Diagnostic Immunohistochemistry Selected Topics

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IMMUNOHISTOCHEMISTRY

Selected Topics

- General Issues
- Breast Carcinoma
- GI Tract Tumors
- Tumors in the Liver
- Male GU Tract Tumors

Ground Rules

- Immunohistochemistry integrates, not replaces, H&E histology
- Conclusions and recommendations based on personal experience and selected published literature
- Not all published literature is equally valid

General Issues

- Standardization: are we there yet?
- Why do antibodies start out specific and end up not so specific?
- How do you decide what the cutoff is for positivity?

What Could Could Be Standardized

- Interval between time tissue removed from patient and immersion into fixative
- Fixative composition - including buffer, tonicity, pH, temperature
- Ratio of tissue to fixative
- Duration and temperature of fixation
- Tissue processing times, reagents
- Heating, drying conditions of slides
- Length of time of slide storage before use
- Epitope retrieval buffer, pH, duration, temperature
- Cooling time following epitope retrieval
- Primary antibody, diluent, duration of incubation
- Detection system
- Instrumentation
- Chromogen (type, length of incubation)

J Clin Oncol 25:118-45, 2007

American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Rec tor 2 Testing in Breast Cancer

Antonio C. Wolff, M. Elizabe Hammond, Jar wartz, Karen L. rty, D. Craig Allred, Richard J. Cote, Mitchell Dows L. Fitz dad M. ny Langer, Lisa M. McShane, Soonmyung Paik, Mark D. Pegr Rhodes, Catharine Sturgeon, Sheila E. Taube, Raymond Tubbs, nas M. Wheeler, and Daniel F. Hayes

Attempt at Standardization

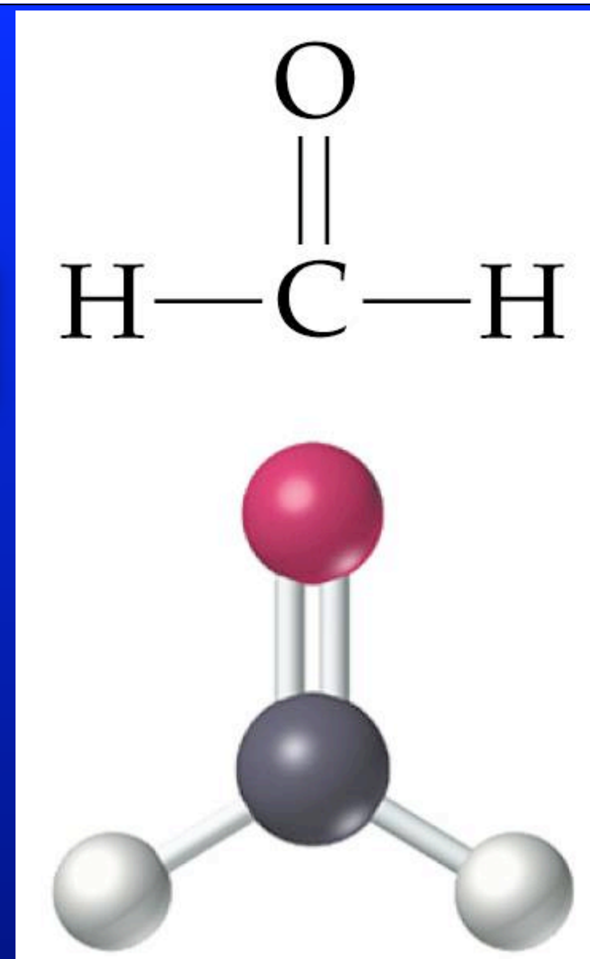
Lab Med 131:18-43, 2007

America College of American Pathologis ecommendations for Human Epider Growth ctor Receptor 2 Testi g in Breast C ncer

Antonio C. Wolff, M. Elizabeth H. Hammond, Jared N. Schwartz, Karen L. Hagerty, D. Craig Allred, Richard J. Cote, Mitchell Dowsett, Patrick L. Fitzgibbons, Wedad M. Hanna, Amy Langer, Lisa M. McShane, Soonmyung Paik, Mark D. Pegram, Edith A. Perez, Michael F. Press, Anthony Rhodes, Catharine Sturgeon, Sheila E. Taube, Raymond Tubbs, Gail H. Vance, Marc van de Vijver, Thomas M. Wheeler, Daniel F. Hayes

FORMALIN


Surgipath
FORMALIN
CONCENTRATE
FIRST AID
Surgipath
FORMALIN
CONCENTRATE




DANGER
FORMALDEHYDE
IRRITANT AND POTENTIAL
CANCER HAZARD
AUTHORIZED PERSONNEL ONLY

Fixation Requirements

- 10% neutral buffered formalin*
- Fixed for no less than 6 and no more than 48 hours**

*Not exclusion criterion, but other fixatives must be validated

*Not exclusion criterion, but if not met and test negative, disclaimer should appear

What Could Could Be Standardized

- Interval between time tissue removed from patient and immersion into fixative
- Fixative composition - including buffer, tonicity, pH, temperature
- Ratio of tissue to fixative
- Duration and temperature of fixation
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- Instrumentation
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Is Standardization Feasible?

(Water boils below 95º C. above 5000 feet)

HER2/neu Testing at Altitudes Above 5000 Feet

Patsy Ruegg[1]*, and LuAnne Lupfer[2]
[1]Pathology Department, University of Colorado Health Sciences Center, Denver, Colorado
[2]Dako Corporation, Carpinteria, CA

J Histotechnol 24:129-30, 2001

A Radical but Provable Hypothesis

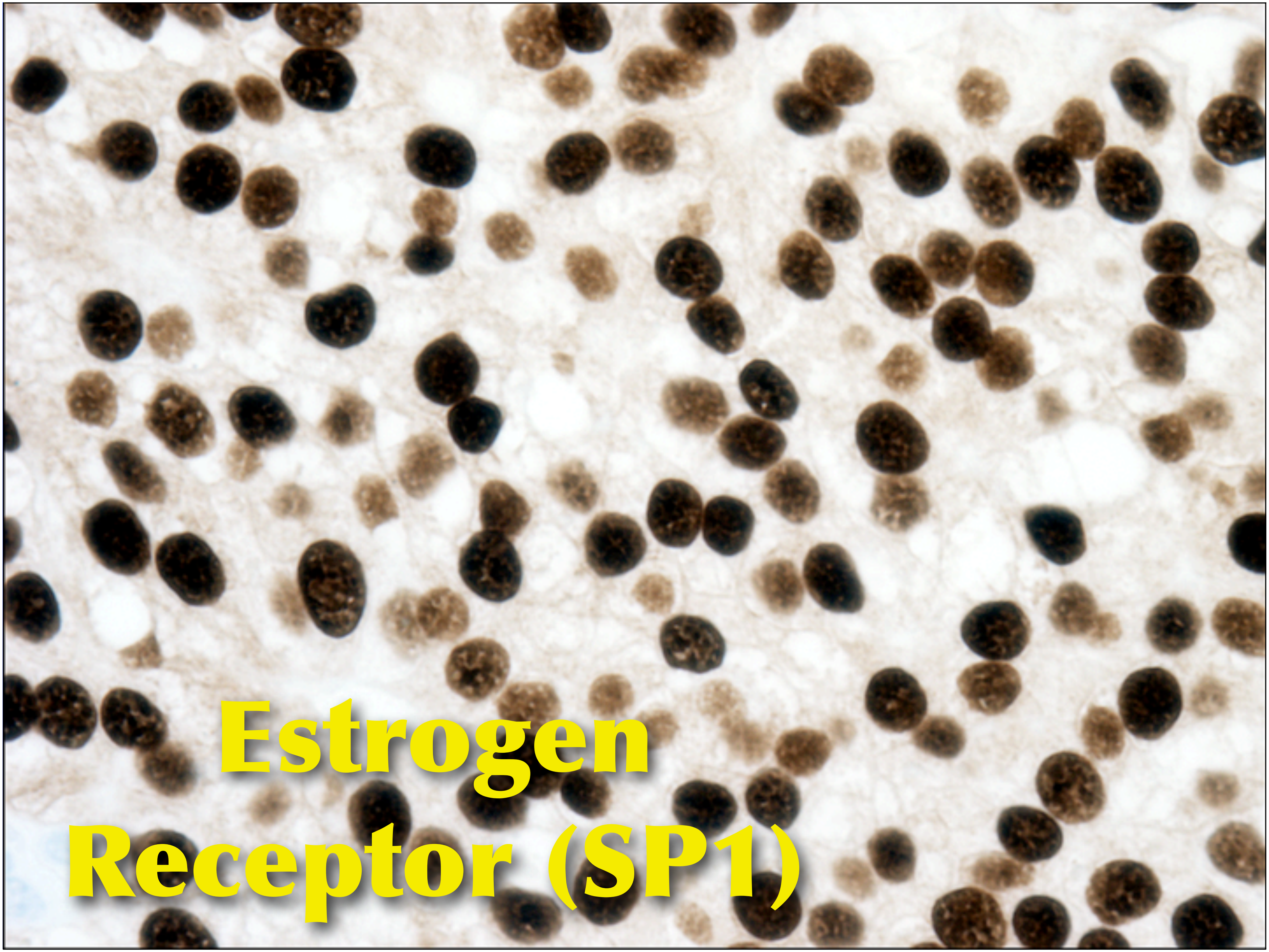
- It is not **feasible** (or perhaps even possible) to standardize all facets of immunohistochemistry
- It is not **necessary** to standardize all facets of immunohistochemistry to achieve high levels of test accuracy and precision

Mandate Standardized Results, not Standardized Methods

- Use **robust** methods that overcome and/or override methodological variations

Examples

- Use antibodies that are (relatively) forgiving of vagaries of tissue fixation - SP1 v 1D5
- Use image analysis
- Use normalization where appropriate


Estrogen
Receptor (SP1)

Immunohistochemical Detection Using the New Rabbit Monoclonal Antibody SP1 of Estrogen Receptor in Breast Cancer Is Superior to Mouse Monoclonal Antibody 1D5 in Predicting Survival

J Clin Oncol 24:5637-44, 2006

Maggie C.U. Cheang, Diana O. Treaba, Caroline H. Speers, Ivo A. Olivotto, Chris D. Bajdik, Stephen K. Chia, Lynn C. Goldstein, Karen A. Gelmon, David Huntsman, C. Blake Gilks, Torsten O. Nielsen, and Allen M. Gown

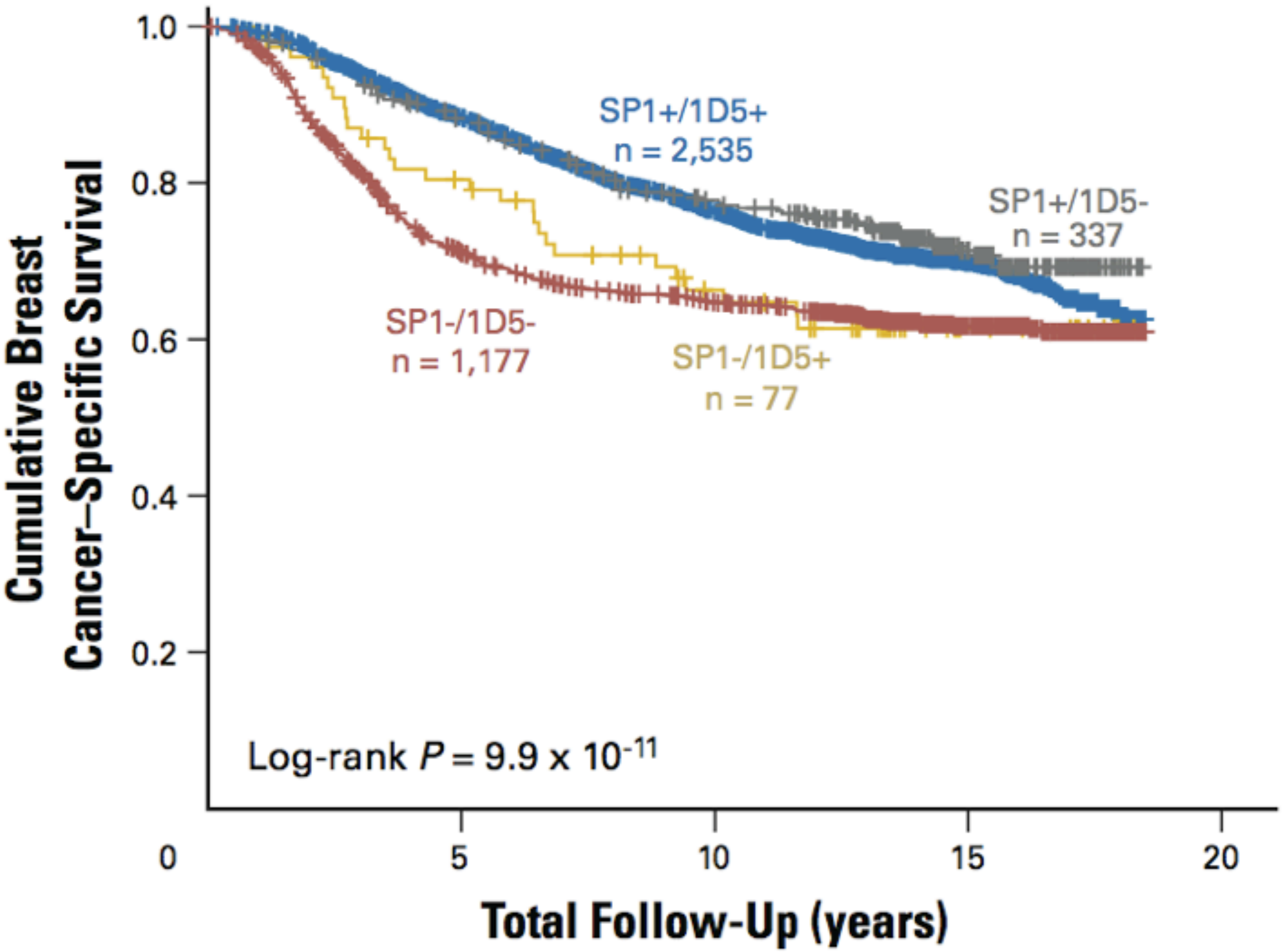
- TMA based study from BCCA patients
- N = 4150
- Median follow-up 12.4 years
- Compared 1D5 with SP1 in predicting outcome and tamoxifen response

Immunohistochemical Detection Using the New Rabbit Monoclonal Antibody SP1 of Estrogen Receptor in Breast Cancer Is Superior to Mouse Monoclonal Antibody 1D5 in Predicting Survival

J Clin Oncol 24:5637-44, 2006

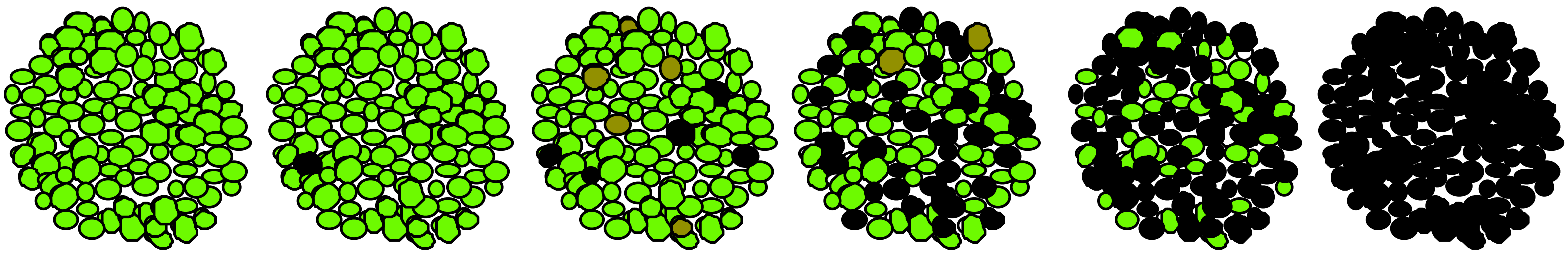
Maggie C.U. Cheang, Diana O. Treaba, Caroline H. Speers, Ivo A. Olivotto, Chris D. Bajdik, Stephen K. Chia, Lynn C. Goldstein, Karen A. Gelmon, David Huntsman, C. Blake Gilks, Torsten O. Nielsen, and Allen M. Gown

- Higher positivity rate (SP1 = 69% vs. 1D5 = 62%)
- SP1 better predicts outcome
- SP1 better predicts response to tamoxifen
- SP1 correlates better with ligand binding



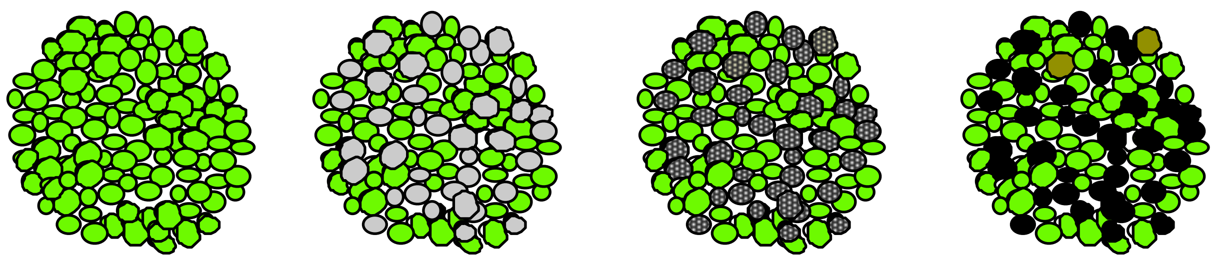
Cheang MCU et al., J Clin Oncol 24:5637-44, 2006

Allred Score



Proportion Score (PS)

0 | **1** 1/100 | **2** 1/10 | **3** 1/3 | **4** 2/3 | **5** 1



Intensity Score (IS)

0 = negative | **1** = weak | **2** = intermed | **3** = strong

Total Score = PS + IS (score from 0 to 8)

from Mohsin, SK

Estrogen Receptor Status by Immunohistochemistry Is Superior to the Ligand-Binding Assay for Predicting Response to Adjuvant Endocrine Therapy in Breast Cancer

Jennet M. Harvey, Gary M. Clark, C. Kent Osborne, and D. Craig Allred

Defining ER Positivity

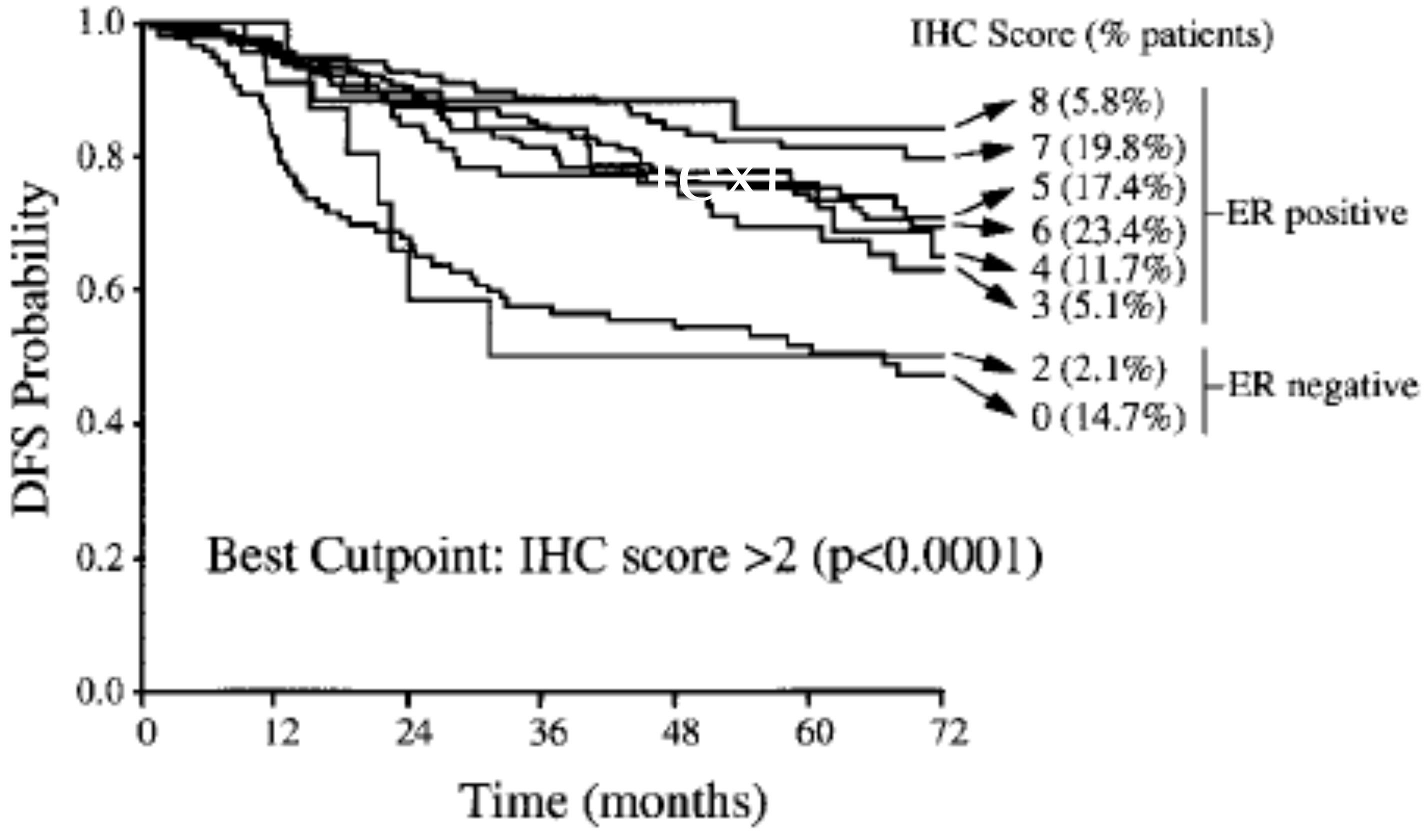
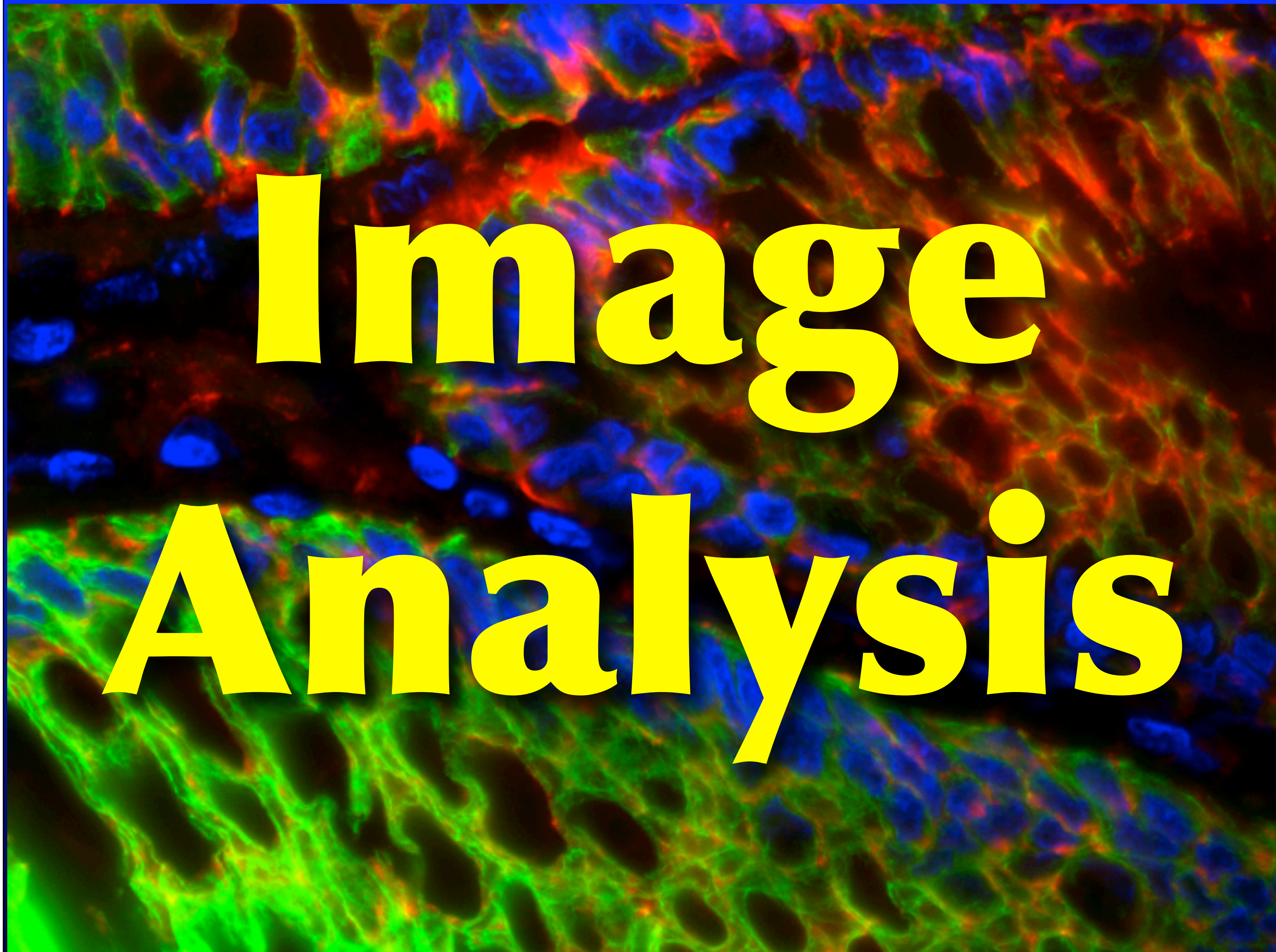


Image Analysis



Br Cancer Treat Res 110:417-26, 2008

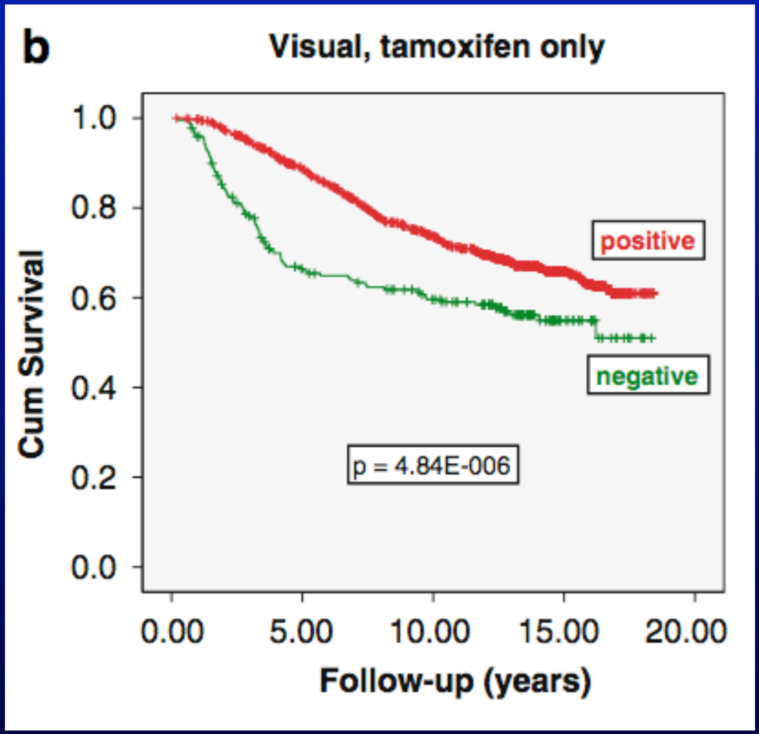
Automated quantitative analysis of estrogen receptor expression in breast carcinoma does not differ from expert pathologist scoring: a tissue microarray study of 3,484 cases

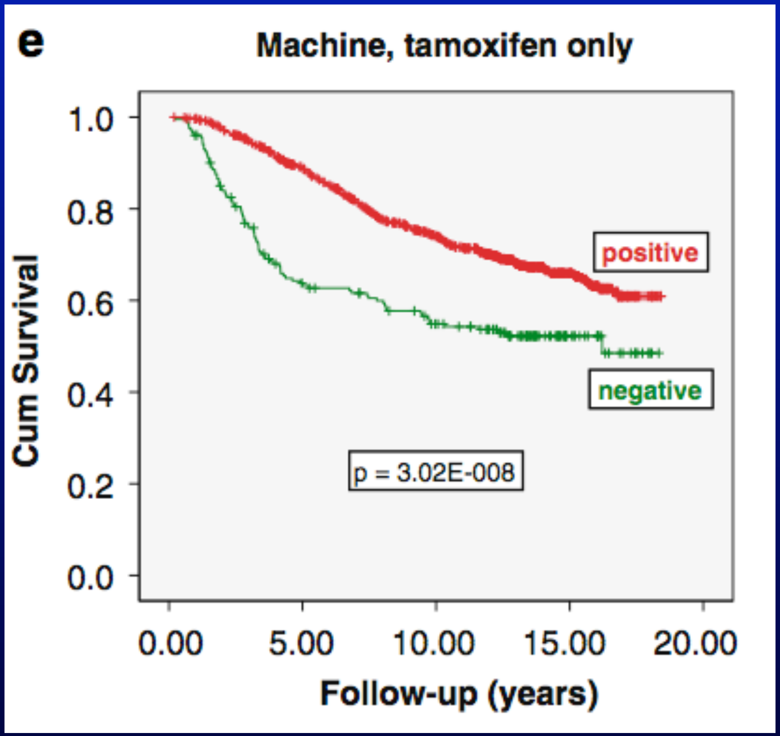
Dmitry A. Turbin · Samuel Leung · Maggie C. U. Cheang · Hagen A. Kennecke · Kelli D. Montgomery · Steven McKinney · Diana O. Treaba · Niki Boyd · Lynn C. Goldstein · Sunil Badve · Allen M. Gown · Matt van de Rijn · Torsten O. Nielsen · C. Blake Gilks · David G. Huntsman

- Two ... nce (kap...
- Two ... rdance (kap...
- Ariol ... rdance (kap...

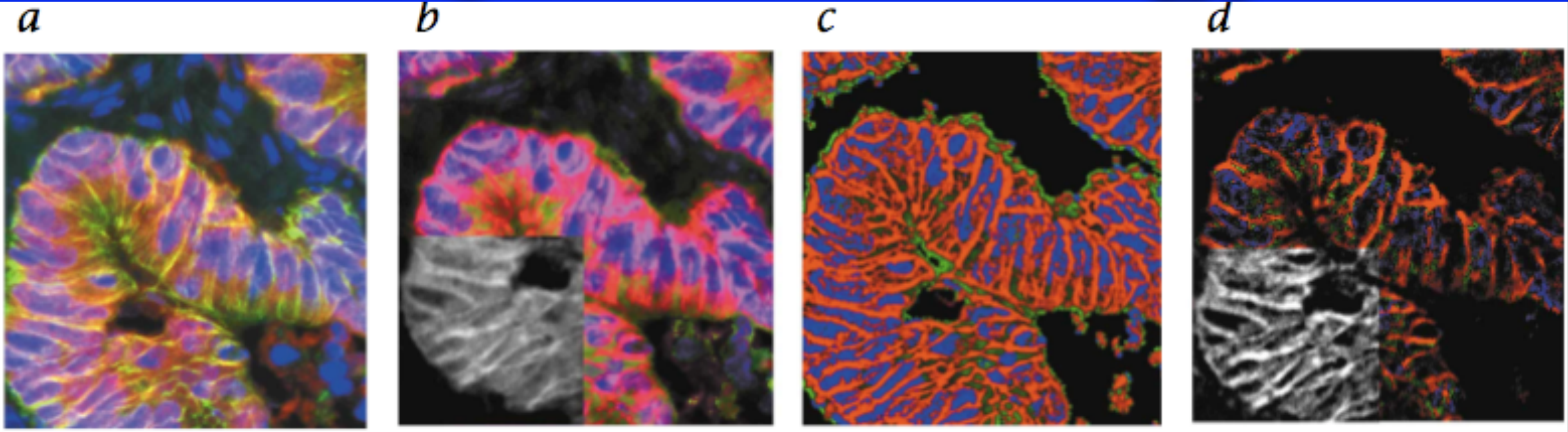
Turbin DA et al., Br Cancer Treat Res 110:417-26, 2008

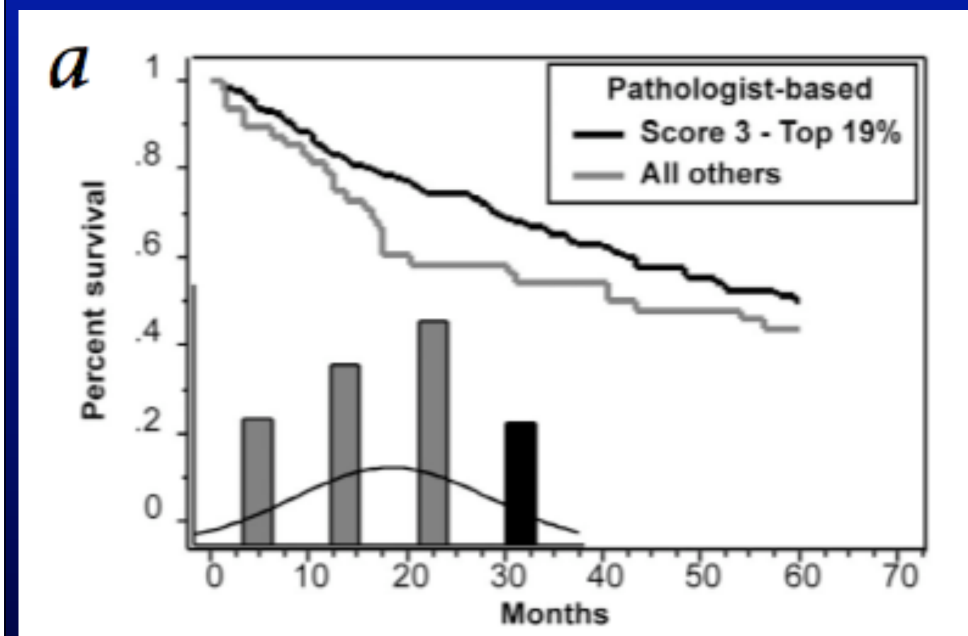
- Optimal cut-point for Ariol using X-tile software was **0.4%**
- No difference in prognostic significance of ER positivity by Ariol vs. pathologist

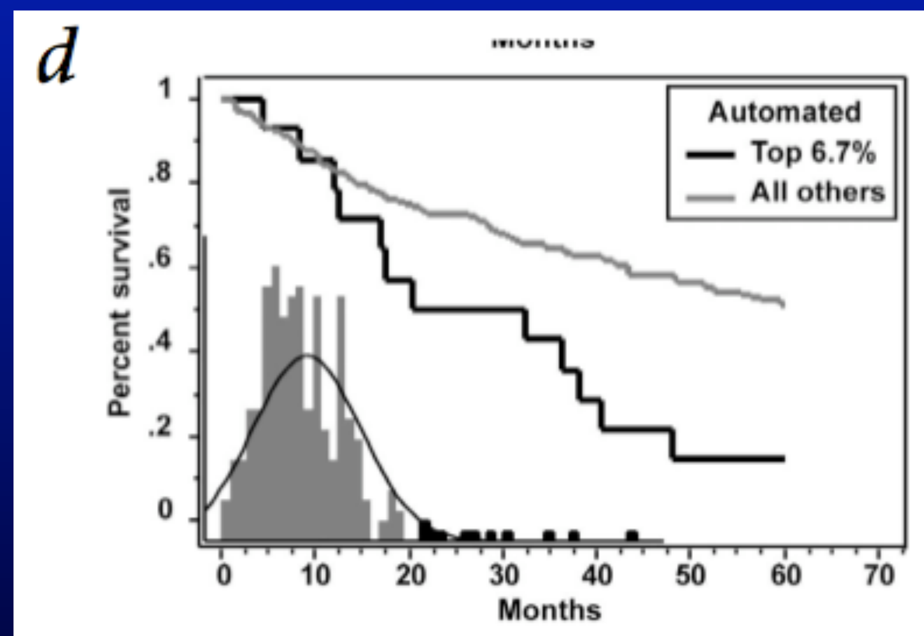




AQUA Technology

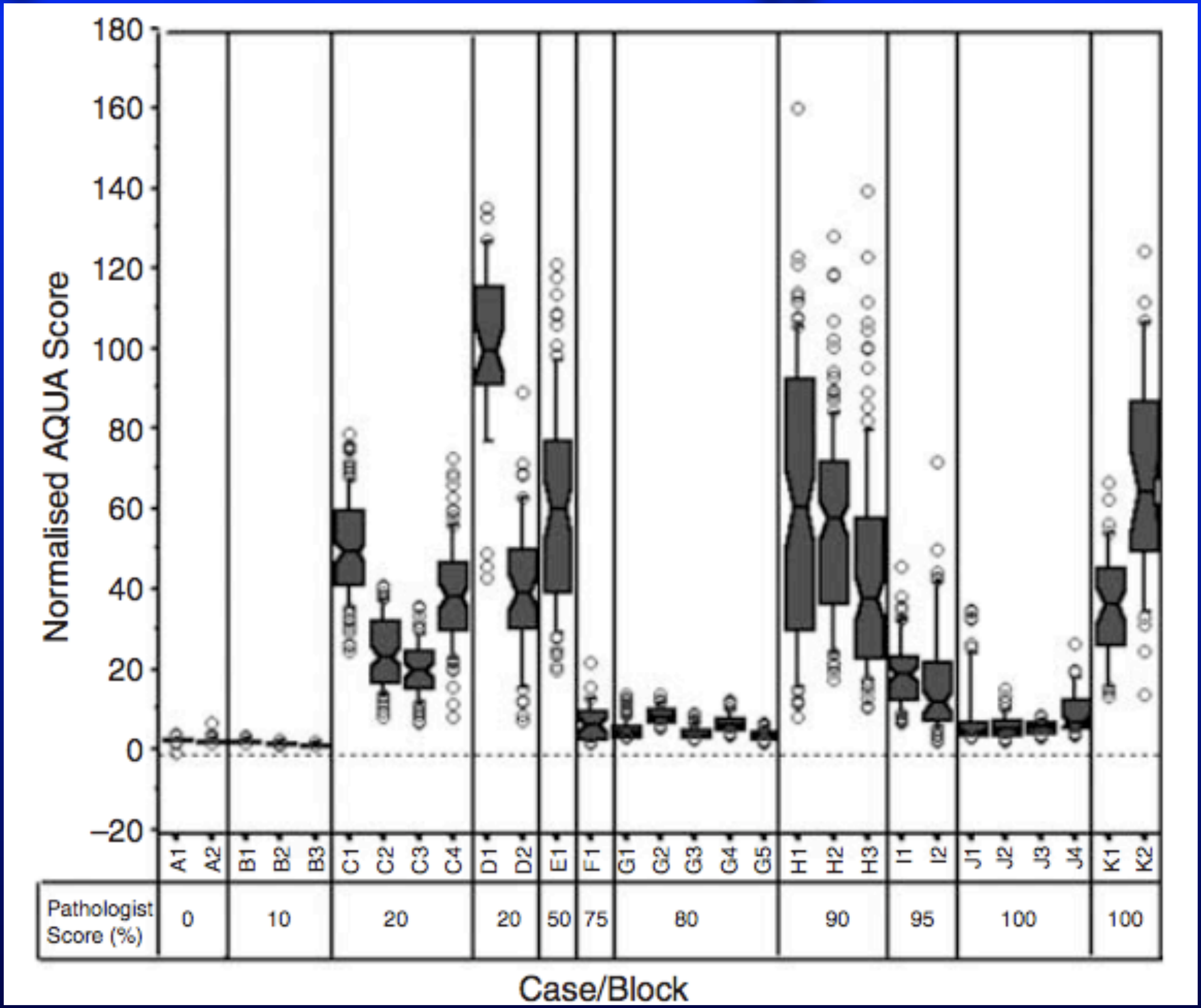






Camp RL et al., Nature Med 8:1323-7, 2002

AQUA v. Pathologist Score



Chung GG et al., Lab Invest 87:662-669, 2007

Lab Invest 87:662-669, 2007

Quantitative analysis of estrogen receptor heterogeneity in breast cancer

Gina G Chung[1], Maciej P Zerkowski[2], Sriparna Ghosh[1], Robert L Camp[3] and David L Rimm[3]

- Reasonable correlation (73%) with traditional ‘binary’ ER/PR assessment by IHC using 10% cutoff
- However, there was significant slide-to-slide tumor heterogeneity seen in a majority of cases when continuous scores analyzed
- Is single slide assessment of biomarkers such as ER sufficient?

Oncotype DX
An Example of a Highly Robust Assay

- No attention paid to fixation or other preanalytical factors (e.g., CAP-ASCO)
- Exceedingly high performance characteristics
- There are built in controls and normalization

A Robust Assay

Analytical Validation of the *Oncotype* DX Genomic Diagnostic Test for Recurrence Prognosis and Therapeutic Response Prediction in Node-Negative, Estrogen Receptor–Positive Breast Cancer

Clin Chem 53:1084-91, 2007

Maureen Cronin,* Chithra Sangli, Mei-Lan Liu, Mylan Pho, Debjani Dutta, Anhthu Nguyen, Jennie Jeong, Jenny Wu, Kim Clark Langone, and Drew Watson

- 21 genes (16 + 5 reporter genes)
- Amplification efficiency, linearity, quantification limits, dynamic range, analytical precision, reproducibility

Cronin M et al., Clin Chem 53:1084-91, 2007

Table 5. Analytical reproducibility for normalized expression measurements and restricted maximum likelihood (REML) estimates of the variance components for all 21 genes and RS in the Onco*type* DX assay.

		REML estimates, C_T units			
Official gene symbol	Wells	Between-day SD	Between-plate SD	Within-plate SD	Total SD
ACTB	114	0.009	0.000	0.057	0.057
BAG1	114	0.053	0.000	0.119	0.130
BCL2	114	0.000	0.090	0.079	0.120
CCNB1	114	0.018	0.047	0.095	0.108
CD68	114	0.001	0.000	0.125	0.125
SCUBE2	114	0.000	0.000	0.069	0.069
CTSL2	113	0.000	0.026	0.147	0.150
ESR1	113	0.035	0.051	0.076	0.098
GAPDH	114	0.048	0.056	0.059	0.094
GRB7	114	0.000	0.000	0.088	0.088
GSTM1	114	0.030	0.049	0.111	0.125
GUSB	114	0.000	0.000	0.103	0.103
ERBB2	114	0.018	0.019	0.057	0.062
MKI67	114	0.055	0.048	0.094	0.119
MYBL2	114	0.026	0.007	0.092	0.096
PGR	114	0.040	0.025	0.078	0.091
RPLPO	114	0.000	0.000	0.057	0.057
AURKA	114	0.000	0.000	0.087	0.087
MMP11	114	0.033	0.000	0.073	0.080
BIRC5	114	0.000	0.000	0.079	0.079

J Clin Oncol 24:3032-8, 2006

HER2 Testing by Local, Central, and Reference Laboratories in Specimens From the North Central Cancer Treatment Group N9831 Intergroup Adjuvant Trial

Edith A. Perez, Vera J. Suman, Nancy E. Davidson, Silvana Martino, Peter A. Kaufman, Wilma L. Lingle, Patrick J. Flynn, James N. Ingle, Daniel Visscher, and Robert B. Jenkins

- Initially, eligibility required HER2 positivity by IHC (3+) or FISH (> 2) by either local or central laboratory
- N = 2547
- **18.4%** of IHC (HercepTest) at local laboratories could not be confirmed in central laboratory testing

Clin Cancer Res 11:6598-607, 2005

Diagnostic Evaluation of HER-2 as a Molecular Target: An Assessment of Accuracy and Reproducibility of Laboratory Testing in Large, Prospective, Randomized Clinical Trials

Michael F. Press,[1,2] Guido Sauter,[5] Leslie Bernstein,[1,3] Ivonne E. Villalobos,[2] Martina Mirlacher,[5] Jian-Yuan Zhou,[2] Rooba Wardeh,[2] Yong-Tian Li,[2] Roberta Guzman,[2] Yanling Ma,[2] Jane Sullivan-Halley,[3] Angela Santiago,[2] Jinha M. Park,[4] Alessandro Riva,[6] and Dennis J. Slamon[4]

- N = 2,600
- Assessment for entry into BCIRG clinical trials
- Overall 77.5% agreement in community lab HER2 IHC vs. central lab FISH

Press MF et al., 2005

Clin Cancer Res 11:6598-607, 2005

	IHC Scoring of Outside Labs			
	0	**1+**	**2+**	**3+**
FISH-NEG	96.4%	93.9%	83.3%	21.9%
FISH-POS	3.6%	6.1%	16.7%	78.1%

Effect of Prolonged Formalin Fixation on the Immunohistochemical Reactivity of Breast Markers

Daniel A. Arber, M.D.

Appl Immunohistochem Mol Morphol 10:183-6, 2002

5/9 "POSITIVE" (2+ or 3+) specimens became NEGATIVE

2+ to 1+	20 days, 42 days
2+ to 0	49 days
3+ to 1+	42 days, 99 days

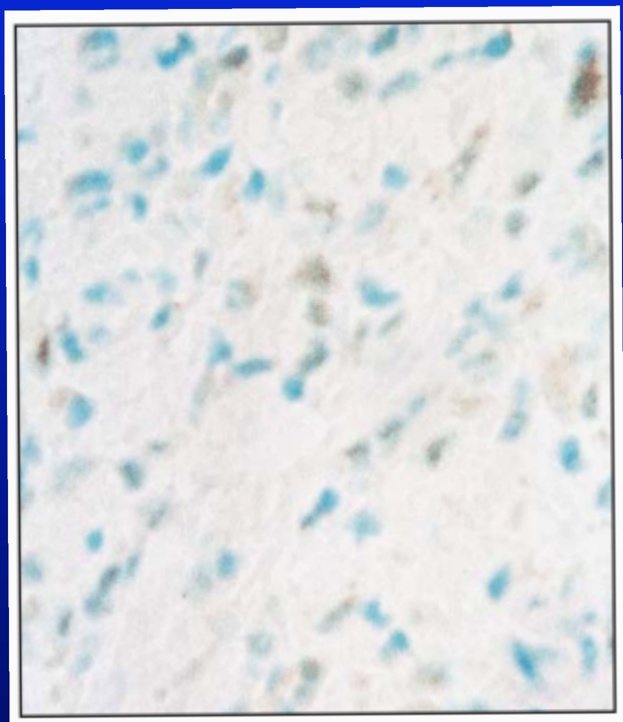
Am J Clin Pathol 120:86-90, 2003

Minimum Formalin Fixation Time for Consistent Estrogen Receptor Immunohistochemical Staining of Invasive Breast Carcinoma

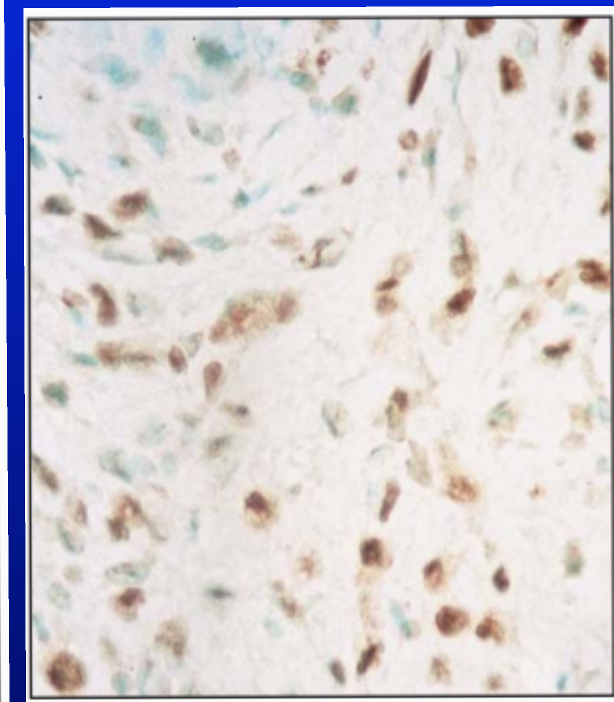
Neal S. Goldstein, MD, Monica Ferkowicz, MT(ASCP), PathA(AAPA), Eva Odish, HTL(IHQ), Anju Mani, MD, and Farnaz Hastah, MD

- What is the minimum time necessary for consistent ER IHC results?
- N = 24
- Fixed for 3, 6, 8, 12 hrs, and 1, 2 and 7 days
- ER quantified using 'Q' score (0-7)

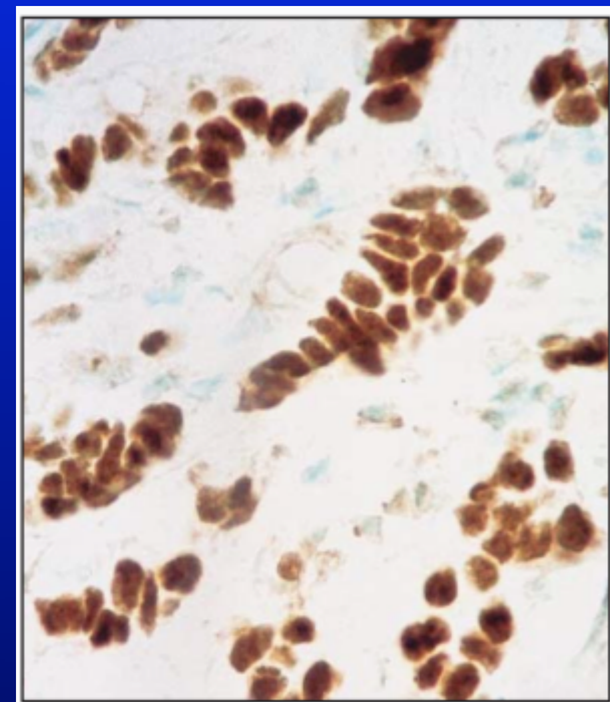
Goldstein NS, et al. Am J Clin Pathol 120:86-90, 2003



3 hrs



6 hrs



8 hrs

Goldstein NS, et al. Am J Clin Pathol 120:86-90, 2003

- Tissue specimens need to be fixed 6-8 hours before being loaded onto tissue processors for consistent and reproducible ER immunostains
- Regardless of length of epitope retrieval
- Regardless of specimen size
- Only cases with strong, uniform ER positivity used - what about low levels of ER?

Does Estrogen Receptor Expression Vary With Fixation Time?

Julio A. Ibarra, M.D. and Lowell W. Rogers, M.D.

MemorialCare Breast Centers at Orange Coast Memorial Medical Center, Fountain Valley, California and Long Beach Memorial Medical Center, Long Beach California

With SP1, No Effect of Fixation Time on ER Immunostaining!

Reasons for Discordances in Reported Antibody Sensitivities and Specificities

1

- Different antibodies employed (e.g., different monoclonal antibody clones)
- Different IHC detection systems
- Different HIER or tissue pretreatments
- Different tissue fixation or processing

34ßE12 vs p63

Prostatic Basal Cell Match

Am J Surg Pathol 26:1161-8, 2002

Comparison of the Basal Cell-Specific Markers, 34βE12 and p63, in the Diagnosis of Prostate Cancer

Rajal B. Shah, M.D., Ming Zhou, M.D., Ph.D., Michele LeBlanc, B.S., Matthew Snyder, M.D., and Mark A. Rubin, M.D.

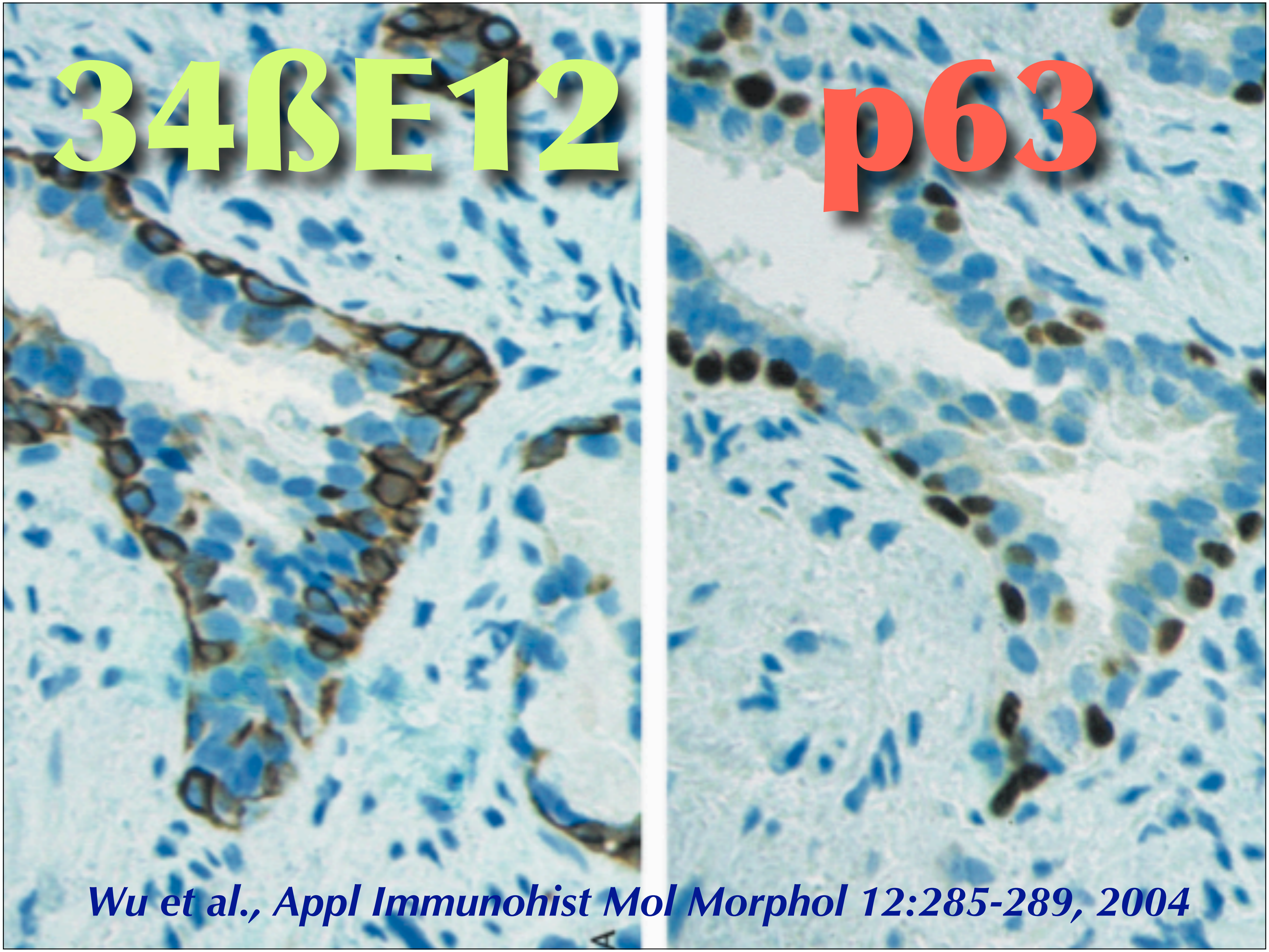
34ßE12

p63

Appl Immunohist Mol Morph 12:285-9, 2004

Comparison of 34βE12 and P63 in 100 Consecutive Prostate Carcinoma Diagnosed by Needle Biopsies

Howard Her-Juing Wu, MD,† Odeta Lapkus, MD,* and Mykim Corbin, HT (ASCP)†*


34ßE12
p63
Wu et al., Appl Immunohist Mol Morphol 12:285-289, 2004

Shah et al, 2002

"45/108 (41%) of prostate NBX cores from 78 cases demonstrated a higher percentage of p63 basal cell staining...p63 is more sensitive than 34ßE12 in staining basal cells..."

Wu et al, 2004

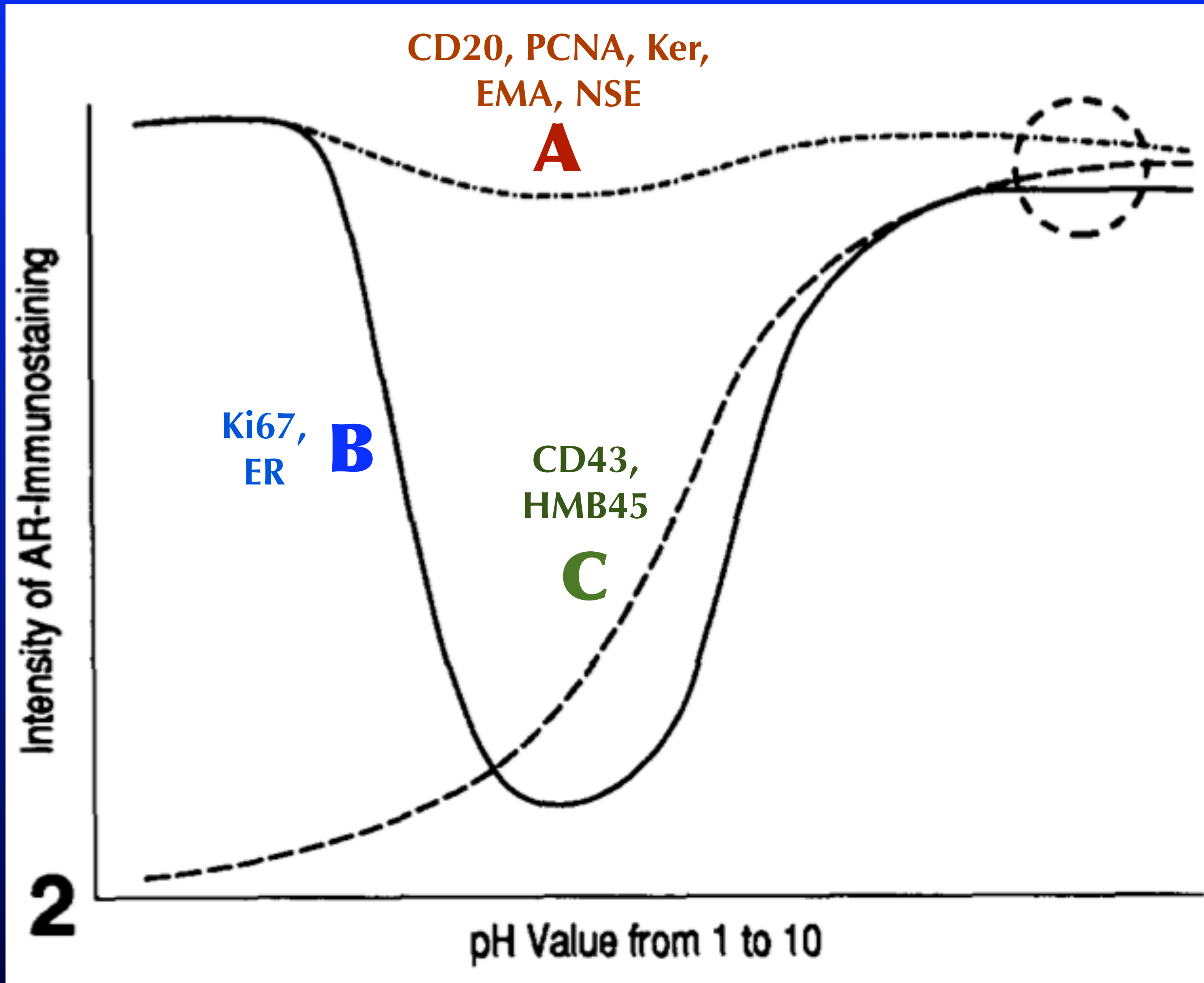
"The overall sensitivity in identifying basal cells in benign glands was 99.48% and 99.44% for 34ßE12 and p63 respectively. Basal cell density was higher for 34ßE12 in comparison with p63 (92% vs. 87%)."

34ßE12  p63

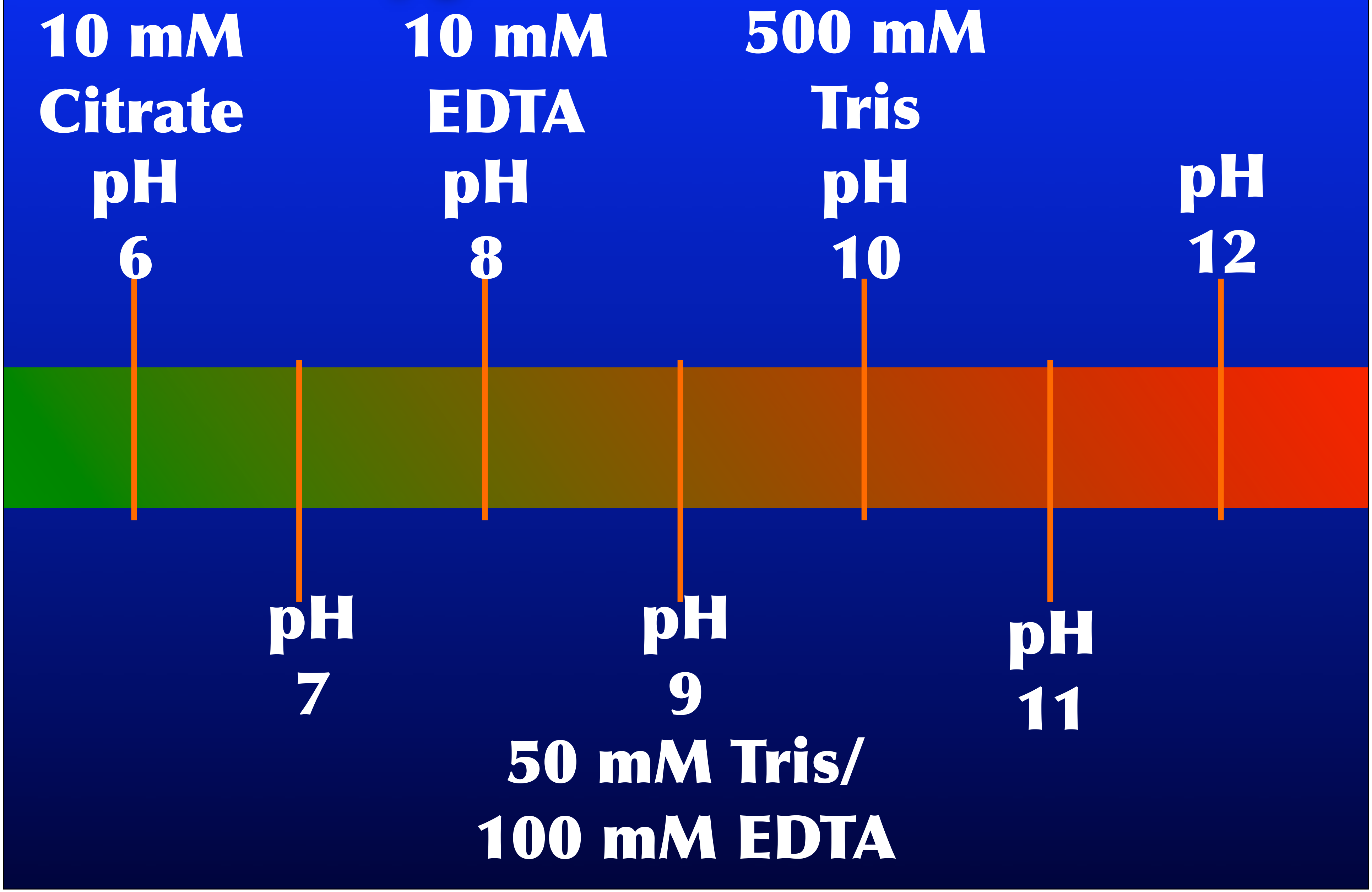
	AR Time	AR Buffer	Antibody dilution
Shah et al 2002	15 min	10 mM citrate	1:100
Wu et al 2004	21 min	Dako Target Retrieval	1:50

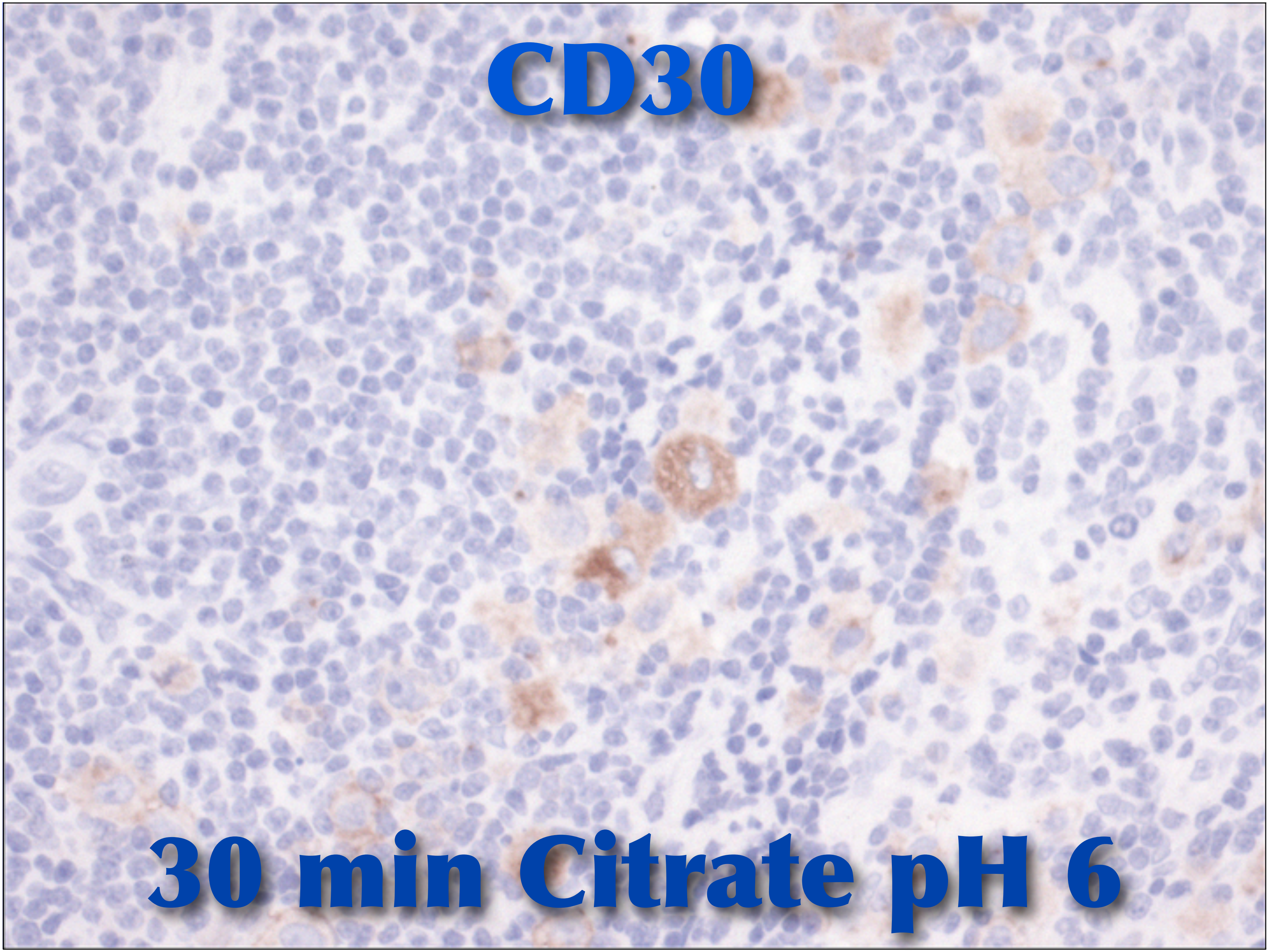
Can these differences yield significant apparent antibody sensitivities

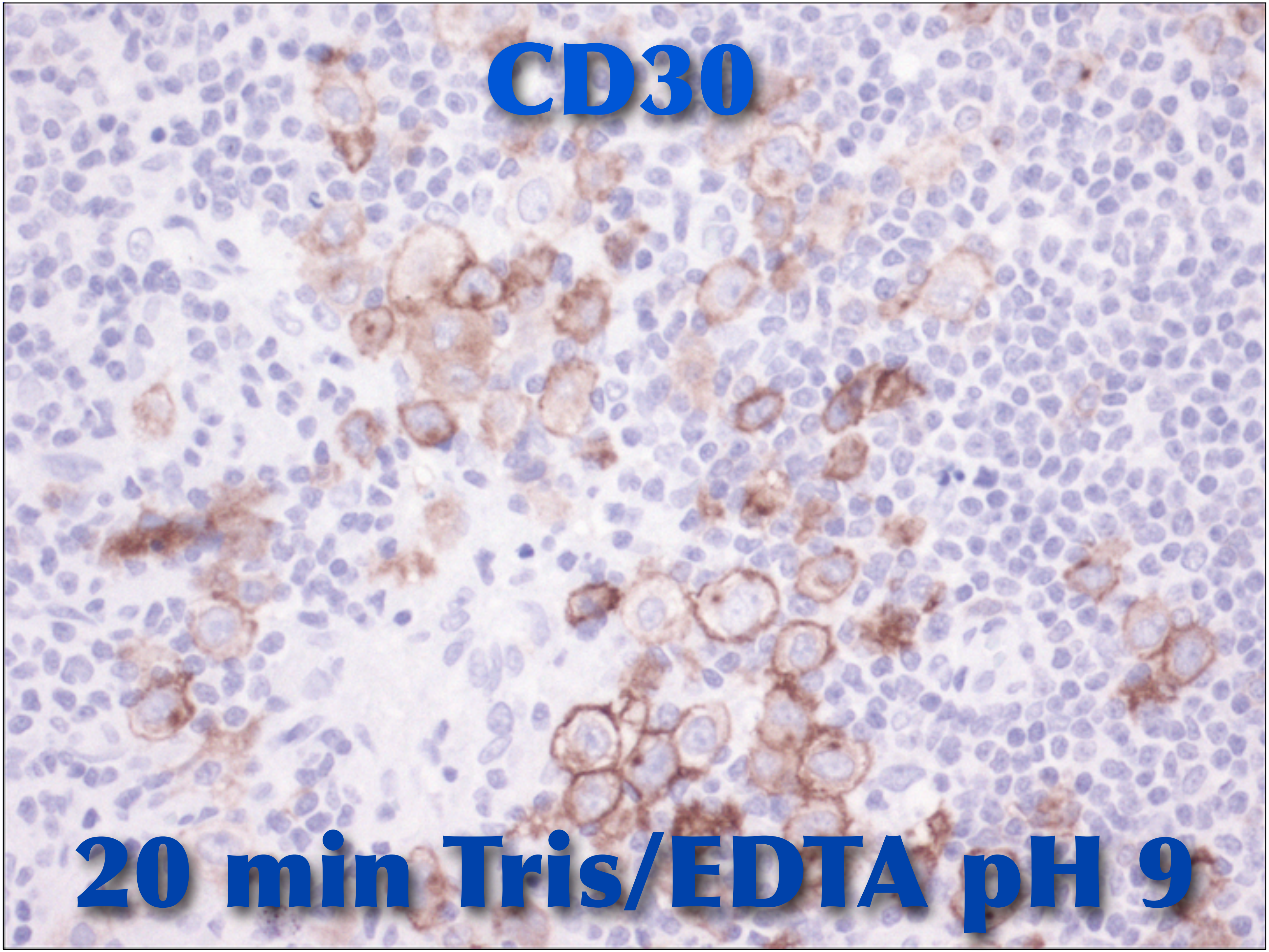
Shi S-R, et al. J Histochem Cytochem 43:193-201, 1995

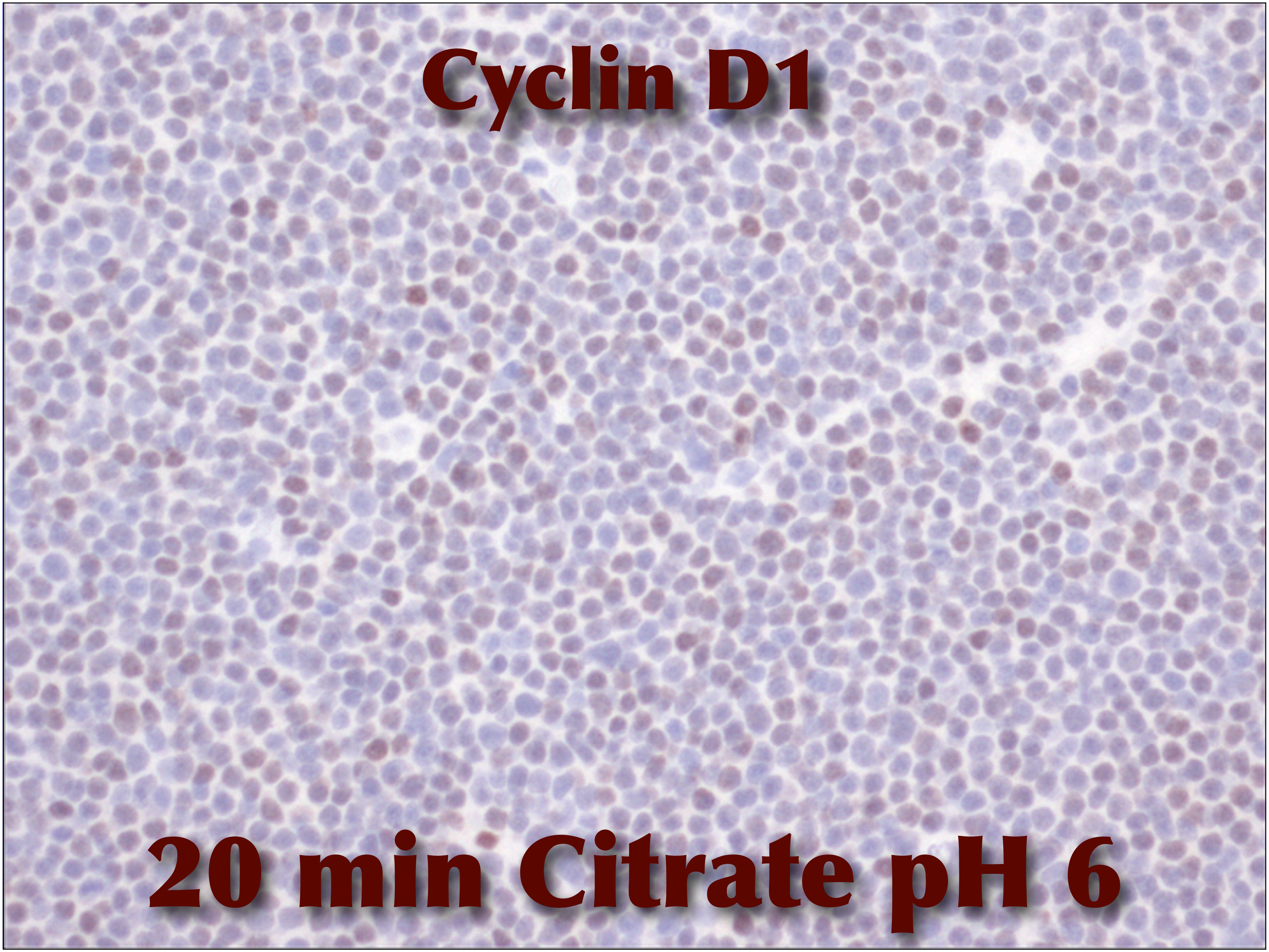


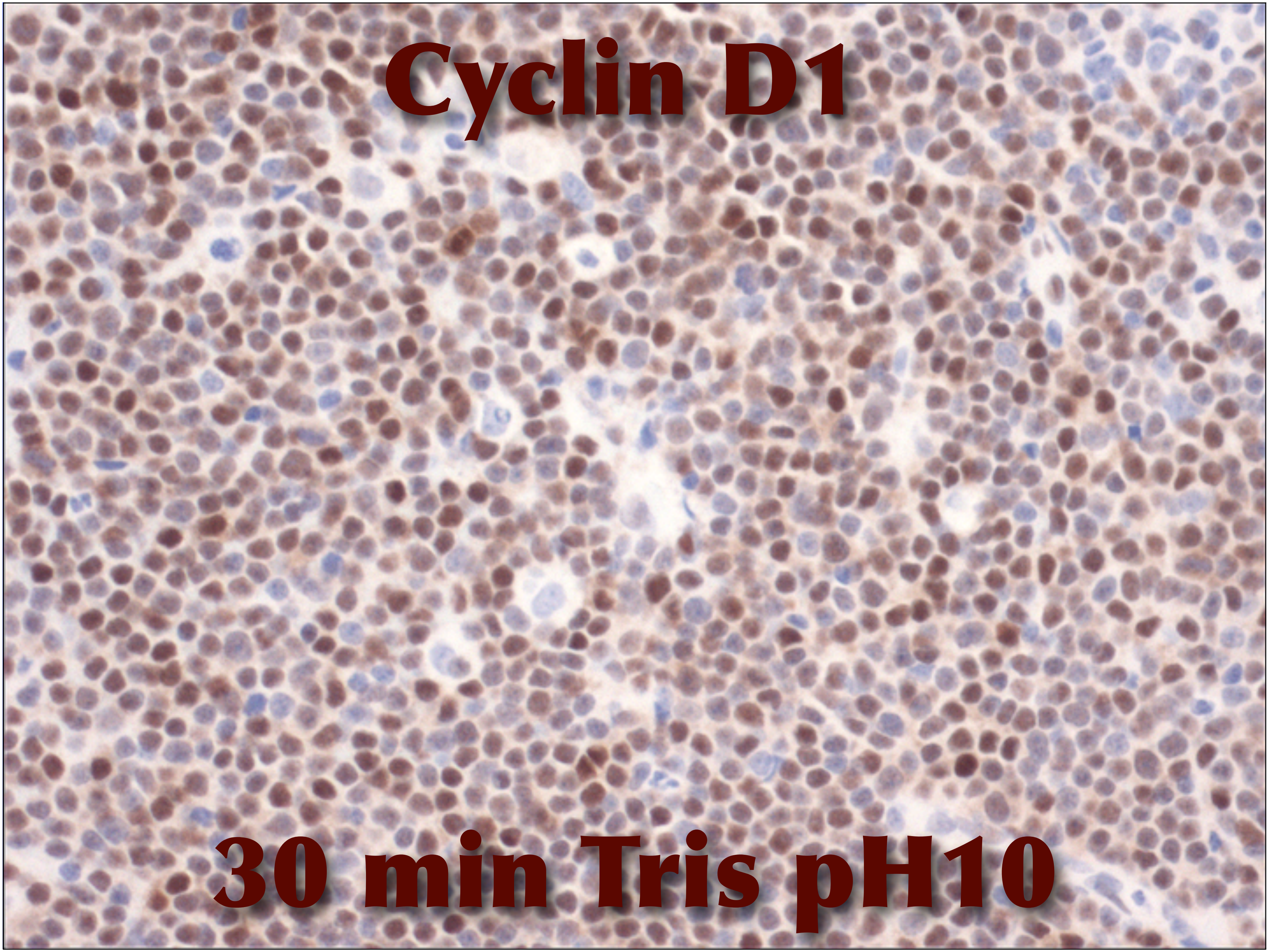
Types of Buffers

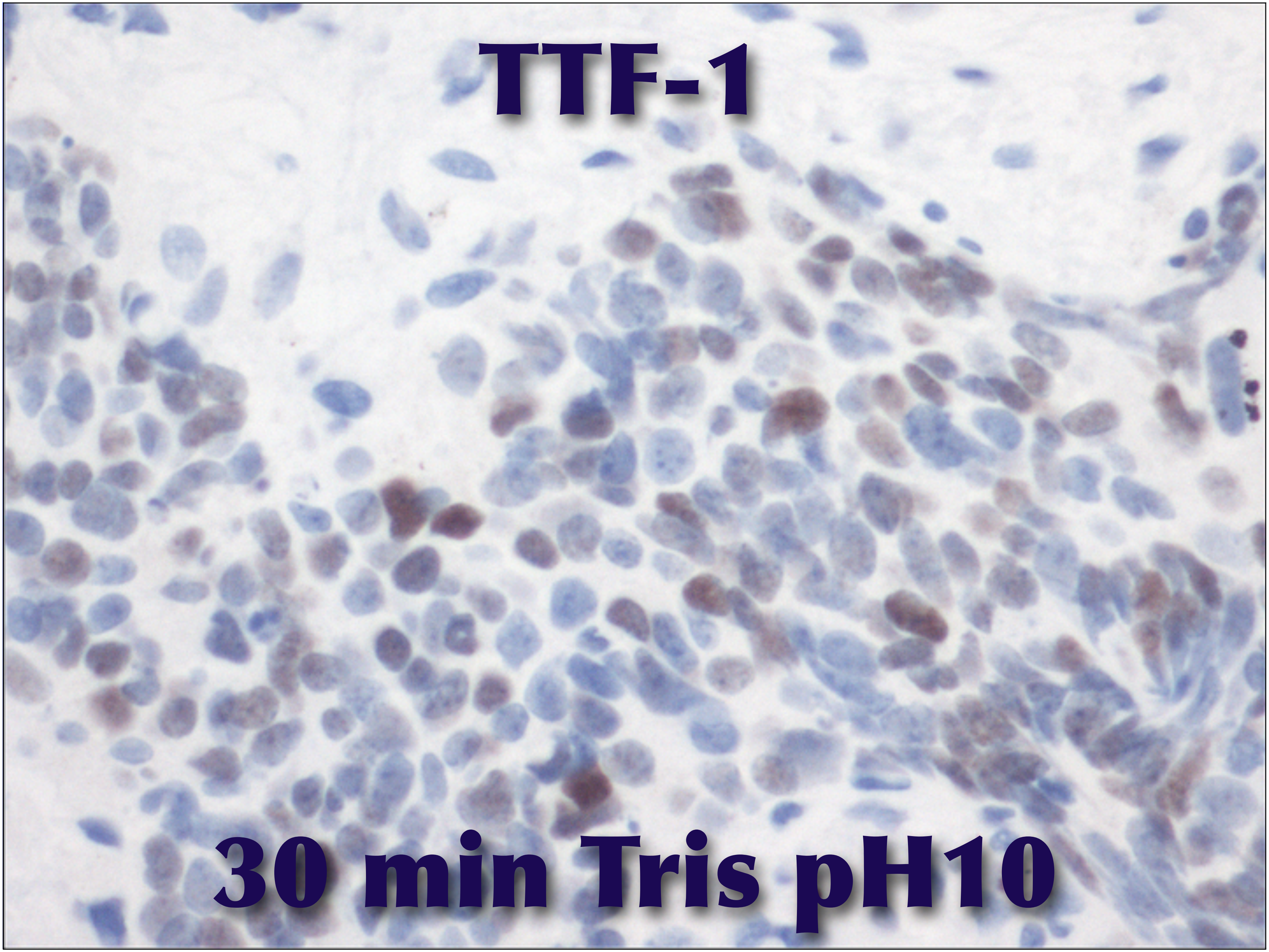


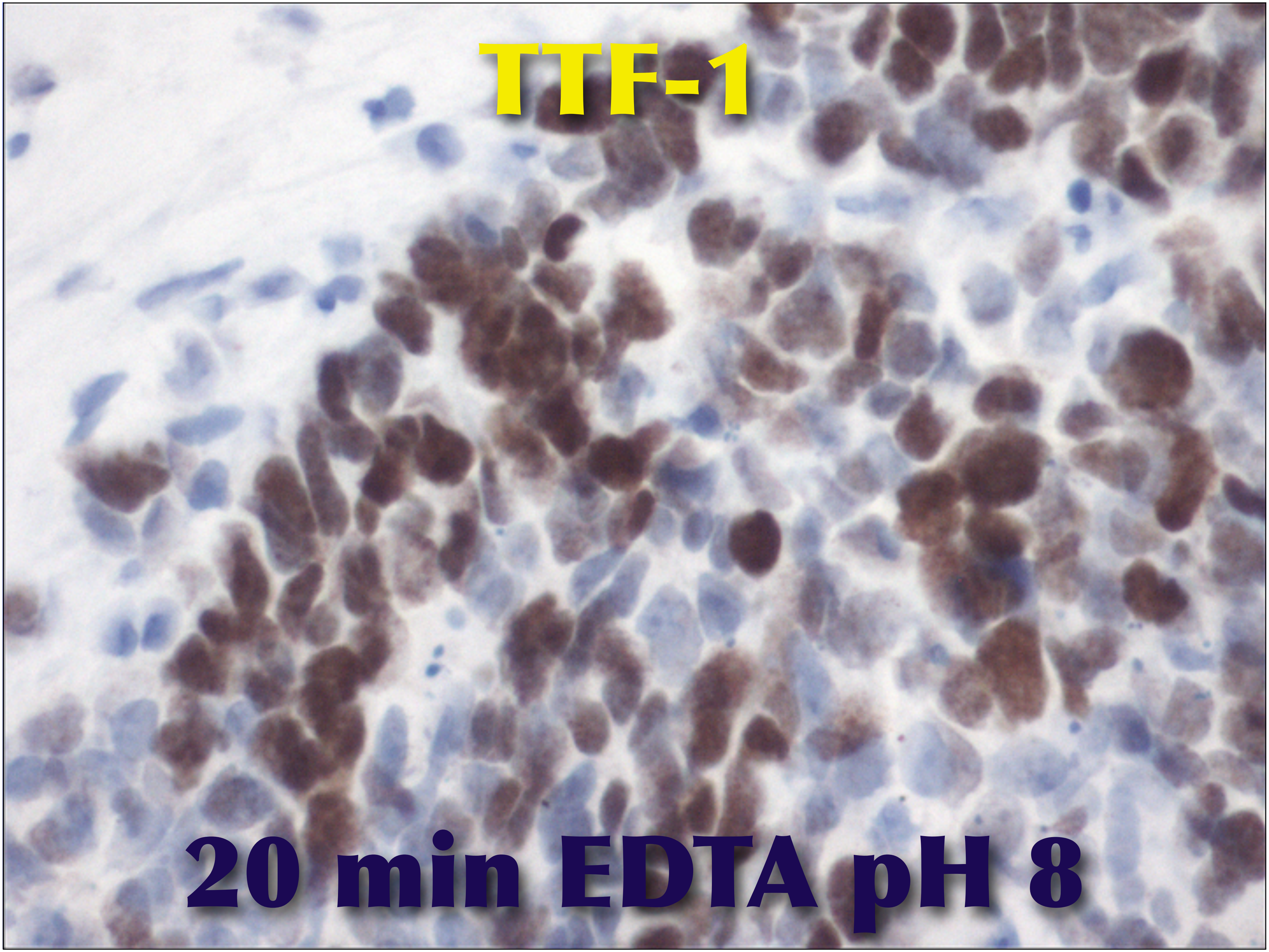

CD30
30 min Citrate pH 6

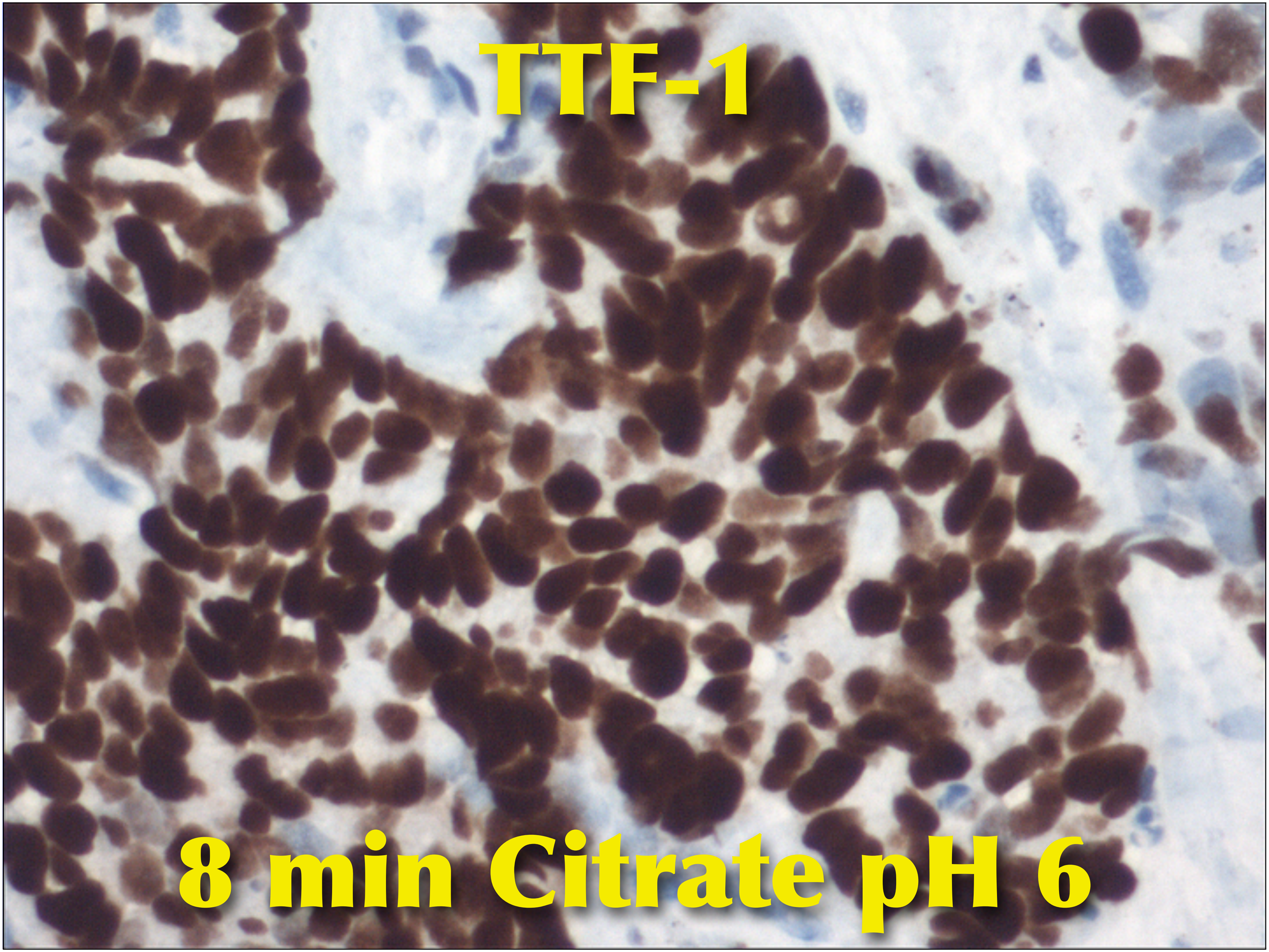

CD30
20 min Tris/EDTA pH 9


Cyclin D1
20 min Citrate pH 6


Cyclin D1
30 min Tris pH10


TTF-1
30 min Tris pH10


TTF-1
20 min EDTA pH 8


TTF-1
8 min Citrate pH 6

Reasons for Discordances in Reported Antibody Sensitivities and Specificities

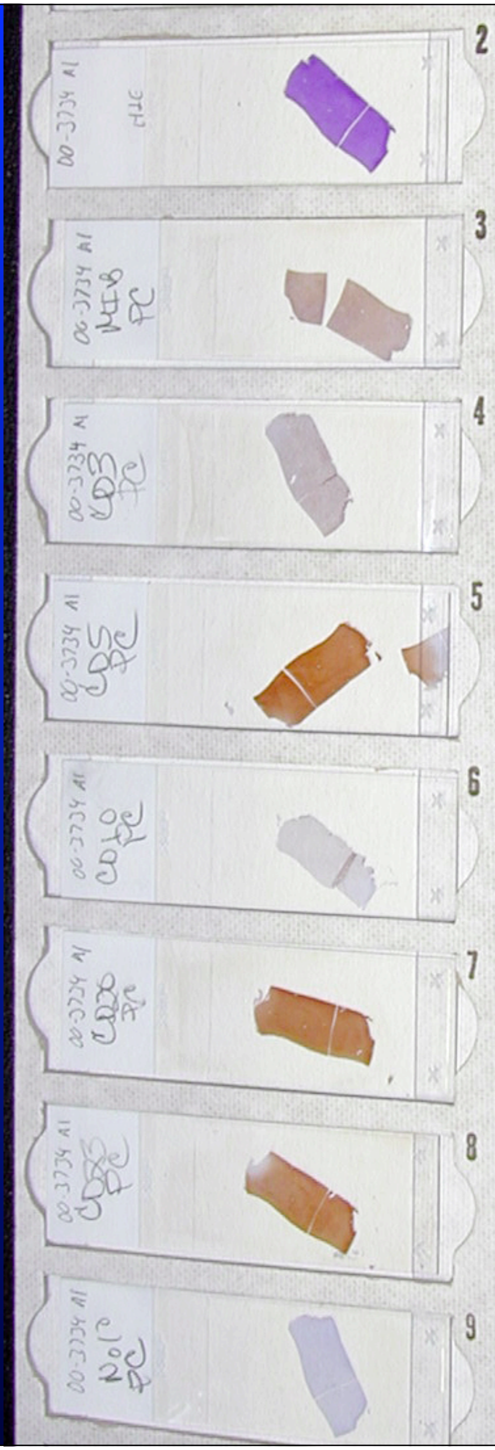
2

- Different definitions of tumor
- Different cutoffs for IHC positivity
- Small sample size

