

The Evolving IHC Landscape

Markers Come, Markers Go, Panels remain

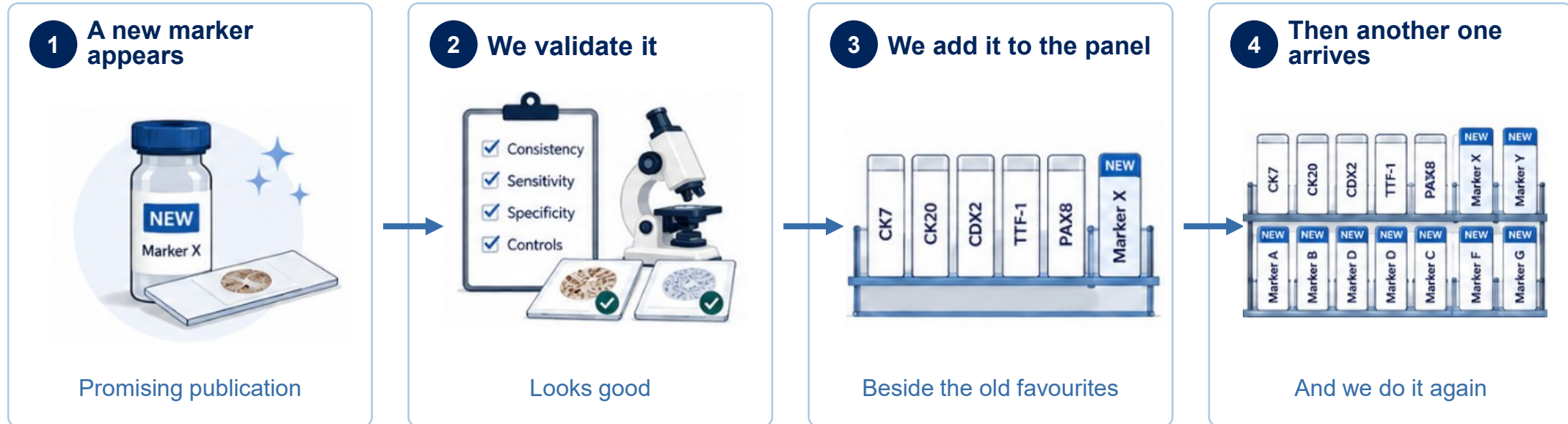
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Markers come. Markers stay?

We are often more enthusiastic about adding new IHC assays than retiring old ones.



! Eventually, the panel begins to resemble a **very expensive antibody collection** rather than a carefully designed diagnostic tool.

Good panels are designed, not just accumulated.

Why do we use supplemental IHC assays?

Additional stains should reduce diagnostic uncertainty, not merely enlarge the panel.



Only if they answer a **defined** diagnostic question.

		Assay result	
		Positive	Negative
Actual state	Target present	✓ True positive <i>correctly detected</i>	— False negative <i>sensitivity problem</i>
	Target absent	+ False positive <i>specificity problem</i>	— True negative <i>correctly excluded</i>

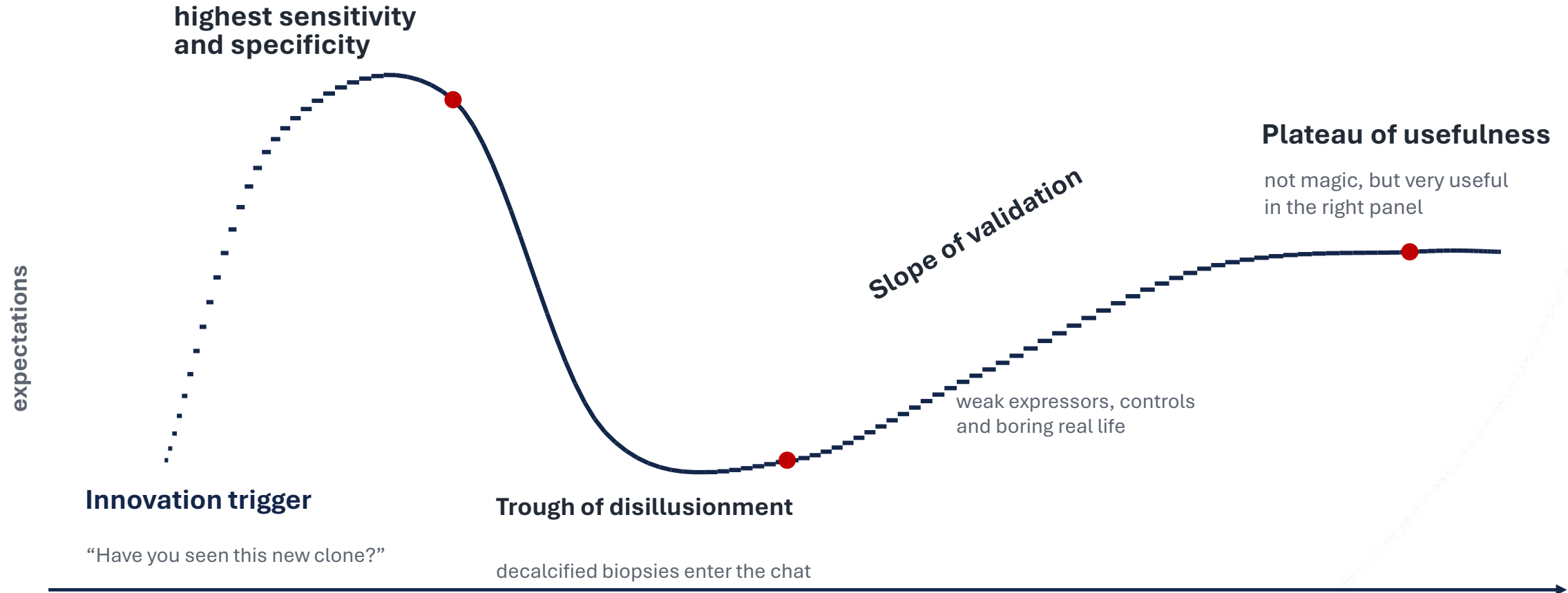
Supplemental IHC should solve a specific problem:

- improve diagnostic sensitivity
- improve diagnostic specificity
- resolve a known diagnostic pitfall

The goal is not more antibodies, but better answers.

The New IHC Marker Hype Curve

“100% sensitivity, 100% specificity” is just a phase



DIAGNOSTIC PANEL

Colorectal carcinoma

Colorectal origin in CUP: which markers add value?

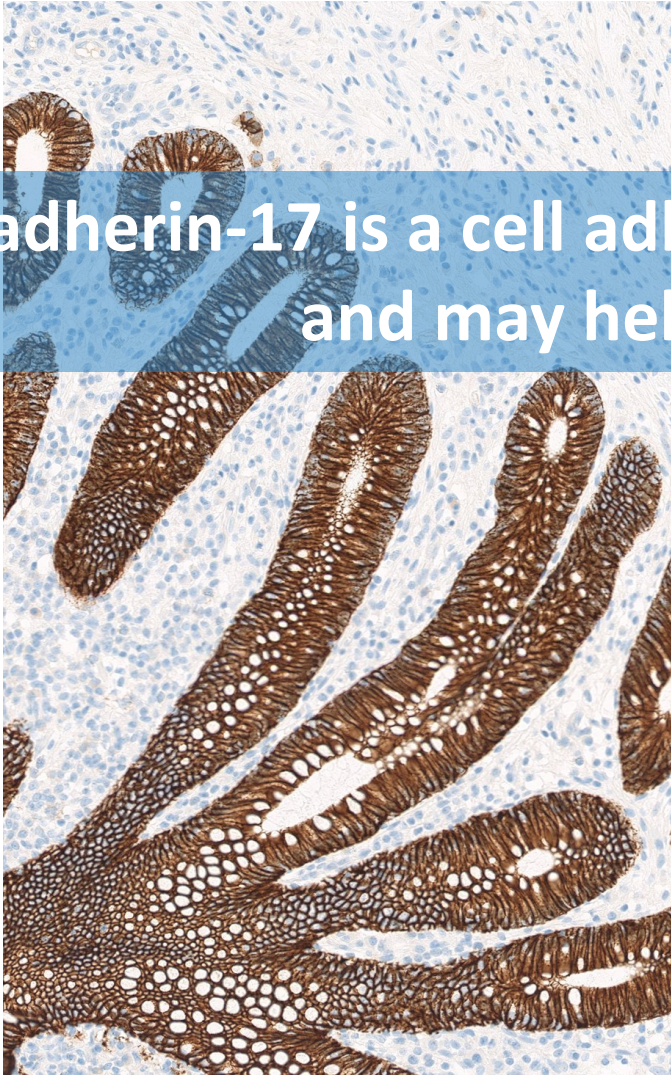
	To stay	Sensitivity	Comments
CK20	Yes	80-95%	MSI-H carcinomas can be negative - Also seen in many other carcinomas
CDX2	Yes	80-95%	MSI-H carcinomas can be negative – Intestinal lineage marker
mCEA	?	90-100%	Not specific for colorectal carcinoma; Might be useful for gastric adenocarcinomas
Villin	No	70-90%	Less sensitive and less specific
SATB2	"New"	75-90%	Lower GI tract and rectal/appendiceal neuroendocrine tumours
Cadherin-17	"New"	85-95%	Publications indicate superior sensitivity compared to CDX2 and not a lineage marker

Combined CK20 and CDX2 sensitivity: 97% in MSS CRC and 88% in MSI-H CRC

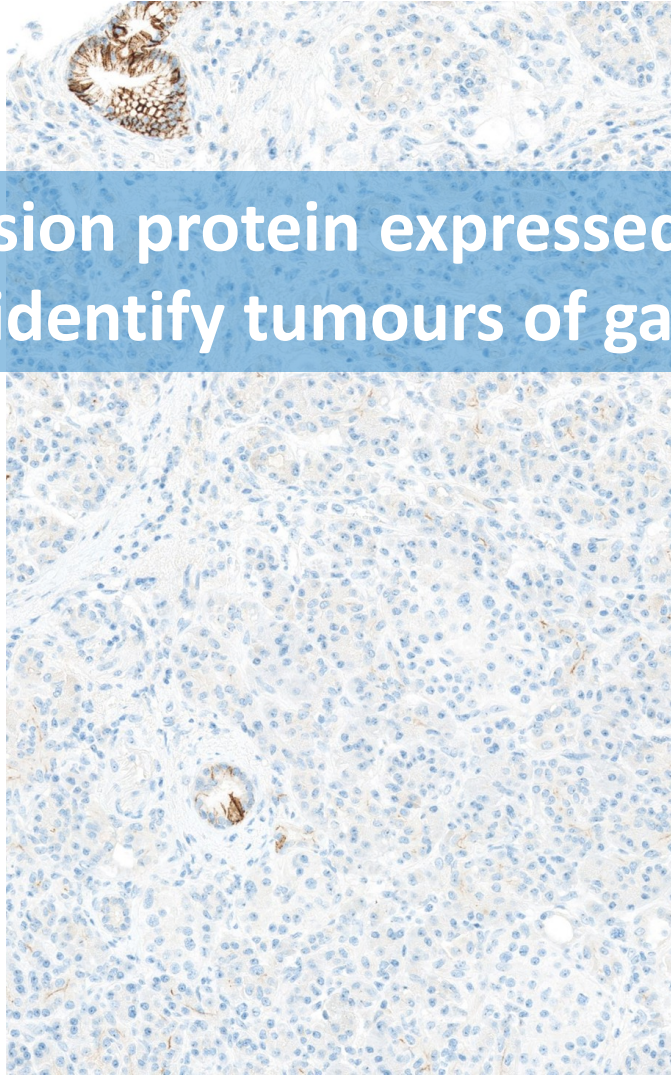
* CUP; Cancer of unknown primary origin

Cadherin 17: does it earn a place in the colorectal CUP panel?

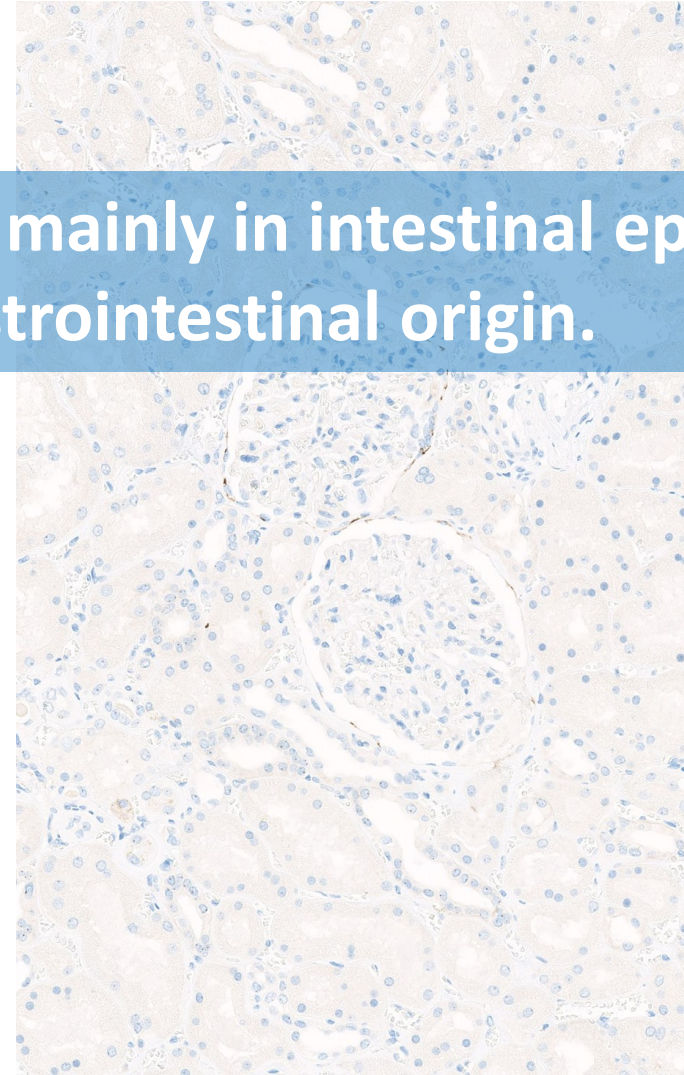
Cadherin-17 is a cell adhesion protein expressed mainly in intestinal epithelium and may help identify tumours of gastrointestinal origin.



Colon/Appendix



Pancreas



Kidney

Cadherin 17: does it earn a place in the colorectal CUP panel?

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

Cadherin 17: does it earn a place in the colorectal CUP panel?

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 (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.

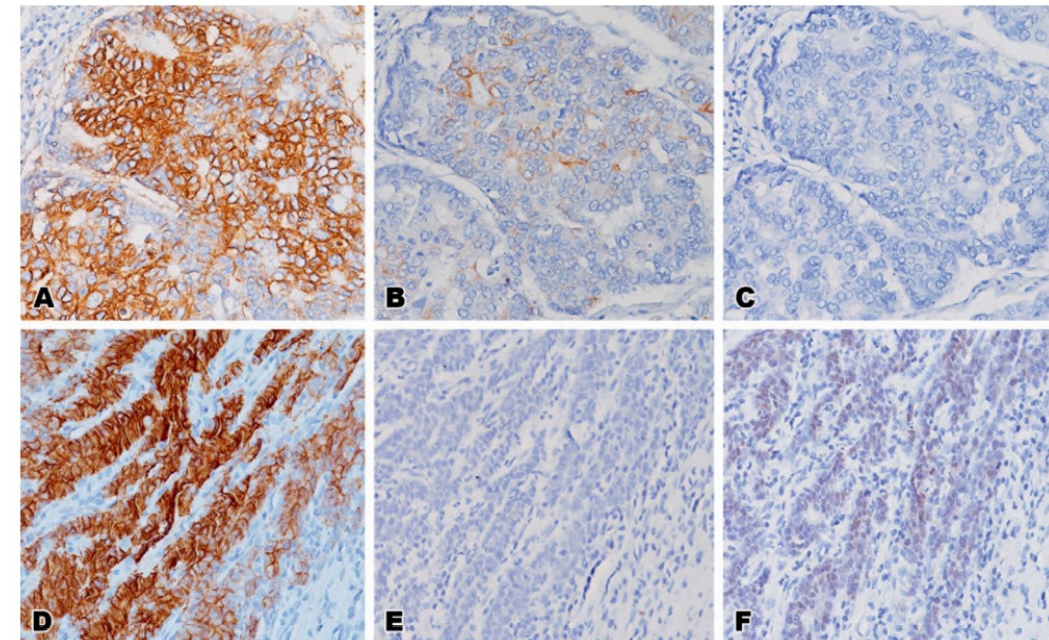


Figure 3. Staining results in metastatic colon adenocarcinoma. A and D, Strong, positive staining was observed in a high percentage of specimens with CDH17. B, Focal staining was observed in CK20-positive tissue; and in specimens considered negative, CK20 was completely absent (E). Representative negative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

Cadherin 17: does it earn a place in the colorectal CUP panel?

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Pathology - Research and Practice 256 (2024) 155175

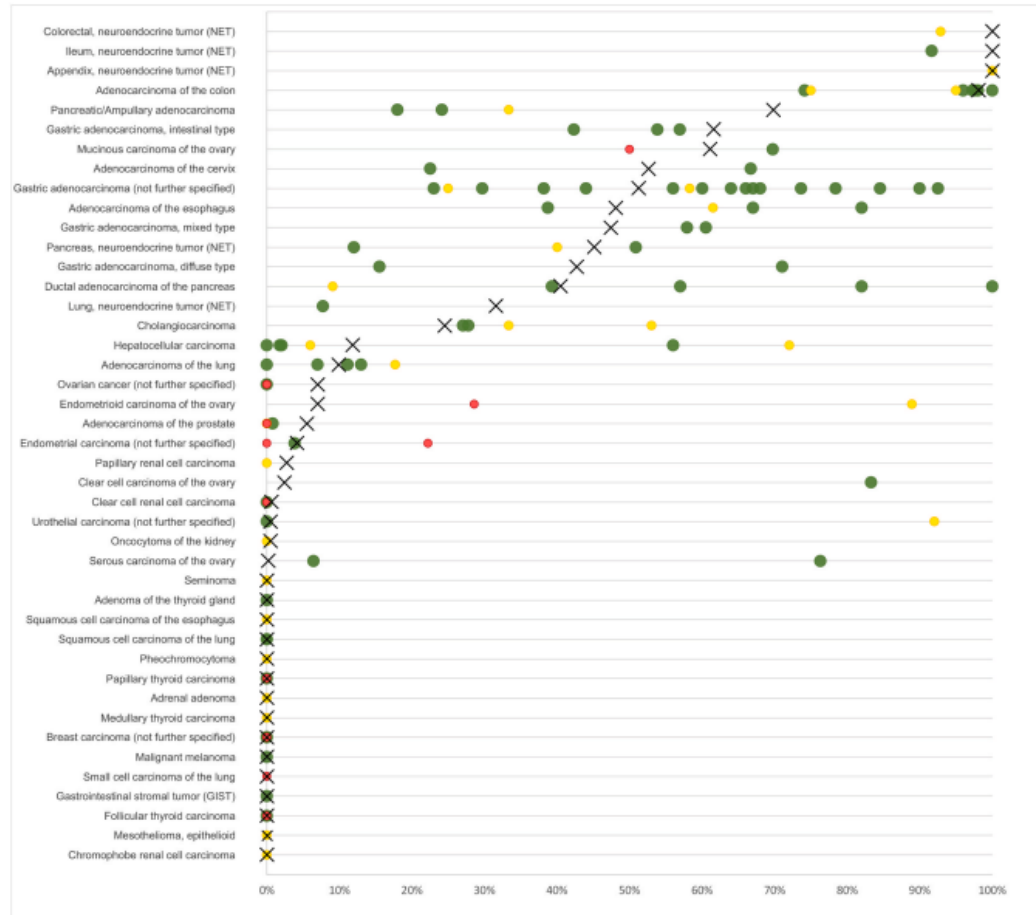


Fig. 5. Comparison with previous CDH17 literature. An „X“ indicates the fraction of CDH17 positive cancers in the present study, dots indicate the reported frequencies from the literature for comparison: red dots mark studies with ≤ 10 analyzed tumors, yellow dots mark studies with 11 to 25 analyzed tumors, and green dots mark studies with > 25 analyzed tumors.

Potential diagnostic roles of CDH17

1. Supporting a gastrointestinal or biliopancreatic origin

Metastatic GI and biliopancreatic adenocarcinomas are often CDH17-positive, whereas primary pulmonary adenocarcinomas are usually negative.

2. Demonstrating intestinal differentiation

CDH17 may help identify intestinal-type differentiation in adenocarcinomas of the oesophagus, stomach and endocervix.

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Cadherin-17 (CDH17) expression in human cancer: A tissue microarray study on 18,131 tumors

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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



Cadherin 17: does it earn a place in the colorectal CUP panel?

Useful as part of a panel, but not specific for colorectal origin.

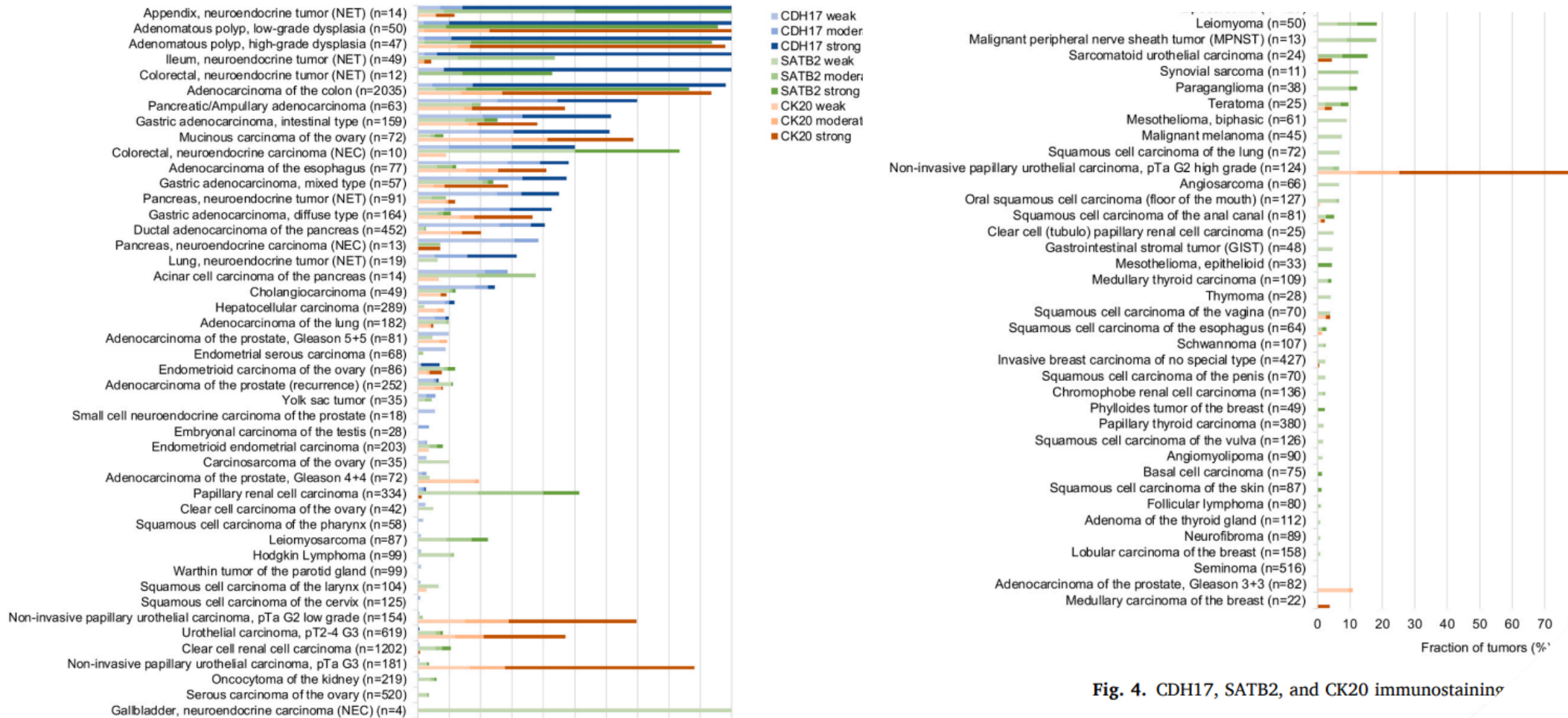


Fig. 4. CDH17, SATB2, and CK20 immunostaining

Cadherin-17 (CDH17) expression in human cancer: A tissue microarray study on 18,131 tumors

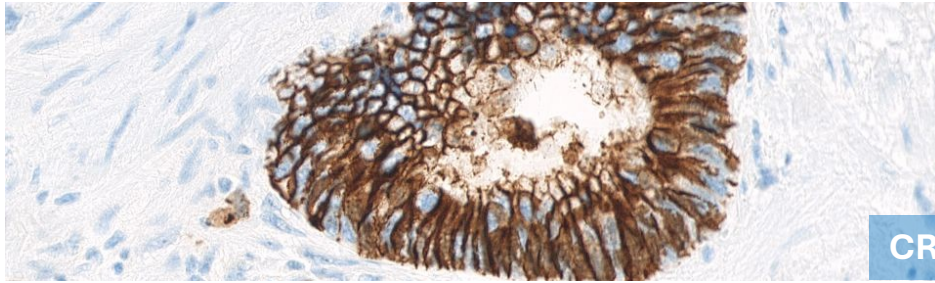
Frank Jacobsen et al, Pathology - Research and Practice 256 (2024) 155175

Candidate; clone MSVA-517M, MS Validated Antibodies

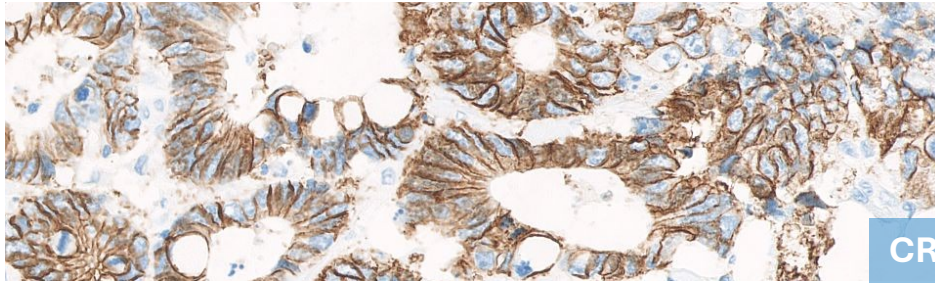
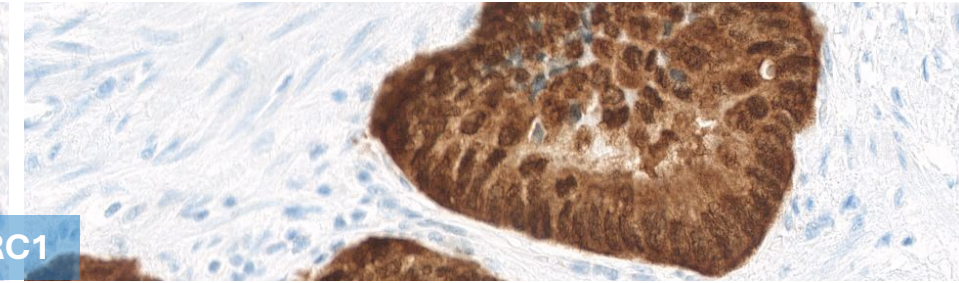
Cadherin 17: does it earn a place in the colorectal CUP panel?

