

IHC in the time of molecular era –

Predictive, diagnostic
and prognostic markers

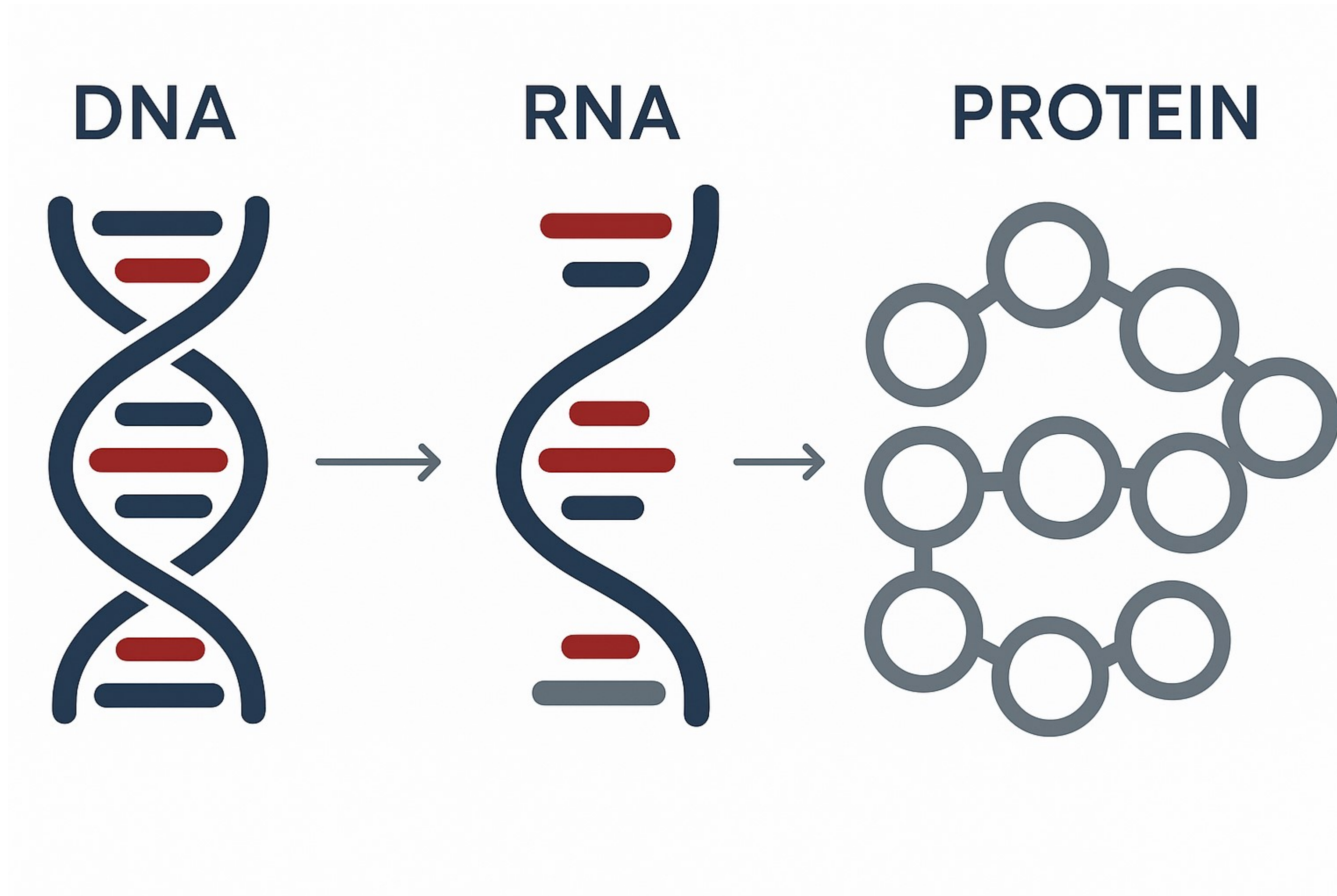
Kristi Bøgh Anderson MD, Scientific Associate @NordiQC

kristi.anderson@rn.dk

Søren Nielsen, Director @NordiQC

Henrik Hager MD, Assessor @NordiQC

Central dogma



GOOD COP

Tumor suppressor gene



BAD COP

Oncogene



Oncogene vs tumor suppressor gene

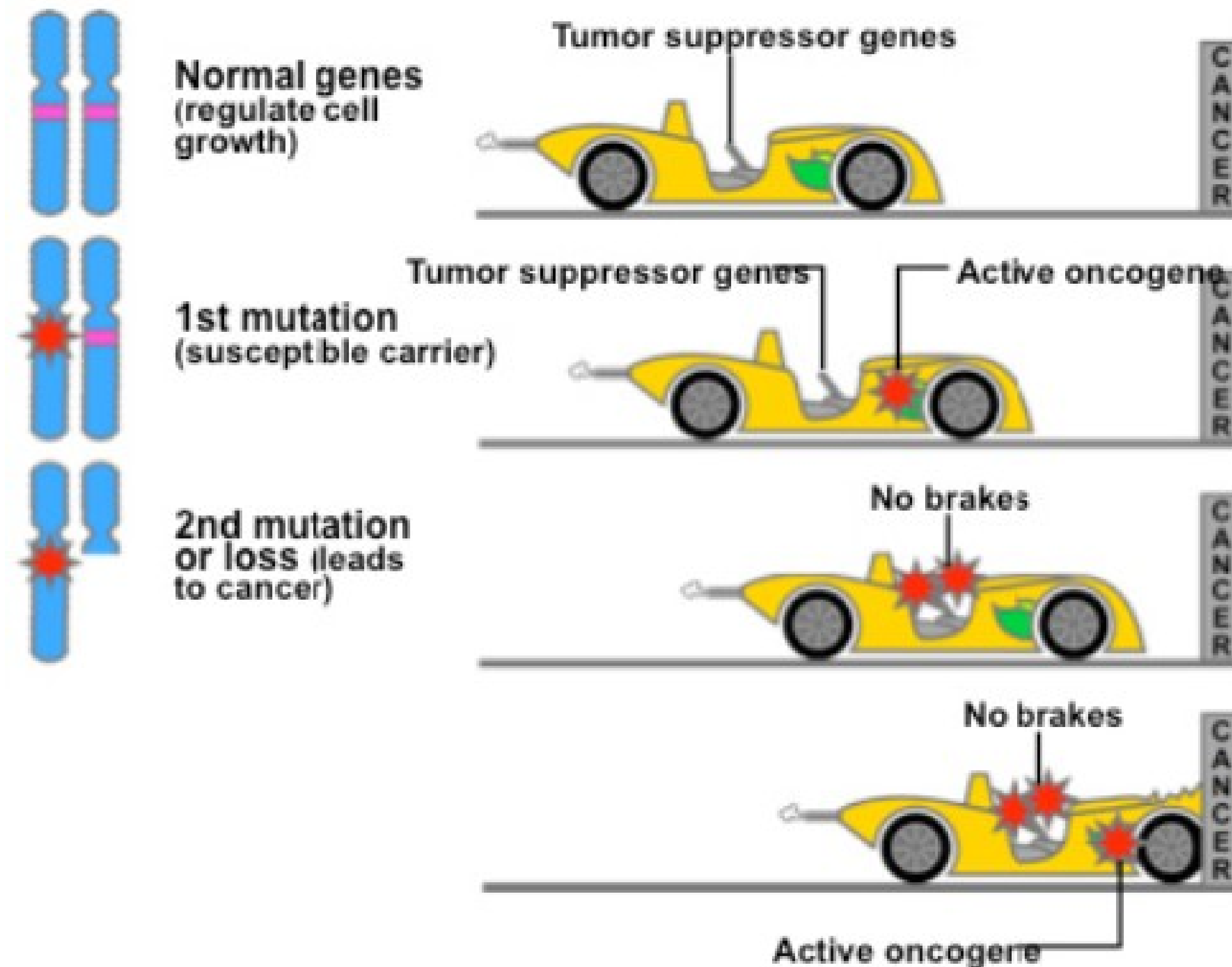


Image courtesy of the National Cancer Institute

Line of differentiation

Epithelial

Smooth muscle

Skeletal muscle

Melanocytic/
nerve sheath lineage

Vascular

Hematopoietic

Immunohistochemistry

Keratins

SMA, Desmin

Desmin, Myogenin,
MyoD1

S100, SOX10

CD31, ERG

CD45

Molecular Methods

Mutation

Translocation

Deletion

Amplification

Methylation

Immunohistochemistry

Changed protein

Absence of protein

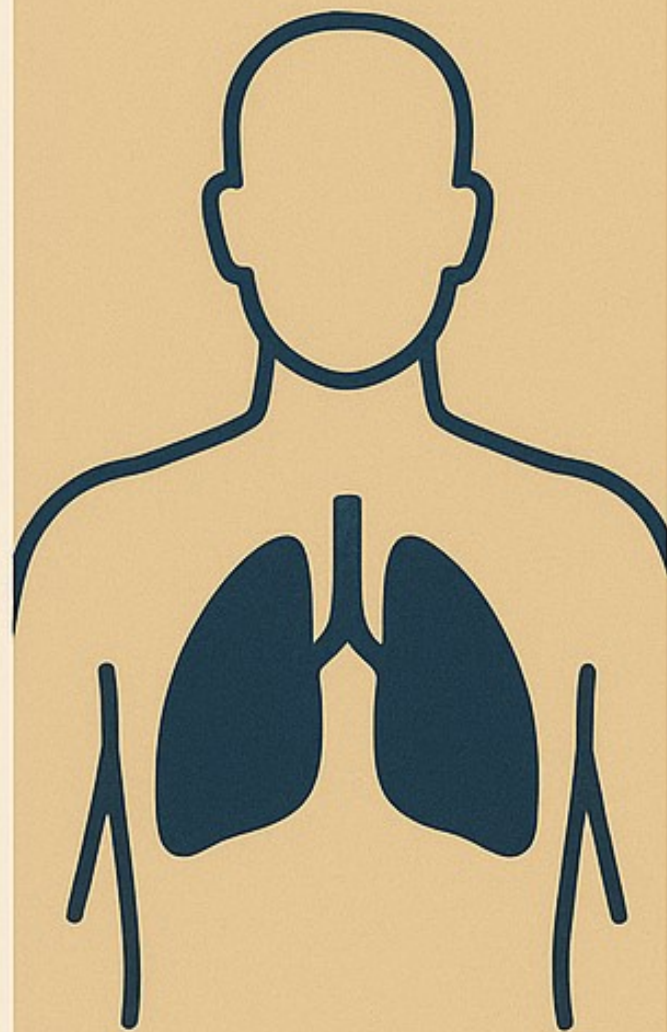
Abnormal localization

Over expression

Fusion protein



DIAGNOSTIC



PROGNOSTIC

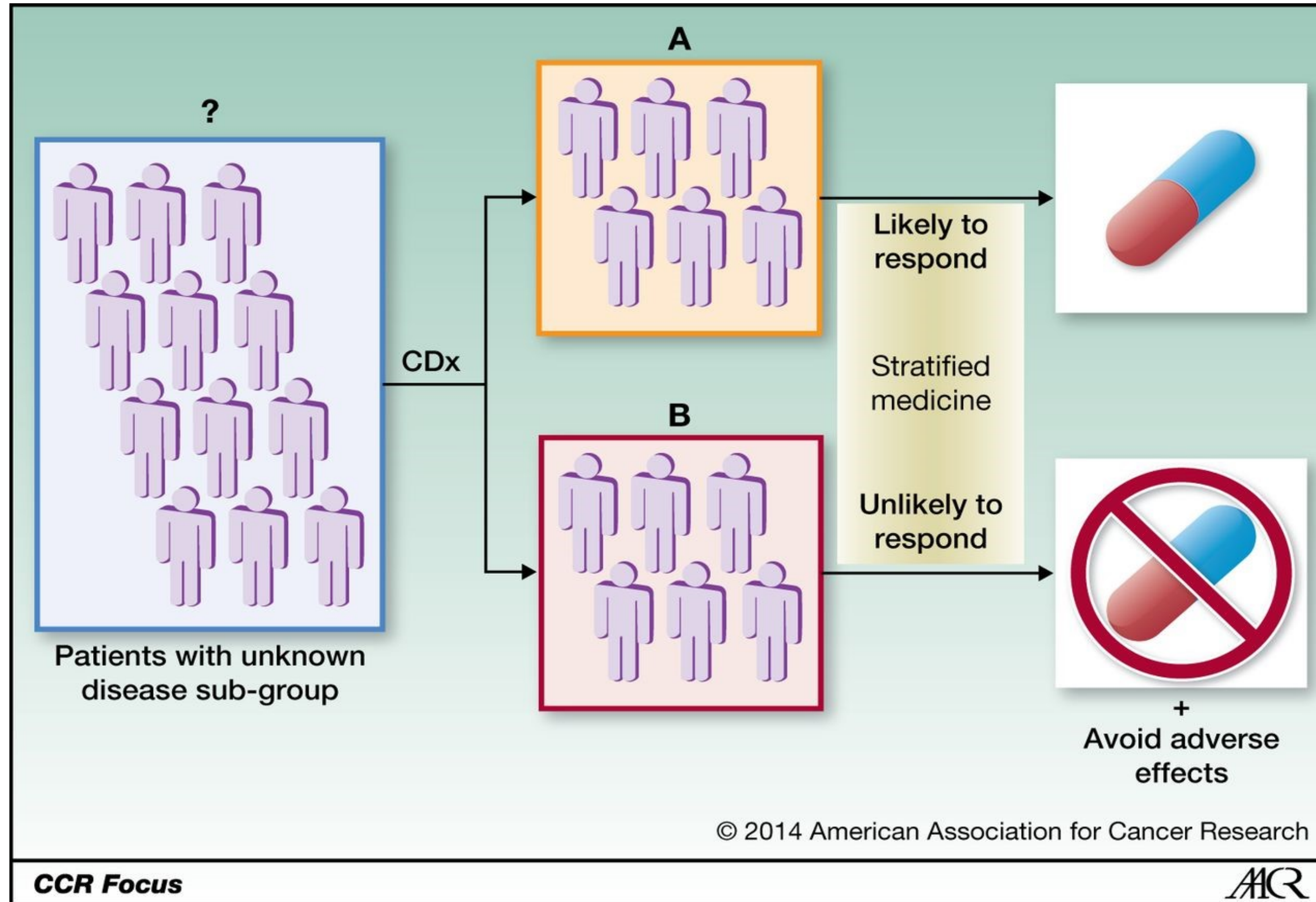


PREDICTIVE



Drug and biomarker alignment

CDx assays for personalized medicine



What is CDx (Companion diagnostics) and why to use it?

Definition*

CDx is a medical device, often in vitro, that provides information essential for safe and effective use of a drug or biological product

Application*

- Identify those patients most likely to benefit from treatment
- Identify patients at increased risk of adverse events
- Monitor treatment response and adjust dose/regimen
- Identify populations with adequate safety and efficacy data

* Per the US Food and Drug Administration and EU European Medicines Agency

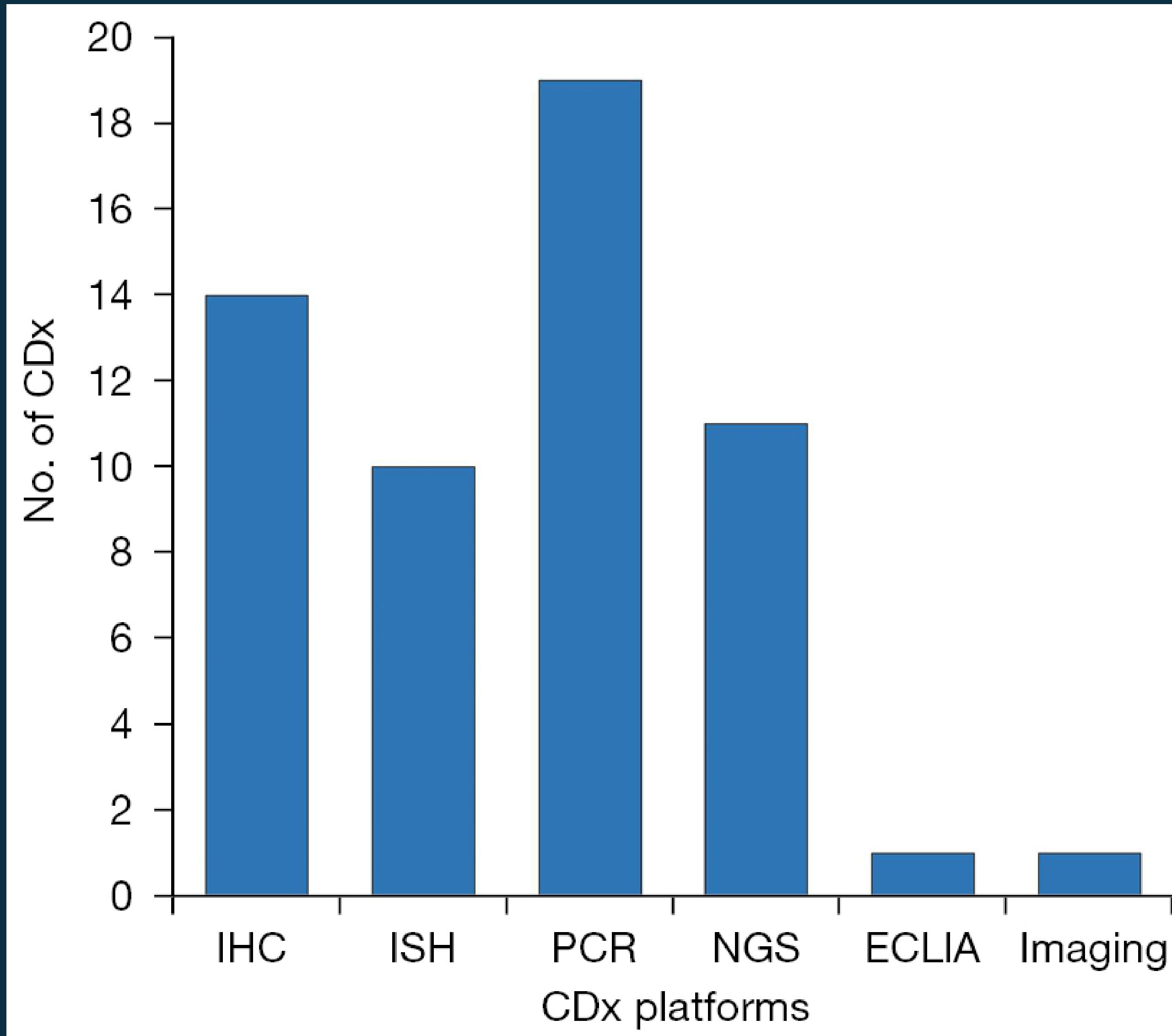


Figure FDA approved companion diagnostic platforms by August 2023 ([11](#)). IHC, immunohistochemistry; ISH, in situ hybridization; PCR, polymerase chain reaction; NGS, next-generation sequencing; ECLIA, electrochemiluminescence immunoassay; CDx, companion diagnostic; FDA, Food and Drug Administration.

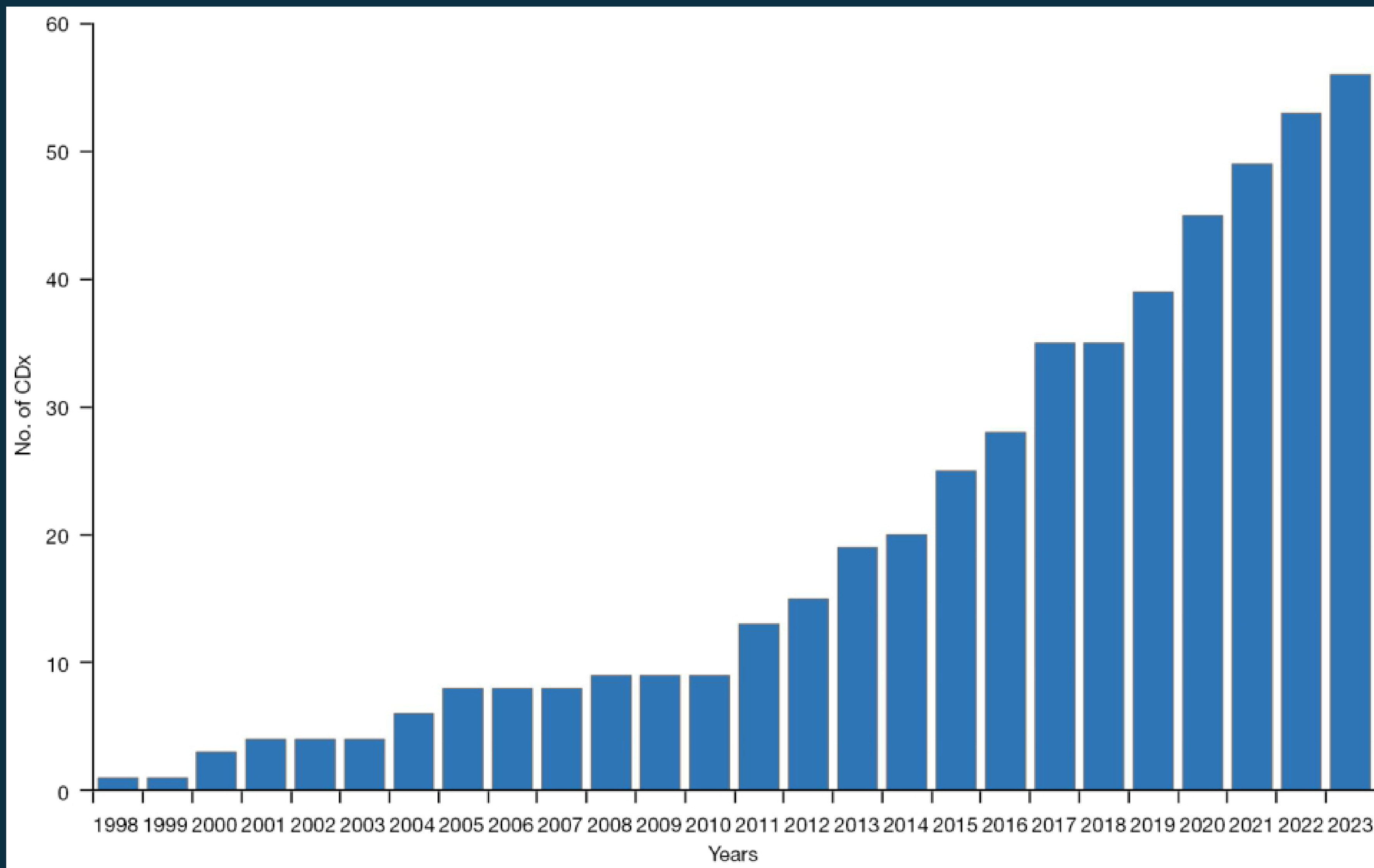
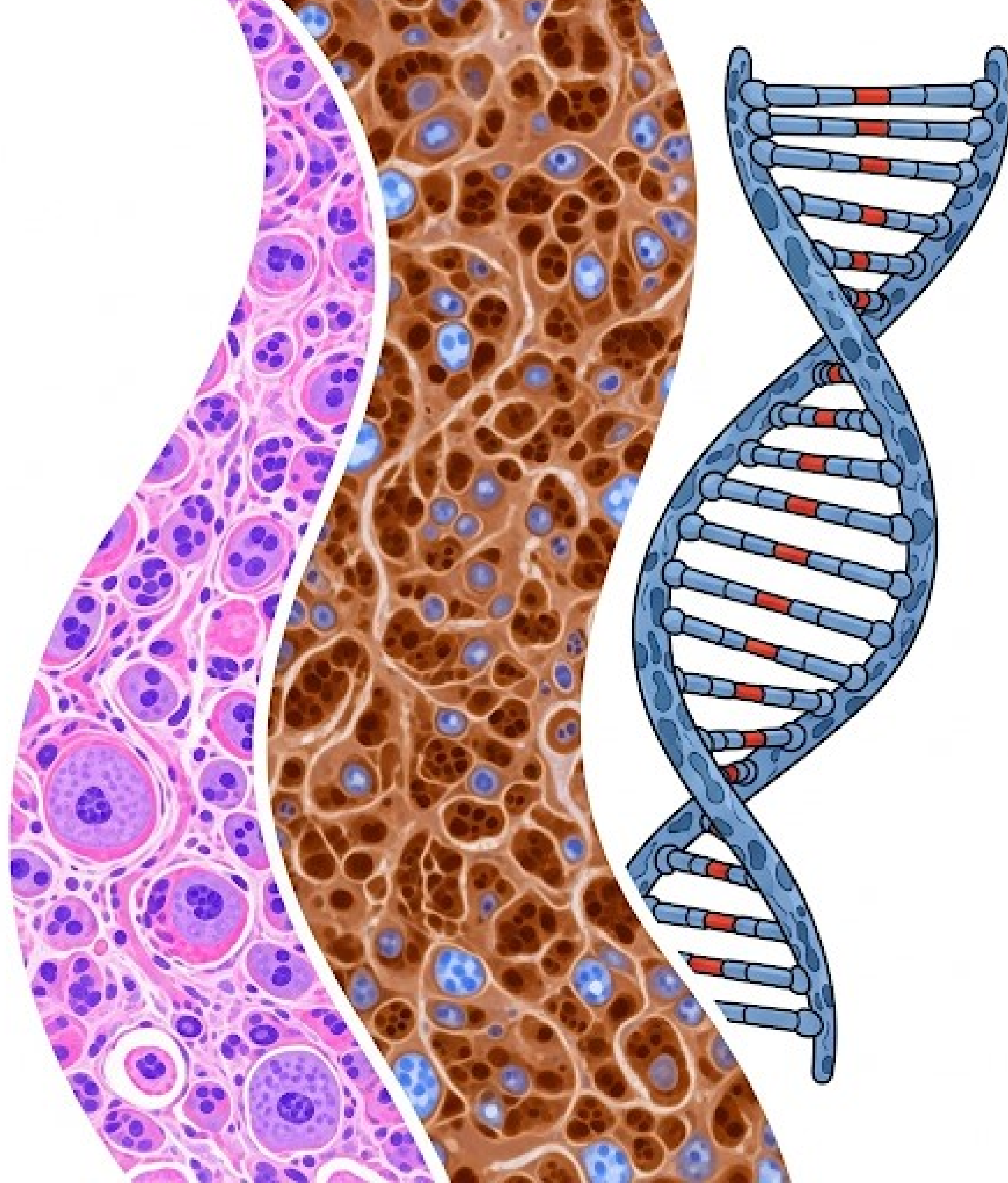


Figure Cumulative number of FDA approved companion diagnostic assays by year. By August 2023, the total number of companion diagnostic assays had reached 56. CDx, companion diagnostics; FDA, Food and Drug Administration.



