



**Seminar in Diagnostic Immunohistochemistry  
Aalborg University Hospital, September 17-19 2025**

# ***The Antibody Graveyard*** **-** ***Goodbye and Hello Markers***

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Director, Scientific lead  
NordiQC*

# The antibody graveyard....

FVIII

Villin

RCC

Thrombomodulin

ER

Tyrosinase

PLAP

NSE

CA125

UPIII

AFP

PSAP

MITF

CEA

CD99

# Next generation antibodies..

Cadh-4

SOX17

SATB2

ISL1

Merlin

HEG1

TPRS1

INSM1

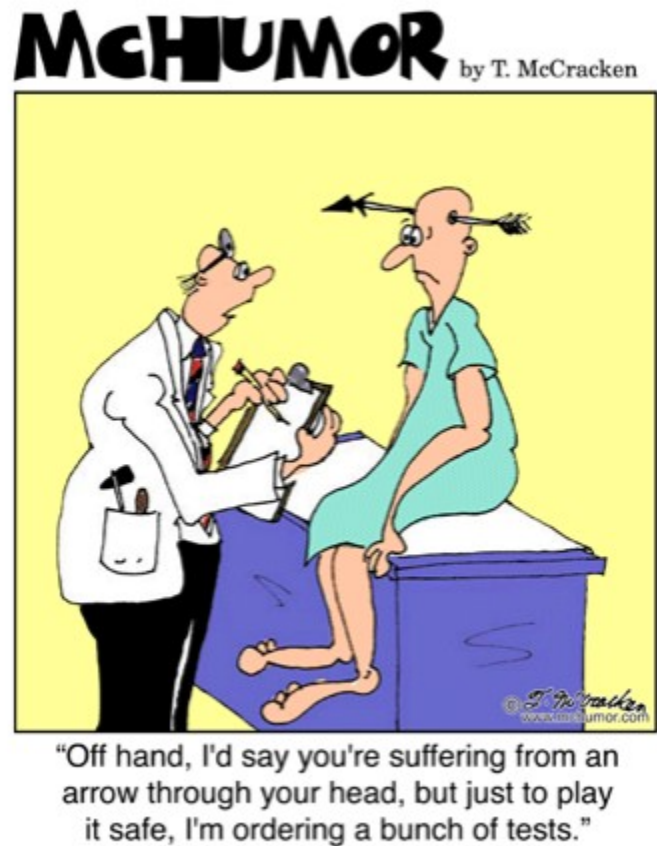
MTAP

Cadh-17

BAP1



# Replacements, supplemental IHC assays – why?

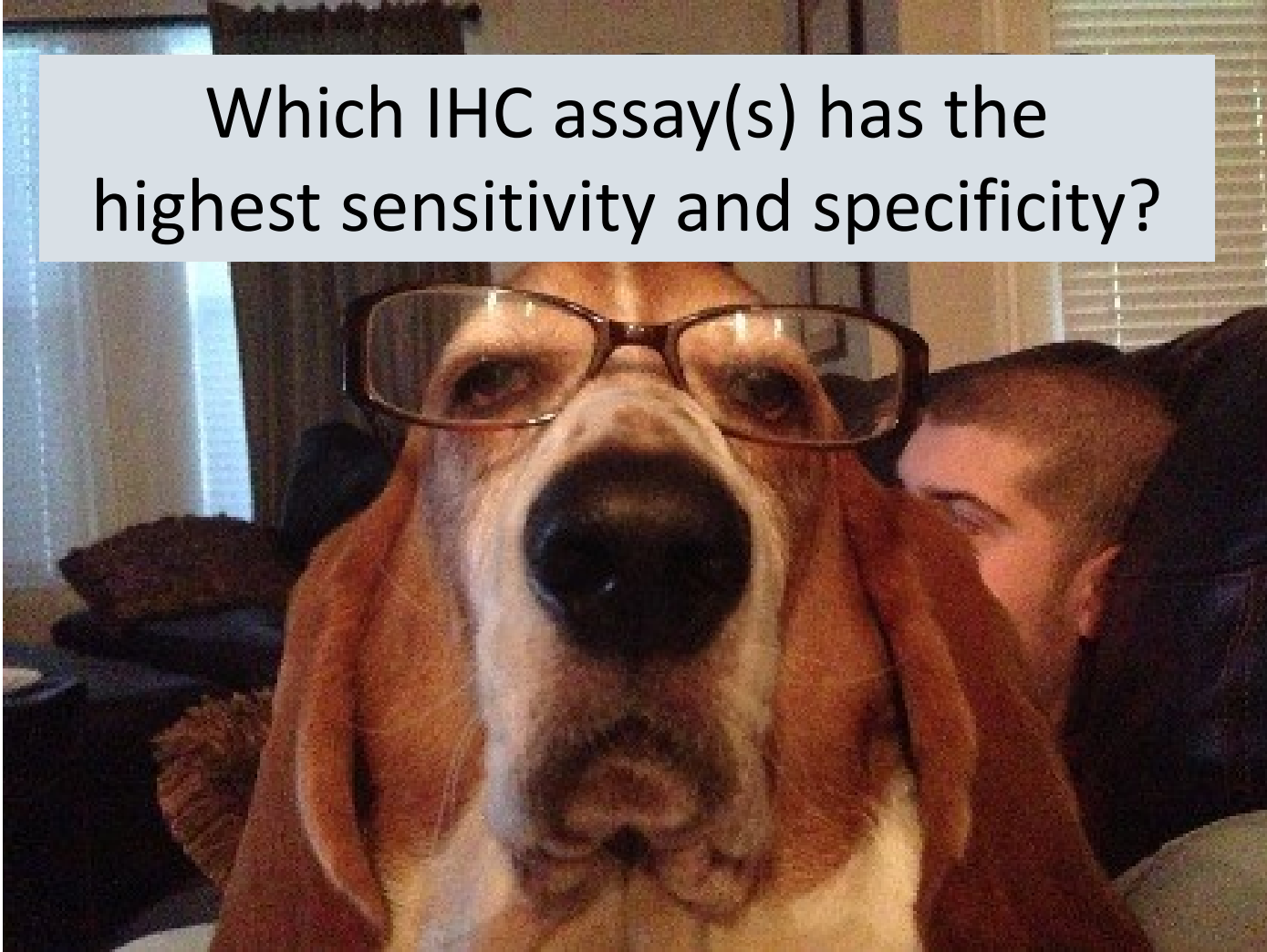


|        |          | Predicted                                                                                                                                         |                                                                                                                                                   |
|--------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
|        |          | Positive                                                                                                                                          | Negative                                                                                                                                          |
| Actual | Positive | <p>You are Dog</p>  <p>True positive</p>                       | <p>You are Cat</p>  <p>Type II error</p> <p>False negative</p> |
|        | Negative | <p>You are Dog</p>  <p>Type I error</p> <p>False positive</p> | <p>You are not Dog</p>  <p>True negative</p>                  |

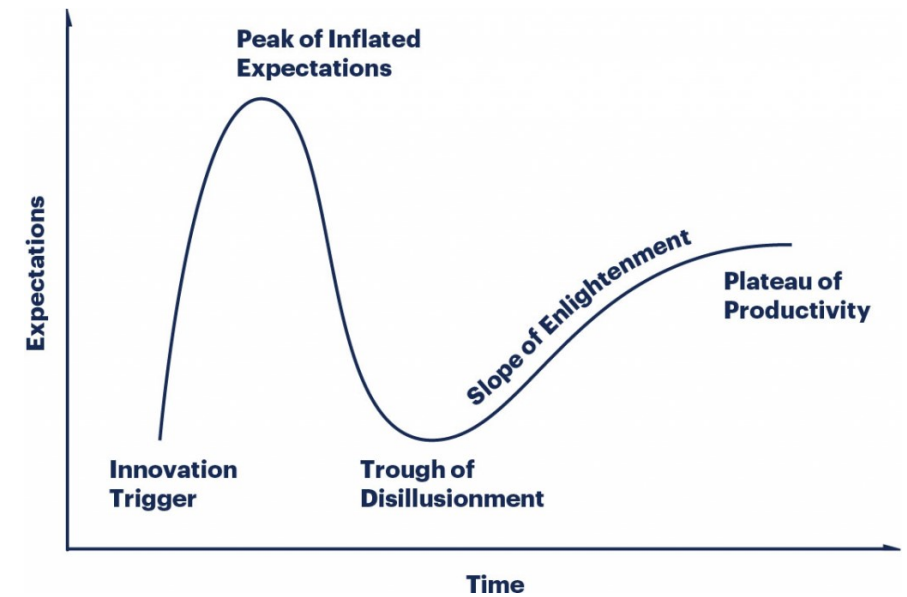


## Sensitivity and Specificity

Which IHC assay(s) has the highest sensitivity and specificity?



## A NEW BIOMARKER



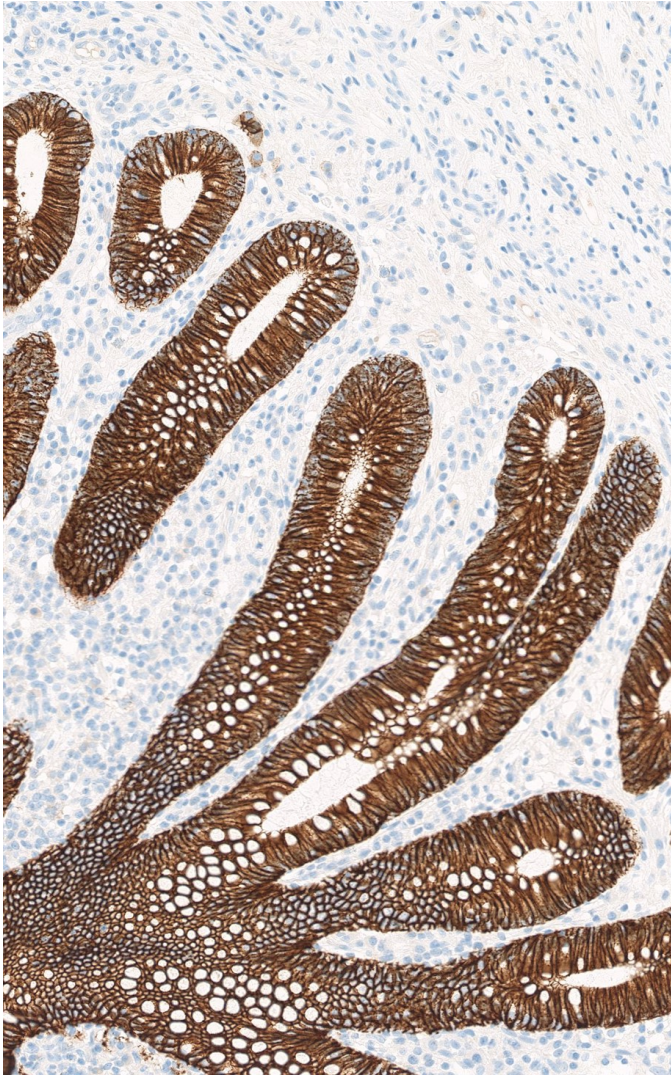
## The antibody graveyard – CUP\* - Colorectal carcinoma\*\* markers

|             | To stay | Sensitivity | Comments                                                                             |                                                  |
|-------------|---------|-------------|--------------------------------------------------------------------------------------|--------------------------------------------------|
| CK20        | Yes     | 80-95%      | MSI-H carc. can be neg. - Also seen in many other carcinomas                         | By both 97% & 88% sens.<br>for MSS and MSI-H CRC |
| CDX2        | Yes     | 80-95%      | MSI-H carc. can be neg. – Intestinal lineage marker                                  |                                                  |
| mCEA        | ?       | 90-100%     | Not specific for CRC - Might be useful for gastric adenocarcinomas                   |                                                  |
| Villin      | No      | 70-90%      | Less sensitive and less specific                                                     |                                                  |
| SATB2       | "New"   | 75-90%      | Lower GI tract and rectal/appendiceal neuroendocrine tumours                         |                                                  |
| Cadherin-17 | "New"   | 85-95%      | Publications indicate superior sensitivity compared to CDX2 and not a lineage marker |                                                  |

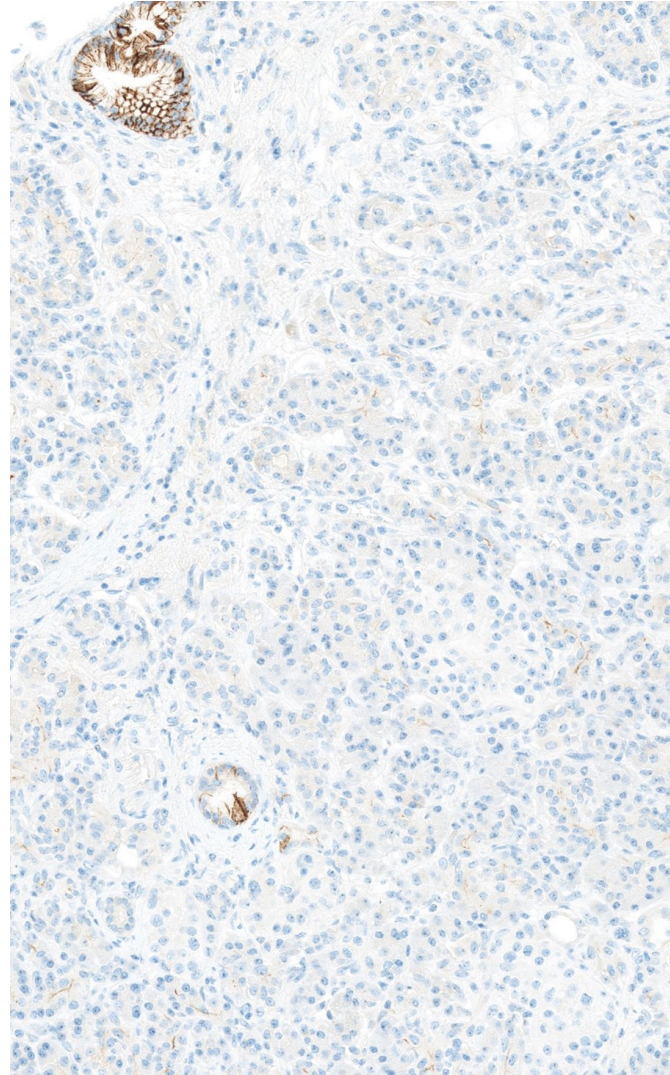
\* CUP; Cancer of unknown primary origin \*\* CRC



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



Colon/Appendix



Pancreas



Kidney

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



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

**Table 3. Primary Colon Cancer Versus Metastasis**

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

Abbreviation: CK, cytokeratin.

<sup>a</sup>  $P > .05$ ; primary CK20:  $P = .10$ , CDX2:  $P = .18$ ; metastasis into lymph node CDX2:  $P = .15$ .

<sup>b</sup> The origin of metastatic carcinomas was determined by a board-certified pathologist before receiving the tissue for testing.

<sup>c</sup>  $P < .05$ ; 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) <sup>a</sup>  | 46 (64/139) <sup>b</sup> |
| Metastasis <sup>c</sup> | 66.7 (24/36)   | 30.5 (11/36) <sup>b</sup> | 50 (18/36) <sup>d</sup>  |

Abbreviation: CK, cytokeratin.

<sup>a</sup>  $P < .001$ .

<sup>b</sup>  $P < .05$ ; primary CDX2:  $P = .004$ ; metastasis CK20:  $P = .002$ .

<sup>c</sup> The origin of metastatic carcinomas was determined by a board-certified pathologist before receiving the tissue for testing.

<sup>d</sup>  $P > .05$ ; metastasis CDX2:  $P = .15$ .

CDH17 Is a More Sensitive Marker for Gastric Adenocarcinoma Than CK20 and CDX2

David Altree-Tacha et al, Arch Pathol Lab Med. 2017;141:144–150

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

Table 2. Neoplastic Tissues (n = 884)

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

Abbreviation: CK, cytokeratin.

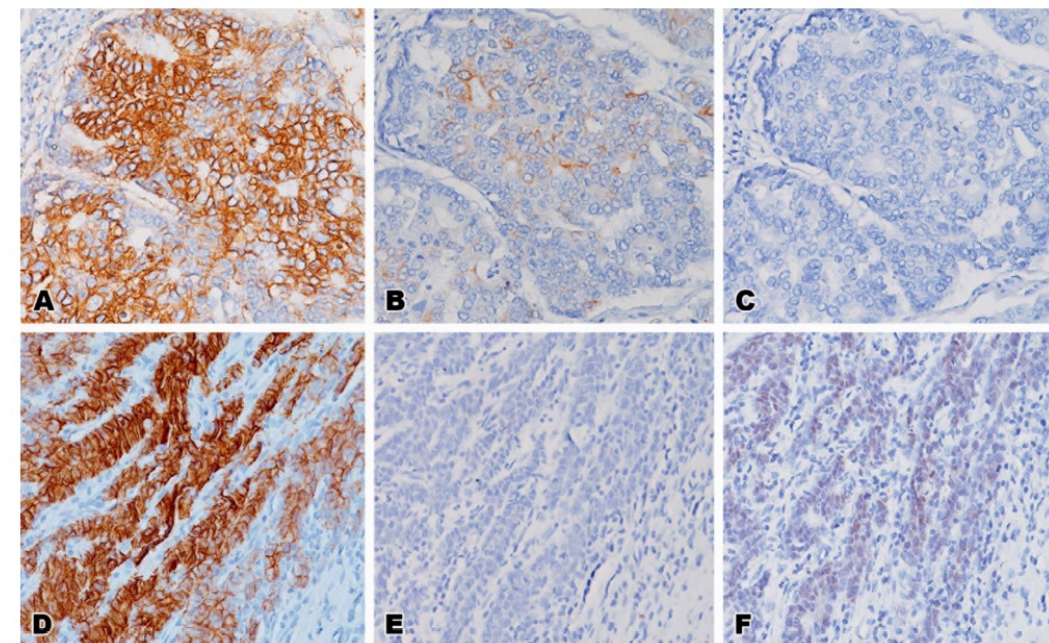


Figure 3. Staining results in metastatic colon adenocarcinoma. A and D, Strong, positive staining was observed in a high percentage of specimens with CDH17. B, Focal staining was observed in CK20-positive tissue; and in specimens considered negative, CK20 was completely absent (E). Representative negative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

CDH17 Is a More Sensitive Marker for Gastric Adenocarcinoma Than CK20 and CDX2

David Altree-Tacha et al, Arch Pathol Lab Med. 2017;141:144–150



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

F. Jacobsen et al.

Pathology - Research and Practice 256 (2024) 155175

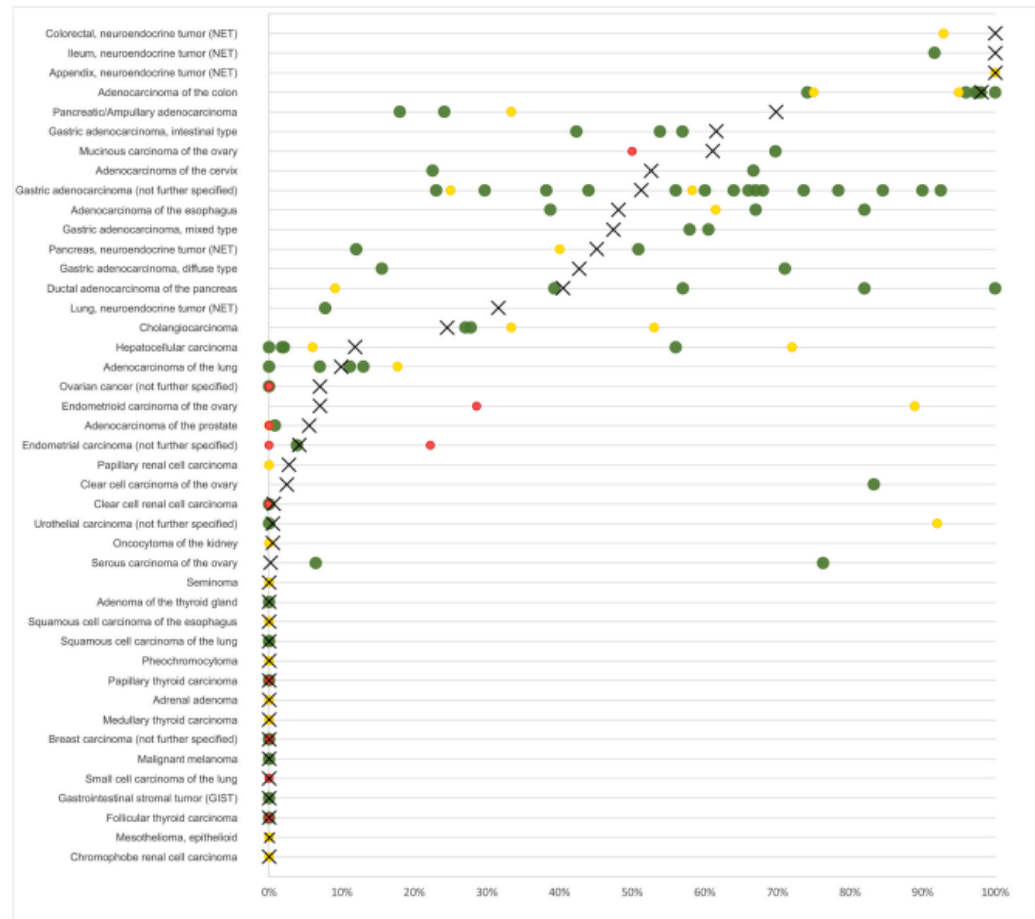


Fig. 5. Comparison with previous CDH17 literature. An „X“ indicates the fraction of CDH17 positive cancers in the present study, dots indicate the reported frequencies from the literature for comparison: red dots mark studies with  $\leq 10$  analyzed tumors, yellow dots mark studies with 11 to 25 analyzed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

*“These findings suggest a diagnostic utility of CDH17 IHC – as part of panels –*

1. for the distinction of metastatic gastrointestinal adenocarcinomas from primary pulmonary adenocarcinomas and
2. for the identification of intestinal subtypes in adenocarcinomas from the esophagus, stomach and the endocervix.”

Pathology - Research and Practice 256 (2024) 155175



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

Frank Jacobsen<sup>a</sup>, Ramesh Pushpadevan<sup>a</sup>, Florian Viehweger<sup>a</sup>, Morton Freytag<sup>a</sup>, Ria Schlichter<sup>a</sup>, Natalia Gorbokov<sup>a</sup>, Franziska Büscheck<sup>a</sup>, Andreas M. Luebke<sup>a</sup>, Devita Putri<sup>a</sup>, Martina Kluth<sup>a</sup>, Claudia Hube-Magg<sup>a</sup>, Andrea Hinsch<sup>a</sup>, Doris Höflmayer<sup>a</sup>, Christoph Fraune<sup>a</sup>, Christian Bernreuther<sup>a</sup>, Patrick Lebok<sup>a,b</sup>, Guido Sauter<sup>a</sup>, Sarah Minner<sup>a</sup>, Stefan Steurer<sup>a</sup>, Ronald Simon<sup>a</sup>, Eike Burandt<sup>a</sup>, David Dum<sup>a</sup>, Florian Lutz<sup>a</sup>, Andreas H. Marx<sup>c</sup>, Till Krech<sup>a,b</sup>, Till S. Clauditz<sup>a</sup>

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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*

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

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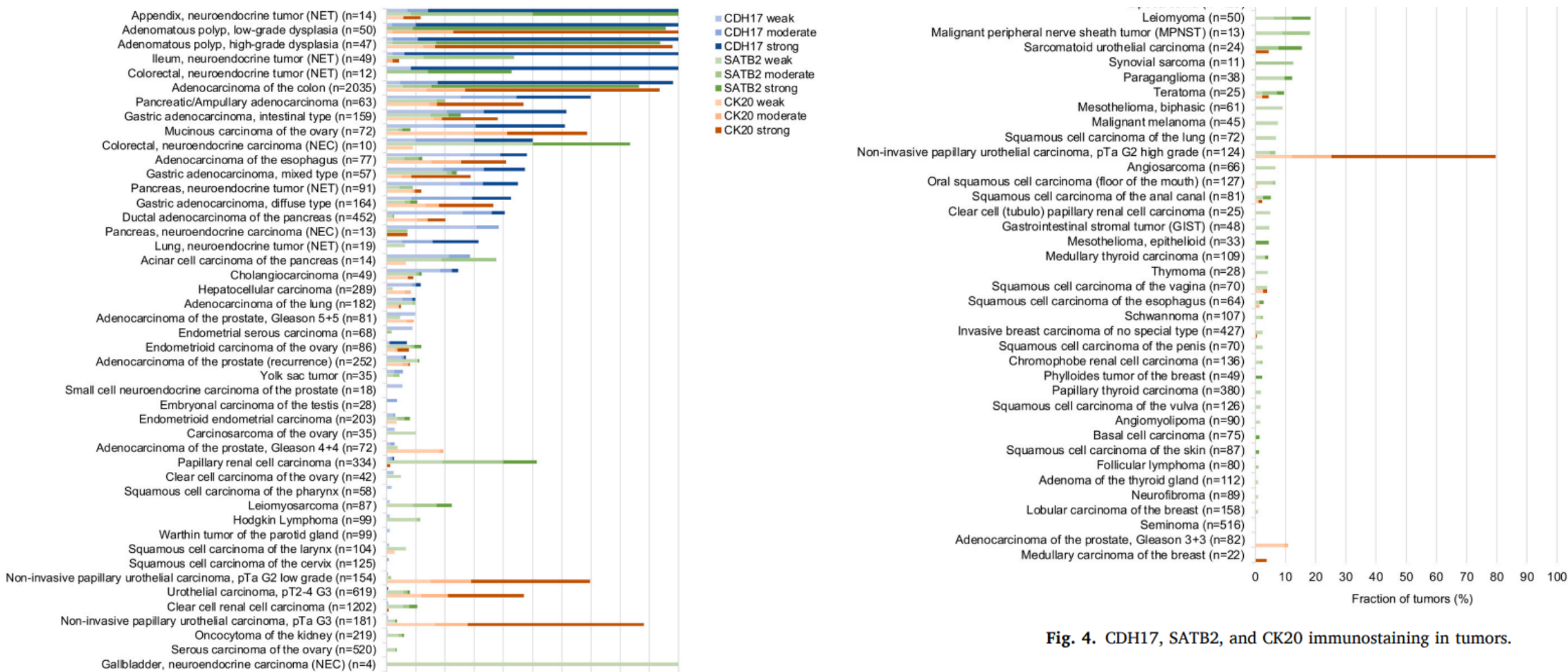


Fig. 4. CDH17, SATB2, and CK20 immunostaining in tumors.

