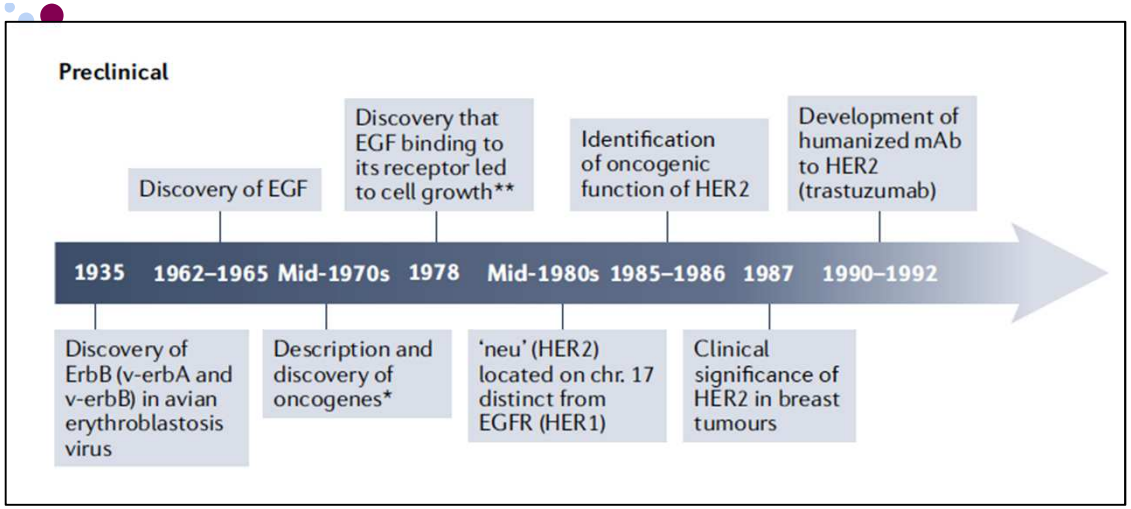
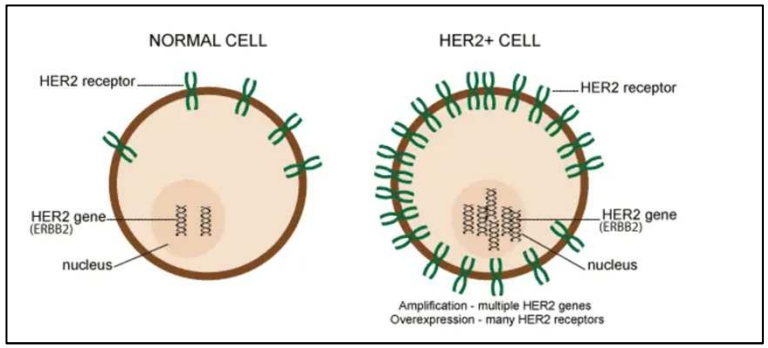
HER2-low in borstkanker: verschillende testen, impact, EQA,…

Gert Van den Eynden, MD, PhD
Heilig Hartziekenhuis Lier

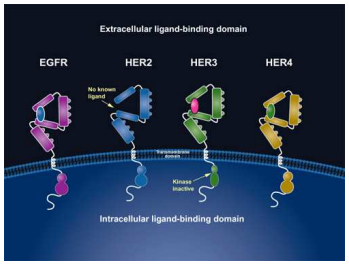
HER2 wat?



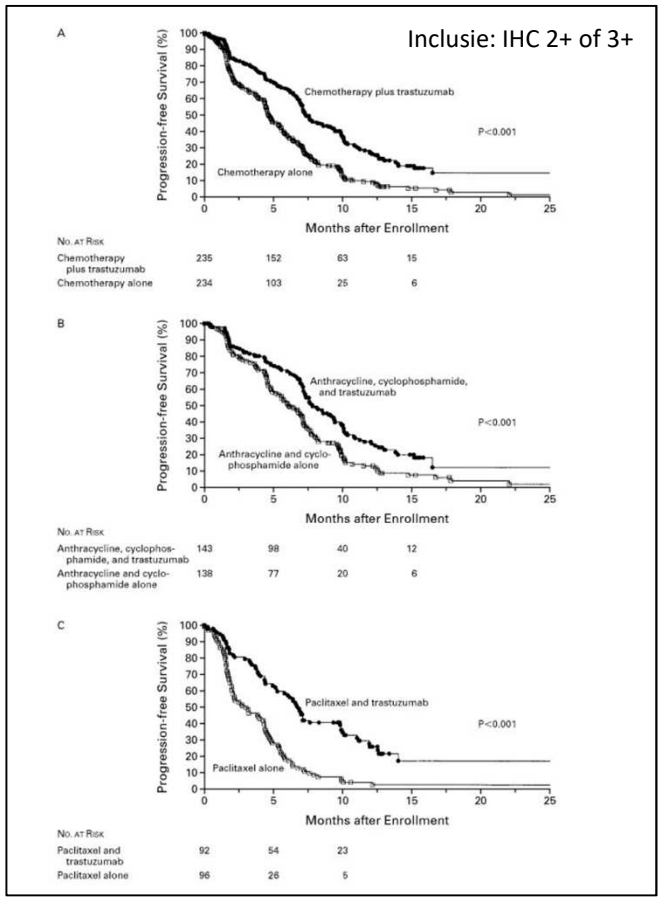
Swain S et al. Nature Reviews Drug Discovery volume 22, pages101–126 (2023)