CAD-17

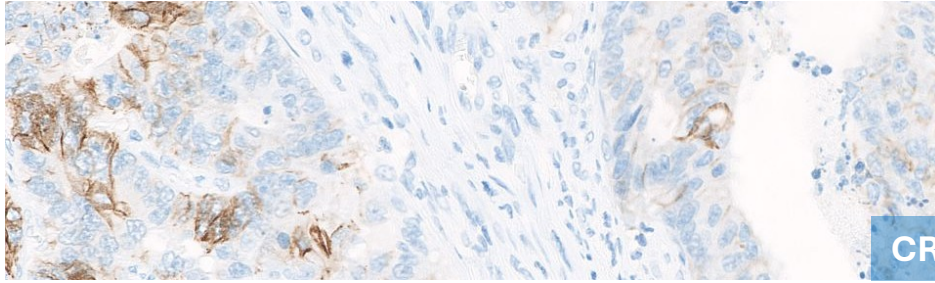
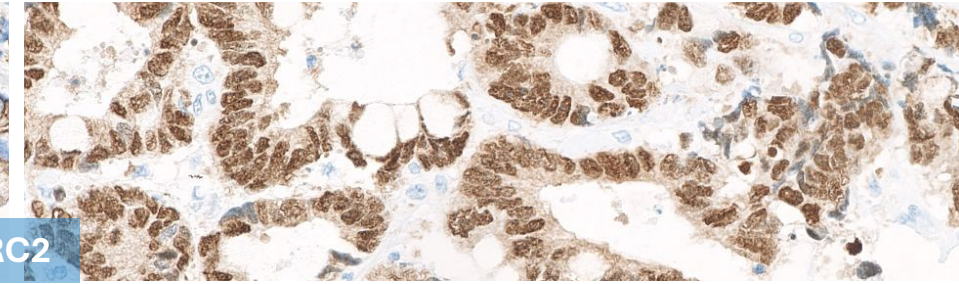
CDX2



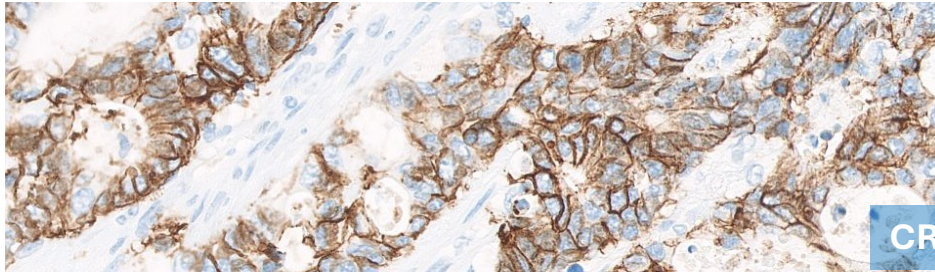
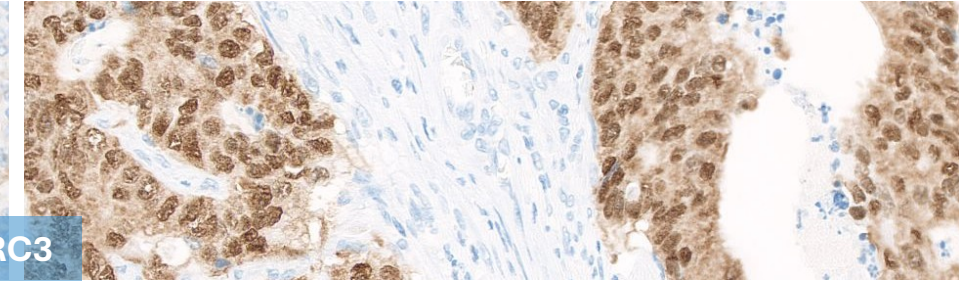
CRC1



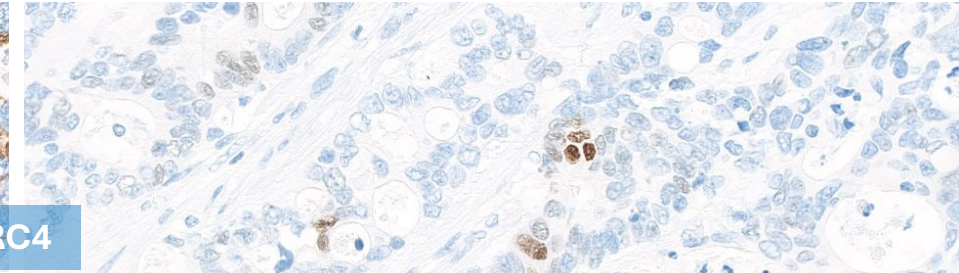
CRC2



CRC3



CRC4



rmAb
SP183

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

Cadherin 17: from diagnostic marker to therapeutic target

Cell Reports Medicine

CellPress
OPEN ACCESS

Article

Overcoming multidrug resistance in gastrointestinal cancers with a CDH17-targeted ADC conjugated to a DNA topoisomerase inhibitor

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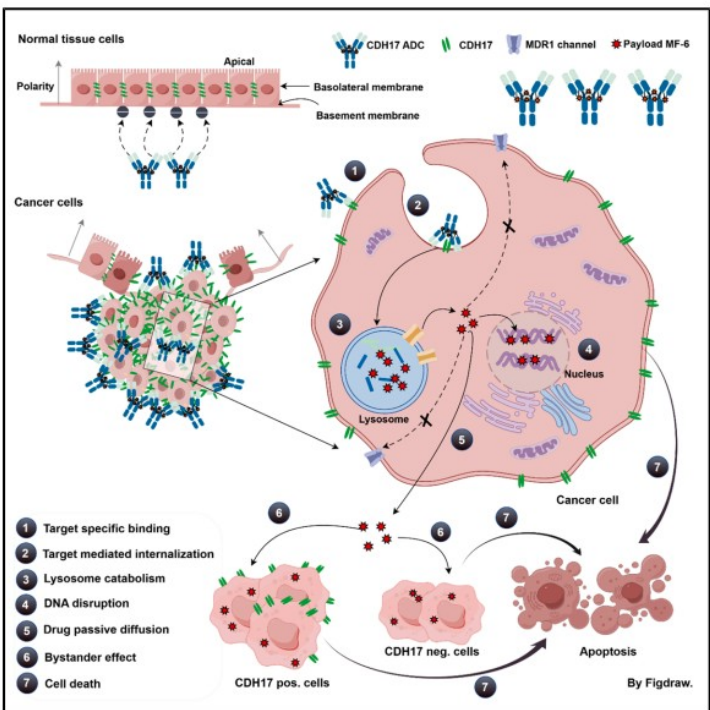
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Blog
CDH17 ADCs blooms in 2025 AACR

POST ON: 2025-04-17

American Association for Cancer Research (AACR)

The 116th AACR Annual Meeting will be held from April 25 to 30, 2025, at the McCormick Place Convention Center in Chicago, USA. Among the 6,470 abstracts released for the 2025 AACR, there are 340 related to antibody-drug conjugates (ADCs), covering various types such as single-target ADCs, dual-target ADCs, and dual-payload ADCs. These involve numerous popular targets, including CDH17 (12 candidates), DLL3 (10 candidates), and TROP2 (54 candidates). In this article, we review the CDH17 ADC drug development pipeline and the key players in the field.

Drug Name	Company	The title of Abstract	Disease
7MW4911	Mabwell	7MW4911, a novel cadherin 17-targeting ADC, demonstrates potent efficacy in preclinical models of gastrointestinal cancers	CC; GC; PC
SCR-A008	Simcere Pharmaceutical	Preclinical development of SCR-A008: A CDH17-targeted ADC for gastrointestinal cancer	GC, CC, PC; Lung/Ovarian/Liver cancer, Solid tumors
YL217	MediLink Therapeutics	Preclinical development of a next generation antibody drug conjugate (ADC) targeting CDH17 for treatment of solid tumors	Solid tumors; Cancer
LM-350	LaNova Medicines	Preclinical evaluation of LM-350: An innovative anti-CDH17 antibody drug conjugate for gastrointestinal cancer therapy	CC; GC; PC
VBC108; CMG901	VelaVigo	VBC108: a first-in-class CDH17/CLDN18.2 targeted bispecific antibody drug conjugate (ADC) to overcome tumor heterogeneity of gastrointestinal cancers (GC, PADG, CRC, etc.)	GC; PC; CC
BR116	BioRay Pharmaceutical	BR116: An antibody drug conjugate targeting CDH17 for gastrointestinal cancer therapy	GC; CC; PC; Biliary tract cancer; Cholangiocarcinoma; Hepatocellular carcinoma
BSI-721	Biosion	Development and preclinical activity of BSI-721, a novel antibody-drug conjugate (ADC) targeting cadherin 17 (CDH17) in gastrointestinal cancers	CC; GC; PC; Esophageal cancer
LBL-054	Nanjing Leadsbiolabs	Novel hydrophilic CDH17-targeting antibody-drug conjugate exhibits anti-tumor efficacy in preclinical xenograft models	Gastrointestinal cancer
SOT109	SOTIO Biotech	Preclinical safety and efficacy of SOT109, an antibody-drug conjugate targeting cadherin 17 (CDH17) for the treatment of colorectal and other gastrointestinal tract tumors	CC; GC; PC; Cancer; Tumor
TAVO307	Tavotek Biotherapeutics	TAVO307, a novel ADC targeting CDH17, for the therapy of gastrointestinal cancers	GC; PC; Liver/Biliary tract/Colon cancer, Cholangiocarcinoma
MRG007	Concept to Medicine Biotech	MRG007, a cadherin 17-targeted, glycan-linked, exatecan-based antibody-drug conjugate, demonstrated potent anti-tumor activity and a good safety profile in preclinical studies	CC
HDM2017	Huadong Medicine	Discovery of HDM2017, a CDH17-targeting ADC for colorectal cancers	CC; GC

***Colorectal cancer: CC; Gastric cancer: GC; Pancreatic cancer: PC

Meeting Abstract: 2025 ASCO Annual Meeting I

FREE ACCESS | Developmental Therapeutics—Molecularly Targeted Agents and Tumor Biology | May 28, 2025

X in f e b

A phase 1, first-in-human study of AMT-676, an anti-CDH17 antibody-drug conjugate, in patients with advanced gastrointestinal tumors.

Authors: [Charlotte Rose Lemech](#), [Warren Lance Joubert](#), [Andrew Ohyama Parsonson](#), [Rui-Hua Xu](#), [Rajiv Shinde](#), [Prachi Bhawe](#), [Junlii Xue](#), [Yaling Huang](#), [Xun Meng](#), and [Shu-Hui Liu](#) | [AUTHORS INFO & AFFILIATIONS](#)

Publication: Journal of Clinical Oncology • Volume 43, Number 16 suppl • https://doi.org/10.1200/JCO.2025.43.16_suppl.TPS3161

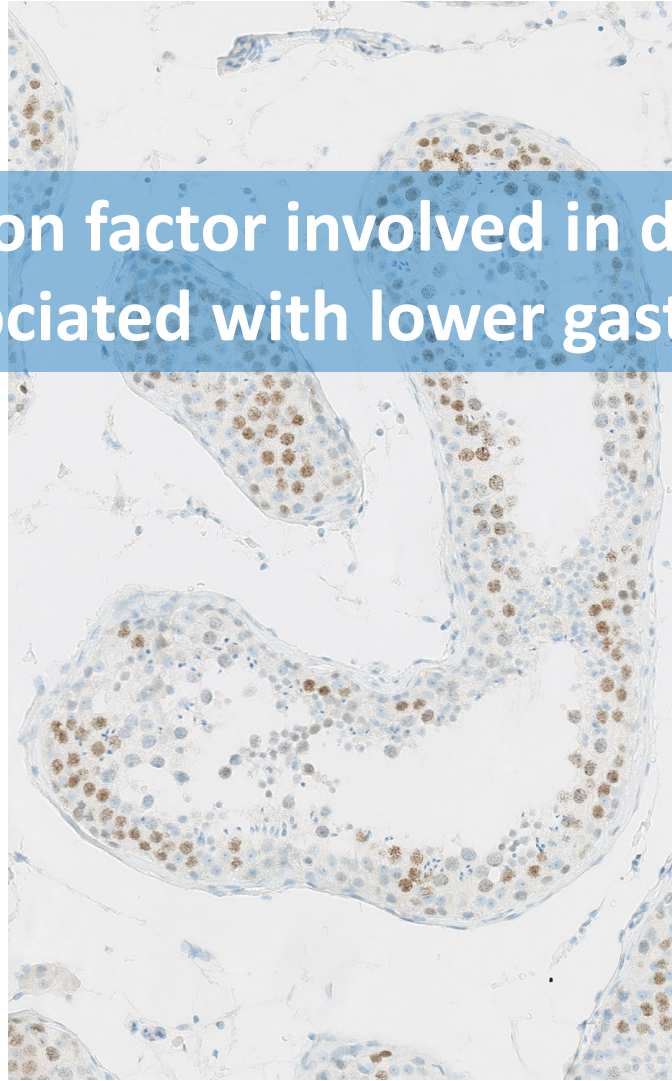
NordiIQC

SATB2: does it earn a place in the colorectal CUP panel?

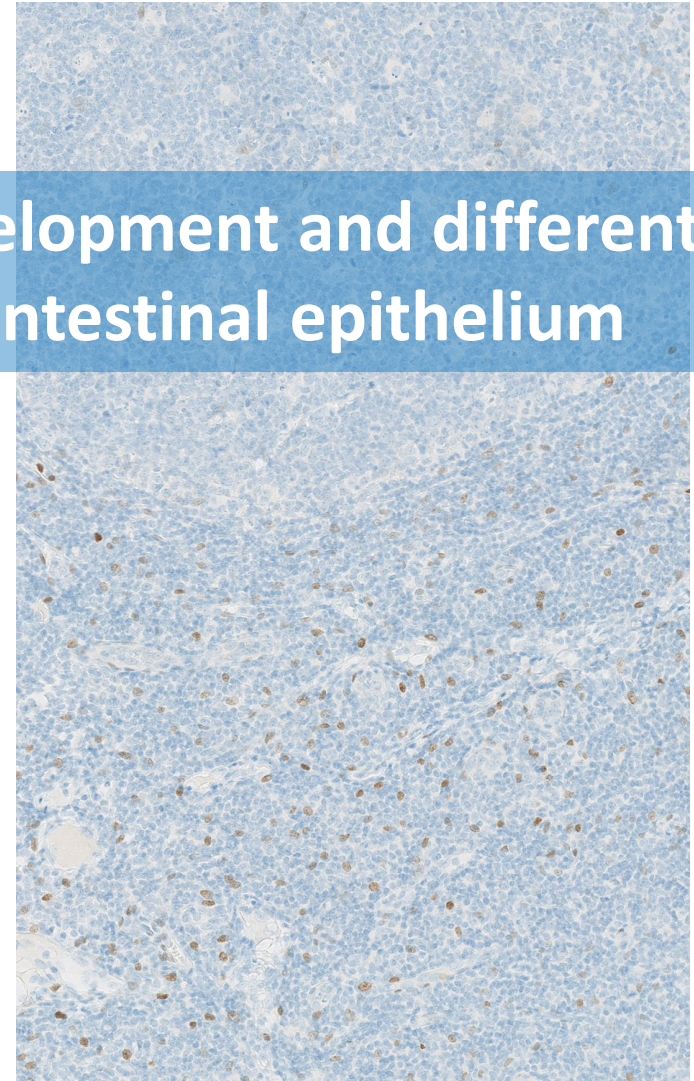
SATB2 is nuclear transcription factor involved in development and differentiation and is strongly associated with lower gastrointestinal epithelium



Colon/Appendix



Testis



Tonsil (EP281 - Clone dependent)

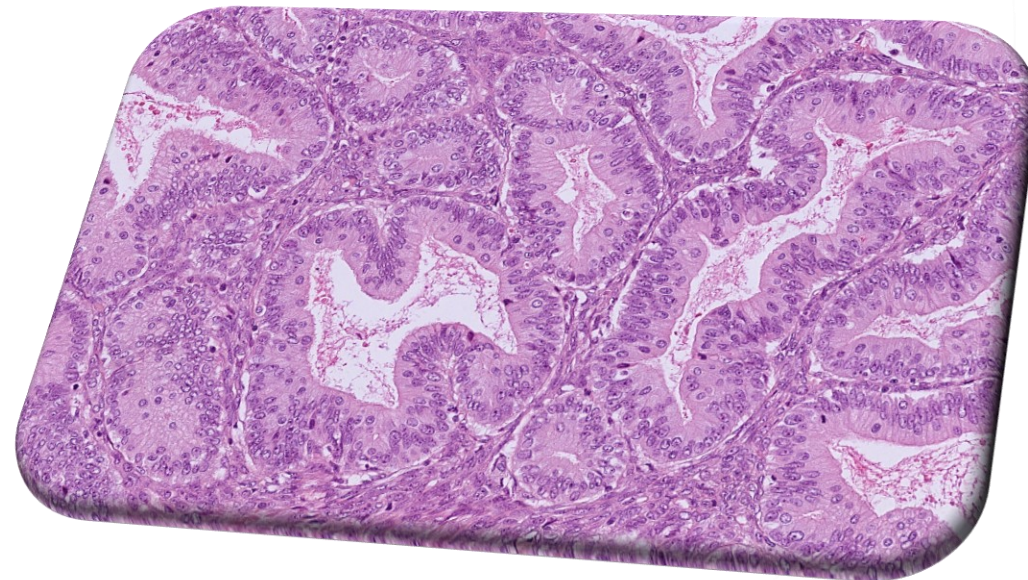
SATB2: does it earn a place in the colorectal CUP panel?

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 is more sensitive for colorectal adenocarcinomas

SATB2 is more specific for colorectal adenocarcinomas



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%,

SATB2: does it earn a place in the colorectal CUP panel?

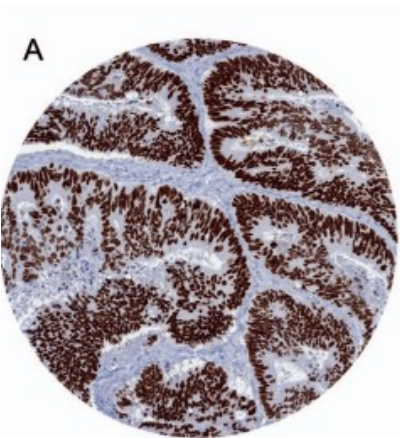
SATB2 Expression in Human Tumors

Arch Pathol Lab Med—Vol 147, April 2023

A Tissue Microarray Study on More Than 15 000 Tumors

David Dum, MD; Daniela Kromm; Maximilian Lennartz, MD; Noémi De Wispelaere; Franziska Büscheck, MD; Andreas M. Luebke, MD; Eike Burandt, MD; Anne Menz, MD; Martina Kluth, PhD; Claudia Hube-Magg, PhD; Andrea Hinsch, MD; Doris Höflmayer, MD; Sören Weidemann, MD; Christoph Fraune, MD; Katharina Möller, MD; Patrick Lebok, MD; Guido Sauter, MD; Ronald Simon, PhD; Ria Uhlig, MD; Waldemar Wilczak, MD; Sarah Minner, MD; Rainer Krech, MD; Christian Bernreuther, MD; Andreas Marx, MD; Stefan Steurer, MD; Frank Jacobsen, MD; Till Clauditz, MD; Till Krech, MD

SATB2 may occur in many tumours, but diffuse expression in >70% of cells strongly favours colorectal origin.



Diagnostic pitfall:
SATB2 may be weak or lost in right-sided, MMR-deficient and BRAF-mutated colorectal carcinomas.

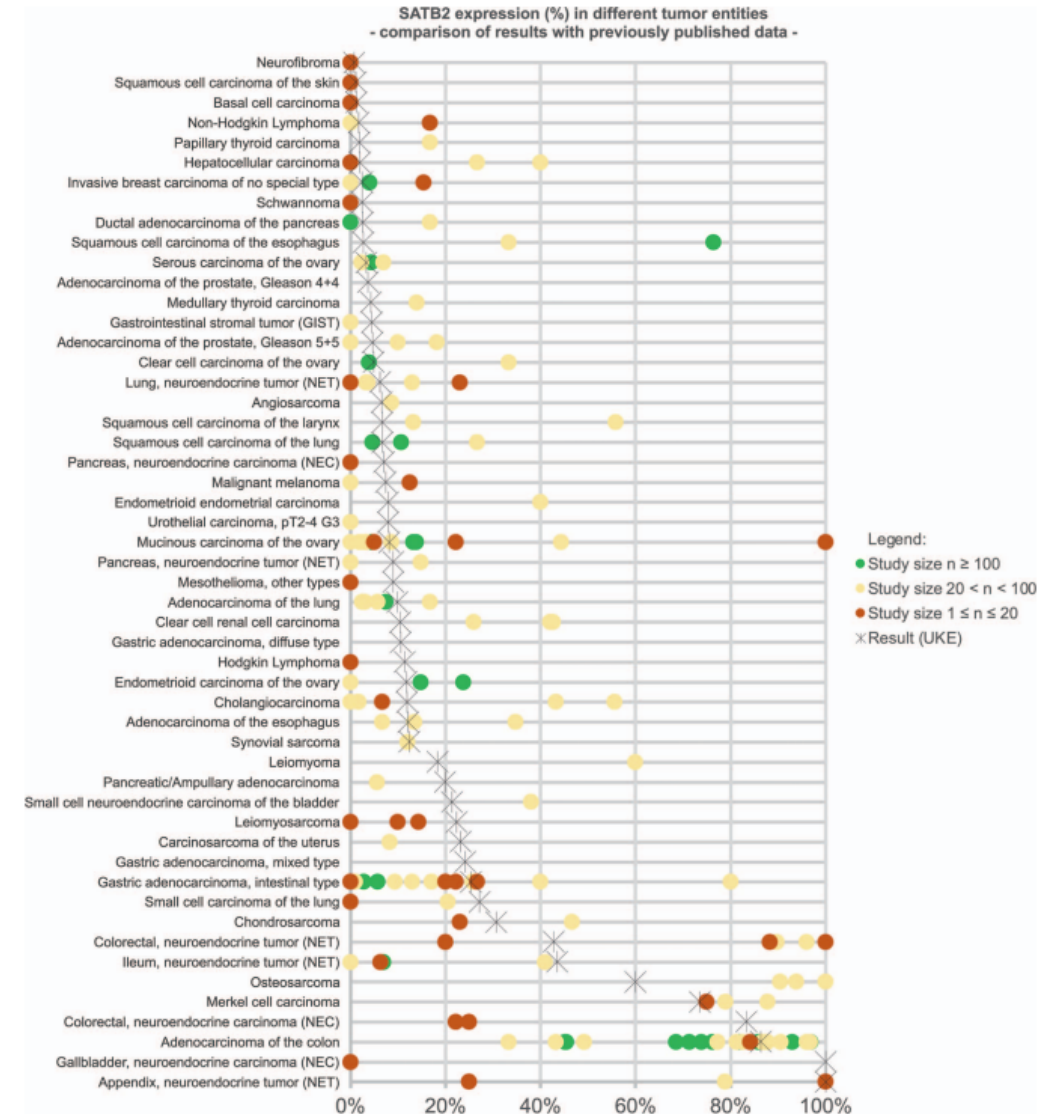


Figure 5. Graphical comparison of SATB2 data from this study (X) in comparison with the previous literature. Colored dots represent the numbers of analyzed tumors in these studies: red: $n \leq 20$; yellow: $n = 21$ to 100 ; green: $n > 100$.

SATB2: does it earn a place in the colorectal CUP panel?

TABLE 1. Clinicopathologic Characteristics of CRCs Classified by SATB2 and CDX2 Expressions

	Total no. 269 (100%)	SATB2+/CDX2+ 217 (81%)	SATB2-/CDX2+ 23 (9%)	SATB2-/CDX2- 15 (6%)	SATB2+/CDX2- 14 (5%)	P
Sex, n (%)						
Male	143 (53)	118 (54)	11 (48)	9 (60)	5 (36)	0.50*
Female	126 (47)	99 (46)	12 (52)	6 (40)	9 (64)	
Age	68.6 ± 12.6	68.4 ± 12.1	65.3 ± 15.3	72.3 ± 16.1	73.7 ± 10.7	0.16†
Size	5.0 ± 2.6	4.84 ± 2.37	4.50 ± 2.26	6.65 ± 3.26	6.57 ± 3.96	0.0039†§
Tumor location, n (%)						
Right-sided colon	124 (46)	87 (40)	16 (69)	12 (80)	9 (64)	0.00097‡
Left-sided colon	86 (32)	77 (36)	2 (9)	2 (13)	5 (36)	
Rectum	59 (22)	53 (24)	5 (22)	1 (7)	0 (0)	
pT stage, n (%)						
T2	36 (13)	30 (14)	3 (13)	2 (13)	1 (7)	0.30*
T3	189 (70)	154 (71)	16 (70)	7 (47)	12 (86)	
T4	44 (16)	33 (15)	4 (17)	6 (40)	1 (7)	
Histologic differentiation						
Well to moderately	242 (90)	210 (97)	17 (74)	6 (40)	9 (64)	<0.00001*¶
Poorly	27 (10)	7 (3)	6 (26)	9 (60)	5 (36)	
Mucus production, n (%)						
Positive	14 (5)	6 (3)	4 (17)	3 (20)	1 (7)	0.0019*
Negative	255 (95)	211 (97)	19 (83)	12 (80)	13 (93)	
Lymph node metastasis, n (%)						
Positive	124 (49)	97 (48)	11 (52)	11 (73)	5 (38)	0.24*
Negative	129 (51)	105 (52)	12 (48)	4 (27)	8 (62)	

SATB2 and CDX2 are complementary markers. The combination improves recognition of colorectal origin.

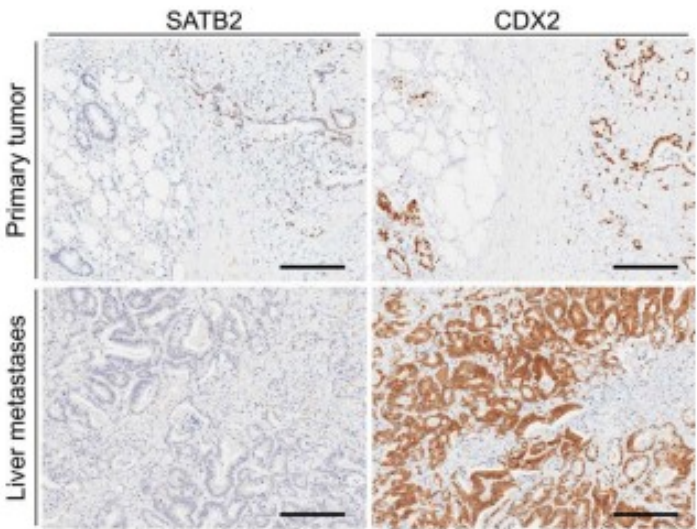


FIGURE 3. A CRC case showing differing SATB2 and CDX2 immunoreactivity in the primary tumor and its metastasis. A CRC case showing discrepant SATB2 expression in the primary and metastatic lesions. Note that while SATB2 was heterogeneously expressed in the primary tumor, the metastatic tumor showed no expression. CDX2 was diffusely expressed at both primary and metastatic sites. Scale bars, 200 μ m.

SATB2 in NETs: a marker of rectal and appendiceal origin



HHS Public Access

Author manuscript

Hum Pathol. Author manuscript; available in PMC 2021 February 01.

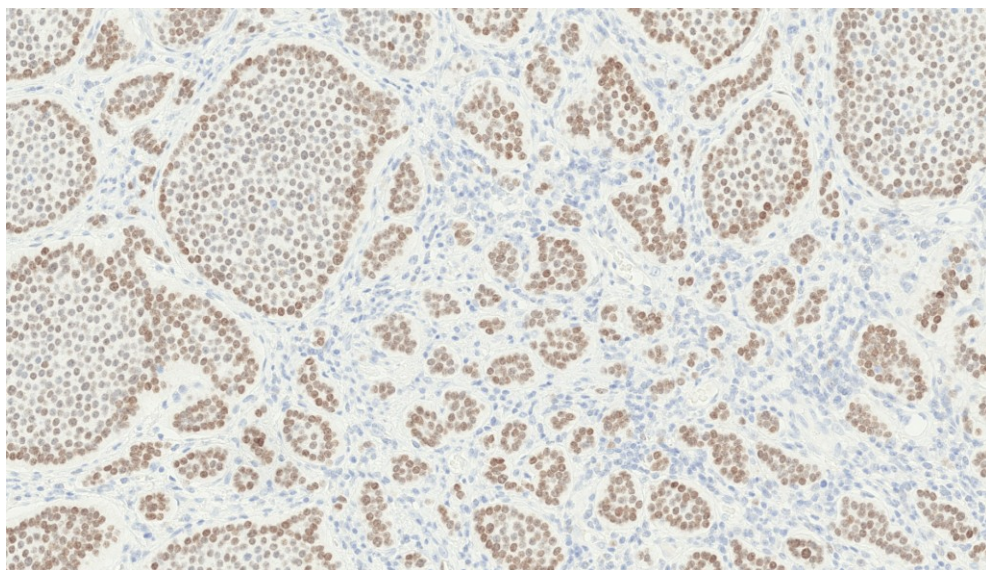
Published in final edited form as:

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*

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NETs: Neuroendocrine tumors

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

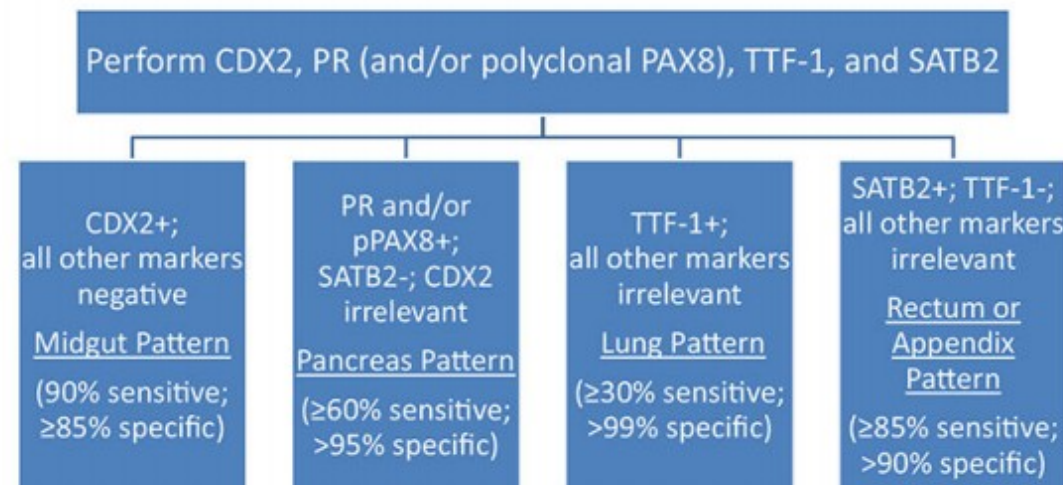


Figure 11. Simplified Immunohistochemical Algorithm for Well-Differentiated Neuroendocrine Tumor Site of Origin.