Thresholds for Positivity

"The devil is in the details"

- Based on fraction of cells positive?
- Based on what fraction? 10%? 50% 90%?
- Based on intensity of immunostaining signal? (1+ out of 3+, 2+ out of 4+?)
- Based on combination of signal intensity and fraction of cells positive (e.g., Allred score)


FedEx
FedEx
Federal Express

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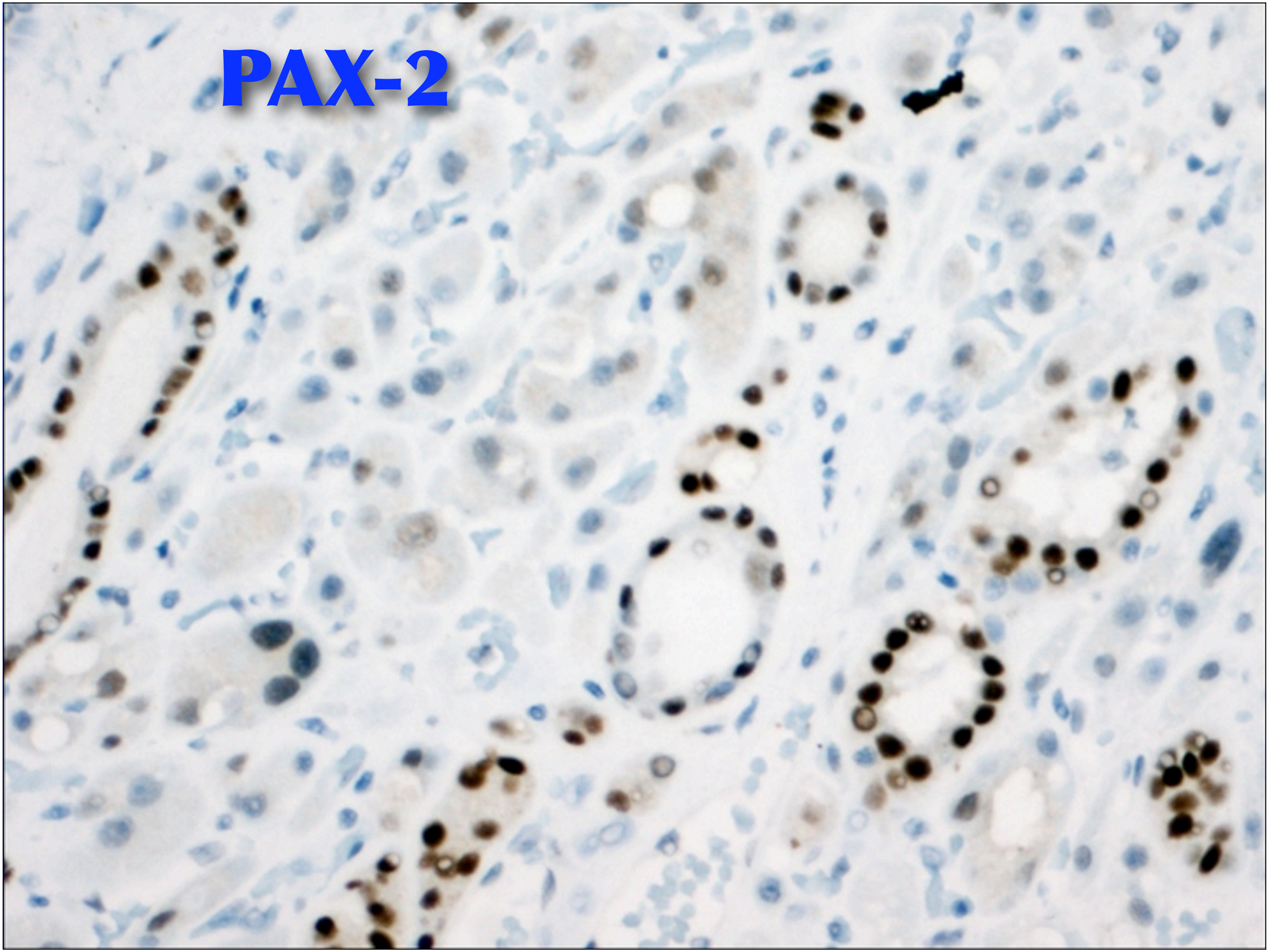
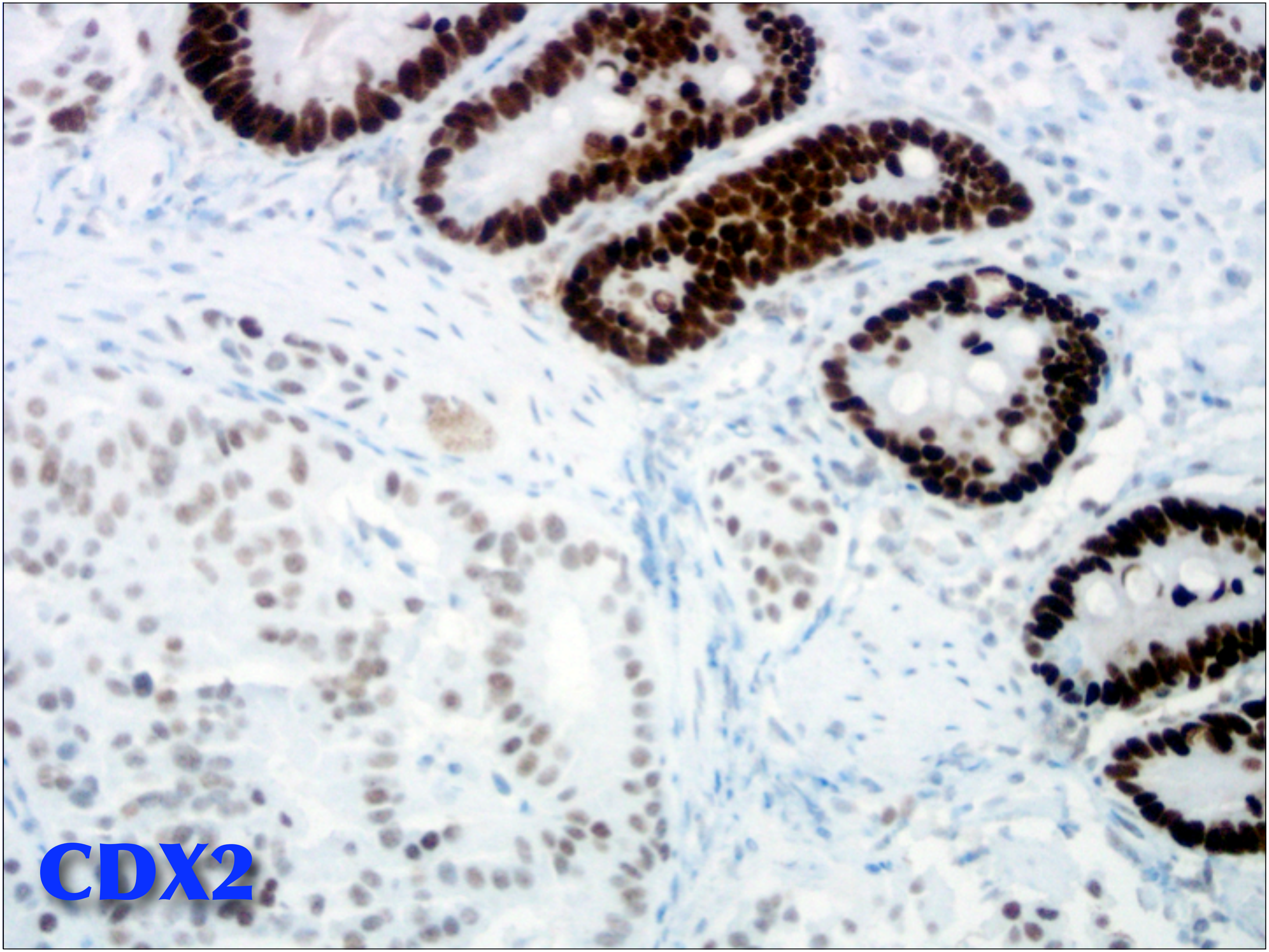
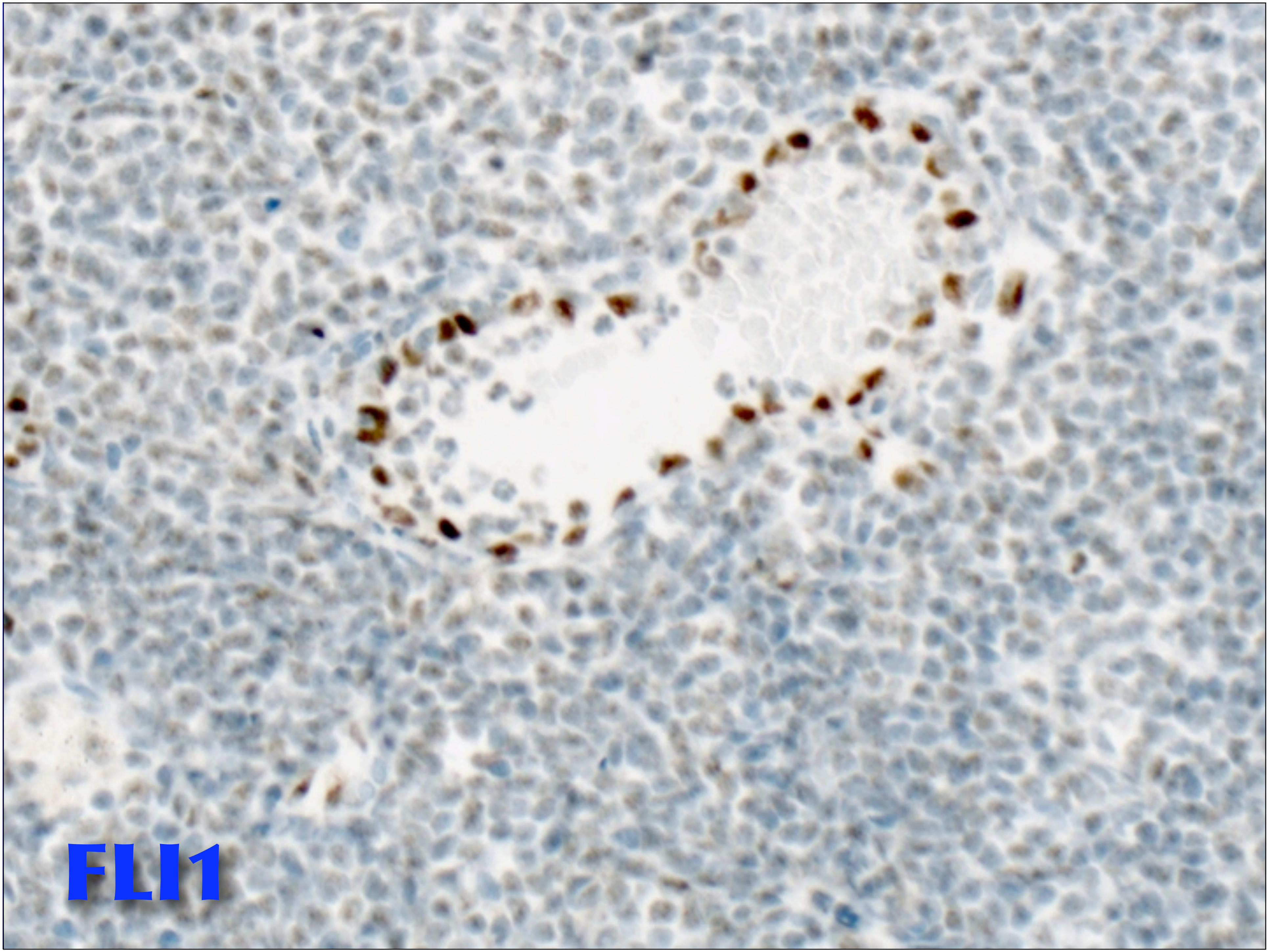

PAX-2

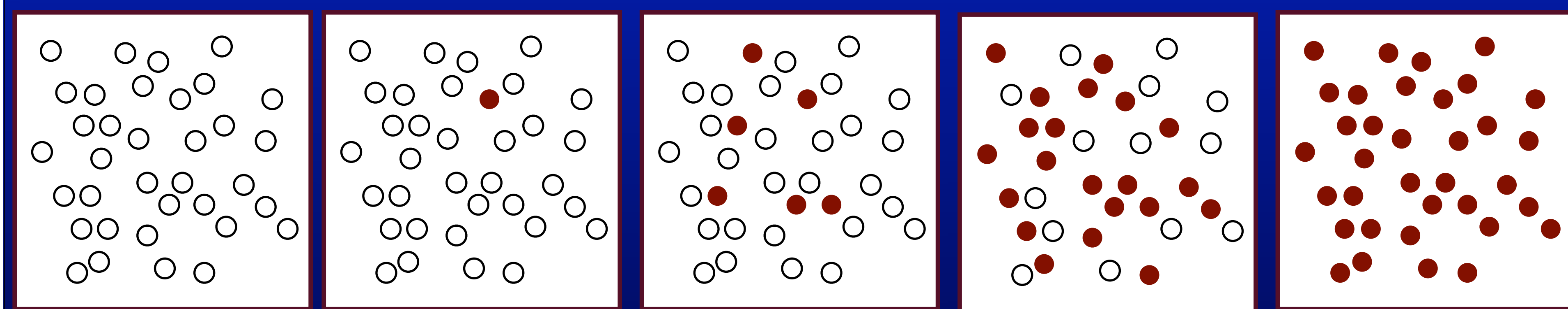

CDX2


FLI1

PhenoPath

LABORATORIES

Semiquantitative IHC Scoring System



0%	<1%	1-25%	25-75%	>75%
Negative	Rare cells Positive	Focally Positive	Variably Positive	Uniformly Positive

Appl Immunohistochemistry 3:99-107,1995

Coordinate Expression of Cytokeratins 7 and 20 Defines Unique Subsets of Carcinomas

Nan Ping Wang, M.D., Ph.D., Sui Zee, M.D.,
Richard J. Zarbo, M.D., Carlos E. Bacchi, M.D., and
Allen M. Gown, M.D.

N=384

Cytokeratin 7 and Cytokeratin 20 Expression in Epithelial Neoplasms: A Survey of 435 Cases

Peiguo Chu, M.D., Ph.D., Emerald Wu, B.S., Lawrence M Weiss, M.D.
Division of Pathology, City of Hope National Medical Center, Duarte, California

N=435

Mod Pathol 13:962-72,2000

CK7 and CK20 Coordinate Expression

Chu et al. vs. Wang et al.

- Overall results very similar
- Some exceptions: e.g., CK20 expression in bladder tumors 89% Wang et al., 29% Chu et al.
- Different "cutoffs" used for positivity (Wang et al - 1%; Chu et al - 5%)
- Different cytokeratin 20 antibodies

Examples of Markers with Unique Quantification

- Ki67 [MIB1]: deciles
- Nuclear beta catenin: >30%
- HER2 (0, 1+, 2+, 3+)

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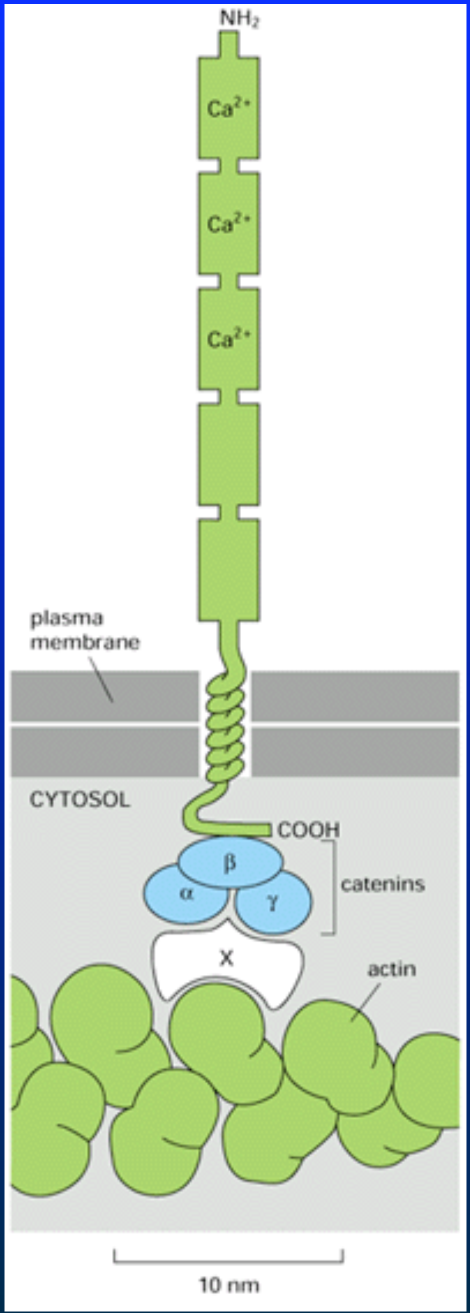
Selected Topics

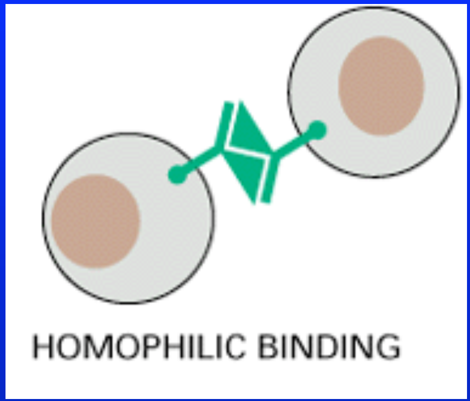
- General Issues
- Breast Carcinoma
- GI Tract Tumors
- Tumors in the Liver
- Male GU Tract Tumors

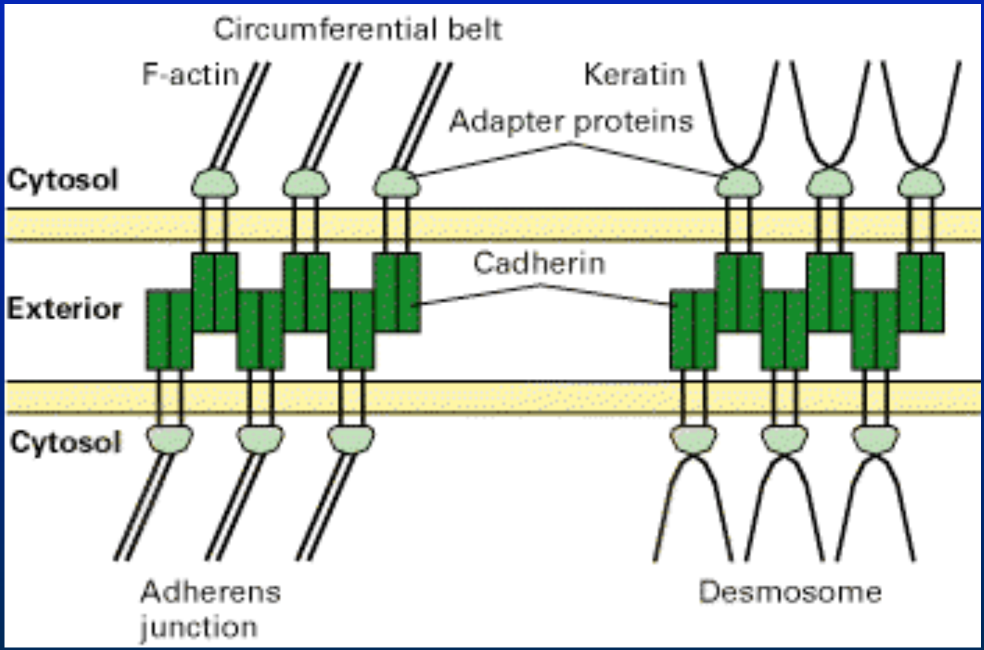
Breast Carcinoma

- E-cadherin in distinguishing lobular v. ductal carcinomas
- Myoepithelial markers in distinguishing in situ from invasive carcinoma
- Markers of metastatic breast cancer

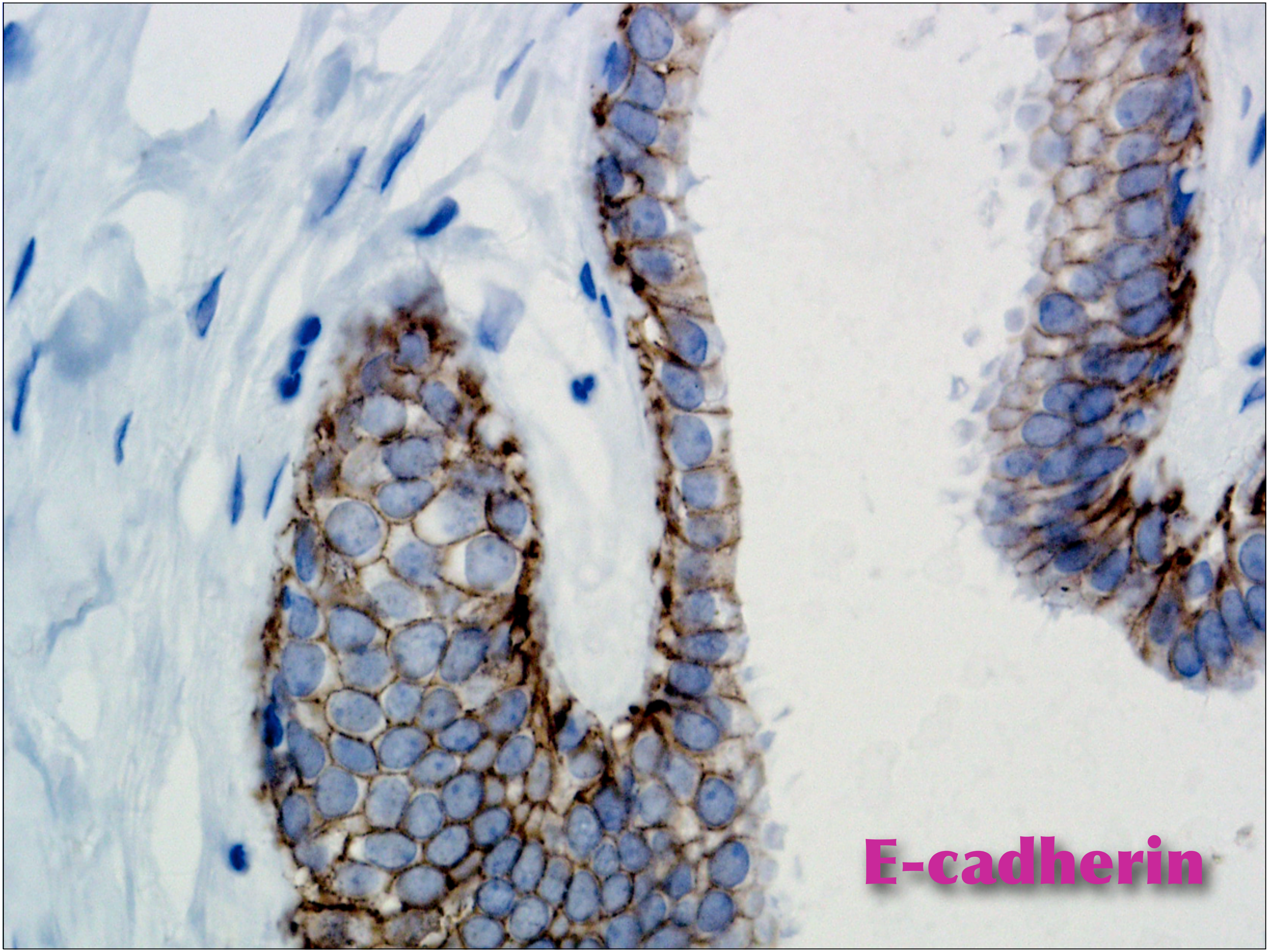
Cadherins Mediate 'Homophilic Binding' Between Cells







from Alberts, B et al. Molecular Biology of the Cell, 3rd Ed, Garland, NY, 1994, and Lodish H et al., Molecular Cell Biology 4th Ed., WH Freeman, NY, 2000.


E-cadherin

E-Cadherin and Lobular Carcinoma

Loss of expression is signature phenotype, and can occur by multiple methods

- Mutations (insertions, deletions, nonsense) resulting in stop codons and loss of expression
- Allelic loss (Loss of heterozygosity)
- Methylation of E-cadherin promoter gene

E-Cadherin And Lobular Carcinoma of the Breast

- Immunohistochemical localization “integrates” what happens at the genomic level
- A myriad of genetic alterations have one “common final pathway” of loss of E-cadherin expression

Moll et al. Am J Pathol 143, 1731-1742, 1993

Infiltrating Duct Carcinoma	67/67 positive
Infiltrating Lobular Carcinoma	0/32 positive

(frozen sections)

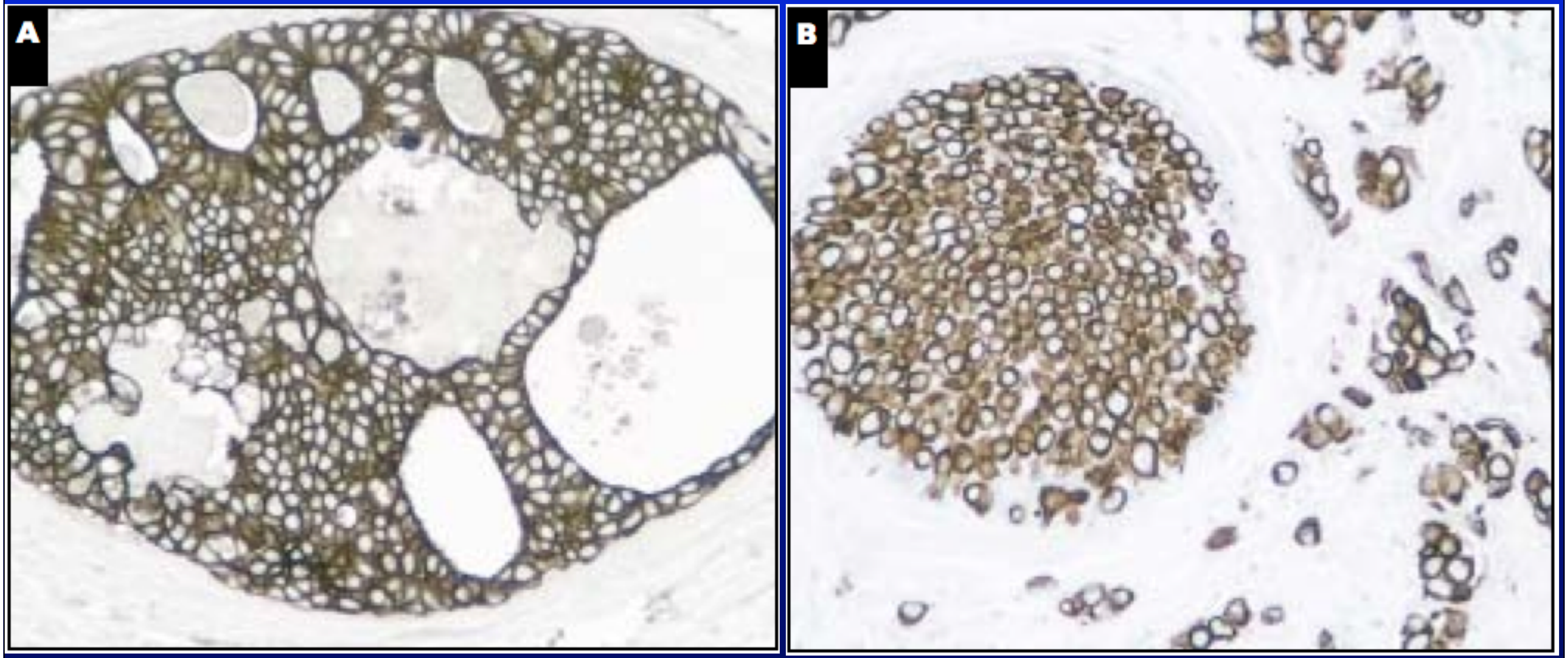
Am J Clin Pathol 114:190-6, 2000

Cytokeratin 8 Immunostaining Pattern and E-Cadherin Expression Distinguish Lobular From Ductal Breast Carcinoma

Hans-Anton Lehr, MD, PhD,[1,3] Andrew Folpe, MD,[2] Hadi Yaziji, MD,[3] Friedrich Kommoss, MD, PhD,[1] and Allen M. Gown, MD[3]

- 33/33 IDCs E-cadherin positive
- 15/15 ILCs E-cadherin negative
- Associated with “bag of marbles” appearance with anti-CK8 antibodies

Lehr H-A, et al., Am J Clin Pathol 114:190-6,

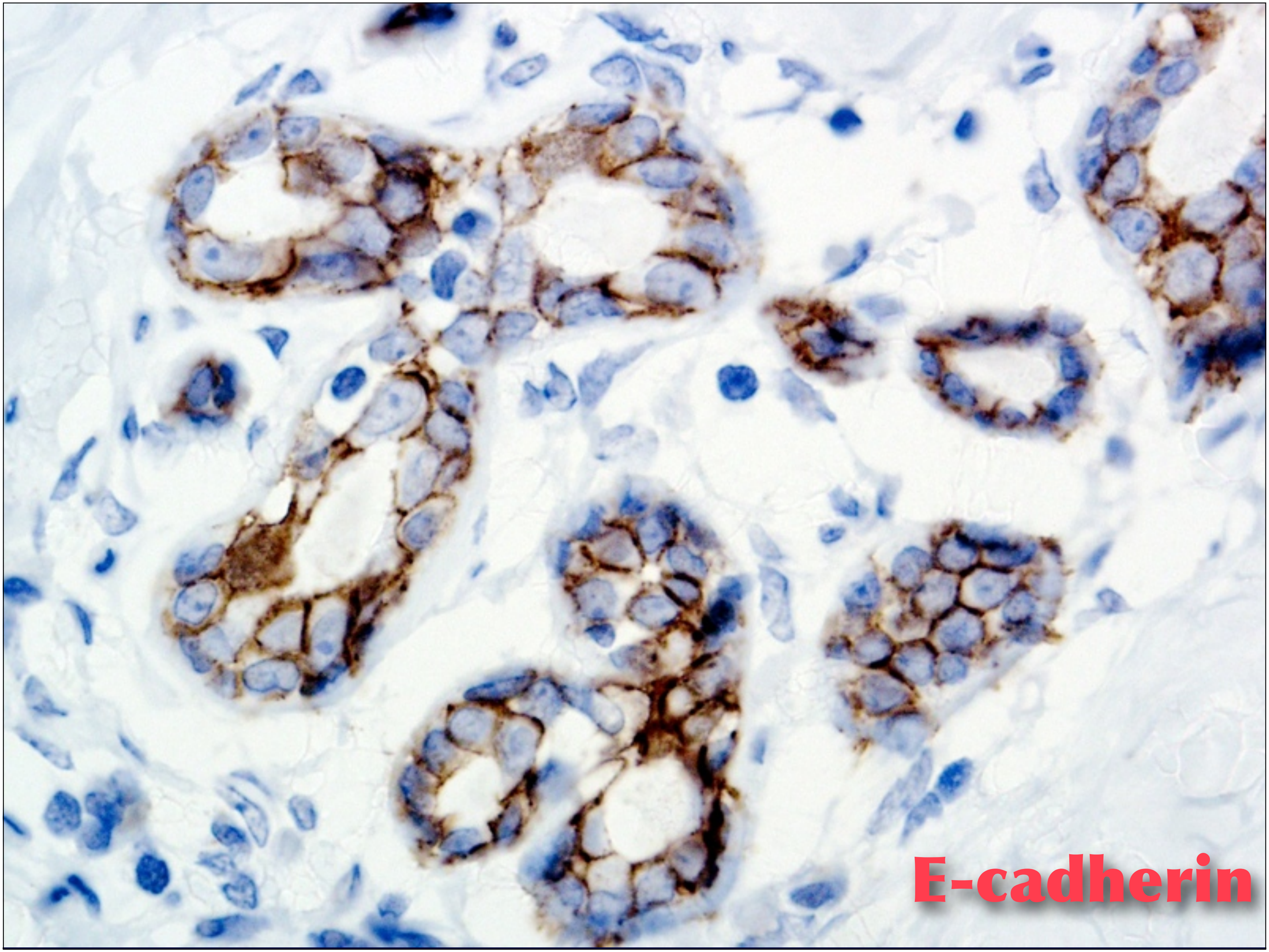


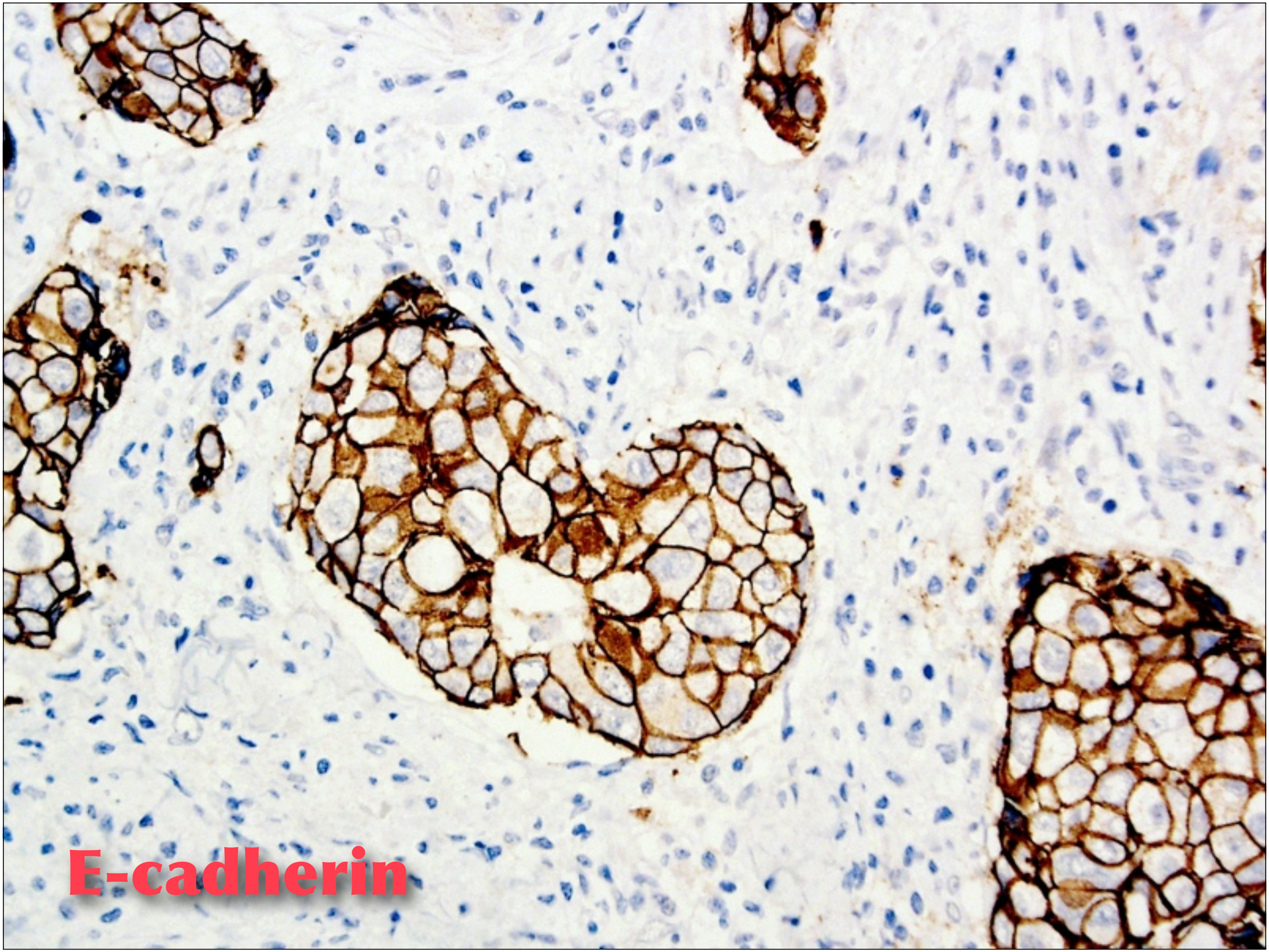
Anti-cytokeratin 8 [35ßH11]

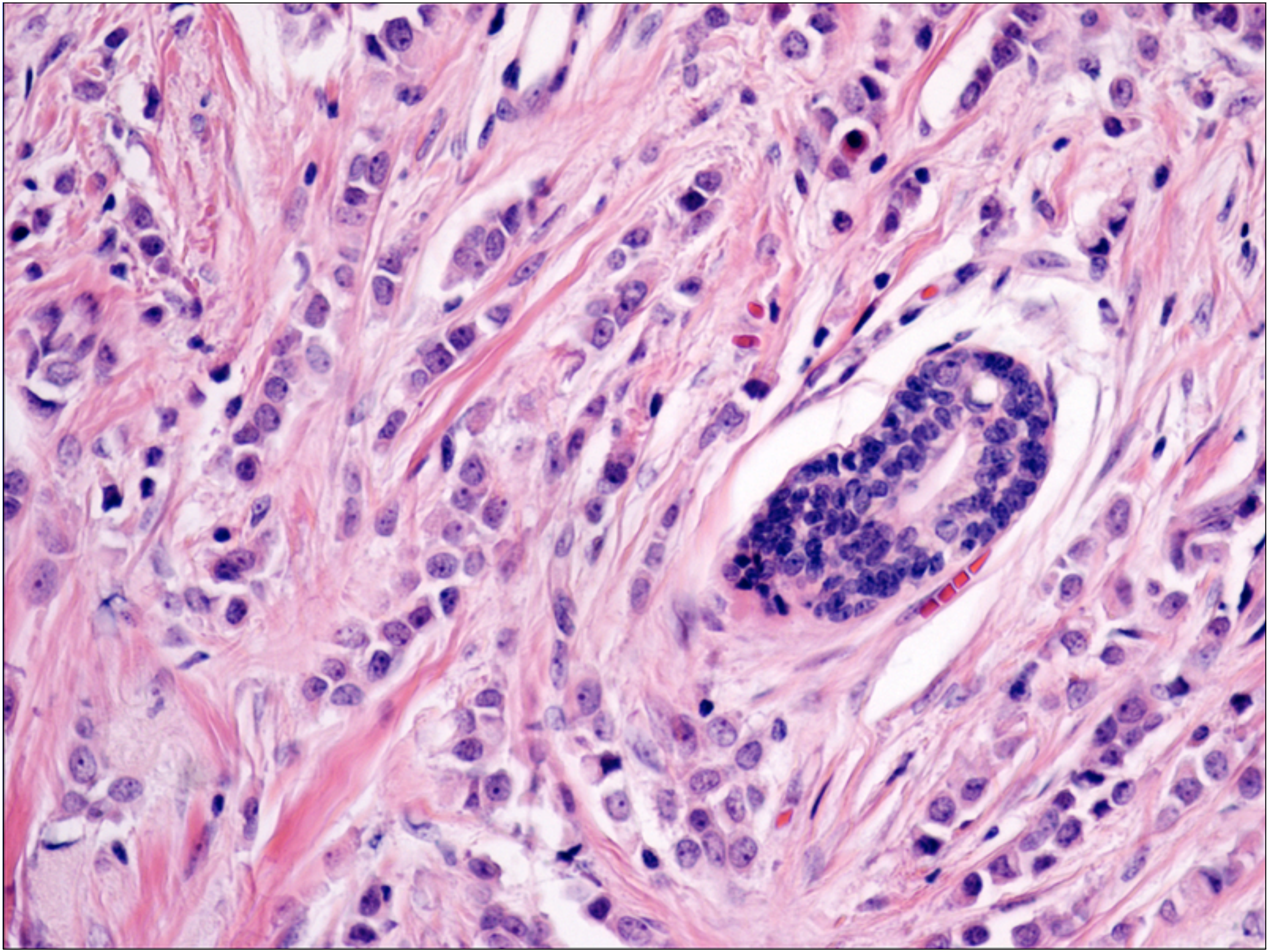
E-Cadherin Expression: Ductal and Lobular Neoplasia

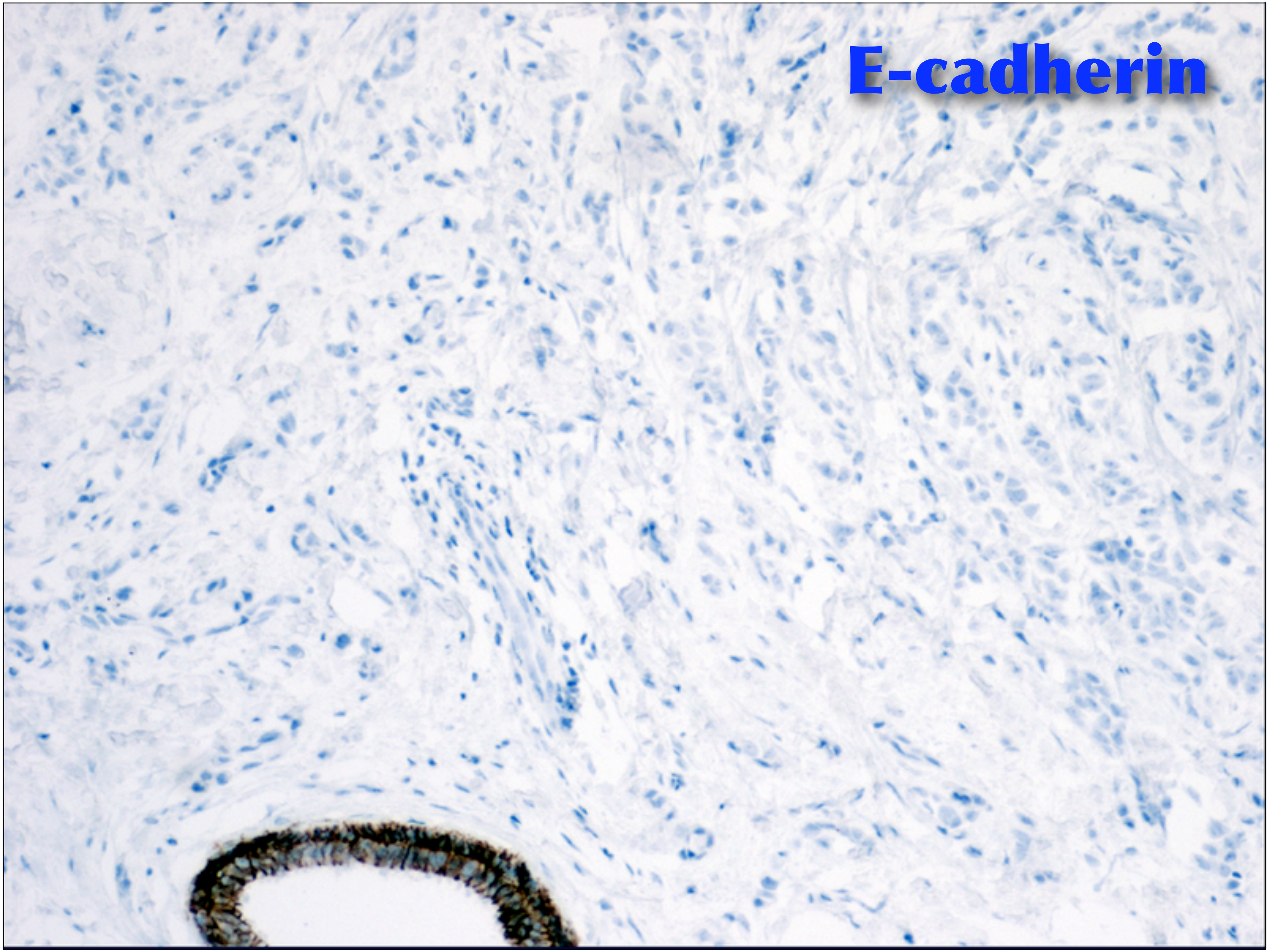
Goldstein et al. Am J Clin Pathol 115:534-542, 2001

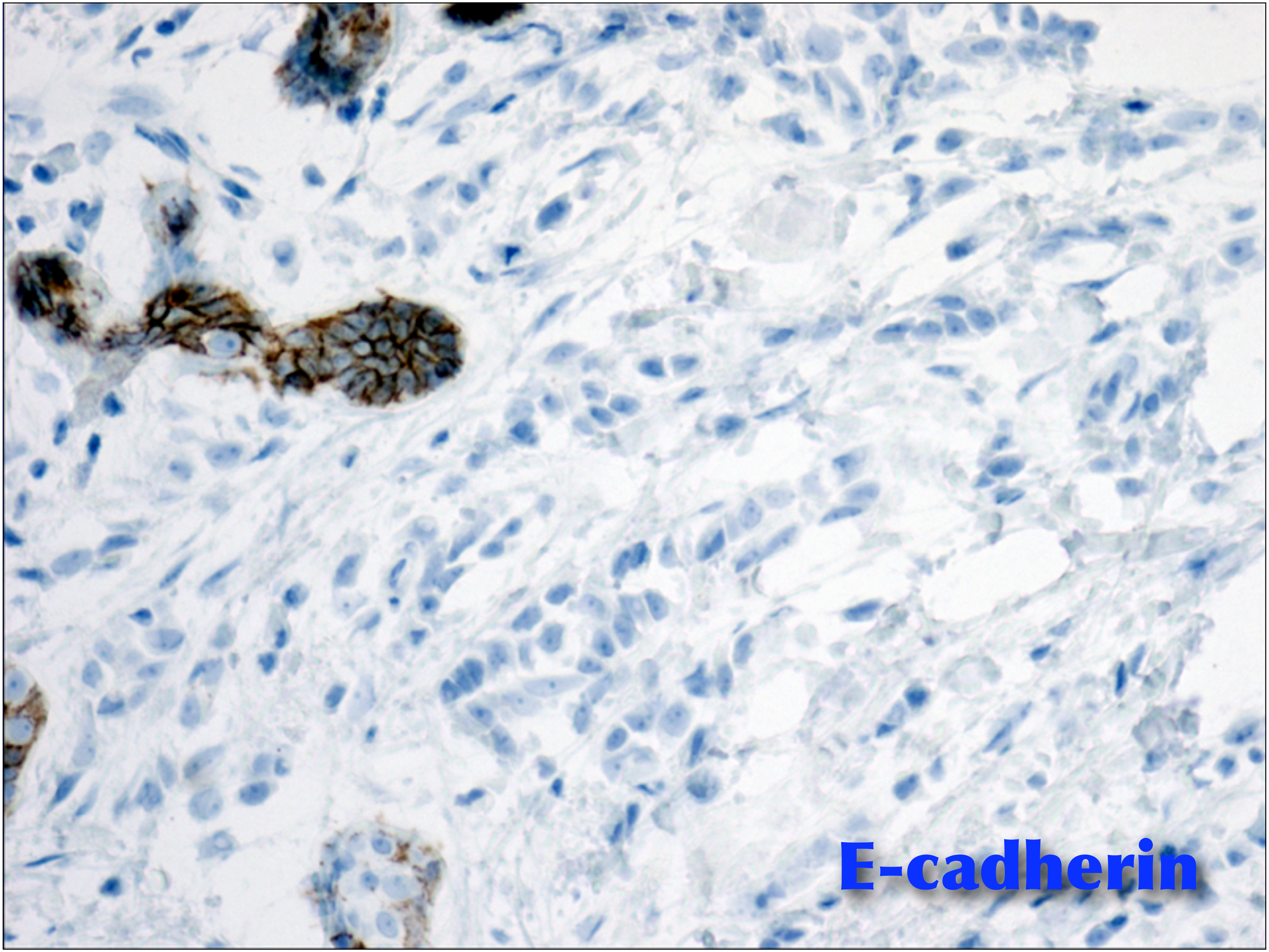
Normal, nonproliferative ductal cells	95 cases; all strongly positive
DCIS and ADH	37 cases; all strongly positive
ALH or LCIS	22 cases: 20 negative, 2 weak

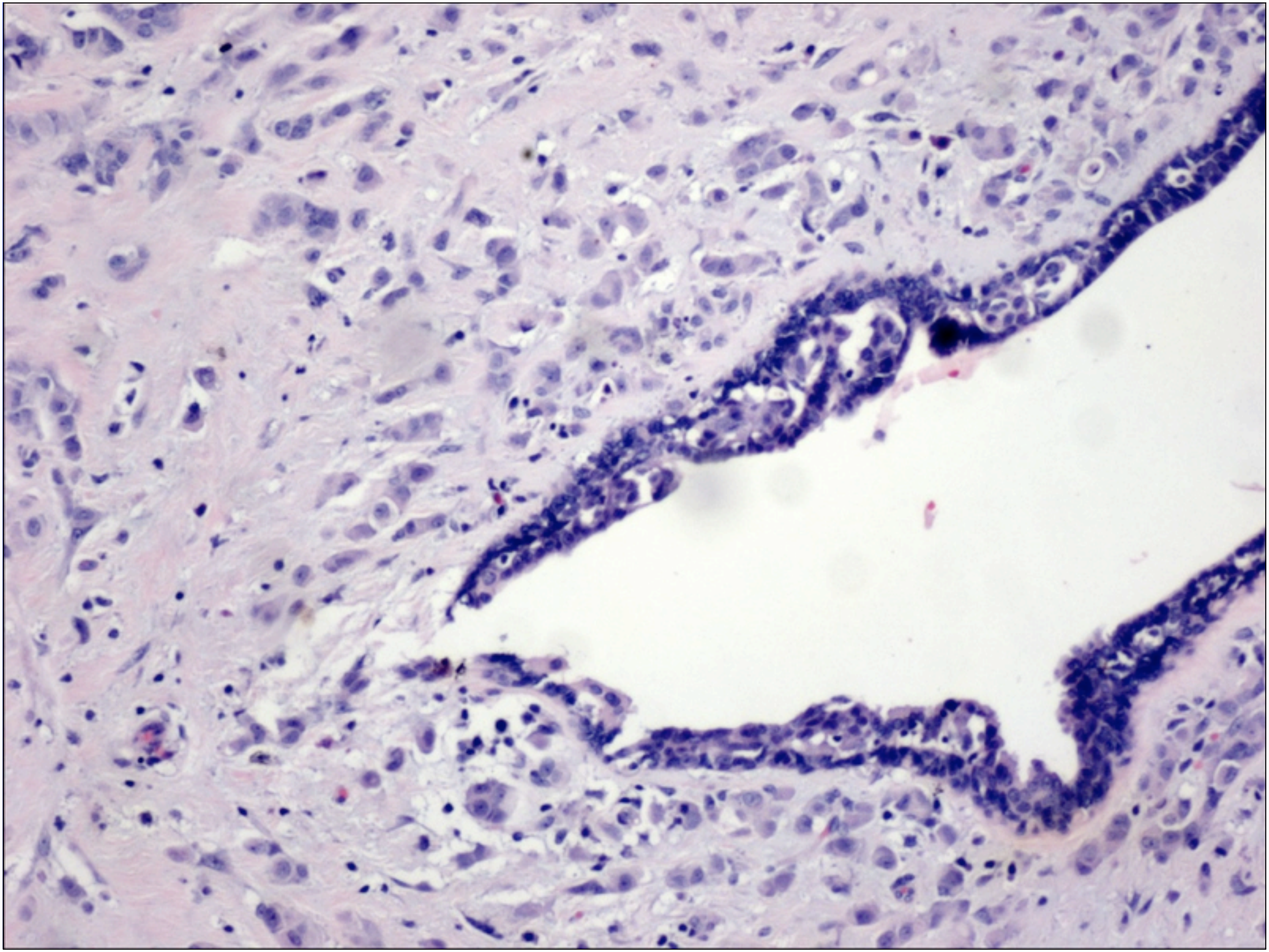

E-cadherin

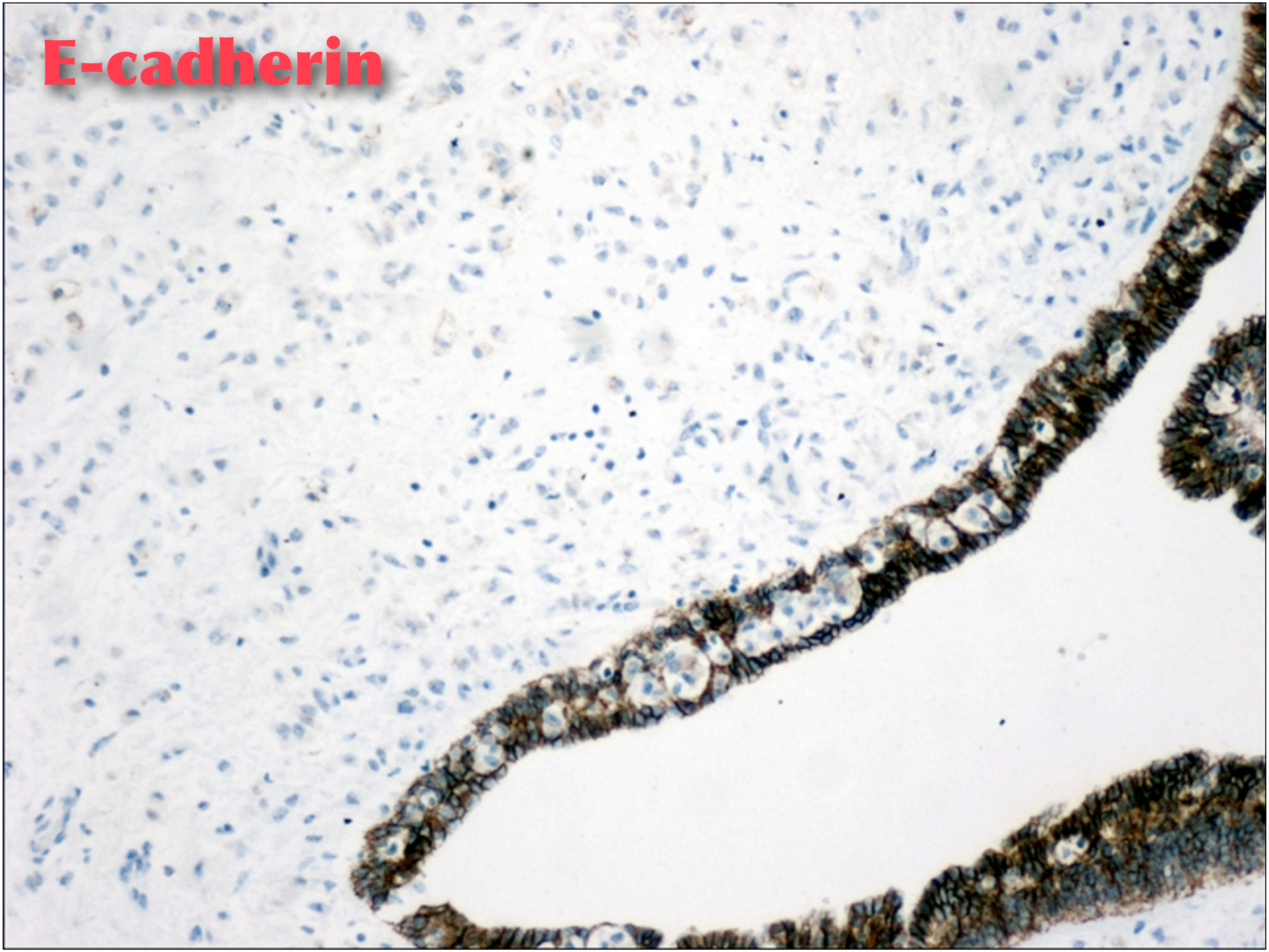

E-cadherin

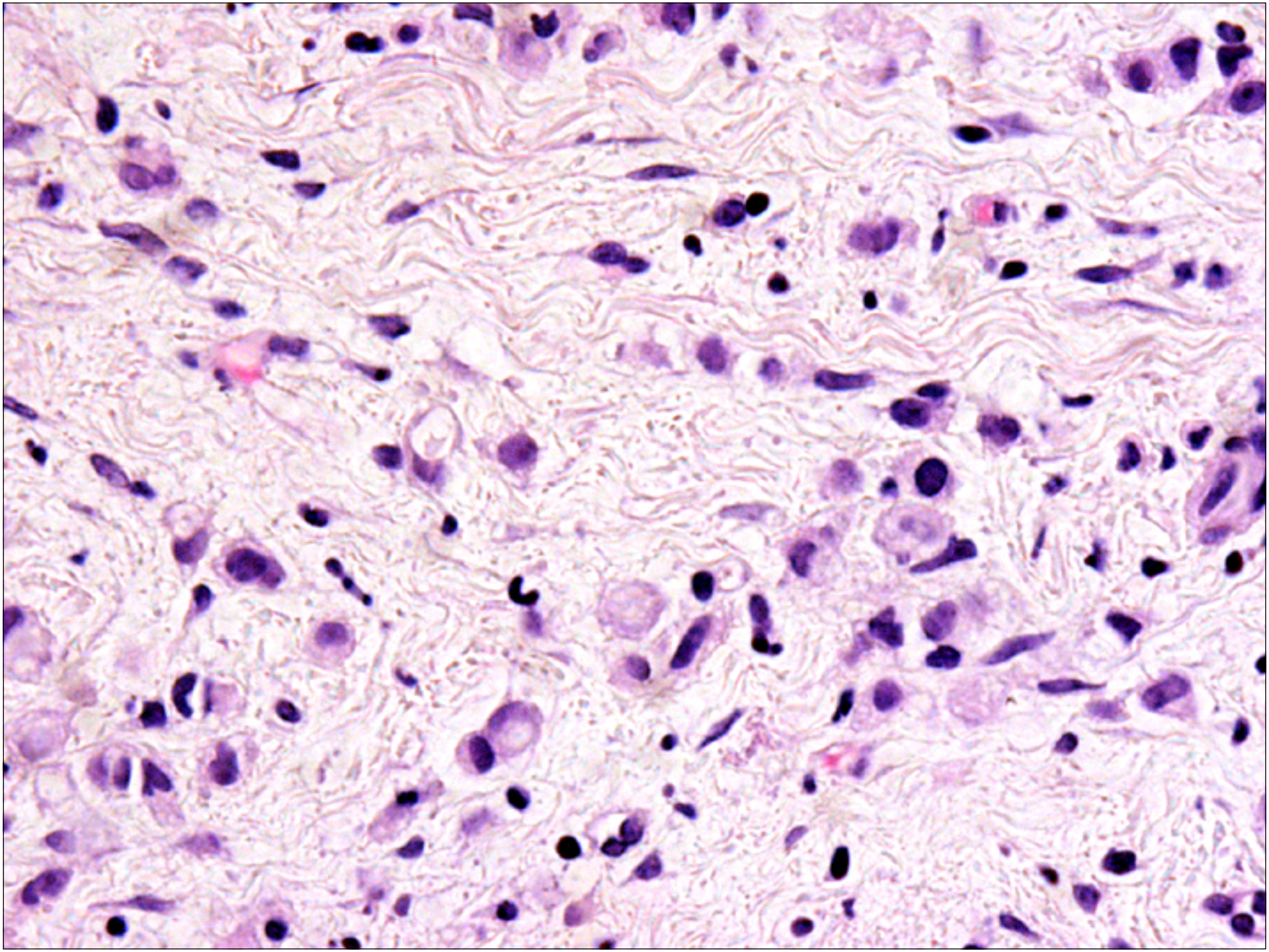


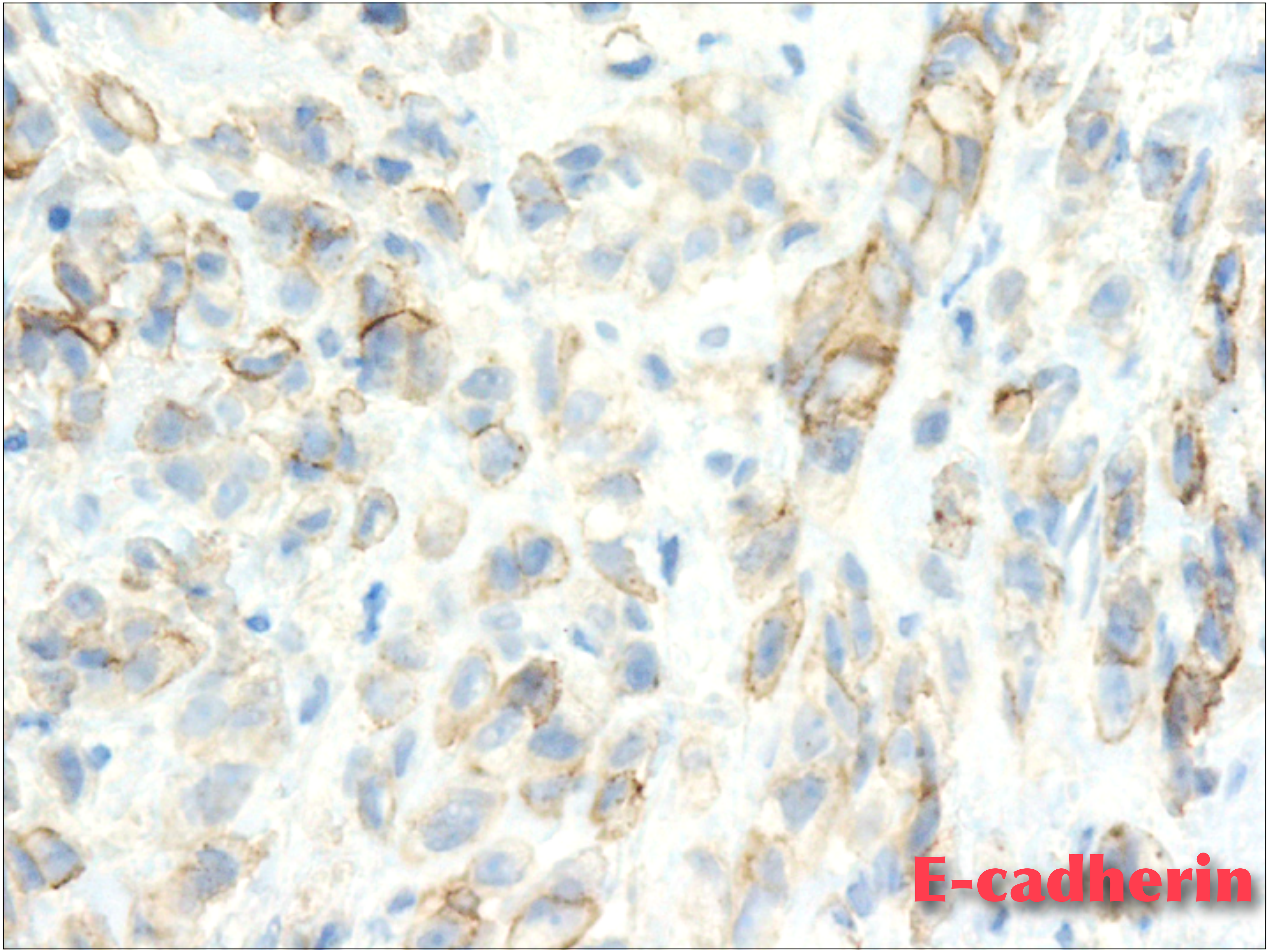

E-cadherin

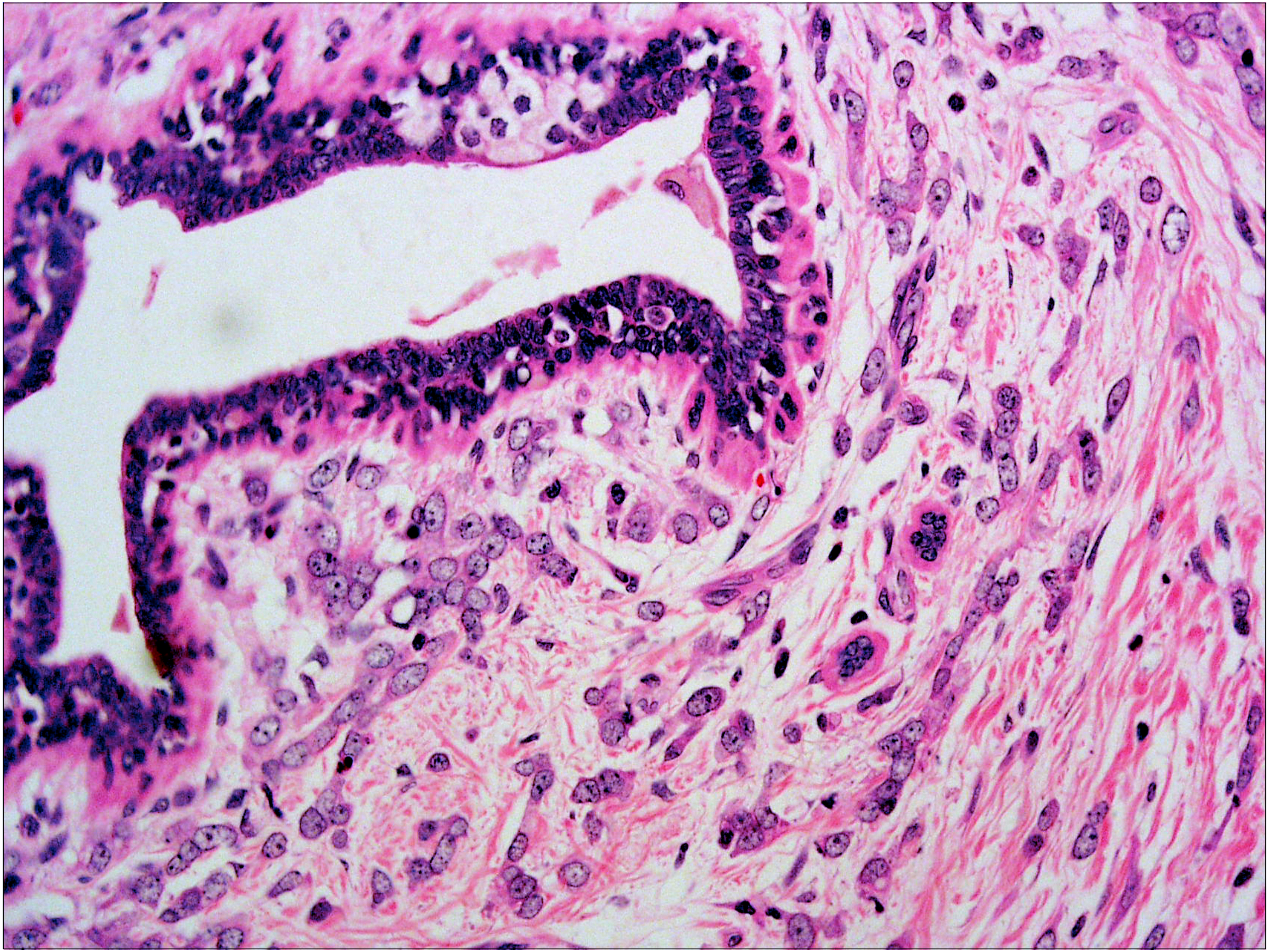

E-cadherin

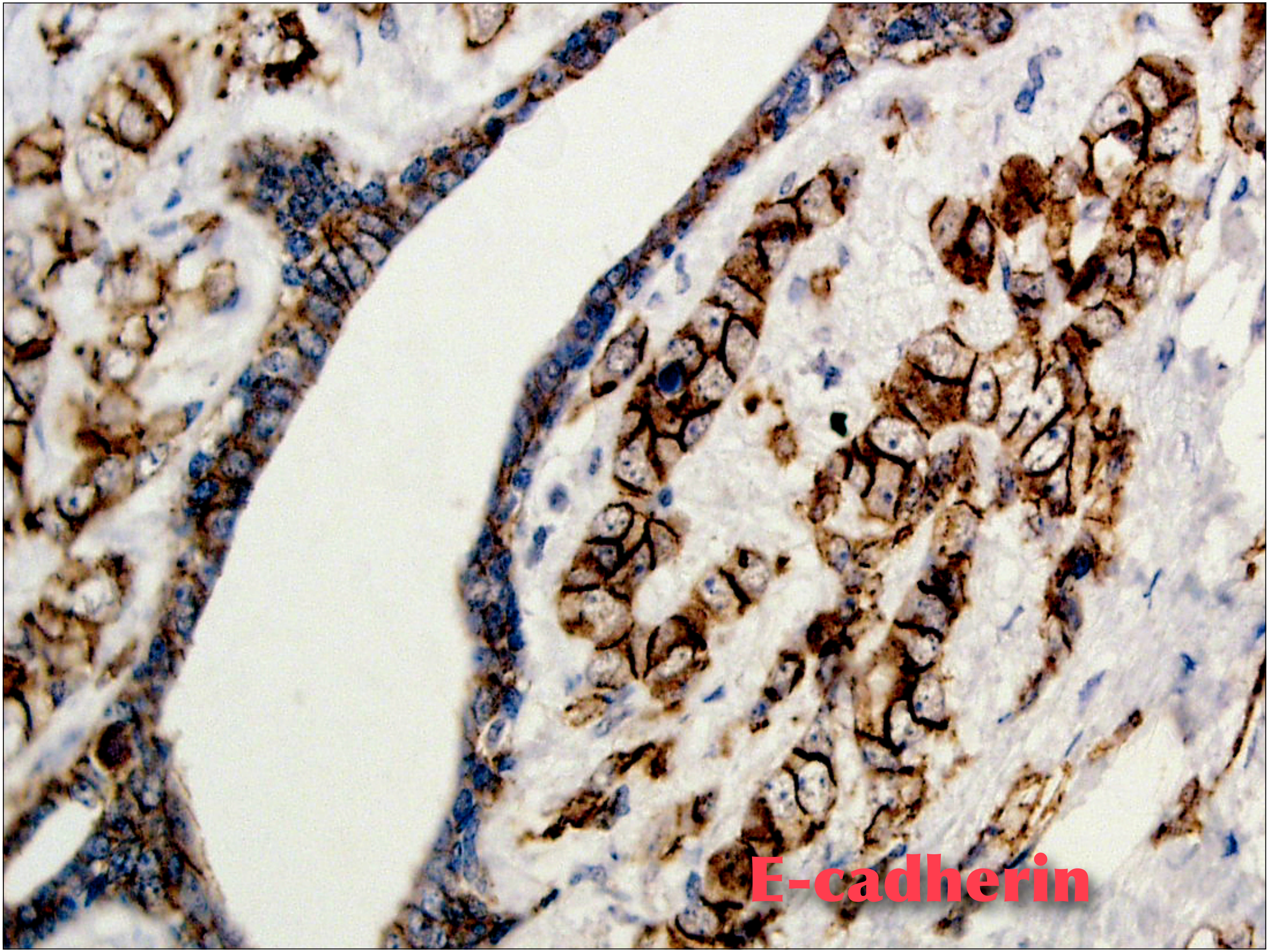


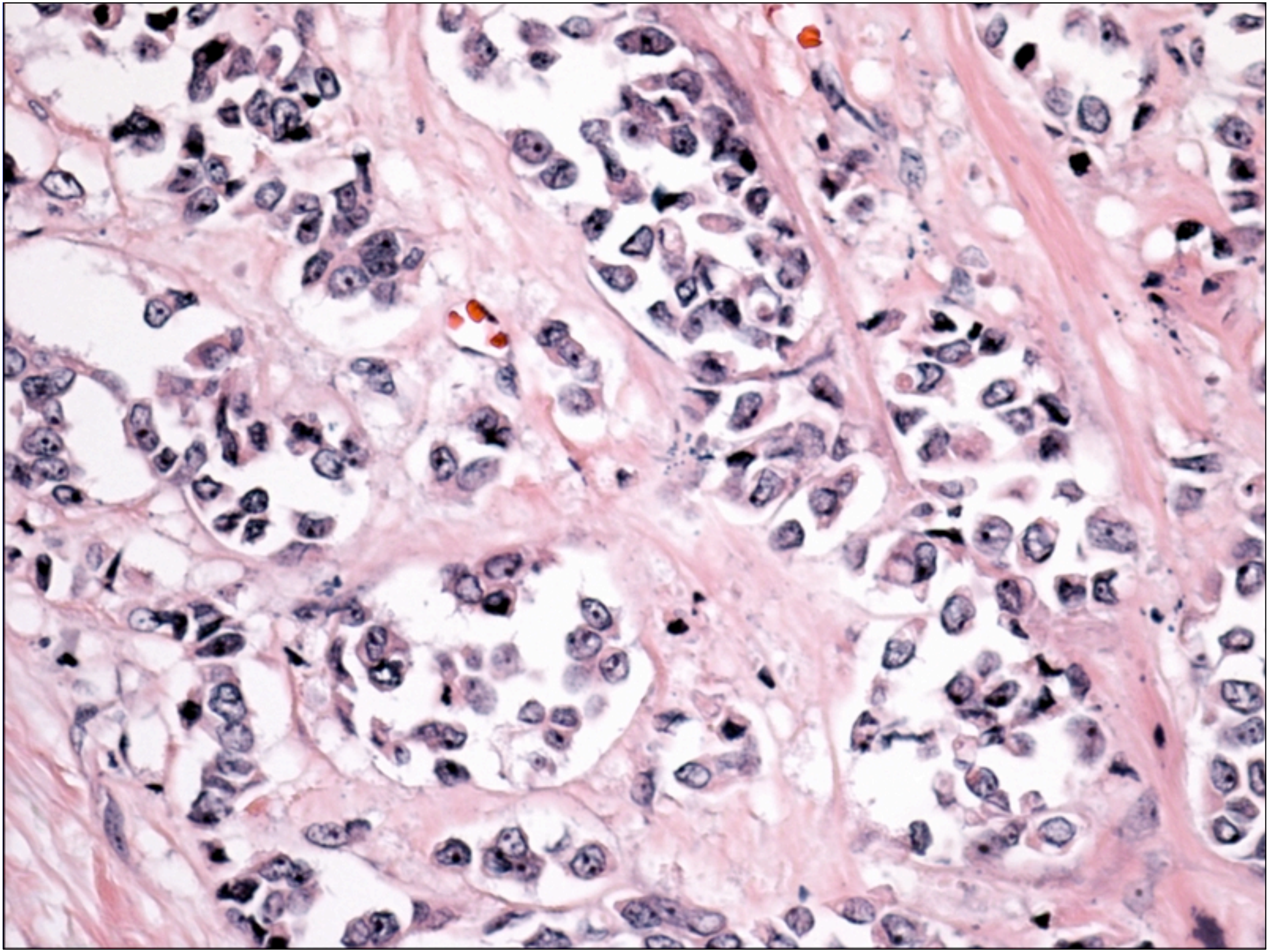

E-cadherin

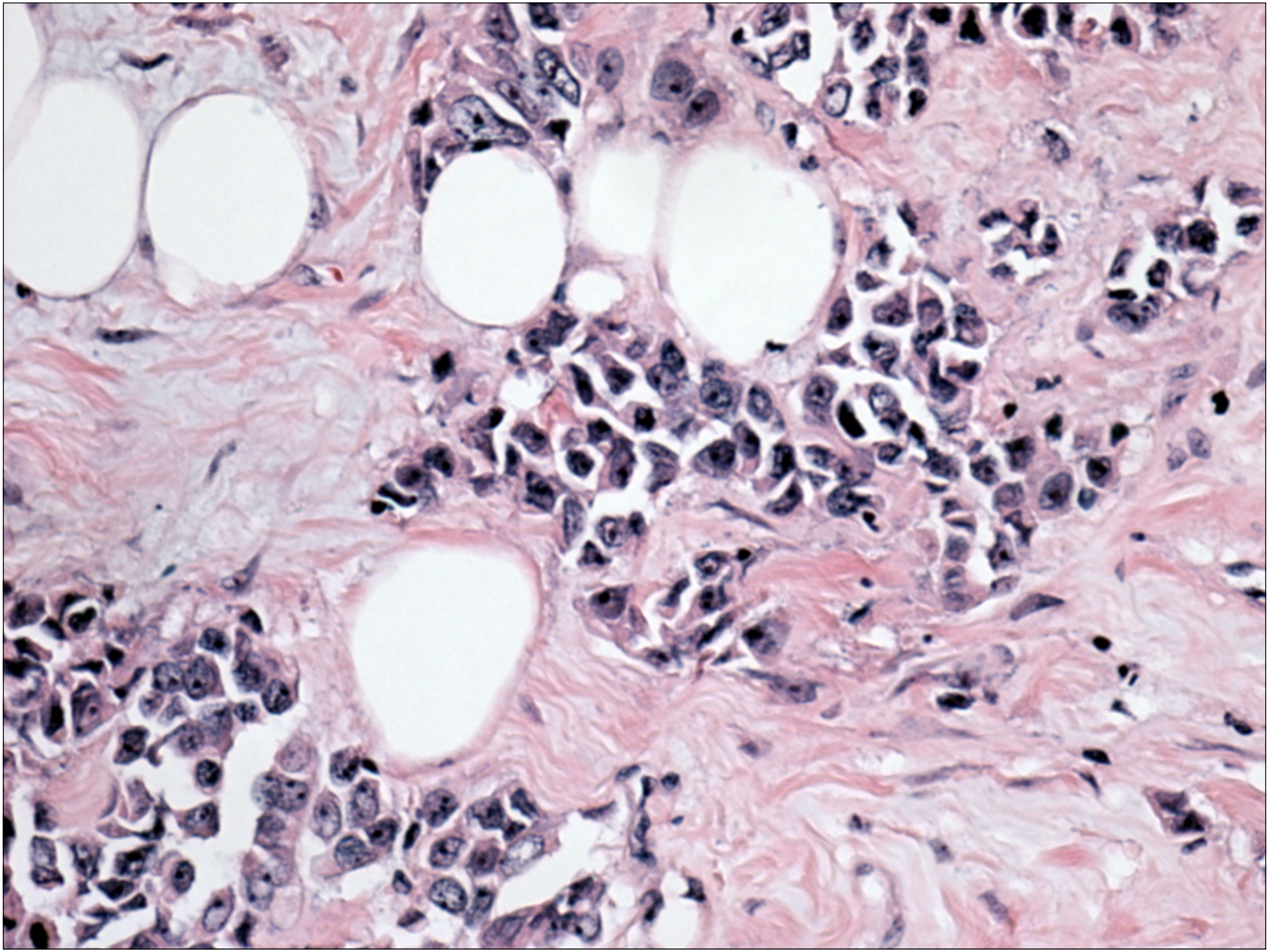


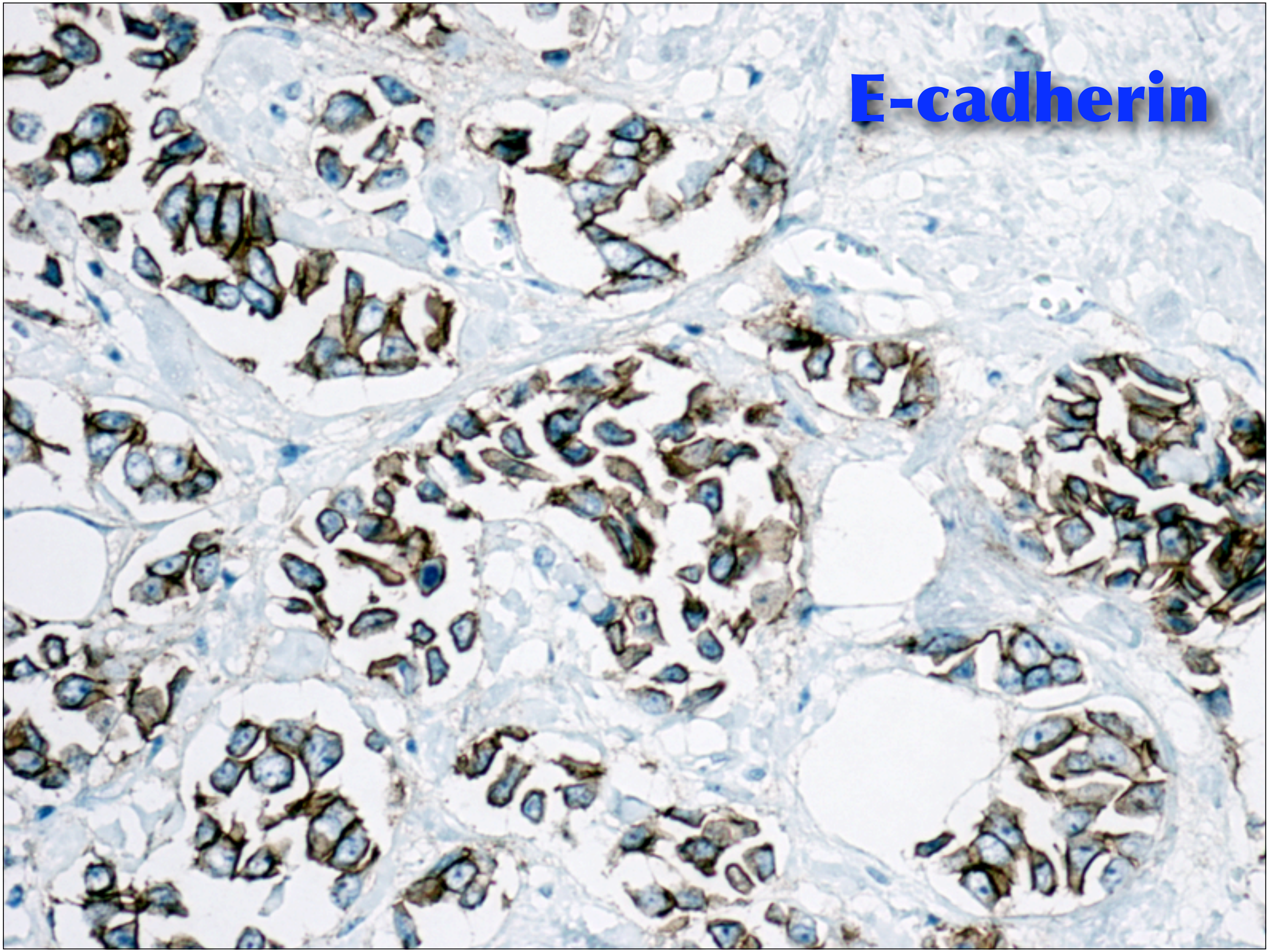

E-cadherin

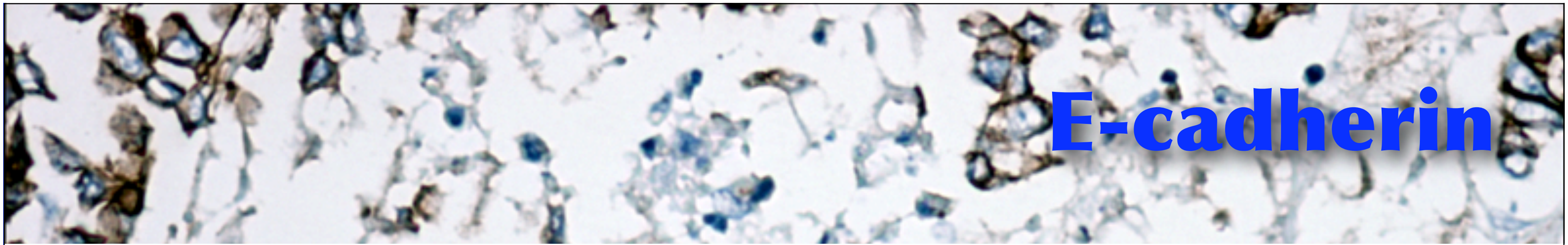



E-cadherin

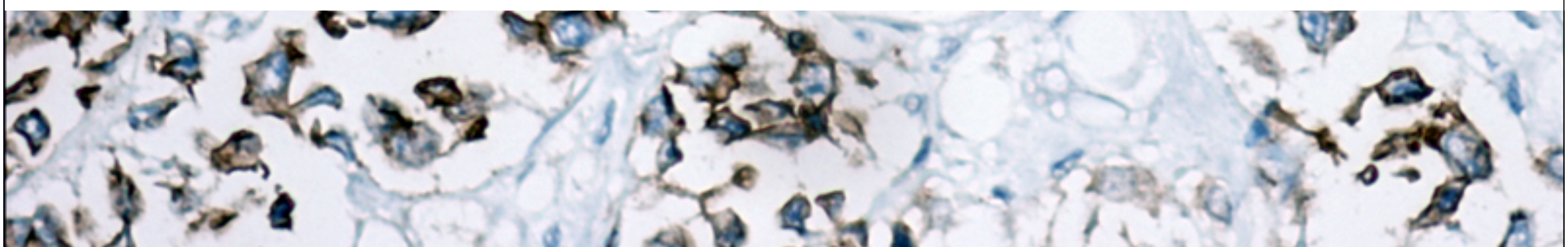


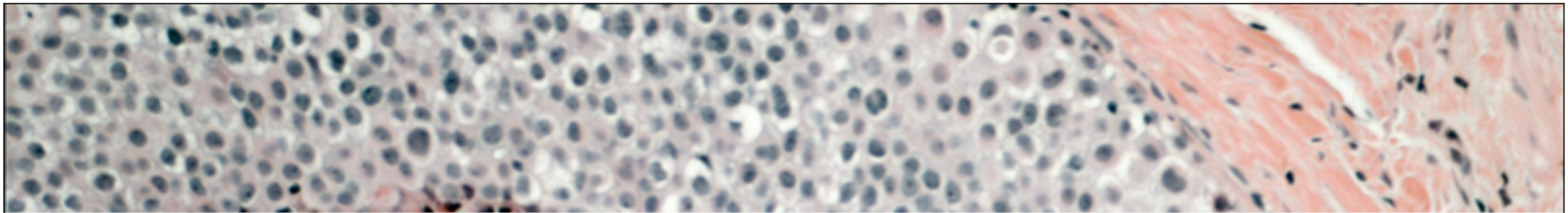



E-cadherin

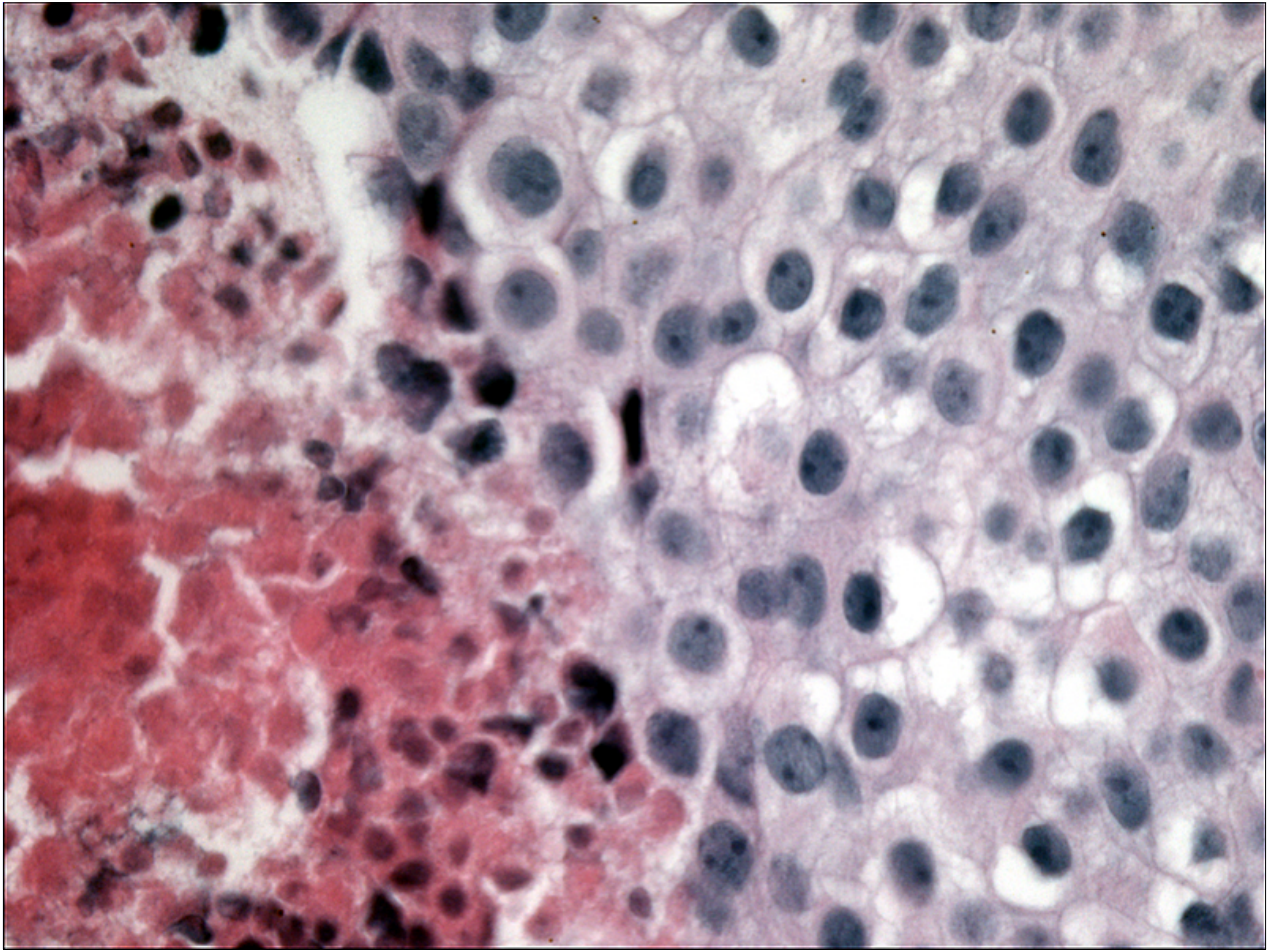


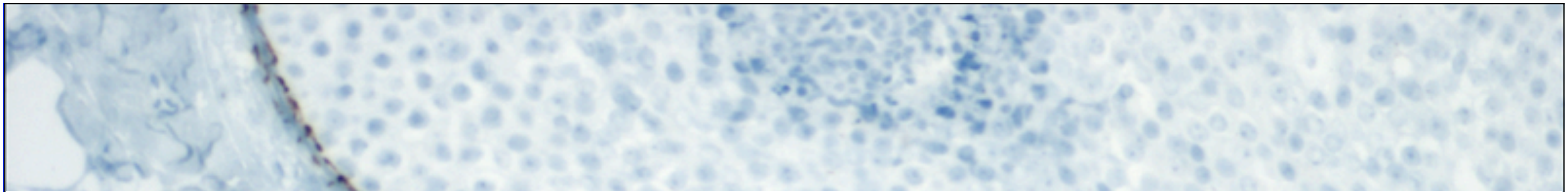
“Pseudolobular” Infiltrating Ductal Carcinoma



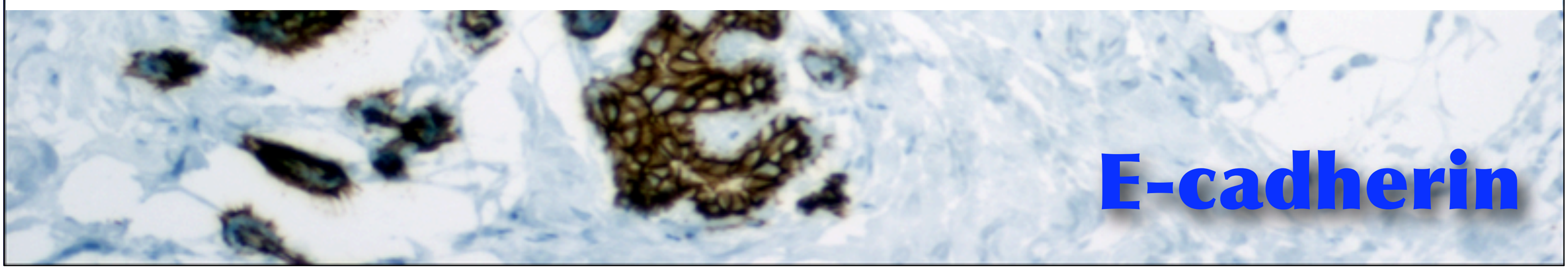


Just another case of DCIS with comedo necrosis…
…or is it?





