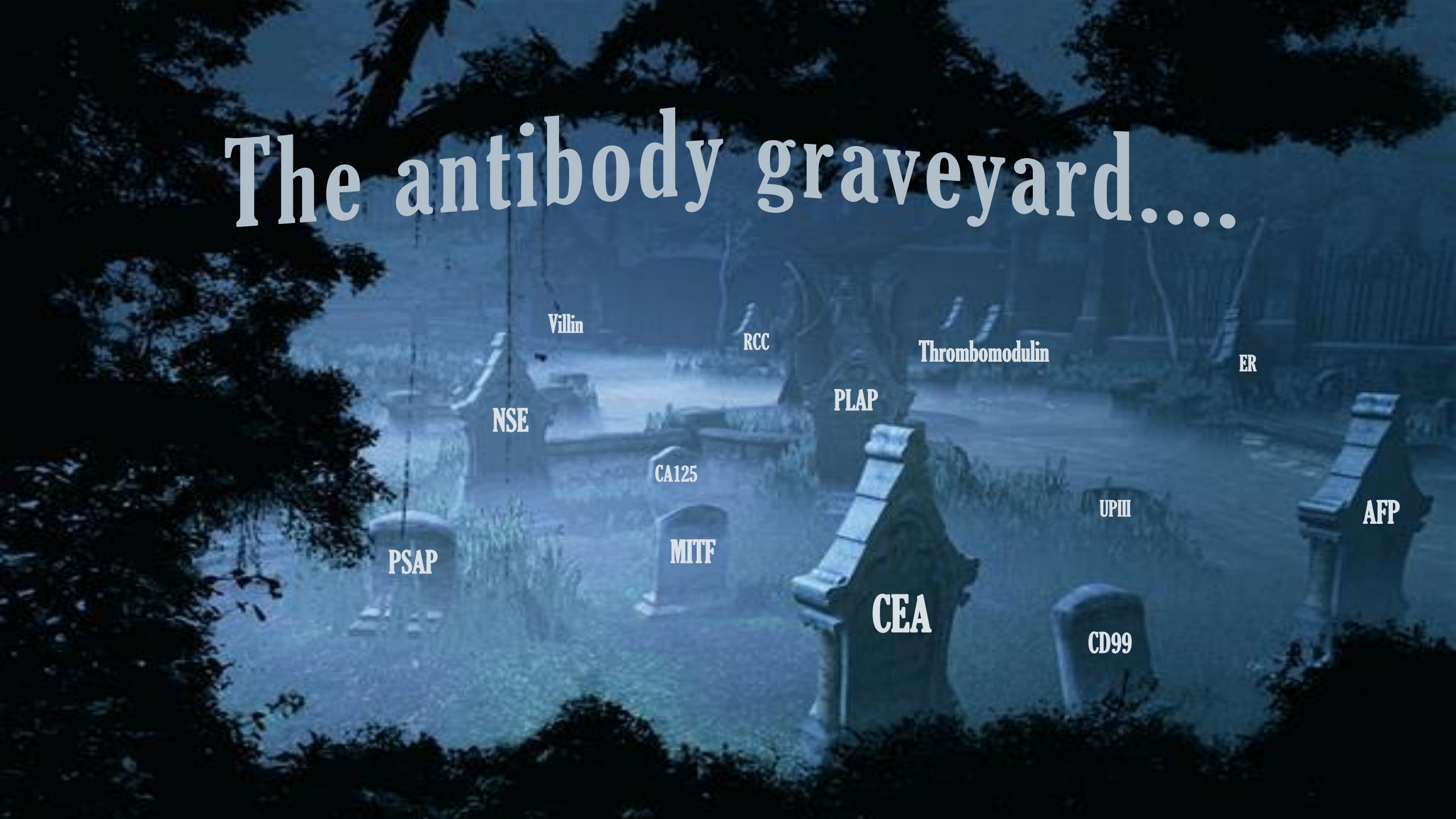
Seminar in Diagnostic Immunohistochemistry
Aalborg University Hospital, October 2-4 2024

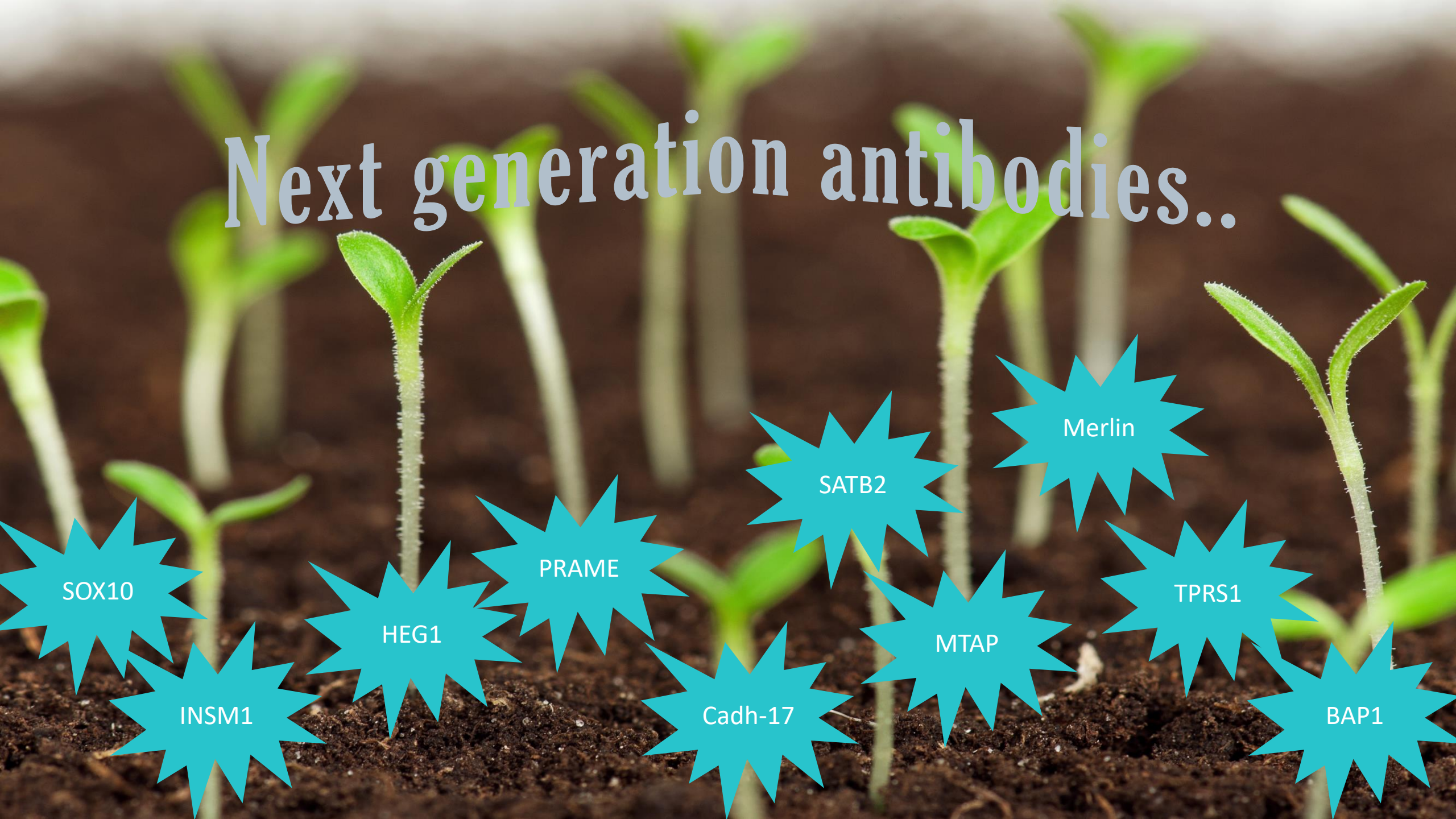
The Antibody Graveyard

-

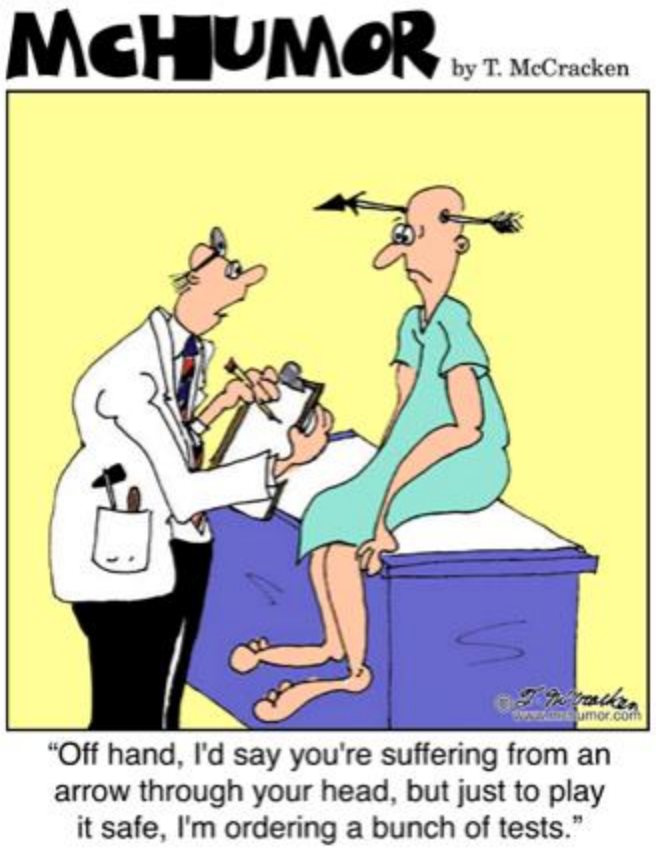
Goodbye and Hello Markers

Søren Nielsen,
Director
NordiQC

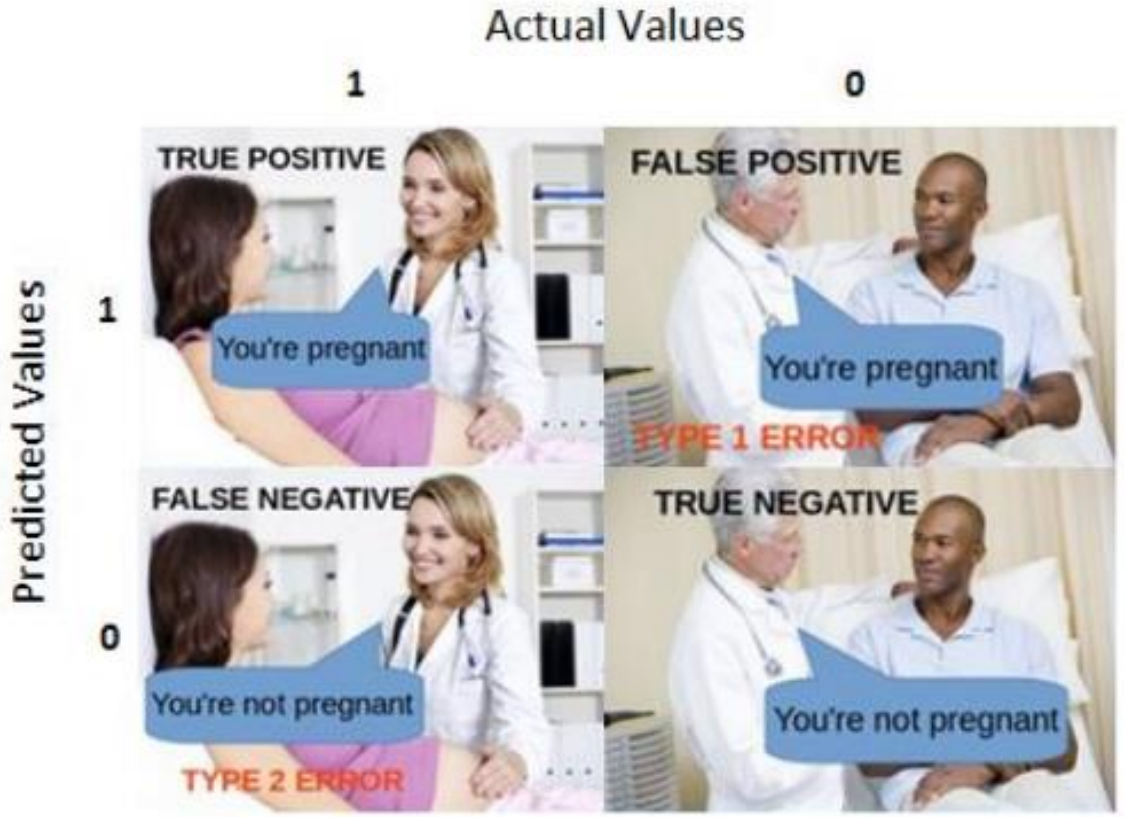

The antibody graveyard....
Villin
RCC
Thrombomodulin
ER
NSE
PLAP
CA125
UPIII
AFP
PSAP
MITF
CEA
CD99


Next generation antibodies..
SOX10
INSM1
HEG1
PRAME
Cadh-17
SATB2
MTAP
Merlin
TPRS1
BAP1

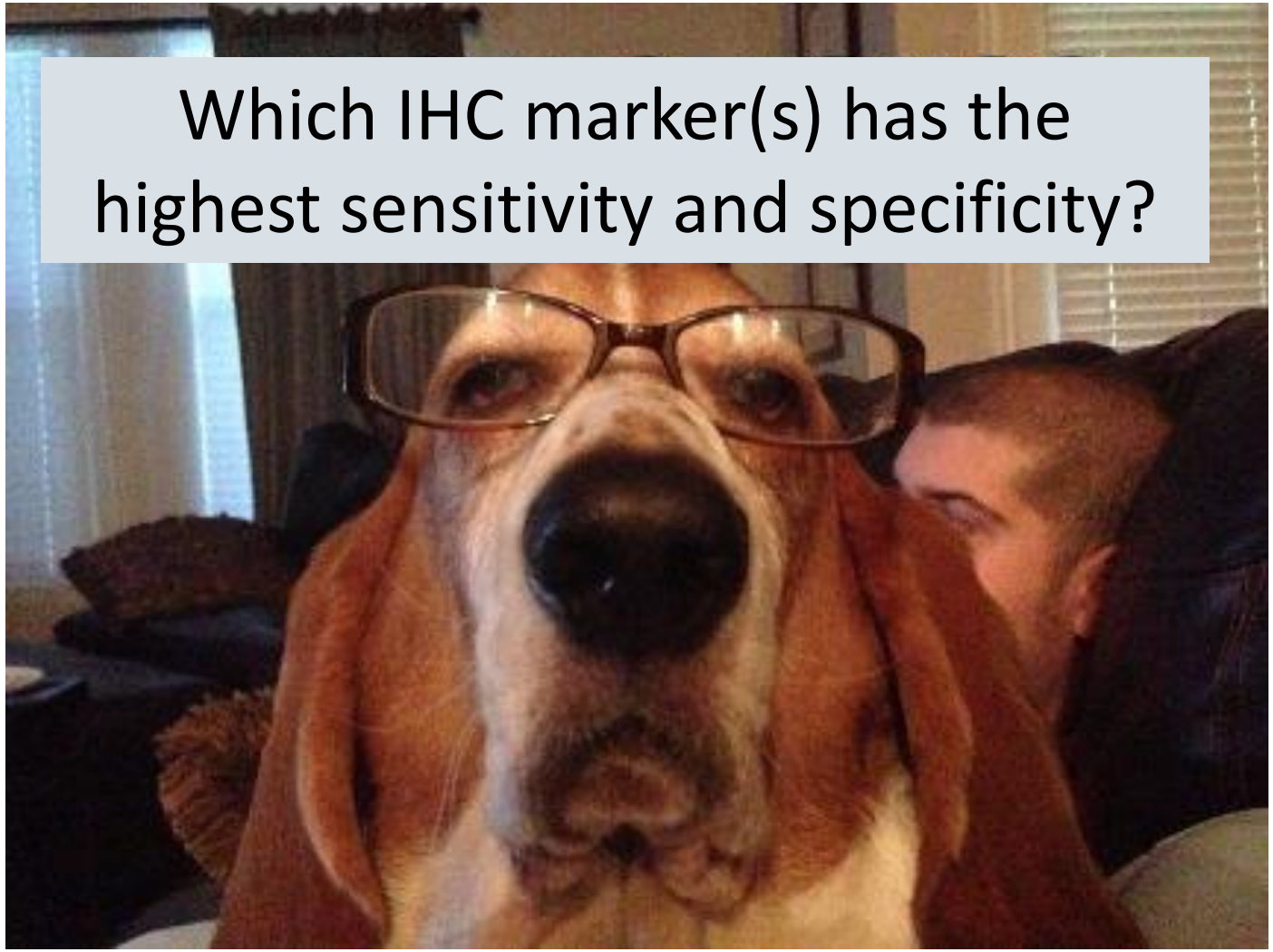
Replacements, supplemental IHC markers – why?



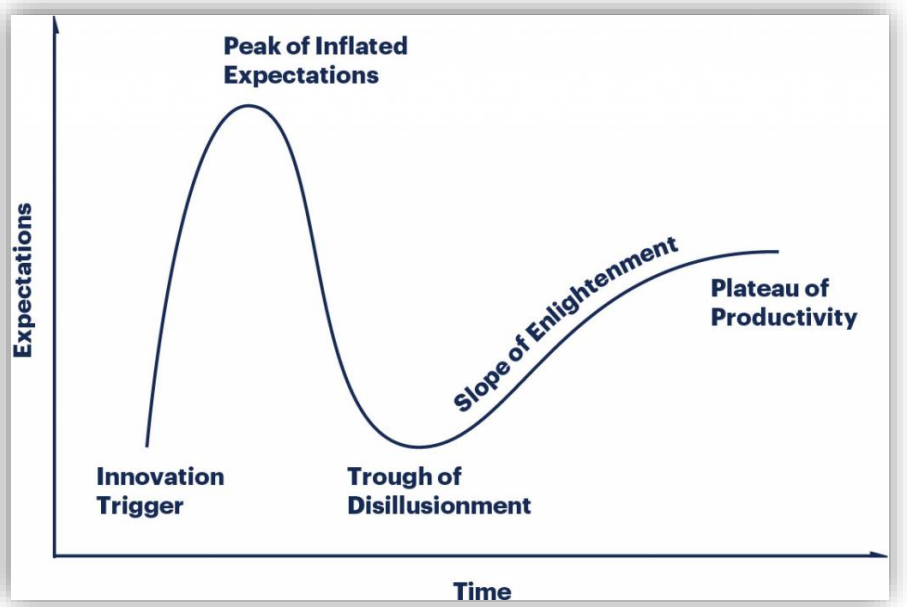
"Off hand, I'd say you're suffering from an arrow through your head, but just to play it safe, I'm ordering a bunch of tests."



Sensitivity and Specificity



A NEW BIOMARKER



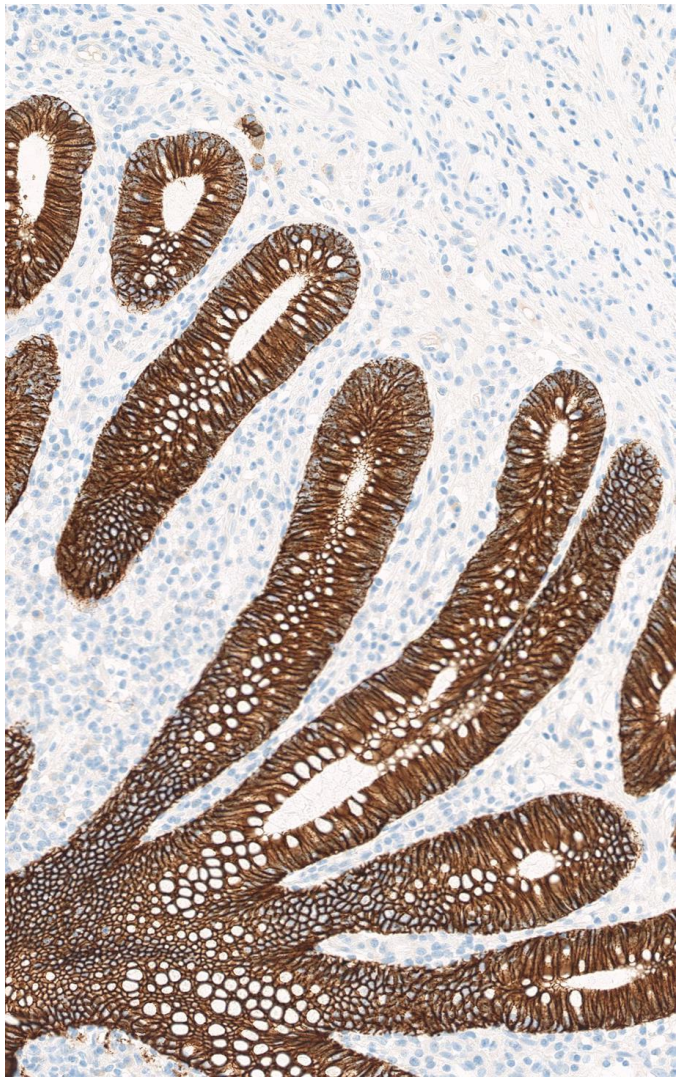
The antibody graveyard – CUP* - Colorectal carcinoma** markers

	To stay	Sensitivity	Comments
CK20	Yes	80-95%	MSI-H carc. can be neg. - Also seen in many other carcinomas
CDX2	Yes	80-95%	MSI-H carc. can be neg. – Intestinal lineage marker
mCEA	?	90-100%	Not specific for CRC - 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

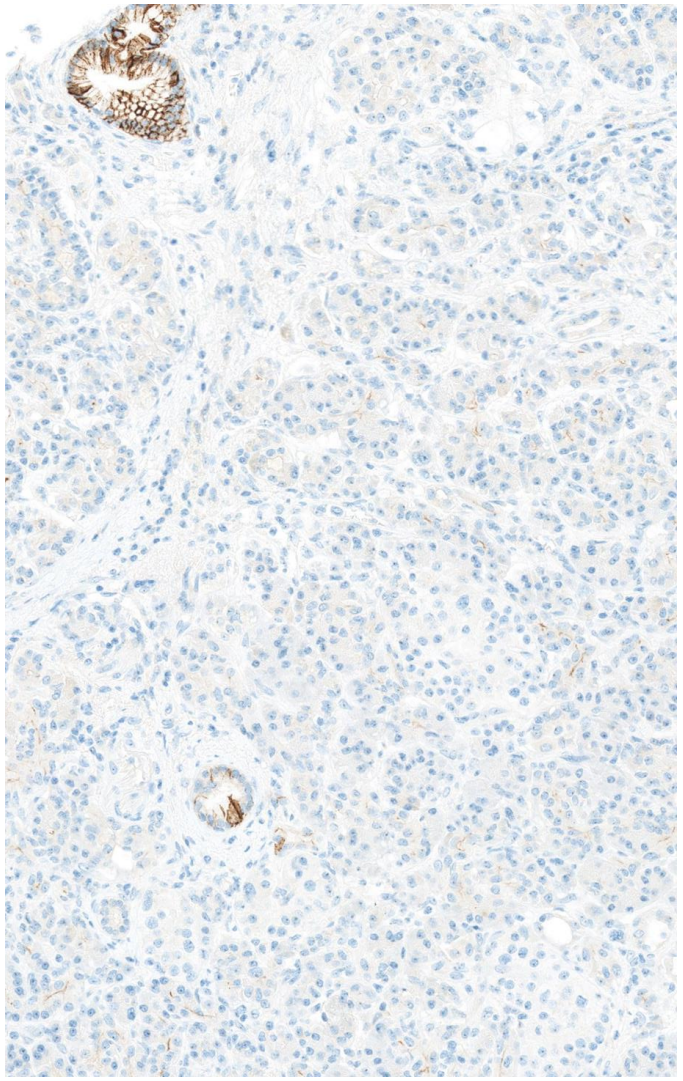
By both 97% & 88% sens. for MSS and MSI-H CRC

* CUP; Cancer of unknown primary origin ** CRC

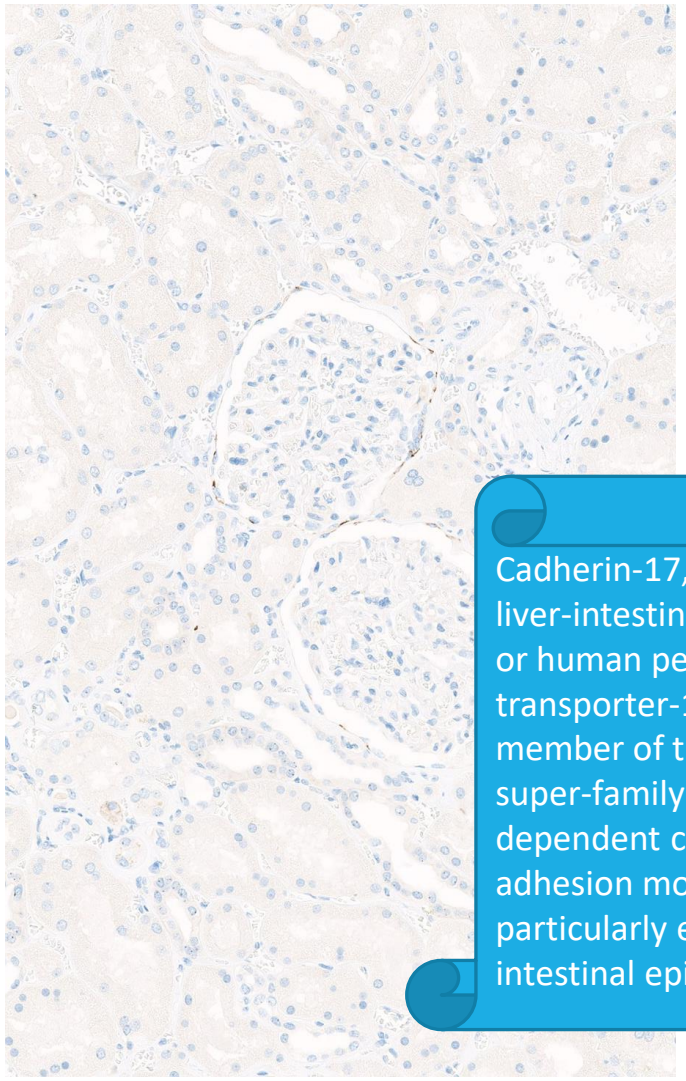
The antibody graveyard – CUP - Colorectal carcinoma markers – Cadherin 17



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.

