

Old tests – New challenges

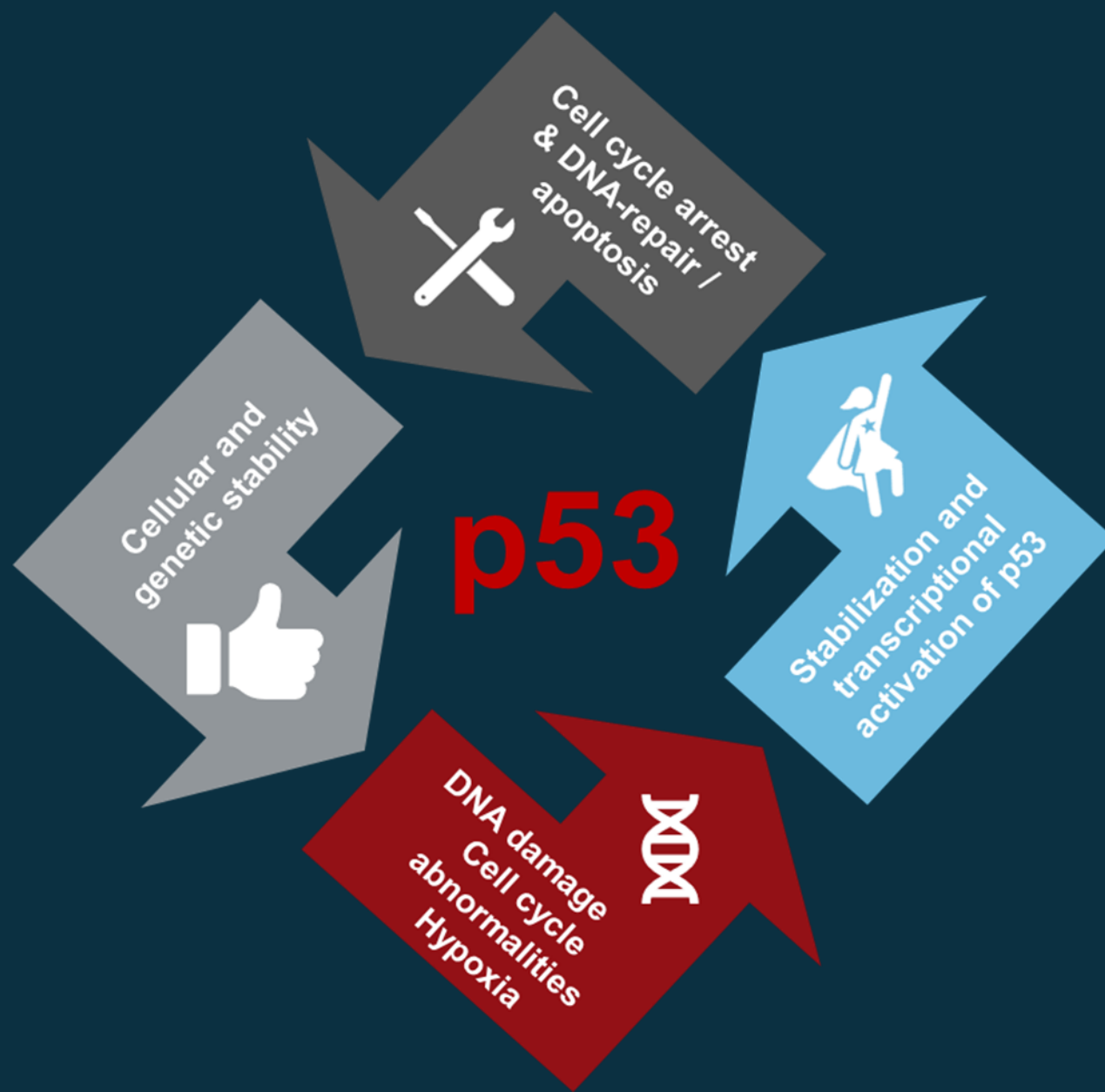
Kristi Bøgh Anderson MD, Scientific associate & PhD-fellow, NordiQC

kristi.anderson@rn.dk

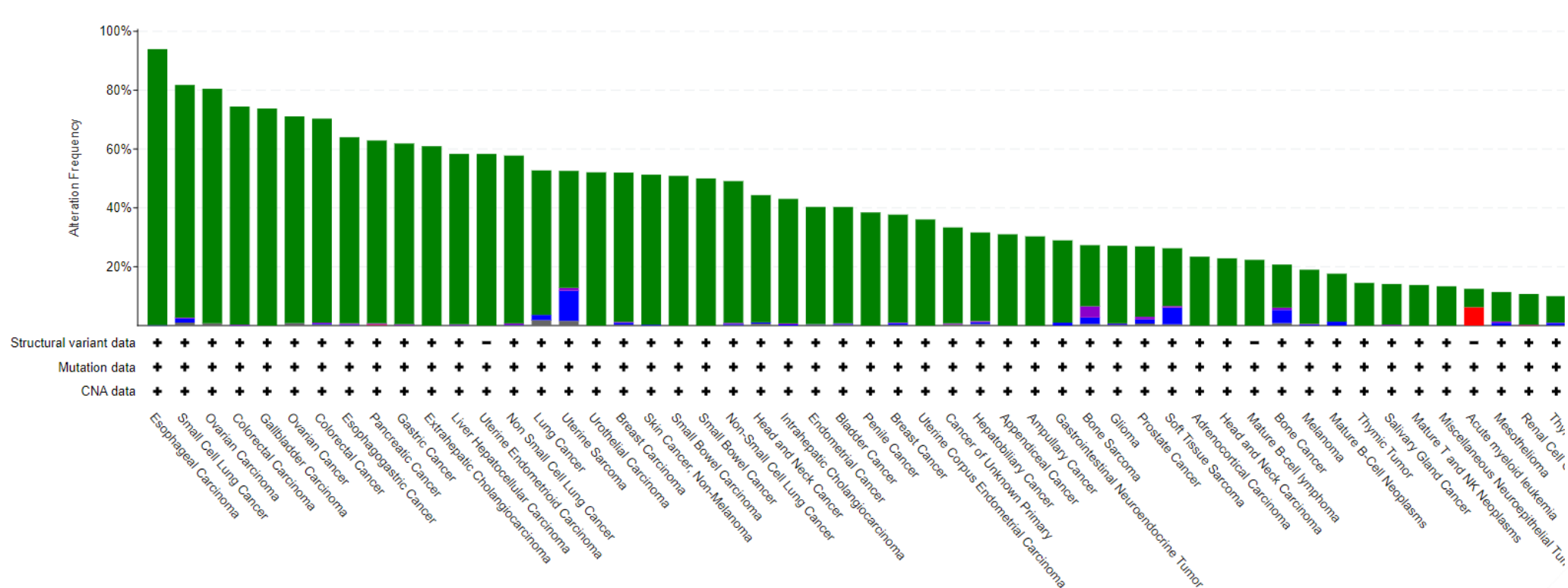
Søren Nielsen, Consultant, NordiQC

P53

When knowledge shifts, so must we



TP53 is mutated in 50% of all cancer



Increased expression of mutant forms of p53 oncogene in primary lung cancer

R. Iggo, MRCP^a · J. Bartek, MD^a · D. Lane, PhD^a · K. Gatter, MRCPath^b · A.L. Harris, FRCP^c · J. Bartek^d

[Affiliations & Notes](#) [Article Info](#)

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[Oncogene](#). 1990 Jun;5(6):893-9.

Genetic and immunochemical analysis of mutant p53 in human breast cancer cell lines

Abstract

Show Outline

Primary lung cancer samples of the J Bartek¹, R Iggo, J Gannon, D P Lane

[Proc Natl Acad Sci U S A](#). 1990 Oct;87(19):7555-9. doi: 10.1073/pnas.87.19.7555.

p53 mutations in colorectal cancer

N R Rodrigues¹, A Rowan, M E Smith, I B Kerr, W F Bodmer, J V Gannon, D P Lane

Affiliations [+ expand](#)

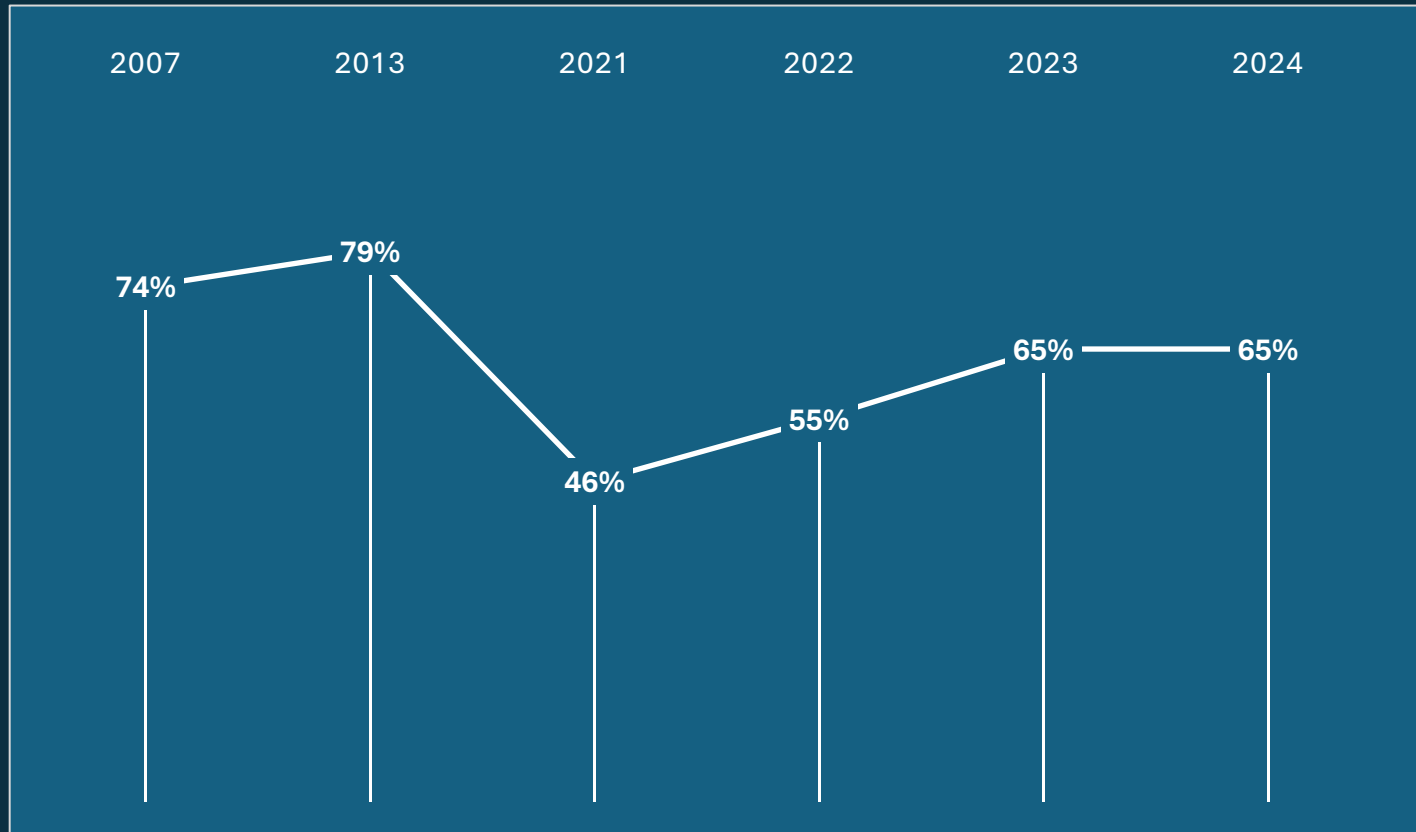
PMID: 1699228 PMCID: [PMC54786](#) DOI: [10.1073/pnas.87.19.7555](#)

Abstract

Immunohistological staining of primary colorectal carcinomas with antibodies specific to p53 demonstrated gross overexpression of the protein in approximately 50% of the malignant tumors examined. Benign adenomas were all negative for p53 overexpression. To determine the molecular

ne mutations were G
p53 gene is the most
al methods can

p53 Assessments in NordiQC



Optimized p53 IHC of TP53 mutations

Martin Köbel,^{1†} Anna M Piskunova,¹
Nitzan Rosenfeld,² Anne-Maria

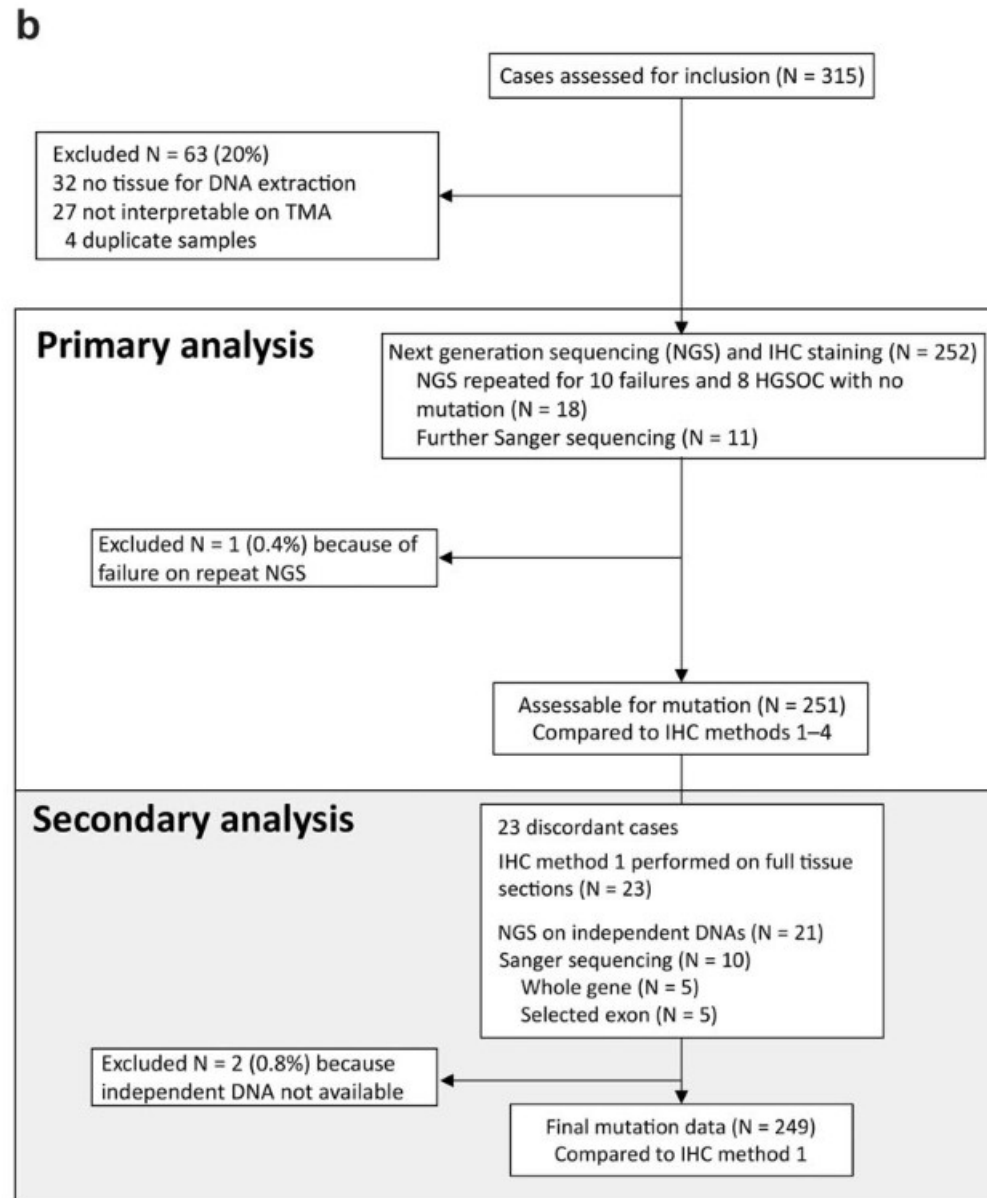
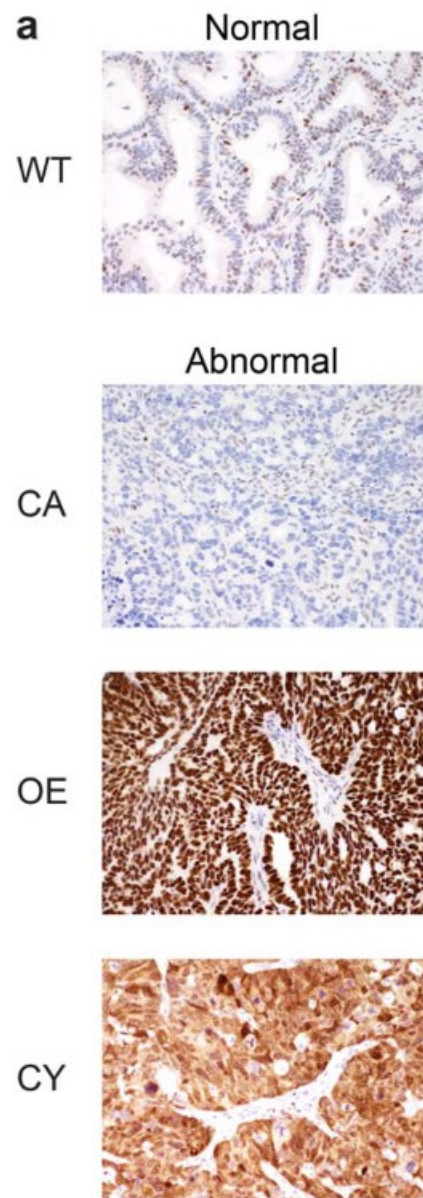
¹ Department of Pathology and Laboratory Medicine

² Cancer Research UK Cambridge Centre

³ Centre de recherche du Centre hospitalier de l'Université de Montréal

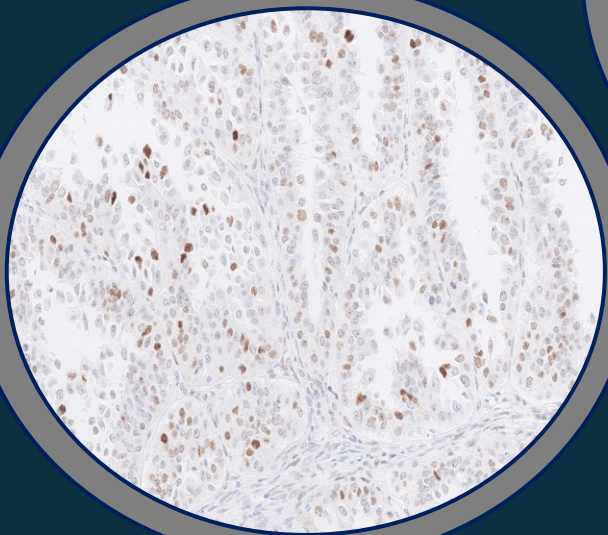
⁴ Institut du cancer de Montréal, Montréal, Québec

