Practice Updates in Genitourinary Molecular Oncology: A Case Based Approach

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35th Annual Park City Anatomic Pathology Update

Learning Objectives

- Outline the standard of care molecular testing practices in genitourinary tumors
- Provide an overview of molecular alterations seen in genitourinary tumors
- Discuss using case scenarios how pathologists can use molecular testing for
  - Purpose of diagnosis
  - Suggesting hereditary predisposition and testing
  - Integrate information in molecular tests into pathology reports

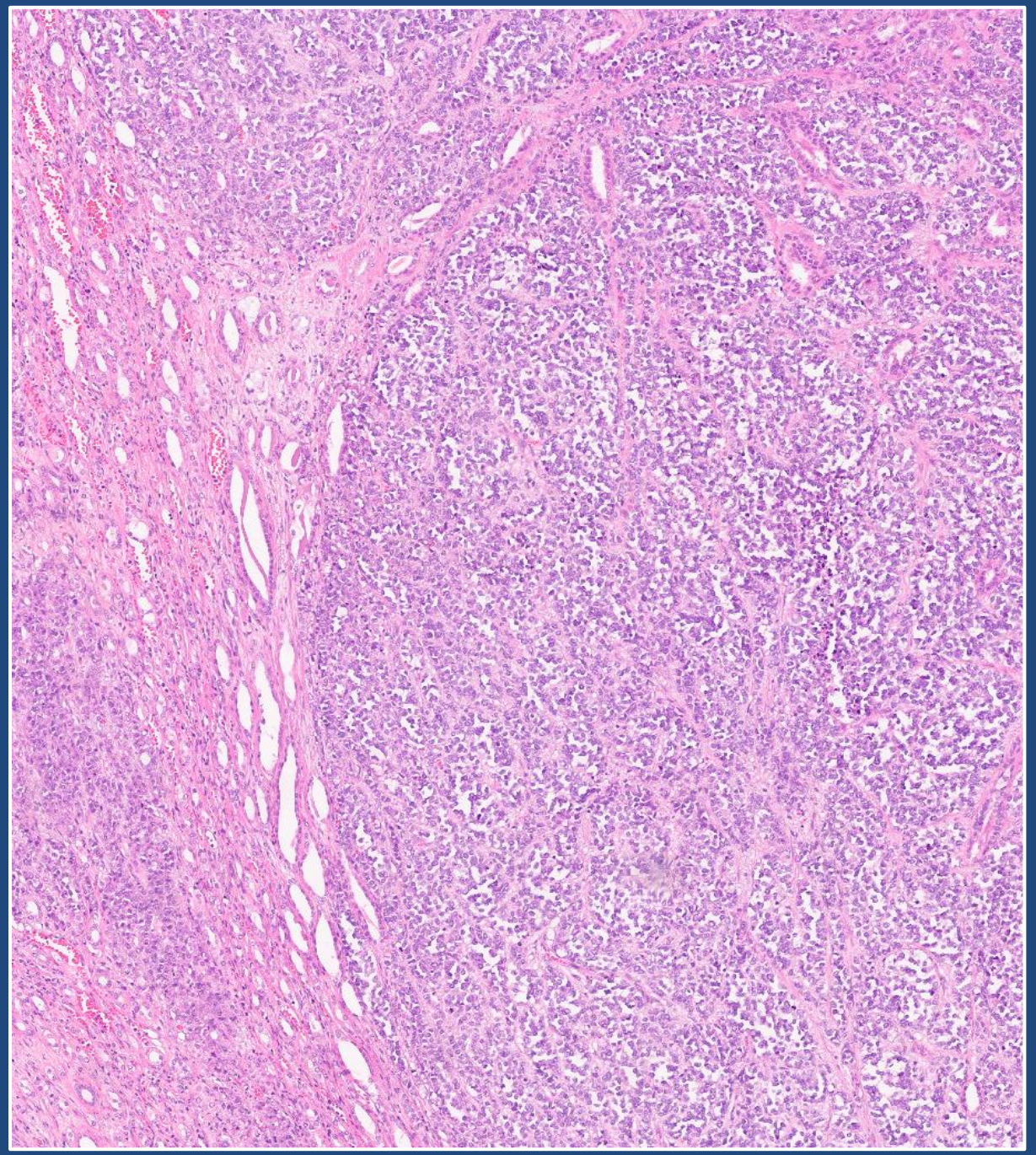
Outline

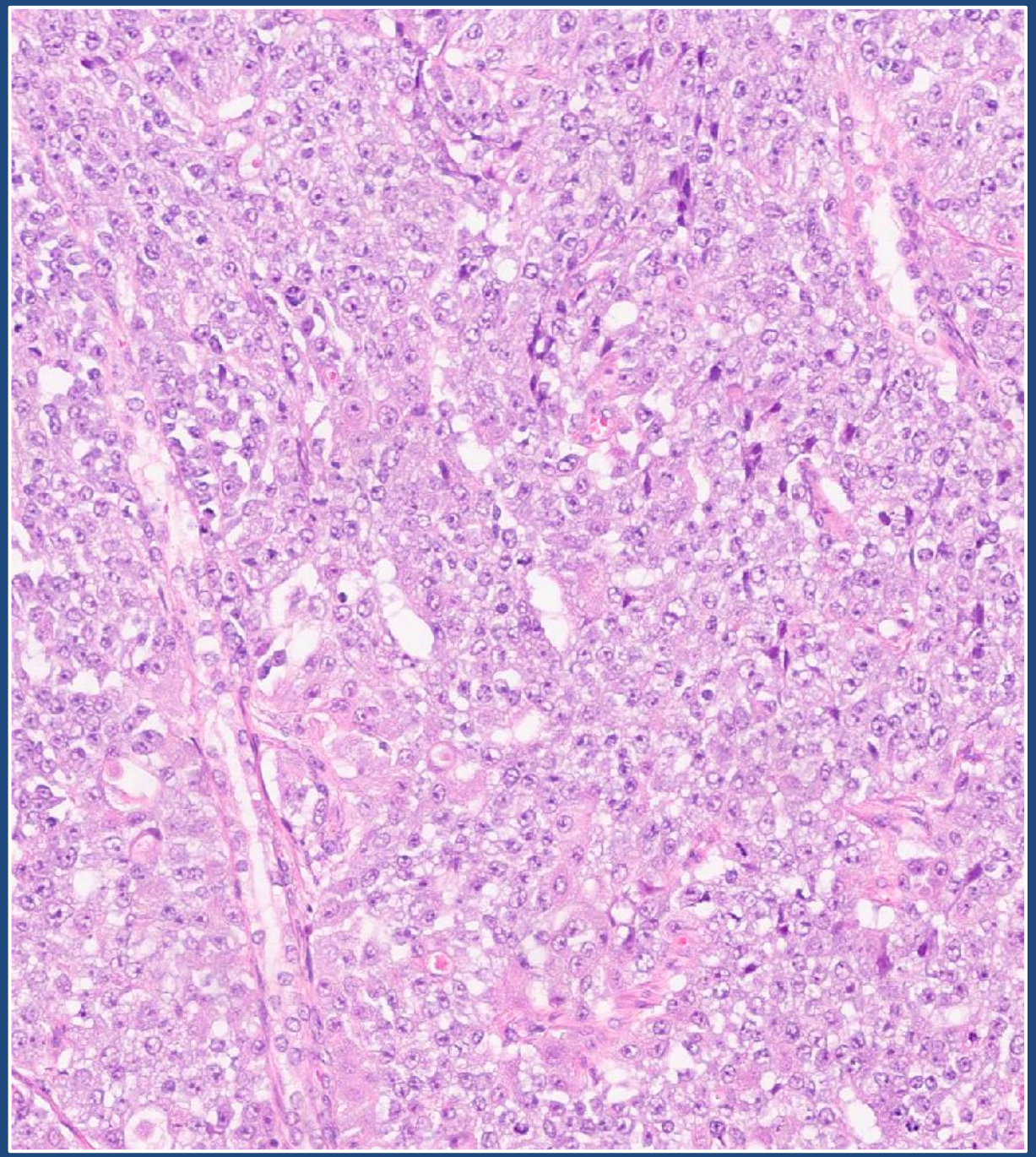
- Kidney tumors
  - Diagnostic biomarkers
  - Hereditary predisposition
- Bladder and Prostate
  - Histogenesis biomarkers- diagnostic
  - Germline and somatic testing for prostate cancer
- Testicular Tumors
  - Diagnostic biomarkers
- Penile carcinomas
  - Viral association
- Immunotherapy Biomarkers

Kidney

Case 1

- 37F, right flank pain
- Family History:
  - Paternal grandmother- Uterine and stomach carcinoma
  - Brother-leukemia
- Ultrasound- 5 cm renal mass with renal vein thrombus extending into inferior vena cava, intracaval lymph nodes
- Radical nephrectomy with retroperitoneal lymphadenectomy





Case 1: IHC/ FISH

Positive

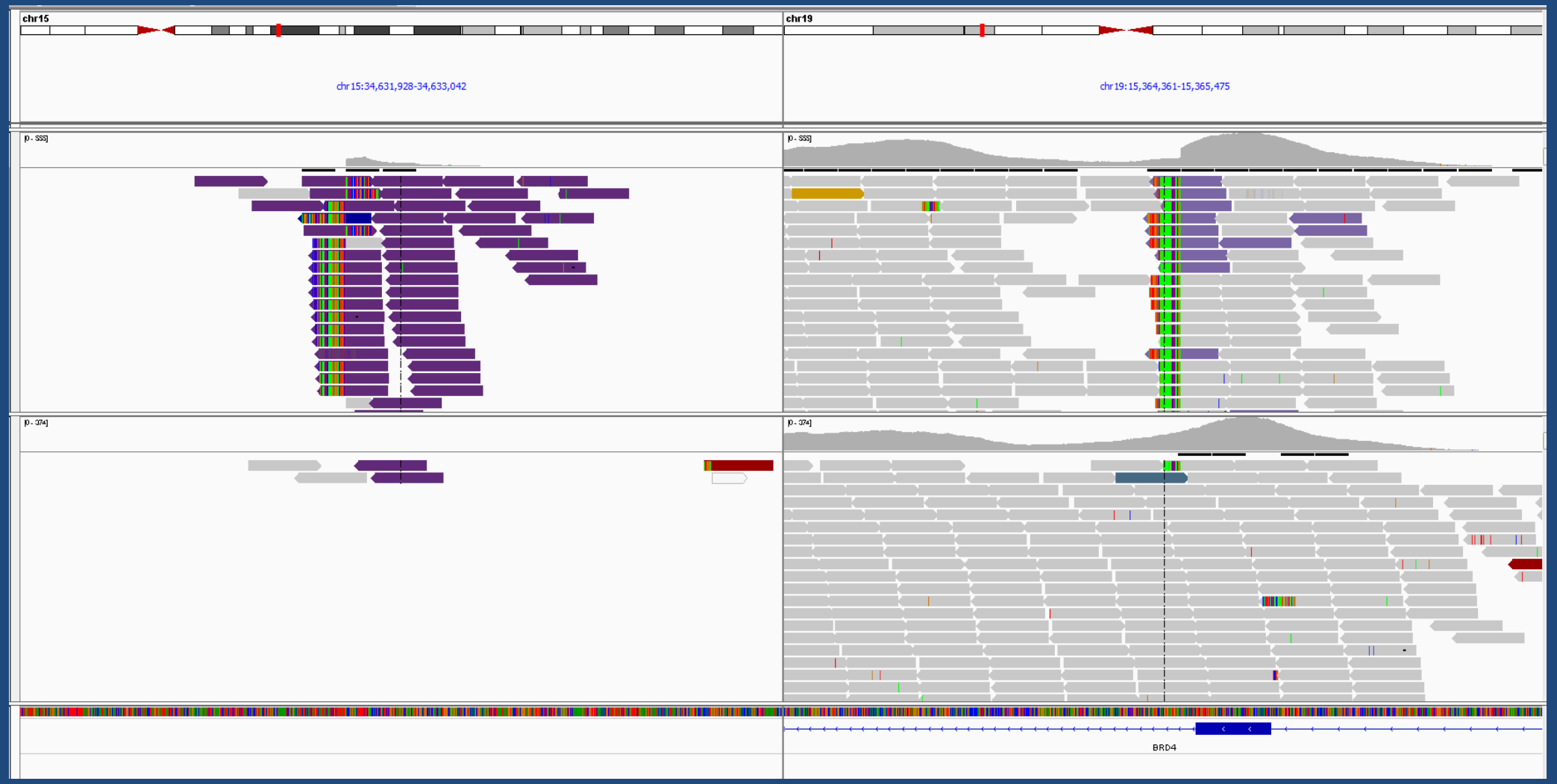
- CK7
- INI1
- SMARCA4
- CA-IX
- CK20 (focal)
- 34BE12 (focal)
- P63 (focal)
- PAX8 (focal)

Negative

- Vimentin
- GATA3
- UP
- PAX2
- RCC
- ALK
- OCT4
- SALL4
- ER
- TTF-1
- ER
- Synaptophysin
- Chromogranin
- WT-1
- SOX-10
- Desmin
- H3
- BCOR
- EWS
- FUS
- SYT, BCOR , YWHAE gene abnormalities

Case 1

- Poorly differentiated carcinoma, possibly of distal nephron origin, invading into renal vein
- pT3aN1
- Genetic testing: diagnostic or predictive biomarkers

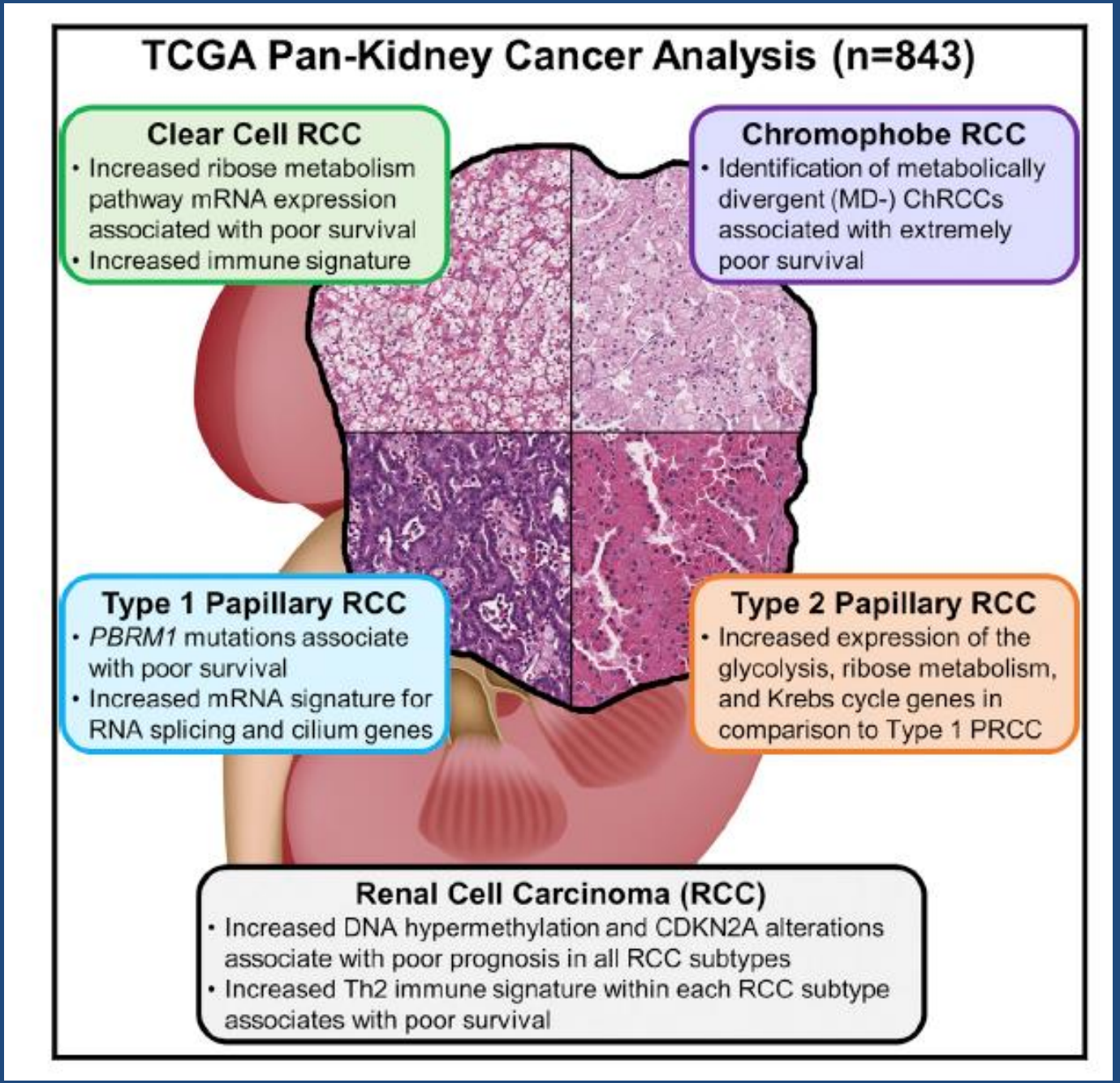


BRD4-NUTM1* fusion** → ***NUT rearranged carcinoma

1 month later….

- Extensive hypermetabolic soft tissue/ LN in nephrectomy bed, retroperitoneum
- Multiple bilateral pulmonary nodules
- Extensive staging work up did not reveal any other primary
- GSK BET inhibitor
- Decrease in size of most target lesions with growth of non-target lesions in peritoneum.
- Immunotherapy
- DOD-6m

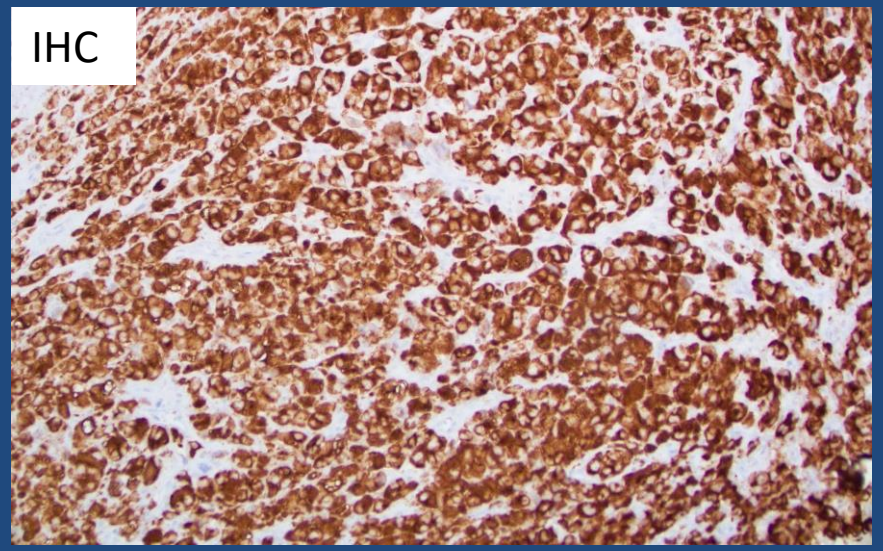
Sirohi D et al. Histopathology. 2018 Feb;72(3):528-530



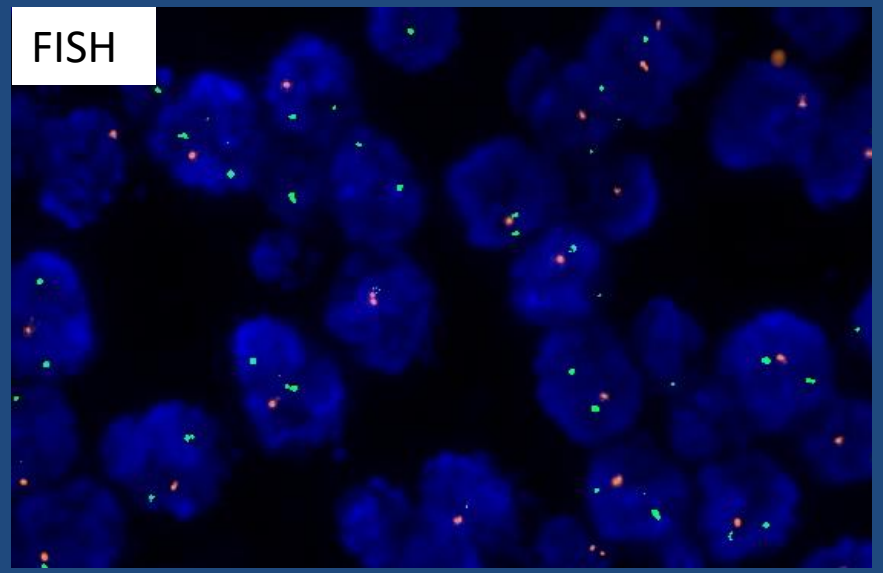
Ricketts et al. Cell Reports 2018;23:313–326

Common Molecular Testing Methodologies

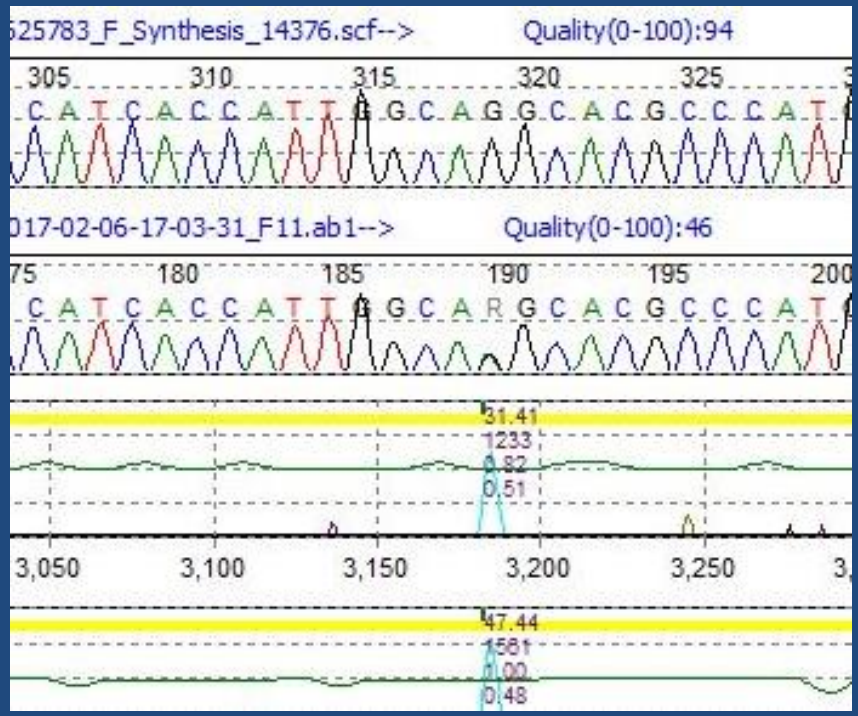
IHC

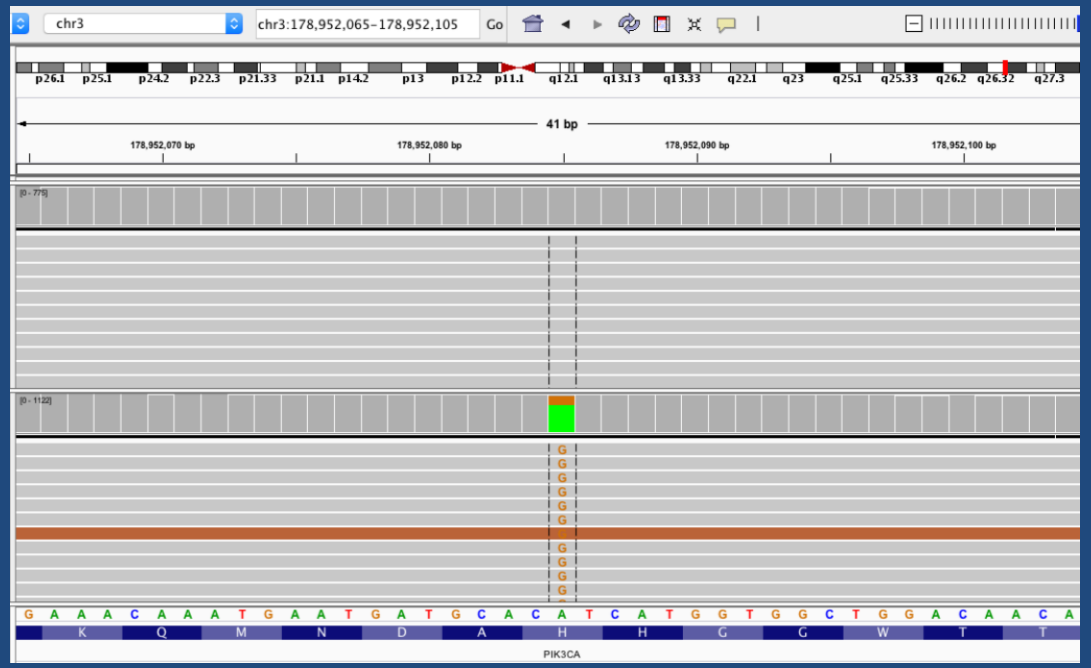


FISH



Sequencing





Tumor Type	Diagnostic Biomarkers	Testing Methodology
Clear Cell RCC	VHL, Loss of 3p BAP1, SETD2, PBRM1	Sequencing, FISH, methylation assays, chromosomal microarrays
Papillary RCC type 1	Gains of chr 7, 17, loss of Y MET	Sequencing, FISH, chromosomal microarrays
Chromophobe RCC	Loss of chr 1, 2, 6, 10, 13, and 17 TP53, PTEN, mTOR, FLCN	Sequencing, chromosomal microarrays
MIT Family associated RCC	TFE3, TFEB	IHC, FISH, sequencing
SDH deficient RCC	SDHB, SDHA, SDHC, SDHD	IHC, sequencing
FH deficient RCC	FH, 2SC	IHC, sequencing
Angiomyolipoma	TSC1, TSC2	Sequencing
Renal Adenomyomatous Tumor	TCEB1, TSC1, TSC2, mTOR	Sequencing
Renal Medullary Carcinoma RCC unclassified with medullary phenotype	SMARCB1	IHC, FISH, sequencing
ALK rearranged RCC	ALK	IHC, FISH, sequencing
Hemangioblastoma	VHL	Sequencing, methylation assays