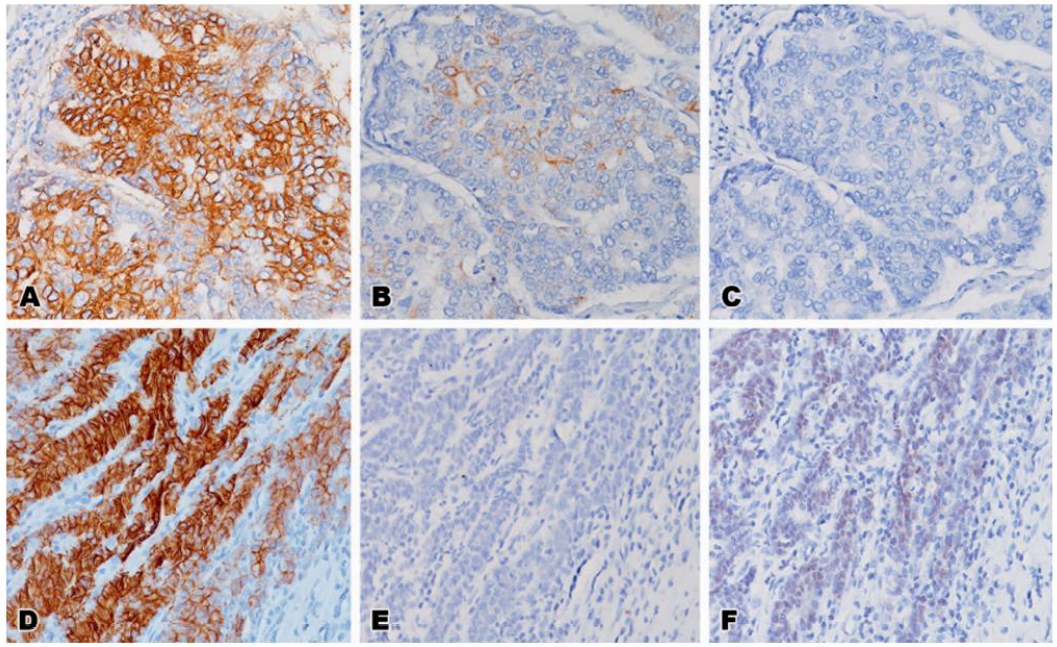


Figure 3. *Staining results in metastatic colon adenocarcinoma. A and D, Strong, positive staining was observed in a high percentage of specimens with CDH17. B, Focal staining was observed in CK20-positive tissue; and in specimens considered negative, CK20 was completely absent (E). Representative negative (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

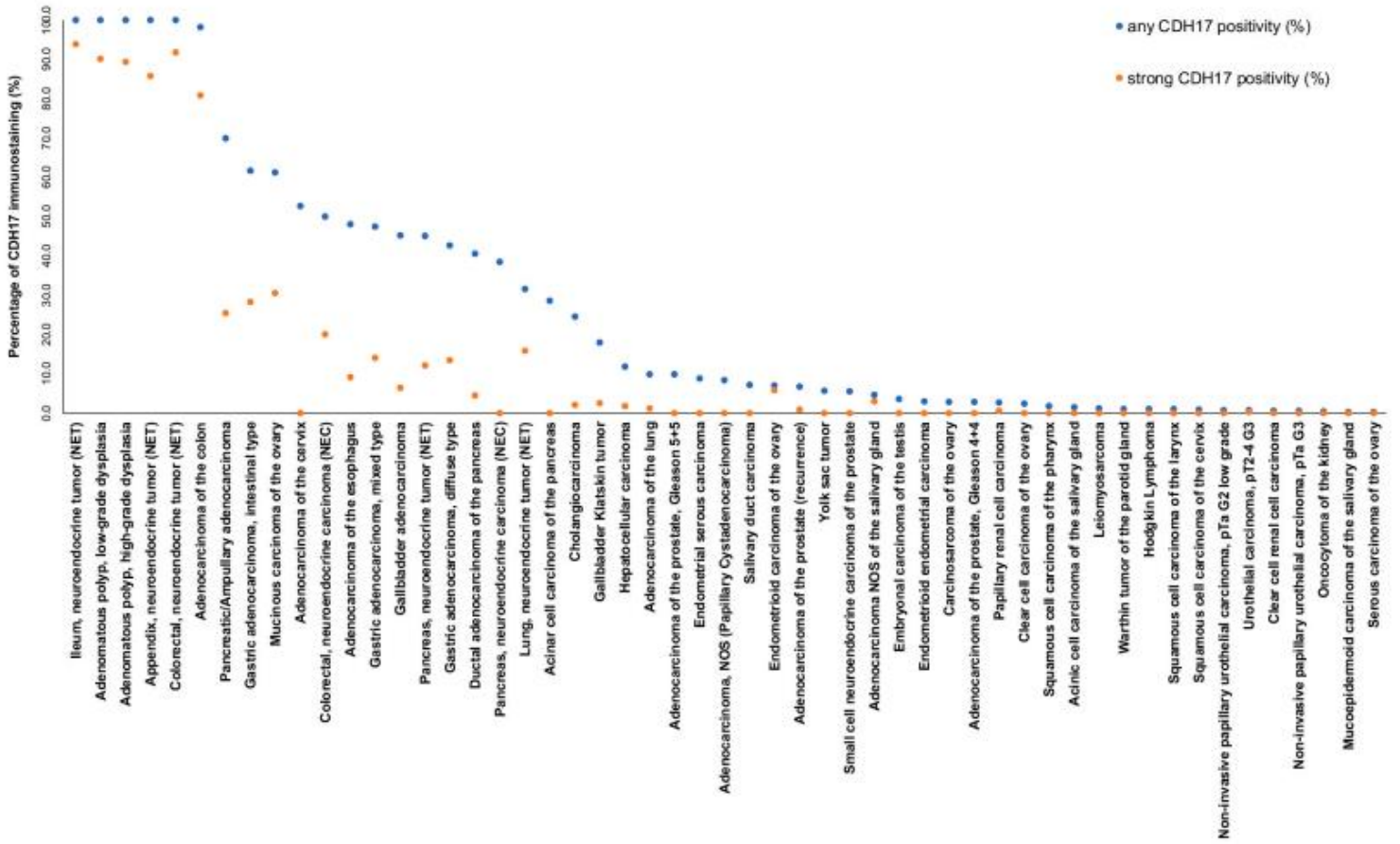


Fig. 3. Ranking order of CDH17 immunostaining in tumors. Both the percentage of positive cases (blue dots) and the percentage of strongly positive cases (orange dots) are shown.

Cadherin-17 (CDH17) expression in human cancer: A tissue microarray study on 18,131 tumors
Frank Jacobsen et al, Pathology - Research and Practice 256 (2024) 155175

Candidate; clone MSVA-517M, MS Validated Antibodies

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

Pathology - Research and Practice 256 (2024) 155175

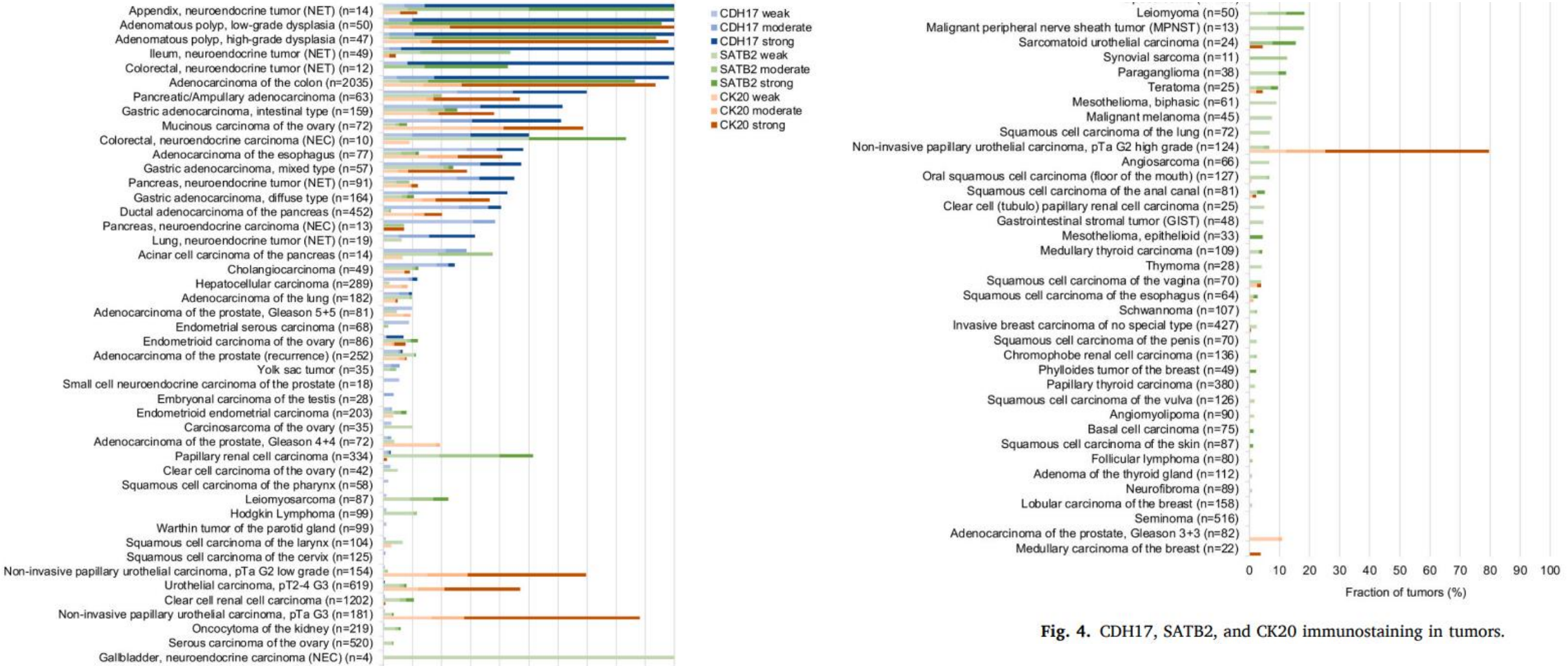


Fig. 4. CDH17, SATB2, and CK20 immunostaining in tumors.

Cadherin-17 (CDH17) expression in human cancer: A tissue microarray study on 18,131 tumors
Frank Jacobsen et al, Pathology - Research and Practice 256 (2024) 155175

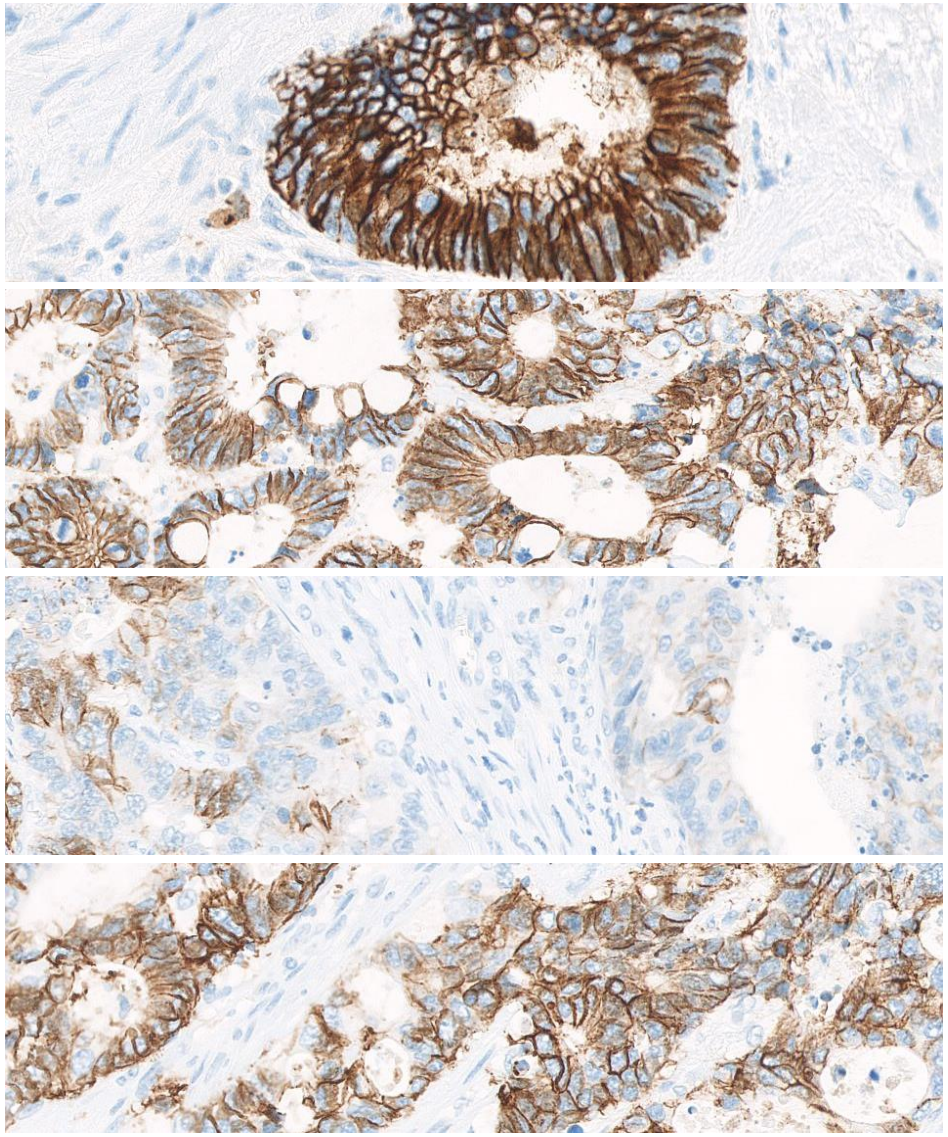
Candidate; clone MSVA-517M, MS Validated Antibodies

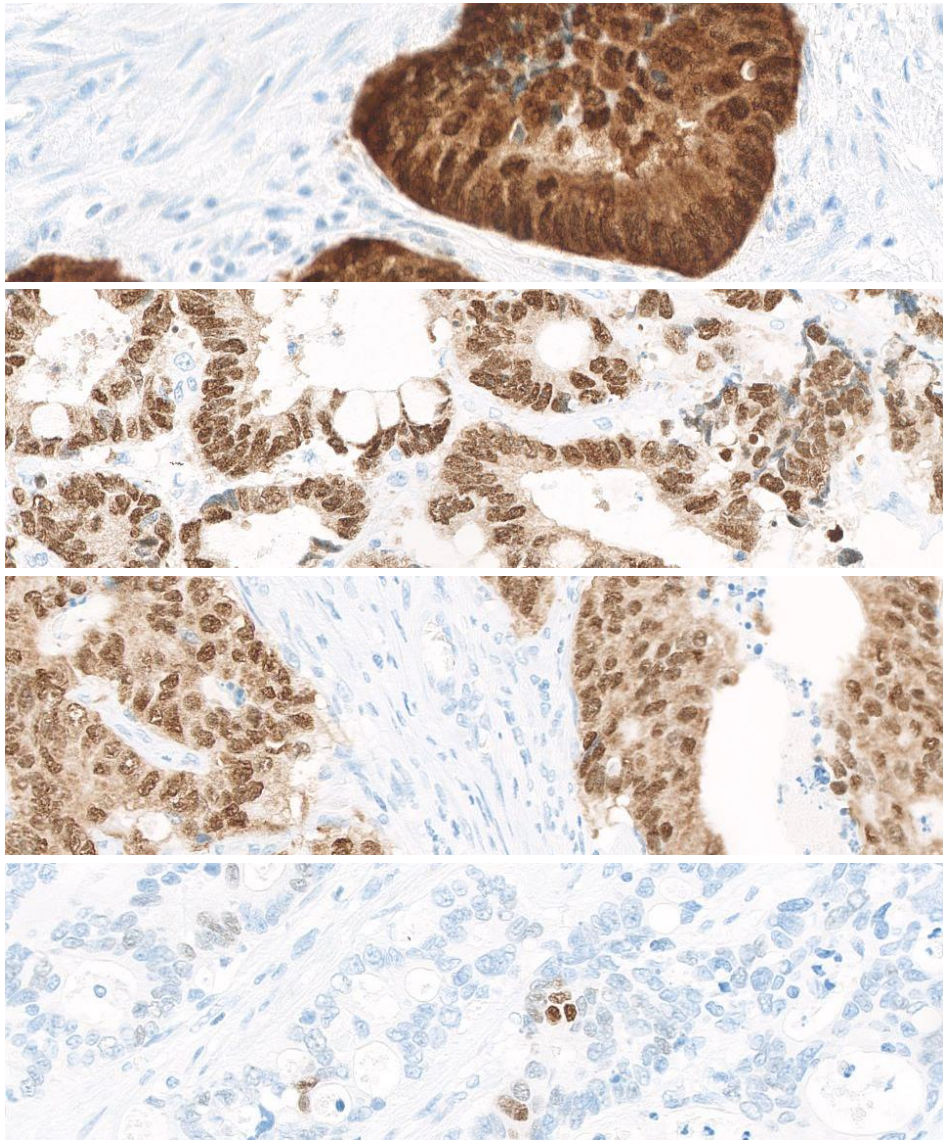
The antibody graveyard – CUP - Colorectal carcinoma markers – Cadherin 17

CAD-17

rmAb
SP183

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

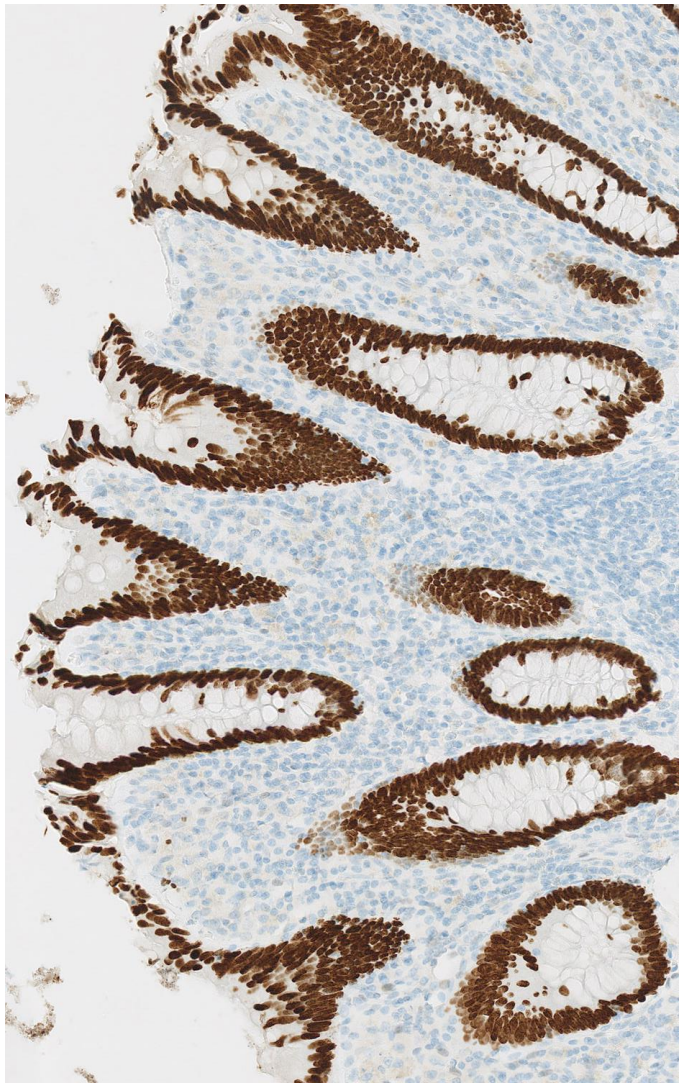




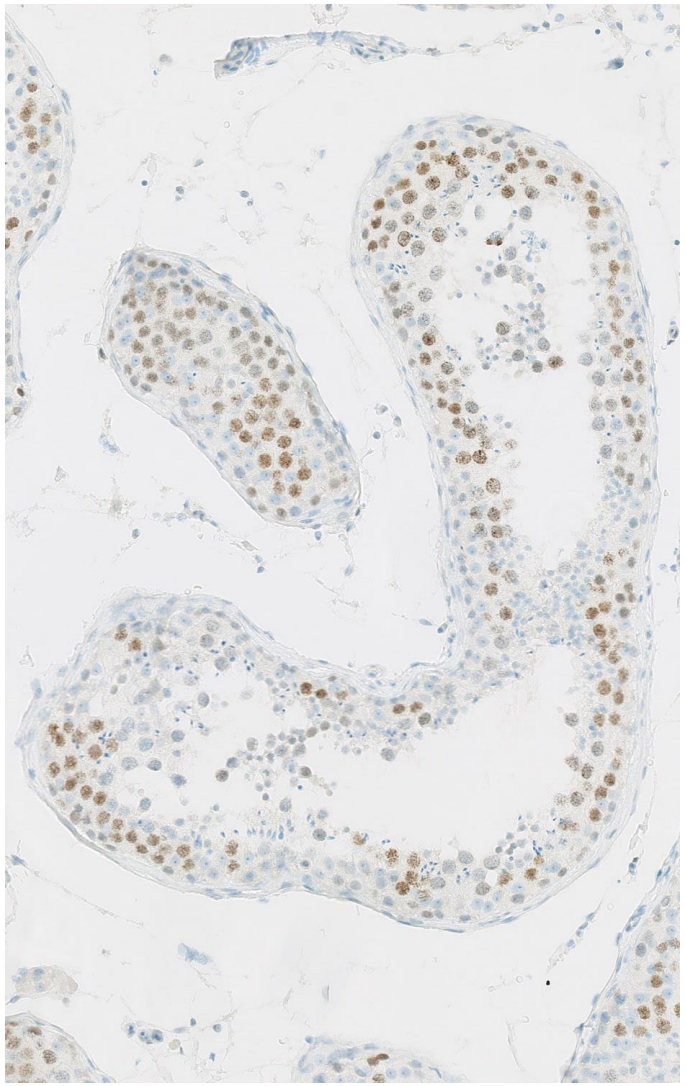
CDX2

The antibody graveyard – CUP - Colorectal carcinoma markers – SATB2

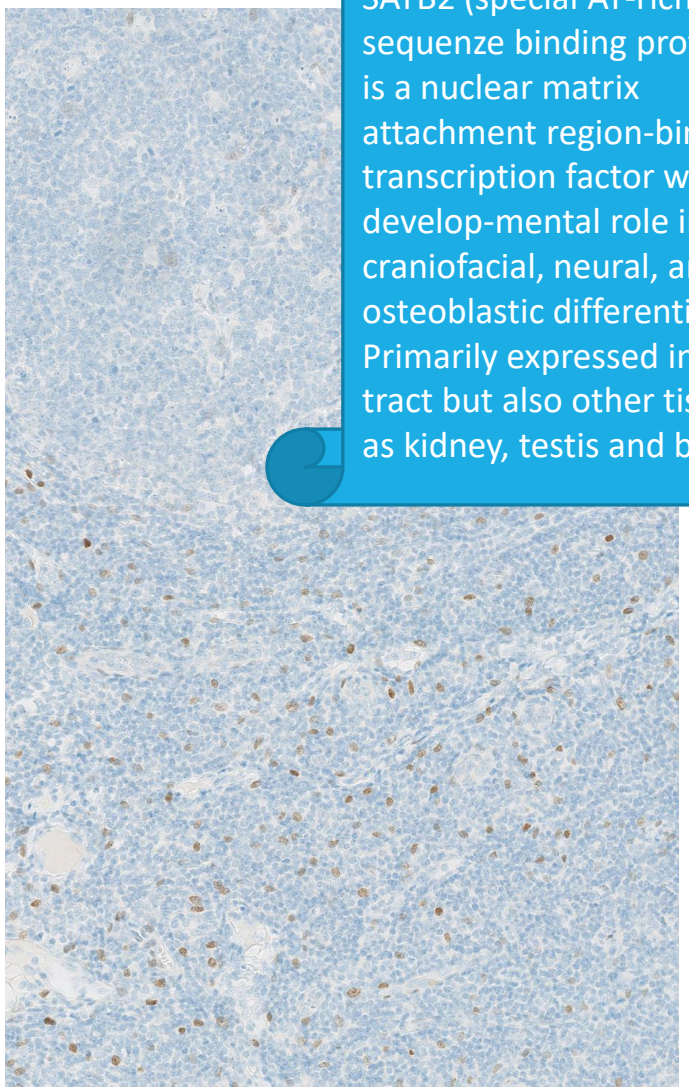
SATB2 (special AT-rich sequenze binding protein 2) is a nuclear matrix attachment region-binding transcription factor with develop-mental role in craniofacial, neural, and osteoblastic differentiation. Primarily expressed in GI tract but also other tissues as kidney, testis and brain.



