BEST PRACTICES IN THE APPLICATION OF IMMUNOHISTOCHEMISTRY TO DIAGNOSTIC UROLOGIC PATHOLOGY:

LESSONS FROM USES & ABUSES

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Toward Best Practice IHC use in routine practice

- **When IHC stains exceed H&E stain**
  - **Complex case *OR***
  - **Lack of best practice approach**

Toward Best Practice IHC use in routine practice

Surgical Pathology

- Foundation is the integration of clinical history, gross examination & microscopy
- Cornerstone is still the H&E with appropriate and judicious IHC support – *IHC guides; does not dictate the diagnosis*
- Practice made considerably more objective by ancillary techniques e.g. IHC

Toward Best Practice IHC use in routine practice

- Serious misdiagnoses are made by inappropriate use of IHC or incomplete knowledge of antibody/ies
  - *More is not necessarily better*
- IHC adjunctive method, histology key
  - *If you have no idea, don't mark it*
- Start with a question based on morphology
- Apply a judiciously constructed panel based on the differential diagnosis generated by the case

Toward Best Practice IHC use in routine practice

- Panel should include expected positive and expected negatives
- There are no absolutely specific or sensitive antibodies
  - *Anomalous stuff happens*
  - *Sensitivity and specificity is not inherent to the antibody, but to the antibody applied in a given setting*
- Evaluate the stain paying attention to pattern *(nuclear, cytoplasmic, membranous, etc.)*
- ALWAYS evaluate the controls *(positive and negative)*
- Diagnose the case after review of IHC only in the context of the morphology and the clinical situation

GOWN'S LAWS OF IMMUNOCYTOCHEMISTRY

- There is no perfect marker of any tumor
- There is no perfect fixative for all antibodies
- If everything in the tissue section appears positive, nothing is actually positive
- All that turns brown (or black, or red, etc.) on the slide is not positive
- Under inappropriate conditions, any antibody can be made to appear positive on any tissue
- In any given immunocytochemical run involving multiple slides, tissue will fall off the slide corresponding to the most critical antibody
- The diagnostic power of any immunocytochemical preparation is no greater than the knowledge and wisdom of the pathologist interpreting it

Best "Special Studies" in Surgical Pathology

- Good thin section and well stained H&E slides
- Additional sections, recuts and levels
- A phone call to the clinician (or reviewing the electronic medical records)
- Another trust-worthy pair of eyes (colleague)
- Placing the diagnostic dilemma in context of the clinical situation and management considerations
- Having a best practice approach immunohistochemistry

SELECT BEST PRACTICE IHC APPLICATIONS IN UROLOGIC PATHOLOGY

- **Bladder:**
  - Proving origin/differentiation in unusual primary or at a metastatic site
  - IHC in flat intraepithelial lesions
- **Prostate:**
  - Proving origin at a metastatic site
  - Issues related to triple cocktail use in prostate biopsies
- **Kidney:**
  - Proving renal origin at a metastatic site
- **Testis:**
  - Screening panels for tumors involving testis – primary or metastatic sites
  - Characterizing the various germ cell components

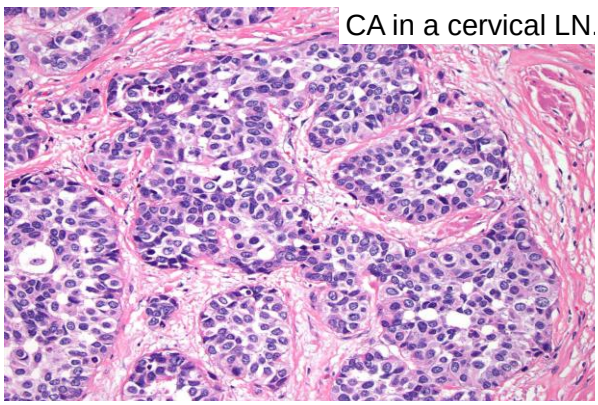
PROVING UROTHELIAL DIFFERENTIATION

Carcinoma of unknown origin or patient with history of bladder/renal cancer:
- Lymph node
- Lung
- Liver
- Bone
- Prostate

"Unusual carcinoma" in the bladder

Metastatic tumors to the bladder:
- Melanoma
- Prostate
- Colorectal
- Cervix
- Ovary
- Renal

Primary urothelial carcinoma:
- UCa with small tubules
- Plasmacytoid
- Micropapillary
- Etc



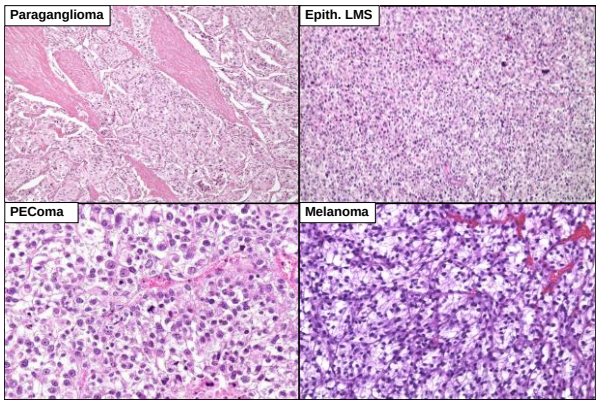
CA in a cervical LN.

UROTHELIAL CARCINOMA (Prim. or Metastatic site)

Challenges:
- Poorly differentiated carcinoma
- "Characterless": solid, nested & trabecular architecture

Hallmarks:
- *Frequent squamous and / or glandular diff.*
- *Cells with nuclear grooves*
- *Nuclear atypia obvious +/- anaplasia*

Approach
- *Clinical history (invasive, usually high stage carcinoma)*
- *Compare with primary*
- *Judicious IHC: ? Best markers*



URINARY BLADDER - IHC

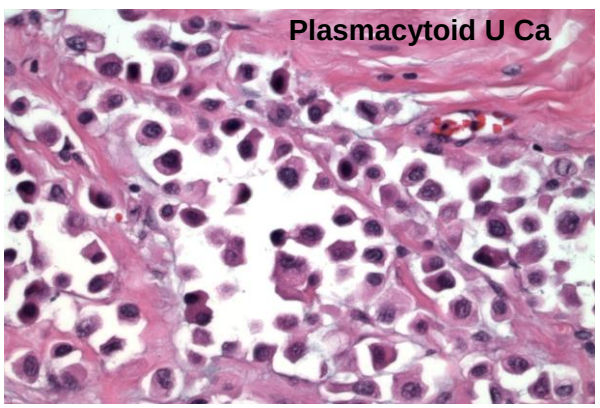
- Diagnosis of metastatic urothelial cancer
  - CK7 (+) (>90%)
  - CK20 (+) (40-70%)
  - p63 (+) (60-90%)
  - High molecular weight cytokeratin 34ßE12 (+) (60-90%)

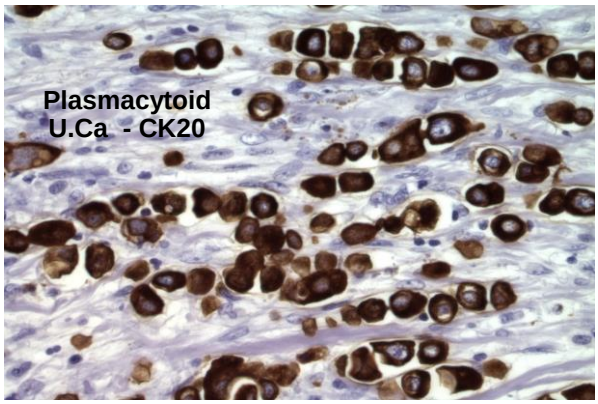
Traditional, Broad Markers

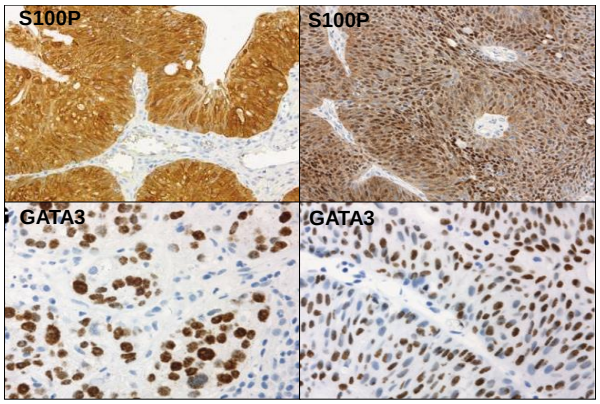
  - GATA3 (60-70%)
  - Uroplakin II (+) (50-80%)
  - S100P (70- 80%)

Histogenesis-associated markers

- *Uroplakin III (+) (20-50%)*
  - *Thrombomodulin (+) (60-75%)*
  - *CEA, Leu-M1 (±) (minimal value)*

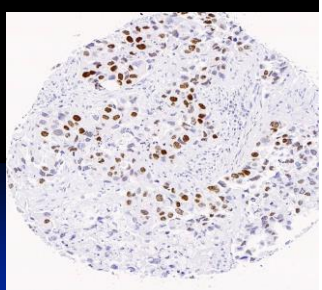






GATA3

- Nuclear staining
- lower sensitivity but higher specificity than S100P for urothelium



GATA3 – Wide Range of Expression

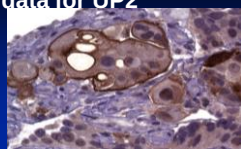
- **Positive in**
  - **Breast, trophoblastic tumors, paragangliomas, salivary gland neoplasms, squamous carcinomas, basal cell carcinomas, yolk sac tumors, pancreatic ductal adenocarcinomas**

- ***Mietinnen et al. Am J Surg Pathol 2013***

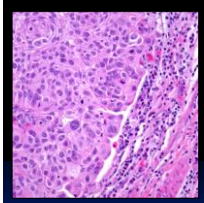
Uroplakins – II and III

- **Protein constituents of the urothelial plaques in vesicles of urothelium**
- **Vital role in expansion and contraction through vesicle cycling**
- **Subunits uroplakins Ia, Ib, II, and IIIa**
- **Unique and characteristic feature of urothelium**
- **Previous data for UP3, new data for UP2**

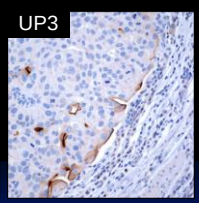
Uroplakin 3



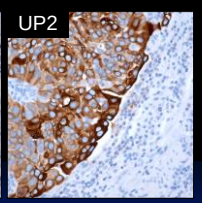
Uroplakin 2 versus Uroplakin 3



UP3



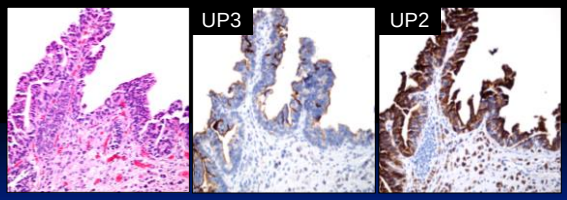
UP2



. Among UC metastases, UP2 showed greater intensity and proportion, (both p<0.001), with higher sensitivity (73% vs 37%, respectively, p=0.001).