“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”.

SATB2: specificity has its limits

Appl Immunohistochem Mol Morphol • Volume 32, Number 10, November/December 2024

Immunohistochemical Expression of SATB2 in Malignant Melanomas

Rasmus Røge, MD, PhD,† Birgit Truumees, MSc,* and Søren Nielsen, BMS**

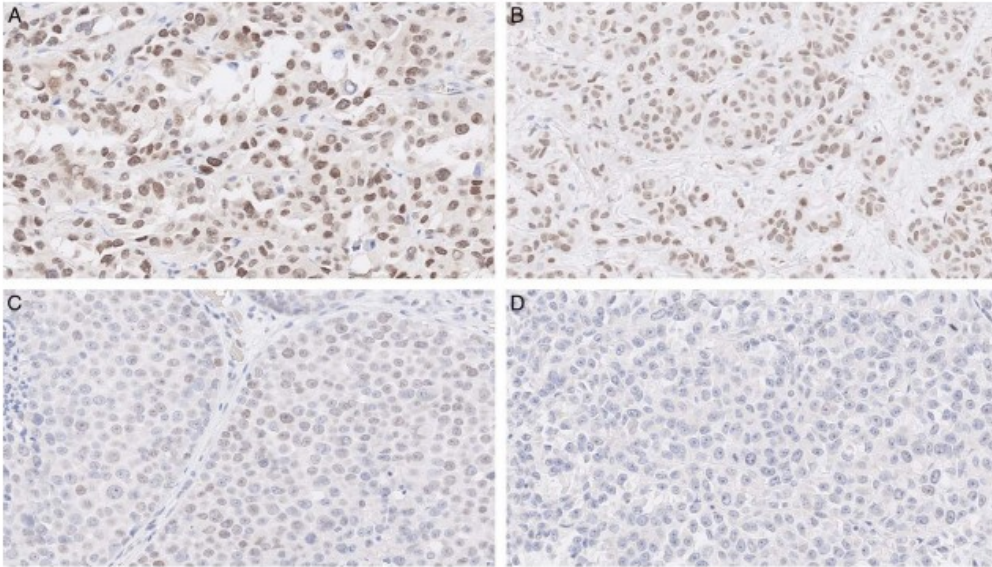


FIGURE 1. Nuclear staining for SATB2 in different malignant melanomas (MM). A–D, Moderate to strong, moderate, weak, and negative staining.

H-scores were calculated as (1x% of cells stained weakly)+(2x% of cells stained moderately)+(3x% of cells stained strongly). This yielded a score ranging from 0 to 300. An H-score of 20 or above was considered as positive.

SATB2 was positive in 30 of 63 melanomas (48%). Most positive cases showed only weak to moderate staining.

Diffuse strong staining still favours colorectal origin, but focal or weak positivity is not specific.

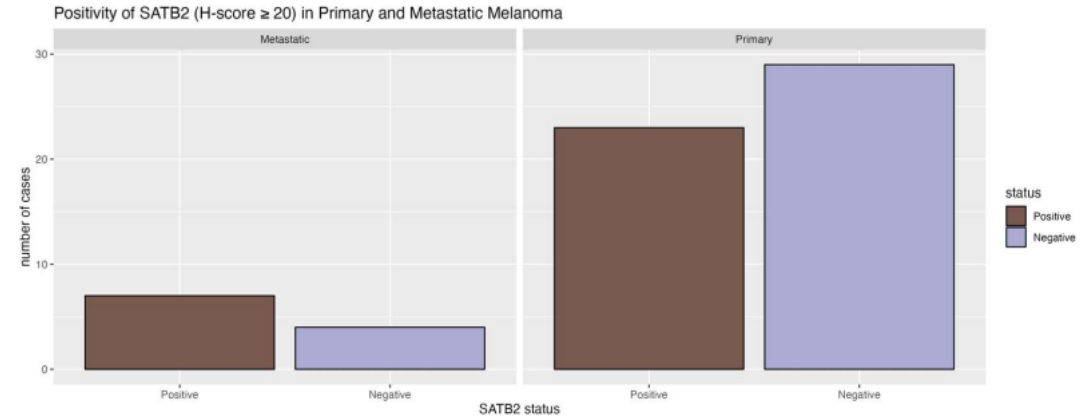


FIGURE 2. The number of SATB2 positive (H-score ≥ 20) and negative cases in primary and metastatic malignant melanoma.

SATB2: performance depends on clone and protocol

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 VRPS³	4	Ventana/Roche	2	2	0	0	-	-
rmAb clone EP281 760-6075 VRPS⁴	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 assessed protocols).

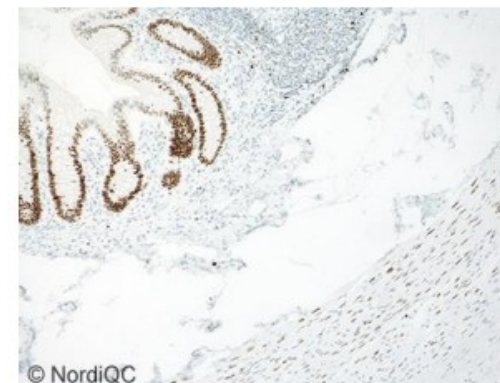
2) Proportion of Optimal Results (≥5 assessed protocols).

3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).

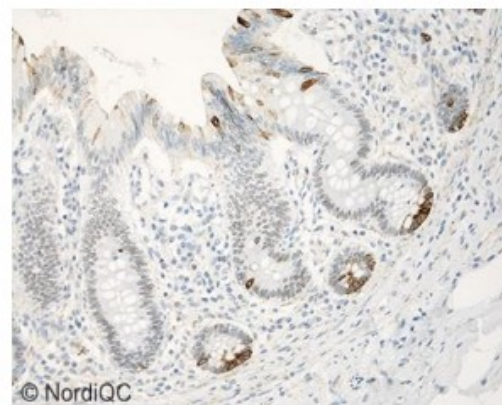
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).



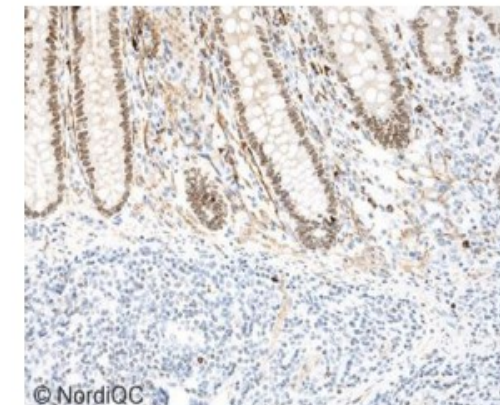
EP281



CL0276

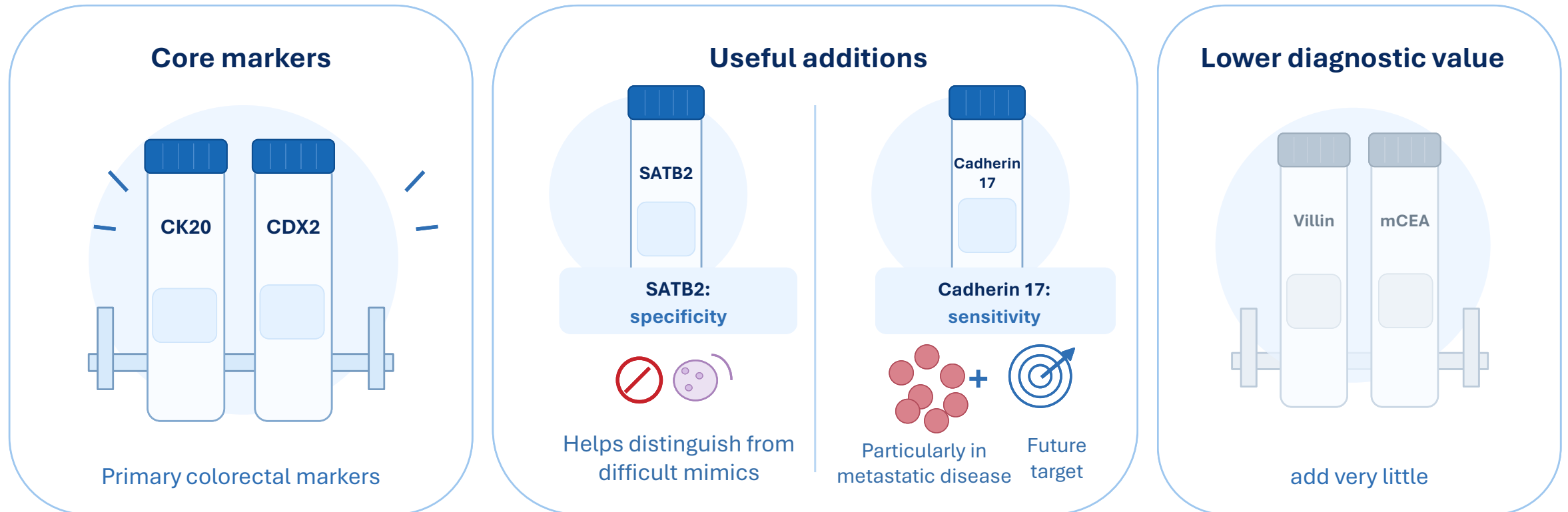


Ab69995



HPA001042

Colorectal origin in CUP: what stays, what adds value?



CK20 and CDX2 stay central. **SATB2 and Cadherin 17** can add value. **Villin and mCEA** often add relatively little.

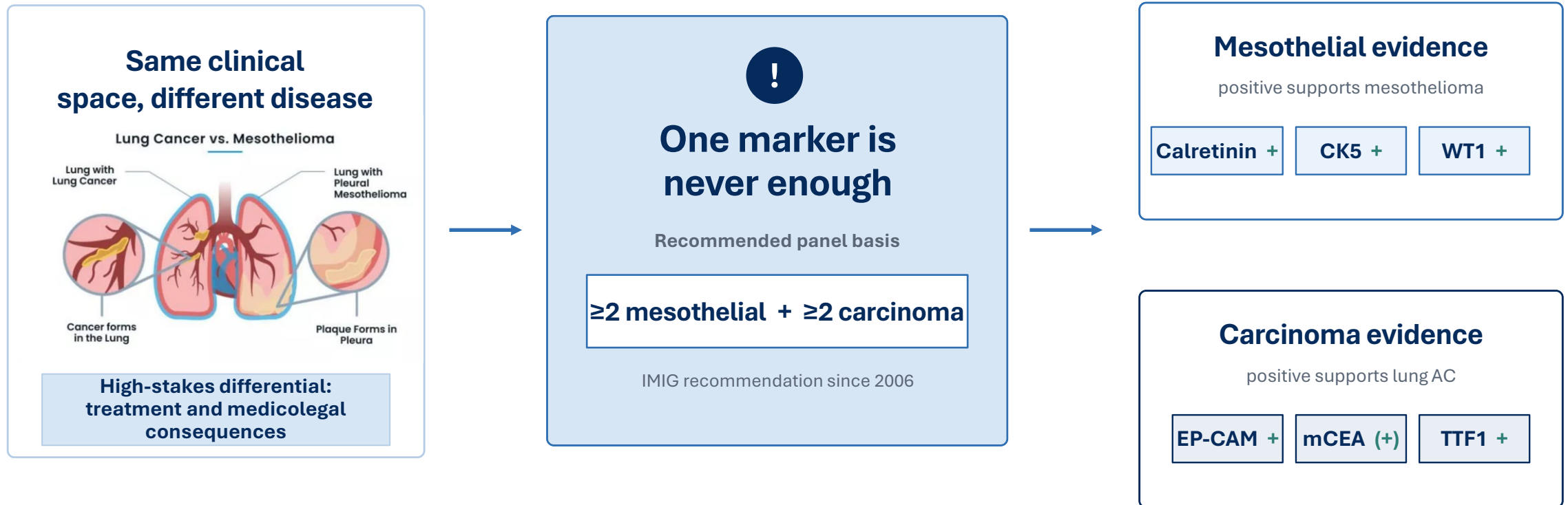
Good panels are designed, not just accumulated.

DIAGNOSTIC PANEL

Mesothelioma vs lung adenocarcinoma

Mesothelioma versus lung adenocarcinoma

A panel is the safety mechanism: one marker can point in the wrong direction.



* EP-CAM is not an exclusion marker by itself: up to 30% of mesotheliomas can be positive.

Use a balanced panel, not a favourite marker.

Mesothelioma-positive markers: which ones still earn their place?

	To stay	Sensitivity	Comments
Calretinin	Yes	85-95%	High sensitivity, but focal expression may occur in carcinomas
CK5	Yes	90-95%	High sensitivity, but also expressed in squamous cell carcinoma
WT1	Yes	85-95%	High sensitivity, but also expressed in serous carcinoma
Thrombomodulin	No	60-70%	Lower sensitivity than the established markers
CA125	No	70-80%	Limited specificity and expressed in several carcinomas
Mesothelin	No	60-80%	Limited sensitivity and specificity

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.

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Mesothelioma-positive markers: new options

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CK5	Yes	90-95%	High sensitivity, but also expressed in squamous cell carcinoma
WT1	Yes	85-95%	High sensitivity, but also expressed in serous carcinoma
Uroplakin 3b	NEW	80%	useful additional marker, but not mesothelioma-specific Also seen in Urothelial carcinoma (15-60%) and Ovarian carc. (10%)
HEG1	NEW	85-95%	High sensitivity across mesothelioma subtypes Also seen in few non-mesotheliomas

Uroplakin IIIB: useful for epithelioid mesothelioma, but not universal



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 Reischwich 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

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

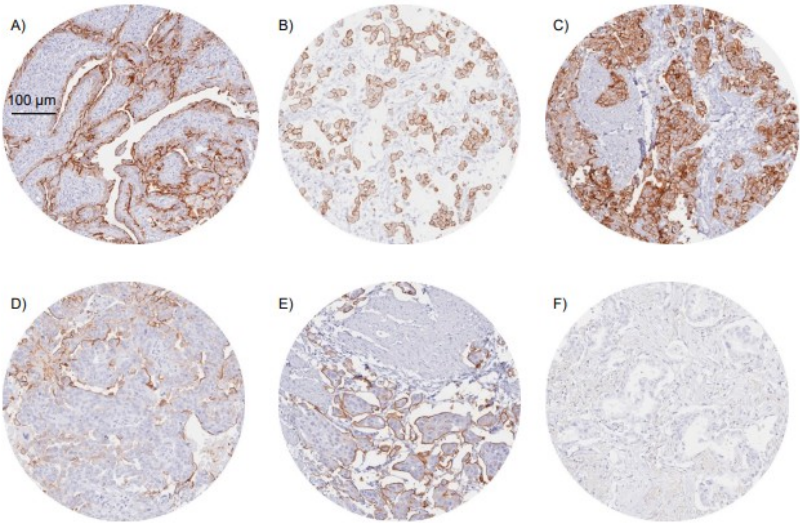


Figure 3. Examples of Upk3b immunostaining in selected cancers. The panels show a strong membranous Upk3b staining in samples of two epithelioid mesotheliomas (A,B), and a muscle-invasive urothelial carcinoma of the urinary bladder (C). Upk3b staining is more focal and predominantly seen on surface membranes 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).

HEG1: high sensitivity across mesothelioma subtypes

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

	N	P	(%)	Staining score					Staining pattern			
				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)

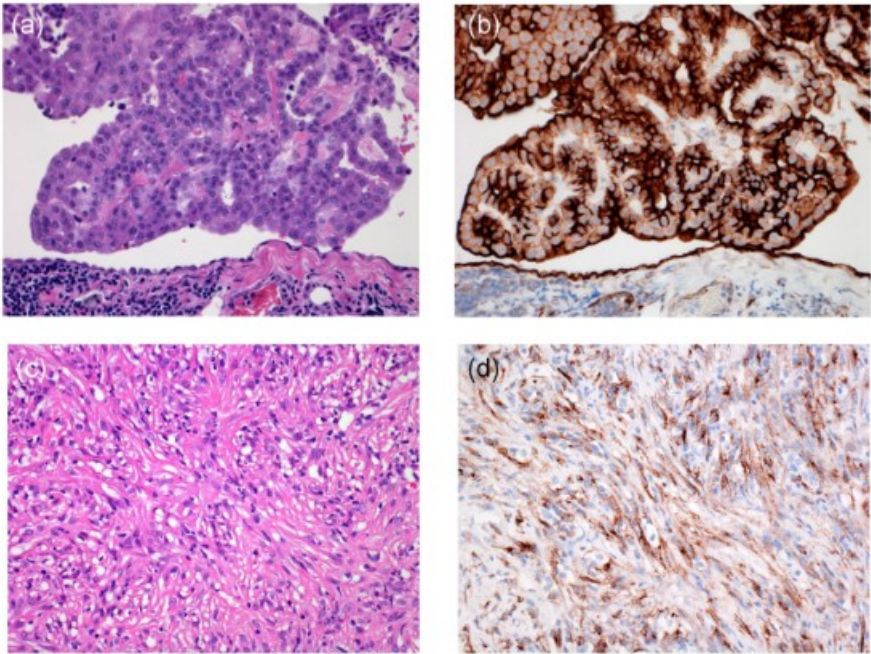


TABLE 1 Expression frequency of HEG1 and conventional mesothelial markers in mesotheliomas

	EM		BM		SM	
	No. /T	(%)	No. /T	(%)	No. /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. /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 Wu² | Eitetsu Koh⁴ | Yasuo Sekine⁴ | Daisuke Ozaki⁵ | Toshikazu Yusa⁶ | Tadao Nakazawa² | Shoutaro Tsuji⁷ | Yohei Miyagi⁸ | Ann E. Walts⁹ | Alberto M. Marchevsky⁹ | Aliya N. Husain¹⁰ | Kohzoh Imai¹¹

HEG1 and Uroplakin IIIB: partners rather than competitors

Human Pathology (2023) 141, 64–68



Original contribution

Uroplakin-IIIb as a novel immunohistochemical marker for mesothelioma☆☆☆

Derek M. van Pel MD^a, Simon Cheung BSc^b, Diana N. Ionescu MD^{a,c}, Andrew Churg MD^{a,b,*}

^aDepartment of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, V5Z 1M9, Canada

^bDivision of Anatomic Pathology, Vancouver General Hospital, Vancouver, BC, V5Z 1M9, Canada

^cDepartment of Pathology, BC Cancer Agency, Vancouver, BC, V5Z 1M9, Canada

HEG1 is the stronger first-line marker for epithelioid mesothelioma.
Uroplakin IIIB identifies some HEG1-negative cases.
Combined sensitivity: 89% → 93%

Table 1 Staining of mesotheliomas with antibodies directed against UpIIIb and HEG1.

Marker	Mesothelioma type	Positive, n (%)	Negative, n (%)	Total cases, n
UpIIIb	Epithelioid	37 (78.7)	10 (21.3)	47
	Sarcomatoid	0 (0)	24 (100)	24
HEG1	Epithelioid	41 (89.1)	5 (10.9)	46
	Sarcomatoid	9 (37.5)	15 (62.5)	24

Table 2 Correlation of staining by UPIIIb and HEG1 in 46 cases of epithelioid mesothelioma.

		UpIIIb	
		Positive, n	Negative, n
HEG1	Positive, n	34	7
	Negative, n	2	3



Carcinoma markers: which ones best exclude mesothelioma?

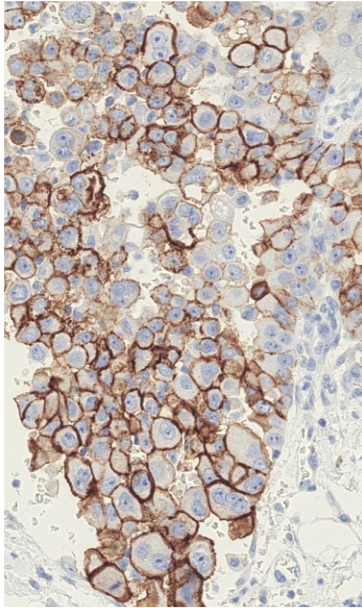
	To stay	Sensitivity*	Comments
Claudin-4	Yes	0-5%	Preferred negative marker Highly reliable for carcinoma and usually absent in mesothelioma
EP-CAM – Ber-EP4	(Yes)	10-35%	Less reliable Mesotheliomas may show focal positivity
EP-CAM – MOC31	(Yes)	10-35%	Less reliable Mesotheliomas may show focal positivity
mCEA	?	0%	Less reliable Very specific when positive, but too insensitive to exclude carcinoma when negative

* Positivity seen in mesotheliomas (epithelial subtype)

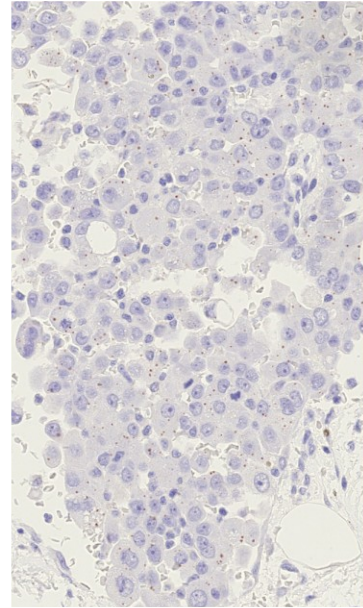
PRACTICAL PANEL

- Positive mesothelial marker: **HEG1**
- Additional mesothelial marker: **calretinin** or **Uroplakin IIIB**
- Carcinoma marker: **Claudin-4**
- Optional second carcinoma marker: **EP-CAM**

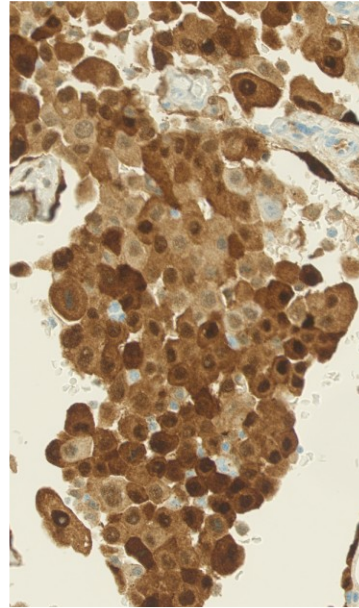
Mesothelioma



EP-CAM (EP4)



Claudin 4

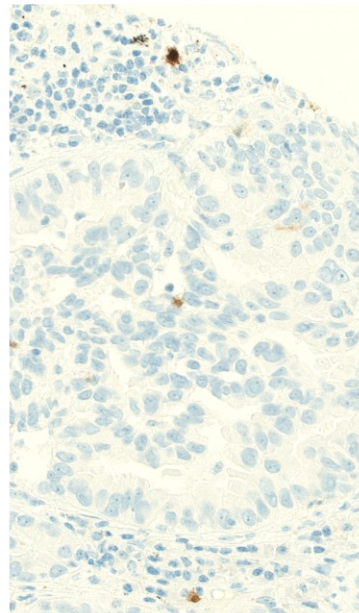
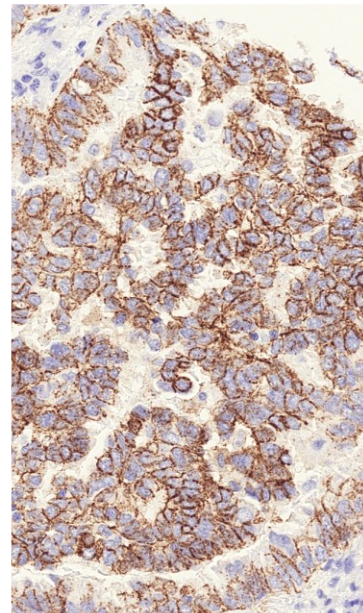
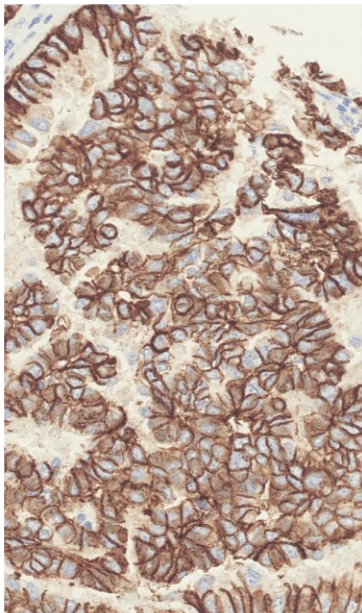


Calretinin

Mesothelioma:
Claudin-4 negative

Lung adenocarcinoma: Claudin-4
strongly positive

Lung
AC



**Compared with EP-CAM,
Claudin-4 gives
a clearer separation between
Carcinoma (pos.) and
mesothelioma (neg.).**

HEG1 and Claudin-4: a two-marker shortcut

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.