TABLE 1. Hereditary RCC Syndromes

Syndromes	Gene	Histologic Types of Renal Tumors	Incidence of Renal Cancer and Mean Age at Diagnosis
VHL disease	*VHL* 3p25-26	Clear cell RCC	25%-45% 40 y
Hereditary papillary RCC	*MET* 7q31	Papillary RCC type 1	Unknown < 60 y
BHD syndrome	*BHD* 17p11.2	Hybrid oncocytic/chromophobe RCC Oncocytoma Clear cell RCC Papillary RCC	34% 50 y
HLRCC	*FH* 1q42-43	Heterogenous, but predominantly papillary RCC type 2-like	2%-21% 46 y
TSC	*TSC1*/*TSC2* 9q34/16p13	AML Renal cysts Papillary RCC Clear cell RCC Oncocytoma	2%-4% 30 y
Hereditary paraganglioma-pheochromocytoma syndrome	*SDHB*/*SDHC*/*SDHD* 1p36/1q21/11q23	Clear cell RCC	5%-15% 30 y
Hereditary sickle cell hemoglobinopathy and medullary RCC		Medullary RCC	10-30 y
Germline PTEN mutation Cowden syndrome	*PTEN* 10q22-23	Clear cell RCC Papillary RCC Chromophobe RCC	34% 40 y
Hyperparathyroidism-jaw tumor syndrome	*HRPT2* 1q21-32	Mixed epithelial and stromal tumor Papillary RCC Wilms tumor	—
BAP1 mutations and familial kidney cancer	*BAP1* 3p21	Clear cell RCC	—
Constitutional chromosome 3 translocation RCC	Unknown chromosome 3	Clear cell RCC	Unknown

Adeniran et al. Am J Surg Pathol 2015;39:e1–e18

CRITERIA FOR FURTHER GENETIC RISK EVALUATION FOR HEREDITARY RCC SYNDROMES[a]

- An individual with a close blood relative[b] with a known pathogenic/likely pathogenic variant in a cancer susceptibility gene

- An individual with RCC with any of the following criteria:
  - Diagnosed at age ≤46 y
  - Bilateral or multifocal tumors
  - ≥1 first- or second-degree relative[b] with RCC

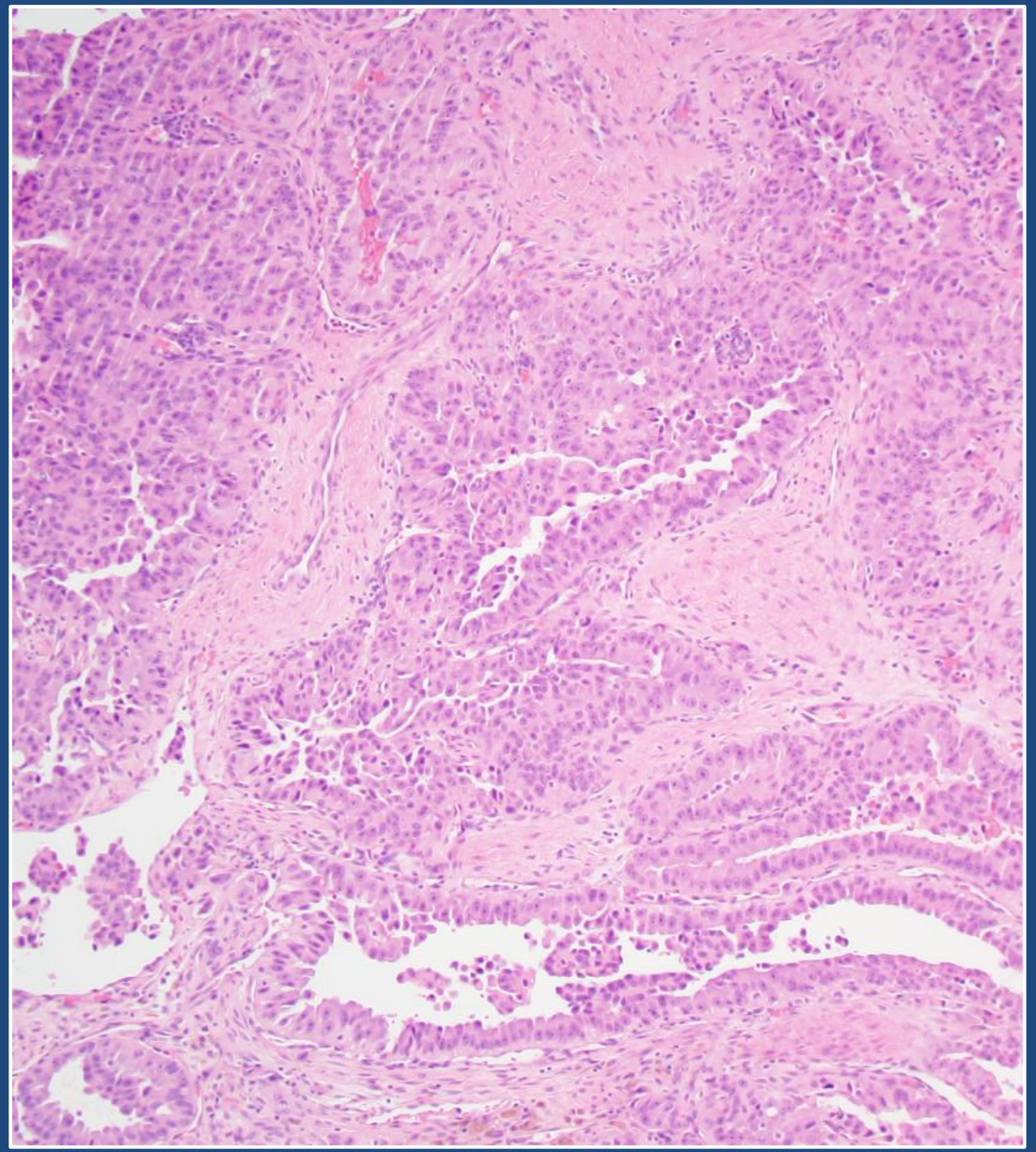
- An individual whose tumors have the following histologic characteristics:
  - Multifocal papillary histology
  - Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)-associated RCC, RCC with fumarate hydratase (FH) deficiency or other histologic features associated with HLRCC
  - Birt-Hogg-Dubé syndrome (BHDS)-related histology (multiple chromophobe, oncocytoma, or oncocytic hybrid)
  - Angiomyolipomas of the kidney and one additional tuberous sclerosis complex criterion in the same person (See Table 1)
  - Succinate dehydrogenase (SDH)-deficient RCC histology[c]

NCCN Guidelines Kidney Version 4.2022

Case 2

- 31 y M
- Diagnosed with renal cell carcinoma
- Nephrectomy in Turkey
- Widespread metastatic disease
- Compressive disease L2- nerve root decompression

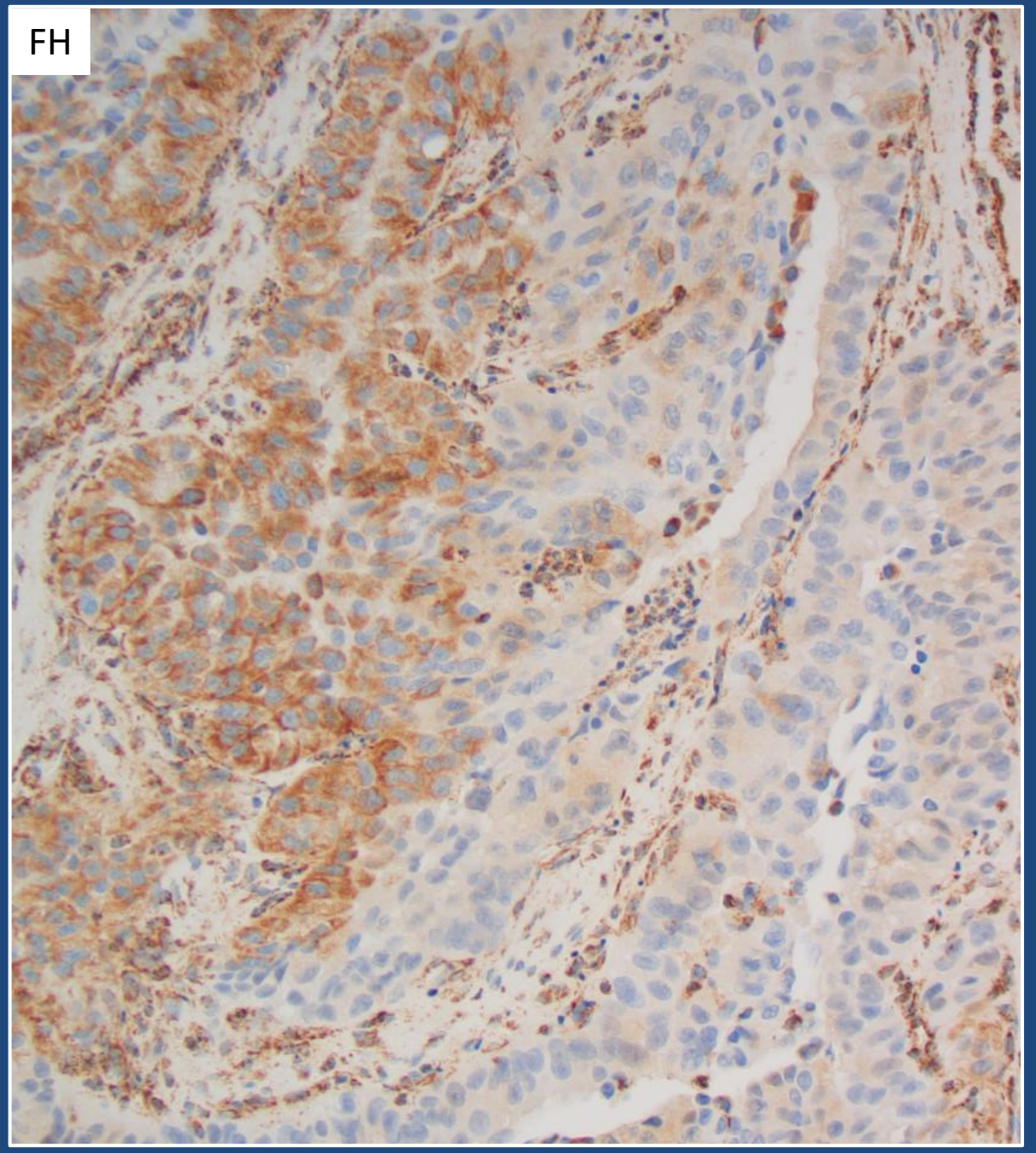
Case 2



Case 2: Pathology

- Consistent with metastatic RCC
- Additional testing
  - *TFE3* FISH: negative
  - FH stain: loss of FH in 40% tumor

- Heterogeneous loss: compatible with FH deficient RCC



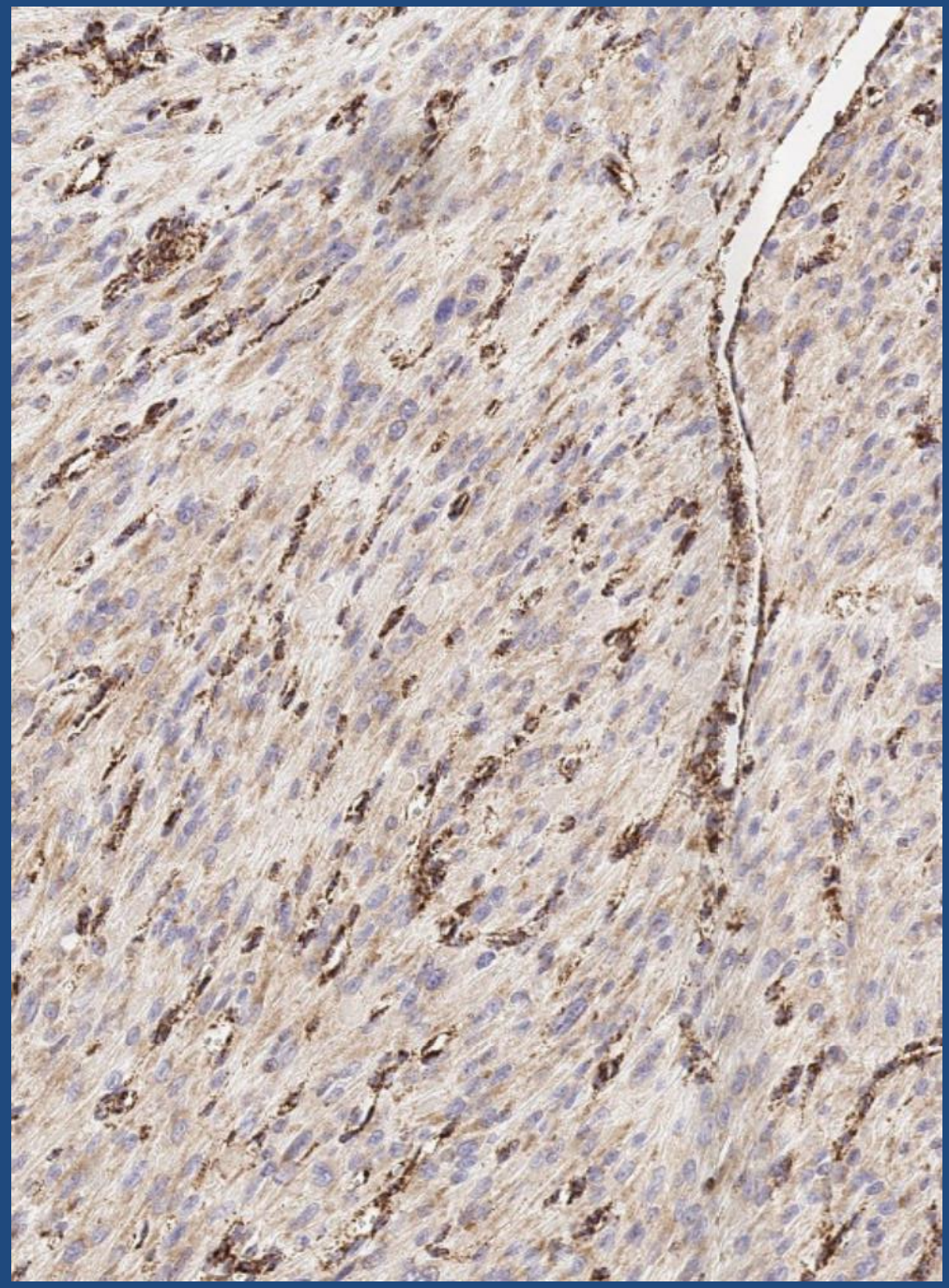
Anderson WJ et al. Int J Surg Pathol. 2022 Online ahead of print

Case 2

- Genetic testing: *FH* c.697C>T p.R233C mutation, heterozygous
- Treatment with Bevacizumab/Erlotinib based on data presented at ASCP 2020
- Tolerated well, interim decrease in metastatic disease
- Hospice
- Disease recurrence
- DOD 1 year later

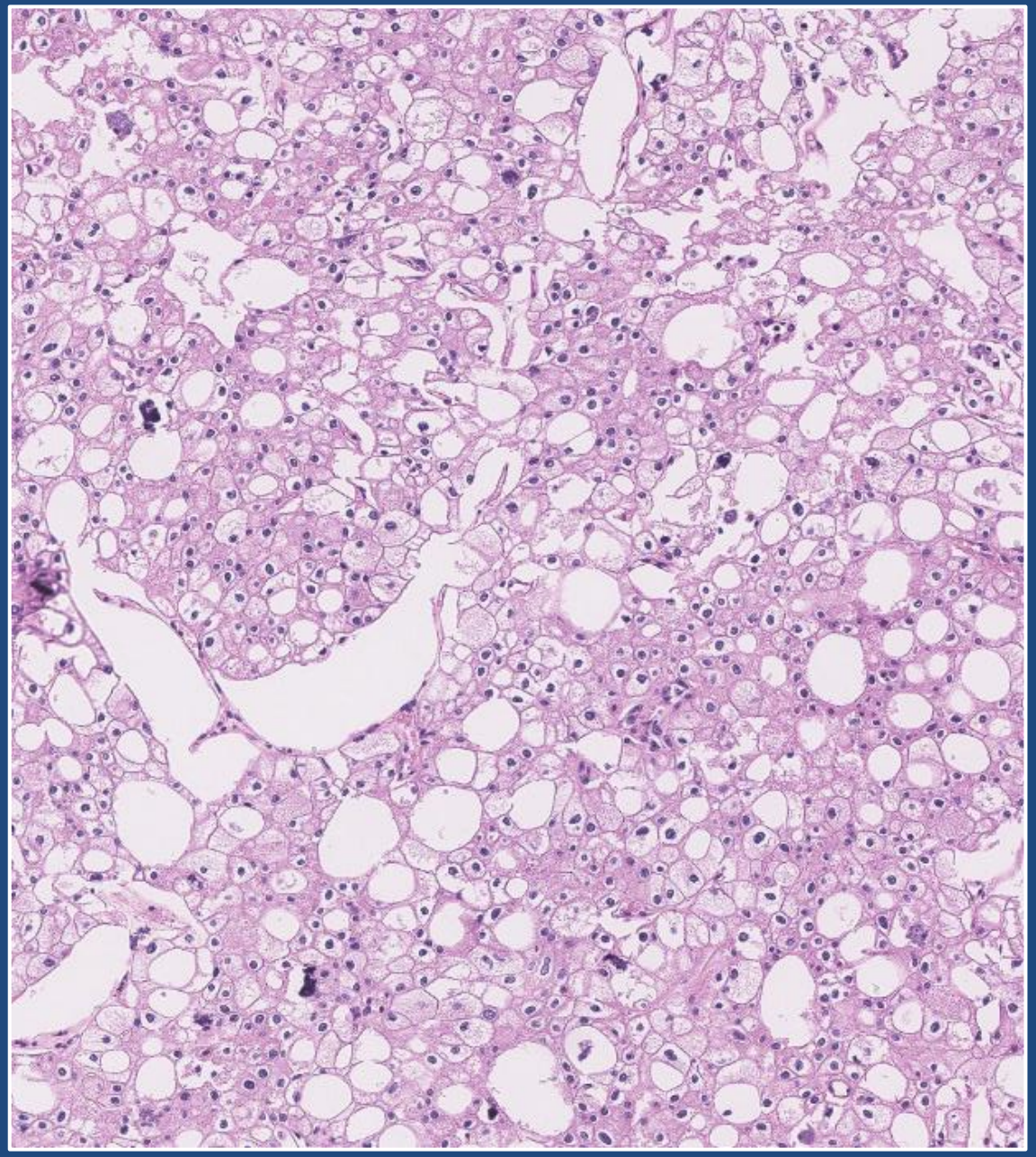
FH staining interpretation

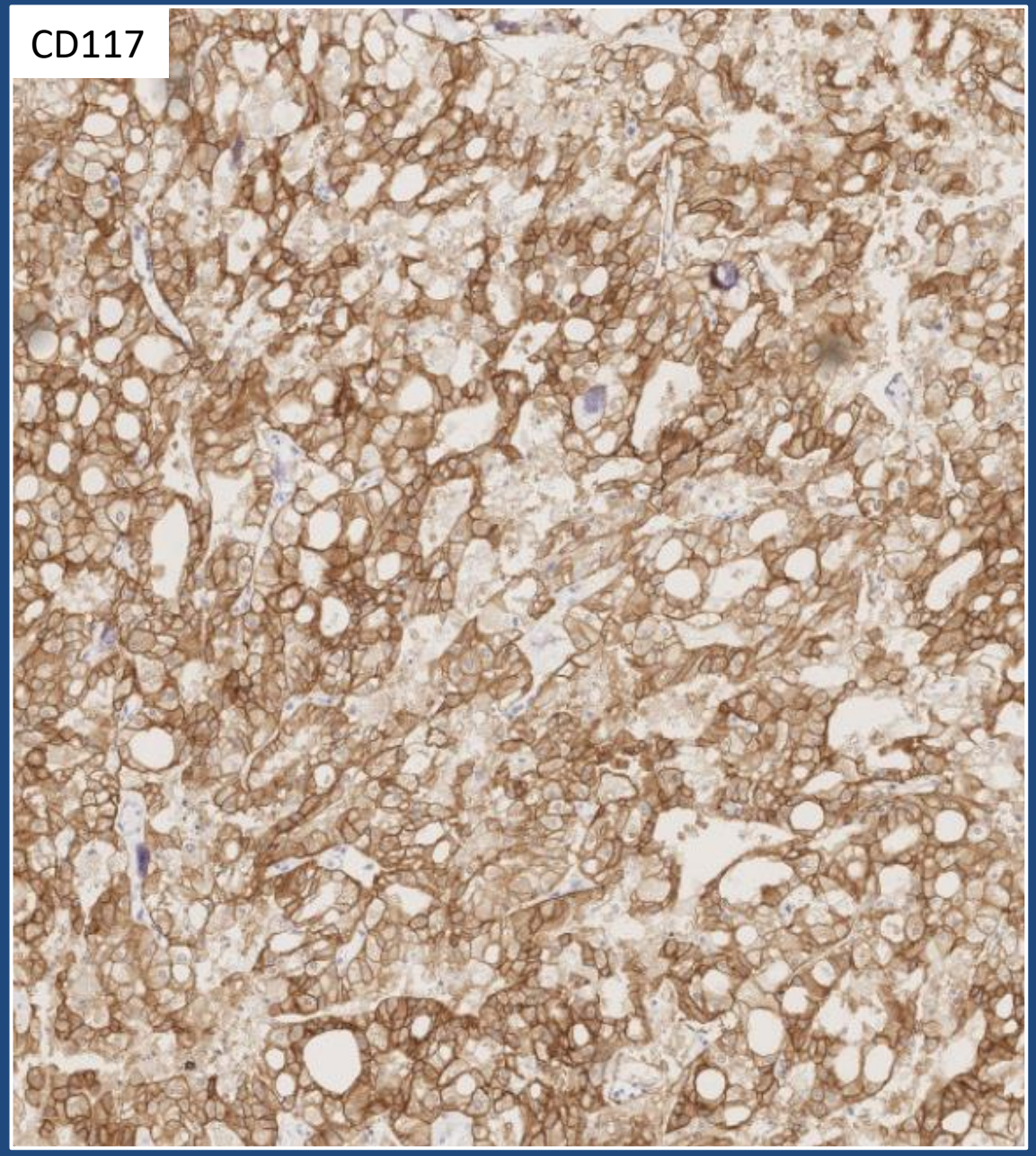
- Sensitivity: 80-90%
- 2SC IHC stain: greater sensitivity, not widely available



Case 3

- 36 y M with renal mass
- Nephrectomy performed at outside hospital
- Family history-
  - Maternal grandfather- testicular cancer

