Mastering Immunohistochemistry: Historical Perspectives, Current Challenges, and Future Horizons

Understanding its Past, Present, and Future in Pathology

Emilia Andersson
Medical & Government Affairs, Oncology assays lead
Roche Diagnostics

Disclosures

Full time employee of Roche Diagnostics Oncology Assays EMEA-LATAM

Board member of SvFP

Consultant for Unilabs and LÖF

Historical Perspectives

Immunohistochemistry: Key Milestones at a Glance

Dawn of IHC

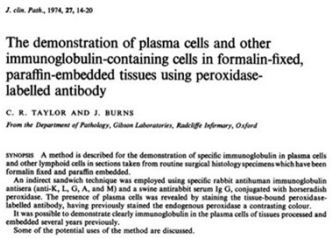
1941-42: Coons et al. pioneer immunofluorescence (FITC), enabling specific antigen detection in tissues.

Routine Application & FFPE Breakthrough

Widespread adoption with HRP/DAB.

Taylor & Burns enable IHC on FFPE tissues, unlocking archival samples.

1975: Hybridoma technology developed to create Monoclonal Antibodies



J. clin. Path., 1974, 27, 14-20

The demonstration of plasma cells and other immunoglobulin-containing cells in formalin-fixed, paraffin-embedded tissues using peroxidase-labelled antibody

C. R. TAYLOR AND J. BURNS

From the Department of Pathology, Gibson Laboratories, Radcliffe Infirmary, Oxford

SYNOPSIS A method is described for the demonstration of specific immunoglobulin in plasma cells and other lymphoid cells in sections taken from routine surgical histology specimens which have been formalin fixed and paraffin embedded.

An indirect sandwich technique was employed using specific rabbit antihuman immunoglobulin antisera (anti-K, L, G, A, and M) and a swine antirabbit serum Ig G, conjugated with horseradish peroxidase. The presence of plasma cells was revealed by staining the tissue-bound peroxidase-labelled antibody, having previously stained the endogenous peroxidase a contrasting colour.

It was possible to demonstrate clearly immunoglobulin in the plasma cells of tissues processed and embedded several years previously.

Some of the potential uses of the method are discussed.

Unmasking & First Companion Diagnostic

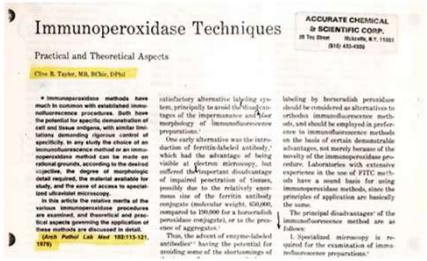
1991: Antigen Retrieval (HIER/PIER) dramatically improves FFPE IHC.

1998: FDA approves HercepTest™ (HER2 IHC) as the first companion diagnostic, Introducing personalized medicine.

1941-42 | 1966-67 | 1974 | 1990s | 2000s-present

Enhanced Visibility

Introduction of enzyme labels (HRP, AP) allows light microscopy, broadening IHC's use.



Immunoperoxidase Techniques

Practical and Theoretical Aspects

ACCURATE CHEMICAL & SCIENTIFIC CORP.

Specificity & Early Automation

Monoclonal Antibodies (Nobel Prize 1984): Revolutionize specificity.

First automated IHC stainers improve consistency and throughput.

"Monoclonal Antibodies"

Predictive Powerhouse & Advanced Techniques

Routine Diagnostic: Essential for cancer diagnosis, classification, and prognostication.

Quantitative & Digital Pathology: Enables objective analysis.

Multiplex IHC: Simultaneous detection of multiple markers.

Predictive Biomarkers: Critical for guiding targeted therapies (e.g., ER/PR, PD-L1 for immunotherapy).

Ongoing CDx Development: IHC remains central to precision medicine.

HRP: Horseradish peroxidase AP Alkaline phosphatase FFPE: Formalin-Fixed Paraffin-Embedded
HIER/PIER: Heat/Proteolytic epitope retrieval

Comparison of Antibody Types for Immunohistochemistry

Binding Characteristics & Applications

Feature	Monoclonal Antibodies (mAbs)	Polyclonal Antibodies (pAbs)	Recombinant Antibodies (rAbs)
Specificity	High (recognize a single epitope)	Lower (recognize multiple epitopes on the same antigen)	Very High (recognize a single, defined epitope; sequence defined)
Sensitivity	Can be lower, especially if the single epitope is masked or altered by processing.	Generally higher due to binding multiple epitopes; more robust to some epitope variations.	Can be engineered for high affinity and sensitivity; still targets a single epitope, so masking can be an issue.
Application	When high specificity is paramount; diagnostic applications; companion diagnostics.	Research applications; detection of low abundance or altered targets; when a broader epitope recognition is beneficial.	Diagnostics, therapeutics, research; situations requiring high specificity, consistency, and scalability; engineered antibody formats.

Comparison of Antibody Types for Immunohistochemistry

Performance & Reliability & Production

Feature	Monoclonal Antibodies (mAbs)	Polyclonal Antibodies (pAbs)	Recombinant Antibodies (rAbs)
Background	Typically lower due to high specificity.	Can be higher due to potential cross-reactivity with other proteins or multiple epitope recognition.	Typically very low due to high specificity and defined nature; reduced non-specific binding.
Reproducibility/ Consistency	Generally good lot-to-lot consistency (from hybridomas).	Can have greater lot-to-lot variability due to different immune responses in animals.	Excellent lot-to-lot consistency due to defined genetic sequence and controlled *in vitro* production; not reliant on animals or hybridoma stability.
Robustness	May be more susceptible to epitope modification by fixation/processing as they target a single site.	Often more robust to minor variations in antigen presentation or epitope modification.	Susceptible to modification of the single target epitope; however, can be engineered for improved stability.
Production	Hybridoma technology (fusion of B-cells with myeloma cells).	Animal immunization.	Recombinant DNA technology (genes cloned into expression systems like mammalian cells, bacteria, yeast); animal-free.

Comparison of Antibody Types for Immunohistochemistry

Production & Practicalities

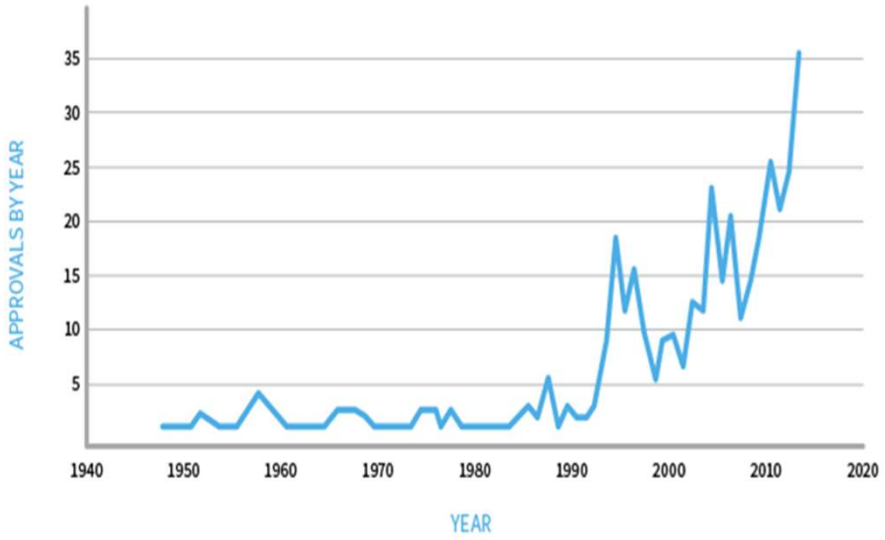
Feature	Monoclonal Antibodies (mAbs)	Polyclonal Antibodies (pAbs)	Recombinant Antibodies (rAbs)
Cost	Often higher to produce initially (hybridoma development).	Generally lower to produce initially (animal immunization).	Initial development (gene cloning, expression system setup) can be costly, but large-scale production can be cost-effective and highly scalable.
Production	Hybridoma technology (fusion of B-cells with myeloma cells).	Animal immunization.	Recombinant DNA technology (genes cloned into expression systems like mammalian cells, bacteria, yeast); animal-free.
Engineering Potential	Limited post-production.	None.	High; can be easily engineered (e.g., fragments, chimerization, humanization, bispecifics, fusion proteins).

Current Challenges (Present-Day Applications)

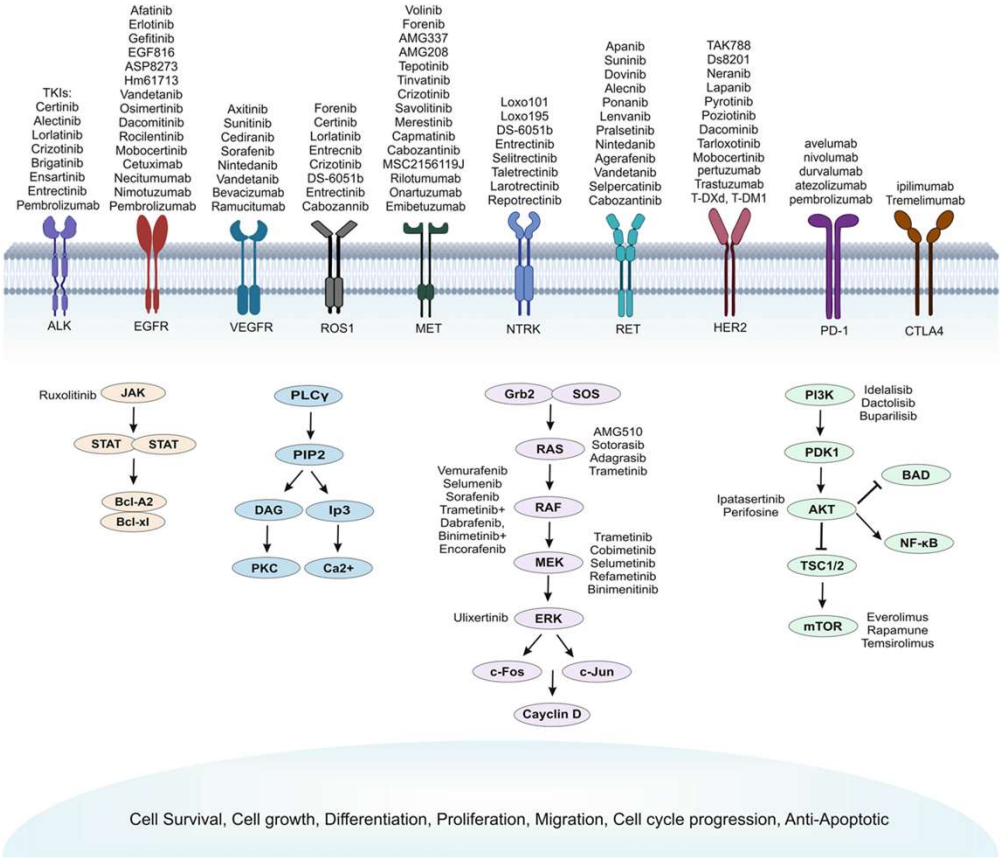
Targeted Therapies Drive Companion Diagnostics Demand

Multiple therapeutic targets in lung cancer

Rapid growth of FDA approvals of targeted therapy for cancer in the last 30+ years



Multiple therapeutic targets in lung cancer

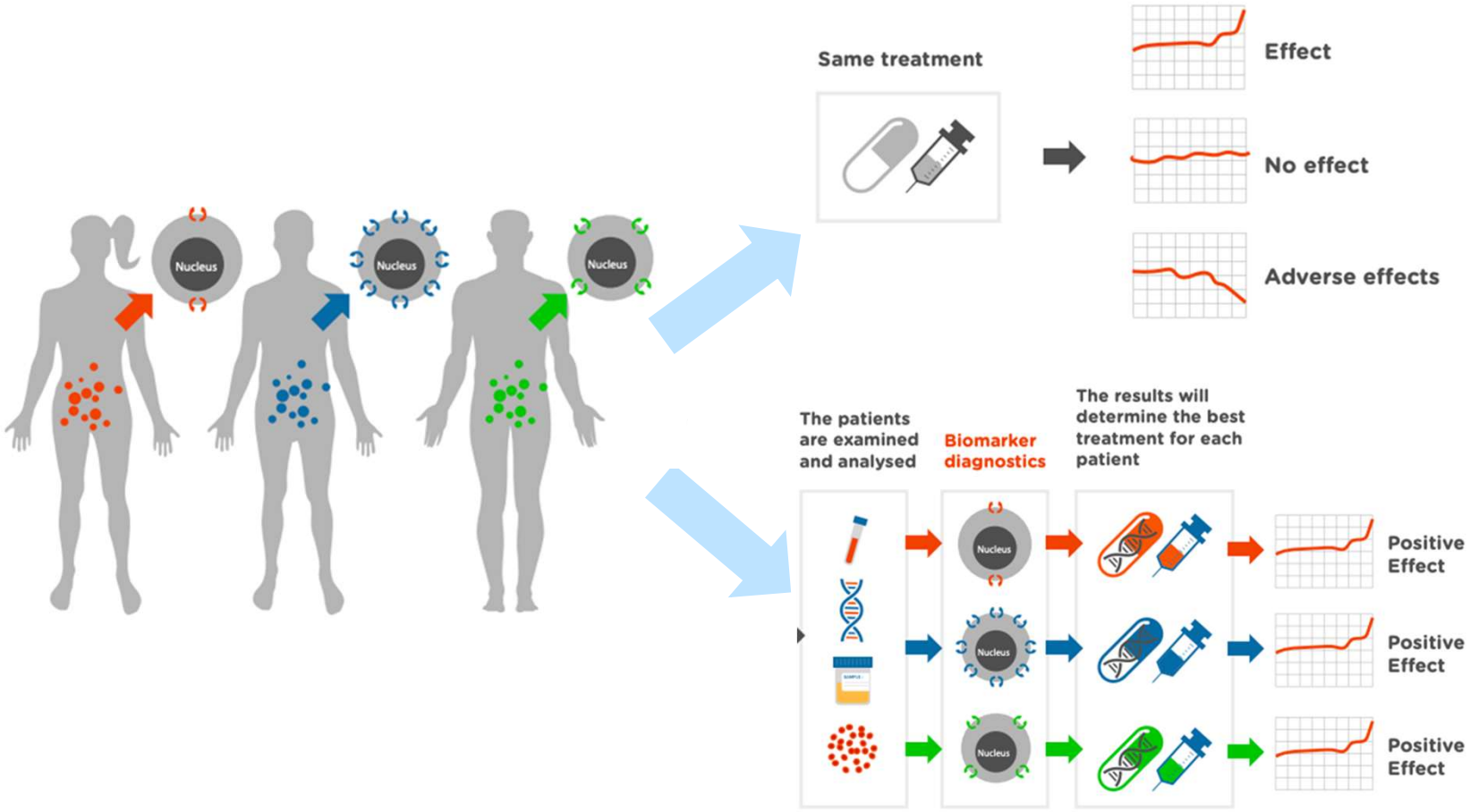


Ref 1. https://medium.com/what-will-it-take-to-end-cancer/evolution-and-future-of-cancer-treatments-7241c4a005a5

Ref 2. Araghi, M., Mannani, R., Heidarnejad maleki, A. *et al.* Recent advances in non-small cell lung cancer targeted therapy; an update review. *Cancer Cell Int* 23, 162 (2023). https://doi.org/10.1186/s12935-023-02990-y

Traditional vs personalised medicine

Treat all or treat informed?



