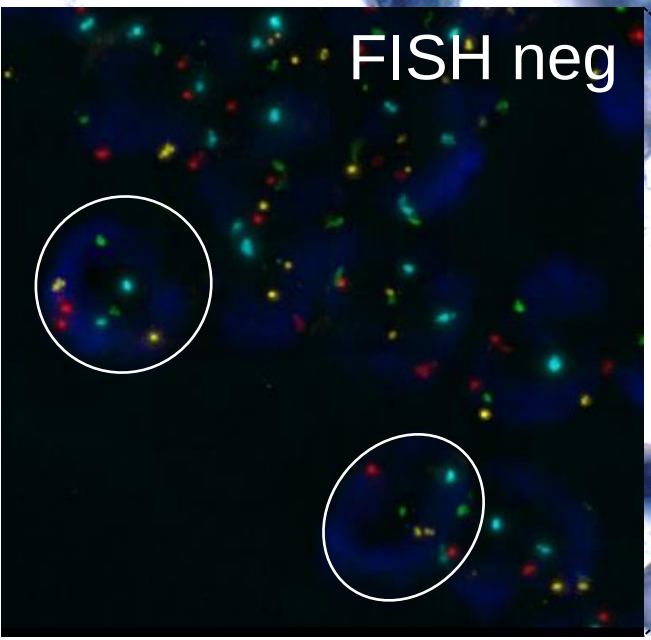

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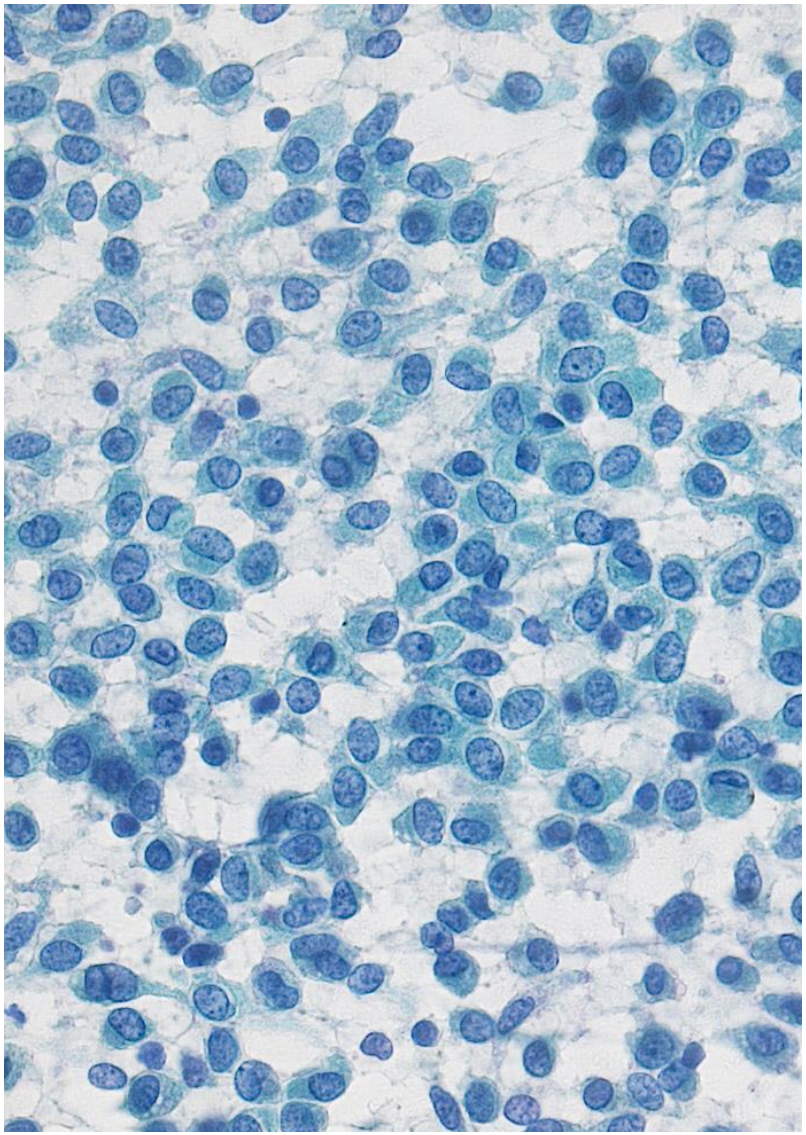
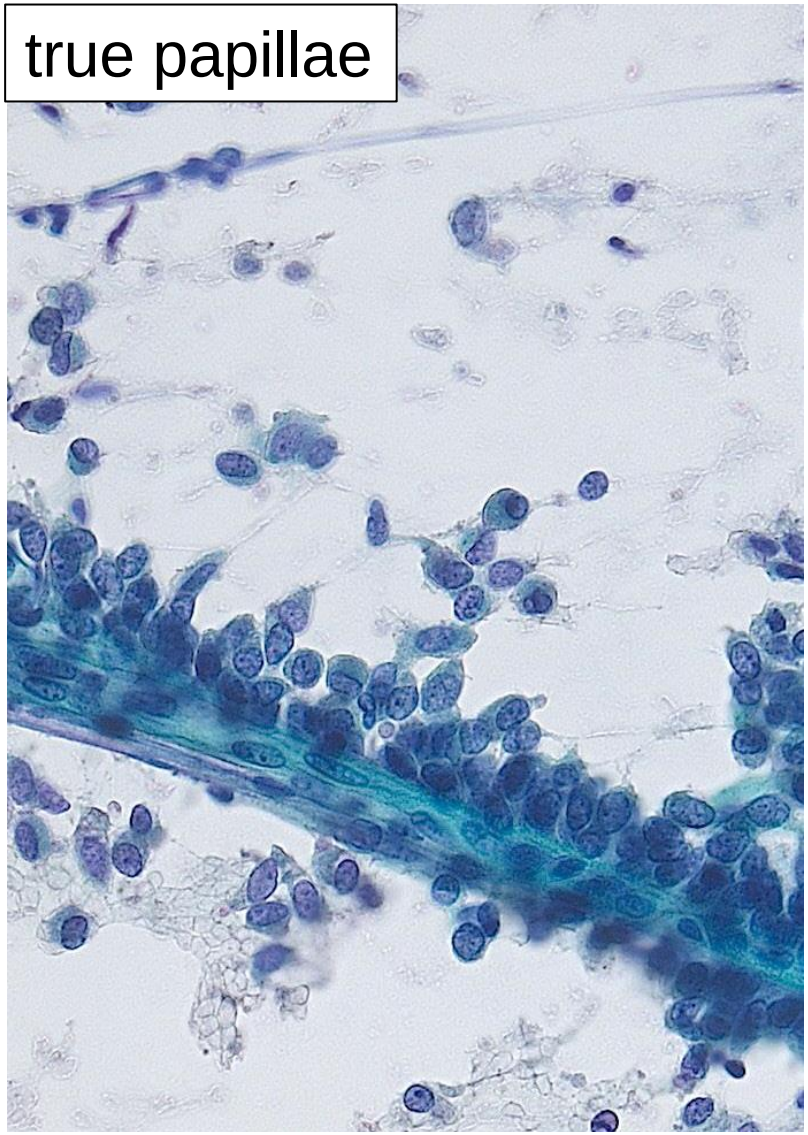
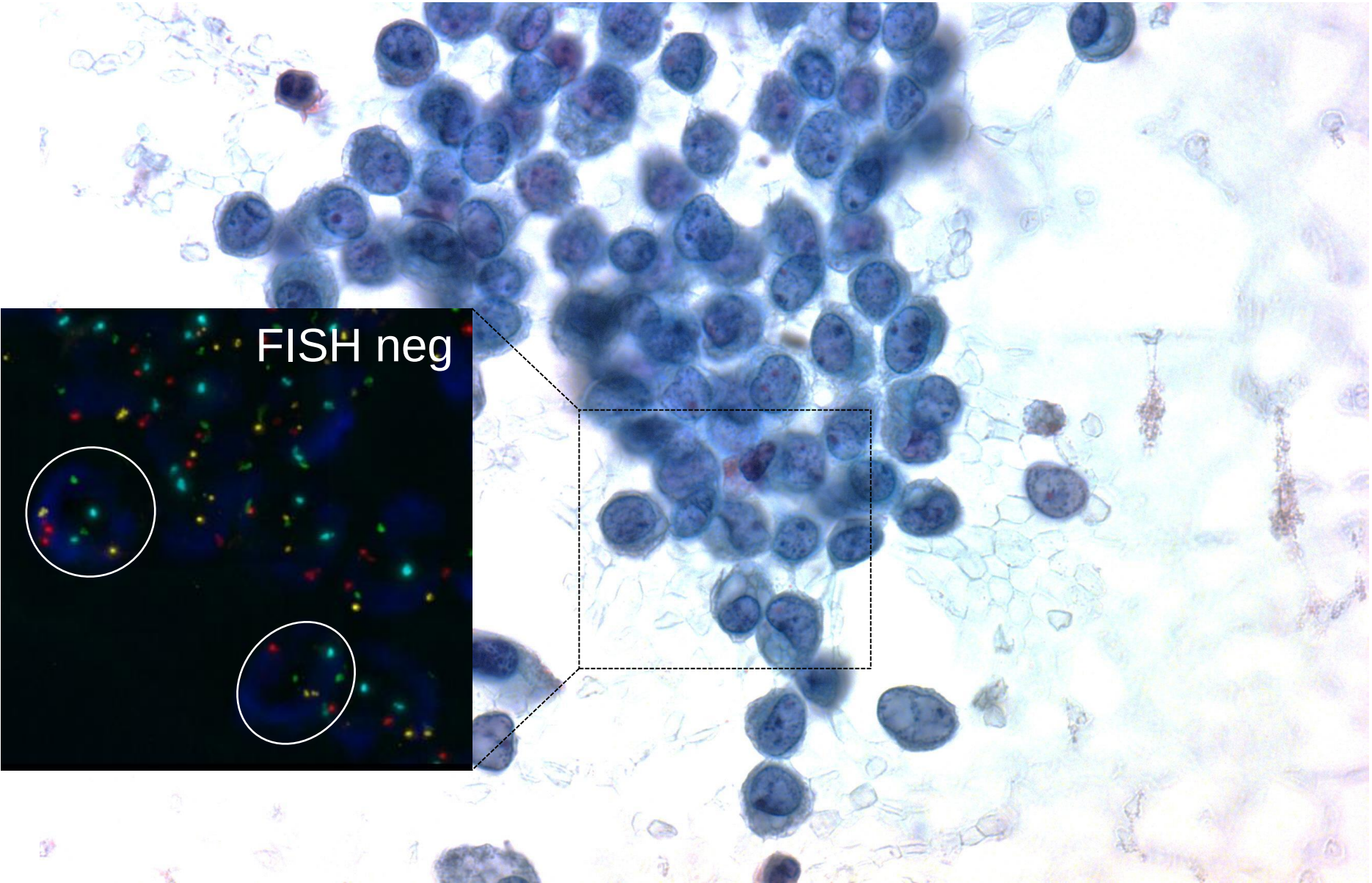




LGUN

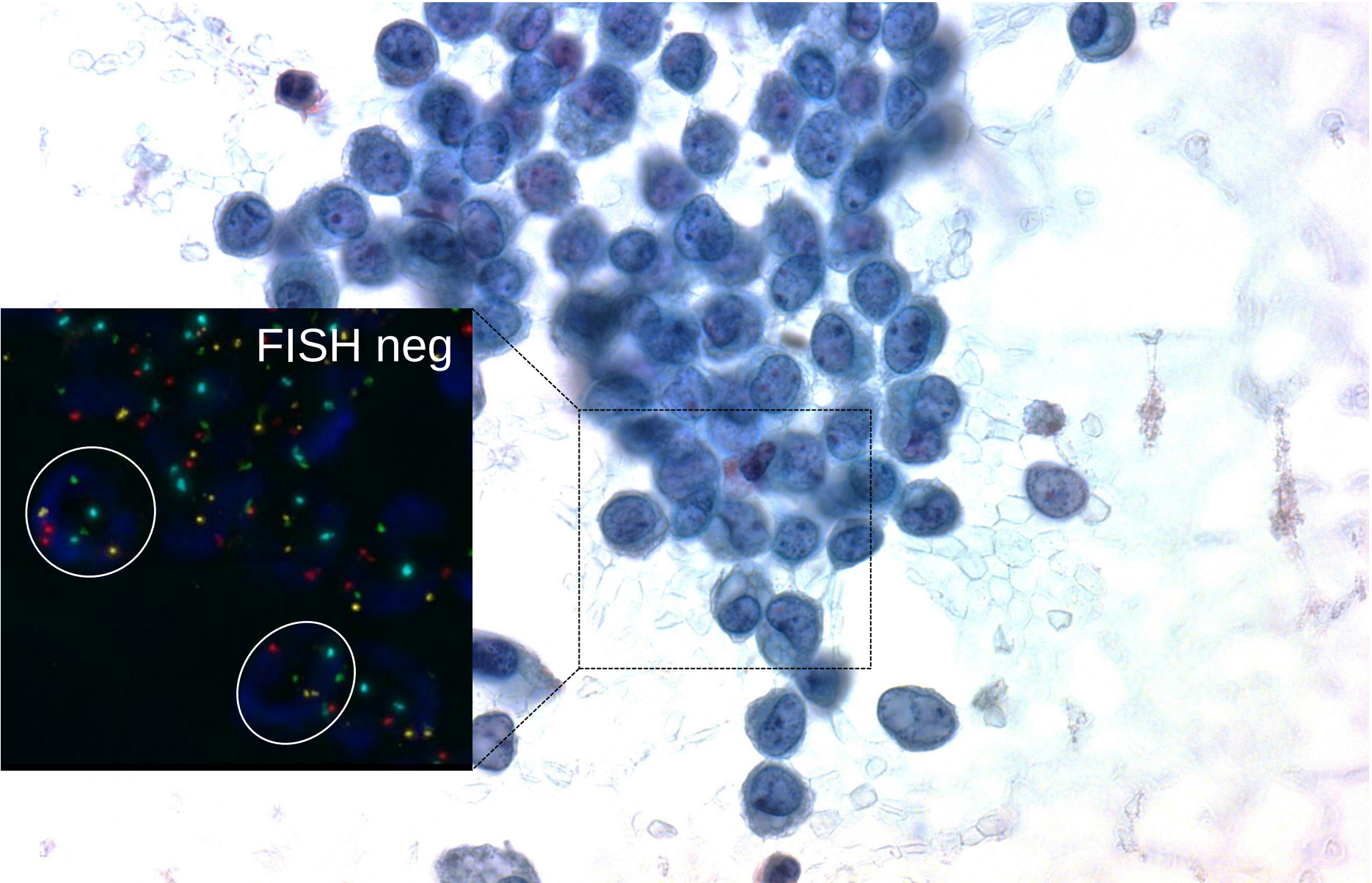
3

20-30% of LGUN FISH neg.



