NordiQC

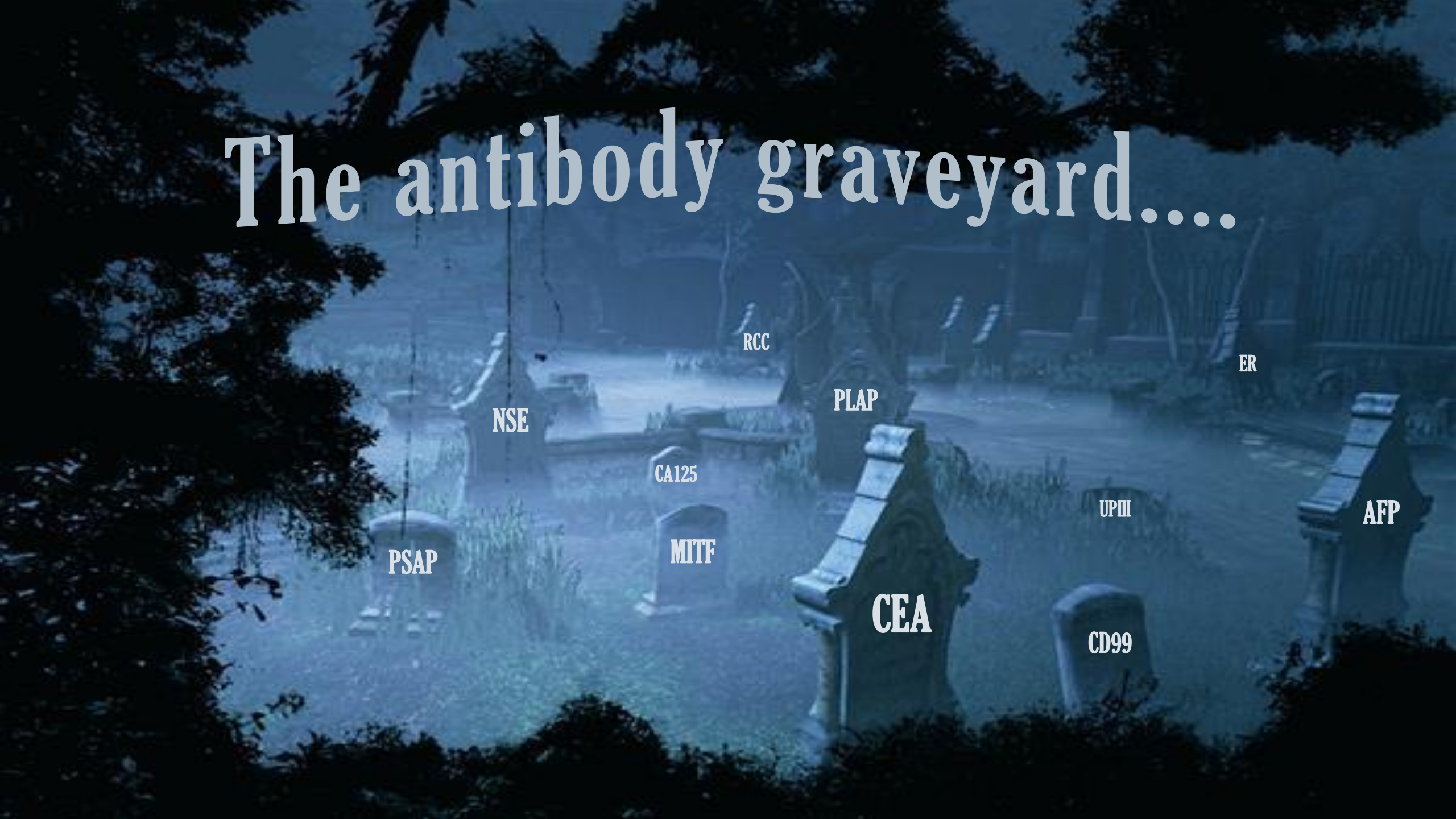
Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, October 5-7th 2022

The Antibody Graveyard

-

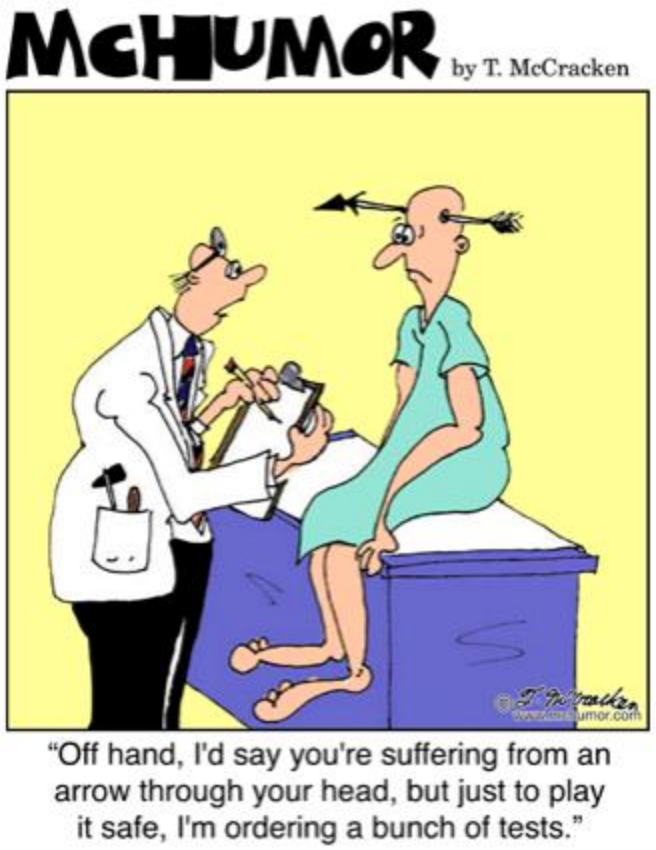
Goodbye and Hello Markers

Søren Nielsen,
Director
NordiQC

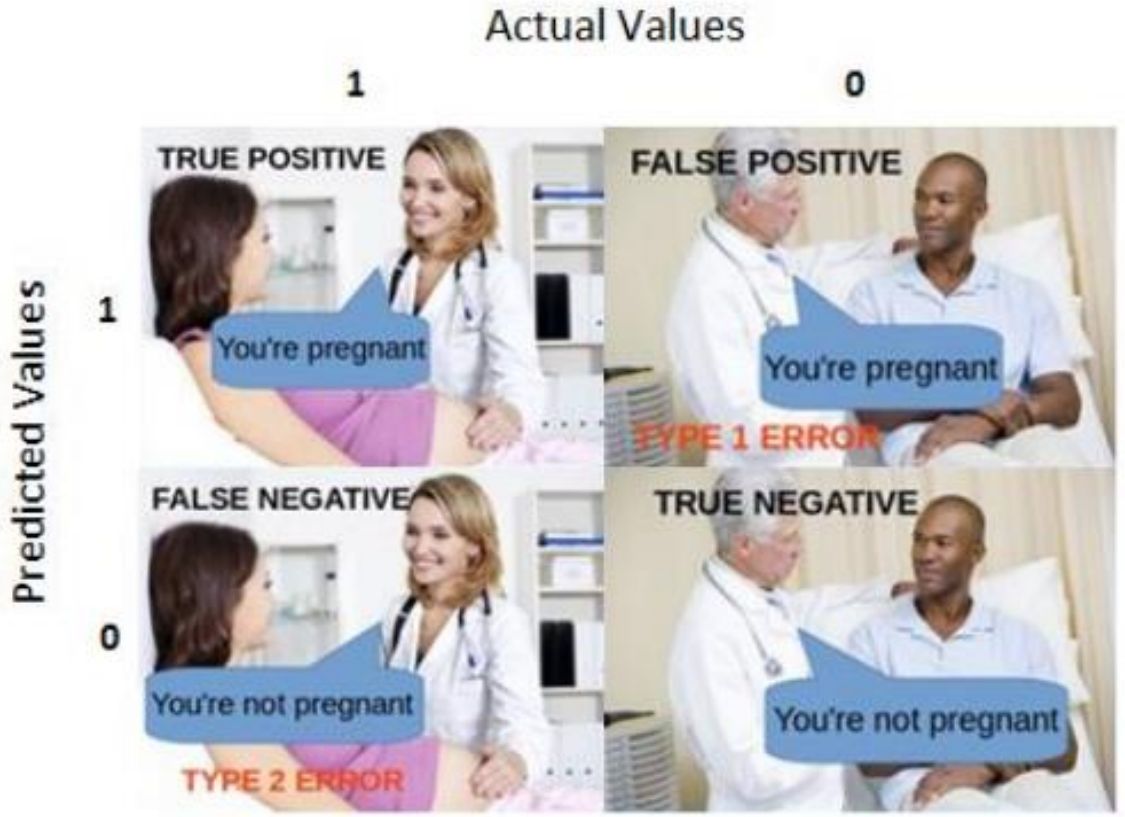

The antibody graveyard....
RCC
ER
PLAP
NSE
CA125
UPIII
AFP
PSAP
MITF
CEA
CD99


Next generation antibodies..
SOX10
INSM1
NKX2.2
PRAME
SATB2
Cadh-17
SALL4
TPRS1
BAP1

Replacements, supplemental IHC markers – why?



"Off hand, I'd say you're suffering from an arrow through your head, but just to play it safe, I'm ordering a bunch of tests."

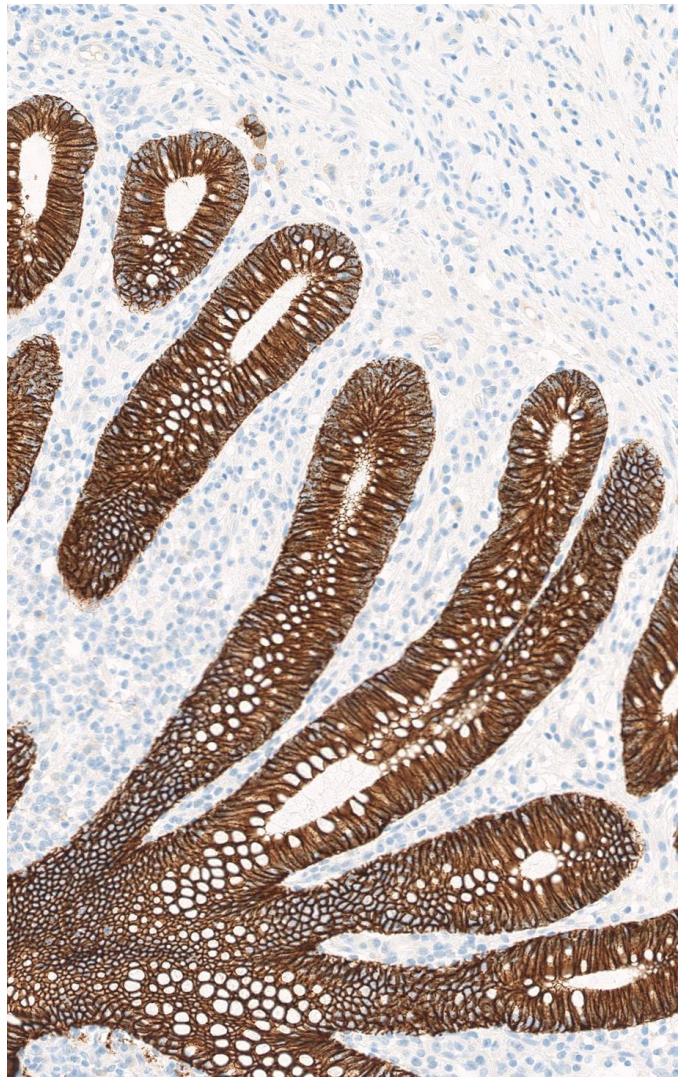


The antibody graveyard – CUP* - Colorectal carcinoma markers

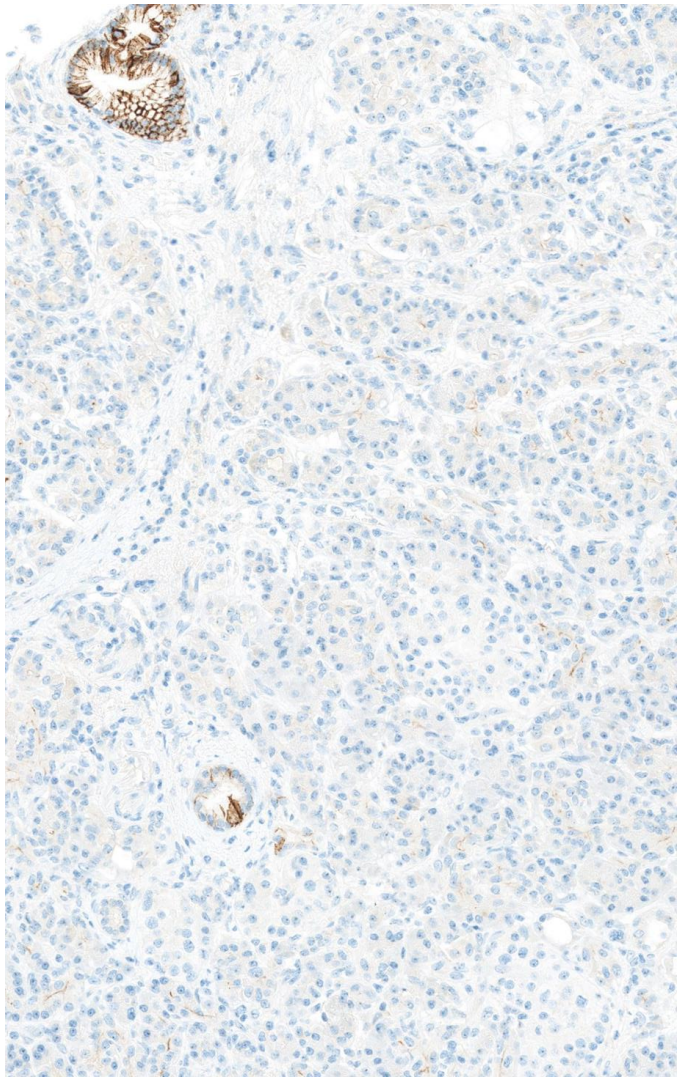
	To stay	Sensitivity	Comments	
CK20	Yes	80-95%	MSI-H carc. can be neg. - Also seen in many other carcinomas	By both 97% & 88% sens. for MSS and MSI-H CRC
CDX2	Yes	80-95%	MSI-H carc. can be neg. – Intestinal lineage marker	
mCEA	?	90-100%	Might be useful for gastric adenocarcinomas	
Villin	No	70-90%	Less sensitive and less specific	
SATB2	”New”	75-90%	Lower GI tract and rectal/appendiceal neuroendocrine tumours	
Cadherin-17	”New”	90-95%	Publications indicate superior sensitivity comp. to CDX2 and not a lineage marker	

* CUP; Cancer of unknown primary origin

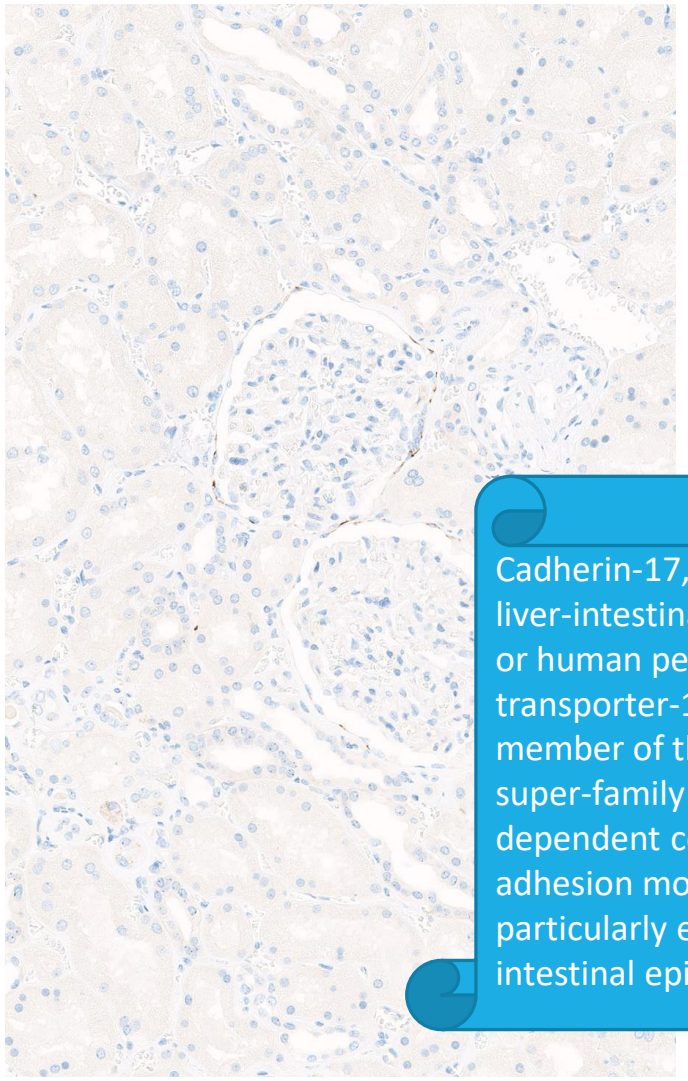
The antibody graveyard – CUP* - Colorectal carcinoma markers – Cadherin 17



Colon/Appendix



Pancreas



Kidney

Cadherin-17, also called liver-intestinal cadherin or human peptide transporter-1, is a member of the cadherin super-family and is a Ca^{2+}-dependent cell–cell adhesion molecule particularly expressed on intestinal epithelial cells

The antibody graveyard – CUP* - Colorectal carcinoma markers – Cadherin 17

Table 3. Primary Colon Cancer Versus Metastasis

Colon Cancer	CDH17, % (No.)	CK20, % (No.)	CDX2, % (No.)
Primary	99.1 (116/117)	95.7 (112/117)[a]	96.6 (113/117)[a]
Metastasis into lymph node[b]	90.6 (29/32)	59.4 (19/32)[c]	81.3 (26/32)[a]

Abbreviation: CK, cytokeratin.

[a] $P > .05$; primary CK20: $P = .10$, CDX2: $P = .18$; metastasis into lymph node CDX2: $P = .15$.
[b] The origin of metastatic carcinomas was determined by a board-certified pathologist before receiving the tissue for testing.
[c] $P < .05$; metastasis into lymph node CK20: $P = .004$.

Table 4. Primary Stomach Adenocarcinoma Versus Metastasis

Stomach Cancer	CDH17, % (No.)	CK20, % (No.)	CDX2, % (No.)
Primary	63.3 (88/139)	23 (32/139)[a]	46 (64/139)[b]
Metastasis[c]	66.7 (24/36)	30.5 (11/36)[b]	50 (18/36)[d]

Abbreviation: CK, cytokeratin.

[a] $P < .001$.
[b] $P < .05$; primary CDX2: $P = .004$; metastasis CK20: $P = .002$.
[c] The origin of metastatic carcinomas was determined by a board-certified pathologist before receiving the tissue for testing.
[d] $P > .05$; metastasis CDX2: $P = .15$.

CDH17 Is a More Sensitive Marker for Gastric Adenocarcinoma Than CK20 and CDX2
David Altree-Tacha et al, Arch Pathol Lab Med. 2017;141:144–150

The antibody graveyard – CUP* - Colorectal carcinoma markers – Cadherin 17

Table 2. Neoplastic Tissues (n = 884)

Cancer Type	CDH17, % (No.)	CK20, % (No.)	CDX2, % (No.)
Colon adenocarcinoma	97.3 (145/149)	88.6 (132/149)[a]	93.3 (139/149)[b]
Stomach adenocarcinoma	64.0 (112/175)	24.6 (43/175)[c]	46.9 (82/175)[a]
Esophageal cancer (n = 54)			
Esophageal adenocarcinoma	38.7 (12/31)	25.8 (8/31)[b]	29 (9/31)[b]
Esophageal squamous cell carcinoma	0 (0/23)	0 (0/23)	0 (0/23)
Appendiceal cancer (n = 5)			
Adenocarcinoma	2/2	2/2	2/2
Undifferentiated carcinoma	0/2	0/2	0/2
Pancreatic cancer (n = 57)			
Pancreatic ductal adenocarcinoma	39.3 (11/28)	10.7 (3/28)[a]	0 (0/28)[c]
Pancreatic adenocarcinoma	24.1 (7/29)	13.8 (4/29)[b]	6.9 (2/29)[b]
Hepatocellular carcinoma	1.8 (1/57)	7 (4/57)	0 (0/57)
Cholangiocarcinoma	33.3 (4/12)	33.3 (4/12)	8.3 (1/12)
Ovarian cancer (n = 60)			
Serous papillary cystadenocarcinoma	6.4 (3/47)	8.5 (4/47)	4.4 (2/47)
Endometrioid adenocarcinoma	28.6 (2/7)	28.6 (2/7)	14.3 (1/7)
Mucinous adenocarcinoma	50 (3/6)	50 (3/6)	66.7 (4/6)
Endometrial adenocarcinoma	28.6 (2/7)	57.1 (4/7)	0 (0/7)
Lung cancer (n = 78)			
Adenocarcinoma	11.1 (4/36)	5.6 (2/36)	2.8 (1/36)
Squamous cell carcinoma	0 (0/29)	0 (0/29)	0 (0/29)
Small cell carcinoma	0 (0/5)	0 (0/5)	0 (0/5)
Large cell carcinoma	0 (0/5)	0 (0/5)	0 (0/5)
Neuroendocrine carcinoma	0 (0/3)	0 (0/3)	0 (0/3)
Prostate adenocarcinoma	0 (0/20)	0 (0/20)	0 (0/20)
Breast cancer (infiltrating ductal)	0 (0/73)	2.7 (2/73)	0 (0/73)
Bladder cancer (n = 63)			
Urothelial carcinoma	0 (0/61)	52.5 (32/61)	4.9 (3/61)
Bladder adenocarcinoma	100 (2/2)	100 (2/2)	(0/2)
Clear cell renal cell carcinoma	0 (0/10)	0 (0/10)	0 (0/10)
Thyroid cancer (n = 12)			
Papillary carcinoma	0 (0/10)	0 (0/10)	0 (0/10)
Follicular carcinoma	0 (0/2)	0 (0/2)	0 (0/2)
Seminoma	0 (0/23)	0 (0/23)	0 (0/23)
Brain cancer (astrocytoma)	0 (0/12)	0 (0/12)	0 (0/12)
Melanoma (classic)	0 (0/6)	0 (0/6)	0 (0/6)
Lymphoma (n = 11)			
B-cell lymphoma	0 (0/8)	0 (0/8)	0 (0/8)
T-cell lymphoma	0 (0/3)	0 (0/3)	0 (0/3)

Abbreviation: CK, cytokeratin.

