PROSTATE CANCER EVOLUTION: WHAT THE PATHOLOGIST NEEDS TO KNOW

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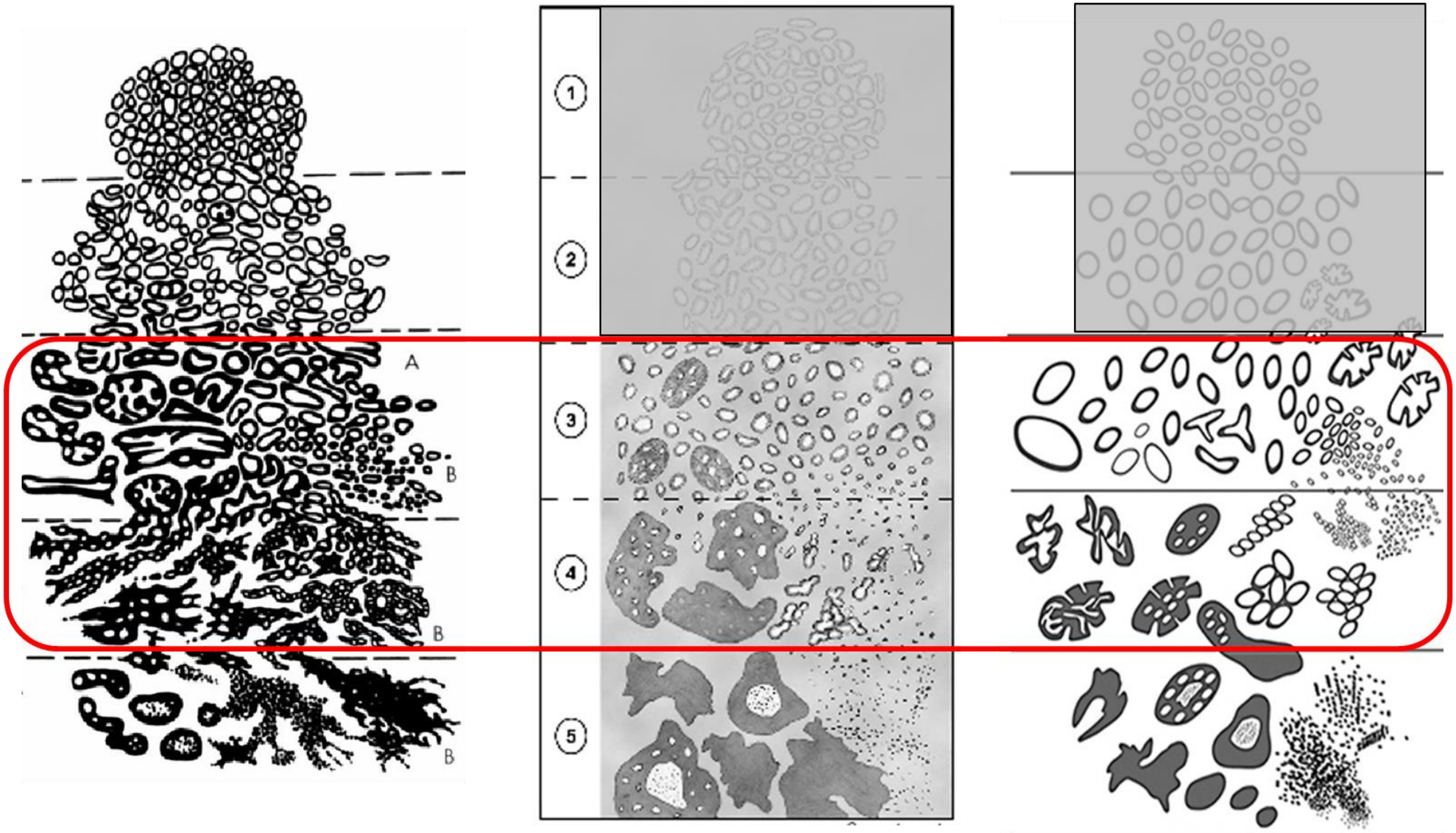
SCHOOL OF MEDICINE

OBJECTIVES

- Discuss relevant modifications in grading, staging & reporting
- Briefly discuss value/limitations of genomic tests and MRI
- Challenges & perspectives

UPDATE ON GRADING

EVOLUTION OF GLEASON GRADING OF PROSTATIC CARCINOMA

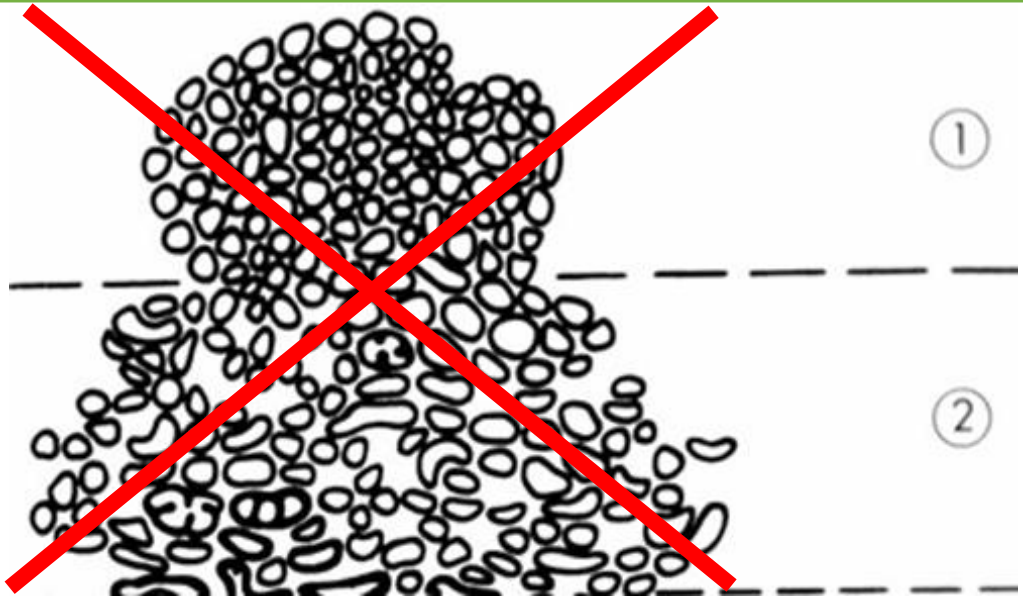


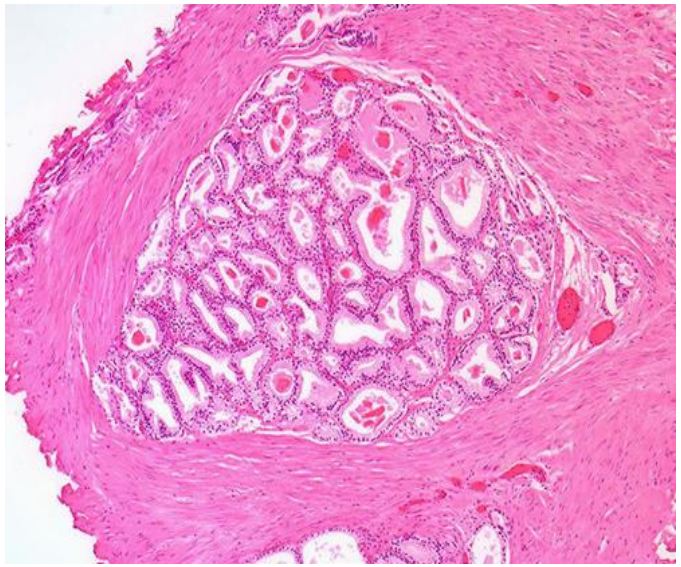
Original Gleason scheme 1967

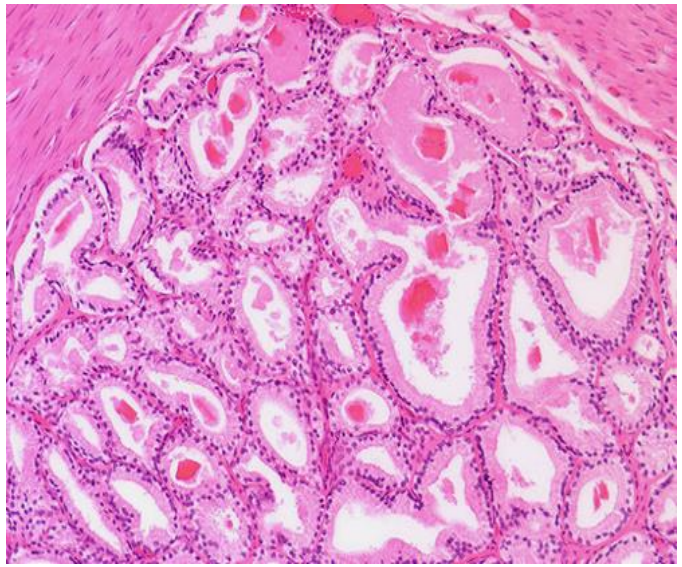
ISUP modified 2005

ISUP modified 2014

EVOLUTION OF GLEASON PATTERNS 1 AND 2







Diagnosis of GS 2-4 PCA should NOT be made on needle Bx

- Poor reproducibility
- Poor correlation with RP grade
- GS 2-4 PCA may misguide clinicians/patients into believing that tumor is indolent

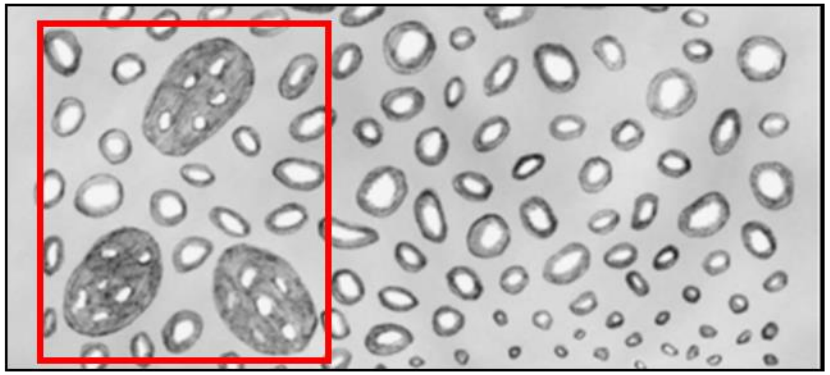
Most GS 2-4 diagnosed in the past represented **adenosis**

JI Epstein AJSP 2000; JI Epstein et al. AJSP, 2005

EVOLUTION OF GLEASON PATTERN 3

2005 ISUP modified

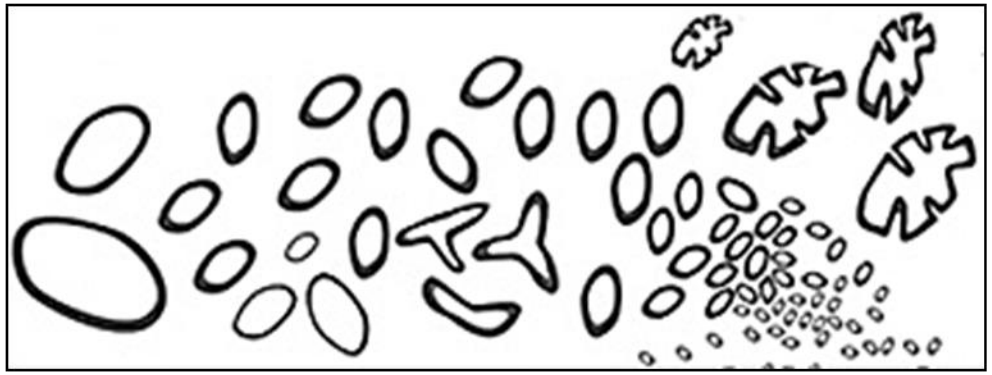
Epstein et al. AJSP 29, 2005



- Small well-formed glands
- Small cribriform lesions: exclude HGPIN with IHC
- ~~Medium to large sized cribriform glands eliminated~~

2014 ISUP modified

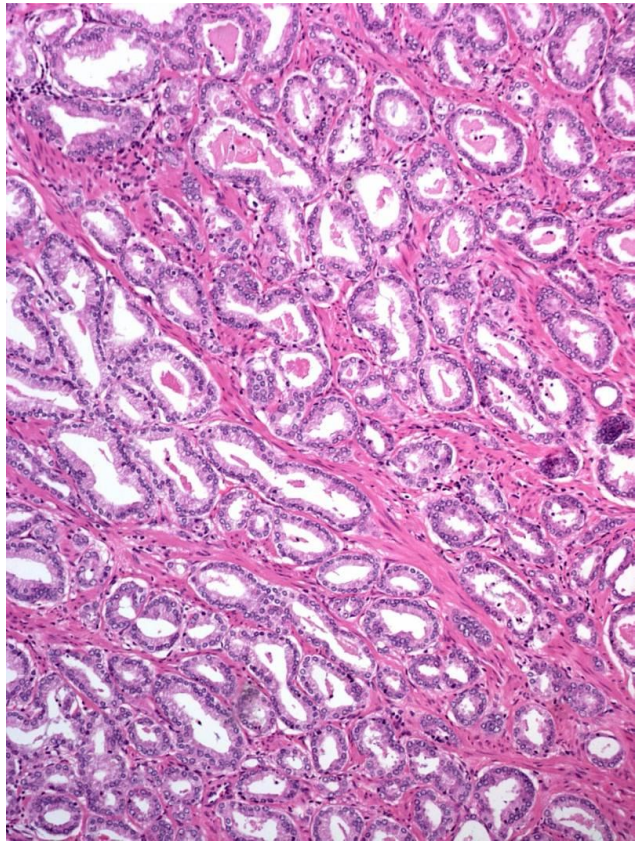
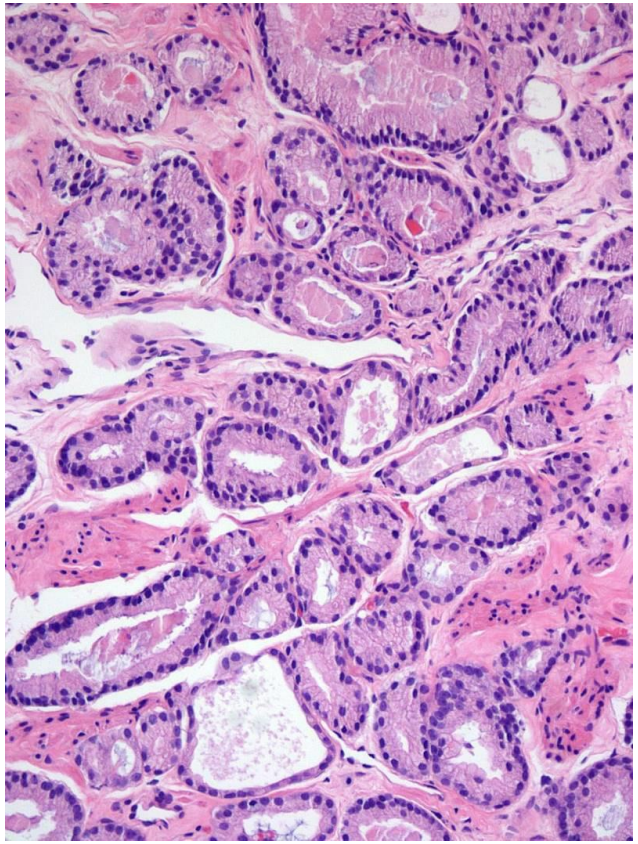
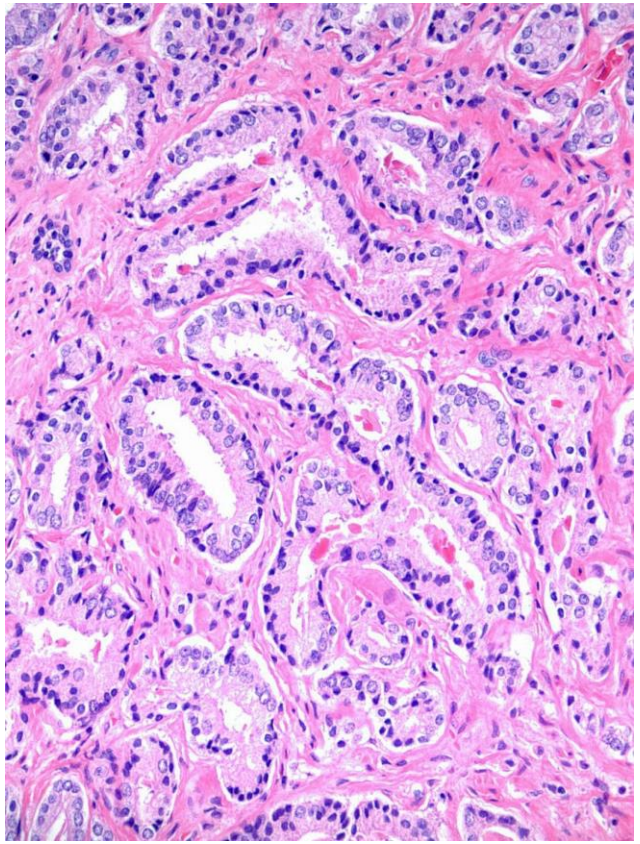
Epstein et al. AJSP 40, 2016



- Small well-formed glands
- Branched glands are allowed
- ~~Small cribriform glands~~

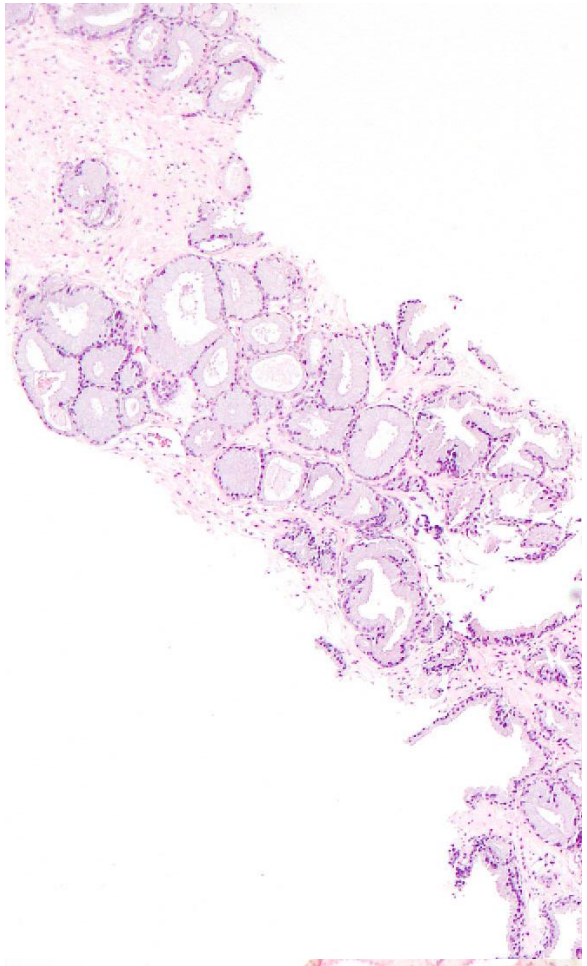
All cribriform glands should be graded as pattern 4 - ISUP, prostate cancer grading, Chicago, 2014 – 100% consensus

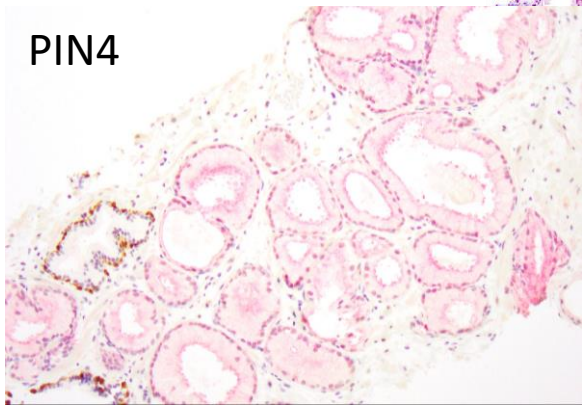
2014 ISUP MODIFIED GLEASON SYSTEM – PATTERN 3

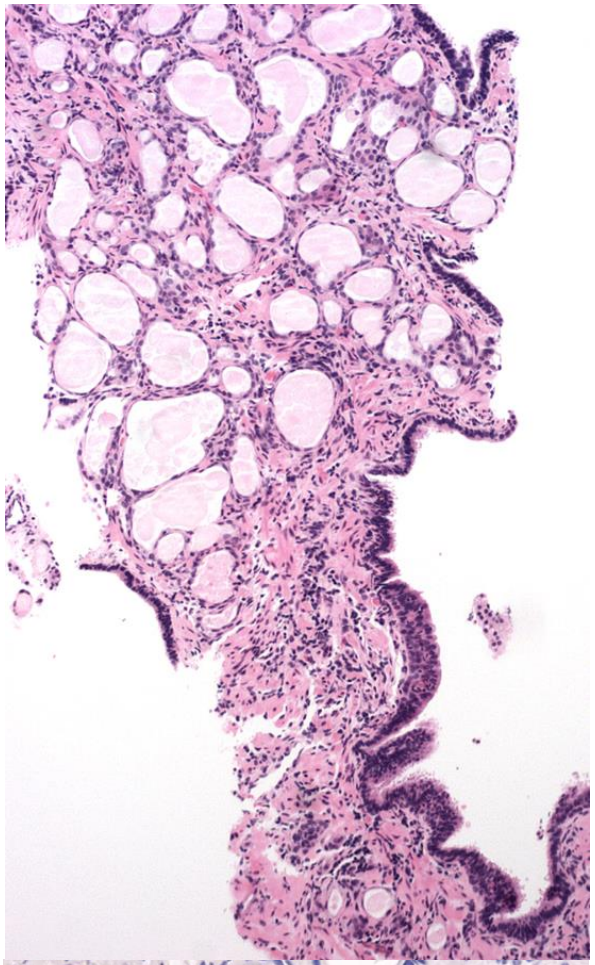
- Well-formed individual glands, discrete unit
- Variation is size and shape (microcystic and pseudohyperplastic)
- Branching glands are allowed in pattern 3

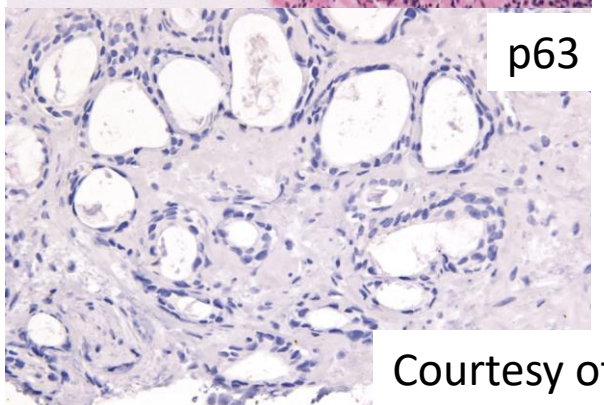
Foamy



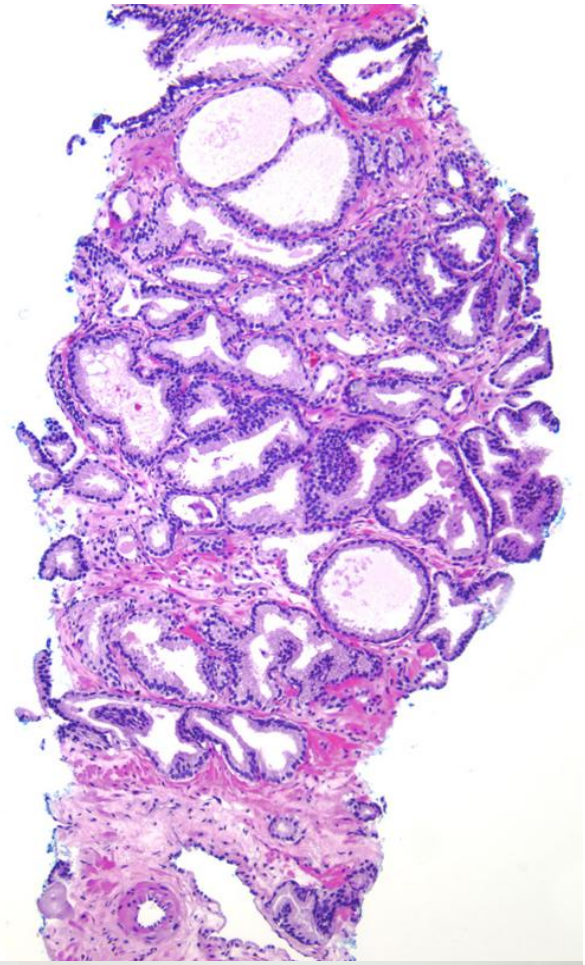


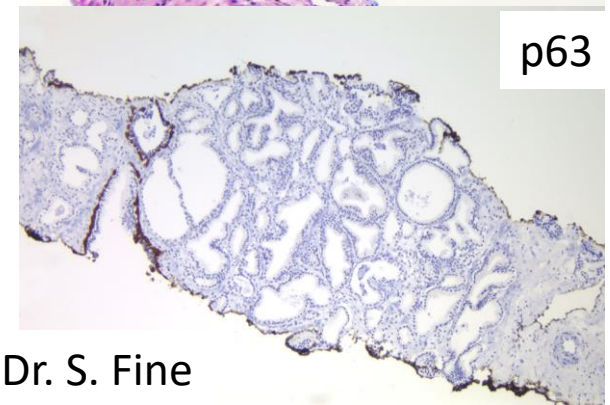
Atrophic



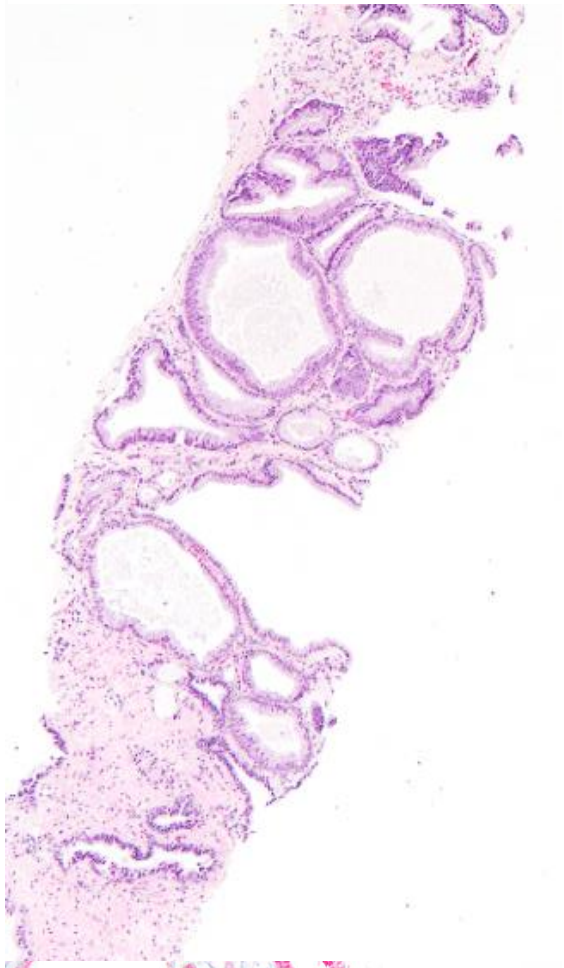


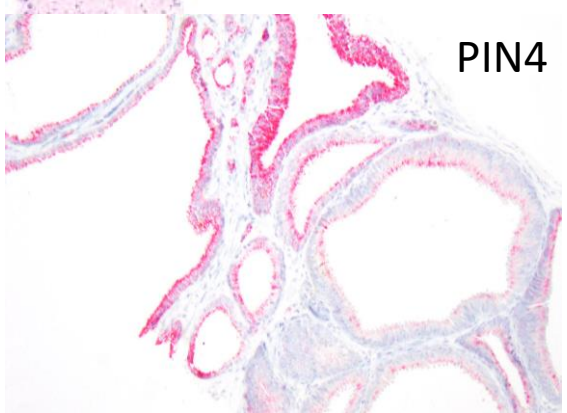
Pseudohyperplastic





Microcystic



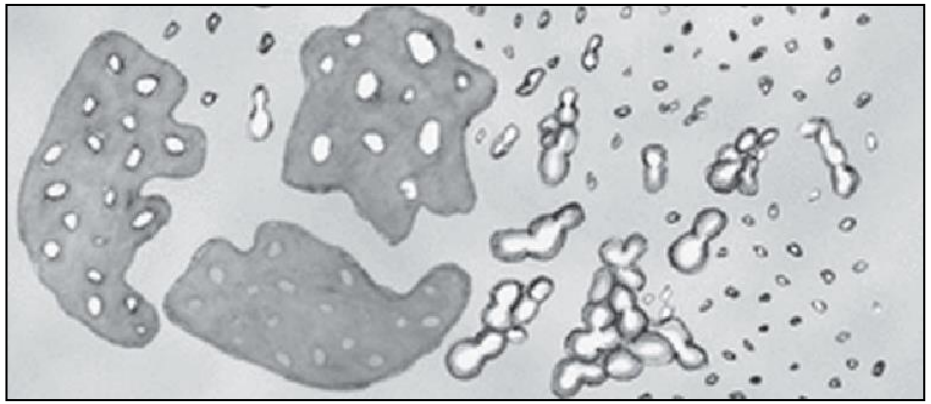


Courtesy of Dr. S. Fine

EVOLUTION OF GLEASON PATTERN 4

2005 ISUP modified

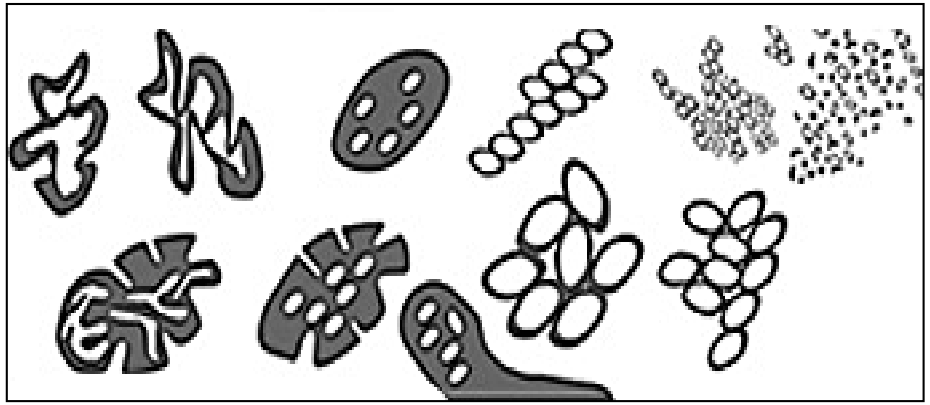
Epstein et al. AJSP 29, 2005



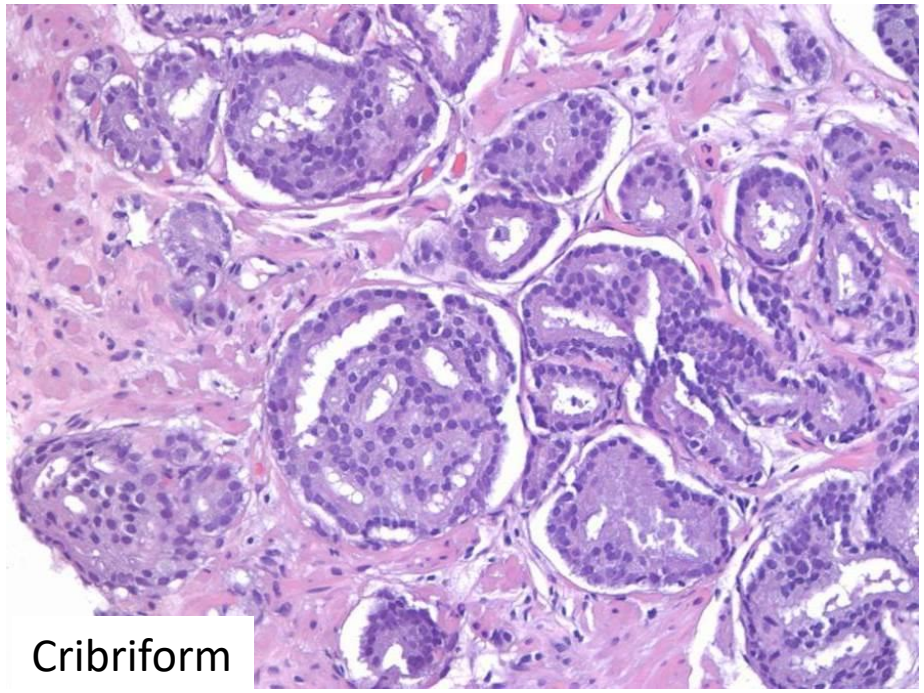
- Large cribriform glands
- Ill-defined glands with poorly-formed lumens
- Fused microacinar glands
- Hypernephroma-like tumors