https://www.whathealth.com/breastcancer/her2receptor.html



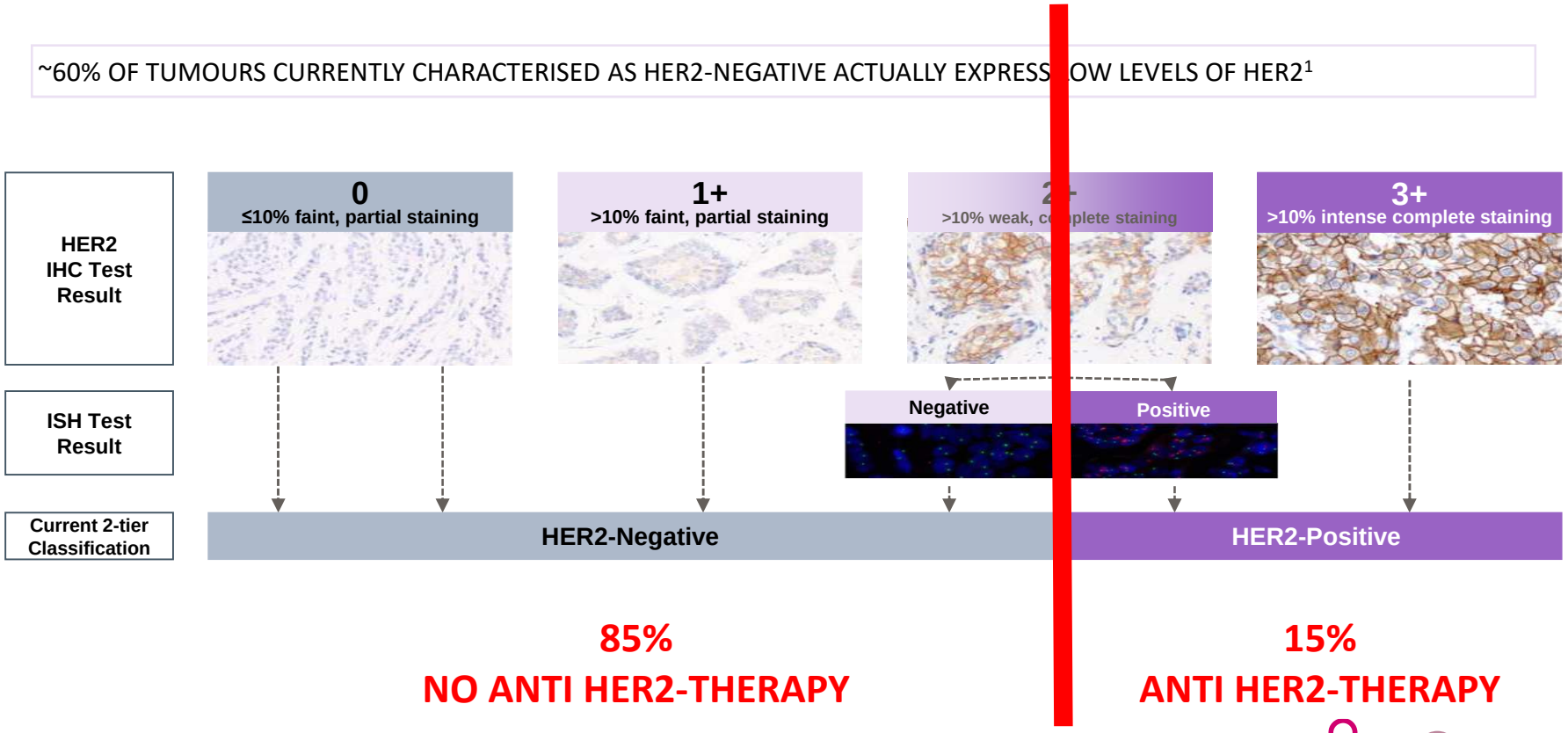
https://proteopedia.org/



D. Slamon et al. N Engl J Med 2001; 344:783-792

HER2 classification has historically been binary, but HER2 expression is a continuum and patients may demonstrate varying levels of HER2 expression

~60% OF TUMOURS CURRENTLY CHARACTERISED AS HER2-NEGATIVE ACTUALLY EXPRESS LOW LEVELS OF HER2[1]



85%
NO ANTI HER2-THERAPY

15%
ANTI HER2-THERAPY

HER2, human epidermal growth factor receptor 2; IHC, Immunohistochemistry; ISH, In situ hybridization.
1. Schettini F et al. Poster presented at ESMO Virtual Congress 2020; September 19-21, 2020. 2. Wolff AC, et al. J Clin Oncol. 2018;36:2105–22. Images: Rüschoff et al. Manuscript in prep.