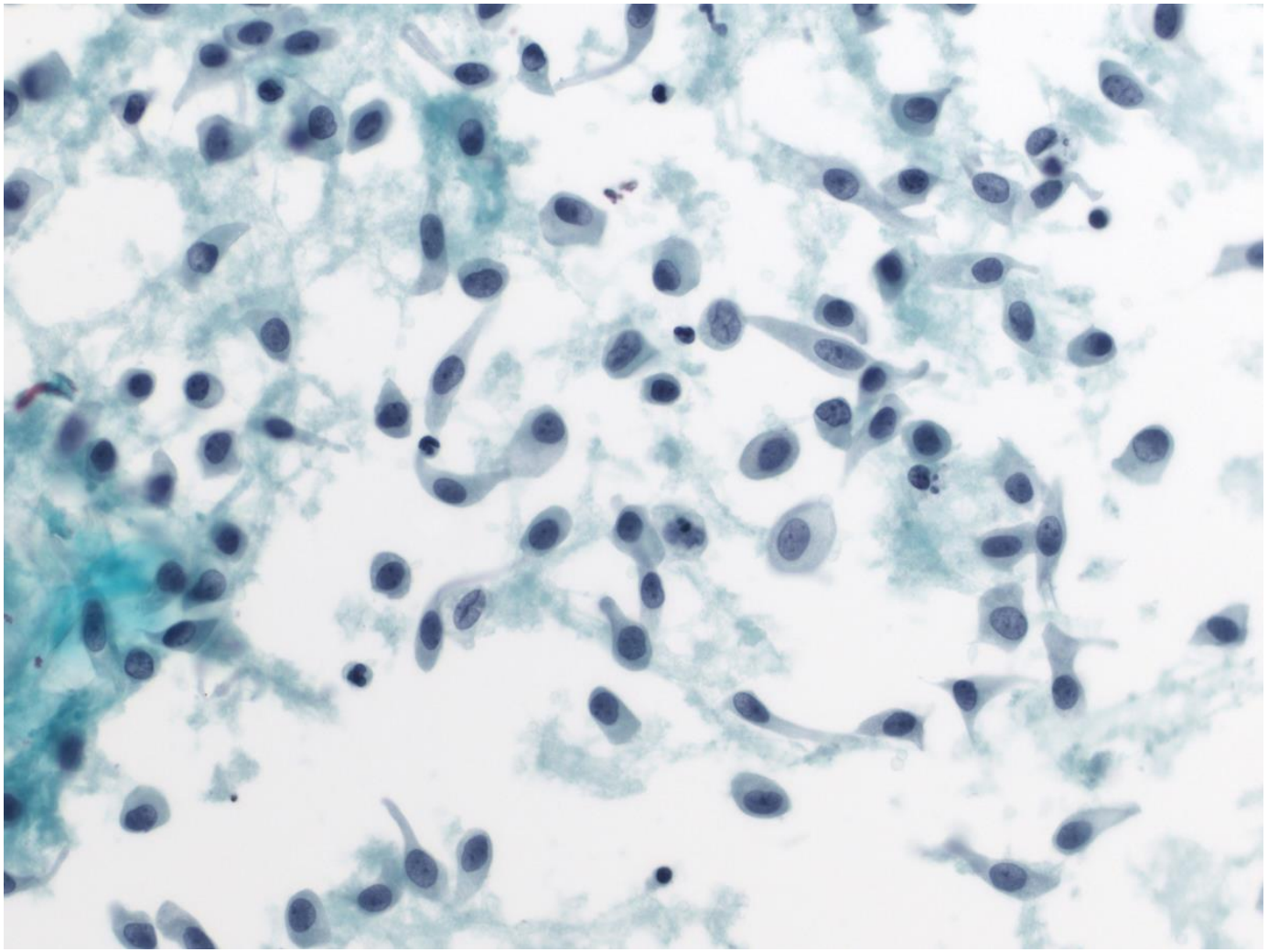
3

Re-cystoscopy: pTaG2, low grade



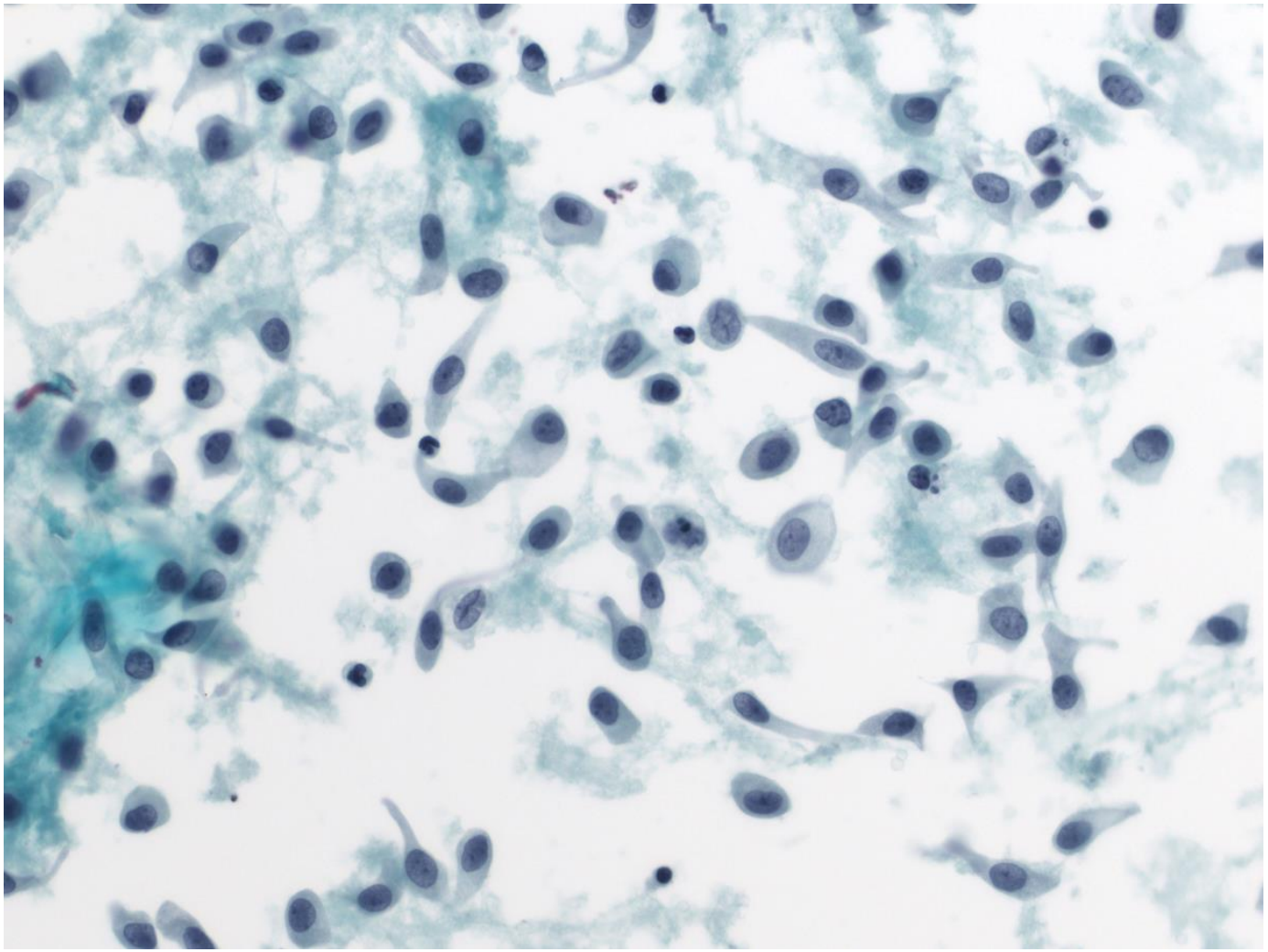
4

70y, renal pelvic washing
Renal pelvis with suspicious lesion



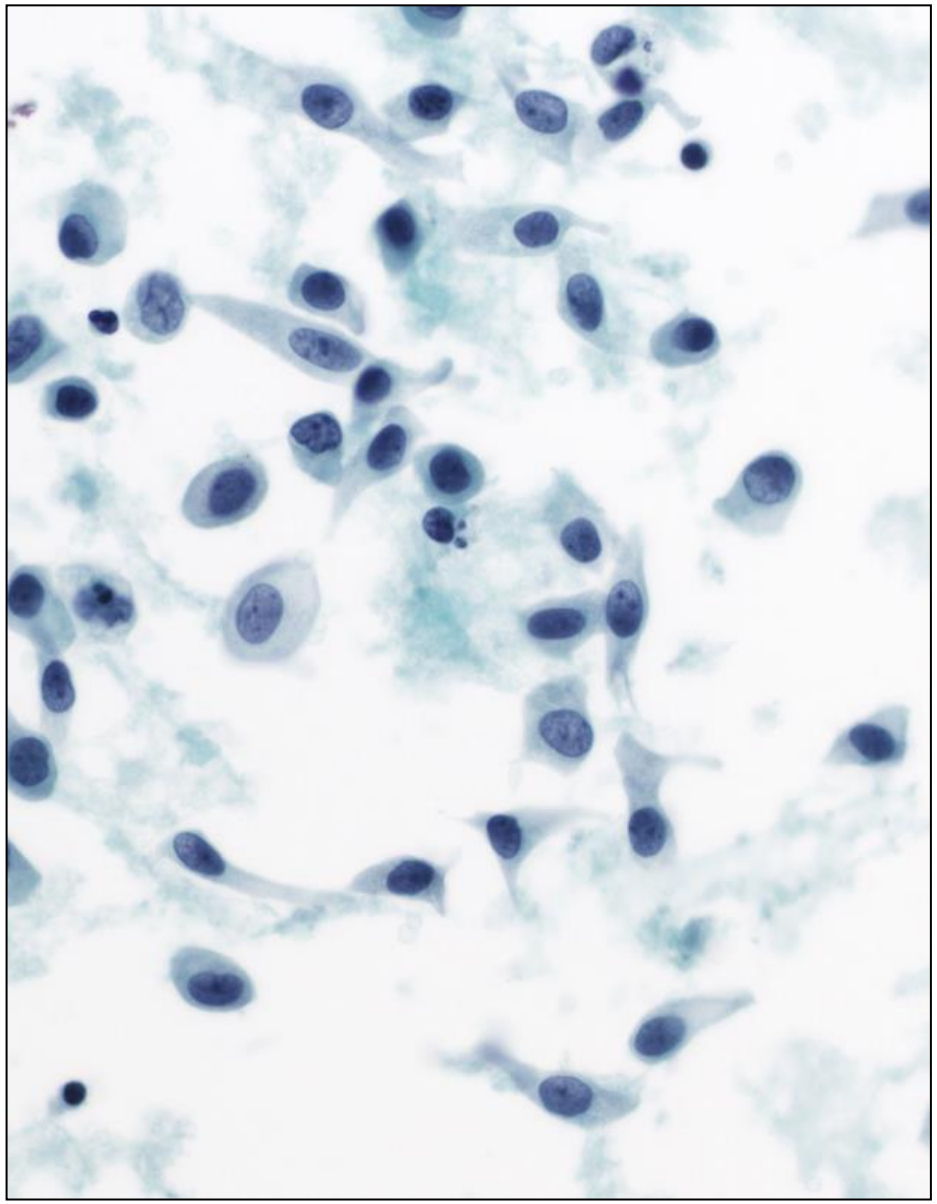
4

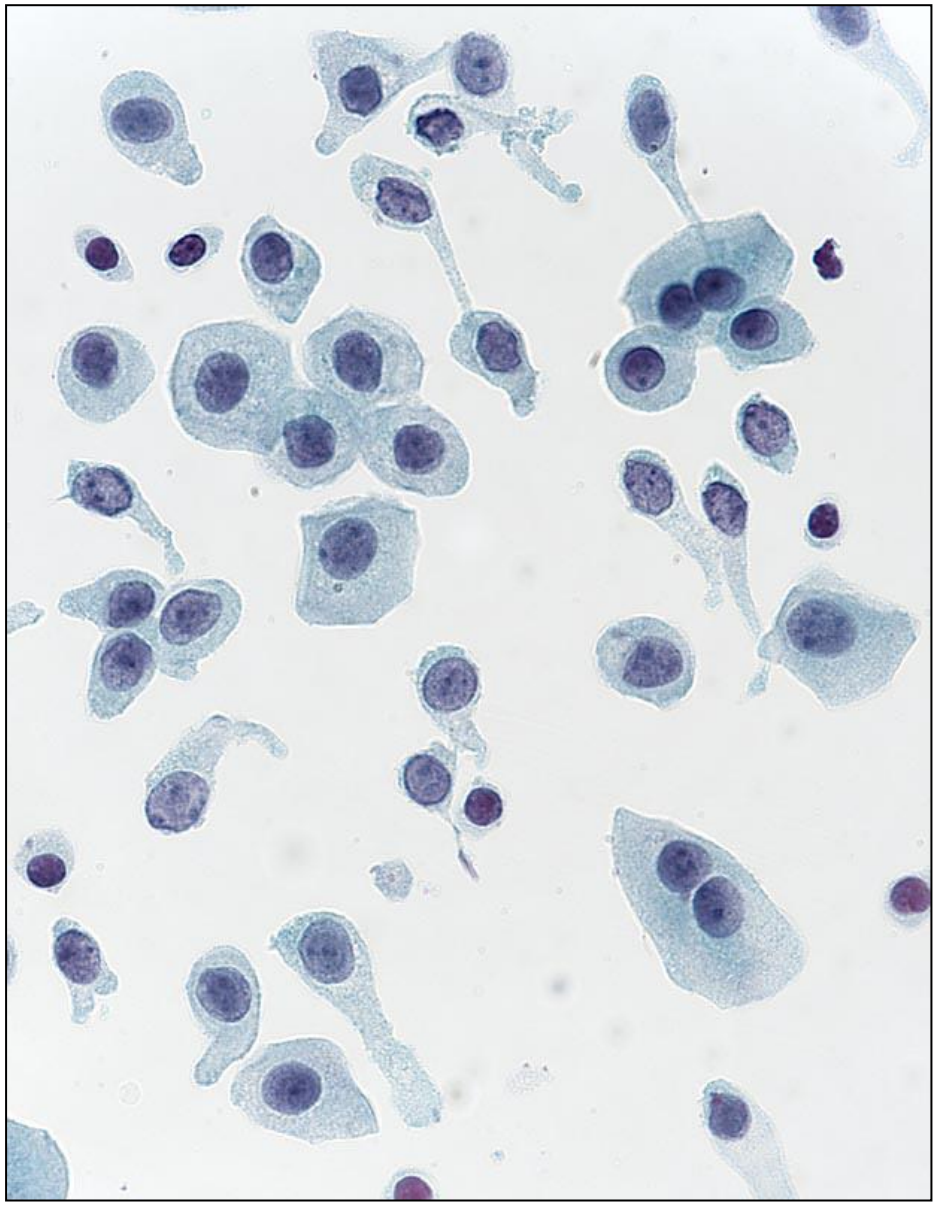
70y, renal pelvic washing
Renal pelvis with suspicious lesion



4

Negative cytology





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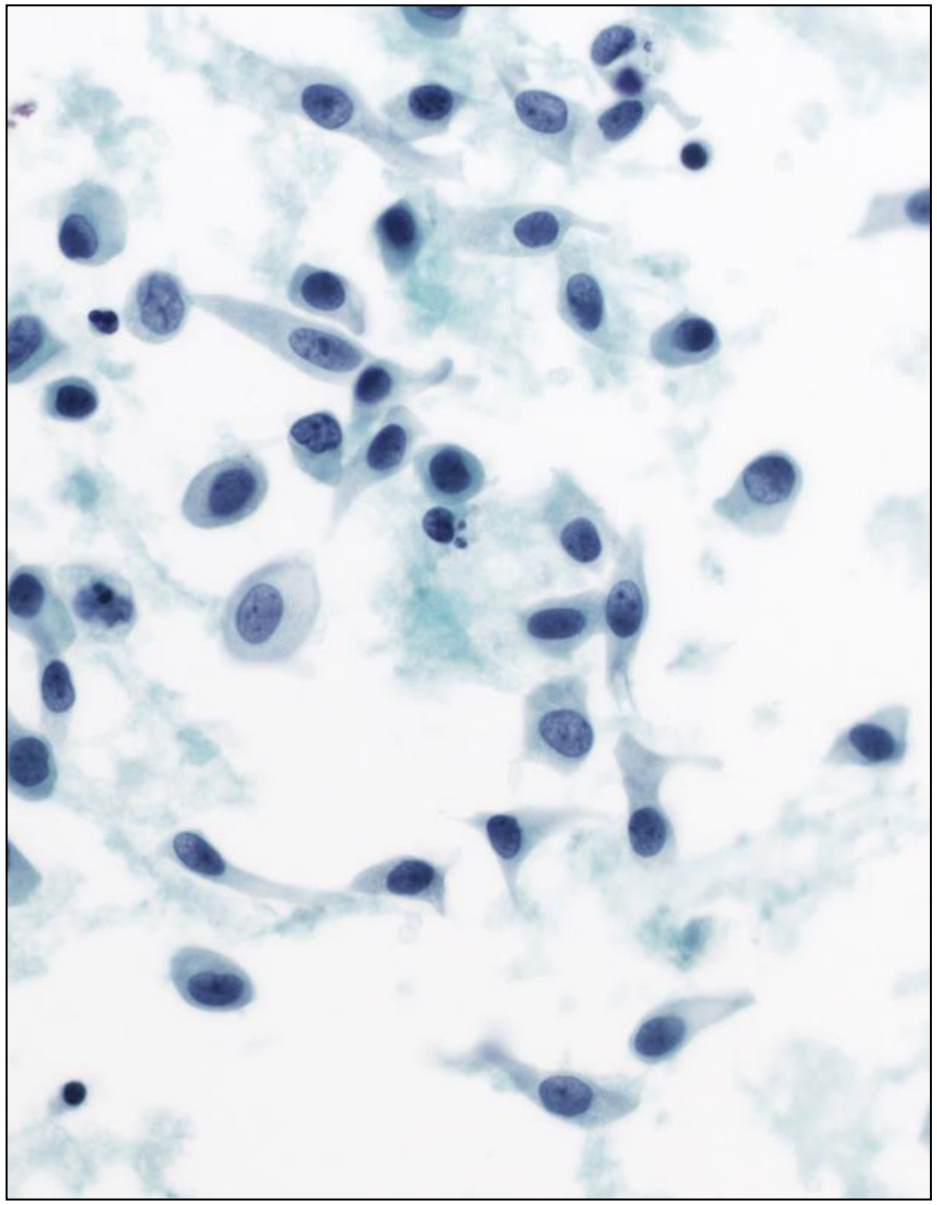
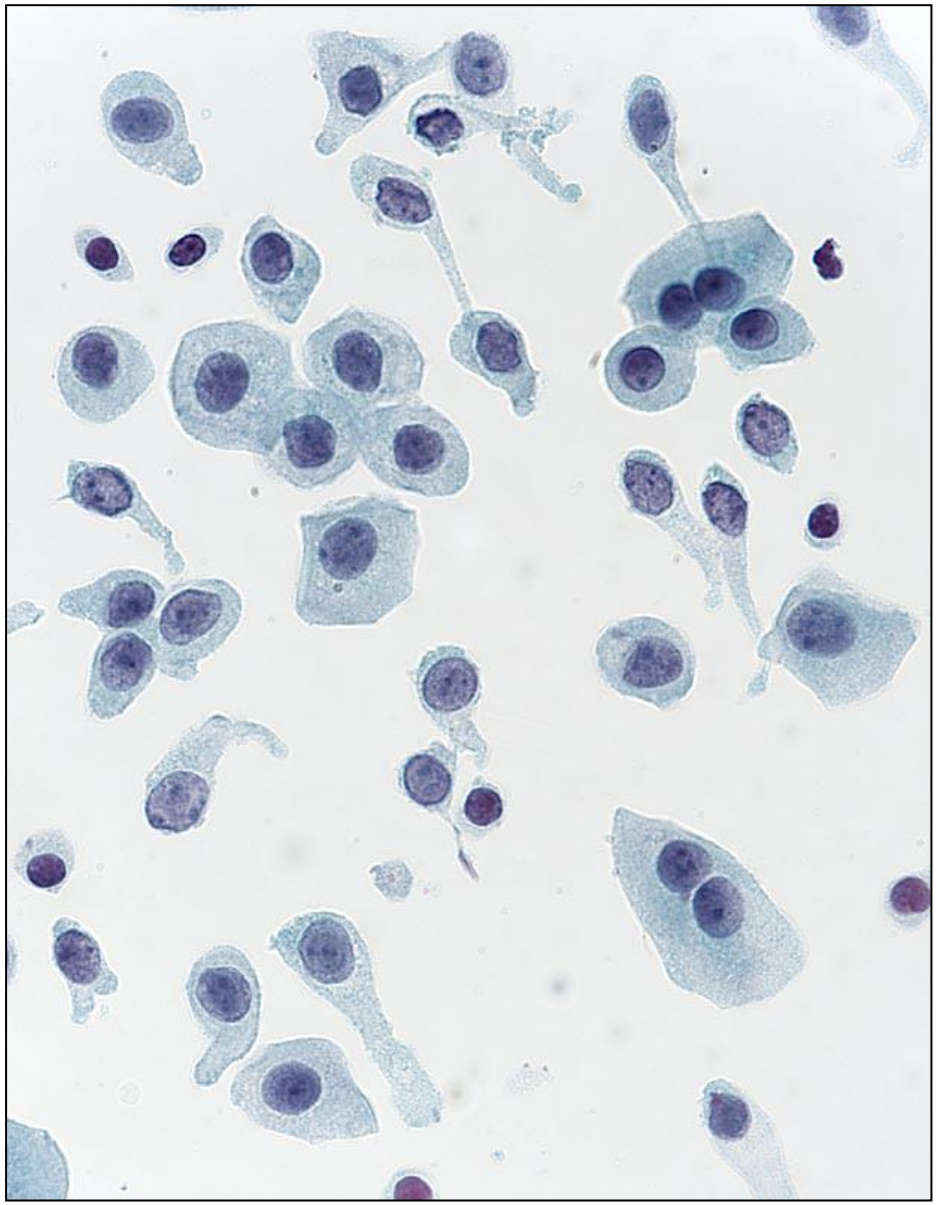
AUC



Negative cytology



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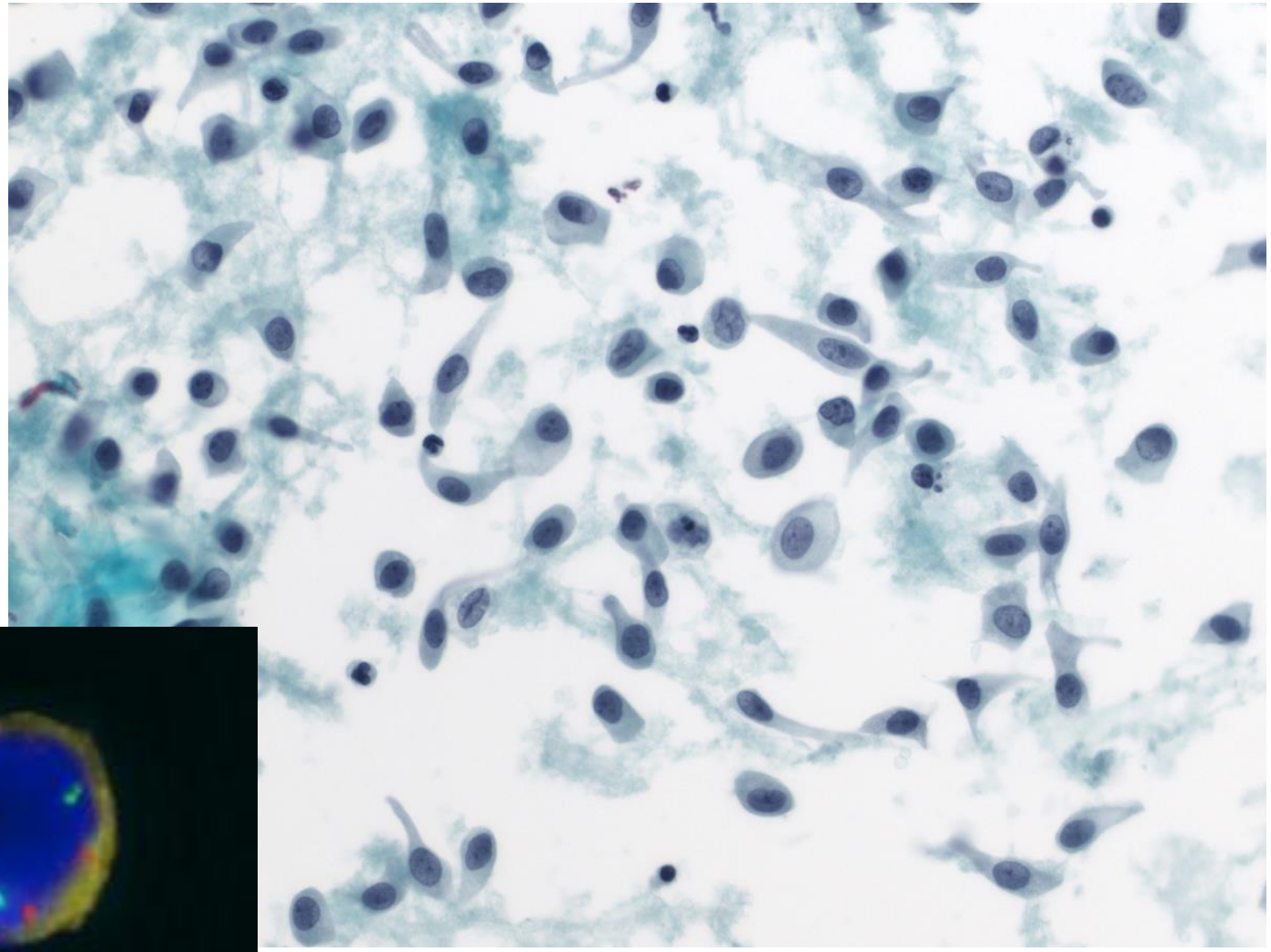
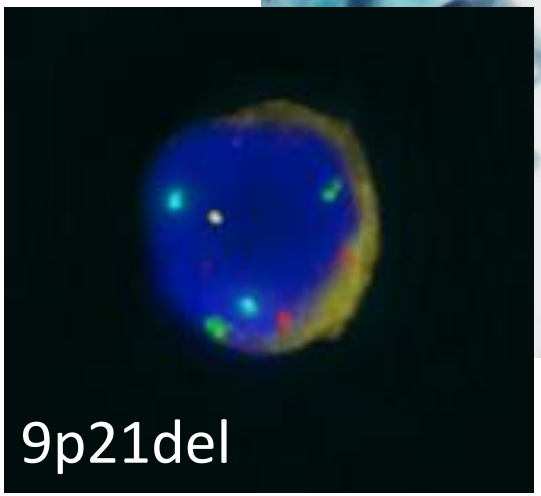
AUC





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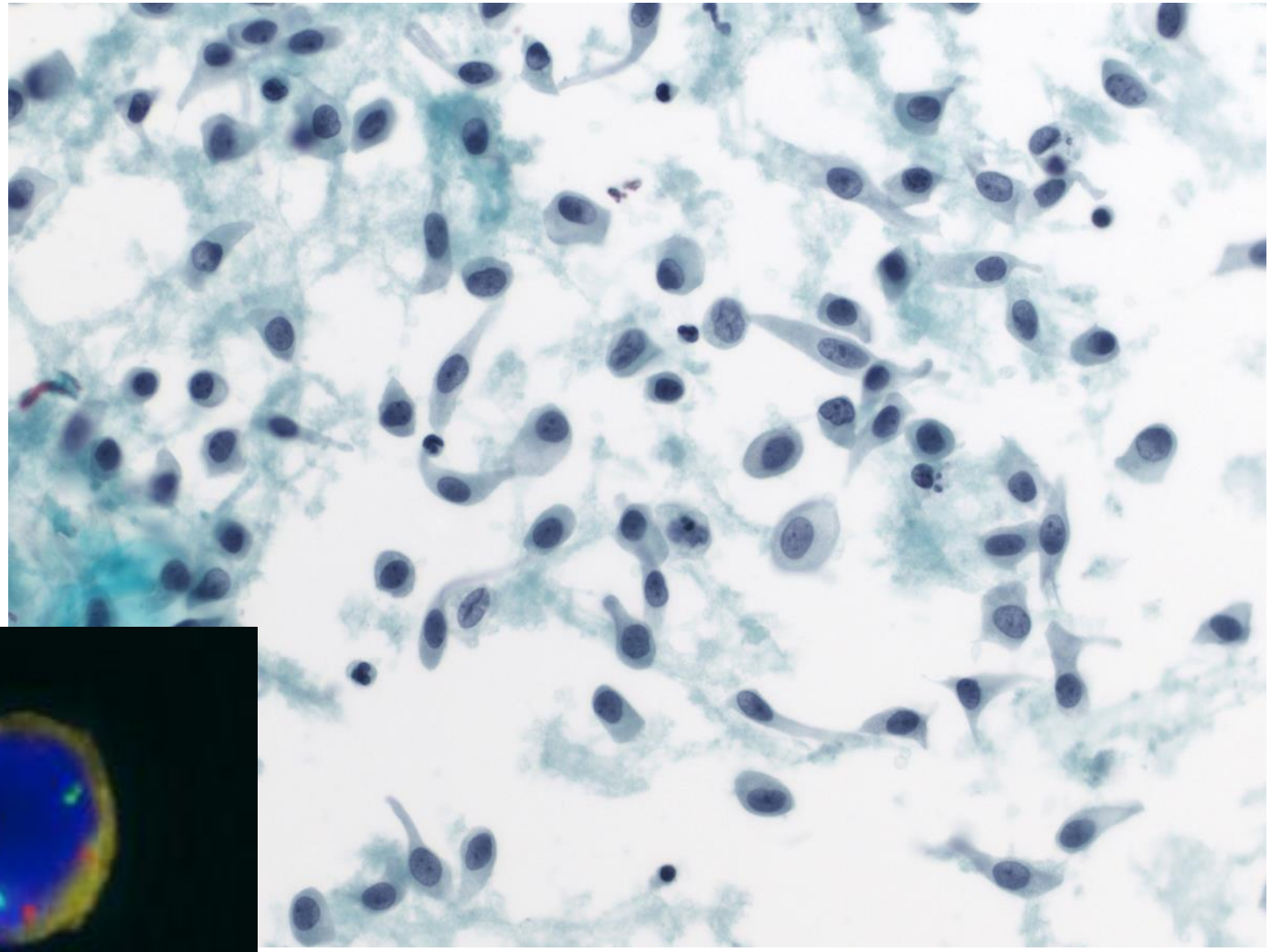
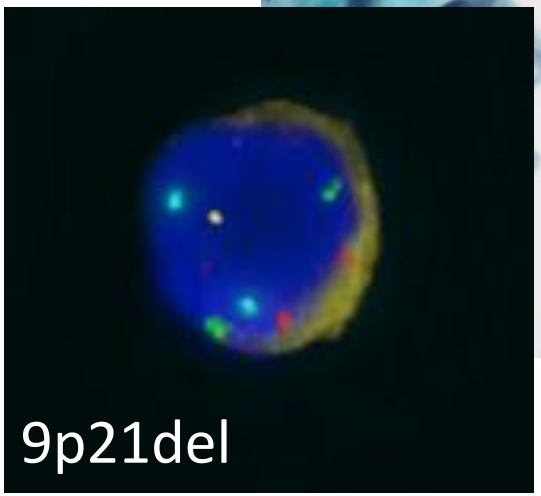
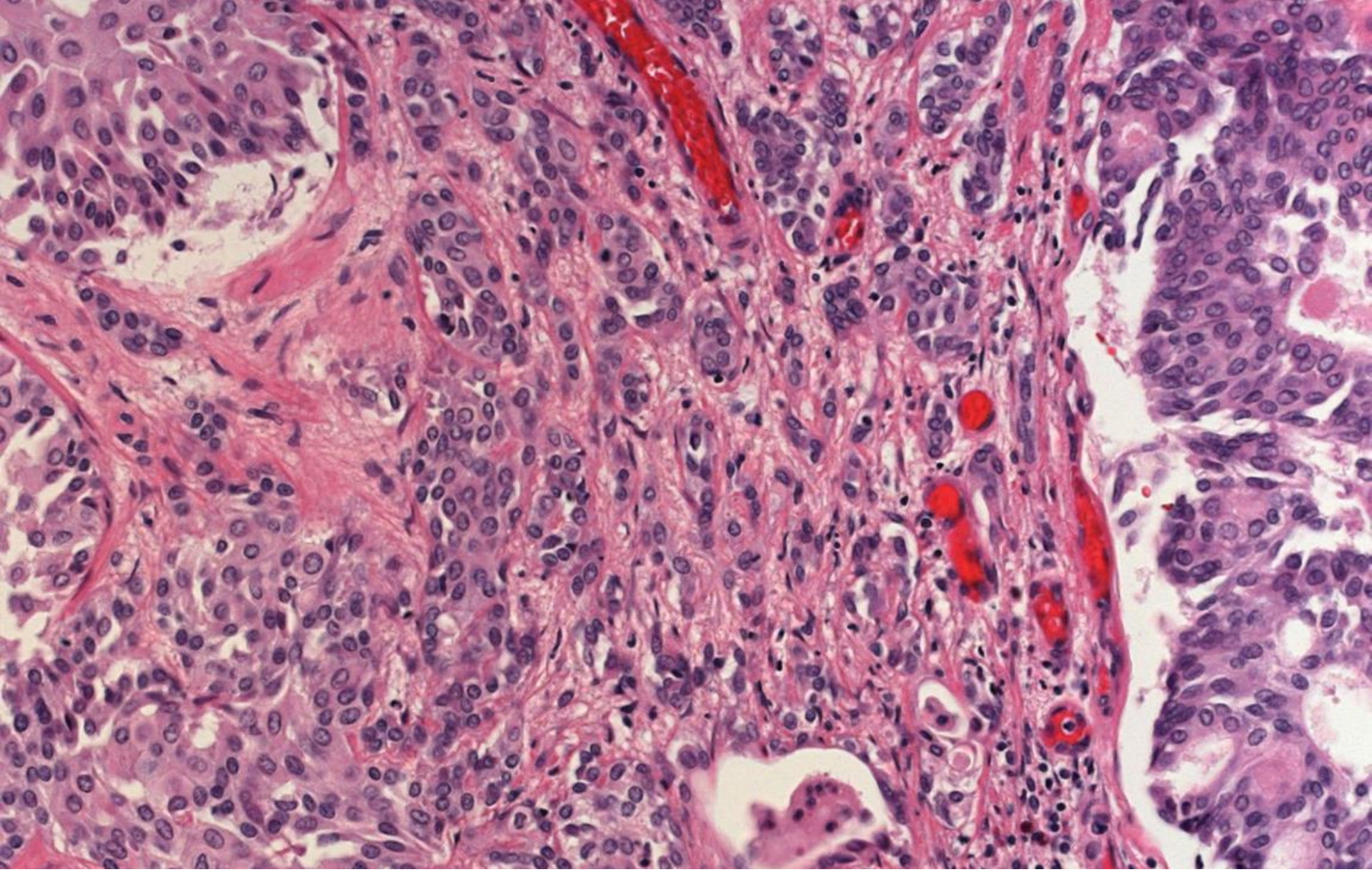
AUC: Urothelial neoplasm