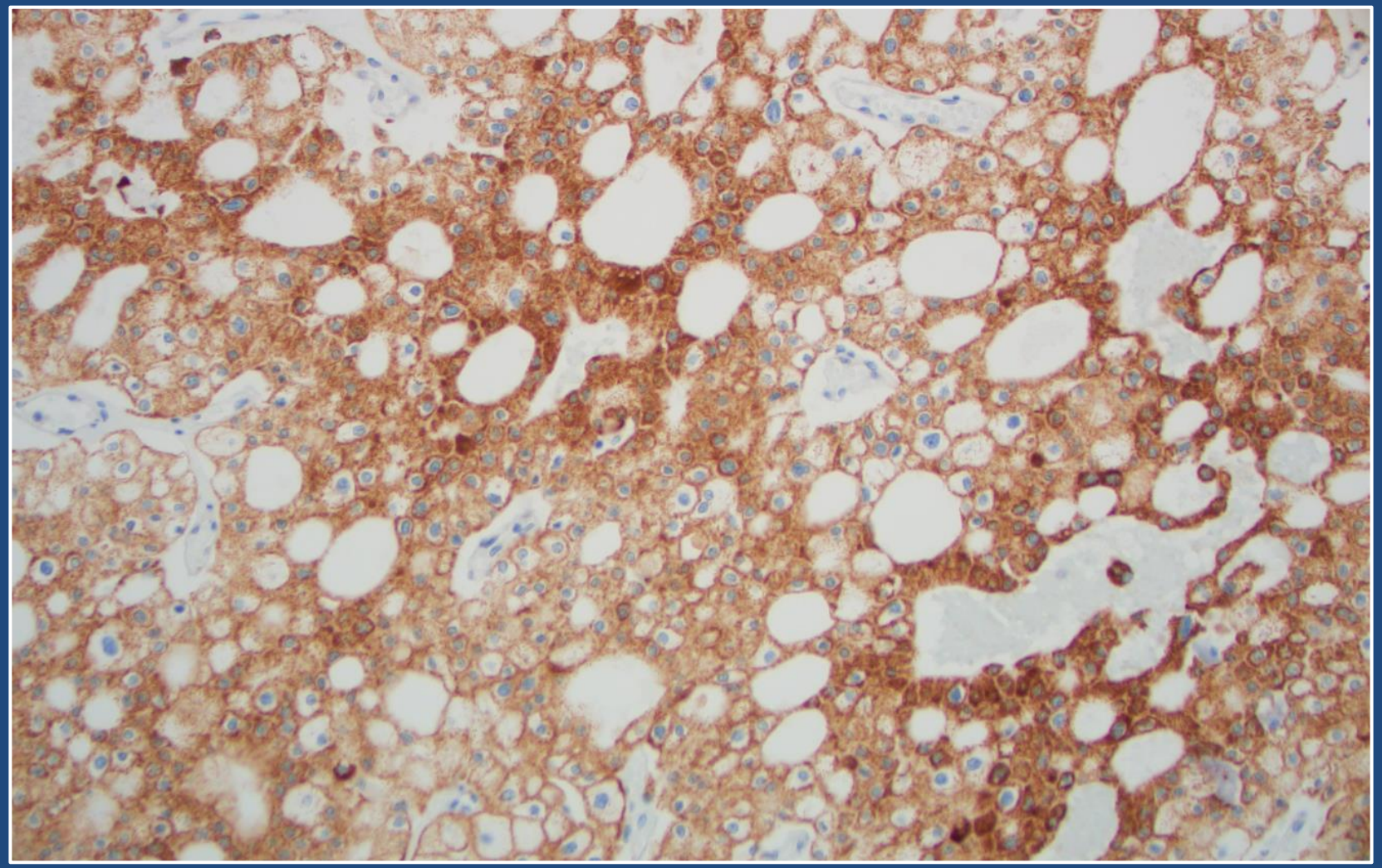



CD117

Case 3

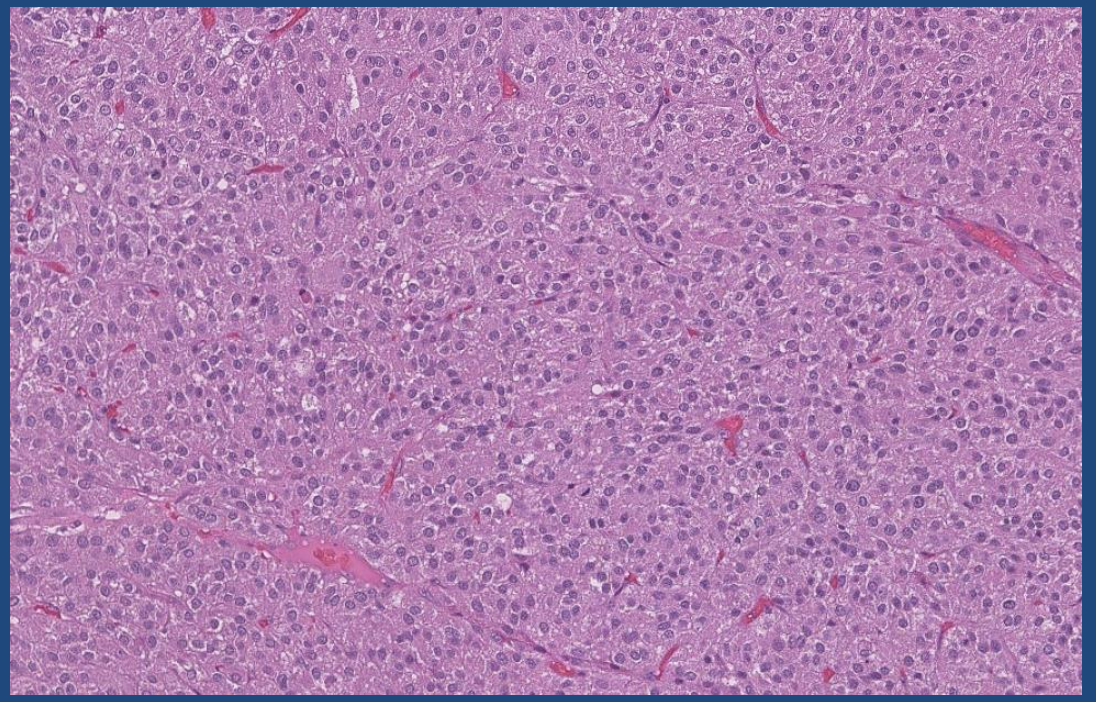
- Additional history:
  - Spontaneous pneumothorax in childhood
  - Skin lesions
- Genetic testing done because of age and associated clinical stigmata
- Likely pathogenic mutation: *FH* c.1431_1433dup p.Lys477dup
- Request to re-evaluate pathology for FH deficient RCC

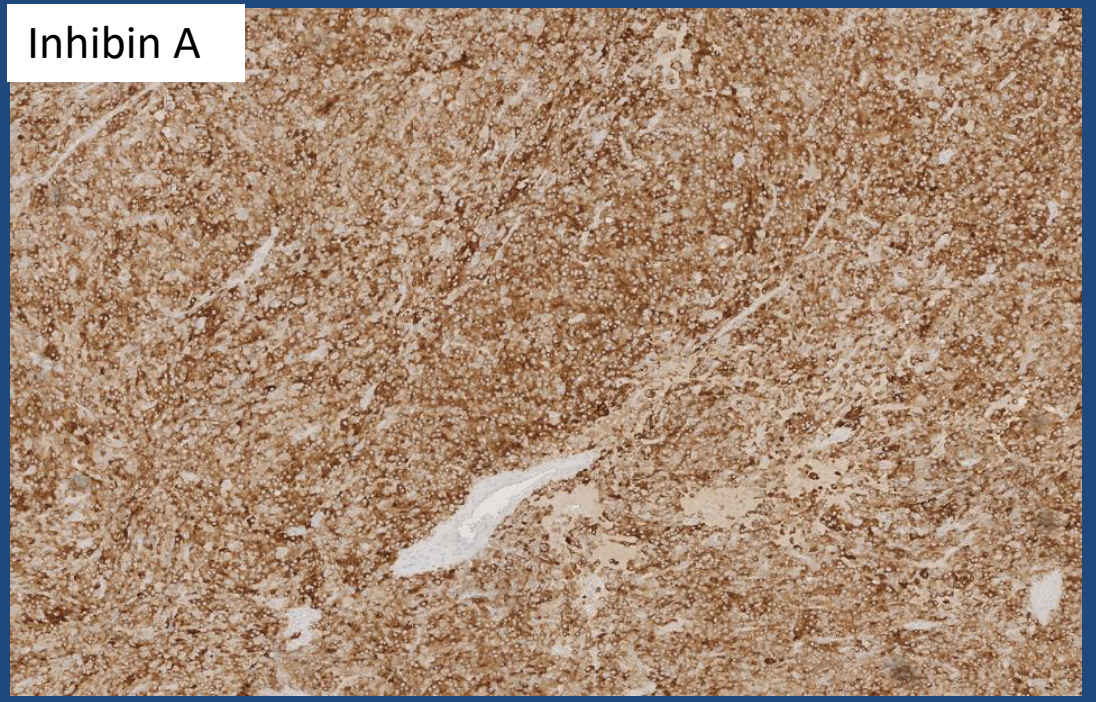
FH IHC



FH alterations also seen in

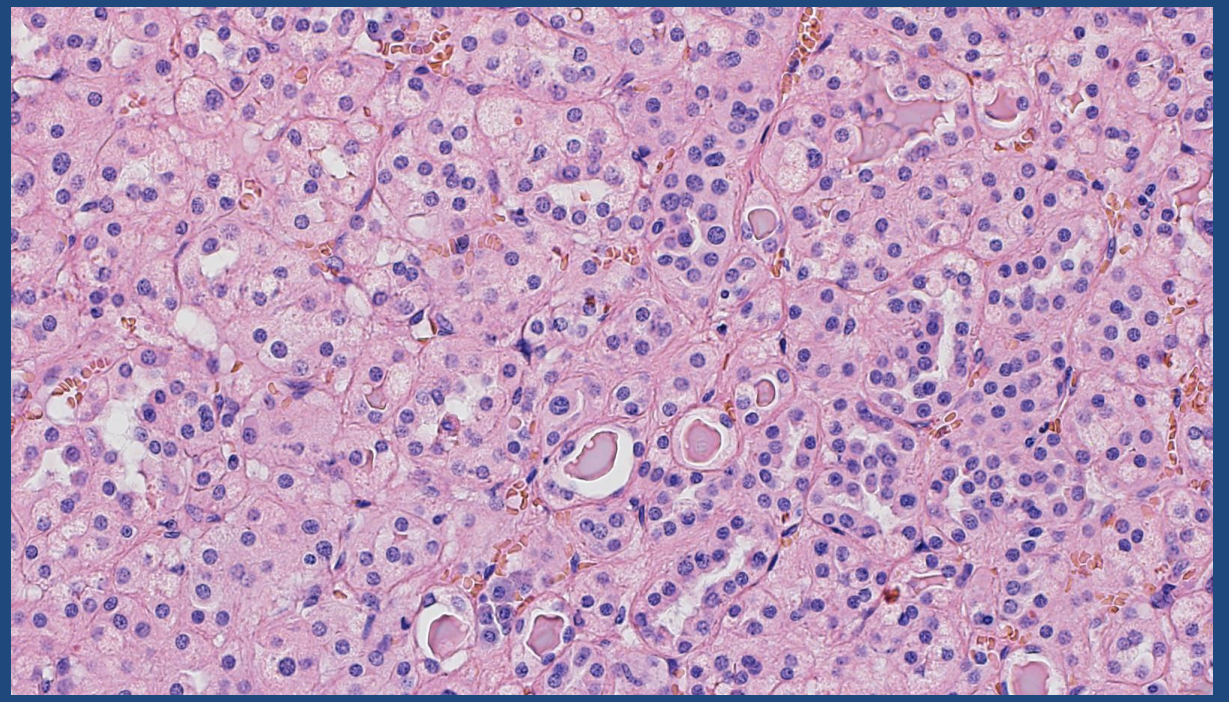
- Leydig cell tumor

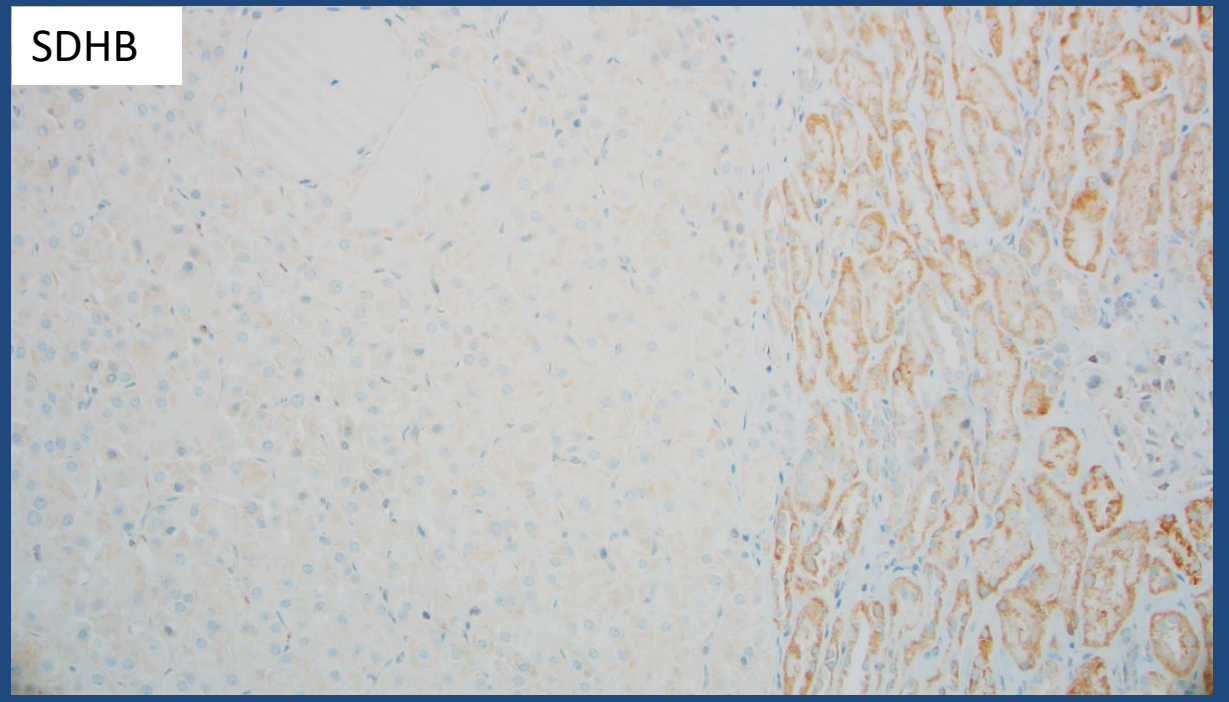




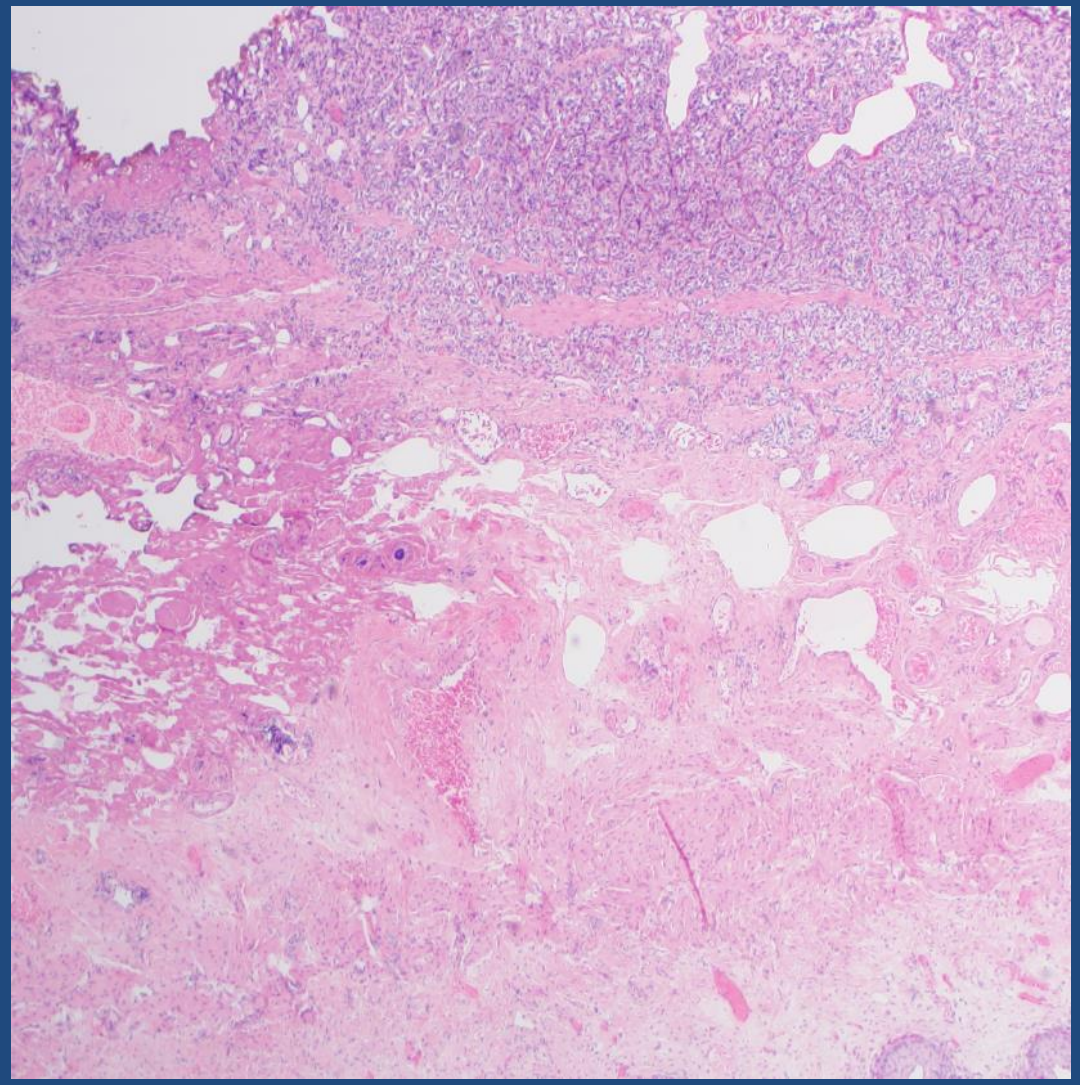
SDHB

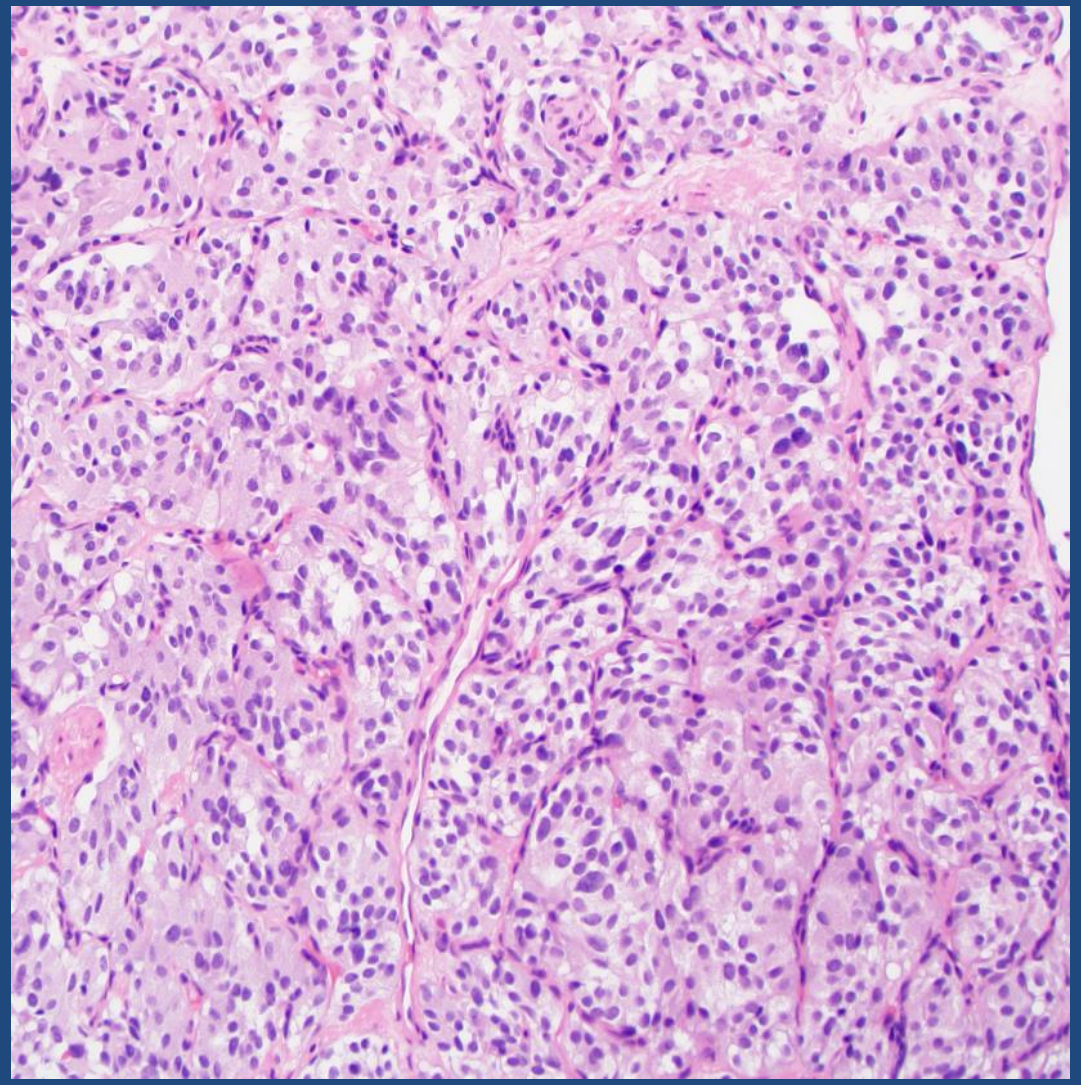
- SDH complex- mitochondrial complex II
  - Four subunits (SDHA, B, C, and D)
- Pheochromocytoma/paraganglioma
- GIST
- Rarely RCC
- SDHB- 80%
- SDHB IHC: screening
  - May not detect SDHA deficient cases
  - SDHD- may show weak staining





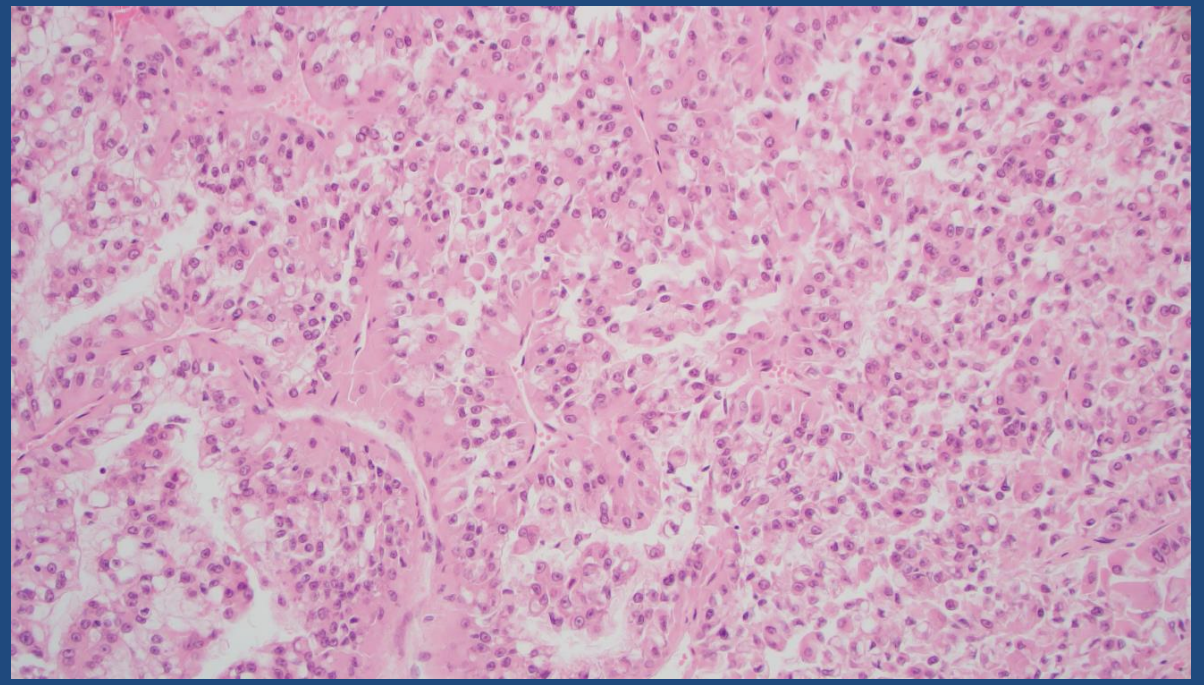
SDH deficiency also seen in….. Paragangliomas