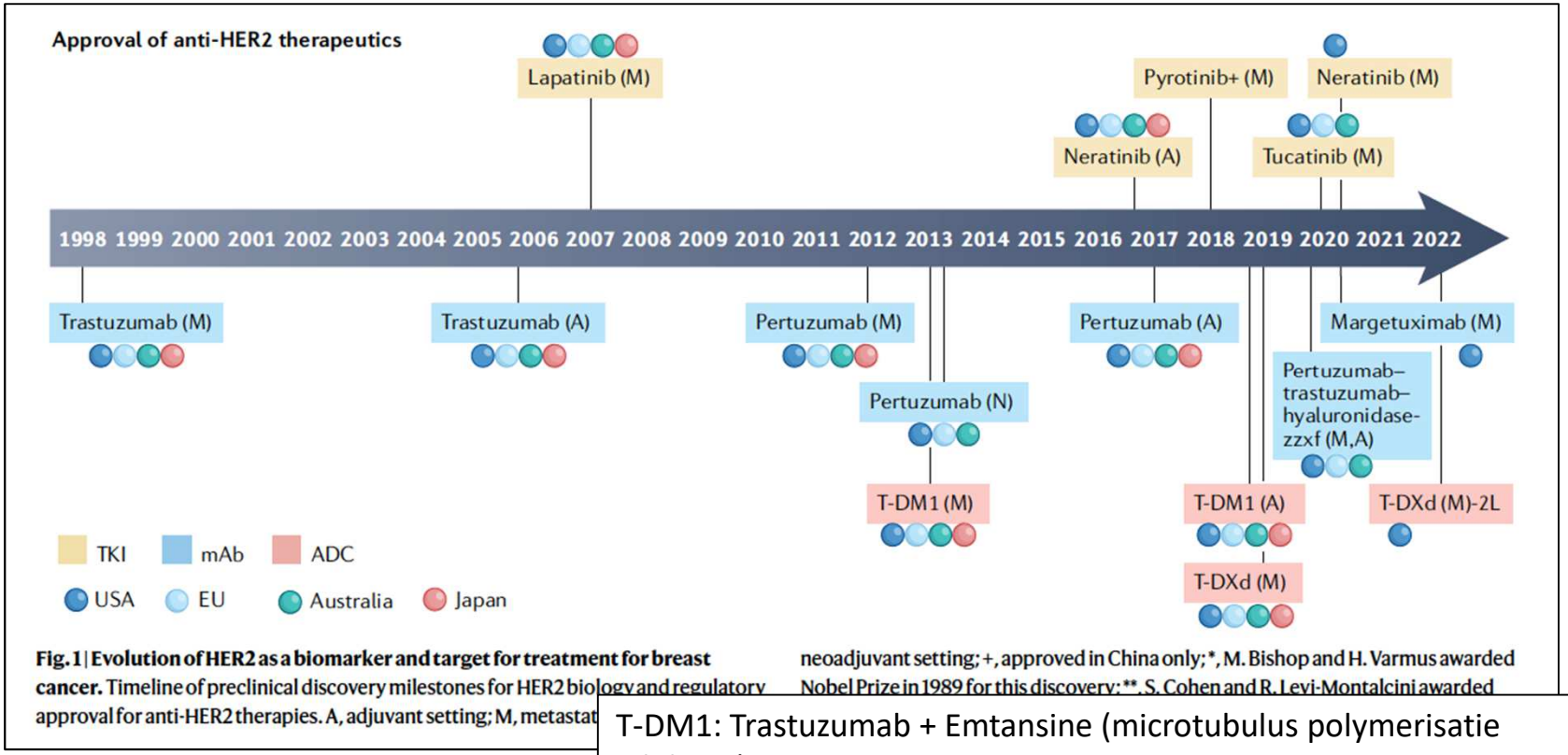
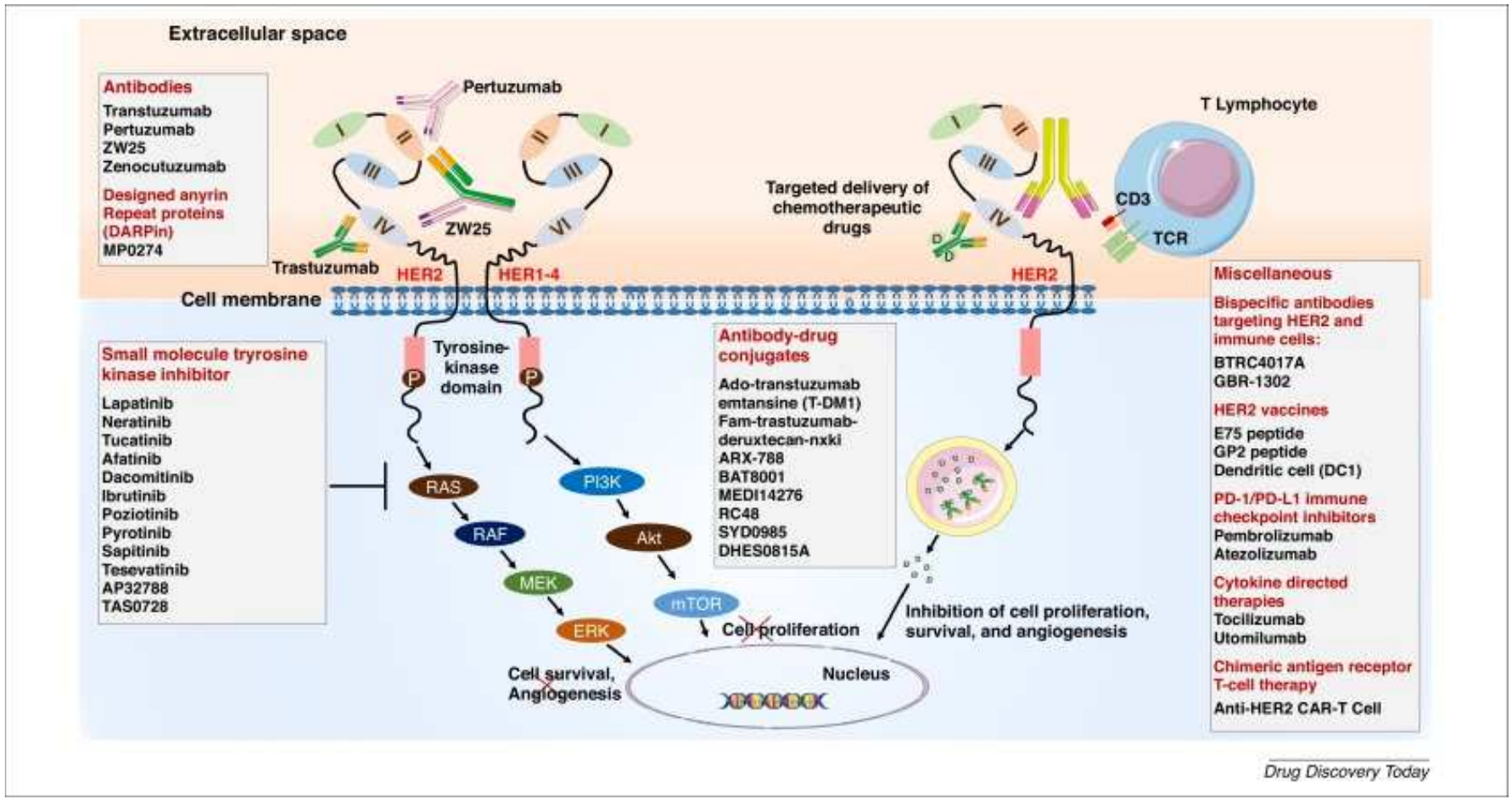


Fig. 1 | Evolution of HER2 as a biomarker and target for treatment for breast cancer. Timeline of preclinical discovery milestones for HER2 biology and regulatory approval for anti-HER2 therapies. A, adjuvant setting; M, metastat neoadjuvant setting; +, approved in China only; *, M. Bishop and H. Varmus awarded Nobel Prize in 1989 for this discovery; **, S. Cohen and R. Levi-Montalcini awarded

Swain S et al. Nature Reviews Drug Discovery volume 22, pages101–126 (2023)

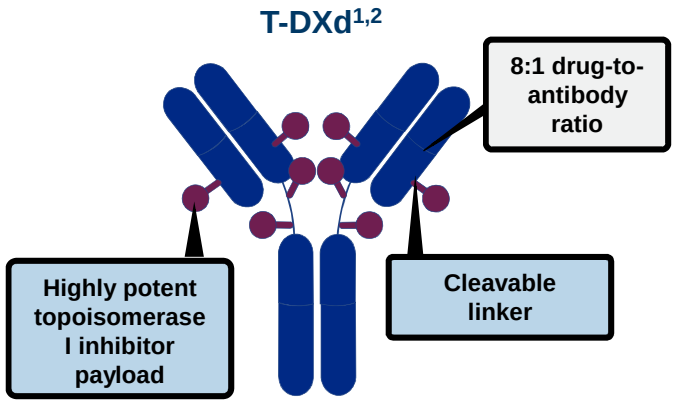
T-DM1: Trastuzumab + Emtansine (microtubulus polymerisatie inhibitor)
T-Dxd: Trastuzumab + Deruxtecan (topoisomerase inhibitor)