*Correspondence to: Dr. James D Brenton, Cancer Research UK
Genomics of Ovarian Cancer Laboratory, 4th Floor, Biomedical Research
Building, 76-78, Charlotte Avenue, Cambridge CB2 0RE, UK. e-mail: james.brenton@cr.uk

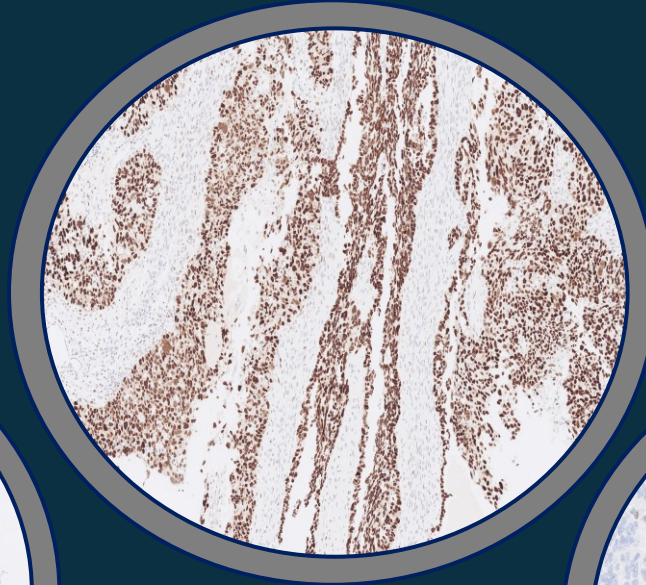


Overexpression

**Wild
type**

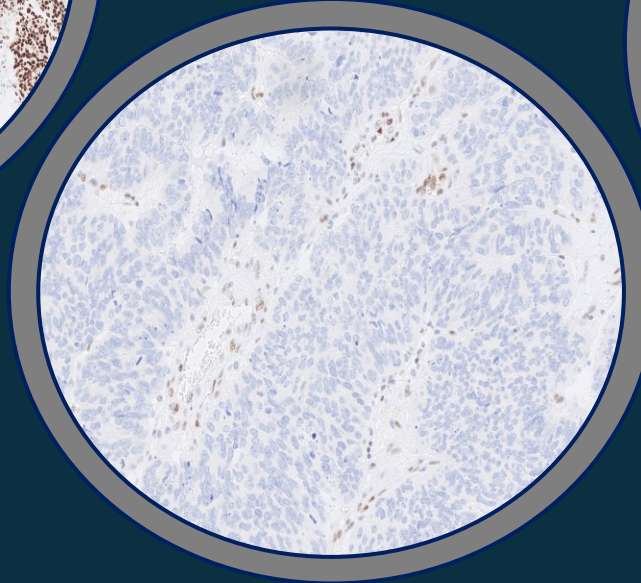


No TP53 mutation



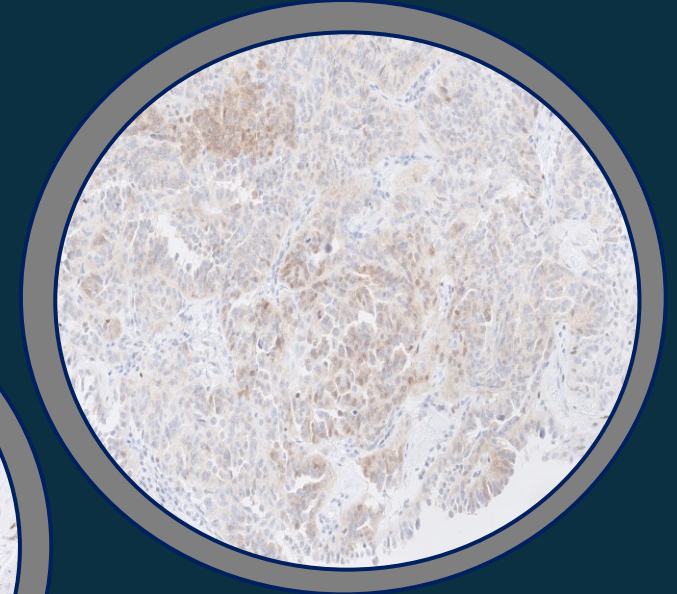
TP53 missense
mutation

0-phenotype

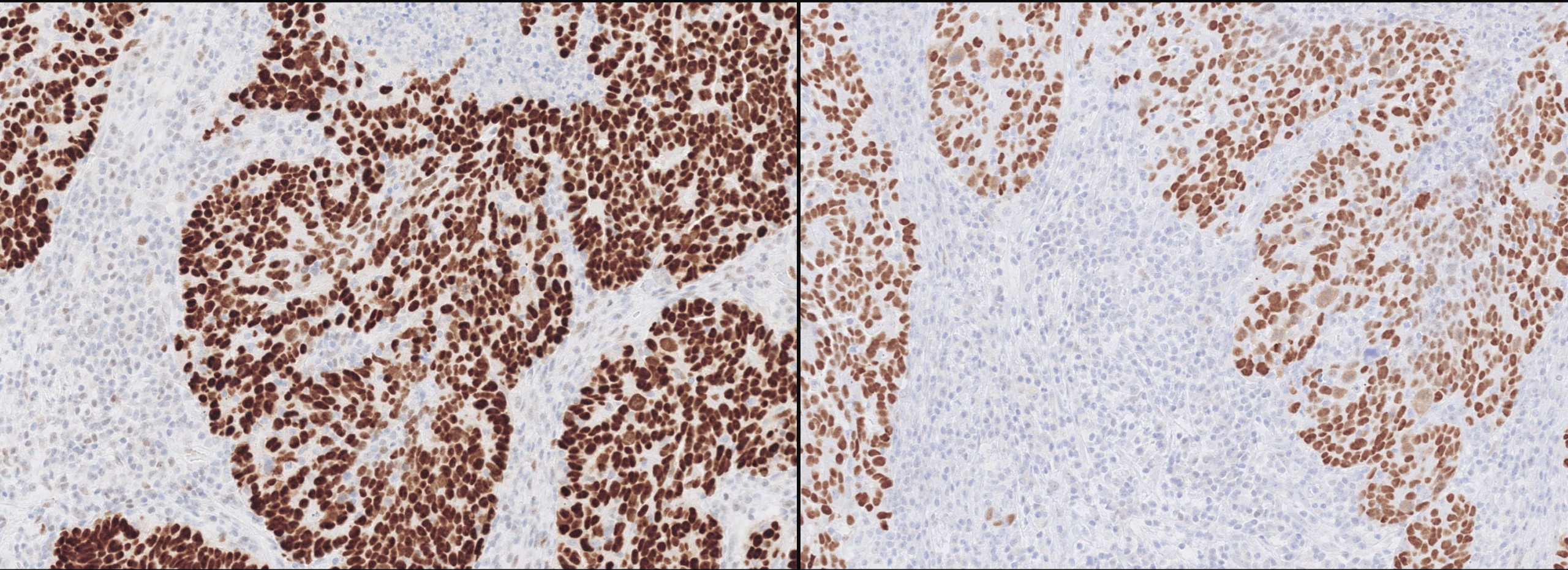


TP53 mutations resulting in
absent p53 protein

Cytoplasmatic p53



TP53 mutations affecting the
nuclear localization domain or
the nuclear export signal



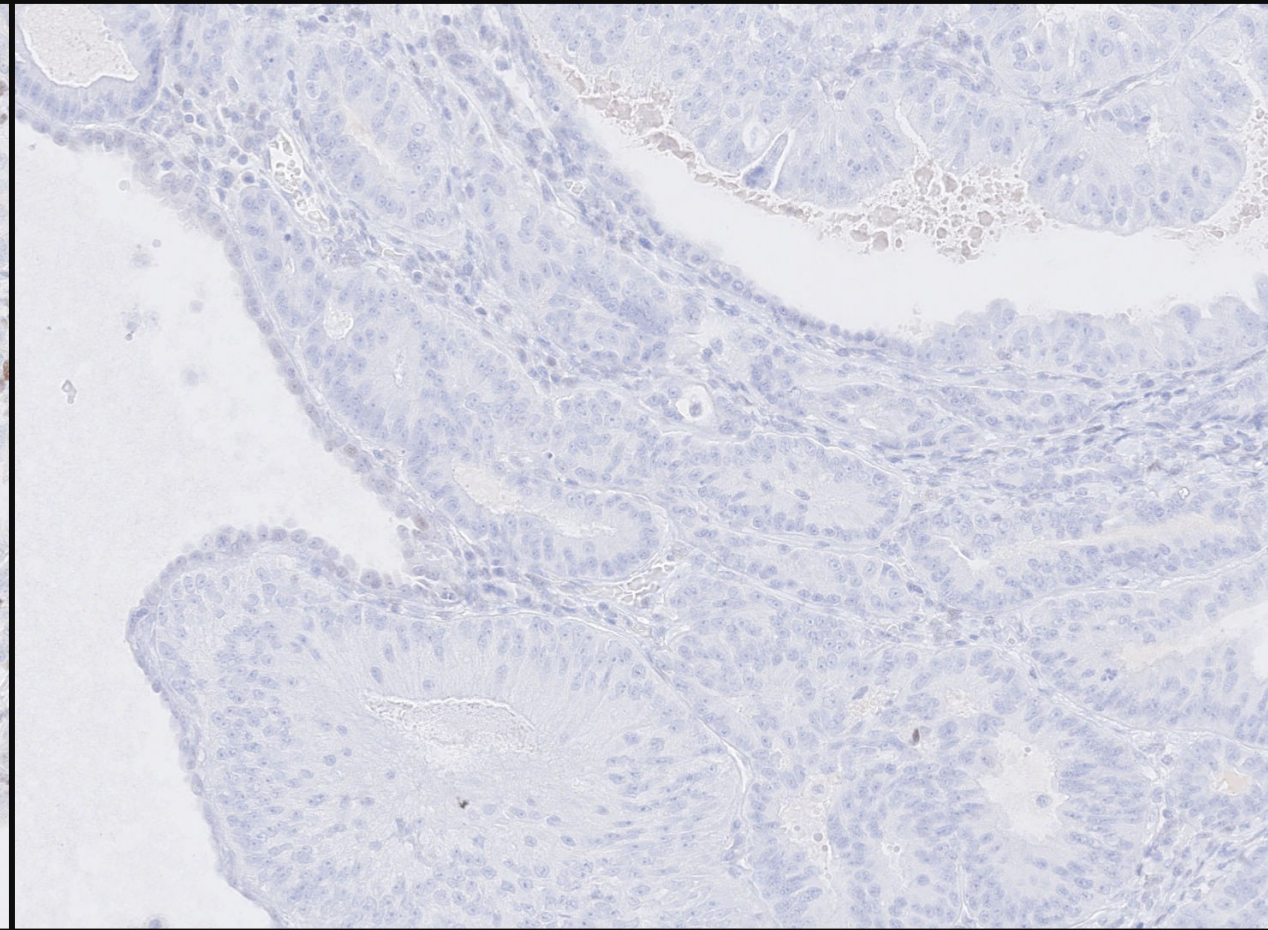
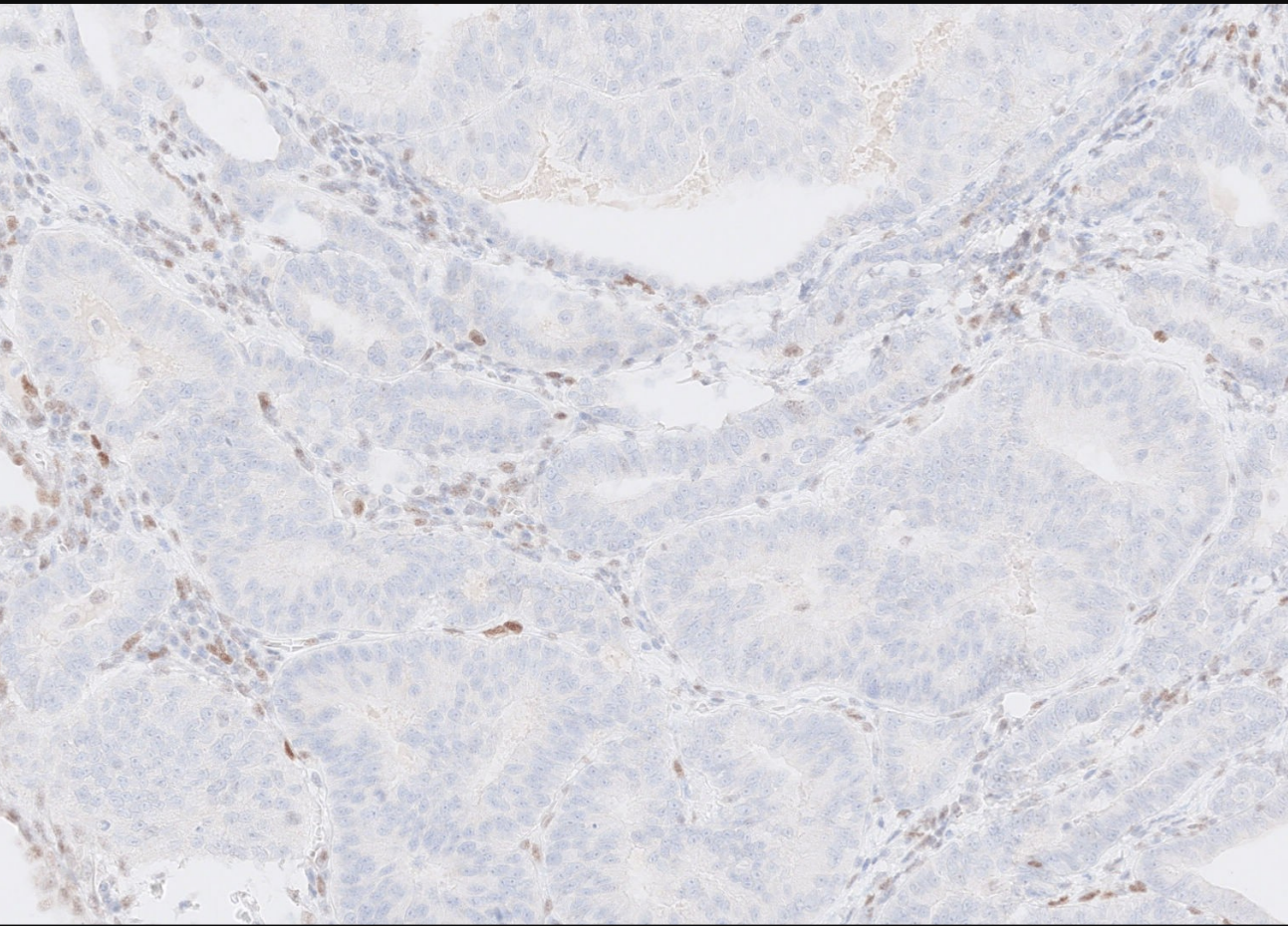
The endometrial serous carcinoma with p53 overexpression

Protocol A:

mAb clone DO-7, RTU for Benchmark Ultra, Ventana/Roche, modified protocol settings with HIER for 64 min. in CC1, 32 min. Ab incubation and OptiView as detection system.

Protocol B:

mAb clone DO-7, RTU for Benchmark Ultra, Ventana/Roche, the recommended protocol settings with HIER for 32 min. in CC1, 16 min. Ab incubation and OptiView as detection system.



