Diagnostic Immunohistochemistry:

Case Studies

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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

Immunophenotype Heat Maps

>85% pos	**70-85% pos**	**NS**	**70-85% neg**	**>85% neg**

Case 1

63 year old female with no prior medical history presents with 6 cm mass at ileocecal valve

Case 1

- **Colorectal adenoCA**
- **Neuroendocrine CA**
- **Metastatic CA, eg., from ovary**

Case 1

Cytokeratins	**Uniformly positive**
Cytokeratin 7	**Negative**
Cytokeratin 20	**Negative**
CDX2	**Negative**
Villin	**Focally positive**
Synaptophysin	**Negative**

Case 1

Continued

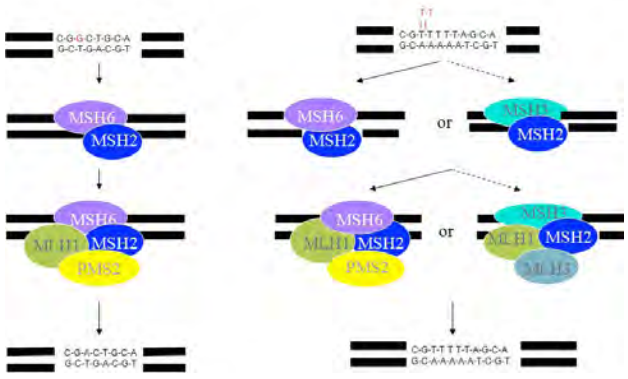
MLH1	**Loss of expression**
MSH2	**No loss of expression**
MSH6	**No loss of expression**
PMS2	**Loss of expression**

FINAL DX: Primary colorectal adenocarcinoma, MSI type

DNA Mismatch Repair System

- MLH1
- PMS2
- MLH2
- MSH6

DNA Mismatch Repair



DNA mismatch repair promotes genomic stability by correcting base-base and small insertion/deletion mispairs that arise during DNA replication and recombination

http://www.helsinki.fi/bioscience/mmrandcancer/mmrgenetics.html

What Is Microsatellite Instability (MSI)?

- The presence of a discrepancy between the size of microsatellites in DNA from tumor compared with nontumor tissue
- Usually results from loss of gene expression of one or more MMR genes that would normally correct these errors

What Are Microsatellites?

- Repetitive segments of DNA two to five nucleotides in length scattered throughout the genome both in noncoding as well as coding regions
- Regions are inherently unstable and susceptible to mutations

HNPCC (Lynch Syndrome)

Hereditary Non-polyposis Colorectal Cancer

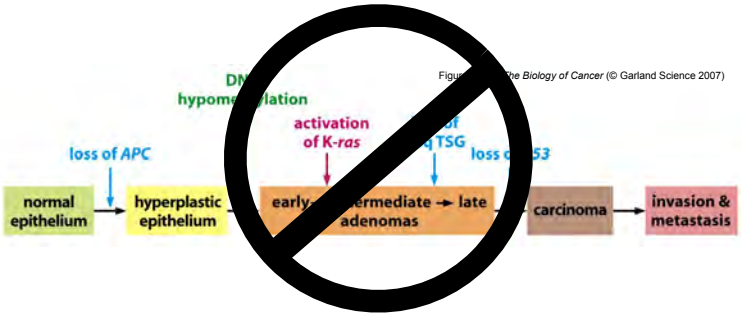
- Autosomal dominant
- Accounts for 2-5% of colorectal adenocarcinoma
- Tumors develop at early age, usually found on right side
- Also develop endometrial adenocarcinoma
- Synchronous and metachronous colorectal cancers: 40% develop within 10 years without total colonic resection

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

Classical 'Vogelstein' Pathway of Colonic Adenocarcinoma Progression



?Through sessile adenoma pathway?

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)

IHC vs. MSI Testing

	IHC	MSI
Cost	$$	$$$
Analyte	Protein	DNA
How much tumor required	Very little	Very little
Requirements	Tumor only	Tumor + normal
Possibility of contamination by normal	No	Yes
Turnaround	Next day	2-7 days
Identifies involved gene	Yes	No
Assay sensitive to fixation	Yes	No

adapted from Bellizzi AM and Frankel WL, Adv Anat Pathol 16:405-17, 2009

IHC v. MSI Testing

- Concordance high in most studies
- High concordance possible even with just two antibodies (e.g., MLH1, MSH2) but even higher with four (MLH1, MSH2, PMS2, MSH6)
- Potential shortcoming if IHC is inability to detect missense mutations that nevertheless result in immunoreactive but nonfunctional protein

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,[9] 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,[8] 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,[28] 2002	1144	hMLH1, hMSH2	92	100	100	97
Lanza et al,[13] 2002	305	hMLH1, hMSH2	91	100	100	94
Plaschke et al,[12] 2002	228	hMLH1, hMSH2, hMSH6, hPMS2	84†	100†	100†	96†
			95‡	100‡	100‡	99‡
			98§	100§	100§	99§
Present series	204	hMLH1, hMSH2, hMSH6, hPMS2	93†	100†	100†	99†
			100‡	100‡	100‡	100‡
			100§	100§	100§	100§
Total of 16 series‖	3494	. . .	92.4	99.6	98.5	97.8

MMR IHC and Colorectal Adenocarcinoma

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

Reasons for MMR IHC

- Identifying Lynch Syndrome patients
- Identifying patients with sporadic 'MSI tumors' who may not require FU-based chemotherapy
- Identifying 'carcinomas of unknown primary' that are 'minimally differentiated' colorectal adenocarcinoma

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

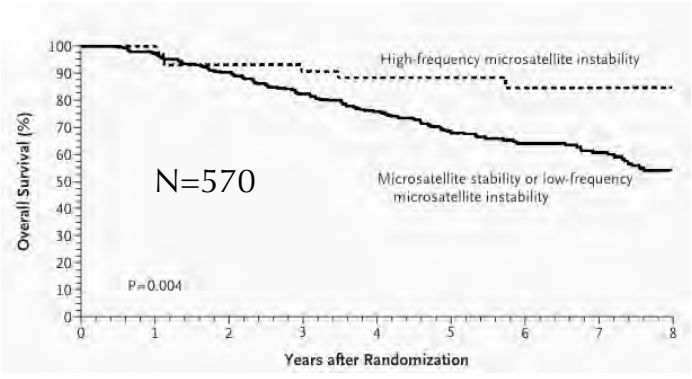
- Prognostic factor (patients with MSI-H tumors have significantly lower mortality rate independent of tumor stage)
- Predictive factor (patients with MSI-H tumors do more poorly with fluorouracil-based adjuvant chemotherapy)
- Can alert clinician to possibility of unrecognized HNPCC

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

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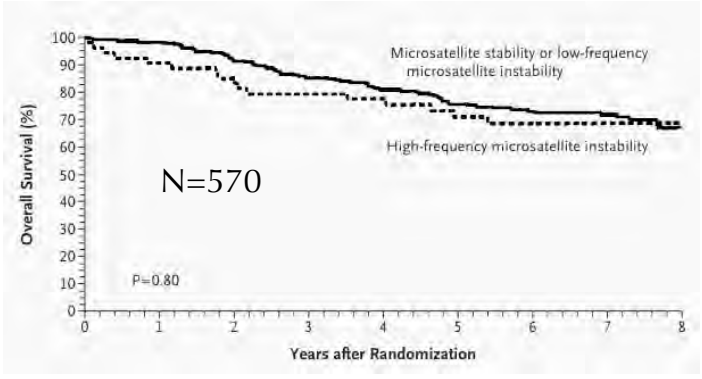


Ribic CM et al. NEJM 349:247-57, 2003

Value of Mismatch Repair, *KRAS*, and *BRAF* Mutations in Predicting Recurrence and Benefits From Chemotherapy in Colorectal Cancer

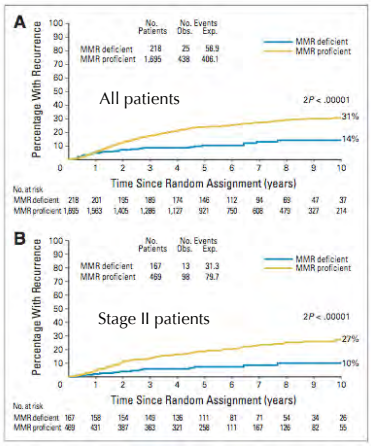
J Clin Oncol 29:1261-70, 2011

Gordon Hutchins, Katie Southward, Kelly Handley, Laura Magill, Claire Beaumont, Jens Stahlschmidt, Susan Richman, Philip Chambers, Matthew Seymour, David Kerr, Richard Gray, and Philip Quirke

- Do modest benefits of adjuvant chemotherapy in stage II colorectal adenocarcinoma justify toxicity, cost, inconvenience?
- N = 1013 patients randomly assigned between 5FU and folinic acid chemotherapy vs. no chemotherapy
- Ten year outcome data

Hutchins G et al. J Clin Oncol 29:1261-70, 2011

- 11% of tumors dMMR
- Risk of recurrence of dMMR half that of pMMR
- Strongly recommend IHC assessment of MMR status routine clinical practice



Reasons for MMR IHC

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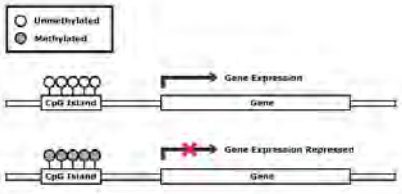
- Identifying 'carcinomas of unknown primary' that are 'minimally differentiated' colorectal adenocarcinoma

MSI-Type Colorectal Adenocarcinomas



- Hypermethylation of MLH1 promoter CpG islands
- But such tumors may be better characterized as "CpG Island Methylator Phenotype High" (CIMP-H)

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

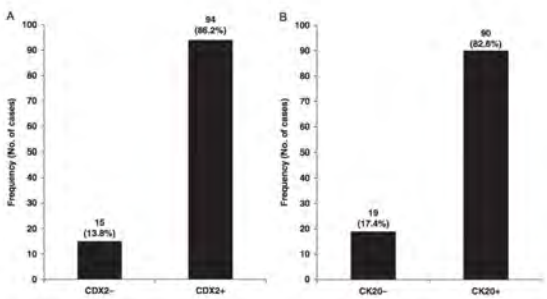
Takao Hinoi,* Masachika Tani,*† Peter C. Lucas,‡ Karel Caca,* Rodney L. Dunn,§ Ettore Macri,† Massimo Loda,† Henry D. Appelman,‡ Kathleen R. Cho,*‡§ and Eric R. Fearon*‡‖§

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

Am J Clin Pathol 140:561-6, 2013

Loss of CDX2/CK20 Expression Is Associated With Poorly Differentiated Carcinoma, the CpG Island Methylator Phenotype, and Adverse Prognosis in Microsatellite-unstable Colorectal Cancer

Jung Ho Kim, MD, PhD,† Ye-Young Rhee, MD,* Jeong Mo Bae, MD,* Nam-Yun Cho, MSc,‡ and Gyeong Hoon Kang, MD, PhD*‡*



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Jung Ho Kim, MD, PhD,† Ye-Young Rhee, MD,* Jeong Mo Bae, MD,* Nam-Yun Cho, MSc,‡ and Gyeong Hoon Kang, MD, PhD*‡*