Molecular Methods

Mutation

Translocation

Deletion

Amplification

Methylation



Immunohistochemistry

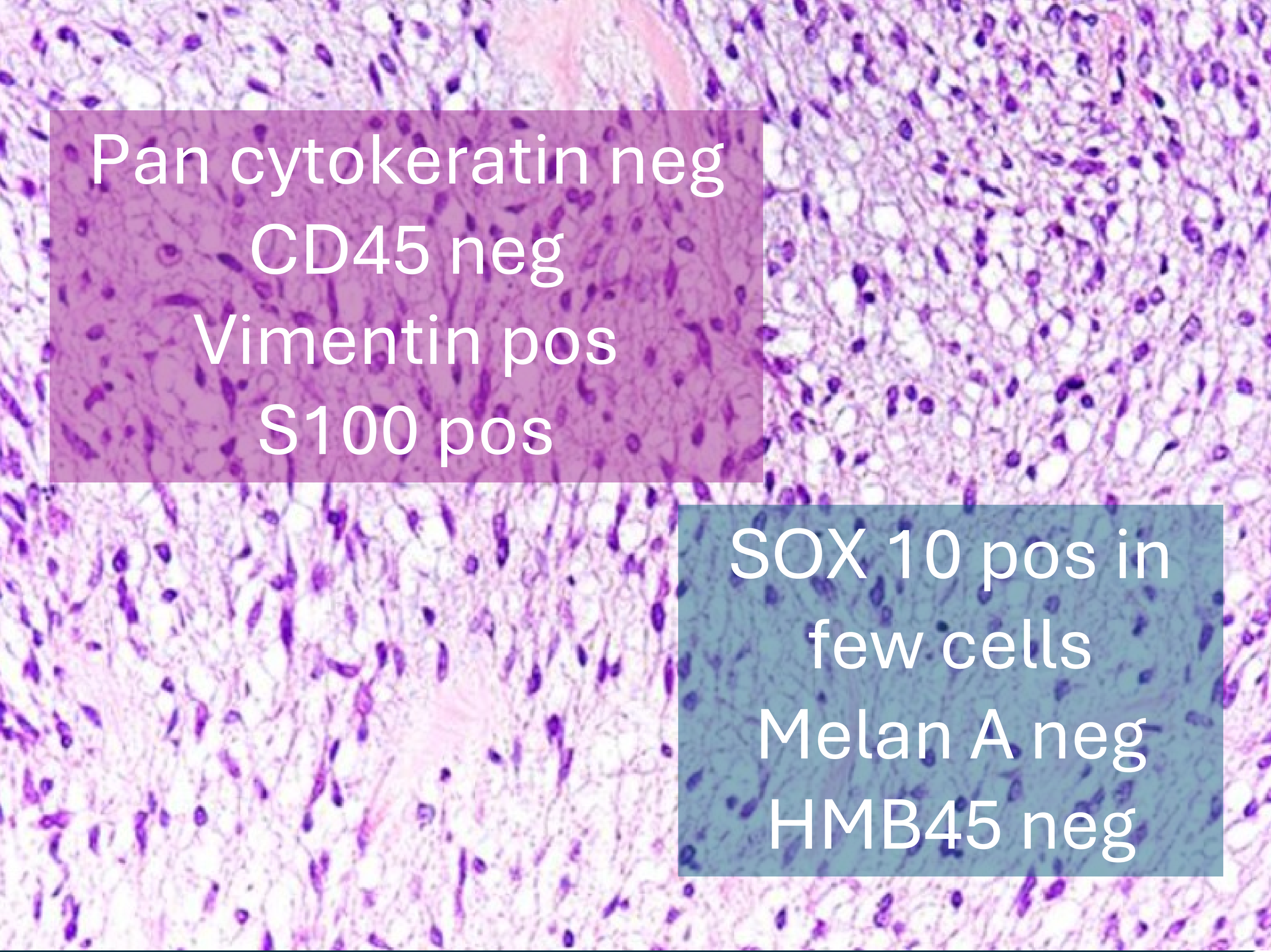
Changed protein

Absence of protein

Abnormal localization

Over expression

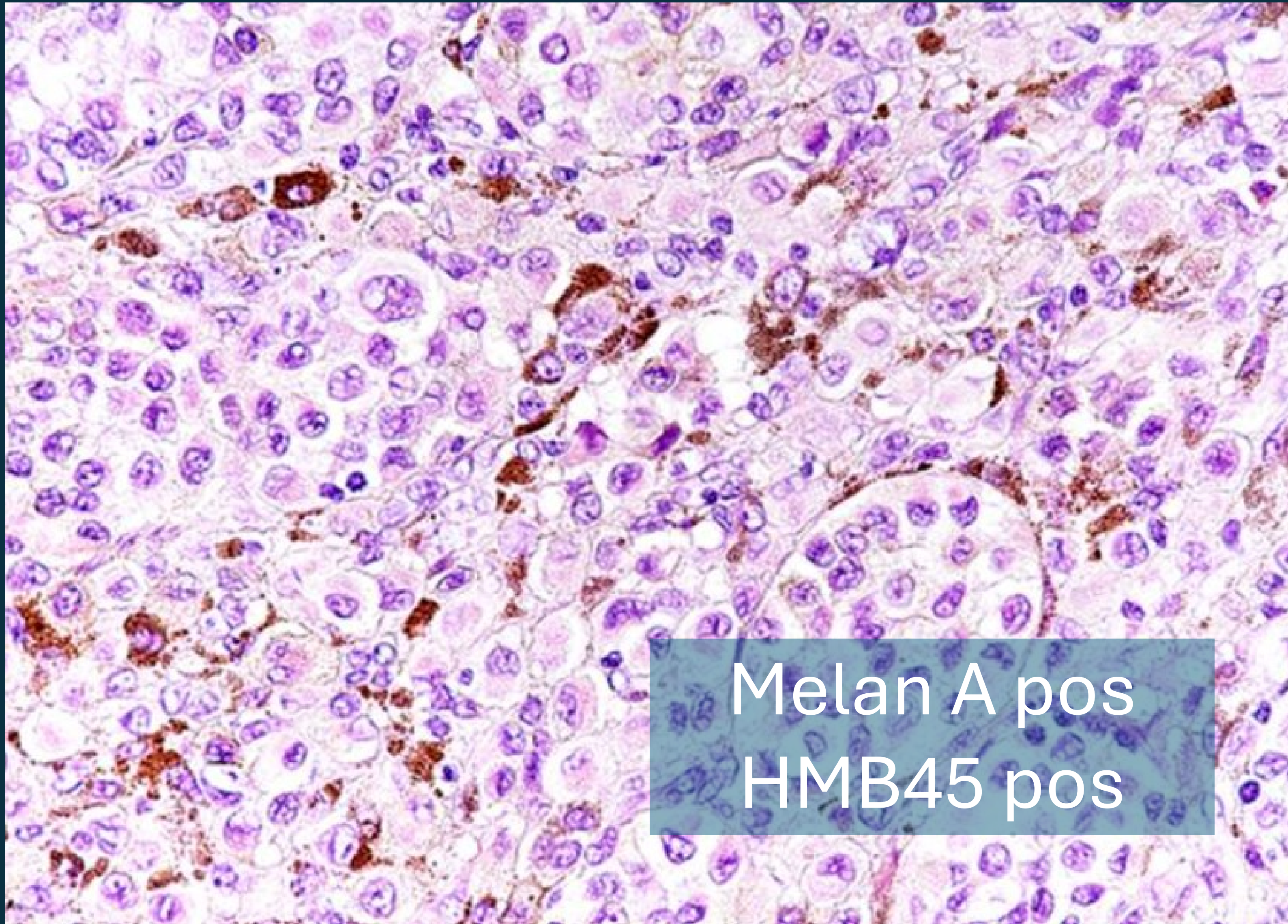
Fusion protein



Pan cytokeratin neg
CD45 neg
Vimentin pos
S100 pos

The background image is a histological slide showing a dense population of cells with purple nuclei and pink cytoplasm/extracellular matrix. A semi-transparent purple box is overlaid on the left side, and a semi-transparent teal box is overlaid on the right side.

SOX 10 pos in
few cells
Melan A neg
HMB45 neg



Melan A pos
HMB45 pos



Original Article

***BRAF* mutation in ‘sarcomas’: a possible method to detect de-differentiated melanomas**

Nicole A Cipriani ✉, Igor Letovanec, Francis J Hornicek, John T Mullen, Zhenfeng Duan, Darrell R Borger, Gunnlaugur Petur Nielsen

First published: 05 October 2013 | <https://doi.org/10.1111/his.12305> | Citations: 34



PDF



TOOLS



SHARE

Kamata M, Minamisaka T, Imura J, Saitoh K, Shimomura A, Noguchi A. A Case of Dedifferentiated Melanoma With Lymph Node Metastasis Where Molecular Biological Tests Were Useful for Diagnosis. Cureus. 2022 Jan 26;14(1):e21644. doi: 10.7759/cureus.21644. PMID: 35233321; PMCID: PMC8881242.

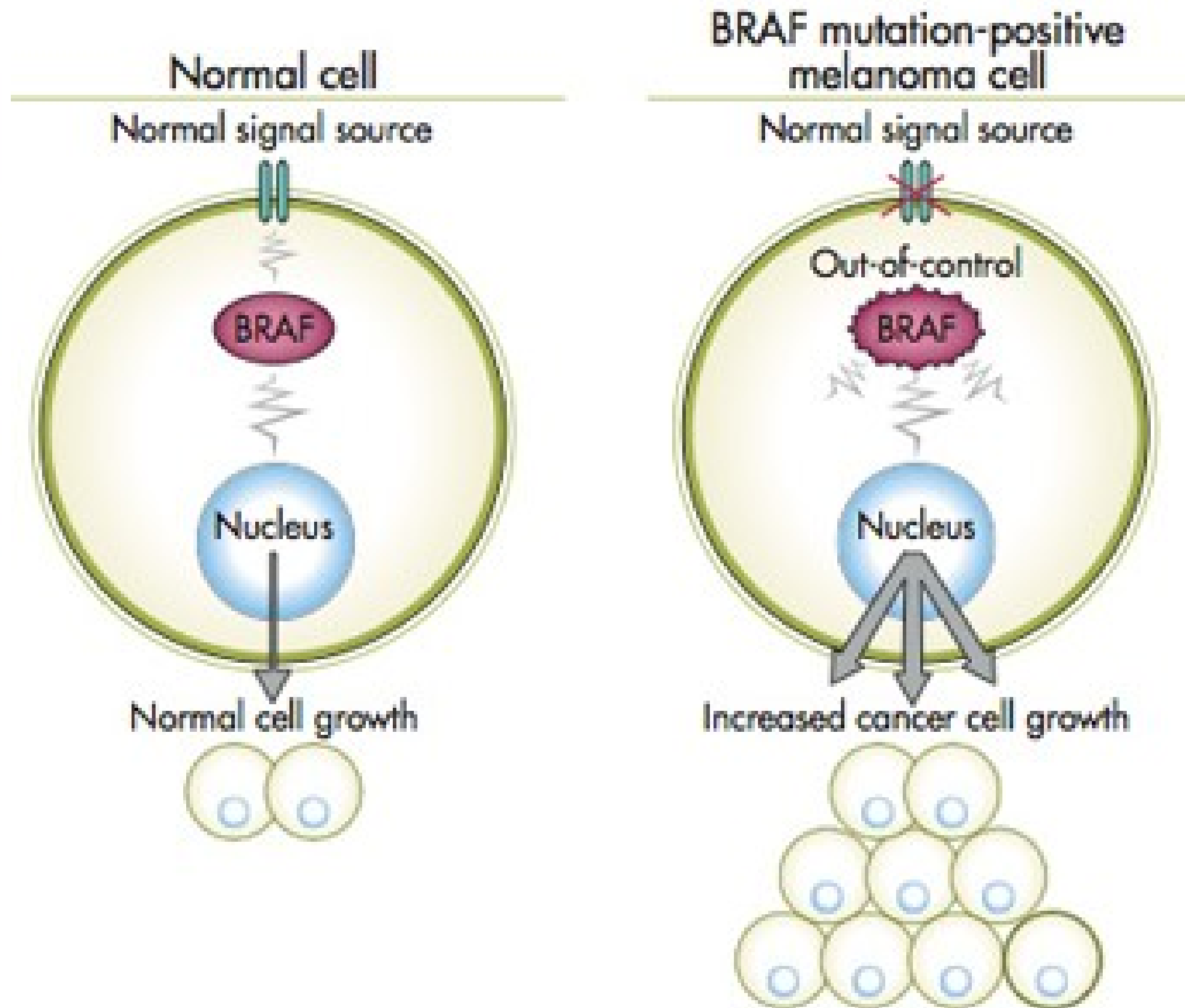
What to Know About BRAF Mutations

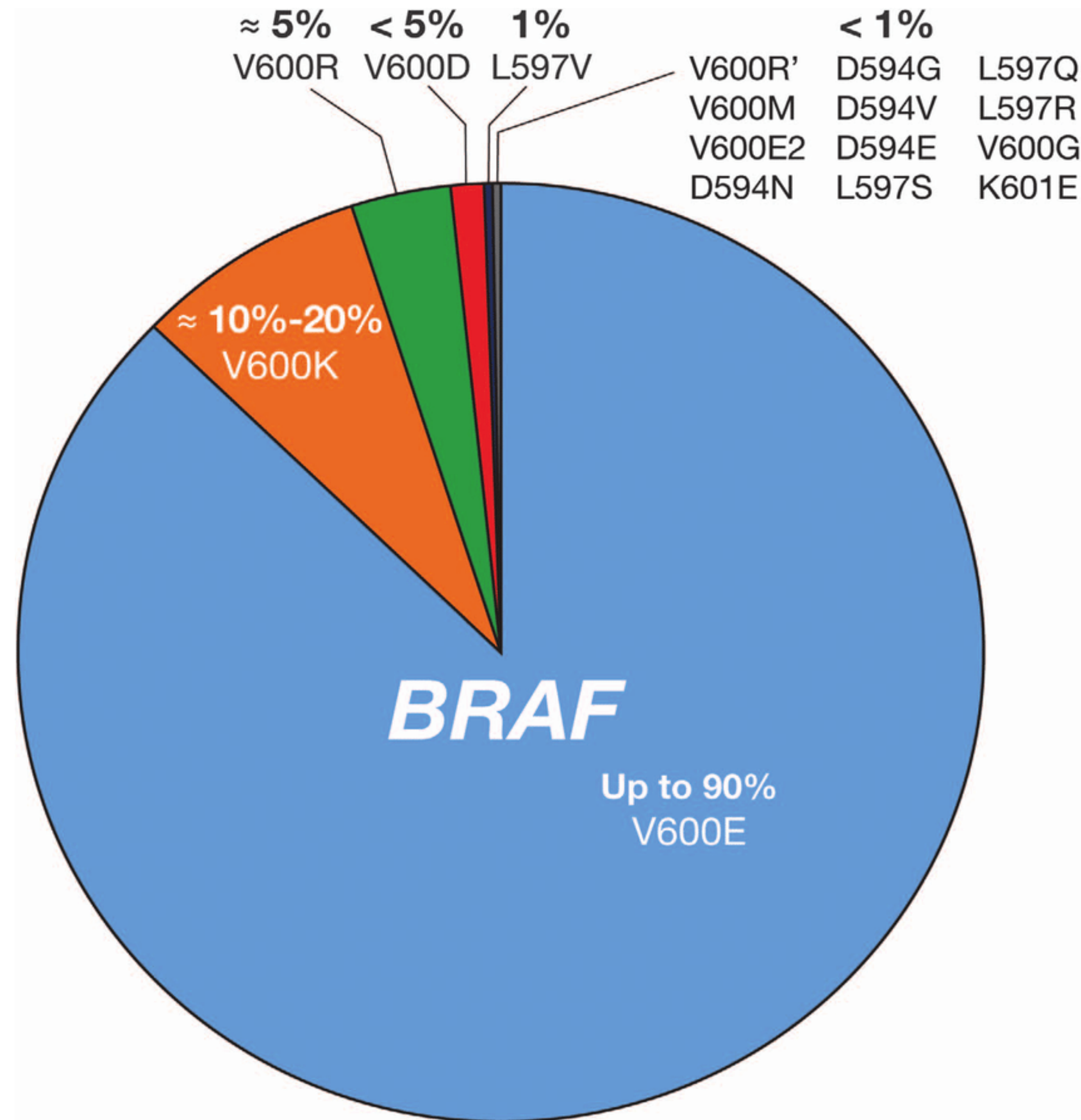
The BRAF gene is responsible for cell growth

A BRAF gene mutation can contribute to cancer development

Found in almost half of melanomas

Targeted therapy and/or immunotherapy may be considered



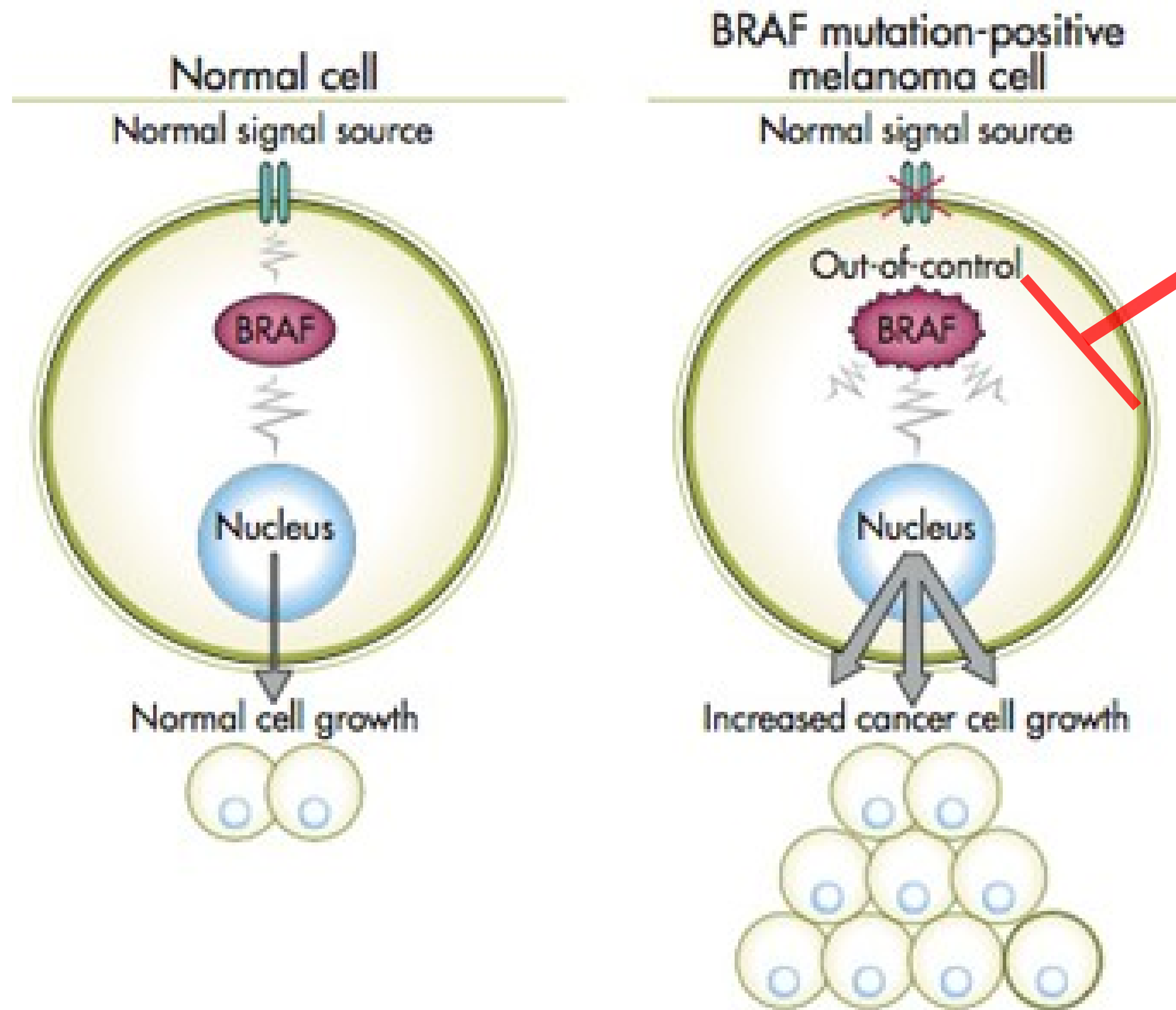


WT: GTG (valin)

V600E: GAG (glutamat)

V600K: AAG (lysin)

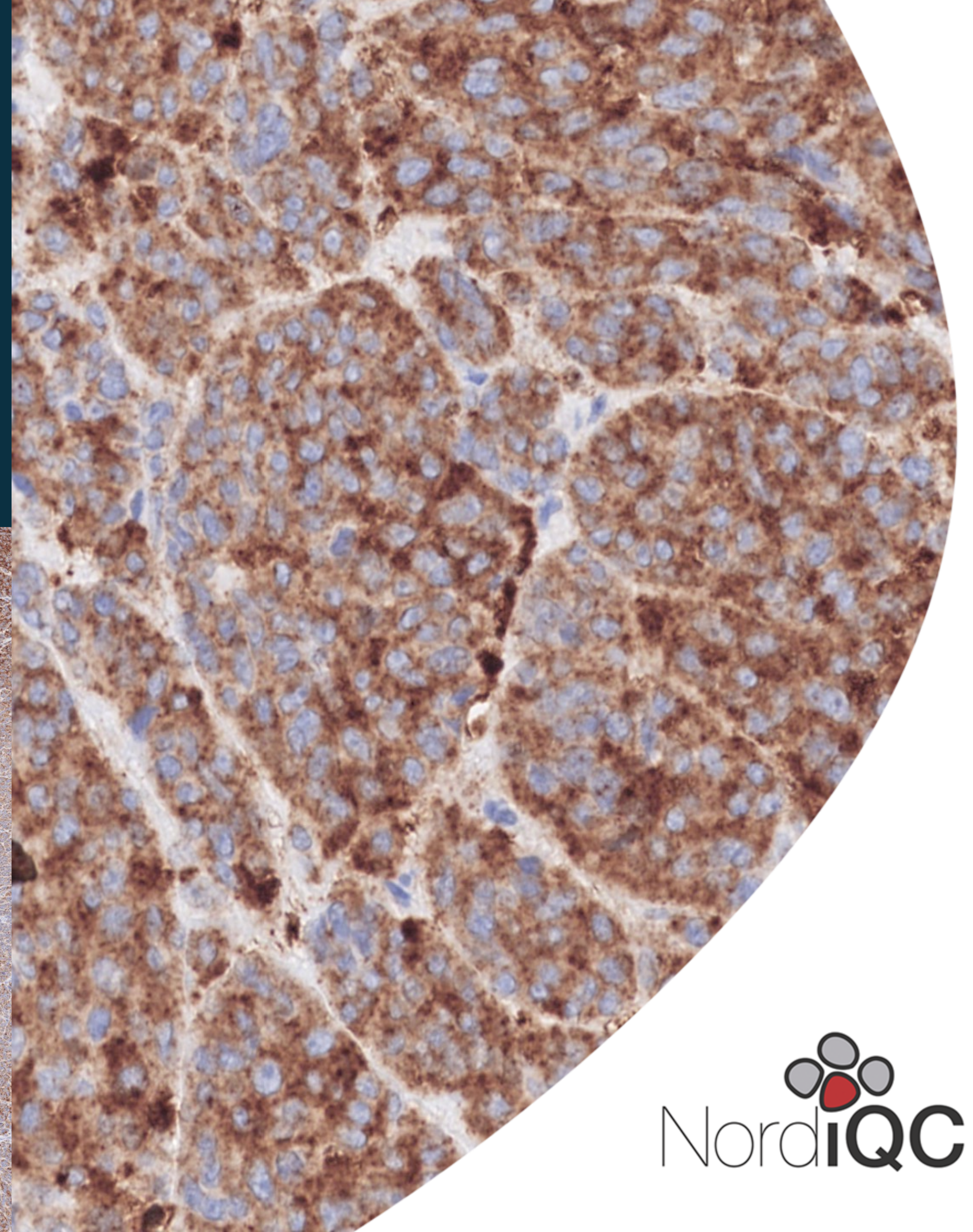
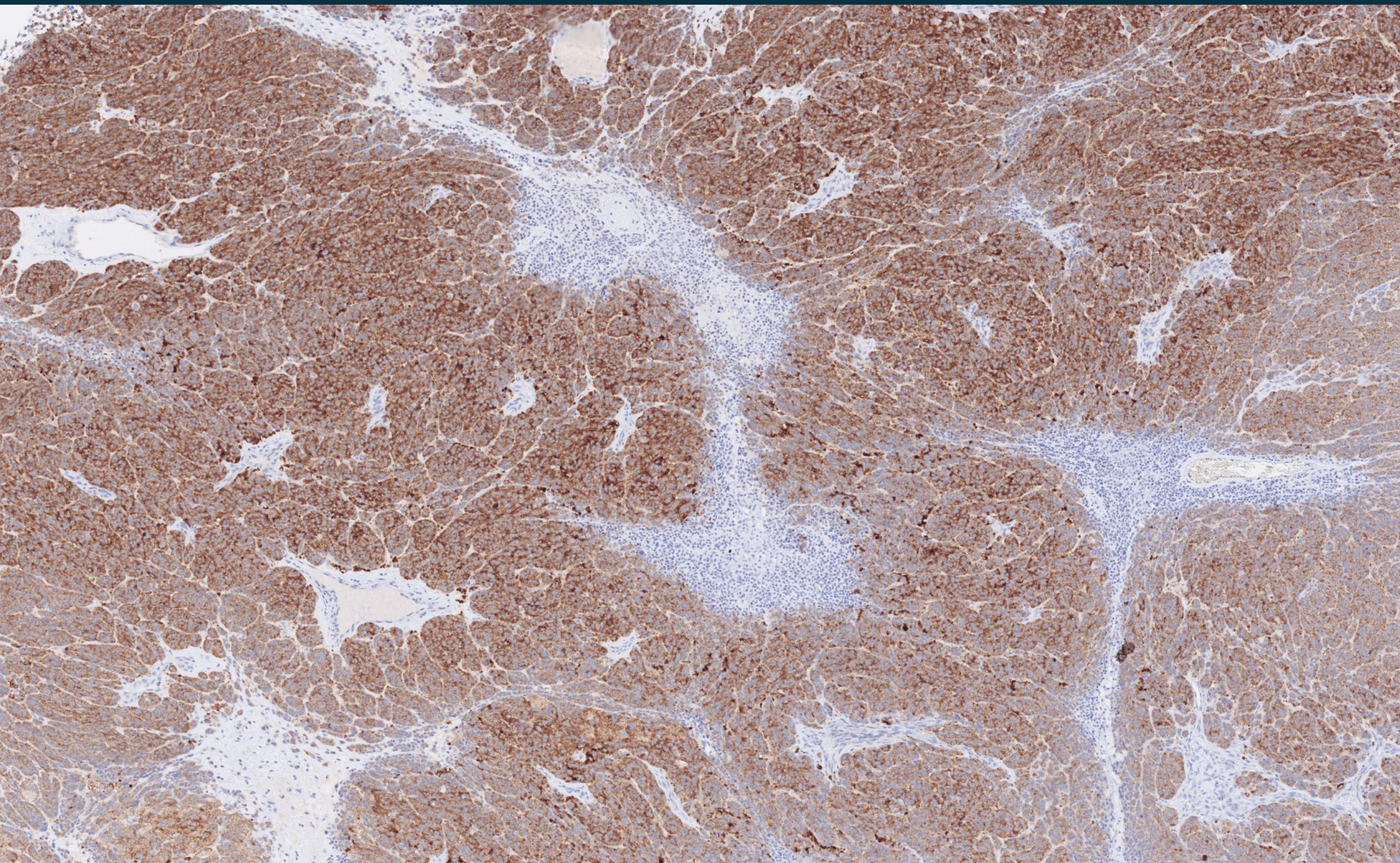
V600R: AGG (Arginin)