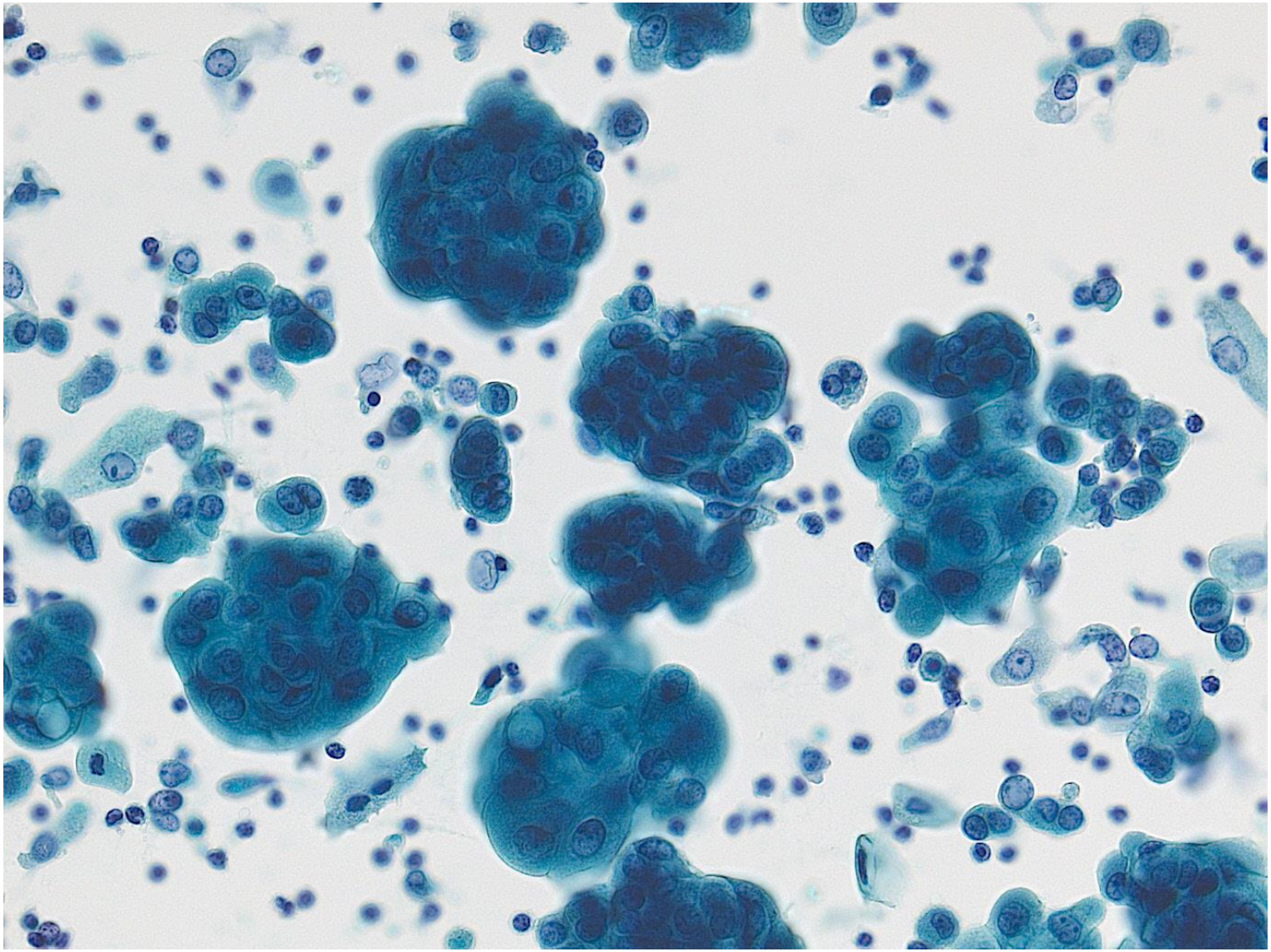




pT3, G2 urothelial carcinoma

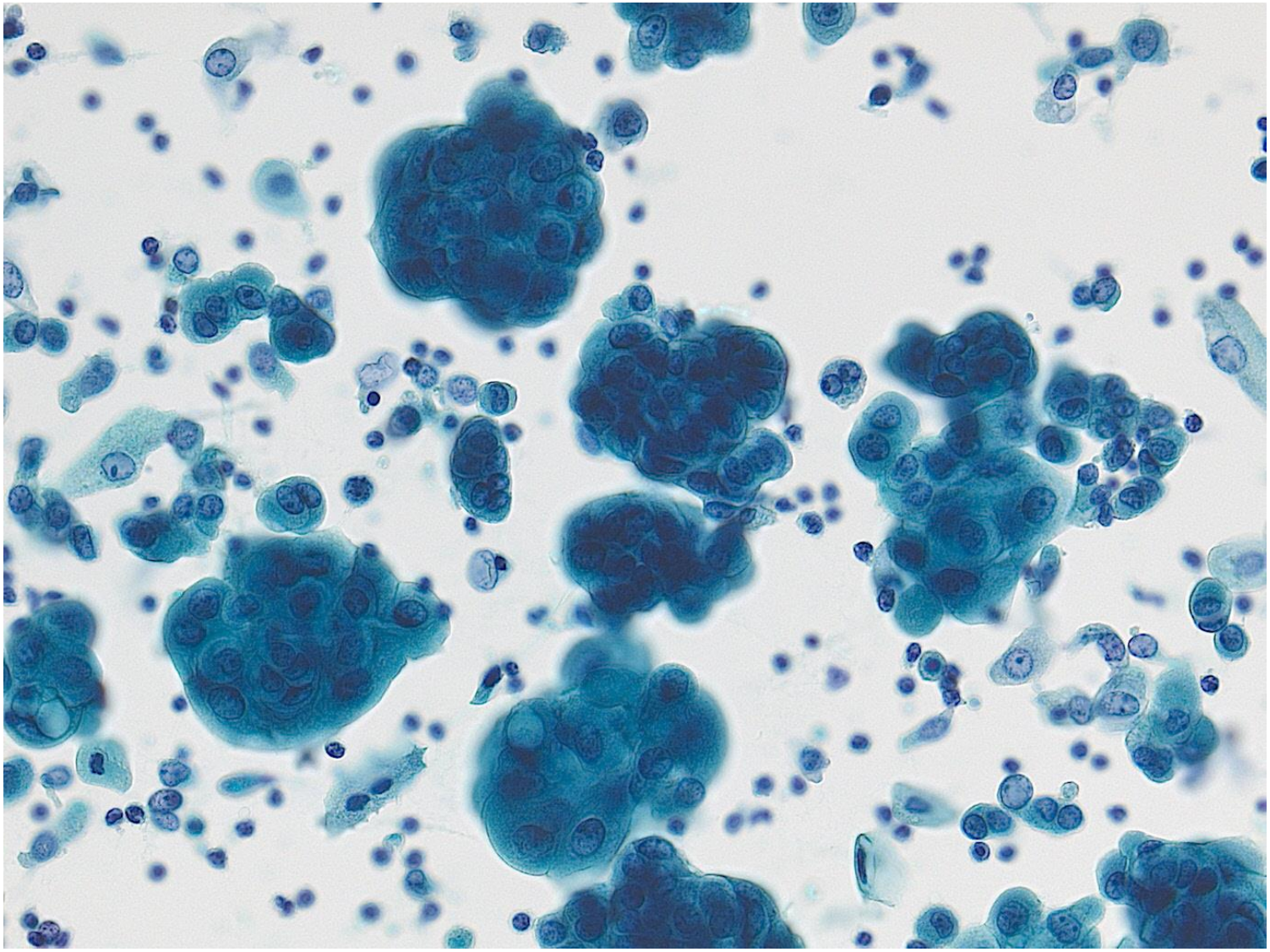
5

68y, history of pT1G3+CIS, cyst.: inconspicuous



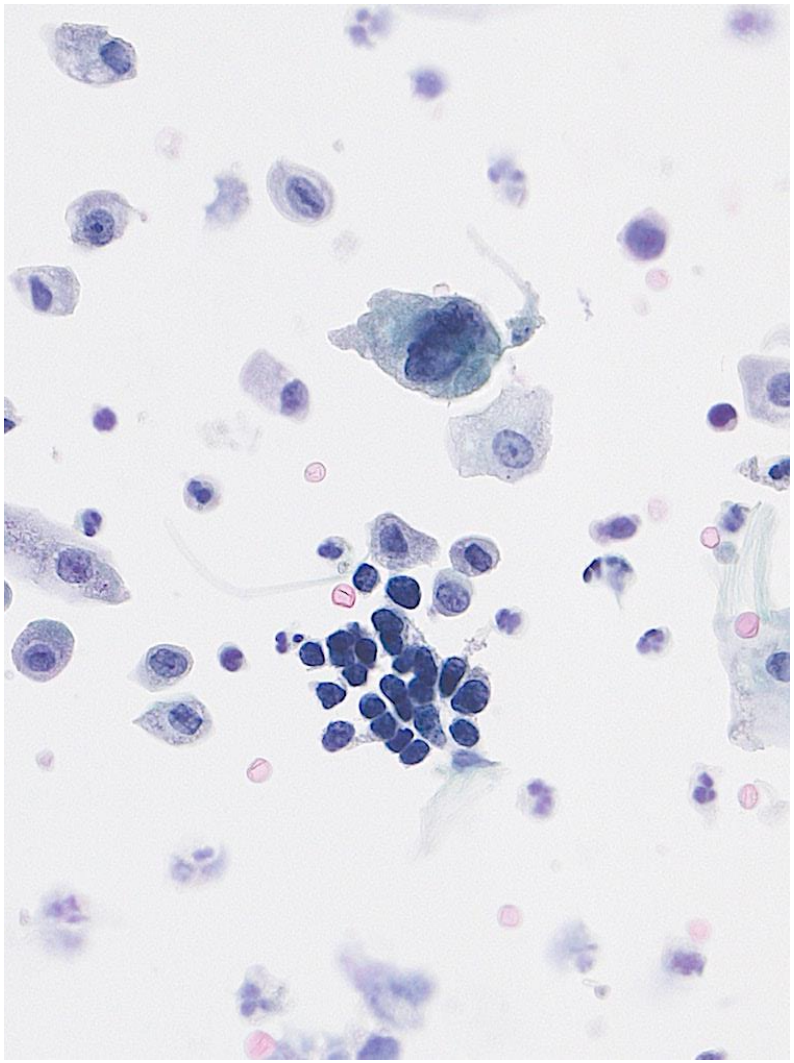
5

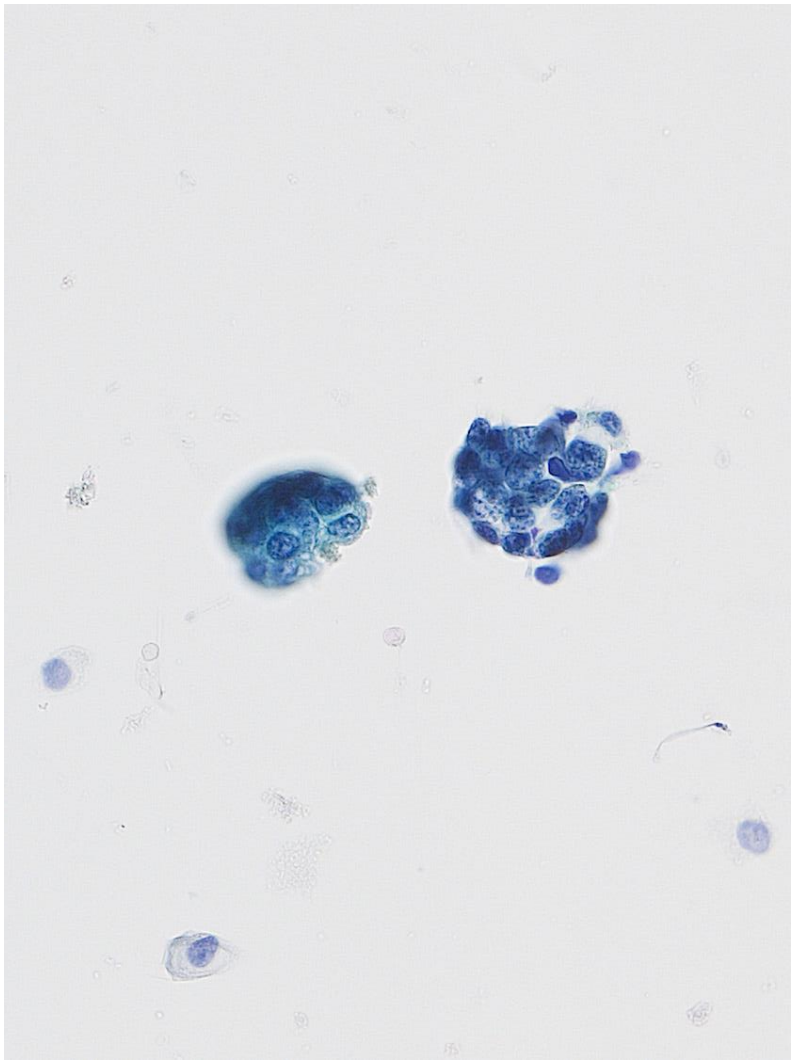
Benign
Paris: Negative for HGUC



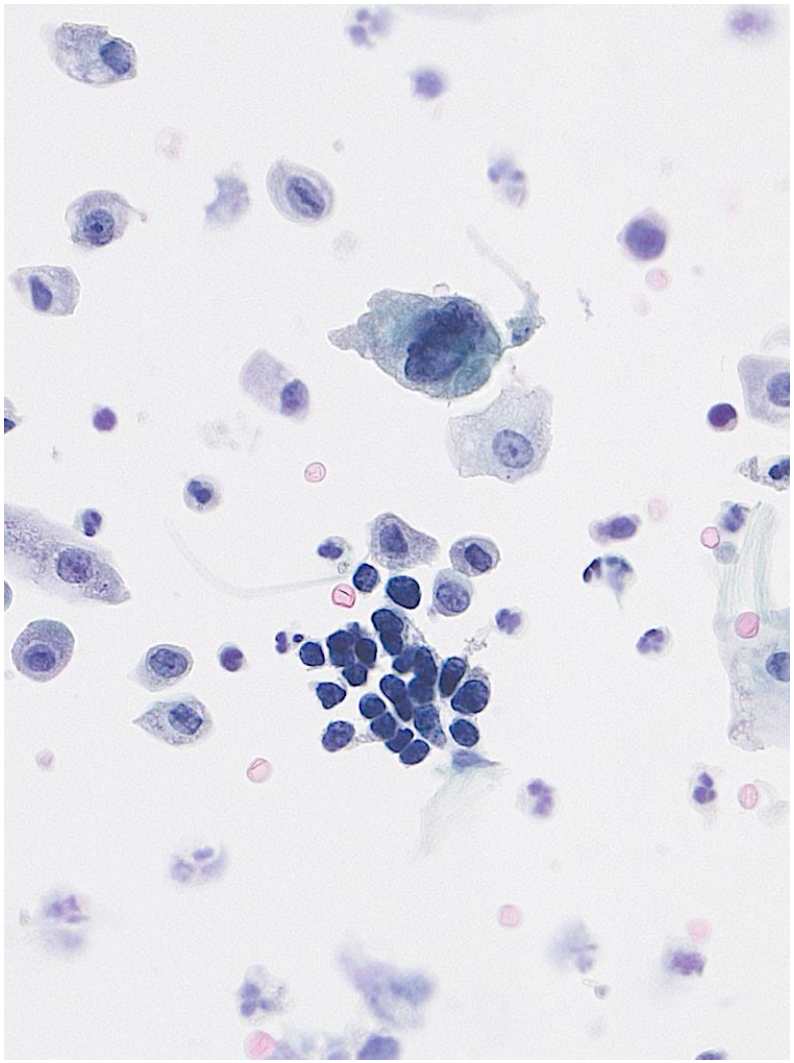
6

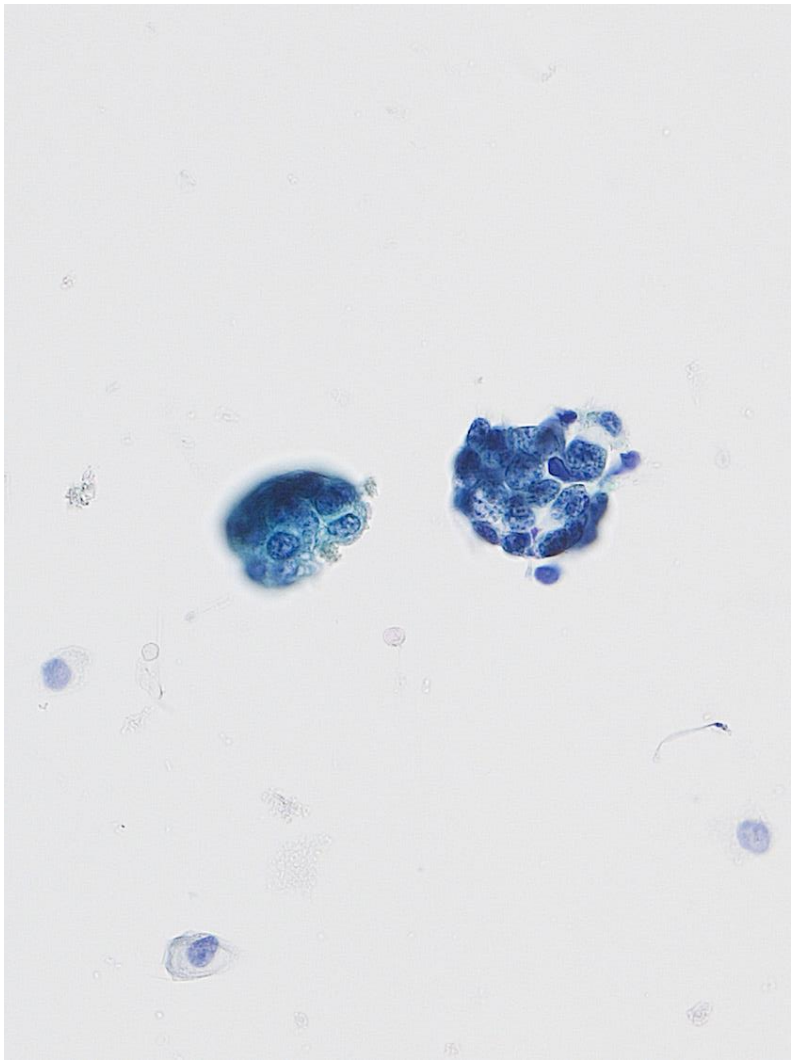
70y, history of pT1G3+CIS



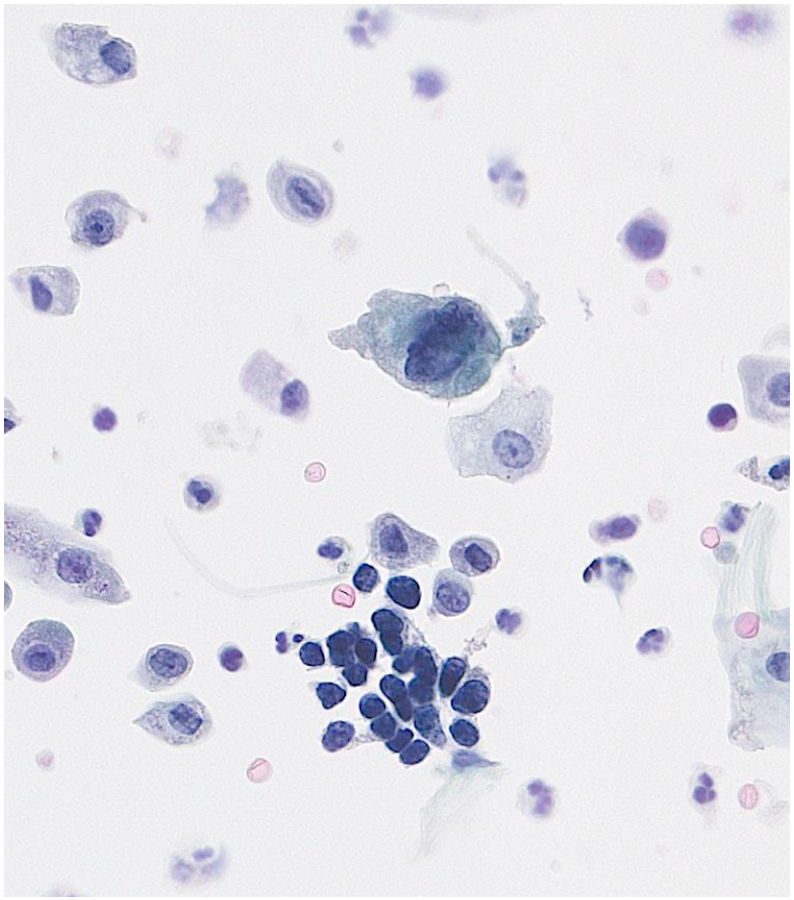


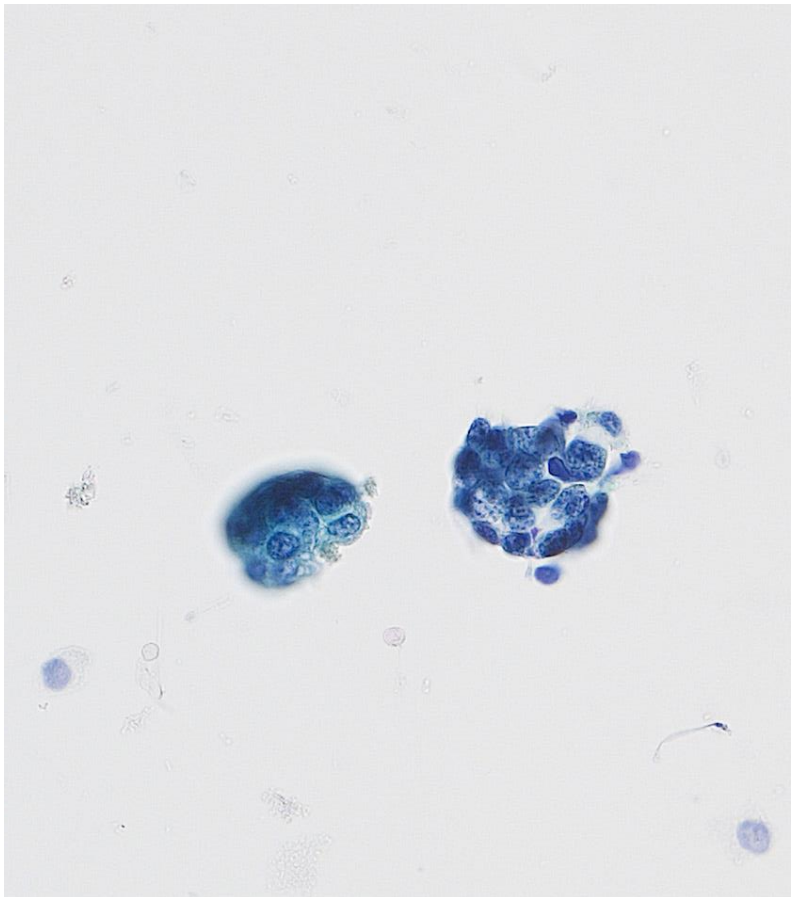