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

Candidate; clone MSVA-517M, MS Validated Antibodies



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

CAD-17

rmAb

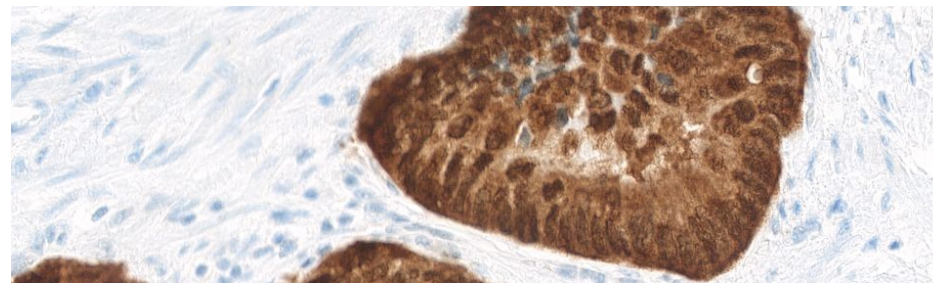
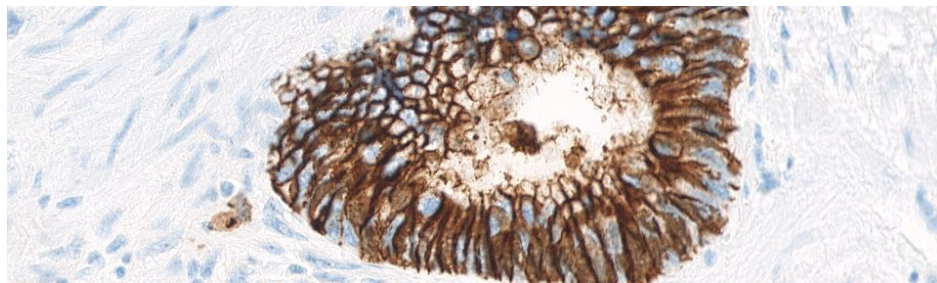
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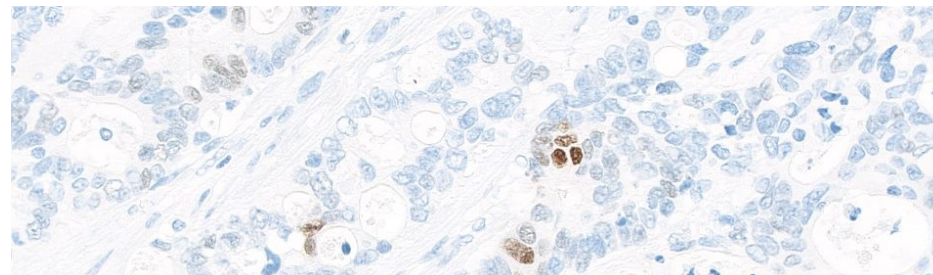
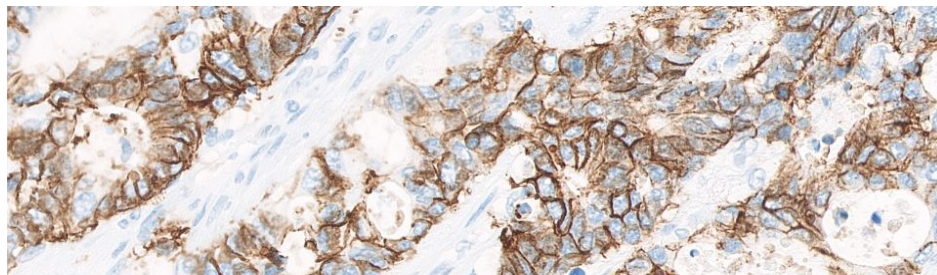
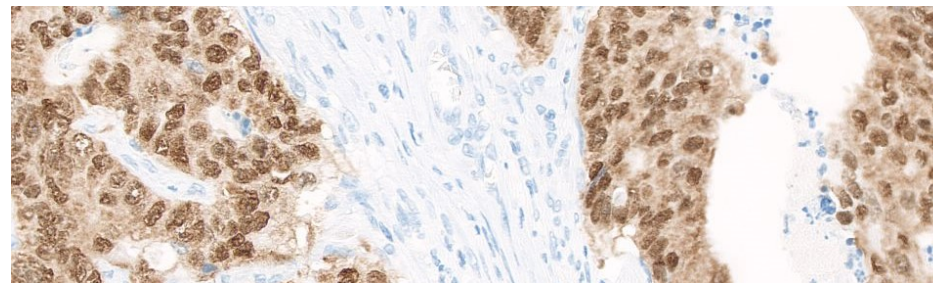
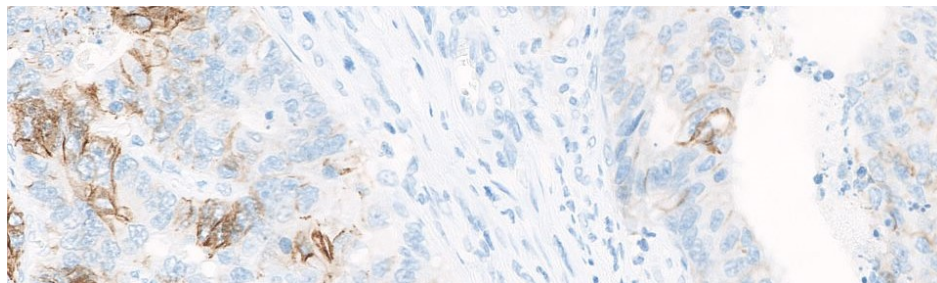
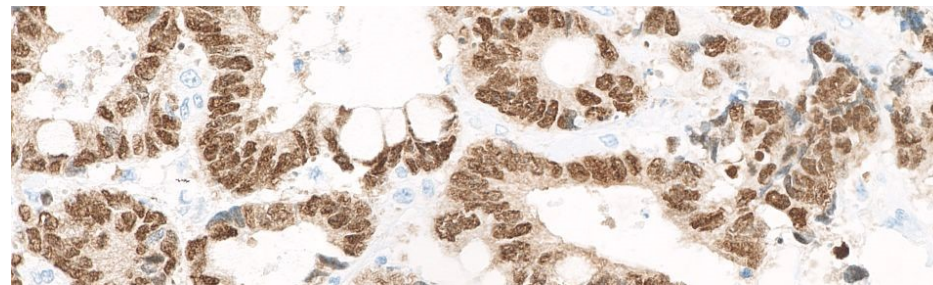
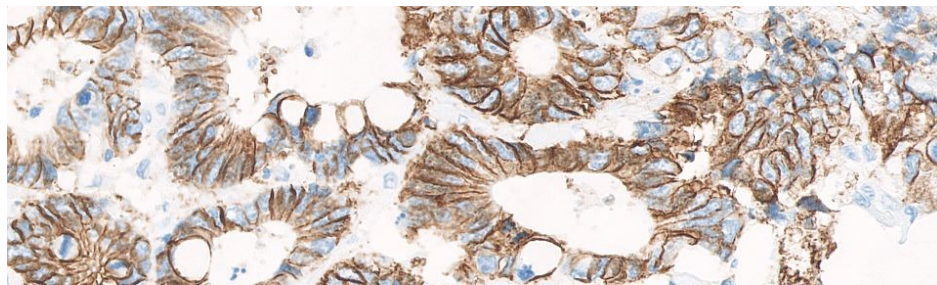
CC1, 48M

OP-DAB

VMS Ultra



CDX2





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

Article

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

Rui Wang,<sup>1,5</sup> Peng Fang,<sup>1,5</sup> Xi Chen,<sup>1,2,5</sup> Jiali Ji,<sup>1</sup> Dongan Yu,<sup>1,3</sup> Fei Mei,<sup>1,3</sup> Zhenzhen Wang,<sup>1</sup> Wei Zhou,<sup>1</sup> Wenjing Peng,<sup>1</sup> Rongjuan Wang,<sup>1,4</sup> Jian Wang,<sup>1</sup> Cuicui Guo,<sup>1</sup> Hai Wu,<sup>1</sup> Datao Liu,<sup>1,2,\*</sup> Xiaoding Tan,<sup>1,3,\*</sup> and Xun Gui<sup>1,6,\*</sup>

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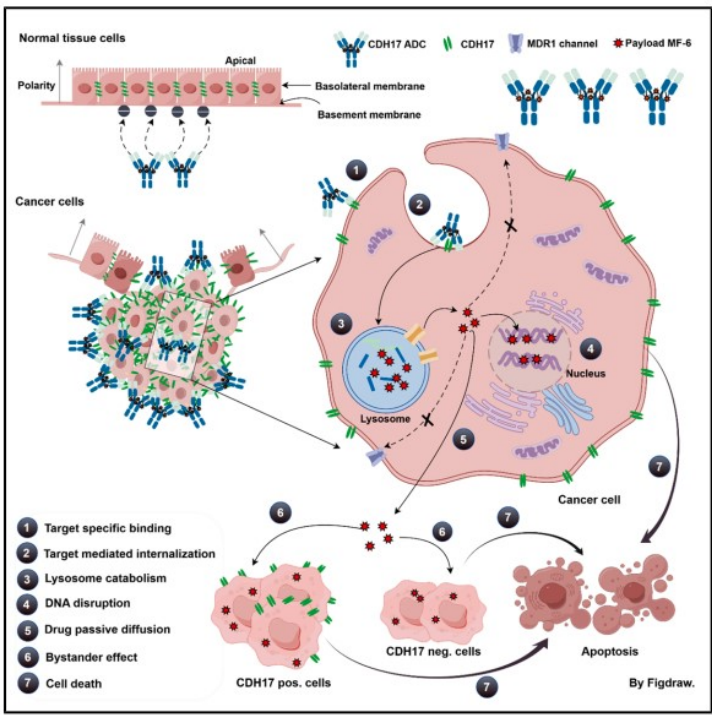
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<https://doi.org/10.1016/j.xcrm.2025.102213>



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; CMG801 | VelaVigo                    | VBC108: a first-in-class CDH17/CLDN18.2 targeted bispecific antibody drug conjugate (ADC) to overcome tumor heterogeneity of gastrointestinal cancers (GC, PADC, 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

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

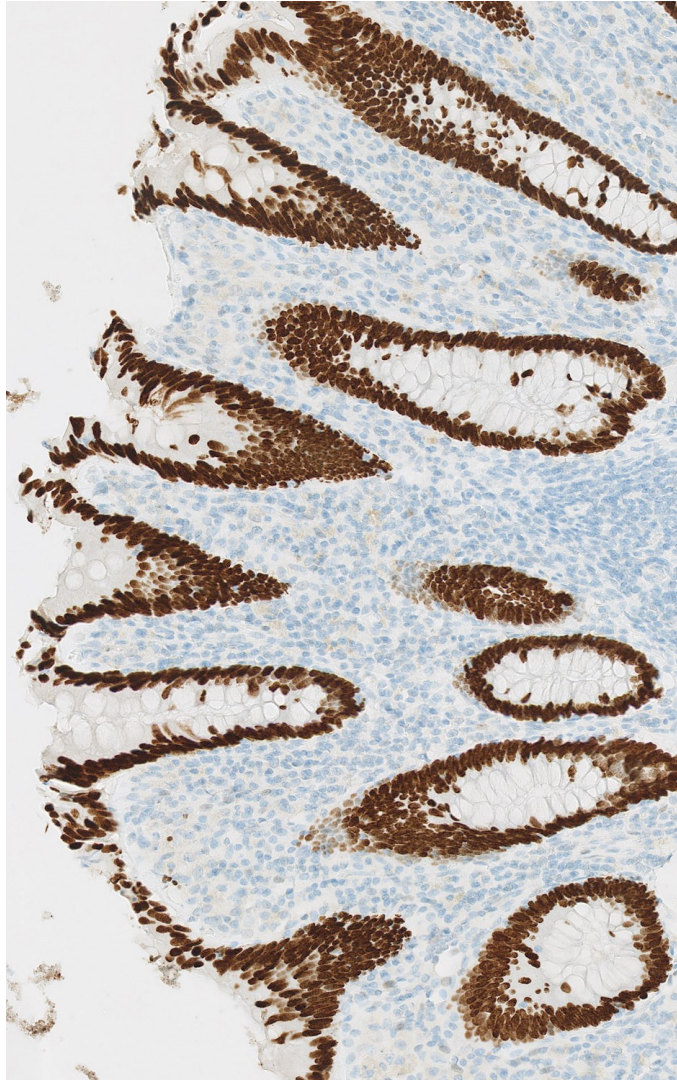
Authors: Charlotte Rose Lemech, Warren Lance Joubert, Andrew Ohyama Parsonson, Rui-Hua Xu, Rajiv Shinde, Prachi Bhawe, Junli Xue, Yaling Huang,

Xun Meng, and Shu-Hui Liu | [AUTHORS INFO & AFFILIATIONS](#)

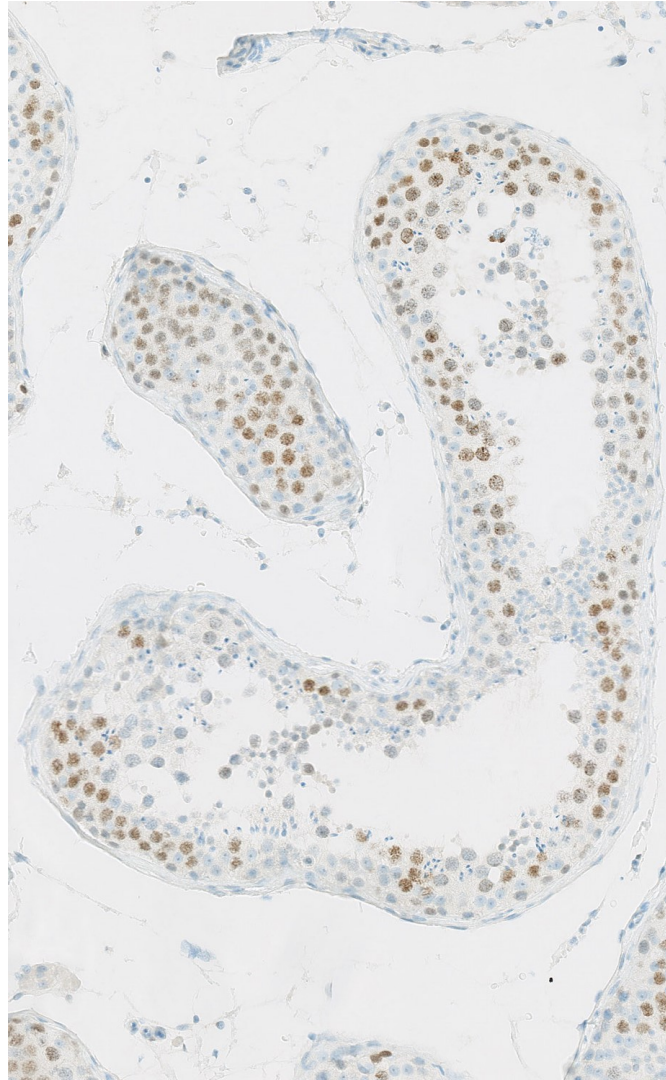
Publication: Journal of Clinical Oncology • Volume 43, Number 16 suppl • [https://doi.org/10.1200/JCO.2025.43.16\\_suppl.TPS3161](https://doi.org/10.1200/JCO.2025.43.16_suppl.TPS3161)



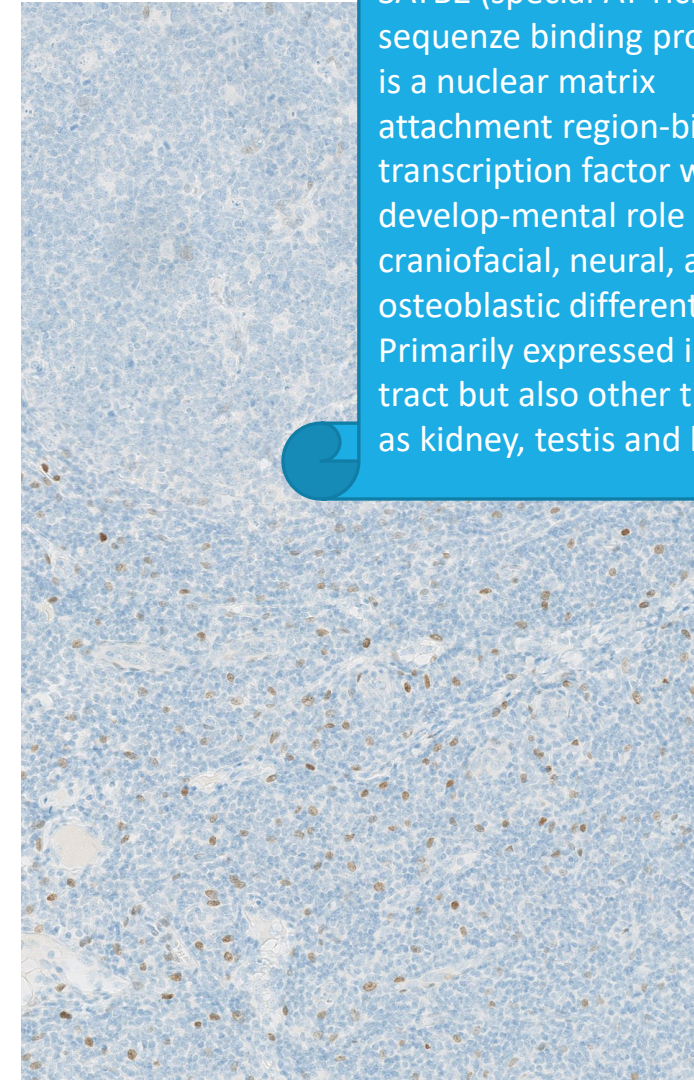
## The antibody graveyard – CUP - Colorectal carcinoma markers – SATB2



Colon/Appendix



Testis



Tonsil (EP281 - Clone dependent)

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



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

Table 1. Any SATB2 Expression in Primary Mucinous Tumors

| Score        | Site, No. %            |                   |                    |                  |                    |                      |                                      |
|--------------|------------------------|-------------------|--------------------|------------------|--------------------|----------------------|--------------------------------------|
|              | Colorectum<br>(n = 44) | Ovary<br>(n = 60) | Breast<br>(n = 31) | Lung<br>(n = 26) | Uterus<br>(n = 28) | Pancreas<br>(n = 15) | Stomach and<br>Esophagus<br>(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 | <u>39 (88.6)</u>       | <u>3 (5.0)</u>    | 5 (16.1)           | 0 (0)            | 1 (3.6)            | <u>0 (0)</u>         | 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)                             |

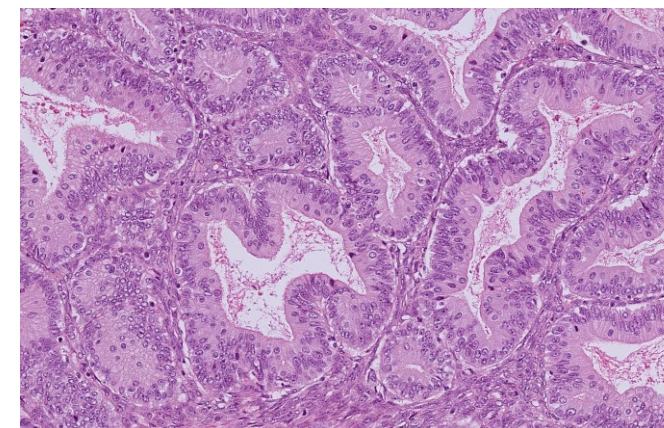
CDX2 is more sensitive for colorectal adenocarcinomas

SATB2 is more specific for colorectal adenocarcinomas

Differential diagnosis of ovarian, lung or colorectal carc.

Table 2. Any CDX2 Expression in Primary Mucinous Tumors

| Score        | Site, No. %            |                   |                    |                  |                    |                      |                                      |
|--------------|------------------------|-------------------|--------------------|------------------|--------------------|----------------------|--------------------------------------|
|              | Colorectum<br>(n = 44) | Ovary<br>(n = 60) | Breast<br>(n = 31) | Lung<br>(n = 26) | Uterus<br>(n = 28) | Pancreas<br>(n = 15) | Stomach and<br>Esophagus<br>(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 | <u>44 (100)</u>        | <u>48 (80.0)</u>  | 0 (0)              | 14 (53.8)        | 5 (17.9)           | <u>14 (93.3)</u>     | 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)                             |



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

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

Arch Pathol Lab Med—Vol 147, April 2023

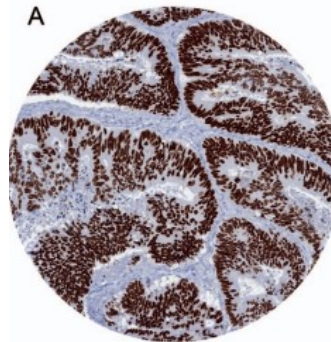
## SATB2 Expression in Human Tumors

### 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

“In summary, our data demonstrate that SATB2 immunostaining can occur in many different tumor entities.

Within adenocarcinomas, a high level (>70% of cells) of SATB2 expression is a strong argument for a colorectal tumor origin”



Low level SATB2 in colorectal tumors with MMRd , BRAF mutation, pT stage 4 and right side located

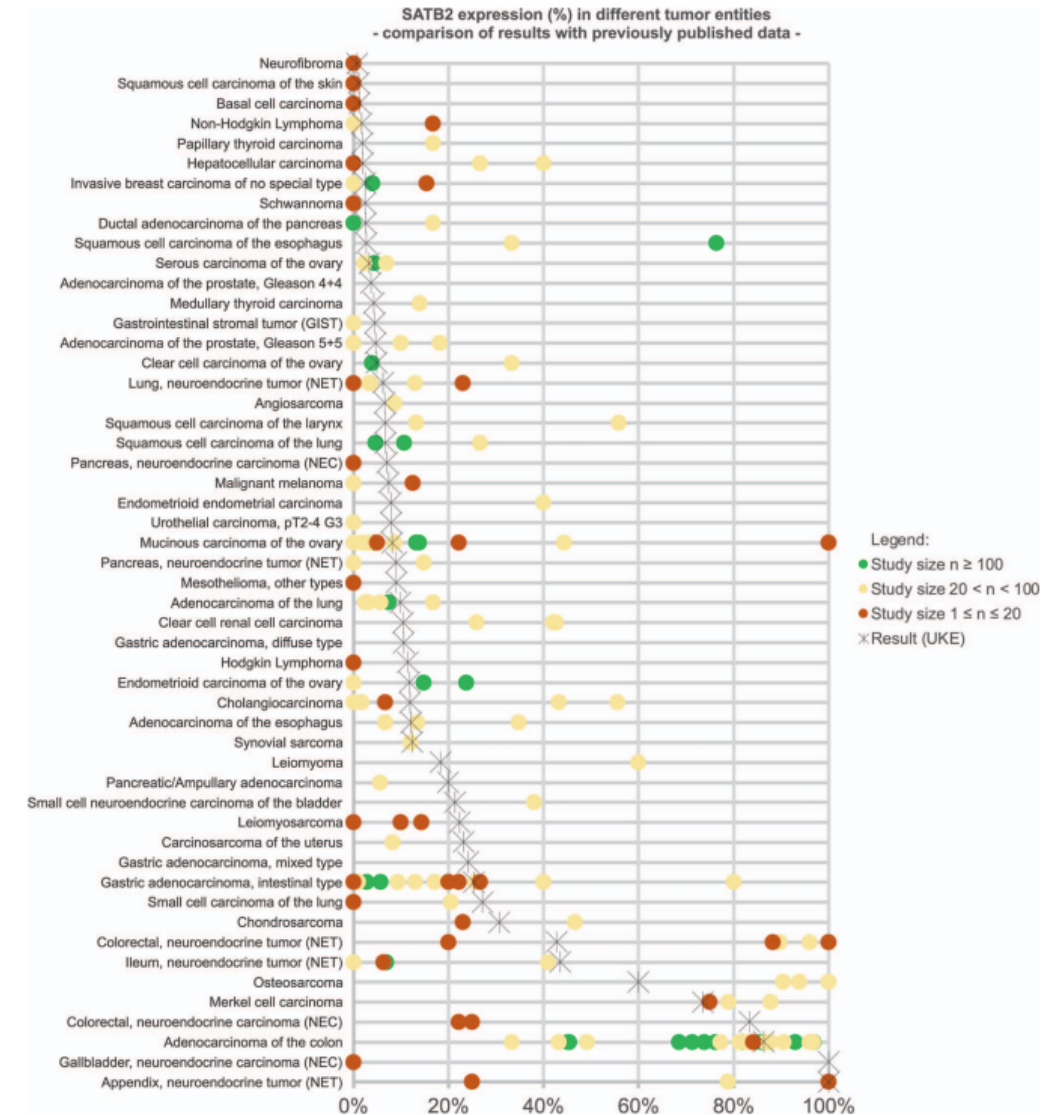


Figure 5. Graphical comparison of SATB2 data from this study (X) in comparison with the previous literature. Colored dots represent the numbers of analyzed tumors in these studies: red:  $n \leq 20$ ; yellow:  $n = 21$  to 100; green:  $n > 100$ .



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

| TABLE 1. Clinicopathologic Characteristics of CRCs Classified by SATB2 and CDX2 Expressions |                         |                           |                         |                         |                         |             |
|---------------------------------------------------------------------------------------------|-------------------------|---------------------------|-------------------------|-------------------------|-------------------------|-------------|
|                                                                                             | Total no.<br>269 (100%) | SATB2+/CDX2+<br>217 (81%) | SATB2-/CDX2+<br>23 (9%) | SATB2-/CDX2-<br>15 (6%) | SATB2+/CDX2-<br>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 in panel with CDX2...