Lobular Carcinoma-In-Situ



Am J Surg Pathol 30:1445-53, 2006

Lobular Intraepithelial Neoplasia [Lobular Carcinoma In Situ] With Comedo-type Necrosis

A Clinicopathologic Study of 18 Cases

Oluwole Fadare, MD,†‡ Farnaz Dadmanesh, MD,§ Isabel Alvarado-Cabrero, MD,‖ Robert Snyder, MD,¶ J. Stephen Mitchell, MD,¶ Tibor Tot, MD,# Sa A. Wang, MD,** Mohiedean Ghofrani, MD,* Vincenzo Eusebi, MD,†† Maritza Martel, MD,* and Fattaneh A. Tavassoli, MD**

- Occurs in older age group
- Commonly associated with invasive carcinoma more frequently lobular
- Usually HMW-CK+, ER+, PR+, HER-2-
- E-cadherin negative
- Long term follow-up required, ?re-excision recommended

Am J Surg Pathol 25:229-36, 2001

Carcinomas In Situ of the Breast With Indeterminate Features

Role of E-Cadherin Staining in Categorization

Timothy W. Jacobs, M.D., Natasha Pliss, B.S., George Kouria, M.D., and Stuart J. Schnitt, M.D.

LCIS: 28 cases

DCIS: 33 cases

CIS-IF: 28 cases

Jacobs TW et al., Am J Surg Pathol 25:229-36, 2001

CIS-IF: 28 cases

- **100% E-cadherin-negative**
- Group 2: CIS with small, uniform cells either solid with focal microacinar structures and dyscohesion, or cohesive but with vacuoles
- **100% E-cadherin-negative**

Group 2

- 35.3% E-cadherin negative
- 29.4% E-cadherin positive
- 35.3% Heterogeneous

Caution: Little clinical outcome data on histologically ambiguous lesions

Distinction of Lobular vs. Ductal

- *Peiro G et al., Breast Cancer Res Treat 59:49-54, 2000 (93 lobulars vs. 1089 ductals)* Stage I or II breast cancer - no difference in outcome of ductal v. lobular (multiple regression analysis) breast-conserving surgery and radiation

- *Molland JG et al., Breast 13:389-96, 2004 (182 lobulars vs. 1612 ductals)* Mastectomy more likely necessary to obtain clear margins in lobular, but overall survival identical

Distinction of Lobular vs. Ductal Carcinoma: Is It Important?

- In situ carcinoma: distinction between LCIS and DCIS has important therapeutic implications
- Patients with LCIS managed with careful observation (and tamoxifen)
- Patients with DCIS treated with excision, radiation therapy, or mastectomy

Clinicopathologic Implications of E-Cadherin Reactivity in Patients with Lobular Carcinoma In Situ of the Breast

Neal S. Goldstein, M.D.[1]
Larry L. Kestin, M.D.[2]
Frank A. Vicini, M.D.[2]

[1] Department of Anatomic Pathology, William Beaumont Hospital, Royal Oak, Michigan.

[2] Department of Radiation Oncology, William Beaumont Hospital, Royal Oak, Michigan.

Cancer 92:738-47, 2001

- 82 consecutive ‘LCIS’ patients 1955-1976
- 486 sections immunostained for E-cadherin
- E-cadherin expression correlated with clinicopathologic features and outcome

Goldstein NS et al., Cancer 92:738-47, 2001

- 9 (10.9%) LCIS cases had E-cadherin expression (focal)
- Patients with E-cadherin-positive ‘LCIS’ more frequently developed a subsequent ipsilateral carcinoma that had a ductal component (55.5% vs. 12.3%; $P < 0.01$)
- **E-cadherin reactivity appears to identify subset of LCIS patients with risk factors for subsequent carcinoma similar to patients with low-grade DCIS**

Cautions in Interpreting E-cadherin

- Expression in lobular carcinomas may be markedly reduced but not completely absent
- Always look for strong positive internal controls
- Cells in question should be cytokeratin positive (r/o macrophages, plasma cells, etc.)

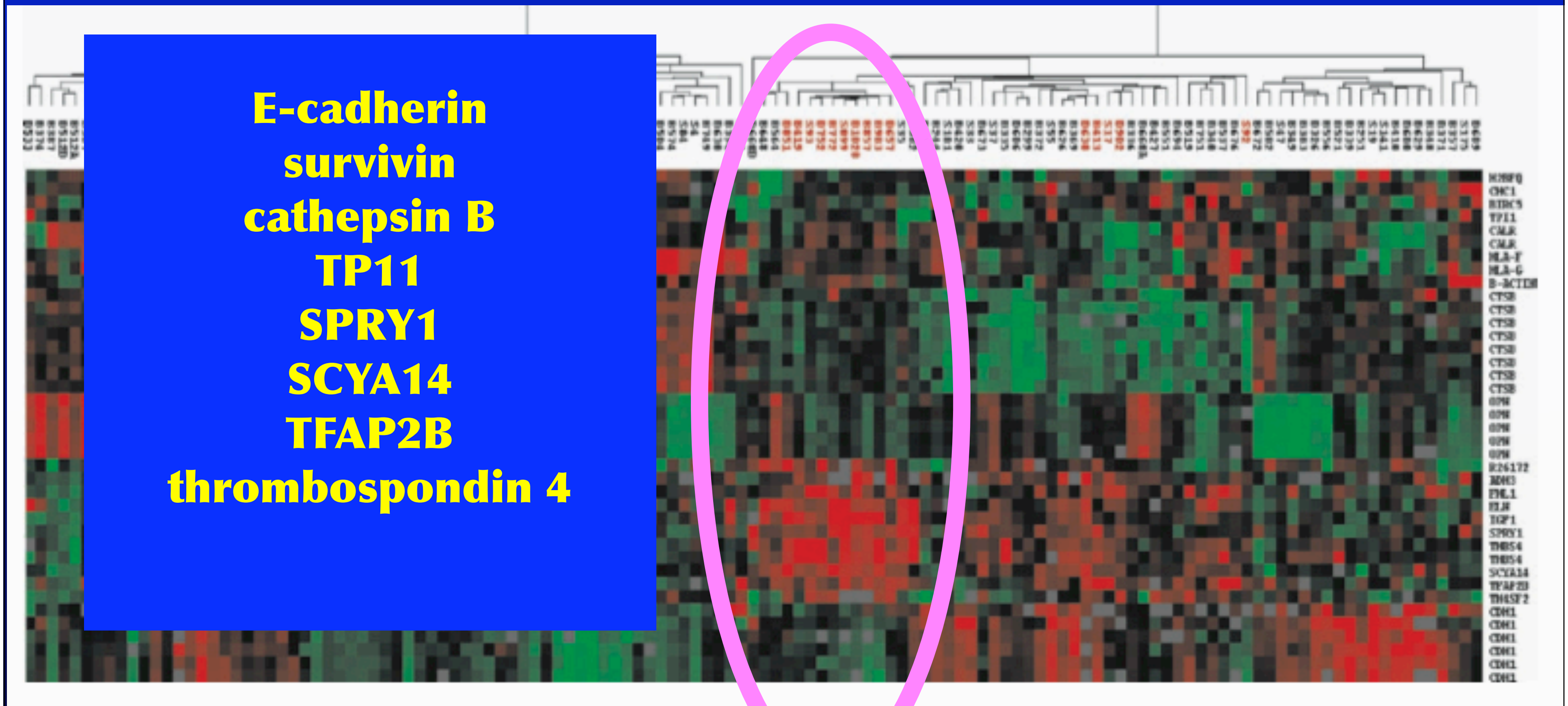
When Histology Says Ductal, but Immunohistochemistry Says Lobular

?

Differentiation of Lobular *versus* Ductal Breast Carcinomas by Expression Microarray Analysis[1]

James E. Korkola,[2] Sandy DeVries, Jane Fridlyand, E. Shelley Hwang, Anne L. H. Estep, Yunn-Yi Chen, Karen L. Chew, Shanaz H. Dairkee, Ronald M. Jensen, and Frederic M. Waldman

Comprehensive Cancer Center [J. E. K., S. D., J. F., A. L. H. E., K. L. C., R. M. J., F. M. W.], and Departments of Surgery [E. S. H.], Pathology [Y-Y. C.], and Laboratory Medicine [R. M. J., F. M. W.], University of California San Francisco, San Francisco, California 94143-0808, and Geraldine Brush Cancer Research Institute, California Pacific Medical Center, San Francisco, California 94115 [S. H. D.]



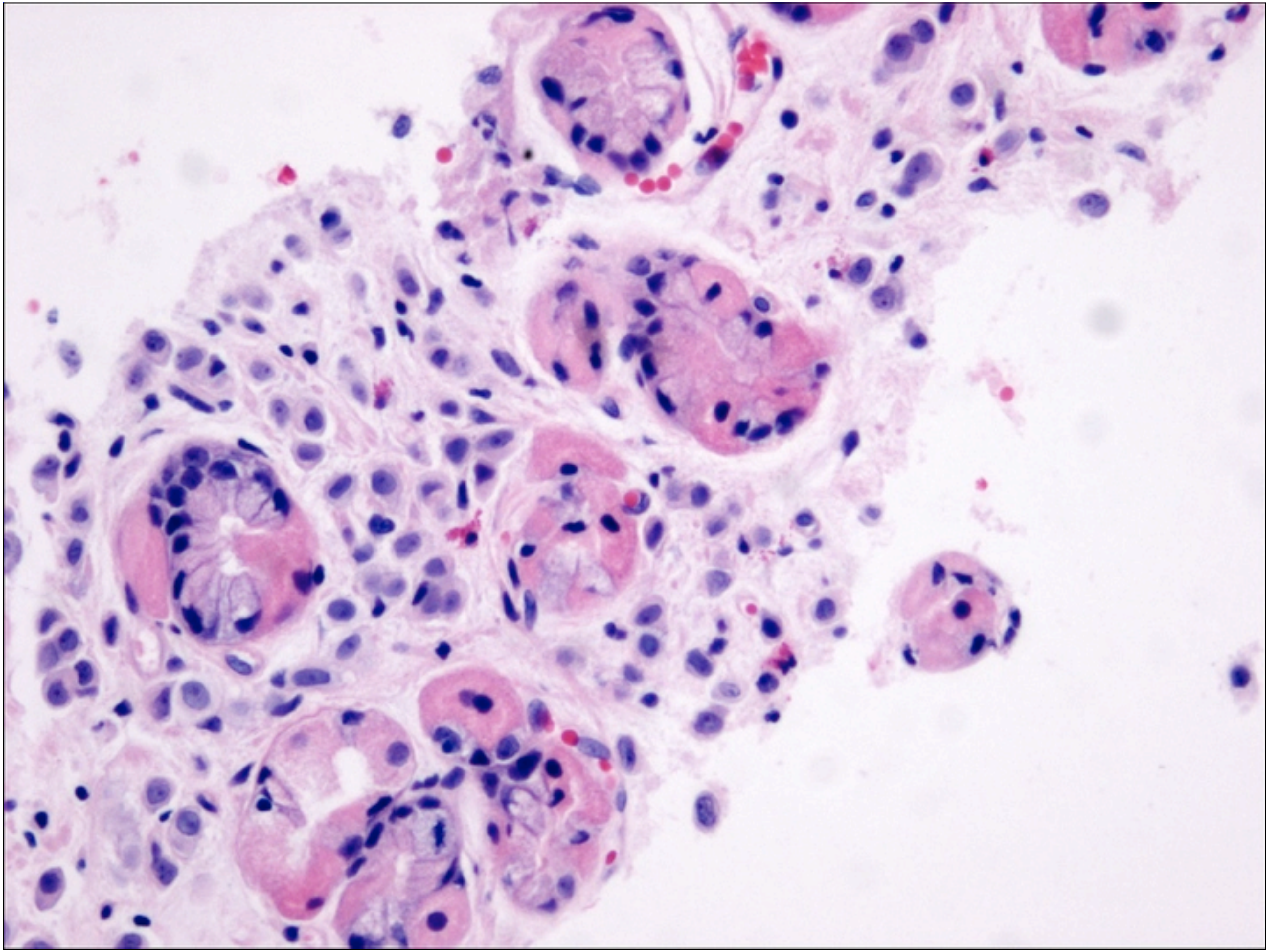
E-cadherin
survivin
cathepsin B
TP11
SPRY1
SCYA14
TFAP2B
thrombospondin 4

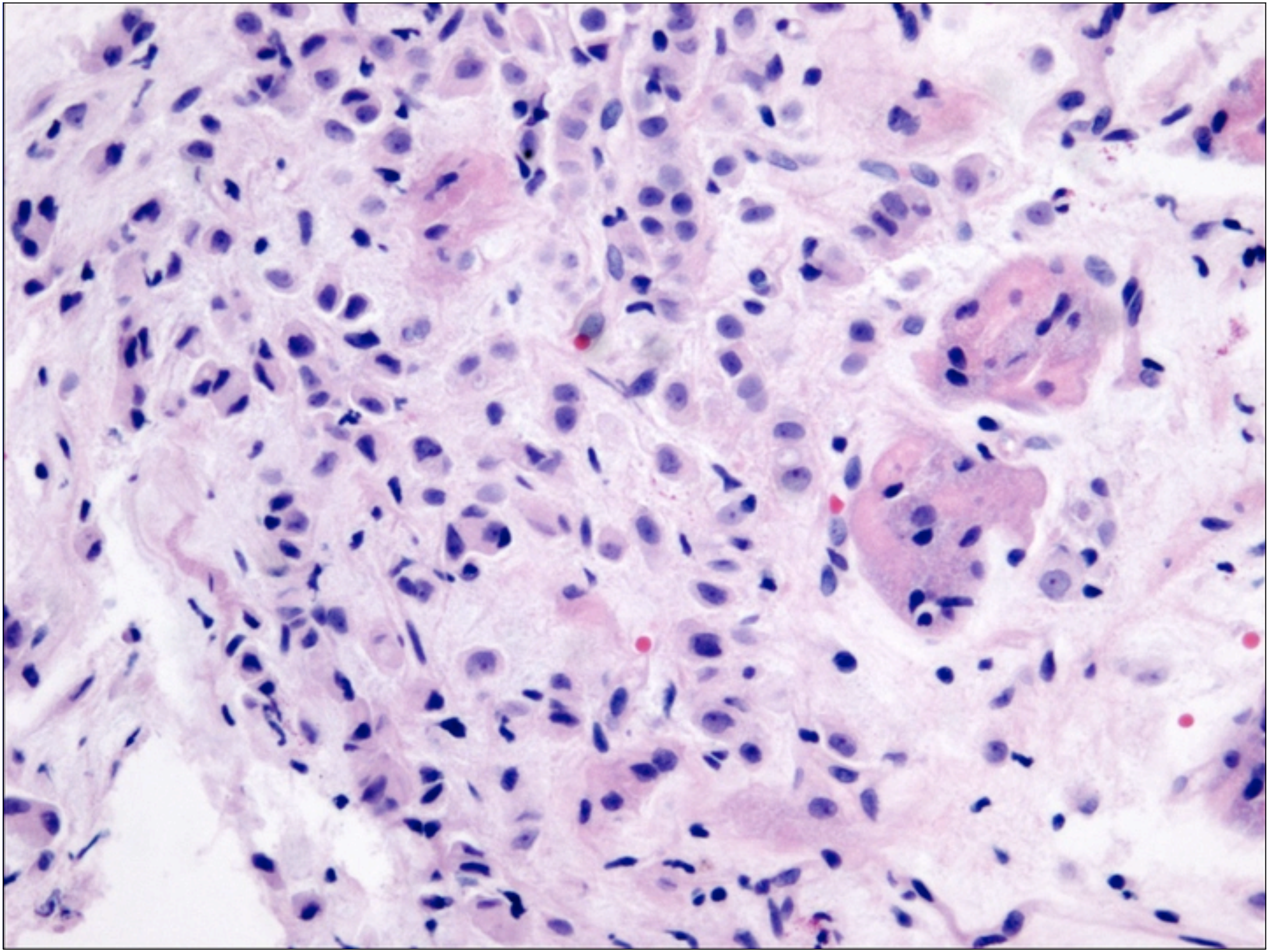
Breast Carcinoma

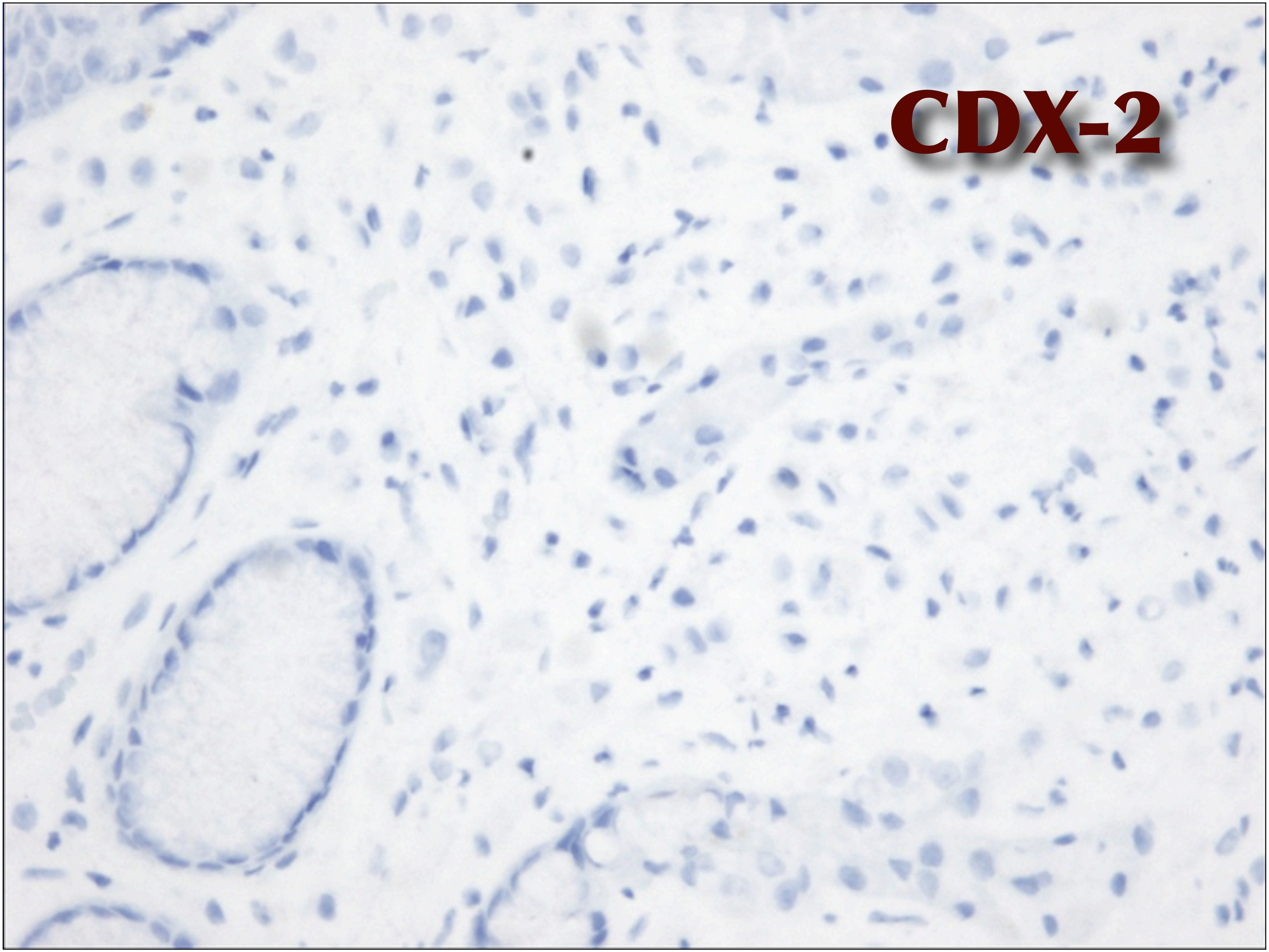
- E-cadherin in distinguishing lobular v. ductal carcinomas
- Myoepithelial markers in [illegible] carcinoma
- Markers of metastatic breast cancer

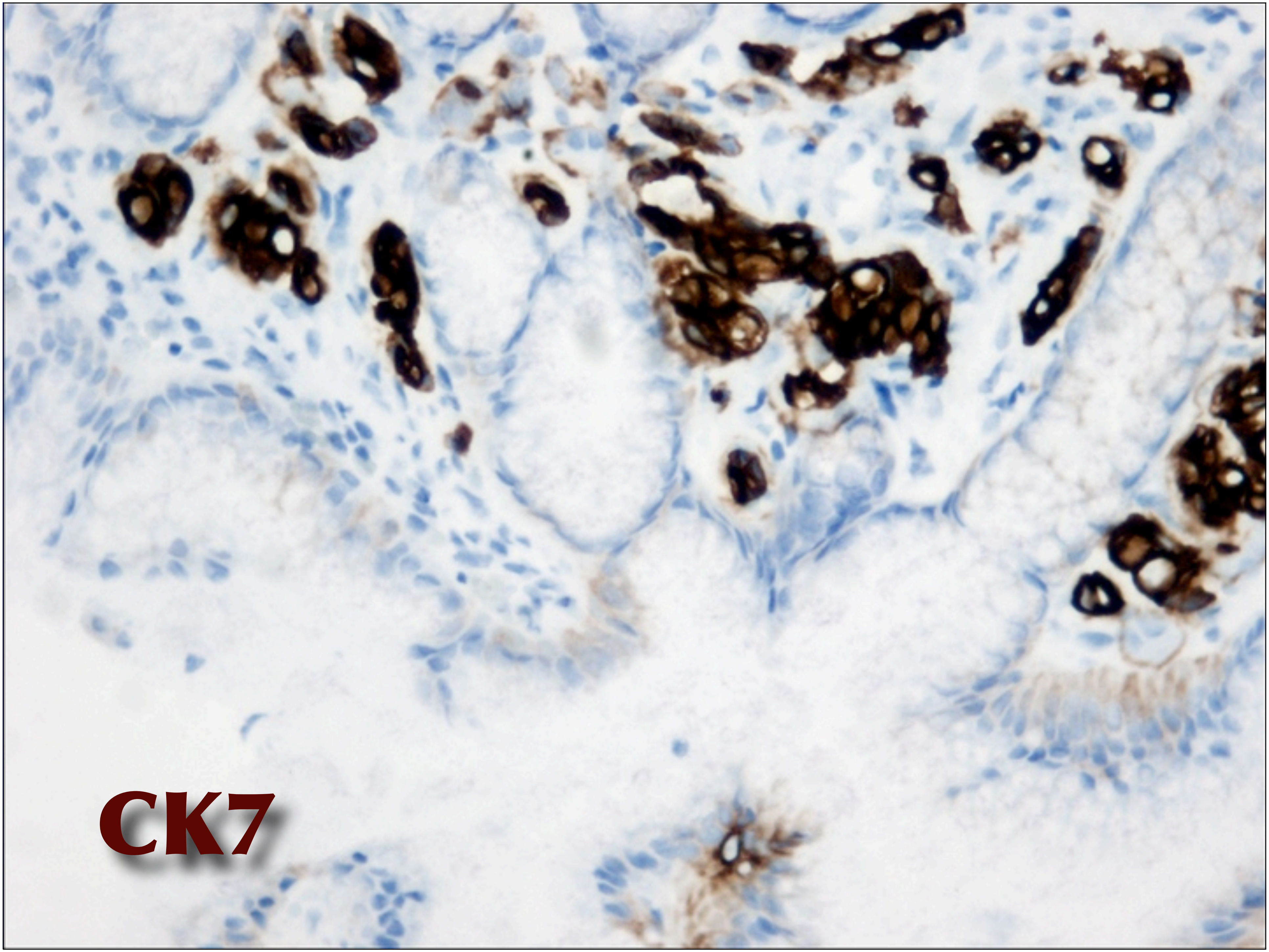
Case 2

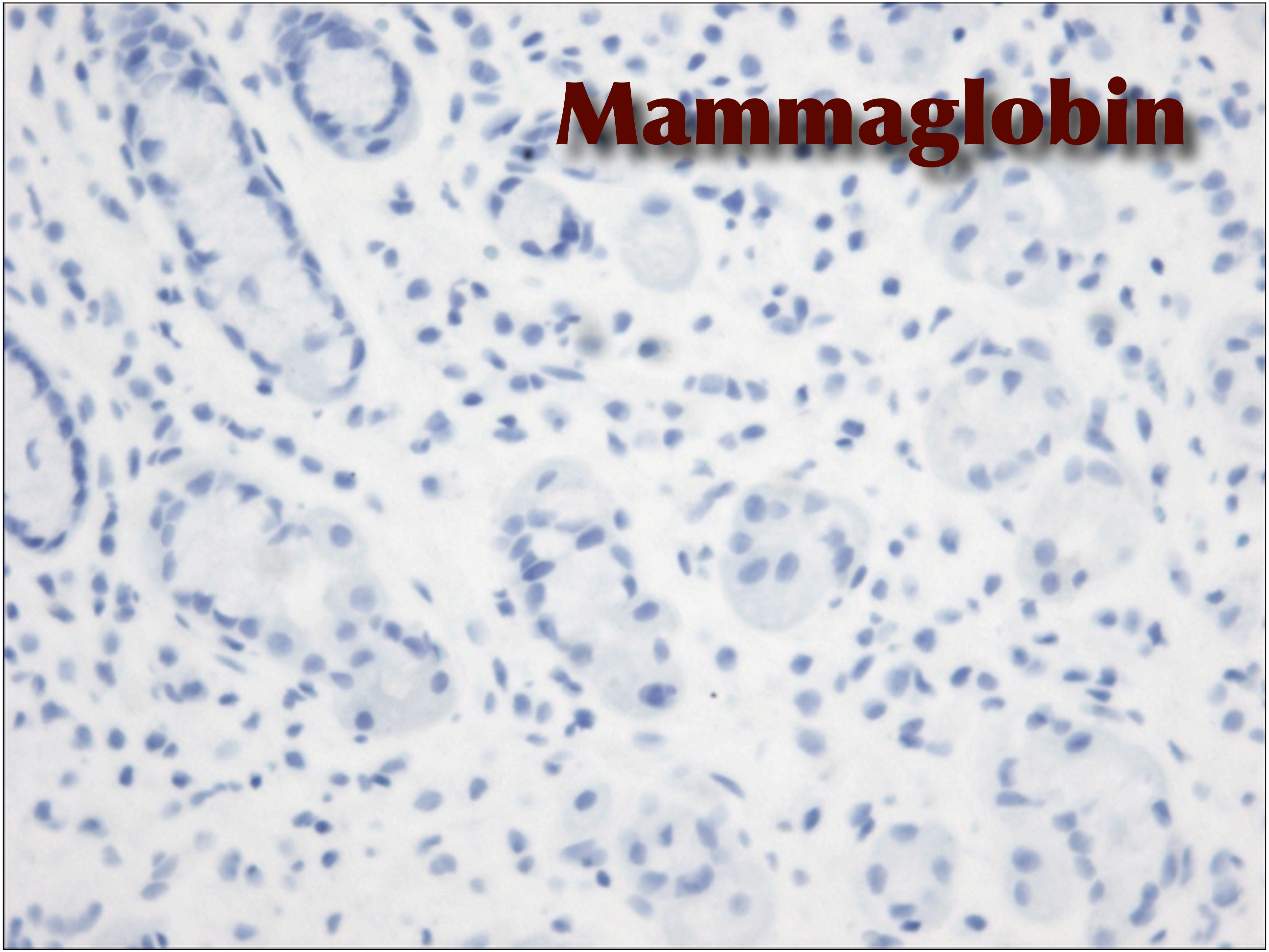
72 year old female with no prior history presents with gastric thickening

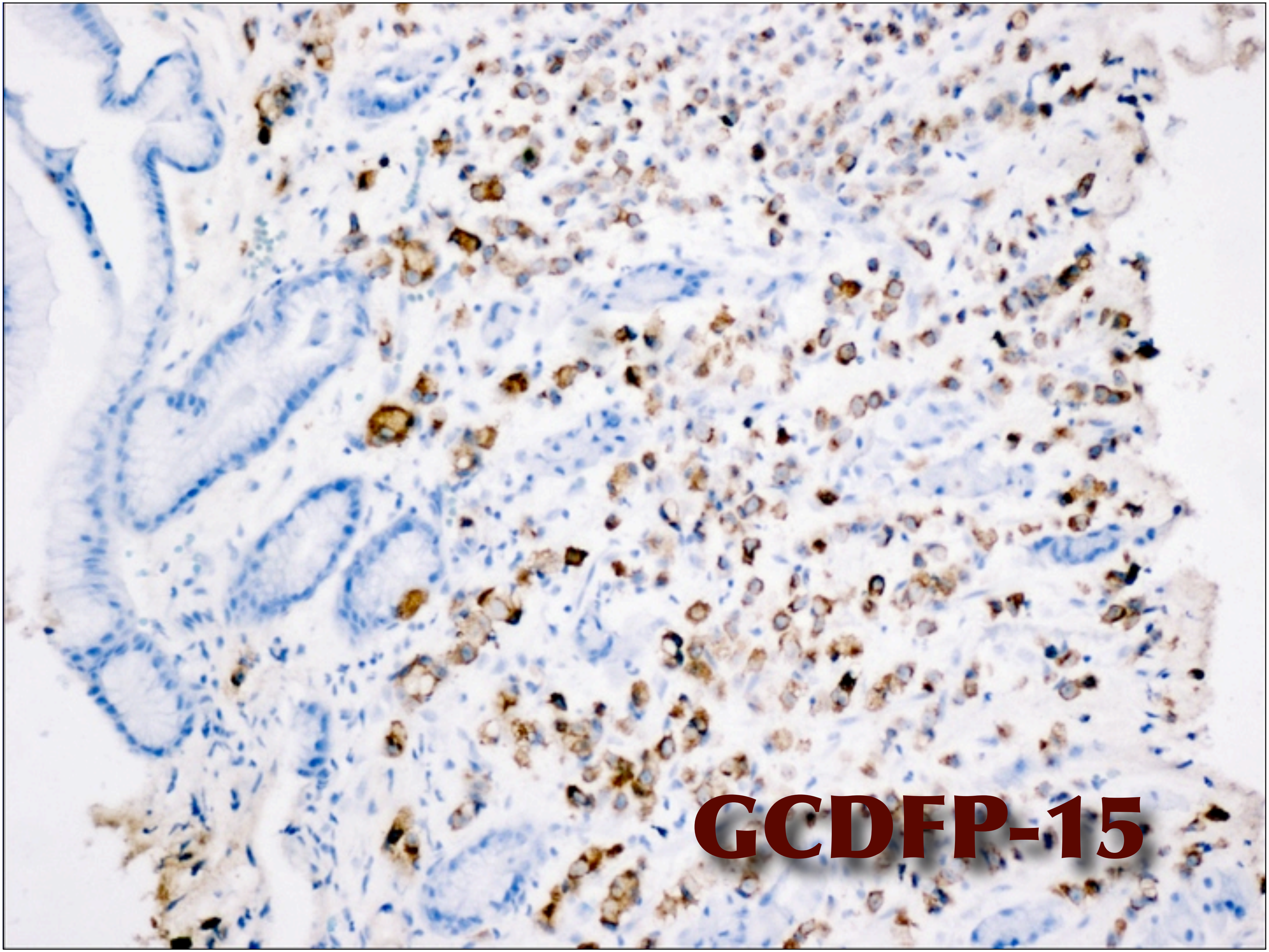





CDX-2


CK7


Mammaglobin


GCDFP-15

FINAL DX:

Metastatic lobular breast cancer to stomach

Markers of Breast Carcinoma: GCDFP-15 & Mammaglobin

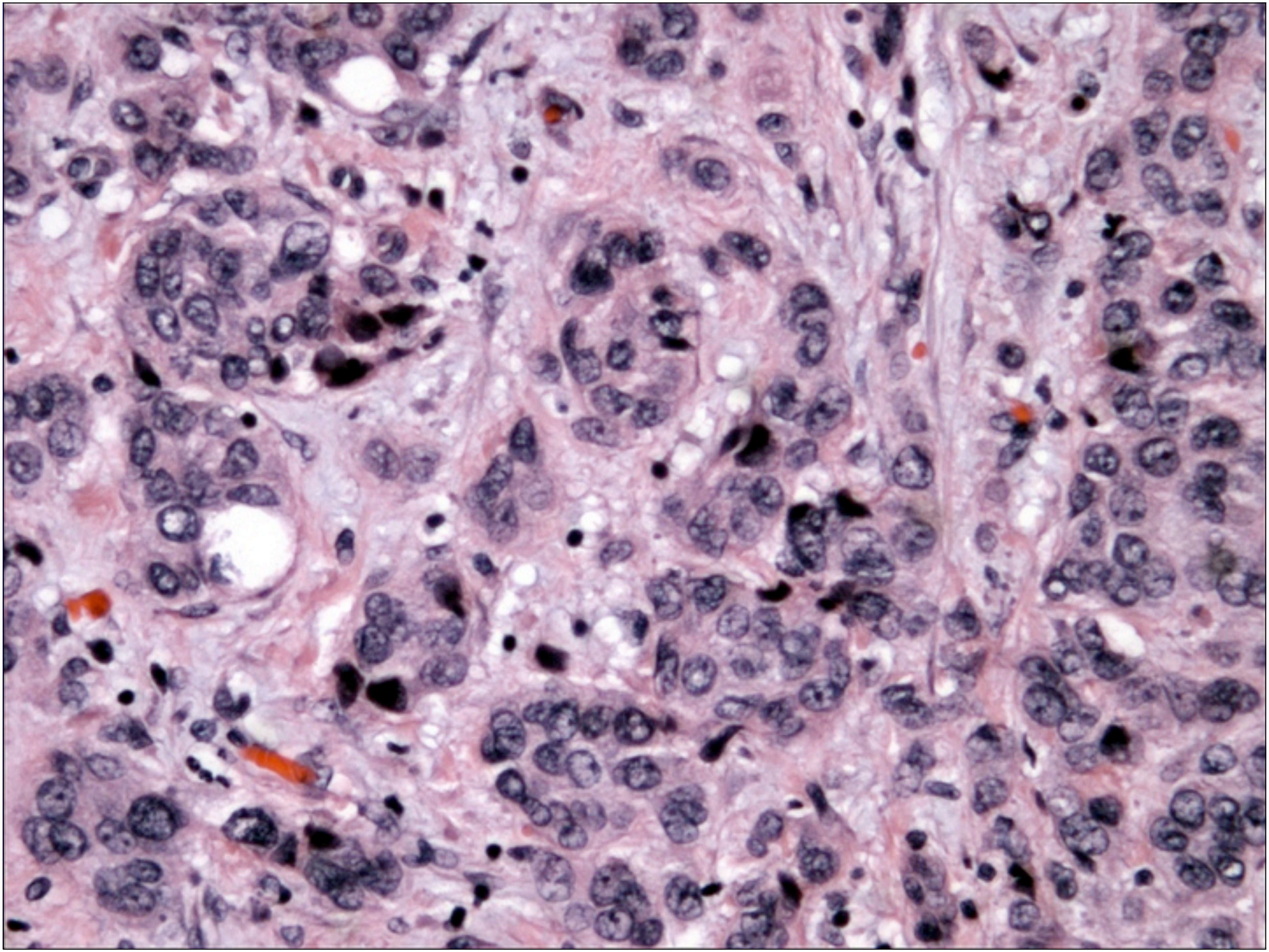
	GCDFP-15	*Mammaglobin*
Molecular weight	15 kd	10 kd
Function	Aspartyl protease	unknown
Location in cells	cytoplasm	cytoplasm

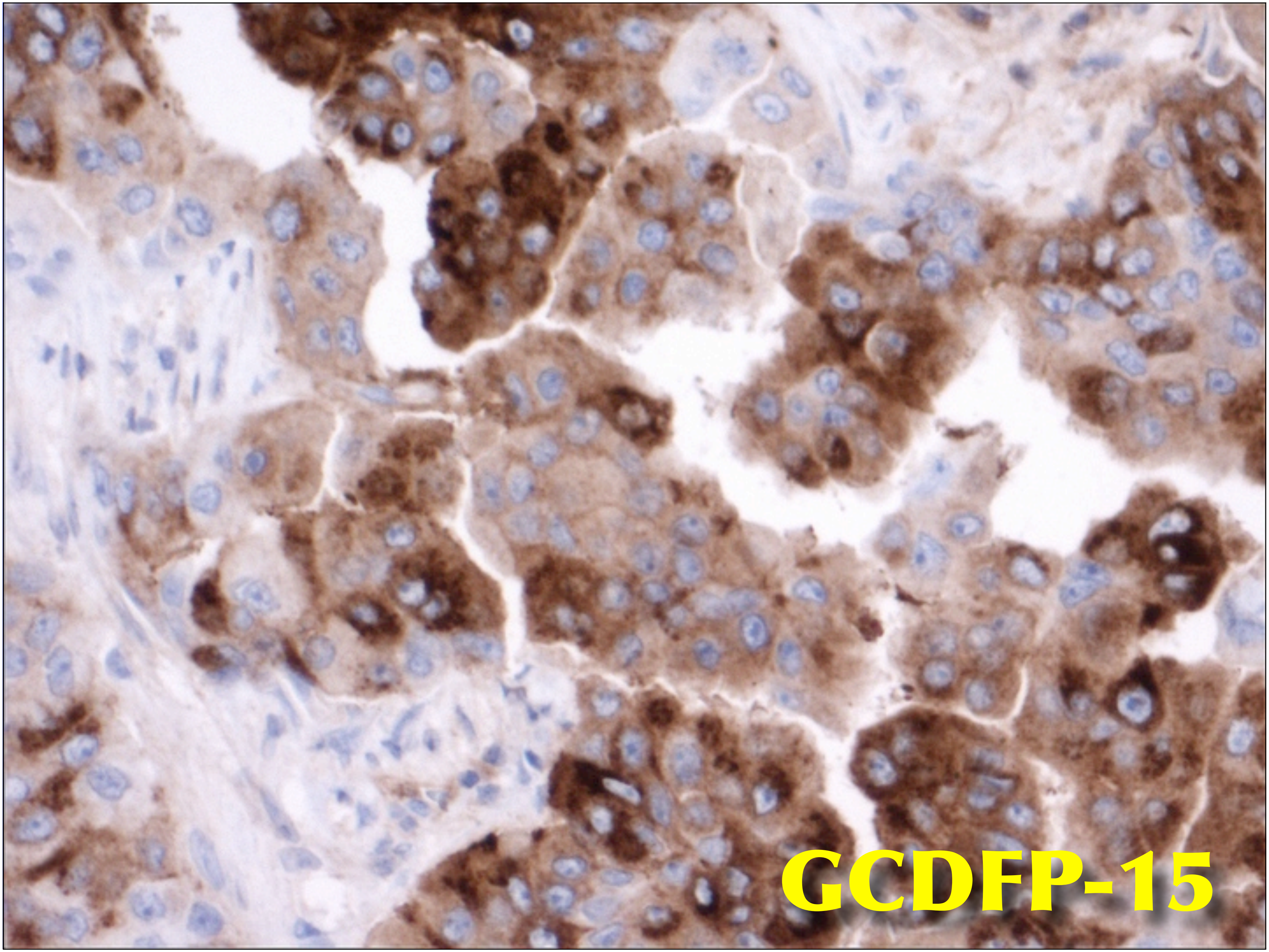
Mammaglobin

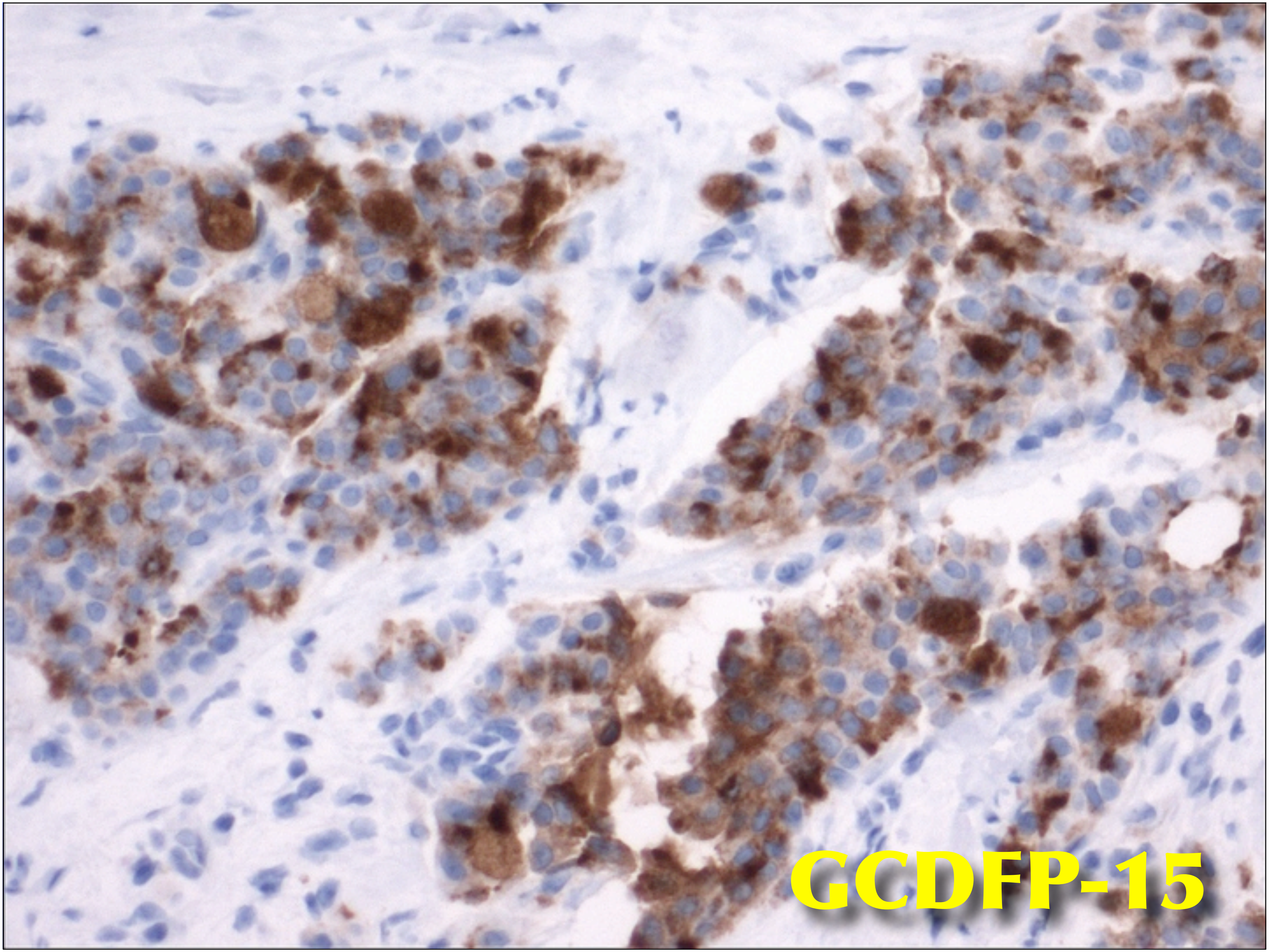
- 10 kd glycoprotein identified by differential screening techniques
- Function unknown
- Expression highly restricted to breast cancers
- Watson MA et al (Cancer Res 59:3028-31, 1999) showed relatively high levels of expression in >80% of breast cancers

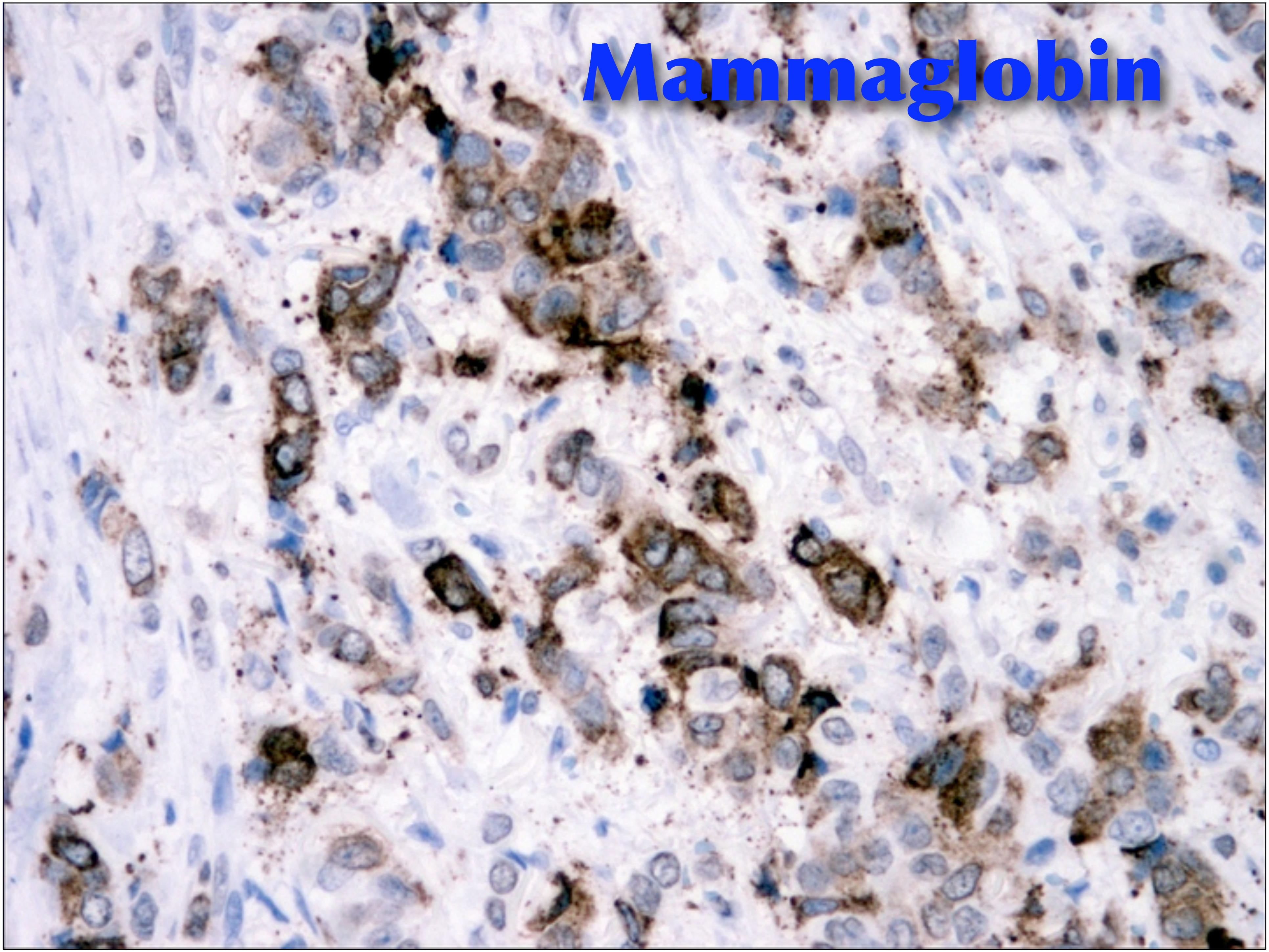
Previously Published Sensitivity Studies

	GCDFP-15	*Mammaglobin*
Majouzian et al. 1989 N=562	**55%** Rabbit Polyclonal	N.D.
Bhargava et al. 2007 N=121	**23.1%** 23A3	**55.4%** 31A5
Sasaki et al. 2007 N=238	N.D.	**48%** 304-1A5
Fritzsche et al. 2007 N=165	**73.3%** D6	**72.1%** CU-18
Takeda et al. 2008 N=20	**45%** D6	**50%** 304-1A5




GCDFP-15

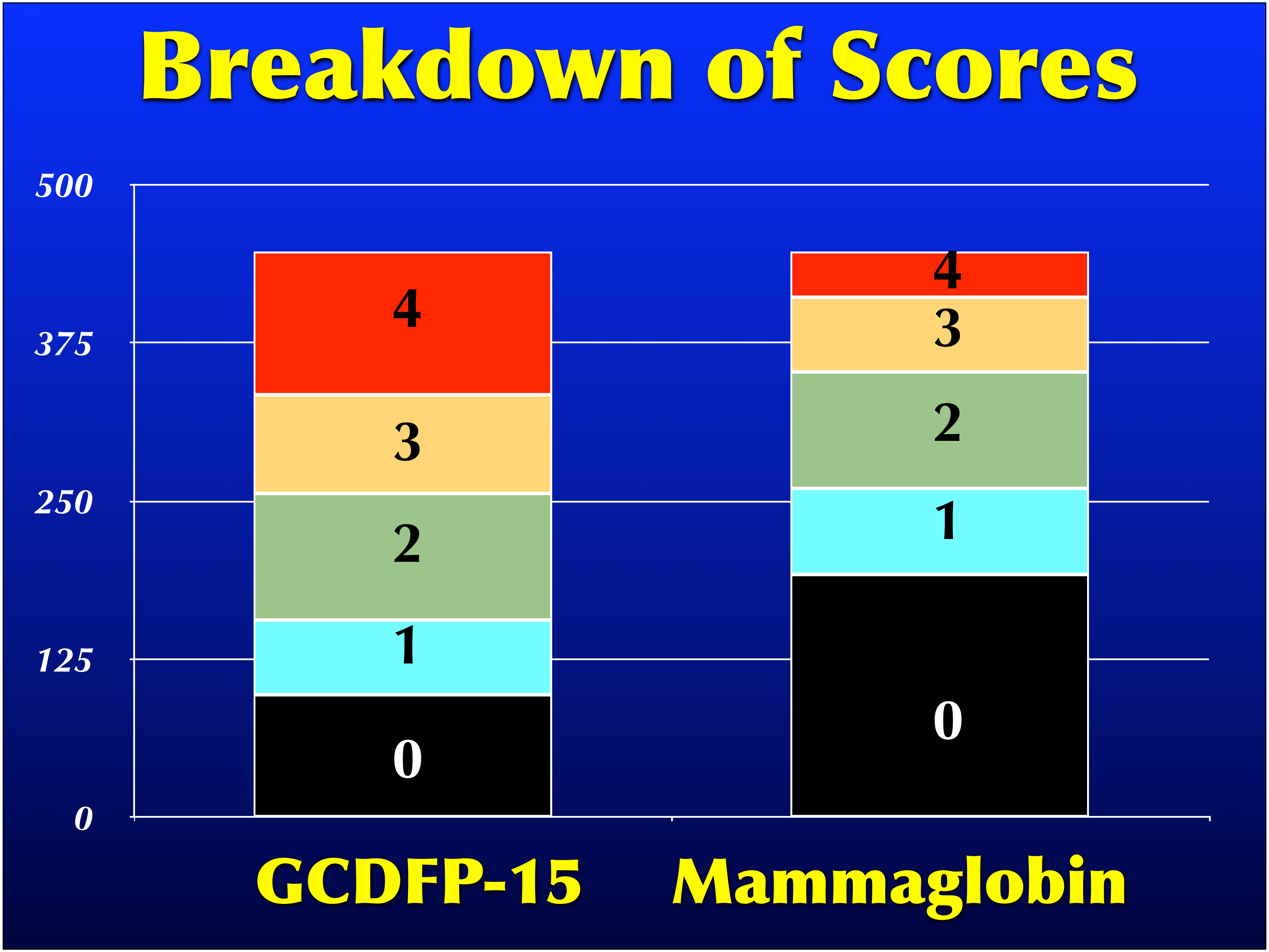

GCDFP-15


Mammaglobin

Mammaglobin v. GCDFP-15

Shaw A, et al., USCAP '09

N=447	Mammaglobin Positive	Mammaglobin Negative
GCDFP-15 Positive	223 (49.9%)	127 (28.4%)
GCDFP-15 Negative	32 (7.2%)	65 (14.5%)


Breakdown of Scores
500
375
250
125
0
4
3
2
1
0
4
3
2
1
0
GCDFP-15
Mammaglobin

Mammaglobin v GCDFP-15

- Overall sensitivity of GCDFP-15 alone **78.3%**
- Overall sensitivity of mammaglobin alone **57.0%**
- 32/447 (7.2%) cases were GCDFP-15 negative and mammaglobin positive
- **Combined sensitivity of 86%**

Percentage of non-breast primary carcinomas positive

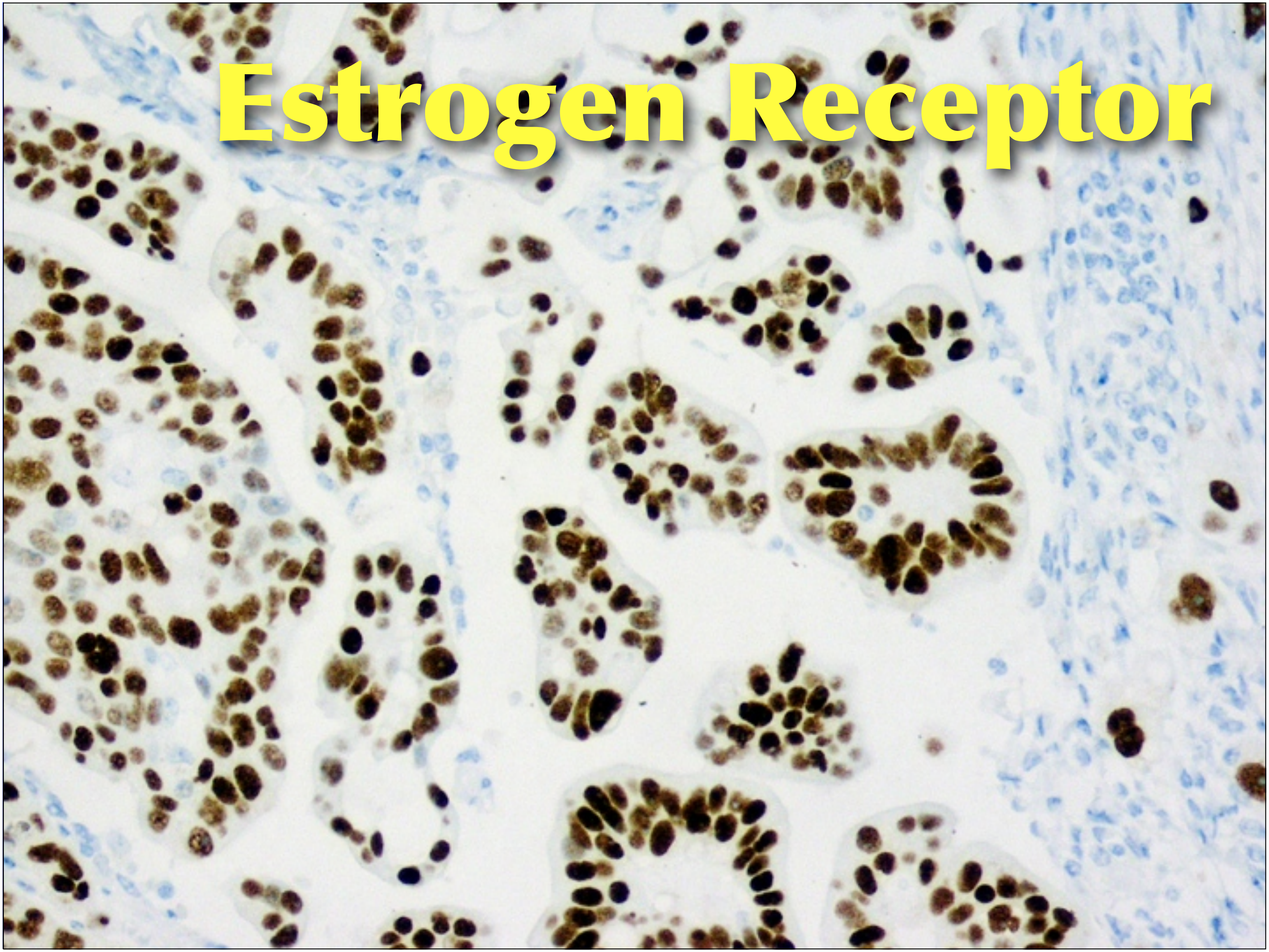
	GCDFP-15	*Mammaglobin*
Lung	4/30 (13.3%)	0/30 (0%)
Ovarian	3/30 (10%)	4/30 (13.3%)
Colorectal	0/30 (0%)	0/30 (0%)
Pancreatic	1/10 (10%)	1/10 (10%)
Salivary	4/8 (50%)	4/8 (50%)
Stomach	0/58 (0%)	0/58 (0%)
Adnexal	17/78 (21.8%)	17/76 (22.4%)
OVERALL SPECIFICITY	**88%**	**89%**

Breast-"Specific" Markers

- There is no breast-specific marker that cannot also be expressed by sweat gland tumors
- ER, PR, GCDFP-15, mammaglobin, etc.

What about Estrogen Receptor?

- Subset of carcinomas can manifest ER/PR expression
- Even in “positive tumors” only a subset actually positive (e.g., breast, endometrium)
- Most useful in restricted clinical settings (e.g., breast vs. lung)


Estrogen Receptor

ER-positive

(Sometimes)

- Breast carcinoma
- Ovarian carcinoma
- Endometrial adenocarcinoma
- Cervical squamous cell carcinoma
- Sweat gland carcinoma
- Thyroid carcinoma
- Neuroendocrine carcinoma

ER-negative

(Almost always)

Using sensitive techniques and antibodies ~7% of lung cancers ER-positive (Hing AW et al., USCAP 2004)

Appl Immunohistochem Mol Morphol 14:83-7, 2006

Immunohistochemical Expression of Estrogen Receptor in Pulmonary Adenocarcinoma

Sean K. Lau, MD, Peiguo G. Chu, MD, PhD, and Lawrence M. Weiss, MD

	ER	TTF-1
Lung adenocarcinoma	10/55 **(18%)**	46/55 **(84%)**
Breast carcinoma	36/50 **(72%)**	0/55 **(0%)**

ER Expression in Lung Cancers

Almost always <50%, usually <25% of cells positive

TABLE 2. Features of ER-Positive Lung Adenocarcinomas

				ER Immuno-reactivity		TTF-1 Immuno-reactivity	
Pt. No.	Sex	Age	History of Breast Carcinoma	% of Reactive Cells	Intensity	% of Reactive Cells	Intensity
1	M	76	No	5–25	1+	> 75	3+
2	F	64	No	5–25	2+	> 75	3+
3	F	75	No	51–75	3+	> 75	3+
4	F	74	Yes	< 5	1+	> 75	3+
5	F	77	No	< 5	1+	> 75	3+
6	M	65	No	5–25	2+	> 75	3+
7	F	57	No	5–25	1+	> 75	3+
8	F	69	No	51–75	3+	> 75	3+
9	F	80	No	5–25	2+	> 75	3+
10	F	63	Yes	26–50	2+	> 75	3+

Lau SK et al., Appl Immunohistochem Mol Morphol 14:83-7, 2006

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Selected Topics

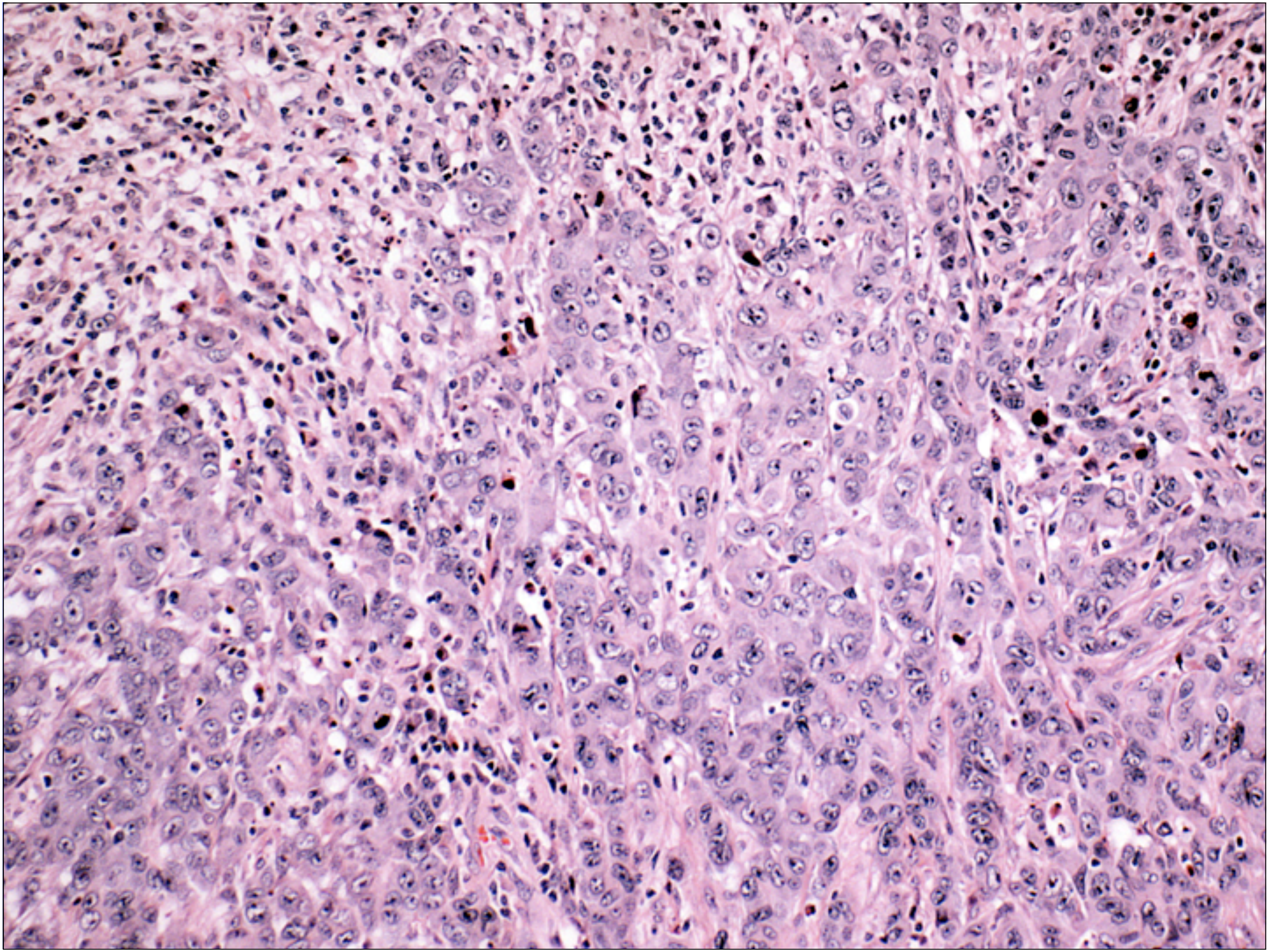
- General Issues
- Breast Carcinoma
- GI Tract Tumors
- Tumors in the Liver
- Male GU Tract Tumors

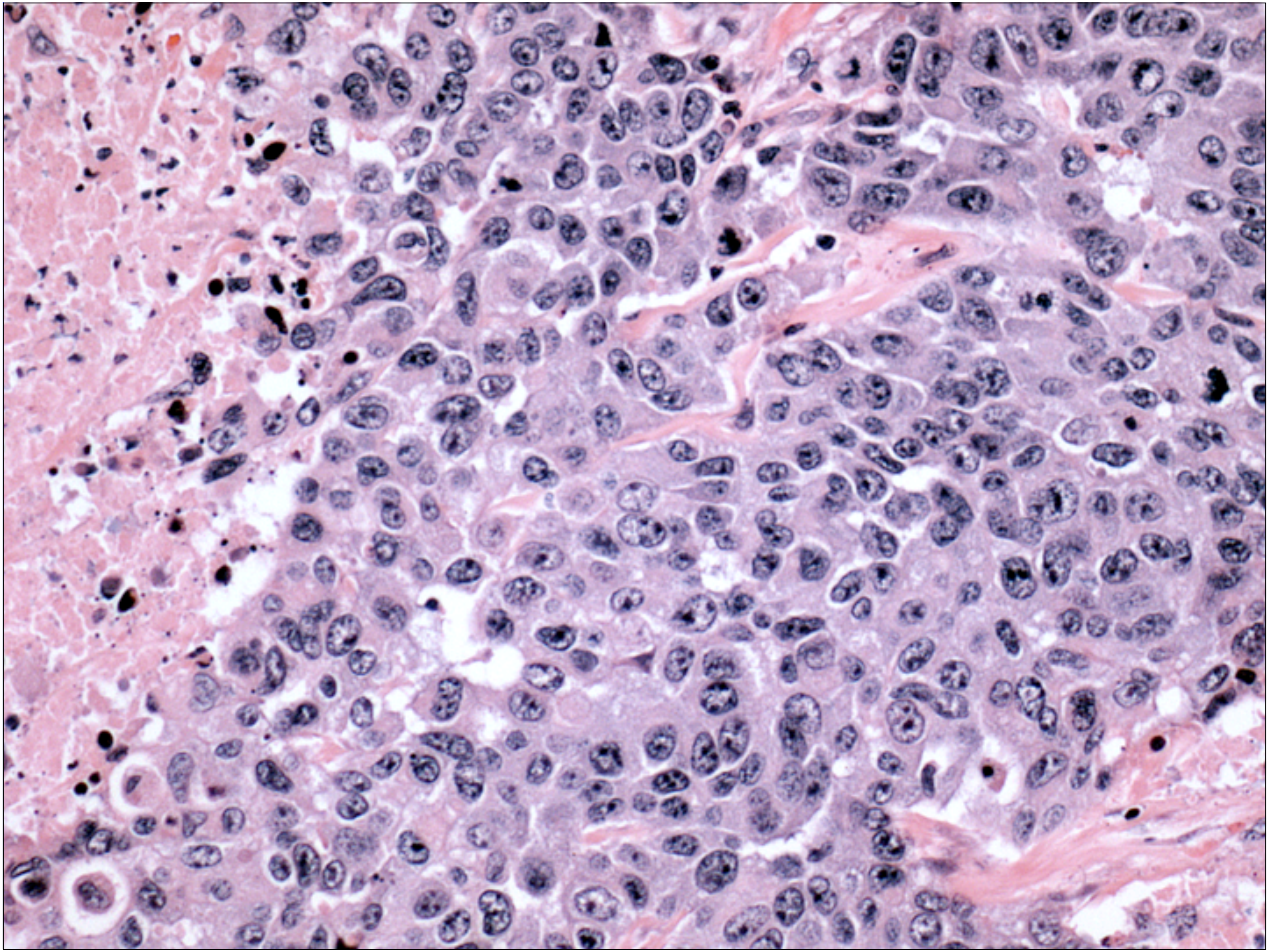
GI Tract

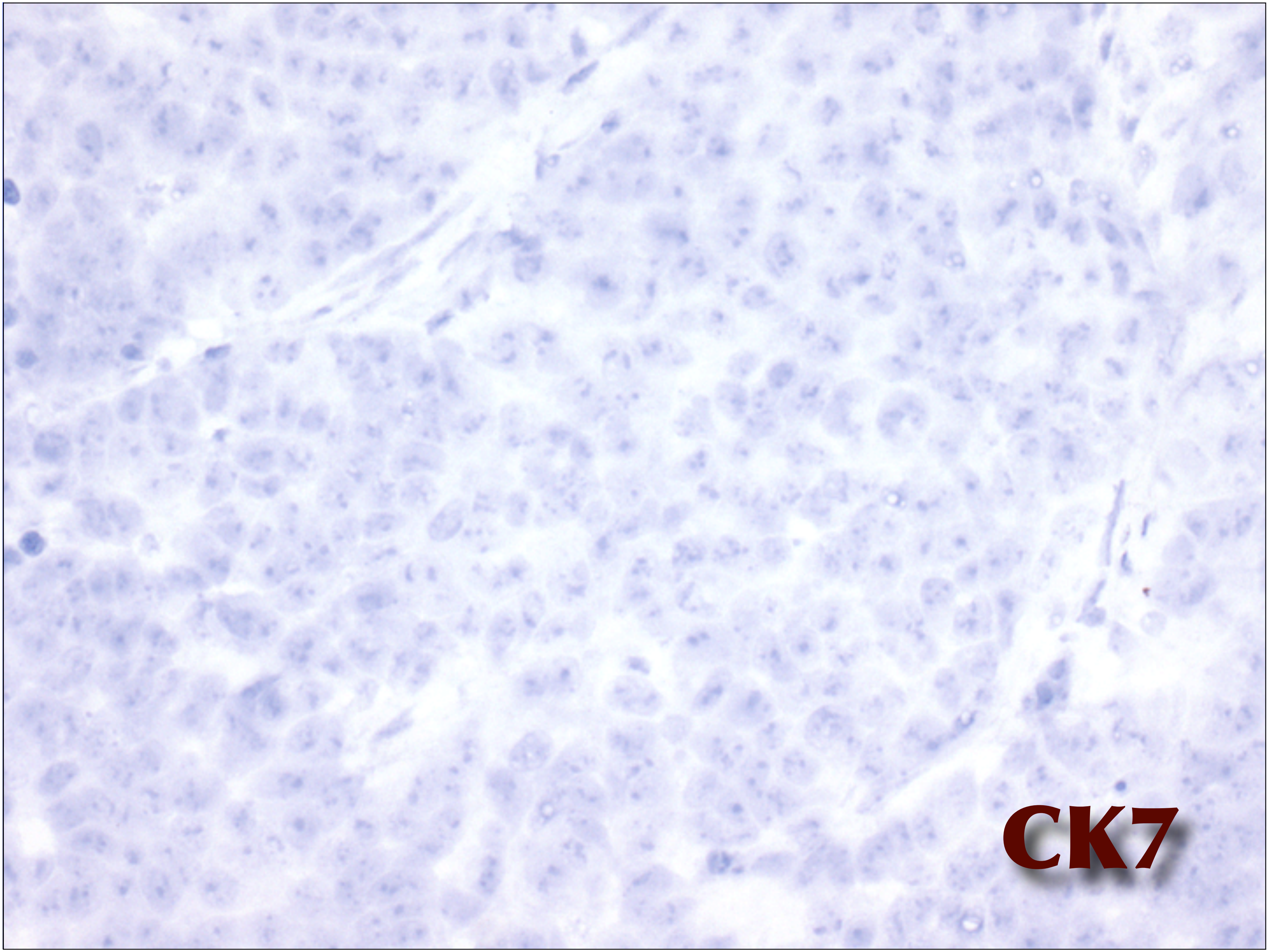
Loss of MMR Enzymes in Colorectal Adenocarcinoma

Case 4

63 year old female with 8 cm right colonic mass



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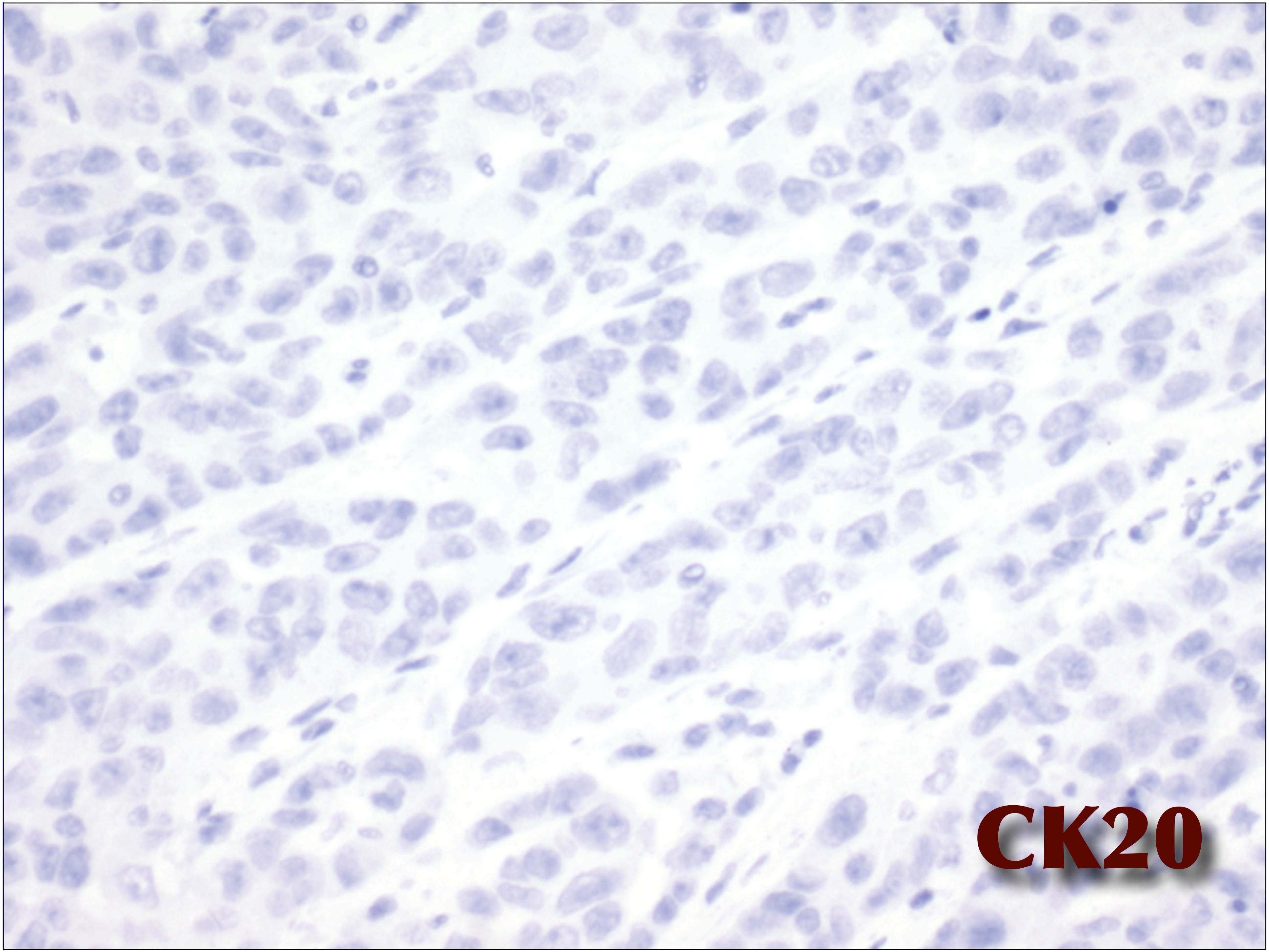
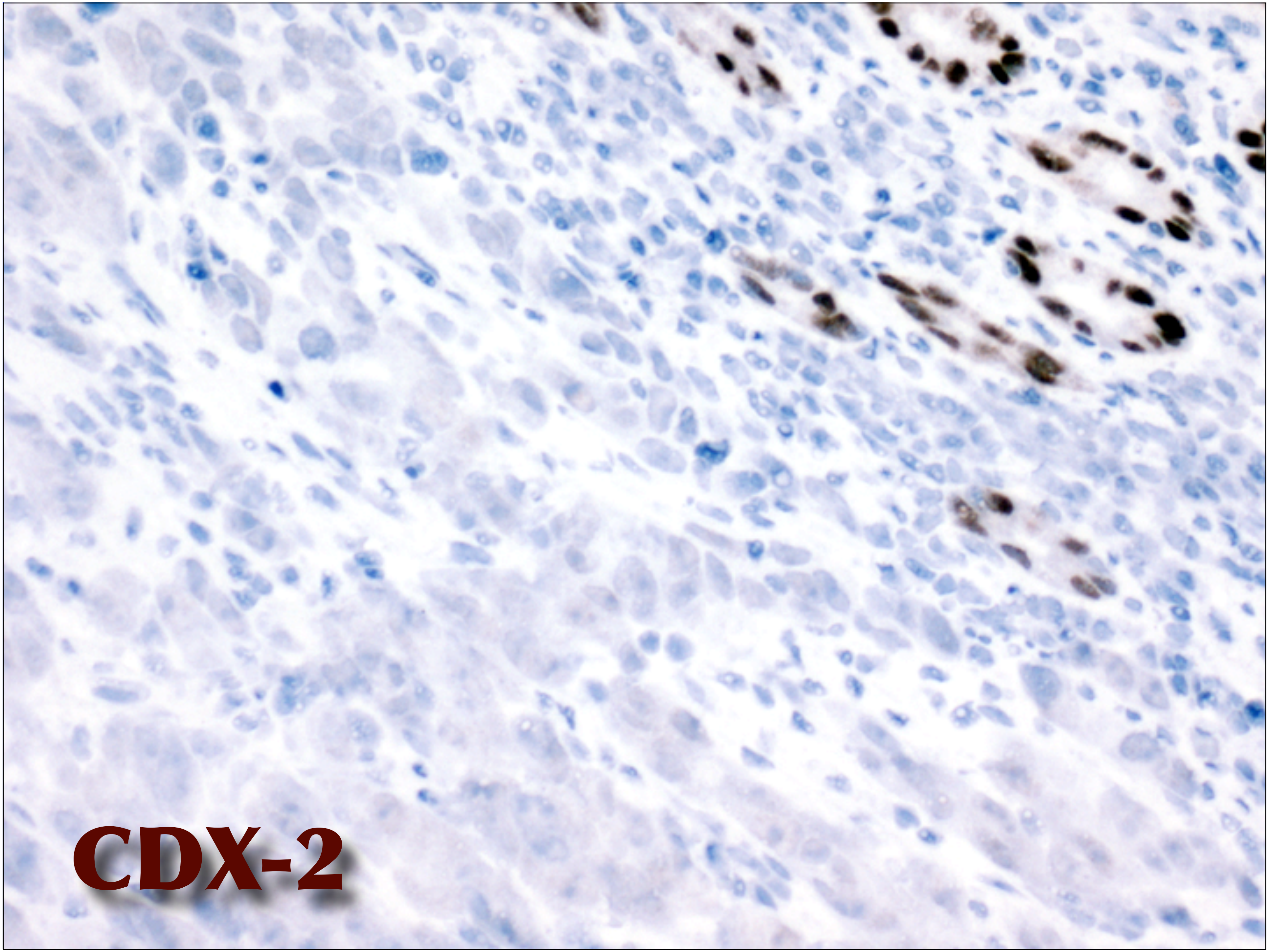