Before Personalised Healthcare,an average of only

25%

of patients responded to their cancer chemotherapy

Today, up to

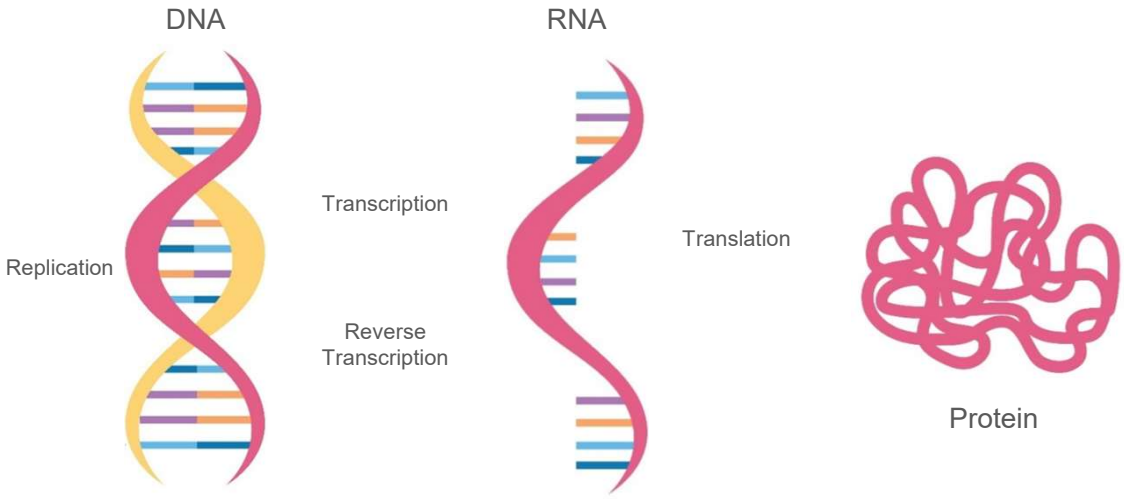
80%

of patients respond to their targeted therapies

https://www.efpia.eu/about-medicines/development-of-medicines/precision-medicine/ (retrieved March 17, 2025)
Spear BB, et al. Trends Mol Med. 2001:(7)5:201–204. Peters S, et al. N Engl J Med. 2017;377(9):829–838;
Kato, S. Nat Commun. 2020;11(1):4965. 5. Naidoo, J. et al. molecular Diagnostic testing in Non-small Cell Lung Cancer. [online] Available at: https://gotoper-com.s3.amazonaws.com/_media/_pdf/AJHO14Sept_01_NSCLC.pdf.

Central dogma: Cancer is caused by genomic alterations

What's the role of IHC in the future?

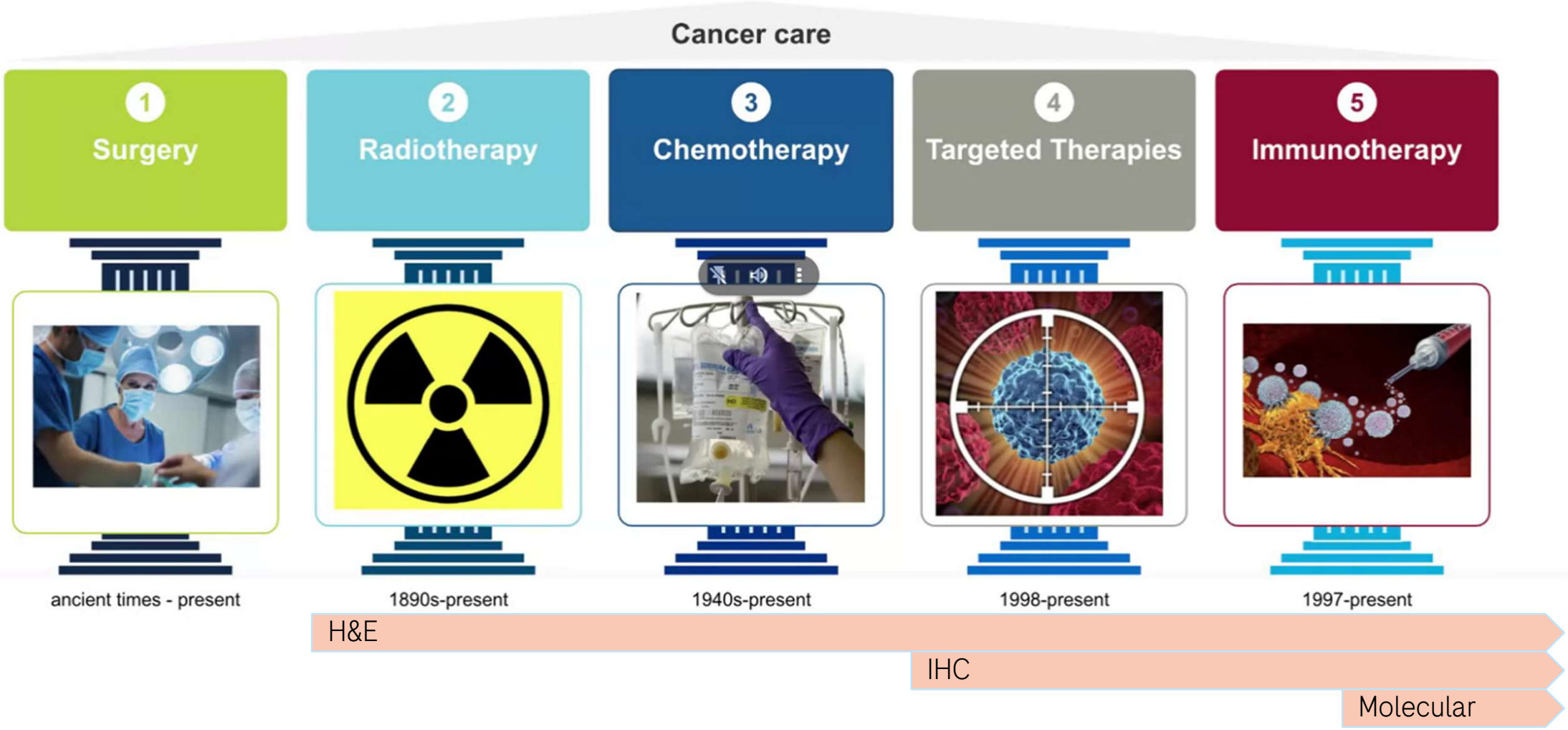


Diagnostic tests	**DNA ISH (FISH or CISH)** **PCR** **NGS**	**RNA (FISH or CISH)** **PCR** **NGS**	**IHC** **(Mass spectrometry)**

| DNA: deoxyribonucleic acid; ISH: in-situ hybridization; FISH: fluorescent *in-situ* hybridization; CISH: chromogenic *in-situ* hybridization; PCR: polmerase chain reaction; NGS: next-generation sequencing; RNA: ribonucleic acid; IHC: immunohistochemistry

Need to understand the Drug mode of action

Clinical utility of testing method.

