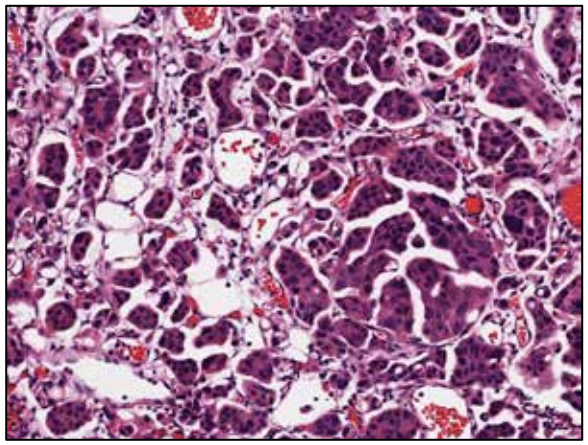
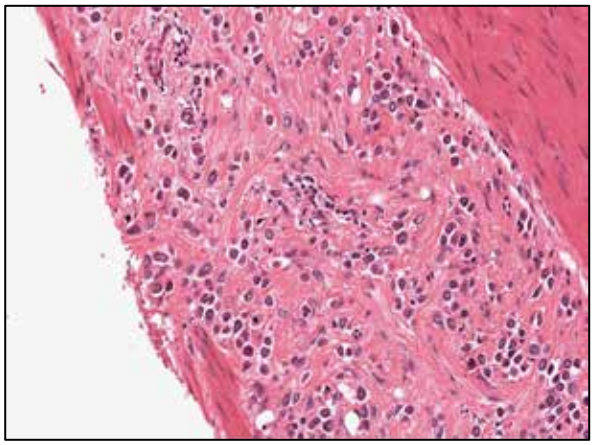
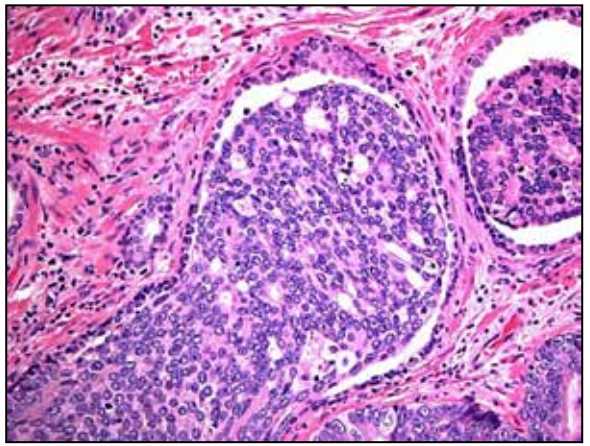
Immunohistochemistry and Bladder Tumours

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Objectives

- Review markers of urothelial differentiation
- Review situations where immunohistochemistry (IHC) is/is not helpful in the assessment of bladder lesions.
- Review IHC panels designed to sort out specific differential diagnoses.
- Illustrate the above with selected cases.

Best Practices Recommendations in the Application of Immunohistochemistry in the Bladder Lesions

Report From the International Society of Urologic Pathology Consensus Conference

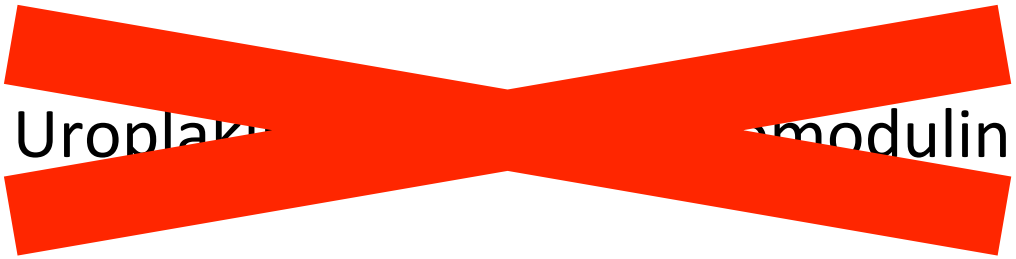
Mahul B. Amin, MD, Kiril Trpkov, MD,† Antonio Lopez-Beltran, MD,‡ David Grignon, MD,§ and Members of the ISUP Immunohistochemistry in Diagnostic Urologic Pathology Group*

(*Am J Surg Pathol* 2014;38:e20–e34)

- ISUP consensus conference on the use of IHC in urological pathology
- March 2013, Baltimore, MD
- Useful IHC markers of urothelial differentiation
- Specific applications for urothelial markers (and their pitfalls)
- Evidence-based recommendations on what markers to use and when

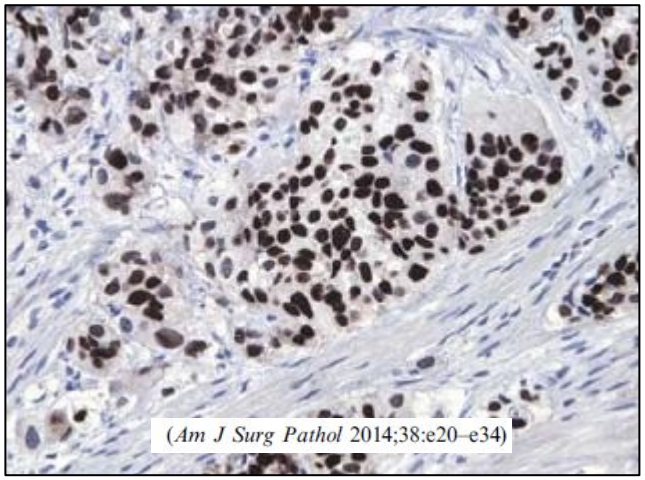
Markers of Urothelial Differentiation: Does an Ideal Panel Exist?

- Cytokeratin 7 (± cytokeratin 20)
- High molecular weight cytokeratin (34βE12)
- Cytokeratin 5/6
- p63
- GATA3
- Uroplak[illegible]modulin



- *S100P and uroplakin II*

GATA3



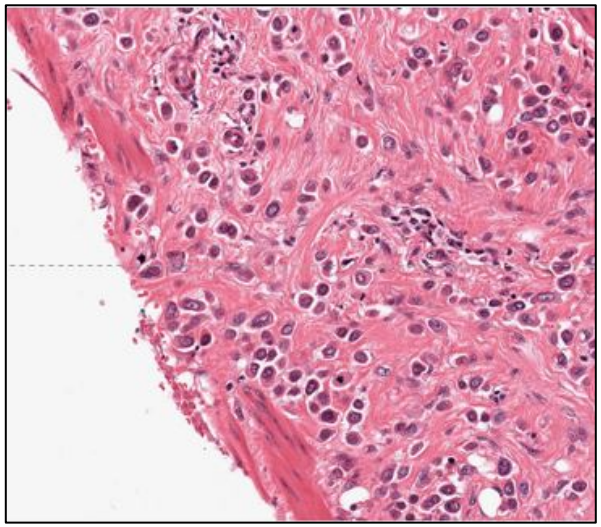
- Nuclear transcription factor
- Identified through cDNA microarray studies (2007)
- 67-90% of urothelial carcinomas
- It also stains lymphocytes
- It also stains other tumours:
  - breast (ductal and lobular carcinoma)
  - paraganglioma
  - trophoblastic tumours
  - mesothelioma
  - some squamous carcinomas

Case-Specific Applications of IHC for Bladder Lesions

- Confirming urothelial differentiation
- Distinction of urothelial atypia from dysplasia/ carcinoma in situ
- Staging bladder cancer (pT)
- Work-up of spindle cell lesions
- Prognosis and selection of therapy

Confirming Urothelial Differentiation

- Urothelial carcinoma can have a wide variety of histologic manifestations (divergent differentiation)
  - plasmacytoid
  - lymphoepithelioma-like
  - sarcomatoid
- Direct invasion of the bladder by tumours in adjacent organs
  - prostate
  - colon/rectum
  - cervix
- Hematogenous metastases to the bladder
  - lung
  - breast
  - stomach
  - kidney
  - melanoma



Adenocarcinoma: Primary Bladder (Urachal/Non-Urachal) vs Colorectal

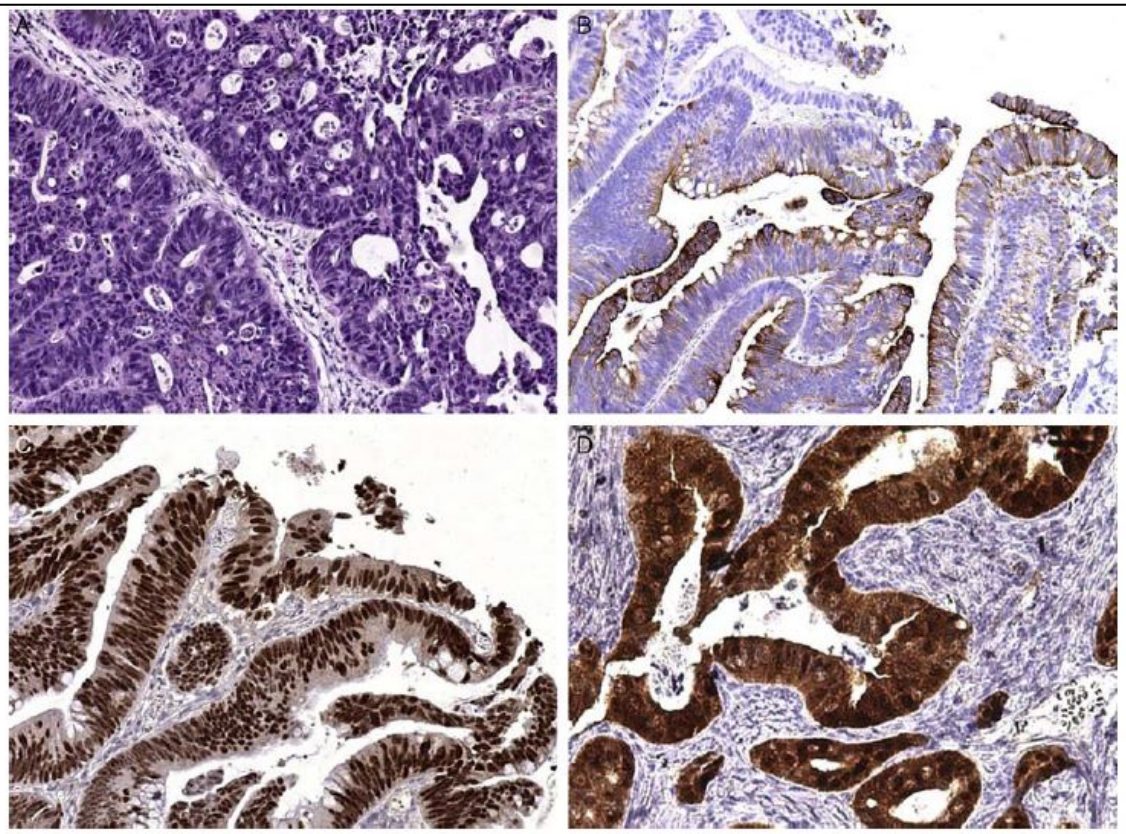


FIGURE 3. Metastatic adenocarcinoma of the colon to the urinary bladder. A, H&E micrograph of a moderately differentiated adenocarcinoma with enteric differentiation could represent either a primary bladder or colorectal cancer. CK20 (B) and CDX2 (C) positivity in this lesion is of no diagnostic value in this differential. D, In contrast, diffuse nuclear positivity for β-catenin (in this case, both nuclear and cytoplasmic positivity) argues for a colorectal primary in the appropriate clinical context.

- CK20 - no role
- CDX2 - no role
- GATA3, p63, 34βE12 are frequently –'ve in bladder adenocarcinoma
- **β-catenin**
- *Nuclear* in colorectal (>90%)
- *Membranous* in bladder (>90%)
- Proper clinical context required

Reactive Atypia vs Dysplasia/CIS

TABLE 1. Typical Immunoreactivity for Commonly Used Markers in Flat Urothelial Lesions

	Normal Urothelium	Reactive Atypia	CIS
CK20	Limited to umbrella cells	Limited to umbrella cells	Aberrant expression through all cell layers. May be full thickness
CD44(s)	Limited to basal cells	Increased reactivity in all cell layers	Absent in atypical cells
p53	Absent	Absent	Strong and intense positivity in atypical cells

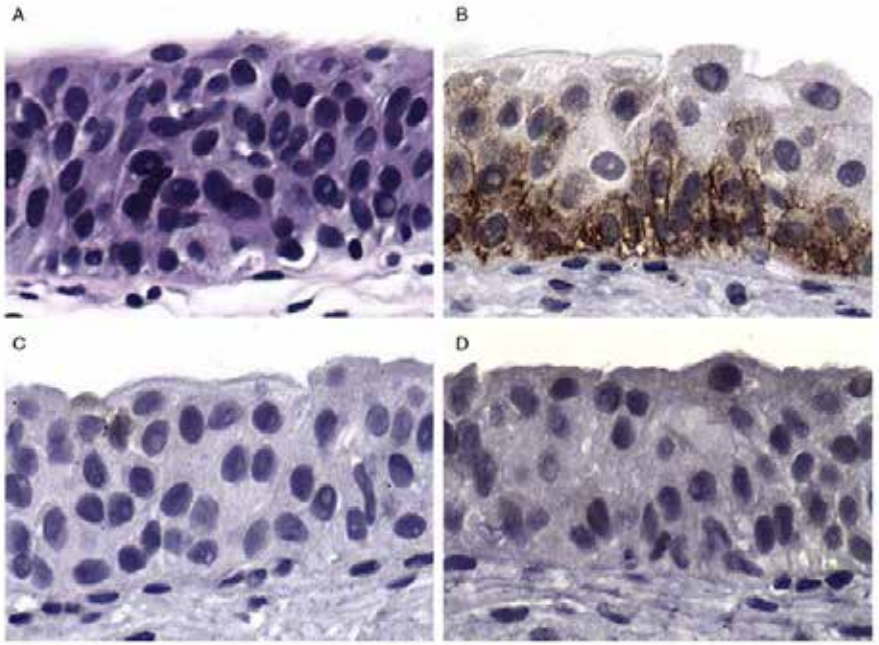


FIGURE 4. Use of IHC in reactive urothelium versus CIS. A, H&E micrograph of a flat urothelial lesion defined by moderate cellular atypia, hyperchromasia, and nuclear enlargement, with generally preserved polarity raises consideration of reactive atypia versus nonpleomorphic CIS. B, CD44 immunostain shows basal and parabasal expression, whereas expression of CK20 (C) and p53 (D) is absent, in keeping with a "reactive" immunoprofile.