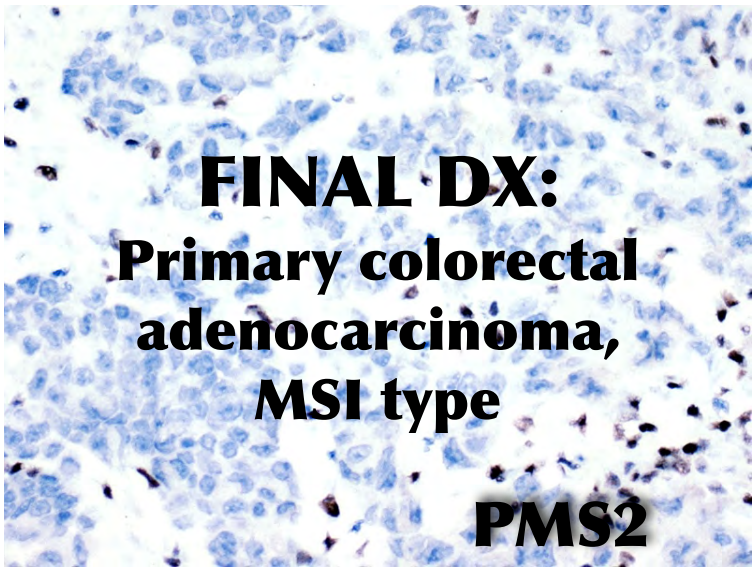
- CDX2-/K20- phenotype associated with older age, higher stage, LN metastases, "medullary" histology, BRAF mutation, CIMP-H status.
- Patients have worse survival compared with those expressing CDX2 and/or K20
- This is a poor prognosis subgroup

MMR IHC Interpretation Caveats

- There must be complete loss of MMR expression in the tumor cell population
- There can be variegated and incomplete immunostaining owing to fixation issues as well as intrinsic variation (e.g., MSH6)
- Don't overcall dMMR if there is no staining within the non-neoplastic elements

MMR Enzyme IHC

- IHC testing for loss of expression of MMR enzymes is a surrogate for the identification of microsatellite instability (MSI)
- IHC testing for loss of expression of (usually) pair of MMR enzymes useful in screening for MSI tumors that characterize Lynch Syndrome
- IHC testing for loss of expression of (usually) pair of MMR enzymes useful in identifying the subset (10-15%) of sporadic colorectal adenocarcinomas with MSI



Case 2

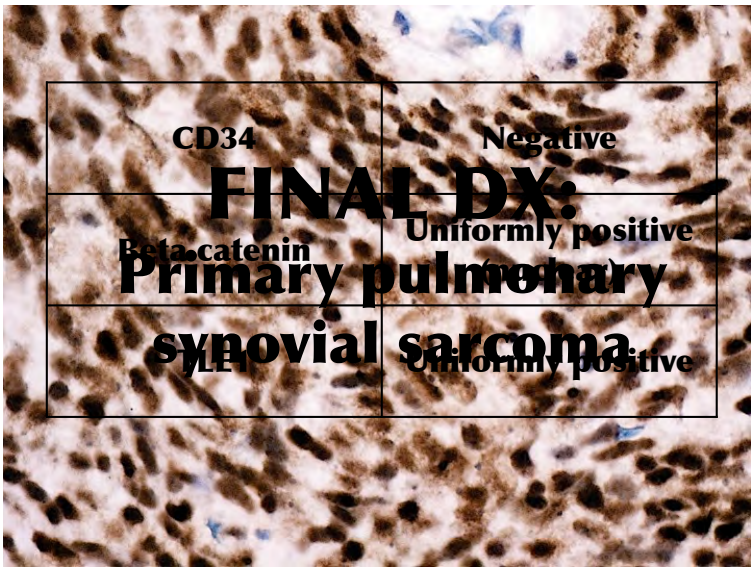
85 year old female with history of lobular breast caner and renal cell carcinoma presents with solitary LLL mass

Case 2

- **Primary lung CA**
- **Metastatic RCC**
- **Metastatic breast CA**
- **Mesenchymal tumor**

Case 2

SM Actins	Negative
Desmin	Negative
S100	Focally positive
CD34	Negative
bcl2	Uniformly positive
CD99	Uniformly positive
ß-catenin	Uniform nuclear



Making the Diagnosis of Synovial Sarcoma

- 'Traditional' IHC markers, e.g., bcl-2 and CD99, with focal keratin expression
- FISH studies for translocation involving SYT gene
- Novel IHC markers such as beta catenin and TLE-1

Synovial Sarcoma

MOLECULAR FINDINGS

- >90% translocatioln involving chromosomes X and 18
- Most common is t(X;18)(p11.2;q11.2) involving SYT gene on chromosome 18
- Creates SYT-SSX1, SYT-SSX2, or SYT-SSX4 fusion gene
- **Can be detected by PCR or FISH**

Diagn Mol Pathol 14:77-82, 2005

Fluorescence In Situ Hybridization for the Detection of t(X;18)(p11.2;q11.2) in a Synovial Sarcoma Tissue Microarray Using a Breakapart-Style Probe

Jefferson Terry, MD, Todd S. Barry, MD, PhD,† Douglas E. Horsman, MD,* Forrest D. Hsu, MD,* Allen M. Gown, MD,† David G. Huntsman, MD,* and Torsten O. Nielsen, MD, PhD**

- Breakapart probe to detect disruption of SYT gene
- 22/23 synovial sarcomas positive
- 100% specificity

FISH Breakapart Probe for Detection of t(X;18)of Synovial Sarcoma

5′ | SY T | 3′

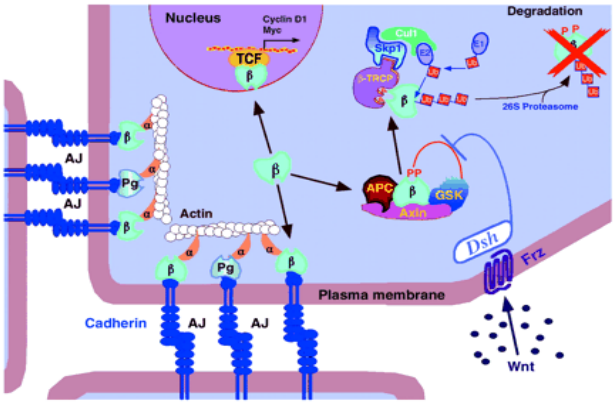
Chromosome 18

FISH WITH BREAKAPART PROBES

No Translocation

Translocation

ß-Catenin: Role in Cell Adhesion and Signaling



ß-Catenin and Fibromatoses

- Montgomery et al, AJSP, 2002
- Fibromatoses have mutation in APC/ ß-catenin pathway
- Abnormal nuclear accumulation of ß-catenin protein
- Studied expression by IHC in mesenteric fibromatosis, GIST, and sclerosing mesenteritis

Is Nuclear ß-catenin Expression Found in Other Tumors?

Nuclear beta-catenin in mesenchymal tumors

Tony L Ng[1], Allen M Gown[2], Todd S Barry[2], Maggie CU Cheang[1], Andy KW Chan[1], Dmitry A Turbin[1], Forrest D Hsu[1], Robert B West[3] and Torsten O Nielsen[1]

[1]*Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, British Columbia, Canada;* [2]*PhenoPath Laboratories, Seattle, Washington, USA and* [3]*Department of Pathology, Stanford University Medical Center, Stanford, CA, USA*

Modern Pathology 18:68-74, 2005

Ng TL et al., Modern Pathology 18:68-74, 2005

- Tissue microarray based study
- N = 549
- 4 separate microarrays
- Wide spectrum of soft tissue tumors represented

Ng TL et al., Modern Pathology 18:68-74, 2005

Tumors NEGATIVE for high level nuclear ß-catenin expression

- Adipocytic (liposarcoma, etc.)
- Fibrohistiocytic (MFH, GCT, AFX, etc.)
- Nerve sheath (NF, NB, schwannoma, PNET, etc.)
- Muscle (LMS, RMS)

Ng TL et al., Modern Pathology 18:68-74, 2005

Tumors POSITIVE for high level nuclear ß-catenin expression

- Desmoid type fibromatosis (71%)
- Solitary fibrous tumor (40%)
- Endometrial stromal sarcoma (40%)
- Synovial sarcoma (28%)

Am J Surg Pathol 31:240-246, 2007

TLE1 as a Diagnostic Immunohistochemical Marker for Synovial Sarcoma Emerging From Gene Expression Profiling Studies

Jefferson Terry, MD, Tsuyoshi Saito, MD, PhD,† Subbaya Subramanian, PhD,‡ Cindy Ruttan, MSc,* Cristina R. Antonescu, MD,† John R. Goldblum, MD,§ Erinn Downs-Kelly, MD,§ Christopher L. Corless, MD, PhD,‖ Brian P. Rubin, MD, PhD,¶ Matt van de Rijn, MD, PhD,‡ Marc Ladanyi, MD,† and Torsten O. Nielsen, MD, PhD**

- Gene expression profiling studies identified TLE1 as excellent discriminator of synovial sarcoma from other sarcomas, inclucing MPNST
- TLE proteins are transcriptional corepressors that inhibit Wnt signalling and other cell fate determination signals, i.e., represssing differentiation

Rush To Judgment?

Mod Pathol 22:872-8, 2009

TLE1 expression is not specific for synovial sarcoma: a whole section study of 163 soft tissue and bone neoplasms

Kemal Kosemehmetoglu[1], Julie A Vrana[2] and Andrew L Folpe[2]

- N = 163 soft tissue and bone tumors (whole sections, not TMAs)
- 18/20 synovial sarcomas, almost all 3+ positive
- TLE1 expression also seen in 53/143 (37%) non-synovial sarcoma, with 36 such cases (25%) showing 2–3+ positivity.
- TLE1 most common in nerve sheath tumors

The Empire Strikes Back

Am J Surg Pathol 33:1743-51, 2009

Prospective Evaluation of TLE1 as a Diagnostic Immunohistochemical Marker in Synovial Sarcoma

Amanda Jagdis, MD, Brian P. Rubin, MD, PhD,† Raymond R. Tubbs, MD,† Marina Pacheco, MD,* and Torsten O. Nielsen, MD, PhD, FRCPC**

- Reconfirms very high sensitivity and specificity from original 2008 paper, but using whole sections
- Positive on 34/34 synovial sarcomas
- Positive on only 3/73 other sarcomas (1 MPNST, 1 fibrosarcoma, 1 pleomorphic sarcoma)

Who is Correct? Why Such Different Results?

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- Different tumors
- Different methodologies (e.g., detection systems)
- Prospective v. retrospective studies (TLE1 employed before final diagnosis known in Jagdis et al.)

Tie breaker?

Immunohistochemical Staining for TLE1 Distinguishes Synovial Sarcoma From Histologic Mimics

Wai Chin Foo, MD,[1] Michael W. Cruise, MD, PhD,[2] Mark R. Wick, MD,[2] and Jason L. Hornick, MD, PhD[1]

Am J Clin Pathol 135:839-44, 2011

- N = 212 tumors
- 60/73 (82%) of synovial sarcomas TLE1 positive
- Only 15% MPNSTs and 8% SFTs TLE1 positive, most of them only weakly
- TLE1 is a sensitive and specific marker of synovial sarcoma, particularly if moderate or strong immunostaining observed

Moral of the TLE1 Story

- Newly described markers generally display the highest level of specificity during the first six months following their description
- No marker shows 100% sensitivity and specificity
- Some markers end up showing better negative than positive predictive value

Case 3

Gastric biopsy from 75 year old female with remote history of breast cancer presents with nausea, vomiting, weight loss and postprandial bloating

Case 3

- **Gastric carcinoma**
- **Metastatic breast carcinoma**

Breast v. GI Tract Markers

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

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**

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

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%)**

Mammaglobin A 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 A positive
- **Combined sensitivity of 86%**

GCDFP-15 and Mammaglobin A Expression

	ER Expression	*HER2 Overexpress*	*Tumor Grade*
GCDFP-15	r = 0.043 *(p = 0.434)*	r = -0.069 *(p = 0.213)*	**r = -0.297** *(p<0.000001)*
Mammaglobin A	**r = 0.230** *(p = 0.00002)*	r = -0.063 *(p = 0.256)*	**r = -0.187** *(p = 0.00045)*

Statistical correlation calculated using Pearson-Product-Moment Correlation coefficient (Gown AM et al., USCAP 2010)

GCDFP-15 and Mammaglobin A

Warning!