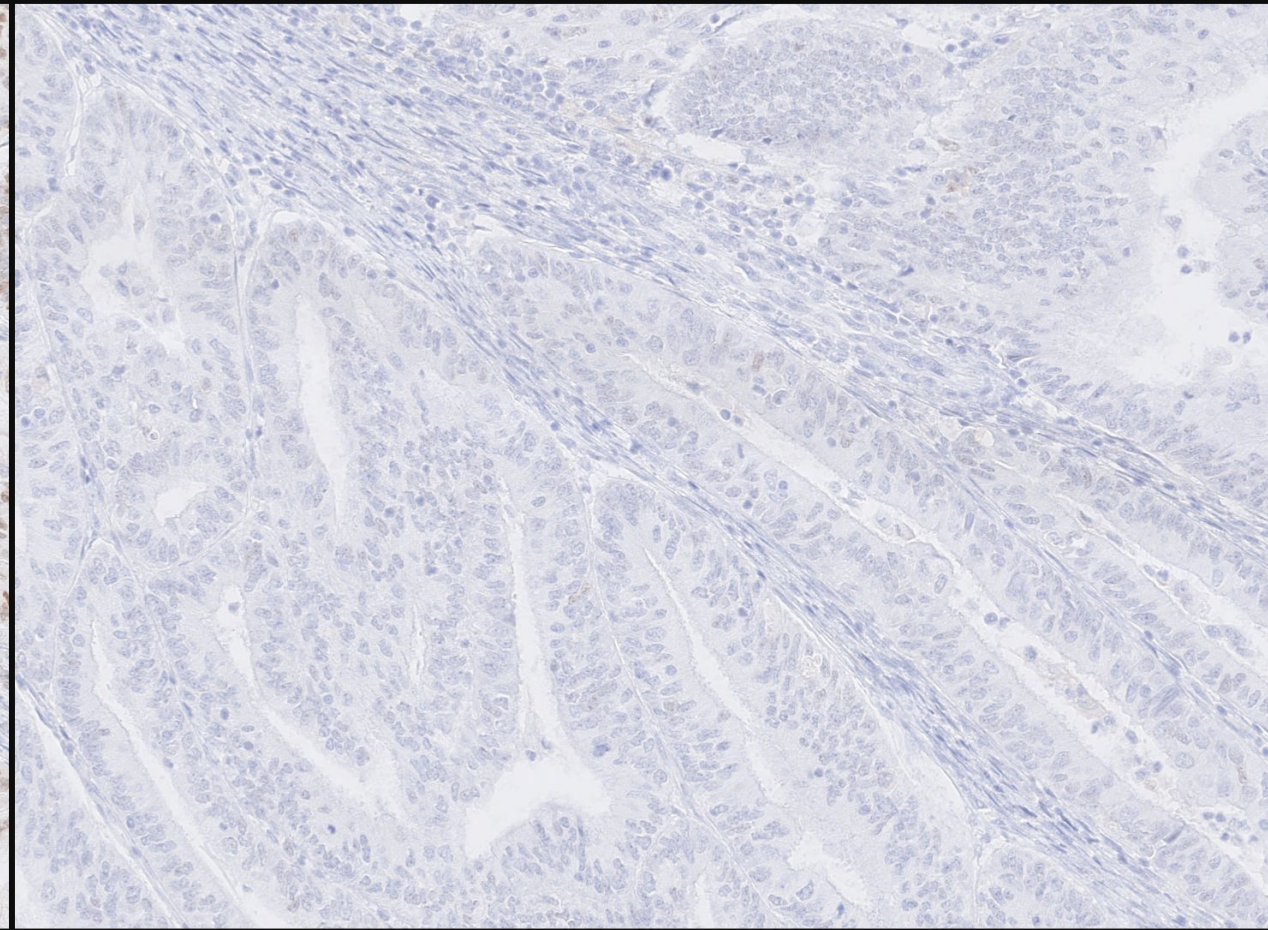
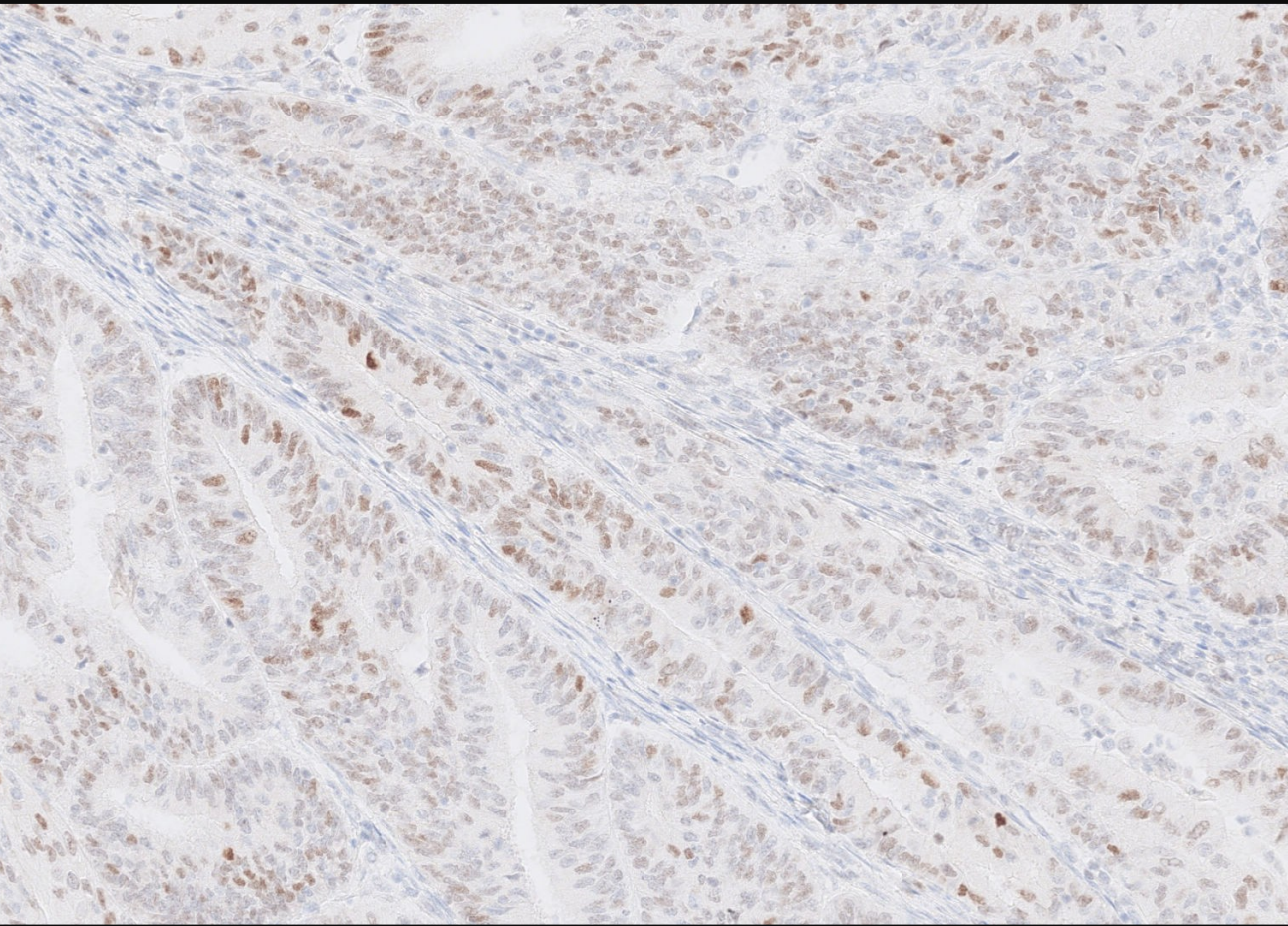
The endometrial serous carcinoma with absence of p53

Protocol A:

mAb clone DO-7, RTU for Benchmark Ultra, Ventana/Roche, modified protocol settings with HIER for 64 min. in CC1, 32 min. Ab incubation and OptiView as detection system.

Protocol B:

mAb clone DO-7, RTU for Benchmark Ultra, Ventana/Roche, the recommended protocol settings with HIER for 32 min. in CC1, 16 min. Ab incubation and OptiView as detection system.



The low grade endometrial carcinoma with p53 wild-type pattern

Protocol A:

mAb clone DO-7, RTU for Benchmark Ultra, Ventana/Roche, modified protocol settings with HIER for 64 min. in CC1, 32 min. Ab incubation and OptiView as detection system.

Protocol B:

mAb clone DO-7, RTU for Benchmark Ultra, Ventana/Roche, the recommended protocol settings with HIER for 32 min. in CC1, 16 min. Ab incubation and OptiView as detection system.

Results of p53 assessments in NordiQC

YEAR	Concentration of antibodies	YEAR	RTU system in recommended protocol Assessment-settings	RTU system in laboratory modified protocol settings
PASS RATE		PASS RATE		E
2024	71%	2024	30%	70%
2023	76%	2023	41%	68%
2022	62%	2022	33%	59%
2021	55%	2021	18%	60%
2013	75%	2013	100%	76%
2007	75%	2007	60%	NA

Results of p53 assessments in NordiQC

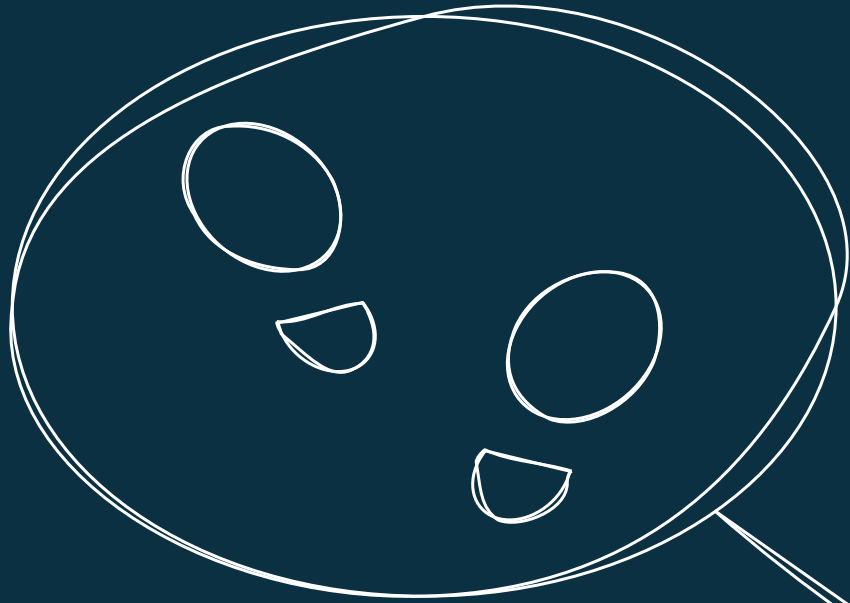
Assessment year	Concentrated antibodies	RTU system
2024	24%	76%
2023	26%	74%
2022	27%	73%
2021	36%	64%
2013	63%	37%
2007	94%	6%

P53

When knowledge shifts, so must we

ALK

new purpose, new
expectations



Unlock ALK anaplastic lymphoma kinase

> [Science](#). 1994 Mar 4;263(5151):1281-4. doi: 10.1126/science.8122112.

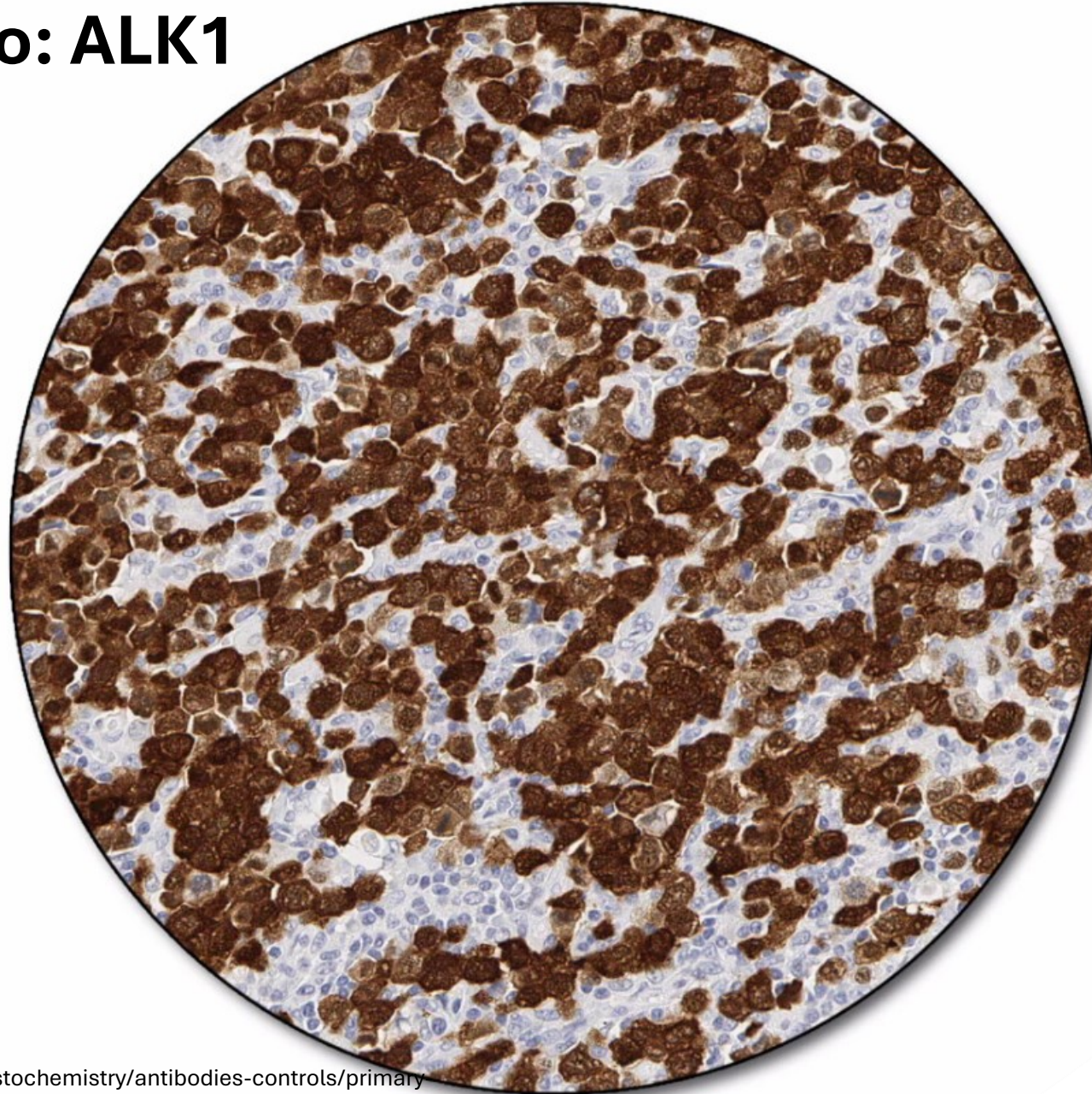
Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma

S W Morris ¹, M N Kirstein, M B Valentine, K G Dittmer, D N Shapiro, D L Saltman, A T Look

Affiliations + expand

PMID: 8122112 DOI: [10.1126/science.8122112](#)

The First Hero: ALK1



[nature](#) > [articles](#) > article

Article | Published: 11 July 2007

Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer

[Manabu Soda](#), [Young Lim Choi](#), [Munehiro Enomoto](#), [Shuji Takada](#), [Yoshihiro Yamashita](#), [Shunpei Ishikawa](#), [Shin-ichiro Fujiwara](#), [Hideki Watanabe](#), [Kentaro Kurashina](#), [Hisashi Hatanaka](#), [Masashi Bando](#), [Shoji Ohno](#), [Yuichi Ishikawa](#), [Hiroyuki Aburatani](#), [Toshiro Niki](#), [Yasunori Sohara](#), [Yukihiko Sugiyama](#) & [Hiroyuki Mano](#) 

U.S. Food And Drug Administration Approves Pfizer's XALKORI® (crizotinib) As First And Only Therapy Specifically For Patients With Locally Advanced Or Metastatic ALK-Positive Non-Small Cell Lung Cancer

Friday, August 26, 2011 - 07:24am

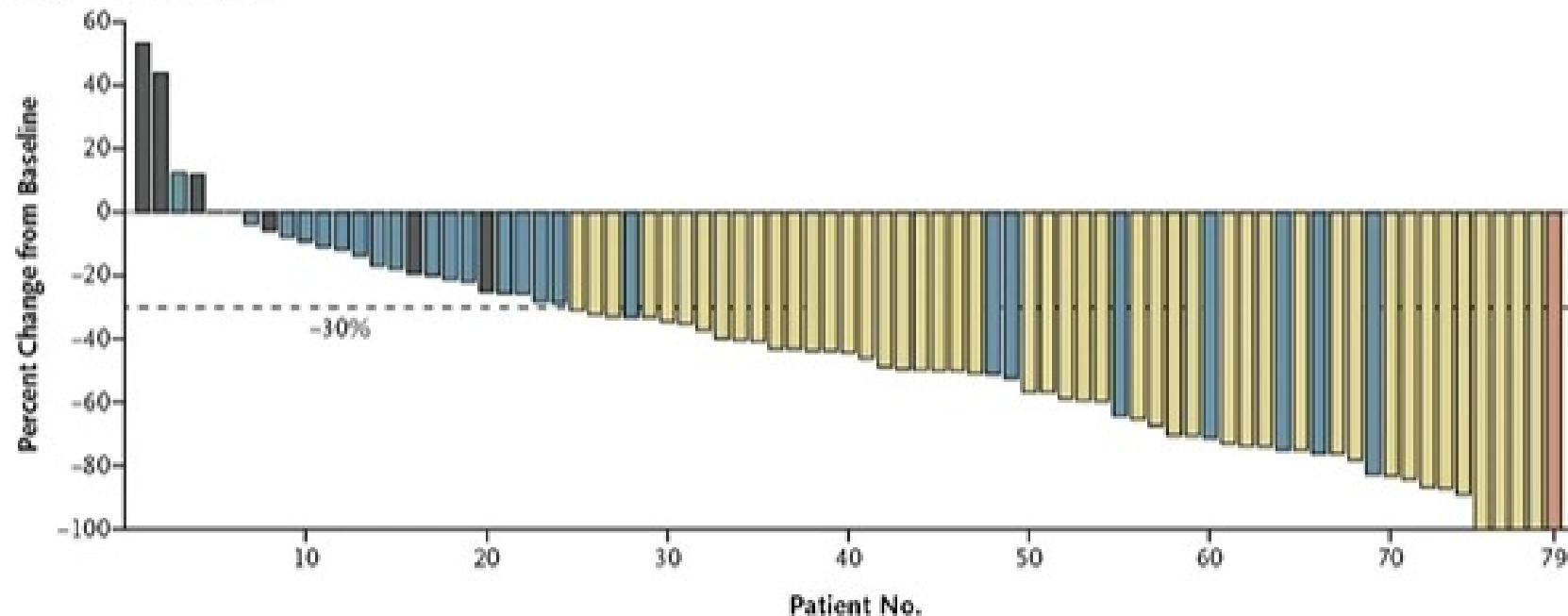


Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

Eunice L. Kwak, M.D., Ph.D., Yung-Jue Bang, M.D., Ph.D., D. Ross Camidge, M.D., Ph.D., Alice T. Shaw, M.D., Ph.D., Benjamin Solomon, M.B., B.S., Ph.D., Robert G. Maki, M.D., Ph.D., Sai-Hong I. Ou, M.D., Ph.D., Bruce J. Dezube, M.D., Pasi A. Jänne, M.D., Ph.D., Daniel B. Costa, M.D., Ph.D., Marileila Varela-Garcia, Ph.D., Woo-Ho Kim, M.D., Thomas J. Lynch, M.D., Panos Fidas, M.D., Hannah Stubbs, M.S., Jeffrey A. Engelman, M.D., Ph.D., Lecia V. Sequist, M.D., M.P.H., WeiWei Tan, Ph.D., Leena Gandhi, M.D., Ph.D., Mari Mino-Kenudson, M.D., Greg C. Wei, Ph.D., S. Martin Shreeve, M.D., Ph.D., Mark J. Ratain, M.D., Jeffrey Settleman, Ph.D., James G. Christensen, Ph.D., Daniel A. Haber, M.D., Ph.D., Keith Wilner, Ph.D., Ravi Salgia, M.D., Ph.D., Geoffrey I. Shapiro, M.D., Ph.D., Jeffrey W. Clark, M.D., and A. John Iafrate, M.D., Ph.D.