Vemurafenib



IHC BRAF V600E



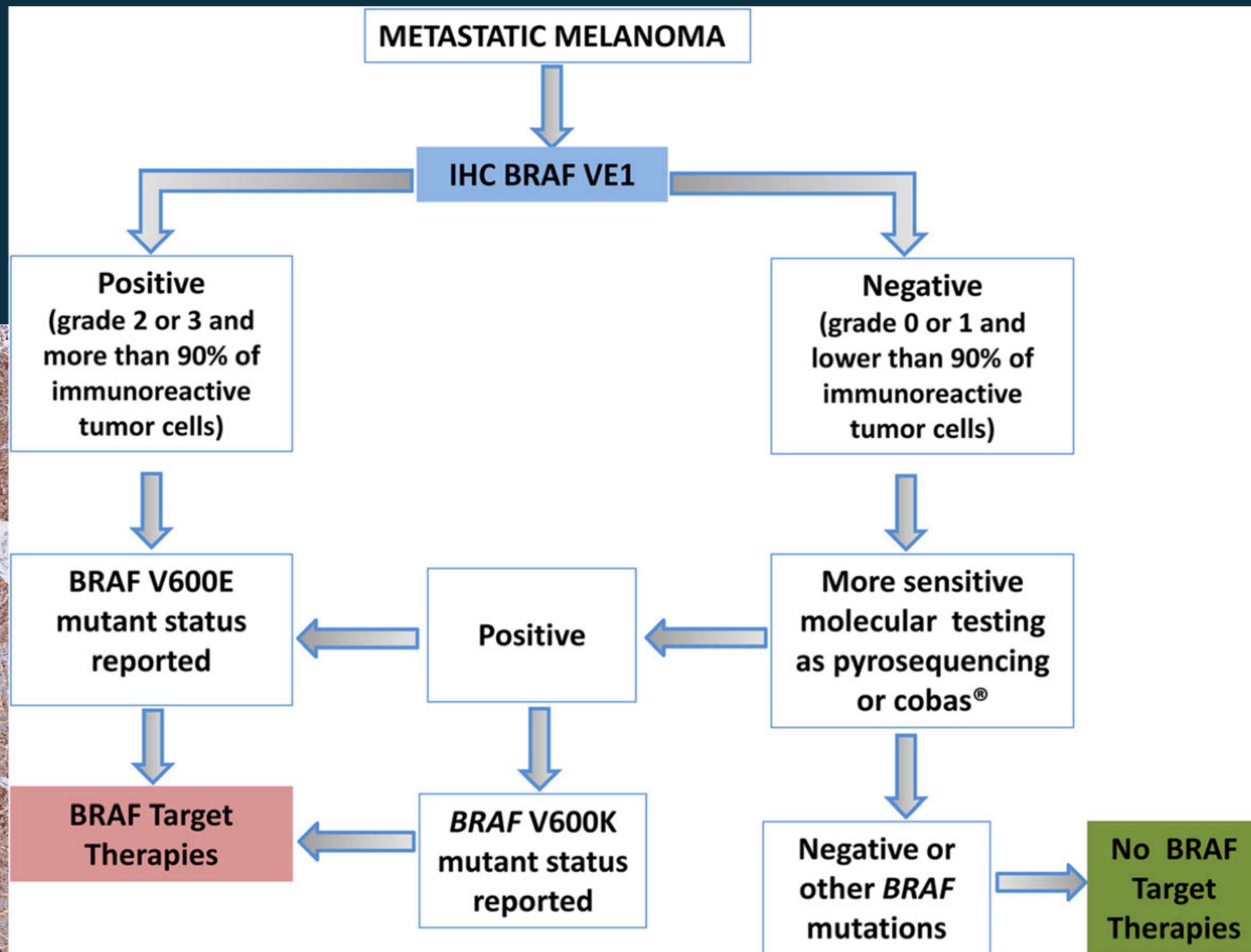
IHC BRAF V600E

Assessment Run 74 2025
BRAF (BRAF V600E)

KEY POINTS FOR BRAF V600E IMMUNOASSAYS

- A high overall pass rate of 81% was observed in this 2' assessment focusing on BRAF V600E mutations in colon adenocarcinomas and melanomas.
- The mAb clone **VE1** was used by 91% of all participants.
- The VE1 **RTU** system 760-5095 from Ventana/Roche applied by vendor recommended protocol settings was most successful giving pass rates of 97%.
- The performance of the Ventana/Roche VE1 RTU system 760-5095 was also found successful when optimized by using OptiView with amplification.

IHC BRAF V600E



Oncogene specific antibodies

Gene	Mutation (antibody clone)	Tumor Type	Detection method
BRAF	V600E (VE1)	Melanoma and many others	IHC /Molecular
EGFR	L858R (43B2, SP125 &GFR/4564R), E746-A750del (D6B6)	NSCLC	Molecular (IHC)
IDH1	R132H (H09, IMab-1)	Gliomas	IHC / Molecular
KRAS	G12D (HL10) G12C (AbAbKRAS01)	NSCLC, Colorectal carcinoma, Pancreatic carcinoma	Molecular
NRAS	Q61R (SP174)	Melanoma, Colorectal carcinoma, acute myeloid leukemia	Molecular
H3F3A	K27M (H3K27me3)	Diffuse intrinsic pontine glioma	IHC / Molecular
H3F3A	G34W (RM263)	Giant cell tumor of bone	IHC/ Molecular
H3F3B	K36M (RM193)	Chondroblastoma	IHC / Molecular
....	...	...	

Molecular Methods

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Amplification

Methylation

Immunohistochemistry

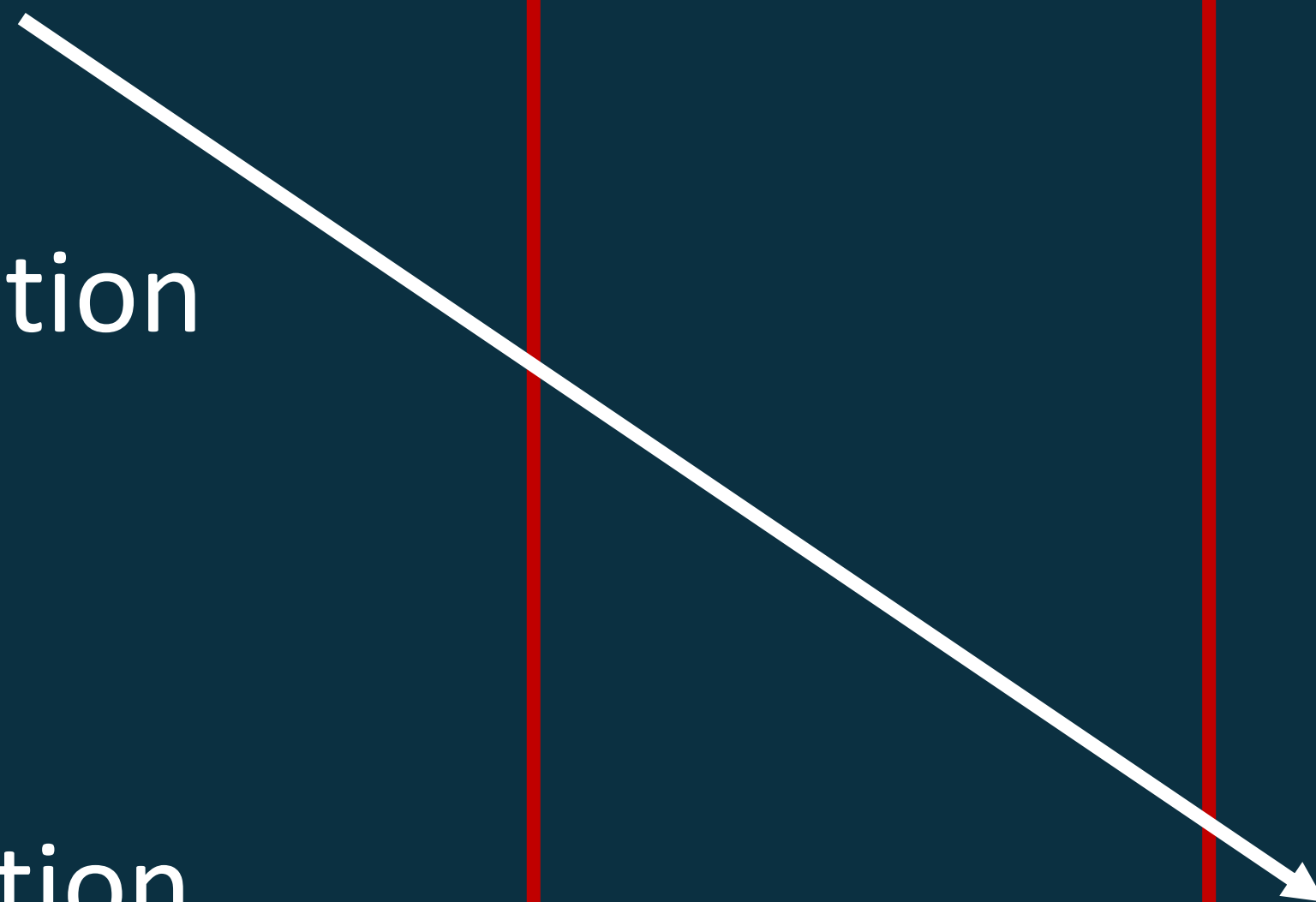
Changed protein

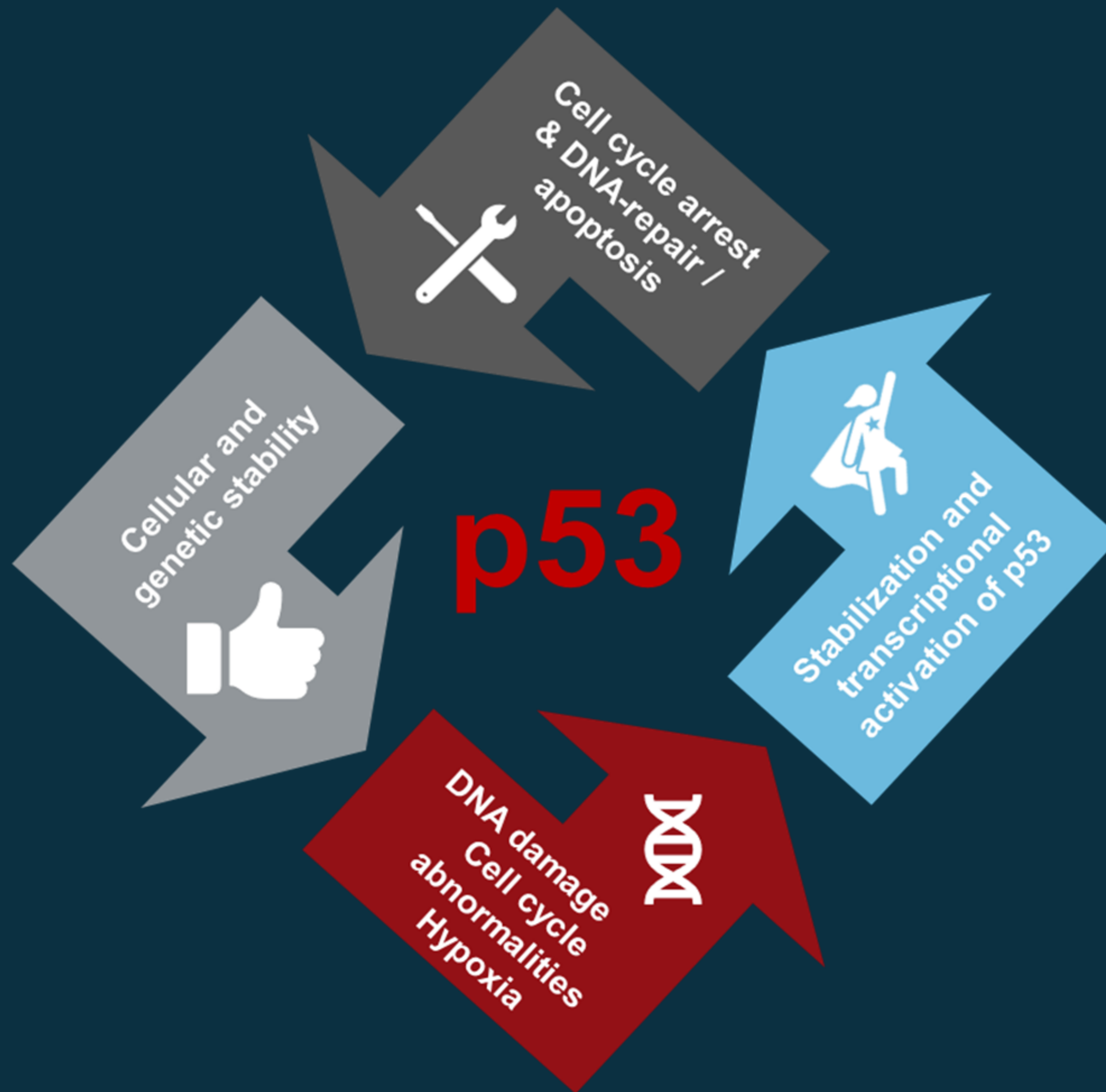
Absence of protein

Abnormal localization

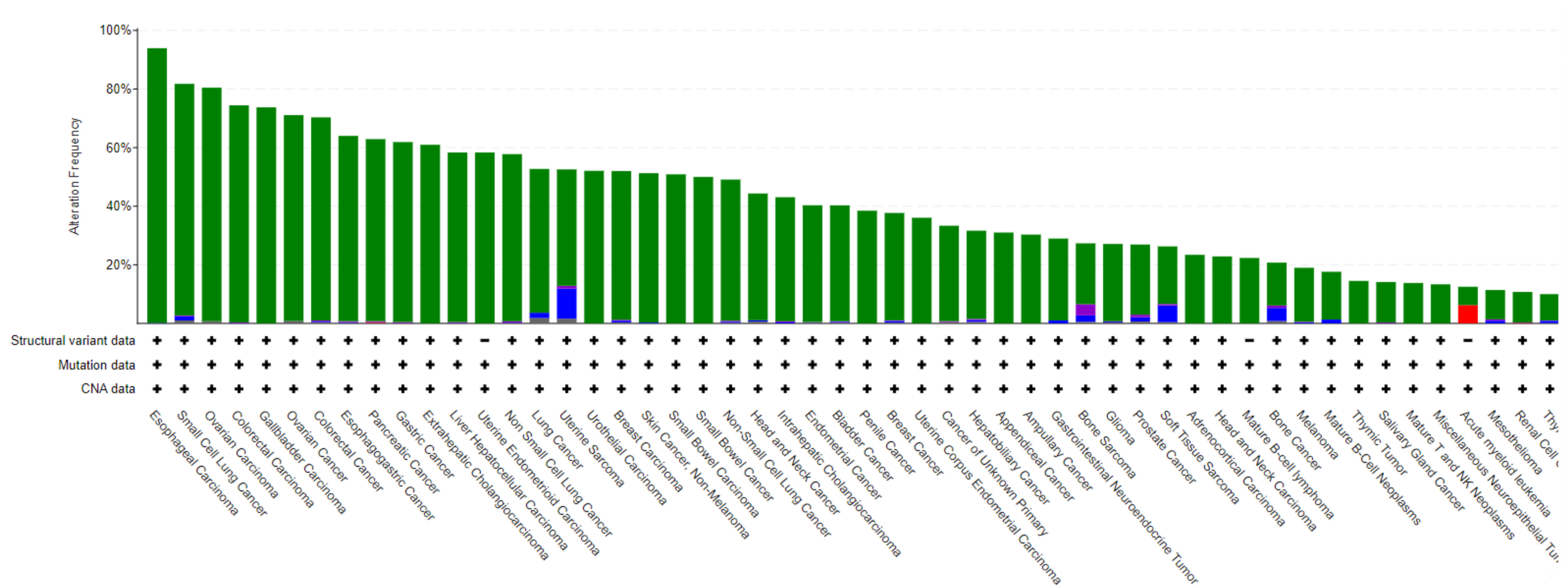
Over expression

Fusion protein





TP53 is mutated in 50% of all cancer

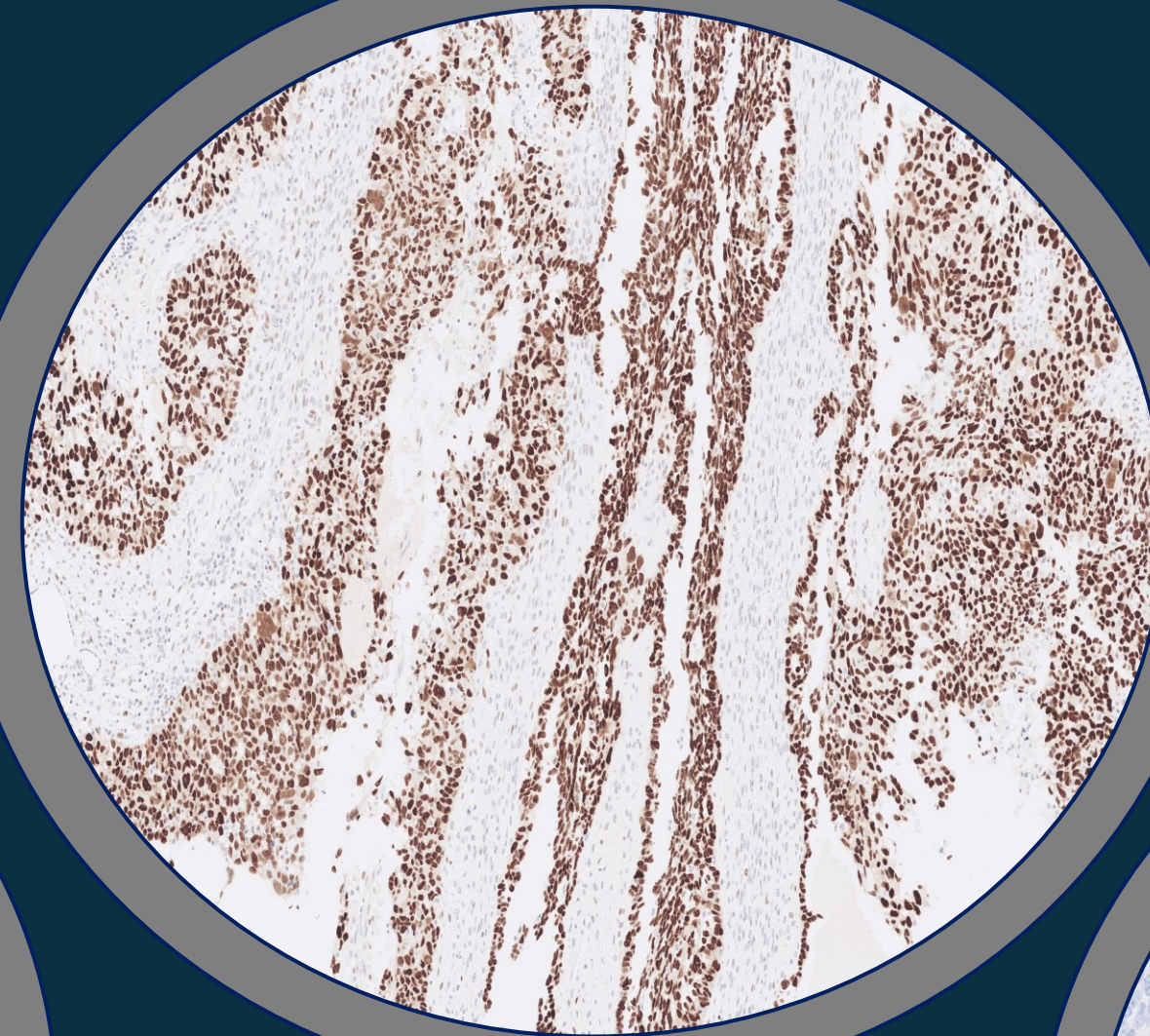
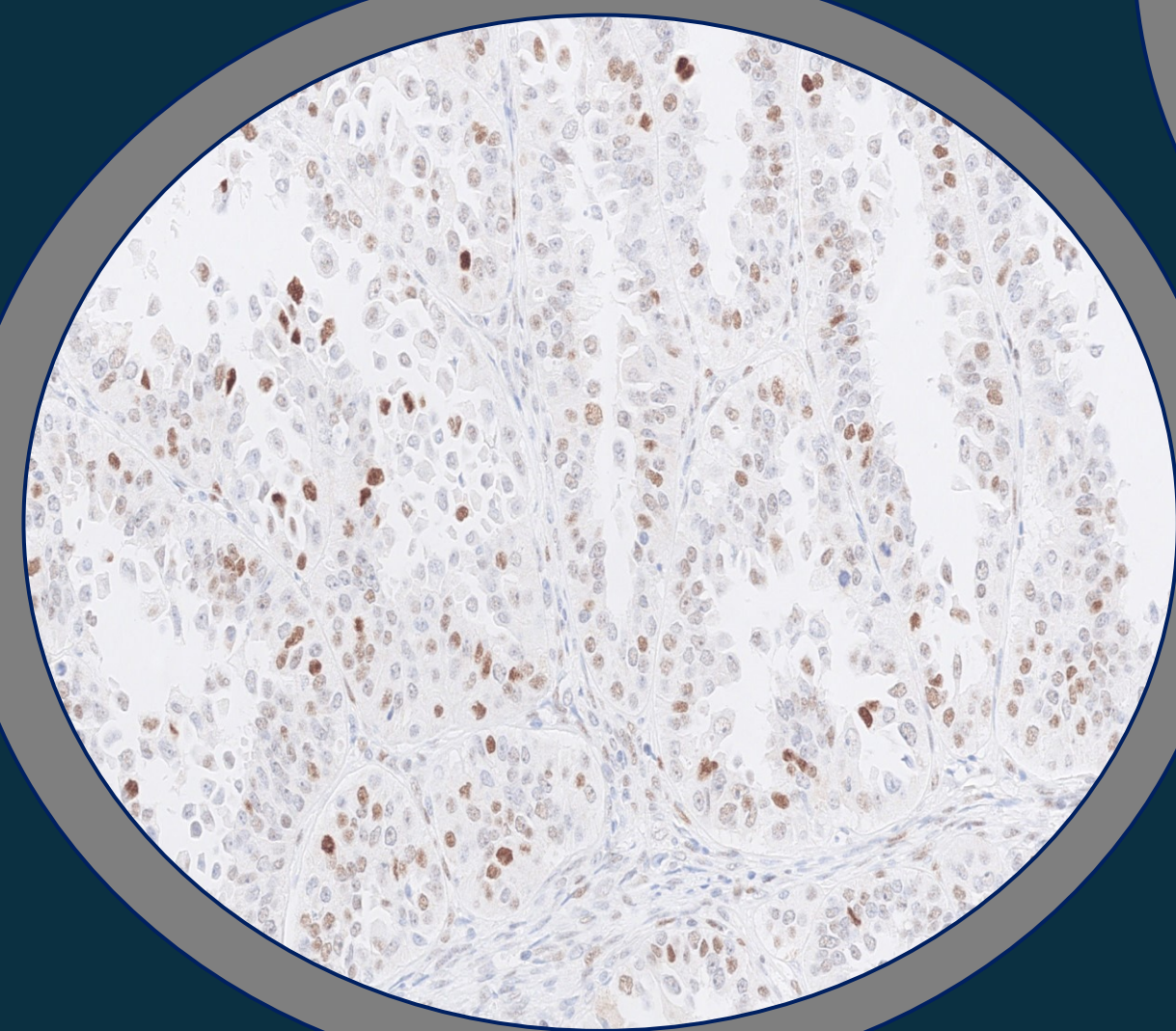