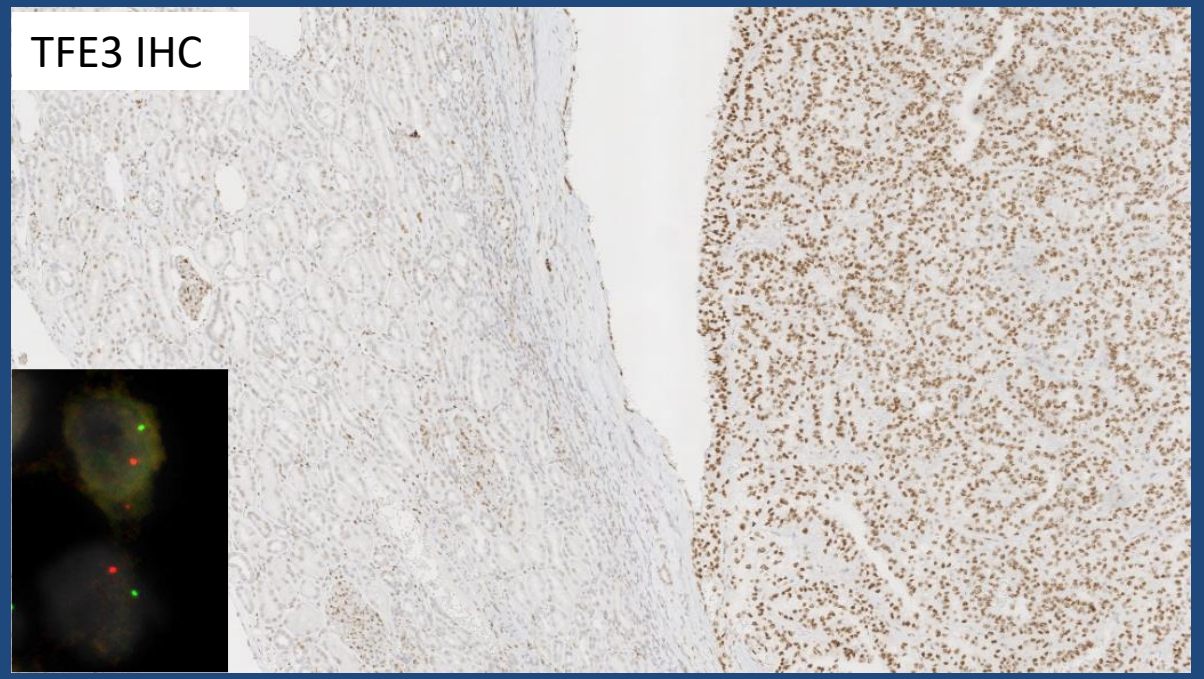




MiT Family Translocation RCC

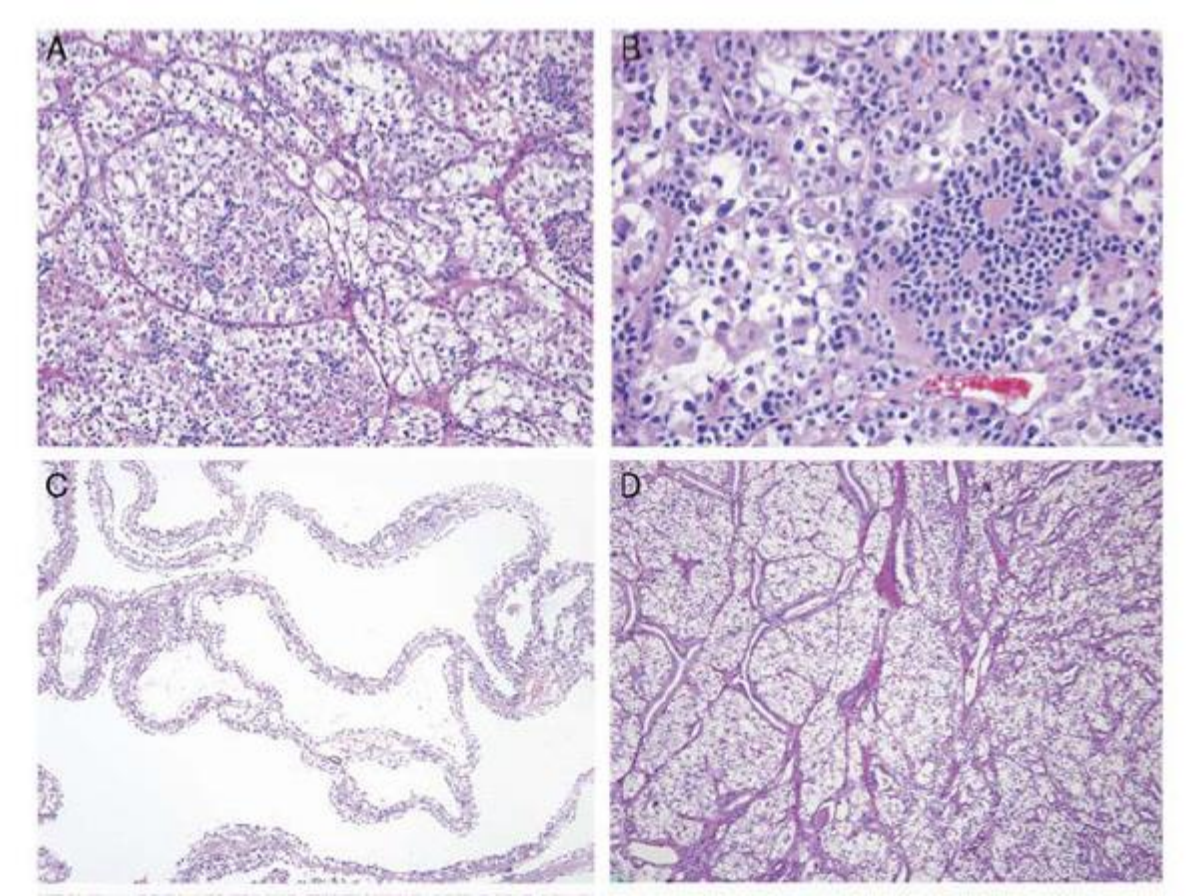
- *TFE3, TFEB, TFEC*, and *MiTF*
- Xp11.2 translocation RCC
  - *TFE3* gene on chromosome Xp11.2
  - Partner genes: *PRCC, ASPL*, others
  - Over-expression of the *TFE3* protein
  - Chimeric *TFE3* fusion protein bind to *MET* promoter





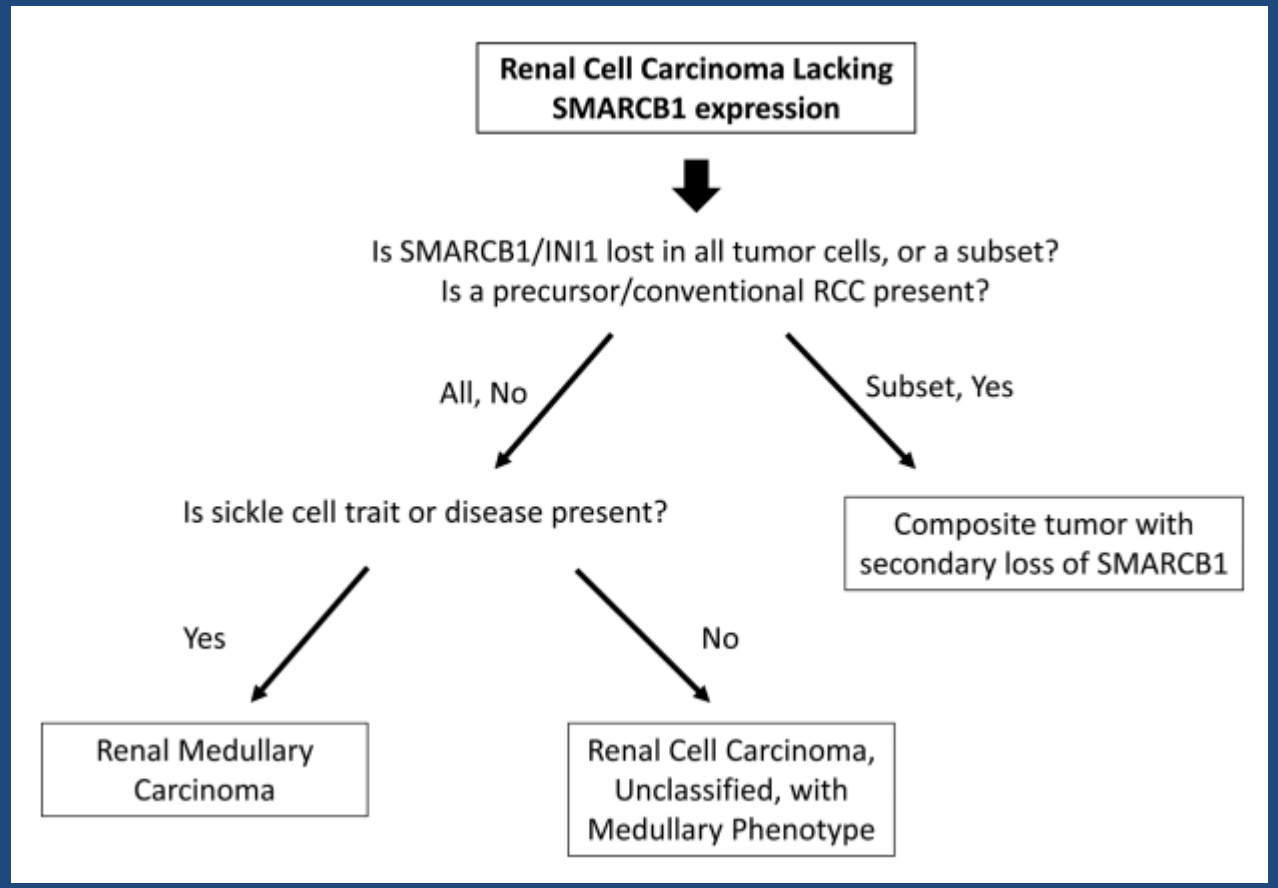
MiT Family Translocation RCC

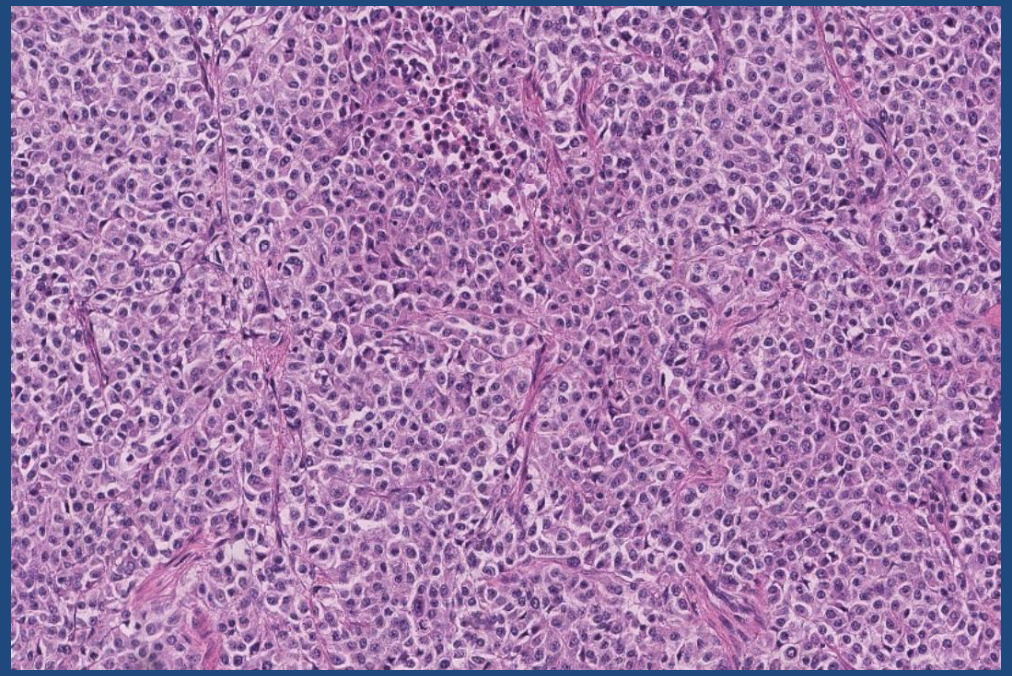
- t(6;11)(p21;q12): *Alpha-TFEB* fusion
  - Over-expression of TFEB transcriptional factor gene
  - TFEB- FISH
- Rare cases with *TFEB* amplification also reported

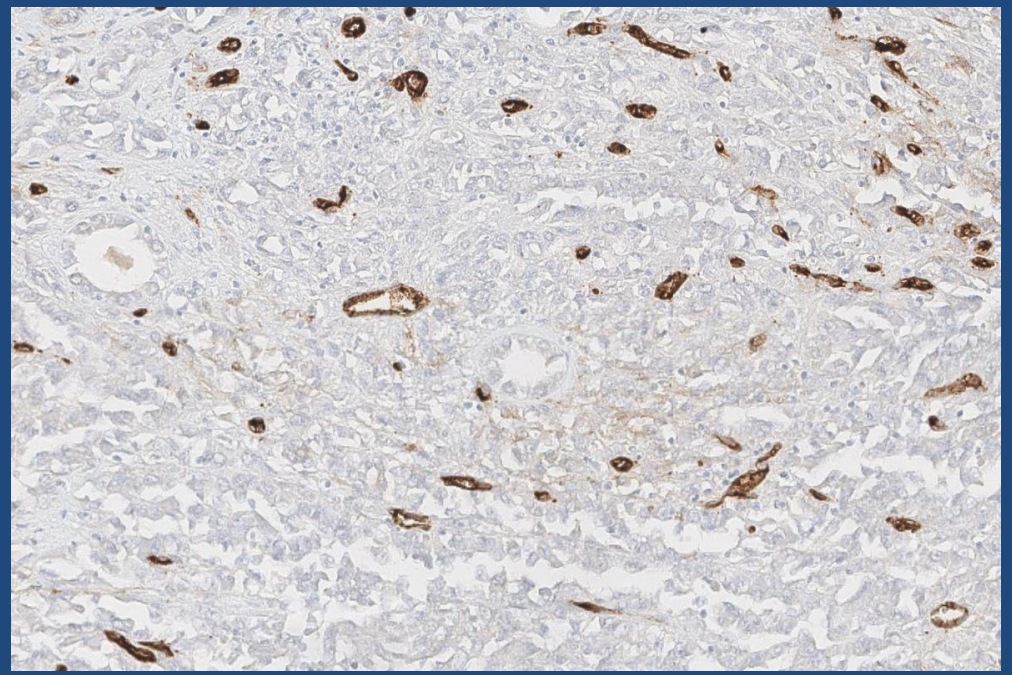


Smith NE et al. Am J Surg Pathol. 2014; 38(5): 604–614
Argami P et al. Am J Surg Pathol. 2016; 40(11): 1484-1495

SMARCB1



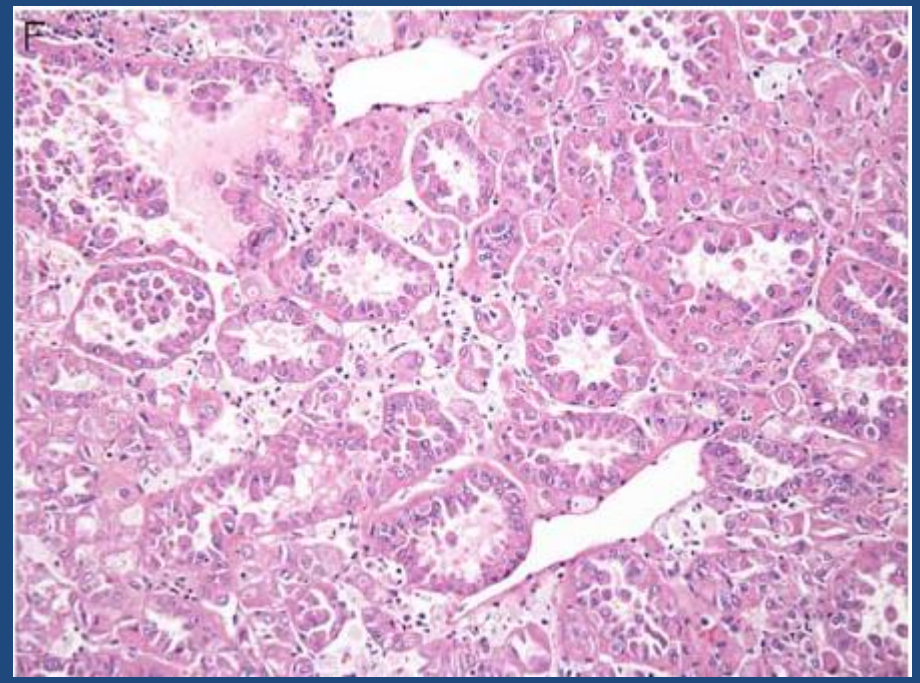


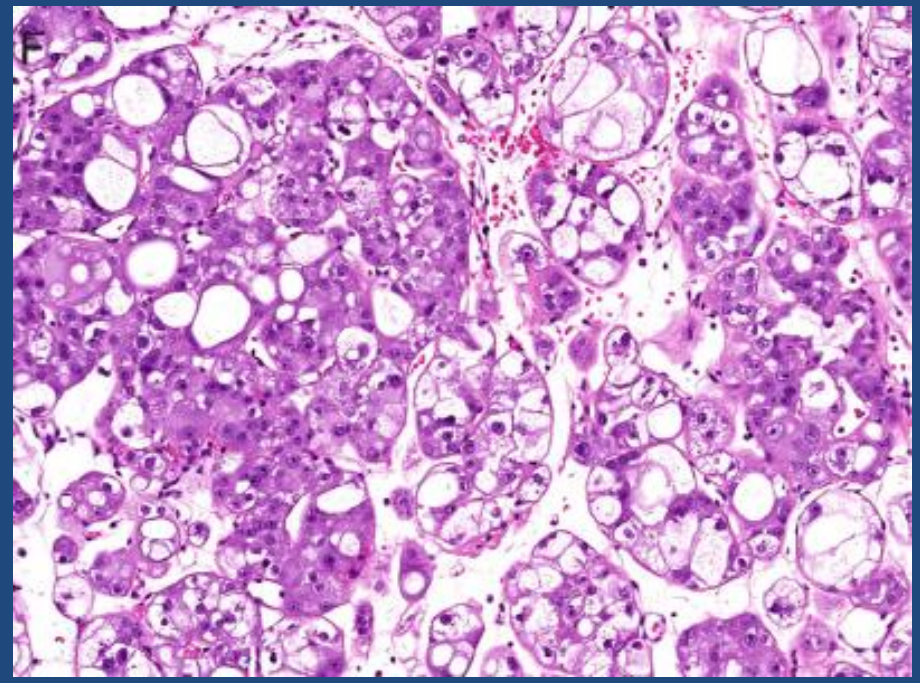


Sirohi D et al. Seminars in Diagnostic Pathology 38(2021):212-221

RCCs with *TSC/MTOR* mutations

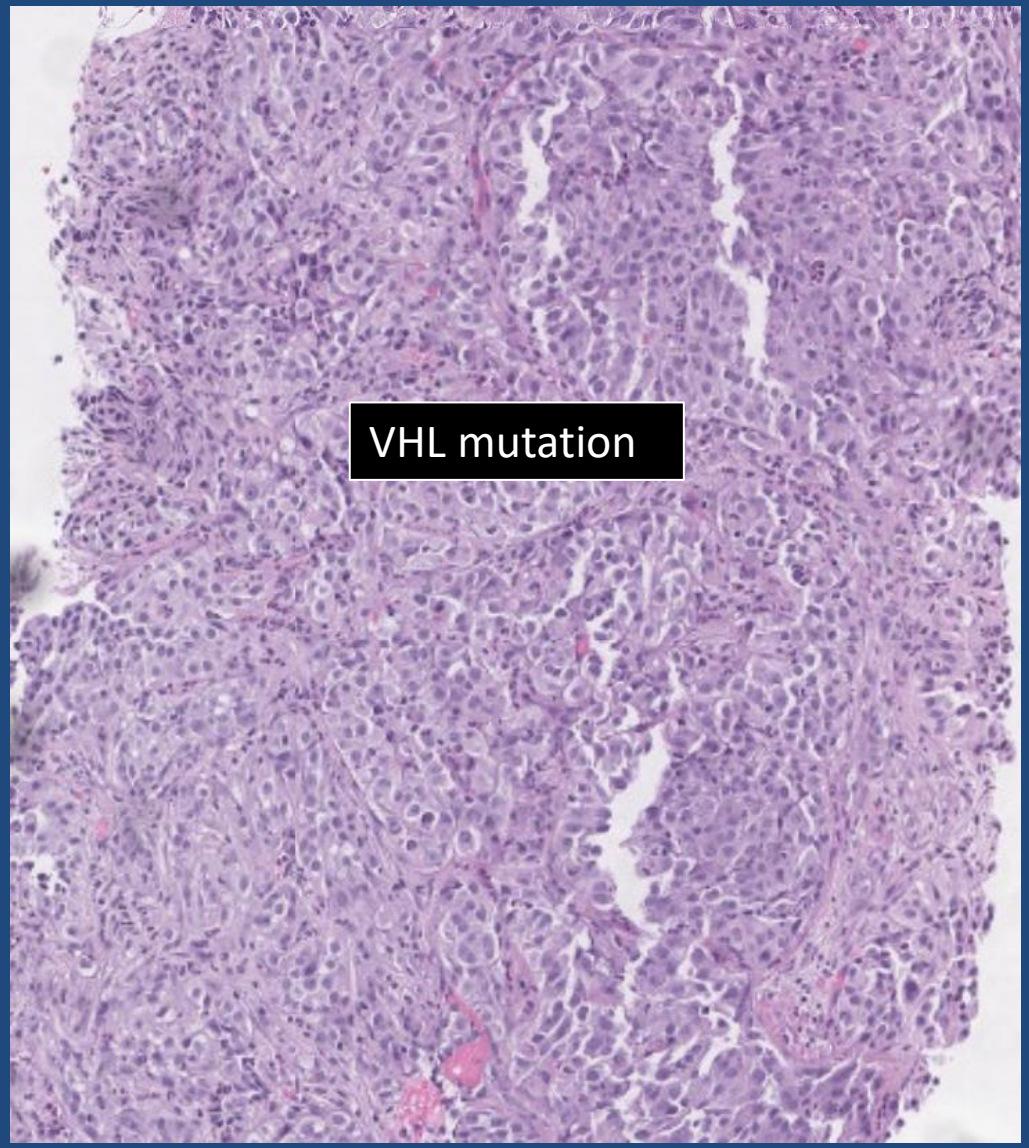
- Renal adenomyomatous tumor
- Eosinophilic solid and cystic RCC
- Eosinophilic and vacuolated tumor