■ Disease progression ■ Stable disease ■ Partial response ■ Complete response

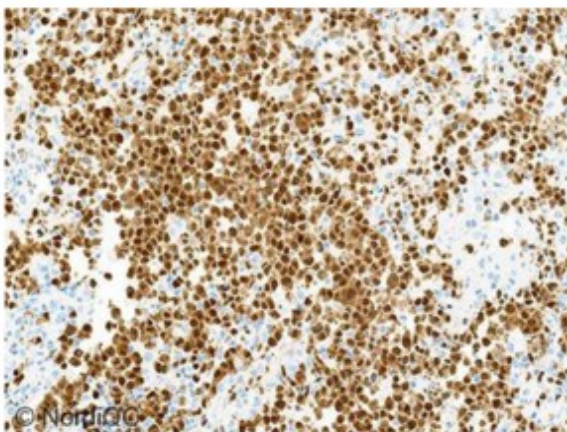
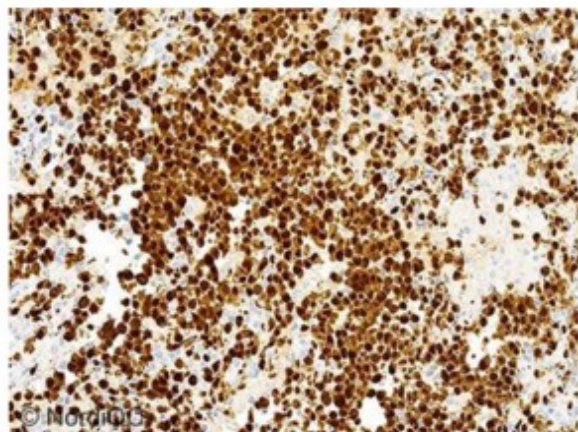
A Percent Change in Tumor Burden



The quality of IHC precision testing

Fit-For-Purpose IHC - ALK

ALCL



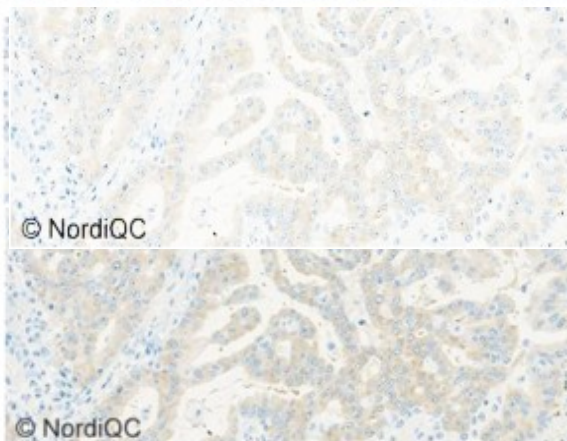
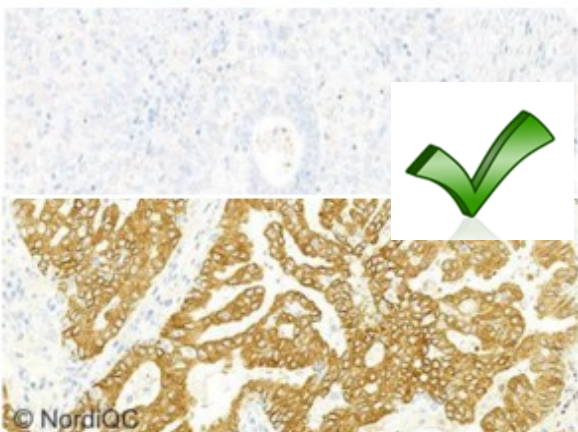
Purpose 1

Typically high
antigen
expression level

- ALK

Lung Ca

+ALK



Purpose 2

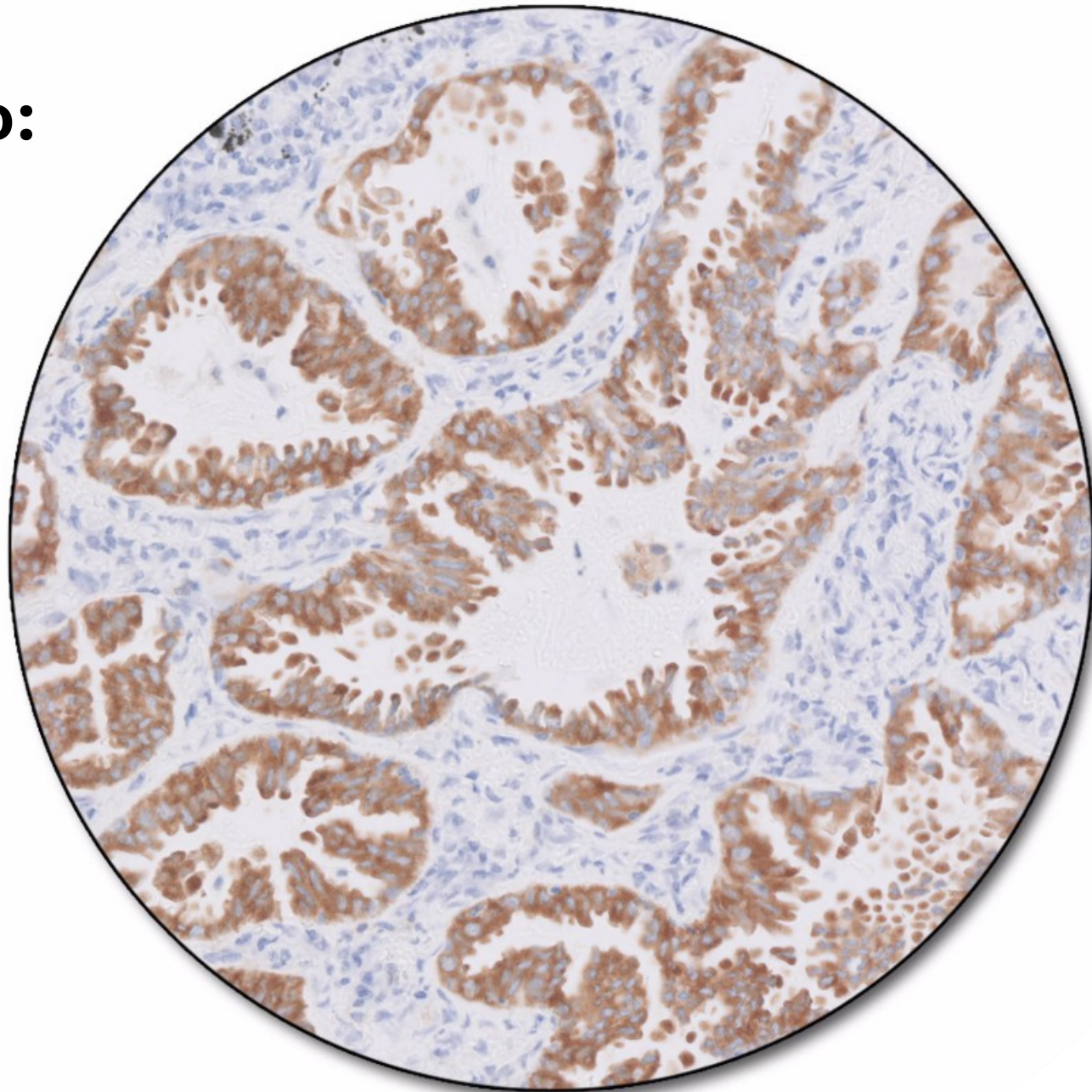
Typically low
antigen
expression level

IHC protocol 1

IHC protocol 2

Slide from Søren Nielsen

The Second Hero: super-sensitive ALK antibody clones



Purpose

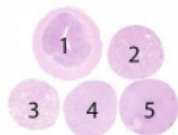
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among NordiQC participants for EML4-ALK translocations in lung adenocarcinomas. The EML4-ALK translocation occurs through a paracentric inversion between EML4 and ALK genes located in the short arm of chromosome 2 and induces an EML4-ALK fusion protein being expressed in 2–4% of lung adenocarcinomas and is a target for ALK tyrosine inhibitors as crizotinib, ceritinib, and alectinib.

Material

The slide to be stained for ALK (lung) comprised:

1. Appendix, 2. Lung adenocarcinoma without EML4-ALK translocation*, 3. Lung adenocarcinoma with EML4-ALK translocation*, 4. Merkel cell carcinoma,
5. Anaplastic large cell lymphoma (ALCL) with ALK translocation*.

*confirmed by Next Generation Sequencing (NGS)



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing ALK (lung) staining as optimal included:

- A distinct, moderate to strong nuclear and cytoplasmic staining reaction of virtually all neoplastic cells in the anaplastic large cell lymphoma (ALCL).
- An at least weak to moderate granular cytoplasmic staining reaction of virtually all neoplastic cells in the lung adenocarcinoma with EML-ALK translocation.
- An at least weak to moderate granular cytoplasmic staining reaction with membrane accentuation of virtually all neoplastic cells in the Merkel cell carcinoma.
- An at least weak to moderate granular cytoplasmic staining reaction of ganglion cells and dispersed axons in the appendix.
- No staining of neoplastic cells in the lung adenocarcinoma without ALK rearrangement.
- No staining of epithelial cells in the appendix and tonsil.

KEY POINTS FOR ALK (lung) IMMUNOASSAYS

- The mAb clone **OTI1A4** is recommendable both as a concentrated Ab and a RTU.
- The mAb clone **ALK-1** should not be used for lung adenocarcinomas.
- Appendix is a recommendable, external positive tissue control

Participation

Number of laboratories registered for ALK (lung), run 73	330
Number of laboratories returning slides	307 (93%)

Results

At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

307 laboratories participated in this assessment. 180 (59%) achieved a sufficient mark (optimal or good), see Table 1a (see page 3). Tables 1b and 1c summarizes the antibodies (Abs) used and assessment marks (see page 3-4).

The most frequent causes of insufficient staining reactions were:

- Unexpected and aberrant cytoplasmic staining reaction on the BenchMark platform.
- Less successful primary antibodies (mAb clone ALK1)

Concentrates:

OTI1A4

5A4

RTU-systems:

OTI1A4

5A4

D5F3

~~ALK1~~

Novel ALK immunohistochemistry assay (clone OTI1A4, Dako) is a sensitive, reliable marker for identifying *ALK* rearrangements in lung adenocarcinomas

A validation study

Ozgur Can Eren, MD,^{1,2,3} Cisel Aydin Mericoz, MD,¹ Emine Bozkurtlar, M. Pinar Bulutay, MD,¹ Arzu Baygul, PhD,⁵ Ibrahim Kulac, MD^{1,6,●}

¹Department of Pathology, Koç University School of Medicine, Istanbul, Turkey; ²Department of Immunology, Graduate School of Health Sciences, Koç University, Istanbul, Turkey; ³Koç University IsBank Research Center for Infectious Disease, Istanbul, Turkey; ⁴Department of Pathology, Marmara University School of Medicine, Istanbul, Turkey; ⁵Department of Biostatistics, Koç University School of Medicine, Istanbul, Turkey; ⁶Research Center for Translational Medicine, Koç University, Istanbul, Turkey

ABSTRACT

Objectives: We present the first study validating the recent Dako ALK assay (clone OTI1A4, in vitro diagnostic) for detecting *ALK* rearrangements in lung adenocarcinoma.

Methods: Lung adenocarcinoma cases between 2011 and 2023 were retrospectively collected to create a cohort of 203 samples. Cases were stained with Dako ALK OTI1A4 and Ventana ALK D5F3 and reviewed by 3 pathologists independently. Correlation between assays, including their sensitivity and specificity, was evaluated.

Results: The cohort (n = 203) consisted of resections, core needle biopsies, and cell blocks. Agreement between Dako ALK OTI1A4 and Ventana ALK D5F3 assays was “almost perfect,” with $\kappa = 0.89$. The sensitivity and specificity of the Dako ALK OTI1A4 assay were 93.3% and 96%, respectively, in a subgroup of 55 molecularly confirmed cases (n = 30 with and n = 25 without *ALK* rearrangement).



Assessment Run 73 2025 Lung Anaplastic Lymphoma Kinase (ALK (lung))

Purpose

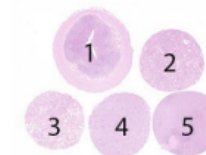
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among NordiQC participants for EML4-ALK translocations in lung adenocarcinomas. The EML4-ALK translocation occurs through a paracentric inversion between EML4 and ALK genes located in the short arm of chromosome 2 and induces an EML4-ALK fusion protein being expressed in 2–4% of lung adenocarcinomas and is a target for ALK tyrosine inhibitors as crizotinib, ceritinib, and alectinib.

Material

The slide to be stained for ALK (lung) comprised:

1. Appendix, 2. Lung adenocarcinoma without EML4-ALK translocation*, 3. Lung adenocarcinoma with EML4-ALK translocation*, 4. Merkel cell carcinoma, 5. Anaplastic large cell lymphoma (ALCL) with ALK translocation*.

*confirmed by Next Generation Sequencing (NGS)



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing ALK (lung) staining as optimal included:

- A distinct, moderate to strong nuclear and cytoplasmic staining reaction of virtually all neoplastic cells in the anaplastic large cell lymphoma (ALCL).
- An at least weak to moderate granular cytoplasmic staining reaction of virtually all neoplastic cells in the lung adenocarcinoma with EML-ALK translocation.
- An at least weak to moderate granular cytoplasmic staining reaction with membrane accentuation of virtually all neoplastic cells in the Merkel cell carcinoma.
- An at least weak to moderate granular cytoplasmic staining reaction of ganglion cells and dispersed axons in the appendix.
- No staining of neoplastic cells in the lung adenocarcinoma without ALK rearrangement.
- No staining of epithelial cells in the appendix and tonsil.

KEY POINTS FOR ALK (lung) IMMUNOASSAYS

- The mAb clone **OTI1A4** is recommendable both as a concentrated Ab and a RTU.
- The mAb clone **ALK-1** should not be used for lung adenocarcinomas.
- Appendix is a recommendable, external positive tissue control



ALK

new purpose, new
expectations

Melan A

New clones, new standards

Purpose

Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for MLA, identifying malignant melanomas in the characterization of tumours of unknown origin. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for MLA (see below). This was the second NordiQC assessment of MLA, excluding steroid hormone producing cells and corresponding tumours (only applicable for mAb clone A103), focusing only on intended use in relation to the diagnosis of malignant melanomas.

Material

The slide to be stained for MLA comprised:

1. Skin, 2. Kidney, 3. Colon Adenocarcinoma, 4-5. Malignant melanoma

All tissues were fixed in 10% neutral buffered formalin.

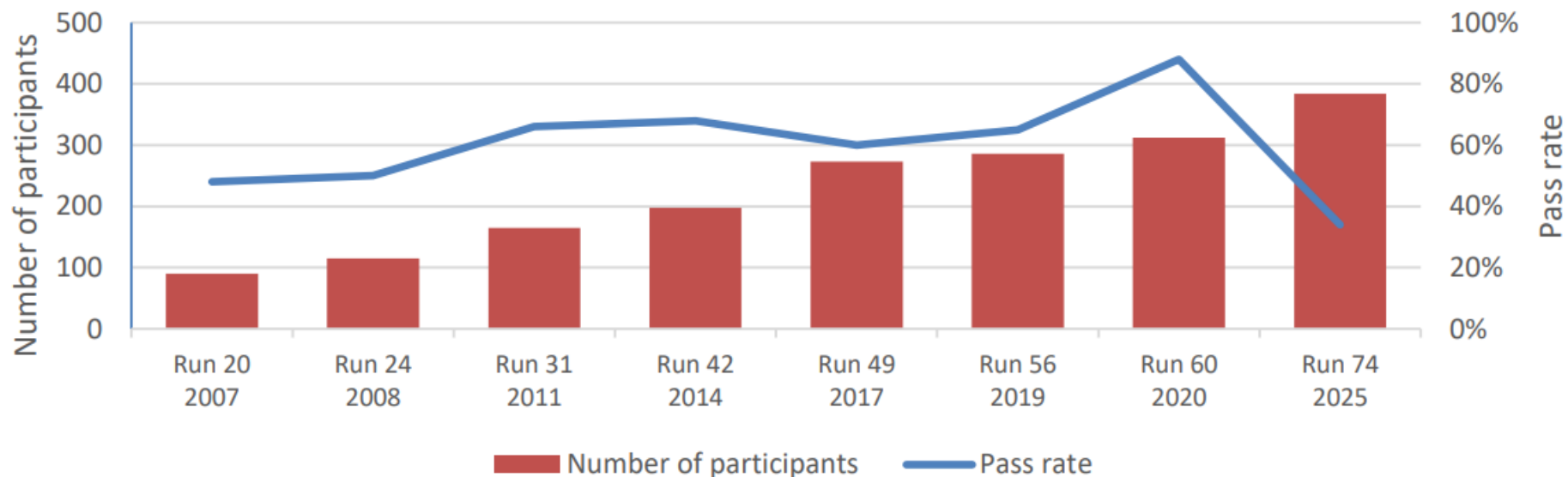
Criteria for assessing a MLA staining as optimal included:

- A moderate to strong, distinct cytoplasmic staining reaction of virtually all melanocytes in the skin. The dendrites of melanocytes should display a crisp and precise staining reaction.



Pass rate 34%

MLA performance in NordiQC assessments



Purpose

Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for MLA, identifying malignant melanomas in the characterization of tumours of unknown origin. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for MLA (see below). This was the second NordiQC assessment of MLA, excluding steroid hormone producing cells and corresponding tumours (only applicable for mAb clone A103), focusing only on intended use in relation to the diagnosis of malignant melanomas.

Material

The slide to be stained for MLA comprised:

KEY POINTS FOR MLA IMMUNOASSAYS

- A historically low pass rate of 34% was observed during Run 74 with only 9.5% being optimal.
- The mAb clone **A103** was used by 90% of all participants either as a concentrate or RTU product.
- The mAb clone **A103** seemed to have less affinity for low-level MLA expression in malignant melanomas compared to other clones.
- The LD assays using mAb clone **BS52** and rmAb clone **EP43** had the highest pass rate and optimal result could be obtained on all main fully automated IHC platforms.

Participation

Number of laboratories registered for MLA, run 74	430
Number of laboratories returning slides	391 (91%)

Results

At the date of assessment, 91% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

391 laboratories participated in this assessment, however 7 laboratories submitted inappropriate antibodies, and the data were not included in this report. 34% achieved a sufficient mark (optimal or good). Table 1a-c summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

NordiQC internal study

MelanA Diagnostic Performance: Clone A103 vs. Newer Clones

71 melanomas + 36 non-melanomas

A103 shows lower sensitivity: The widely used **A103 clone** has a significantly lower diagnostic sensitivity for melanomas compared to newer clones. **Newer clones increased diagnostic specificity.**

A103: 82% sensitivity

EP43: 94% sensitivity

BS52: 93% sensitivity

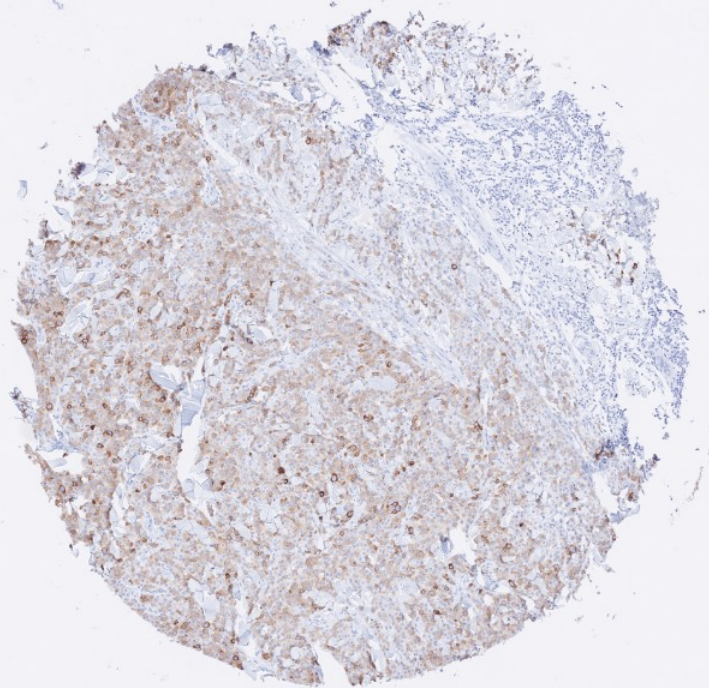
A103 has weaker staining intensity: A103 also showed weaker H-scores, indicating a less intense stain and fewer positive cells, increasing the risk of **misdiagnosis**.