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}
¹Department of Pathology, Vancouver General Hospital, and ²Department of Pathology, University of British Columbia, Vancouver, BC, Canada

HEG1 and Claudin-4: a two-marker shortcut

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

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

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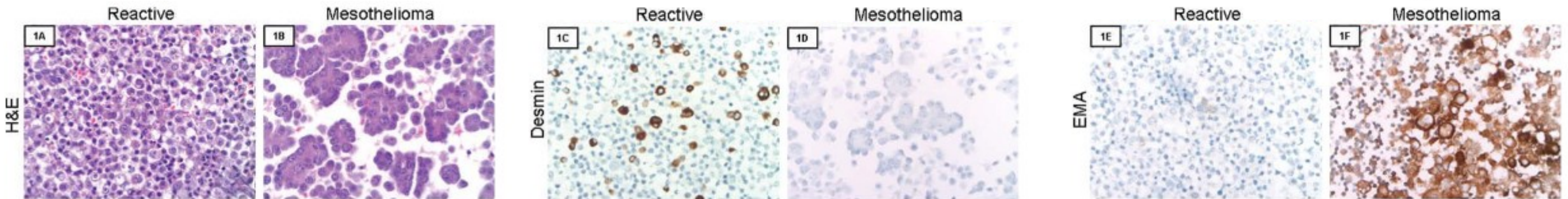
¹Department of Pathology, Vancouver General Hospital, and ²Department of Pathology, University of British Columbia, Vancouver, BC, Canada

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%)

Mesothelioma versus reactive mesothelium: loss is the clue

	To stay	Sensitivity	Comments
BAP1	"New"	60%	Nuclear loss supports malignancy
MTAP	"New"	50%	Cytoplasmic loss supports CDKN2A-region deletion
Merlin	New	50%	Loss may support NF2 alteration
Desmin	No	50%	About 50% of reactive mesothelial proliferations and 95% of mesotheliomas are Desmin negative
EMA	No	60-80%	Strong membranous staining may support malignancy, but lacks specificity



BAP1: when loss supports malignancy

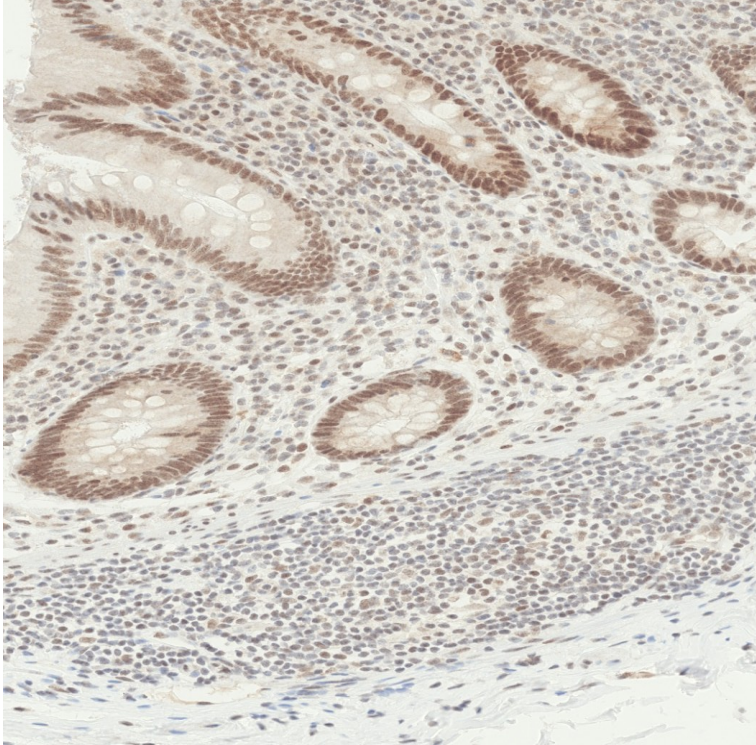
Clone C-4, Santa Cruz

Or

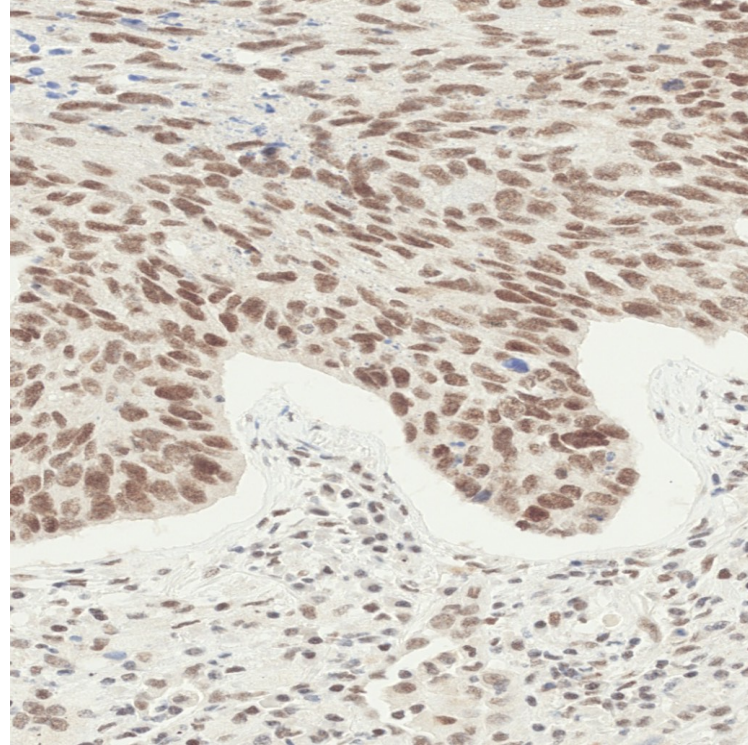
Clone EPR22826, Abcam

Clone BSB-109, BioSp

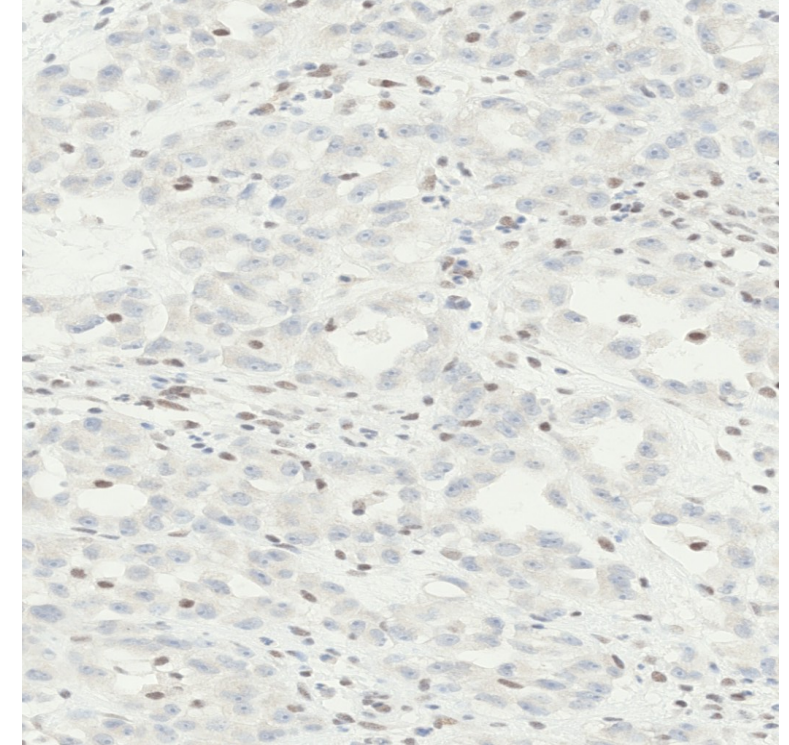
Colon/Appendix



Tumour without mutation



Mesothelioma with lost BAP1



BAP1 is a nuclear tumour-suppressor protein.

Loss of nuclear staining strongly supports malignant mesothelioma over a reactive mesothelial proliferation.

High specificity, but limited sensitivity: retained BAP1 does not exclude mesothelioma.

BAP1 in cytology: highly specific, but not highly sensitive

Review Article

Diagnostic Mesothelioma Biomarkers in Effusion Cytology

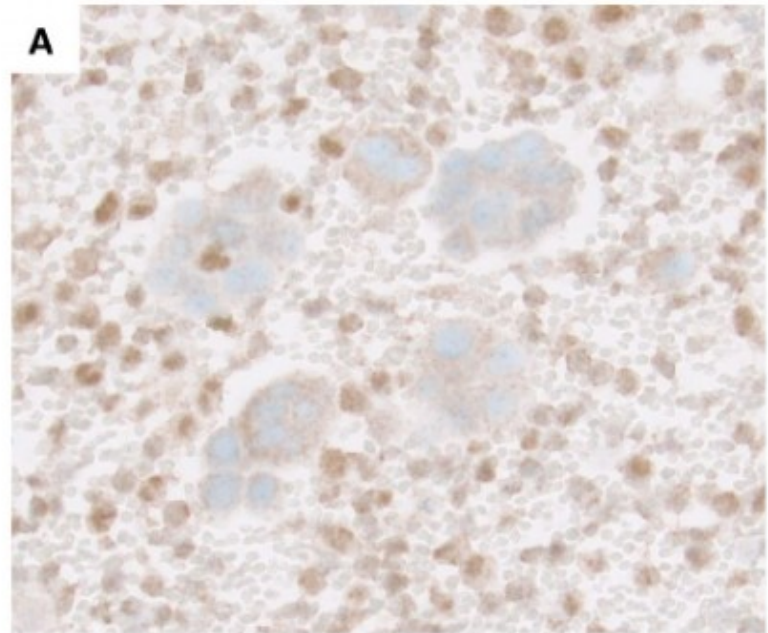
Albino Eccher, MD ¹; Ilaria Girolami, MD ²; Ersilia Lucenteforte, MD³; Giancarlo Troncone, MD ⁴; Aldo Scarpa, MD¹; and Liron Pantanowitz, MD ⁵

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

Marker	Sensitivity and Specificity in Systematic Reviews		Notes
	Sensitivity (CI)	Specificity (CI)	
Soluble Mesothelin/SMRP Fibulin-3	0.79 (0.75-0.83) ²⁷	0.85 (0.83-0.87) ²⁷	• Different cutoffs of the studies included • No subgroup analysis for different MPM subtypes • Diagnostic performance is usually studied in differential against both lung cancer and reactive atypical mesothelium
	0.69 (0.64-0.72) ²⁸	0.90 (0.85-0.94) ²⁸	
	0.73 (0.54-0.86) ³¹	0.80 (0.60-0.91) ³¹	
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
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	• Few studies dealing with cytology^{37,38} • 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



BAP1: a negative result that requires a positive control

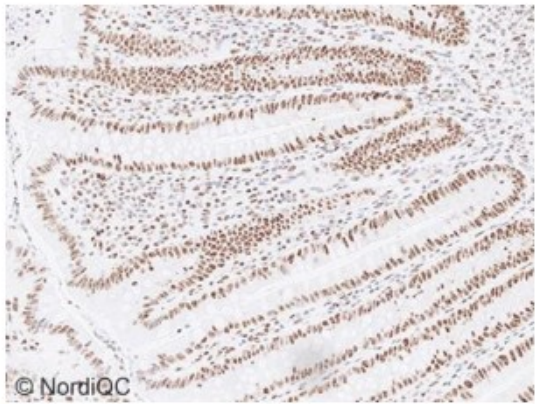


Fig. 1a
Optimal BAP1 staining of appendix using the mAb clone C-4 - diluted, 1:25 (40 min. incubation), epitope retrieval using HIER in CC1 (32 min.), a 3-step multimer based detection system (OptiView) with tyramide amplification (OptiView Amplification) and performed on BenchMark (Ventana/Roche). Virtually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

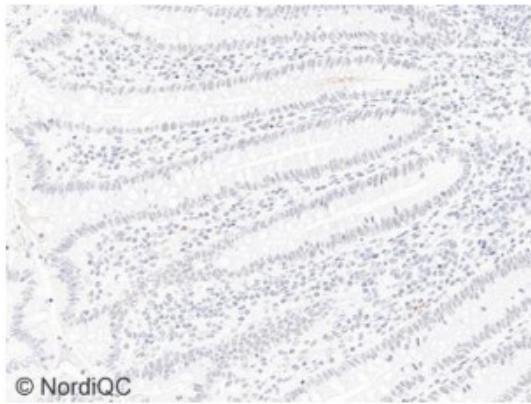


Fig. 1b
Insufficient BAP1 staining of the appendix using same clone and similar protocol settings as in Fig. 1a, but with a less sensitive detection system (UltraView). Only scattered epithelial cells show a faint nuclear staining reaction. Virtually all lymphocytes/stromal cells are negative. Same protocol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

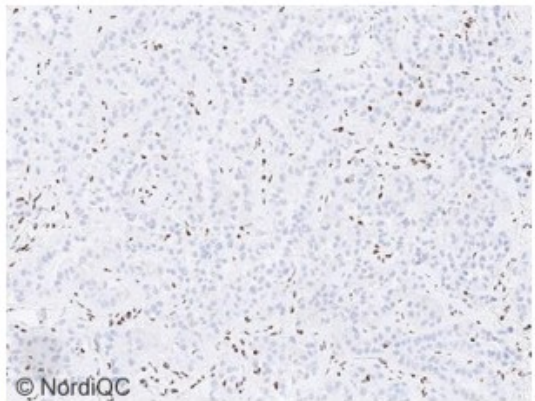


Fig. 4a
Optimal BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1a - 3a. All neoplastic cells are negative, whereas stromal cells show a distinct, weak to moderate nuclear staining reaction.

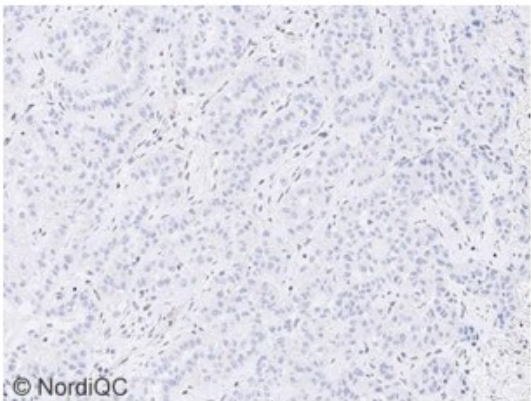
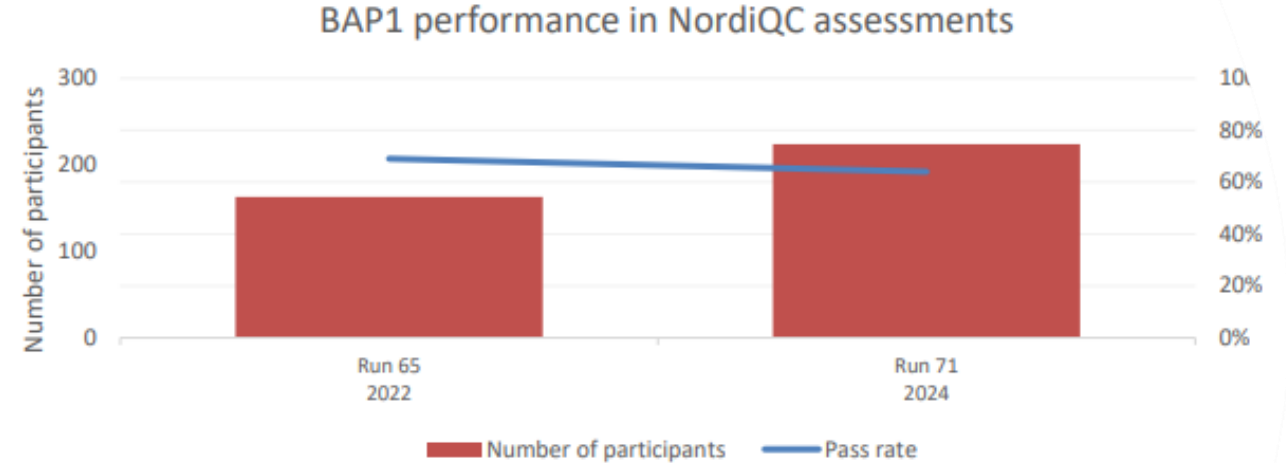


Fig. 4b
Insufficient BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1b - 3b. The neoplastic cells are negative as expected. However, also the stromal cells, expected to be positive serving as internal positive tissue control, are negative.



Loss of tumour nuclear staining
supports BAP1 alteration

Non-neoplastic cells must retain nuclear staining
Internal positive controls are essential

BAP1: not every negative result is a true loss

BAP1 assays are clone- and protocol-dependent:
Some BAP1 clones require highly sensitive protocols. Insufficient optimisation may produce weak controls, background staining or false loss

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 assessed protocols).

2) Proportion of Optimal Results (OR).

* including distributed by Genova (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%	

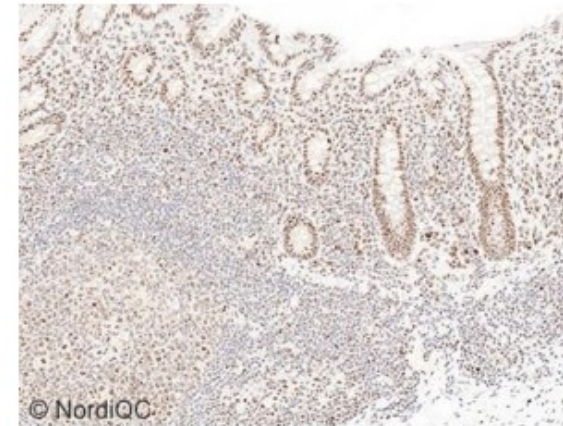


Fig. 2a

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.

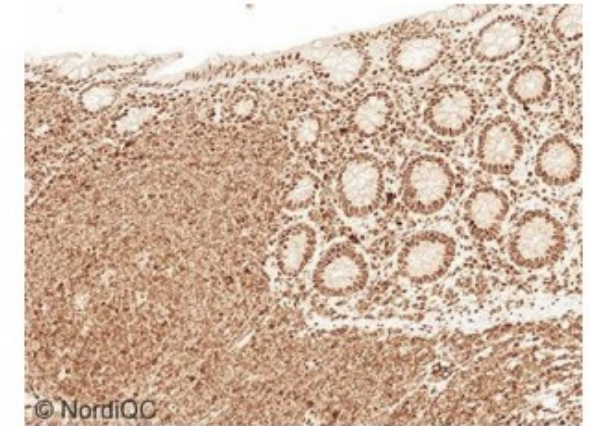


Fig. 2b

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.

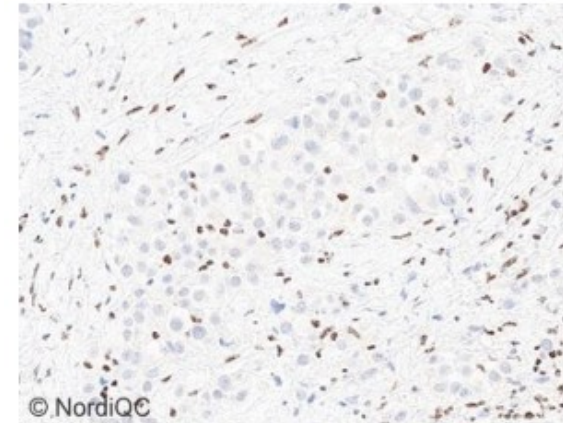


Fig. 4a

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.

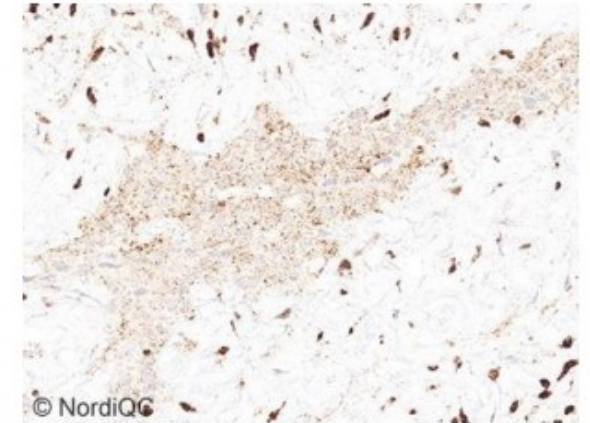


Fig. 4b

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.

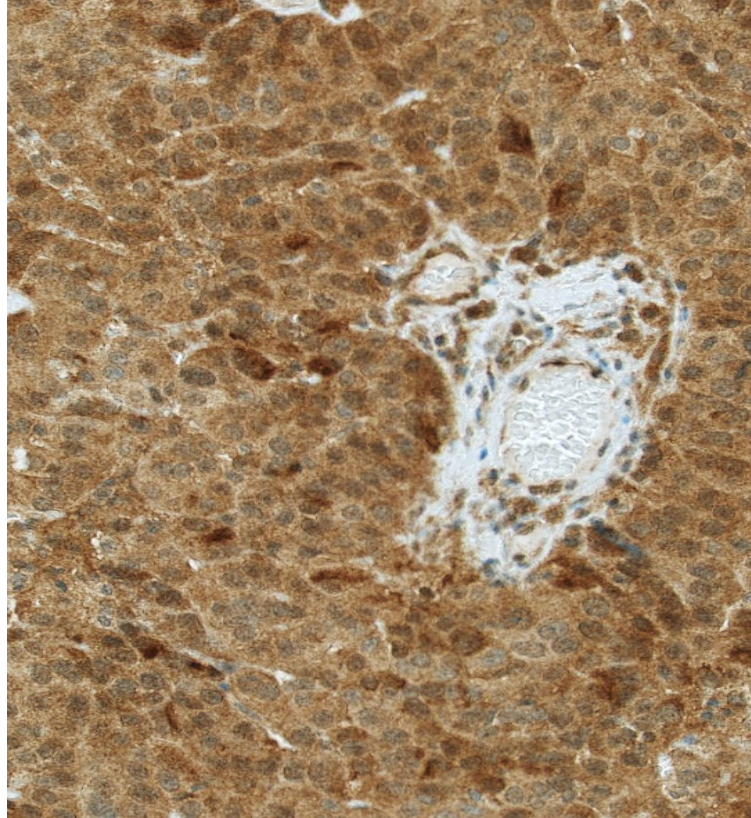
MTAP: loss as a surrogate for CDKN2A deletion

Clone EPR6893

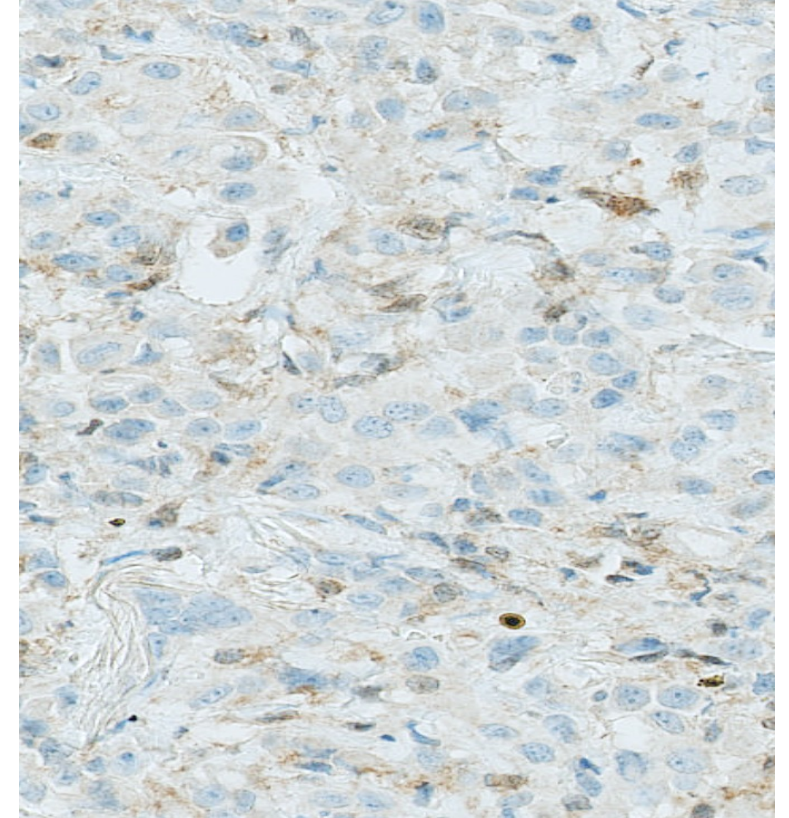
Adrenal gland



Tumour with intact CDKN2A



Mesothelioma with CDKN2A del



MTAP is normally expressed in mesothelial cells.

Loss of cytoplasmic staining supports malignancy and may indicate CDKN2A homozygous deletion.

Highly specific, but not all mesotheliomas show loss.

Best interpreted alongside BAP1

MTAP loss across human cancers: useful, but not mesothelioma-specific

ORIGINAL ARTICLE

OPEN

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 Reischwich, 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*

(Am J Surg Pathol 2024;00:000–000)

MTAP (MSVA-741R)

Sensitive and specific marker for homozygous 9p21 deletions.

MS Validated antibodies

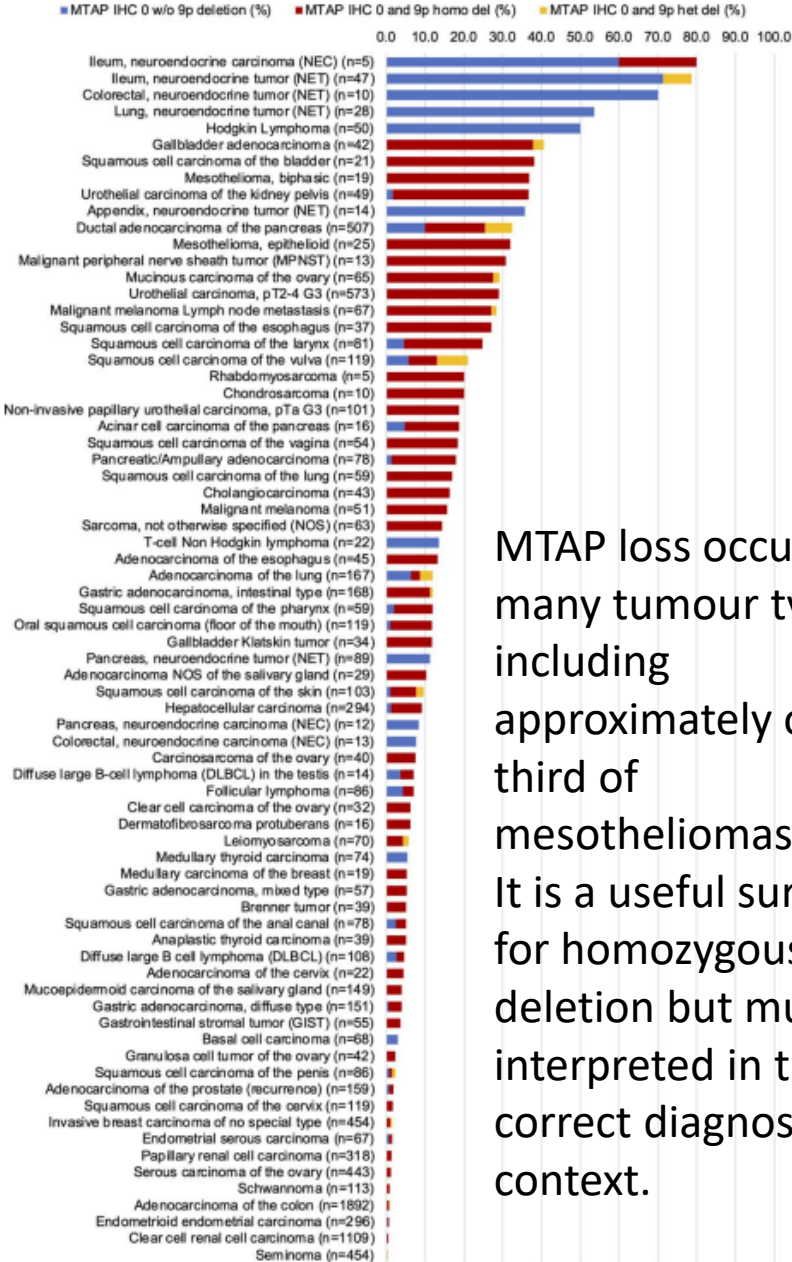
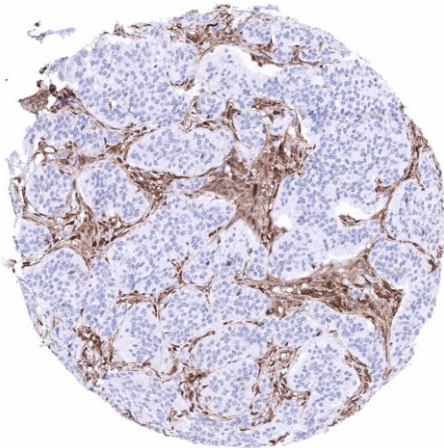


FIGURE 4. Concordance rate of MTAP IHC and 9p21 copy number analysis.

MTAP loss occurs in many tumour types, including approximately one-third of mesotheliomas. It is a useful surrogate for homozygous 9p21 deletion but must be interpreted in the correct diagnostic context.

MTAP loss is not mesothelioma-specific

Mod Pathol 37 (2024) 100495

MODERN PATHOLOGY



Journal homepage: <https://modernpathology.org/>

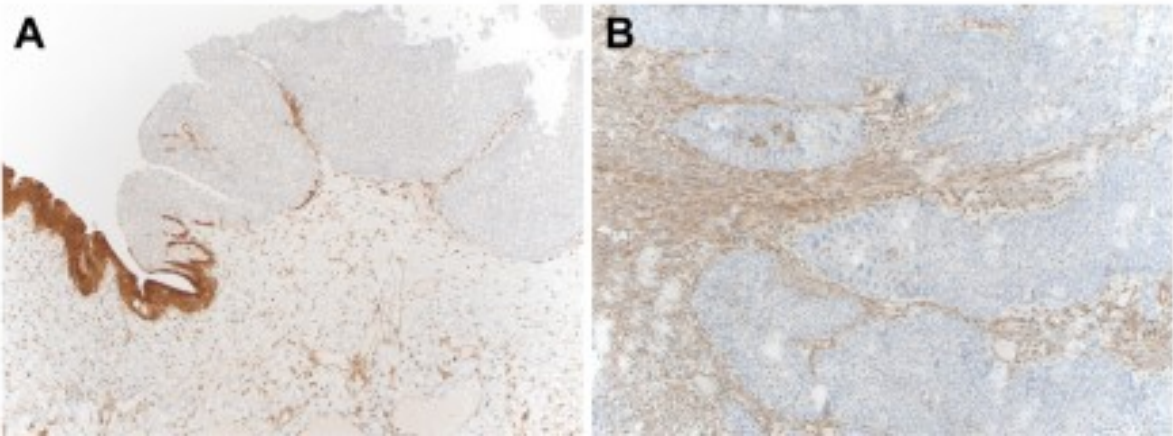
Research Article

Loss of MTAP Expression by Immunohistochemistry Is a Surrogate Marker for Homozygous 9p21.3 Deletion in Urothelial Carcinoma

Tatjana Vlajnic^{a,*}, Obinna Chijioke^a, Luca Roma^a, Spasenija Savic Prince^a, Tobias Zellweger^b, Cyrill A. Rentsch^c, Lukas Bubendorf^a

^a Institute of Medical Genetics and Pathology, University Hospital Basel, University of Basel, Basel, Switzerland; ^b Division of Urology, St. Clara Hospital, Basel, Switzerland; ^c Department of Urology, University Hospital Basel, Basel, Switzerland

Tatjana Vlajnic et al. / Mod Pathol 37 (2024) 100495



Annals of Diagnostic Pathology 79 (2025) 152524

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journal homepage: www.elsevier.com/locate/anndiagpath



ELSEVIER



MTAP and p16 as immunohistochemical surrogates of CDKN2A/B homozygous deletion in central nervous system tumors: A multicentre Italian experience

Serena Salzano^a, Rosario Caltabiano^a, Gaetano Magro^a, Antonio D'Amati^{b,c}, Cristina Pizzimenti^d, Andrea Maugeri^e, Antonella Agodi^e, Giuseppe Barbagallo^f, Francesco Certo^f, Francesco Fiorentino^g, Giovanni Tuccari^h, Maurizio Martini^h, Antonio Ieni^h, Valeria Barresi^{i,j}, Giuseppe Broggi^{a,*}

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MTAP loss reflects 9p21.3 deletion, not mesothelial origin.