Overexpression

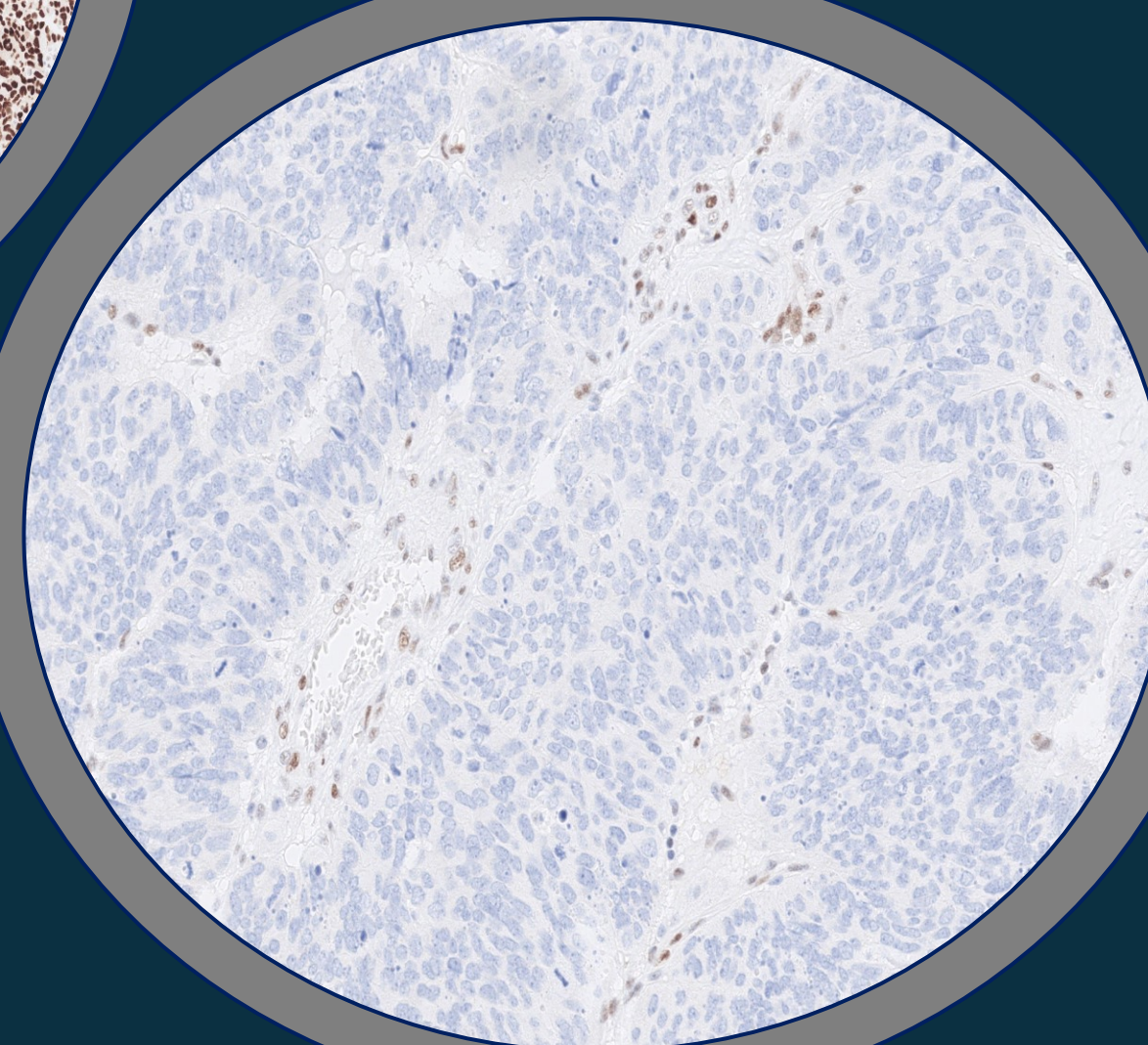
Cytoplasmatic p53

**Wild
type**

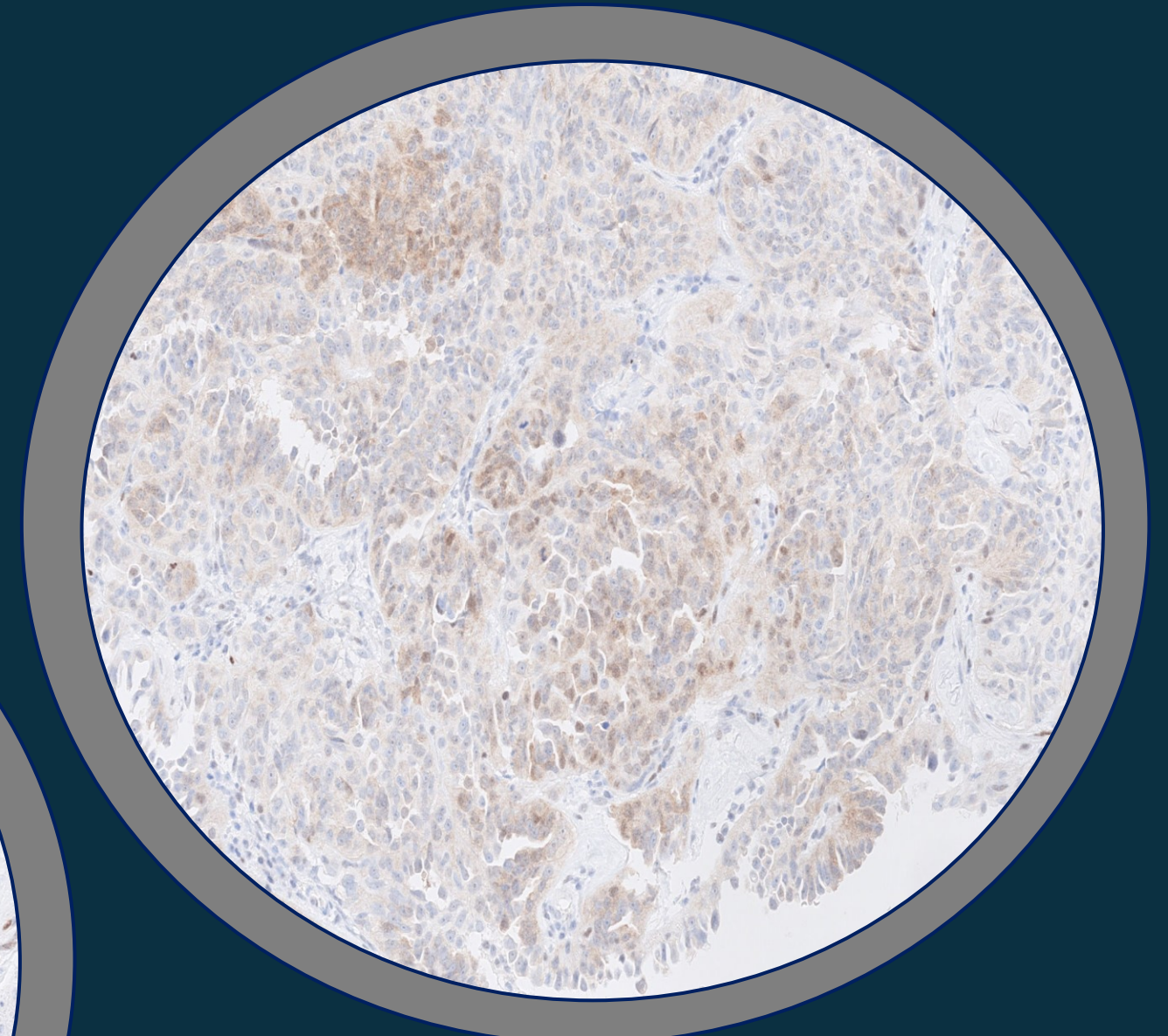
0-phenotype



TP53 missense
mutation

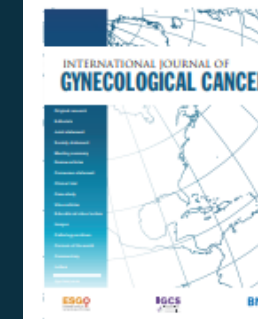


TP53 mutations resulting in
absent p53 protein



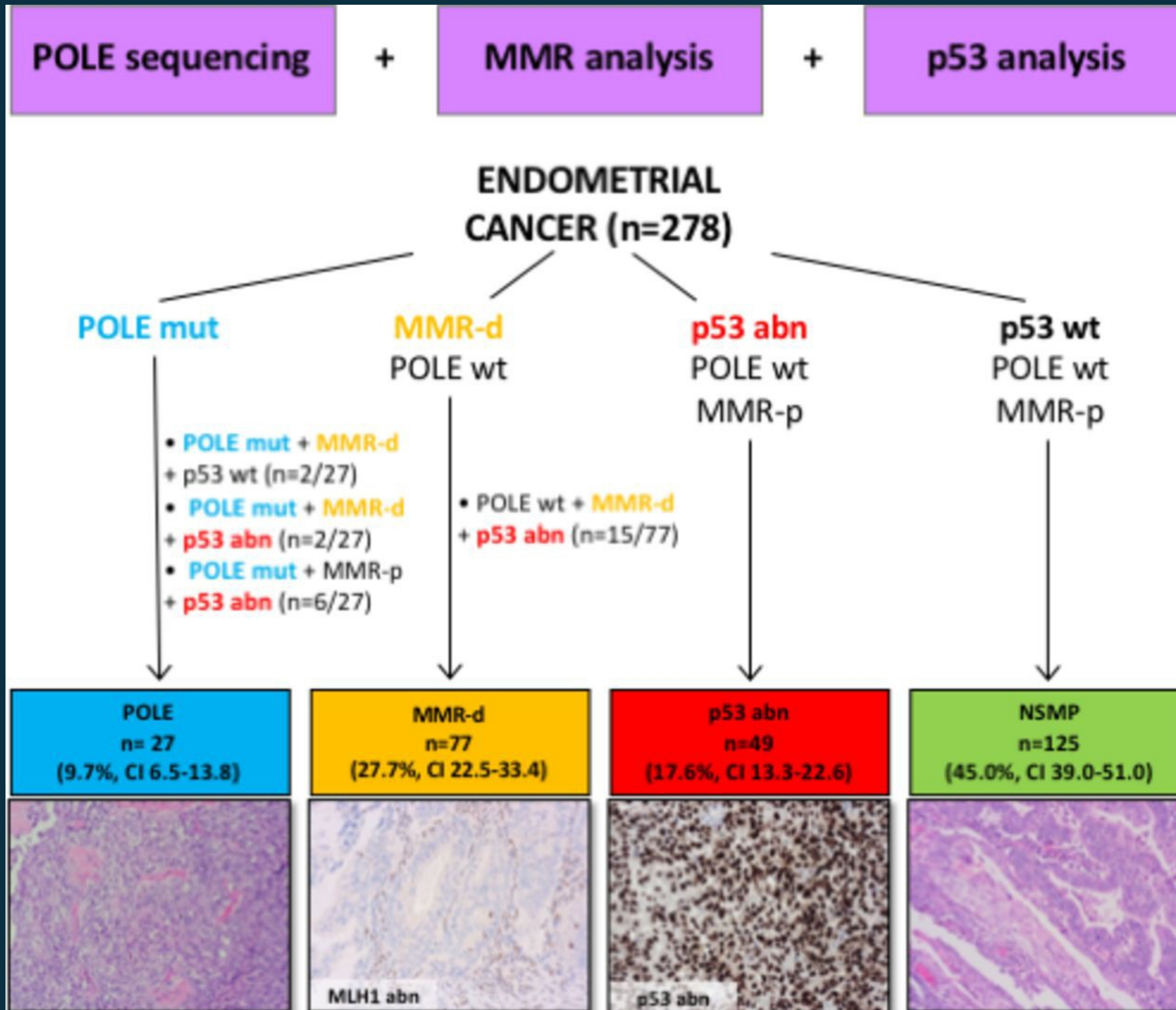
TP53 mutations affecting the
nuclear localization domain or
the nuclear export signal

No TP53 mutation



A novel algorithm to implement the molecular classification according to the new ESGO/ESTRO/ESP 2020 guidelines for endometrial cancer

Ilaria Betella ¹, Caterina Fumagalli ^{2,3}, Paola Rafaniello Raviele ⁴, Gabriella Schivardi ^{1,5}, Luigi Antonio De Vitis ¹, Maria Teresa Achillarre ¹, Alessia Aloisi ¹, Annalisa Garbi ¹, Matteo Maruccio ¹, Vanna Zanagnolo ¹, Giovanni Aletti ^{1,6}, Elena Guerini-Rocco ^{4,6}, Andrea Mariani ⁵, Angelo Maggioni ¹, Massimo Barberis ⁴, Nicoletta Colombo ^{1,7}, Francesco Multinu ¹



Molecular Methods

Mutation

Translocation

Deletion

Amplification

Methylation

Immunohistochemistry

Changed protein

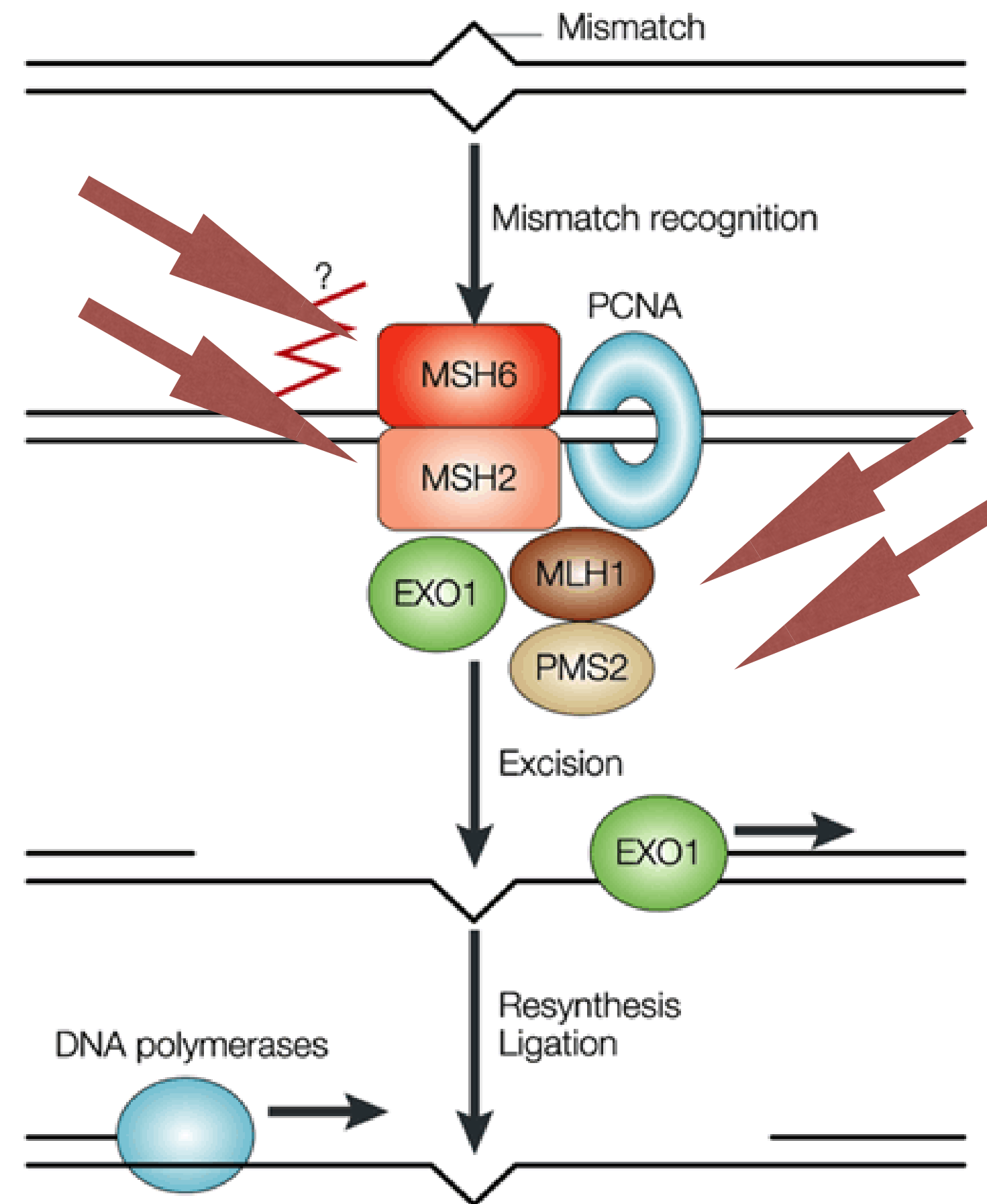
Absence of protein

Abnormal localization

Over expression

Fusion protein

MMR-mutation or methylation



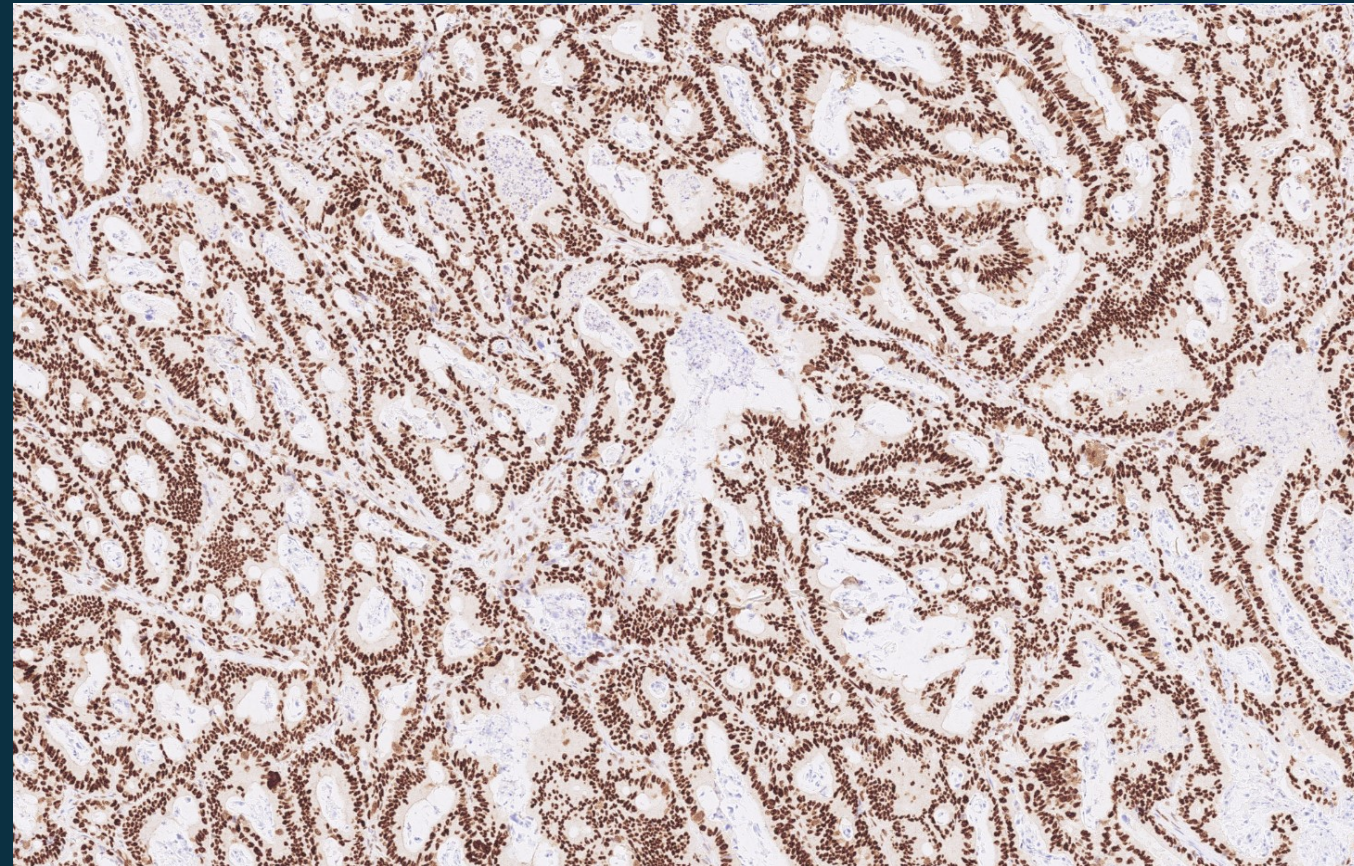
Immune
checkpoint
inhibitors

Nature Reviews | Immunology

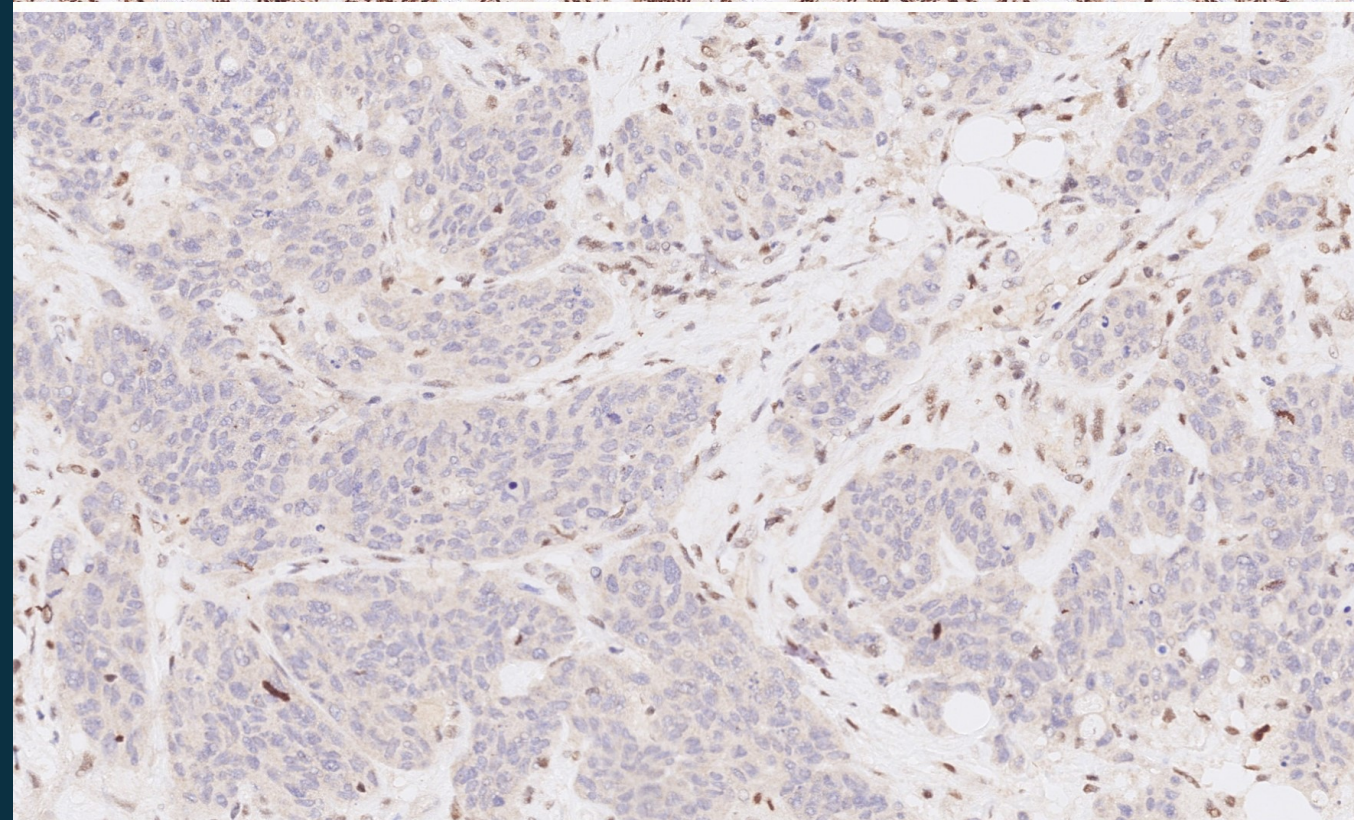
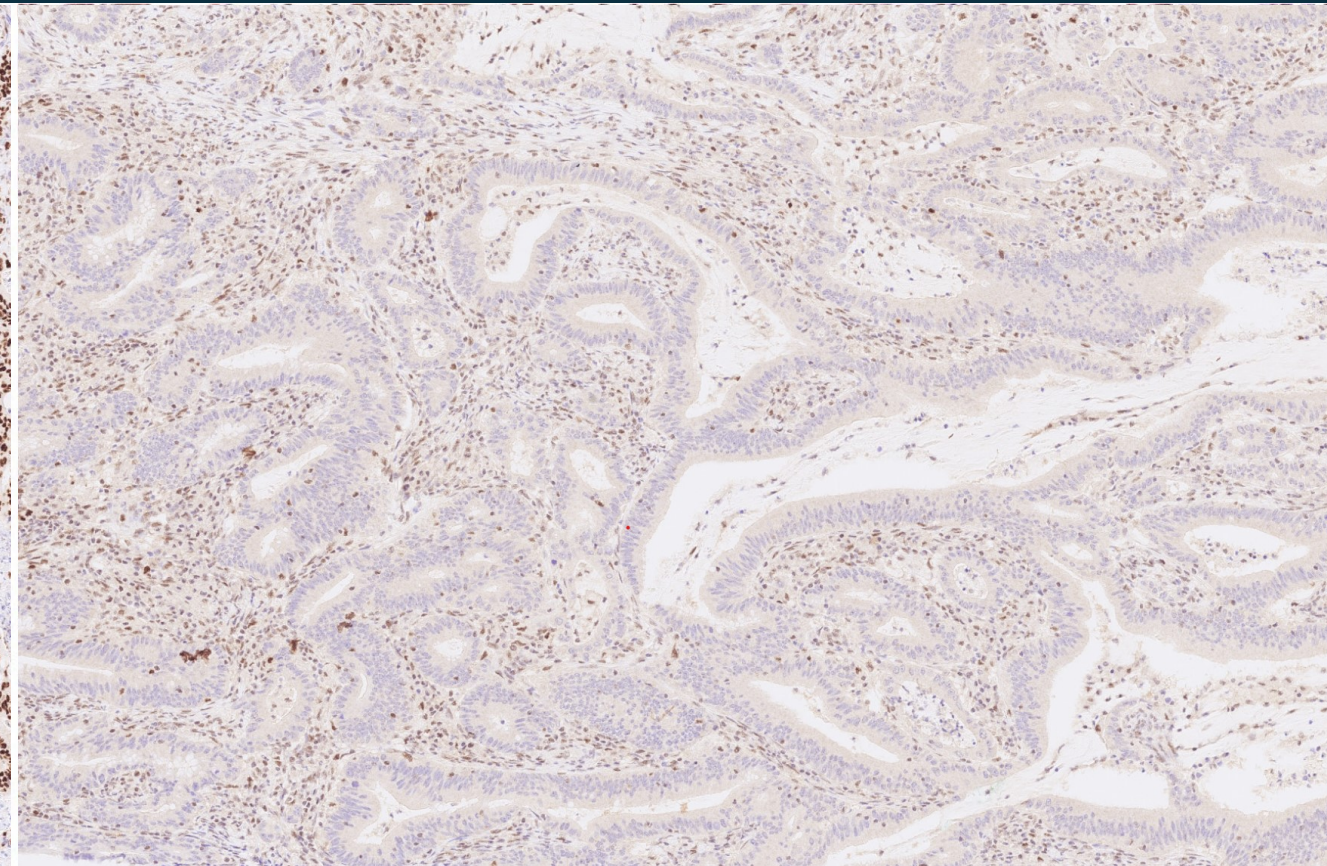
NordiQC