- Usually negative in the setting of triple negative breast cancers
- Use other markers of 'basal-like' breast cancer (EGFR, CK5, p63, p53, c-kit, etc.)

Histopathology 2012 DOI: 10.1111/j.1365-2559.2012.04344.x

Gross cystic disease fluid protein-15 and mammaglobin A expression determined by immunohistochemistry is of limited utility in triple-negative breast cancer

Lei Huo, Jinxia Zhang, Michael Z Gilcrease, Yun Gong, Yun Wu, Hong Zhang, Erika Resetkova, Kelly K Hunt[1] & Michael T Deavers

Department of Pathology, The University of Texas MD Anderson Cancer Centre, Houston, TX, USA, and [1]Department of Surgical Oncology, The University of Texas MD Anderson Cancer Centre, Houston, TX, USA

	Primary Triple Negative	*Metastatic Triple Negative*
Mammaglobin A	25%	41%
GCDFP-15	14%	21%
Mammaglobin A OR GCDFP-15	30%	43%

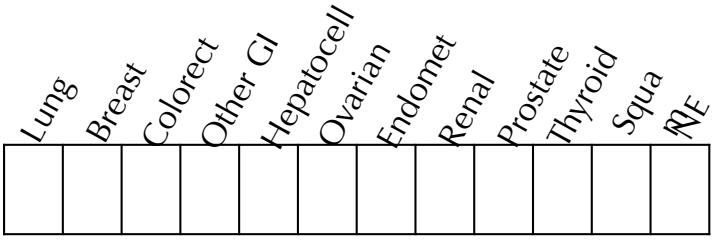
Percentage of non-breast primary carcinomas positive

	GCDFP-15	*Mammaglobin A*
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%)
Gastric	0/58 (0%)	0/58 (0%)
Adnexal	17/78 (21.8%)	17/76 (22.4%)
OVERALL SPECIFICITY	**88%**	**89%**

Breast-"Specific" Markers

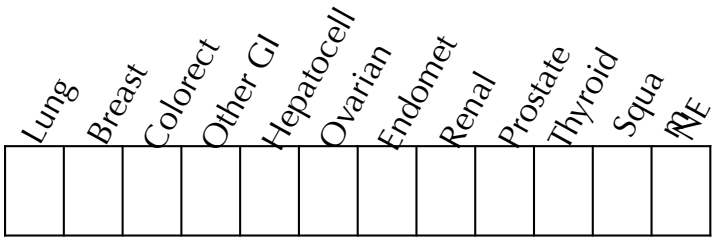
- There is no breast-specific marker that cannot also be expressed by sweat gland tumors
- ER, PR, GCDFP-15, mammaglobin, GATA-3

Immunophenotype Band



GCDFP-15

Immunophenotype Band



Mammaglobin A

Am J Clin Pathol 138:57-64, 2012

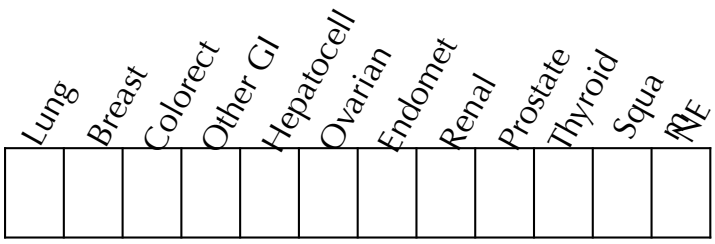
Immunohistochemical Evaluation of GATA3 Expression in Tumors and Normal Tissues

A Useful Immunomarker for Breast and Urothelial Carcinomas

Haiyan Liu, MD, Jianhui Shi, MD, PhD, Myra L. Wilkerson, MD, and Fan Lin, MD, PhD

- GATA binding protein 3 to DNA sequence [A/T]GATA[A/G]
- One of six members of a zinc finger transcription factor family
- Plays key role in regulation of cell proliferation, development, and differentiation in mammary epithelium (as well as T lymphocytes, adipose tissue, kidney, hair follicles among others)

Immunophenotype Band



GATA3

Bladder

GATA3 Expression

- Highly sensitive marker of breast infiltrating ductal carcinoma (91%; N = 99)
- Highly sensitive marker of breast infiltrating lobular carcinoma (100%; N = 48)
- Highly sensitive marker of urothelial carcinoma (88%; N = 72)
- Negative on all lung carcinomas (N = 122), ovarian serous carcinomas (N = 96), gastric and esophageal carcinoma (N = 51)

Liu H, et al., Am J Clin Pathol 138:57-64, 2012

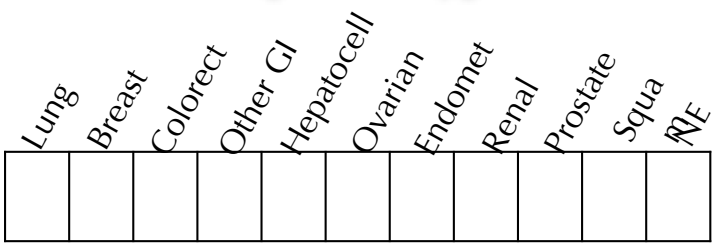
Relative Sensitivities of GATA3 vs. Mammaglobin A vs. GCDFP-15?

- No published data; large study currently underway at PhenoPath Laboratories
- Prior publications (e.g., Voduc D et al., 2008) suggest strong correlation between GATA3 expression and estrogen receptor expression
- GATA3 ay be superior marker in context of triple negative breast cancers

What about Estrogen Receptor?

- Subset of carcinomas can manifest ER 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)

Immunophenotype Band



ER

Cervical

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%)**

Villin

- 95 kd actin-binding protein, found preferentially in microvilli
- Expression in normal tissues largely restricted to epithelial cells of the GI tract
- Highly sensitive marker of colorectal adenocarcinomas (West AB et al., Gastroenterol 94:343-52, 1988)

Patterns of Villin Expression

- Cytoplasmic
- 'Chicken wire' membranous
- Brush border

Villin

Werling RW et al., Am J Surg Pathol 27:303-10, 2003

- 0% renal cell carcinomas positive
- 5% lung carcinomas positive ("rootlet type" adenocarcinomas)
- 0% head and neck carcinomas (squamous cell, thyroid, salivary gland) positive
- 0% breast carcinomas positive
- 0% ovarian serous carcinomas positive

CDX2

- Intestinal-specific nuclear transcription factor
- Regulates proliferation and differentiation of intestinal epithelial cells
- Expression may be reduced compared with normal in colorectal adenocarcinoma
- Extremely sensitive marker of colorectal adenocarcinoma

Am J Surg Pathol 27:303-10, 2003

CDX2, a Highly Sensitive and Specific Marker of Adenocarcinomas of Intestinal Origin

An Immunohistochemical Survey of 476 Primary and Metastatic Carcinomas

Robert W. Werling, M.D., Hadi Yaziji, M.D., Carlos E. Bacchi, M.D., and Allen M. Gown, M.D.

Surveys of CDX-2 Expression

Moskaluk, et al., Mod Pathol 16:913-9, 2003

Cdx2 Protein Expression in Normal and Malignant Human Tissues: An Immunohistochemical Survey Using Tissue Microarrays

Christopher A. Moskaluk, M.D., Ph.D., Hong Zhang, M.D., Steven M. Powell, M.D., Lisa A. Cerilli, M.D., Garret M. Hampton, Ph.D., Henry F. Frierson, Jr., M.D.

Departments of Pathology (CAM, LAC, HFF), of Biochemistry and Molecular Genetics (CAM), and of Medicine (SMP), University of Virginia Health System, Charlottesville, Virginia; Department of Pathology (HZ), Anhui Medical University, Hefei, China; and Genomics Institute of the Novartis Research Foundation (GMH), San Diego, California

De Lott et al., Arch Pathol Lab Med 129:1100-1105, 2005:913-9, 2005

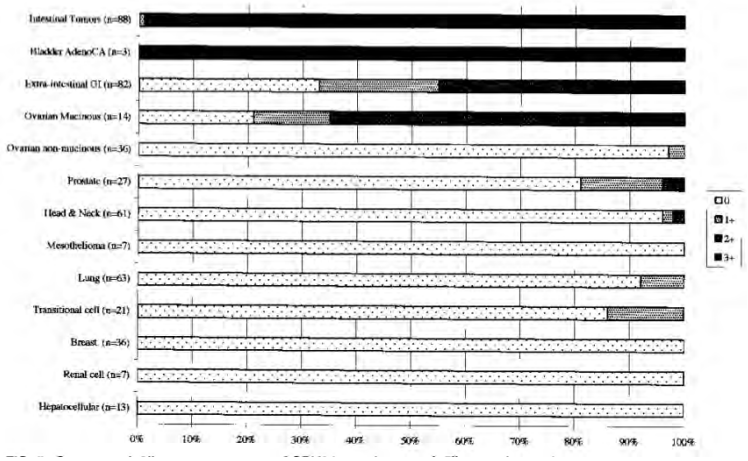
CDX2 Is a Useful Marker of Intestinal-Type Differentiation

A Tissue Microarray–Based Study of 629 Tumors From Various Sites

Lindsey B. De Lott, BS; Carl Morrison, MD, DVM; Saul Suster, MD; David E. Cohn, MD; Wendy L. Frankel, MD

CDX-2 Expression in Normal Tissues

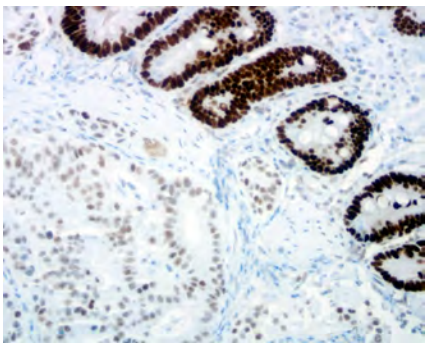
Esophagus	
Stomach	
Small Intestine	
Large Intestine	
Liver	
Pancreas	**???**
Gallbladder	
Lung	
Breast	



Werling RW et al., Am J Surg Pathol 27:303-10, 2003

CDX2

- Positive on adenocarcinoma as well as neurendocrine carcinoma



CDX2

- Outstanding marker of colorectal adenocarcinoma (e.g., distinguish metastatic colorectal CA from primary lung adenocarcinoma)
- Also marker of non-colorectal GI adenocarcinomas (heterogeneous pattern)
- Enteric type tumors: mucinous ovarian, bladder adenocarcinomas, sinonasal

WARNING!