CK20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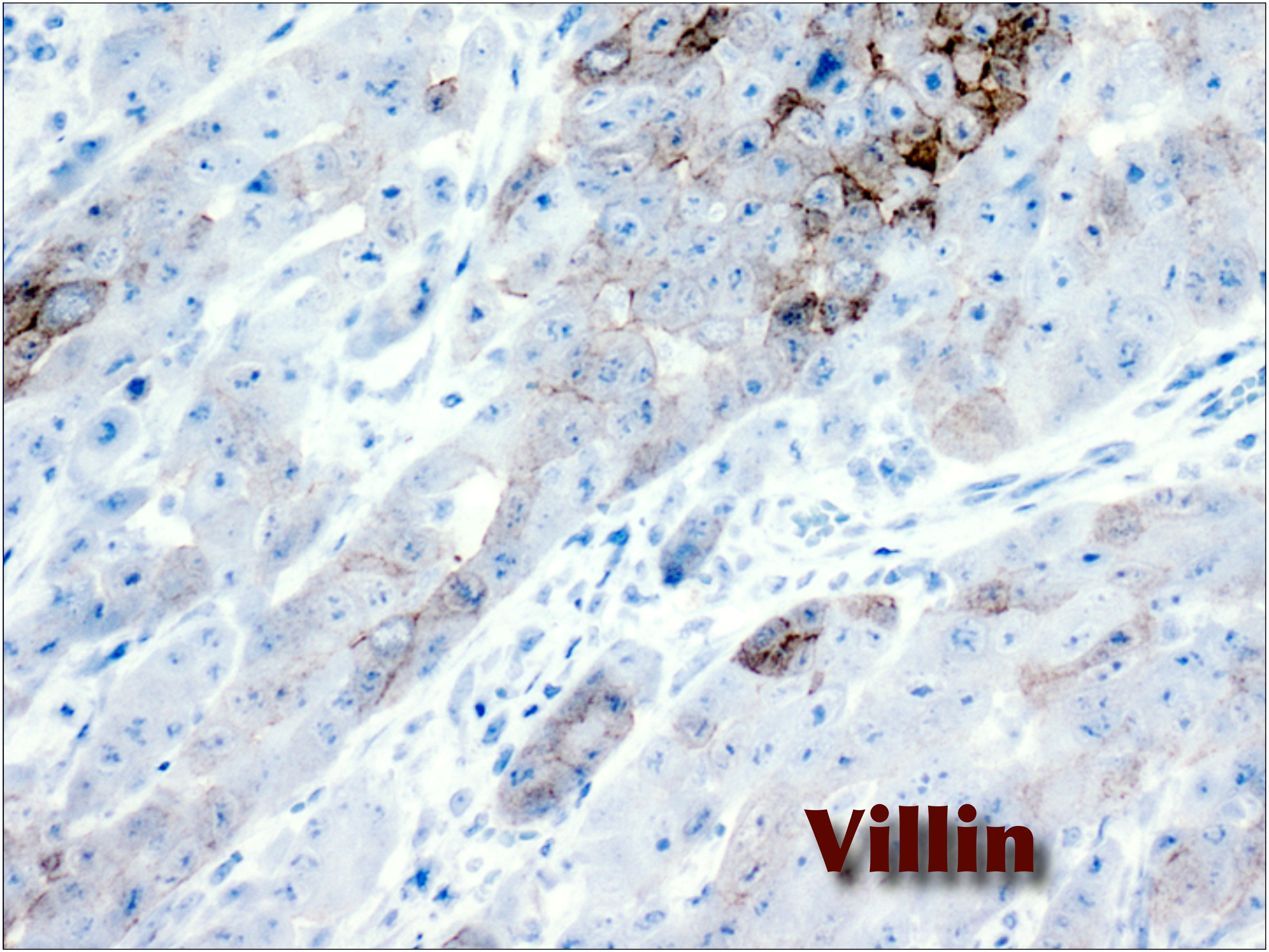
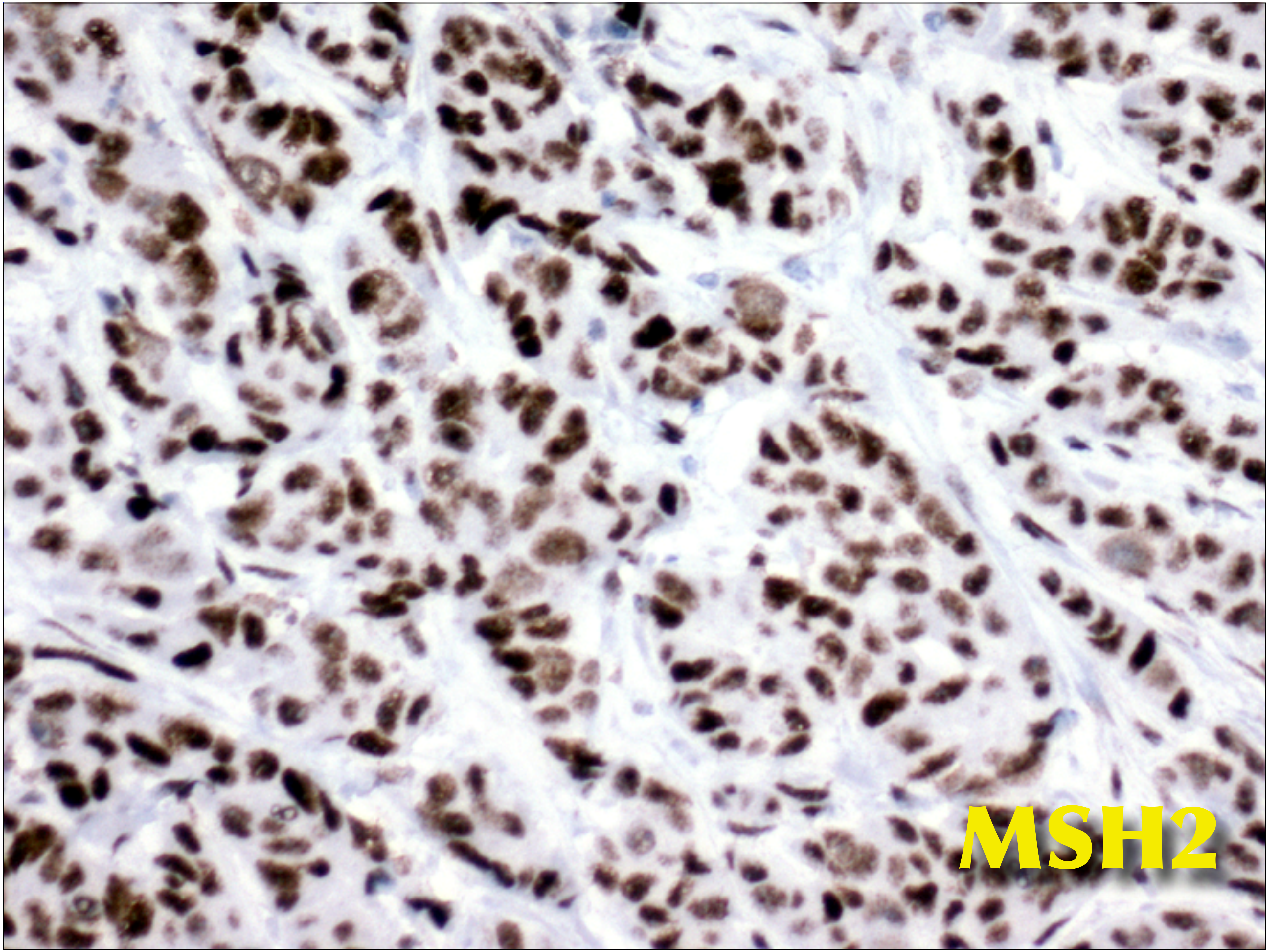
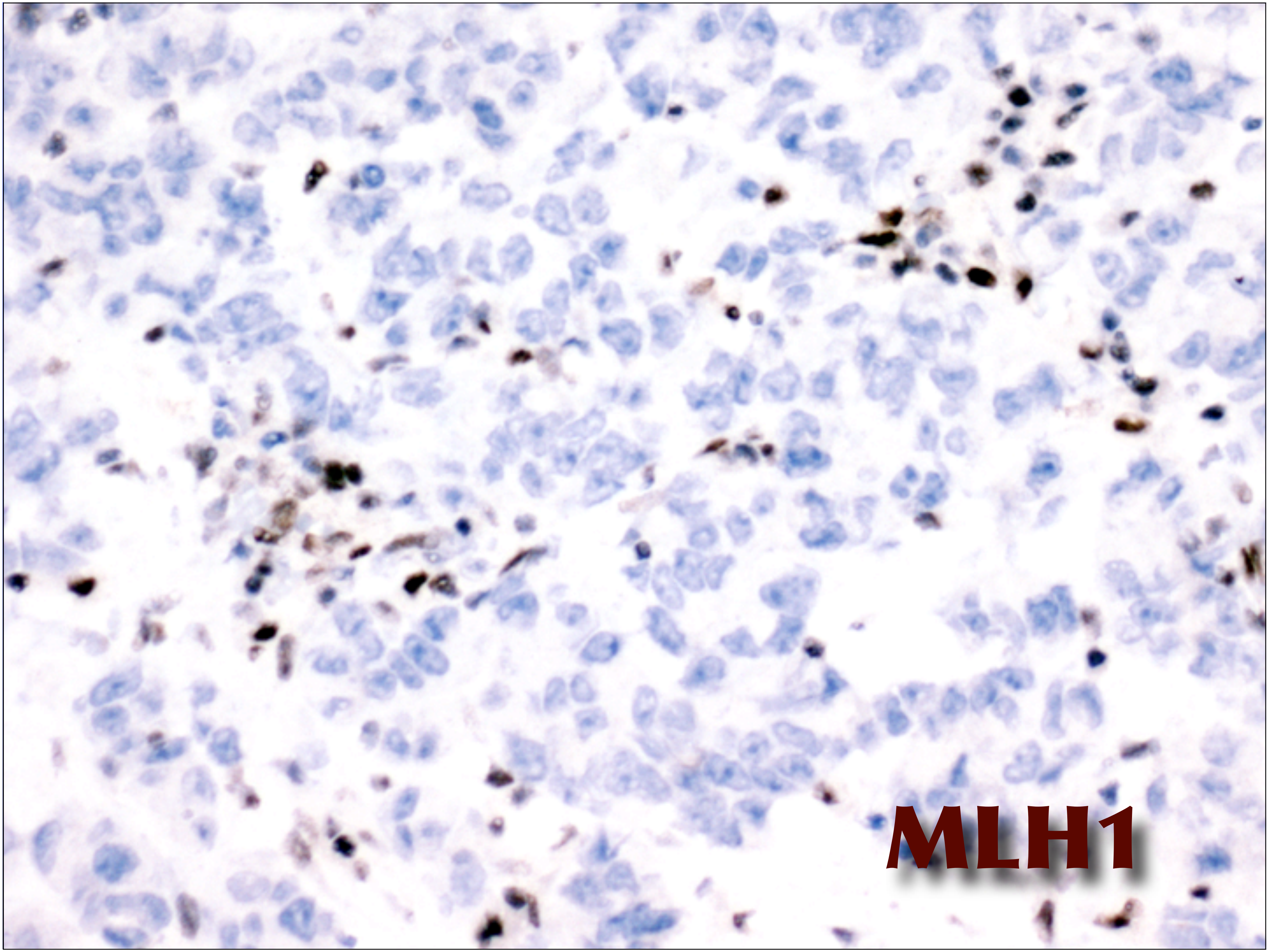

Villin


MSH2


MLH1

FINAL DX:

Primary colorectal adenocarcinoma, MSI type

Microsatellite DNA

- Repetitive sequences of 1-6 bases scattered throughout genome
- Most commonly $(CA)_n$
- Replication machinery slips more frequently on repetitive (vs. nonrepetitive) sequences
- Microsatellites accumulate mutations in MMR-deficient cells, resulting in microsastellite instability (MSI)

DNA Polymerase Errors and Mismatch Repair

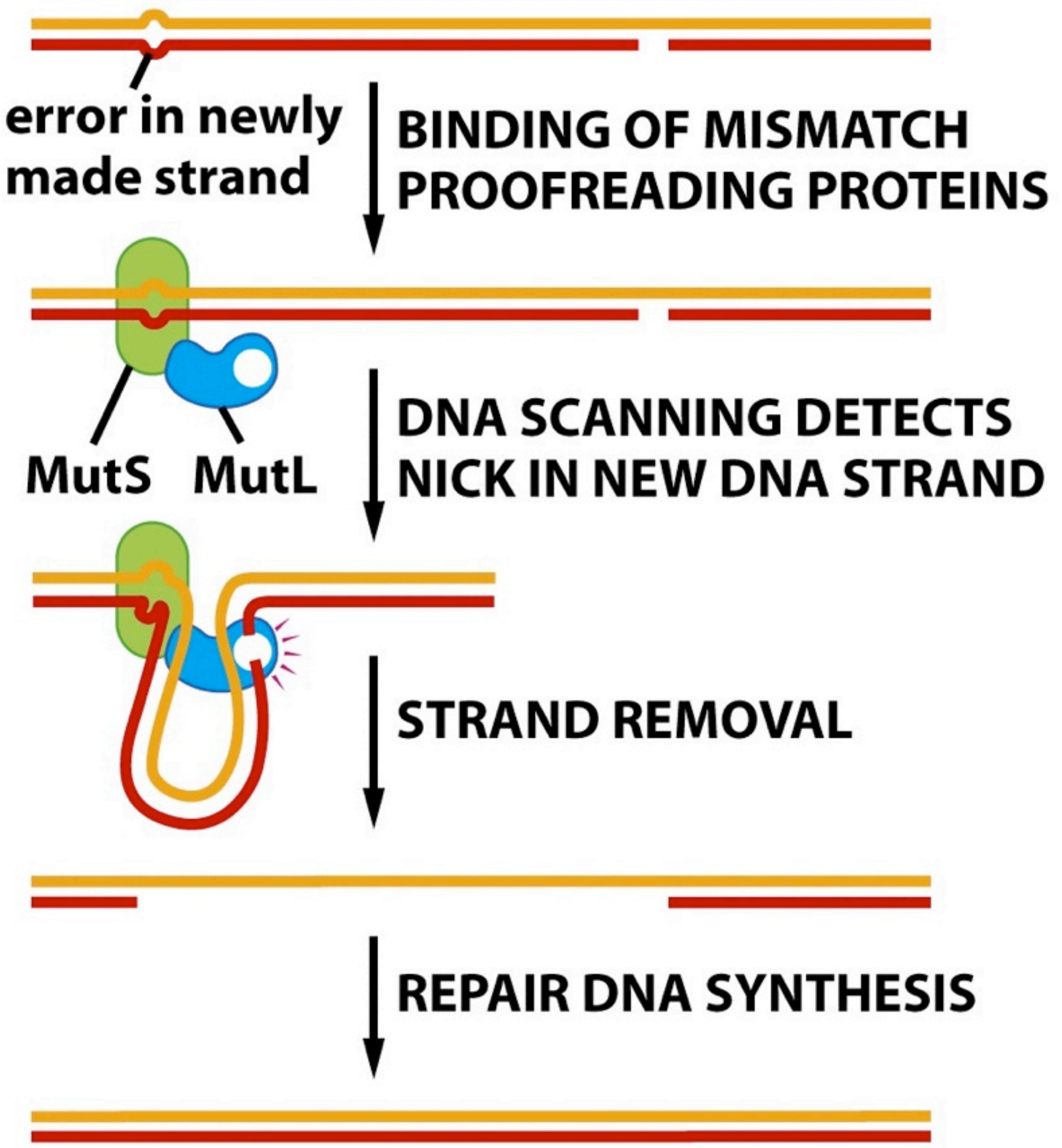


Figure 12-8c The Biology of Cancer (© Garland Science 2007)

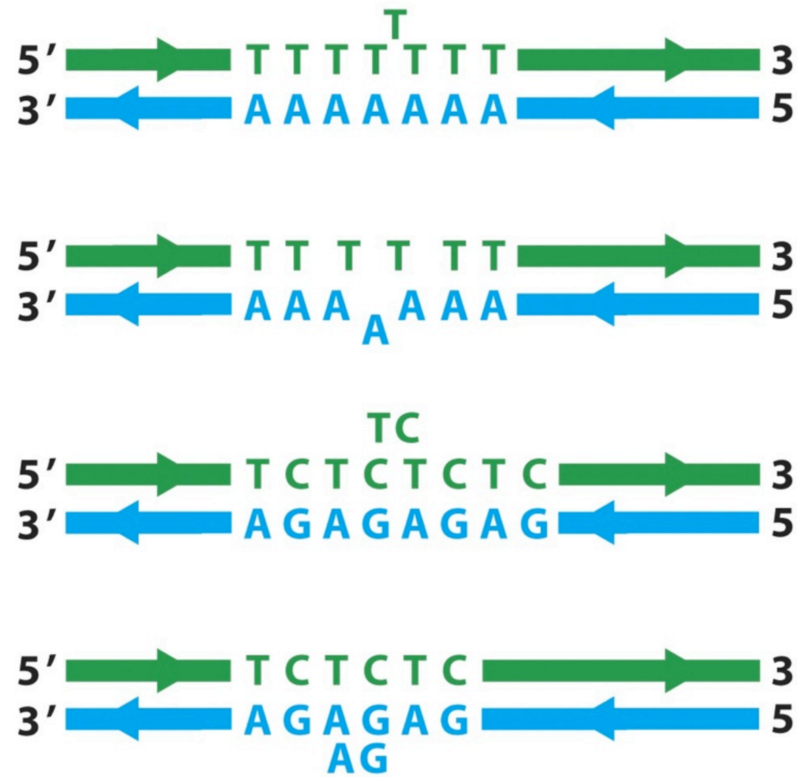
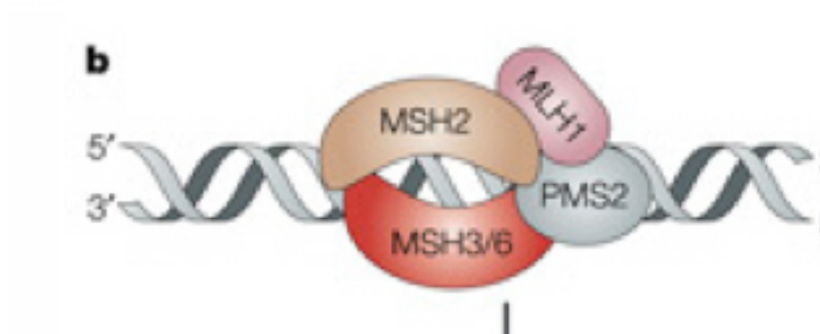


Figure 12-8a The Biology of Cancer (© Garland Science 2007)



DNA Mismatch Repair System

- MLH1
- PMS2
- MLH2
- MSH6

HNPCC (Lynch Syndrome)

Hereditary Non-polyposis Colorectal Cancer

- Autosomal dominant
- Accounts for 2-5% of colorectal adenocarcinoma
- Tumors develop at early age, usually found on right side
- Also develop endometrial adenocarcinoma
- Synchronous and metachronous colorectal cancers: 40% develop within 10 years without total colonic resection

HNPCC (Lynch Syndrome)

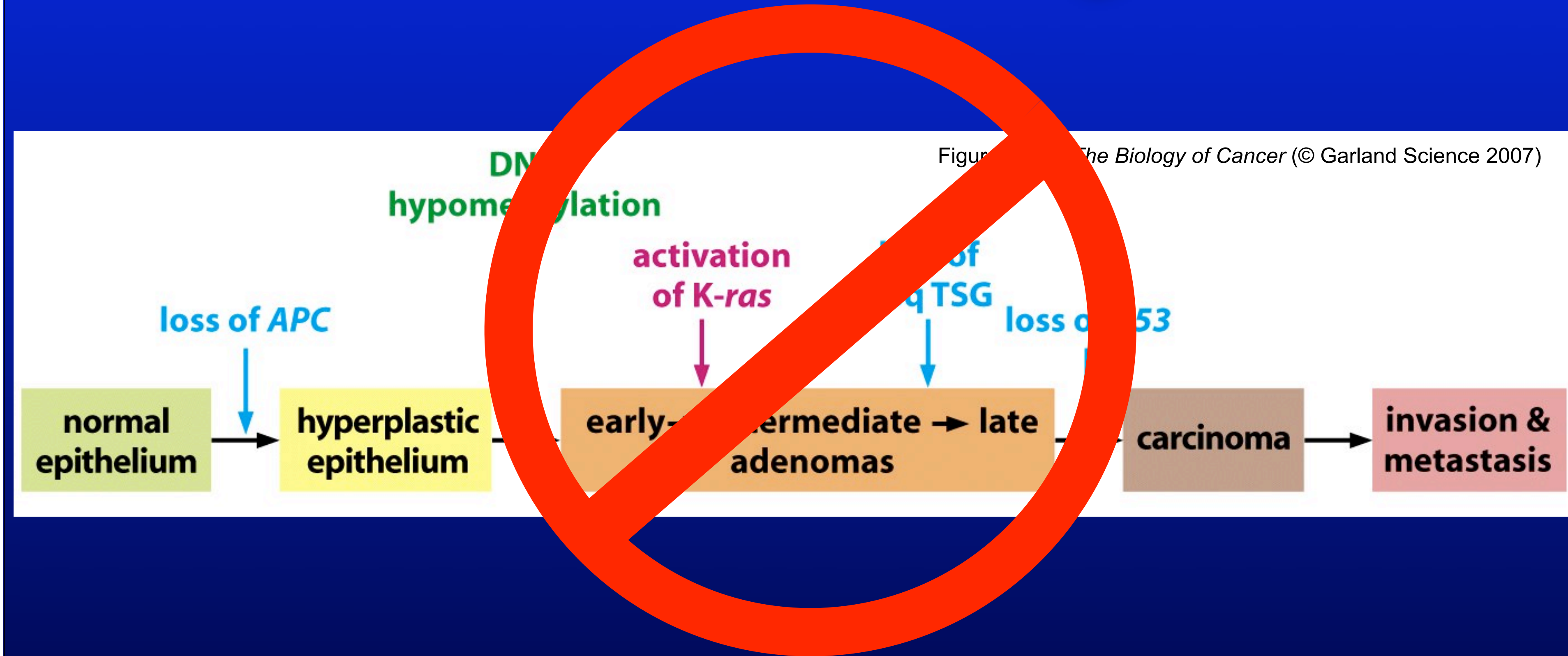
Hereditary Non-polyposis Colorectal Cancer

- Vast majority have germline mutations in hMSH2, hMLH1, or hMSH6 genes
- Second functional copy of gene may be inactivated by allele loss, hypermethylation of promoter region, or further mutation

Two Reasons to Do IHC for MMR Enzyme Loss

- Screen for HNPCC (Lynch Syndrome)
- Look for sporadic MSI tumors

Classical ‘Vogelstein’ Pathway of Colonic Adenocarcinoma Progression



Lindor NM et al. J Clin Oncol 20:1043-8, 2002

- 350 classified as MSI-H by MSI testing
- 323 showed absence of either MLH1(70.6%) or MSH2 (29.4%) by IHC
- IHC sensitivity 92.3%
- IHC specificity 100%
- Predictive value of normal expression of both proteins for MSS/MSI-L status 96.7
- IHC testing much more rapid, less expensive, useful in small samples, and can guide genetic testing

Rigau V et al. Arch Pathol Lab Med 127:694-700, 2003

- Loss of expression of at least 1 protein present in 17% of cases
- 100% of MSI-H tumors showed expression of either hMLH1, hMSH2, or hMSH6
- Loss of expression of 2 proteins present in 59.4% of cases (hMLH1/hPMS2 and hMSH2/hMSH6)
- Isolated loss of hMSH6 in 6 cases

Rigau V et al. Arch Pathol Lab Med 127:694-700, 2003

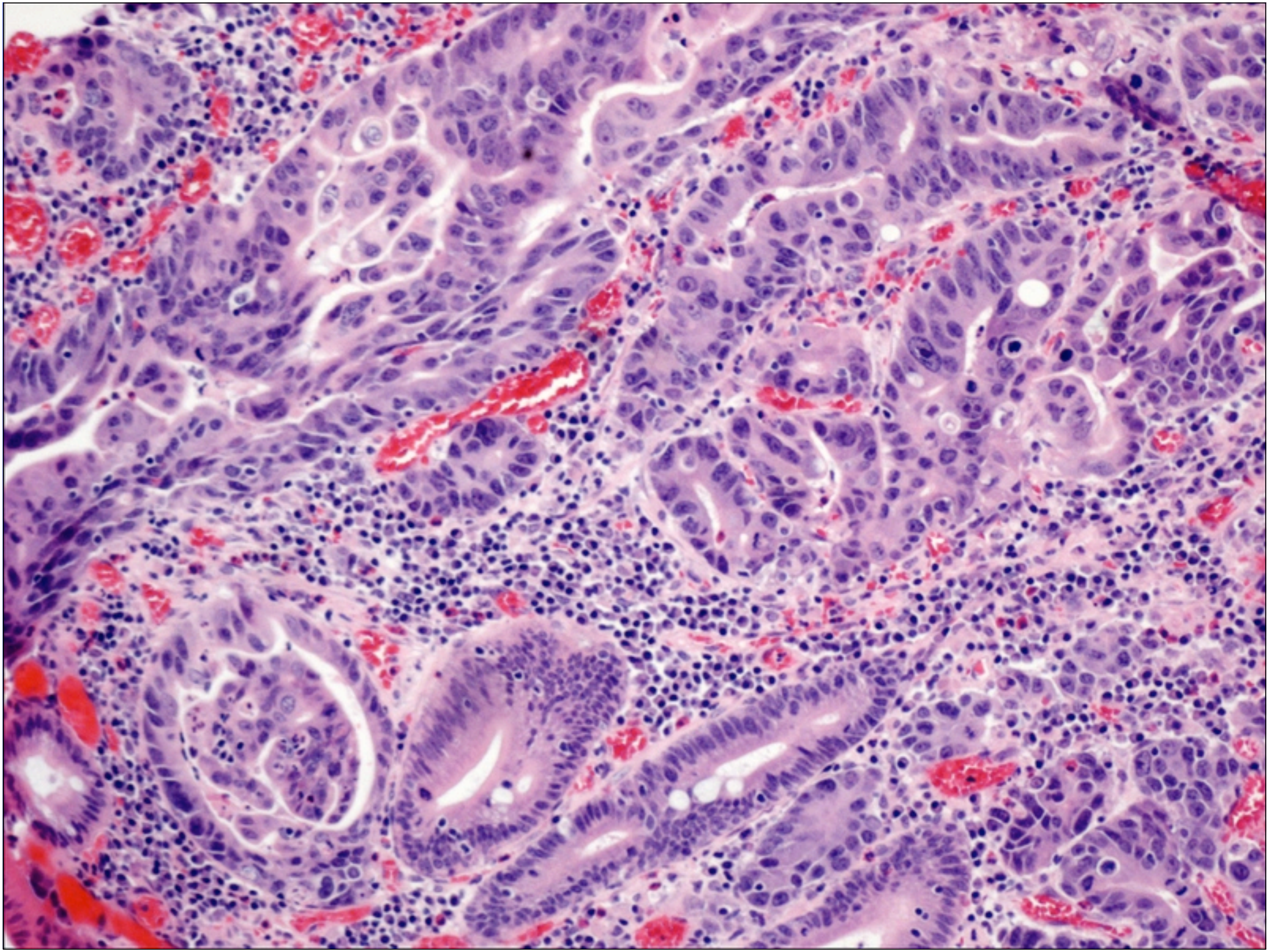
Table 3. Results of Immunohistochemistry of Mismatch Repair (MMR) Proteins to Assess Microsatellite Instability in Previously Documented Series and in the Present Series*

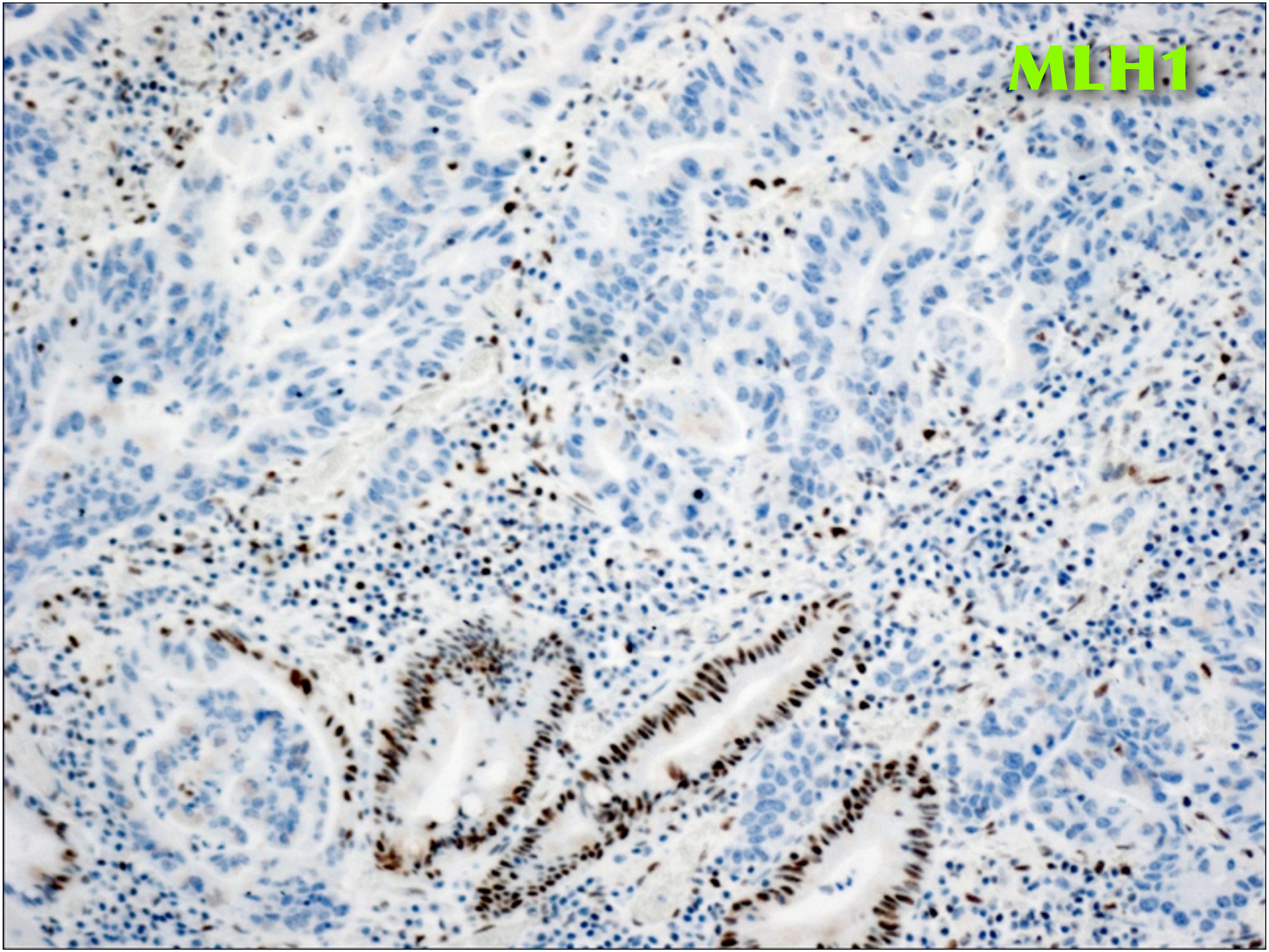
Reference, y	No. of Cases	MMR Proteins Studied by Immunohistochemistry	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %
Thibodeau et al,[16] 1996	188	hMLH1, hMSH2	95	100	100	99
Dietmaier et al,[5] 1997	58	hMLH1, hMSH2	93	100	100	98
Cawkwell et al,[17] 1999	502	hMLH1, hMSH2	100	100	100	100
Marcus et al,[18] 1999	72	hMLH1, hMSH2	97	100	100	97
Chaves et al,[19] 2000	76	hMLH1, hMSH2	75	95	66	97
Cawkwell et al,[20] 2000	46	hMLH1, hMSH2	100	100	100	100
Dieumegard et al,[21] 2000	31	hMLH1, hMSH2	77	100	100	85
Jass,[22] 2000	83	hMLH1, hMSH2	96	100	100	98
Iino et al,[24] 2000	129	hMLH1, hMSH2	94	96	98	96
Ward et al,[9] 2001	310	hMLH1, hMSH2	81	99.6	96	98
Young et al,[11] 2001	169	hMLH1, hMSH2, hMSH6, hPMS2	92† 93‡ 96§	NA	NA	NA
Stone et al,[25] 2001	46	hMLH1, hMSH2	96	100	100	96
Salahshor et al,[26] 2001	50	hMLH1, hMSH2	76	NA	NA	NA
Chiaravelli et al,[27] 2001	72	hMLH1, hMSH2	91	100	100	96
Lindor et al,[29] 2002	1144	hMLH1, hMSH2	92	100	100	97
Lanza et al,[31] 2002	305	hMLH1, hMSH2	91	100	100	94
Plaschke et al,[32] 2002	228	hMLH1, hMSH2, hMSH6, hPMS2	84† 95‡ 98§	100† 100‡ 100§	100† 100‡ [illegible]	96† 99‡ 99§
Present series	204	hMLH1, hMSH2, hMSH6, hPMS2	93† 100‡ 100§	100† 100‡ 98§	100† 100‡ [illegible]	99† 100‡ 100§
Total of 16 series‖	**3494**	. . .	**92.4**	**99.6**	**98.5**	**97.8**

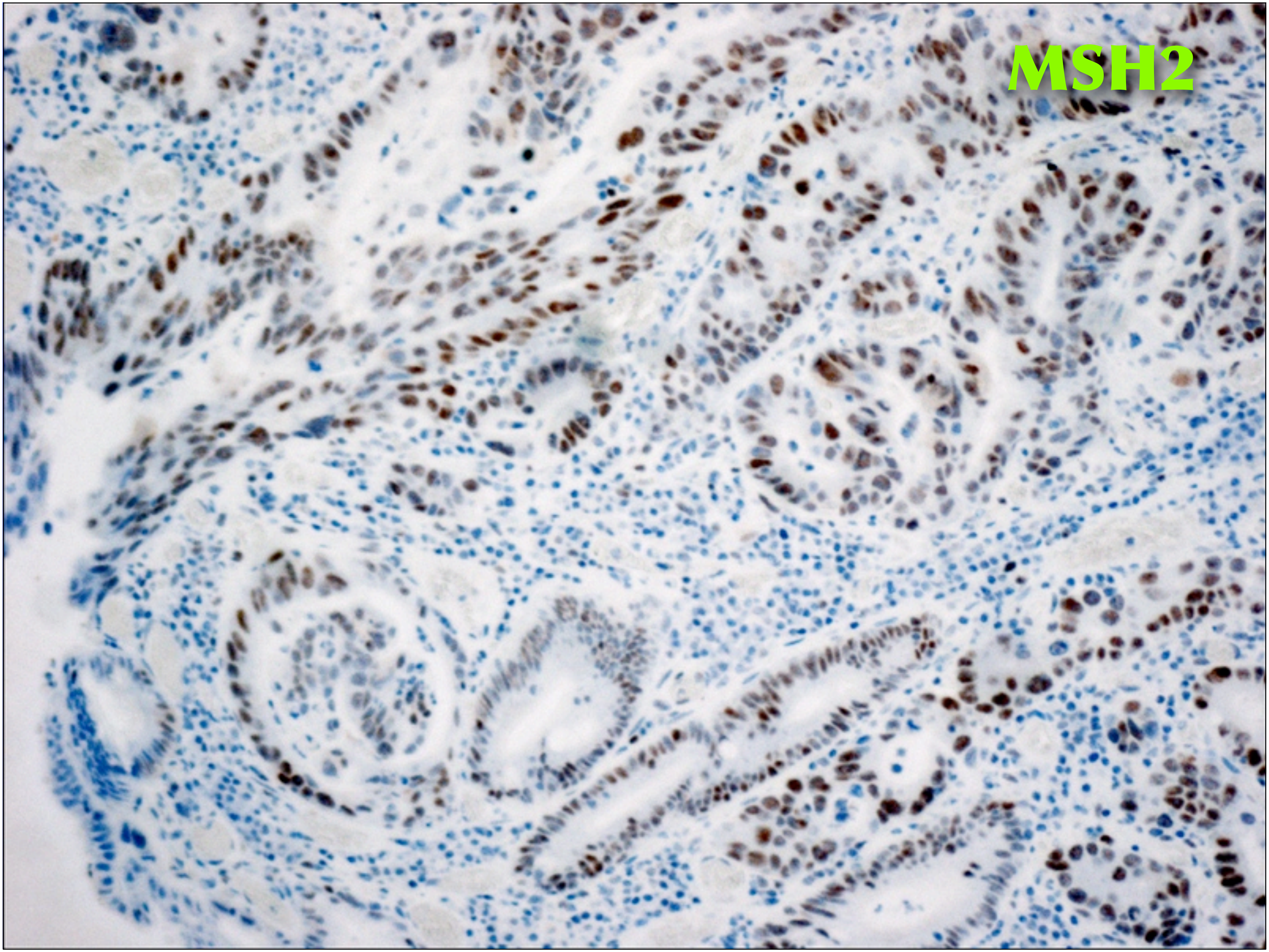
* NA indicates not available.

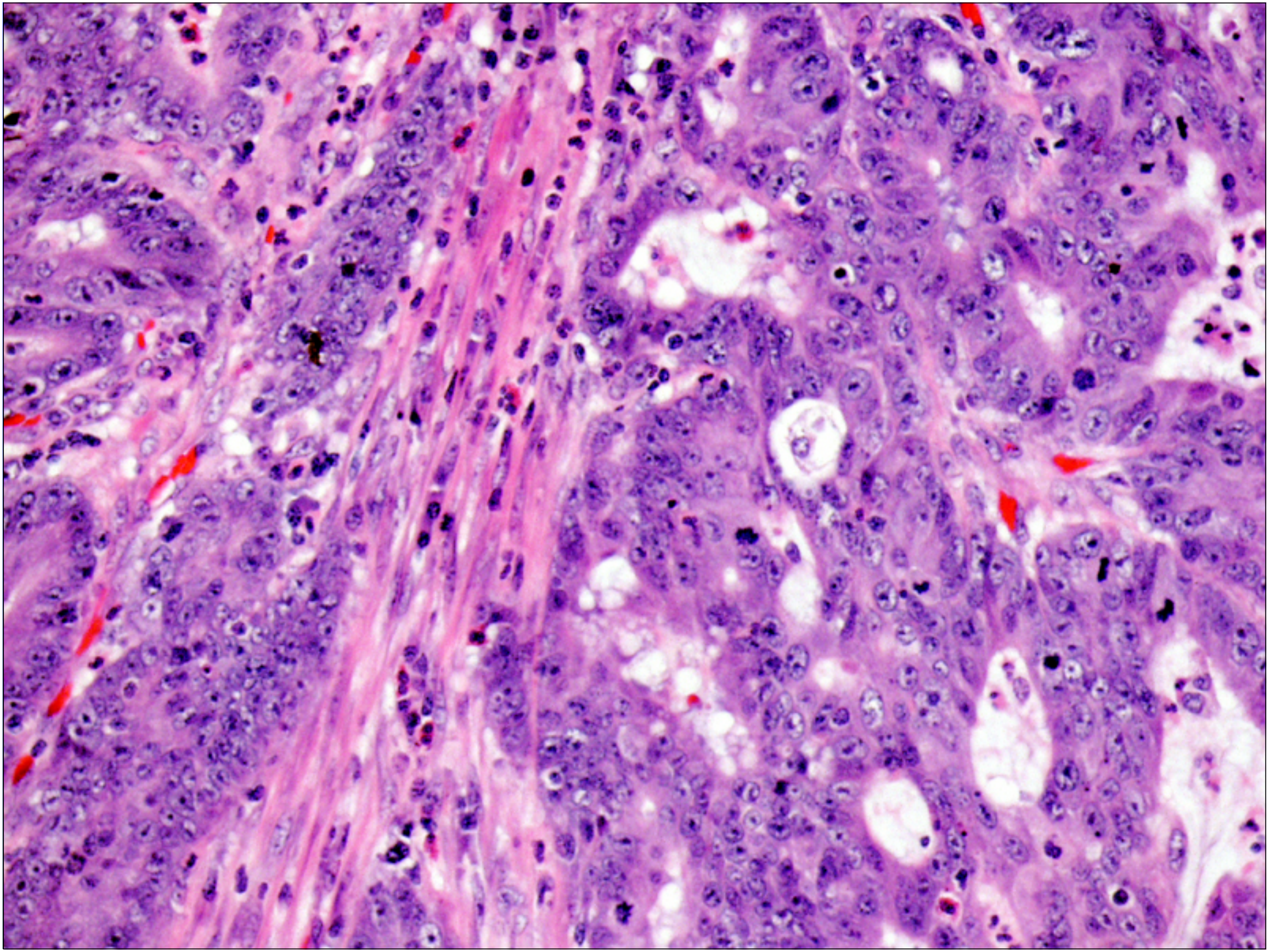
Are MSI-H tumors distinct?

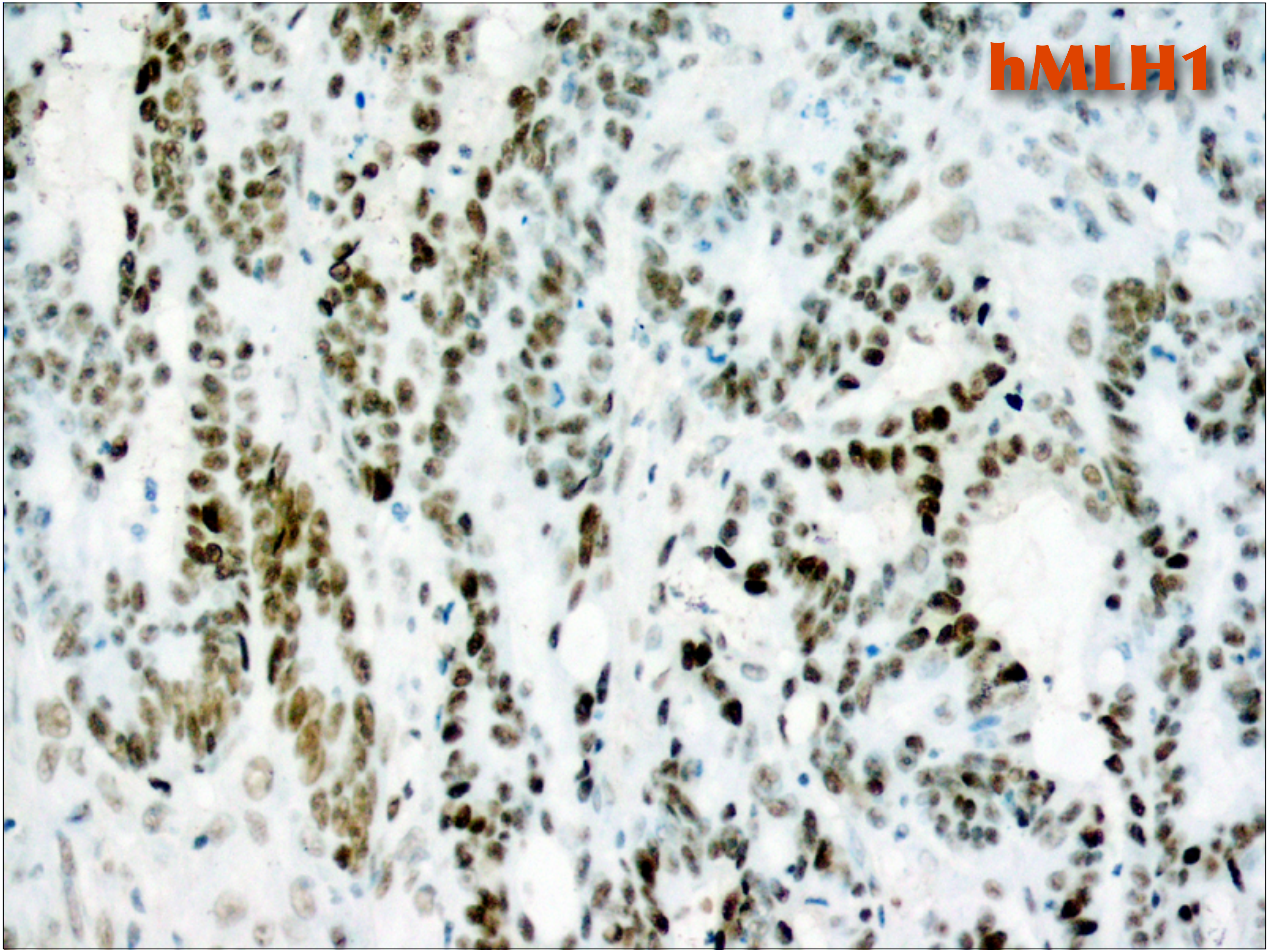
- MSI-H tumors more likely arise on the right side
- MSI-H tumors more likely to occur in people with positive family history of colorectal cancer
- MSI-H tumors more likely to be cribriform, solid, signet ring, high grade ('medullary'), mucinous
- Lymphocytic infiltration most important feature for predicting MSI-H (nodular "Crohn-like" peritumoral or TIL)

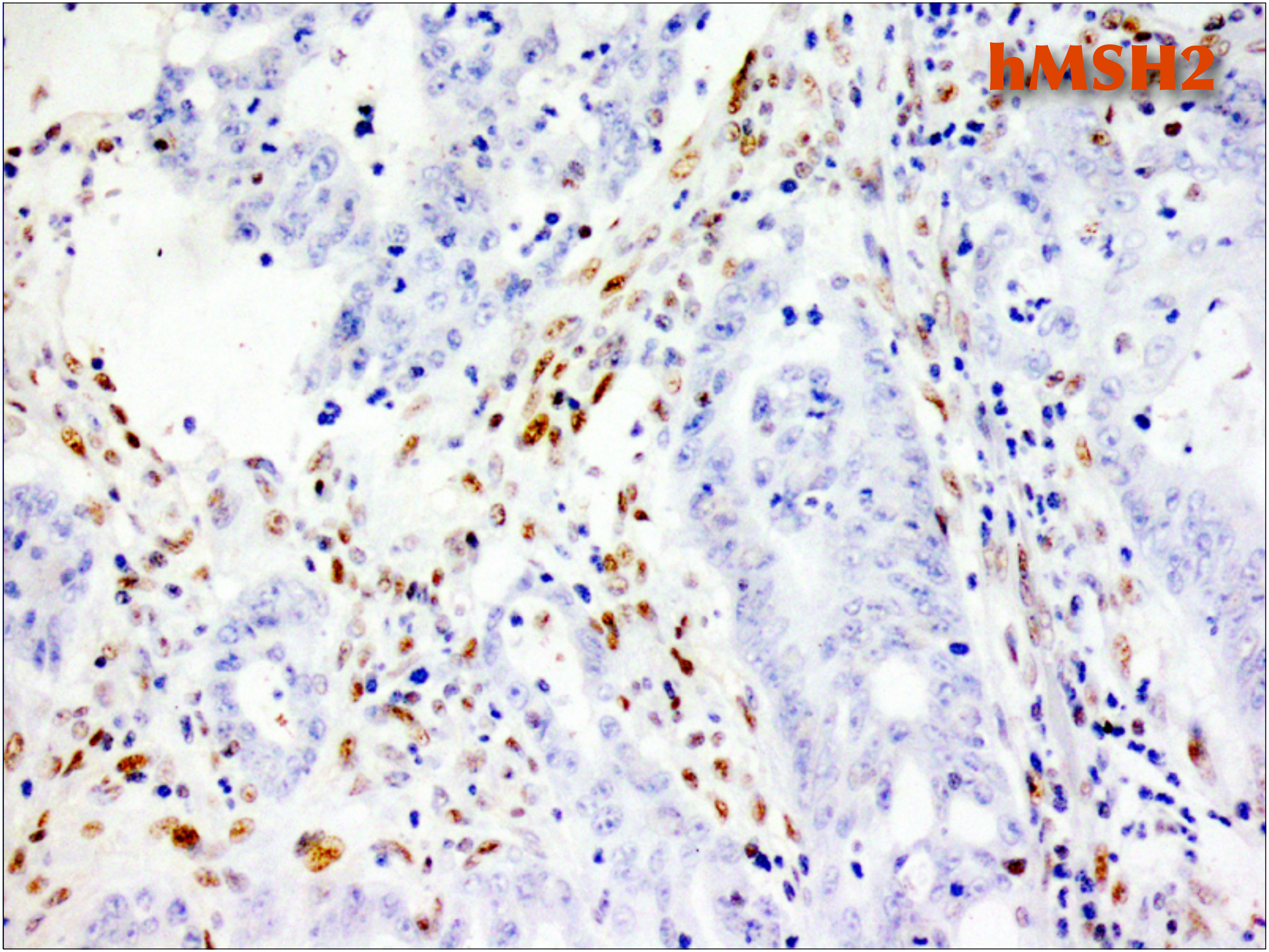


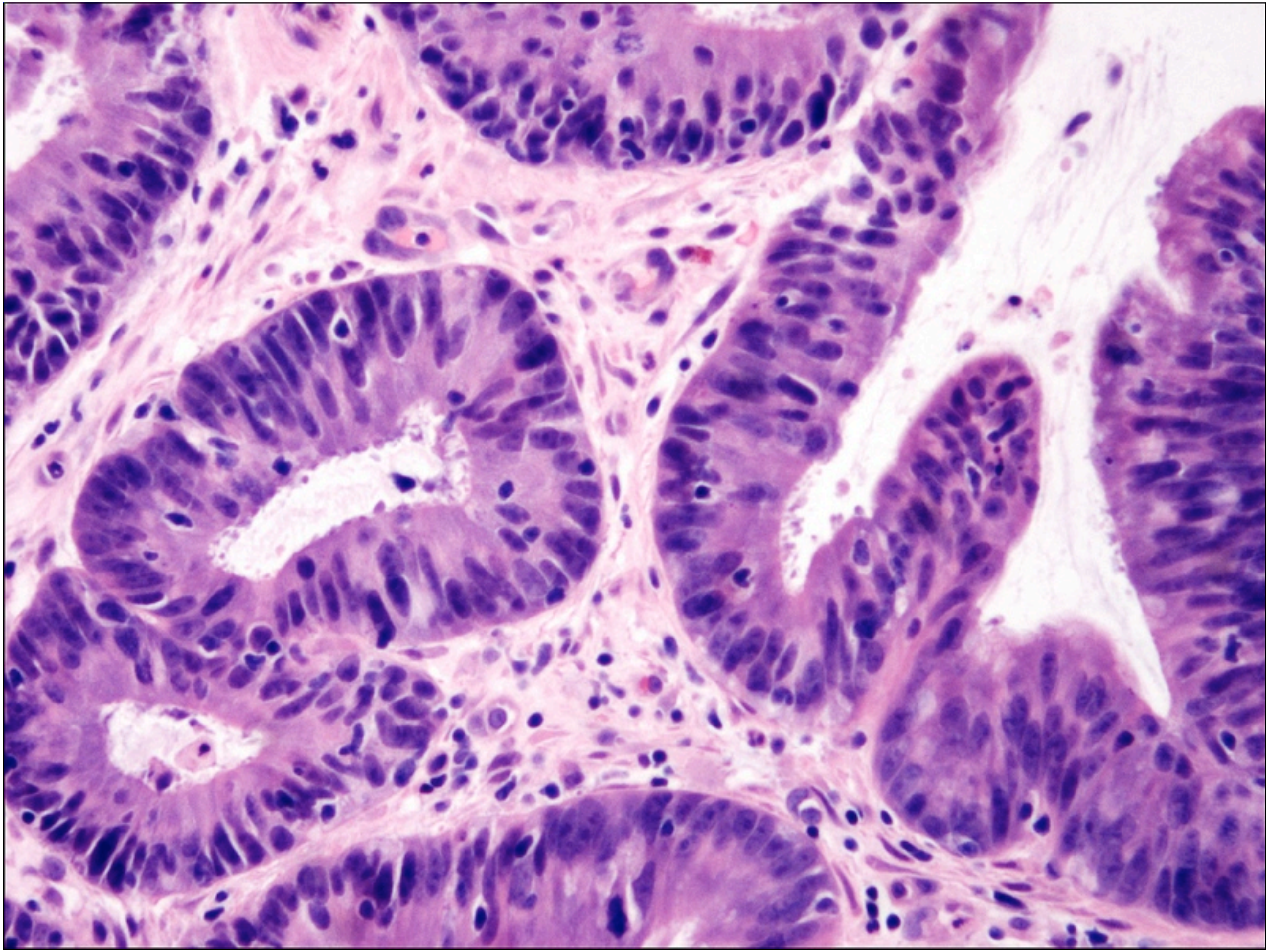

MLH1

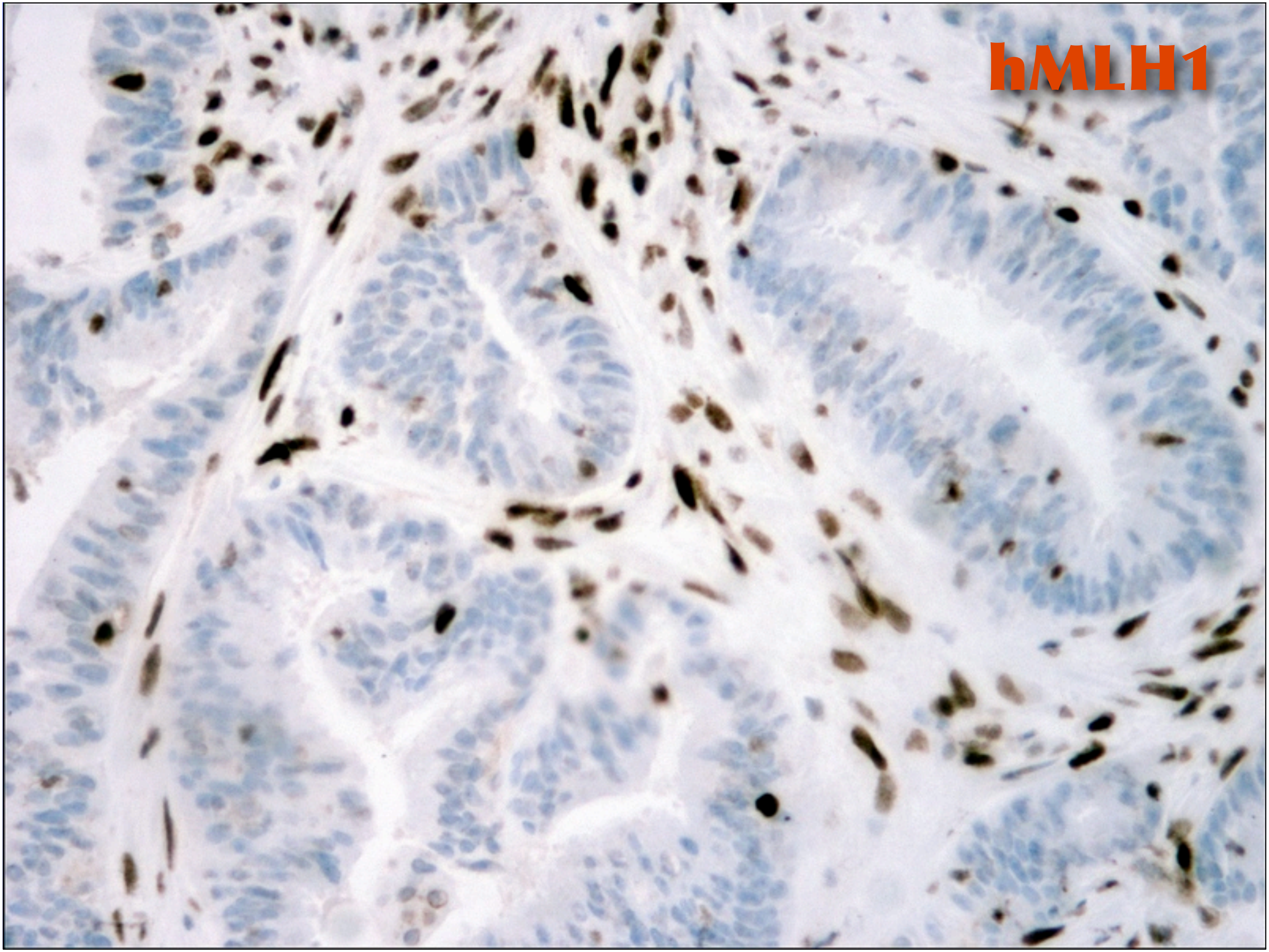

MSH2

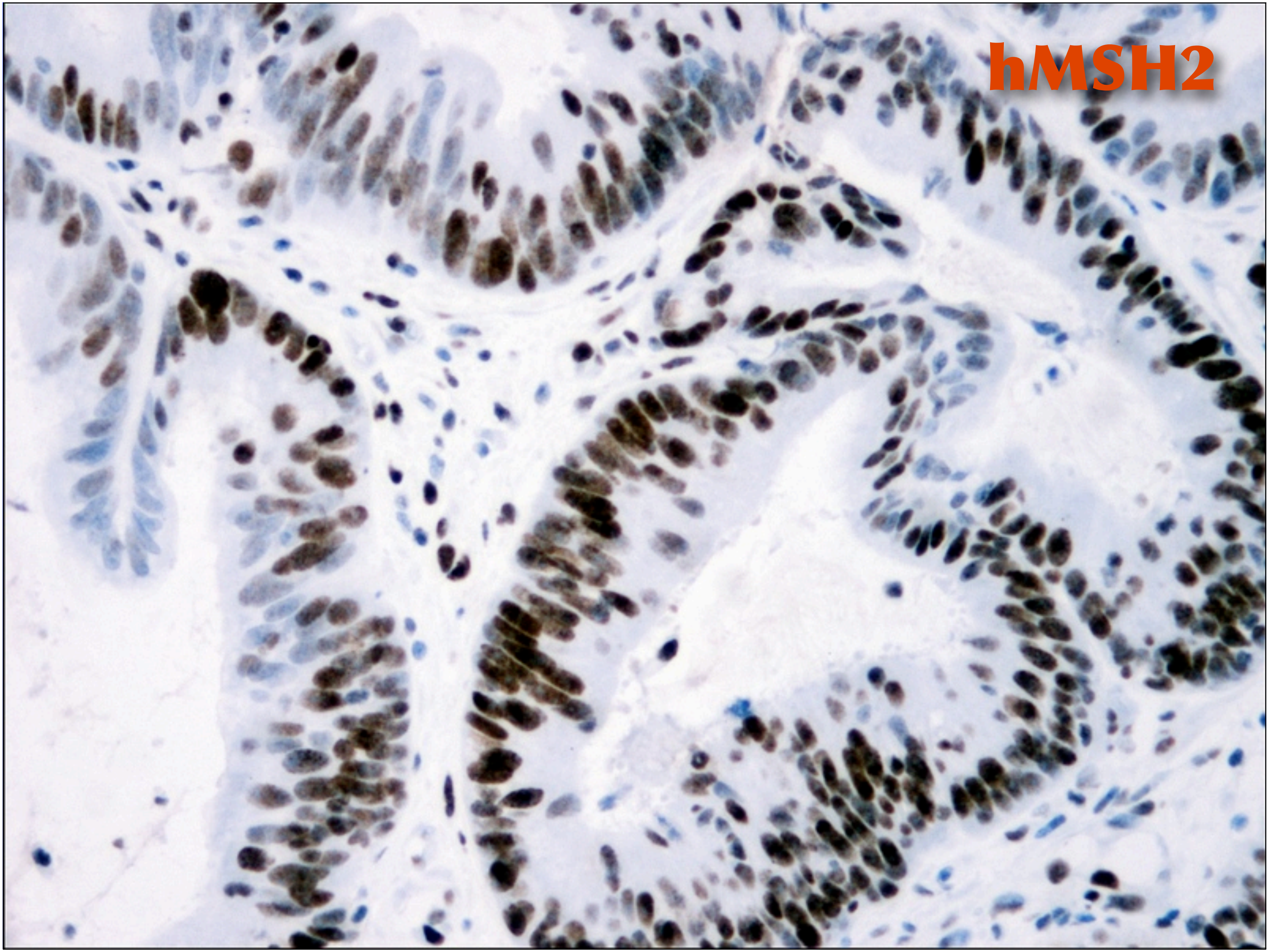


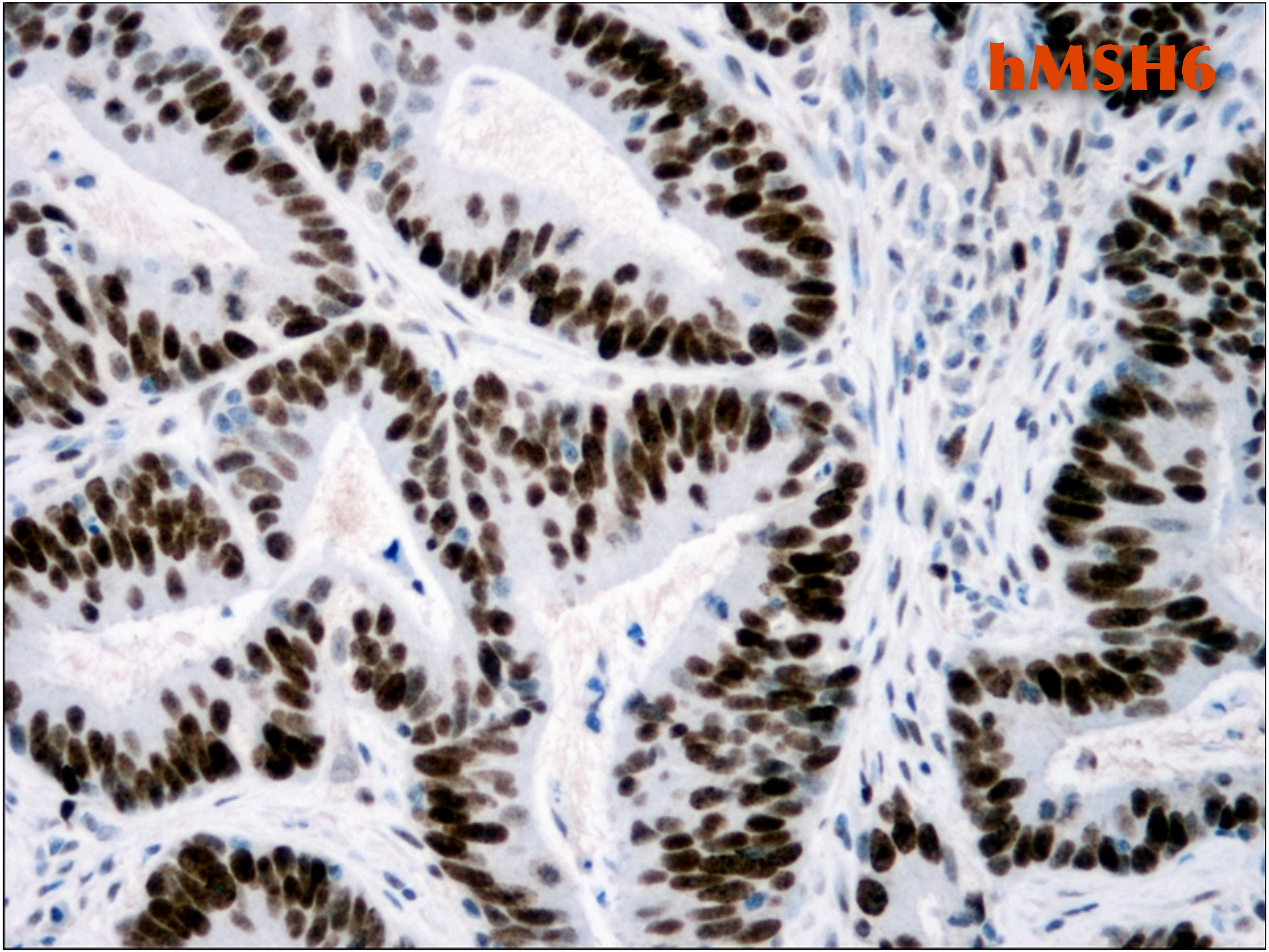

hMLH1

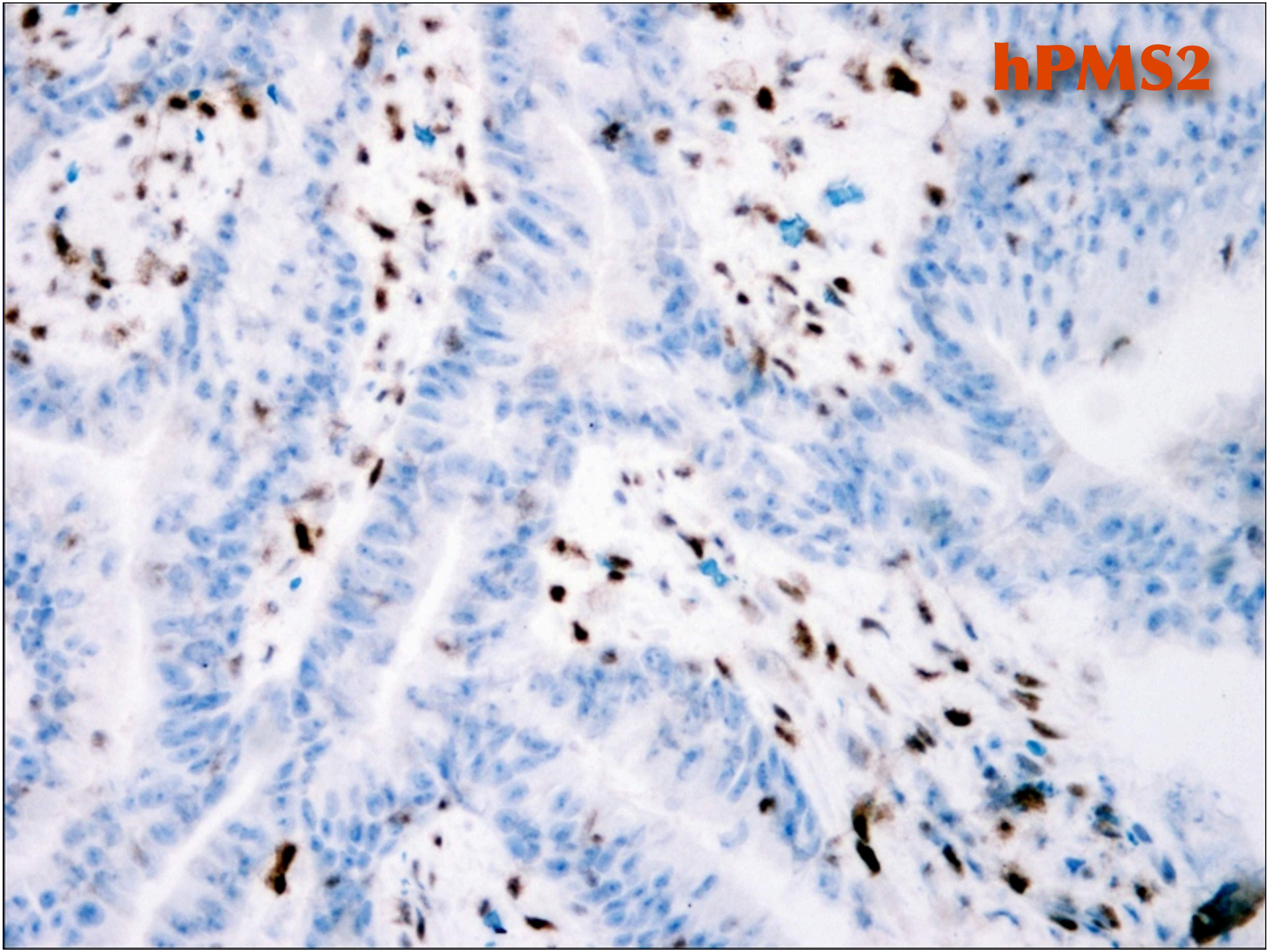

hMSH2

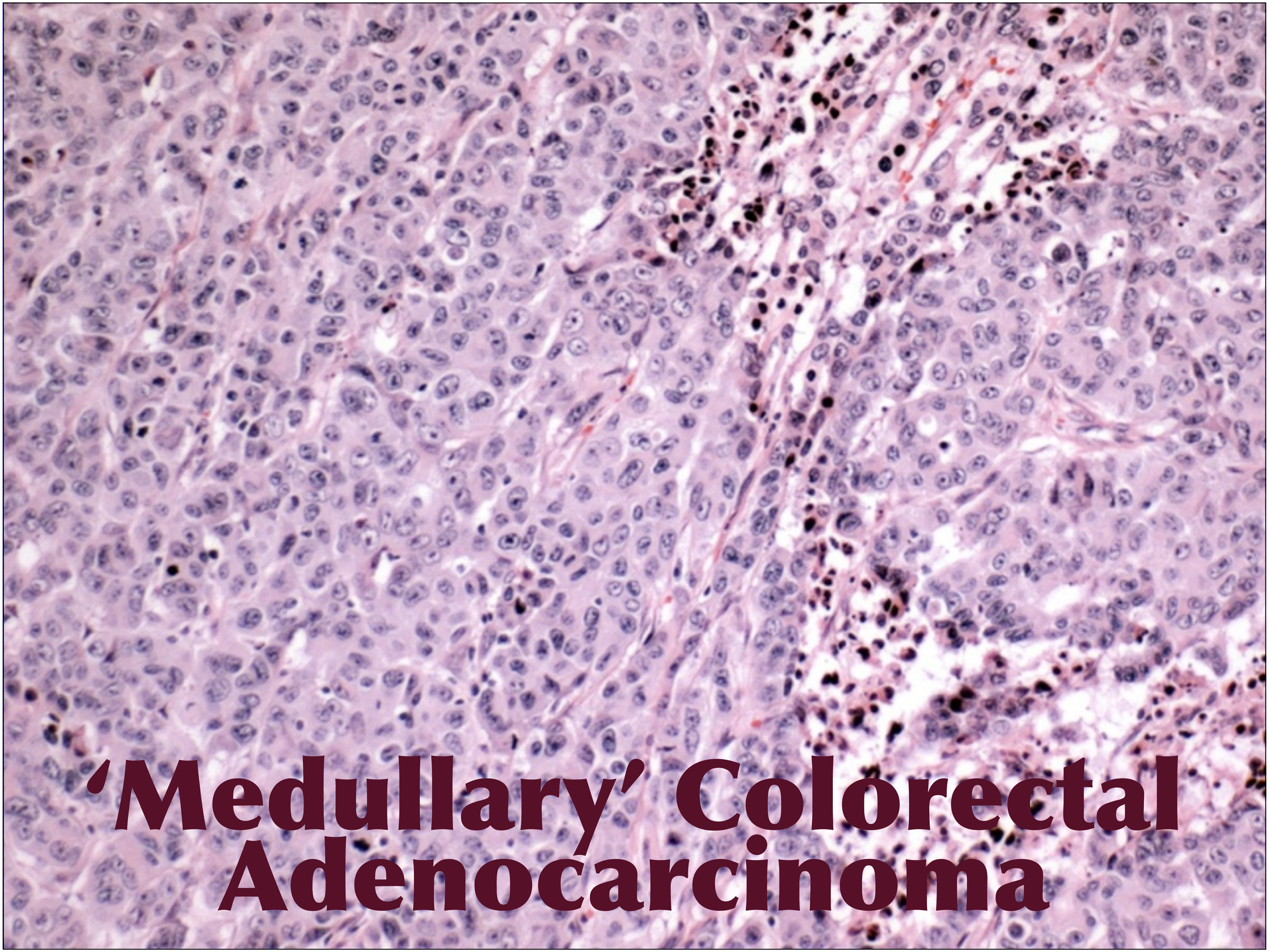


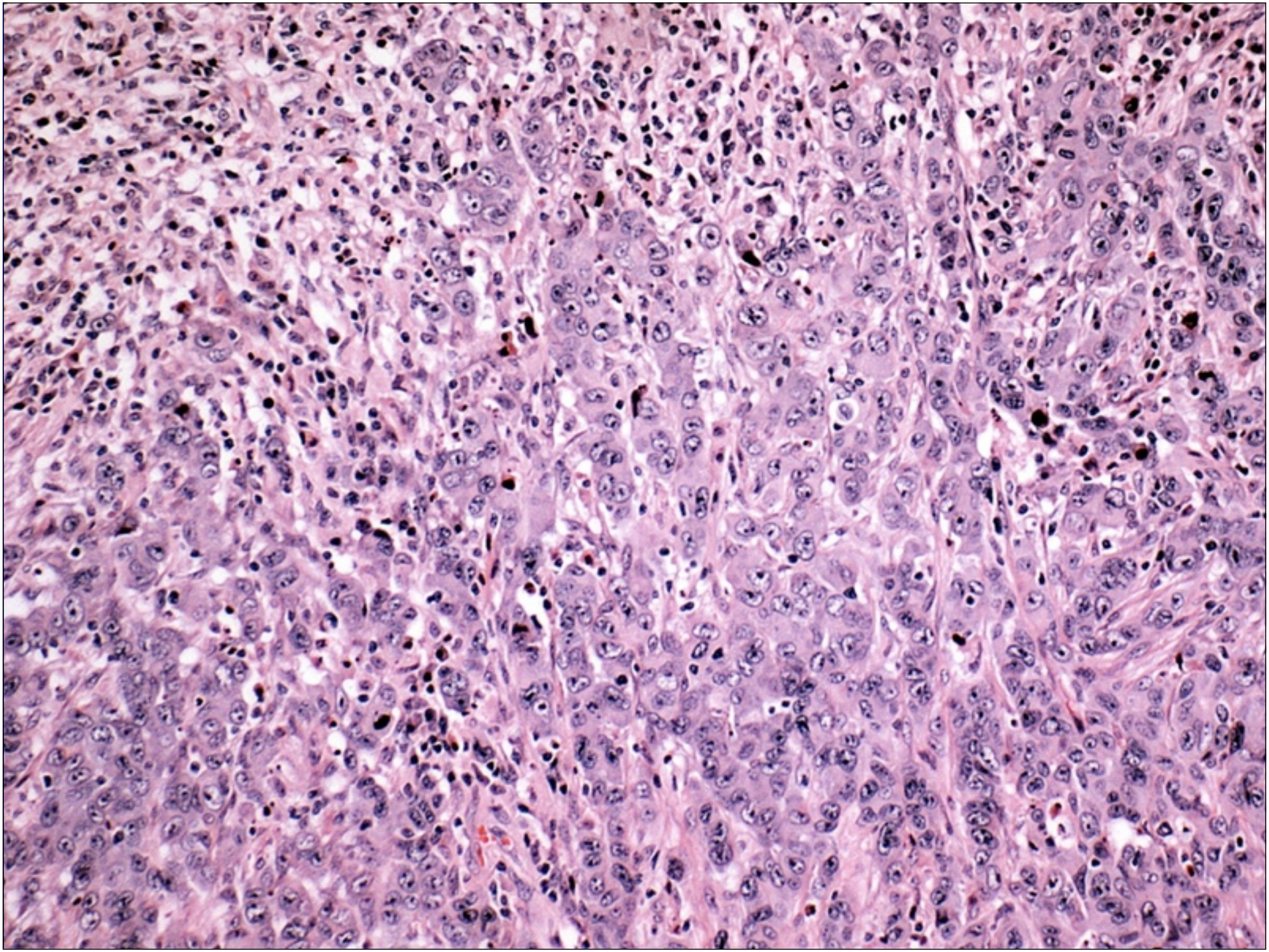

hMLH1

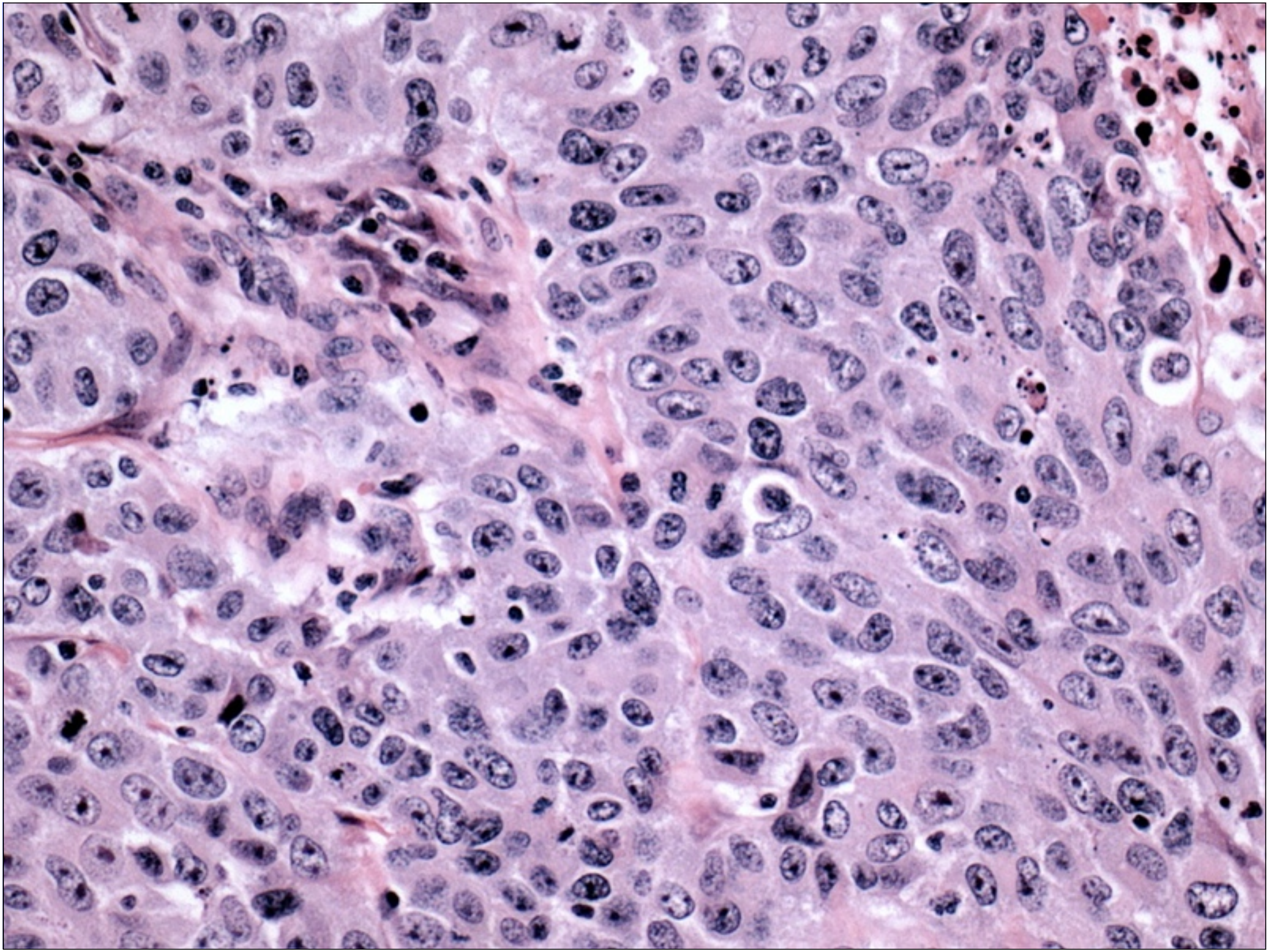

hMSH2

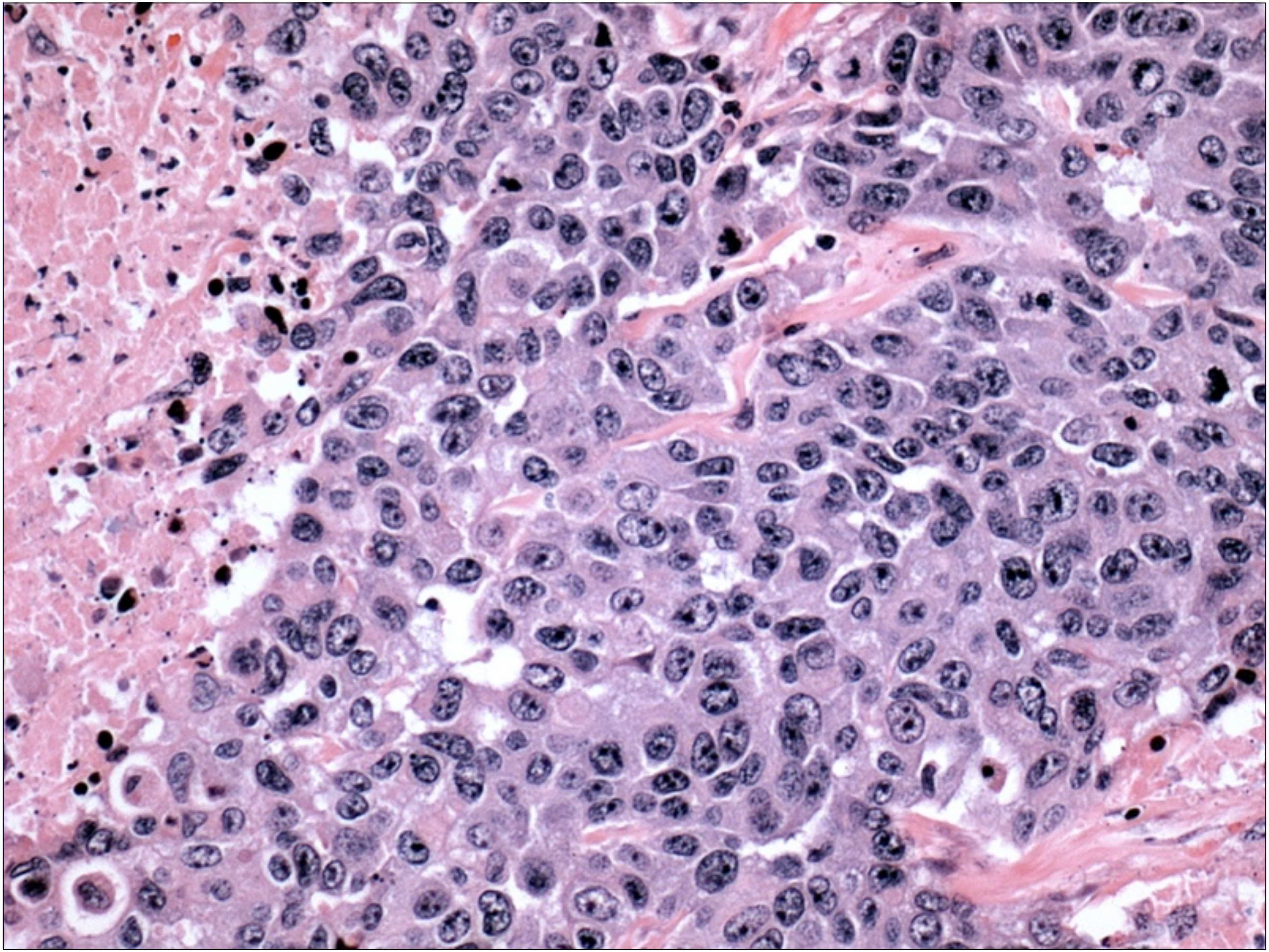

hMSH6

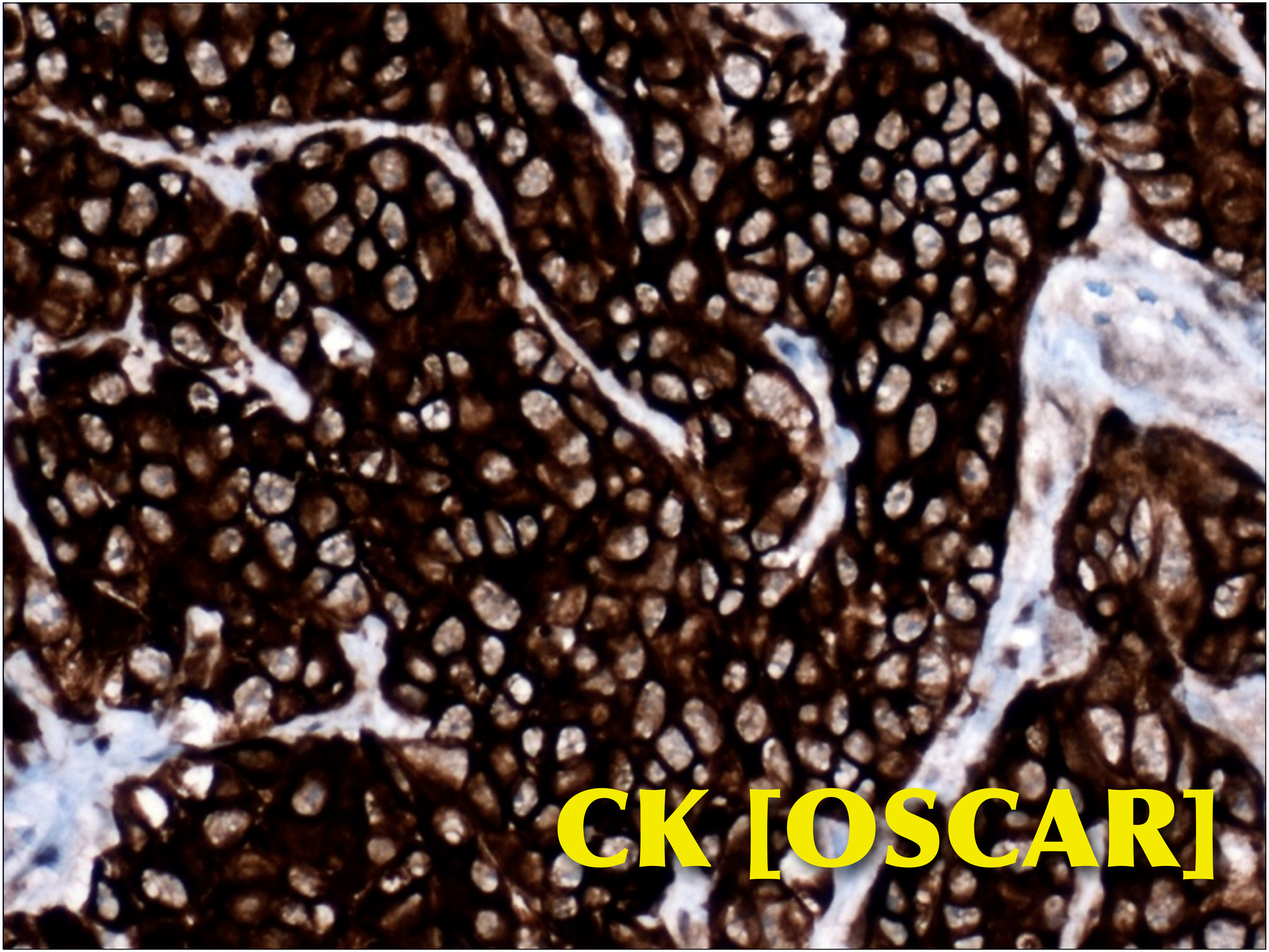

hPMS2

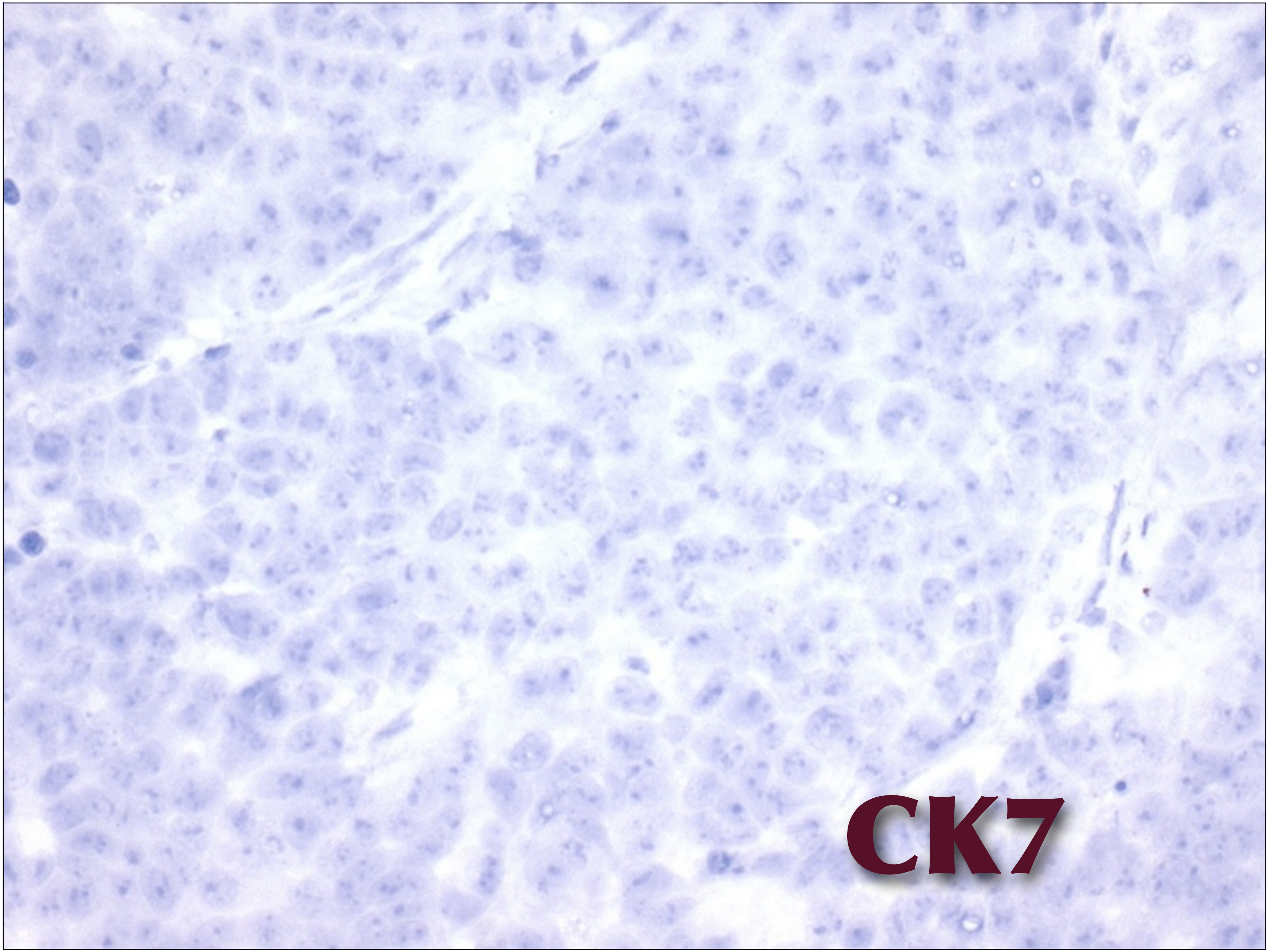

‘Medullary’ Colorectal
Adenocarcinoma

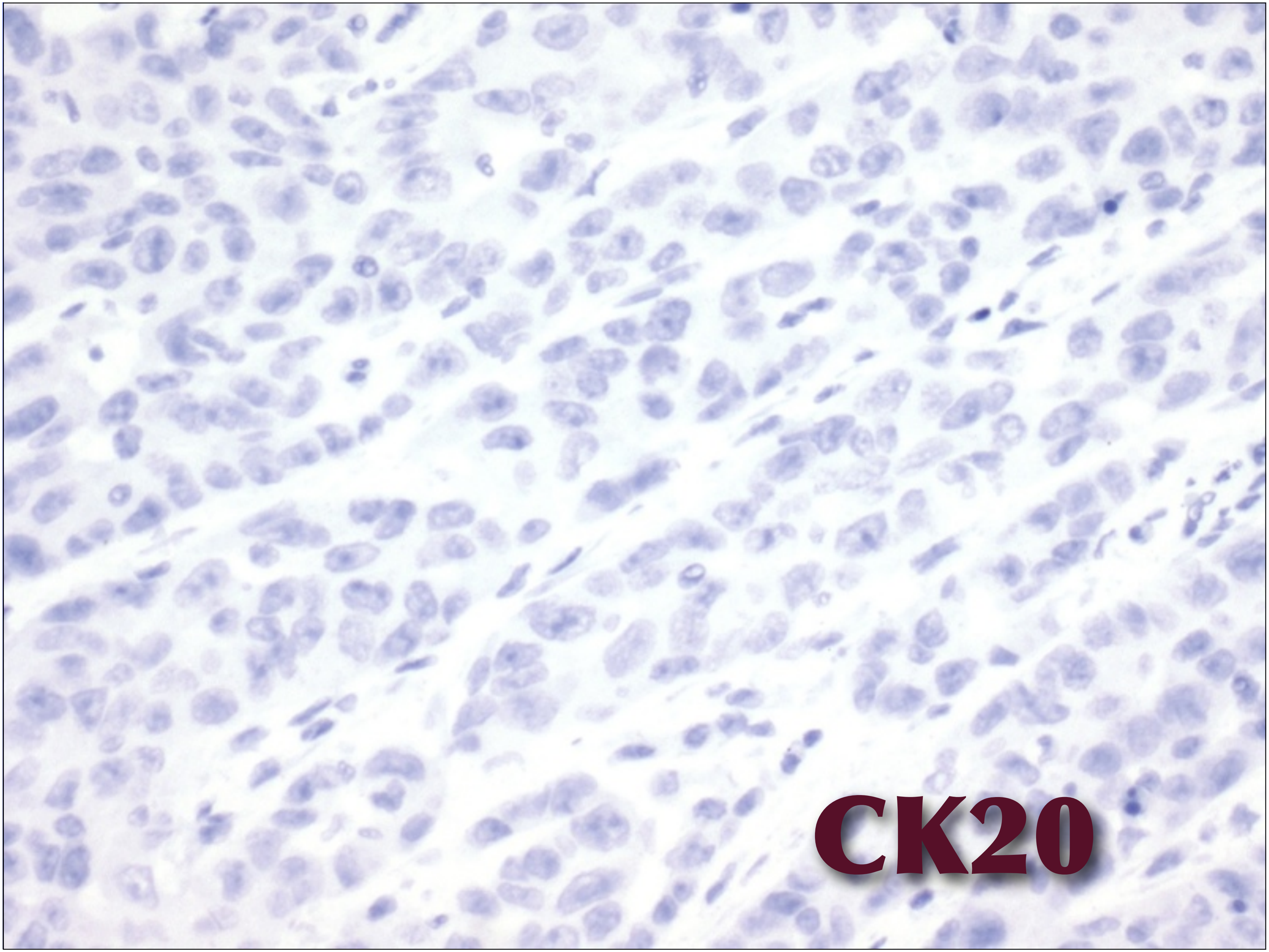







CK [OSCAR]