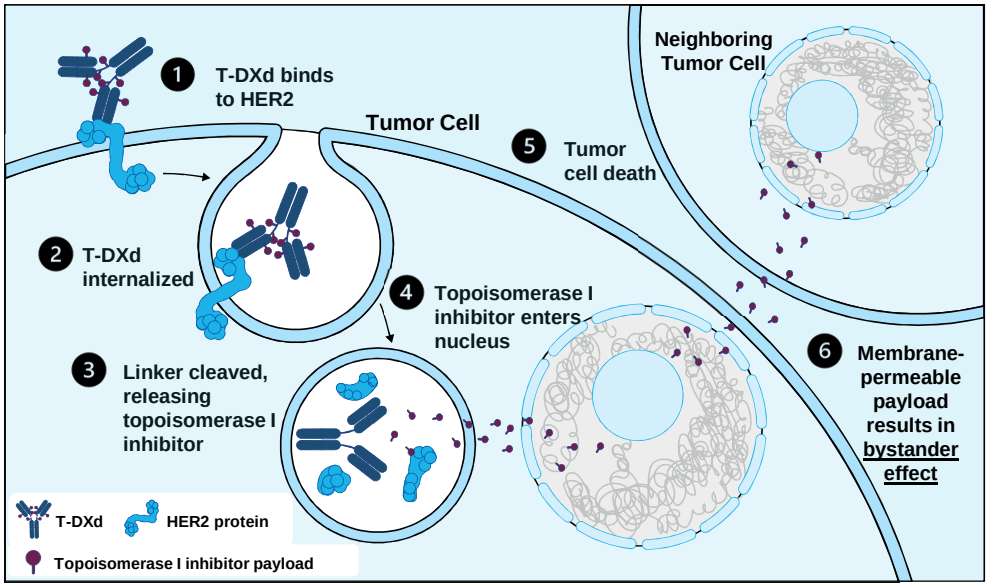
https://doi.org/10.1016/j.drudis.2020.12.014

T-DXd MOA, Bystander Effect, and Rationale for Targeting HER2-low mBC

T-DXd MOA, Bystander Effect, and Rationale for Targeting HER2-low mBC



Internalization of T-DXd leads to release of the DXd payload and subsequent cell death in the target tumor cell and neighboring tumor cells through the bystander effect[1,2]



Adapted with permission from Modi S, et al. *J Clin Oncol* 2020;38:1887-96. CC BY ND 4.0.

1. Nakada T, et al. *Chem Pharm Bull*. 2019;67:173-85. 2. Ogitani Y, et al. *Clin Cancer Res.* 2016;22:5097-108. 3. Modi S, et al. *J Clin Oncol* 2020;38:1887-96.

T-DXd is approved for the treatment of adult patients with unresectable or metastatic HER2 positive breast cancer who have received one or more prior anti-HER2-based regimens. T-DXd is not reimbursed in Belgium.

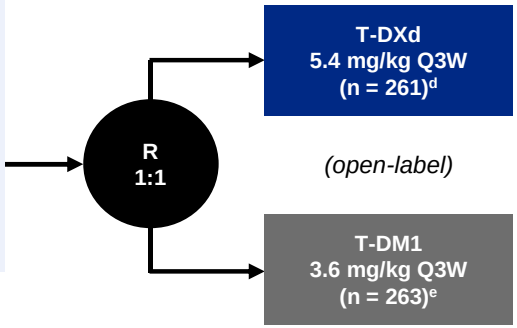
ADC/22/0540 Date of last revision: October 2022

DESTINY-Breast03: Study Design

An Open-label, Multicenter, Phase 3 Study (NCT03529110)[1-7]

Patients (N = 524)

- Unresectable or metastatic HER2 positive[a] breast cancer that has been previously treated with trastuzumab and taxane[b]
- Could have clinically stable, treated brain metastases[c]
  - ≥2 weeks between end of whole-brain radiotherapy and study enrollment[3]



Primary endpoint

- PFS (BICR)

Key secondary endpoint

- OS[f]

Secondary endpoints

- ORR (BICR and investigator)
- DoR (BICR)
- PFS (investigator)
- Safety
- HEOR outcomes (PROs and hospitalization rates)

Exploratory subgroup analysis

Disease history

- De novo or recurrent metastatic disease at diagnosis
- Presence or absence of visceral disease at baseline

Setting for 1 prior line of therapy[g]

- Metastatic
- (Neo)adjuvant (early progression)

Prior anti-HER2 therapy

- 1 line, ≥2 lines, 1 or 2 lines, ≥3 lines
- Prior pertuzumab

Stratification factors

- Hormone receptor status
- Prior treatment with pertuzumab
- History of visceral disease

- BMs were measured at baseline by CT or MRI and BM progression was monitored throughout the study[3]