Pulmonary Adenocarcinomas With Enteric Differentiation

Histologic and Immunohistochemical Characteristics Compared With Metastatic Colorectal Cancers and Usual Pulmonary Adenocarcinomas

Kentaro Inamura, MD,† Yukitoshi Satoh, MD, PhD,*‡ Sakae Okumura, MD,‡ Ken Nakagawa, MD,‡ Eiju Tsuchiya, MD, PhD,§ Masashi Fukayama, MD, PhD,† and Yuichi Ishikawa, MD, PhD**

- Adenocarcinomas with enteric immunophenotype can be primary to the lung and other sites
- Can show expression of all colorectal-type markers (CK20, CDX-2, villin)
- May or may not be TTF-1 positive

Sinonasal Adenocarcinoma, Intestinal Type

ORIGINAL ARTICLE

Expression pattern of CK7, CK20, CDX-2, and villin in intestinal-type sinonasal adenocarcinoma

M T Kennedy, R C K Jordan, K W Berean, B Perez-Ordoñez

J Clin Pathol 2004;**57**:932–937. doi: 10.1136/jcp.2004.016964

Table 2 Histological types and immunohistochemical profile

Case	Histology	CK7	CK20	Villin	CDX-2
1	Colonic, grade 3	Negative	3+	3+	3+
2	Papillary, grade 3	Negative	3+	3+	3+
3	Mixed (papillary with mucinous component), grade 3	Negative	3+	3+	3+
4	Colonic grade 2	Negative	1+	3+	3+
5	Papillary, grade 2	1+	3+	3+	3+
6	Papillary, grade 2	1+	3+	3+	3+
7	Colonic, grade 2	1+	3+	3+	3+
8	Colonic, grade 2	2+	3+	3+	3+
9	Papillary, grade 2	3+	2+	3+	2+
10	Colonic, grade 2	3+	3+	3+	3+
11	Papillary nasopharyngeal adenocarcinoma	3+	Negative	Negative	Negative
12	Sinonasal papillary low grade adenocarcinoma	3+	Negative	Negative	Negative

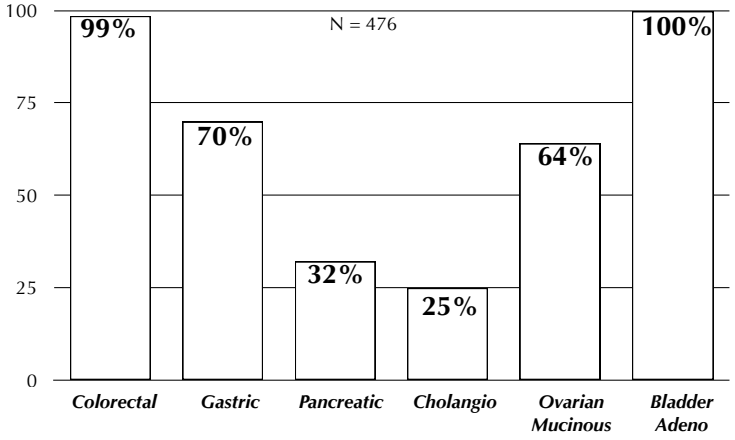
CK, cytokeratin.

"Anomalous" CDX-2 Expression

- A subset of lung adenocarcinomas
- Endometrial 'squamous morules' and endometrial adenocarcinomas
- Endometrial adenocarcinoma
- Prostatic adenocarcinoma

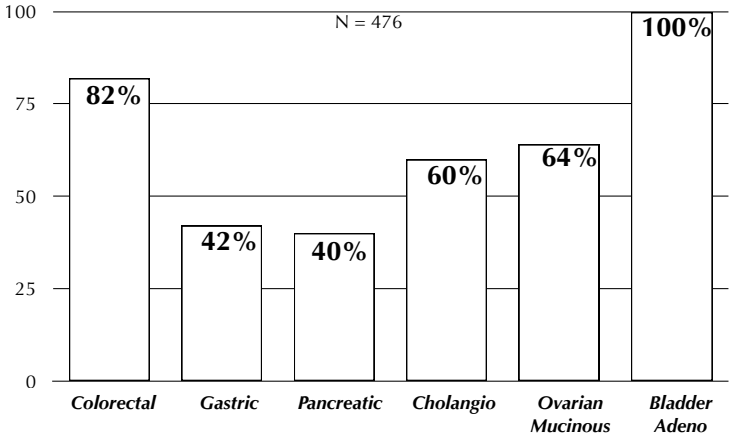
CDX-2

Werling RW et al., Am J Surg Pathol 27:303-10, 2003

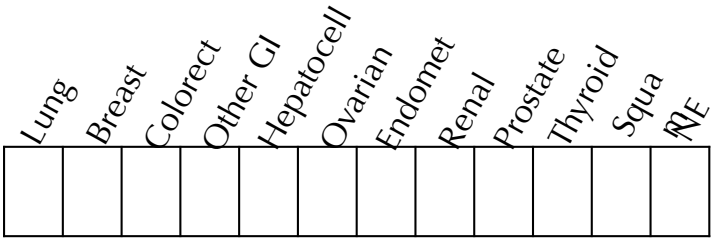


Villin

Werling RW et al., Am J Surg Pathol 27:303-10, 2003



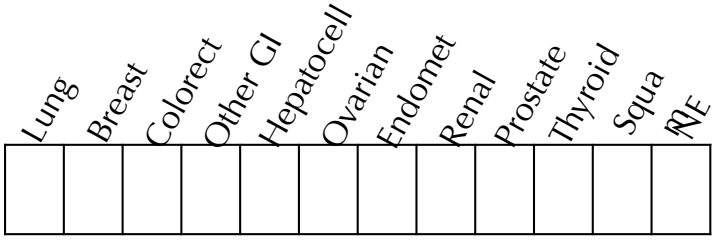
Immunophenotype Band



Mucinous

CDX2

Immunophenotype Band



Villin

Different pattern

Case 3

Cytokeratins	Uniformly positive
Cytokeratin 7	Uniformly positive
Cytokeratin 20	Negative
CDX2	Negative
Villin	Negative
Estrogen receptor	Uniformly positive
GCDFP-15	Uniformly positive
Mammaglobin	Negative

Metastatic (lobular) breast cancer to stomach

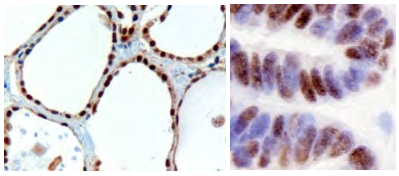
Case 4

64 year old female presents with DVT and mediastinal and periaortic LN enlargement. History of breast cancer as well as history of atypical parathyroid lesion. Bx of periaortic LN

Case 4

- **Metastatic breast CA**
- **Metastatic GI tract CA**
- **Metastatic GYN tract CA**
- **Metastatic parathyroid tx**

PAX8



~48 kd nuclear transcription factor

Member of paired box (PAX) gene family (PAX1 through PAX9) that code key regulators of tissue development and cellular differentiation

Embryonal: developing eye, thyroid, kidney, reproductive structures

PAX 8 expression in non-neoplastic tissues, primary tumors, and metastatic tumors: a comprehensive immunohistochemical study

Ayhan Ozcan[1,2,3], Steven S Shen[1,2,4], Candice Hamilton[1], Kundu Anjana[1], Donna Coffey[1,2,4], Bhuvaneswari Krishnan[5] and Luan D Truong[1,2,4,5]

Mod Pathol 24:751-64, 2011

A Comprehensive Analysis of PAX8 Expression in Human Epithelial Tumors

Anna R. Laury, MD,† Ruth Perets, MD, PhD,‡ Huiying Piao,‡ Jeffrey F. Krane, MD, PhD,*† Justine A. Barletta, MD,*† Christopher French, MD,*† Lucian R. Chirieac, MD,*† Rosina Lis, MD,‡ Massimo Loda, MD,*†‡ Jason L. Hornick, MD, PhD,*† Ronny Drapkin, MD, PhD,*†‡ and Michelle S. Hirsch, MD, PhD*†§*

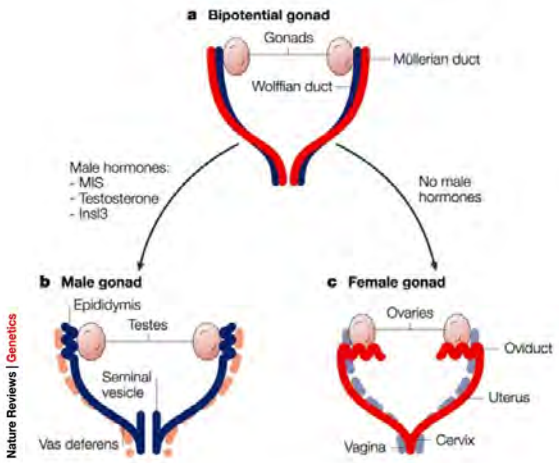
Am J Surg Pathol 35:816-26, 2011

Expression of PAX8 in Normal and Neoplastic Tissues - *A Comprehensive Immunohistochemical Study*

David Tacha, PhD, Ding Zhou, BS,* and Liang Cheng, MD†*

Appl Immunohist Mol Morphol 19:293-9, 2011

PAX8 Dependent Development



PAX 8 In Normal Tissues

Renal tubular epithelium (but not glomeruli)

Thyroid epithelial cells

Fallopian tubal, endometrial, endocervical epithelium (but not stromal cells)

Epididymal and seminal vesicle epithelium (but not Sertoli, Leydig cells or seminiferous tubules)

- *Lymphoid cells**
- *Pancreatic islet cells***

**probably PAX5*
***probably PAX6*

PAX 8 In Human Tumors

Renal carcinoma (all except rhabdoid)

Thyroid carcinoma (follicular and papillary but not medullary)

Endometrioid, serous, (some) mucinous, clear cell carcinoma of female genital tract

Epididymal tumors, carcinoma rete testis, endometrioid adenoCA of seminal vesicle

Thymic carcinoma

- *Lymphoma**
- *Pancreatic islet cell tumors***

**probably PAX5*

***probably PAX6*

PAX 8 NOT Expressed

Breast carcinoma

Cervical squamous cell carcinoma

Urothelial carcinoma of bladder

Lung carcinoma (including squamous cell carcinoma)

Gastrointestinal tract adenocarcinoma

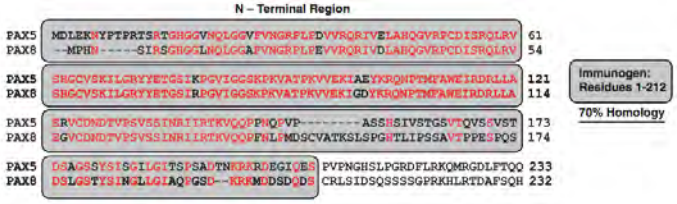
Mesothelioma

Mod Pathol 25:231-6, 2012

N-terminal PAX8 polyclonal antibody shows cross-reactivity with N-terminal region of PAX5 and is responsible for reports of PAX8 positivity in malignant lymphomas

Lucas Moretti[1], L Jeffrey Medeiros[1], Kranthi Kunkalla[1], Michelle D Williams[2], Rajesh R Singh[1] and Francisco Vega[1]

[1]*Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA and* [2]*Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA*



Expression of Pax8 as a Useful Marker in Distinguishing Ovarian Carcinomas From Mammary Carcinomas

Daisuke Nonaka, MD, Luis Chiriboga, PhD,* and Robert A. Soslow, MD†*

Am J Surg Pathol 32:1566-71, 2008

PAX8 a critical transcription factor for thyroid and lung; also expressed in brain and Mullerian duct

PAX8 expressed in nonciliated mucosal cells of fallopian tubes, not on surface epithelium

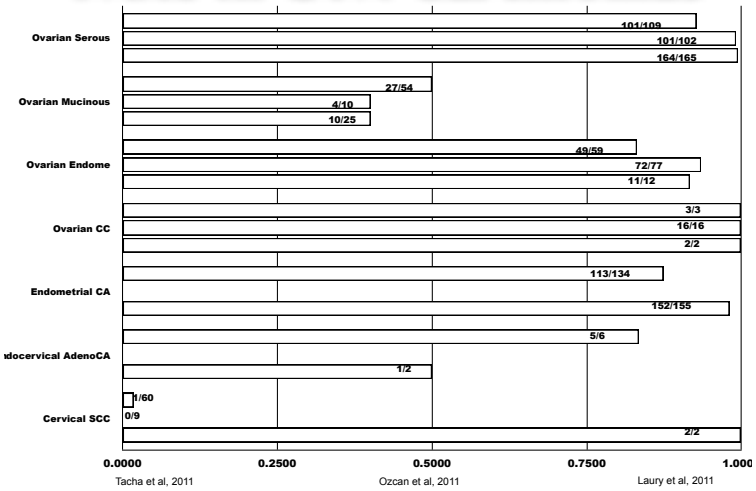
Distribution similar but nonidentical to PAX2

PAX8 as Marker of Ovarian Carcinomas

Nonaka D et al., Am J Surg Pathol 32:1566-71, 2008

Ovarian serous	81/84 (96%)
Ovarian endometrioid	16/18
Ovarian clear cell	10/10 (100%)
Ovarian mucinous	1/12 (8%)
Breast carcinoma	0/65 (0%)

PAX8 in GYN Carcinomas

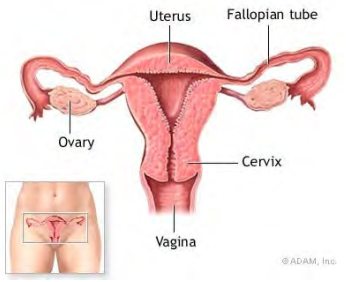


What is Relationship of PAX8 to PAX2 and WT1?