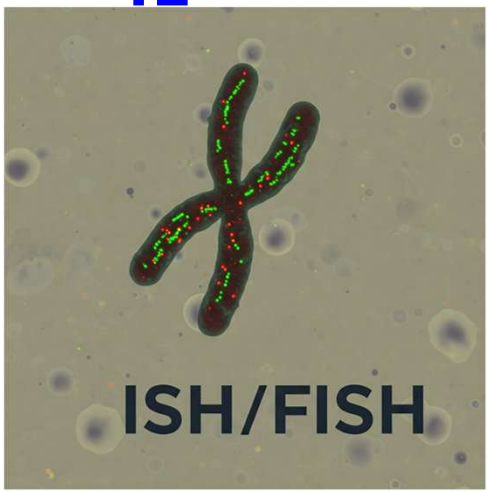


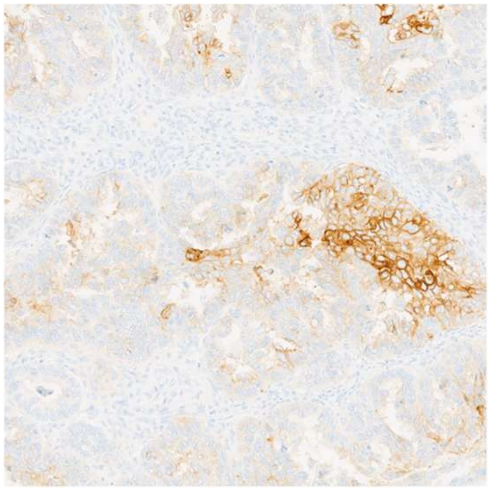
Recent CDx approvals in numbers

Based on FDA CDx list early 2024 related to oncology

19

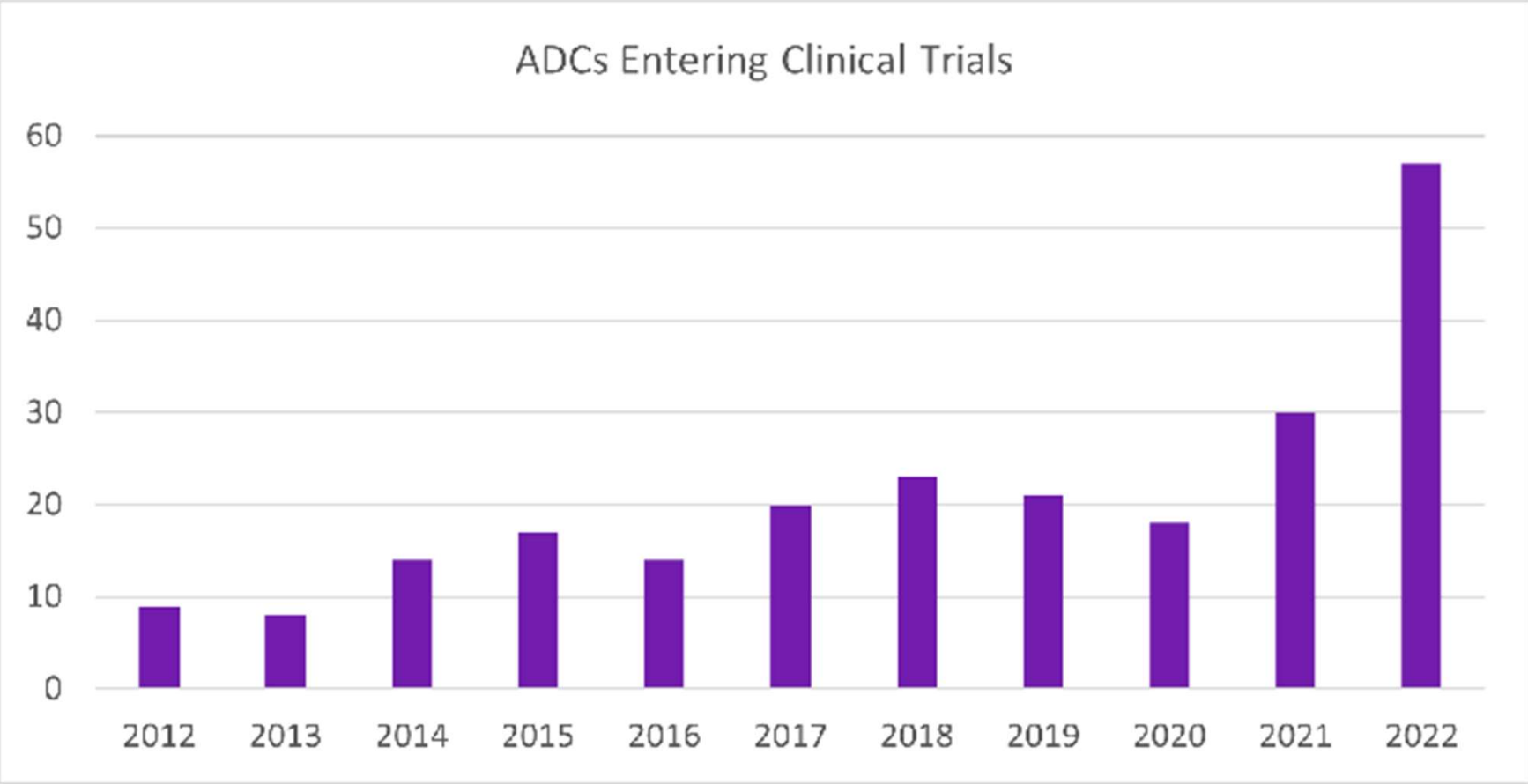
12





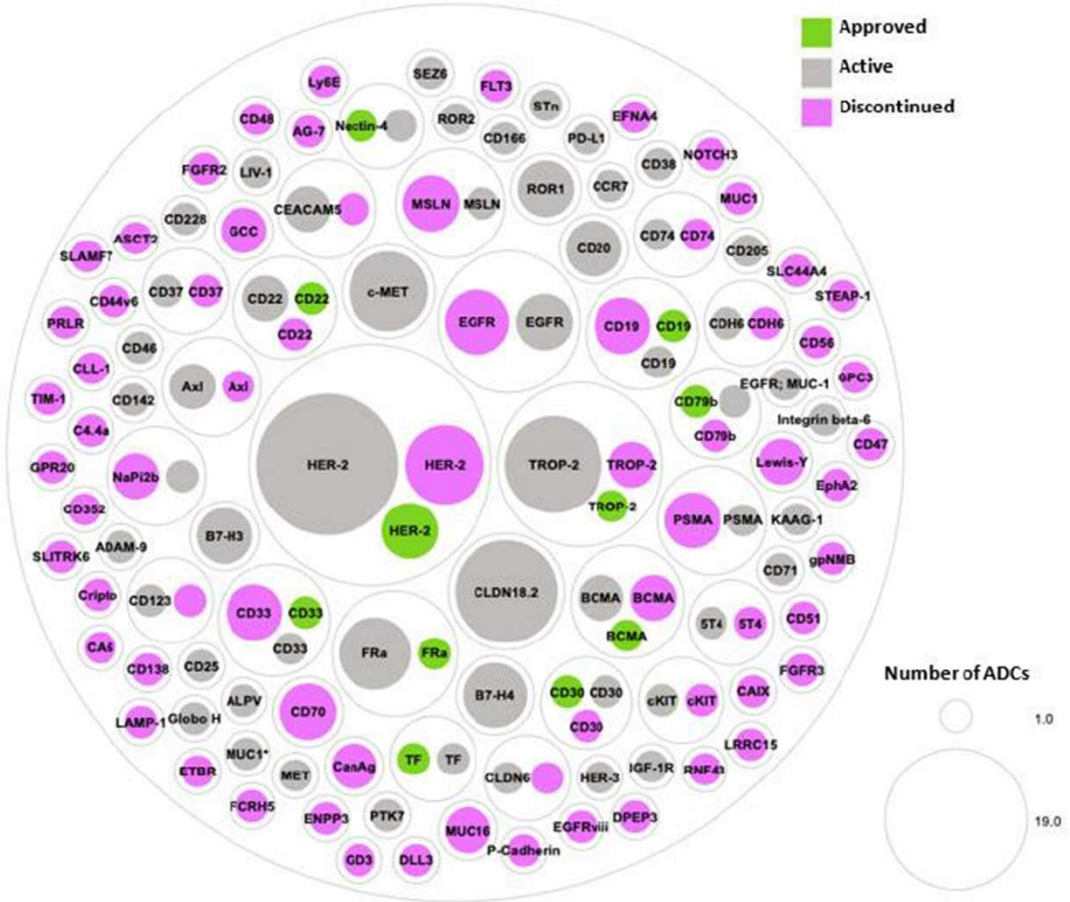
Antibody based therapies are increasing

So most likely will the need for IHC increase too…

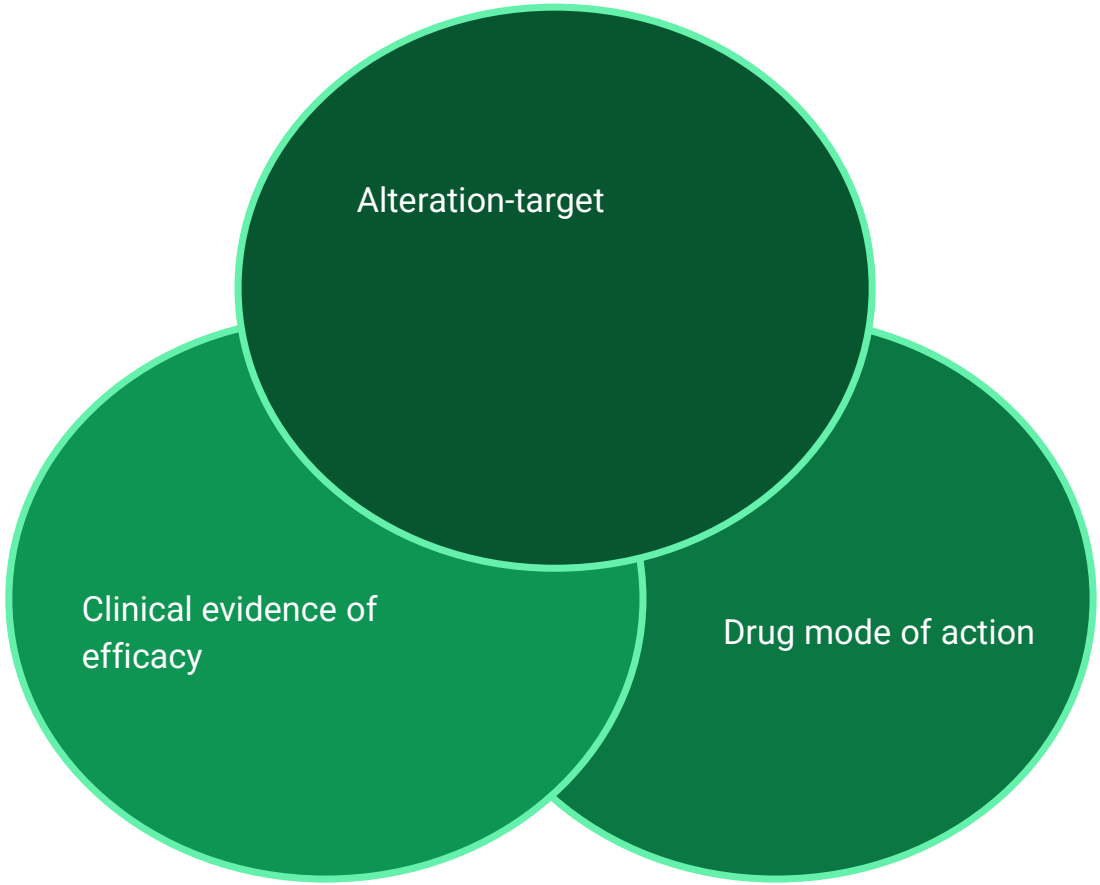


https://www.clinicalleader.com/doc/the-clinical-landscape-of-adcs-in-diverse-technologies-narrow-target-0001

Predictive biomarkers in development



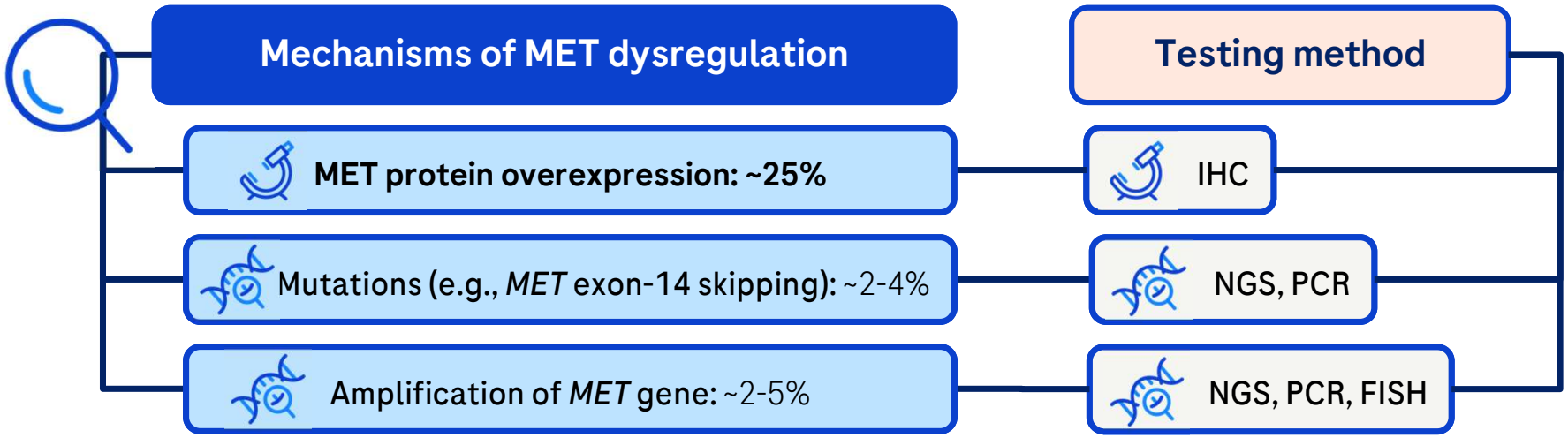
How is a CDx testing method decided?



Mechanisms of dysregulation/ alteration & testing methods

Example MET

Different testing methods, like IHC and NGS, are needed to detect MET aberrations



There is **minimal overlap between MET aberrations**

Park S, et al. *Histol Histopathol.* 2012; Michaels E, et al. *Front Oncol.* 2022

Drug mode of action

Targeting MET with an Antibody Drug Conjugate (ADC)

Preclinical Data

- Telisotuzumab vedotin antitumor efficacy correlates with *MET protein expression levels, irrespective of MET gene amplification*

- Minimal inhibitory effects were observed on MET-expressing normal cells

MET overexpression was measured by immunohistochemistry (IHC) using **VENTANA SP44** antibody

Table 2. c-Met expression on tumor cells *in vitro* and sensitivity to ABBV-399

Cell Line	c-Met Expression[a]	Maximal Killing[b]	ABBV-399 $IC_{50} \pm SD$[c]
Lung cancer			
NCI-H1650	4,500	13%	47.9 ± 8.5
A549	43,000	22%	1.6 ± 1.1
NCI-H1573[d]	116,000	18%	18 ± 14
NCI-H441	197,000	56%	0.06 ± 0.05
EBC-1[d]	233,000	96%	0.06 ± 0.03
NCI-H820	320,000	87%	0.20 ± 0.07
Nontumor cell lines			
NHBE (bronchial epithelial)	40,000	10%	NA
HUVEC (vascular endothelial	16,000	6%	NA
HMEC (mammary epithelial)	ND	0%	NA
PrEC (prostate epithelial)	65,000	0%	NA
NHDF (dermal fibroblasts)	1,600	0%	NA

Clinical evidence of efficacy

Targeting MET with telisotuzumab vedotin

LUMINOSITY Results

	Non-squamous *EGFR* WT NSCLC cohort (n=161)	
	c-MET intermediate (n=83) ≥ 25% to < 50% TC, 3+ intensity	**c-MET high (n=78)** ≥ 50% TC, 3+ intensity
ORR	**22.9%** [95% CI, 14.4–33.4]	**34.6%** [95% CI, 24.2–46.2]
DoR	**7.2 months** [95% CI, 5.3–11.5]	**9.0 months** [95% CI, 4.2–13.0]
DCR	**57.8%** [95% CI, 46.5-68.6]	**60.3%** [95% CI, 48.5-71.2]
PFS	**6.0 months** [95% CI, 4.5–8.1]	**5.5 months** [95% CI, 4.1– 8.3]
OS	**14.2 months** [95% CI, 9.6–16.6]	**14.6 months** (95% CI, 9.2–25.6)

Conclusion:

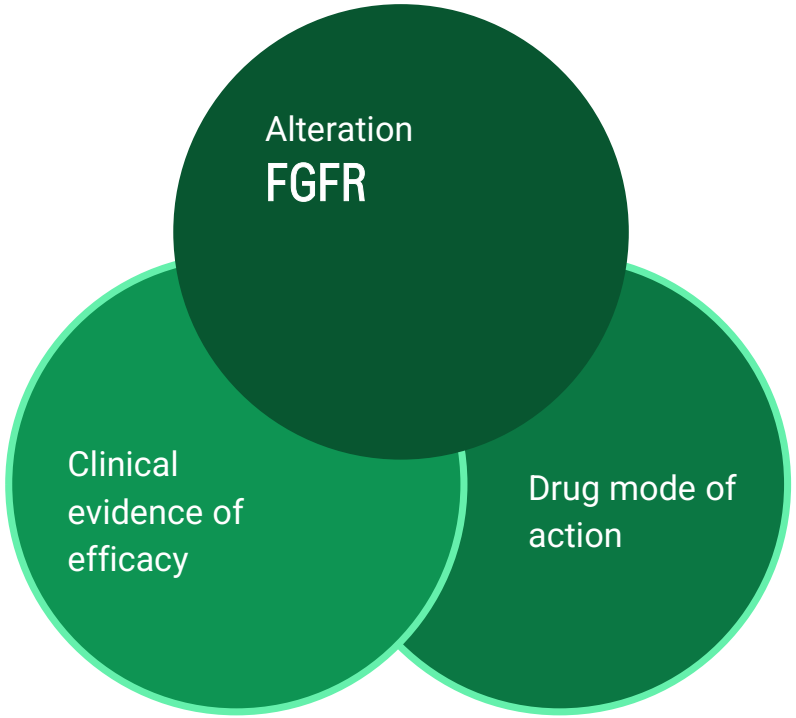
In this single arm Ph2 trial, outcome data was favorable for patients with previously treated locally advanced/ metastatic NSQ *EGFR* WT NSCLC with c-MET protein expression, **especially in c-MET high patients** who had a partial or complete response (ORR) of 34.6% to telisotuzumab vedotin therapy. Ph3 trial ongoing (NCT04928846).

Int, intermediate; NQS, nonsquamous; NSCLC; non-small cell lung cancer; TC, tumor cells; WT, wild-type

Camidge DR, et al. J Clin Oncol. 2024 Jun 6:JCO2400720. doi: 10.1200/JCO.24.00720.

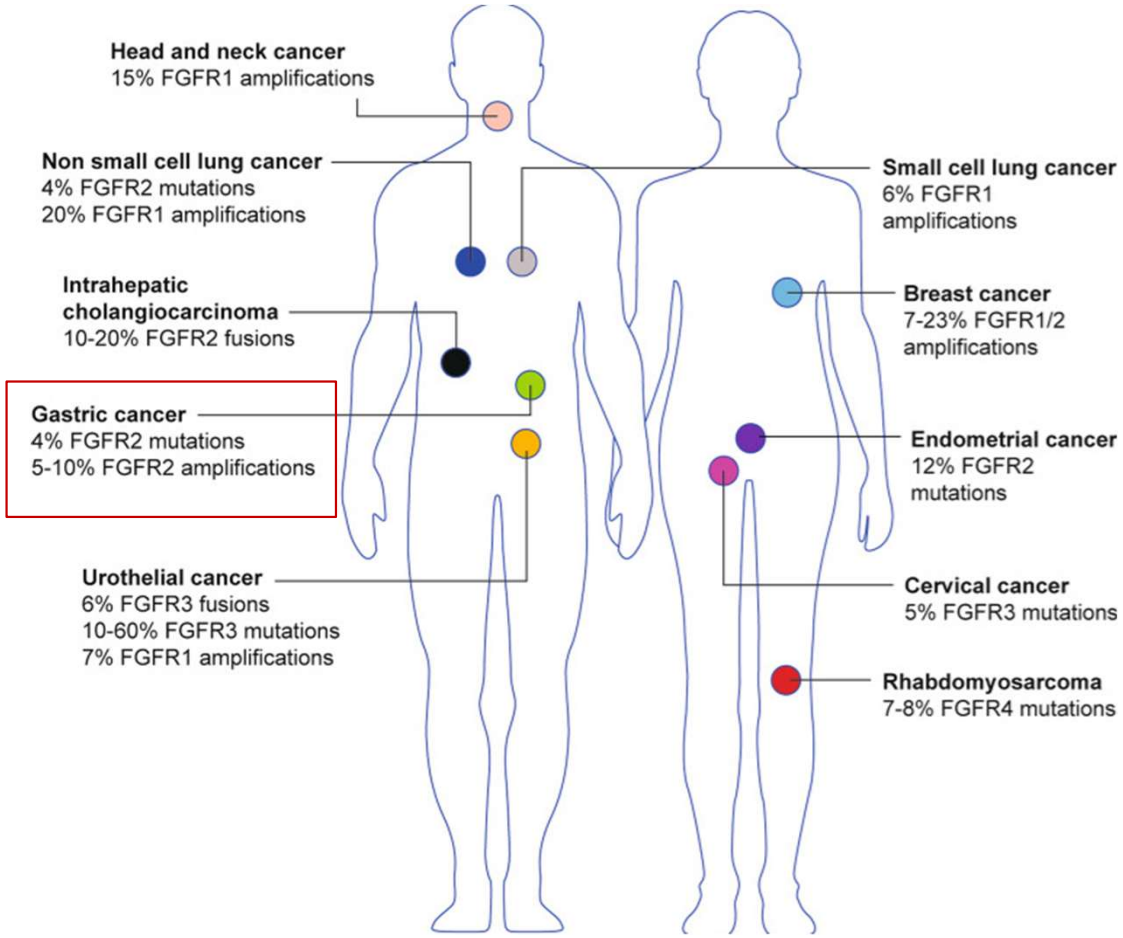
How is a CDx testing method decided?