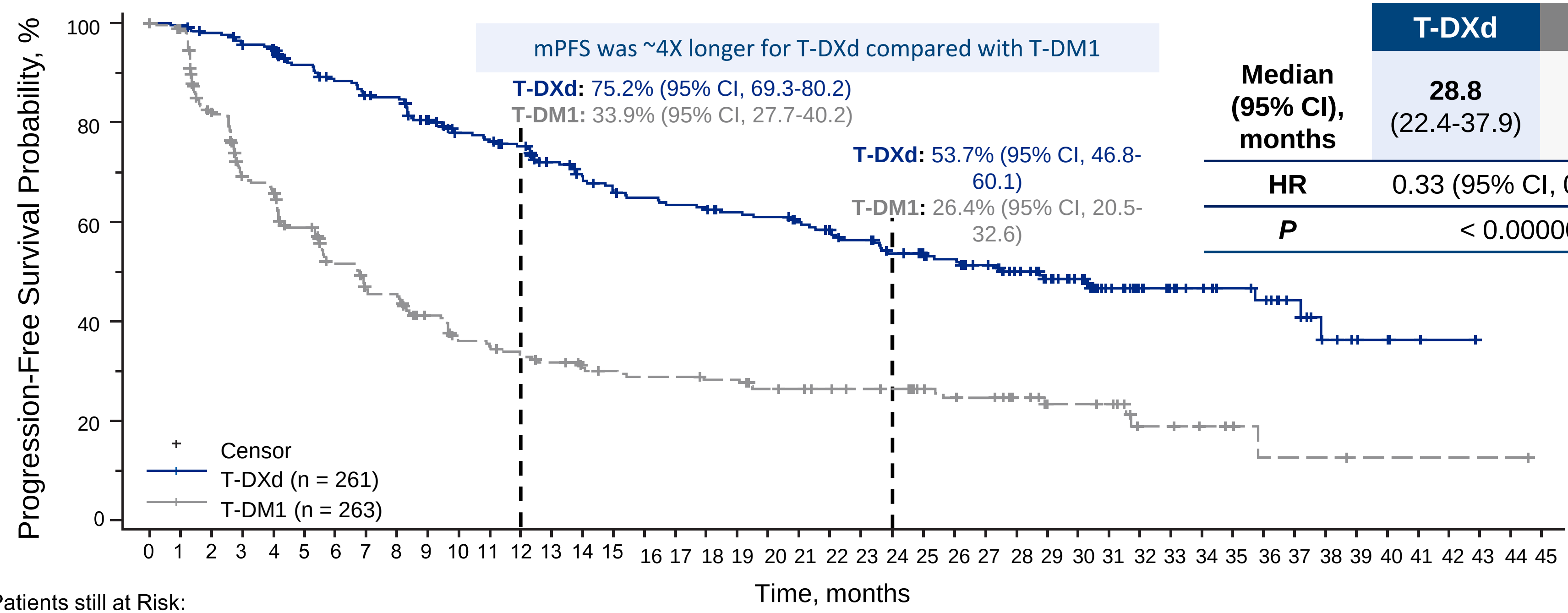
[a]HER2 IHC 3+ or IHC 2+/ISH+ based on central confirmation. [b]Progression during or <6 months after completing neoadjuvant or adjuvant therapy involving trastuzumab or a taxane. [c]Before protocol amendment, patients with stable, untreated BM were eligible. [d]4 patients were randomly assigned but not treated. [e]2 patients were randomly assigned but not treated. [f]80% powered at 2-sided significance level of 5%. [g]In patients with exactly 1 prior line of therapy in the metastatic setting, excluding hormone therapy.

Cortés J et al. *N Engl J Med.* 2022;386:1143-1154. 2. Cortés J et al. *N Engl J Med.* 2022;386:1143-1154 [supplement]. 3. Hurvitz SA et al. Presented at: San Antonio Breast Cancer Symposium 2021; December 7-10, 2021; San Antonio, TX, USA. Presentation GS3-01. 4. Cortés J et al. Presented at: ESMO Virtual Congress 2022; September 9-13, 2022. Poster 236P. 5. Curigliano G et al. Presented at: European Society for Medical Oncology Breast Cancer 2022; May 3-5, 2022; Berlin, Germany. Presentation 163O. 6. Hurvitz SA et al. Presented at: San Antonio Breast Cancer Symposium 2022; December 6-10, 2022; San Antonio, TX, USA. Presentation GS2-02. 7. Hurvitz SA et al. *The Lancet*. 2022;400 [in press].

https://medicines.astrazeneca.be/content/dam/multibrand/nl/nl/products/bijsluiter/Enhertu-bijsluiter.pdf

DESTINY-Breast03: July 25, 2022, DCO

Updated Primary Endpoint: PFS by BICR[1,2]



	T-DXd	T-DM1
Median (95% CI), months	**28.8** (22.4-37.9)	**6.8** (5.6-8.2)
HR	0.33 (95% CI, 0.26-0.43)	
P	< 0.000001[a,b]	

Patients still at Risk:

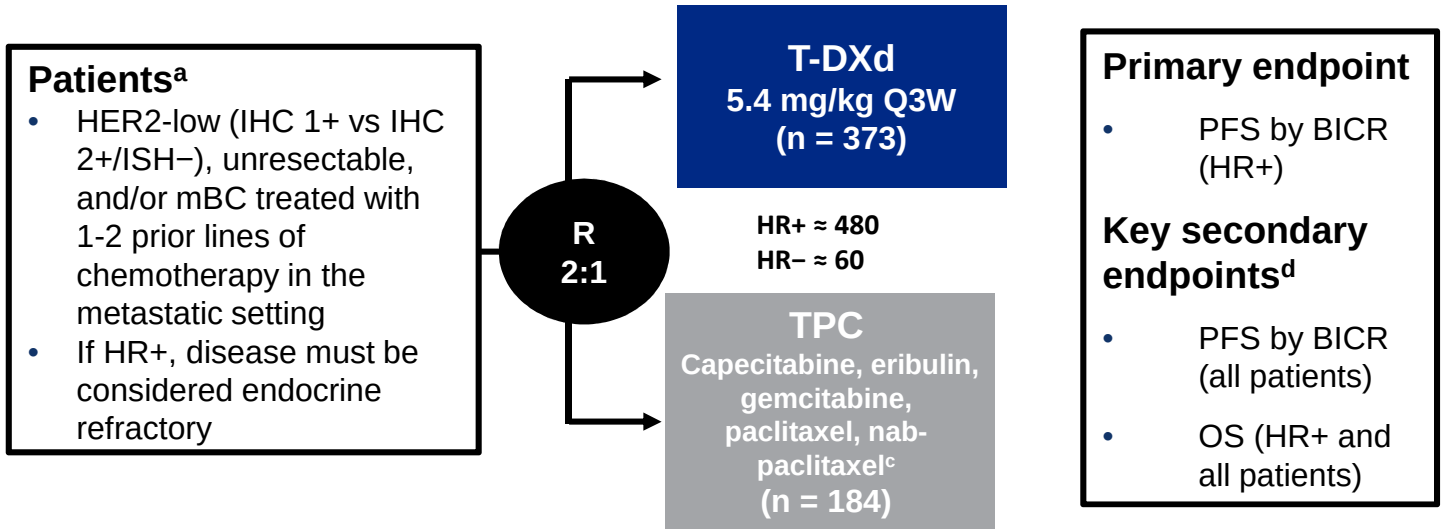
T-DXd 261 256 250 244 240 225 216 207 205 191 176 173 167 154 146 140 134 131 130 125 123 117 113 107 99 96 90 82 73 64 55 41 32 28 23 20 18 13 7 5 4 2 1 0

T-DM1 263 253 201 164 156 134 111 99 96 81 69 67 63 58 54 51 49 49 47 47 42 41 39 37 36 32 28 27 22 19 15 14 8 7 6 4 2 2 2 1 1 1 1 1 1 0

[a]Two-sided, from stratified log-rank test. [b]Nominal *P* value.