CK7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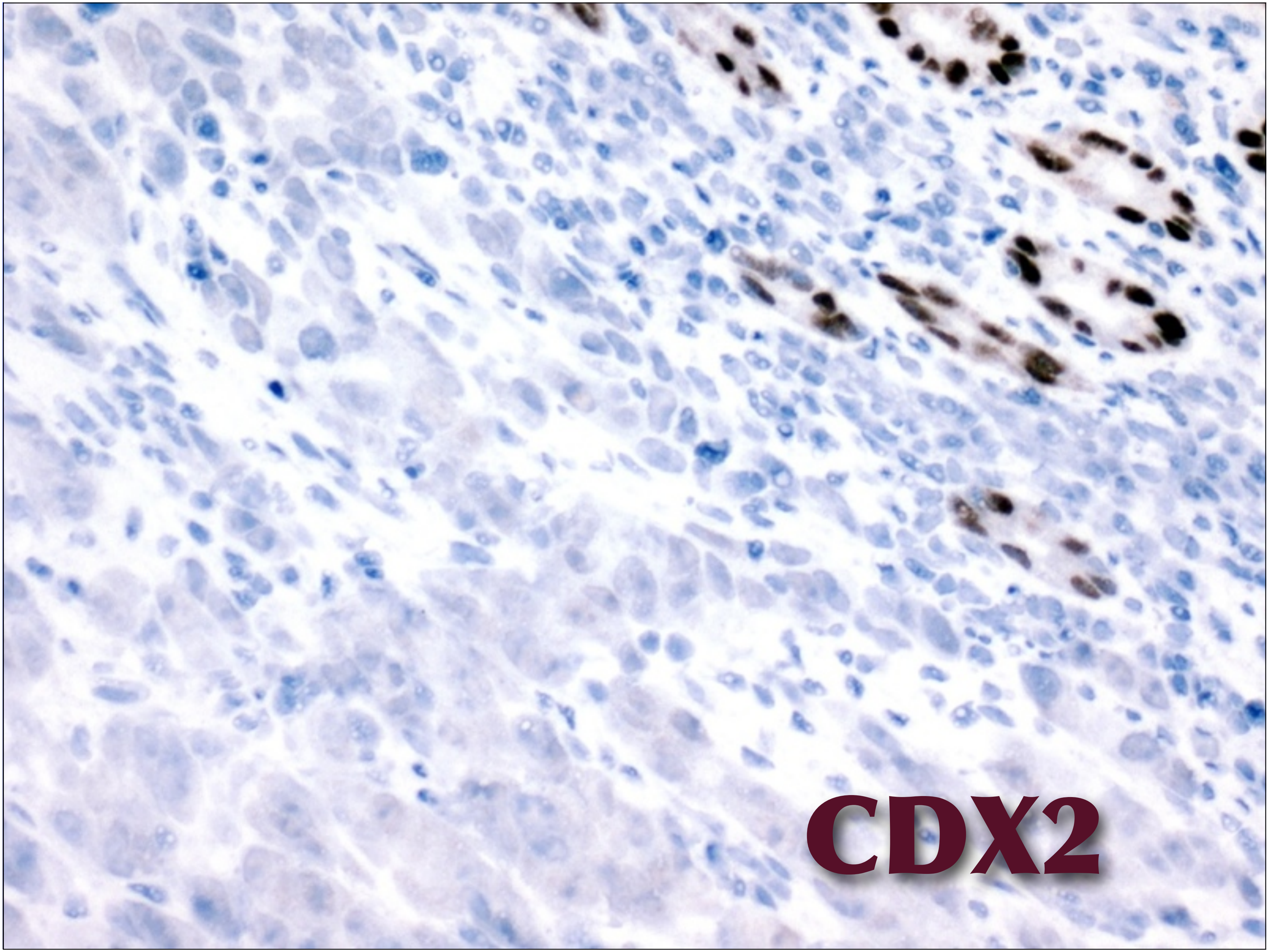
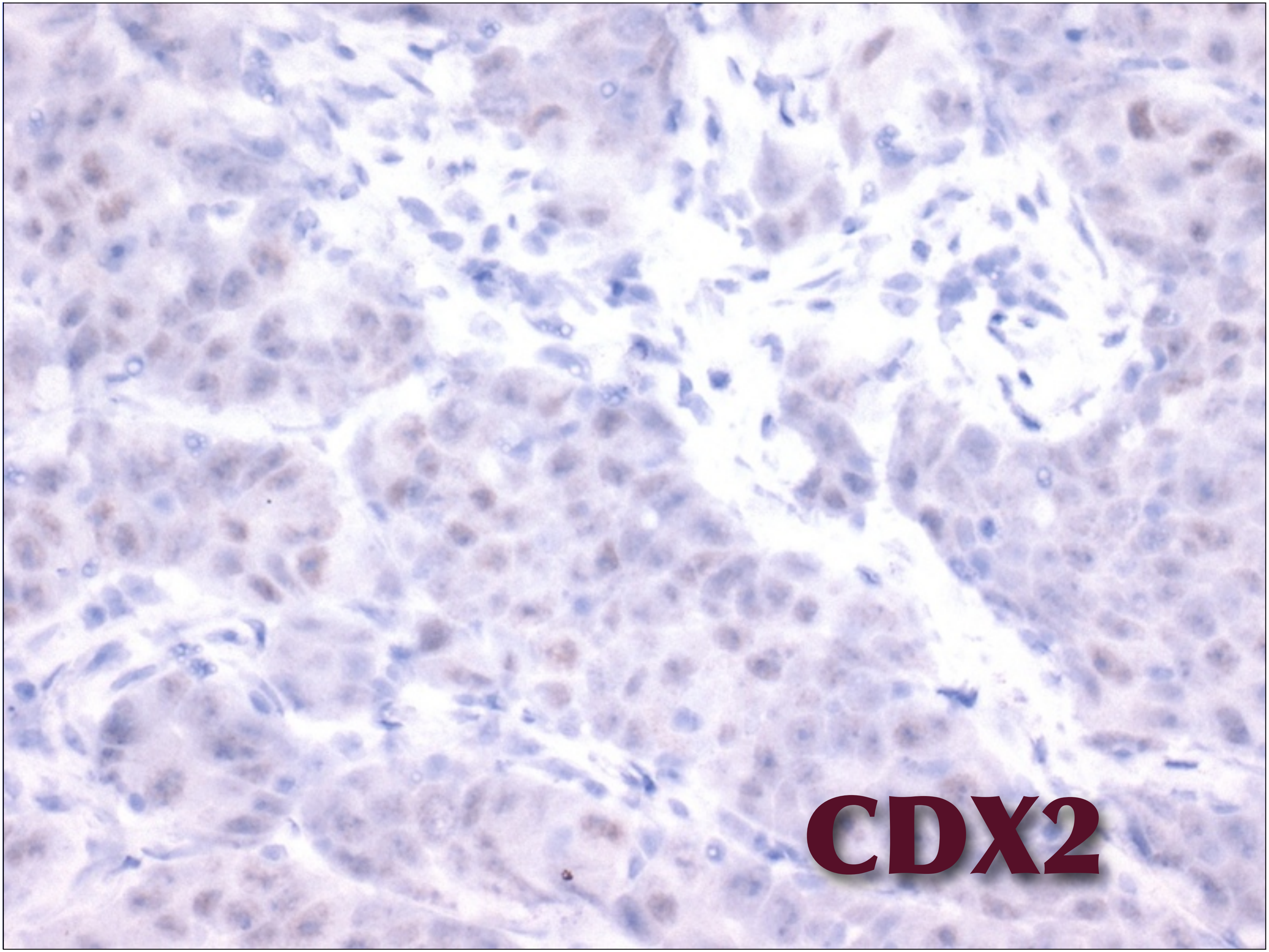
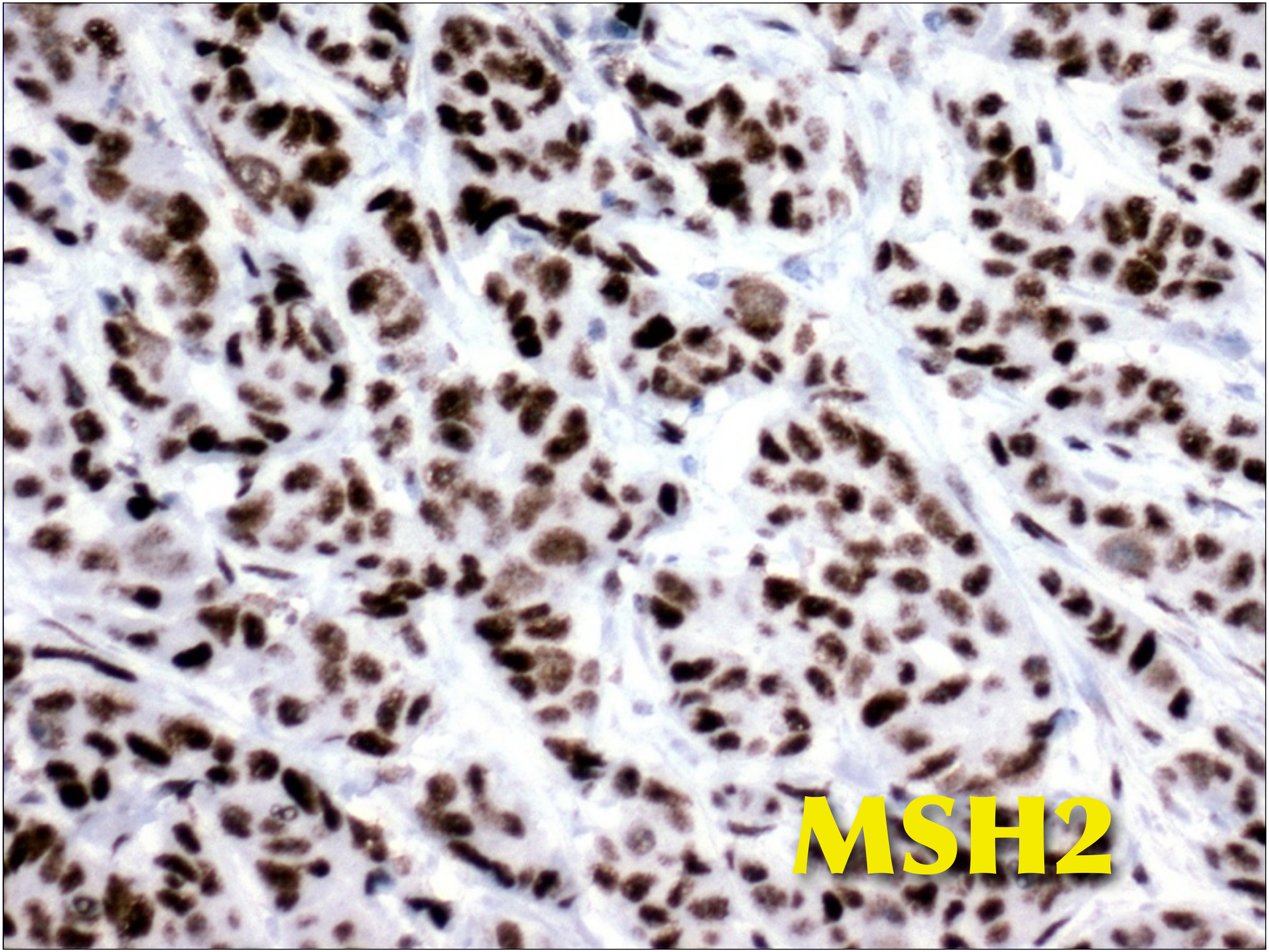
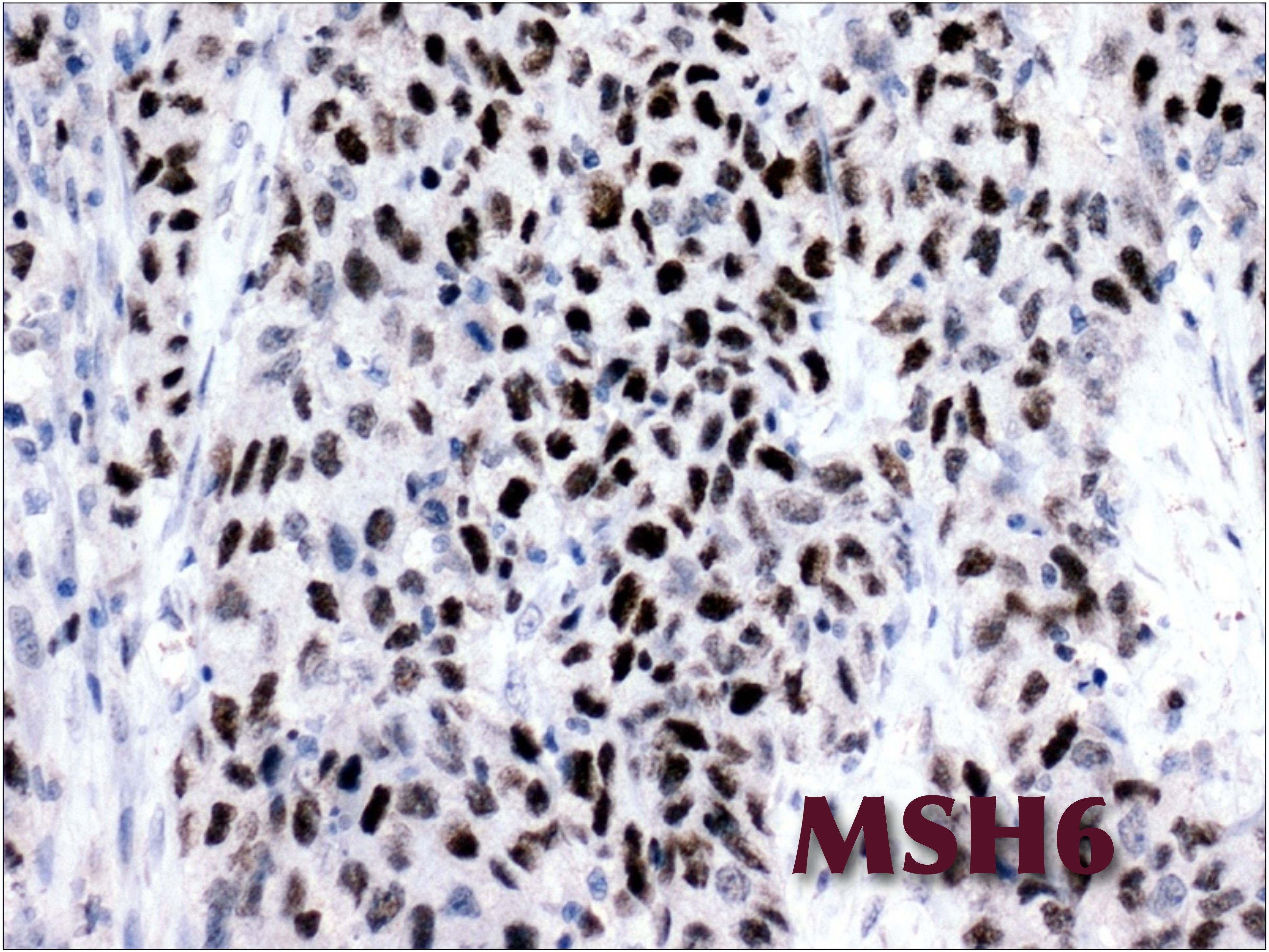
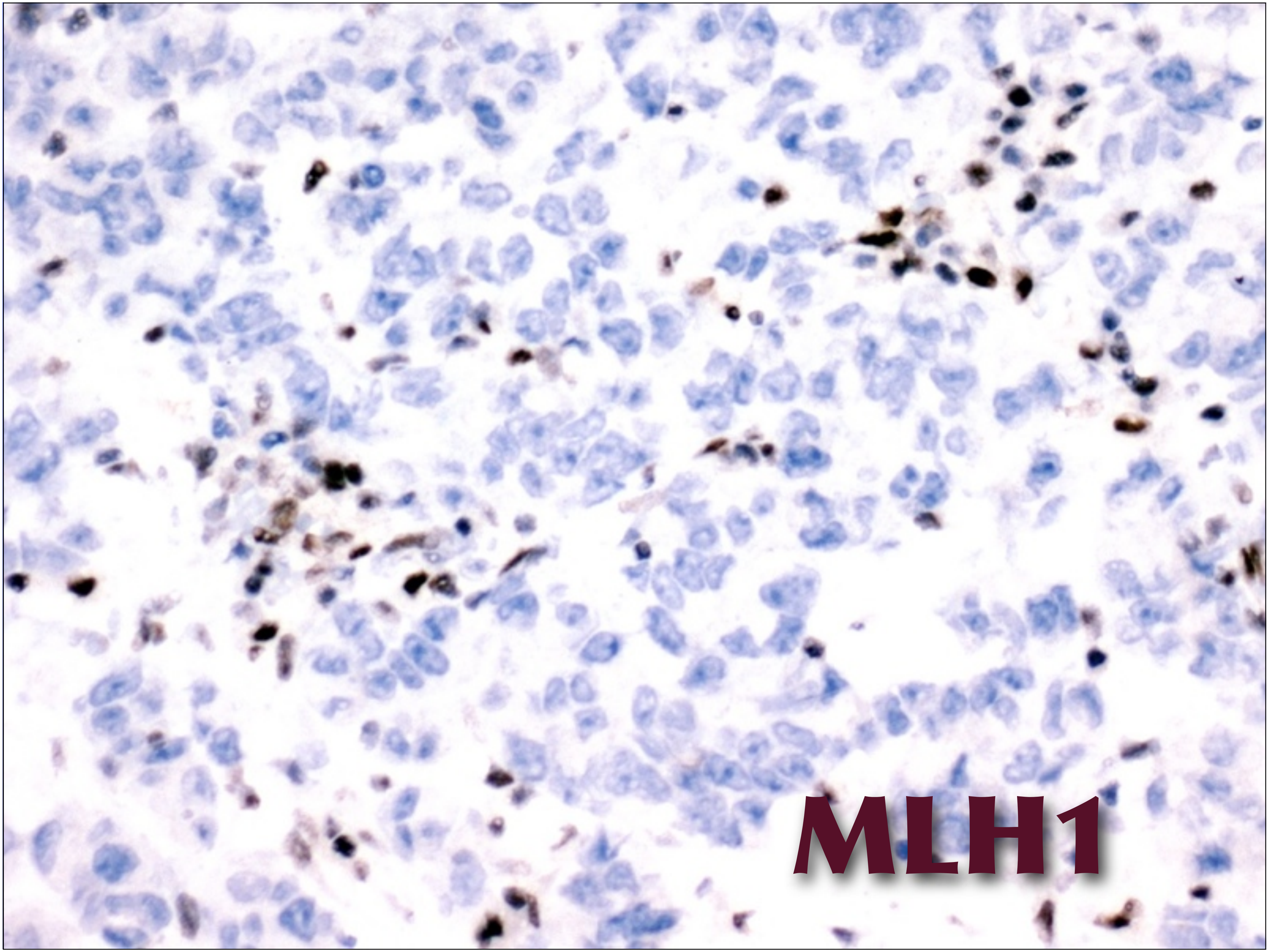
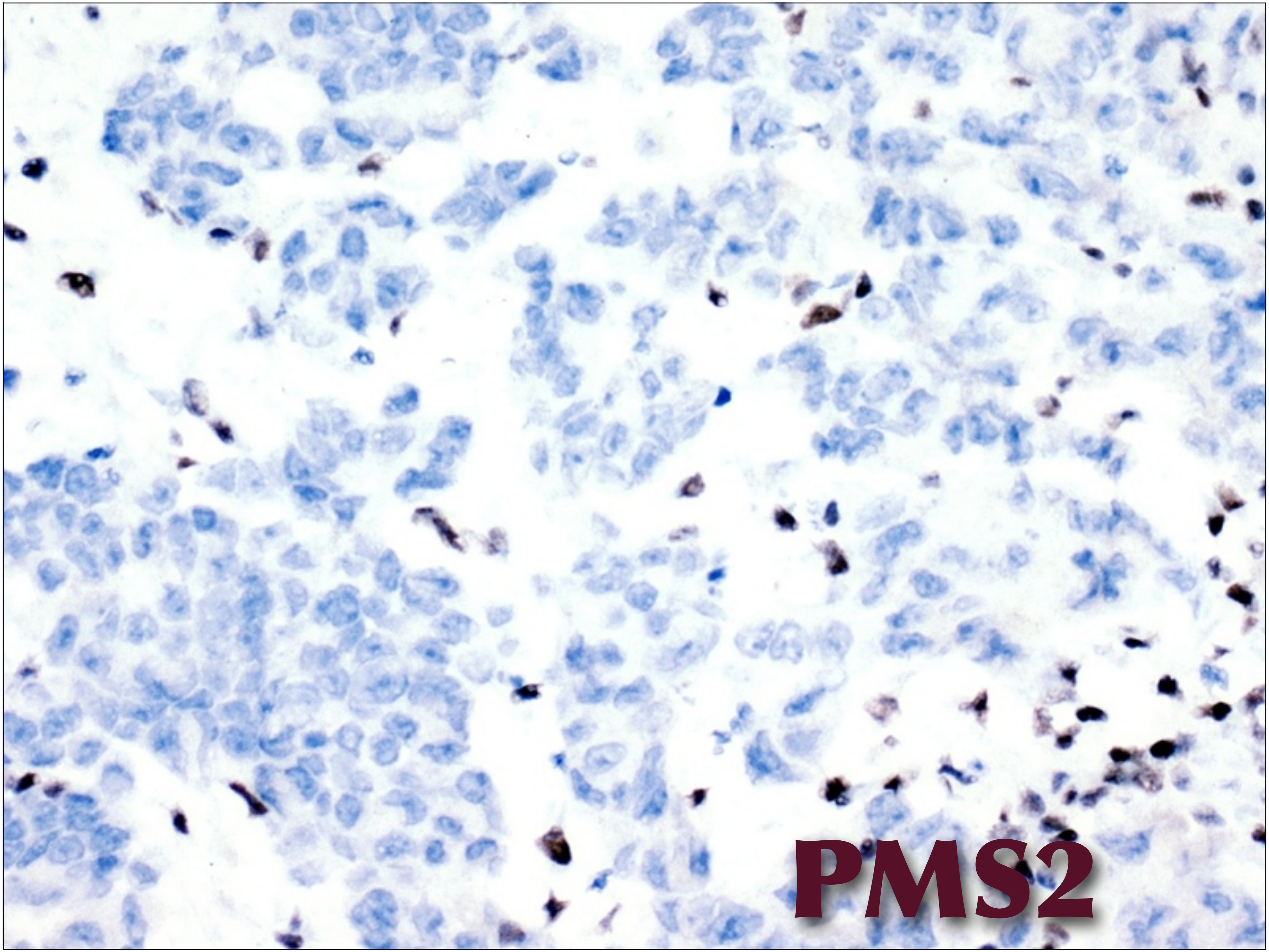

CDX2


CDX2


MSH2


MSH6


MLH1


PMS2

Am J Pathol 159:2239-2248, 2001

Loss of CDX2 Expression and Microsatellite Instability Are Prominent Features of Large Cell Minimally Differentiated Carcinomas of the Colon

Takao Hinoi,* Masachika Tani,*† Peter C. Lucas,‡
Karel Caca,* Rodney L. Dunn,§ Ettore Macri,¶
Massimo Loda,¶ Henry D. Appelman,‡
Kathleen R. Cho,*‡§ and Eric R. Fearon*‡||§

- "Minimally differentiated" or "medullary" carcinoma
- 87% show reduced or absent CDX2
- 60% showed MSI phenotype

Why Test Sporadic Colorectal Adenocarcinomas for Loss of Expression of Mismatch Repair Enzymes?

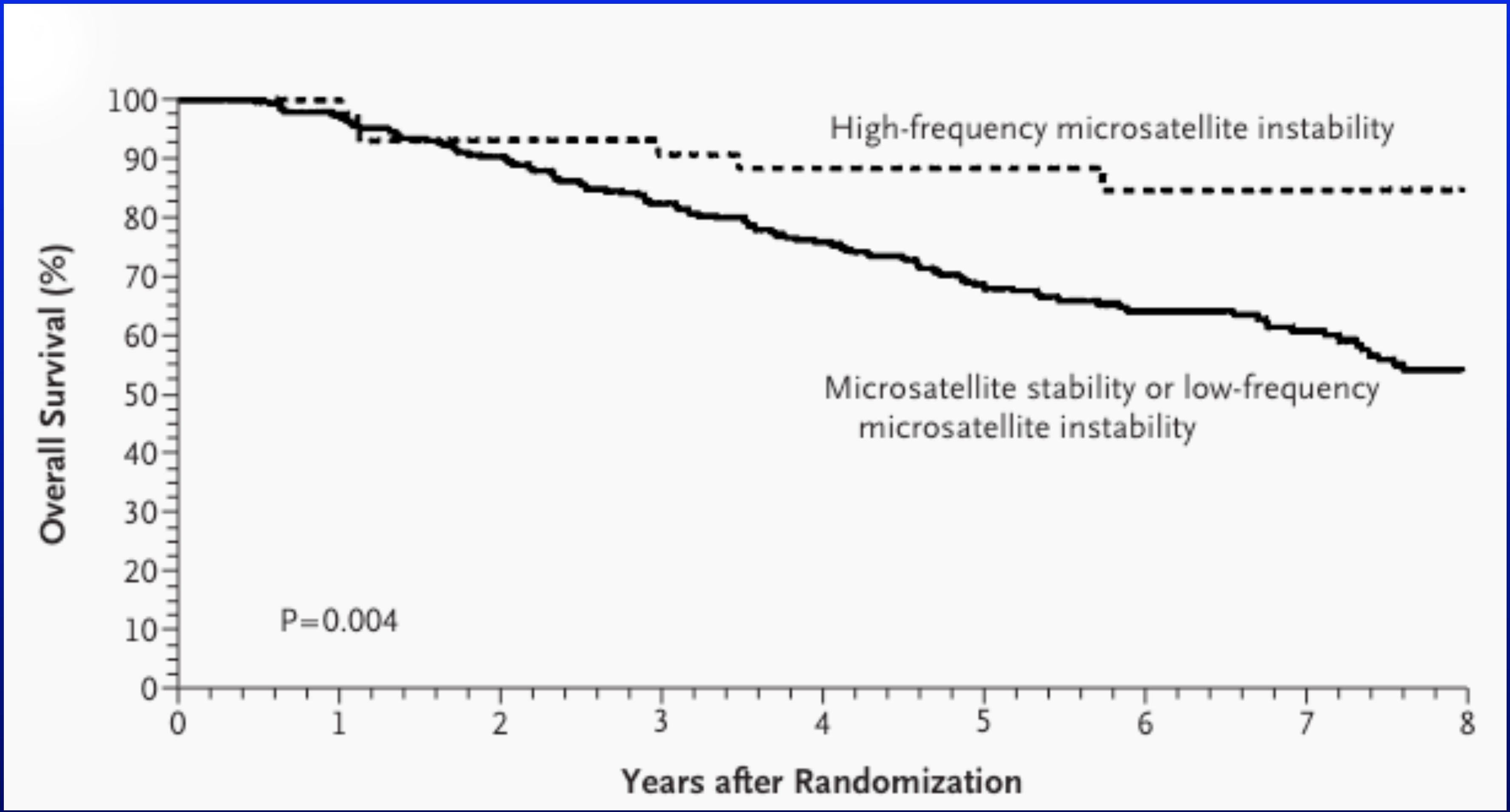
- Prognostic factor (patients with MSI-H tumors have significantly lower mortality rate independent of tumor stage)
- Predictive factor (patients with MSI-H tumors do more poorly with fluorouracil-based adjuvant chemotherapy)
- Can alert clinician to possibility of unrecognized HNPCC

Ribic CM et al. NEJM 349:247-57, 2003

- N = 570; 16.7% displayed MSI-H
- Patients with MSI-H tumors had better overall 5 year survival (HR = 0.31)
- Among patients receiving adjuvant chemotherapy*, 5 year survival benefit disappeared
- Adjuvant chemotherapy* improved survival among patients with MSI-S or MSI-L but not MSI-H tumors

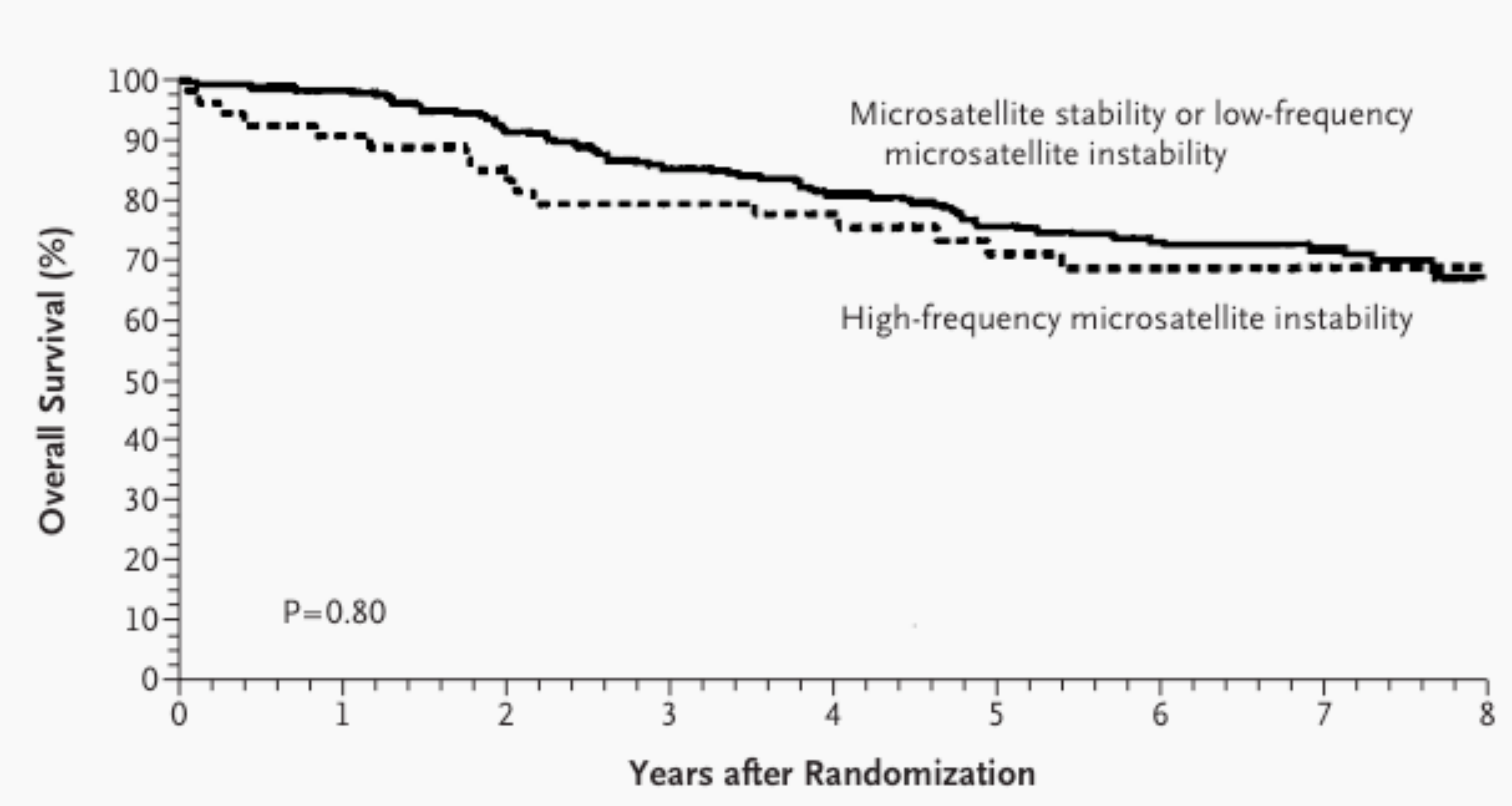
**fluorouracil + levamisole or leucovorin*

NO ADJUVANT CHEMOTHERAPY



Ribic CM et al. NEJM 349:247-57, 2003

ADJUVANT CHEMOTHERAPY



Ribic CM et al. NEJM 349:247-57, 2003

VOLUME 27 · NUMBER 11 · APRIL 10 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

N = 1,264

Microsatellite Instability Predicts Improved Response to Adjuvant Therapy With Irinotecan, Fluorouracil, and Leucovorin in Stage III Colon Cancer: Cancer and Leukemia Group B Protocol 89803

Monica M. Bertagnolli, Donna Niedzwiecki, Carolyn C. Compton, Hejin P. Hahn, Margaret Hall, Beatrice Damas, Scott D. Jewell, Robert J. Mayer, Richard M. Goldberg, Leonard B. Saltz, Robert S. Warren, and Mark Redston

- Patients treated with FU/leucovorin (FU/LV) or irinotecan, FU and leucovorin (IFL)
- MLH1, MSH2 assessed by IHC
- Endpoint OS and DFS

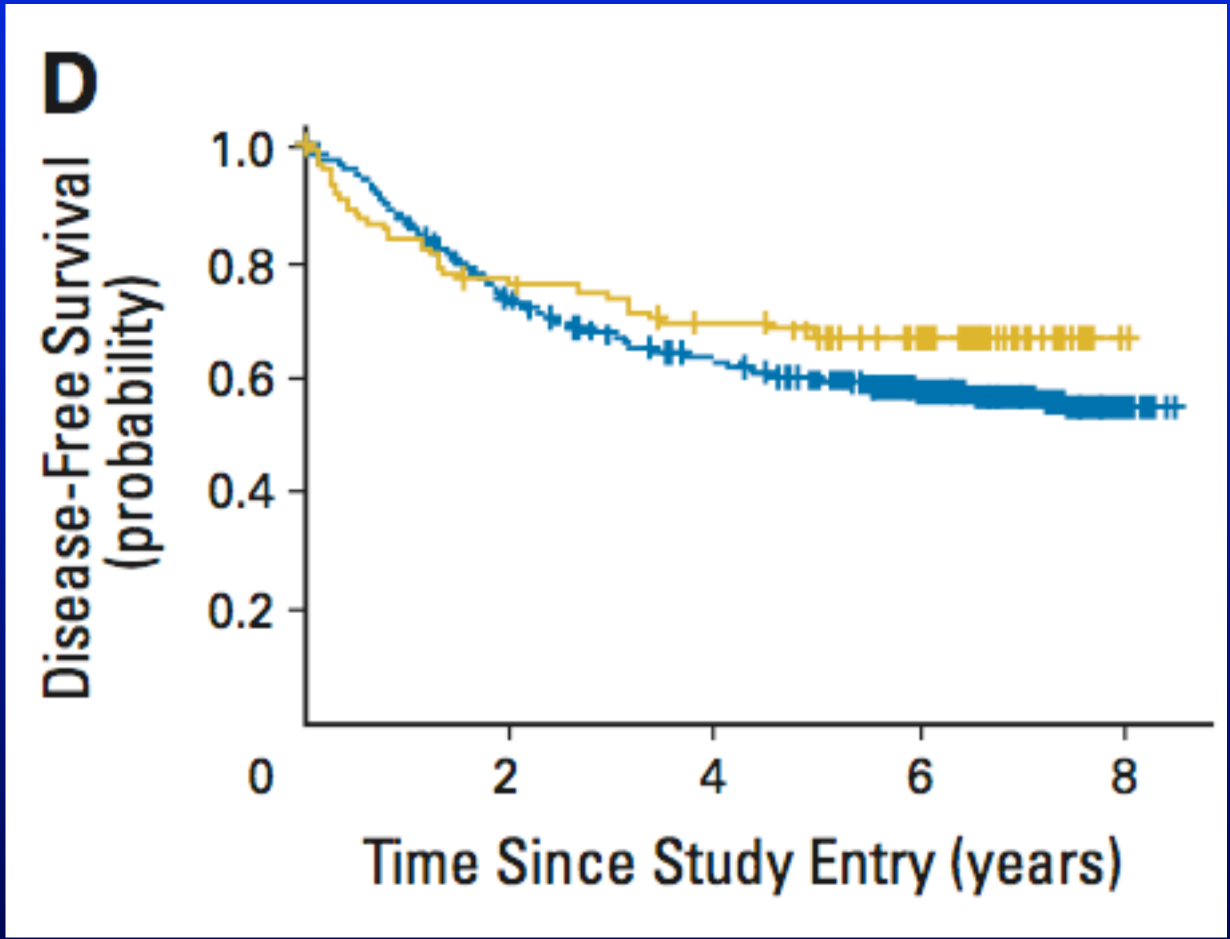
Bertagnolli MM et al, J Clin Oncol 1814-21, 2009

13.3% of tumors were MMR-D/MSI

	MMR-D	MMR-I
MSI-H	96	17
MSI-L/S	4	606

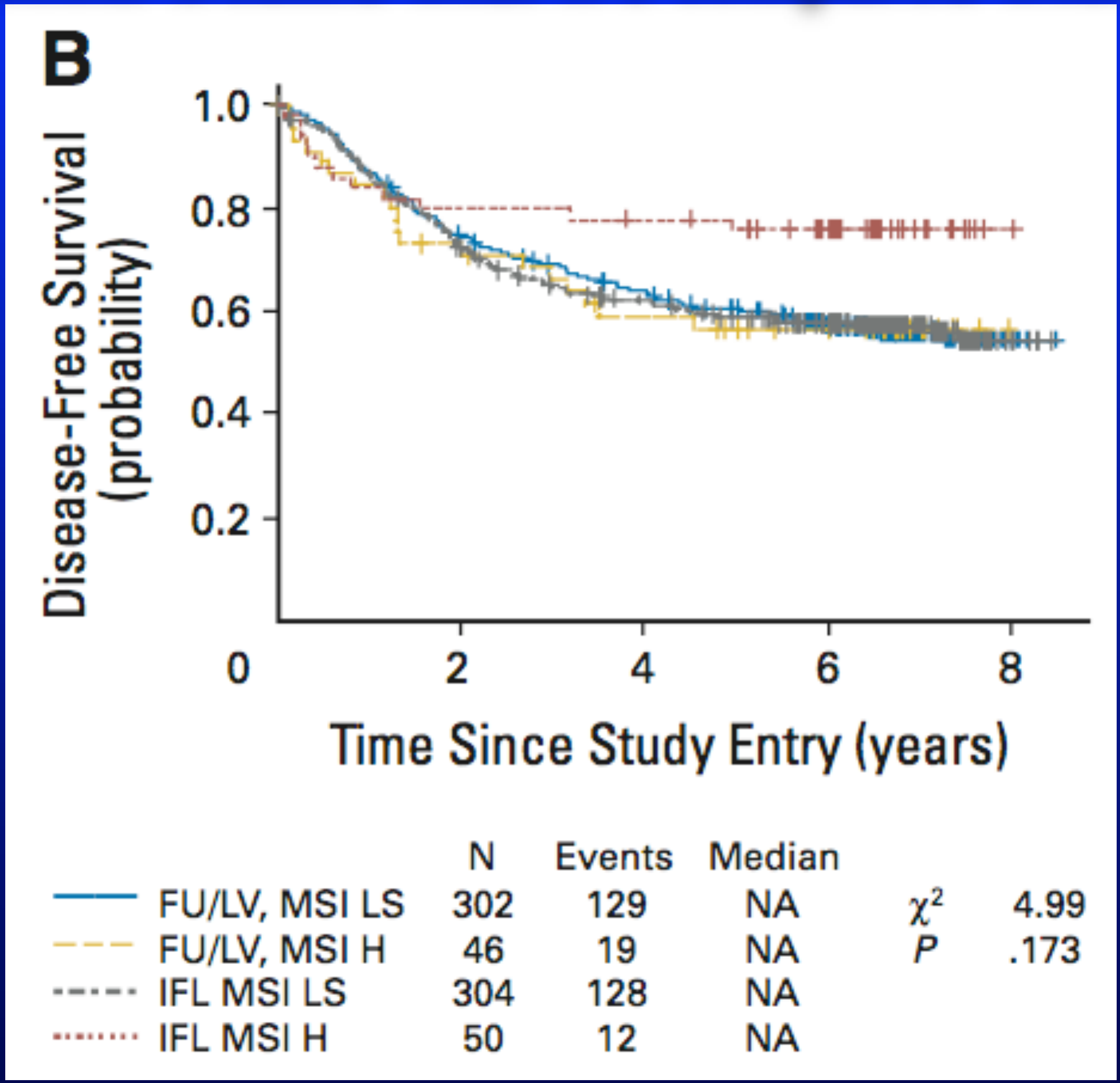
Overall concordance: 97.1%

No difference in survival, MMR-D and MMR-I



Bertagnolli MM et al, J Clin Oncol 1814-21, 2009

Loss of expression of MMR predicts improved outcome in patients treated with IFL compared with FU/LV



Bertagnolli MM et al, J Clin Oncol 1814-21, 2009

MMR IHC and Colorectal Adenocarcinoma

- Immunohistochemical localization “integrates” what happens at the genomic level to MMR genes
- Identifies genotypically distinct vairiants of colorectal adenocarcinoma with important clinical implications

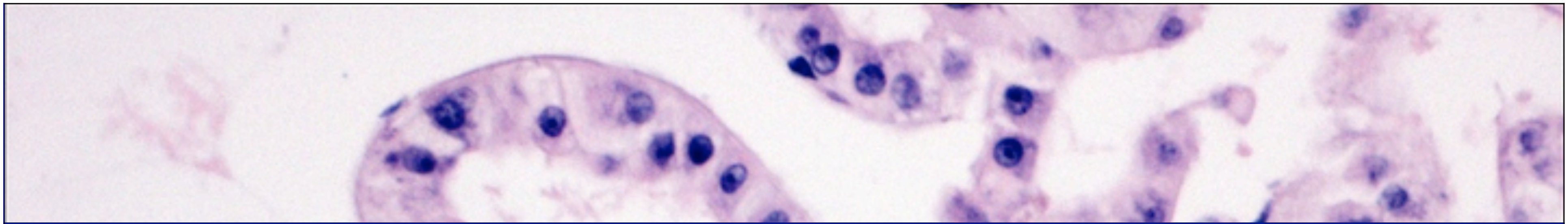
IMMUNOHISTOCHEMISTRY

Selected Topics

- General Issues
- Breast Carcinoma
- GI Tract Tumors
- Tumors in the Liver
- Male GU Tract Tumors

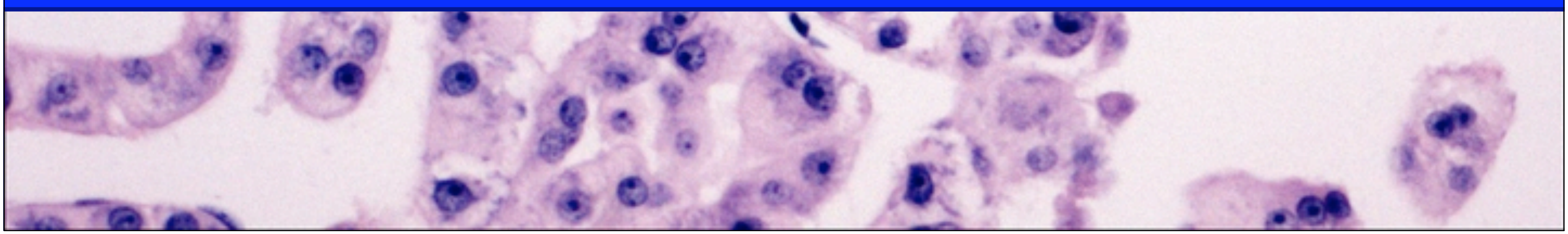
Tumors in the Liver

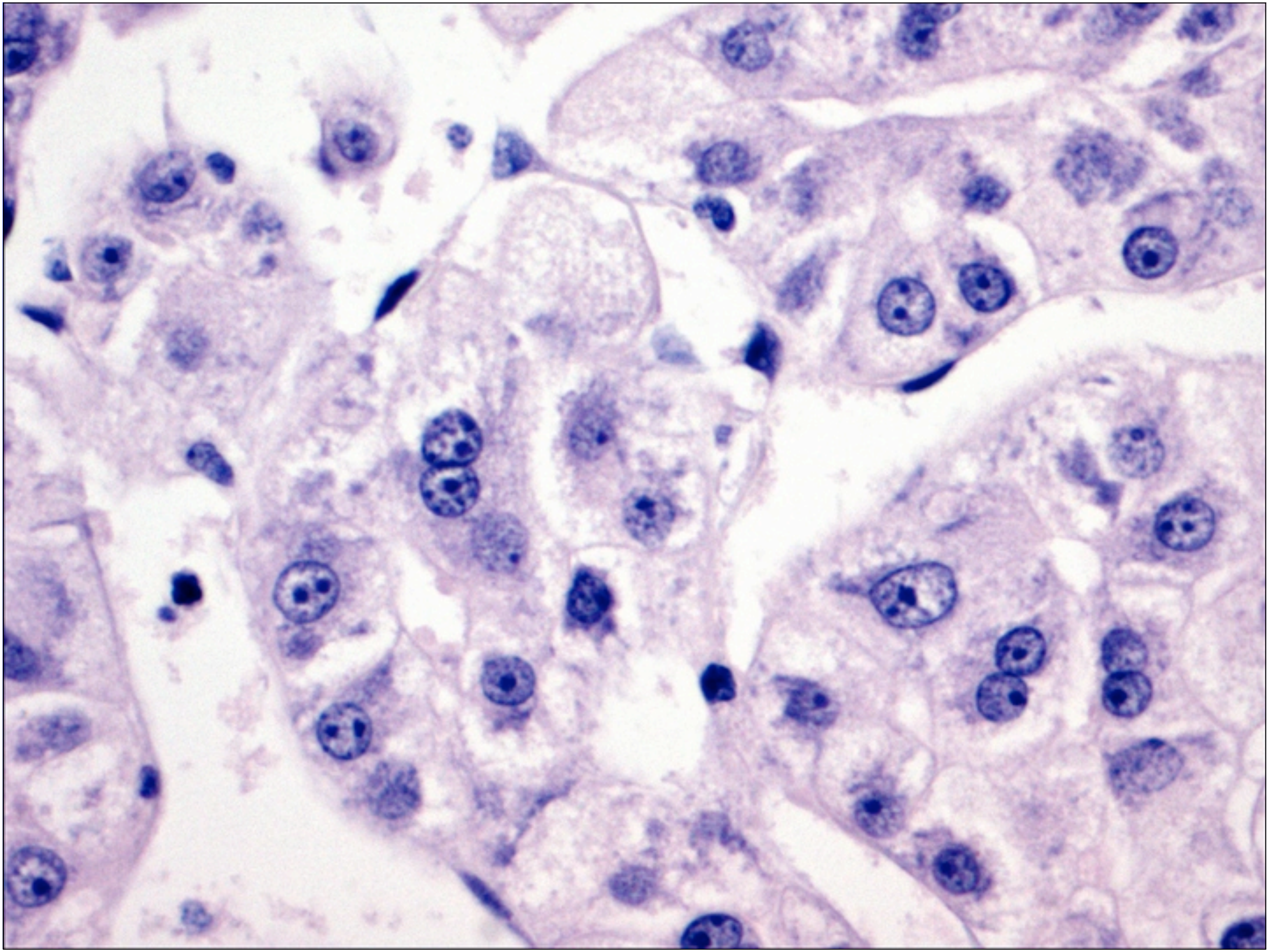
- Markers of hepatocellular CA, including HepPar1 antibody update
- Hepatocellular carcinoma v. metastatic carcinoma markers

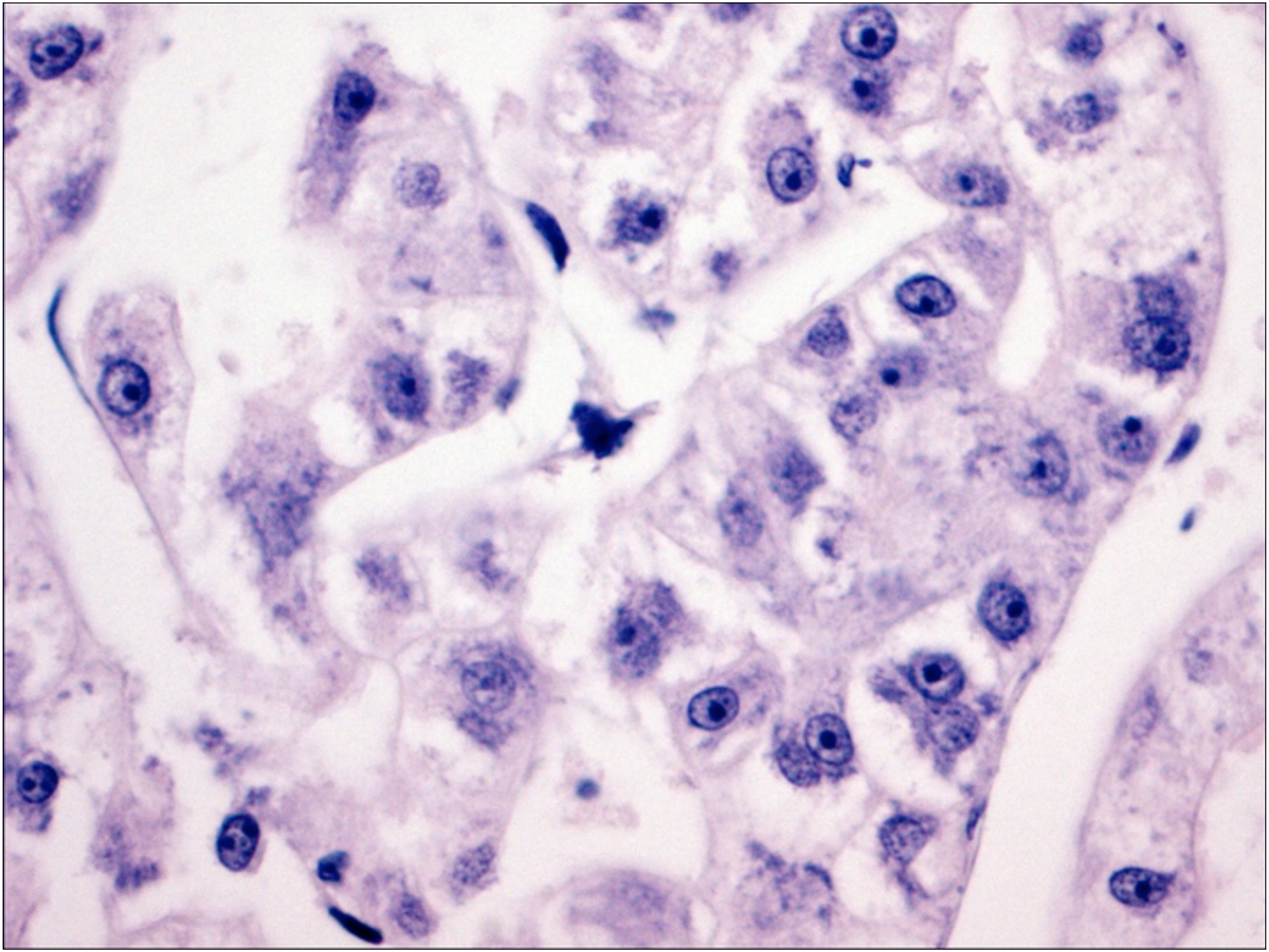


Case 6

Liver Biopsy from 48 year old male with ? Pancreatic Mass

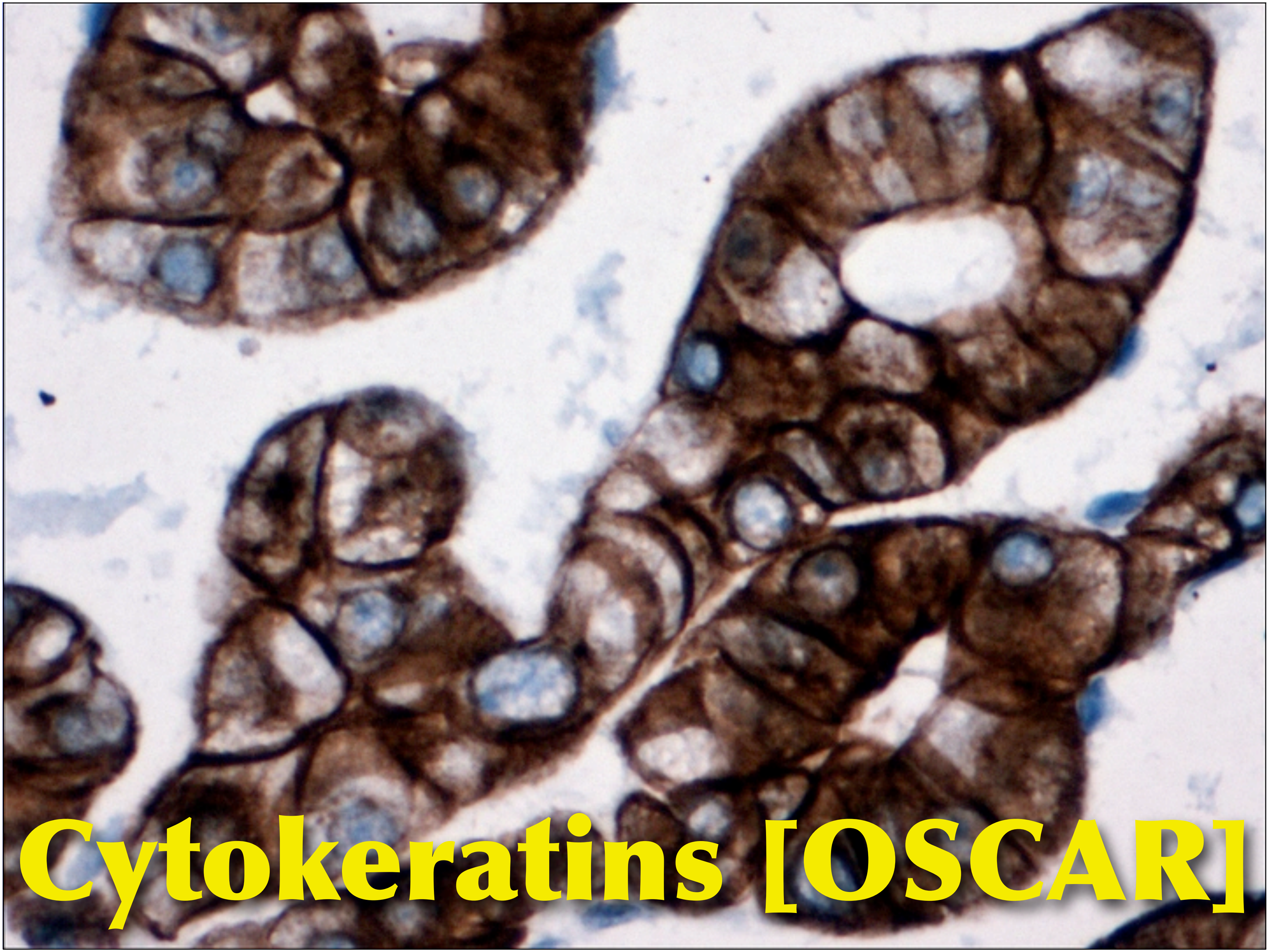


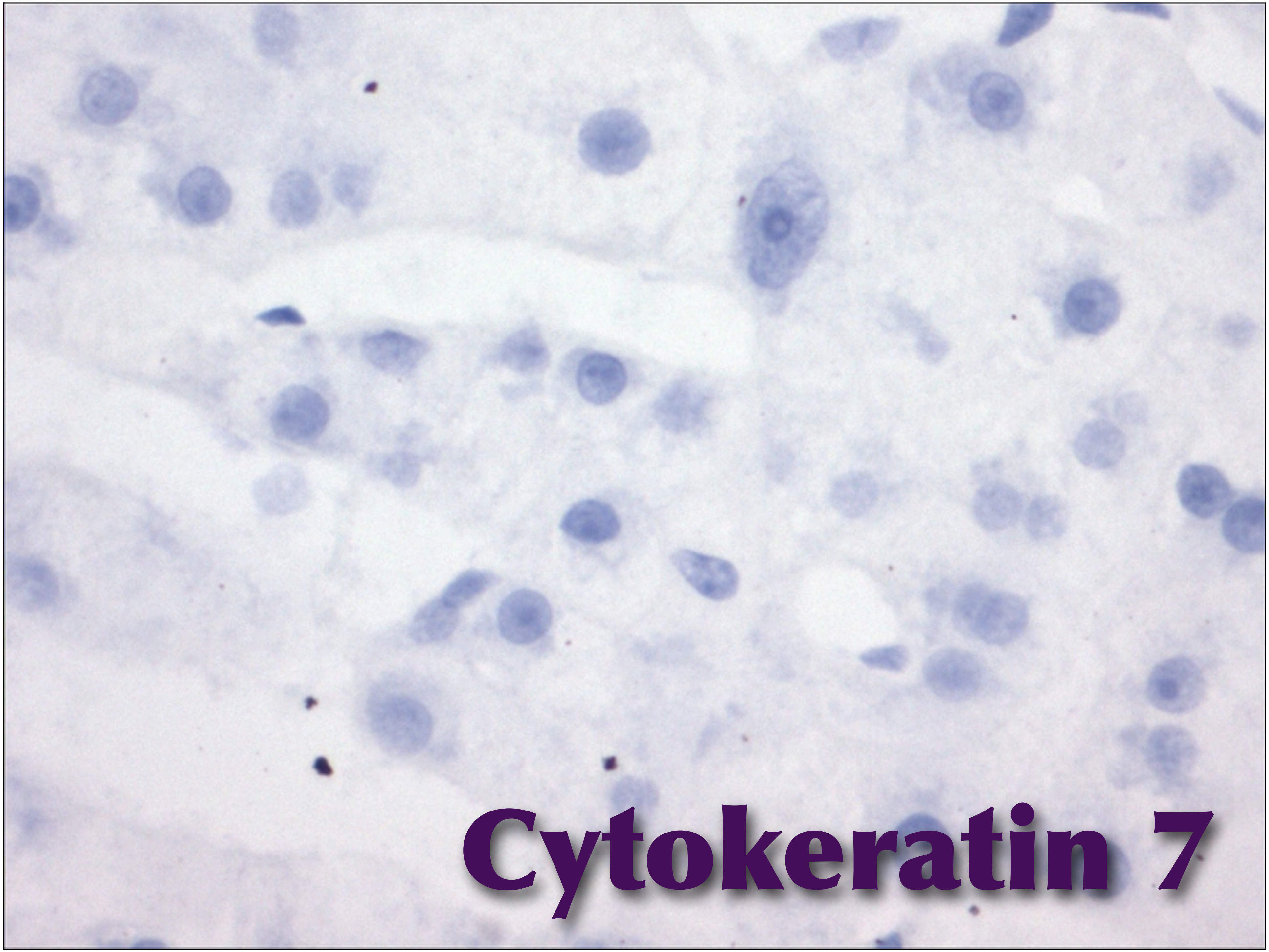


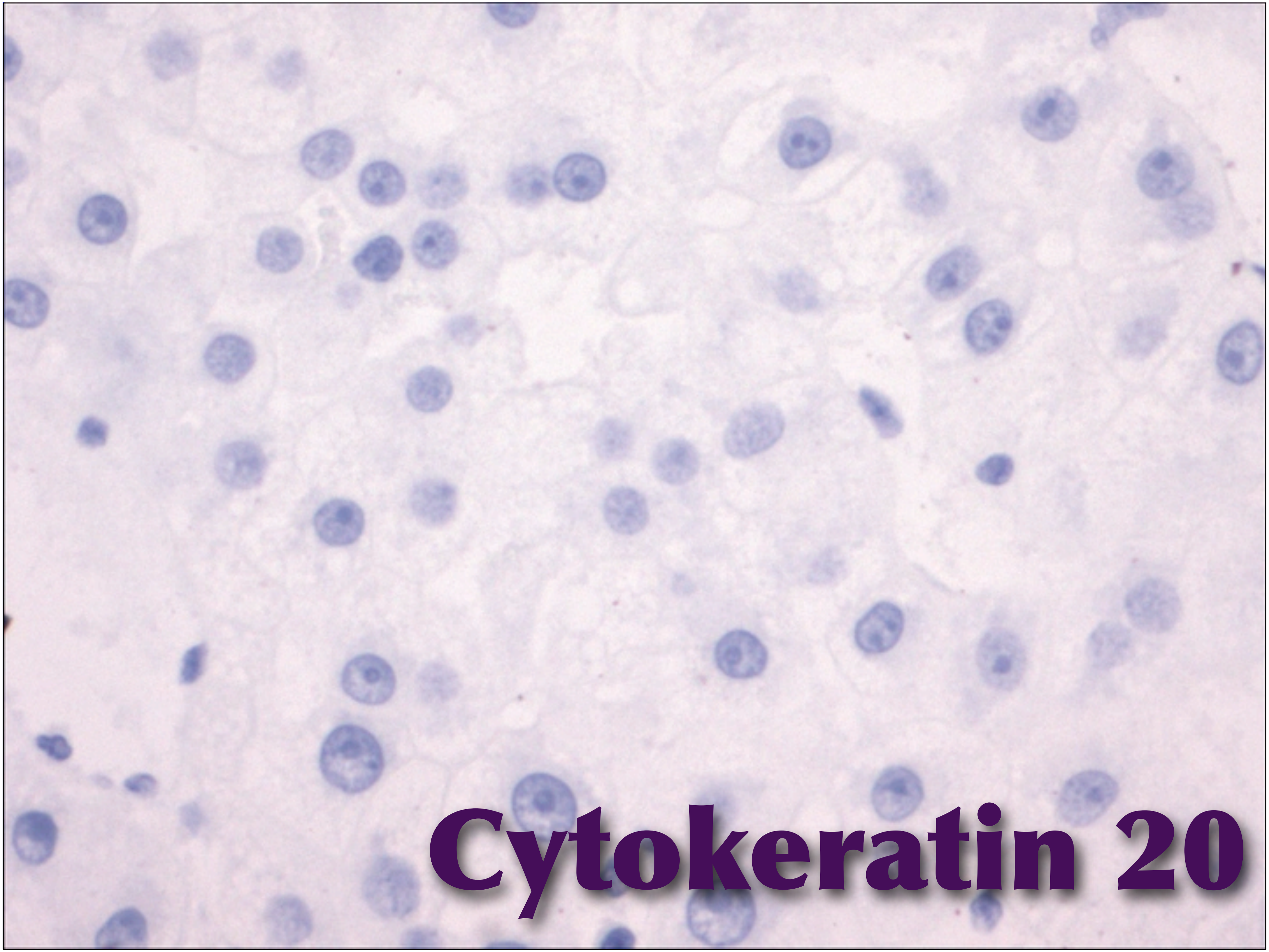


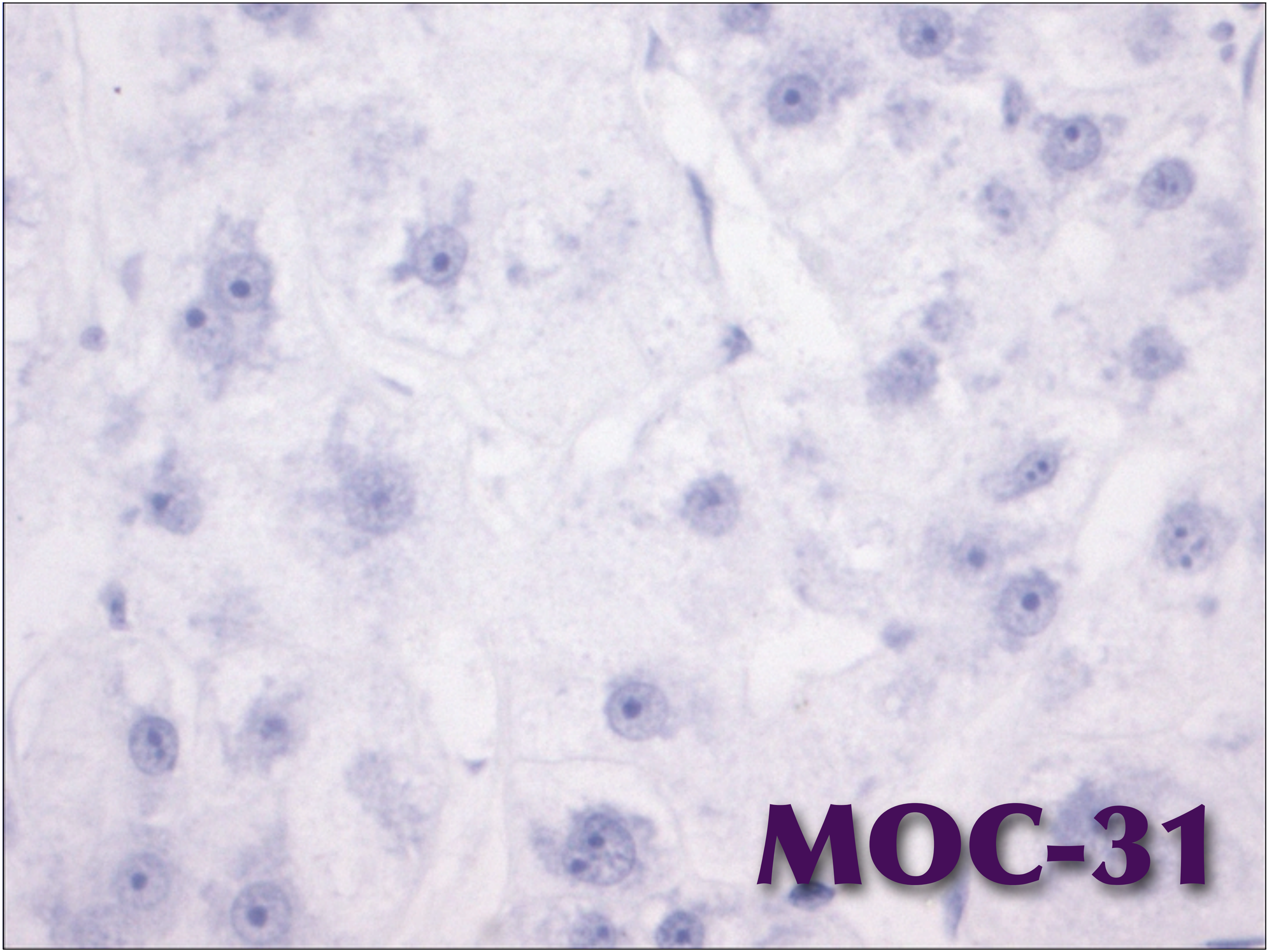
Hepatocellular vs. Metastatic Pancreatic CA

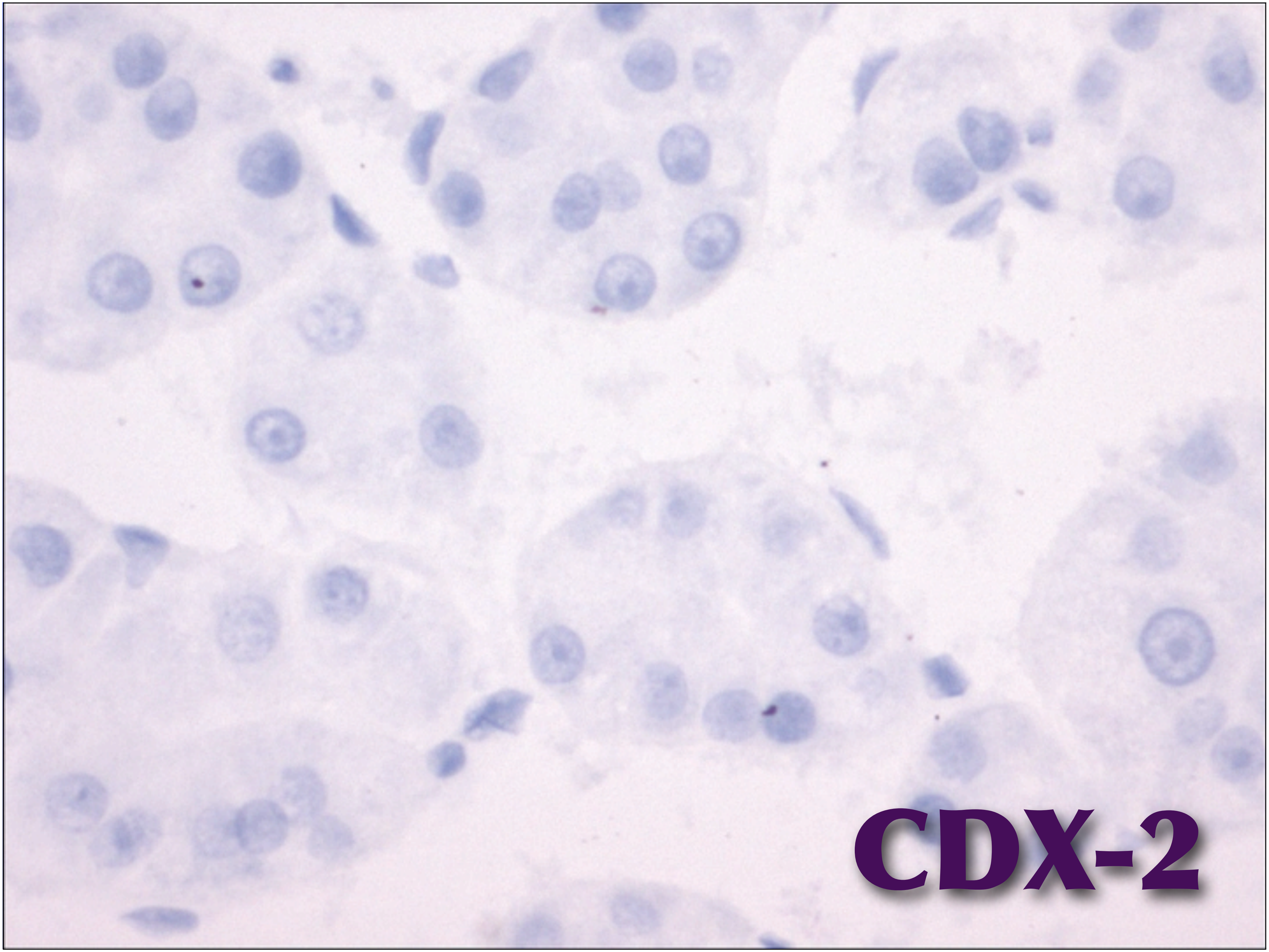
	HCC	Pancreat
Cytokeratin 7	❍	❋
Cytokeratin 20	❍	❋
HepPar1	●	❍
Bile canalicular 'CEA'	●	❍
Villin, CDX-2	❍	❋
Cytokeratin 17	❍	❋

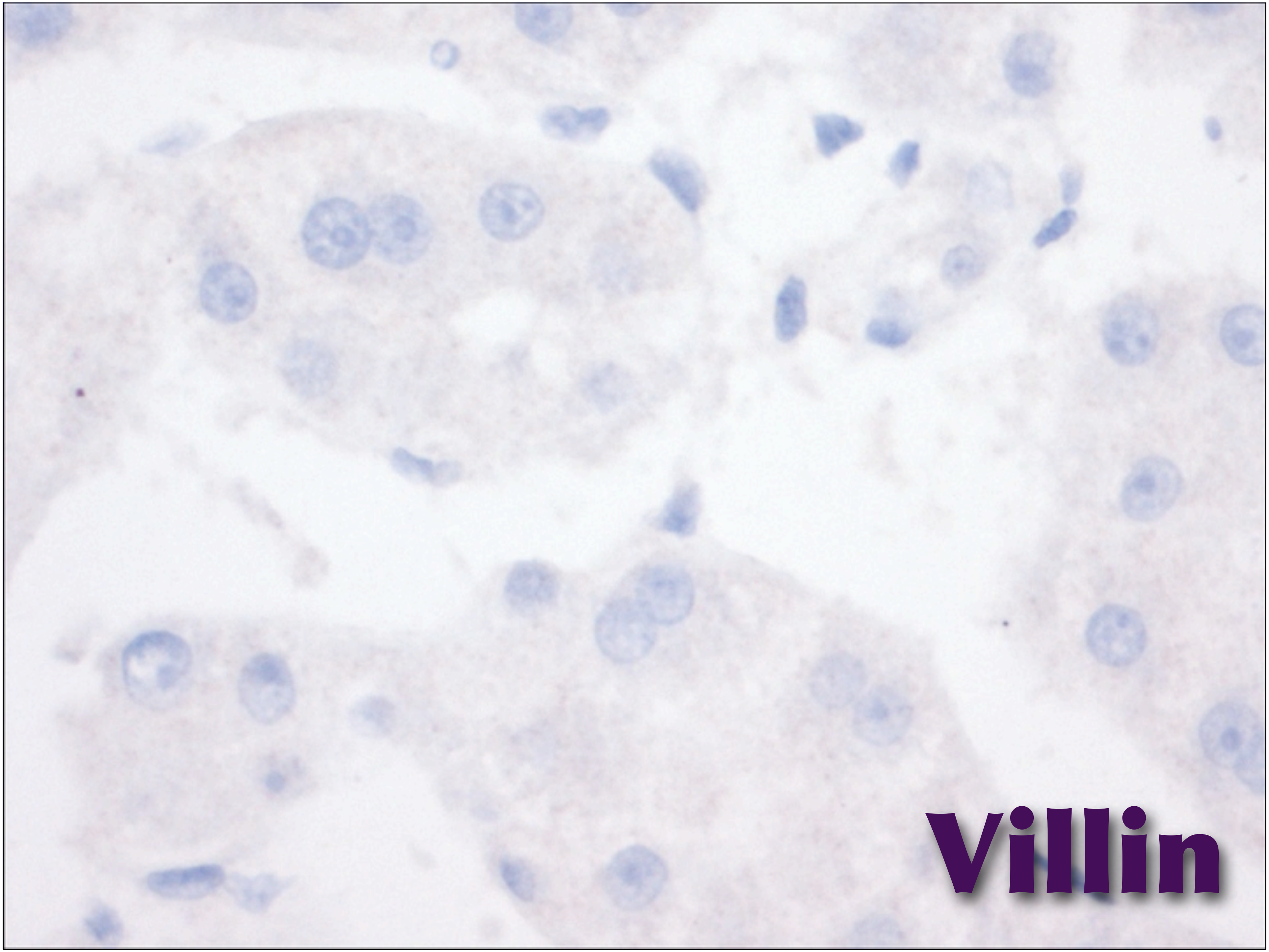

Cytokeratins [OSCAR]

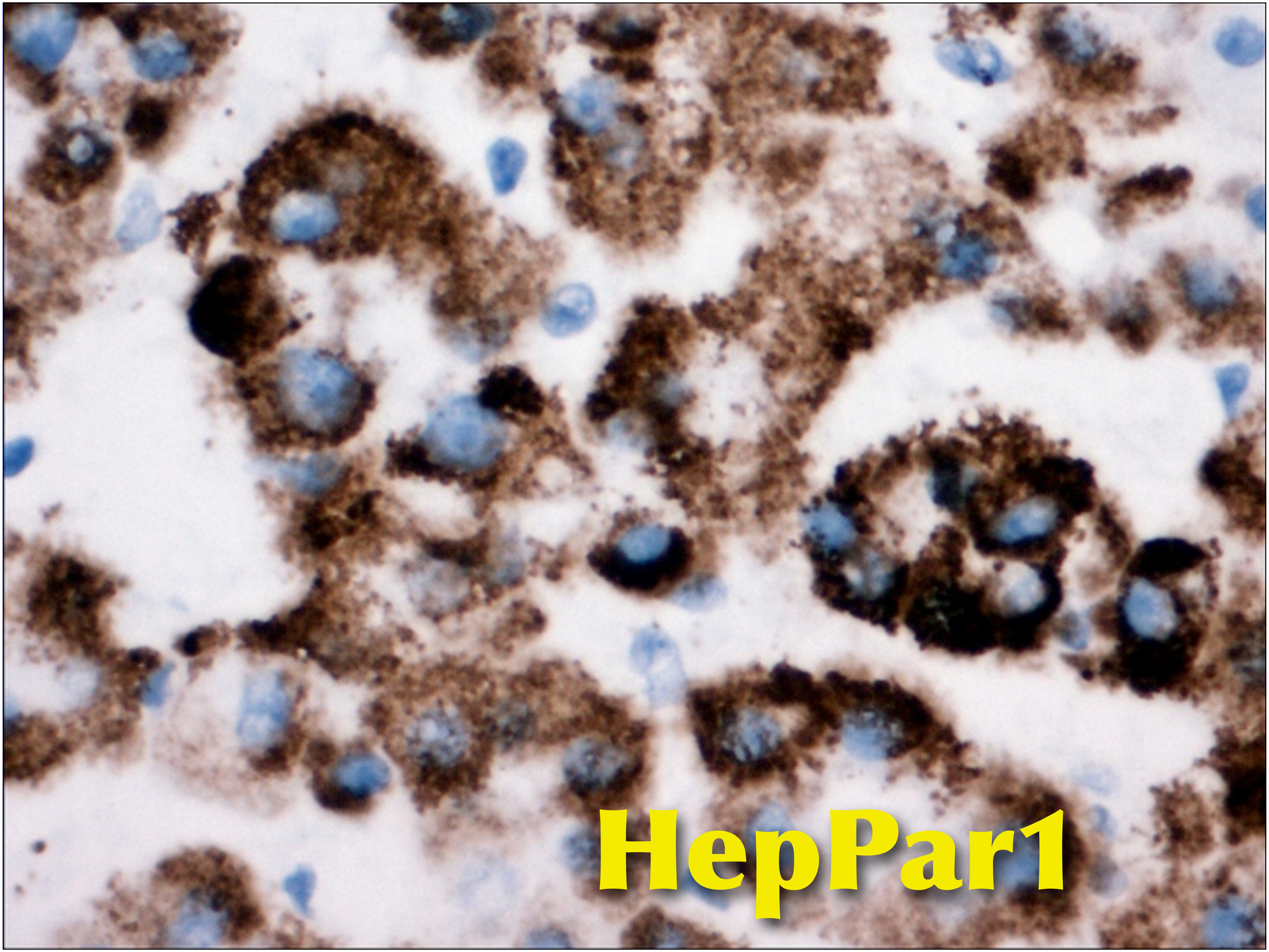

Cytokeratin 7


Cytokeratin 20


MOC-31


CDX-2


Villin


HepPar1

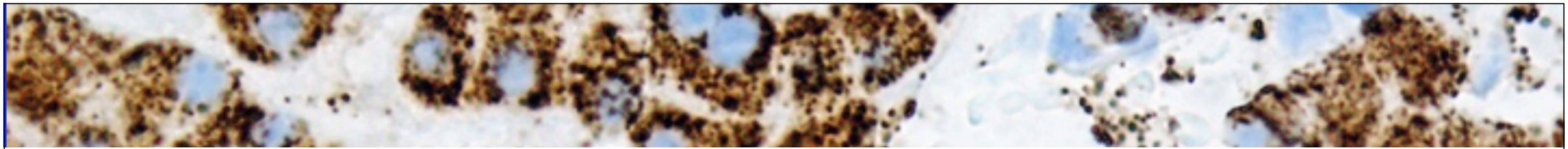
Hepatocellular Carcinoma

Hepatocellular Carcinoma Markers

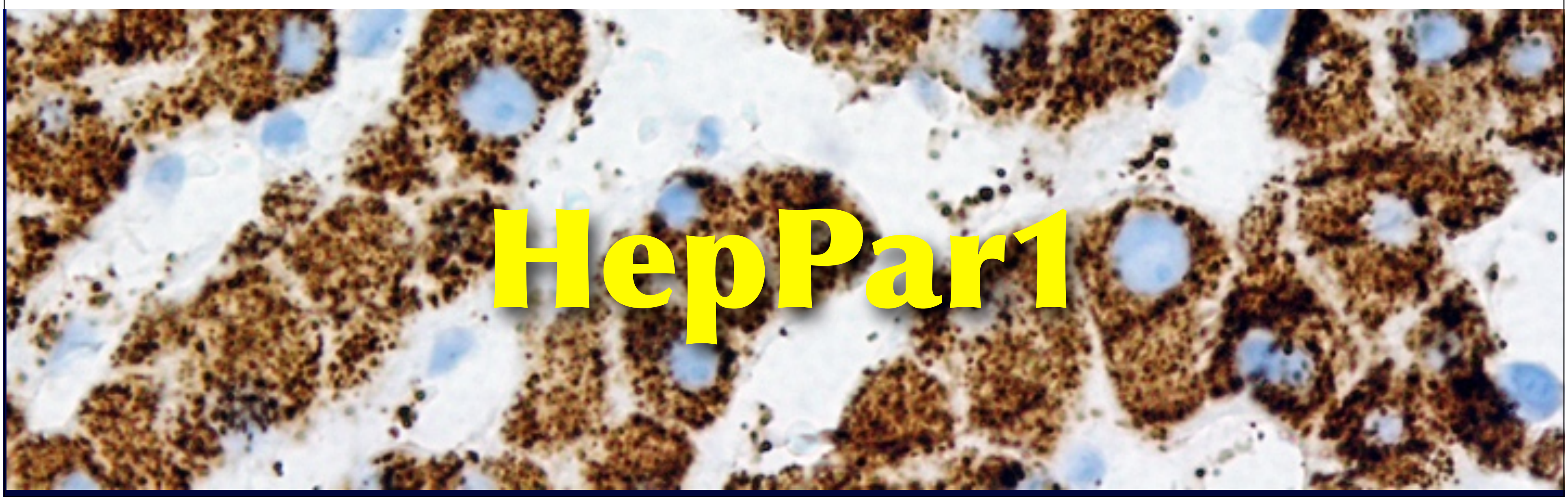
- Cytokeratin 7-negative, cytokeratin 20-negative
- CSP-1 positive (HepPar1)
- Presence of CEA+, CD10+ bile canaliculi
- Presence of CD34+ sinusoidal lining cells
- ~~Alpha fetoprotein~~

Cytokeratin 7-Negative Cytokeratin 20-Negative Carcinomas

- Hepatocellular carcinoma
- Renal cell carcinoma
- Prostatic adenocarcinoma
- Neuroendcrine carcinoma
- Squamous cell carcinoma
- Subset of noncolorectal GI adenoCA



Hepatocellular CA Marker



HepPar1

- Liver-specific marker defined by antibody HepPar1
- Minervi et al (1997) sensitivity 82%, specificity 90%
- Helpful in distinguishing metastatic carcinomas to liver from primary hepatocellular carcinoma

HepPar1

CAVEATS

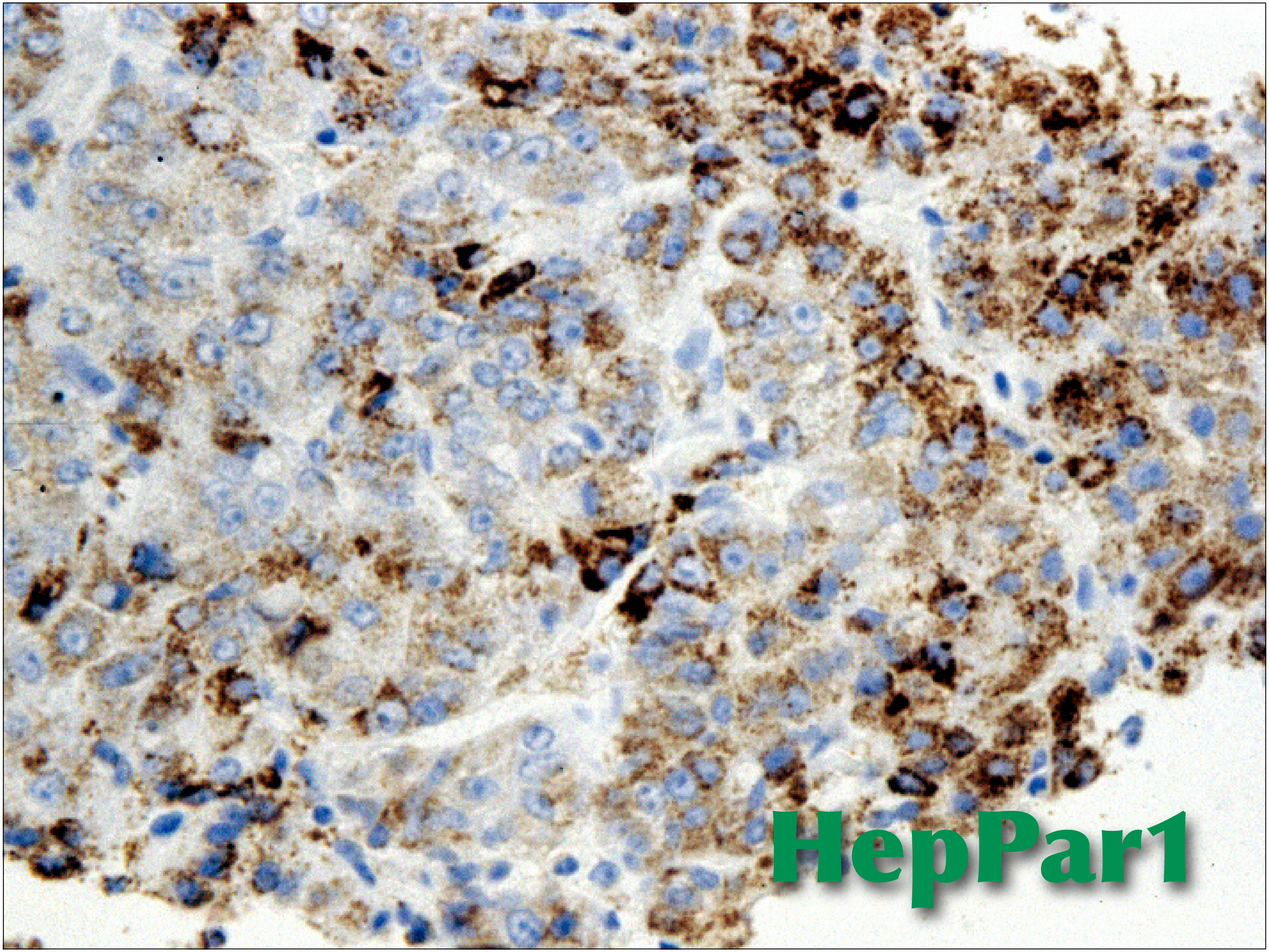
- More likely to be negative in poorly differentiated and sclerosing hepatocellular carcinomas
- Patchy immunostaining seen in about 20% of hepatocellular carcinomas - beware of false negatives in needle core biopsies

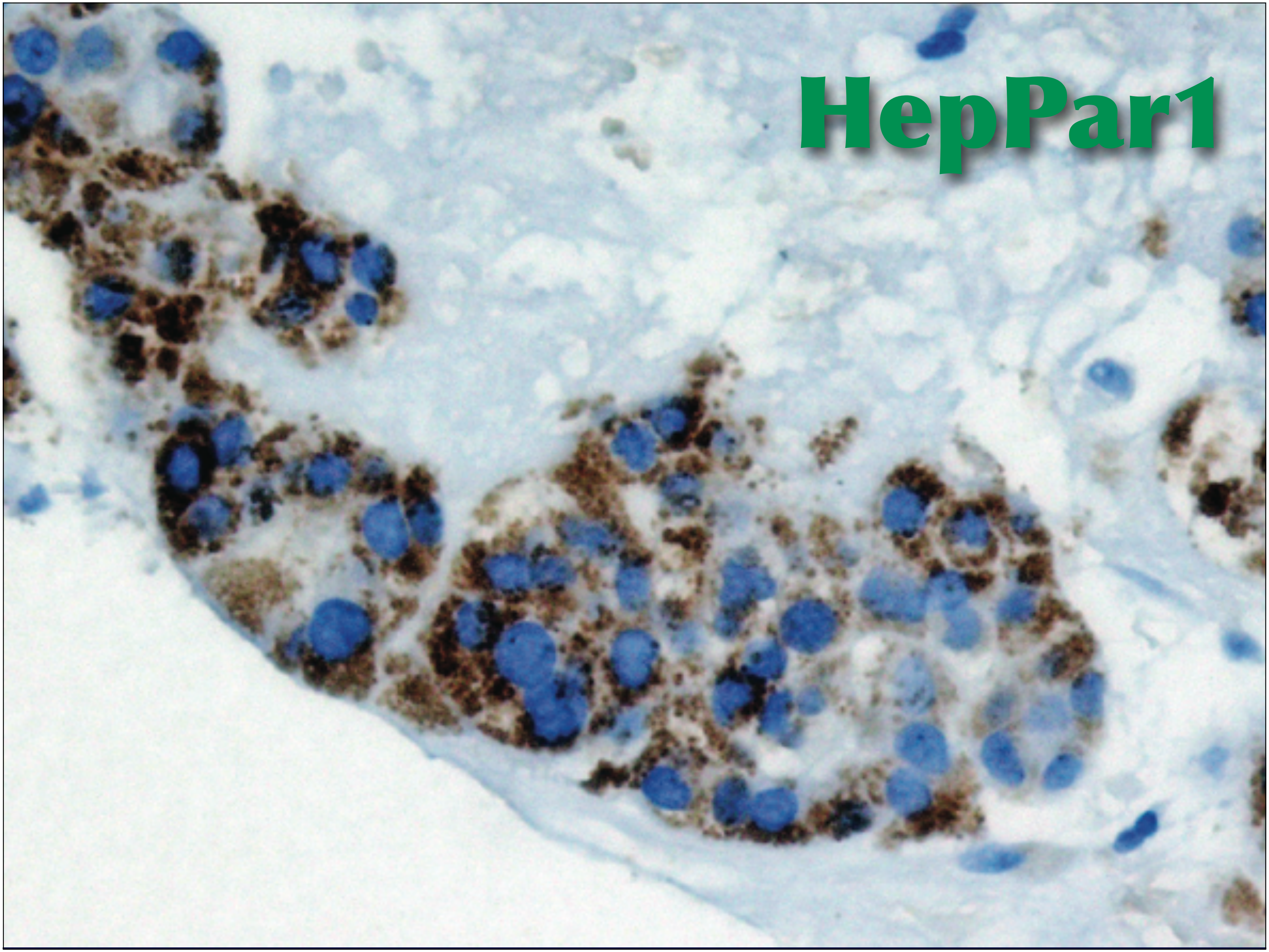
Lab Invest 88:78-88, 2008

The antigen for Hep Par 1 antibody is the urea cycle enzyme carbamoyl phosphate synthetase 1

Samantha L Butler*, Huijia Dong*, Diana Cardona, Minghong Jia, Ran Zheng, Haizhen Zhu, James M Crawford and Chen Liu

- 165 kd protein
- CSP1 (carbamoyl phosphate synthase 1), rate limiting enzyme in urea cycle (converting ammonia to urea is essential function of liver)
- Identified by immunoprecipitation and Western blot analyses, IHC with anti-CSP1


HepPar1


HepPar1

"False Positive" HepPar1 Immunostaining

- Gastric, esophageal, and lung adenocarcinomas can show strong positive immunostaining in minority of cases
- Given high frequency of metastatic CA v. primary hepatocellular CAs, predictive value of HepPar1 by itself not very high

Am J Surg Pathol 26:978-88, 2002

Hepatocyte Antigen as a Marker of Hepatocellular Carcinoma

An Immunohistochemical Comparison to Carcinoembryonic Antigen, CD10, and Alpha-Fetoprotein

Peiguo G. Chu, M.D., Ph.D., Shin Ishizawa, M.D.,
Emerald Wu, B.S., HT (ASCP), and Lawrence M. Weiss, M.D.