Colon/Appendix



Testis



Tonsil (EP281 - Clone dependent)

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)

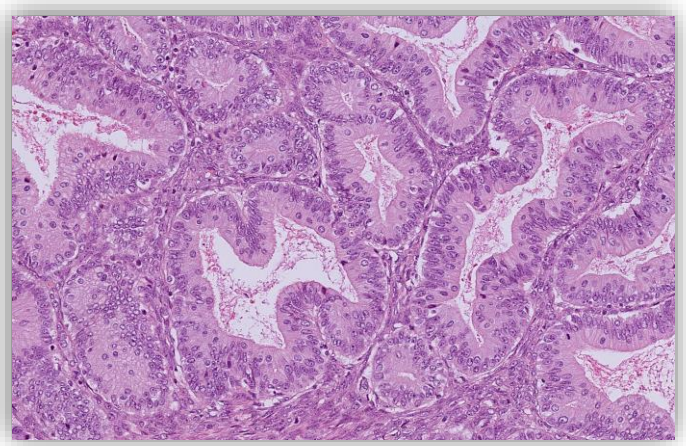
Table 2. Any CDX2 Expression in Primary Mucinous Tumors

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.



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%,

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

The antibody graveyard – CUP - Colorectal carcinoma markers – SATB2

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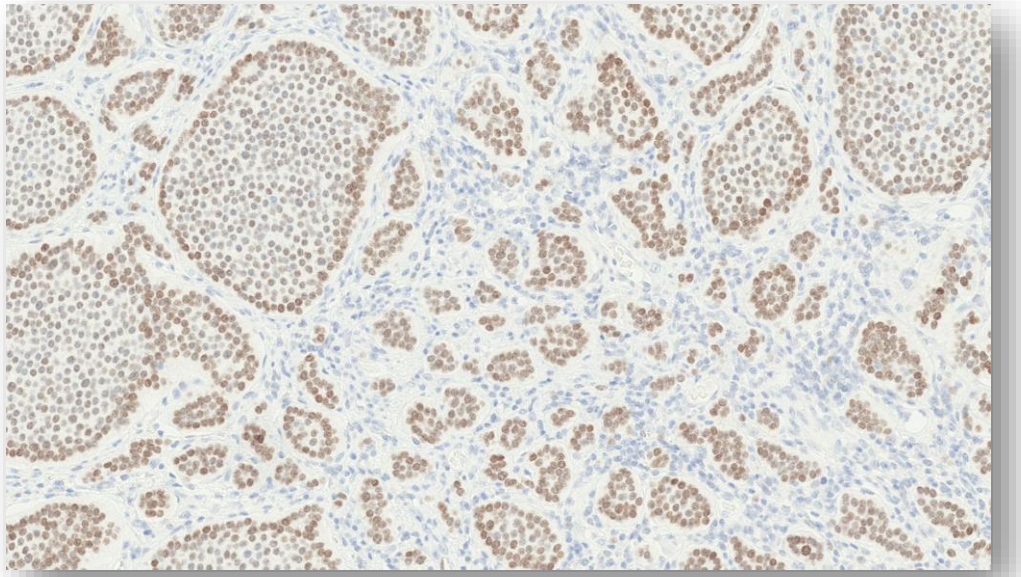
Immunohistochemistry in the diagnosis and classification of neuroendocrine neoplasms: what can brown do for you?⋆

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

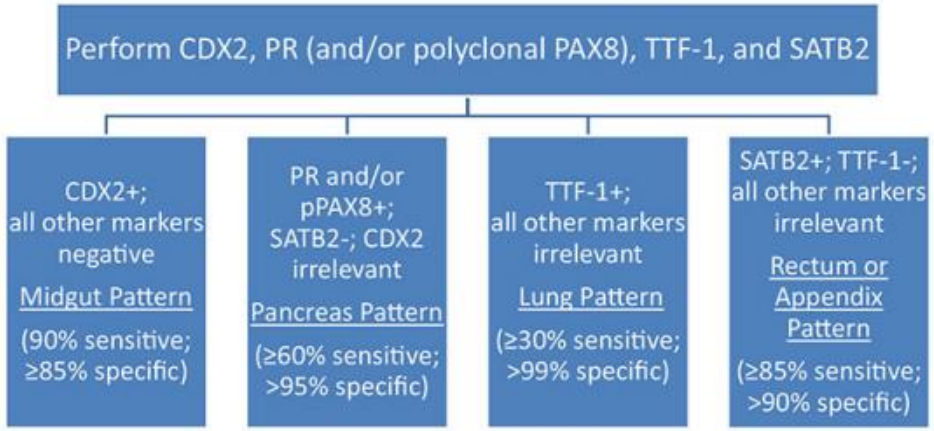


Figure 11. Simplified Immunohistochemical Algorithm for Well-Differentiated Neuroendocrine Tumor Site of Origin.

"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….

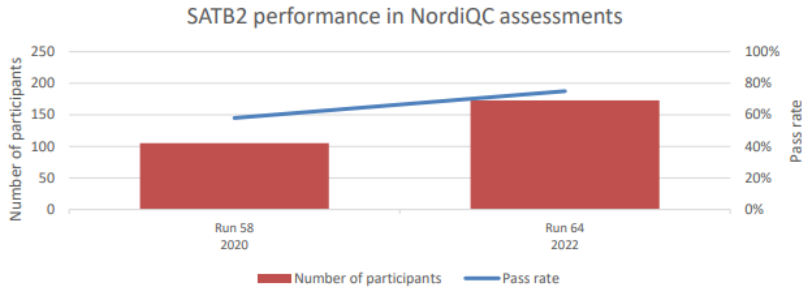
Table 1. **Antibodies and assessment marks for SATB2, run 64**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **SATBA4B10**	1 3 3	Abcam Santa Cruz Zytomed Systems	0	1	6	0	14%	0%
rmAb clone **EP281**	13 66 1 3 1 5 3	Epitomics Cell Marque Diagnostic BioSystems BioSB Biocare Medical Gennova Scientific Zeta Corporation	48	28	8	8	83%	52%
rmAb clone **SP281**	4 1	Abcam Zytomed Systems	0	4	0	1	80%	0%
rmAb clone **QR023**	1	Quartett	1	0	0	0	-	-
rmAb clone **ZR167**	1	Zeta Corporation	0	0	0	1	-	-
pAb **HPA001042**	4	Sigma Aldrich	0	1	1	2	-	-
pAb **Ab69995**	1	Abcam	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **IHC660**	1	GenomeMe	0	0	1	0	-	-
rmAb clone **EP281 384R-17/18**	19	Cell Marque	9	8	2	0	89%	47%
rmAb clone **EP281 760-6075 VRPS[3]**	4	Ventana/Roche	2	2	0	0	-	-
rmAb clone **EP281 760-6075 VRPS[4]**	23	Ventana/Roche	11	9	1	2	87%	48%
rmAb clone **EP281 PR/HAR239**	3	PathnSitu	0	1	2	0	-	-
rmAb clone **EP281 API3225**	3	Biocare Medical	0	2	1	0	-	-
rmAb clone **EP281 MAD-000747QD**	3	Máster Diagnostica	0	0	3	0	-	-
rmAb clone **EP281 BSB319 7/8/9**	2	BioSB	0	1	1	0	-	-
rmAb clone **EP281 CSR-0140**	1	Celnovte	1	0	0	0	-	-
rmAb clone **EP281 Z2321RP**	2	Zeta Corporation	1	0	1	0	-	-
rmAb clone **EP281 RMPD112**	1	Diagnostic BioSystems	0	0	0	1	-	-
Total	173		73	57	27	16		
Proportion			42%	33%	16%	9%	75%	

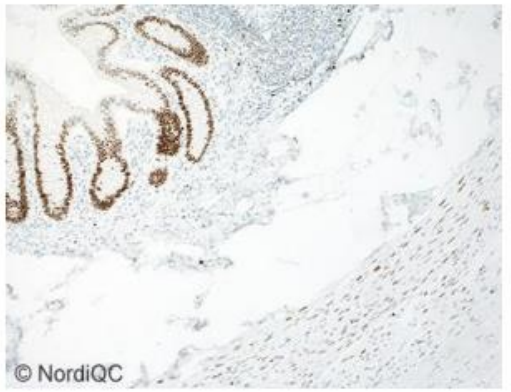
1) Proportion of sufficient stains (optimal or good) (≥5 asessed protocols).
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).

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).

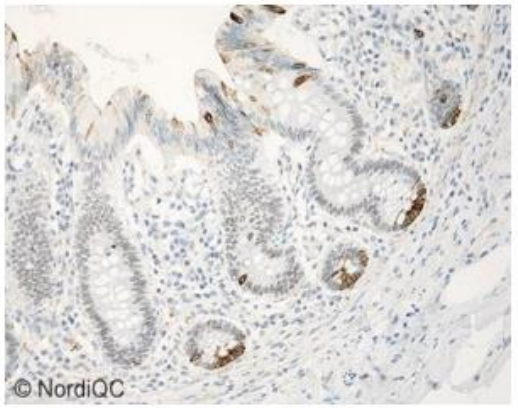
Graph 1. **Proportion of sufficient results for SATB2 in the NordiQC runs performed**



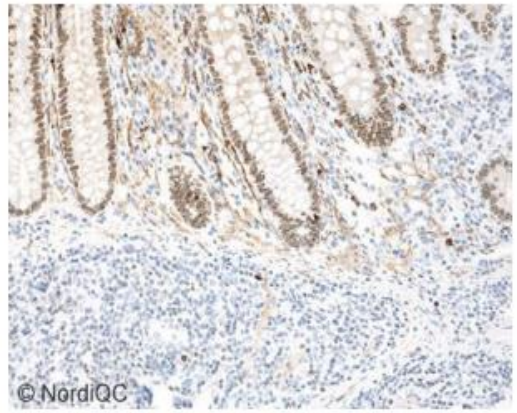
EP281



CL0276



Ab69995



HPA001042

The antibody graveyard – CUP - Colorectal carcinoma markers

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

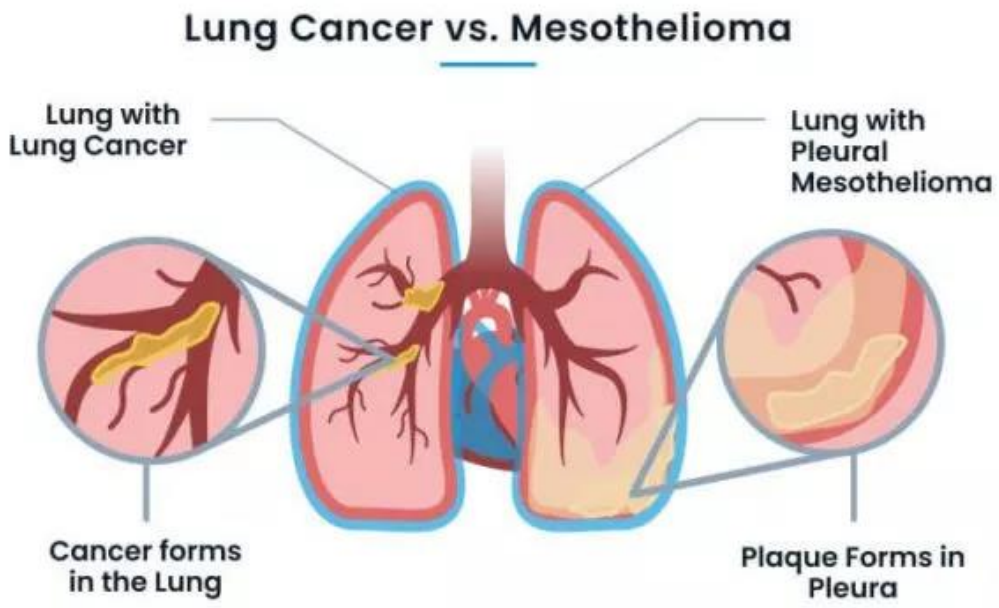
Cadherin 17 might be superior to CK20 due to higher analytical sensitivty in metastatic lesions, but the wide publication history of CK20 challenges the position of Cadherin 17 as primary marker – Cadherin 17 an ad-on??

SATB2 to used in the differential diagnosis of mucinous ovarian and CRC adenocarcinoma – superior to CDX2

Villin and mCEA of less diagnostic value for CRC

Novel biomarkers for the diagnosis and prognosis of colorectal cancer
Intest Res. 2020;18(2):168-183. Published online November 30, 2019
DOI: https://doi.org/10.5217/ir.2019.00080
Hyung-Hoon Oh et al.

The antibody graveyard – Mesothelioma versus (lung-) adenocarcinoma

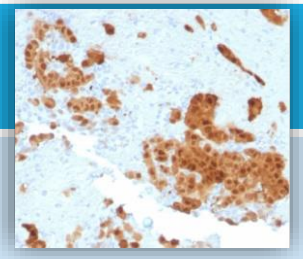


The accurate diagnosis of malignant pleural mesothelioma (MPM) is essential for therapeutic and legal reasons. In 2006 and consolidated in 2020 the International Mesothelioma Panel advocated the use of a panel, including **two mesothelial** and **two non-mesothelial** immunohistochemical (IHC)markers.

	Lung adenocarcinoma	Mesothelioma epithelial
Calretinin	-	+
CK5	-	+
WT1	-	+
EP-CAM	+	-*
CEA	(+)	-
TTF1	+	
…….		

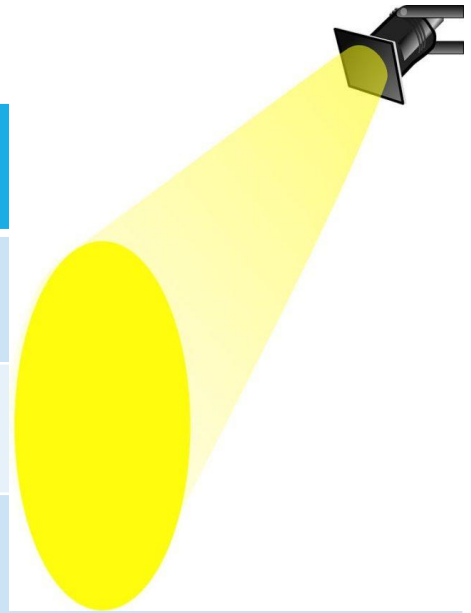
The antibody graveyard – Mesothelioma – positive markers

	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
WT1	Yes	85-95%	Also seen in serous ovarian 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



Nolwenn Le Stang et al;
Differential Diagnosis of Epithelioid Malignant Mesothelioma With Lung and Breast Pleural Metastasis:
A Systematic Review Compared With a Standardized Panel of Antibodies—A New Proposal That May Influence Pathologic Practice. *Arch Pathol Lab Med* 1 April 2020; 144 (4): 446–456.

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
WT1	Yes	85-95%	Also seen in serous ovarian carcinomas
Uroplakin 3b	NEW	80%	Also seen in Urothelial carcinoma (15-60%) and Ovarian carc. (10%)
HEG1	NEW	85-95%	High sensitivity in all mesothelioma subtypes - Also seen in few non-mesotheliomas
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

The antibody graveyard – Mesothelioma – positive markers

diagnostics

MDPI

Article

Analysis of More than 16,000 Human Tumor and Normal Tissues Identifies Uroplakin 3B as a Useful Diagnostic Marker for Mesothelioma and Normal Mesothelial Cells

Maximilian Lennartz [1], Dennis Atug [1], Sebastian Dwertmann Rico [1], Viktor Reiswich [1], Florian Viehweger [1], Franziska Büscheck [1], Martina Kluth [1], Claudia Hube-Magg [1], Andrea Hinsch [1], Christian Bernreuther [1], Guido Sauter [1], Eike Burandt [1], Andreas H. Marx [2], Till Krech [1,3], Ronald Simon [1,*], Sarah Minner [1], Till S. Clauditz [1], Frank Jacobsen [1], Patrick Lebok [1,3], Natalia Gorbokon [1], Katharina Möller [1], Stefan Steurer [1,†] and Christoph Fraune [1,†]

	Tumor Entity	on TMA (n)	int. (n)	Upk3b Immunostaining Result neg. (%)	weak (%)	mod. (%)	str. (%)
Tumors of the lung, pleura, and thymus	Adenocarcinoma of the lung	196	184	100.0	0.0	0.0	0.0
	Squamous cell carcinoma of the lung	80	75	100.0	0.0	0.0	0.0
	Small cell carcinoma of the lung	16	16	100.0	0.0	0.0	0.0
	Mesothelioma, epithelioid	40	28	17.9	25.0	14.3	42.9
	Mesothelioma, biphasic	77	52	69.2	11.5	5.8	13.5

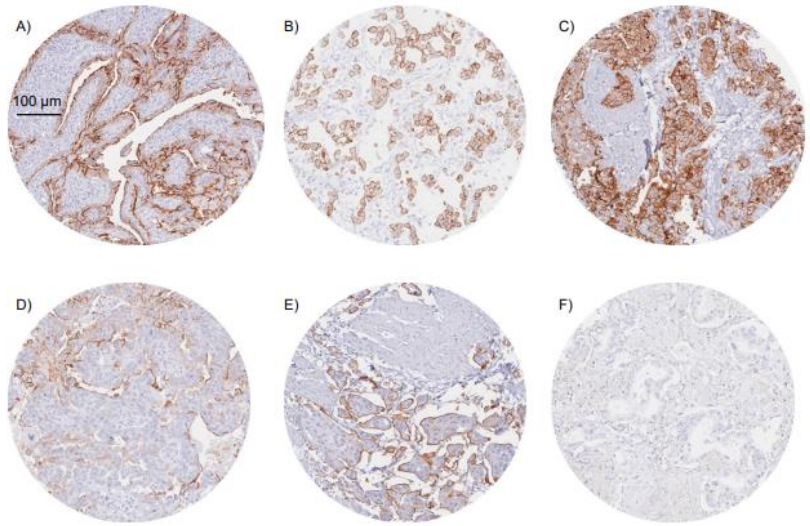


Figure 3. Examples of Upk3b immunostaining in selected cancers. The panels show a strong membranous Upk3b staining in samples of two epithelioid mesotheliomas (**A**,**B**), and a muscle-invasive urothelial carcinoma of the urinary bladder (**C**). Upk3b staining is more focal and predominantly seen on surface membranes in a serous high-grade ovarian carcinoma (**D**) and an invasive micropapillary urothelial cancer (**E**). Upk3b staining is absent in an adenocarcinoma of the lung (**F**).

Urothelial carcinoma, ovarian clear cell / serous type were also reported positive

The antibody graveyard – Mesothelioma – positive markers

Go to Uroplakin 3B Normal Tissue Gallery

Go to Uroplakin 3B Cancer Tissue Gallery

The antibody graveyard – Mesothelioma – positive markers; HEG1 clone SKM9-2

TABLE 2 HEG1 immunostaining in tumors analyzed in this study (whole sections)

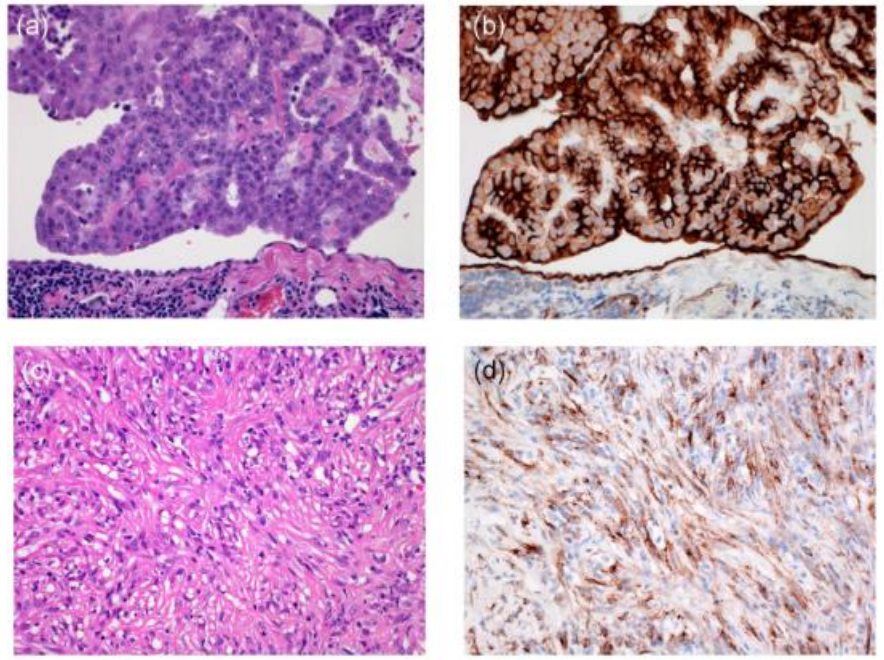
				Staining score					Staining pattern			
	N	P	(%)	0–2	3	4	5	6	m	(%)	c	(%)
Mesotheliomas	122	112	(91.8)	10	5	5	21	81	81	(66.4)	31	(25.4)
Epithelioid and biphasic	89	86	(96.6)	3	1	0	12	73	79	(88.8)	7	(7.9)
Epithelioid	57	56	(98.2)	1	1	0	7	48	53	(93.0)	3	(5.3)
Biphasic (epithelioid component)	32	30	(93.8)	2	0	0	5	25	26	(81.3)	4	(12.5)
Sarcomatoid	25	20	(80.0)	5	4	4	7	5	0	(0.0)	20	(80.0)
Desmoplastic	3	2	(66.7)	1	0	0	2	0	0	(0.0)	2	(66.7)
Transitional	3	3	(100.0)	0	0	1	0	2	2	(66.7)	1	(33.3)
Heterologous elements	1	0	(0.0)	1	0	0	0	0	0	(0.0)	0	(0.0)
Lymphohistiocytoid	1	1	(100.0)	0	0	0	0	1	0	(0.0)	1	(100.0)

TABLE 1 Expression frequency of HEG1 and conventional mesothelial markers in mesotheliomas

	EM		BM		SM	
	No. I/T	(%)	No. I/T	(%)	No. I/T	(%)
TMA						
m + cHEG1	66/69	95.7	34/36	94.4	2/2	100
mHEG1	59/69	85.5	25/36	69.4	0/2	0
Calretinin	64/70	91.4	19/36	52.8	0/2	0
WT1	45/70	64.3*	10/36	27.8*	0/2	0
Podoplanin	47/70	67.1*	10/36	27.8*	0/2	0
Whole sections						
m + cHEG1	56/57	98.2	30/32	93.8	20/25	80.0
mHEG1	53/57	93.0	26/32	81.3	0/25	0
Calretinin	41/43	95.3	16/19	84.2	NA	
WT1	30/38	78.9	10/17	58.8	NA	
Podoplanin	36/42	85.7	14/17	82.4	NA	

Abbreviations: BM, biphasic mesothelioma; EM, epithelioid mesothelioma; m + cHEG1, membranous and/or cytoplasmic HEG1 staining; mHEG1, membranous HEG1 staining; NA, not analyzed; No. I/T, number of immunoreactive/total cases; SM, sarcomatoid mesothelioma; TMA, tissue microarray.

*Statistically significant difference between expression frequency of membranous HEG1 staining and that of conventional mesothelial marker ($p < 0.05$).



Membranous HEG1 expression is a useful marker in the differential diagnosis of epithelioid and biphasic malignant mesothelioma versus carcinomas

Kenzo Hiroshima[1,2,3] | Di Wu[2] | Eitetsu Koh[4] | Yasuo Sekine[4] | Daisuke Ozaki[5] | Toshikazu Yusa[6] | Tadao Nakazawa[2] | Shoutaro Tsuji[7] | Yohei Miyagi[8] | Ann E. Walts[9] | Alberto M. Marchevsky[9] | Aliya N. Husain[10] | Kohzoh Imai[11]

Pathology International. 2021;71:604–613.