2014 ISUP modified

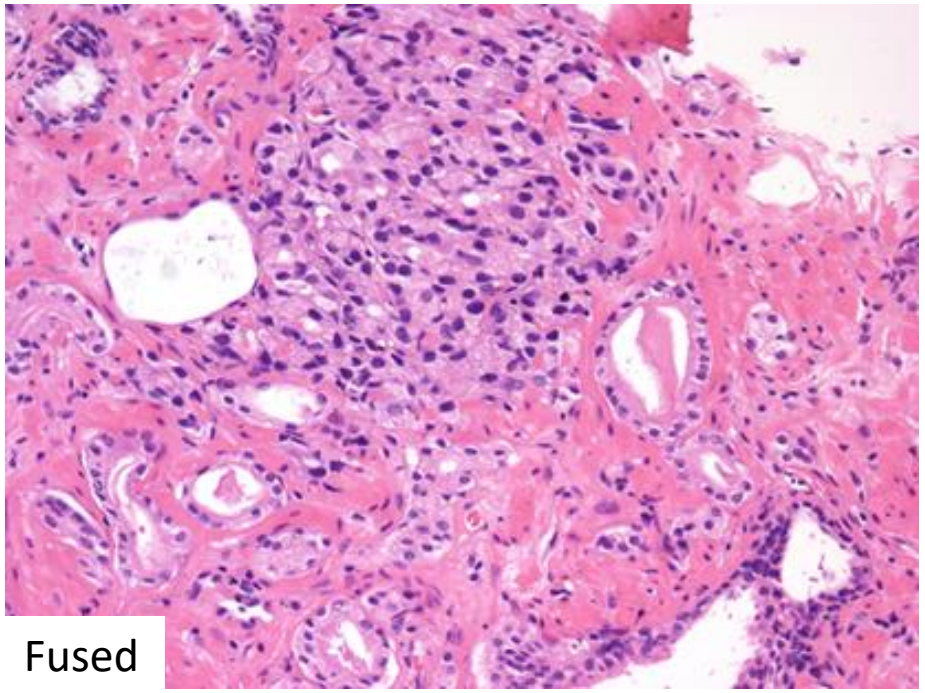
Epstein et al. AJSP 40, 2016



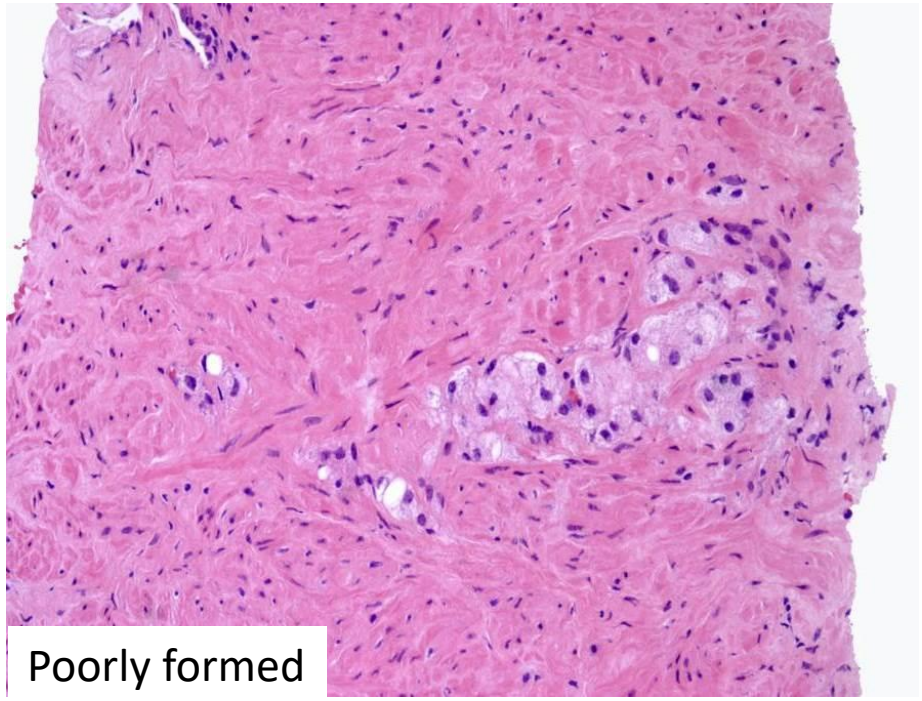
- Cribriform (small & large)
- Fused
- Poorly formed
- Glomeruloid glands
- ~~Hypernephromatoid term~~



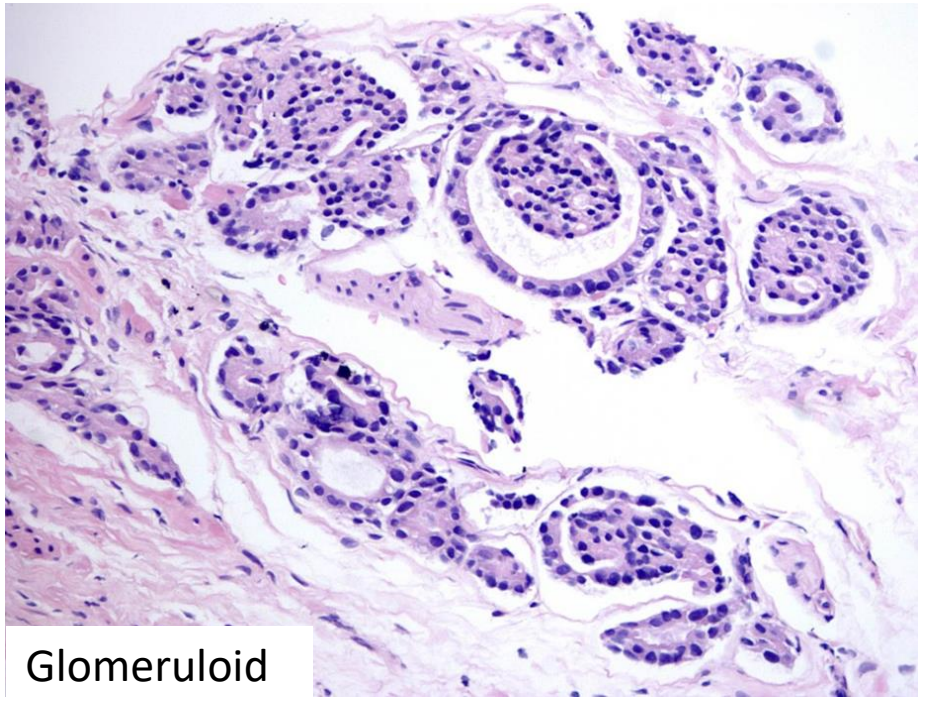
Cribriform



Fused



Poorly formed

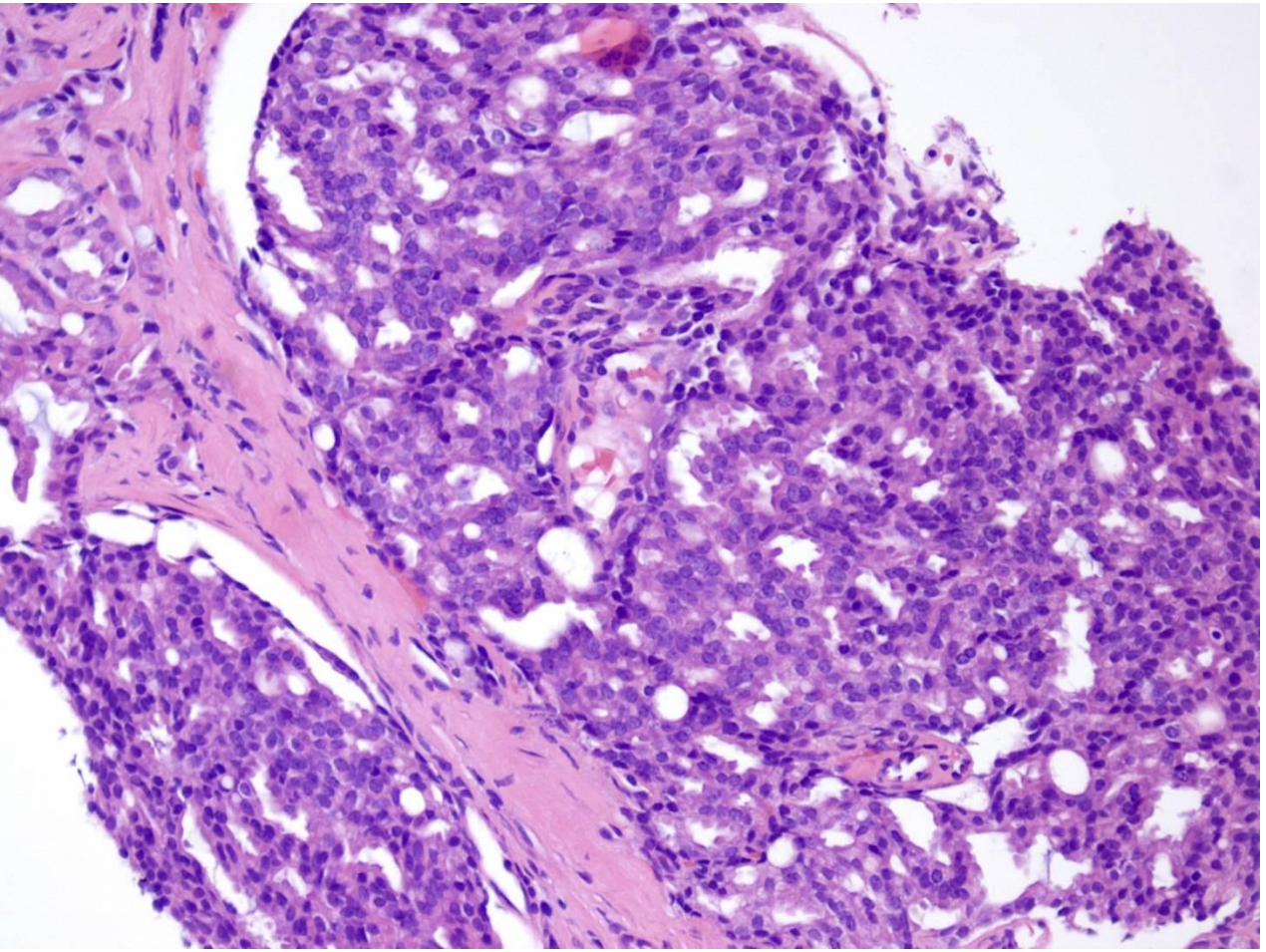


Glomeruloid

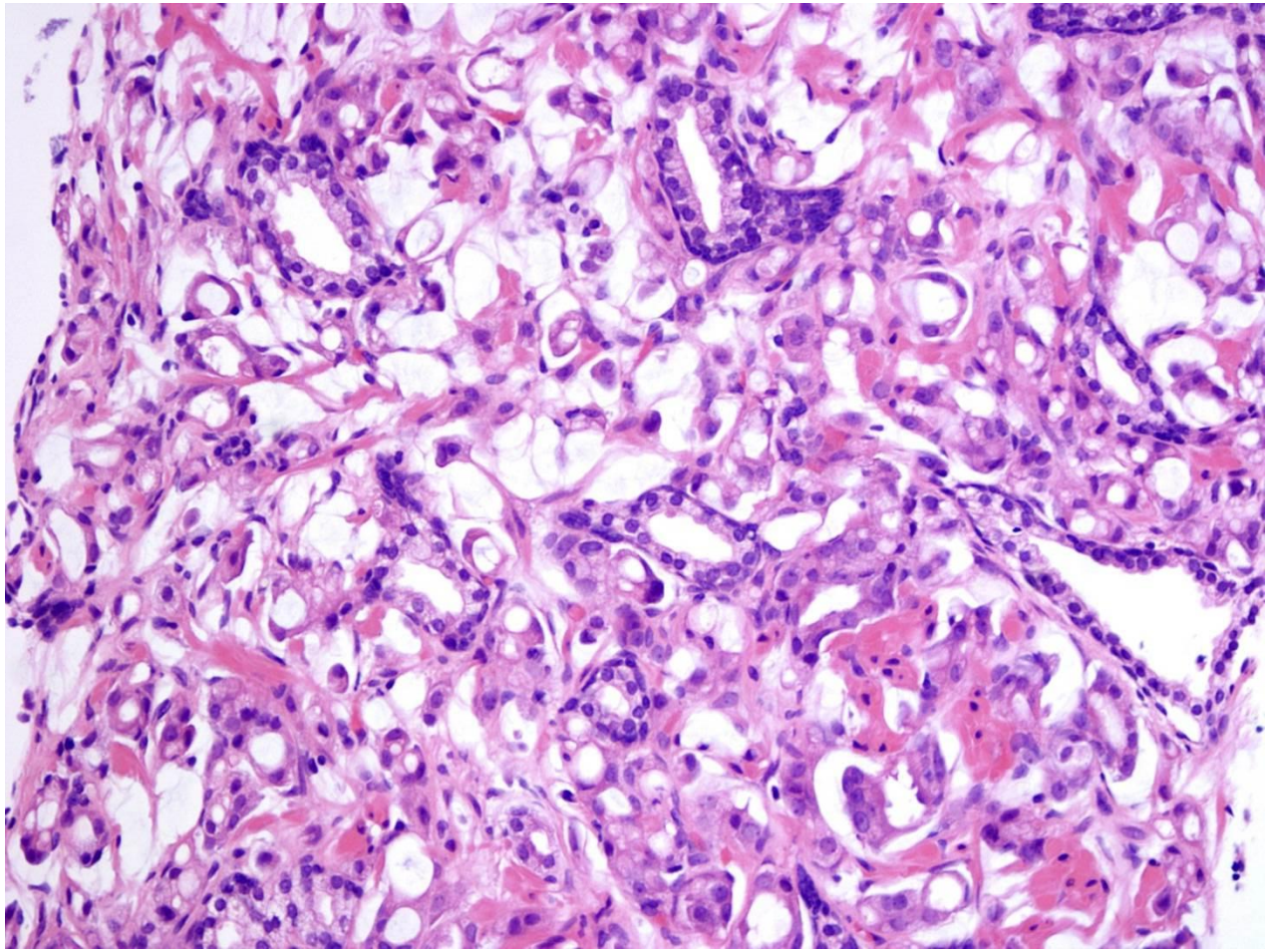
2014 ISUP modified Gleason system

pattern 4

MODIFIED GLEASON PATTERN 4 - CRIBRIFORM & FUSED GLANDS

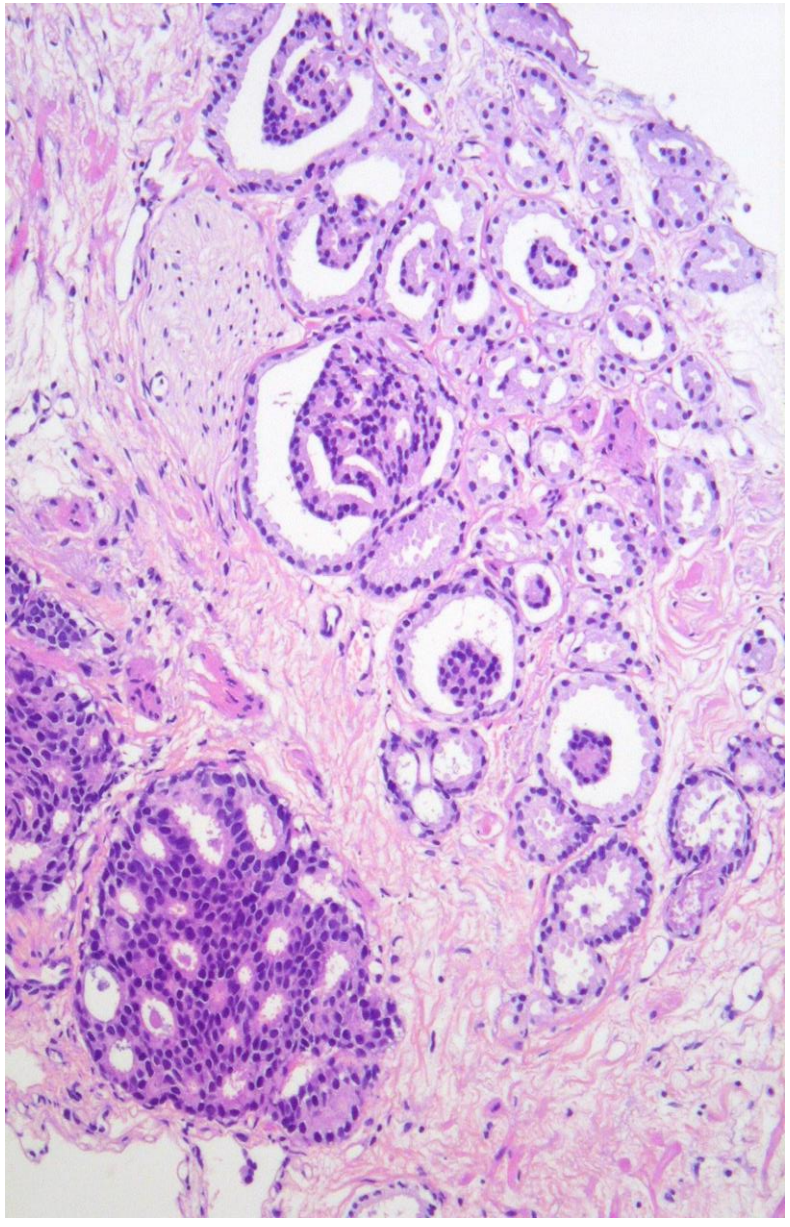


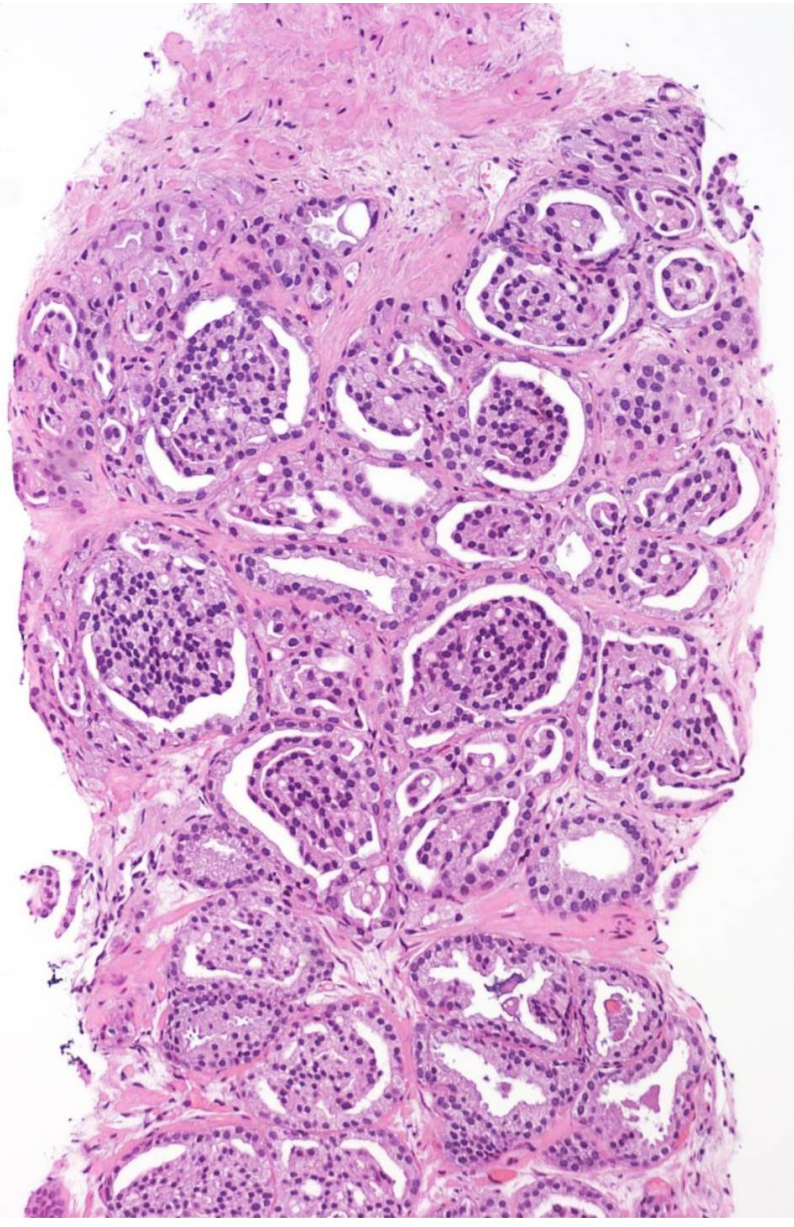
Large cribriform

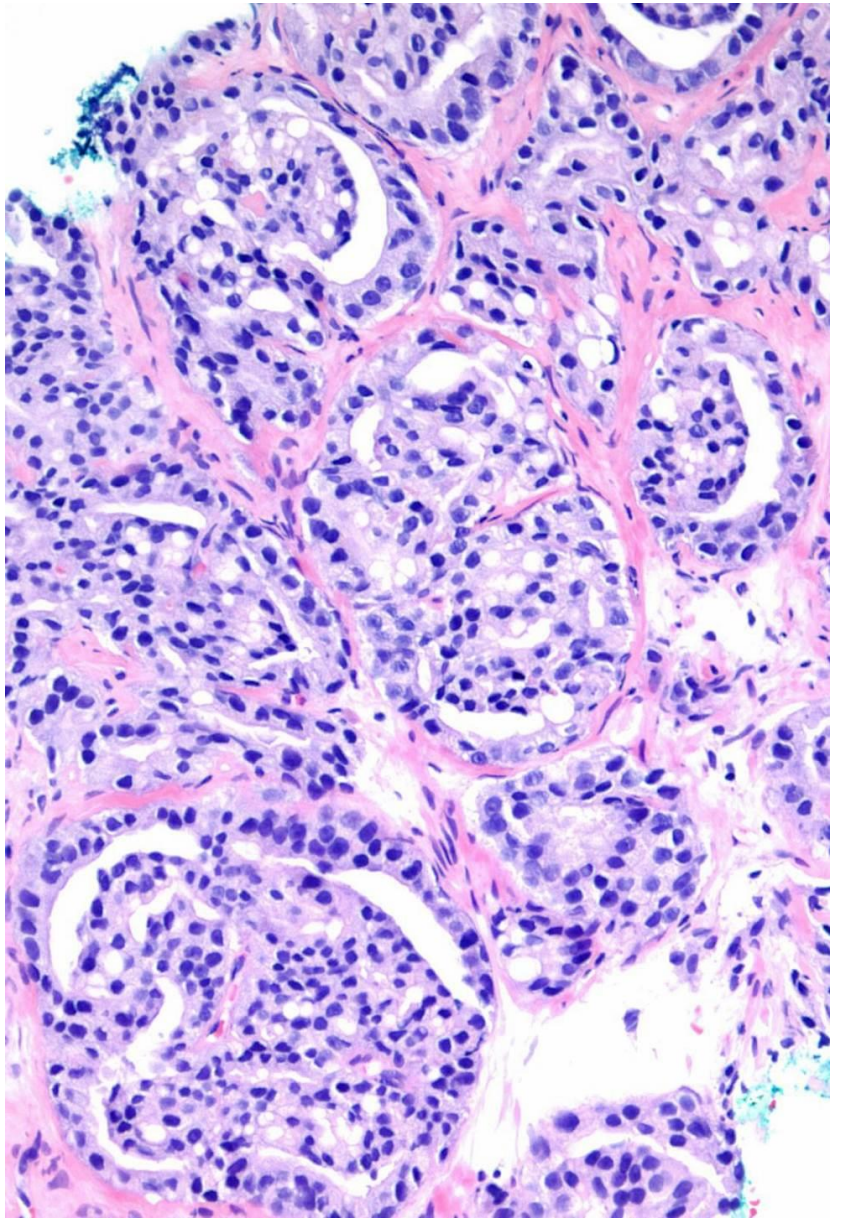


Fused microacinar

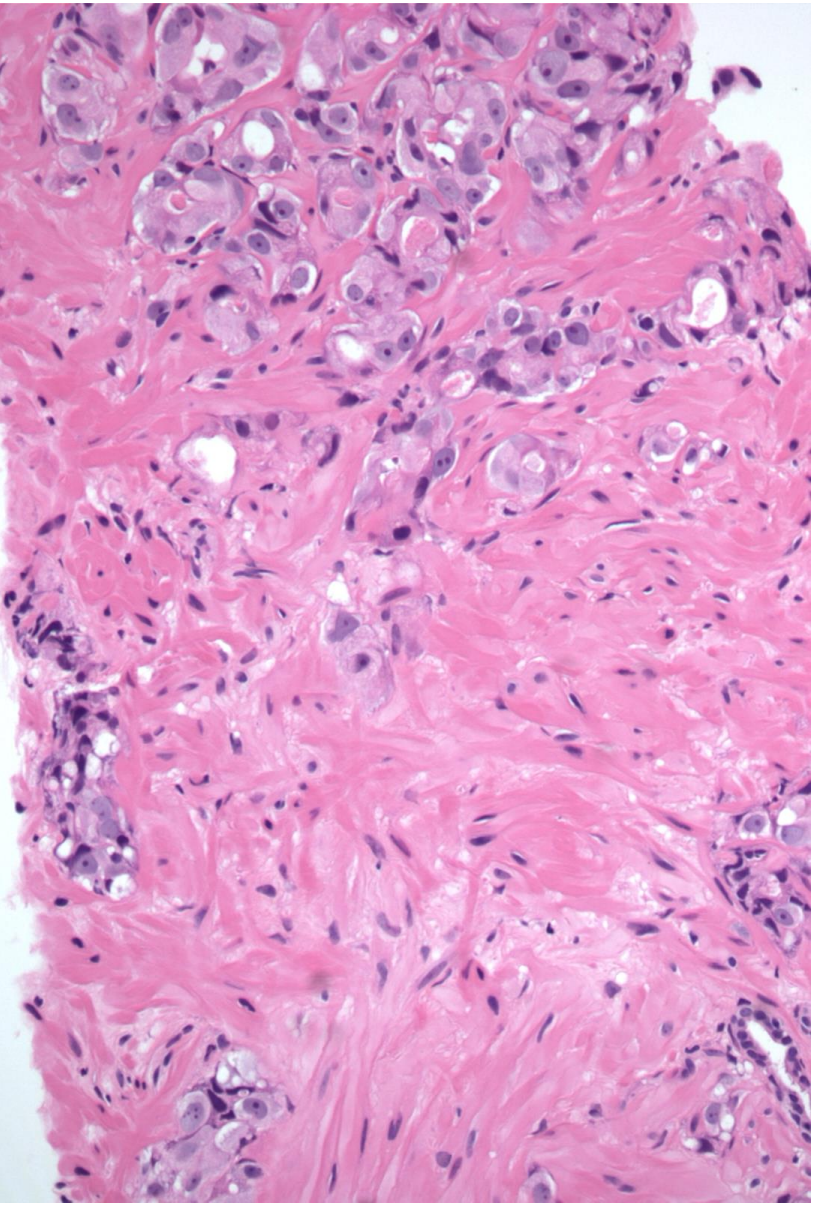
MODIFIED GLEASON PATTERN 4 - GLOMERULOID & CRIBRIFORM