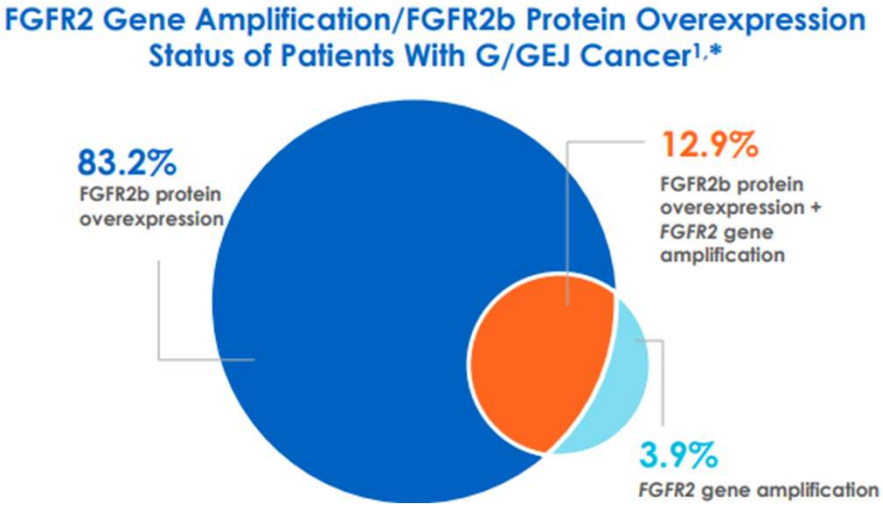
Another example



How is a CDx testing method decided?

Eg FGFR alterations



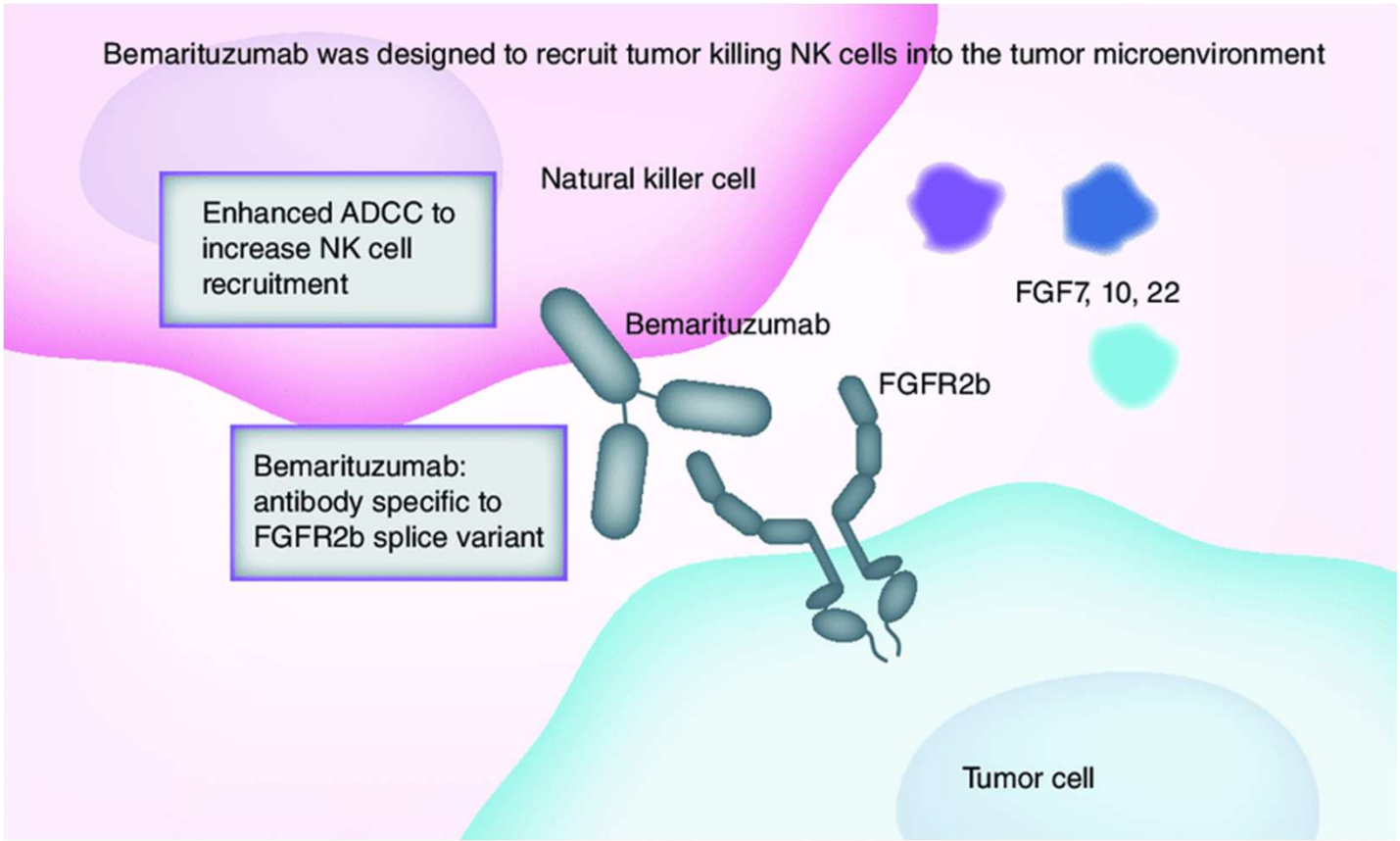


FGFR2b PROTEIN OVEREXPRESSION CAN BE INDEPENDENT OF FGFR2 GENE AMPLIFICATION

*Data from a 2021 randomized, double-blind, placebo-controlled, phase 2 study of patients (N = 155) with metastatic G/GEJ cancer. Wainberg ZA, et al. Presented at: American Society of Clinical Oncology Gastrointestinal Cancer Symposium; January 15-17, 2021; Online Virtual Scientific Program. Abstract LBA160

Drug mode of action

Targeting FGFR2b with Antibody dependent cell mediated cytotoxicity - bemarituzumab

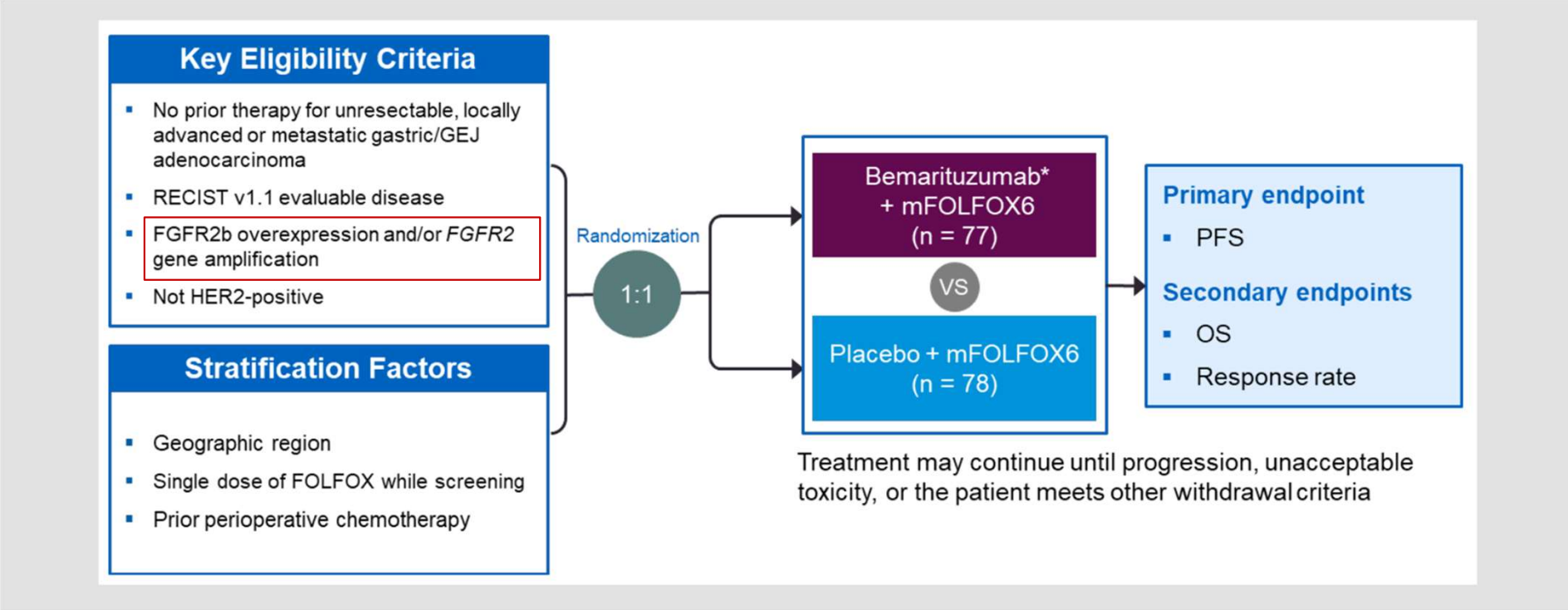


ADCC: Antibody-dependent cell-mediated cytotoxicity

Clinical evidence of efficacy

Targeting FGFR2b with bemarituzumab

FIGHT phase 2 study design



*Bemarituzumab dosing: 15 mg/kg Q2W beginning cycle 1 day 1 (plus 1 dose of 7.5 mg/kg on day 8 of cycle 1 only). FOLFOX6 dosing: standard fixed doses Q2W.
FGFR2b = fibroblast growth factor receptor 2b

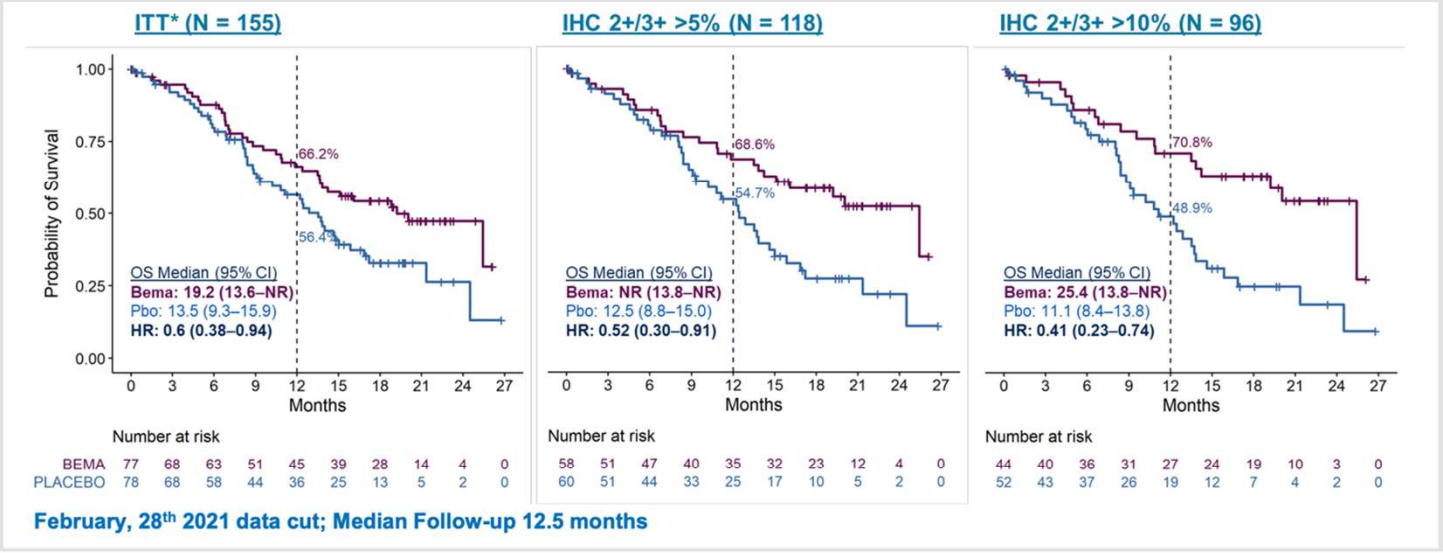
FGFR2b overexpression= any 2+/3+ IHC staining FGFR2 amplification by ctDNA

Clinical evidence of efficacy

Targeting FGFR2b with bemarituzumab

FIGHT phase 2 study results

Addition of Bemarituzumab Showed a +5.7 Month Improvement in Median OS



February, 28th 2021 data cut; Median Follow-up 12.5 months

*ITT = includes 149 patients with IHC 2+/3+ and 6 with IHC <2+ or not available who were enrolled based on ctDNA alone; NR = not reached