The antibody graveyard – Mesothelioma – negative markers

	To stay	Sensitivity*	Comments
EP-CAM – Ber-EP4	(Yes)	10-35%	Less specific/selective due to increased analytical sensitivity with optimized protocols
EP-CAM – MOC31	(Yes)	10-35%	Less specific/selective due to increased analytical sensitivity with optimized protocols
mCEA	?	0%	Less sensitive in adenocarcinomas – about 50-60% and thus neg result not conclusive
Claudin-4	Yes	0-5%	Shown to be more reliably negative in mesotheliomas and thus preferred negative marker

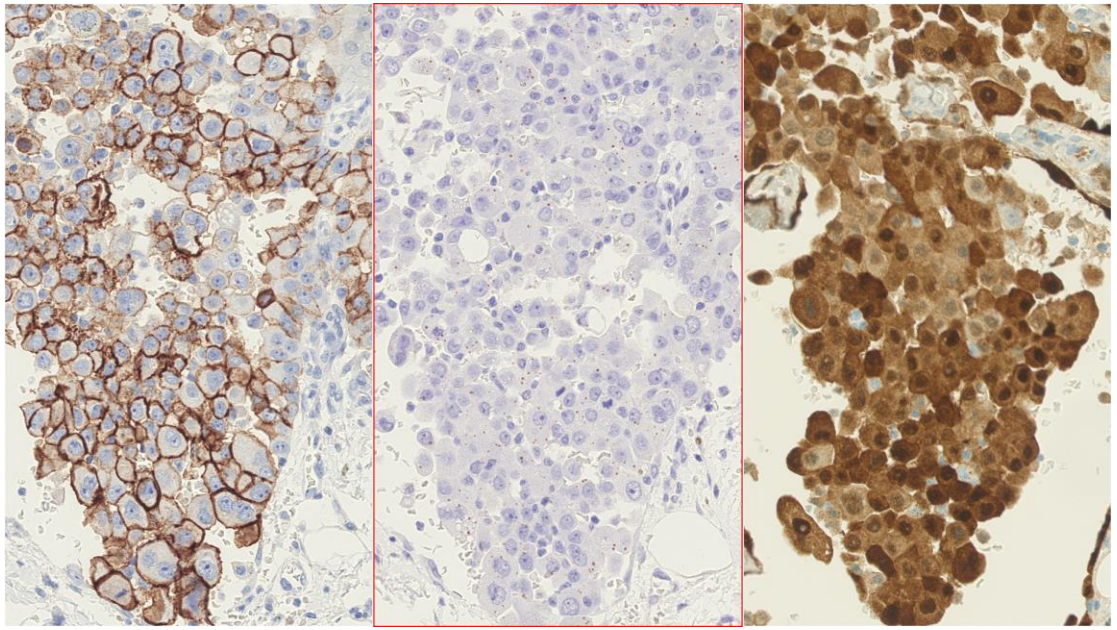
* Positivity seen in mesotheliomas (epithelial subtype)

Typically 1-2 ”positive markers” in combination with 1-2 ”negative markers” used to differentiate mesothelioma and carcinoma

	Lung adenocarcinoma	Mesothelioma epithelial
Calretinin	-	+
CK5	-	+
EP-CAM	+	-*
CEA	(+)	-

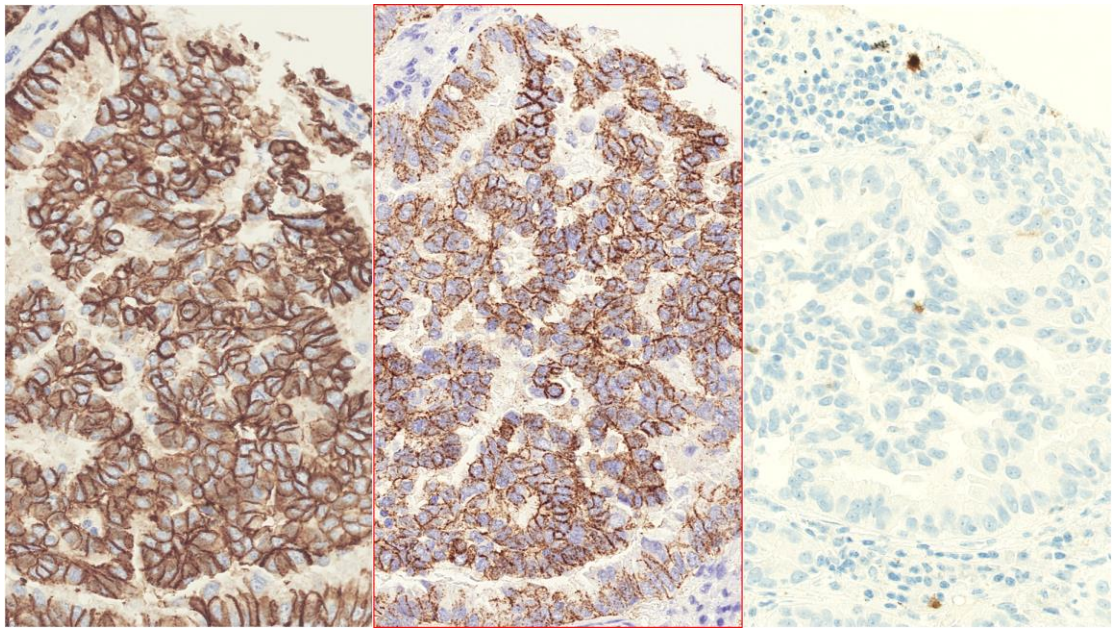
The antibody graveyard – Claudin 4

Mesothelioma



EP-CAM (EP4) Claudin 4 Calretinin

Lung adenocarcinoma



The antibody graveyard – Claudin 4 and HEG1; the magic combo for NSCLC vs mesothelioma

Table 1. HEG1: mesothelioma versus non-small-cell lung carcinoma staining

Report	Epithelioid and biphasic (membrane staining)	Sarcomatoid (membrane or cytoplasmic staining)	Non-small-cell lung carcinoma (membrane staining)
Tsuji[7,*]	108/112 (96%)	11/16 (69%)	0/98 (0%)
Naso[8,*]	65/69 (94%)	14/32 (44%)	0/167 (0%)
Hiroshima[9,**]	79/89 (89%)	20/25 (80%)	1/75 (1.3%)
Hiroshima[9,*]	84/105 (80%)	2/2 (100%)	ND
Itami[10,**]	34/34 (100%)	ND	ND
This report**	23/25 (92%)	ND	0/20
Total	393/434 (91%)	47/75 (63%)	1/360 (0.3%)

ND, not done.
*Based on TMAs.
**Based on whole sections.

Table 3. Suggested scheme for interpretation of cases stained for HEG1 and claudin-4

HEG1	Claudin-4	Interpretation
Positive	Negative	Mesothelioma
Negative	Positive	Carcinoma
Positive	Positive	Carcinoma, serous most common
Negative	Negative	Non-informative. Run other markers

Table 2. Claudin-4: mesothelioma versus carcinoma staining

Report	Epithelioid and biphasic mesothelioma (membrane staining)	Sarcomatoid mesothelioma (membrane or cytoplasmic staining)	NSCLC (adeno, squamous, adenosquamous, large cell)	All carcinomas tested (serous carcinomas)
Facchetti[3]	0/71	0/9	63/63 (100%)	302/336 (90%) (10/10 serous)
Ordonez[4]	0/50	0/10	44/45 (98%)	169/185 (91%) (44/45 serous)
Kawai[14]	1/27 (6%) peritoneal tumours			11/11 (100%) all serous
Ohta[15]	0/18		44/44 (100%)	59/59 (100%) (Serous 15/15)
Ordonez[11]	0/40		ND	44/45 (98%) all serous
Naso[13]	0/68	0/31	103/126 (82%)	103/126 (82%)
Devins[16]	0/17 peritoneal tumours	0/1	ND	49/49 (100%) all serous
Kai[17]	2/70 (2.8%)		68/70 (97%)	68/70 (97%)
Mawas[18]	0/65		112/116 (97%)	112/116 (97%)
Kushitani[19]	2/36 (5.5%)		35/38 (92%)	35/38 (92%)
Total	5/463 (0.1%) (4 pleural, 1 peritoneal)	0/51	469/502 (93%)	953/1035 (92%) (Serous 173/174, 99%)

Histopathology 2023, **82**, 385–392. DOI: 10.1111/his.14783

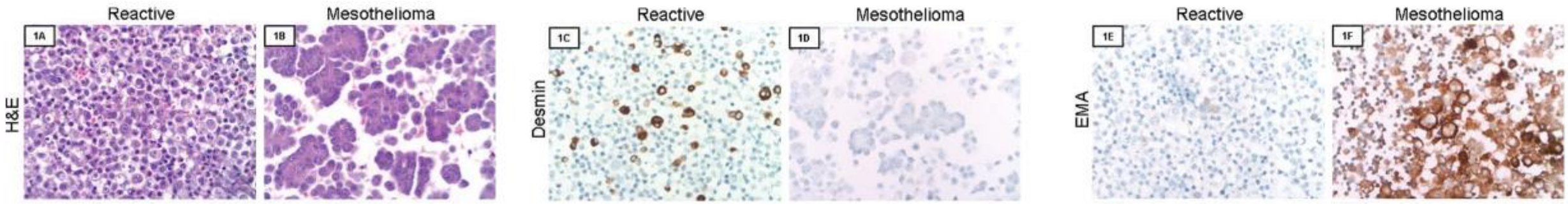
REVIEW

Hypothesis: HEG1 and claudin-4 staining will allow a diagnosis of epithelioid and biphasic mesothelioma versus non-small-cell lung carcinoma with only two stains in most cases

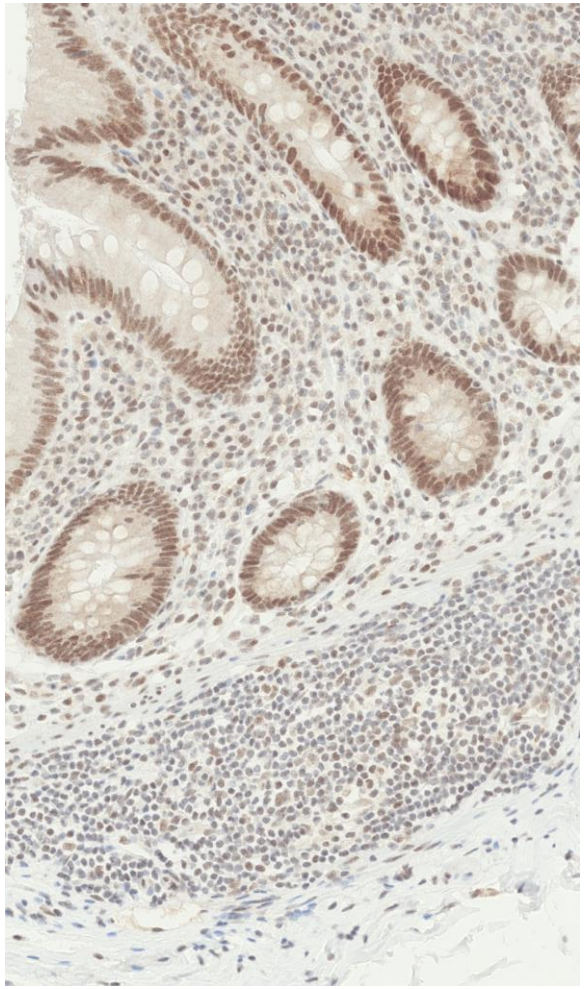
Andrew Churg[1,2] & Julia R Naso[1,2]
[1]*Department of Pathology, Vancouver General Hospital, and* [2]*Department of Pathology, University of British Columbia, Vancouver, BC, Canada*

The antibody graveyard – Mesothelioma versus reactive mesothelial cells

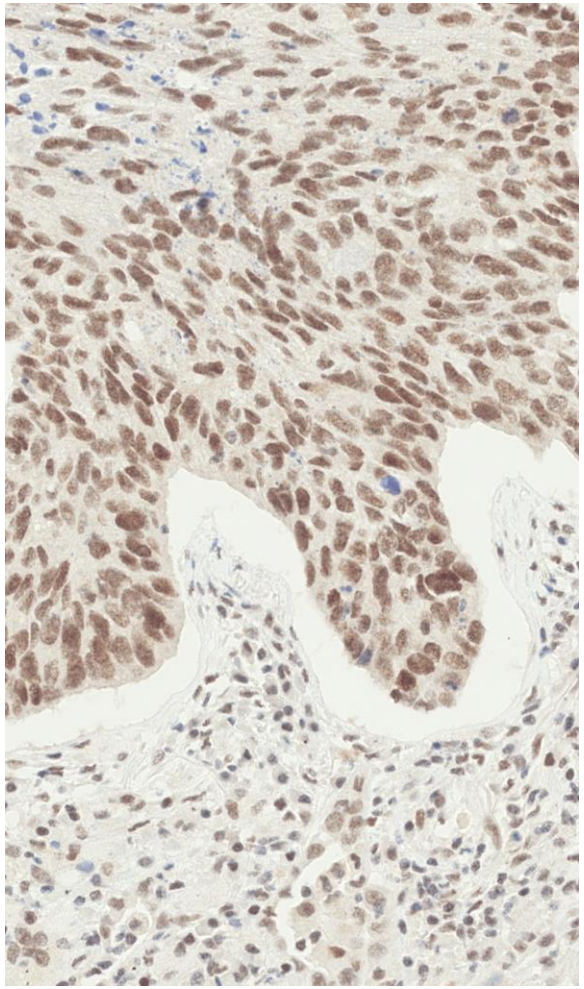
	To stay	Sensitivity	Comments
BAP1	New	60%	BRAC1 associated protein; mutation in BAP1 gene seen in mesothelioma (app 60%)
MTAP	New	50%	MTAP (methylthioadenosine phosphorylase); deficient expression seen in mesothelioma (app 50%)
Merlin	New	50%	Merlin; protein encoded by NF-2 gene (neurofibromatosis 2); deficient expression seen in mesothelioma (app 50%)
Desmin	No	50%	About 50% of reactive mesothelial proliferations are Desmin negative – Mesotheliomas 95% neg
EMA	No	60-80%	”3+ membrane reaction in mesothelial cells support mesothelioma” – not specific and protocol rel.



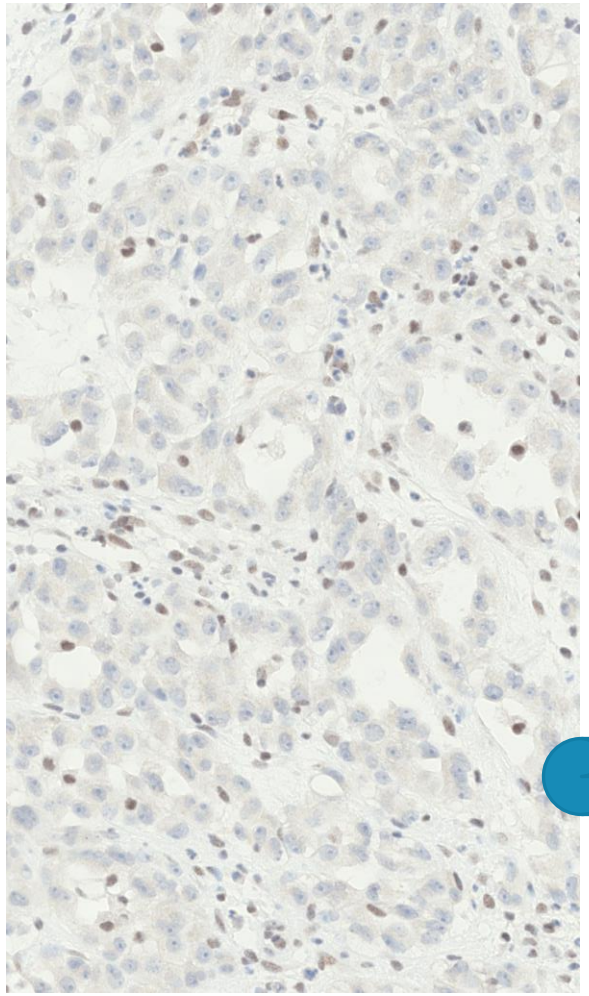
The antibody graveyard – Mesothelioma – BAP1



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 due to gene mutation 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

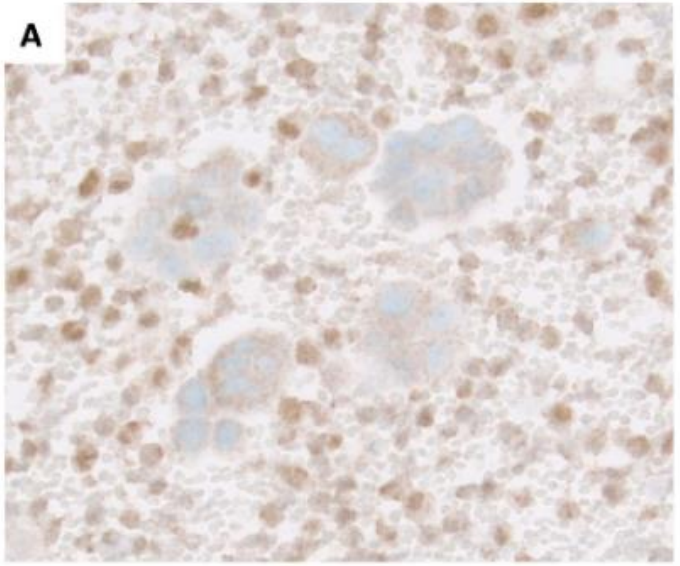
Albino Eccher, MD [1]; Ilaria Girolami, MD [2]; Ersilia Lucenteforte, MD[3]; Giancarlo Troncone, MD [4]; Aldo Scarpa, MD[1]; and Liron Pantanowitz, MD [5]

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*.

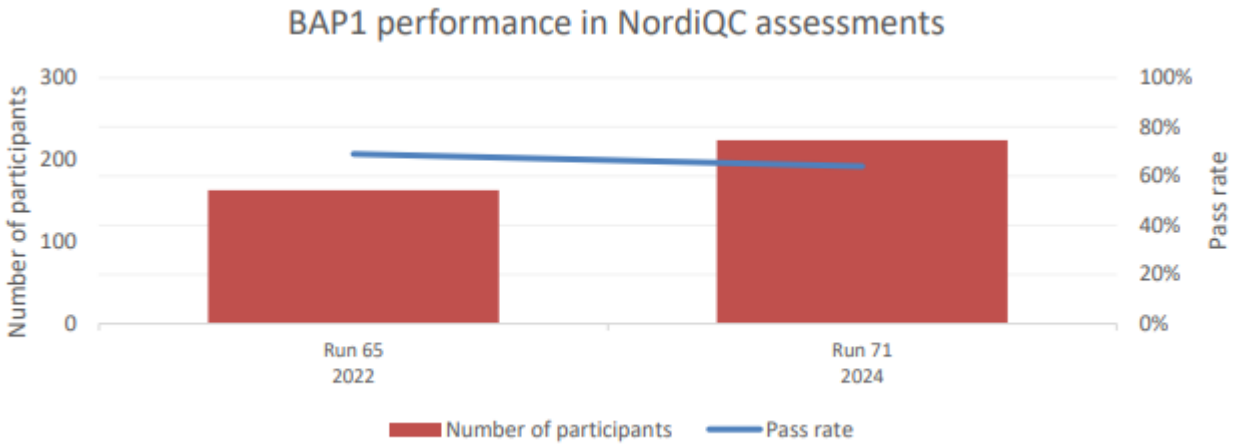
KEY WORDS: biomarker; cytology; immunohistochemistry; mesothelioma; mesothelium; pleural effusion.

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 data



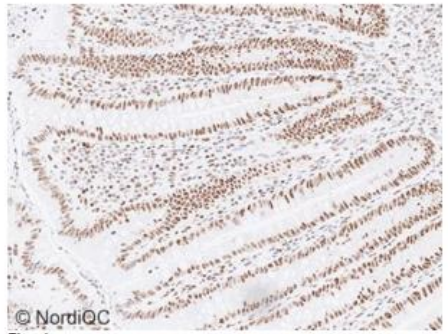


Fig. 1a
Optimal BAP1 staining of appendix using the mAb clone C-4 - diluted, 1:25 (40 min. incubation), epitope retrieval using HIER in CC1 (32 min.), a 3-step multimer based detection system (OptiView) with thyramide amplification (OptiView Amplification) and performed on BenchMark (Ventana/Roche).
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.

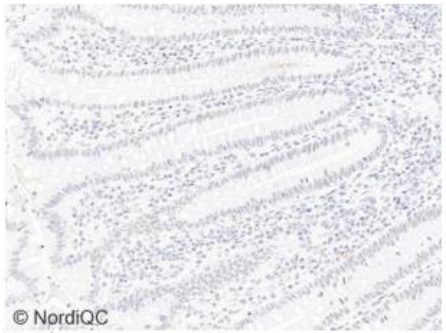


Fig. 1b
Insufficient BAP1 staining of the appendix using same clone and similar protocol settings as in Fig. 1a, but with a less sensitive detection system (UltraView).
Only scattered epithelial cells show a faint nuclear staining reaction. Virtually all lymphocytes/stromal cells are negative.
Same protocol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

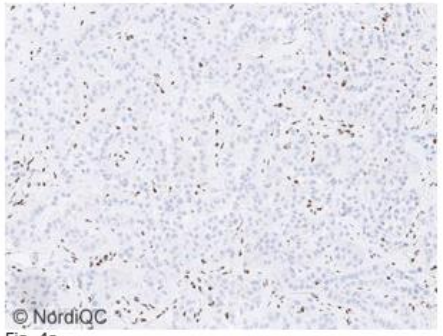


Fig. 4a
Optimal BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1a – 3a. All neoplastic cells are negative, whereas stromal cells show a distinct, weak to moderate nuclear staining reaction.

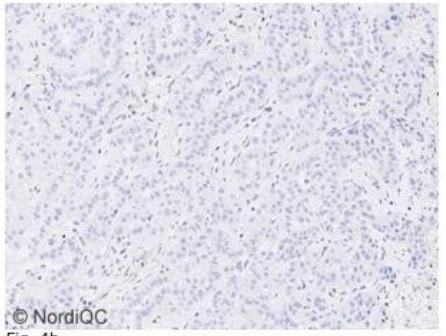


Fig. 4b
Insufficient BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1b – 3b. The neoplastic cells are negative as expected. However, also the stromal cells, expected to be positive serving as internal positive tissue control, are negative.

Mutation identified by negative IHC;

Internal positive tissue control essential!
”MMR similar”

The antibody graveyard – Mesothelioma – BAP1 – NordiQC data

Table 1a. **Overall results for BAP1, run 71**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR.[2]
Concentrated antibodies	183	48	69	56	10	64%	26%
Ready-To-Use antibodies	41	7	18	11	5	61%	17%
Total	224	55	87	67	15		
Proportion		25%	39%	30%	7%	63%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for BAP1, Run 71**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb **C-4**	124	Santa Cruz	33	48	36	7	65%	27%
	10	Immunologic	2	7	1	0	90%	20%
	4	Histopathology	0	1	3	0	-	-
	3	Zeta Corporation	1	0	1	1	-	-
	2	Nordic Biosite	0	0	2	0	-	-
	1	Monosan	1	0	0	0	-	-
mAb **BSB-109**	24	BioSB*	4	11	9	0	63%	17%
	3	LSBio	3	0	0	0	-	-
mAb **IHC761**	1	GenomeMe	1	0	0	0	-	-
rmAb **EPR22826-65**	7	Abcam	3	1	3	0	57%	43%
rmAb **QR119**	1	Quartett	0	1	0	0	-	-
rmAb **ZR454**	1	Histopathology	0	0	1	0	-	-
pAb **AB199396**	1	Abcam	0	0	0	1	-	-
pAb **HPA026803**	1	Sigma-Aldrich	0	0	0	1	-	-
Conc total	183		48	69	56	10		
Proportion			26%	38%	31%	5%	64%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
* including distributed by Gennova (n=6)

Table 1c. **Ready-To-Use antibodies and assessment marks for BAP1, Run 71**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR.[2]
mAb clone **BSB-109 BSB 3300/1/2**	28	Bio SB	7	12	7	2	68%	25%
mAb clone **C-4 PDM595**	5	Diagnostic BioSystems	0	4	1	0	80%	0%
mAb clone **C-4 Z2318MP**	2	Zeta Corporation	0	1	1	0	-	-
mAb clone **C-4 MAB-1143**	1	Fuzhou Maixin Biotech	0	0	0	1	-	-
rmAb clone **BAP1/8959R AND45**	1	BioGenex	0	0	1	0	-	-
rmAb clone **EPR22826-65 8353-C010**	1	Sakura Finetek	0	1	0	0	-	-
pAb **PA525**	1	Abcarta	0	0	1	0	-	-
pAb **API 3247 AA**	2	Biocare Medical	0	0	0	2	-	-
RTU total	41		7	18	11	5		
Proportion			17%	44%	27%	12%	61%	

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

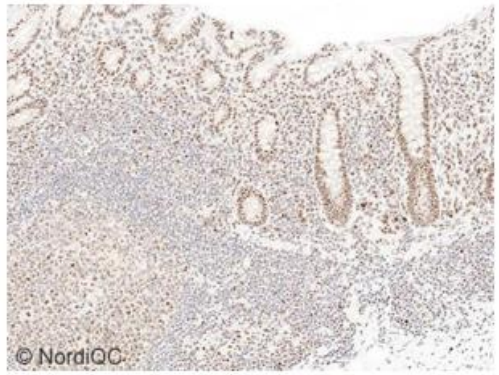


Fig. 2a
Optimal BAP1 staining of appendix using same protocol as in Fig. 1a. Virtually all epithelial cells display a moderate to strong nuclear staining reaction, and the vast majority of lymphocytes/stromal cells show a weak to moderate nuclear staining reaction.