ISUP Recommendation

- Morphology remains the gold standard
- CK20/CD44(s)/p53 can be misleading
- No role in dysplasia vs CIS or grading papillary tumours

Staging Bladder Cancer (pT)

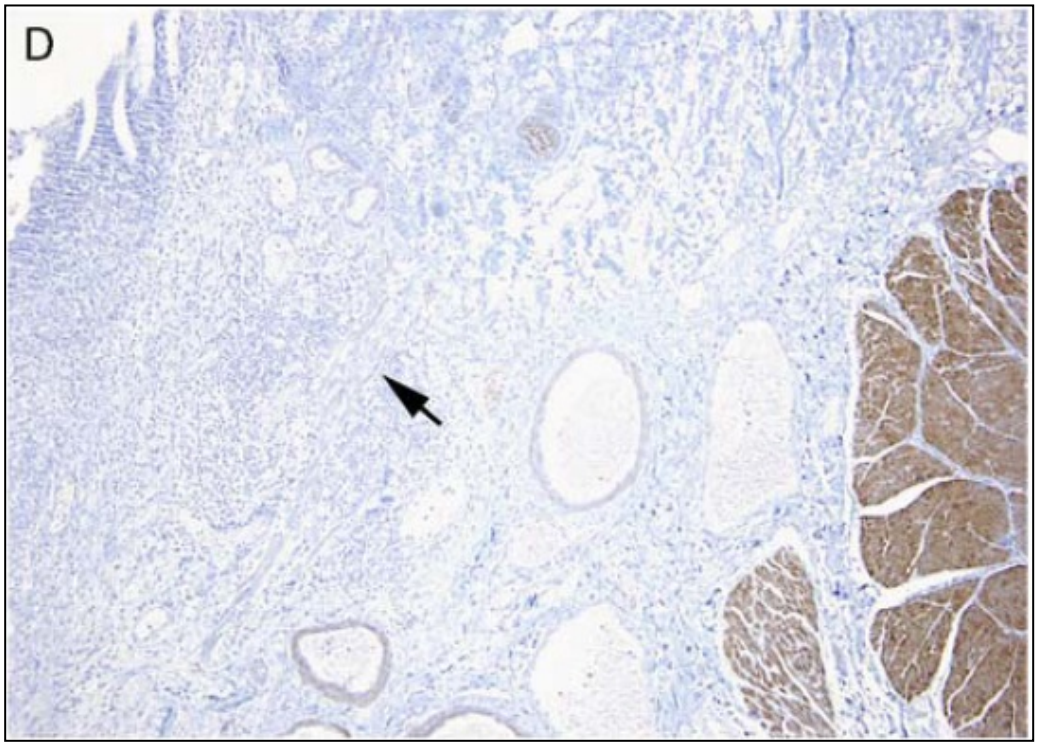
- 3 potential roles for IHC
  1) Early lamina propria invasion as small nests/single cells
  2) Distinguishing muscularis propria from muscularis mucosa and/or vascular smooth muscle
  3) Distinguishing muscularis propria from desmoplastic stroma

ISUP Recommendation

- IHC has limited applications in this setting
- Insufficient evidence for smoothelin

Smoothelin

- Specific to terminally differentiated smooth muscle - "contractile phenotype" (ie: muscularis propria)



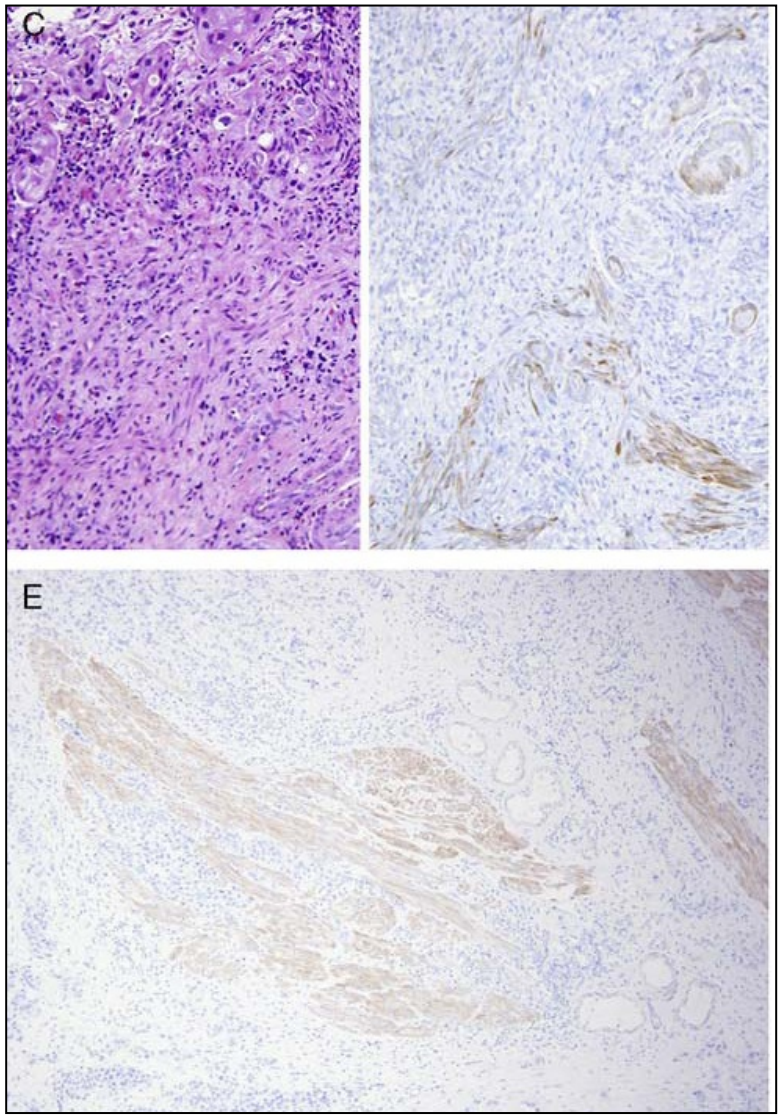
Cystectomy Specimens

- Muscularis Propria
  - 86% strong/diffuse
- Muscularis Mucosae
  - 88% negative/weak

Paner et al, Am J Surg Pathol 33:91-98, 2009

Pitfalls in the Use of Smoothelin to Identify Muscularis Propria Invasion by Urothelial Carcinoma

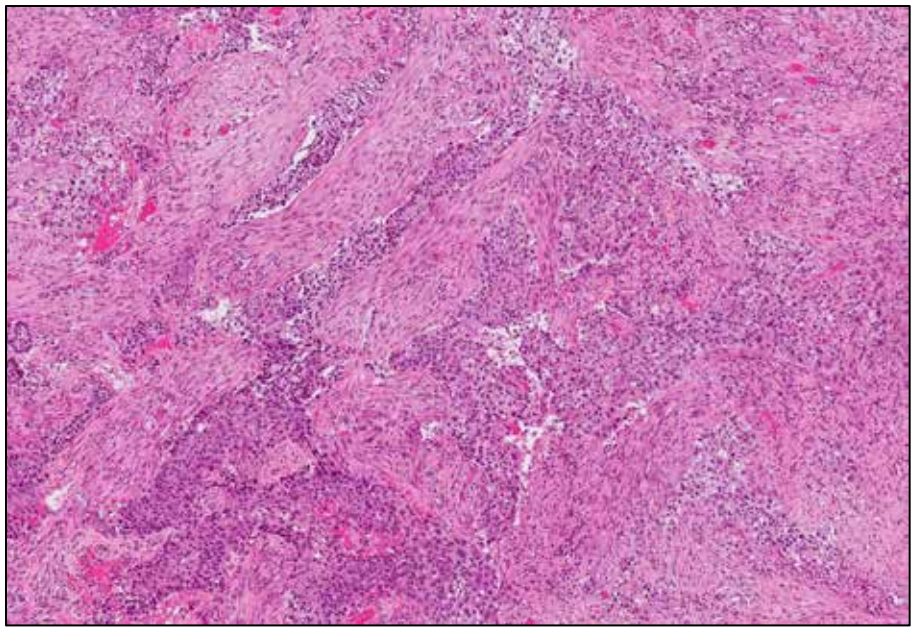
Hiroshi Miyamoto, MD, PhD, Rajni B. Sharma, PhD,*
Peter B. Illei, MD,* and Jonathan I. Epstein, MD*† ‡*



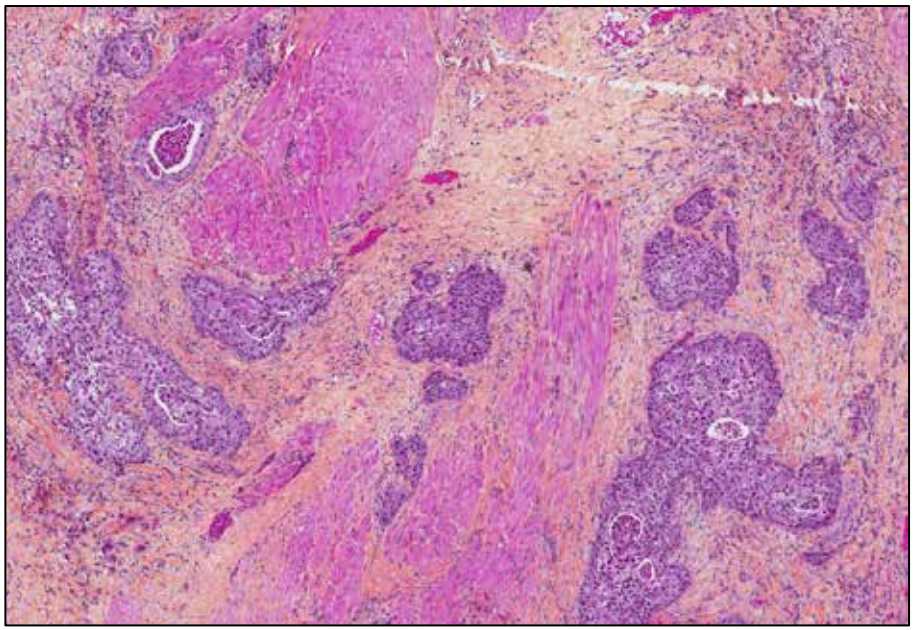
- 34 consultation TURBT's
- outside pathologist ? pT2
- Hopkins H&E Dx – 18 pT1, 16 pT2
- Smoothelin in MM pT1's
  - negative in 57%
  - weak in 29%
  - moderate in 14%
- Smoothelin in MP in pT2's
  - weak in 6%
  - moderate in 19%
  - moderate-strong in 75%

Miyamoto et al, Am J Surg Pathol 34:418-422, 2010

Stromal Desmoplasia vs Muscularis Propria?



H&E



HPS

Spindle Cell Lesions

- PMP/IMT
- Leiomyosarcoma
- Sarcomatoid urothelial carcinoma
- Rhabdomyosarcoma

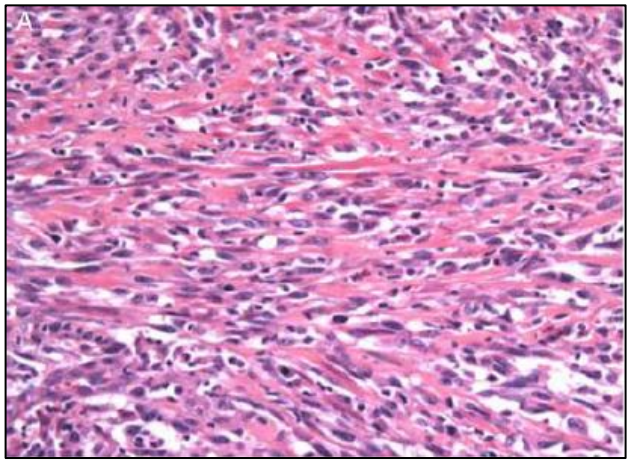


TABLE 3. IHC Marker Positivity by Type of Spindle Cell Proliferation of the Urinary Bladder

Antibody	PMP* (%)	Sarcomatoid Carcinoma (%)	Leiomyosarcoma (%)	Rhabdomyosarcoma (%)
ALK1	20-89	0	0-10	20
Muscle-associated markers				
SMA	63-100	15-80	43-100	97
Desmin	27-80	0-40	0-60 (35-75)	97-100
h-caldesmon	0-67	N/A	100 (18-57)	0
Calponin	89	N/A	100 (57-90)	0
MyoD1	0	0†	NA (0)	100
Myogenin	0	0†	0	76-100
Epithelium-associated markers				
Pan cytokeratin (AE1/AE3)	36-89	67-100	0-58	NA (6 [focal])
EMA/MUC1	0-50	50-100	0-12	NA (6 [focal])
P63	0	50-58	23	NA (5 weak or focal)
HMWCK (34βE12)	0	0-27	0	NA
CK5/6	0	27-65	0	NA
OSCAR	70	68	54	NA

*PMP includes IMT, pseudosarcomatous fibromyxoid tumor, inflammatory pseudotumor, and pseudosarcomatous spindle cell proliferation.
†Excluding any rhabdomyosarcomatous component.
Values in parentheses indicate percentages given for non–bladder-specific series.
NA indicates not available.

Uncommon tumours in the adult bladder: rare entities with recognized diagnostic pitfalls

Andrew J Evans
Brendan C Dickson

Volume 19:10 October 2013

Diagnostic histopathology

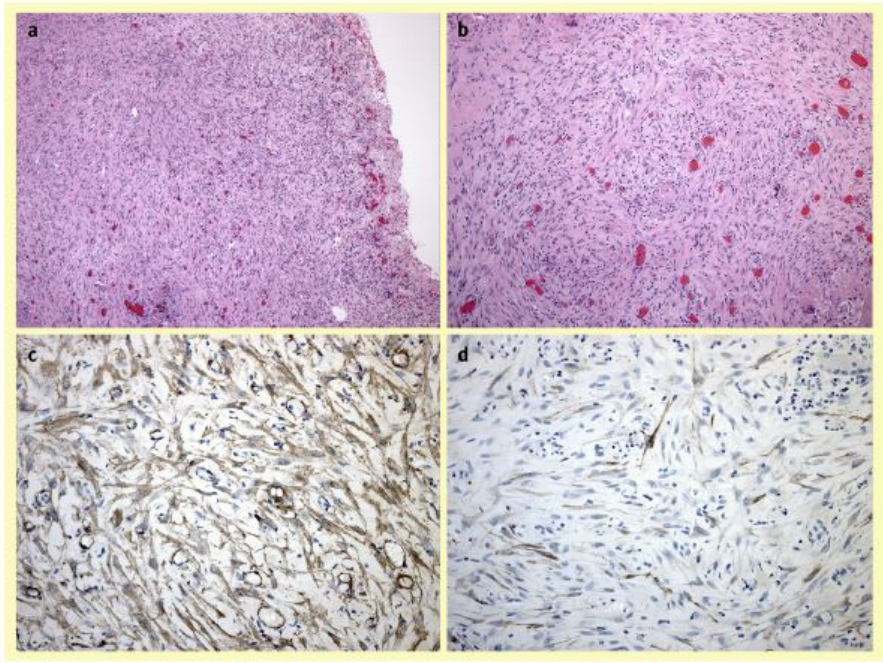


Figure 1 Pseudosarcomatous myofibroblastic proliferation. Tumour resected from the posterior bladder wall of a 59-year-old woman with a history of pT1 bladder cancer, BCG therapy and recent TURBT. (**a**) Spindle cell proliferation with a fasciitis-like pattern (H & E: ×100). (**b**) Note the prominent thin-walled vasculature, mucosal ulceration and scattered granulomas, (H & E: ×200). (**c**) The tumour is diffusely positive for smooth muscle actin (×200), (**d**) with focal immunoreactivity for desmin (×200), but negative for ALK1, S100, CD34, and keratin.