SATB2 IHC reduced in metastatic lesions compared to CDX2 IHC

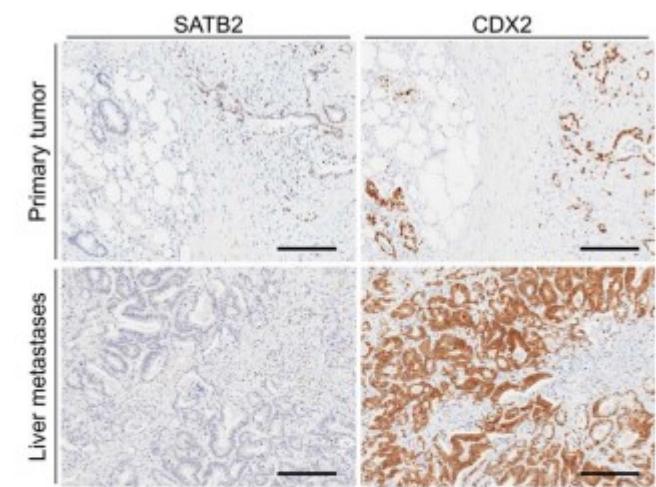


FIGURE 3. A CRC case showing differing SATB2 and CDX2 immunoreactivity in the primary tumor and its metastasis. A CRC case showing discrepant SATB2 expression in the primary and metastatic lesions. Note that while SATB2 was heterogeneously expressed in the primary tumor, the metastatic tumor showed no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

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



## HHS Public Access

Author manuscript

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

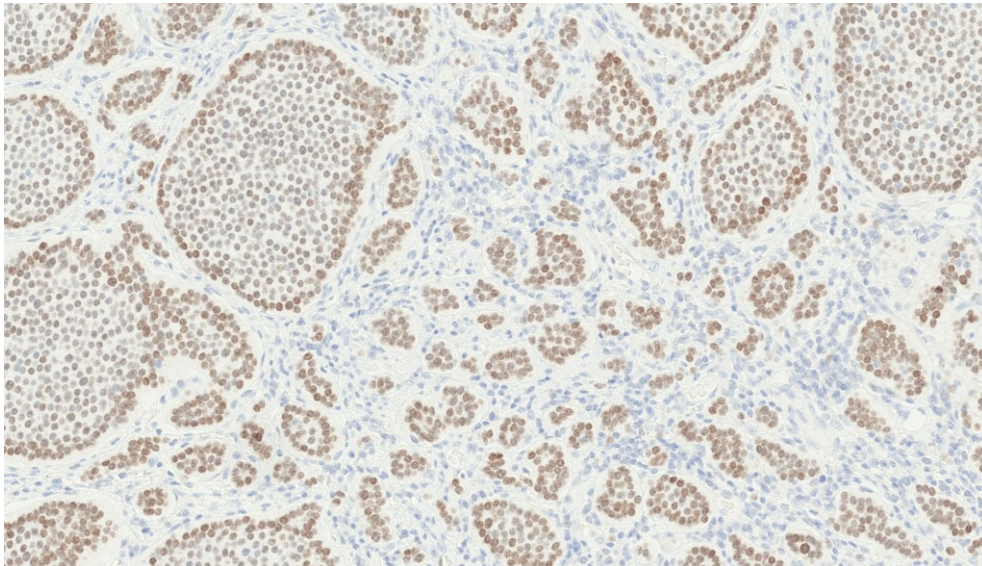
Published in final edited form as:

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\*

Department of Pathology, University of Iowa Hospitals and Clinics and Carver College of Medicine, Iowa City, IA, USA University of Iowa Neuroendocrine Cancer Program, University of Iowa Hospitals and Clinics and Holden Comprehensive Cancer Center, Iowa City, IA 52242, USA



## Well-Differentiated Neuroendocrine Tumor Classifier For the Real World:

Assumes Positivity for Broad-Spectrum Epithelial Marker and Diffuse, Strong Positivity for Chromogranin A and/or Synaptophysin

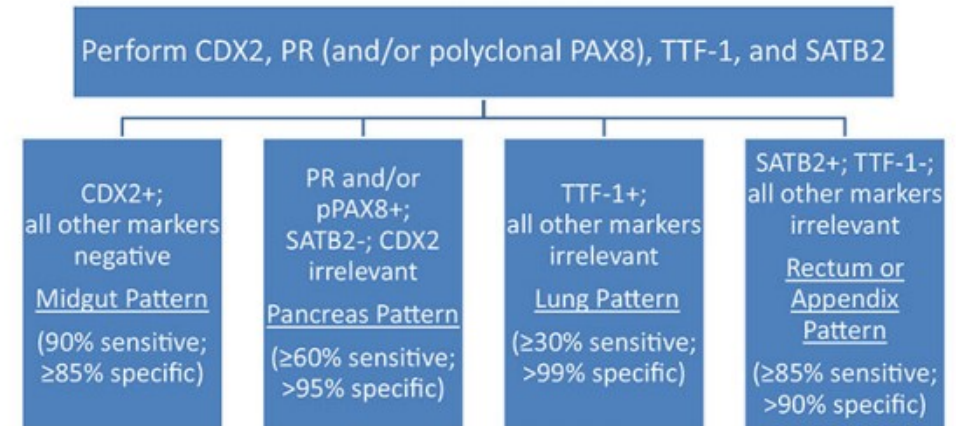


Figure 11. Simplified Immunohistochemical Algorithm for Well-Differentiated Neuroendocrine Tumor Site of Origin.

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

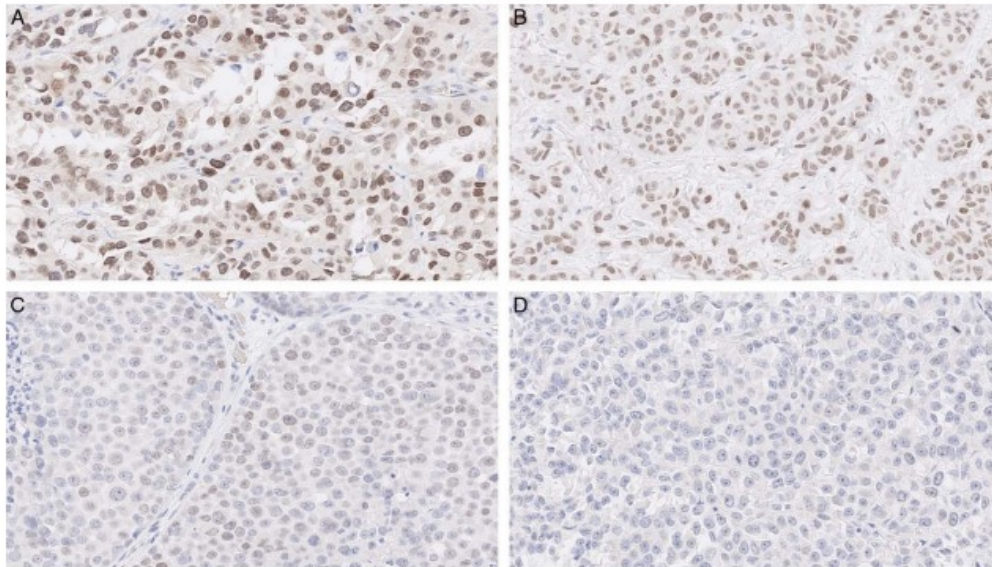


# The antibody graveyard – CUP - Colorectal carcinoma markers – SATB2

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

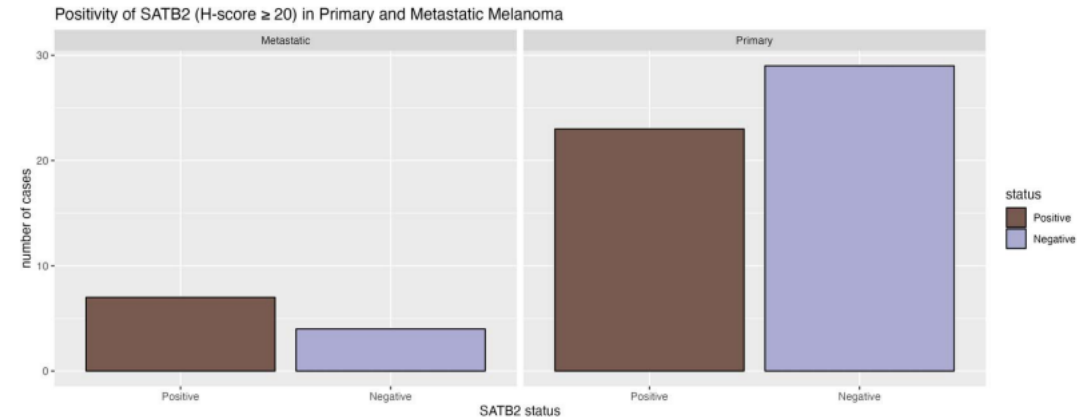
## Immunohistochemical Expression of SATB2 in Malignant Melanomas

*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.

On the basis of an H-score cutoff of 20, 30/63 (48%) of MM were positive for SATB2. The H-score in positive cases ranged from 20 to 210 (median 45) indicating that most positive cases were weakly to moderately stained.



**FIGURE 2.** The number of SATB2 positive ( $H\text{-score} \geq 20$ ) and negative cases in primary and metastatic malignant melanoma.

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.

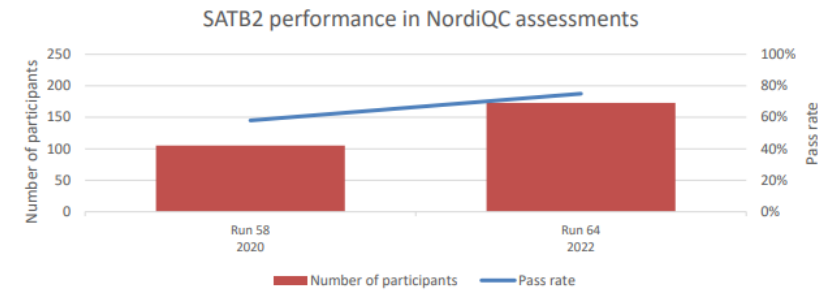
# The antibody graveyard – CUP - Colorectal carcinoma markers – SATB2 – the Ab....

| Concentrated antibodies                     | n   | Vendor                | Optimal | Good | Borderline | Poor | Suff. <sup>1</sup> | OR <sup>2</sup> |
|---------------------------------------------|-----|-----------------------|---------|------|------------|------|--------------------|-----------------|
| mAb clone SATBA4B10                         | 1   | Abcam                 | 0       | 1    | 6          | 0    | 14%                | 0%              |
|                                             | 3   | Santa Cruz            |         |      |            |      |                    |                 |
|                                             | 3   | Zytomed Systems       |         |      |            |      |                    |                 |
| rmAb clone EP281                            | 13  | Epitomics             | 48      | 28   | 8          | 8    | 83%                | 52%             |
|                                             | 66  | Cell Marque           |         |      |            |      |                    |                 |
|                                             | 1   | Diagnostic BioSystems |         |      |            |      |                    |                 |
|                                             | 3   | BioSB                 |         |      |            |      |                    |                 |
|                                             | 1   | Biocare Medical       |         |      |            |      |                    |                 |
| rmAb clone SP281                            | 5   | Gennova Scientific    | 0       | 4    | 0          | 1    | 80%                | 0%              |
|                                             | 3   | Zeta Corporation      |         |      |            |      |                    |                 |
|                                             | 4   | Abcam                 |         |      |            |      |                    |                 |
| rmAb clone QR023                            | 1   | Quartett              | 1       | 0    | 0          | 0    | -                  | -               |
| rmAb clone ZR167                            | 1   | Zeta Corporation      | 0       | 0    | 0          | 1    | -                  | -               |
| pAb HPA001042                               | 4   | Sigma Aldrich         | 0       | 1    | 1          | 2    | -                  | -               |
| pAb Ab69995                                 | 1   | Abcam                 | 0       | 0    | 0          | 1    | -                  | -               |
| Ready-To-Use antibodies                     |     |                       |         |      |            |      |                    |                 |
| mAb clone IHC660                            | 1   | GenomeMe              | 0       | 0    | 1          | 0    | -                  | -               |
| rmAb clone EP281 384R-17/18                 | 19  | Cell Marque           | 9       | 8    | 2          | 0    | 89%                | 47%             |
|                                             |     |                       |         |      |            |      |                    |                 |
| rmAb clone EP281 760-6075 VRPS <sup>3</sup> | 4   | Ventana/Roche         | 2       | 2    | 0          | 0    | -                  | -               |
| rmAb clone EP281 760-6075 VRPS <sup>4</sup> | 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%                |                 |

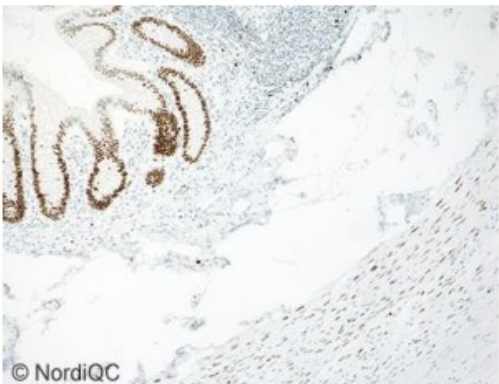
1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).  
 2) Proportion of Optimal Results (≥5 assessed protocols).  
 3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).  
 4) Laboratory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

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

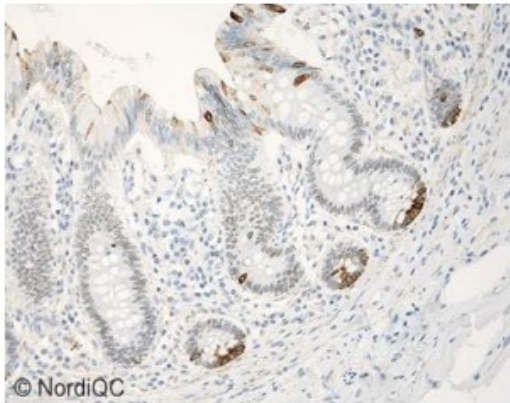
Graph 1. Proportion of sufficient results for SATB2 in the NordiQC runs performed



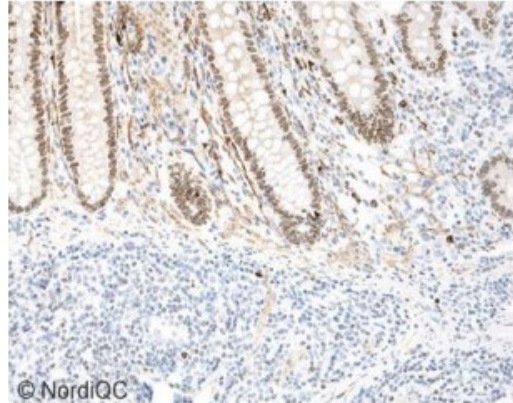
EP281



CL0276



Ab69995



HPA001042



# The antibody graveyard – CUP - Colorectal carcinoma markers

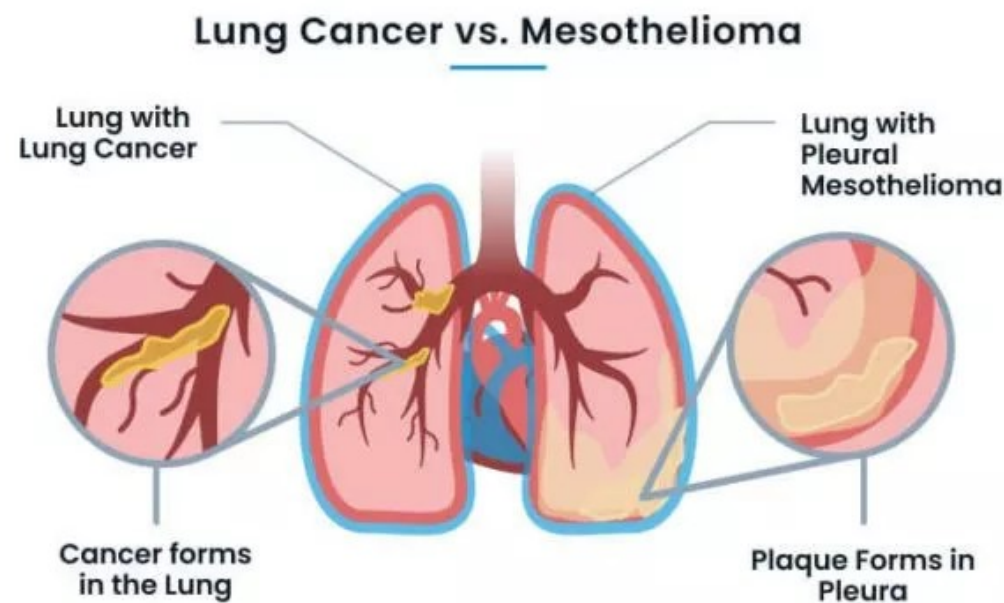
CK20 and CDX2; the two primary markers for identification of colorectal (CRC) adenocarcinoma

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

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

Villin and mCEA of less diagnostic value for CRC

# The antibody graveyard – Mesothelioma versus (lung-) adenocarcinoma



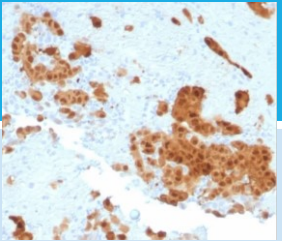
|            | Lung adenocarcinoma | Mesothelioma epithelial |
|------------|---------------------|-------------------------|
| Calretinin | -                   | +                       |
| CK5        | -                   | +                       |
| WT1        | -                   | +          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

\* Up to 30% mesotheliomas can be positive

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



## The antibody graveyard – Mesothelioma – positive markers

|                | To stay | Sensitivity | Comments                                                                                  |  |
|----------------|---------|-------------|-------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Calretinin     | Yes     | 85-95%      | Also seen in some carcinomas, but typically focal                                         |                                                                                     |
| CK5            | Yes     | 90-95%      | Also seen in squamous cell carcinomas                                                     |                                                                                     |
| WT1            | Yes     | 85-95%      | Also seen in serous ovarian carcinomas                                                    |                                                                                     |
| Thrombomodulin | No      | 60-70%      | Less sensitive                                                                            |                                                                                     |
| CA125          | No      | 70-80%      | Less sensitive and less specific (breast carc., pancreas carc, ovarian serous carc. ....) |                                                                                     |
| Mesothelin     | No      | 60-80%      | Less sensitive and less specific                                                          |                                                                                     |

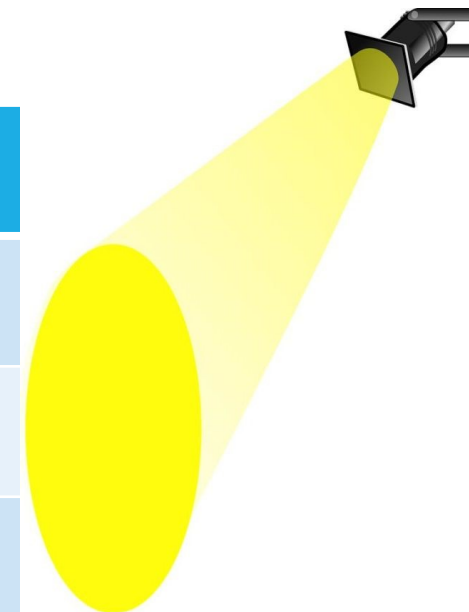
Nolwenn Le Stang et al;

Differential Diagnosis of Epithelioid Malignant Mesothelioma With Lung and Breast Pleural Metastasis:

A Systematic Review Compared With a Standardized Panel of Antibodies—A New Proposal That May Influence Pathologic Practice. *Arch Pathol Lab Med* 1 April 2020; 144 (4): 446–456.

## The antibody graveyard – Mesothelioma – positive markers

|                | To stay | Sensitivity | Comments                                                                                  |
|----------------|---------|-------------|-------------------------------------------------------------------------------------------|
| Calretinin     | Yes     | 85-95%      | Also seen in some carcinomas, but typically focal                                         |
| CK5            | Yes     | 90-95%      | Also seen in squamous cell carcinomas                                                     |
| WT1            | Yes     | 85-95%      | Also seen in serous ovarian carcinomas                                                    |
| Uroplakin 3b   | NEW     | 80%         | Also seen in Urothelial carcinoma (15-60%) and Ovarian carc. (10%)                        |
| HEG1           | NEW     | 85-95%      | Highly sensitivity in all mesothelioma subtypes - Also seen in few non-mesotheliomas      |
| Thrombomodulin | No      | 60-70%      | Less sensitive                                                                            |
| CA125          | No      | 70-80%      | Less sensitive and less specific (breast carc., pancreas carc, ovarian serous carc. ....) |
| Mesothelin     | No      | 60-80%      | Less sensitive and less specific                                                          |





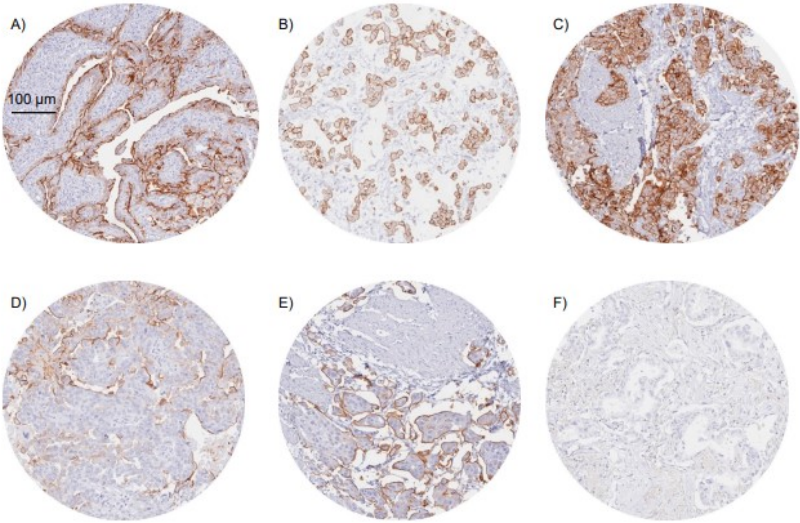
# The antibody graveyard – Mesothelioma – positive markers

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 <sup>1,✉</sup>, Dennis Atug <sup>1</sup>, Sebastian Dwertmann Rico <sup>1</sup>, Viktor Reischwich <sup>1</sup>, Florian Viehweger <sup>1</sup>, Franziska Büschek <sup>1</sup>, Martina Kluth <sup>1</sup>, Claudia Hube-Magg <sup>1</sup>, Andrea Hinsch <sup>1</sup>, Christian Bernreuther <sup>1</sup>, Guido Sauter <sup>1</sup>, Eike Burandt <sup>1</sup>, Andreas H. Marx <sup>2</sup>, Till Krech <sup>1,3</sup>, Ronald Simon <sup>1,\*</sup>, Sarah Minner <sup>1</sup>, Till S. Clauditz <sup>1,✉</sup>, Frank Jacobsen <sup>1</sup>, Patrick Lebok <sup>1,3</sup>, Natalia Gorbokon <sup>1</sup>, Katharina Möller <sup>1</sup>, Stefan Steurer <sup>1,†</sup> and Christoph Fraune <sup>1,†</sup>

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



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

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

# The antibody graveyard – Mesothelioma – positive markers



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## Uroplakin 3B (MSVA-736M)

Marker for mesothelial cells and umbrella cells

Normal tissue gallery

Cancer tissue gallery

Delivery Time: In Stock

Format

Concentrate

Volume

0,1 ml

0,5 ml

1 ml

Clear

295,00 €

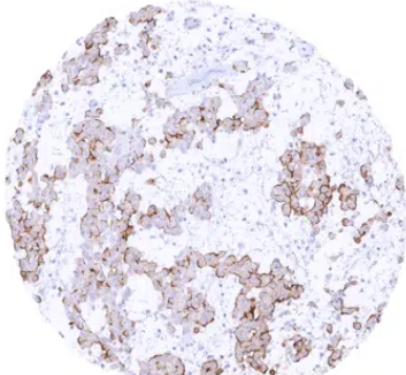
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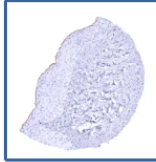
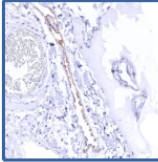
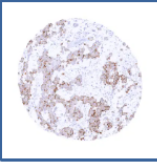
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Categories: [Antibodies with Performance Data](#), [Primary antibodies](#)



Epitheloid mesothelioma with membranous Upk3b staining of variable intensity in most tumor cells



[Go to Uroplakin 3B Normal Tissue Gallery](#)

[Go to Uroplakin 3B Cancer Tissue Gallery](#)



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

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

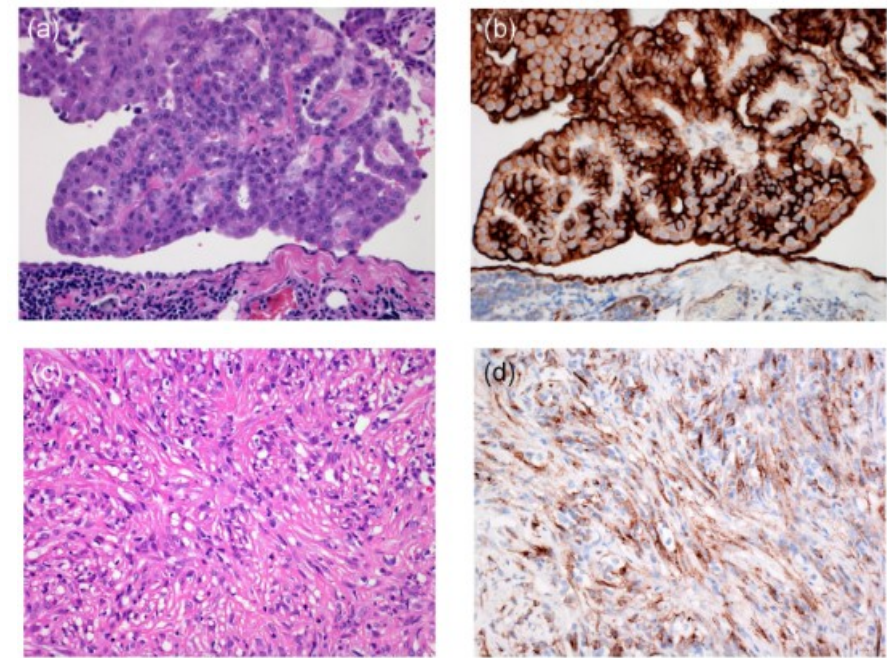
|                                  | N   | P   | (%)     | Staining score |   |   |    |    | Staining pattern |        |    |         |
|----------------------------------|-----|-----|---------|----------------|---|---|----|----|------------------|--------|----|---------|
|                         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

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

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



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

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

*Pathology International*. 2021;71:604–613.

# The antibody graveyard – Mesothelioma – positive markers

Human Pathology (2023) 141, 64–68



Original contribution

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

Derek M. van Pel MD<sup>a</sup>, Simon Cheung BSc<sup>b</sup>, Diana N. Ionescu MD<sup>a,c</sup>, Andrew Churg MD<sup>a,b,\*</sup>

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<sup>c</sup> Department of Pathology, BC Cancer Agency, Vancouver, BC, V5Z 1M9, Canada



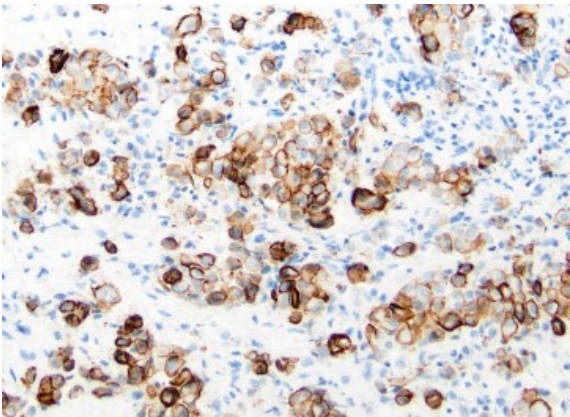
Overall, we find that, when the differential is epithelioid mesothelioma versus NSCLC, HEG1 is probably the first choice of stain, but UpIIIb is of potential value because it does stain a few epithelioid mesotheliomas that are missed by HEG1, here increasing the sensitivity from 89% for HEG1 alone to 93% for both markers.