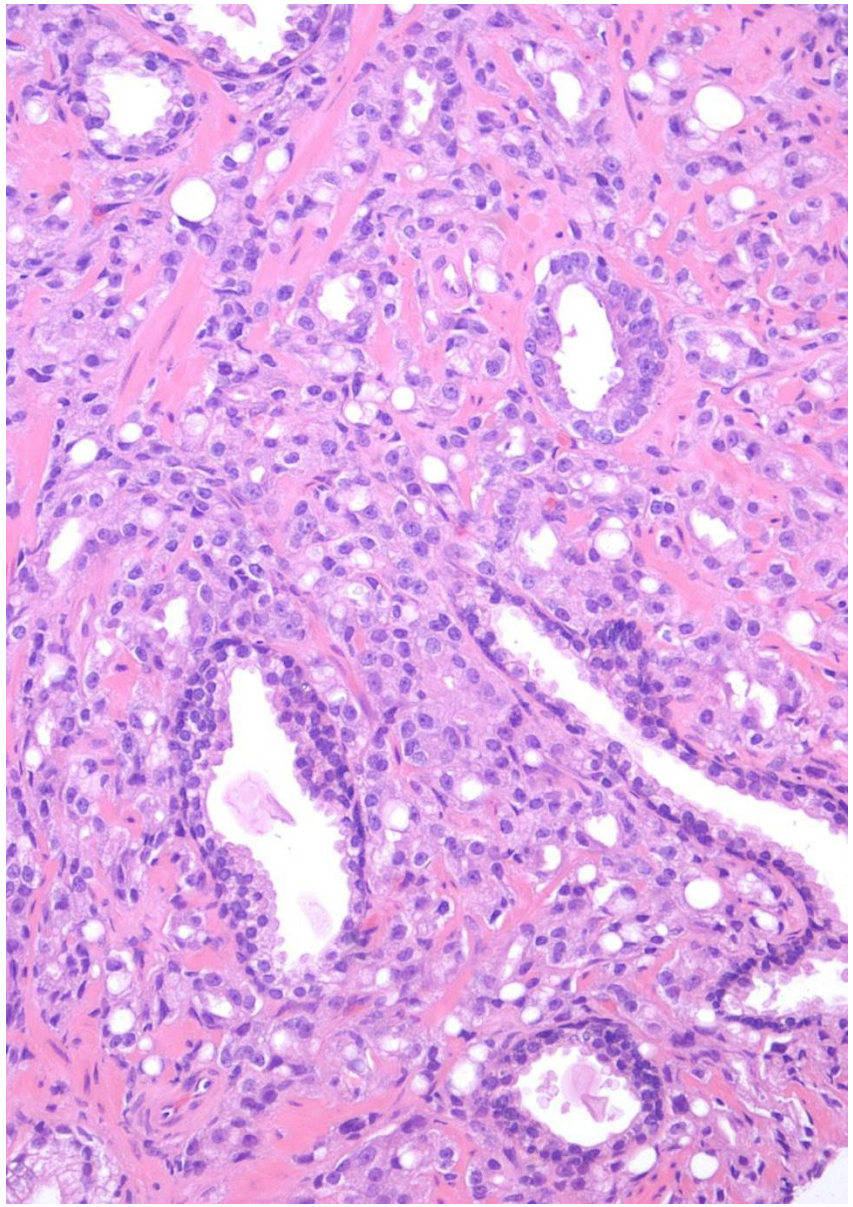


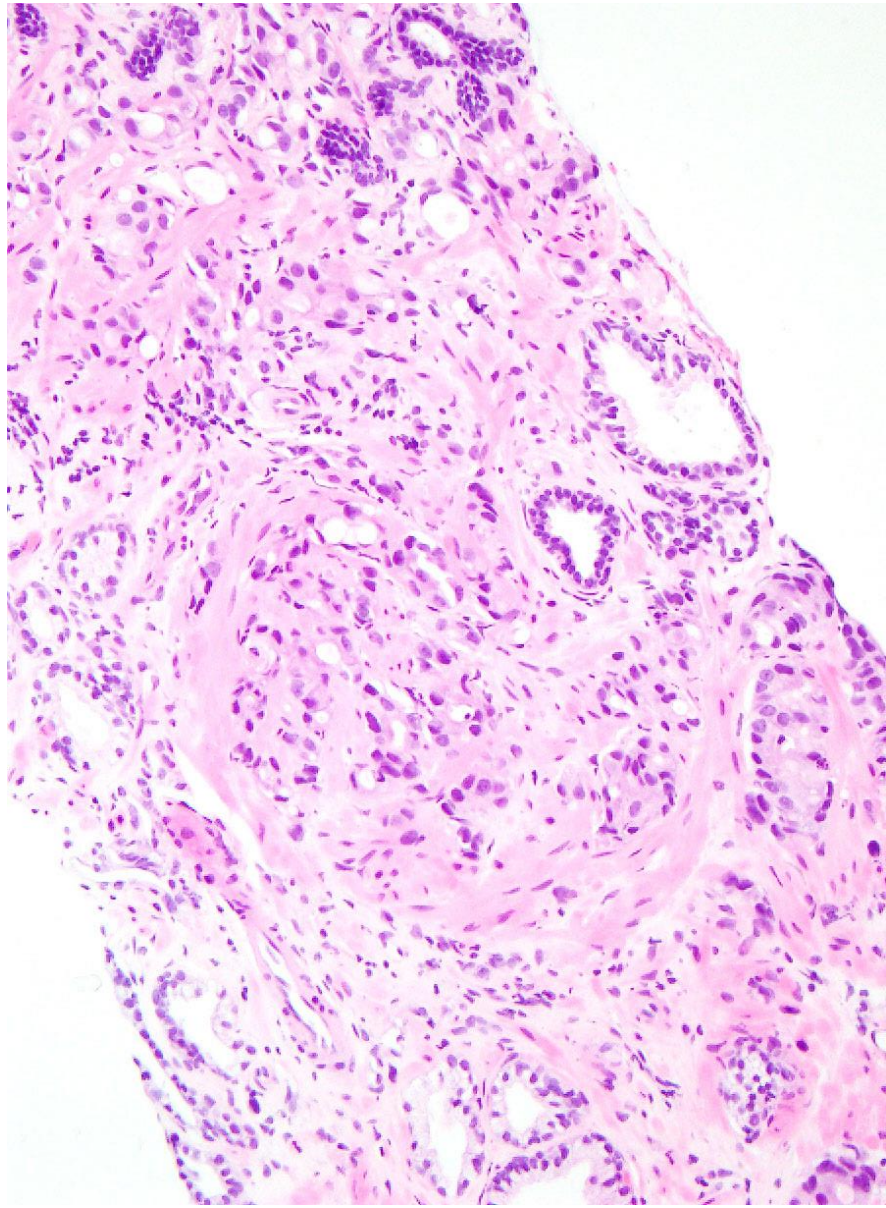




MODIFIED GLEASON PATTERN 4 - POORLY FORMED GLANDS



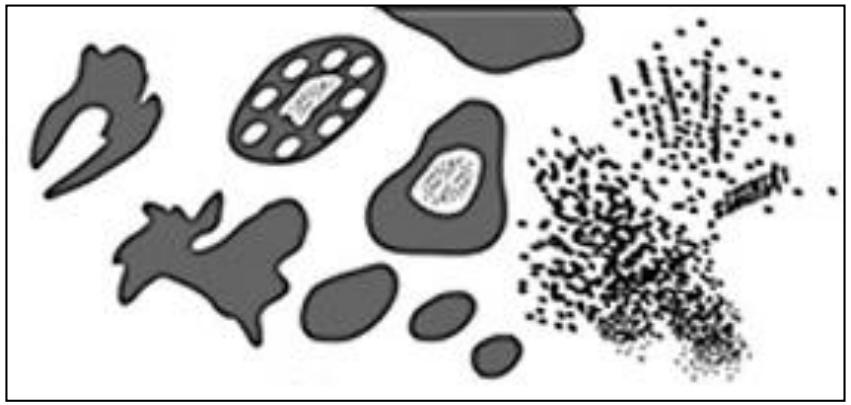




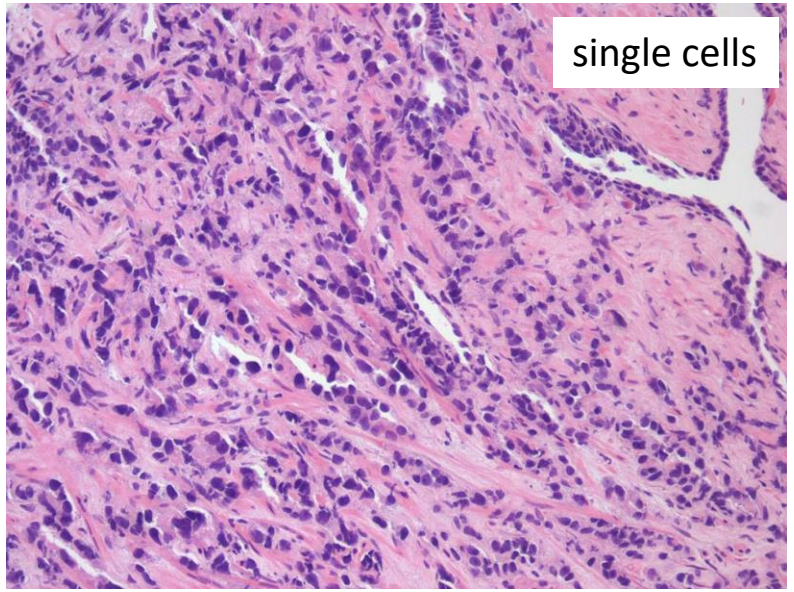
EVOLUTION OF GLEASON PATTERN 5

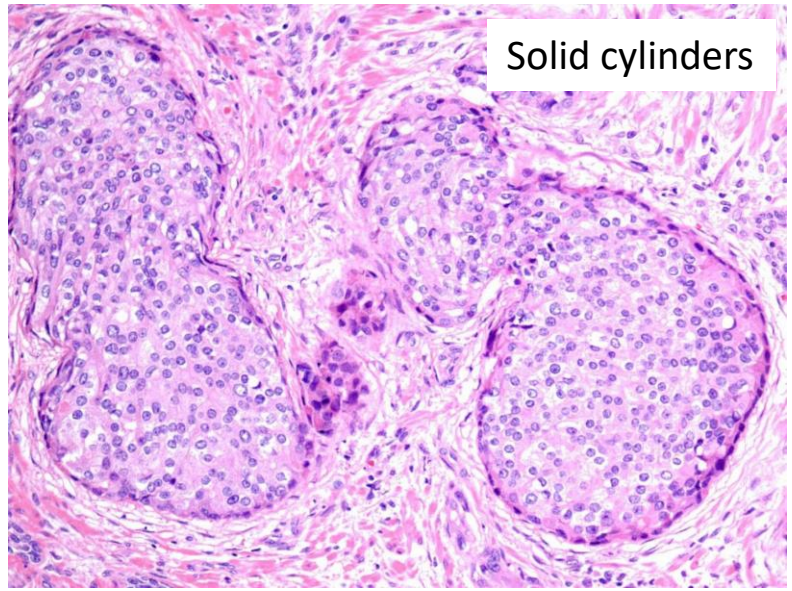
2014 ISUP modified

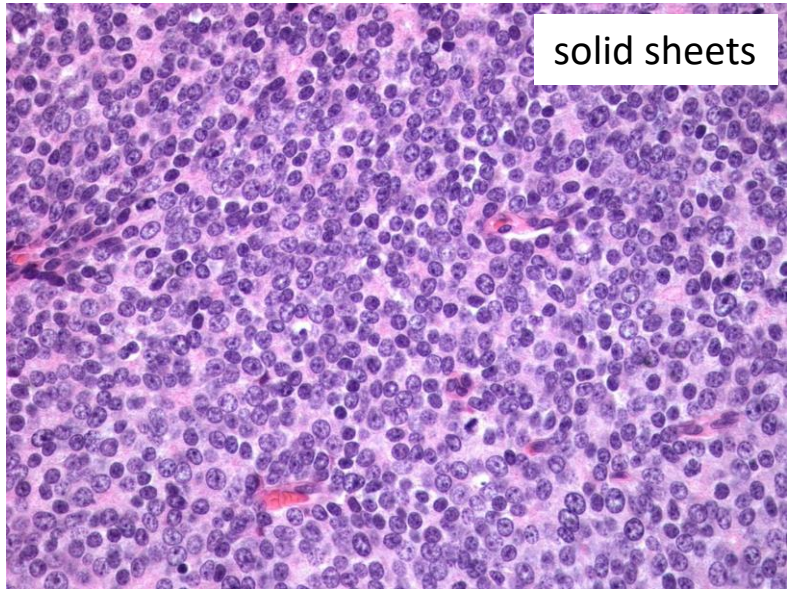
Epstein et al. AJSP 40, 2016

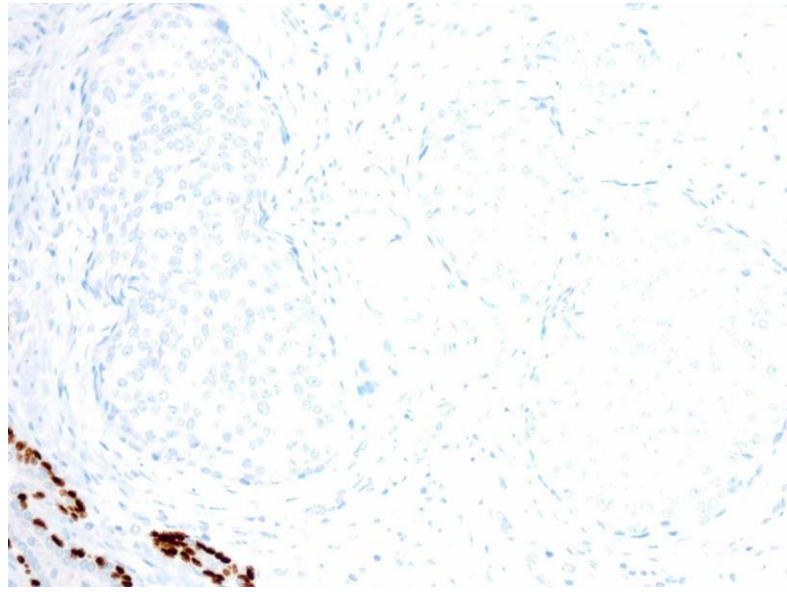


- Small solid cylinders
- Solid medium to large nests with rosette-like spaces
- Comedonecrosis, even focal
- Single cells







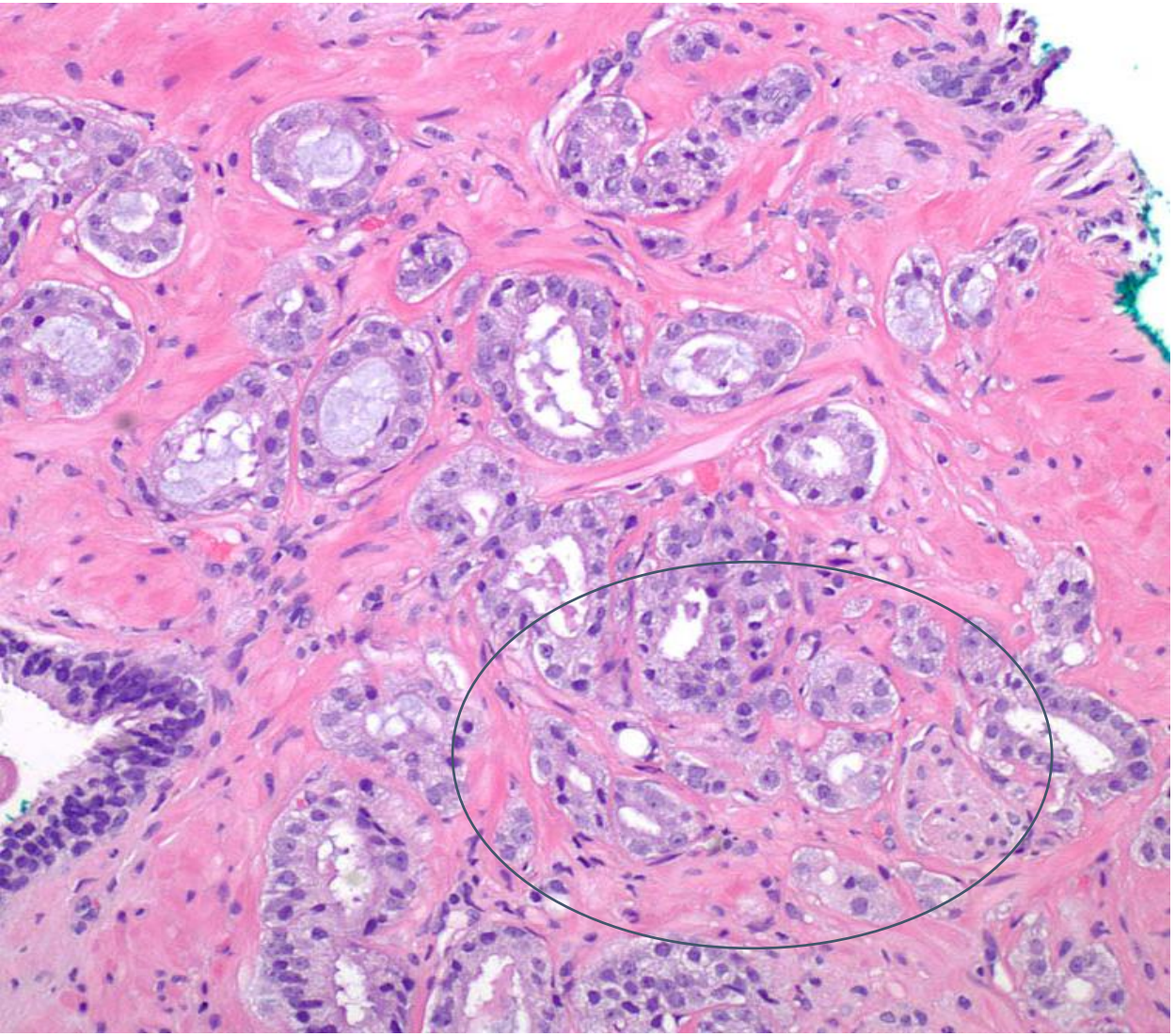


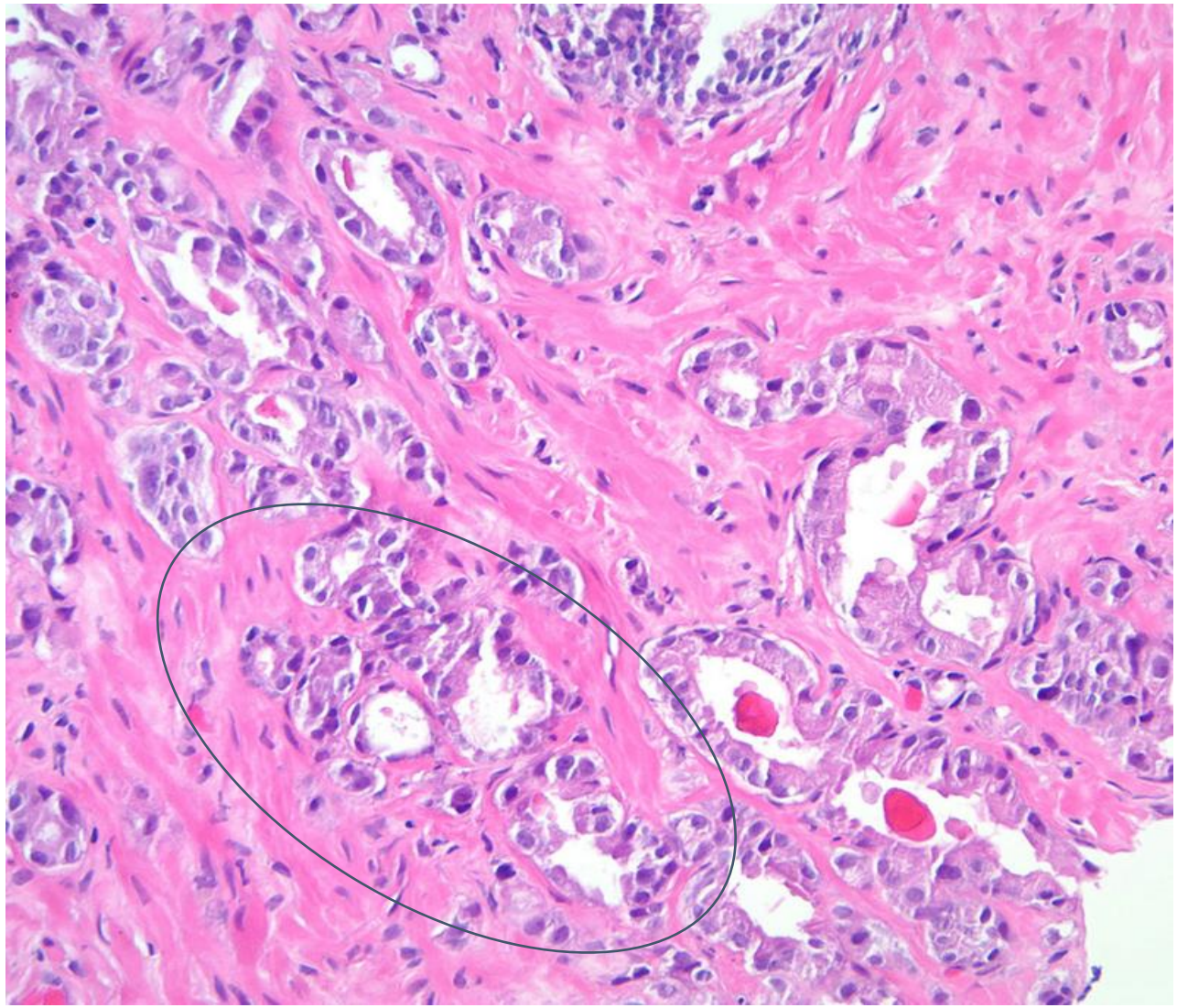
PITFALLS

- **Fused glands** (modified Gleason pattern 4):
  - May be under-graded when present in small foci
  - On the other hand, careful evaluation of multiple tissue levels may be necessary to determine whether few glands are truly fused or simply tangentially cut

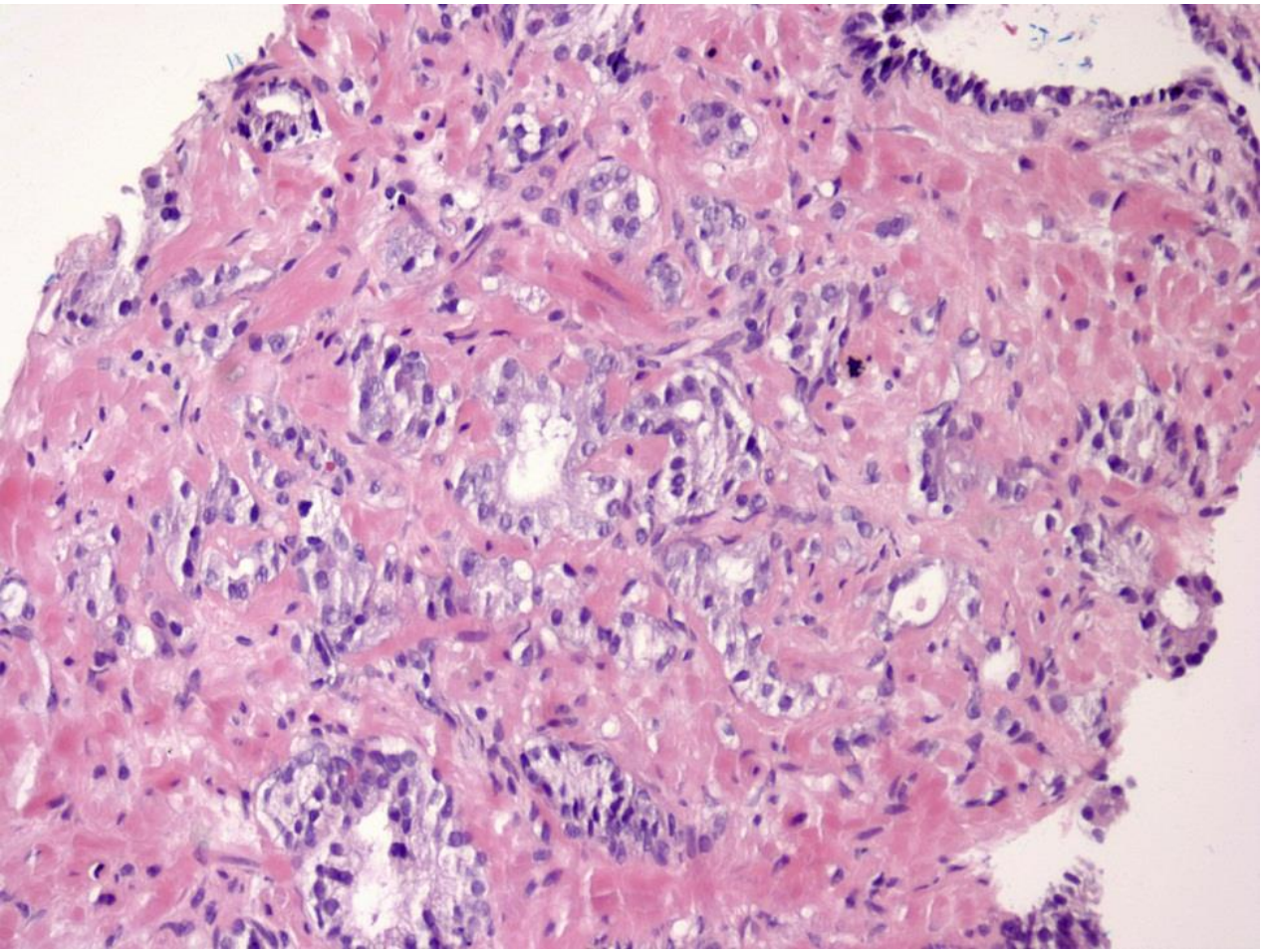
- **Ill-defined glands**:
  - Ill-defined glands with poorly formed glandular lumina should be graded as pattern 4
  - Caution should be applied in distinguishing them from very small “well-formed” glands (modified Gleason pattern 3)

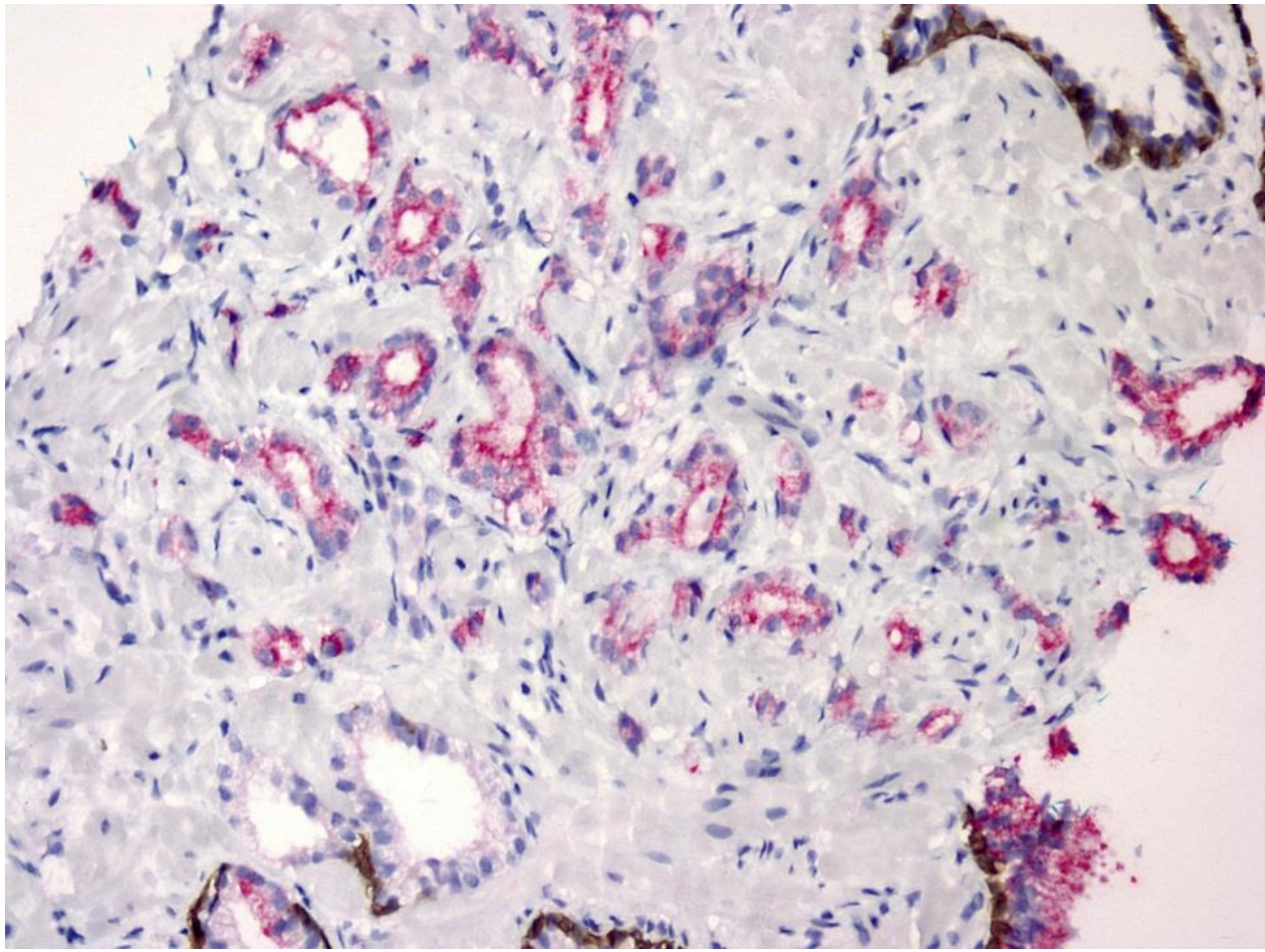
GS 3+3=6 VS. GS 3+4=7



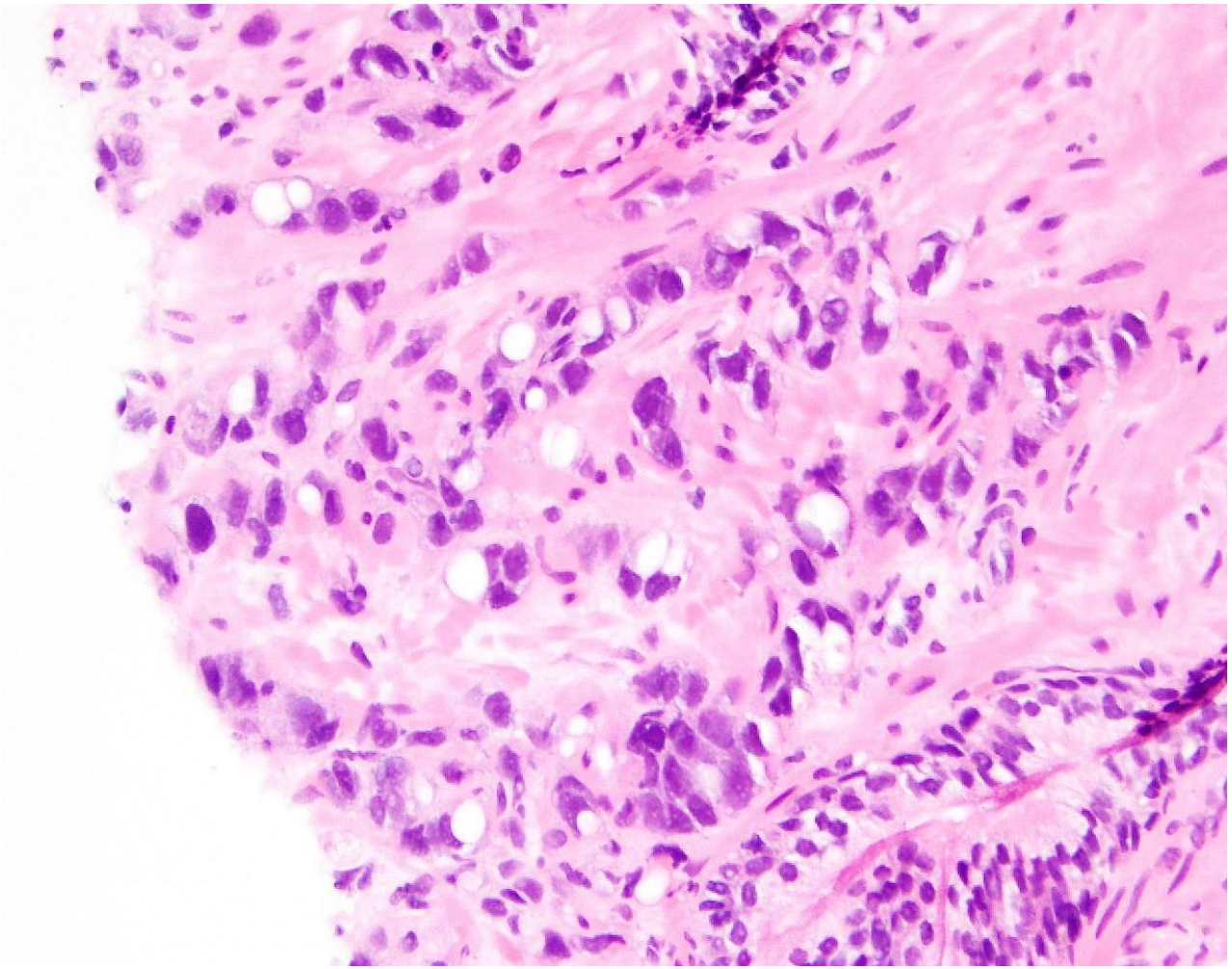


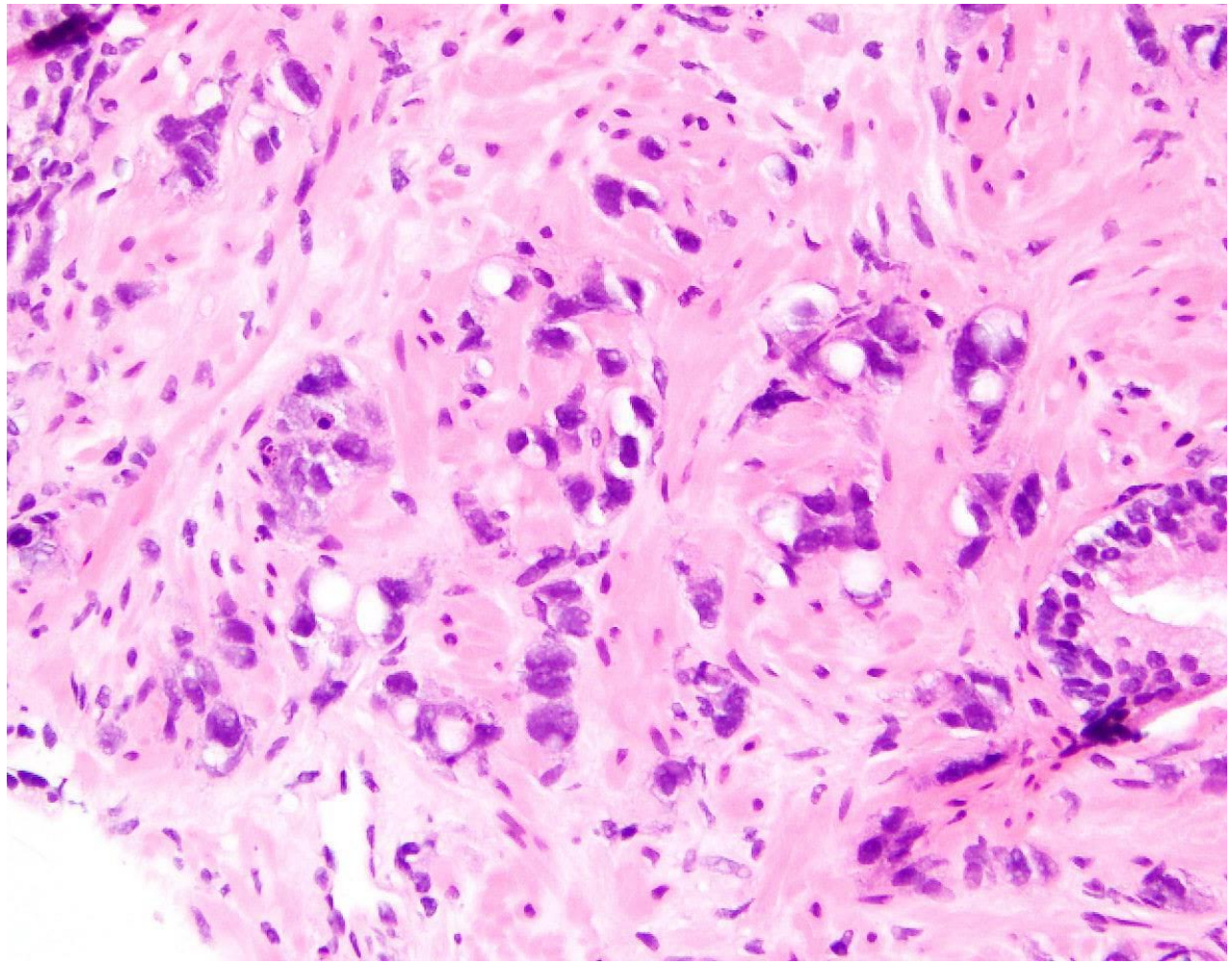
GS 3+3=6 VS. GS 3+4=7





POORLY FORMED GLANDS (PATTERN 4) V. SINGLE CELLS (PATTERN 5)?





Evaluate multiple levels!!!