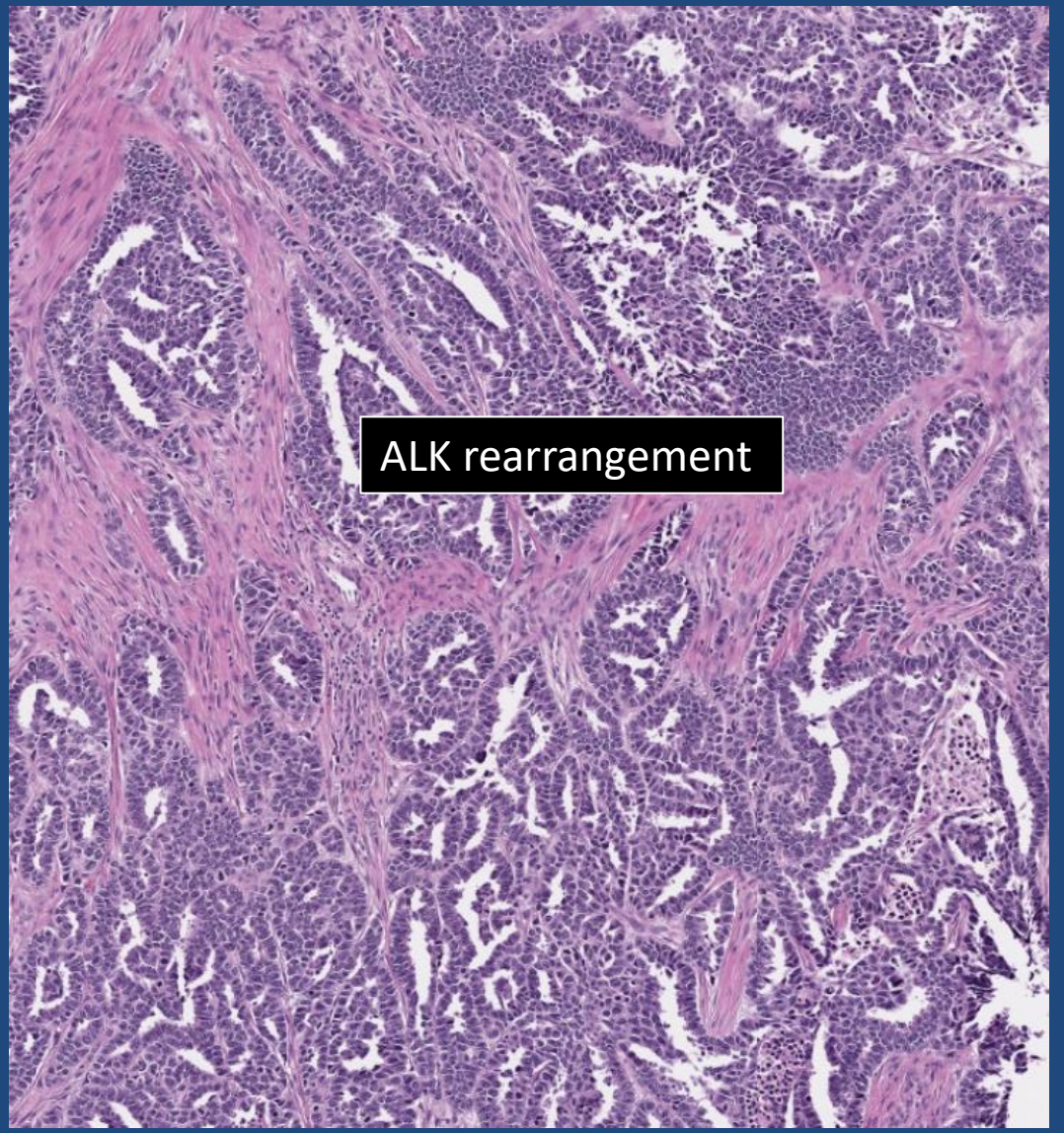




Trpkov et al. Am J Surg Pathol 2017;41:1299–1308
Chen Y et al. Am J Surg Pathol. 2019 January ; 43(1): 121–131

Unclassified RCC





Histopathology	Diagnostic marker	Diagnosis	Significance
Unclassified	VHL p.C77*	ccRCC	Diagnostic, germline HNF1A-risk factor
Favor Sarcomatoid Chromophobe	TP53 p.M237K with LOH	Chromophobe	Diagnostic
Poorly differentiated	TERT promoter, VHL p.I151T	ccRCC	Diagnostic
CDC Vs Urothelial Vs RCC	NF2 p.E530fs- hemizygous	pRCC	Diagnostic
Possible distal nephron origin	BRD4-NUTM1 fusion	NUT midline carcinoma	Diagnostic, therapeutic
Metastatic carcinoma- favor renal primary	FH c.267+1G>T, hemizygous	FH deficient RCC	Diagnostic, germline ATM
Unclassified	None	Unclassified	Unclassified
Type II pRCC	TFE3-PRCC fusion	TFE3-RCC	Diagnostic
Unclassified	Somatic- TSC2 c.3132-2A>G Germline TSC2 p.A614D	TSC-RCC	Diagnostic
Unclassified	TFE3-PRCC fusion	TFE3-RCC	Diagnostic

Aggressive Unclassified RCCs

55%	45%

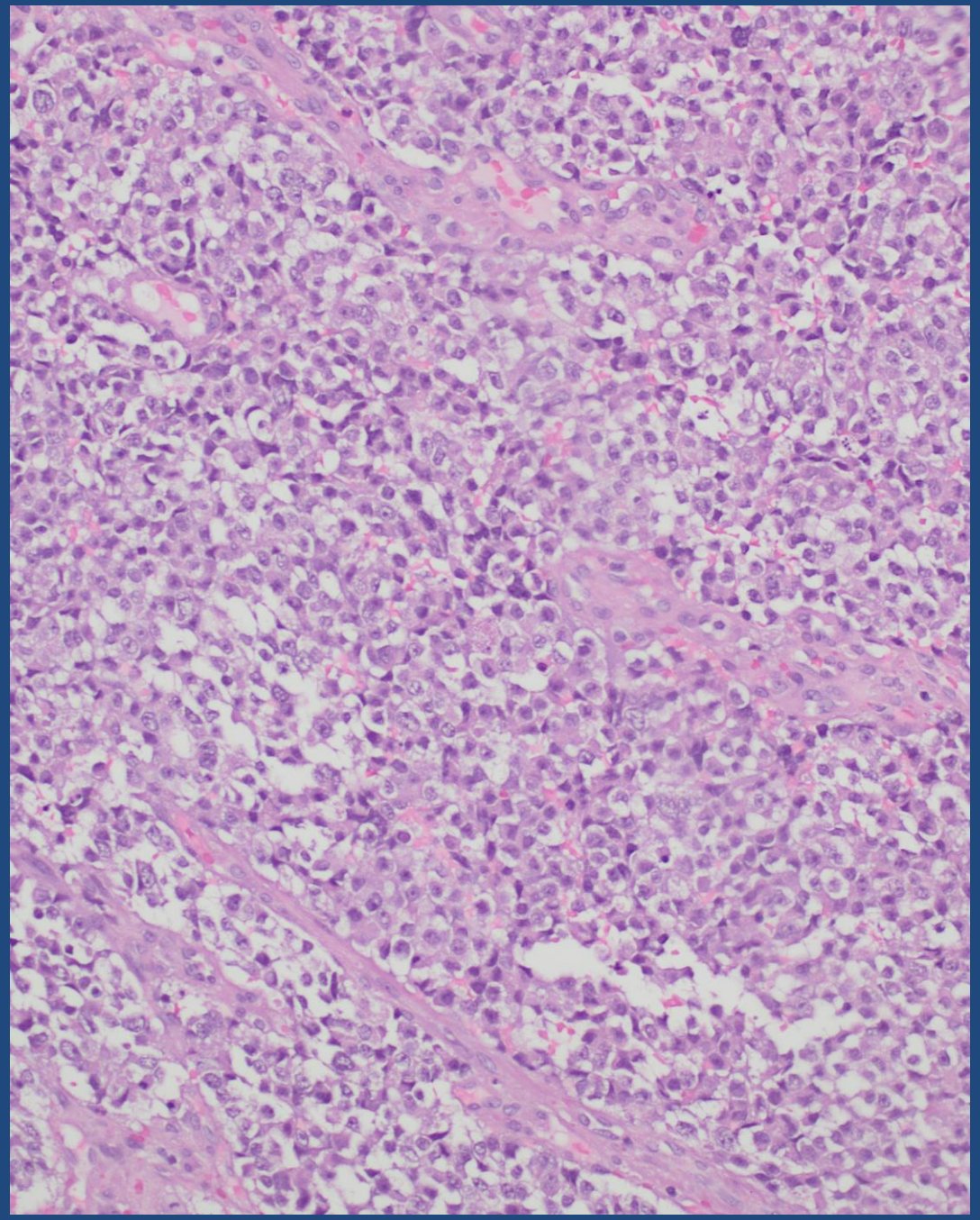
• NF2 loss and dysregulated Hippo–YAP signaling (18%)
  • Worse outcomes
• Hyperactive mTORC1 signaling (26%)
  • Better outcomes, therapeutic target
  • MTOR, TSC1, TSC2, PTEN
• FH: worse outcomes
• ALK
	• Chromatin modulation (13%)
	  • Intermediate outcomes
	  • (SETD2, BAP1, KMT2A/C/D, PBRM1)
	• DNA damage response (8%)
	  • (TP53, CHEK2, BRCA2)
	• No recurrent molecular features (24%)

Chen Y-B et al. Nat Commun. 2016;7:13131

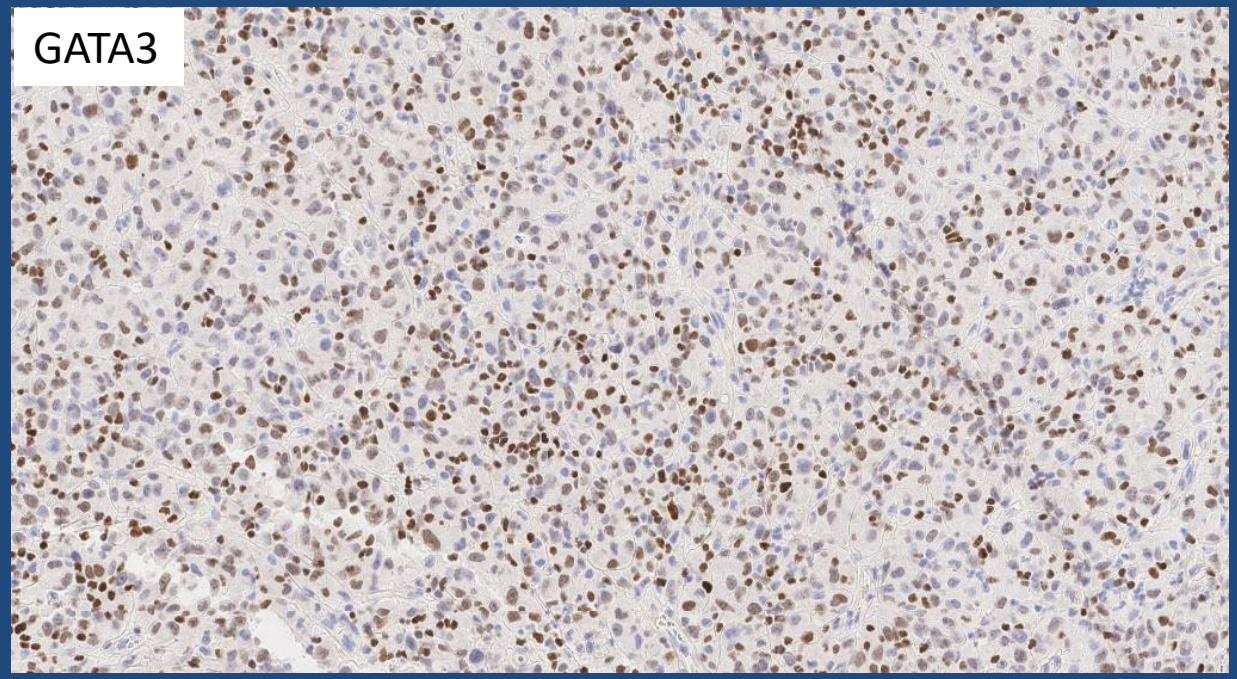
Bladder and Prostate

Case 4

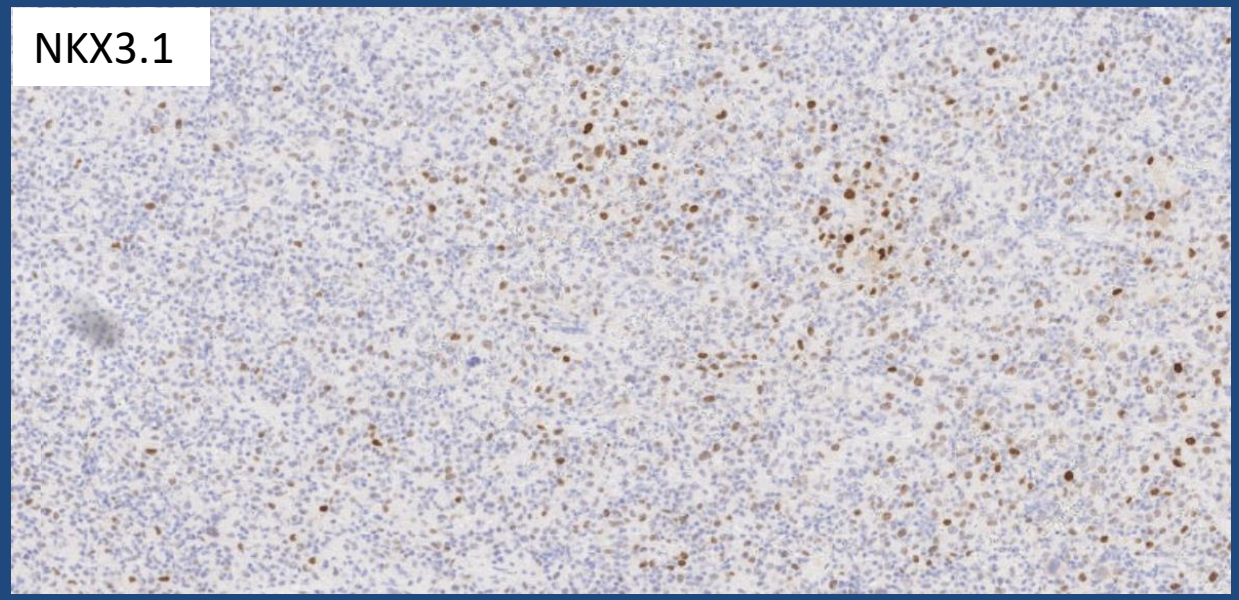
- 64 y M
- History of prostate carcinoma, status post radiation
- Bladder mass
- Transurethral resection

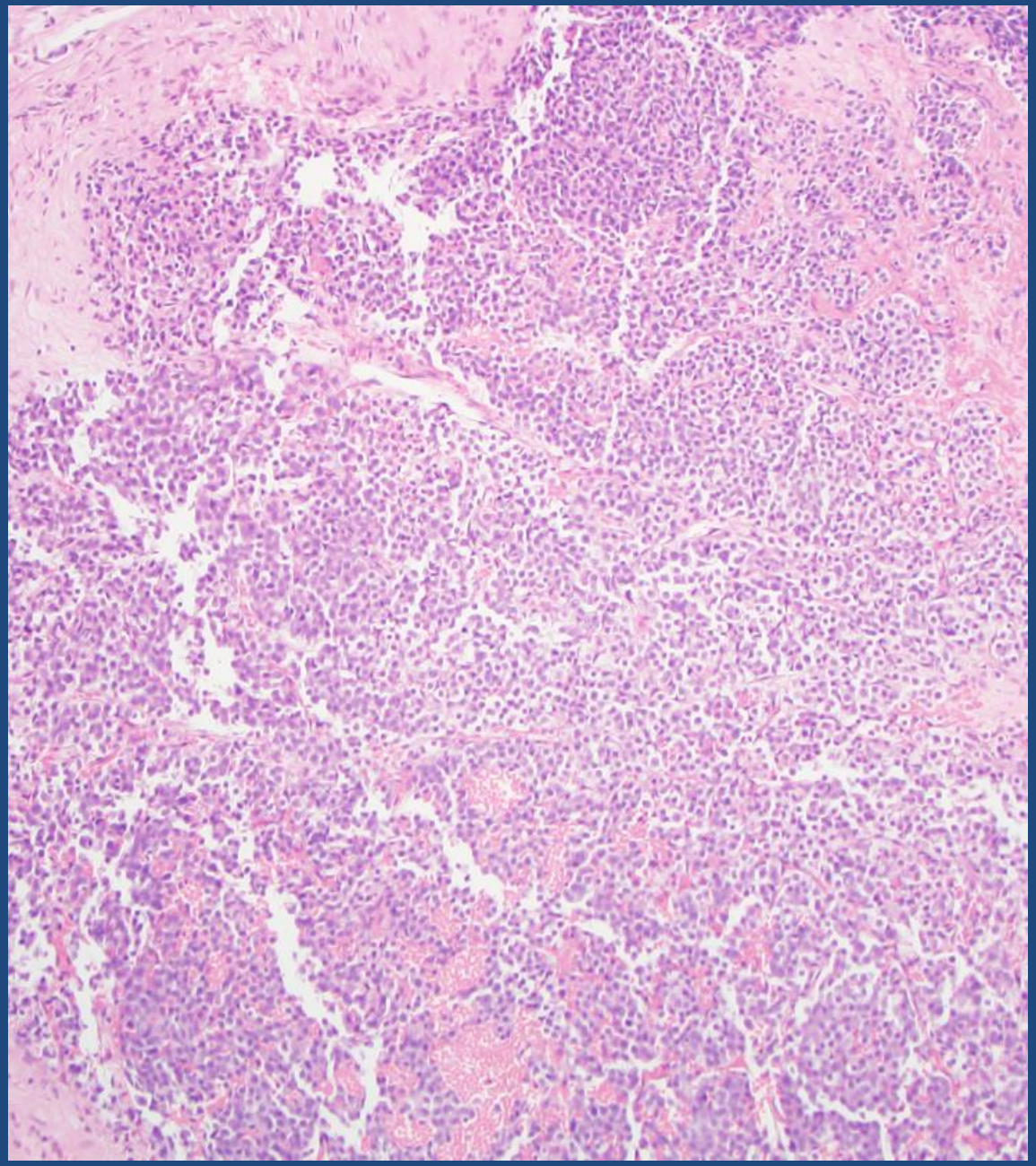


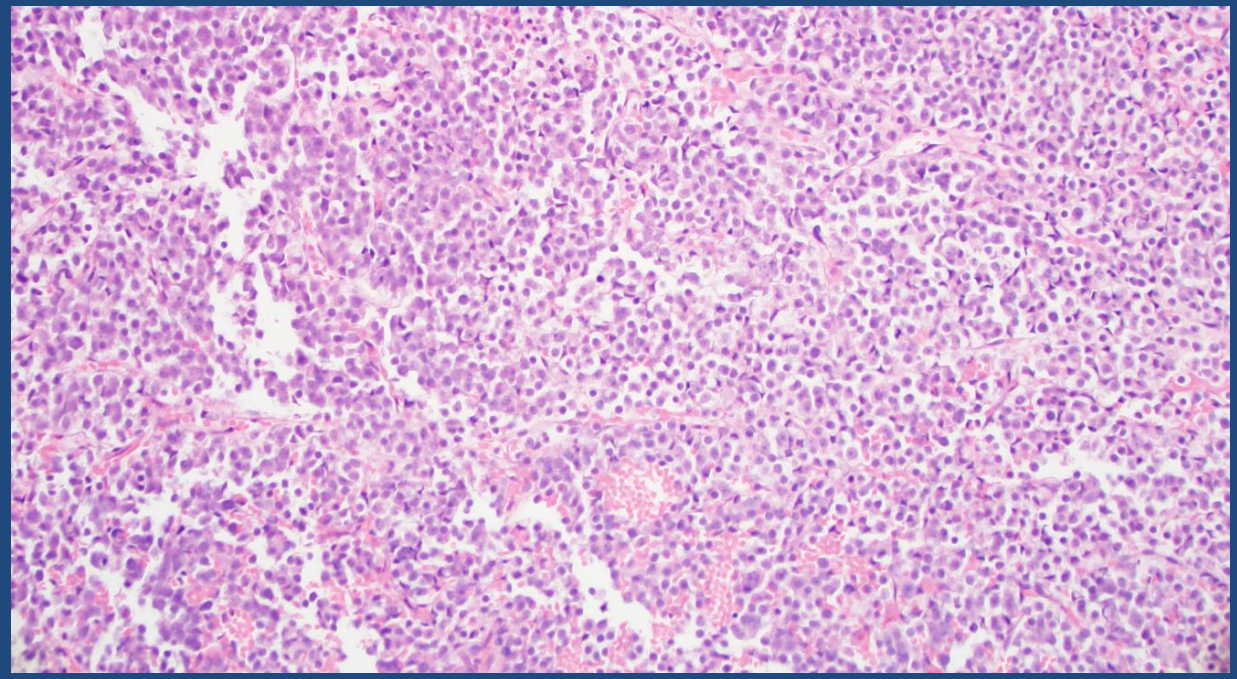
GATA3

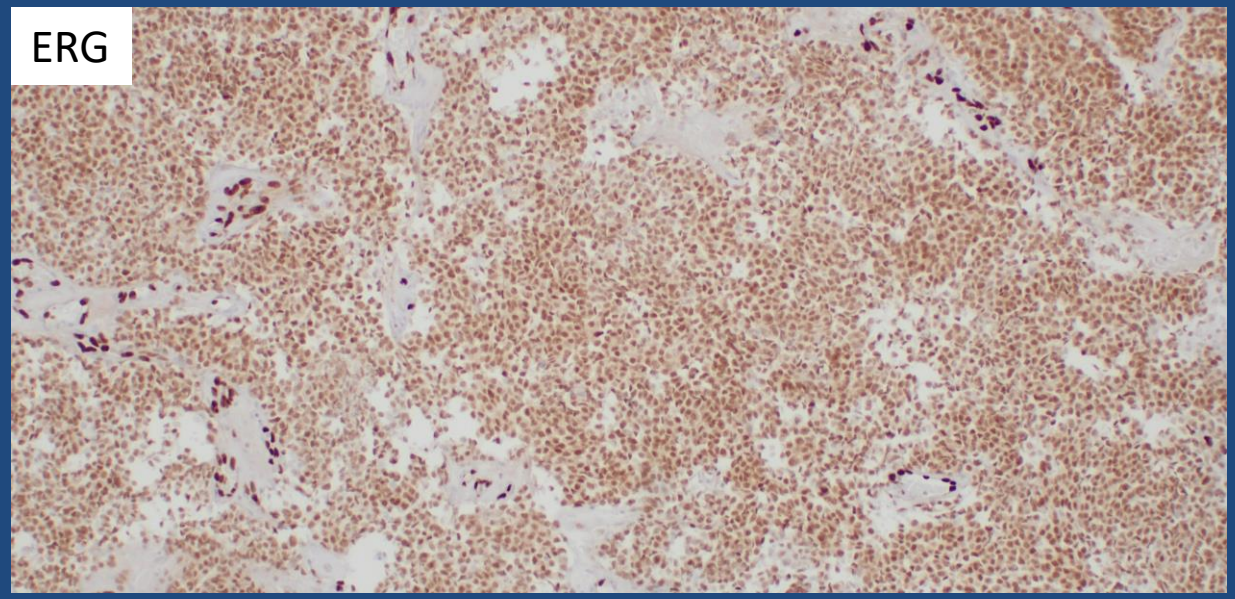


NKX3.1








ERG

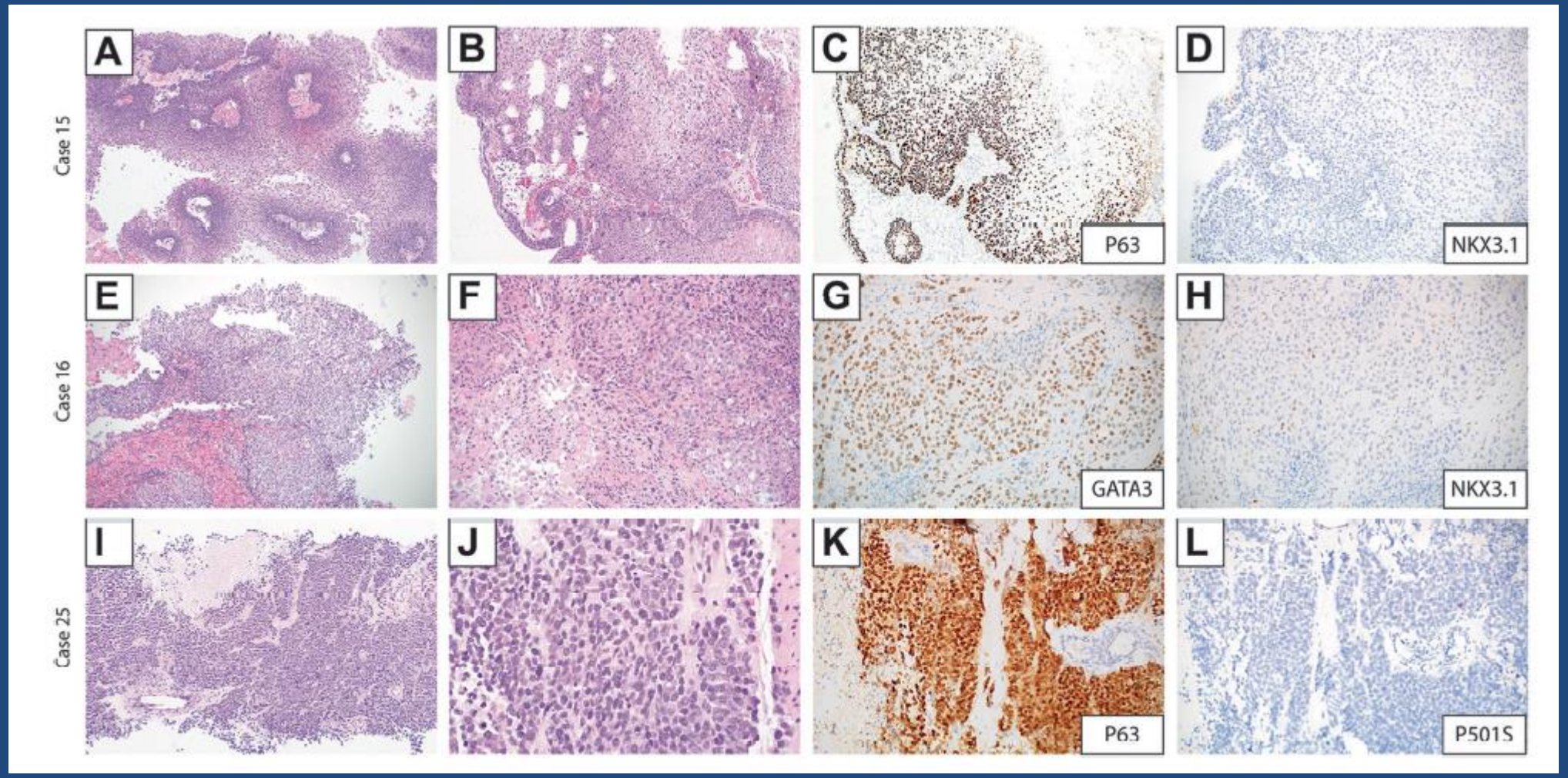
Case 4

- Genetic testing: *TMPRSS2-ERG* rearrangement
- Diagnostic of prostate carcinoma