Small cell carcinoma





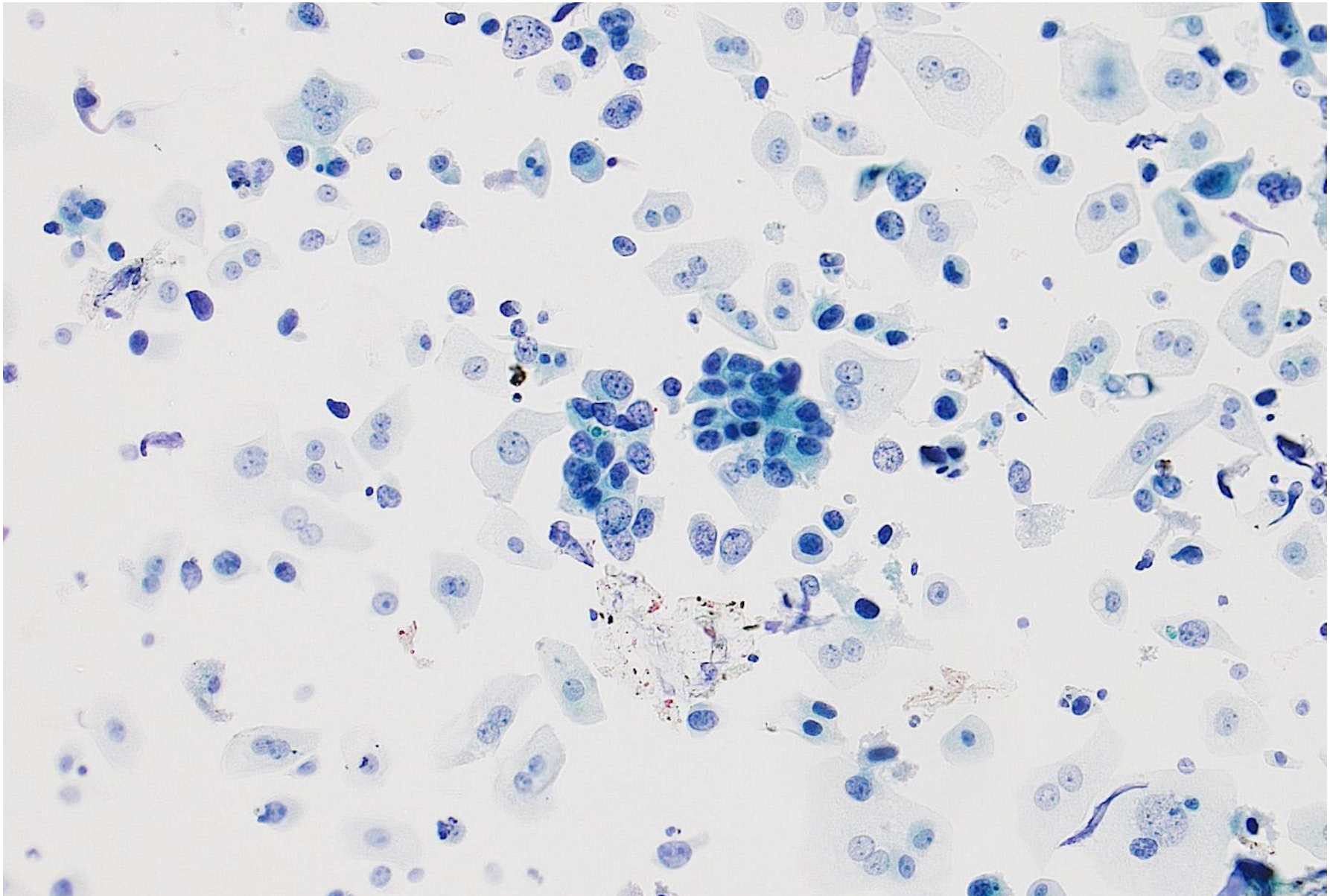
Small cell carcinoma





TTF1 does not help

7 61y, history of pTaG3, cyst.: red patch



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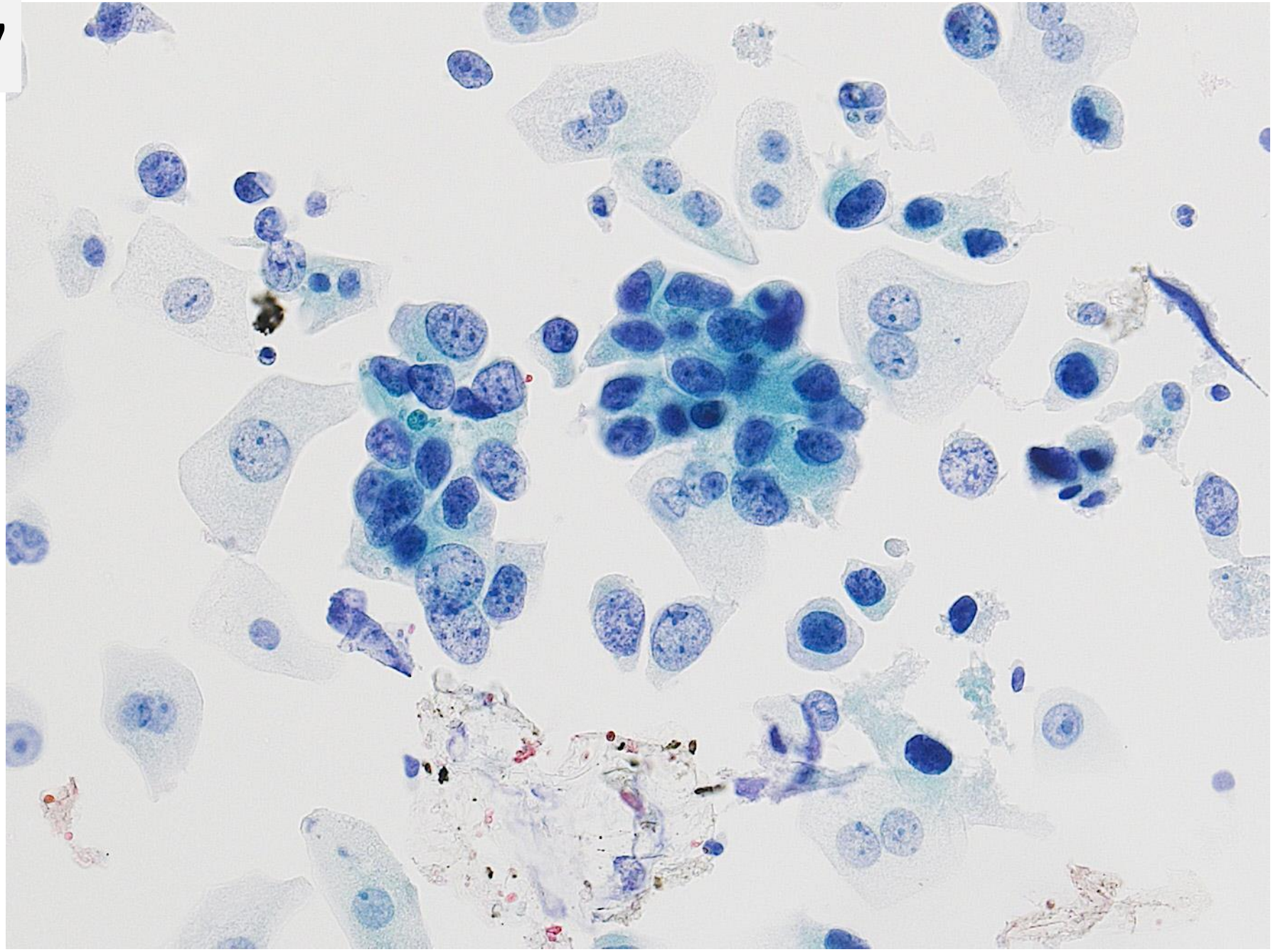

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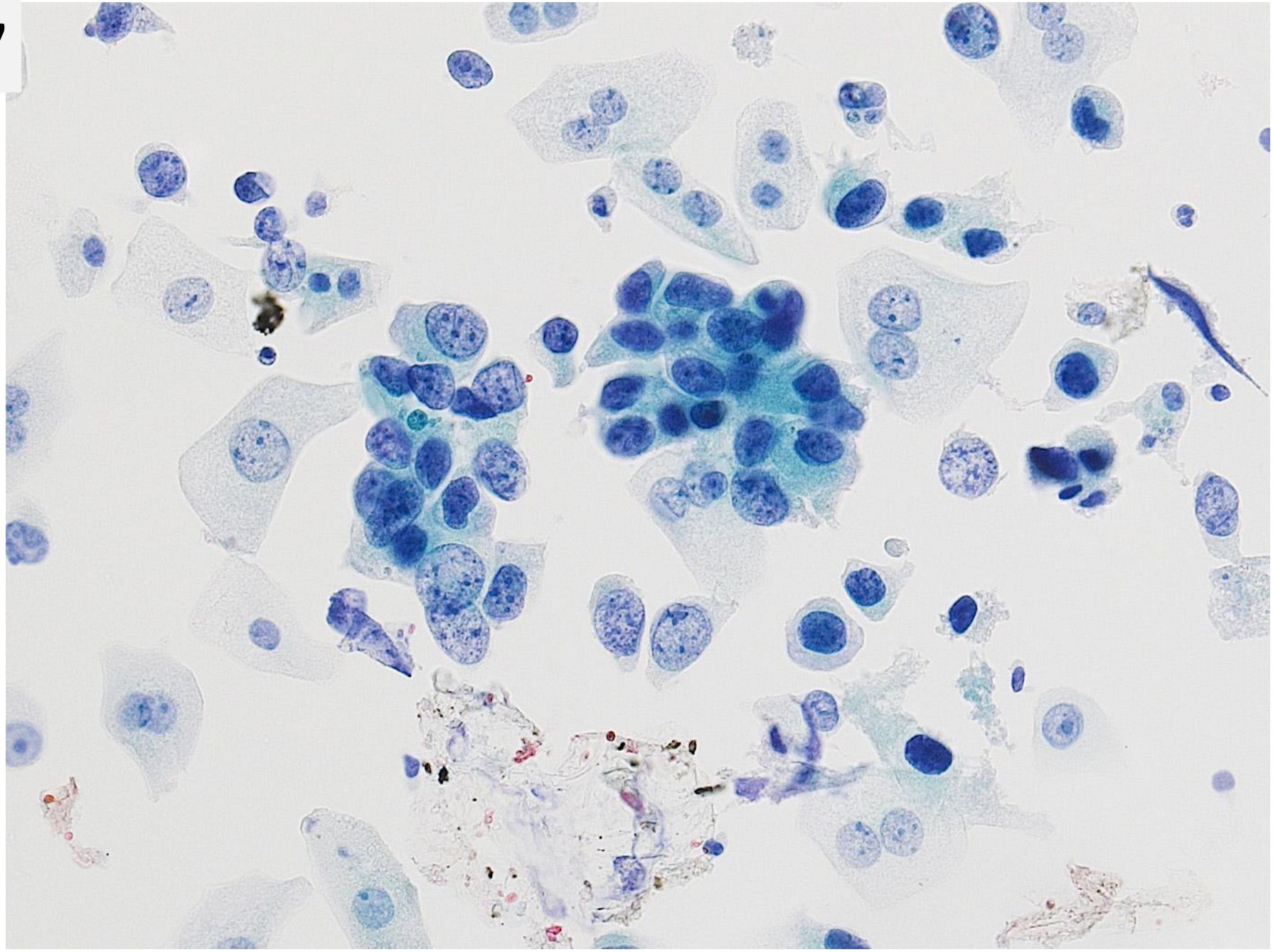

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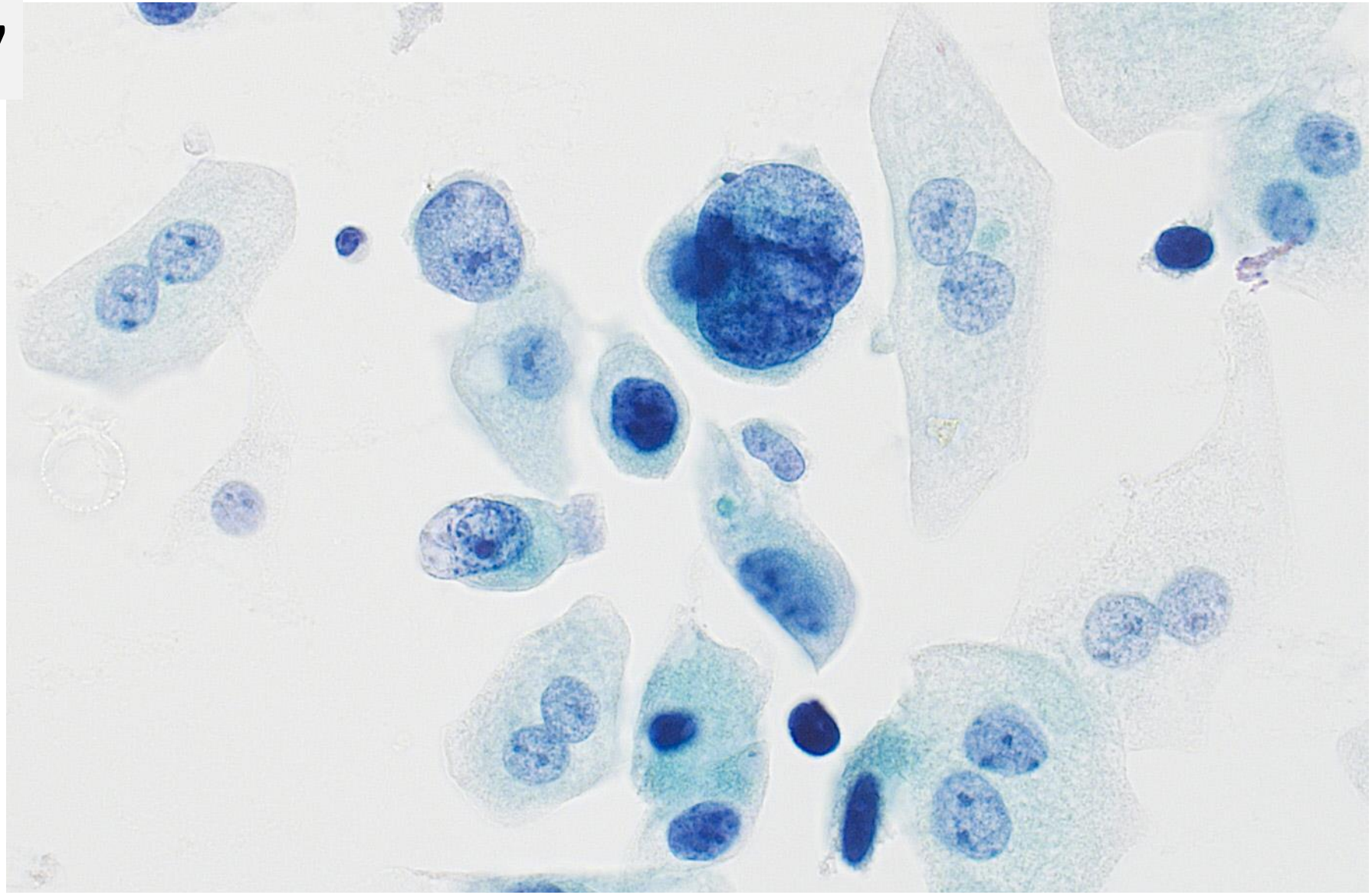
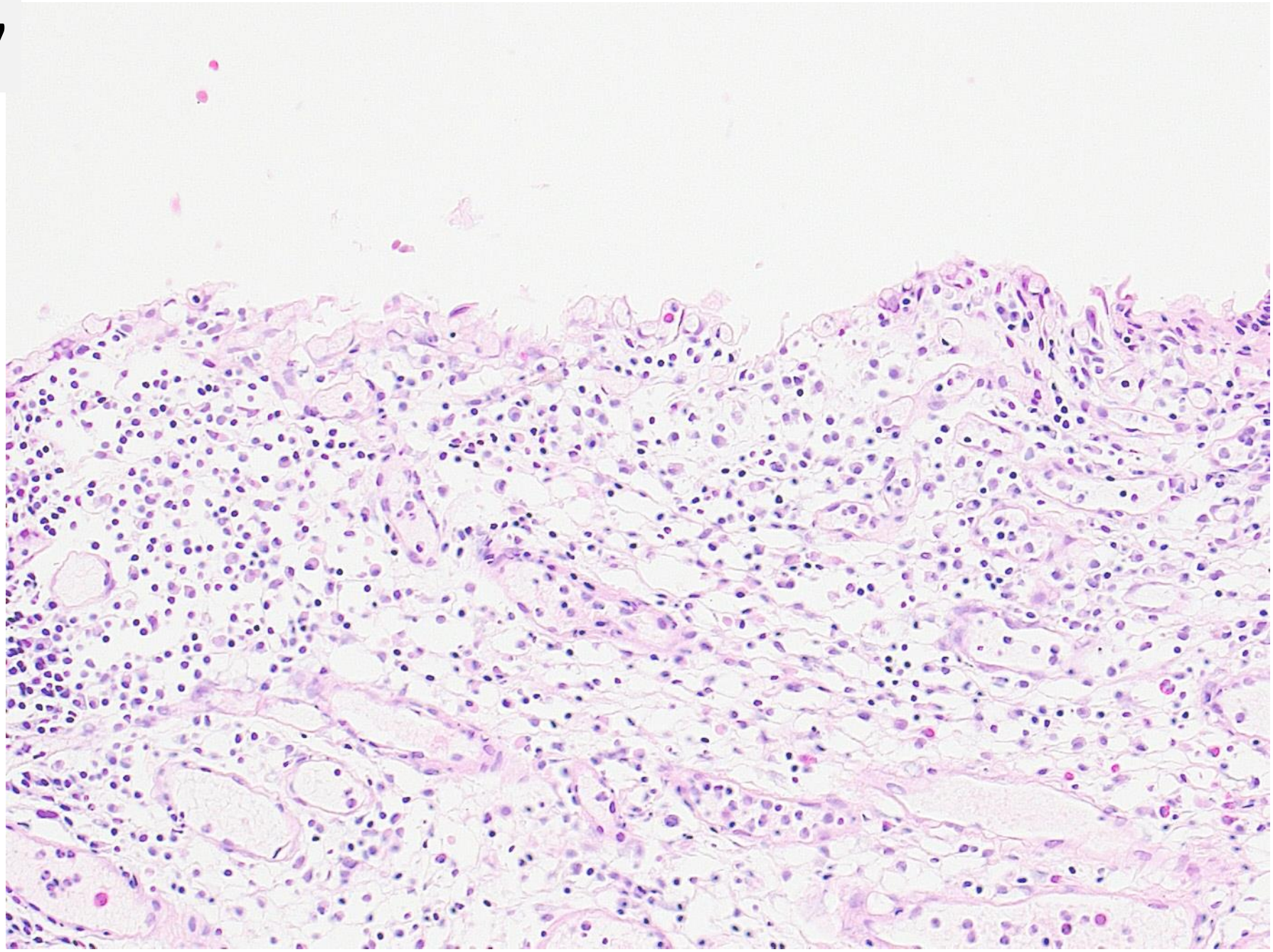