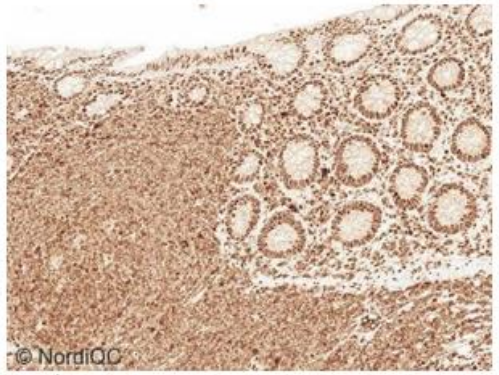


Fig. 2b
Insufficient BAP1 staining of the appendix using same protocol as in Fig. 1b. Similar to Fig. 1b, separation of cells is hindered due to a prominent aberrant granular cytoplasmic staining reaction most likely caused by the tyramide-based amplification of the signal – compare with Fig. 2a, same tissue.

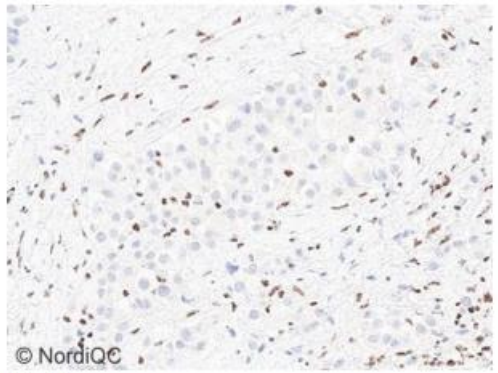


Fig. 4a
Optimal BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1a–3a. All neoplastic cells are negative, whereas stromal cells show a distinct, moderate to strong nuclear staining reaction serving as internal positive tissue control verifying the loss of BAP1 expression in the tumor.

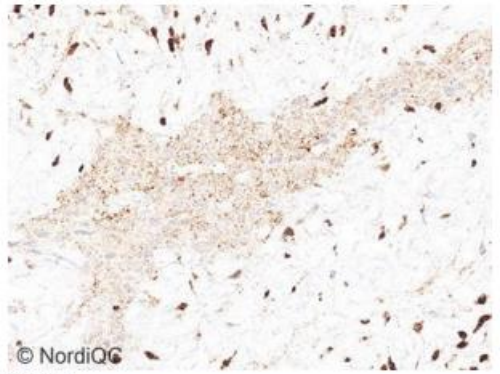
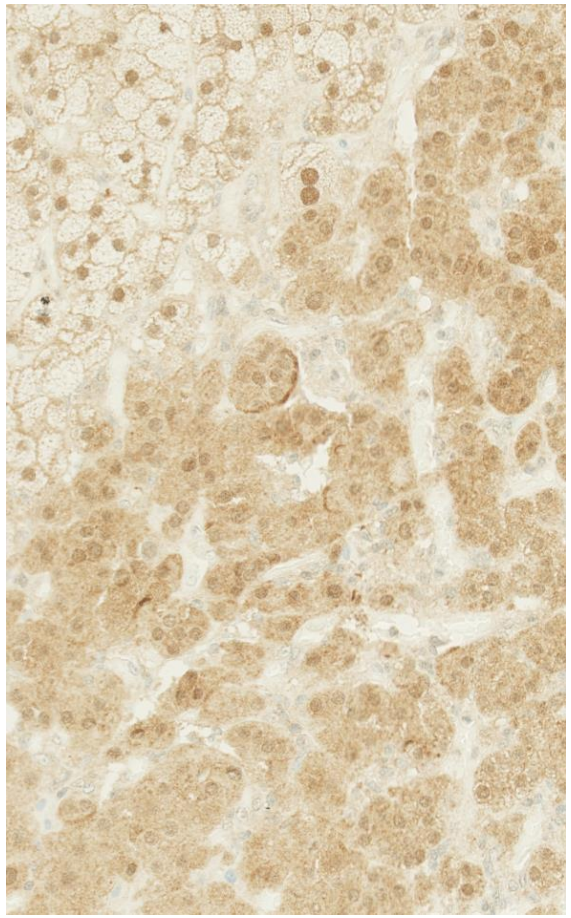


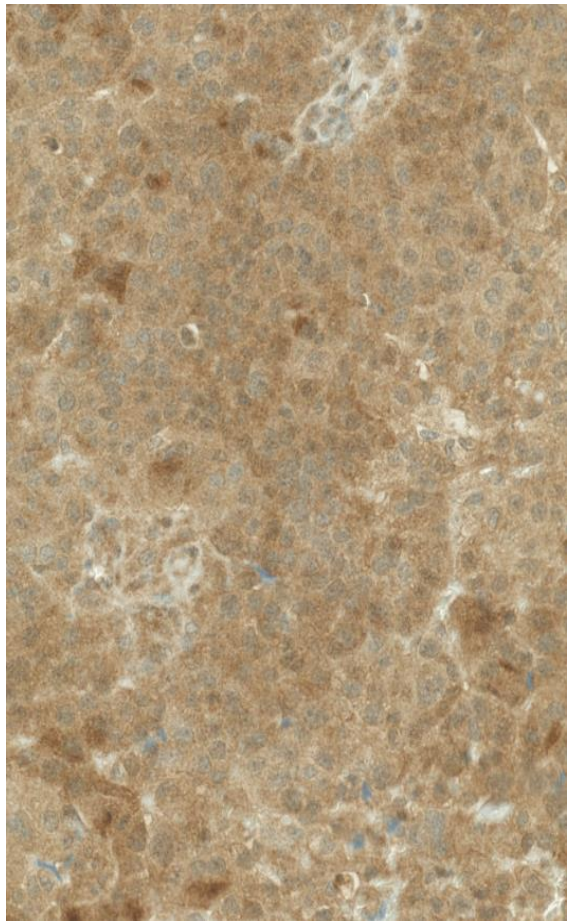
Fig. 4b
Insufficient BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1b – 3b. The stromal cells display a strong nuclear staining reaction, whereas specifically the tumor cells harbor a distinct mostly cytoplasmic but also nuclear aberrant granular staining reaction complicating the interpretation – compare with Fig. 4a, same tissue.

Low affinity clones requiring “utmost highly sensitive” protocols

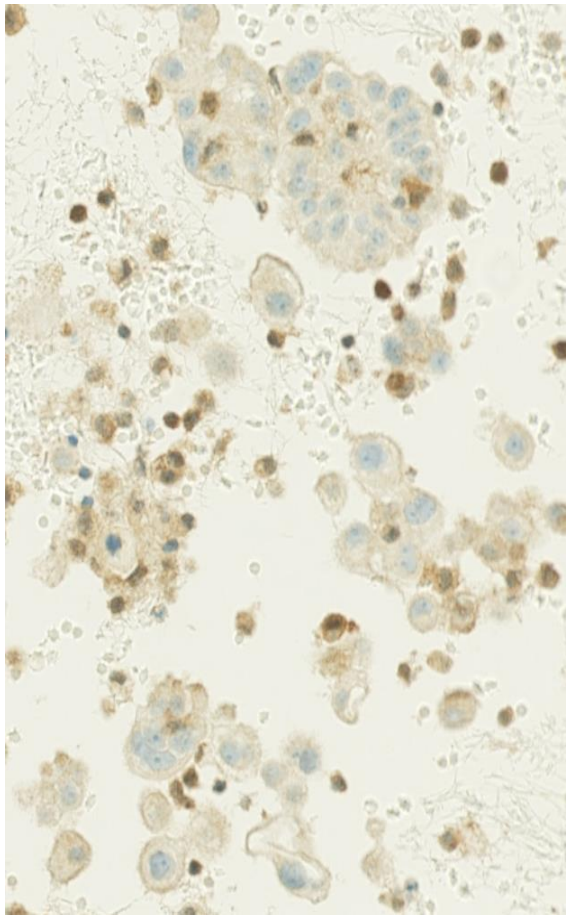
The antibody graveyard – Mesothelioma – MTAP



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 – Mesothelioma – MTAP

ORIGINAL ARTICLE

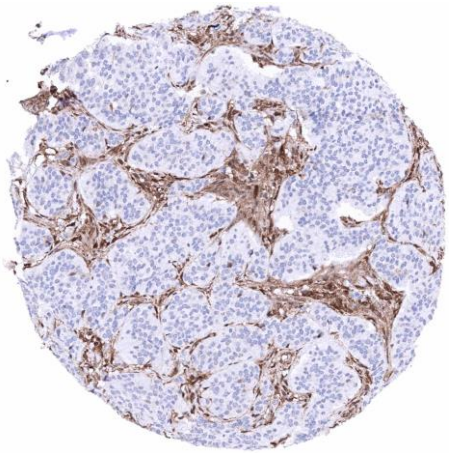
OPEN

(*Am J Surg Pathol* 2024;00:000–000)

Prevalence of S-methyl-5′-thioadenosine Phosphorylase (MTAP) Deficiency in Human Cancer

A Tissue Microarray Study on 13,067 Tumors From 149 Different Tumor Types

Natalia Gorbokon, MD, Niklas Wößner,* Maximilian Lennartz, MD,* Sebastian Dwertmann Rico, MD,* Simon Kind, MD,* Viktor Reiswich, MD,* Florian Viehweger, MD,* Florian Lutz, MD,* Christoph Fraune, MD,*† Andreas M. Luebke, MD,* Claudia Hube-Magg, PhD,* Anne Menz, MD,* Ria Schlichter, MD,* Till Krech, MD,*† Andrea Hinsch, MD,* Eike Burandt, MD,* Guido Sauter, MD,* Ronald Simon, PhD,* Stefan Steurer, MD,* Andreas H. Marx, MD,*‡ Patrick Lebok, MD,*† David Dum, MD,* Sarah Minner, MD,* Frank Jacobsen, MD,* Till S. Clauditz, MD,* Thilo Hackert, MD,§ Faik G. Uzunoğlu, MD,§ Lukas Bubendorf, MD,‖ Christian Bernreuther, MD,* and Martina Kluth, PhD**



Rabbit monoclonal

MTAP (MSVA-741R)

Sensitive and specific marker for homozygous 9p21 deletions.

MS Validated antibodies

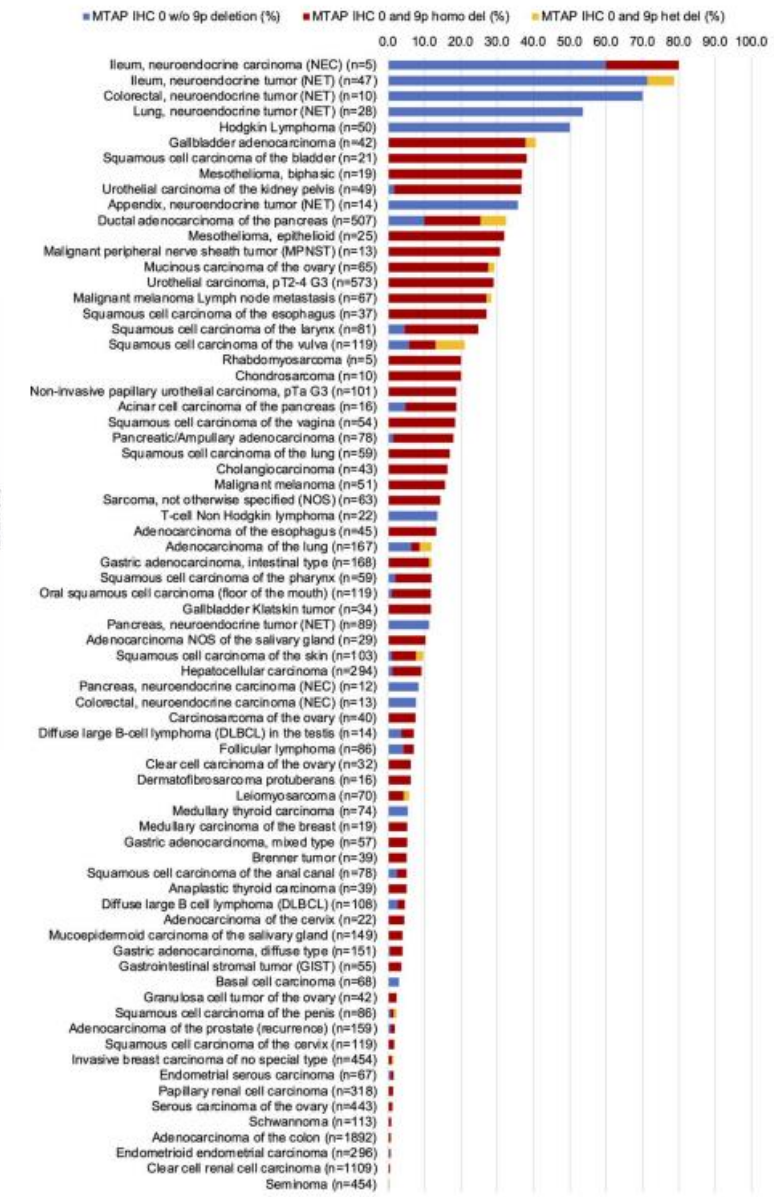


FIGURE 4. Concordance rate of MTAP IHC and 9p21 copy number analysis.

The antibody graveyard – MTAP

Annals of Oncology
Available online 16 September 2024
In Press, Journal Pre-proof What's this?

Original Article

First-in-human study of AMG 193, an MTA-cooperative PRMT5 inhibitor, in patients with *MTAP*-deleted solid tumors: results from phase 1 dose exploration

J. Rodon [1], H. Prenen [2], A. Sacher [3], M. Villalona-Calero [4], N. Penel [5], A. El Helali [6], S. Rottey [7], N. Yamamoto [8], F. Ghiringhelli [9], M.E. Goebeler [10], T. Doi [11], S. Postel-Vinay [12], C.-C. Lin [13], C. Liu [14], C.-H. Chuang [14], K. Keyvanjah [14], T. Eggert [14], B.H. O'Neil [15]

nature COMMUNICATIONS

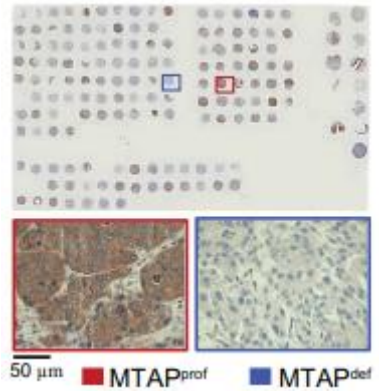
NATURE COMMUNICATIONS | (2022)13:1797 | https://doi.org/10.1038/s41467-022-29397-z | www.nature.com/naturecommunications

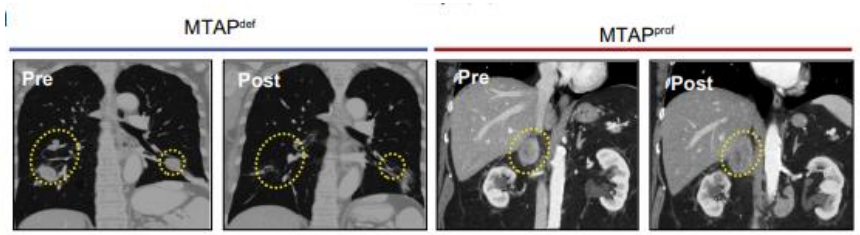
ARTICLE

https://doi.org/10.1038/s41467-022-29397-z OPEN

MTAP deficiency creates an exploitable target for antifolate therapy in 9p21-loss cancers

Omar Alhalabi [1,12], Jianfeng Chen[1,12], Yuxue Zhang[1,12], Yang Lu [2,12], Qi Wang [3], Sumankalai Ramachandran[1], Rebecca Slack Tidwell [4], Guangchun Han [5], Xinmiao Yan[5], Jieru Meng[1], Ruiping Wang[5], Anh G. Hoang[1], Wei-Lien Wang [1], Jian Song[1], Lidia Lopez[1], Alex Andreev-Drakhlin[1], Arlene Siefker-Radtke [1], Xinqiao Zhang[1], William F. Benedict[1], Amishi Y. Shah [1], Jennifer Wang[1], Pavlos Msaouel[1], Miao Zhang[6], Charles C. Guo[6], Bogdan Czerniak[6], Carmen Behrens[7], Luisa Soto [8], Vassiliki Papadimitrakopoulou[7], Jeff Lewis[4], Waree Rinsurongkawong[4], Vadeerat Rinsurongkawong[4], Jack Lee [4], Jack Roth [9], Stephen Swisher [9], Ignacio Wistuba[6], John Heymach [7], Jing Wang [3], Matthew T. Campbell [1], Eleni Efstathiou[1], Mark Titus[1], Christopher J. Logothetis [1], Thai H. Ho [10,13], Jianjun Zhang [7,13], Linghua Wang [5,11,13]✉ & Jianjun Gao [1,13]✉





IHC for MTAP ”forecast”;

Type I IHC assay for e.g. mesothelioma

Type II IHC assay for solid tumours

The antibody graveyard – Mesothelioma – Merlin

ARTICLE

Check for updates

Clinical and molecular validation of BAP1, MTAP, P53, and Merlin immunohistochemistry in diagnosis of pleural mesothelioma

David B. Chapel[1,2], Jason L. Hornick[1], Julianne Barlow[3], Raphael Bueno[3] and Lynette M. Sholl[1,4]

Modern Pathology (2022) 35:1383 – 1397

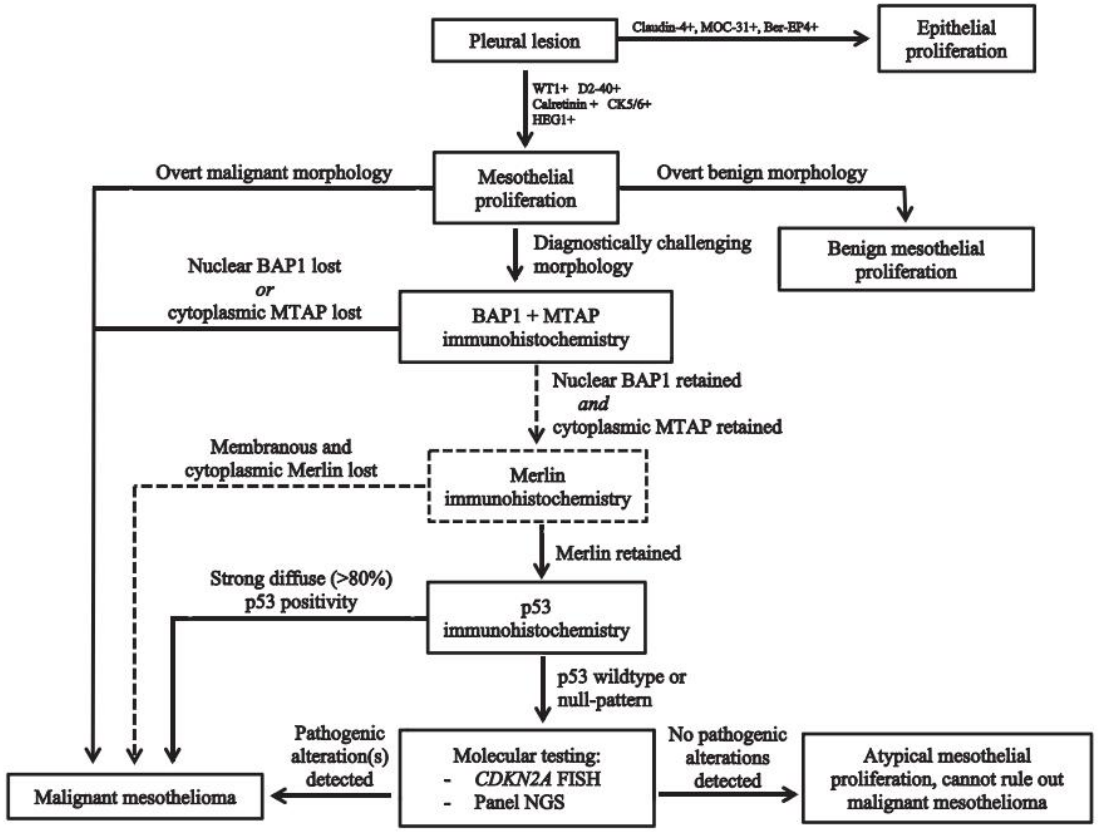


Fig. 5 Proposed diagnostic algorithm. Dashed lines indicate that Merlin immunohistochemistry is a provisional diagnostic marker of malignant mesothelioma, pending independent validation in subsequent studies. FISH, fluorescence in situ hybridization; NGS, next-generation sequencing. Figure adapted from Chapel DB, et al. Mod Pathol. 2020;33:245–254.

Merlin is the protein product of NF2 gene and one of the most versatile tumor suppressors capable of integrating different mechanisms that **regulate cell proliferation, motility, survival and signaling pathways** underlying and governing those mechanisms. Merlin is considered a member of the band 4.1 families of cytoskeleton-associated proteins also called ERM family and acts as **tumor suppressor.**

The antibody graveyard – Mesothelioma – Merlin

Clone D1D8, Cell Signaling.

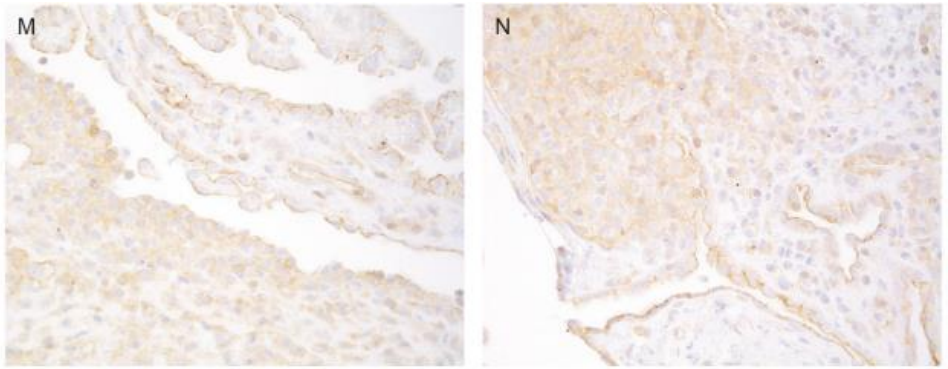
ARTICLE

Check for updates

Clinical and molecular validation of BAP1, MTAP, P53, and Merlin immunohistochemistry in diagnosis of pleural mesothelioma

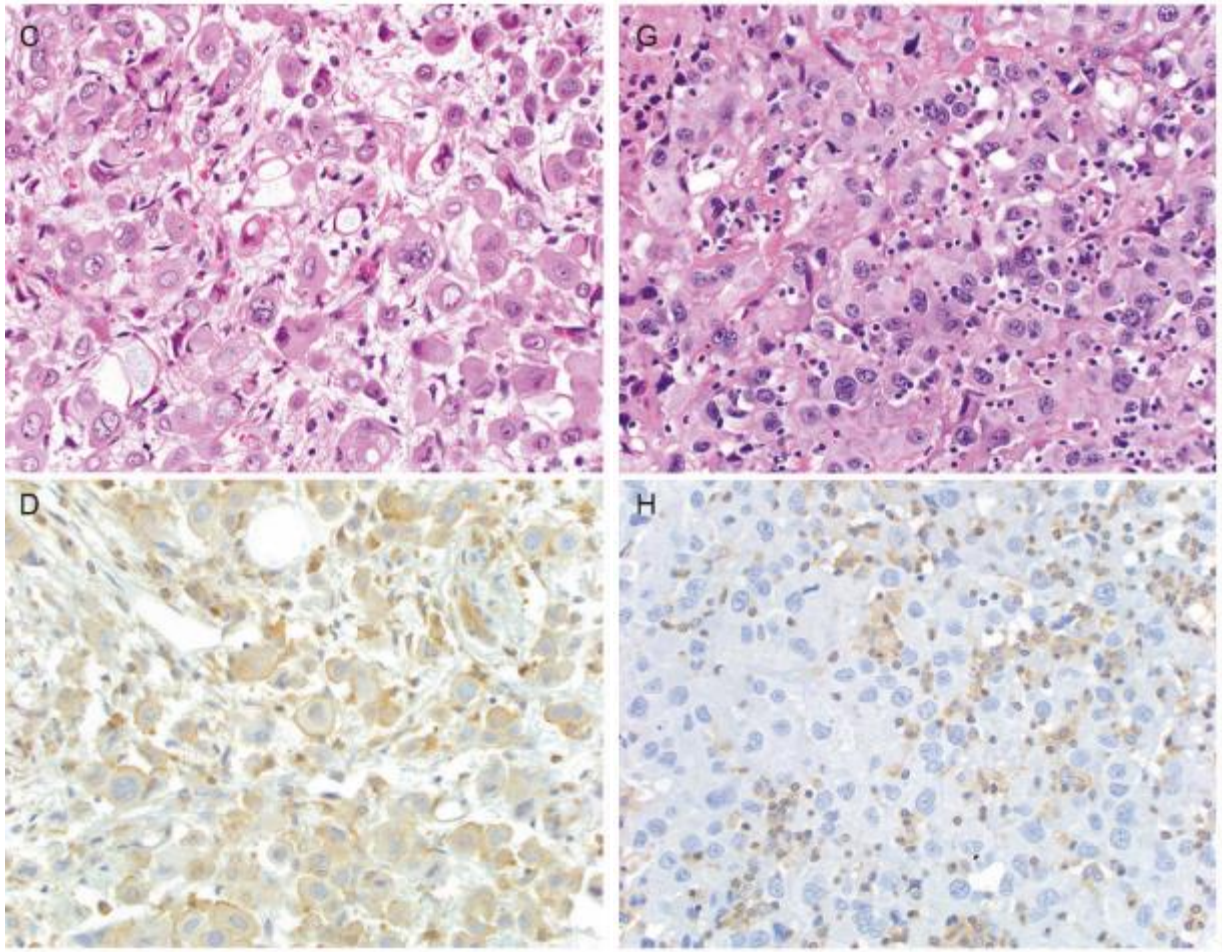
David B. Chapel[1,2], Jason L. Hornick[1], Julianne Barlow[3], Raphael Bueno[3] and Lynette M. Sholl[1,4]

Merlin expression in normal/reactive mesothelial cells is characterized by weak to moderate membranous staining reaction and/or as a granular cytoplasmic positivity.