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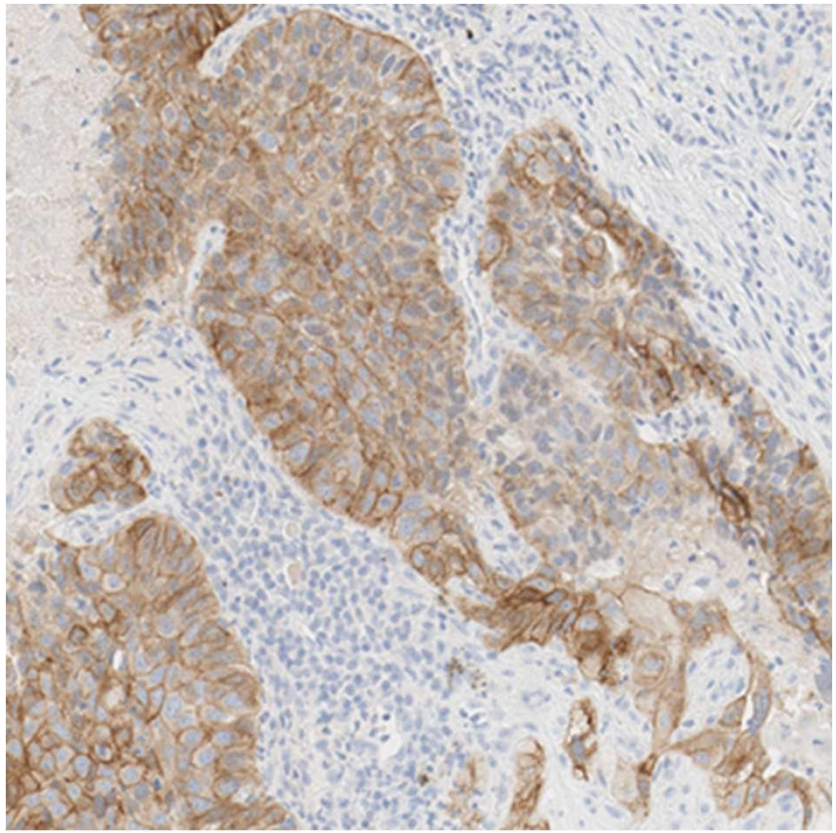
Conclusion:

Treatment benefit was more pronounced in patients with higher FGFR2b expression

Benefit was observed regardless of FGFR2b amplification

Pathologist challenge

Cutoff based on intensity and percentage



MET

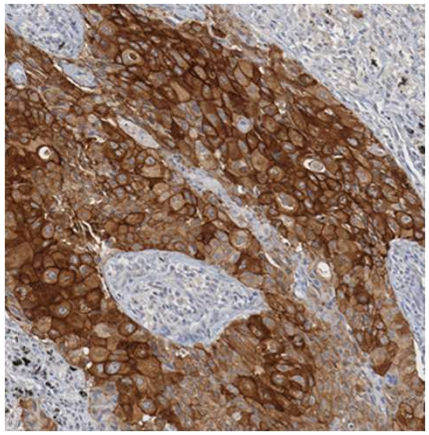
Criteria

Intensity: 3+ membrane and/or cytoplasmic

Percentage: > 25% or 50% (remains to be decided)

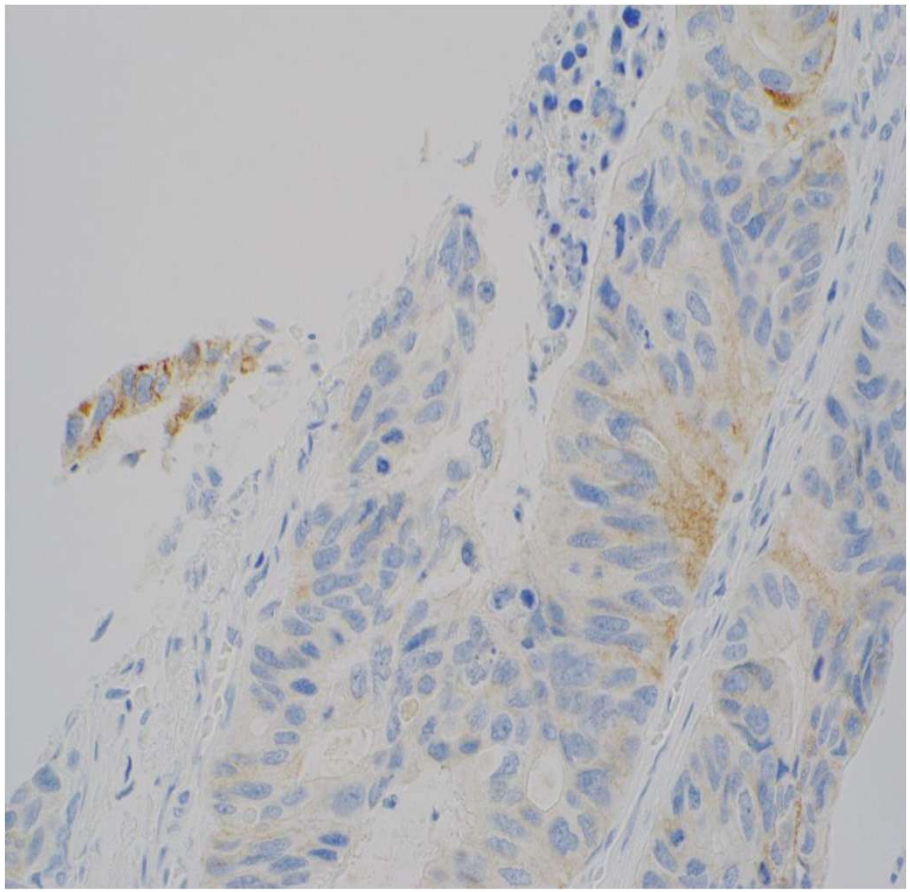
Score

Negative



Pathologist challenge

Cutoff based on intensity and percentage



FGFR2b

Criteria

Intensity: 2+/3+

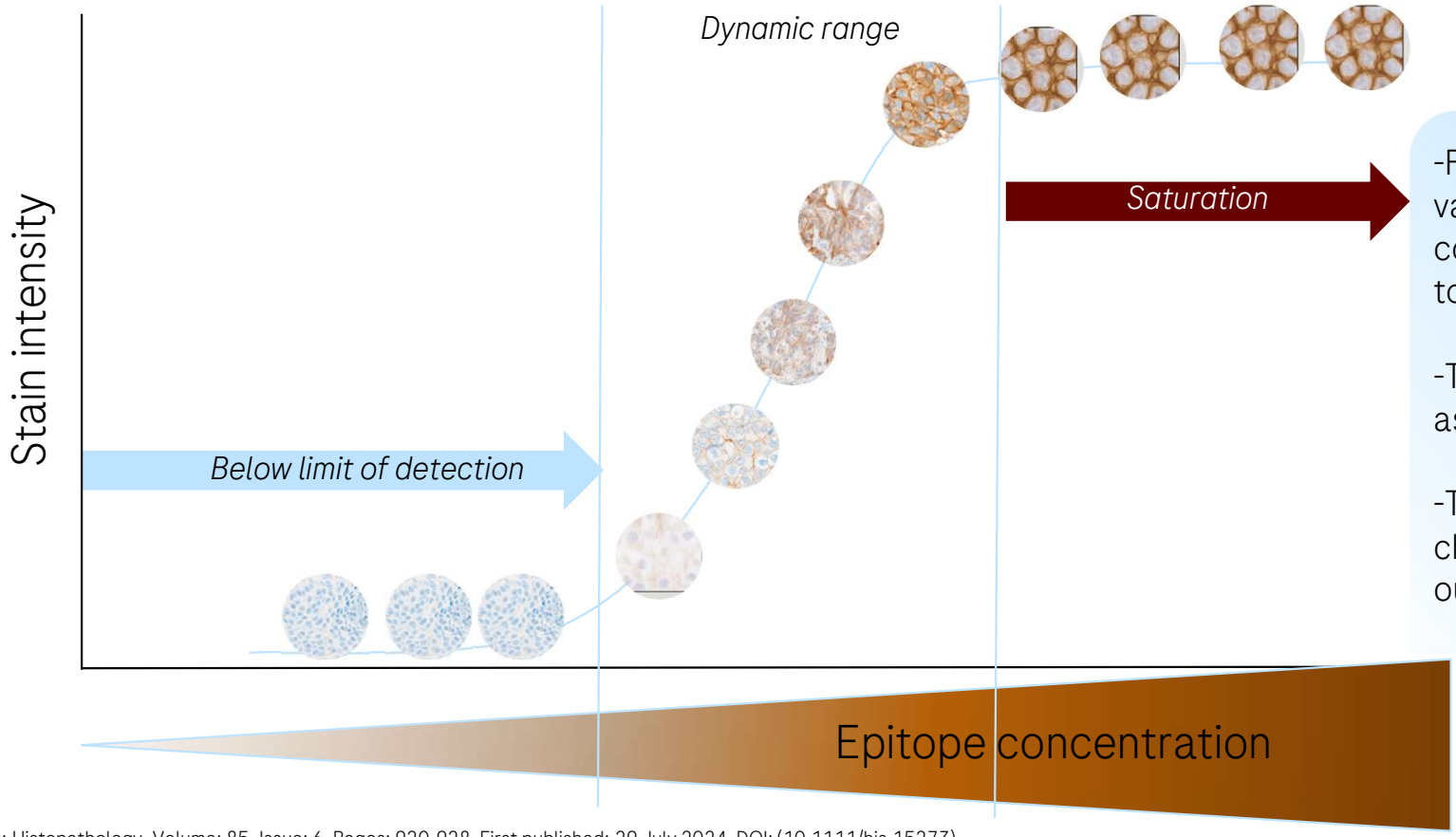
Percentage: > any, >5% or >10% (remains to be decided)

Score

?

Dynamic range

From Limit of Detection to Saturation



-Protein expression on a cell can vary up to four logs, corresponding to a few hundred to millions of molecules per cell.

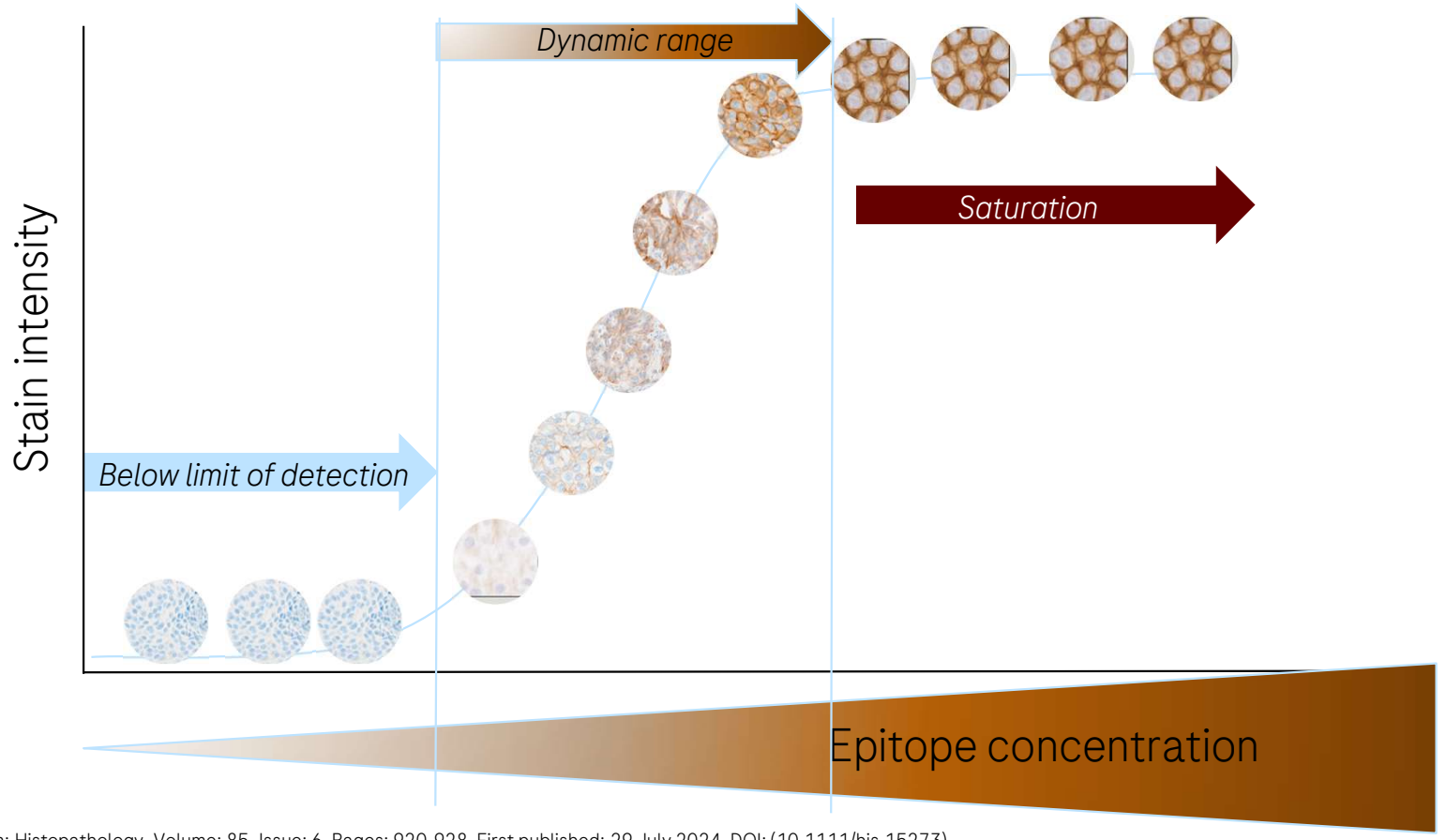
-The dynamic range of any IHC assay is about one to two logs.

-The IHC method will not reflect changes in staining intensity outside the dynamic range

Image adapted from: Histopathology, Volume: 85, Issue: 6, Pages: 920-928, First published: 29 July 2024, DOI: (10.1111/his.15273)
Lohse J,et al. Improved catalyzed reporter deposition, iCARD. Bioconjugate Chemistry. 2014/06/18 2014;25(6):1036-1042.
Rimm DL. What brown cannot do for you. Nat Biotech. 2006;24(8):914-916.