Median H-scores:

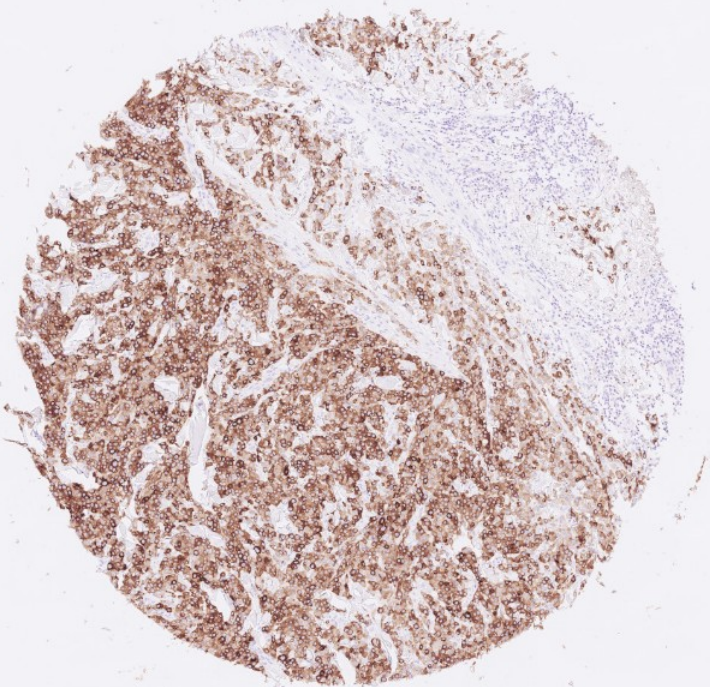
EP43 & BS52: 260

A103: 160

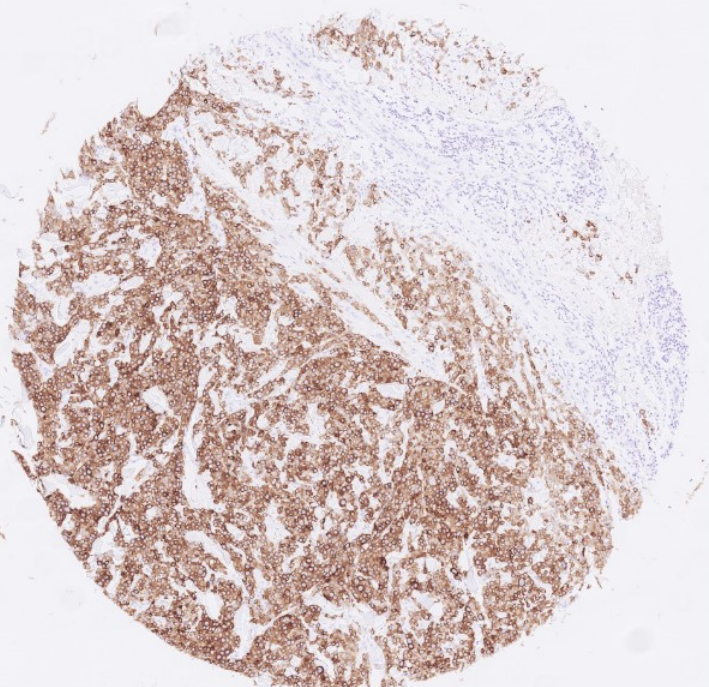
Positive cut-off; H-score 50



A103 (Roche VRPS)

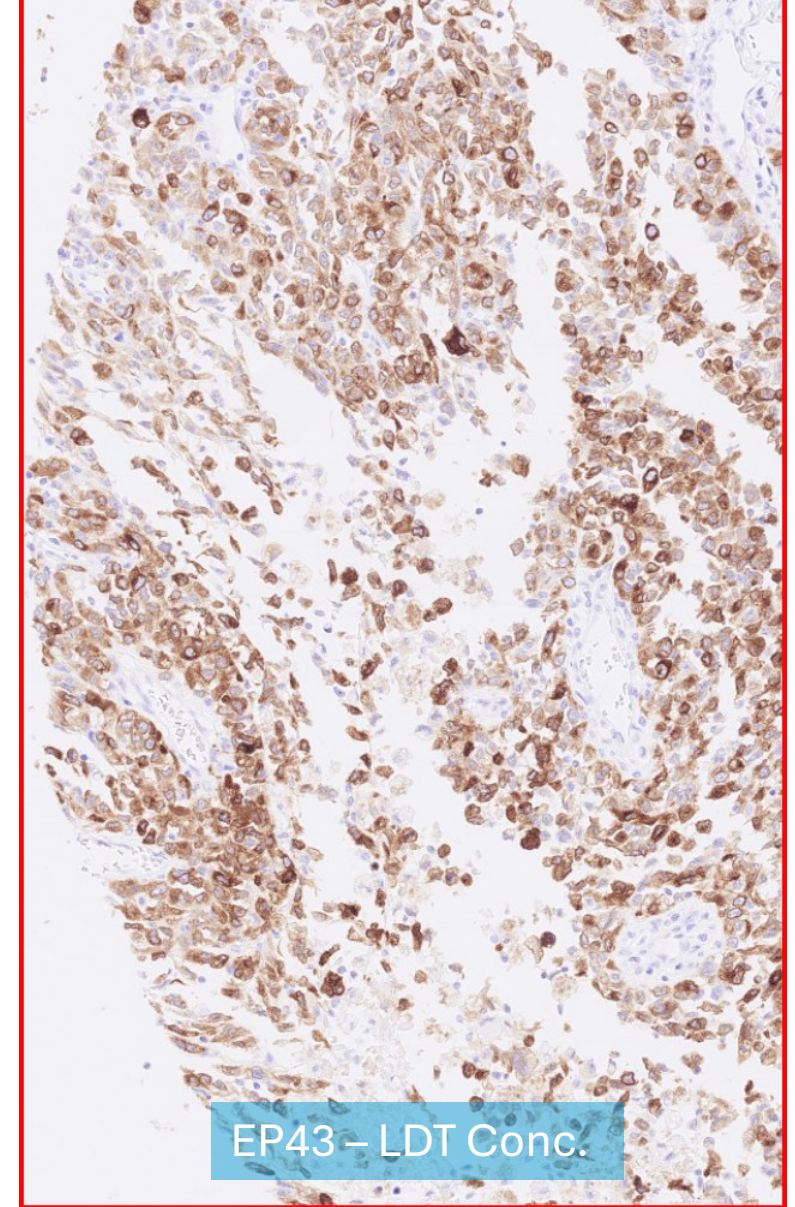
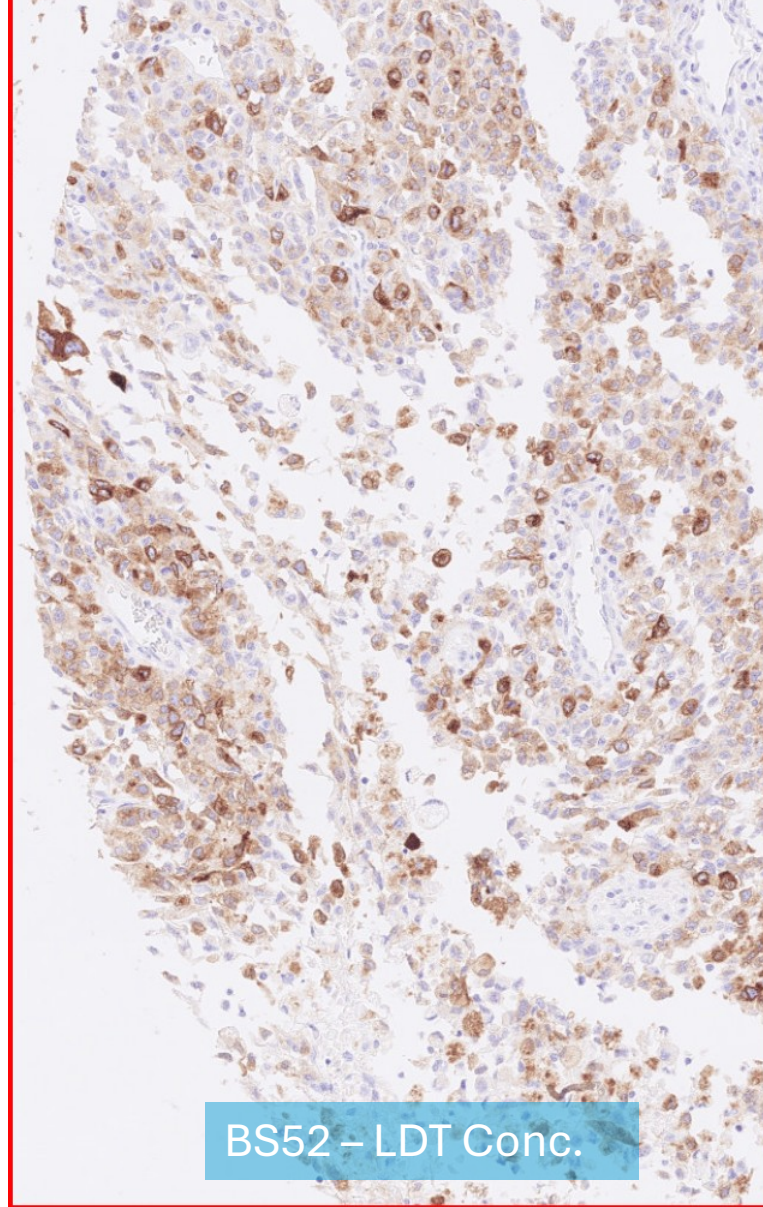
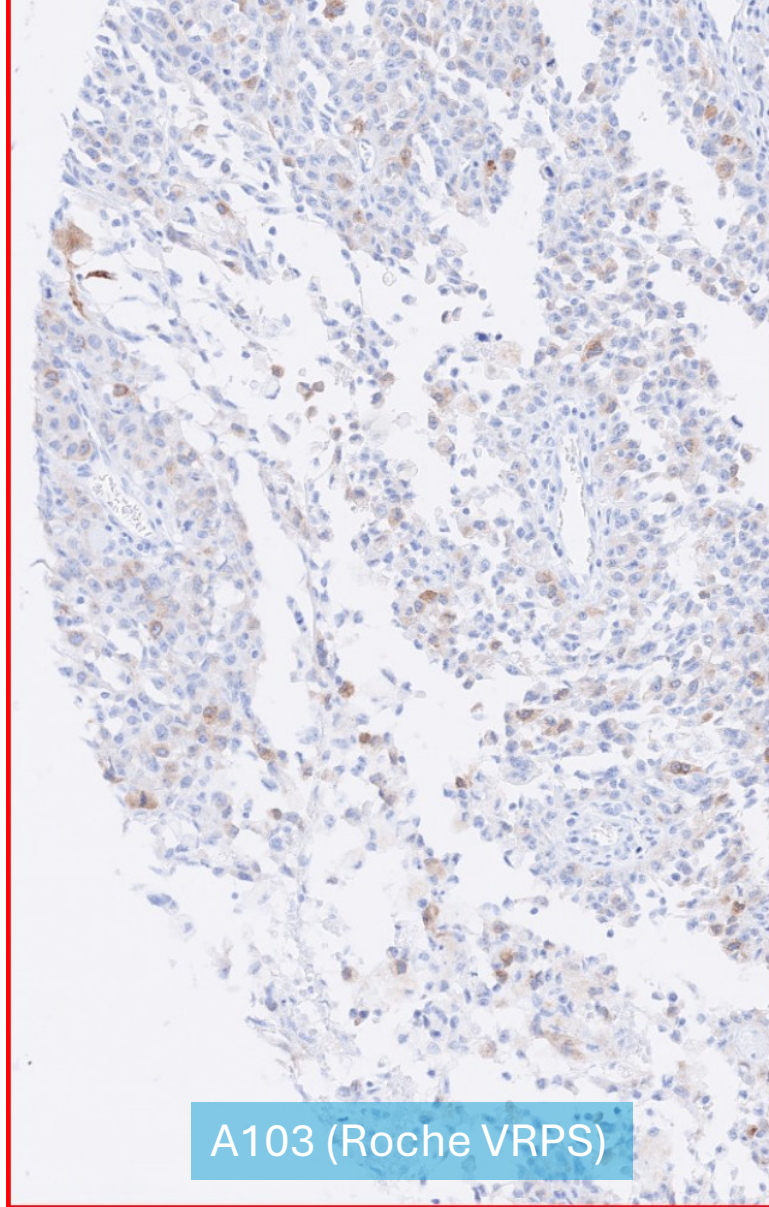


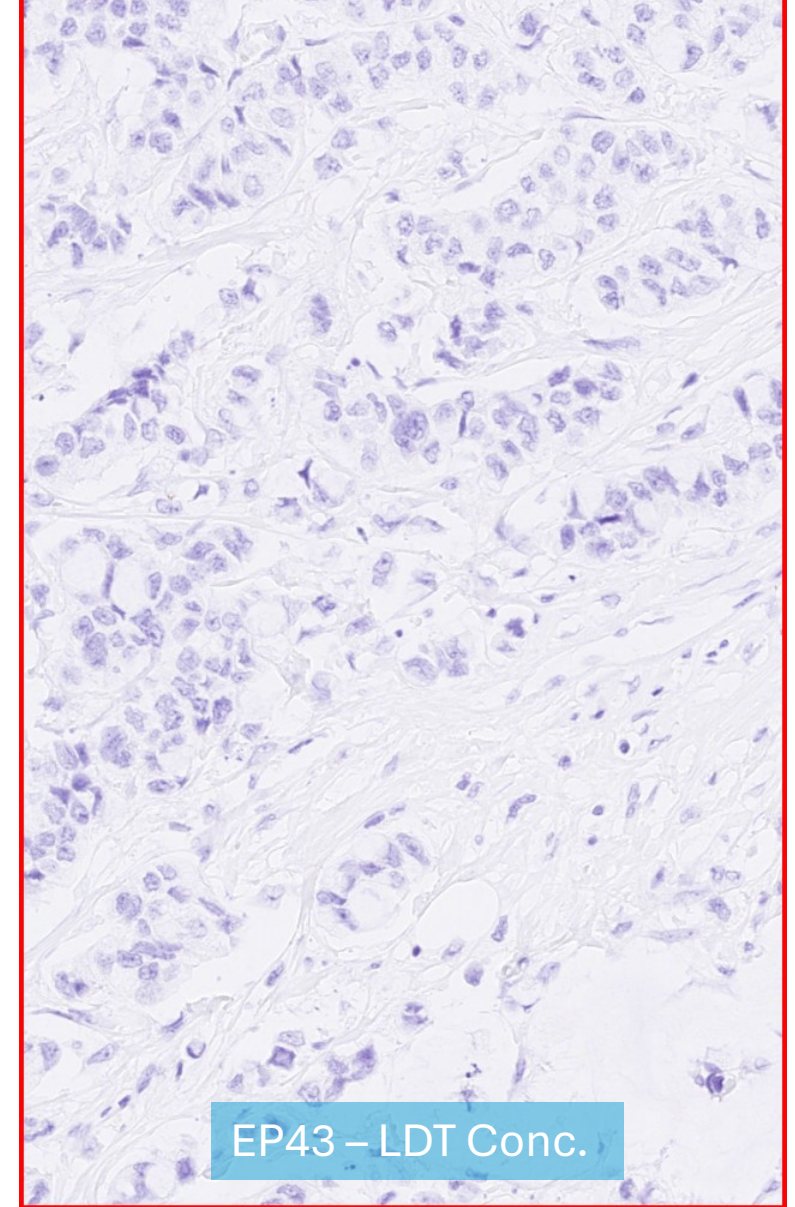
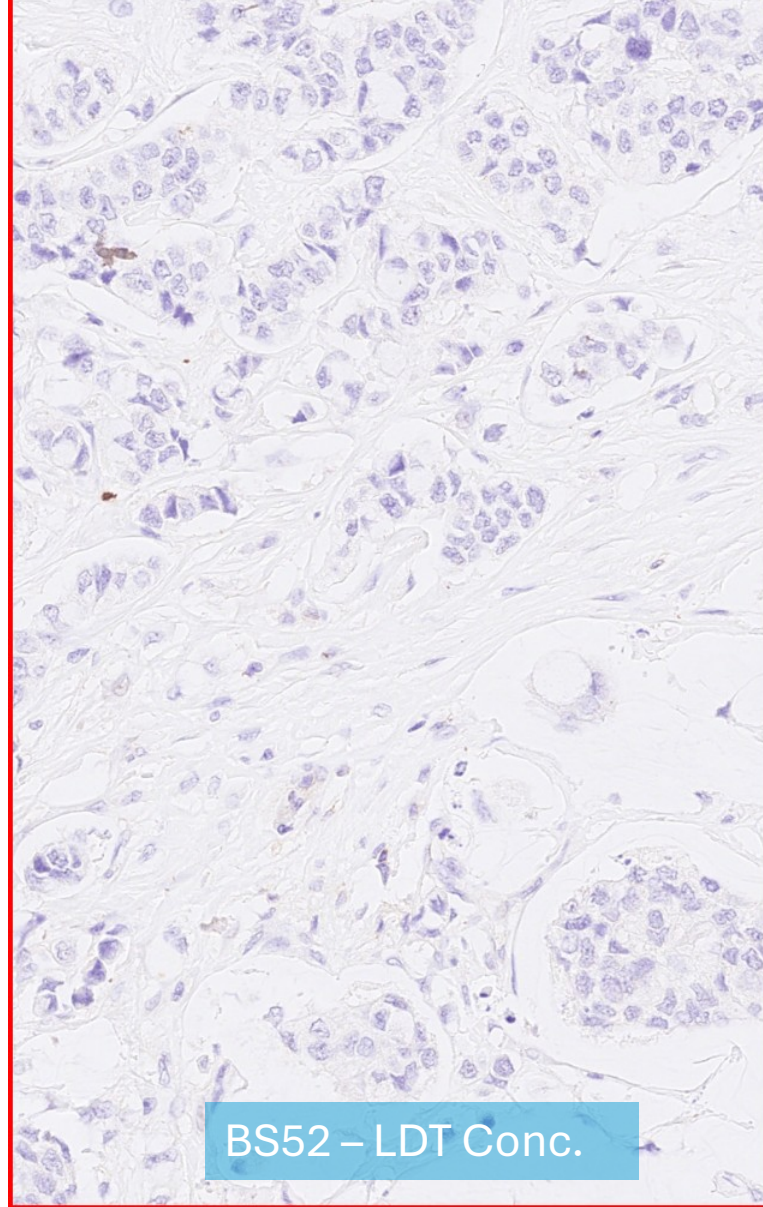
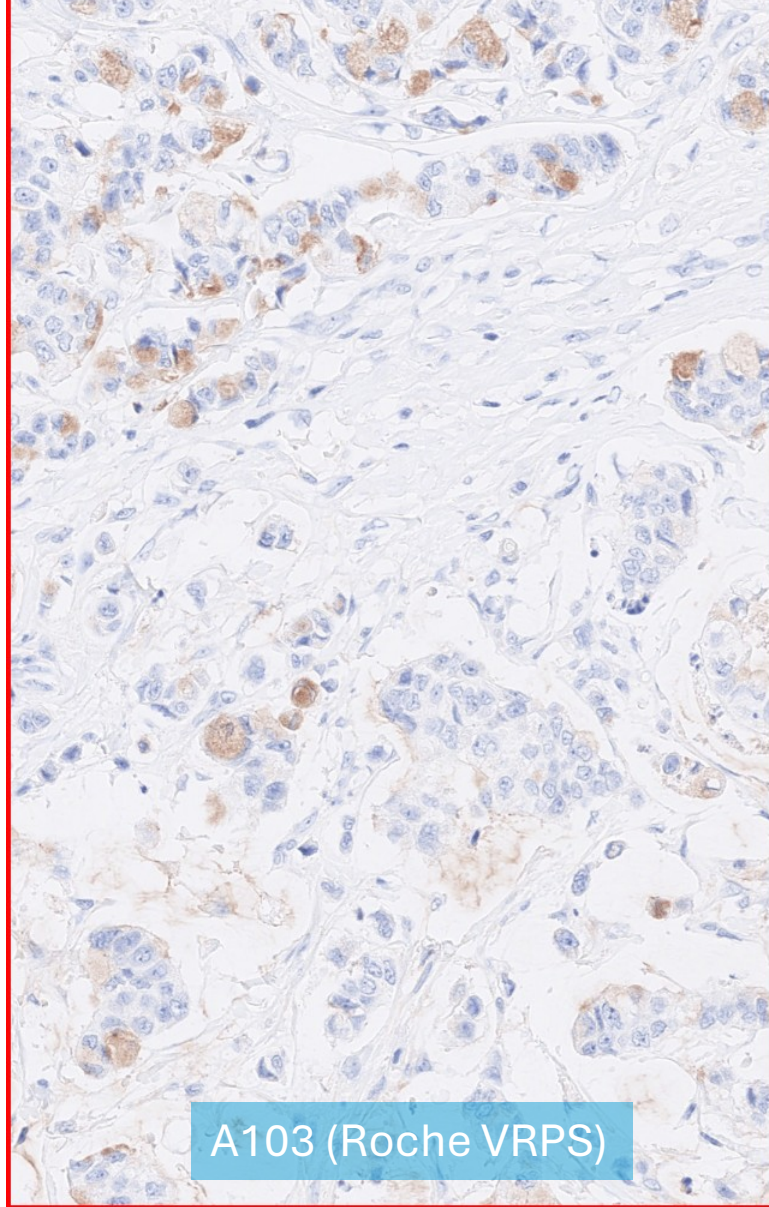
BS52 – LDT Conc.