2014 ISUP CONSENSUS CONFERENCE ON GLEASON GRADING

Recommendations

- For a diagnosis of Gleason pattern 4, it needs to be seen at x10 lens magnification
  - Vote: 78% yes

- Occasional/seemingly poorly formed or fused glands between well-formed glands is insufficient for a diagnosis of pattern 4
  - Vote: 85% yes

- In cases with borderline morphology between pattern 3 and 4 and crush artifacts, the **lower grade** should be favored
  - Vote: 98% yes

IMPACT OF MODIFIED GLEASON GRADING SYSTEM

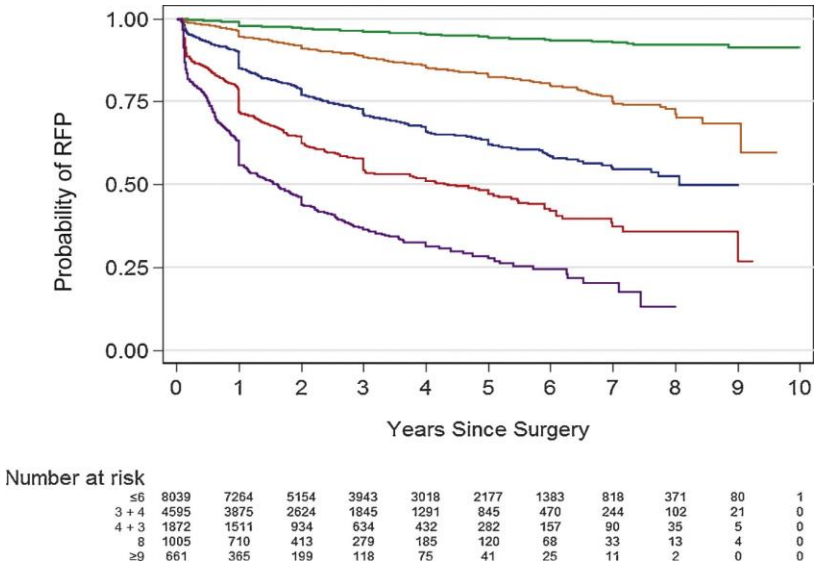
- GS6 is lowest score on PBx (confusing for patients and clinicians)
- Current GS6 has little propensity to recur or metastasize
- Several morphologies previously considered pattern 3 are currently assigned a pattern 4
    - GS6 tumors have decreased
    - GS7 tumors have increased
- Inter-observer reproducibility and Bx–PR concordance have improved
- GS7 includes patients with different prognosis:
    - 3+4=7 have better prognosis than 4+3=7

NEW 5-GRADE GROUP SYSTEM (ISUP & WHO)

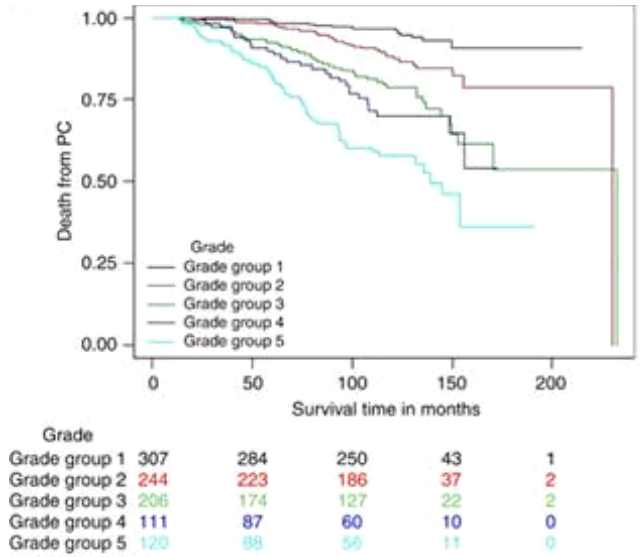
Grade Group 1	**GS ≤6**	Only individual discrete well-formed glands
Grade Group 2	**GS 3+4=7**	Predominantly well-formed glands with lesser component of poorly-formed/fused/cribriform glands
Grade Group 3	**GS 4+3=7**	Predominantly poorly-formed/fused/cribriform glands with a lesser component of well-formed glands
Grade Group 4	**GS 4+4=8** **GS 3+5=8** **GS 5+3=8**	Only poorly-formed/fused/cribriform glands Predominantly well-formed glands with a lesser component lacking glands Predominantly lacking glands with a lesser component of well-formed glands
Grade Group 5	**GS 9/10**	Lacks gland formation (or with necrosis) with or w/o poorly-formed/fused/cribriform glands

Pierorazio et al. BJU Int 2013; Epstein JI et al. AJSP 2016

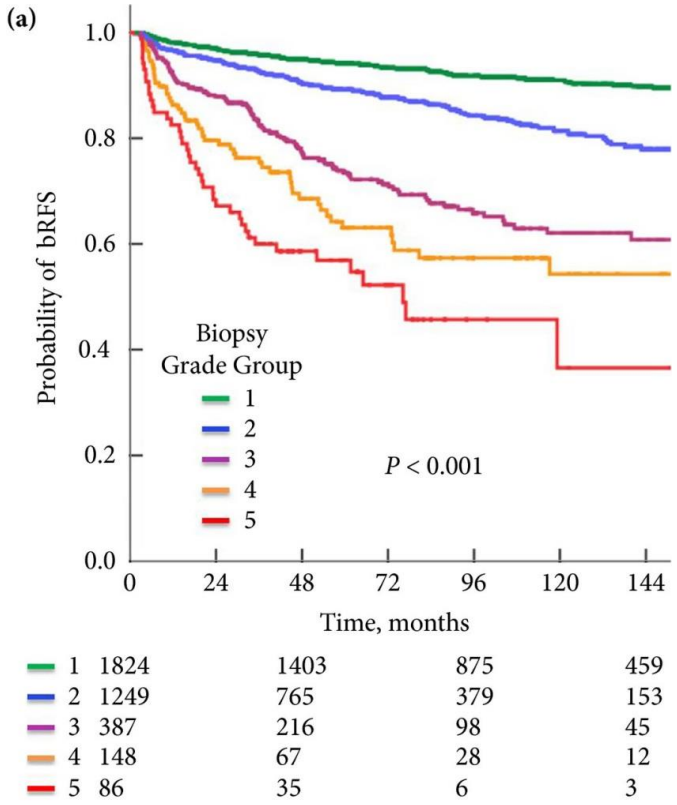
5-grade groups (GG) system validation



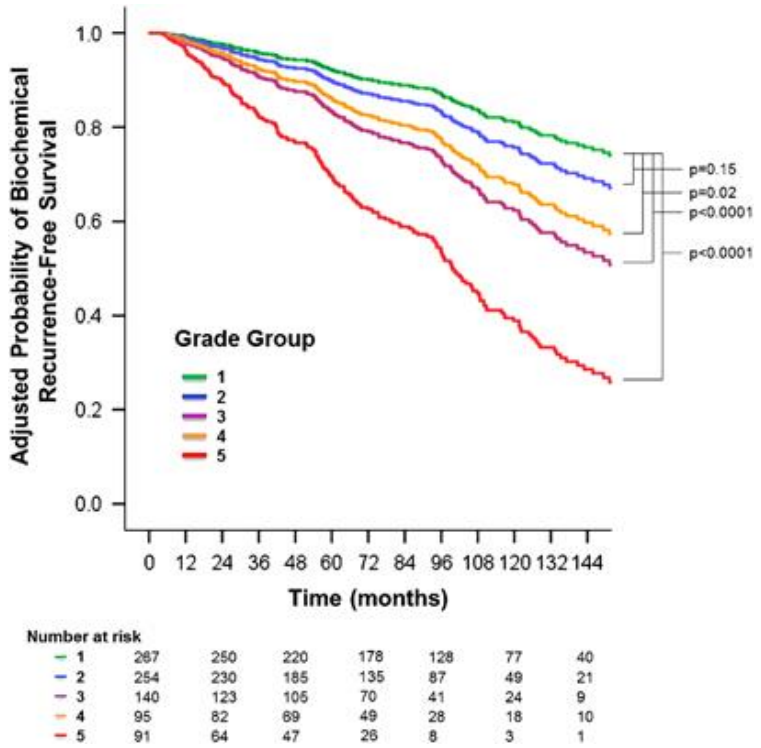
Epstein JI et al. Eur Urol 2016 (20,800 men)



Berney et al. British Journal of Cancer 2016 (988 men treated conservatively)

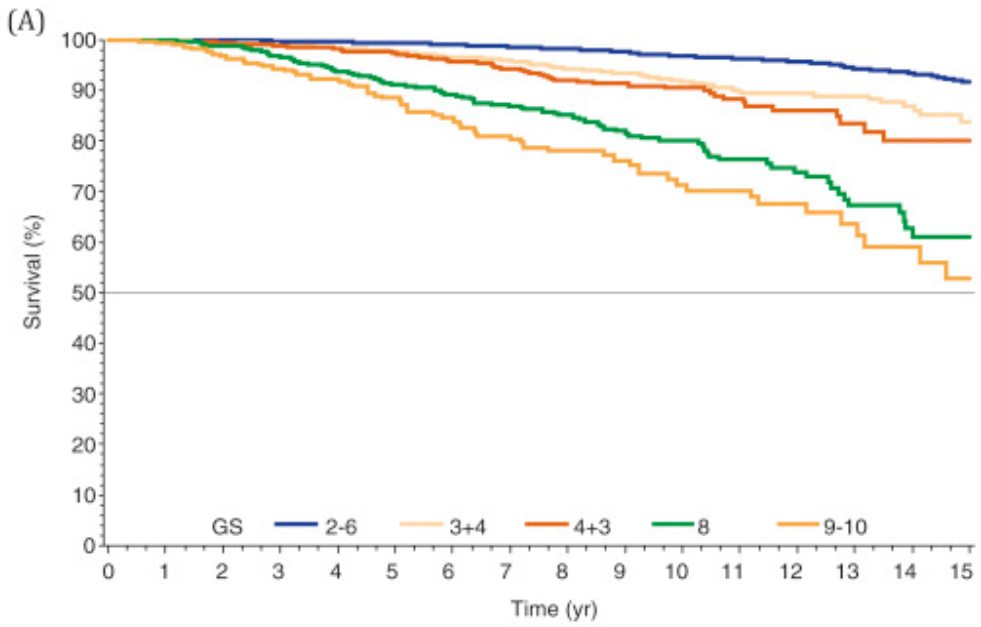


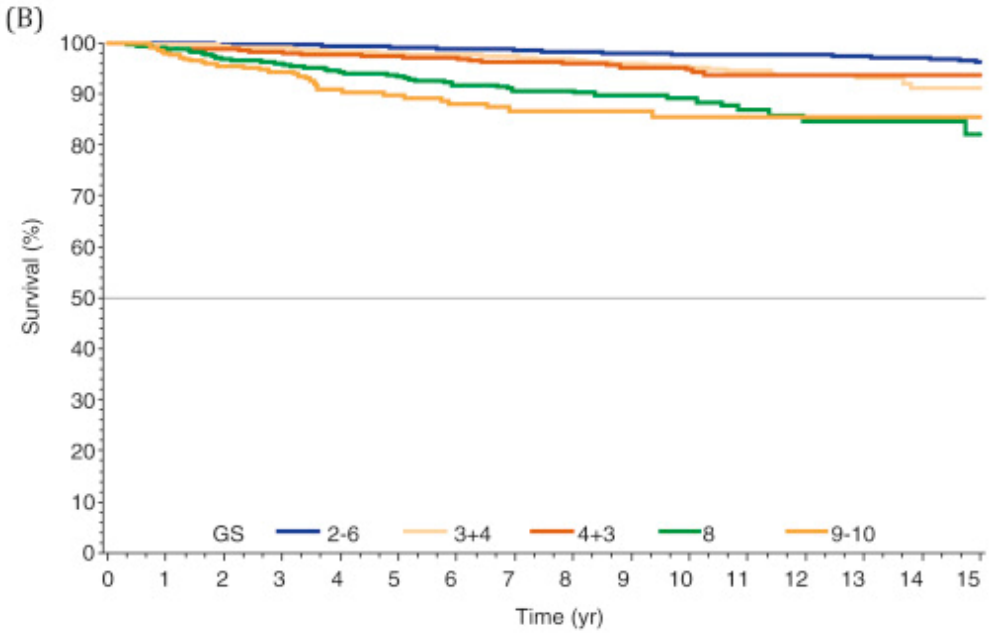
Spratt et al. BJUI 2016 (3,700 cases)



Spratt et al. Prostate Cancer Prostatic Dis 2016 (847 radiation-treated)

GG SYSTEM ASSOCIATED WITH RISK OF PROSTATE CANCER-SPECIFIC MORTALITY AND BONE METASTASIS PROGRESSION





Biopsy Gleason grade	Total patients at risk (*n*)	Patients at risk at 1 yr (*n*)	CEs at 1 yr (*n*)	Patients at risk at 5 yr (*n*)	CEs at 5 yr (*n*)	Patients at risk at 10 yr (*n*)	CEs at 10 yr (*n*)	Patients at risk at 15 yr (*n*)	CEs at 15 yr (*n*)
I (2–6)	6776	6456	4	4409	38	2121	116	558	172
II (3 + 4)	1773	1654	4	988	31	396	72	67	87
III (4 + 3)	955	874	3	503	18	190	44	23	55
IV (8)	636	600	1	362	42	141	73	31	92
V (9–10)	389	350	3	195	32	68	59	14	68

Biopsy Gleason grade	Total patients at risk (*n*)	Patients at risk at 1 yr (*n*)	CEs at 1 yr (*n*)	Patients at risk at 5 yr (*n*)	CEs at 5 yr (*n*)	Patients at risk at 10 yr (*n*)	CEs at 10 yr (*n*)	Patients at risk at 15 yr (*n*)	CEs at 15 yr (*n*)
I (2–6)	6722	6367	5	4346	50	2063	94	538	108
II (3 + 4)	1757	1607	6	971	28	383	46	64	54
III (4 + 3)	943	840	10	479	21	184	29	22	31
IV (8)	617	565	7	338	33	135	44	27	50
V (9–10)	368	308	7	173	27	65	33	13	33

Leapman et al. Eur Urol 2017

IMPACT OF THE MODIFIED GLEASON GRADING AND GRADE GROUPS

- Provides clearer labels for patient understanding
- Defines a more homogenous low-risk group (i.e. Grade Group 1)
- Distinguish Grade Group 2 (3+4=7) (AS eligible) from Grade Group 3 (4+3=7) (AS non-eligible)
- Re-definition of Gleason pattern 4 might reduce upgrading from Bx to RP specimen

UPDATE ON STAGING

PROSTATE CANCER STAGING (AJCC 8TH EDITION)

Pathologic stage

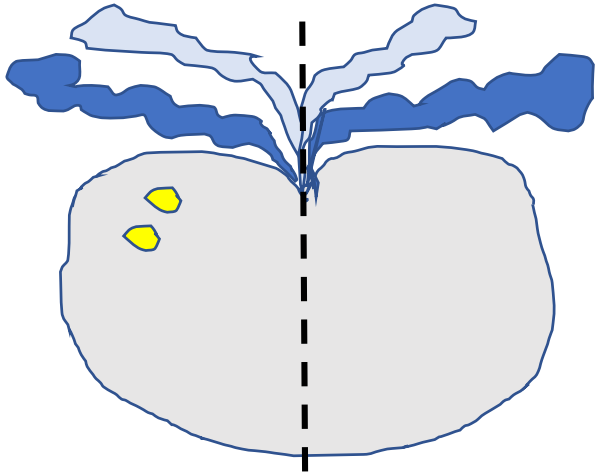
Stage	Description
T0	No evidence of residual tumor
No T1	
T2	Tumor confined within prostate
T3	Tumor through prostate capsule
T3a	Extraprostatic extension
T3b	Seminal vesicle invasion
T4	Tumor invades adjacent structures
N1	Regional lymph nodes involvement
M1a	Non-regional lymph node involvement
M1b	Bone involvement
M1c	Visceral sites involvement

Prognostic stage groups

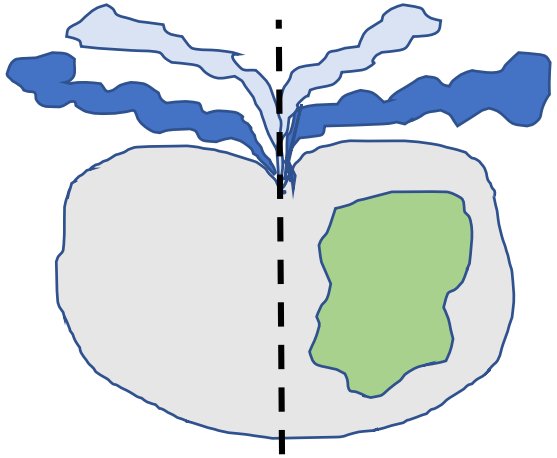
When T is	And N is	And M is	And PSA is	And Grade Group is	Then stage group is
cT1a-c, cT2a	N0	M0	<10	1	I
pT2	N0	M0	<10	1	I
cT1a-c, cT2a	N0	M0	≥10 <20	1	IIA
cT2b-c	N0	M0	<20	1	IIA
T1-2	N0	M0	<20	2	IIB
T1-2	N0	M0	<20	3	IIC
T1-2	N0	M0	<20	4	IIC
T1-2	N0	M0	≥20	1-4	IIIA
T3-4	N0	M0	Any	1-4	IIIB
Any T	N0	M0	Any	5	IIIC
Any T	N1	M0	Any	Any	IVA
Any T	N0	M1	Any	Any	IVB

ORGAN-CONFINED DISEASE (PT2)

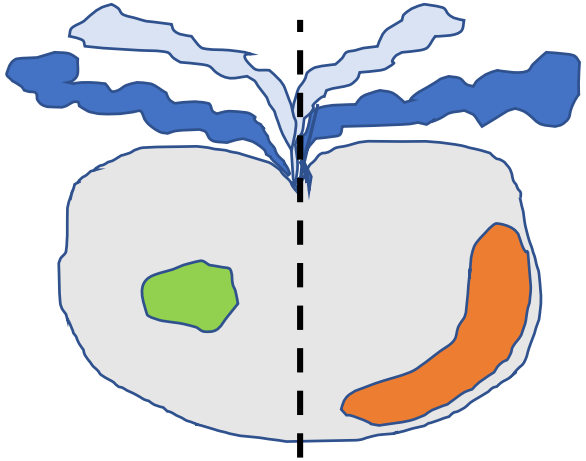
- No longer subclassified by extent of involvement or laterality



pT2



pT2



pT2

AJCC 8th edition

Does Subclassification of Pathologically Organ Confined (pT2) Prostate Cancer Provide Prognostic Discrimination of Outcomes after Radical Prostatectomy?

(Nguyen DP et al. J Urol. 199, 2018)

- 15,305 patients with T2 disease at RP from MSKCC & Mayo Clinic between 1985-2016 (median FU 6.0 yrs)
- Univariate analysis: pT2 subclassification was associated with BCR and distant metastasis, but NOT with overall mortality and death from disease
- Multivariate analysis: NO association between pT2 subclassification and BCR or distant metastasis

pT2 subclassification is not a prognostic indicator of survival related outcome

Independent Validation of the American Joint Committee on Cancer 8th Edition Prostate Cancer Staging Classification

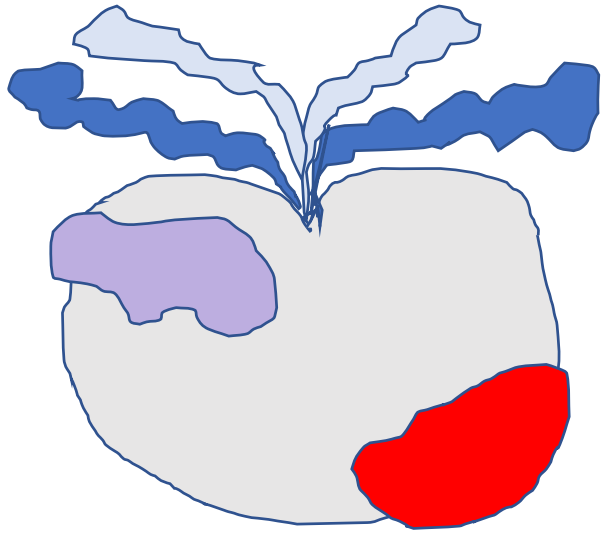
(Bhindi B et al. J Urol. 198, 2017)

- 13,839 RP patients from Mayo Clinic (1987-2011):
  - 11,031 pT2 (median FU 10.5 yrs)
- pT2 subclassification demonstrated limited discrimination for BRFS, MFS, PCSS
- Supported prognostic Stage Group reclassification:
  - PSA ≥ 20 ng/ml (T1-2) ---------- Stage Group III A
  - Grade Group 5 (any T) ---------- Stage Group III C

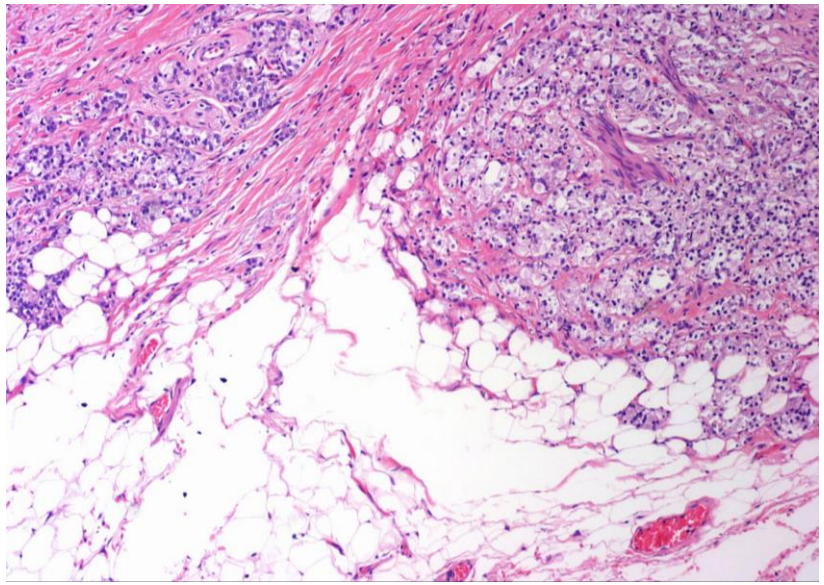
Data support changes in new AJCC classification

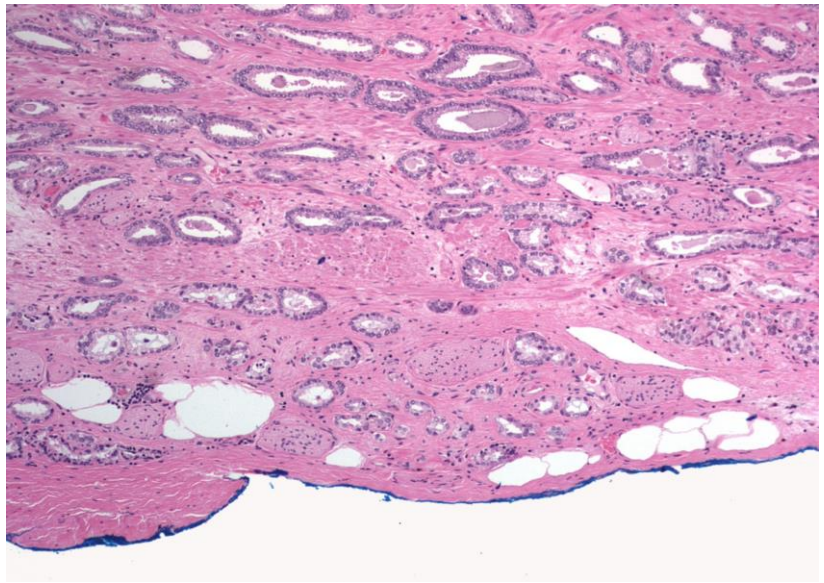
EXTRAPROSTATIC EXTENSION (PT3A)

- Tumor beyond confines of gland
- Admixed with periprostatic adipose tissue; easily recognized in posterolateral, posterior, lateral regions
- Tumor in skeletal muscle does **NOT** constitute EPE
- **Extent** (focal/nonfocal) and **location** of EPE should be documented

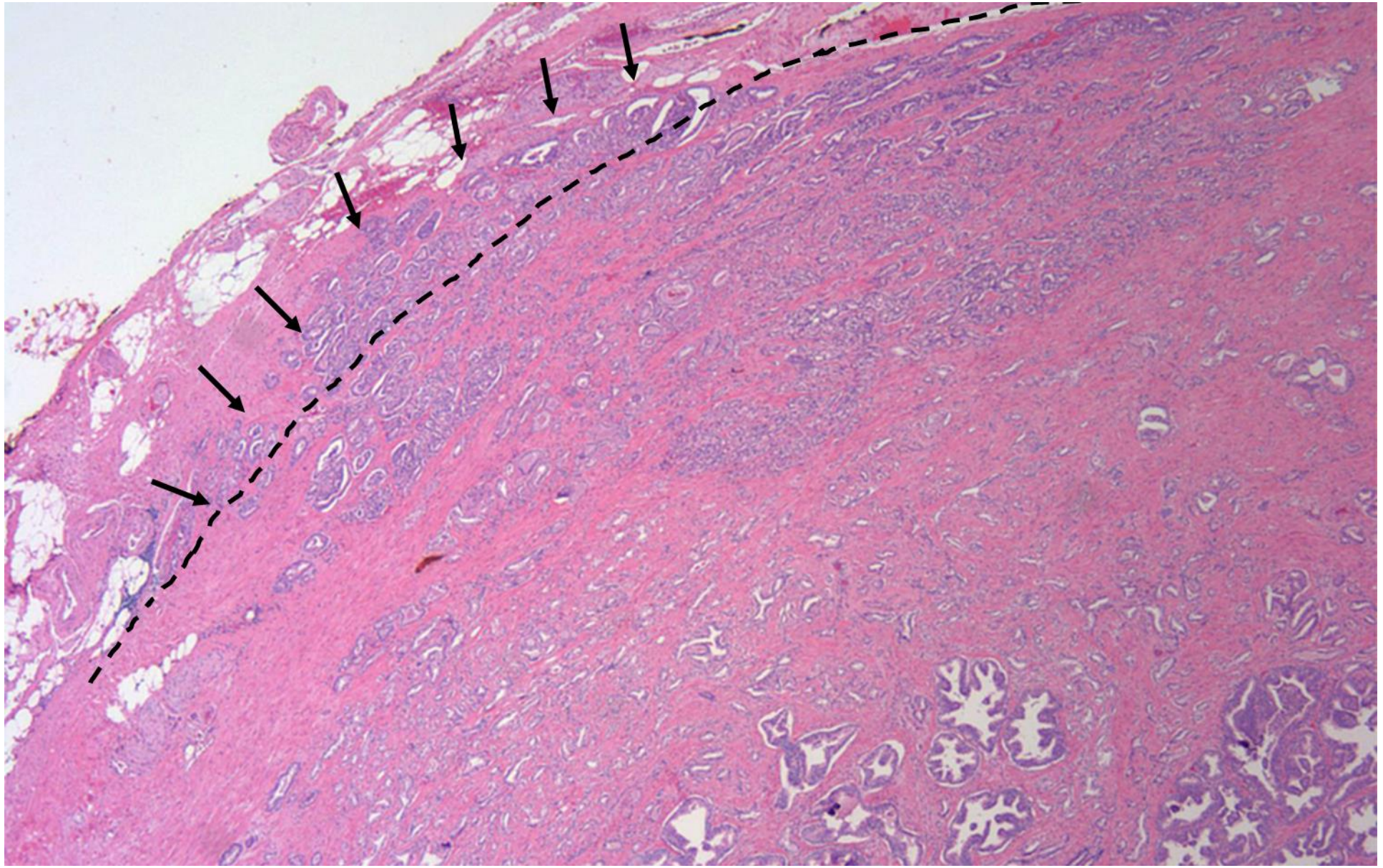


pT3a

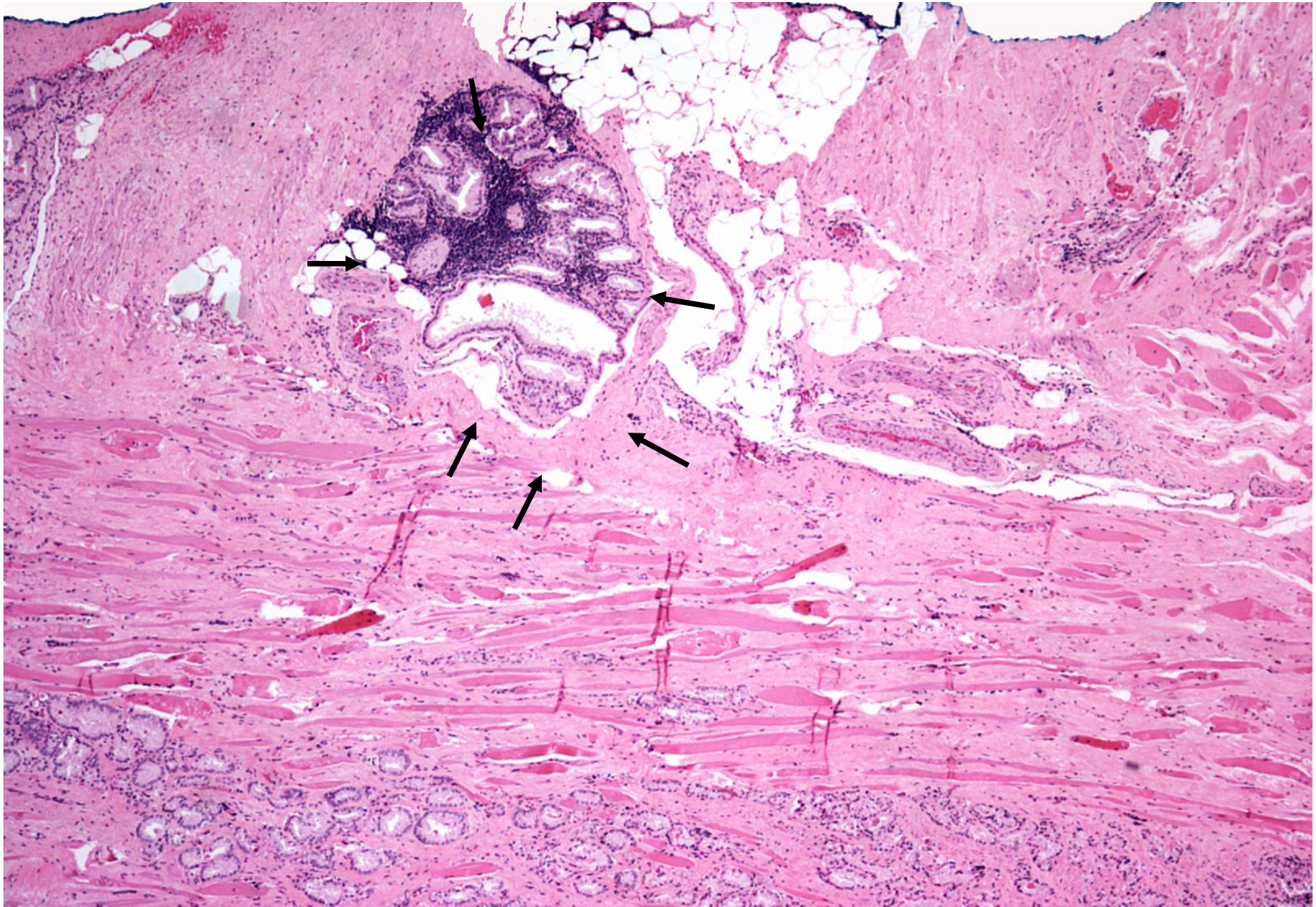




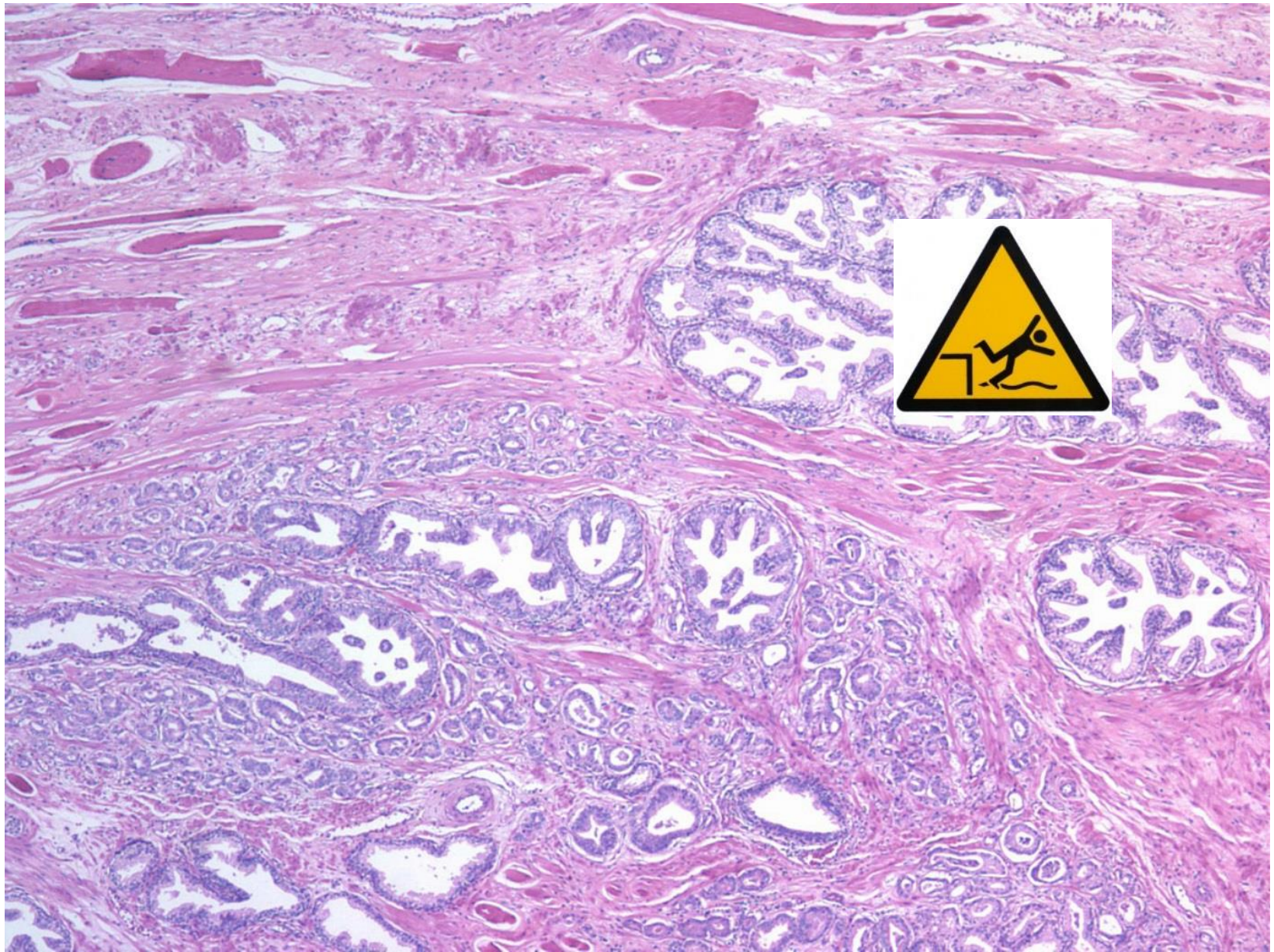
EPE -TUMOR BULGING BEYOND NORMAL PROSTATE CONTOUR