MMR-mutation or methylation

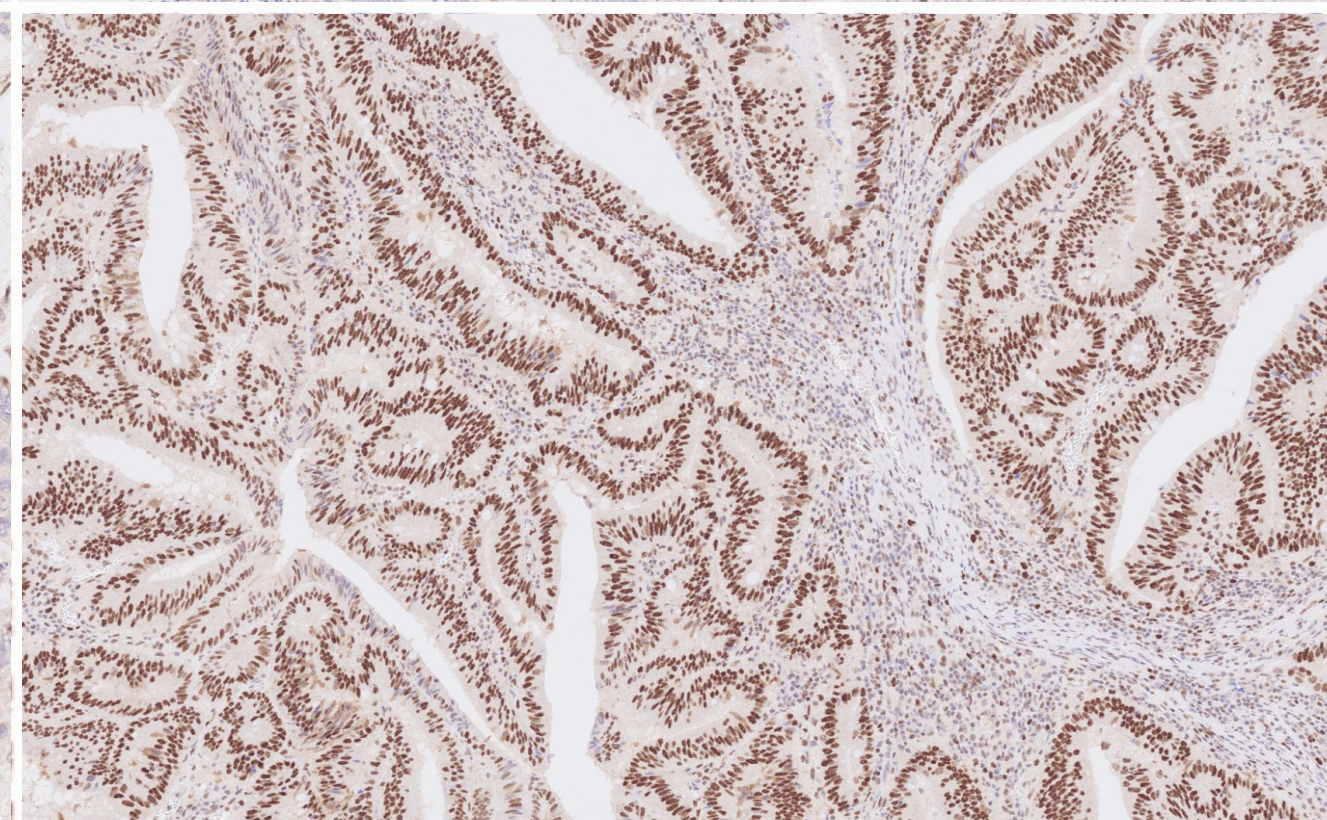
MSH6



MLH1



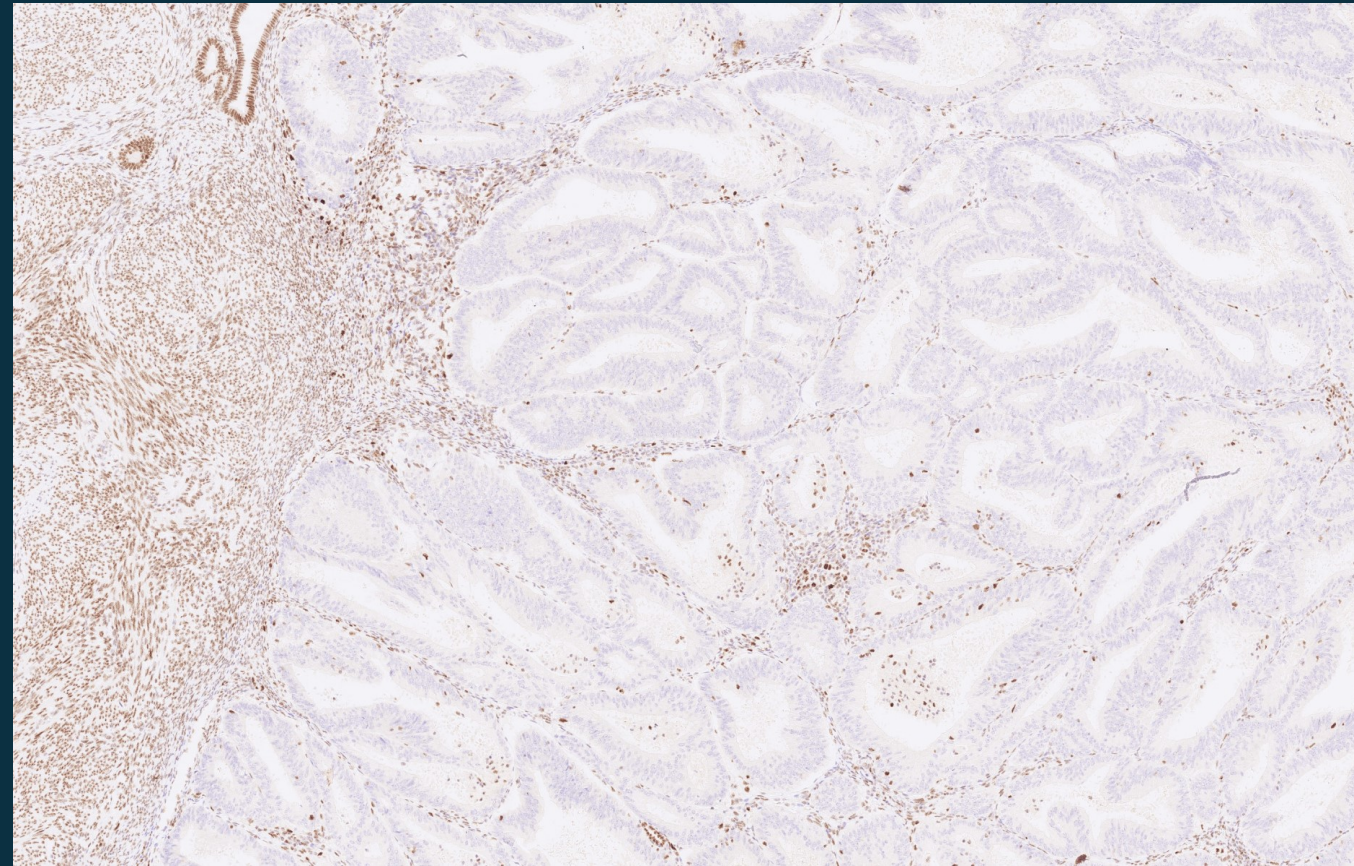
PMS2



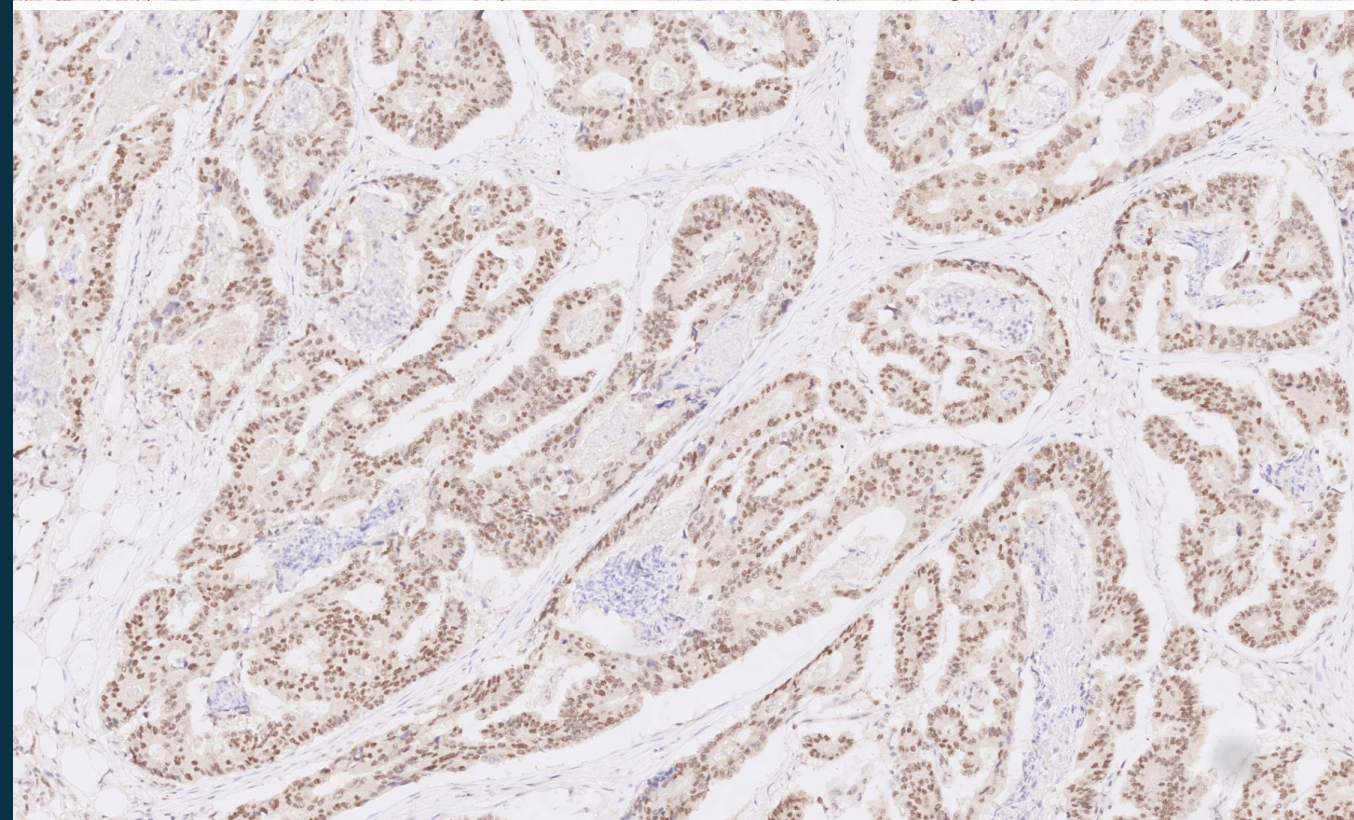
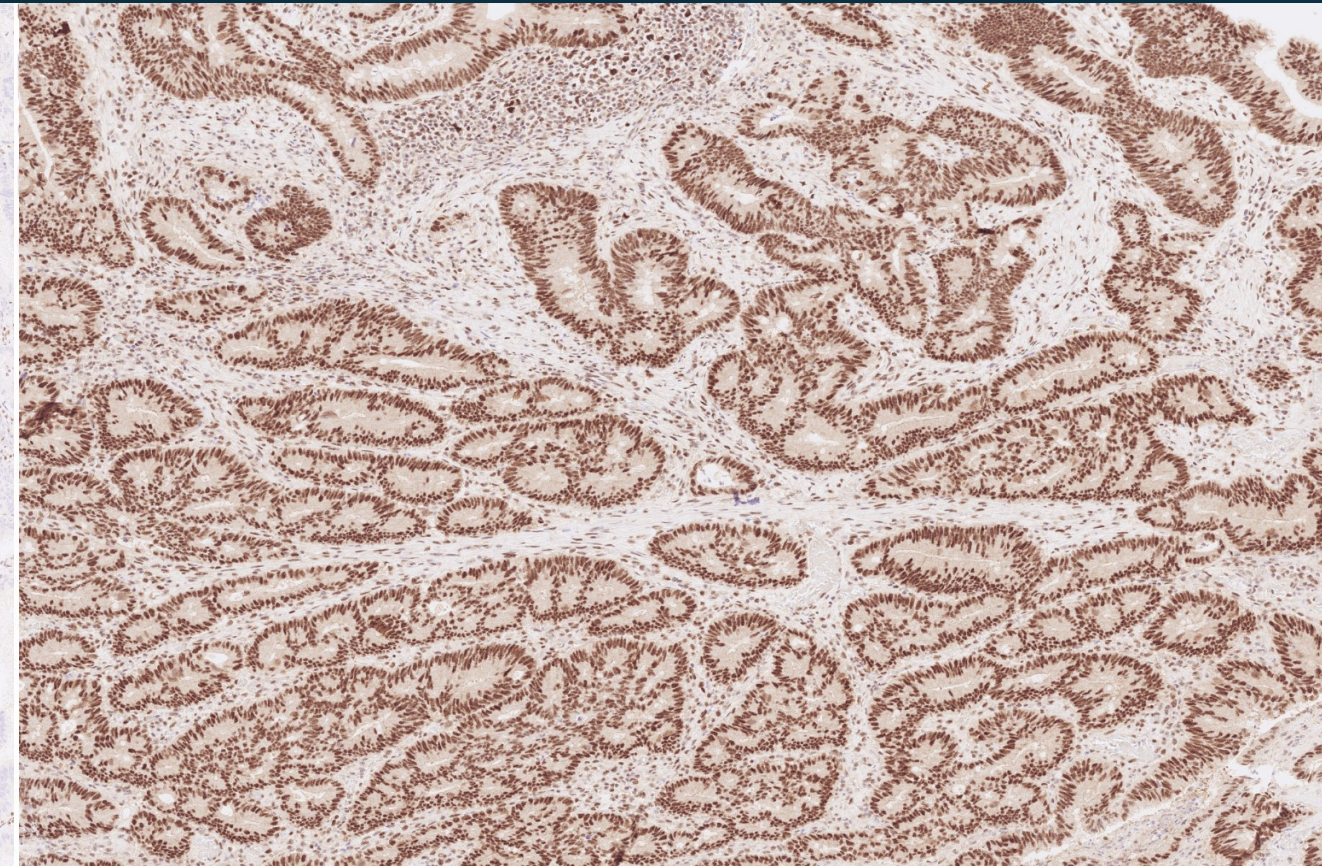
MSH2

MMR-mutation or methylation

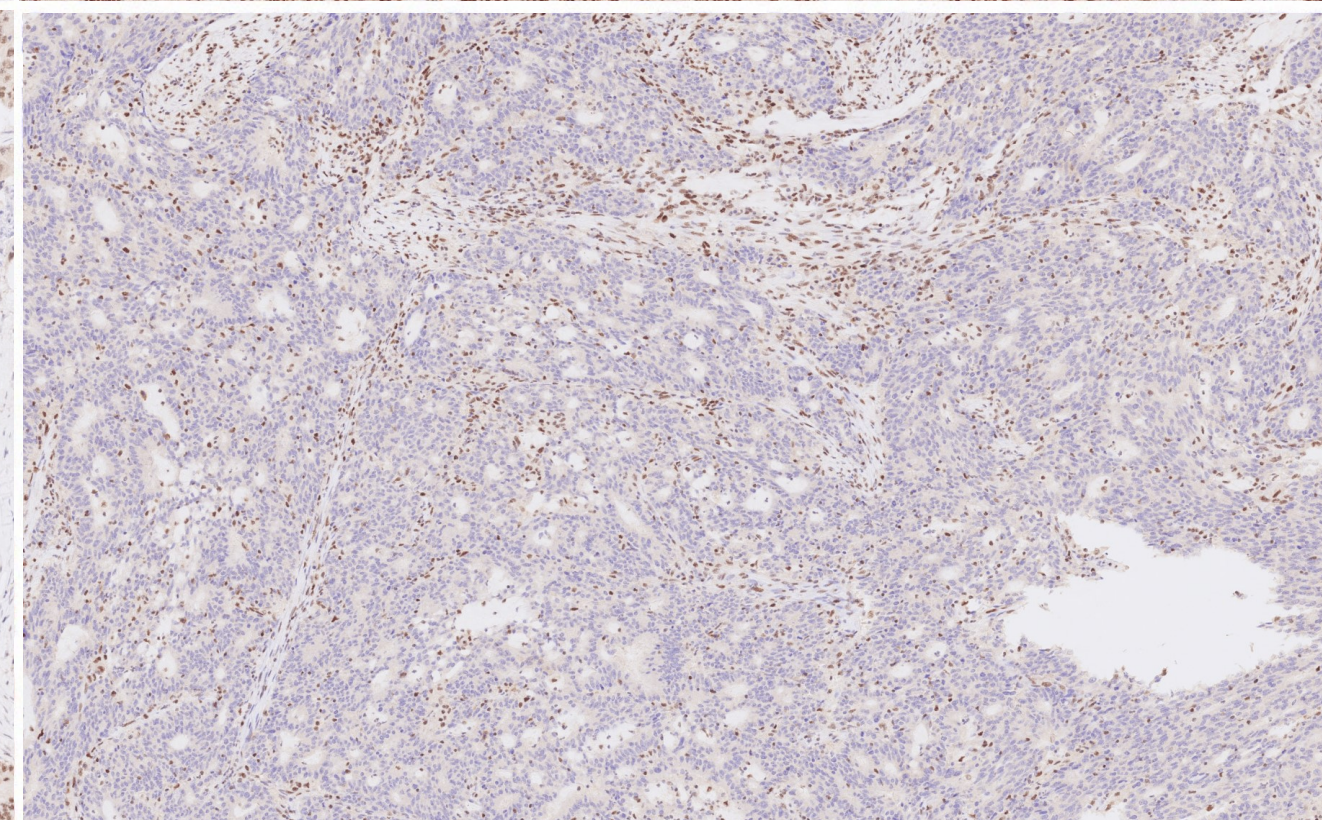
MSH6



MLH1



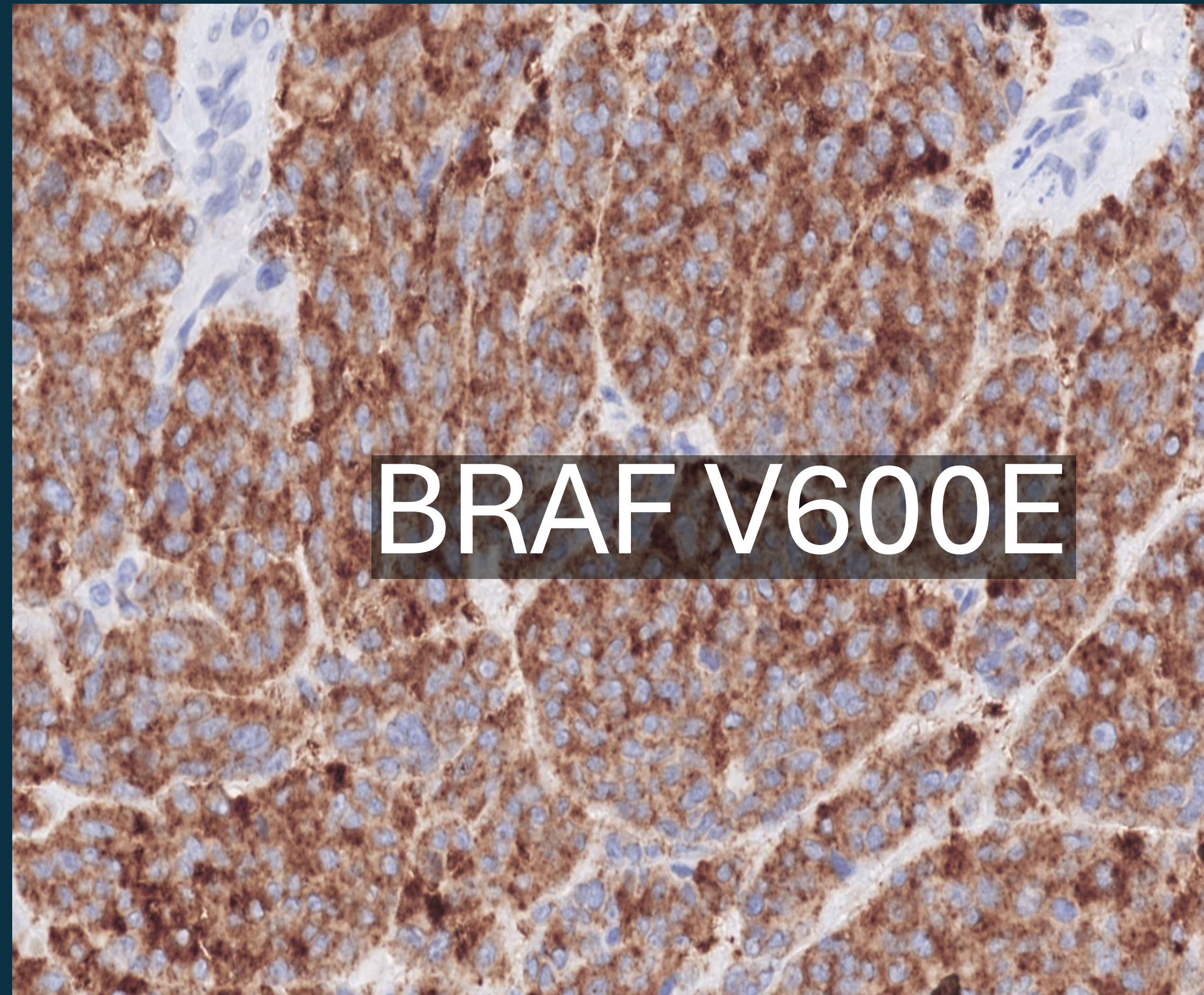
PMS2



MSH2

MMR-mutation or methylation

Is IHC enough?