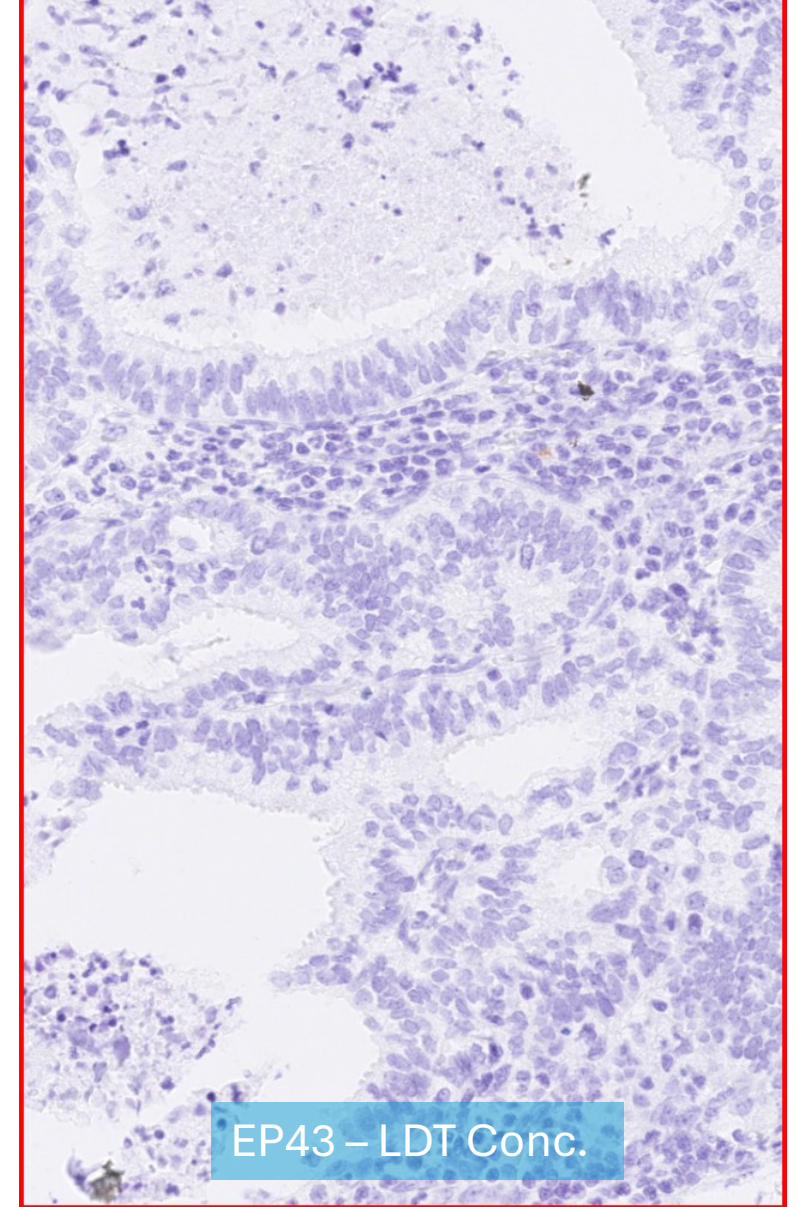
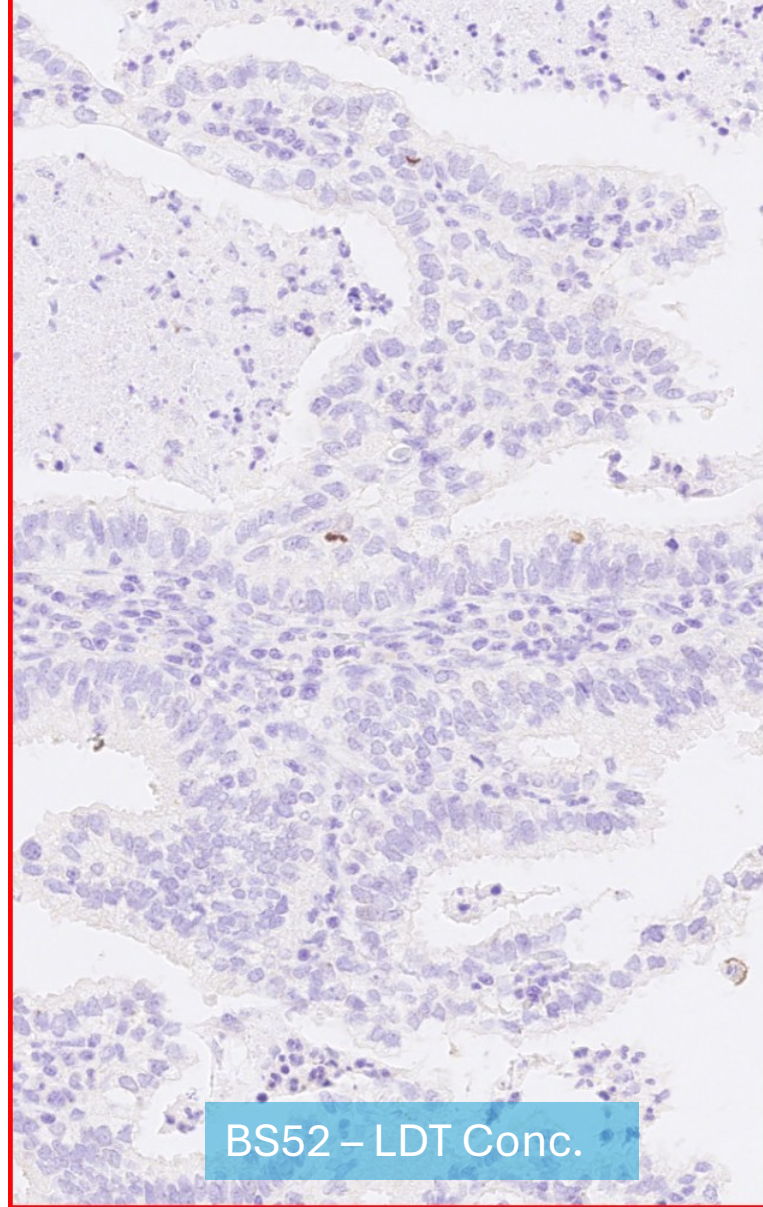
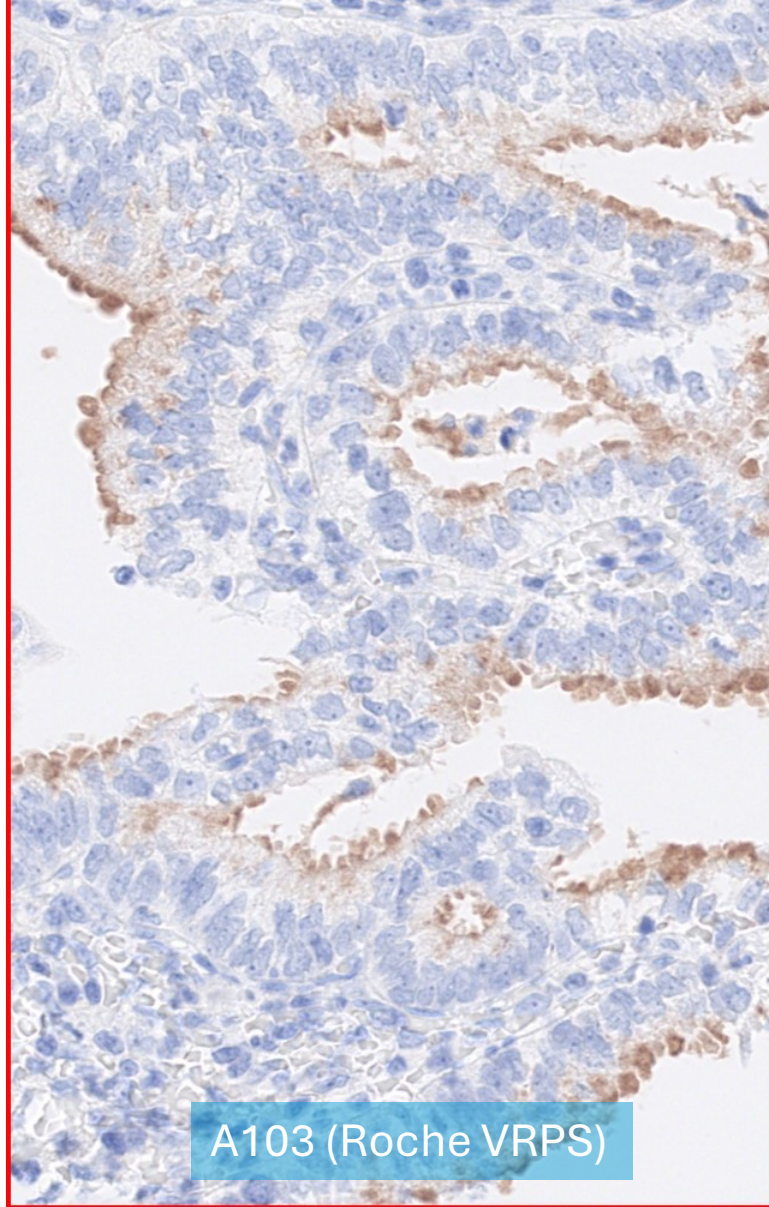
EP43 – LDT Conc.

Melanoma I





Ductal breast carcinoma



Uterine endocervical carcinoma

Melan-A expression in non-melanocytic carcinoma: A potential diagnostic pitfall

Linwei Zuo, Huiyan You, Zhe Cai, Shousheng Liao, Xiangtong Lu, Lixiang Li* and Wenyong Huang*

Department of Pathology, The Second Affiliated Hospital of Nanchang University, Nanchang, China

*These authors contributed to the work equally and should be regarded as co-corresponding authors

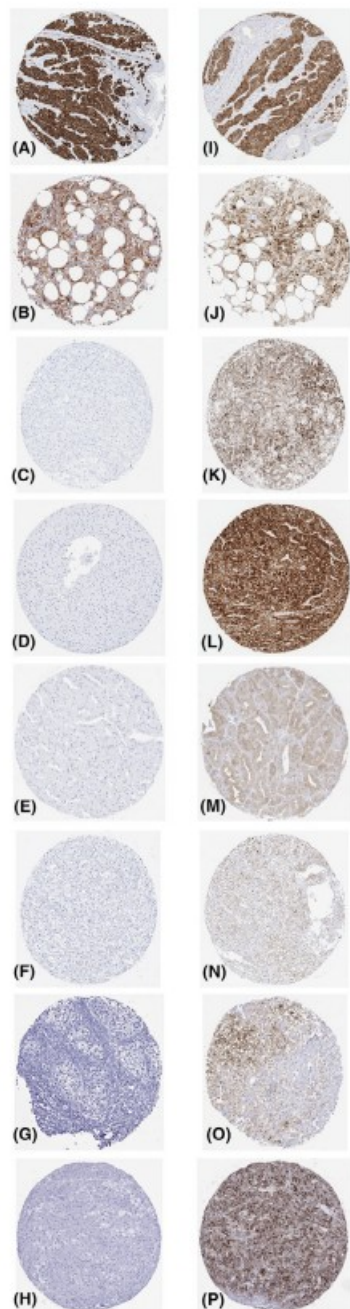
stained with A103

Results. In 87 non-melanocytic carcinoma tissue samples, Melan-A was positive in six (6.89%) cases, of which four (66.7%) were male and two (33.3%) were female, with a mean age of 60 years (range 21-82 years). Five (83.3%) of the Melan-A-positive cases had distant metastases. Compared with Melan-A negative cases, Melan-A positive non-melanocytic carcinomas were significantly associated with poor prognosis ($P=0.0023$).

Table 1. Summary of clinicopathologic characteristics of six Melan-A-positive non-melanocytic carcinoma cases.

Case #	Age (y)/sex	Site	Treatment	IHC					Diagnosis	Follow-up
				Melan-A	HMB45	S100	CK	inhibin		
1	77/F	Lung	Biopsy	+	-	-	+	-	NSCLC	2Ms, dead
2	82/M	Lung	WE	+(p)	-	-	+	-	NSCLC	3Ms, dead
3	47/M	Nasal cavity	Biopsy+CT	+(w)	-	-	+	-	NK-NPC	7Ms, alive
4	70/M	Stomach	Biopsy	+(w)	-	-	+	-	AC	6Ms, dead
5	21/F	Kidney	WE	+(f)	-	-	+	-	RCC	12Ms, alive
6	64/M	Liver	TACE	+(f)	-	-	+	-	HCC	8Ms, dead

+ indicates positive; -, negative; F, female; M, male; CT, chemotherapy; NSCLC, non-small cell carcinoma; NK-NPC, non-keratinizing nasopharyngeal carcinoma; AC, adenocarcinoma; RCC, renal cell carcinoma; HCC, hepatocellular carcinoma; WE, wide excision; TACE, transarterial chemoembolization; Ms, months; p, partial; w, weak; f, focal.



Melanoma

Renal
angiomyolipoma

Adrenocortical
adenoma

Adrenocortical
carcinoma

Follicular adenoma
of thyroid

Clear cell renal
cell carcinoma

Urothelial
Carcinoma

Testicular Leydig
cell tumor

Staining pattern of specific and cross-reacting Melan-A antibodies: A comparative study on 15,840 samples from 133 human tumor types

SHIVA BOROOJERDI,^{1,†} SÖREN WEIDEMANN,^{1,†} ANNE MENZ,¹ MAXIMILIAN LENNARTZ,¹ ID SEBASTIAN DWERTMANN RICO,¹ RIA SCHLICHTER,¹ SIMON KIND,¹ VIKTOR REISWICH,¹ FLORIAN VIEHWEGE,¹ AHMED ABDULWAHAB BAWAHAB,^{1,2} DORIS HÖFLMEYER,¹ CHRISTOPH FRAUNE,¹ NATALIA GORBOKON,¹ ANDREAS M. LUEBKE,¹ CLAUDIA HUBE-MAGG,¹ ID FRANZISKA BÜSCHECK,¹ TILL KRECH,^{1,3} ANDREA HINSCH,¹ FRANK JACOBSEN,¹ EIKE BURANDT,¹ ID GUIDO SAUTER,¹ RONALD SIMON,^{1,*} ID MARTINA KLUTH,¹ STEFAN STEURER,¹ SARAH MINNER,¹ ANDREAS H. MARX,⁴ CHRISTIAN BERNREUTHER,¹ ID TILL S. CLAUDITZ,¹ DAVID DUM¹ and PATRICK LEBOK^{1,3}

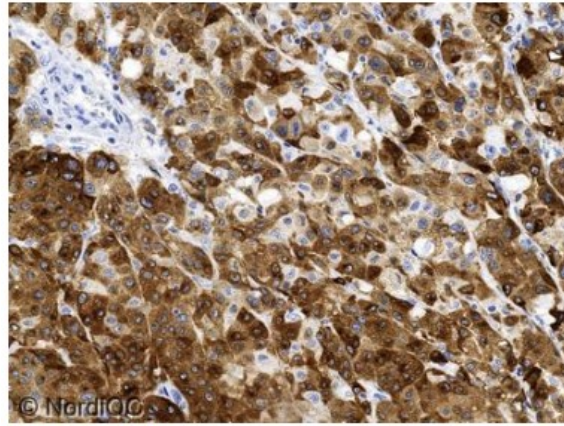
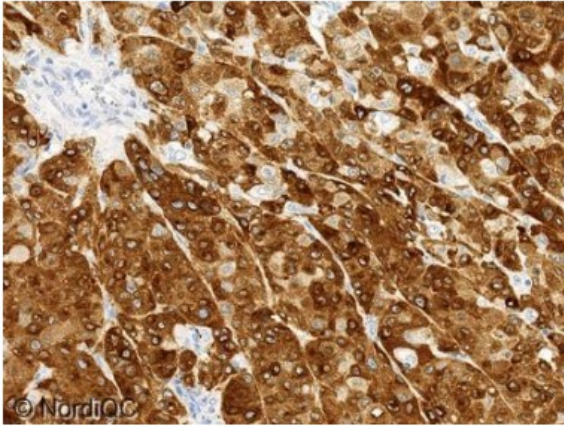
¹Institute of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ²Department of Pathology, Faculty of Medicine, University of Jeddah, Jeddah, Saudi Arabia; ³Institute of Pathology, Clinical Center Osnabrueck, Osnabrueck; and ⁴Department of Pathology, Academic Hospital Fuerth, Fuerth, Germany

Fig. 2. Melan-A immunostaining of selected tumors by specific and 'cross-reactive' Melan-A antibodies. Using the Melan-A-specific antibody MSVA-900M (Melan-A specific), a distinct Melan-A positivity is seen in cases of cutaneous melanoma (A) and renal angiomyolipoma (B) while staining is absent in samples from adrenocortical adenoma (C) and carcinoma (D), follicular adenoma of the thyroid (E), clear cell renal cell carcinoma (F), muscle-invasive urothelial carcinoma (G), and a testicular Leydig cell tumor (H). Using the 'cross-reactive' clone MSVA-901M+ (Melan-A+) on the same tumors as depicted in A–H, 'Melan-A' positivity is seen at variable intensity in melanoma (I), renal angiomyolipoma (J), adrenocortical adenoma (K) and carcinoma (L), follicular adenoma of the thyroid (M), clear cell renal cell carcinoma (N), muscle-invasive urothelial carcinoma (O), and testicular Leydig cell tumor (P).