HGUC

7



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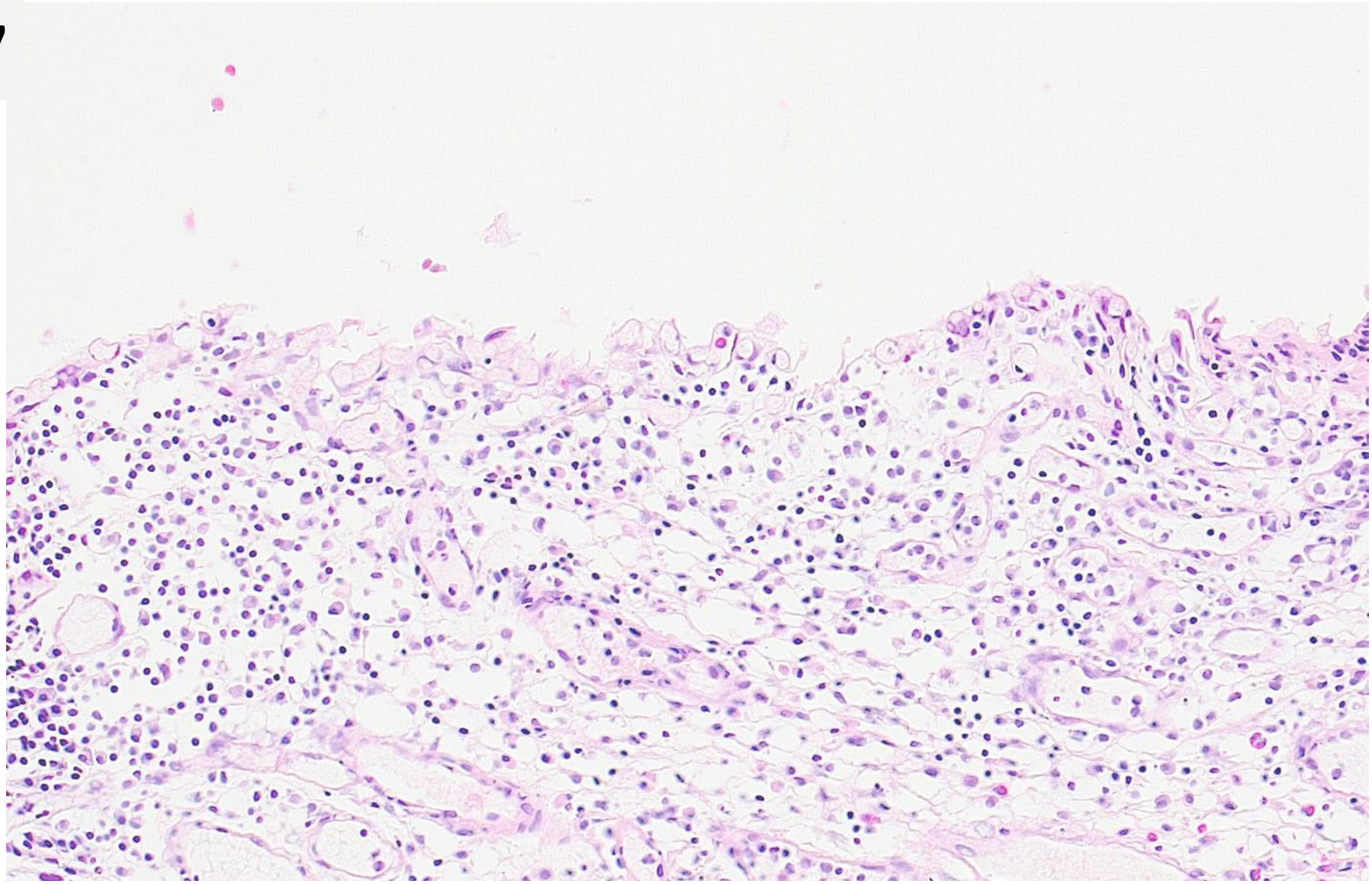
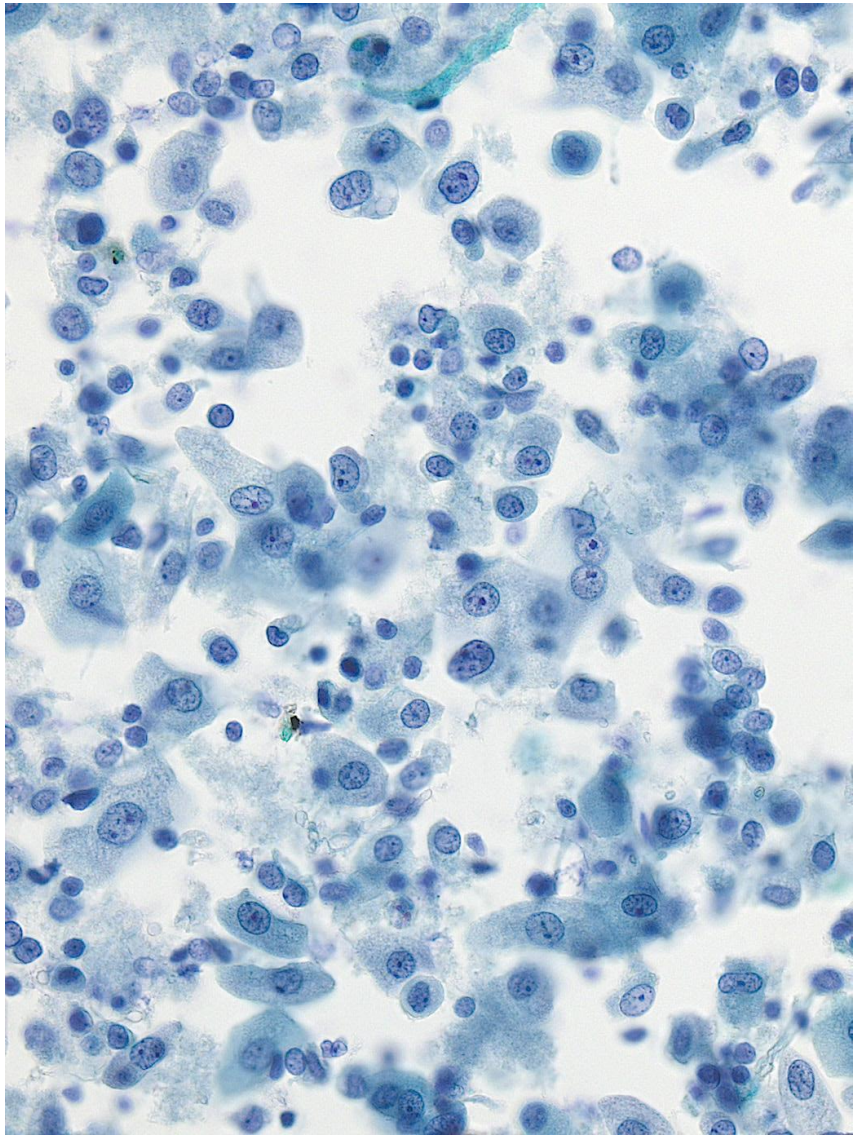
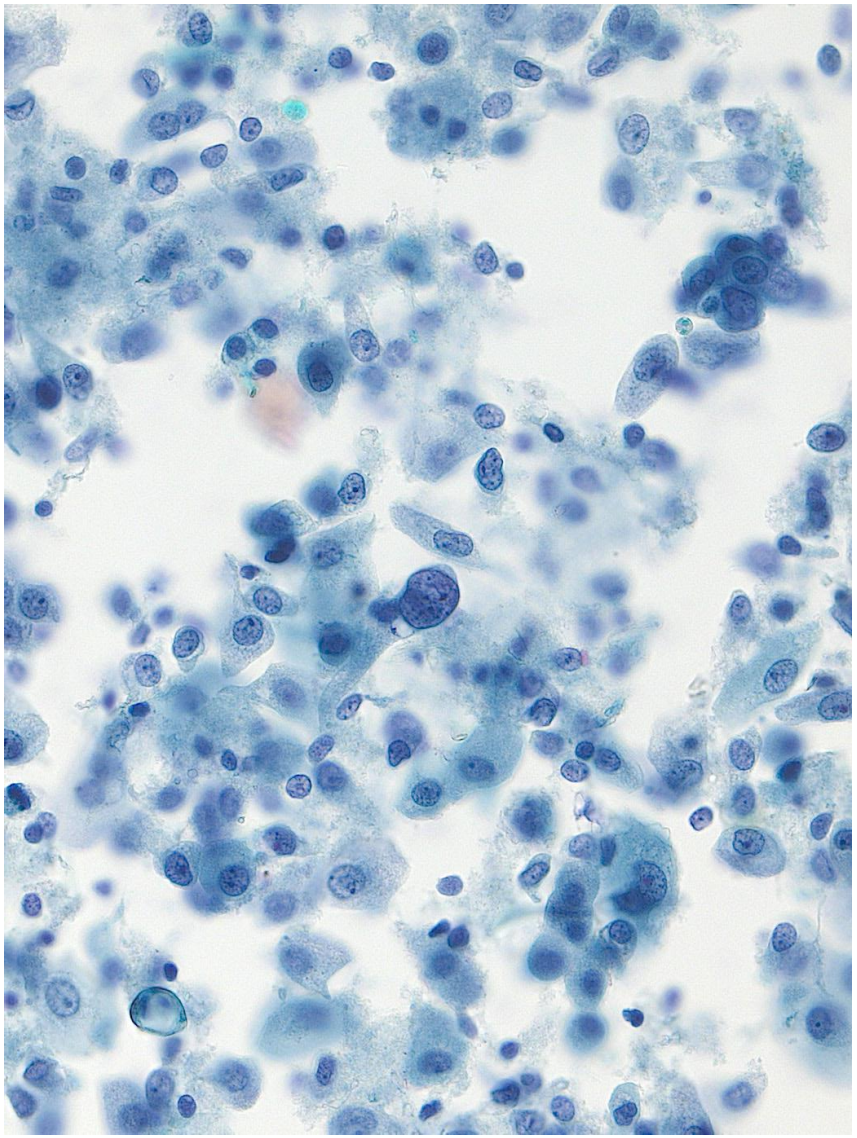


Urothelial CIS → BCG

8

90y, history of pT1G3 +CIS
Bladder washing after BCG





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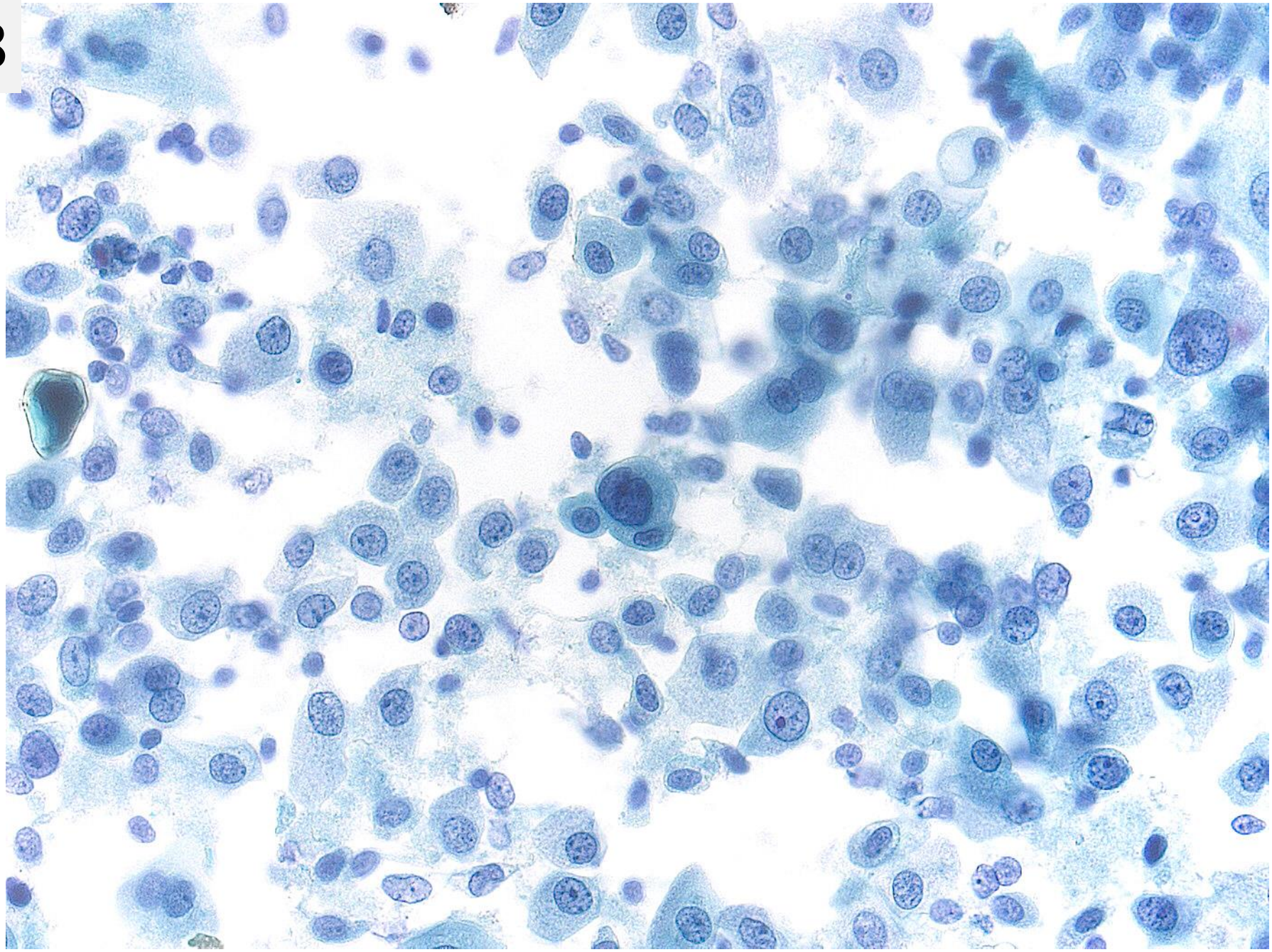

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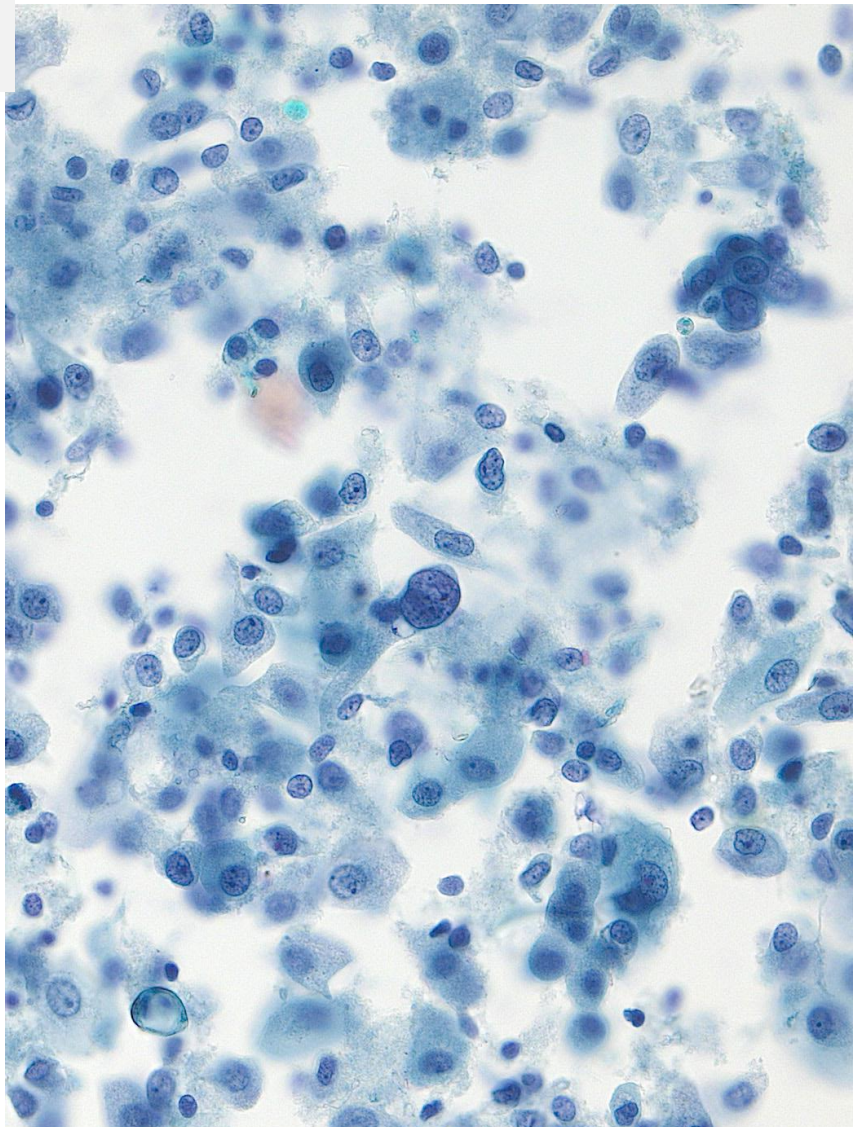
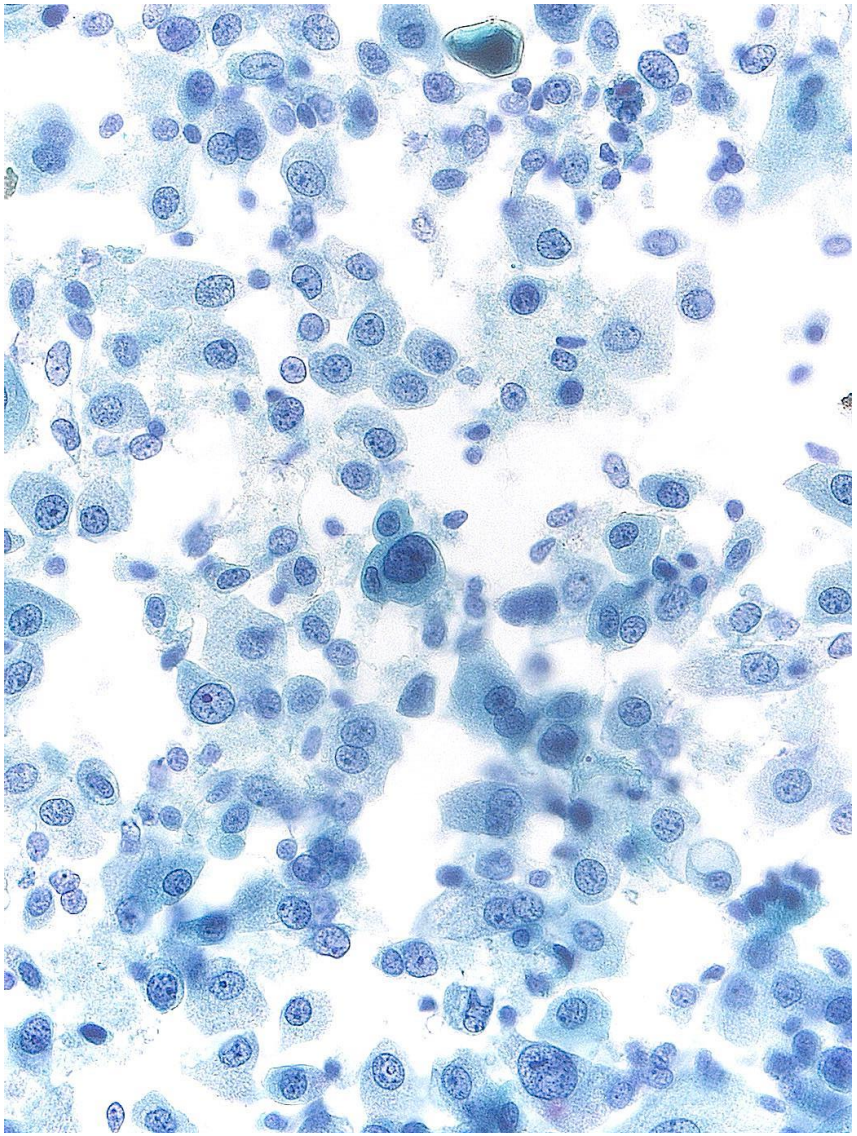
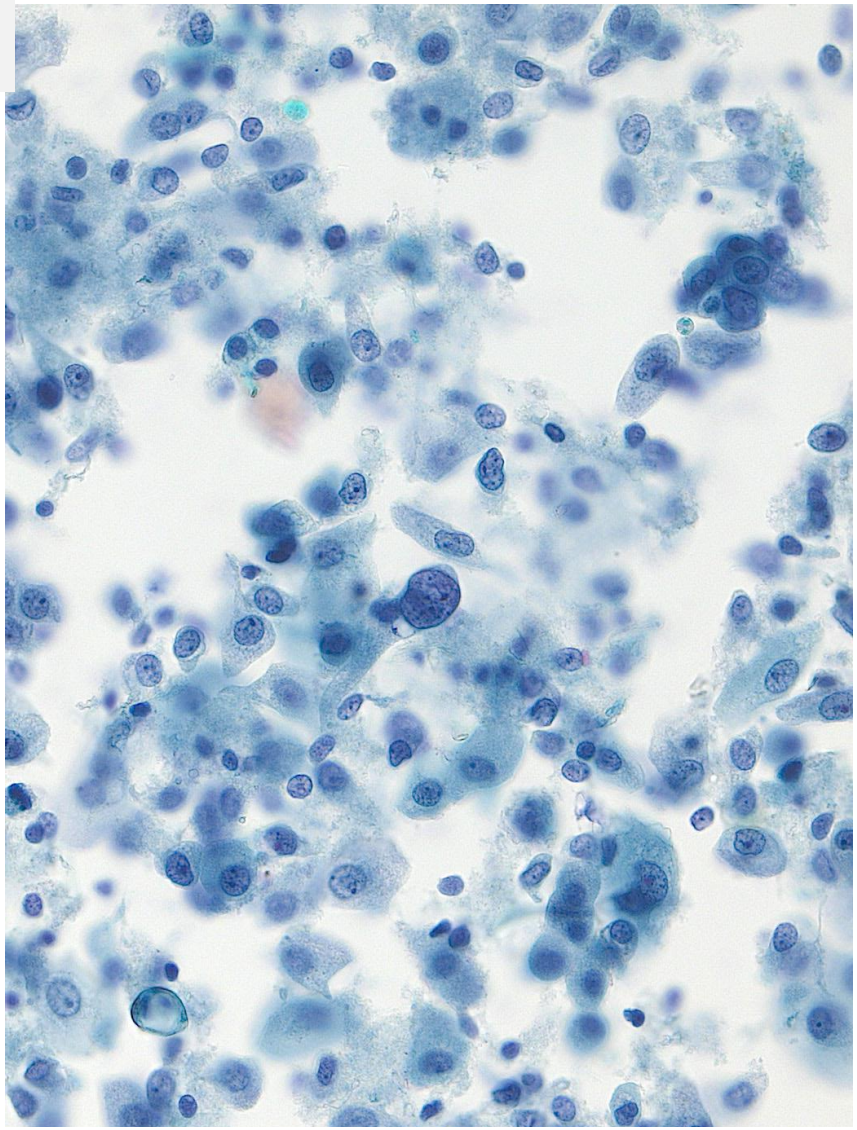
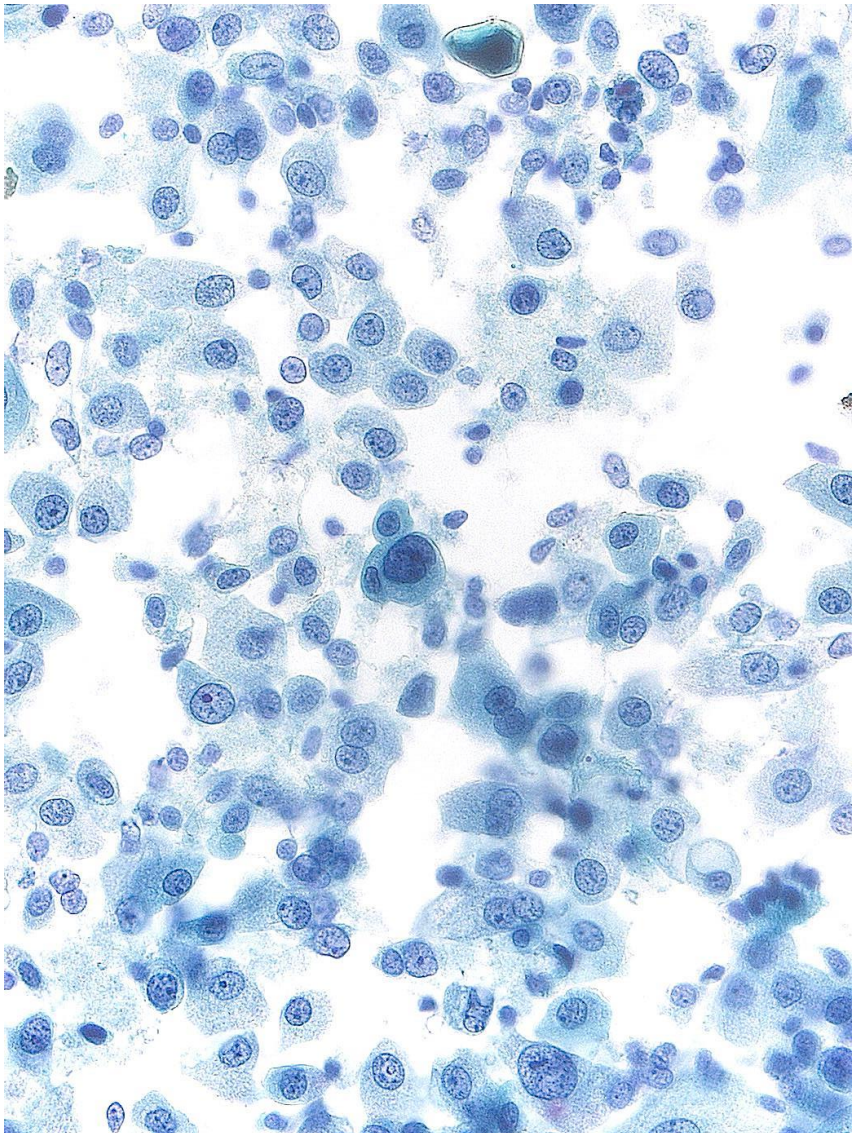