Prognosis and Therapy Selection

TABLE 4. Emerging Prognostic Markers in Bladder Cancer

Biomarker	Normal Function	Abnormality	Prognostic Role in: Non–muscle-invasive Bladder Cancer	Prognostic Role in: Muscle-invasive Bladder Cancer
Cell cycle				
p53*	Tumor suppressor	Inactivation/accumulation	Yes (multimarker)/No	Yes/No
p21	CDK inhibitor	Downregulated expression/loss	Yes (multimarker)/No	Yes
p27	CDK inhibitor	Downregulated expression	Yes (multimarker)/No	Yes/No
p16	CDK inhibitor	Altered expression	No	Yes/No
pRB	Tumor suppressor	Deletion/mutation	Yes (multimarker)/No	Yes/No
Ki-67*	Cell proliferation	Increased expression	Yes (multimarker, mG)	Yes
Cyclins				
D1, D3, E1	Phosphorylate Rb	Increased expression	Yes/No	No
Apoptosis				
Survivin	Inhibitor of apoptosis	Increased expression	Yes/No	Yes
Angiogenesis				
MVD	Marker of angiogenesis	Increased density	Yes/No	Yes/No
VEGF, HIF1A	Promote angiogenesis	Increased expression	Yes/No	Yes/No
Signaling proteins				
FGFR3*	Tyrosine kinase receptor	Mutation/overexpression	Yes	No
Hormone receptors				
HER2	Tyrosine kinase receptor	Amplification	No	Yes/No
AR	Nuclear receptor	Loss of expression	No	No
ER	Nuclear receptor	Downregulated expression	No	No
Cell adhesion				
E-cadherin	Cell adhesion	Loss	Yes	Yes/No

Compiled from Amin et al.[66,103–106]

*p53, FGFR3, and Ki-67 have the strongest evidence as prognostic markers in bladder cancer.

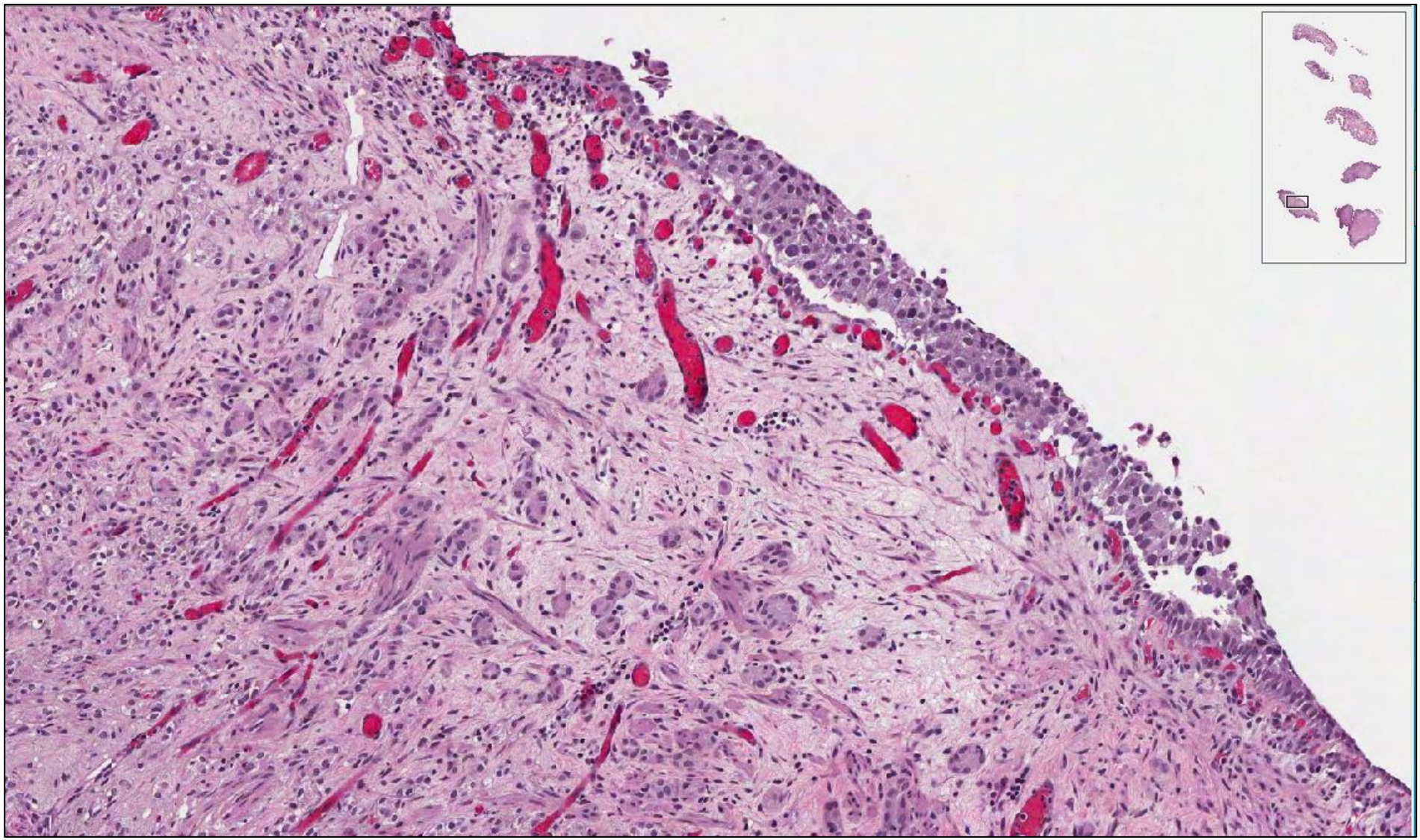
AR indicates androgen receptor; CDK, cyclin-dependent kinase; ER, estrogen receptor; FGFR, fibroblast growth factor receptor; HER2, human epidermal growth receptor 2; HIF1A, hypoxia-induced factor 1A; mG, molecular grade; MVD, microvessel density; VEGF, vascular endothelial growth factor.

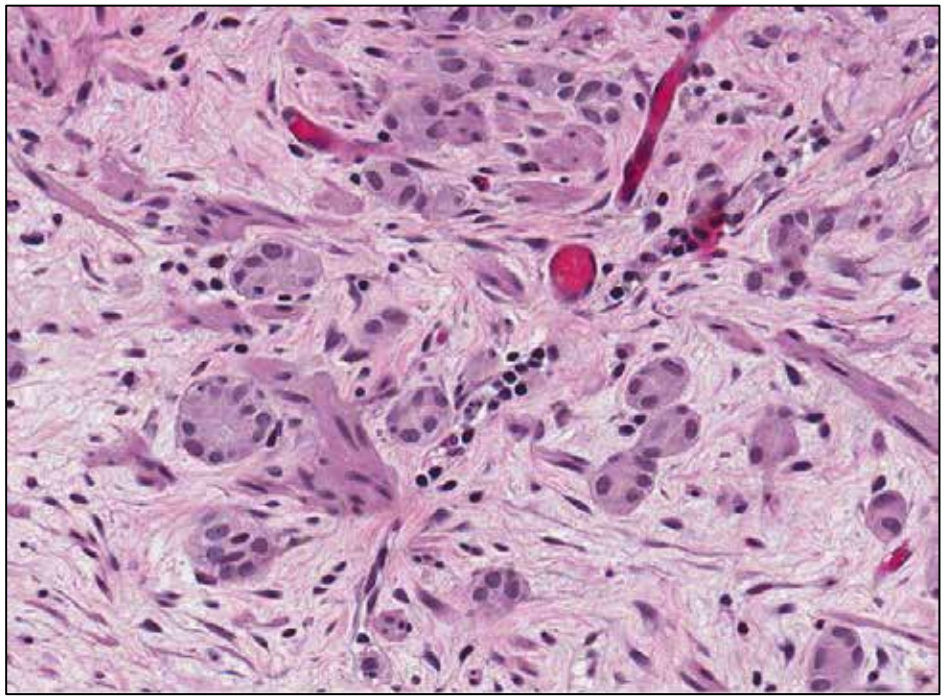
ISUP Recommendation

- There are no prognostic IHC markers ready for routine use

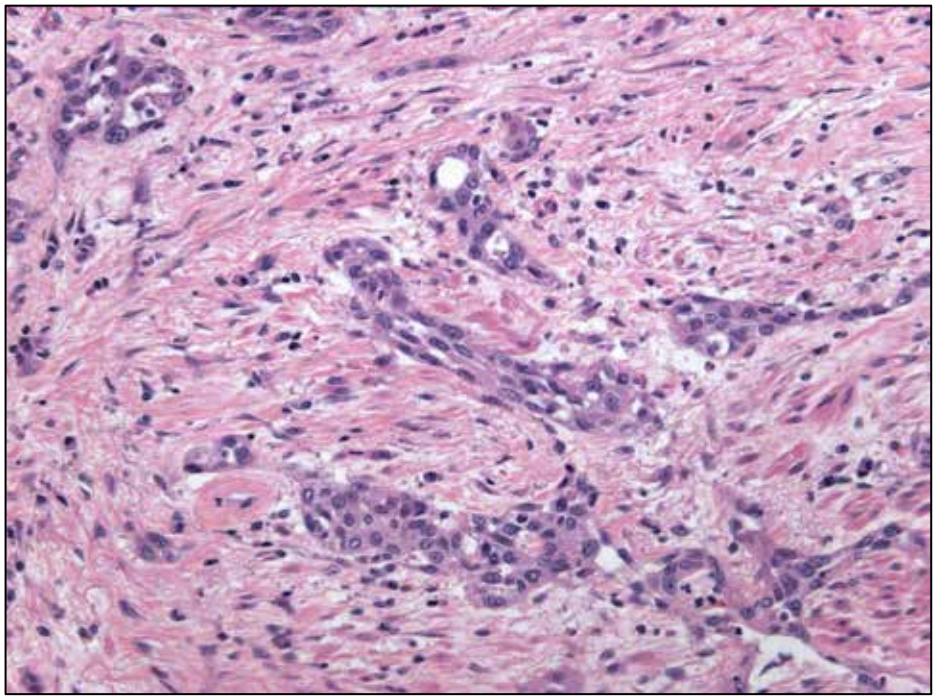
Case #1

- 64 year-old man with pTa high-grade urothelial carcinoma and CIS
- TURBT showing recurrent CIS and ? invasive urothelial carcinoma (pT1) with nested variant features.