EPE -Tumor extend beyond contour of normal prostate (apex)



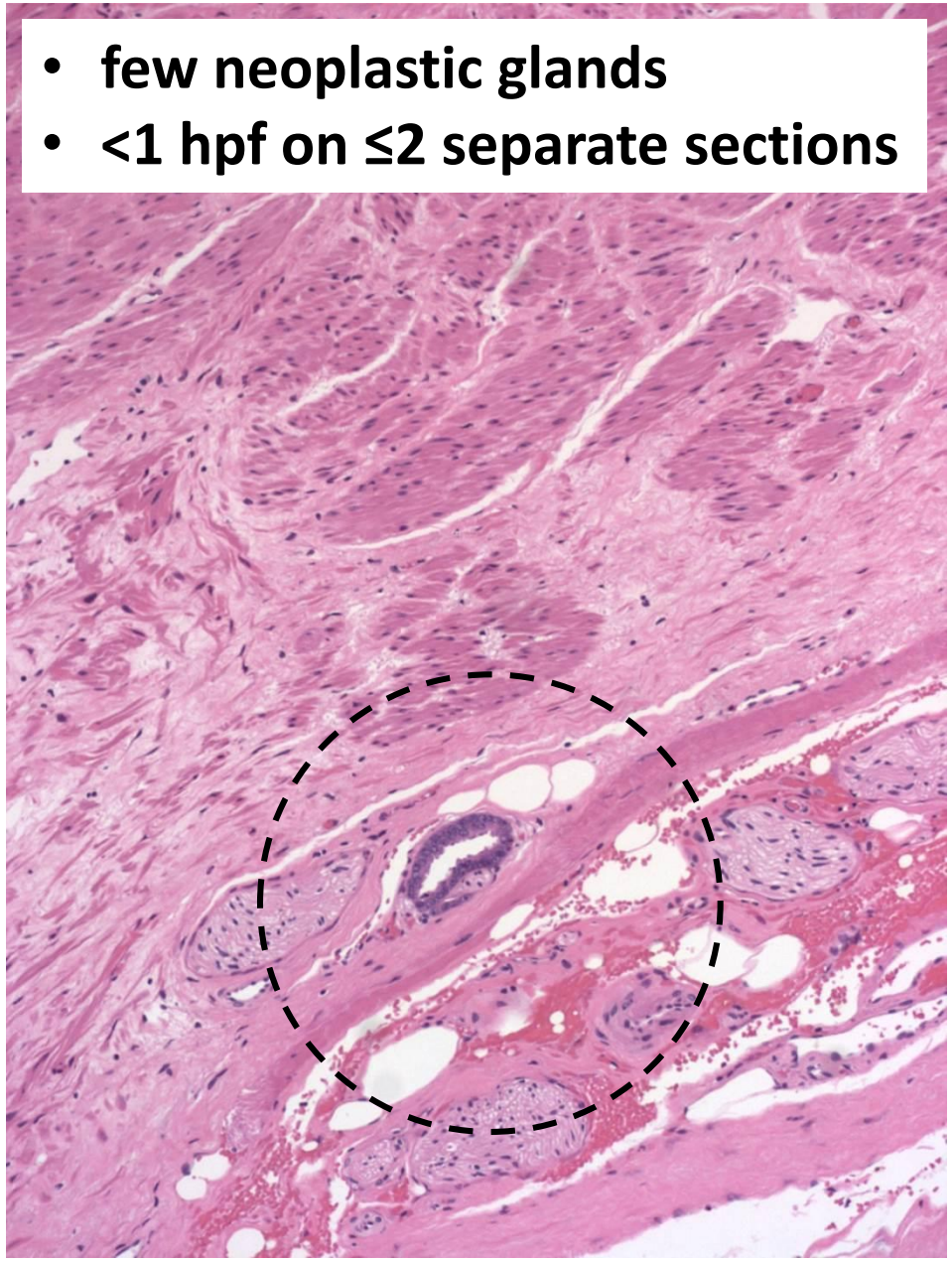
TUMOR IN SKELETAL MUSCLE – NO EPE



Epstein Wheeler

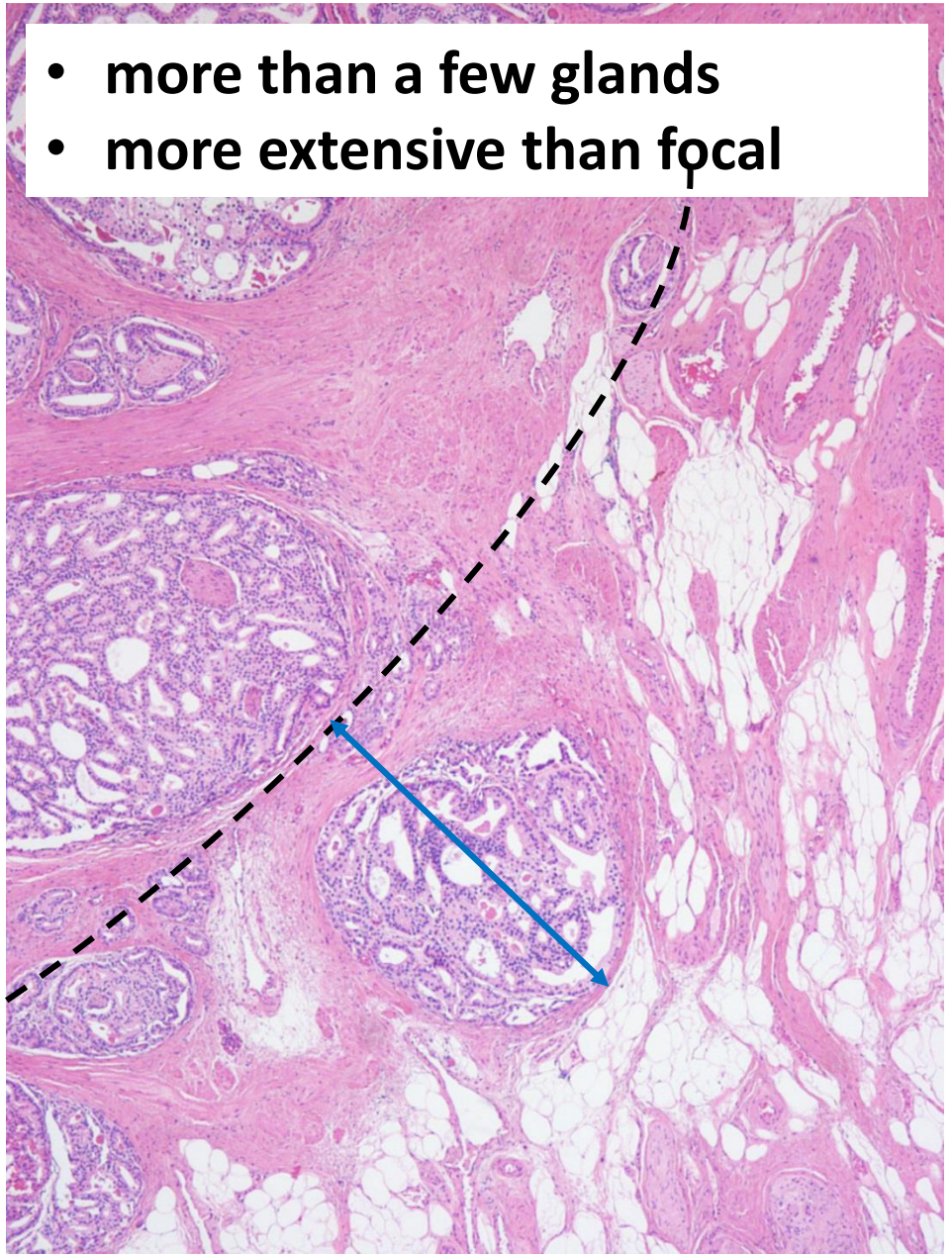
Focal EPE

- **few neoplastic glands**
- **<1 hpf on ≤2 separate sections**



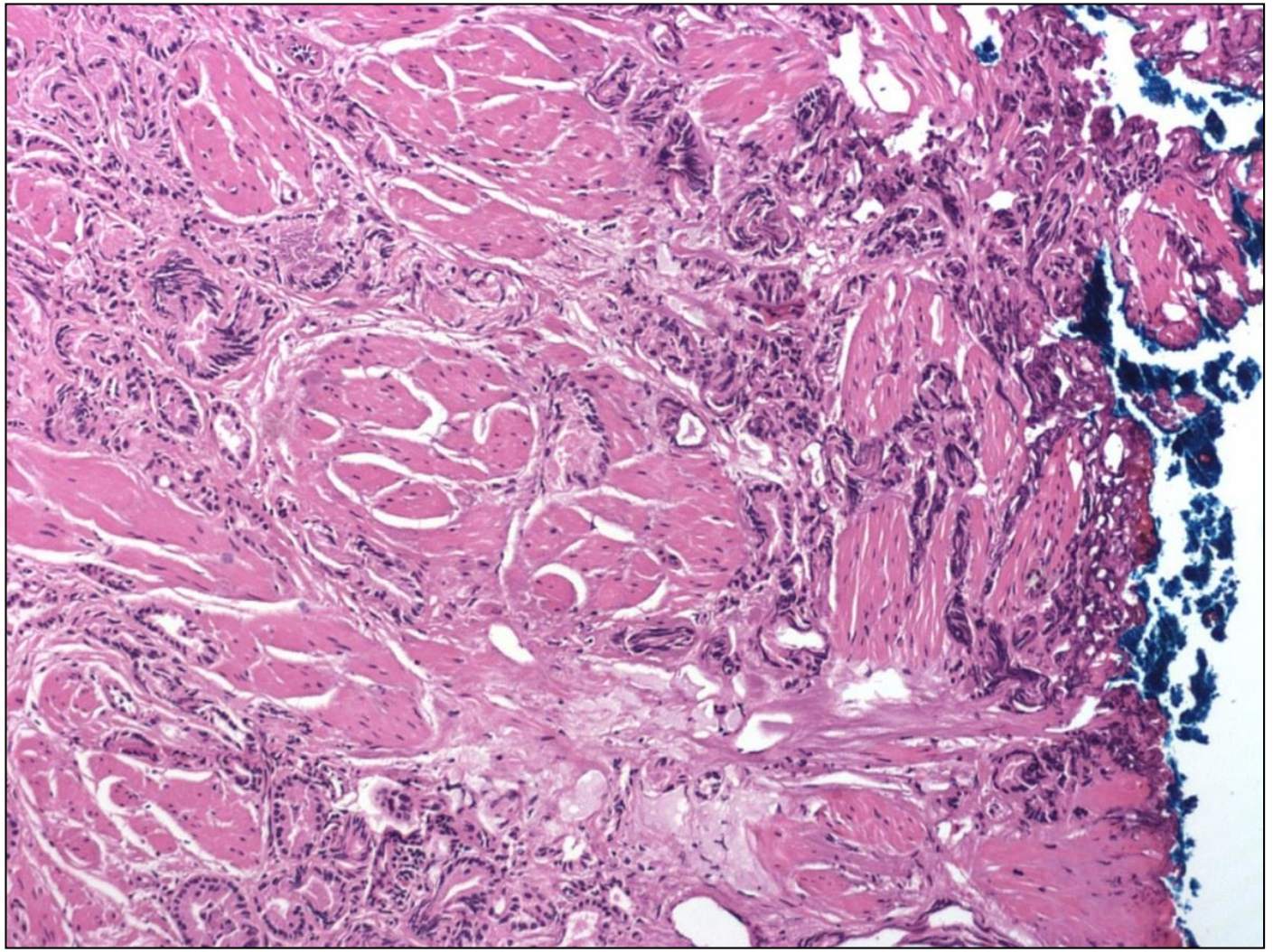
Nonfocal EPE

- **more than a few glands**
- **more extensive than focal**



MICROSCOPIC BLADDER NECK INVOLVEMENT (PT3A)

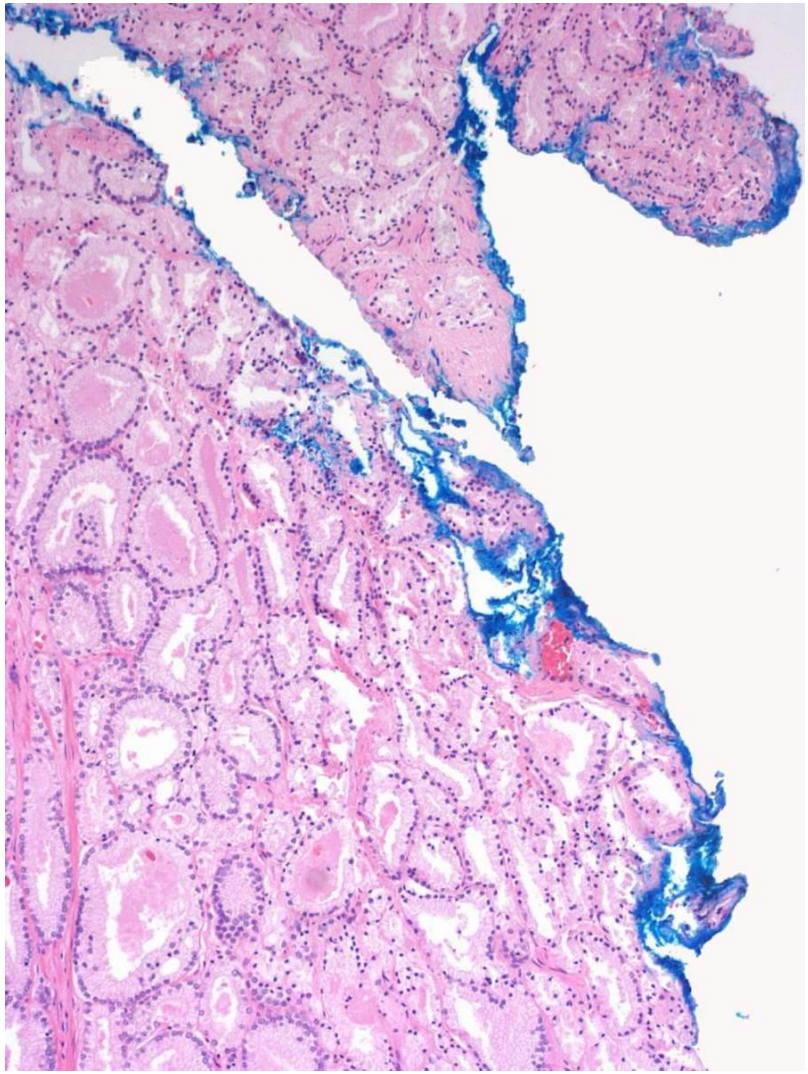
- Neoplastic cells within smooth muscle bundles of bladder neck in absence of benign glandular tissue
- Staged as **pT3a,** not pT4

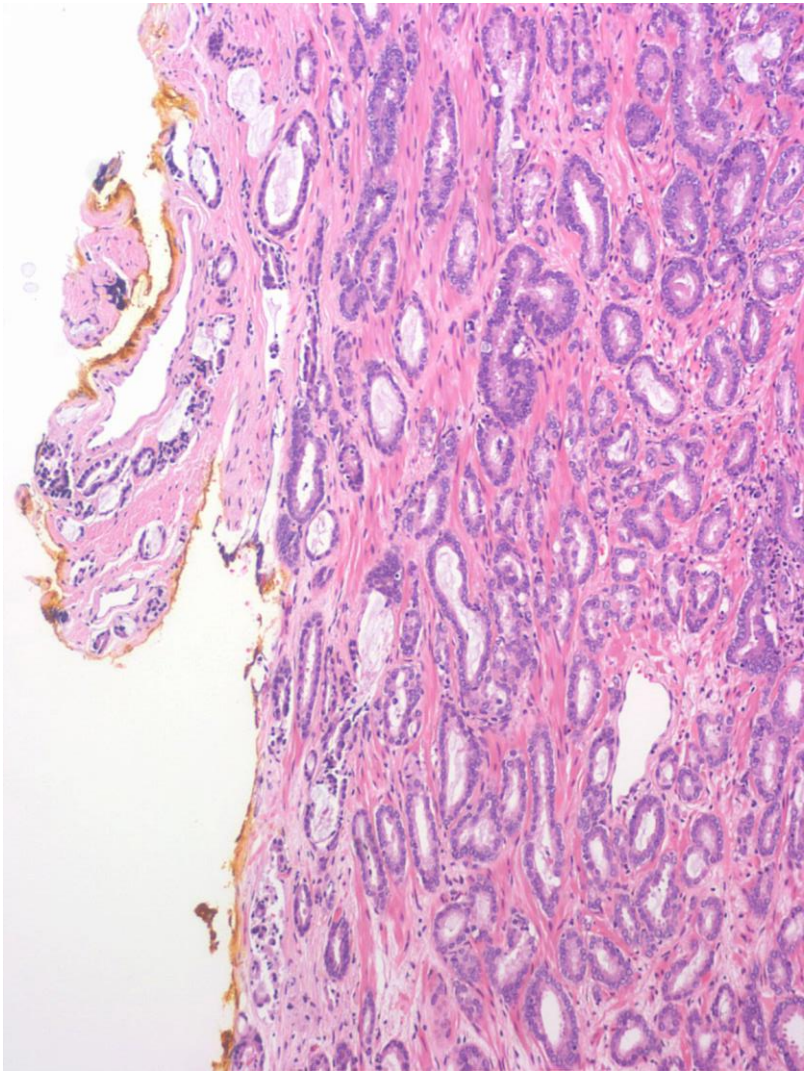


Magi-Galluzzi et al. Mod Pathol 24, 2011

SURGICAL MARGIN INVOLVEMENT

- Tumor extends (extraprostatic or intraprostatic) to inked surface of prostate
- Document location and extent of positive margins (linear length, <3 or ≥3 mm)
- Document Gleason score (Grade Group) @ margins





Tan et al. Mod Pathol 24, 2011

Short (≤ 1 mm) positive surgical margin and risk of biochemical recurrence after radical prostatectomy (RP)

(Shikanov et al. BJU Int. 111, 2013)

- In pT3 or GS≥7 tumors, short positive surgical margin (PSM) ≤1mm had significant adverse impact on BCR

The length of a positive surgical margin is of prognostic significance in patients with clinically localized prostate cancer treated with RP

(Servoll et al. Urol Int. 93, 2014)

- PSM >3mm is independent predictor of clinical failure after RP

Positive margin length and highest Gleason grade of tumor at margin predict for BCR after RP in patients with organ-confined PCA

(Chapin et al. Prostate Cancer Prostatic Dis. 2017 Dec 11)

- pT2 with PSM >1mm or GG ≥4 at margin have elevated risk for BCR

Impact of multifocality and multilocation of positive surgical margin (PSM) after RP on predicting oncological outcome

(Wu et al. Clinical Genitourinary Cancer 2019)

- Multilocation is an independent prognostic factor for BCR
- Multifocality + multilocation PSM shows added prognostic value on predicting BCR-free survival, but not on MFS or OS

Importance of Reporting the Gleason Score at the Positive Surgical Margin Site: Analysis of 4,082 Consecutive RP Cases

(Kates et al. J Urol 2016)

- Lower GS at positive margin is independently associated with shorter margin length and decreased risk of early BCR

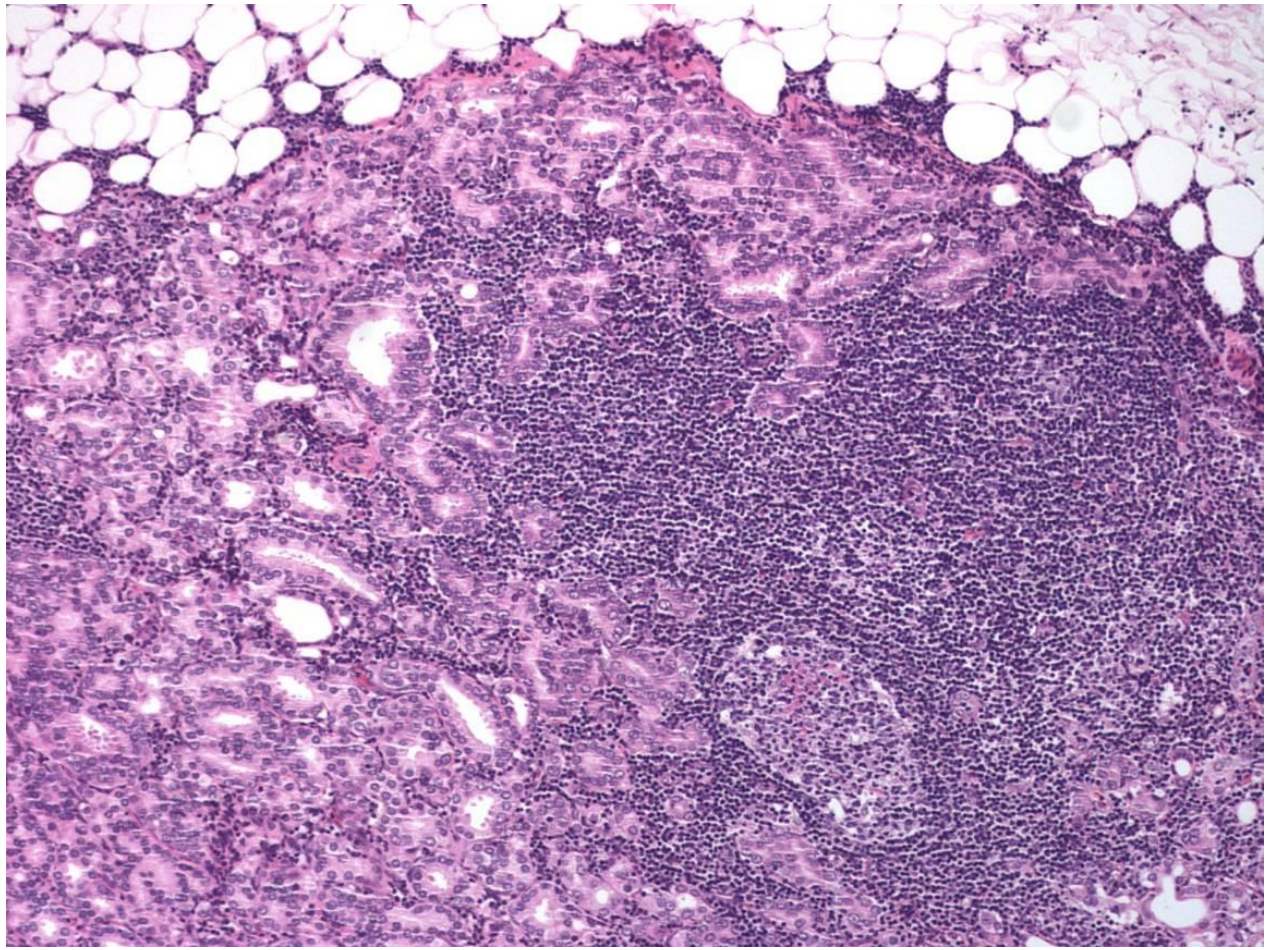
Importance of Reporting the Gleason Score at the Positive Surgical Margin Site: Analysis of 4,082 Consecutive RP Cases

(Iremashvili et al. Am J Surg Path 2019)

- Reporting presence of Gleason pattern 4/5 at SM may be most practical

LYMPH NODE(S) INVOLVEMENT (N1)

- Important for adequate staging
- # and diameter of largest metastatic focus are independent predictors of early BCR [Passoni et al. BJU Int 2014]
- Extranodal extension is associated with significantly higher risk of BCR and “global” recurrence [Luchini et al. Sci Rep 2017]



UPDATE ON REPORTING

REPORTING MINOR HIGH-GRADE (TERTIARY PATTERN) ON RP

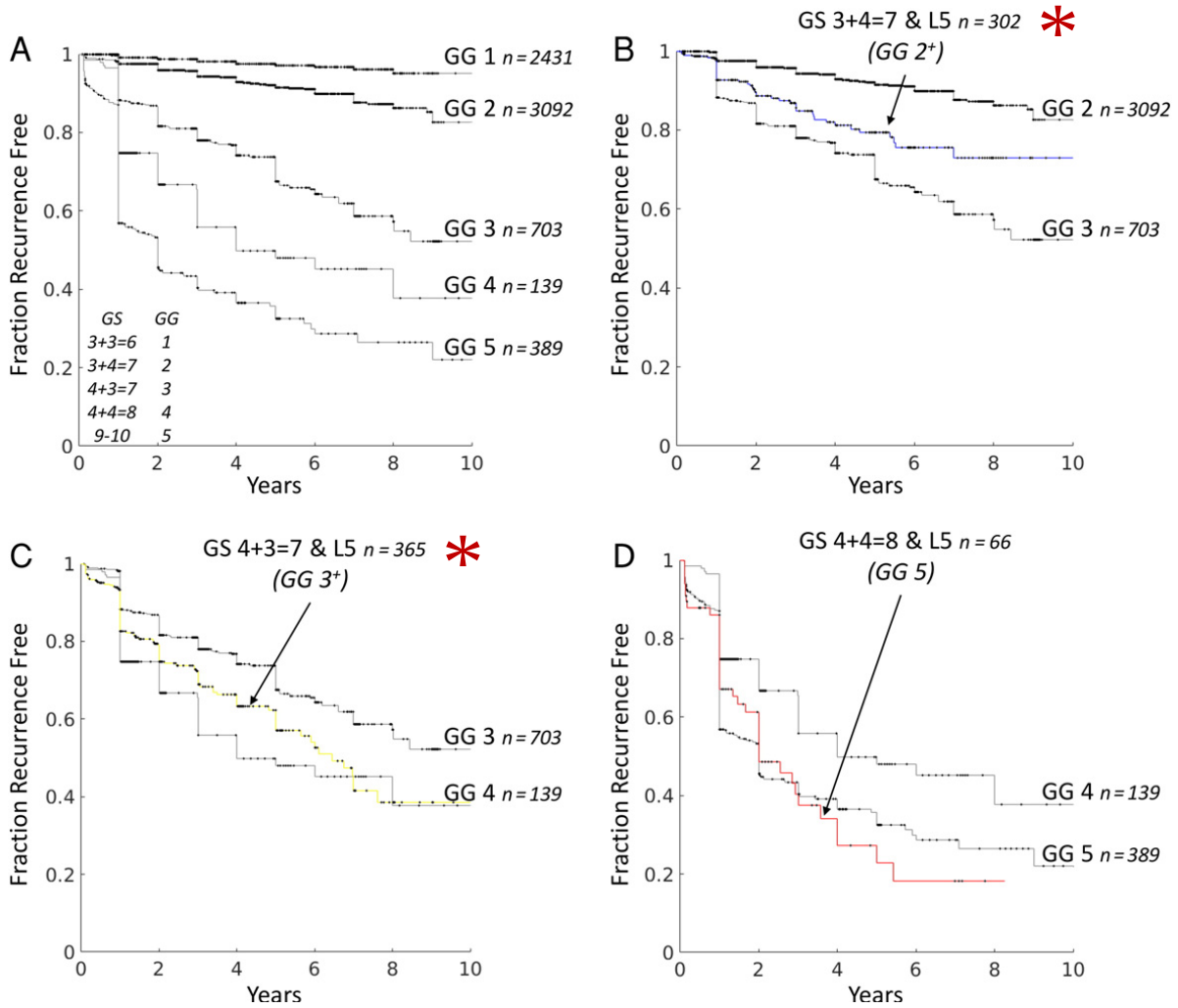
- If tertiary pattern 5 is **>5%** on RP, it is assigned as secondary pattern, rather than tertiary pattern:
  - Gleason pattern 4 (60%) + pattern 3 (30%) + pattern 5 (10%) = **Gleason score 4+5=9**

- If tertiary pattern 5 is **≤5%** on RP, it is assigned as tertiary pattern:
  - Gleason pattern 4 (70%) + pattern 3 (25%) + pattern 5 (5%) = **Gleason score 4+3=7 with tertiary pattern 5**

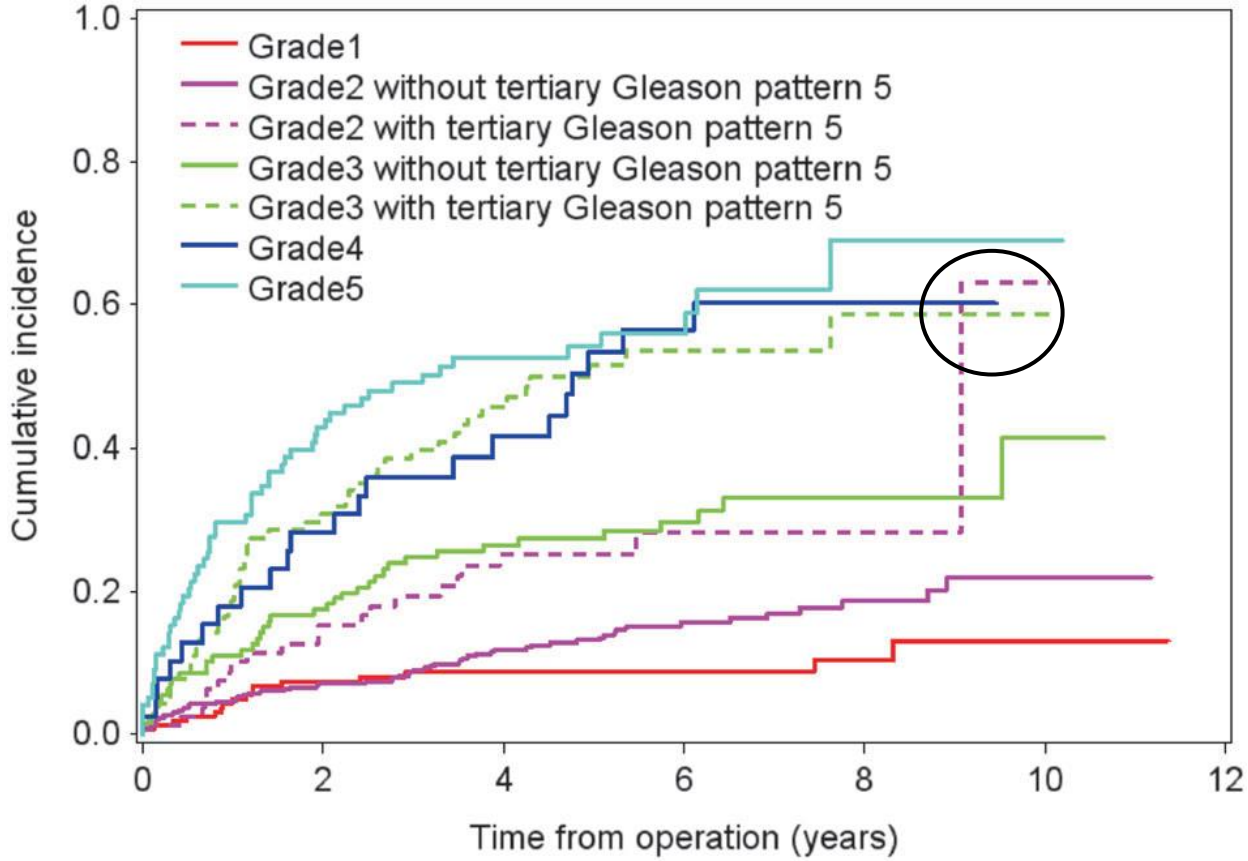
- Tertiary pattern does not impact Grade Groups