MTAP

from diagnostic surrogate to therapeutic biomarker



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



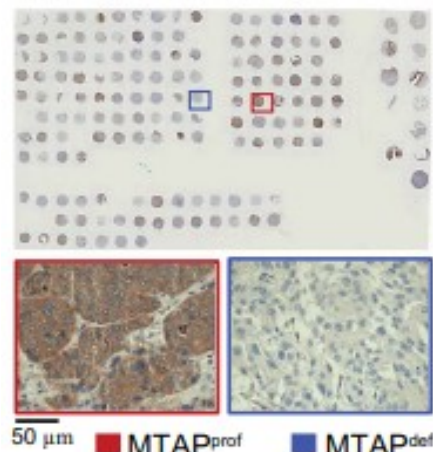
Original Article

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. Rodon¹ , H. Prenen², A. Sacher³, M. Villalona-Calero⁴, N. Penel⁵, A. El Helali⁶, S. Rottey⁷, N. Yamamoto⁸, F. Ghiringhelli⁹, M.E. Goebeler¹⁰, T. Doi¹¹, S. Postel-Vinay¹², C.-C. Lin¹³, C. Liu¹⁴, C.-H. Chuang¹⁴, K. Keyvanjah¹⁴, T. Eggert¹⁴, B.H. O'Neil¹⁵

Current role: Type I IHC assay Supports malignancy in a mesothelial proliferation

Potential future role: Type II predictive assay Identifies *MTAP*-deficient tumours that may benefit from targeted therapy



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

ARTICLE

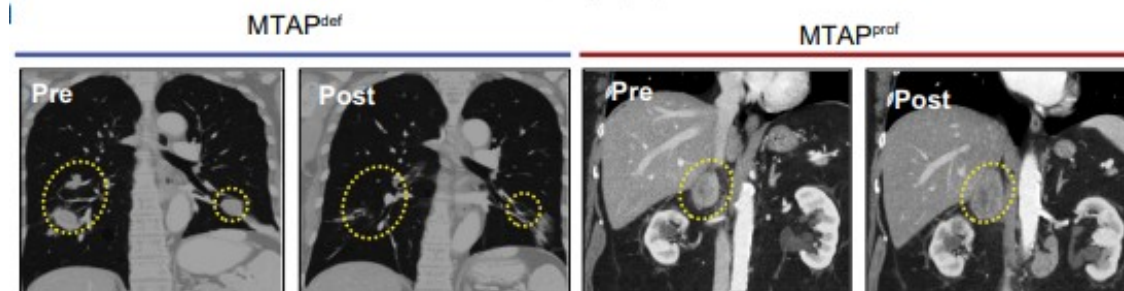
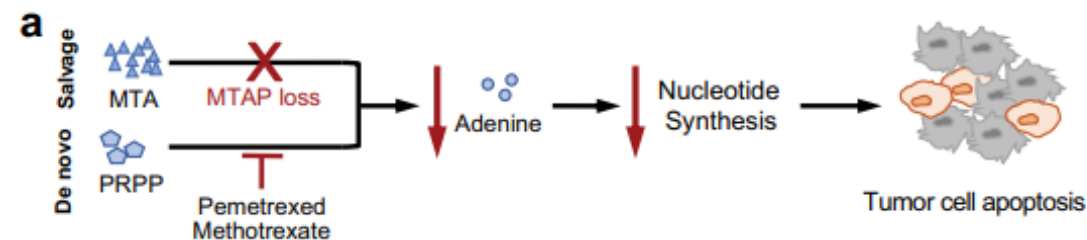
<https://doi.org/10.1038/s41467-022-29397-z>

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 Wang³, Sumankalai Ramachandran¹, Rebecca Slack Tidwell⁴, Guangchun Han⁵, Xinmiao Yan⁵, Jieru Meng¹, Ruiping Wang⁵, Anh G. Hoang¹, Wei-Lien Wang¹, Jian Song¹, Lidia Lopez¹, Alex Andreev-Drakhlin¹, Arlene Siefker-Radtke¹, Xinqiao Zhang¹, William F. Benedict¹, Amishi Y. Shah¹, Jennifer Wang¹, Pavlos Msaouel¹, Miao Zhang⁶, Charles C. Guo⁶, Bogdan Czerniak⁶, Carmen Behrens⁷, Luisa Soto⁸, Vassiliki Papadimitrakopoulou⁷, Jeff Lewis⁴, Waree Rinsurongkawong⁴, Vadeerat Rinsurongkawong⁴, Jack Lee⁹, Jack Roth⁹, Stephen Swisher⁹, Ignacio Wistuba⁶, John Heymach⁷, Jing Wang³, Matthew T. Campbell¹, Eleni Efsthathiou¹, Mark Titus¹, Christopher J. Logothetis¹, Thai H. Ho^{10,13}, Jianiun Zhang^{7,13}, Linghua Wang^{5,11,13} & Jianiun Gao^{1,13}



Merlin: the next step when BAP1 and MTAP are retained

ARTICLE

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

David B. Chapel^{1,2}, Jason L. Hornick¹, Julianne Barlow³, Raphael Bueno³ and Lynette M. Sholl^{1,4}

Modern Pathology (2022) 35:1383 – 1397

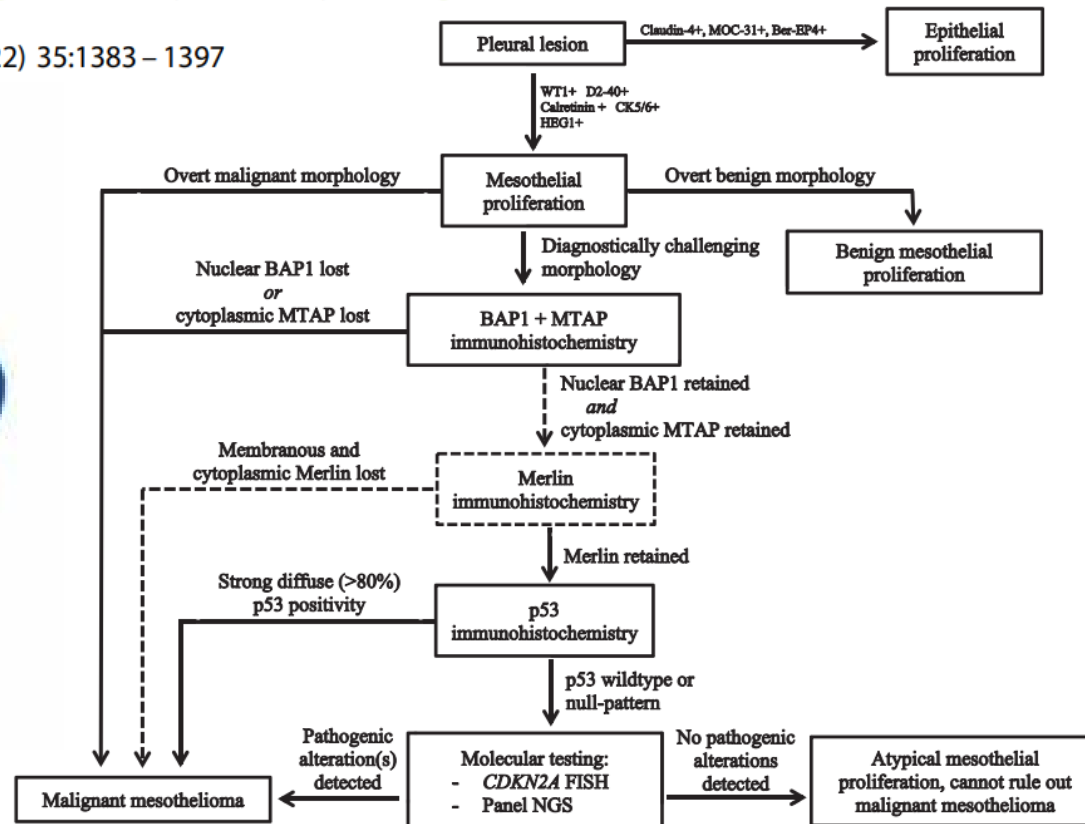


Fig. 5 Proposed diagnostic algorithm. Dashed lines indicate that Merlin immunohistochemistry is a provisional diagnostic marker of malignant mesothelioma, pending independent validation in subsequent studies. FISH, fluorescence in situ hybridization; NGS, next-generation sequencing. Figure adapted from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

Merlin is the tumour-suppressor protein encoded by NF2.
Loss of membranous and cytoplasmic staining may support malignant mesothelioma.

Potential value:
identifies additional cases not detected by BAP1 or MTAP loss.

Merlin: loss of expression may support NF2 alteration

ARTICLE

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

David B. Chapel^{1,2}, Jason L. Hornick¹, Julianne Barlow³, Raphael Bueno³ and Lynette M. Sholl^{1,4}

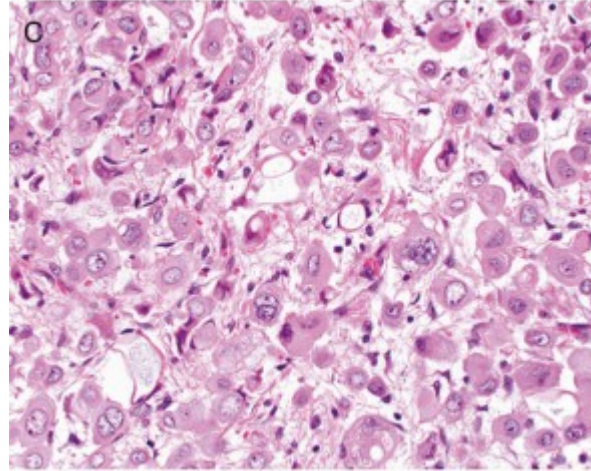
Modern Pathology (2022) 35:1383–1397

Normal and reactive mesothelial cells show weak to moderate membranous and granular cytoplasmic Merlin staining.

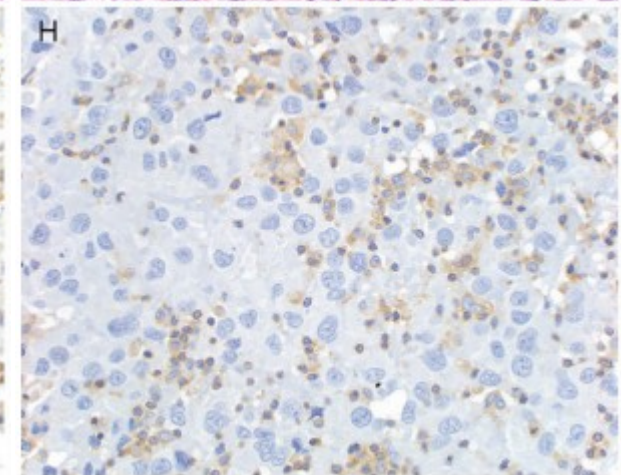
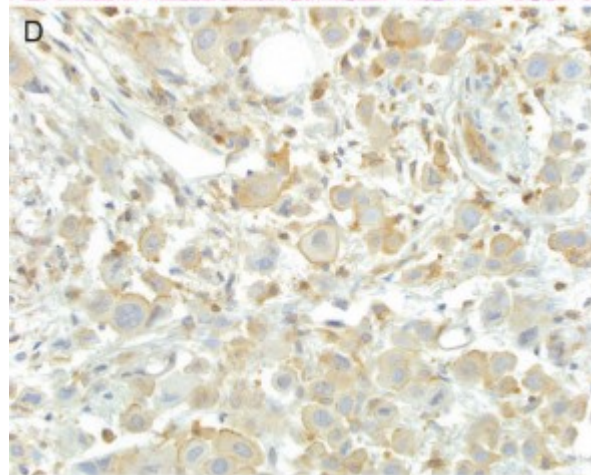
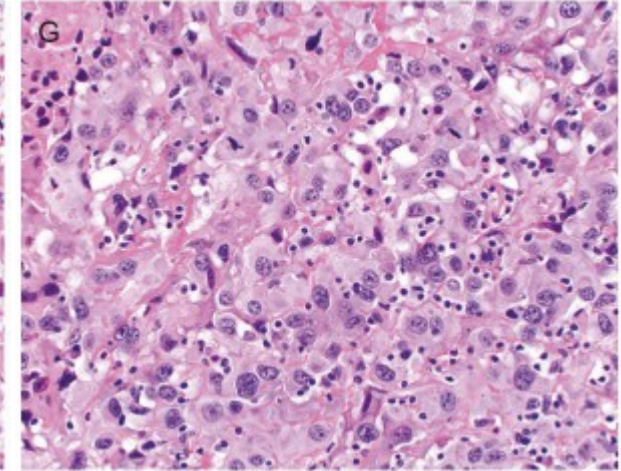
Loss of this pattern may support malignant mesothelioma and an underlying NF2 alteration.



Pleural mesothelioma 1

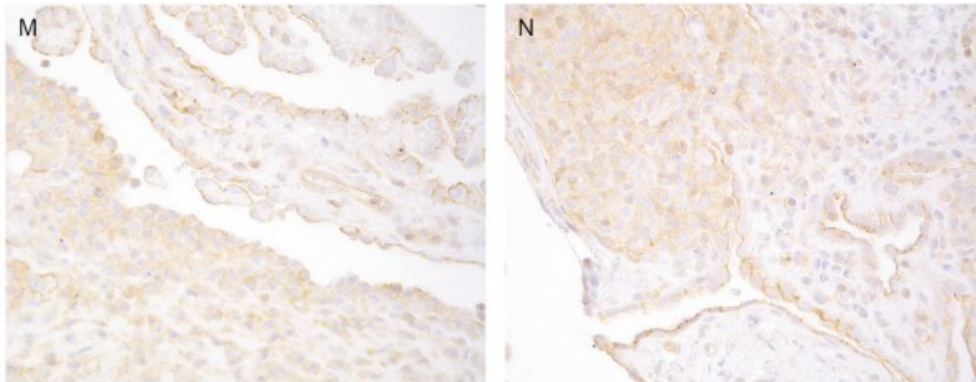


Pleural mesothelioma 2



Merlin retained

Merlin lost



Loss correlates with NF2 alteration, but IHC and molecular testing are not fully concordant (IHC and NGS agreement 77%)

Clone D1D8,
Cell Signaling.

DIAGNOSTIC PANEL

Neuroendocrine neoplasia

The neuroendocrine panel: core markers and new

	To stay	Sensitivity [#]	Comments
Chromogranin A	Yes	50-85%	Very specific but less sensitive, especially in poorly differentiated NEC
Synaptophysin	Yes	60-90%	Sensitive core marker, but not entirely specific
NSE	No	60-70%	Neuron Specific Enolase, insufficient specificity
CD56	?	70-90%	Sensitive but very non-specific, use selectively
INSM1*	"New"	85-95%	Insulinoma-associated protein 1, sensitive nuclear marker, strong candidate for the core panel
ISL1**	New	15-100%	Supports pancreatic or lower intestinal origin; usually low or absent in upper gastric NETs.
Secretagogen**	New	55-85%	Promising supplemental marker for NET/NEC, performance varies by tumour type

[#] Reported sensitivity varies by tumour differentiation, anatomical site and study cohort. Values for NETs and NECs are not directly comparable.

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

Andrew Bellizzi; Human Pathol 2020; Feb;96:8-33

**Second-Generation Neuroendocrine Immunohistochemical Markers: Reflections from Clinical Implementation

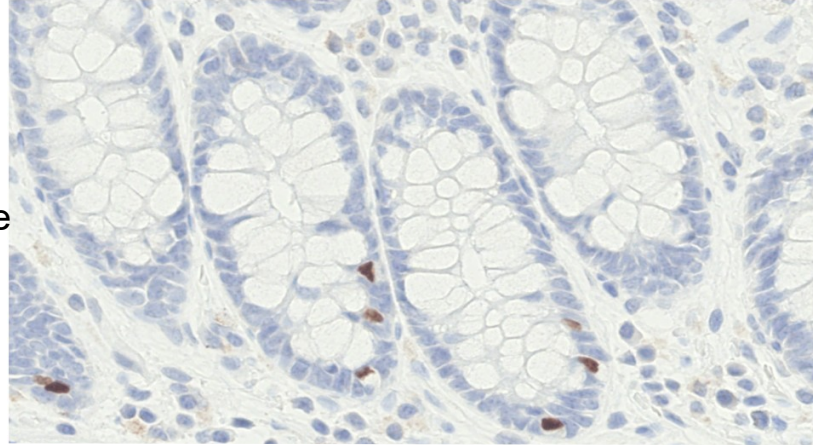
Juhlin C. C; Biology 2021, 10, 874. <https://doi.org/10.3390/biology10090874>

INSM1: a nuclear partner for synaptophysin

Established clone: A-8
Newer alternatives:
MRQ-70, MSVA-465R,
BSB-123

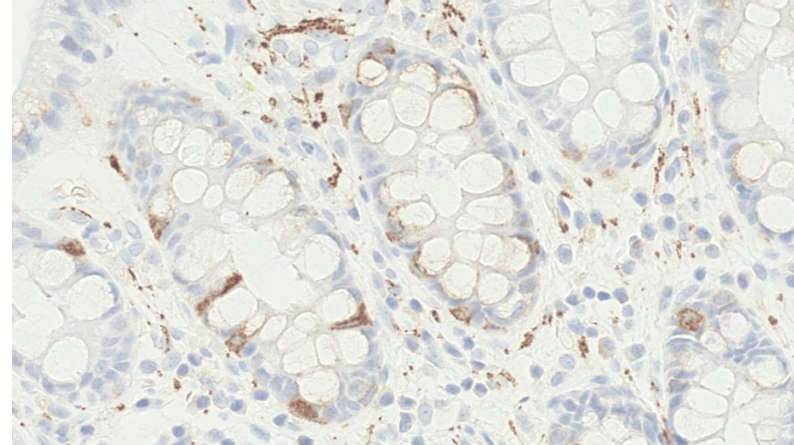
INSM1

Nuclear staining

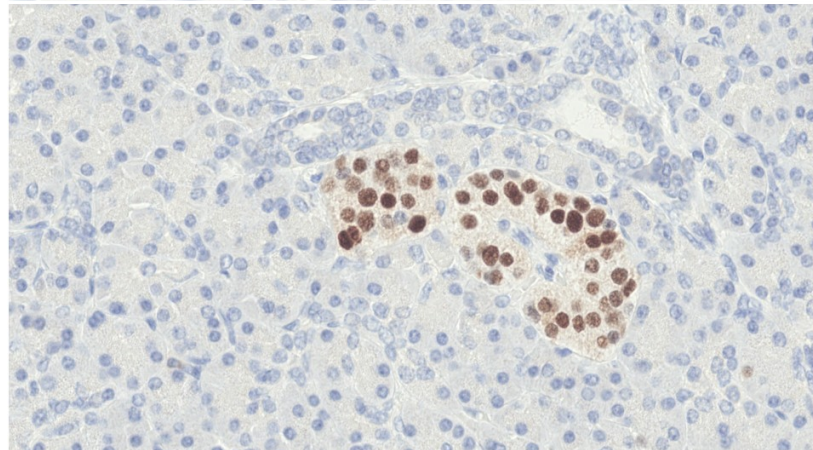


SYP

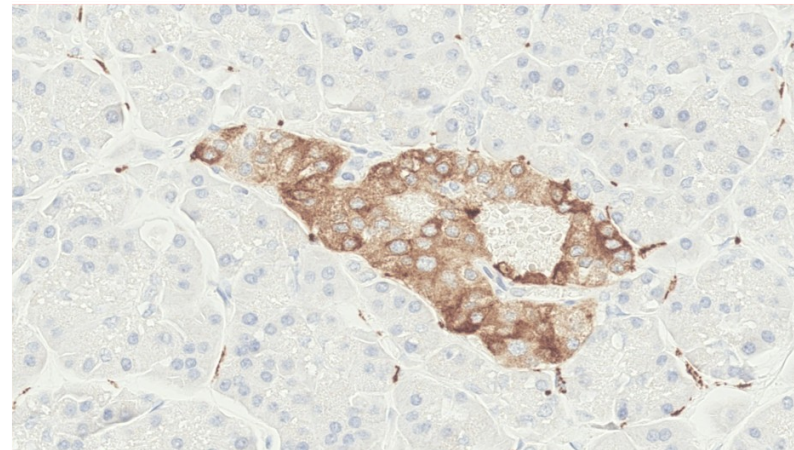
Cytoplasmic staining



Colon
scattered
neuroendocrine
cells

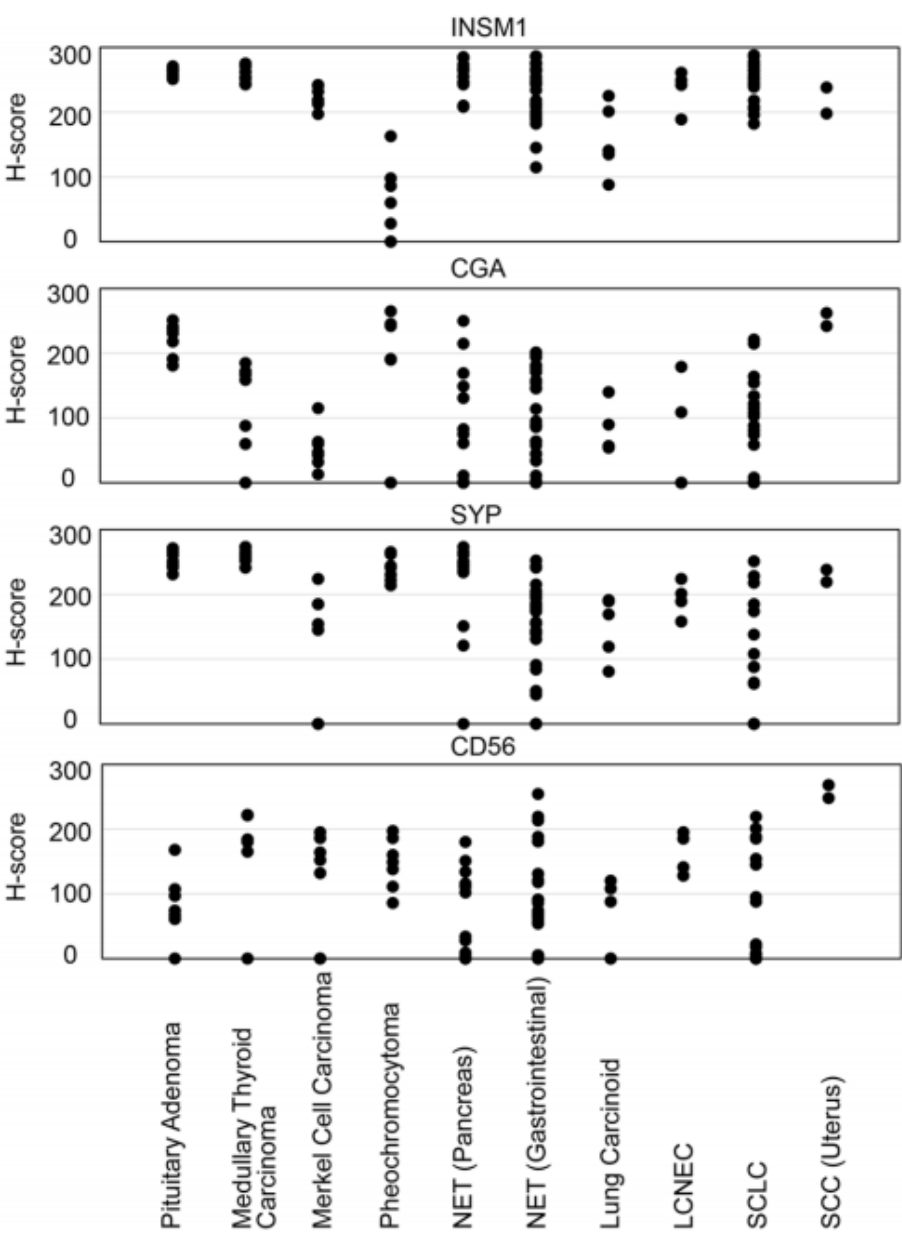
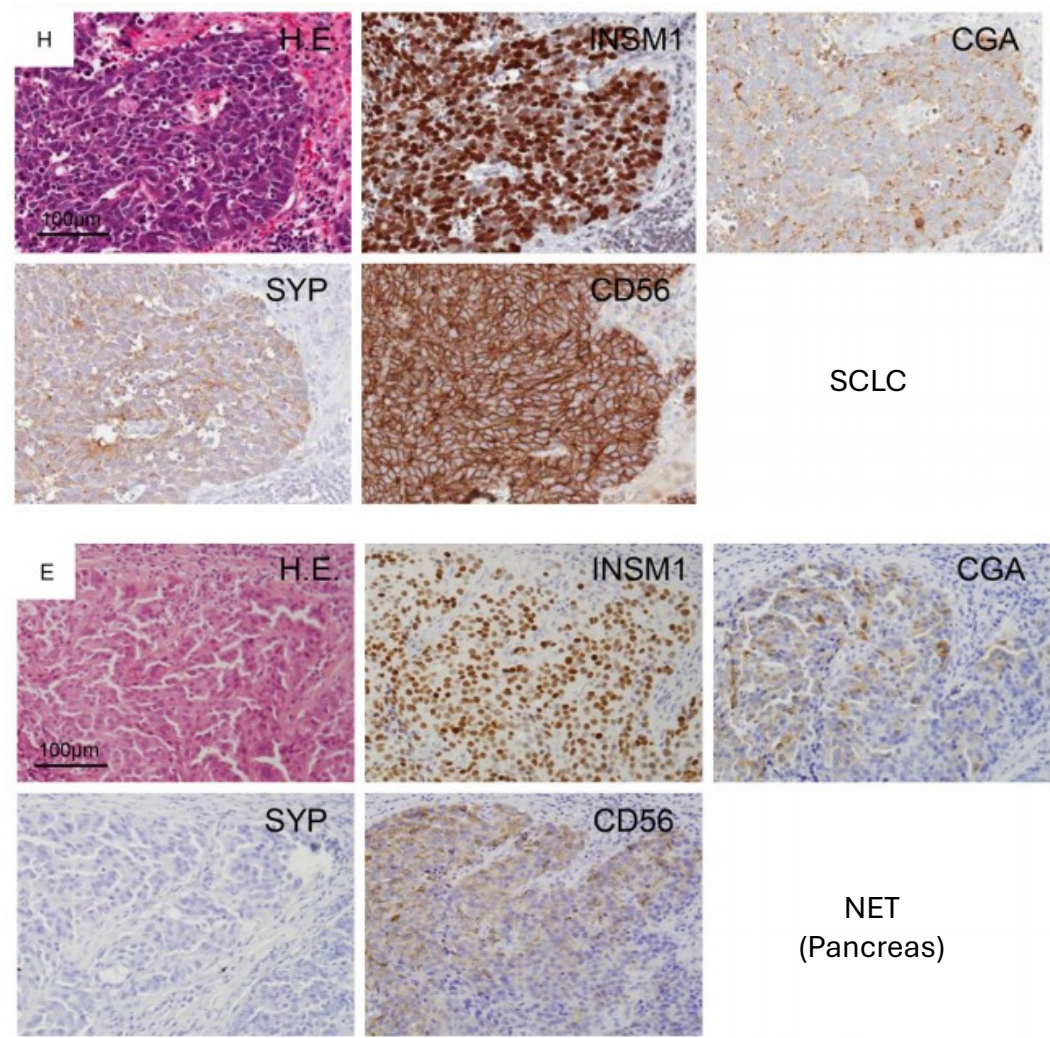


Pancreas
islet cells



INSM1 is a nuclear transcription factor associated with neuroendocrine differentiation.
It is expressed in normal and neoplastic neuroendocrine cells across multiple anatomical sites.

INSM1: does it outperform the established 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

INSM1 in lung neuroendocrine tumours: broad nuclear expression

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*	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.

* Synaptophysin clone SP11 was used for most of the extra small cell lung carcinoma 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.