ERG

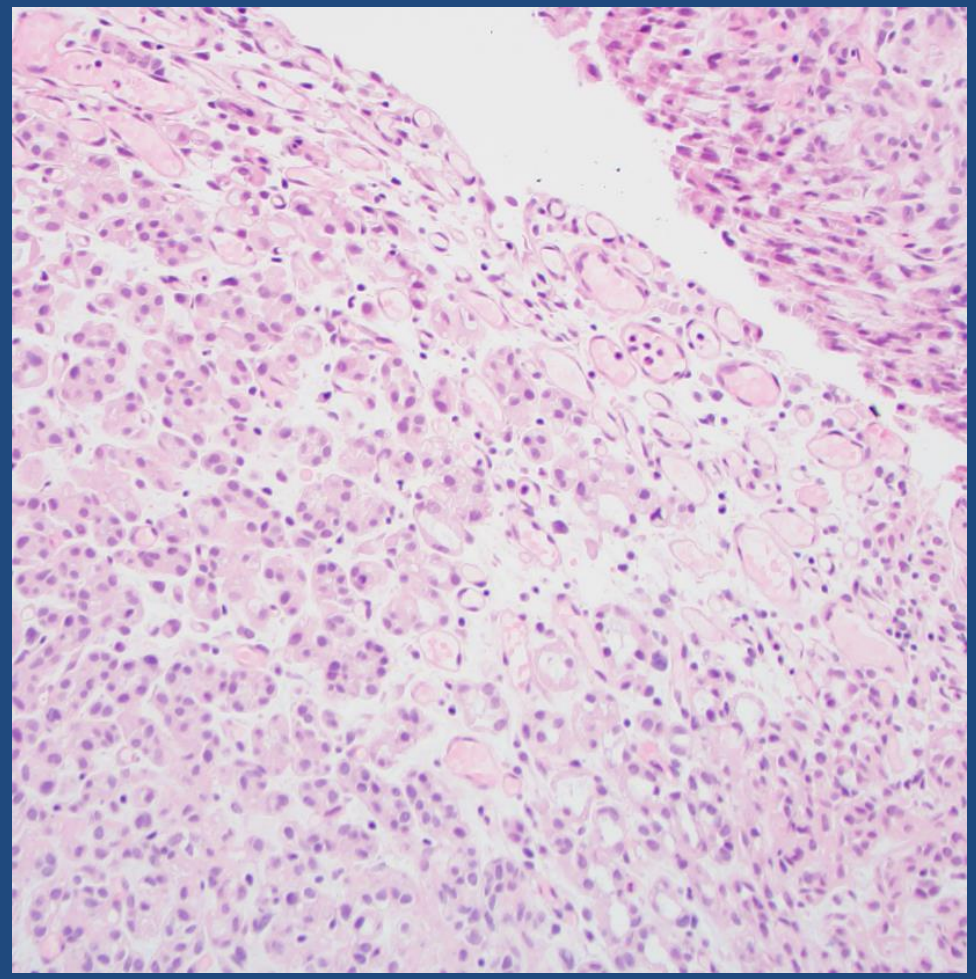
- *ERG* rearrangements in ~50% of prostate carcinoma
- Also seen in high grade prostatic intraepithelial neoplasia
- In Castration resistant prostate carcinoma (CRPC)- protein expression may be reduced/absent
- Testing methods: IHC, FISH, sequencing

IHC and Molecular Discordances

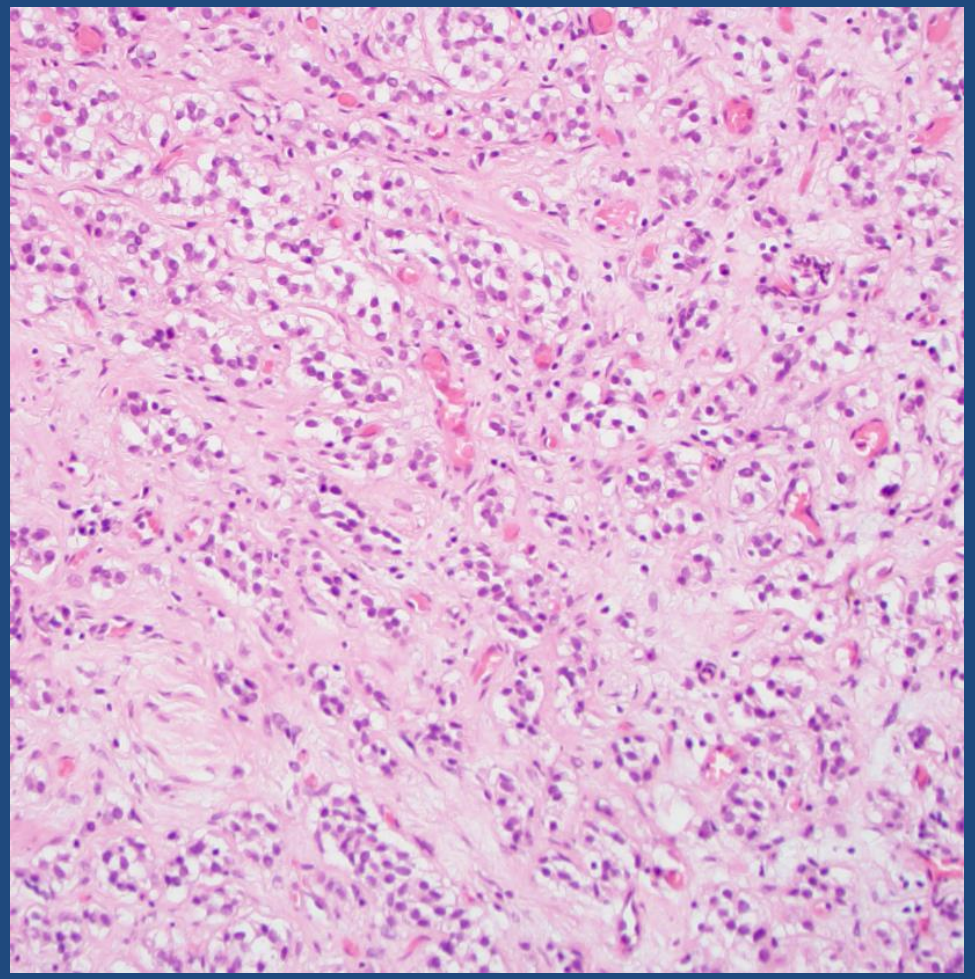


Chen E at al. Mod Pathol 2020;33(9):1802-1810

Bladder: Reactive Vs Neoplastic



Nephrogenic Adenoma



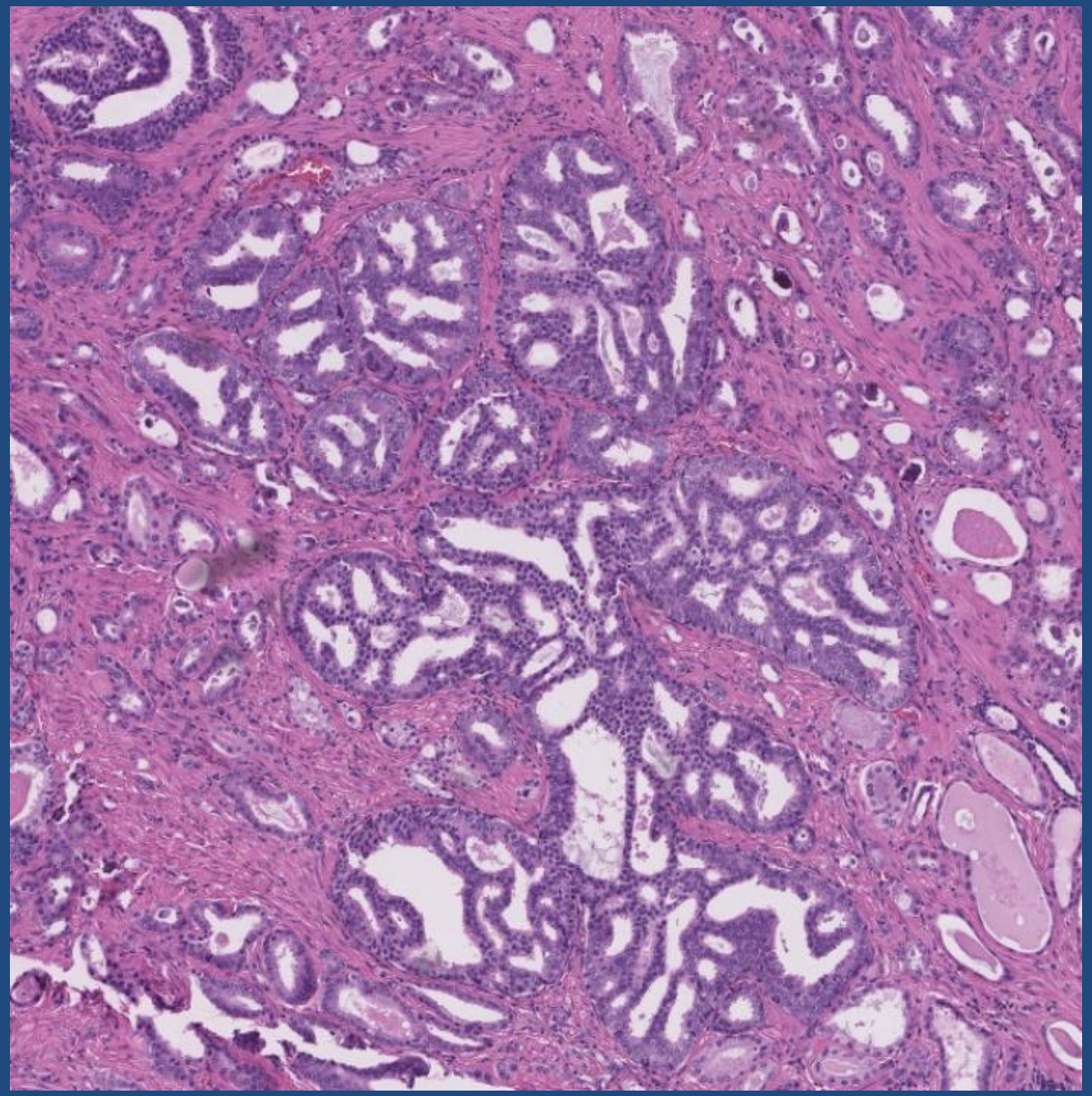
Urothelial carcinoma, nested variant

Bladder: Reactive Vs Neoplastic

- *TERT* promoter mutations
  - 60-80% of urothelial carcinomas
  - Early event
  - Seen in both benign and malignant urothelial neoplasms
- Rare in prostate carcinoma
- Utility
  - Reactive vs neoplastic
  - Urothelial carcinoma vs prostate carcinoma
  - Urine screening for disease recurrence

Case 5

- 65 y M
- 5/2021: Presented with 9 months history of progressive left hip pain
  - Imaging: 6 cm prostate, destructive femoral lesion
  - Left femur biopsy



Case 5

- Treatment: Bicalutamide
- 8/2021: Pulmonary nodules
  - Tumor genetic testing: *BRCA2* copy number loss
- 10/2021: clinical trial for DDR mutated gene
- 12/2021: improving pelvic adenopathy

Somatic Tumor Testing: NCCN Recommendations

- Purpose
  - Treatment decisions
  - Genetic counseling
  - Clinical trial eligibility
- Testing recommendations
  - Metastatic/regional cancer
    - HRD genes: *BRCA1, BRCA2, ATM, PALB2,FANCA, RAD51D, CHEK2, CDK12*
  - Metastatic CRPC, castration naïve metastatic/regional cancer
    - Microsatellite instability
  - Metastatic CRPC
    - Tumor mutational burden

NCCN Guidelines Prostate Version 3.2022

Germline testing: NCCN Recommendations

PRINCIPLES OF GENETICS AND MOLECULAR/BIOMARKER ANALYSIS

Germline testing is recommended *in patients with a personal history of prostate cancer* in the following scenarios:

- By Prostate Cancer Stage or Risk Group (diagnosed at any age)
  - Metastatic, regional (node positive), very-high risk localized, high-risk localized prostate cancer
- By Family History[a] and/or Ancestry
  - ≥1 first-, second-, or third-degree relative with:
    - ◊ breast cancer at age ≤50 y
    - ◊ colorectal or endometrial cancer at age ≤50 y
    - ◊ male breast cancer at any age
    - ◊ ovarian cancer at any age
    - ◊ exocrine pancreatic cancer at any age
    - ◊ metastatic, regional, very-high-risk, high-risk prostate cancer at any age
  - ≥1 first-degree relative (father or brother) with:
    - ◊ prostate cancer[b] at age ≤60 y
  - ≥2 first-, second-, or third-degree relatives with:
    - ◊ breast cancer at any age
    - ◊ prostate cancer[b] at any age
  - ≥3 first- or second-degree relatives with:
    - ◊ Lynch syndrome-related cancers, especially if diagnosed <50 y: colorectal, endometrial, gastric, ovarian, exocrine pancreas, upper tract urothelial, glioblastoma, biliary tract, and small intestinal cancer
  - A known family history of familial cancer risk mutation (pathogenic/likely pathogenic variants), especially in: *BRCA1, BRCA2, ATM, PALB2, CHEK2, MLH1, MSH2, MSH6, PMS2, EPCAM*
  - Ashkenazi Jewish ancestry
- Personal history of breast cancer

Germline testing may be considered *in patients with a personal history of prostate cancer* in the following scenarios:

- By Prostate Cancer Tumor Characteristics (diagnosed at any age)
  - ◊ intermediate-risk prostate cancer with intraductal/cribriform histology[c]
- By prostate cancer[b] AND a prior personal history of any of the following cancers:
  - ◊ exocrine pancreatic, colorectal, gastric, melanoma, pancreatic, upper tract urothelial, glioblastoma, biliary tract, and small intestinal

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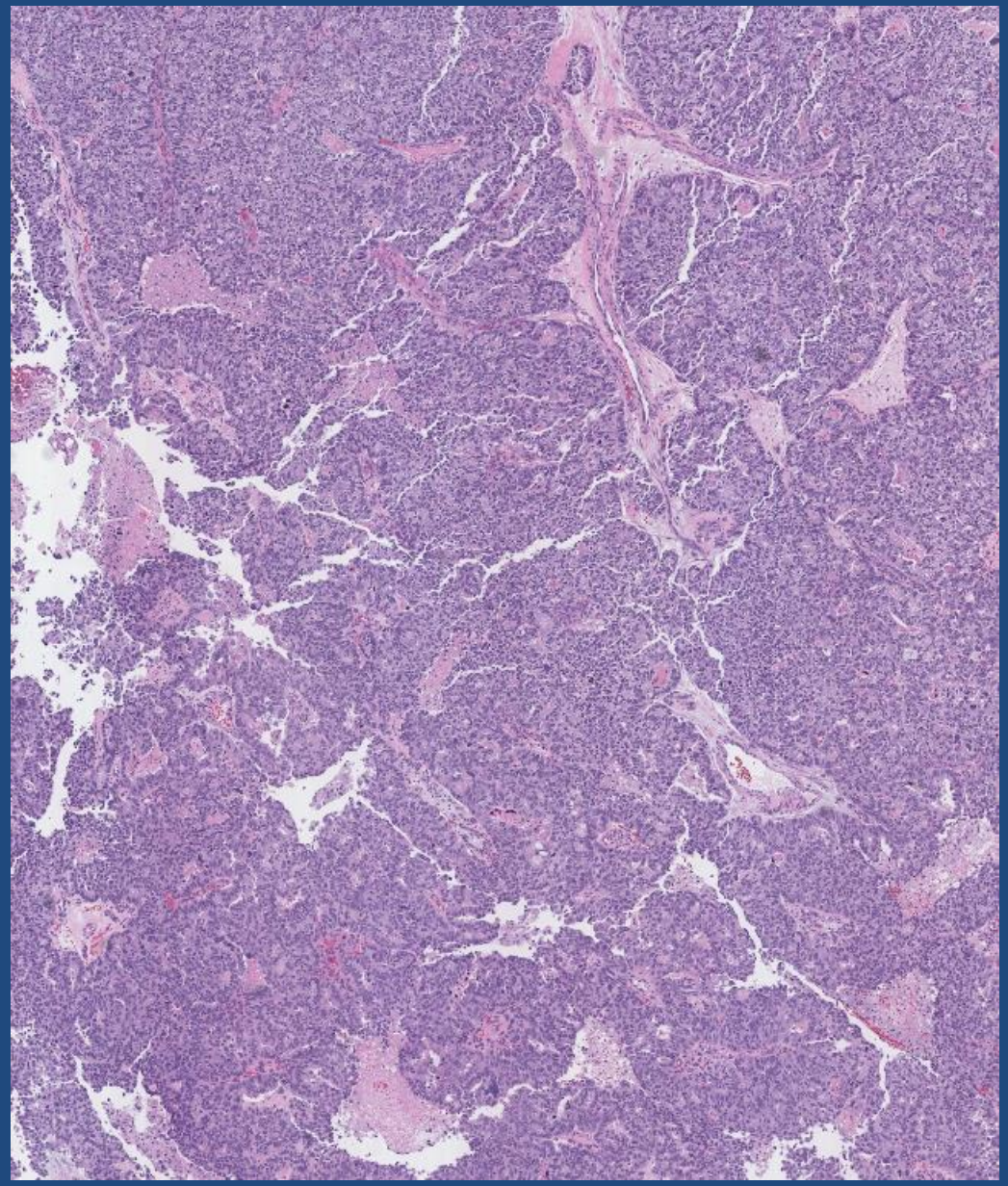
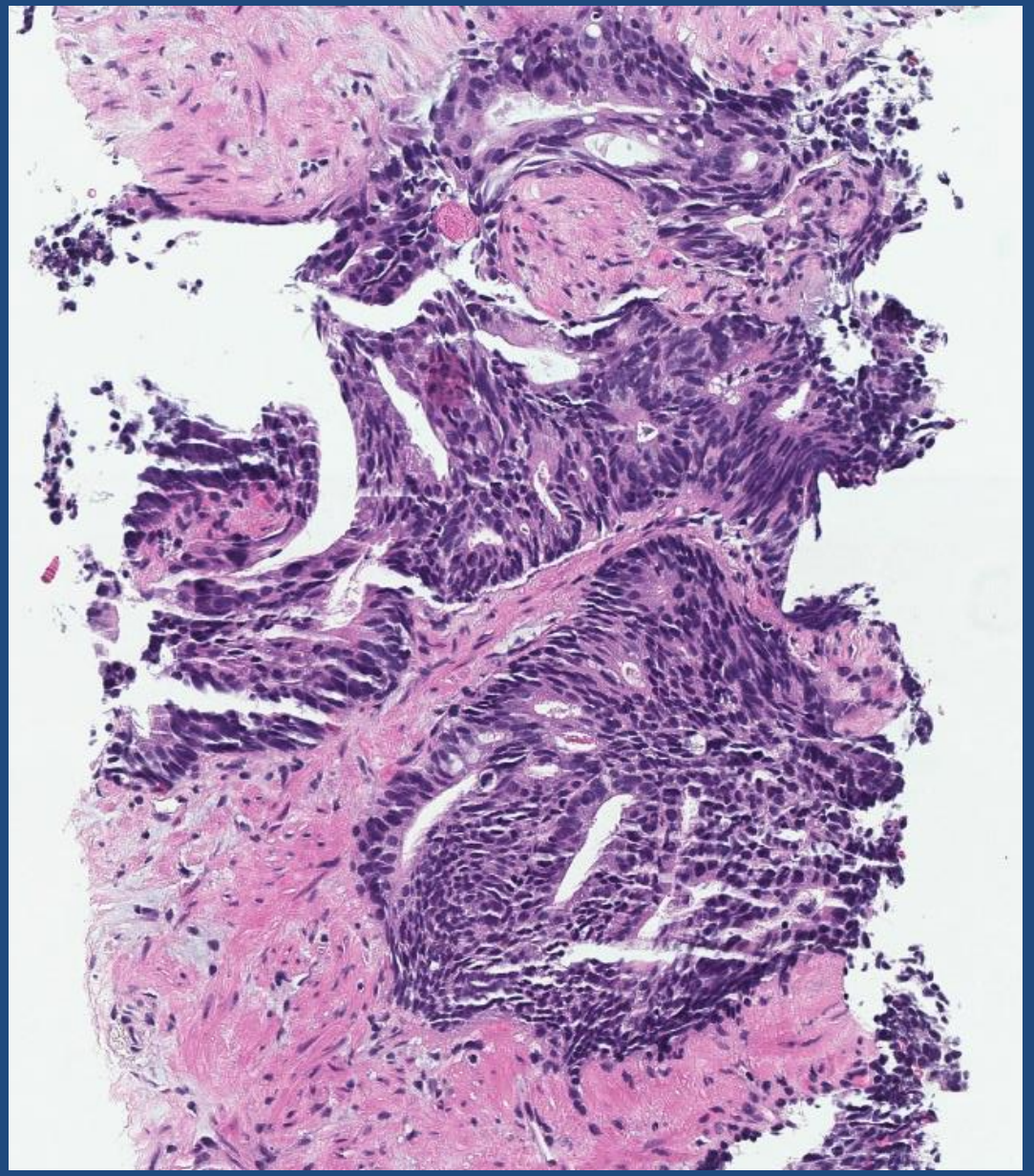