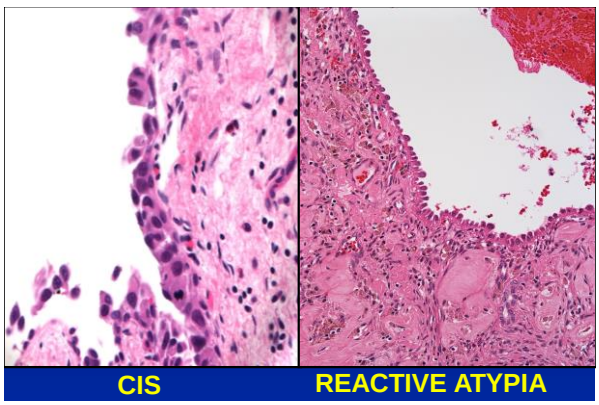
Smith et al. Histopathology. In press

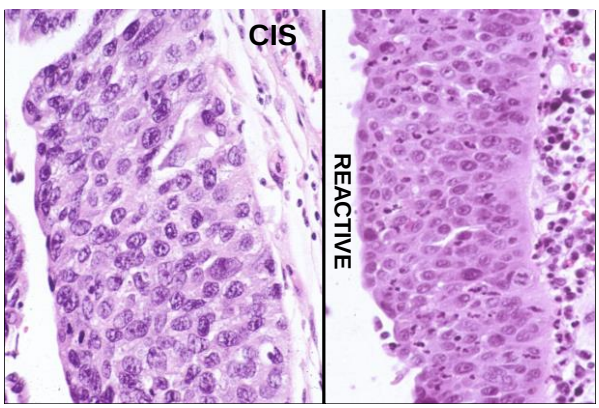
Uroplakin 2 versus Uroplakin 3



Villoglandular variant simulates colorectal carcinoma

Smith et al. Histopathology. In press





IMMUNOHISTOCHEMISTRY IN FLAT LESIONS OF THE BLADDER

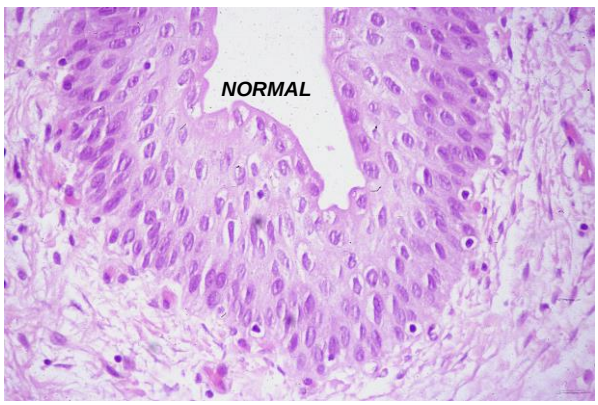
Panel: p53, CD44 (standard isoform), CK20

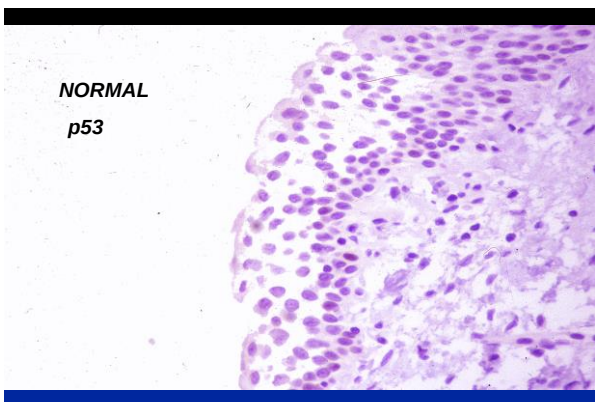
Indications:

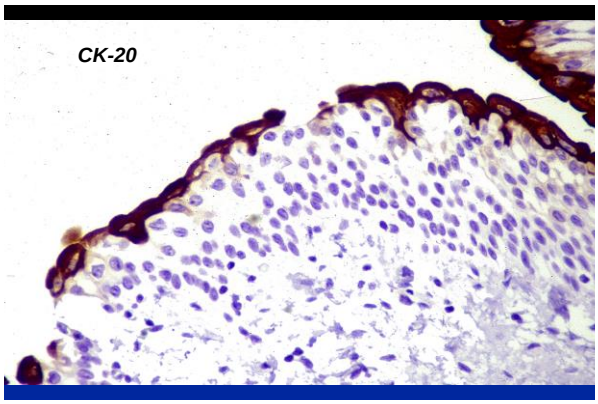
- Marked denudation – residual basal cells vs "clinging" CIS
- Distinction between reactive atypia and CIS (large cell non-pleomorphic or "small" cell)
- Pathologist favors CIS but has reservations making diagnosis
- CIS with unusual morphology – Pagetoid, undermining, etc.

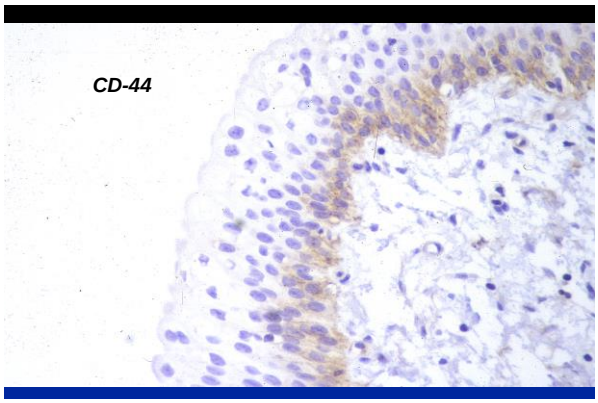
Caveats:

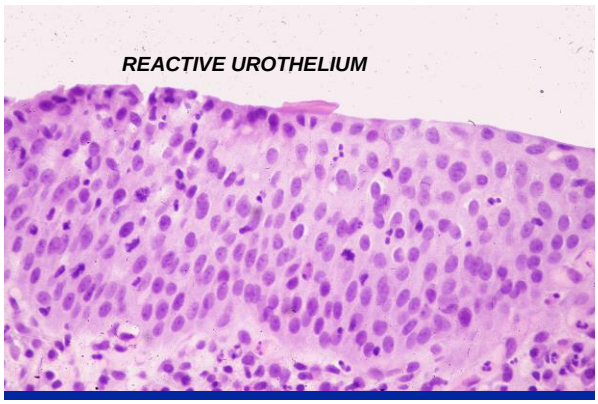
- Not applicable for dysplasia vs CIS
- Greater caution while evaluaiting post-treatment biopsies