Pleural mesothelioma 1

Pleural mesothelioma 2



IHC and NGS agreement; 77%

No NF2 gene mutation

NF2 gene mutation (+/-50%)

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%	Preferred by many pathologists due to increased sensitivity, but unspecific….
INSM1	New	85-95%	Insulinoma-associated protein 1

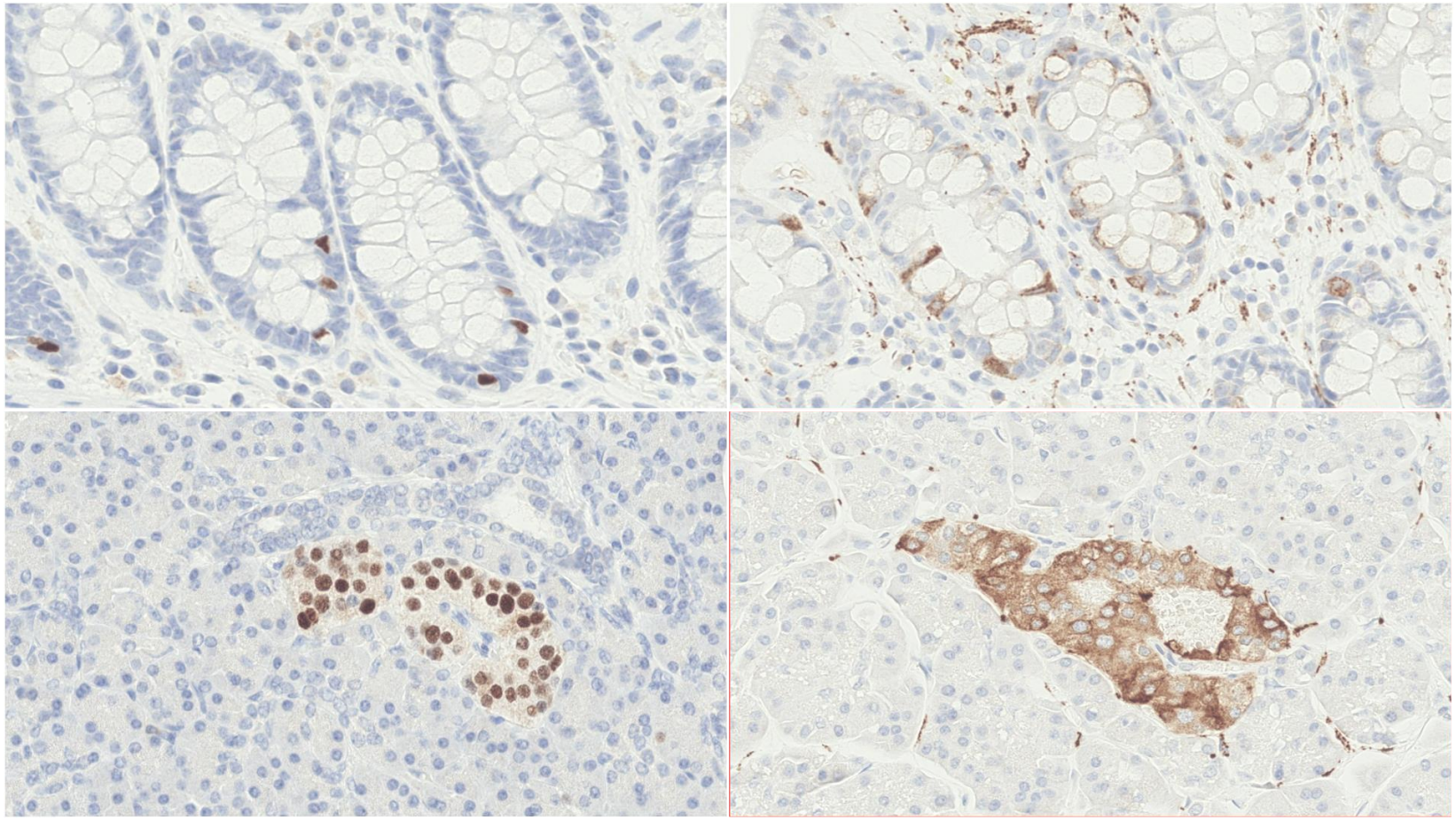
INSM1 is the best marker for the diagnosis of neuroendocrine tumors: comparison with CGA, SYP and CD56
Kosuke Fujino et al; Int J Clin Exp Pathol 2017;10(5):5393-5405

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

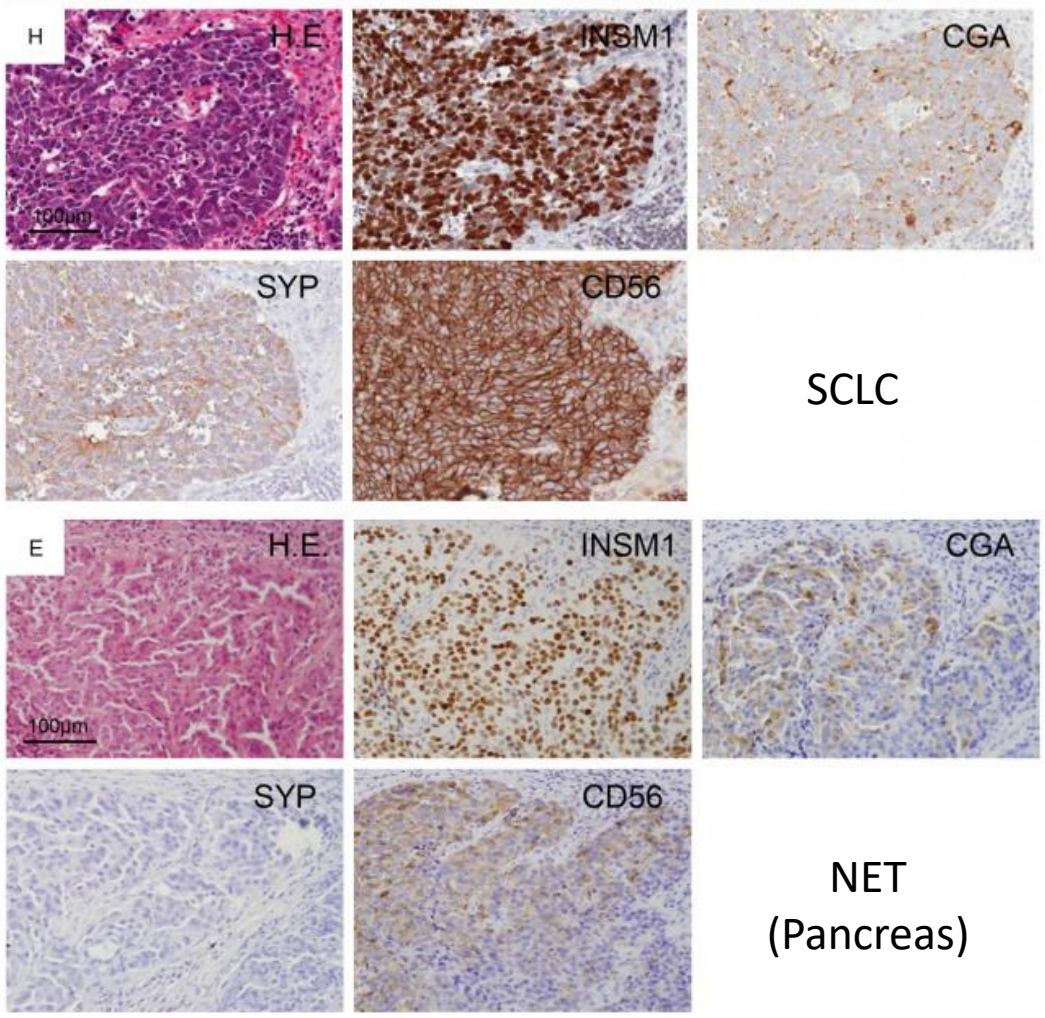
SYP

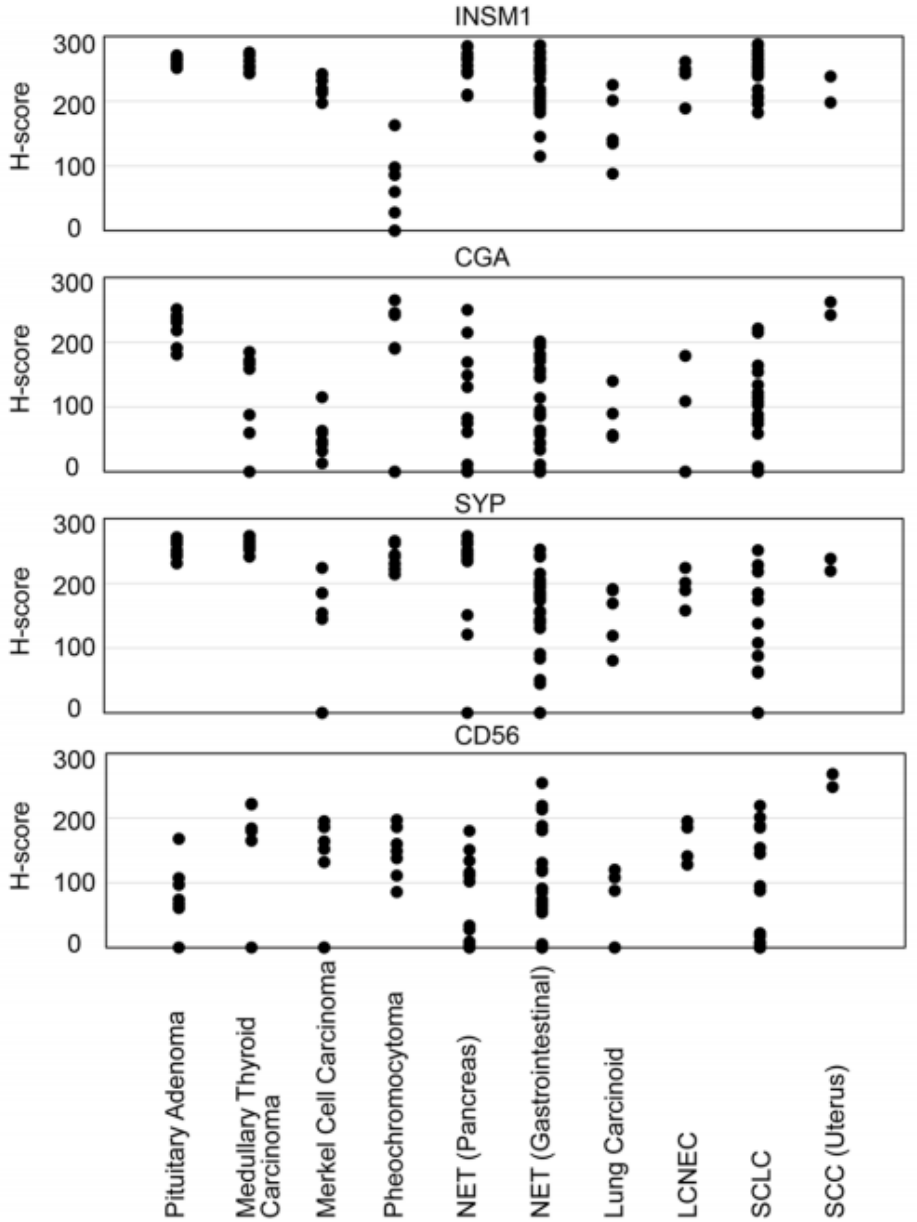
Insulinoma-associated protein 1 (INSM1) is a transcription factor that has recently emerged as a useful diagnostic marker of NE differentiation. INSM1 expression has been tightly coupled to NE differentiation in normal and neoplastic tissues across a wide range of anatomic sites including pancreas, gastrointestinal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

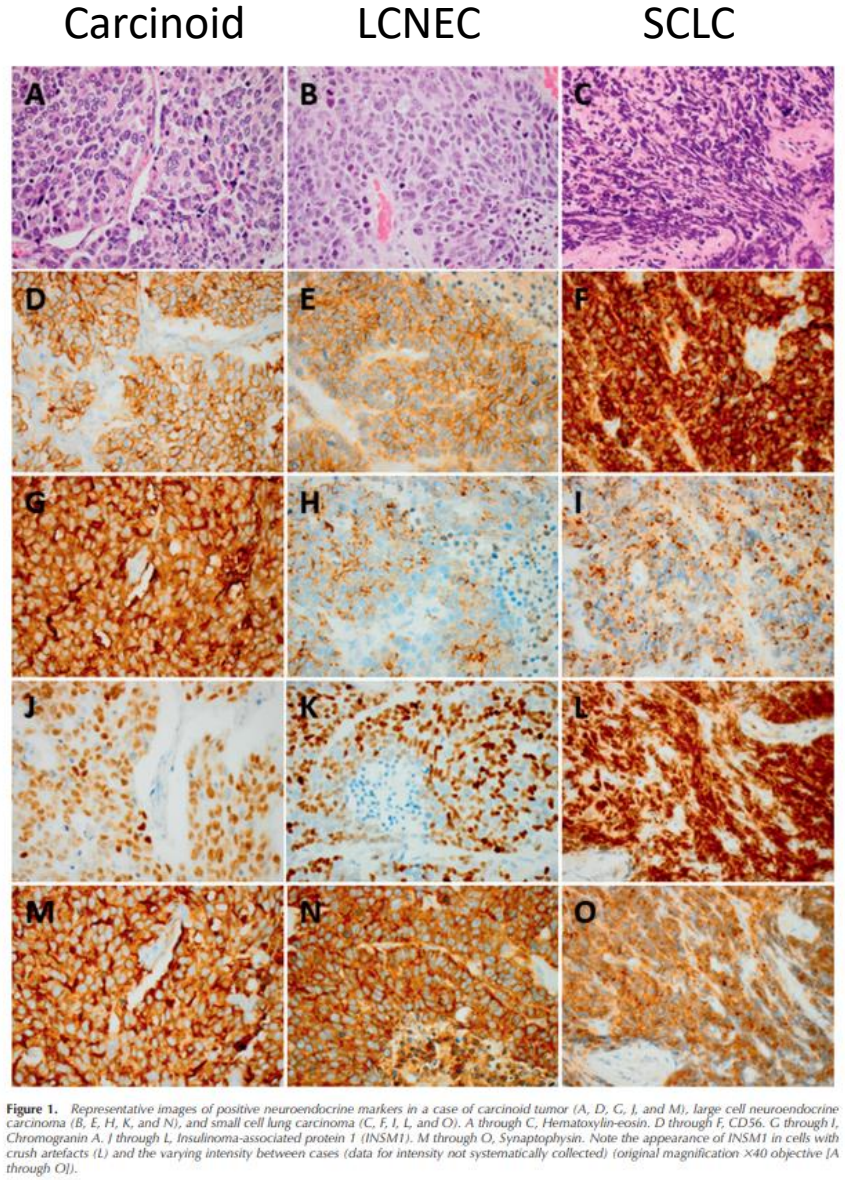


Figure 1. Representative images of positive neuroendocrine markers in a case of carcinoid tumor (A, D, G, J, and M), large cell neuroendocrine carcinoma (B, E, H, K, and N), and small cell lung carcinoma (C, F, I, L, and O). A through C, Hematoxylin-eosin. D through F, CD56. G through I, Chromogranin 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 – Neuroendocrine markers

Journal of *Molecular Pathology*

MDPI

Review

Insulinoma-Associated Protein 1 (INSM1): Diagnostic, Prognostic, and Therapeutic Use in Small Cell Lung Cancer

Renato Rocha [1] and Rui Henrique [2,3,4,*]

J. Mol. Pathol. 2022, 3, 140–167. https://doi.org/10.3390/jmp3030013

An extensive bibliographic search was conducted in PubMed® focusing on articles published since 2015 (n=14).

According to the literature, INSM1 is a highly sensitive (75–100%) and specific (82–100%) neuroendocrine immunohistochemical marker for SCLC diagnosis.

It can be used in histological and cytological samples. Although advantageous, its standalone use is currently not recommended.

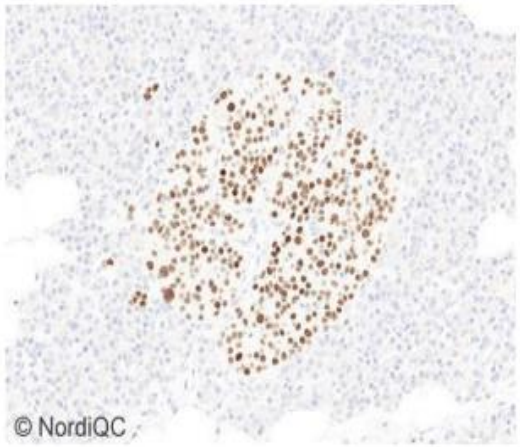
The antibody graveyard – Neuroendocrine markers – INSM1 clone choice….

Conclusion
The rmAb clone **MRQ-70** was the most successful Ab for the demonstration of INSM1. As concentrated (conc.) format within a laboratory developed assay, optimal results were obtained on all four main stainer platforms. Efficient HIER in an alkaline buffer and carefully calibrated primary Ab together with a sensitive detection system were the most important prerequisites for a sufficient staining. The mAb clone A-8 gave overall an inferior and less reproducible performance.

Table 1a. **Overall results for INSM1, run 71**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	104	39	31	20	14	68%	38%
Ready-To-Use antibodies	52	16	26	4	6	81%	31%
Total	156	55	57	24	20		
Proportion		35%	37%	15%	13%	72%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.



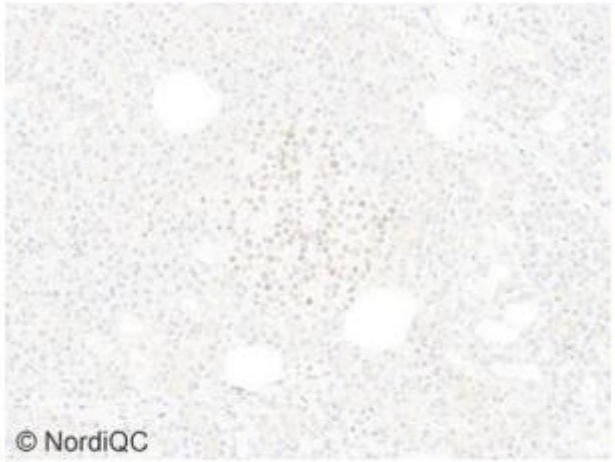


Fig. 6a
Insufficient INSM1 staining reaction of pancreas using exactly same protocols as in Figs. 1b – 5b, But in a different laboratory.
A too weak staining reaction is seen in the endocrine islet cells. Compare with optimal result in Fig. 2a.
The mAb clone A-8 was found to give less reproducible results among the participants. Same protocol settings could give the expected results, results characterized as false positive or false negative.

Table 1b. **Concentrated antibodies and assessment marks for INSM1, run 71**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **A-8**	1	Diagnostic Biosystems	1	-	-	-	-	-
	5	Gennova	1	2	1	1	60%	20%
	1	Master Diagnostica	-	-	1	-	-	-
	1	Monosan	1	-	-	-	-	-
	2	Nordic Biosite	-	1	1	-	-	-
	50	Santa Cruz	10	15	13	12	50%	20%
	5	Zeta Corporation	4	1	-	-	100%	80%
mAb clone **BSB-123**	3	Bio SB	-	3	-	-	-	-
	1	LS Bio	-	1	-	-	-	-
rmAb clone **MSVA-456R**	1	MS Validated Antibodies	-	1	-	-	-	-
rmAb clone **BP6240**	1	Biolynx Biotechnology	1	-	-	-	-	-
rmAb clone **EPR23199-37-6-1**	1	Abcam	-	-	1	-	-	-
rmAb clone **MRQ-70**	27	Cell Marque	19	6	2	-	93%	70%
rmAb clone **QR116**	1	Quartett	-	-	-	1	-	-
rmAb clone **RBT-INSM1**	2	Bio SB	-	1	1	-	-	-
rmAb clone **ZR395**	2	Zeta Corportation	2	-	-	-	-	-
Total	104		39	31	20	14		
Proportion			38%	30%	19%	13%	68%	

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

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%	Moderate to highly sensitive but not specific
GATA3	Yes	90-95%	Highly sensitive for ER+ breast carcinoma – 20-60% positivity in TNBC and metaplastic type Low specificity – ”a selective marker”
TNBC			
SOX10	?	40-60%	Moderately sensitive and specific (obs melanoma)
TRPS1*	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.

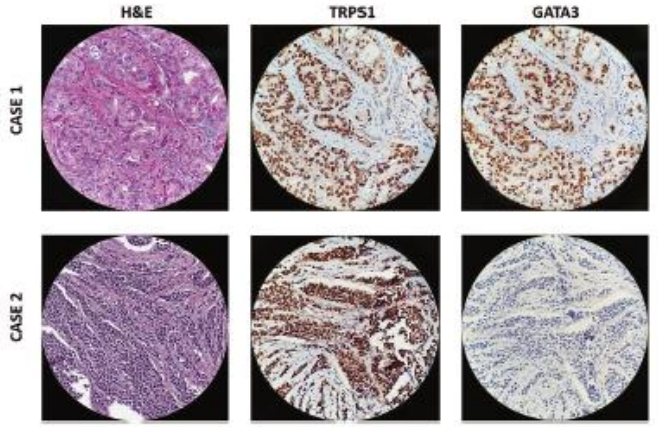
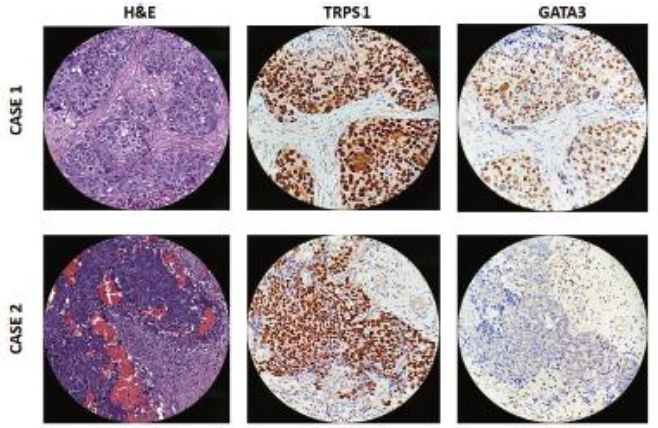


Fig. 4 TRPS1 and GATA3 expression in representative nonmetaplastic TNBC cases. Case 1 shows a poorly differentiated carcinoma with high expression of TRPS1 and intermediate to high expression of GATA3. Case 2 shows a poorly differentiated carcinoma with high expression of TRPS1 and negative GATA3.