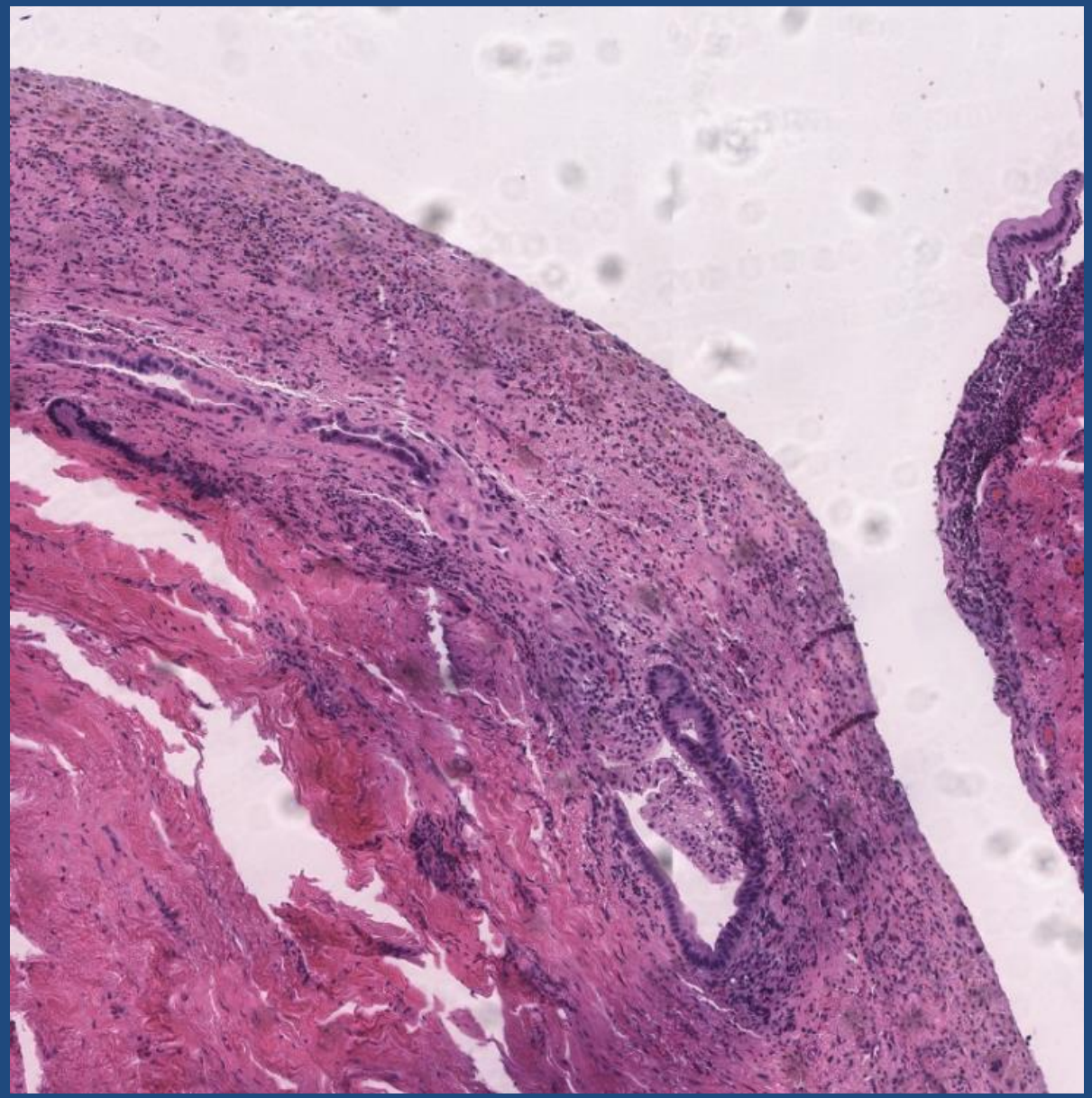
BRCA alterations: more frequent in ductal and cribriform histology

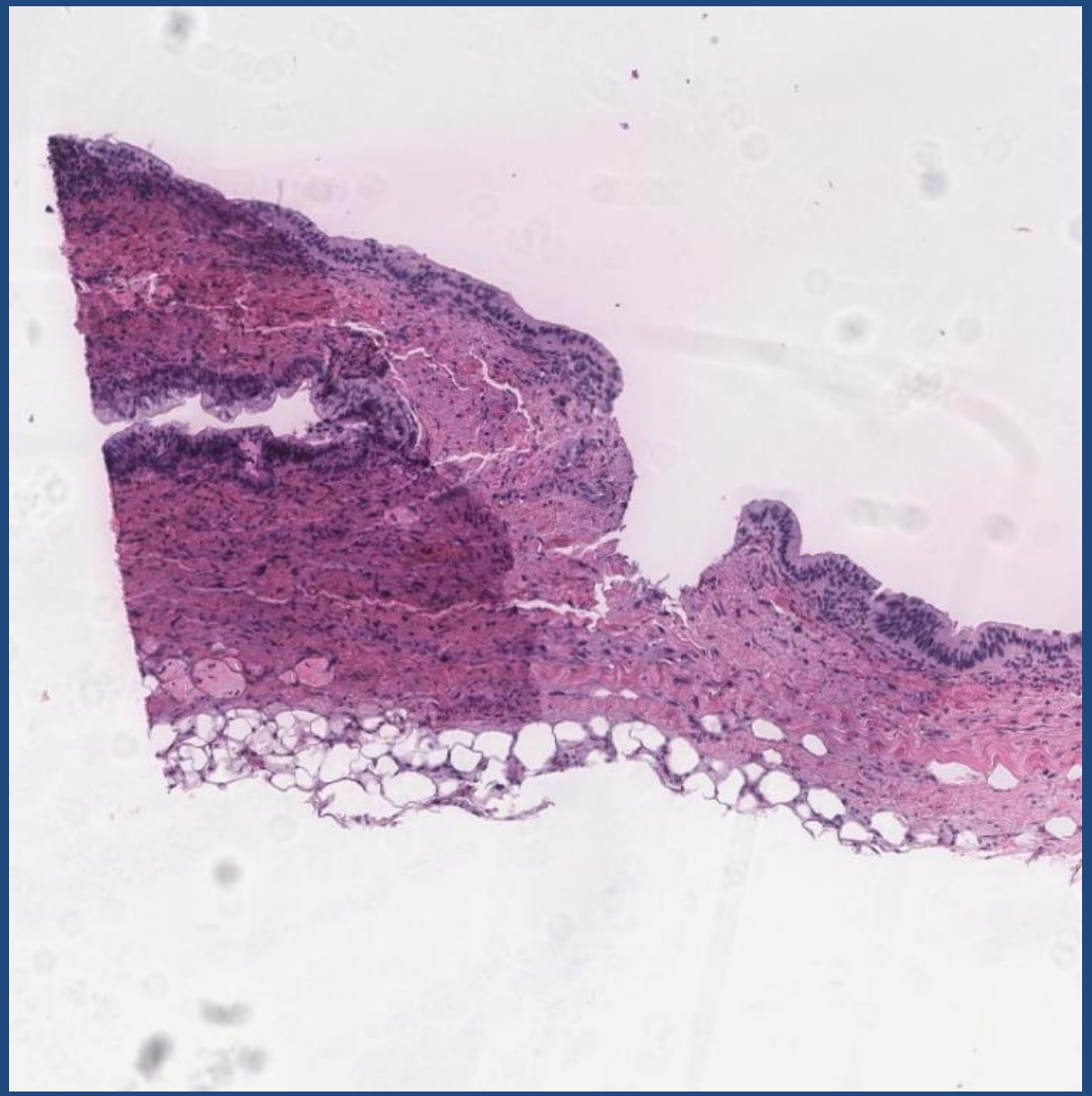
Velho PI et al. Prostate. 2018;78(5):401-407

Testicular Tumors

Case 6

- 39 y M
- 2/2019:
  - Abdominal pain while on a vacation
  - CT scan at ED: 12 cm retroperitoneal mass
  - Testicular exam: negative
  - Elevated AFP
  - Biopsy: Yolk Sac Tumor
  - Bleomycin, etoposide, cisplatinum
- 6/2019: residual disease
  - Retroperitoneal mass excision



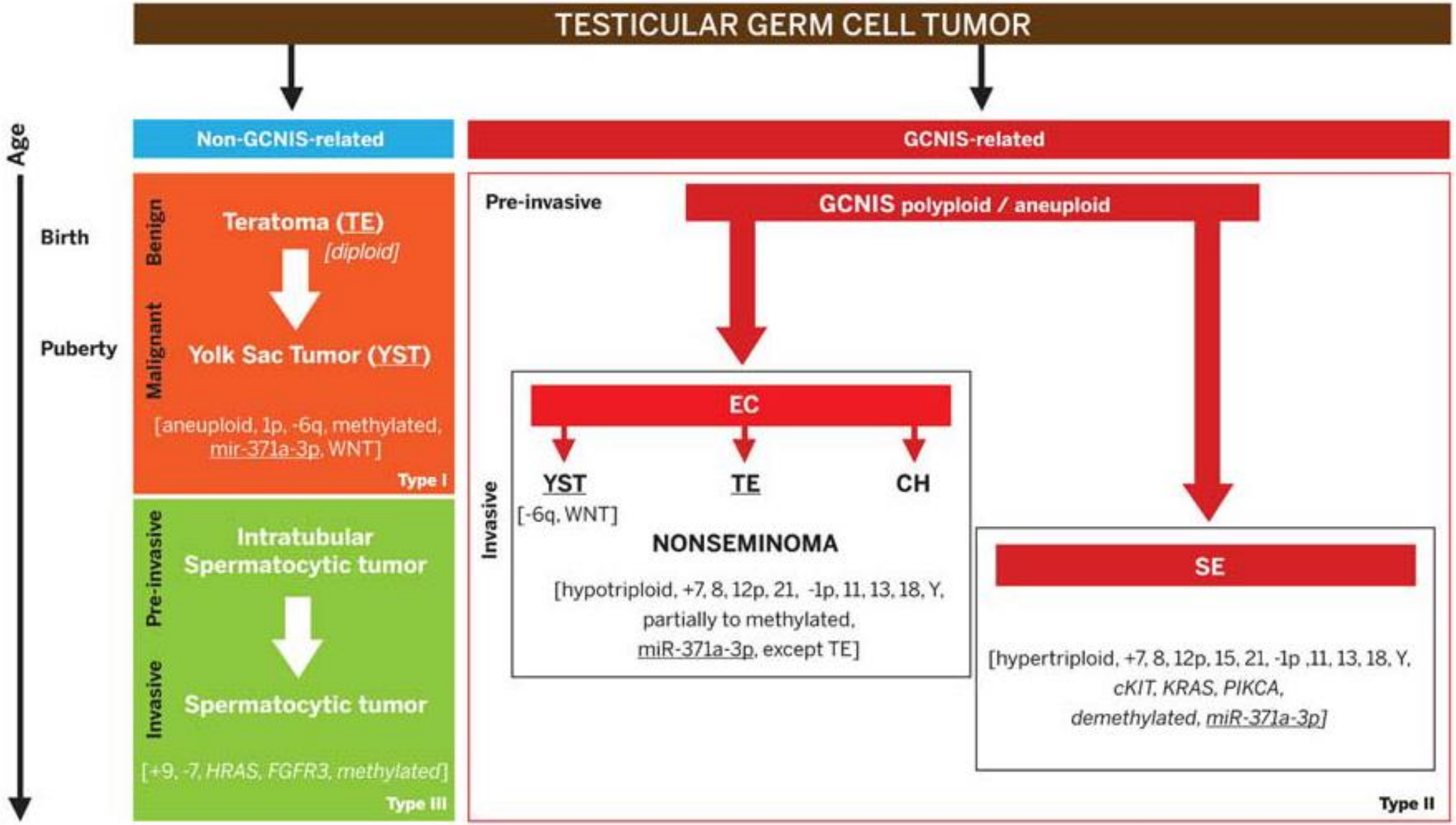


Case 6

- SALL4: focally positive
- Cyst with enteric lining compatible with residual teratoma
- Isochromosome 12p (FISH): Positive

Isochromosome 12p

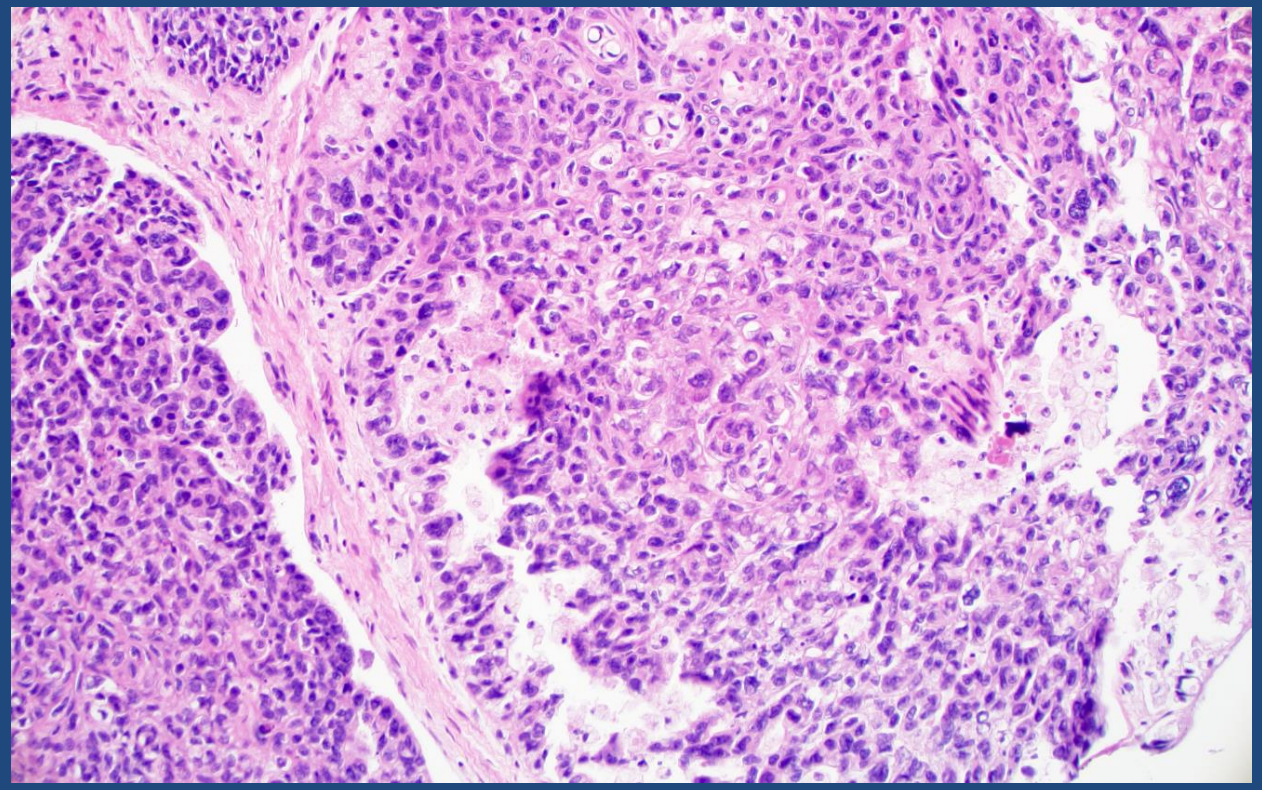
- Present in ~80% of germ cell tumors
- Testing methods: FISH, sequencing, genomic array
- Utility
  - Prepubertal vs postpubertal teratoma
  - Characterizing somatic type malignancy as of germ cell origin
- Also seen in ovarian and extragonadal germ cell tumors

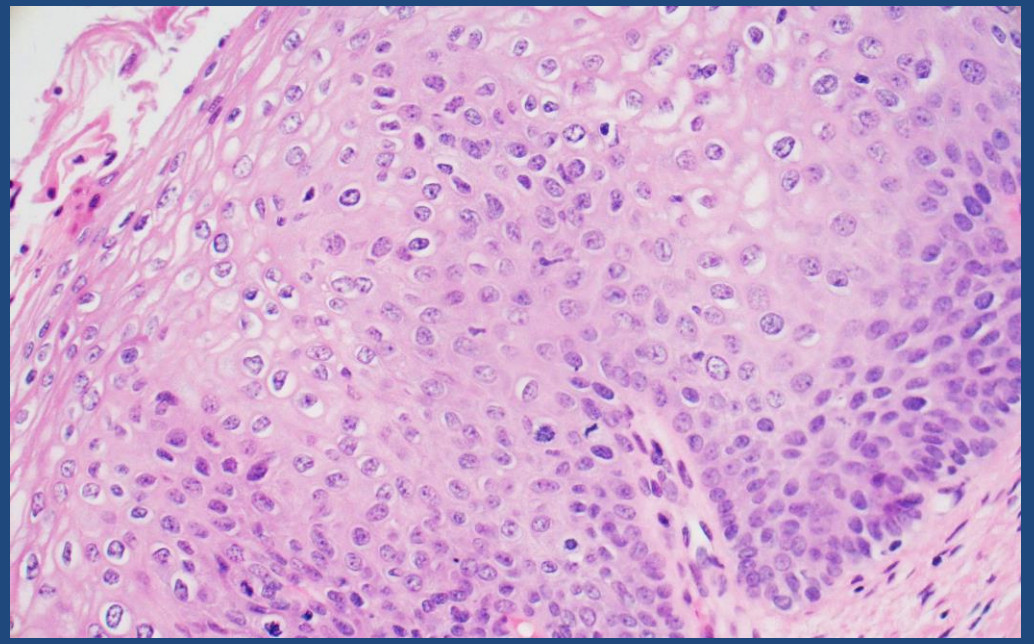


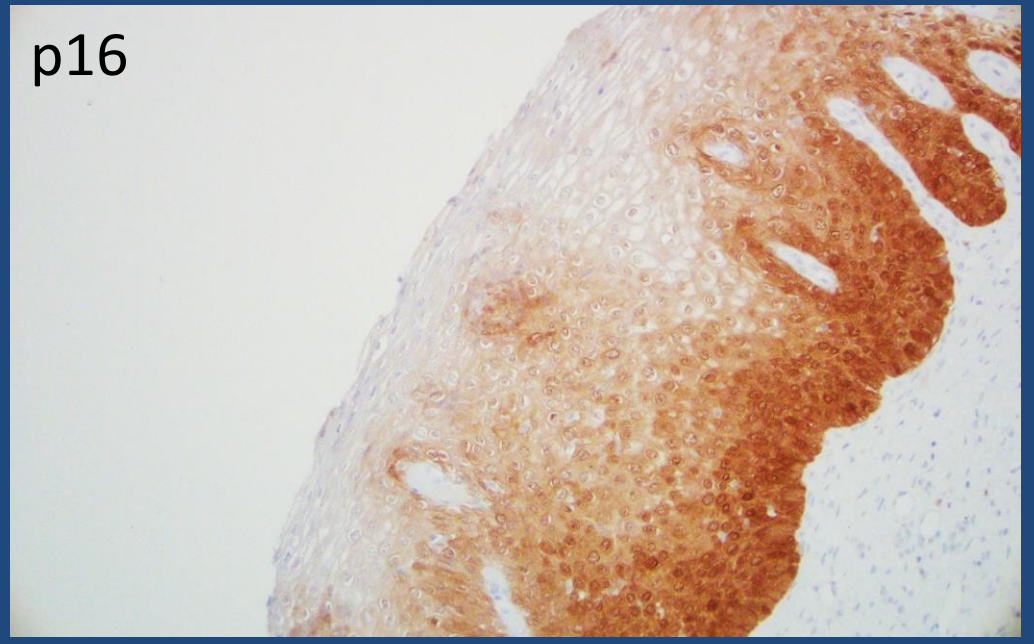
Looijenga LH et al. Am J Surg Pathol 2020;44:e66–e79

Penile Carcinomas

HPV Related Changes







HPV driven Penile carcinomas

- ~ 50%
- HPV driven- better prognosis
- Testing methods:
  - H&E morphology
  - p16 IHC
  - HPV ISH
  - PCR

TABLE 2. WHO Classification of PeIN

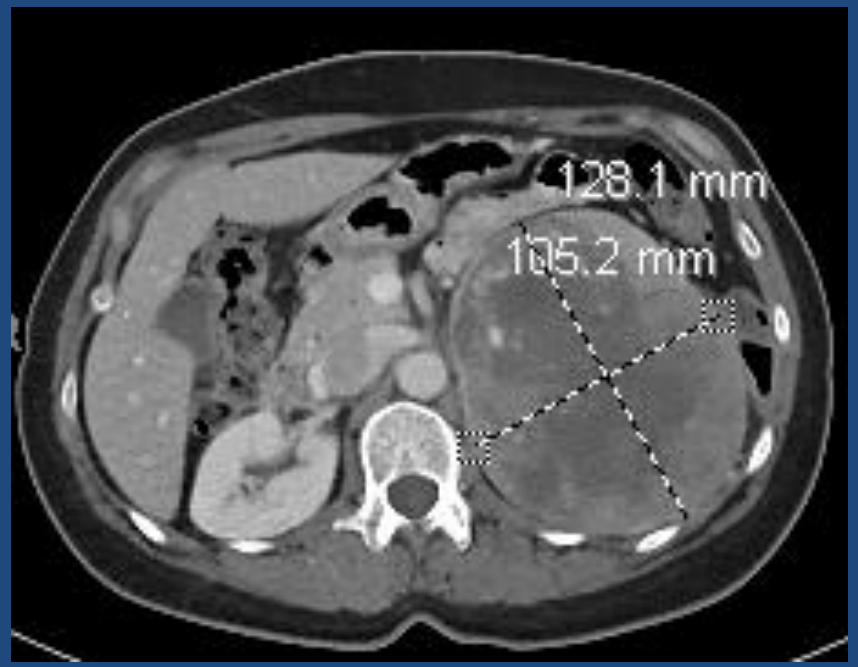
Non–HPV-related
Differentiated
HPV-related
Basaloid
Warty
Warty-basaloid
Other
Pleomorphic
Spindle
Clear cell
Pagetoid

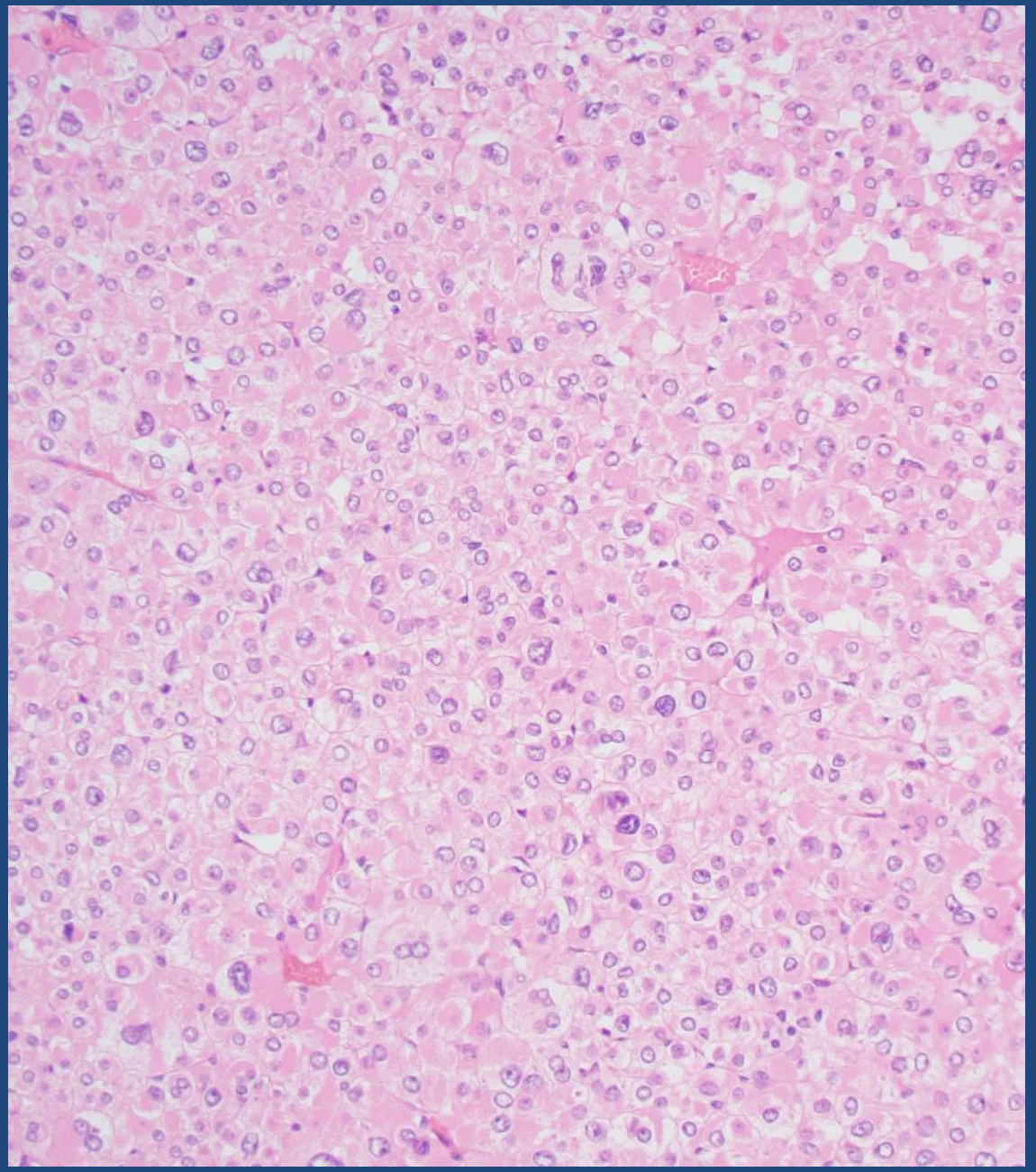
Canete-Portillo S et al. Am J Surg Pathol 2020;44:e80–e86