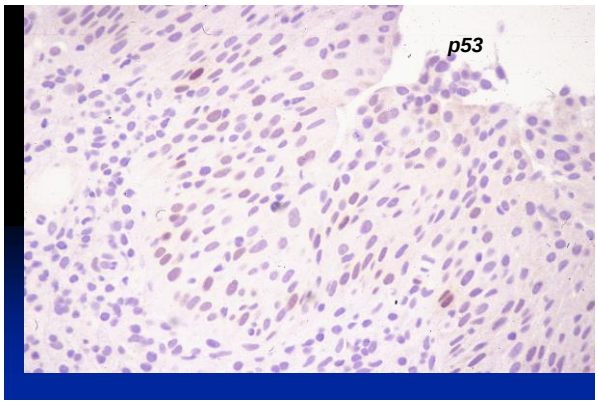


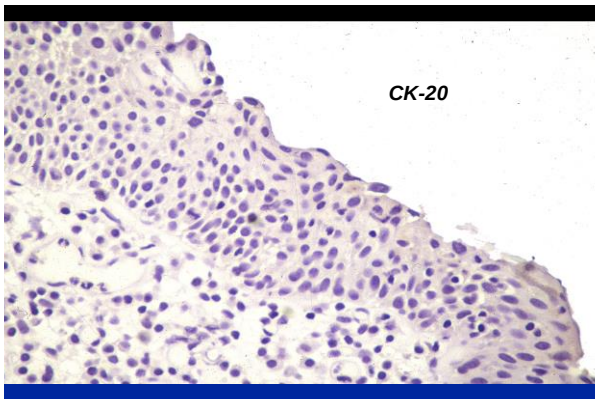


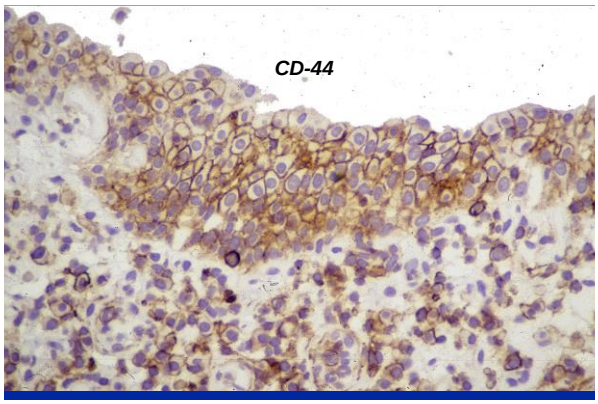

CK-20

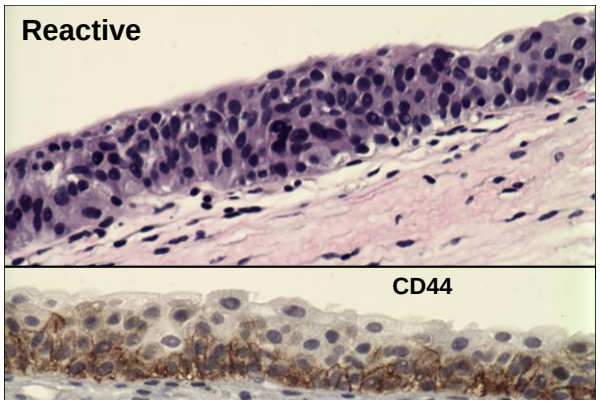

CD-44

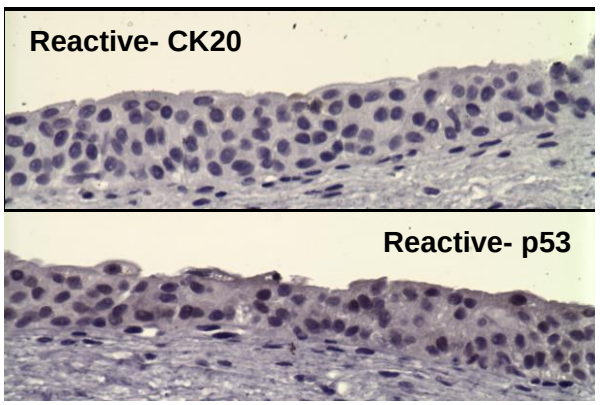

REACTIVE UROTHELIUM

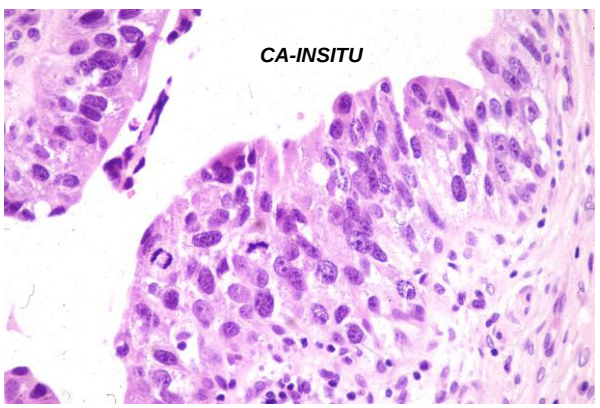

p53


CK-20

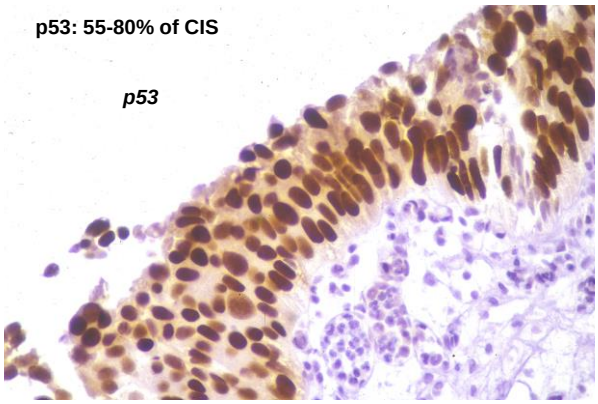

CD-44

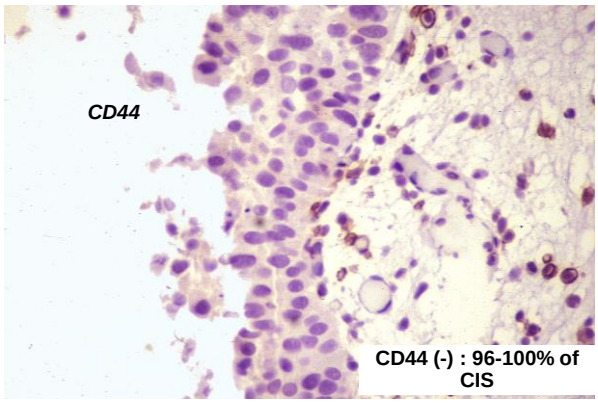

Reactive
CD44

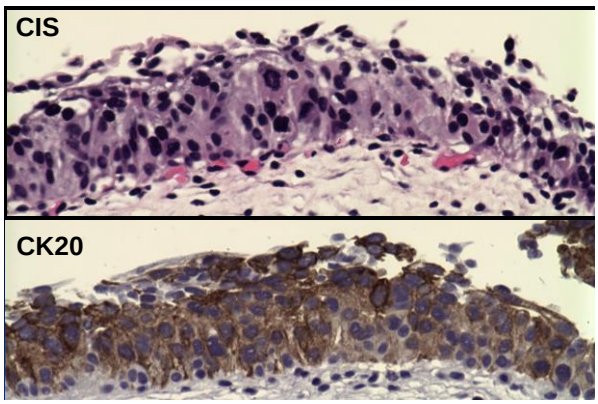

Reactive- CK20
Reactive- p53

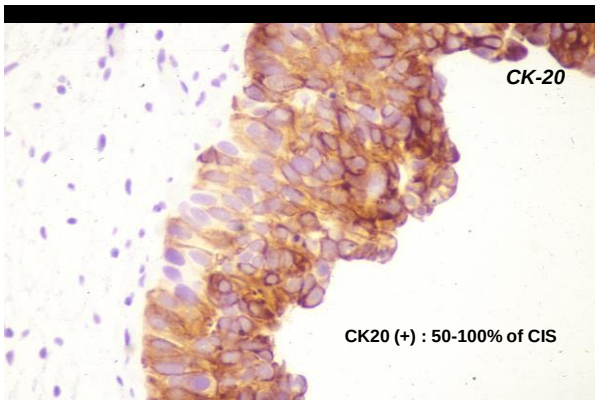

CA-INSITU

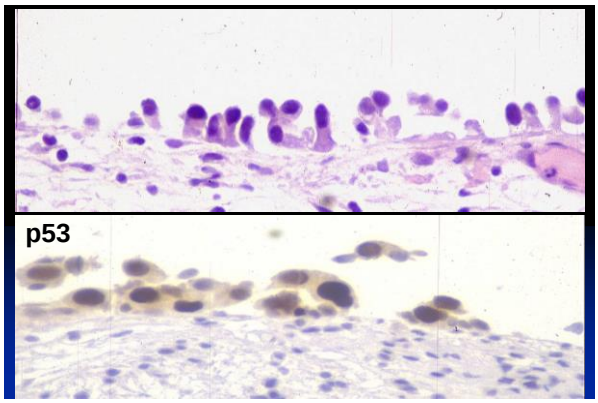
p53: 55-80% of CIS

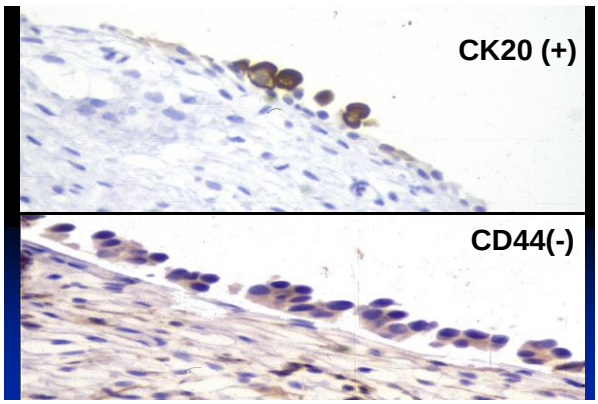


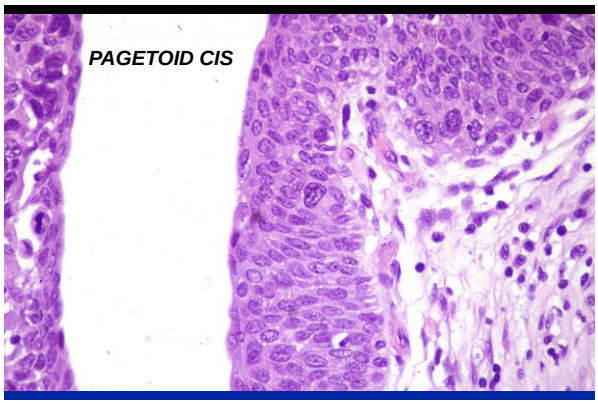


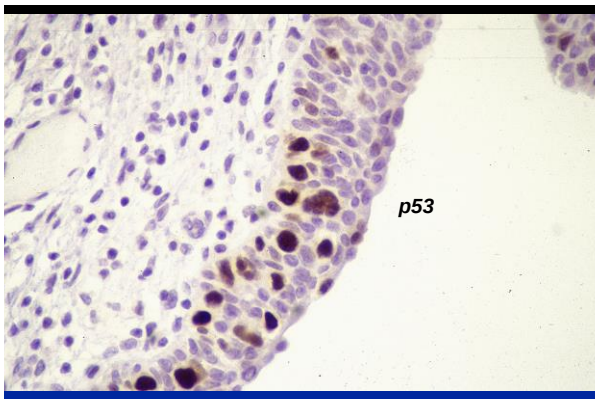


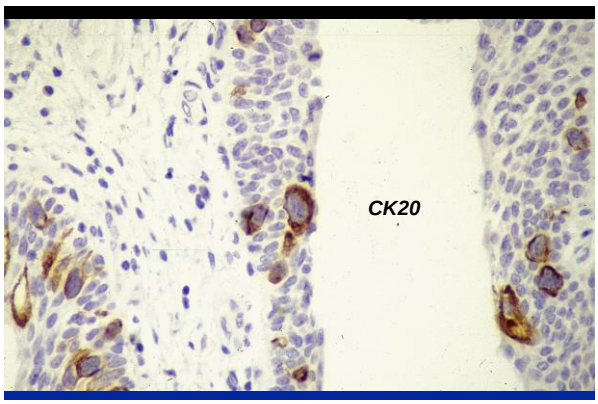

CK-20
CK20 (+) : 50-100% of CIS

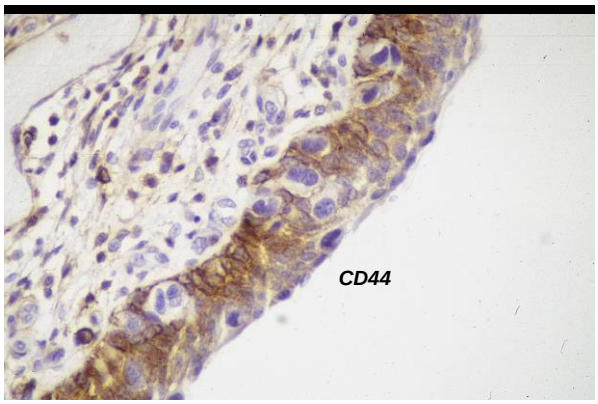

p53


CK20 (+)
CD44(-)