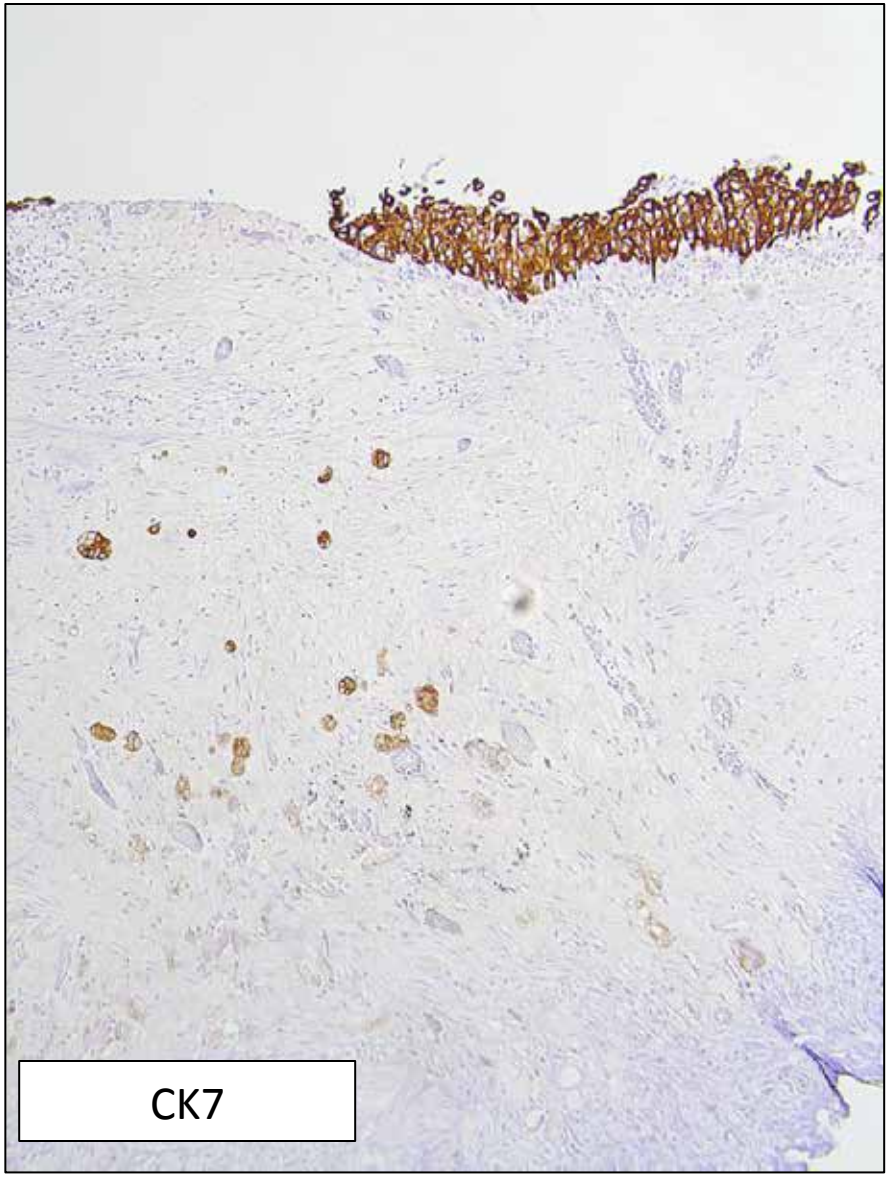


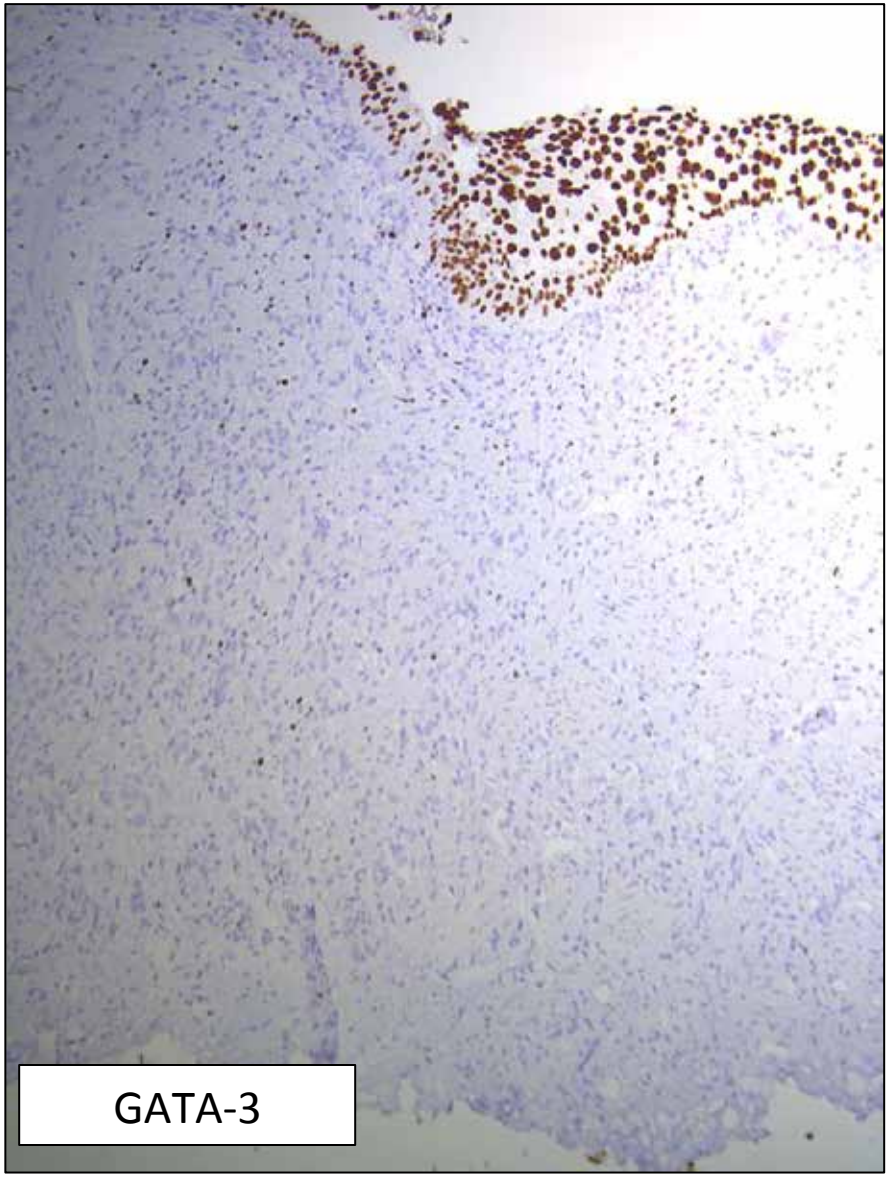
Lesion in Current
Case NYD

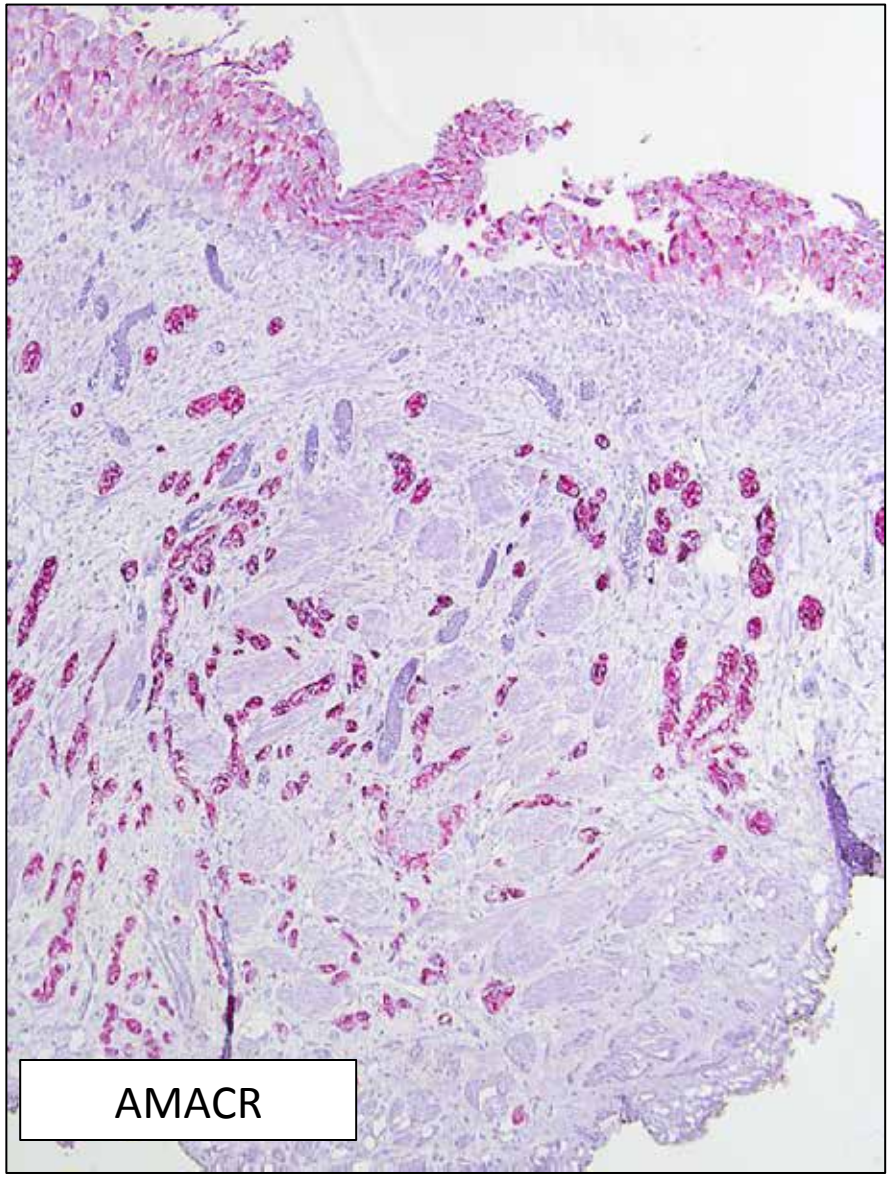


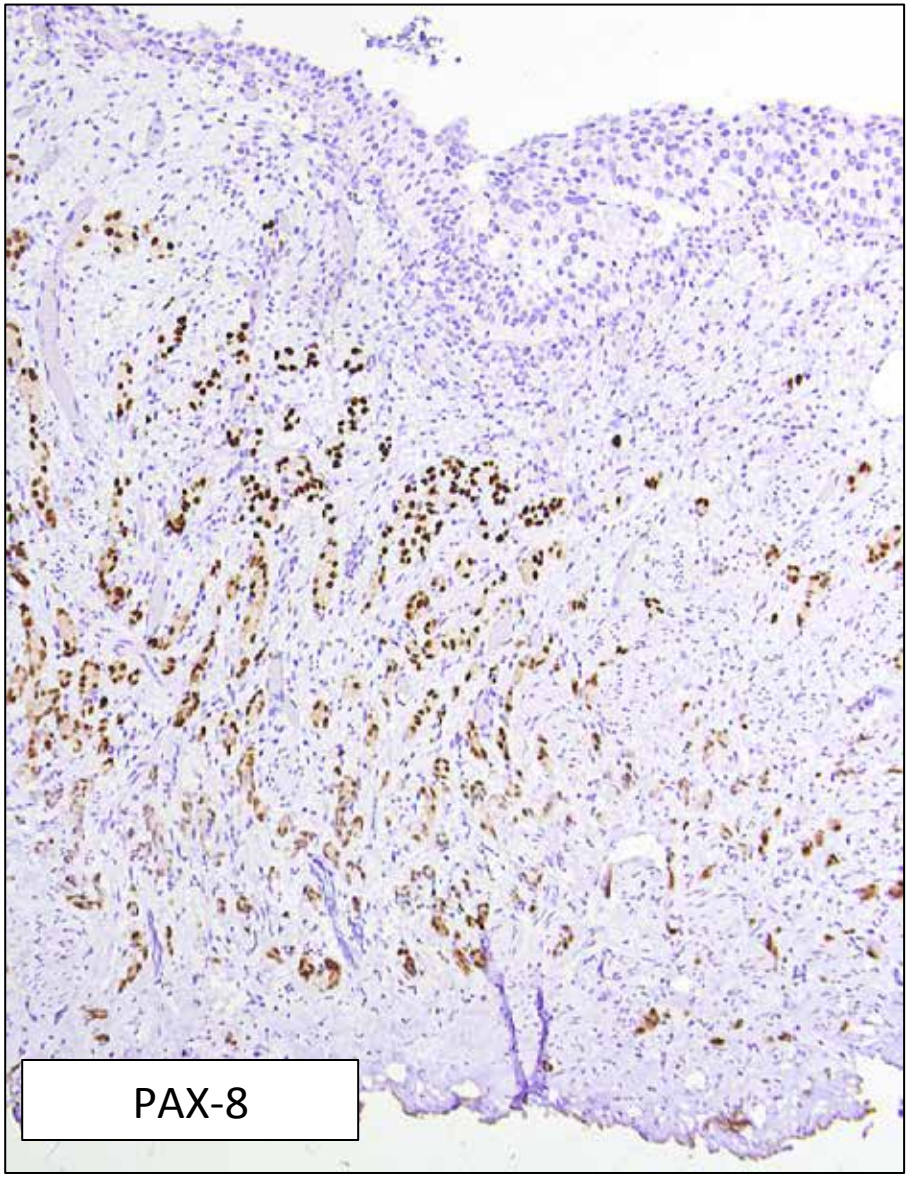
Nested Variant
Urothelial Carcinoma

- Nested variant urothelial carcinoma – CK7, 34βE12, p63, GATA3
- Prostatic adenocarcinoma – PSA, PSAP, AR
- Nephrogenic adenoma/metaplasia – AMACR, PAX-8


CK7


GATA-3


AMACR


PAX-8

Annals of Diagnostic Pathology 17 (2013) 41–44

Contents lists available at SciVerse ScienceDirect

Annals of Diagnostic Pathology

Immunohistochemical markers for the differential diagnosis of nephrogenic adenomas