**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           |



UpIIIb in epithelial mesothelioma

## The antibody graveyard – Mesothelioma – negative markers

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

\* Positivity seen in mesotheliomas (epithelial subtype)

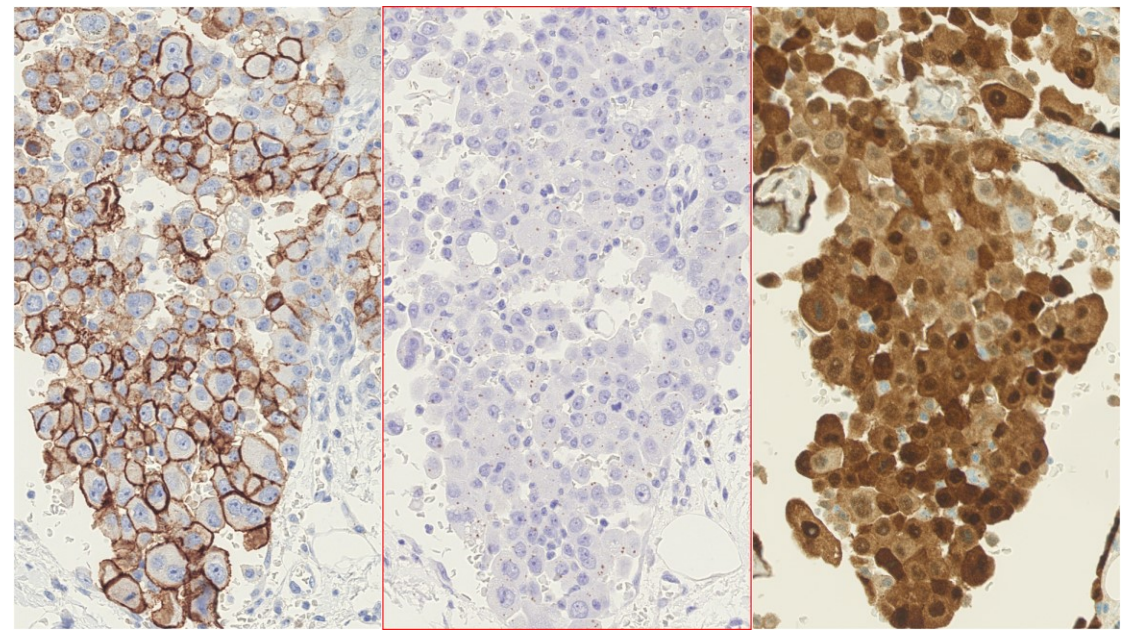
Typically 1-2 "positive markers" in combination with 1-2 "negative markers" used to differentiate mesothelioma and carcinoma

|                              | Lung adenocarcinoma | Mesothelioma epithelial |
|------------------------------|---------------------|-------------------------|
| HEG1                         | -                   | +                       |
| Calretinin or Uroplakin IIIb | -                   | +                       |
| EP-CAM                       | +                   | _*                      |
| Claudin-4                    | +                   | -                       |



## The antibody graveyard – Claudin 4

Mesothelioma

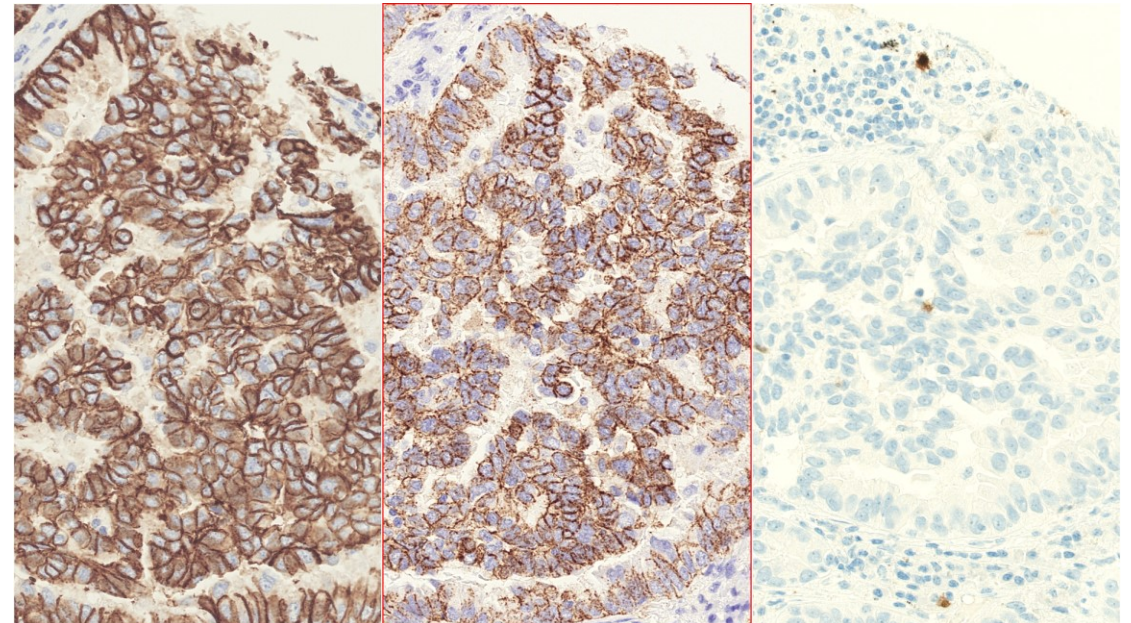


EP-CAM (EP4)

Claudin 4

Calretinin

Lung adenocarcinoma



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

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

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

ND, not done.

\*Based on TMAs.

\*\*Based on whole sections.

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

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

**Table 2.** Claudin-4: mesothelioma versus carcinoma staining

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

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

## REVIEW

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

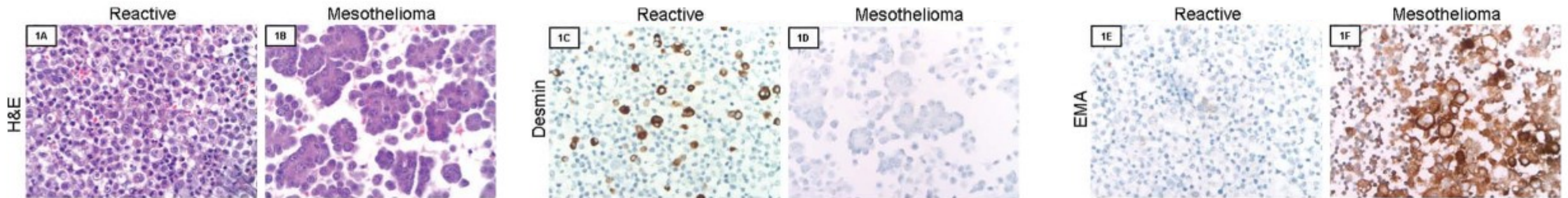
Andrew Churg<sup>1,2</sup> & Julia R Naso<sup>1,2</sup>

<sup>1</sup>Department of Pathology, Vancouver General Hospital, and <sup>2</sup>Department of Pathology, University of British Columbia, Vancouver, BC, Canada



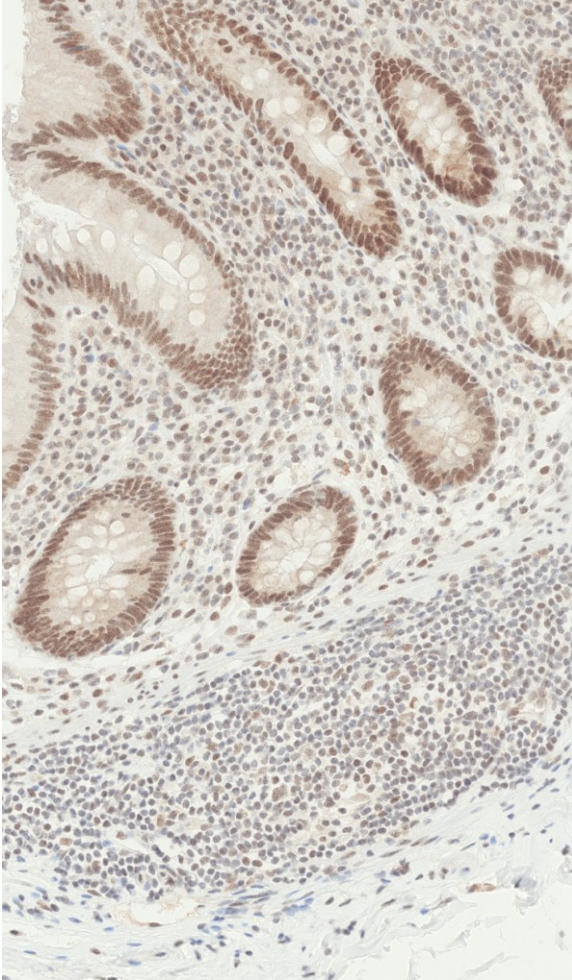
# The antibody graveyard – Mesothelioma versus reactive mesothelial cells

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

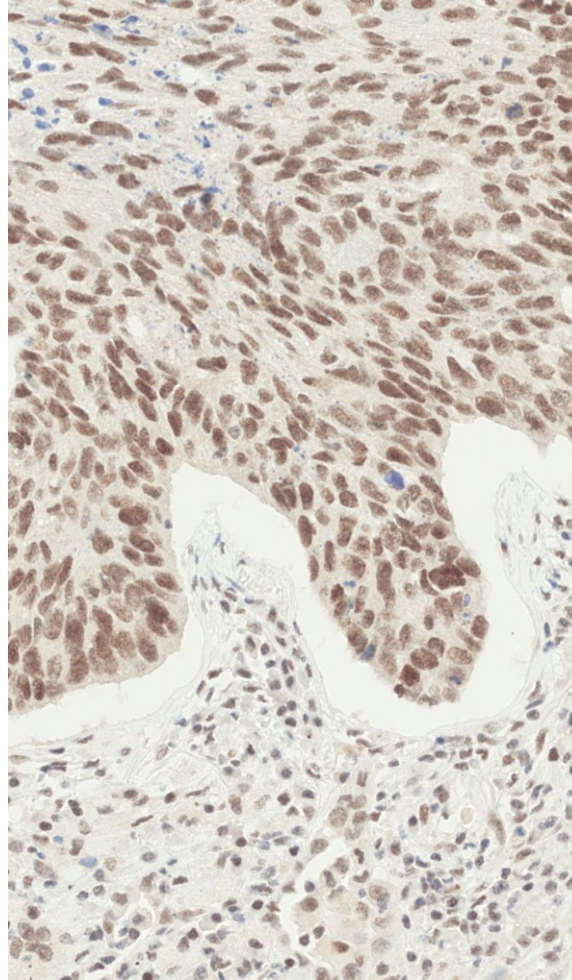




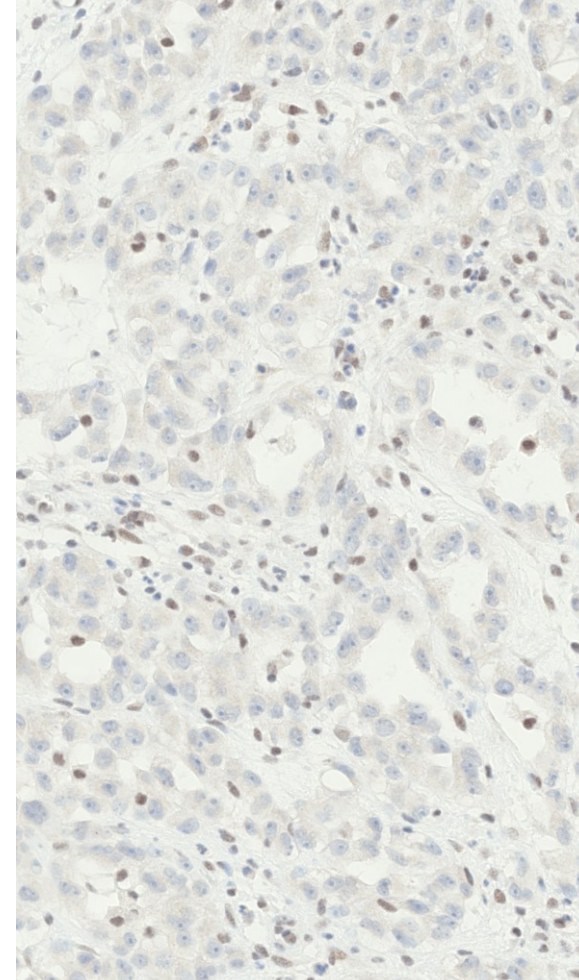
## The antibody graveyard – Mesothelioma – BAP1



Colon/Appendix



Tumour no mutation



Mesothelioma + mutation

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

Clone C-4, Santa Cruz

Or

Clone EPR22826, Abcam

Clone BSB-109, BioSp

Not beautiful, but ok 😊



# The antibody graveyard – Mesothelioma – BAP1

Review Article

## Diagnostic Mesothelioma Biomarkers in Effusion Cytology

Albino Eccher, MD <sup>1</sup>; Ilaria Girolami, MD <sup>2</sup>; Ersilia Lucenteforte, MD<sup>3</sup>; Giancarlo Troncone, MD <sup>4</sup>; Aldo Scarpa, MD<sup>1</sup>; and Liron Pantanowitz, MD <sup>5</sup>

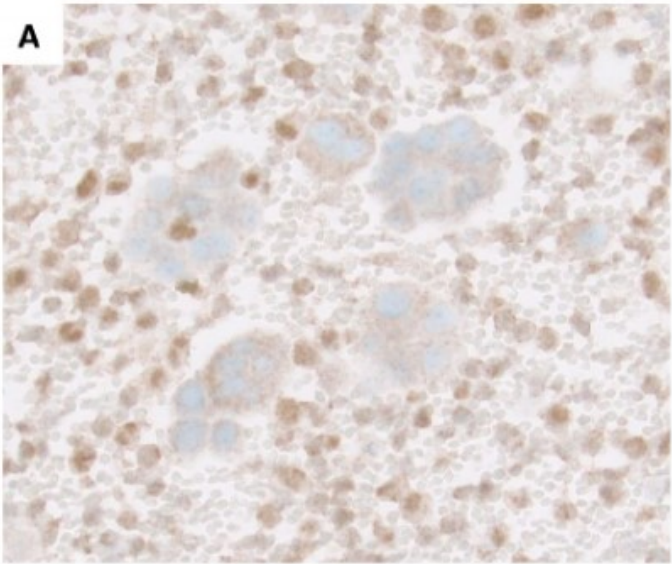
Malignant mesothelioma is a rare malignancy with a poor prognosis whose development is related to asbestos fiber exposure. An increasing role of genetic predisposition has been recognized recently. Pleural biopsy is the gold standard for diagnosis, in which the identification of pleural invasion by atypical 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.

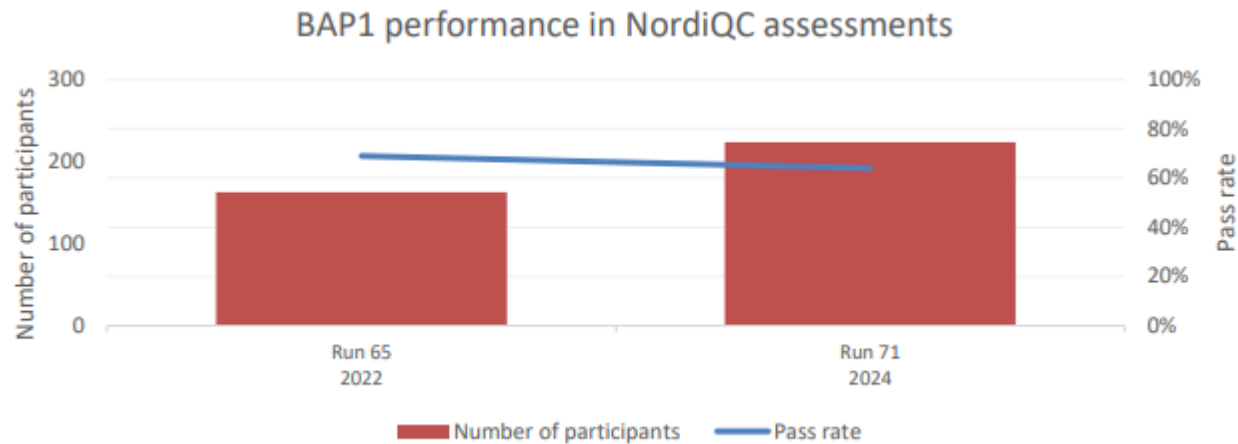
**TABLE 1.** Systematic Evidence on Diagnostic Performance of Malignant Pleural Mesothelioma Markers

| Marker          | Sensitivity and Specificity in Systematic Reviews                   |                                                                     | Notes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-----------------|---------------------------------------------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                 | Sensitivity (CI)                                                    | Specificity (CI)                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Soluble         |                                                                     |                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Mesothelin/SMRP | 0.79 (0.75-0.83) <sup>27</sup><br>0.69 (0.64-0.72) <sup>28</sup>    | 0.85 (0.83-0.87) <sup>27</sup><br>0.90 (0.85-0.94) <sup>28</sup>    | <ul style="list-style-type: none"><li>• Different cutoffs of the studies included</li><li>• No subgroup analysis for different MPM subtypes</li><li>• Diagnostic performance is usually studied in differential against both lung cancer and reactive atypical mesothelium</li></ul>                                                                                                                                                                                                                                                                                              |
| Fibulin-3       | 0.73 (0.54-0.86) <sup>31</sup>                                      | 0.80 (0.60-0.91) <sup>31</sup>                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| IHC and FISH    |                                                                     |                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| GLUT1           | 0.83 (0.71-0.90) <sup>36</sup>                                      | 0.90 (0.79-0.96) <sup>36</sup>                                      | <ul style="list-style-type: none"><li>• Marker of malignancy, not of MPM</li><li>• Informative only when positive</li><li>• Stains also red blood cells</li><li>• Oncofetal protein used as marker of malignancy, not of MPM</li><li>• Few studies dealing with cytology<sup>27,38</sup></li><li>• The sensitivity is reported to be higher in epithelioid mesothelioma and very low (0-0.22) in sarcomatoid mesothelioma</li><li>• Some carcinomas and melanoma could also show BAP1 loss</li><li>• Reliable to assess in cytology specimens, particularly cell blocks</li></ul> |
| IMP3            | No systematic review; reported values ranging 37-94%                |                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| BAP1            | 0.58 (0.50-0.65) <sup>44</sup><br>0.547 (0.512-0.716) <sup>45</sup> | 0.96 (0.89-0.99) <sup>44</sup><br>0.957 (0.939-0.971) <sup>45</sup> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

**A**



# The antibody graveyard – Mesothelioma – BAP1 – NordiQC data



Mutation identified by negative IHC;

Internal positive tissue control essential!

*"MMR similar"*

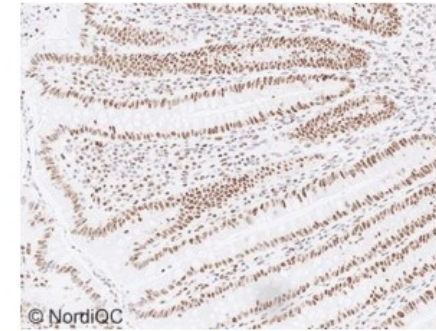


Fig. 1a  
Optimal BAP1 staining of appendix using the mAb clone C-4 - diluted, 1:25 (40 min. incubation), epitope retrieval using HIER in CC1 (32 min.), a 3-step multimer based detection system (OptiView) with tyramide amplification (OptiView Amplification) and performed on BenchMark (Ventana/Roche).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

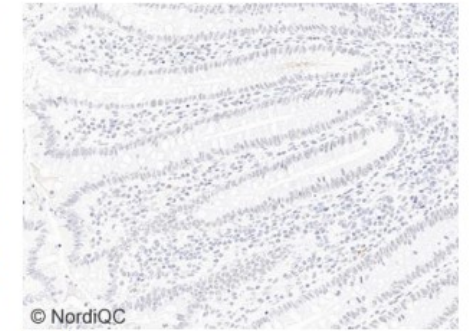


Fig. 1b  
Insufficient BAP1 staining of the appendix using same clone and similar protocol settings as in Fig. 1a, but with a less sensitive detection system (UltraView). Only scattered epithelial cells show a faint nuclear staining reaction. Virtually all lymphocytes/stromal cells are negative. Same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

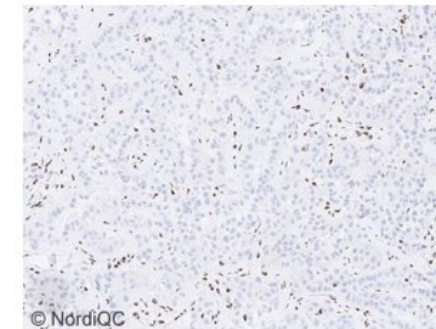


Fig. 4a  
Optimal BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1a - 3a. All neoplastic cells are negative, whereas stromal cells show a distinct, weak to moderate nuclear staining reaction.

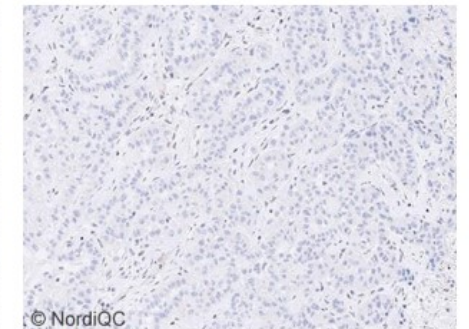


Fig. 4b  
Insufficient BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1b - 3b. The neoplastic cells are negative as expected. However, also the stromal cells, expected to be positive serving as internal positive tissue control, are negative.



# The antibody graveyard – Mesothelioma – BAP1 – NordiQC data

Table 1a. Overall results for BAP1, run 71

|                         | n   | Optimal | Good | Borderline | Poor | Suff. <sup>1</sup> | OR. <sup>2</sup> |
|-------------------------|-----|---------|------|------------|------|--------------------|------------------|
| 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. <sup>1</sup> | OR. <sup>2</sup> |
|-------------------------|-----|------------------|---------|------|------------|------|--------------------|------------------|
| 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 Gennova (n=6)

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

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

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

2) Proportion of Optimal Results (OR).

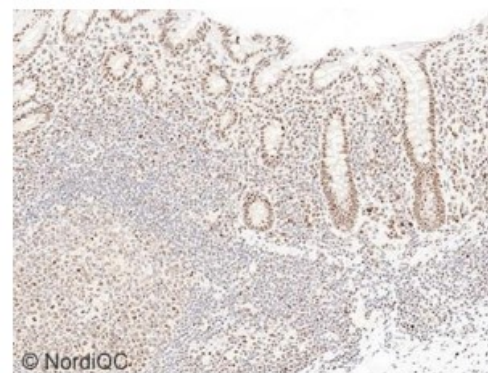


Fig. 2a  
Optimal BAP1 staining of appendix using same protocol as in Fig. 1a. Virtually all epithelial cells display a moderate to strong nuclear staining reaction, and the vast majority of lymphocytes/stromal cells show a weak to moderate nuclear staining reaction.

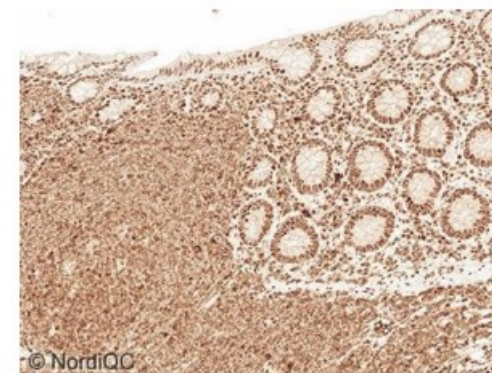


Fig. 2b  
Insufficient BAP1 staining of the appendix using same protocol as in Fig. 1b. Similar to Fig. 1b, separation of cells is hindered due to a prominent aberrant granular cytoplasmic staining reaction most likely caused by the tyramide-based amplification of the signal – compare with Fig. 2a, same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

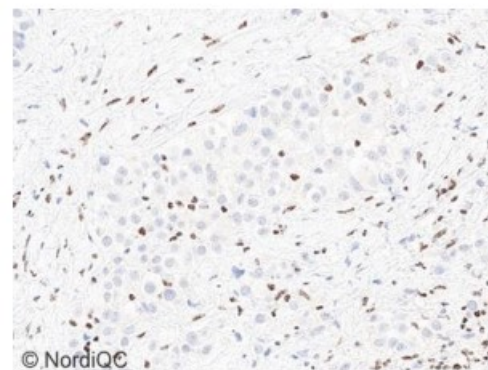


Fig. 4a  
Optimal BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1a–3a. All neoplastic cells are negative, whereas stromal cells show a distinct, moderate to strong nuclear staining reaction serving as internal positive tissue control verifying the loss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

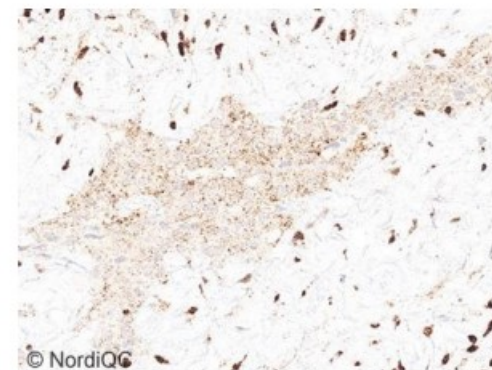
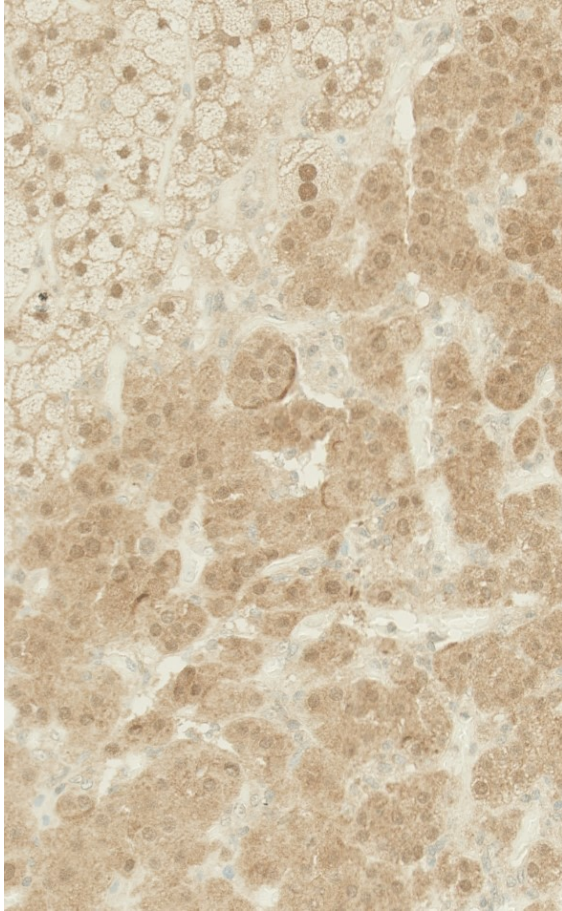


Fig. 4b  
Insufficient BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1b – 3b. The stromal cells display a strong nuclear staining reaction, whereas specifically the tumor cells harbor a distinct mostly cytoplasmic but also nuclear aberrant granular staining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