PAGETOID CIS


p53


CK20



UROTHELIAL ASSOCIATED-MARKERS

Prostate vs. Urothelial Carcinoma

- Often in bladder neck specimens

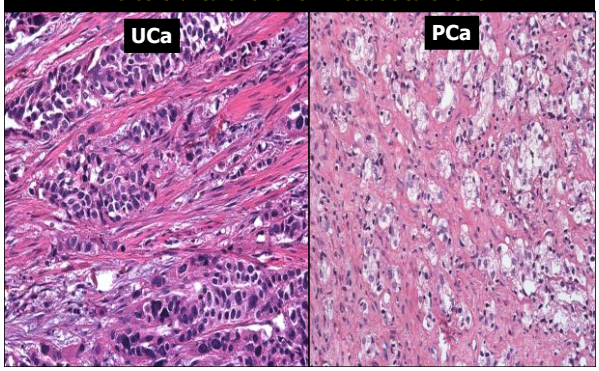
- Therapeutically critical differential

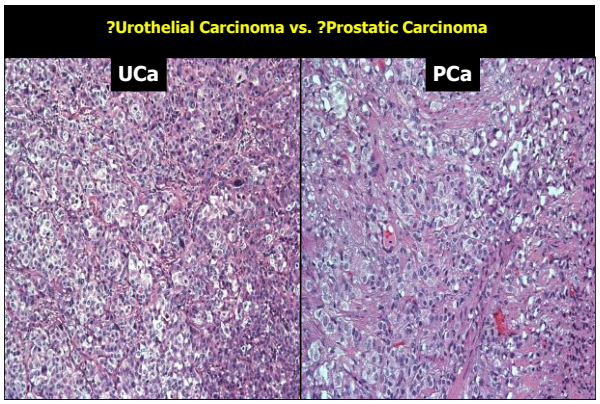
- *PSA*
- *PSAP*
- *NKX1.3*
- *Prostein (P501S)*
- *ERG-TMPRSS2*
- *PSMA*

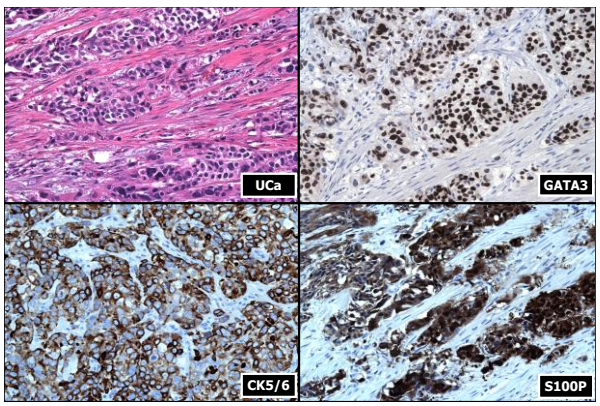
- CK20
- P63 or MWCK
- *GATA3*
- *Uroplakin 2*
- *S100p*
- *Uroplakin 3*

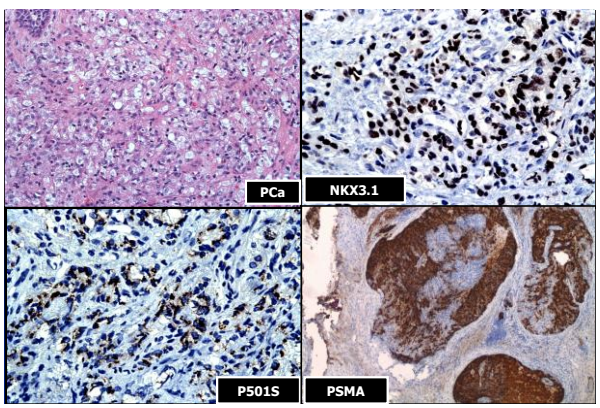
CAUTION: Both may coexist!

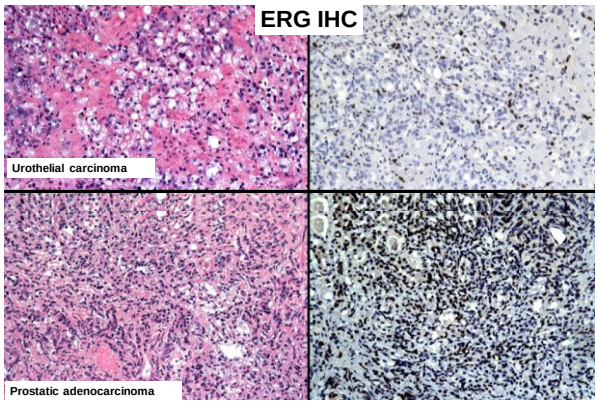
?Urothelial Carcinoma vs. ?Prostatic Carcinoma



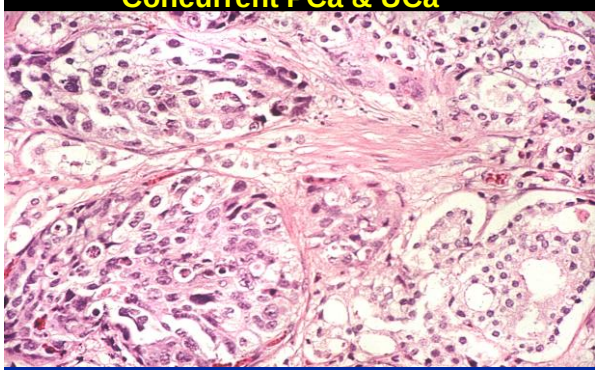

?Urothelial Carcinoma vs. ?Prostatic Carcinoma
UCa
PCa


UCa
GATA3
CK5/6
S100P


PCa
NKX3.1
P501S
PSMA



Concurrent PCa & UCa

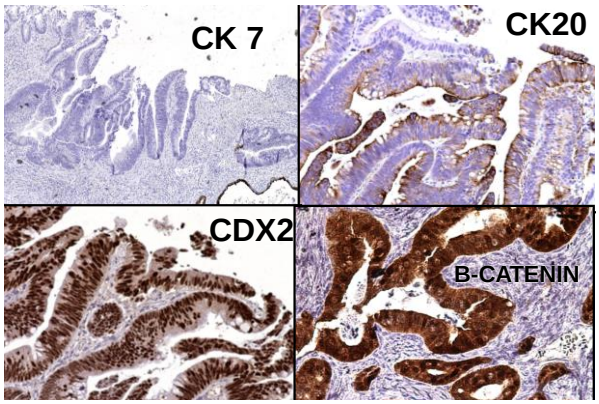


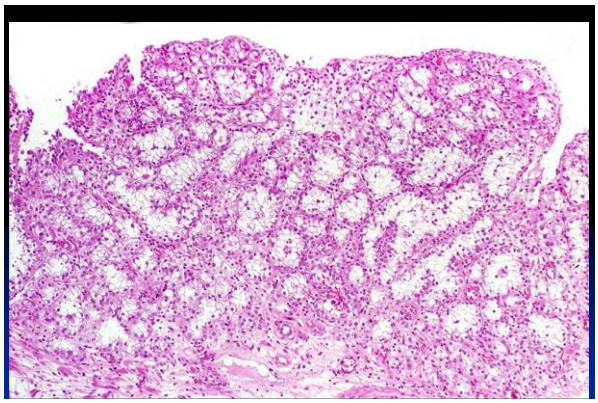
METASTATIC ADENOCARCINOMA TO THE BLADDER

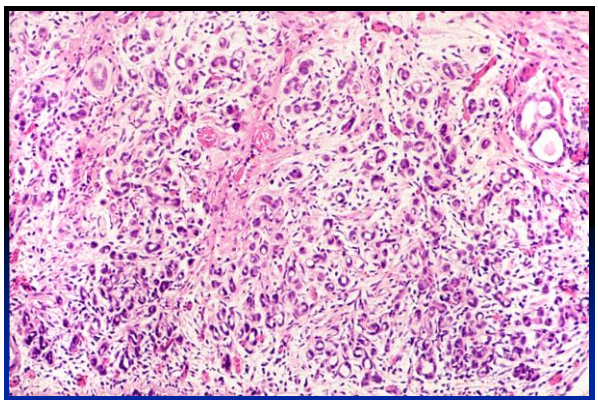
Virtually any tumor from the body can spread to the bladder on occasion. Problem areas:

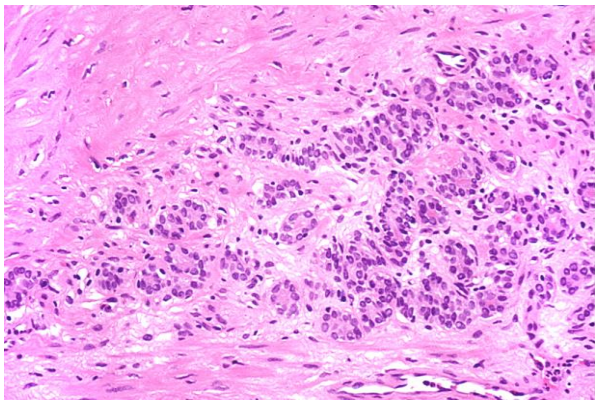
Enteric morphology: Colon and appendiceal primary vs. bladder primary

- Morphologically identical
- May have a surface well-differentiated "villous adenoma" surface component
- Helpful features:
  - Clinical history of high-stage colon cancer
  - Absence of intestinal metaplasia
- Immunohistochemistry (CK7, CK20, CDX2) not helpful (β-catenin, nuclear positivity, limited role)