William Quinones, MD, Amy Ziober, JD, Yuan Yao, MD, Zhanyong Bing, MD, PhD *

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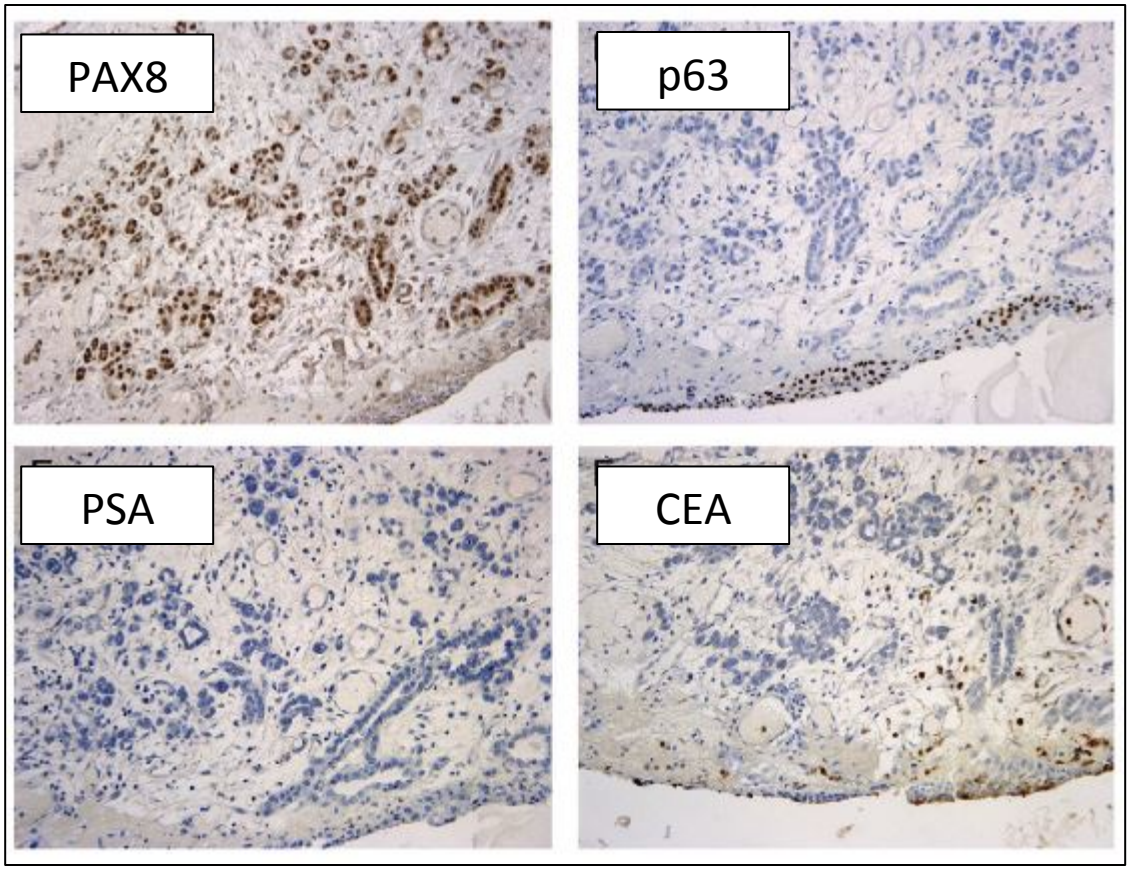
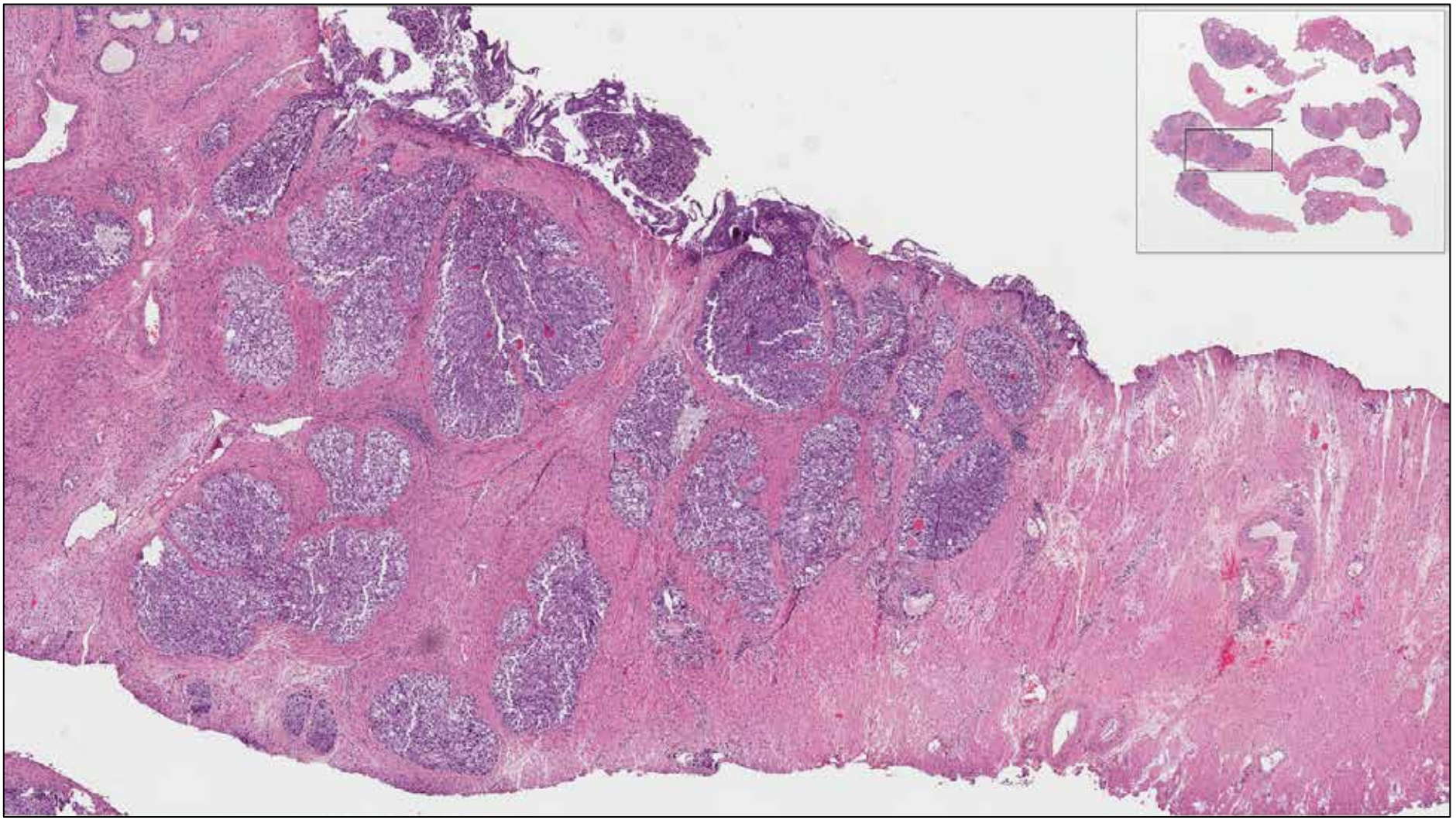
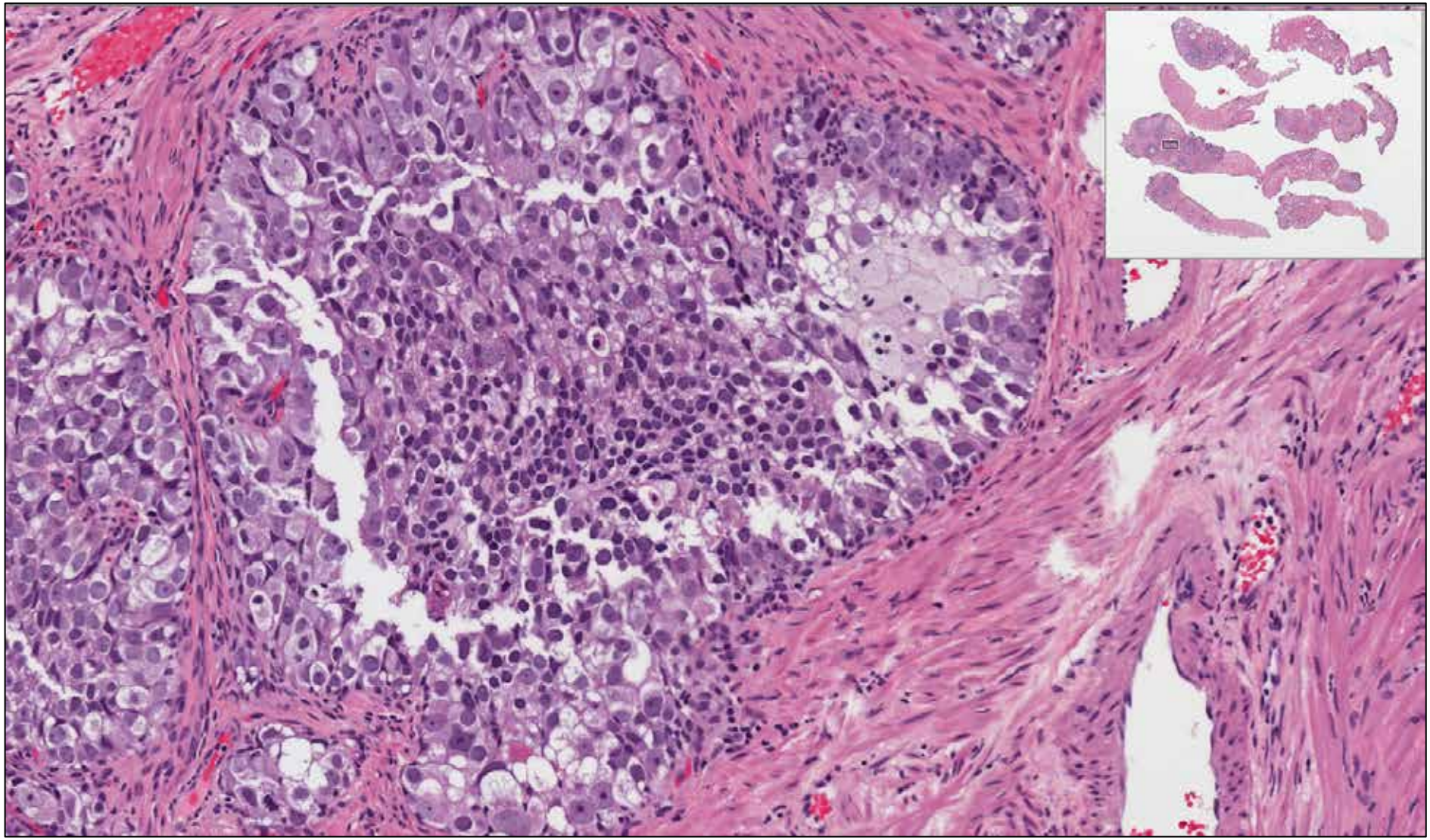
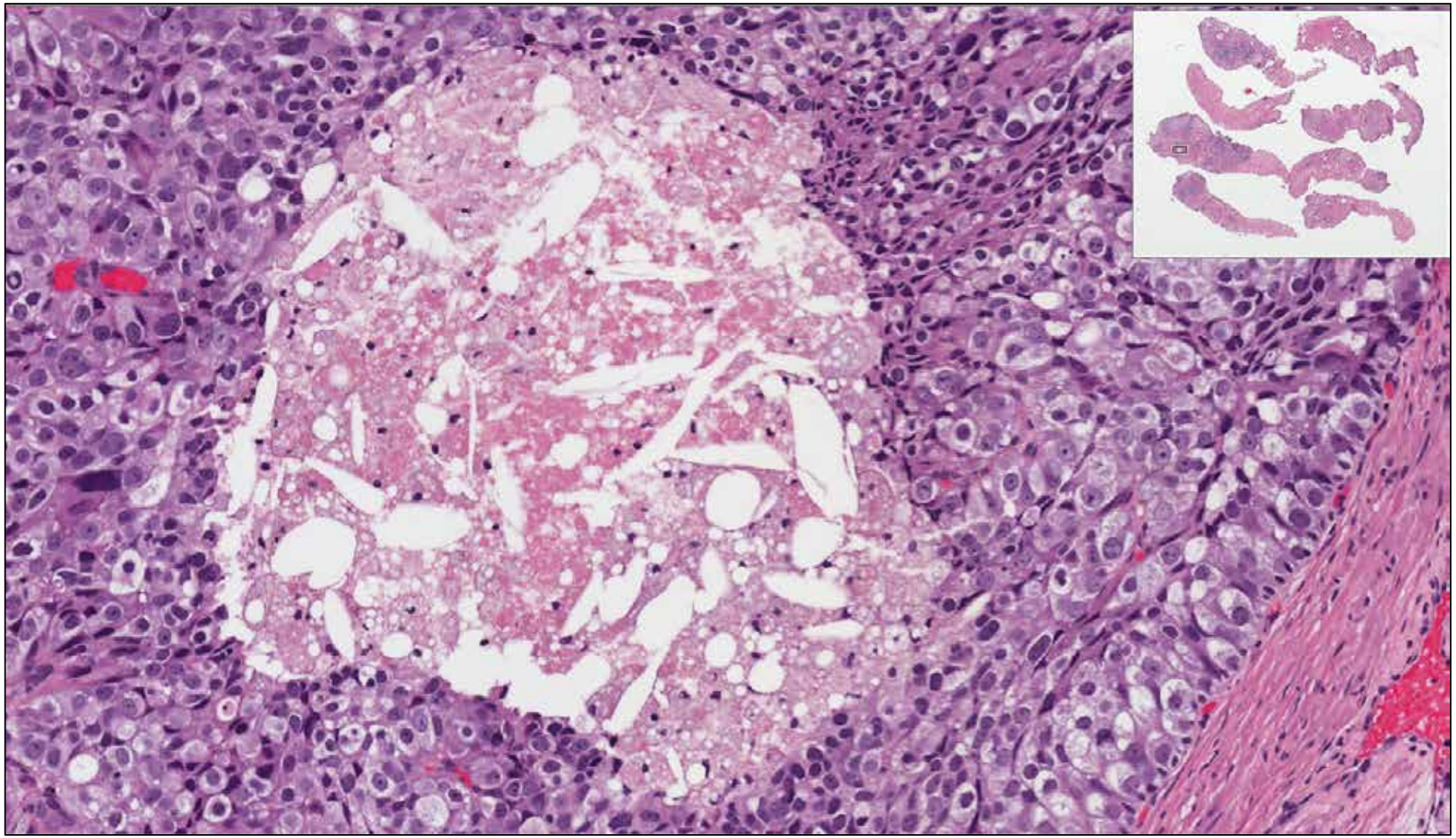


Case #2

- 77 year-old male
- Urinary retention with acute renal failure
- TURP performed for bladder neck lesion/stricture
- No imaging of upper tracts and no serum PSA or history of bladder/prostate cancer







- Intraductal carcinoma of prostate
- Urothelial carcinoma in situ involving prostatic ducts

IHC Panel

- PSA
- PSAP
- AR

Positive

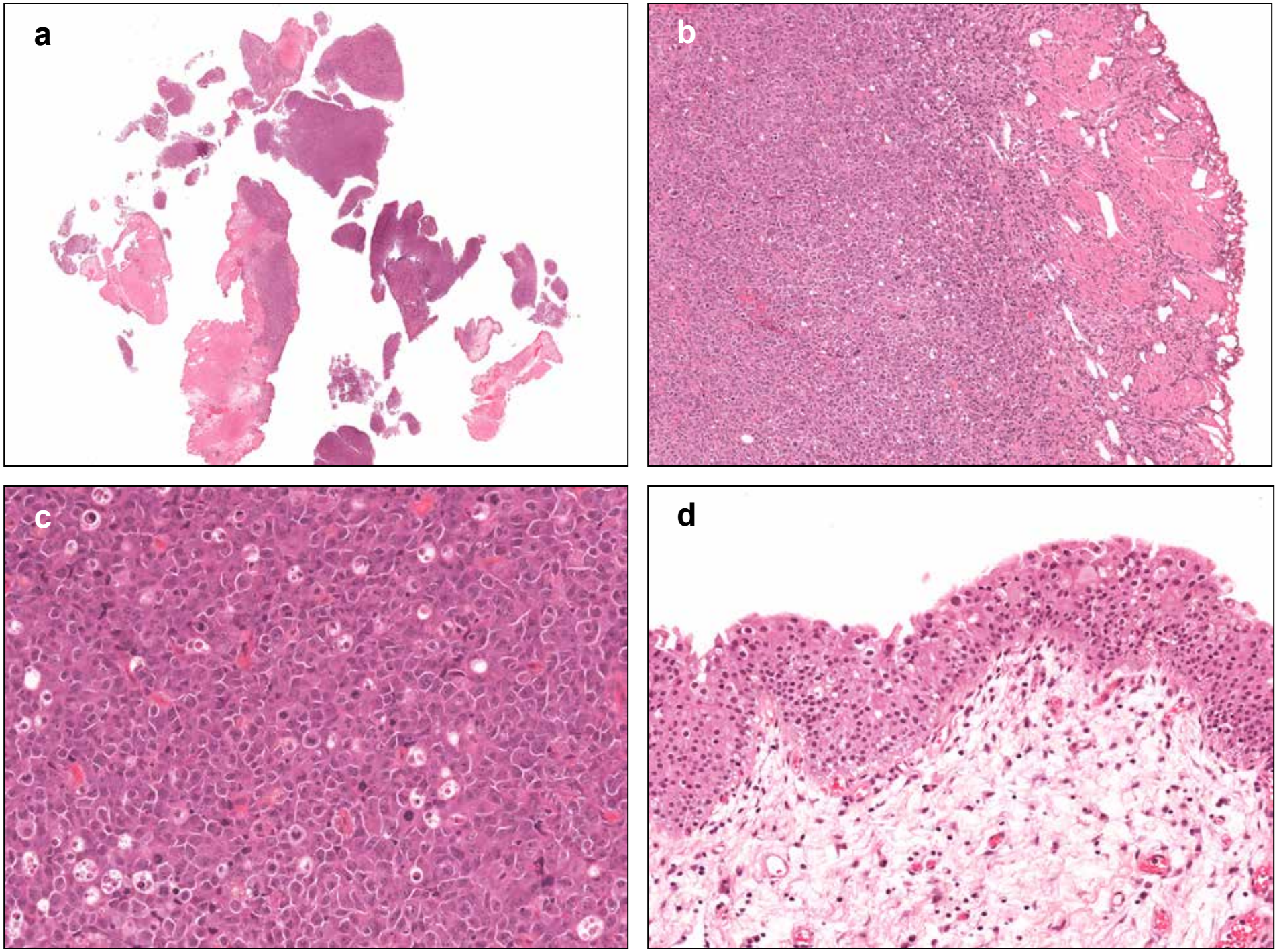
- 34βE12
- p63
- CK7
- GATA3

Negative

- AMACR – no role in this differential diagnosis

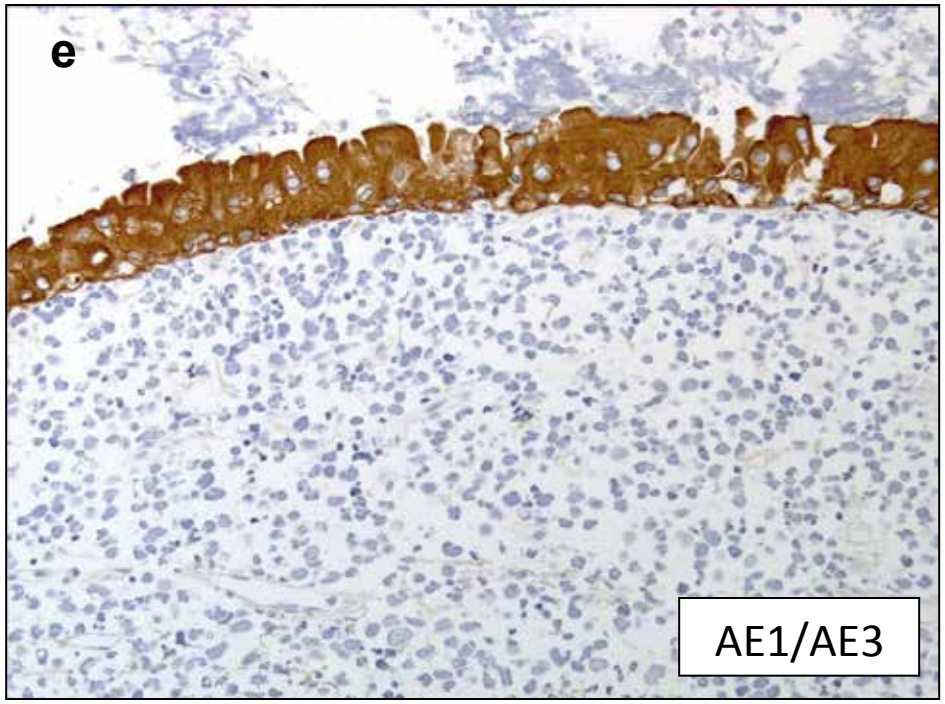
Case #3

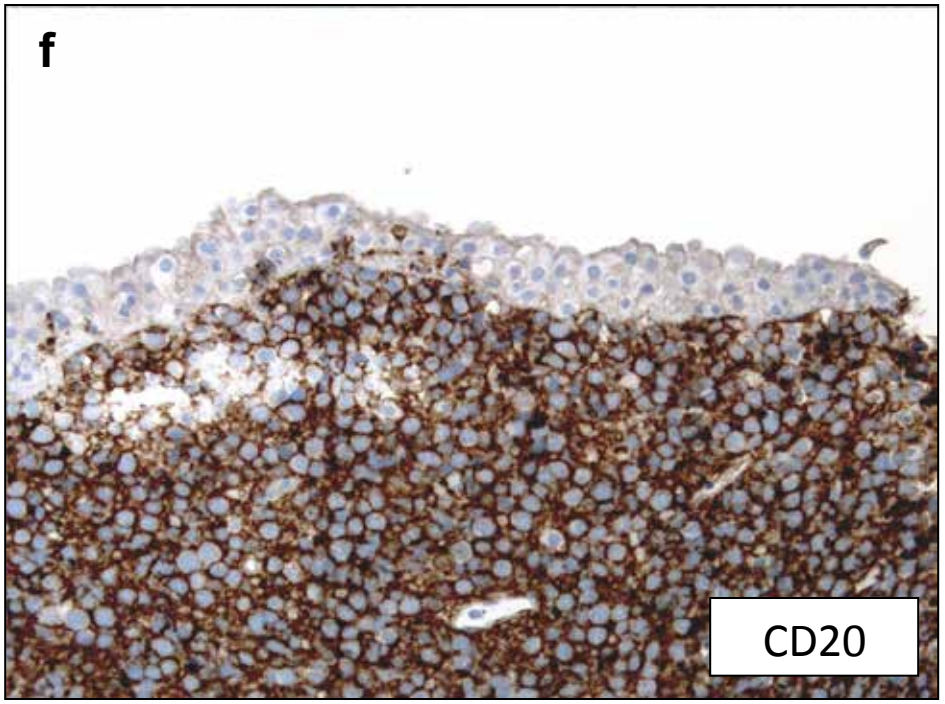
- 69 year-old male with gross hematuria, feeling generally unwell x months with weight loss
- History otherwise unremarkable
- TURBT for a large submucosal mass