MMR- mutation or methylation

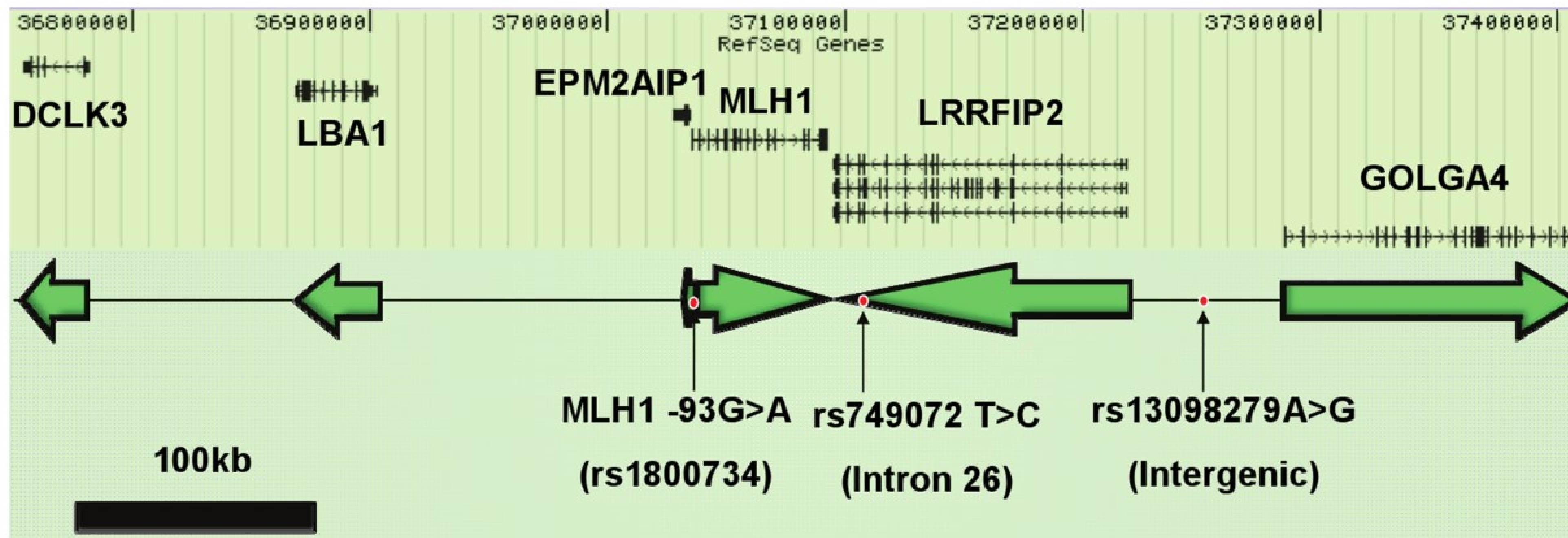
BRAFV600E immunohistochemistry facilitates universal screening of colorectal cancers for Lynch Syndrome

[Christopher W Toon](#)^{1,2,*}, [Michael J Walsh](#)^{3,4,*}, [Angela Chou](#)^{5,6}, [David Capper](#)^{7,8}, [Adele Clarkson](#)¹, [Loretta Sioson](#)¹, [Stephen Clarke](#)^{2,12}, [Scott Mead](#)^{5,6}, [Rhiannon J Walters](#)⁴, [Mark Clendenning](#)⁴, [Christophe Rosty](#)^{4,9,10}, [Joanne P Young](#)⁴, [Aung Ko Win](#)¹¹, [John L Hopper](#)¹¹, [Ashley Crook](#)¹², [Andreas von Deimling](#)^{7,8}, [Mark A Jenkins](#)¹¹, [Daniel B Buchanan](#)^{4,#}, [Anthony J Gill](#)^{1,2,13,#}

Activating mutations in *BRAF* are found in 5-25% of CRC, with the vast majority being the *BRAFV600E* mutation.[[10-14](#)] *BRAFV600E* mutation occurs in two thirds of CRC with *MLH1* silencing due to somatic hyper-methylation but virtually never in CRC with MSI due to LS.[[4](#)] Therefore *BRAFV600E* mutation is used as a proxy marker for hyper-methylation in *MLH1* IHC negative tumors. Further testing for LS is commonly offered on *MLH1* negative cases only if they are *BRAF* wild type.[[9](#)]

MMR-mutation or methylation

Is IHC enough?



doi: <https://doi.org/10.1371/journal.pone.0013314.g002>

MMR-mutation or methylation

> [Am J Surg Pathol](#). 2022 Mar 1;46(3):376-382. doi: 10.1097/PAS.0000000000001832.

EPM2AIP1 Immunohistochemistry Can Be Used as Surrogate Testing for MLH1 Promoter Methylation in Endometrial Cancer

[Miralem Mrkonjic](#)¹, [Gulisa Turashvili](#)

Affiliations + expand

PMID: 34772843 DOI: [10.1097/PAS.0000000000001832](#)

> [Virchows Arch](#). 2025 Jun 5. doi: 10.1007/s00428-025-04132-3. Online ahead of print.

EPM2 AIP1 immunohistochemistry as a surrogate of promoter methylation analysis in endometrial carcinoma

[Sonia Gatus](#)¹, [Marta Vaquero](#)², [Oliver Scheiber](#)³, [Ana Velasco](#)², [Dolors Cuevas](#)², [Karl Kashofer](#)⁴,
[Maria Santacana](#)⁵, [Núria Eritja](#)⁶, [Sigurd Lax](#)^{3 7}, [Xavier Matias-Guiu](#)⁸

> [Hum Pathol](#). 2024 Aug;150:74-77. doi: 10.1016/j.humpath.2024.06.017. Epub 2024 Jun 28.

EPM2AIP1 immunohistochemistry is inadequate as a surrogate marker for MLH1 promoter hypermethylation testing in colorectal cancer

[Bindu Challa](#)¹, [Wendy L Frankel](#)¹, [Deborah Knight](#)¹, [Rachel Pearlman](#)¹, [Heather Hampel](#)²,
[Wei Chen](#)³

Affiliations + expand

PMID: 38945374 DOI: [10.1016/j.humpath.2024.06.017](#)

[7/s00428-025-04132-3](#)

Setting	Intended use	Clone	Platform / Retrieval	Readout	Performance	Notes
Endometrial carcinoma (retrospective, n=119)	Surrogate for MLH1 promoter methylation	OTI2G3, OriGene (1:900)	Detection MACH4 universal HRP-polymer (Biocare) on LabVision Autostainer 720 (Thermo Scientific)	Nuclear loss = methylated	Sensitivity 94.5%; PPV 98.1% ; overall ~95% concordance with MLH1 methylation	Internal nuclei in benign endometrium & myometrium positive; proposed as cost-effective surrogate. (PubMed)
Endometrial carcinoma (Series 1: 70 MLH1-deficient EC + 30 MLH1-positive controls; Series 2: ~29–30 MLH1-deficient EC (abstract says 29; methods list 30) + 30 controls)	Surrogate for MLH1 promoter methylation	OTI2A2, OriGene	Series 1: Dako PT-Link pH9, EnVision DAB; Series 2: Ventana Benchmark Ultra, CC2, OptiView DAB	Nuclear loss = methylated	1: 100% concordance with methylation after re-assessing 2. 96.5% concordance with methylation, and 1/30 MMR-proficient controls showed EPM2AIP1 loss (a false positive).	Prealanalytical sensitivity: staining better on curettage/biopsy vs hysterectomy due to fixation delays; consider tumor-cell enrichment when methylation scores near cutoff. (PubMed)
Colorectal carcinoma (MSI/MMRd cohort, 101 cases)	Surrogate for MLH1 promoter methylation	OTI2G3, OriGene (1:900)	Leica Bond III; Bond Polymer Refine (DS9800)	Nuclear loss = methylated	Sensitivity 64%, specificity 67% , accuracy 64% → not recommended	Unacceptable false loss in MPH-negative tumors (incl. some Lynch cases). Authors caution clone/platform issues may contribute. (PubMed)

Absence of protein

Tumor suppressor inactivation

Gene	Tumor type	Detection method
TP53	Many cancers (high-grade serous carcinoma of ovary, serous carcinoma of endometrium, etc.)	IHC/Molecular
MMR	Colorectal, gastric, endometrial, ovarian, etc.	IHC/Molecular
BAP1	Mesothelioma, melanoma, renal cell carcinoma	IHC/Molecular
SMAD4	Pancreatic adenokarcinoma	IHC/Molecular
RB1 (Retinoblastoma protein)	Retinoblastoma, osteosarcoma, SCLC	IHC/Molecular
PTEN	Endometrial carcinoma, glioblastoma, prostate carcinoma	IHC/Molecular
SMARCB1 (INI1)	Malignant rhabdoid tumor, epitheloid sarcoma	IHC/Molecular
SMARCA4 (BRG1)	Ovarian rhabdoid tumor, malignant rhabdoid tumor of the uterus, Poorly differentiated chordoma	IHC/Molecular
CDC73 (parafibromin)	Parathyroid carcinoma	IHC/Molecular
...	...	

Molecular Methods

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Methylation

Immunohistochemistry

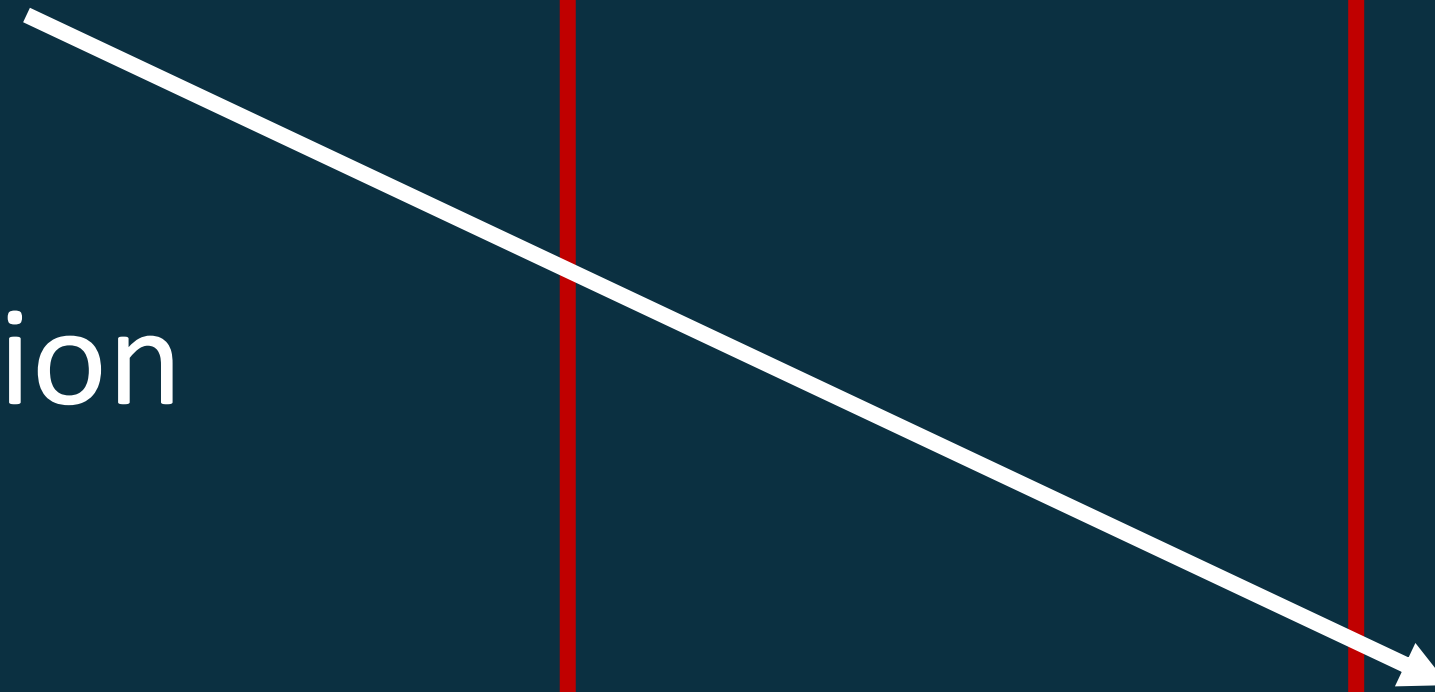
Changed protein

Absence of protein

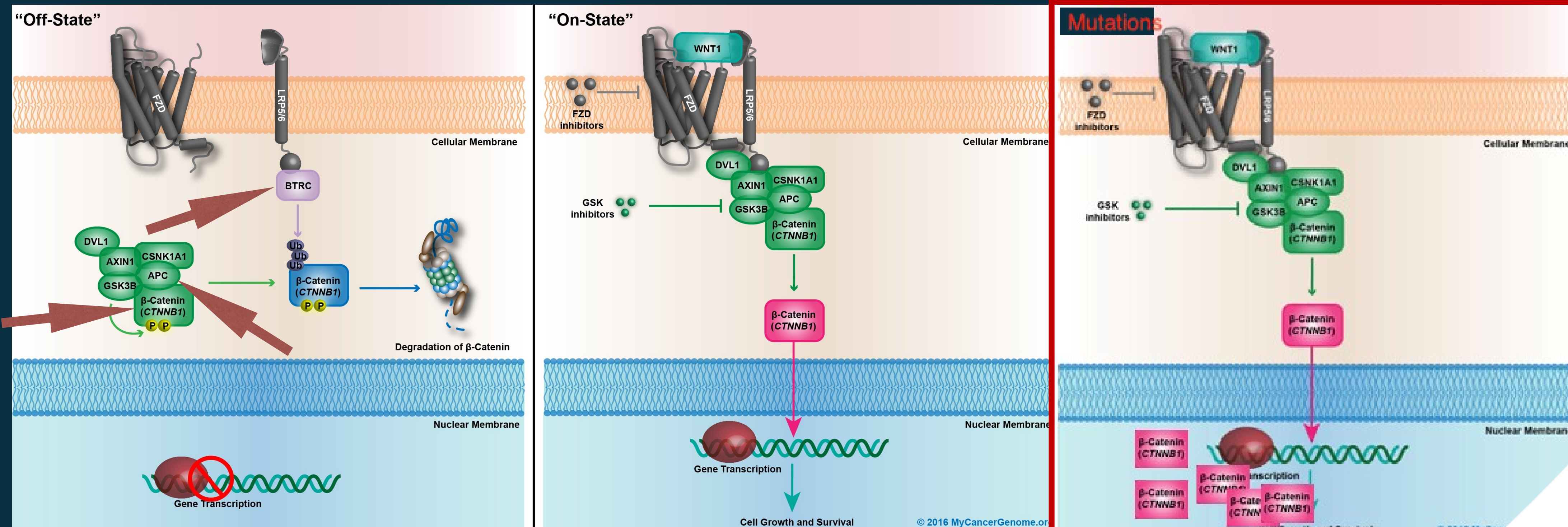
Abnormal localization

Over expression

Fusion protein



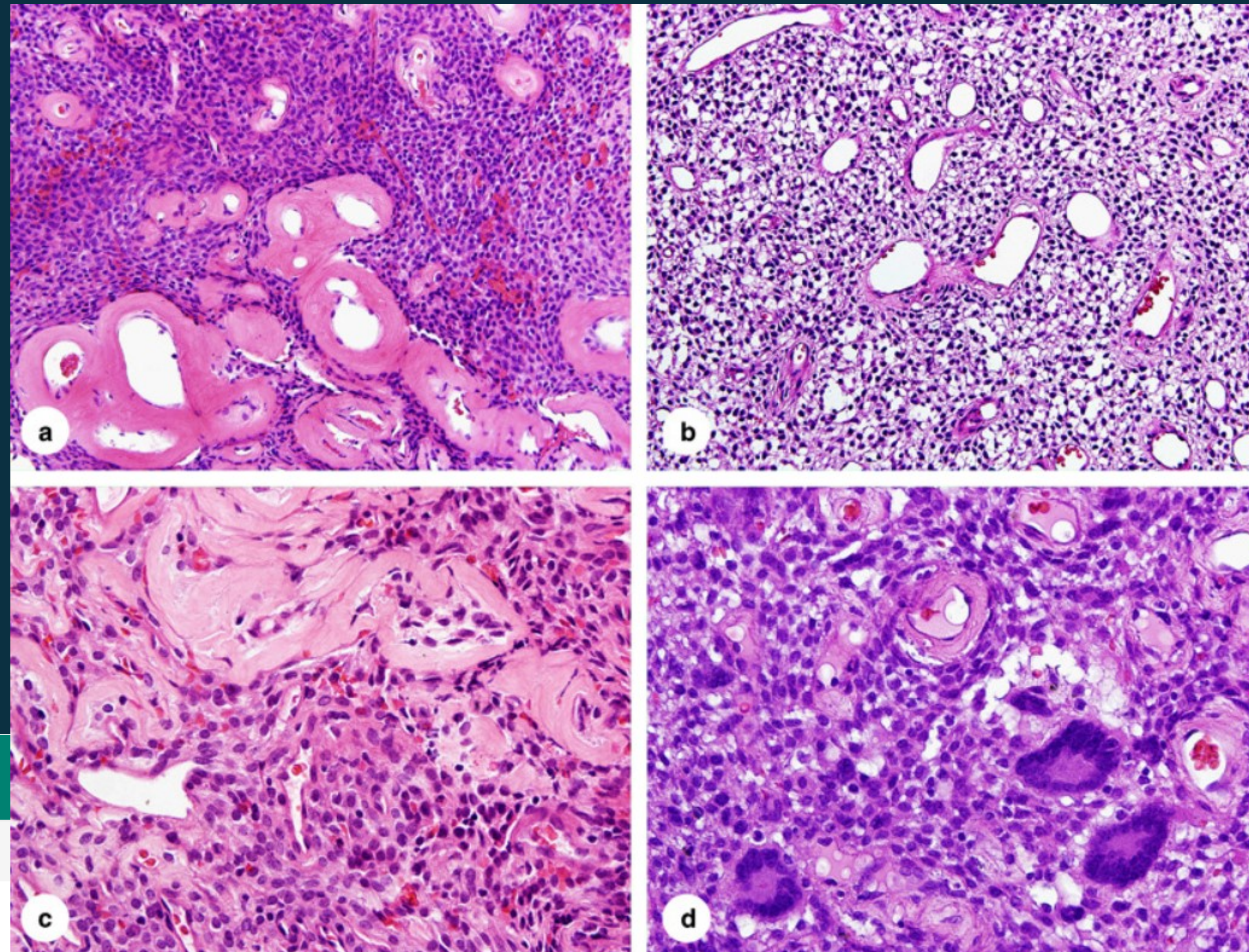
β -catenin nuclear reaction



Glomangiopericytoma

Sinonasal Hemangiopericytoma

- Polypoid mass in nasal cavity or sinuses
- Benign, but shows local recurrence in 30%



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ARTICLE · Volume 28, Issue 5, P715-720, May 2015 · [Open Access](#)

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Nuclear expression and gain-of-function β -catenin mutation in glomangiopericytoma (sinonasal-type hemangiopericytoma): insight into pathogenesis and a diagnostic marker

[Jerzy Lasota](#)¹ [✉](#) · [Anna Felisiak-Golabek](#)¹ · [F Zahra Aly](#)² · [Zeng-Feng Wang](#)¹ · [Lester D R Thompson](#)³ · [Markku Miettinen](#)¹

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

Glomangiopericytoma Sinonasal Hemangiopericytoma

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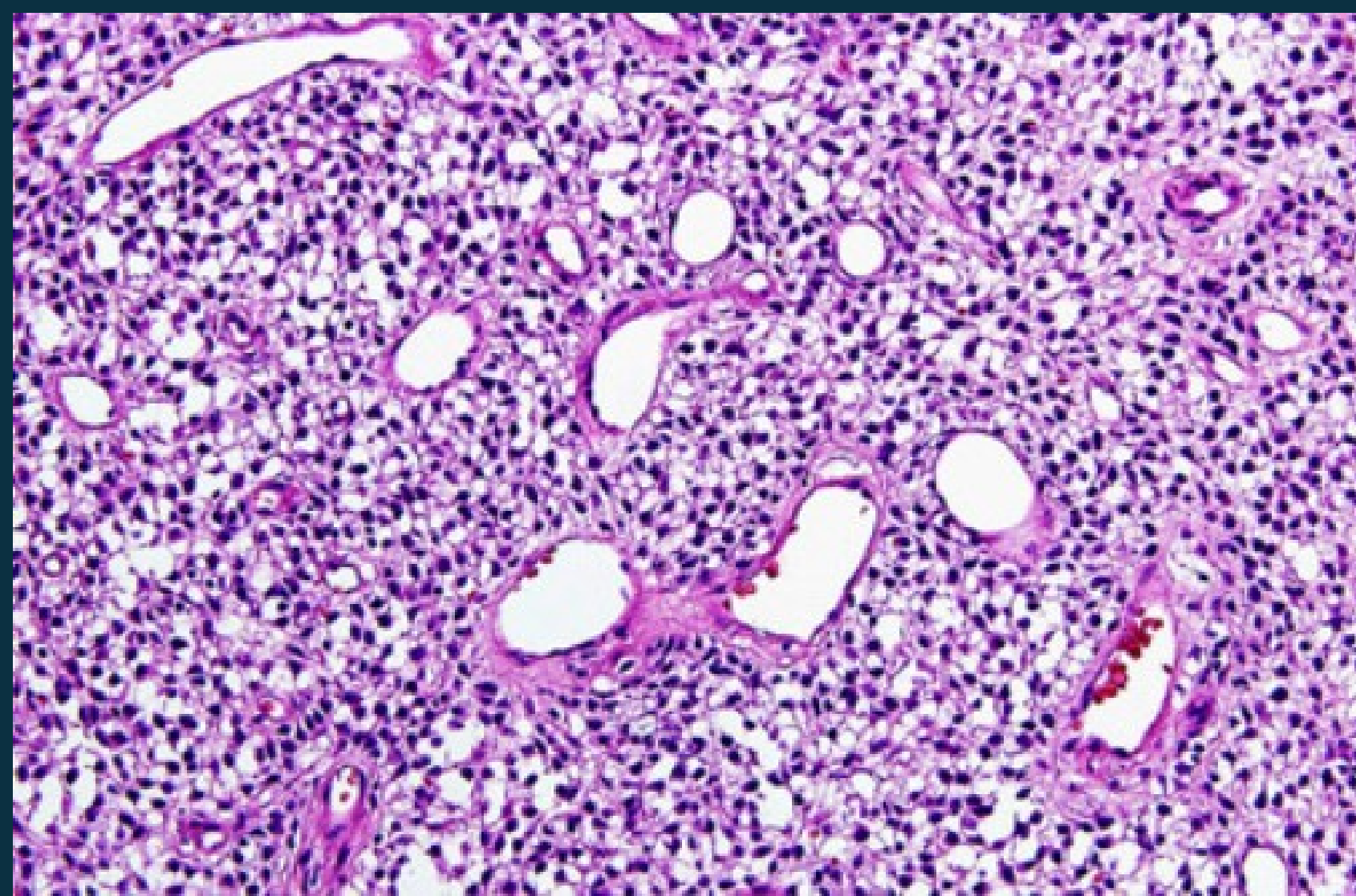
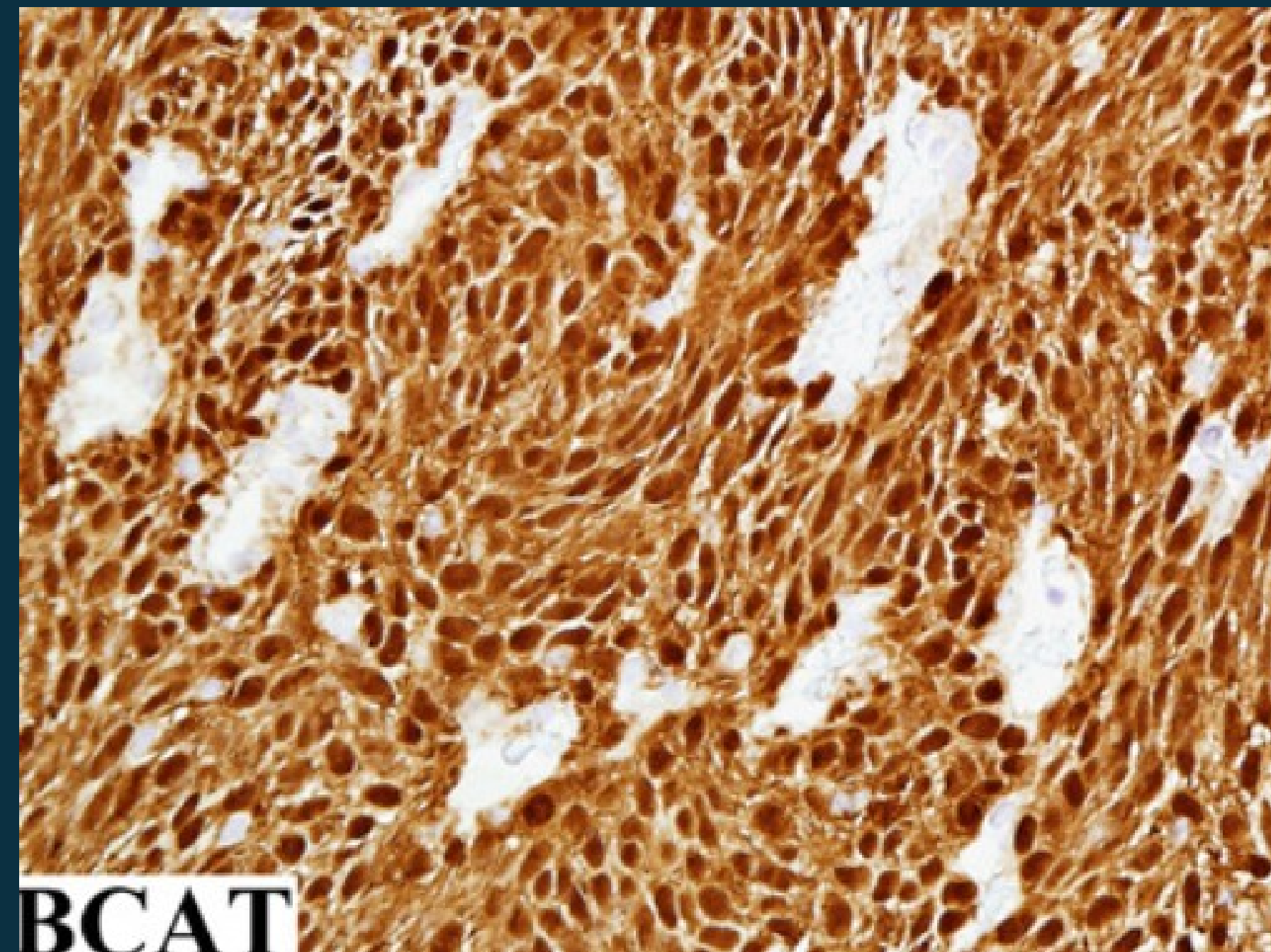
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Nuclear expression and gain-of-function β -catenin mutation in glomangiopericytoma (sinonasal-type hemangiopericytoma): insight into pathogenesis and a diagnostic marker