CK 7
CK20
CDX2
B-CATENIN





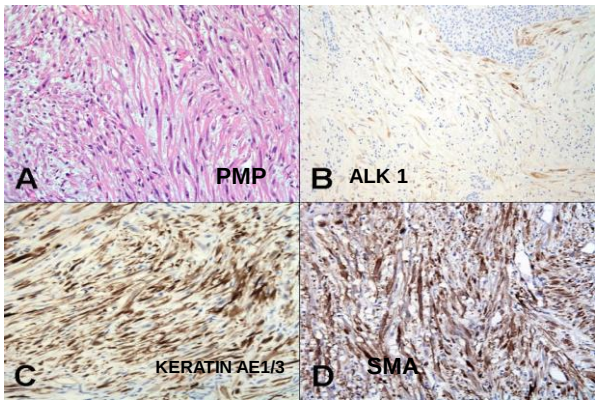


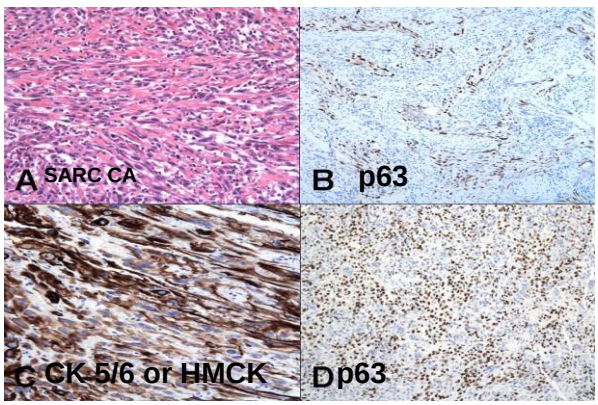
	Nephrogenic adenoma	Clear cell adenoCa of bladder	Urothelial Ca with glandular morphology	Prostatic adenoCa
Pax2/8	90%	10-20%	0%	0%
AMACR	100%	75%	Frequently positive	70-100%
S100A1	94%	10%	0%	0%
Ki67 % + nuclei	2-5%	40-50%	30-40%	2-25%
PSA	0 -2%	0	0	70-100%

Spindle cell lesions

Benign (PMP) vs. Malignant - H&E diagnosis

- PMP / PSFMT
  - keratin(+/-),SMA(+), desmin(+/-), p63(-), Alk-1(+)
- Sarc. Ca
  - keratin (+/-), SMA(-), desmin(-), p63(+/-), Alk-1 (-), HMCK & CK5/6 (+)
- LMS
  - keratin (-/+),SMA(+), desmin(+), Alk1(-/+),p63(-)





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		support this diagnosis.
(15A)	Right Lat Base	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(16A)	Right Base	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(17A)	Right Lat Lat Mid	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(18A)	Right Lat Medial Mid	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(19A)	Right Lat Mid	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(20A)	Right Mid	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(21A)	Right Lat Lat Apex	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(22A)	Right Lat Medial Apex	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(23A)	Right Lat Apex	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(24A)	Right Apex	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(25A)	Left Base Margin	Benign fibromuscular tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(26A)	Right Apex Margin	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(27A)	Left Apex Margin	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.
(28A)	Right Base Margin	Benign prostatic tissue. Immunostains for basal cell-specific high molecular weight keratin (34BE12), p63, c-myc and racemase support this diagnosis.

Indications for IHC – Needle Biopsy

Atypical small cell proliferations

- To confirm focus as cancer
- Confirm benignity in ASAP felt to be benign
- Unusual patterns
  - Atrophic
  - Pseudohyperplastic
  - Double – layer
  - PIN-like

Atypical large acinar proliferations (intraductal patterns)

Post – treatment setting

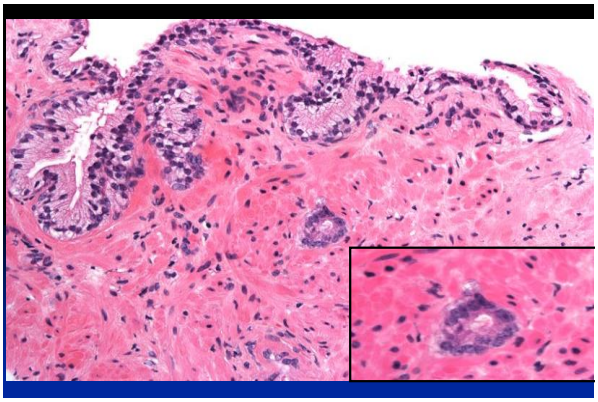
IHC in Prostate Needle Bxs.

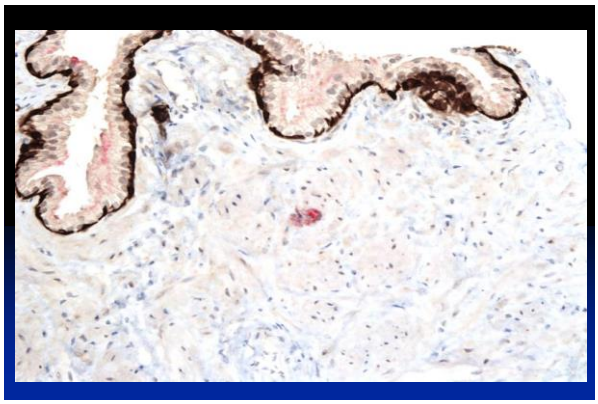
- Basal cell cocktail
  - p63 and 34βE12
- Triple cocktail "PIN cocktail"
  - p63/34βE12/AMACR
- ERG immunohistochemistry
  - Additional marker, only if triple not conclusive

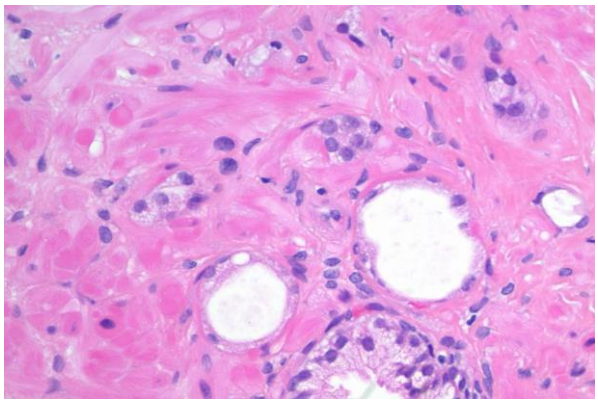
PSA – to prove prostate origin – NA, Cowper's glands

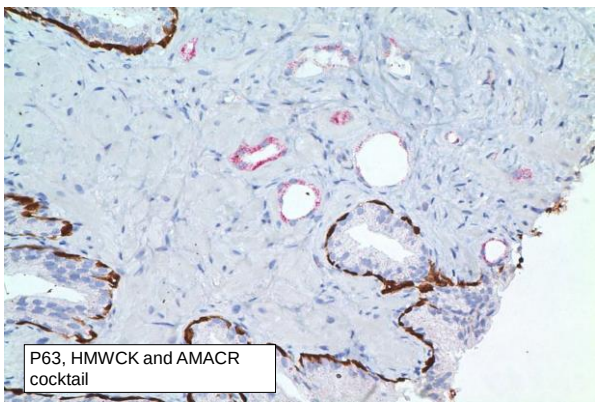
Triple cocktail

- Expected reactions
  - PCa: p63(-), HMCK(-), AMACR(+)
  - Benign small cancer mimics: p63, HMCK(+), AMACR(-)
  - HGPIN: p63, HMCK(+), AMACR(-/+)
  - Ductal cancer:
    - *Invasive component: p63, HMCK(-), AMACR(+)*
    - *Intraductal component: p63, HMCK(+), AMACR(+)*
  - Urothelial cancer: p63, HMCK(+/-), AMACR(+)





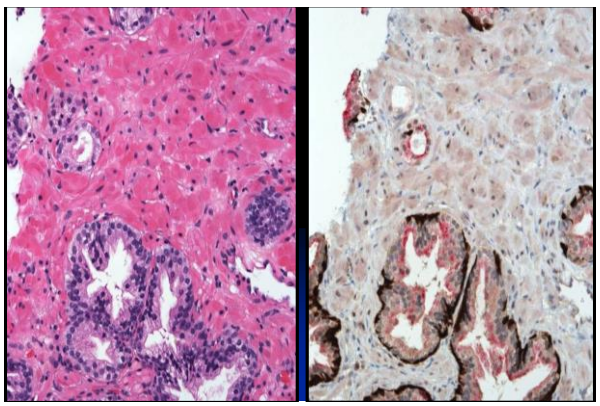


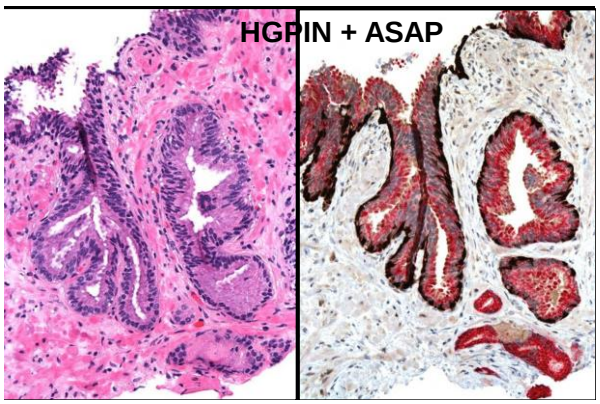

P63, HMWCK and AMACR cocktail

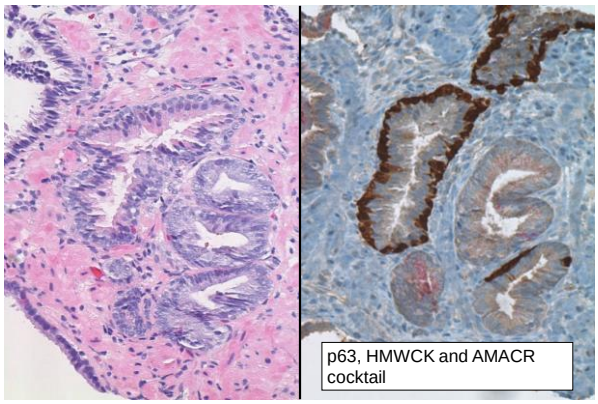
EQUIVOCAL IHC

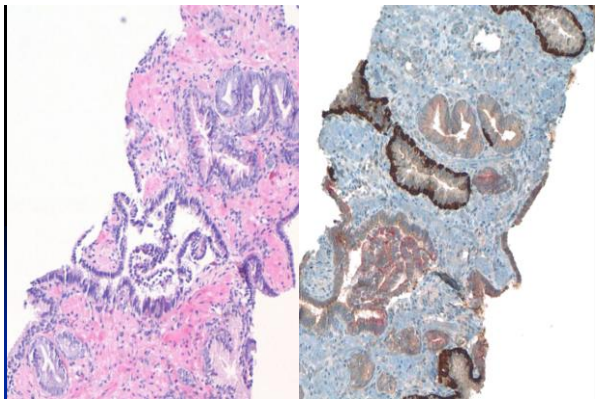
- Results not entirely complimentary

- Unexpected basal cell layer staining
- Results supportive but all glands in an already small or difficult focus not represented in the IHC