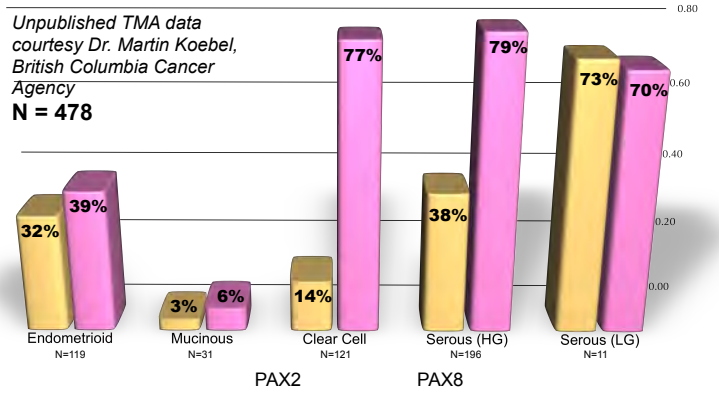
Ovarian tumors

Endometrial tumors

Cervical tumors



PAX2 v. PAX8 as Markers of Ovarian Cancers



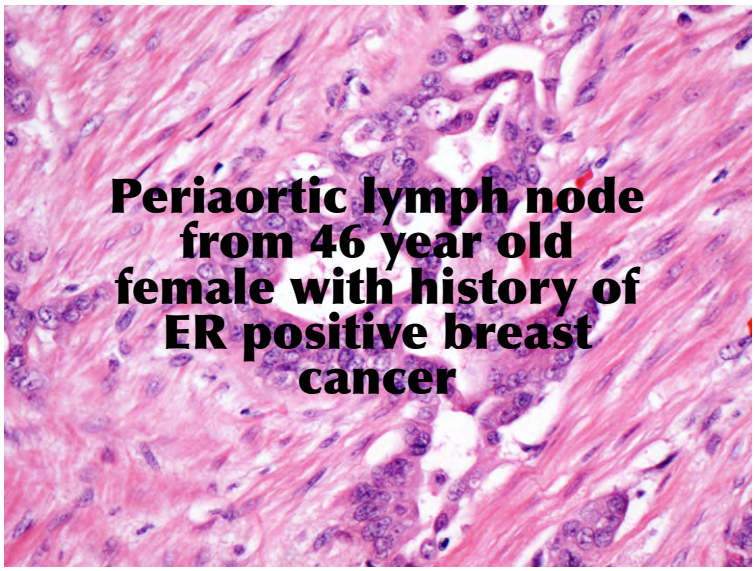
	Lung	Breast	Colorect	Other GI	Hepatocell	Ovarian	Endomet	Renal	Prostate	Thyroid	Squam	NE
PAX8												
PAX2												
WT1												

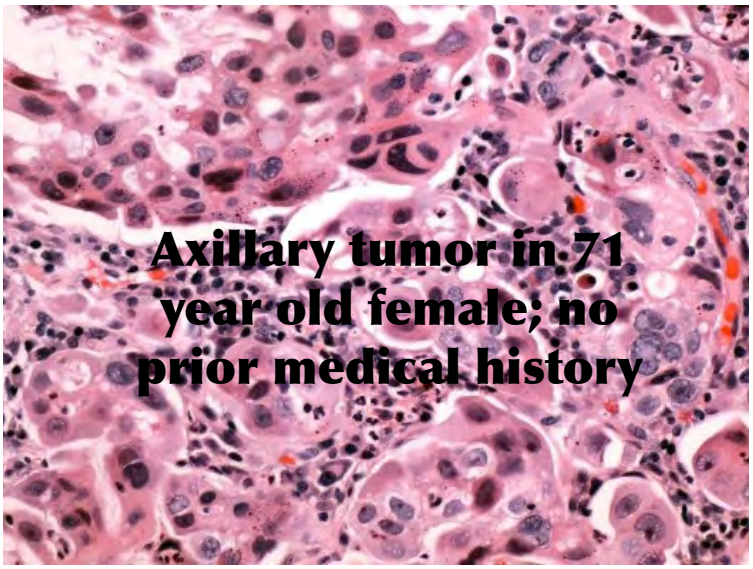
PAX2 v. PAX8 Expression in Carcinomas

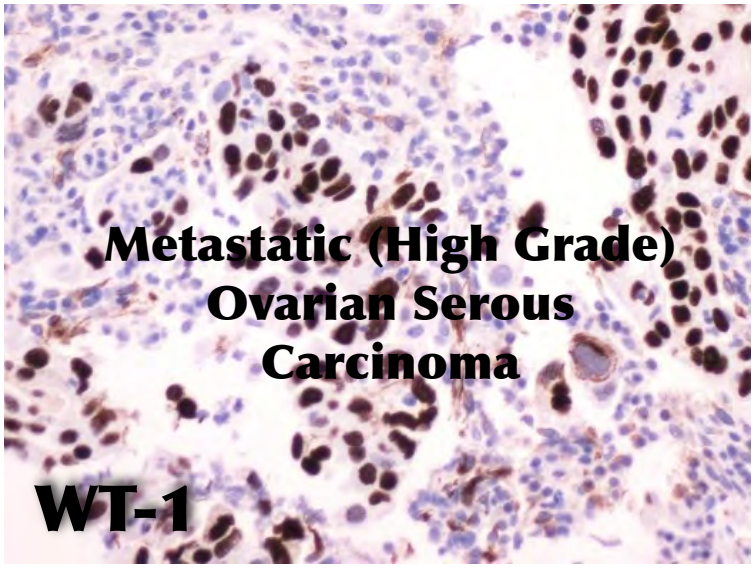
	PAX2	PAX8
Ovarian serous		
Ovarian mucinous		
Ovarian endometrioid		
Ovarian clear cell		
Breast carcinoma		
Renal cell carcinoma		
Thyroid carcinoma		

Comparative Immunopheno-types: Ovarian Carcinomas

	PAX8	WT1	ER	
Ovarian serous				ER lost high grade
Ovarian mucinous				CDX-2
Ovarian endometrioid				Nuclear ß-catenin
Ovarian clear cell				Napsin A
Breast carcinoma				GCDFP -15







Am J Surg Pathol 35:1305-10, 2011

ORIGINAL ARTICLE

Pax8 Expression in Thymic Epithelial Neoplasms: An Immunohistochemical Analysis

Annikka Weissferdt, MD and Cesar A. Moran, MD

TABLE 1. Immunohistochemical Results for Pax8 in Thymic Carcinoma and Thymoma

Tumor	n	0	+1	+2	+3	+4	Positive Cases	Percent
Thymic carcinoma	31	7	2	0	1	21	24	77
WHO type A thymoma	30	0	0	0	0	30	30	100
WHO type B thymoma (B1-B3)	30	2	1	0	5	22	28	93

0 indicates negative; 1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; 4+, 76%-100%; n, sample size.

Utility of PAX8 IHC

- Identification of ovarian carcinomas
- Identification of other GYN epithelial tumors
- Identification of renal cell carcinomas (all variants)
- ?Identification of thymic epithelial neoplasms
- Identification (with TTF-1) of metastatic thyroid carcinomas

Case 4

Keratins	**Uniformly positive**
Keratin 7	**Uniformly positive**
Keratin 20	**Negative**
CDX2	**Negative**
Villin	**Negative**
WT-1	**Negative**
Estrogen receptor	**Negative**

Case 4

CDX-2	**Negative**
Villin	**Negative**
GCDFP-15	**Negative**
Mammaglobin A	**Negative**
WT-1	**Negative**
ER	**Negative**
PAX8	**Uniformly positive**

Comparative Immunophenotypes: Ovarian Carcinomas

	PAX8	WT1	ER	
Ovarian serous				ER lost high grade
Ovarian mucinous				CDX-2
Ovarian endometrioid				Nuclear ß-catenin
Ovarian clear cell				Napsin A
Breast carcinoma				GCDFP-15

Case 4

Continued

GCDFP-15	Negative
Mammaglobin	Negative
WT-1	Negative
PAX2	Negative
PAX8	Uniformly positive

FINAL DX: Metastatic clear cell ovarian carcinoma

Case 5

57 year old female with large abdominal and pelvic retroperitoneal masses (19 cm), periaortic masses involving psoas muscle and retrocrural and upper retroperitoneal lymph nodes. Also has small pulmonary nodules and 2.2 cm mass in the right adrenal gland. Biopsy of retroperitoneal mass.

Case 5

- **Smooth muscle tx**
- **GIST**
- **Spindle cell carcinoma**
- **Germ cell tumor**

Case 6: Differential Dx

	SM Actins	Desmin	DOG-1	Keratins	Inhibin alpha	SALL4
SMC Tumor						
GIST						
Spindle cell CA						
Germ cell tx						
Adrenal cortical tx						

Case 5

SM Actins	Negative
Desmin	Negative
Keratins [OSCAR]	Negative
PAX8	Negative
DOG1	Negative
S100	Negative
Inhibin	Negative

Case 5

Melan A	**Variably positive**
gp100 [HMB45]	**Uniformly positive**

Case 5

FINAL DX: Epithelioid variant of angiomyolipoma

Perivascular Epithelioid Cell Family of Tumors ("PEComas")

- Angiomyolipoma
- Malignant angiomyolipoma
- Sugar tumor of lung, pancreas, uterus
- Lymphangioleiomyomatosis
- Clear cell myomelanocytic tumor

Human Pathol 41:1-15, 2010

Perivascular epithelioid cell neoplasms: pathology and pathogenesis

Andrew L. Folpe MD[a,*], David J. Kwiatkowski MD, PhD[b]

[a]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, USA
[b]Translational Medicine Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

- Mesenchymal neoplasms ("PEComas") composed of histologically and immunohistochemically distinctive perivascular epithelioid cells
- First described by Bonetti et al (1992) noting that angiomyolipoma and sugar tumor of lung share unusual cell type expressing melanocytic markers, epithelioid morphology, clear-acidophilic cytoplasm anda perivascular distribution

Perivascular epithelioid cell

- PECs may accumulate large amounts of lipid, mimicking appearance of adipocytes or lipoblasts
- Great variation in relative proportion of epithelioid, spindled, lipid distended cells
- These tumors typically confused with carcinomas, smooth muscle tumors, and adipocytic tumors
- No normal counterpart cell

PEComa Immunophenotype

- *Unique cell type showing smooth muscle and melanocytic differentiation*

Cytokeratins	**Negative**
S100	**Negative**
gp100	**Positive**
MelanA	**Positive**
SM Actins	**Positive**
Desmin	**Negative**
CD68	**Positive**
TFE3	**(subset) Positive**

PEComas

- Angiomyolipoma (e.g., kidney, liver, etc.), including 'atypical AML' and 'epithelioid AML'
- Pulmonary lymphangioleiomatosis
- Clear cell ("sugar") tumor of the lung
- Clear cell myelomonocytic tumor of falciform ligament
- Malignant angiomyolipoma

Poor Prognostic Features of PEComas

- Size > 5 cm
- Infiltrative growth pattern
- High nuclear grade
- Necrosis
- High proliferative rate

Case History

49 year old female with liver mass.