Identification of purpose of the test

E Torlakovic et al. AIMM 2017;25:4-11
Evolution of Quality Assurance for Clinical
Immunohistochemistry in the Era of
Precision Medicine: Part 1

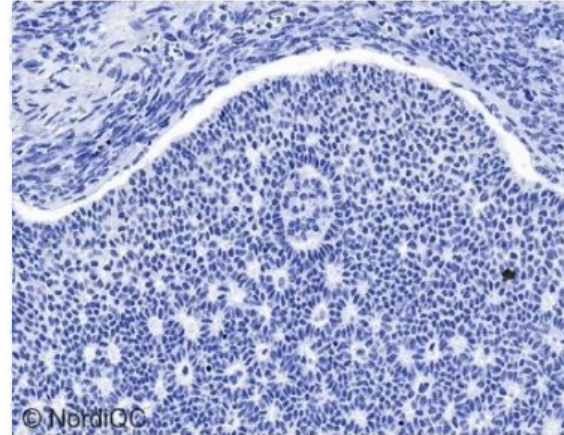
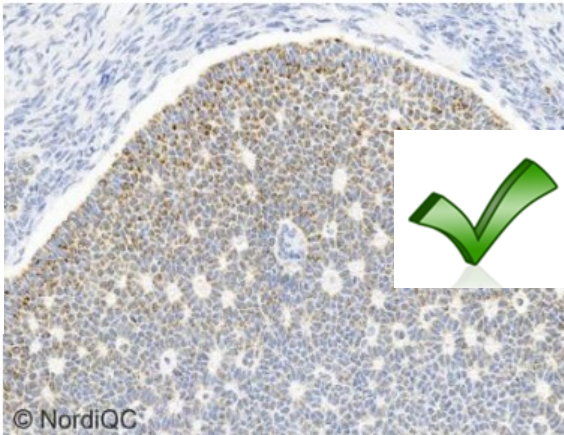
Melanoma



Purpose 1

Typically **mod.- high**
antigen expression
level

Sex cord
tumour



Purpose 2

Typically **low – mod.**
antigen expression
level and only for
clone A103

IHC method 1

IHC method 2

IHC for Melan A

Melan A

New clones, new standards

HER 2

Therapy drives IHC

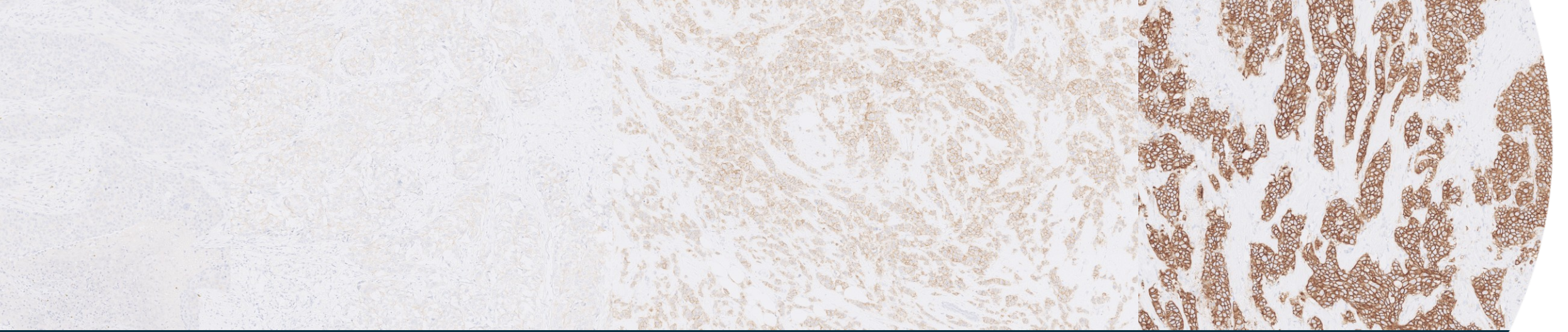
Traditional Breast HER2-scoring

0

1+

2+

3+

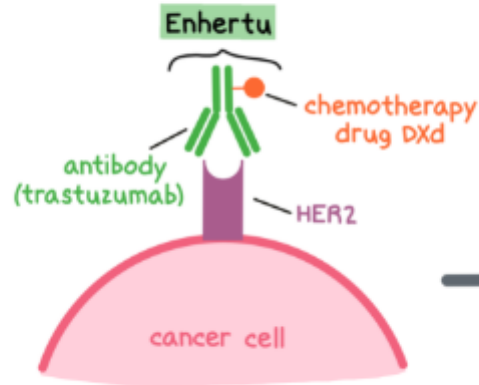


HER2 No-overexpression:
0, 1+ or 2+ISH non-
amplified

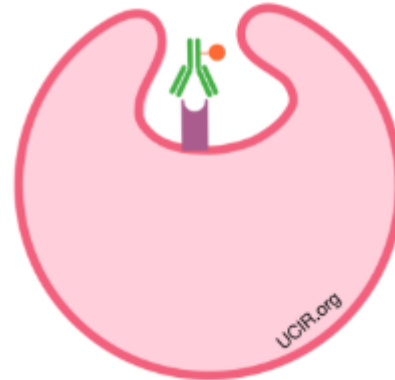
HER2 2+:
Send ISH

HER2 Overexpression:
3+ or 2+ISH amplified

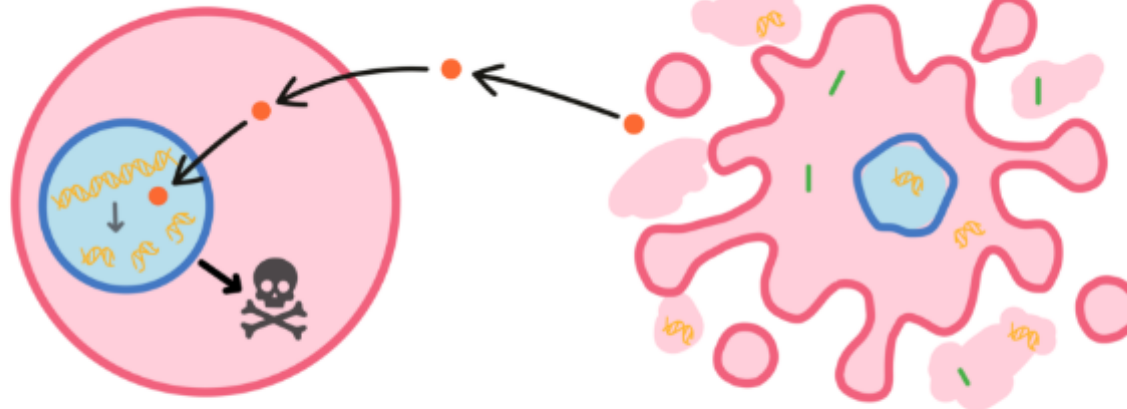
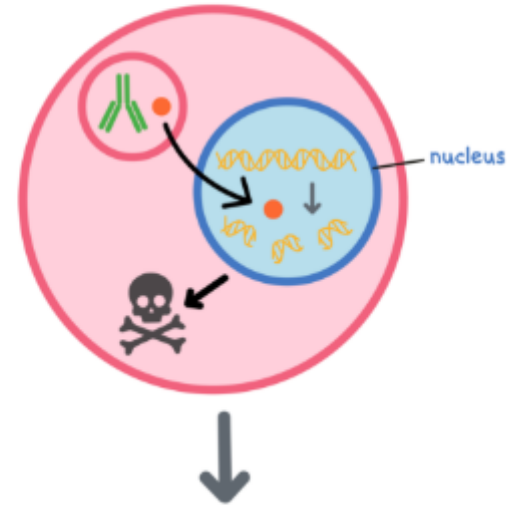
Using its **antibody** part, **Enhertu** binds to **HER2** on the surface of a **cancer cell**...



...and enters the cell.



Inside the cell, the **chemotherapy drug DXd** gets released and travels to the **nucleus** of the cell, where it leads to **DNA damage** and results in **cell death**



when the **cancer cell** dies, the **chemotherapy drug DXd** may be freed, going on to kill neighboring **cancer cells**

**Trastuzumab
deruxtecan**

Breast HER2-scoring in 2020's

0

1+

2+

3+

HER2 low expression:
1+ or 2+ISH non-amplified

HER2 No-overexpression:
0, 1+ or 2+ISH non-amplified

HER2 2+:
Send ISH

HER2 Overexpression:
3+ or 2+ISH amplified

Breast HER2-scoring in 2025

0

1+

2+

3+



HER2 ultra-low:
0+

HER2- low:
1+ or 2+ISH non-amplified

HER2 No-overexpression:
0, 1+ or 2+ISH non-amplified

HER2 2+:
Send ISH

HER2 Overexpression:
3+ or 2+ISH amplified

Are HER2 assays comparable?



Impact of different CE-IVD IHC assays on HER2 status in breast cancer with focus on HER2-low & HER2-ultralow



Kristi Bøgh Anderson, Søren Nielsen, Birgit Truumees, Rasmus Røge
NordIQC, Aalborg University Hospital, Denmark

Background and objective

The European Medicines Agency (EMA) recently approved trastuzumab deruxtecan for treating patients with unresectable or metastatic HR-positive breast cancer (BC) that is HER2-low or HER2-ultralow. HER2 status is at present determined by immunohistochemistry (IHC) most frequently using a CE-IVD-approved assay. While staining differences among IHC-assays are well known, a direct comparison of the three main CE-IVD-approved HER2 IHC assays is lacking. Additionally, previously published comparisons provide limited information on analytical performance and inter-assay concordance due to post-analytical variability in the studies.

This study evaluates the analytical performance of three most frequently used CE-IVD-approved HER2 IHC assays and their clinical implications for categorizing HER2-low and HER2-ultralow BC.

Methods

Two tissue microarrays (TMAs) were constructed for other quality control studies, including triple-negative and ductal BCs. Serial sections, minimizing tumor heterogeneity, were stained with three CE-IVD approved HER2 IHC assays: PATHWAY rmAb 4B5, 790-2991 (Ventana/Roche), HercepTest pAb, SK001 (Dako/Agilent) and HercepTest rmAb DG44, GE001 (Dako/Agilent). All BCs were scored by three observes and classified as HER2 0, ultralow, 1+, 2+ or 3+. The amplification status was available for HER2 2+ cases based on fluorescence in situ hybridization of the original tissue.

Results

In total 109 BCs were analyzed. A 97% concordance was observed across all three assays, classifying samples into the classical two groups: HER2 negative (0, ultralow, 1+, 2+ unamplified) and HER2 positive (3+, 2+ amplified) (Table 1). For HER2-low and HER2-ultralow a low inter-assay agreement was seen as the HER2 0 classification differed between assays: PATHWAY rmAb 4B5 classified 34% (37 samples) as HER2 0, compared to 31% (34 samples) with HercepTest pAb SK001 and 25% (27 samples) with HercepTest rmAb DG44 (Graph 1, Figure 1).

Table 1. Frequency of Different HER2 Scores Across CE-IVD-approved Assays.

HER2 Group	PATHWAY 4B5	HercepTest SK001	HercepTest DG44
HER2-negative	88	90	87
HER2-positive	21	19	22
Total	109	109	109

Graph 1. HER2 Status Across CE-IVD-approved IHC-assays.

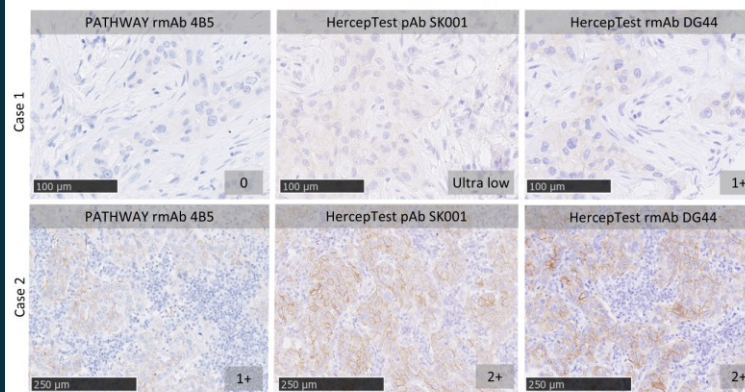
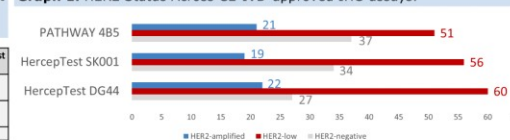


Figure 1. HER2 IHC on three serial sections of two different breast carcinoma cases using three different IHC assays: PATHWAY rmAb 4B5, HercepTest pAb SK001 and HercepTest rmAb DG44. All assays were performed using vendor-recommended protocol settings (VRPS).

Top row: Case 1, showing HER2 expression score "0" using PATHWAY rmAb 4B5, "ultralow" with HercepTest pAb SK001 and "1+" with HercepTest rmAb DG44.

Bottom row: Case 2, showing HER2 expression score "1+" using PATHWAY rmAb 4B5, "2+" with HercepTest pAb SK001 and "2+" with HercepTest rmAb DG44.

Scale bars: 100 µm (top row), 250 µm (bottom row).

Conclusion

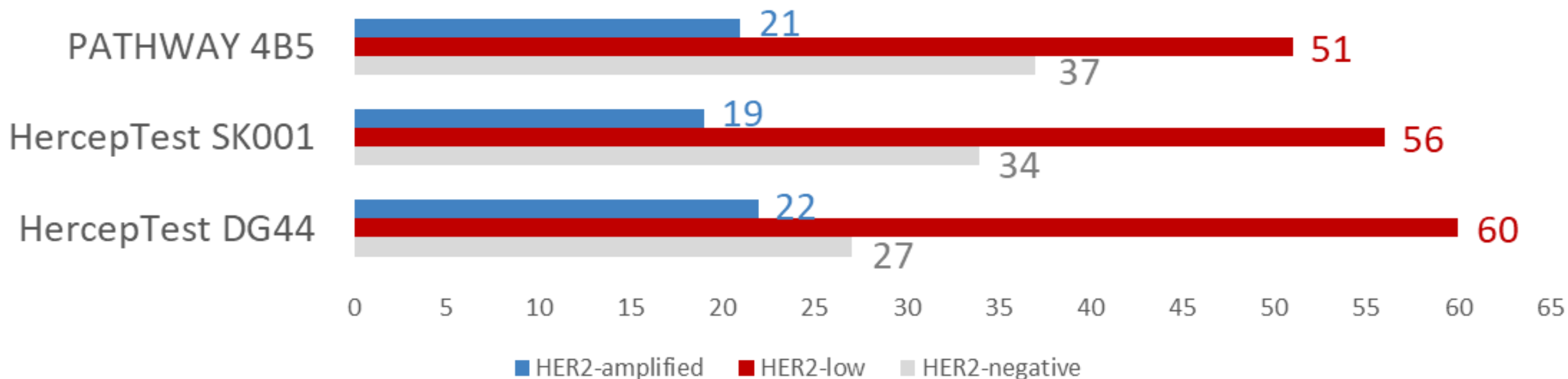
This study shows strong agreement among the three IHC assays when using the classical interpretation that classifies BC as either HER2-positive or HER2-negative. However, under the new treatment approach, where only HER2 0 patients do not receive therapy, the choice of assay becomes important. The PATHWAY rmAb assay identifies more patients as HER2 0 compared to the two HercepTest assays, potentially influencing treatment decisions.



← Download the poster here!

The authors have no conflict of interest related to the content of this work.
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Are HER2 assays comparable?



HER2 Group	PATHWAY 4B5	HercepTest SK001	HercepTest DG44
HER2-negative	88	90	87
HER2-positive	21	19	22
Total	109	109	109

Two TMAs: triple-negative and ductal BC

Three CE-IVD approved IHC-assays:
 PATHWAY rmAb 4B5, 790-2991 (Ventana/Roche) HercepTest pAb, SK001 (Dako/Agilent)
 HercepTest rmAb DG44, GE001 (Dako/Agilent)

Scored as HER2 0, ultralow, 1+, 2+ or 3+

Amplification FISH status for HER2 2+

Case 1

PATHWAY rmAb 4B5

HercepTest pAb SK001

HercepTest rmAb DG44

100 μ m

0

100 μ m

Ultra low

100 μ m

1+

Case 2

PATHWAY rmAb 4B5

HercepTest pAb SK001

HercepTest rmAb DG44

250 μ m

1+

250 μ m

2+

250 μ m

2+

HER2 IHC

What will HER2 low change?

A key finding was the large group of *Good* (rather than *Optimal*) results — 32% of all stains

Figs. 3a and 3b – **staining result assessed as Optimal versus Good**
expected result for HER2 overexpression, but failed HER2 Low.
3a left and 3b left same protocol (assessed as Optimal).
3a right and 3b right same protocol (assessed as Good).