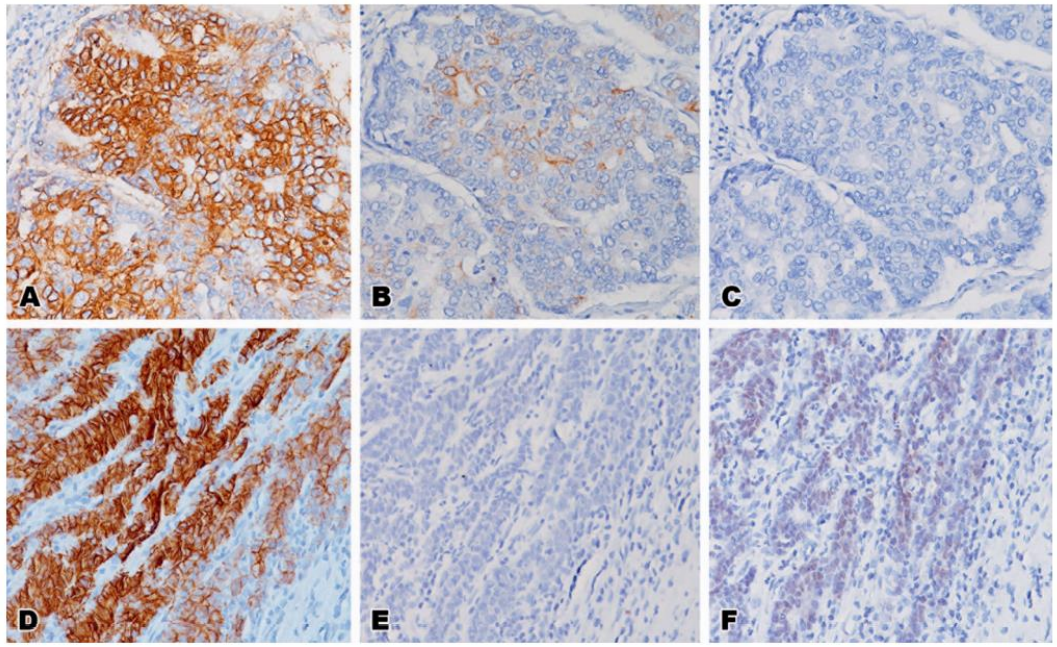


Figure 3. *Staining results in metastatic colon adenocarcinoma. A and D, Strong, positive staining was observed in a high percentage of specimens with CDH17. B, Focal staining was observed in CK20-positive tissue; and in specimens considered negative, CK20 was completely absent (E). Representative negative (C) and moderate positive (F) staining for CDX2 (original magnification ×20 [A through F]).*

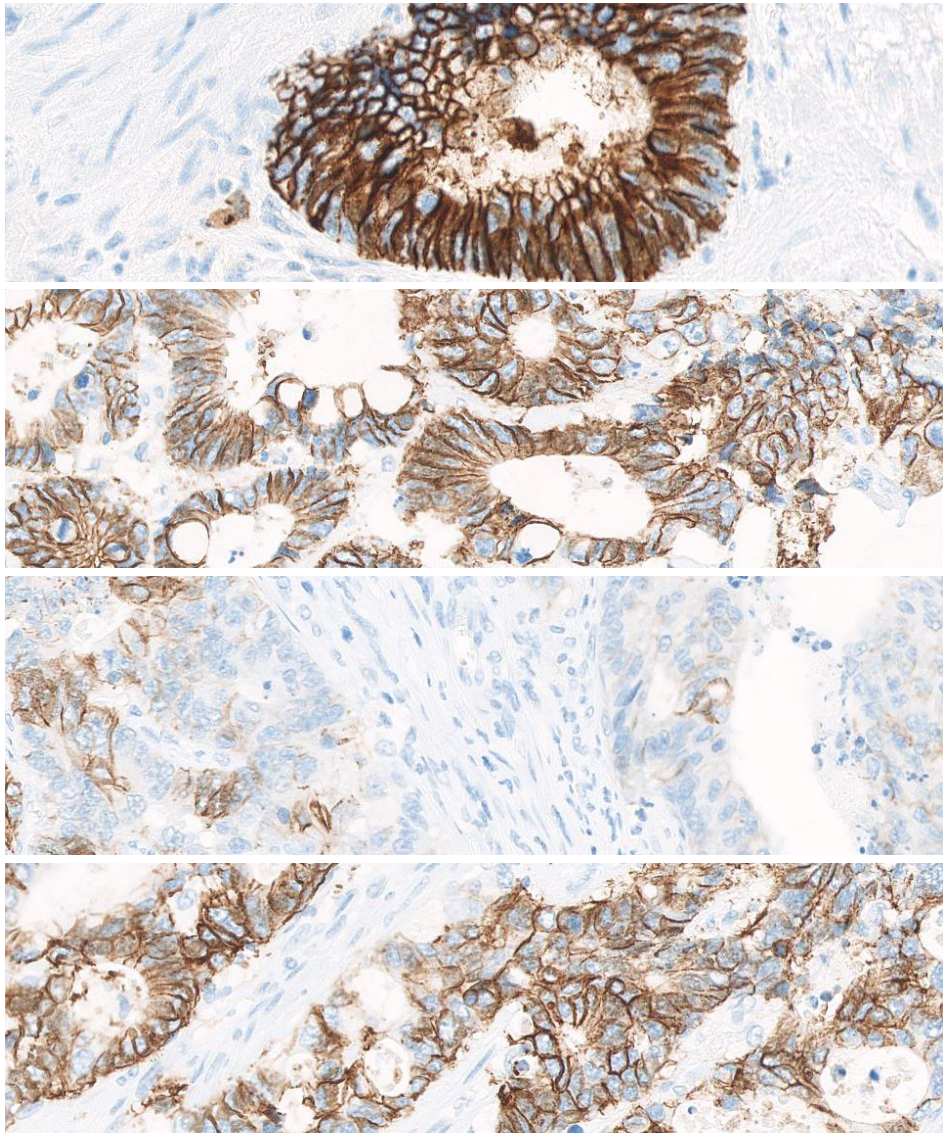
CDH17 Is a More Sensitive Marker for Gastric Adenocarcinoma Than CK20 and CDX2
David Altree-Tacha et al, Arch Pathol Lab Med. 2017;141:144–150

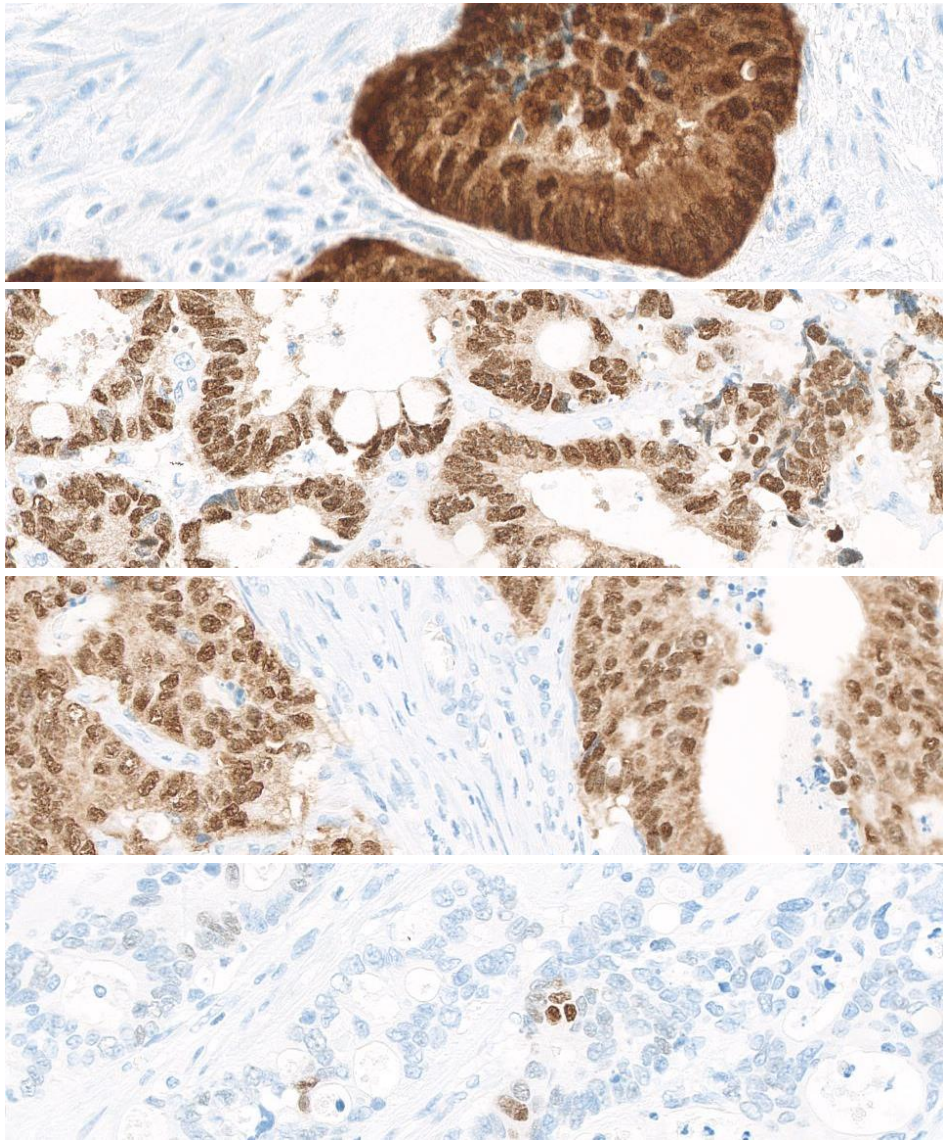
The antibody graveyard – CUP* - Colorectal carcinoma markers – Cadherin 17

CAD-17

rmAb
SP183

1:100, 32M
CC1, 48M
OP-DAB
VMS Ultra

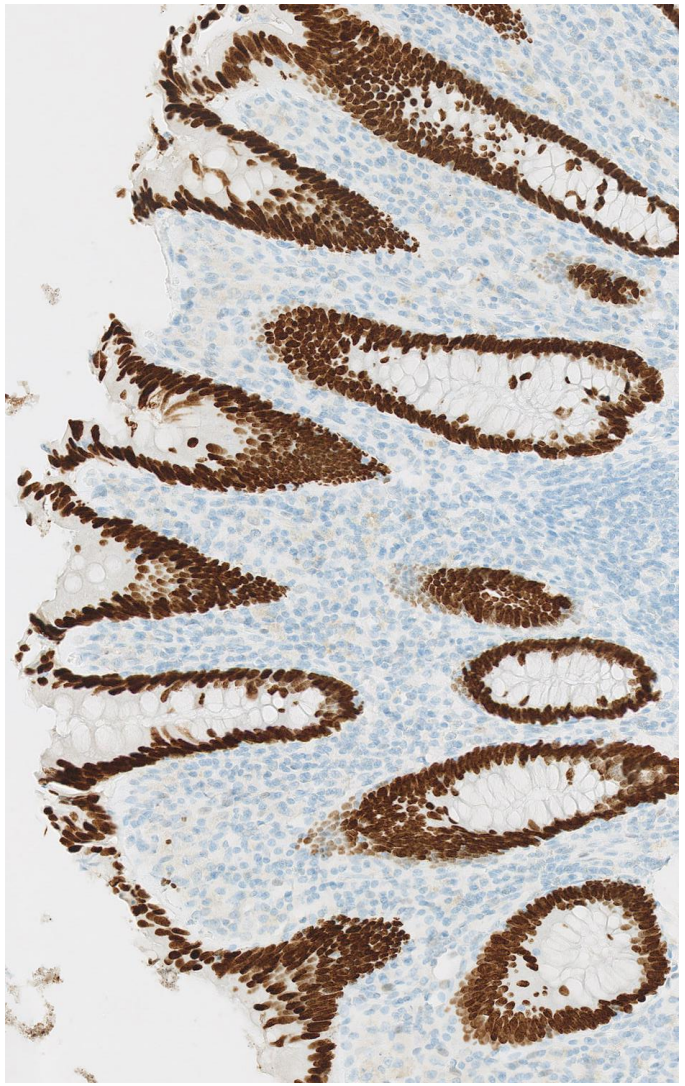




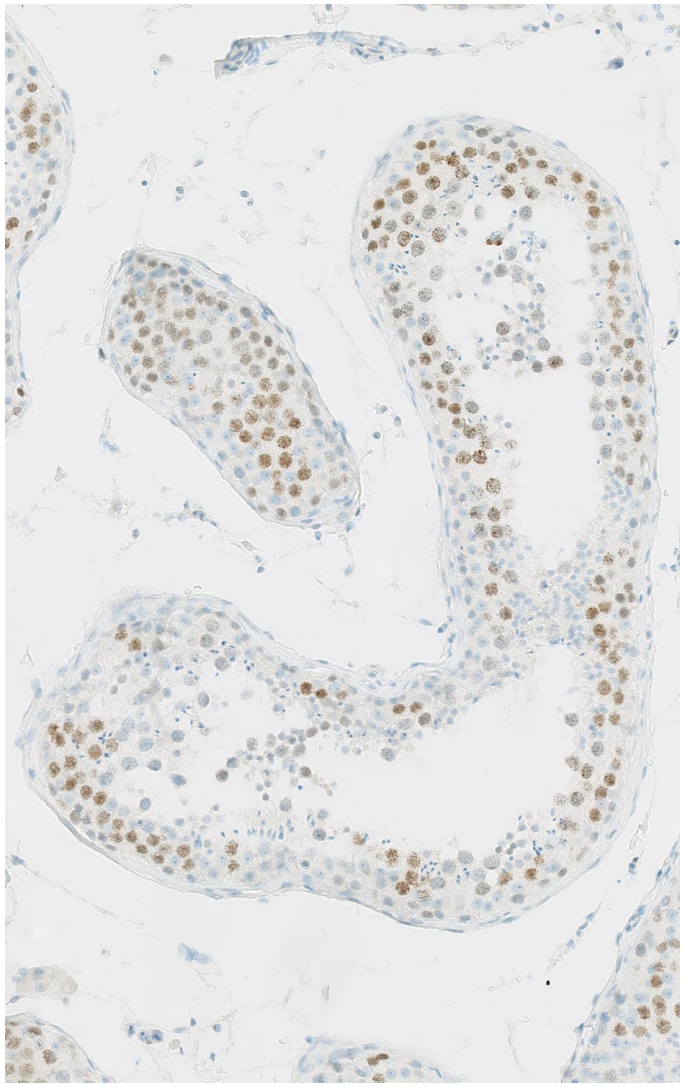
CDX2

The antibody graveyard – CUP* - Colorectal carcinoma markers – SATB2

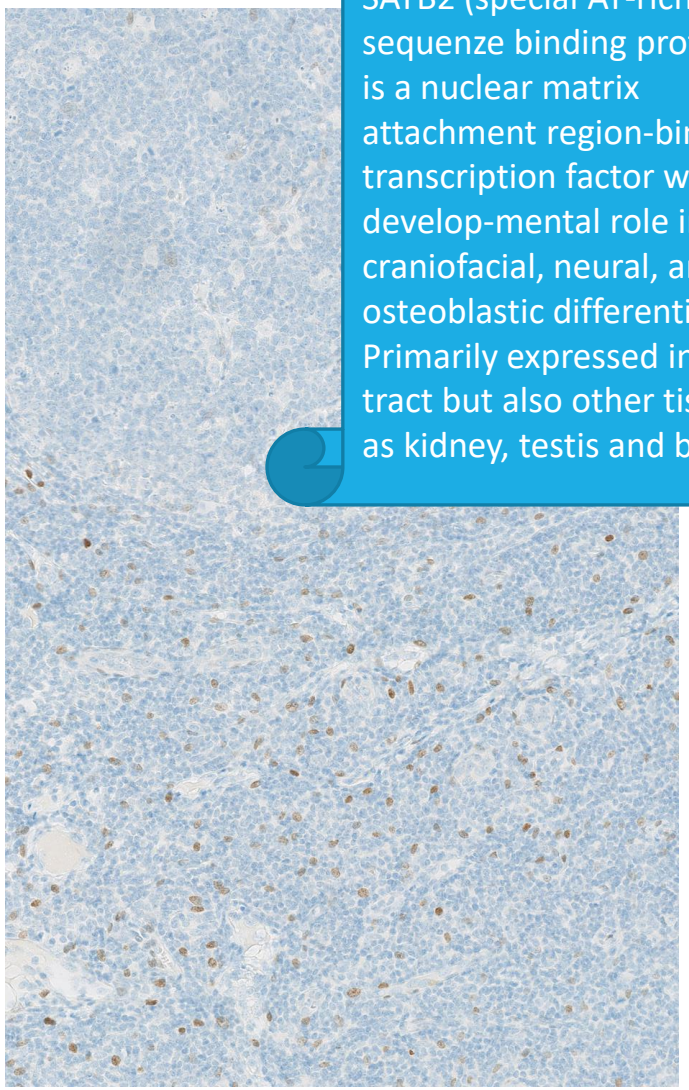
SATB2 (special AT-rich sequenze binding protein 2) is a nuclear matrix attachment region-binding transcription factor with develop-mental role in craniofacial, neural, and osteoblastic differentiation. Primarily expressed in GI tract but also other tissues as kidney, testis and brain.



Colon/Appendix



Testis



Tonsil

The antibody graveyard – CUP* - Colorectal carcinoma markers – SATB2

Table 1. Any SATB2 Expression in Primary Mucinous Tumors

Score	Site, No. %						
	Colorectum (n = 44)	Ovary (n = 60)	Breast (n = 31)	Lung (n = 26)	Uterus (n = 28)	Pancreas (n = 15)	Stomach and Esophagus (n = 15)
Intensity							
1	8 (18.2)	1 (1.7)	2 (6.5)	0 (0)	1 (3.6)	0 (0)	1 (6.7)
2	18 (40.9)	2 (3.3)	3 (9.7)	0 (0)	0 (0)	0 (0)	3 (20.0)
3	13 (29.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
All positive	39 (88.6)	3 (5.0)	5 (16.1)	0 (0)	1 (3.6)	0 (0)	4 (26.7)
Percentage							
0	1 (2.3)	0 (0)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)
1	4 (9.1)	2 (3.3)	3 (9.7)	0 (0)	1 (3.6)	0 (0)	0 (0)
2	34 (77.3)	1 (1.7)	1 (3.2)	0 (0)	0 (0)	0 (0)	4 (26.7)

Table 2. Any CDX2 Expression in Primary Mucinous Tumors

Score	Site, No. %						
	Colorectum (n = 44)	Ovary (n = 60)	Breast (n = 31)	Lung (n = 26)	Uterus (n = 28)	Pancreas (n = 15)	Stomach and Esophagus (n = 15)
Intensity							
1	0 (0)	6 (10.0)	0 (0)	9 (34.6)	2 (7.1)	2 (13.3)	7 (46.7)
2	8 (18.2)	32 (53.3)	0 (0)	5 (19.2)	1 (3.6)	8 (53.3)	1 (6.7)
3	36 (81.8)	10 (16.7)	0 (0)	0 (0)	2 (7.1)	4 (26.7)	7 (46.7)
All positive	44 (100)	48 (80.0)	0 (0)	14 (53.8)	5 (17.9)	14 (93.3)	15 (100)
Percentage							
0	0 (0)	5 (8.3)	0 (0)	2 (7.7)	3 (10.7)	1 (6.7)	0 (0)
1	0 (0)	11 (18.3)	0 (0)	4 (15.4)	1 (3.6)	5 (33.3)	6 (40.0)
2	44 (100)	32 (53.3)	0 (0)	8 (30.8)	1 (3.6)	8 (53.3)	9 (60.0)

CDX2 more sensitive for colorectal adenocarcinomas

SATB2 more specific for colorectal adenocarcinomas

Differential diagnosis of ovarian, lung or colorectal carc.