Diagnosis

PEComa

(Clear Cell Myomelanocytic Tumor of Falciform Ligament/Ligamentum Teres)

Clear Cell Myomelanocytic Tumor of Falciform Ligament/Ligamentum Teres

- Predominantly children and youg adults (ages 3-29, median 11 yrs)
- Tumor size 5-20 cm (median, 8 cm)
- Clear to faintly eosinophilic spindled cells arranged in fascicular and nested patterns
- Low mitotic activity

Folpe AL et al. Am J Surg Pathol 24:1239-46, 2000

Epithelioid Variant of Angiomyolipoma

- First described by Martignoni et al (1995) and Eble et al (1997) as variant of angiomyolipoma of kidney
- More often associated with TSC
- Considered potentially malignant
- Composed predominantly or exclusively of epithelioid cells

Epithelioid Angiomyolipoma

Tumors in the Differential Diagnosis

- Renal cell carcinoma
- Melanoma
- Epithelioid smooth muscle tumor
- Epithelioid peripheral nerve sheath tumor
- Adrenal cortical neoplasm
- GIST
- Hepatocellular carcinoma

Case 5

FINAL DX:

Epithelioid variant of angiomyolipoma

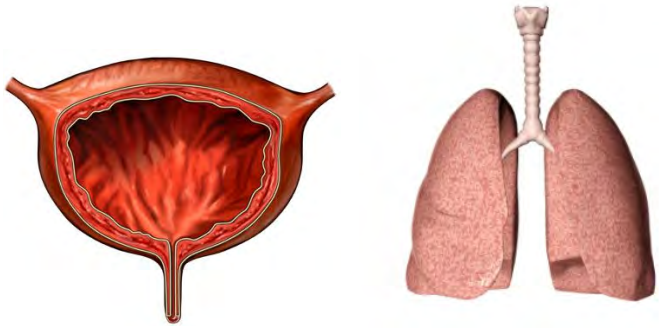
Case 6

65 year old female presents with solitary left upper lobe nodule and history of renal cell carcinoma and 3 cm bladder cancer. Lung biopsy.

Case 6

- **Primary lung squamous cell CA**
- **Metastatc renal cell carcinoma**
- **Metastatic TCC**

Urothelial v. Lung Markers



Markers of Transitional Cell Carcinoma

- p63
- Keratin 5
- Keratin 20
- Uroplakin
- GATA-3

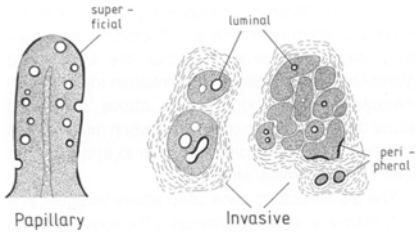
Specific Marker of Bladder Transitional Cell Carcinoma?

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

- 21/35 (60%) primary TCCs positive
- 17/32 (53%) metastatic TCCs
- Overall sensitivity 57%
- Specificity 100%

Real World Sensitivity in Metastatic/High grade setting: <20%

Am J Clin Pathol 138:57-64, 2012

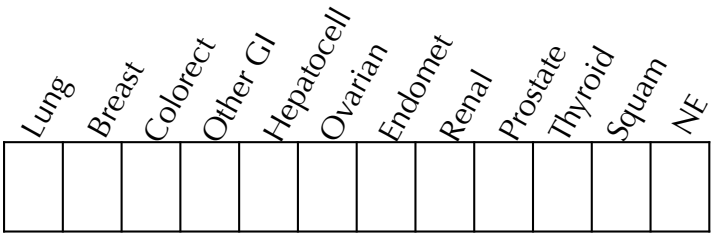
Immunohistochemical Evaluation of GATA3 Expression in Tumors and Normal Tissues

A Useful Immunomarker for Breast and Urothelial Carcinomas

Haiyan Liu, MD, Jianhui Shi, MD, PhD, Myra L. Wilkerson, MD, and Fan Lin, MD, PhD

- GATA binding protein 3 to DNA sequence [A/T]GATA[A/G]
- One of six members of a zinc finger transcription factor family
- Plays key role in regulation of cell proliferation, development, and differentiation in mammary epithelium (as well as T lymphocytes, adipose tissue, kidney, hair follicles among others)

Immunophenotype Band



GATA3

Bladder

GATA3 Expression

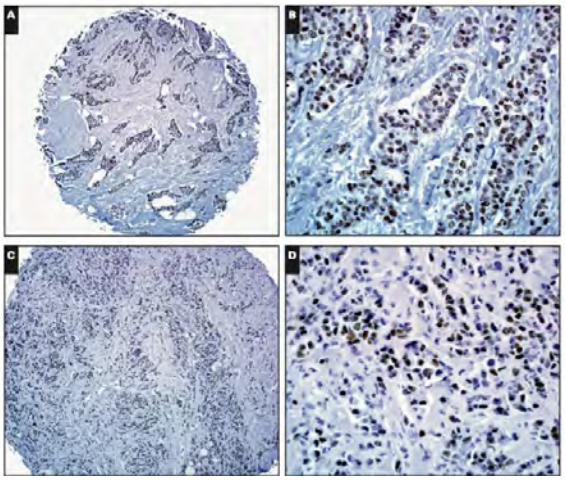
- Highly sensitive marker of breast infiltrating ductal carcinoma (91%; N = 99)
- Highly sensitive marker of breast infiltrating lobular carcinoma (100%; N = 48)
- Highly sensitive marker of urothelial carcinoma (88%; N = 72)
- Negative on all lung carcinomas (N = 122), ovarian serous carcinomas (N = 96), gastric and esophageal carcinoma (N = 51)

Liu H, et al., Am J Clin Pathol 138:57-64, 2012

Liu H, et al., Am J Clin Pathol 138:57-64, 2012

IDC

ILC



Markers of Squamous v. Transitional Cell v. Renal Cell CA

	p63	Ker 5	Ker 20	Uroplakin	GATA-3	PAX-8
Lung Squamous Cell CA						
Bladder TCC						
Renal Cell CA						

Arch Pathol Lab Med 136:1339-46, 2012

Selective Immunohistochemical Markers to Distinguish Between Metastatic High-Grade Urothelial Carcinoma and Primary Poorly Differentiated Invasive Squamous Cell Carcinoma of the Lung

Aaron M. Gruver, MD, PhD; Mahul B. Amin, MD; Daniel J. Luthringer, MD; Danielle Westfall, MD; Komal Arora, MD; Carol F. Farver, MD; Adeboye O. Osunkoya, MD; Jesse K. McKenney, MD; Donna E. Hansel, MD, PhD

Table 1. Details of the Immunohistochemical Stains

Antibody	Company	Clone	Species	Dilution	Pretreatment
CK7	Dako	OV-TL 12/30	Mouse	1:40	Protease
CK20	Dako	Ks20.8	Mouse	1:20	Protease
HMCK	Enzo	34βE12	Mouse	Prediluted	Protease
GATA-3	Santa Cruz	HG3-35	Mouse	1:10	Citrate
Napsin A	Leica	IP64	Mouse	1:200	High pH
p63	Dako	A4A	Mouse	1:80	EDTA
S100A1	Abnova[a]	ID5	Mouse	1:250	Low pH
S100P	BD	16/S100P	Mouse	1:400	Protease
Surfactant protein A	Leica	32E12	Mouse	1:200	High pH
Thrombomodulin	Dako	1009	Mouse	1:50	High pH
TTF-1	Dako	8G7G3/1	Mouse	1:40	EDTA
Uroplakin III	Cell Marque[b]	AU-1	Mouse	Prediluted	High pH
CK14	Cell Marque[b]	LL002	Mouse	Prediluted	High pH
Desmoglein-3	Abcam[c]	3G133	Mouse	1:20	High pH

Comparative Immunophenotypes: Bladder TCC v. Lung Squamous Cell CA

	Bladder TCC	Lung Sq Cell CA
Keratin 7	100%	33%
Keratin 20	54%	7%
p63	78%	93%
Uroplakin	14%	0%
TTF-1	0%	3%
GATA-3	78%	23%

Gruver AM, Arch Pathol Lab Med 136:1339-46, 2012

Case 6

p63	Uniformly positive
Keratin 5	Variably positive
Keratin 7	Uniformly positive
GATA-3	Uniformly positive
Napsin A	Negative
TTF-1	Negative
PAX8	Variably positive

Mod Pathol 22:1218-27, 2009

Expression of *PAX8* in normal and neoplastic renal tissues: an immunohistochemical study

Guo-Xia Tong, Woojin M Yu, Nike T Beaubier, Erin M Weeden, Diane Hamele-Bena, Mahesh M Mansukhani and Kathleen M O'Toole

Department of Pathology, Columbia University College of Physicians and Surgeons, New York, NY, USA

TCC of Bladder	**0/40**
TCC of Renal pelvis	**4/17**

Case 6

FINAL DX:

Metastatic transitional cell CA from renal pelvis

TTF-1	**Negative**

Case 7

69 year old male with tumor involving multiple ribs and lung parenchyma with compression of pulmonary artery

Case 7

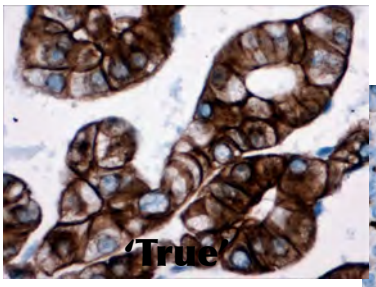
Metastatic carcinoma

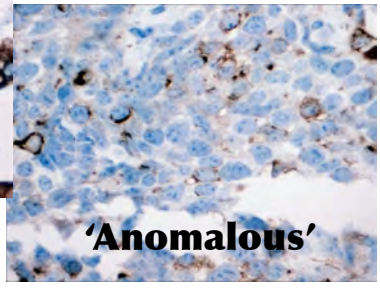
- **Lung?**
- **Prostate?**
- **Other?**

RULING IN OR OUT THE DIAGNOSIS OF CARCINOMA

- Uniform expression of keratin usually points to diagnosis of carcinoma
- Negative keratin expression rules out carcinoma
- "Incomplete" pattern of keratin expression suggests melanoma or sarcoma

Two distinct patterns of keratin expression





Patterns of Keratin Expression

- Strong, uniform = Carcinoma
- Focal, compartmentalized = "Anomalous"
- Exceptions: Neuroendocrine tumors with 'dot-like' keratin expression
- Caveat: Loss of tissue reactivity can result in simulation of 'anomalous' pattern in true carcinomas

Potential Pitfalls in Use of Anti-Keratin Antibodies to Identify Carcinoma

- For identification of epithelial differentiation, **do not** use antibodies to "low MW" keratins such as 35ßH11 or CAM5.2
- For identification of epithelial differentiation, **do** use antibodies to broad spectrum of keratins ("pankeratin" antibodies*) such as AE1/AE3 cocktail or OSCAR
- Be aware of significance of different patterns of keratin expression (uniform v. 'anomalous')

AE1/AE3 is an Antibody Cocktail

- Should be optimized as an antibody cocktail, not as a single clone
- Need to demonstrate that both clones are optimized
- Tissues with low level expression best to optimize antibody

J Clin Pathol 64:220-5, 2011

Inappropriate calibration and optimisation of pan-keratin (pan-CK) and low molecular weight keratin (LMWCK) immunohistochemistry tests: Canadian Immunohistochemistry Quality Control (CIQC) experience

Maria Copete,[1] John Garratt,[2] Blake Gilks,[3] Dragana Pilavdzic,[4] Richard Berendt,[5,6] Gilbert Bigras,[5,6] Sarah Mitchell,[5,6] Leslie Ann Lining,[1] Carol Cheung,[7] Emina E Torlakovic[1,7]

70 sample TMA for challenge 1, 30 sample TMA for challenge 2

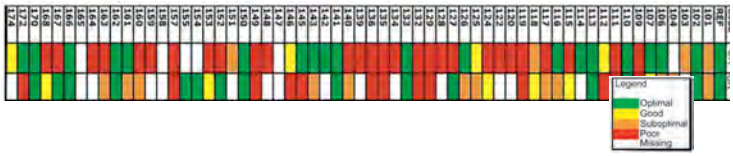
N = 13 challenge 1, N = 62 challenge 2

J Clin Pathol 64:220-5, 2011

Inappropriate calibration and optimisation of pan-keratin (pan-CK) and low molecular weight keratin (LMWCK) immunohistochemistry tests: Canadian Immunohistochemistry Quality Control (CIQC) experience

Maria Copete,[1] John Garratt,[2] Blake Gilks,[3] Dragana Pilavdzic,[4] Richard Berendt,[5] Gilbert Bigras,[5,6] Sarah Mitchell,[5,6] Leslie Ann Lining,[1] Carol Cheung,[7] Emina E Torlakovic[1,7]

False negative rate 20-80%!!