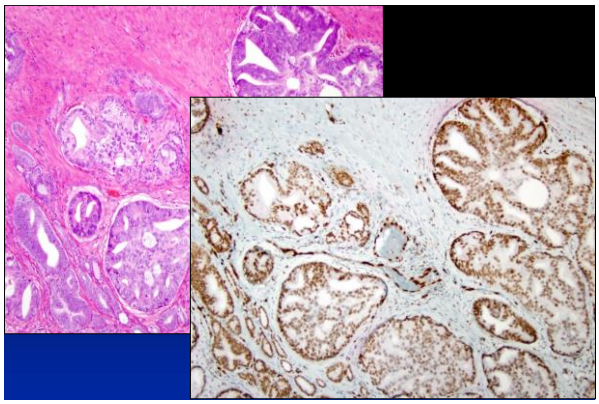


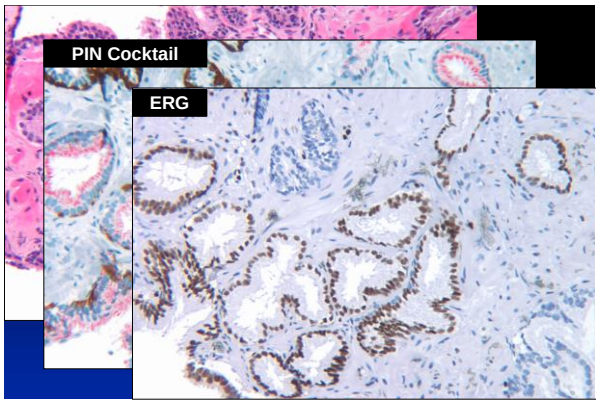




ERG Immunohistochemistry

- 60% of PCa harbor any ETS-rearrangement
- 50% of PCa – TMPRSS2-ERG
- Detection by IHC or FISH
  - High concordance in hormone naive
- IHC detection in ~30% in needle setting
- Do we need a 4th marker?
  - – Helps in about 5% of cases with equivocal triple cocktail
- Additional: Marker of prostate histogenesis





IHC in a pt. with one (+) core

- **Confirm bilaterality-** clinical staging - almost 50% patients with prostate cancer treated with RT
- Accurate assessment of # of cores involved – **Active surveillance**
- Quantitation of cancer – **Active surveillance (>50% may exclude)**

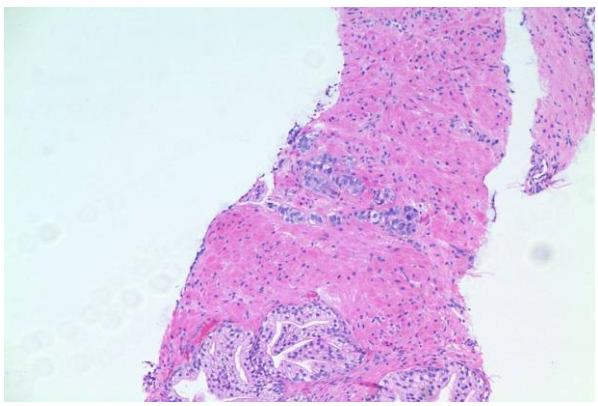
Work-up of Atypical Foci with Definite Cancer in Other Parts

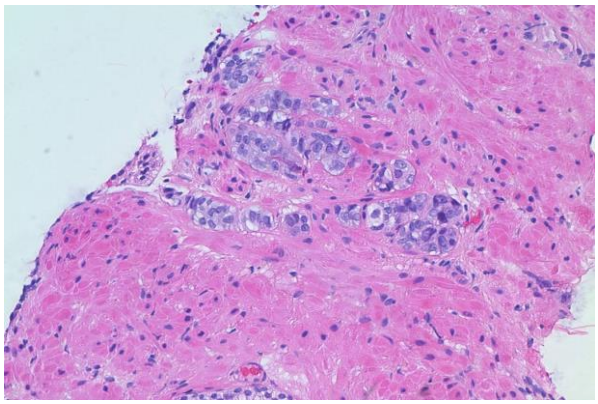
Patient with Gleason score 3+4 or higher grade cancer on at least one part.

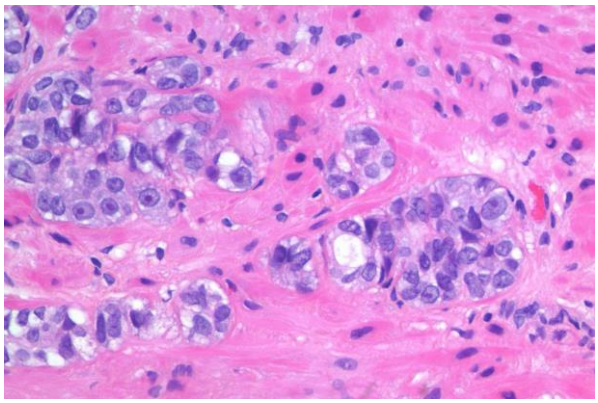
? Work up other parts with small foci of possible 3+3=6

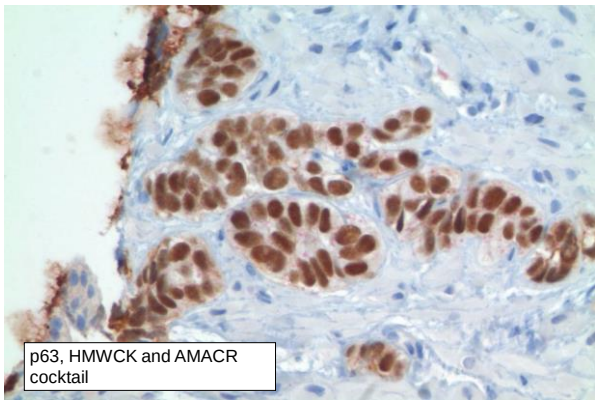
Generally, not indicated, as additional IHC confirmation will likely not change management

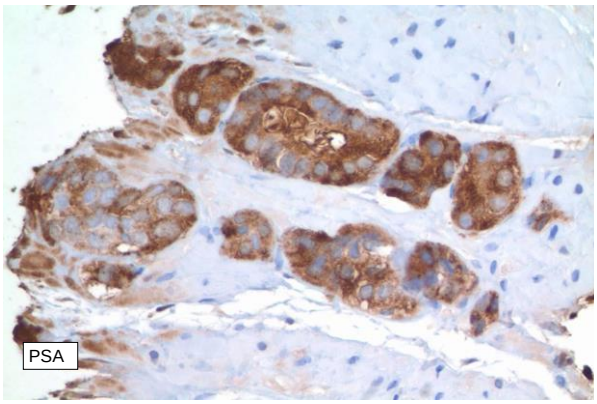
Abberant expression p63 in Prostate cancer








p63, HMWCK and AMACR cocktail



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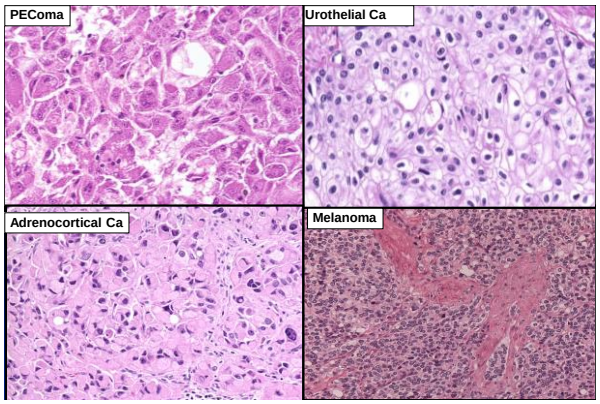
IHC IN KIDNEY SURGICAL PATHOLOGY

- **Confirming Renal origin**
- ***Histologic subtyping of RCC***

Metastatic sites
Primary tumors
Small biopsies and FNAS



CONFIRMING RENAL ORIGIN

Carcinoma of unknown origin or patient with history of RCC:

- Lymph node
- Lung
- Liver
- Bone
- Other

"Unusual carcinoma" in the kidney

- Epithelioid PEComa
- Urothelial Carcinoma
- Metastatic carcinoma to the kidney

versus

- Poorly differentiated, high grade RCC (unclassified)

versus

- Lymphoma, sarcoma, melanoma, other

APPROACH TO APPLICATION OF IHC IN RENAL TUMORS

Is the neoplasm a carcinoma?:
rule out Epi AML (PEComa), lymphoma, sarcoma, melanoma etc

↓

Is the carcinoma a renal primary?:
rule out urothelial carcinoma, metastasis

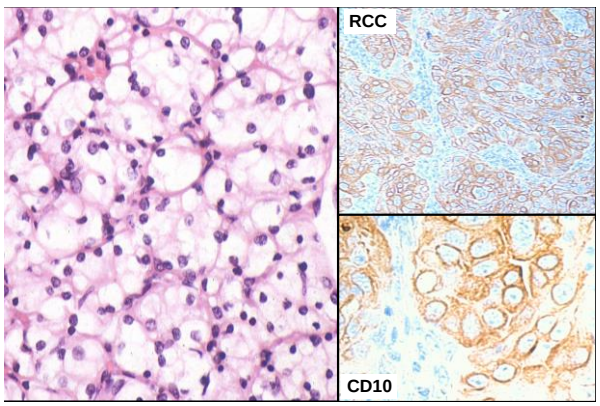
↓

Can you subtype the renal cell carcinoma?:
Clear cell vs papillary vs chromophone vs oncocytoma vs translocation associated Ca …..