SHGUC

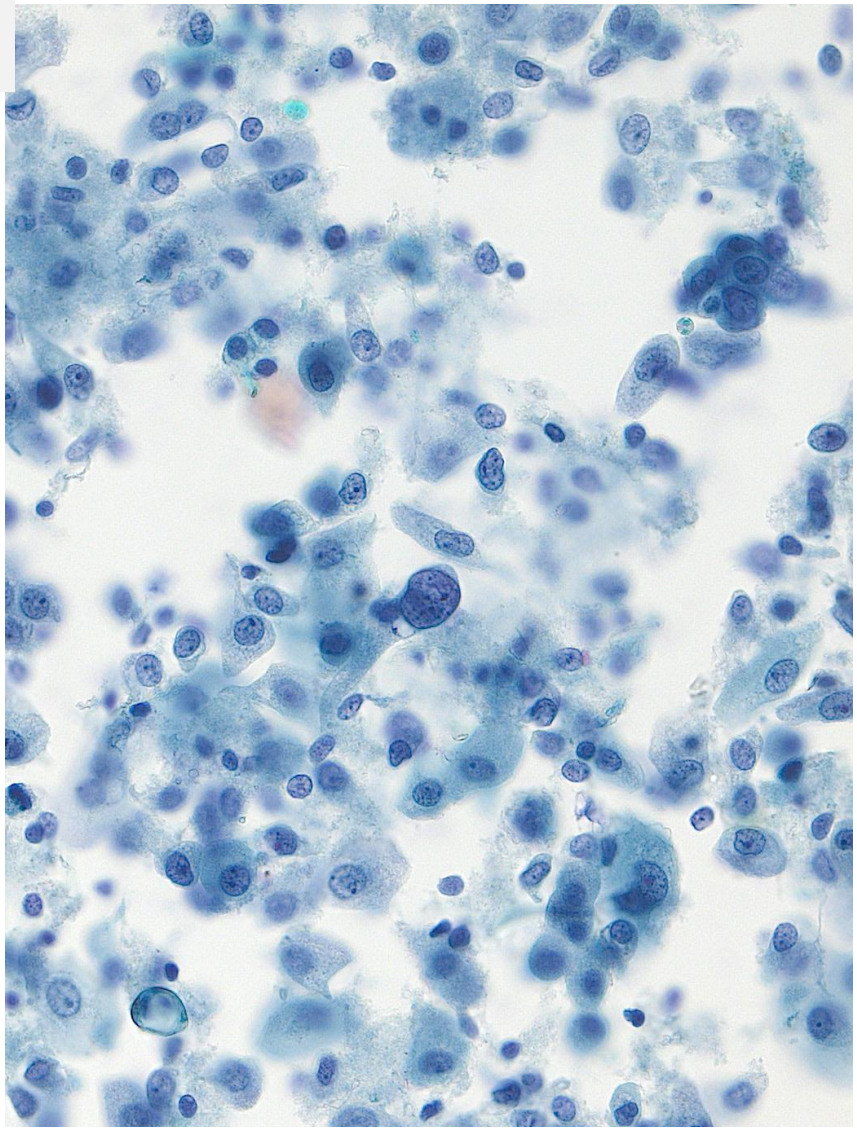
8

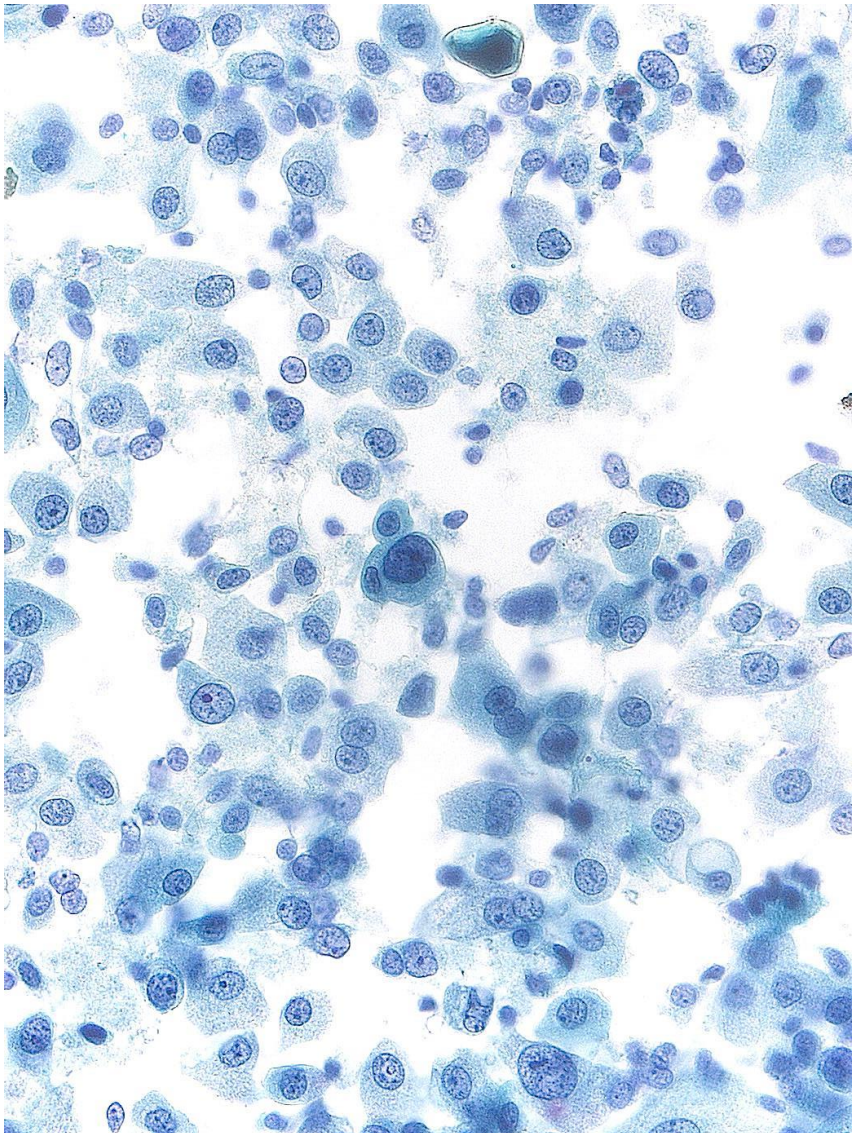




FISH pos.

8

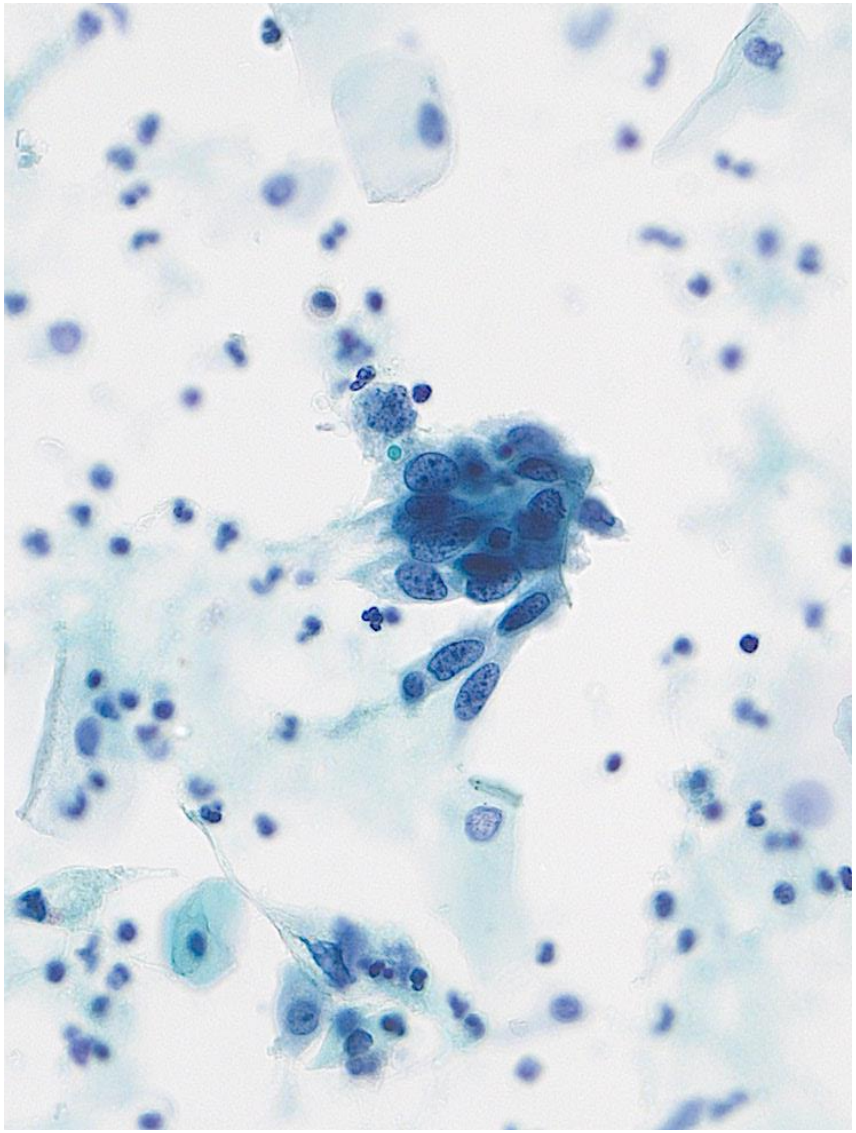


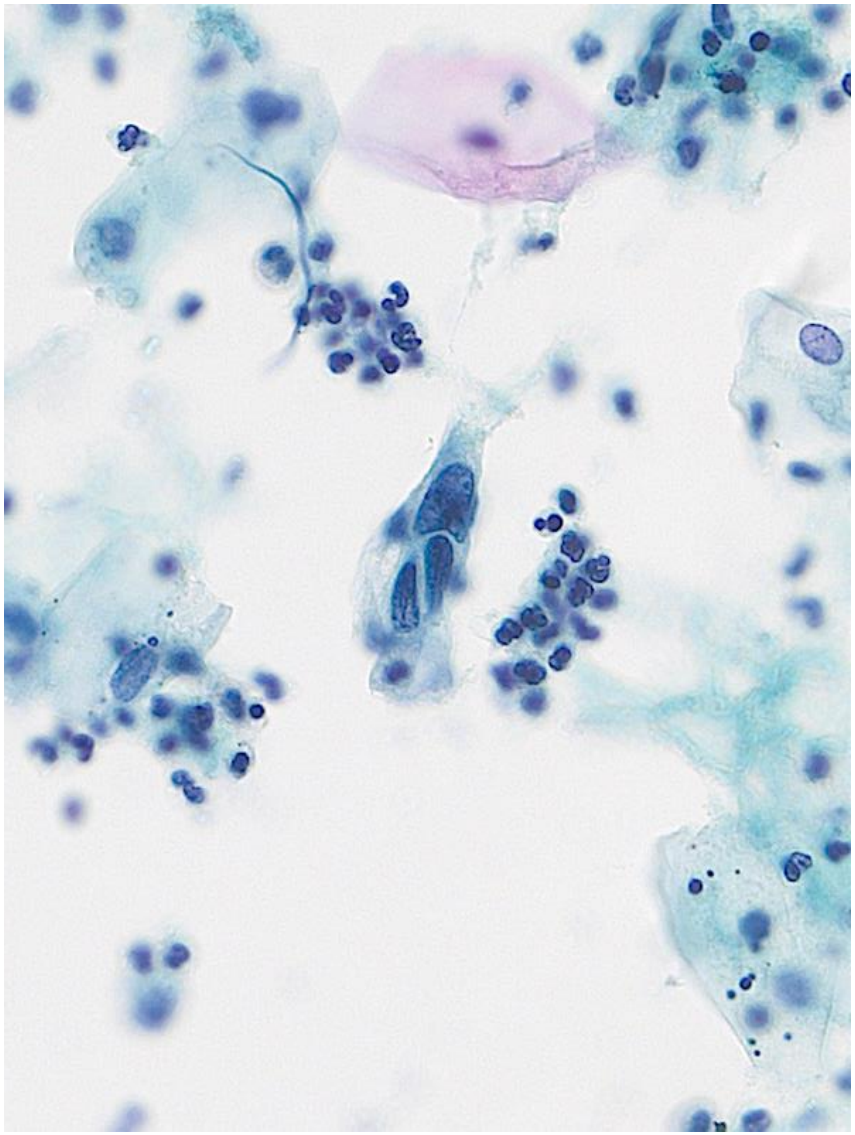


HGUC: Persistent CIS after BCG

9

63y, history of CA of the uterine cervix, cyst.: severe redness





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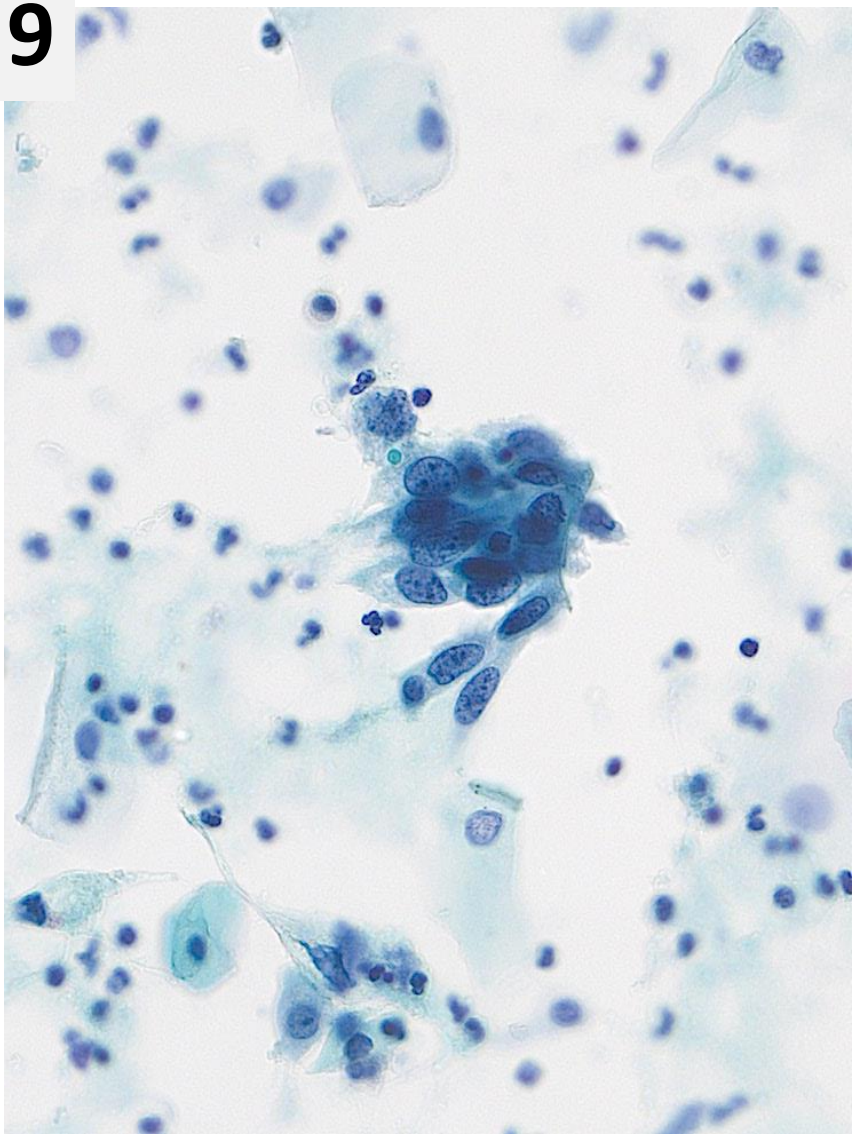
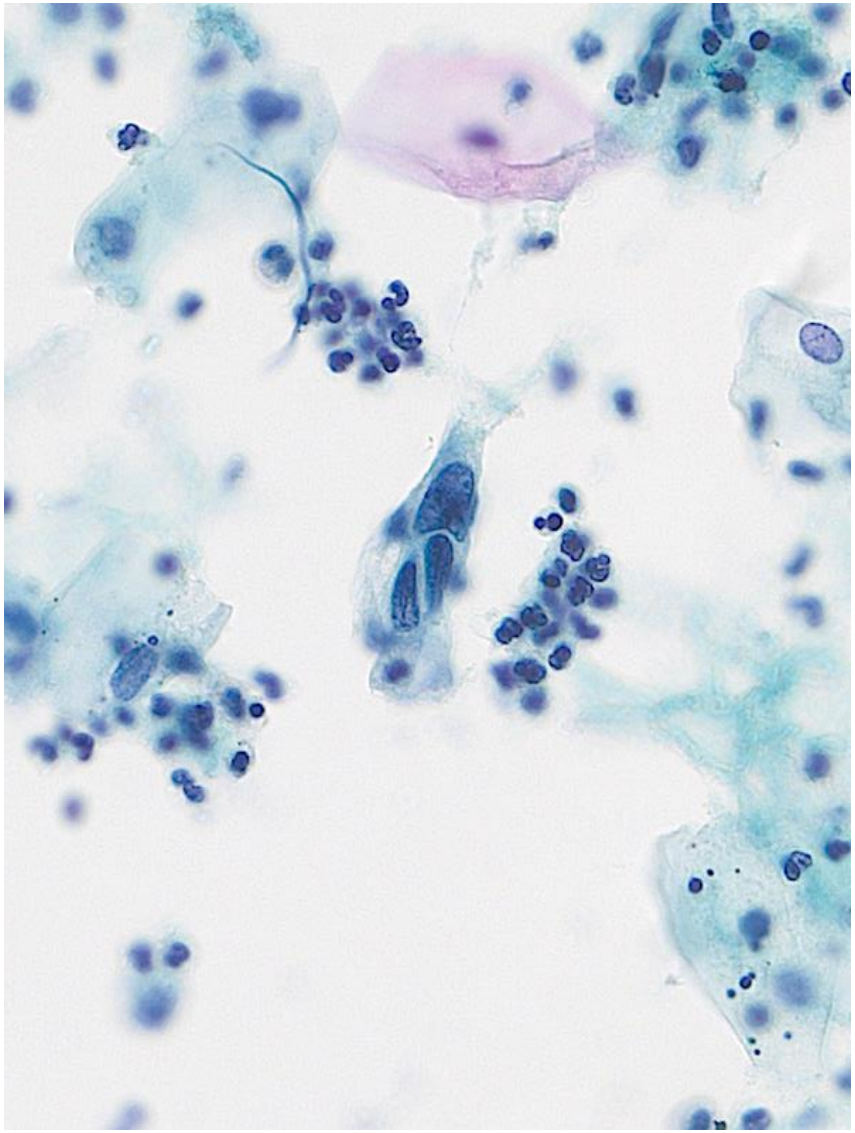
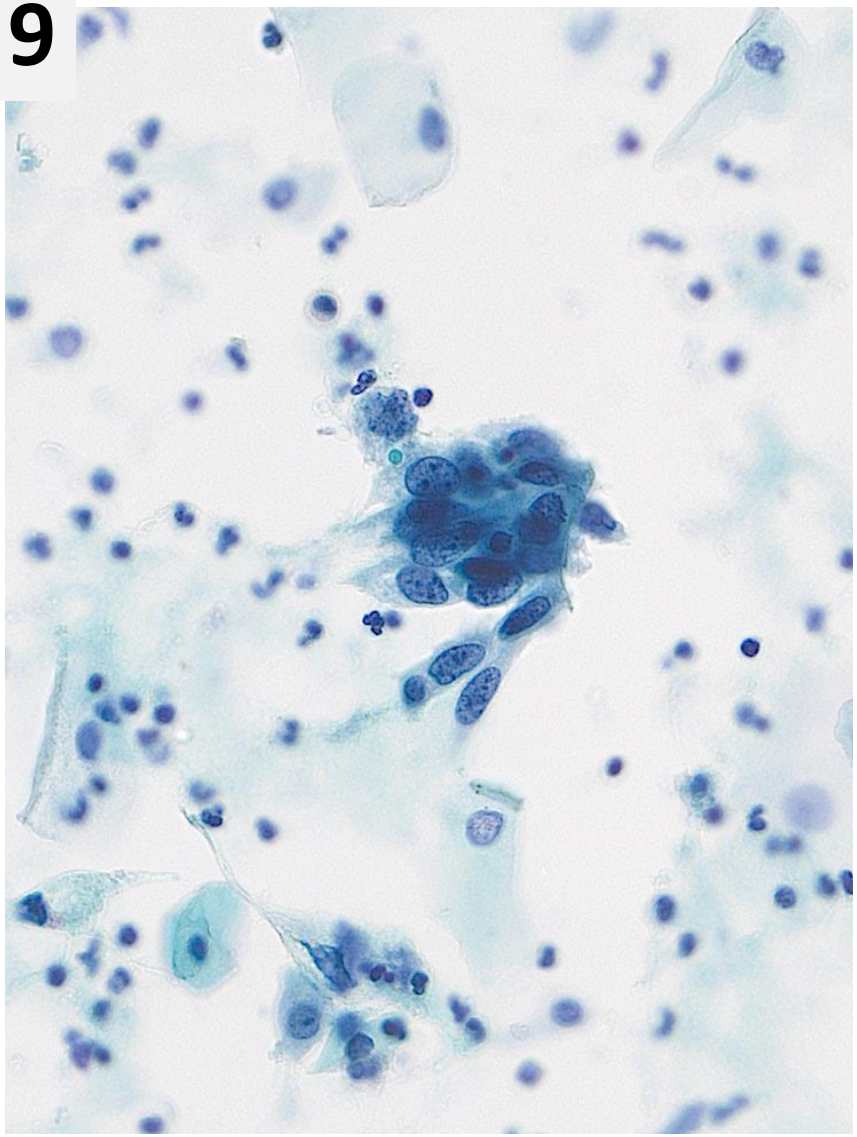
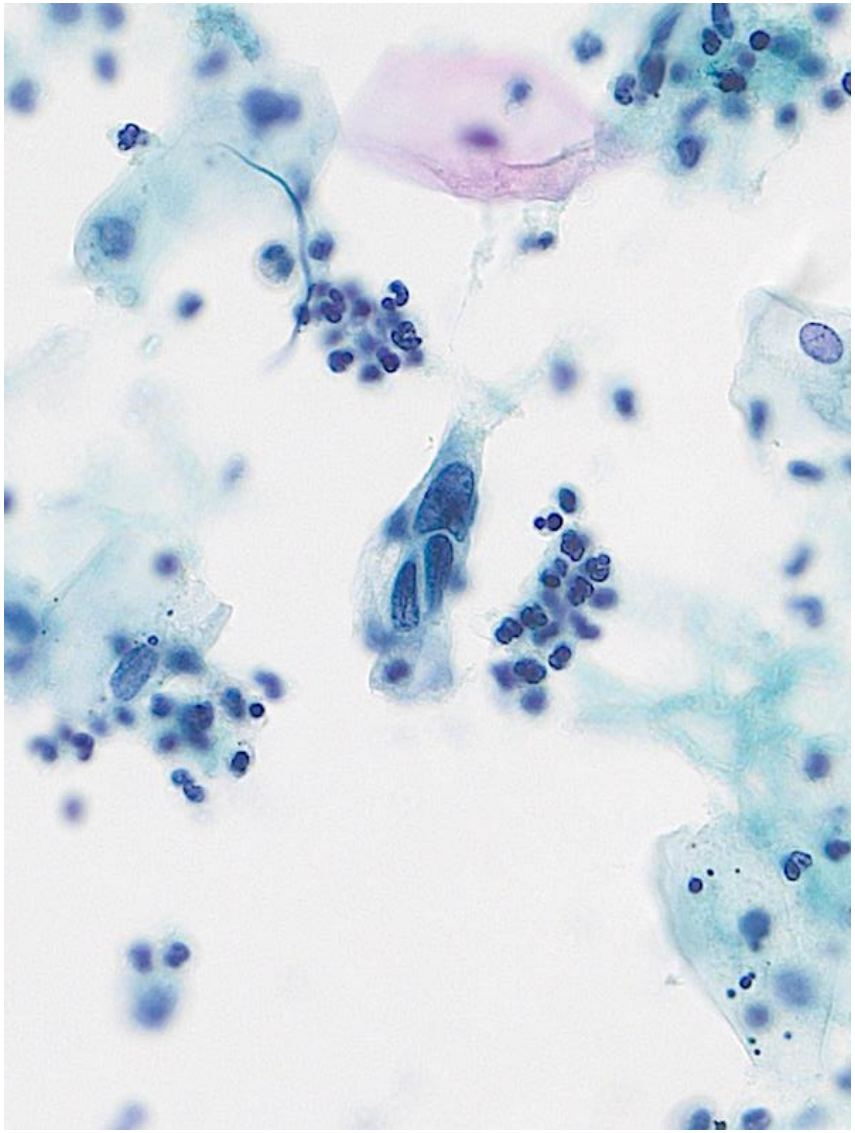