Hurvitz SA et al. *The Lancet*. 2022;400 [in press]. 2. Hurvitz SA et al. Presented at: San Antonio Breast Cancer Symposium 2022; December 6-10, 2022; San Antonio, TX, USA. Presentation GS2-02.
https://medicines.astrazeneca.be/content/dam/multibrand/nl/nl/products/bijsluiter/Enhertu-bijsluiter.pdf

DESTINY-Breast04: Study Design (NCT03734029)



Stratification factors

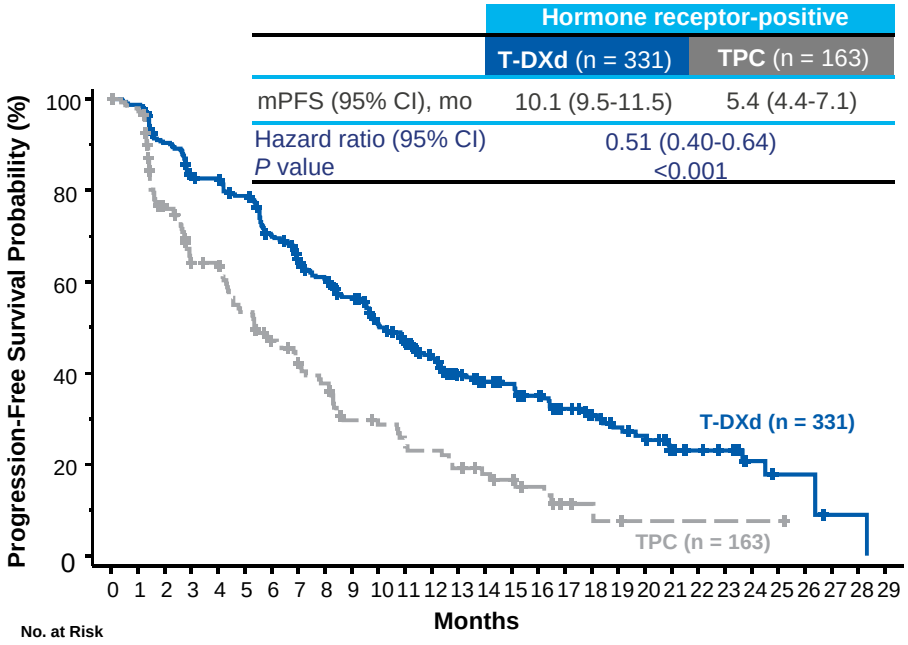
- Centrally assessed HER2 status[b] (IHC 1+ vs IHC 2+/ISH−)
- 1 vs 2 prior lines of chemotherapy
- HR+ (with vs without prior treatment with CDK4/6 inhibitor) vs HR−

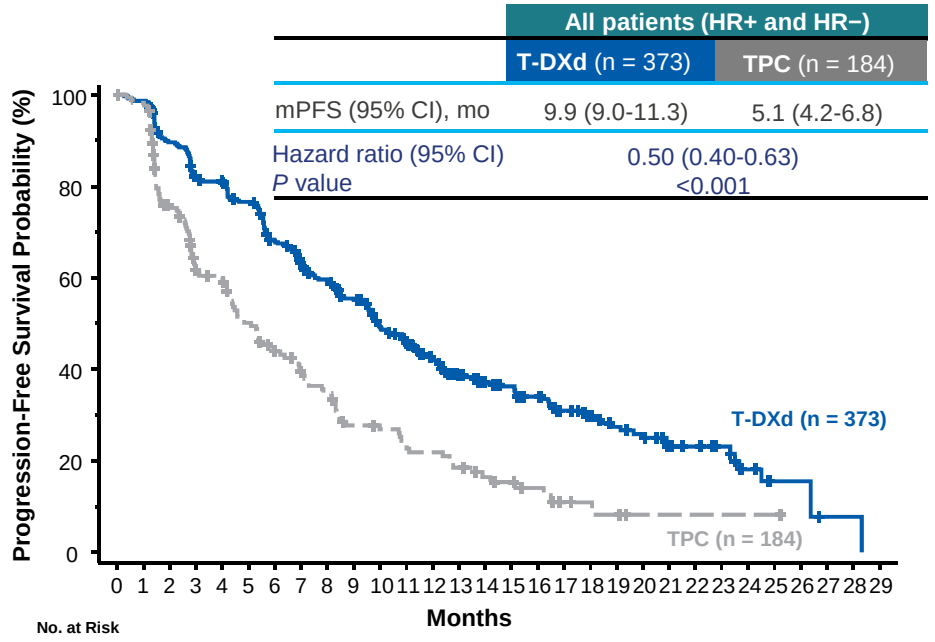
[a]If patients had HR+ mBC, prior endocrine therapy was required. [b]Performed on adequate archived or recent tumor biopsy per ASCO/CAP guidelines using the VENTANA HER2/neu (4B5) investigational use only Assay system. [c]TPC was administered according to the label.
[d]Other secondary endpoints included ORR (BICR and investigator), DOR (BICR), and PFS (investigator) in the HR+ cohort and in all patients (HR+ and HR−), and safety in all treated patients; efficacy in the HR− cohort was an exploratory endpoint.

1. Modi S et al. *N Engl J Med*. 2022. doi: 10.1056/NEJMoa2203690. 2. Modi S et al et al. Oral presentation at American Society of Clinical Oncology (ASCO) 2022, June 5 (2022b, LBA3).

T-DXd is approved for the treatment of adult patients with unresectable or metastatic HER2 positive breast cancer who have received one or more prior anti-HER2-based regimens, within this indication TDXd is reimbursed in Belgium. In Belgium no reimbursement is available within the DESTINY-Breast04 indication.
https://medicines.astrazeneca.be/content/dam/multibrand/nl/nl/products/bijsluiter/Enhertu-bijsluiter.pdf
ADC/22/0540 Date of last revision: October 2022

DESTINY-Breast04: January 11, 2022 DCO
PFS in HR+ and All Patients





PFS by blinded independent central review.