Dynamic range

An assay can be optimized to a lower range to increase sensitivity



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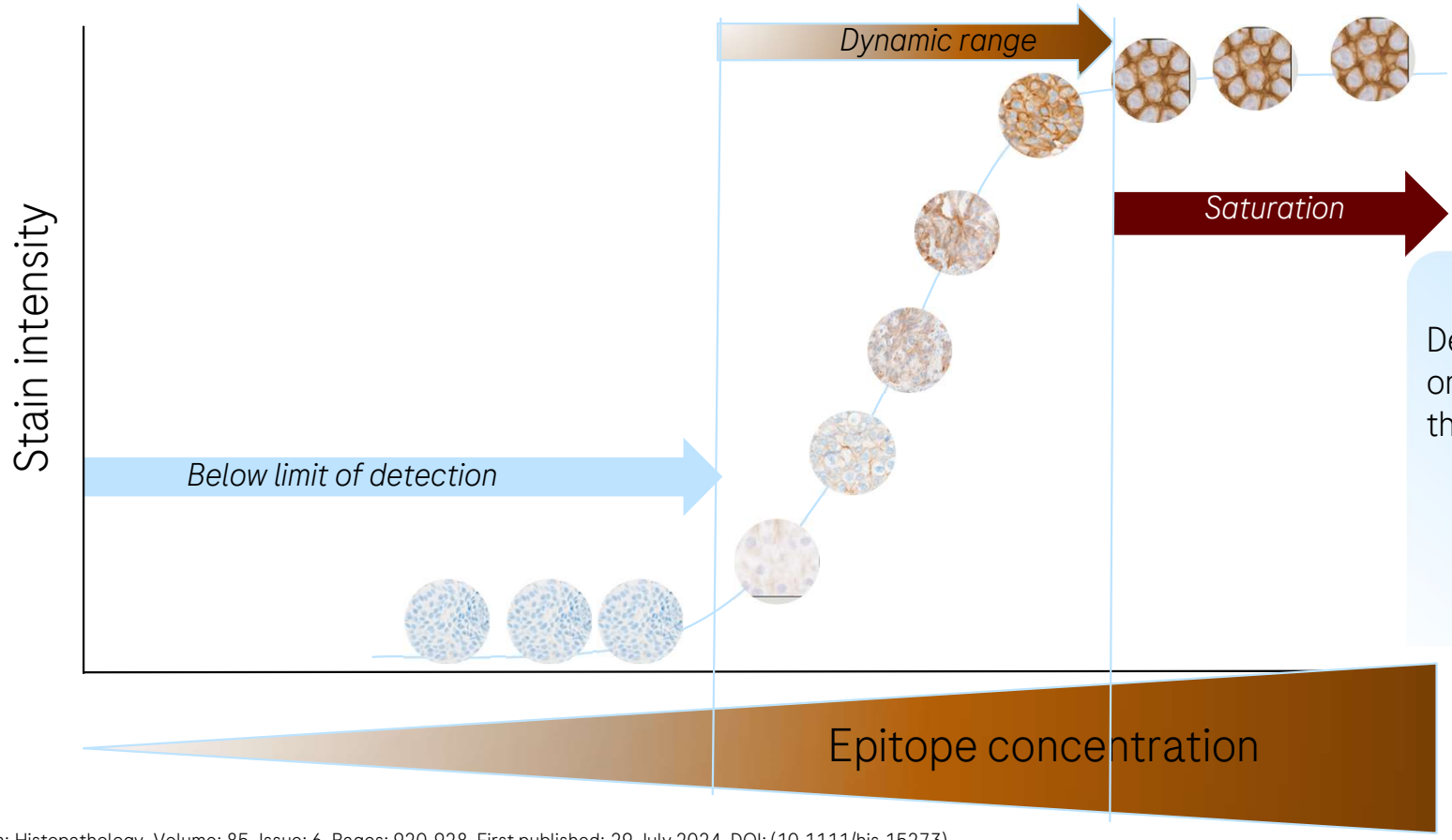
Rimm DL. What brown cannot do for you. Nat Biotech. 2006;24(8):914-916.

Dynamic range

Or a higher range, which decreases the sensitivity but reflect a different spectrum of epitope concentration

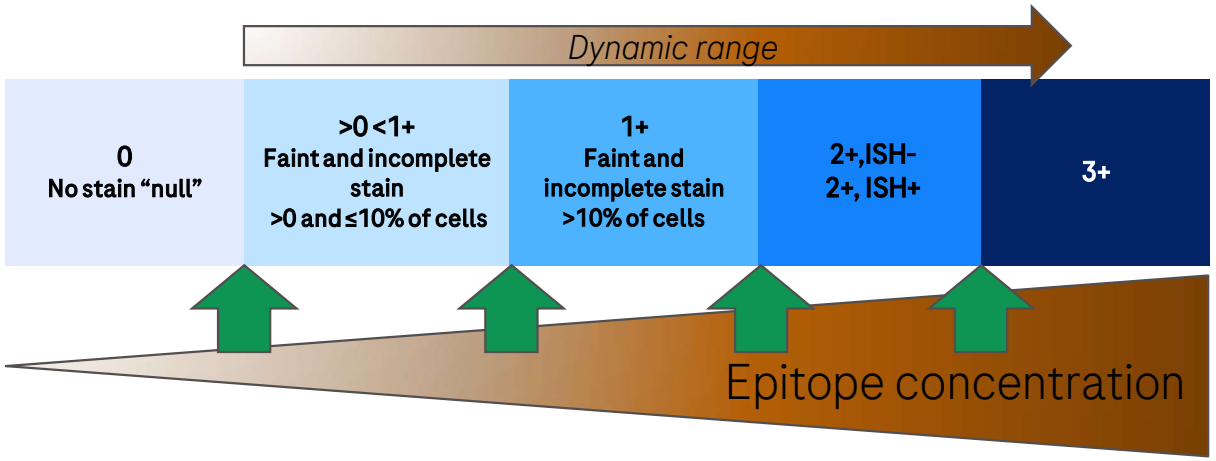


Decision should be made based on the clinically relevant threshold

Image adapted from: Histopathology, Volume: 85, Issue: 6, Pages: 920-928, First published: 29 July 2024, DOI: (10.1111/his.15273)
Lohse J,et al. Improved catalyzed reporter deposition, iCARD. Bioconjugate Chemistry. 2014/06/18 2014;25(6):1036-1042.
Rimm DL. What brown cannot do for you. Nat Biotech. 2006;24(8):914-916.

Dynamic range and the example of HER2

Clinically relevant thresholds across the dynamic range



Normal breast epithelial cells with 2 copies of the HER2 gene express approximately 20 000 HER2 receptor molecules per cell

IHC 0 → approximately ≥20 000 molecules per cell
IHC 1+ → approximately >100 000 molecules per cell
IHC 2+ → approximately >500 000 molecules per cell
IHC 3+ → approximately >2 000 000 molecules per cell

Send to ISH

Treat directly without ISH confirmation

HER2 low (FDA/EMA, not UK)

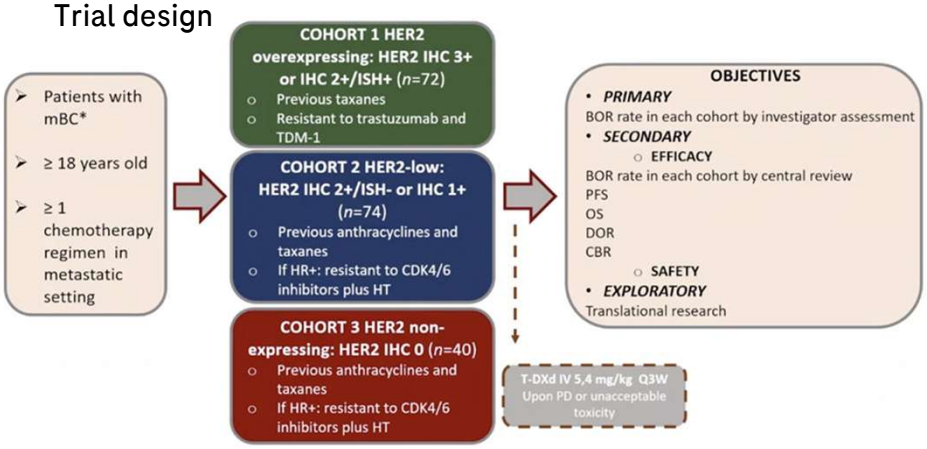
HER2 ultralow (only FDA)

Zhang and Peng (2023) Cancers 15(1):126 Accessed 9/18/2023

Will it be clinically relevant to push the dynamic range lower?

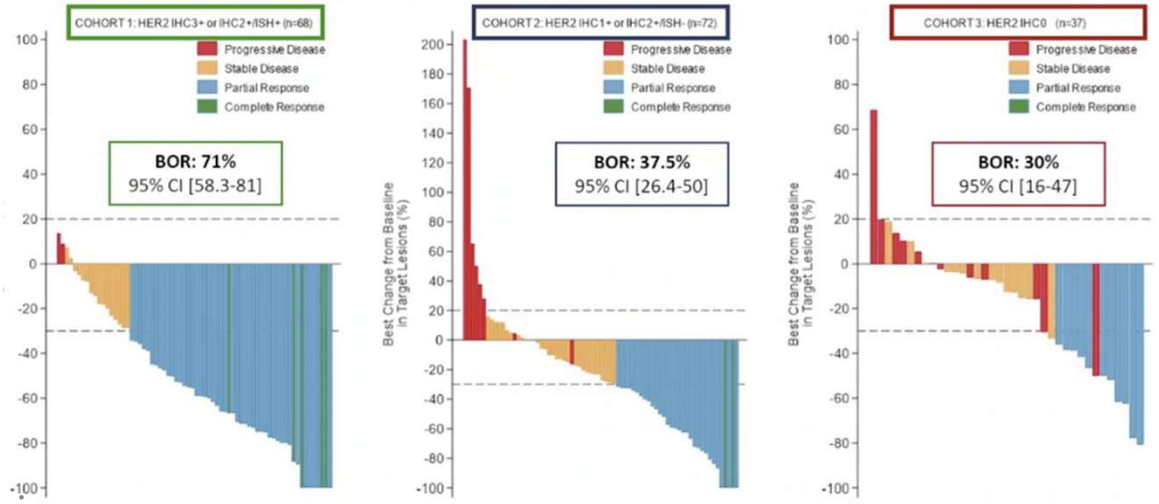
The therapy is not targeting a “score”, but rather the amount of epitopes correlating to a score of a specific assay = CDx

Trial design



DAISY: Investigating T-DXd at different levels of HER2 expression
An open label, multicenter, phase 2 study (NCT04132960)

BOR rate decreases with lower levels of HER2 expression



THE BOR RATE IS DEFFERENT BETWEEN THE THREE COHORTS p <0.0001

FDA/EMA/NICE will need to Consider the benefit vs risk profile as well as cost/benefit when approving.

BOR: Best Objective Response
M.F Mosele et al. Annals of Onc. 2022

HER2

Used to be relatively straightforward, but not so much anymore…

Tumor Type	HER2 Targeted Therapy	Testing Method Require	Eligibility Definition	Approval Status	CDx Required
Breast Cancer	Trastuzumab	IHC & ISH	IHC 3+ OR (IHC 2+ AND ISH positive[1])	Approved	Any Validated
	Pertuzumab (in combination)				
	Ado-trastuzumab emtansine (T-DM1)				
	Lapatinib				
	Neratinib				
	Tucatinib (in combination)				
	Trastuzumab deruxtecan (T-DXd)		IHC 3+ OR (IHC 2+ AND ISH positive[1])		
			For HER2-Low: IHC 1+ OR (IHC 2+ AND ISH negative[1])		Ventana(4B5) explicitly linked to approval for HER2-low/ultralow determination[14]
Gastric / GEJ Adenocarcinoma	Trastuzumab	IHC & ISH	IHC 3+ OR (IHC 2+ AND ISH positive[2])	Approved	Any Validated
	Trastuzumab deruxtecan (T-DXd)		HER2-Low: IHC 1+ OR (IHC 2+ AND ISH negative[2]). (Efficacy shown, but specific "HER2-low" label may vary)	Investigational[9]	Investigational
Non-Small Cell Lung Cancer (NSCLC)	Trastuzumab deruxtecan (T-DXd)	NGS[3]	Activating HER2 (ERBB2) mutation	Approved	FoundationOne®CDx, Thermo Fisher Oncomine™ Dx
		IHC	HER2 Overexpression: IHC 3+ (based on criteria used in trials like DESTINY-Lung02)[4]	Approved	Any Validated
Colorectal Cancer (CRC) (metastatic)	Trastuzumab + Pertuzumab	IHC (& ISH)	IHC 3+ OR (IHC 2+ AND ISH positive[5]). Often restricted to RAS Wild-Type.	Guideline Recommended / Off-Label[11]	NA
	Trastuzumab + Tucatinib			Approved	Any Validated
	Trastuzumab deruxtecan (T-DXd)	IHC & ISH	HER2-Positive: IHC 3+ OR (IHC 2+ AND ISH positive[5]).	Approved	Any Validated
			HER2-Low: IHC 1+ OR (IHC 2+ AND ISH negative[5]).	Investigational	Investigational
Biliary Tract Cancer (BTC)	Trastuzumab + Pertuzumab	IHC (& ISH)	IHC 3+ OR (IHC 2+ AND ISH positive[6])	Guideline Recommended / Off-Label[11]	NA
	Trastuzumab deruxtecan (T-DXd)	IHC	IHC 3+	Approved	Ventana (4B5)
Salivary Gland Cancer	Trastuzumab (often in combination)	IHC (& ISH)	IHC 3+ (HER2 amplification may also be considered)	Guideline Recommended / Off-Label[11]	NA
	Ado-trastuzumab emtansine (T-DM1)				
Pan-Tumor Solid Tumors	Trastuzumab deruxtecan (T-DXd)	IHC	IHC 3+	Approved	Any Validated
	Tucatinib + Trastuzumab	IHC & ISH	HER2-Amplified/Overexpressed: IHC 3+ OR ISH positive (under investigation/specific contexts)[8]	Investigational	Investigational

Disclaimer: Approval status is dynamic and can differ by region (e.g., FDA vs. EMA vs. local Swedish regulations) and specific clinical circumstances (e.g., lines of therapy). This information is based on generally known major approvals as of April 25, 2025. Always verify with current, local regulatory information and clinical guidelines.