Minor Gleason pattern (GP) 5 on RP

Baras et al. Hum Pathol 2017;63:27-32



Kato et al. Mod Pathol 2019;32:122-127

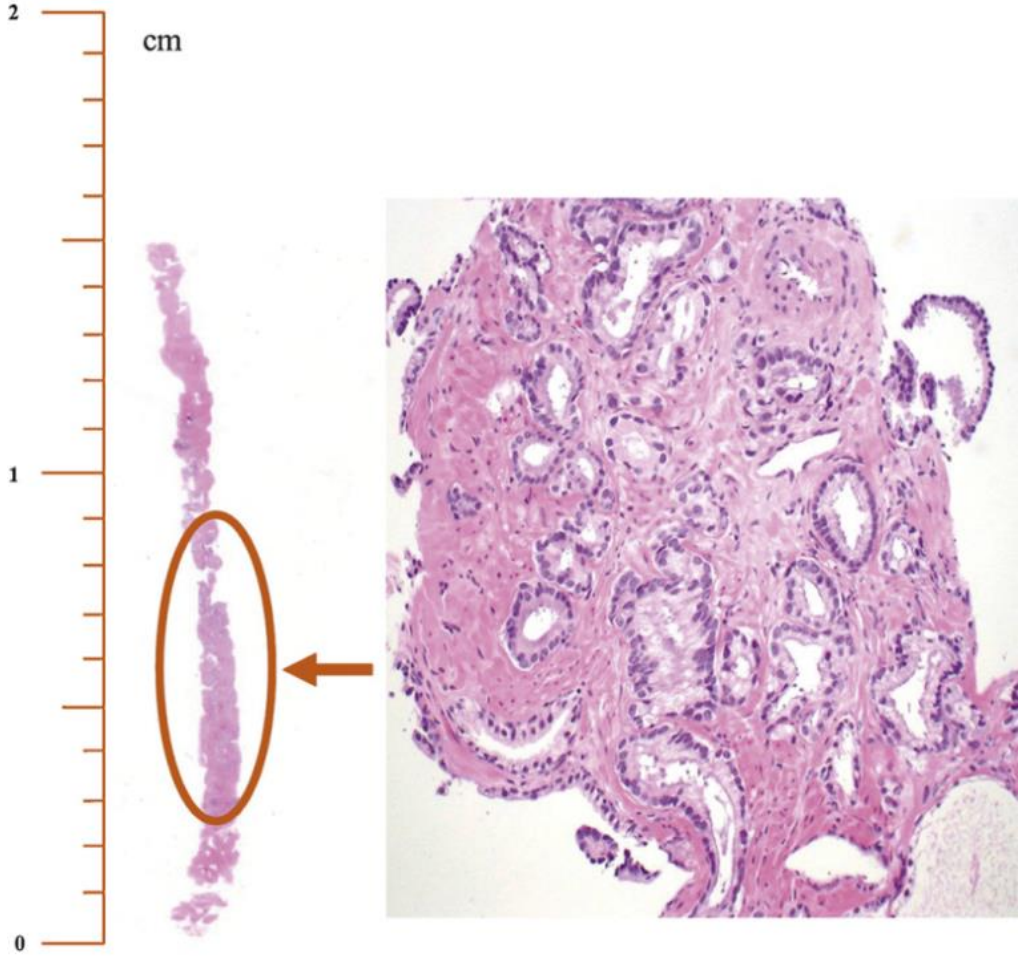


Integrating tertiary GP5 into GG, PSA, SM status predicted BCR

* Minor GP5 imparts intermediate prognosis relative to next GG

TUMOR QUANTIFICATION ON PROSTATE BX

- # of positive cores involved by PCA out of total
- **Linear extent (% and/or mm) of cancer length in each core**
- Total % or length of cancer in all biopsy cores
- Greatest % or length of cancer involvement
- Amount of cancer in single core with largest amount of tumor

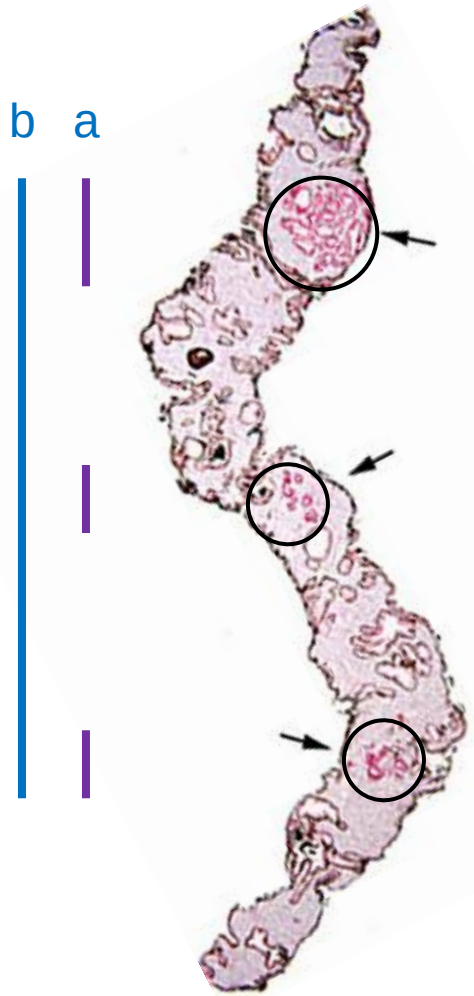


^fragmentation may preclude accurate assessment

From Montironi el al. Eur Urol 2012

REPORTING DISCONTINUOUS FOCI OF PCA

- Involvement by multiple PCA foci separated by BPT
- No consensus on quantification method:
  a. Adding foci, ignoring intervening BPT (additive quantification)
  b. Assessing discontinuous foci as single focus (linear quantification, end-to-end measurement)
- Both methods showed excellent correlation with tumor at RP; linear quantification improved prediction of PCA extent
- 78% of discontinuous tumors on PBx results from single tumor nodule



Schultz et al. AJSP 2013; Arias-Stella et al AJSP 2015

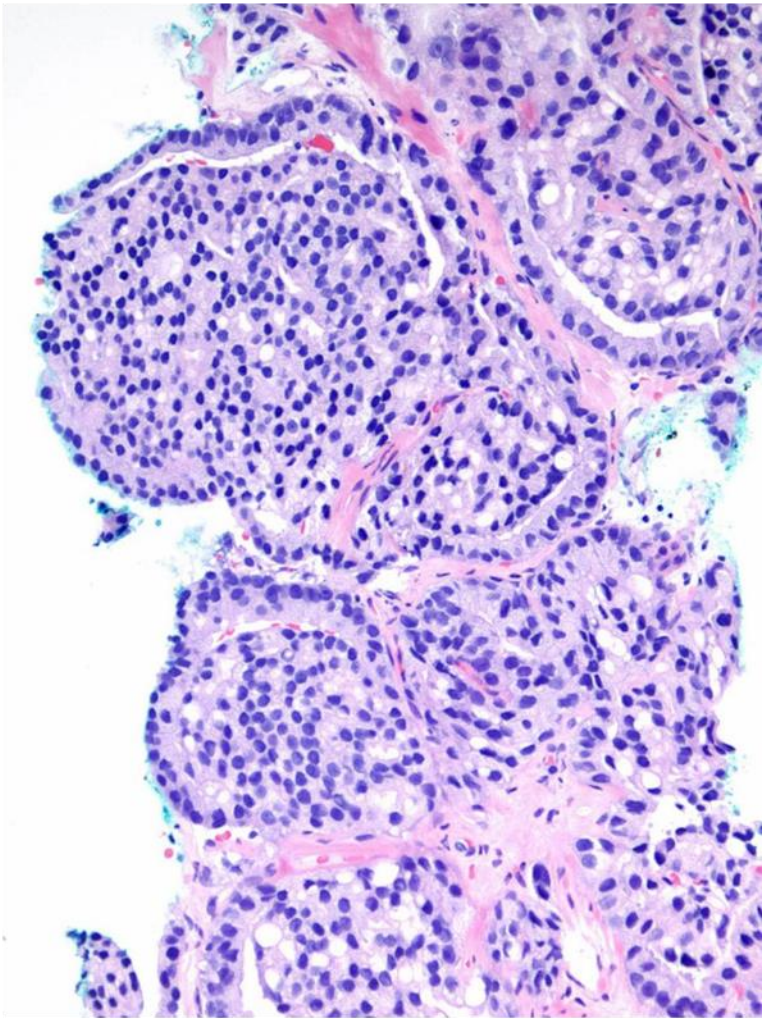
REPORTING % PATTERN 4 AND CRIBRIFORM MORPHOLOGY

- ISUP 2014 recommended reporting **% GP4** in **GS7 tumors** (implications for surveillance, XRT)
  - 3+4=7 PCA with <5% pattern 4 is associated with low-risk tumor on RP

  [Huang et al. AJSP 2014, Kir at al. Ann Diagn Pathol 2016]

- **Cribriform morphology** is associated with adverse outcome
  - meta-analysis of 14 publications determined an OR of 11.37 for adverse outcome in cribriform PCA

  [Luo X et al. Mod Pathol 2017, Iczkowski KA et al. Adv Anat Pathol 2017]

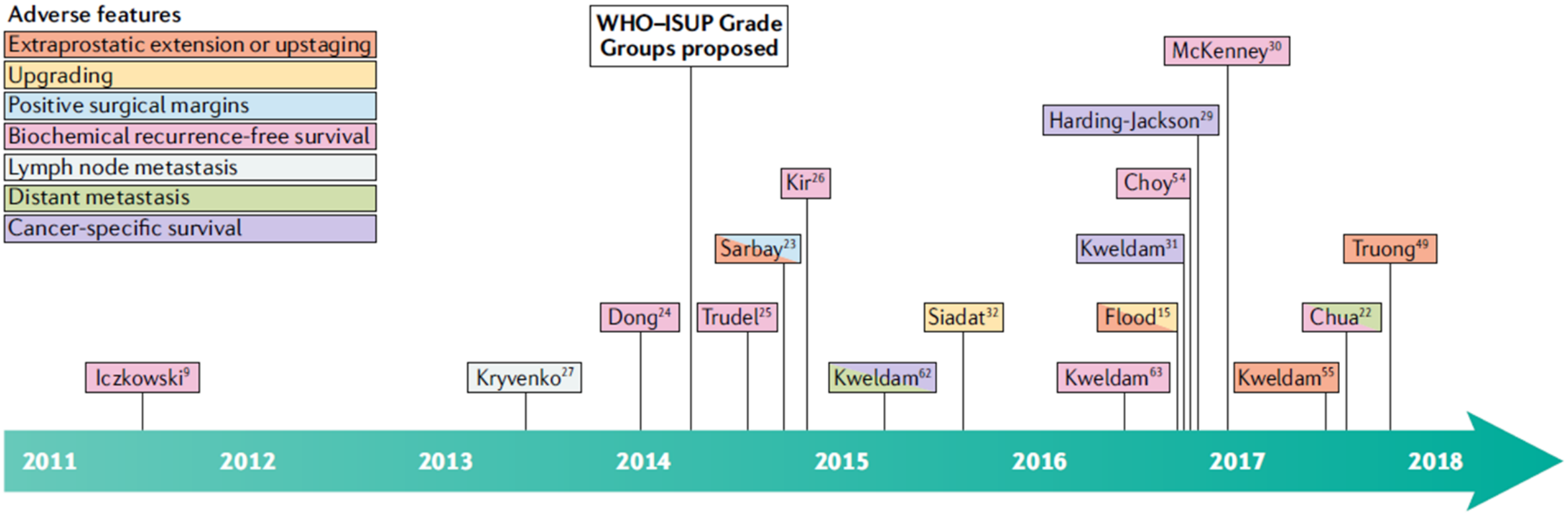


REPORTING PERCENTAGE PATTERN 4 IN GS7 TUMORS

Relationship between % GP4 and prognosis of PCA patients undergoing RP				
study [author, yr.]	**# of patients**	**specimen type**	**separation method of GP4**	**significance of RP %GP4**
Cheng et al. 2007	504	RP	0%/1-20/>20% (GP4, GP5, or both)	indep. predictor of CSS
Huang et al. 2014	256	Bx	≤5/6–50%	predictor of pT3 on RP
Choy et al. 2016	585	RP	1-5/6-10/11-20/2-30/31-40/41-50/51-60/61-70/71-80/81-90%	indep. predictor of BCR
Cole et al. 2016	1,691	Bx	1-9.9/10-19.9/20-39.9/40-59.9/60-79.9/80-100%	indep. predictor of BCR
Kir et al. 2016	372	Bx	<6/6–25/26/49/≥50%	indep. predictor of BCR
Sauter et al. 2016	12,823	Bx and RP	(I) ≤25/26-49/50-74/≥75&; (II) ≤5/6-10/11-20/21-30/31-49/50-60/61-80/>80%	predictor of BCR
Perlis et al 2017	1,255	Bx	1-5/6-10/11-20/21-49%	predictor of pT3 on RP

Sharma & Miyamoto Transl Androl Urol 2018

CRIBRIFORM MORPHOLOGY



- Cribriform morphology is recognized as most aggressive GP4 subtype
- Routine reporting of cribriform morphology on Bx should be encouraged

Truong et al. Nature Reviews. Urology 2018

Cribriform growth is highly predictive for postoperative metastasis and disease-specific death in Gleason score 7 prostate cancer

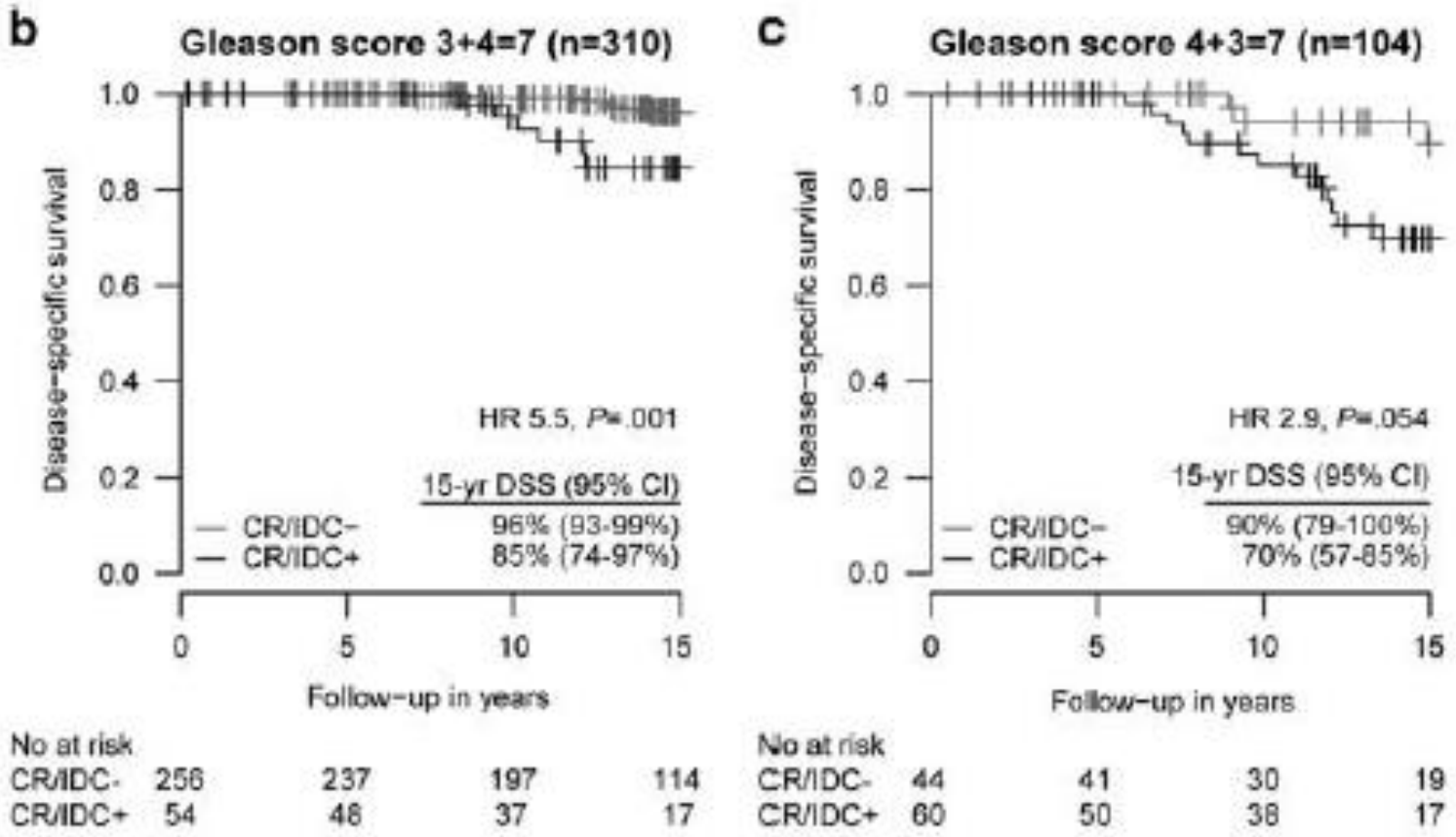
Charlotte F Kweldam[1], Mark F Wildhagen[2,3], Ewout W Steyerberg[4], Chris H Bangma[3], Theodorus H van der Kwast[5] and Geert JLH van Leenders[1]

Modern Pathology 2015

Disease-specific survival of patients with invasive cribriform and intraductal prostate cancer at diagnostic biopsy

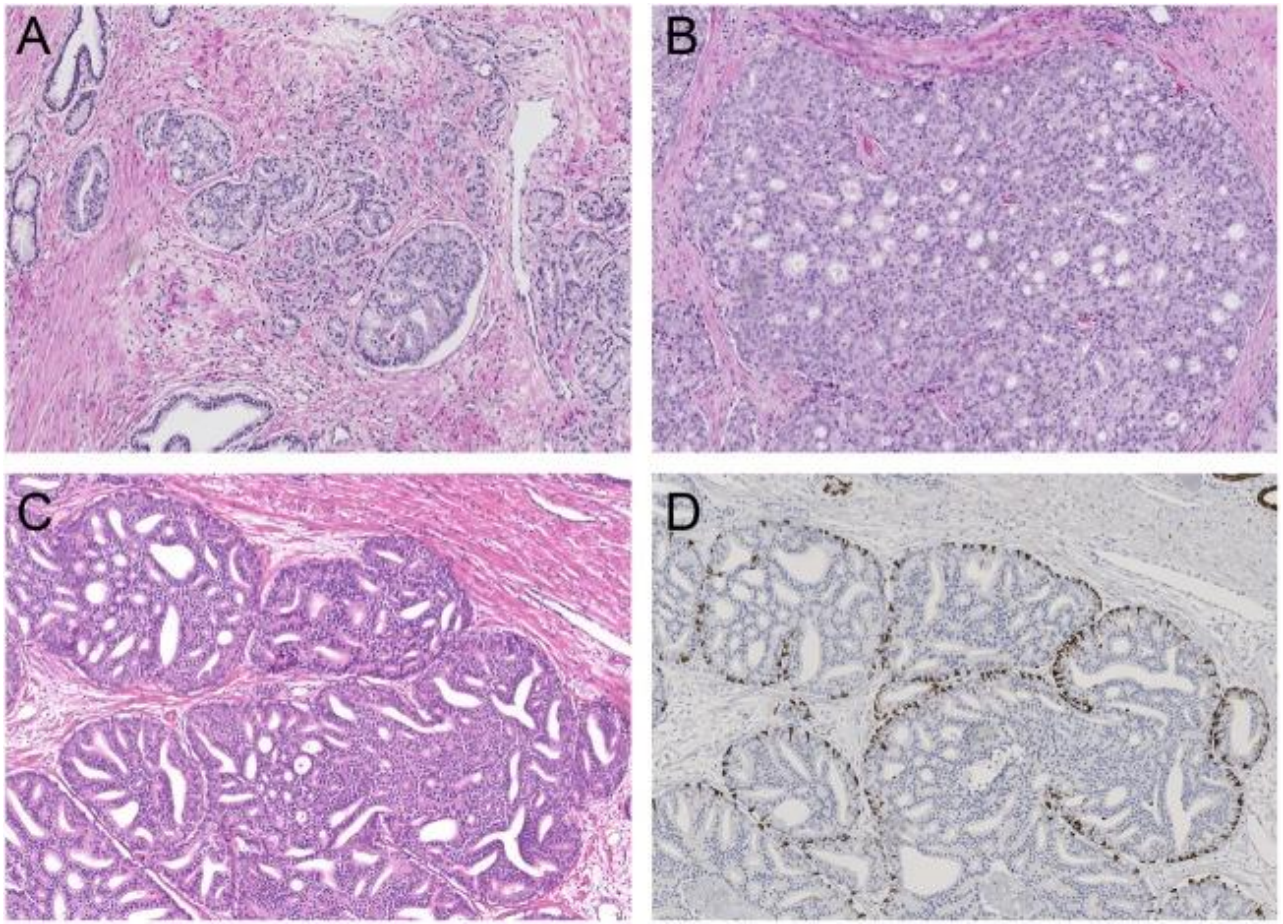
Charlotte F Kweldam[1], Intan P Kümmerlin[1], Daan Nieboer[2], Esther I Verhoef[1], Ewout W Steyerberg[2], Theodorus H van der Kwast[3], Monique J Roobol[4] and Geert J van Leenders[1]

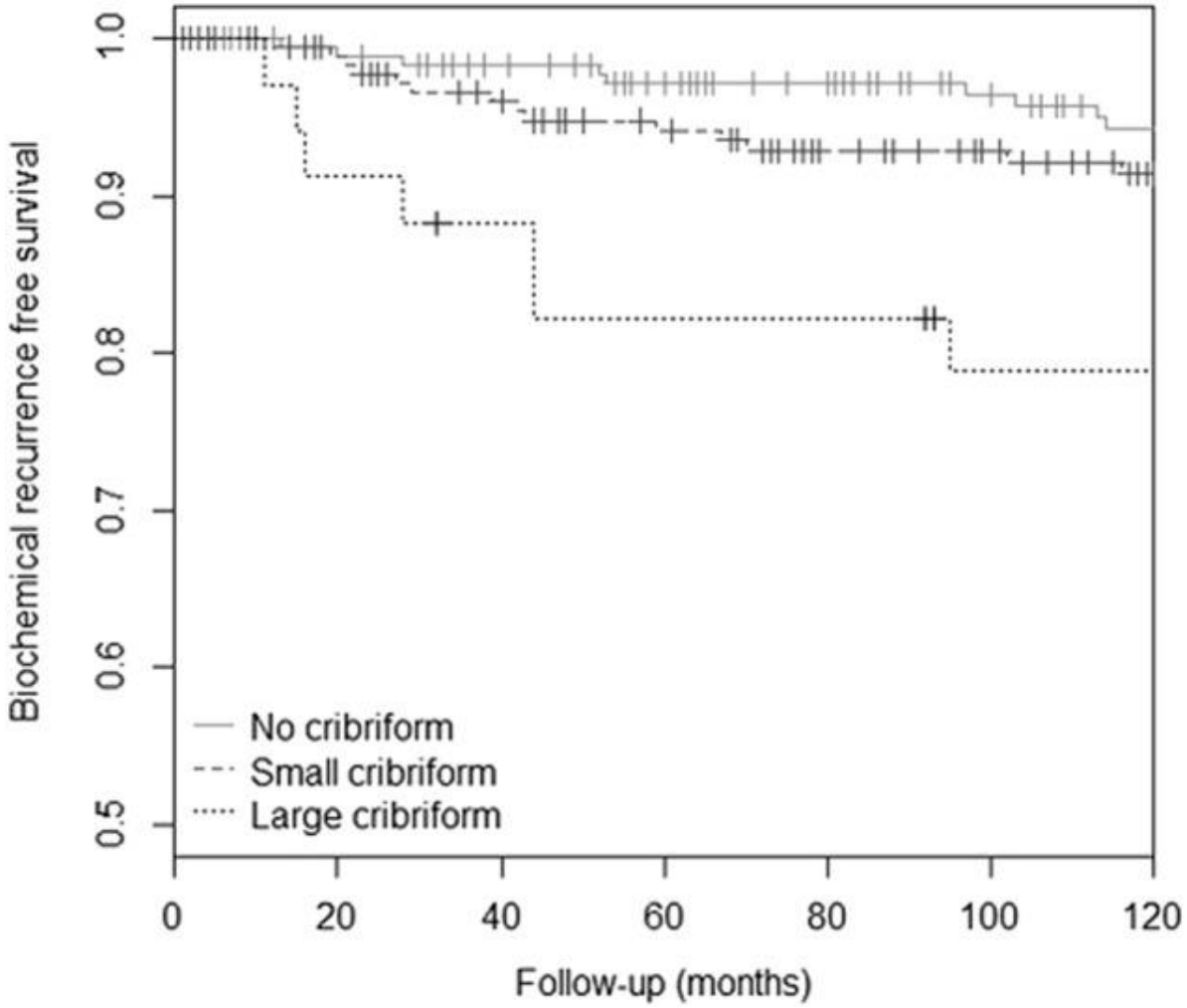
Modern Pathology 2016



Large cribriform growth pattern identifies ISUP grade 2 prostate cancer at high risk for recurrence and metastasis

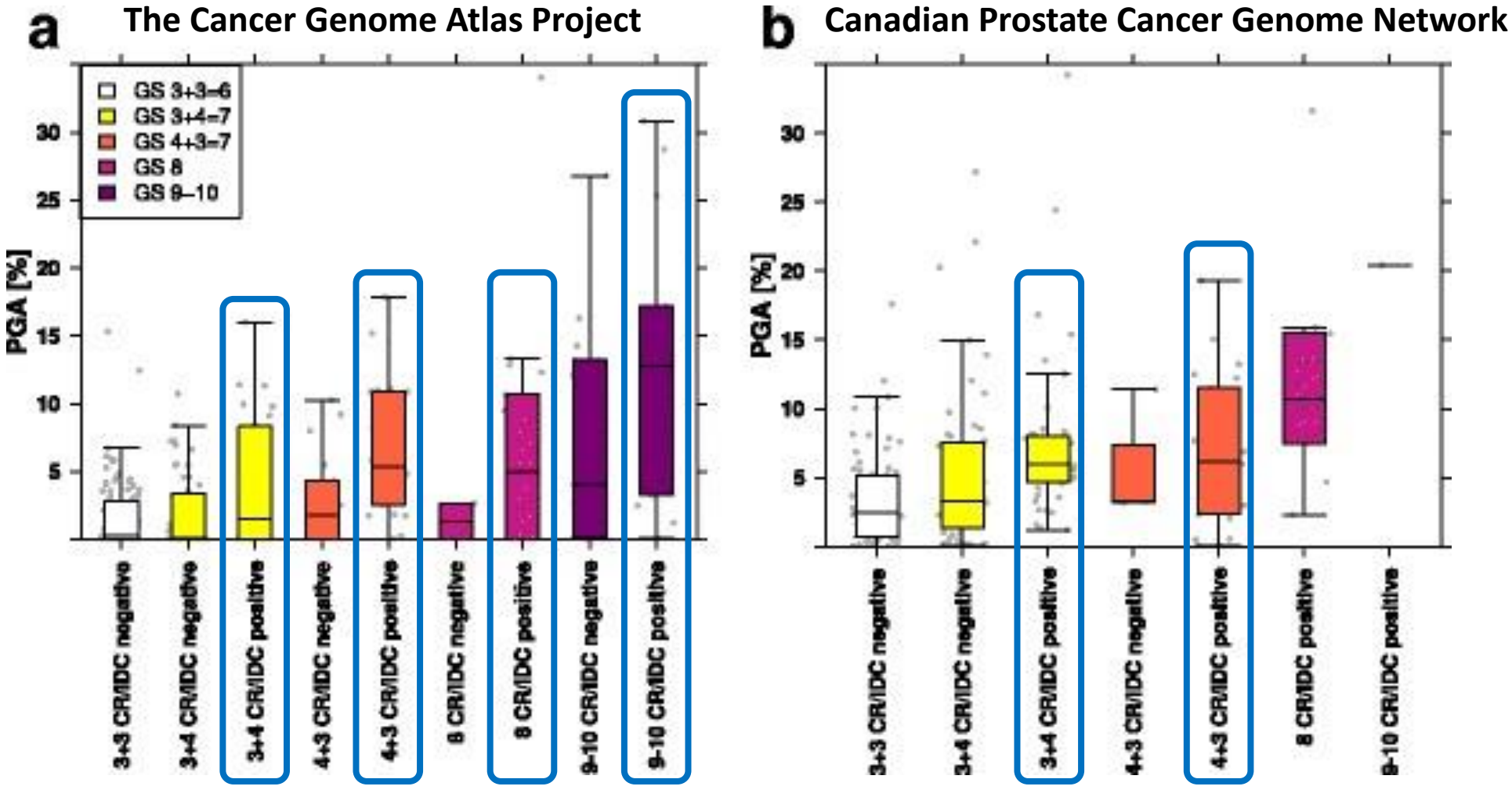
(Hollemans et al. Mod Pathol 2019)





Cribriform and intraductal prostate cancer are associated with increased genomic instability and distinct genomic alterations

(Bottcher et al. BMC Cancer 2018)



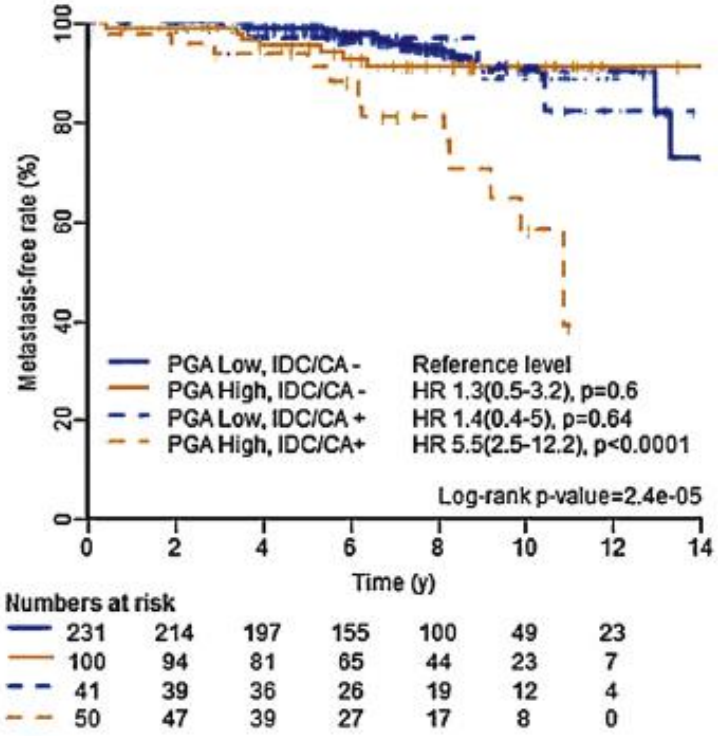
A Prostate Cancer "Nimbosus": Genomic Instability and SChLAP1 Dysregulation Underpin Aggression of Intraductal and Cribriform (CR) Subpathologies