Modi S et al. *N Engl J Med*. 2022. doi: 10.1056/NEJMoa2203690.

DESTINY-Breast04: January 11, 2022 DCO; T-DXd is approved for the treatment of adult patients with unresectable or metastatic HER2 positive breast cancer who have received one or more prior anti-HER2-based regimens, within this indication TDXd is reimbursed in Belgium. In Belgium no reimbursement is available within the DESTINY-Breast04 indication.

ADC/22/0540 Date of last revision: October 2022

https://medicines.astrazeneca.be/content/dam/multibrand/nl/nl/products/bijsluiter/Enhertu-bijsluiter.pdf

FDA Approval on August 5th, 2022

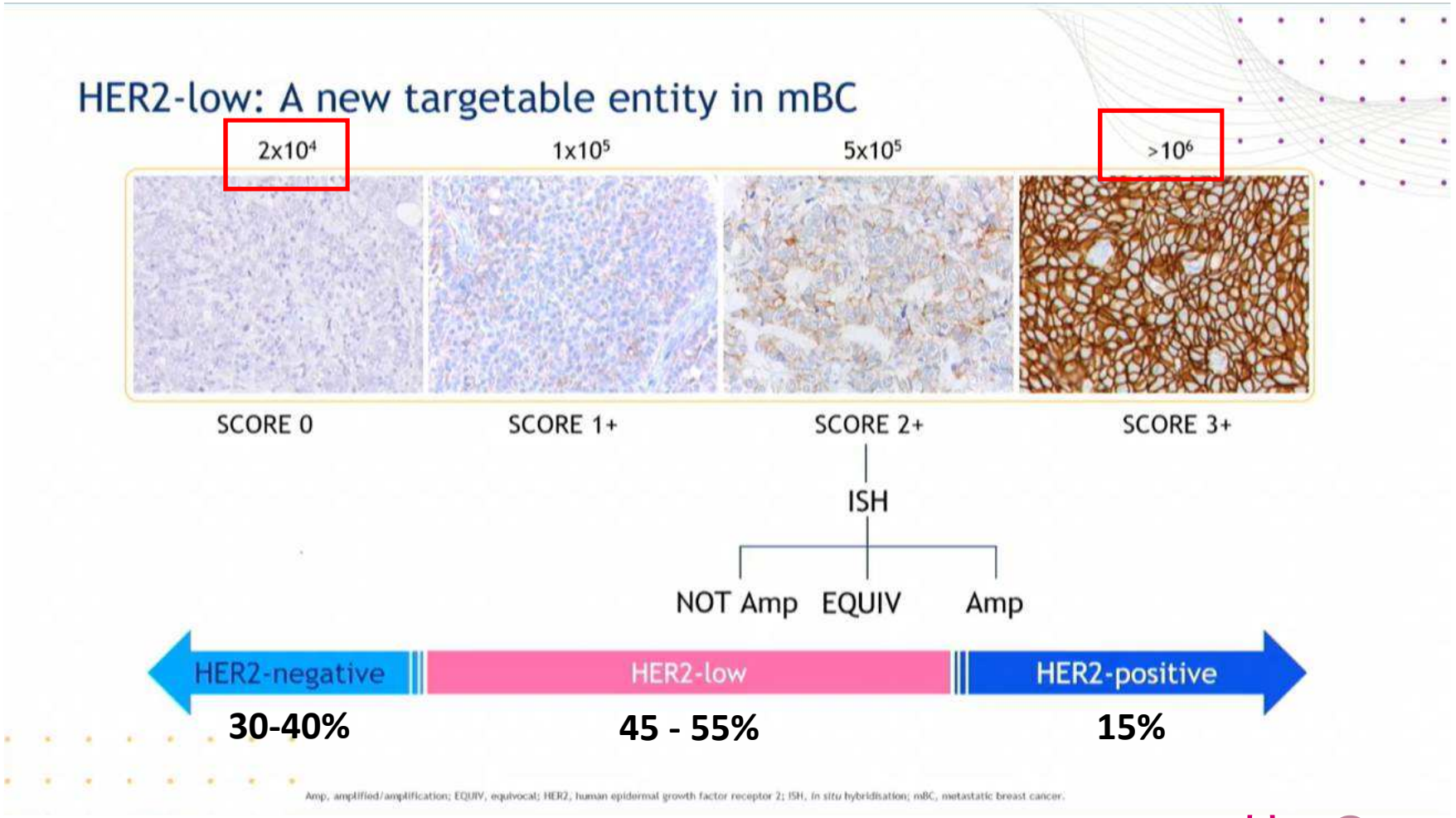
FDA NEWS RELEASE

FDA Approves First Targeted Therapy for HER2-Low Breast Cancer

Today, the U.S. Food and Drug Administration approved Enhertu (fam-trastuzumab-deruxtecan-nxki), an IV infusion for the treatment of patients with unresectable (unable to be removed) or metastatic (spread to other parts of the body) HER2-low breast cancer. This is the first approved therapy targeted to patients with the HER2-low breast cancer subtype, which is a newly defined subset of HER2-negative breast cancer.

SUSAN F. SMITH CENTER FOR WOMEN'S CANCERS

Dana-Farber Cancer Institute | Brigham and Women's Hospital | Harvard Medical School Teaching Hospital



Source: Daiichi Sankyo satellite symposium ECP 2023, Dublin

HER2 classification has historically been binary, but HER2 expression is a continuum and patients may demonstrate varying levels of HER2 expression