SHGUC

9

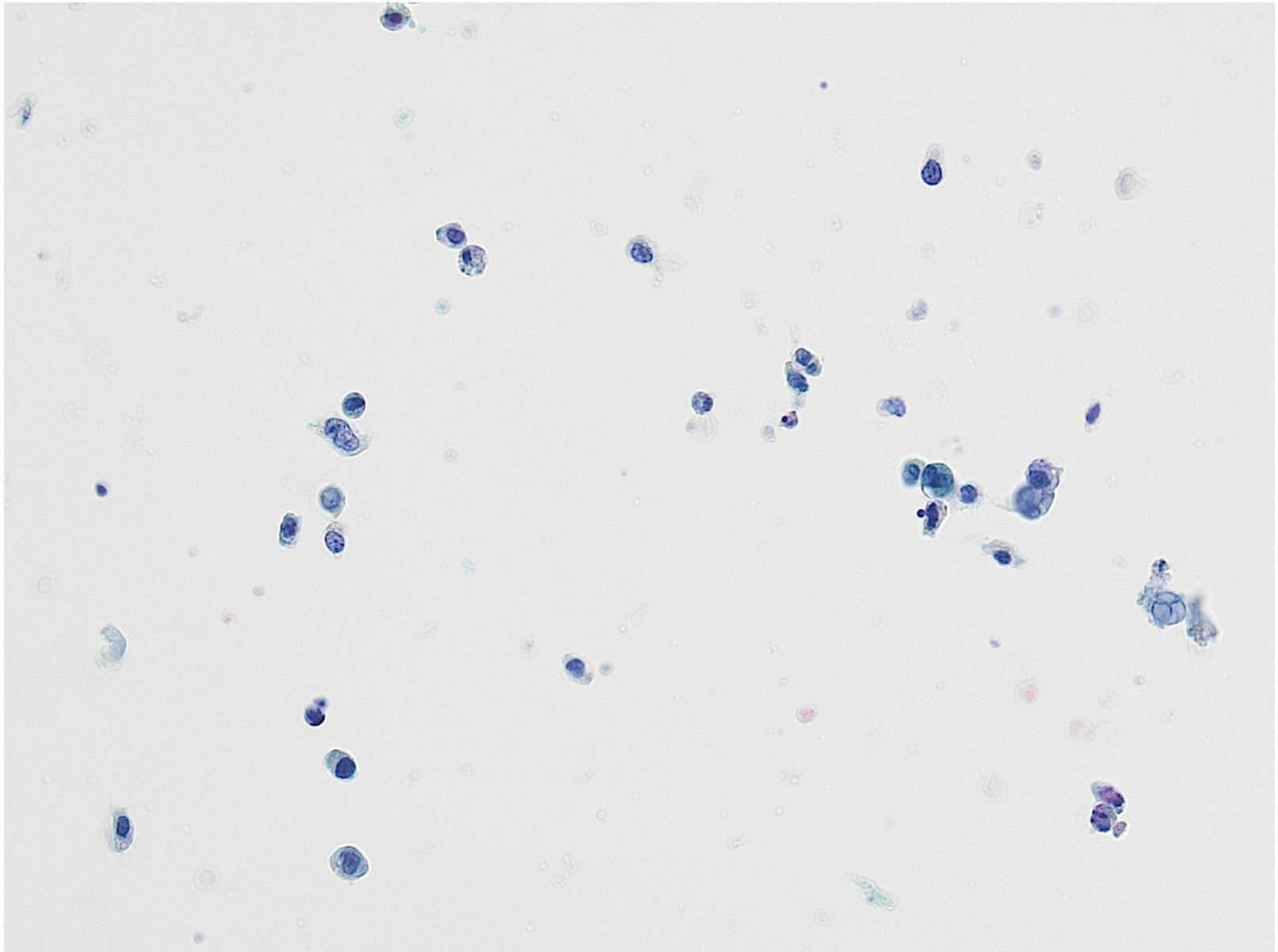




Radiation Cystitis

10

39y, dysuria, cyst.: papillary tumor



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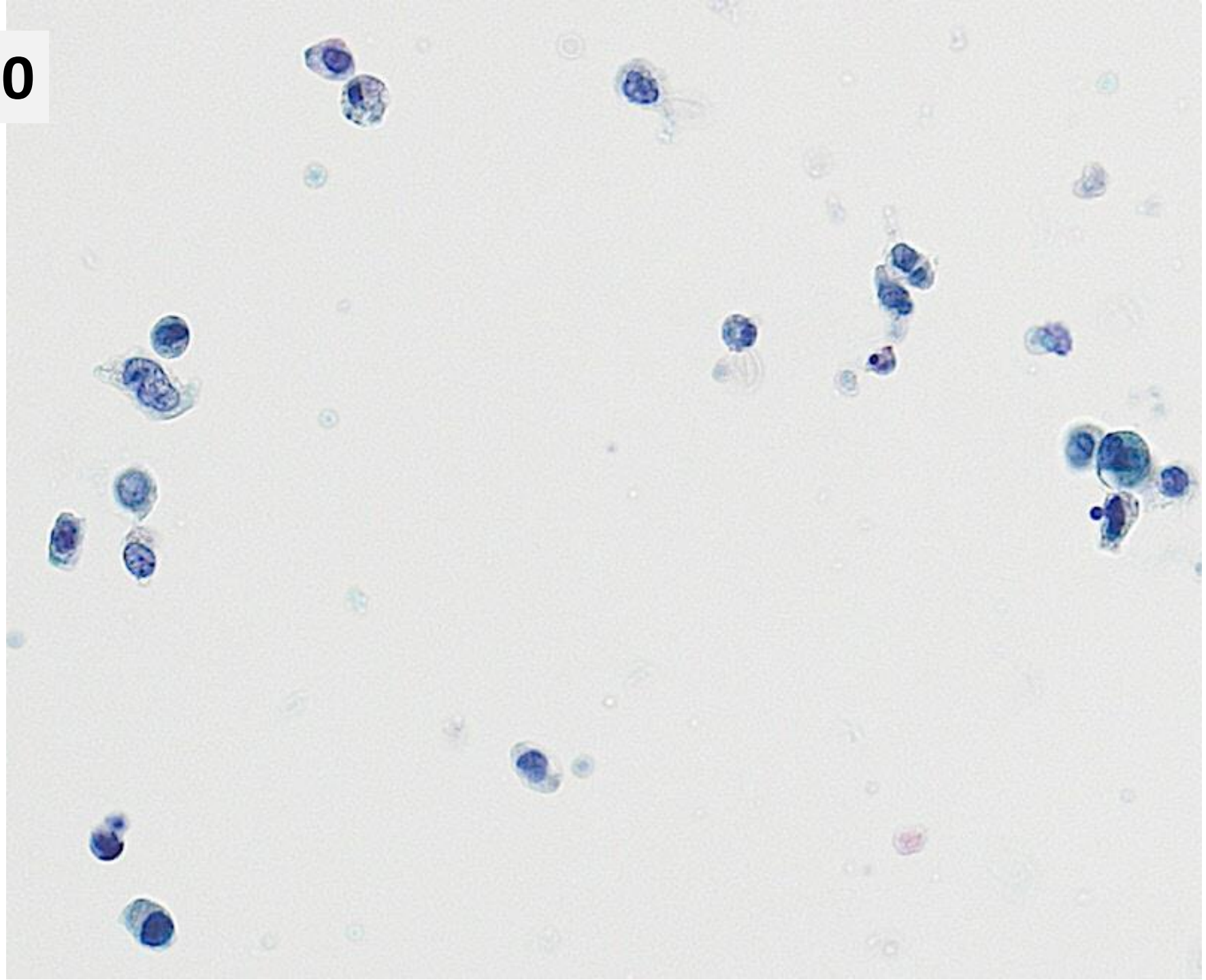

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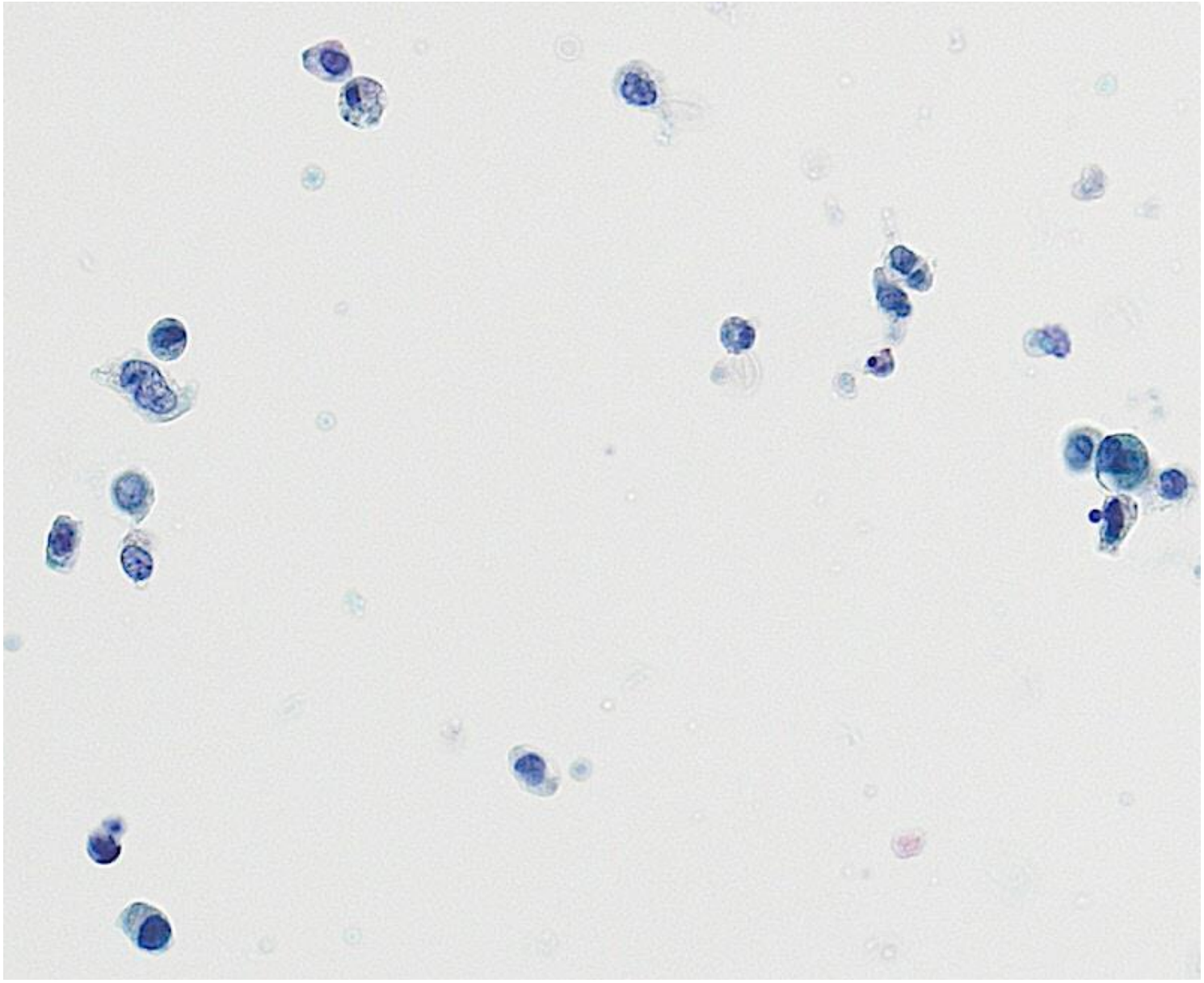
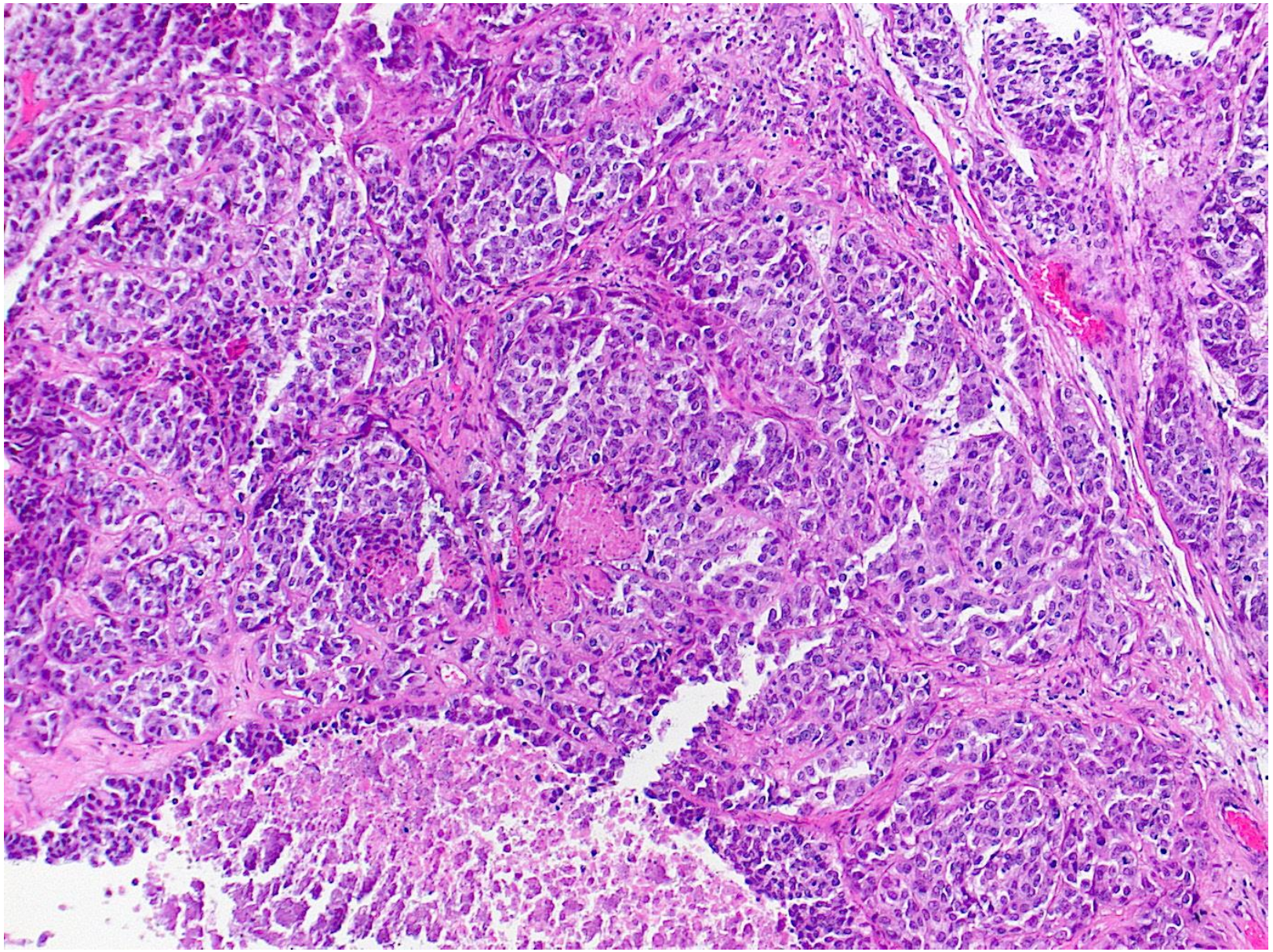