SATB2 Versus CDX2 - A Battle Royale for Diagnostic Supremacy in Mucinous Tumors
Arch Pathol Lab Med. 2019;143:1119–1125

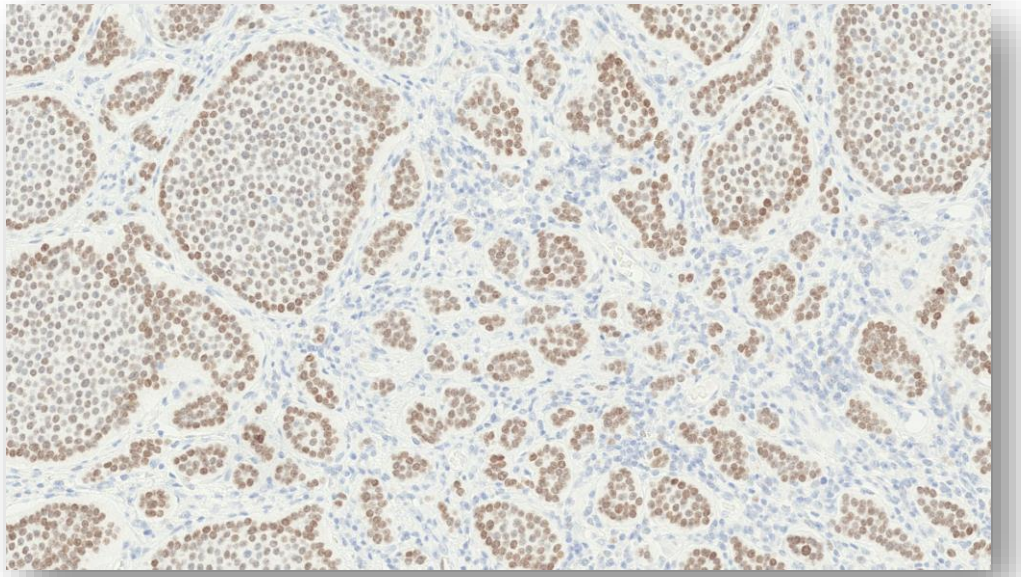
Intensity of SATB2/CDX2 staining was scored as; negative, 0; weak, 1; moderate, 2; or strong, 3
Percentage of tumor staining was scored as 0; <5%, 1; 5%–49 and 2; ≥50%,

The antibody graveyard – CUP* - Colorectal carcinoma markers – SATB2

Published in final edited form as:
Hum Pathol. 2020 February ; 96: 8–33. doi:10.1016/j.humpath.2019.12.002.

Immunohistochemistry in the diagnosis and classification of neuroendocrine neoplasms: what can brown do for you?⋆

Andrew M Bellizzi, MD[*]
Department of Pathology, University of Iowa Hospitals and Clinics and Carver College of Medicine, Iowa City, IA, USA University of Iowa Neuroendocrine Cancer Program, University of Iowa Hospitals and Clinics and Holden Comprehensive Cancer Center, Iowa City, IA 52242, USA



Well-Differentiated Neuroendocrine Tumor Classifier For the Real World:
Assumes Positivity for Broad-Spectrum Epithelial Marker and
Diffuse, Strong Positivity for Chromogranin A and/or Synaptophysin

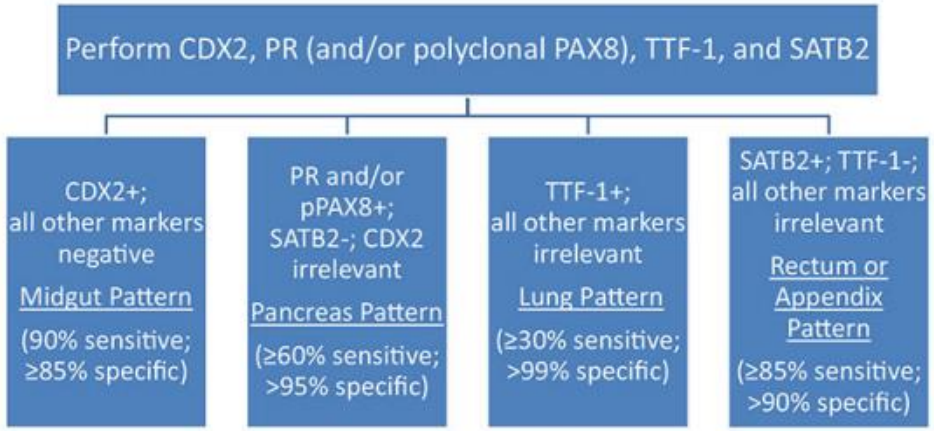


Figure 11. Simplified Immunohistochemical Algorithm for Well-Differentiated Neuroendocrine Tumor Site of Origin.

"A rectal origin is suggested by morphology and can be confirmed with SATB2-positivity (strongly positive in nearly all [96%] rectal NETs and never strongly expressed by pancreatic tumors); incidentally, SATB2 is also expressed by most (79%) appendiceal NETs"].

The antibody graveyard – CUP* - Colorectal carcinoma markers – SATB2 – the Ab….

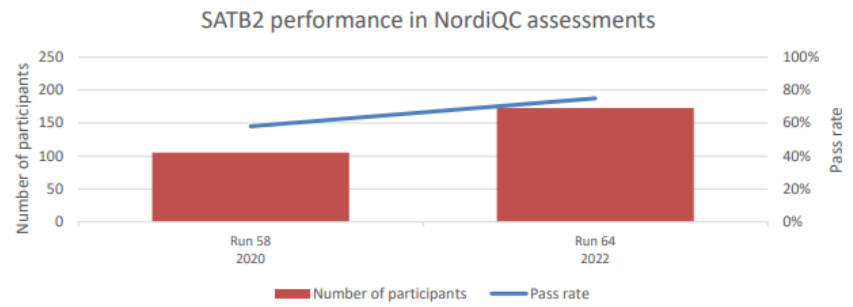
Table 1. **Antibodies and assessment marks for SATB2, run 58**

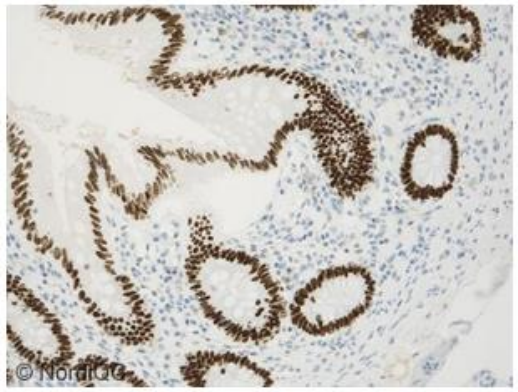
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **CL0276**	5 2 1	Atlas Antibodies Sigma Aldrich Novus Biologicals	0	0	0	8	0%	0%
mAb clone **CL0320**	1	Atlas Antibodies	0	0	1	0	-	-
mAb clone **SATBA4B10**	3 2 2	Abcam Santa Cruz Zytomed Systems	0	0	2	5	0%	0%
mAb clone **OTI5H7**	1	ZSBio	1	0	0	0	-	-
rmAb clone **EP281**	30 12 1 1 1 1	Epitomics Cell Marque Immunologic BioSB Biocare Medical Unknown	22	14	4	6	78%	82%
rmAb clone **SP281**	4 1	Abcam Spring Bioscience	2	1	1	1	60%	40%
rmAb clone **ZR167**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **EPNCIR130A**	5	Abcam	0	0	0	5	0%	0%
pAb **HPA001042**	5	Sigma Aldrich	0	0	2	3	0%	0%
pAb **Ab69995**	1	Abcam	0	0	0	1	-	-
Ready-To-Use antibodies							Suff.[1]	OR[2]
rmAb clone **EP281 384R-17/18**	19	Cell Marque	7	10	1	1	89%	37%
rmAb clone **EP281 PR/HAR239**	2	PathnSitu	2	0	0	0	-	-
rmAb clone **EP281 API3225**	1	Biocare Medical	0	1	0	0	-	-
rmAb clone **EP281 MAD-000747QD**	1	Máster Diagnostica	0	0	1	0	-	-
rmAb clone **EP281 BSB3199**	2	BioSB	0	0	0	2	-	-
Total	105		35	26	12	32	-	
Proportion			33%	25%	11%	31%	58%	

1) Proportion of sufficient stains (optimal or good). (≥5 asessed protocols)
2) Proportion of Optimal Results (OR)

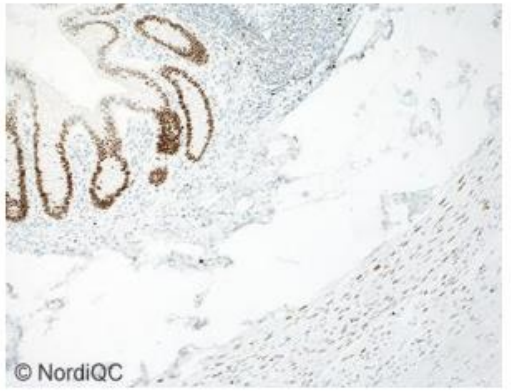
Performance history
This was the second NordiQC assessment of SATB2. The pass rate increased significantly from 58% in the first run 58 to 75% in this run 64 (see Graph 1).

Graph 1. **Proportion of sufficient results for SATB2 in the NordiQC runs performed**





EP281



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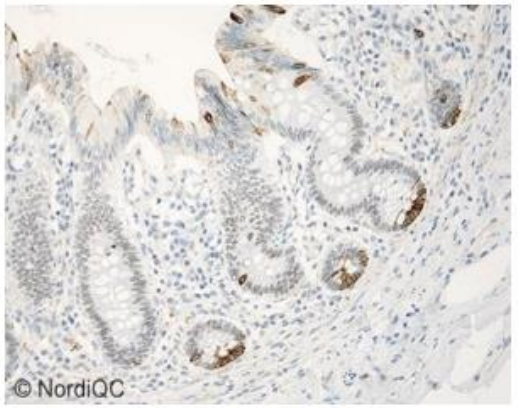
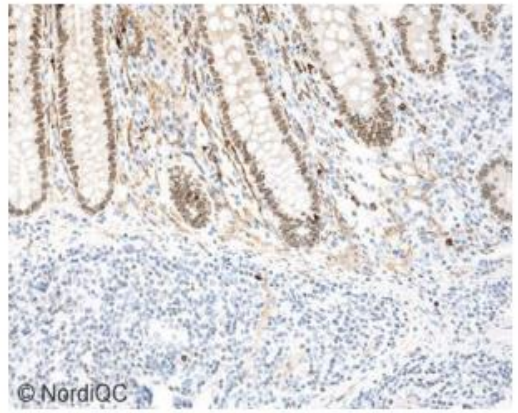


Ab69995



HPA001042

The antibody graveyard – CUP - Colorectal carcinoma markers

CK20 and CDX2; the two primary markers for identification of colorectal (CRC) adenocarcinoma

Cadherin 17 might be superior to CK20, but the wide publication history of CK20 challenges the position of Cadherin 17 as primary marker

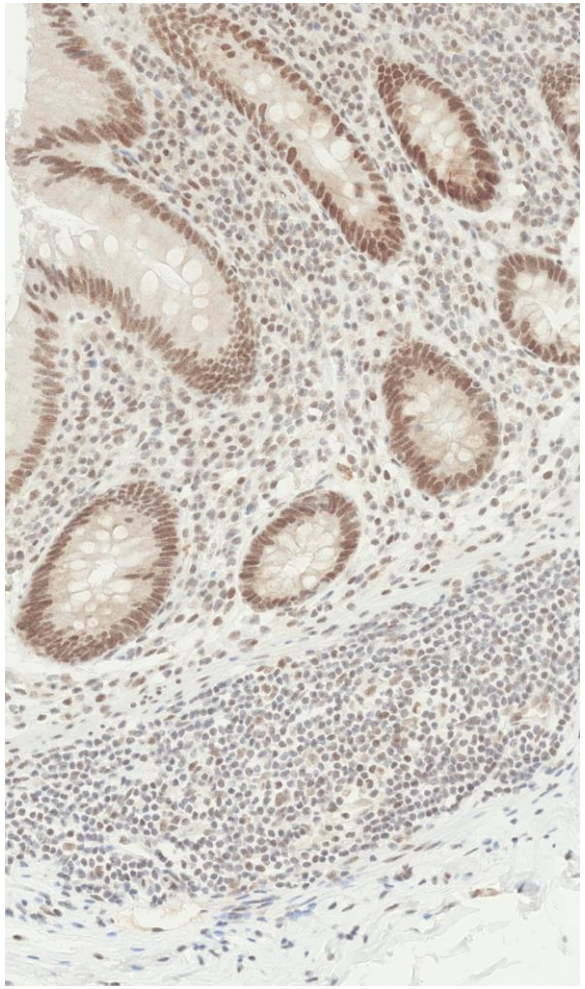
SATB2 to used in the differential diagnosis of mucinous ovarian and CRC adenocarcinoma

Villin and mCEA of less diagnostic value for CRC

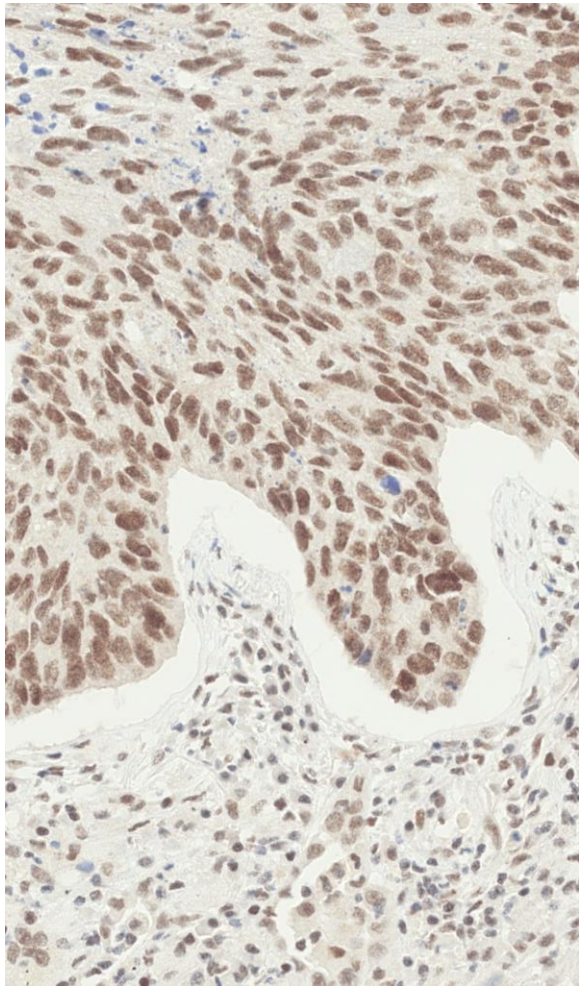
The antibody graveyard – Mesothelioma – positive markers

	To stay	Sensitivity	Comments
Calretinin	Yes	85-95%	Also seen in some carcinomas, but typically focal
CK5	Yes	90-95%	Also seen in squamous cell carcinomas
Thrombomodulin	No	60-70%	Less sensitive
CA125	No	70-80%	Less sensitive and less specific (breast carc., pancreas carc, ovarian serous carc. ….)
Mesothelin	No	60-80%	Less sensitive and less specific
Mesothelioma versus reactive mesothelial cells			
BAP1	*New*	*60%*	*BRAC1 associated protein; mutation in BAP1 gene seen in mesothelioma (app 60%)*
MTAP	*New*	*50%*	*MTAP (methylthioadenosine phosphorylase); deficient expression seen in mesothelioma (app 50%)*

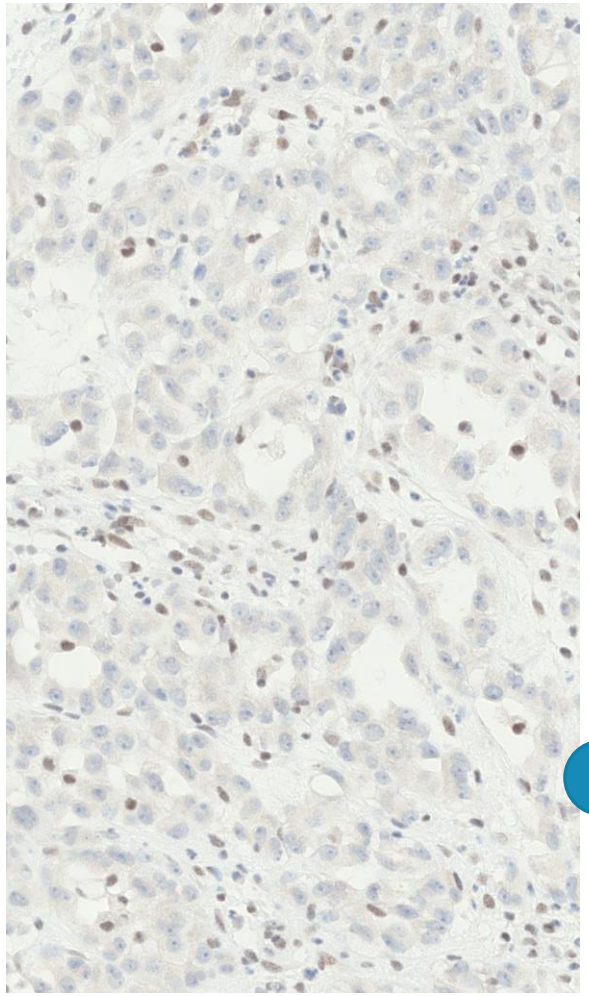
The antibody graveyard – Mesothelioma – BAP1



Colon/Appendix



Tumour no mutation



Mesothelioma + mutation

BRCA1-associated protein 1 (BAP1) is a tumor suppressor gene that regulates several cellular functions such as chromatin remodeling, cellular differentiation, DNA damage response, growth suppression, and apoptosis. BAP1 loss has emerged in recent years as a virtually 100% specific marker of malignancy in mesothelial proliferations

Clone C-4, Santa Cruz

Not beautiful, but ok☺

The antibody graveyard – Mesothelioma – BAP1

Review Article

Diagnostic Mesothelioma Biomarkers in Effusion Cytology

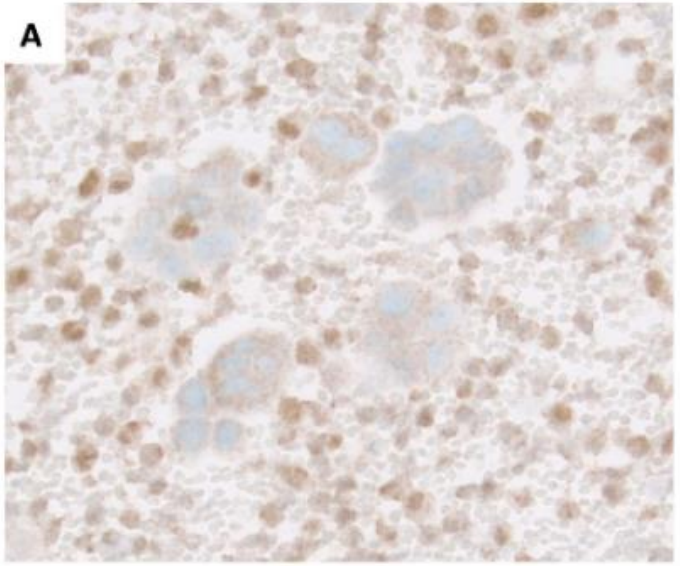
Albino Eccher, MD [1]; Ilaria Girolami, MD [2]; Ersilia Lucenteforte, MD[3]; Giancarlo Troncone, MD [4]; Aldo Scarpa, MD[1]; and Liron Pantanowitz, MD [5]

Malignant mesothelioma is a rare malignancy with a poor prognosis whose development is related to asbestos fiber exposure. An increasing role of genetic predisposition has been recognized recently. Pleural biopsy is the gold standard for diagnosis, in which the identification of pleural invasion by atypical mesothelial cell is a major criterion. Pleural effusion is usually the first sign of disease; therefore, a cytological specimen is often the initial or the only specimen available for diagnosis. Given that reactive mesothelial cells may show marked atypia, the diagnosis of mesothelioma on cytomorphology alone is challenging. Accordingly, cell block preparation is encouraged, as it permits immunohistochemical staining. Traditional markers of mesothelioma such as glucose transporter 1 (GLUT1) and insulin-like growth factor 2 mRNA-binding protein 3 (IMP3) are informative, but difficult to interpret when reactive proliferations aberrantly stain positive. BRCA1-associated protein 1 (BAP1) nuclear staining loss is highly specific for mesothelioma, but sensitivity is low in sarcomatoid tumors. Cyclin-dependent kinase inhibitor 2A (CDKN2A)/p16 homozygous deletion, assessed by fluorescence in situ hybridization, is more specific for mesothelioma with better sensitivity, even in the sarcomatoid variant. The surrogate marker methylthioadenosine phosphorylase (MTAP) has been found to demonstrate excellent diagnostic correlation with p16. The purpose of this review is to provide an essential appraisal of the literature regarding the diagnostic value of many of these emerging biomarkers for malignant mesothelioma in effusion cytology. ***Cancer Cytopathol* 2021;129:506-516**. © *2021 American Cancer Society*.

KEY WORDS: biomarker; cytology; immunohistochemistry; mesothelioma; mesothelium; pleural effusion.