- N = 96 cases of hepatocellular carcinoma
- N = 311 cases of nonhepatic epithelial tumors

HepPar1 Positive Tumors

Hepatocellular CA	88/96	92%
Lung, liver GI neuroendocrine	4/9	44%
Gastric CA	4/13	31%
Lung CA	5/21	24%
Ovarian CA	4/24	16%
Lung and GI carcinoid	1/10	10%
Pancreatic CA	1/13	8%
CholangioCA	1/14	7%

Chu et al., Am J Surg Pathol 26:978-88, 2002

HepPar1 Negative Tumors

Breast CA	0/9	0%
Colon CA	0/10	0%
Renal Cell CA	0/10	0%
Germ Cell Tumor	0/14	0%
Lung, Skin Small Cell CA	0/15	0%
Salivary Gland CA	0/19	0%
Mesothelioma	0/16	0%
Prostate	0/7	0%

Chu et al., Am J Surg Pathol 26:978-88, 2002

Comparative Sensitivities

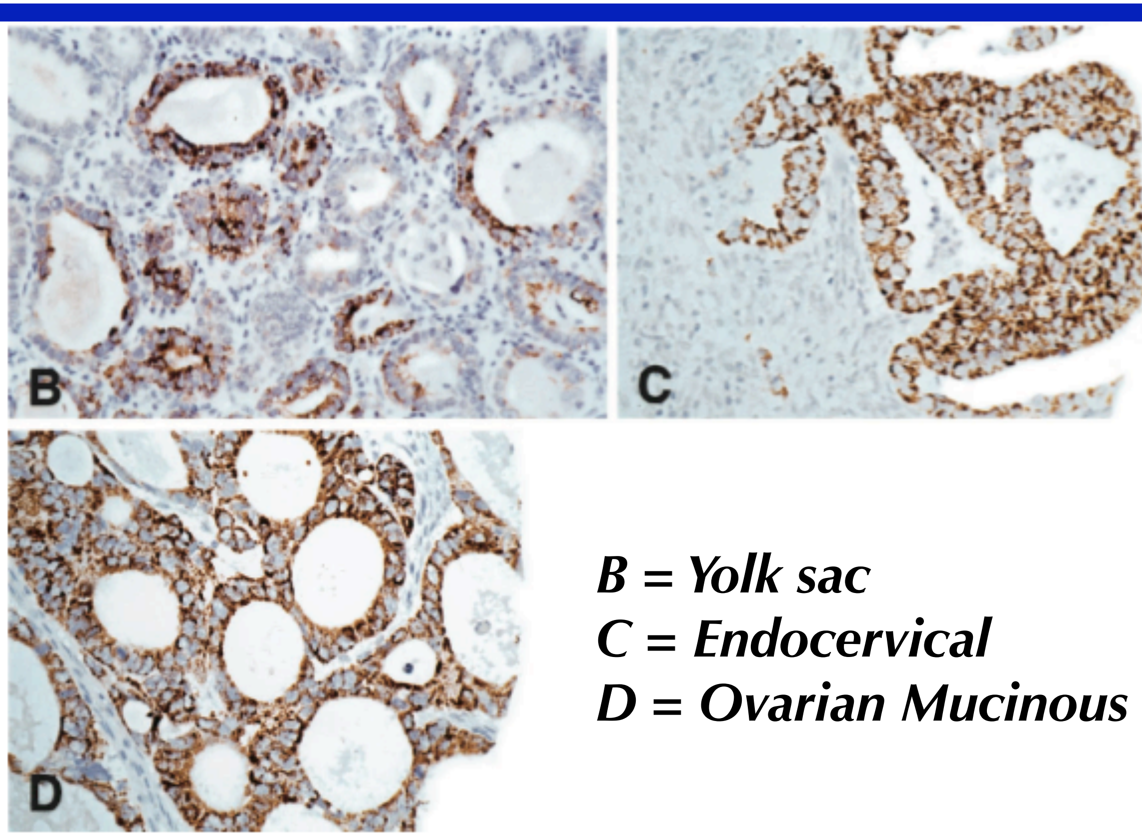
HepPar1	92%
CEA - bile canaliculi	76%
AFP	31%

Chu et al., Am J Surg Pathol 26:978-88, 2002

Mod Pathol 16:137-44, 2003

Hep Par 1 Antibody Stain for the Differential Diagnosis of Hepatocellular Carcinoma: 676 Tumors Tested Using Tissue Microarrays and Conventional Tissue Sections

Zhen Fan, M.D., Matt van de Rijn, M.D., Ph.D., Kelli Montgomery, Robert V. Rouse, M.D.
Deparent of Pathology, Stanford University Medical Center, Stanford, California



B = Yolk sac
C = Endocervical
D = Ovarian Mucinous

HepPar1 Positive Tumors

Hepatocellular CA	18/19	95%
Gastric CA	16/34	47%
Lung Adenocarcinoma	2/34	6%
Ovarian CA (CC, Muc)	5/13	38%

Fan Z et al., Mod Pathol 16:137-44, 2003

Fan Z et al., Mod Pathol 16:137-44, 2003

- HepPar1 a very useful marker in differential diagnosis of hepatocellular carcinoma
- HepPar1 has significant limitations
- Best used in panel with other antibodies, e.g., to alpha fetoprotein, CD10, CEA

Arch Pathol Lab Med 131:1648-54

Best Practices in Diagnostic Immunohistochemistry

Hepatocellular Carcinoma Versus Metastatic Neoplasms

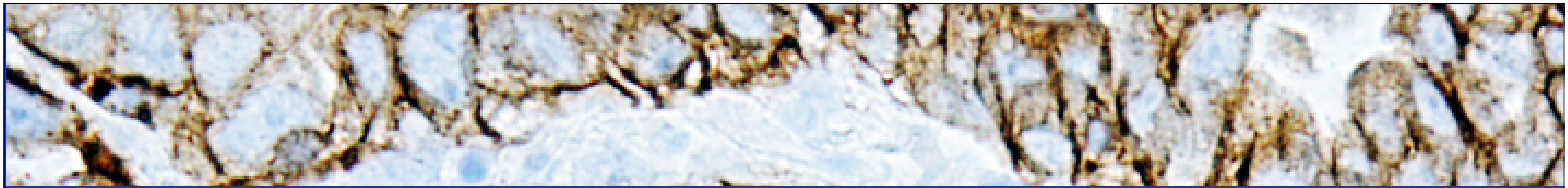
Sanjay Kakar, MD; Allen M. Gown, MD; Zachary D. Goodman, MD; Linda D. Ferrell, MD

HepPar ● MOC31 ○

Hepatocellular carcinoma

HepPar ○ MOC31 ●

Metastatic carcinoma



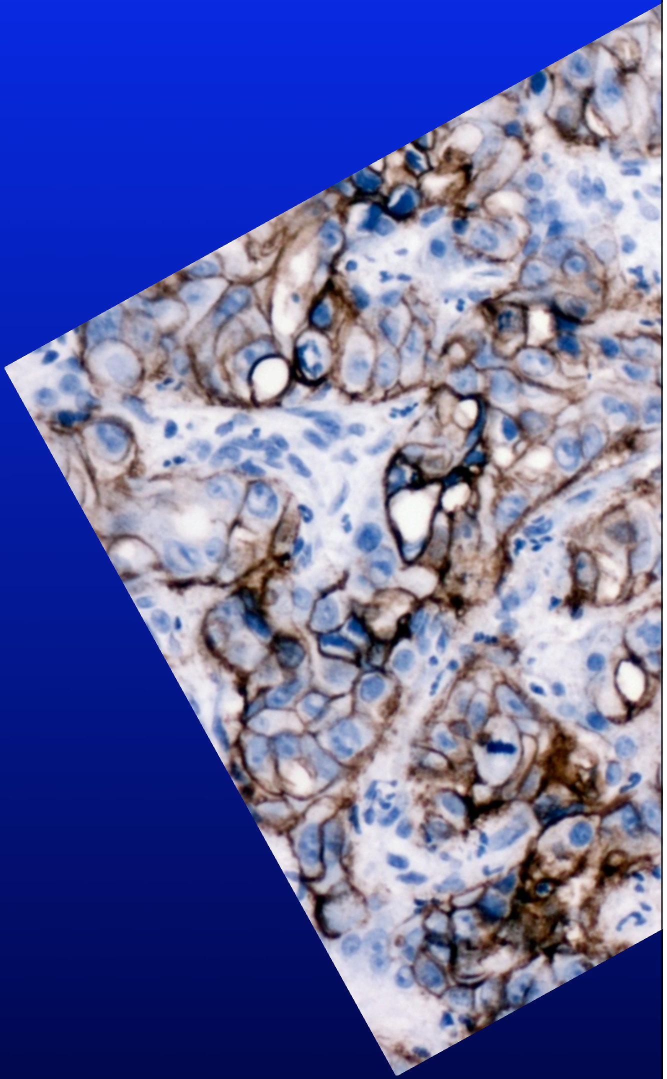
MOC-31 Antibody

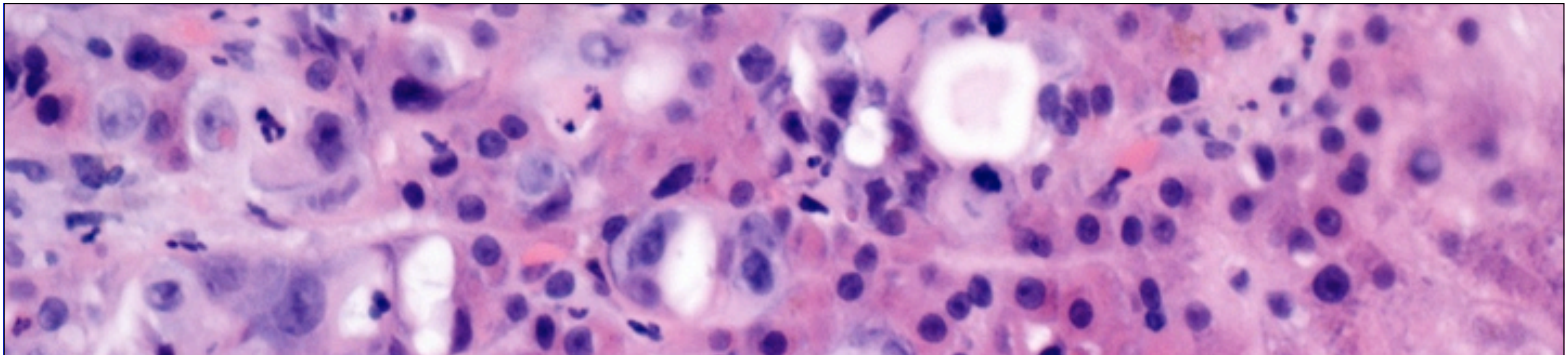
A useful adjunct to distinguish metastatic carcinomas to the liver (positive) from primary hepatocellular carcinomas (negative)

- **Niemann TH et al., Cancer 87:295-8, 1999 (87% specificity)**
- **Morrison C et al., Mod Pathol 15:1279-87, 1999 (96% specificity)**
- **Lau SK et al., Hum Pathol 33:1175-81, 2002**

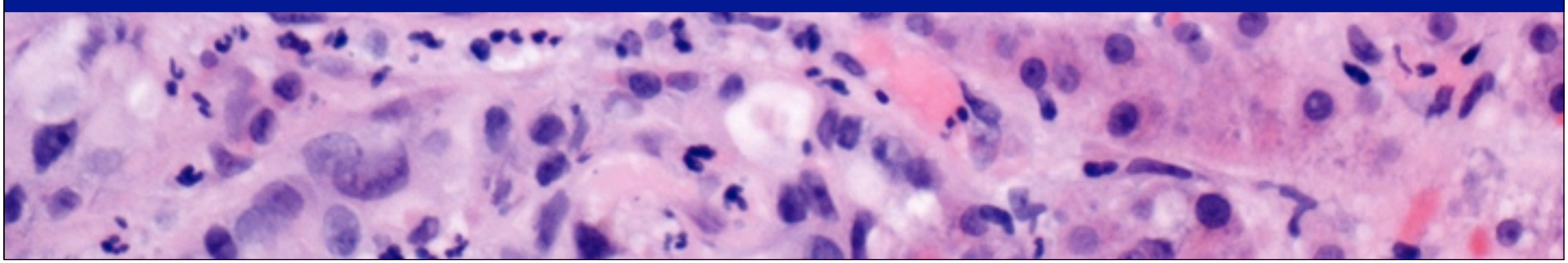
What is MOC-31?

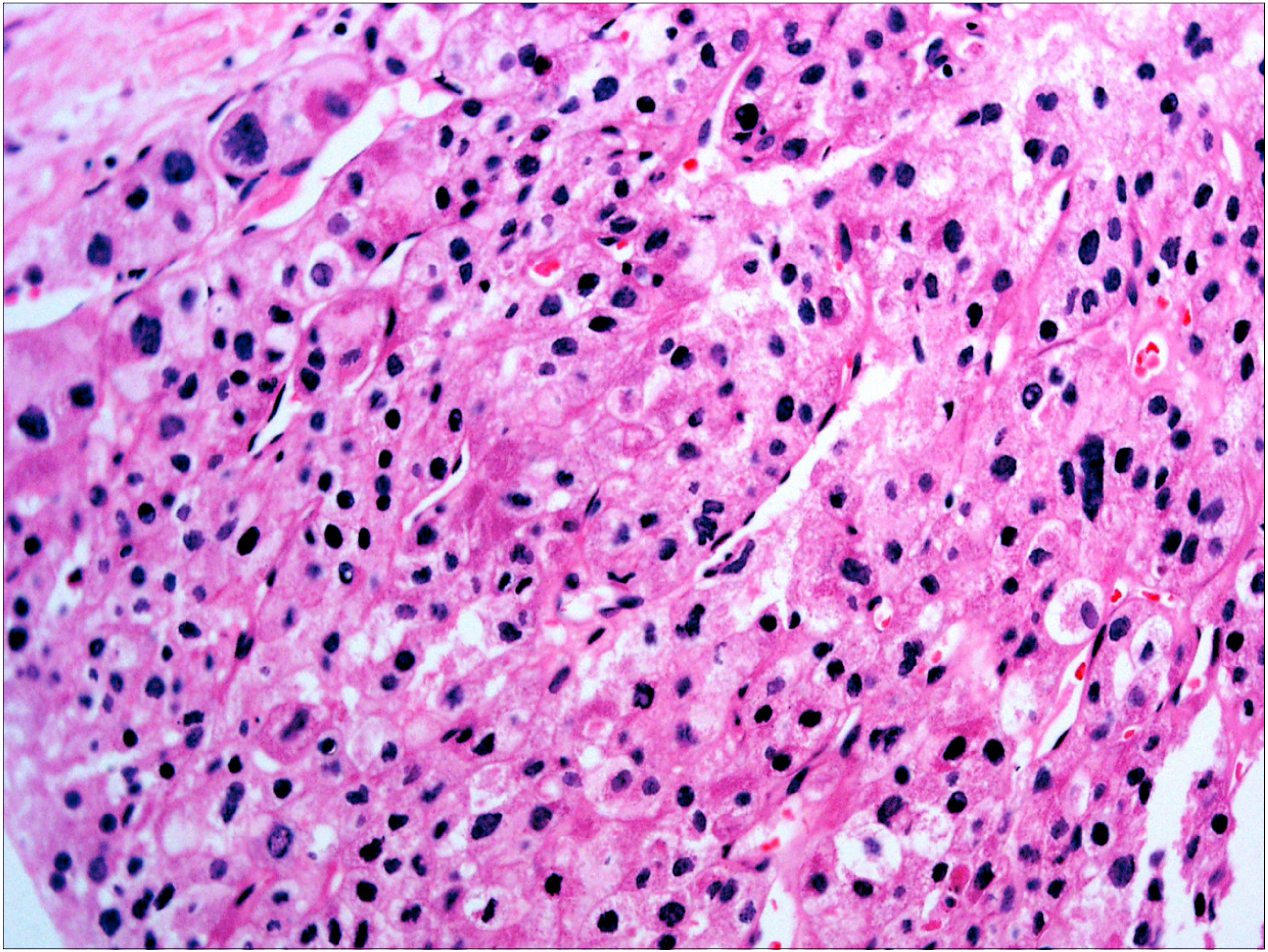
- Not a target molecule but the clone name of a hybridoma
- Categorized as "small cell lung carcinoma-cluster 2" antibody
- Target molecule is 38 kd transmembrane glycoprotein known as "epithelial glycoprotein 2" or EGP-2
- Function of protein unknown

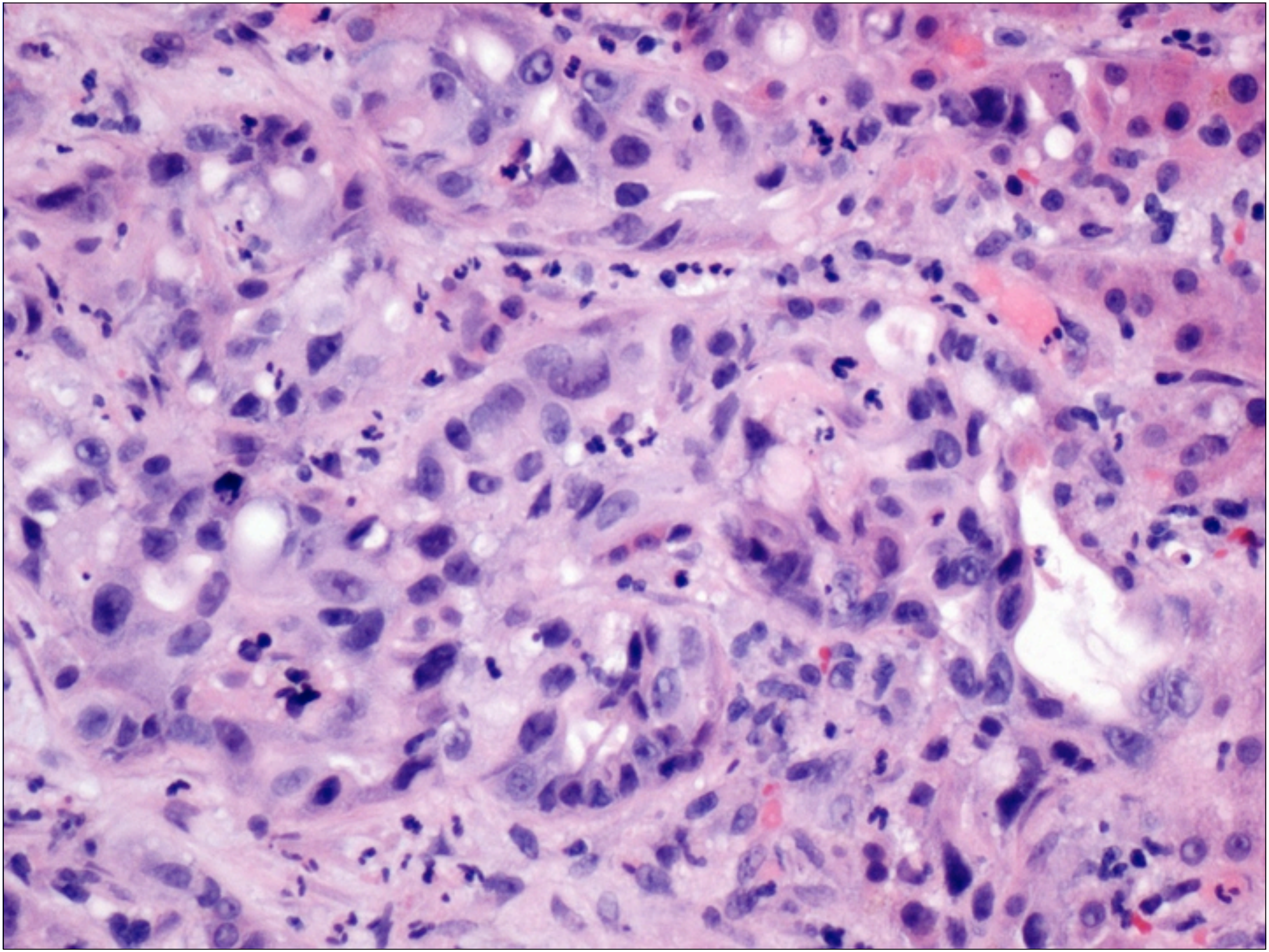


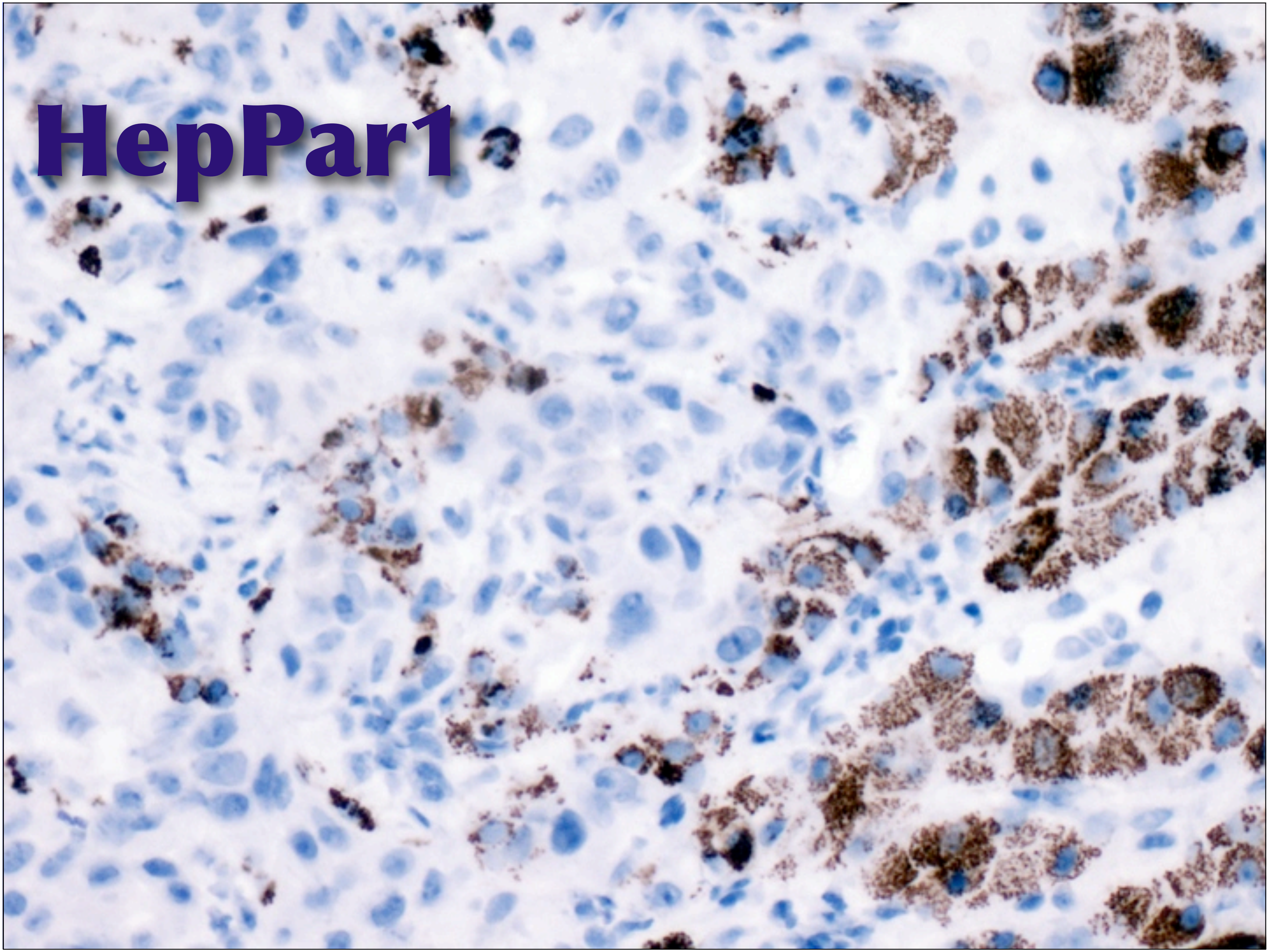


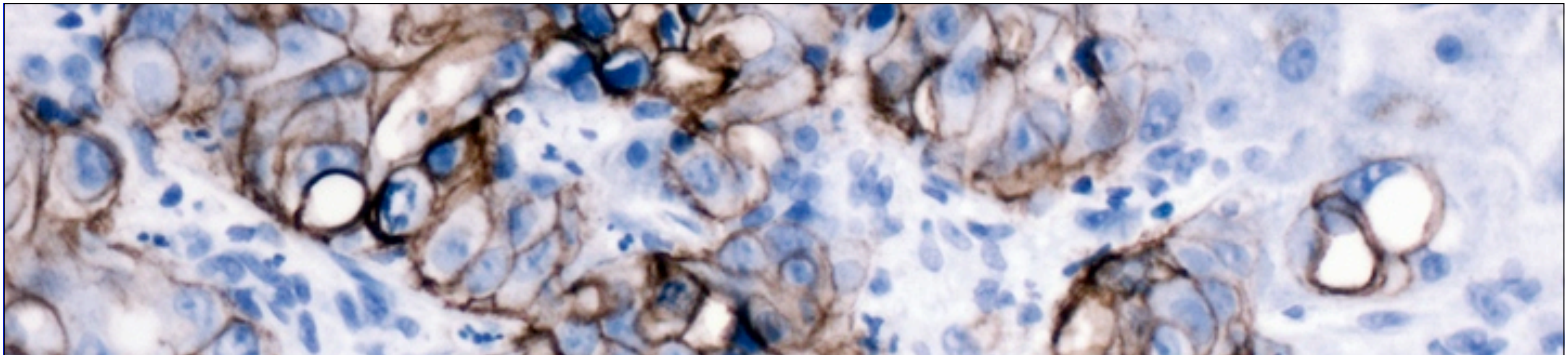
Solitary liver tumor in female with Hepatitis C



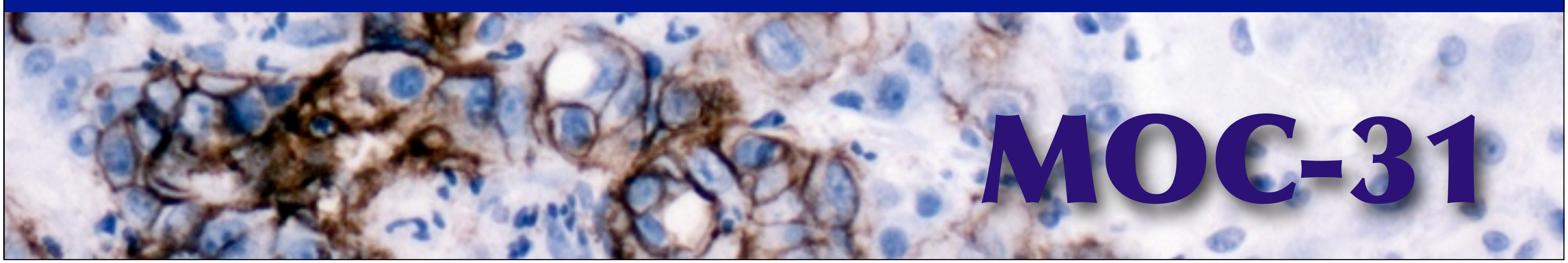


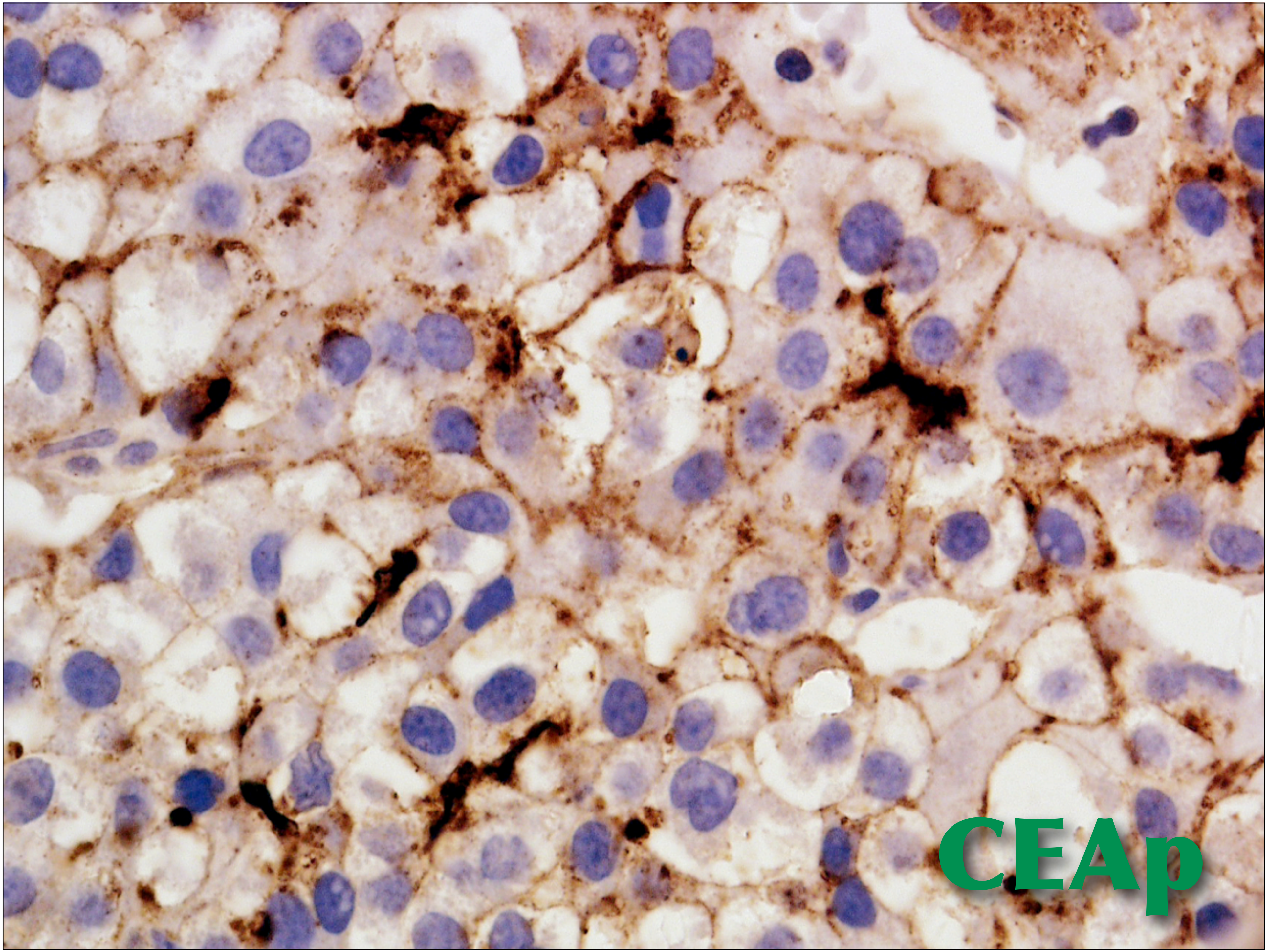


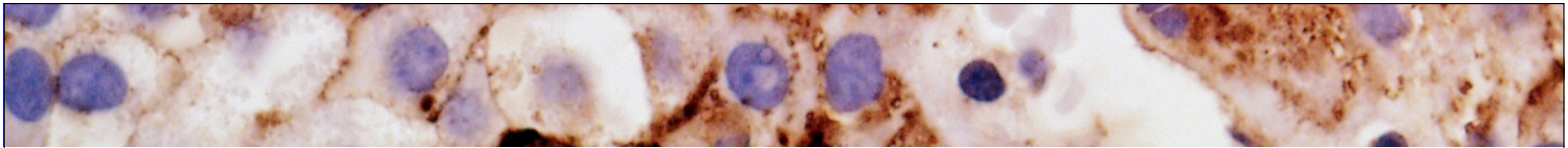

HepPar1



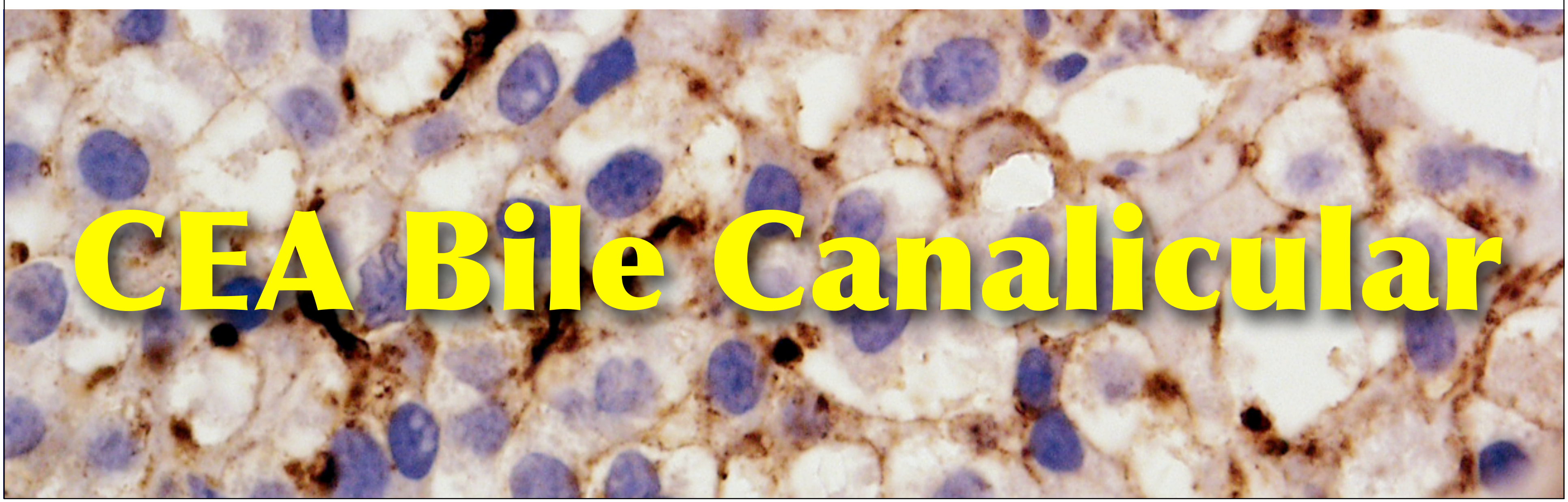
NOT Hepatocellular CA (Metastatic carcinoma)




CEAp



Hepatocellular CA Marker



Bile Canaliculi

- Can be identified with antibodies to CEA (actually BGP not 'true' CEA)
- Can be identified with antibodies to CD10
- Highly specific for hepatocellular carcinoma
- Must not confuse with surface CEA
- Fairly low sensitivity (well below 50%)

Hepatocellular CA Marker

Sinusoidal Pattern of CD34 Positive Vessels

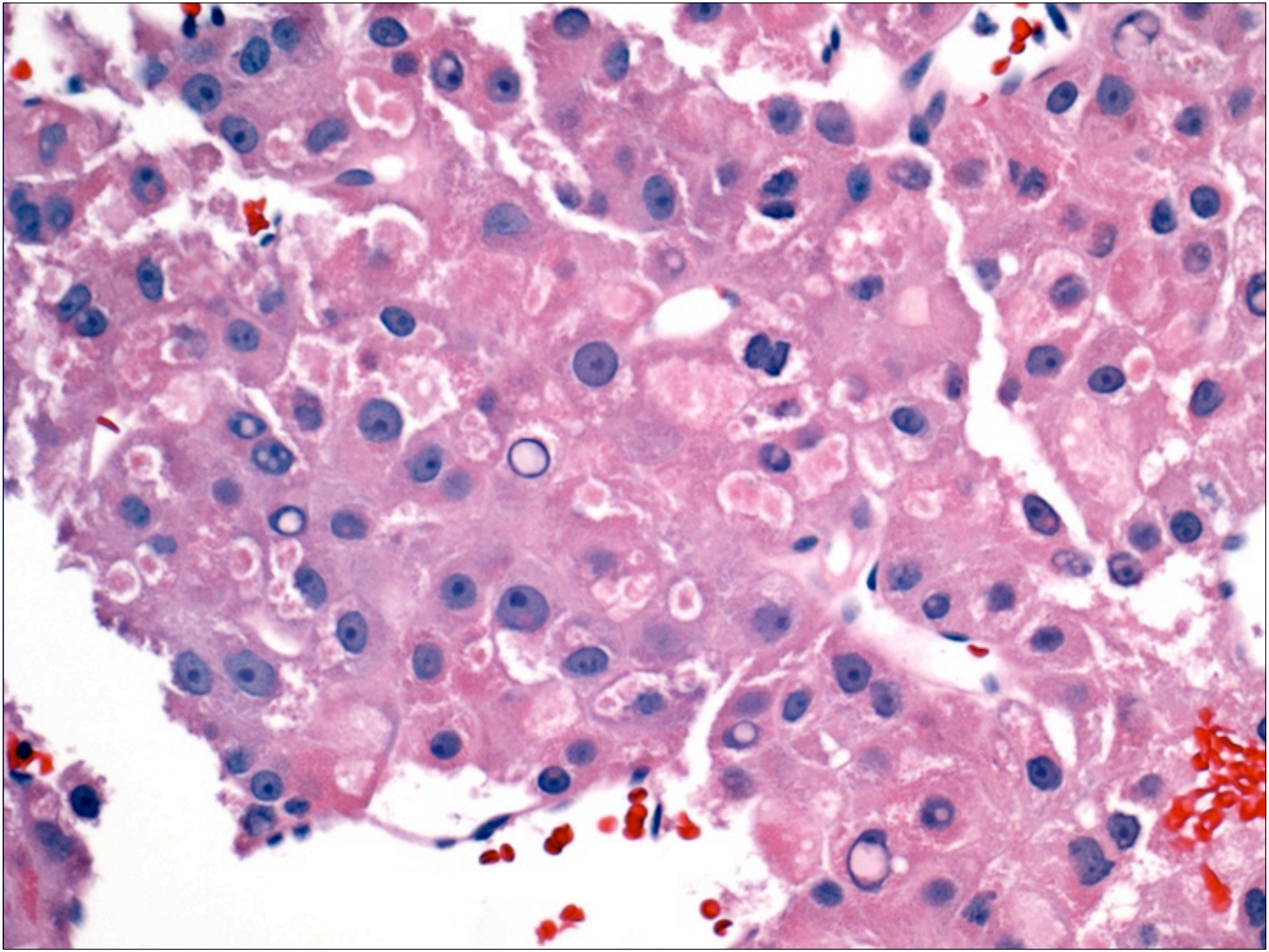
CD34+ Sinusoidal Cells as Marker of Hepatocellular Carcinoma

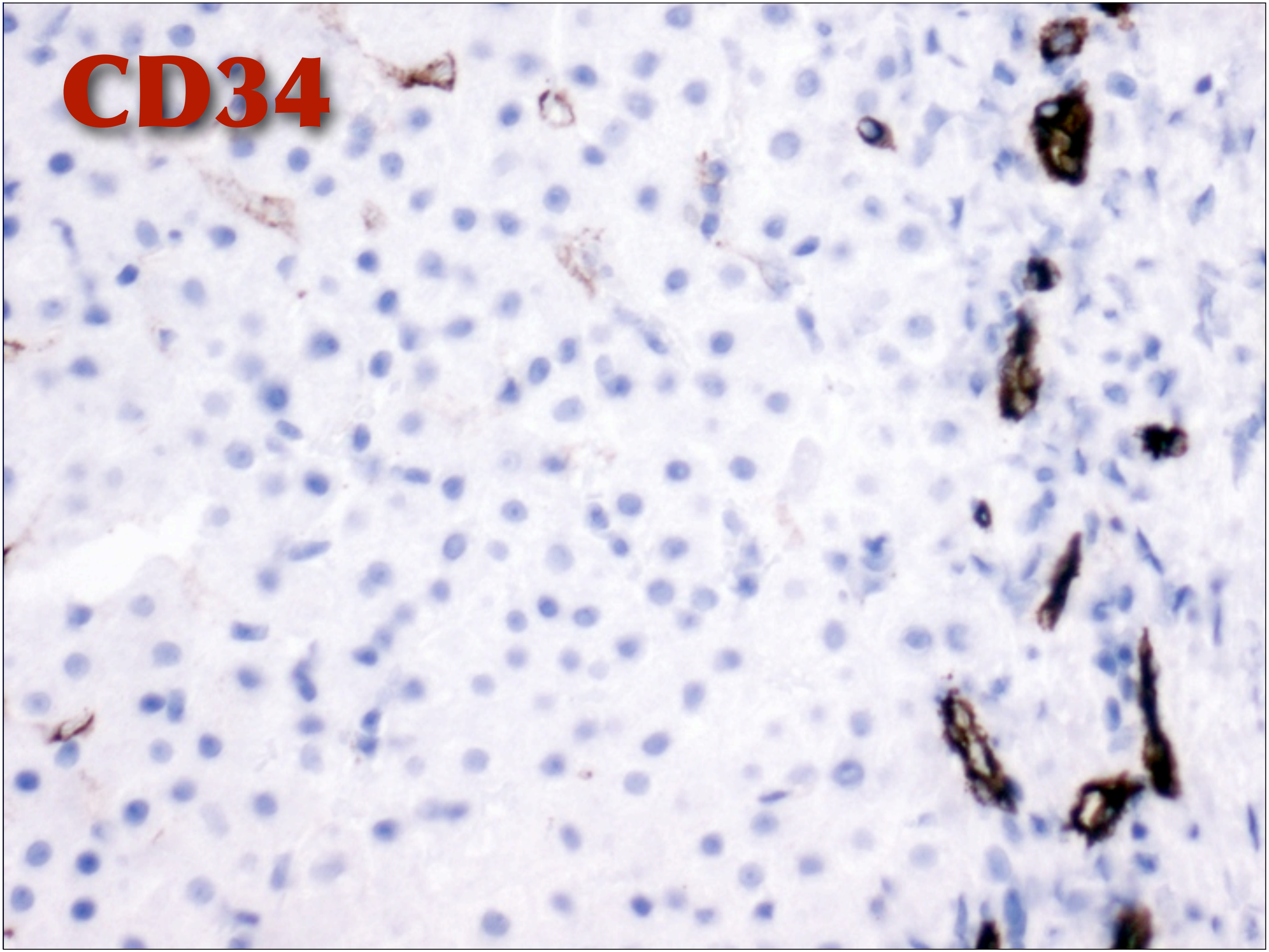
Ruck P et al., Arch Pathol Lab Med 119:173-8, 1995

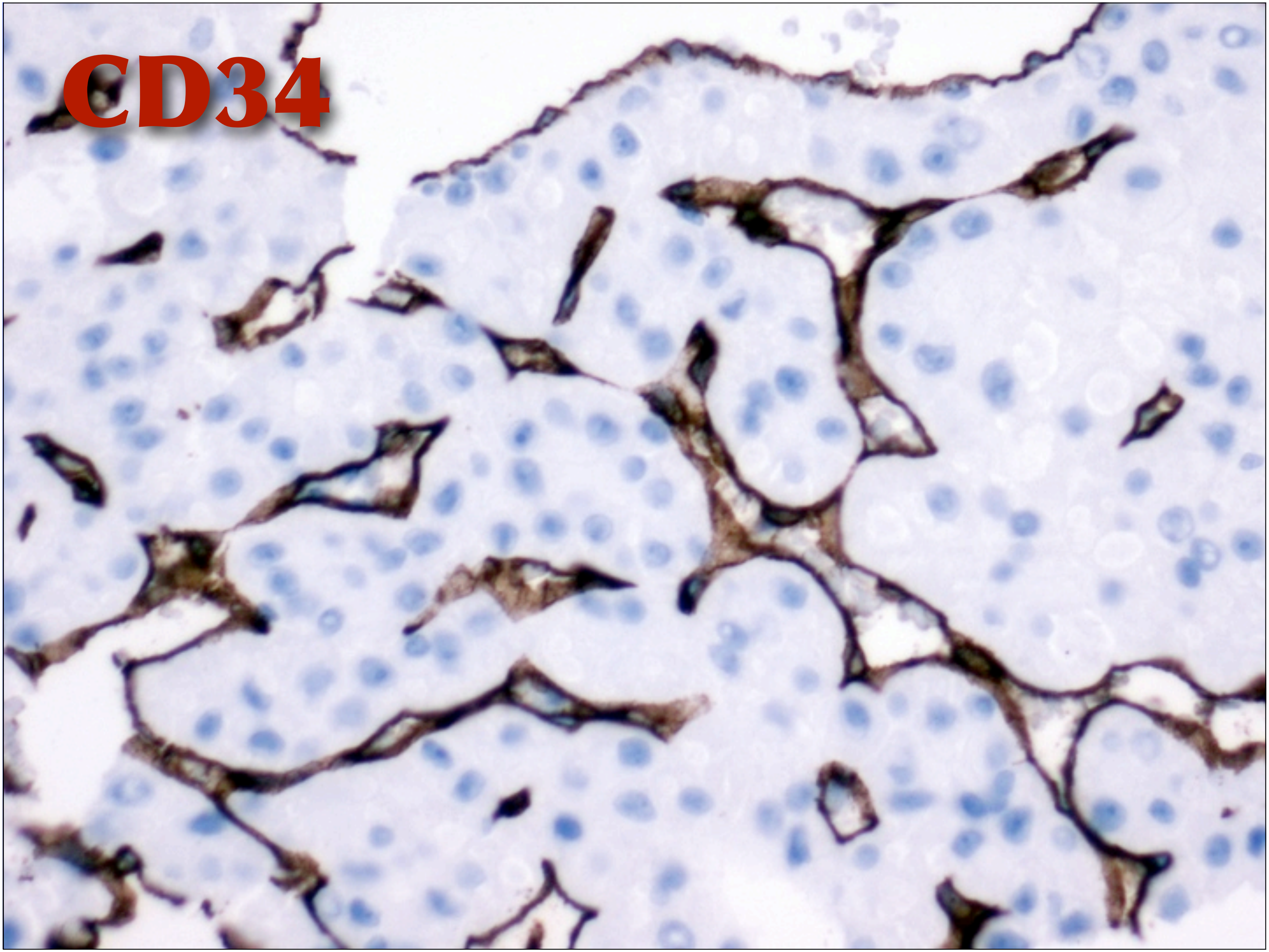
Park YN et al., Am J Surg Pathol 22:656-62, 1998

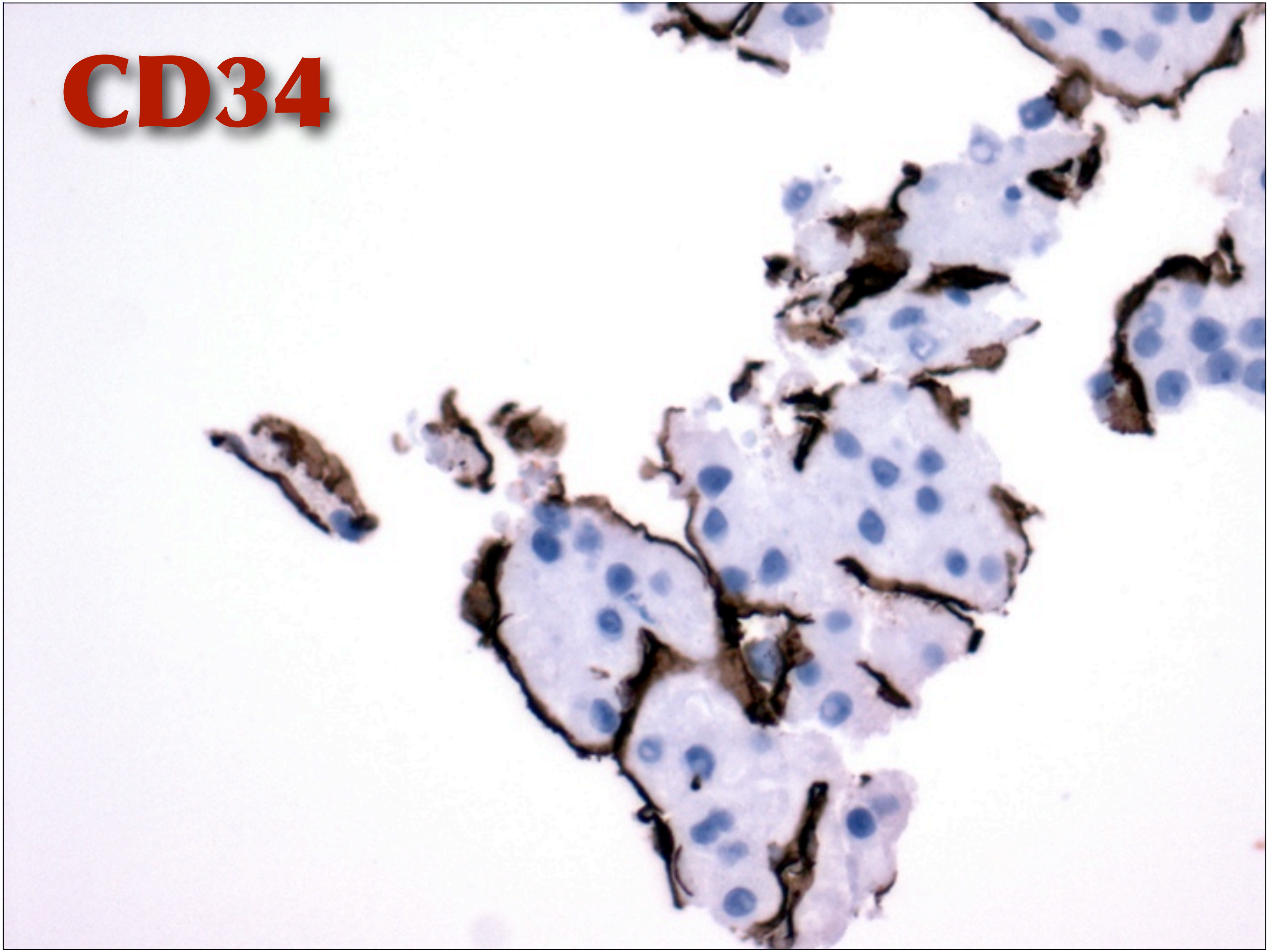
CD34

- Negative on normal hepatocyte sinusoidal endothelial cells
- Positive on small subset of endothelial cells in adenomatous nodule, cirrhotic nodule
- Positive on significant subset of endothelial cells in hepatocellular carcinoma




CD34


CD34


CD34

In Addition....
Hepatocellular Carcinomas Rarely Express

- Cytokeratin 7
- Cytokeration 20
- Cytokeratin 5

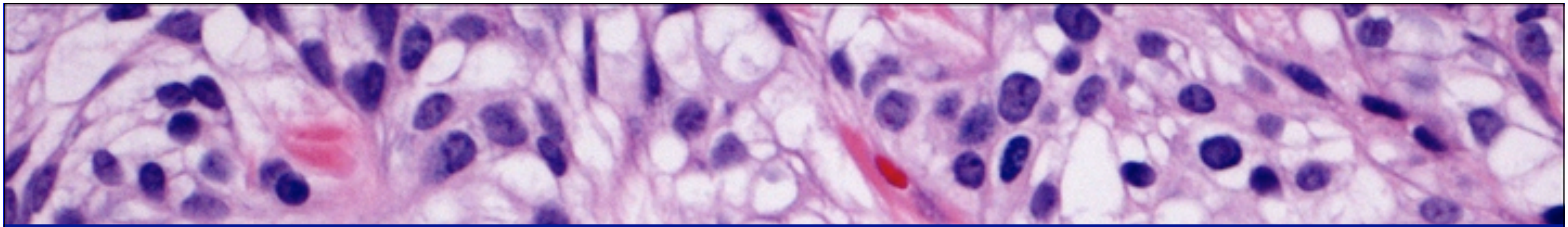
IMMUNOHISTOCHEMISTRY

Selected Topics

- General Issues
- Breast Carcinoma
- GI Tract Tumors
- Tumors in the Liver
- Male GU Tract Tumors

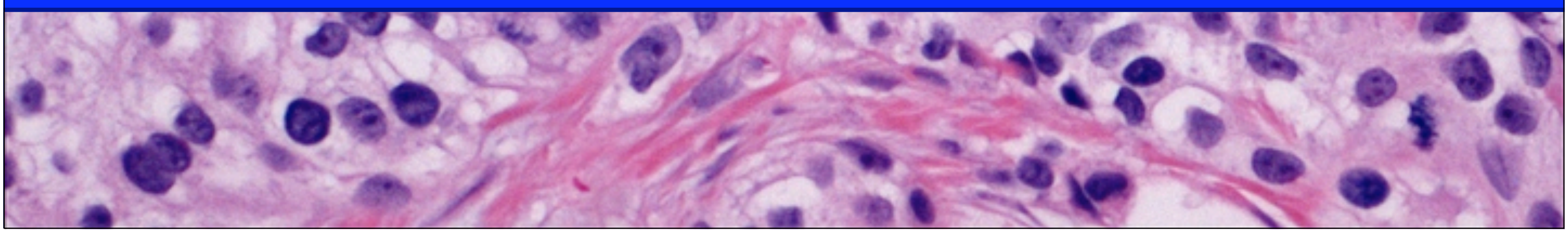
Male GU Tract Tumors

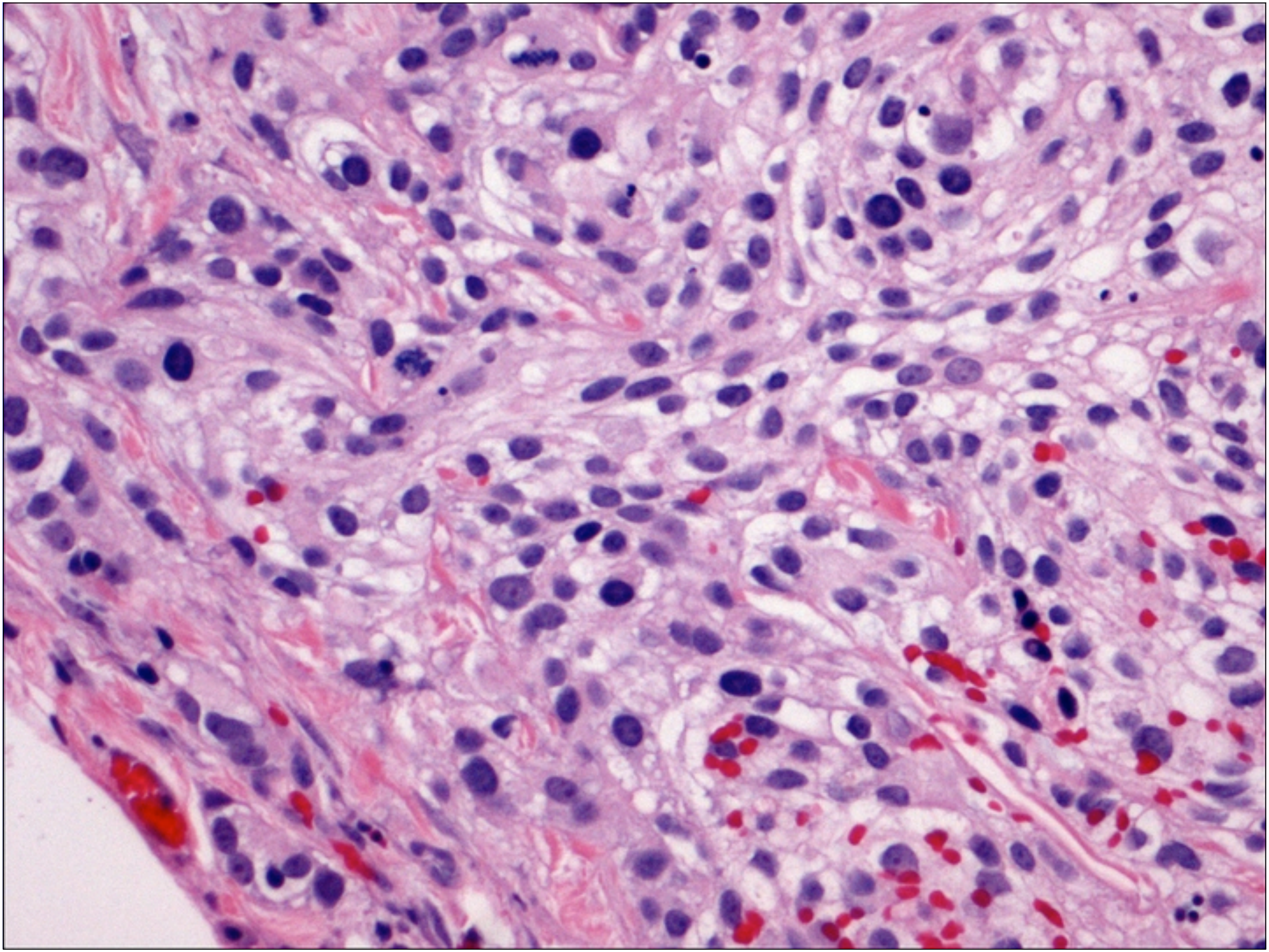
- IHC markers to distinguish prostatic adenocarcinoma from transitional cell
- Prostatic adenocarcinoma caveats

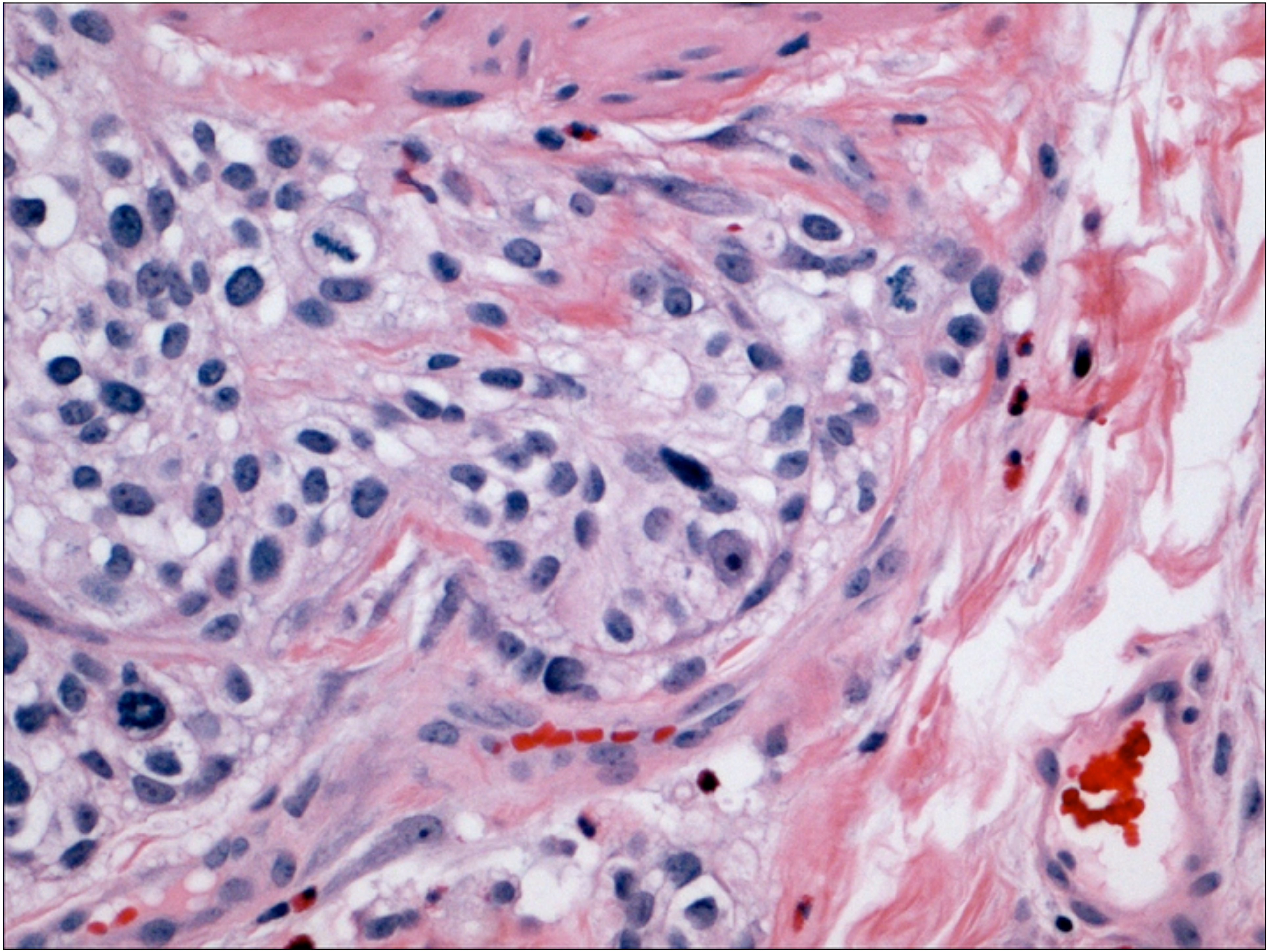


Case 8

Bladder biopsy from 81 year old male with history of prostatic adenocarcinoma

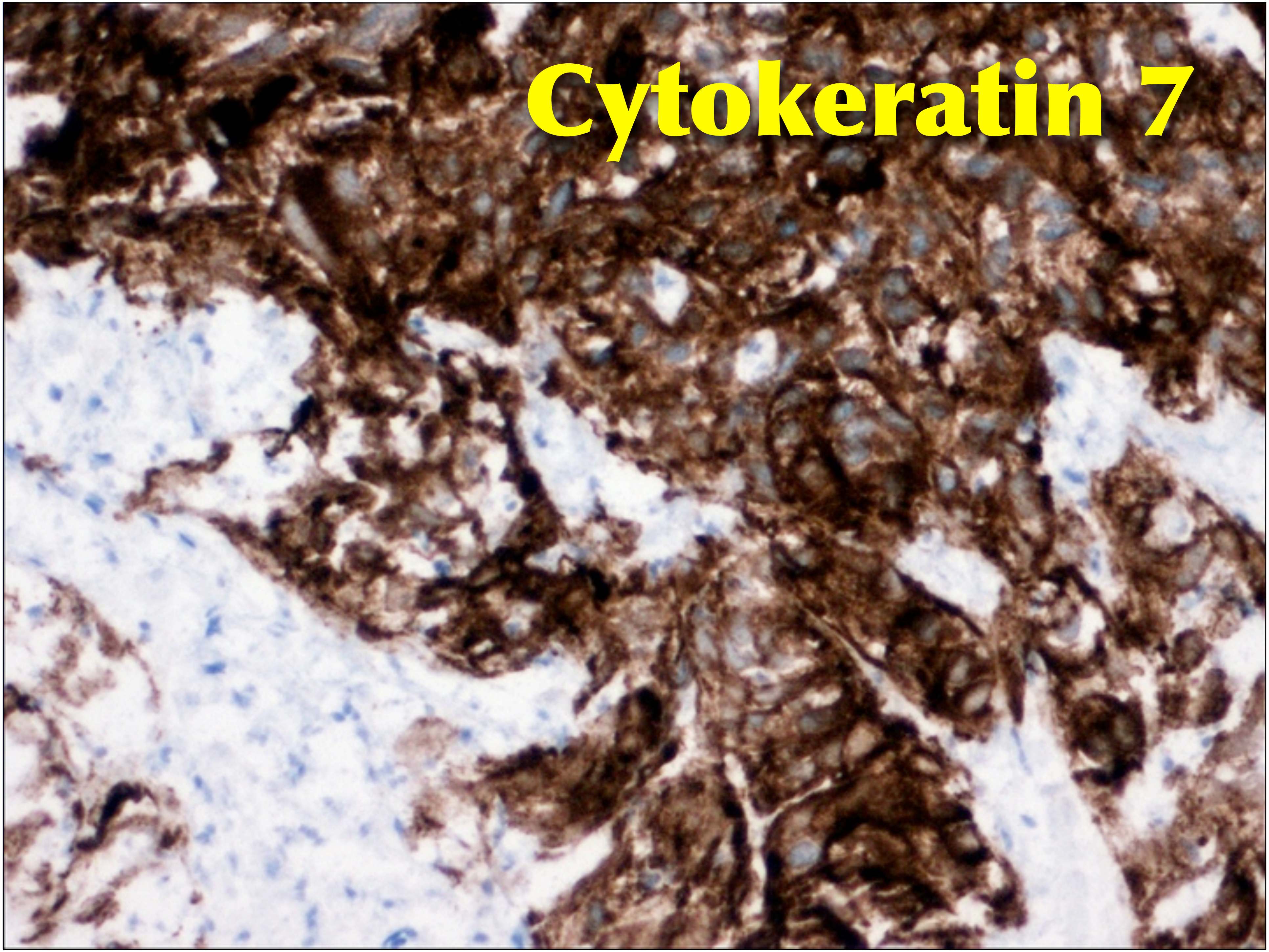


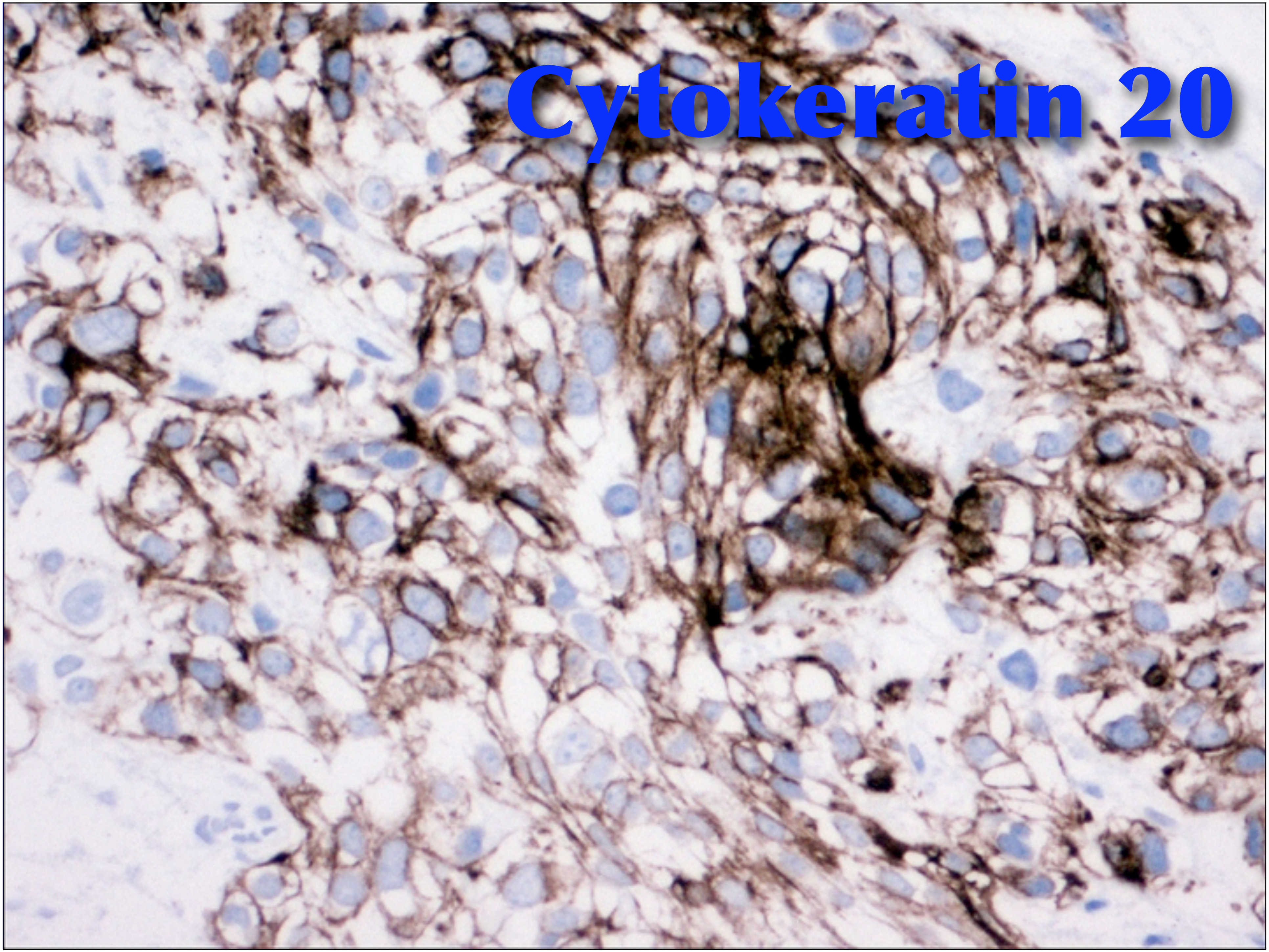


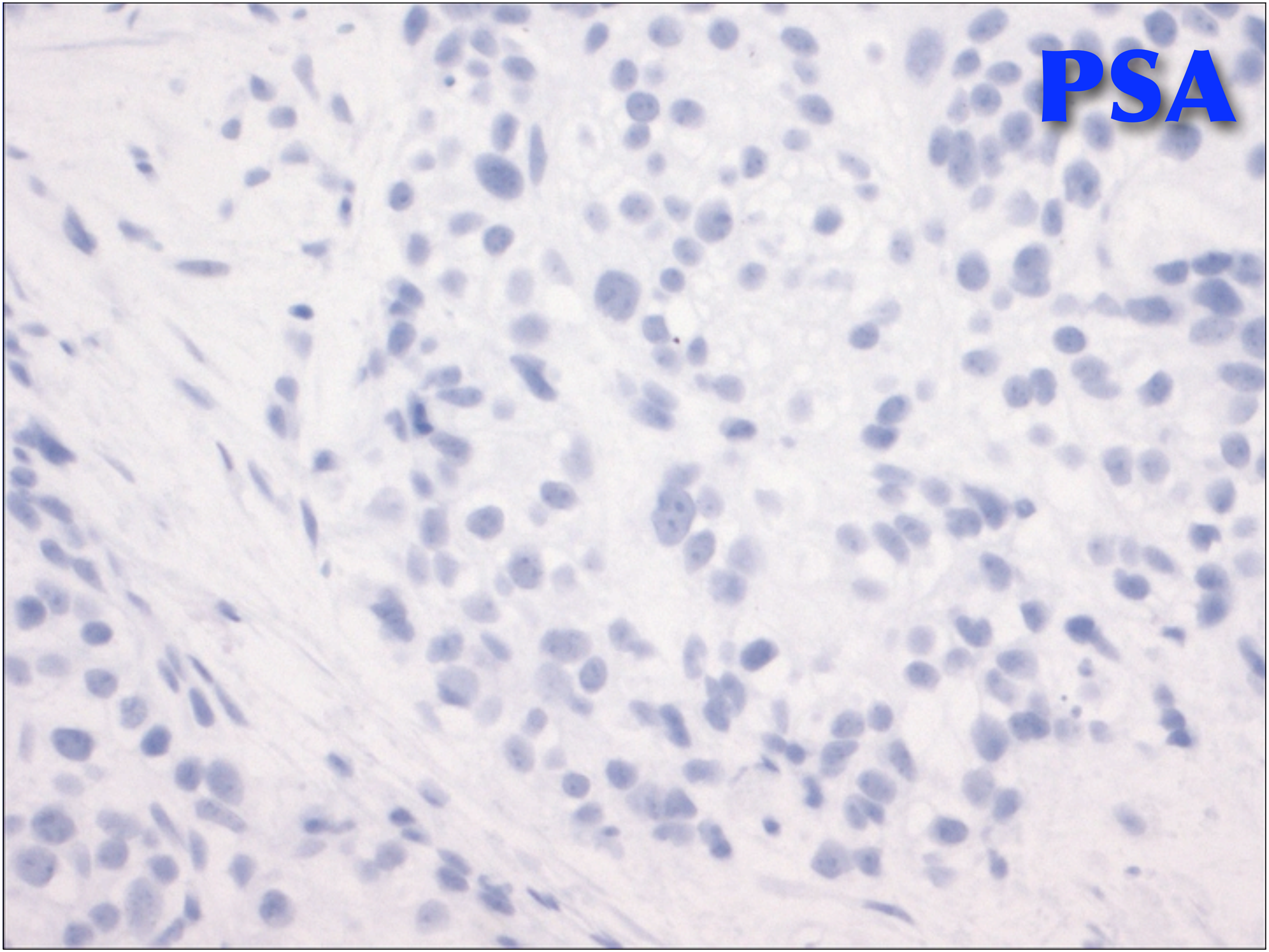


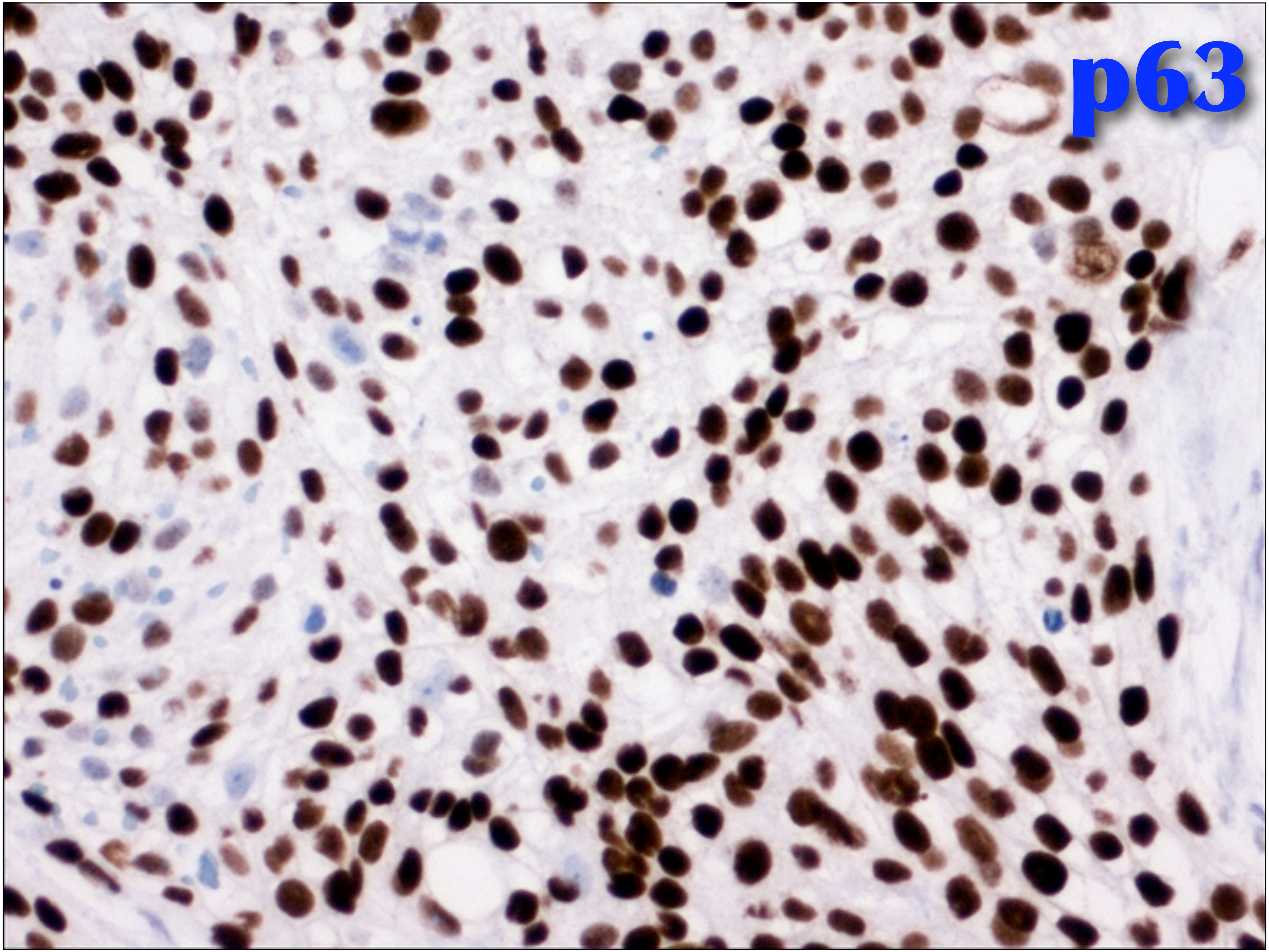
Bladder Transitional Cell CA vs. Prostatic Adenocarcinoma

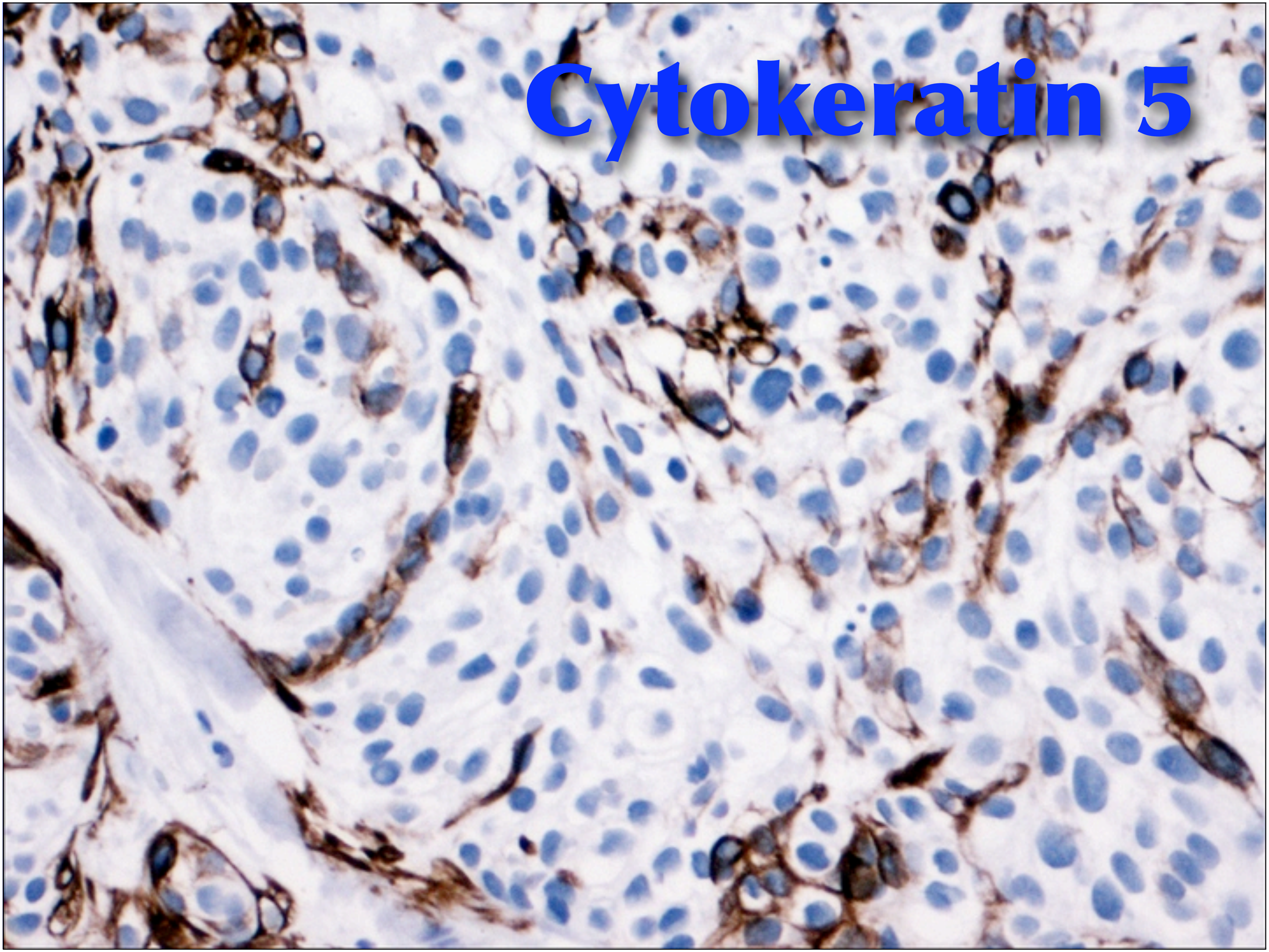
	Bladder	Prostate
Cytokeratin 7	●	❍
Cytokeratin 20	❋	❍
PSA	❍	●
p63	●	❍
Cytokeratin 5	❋	❍
Uroplakin	❋	❍


Cytokeratin 7


Cytokeratin 20


PSA


p63


Cytokeratin 5

Bladder Transitional Cell Carcinoma

Bladder Transitional Cell Carcinoma Markers

- Cytokeratin 7 and Cytokeratin 20 co-expression
- p63 expression
- Uroplakin expression

Coordinate Expression of Cytokeratins 7 and 20

- Partially overlapping but unique distribution in normal tissues

- Normal tissue distribution reflected in corresponding tumor specificity (ex: CK20 in normal colonic epithelium and colorectal carcinoma)