KERATIN EXPRESSION EXCEPTIONS

- Adrenal cortical carcinomas (expression can be vanishingly low)
- Spindle cell carcinomas, e.g., of bladder and head and neck (expression can be variable and low)
- Neuroendocrine carcinomas can show low levels of keratin expression

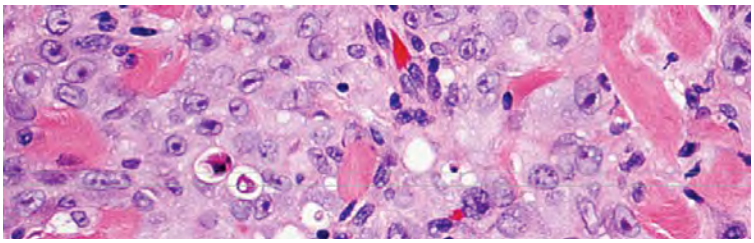
If it LOOKS like carcinoma but is keratin negative, think of:

- Melanoma
- Epithelioid angiosarcoma
- Epithelioid peripheral nerve sheath tumor
- Epithelioid leiomyosarcoma
- Epithelioid sarcoma

	Keratins	CD34	S100	Desmin	INI1 Loss	ERG
PD Carcinoma						
Melanoma						
Epithelioid sarcoma						
Epithelioid LMS						
Epithelioid MPNST						
Epithelioid angiosarcoma						

Epithelioid Angiosarcoma

- Conventional angiosarcomas often show epithelioid foci; nomenclature reserved for tumors almost entirely epithelioid



Epithelioid Angiosarcoma

- Most commonly arise in deep soft tissue,but can be primary to adrenal, bone, thyroid, skin, etc.
- Male predilection
- May present with bleeding disorders (ecchymoses, hemothoraces, etc.)
- Can occur within other tumors (schwannomas) or following trauma

Epithelioid Angiosarcoma

- Early nodal and solid organ metastases (especially lungs, bone, skin, soft tissues)
- High mortality rate (50% dead at 3 years)
- Abundant eosinophilic cytoplasm
- Histologic clues: vacuolated cytoplasm, formation of primitive vascular spaces, blood

Epithelioid Angiosarcoma

Immunophenotype

- Keratin expression may be negative, focal (“anomalous” pattern), or high levels mimicking carcinoma
- Requires use of endothelial-restricted markers such as CD34, CD31, FLI-1, or ERG for positive identification

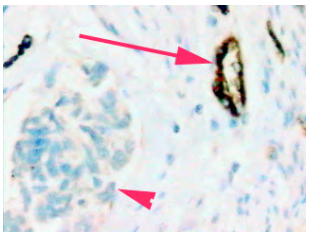
Endothelial Markers

vWF **CD31**

CD34 **ERG**

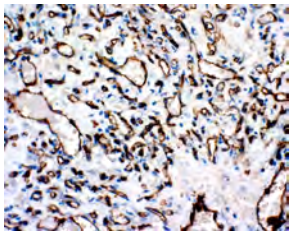
Endothelial Markers

vWF



- Often erroneously referred to as "F. VIII" (actually F.VIII-related antigen)
- Highly specific for endothelial cells and angiosarcoma
- Low sensitivity; not recommended

Endothelial Markers



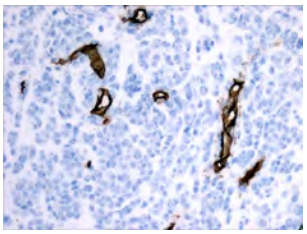
CD31

- **P**latelet **E**ndothelial **C**ell **A**dhesion **M**olecule
- Present on monocytes, platelets, granulocytes, lymphocyte subset
- Highly expressed endothelial cells

Endothelial Markers

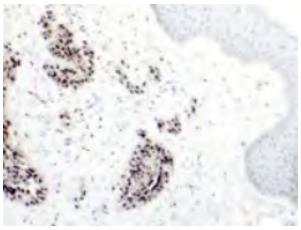
- Cell surface glycoprotein cell adhesion molecule
- Highly expressed on vascular (not lymphatic) endothelial cells
- Also expressed in megakaryocytes, hematopoietic progenitor stem cells, many mesenchymal tumors (GIST, DFSP, hemangiopericytoma, etc.)

CD34



Endothelial Markers

- avian v-ets erythroblastosis virus E26 oncogene homolog
- Member of ETS transcription factor family (along with ETS-1, FLI-1, NERF-2, TEL)
- Consitually express in endothelial cells
- Also expressed in subset of prostatic adenocarcinomas showing TMPRSS2-ERG fusion



ERG

ERG Transcription Factor as an Immunohistochemical Marker for Vascular Endothelial Tumors and Prostatic Carcinoma

Markku Miettinen, MD, Zeng-Feng Wang, PhD,* Anders Paetau, MD,† Shyh-Han Tan, PhD,‡§ Albert Dobi, PhD,‡§ Shiv Srivastava, PhD,‡§ and Isabell Sesterhenn, MD**

Angiosarcoma	**96/100**
Mesenchymal non-vascular tumors	**9/973***
Carcinomas	**32/637****

*PNET/ES, EMMT

**Prostatic AdenoCA

Real World Performance of Vascular Markers

Best CD31	**ERG**
Better	**CD31**
Good	**CD34**

Case 7

FINAL DX:

Epithelioid Angiosarcoma

Case 8

28 year old male in excellent health presents with large deep perineal mass

	Keratins	CD34	S100	Desmin	INI1 Loss	ERG
PD Carcinoma						
Melanoma						
Epithelioid sarcoma						
Epithelioid LMS						
Epithelioid MPNST						
Epithelioid angiosarcoma						

Case 8

Epithelioid Sarcoma

Epithelioid Sarcoma

- Classic (peripheral) variant typically presents in fingers, hands, forearms of young adults
- "Proximal" variant more recently described occurs in pelvic, perineal and pubic regions of older individuals

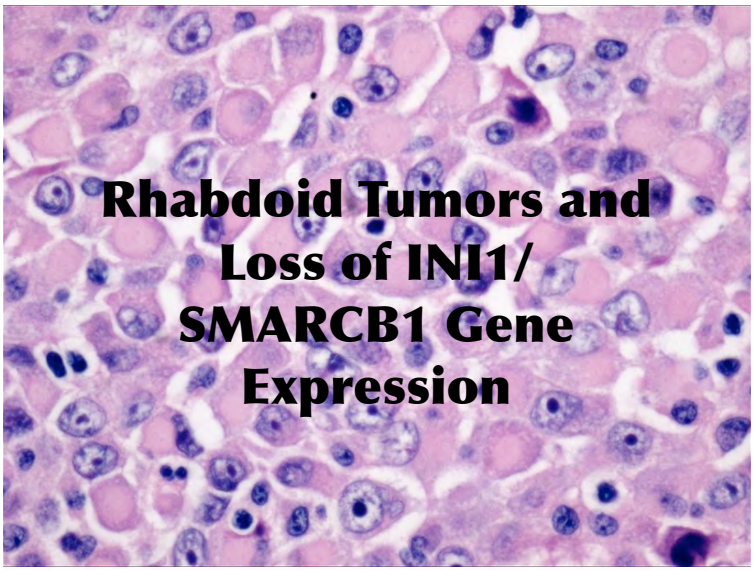
Proximal-Type Epithelioid Sarcoma

- First described by Guillou et al., 1997
- Age range 13-80 (mean, 40)
- Location: pelvis and perineal region (6), pubic region and vulva (4), buttocks (3)
- Shows rhabdoid features, usually without granuloma-like features of peripheral variant
- Follow-up study by Hasegawa et al (2001)

Proximal-Type Epithelioid Sarcoma

Differential Diagnosis

- Poorly differentiated carcinoma
- Rhabdoid tumor
- Epithelioid angiosarcoma
- Epithelioid malignant peripheral nerve sheath tumor
- Langerhans cell sarcoma



Rhabdoid Tumor

- Controversial entity since description as variant of Wilms tumor by Beckwith and Palmer (1978)
- Most frequent in kidney of infants and children
- Has been described as primary tumor in liver, soft tissue, mediastinum, pancreas, GI tract, uterus, skin, bladder, etc.
- In CNS, called atypical teratoid/rhabdoid tumor

Rhabdoid Tumor

- Usually show cytokeratin and vimentin co-expression (to inclusions)
- No other consistent findings
- Descriptions of focal expression of synaptophysin, neurofilament, desmin, muscle actins, S-100, CD57, CD99, WT-1

INI1/SMARCB1 and Rhabdoid Tumors

- Deletions of 22q11.22
- Loss of expression of INI1 (hSNF5, SMARCB1, BAF47), candidate tumor suppressor gene
- CNS tumors have high frequency of monosomy 22
- Extra-renal tumors have high incidence of homozygous deletion
- 20% of tumors show no (apparent) alterations in INI1

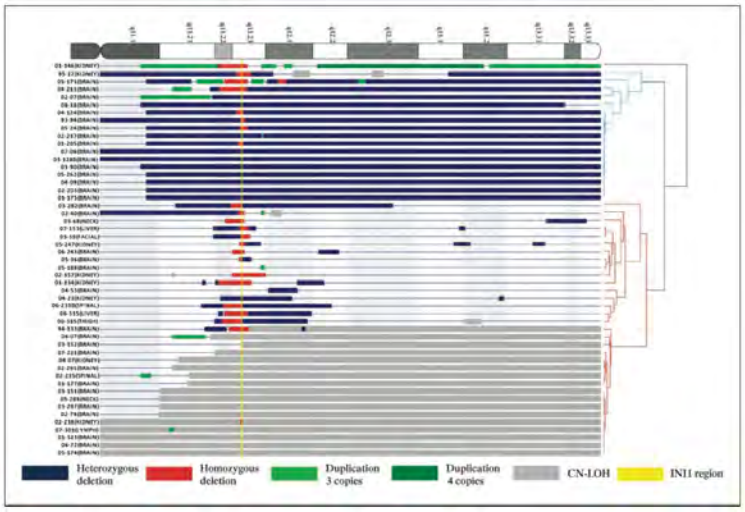
Clin Cancer Res 15:1923-30, 2009

Genomic Analysis Using High-Density Single Nucleotide Polymorphism-Based Oligonucleotide Arrays and Multiplex Ligation-Dependent Probe Amplification Provides a Comprehensive Analysis of *INI1/SMARCB1* in Malignant Rhabdoid Tumors