TABLE 1. Systematic Evidence on Diagnostic Performance of Malignant Pleural Mesothelioma Markers

	Sensitivity and Specificity in Systematic Reviews		
Marker	Sensitivity (CI)	Specificity (CI)	Notes
Soluble			
Mesothelin/SMRP	0.79 (0.75-0.83)[27] 0.69 (0.64-0.72)[28]	0.85 (0.83-0.87)[27] 0.90 (0.85-0.94)[28]	• Different cutoffs of the studies included • No subgroup analysis for different MPM subtypes
Fibulin-3	0.73 (0.54-0.86)[31]	0.80 (0.60-0.91)[31]	• Diagnostic performance is usually studied in differential against both lung cancer and reactive atypical mesothelium
IHC and FISH			
GLUT1	0.83 (0.71-0.90)[36]	0.90 (0.79-0.96)[36]	• Marker of malignancy, not of MPM • Informative only when positive • Stains also red blood cells
IMP3	No systematic review; reported values ranging 37-94%		• Oncofetal protein used as marker of malignancy, not of MPM • Few studies dealing with cytology[37,38]
BAP1	0.58 (0.50-0.65)[44] 0.547 (0.512-0.716)[45]	0.96 (0.89-0.99)[44] 0.957 (0.939-0.971)[45]	• The sensitivity is reported to be higher in epithelioid mesothelioma and very low (0-0.22) in sarcomatoid mesothelioma • Some carcinomas and melanoma could also show BAP1 loss • Reliable to assess in cytology specimens, particularly cell blocks



The antibody graveyard – Mesothelioma – BAP1 – NordiQC run 65 2022

Table 1. **Antibodies and assessment marks for BAP1, run 65**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb **BSB-109**	14 3	BioSB Gennova	6	7	4	0	77%	35%
mAb **C-4**	5 2 103 12	Nordic Biosite Immunologic Santa Cruz Zeta Corporation	52	31	25	14	68%	43%
rmAb **EPR22826-65**	1	Abcam	1	0	0	0	-	-
pAb	1	Abcam	0	0	0	1	-	-
pAb	1	Biocare Medical	0	0	0	1	-	-
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb **BSB-109** **BSB 3300/3301/3302**	10	BioSB	6	2	1	1	80%	60%
mAb **C-4** **AZC-E0R3F3**	2	Nordic Biosite	2	0	0	0	-	-
mAb **C-4** **PDM595**	1	Diagnostic BioSystems	0	0	0	1	-	-
mAb **C-4** **Z2318MP**	7	Zeta Corporation	2	4	0	1	86%	29%
pAb **API 3247 AA**	1	Biocare Medical	0	0	0	1	-	-
Total	163		69	44	30	20		
Proportion			42%	27%	19%	12%	69%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).

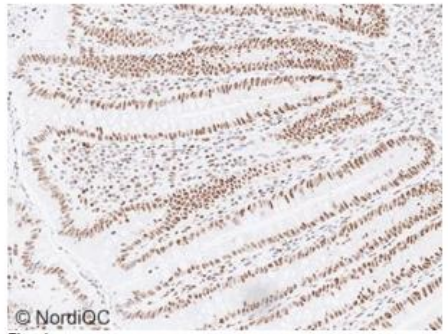


Fig. 1a
Optimal BAP1 staining of appendix using the mAb clone C-4 - diluted, 1:25 (40 min. incubation), epitope retrieval using HIER in CC1 (32 min.), a 3-step multimer based detection system (OptiView) with thyramide amplification (OptiView Amplification) and performed on BenchMark (Ventana/Roche).
Virtually all epithelial cells display a moderate nuclear staining reaction, and the vast majority of lymphocytes/stromal cells show a weak nuclear staining reaction. Same protocol used in Figs. 2a-4a.

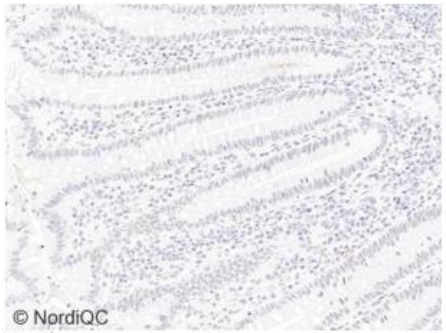


Fig. 1b
Insufficient BAP1 staining of the appendix using same clone and similar protocol settings as in Fig. 1a, but with a less sensitive detection system (UltraView).
Only scattered epithelial cells show a faint nuclear staining reaction. Virtually all lymphocytes/stromal cells are negative.
Same protocol used in Figs. 2b-4b.

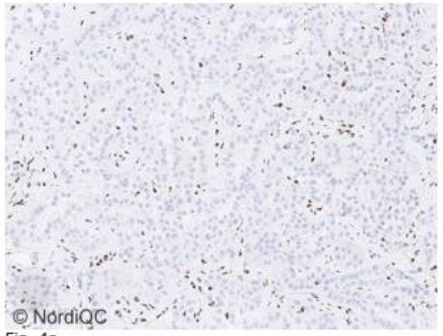


Fig. 4a
Optimal BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1a – 3a. All neoplastic cells are negative, whereas stromal cells show a distinct, weak to moderate nuclear staining reaction.

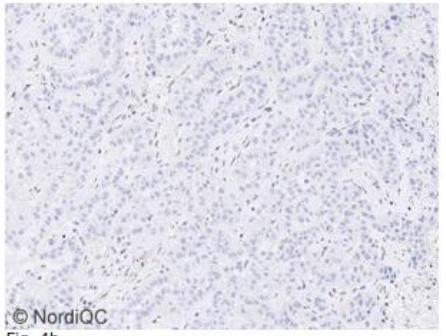
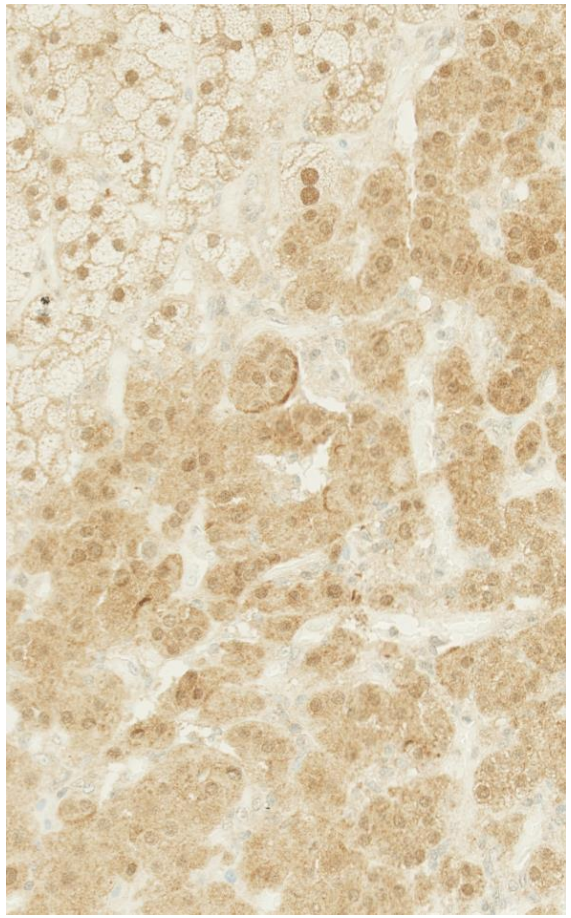


Fig. 4b
Insufficient BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1b – 3b. The neoplastic cells are negative as expected. However, also the stromal cells, expected to be positive serving as internal positive tissue control, are negative.

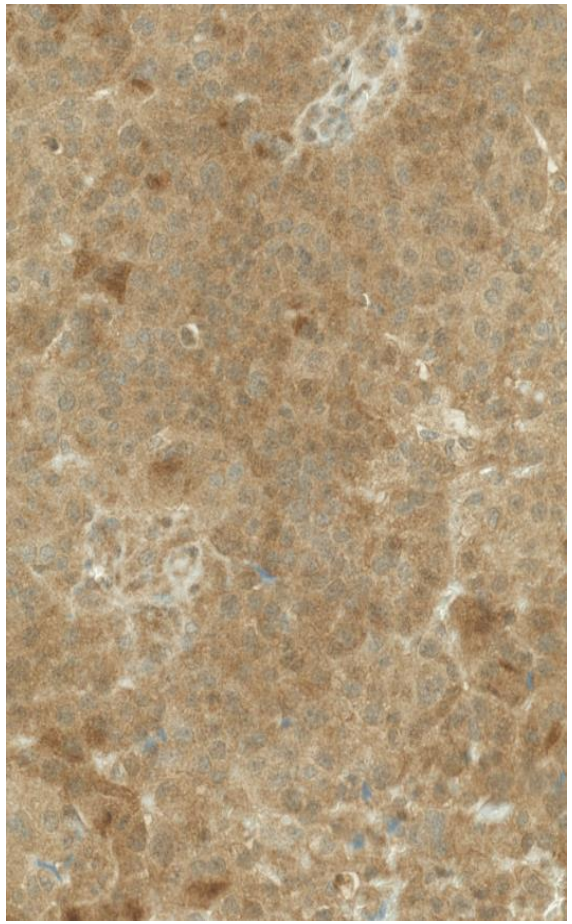
Mutation identified by negative IHC;

Internal positive tissue control essential!
”MMR similar”

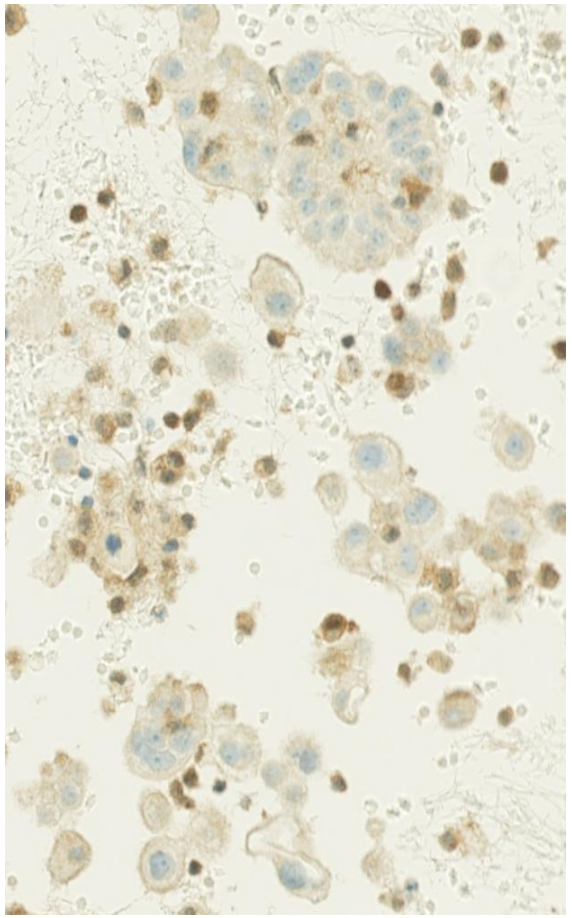
The antibody graveyard – Mesothelioma – MTAP



Adrenal gland



Tumour no mutation



Mesothelioma + mutation

Methylthioadenosine phosphorylase (MTAP), a purine metabolic enzyme, is abundant in normal tissues but deficient in many cancers including mesothelioma.
Reported as valuable to differentiate reactive mesothelium (positive) versus mesothelioma (negative in about 50%).
In panel with BAP1.

Clone EPR6893

Not beautiful, but ok☺

The antibody graveyard – Neuroendocrine markers - general

	To stay	Sensitivity	Comments
Chromogranin A	Yes	50-85%	Traditionally the most specific NE marker
Synaptophysin	Yes	60-90%	Superior sensitivity compared to CGA, but less specific
NSE	No	60-70%	”Non Specific Enolase” instead of Neuron Specific Enolase
CD56	?	70-90%	Prefered by many pathologists due to increased sensitivity, but unspecific….
INSM1	New	85-95%	Insulinoma-associated protein 1

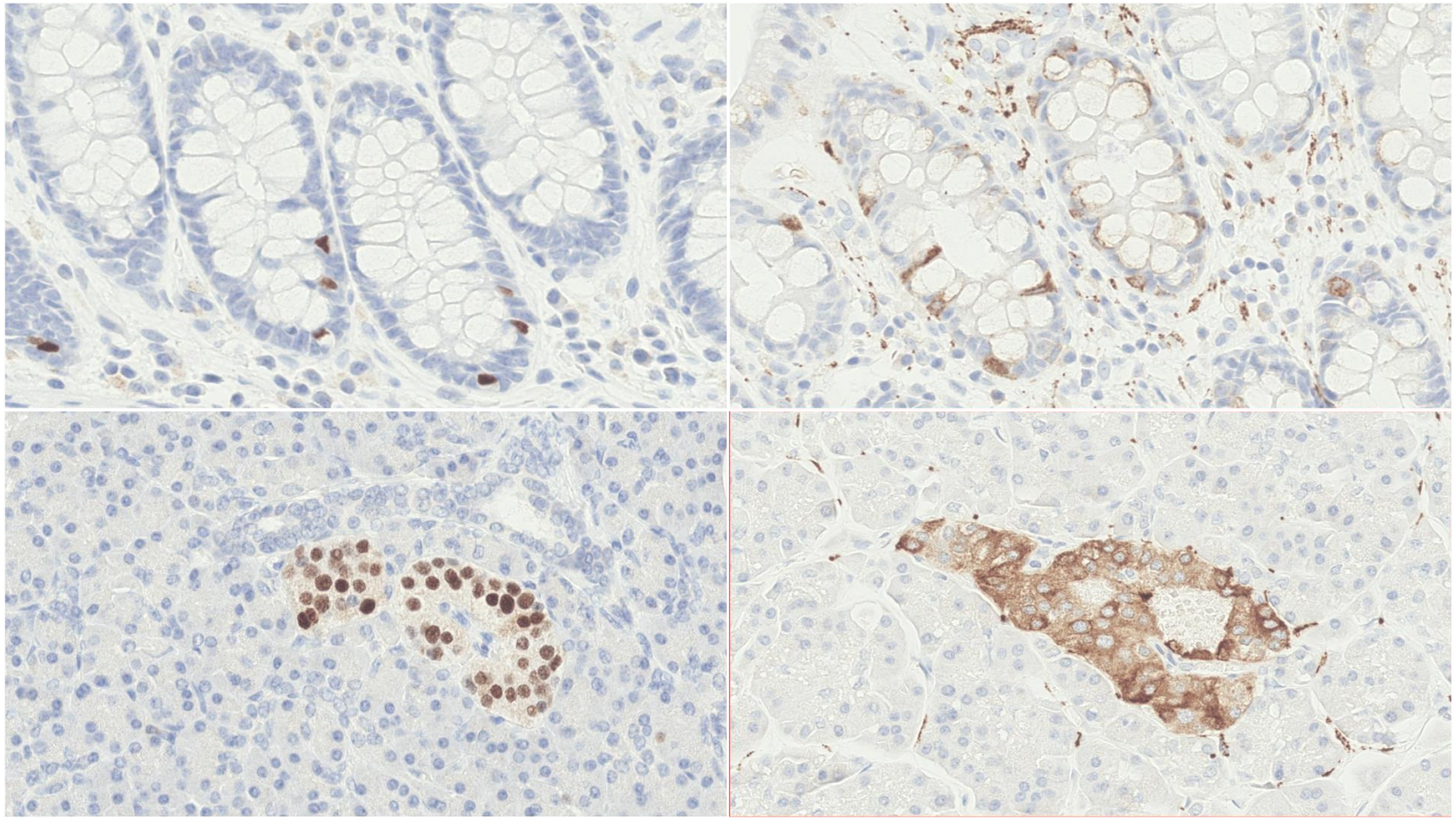
INSM1 is the best marker for the diagnosis of neuroendocrine tumors: comparison with CGA, SYP and CD56
Kosuke Fujino et al; Int J Clin Exp Pathol 2017;10(5):5393-5405

Immunohistochemistry in the diagnosis and classification of neuroendocrine neoplasms: what can brown do for you?
Andrew Bellizzi; Human Pathol 2020; Feb;96:8-33

The antibody graveyard – Neuroendocrine markers - general

Colon

Pancreas



INSM1

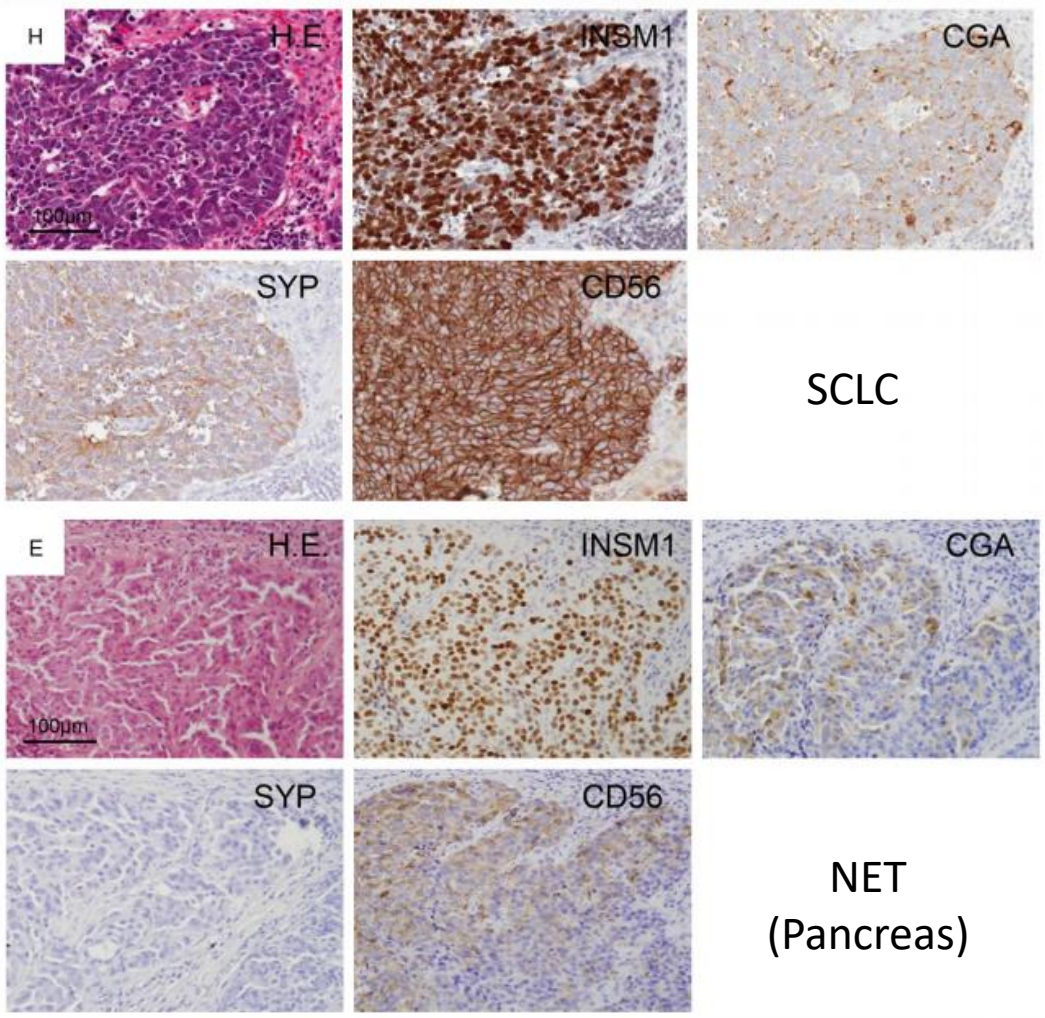
SYP

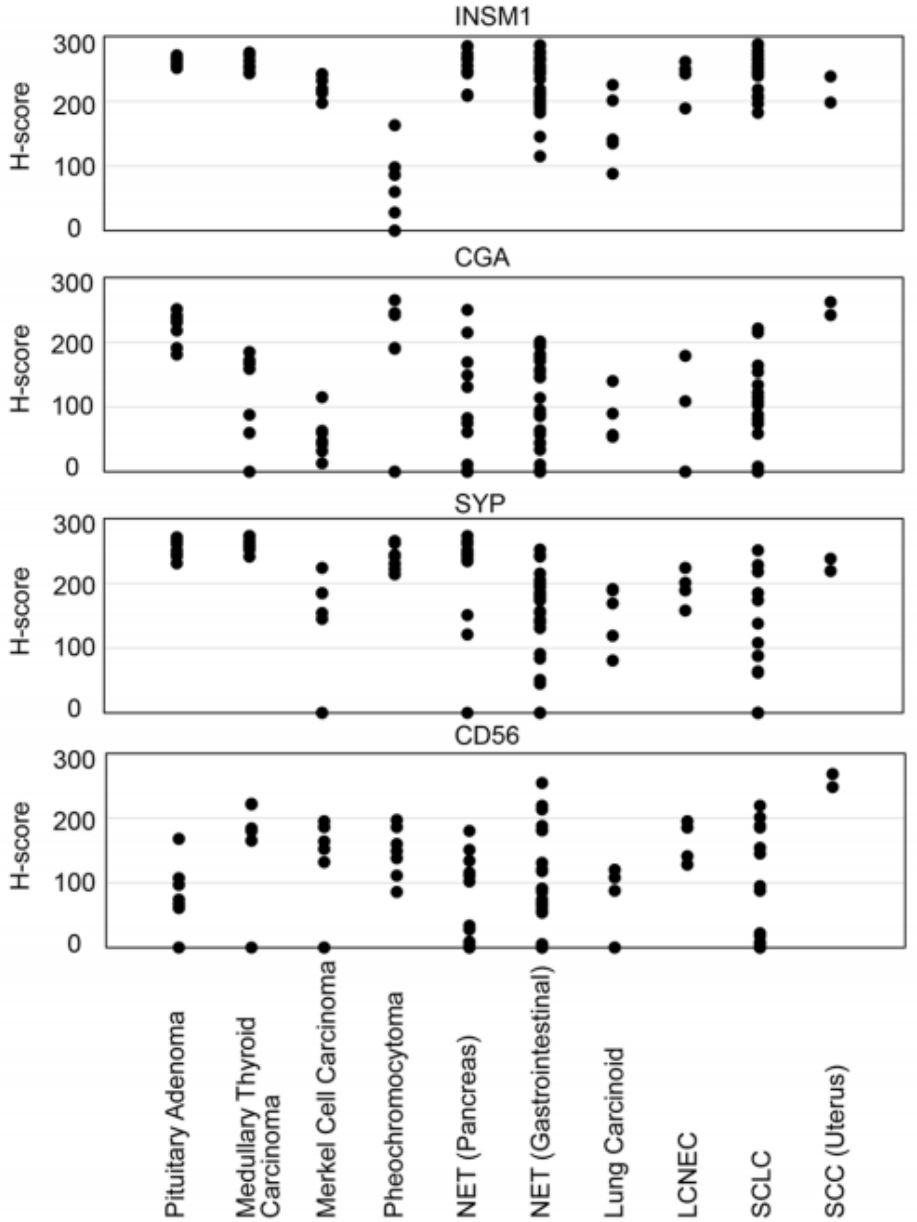
Insulinoma-associated protein 1 (INSM1) is a transcription factor that has recently emerged as a useful diagnostic marker of NE differentiation. INSM1 expression has been tightly coupled to NE differentiation in normal and neoplastic tissues across a wide range of anatomic sites including pancreas, gastrointestinal tract, lung, central and peripheral nervous system.