a
b
c
d

IHC Panel

- AE1/AE3
- CAM 5.2
- CK7
- p63
- 34βE12
- Chromogranin A
- Synaptophysin
- CD56
- LCA


e
AE1/AE3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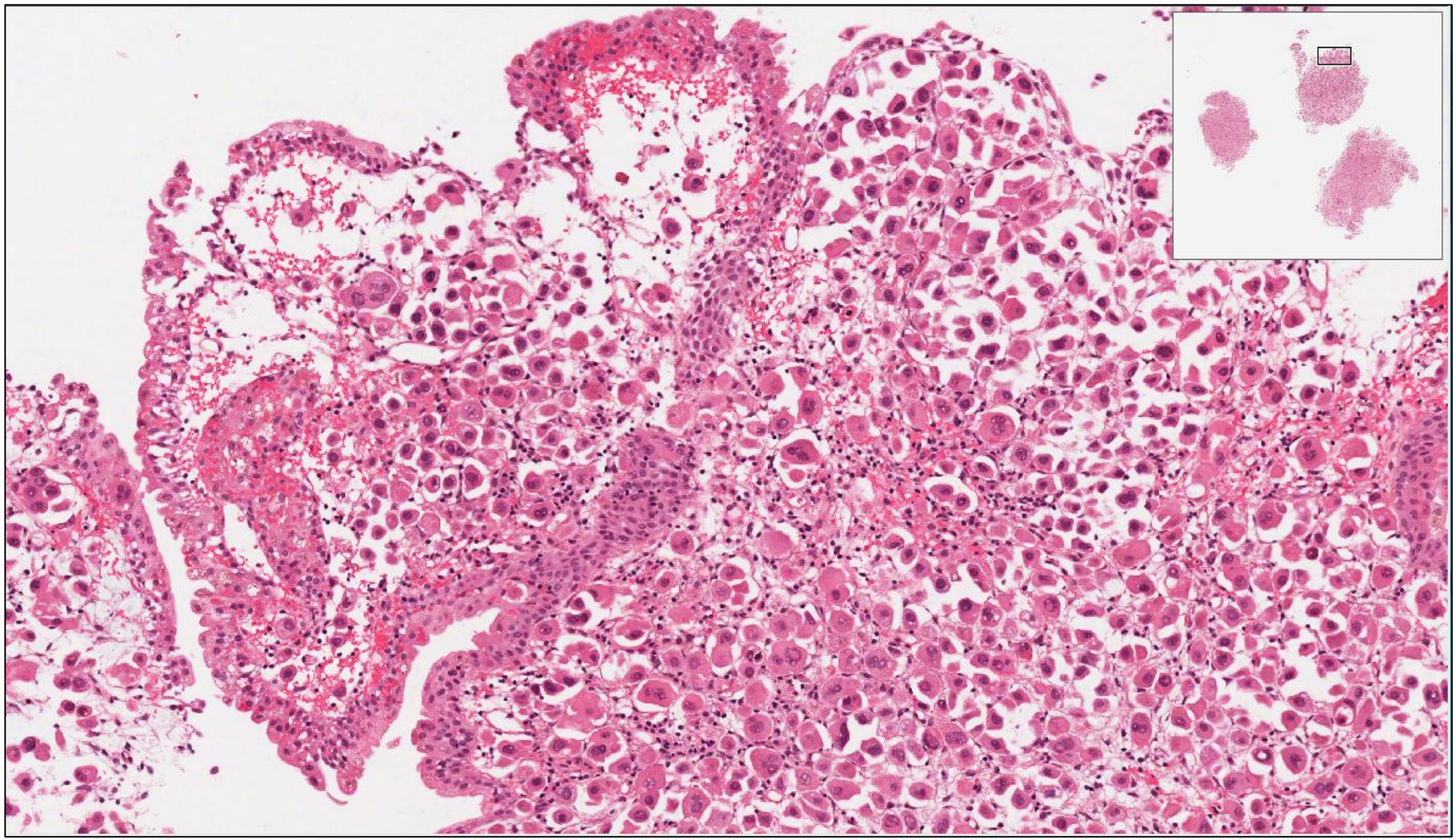
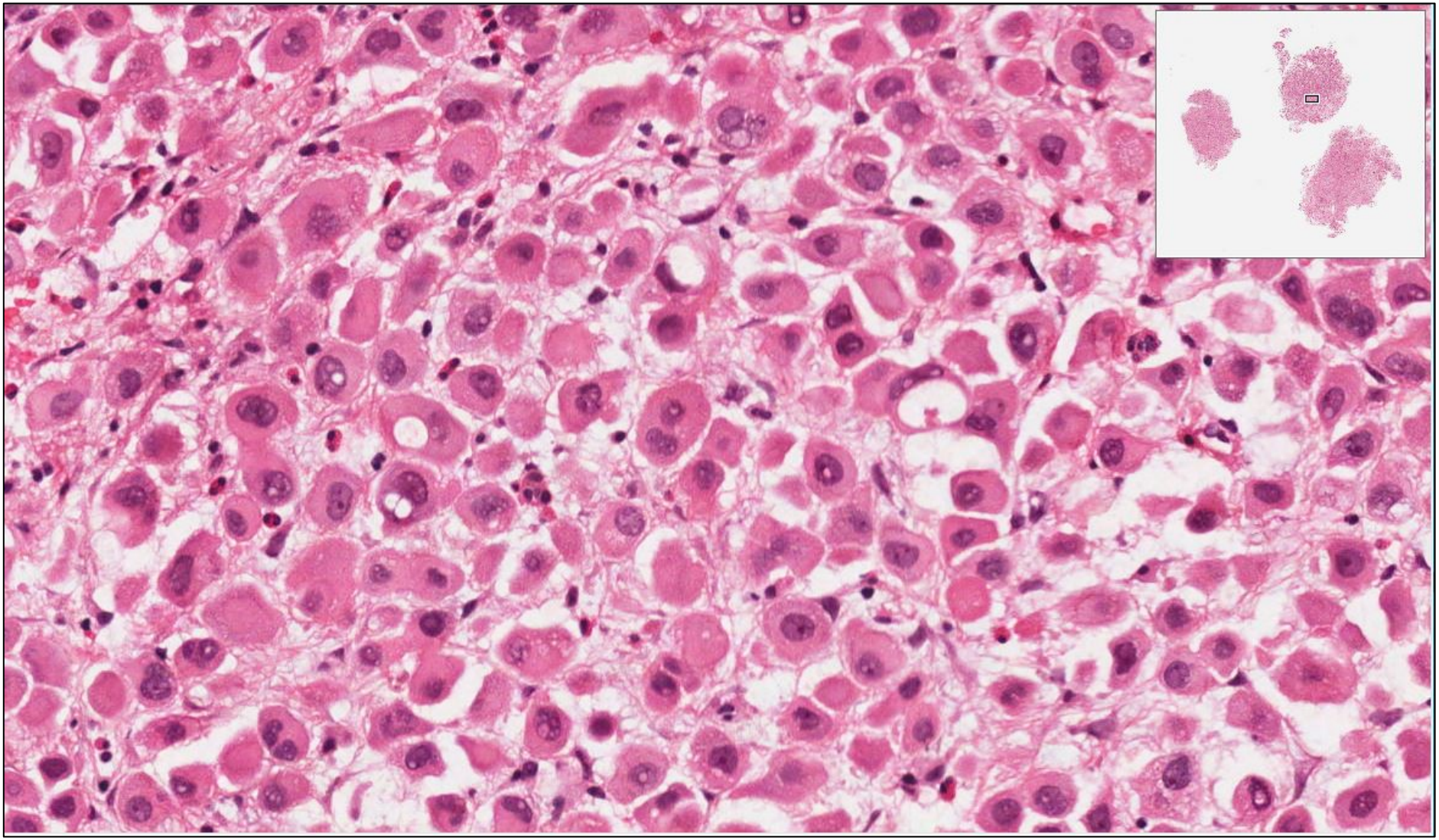
CD20

Case #4

- 72 year-old female with chronic hepatitis B x 20 years
- 3 month history of diffuse bone pain and new onset gross hematuria
- Bone scan shows “innumerable metastases”
- Cystoscopy reveals polypoid bladder lesion
- TURBT to confirm bladder cancer





IHC Panel

- AE1/AE3
- CK7
- CK20

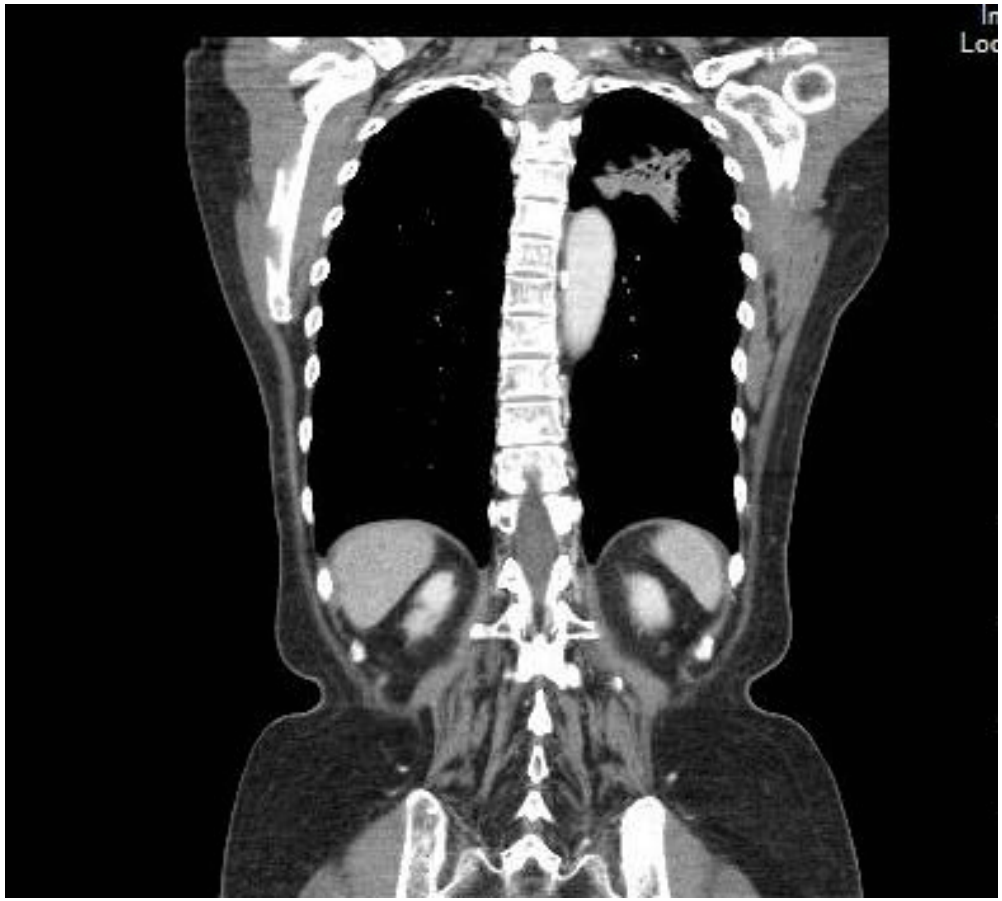
Positive

- p63
- 34βE12
- GATA3
- ER/PR/BRST-2
- CDX2/Villin/β-catenin
- TTF-1

Positive

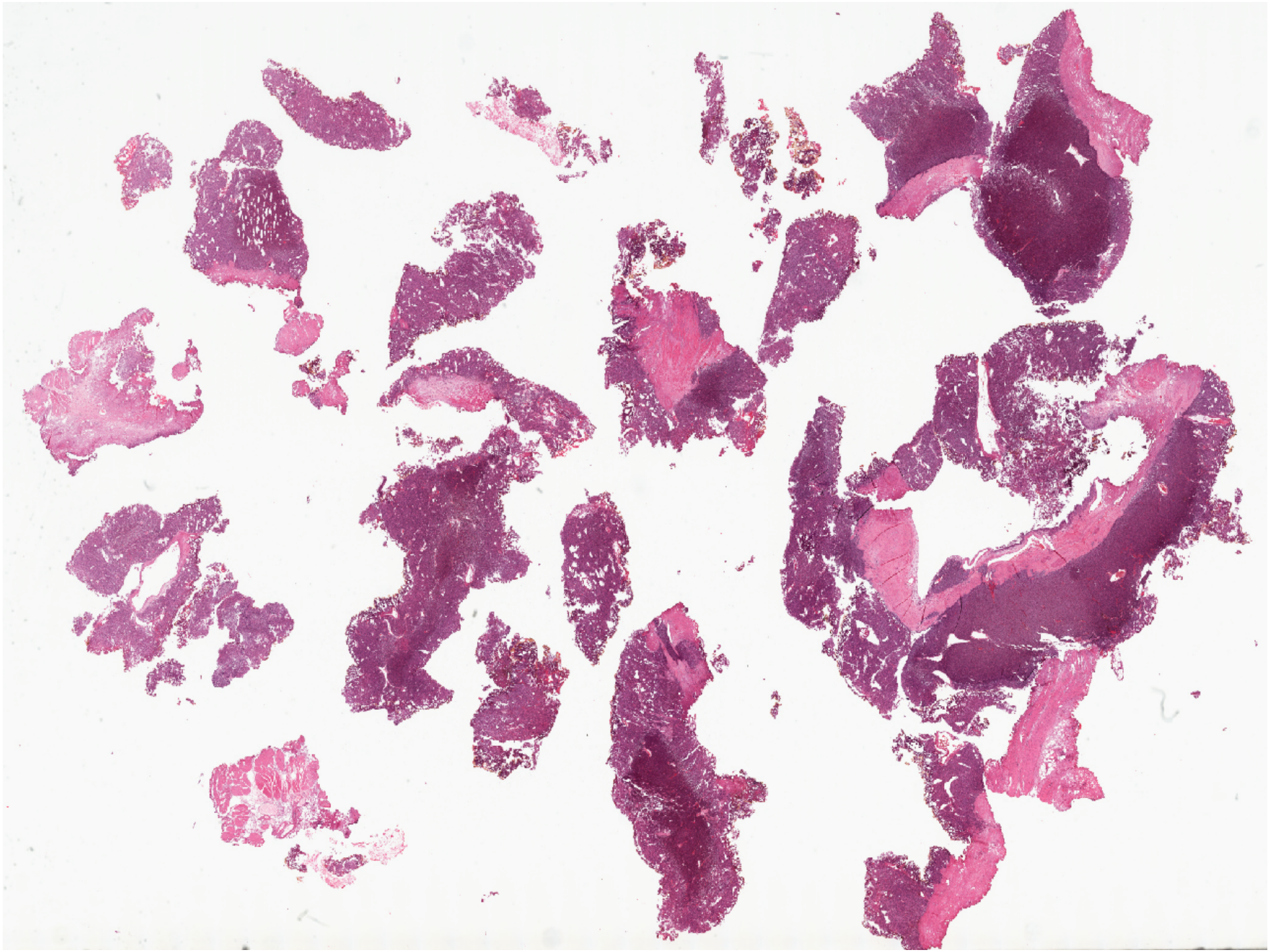
What is Going On in the Lungs?