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

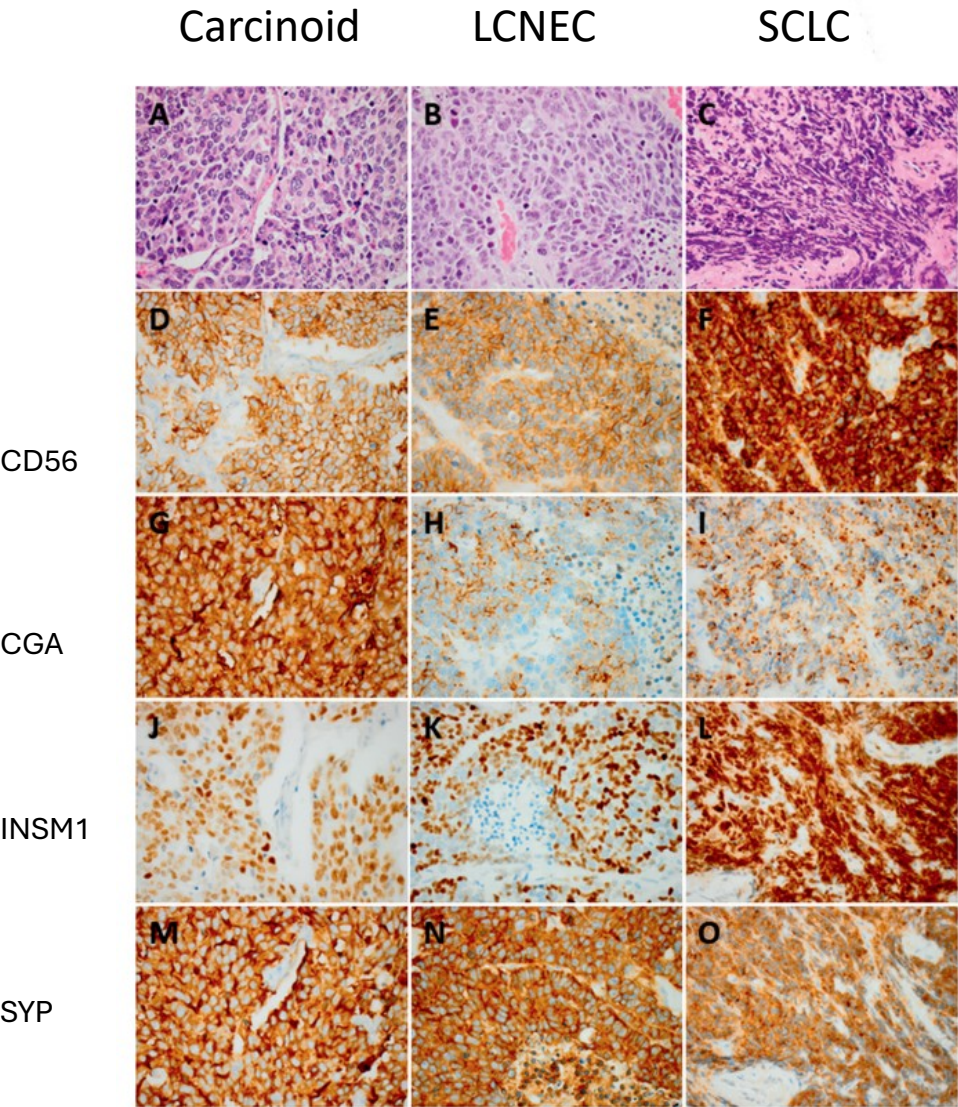


Figure 1. Representative images of positive neuroendocrine markers in a case of carcinoid tumor (A, D, G, J, and M), large cell neuroendocrine carcinoma (B, E, H, K, and N), and small cell lung carcinoma (C, F, I, L, and O). A through C, Hematoxylin-eosin. D through F, CD56. G through I, Chromogranin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

INSM1 in lung neuroendocrine tumours: broad nuclear expression

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.



INSM1 in SCLC: diagnostic marker today, therapeutic target tomorrow?



Journal of
Molecular Pathology



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>

Diagnostic

High sensitivity (75–100%) and specificity for SCLC (82–100%)

Clear nuclear staining in histology and cytology

Best used together with morphology and other markers

Beyond diagnosis

Prognostic evidence remains inconsistent

INSM1-related pathways are being explored as therapeutic targets

INSM1 has earned a place in the diagnostic panel,
but its future role may extend beyond diagnostic use



INSM1 clone choice: MRQ-70 brings reproducibility

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.

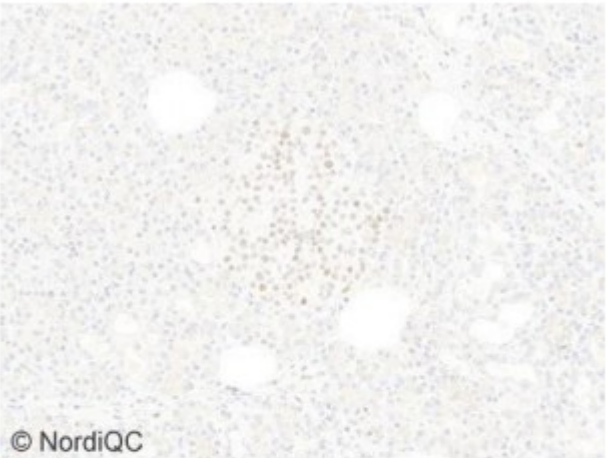


Fig. 6a
Insufficient INSM1 staining reaction of pancreas using exactly same protocols as in Figs. 1b – 5b, But in a different laboratory.
A too weak staining reaction is seen in the endocrine islet cells. Compare with optimal result in Fig. 2a. The mAb clone A-8 was found to give less reproducible results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

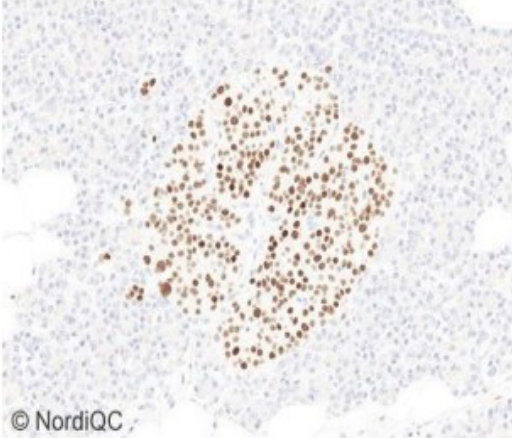


Table 1b. Concentrated antibodies and assessment marks for INSM1, run 71

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 Corporation	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).

MRQ-70 showed the most consistent performance across laboratories and platforms.
A-8 produced more variable results, including both weak staining and false positivity.

Second-generation neuroendocrine markers: when the first-generation panel is uncertain



Commentary

Second-Generation Neuroendocrine Immunohistochemical Markers: Reflections from Clinical Implementation

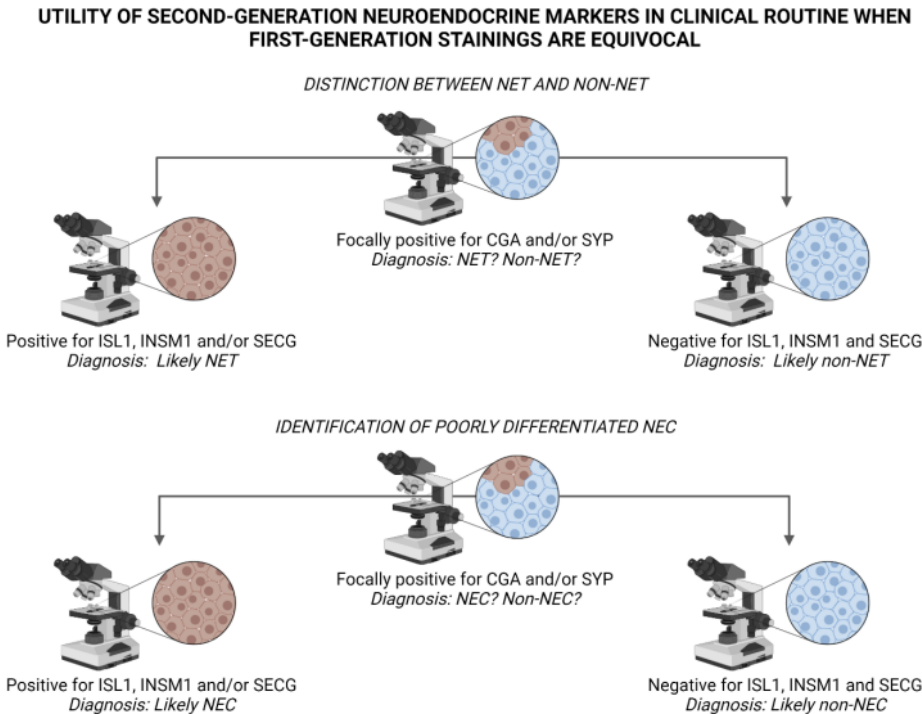
Carl Christofer Juhlin ^{1,2}

Table 1. Schematic overview of tissue-specific expression patterns in NET for first- and second-generation neuroendocrine markers.

	CGA	SYP	ISL1	INSM1	SECG
Lung	+	+	+	+	+
Pancreas	+	+	+	+	+
Small intestine	+	+	-	+	+
PPGL	+	+	+	+	-
Colorectum	-	+	+	+	+

NET: neuroendocrine tumor; CGA: Chromogranin A; SYP: Synaptophysin; ISL1: ISL LIM Homeobox 1; INSM1: INSM Transcriptional Repressor 1; SECG: Secretagogen; PPGL: Pheochromocytoma/paraganglioma.

INSM1, ISL1 and Secretagogen are most useful as problem-solving markers when chromogranin A and synaptophysin are weak, focal or inconclusive.



When chromogranin and synaptophysin overcall neuroendocrine differentiation

CGA and synaptophysin may be expressed in non-neuroendocrine tumours

Negative INSM1, ISL1 and Secretagoin can argue against a true neuroendocrine neoplasm

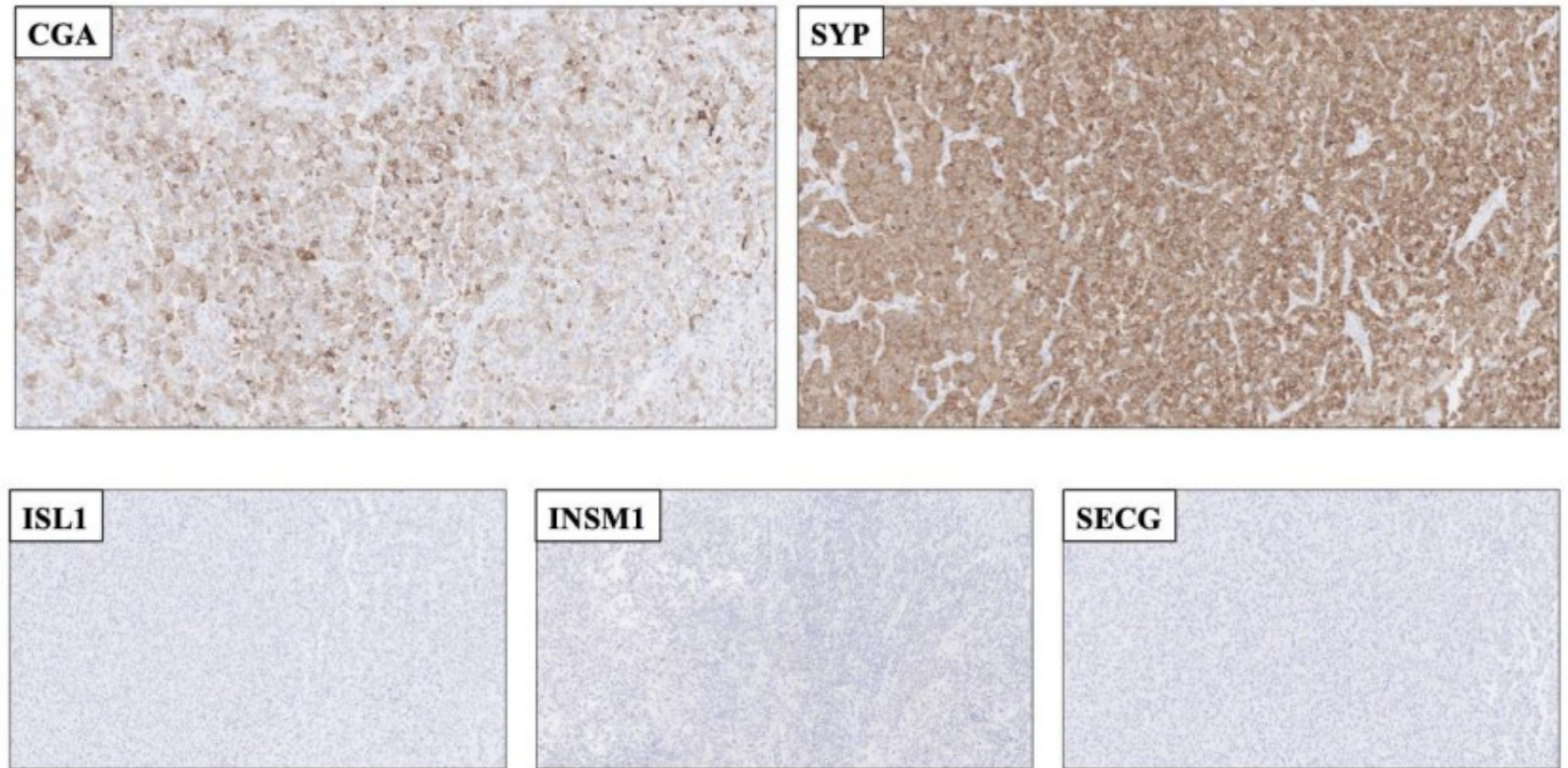


Figure 2. Application of second-generation neuroendocrine markers to rule out a neuroendocrine tumor as a plausible differential diagnosis. **Top row:** Resected cerebral metastasis of a tumor with unknown primary, with focal Chromogranin A (CGA) and diffuse Synaptophysin (SYP) staining. **Bottom row:** The metastasis was completely absent of ISL LIM Homeobox 1 (ISL1), INSM Transcriptional Repressor 1 (INSM1 and Secretagoin (SECG) immunoreactivity. As of this, the second-generation markers aided the pathologist in ruling out this lesion as a *bona fide* neuroendocrine tumor. The lesion was later diagnosed as a Ewing sarcoma exhibiting an *ESW1-ATF1* gene fusion (data not shown).

Second-generation markers can prevent a false neuroendocrine diagnosis

The antibody graveyard – Neuroendocrine markers

Original Article

Islet-1 Is Differentially Expressed Among Neuroendocrine and Non-Neuroendocrine Tumors and Its Potential Diagnostic Implication

Yan Xiang, MD¹ , Faizan Malik, MD² , and Paul J. Zhang, MD²

International Journal of Surgical Pathology
2023, Vol. 31(7) 1294–1301
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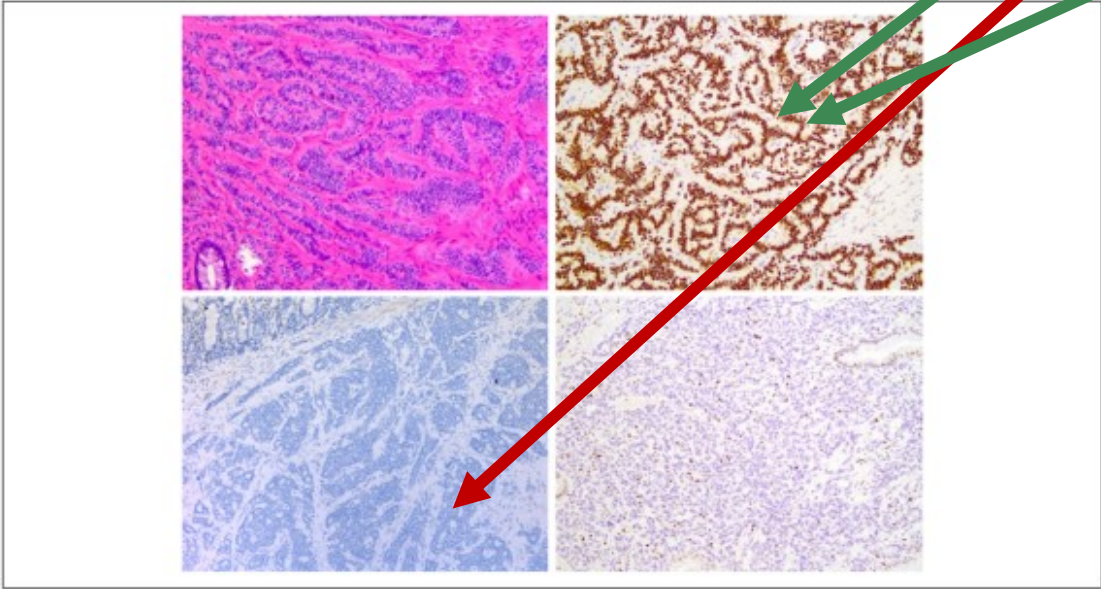


Figure 2. Rectal grade 1 neuroendocrine tumor (top left) with diffuse and strong (3+) ISL1 nuclear expression (top right); ISL1 was negative (0) in neuroendocrine tumors of small bowel (bottom left) and stomach (bottom right).

Table 1. Immunohistochemical expression of ISL1 in different tumor types

Tumor Types		Total Cases	Score 0	Score 1 +	Score 2 +	Score 3 +	Positive Cases (%)
NET	Pancreatic NET	27	3	3	0	21	78
	Upper GI NET	21	21	0	0	0	0
	Small intestine	9	9	0	0	0	0
	Stomach	8	7	1	0	0	0
	Appendix	1	1	0	0	0	0
	Cecum	3	3	0	0	0	0
	Lower GI NET	14	1	0	0	13	93
	Rectum	7	0	0	0	7	100
	Colon	7	1	0	0	6	86
	Lung carcinoid tumor	23	17	2	2	2	17
	Lung small cell carcinoma	13	6	1	1	5	46
	Small cell carcinoma of Ovary	2	0	0	0	2	100
	Merkel cell carcinoma	9	0	0	0	9	100
	Pituitary NET	6	3	0	0	3	50
	Head, neck, and salivary gland neuroendocrine carcinoma	5	1	0	0	4	80
	GU neuroendocrine carcinoma	7	2	0	1	4	71
	Parathyroid adenoma	7	7	0	0	0	0
Non-NET	Medullary thyroid carcinoma	5	0	0	0	5	100
	Paraganglioma/ Pheochromocytoma	8	0	0	0	8	100
	Thymoma	27	2	3	6	16	81
	Lung adenocarcinoma	11	11	0	0	0	0
	Breast carcinoma	6	5	1	0	0	0
	Renal cell carcinoma	2	2	0	0	0	0
	Pancreatic ductal adenocarcinoma	7	7	0	0	0	0
	Pancreatic IPMN	2	2	0	0	0	0
	Pancreatic acinar cell carcinoma	4	4	0	0	0	0
	SCC of head, neck, and skin	21	12	4	4	1	24
	Papillary thyroid carcinoma	5	5	0	0	0	0
	Gastric adenocarcinoma	5	1	2	2	0	40
	Adrenocortical carcinoma	6	5	0	1	0	17
	Mesothelioma	14	10	0	4	0	29

Abbreviations: NET, neuroendocrine tumor; GI, gastrointestinal tract; GU, genitourinary tract, IPMN, intraductal papillary mucinous neoplasm of the pancreas

ISL1 expression depends strongly on tumour site: High in pancreatic and lower gastrointestinal NETs; Usually absent in upper gastrointestinal NETs

Secretagogin in SCLC: supplemental value

Virchows Archiv (2022) 481:31–39
<https://doi.org/10.1007/s00428-022-03312-9>

ORIGINAL ARTICLE

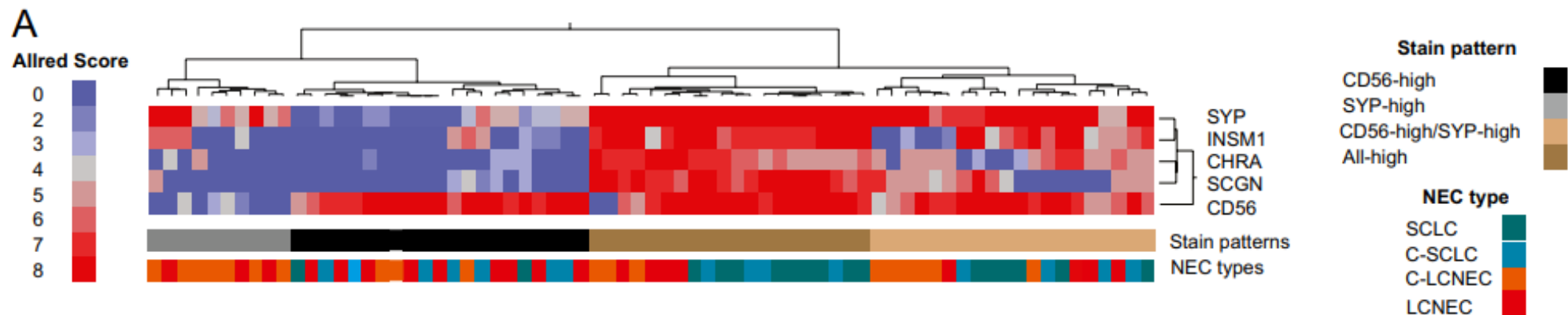
Utility of secretagogin as a marker for the diagnosis of lung neuroendocrine carcinoma

Yigit Baykara¹ · Ying Xiao¹ · Dongfang Yang¹ · Evgeny Yakirevich¹ · Sara Maleki¹ · Maria Garcia-Moliner¹ · Li Juan Wang¹ · Chiung-Kuei Huang² · Shaolei Lu¹ 

Received: 23 November 2021 / Revised: 22 February 2022 / Accepted: 14 March 2022 / Published online: 31 March 2022
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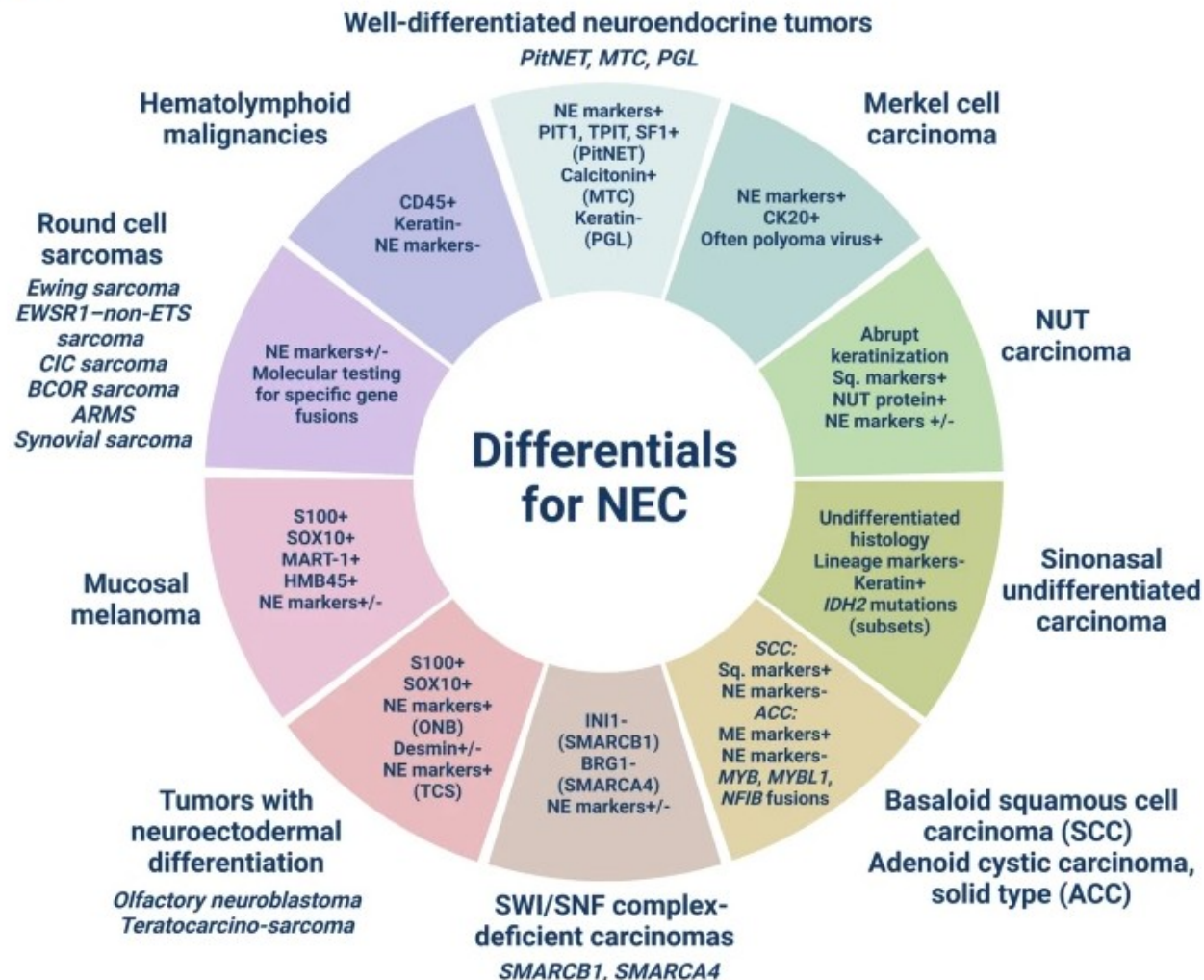
In SCLC, sensitivity was slightly higher than chromogranin A: 71% versus 68%, and positive tumours also tended to show a greater proportion of stained cells.

Potential role: a supplemental marker when chromogranin A is weak or negative, or when the conventional stains give a discordant result.



The neuroendocrine panel: a focused core, not an antibody collection

Fig. 2



Core markers for NET/NEC:

Chromogranin A
Synaptophysin
INSM1

Problem-solving marker:

ISL1 when site of origin is uncertain

Still under evaluation:

Secretagoin

DIAGNOSTIC PANEL

Breast carcinoma

With a focus on triple-negative disease

Breast origin in TNBC: which markers add value?

	To stay	Sensitivity	Comments
GCDFP15	Yes	50-70%	Highly specific for breast carcinoma, but low sensitivity in TNBC
Mammaglobin	Yes	40-60%	Highly specific for breast carcinoma, but low sensitivity in TNBC
ER	No	80%	Moderate to highly sensitive but not specific. Defines receptor status rather than confirming breast origin
GATA3	Yes	90-95%	Highly sensitive for ER+ breast carcinoma – 20-60% positivity in TNBC and metaplastic type Low specificity
TNBC			
SOX10	?	40-60%	Moderately sensitive and specificity. Complements GATA3 in TNBC, but also stains melanoma, salivary tumours and peripheral nerve-sheath tumours.
TRPS1*	Yes	80-98%	Highly sensitive and relatively specific for TNBC

* Trichorhinophalangeal syndrome type 1 (TRPS1) gene

TRPS1: a more sensitive breast marker for TNBC

Table 1 TRPS1 and GATA3 expression in breast cancers.

Breast carcinoma		Negative	Positive			Total
			Low	Intermediate	High	
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			Total
			Low	Intermediate	High	
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.

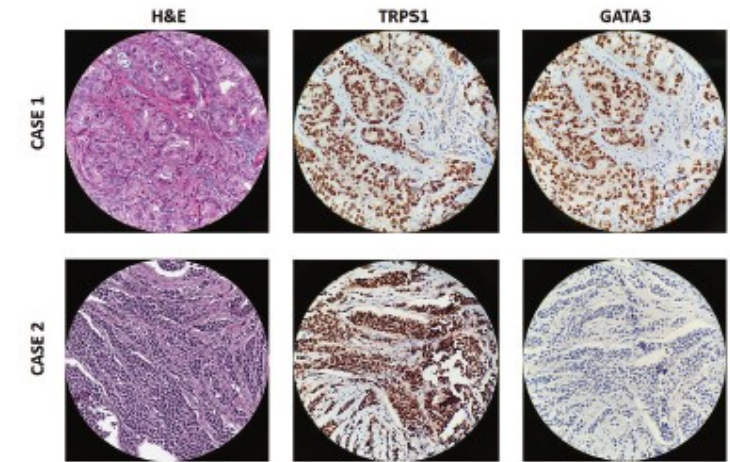
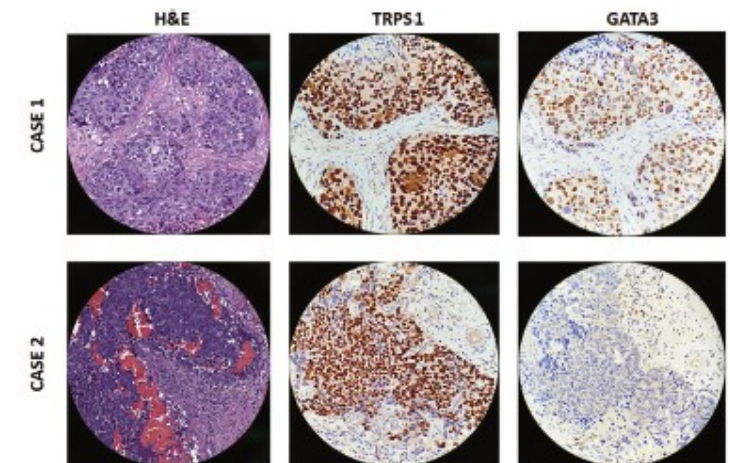


Fig. 4 TRPS1 and GATA3 expression in representative nonmetaplastic TNBC cases. Case 1 shows a poorly differentiated carcinoma with high expression of TRPS1 and intermediate to high expression of GATA3. Case 2 shows a poorly differentiated carcinoma with high expression of TRPS1 and negative GATA3.



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).

TRPS1 is retained in most triple-negative breast carcinomas, including many GATA3-negative cases.
Potential role: improving recognition of breast origin when traditional markers are lost.