Clone A-8, Santa Cruz
Most cited –

MRQ-70 CM new
MSVA-465R new
BSB-123 new…..

The antibody graveyard – Neuroendocrine markers





INSM1 is the best marker for the diagnosis of neuroendocrine tumors: comparison with CGA, SYP and CD56
Kosuke Fujino et al; Int J Clin Exp Pathol 2017;10(5):5393-5405

The antibody graveyard – Neuroendocrine markers

Table 1. Staining Specifications

Antibody	Clone	Vendor	Dilution	Pretreatment	Control Tissue	Location
CD56	123C3	Agilent/Dako (Glostrup, Denmark)	1:50	CC1 + Amp	Appendix, tonsil, liver	Predominantly membranous
Chromogranin A	LK2H10	Cell Marque (Rocklin, California)	1:50	CC2	Pancreas, small intestine, tonsil	Cytoplasmic
INSM1	A-8	Santa Cruz Biotechnology (Dallas, Texas)	1:100	CC1	Pancreas, small intestine	Nuclear
Synaptophysin	MRQ-40[a]	Ventana Medical Systems (Tucson, Arizona)	RTU	CC1 + Amp	Pancreas, small intestine, tonsil	Cytoplasmic

Abbreviations: Amp, amplification; CC1, Ventana Cell Conditioning 1 (EDTA, pH 8); CC2, Ventana Cell Conditioning 2 (citrate, pH 6); INSM1, insulinoma-associated protein 1; RTU, ready-to-use.

[a] Synaptophysin clone SP11 was used for most of the extra small cell lung carinoma cases (not in tissue microarrays).

Objective.—To determine the diagnostic value of insulinoma-associated protein 1 (INSM1), in comparison with established NE markers, in pulmonary tumors.

Design.—Fifty-four pulmonary NE tumors and 632 NSCLCs were stained for INSM1, CD56, chromogranin A, and synaptophysin. In a subset, gene expression data were available for analysis. Also, 419 metastases to the lungs were stained for INSM1. A literature search identified 39 additional studies with data on NE markers in lung cancers from the last 15 years. Seven of these included data on INSM1.

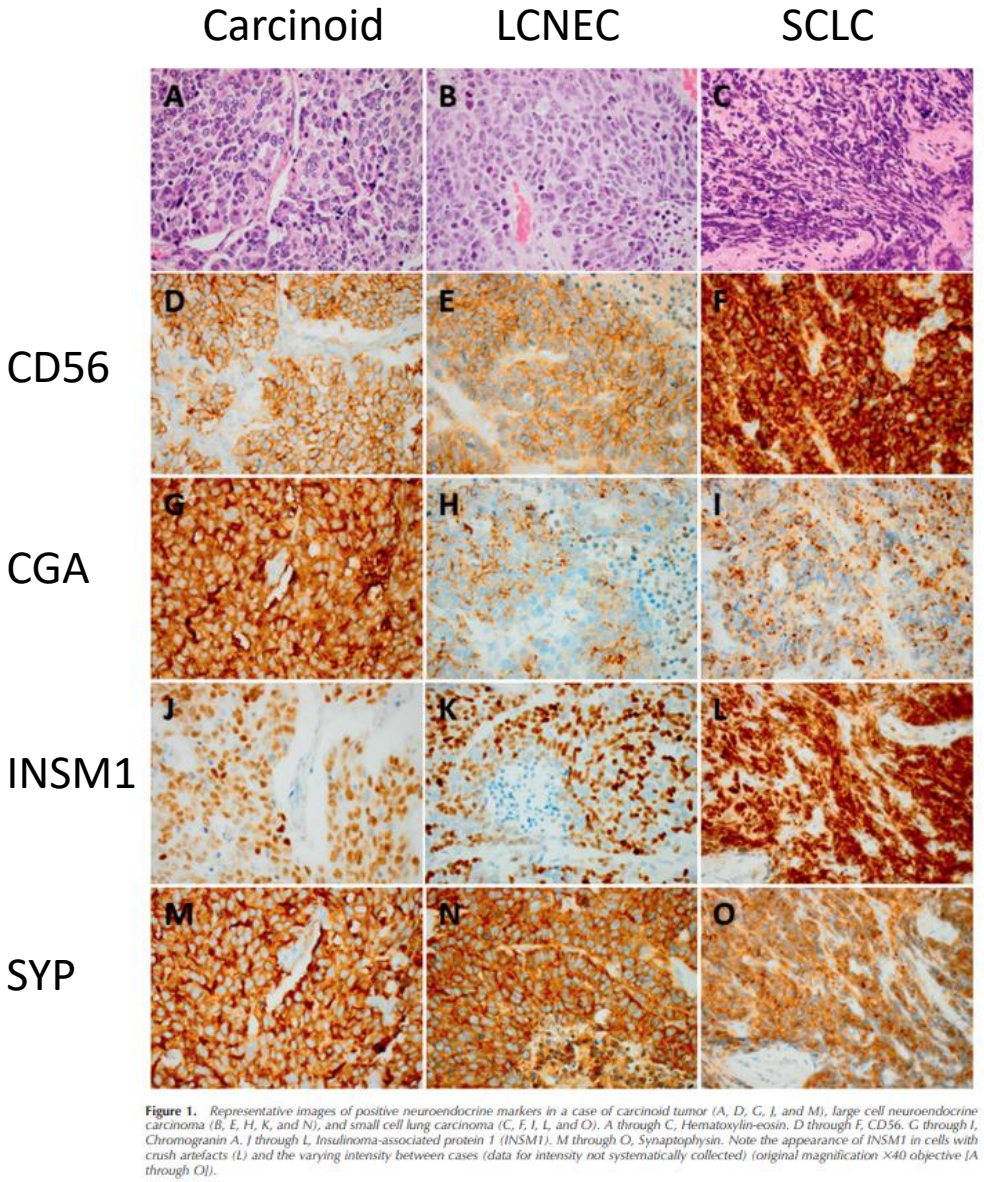


Figure 1. Representative images of positive neuroendocrine markers in a case of carcinoid tumor (A, D, G, J, and M), large cell neuroendocrine carcinoma (B, E, H, K, and N), and small cell lung carcinoma (C, F, I, L, and O). A through C, Hematoxylin-eosin. D through F, CD56. G through I, Chromogranin A. J through L, Insulinoma-associated protein 1 (INSM1). M through O, Synaptophysin. Note the appearance of INSM1 in cells with crush artefacts (L) and the varying intensity between cases (data for intensity not systematically collected) (original magnification ×40 objective [A through O]).

Diagnostic Value of Insulinoma-Associated Protein 1 (INSM1) and Comparison With Established Neuroendocrine Markers in Pulmonary Cancers: A Comprehensive Study and Review of the Literature. Johan Staff et al. Arch Pathol Lab Med (2020) 144 (9): 1075–1085

The antibody graveyard – Neuroendocrine markers

Table 4. Neuroendocrine Markers in Lung Cancer, With Positive/Total Number of Cases and (in Parentheses) Number of Studies and Range in Individual Investigations From 15 Years (Studies With INSM1 Published in 2015–2019)

Marker	CD56	Chromogranin A	INSM1	Synaptophysin
Without regard to cutoff for positive staining				
CT	516/552 = 93% (8; 83%–100%)	546/558 = 98% (8; 93%–100%)	224/256 = 88% (3; 79%–100%)	516/526 = 98% (7; 94%–100%)
LCNEC	379/440 = 86% (8; 61%–94%)	243/440 = 55% (8; 42%–85%)	85/147 = 58% (4; 42%–91%)	301/440 = 68% (8; 55%–88%)
SCLC	643/712 = 90% (15; 63%–100%)	350/633 = 55% (14; 4%–83%)	419/471 = 89% (8; 75%–100%)	497/632 = 79% (14; 52%–100%)
NSCLC (any type)	321/3936 = 8% (21; 0%–28%)	332/4296 = 8% (24; 0%–66%)	18/1202 = 1% (6; 0%–4%)	514/4494 = 11% (24; 0%–69%)
AC	73/1505 = 5% (14; 0%–22%)	45/1654 = 3% (16; 0%–41%)	12/738 = 2% (5; 0%–3%)	231/1716 = 13% (16; 0%–72%)
SqCC	142/1495 = 9% (15; 0%–20%)	59/1573 = 4% (17; 0%–26%)	5/414 = 1% (5; 0%–4%)	88/1691 = 5% (17; 0%–43%)
10% positive tumor cells as cutoff for positive staining				
CT	No data (all <20 cases)	No data (all <20 cases)	56/64 = 88% (1; 88%)	No data (all <20 cases)
LCNEC	62/70 = 89% (2; 83%–91%)	52/70 = 74% (2; 52%–85%)	21/47 = 45% (2; 29%–61%)	46/70 = 66% (2; 55%–87%)
SCLC	111/122 = 91% (4; 88%–95%)	54/102 = 53% (3; 36%–63%)	71/88 = 81% (2; 75%–83%)	66/103 = 64% (3; 57%–79%)
NSCLC (any type)	40/1058 = 4% (6; 0%–13%)	75/1231 = 6% (7; 0%–66%)	6/786 = 0.8% (0%–1%)	220/1551 = 14% (8; 1%–69%)
AC	13/503 = 3% (3; 0%–6%)	7/616 = 1% (4; 0%–3%)	5/544 = 1% (2; 0%–1%)	103/741 = 14% (5; 4%–33%)
SqCC	4/251 = 2% (3; 0%–2%)	0/298 = 0% (4; 0%)	0/228 = 0% (2; 0%)	41/461 = 9% (5; 0%–21%)
1% or any positive tumor cells as cutoff for positive staining				
CT	412/437 = 94% (5; 83%–100%)	430/437 = 98% (5; 94%–100%)	224/256 = 88% (3; 79%–100%)	441/448 = 98% (97%–100%)
LCNEC	180/210 – 86% (5; 61%–94%)	104/210 – 50% (5; 42%–57%)	91/147 – 62% (4; 42%–91%) ★	145/210 – 69% (5; 61%–100%)
SCLC	351/378 = 93% (8; 70%–100%)	235/371 = 63% (7; 34%–83%)	396/444 = 89% (7; 81%–98%)	305/371 = 82% (7; 52%–100%)
NSCLC (any type)	184/1973 = 9% (8; 4%–28%) ★	102/2162 = 5% (10; 0%–33%)	22/1069 = 2% (5; 0%–4%) ★	211/2082 = 10% (10; 3%–56%) ★
AC	50/821 = 6% (5; 3%–15%)	35/861 = 4% (6; 0%–41%)	18/652 = 3% (4; 2%–3%)	142/785 = 18% (6; 7%–72%)
SqCC	130/1052 = 12% (6; 5%–20%)	53/1081 = 5% (7; 0%–26%)	6/367 = 2% (4; 0%–4%)	60/1059 = 6% (7; 1%–43%)

Abbreviations: AC, adenocarcinoma; CT, carcinoid tumor; INSM1, insulinoma-associated protein 1; LCNEC, large cell neuroendocrine carcinoma; NSCLC, non–small cell lung carcinoma; SCLC, small cell lung carcinoma, SqCC, squamous cell carcinoma.

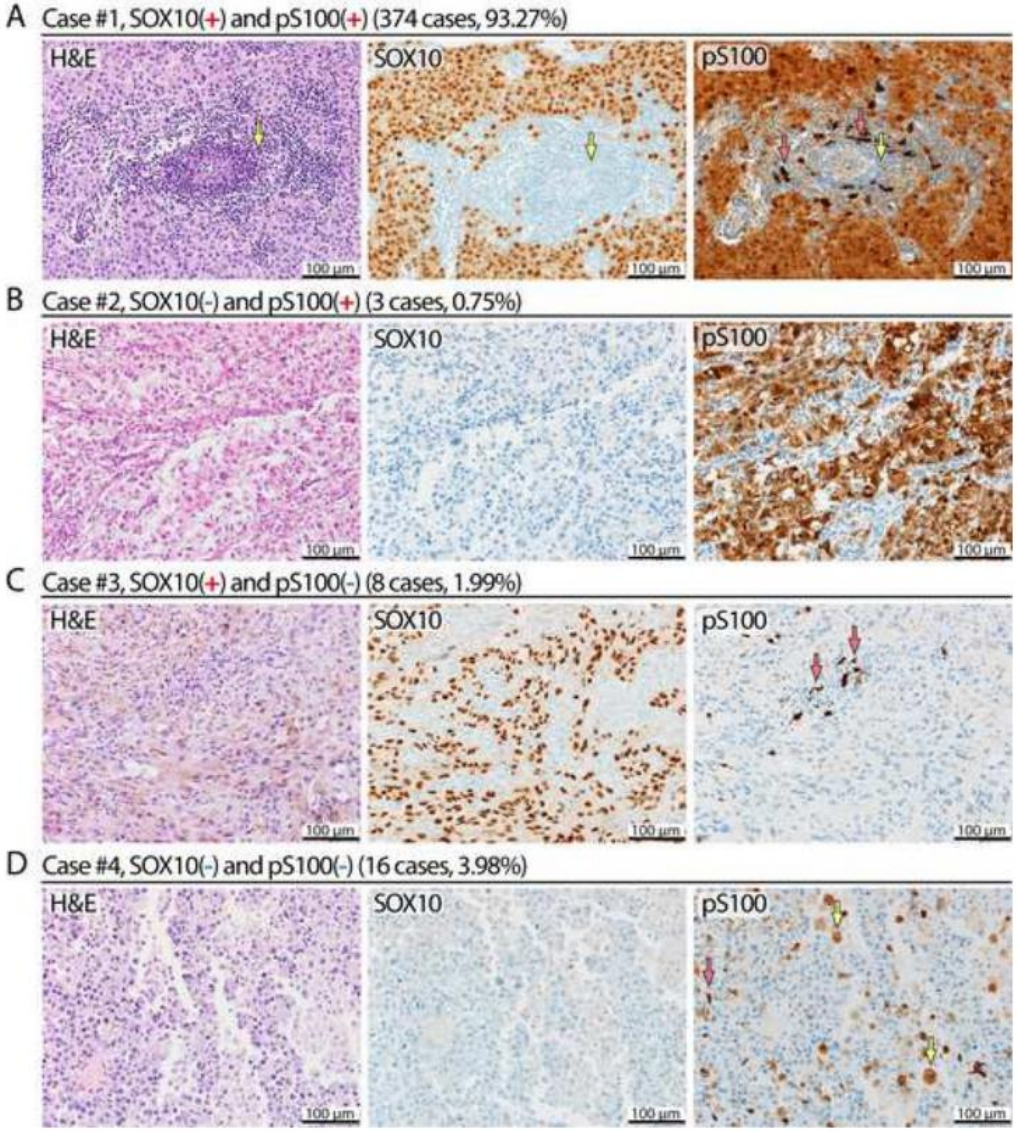
Note: Only studies with at least 20 cases of a specific histologic type are included, and only studies reporting 10% or any/1% positive tumor cells as cutoff are included in the mid and lower parts of the table, respectively.

Diagnostic Value of Insulinoma-Associated Protein 1 (INSM1) and Comparison With Established Neuroendocrine Markers in Pulmonary Cancers:
A Comprehensive Study and Review of the Literature. Johan Staff et al. Arch Pathol Lab Med (2020) 144 (9): 1075–1085

The antibody graveyard – Melanoma markers

	To stay	Sensitivity	Comments
Mel. A	Yes	80-95%	Highly sensitive and specific (CUP, sentinel node and melanoma extension)
HMB45	Yes	75-90%	Moderate to high sensitivity and primarily used to differentiate nevi from melanoma (HMB45 typically expressed in deeper parts in melanoma, while neg in nevi)
SOX10	YES	90-100%	Highly sensitive (incl desmoplastic) and specific (CUP, sentinel node and melanoma extension)
S100	?	90-100%	Highly sensitive, moderate specificity causing challenges eg sentinel node
MITF	No	70-90%	Moderate to high sensitivity, reduced specificity
Tyrosinase	No	75-90%	Moderate to high sensitivity – out-performed by SOX10
PRAME	New	90-95%	Highly sensitive for melanoma and beneficial to differentiate nevi from melanoma

The antibody graveyard – Melanoma markers – SOX10 versus S100; the battle…



S100 and SOX10 similar sensitivity for melanoma in sentinel node
94-96% positive

S100 can give read-out challenges in node-negative pts

Sentinel lymph node (SLN) biopsy remains crucial for melanoma staging. The European Organisation for Research and Treatment of Cancer Melanoma Group recommends IHC for reproducible identification of melanoma metastases. S100 standard for years, but give read-out challenges…

EJC
EUROPEAN JOURNAL OF CANCER

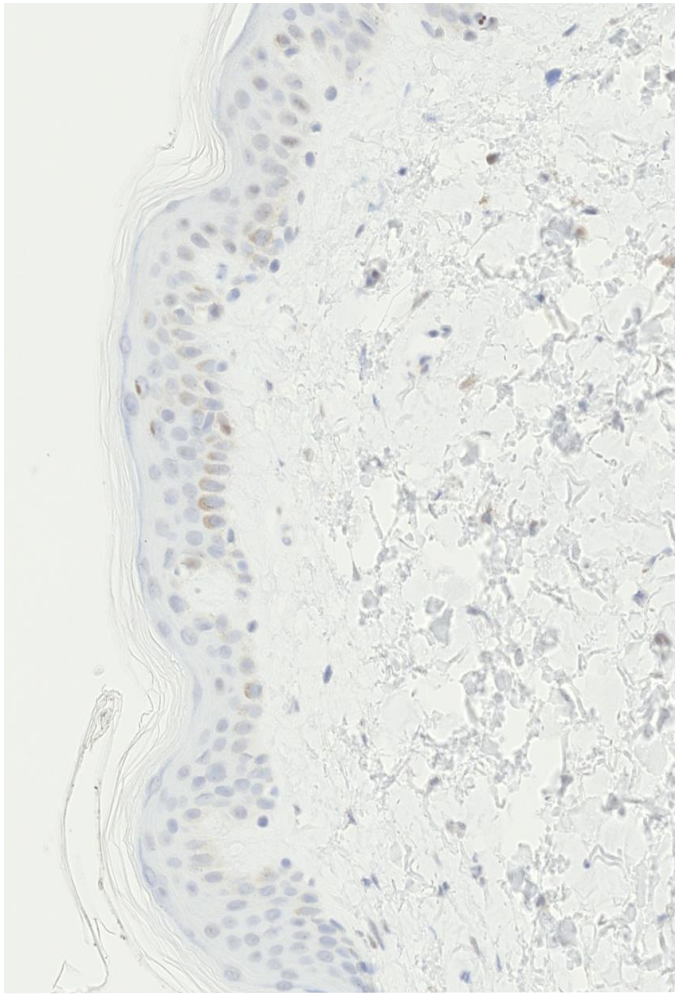
ORIGINAL RESEARCH | VOLUME 137, P175-182, SEPTEMBER 01, 2020

SOX10 is as specific as S100 protein in detecting metastases of melanoma in lymph nodes and is recommended for sentinel lymph node assessment

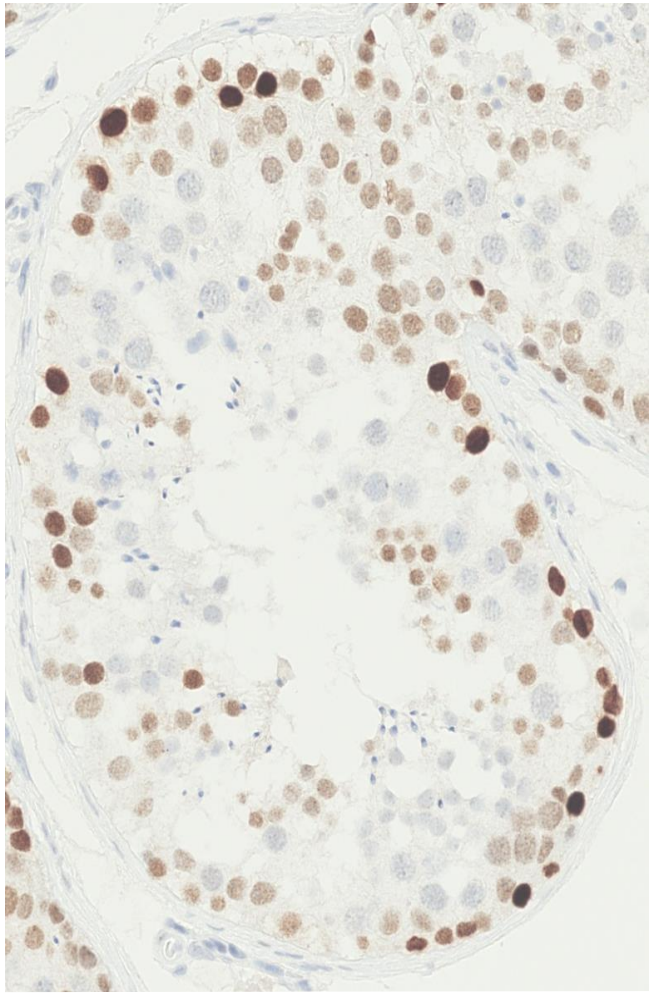
Anna Szumera-Ciećkiewicz • Francesca Bosisio • Paweł Teterycz • … Martin Cook • Daniela Massi • EORTC Melanoma Group • Show all authors

Published: August 08, 2020 • DOI: https://doi.org/10.1016/j.ejca.2020.06.037 • Check for updates

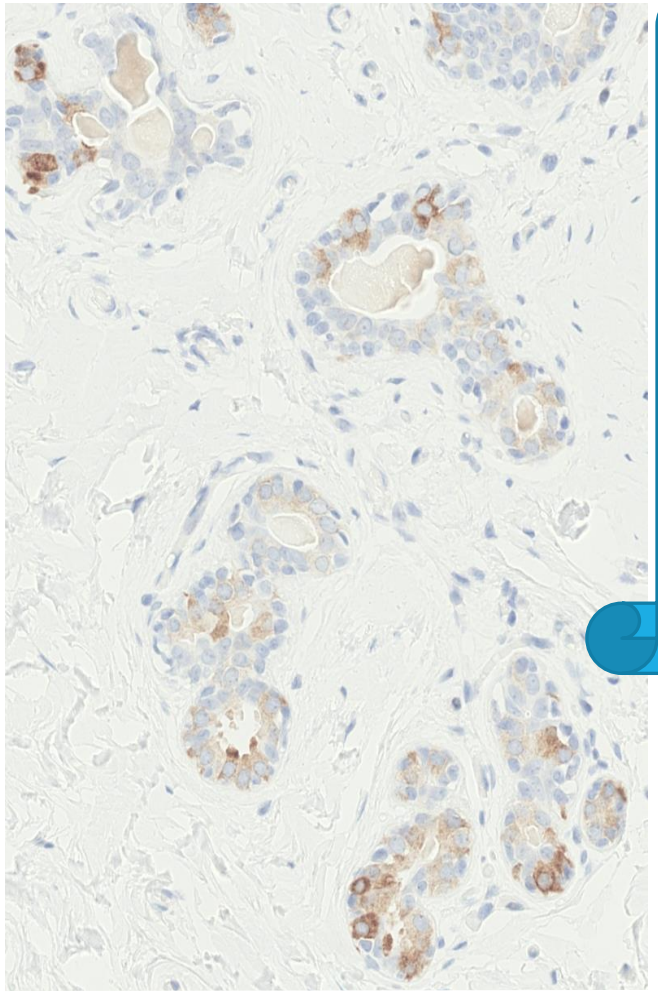
The antibody graveyard – Melanoma markers – PRAME



Normal skin



Testis



Breast

PRAME (preferential expressed antigen in melanoma) is a member of the cancer testis antigen family that has normal expression in the testis, ovaries, adrenals, endometrium, and placenta. These proteins encode antigens that are subsequently recognized by T lymphocytes.