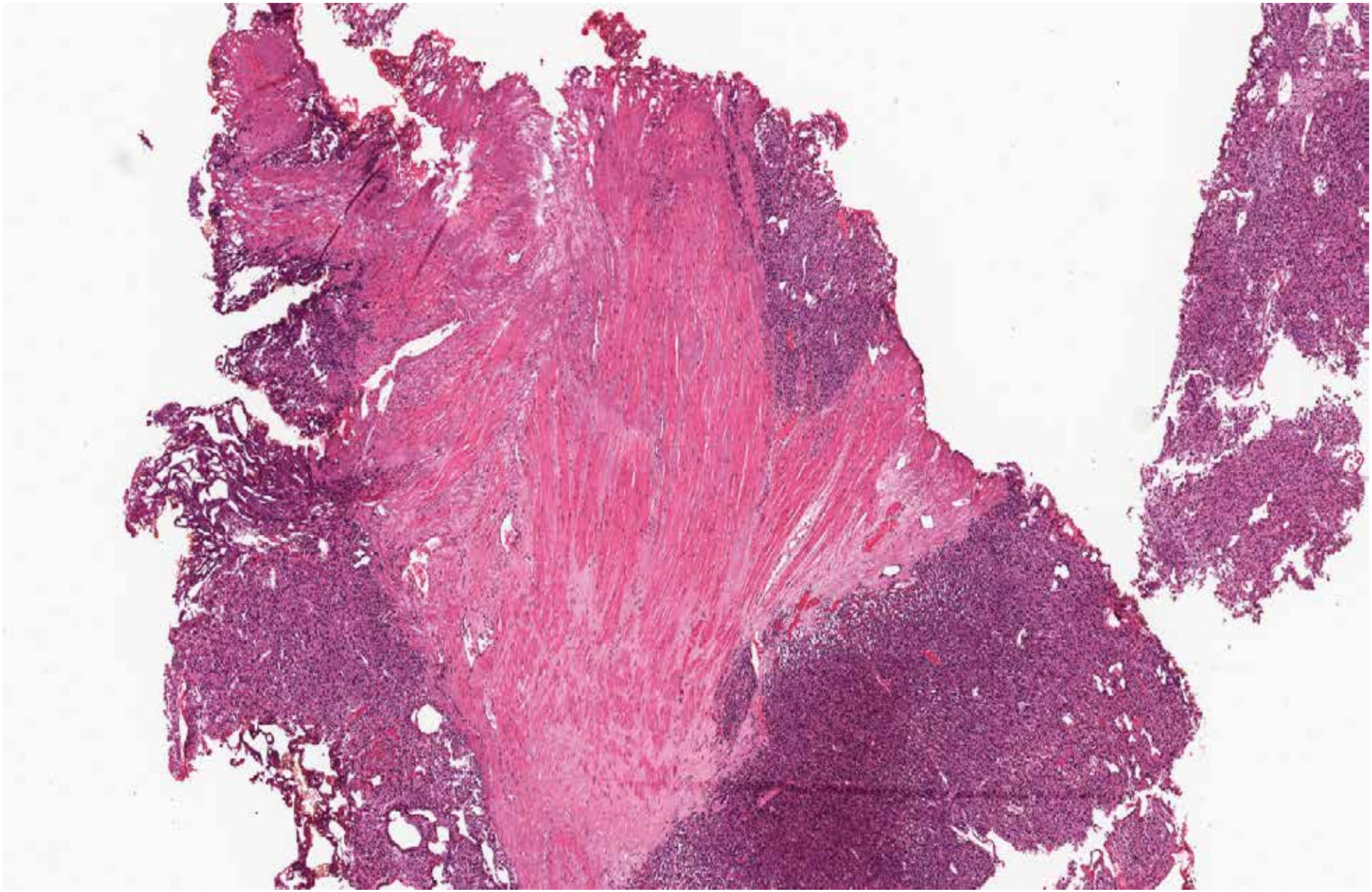
- No imaging above the diaphragm in the EPR
- Suggest chest imaging re: lung primary

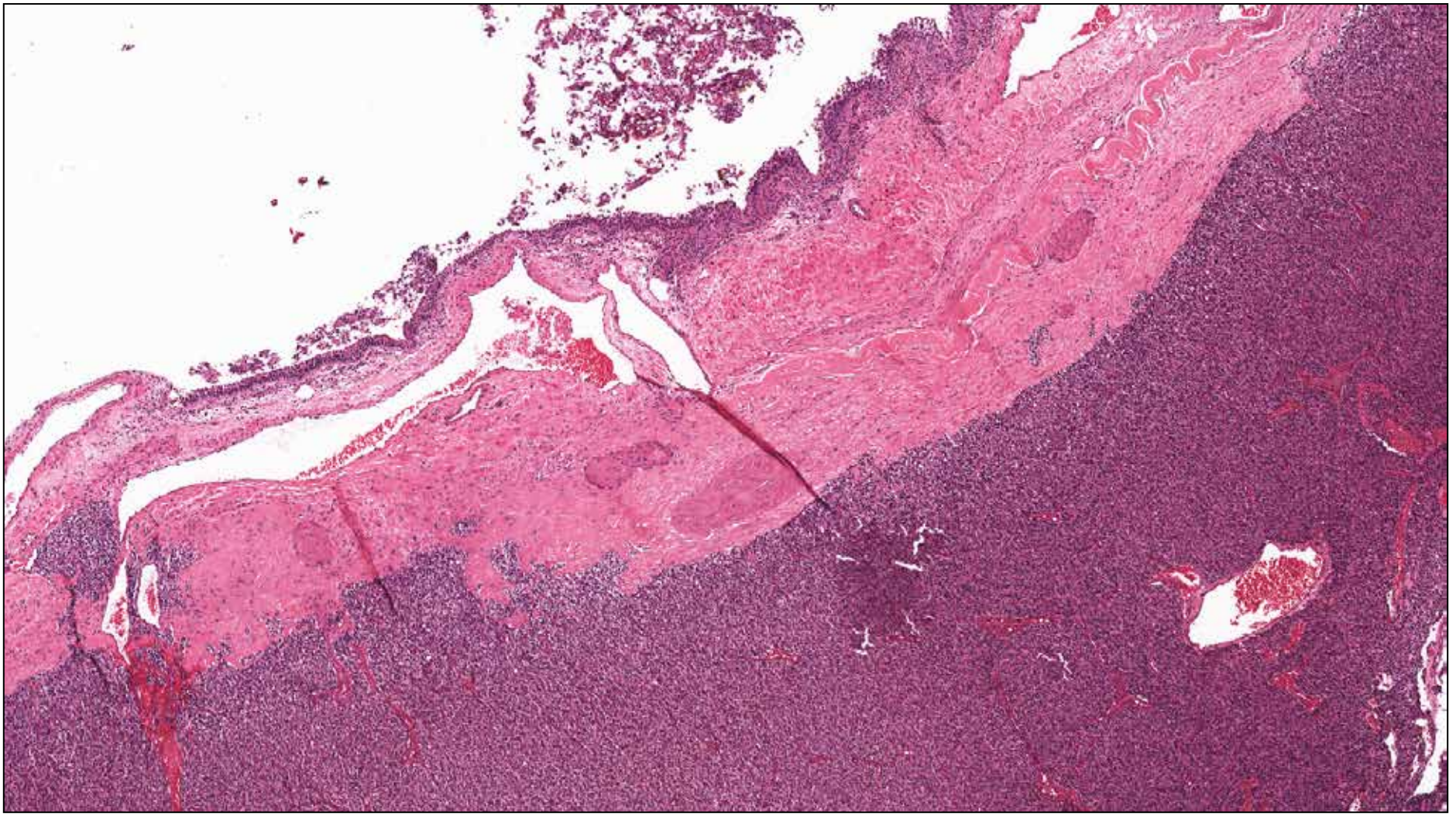


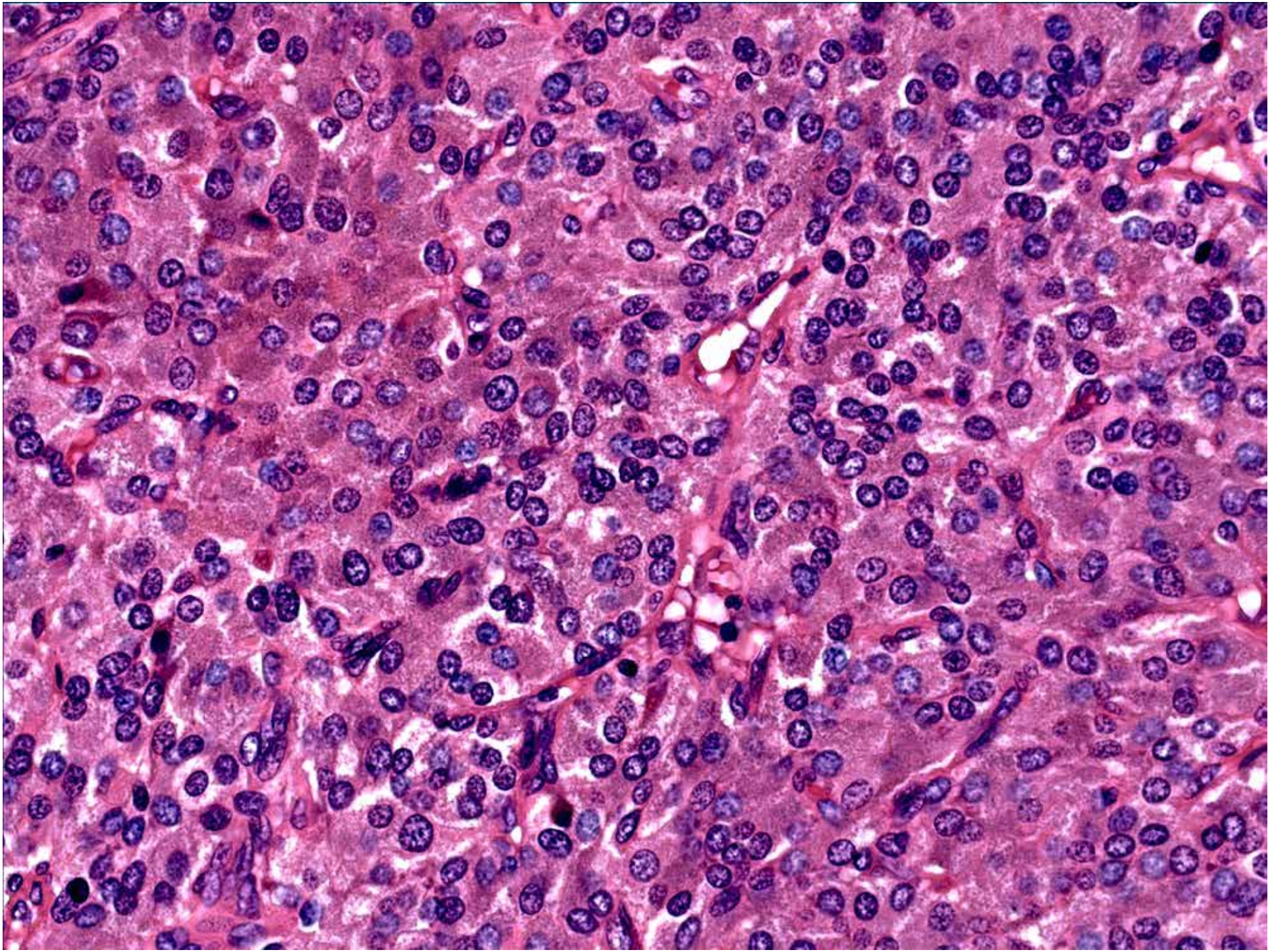
Case #5

- 47 year-old male, painless gross hematuria, sudden onset
- Cystoscopy/TURBT
  - 2 cm dome-shaped nodule, right lateral wall
  - no ulceration/active bleeding
- Urine cytology - negative









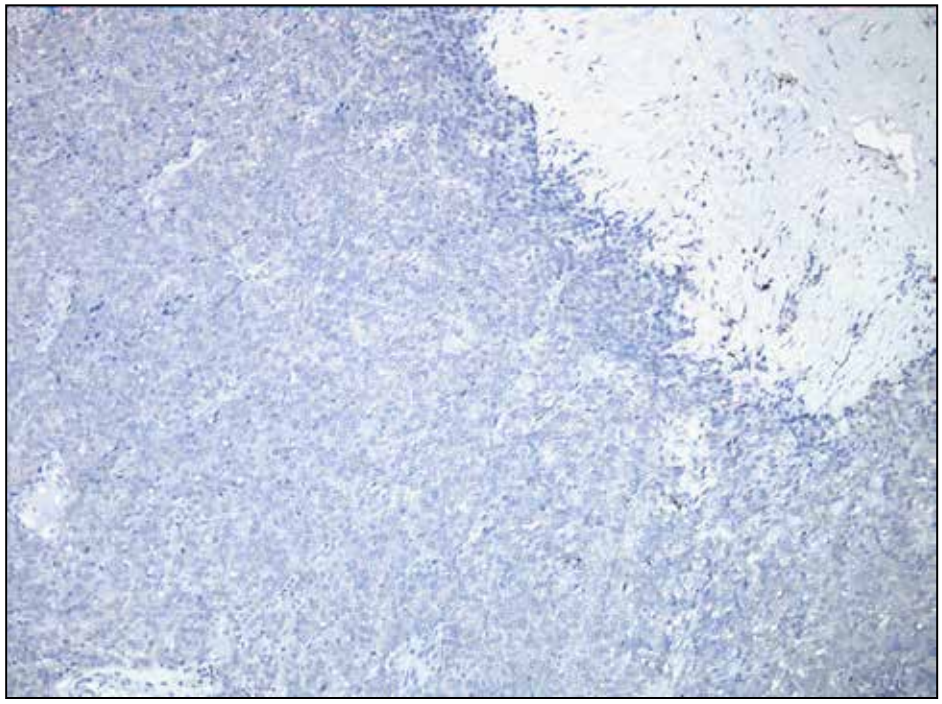
Histologic Differential Diagnosis

- Urothelial carcinoma
- Paraganglioma with a diffuse growth pattern (no nests or “zellballen”)
- Low-grade neuroendocrine carcinoma (carcinoid), primary or metastatic.
- Granular cell tumour