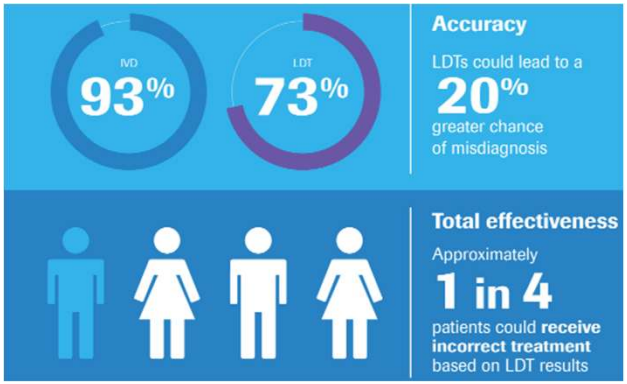
LDT potential Impact to patients

Potential for inappropriate care

Intensity based LDTs have a risk of staining at different levels then the clinically validated CDx, resulting potentially identifying the wrong patient for therapy

Lessons learned from PD-L1 and HER2 4B5

- Deviations from recommended staining procedure affected the HER2 IHC score[1]
- In NSCLC, use of PD-L1 IVD was substantially more effective, leading to improved outcomes and a reduction in overall healthcare costs
- Usage of LDTs can provide challenges to reimbursement and potential legal exposure



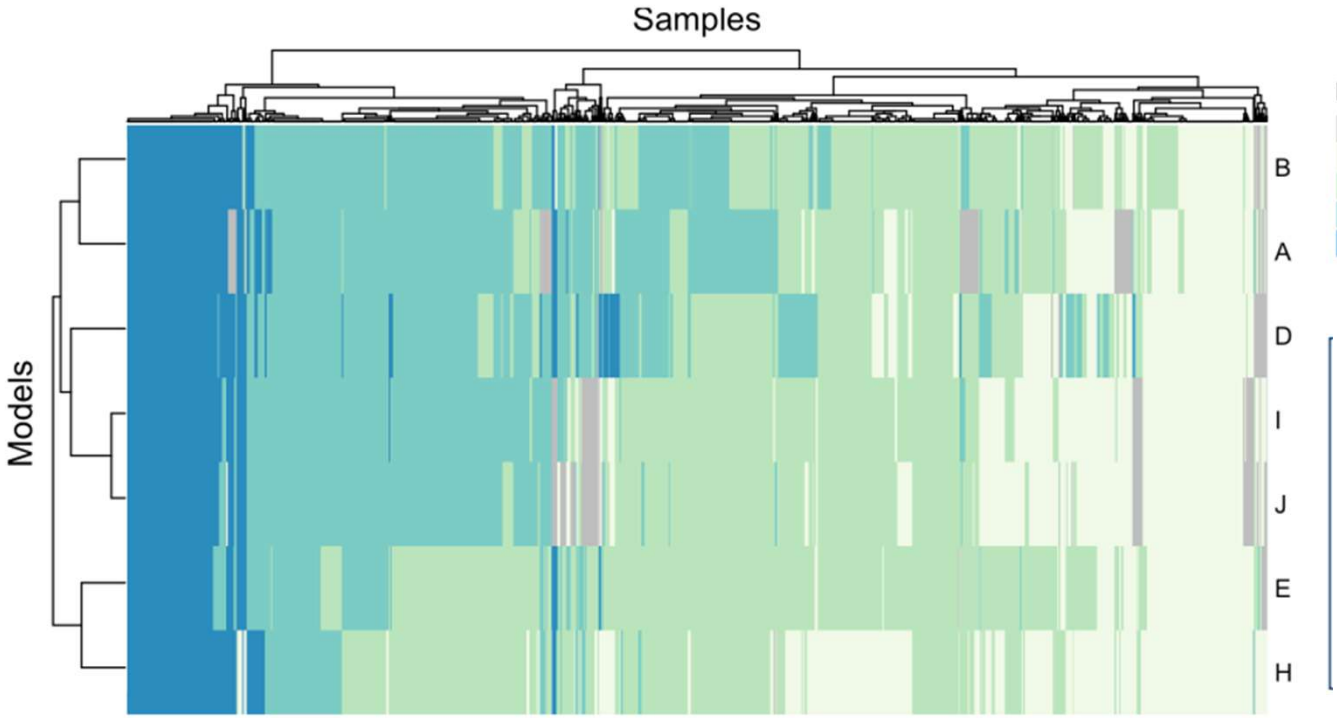
Only clinically validated test

CDx (complete system: Platform, antibody, detection system, protocol)
LDT-validated against a CDx ***at the clinically relevant threshold(s)!***

1 Garrido, Charo, et al. "Analytical and clinical validation of PATHWAY Anti-HER-2/neu (4B5) antibody to assess HER2-low status for trastuzumab deruxtecan treatment in breast cancer." Virchows Archiv (2023): 1-10.
2 Hurwitz, Jason T., et al. "Cost-Effectiveness of PD-L1 Testing in Non-Small Cell Lung Cancer (NSCLC) Using In Vitro Diagnostic (IVD) Versus Laboratory-Developed Test (LDT)." Oncology and therapy 10.2 (2022): 391-409.

Clinical validation is equally important for algorithms.

1100+ breast cancer cohort stained with Roche HER2 (4B5) IHC assay, scanned, and subject to 10 different HER2 IHC algorithms



No case-level ("Sample") ground truth (manual score) provided

7 algorithms ("Models") provided IHC score according to ASCO/CAP

2+/3+ ("positive" / "negative") discrimination good

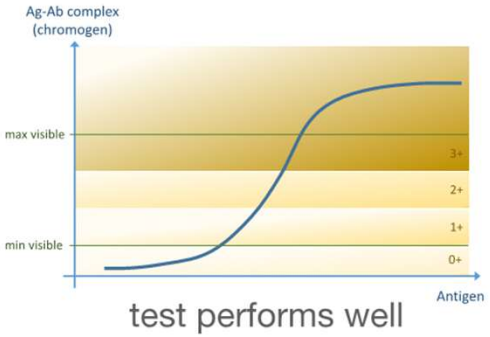
0/1+ and 2+/1+ comparable to interpathologist variation

Friends of Cancer Research DigitalPATH project

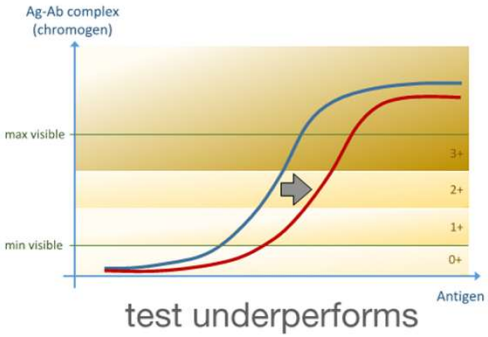
McKelvey et al., Agreement Across 10 Artificial Intelligence Models in Assessing HER2 in Breast Cancer Whole Slide Images: Findings from the Friends of Cancer Research Digital PATH Project. SABCS Abstract P2-02-18

The importance of On-slide controls

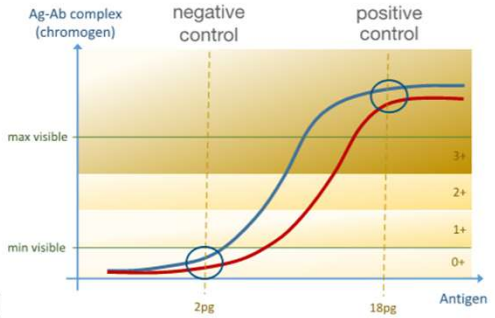
On slide controls for IHC: monitoring test performance



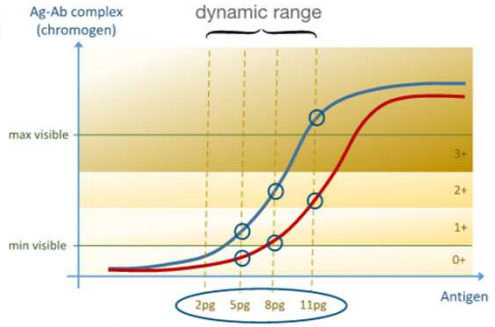
test performs well



test underperforms



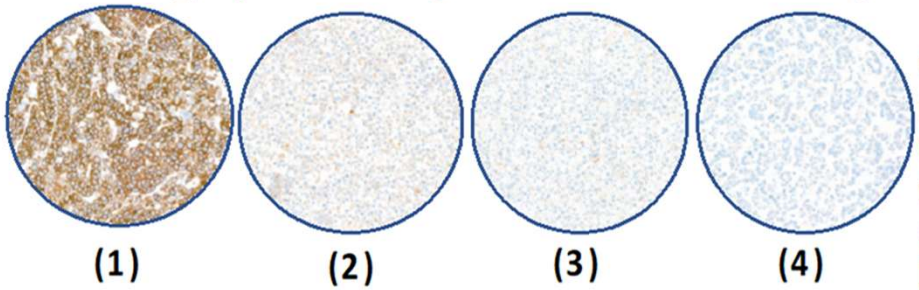
unaware of underperformance



underperformance detected!

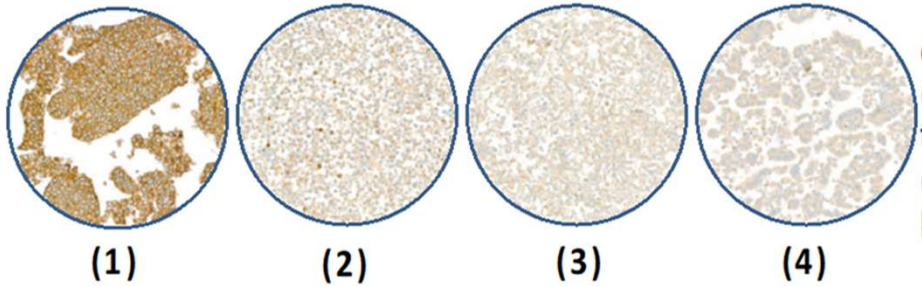
On slide controls should be within the dynamic range

Ensure appropriate staining of on-slide controls before proceeding



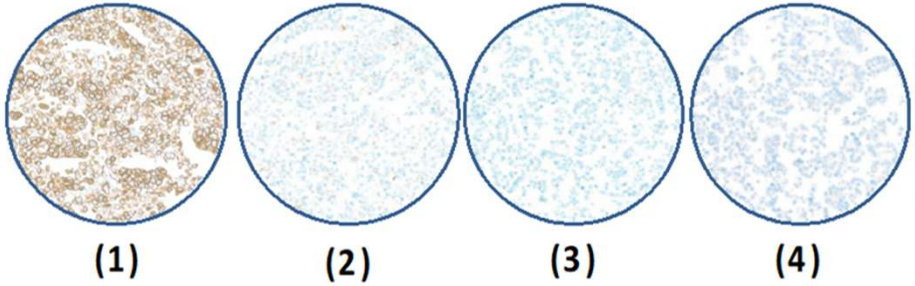
Good analytical performance: There is good staining in the strong core(1) and no staining in the negative core (4). There is little staining in the moderate core (2) and staining in the weak core (3) can only be seen at 20x

Proceed with assessment



Over-staining: The moderate and weak cores (2,3) are over-stained and there is staining in the negative core (4). Note that the strong core (1) has no perceptible over-staining.

Repeat the test



Under-staining: There is poor staining in the strong core (1) and almost no staining in the moderate and weak cores (2,3)

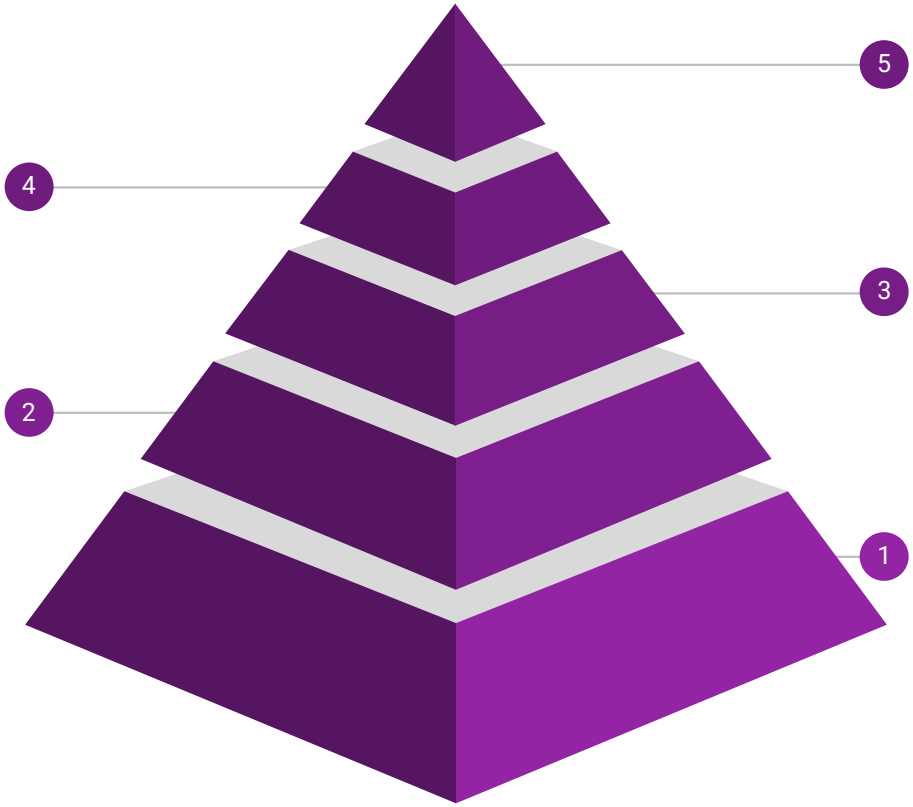
Repeat the test

Slide at the courtesy of Dr D'Arrigo

Future Horizons

The need for quantitative measurements.

The quest for higher sensitivity, improved quantification, broader dynamic range, greater stability, and expanded multiplexing capabilities



Novel detection modalities

Nanoparticles and mass spectrometry detection

Multiplex

Detecting multiple (3 to 100+) biomarkers for a deeper understanding of cellular interactions and spatial interaction in complex tissue microenvironments.

Preanalytics

The more advanced sensitivity, specificity and broader dynamic range, the pre-analytics grows in importance.

Translucent chromogens

To enable multiplexing and co-localization analysis.