Clone EPR20330, Abcam mostly cited

New EP461, Cell Marque

The antibody graveyard – Melanoma markers – PRAME

TABLE 1. Primary Cutaneous Melanomas With Diffuse (4+) PRAME IHC Expression

Melanoma Type	In Situ Only	Invasive	Total
Superficial spreading	12/12	37/41	49/53
Lentigo maligna	24/27	15/17	39/44
Acral	7/7	10/11	17/18
Nodular	NA	9/10	9/10
Other*	2/2	6/8	8/10
Subtotal†	45/48	77/87	122/135
Desmoplastic‡	NA	7/20	7/20
Total	45/48	84/107	129/155

*This category includes (proportion of cases with 4+ PRAME): lentiginous vulvar in situ melanomas (2/2), nevoid melanoma (2/2), malignant melanoma ex-blue nevus (0/1), cutaneous paramucosal (3/3), and unclassified invasive melanomas (1/2).

†Subtotal = all melanomas except for desmoplastic melanomas.

‡This category comprises (proportion of cases with 4+ PRAME): spindle cell melanomas with variable desmoplasia, including pure (0/4) and mixed (6/14) desmoplastic melanomas, and spindle cell neurotropic (1/2) melanomas.

NA indicates not available.

1;1-25%, 2;26-50%, 3;51-75%, 4;76-100%

PRAME Expression in Melanocytic Tumors
Cecilia Lezcano et al. Am J Surg Pathol 2018;42:1456–1465

TABLE 3. PRAME IHC Expression in Melanocytic Nevi

Type of Melanocytic Nevus	Diffuse (4+) IHC PRAME Expression	Focal (1 or 2+) IHC PRAME Expression
Common acquired nevus	0/40	4/40 (1+)
Dysplastic (Clark's) nevus	0/60	10/60 (1+)
		1/60 (2+)
Blue nevus	0/10	0/10
Spitz nevus	1/10	1/10 (1+)
Deep penetrating nevus	0/3	0/3
Traumatized/ recurrent nevus	0/15	1/15 (2+)
		1/15 (1+)
Congenital nevus	0/2	0/2
Nodal nevus	0/5	0/5
Total	1/145	18/145

"Diffuse nuclear immunoreactivity for PRAME was found in 87% of metastatic and 83.2% of primary melanomas.

Of the 140 cutaneous melanocytic nevi, 86.4% were completely negative for PRAME. Immunoreactivity for PRAME was seen, albeit usually only in a minor subpopulation of lesional melanocytes, in 13.6% of cutaneous nevi, including dysplastic nevi, common acquired nevi, traumatized/recurrent nevi, and Spitz nevi."

The antibody graveyard – Melanoma markers – PRAME

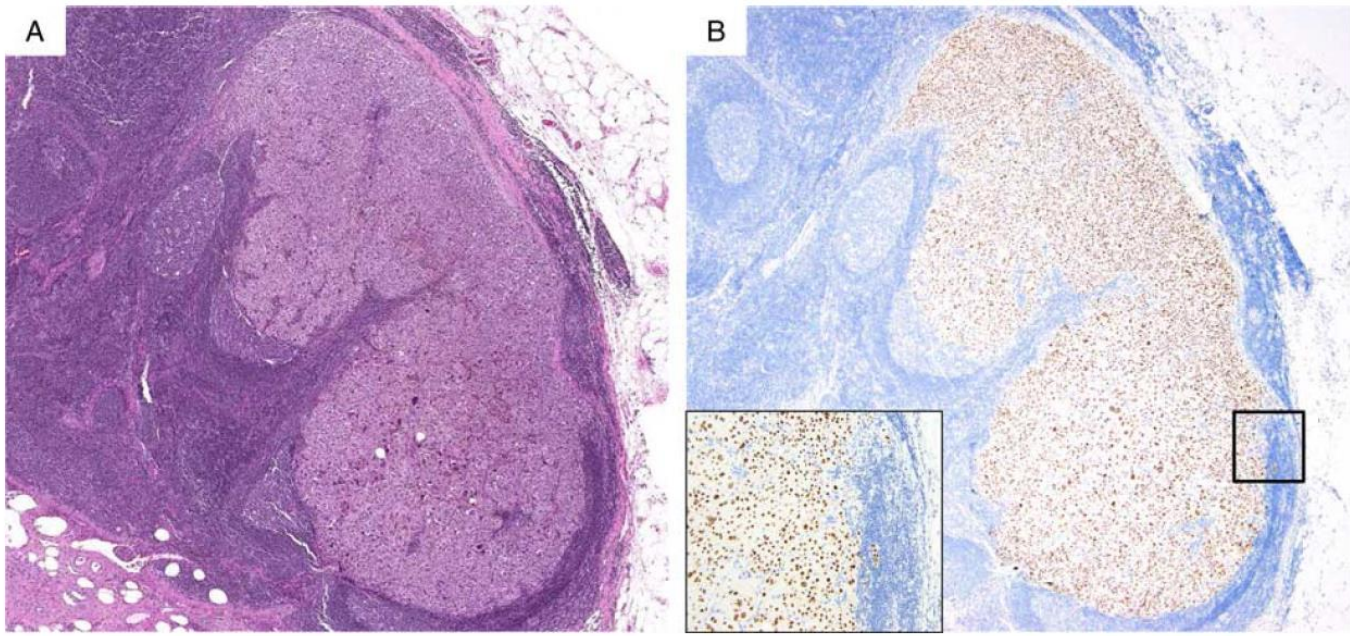


FIGURE 1. A, Metastatic melanoma in lymph node (H&E-stain). B, The tumor cells are diffusely immunopositive for PR (nuclear labeling). Inset highlights PRAME labeling is nuclear.

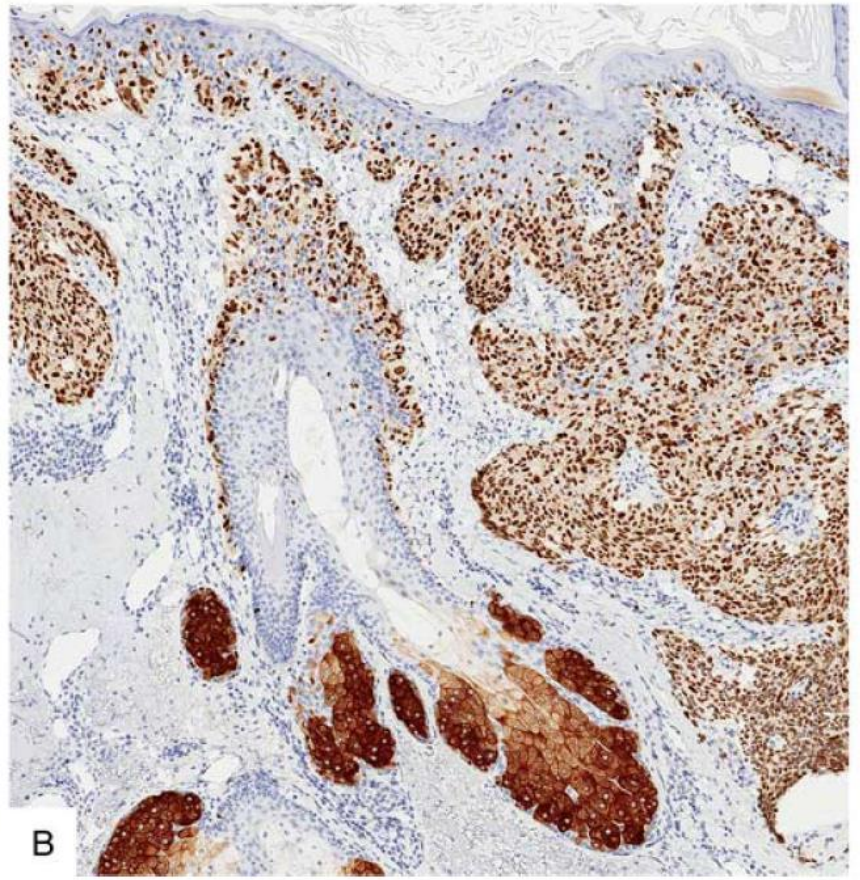
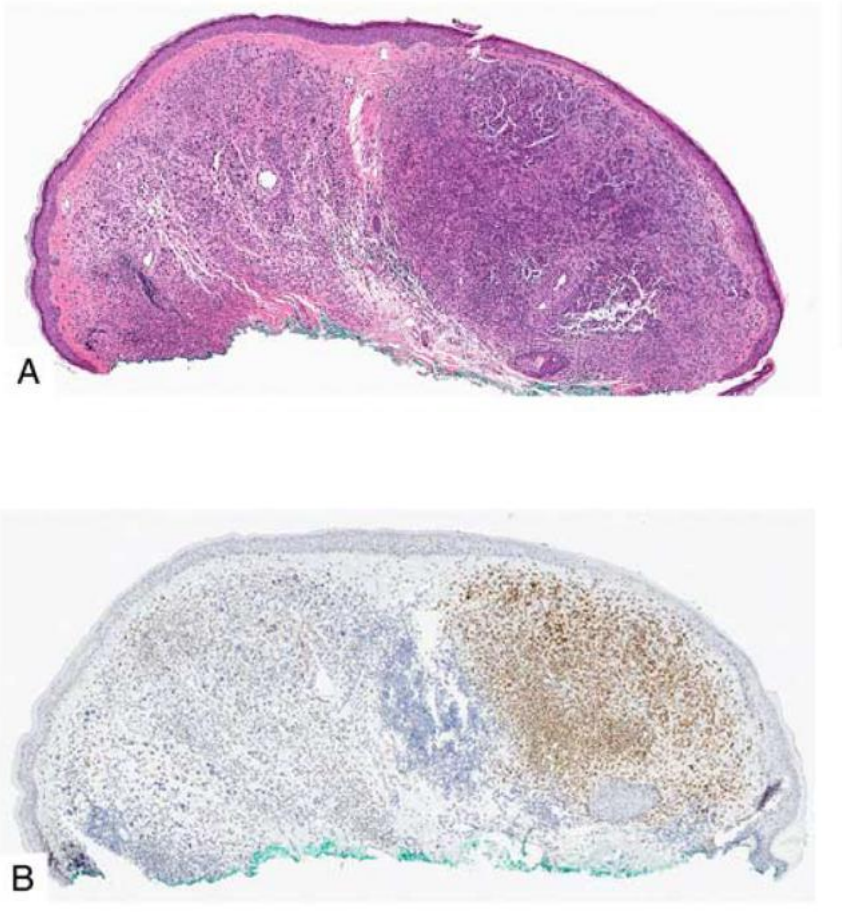


FIGURE 3. Primary melanoma from the scalp of a 75-year-old man. A, Both in situ and invasive melanoma are equally strongly immunoreactive for PRAME. There is prominent follicular involvement by melanoma. B, The melanocytes show nuclear labeling for PRAME. The sebaceous glands show cytoplasmic labeling.

PRAME Expression in Melanocytic Tumors
Cecilia Lezcano et al. Am J Surg Pathol 2018;42:1456–1465

The antibody graveyard – Melanoma markers – PRAME



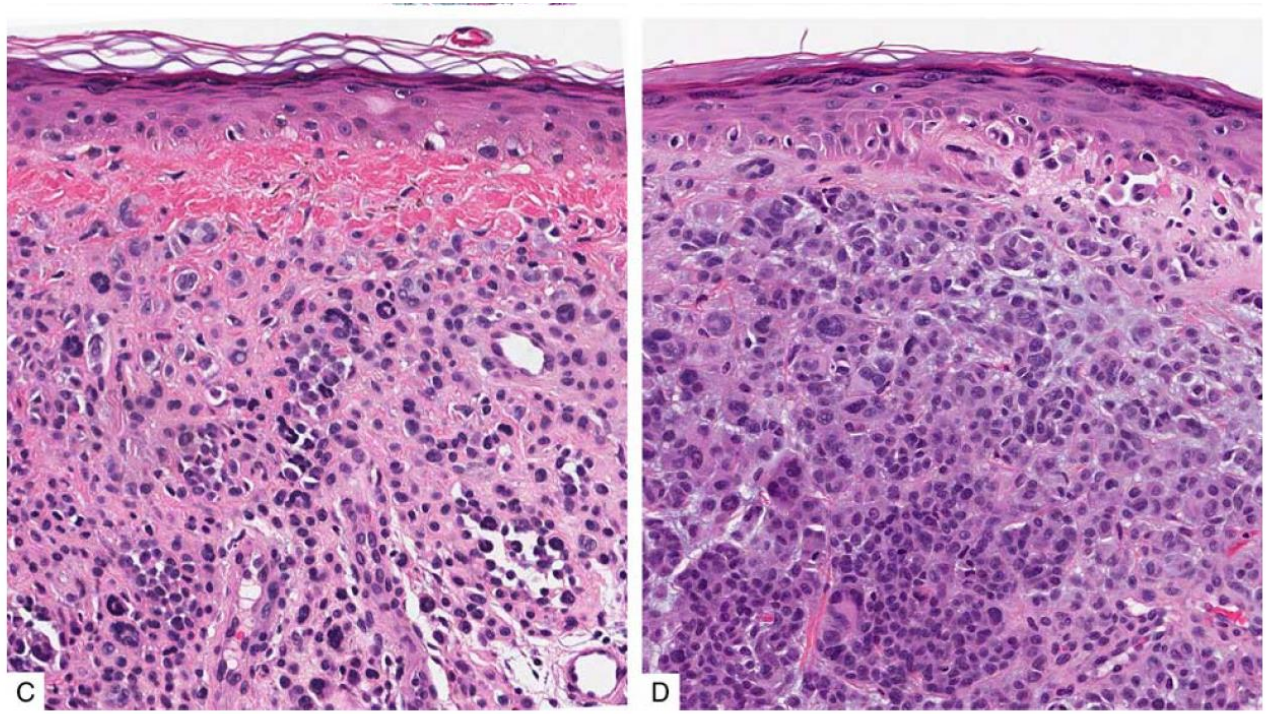


FIGURE 4. Melanoma associated with a melanocytic nevus in the ear of a 63-year-old man. A, Nodular silhouette of the lesion with a more densely cellular tumor cell population on the right side of the lesion. B, IHC for PRAME stains only the densely cellular nodule. C, The less cellular area shows cytologic features of a melanocytic nevus. D, The PRAME-positive tumor cells are cytologically atypical. Cytogenetic analysis of the tumor cells revealed a number of chromosomal aberrations, including loss of 9p and gain of 8q (not shown).

PRAME Expression in Melanocytic Tumors
Cecilia Lezcano et al. Am J Surg Pathol 2018;42:1456–1465

The antibody graveyard – Melanoma markers – PRAME

TABLE 1. Summary of Characteristics of 110 Ambiguous Melanocytic Tumors

	Sex (%)	Age, Range (Mean; Median)	PRAME IHC	FISH	SNP-array	Dx	PRIHC and FISH/ SNP-array Agreement (%)	PRIHC and Dx Agreement (%)
Spitzoid neoplasm (n = 42)	F: 23 (54.8) M: 19 (45.2)	2-78 (27.3; 19)	4+: 6 0-3+: 36	Pos: 4 Neg: 12	Pos: 4 Neg: 17 Ab: 8	MM: 7 Ind: 35	31/34 (91.2)	39/42 (92.9)
DysN vs. MM (n = 26)	F: 9 (34.6) M: 17 (65.4)	19-81 (50.6; 47.5)	4+: 5 0-3+: 21	Pos: 5 Neg: 20	Pos: 0 Neg: 0 Ab: 1	MM: 6 Ind: 20	23/25 (92)	25/26 (96.2)
Nevoid (n = 33)	F: 19 (57.6) M: 14 (42.4)	13-90 (50.5; 51)	4+: 9 0-3+: 24	Pos: 11 Neg: 18	Pos: 4 Neg: 4 Ab: 0	MM: 13 Ind: 20	28/33 (84.8)	29/33 (87.9)
Combined nevus vs. MM (n = 3)	F: 3 (100)	5-31 (21.3; 28)	4+: 0 0-3+: 3	Pos: 0 Neg: 2	Pos: 0 Neg: 0 Ab: 1	MM: 0 Ind: 3	2/2	3/3
DPN vs. MM (n = 2)	F: 1 M: 1	35, 73	4+: 1 0-3+: 1	Pos: 1 Neg: 1	Pos: 0 Neg: 0 Ab: 0	MM: 1 Ind: 1	2/2	2/2
PEM vs. MM (n = 2)	F: 1 M: 1	25, 81	4+: 1 0-3+: 1	Pos: 0 Neg: 1	Pos: 1 Neg: 0 Ab: 0	MM: 1 Ind: 1	2/2	2/2
Acral nevus vs. MM (n = 1)	F: 1	46	4+: 0 0-3+: 1	Pos: 0 Neg: 1	Pos: 0 Neg: 0 Ab: 0	MM: 0 Ind: 1	1/1	1/1
Blue nevus vs. MM (n = 1)	M: 1	67	4+: 0 0-3+: 1	Pos: 0 Neg: 1	Pos: 0 Neg: 0 Ab: 0	MM: 0 Ind: 1	1/1	1/1
Total (n = 110)	F: 57 (51.8) M: 53 (48.2)	2-90 (41.1; 41.5)	4+: 22 0-3+: 88	Pos: 21 Neg: 56	Pos: 9 Neg: 21 Ab: 10*	MM: 28 Ind: 82	90/100 (90)*	102/110 (92.7)

*Ten cases with abnormal SNP-array results of uncertain significance are excluded from agreement calculations between PRAME IHC and cytogenetic test results.
Ab indicates abnormal SNP-array result of uncertain significance; DPN, deep penetrating nevus; Dx, diagnosis; DysN, dysplastic nevus; F, female; Ind, indolent (including nevi and low risk AST); M, male; MM, malignant melanoma; Neg, negative; PEM, pigmented epithelioid melanocytoma; Pos, positive; PRIHC, immunohistochemistry for PRAME.