AUC

Note: cannot exclude HGUC

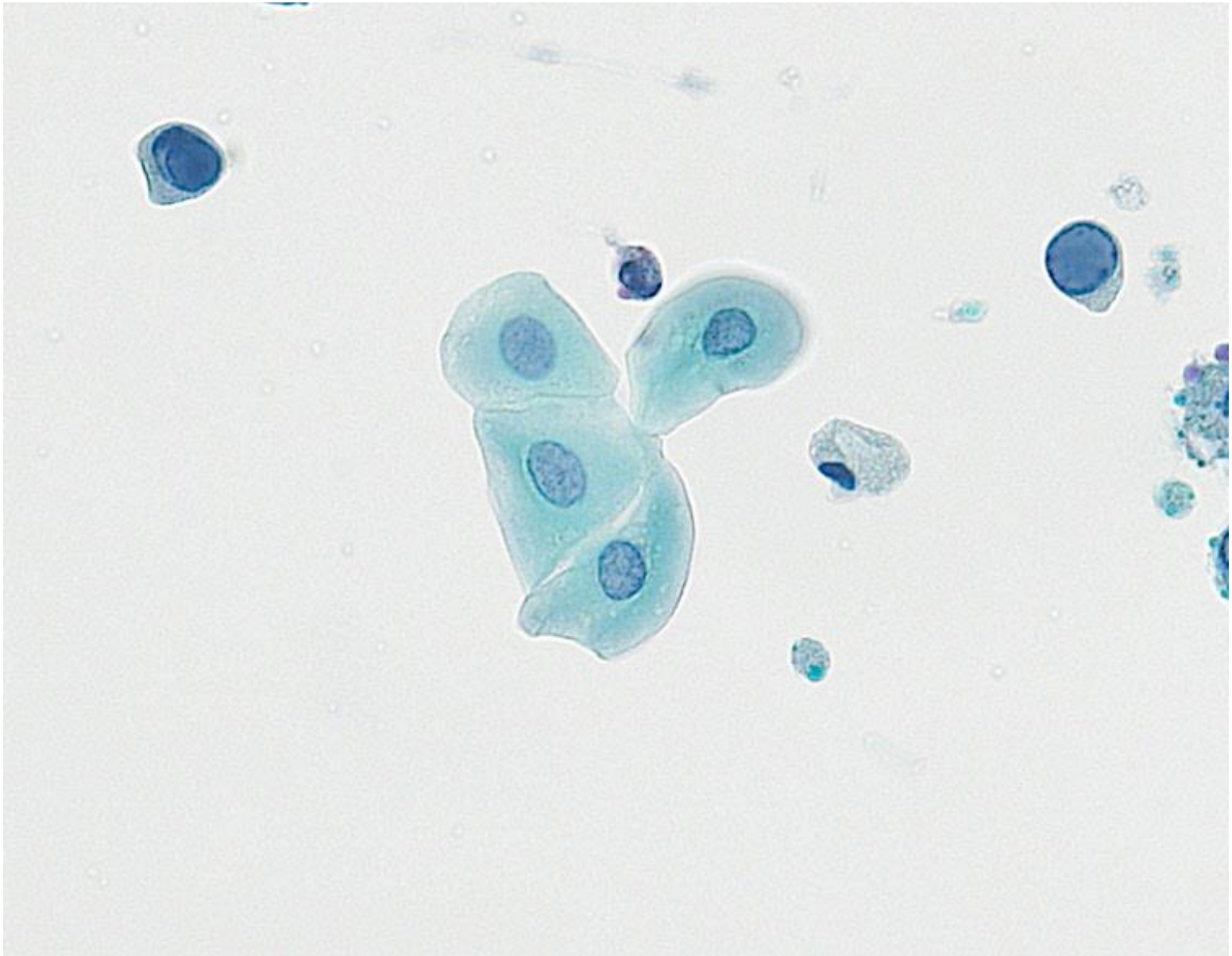
10



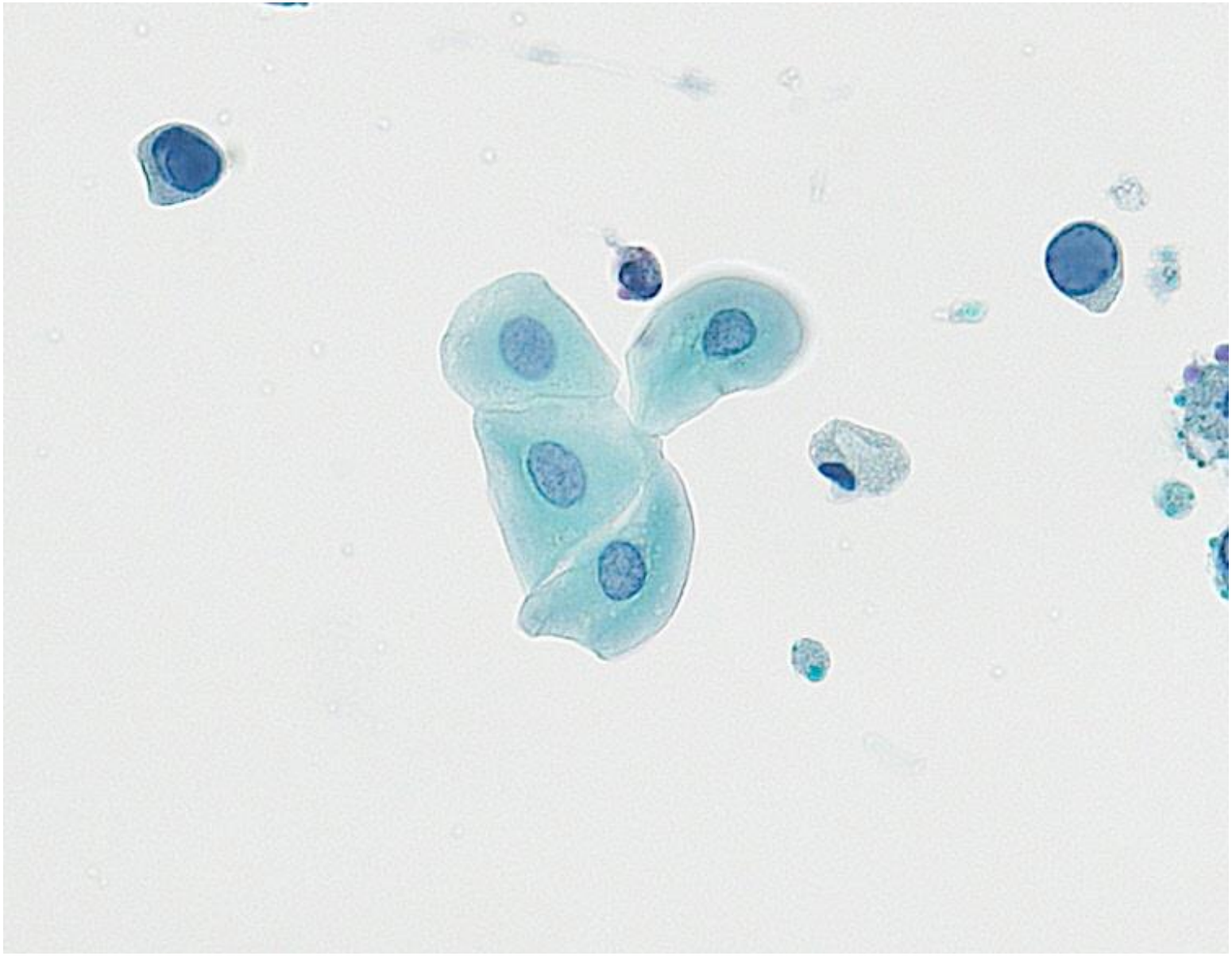
pT2, G3

11

55y, dysuria, cyst.: inconspicuous

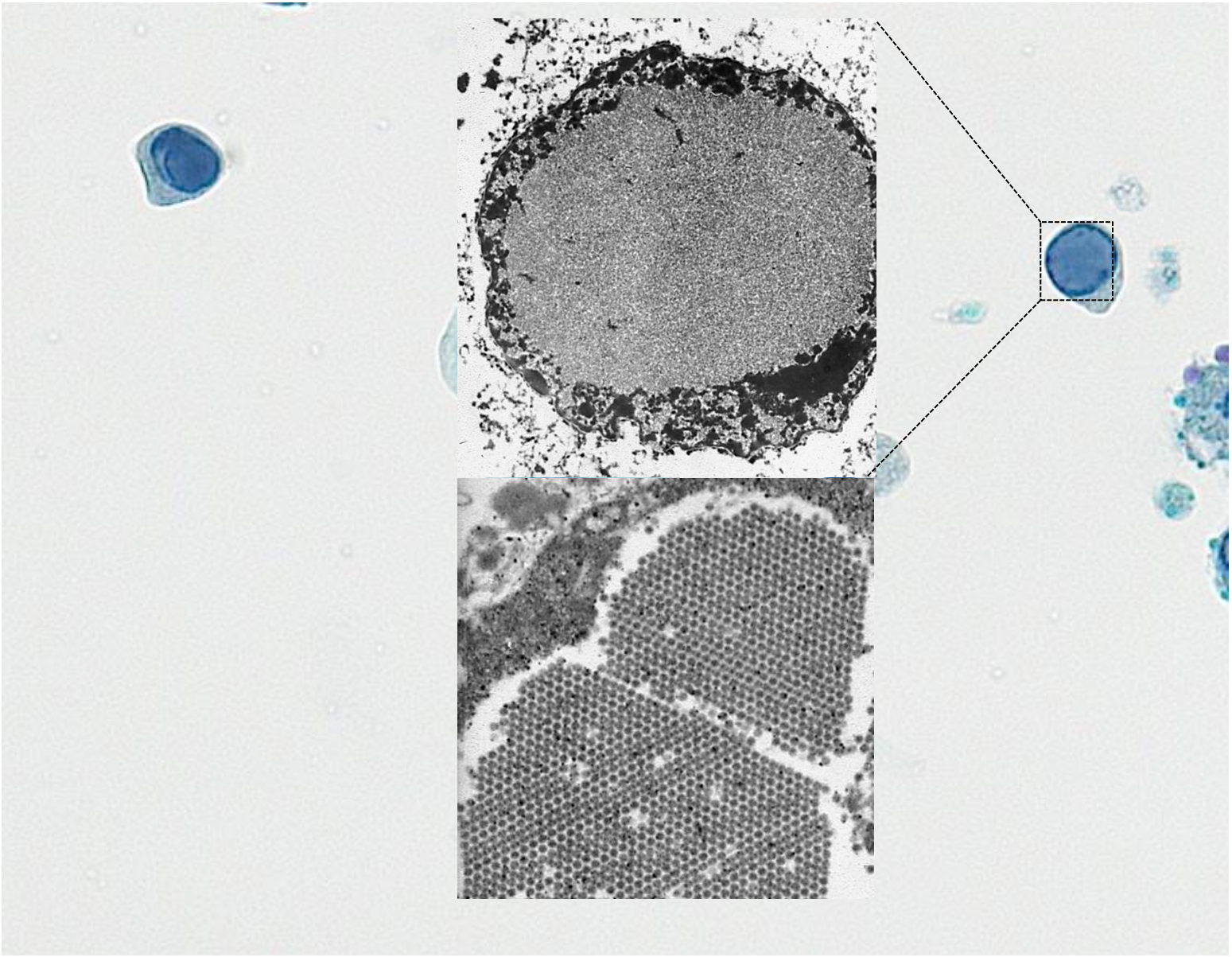


11



Decoy Cells

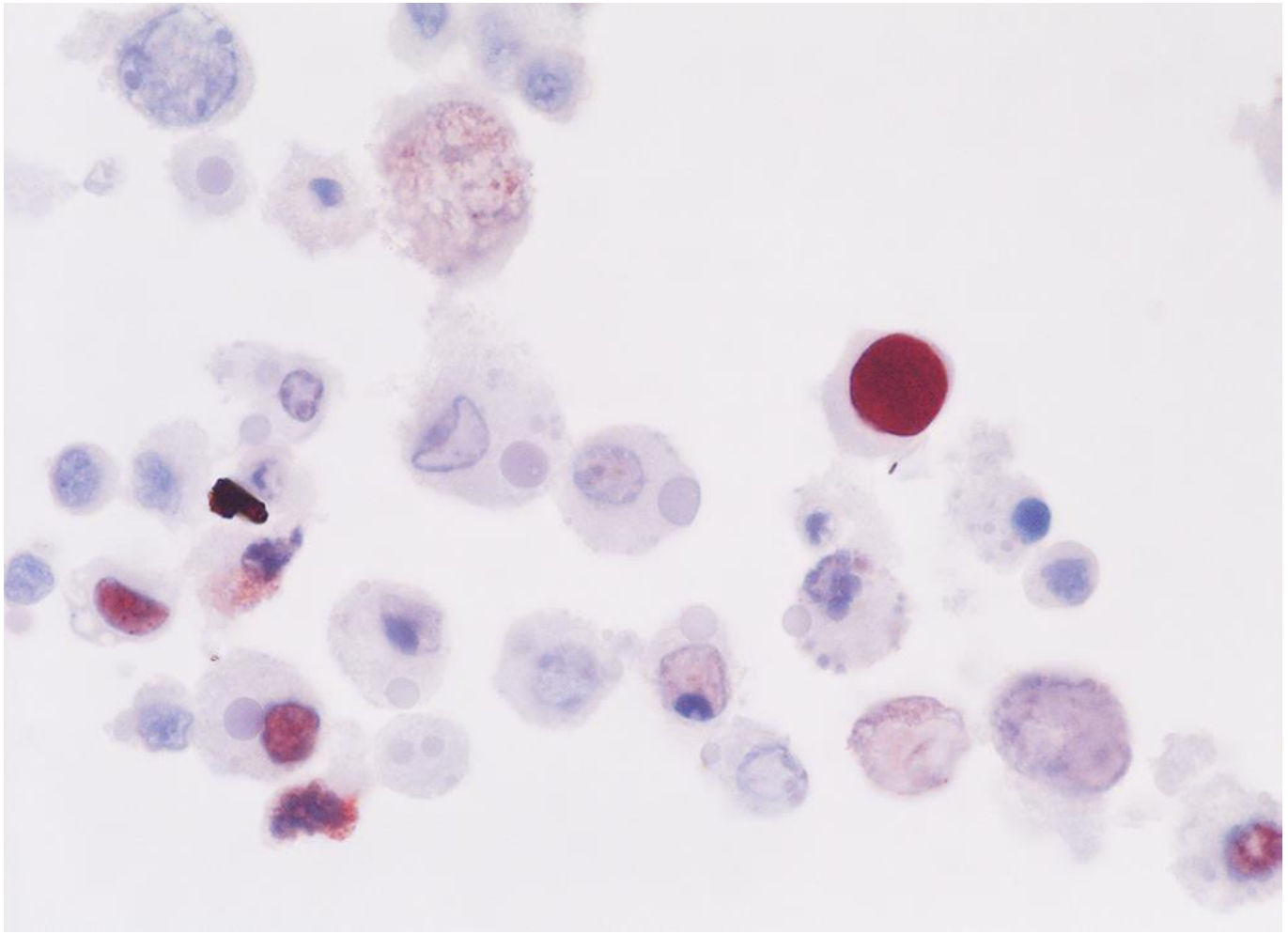
Paris: Negative

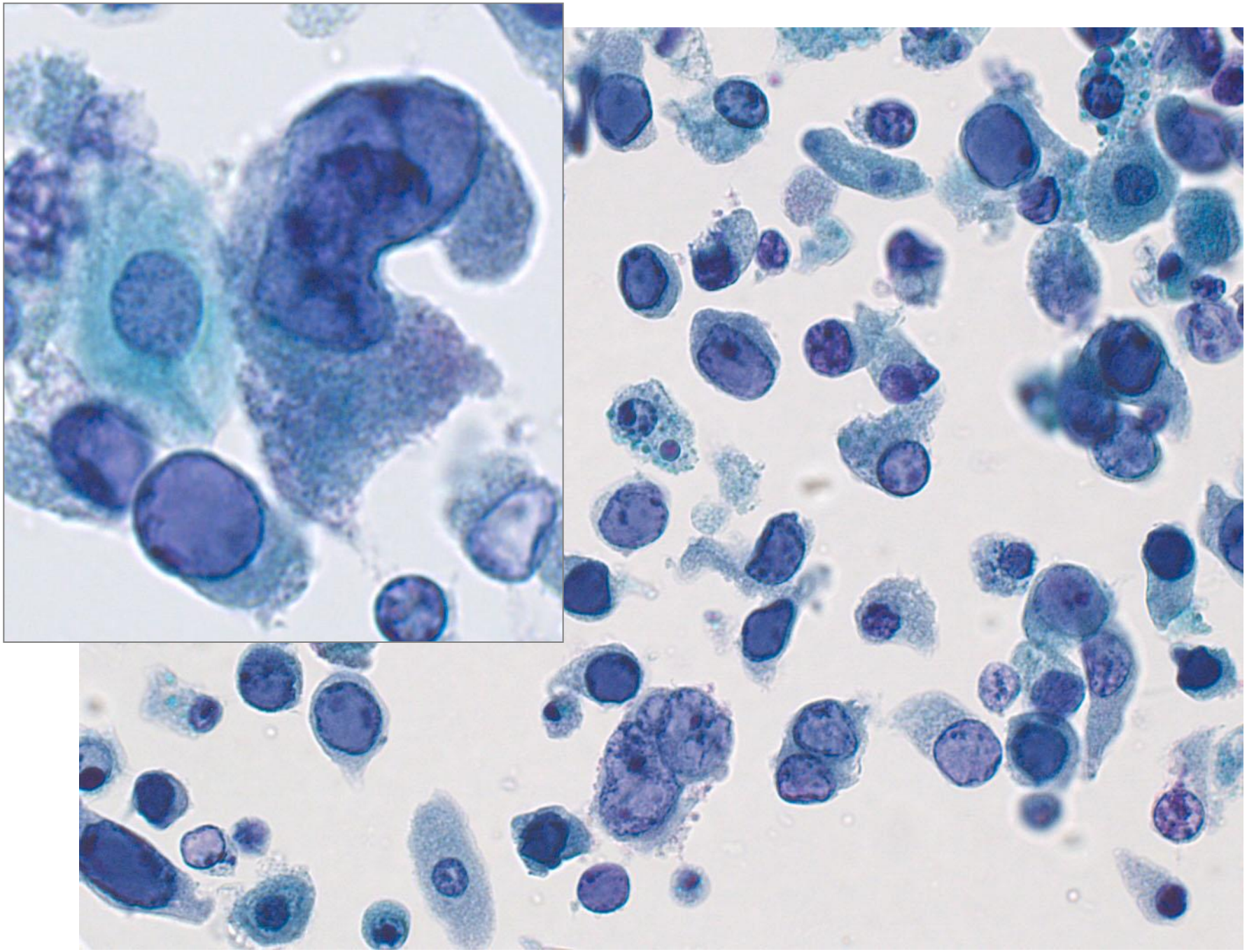


Decoy Cells

11

SV40 ICC





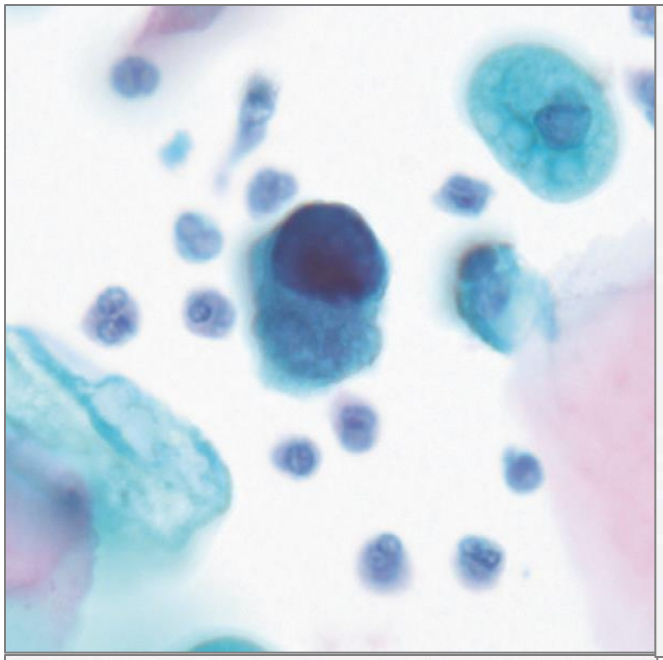
Decoy Cells

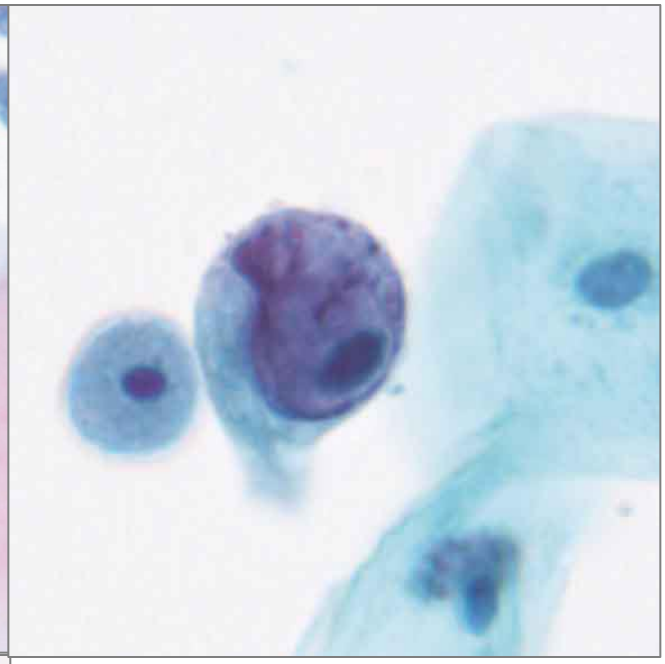
- 1960s Andrew Ricci:

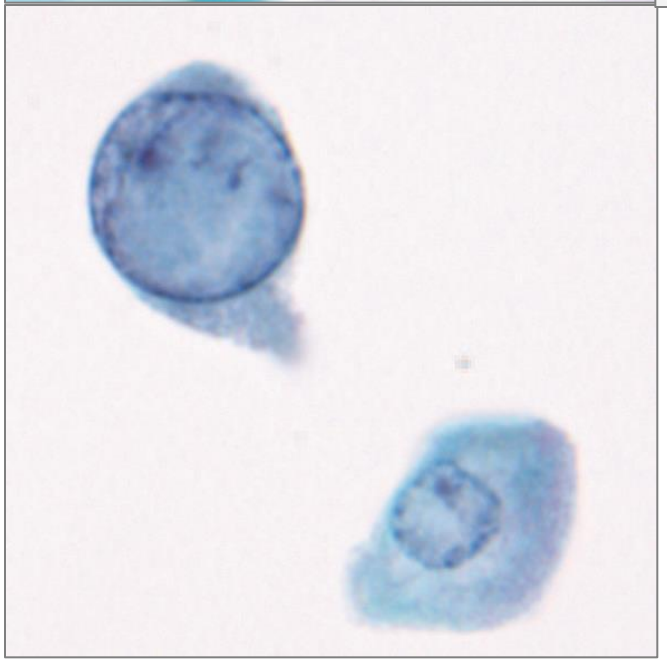
  „Decoy cells“ in urinary tract may be mistaken for carcinoma cells

HAVRE DE GRACE
DECOY MUSEUM

Havre de Grace, Maryland
the “Decoy Capital of the World!”







Decoy Cells

Polyomavirus reactivation

BK Virus

- Seroprevalence 65-90%
  - Transmission during childhood
  - Persists in the renourinary tract
- Immunocompetent adults
  - Life-long latency, no disease
  - Transient, asymptomatic and self-limiting activation

→ **Decoy cells**

0.5% urinary cytology specimens
3% pregnant women
3% patients with diabetes mellitus
13% cancer patients

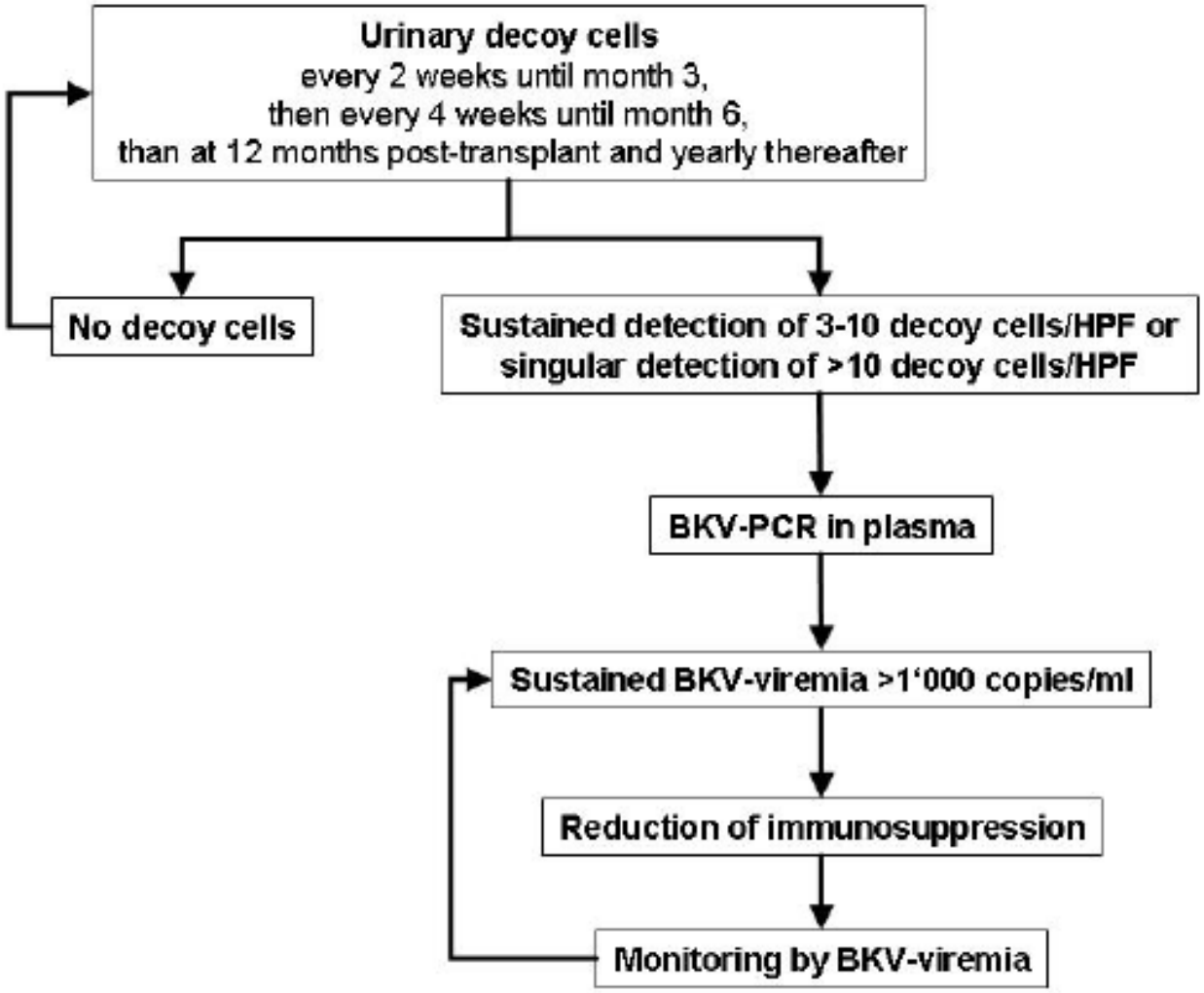
BK Virus

- Immunocompromised patients
  - Polyomavirus BK-associated Nephropathy (PVAN)
    - Renal transplant recipients
  - Hemorrhagic cystits
    - Bone marrow transplant recipients

→ **Decoy cells**

23% renal transplant recipients

11 Screening for Decoy Cells in Voided Urine



Summary

- New classification (Paris)
- Focus on the detection of HGUC
- Cave: benign atypia (degenerative, reactive, Decoy)
- Keep “atypia” low (AUC)
- Use FISH only with clear indication and under morphological control