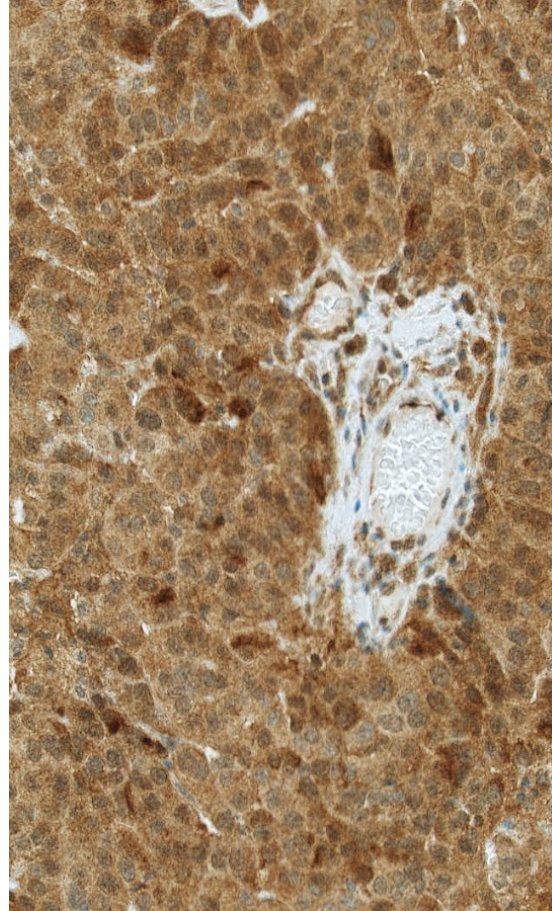
Low affinity clones requiring “utmost highly sensitive” protocols



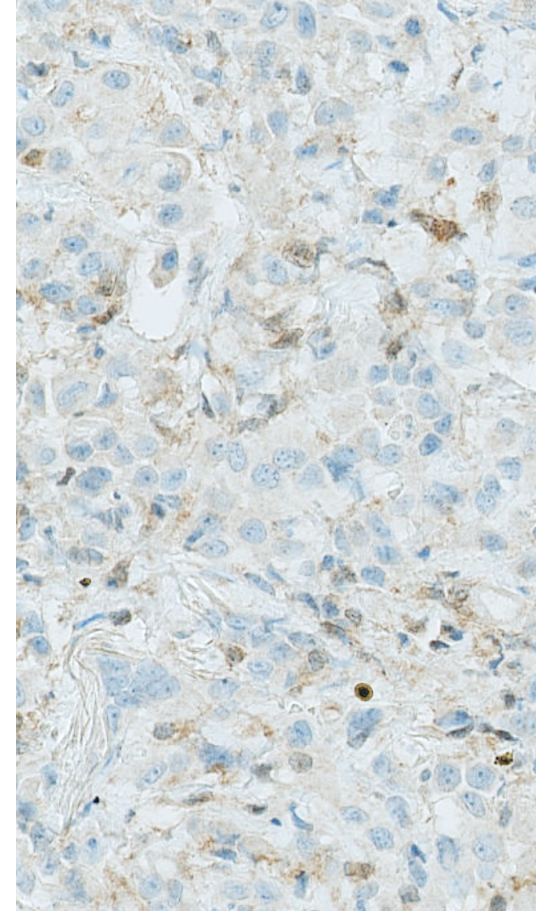
## The antibody graveyard – Mesothelioma – MTAP



Adrenal gland



Mesothelioma no mutation



Mesothelioma + mutation

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

Clone EPR6893

Not beautiful, but ok 😊



# The antibody graveyard – Mesothelioma – MTAP

ORIGINAL ARTICLE

OPEN

(Am J Surg Pathol 2024;00:000–000)

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

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

Natalia Gorbokon, MD,\* Niklas Wößner,\* Maximilian Lennartz, MD,\* Sebastian Dwertmann Rico, MD,\* Simon Kind, MD,\* Viktor 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 Lebock, 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\*

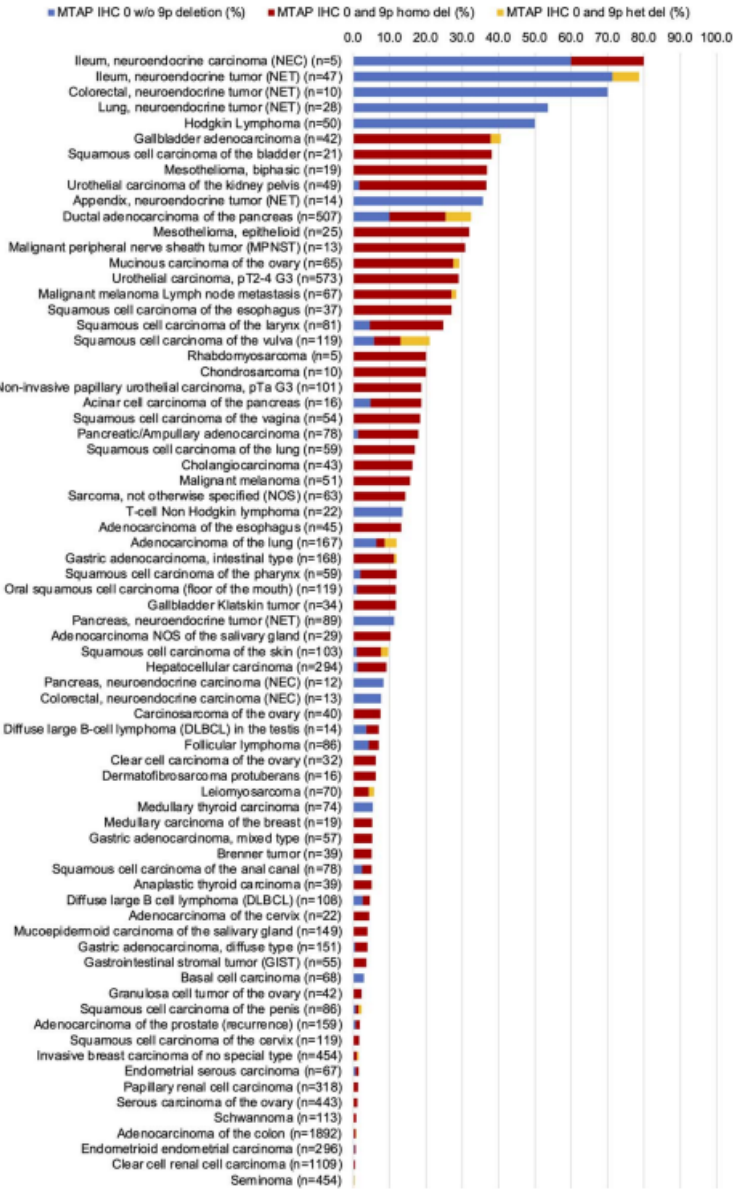
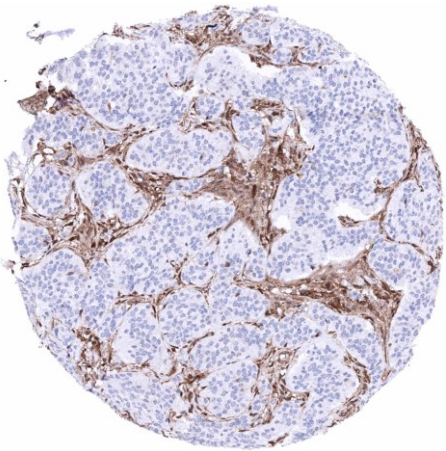


FIGURE 4. Concordance rate of MTAP IHC and 9p21 copy number analysis.

Rabbit monoclonal

**MTAP (MSVA-741R)**

Sensitive and specific marker for homozygous 9p21 deletions.

MS Validated antibodies

# The antibody graveyard – MTAP



Annals of Oncology

Available online 16 September 2024

In Press, Journal Pre-proof [What's this?](#)



Original Article

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

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

IHC for MTAP “forecast”;

Type I IHC assay for e.g. mesothelioma

Type II (CDx) IHC assay for solid tumours



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

ARTICLE

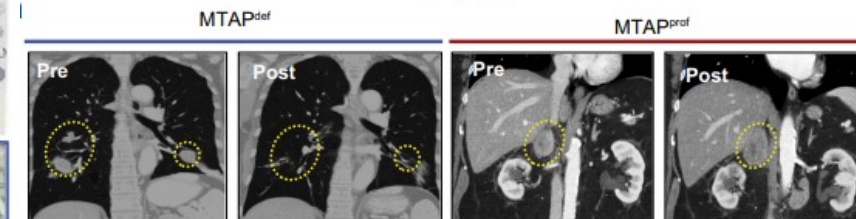
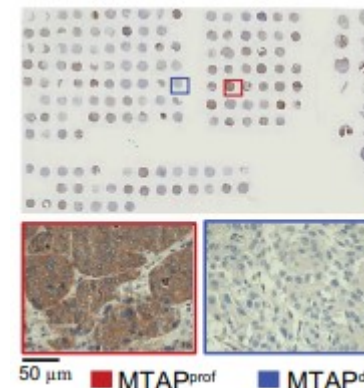
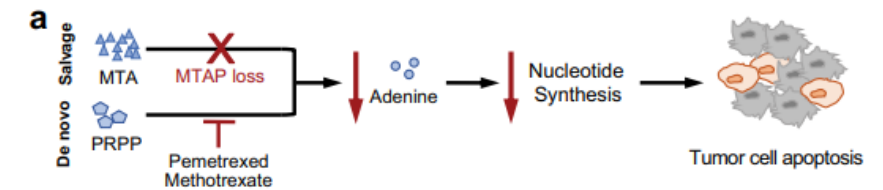
<https://doi.org/10.1038/s41467-022-29397-z>

OPEN



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

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





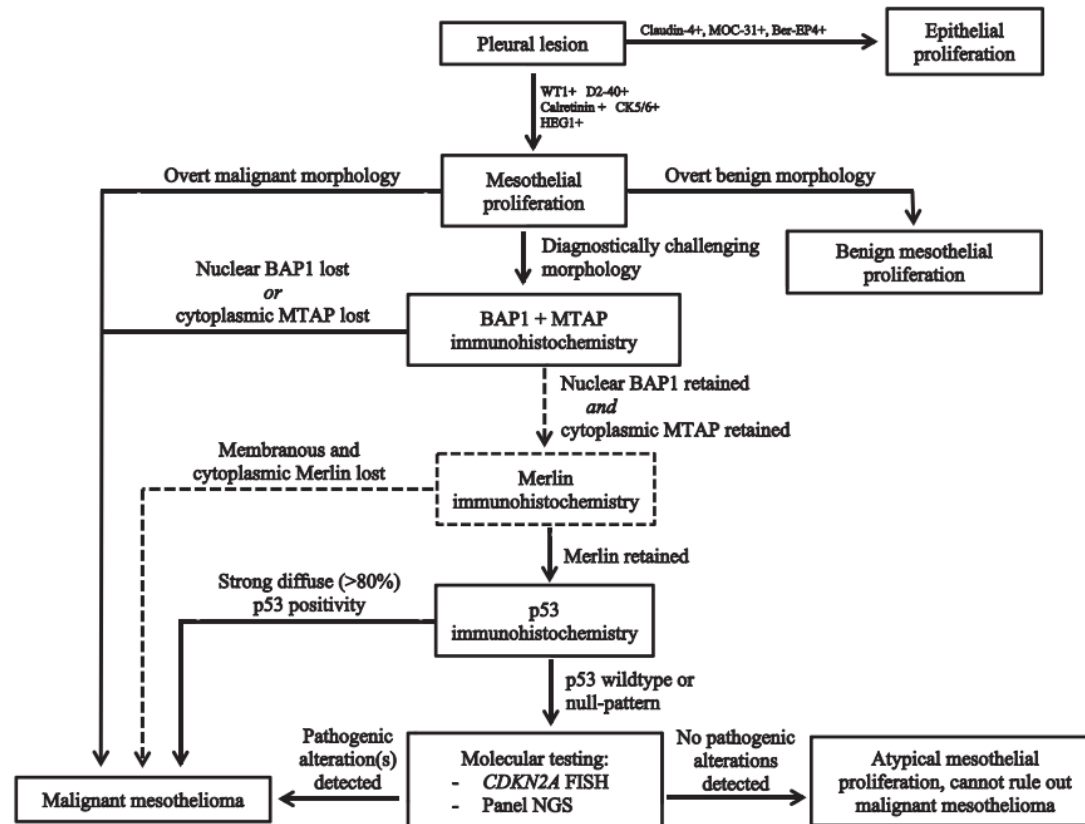
# The antibody graveyard – Mesothelioma – Merlin

## ARTICLE

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

David B. Chapel<sup>1,2</sup>, Jason L. Hornick<sup>1</sup>, Julianne Barlow<sup>3</sup>, Raphael Bueno<sup>3</sup> and Lynette M. Sholl<sup>1,4</sup>

Modern Pathology (2022) 35:1383 – 1397



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

**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.

# The antibody graveyard – Mesothelioma – Merlin

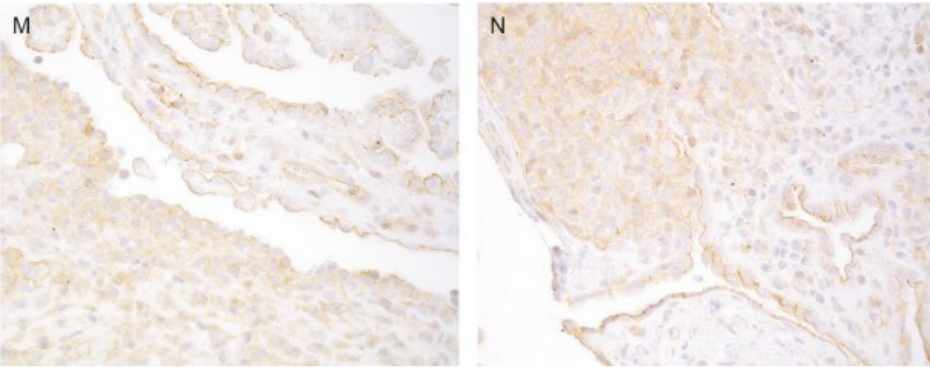
## ARTICLE

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

David B. Chapel<sup>1,2</sup>, Jason L. Hornick<sup>1</sup>, Julianne Barlow<sup>3</sup>, Raphael Bueno<sup>3</sup> and Lynette M. Sholl<sup>1,4</sup>

Modern Pathology (2022) 35:1383 – 1397

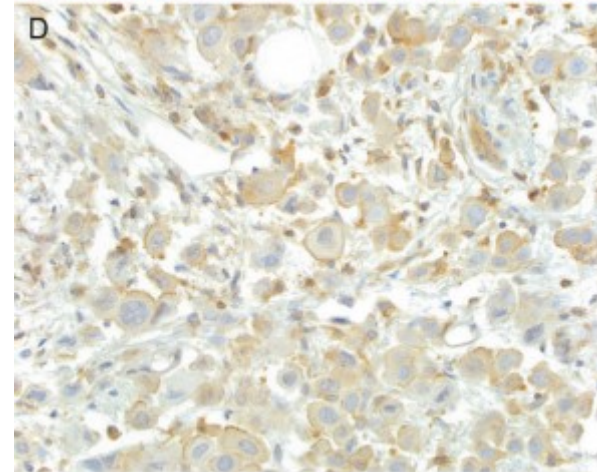
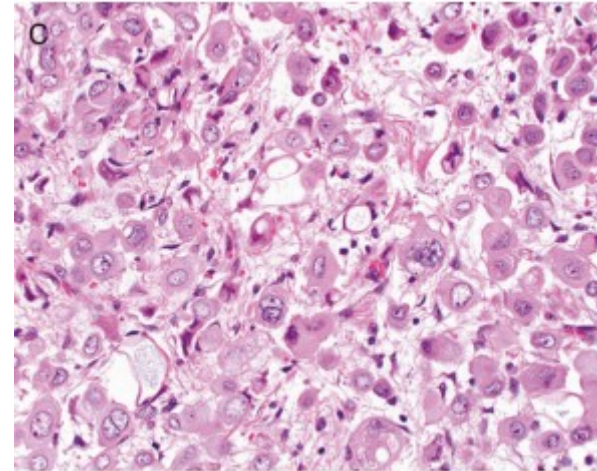
Merlin expression in normal/reactive mesothelial cells is characterized by weak to moderate membranous staining reaction and/or as a granular cytoplasmic positivity.



IHC and NGS agreement; 77%

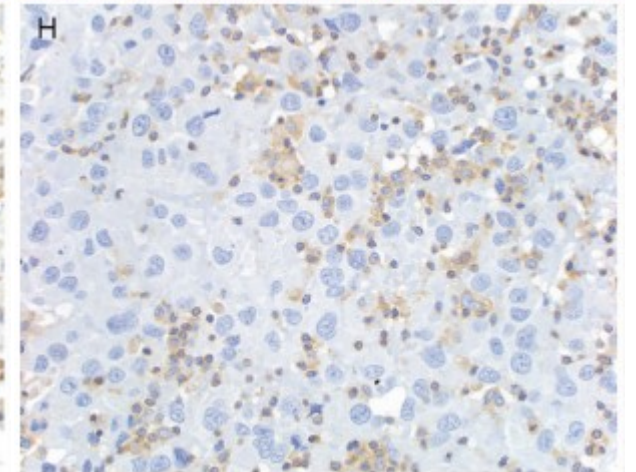
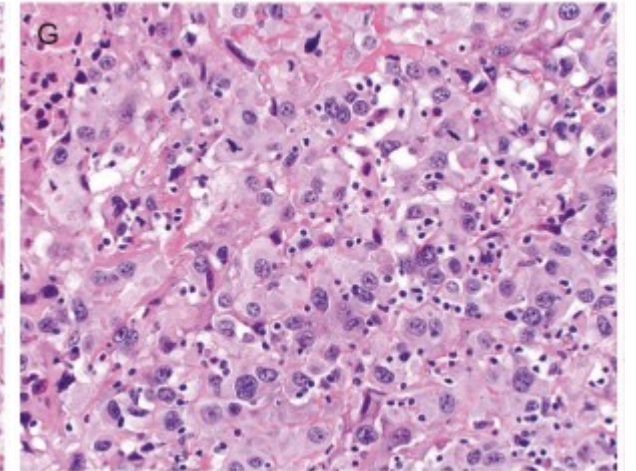
Clone D1D8, Cell Signaling.

Pleural mesothelioma 1



No NF2 gene mutation

Pleural mesothelioma 2



NF2 gene mutation (+/-50%)



## The antibody graveyard – Neuroendocrine markers - general

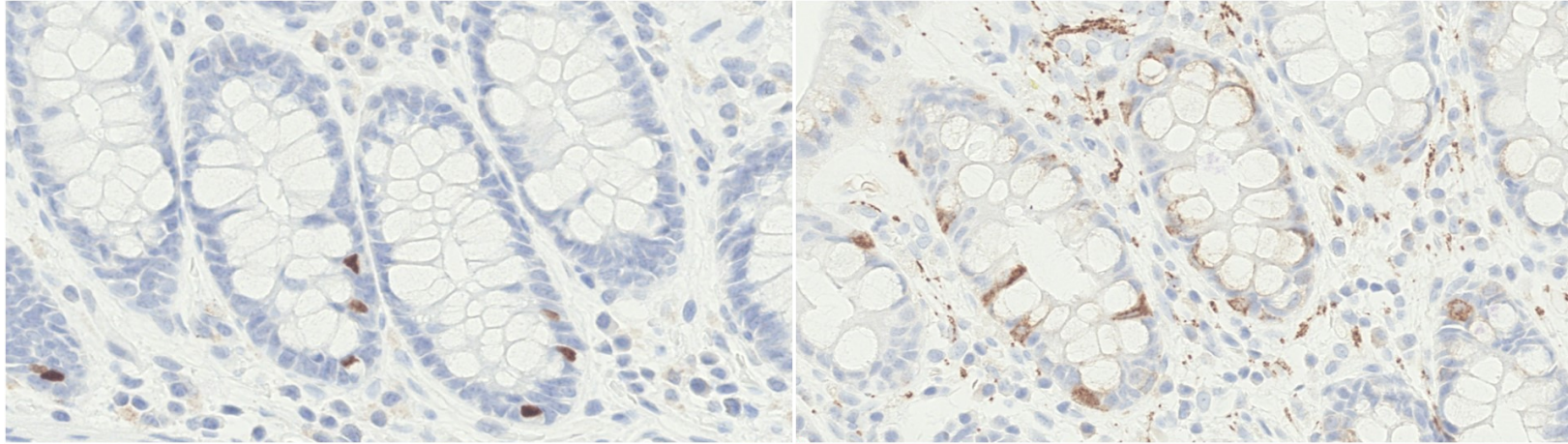
|                       | To stay    | Sensitivity    | Comments                                                                                                                |
|-----------------------|------------|----------------|-------------------------------------------------------------------------------------------------------------------------|
| Chromogranin A        | Yes        | 50-85%         | Traditionally the most specific NE marker                                                                               |
| Synaptophysin         | Yes        | 60-90%         | Superior sensitivity compared to CGA, but less specific                                                                 |
| NSE                   | No         | 60-70%         | "Non Specific Enolase" instead of Neuron Specific Enolase                                                               |
| CD56                  | ?          | 70-90%         | Preferred by many pathologists due to increased sensitivity, but unspecific....                                         |
| INSM1*                | "New"      | 85-95%         | Insulinoma-associated protein 1                                                                                         |
| <i>ISL1**</i>         | <i>New</i> | <i>15-100%</i> | <i>Islet-1 - High expression in NET from pancreas and lower intestinal tract – Negative or low in upper gastric NET</i> |
| <i>Secretagonin**</i> | <i>New</i> | <i>55-85%</i>  | <i>Generally broadly expressed in NET/NEC</i>                                                                           |

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

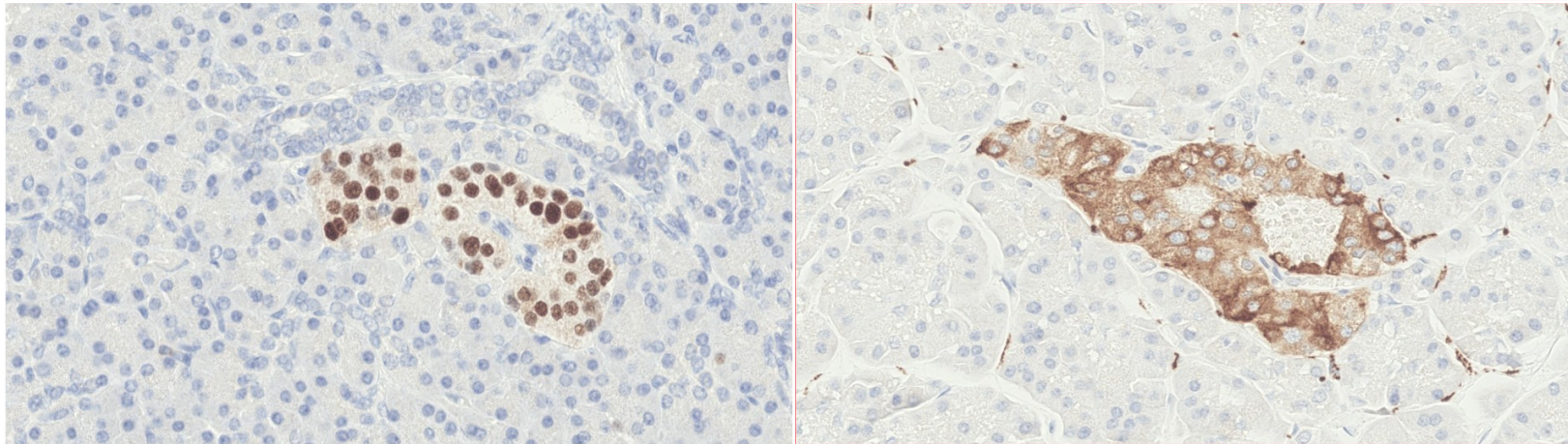
\*\*Second-Generation Neuroendocrine Immunohistochemical Markers: Reflections from Clinical Implementation  
Juhlin C. C.; Biology 2021, 10, 874. <https://doi.org/10.3390/biology10090874>