TABLE 2. Correlation of PRAME IHC With FISH and/or SNP-array Results

	FISH/SNP-array Positive	FISH/SNP-array Negative	Total Cases	
PRAME IHC			100	Intertest agreement 90%
4+	18	2		
0-3+	8	72		

Comparison of Immunohistochemistry for PRAME With Cytogenetic Test Results in the Evaluation of Challenging Melanocytic Tumors. Cecilia Lezcano et al. Am J Surg Pathol 2020;44:893–900

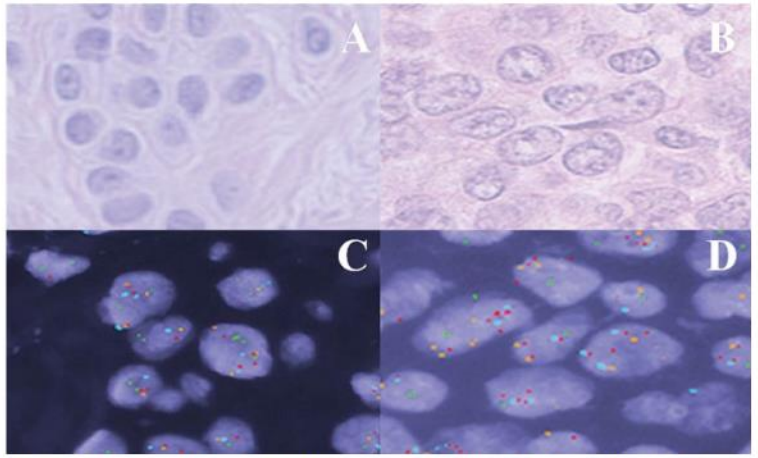


Figure 2: (**A**) H&E stained tissue sample of a benign nevus showing a nest of melanocytes; (**B**) H&E stained tissue section of metastatic melanoma cells within a lymph node. Melanocytes show enlarged nuclei with irregular contours and coarse chromatin; (**C**) Benign nevus tissue probed with melanoma FISH probe set; (**D**) Malignant melanoma cells probed with melanoma FISH probe set.

Table 1: Summary of FISH findings for 21 benign nevi. None of the benign nevi were FISH positive.

Calculation	Mean	Standard Deviation	Range
Mean RREB1 per cell	1.83	0.06	1.73-1.92
% Abnormal RREB1	20.3	6.9	8.5-35.0
Mean MYB per cell	1.68	0.08	1.55-1.82
% cells MYB < CEP6	10.9	4.40	3.15-18.0
Mean CCND1 per cell	1.73	0.10	1.63-1.88
Mean CEP6 per cell	1.57	0.09	1.38-1.72

Table 3: Frequency of each of the four FISH positive criteria in our 20 metastatic melanomas.

Criteria for FISH Positivity	Number of Melanoma Cases Meeting Criteria/Total N (%)
Abnormal RREB1 % > 63	14/20 (70%)
Mean MYB signal # >2.5	1/20 (5%)
Mean CCND1 signal # > 2.5	5/20 (25%)
MYB loss (MYB < CEP6) % > 31	9/20 (45%)

Fluorescence in Situ Hybridization (FISH) Copy Number Abnormalities at 6p (RREB1), 6q (MYB), and 11q (CCND1) Reliably Distinguish Metastatic Versus Benign Melanocytic Lesions Hindi et al. J Dermatol Res Ther 2016, 2:017

The antibody graveyard – Sarcoma markers; Rhabdomyosarcoma and Ewing

	To stay	Sensitivity	Comments
Myogenin	Yes	60-80%	Highly sensitive and specific for alveolar and embryonal rhabdomyosarcoma
MYOD1	No	40-70%	Moderate sensitivity for rhabdomyosarcoma & enhanced cytoplasmic staining
PAX7	New	60-90%; Rhabdo. 90-95%; Ewing	Highly sensitive and ”specific” for the two different entities
NKX2.2	New	90-95%	Highly sensitive and moderate to high specificity for Ewing
CD99	Yes	100%	Sensitive for Ewing – but unspecific….

The antibody graveyard – Sarcoma markers; Rhabdomyosarcoma and PAX7

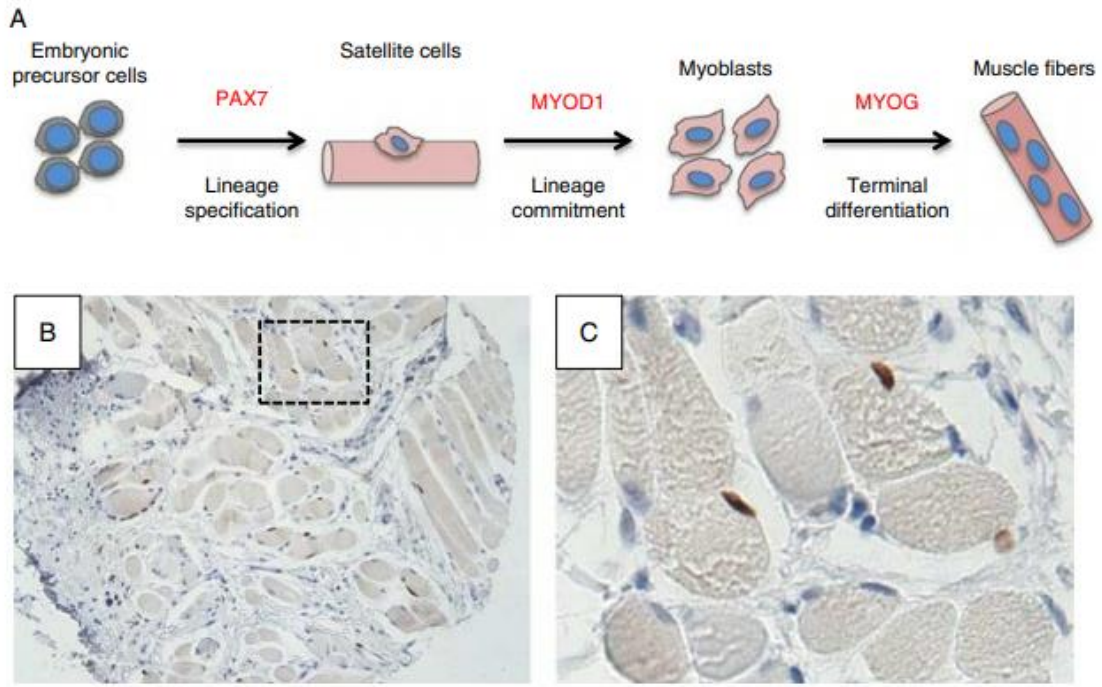


FIGURE 1. PAX7 expression in skeletal muscle satellite cells. A, Schematic showing transcriptional regulation of mammalian myogenesis by PAX7, MYOD1, and MYOG. B, Representative image showing PAX7 expression localized to satellite cells in adult skeletal muscle (tongue) by immunohistochemistry. C, Magnified image of area highlighted by black dashed line in (B), showing PAX7 expression localized to satellite cells in adult skeletal muscle.

The PAX-7 transcription factor has important functions in myogenesis and early neural development, with a crucial role in specification and self-renewal of skeletal muscle tissue. The expression of PAX-7 is highly restricted in normal adult tissues in scattered satellite cells of the skeletal muscle and absent in both visceral smooth muscle and cardiac muscle as well as in most other non-neoplastic tissues.

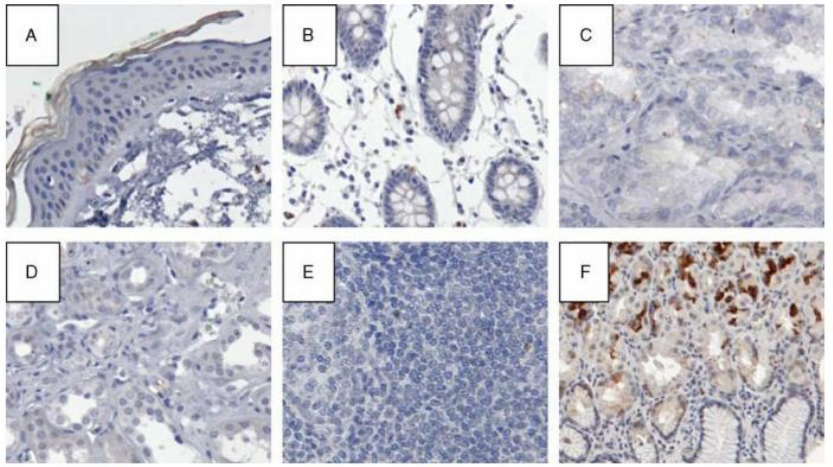


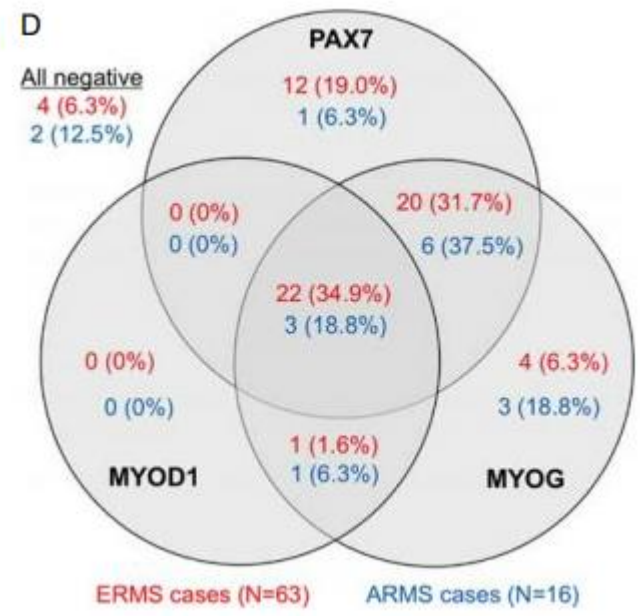
FIGURE 2. Limited PAX7 expression in non-neoplastic tissues. Representative immunohistochemical detection of PAX7 expression in skin (A), colon (B), seminal vesicle (C), kidney (D), tonsil (E), and stomach (F).

PAX7 Expression in Rhabdomyosarcoma, Related Soft Tissue Tumors, and Small Round Blue Cell Neoplasms
Charville Gregory W. et al. The American Journal of Surgical Pathology: October 2016 - Volume 40 - Issue 10 - p 1305-1315

The antibody graveyard – Sarcoma markers; Rhabdomyosarcoma and PAX7

	MyoD1	Myogenin	PAX7
ERMS	36,5%	75%	86%
ARMS	25%	81%	55%

ERMS; Embryonal rhabdomyosarcoma
ARMS; Alveolar rhabdomyosarcoma



Myogenin and PAX7 in panel;
Few cases PAX7 neg and Myogenin pos
Myogenin often only focal

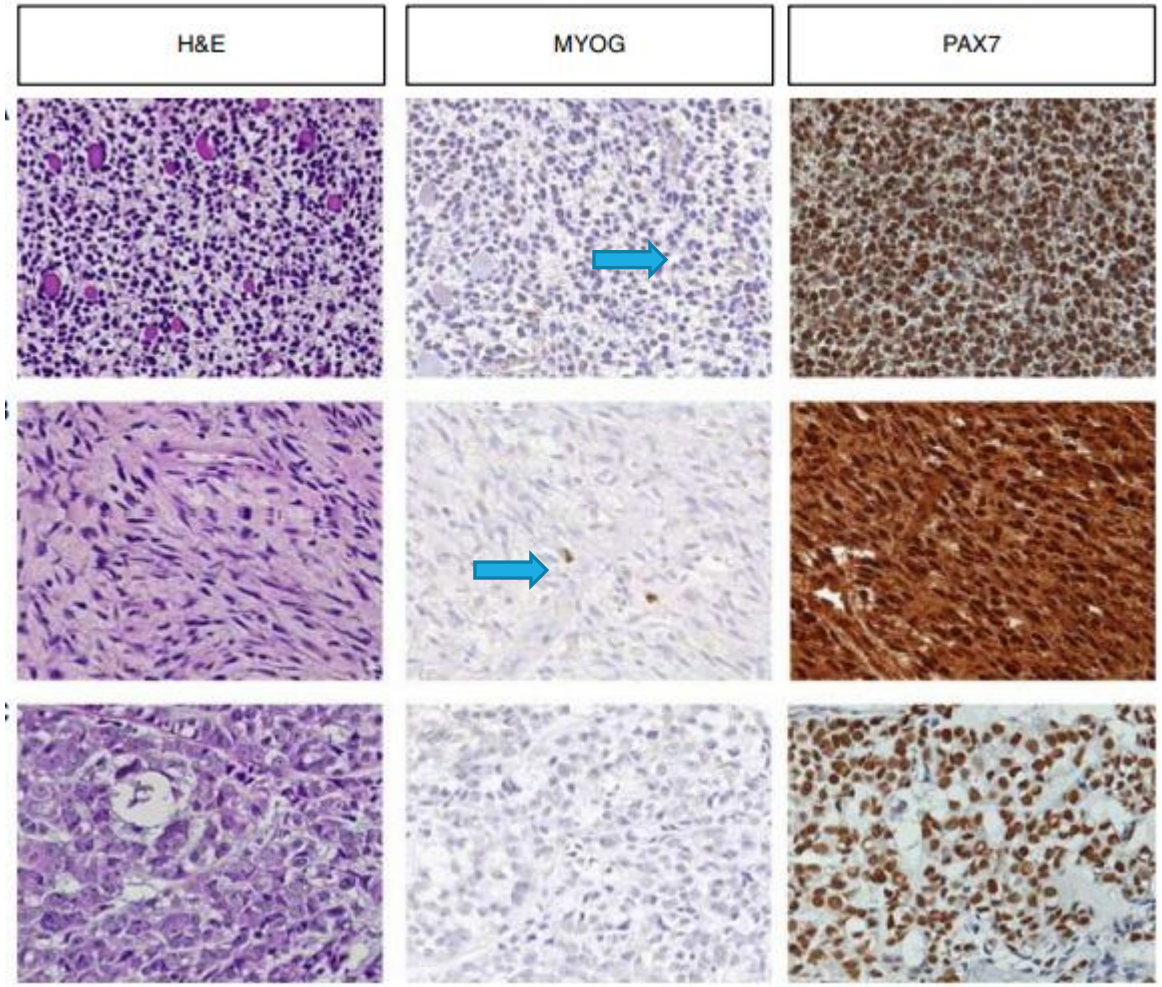
TABLE 1. Summary of PAX7 Expression in Rhabdomyosarcomas, Small Round Blue Cell Neoplasms, and Other Soft Tissue Tumors

Tumor Type	Total Cases	PAX7 Expressing (n [%])
ERMS	63	54 (86)
ARMS*	31	17 (55)
Spindle cell rhabdomyosarcoma	8	6 (75)
Pleomorphic rhabdomyosarcoma	7	5 (71)
Leiomyosarcoma	62	0 (0)
Ewing sarcoma	7	7 (100)
Gastrointestinal stromal tumor	51	0 (0)
Neuroblastoma	4	0 (0)
Atypical lipomatous tumor	10	0 (0)
Glomus tumor	11	0 (0)
Angiosarcoma	10	0 (0)
Osteosarcoma	13	0 (0)
Myxoid liposarcoma	10	0 (0)
Hemangioendothelioma	8	0 (0)
Leiomyoma	20	0 (0)
Dedifferentiated liposarcoma	10	0 (0)
Desmoplastic small round cell tumor	6	0 (0)
Extraskeletal myxoid chondrosarcoma	11	0 (0)
Solitary fibrous tumor	7	0 (0)
Dermatofibrosarcoma protuberans	10	0 (0)
Desmoid-type fibromatosis	19	0 (0)
Synovial sarcoma†	22	2 (9)
Ovarian fibroma	9	0 (0)
Nodular fasciitis	9	0 (0)
Granular cell tumor	20	0 (0)
Schwannoma	22	0 (0)
Sarcoma with *CIC-DUX4* translocation	1	0 (0)
Mesenchymal chondrosarcoma	5	0 (0)
Leukemia/lymphoma‡	311	0 (0)
Tenosynovial giant cell tumor	29	0 (0)

*Including cases from both ARMS cohorts used in this study.
†Cases of synovial sarcoma used in this study have *not* been molecularly defined by the presence of t(X;18).
‡Including 89 diffuse large B-cell lymphomas, 35 grade 1 follicular lymphomas, 44 grade 2 follicular lymphomas, 54 grade 3 follicular lymphomas, 19 marginal zone lymphomas, 11 mantle cell lymphomas, 26 chronic lymphocytic leukemias, 12 lymphoblastic lymphomas (7 T cell and 5 B cell), 8 peripheral T-cell lymphomas, 3 angioimmunoblastic lymphomas, 5 anaplastic large cell lymphomas, and 5 lymphoplasmacytic lymphomas.

PAX7 Expression in Rhabdomyosarcoma, Related Soft Tissue Tumors, and Small Round Blue Cell Neoplasms
Charville Gregory W. et al. The American Journal of Surgical Pathology: October 2016 - Volume 40 - Issue 10 - p 1305-1315

The antibody graveyard – Sarcoma markers; Rhabdomyosarcoma and PAX7



Myogenin and PAX7 in panel;
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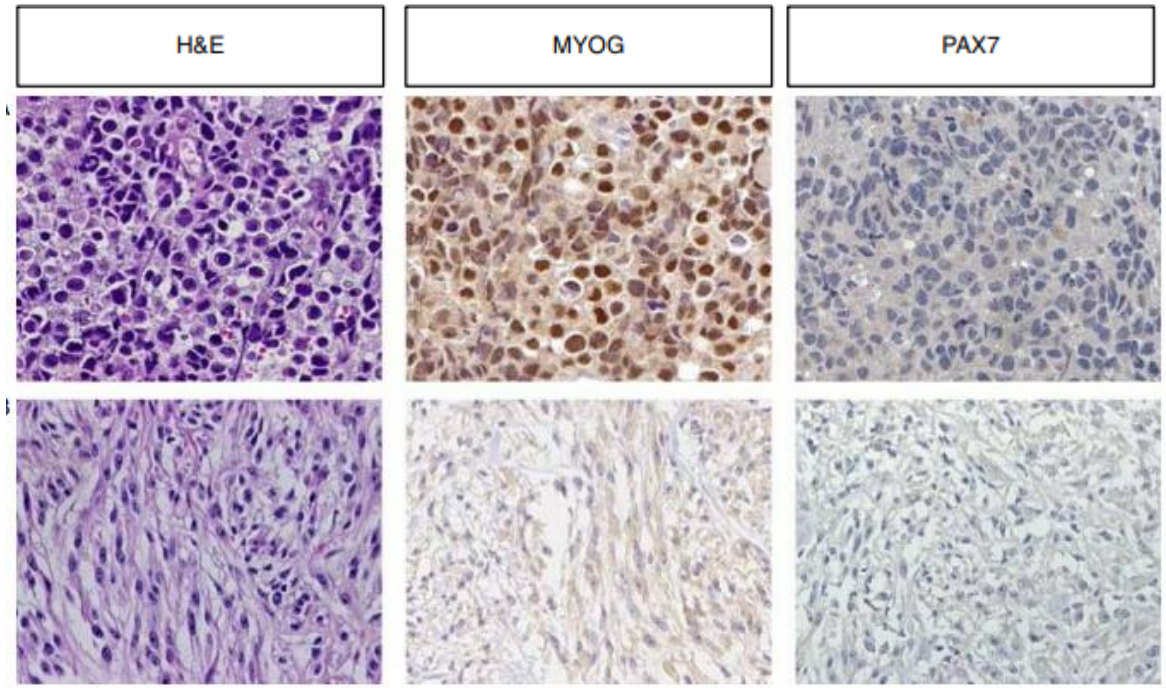


PAX7 Expression in Rhabdomyosarcoma, Related Soft Tissue Tumors, and Small Round Blue Cell Neoplasms
Charville Gregory W. et al. The American Journal of Surgical Pathology: October 2016 - Volume 40 - Issue 10 - p 1305-1315

The antibody graveyard – Sarcoma markers; Rhabdomyosarcoma and PAX7

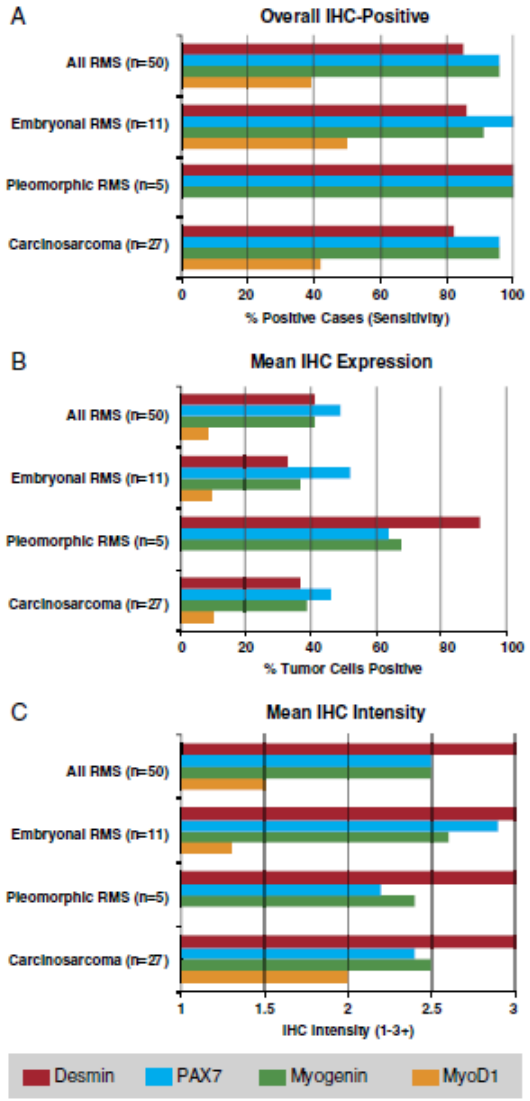


FIG. 1. (A–C) Summary of myogenic marker immunohistochemical (IHC) performance by rhabdomyosarcoma (RMS) subgroup.