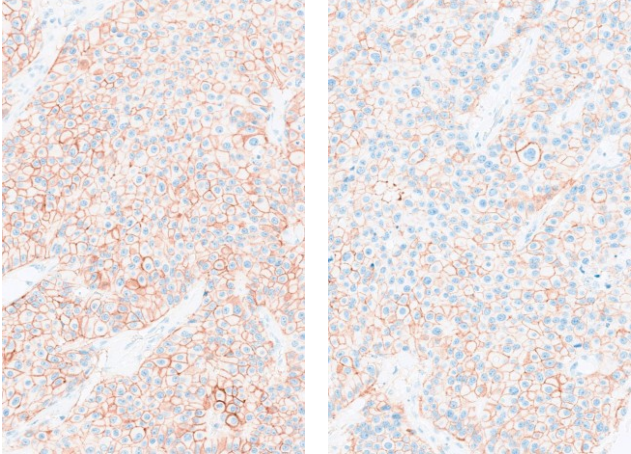


Fig. 3a.
Left: Expected staining result for HER2 of the breast carcinoma, tissue core no. 2, with a HER2/chr17 ratio of 3.33.
>10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.
Right: Staining result for HER2 of the breast carcinoma, tissue core no. 2, with a HER2/chr17 ratio of 3.33.
>10% of the neoplastic cells show a weak but complete membranous staining reaction corresponding to 2+.
The proportion and intensity of cells demonstrated is reduced but still the result is considered acceptable for HER2 classical overexpression.

However, the reduced analytical sensitivity of the protocol applied in Fig. 3a right has an impact on the accuracy for HER-Low, see Fig. 3b.

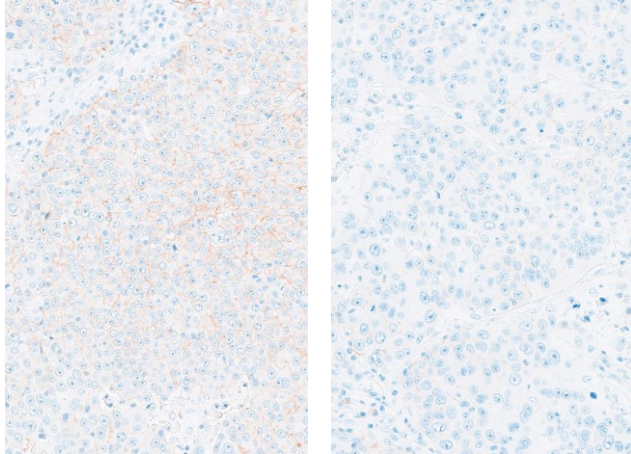


Fig. 3b.
Left: Expected staining result for HER2 of the breast carcinoma, tissue core no. 4, with a ratio HER2/chr17 of 1.35.
>10% of the neoplastic cells show a weak, partial membranous staining reaction corresponding to 1+.
Right: **Insufficient** staining result for HER2 of the breast carcinoma, tissue core no. 4, with a HER2/chr17 ratio of 1.35.
Only very few neoplastic cells show a very faint and partial membranous staining reaction corresponding to a 0 score.

Both the participant and NordiQC scored the result in Fig. 3b right as 0.

The tumour was thus categorized as HER 0 impacting HER2-Low classification as the expected 1+ status changed to 0.

Run 75

410 participating laboratories

Pass rate 89%

In 76% of Good-rated stains, the lower sensitivity would shift the HER2-low classification

CDx assay	Vendor recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark Ultra, Ultra Plus PATHWAY® rmAb 4B5, 790-2991	22/26 (85%)	15/26 (58%)	52/66 (79%)	35/66 (53%)
Ventana BenchMark GX, XT, Ultra, Ultra Plus VENTANA 4B5, 790-4493	35/35 (100%)	24/35 (69%)	79/84 (94%)	60/84 (71%)
Ventana BenchMark GX, XT, Ultra, Ultra Plus VENTANA RxDx 4B5, 790-7167	40/41 (95%)	24/41 (59%)	32/35 (91%)	18/35 (51%)
Dako Autostainer Link 48+ HercepTest™ pAb, SK001	6/7 (86%)	4/7 (57%)	1/1	1/1
Dako Omnis HercepTest™ rmAb DG44, GE001	34/34 (100%)	26/34 (76%)	-	-
Leica Bond MAX, III Oracle™ mAb CB11, TA9145	2/4	0/4	0/1	0/1

* Protocol settings recommended by vendor – Retrieval method & conditions, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.



HER 2

Therapy drives IHC

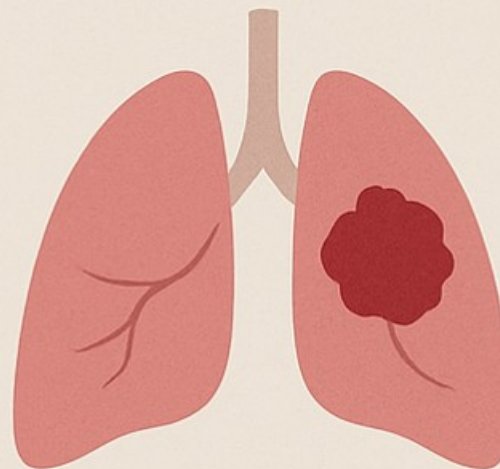
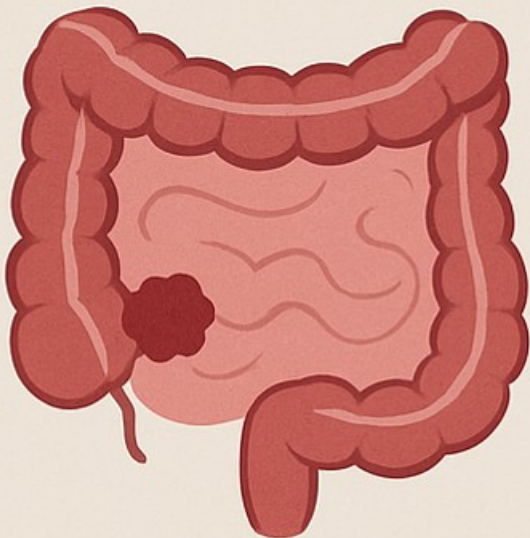


CEA = Carcinoembryonic Antigen

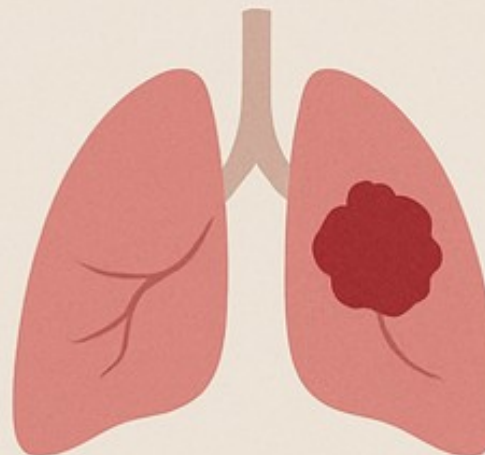
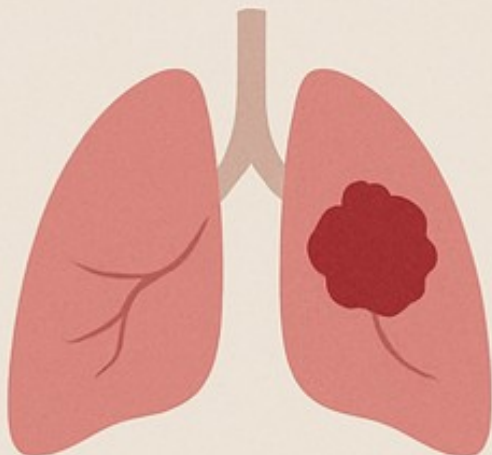
CEA

From tool to target- rebirth of an old marker

If CEA-negative,
tumor unlikely to be
colonic AC



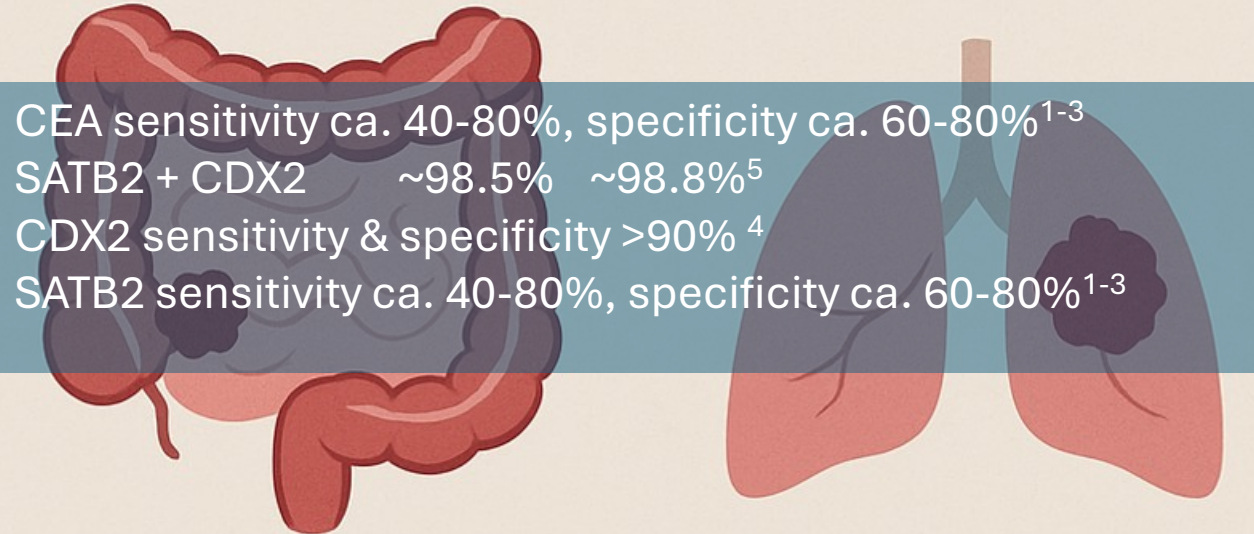
If CEA-positive,
likely lung AC, not
mesothelioma



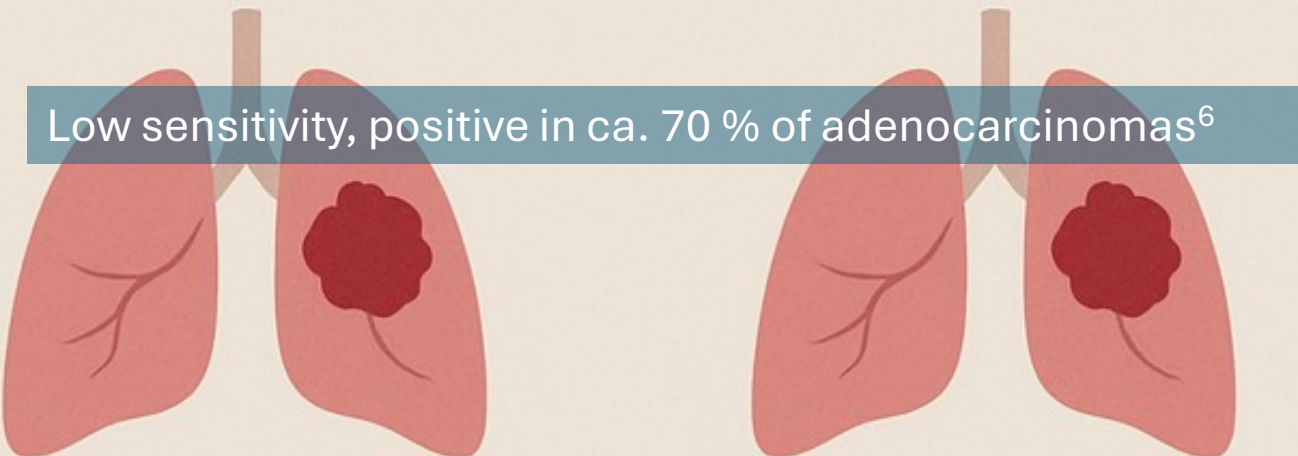


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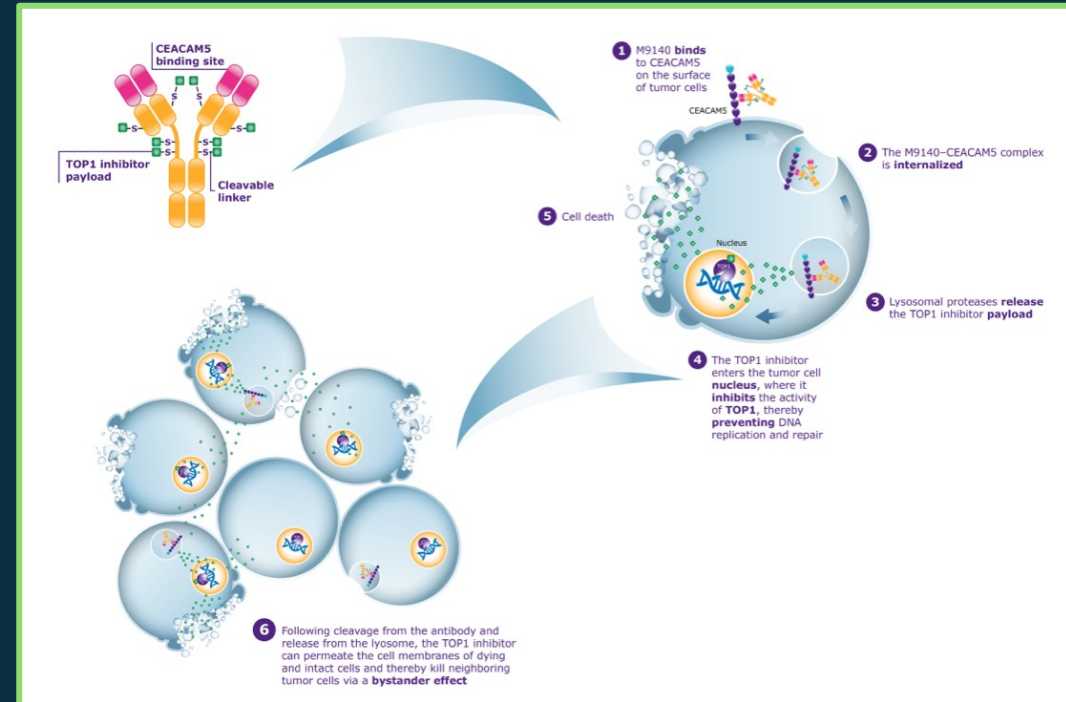
If CEA-negative,
tumor unlikely to be
colonic AC



If CEA-positive,
likely lung AC, not
mesothelioma



CEACAM5 is high in several tumor types, such as gastric, colorectal and pancreatic cancers, expression in normal epithelial tissues is low.¹⁻²



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CARMEN-LC03: Tusamitamab Ravtansine vs Docetaxel in Previously Treated Advanced Nonsquamous NSCLC

By Julia Cipriano, MS

Posted: 10/22/2024 11:30:00 AM

Last Updated: 10/22/2024 10:34:07 AM

 Get Permission



Benjamin Besse, MD,
PhD

The multicenter phase III CARMEN-LC03 trial did not meet its dual primary endpoints of progression-free and overall survival with the **CEACAM5-directed** antibody-drug conjugate tusamitamab ravtansine vs standard chemotherapy with docetaxel in previously treated patients with advanced nonsquamous non–small cell lung cancer (NSCLC), according to the final progression-free survival and first interim overall survival analyses.¹ At the International Association for the Study of Lung Cancer (IASLC) 2024 World Conference on Lung Cancer, **Benjamin Besse, MD, PhD**, of Gustave Roussy, Villejuif, France, and colleagues presented these findings, which led to the termination of the study in December 2023.

Dr. Besse reported trends toward improved overall survival in the overall population and improved progression-free and overall survival in the subgroup of patients with CEACAM5 expression levels of at least 80%, along with a favorable safety profile.

“CEACAM5 is a transmembrane glycoprotein, which is overexpressed in up to 25% of patients with nonsquamous NSCLC,” Dr. Besse explained. He noted that an exploratory analysis aiming to understand the high performance of the control arm, which was also presented during the conference, revealed a potential prognostic role for CEACAM5 expression.²

CARMEN-LC03: Tusamitamab Ravtansine vs Docetaxel in Previously Treated Advanced Nonsquamous NSCLC

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Press Release: Sanofi announces end of program evaluating tusamitamab ravtansine after a 2L NSCLC Phase 3 trial did not meet a primary endpoint

December 21, 2023

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Meeting Abstract: 2025 ASCO Annual Meeting I

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

Precentabart tocentecan (M9140), an anti-CEACAM5 ADC with exatecan payload, in patients with metastatic colorectal cancer (mCRC): Results from the dose optimization of the phase 1 PROCEADE CRC-01 study.

Authors: [Scott Kopetz](#), [Rocio Garcia-Carbonero](#), [Sae-Won Han](#), [Ildefonso Ismael Rodriguez Rivera](#), [Jose Carlos Ruffinelli](#), [Valentina Boni](#), [Maria Vieito Villar](#), ... [SHOW ALL ...](#) , and [Ken Kato](#) | [AUTHORS INFO & AFFILIATIONS](#)

Publication: Journal of Clinical Oncology • Volume 43, Number 16 suppl • https://doi.org/10.1200/JCO.2025.43.16_suppl.3038

224

**Recruiting** ⓘ

Anti-CEACAM5 ADC M9140 in Advanced Solid Tumors (PROCEADE-CRC-01)

ClinicalTrials.gov ID ⓘ NCT05464030**Sponsor** ⓘ EMD Serono Research & Development Institute, Inc.**Information provided by** ⓘ EMD Serono (EMD Serono Research & Development Institute, Inc.) (Responsible Party)**Last Update Posted** ⓘ 2025-07-25

CEACAM5

[Home](#) > [Our Research & Development](#) > [CEACAM5](#)

Antibody-Drug Conjugate (ADC)

CEACAM5

Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) is a cell surface glycoprotein that is overexpressed on tumor cells and is associated with tumor differentiation, invasion, and metastasis.^{1,2} While expression of CEACAM5 is high in several tumor types, such as gastric, colorectal, and pancreatic cancers, expression in normal epithelial tissues is low.^{3,4} Thus, CEACAM5 presents a potential target for anticancer therapy.

M9140 – CEACAM5 ADC

M9140 is an investigational CEACAM5 ADC that is designed to deliver a cytotoxic topoisomerase 1 (TOP1) inhibitor payload to CEACAM5-expressing tumor cells.⁵⁻⁷ The TOP1 inhibitor payload enters the nucleus and disrupts DNA replication and repair, thereby killing CEACAM5-expressing tumor cells.⁵⁻⁷ The payload can also enter and kill neighboring tumor cells via a bystander effect.⁵



CEA = Carcinoembryonic Antigen

CEA

From tool to target- rebirth of an old marker

Thank you for your attention!



Let's connect



Rasmus Røge
Jette Boysen Møller
Heidi Lykke Kristoffersen
Louise Rønnow Holler
Charlotte Papior Carstensen
Tanya Julio
Jacqueline Capion Lemming
Mette Skov Mikkelsen
Lilli Guldager Malaca
Carsten Munck Malmgaard
Jesper Lund Lauridsen
Kristi Bøgh Anderson



AALBORG UNIVERSITY
HOSPITAL DENMARK

With deep appreciation and gratitude to **Søren Nielsen**