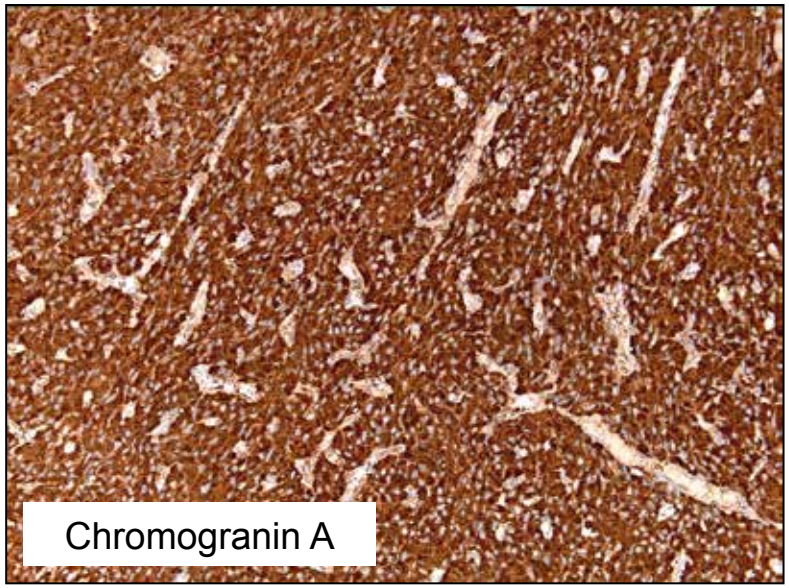
IHC Panel

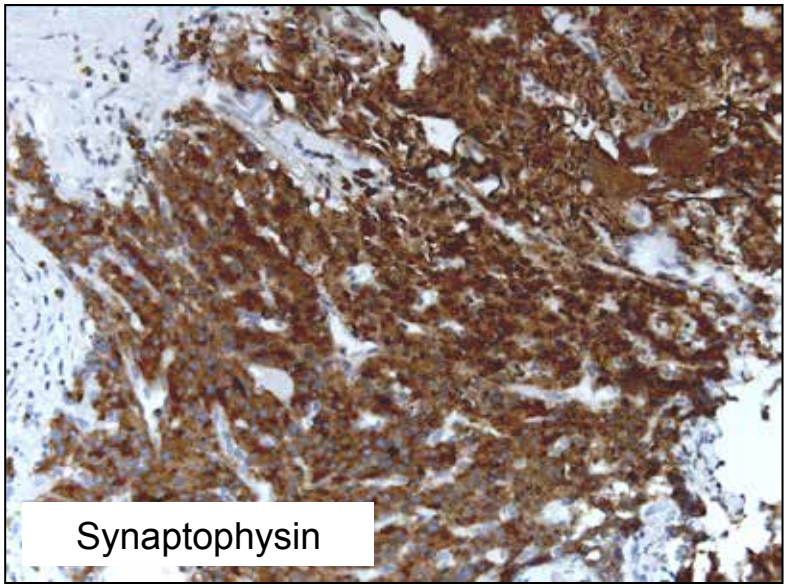
- Urothelial markers
  - AE1/AE3
  - CAM 5.2
  - CK7/CK20
  - HMWK (34βE12)
  - GATA3
- Neuroendocrine markers
  - NSE
  - Chromogranin A
  - Synaptophysin
  - Tyrosine hydroxylase
- S100

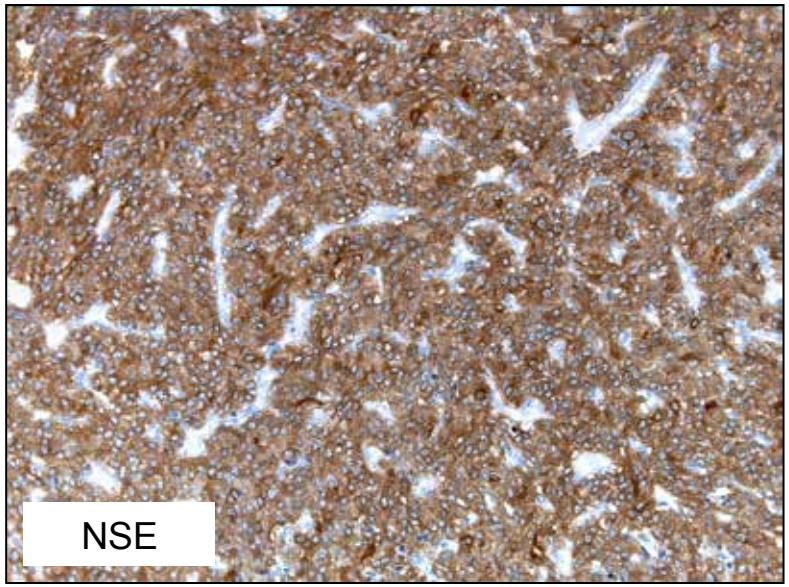
AE1/AE3, CAM 5.2, CK7, 34βE12

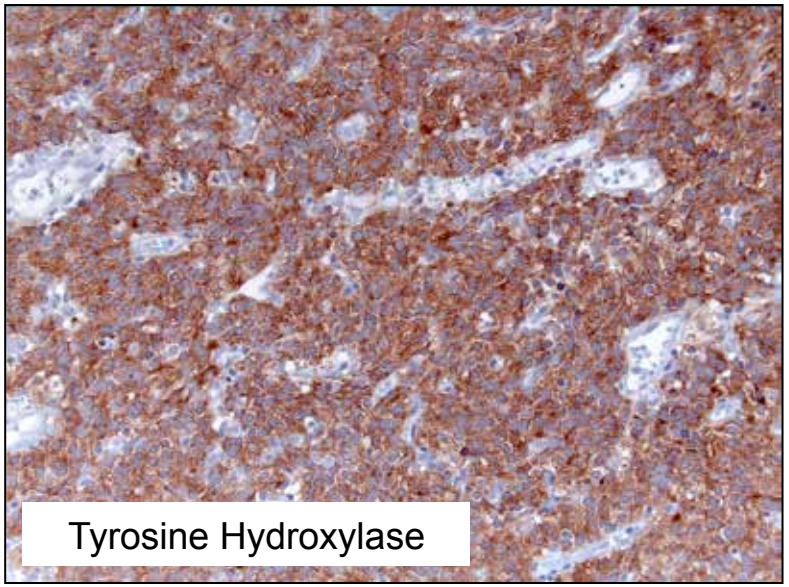


Neuroendocrine Markers









ORIGINAL ARTICLE

Am J Surg Pathol 2004;28:94-100

Paraganglioma of the Urinary Bladder

A Lesion That May Be Misdiagnosed as Urothelial Carcinoma in Transurethral Resection Specimens

Ming Zhou, MD, PhD, Jonathan I. Epstein, MD,* and Robert H. Young, MD†*

- frequent presence in muscularis propria
- morphologic alterations attributed to transurethral resection
- minority of cases presenting with tell-tale symptoms
- failure of pathologists to include it in a differential diagnosis

SDHB Mutations in Paraganglioma

- SDHB mutations are associated with:
  - familial paraganglioma/pheochromocytoma syndrome
    - *1p36.1-p35* (PGL4)
    - autosomal dominant with variable penetrance
  - younger age at presentation
    - mean age 30 years
    - index cases as young as 10 years
  - malignant behavior
  - loss of SDHB immunoreactivity

SDHB Staining

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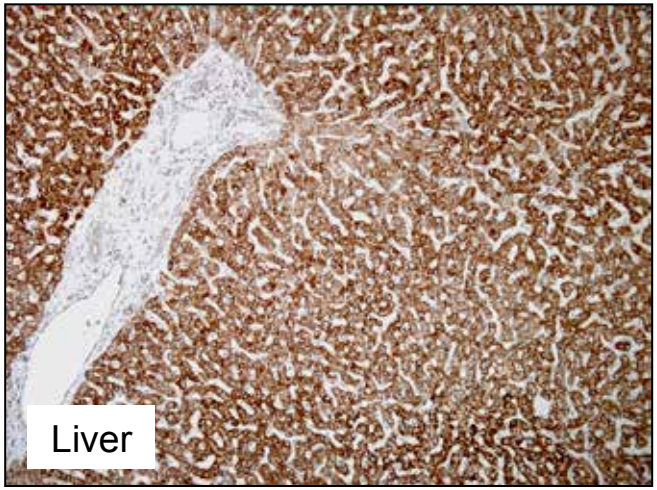

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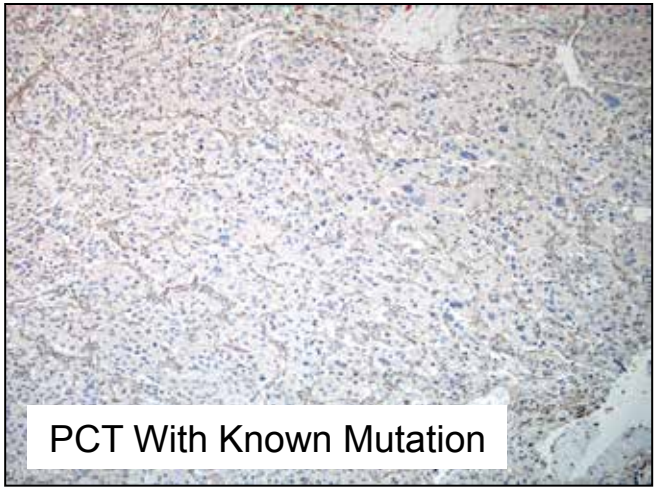
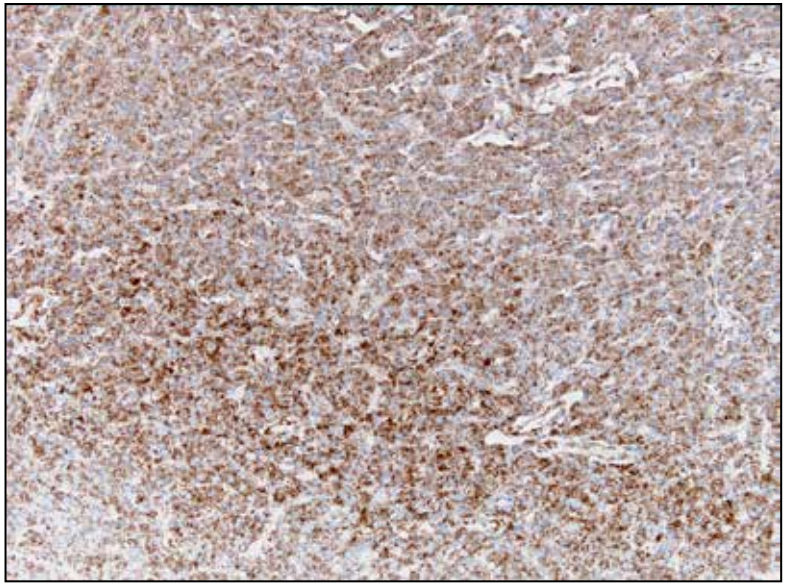
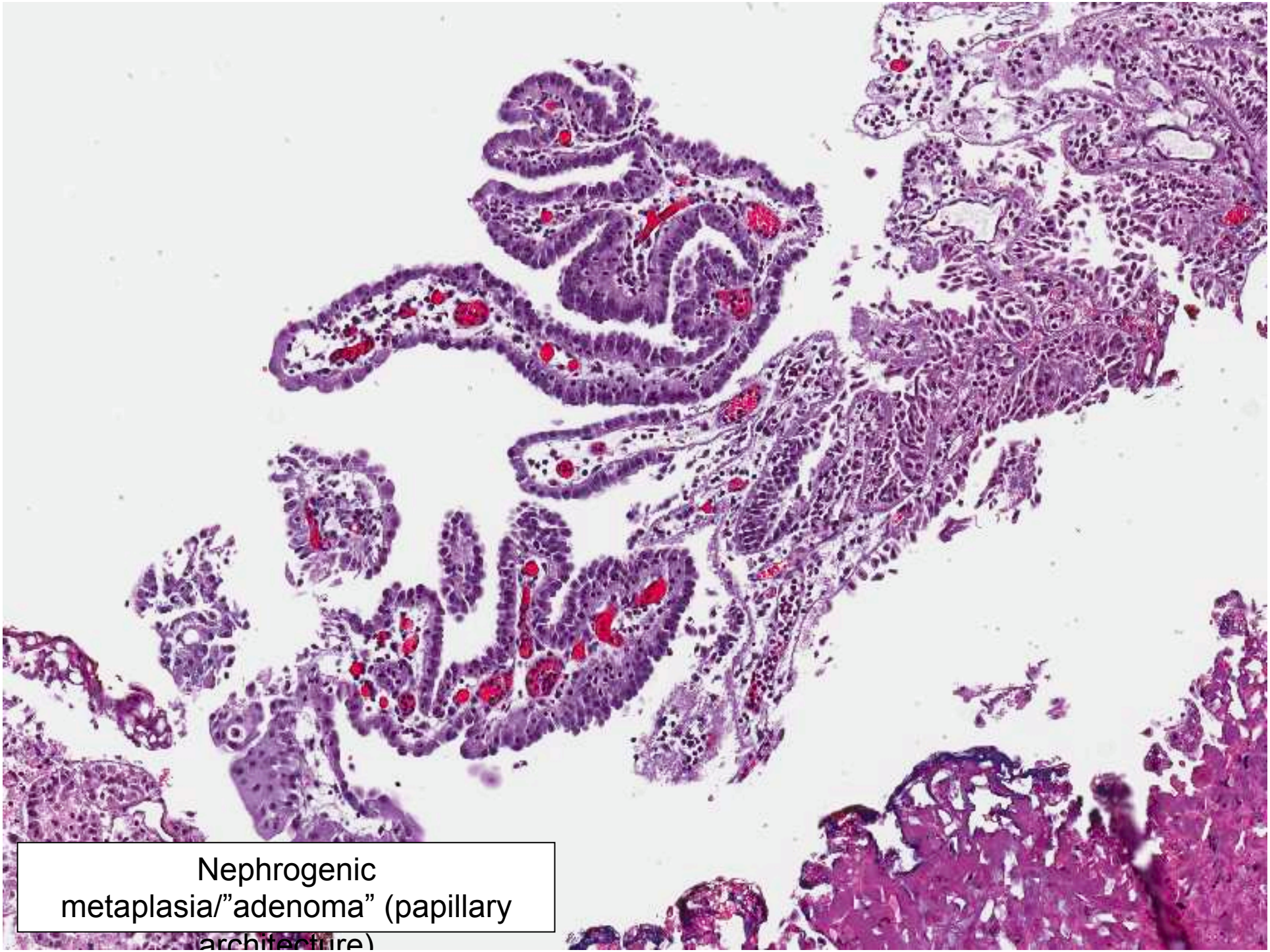


Case



Case #6: A Homer Simpson Moment in Pathology

- 45 year-old male with history of bladder stones and gross hematuria
- Cystoscopy reveals polypoid bladder lesion
- TURBT to rule out bladder cancer


Nephrogenic metaplasia/”adenoma” (papillary architecture)

Summary

- Review markers of urothelial differentiation
- Review situations where immunohistochemistry (IHC) is/is not helpful in the assessment of bladder lesions.
- Review IHC panels designed to sort out specific differential diagnoses.
- Illustrate the above with selected cases.


Thank You!