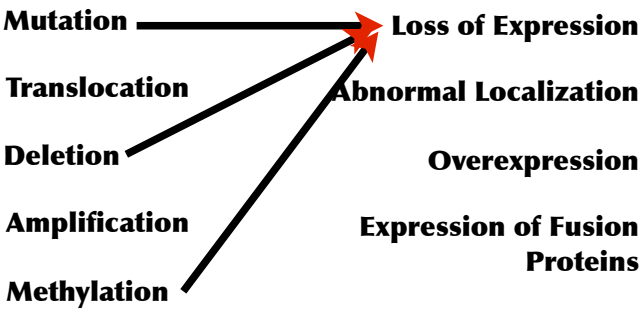
Eric M. Jackson,[1] Angela J. Sievert,[2,3] Xiaowu Gai,[5] Hakon Hakonarson,[2,4] Alexander R. Judkins,[6] Laura Tooke,[4] Juan Carlos Perin,[5] Hongbo Xie,[5] Tamim H. Shaikh,[2,4] and Jaclyn A. Biegel[2,4]

- 51 rhabdoid tumors
- INI1 (SMARCB1) inactivation shown to be via variety of mechanisms, including deletions, mutations, and loss of heterozygosity
- Two (both) inactivating events identified in virtually all

Clin Cancer Res 15:1923-30, 2009



Major Genetic Alterations in Cancer



Am J Surg Pathol 28:1485-91, 2004

Immunohistochemical Analysis of hSNF5/INI1 Distinguishes Renal and Extra-renal Malignant Rhabdoid Tumors From Other Pediatric Soft Tissue Tumors

Andrew C. Hoot, MD, Pierre Russo, MD,* Alexander R. Judkins, MD,* Elizabeth J. Perlman, MD,‡ and Jaclyn A. Biegel, PhD†*

- 27 Rhabdoid tumors with molecular analysis
- 17 Rhabdoid tumors without molecular analysis
- Examined INI1 protein by IHC and compared with PNET/ES, DSRCT, ARMS, ES

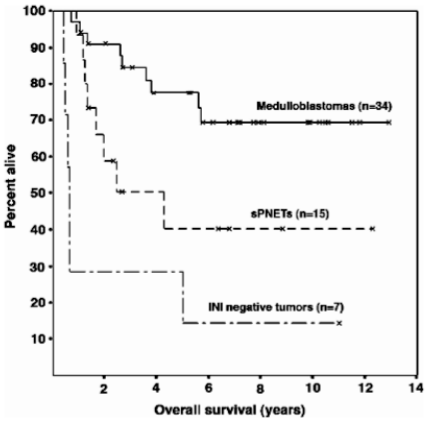
Am J Surg Pathol 30:1462-8, 2006

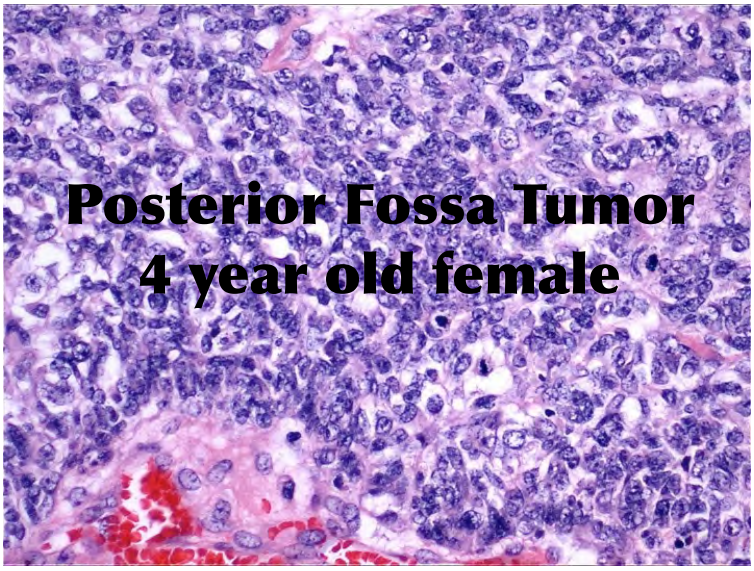
Immunohistochemical Analysis of INI1 Protein in Malignant Pediatric CNS Tumors: Lack of INI1 in Atypical Teratoid/Rhabdoid Tumors and in a Fraction of Primitive Neuroectodermal Tumors without Rhabdoid Phenotype

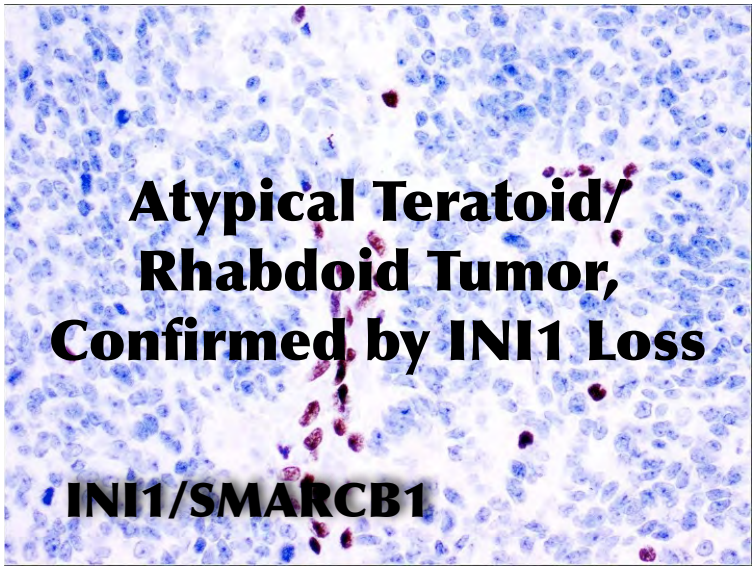
Christine Haberler, MD, Ute Laggner, MD,* Irene Slavc, MD,† Thomas Czech, MD,‡ Inge M. Ambros, MD,§ Peter F. Ambros, PhD,§ Herbert Budka, MD,* and Johannes A. Hainfellner, MD**

- 289 malignant pediatric CNS tumors
- IHC documented loss of INI1 in all cases of AT/RT
- Some medulloblastomas and sPNETs showed loss of INI1

Haberler C et al., Am J Surg Pathol 30:1462-8, 2006







INI1/SMARCB1 and Pediatric CNS Tumors

- IHC preferred to FISH, microsatellite analysis, and mutational analysis
- IHC for INI1 protein should be performed on all embryonal pediatric CNS tumors

Am J Surg Pathol 33:542-50, 2009

Loss of INI1 Expression is Characteristic of Both Conventional and Proximal-type Epithelioid Sarcoma

Jason L. Hornick, MD, PhD, Paola Dal Cin, PhD, and Christopher D.M. Fletcher, MD, FRCPath

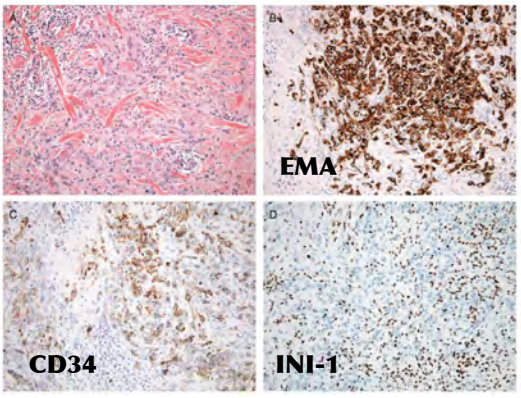


Table 1 Epithelioid sarcoma: patient demographics and SMARCB1 staining

Case	*Diagnosis*	*Sex*	*Age at diagnosis (years)*	*Location*	*SMARCB1*
1	Classical	Female	40	Hand	Negative
2	Classical	Male	18	Leg	Negative
3	Proximal	Male	17	Perineum	Negative
4	Classical	Female	40	Hand	Negative
5	Proximal	Male	45	Unknown	Negative
6	Proximal	Male	37	Unknown	Negative
7	Classical	Male	42	Arm	Negative
8	Classical	Female	40	Hand	Negative
9	Classical	Male	58	Arm	Negative
10	Proximal	Male	49	Groin	Negative
11	Proximal	Male	37	Inguinal	Negative
12	Classical	Male	32	Shoulder	Negative
13	Proximal	Male	20	Scrotum	Negative
14	Classical	Female	24	Thigh	Negative
15	Classical	Female	25	Hand	Negative
16	Classical	Male	38	Forearm	Negative
17	Classical	Male	18	Shin	Negative
18	Classical	Male	56	Arm	Negative
19	Classical	Male	22	Forearm	Negative
20	Proximal	Male	48	Buttock	Positive
21	Classical	Female	72	Foot	Positive

Mod Pathol 26:385-92, 2013

Epithelioid sarcoma is associated with a high percentage of *SMARCB1* deletions

Lisa M Sullivan[1], Andrew L Folpe[2], Bruce R Pawel[1], Alexander R Judkins[3] and Jaclyn A Biegel[1,4]

- 12/19 (63%) of SMARCB1-negative cases had molecular assays performed
- 10/12 (83%) demonstrated homozygous deletions of at least two exons within SMARCB1 gene (but no mutations)
- 2/12 (16%) showed heterozygous deletions of all nine eons

Mod Pathol 21:647-52, 2008

Renal medullary carcinoma: rhabdoid features and the absence of INI1 expression as markers of aggressive behavior

Jason X Cheng, Maria Tretiakova, Can Gong, Saptarshi Mandal, Thomas Krausz and Jerome B Taxy

Department of Pathology, University of Chicago, Chicago, IL, USA

- Rare, highly aggressive primary renal tumor
- Typically affects young patients with sickle cell trait or disease
- Rhabdoid features in some cases
- Relationship to renal rhabdoid tumors?

Am J Surg Pathol 32:1168-74, 2008

SMARCB1/INI1 Protein Expression in Round Cell Soft Tissue Sarcomas Associated With Chromosomal Translocations Involving *EWS*: A Special Reference to SMARCB1/INI1 Negative Variant Extraskeletal Myxoid Chondrosarcoma

Kenichi Kohashi, Sadafumi Tami Tomoaki ... *moto, MD, PhD,* umi, MD, PhD,* MD, PhD**

TABLE 1. BAF47 Expression in Sarcomas Associated With Chromosomal Translocations Involving EWS

Diagnosis	No. Cases	Fusion Gene Positive Cases		BAF47 Negative Cases
EWS/PNET	52	EWS-FLI1 (+)	7	0
		EWS-ERG (+)	1	0
		Not detected	1	0
		Not done	43	0
Extraskeletal myxoid chondrosarcoma	24	EWS-CHN (+)	6	0
		TAF2N-CHN (+)	1	0
		Not detected	7	4
		Not done	10	0
Clear cell sarcoma of soft tissue	14	EWS-ATF1 (+)	5	0
		Not detected	2	0
		Not done	7	0
Desmoplastic small round cell tumor	2	EWS-WT1 (+)	2	0
Myxoid/round cell liposarcoma	1	EWS-CHOP (+)	1	0

Loss of INI-1 Expression

- Renal and extra-renal rhabdoid tumors
- Atypical teratoid/rhabdoid tumors
- Epithelioid sarcoma
- Medullary renal carcinoma
- Extraskeletal myxoid chondrosarcoma
- Epithelioid MPNST
- Myoepithelial carcinoma

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Questions?

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Thank you

Obrigado

Grazie

Gracias

Merci

Mahalo

Danke

Takk