## The antibody graveyard – Neuroendocrine markers - general

Colon



Pancreas



INSM1

SYP

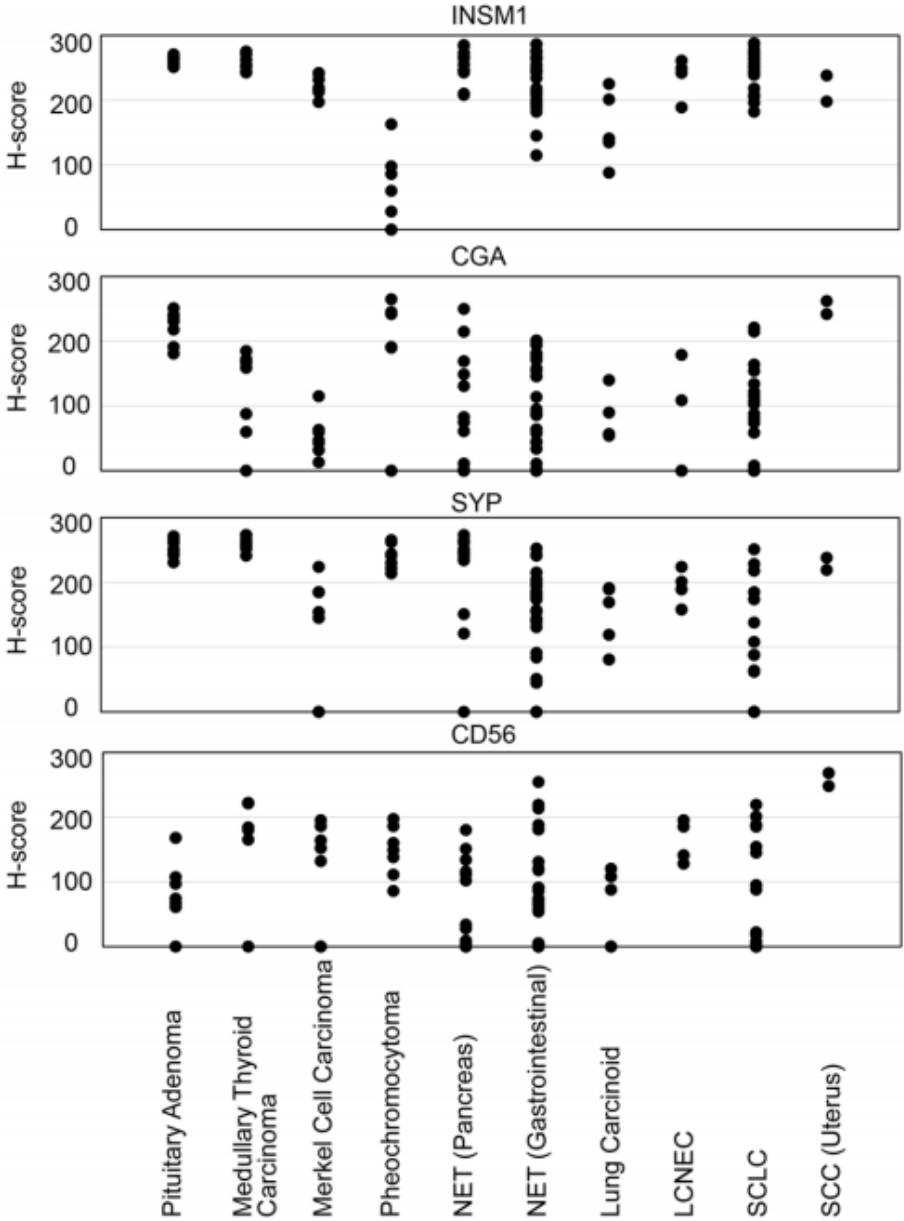
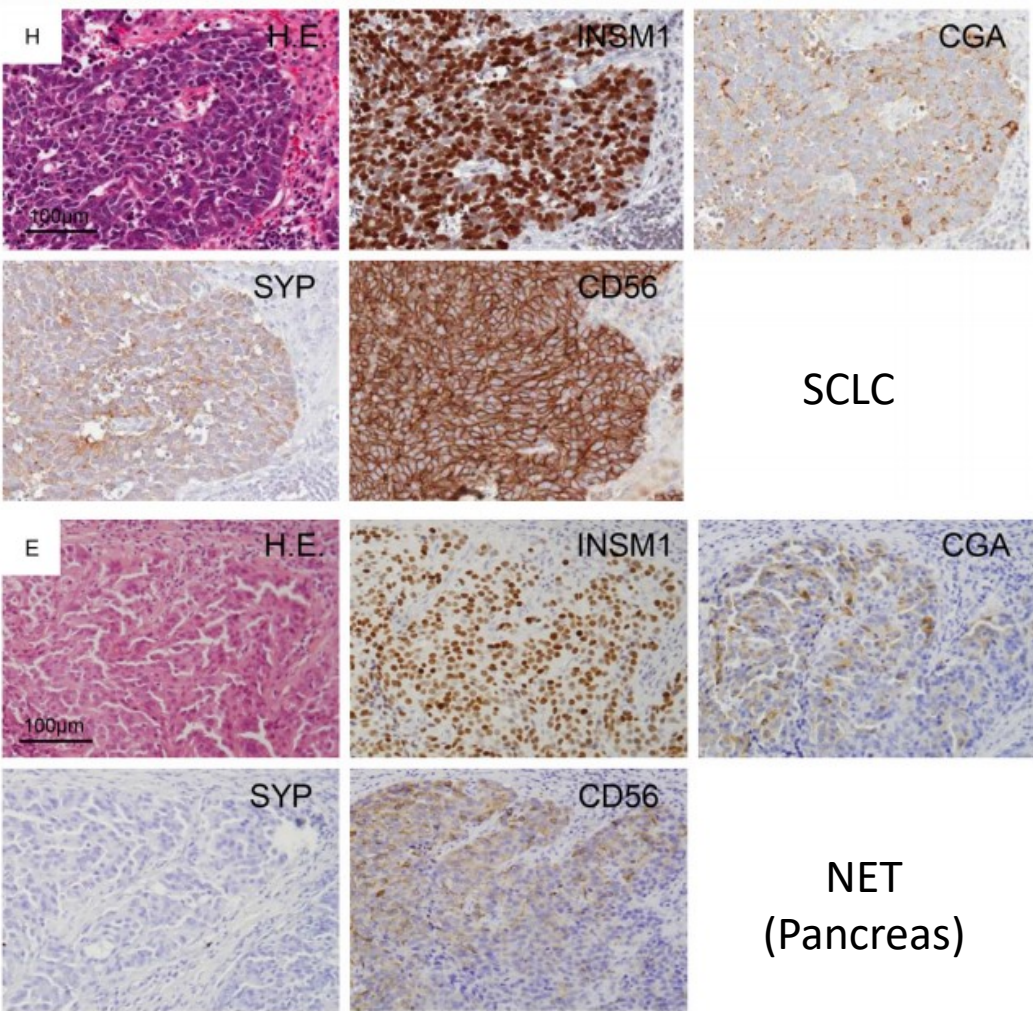
Insulinoma-associated protein 1 (INSM1) is a transcription factor that has recently emerged as a useful diagnostic marker of NE differentiation. INSM1 expression has been tightly coupled to NE differentiation in normal and neoplastic tissues across a wide range of anatomic sites including pancreas, gastrointestinal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

Clone A-8, Santa Cruz  
Most cited –

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



# The antibody graveyard – Neuroendocrine markers



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



# The antibody graveyard – Neuroendocrine markers

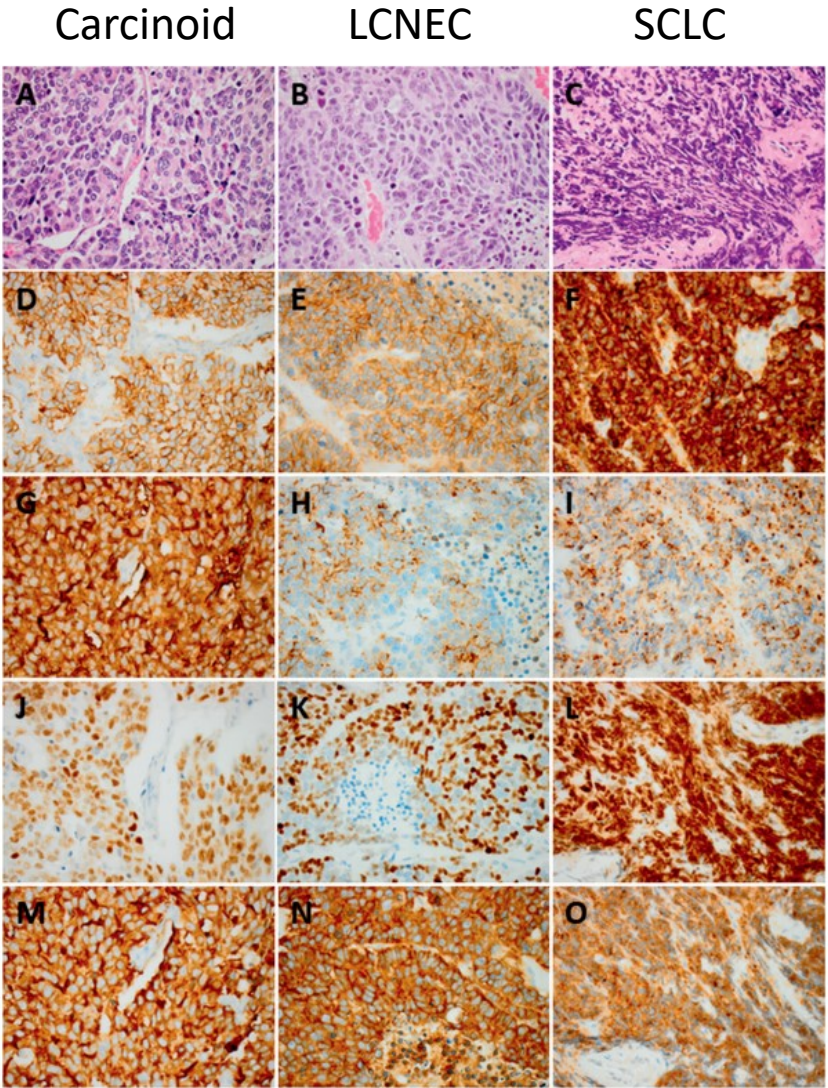
| Table 1. Staining Specifications |         |                                           |          |              |                                   |                          |
|----------------------------------|---------|-------------------------------------------|----------|--------------|-----------------------------------|--------------------------|
| Antibody                         | Clone   | Vendor                                    | Dilution | Pretreatment | Control Tissue                    | Location                 |
| CD56                             | 123C3   | Agilent/Dako (Glostrup, Denmark)          | 1:50     | CC1 + Amp    | Appendix, tonsil, liver           | Predominantly membranous |
| Chromogranin A                   | LK2H10  | Cell Marque (Rocklin, California)         | 1:50     | CC2          | Pancreas, small intestine, tonsil | Cytoplasmic              |
| INSM1                            | A-8     | Santa Cruz Biotechnology (Dallas, Texas)  | 1:100    | CC1          | Pancreas, small intestine         | Nuclear                  |
| Synaptophysin                    | MRQ-40* | 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.



**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]).



# The antibody graveyard – Neuroendocrine markers

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

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

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

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

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

# The antibody graveyard – Neuroendocrine markers



Journal of  
*Molecular Pathology*



*Review*

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

Renato Rocha <sup>1</sup> and Rui Henrique <sup>2,3,4,\*</sup>

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

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

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

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



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

## Conclusion

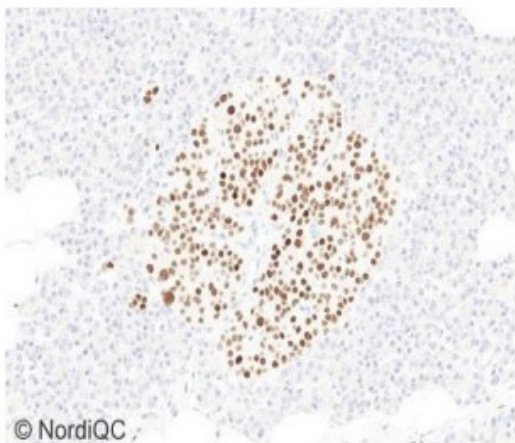
The rmAb clone **MRQ-70** was the most successful Ab for the demonstration of INSM1. As concentrated (conc.) format within a laboratory developed assay, optimal results were obtained on all four main stainer platforms. Efficient HIER in an alkaline buffer and carefully calibrated primary Ab together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

Table 1a. Overall results for INSM1, run 71

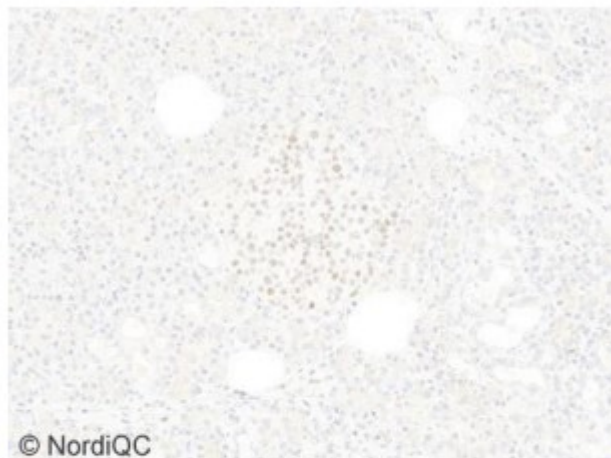
|                         | n   | Optimal | Good | Borderline | Poor | Suff. <sup>1</sup> | OR <sup>2</sup> |
|-------------------------|-----|---------|------|------------|------|--------------------|-----------------|
| Concentrated antibodies | 104 | 39      | 31   | 20         | 14   | 68%                | 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.



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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. <sup>1</sup> | OR <sup>2</sup> |
|-----------------------------------|-----|-------------------------|---------|------|------------|------|--------------------|-----------------|
| mAb clone <b>A-8</b>              | 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 <b>BSB-123</b>          | 3   | Bio SB                  | -       | 3    | -          | -    | -                  | -               |
|                                   | 1   | LS Bio                  | -       | 1    | -          | -    | -                  | -               |
| rmAb clone <b>MSVA-456R</b>       | 1   | MS Validated Antibodies | -       | 1    | -          | -    | -                  | -               |
| rmAb clone <b>BP6240</b>          | 1   | Biolyx Biotechnology    | 1       | -    | -          | -    | -                  | -               |
| rmAb clone <b>EPR23199-37-6-1</b> | 1   | Abcam                   | -       | -    | 1          | -    | -                  | -               |
| rmAb clone <b>MRQ-70</b>          | 27  | Cell Marque             | 19      | 6    | 2          | -    | 93%                | 70%             |
| rmAb clone <b>QR116</b>           | 1   | Quartett                | -       | -    | -          | 1    | -                  | -               |
| rmAb clone <b>RBT-INSM1</b>       | 2   | Bio SB                  | -       | 1    | 1          | -    | -                  | -               |
| rmAb clone <b>ZR395</b>           | 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 seems to be the clone to go-to.....!

A-8 night and day..... Not reproducible!!!!

Commentary

Biology 2021, 10, 874

6 of 9

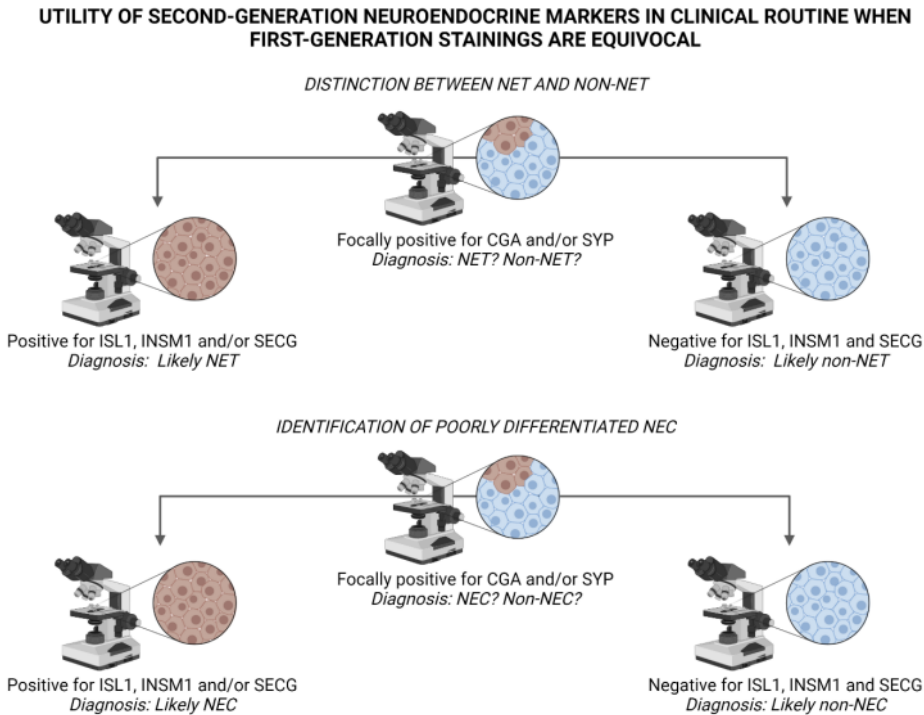
## Second-Generation Neuroendocrine Immunohistochemical Markers: Reflections from Clinical Implementation

Carl Christofer Juhlin <sup>1,2</sup>

**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: Secretagoin; PPGL: Pheochromocytoma/paraganglioma.

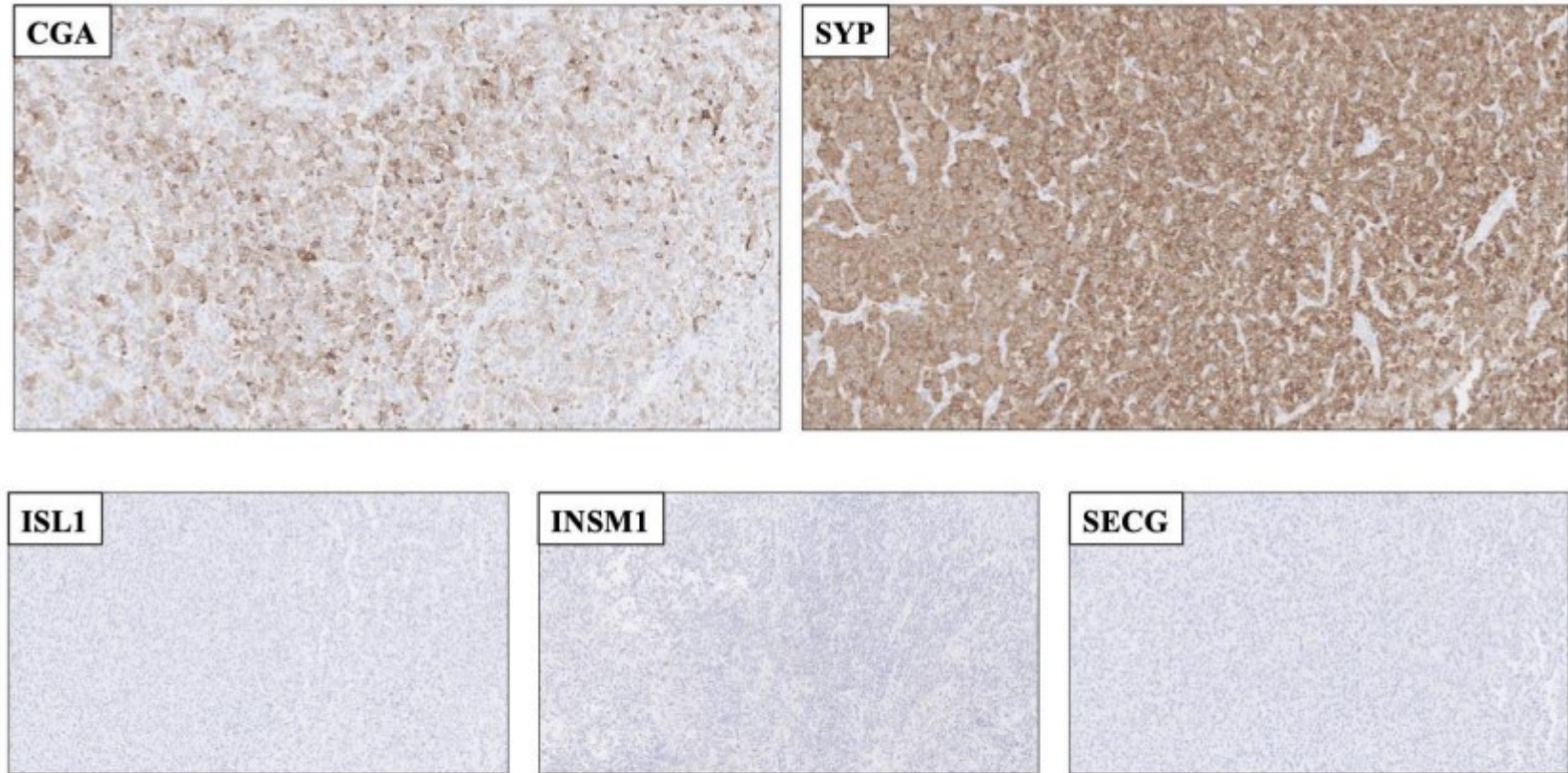




## The antibody graveyard – Neuroendocrine markers

CGA and SYP  
alone may be  
seen in many non-  
NEN

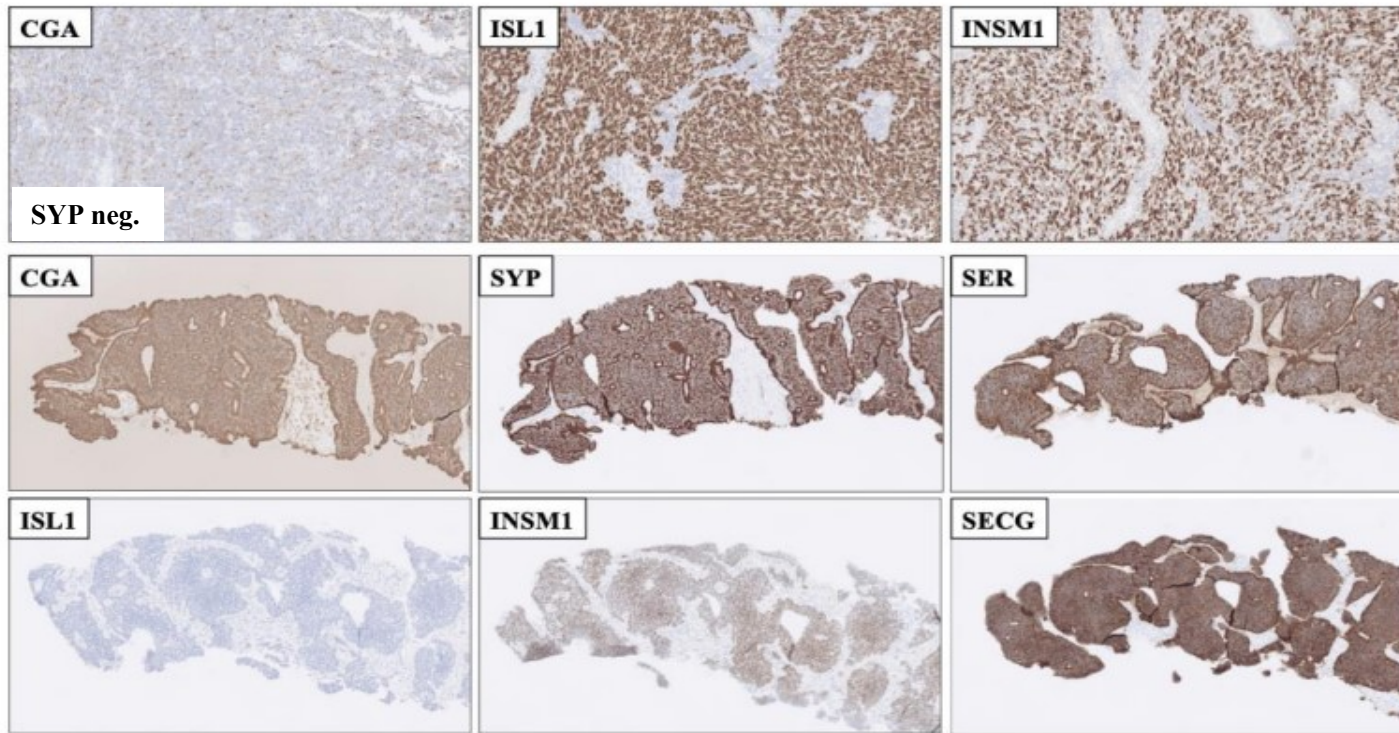
Addition of INSM1  
and ISL1 enhance  
specificity of NEN



**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 Secretagogen (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).



## The antibody graveyard – Neuroendocrine markers



**Figure 3.** Immunohistochemical stainings displaying advantages of employing second-generation neuroendocrine markers compared to using first-generation markers alone. **Top row:** Resected cerebellar metastasis of a poorly differentiated neuroendocrine carcinoma (NEC) of unknown primary with only focal Chromogranin A (CGA) staining. The metastasis was diffusely positive for nuclear ISL LIM Homeobox 1 (ISL1) and INSM Transcriptional Repressor 1 (INSM1). Thus, the second-generation markers helped pinpoint this lesion as neuroendocrine. **Middle and bottom rows:** Core needle biopsy of a well-differentiated neuroendocrine tumor, metastatic to liver. First-generation CGA and Synaptophysin (SYP) were both positive, as was serotonin, but the second-generation marker ISL1 was negative, adjoined by INSM1 and SEGC positivity. This profile (ISL negative, INSM1 and SECG positive) is indicative of a small-intestinal neuroendocrine tumor, which was subsequently found on imaging.

The inclusion of second-generation neuroendocrine markers could

(1) increase the likelihood of correctly pinpoint NETs when a non-NET is among the potential differential diagnoses

2) identify poorly differentiated pancreatic and pulmonary NECs exhibiting absent/focal CGA and SYP immunoreactivity

(3) give important clues regarding the origin of metastatic cases when a primary tumor has not been established through radiology and/or clinical examinations.



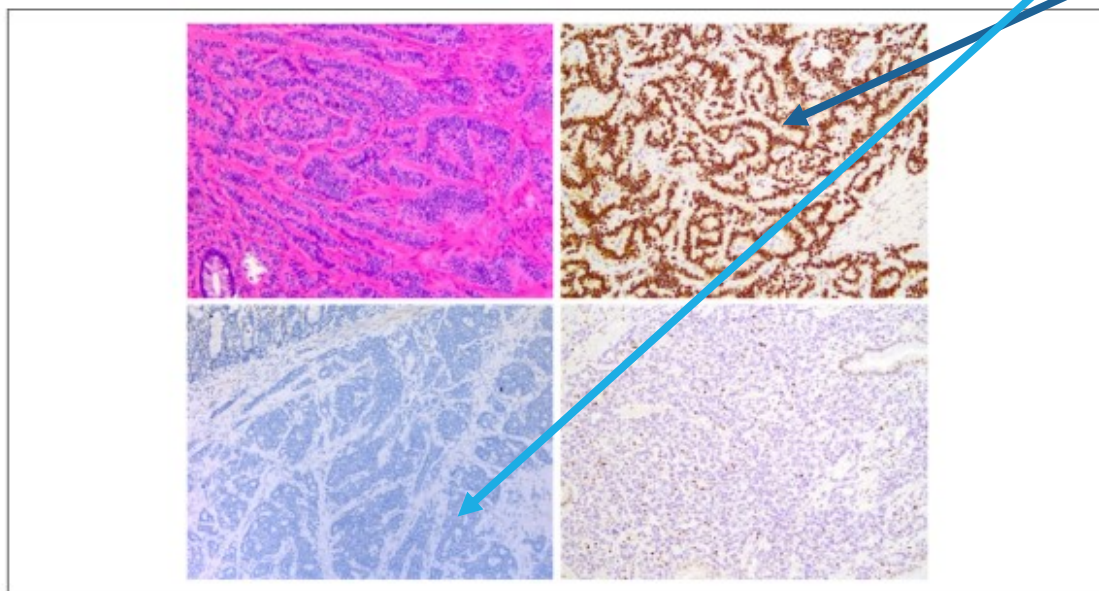
# The antibody graveyard – Neuroendocrine markers

Original Article

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

Yan Xiang, MD<sup>1</sup> , Faizan Malik, MD<sup>2</sup> , and Paul J. Zhang, MD<sup>2</sup>

International Journal of Surgical Pathology  
2023, Vol. 31(7) 1294–1301  
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DOI: 10.1177/10668969231157319  
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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                 |
| Non-NET     | GU neuroendocrine carcinoma                             | 7           | 2       | 0         | 1         | 4         | 71                 |
|             | Parathyroid adenoma                                     | 7           | 7       | 0         | 0         | 0         | 0                  |
|             | 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

# The antibody graveyard – Neuroendocrine markers

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

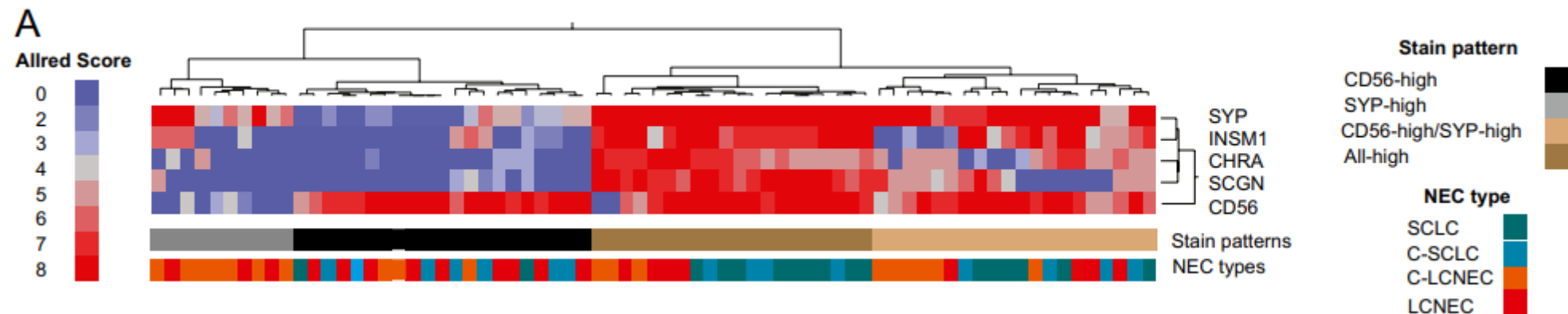
Yigit Baykara<sup>1</sup> · Ying Xiao<sup>1</sup> · Dongfang Yang<sup>1</sup> · Evgeny Yakirevich<sup>1</sup> · Sara Maleki<sup>1</sup> · Maria Garcia-Moliner<sup>1</sup> · Li Juan Wang<sup>1</sup> · Chiung-Kuei Huang<sup>2</sup> · Shaolei Lu<sup>1</sup>

Received: 23 November 2021 / Revised: 22 February 2022 / Accepted: 14 March 2022 / Published online: 31 March 2022  
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The sensitivity of SCGN was no better than the other established neuroendocrine markers based on all NECs combined or LCNECs/c-LCNECs only.