RCC antigen

Monoclonal antibody against brush border of healthy PCT

- **RCC types**
  - Clear cell RCC (85%)
  - Papillary RCC (95%)
  - *Oncocytoma & Chromophome (-/+)*
  - *Collecting duct Ca (-/+)*
- **Other tumors**
  - Breast ca
  - Parathyroid ca
  - Embryonal ca, testis
  - Lung
  - Prostate
  - Ovary
  - Melanoma
  - Epididymal cystadenoma
  - Mesothelioma

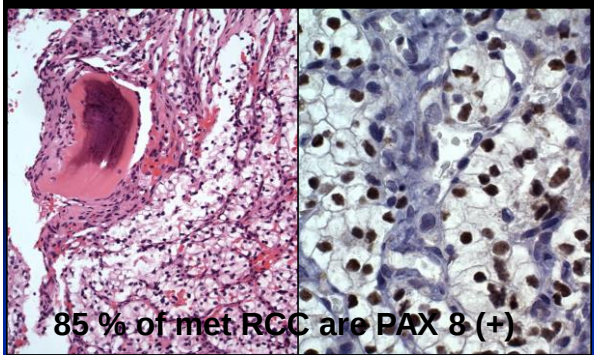


PAX8

Paired box transcription factor, similar to PAX2
Predominantly data from polyclonal antibody – new monoclonal

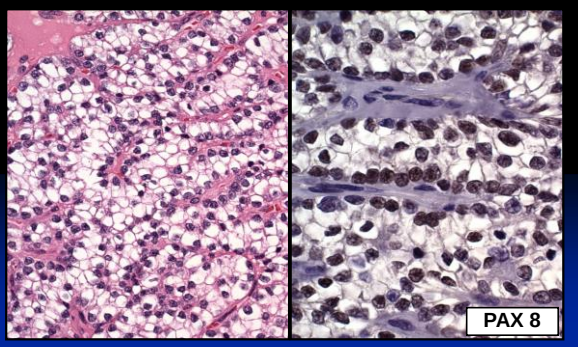
- **RCC types**
  - *Clear cell RCC (>95%)*
  - *Papillary RCC (>95%)*
  - *Wilms tumor*
  - *Metanephric (+) adenoma*
  - *Oncocytoma (+)*
  - *Chromophobe RCC (-/+)*
  - *Collecting duct Ca (-/+)*
  - *Translocation assoc. Ca (-/+)*
- **Other tumors**
  - *Similar to Pax2*
  - *Thyroid neoplasms*
  - *Extensive GYN positivity*

Metastatic Clear cell RCC (Bone)



85 % of met RCC are PAX 8 (+)

PARATHYROID CARCINOMA



S100A1

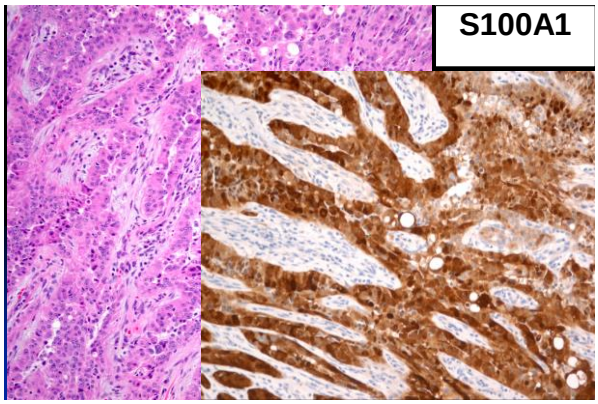
Among the 13 member S100 protein family.
Expressed in numerous cell types, not well studied

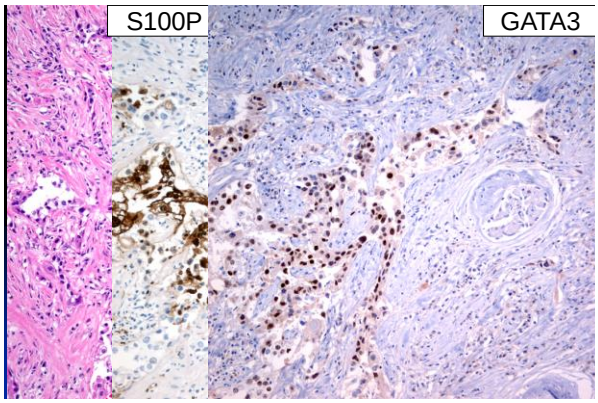
Positive in RCC

- Clear cell RCC (60%)
- Pap RCC (80%)
- Clear cell-pap RCC
- Oncocytoma
- Translocation assoc RCC
- Chromophobe RCC (-)

Other tumors

- Ovarian Ca (serous, clear)
- Endometrial Ca





Carbonic anhydrase IX

•Family of zinc containing metalloproteinase that regulates cell proliferation, adhesion and metastasis

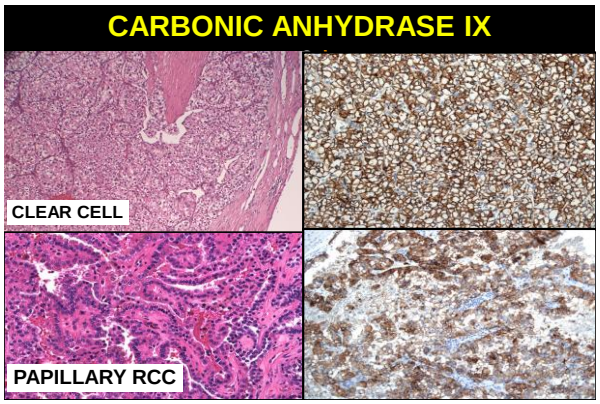
Kidney tumors

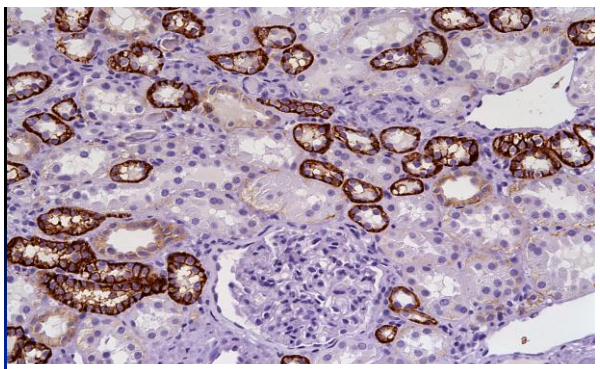
- Clear cell RCC (+)
- Papillary RCC (-/+)
- Chromophobe RCC (-)
- Oncocytoma (-)
- Urothelial Ca (+/-)

Other tumors

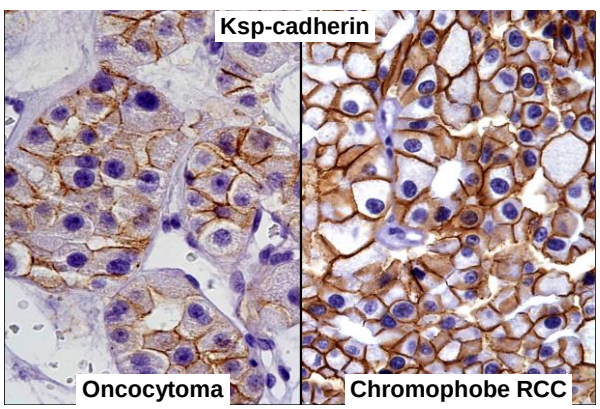
Most carcinomas of endometrium, stomach, lung, cervix, liver, breast etc.

Prognostic utility of CA IX in clear cell RCC





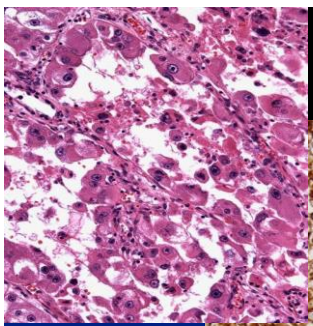
Ksp-cadherin in distal convoluted tubules



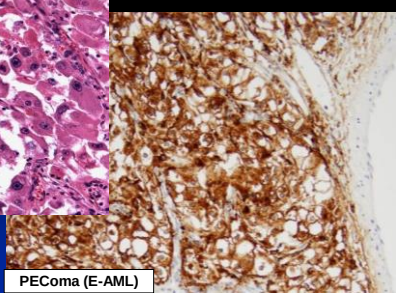
Cathepsin K

- Expression is related to overexpression of MiTF
- PEComas: moderate to strong and diffuse cytoplasmic staining is seen in all variants
  - co-expressed with other melanocytic markers (more diffuse than HMB-45)
- MiTF-TFE3 translocation associated carcinomas
  - t(X;1): >85% cases, diffuse
  - t(X;17): 0%
  - t(6;11): 100% of cases, diffuse

Other renal tumors:
Negative except nonspecific in necrotic areas



Cathepsin K



PEComa (E-AML)

CONFIRMING RENAL ORIGIN

Is the neoplasm a carcinoma?:

- Renal "related"
  - *AE1/AE3 (+)*
  - *EMA (+)*
  - *Vimentin (+)*
  - *CK7 (-), CK20 (-)*

Is the carcinoma a renal primary?:

- Renal associated
  - *"RCC marker" (80%)*
  - *PAX8 (>90%)*
  - *S100A1**
  - *CD10 (+) (94%)*

If history of renal mass and renal histogenesis markers are negative?