TRPS1 in TNBC: covering the cases missed by GATA3 and SOX10

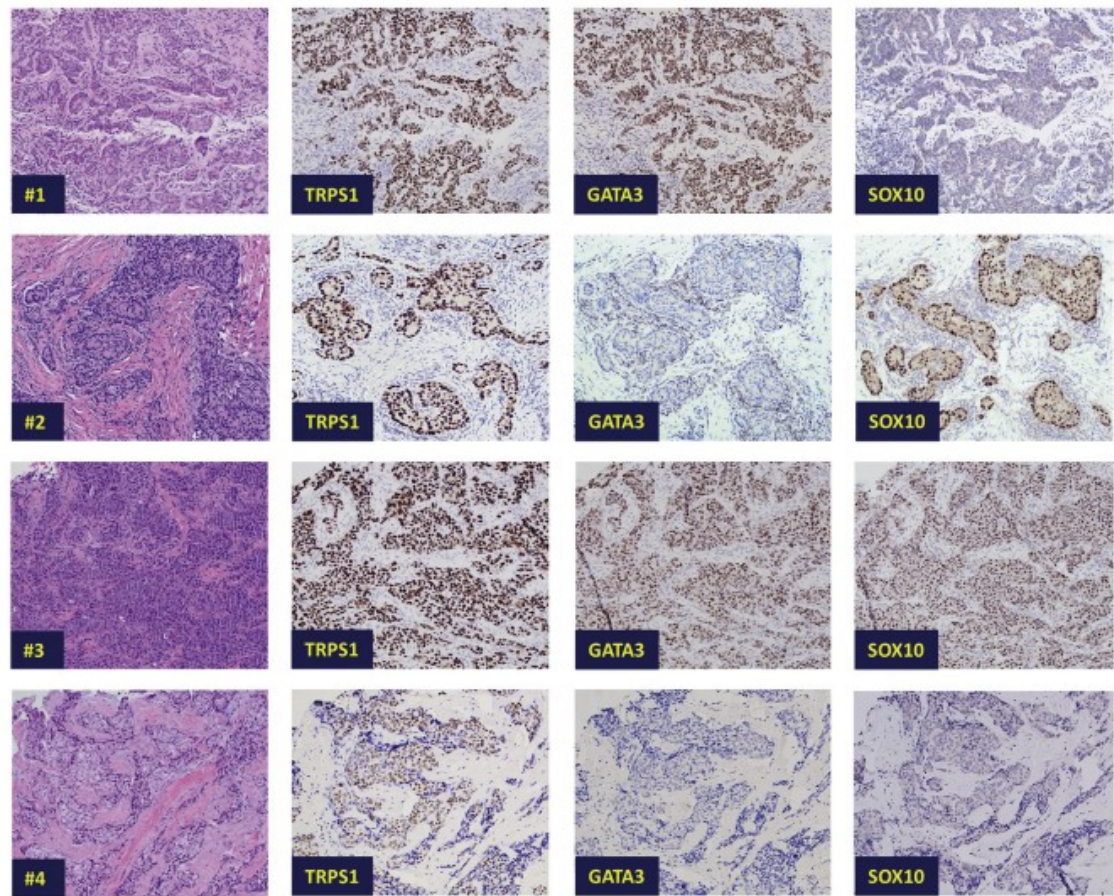


Fig. 1 TRPS1, GATA3, and SOX10 expression (x10) in representative cases of triple-negative invasive breast carcinoma of no special type (IBC-NST). Case 1 shows a triple-negative IBC-NST with high expression of TRPS1 and GATA3 and negative expression of SOX10. Case 2 shows a triple-negative IBC-NST with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

TRPS1 may serve as the
primary breast-lineage marker in TNBC

TRPS1: high sensitivity, but broader expression than first reported

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

TMA 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)

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

Most published data:

pAb PA5-84874

Promising monoclonal alternatives:

EP392, EPR16171 and MSVA-512R

Clone-specific validation required

TRPS1 detects approximately 90% of breast carcinomas, including TNBC.

However, expression also occurs in gynaecological tumours, particularly endometrial carcinoma, usually at lower levels.

TRPS1 assays: comparable performance, but specificity depends on the cut-of

NordiQC poster at ECP 2024

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



Birgit Truumees¹, Mia Korsdal Stensballe², Rasmus Røge¹, Søren Nielsen¹

Affiliation: ¹NordiQC, Department of Pathology, Aalborg University Hospital, Denmark, ²Department 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 $\geq 10\%$ 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

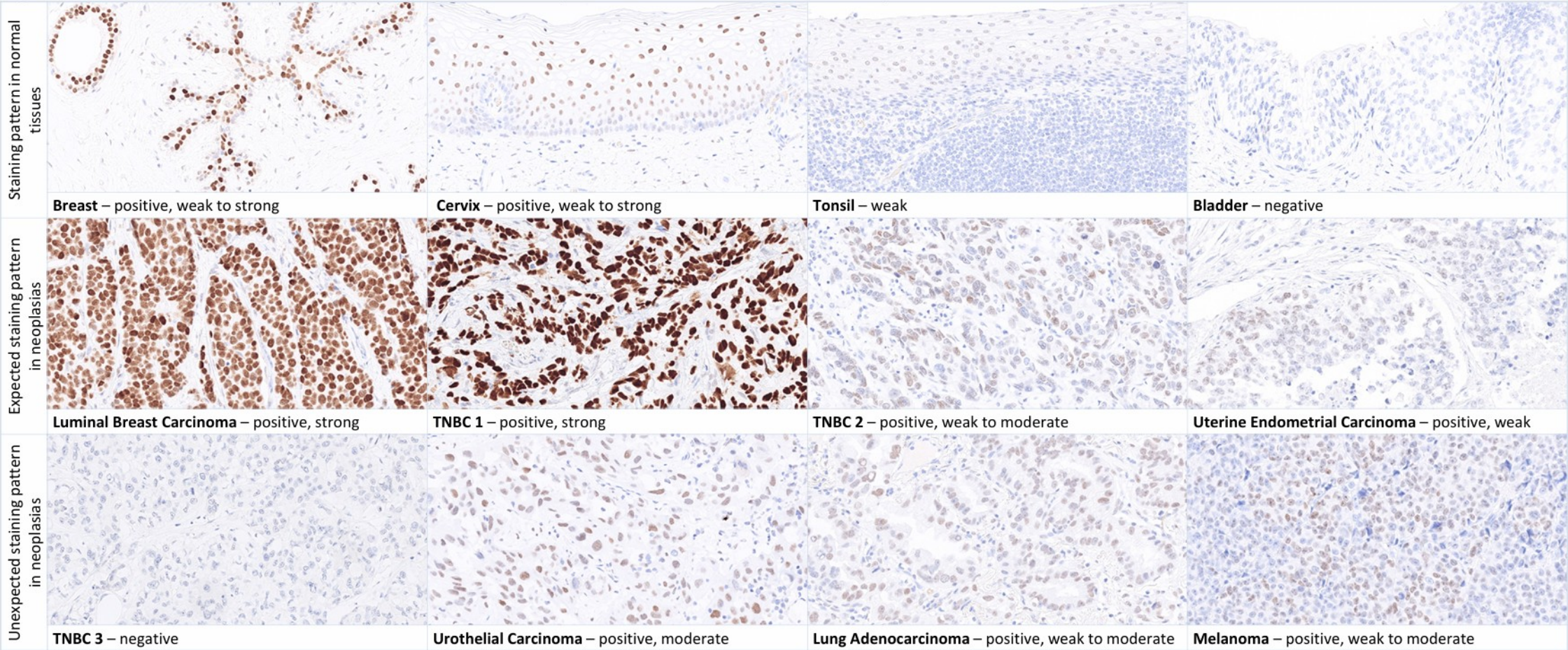
	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
Antibody / vendor	EP392 / BioSB	polyclonal / Invitrogene	MSVA-512R / MS validated	8131R / NeoBiotechnologies	ZR382 / Zeta corporation
Titer / time	1:40 / 30 min	1:100 / 32 min	1:200 / 32 min	1:1000 / 32 min	1:1000 / 32 min
Diluent	Background sniper, Biocare Medical	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

TRPS1: NordiQC poster at ECP 2024

Results

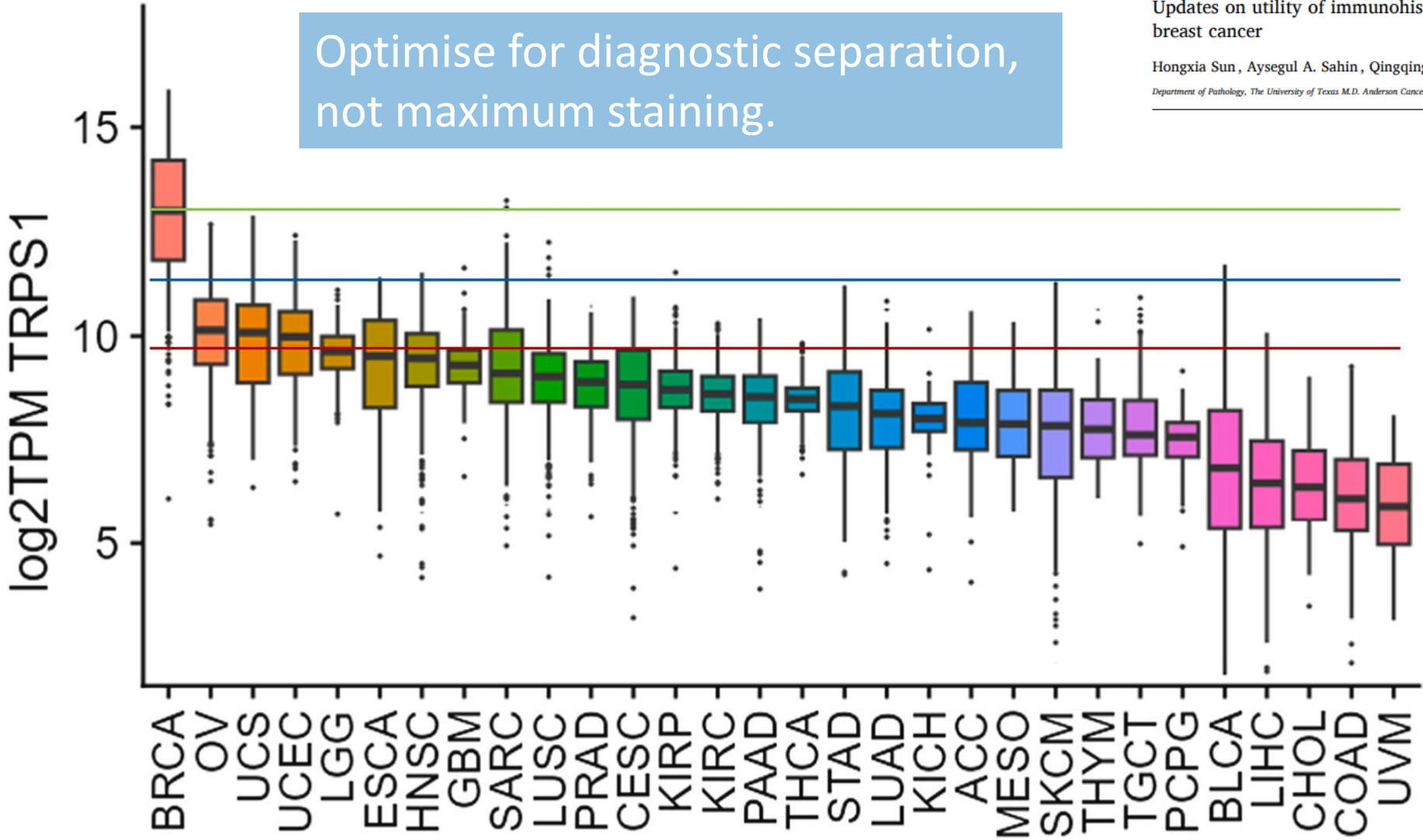
The five different and individually optimized IHC assays provided a fully comparable level of diagnostic sensitivity and specificity (see Table 2 and Graph 1). Positive TRPS1 staining in $\geq 10\%$ of neoplastic cells was seen in a subset of lung, gynecological and urothelial carcinomas with all assays.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

Figure 1. Examples of staining patterns seen in normal and neoplastic tissues with TRPS1 IHC assays



TRPS1: where should we set the threshold?

Optimise for diagnostic separation,
not maximum staining.



Neoplasias with TRPS expression levels

Human Pathology 162 (2025) 105821

Contents lists available at ScienceDirect

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Check for updates

Updates on utility of immunohistochemistry in diagnosis of metastatic breast cancer

Hongxia Sun, Aysegul A. Sahin, Qingqing Ding^{*}

Department of Pathology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, 77030, USA

- B: Conservative assay.**
High specificity, but may miss some breast carcinomas
- A: Balanced assay.**
Best compromise between sensitivity and specificity
- C: Highly sensitive assay.**
Detects more breast carcinomas but increases non-breast positivity.

TRPS1 IHC: clone choice and calibration matter



Assessment Run 72 2024 Trichorhinophalangeal syndrome 1 (TRPS1)

Purpose

Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for TRPS1, typically used to identify breast carcinomas including triple negative breast carcinomas in the diagnostic work-up of carcinomas of unknown primary origin. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for TRPS1 (see below).

Material

The slide to be stained for TRPS1 comprised:

1. Appendix, 2. Breast hyperplasia, 3. Uterine cervix, 4. Non-small cell lung carcinoma (NSCLC) 5-6. Triple negative breast carcinoma (TNBC).

All tissues were fixed in 10% neutral buffered formalin.



KEY POINTS FOR TRPS1 IMMUNOASSAYS

- The rmAb clone **ZR382** is recommendable both as a concentrated Ab and a RTU.
- The widely used rmAb clone **EP392** and pAb **PA5-84874** from **Invitrogen** seem less reproducible overall and more challenging to calibrate properly.
- IHC assays for TRPS1 must be calibrated carefully to provide desired diagnostic sensitivity and specificity as TRPS1 protein is expressed in many cells/neoplasias.

Table 1a. Overall results for TRPS1, run 72

	n	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
Concentrated antibodies	59	22	20	12	5	71%	37%
Ready-To-Use antibodies	30	13	10	2	5	76%	43%
Total	89	35	30	14	10		
Proportion		39%	34%	16%	11%	73%	

1) Proportion of sufficient stains (optimal or good).

2) Proportion of Optimal Results.

Table 1b. Concentrated antibodies and assessment marks for TRPS1, run 72

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
rmAb clone TRPS1/8131R	1	ImmunoLogic	-	1	-	-	-	-
rmAb clone BP6231	1	Biolynx Biotechnology	1	-	-	-	-	-
rmAb clone EP392	16	Bio SB	7	4	3	2	69%	44%
	3	Epitomics	-	-	2	1	-	-
rmAb clone EPR16171	3	Abcam	2	-	-	1	-	-
	1	Diagnostic BioSystems	-	-	1	-	-	-
rmAb clone MSVA-512R	1	MS Validated Antibodies	-	1	-	-	-	-
rmAb clone ZR382	17	Zeta Corporation	12	4	1	-	94%	71%
	1	Atlas Antibodies	-	1	-	-	-	-
	1	Gennova	-	-	-	1	-	-
Polyclonal	14	Invitrogen, PA5-84874	-	9	5	-	64%	0%
Total	59		22	20	12	5		
Proportion			37%	34%	20%	9%	71%	

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

Table 1c. Ready-To-Use antibodies and assessment marks for TRPS1, run 72

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
rmAb clone EP392	7	Bio SB	1	1	1	4	29%	14%
BSB-3790-x								
rmAb clone EPR16171	1	Gene Tech	1	-	-	-	-	-
GT245402								
rmAb clone MXR063	1	Fuzhou Maixin	-	1	-	-	-	-
RMA-1141								
rmAb clone ZR382	1	Sakura Finetek	1	-	-	-	-	-
8359-C010								
rmAb clone ZR382	18	Zeta Corporation	9	8	-	1	94%	50%
Z2673RP								
Ab clone 506I3C7	1	abcarta	-	-	1	-	-	-
PA588								
Ab clone BY193	1	Bioin Biotechnology	1	-	-	-	-	-
BFM-0560								
Total	30		13	10	2	5		
Proportion			43%	33%	7%	17%	76%	

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

2) Proportion of Optimal Results (≥5 assessed protocols).

The three roles of IHC

Different assays answer different clinical questions



1

Diagnostic



What is it?

2

Prognostic



What may happen?

3

Predictive



What may work?

FOR THE LAST 60 SLIDES:

*Never let one stain
make the decision.*

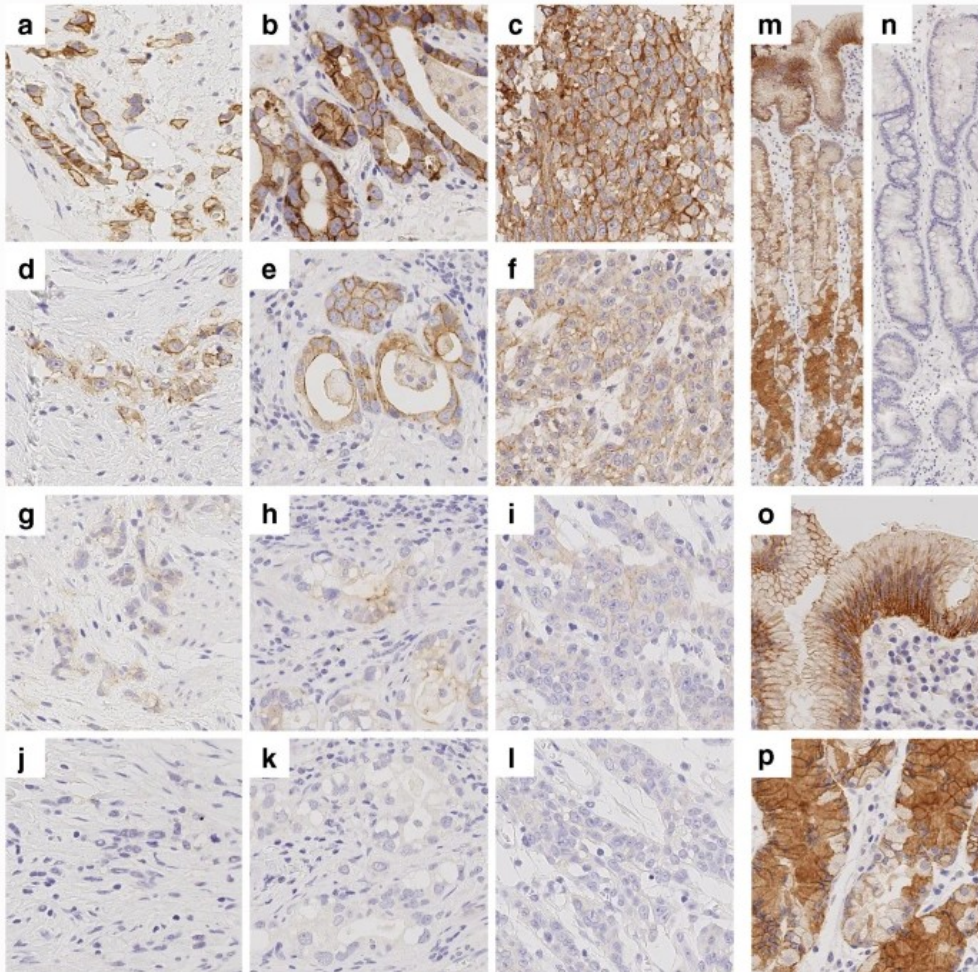
PREDICTIVE IHC:

*Here is one stain
and a treatment cut-off.*

PREDICTIVE PANEL

Claudin 18.2

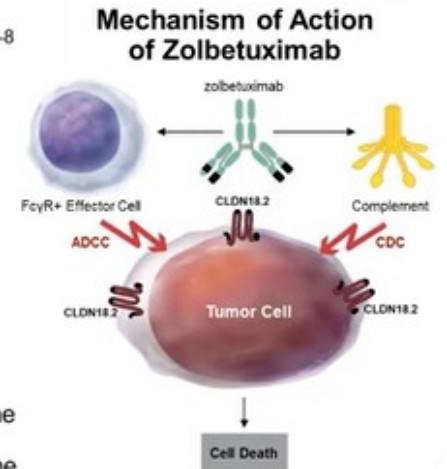
Claudin 18.2: Predictive IHC in gastric cancer



Normal gastric mucosa: strong membranous expression
Intestinal metaplasia: generally weaker and heterogeneous expression

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 cells⁸
 - 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. Nishi 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:vii14-vii57. 4. Sahin U et al. *Eur J Cancer*. 2018;100:17–26. 5. Rhede C et al. *Jpn J*. 2019;49:879–6. 6. Tureci O 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

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Zolbetuximab approved in the EU, 24 September 2024

CLDN18.2 scoring: intensity and extent both matter

Impact of Pre-Analytical Conditions on VENTANA CLDN18 (43-14A) RxDx Assay

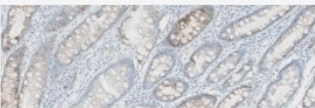
The recommended fixative is 10% NBF for 6-48 hours and outlined in box with blue border.

Claudin18.2 positive = may be eligible for Zolbexitumab

Scoring includes intensity of
membranous staining and
percentage of tumor cells
stained¹



0 NO STAINING



1+ WEAK
STAINING



2+ MODERATE
STAINING

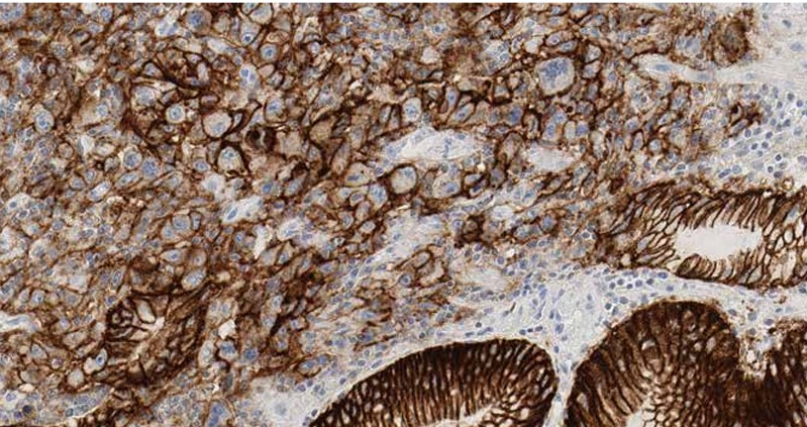


3+ STRONG
STAINING

Due to the heterogeneous cellular composition of G/GEJ tumor cells, CLDN18.2 is reported for both membranous staining intensity and percentage of tumor cells stained.¹

- Tissue slides with tumor present can demonstrate varying levels of CLDN18 membranous staining intensity ranging from no staining to strong staining (0 to 3+)
- Percentage of tumor cells stained should only include cells demonstrating membranous staining

VENTANA CLDN18 (43-14A) RxDx Assay Interpretation Guide for Gastric Adenocarcinoma including Gastroesophageal Junction (GEJ)



Claudin18.2 positive = 75% cells
positive with moderate to strong
intensity

Tumor Cell Scoring	What to Include
Cells Included	Greater than 50 Viable Tumor Cells Only
Staining Intensity	Moderate to Strong
Pattern of Staining	Apical, Circumferential (partial and complete), Basolateral/ Lateral, Microluminal
Denominator for Scoring	Total Number of Viable Tumor Cells

CLDN18.2: predictive accuracy begins with analytical accuracy

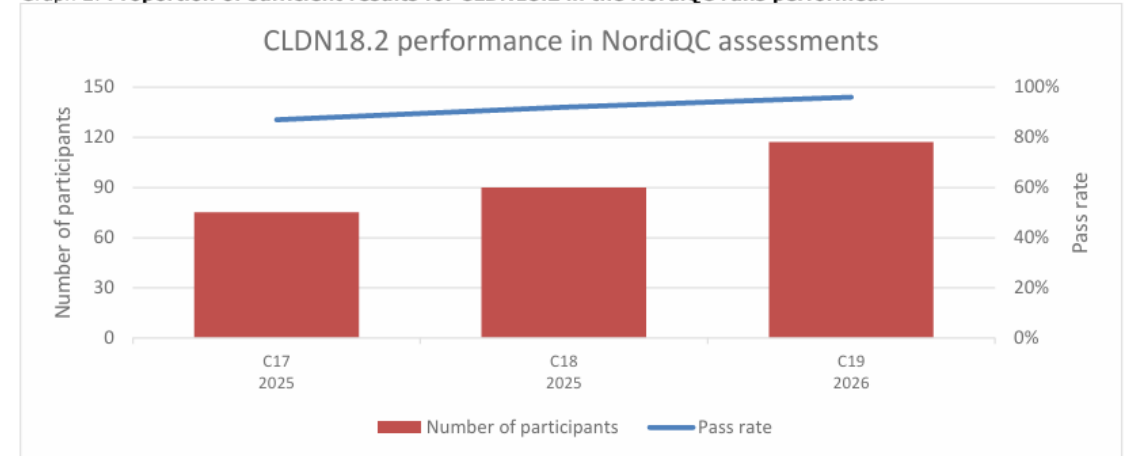


Assessment Run C17 2025 Claudin 18.2 (CLDN18.2)

Purpose

This first assessment in the NordiQC Companion module of CLDN18.2 focused on the evaluation of the analytical accuracy of the IHC assays performed by the NordiQC participants to identify patients with gastric and gastroesophageal junction (G/GEJ) cancer to be treated with VYLOY™. The CLDN18 (43-14A) RxDx Assay (741-6067, Ventana/Roche) was used as reference standard method. Accuracy was evaluated in six carcinomas with the dynamic and critical relevant expression levels of CLDN18.2 characterized by tumor cell scoring (TCS). The assessment mark obtained in NordiQC is indicative of the performance of the IHC tests but due to the limited number and composition of samples, internal validation/verification and extended quality control, e.g. regularly measuring the CLDN18.2 results, is needed.

Graph 2. Proportion of sufficient results for CLDN18.2 in the NordiQC runs performed.



Assessment Run C19 2026 Claudin 18.2 (CLDN18.2)

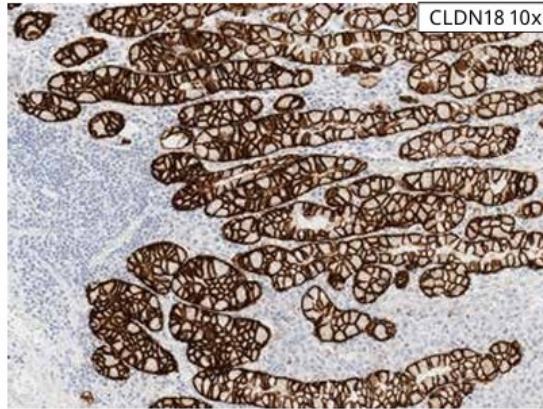
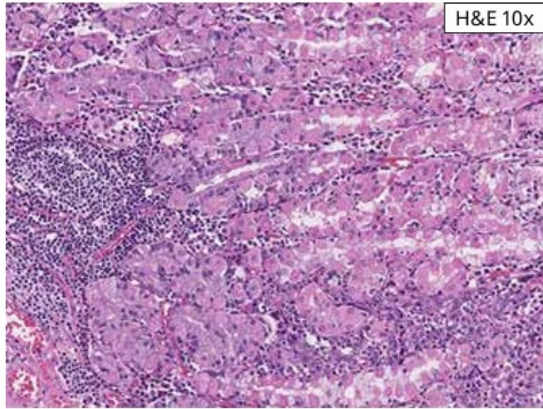
KEY POINTS FOR CLDN18.2 IMMUNOASSAYS

- The Ventana/Roche RTU CLDN18 assays, based on clone 43-14A, provided a 100% pass rate overall.
- Laboratory developed assays based on concentrated Abs gave an inferior pass rate of 60%.
- Insufficient results were mainly caused by too weak or false negative staining reaction in gastric carcinomas expected to be TCS $\geq 75\%$

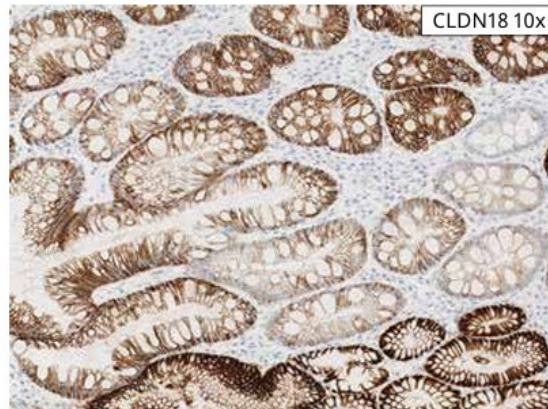
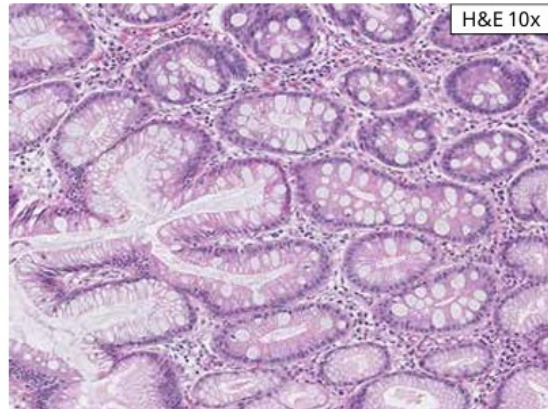


CLDN18.2: in search for a reliable low-expression control

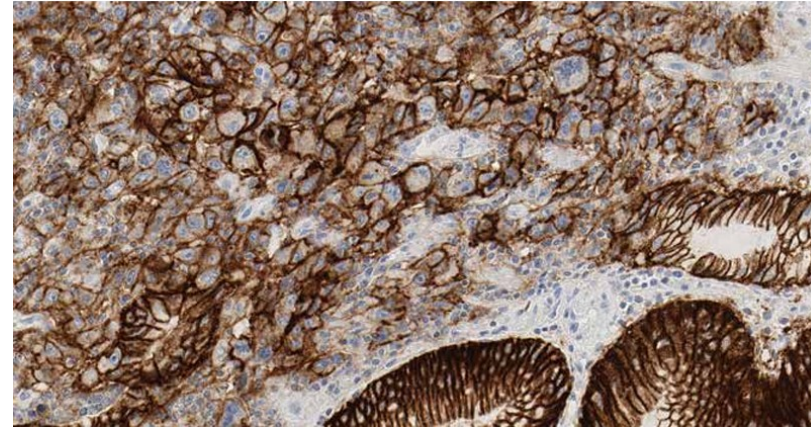
Non-neoplastic Gastric Epithelium: Strong membrane staining is seen in non-neoplastic gastric epithelium that may be present in the tissue section. This is useful as an internal positive control, but IS NOT REQUIRED for assay interpretation. Cytoplasmic staining can also be seen but is not included in the scoring algorithm.