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).

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

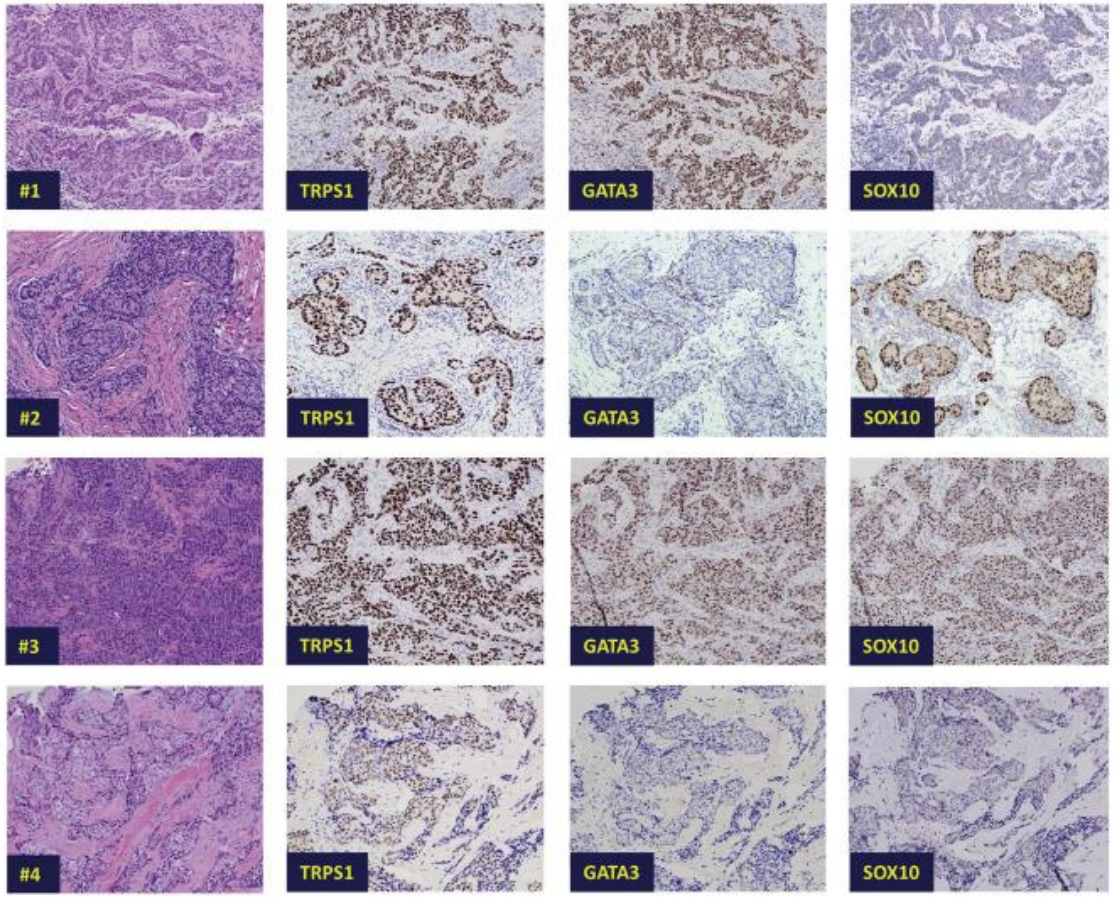


Fig. 1 TRPS1, GATA3, and SOX10 expression (x10) in representative cases of triple-negative invasive breast carcinoma of no special type (IBC-NST). Case 1 shows a triple-negative IBC-NST with high expression of TRPS1 and GATA3 and negative expression of SOX10. Case 2 shows a triple-negative IBC-NST with high expression of TRPS1 and SOX10 and negative expression of GATA3. Case 3 shows a triple-negative IBC-NST with high expression of TRPS1, GATA3, and SOX10. Case 4 shows a triple-negative IBC-NST with high expression of TRPS1 and negative expression of GATA3 and SOX10.

rabbit polyclonal antibody against human TRPS1 (PA5-84874 from Invitrogen/Thermo Fisher Scientific)

Table 3 Correlation between TRPS1 and GATA3 expression in TNBCs (n = 292).

TNBCs (n = 292)		TRPS1, no. (%)		Total, no. (%)
		Intermediate and high positive	Negative and low positive	
GATA3, no. (%)	Intermediate and high positive	128 (43.8)	1 (0.3)	129 (44.2)
	Negative and low positive	145 (49.7)	18 (6.2)	163 (55.8)
Total, no. (%)		273 (93.5)	19 (6.5)	292 (100)

The chi-square statistic is 12.4794. The p-value is 0.000411.

Table 4 Correlation between TRPS1 and SOX10 expression in TNBCs (n = 292).

TNBCs (n = 292)		TRPS1, no. (%)		Total, no. (%)
		Intermediate and high positive	Negative and low positive	
SOX10, no. (%)	Intermediate and high positive	156 (53.4)	2 (0.7)	158 (54.1)
	Negative and low positive	117 (40.1)	17 (5.8)	134 (45.9)
Total, no. (%)		273 (93.5)	19 (6.5)	292 (100)

The chi-square statistic is 15.546. The p-value is 0.000081.

Esther C. Yoon, Gang Wang, Bryce Parkinson, et Al.
TRPS1, GATA3, and SOX10 expression in triple-negative breast carcinoma. Human Pathology, Volume 125, 2022, Pages 97-107.

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

TABLE 1 TRPS1 Expression in Breast, Gynecologic, and Gastrointestinal Carcinomas Under 2 Different Conditions

TMAs and tumors	Condition 1: TRPS1, pH 9.0 (Epitope Retrieval Solution 2, 1:1000), +ve/total (%)	Condition 2:TRPS1, pH 6.0 (Epitope Retrieval Solution 1, 1:300), +ve/total (%)	Condition 1 TRPS1 H-score, median (range; IQR on positive cases)	Condition 2 TRPS1 H-score, median (range; IQR on positive cases)
Breast carcinoma (TNBC, including ER-low)	123/138 (89)	117/138 (85)	230 (1-300; 130-270)	170 (1-300; 100-225)
Breast carcinoma (HR+/ERBB2−)	51/53 (96)	48/53 (91)	180 (1-300; 105-240)	140 (5-280; 85-200)
Breast carcinoma (HR+/ERBB2+)	6/6 (100)	6/6 (100)	190 (110-290; 150-253)	135 (80-280; 80-220)
Endometrial endometrioid adenocarcinoma	49/69 (71)	46/69 (67)	25 (2-170; 10-70)	30 (1-150; 10-58)
Ovarian tumors, all	43/250 (17)	38/250 (15)	15 (1-110; 5-25)	7.5 (1-120; 5-40)
Carcinoma NOS	2/3 (67)	2/3 (67)	15 (10-20; N/A)	3.5 (2-5; N/A)
Clear cell carcinoma	7/25 (28)	5/25 (20)	5 (2-30; 5-18)	10 (1-10; 5-10)
Endometrioid carcinoma	1/5 (20)	2/5 (40)	80 (N/A; N/A)	61 (1-120; N/A)
HG serous carcinoma	29/55 (53)	24/55 (44)	15 (1-110; 5-30)	20 (1-90; 5-46)
LG serous carcinoma	1/24 (4)	2/24 (8)	5 (N/A; N/A)	3 (1-5; N/A)
Serous borderline tumors	3/138 (2)	3/138 (2)	5 (5-5; N/A)	2 (1-5; N/A)
Vulvar squamous cell carcinoma	54/96 (56)	29/97 (30)	30 (1-140; 10-60)	20 (1-120; 10-50)
Pancreatic ductal adenocarcinoma	2/20 (10)	2/20 (10)	22.5 (5-40; 14-31)	22.5 (5-40; 14-31)
Gastric adenocarcinoma	4/12 (33)	0/12 (0)	7.5 (2-10; 4-10)	N/A

ER, estrogen receptor; ERBB2, formerly HER2 or HER2/neu; H-score, histochemical score; HG, high grade; HR, hormone receptor; LG, low grade; N/A, not applicable; NOS, not otherwise specified; TMA, tissue microarray; TNBC, triple-negative breast cancer.

rabbit polyclonal antibody against human TRPS1 (PA5-84874 from Invitrogen/Thermo Fisher Scientific)

pAb PA5-84874 most cited
rmAb EP392 potential
rmAb EPR16171 potential
rmAb MSVA-512R potential

Conclusions:
TRPS1 stains approximately 90% of breast carcinomas but also up to 71% of endometrial carcinomas, albeit with a weaker median expression.

Our data show that although TRPS1 is a highly sensitive marker for TNBCs, it is not as highly specific as previously reported.

Rayan Rammal et al. Utility of TRPS1 immunohistochemistry in confirming breast carcinoma Emphasis on staining in triple-negative breast cancers and gynecologic tumors. Am J Clin Pathol 2023;XX:1-10

The antibody graveyard – TRPS IHC assay optimization and validation…… ECP 2024

TRPS1 in Breast Cancer:
A comparative study of five different IHC assays

Birgit Truumees[1], Mia Korsdal Stensballe[2], Rasmus Røge[1], Søren Nielsen[1]

Affiliation: [1]NordiQC, Department of Pathology, Aalborg University Hospital, Denmark, [2]Department of Pathology, Aalborg University Hospital, Denmark

Disclosures: Birgit Truumees: None; Heidi Lykke Kristoffersen: None; Mia Korsdal Stensballe: None; Rasmus Røge: None; Søren Nielsen: None.

Background & Objective

Immunohistochemistry (IHC) for TRPS1 (Trichorhinophalangeal syndrome 1) is a novel biomarker for breast cancer (BC), especially triple negative breast cancer (TNBC). The aim of the study was to compare the staining patterns of five different TRPS1 IHC assays.

Methods

Five commercially available antibodies for TRPS1 were optimized on TMAs with a range of normal tissues and BCs. Subsequently each IHC assay was validated on TMAs comprising of 135 breast carcinomas (BC), including 64 TNBCs, and 74 various neoplasias. A positive cut-off at ≥10% of neoplastic cells (NCs) with nuclear TRPS1 expression was applied to determine the diagnostic sensitivity and specificity.

Table 1. The five TRPS1 IHC assays used

	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
Antibody / vendor	EP392 / BioSB	polyclonal / Invitrogene	MSVA-512R / MS validated	8131R / NeoBiotechnologies	ZR382 / Zeta corporation
Titer / time	1:40 / 30 min	1:100 / 32 min	1:200 / 32 min	1:1000 / 32 min	1:1000 / 32 min
Diluent	Background sniper, Biocare Medical	Background sniper, Biocare Medical	Antibody diluent, Dako/Agilent	Antibody diluent, Dako/Agilent	Antibody diluent, Dako/Agilent
HIER / time	TRS High, pH 9,0 / 20 min	CC1 pH 8,5 / 48 min	CC1 pH 8,5 / 48 min	CC1 pH 8,5 / 48 min	CC1 pH 8,5 / 48 min
Detection system	EnVision Flex+	OptiView DAB	OptiView DAB	OptiView DAB	OptiView DAB
IHC platform	Dako Omnis	Ventana Benchmark Ultra	Ventana Benchmark Ultra	Ventana Benchmark Ultra	Ventana Benchmark Ultra

The antibody graveyard – TRPS IHC assay optimization and validation…… ECP 2024

Results

The five different and individually optimized IHC assays provided a fully comparable level of diagnostic sensitivity and specificity (see Table 2 and Graph 1). Positive TRPS1 staining in ≥10% of neoplastic cells was seen in a subset of lung, gynecological and urothelial carcinomas with all assays. In addition, two assays also labelled melanomas (20%). If the positive cut-off was changed to ≥1%, TRPS1 was observed in more neoplasias and the diagnostic specificity was reduced to 82-85% (see Graph 1 & 2).

Table 2. Sensitivity of 5 TRPS1 IHC assays for detecting BCs

	Total, n	≥10% NCs Positive, n (%)				
		Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
Luminal BC	71	71 (**100**)	71 (**100**)	71 (**100**)	71 (**100**)	71 (**100**)
TNBC	64	60 (**94**)	60 (**94**)	60 (**94**)	60 (**94**)	60 (**94**)
BCs, total	135	131 (**97**)	131 (**97**)	131 (**97**)	131 (**97**)	131 (**97**)

Graph 1. Specificity of 5 TRPS1 IHC assays with cut-off points at 1% and 10% of tumor cells stained

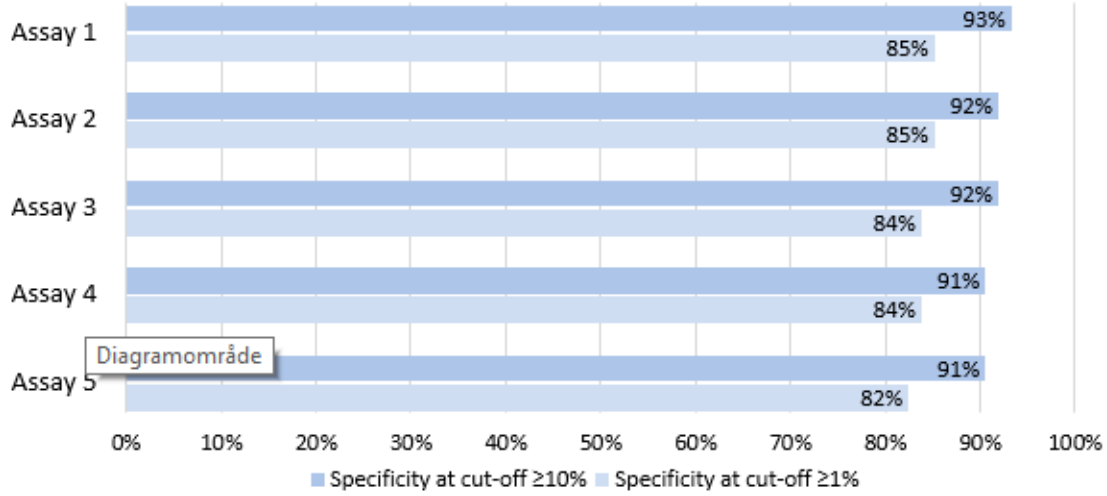
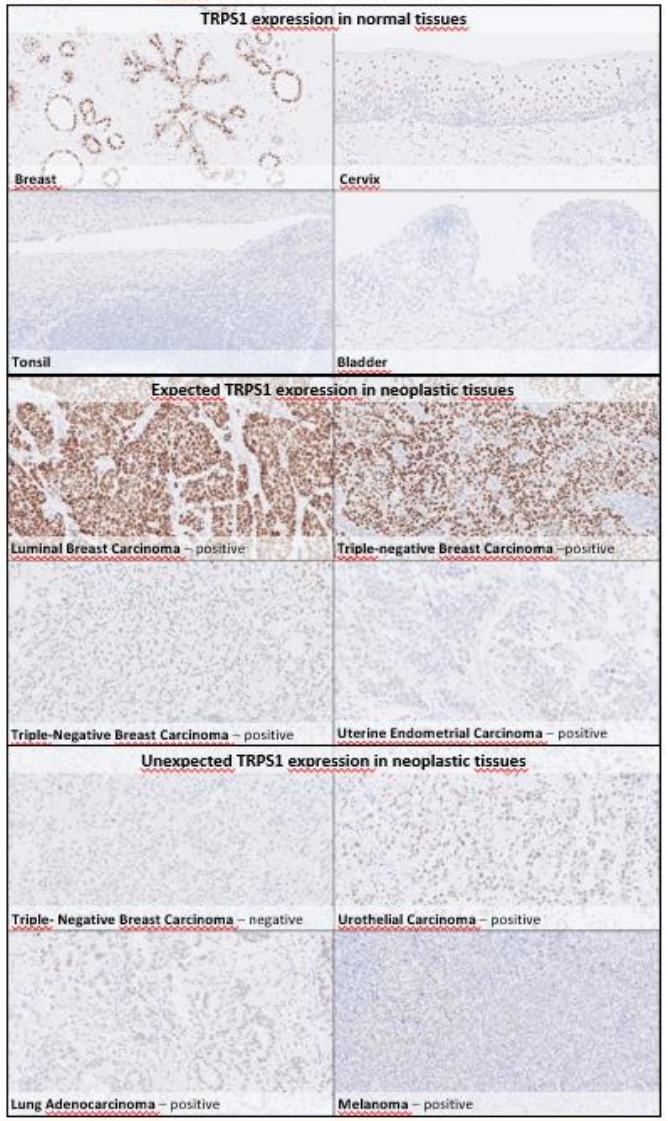


Figure 1 TRPS1 staining patterns in normal and neoplastic tissues



The antibody graveyard – Potential new predictive IHC biomarkers

	To come	Indication	Comments
Claudin 18.2	Yes	GI, Pancreatic carc	IHC based estimate on Claudin 18.2 expression – 75% cut-off potentially

The antibody graveyard – Potential new predictive IHC biomarkers – Gastric carcinoma

nature reviews clinical oncology

https://doi.org/10.1038/s41571-024-00874-2

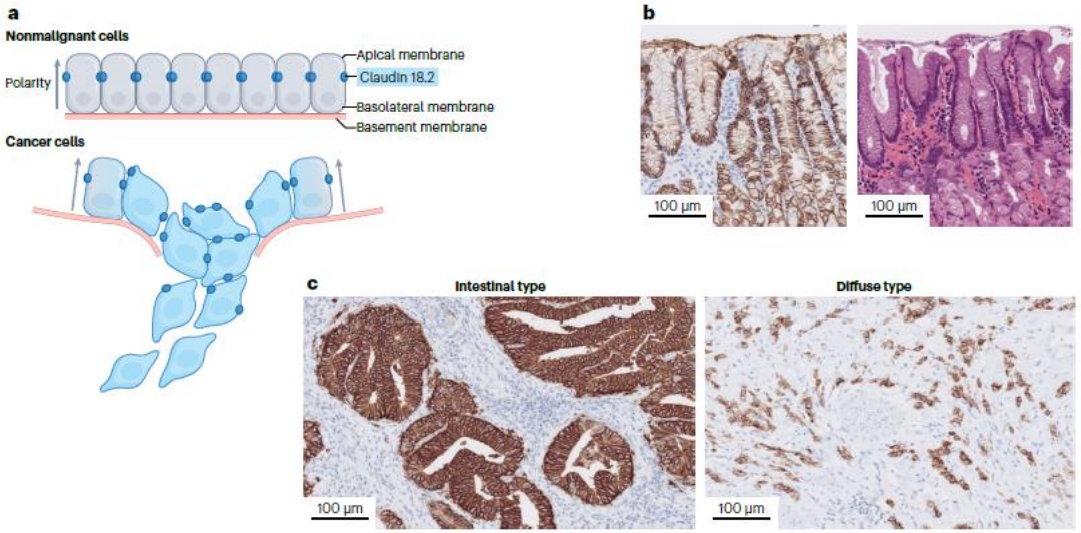
Nature Reviews Clinical Oncology | Volume 21 | May 2024 | 354–369

Review article

Check for updates

Claudin 18.2 as a novel therapeutic target

Izuma Nakayama[1,5], Changsong Qi[2,5], Yang Chen[2], Yoshiaki Nakamura[1,3,4], Lin Shen[2] & Kohei Shitara[1]



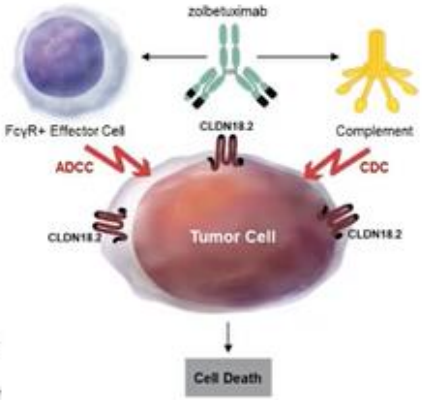
Key points

- Claudin 18.2 is expressed almost exclusively in the gastric mucosa, and no clear evidence exists of a role of this tight-junction protein in the carcinogenesis and/or proliferation of gastric cancer.
- Two pivotal phase III trials to test zolbetuximab, a monoclonal antibody that targets claudin 18.2, have demonstrated statistically significant improvements in both the progression-free and overall survival of patients with unresectable gastric cancer and have established claudin 18.2 as a validated therapeutic target.
- Determination of the optimal treatment sequence, especially for patients who are potentially eligible for several targeted therapies or immunotherapies, as well as the feasibility of biomarker tests for multiple proteins, will be the subject of much debate following the clinical implementation of zolbetuximab.
- Claudin 18.2 can potentially be targeted using a wide range of therapeutic modalities beyond monoclonal antibodies including bispecific antibodies, antibody–drug conjugates, chimeric antigen receptor T cells and mRNA-based approaches.
- The development of claudin 18.2-targeted therapies is expanding and will probably encompass other claudin 18.2-positive cancer types.

The antibody graveyard – Potential new predictive IHC biomarkers

Introduction: Zolbetuximab Targets CLDN18.2

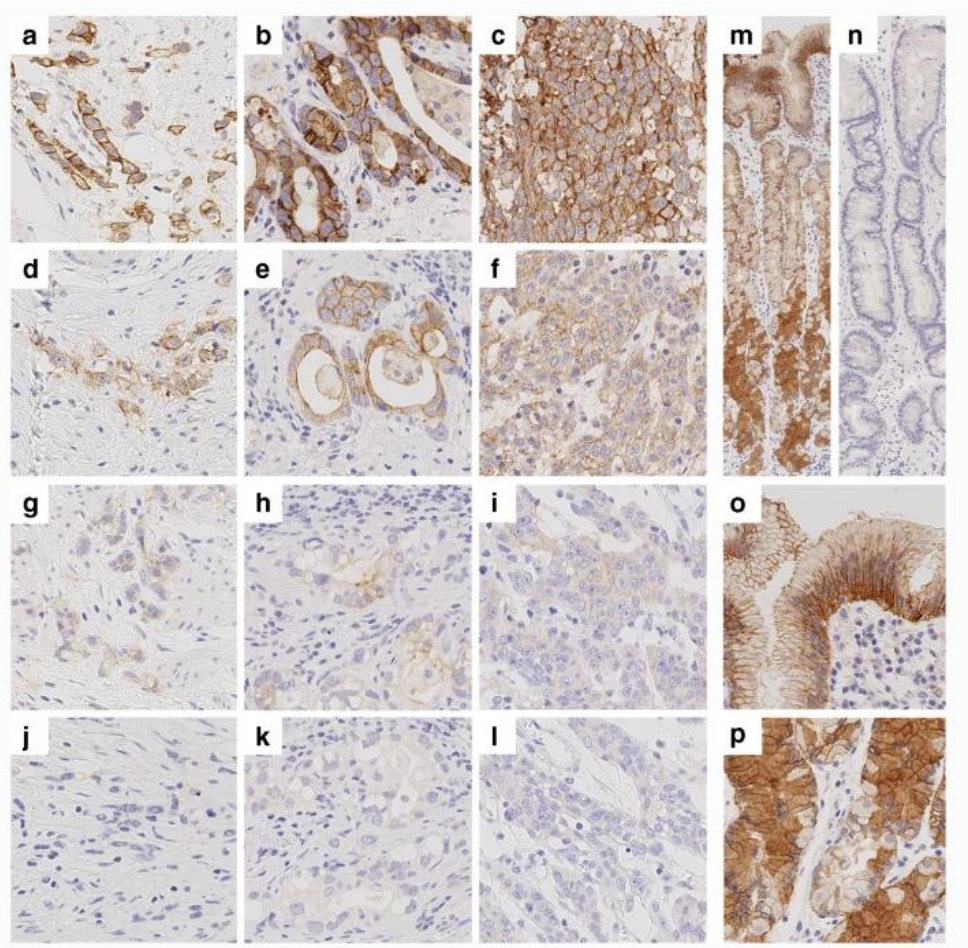
- CLDN18.2 is a tight junction protein normally expressed in gastric mucosa cells and retained in G/GEJ adenocarcinoma[1–8]
- CLDN18.2 may become exposed on the surface of G/GEJ adenocarcinoma cells, making it a promising target[2–8]
- Zolbetuximab is a first-in-class chimeric IgG1 monoclonal antibody that targets CLDN18.2 and induces ADCC/CDC[4–8]
- In the phase 2b FAST study, EOX ± zolbetuximab prolonged survival in a subgroup of patients with higher expression of CLDN18.2 in tumor cells[8]
  - mPFS: 9.0 vs 5.7 months with zolbetuximab + EOX vs EOX alone
  - mOS: 16.5 vs 8.9 months with zolbetuximab + EOX vs EOX alone



1. Niimi T et al. *Mol Cell Biol* 2001;21:7380–90. 2. Sahin U et al. *Clin Cancer Res* 2008;14:7624–34. 3. Moran D et al. *Ann Oncol* 2018;29:viii14-viii57. 4. Sahin U et al. *Eur J Cancer* 2018;100:17–26. 5. Rhode C et al. *Jpn J Clin Oncol* 2019;49:870–6. 6. Türeci Ö et al. *Ann Oncol* 2019;30:1487–95. 7. Pellino A et al. *J Pers Med* 2021, 11(11):1095. 8. Sahin U et al. *Ann Oncol* 2021;32:609–19.