- Consider: Chromophobe carcinoma
  - CD117 (+) and Ksp-Cadherin (+)
- Consider: Epithelioid PEComa and translocation carcinoma
  - Cathepsin K, MelanA/HMB45

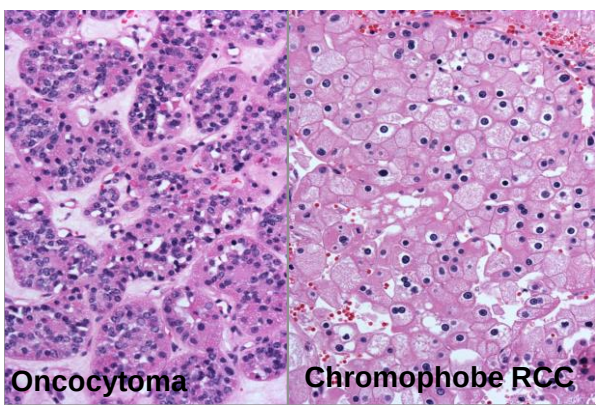
Renal Clear and Papillary Tumors

Clear cell RCC
CA-9 (+)
RCC (+)
Pax8 (+)
Vimentin (+)

Clear –Papillary RCC
CK 7(+)
Racemase (-)
HMCK (+)
RCC, CD10(-)

Papillary RCC
RCC (+)
CK7 (+)
Racemase (+)

Metanephric adenoma
RCC (+)
CK7 (+)
Racemase (+)



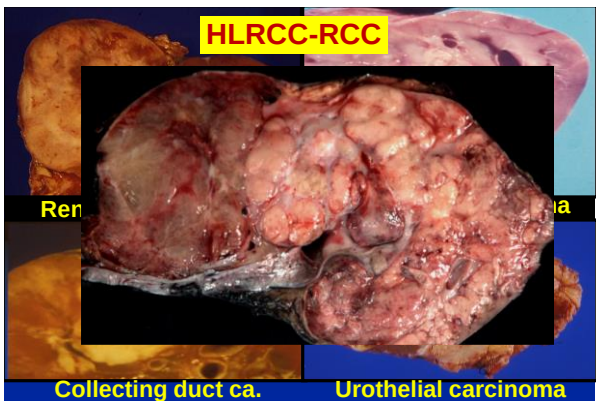
Renal Oncocytic Tumors

Oncocytoma
- CK 7 (- / +)
- S100 A1 (+)
- Barttin (cytoplasmic)

Chromophobe RCC
- CK 7 (+ / -)
- S100A1 (-)
- Barttin (membranous)

Amylase 1A (AMY1A), EPCAM, Claudin and Caveolin 1 - Investigational

**Not adequately studied: preliminary data*
*Not tested in hybrid oncocytic tumors**



IHC FOR HIGH GRADE DISTAL NEPHRON CA

RENAL CELL CA incl. CDC
- PAX8
- RCC
- S100 A1
- CK 7 & 20 (-)

RENAL MEDULLARY CA
- OCT3/4 (+)
- INI1 lost (-)
- PAX8

UROTHELIAL CA
- GATA 3
- S100P
- HMCK
- P63
- Uroplakin 2
- CK 7 & 20 (+)

HLRCC-RCC/FH deficient
- FH lost (-)
- 2SC positive

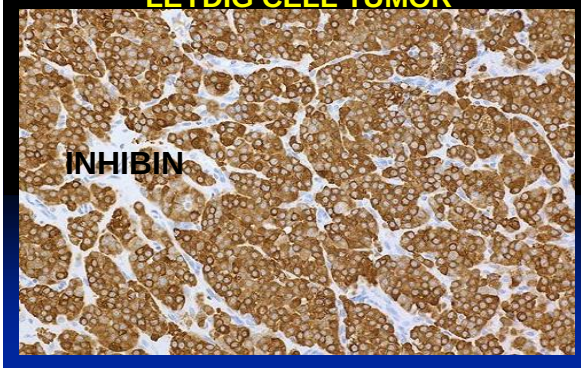
CAIX and Vimentin immunoreactivity can be seen in UCa

TESTIS IHC: Screening panels

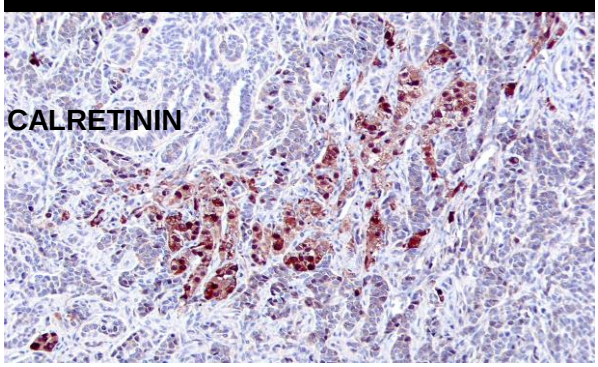
- **Germ cell tumors**
  - *OCT 3/4*
  - *SALL4*
  - *PLAP*
  - *EMA(-)*
  - *Vimentin (-)*
- **Sex cord tumors**
  - *SF1*
  - *Melan A*
  - *Inhibin*
  - *Calretinin*
  - *CD99*
  - *Synaptophysin*
  - *S-100*
  - *FOXL2*

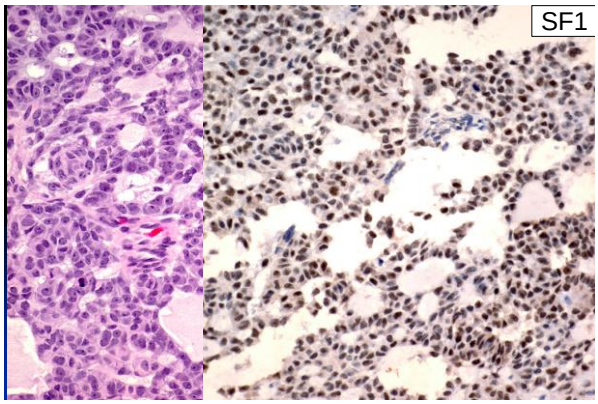
- **Lymphoma:** *CD-45, CD3, L26*
- **Visceral malignancy:** *EMA (+), vimentin (±)*

LEYDIG CELL TUMOR



SERTOLI CELL TUMOR





IHC in characterizing the different germ cell components

- There is no substitute to well (overnight) fixed sections
- Adequate sampling is key - the # of IHCs should ***NEVER*** exceed the H&E slides
- Remember what matters in germ cell tumors

GERM CELL TUMOR – What really matters?

One does not necessarily have to characterize every morphologically different focus

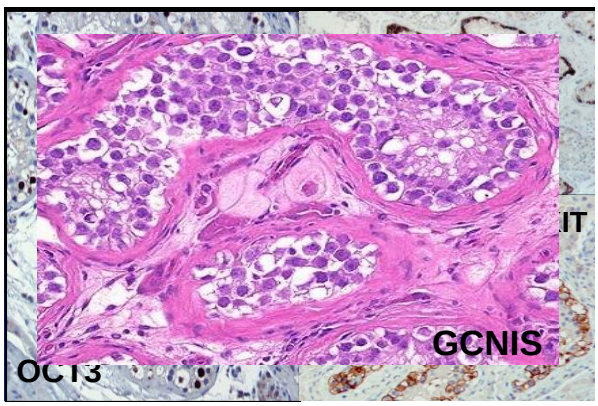
- Pure classic Seminoma vs. non-seminomatous components
- Mixed germ cell tumor
  - -Specify components (as accurately as you can)
  - ->80% or pure embryonal carcinoma (↓)
  - ->50% teratoma (↑)

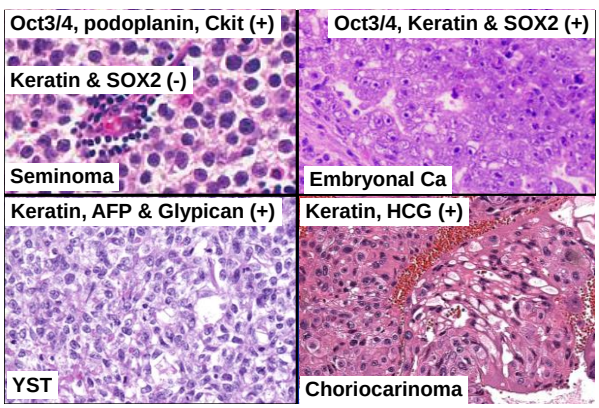
Vascular-lymphatic invasion – pathologic stage
Margin status

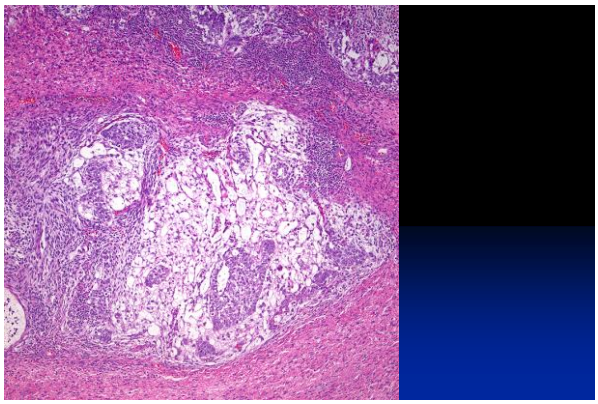
IHC IN GERM CELL TUMORS

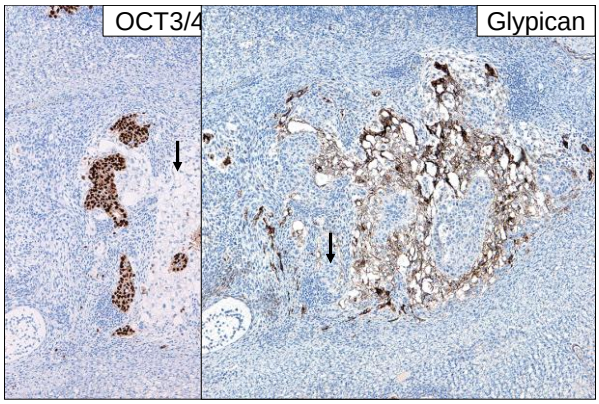
- GCNIS: Oct3/4, c- kit, SALL4, Podoplanin, PLAP - all (+)
- Seminoma: Oct3/4, c-kit, Podoplanin – all (+)
- Embryonal Ca: Oct3/4, CD30, SOX2, Keratin weak, – all (+)
- YST: Glypican, AFP, Keratin strong
- CC: HPL, βHCG, Glypican-syncytiotrophoblasts
- SS: CD117, SAL4 (weak)

Cytokeratin AE1/AE3: E Ca, YST, T, CC
Oct 3/4: Seminoma, E Ca
PLAP: Minimal / no value – except in GCNIS








OCT3/4
Glypican


THANK YOU