However, the sensitivity of SCGN (71%) was higher than chromogranin A (68%) for SCLCs/cSCLCs only. The average proportion of SCGN positive tumor cells was 8% higher than chromogranin A (22% versus 14%,  $P=0.0332$ ) in all NECs and 18% higher for SCLC and c-SCLC cases only (32% versus 13%,  $P=0.0054$ ).

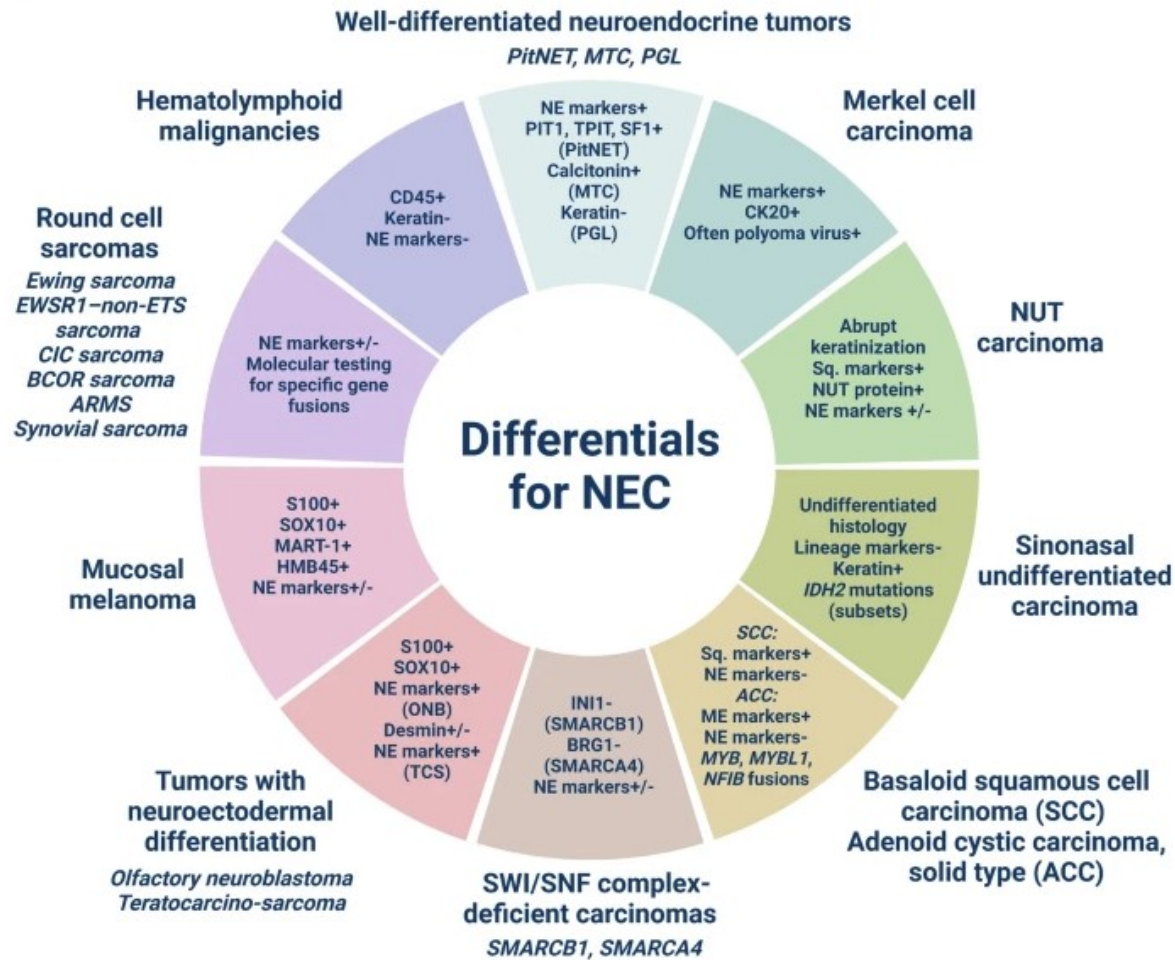
The above data showed that SCGN could be used as a supplemental neuroendocrine marker to diagnose SCLC.





# The antibody graveyard – Neuroendocrine markers

Fig. 2



The "core NET/NEC" IHC markers

CGA

SYP

INSM1

In doubt then ISL1

Secretagonin still to be evaluated more

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

|             | To stay | Sensitivity | Comments                                                                                                                             |
|-------------|---------|-------------|--------------------------------------------------------------------------------------------------------------------------------------|
| GCDFP15     | Yes     | 50-70%      | Highly specific for breast carcinoma                                                                                                 |
| Mammaglobin | Yes     | 40-60%      | Highly specific for breast carcinoma                                                                                                 |
| ER          | No      | 80%         | Moderate to highly sensitive but not specific                                                                                        |
| GATA3       | Yes     | 90-95%      | Highly sensitive for ER+ breast carcinoma – 20-60% positivity in TNBC and metaplastic type<br>Low specificity – “a selective marker” |
| TNBC        |         |             |                                                                                                                                      |
| SOX10       | ?       | 40-60%      | Moderately sensitive and specific (obs melanoma)                                                                                     |
| TRPS1*      | Yes     | 80-98%      | Highly sensitive and relatively specific for TNBC                                                                                    |

\* Trichorhinophalangeal syndrome type 1 (TRPS1) gene



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

Table 1 TRPS1 and GATA3 expression in breast cancers.

| Breast carcinoma |                | Negative | Positive |              |           | 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.

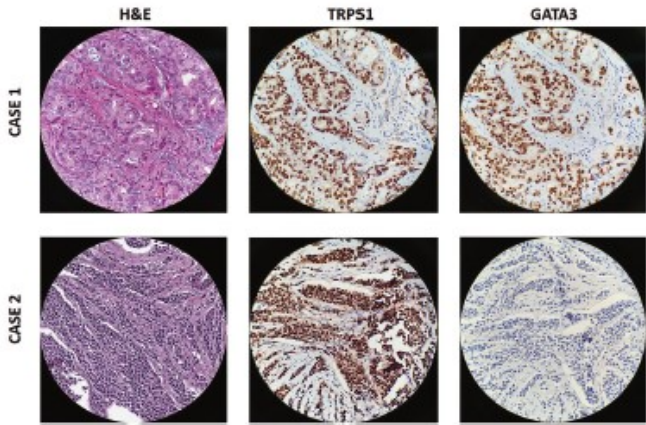
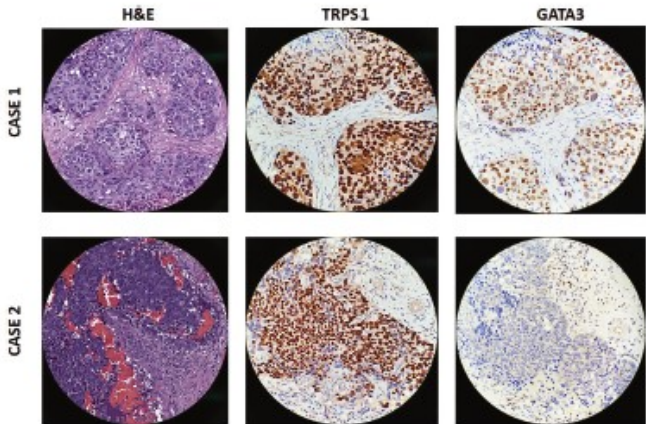
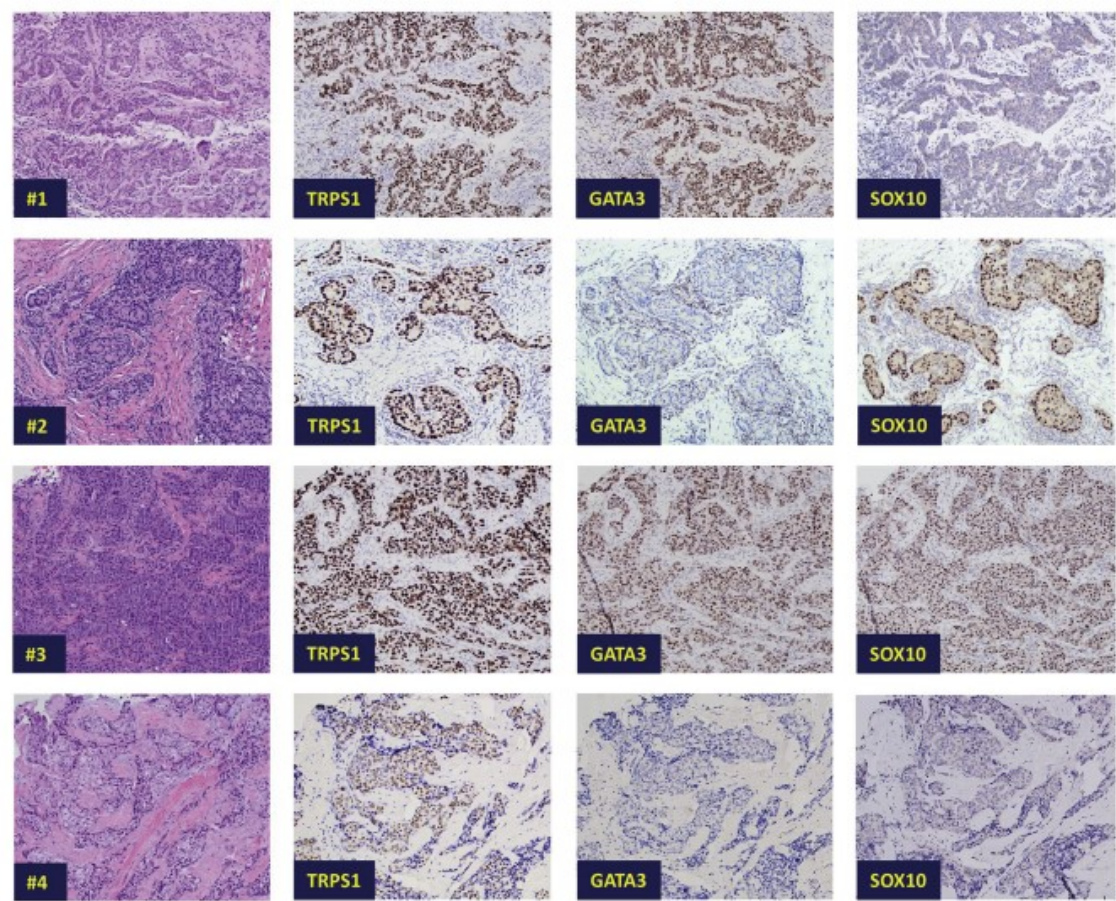


Fig. 4 TRPS1 and GATA3 expression in representative nonmetaplastic TNBC cases. Case 1 shows a poorly differentiated carcinoma with high expression of TRPS1 and intermediate to high expression of GATA3. Case 2 shows a poorly differentiated carcinoma with high expression of TRPS1 and negative GATA3.



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).

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



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

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

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

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

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

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

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

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

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



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

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

| TMA and tumors                                | Condition 1: TRPS1, pH 9.0<br>(Epitope Retrieval Solution<br>2, 1:1000), +ve/total (%) | Condition 2: TRPS1, pH 6.0<br>(Epitope Retrieval Solution<br>1, 1:300), +ve/total (%) | Condition 1 TRPS1<br>H-score, median (range;<br>IQR on positive cases) | Condition 2 TRPS1<br>H-score, median (range;<br>IQR on positive cases) |
|-----------------------------------------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------|
| Breast carcinoma (TNBC,<br>including ER-low)  | 123/138 (89)                                                                           | 117/138 (85)                                                                          | 230 (1-300; 130-270)                                                   | 170 (1-300; 100-225)                                                   |
| Breast carcinoma (HR+/<br>ERBB2-)             | 51/53 (96)                                                                             | 48/53 (91)                                                                            | 180 (1-300; 105-240)                                                   | 140 (5-280; 85-200)                                                    |
| Breast carcinoma (HR+/<br>ERBB2+)             | 6/6 (100)                                                                              | 6/6 (100)                                                                             | 190 (110-290; 150-253)                                                 | 135 (80-280; 80-220)                                                   |
| Endometrial<br>endometrioid<br>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<br>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<br>tumors                   | 3/138 (2)                                                                              | 3/138 (2)                                                                             | 5 (5-5; N/A)                                                           | 2 (1-5; N/A)                                                           |
| Vulvar squamous cell<br>carcinoma             | 54/96 (56)                                                                             | 29/97 (30)                                                                            | 30 (1-140; 10-60)                                                      | 20 (1-120; 10-50)                                                      |
| Pancreatic ductal<br>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.

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

## Conclusions:

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

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

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

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



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

Birgit Truumees<sup>1</sup>, Mia Korsdal Stensballe<sup>2</sup>, Rasmus Røge<sup>1</sup>, Søren Nielsen<sup>1</sup>

**Affiliation:** <sup>1</sup>NordiQC, Department of Pathology, Aalborg University Hospital, Denmark, <sup>2</sup>Department of Pathology, Aalborg University Hospital, Denmark

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

### Background & Objective

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

### Methods

Five commercially available antibodies for TRPS1 were optimized on TMAs with a range of normal tissues and BCs. Subsequently each IHC assay was validated on TMAs comprising of 135 breast carcinomas (BC), including 64 TNBCs, and 74 various neoplasias. A positive cut-off at  $\geq 10\%$  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

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



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

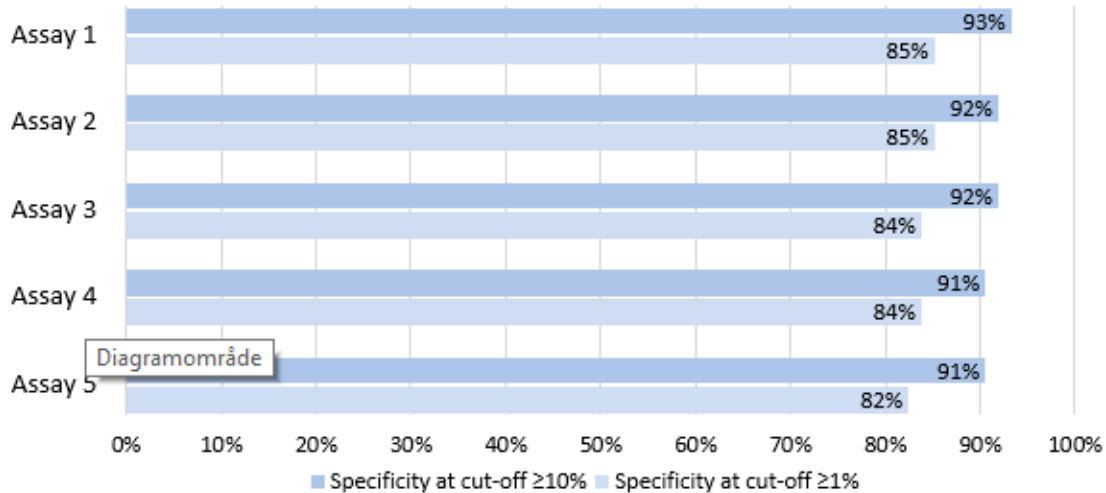
## Results

The five different and individually optimized IHC assays provided a fully comparable level of diagnostic sensitivity and specificity (see Table 2 and Graph 1). Positive TRPS1 staining in  $\geq 10\%$  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).

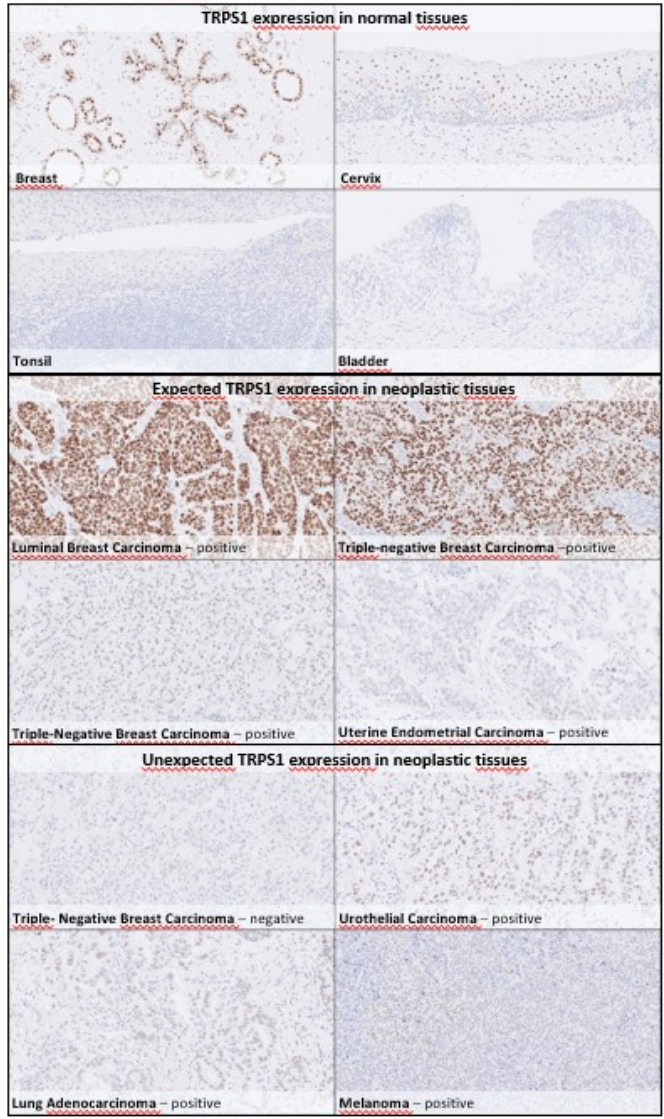
**Table 2.** Sensitivity of 5 TRPS1 IHC assays for detecting BCs

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

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



**Figure 1** TRPS1 staining patterns in normal and neoplastic tissues



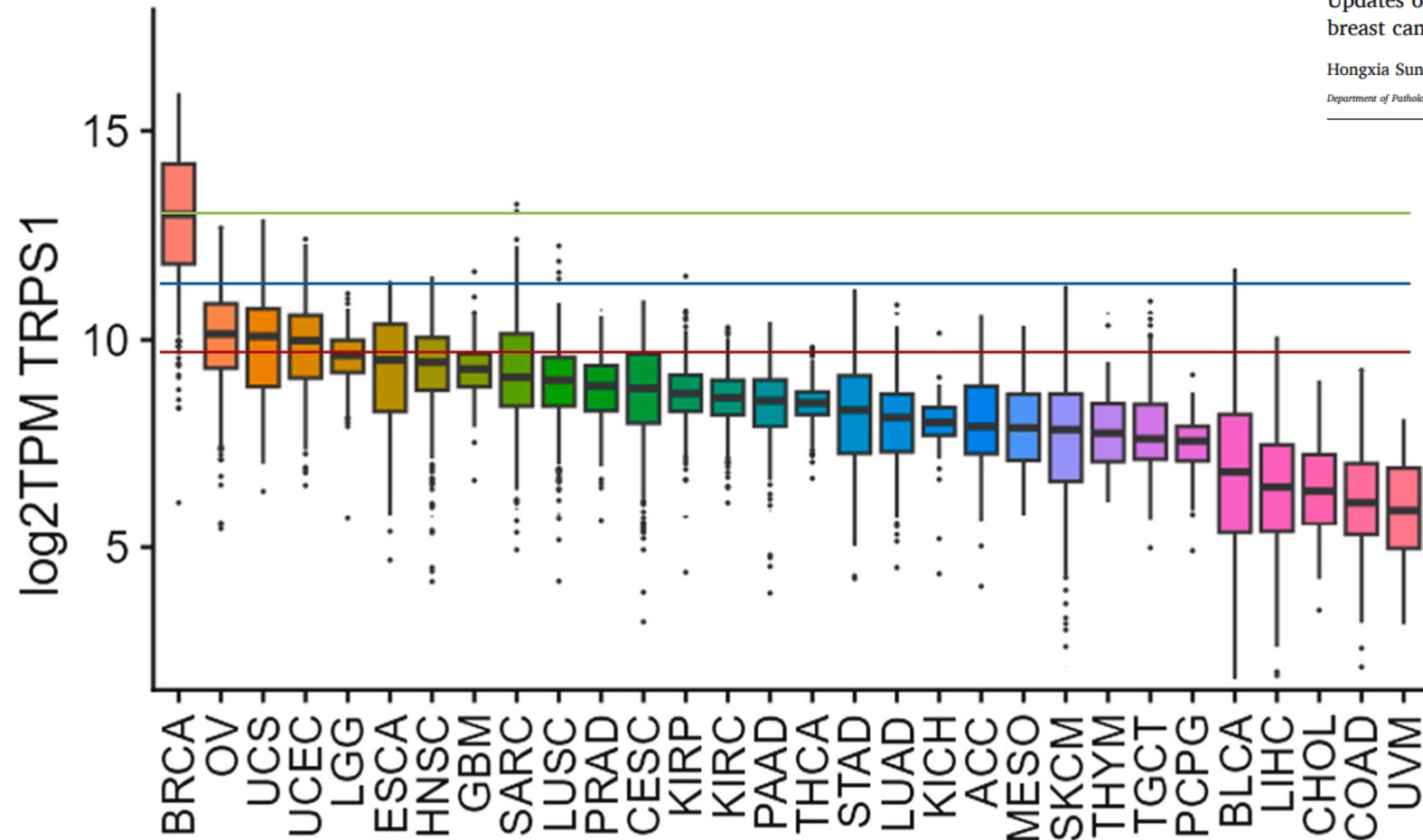
# Sensitivity and Specificity... what to choose???



Updates on utility of immunohistochemistry in diagnosis of metastatic breast cancer

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Neoplasias with TRPS expression levels

- B** B; protocol with low sensitivity and identify most BCs but will miss some
- A**
- C** A; protocol with balance on sensitivity and specificity
- C; protocol with high sensitivity and identify most BCs but will also label many-non-BCs



# The antibody graveyard – TRPS IHC



## 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. <sup>1</sup> | OR <sup>2</sup> |
|-------------------------|----|---------|------|------------|------|--------------------|-----------------|
| 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. <sup>1</sup> | OR <sup>2</sup> |
|-------------------------------|----|-------------------------|---------|------|------------|------|--------------------|-----------------|
| rmAb clone <b>TRPS1/8131R</b> | 1  | ImmunoLogic             | -       | 1    | -          | -    | -                  | -               |
| rmAb clone <b>BP6231</b>      | 1  | Biolynx Biotechnology   | 1       | -    | -          | -    | -                  | -               |
| rmAb clone <b>EP392</b>       | 16 | Bio SB                  | 7       | 4    | 3          | 2    | 69%                | 44%             |
|                               | 3  | Epitomics               | -       | -    | 2          | 1    | -                  | -               |
| rmAb clone <b>EPR16171</b>    | 3  | Abcam                   | 2       | -    | -          | 1    | -                  | -               |
|                               | 1  | Diagnostic BioSystems   | -       | -    | 1          | -    | -                  | -               |
| rmAb clone <b>MSVA-512R</b>   | 1  | MS Validated Antibodies | -       | 1    | -          | -    | -                  | -               |
| rmAb clone <b>ZR382</b>       | 17 | Zeta Corporation        | 12      | 4    | 1          | -    | 94%                | 71%             |
| <b>Polyclonal</b>             | 1  | Atlas Antibodies        | -       | 1    | -          | -    | -                  | -               |
|                               | 1  | Gennova                 | -       | -    | -          | 1    | -                  | -               |
|                               | 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. <sup>1</sup> | OR <sup>2</sup> |
|----------------------------|----|---------------------|---------|------|------------|------|--------------------|-----------------|
| rmAb clone <b>EP392</b>    | 7  | Bio SB              | 1       | 1    | 1          | 4    | 29%                | 14%             |
| <b>BSB-3790-x</b>          |    |                     |         |      |            |      |                    |                 |
| rmAb clone <b>EPR16171</b> | 1  | Gene Tech           | 1       | -    | -          | -    | -                  | -               |
| <b>GT245402</b>            |    |                     |         |      |            |      |                    |                 |
| rmAb clone <b>MXR063</b>   | 1  | Fuzhou Maixin       | -       | 1    | -          | -    | -                  | -               |
| <b>RMA-1141</b>            |    |                     |         |      |            |      |                    |                 |
| rmAb clone <b>ZR382</b>    | 1  | Sakura Finetek      | 1       | -    | -          | -    | -                  | -               |
| <b>8359-C010</b>           |    |                     |         |      |            |      |                    |                 |
| rmAb clone <b>ZR382</b>    | 18 | Zeta Corporation    | 9       | 8    | -          | 1    | 94%                | 50%             |
| <b>Z2673RP</b>             |    |                     |         |      |            |      |                    |                 |
| Ab clone <b>506I3C7</b>    | 1  | abcarta             | -       | -    | 1          | -    | -                  | -               |
| <b>PA588</b>               |    |                     |         |      |            |      |                    |                 |
| Ab clone <b>BY193</b>      | 1  | Bioin Biotechnology | 1       | -    | -          | -    | -                  | -               |
| <b>BFM-0560</b>            |    |                     |         |      |            |      |                    |                 |
| 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 challenge to set-up and implement a "new biomarker" in the lab.

**TABLE 5.** New Biomarkers Introduced in NordiQC, 2017-2021

| Target     | Pass Rate (%) | Participants |
|------------|---------------|--------------|
| BRAF       | 72            | 135          |
| C-MYC      | 57            | 172          |
| ERG        | 67            | 130          |
| NKX3.1     | 65            | 49           |
| SATB2      | 58            | 105          |
| SMAD4      | 42            | 52           |
| URO II/III | 45            | 66           |

How to optimize?

Which antibody to select?

How to verify/validate?

Reaction patterns?

Recommended tissue controls?