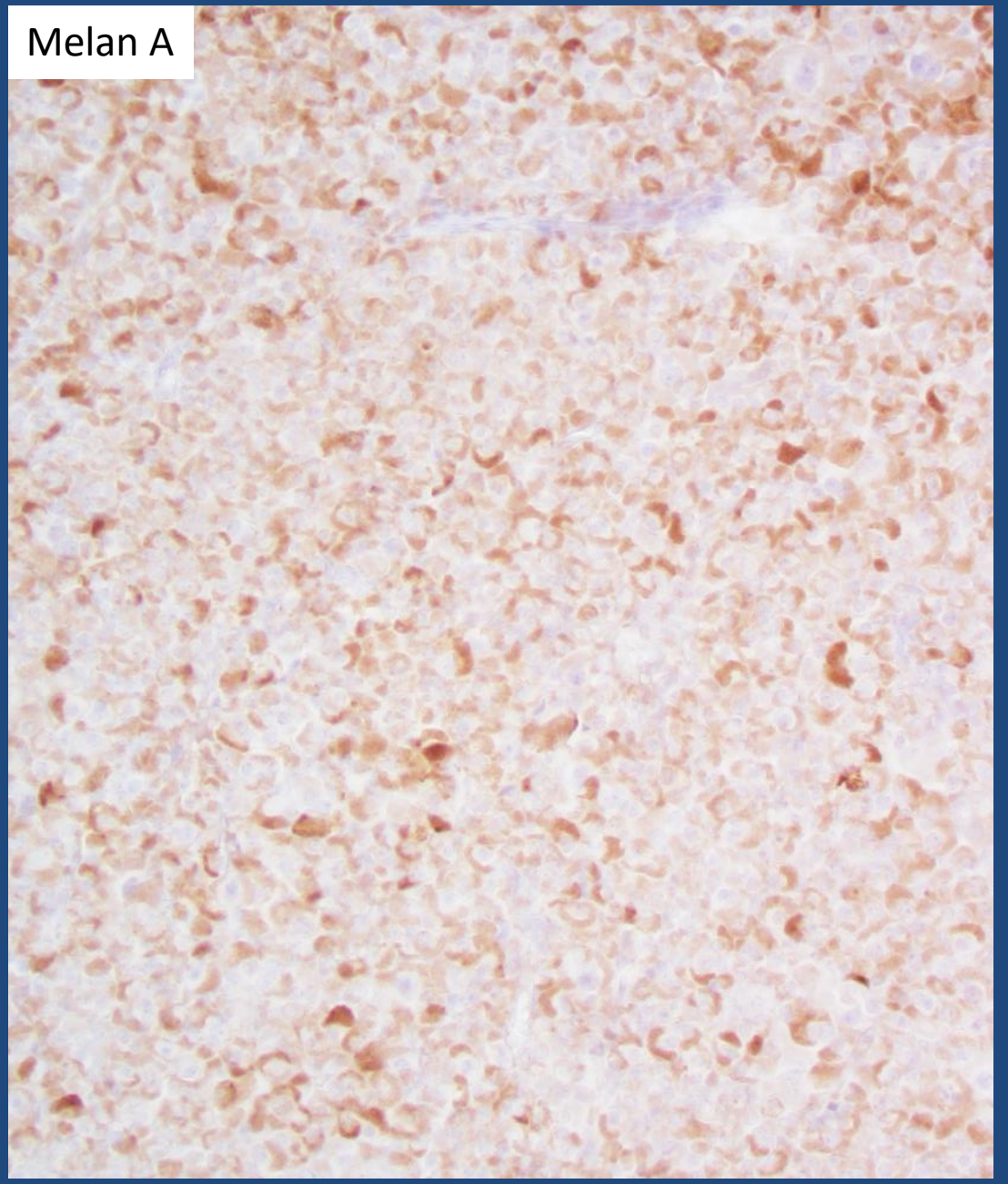
Immunotherapy Biomarkers

Case 7

- 52 y F
- 2017: Pelvic mass- incidentally discovered on abdominal imaging adjacent to upper pole of left kidney
- Family history of cancer: mother, paternal grandfather
- Biopsy, adrenalectomy

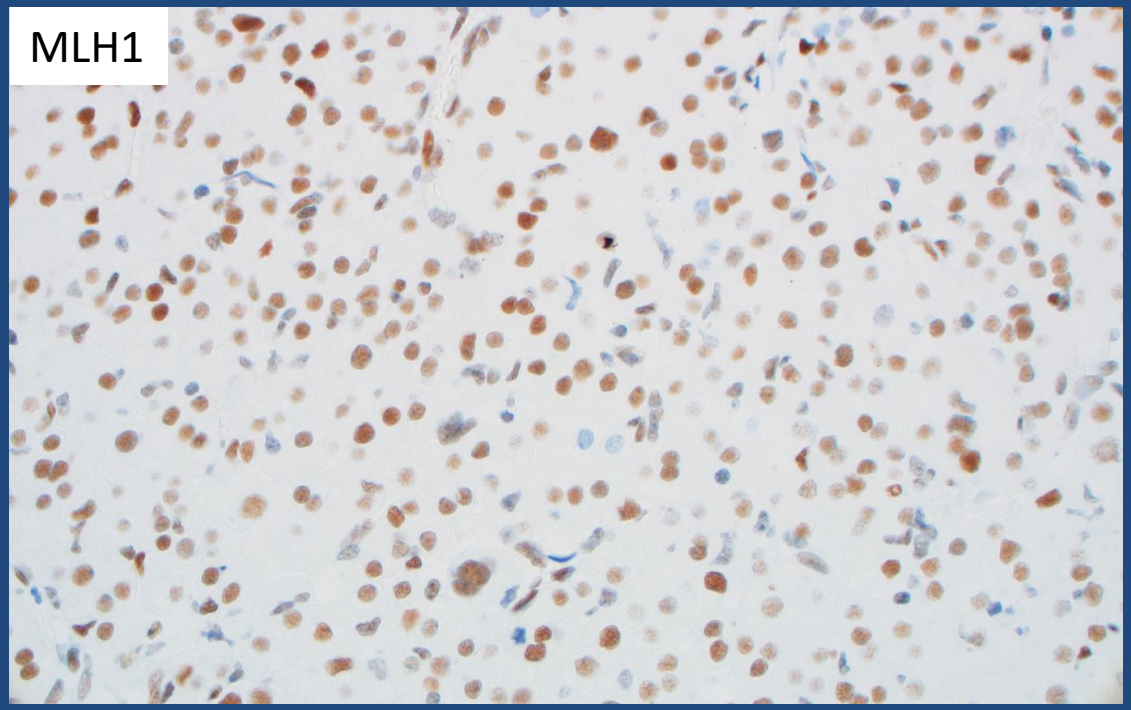


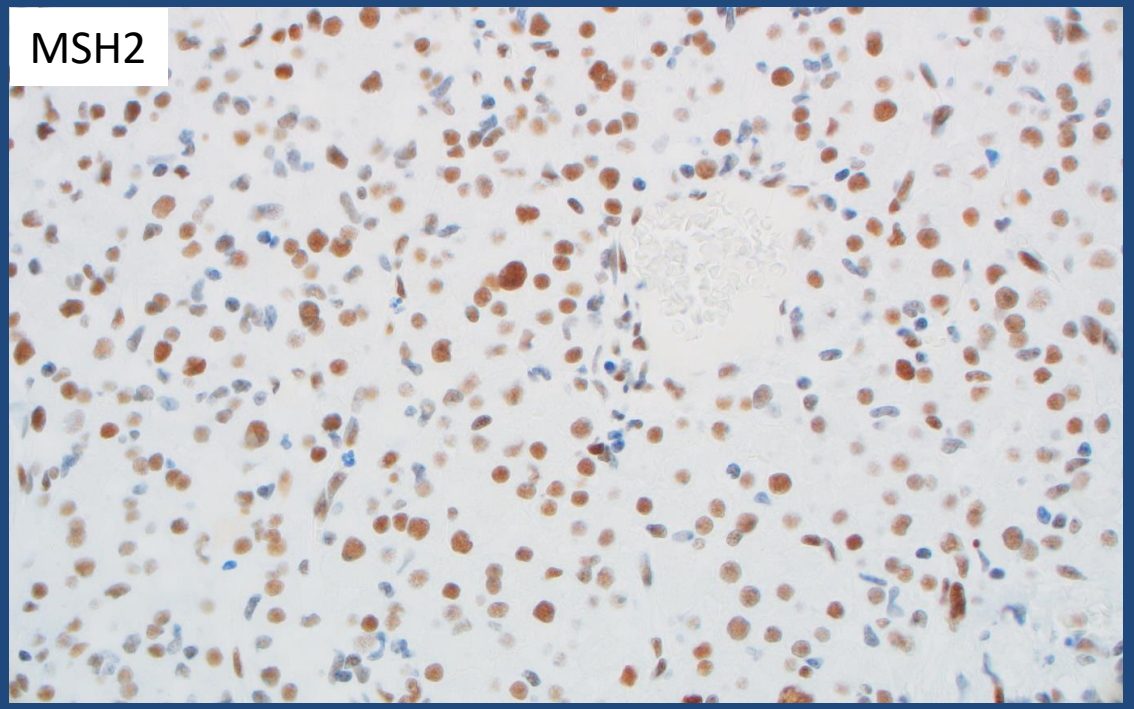


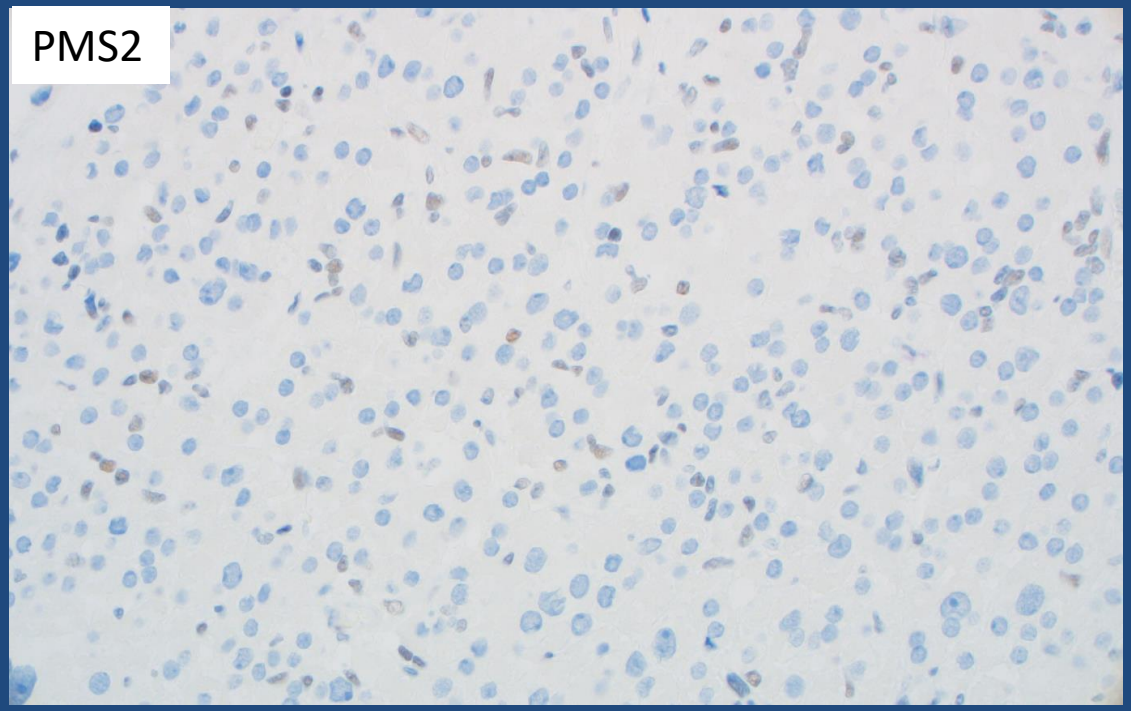

Melan A

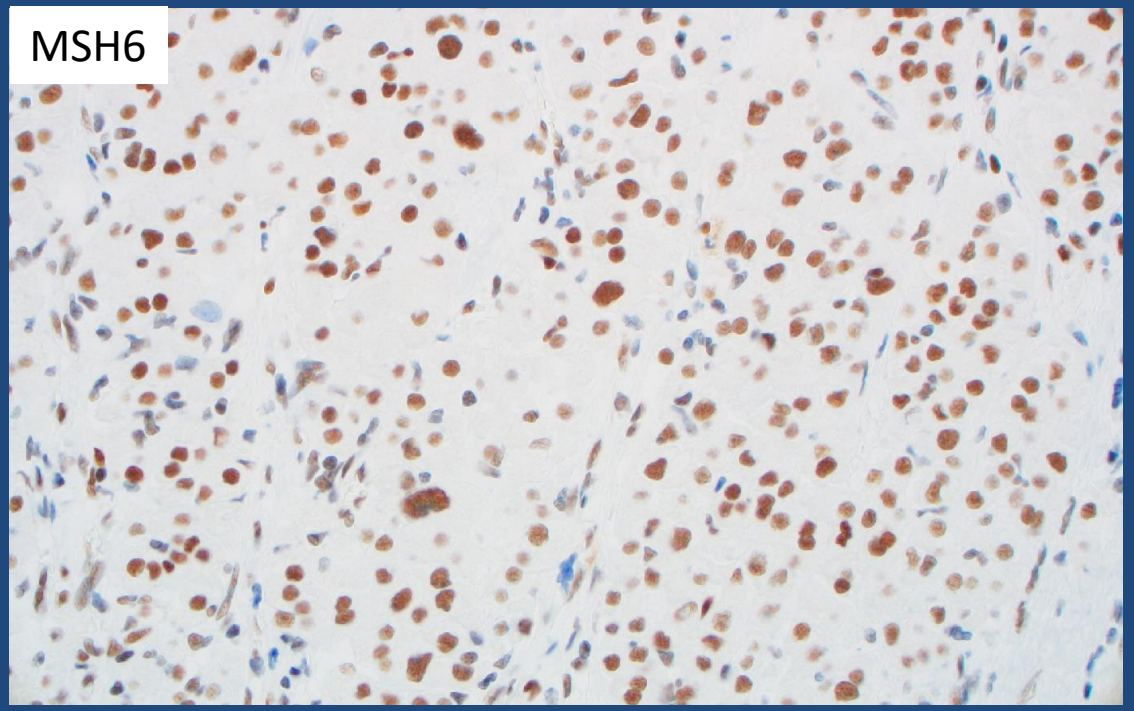
Case 7

- Diagnosis: Adrenal cortical Carcinoma
- 5/2019: Lucent bone lesions vertebral bodies, lung lesions. Bone biopsy- c/w metastatic adrenal cortical carcinoma
- 7/2019: Nivolumab/Ipilumab
- 12/2021: unchanged bone lesions
  - Likely treated disease on bone scans
  - Lung lesions- resolved, no new lesions
  - Ongoing response
- *PMS2* mutation: c.736_741delCCCCCTins11
- Hereditary non-polyposis colorectal cancer/Lynch syndrome


MLH1


MSH2


PMS2

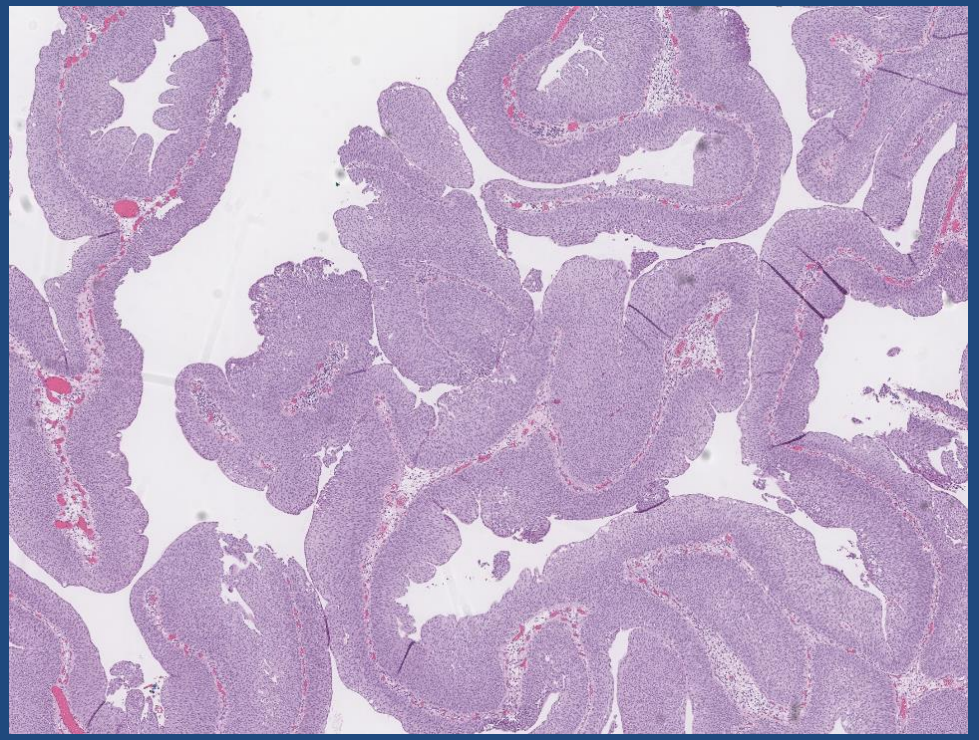

MSH6

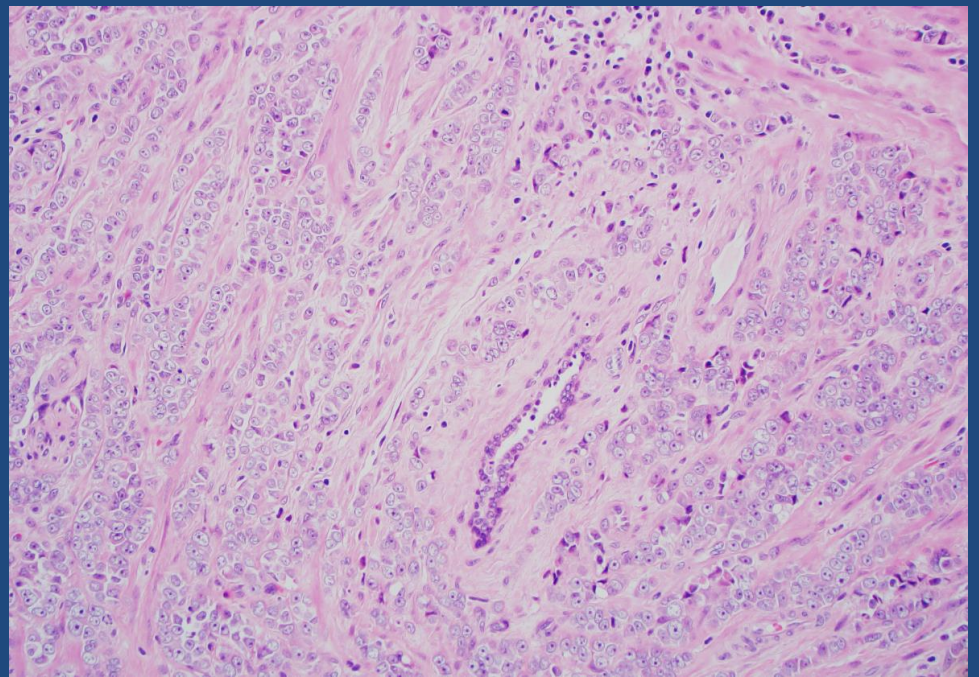
Microsatellite instability/Mismatch Repair Protein Deficiency

- MSI/MMR: eligibility for immunotherapy
- Agnostic of tumor type
- Testing methods:
  - MMR IHC: MLH1/PMS2, MSH2/MSH6
  - Next generation sequencing
  - MSI PCR

Genitourinary tumor in HNPCC syndrome

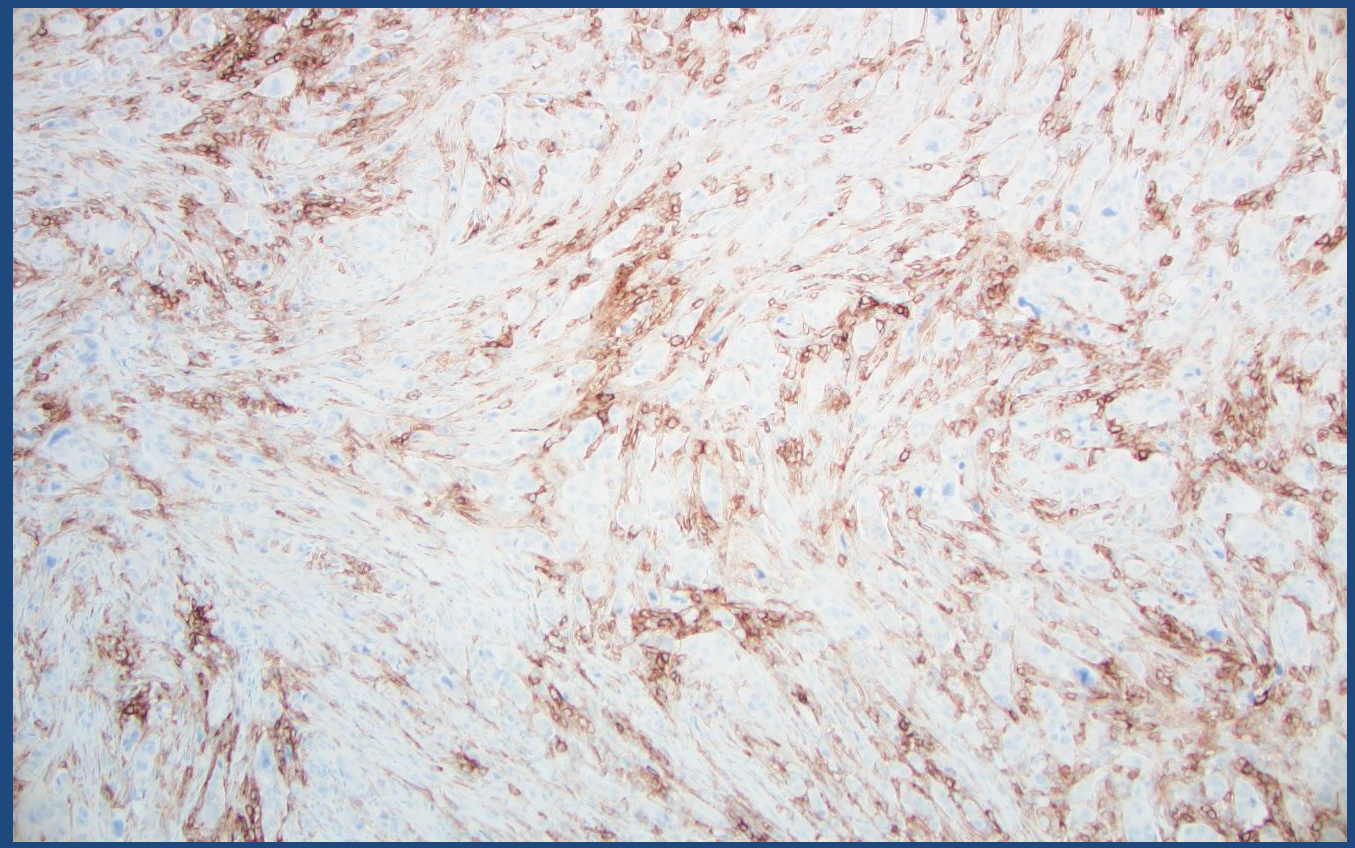
- Upper urothelial tract carcinoma ~ 1-5%
- Prostate carcinoma ~ 2%
- Adrenal cortical carcinoma





PD-L1 and Tumor Mutational Burden

- PD-L1:
  - Urothelial carcinoma
    - PD-L1 28-8: Nivolumab
    - SP-142: Atezolizumab
- Tumor Mutational Burden
  - Lacks standardization
  - High TMB- targetable



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Thank you

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