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β-catenin nuclear reaction

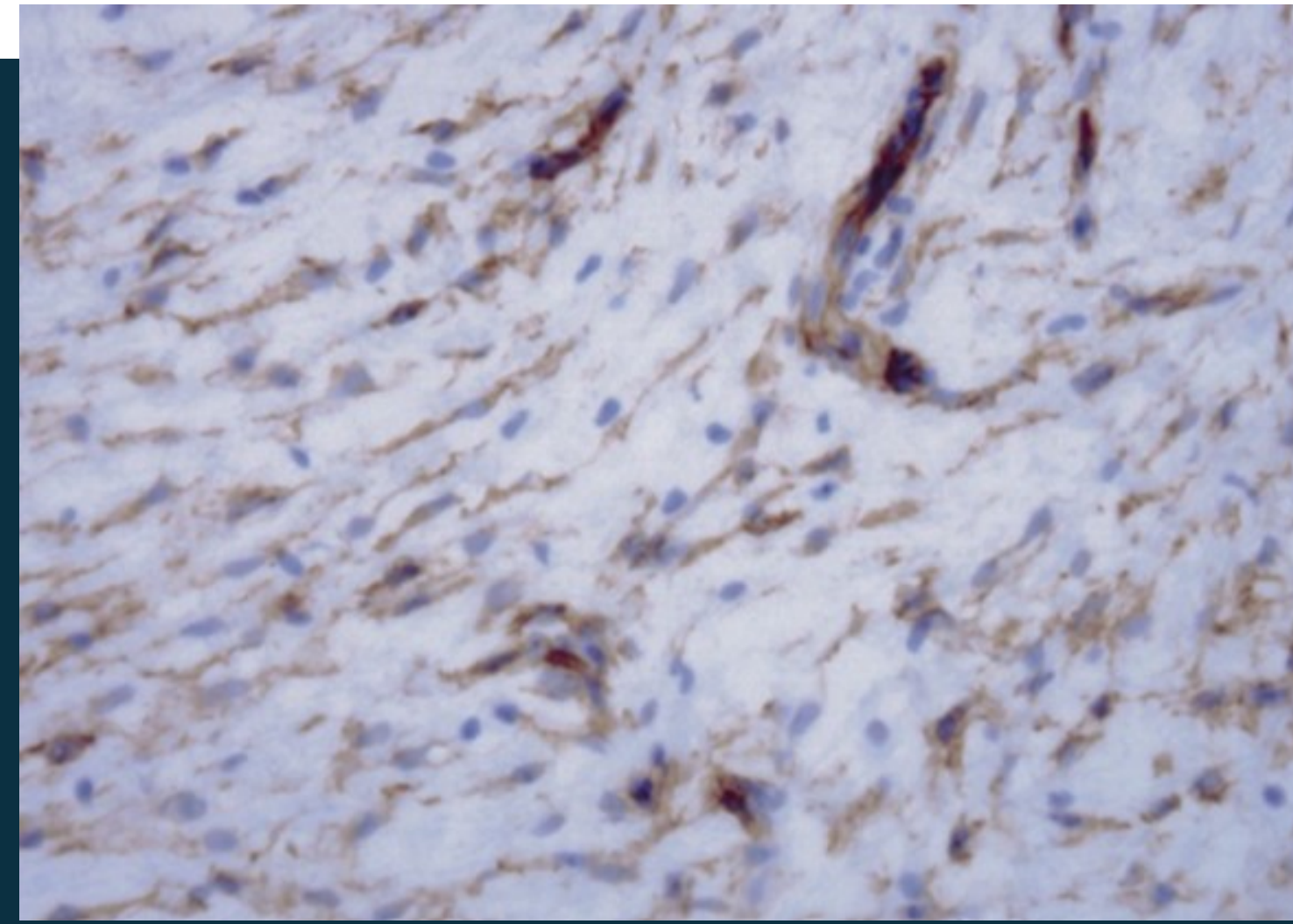
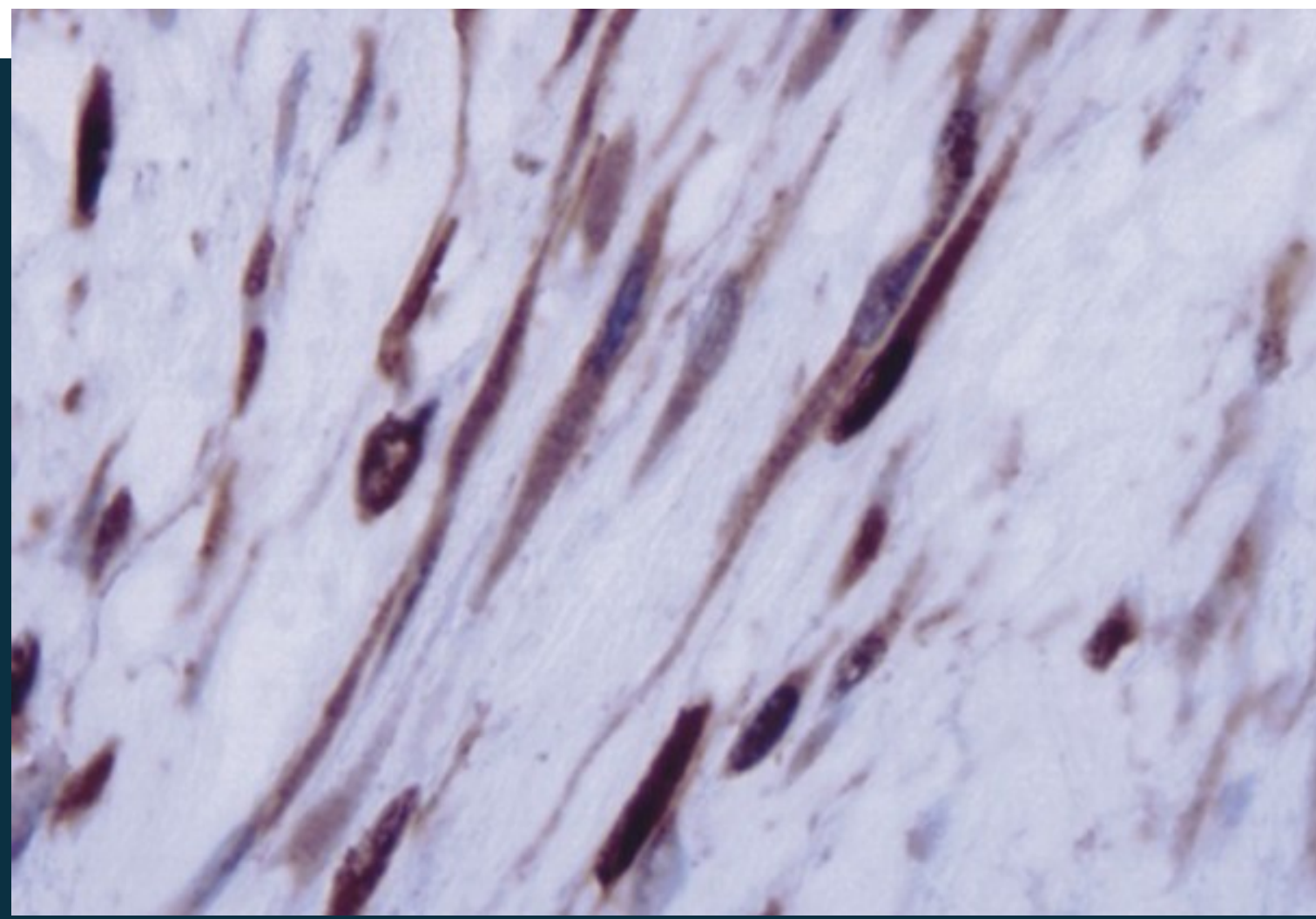
ORIGINAL ARTICLE

Nuclear β-Catenin Expression Distinguishes Deep Fibromatosis From Other Benign and Malignant Fibroblastic and Myofibroblastic Lesions

Bhattacharya, Baishali MD, MPH^{*}; Dilworth, Harrison Parry MD^{*}; Iacobuzio-Donahue, Christine MD, PhD^{*}; Ricci, Francesca MD^{*}; Weber, Kristin MD^{*}; Furlong, Mary A MD[†]; Fisher, Cyril MD, DSc[‡]; Montgomery, Elizabeth MD^{*}

Author Information[☺]

The American Journal of Surgical Pathology 29(5):p 653-659, May 2005. | DOI: 10.1097/01.pas.00000157938.95785.da



β -catenin nuclear reaction

Deep penetrating nevus

Virchows Archiv (2019) 474:535–537

<https://doi.org/10.1007/s00428-019-02552-6>

EDITORIAL



Toward an effective use of β -catenin immunohistochemistry in the evaluation of challenging melanocytic lesions

Emily M. Erstine¹  • Alexander J. Lazar^{1,2,3,4}

Received: 21 February 2019 / Accepted: 26 February 2019 / Published online: 13 March 2019

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β -catenin nuclear reaction Mesenteric fibromatosis

ORIGINAL ARTICLES

β -Catenin Immunohistochemistry Separates Mesenteric Fibromatosis From Gastrointestinal Stromal Tumor and Sclerosing Mesenteritis

Montgomery, Elizabeth M.D.; Torbenson, Michael S. M.D.; Kaushal, Manju M.S.; Fisher, Cyril M.D., D.Sc.; Abraham, Susan C. M.D.

[Author Information](#) 

The American Journal of Surgical Pathology 26(10):p 1296-1301, October 2002.

IHC markers with diagnostic abnormal protein localization

Gene (protein)	Normal localization	Abnormal localization	Tumor
TP53 (p53)	Nucleus	Cytoplasm	Many cancers (high-grade serous carcinoma of ovary, serous carcinoma of endometrium, etc.)
CTNNB1 (β-catenin)	Membrane	Nucleus	Glomangiopericytoma, deep fibromatosis, deep penetrating nevus, mesenteric fibromatosis
NPM1 (NPM1)	Nucleus	Cytoplasm	Acute myeloid leukemia
FOXO3 (FOXO3a)	Nucleus	Cytoplasm	Aggressive subtype of rhabdomyosarcoma
...			...

Molecular Methods

Mutation

Translocation

Deletion

Amplification

Methylation

Immunohistochemistry

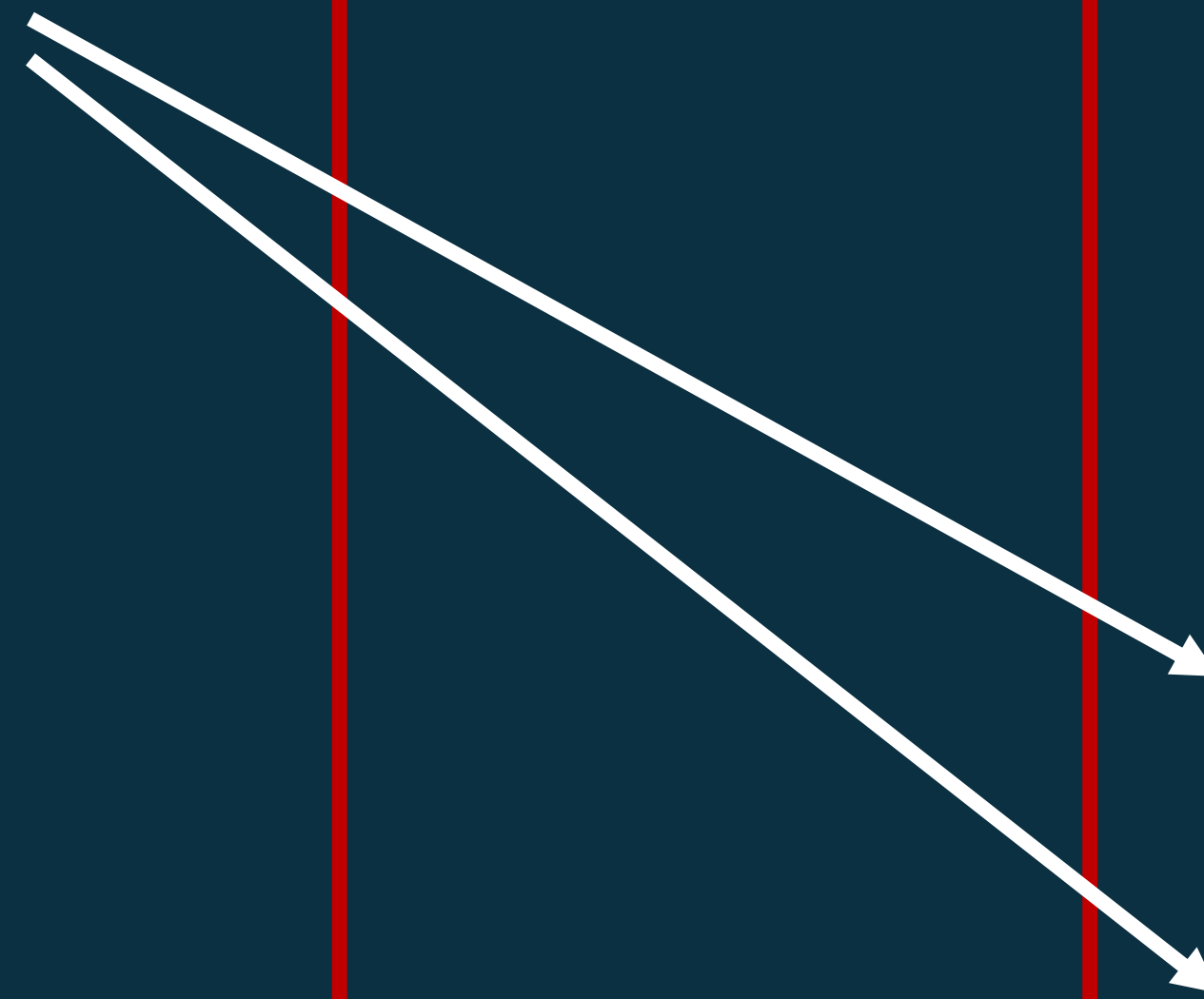
Changed protein

Absence of protein

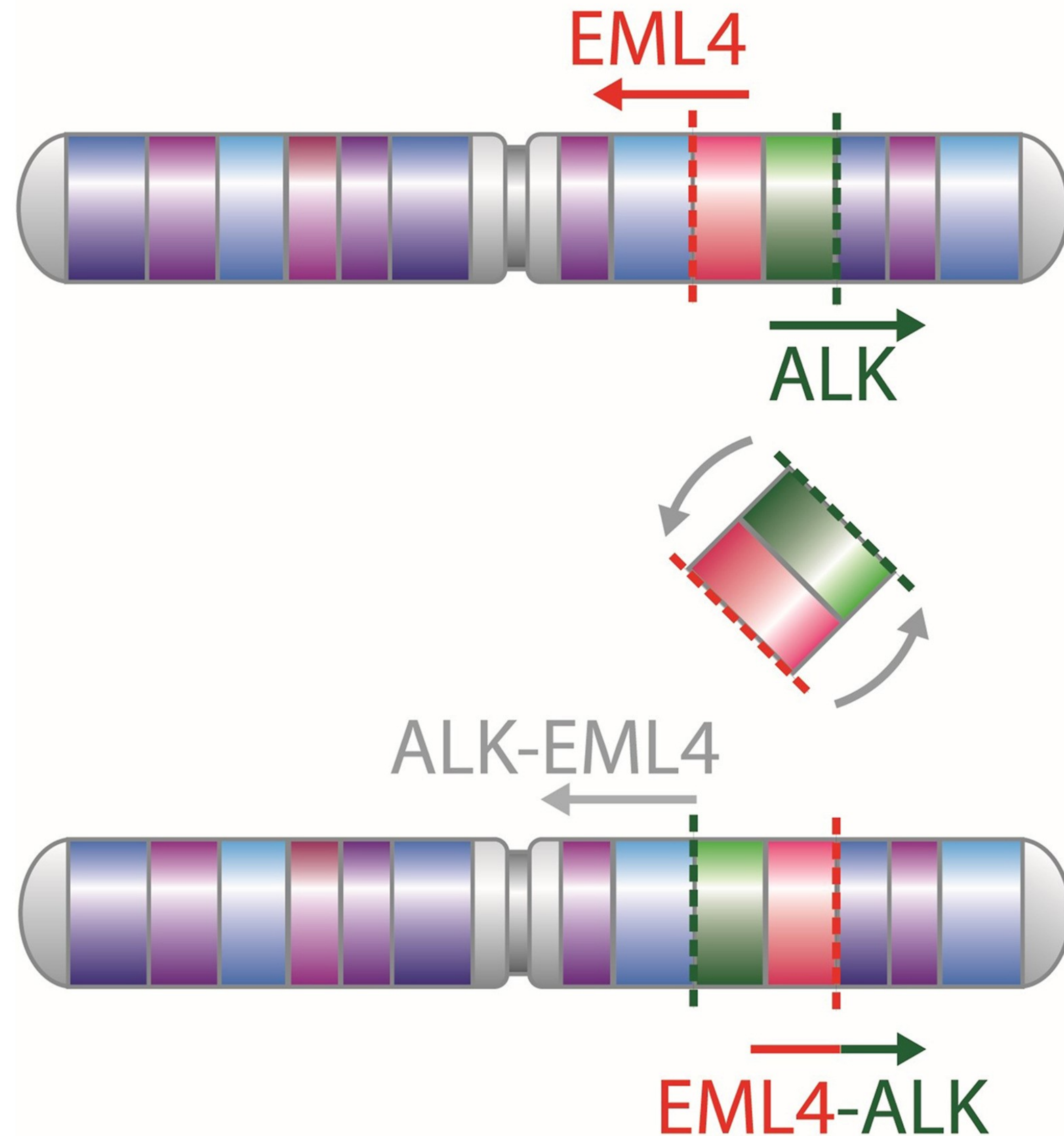
Abnormal localization

Over expression

Fusion protein



Chromosome 2



ALK-inhibitors

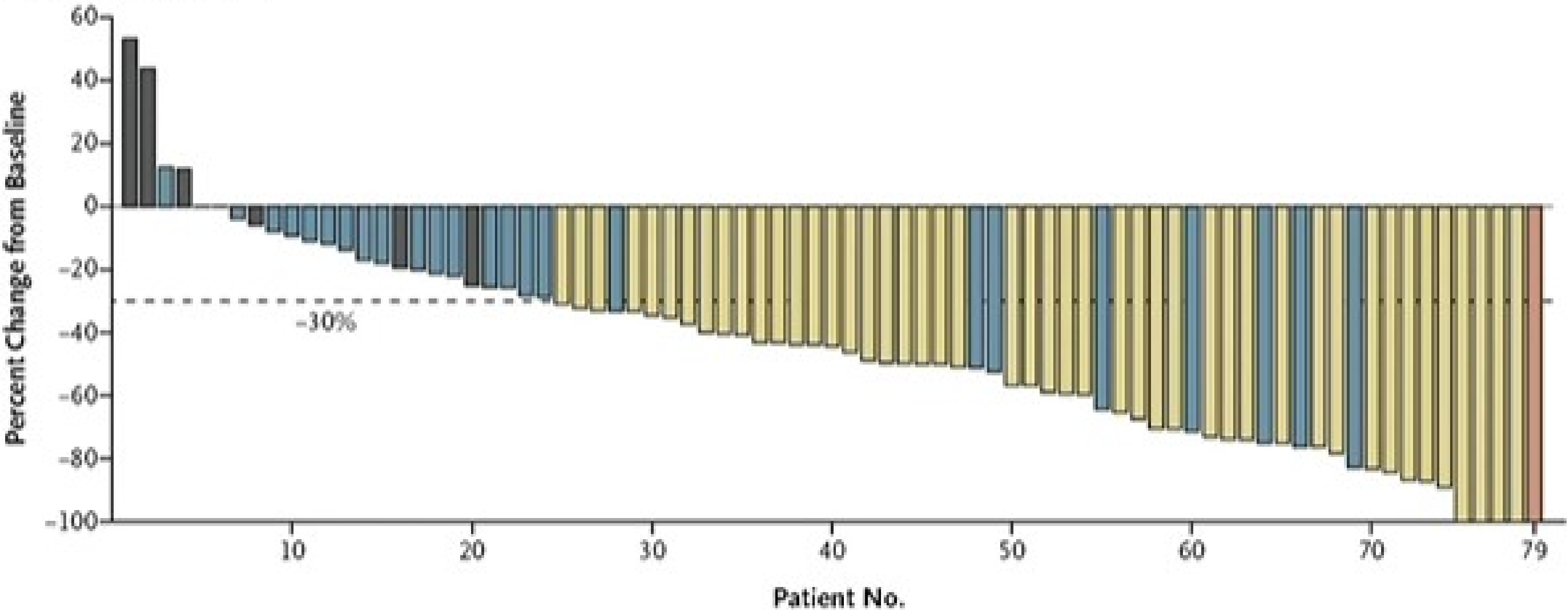
ALK fusion, lung adenocarcinoma

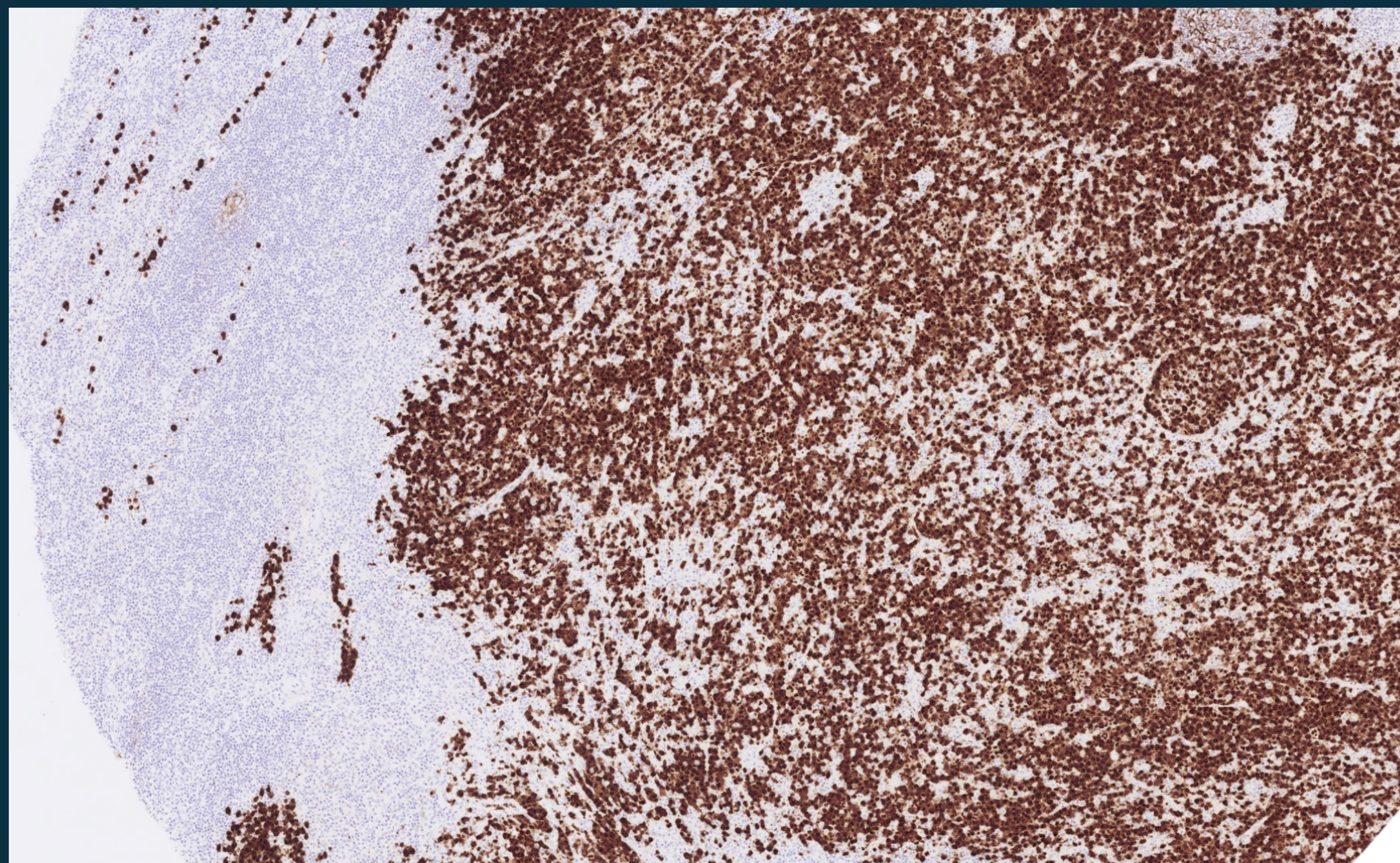
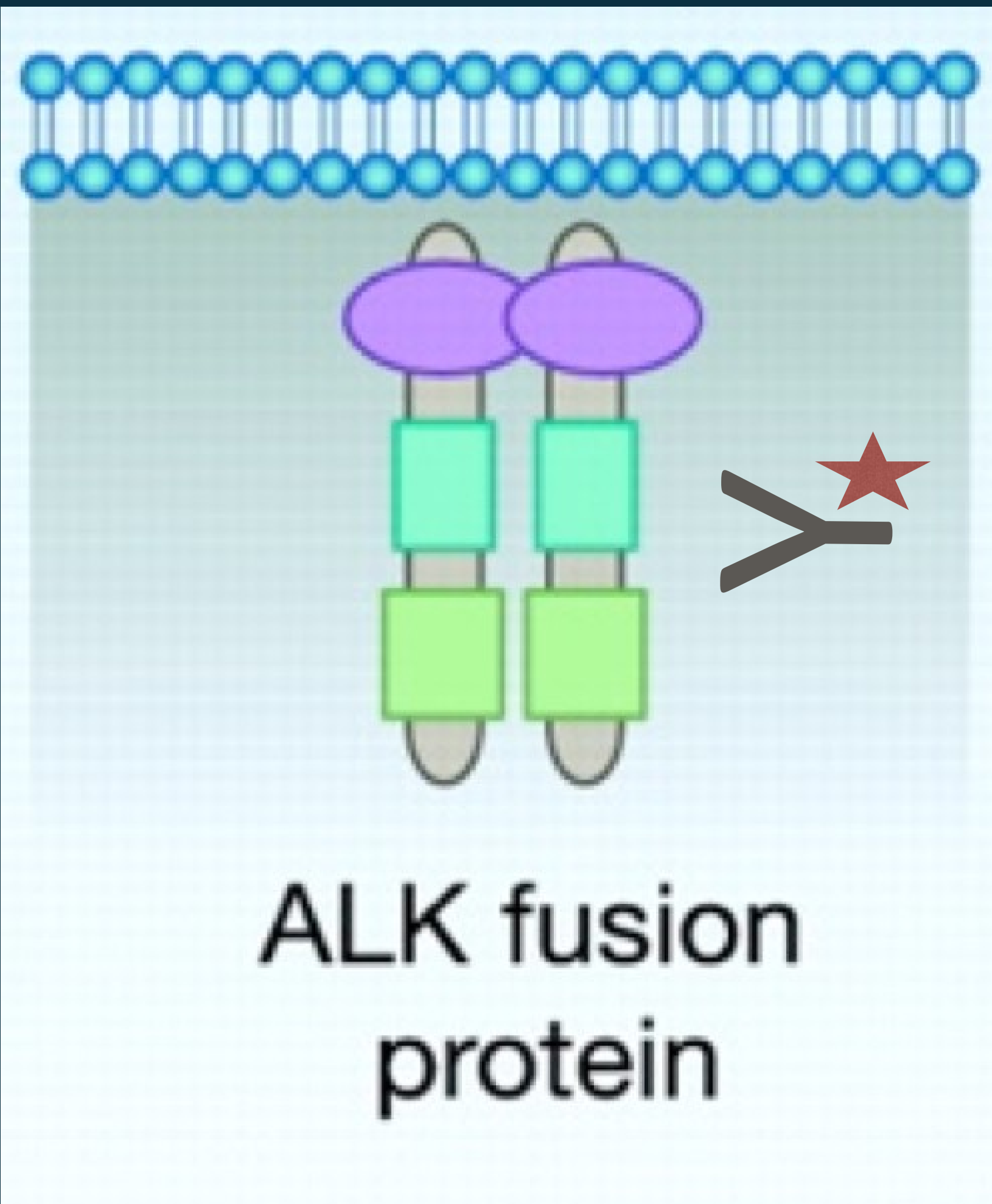
Anaplastic Lymphoma Kinase Inhibition in Non-Small-Cell Lung Cancer

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 Fidias, 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





Novel ALK immunohistochemistry assay (clone OT1A4, 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, MD,⁴ 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 Diseases, 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 OT1A4, 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 OT1A4 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 OT1A4 and Ventana ALK D5F3 assays was “almost perfect,” with $\kappa = 0.89$. The sensitivity and specificity of the Dako ALK OT1A4 assay were 93.3% and 96%, respectively, in a subgroup of 55 molecularly confirmed cases (n = 30 with and n = 25 without *ALK* rearrangement).