CK7 ● CK20 ○

Breast carcinoma

● *Almost always positive*

○ *Almost always negative*

✻ *Sometimes positive*

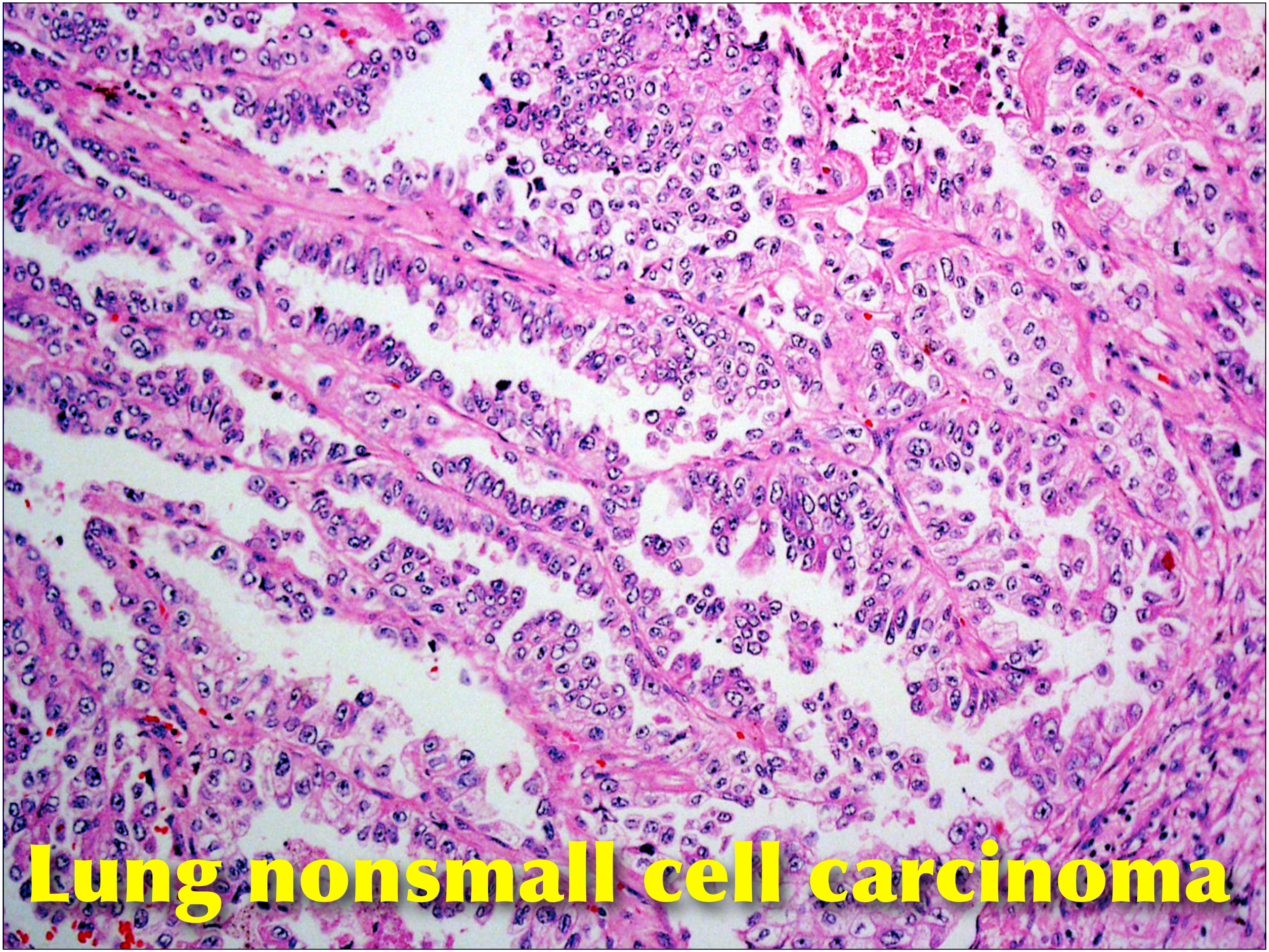
Colorectal adenocarcinoma

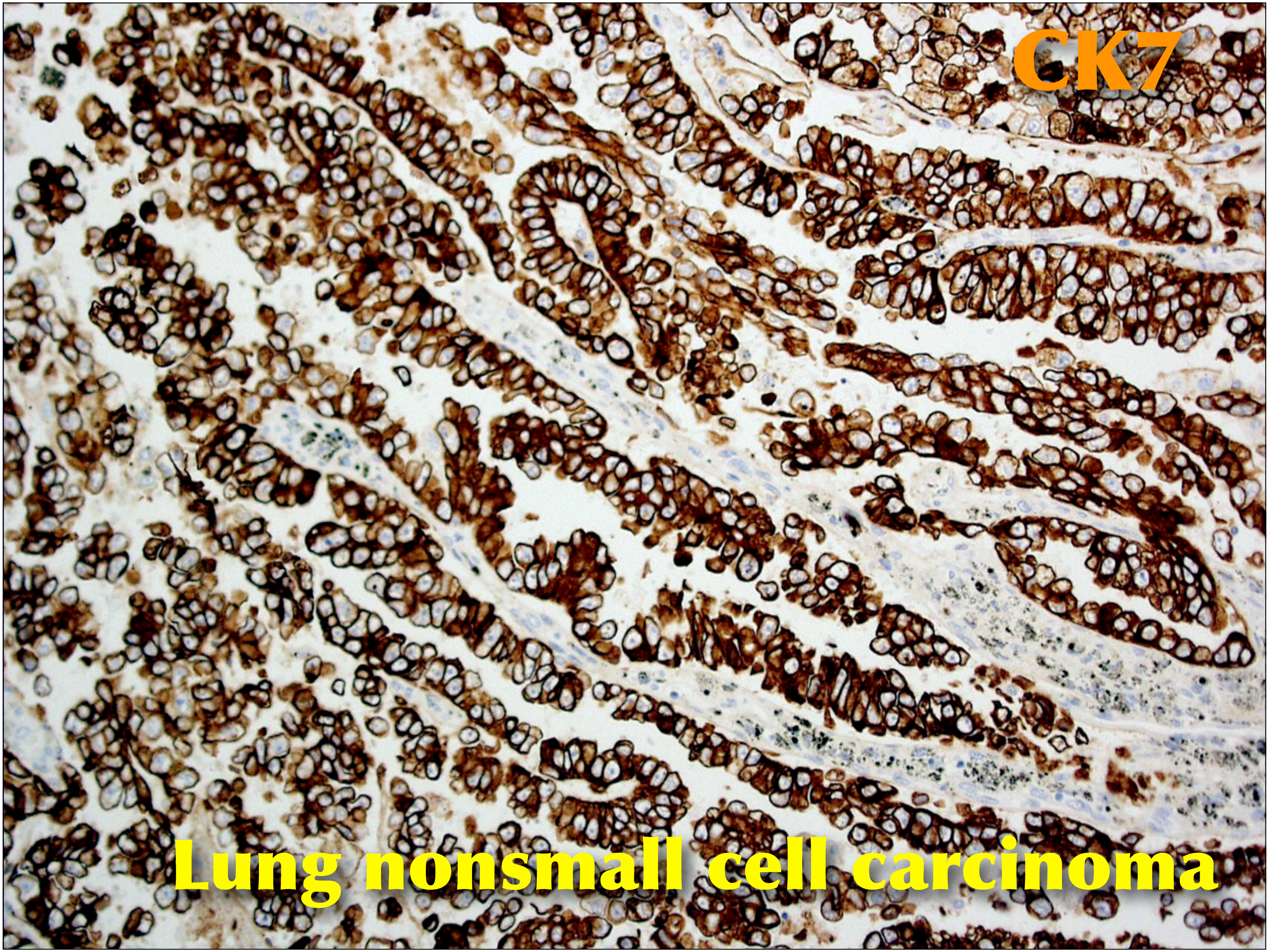
CK7 ● CK20 ●

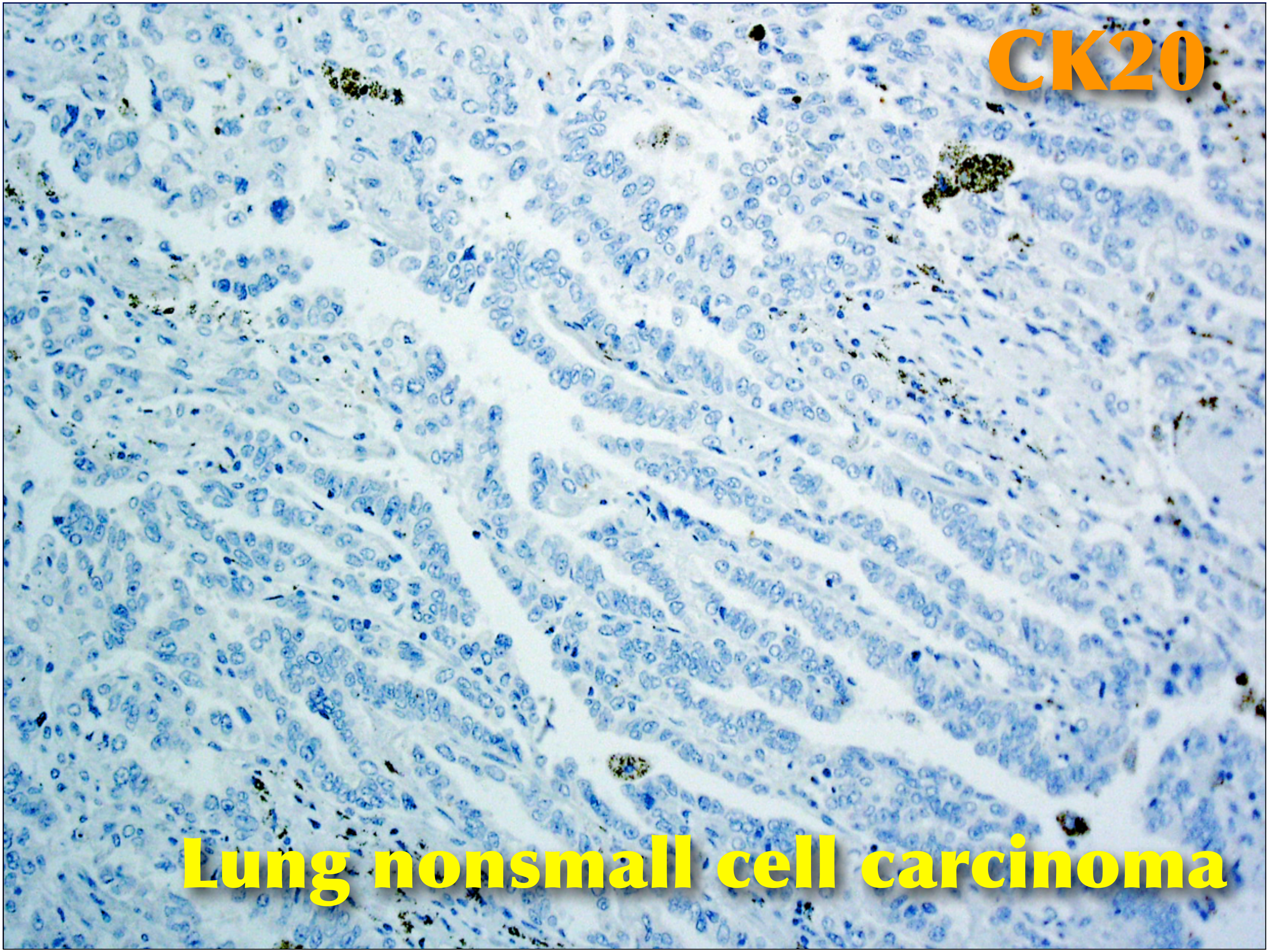
Transitional cell carcinoma
Ovarian carcinoma (mucinous)
Pancreatic carcinoma (subset)

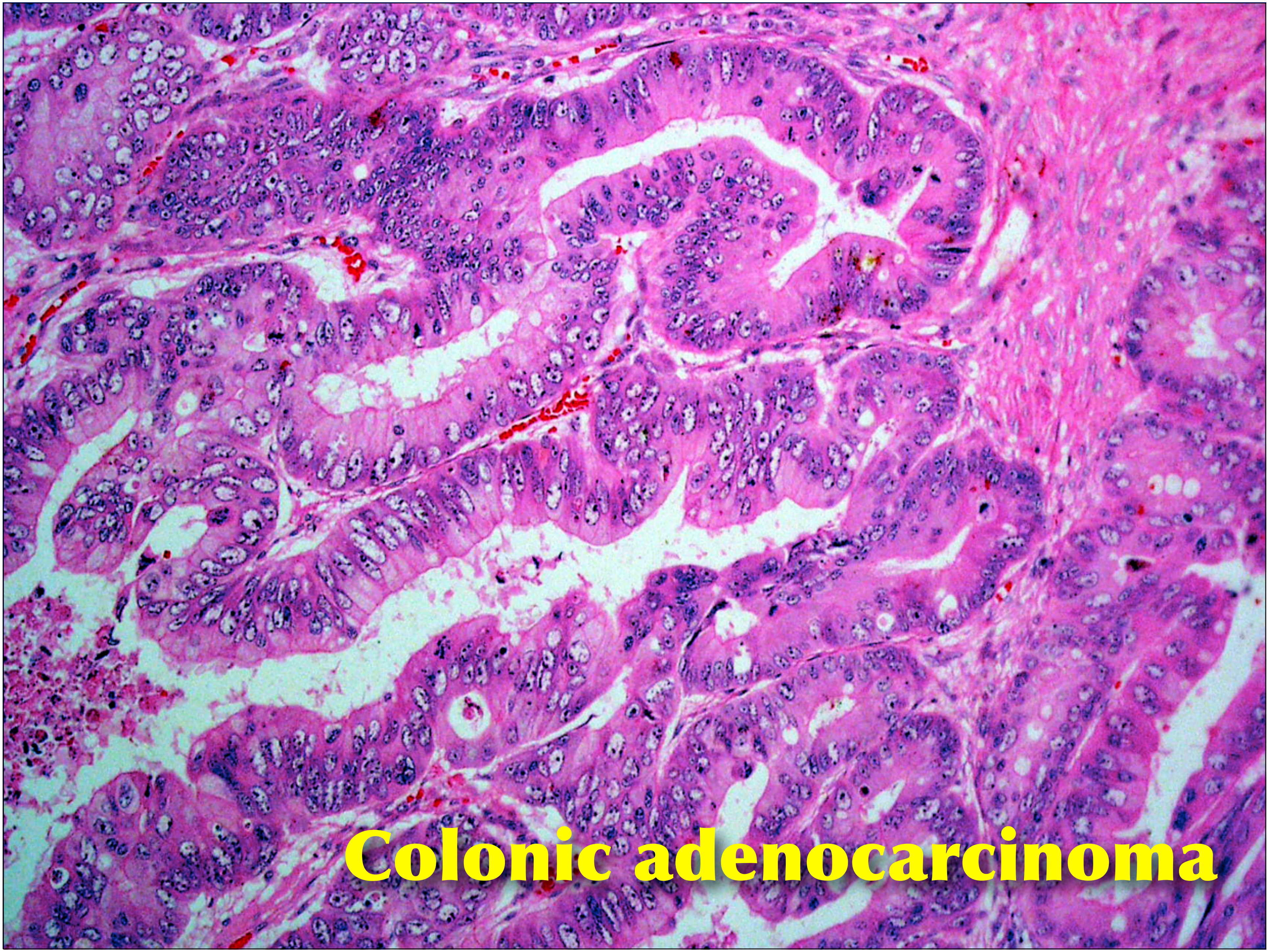
CK7 ○ CK20 ○

Hepatocellular carcinoma
Renal cell carcinoma
Prostatic adenocarcinoma
Neuroendocrine carcinoma
Squamous cell carcinoma

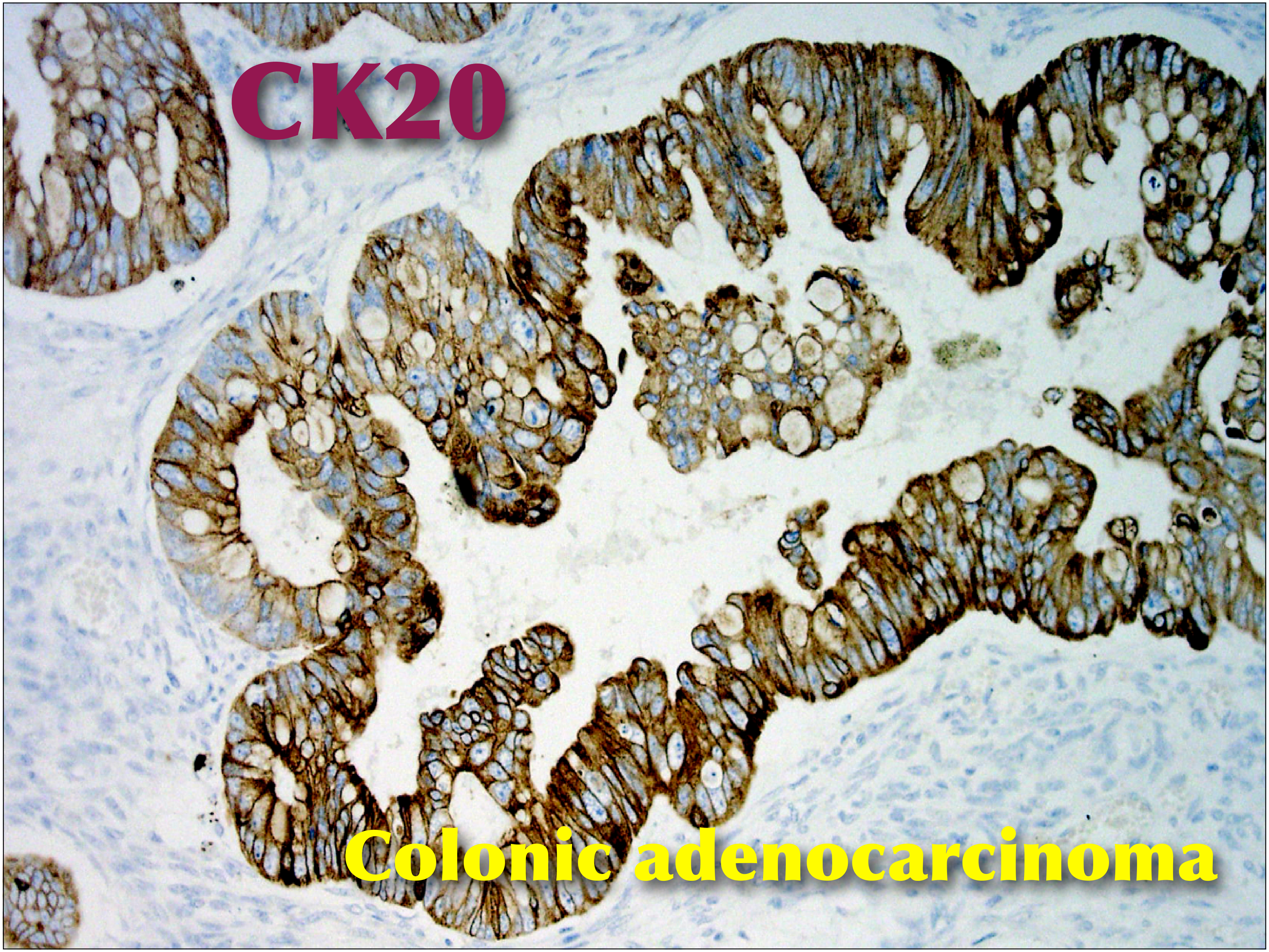

Lung nonsmall cell carcinoma

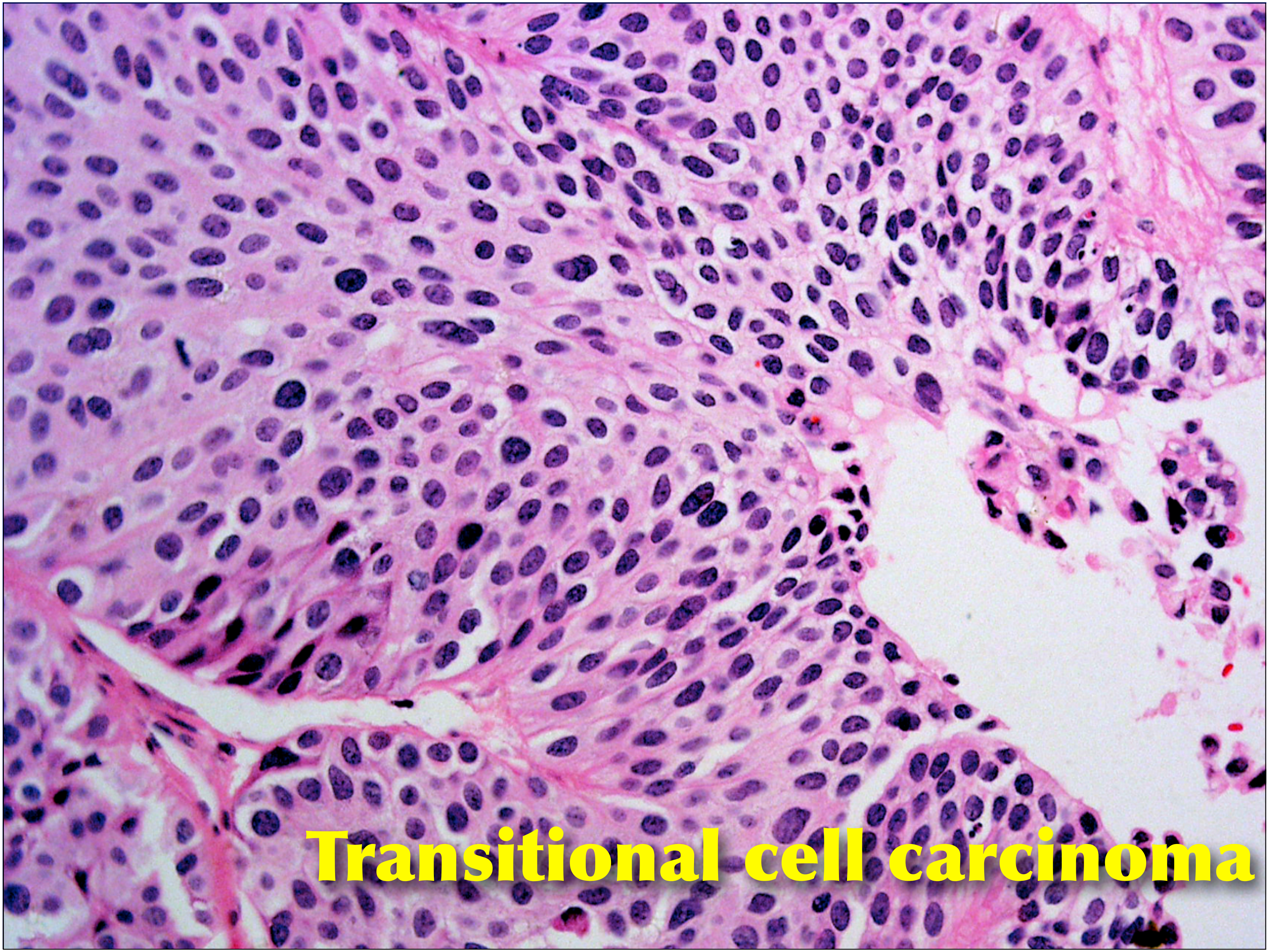

CK7
Lung nonsmall cell carcinoma


CK20
Lung nonsmall cell carcinoma

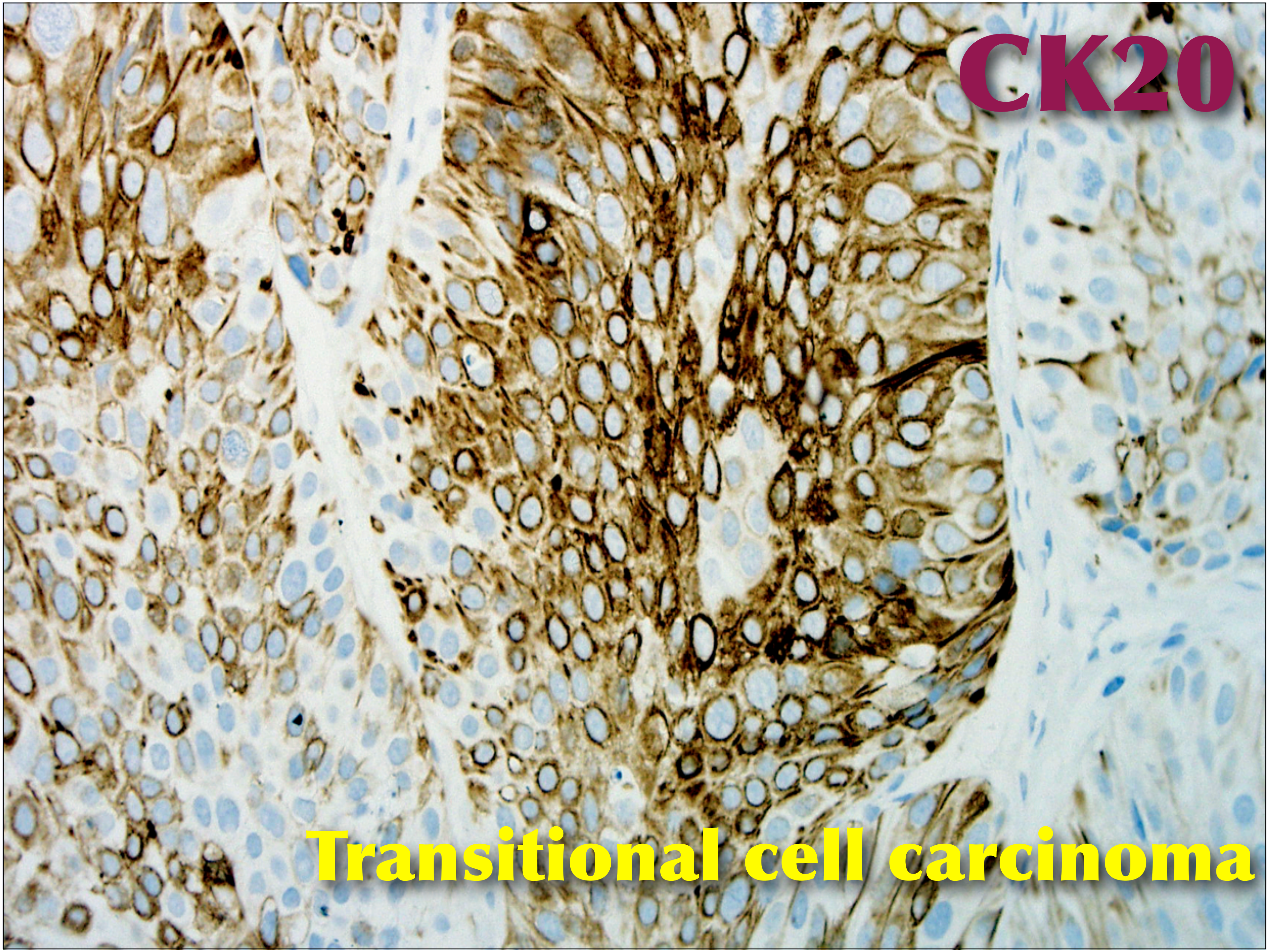

Colonic adenocarcinoma

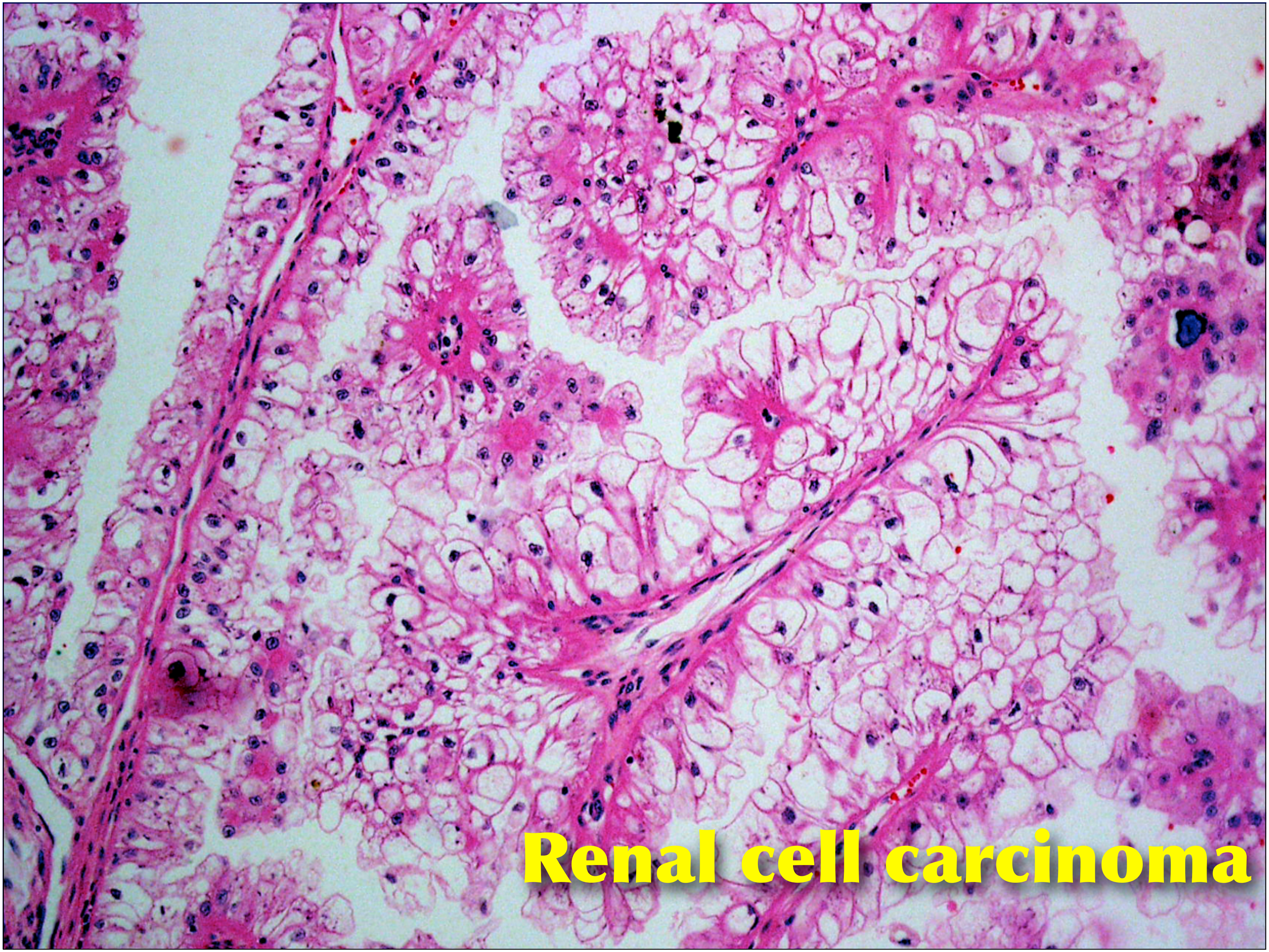

CK7
Colonic adenocarcinoma

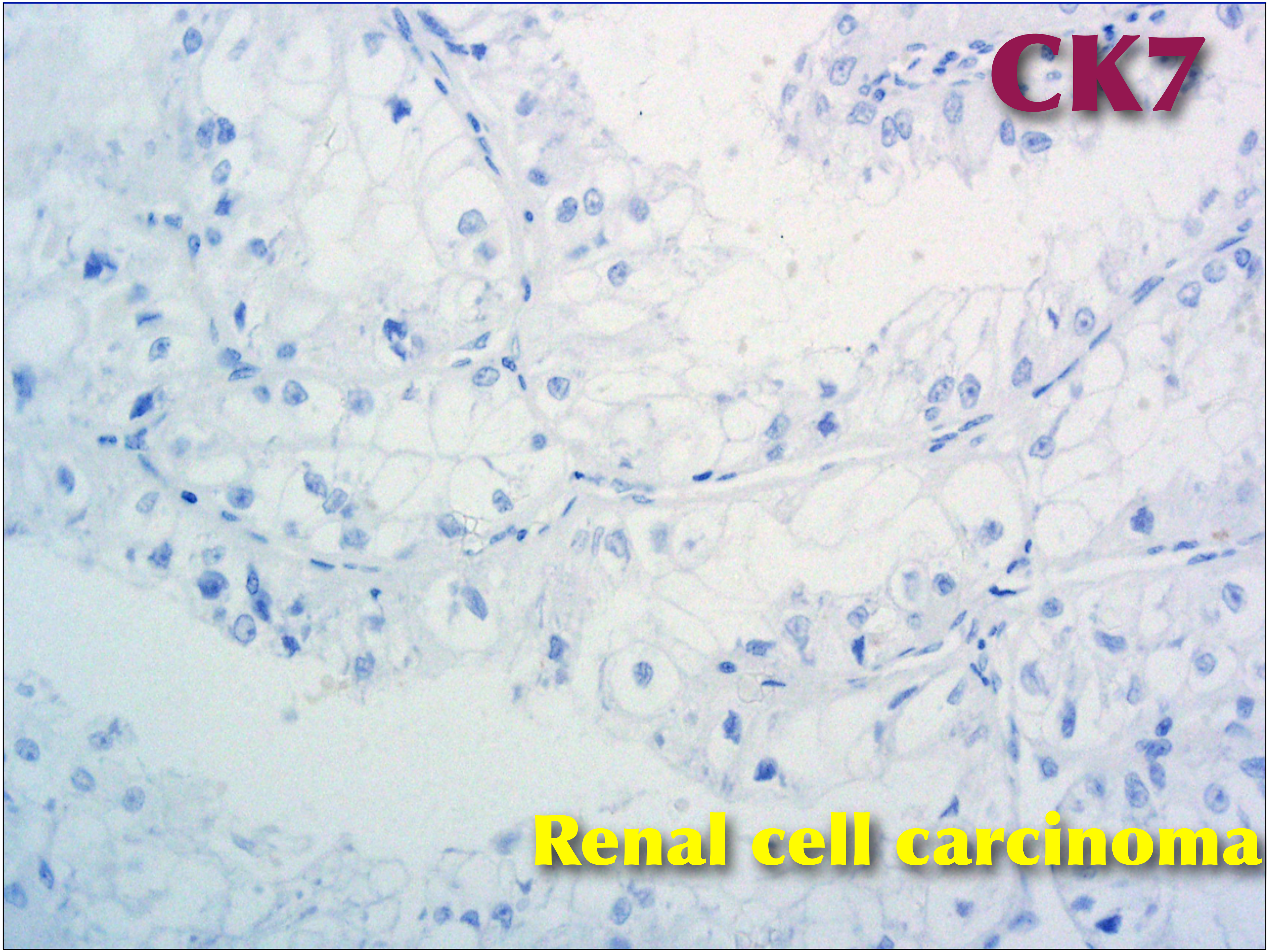

CK20
Colonic adenocarcinoma

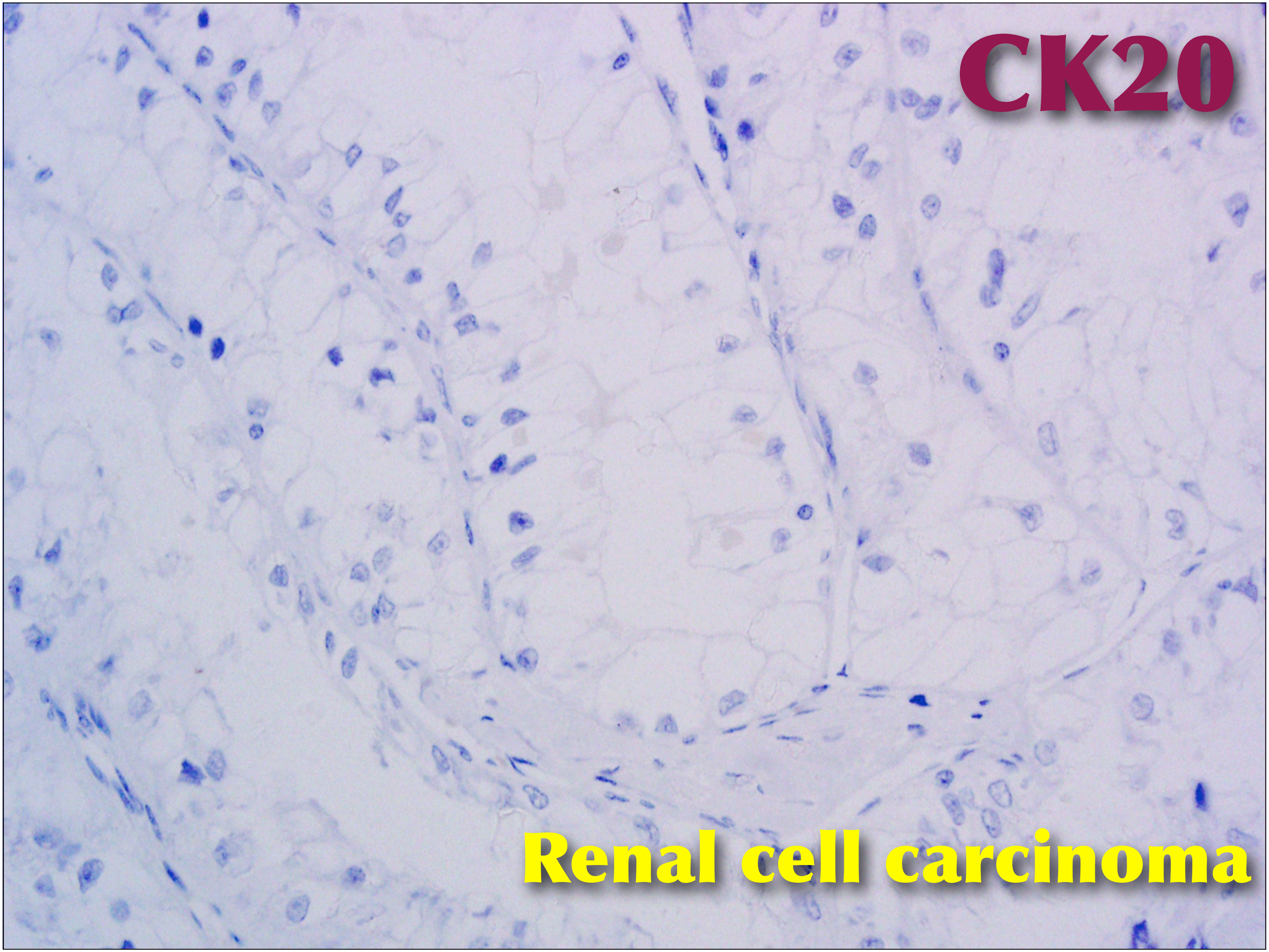

Transitional cell carcinoma


CK7
Transitional cell carcinoma


CK20
Transitional cell carcinoma


Renal cell carcinoma


CK7
Renal cell carcinoma


CK20
Renal cell carcinoma

CK7 and CK20 Coordinate Expression

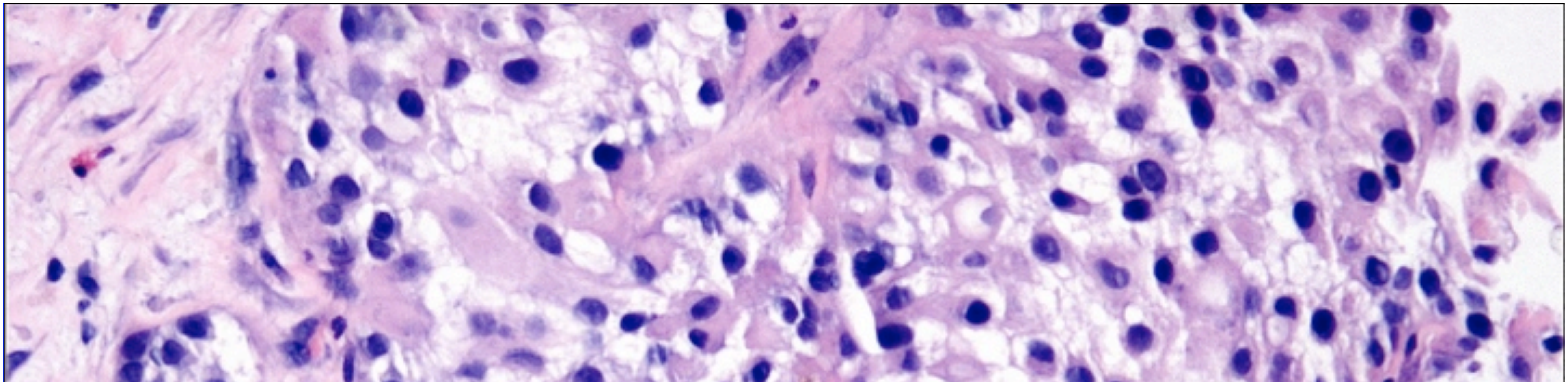
- "Modal" immunophenotypes generally cited
- First order approximation only
- Additional tumor specific markers generally required

Distribution of 'Modal' CK7 and CK20 Immunophenotypes

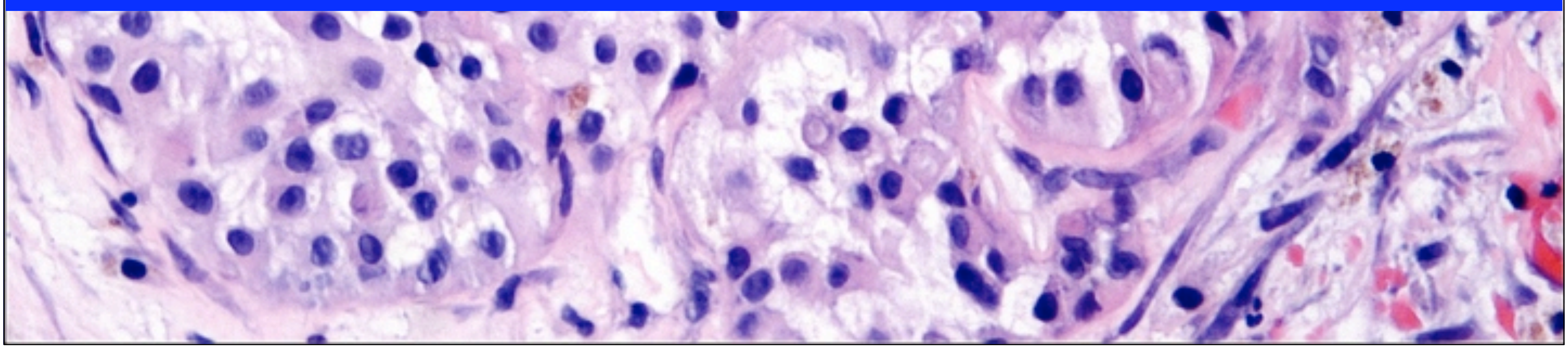
	CK7	*CK20*	
Colorectal adenoCA	○	●	75-95%
Hepatocellular CA	○	○	70-90%
Lung nonsmall cell	●	○	90%
Lung NE carcinoma	○	○	60-80%
Ovarian serous CA	●	○	>90%
Renal cell CA	○	○	70-90%
Lung squam cell CA	○	○	50-90%

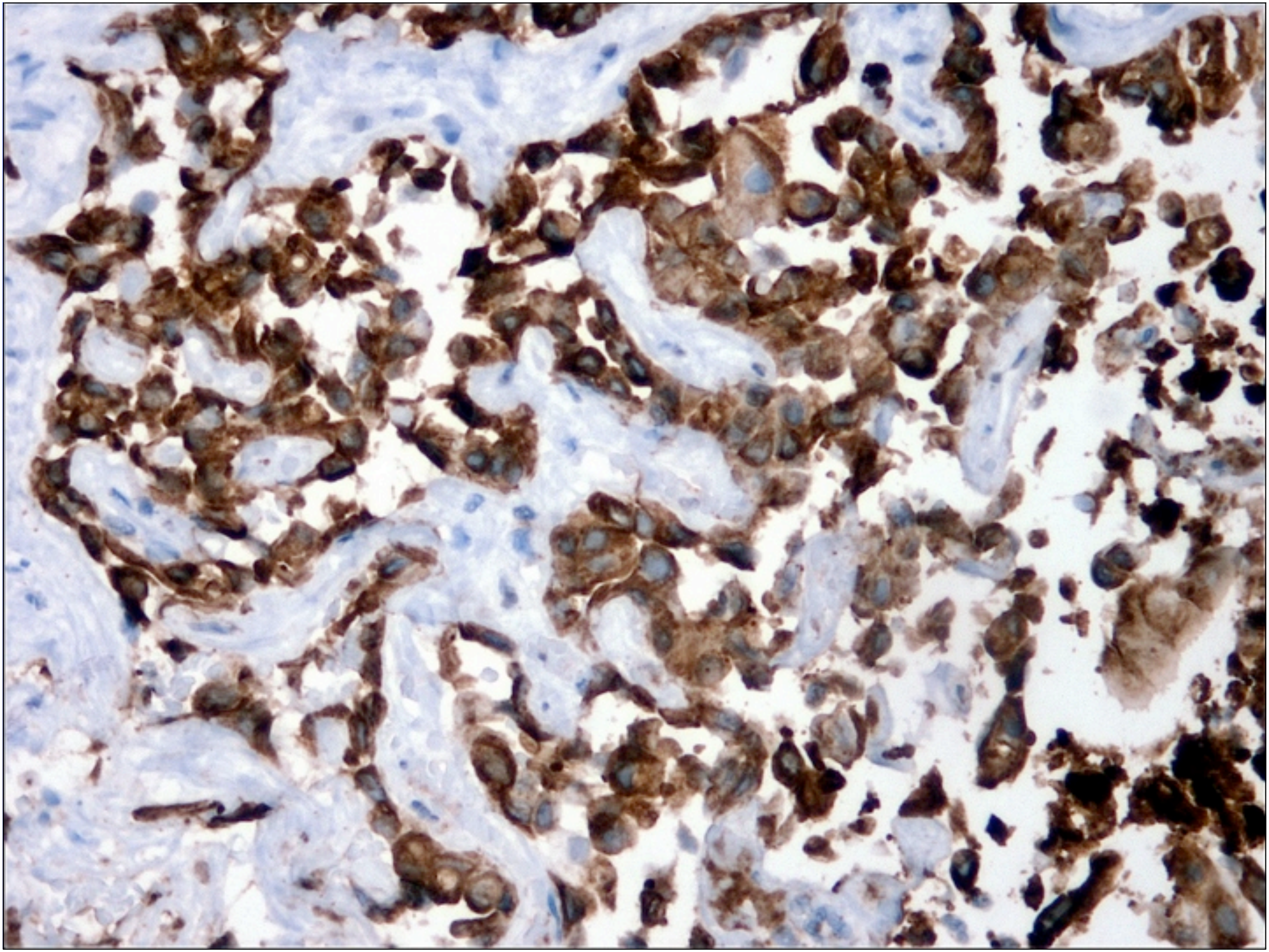
Bladder Transitional Cell Carcinoma

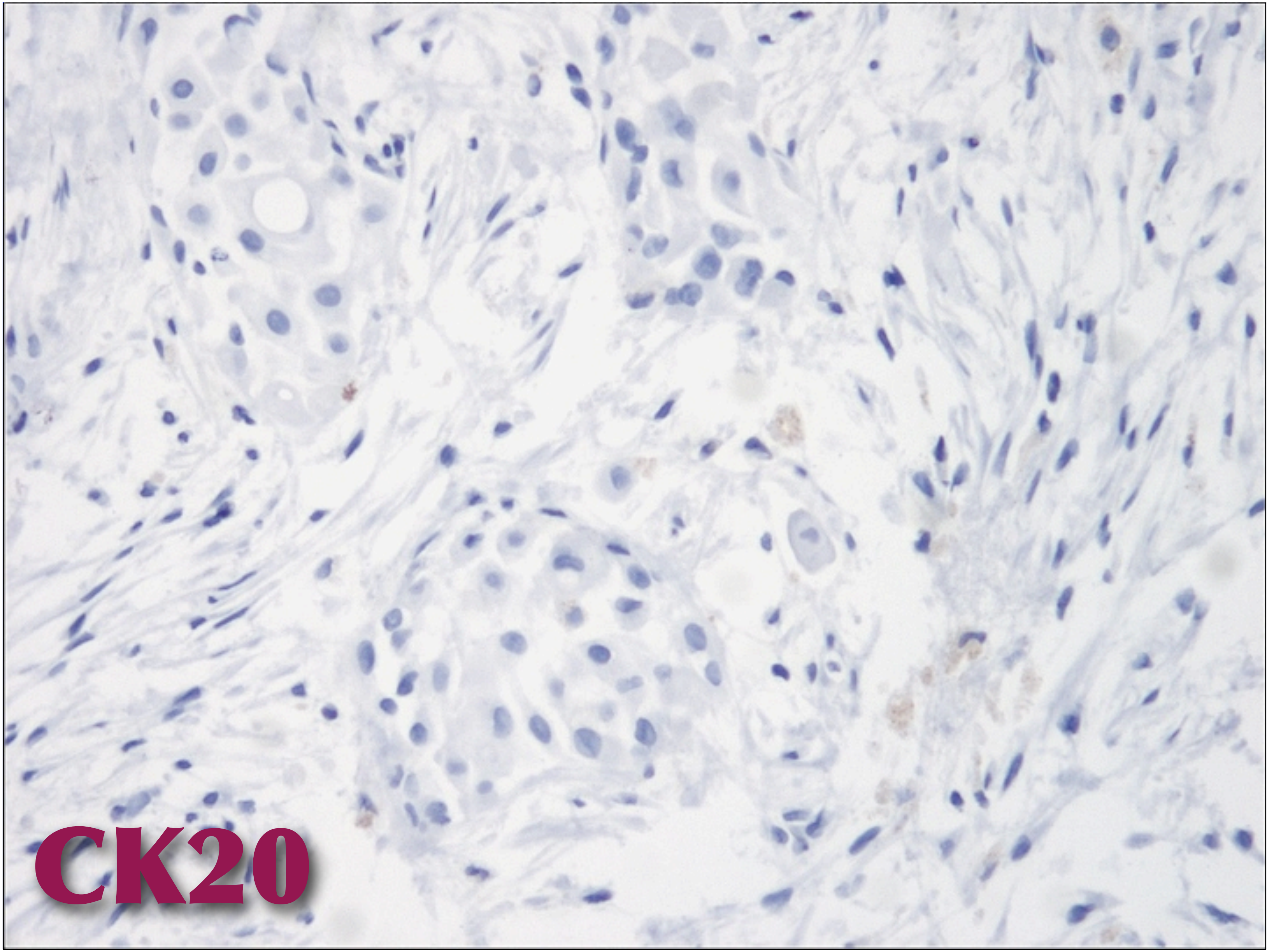
- Usually CK7+, CK20+
- CK20 positivity can be focal
- CK20 can be negative
- Highly unusual to be CK7-negative

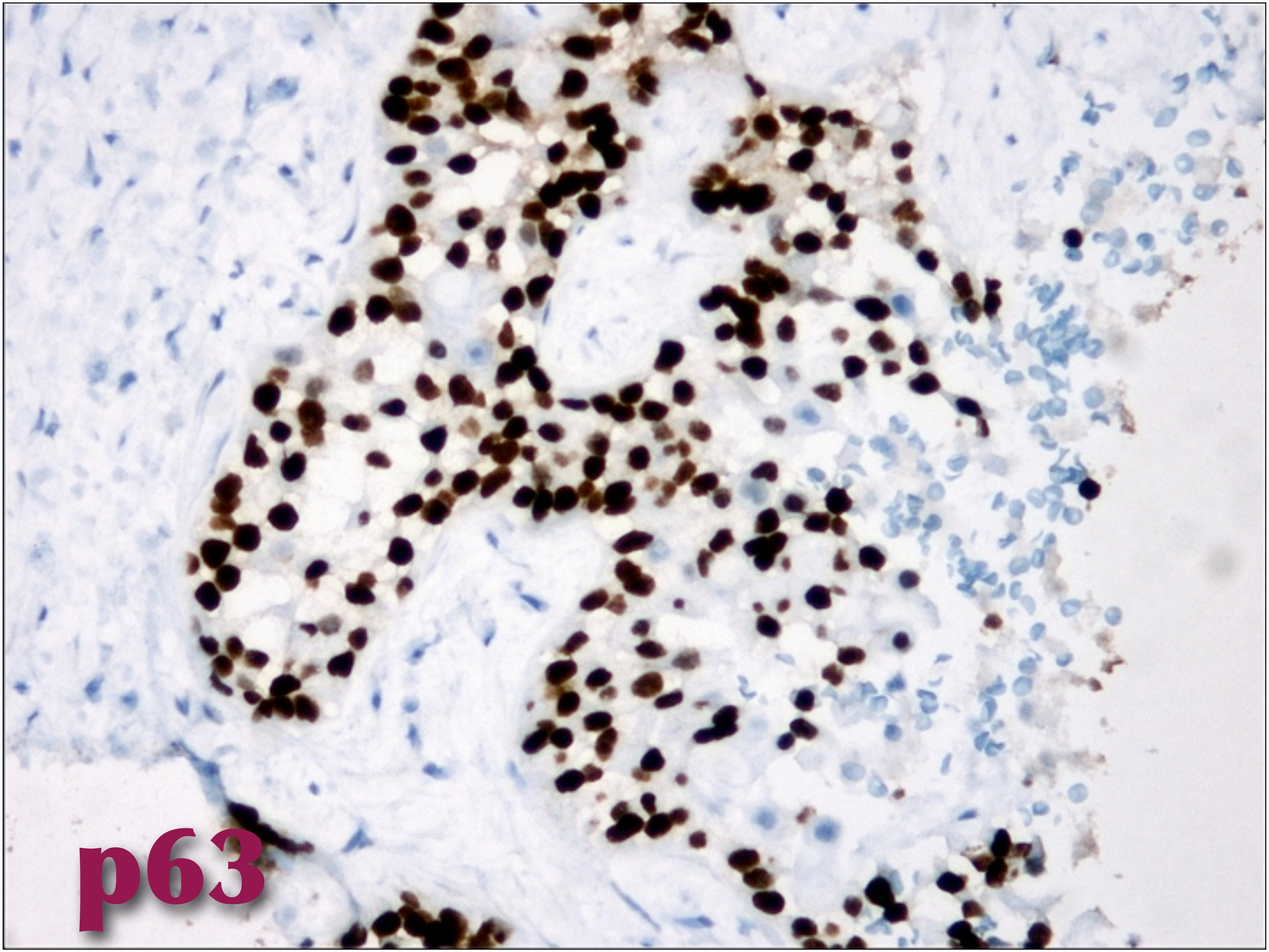


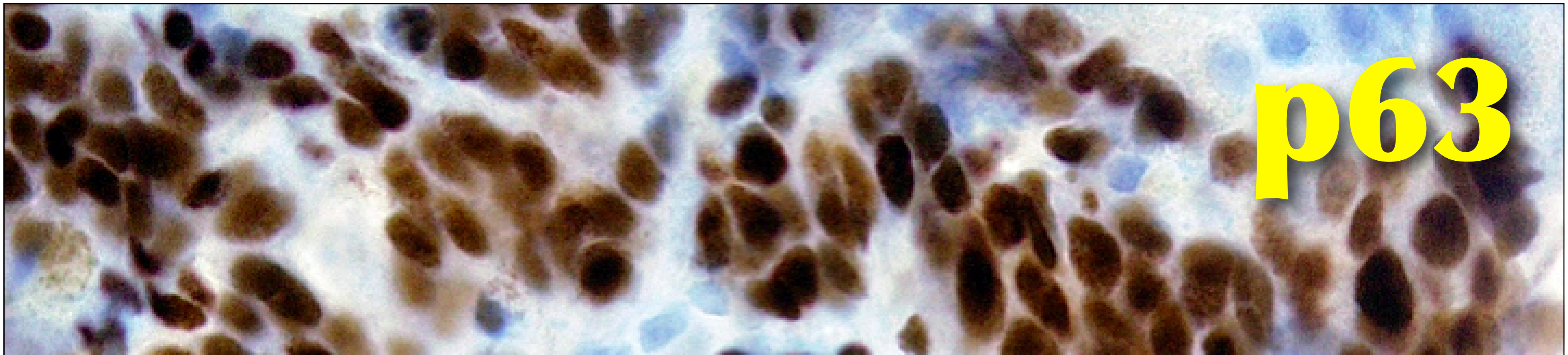
Liver tumor in 62 year old female with history of bladder carcinoma



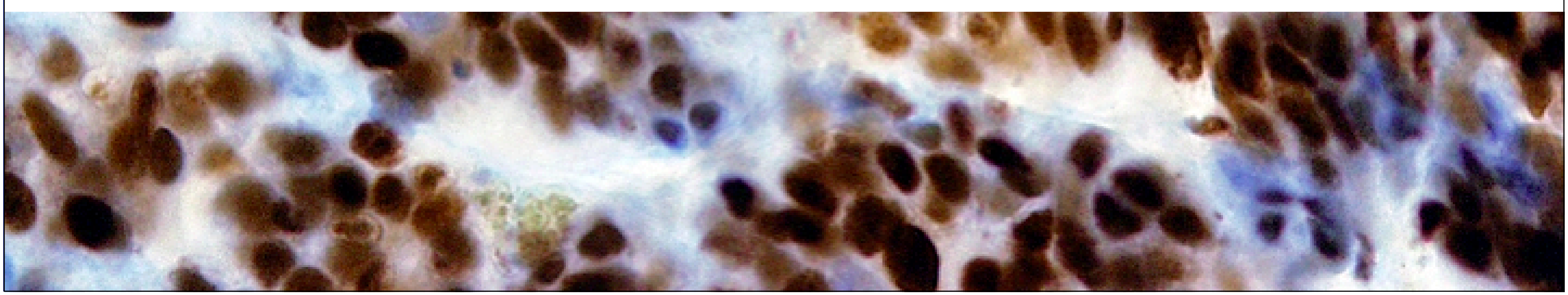


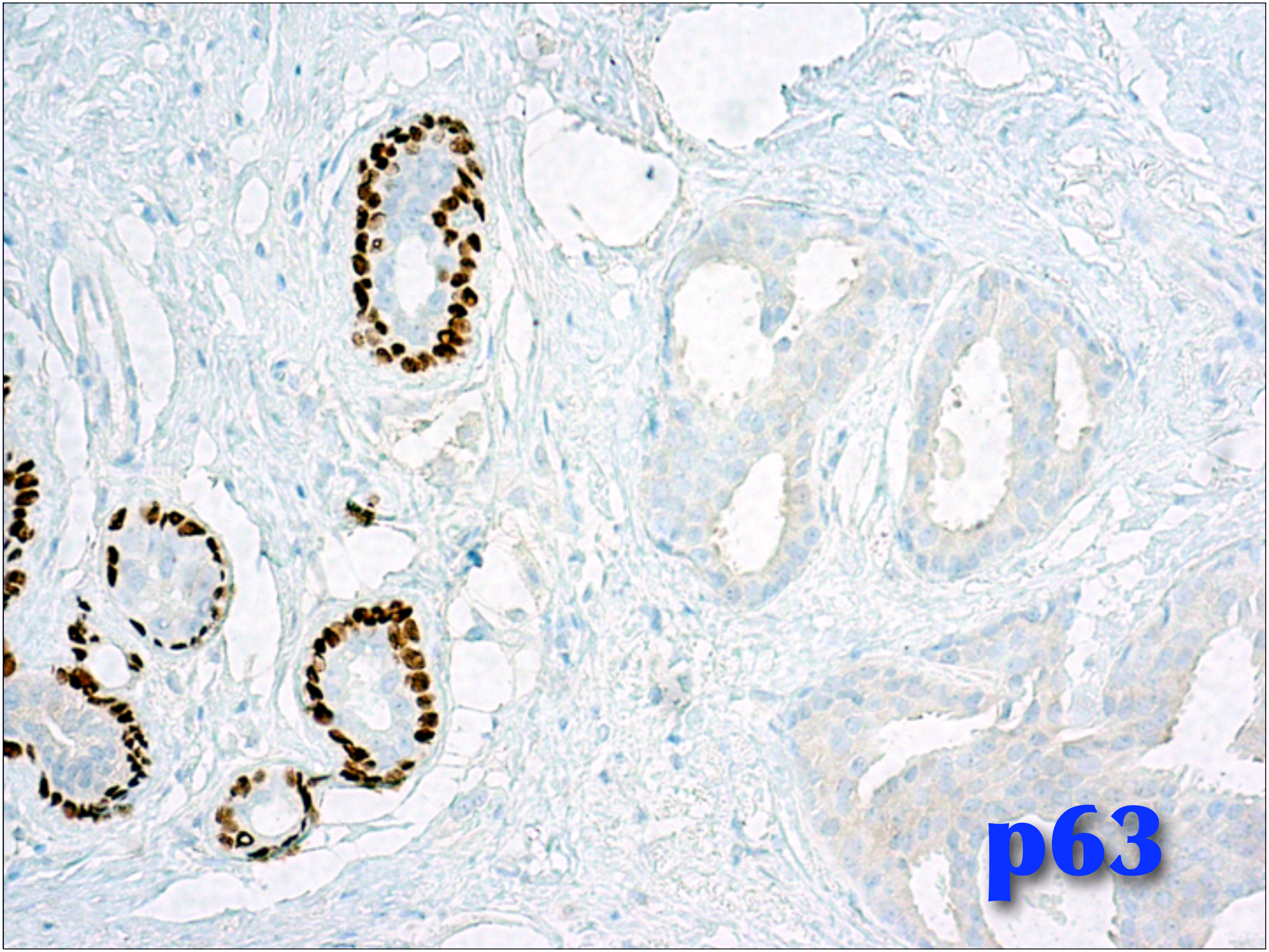

CK20


p63



Squamous/ Transitional Cell Marker

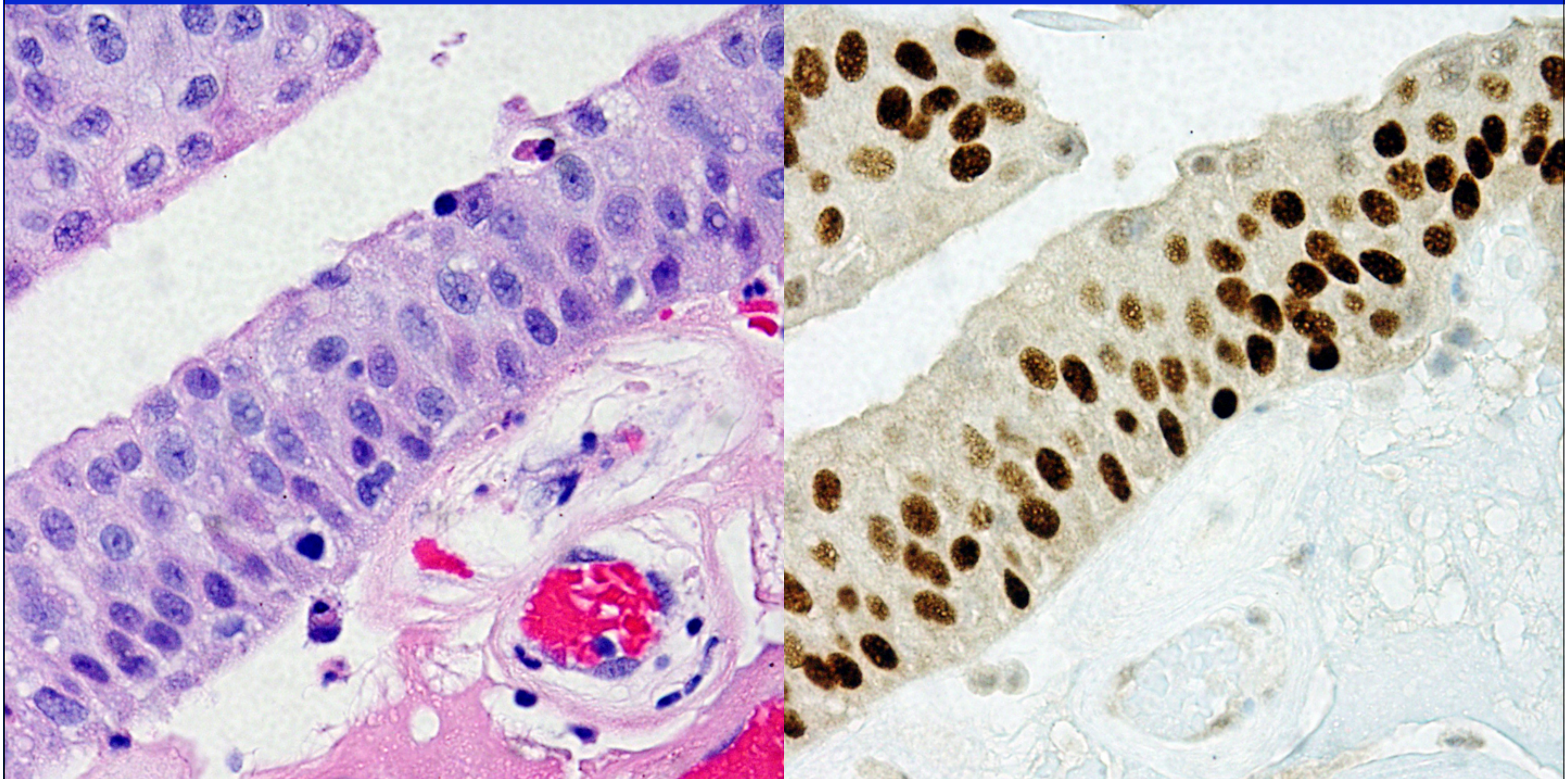



p63

p63 Distribution in Normal Tissues

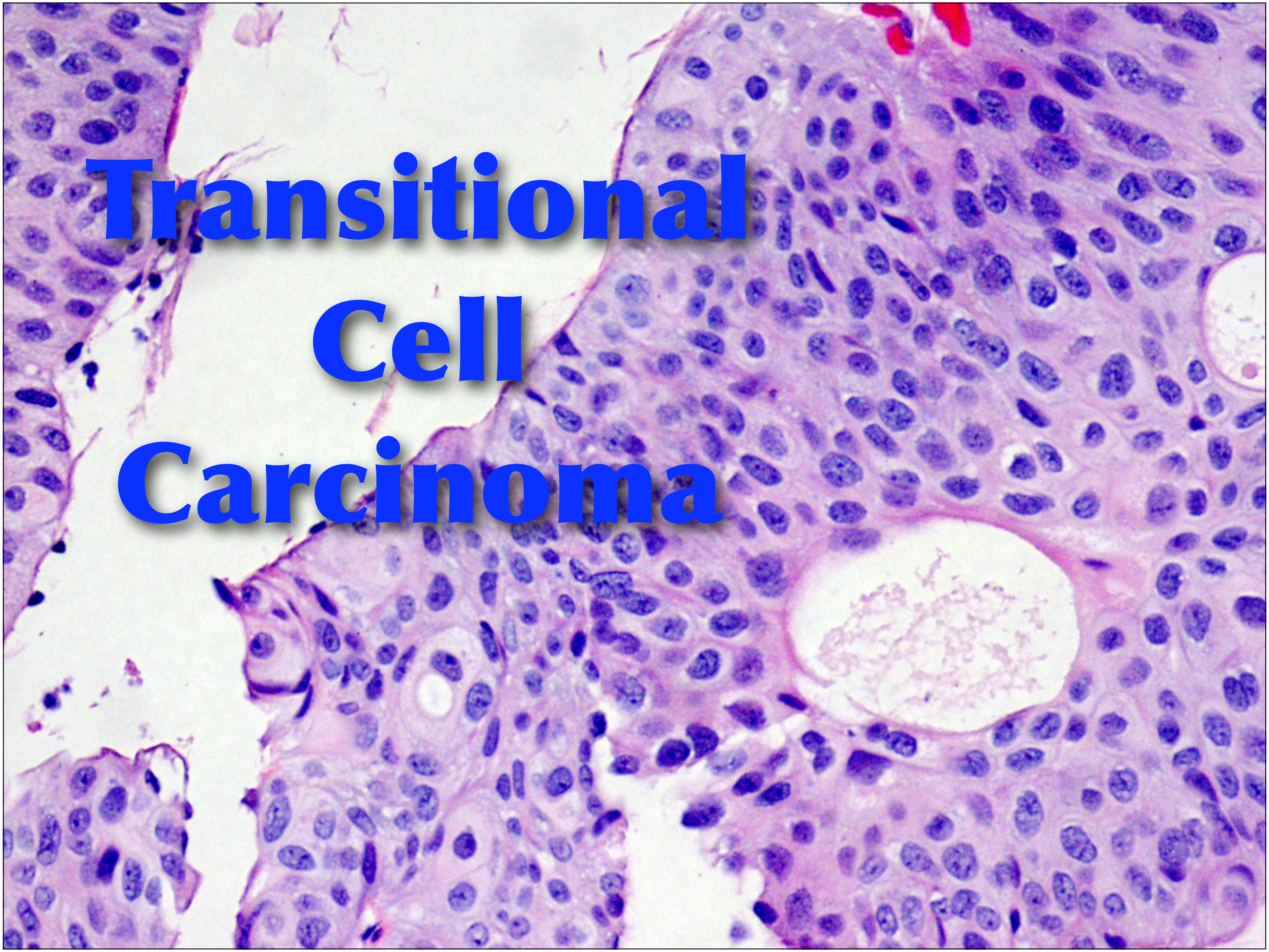
- Squamous epithelium
- Urothelium
- Basal cells of pseudostratified columnar epithelium (e.g., bronchus)
- Reserve cells of endocervix, pancreatic ducts
- Outer cell layer of prostate
- Myoepithelium of breast, salivary gland
- Ovarian oocytes (but not testicular germ cells)

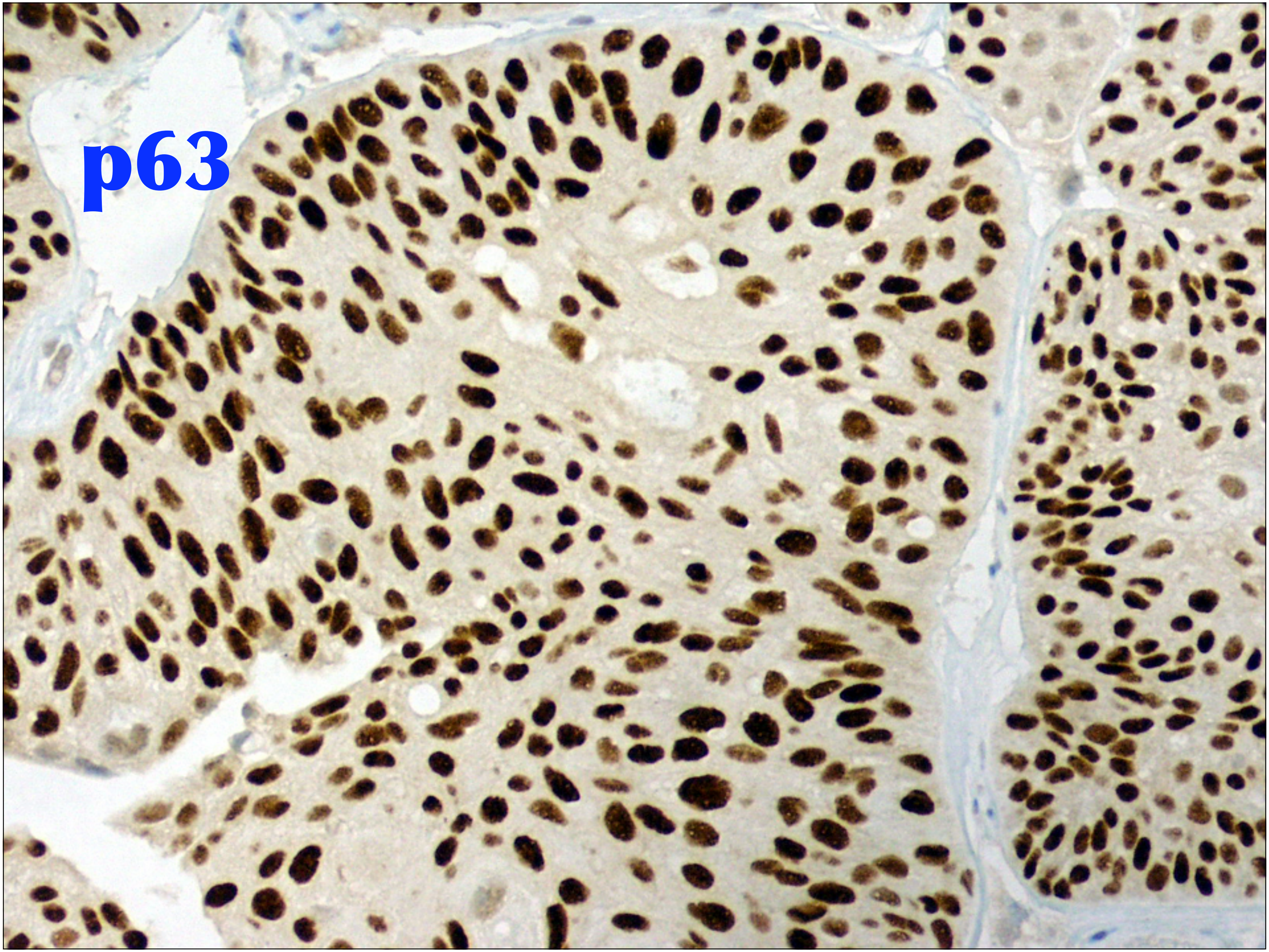
Urothelium Expresses p63



p63 Positive Tumors

- Squamous cell carcinoma
- Basal cell carcinoma
- Transitional cell carcinoma
- Thymic epithelial tumor
- Myoepithelial tumor (e.g., salivary gland)
- Trophoblastic tumors

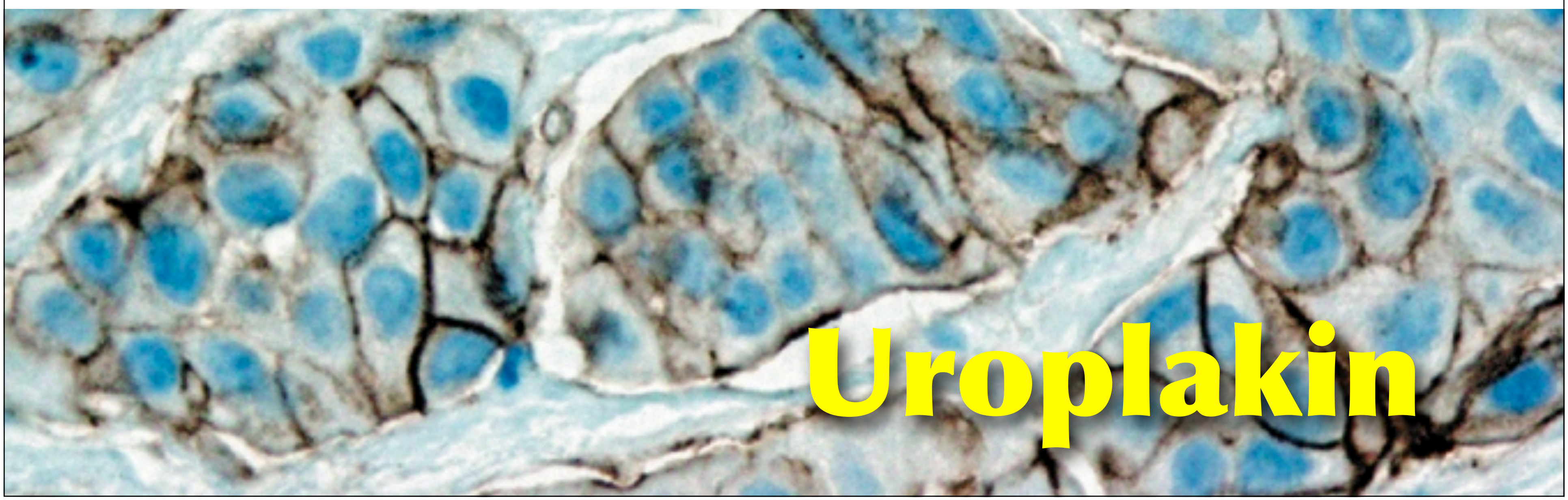

Transitional
Cell
Carcinoma

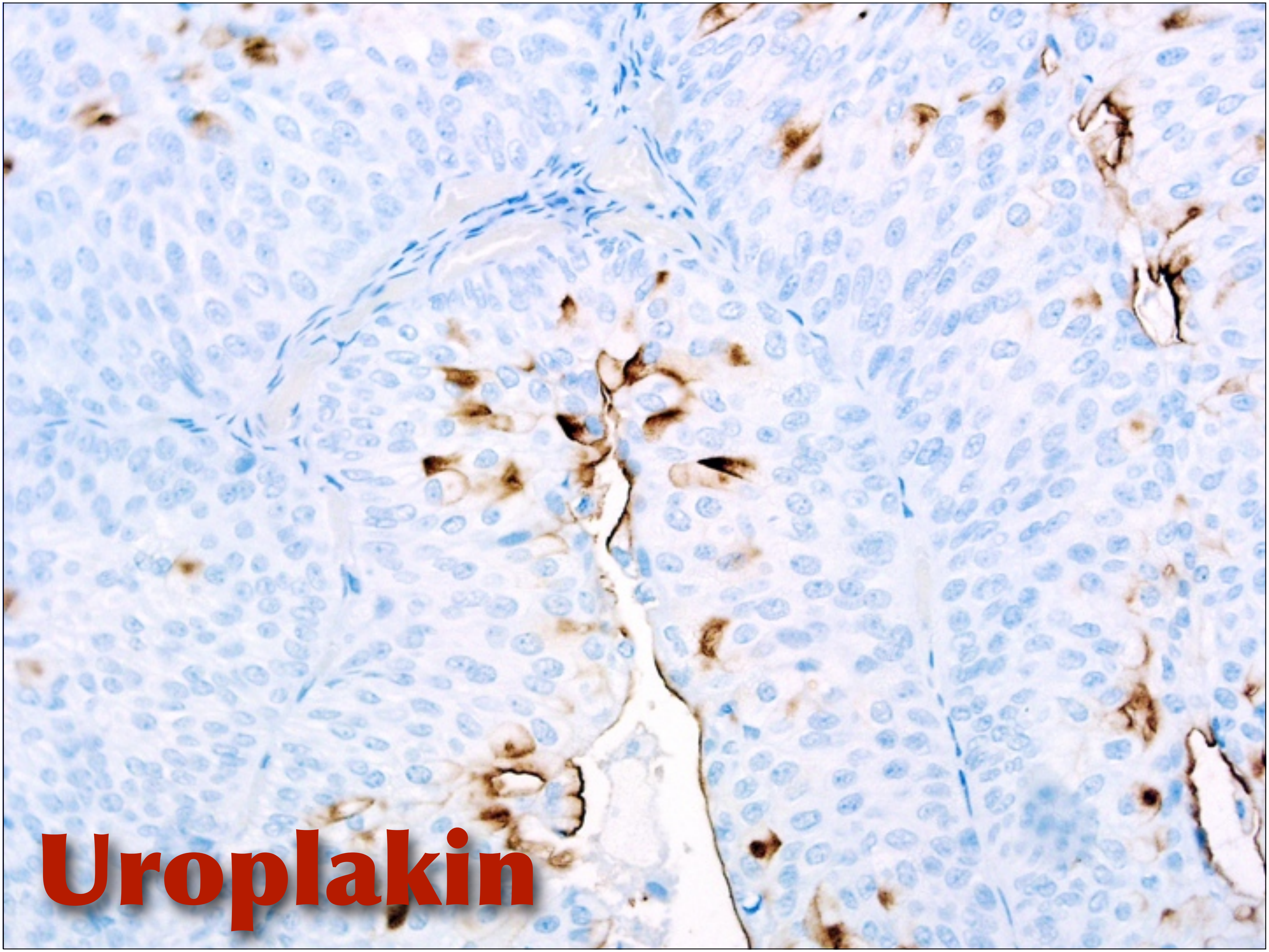

p63

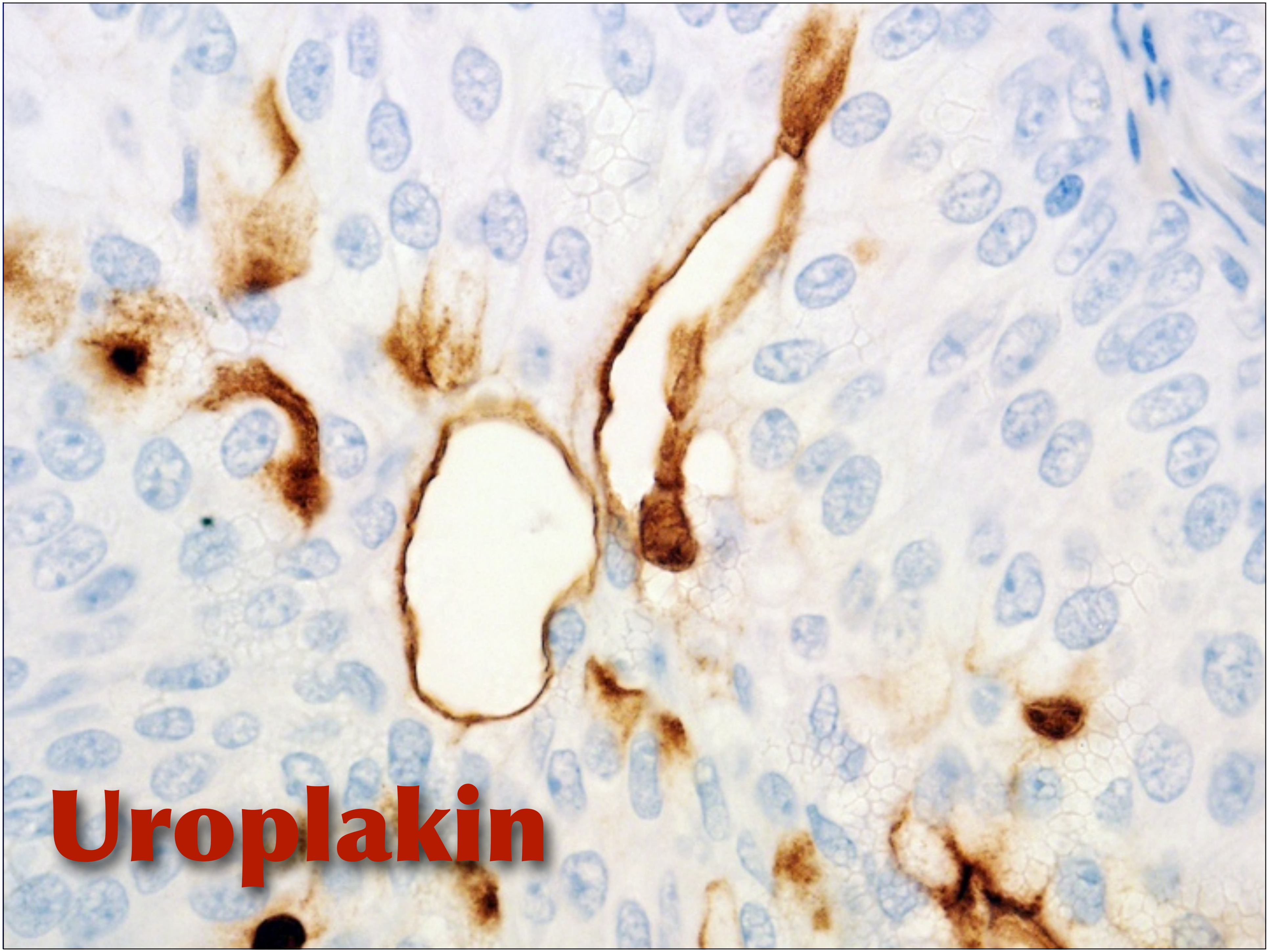
p63 in Transitional Cell Carcinoma

- Very high sensitivity (>90%)
- When positive, generally positive on vast majority of tumor cells
- Beware of tumors showing focal or rare cells positive
- Much more sensitive than CK5

Bladder CA (TCC) Marker




Uroplakin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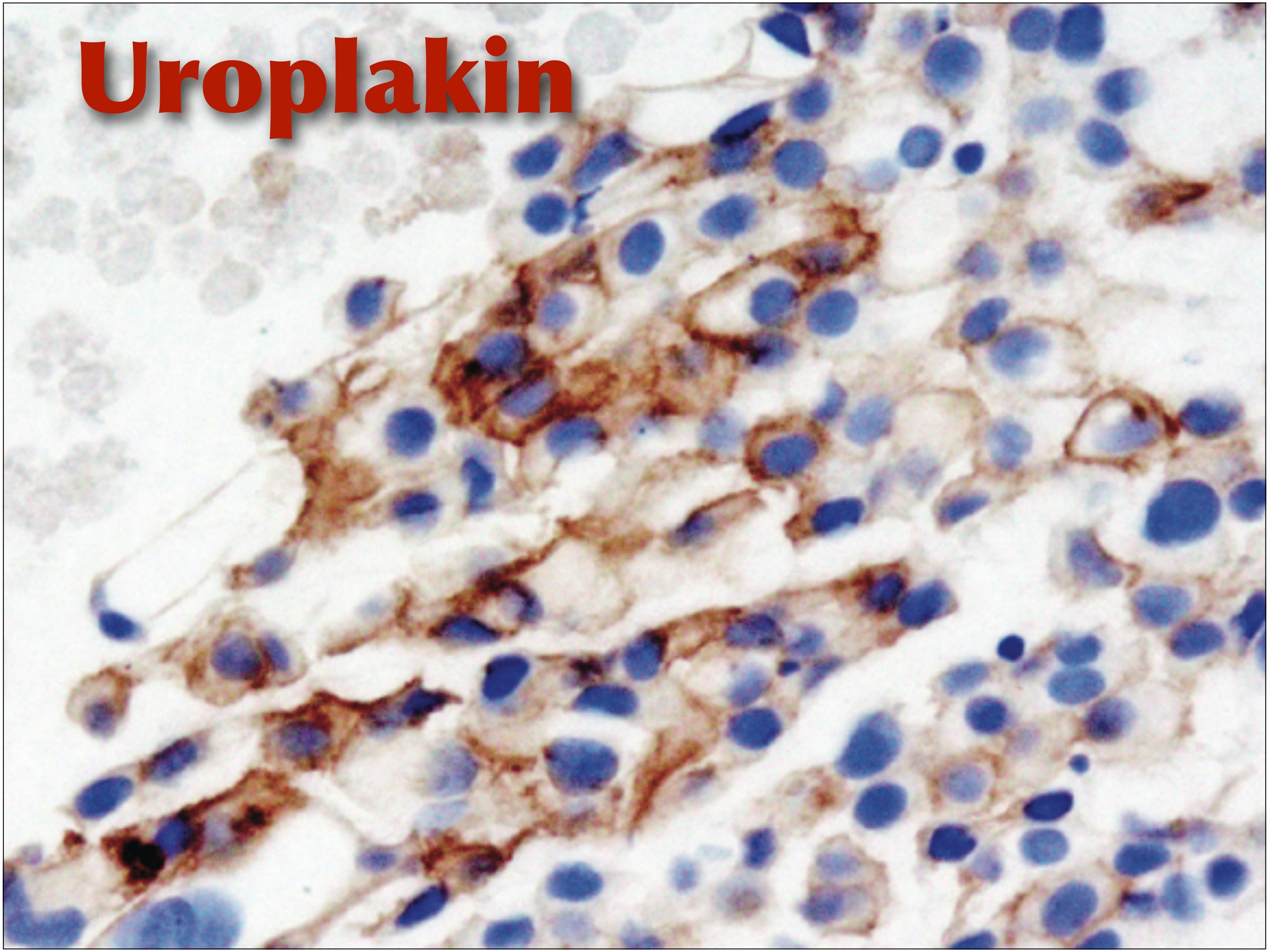
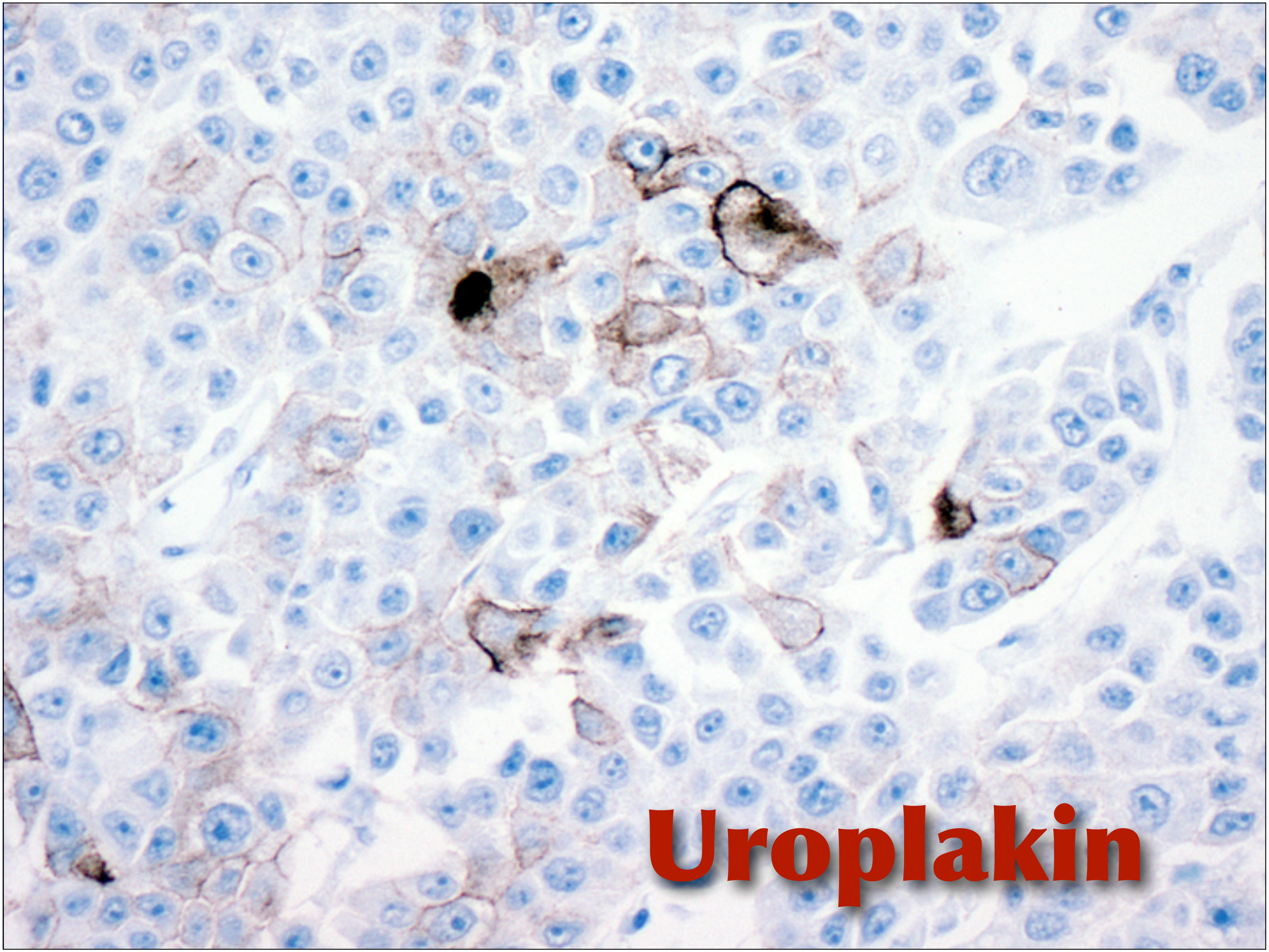
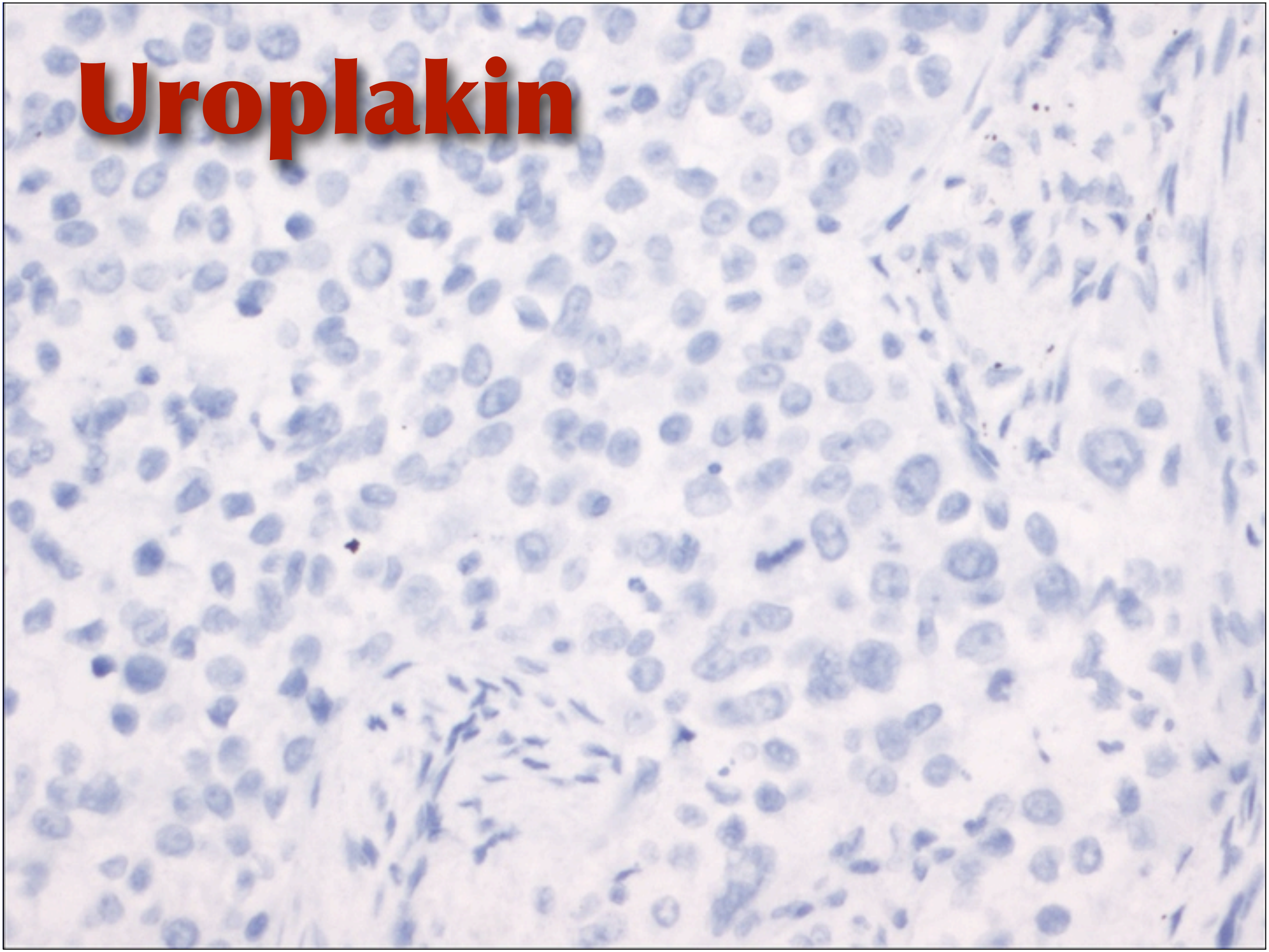

Uroplakin


Uroplakin


Uroplakin

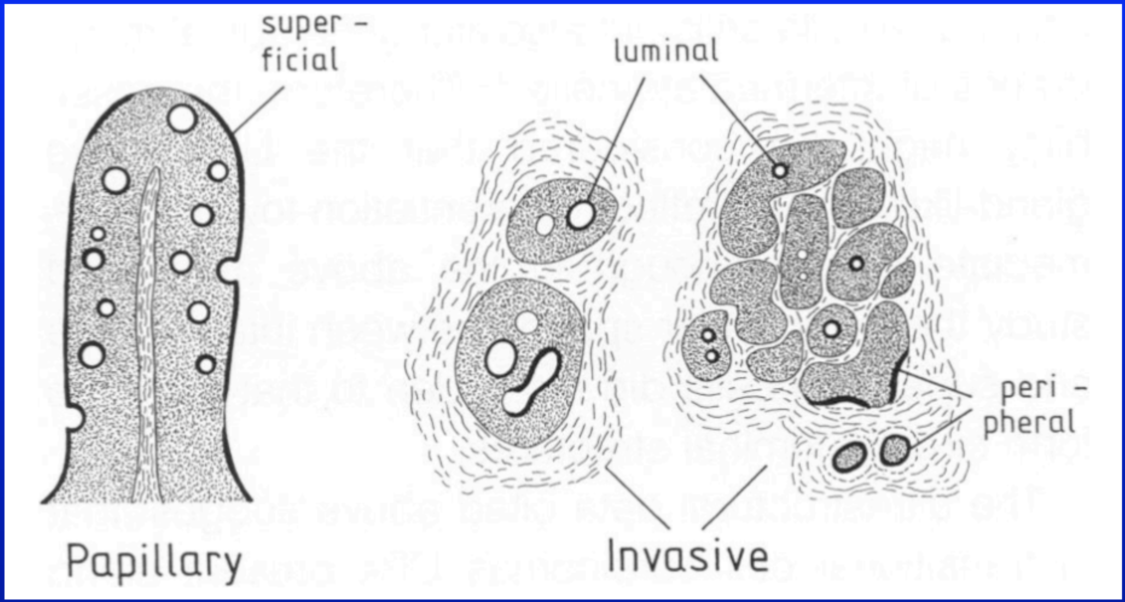
Specific Marker of Bladder Transitional Cell Carcinoma?