#GI23

PRESENTED BY: Dr. Kohei Shitara



Gastric/Gastric esophageal junction/Pancreas carcinoma

75% positive cut-off + intensity 2+ (moderate) or 3+ (strong)

Normal gastric epithelium positive
- Intestinal metaplasia weakly pos.

The antibody graveyard – Potential new predictive IHC biomarkers

Astellas; Zolbetuximab
Approved 24.9.2024

Transcenta; Osemitamab

PD-L1 repeat….???

KEYTRUDA
NIVOLUMAB
TECENTRIQ
IMFINZI
….

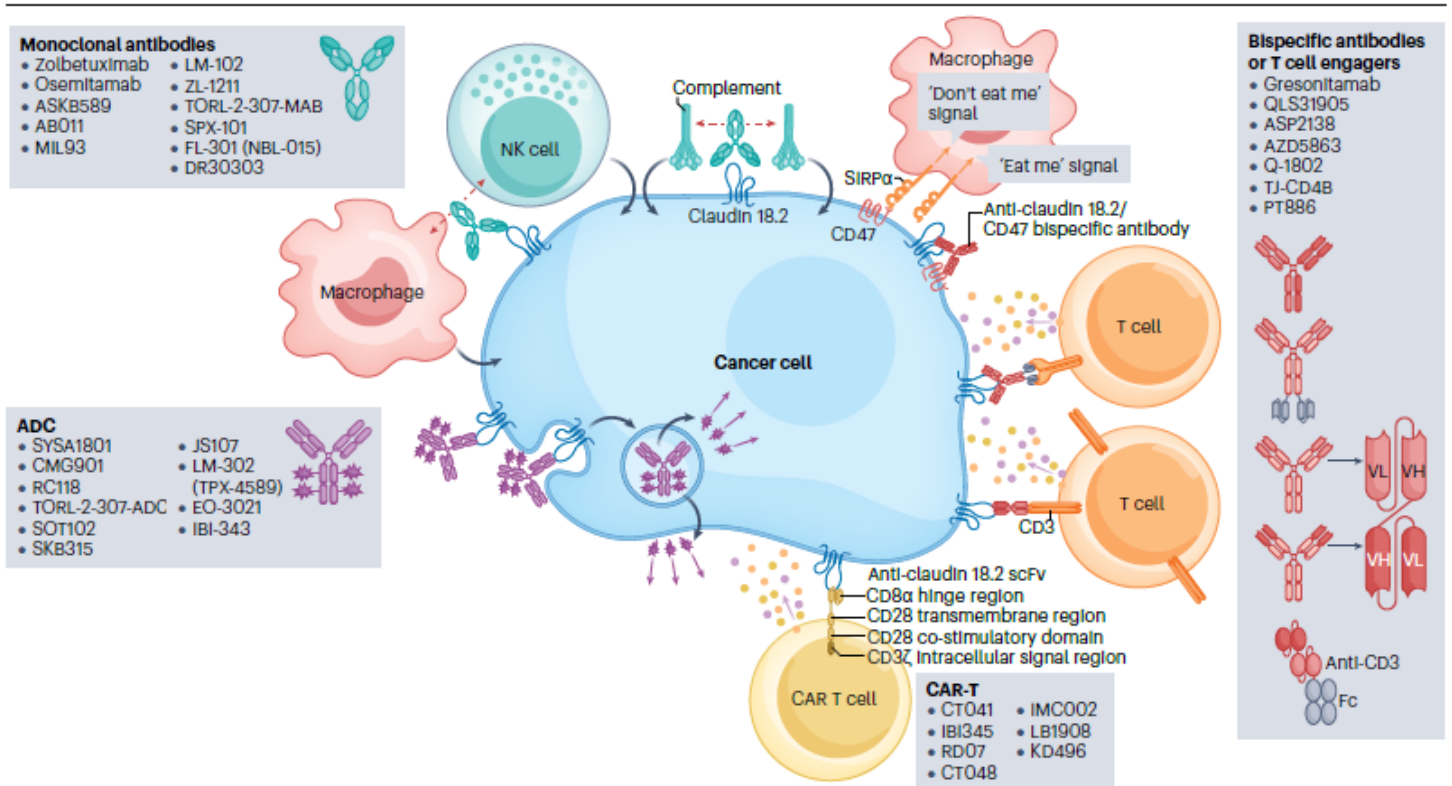
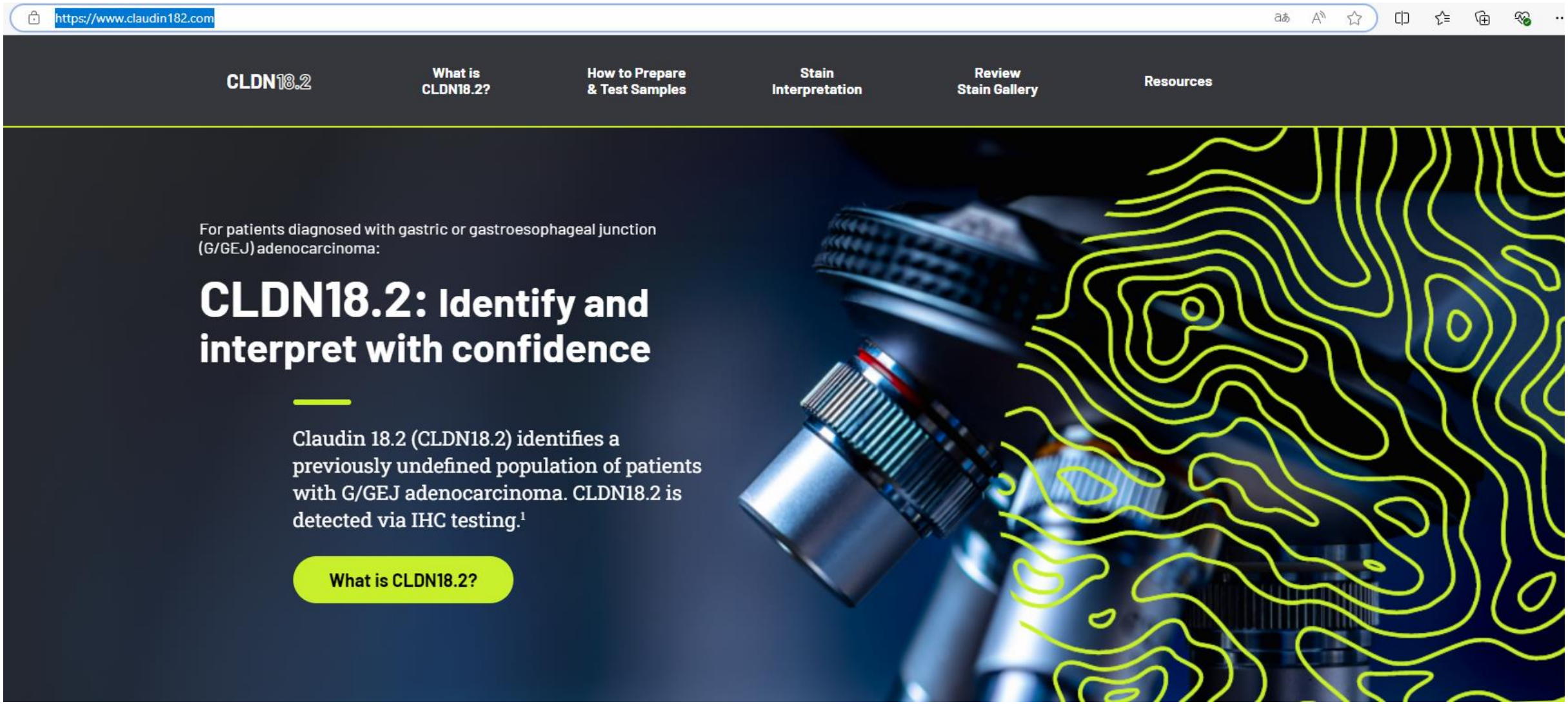


Fig. 4 | Mechanisms of action of the various classes of developmental claudin 18.2-targeted therapies. Various claudin 18.2-targeted therapies have been or are being tested in clinical trials. Owing to lack of clinical activity from direct inhibition of the target protein, monoclonal antibodies such as zolbetuximab are designed to induce antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), leading to cell death. Bispecific T cell engagers or bispecific antibodies are designed to simultaneously bind to claudin 18.2 as well as to other target proteins, such as CD3 with the former, and a range of immune-related targets with the latter, usually with the aim of bringing active immune cells into close proximity with tumour cells. Claudin 18.2-targeted antibody–drug conjugates (ADCs) are designed to deliver highly cytotoxic agents to claudin 18.2-expressing cancer cells, which are then able to release the payload either following endocytosis and digestion within endosomes (noncleavable linkers) or via cleavage of the linker by cancer-cell-specific enzymes (cleavable linkers), resulting in cancer cell death while sparing the surrounding nonmalignant cells. Second-generation chimeric antigen receptor (CAR) T cells that target claudin 18.2 consist of a CD8α hinge region, a CD28 costimulatory domain and a CD3ζ signalling domain. CAR T cells are genetically modified to recognize and eliminate cells that express claudin 18.2. V_H, immunoglobulin heavy chain variable region; V_L, immunoglobulin light chain variable region.

The antibody graveyard – Claudin 18.2 - https://www.claudin182.com/ - Astellas



The antibody graveyard – Claudin 18.2 - https://www.claudin182.com/

https://www.claudin182.com/What-Is-CLDN

CLDN18.2 | What is CLDN18.2? | How to Prepare & Test Samples | Stain Interpretation | Review Stain Gallery | Resources

An emerging biomarker

- Claudins are a family of transmembrane proteins[2,3]
- As a component of tight junctions, claudins are involved in the regulation of permeability, barrier function, and polarity of epithelial layers[2,3]

CLDN18.2: the biomarker that gastric cancer reveals

Preclinical data have shown that CLDN18.2 may become more exposed as gastric tumors develop.[4,6]

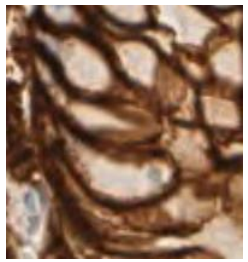
Claudins are present throughout the body, but two specific isoforms of CLDN18 are localized to certain tissue types[4,5]

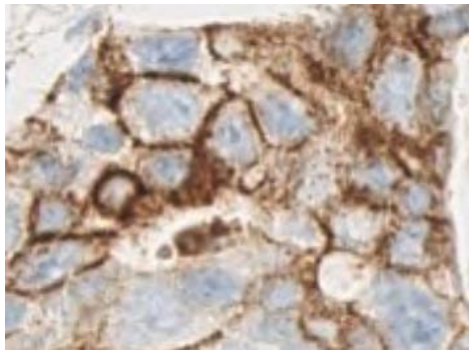
CLDN18.1

CLDN18.1 is the dominant isoform in normal and malignant lung tissue

CLDN18.2

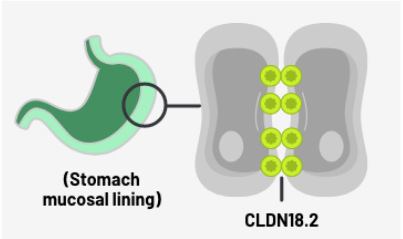
CLDN18.2 is the dominant isoform in normal gastric tissue and is often retained in malignant transformation





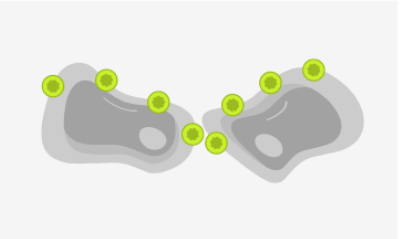
Preclinical data have shown that CLDN18.2 may become more exposed as gastric tumors develop.[4,6]

CONFINED IN HEALTHY TISSUE



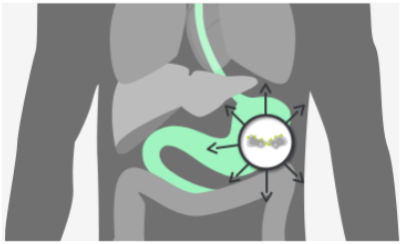
In normal gastric mucosa, CLDN18.2 is typically buried within tight junctions.[4,6]

RETAINED AND EXPOSED IN MALIGNANT TRANSFORMATION



CLDN18.2 is often retained during malignant transformation. CLDN18.2 may be more exposed and accessible to antibodies when cell polarity disruptions and structure loss occur.[4,6,7]

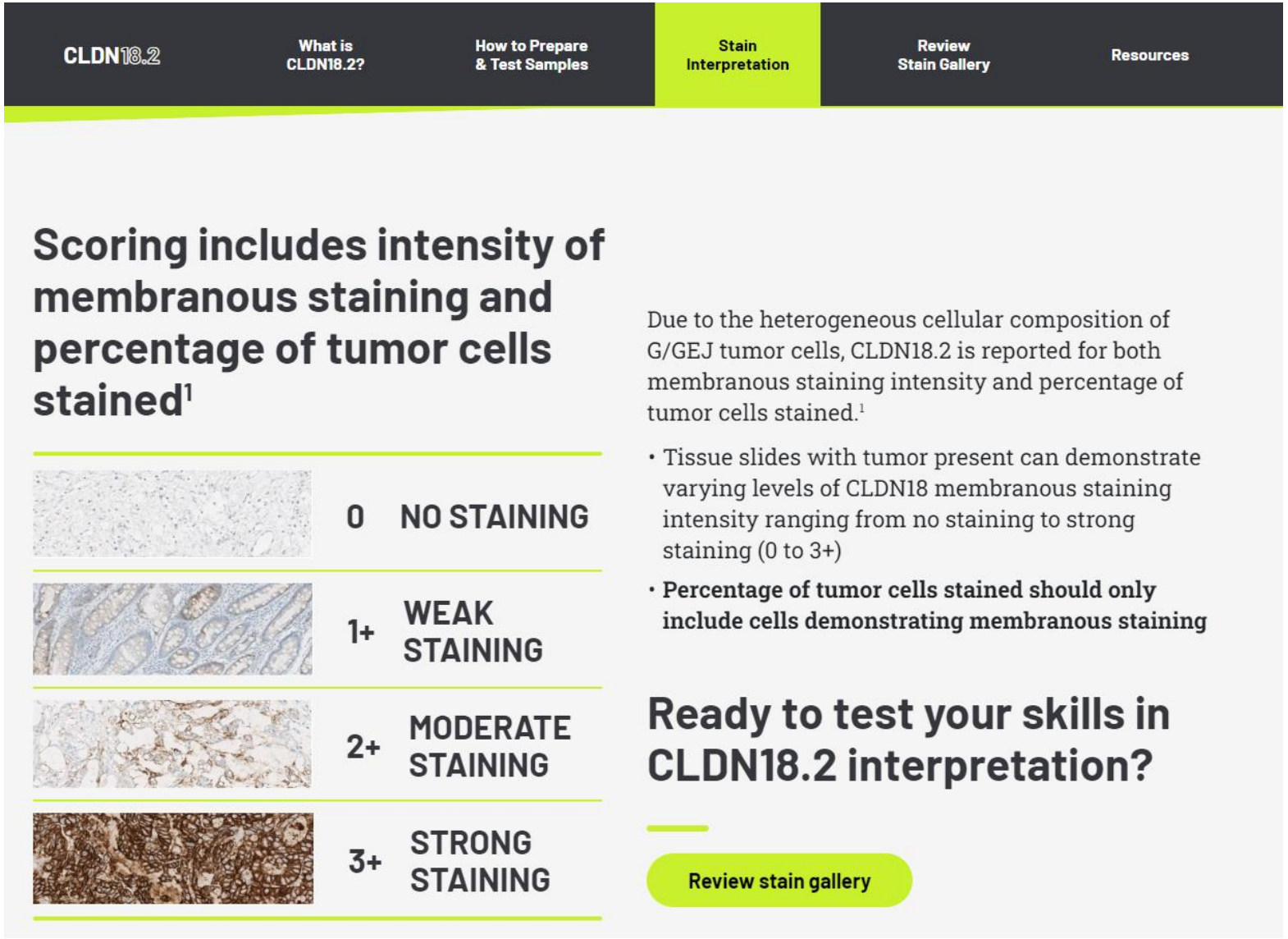
MAINTAINED IN METASTATIC PROGRESSION



CLDN18.2 may also be expressed in lymph node metastases of gastric adenocarcinoma as well as other distant metastatic sites.[1,4,8,9]

The information provided above is based on the current understanding of data.

The antibody graveyard – Claudin 18.2 - https://www.claudin182.com/

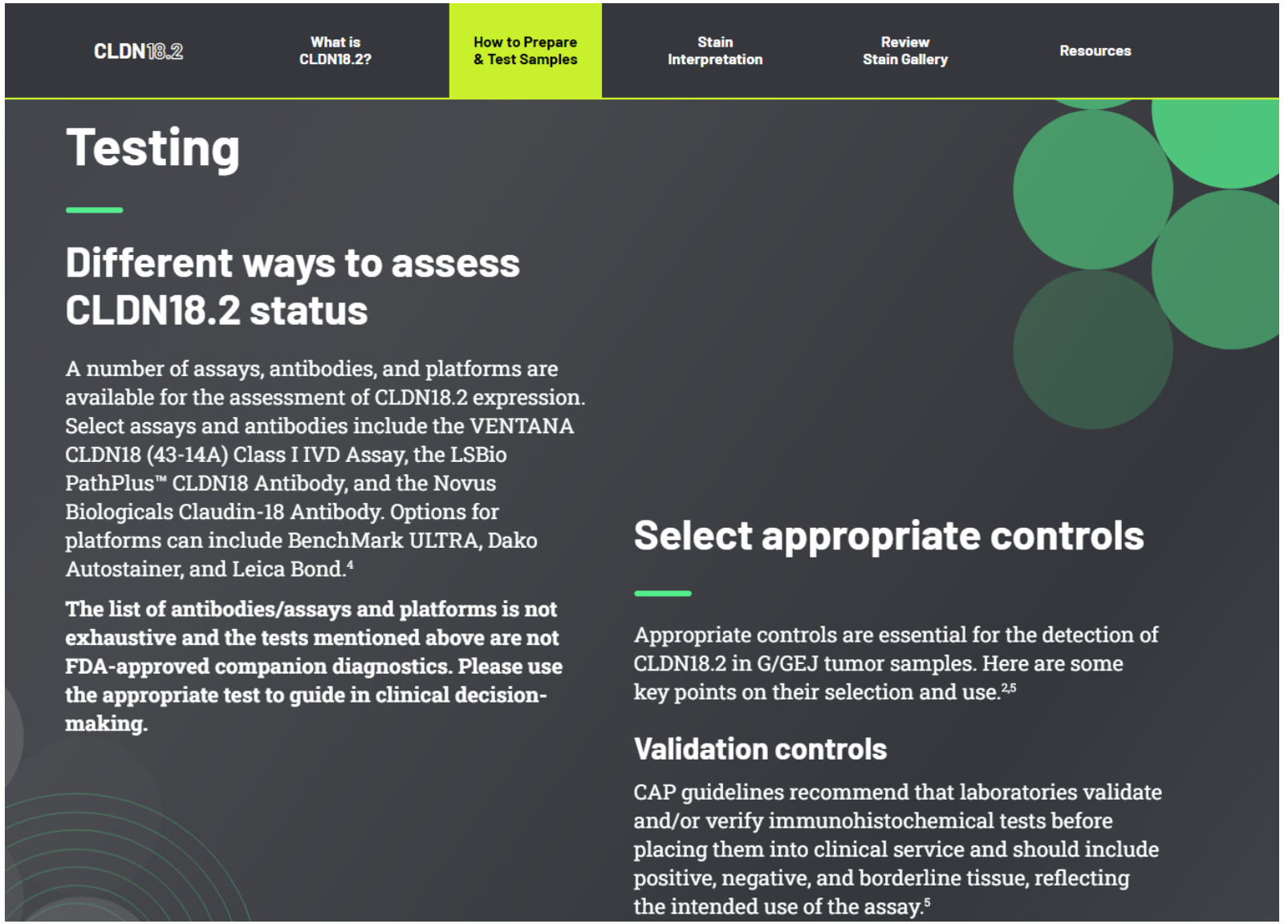


CLDN 18.2 positive and eligible for Zolbexitumab;

75% cells positive with moderate to strong intensity

A kind of combined HER2 and PD-L1 scoring system…

The antibody graveyard – Claudin 18.2 - https://www.claudin182.com/

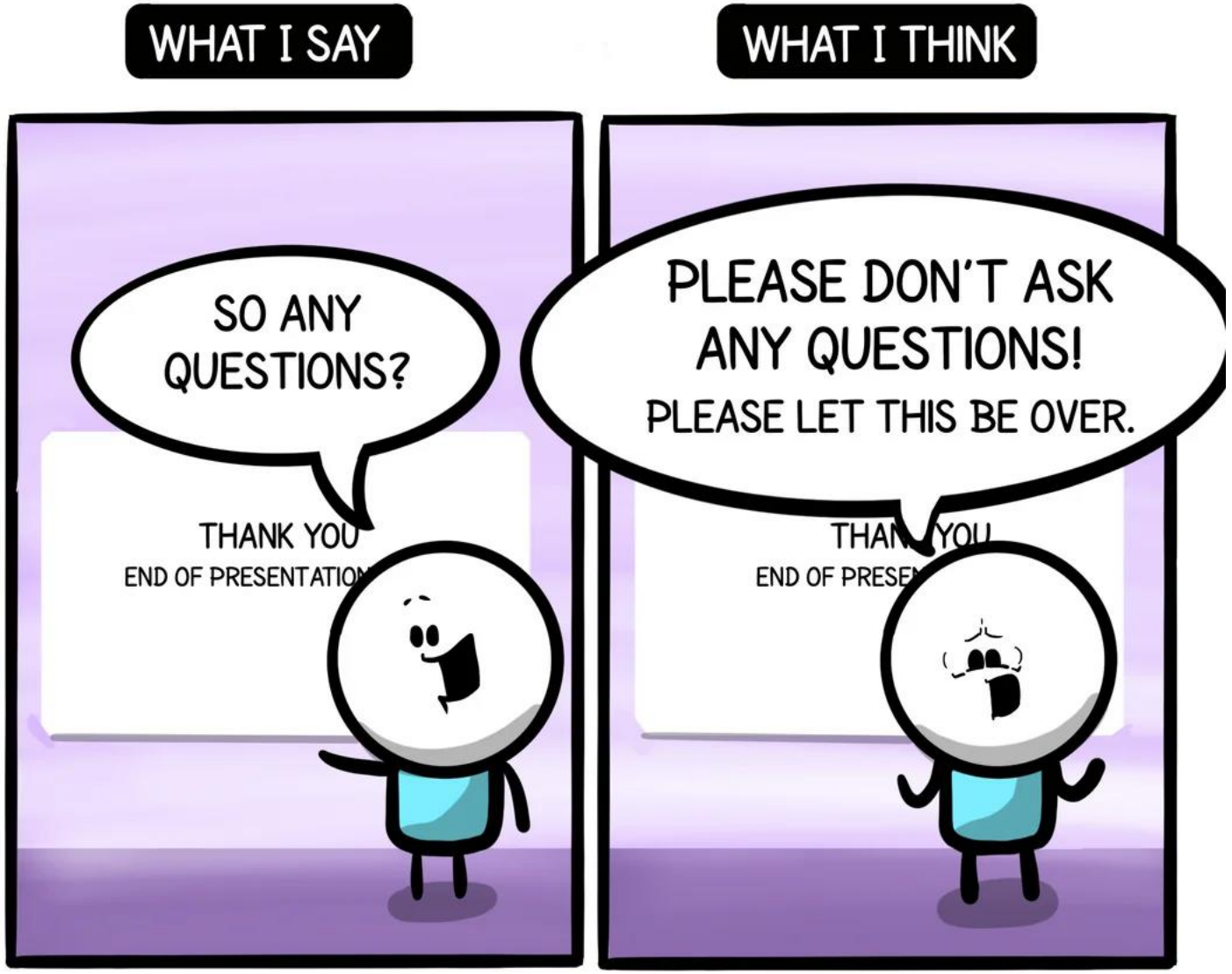


Roche has the IHC asssay (43-14A) used by Astellas/researchers for studies on CLDN18.2 in GI cancers.

Agilent in partnership with Transcenta to launch a CLDN18.2 assay as well (14G11 pharmDX)

No data on inter-assay concordance…….


KEEP
CALM
AND
REMEMBER
TO SKIP


WHAT I SAY
WHAT I THINK
SO ANY QUESTIONS?
THANK YOU
END OF PRESENTATION
PLEASE DON'T ASK ANY QUESTIONS!
PLEASE LET THIS BE OVER.