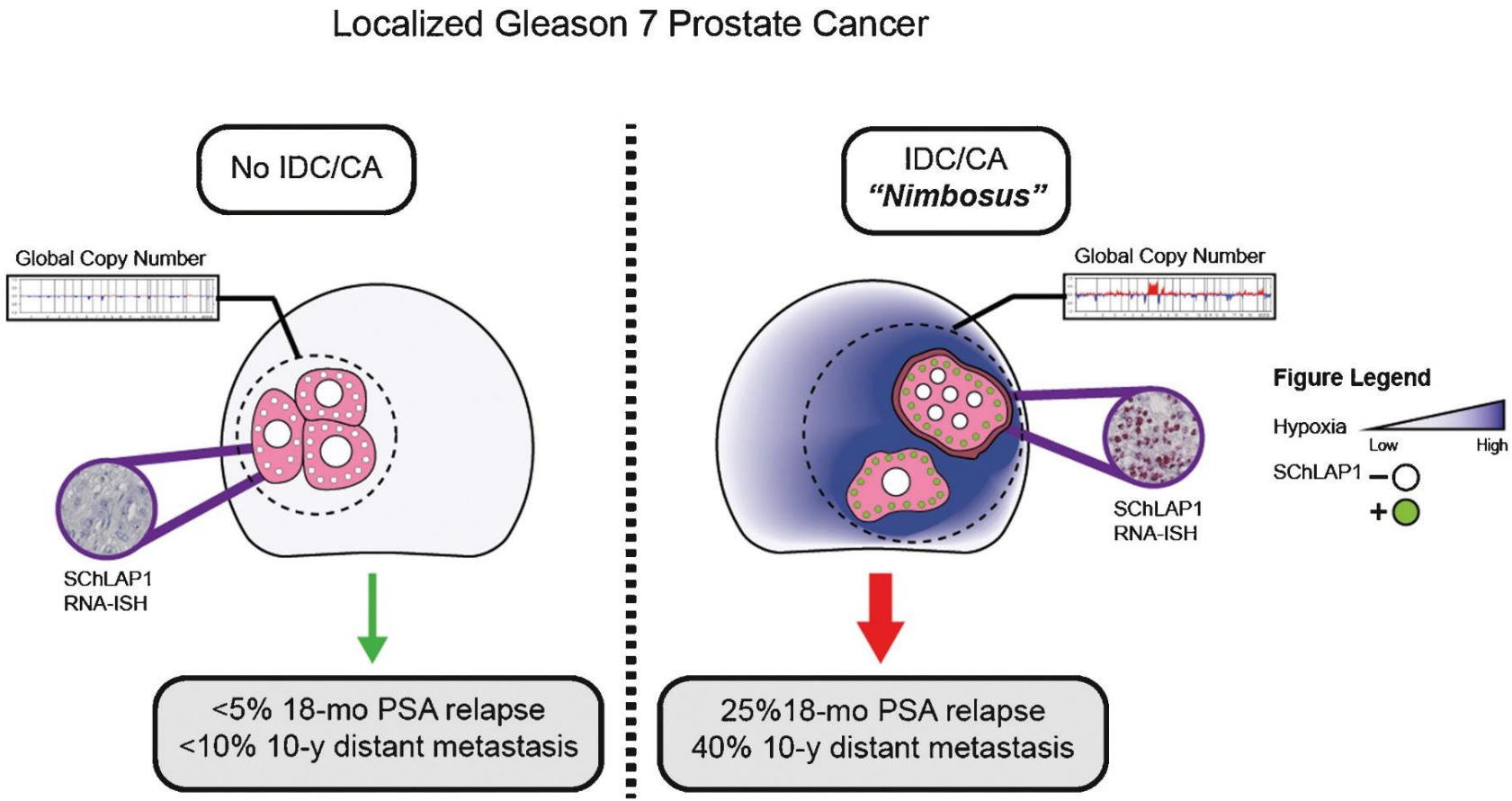
Chua MLK et al. Eur Urol 2017

- 1325 men with NCCN low to high risk PCA treated with RP or radiotherapy
- Evaluated:
  - Pathologic IDC/CR
  - Genomic instability
  - Copy number aberrations
  - Hypoxia
  - SChLAP1 RNA-ISH

- **IDC/CR+**
  - Independently predicted increased risk of BCR and metastasis
  - Increased % of genome alteration and hypoxia
  - SChLAP1 was only gene expressed >3-fold higher in IDC/CR+ than IDC/CR–

"Nimbosus": A constellation of unfavorable molecular characteristics co-occur with intraductal and cribriform subpathologies in PCA



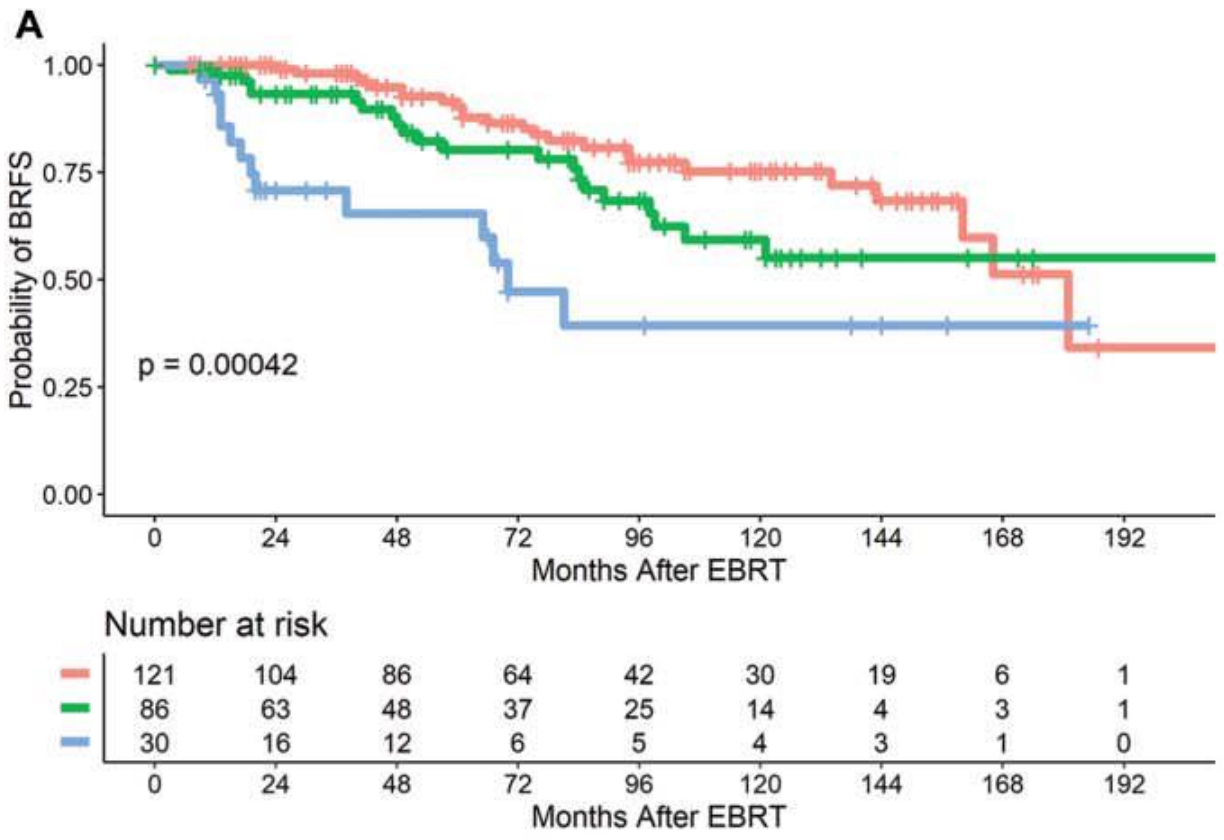


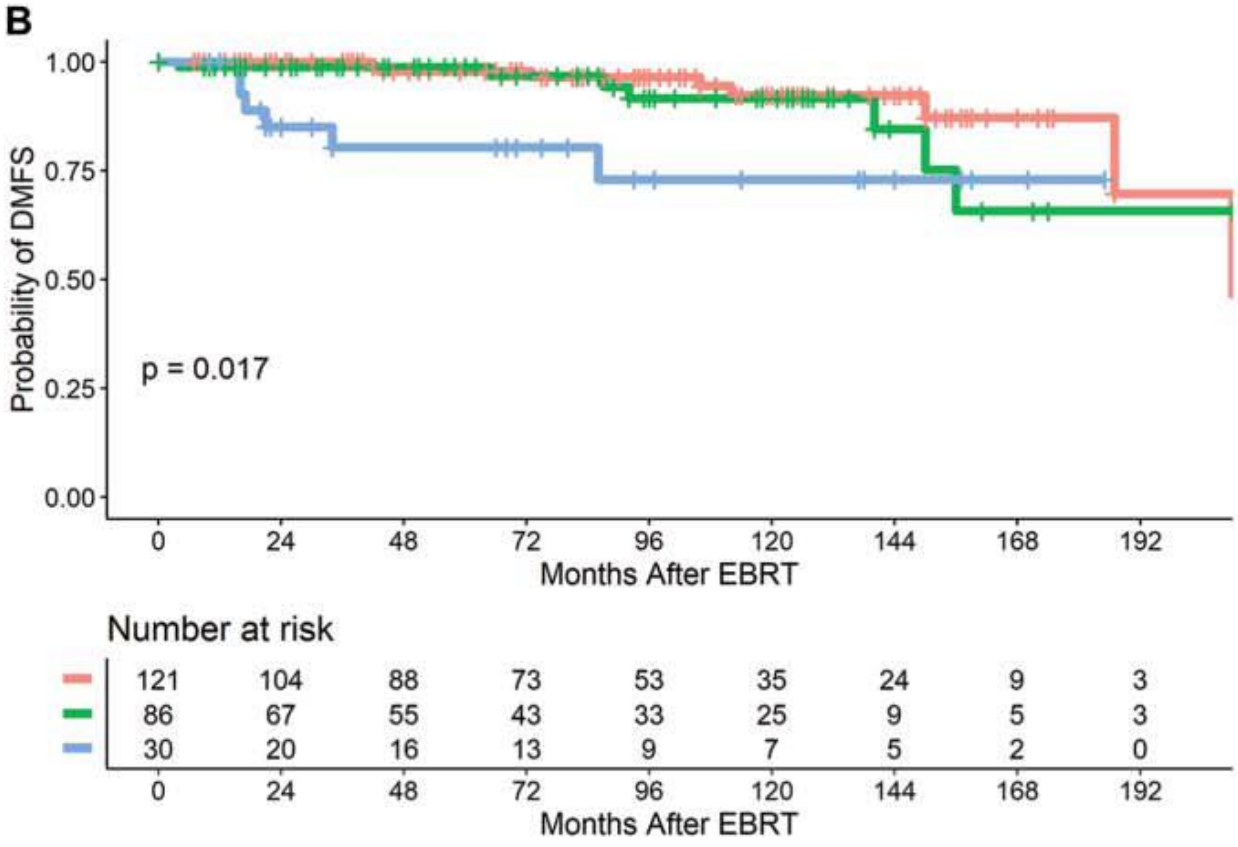
Chua et al. Eur Urol. 2017;72:665-674

Impact of Cribriform Pattern and Intraductal Carcinoma on Gleason 7 Prostate Cancer Treated with External Beam Radiotherapy

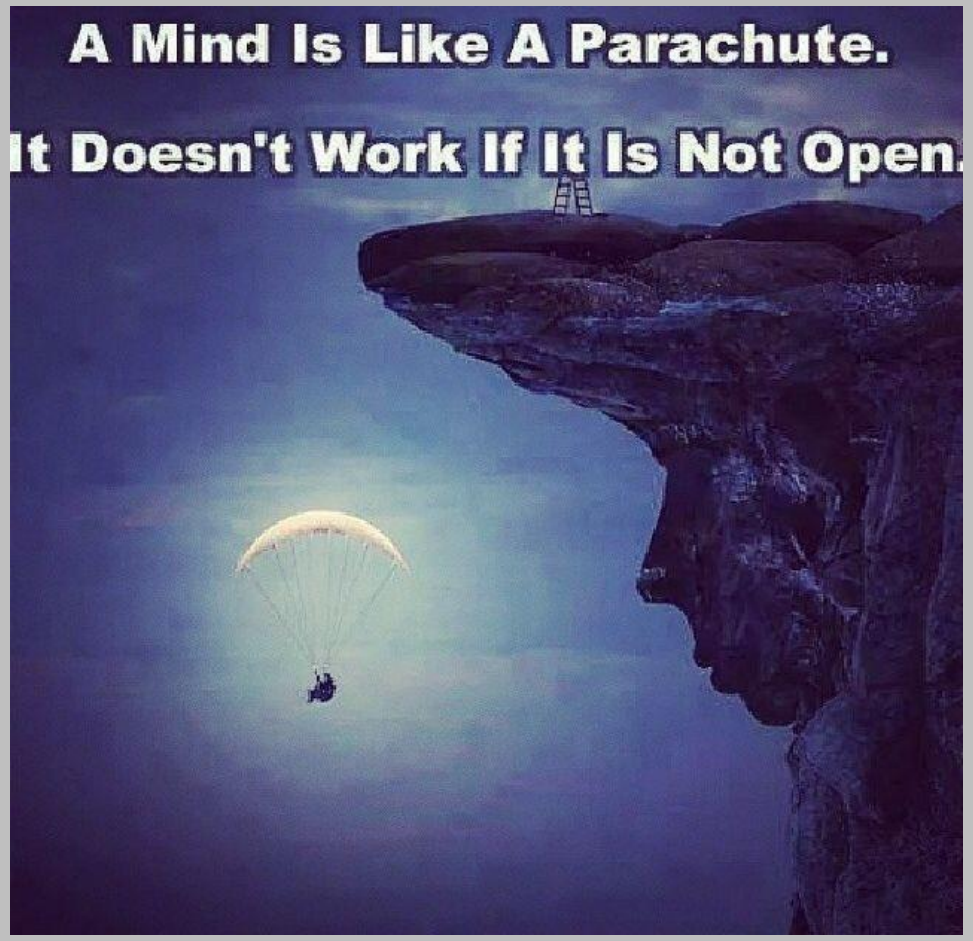
Martin C. Tom,*,† Jane K. Nguyen,* Roberta Lucianò, Omar Y. Mian, Kevin L. Stephans, Jay P. Ciezki, Timothy D. Smile, Wei Wei, Jesse K. McKenney, Cristina Magi-Galluzzi and Rahul D. Tendulkar‡

No cribriform; no IDC
Cribriform without IDC
Cribriform with IDC



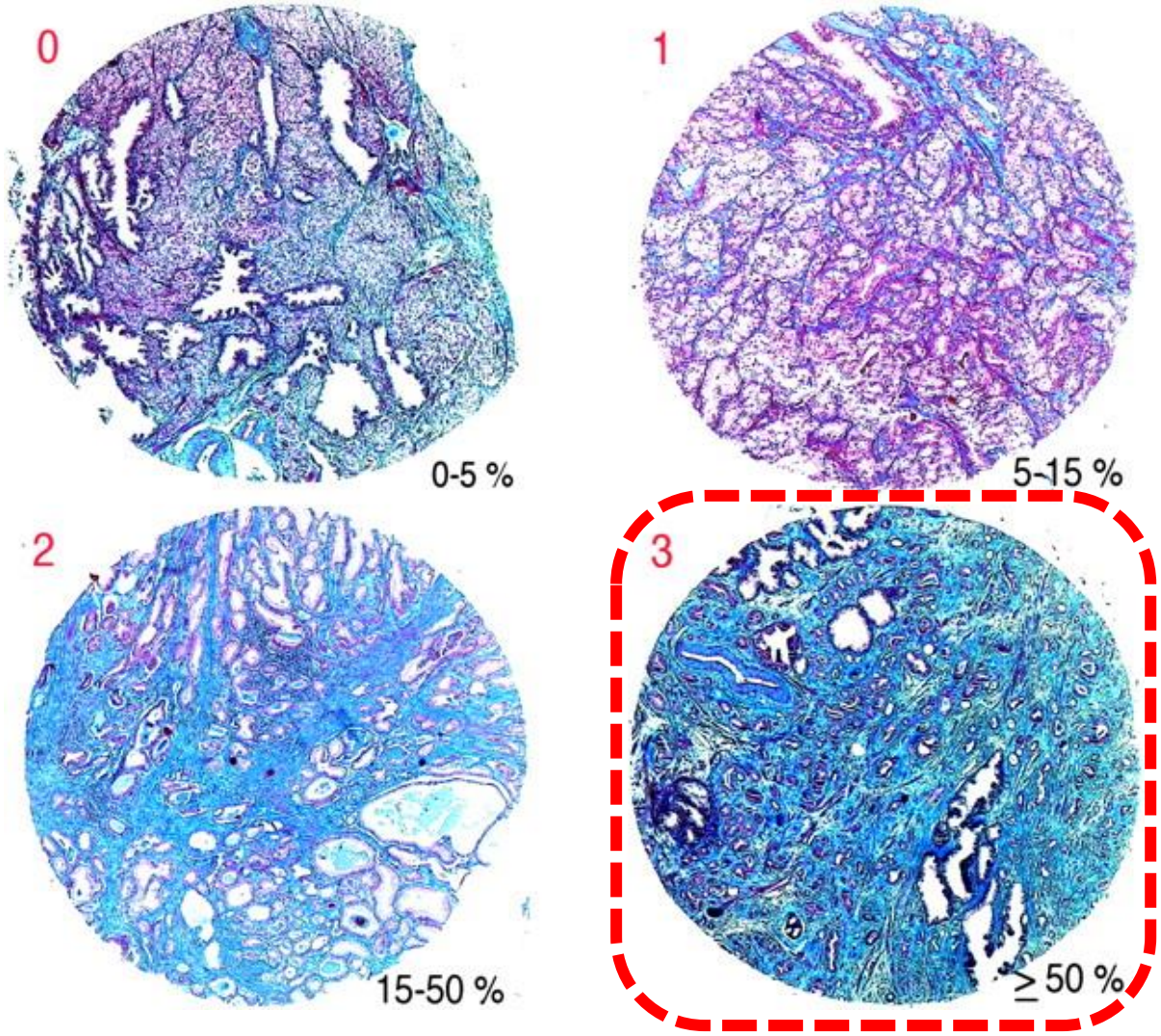


Moving Beyond Gleason Scoring….is it time?

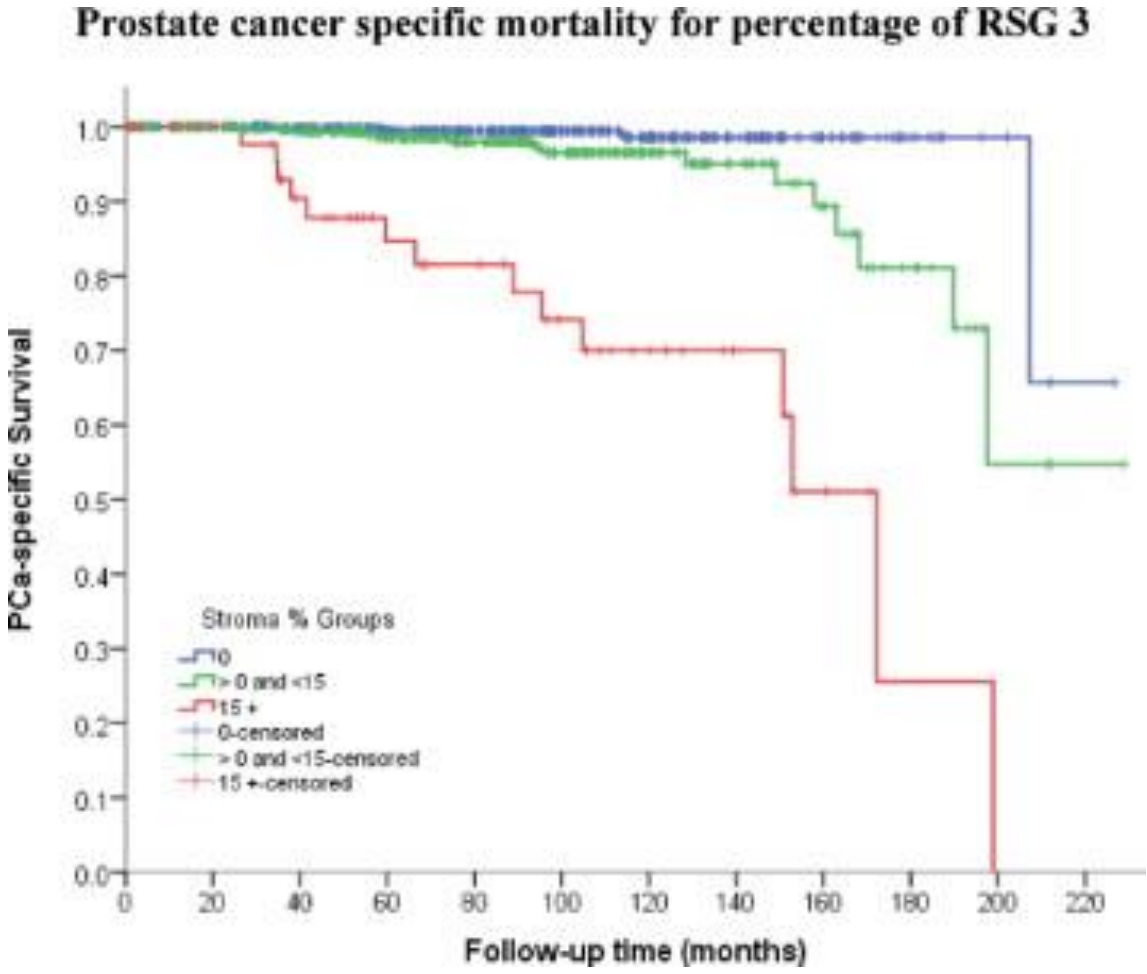


Miles at al. Arch Pathol Lab Med 2019

Reactive stromal grading (RSG) predictive ability for prostate cancer-specific death and biochemical recurrence

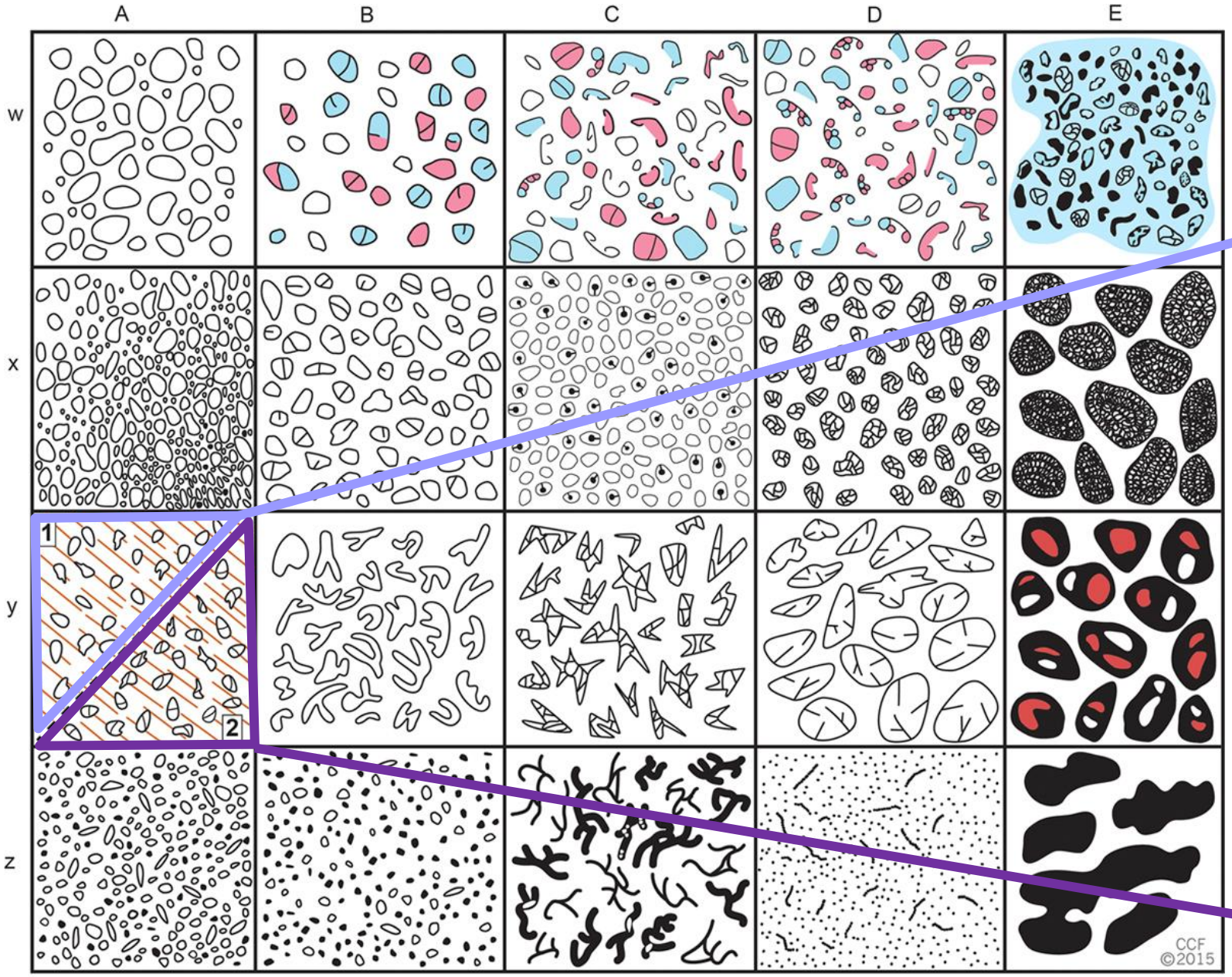


stromogenic carcinoma

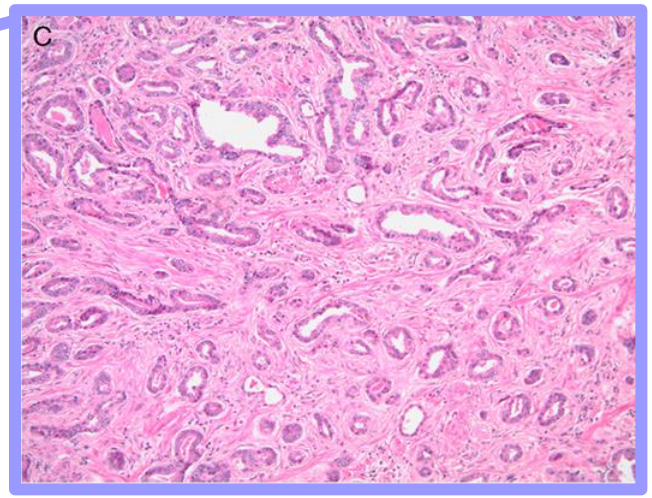


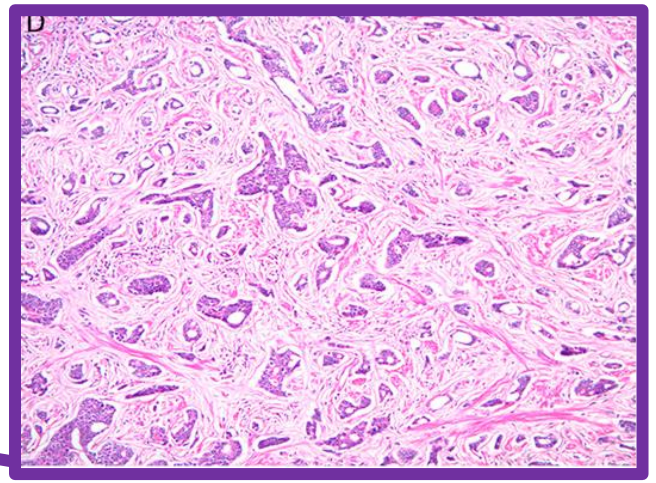
Ayala et al. Clinical Cancer Res 2003; Ayala et al. A. J Pathol 2011

Individual architectural patterns independent of Gleason in 1275 pts



reactive stroma response was associated with worse RFS





McKenney et al. Am J Surg Path 2016

The NEW ENGLAND JOURNAL of MEDICINE

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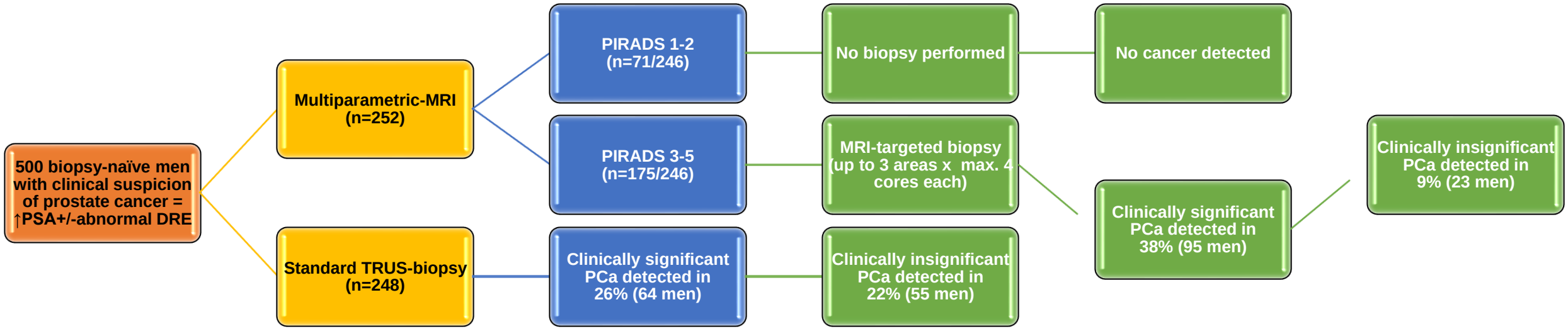
MRI-Targeted or Standard Biopsy for Prostate-Cancer Diagnosis

V. Kasivisvanathan, A.S. Rannikko, M. Borghi, V. Panebianco, L.A. Mynderse, M.H. Vaarala, A. Briganti, L. Budäus, G. Hellawell, R.G. Hindley, M.J. Roobol, S. Eggener, M. Ghei, A. Villers, F. Bladou, G.M. Villeirs, J. Virdi, S. Boxler, G. Robert, P.B. Singh, W. Venderink, B.A. Hadaschik, A. Ruffion, J.C. Hu, D. Margolis, S. Crouzet, L. Klotz, S.S. Taneja, P. Pinto, I. Gill, C. Allen, F. Giganti, A. Freeman, S. Morris, S. Punwani, N.R. Williams, C. Brew-Graves, J. Deeks, Y. Takwoingi, M. Emberton, and C.M. Moore, for the PRECISION Study Group Collaborators*

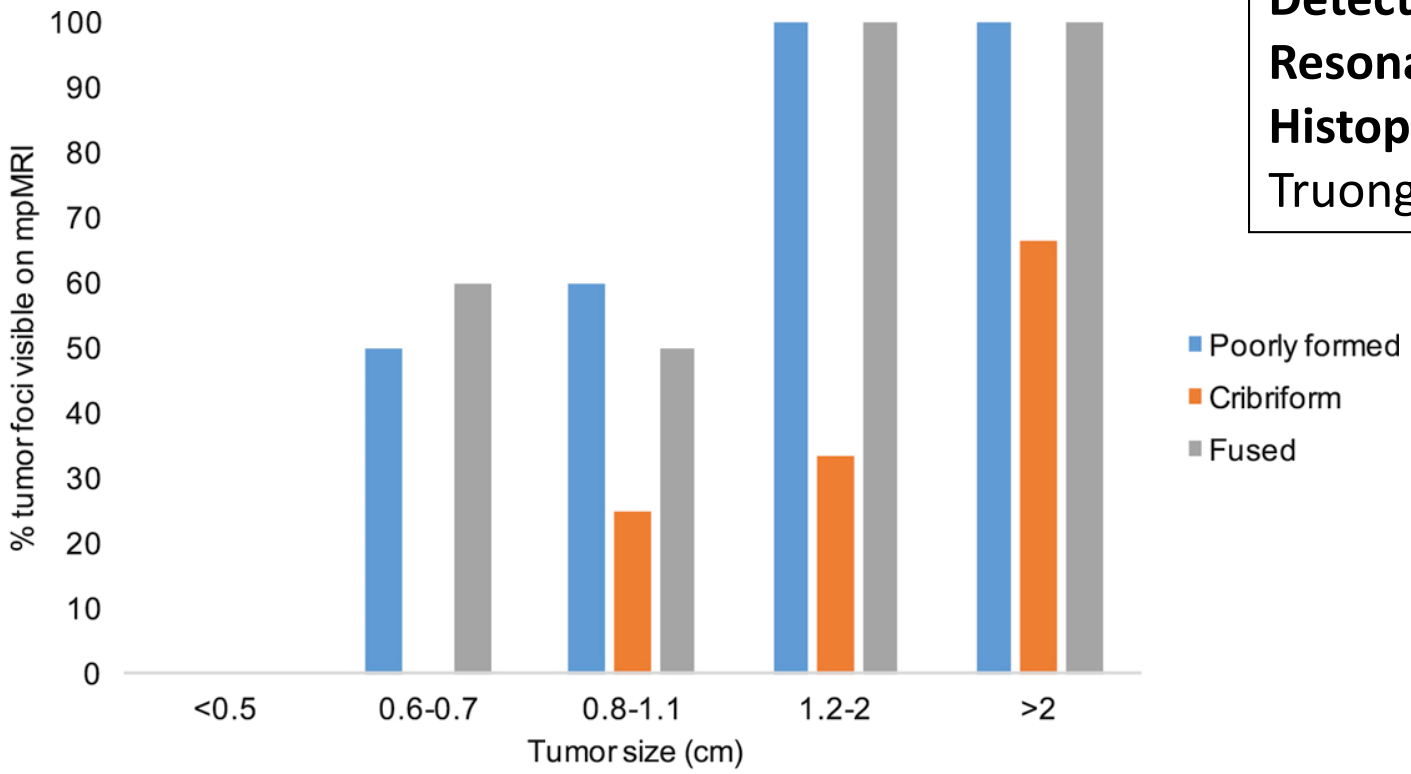
MRI-Targeted Biopsy

Clinically significant PCA: GS 3+4=7
Ø pattern 4 quantitation



Conclusions: 12% increase in clinically significant & 13% decrease in clinically insignificant PCA

Impact of Gleason Subtype on Prostate Cancer Detection Using Multiparametric Magnetic Resonance Imaging: Correlation with Final Histopathology.
Truong, Matthew et al. Journal of Urology 2017



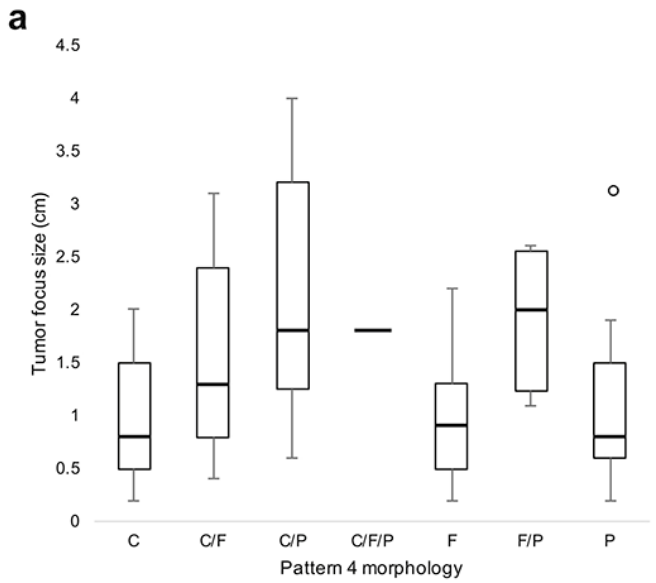
	Tumor size (cm)	<0.5	0.6-0.7	0.8-1.1	1.2-2	>2
Poorly formed	Visible	0	1	3	1	5
	Total	1	2	5	1	5
Cribriform	Visible	0	0	1	2	2
	Total	4	1	4	6	3
Fused	Visible	0	3	1	5	2
	Total	4	5	2	5	2

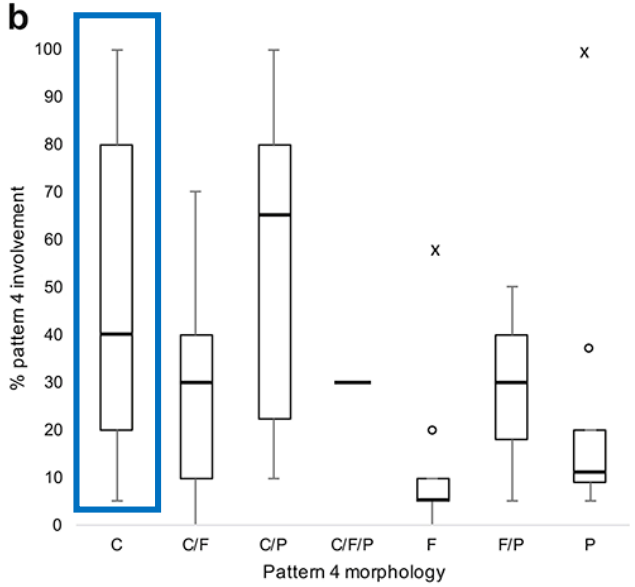
MRI visibility of GP4 subtype stratified by tumor size

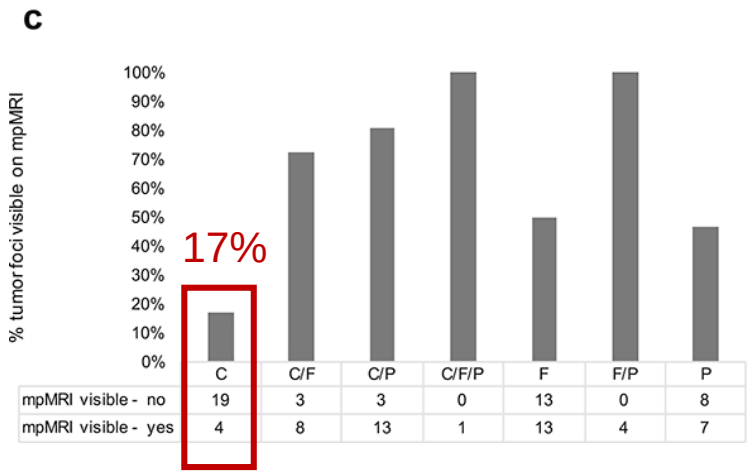
- MRI did not detect Gleason pattern 4 tumors less than 0.5 cm.
- **Visibility of cribriform tumors was lower** than that of other architectural patterns across all tumor sizes.