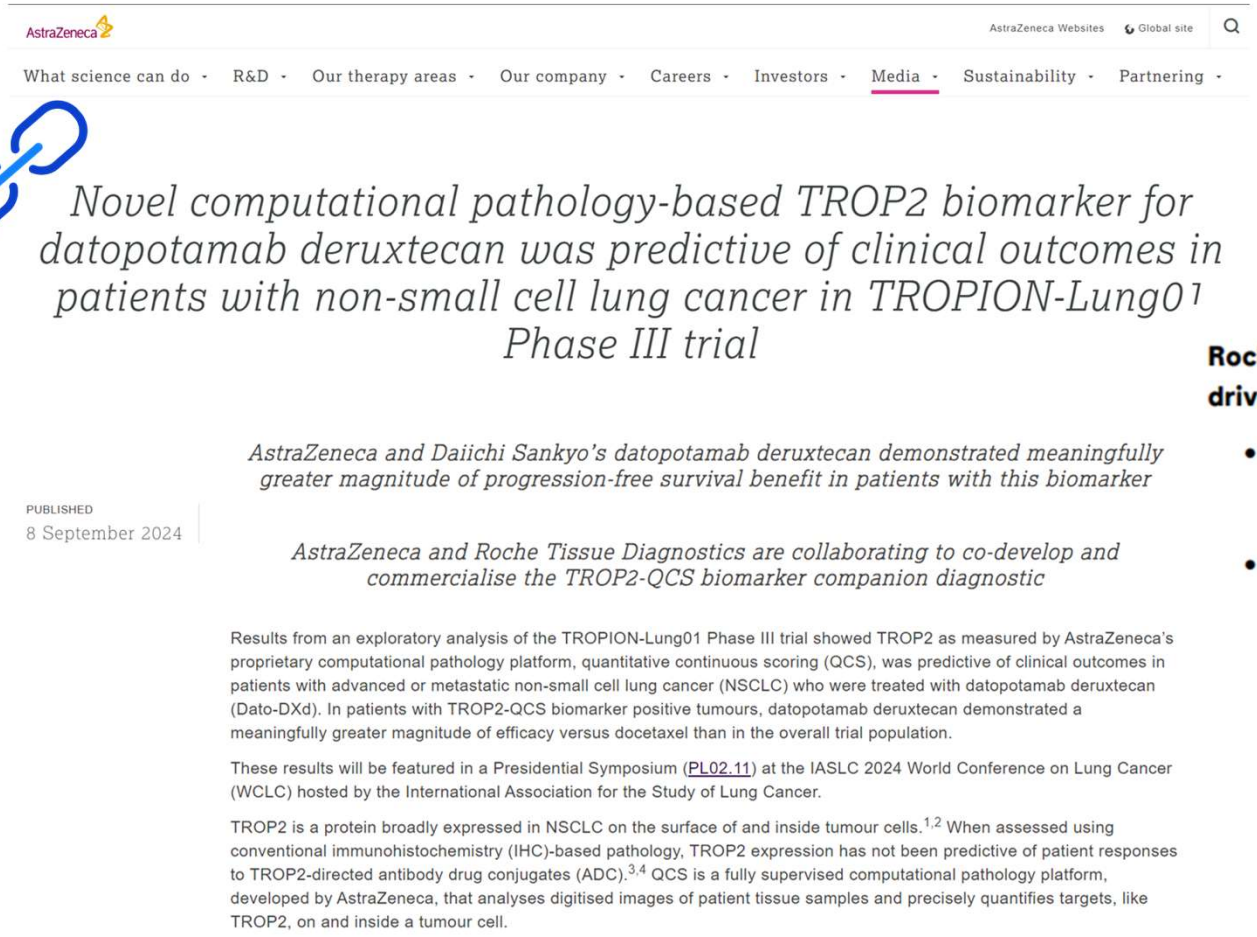
Quantitative IHC and Digital Pathology

More objective and reproducible quantification of IHC staining

Introduction of IHC calibrators and quantitative controls aims to standardize assays and improve inter-laboratory reproducibility.

Press Release for TROP2 NMR-QCS CDx Development

AZ and Roche Tissue Diagnostics collaboration to co-develop TROP2-QCS biomarker CDX



AstraZeneca

AstraZeneca Websites | Global site

What science can do · R&D · Our therapy areas · Our company · Careers · Investors · Media · Sustainability · Partnering

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

AstraZeneca and Daiichi Sankyo's datopotamab deruxtecan demonstrated meaningfully greater magnitude of progression-free survival benefit in patients with this biomarker

PUBLISHED
8 September 2024

AstraZeneca and Roche Tissue Diagnostics are collaborating to co-develop and commercialise the TROP2-QCS biomarker companion diagnostic

Results from an exploratory analysis of the TROPION-Lung01 Phase III trial showed TROP2 as measured by AstraZeneca's proprietary computational pathology platform, quantitative continuous scoring (QCS), was predictive of clinical outcomes in patients with advanced or metastatic non-small cell lung cancer (NSCLC) who were treated with datopotamab deruxtecan (Dato-DXd). In patients with TROP2-QCS biomarker positive tumours, datopotamab deruxtecan demonstrated a meaningfully greater magnitude of efficacy versus docetaxel than in the overall trial population.

These results will be featured in a Presidential Symposium (PL02.11) at the IASLC 2024 World Conference on Lung Cancer (WCLC) hosted by the International Association for the Study of Lung Cancer.

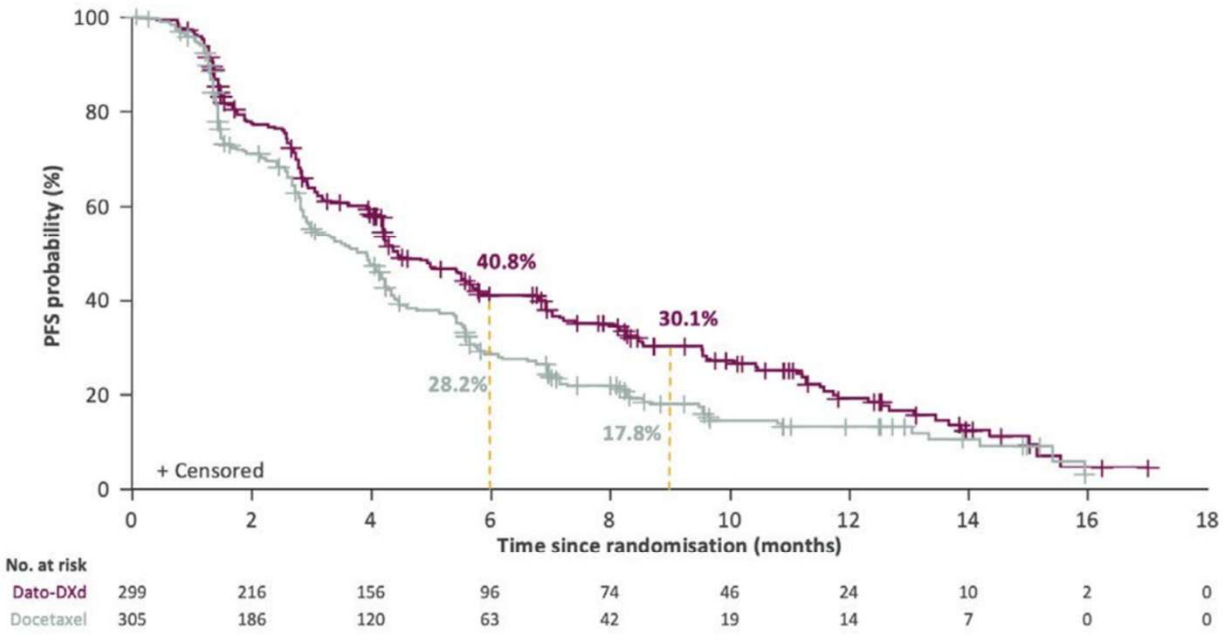
TROP2 is a protein broadly expressed in NSCLC on the surface of and inside tumour cells.[1,2] When assessed using conventional immunohistochemistry (IHC)-based pathology, TROP2 expression has not been predictive of patient responses to TROP2-directed antibody drug conjugates (ADC).[3,4] QCS is a fully supervised computational pathology platform, developed by AstraZeneca, that analyses digitised images of patient tissue samples and precisely quantifies targets, like TROP2, on and inside a tumour cell.

Roche granted FDA Breakthrough Device Designation for first AI-driven companion diagnostic for non-small cell lung cancer

- **The VENTANA TROP2 (EPR20043) RxDx Device is an immunohistochemistry (IHC) assay combined with a digital pathology algorithm to determine patient treatment.**
- **The device uses artificial intelligence-based image analysis with a level of diagnostic precision not possible with traditional manual scoring methods.**

NMR: Normalized Membrane ratio
QCS: Quantitative Continuous Scoring

TROPION-Lung01 – Progression-free survival in ITT



	Dato-DXd	Docetaxel
Median PFS, months (95% CI)[1]	4.4 (4.2, 5.6)	3.7 (2.9,4.2)
HR (95% CI)	**0.75** (0.62, 0.91)	
P-value	0.004	
Prespecified boundary (2-sided)	0.008	

	Dato-DXd	Docetaxel
ORR, % (95% CI)[2]	26.4 (21.5, 31.8)	12.8 (9.3, 17.1)
DOR, mo. (95% CI)	7.1 (5.6, 10.9)	5.6 (5.4, 8.1)

1. Median PFS follow-up time was 10.9 months (95% CI: 9.8, 12.5) and 9.6 months (95% CI: 8.2, 11.9) for Dato-DXd and docetaxel, respectively. 2. Included four CRs and 75 PRs for Dato-DXd and 39 PRs for docetaxel.
PFS = progression-free survival; no. = number; Dato-DXd = datopotamab deruxtecan; HR = hazard ratio; CI = confidence interval; ORR = objective response rate; DOR = duration of response, mo. = months.
Collaboration partner: Daiichi Sankyo (Dato-DXd).

WCLC presentation of TROPION-Lung01 2024

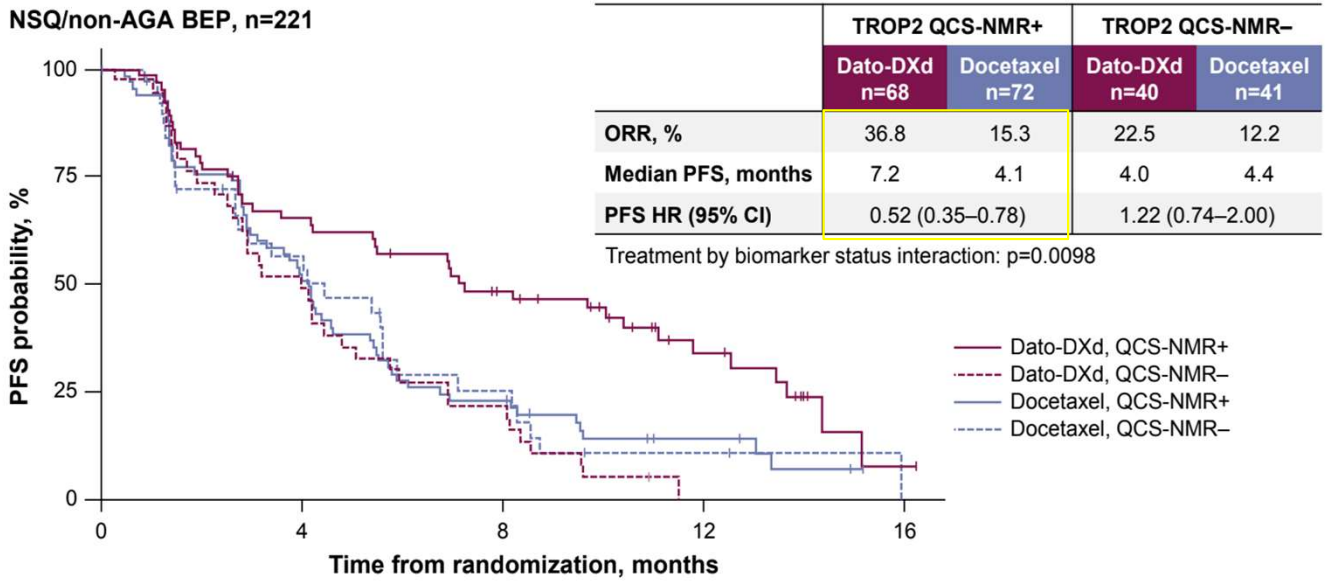
Exploratory analysis demonstrates potential clinical utility of QCS assessment of TROP2

Detection of TROP2 presence or intensity by eye (eg H-score) is insufficient for robust patient stratification.

ASCO Living Guideline, stated that "TROP2 expression by IHC was not associated with response" to datopotamab deruxtecan.

NSQ/non-AGA BEP: Efficacy by TROP2 QCS-NMR Status

TROP2 QCS-NMR positivity is predictive for longer PFS with Dato-DXd in the NSQ/non-AGA biomarker-evaluable population



	TROP2 QCS-NMR+		TROP2 QCS-NMR–	
	Dato-DXd n=68	Docetaxel n=72	Dato-DXd n=40	Docetaxel n=41
ORR, %	36.8	15.3	22.5	12.2
Median PFS, months	7.2	4.1	4.0	4.4
PFS HR (95% CI)	0.52 (0.35–0.78)		1.22 (0.74–2.00)	

Treatment by biomarker status interaction: p=0.0098

Dr Marina Chiara Garassino | Normalized Membrane Ratio of TROP2 by Quantitative Continuous Scoring is Predictive of Clinical Outcomes in TROPION-Lung01

Data cutoff: March 29 2023

PFS HR (95% CI) by TROP2 QCS-NMR status (+ vs -) within treatment: Dato-DXd: 0.40 [0.25-0.64]; Docetaxel:0.94 [0.60-1.49]

1.Garassino, M. C. *et al.* PL02.11 Normalized Membrane Ratio of TROP2 by Quantitative Continuous Scoring is Predictive of Clinical Outcomes in TROPION-Lung 01. *J. Thorac. Oncol.* 19, S2–S3 (2024).

NSQ: non Squamous. non-AGA: non actionable genomic alteration

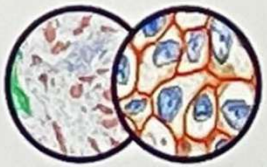
TROP2 Normalized Membrane Ratio (NMR) measured by Quantitative Continuous Scoring (QCS)

QCS is a novel, fully-supervised computational pathology approach that precisely quantifies and locates targets like TROP2

1 IHC with TROP2 Assay

2 Whole Slide Imaging

3 Automated Image Analysis (QCS)



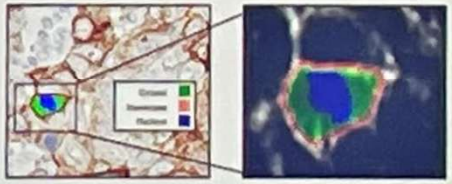
4 Patient Biomarker Status Determination

\+ ≥75% of tumor cells with TROP2 NMR ≤0.56

\- <75% of tumor cells with TROP2 NMR ≤0.56*

Differentiates tumor from non-tumor

Measures OD in each tumor cell



Membrane and cytoplasm optical density (OD)

Calculates TROP2 NMR for every tumor cell

$$\frac{\text{Membrane OD}}{\text{Membrane OD + Cytoplasm OD}}$$

Lower NMR → higher cytoplasm proportion

Dr Marina Chiara Garassino | Normalized Membrane Ratio of TROP2 by Quantitative Continuous Scoring is Predictive of Clinical Outcomes in TROPION-Lung01

OD, optical density (a measure of staining intensity)
*Or >25% of cells with an NMR >0.56

Key take away

IHC: an 85 year long journey

Drive towards higher specificity, sensitivity and broader dynamic range

Shift from qualitative to quantitative- Algorithms and New detection modalities

Pre-analaytics!!!