TABLE 2. *Immunohistochemical expression of skeletal muscle markers and desmin in rhabdomyosarcoma mimics in the female genital tract*

Tumor type	Total cases	PAX7	Myogenin	MyoD1	Desmin
Carcinosarcoma	9	0/9	0/9*	—	0/3
Leiomyosarcoma	9	0/9	0/9	0/1	9/9
High-grade adenocarcinoma with rhabdoid features	5	0/5	0/5	—	—
Squamous cell carcinoma with rhabdoid features	4	0/4	0/4	0/1	0/2
High-grade sarcoma with rhabdoid features	3	0/3	0/3	—	0/2
SMARCA4-deficient uterine sarcoma	3	0/3	0/3	—	—
Dedifferentiated carcinoma	2	0/2	0/2	—	—
Epithelioid sarcoma	2	0/2	0/2	0/2	0/2
Smooth muscle tumor with rhabdoid features	2	0/2	0/2	—	2/2
Malignant PEComa	1	0/1	0/1	—	0/1
Neuroendocrine carcinoma, small cell-type	1	0/1	0/1	—	0/1
Small cell carcinoma, hypercalcemic-type	1	0/1	0/1	—	0/1
Sex cord-stromal tumor	1	0/1	0/1	—	—
Sarcomatoid mesothelioma	1	0/1	0/1	—	0/1
NTRK-rearranged sarcoma	1	0/1	0/1	—	0/1
Sarcomatoid carcinoma arising in mucinous cystic tumor	1	0/1	0/1	—	0/1
Fibroepithelial polyp	1	0/1	0/1	—	—
Endometrial polyp with rhabdoid stromal cells	1	0/1	0/1	—	0/1
LG-ESS with rhabdoid cells	1	0/1	0/1	0/1	0/1
Vulvar mesenchymal tumor	1	0/1	0/1	—	1/1†

*Scattered (1+), <1% of tumor cells in 1/9 cases.
†Focal (2+), 5% of tumor cells.
LG-ESS indicates low-grade endometrial stromal sarcomas.

"PAX7 should be used in combination with other markers of skeletal muscle differentiation, namely myogenin, and may be particularly helpful in cases where myogenin and/or MyoD1 expression is limited".

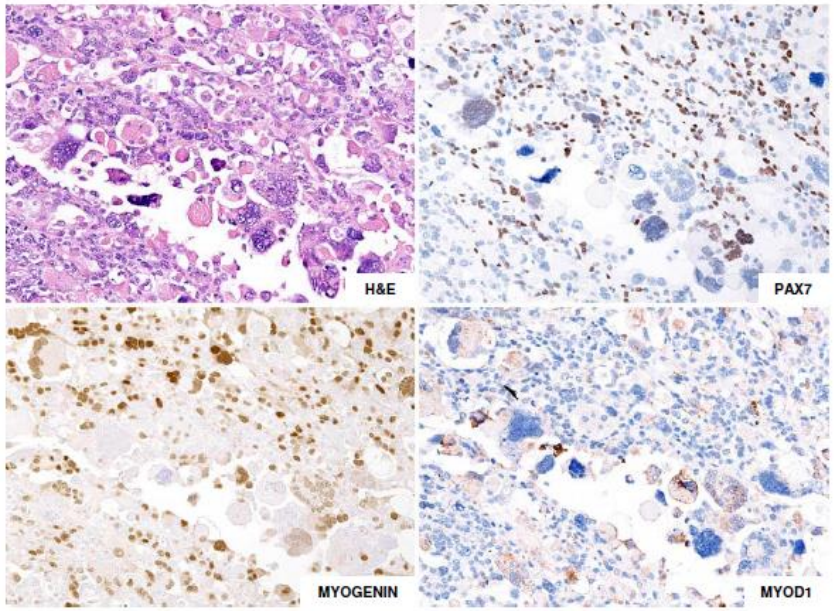


FIG. 3. Uterine "pure" pleomorphic rhabdomyosarcoma. PAX7 IHC highlights predominantly primitive-appearing small, round to spindled rhabdomyoblast nuclei whereas myogenin is localized to the nuclei of primitive cells as well as enlarged, pleomorphic rhabdomyoblastic cells. MyoD1 is expressed in a minor subset of rhabdomyoblast nuclei and shows variable cytoplasmic immunoreactivity (200×).

PAX7 Is a Sensitive Marker of Skeletal Muscle Differentiation in Rhabdomyosarcoma and Tumors With Rhabdomyosarcomatous Differentiation in the Female Genital Tract. Weiel, J. , Kokh, D. , Charville, G. & Longacre, T. International Journal of Gynecological Pathology, 2022; 41 (3), 235-243.

The antibody graveyard – Sarcoma markers; Ewing sarcoma and PAX7

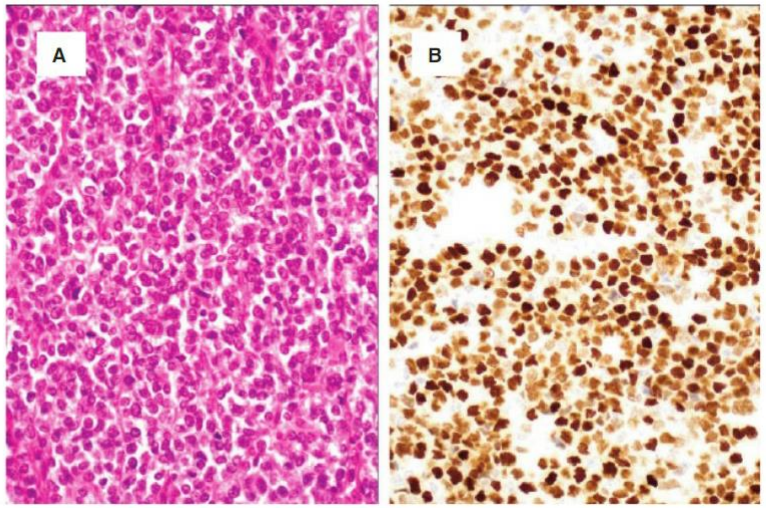


Figure 1. Most of the Ewing sarcomas (90%) were positive for PAX7. Almost all positive cases showed strong diffuse expression (**A**, haematoxylin and eosin; **B**, PAX7 staining).

Table 1. PAX7 comparative immunohistochemistry in small round cell tumours

Tumour type	Positivity	Extent	Intensity
Ewing sarcoma	27/30 (90%)	F1, D26	W0, M1, S26
Non-Ewing small round cell tumour	24/141	F12, D12	W1, M10, S13
Neuroblastoma	0/10 (0%)	–	–
Olfactory neuroblastoma	0/5 (0%)	–	–
Alveolar rhabdomyosarcoma	7/10 (70%)	F6, D1	W0, M4, S3
Small-cell carcinoma	0/10 (0%)	–	–
Lymphoma	0/10 (0%)	–	–
Mesenchymal chondrosarcoma	0/10 (0%)	–	–
Small-cell osteosarcoma	1/5 (20%)	F1, D0	W1, M0, S0
Poorly differentiated synovial sarcoma	7/10 (70%)	F2, D5	W0, M3, S4
Desmoplastic small round cell tumour	1/10 (10%)	F1, D0	W0, M1, S0
Round cell liposarcoma	0/10 (0%)	–	–
Merkel cell carcinoma	0/8 (0%)	–	–
Medulloblastoma	0/3 (0%)	–	–
Retinoblastoma	0/5 (0%)	–	–
Cellular extraskeletal myxoid chondrosarcoma	0/5 (0%)	–	–
Melanoma, small-cell type	0/7 (0%)	–	–
BCOR–CCNB3 sarcoma	8/10 (80%)	F2, D6	W0, M2, S6
CIC-rearrangement sarcoma	0/10 (0%)	–	–
Miscellaneous*	0/3 (0%)	–	–
EWSR1–NFATC2 sarcoma	1/1 (100%)	F0, D1	W0, M0, S1

Reactivity was defined as positive if at least 5% of tumour cells were stained. Staining characteristics are indicated as follows: F, focal (5–50%); D, diffuse (> 50%); W, weak; M, moderate; S, strong.
*This category includes malignant gastrointestinal neuroectodermal tumour, malignant peripheral nerve sheath tumour (small-cell type) and sclerosing epithelioid fibrosarcoma.

PAX7 immunohistochemical evaluation of Ewing sarcoma and other small round cell tumours
Shunichi Toki et al. Histopathology 2018, 73, 645–652

The antibody graveyard – Sarcoma markers; Ewing sarcoma and NKX2.2

”In summary, NKX2-2 is a sensitive but imperfectly specific marker for Ewing sarcoma. Nonetheless, NKX2-2 may be helpful to distinguish Ewing sarcoma from some histologic mimics including CIC-DUX4 and BCOR-CCNB3 sarcomas. Most other EWSR1-associated soft tissue tumors are negative for NKX2-2”.

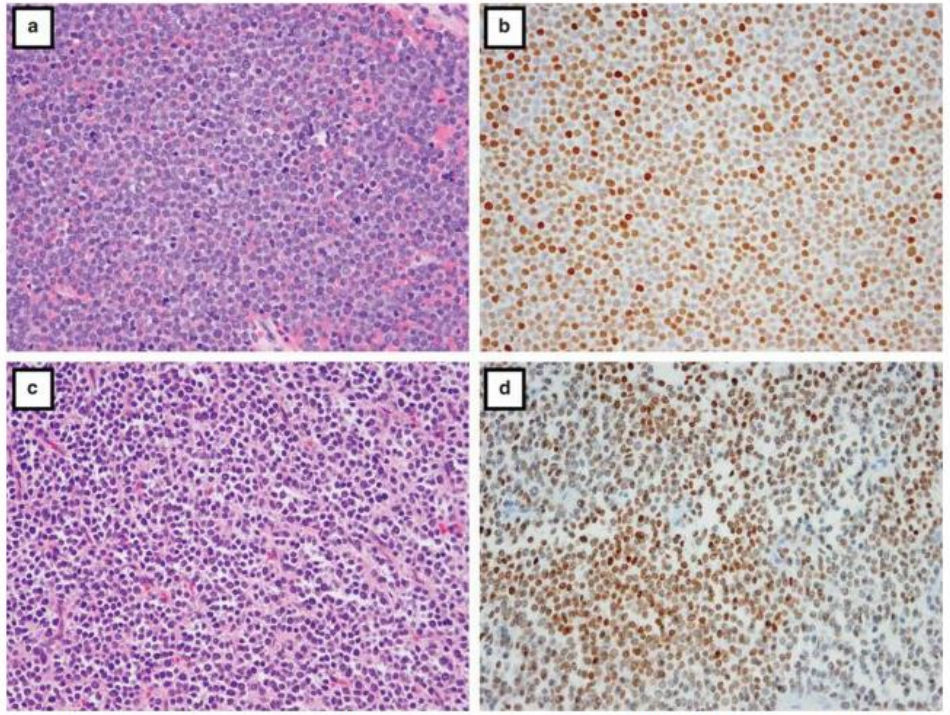


Figure 1 Ewing sarcoma with classic histomorphology composed of uniform small round cells in a solid architecture (**a**) showing diffuse nuclear immunoreactivity for NKX2-2 (**b**). Ewing sarcoma of the uterus with *EWSR1-FLI1* rearrangement (**c**) and diffuse nuclear staining for NKX2-2 (**d**).

Ewing
CD99; 100% - but unspec.
NKX2; 95% - more spec.
Fli-1; 90% - less spec.
PAX7; 95% - role?

Table 1 Summary of immunohistochemical staining for NKX2-2

Tumor type	*Total cases*	*NKX2-2 positive (%)*
Ewing sarcoma	40	37 (93)
Non-Ewing small round blue cell tumors		
CIC-DUX4 sarcoma	20	1 (5)
BCOR-CCNB3 sarcoma	5	0 (0)
Unclassified round cell sarcoma	9	2 (22)
Synovial sarcoma, poorly differentiated	10	1 (10)
Lymphoblastic lymphoma	10	0 (0)
Alveolar rhabdomyosarcoma	10	0 (0)
Embryonal rhabdomyosarcoma	10	0 (0)
NUT midline carcinoma	5	0 (0)
Wilms tumor	10	0 (0)
Merkel cell carcinoma	10	0 (0)
Melanoma	20	0 (0)
Small cell carcinoma	10	3 (30)
Neuroblastoma	10	1 (10)
Olfactory neuroblastoma	10	8 (80)
Mesenchymal chondrosarcoma	12	9 (75)
Other EWSR1-associated tumors		
Angiomatoid fibrous histiocytoma	10	0 (0)
Clear cell sarcoma	10	0 (0)
Gastrointestinal clear cell sarcoma-like tumor	5	0 (0)
Extraskeletal myxoid chondrosarcoma	10	0 (0)
Desmoplastic small round cell tumor	5	1 (20)
Soft tissue and cutaneous myoepitheliomas	10	1 (10)
Myoepithelial carcinoma	19	1 (5)

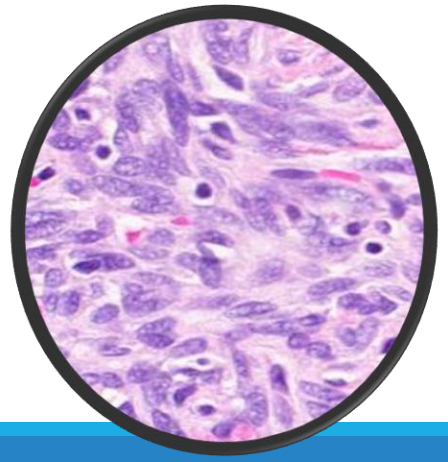
Evaluation of NKX2-2 expression in round cell sarcomas and other tumors with EWSR1 rearrangement: imperfect specificity for Ewing sarcoma. Yin P Hung et al. Modern Pathology (2016) 29, 370–380

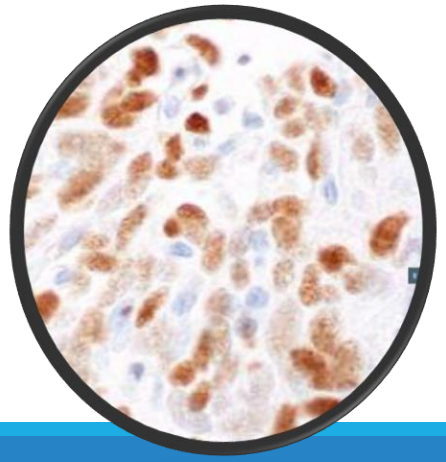
The antibody graveyard – Sarcoma markers; Classical and Next Generation IHC Markers

Classical IHC markers	Diagnosis
ASMA, Desmin	Leiomyosarcoma
Myogenin, Desmin	Rhabdomyosarcoma
CD31, ERG, FLI-1	Angiosarcoma
CD117, DOG-1	Gastrointestinal stromal tumor
CD99, NKX2.2, *FISH*	Ewing sarcoma

Next Generation IHC Markers represent molecular genetic alterations giving a ”protein footprint!

Next Generation IHC Markers		
ALK	H3K27me3	RB1
Beta-Catenin	MDM2	ROS1
BCOR	MUC4	SDHB
CAMTA1	MYC	SMARCA4
CCNB3	NKX2.2	SMARCB1
CDK4	PAX3	STAT6
ETV4	PAX7	TLE1
FOSB	PDGFRA	TRK





Inspired by the lecture by Dr Jason Hornick, USCAP 2019
The Evolution of Immunohistochemistry for soft tissue tumors – From differentiation to molecular genetics

Limited biopsies of soft tissue tumors: the contemporary role of immunohistochemistry and molecular diagnostics
Jason Hornick, Modern Pathology (2019) 32:S27–S37

The antibody graveyard – markers for breast carcinoma with focus on TNBC

	To stay	Sensitivity	Comments
GCDFP15	Yes	50-70%	Highly specific for breast carcinoma
Mammaglobin	Yes	40-60%	Highly specific for breast carcinoma
ER	No	80%	Moderately sensitive but nor specific
GATA3	Yes	90-95%	Highly sensitive for ER+ breast carcinoma – 20-60% positivity in TNBC and metasplastic type Low specificity – A ”selective marker”
TNBC			
SOX10	?	40-60%	Moderately sensitive and specific (obs melanoma)
TPRS1*	Yes	80-90%	Highly sensitive and relatively highly specific for TNBC

* Trichorhinophalangeal syndrome type 1 (TRPS1) gene

The antibody graveyard – markers for breast carcinoma with focus on TNBC

Table 1 TRPS1 and GATA3 expression in breast cancers.

Breast carcinoma		Negative	Positive: Low	Positive: Intermediate	Positive: High	Total
TRPS1						
ER/PR+		3 (2%)	5 (3%)	22 (12%)	146 (83%)	176
HER2+		9 (13%)	5 (8%)	14 (21%)	39 (58%)	67
TNBC	Metaplastic	7 (14%)	3 (5%)	12 (23%)	30 (58%)	52
	Nonmetaplastic	26 (14%)	8 (5%)	41 (22%)	109 (59%)	184
GATA3						
ER/PR+		8 (5%)	7 (4%)	27 (15%)	131 (76%)	173
HER2+		8 (12%)	8 (12%)	22 (33%)	29 (43%)	67
TNBC	Metaplastic	41 (79%)	7 (13%)	3 (6%)	1 (2%)	52
	Nonmetaplastic	90 (49%)	20 (11%)	48 (26%)	26 (14%)	184

Table 2 TRPS1 expression in malignancies of multiple organs.

		Negative	Positive: Low	Positive: Intermediate	Positive: High	Total
Breast	Carcinoma	45 (9%)	21 (4%)	89 (19%)	324 (68%)	479
Bladder	Urothelial carcinoma	113 (98%)	2 (2%)	0	0	115
Lung	Adenocarcinoma	119 (97%)	2 (2%)	1 (1%)	0	122
	Squamous cell carcinoma	58 (75%)	15 (19%)	2 (3%)	2 (3%)	77
Ovary	Serous carcinoma	142 (86%)	17 (10%)	4 (2%)	2 (2%)	165
	Non-serous carcinoma	79 (92%)	4 (5%)	2 (2%)	1 (1%)	86
Head/Neck	Salivary duct carcinoma	132 (76%)	18 (10%)	16 (9%)	7 (4%)	173
Pancreas	Adenocarcinoma	143 (99%)	1 (1%)	0	0	144
Skin	Melanoma	39 (98%)	1 (2%)	0	0	40
Colon	Adenocarcinoma	92 (100%)	0	0	0	92
Stomach	Adenocarcinoma	38 (100%)	0	0	0	38
Kidney	Clear cell carcinoma	49 (100%)	0	0	0	49
	Papillary carcinoma	38 (100%)	0	0	0	38
	Chromophobe carcinoma	25 (100%)	0	0	0	25
Thyroid	Papillary carcinoma	44 (100%)	0	0	0	44
	Follicular carcinoma	20 (100%)	0	0	0	20
	Undifferentiated carcinoma	6 (100%)	0	0	0	6

Fig. 3 TRPS1 and GATA3 expression in representative HER2+ breast cancer cases. Case 1 shows an invasive ductal carcinoma with high expression of both TRPS1 and GATA3. Case 2 shows an invasive carcinoma with high expression of TRPS1 and negative GATA3.

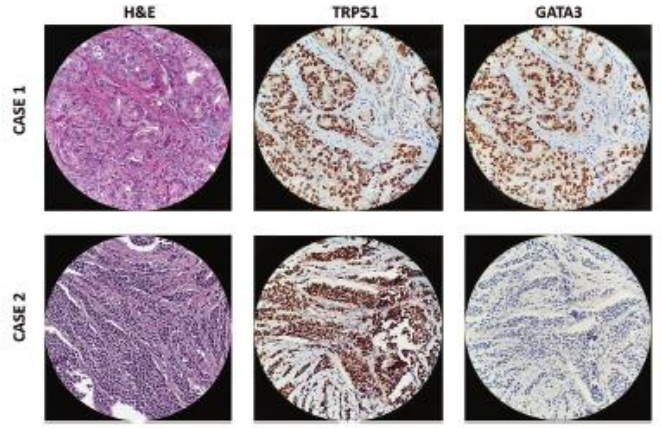
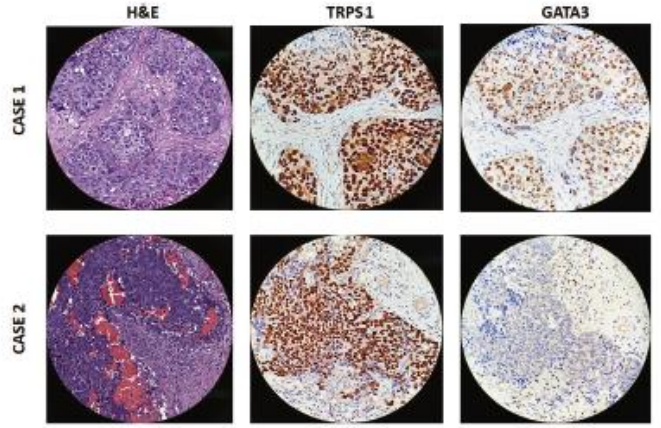


Fig. 4 TRPS1 and GATA3 expression in representative nonmetaplastic TNBC cases. Case 1 shows a poorly differentiated carcinoma with high expression of TRPS1 and intermediate to high expression of GATA3. Case 2 shows a poorly differentiated carcinoma with high expression of TRPS1 and negative GATA3.



Ai, D., Yao, J., Yang, F. et al. TRPS1: a highly sensitive and specific marker for breast carcinoma, especially for triple-negative breast cancer. Mod Pathol 34, 710–719 (2021).


KEEP
CALM
AND
REMEMBER
TO SKIP