Purpose

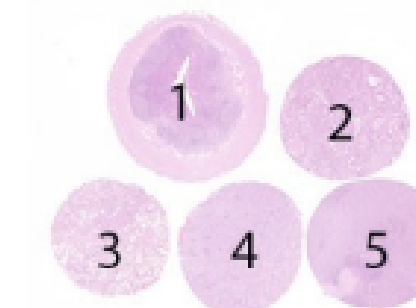
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among NordiQC participants for EML4-ALK translocations in lung adenocarcinomas. The EML4-ALK translocation occurs through a paracentric inversion between EML4 and ALK genes located in the short arm 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.

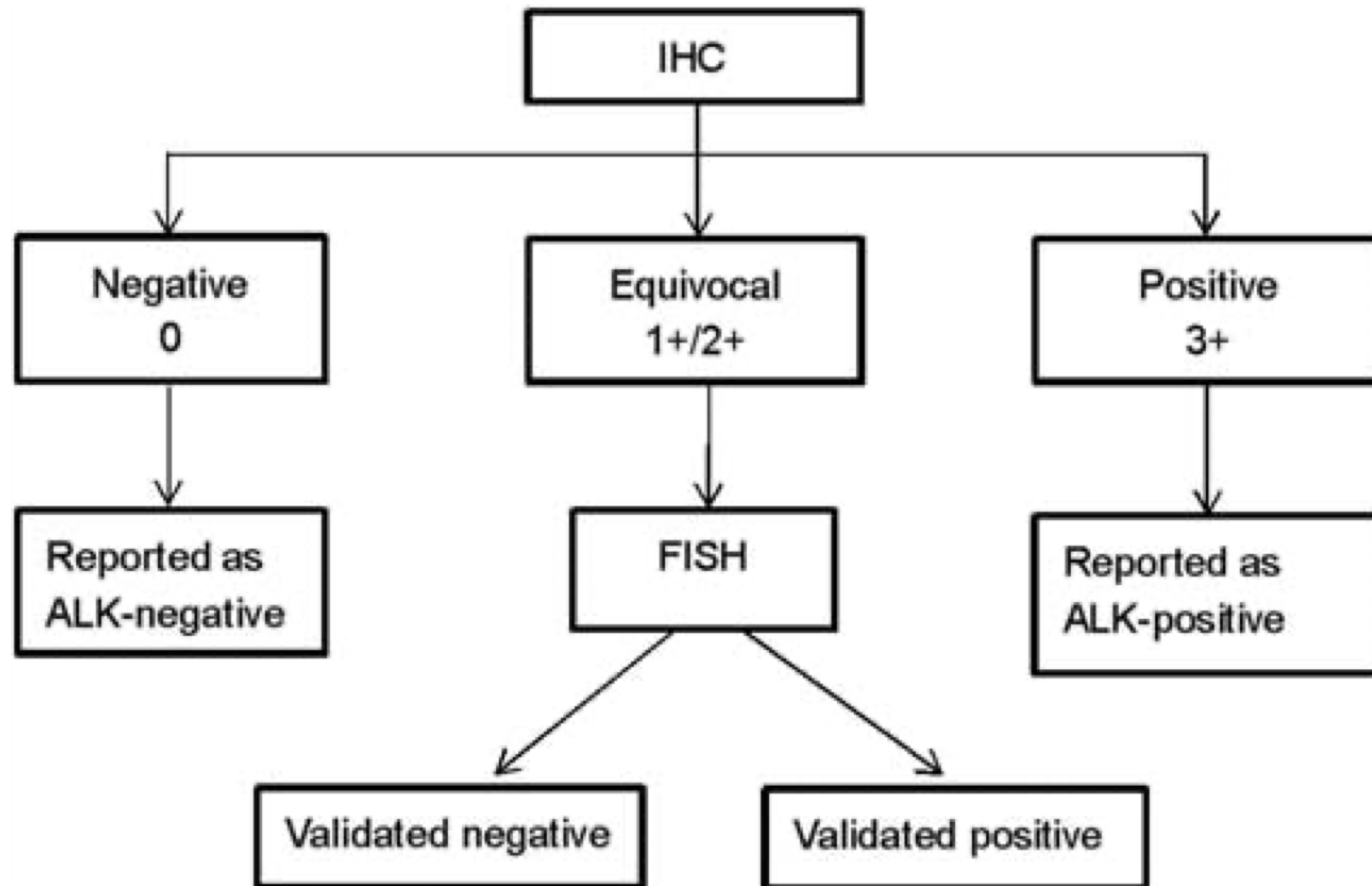
Criteria for assessing ALK (lung) staining as optimal included:

- 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 **OT1A4** 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

provided by the manufacturers.



Improving Selection Criteria for ALK Inhibitor Therapy in Non-Small Cell Lung Cancer

A Pooled-Data Analysis on Diagnostic Operating Characteristics of Immunohistochemistry

Long Jiang, MD, PhD,† Haihong Yang, MD, PhD,‡ Ping He, MD, PhD,§ Wenhua Liang, MD, PhD,‡ Jianrong Zhang, MD,*† Jingpei Li, MD,*† Yang Liu, MD,*† and Jianxing He, MD, PhD, FACS*†*

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).
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- 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.

Correlation between ALK fusion partners and ALK IHC reaction patterns

ALK fusion partner	Tumor type	Reaction pattern
NPM1	ALCL, DLBCL	Cytoplasmic / nuclear /nucleolar
TPM3	ALCL, inflammatory myofibroblastic tumor	Diffus cytoplasmic
EML4	Lung AC, inflammatory myofibroblastic tumor	Diffus cytoplasmic
CLTC	ALCL, DLBCL, inflammatory myofibroblastic tumor	Punctate cytoplasmic
RANBP2	Epithelioid inflammatory Myofibroblastic sarcoma	Nuclear membrane

ALCL, anaplastic large-cell lymphoma; DLBCL, diffuse large B-cell lymphoma

Table from J. Hornick presentation at USCAP 2025

IHC Markers for Tumors Translocations

Target	Tumor type (translocation/fusion protein)	Detection method
ALK	Anaplastic large-cell lymphoma (NPM-ALK), NSCLC (EML4-ALK)	IHC / Molecular
ROS1	NSCLC (CD74-ROS1, EZR-ROS1SDC4-ROS1SLC34A2-ROS1), Glioblastoma (GOPC-ROS1, CEP85L-ROS1)	Molecular (IHC)
pan-TRK (NTRK)	Secretory carcinoma (ETV6-NTRK3), Infantile fibrosarcoma (ETV6-NTRK3), various others	Molecular (IHC)
Bcl-2	Follicular lymphoma (t(14;18) translocation)	IHC (Molecular)
MET	NSCLC (KIF5B-MET)	Molecular
FLI1	Ewings sarcoma (EWSR1-FLI1)	Molecular
...	...	

Molecular Methods

Mutation

Translocation

Deletion

Amplification

Methylation

Immunohistochemistry

Changed protein

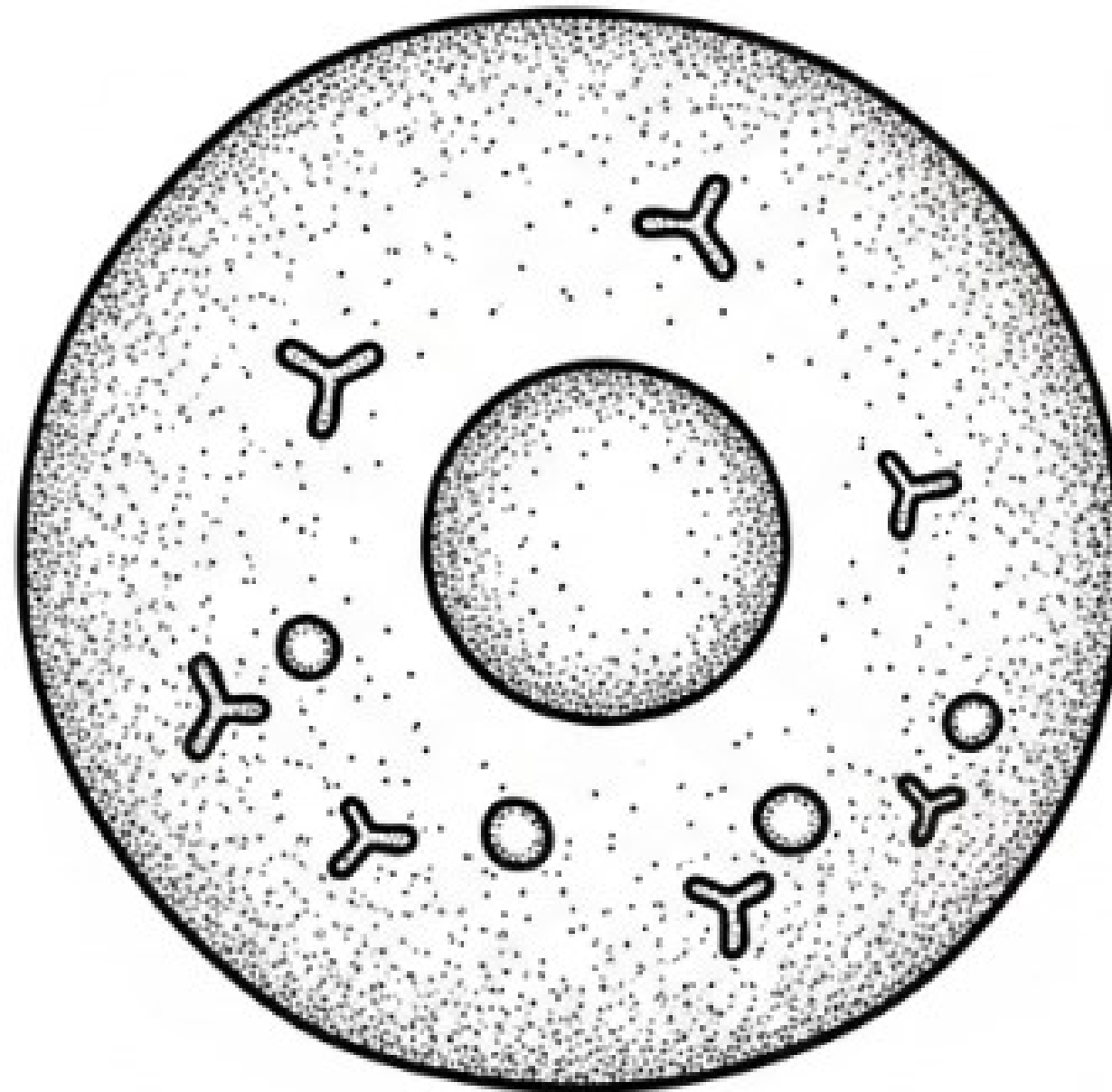
Absence of protein

Abnormal localization

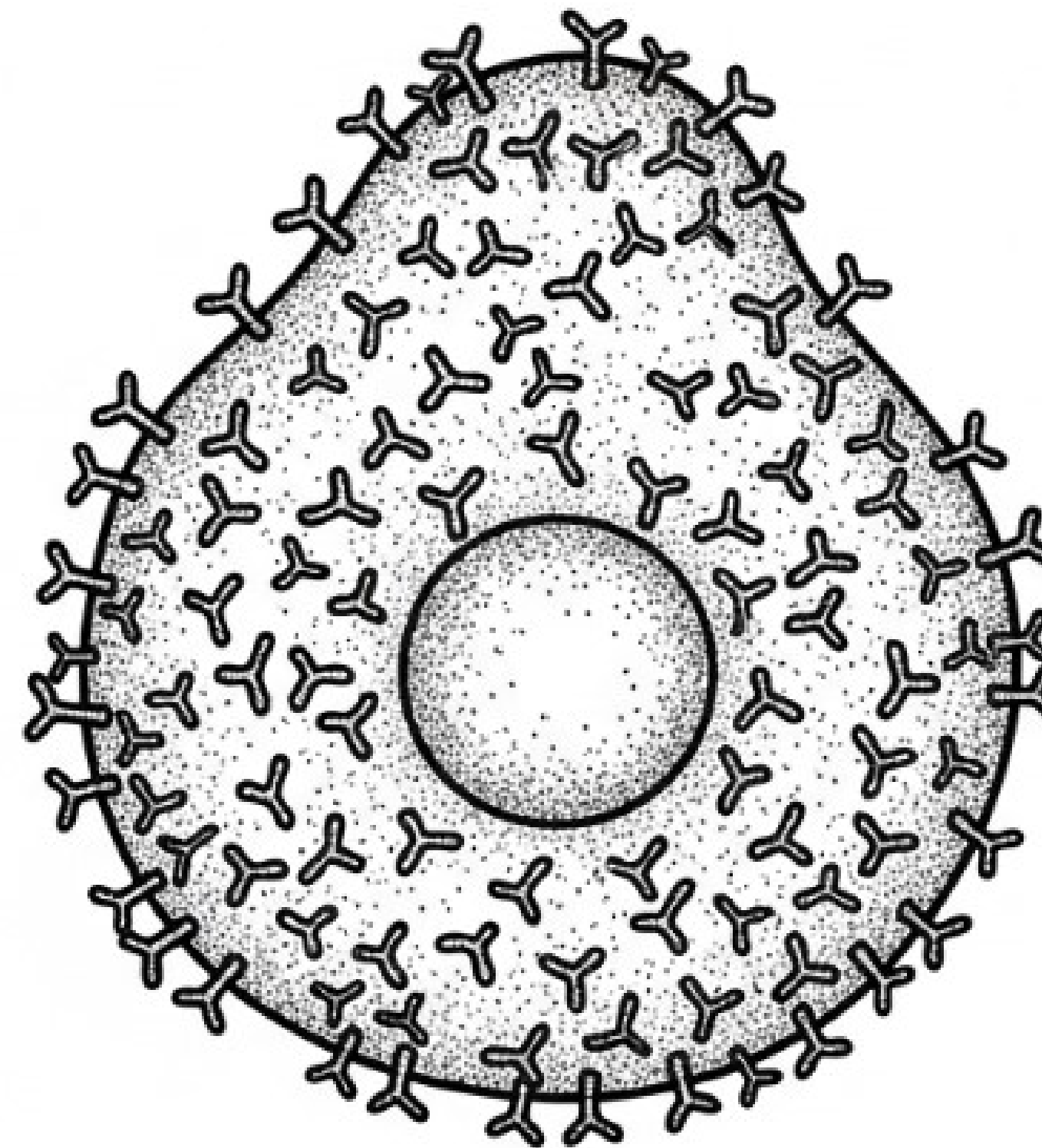
Over expression

Fusion protein





Normal breast cell



HER2-positive cancer

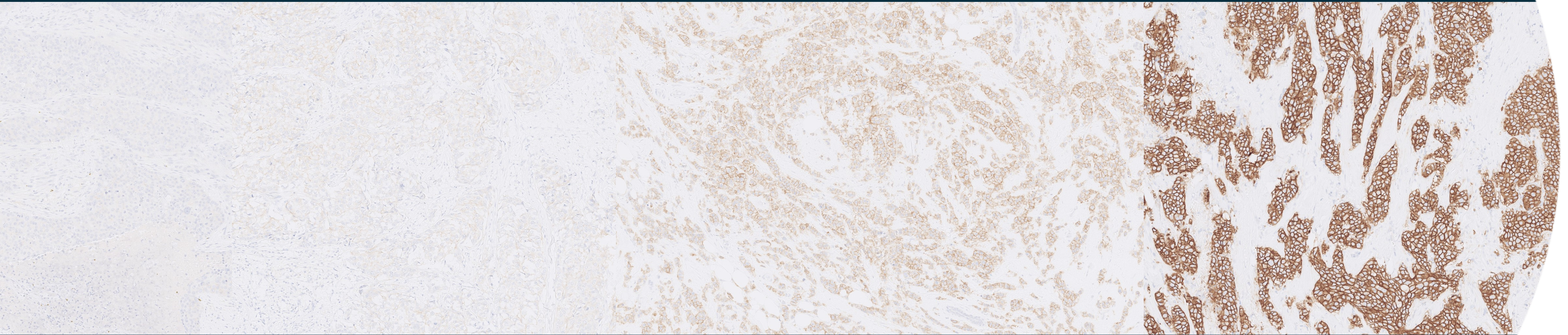
Traditional Breast HER2-scoring

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

IHC markers for tumors with gene amplification

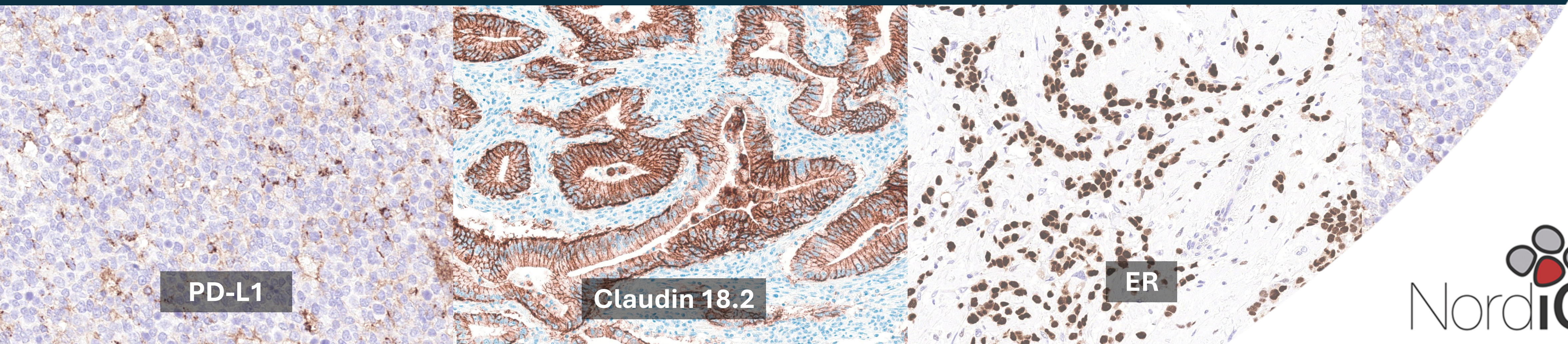
Target	Tumor	Detection method
HER2	Breast cancer, gastric and GEJ-cancer, bladder cancer, endometrial carcinoma, uterine sarcoma	IHC /ISH
MDM2	liposarcoma	IHC /NGS
EGFR	Glioblastoma, NSCLC (as a mechanism of resistance, amplification of the wild-type EGFR gene in patients treated with third-generation EGFR TKIs)	NGS
MET /c-MET	NSCLC	NGS for amplification/translocation (MET tyrosine kinase inhibitors) / IHC for protein overexpression (ADC, telisotuzumab vedotin)
FGFR2b	Gastric cancer	IHC
...		

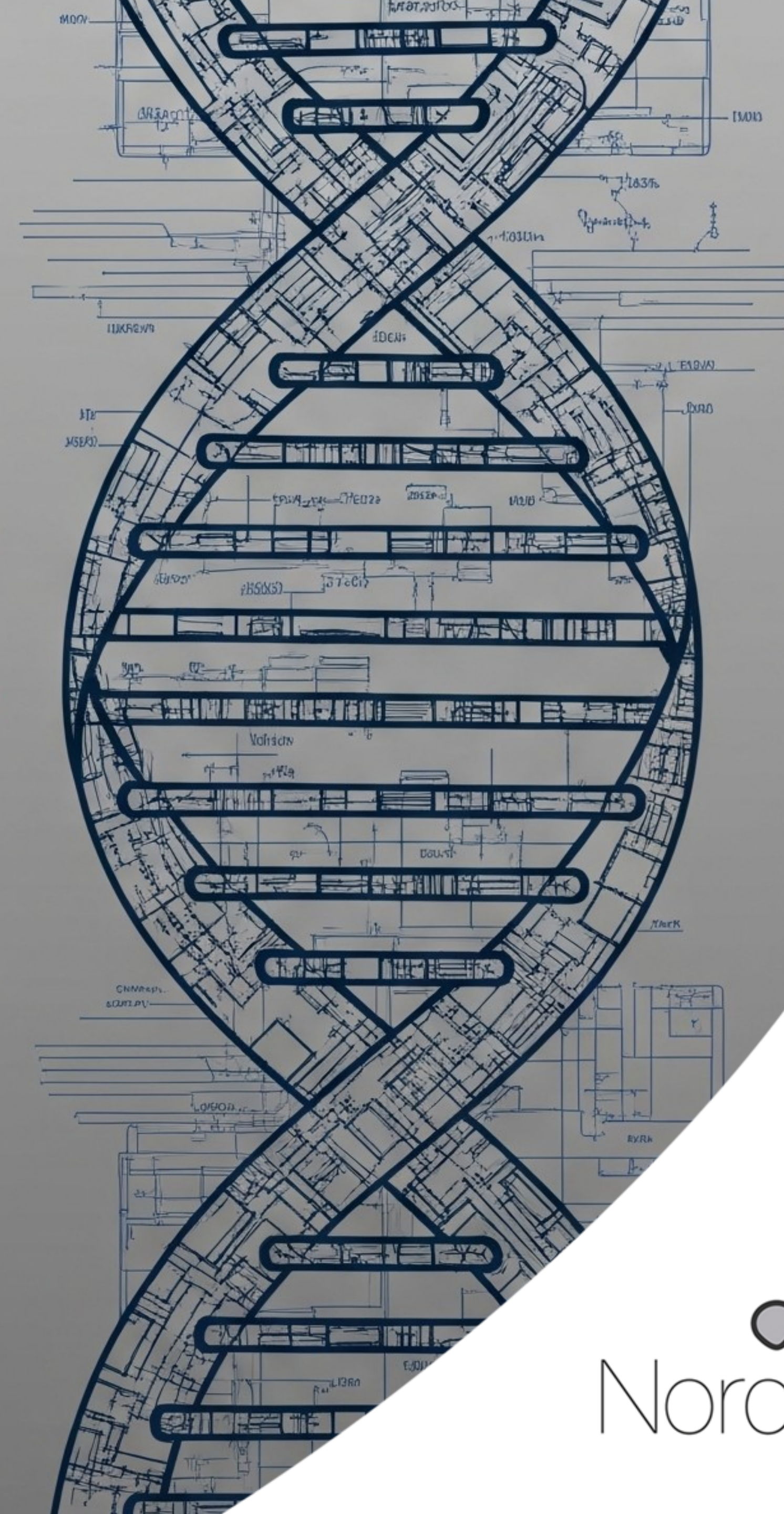
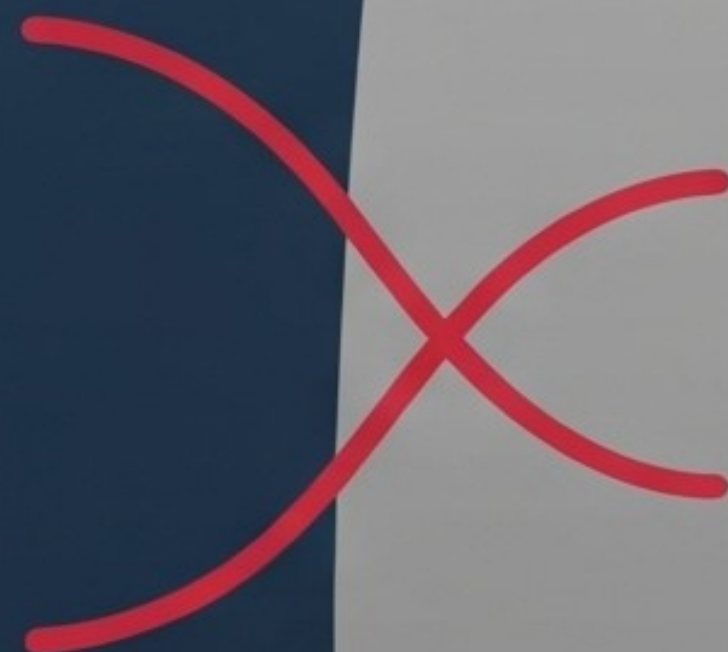
When IHC is gold standard

When the clinical relevance lies in the protein itself, its presence, quantity, or location, rather than a specific, underlying genetic change.

Type I assays

Type II assays, where the protein is the direct target of the drug (ER/PR, PD-L1, Claudin 18.2, Folate receptor alpha)





Thank you!