Intestinal Metaplasia: The full spectrum of CLDN18 staining intensity may be seen in intestinal metaplasia (if present) in the gastric tissue section. Acceptability criteria for CLDN18 staining of intestinal metaplasia apply to the system level control ONLY (Table 2). Intestinal metaplasia is NOT REQUIRED within the gastric tissue section for assay interpretation.



VENTANA CLDN18 (43-14A) RxDx Assay Interpretation Guide for Gastric Adenocarcinoma including Gastroesophageal Junction (GEJ)



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

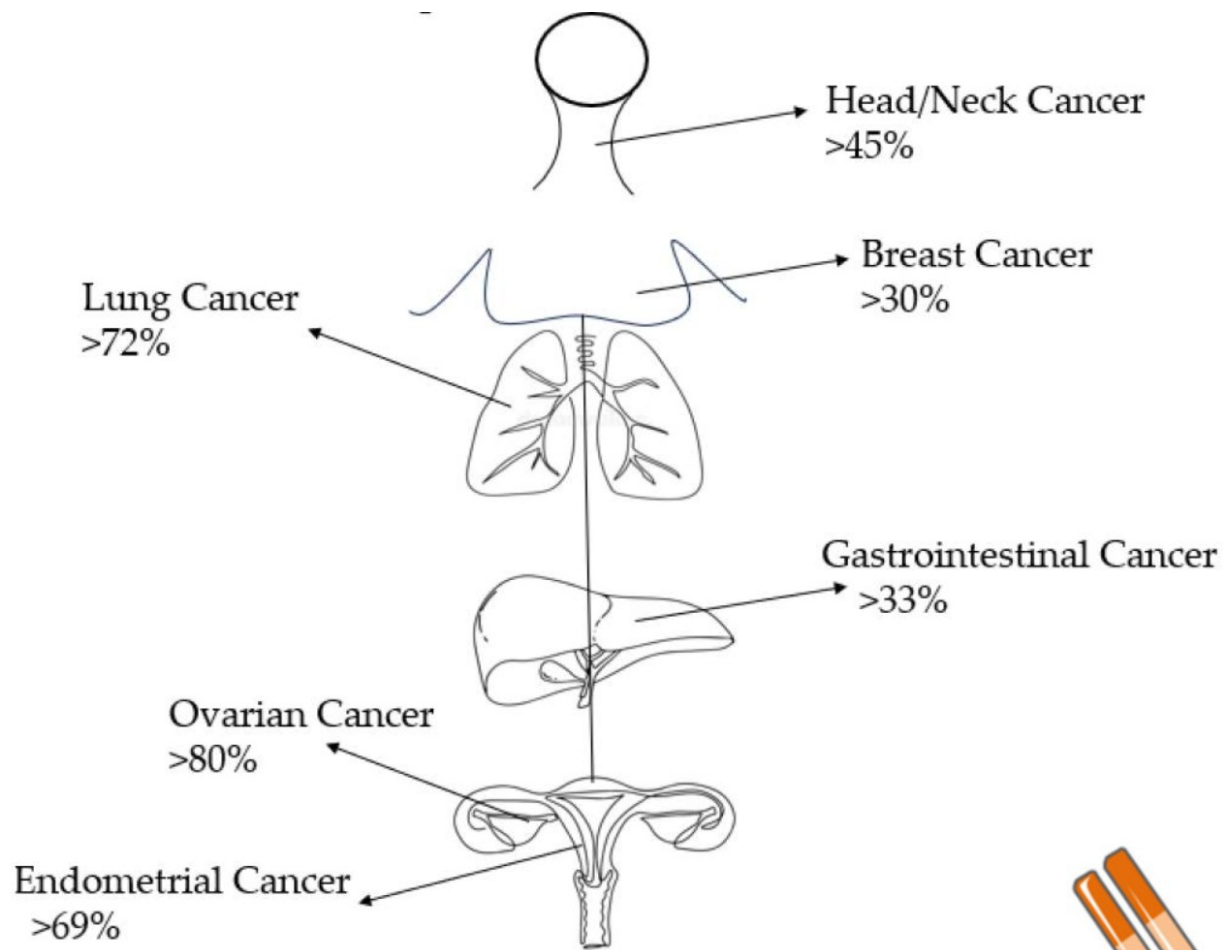
<https://www.claudin182.com/>

Potential alternative: normal lung

Weak membranous staining of pneumocytes with clone 43-14A
Promising as a low-expression control, but requires further validation

PREDICTIVE PANEL

Folate receptor alpha



FR α (pronounced eff-arr-AL-fah) is a folate transport protein that is highly expressed in ovarian cancer.^{3*}

~20,000 women are diagnosed with ovarian cancer in the US each year and will need multiple therapy options^{1,4}

FR α is one of the most prevalent biomarkers in ovarian cancer⁷⁻¹³

70%
are diagnosed at an advanced stage⁵

80%
will experience recurrence despite high CRR to surgery and 1L chemotherapy⁶

~1 in 3
patients with advanced ovarian cancer

express FR α at a level that may make them candidates for ELAHERE monotherapy^{7,14}

Review

Antibody–Drug Conjugates: The Last Decade

Nicolas Joubert ^{1,*} , Alain Beck ² , Charles Dumontet ^{3,4} and Caroline Denevault-Sabourin ¹

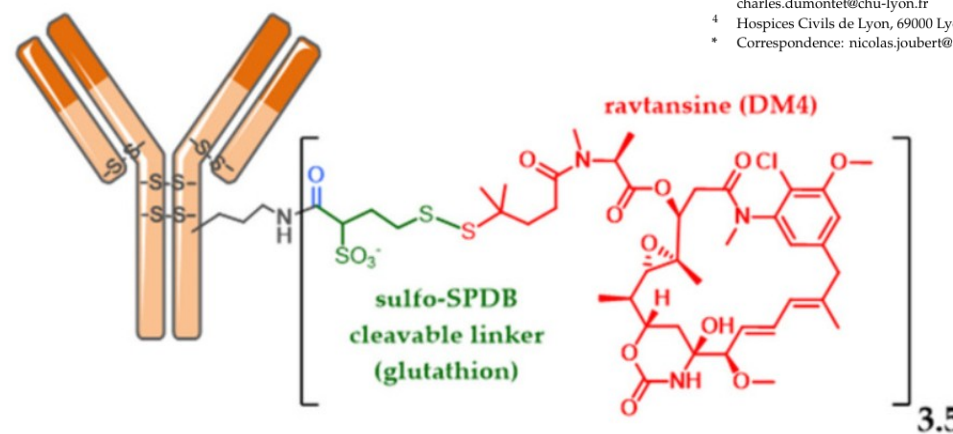
¹ GICC EA7501, Equipe IMT, Université de Tours, UFR des Sciences Pharmaceutiques, 31 Avenue Monge, 37200 Tours, France; caroline.denevault@univ-tours.fr

² Institut de Recherche Pierre Fabre, Centre d'Immunologie Pierre Fabre, 5 Avenue Napoléon III, 74160 Saint Julien en Genevois, France; alain.beck@pierre-fabre.com

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⁴ Hospices Civils de Lyon, 69000 Lyon, France

* Correspondence: nicolas.joubert@univ-tours.fr



► Int J Mol Sci. 2024 Jan 15;25(2):1046. doi: [10.3390/ijms25021046](https://doi.org/10.3390/ijms25021046)

Folate Receptor Alpha—A Novel Approach to Cancer Therapy

Teresita Gonzalez ^{1,*}, Meri Muminovic ¹, Olger Nano ¹, Michel Vulfovich ¹

Editors: Cecilia Garofalo¹, Hisashi Harada¹

► Author information ► Article notes ► Copyright and License information

PMCID: PMC11154542 PMID: [38256120](https://pubmed.ncbi.nlm.nih.gov/38256120/)

FDA approves mirvetuximab soravtansine-gynx for FR α positive, platinum-resistant epithelial ovarian, fallopian tube, or primary peritoneal cancer

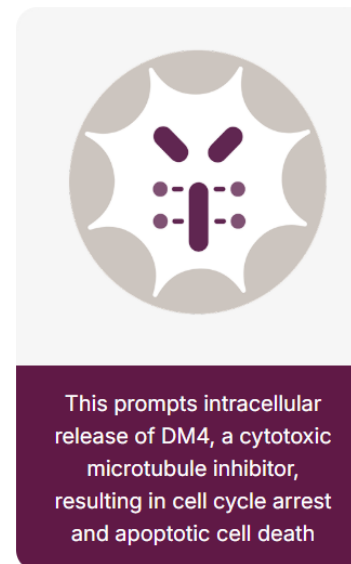
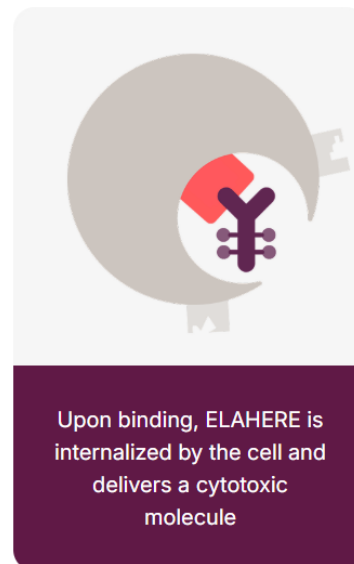
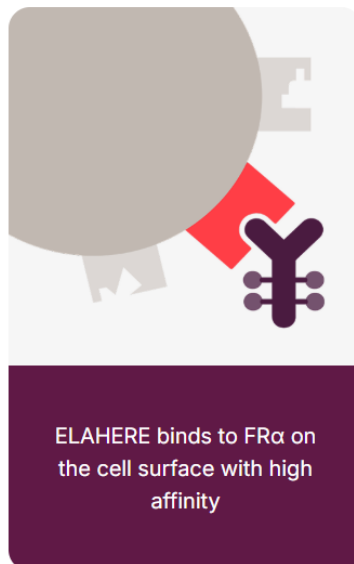
On March 22, 2024, the Food and Drug Administration approved mirvetuximab soravtansine-gynx (Elahere, ImmunoGen, Inc. [now a part of AbbVie]) for adult patients with FR α positive, platinum-resistant epithelial ovarian, fallopian tube, or primary peritoneal cancer, who have [received](#) one to three prior systemic treatment regimens. Patients are selected based on an FDA-approved test. Mirvetuximab soravtansine-gynx previously received accelerated approval for this indication.

November 18, 2024

AbbVie Receives European Commission Approval of ELAHERE® (mirvetuximab soravtansine) for the Treatment of Platinum-Resistant Ovarian Cancer

ELAHERE specifically targets FR α and delivers a cytotoxic DM4 payload¹⁴

ELAHERE is an antibody-drug conjugate (ADC) that binds to cell surface receptor FR α ^{14,17-20}

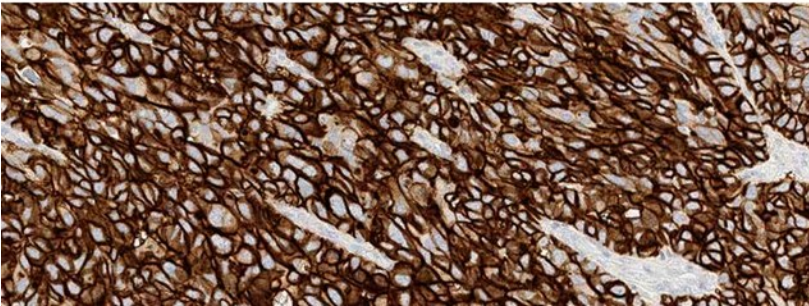


Folate receptor alpha scoring: intensity and extent both matter

FOLR1 positive = may be eligible for Mirvetuximab Soravtansine

**VENTANA FOLR1 (FOLR1-2.1) RxDx
Assay Interpretation Guide for Epithelial
Ovarian, Fallopian Tube, or Primary
Peritoneal Cancer**

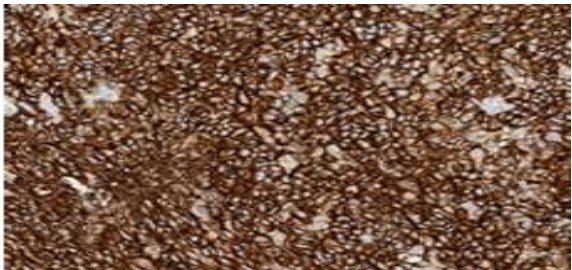
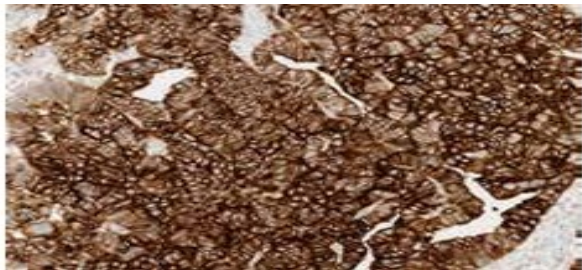
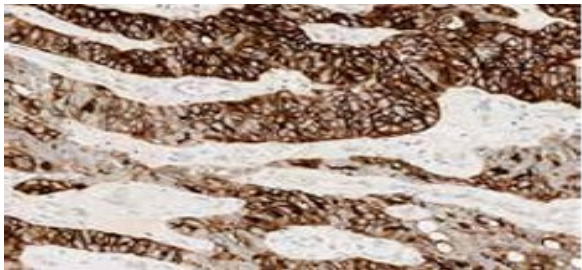
IHC Interpretation	Staining Description
Positive for FOLR1*	≥ 75% of viable tumor cells with moderate (2+) and/or strong (3+) membrane staining
Negative for FOLR1*	< 75% of viable tumor cells with moderate (2+) and/or strong (3+) membrane staining
Not Evaluable	Artifacts making interpretation not possible



Impact of Pre-analytical Conditions on VENTANA FOLR1 (FOLR1-2.1) RxDx Assay

Acceptable Fixation Conditions to Achieve Optimal Staining Results

- **Ventana recommends fixation in 10% NBF for 12-72 hours.** See acceptable fixatives and fixation times in rectangular box below.



Folate receptor alpha: predictive accuracy begins with analytical accuracy

Table 2a. Overall results for FOLR1, run C19

	n	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
CE-IVD / FDA approved FOLR1 assays*	67	59	7	1	-	99%	88%
Antibodies for laboratory developed FOLR1 assays, based on concentrated antibodies	10	1	6	2	1	70%	10%
Ready-To-Use antibodies	6	0	2	4	0	33%	0%
Total	83	60	15	7	1		
Proportion		72%	18%	9%	1%	90%	

1) Proportion of sufficient results (optimal or good) (≥ 5 assessed protocols).

2) Proportion of optimal results (≥ 5 assessed protocols).

* Including all uses of product on non-intended platforms.

Purpose

This first assessment in the NordiQC Companion module of FOLR1 focused on the evaluation of the analytical accuracy of the IHC assays performed by the NordiQC participants to identify patients with epithelial ovarian, fallopian tube, or primary peritoneal cancer to be treated with ELAHERE®. The technical accuracy was evaluated in six carcinomas with the dynamic and clinically relevant expression levels of FOLR1 characterized by tumour cell scoring (TCS). The assessment mark obtained in NordiQC is indicative of the performance of the IHC tests but due to the limited number and composition of samples, internal validation/verification and extended quality control, e.g. regularly measuring the FOLR1 results, are needed.

Material

Table 1. Content of the TMA used for the NordiQC FOLR1 C19 assessment

Tissue controls	FOLR1 IHC reaction pattern
1-2. Fallopian tube	See control section
Carcinomas	TCS score*
3. Ovarian serous adenocarcinoma	$\geq 75\%$ (moderate to strong)
4. Ovarian serous adenocarcinoma	$< 75\%$
5. Ovarian serous adenocarcinoma	$\geq 75\%$ (moderate to strong)
6. Ovarian serous adenocarcinoma	$< 75\%$
7. Ovarian serous adenocarcinoma	$\geq 75\%$ (moderate to strong)
8. Ovarian endometrioid carcinoma	$< 75\%$

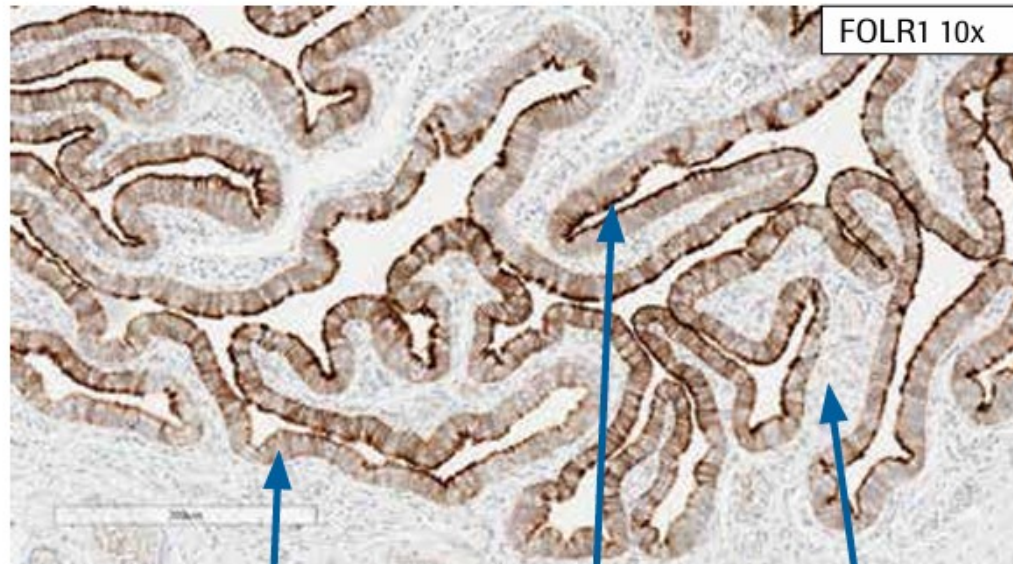


*Tumour Cell Scoring (TCS) determined by FOLR1 (FOLR1-2.1) RxDx Assay (741-5076, Ventana/Roche) performed in NordiQC reference laboratory.

KEY POINTS FOR FOLR1 IMMUNOASSAYS

- The Ventana/Roche RTU FOLR1 assays, based on clone FOLR1-2.1, provided a 100% pass rate when applied on the intended platform.
- mAb clone BN3.2 was the most used LD assay, with a pass rate of 71%.

Folate receptor alpha: in search for a reliable low-expression control



Predominantly moderate (2+) circumferential FOLR1 membrane staining in the epithelial cells

Strong (3+) apical FOLR1 membrane staining

Absence of staining in normal Fallopian tube stroma

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Interpretation Guide for Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer

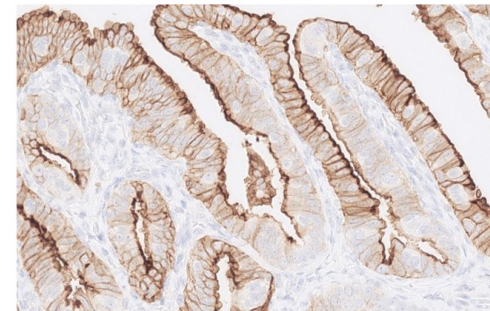
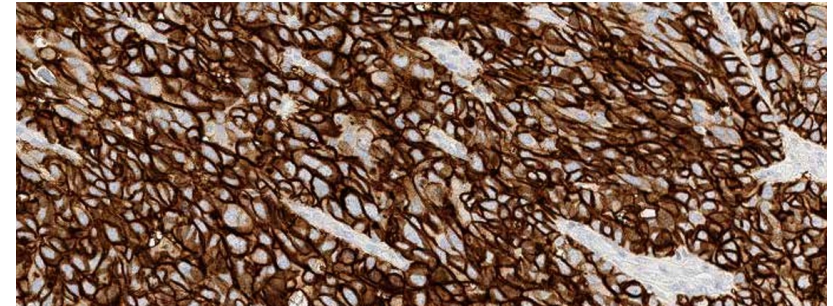


Fig. 1a
Optimal staining result of fallopian tube using the FOLR1 IHC assay 741-5076 from Ventana/Roche, based on the mAb clone FOLR1-2.1 following the recommended protocol settings. Same protocol used in Figs. 2a-5a. Virtually all epithelial cells show an at least weak membranous staining reaction.

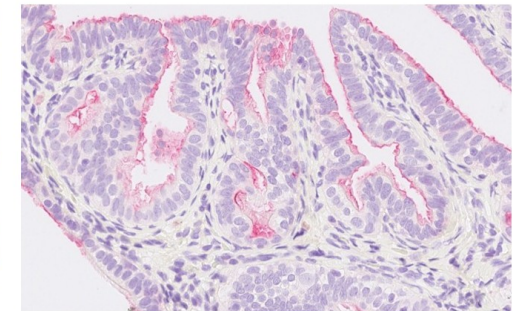


Fig. 1b
Staining result of fallopian tube using the mAb clone BN3.2 as an LD assay. The Ab was diluted 1:100, HIER was performed in a low pH buffer and a 3-layer detection system was used. Overall, a reduced analytical and diagnostic sensitivity was observed. Same protocol used in Figs. 2b-3b. The staining intensity and proportion of positive epithelial cells are significantly reduced as only a luminal staining reaction is seen. Compare to optimal result in Fig. 1a.

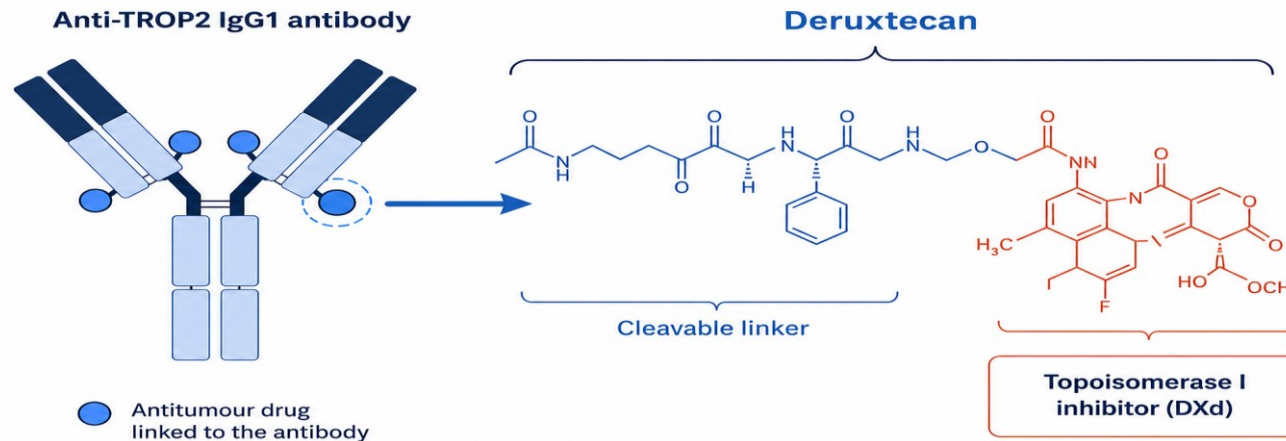
PREDICTIVE PANEL

TROP2

TROP2: a common target, but not yet a useful conventional biomarker

Cell-surface glycoprotein expressed in many epithelial cancers
Target of several antibody-drug conjugates

Datopotamab deruxtecan



The problem

TROP2 is widely expressed, but conventional expression level alone has not consistently predicted benefit from TROP2-directed ADCs.

VENTANA®

VENTANA TROP2 (EPR20043) Rabbit Monoclonal Antibody

REF 790-7280

10599251001

IVD Σ 50

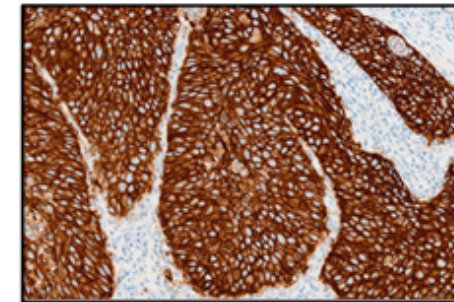


Figure 1. VENTANA TROP2 (EPR20043) Rabbit Monoclonal Antibody staining in Non-Small Cell Lung Cancer (NSCLC).

INTENDED USE

VENTANA TROP2 (EPR20043) Rabbit Monoclonal Antibody is intended for laboratory use for the qualitative immunohistochemical detection of trophoblast cell-surface antigen receptor 2 (TROP2) protein by light microscopy in sections of formalin-fixed, paraffin-embedded (FFPE) neoplastic tissues stained on a BenchMark IHC/ISH Instrument.

This product should be interpreted by a qualified pathologist in conjunction with

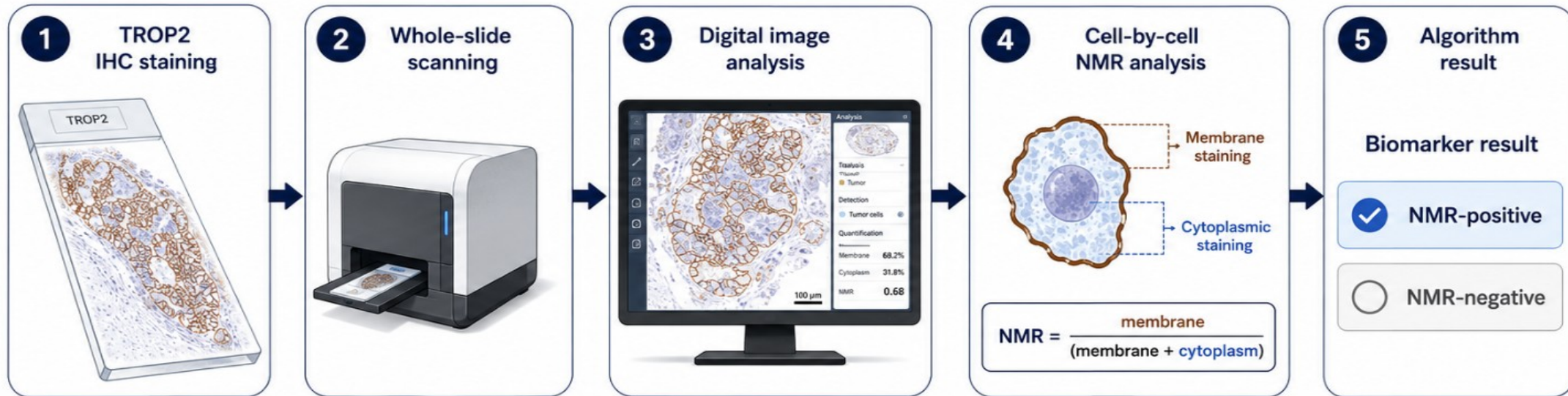
histological examination, relevant clinical information, and proper controls.

This antibody is intended for in vitro diagnostic (IVD) use.

NordiQC

TROP2: predictive IHC without a human read-out

Novel computational pathology-based TROP2 biomarker for datopotamab deruxtecan was predictive of clinical outcomes in patients with non-small cell lung cancer in TROPION-Lung01 Phase III trial





Apparently,
IHC is not only
fit for purpose.

It is also fit for cycling.

Thank you for your attention!



Let's connect



Rasmus Røge
Jette Boysen Møller
Heidi Lykke Kristoffersen
Louise Rønnow Holler
Charlotte Papior Carstensen
Tanya Julio
Jacqueline Capion Lemming
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With deep appreciation and gratitude to **Søren Nielsen**