Uroplakins, Specific Membrane Proteins of Urothelial Umbrella Cells, as Histological Markers of Metastatic Transitional Cell Carcinomas

Roland Moll,* Xue-Ru Wu,†‡ Jun-Hsiang Lin,†
and Tung-Tien Sun†

Am J Pathol 147:1383-97, 1995

UROPLAKIN III EXPRESSION



- Positive on 14/16 noninvasive TCCs
- Positive on 29/55 (53%) invasive TCCs
- Positive on 23/35 (53%) metastatic TCCs
- Non-TCC carcinomas (N = 177) all negative

Moll R et al., Am J Pathol 147:1383-97, 1995

Am J Clin Pathol 113:683-7, 2000

Uroplakin III Is a Highly Specific and Moderately Sensitive Immunohistochemical Marker for Primary and Metastatic Urothelial Carcinomas

Olaf Kaufmann, MD, Jan Volmerig, and Manfred Dietel, MD

Real World Sensitivity in Metastatic/High grade setting:

< 20%

What About Sarcomatous Transitional Cell CAs?

Am J Surg Pathol 33:99-105 2009

Utility of a Comprehensive Immunohistochemical Panel in the Differential Diagnosis of Spindle Cell Lesions of the Urinary Bladder

Danielle E. Westfall, MD, Andrew L. Folpe, MD,† Gladell P. Paner, MD,* Esther Oliva, MD,‡ Lynn Goldstein, MD,§ Randa Alsabeh, MD,* Allen M. Gown, MD,§ and Mahul B. Amin, MD**

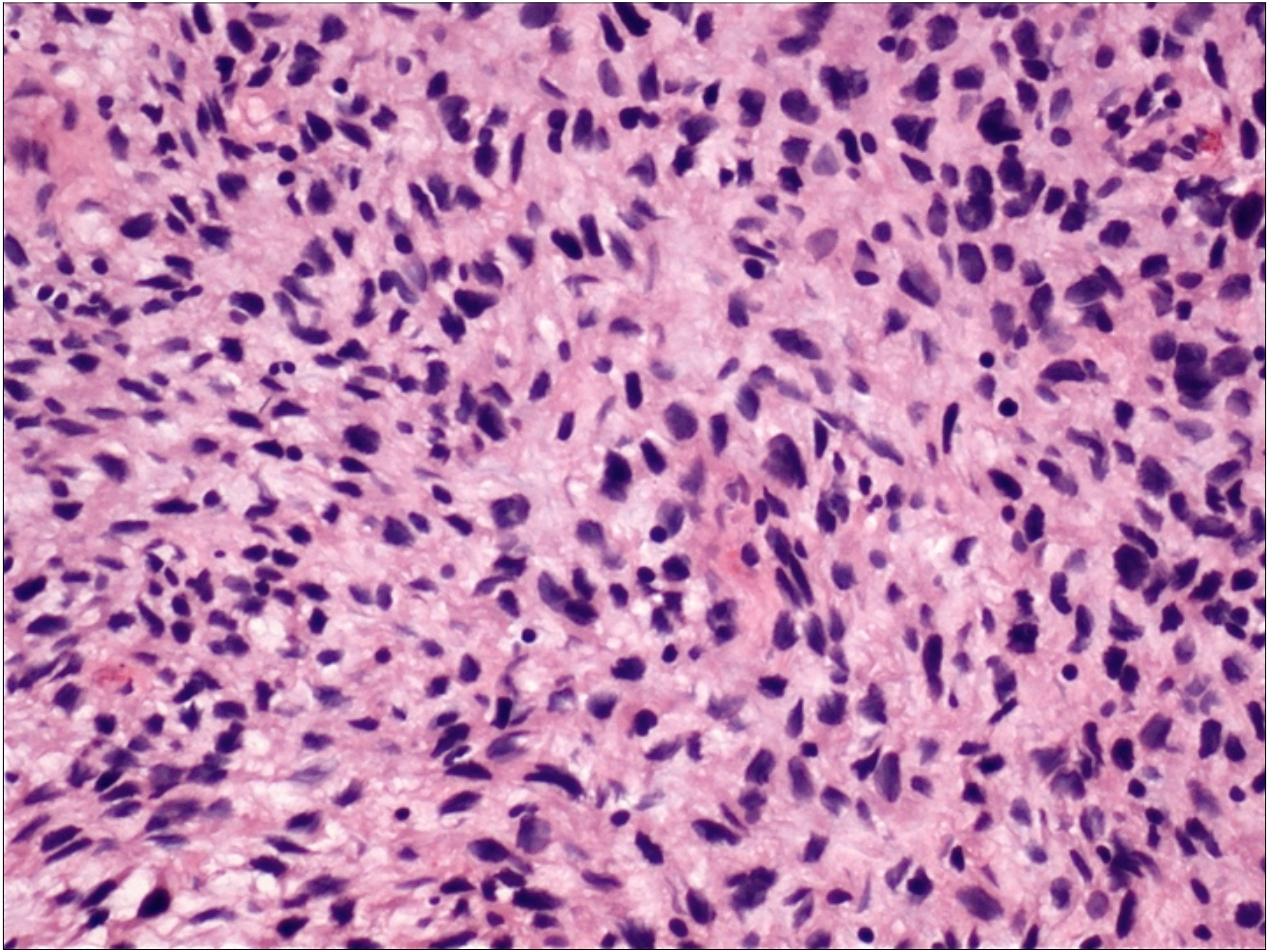
- N = 22 sarcomatous urothelial carcinomas
- Immunophenotype compared with pseudosarcomatous myofibroblastic proliferations, leiomyosarcoma

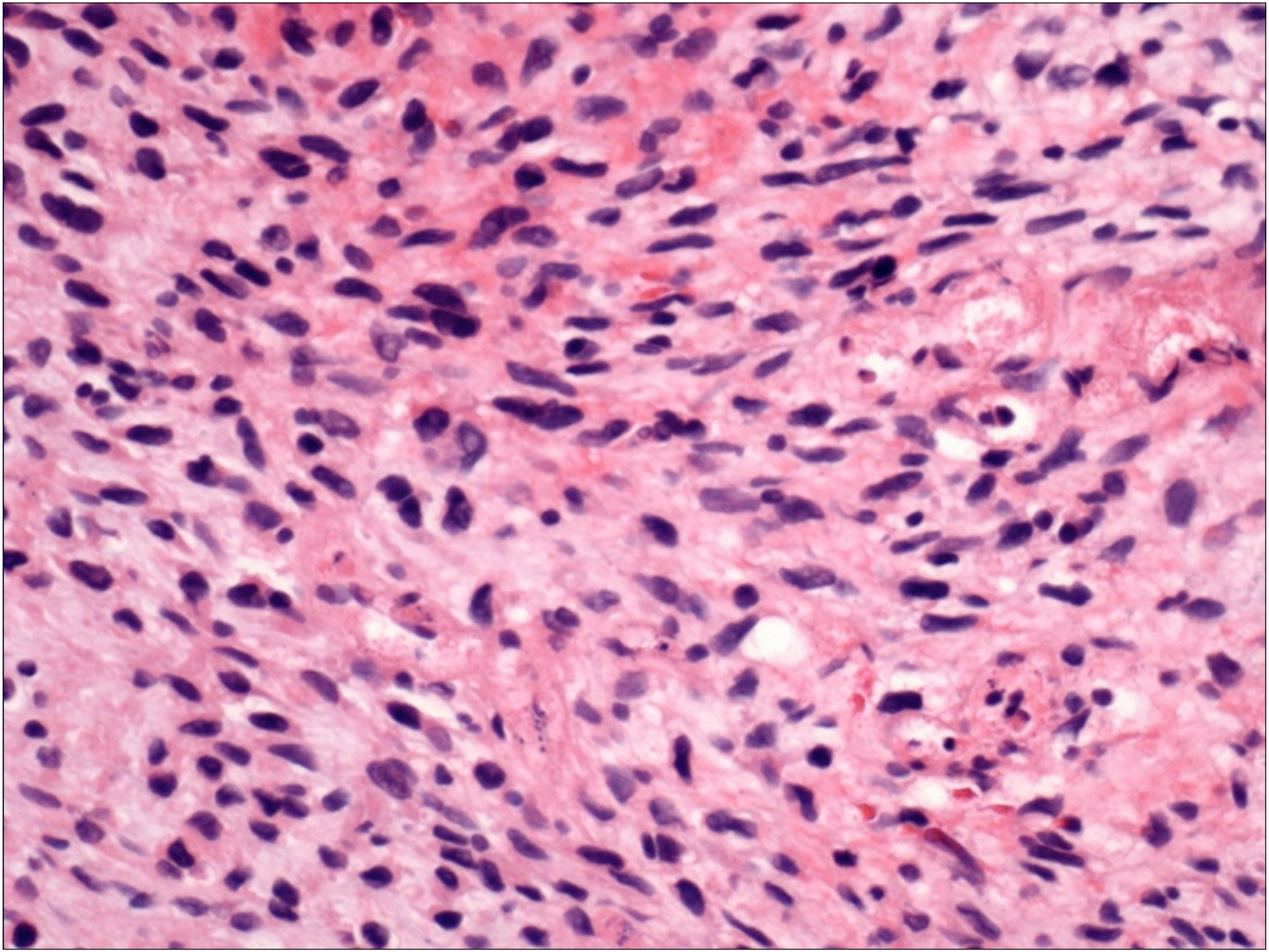
Sarcomatoid Tumors of the Urinary Bladder

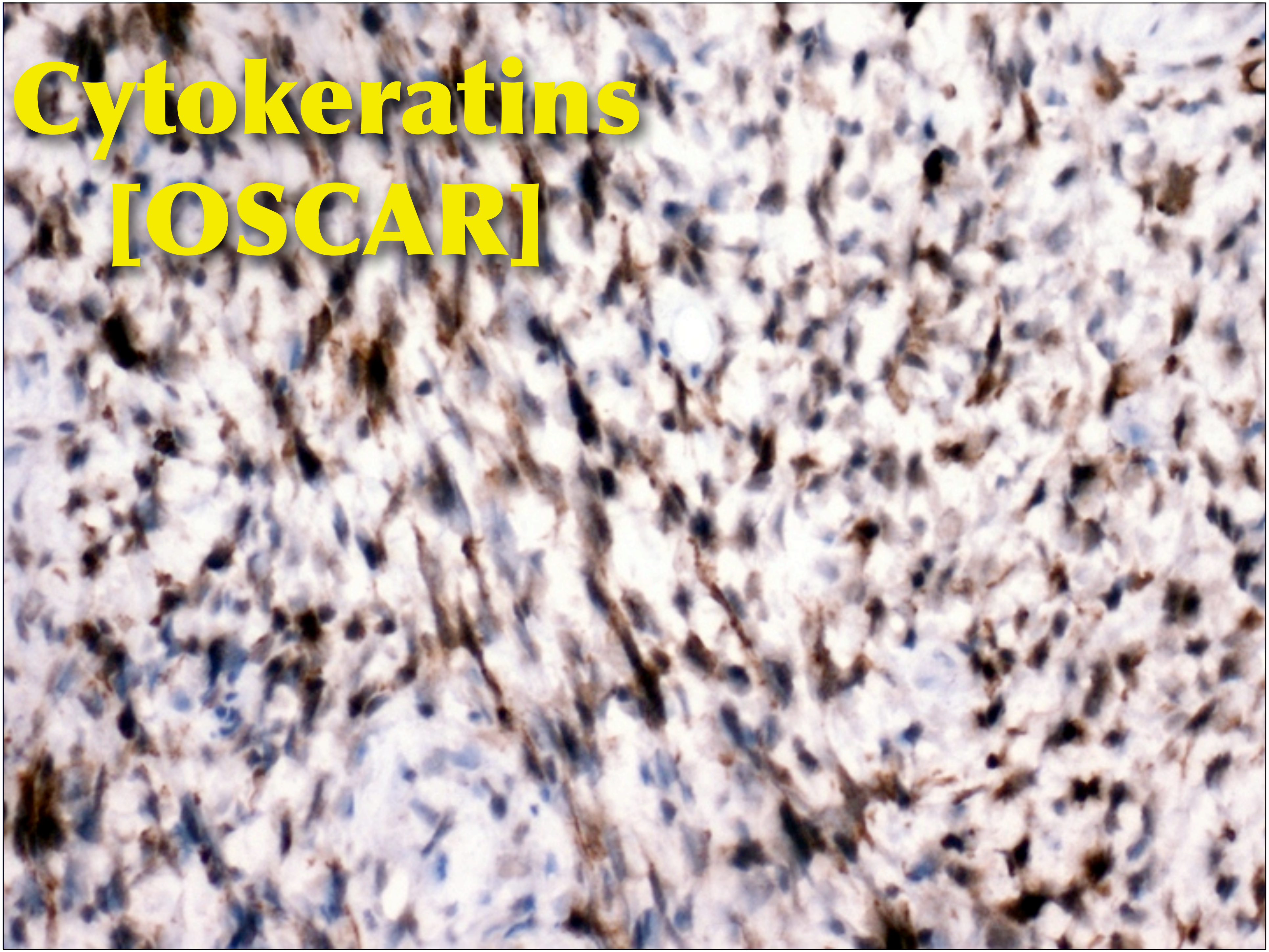
Westfall DE et al., Am J Surg Pathol 33:99-105 2009

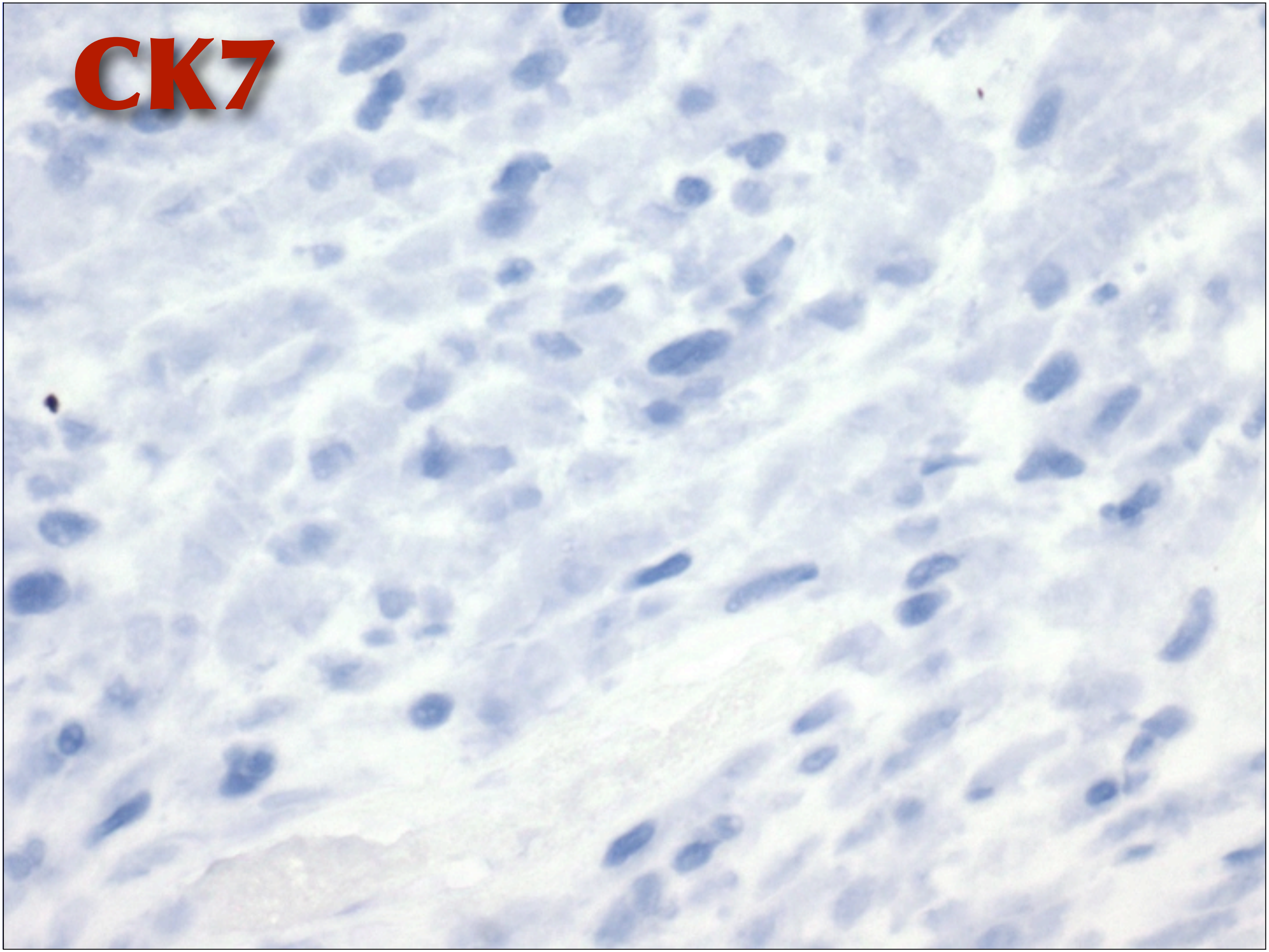
	p63	CKs	CK5	SMA
PMP	0%	78%	0%	100
Sarcom CA	50%	70%	27%	73%
LMS	23%*	58%	0%	85%

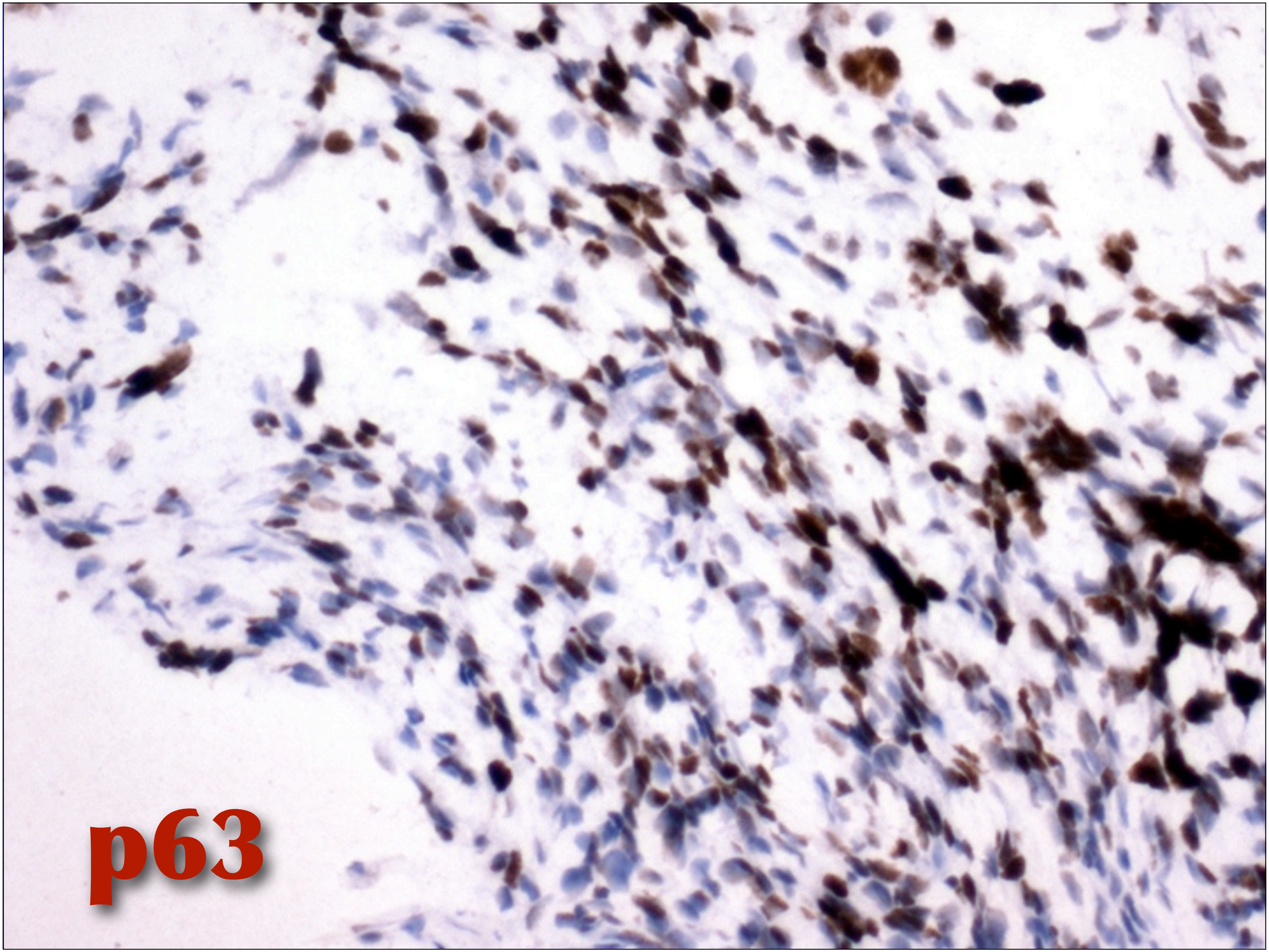
**Usually focal*



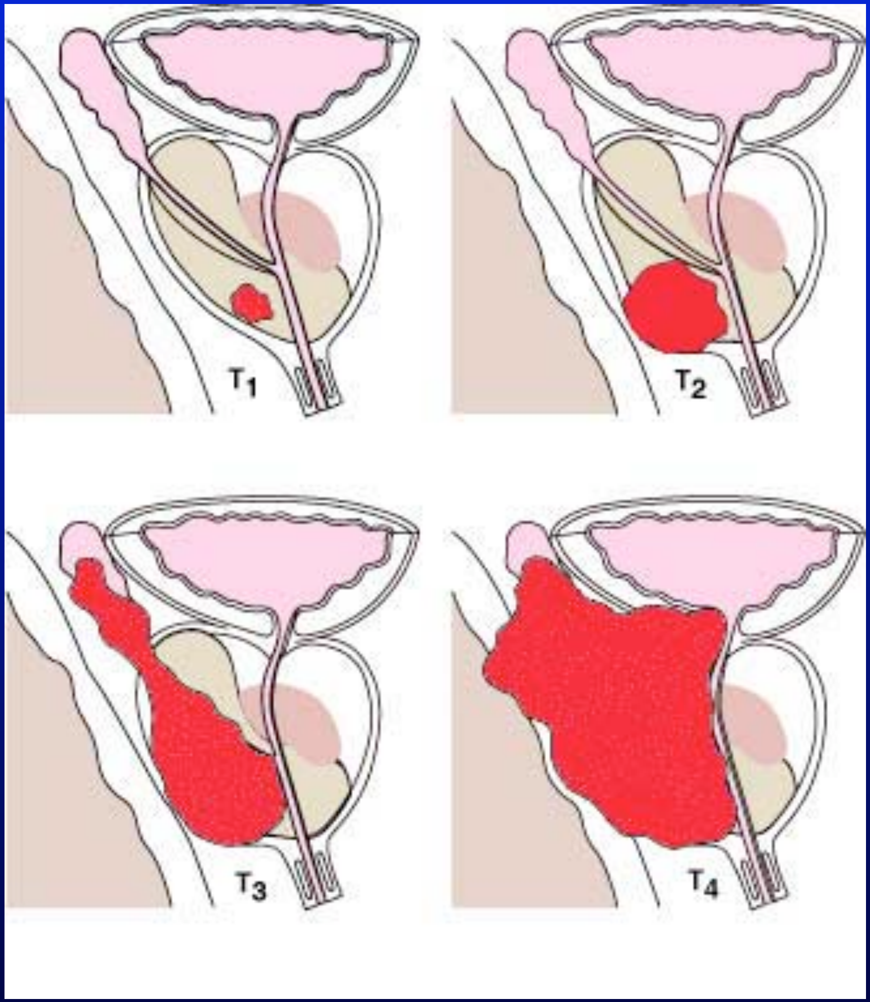



Cytokeratins
[OSCAR]

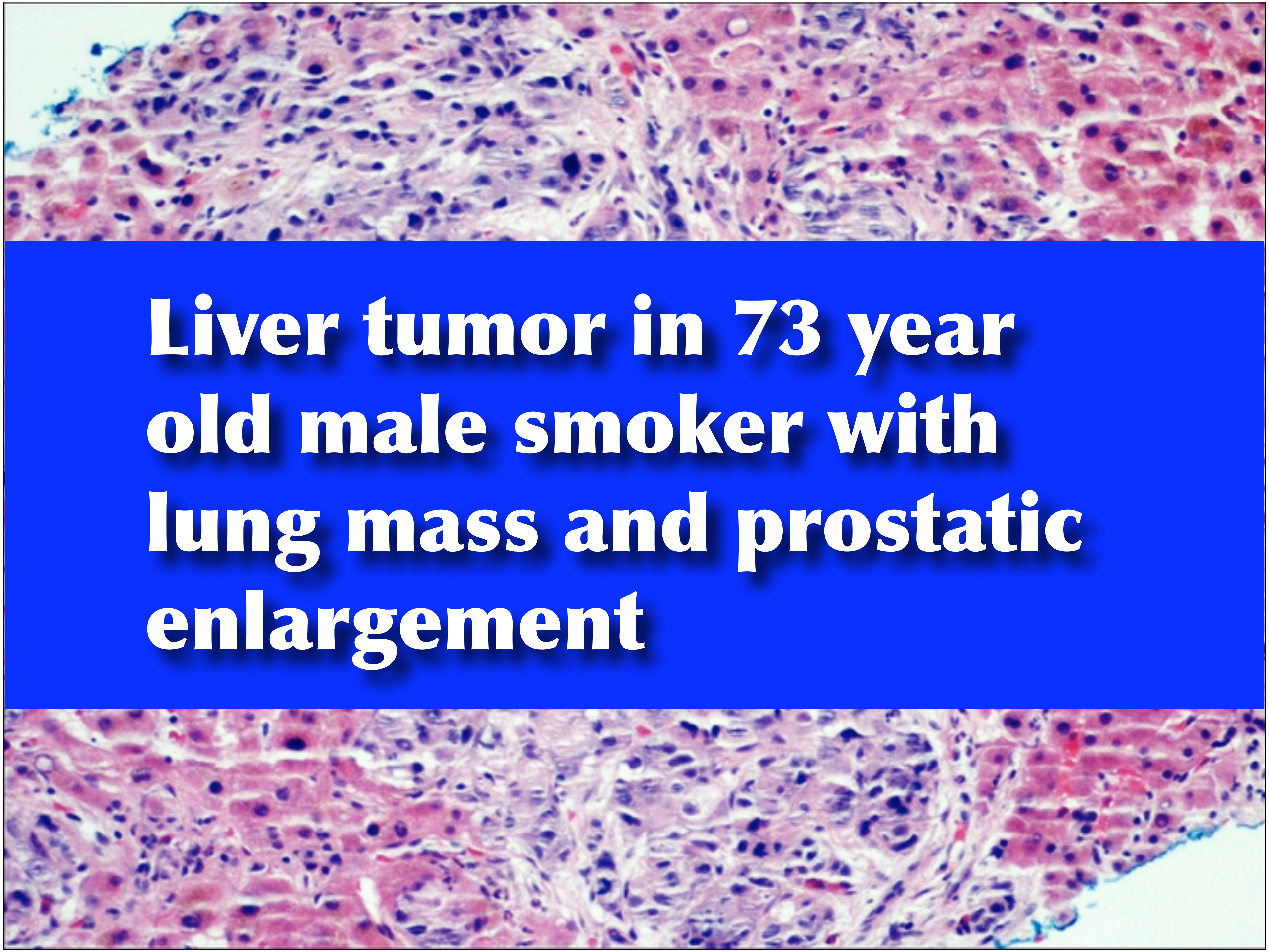

CK7

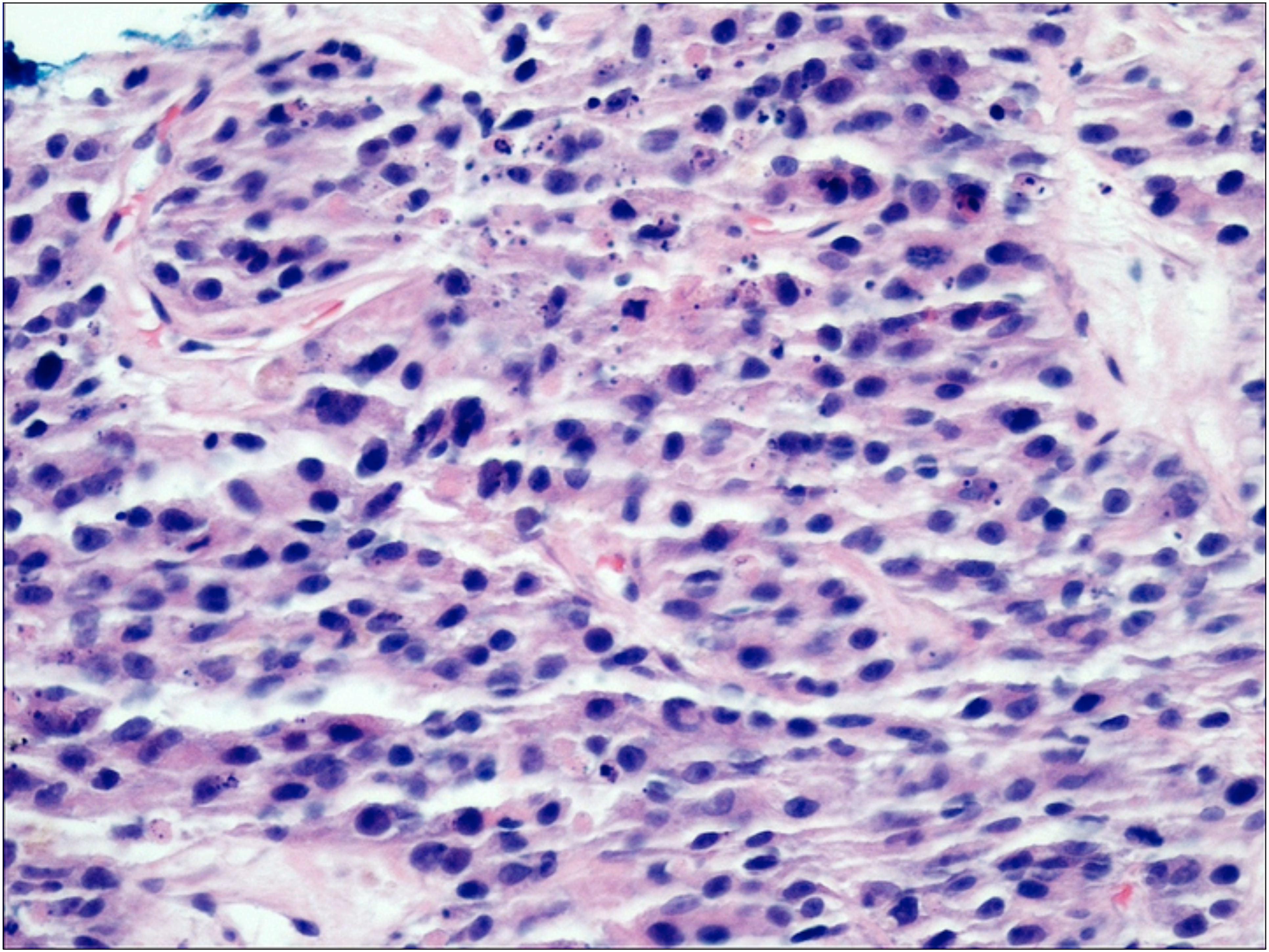

p63

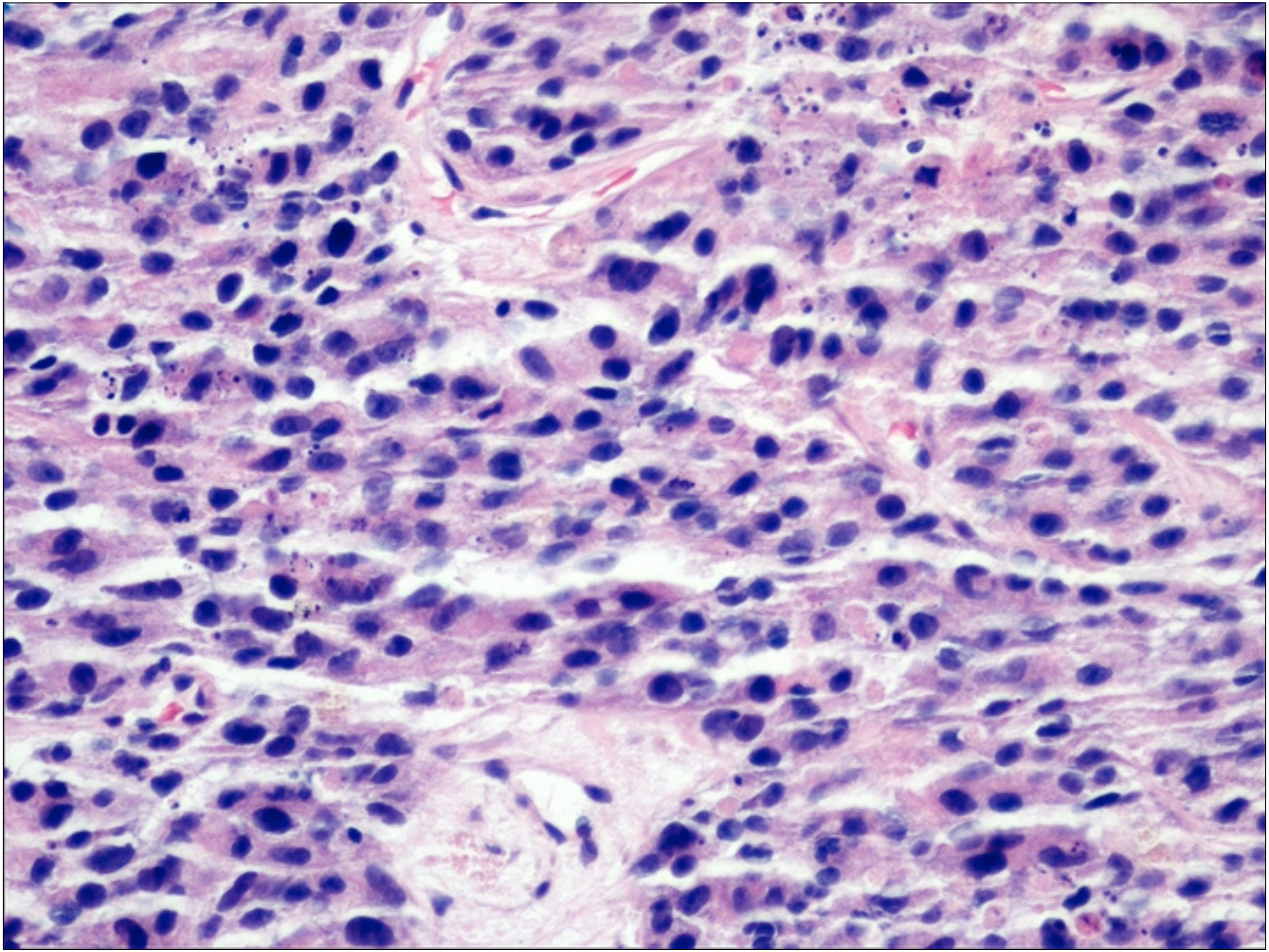
Prostatic Adenocarcinoma
Most Useful Markers

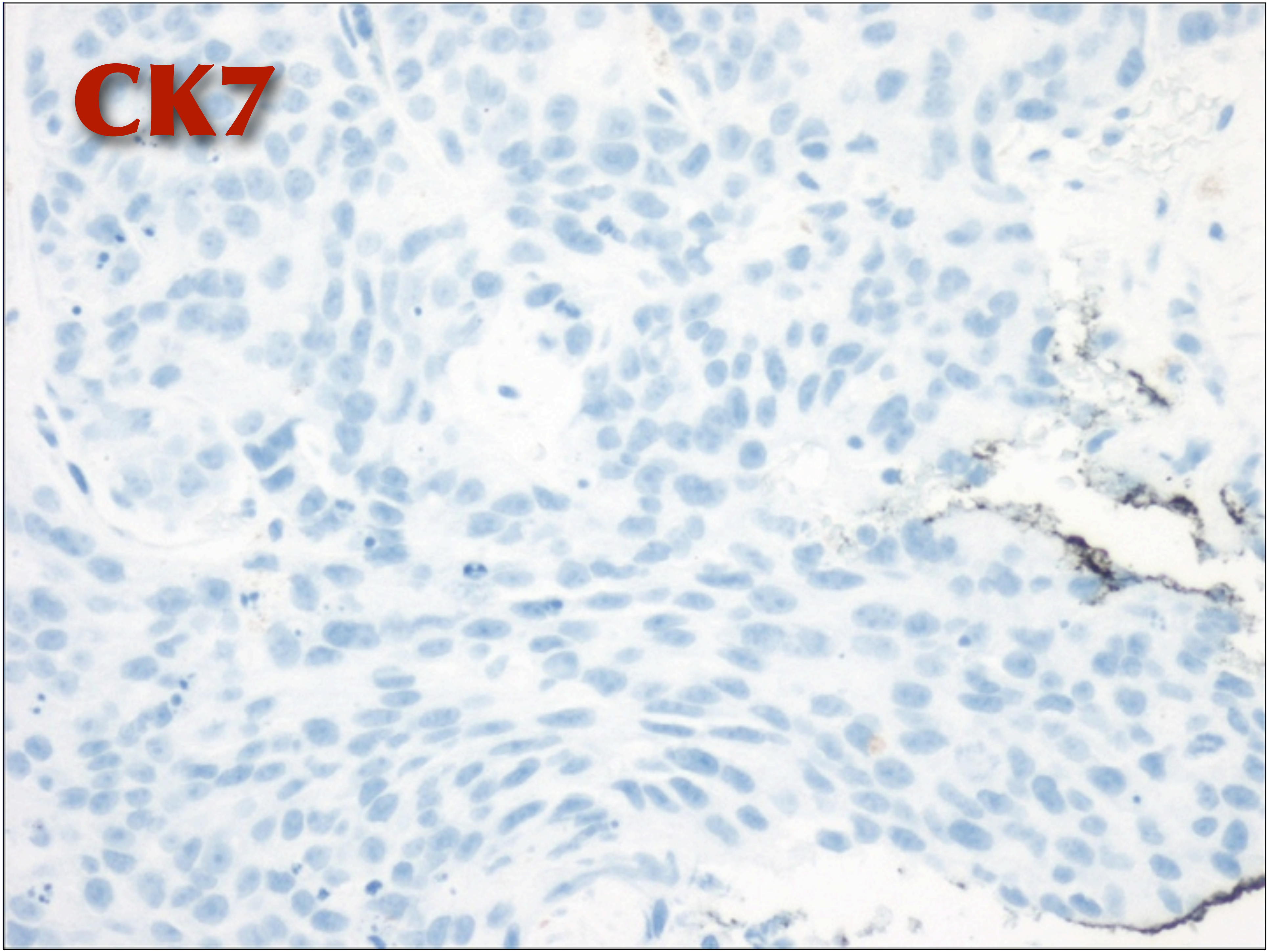


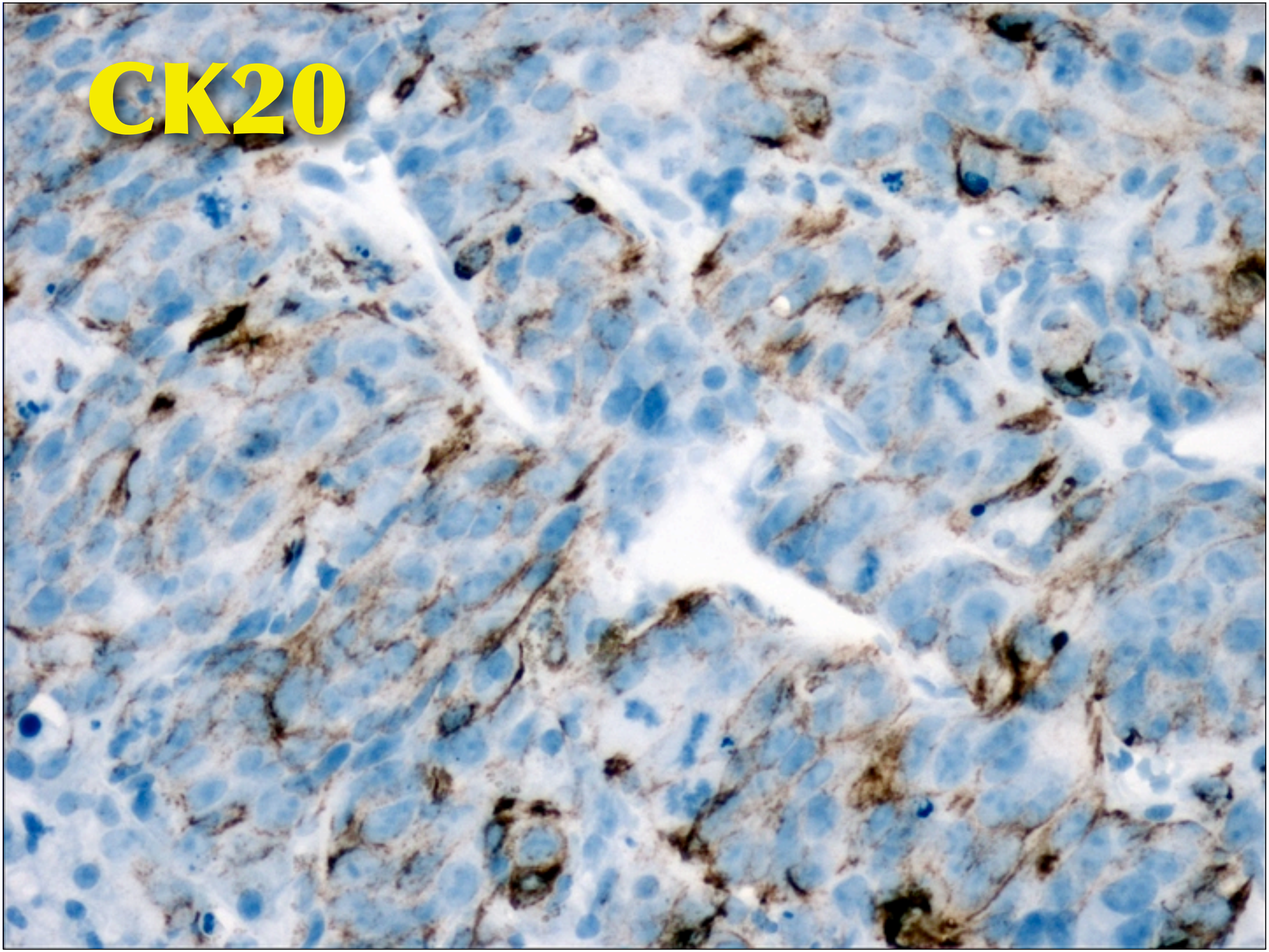
- PSA
- PSA
- PSA
- PSA
- PSA

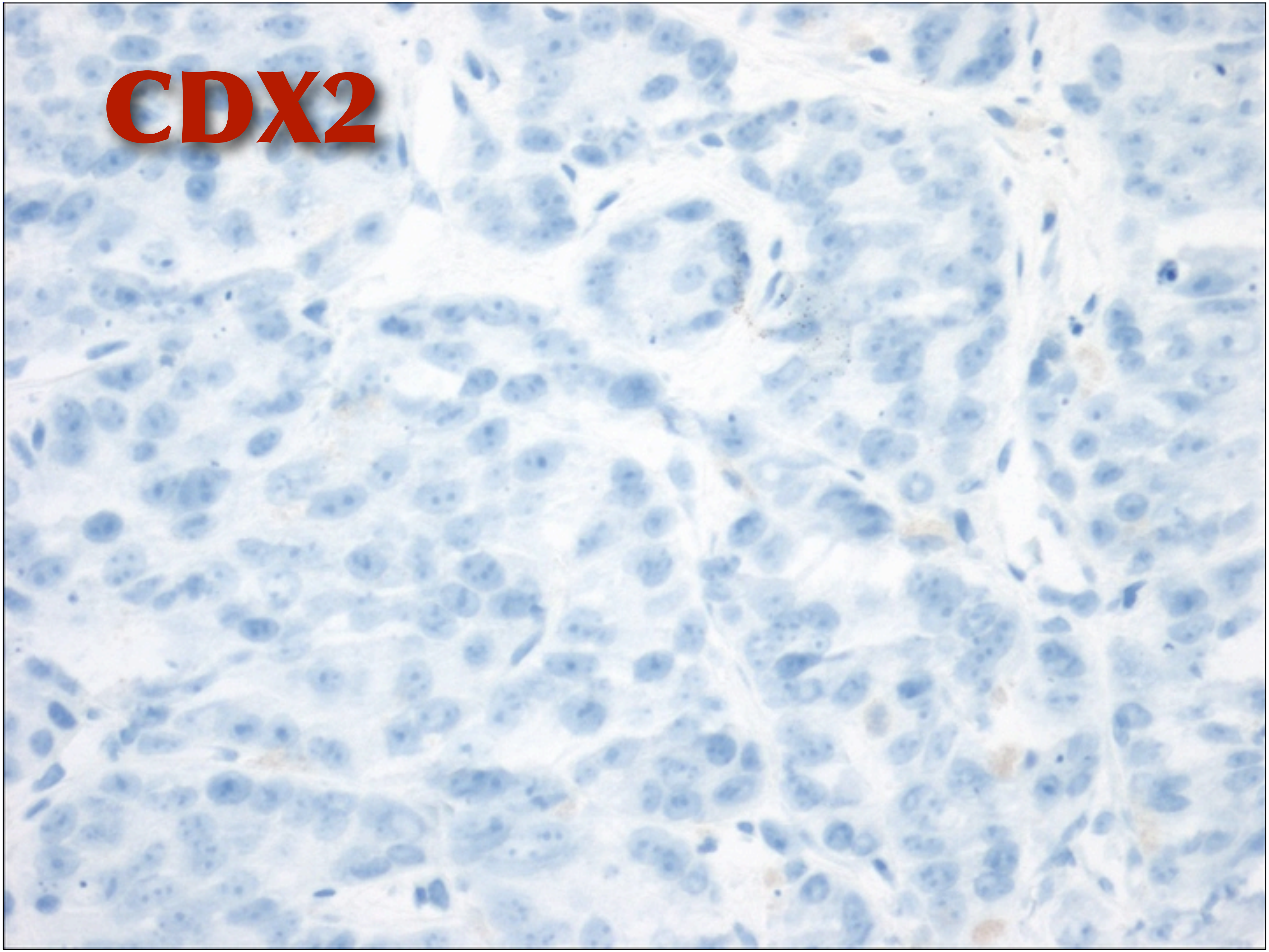

Liver tumor in 73 year old male smoker with lung mass and prostatic enlargement

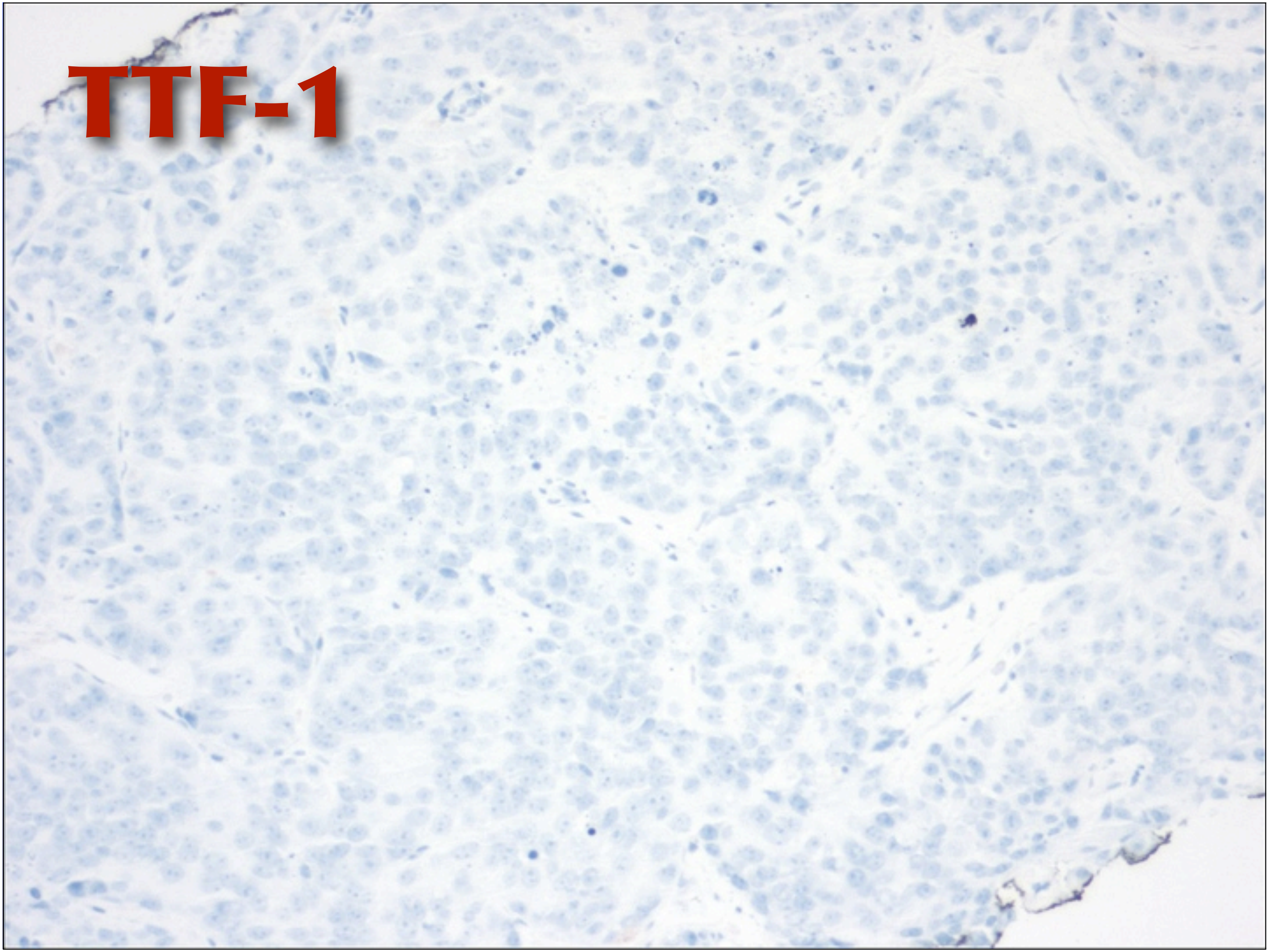


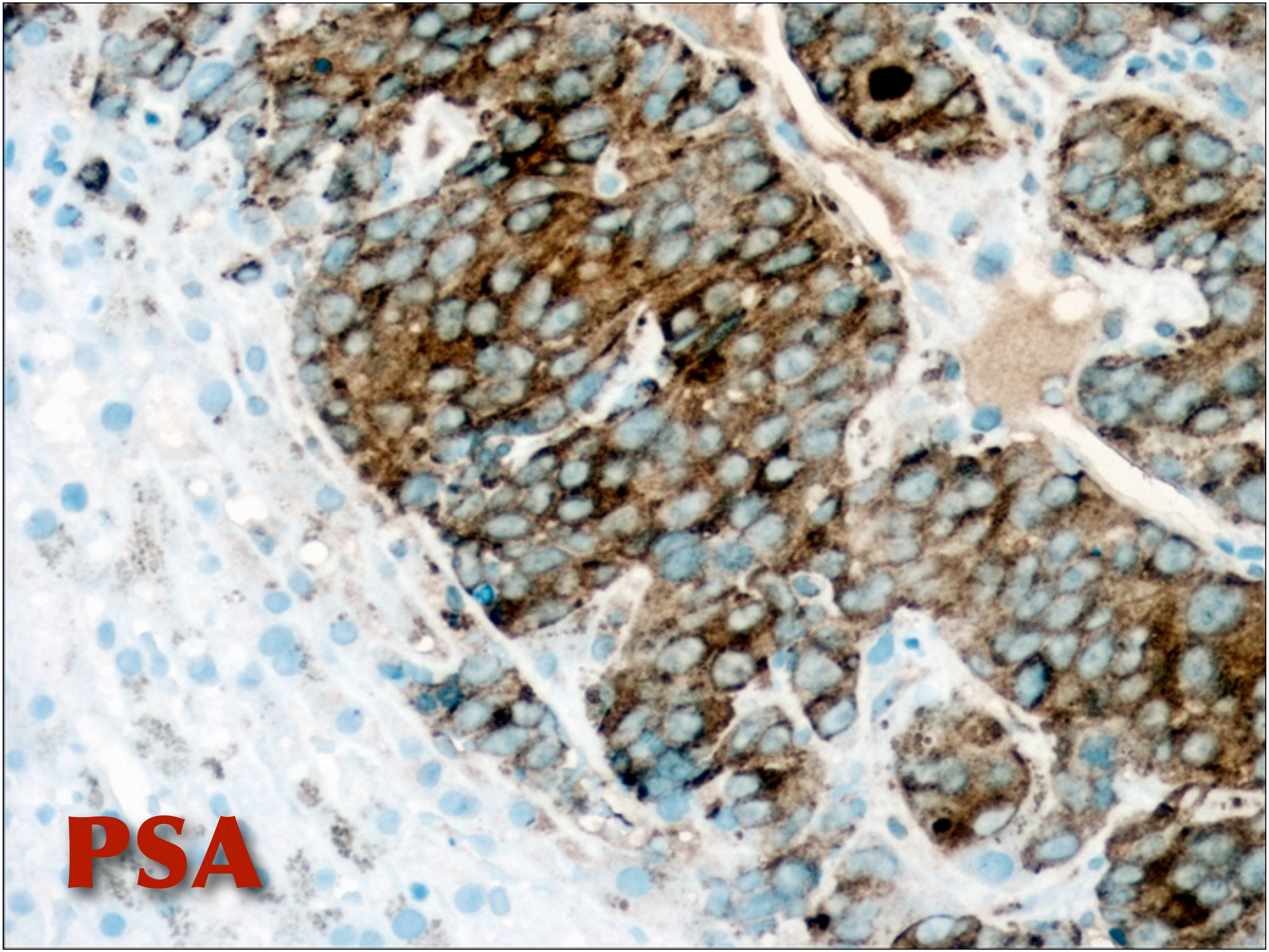


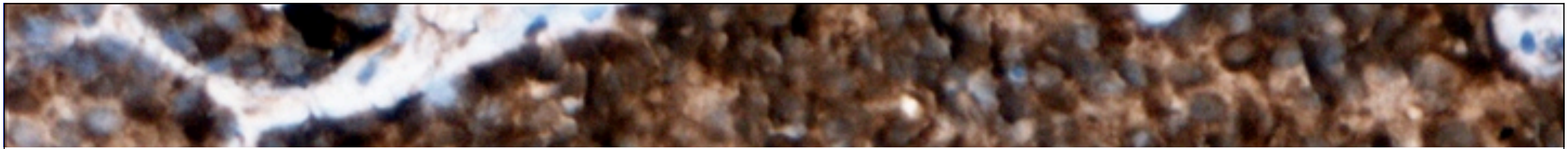

CK7


CK20

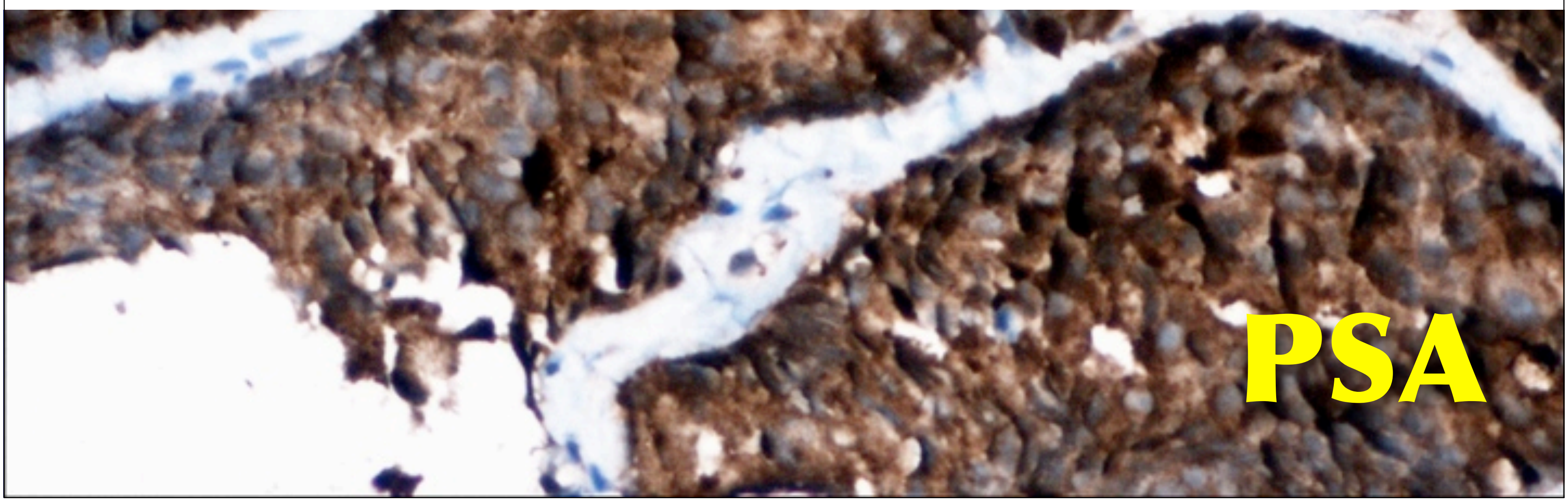

CDX2


TTF-1


PSA



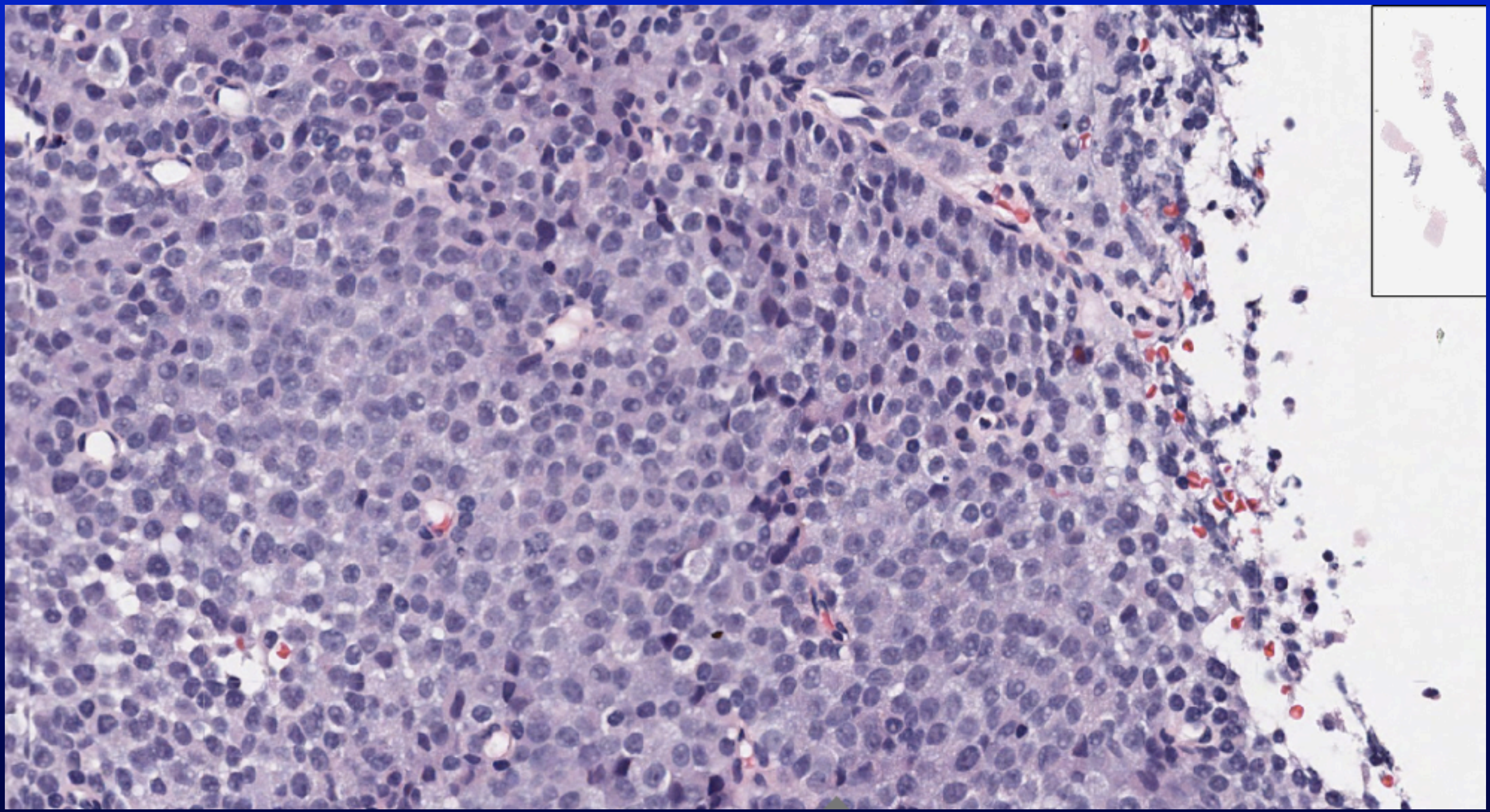
Prostatic AdenoCA Marker

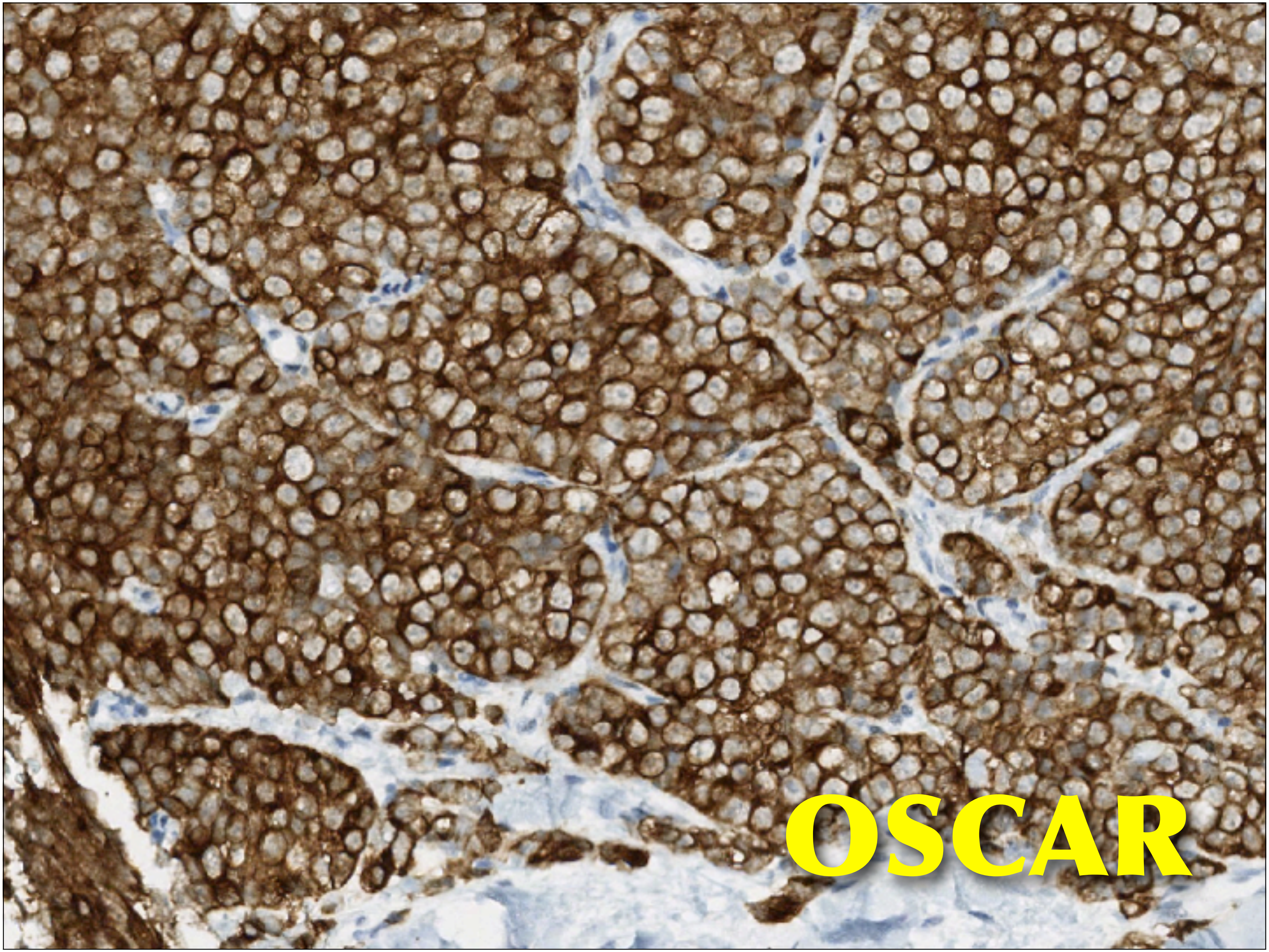


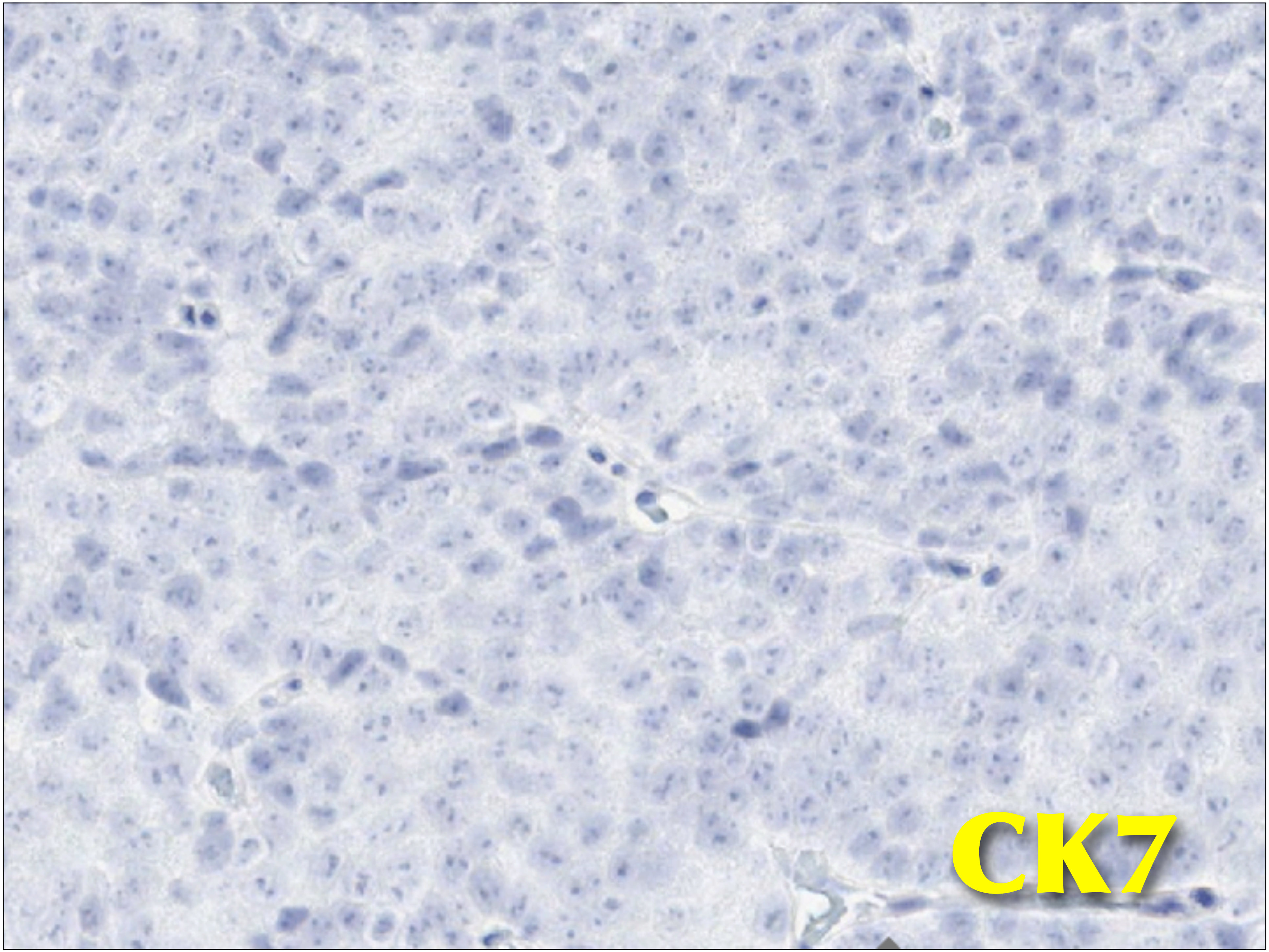
The Early Days of Diagnostic Immunohistochemistry

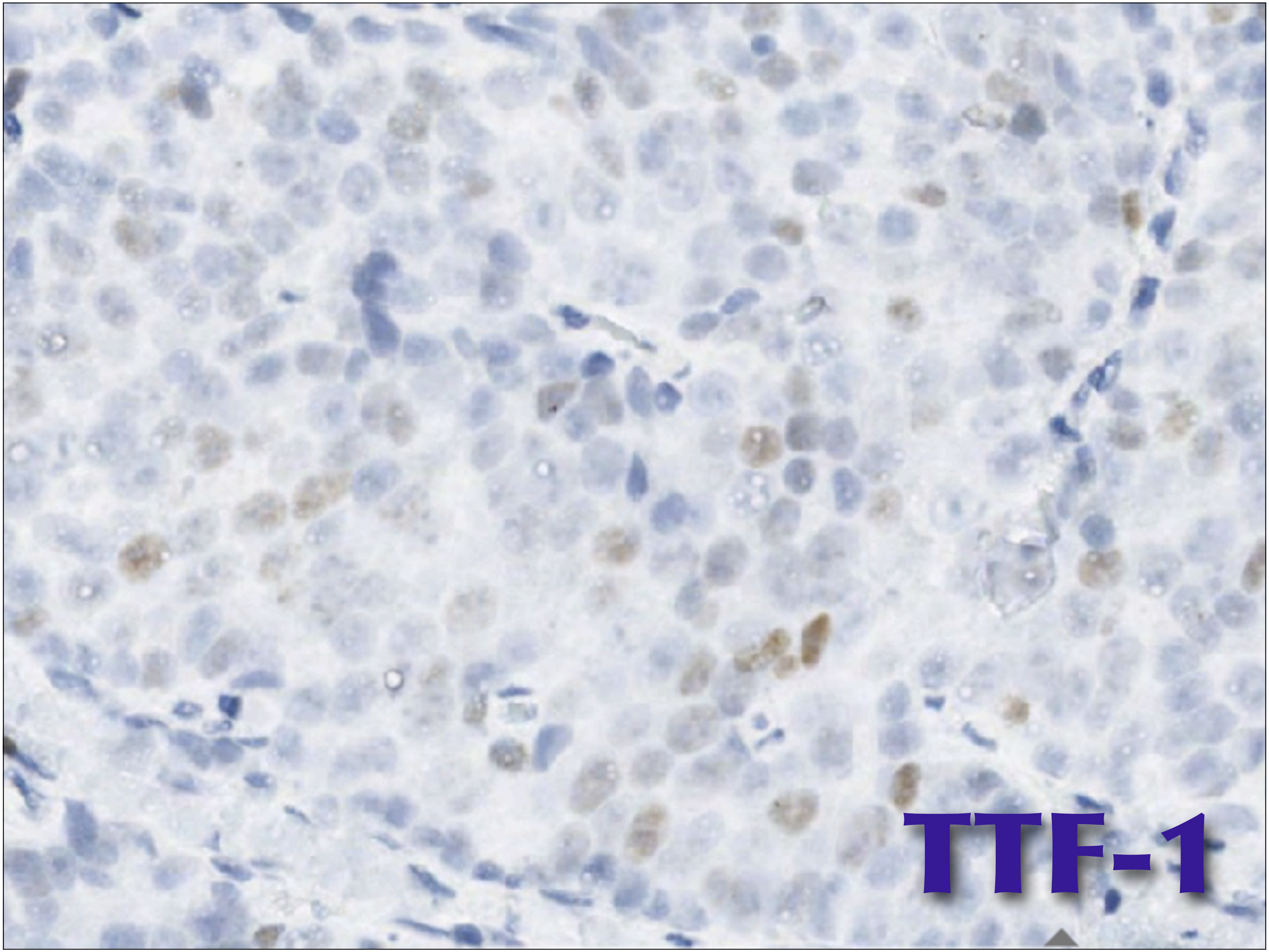
- Nadji M et al., Prostatic origin of tumors. An immunohistochemical study. Am J Clin Pathol 73:735-9, 1980
- Nadji M et al., Prostatic-specific antigen: an imunohistologic marker for prostatic neopalsms. Cancer 48:1229-32, 1981

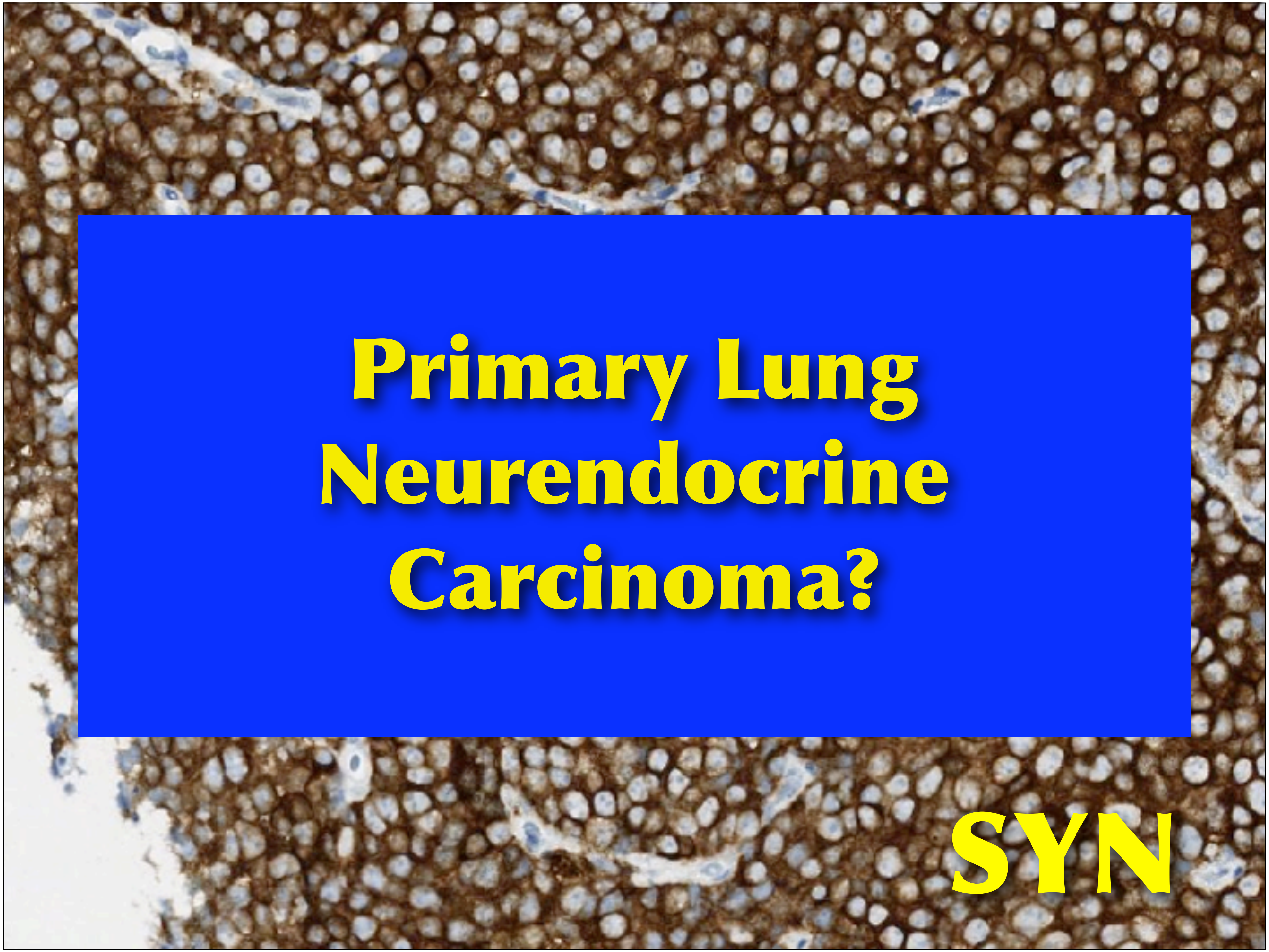
78 Year Old Male Smoker with Tumor in Lung - No Prior History Given




OSCAR


CK7


TTF-1


Primary Lung
Neurendocrine
Carcinoma?
SYN

True or False?

- Expression ... in a car... ...docrine
- ...ession of TTF-1 points unequivocally to lung primary

FALSE!

TTF-1

- A highly sensitive and specific marker of carcinomas of lung (and thyroid) origin, both neuroendocrine and non-neuroendocrine
- Sensitivity and specificity highly dependent on histologic cell type

TTF-1

- Loses fidelity for lung carcinoma in context of high grade (not low grade) neuroendocrine carcinomas
- Cannot be used to identify primary site of metastatic high grade neuroendocrine carcinoma
- Can distinguish Merkel cell vs. metastatic lung small cell (NE) carcinoma


Metastatic Prostatic Adencarcinoma!
PSA

Metastatic Prostatic Adenocarcinoma

- No technical issues - all immunostaining real
- With tumor progression, prostatic adenocarcinomas can acquire neuroendocrine differentiation
- High grade NE CAs can show TTF-1 expression
- Near perfect specificity of PSA confirms Dx

Hum Pathol 34:1001-8, 2003

Tissue Microarray Analysis of Neuroendocrine Differentiation and Its Prognostic Significance in Breast Cancer

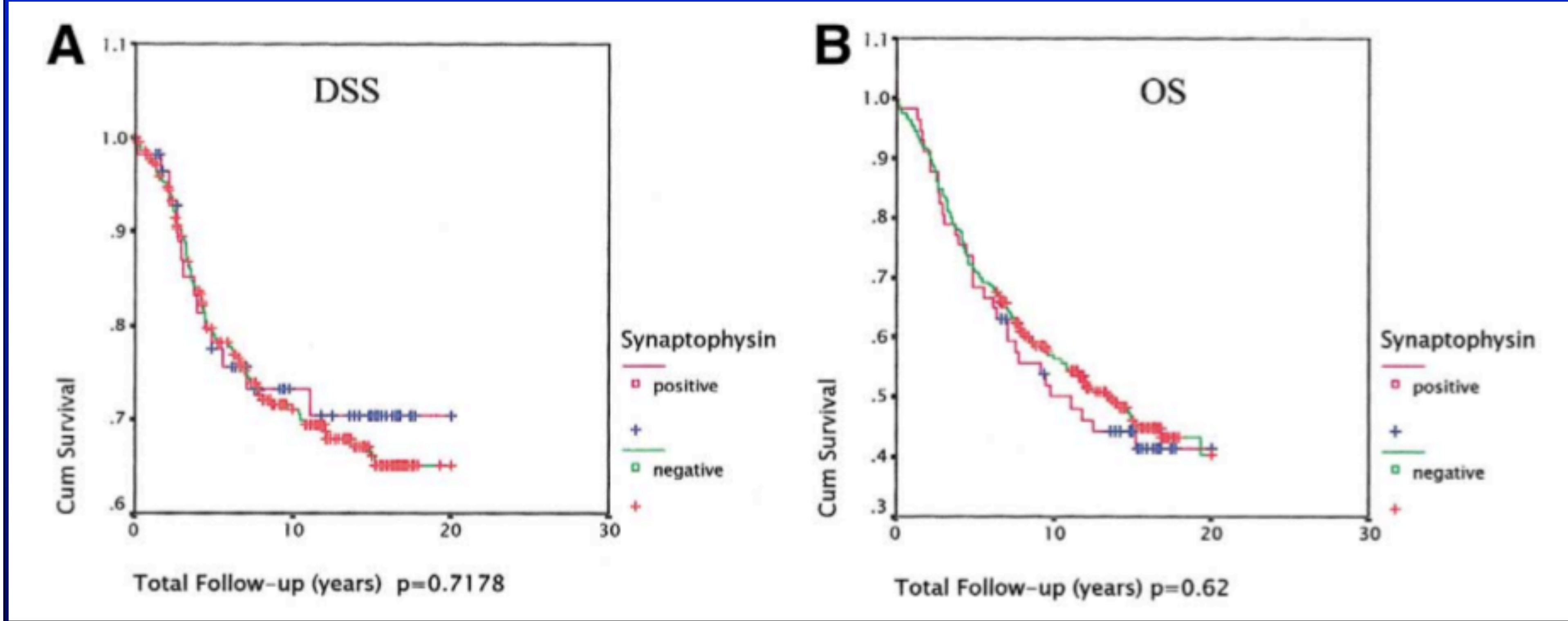
NIKITA MAKRETSOV, MD, PHD, C. BLAKE GILKS, MD, ANDREW J. COLDMAN, PHD, MALCOLM HAYES, MD, AND DAVID HUNTSMAN, MD

- Synaptophysin, Chromogranin A, NSE expression
- N = 334 breast cancers (with clinical outcome)

Synaptophysin only	**14.1%**
Chromogranin only	**0%**
Any NE marker	**19.5%**

Makretsov N et al., Hum Pathol 34:1001-8, 2003

NO RELATIONSHIP TO CLINICAL OUTCOME!



Makretsov N et al., Hum Pathol 34:1001-8, 2003

Thank you for your attention!

Questions?

gown@phenopath.com