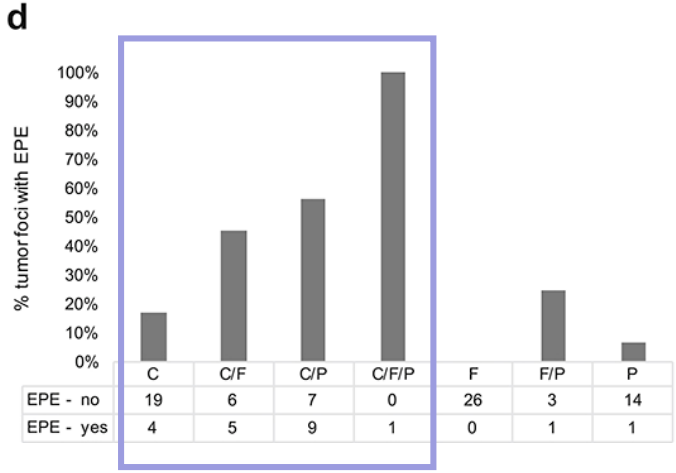
A Comprehensive Analysis of Cribriform Morphology on MRI/Ultrasound Fusion Biopsy Correlated with RP

Truong et al. Journal of Urology 2018









Characteristics of 96 tumor foci containing GP4

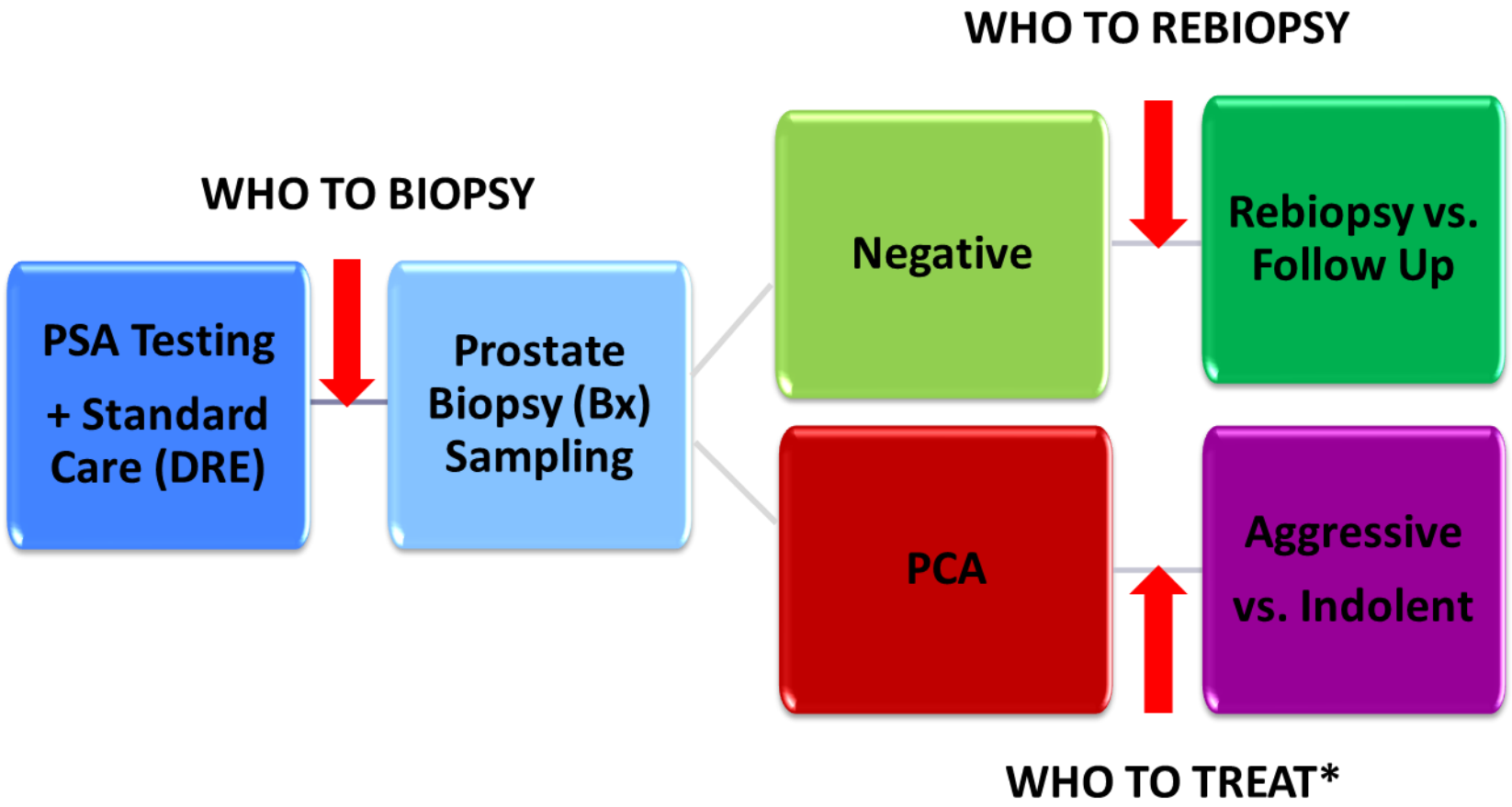
a, pure cribriform (C), fused (F) and poorly formed (P) tumors were smaller than mixed tumors.

b, cribriform tumors had **higher % GP4**.

c, **pure cribriform tumors were frequently missed on MRI;** mixed GP4 tumors with cribriform morphology were less visible than mixed F/P tumors.

d, **EPE was more common in cribriform tumors**, but it was found in 0/26 fused and 1/15 (6.17%) poorly formed tumors.[2]

RATIONALE FOR PCA BIOMARKERS DEVELOPMENT



*Radical treatment vs. AS in Bx setting;

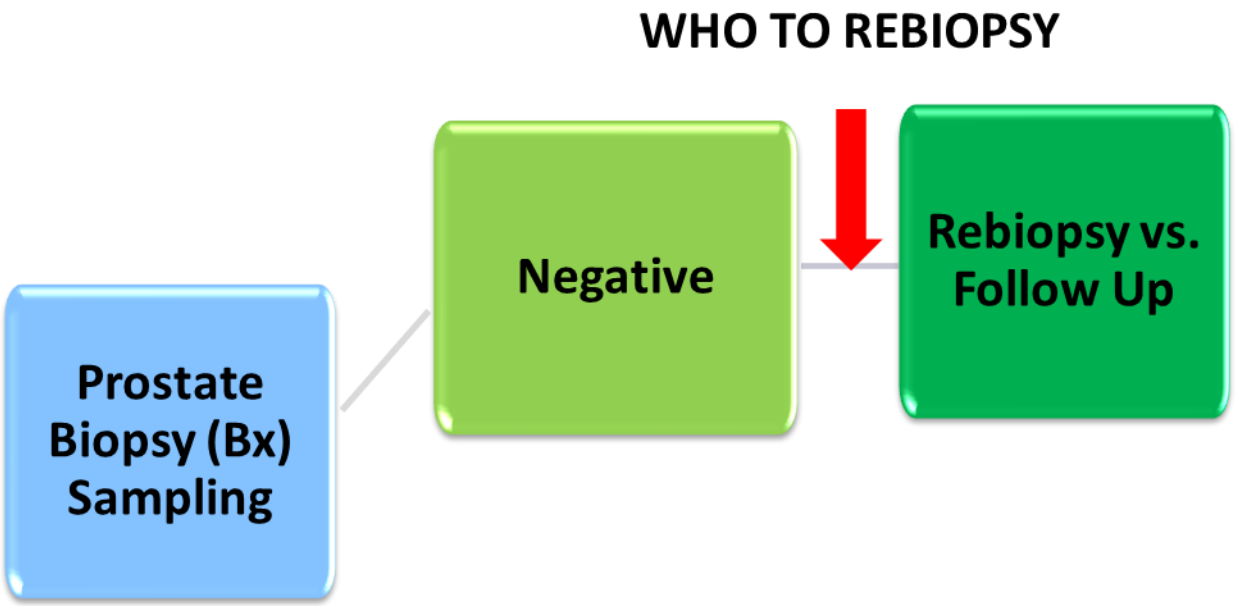
*Multimodal therapy vs. observation in post treatment setting

BIOMARKERS AND GENOMIC TESTS

- Predict biopsy outcome
- Identify clinically significant/aggressive disease
- Predict disease progression/outcome
- Advise management (right treatment at appropriate time)

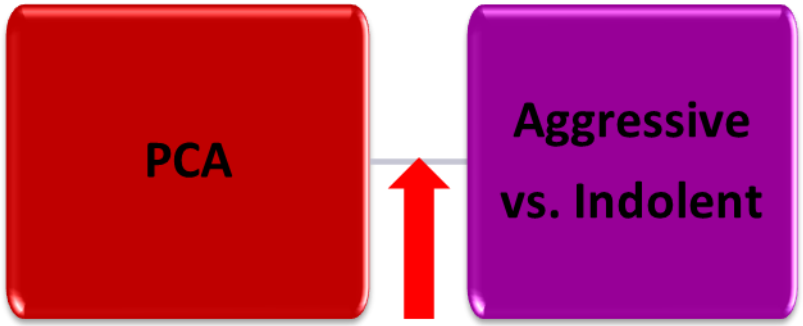
Who to Biopsy	Who to Rebiopsy	Who to Watch or Treat
• PSA • PCA3 • PHI • TMPRSS2-ERG • 4Kscore	• PCA3 • Confirm MDx • Prostate Core Mitomic Test (PCMT)	• OncotypeDX • Prolaris • ProMark • Decipher

PCA TISSUE-BASED GENOMIC TESTS



- PCA3
- Confirm MDx
- Prostate Core Mitomic Test (PCMT)

PCA TISSUE-BASED GENOMIC TESTS



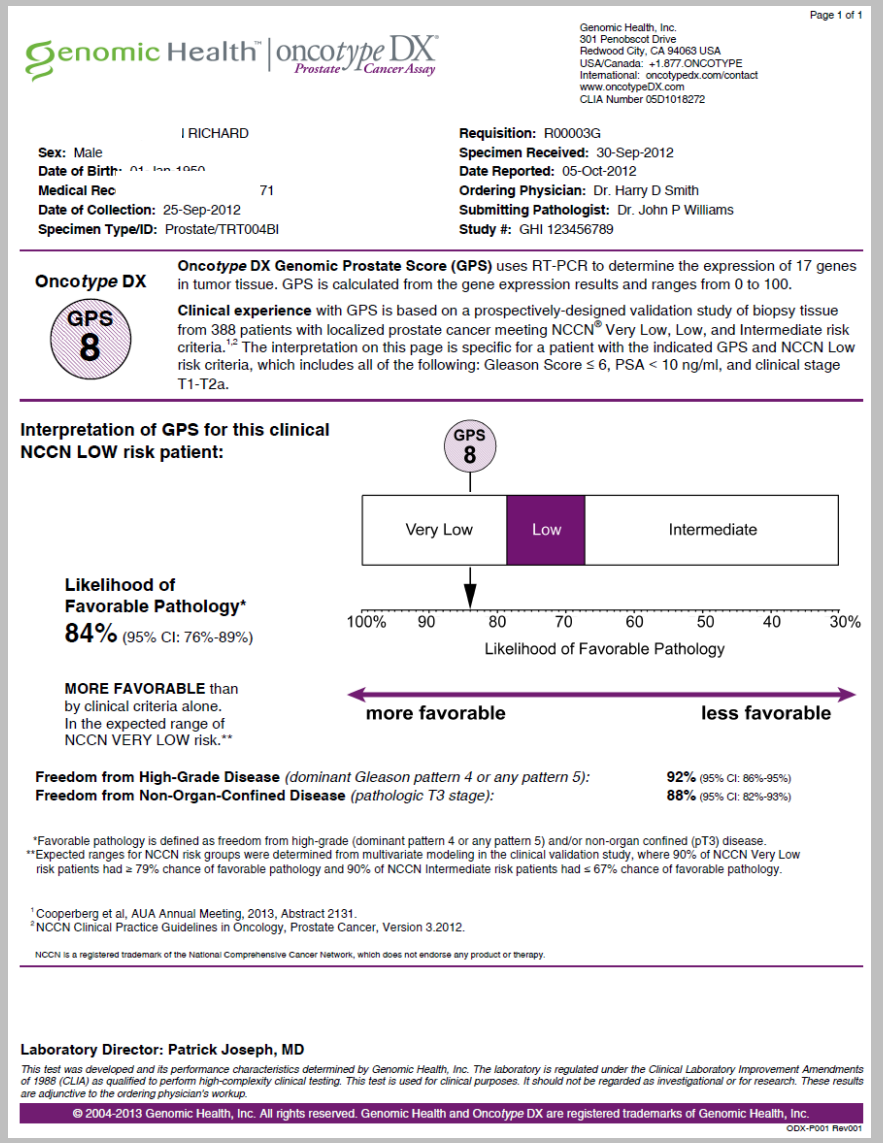
WHO TO TREAT*

- OncotypeDX
- Prolaris
- Decipher

Oncotype DX® Prostate Cancer Assay

- **WHAT is the test?**
  - qRT-PCR measuring 5 reference genes-normalized RNA expression of 12 cancer genes from PCA tissue (≥1 mm)
  - Lower Genomic Prostate Score (GPS, 0-100) indicates higher likelihood of favorable RP pathology (LFP)
- **WHO is the test for?**
  - Men newly diagnosed with low, low-intermediate risk PCA (GS 3+3, low volume 3+4)
- **WHY do the test?**
  - Identify patients for AS or immediate treatment

Clinical Endpoint: Adverse Pathology at RP



Page 1 of 1

Genomic Health™ | oncotype DX® Prostate Cancer Assay

Genomic Health, Inc.
301 Penobscot Drive
Redwood City, CA 94063 USA
USA/Canada: +1.877.ONCOTYPE
International: oncotypedx.com/contact
www.oncotypeDX.com
CLIA Number 05D1018272

I RICHARD
Sex: Male
Date of Birth: 01-Jan-1950
Medical Rec 71
Date of Collection: 25-Sep-2012
Specimen Type/ID: Prostate/TRT004BI

Requisition: R00003G
Specimen Received: 30-Sep-2012
Date Reported: 05-Oct-2012
Ordering Physician: Dr. Harry D Smith
Submitting Pathologist: Dr. John P Williams
Study #: GHI 123456789

Oncotype DX
GPS 8

Oncotype DX Genomic Prostate Score (GPS) uses RT-PCR to determine the expression of 17 genes in tumor tissue. GPS is calculated from the gene expression results and ranges from 0 to 100.

Clinical experience with GPS is based on a prospectively-designed validation study of biopsy tissue from 388 patients with localized prostate cancer meeting NCCN® Very Low, Low, and Intermediate risk criteria.[1,2] The interpretation on this page is specific for a patient with the indicated GPS and NCCN Low risk criteria, which includes all of the following: Gleason Score ≤ 6, PSA < 10 ng/ml, and clinical stage T1-T2a.

Interpretation of GPS for this clinical NCCN LOW risk patient:

GPS 8

Very Low | Low | Intermediate

100% 90 80 70 60 50 40 30%

Likelihood of Favorable Pathology

Likelihood of Favorable Pathology*
84% (95% CI: 76%-89%)

MORE FAVORABLE than by clinical criteria alone. In the expected range of NCCN VERY LOW risk.**

more favorable ↔ less favorable

Freedom from High-Grade Disease *(dominant Gleason pattern 4 or any pattern 5):* **92%** (95% CI: 86%-95%)
Freedom from Non-Organ-Confined Disease *(pathologic T3 stage):* **88%** (95% CI: 82%-93%)

*Favorable pathology is defined as freedom from high-grade (dominant pattern 4 or any pattern 5) and/or non-organ confined (pT3) disease.
**Expected ranges for NCCN risk groups were determined from multivariate modeling in the clinical validation study, where 90% of NCCN Very Low risk patients had ≥ 79% chance of favorable pathology and 90% of NCCN Intermediate risk patients had ≤ 67% chance of favorable pathology.

[1] Cooperberg et al, AUA Annual Meeting, 2013, Abstract 2131.
[2] NCCN Clinical Practice Guidelines in Oncology, Prostate Cancer, Version 3.2012.

NCCN is a registered trademark of the National Comprehensive Cancer Network, which does not endorse any product or therapy.

Laboratory Director: Patrick Joseph, MD

This test was developed and its performance characteristics determined by Genomic Health, Inc. The laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. These results are adjunctive to the ordering physician's workup.

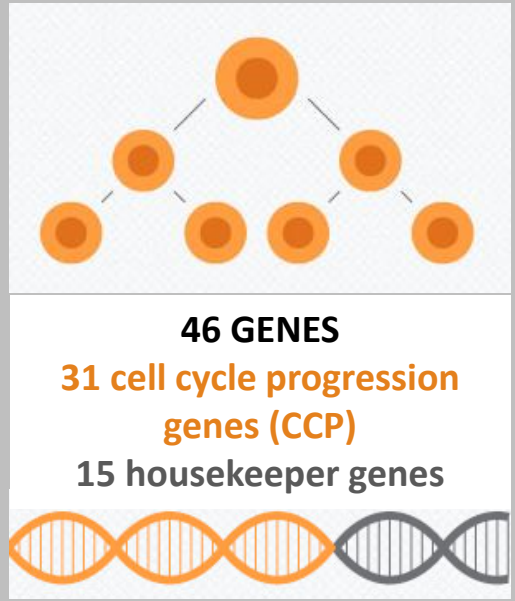
ODX-P001 Rev001

Knezevic et al. BMC Genomics 2013; Klein et al. Eur Urol 2014; Cullen et al. Eur Urol 2015

Prolaris Score

- **WHAT is the test?**
  - qRT-PCR measuring RNA expression of 31 cell cycle progression (CCP) and 15 housekeeping genes (>0.5 mm PCA)
  - Report factors in clinicopathological data to calculate patient's 10-year PCA-specific mortality risk (higher CCP-score=more aggressive disease
- **WHO is the test for?**
  - Men diagnosed with PCA
- **WHY do the test?**
  - Predict disease specific mortality
  - Identify appropriate patients for AS or immediate treatment
  - Add predictive value to postoperative risk models

Clinical Endpoint: Prostate Cancer Death



Prolaris Score	10-year death rate %
<0.0	7
0.0–1.0	15
1.1–2.0	36
>2.0	59

Cuzick et al. Br J Cancer 2012; Bishoff et al. J Urol 2014; Cuzick et al Br J Cancer 2015; Oderda et al. Urology 2017

Decipher Prostate Cancer Test

- **WHAT is the test?**
  - Ribonucleic acid-based genomic classifier (GC) test using 22 RNAs from coding/non-protein coding regions derived from FFPE RP specimens
- **WHO is the test for?**
  - Patients with adverse pathology at RP
- **WHY do the test?**
  - Predict risk of early metastatic (within 5 years) disease and PCA-specific mortality following RP
  - GC 0 -1 (increments of 0.1=10% increase metastatic risk)
  - Select patients who may benefit from multimodal therapy/clinical trial

Clinical Endpoint: Metastasis

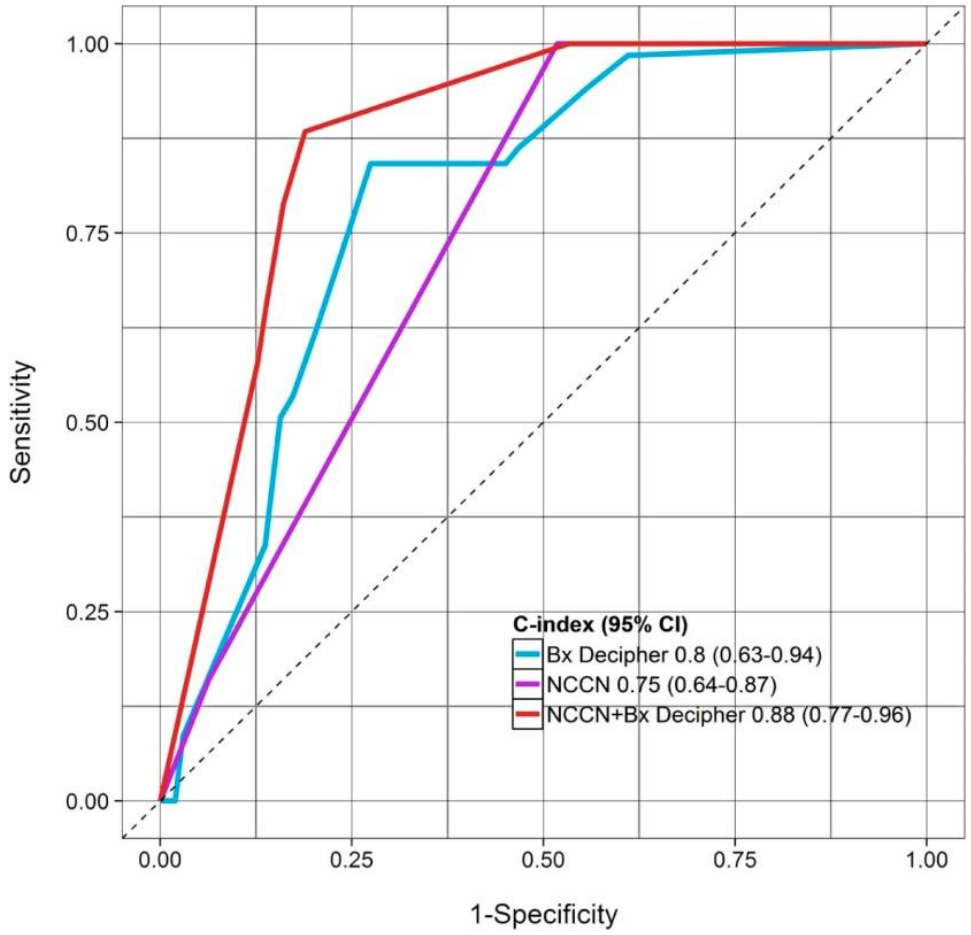
Erho et al. PLoS One 2013; Karnes et al. J Urol 2013; Klein et al. Eur Urol 2014; Knudsen et al. J Mol Diagn 2016

Decipher Genomic Classifier Measured on Prostate Biopsy Predicts Metastasis Risk

Eric A. Klein, Zaid Haddad, Kasra Yousefi, Lucia L. C. Lam, Qiqi Wang, Voleak Choeurng, Beatrix Palmer-Aronsten, Christine Buerki, Elai Davicioni, Jianbo Li, Michael W. Kattan, Andrew J. Stephenson, and Cristina Magi-Galluzzi

		PBx Decipher Risk Group			
	# mets/# patients (row %)	Low (<0.45)	Interm. (0.45-06)	High (>0.6)	Total
NCCN risk group	Low	0/20 (87%)	0/3 (13%)	0/0 (0%)	23
	Interm.	0/13 (48%)	**4/9** (33%)	**2/5** (18%)	27
	High	**1/3** (75%)	**1/1** (25%)	0/0 (0%)	4
	Unknown	0/2 (67%)	0/1 (33%)	0/0 (0%)	3
Total		38	14	5	**57**

Survival C-index @ 10yrs post-RP for PBx variables

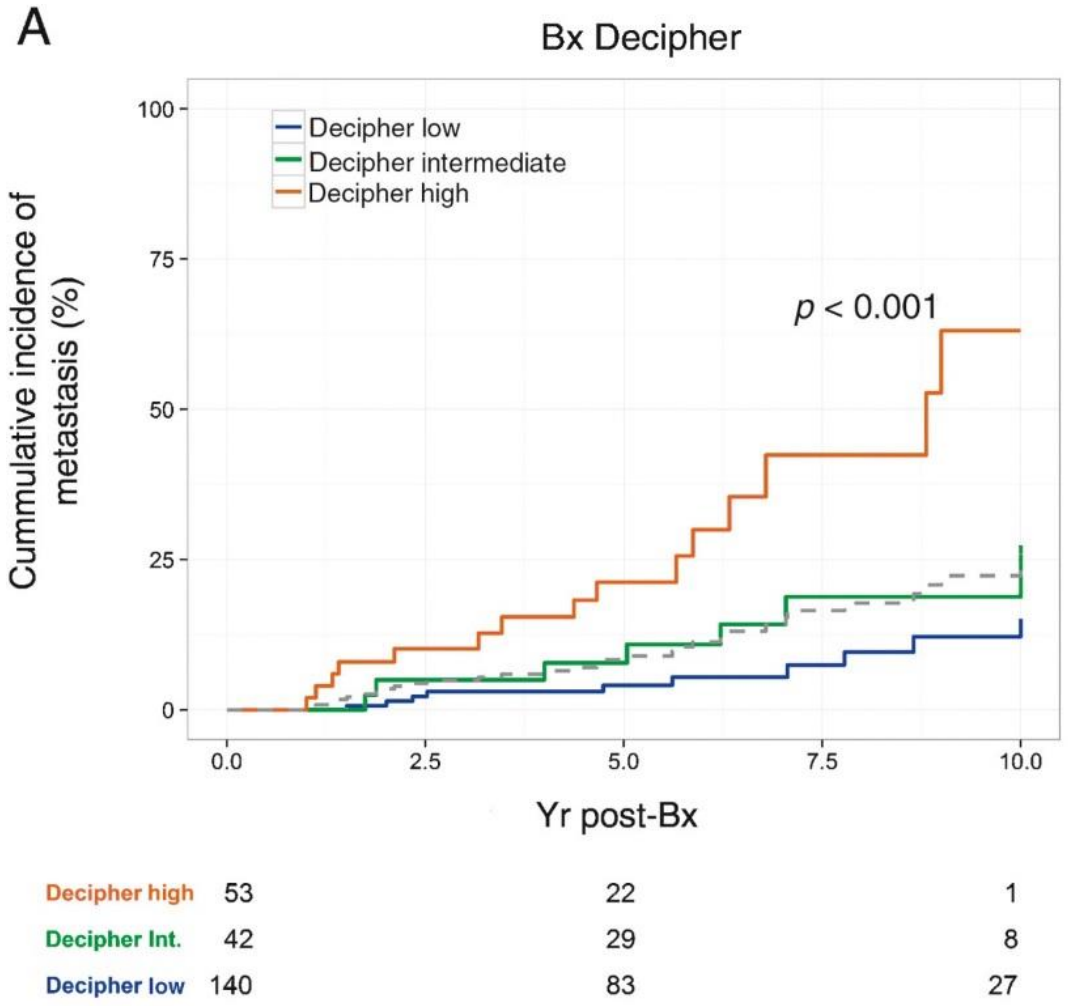


- Median follow-up 8 years; 8 pts. metastasized; 3 DOD
- After adjusting for age, PSA, GS, GC only significant predictor of metastasis

Klein EA et al. Urology 2016

Ability of a Genomic Classifier to Predict Metastasis and PCSM after Radiation or Surgery based on Bx Specimens

- 235 pts. treated with RT±ADT or RP
- Genomic profile from Bx
- Median FU 6 yrs; 34 pts. developed metastases; 11 died of PCA
- Predicting metastasis 5-yr post-Bx:
  - CAPRA score c-index: 0.60
  - CAPRA + **Bx Decipher** c-index: **0.71**
  - NCCN risk group c-index: 0.66
  - NCCN + **Bx Decipher** c-index: **0.74**
- Bx Decipher predicted metastasis and PCSM



Nguyen PL et al. Eur Urology 2017

Prostate cancer risk stratification

Low Risk	GG1	without stromal response
	GG2	without cribriform, or IDC-P, or stromal response
High Risk	GG2	**with cribriform, or IDC-P, or stromal response**
	GG≥3	independent of stromal response

CURRENT CHALLENGES & PERSPECTIVES

- Moving beyond Gleason scoring
  - Stromal response
  - Architectural patterns
- MRI-US fusion biopsy:
  - MRI-invisible PCA
  - Occult cribriform morphology
  - Radiomics & radiogenomics (radiophenotype)
- Tissue-based genomic tests
  - Intra-tumoral heterogeneity

PATHOLOGIST'S ROLE

- In addition to accurate PCA diagnosis, includes reporting elements essential to estimate malignant potential
- Pathological parameters need to be:
  - Accurate
  - Reproducible
  - Consistently reported
- Unfavorable pathology (IDC, cribriform architecture, stromal response) is an important predictor of clinical outcome
- Accurate validation of genomic tests is critical for appropriate integration into clinical practice

THANK YOU!

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SCHOOL OF MEDICINE