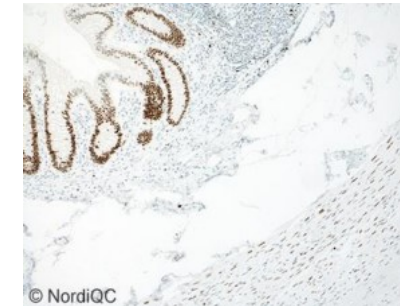
Clone EP281



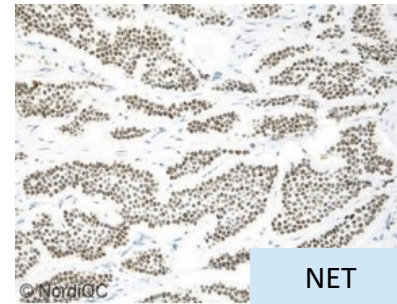
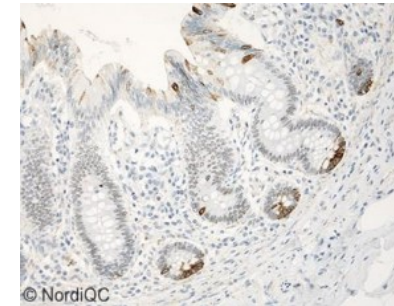
Colon

SATB2 run 58

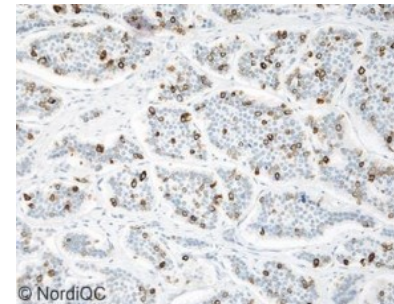
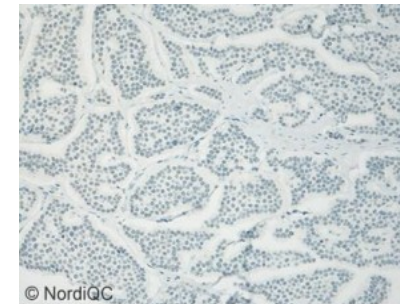
Clone CL0246



pAb 69995



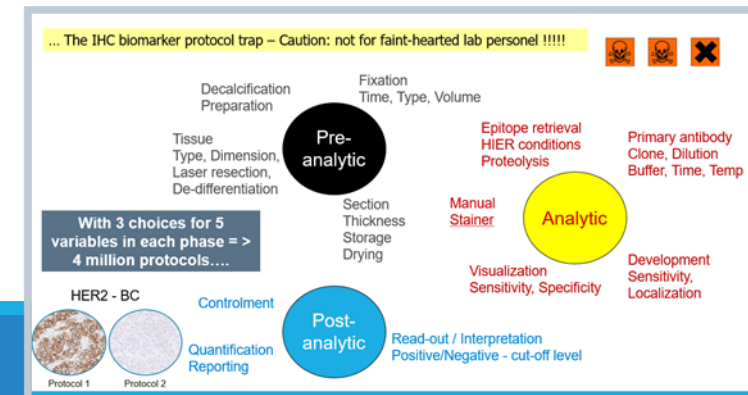
NET



Nielsen, Søren, Bzorek, Michael, Vyberg, Mogens, Røge, Rasmus.

Lessons Learned, Challenges Taken, and Actions Made for "Precision" Immunohistochemistry. Analysis and Perspectives From the NordiQC Proficiency Testing Program.

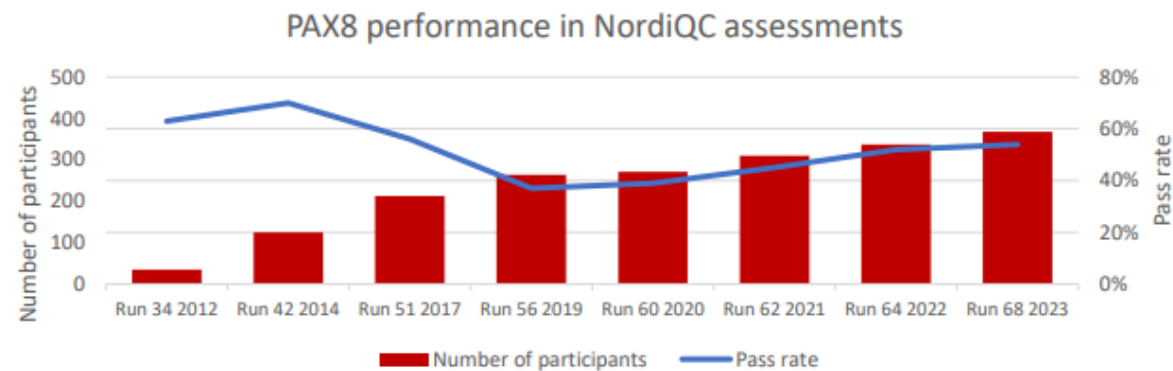
Applied Immunohistochemistry & Molecular Morphology ( ):10.1097/PAI.0000000000001071, October 5, 2022. | DOI: 10.1097







Graph 1. Proportion of sufficient results for PAX8 in the eight NordiQC runs performed

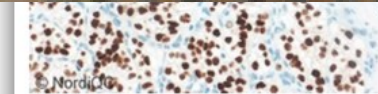
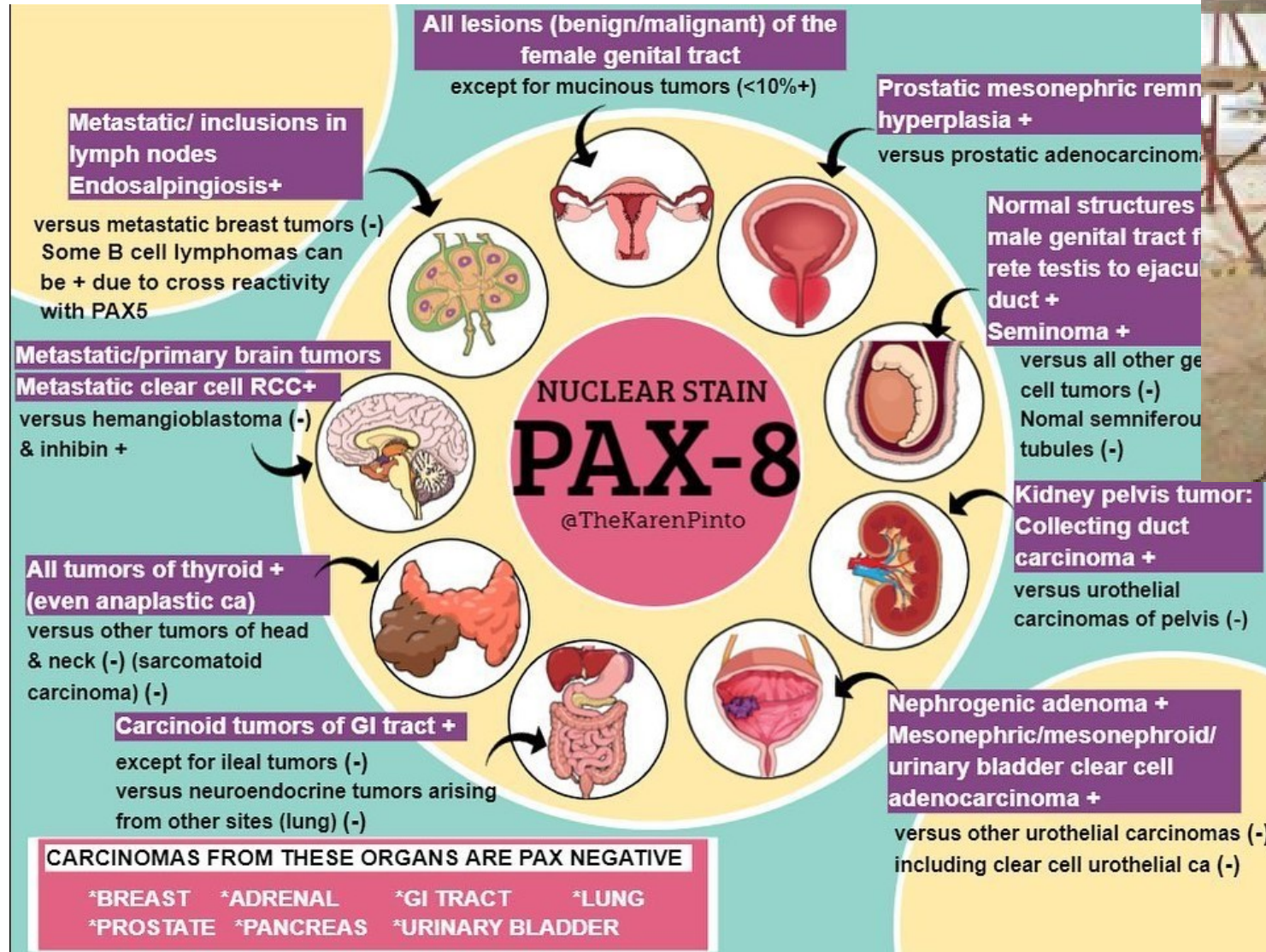


PAX8...

in 2035 a pass rate of 75%.....



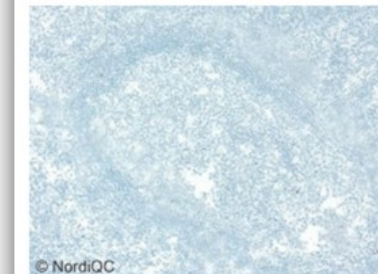
# The potential and challenge of PAX8.....



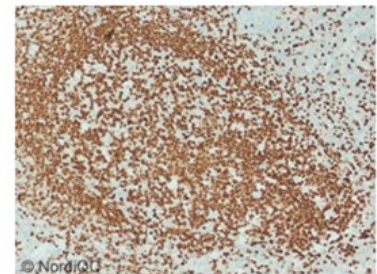
© NordiQC  
Fig. 4a x200  
Optimal PAX8 staining of the RCC using the same protocol as in Figs. 1a-3a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen. Compare with Fig. 4b.



© NordiQC  
Fig. 4b x200  
Insufficient PAX8 staining of the RCC using the same protocol as in Figs. 1b-3b. Only a faint nuclear staining is seen in the vast majority of neoplastic cells. Compare to Fig. 4a.



© NordiQC  
Fig. 5a x100  
PAX8 staining without PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1a-4a. The mAb clone SP348 do not cross-react with PAX5, leaving the B-cells unstained. Compare with Fig. 5b.



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Fig. 5b x100  
PAX8 staining with PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1b-4b. The mAb clone MRQ-50 cross-reacts with PAX5 resulting in nuclear staining reaction in virtually all B-cells. Compare with Fig. 5a.



# SOX17 being the new kid in the class

Received: 24 January 2023 | Revised: 20 March 2023 | Accepted: 23 March 2023  
DOI: 10.1002/ncy.22708

## ORIGINAL ARTICLE

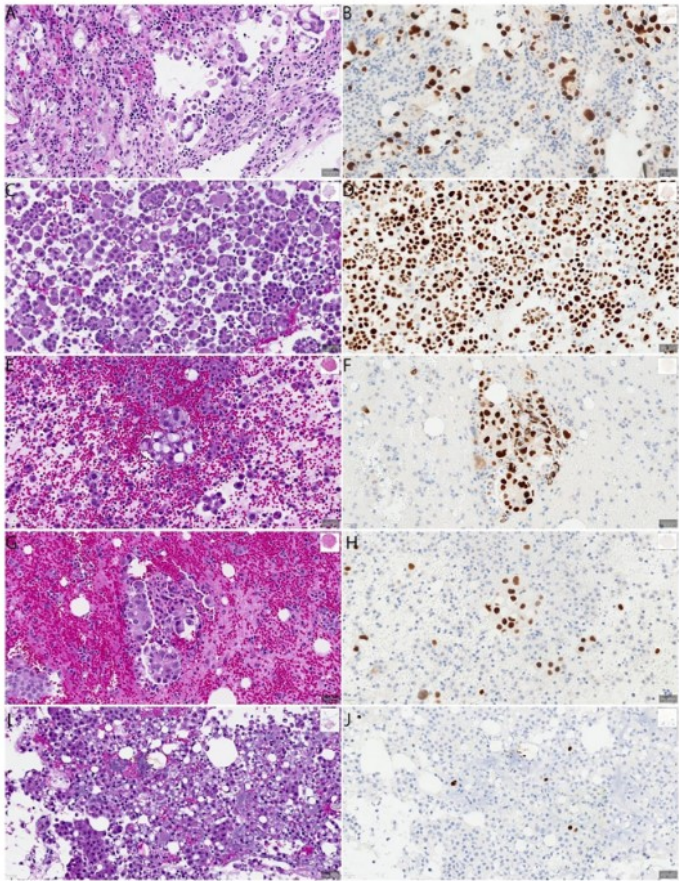
### SOX17 is a highly sensitive and specific marker for metastatic ovarian and endometrial carcinomas in cytology cell block specimens

Swati Satturwar MD<sup>1</sup> | Nada Shaker MD<sup>1</sup> | William Sinclair MD<sup>1</sup> |  
Rulong Shen MD<sup>1</sup> | Anil V. Parwani MD, PhD, MBA<sup>1</sup> | Qingqing Ding MD, PhD<sup>2</sup> |  
Zaibo Li MD, PhD<sup>1</sup>

**TABLE 2** SOX17 immunohistochemistry in metastatic gynecologic and nongynecologic carcinomas (*n* = 84).

|                                                      | SOX17 immunohistochemistry cases, No. (%) |                                 |                      | Intensity score |
|------------------------------------------------------|-------------------------------------------|---------------------------------|----------------------|-----------------|
|                                                      | Low positive (<10%)                       | Intermediate positive (10%–49%) | High positive (>50%) |                 |
| Ovarian high-grade serous carcinoma ( <i>n</i> = 24) | 0                                         | 0                               | 24/24 (100)          | 3               |
| Ovarian low-grade serous carcinoma ( <i>n</i> = 1)   | 0                                         | 0                               | 1/1 (100)            | 3               |
| Ovarian clear cell carcinoma ( <i>n</i> = 1)         | 0                                         | 0                               | 1/1 (100)            | 3               |
| Endometrial endometrioid carcinoma ( <i>n</i> = 1)   | 0                                         | 0                               | 1/1 (100)            | 3               |
| Endometrial serous carcinoma ( <i>n</i> = 2)         | 0                                         | 0                               | 2/2 (100)            | 3               |
| Total gynecologic carcinomas ( <i>n</i> = 29)        | 0                                         | 0                               | 29/29 (100)          | —               |
| Clear cell renal cell carcinoma ( <i>n</i> = 10)     | 0                                         | 0                               | 0                    | NA              |
| Papillary thyroid carcinoma ( <i>n</i> = 10)         | 1/10 (5)                                  | 0                               | 0                    | 2               |
| Colonic adenocarcinoma ( <i>n</i> = 11)              | 0                                         | 0                               | 0                    | NA              |
| Ductal adenocarcinoma of breast ( <i>n</i> = 10)     | 0                                         | 0                               | 0                    | NA              |
| Lung adenocarcinoma ( <i>n</i> = 10)                 | 0                                         | 0                               | 0                    | NA              |
| High-grade urothelial carcinoma ( <i>n</i> = 4)      | 0                                         | 0                               | 0                    | NA              |
| Total nongynecologic carcinomas                      | 1/55 (0.01)                               | 0                               | 0                    | —               |

rmAb clone EPR20684, Abcam



**FIGURE 1** SOX17 immunohistochemistry shows diffuse and strong nuclear expression in metastatic gynecologic carcinomas in cytology specimens. (A,B) High-grade ovarian serous carcinoma. (C,D) Low-grade ovarian serous carcinoma. (E,F) Clear cell carcinoma of the ovary. (G, H) Endometrial serous carcinoma. (I,J) Endometrial endometrioid carcinoma. Magnification ×400. Left panel are examples of metastatic gynecologic carcinomas and right panels are corresponding SOX17 IHC results.

# SOX17 being the new kid in the class

Mod Pathol 36 (2023) 100001

## MODERN PATHOLOGY

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

### Research Article

## SOX17: A Highly Sensitive and Specific Immunomarker for Ovarian and Endometrial Carcinomas

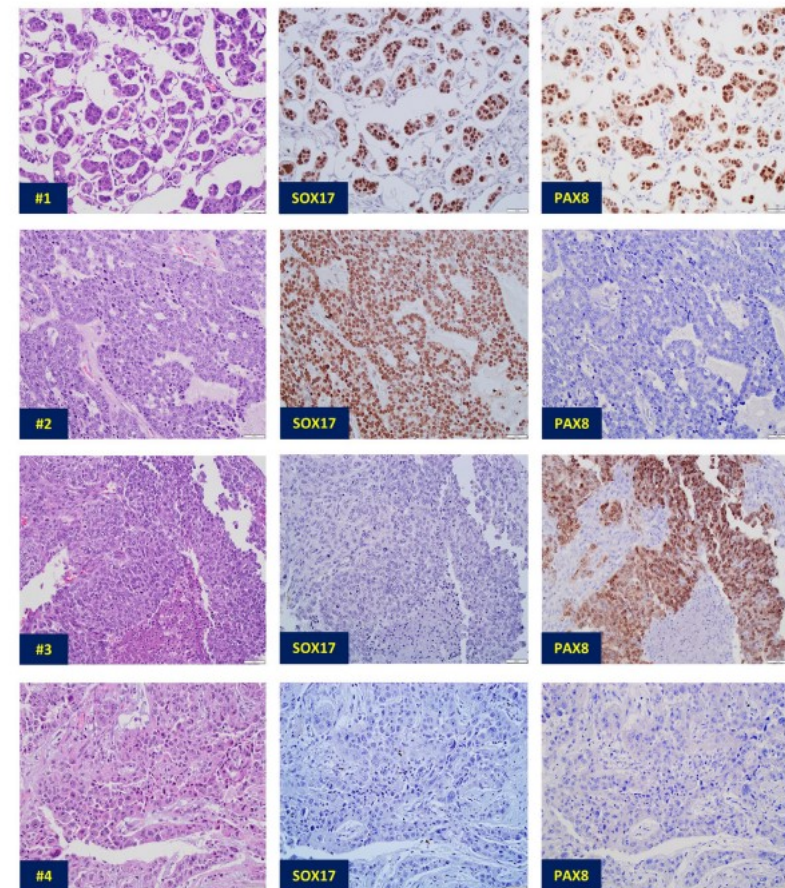
Xudong Zhang<sup>a</sup>, Jun Yao<sup>b</sup>, Na Niu<sup>a</sup>, Xiaoran Li<sup>a</sup>, Yan Liu<sup>a</sup>, Lei Huo<sup>a</sup>, Elizabeth D. Euscher<sup>a</sup>, Huamin Wang<sup>a,c</sup>, Diana Bell<sup>a</sup>, Anil K. Sood<sup>d</sup>, Guoliang Wang<sup>a</sup>, Barrett C. Lawson<sup>a</sup>, Preetha Ramalingam<sup>a</sup>, Anais Malpica<sup>a</sup>, Aysegul A. Sahin<sup>a</sup>, Qingqing Ding<sup>a,c,\*</sup>, Jinsong Liu<sup>a,b,\*</sup>

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**Table 1**

SOX17 and PAX8 expression in ovarian cancers (N = 327)

| Cancer type    | Negative  | Positive |              |             | Total |
|----------------|-----------|----------|--------------|-------------|-------|
|                |           | Low      | Intermediate | High        |       |
| SOX17          |           |          |              |             |       |
| Serous, total  | 7 (2.5%)  | 8 (3%)   | 42 (15%)     | 220 (79.5%) | 277   |
| Low grade      | 0         | 0        | 4            | 12          | 16    |
| High grade     | 7         | 8        | 38           | 208         | 261   |
| Mucinous       | 11 (100%) | 0        | 0            | 0           | 11    |
| Endometrioid   | 2 (10%)   | 0        | 3 (15%)      | 15 (75%)    | 20    |
| Clear cell     | 0         | 0        | 2 (25%)      | 6 (75%)     | 8     |
| Carcinosarcoma | 5 (45%)   | 0        | 0            | 6 (55%)     | 11    |
| PAX8           |           |          |              |             |       |
| Serous, total  | 9 (3%)    | 11 (4%)  | 51 (18%)     | 206 (74%)   | 277   |
| Low grade      | 0         | 0        | 5            | 11          | 16    |
| High grade     | 9         | 11       | 46           | 195         | 261   |
| Mucinous       | 8 (73%)   | 1 (9%)   | 1 (9%)       | 1 (9%)      | 11    |
| Endometrioid   | 2 (10%)   | 1 (5%)   | 6 (30%)      | 11 (55%)    | 20    |
| Clear cell     | 0         | 0        | 0            | 8 (100%)    | 8     |
| Carcinosarcoma | 5 (45%)   | 0        | 1 (9%)       | 5 (45%)     | 11    |



**Figure 2.** SOX17 and PAX8 expression (>200) in representative ovarian serous carcinomas. Case 1 shows a high expression of both SOX17 and PAX8. Case 2 shows a high expression of SOX17 and negative PAX8. Case 3 shows a high expression of PAX8 and negative SOX17. Case 4 shows negative SOX17 and PAX8.

**Table 2**

Comparison of SOX17 and PAX8 expression in ovarian serous carcinoma (N = 277)

|          | SOX17       |          | Total     |
|----------|-------------|----------|-----------|
|          | Positive    | Negative |           |
| PAX8     |             |          |           |
| Positive | 265         | 3        | 268 (97%) |
| Negative | 5           | 4        | 9 (3%)    |
| Total    | 270 (97.5%) | 7 (2.5%) | 277       |



# SOX17 being the new kid in the class

Mod Pathol 36 (2023) 100001

## MODERN PATHOLOGY

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### Research Article

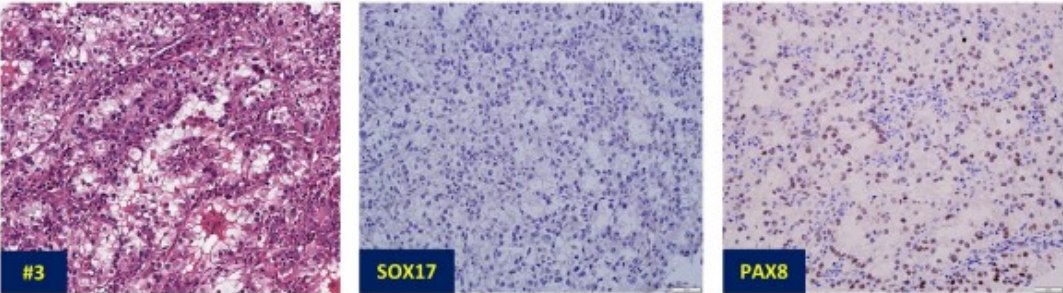
### SOX17: A Highly Sensitive and Specific Immunomarker for Ovarian and Endometrial Carcinomas

Xudong Zhang<sup>a</sup>, Jun Yao<sup>b</sup>, Na Niu<sup>a</sup>, Xiaoran Li<sup>a</sup>, Yan Liu<sup>a</sup>, Lei Huo<sup>a</sup>, Elizabeth D. Euscher<sup>a</sup>, Huamin Wang<sup>a,c</sup>, Diana Bell<sup>a</sup>, Anil K. Sood<sup>d</sup>, Guoliang Wang<sup>a</sup>, Barrett C. Lawson<sup>a</sup>, Preetha Ramalingam<sup>a</sup>, Anais Malpica<sup>a</sup>, Aysegul A. Sahin<sup>a</sup>, Qingqing Ding<sup>a,c,\*</sup>, Jinsong Liu<sup>a,b,\*</sup>

<sup>a</sup> Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas; <sup>b</sup> Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas; <sup>c</sup> Department of Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas; <sup>d</sup> Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas



## Renal clear cell carcinoma; SOX17 and PAX8



**Table 6**  
SOX17 expression in 1960 cases of malignant neoplasm from different organs

| Site        |                    | Negative   | Positive |              |           | Total |
|-------------|--------------------|------------|----------|--------------|-----------|-------|
|             |                    |            | Low      | Intermediate | High      |       |
| Ovary       | Multiple types     | 25 (8%)    | 8 (2%)   | 47 (14%)     | 247 (76%) | 327   |
| Endometrium | Multiple types     | 11 (12%)   | 0        | 17 (19%)     | 61 (69%)  | 89    |
| Cervix      | Adenocarcinoma     | 20 (80%)   | 1 (4%)   | 2 (8%)       | 2 (8%)    | 25    |
|             | Squamous           | 115 (100%) | 0        | 0            | 0         | 115   |
| Thyroid     | Multiple types     | 70 (100%)  | 0        | 0            | 0         | 70    |
| Kidney      | Multiple types     | 52 (100%)  | 0        | 0            | 0         | 52    |
|             | ER+                | 141 (100%) | 0        | 0            | 0         | 141   |
| Breast      | HER2+              | 111 (100%) | 0        | 0            | 0         | 111   |
|             | TNBC               | 112 (100%) | 0        | 0            | 0         | 112   |
| Lung        | Adenocarcinoma     | 193 (100%) | 0        | 0            | 0         | 193   |
|             | Squamous           | 88 (100%)  | 0        | 0            | 0         | 88    |
|             | Small cell         | 40 (100%)  | 0        | 0            | 0         | 40    |
|             | Undifferentiated   | 34 (100%)  | 0        | 0            | 0         | 34    |
| Mesothelium | Mesothelioma       | 35 (100%)  | 0        | 0            | 0         | 35    |
| Head/neck   | Salivary duct      | 77 (100%)  | 0        | 0            | 0         | 77    |
| Bladder     | Urothelial         | 89 (100%)  | 0        | 0            | 0         | 89    |
| Stomach     | Adenocarcinoma     | 138 (100%) | 0        | 0            | 0         | 138   |
| Colon       | Adenocarcinoma     | 70 (100%)  | 0        | 0            | 0         | 70    |
| Pancreas    | Adenocarcinoma     | 86 (100%)  | 0        | 0            | 0         | 86    |
| Liver       | HCC                | 60 (100%)  | 0        | 0            | 0         | 60    |
|             | Cholangiocarcinoma | 8 (100%)   | 0        | 0            | 0         | 8     |

ER, estrogen receptor; HCC, hepatocellular carcinoma; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

**Table 5**  
SOX17 and PAX8 expression in carcinomas of the thyroid (N = 70) and kidney (N = 52)

| Site    | Negative  | Positive |              |          | Total |
|---------|-----------|----------|--------------|----------|-------|
|         |           | Low      | Intermediate | High     |       |
| Thyroid |           |          |              |          |       |
| SOX17   | 70 (100%) | 0        | 0            | 0        | 70    |
| PAX8    | 10 (14%)  | 10 (14%) | 14 (20%)     | 36 (52%) | 70    |
| Kidney  |           |          |              |          |       |
| SOX17   | 52 (100%) | 0        | 0            | 0        | 52    |
| PAX8    | 8 (15%)   | 12 (23%) | 17 (33%)     | 15 (29%) | 52    |





## WHAT I SAY



## WHAT I THINK