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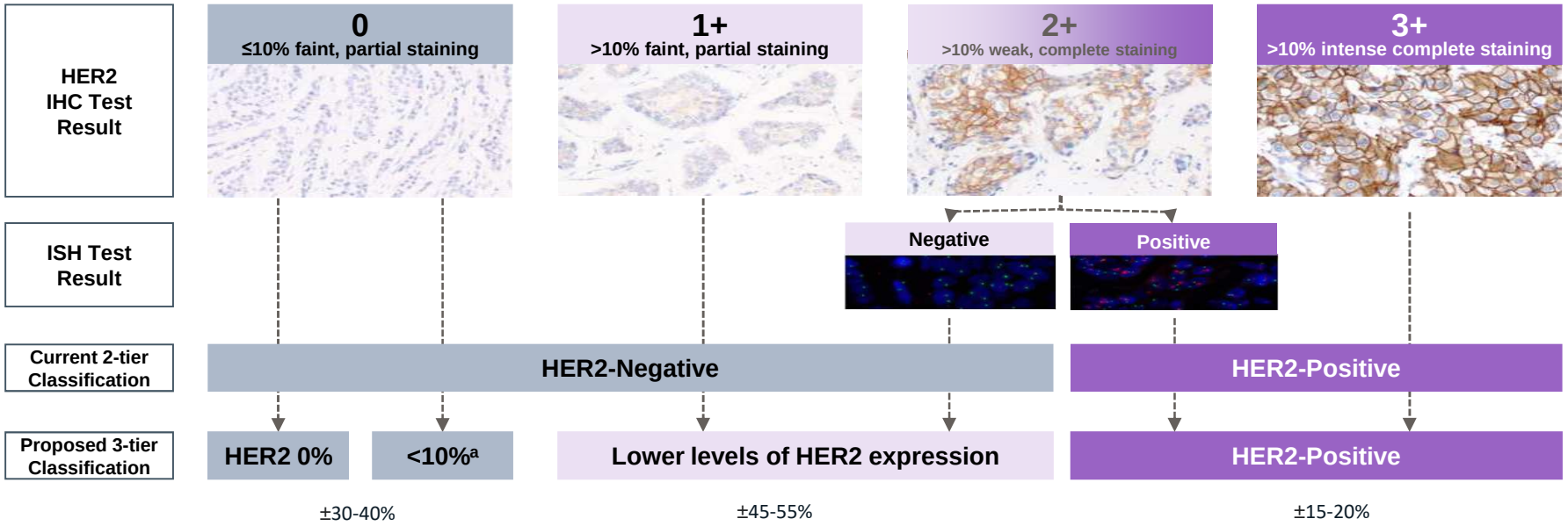


Patients with a lower level of HER2 expression can be identified through existing HER2 assays and scoring guidelines

HER2, human epidermal growth factor receptor 2; IHC, Immunohistochemistry; ISH, In situ hybridization.

1. Schettini F et al. Poster presented at ESMO Virtual Congress 2020; September 19-21, 2020. 2. Wolff AC, et al. J Clin Oncol. 2018;36:2105–22. Images: Rüschoff et al. Manuscript in prep.

HER2-low
praktische aspecten

HER2-low – Praktische Aspecten

- Keuze van het monster
- Pre-analyse
- IHC assay
- Controles
- Aflezen van de assay
- Rapportering

HER2-low – Praktische Aspecten

- **Keuze van het monster**
- Pre-analyse
- IHC assay
- Controles
- Aflezen van de assay
- Rapportering

Diagnostisch biopt vs. chirurgische excisie

Core Biopt

- Noodzakelijk wanneer (mogelijk) neo-adjuvante therapie
- Optimale pre-analytische condities
- Beperkte cellulariteit (mogelijk)
- Beperkt zicht op intra-tumorale heterogeniteit (zeker indien slechts 1 core)

Chirurgisch specimen

- Meer representatief beeld van de tumor cellulariteit en/of heterogeniteit
- Pre-analytische condities kunnen minder optimaal zijn (koude ischemie; fixatie;….)

- ASCO en ESMO raden testing HER2 status in het core biopt aan
- Concordantie van HER2 testing in core biopt vs chirurgisch specimen: 61.5% to 98.8%
- Cytologische preparaten (smears, cytospins) zijn niet geschikt voor IHC testing, FFPE cell blokken wel.

Referenties: 1. Wolff AC et al. J Clin Oncol. 2018 Jul 10;36(20):2105-2122; 2.

Gebruik van biopten kan de identificatie van HER2-low tumoren beïnvloeden

- 5610 opeenvolgende patiënten – biopt vs chirurgische excisie
- Overall concordance was 76.87% in de Her2-negatieve groep
- Overall discordantie ratio was 17.83%

Verandering van HER2 status op resectie t.o.v. analyse op biopt

Discordantie

387/3209 HER2-low	→	**HER2-0**	**6.9%**
530/1066 HER2-0	→	**HER2-low**	**9.4%**

Yujie Lu et al. Cancers 2022, 14(24), 6200; https://doi.org/10.3390/cancers14246200

(Mogelijke) oorzaken van discordantie tussen biopt en excisie

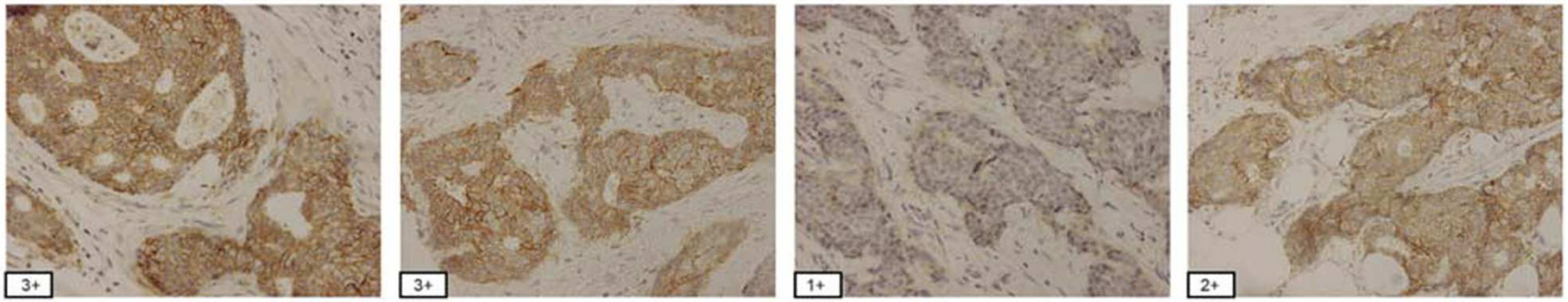
- Pre-analyse
- Beperkte hoeveelheid invasieve tumor op het biopt
- Multipele tumoren, verschillende fenotypes
- Inter-observer variatie
- Behandeling (post-NACT, NAET)
- Tumor heterogeniteit

Wat met (bot)metastasen ?

Metastatic site biopsy - Core needle biopsies (CNB) excisional biopsy[1,2]:

- The biopsy site can make evaluating HER2 status challenging
- The decalcification process, required to process a tissue from bone metastasis, could potentially compromise HER2 staining detectability and affect score (especially Nitric acid)

HER2



Control | EDTA | Christensen's buffer with microwave | Christensen's buffer

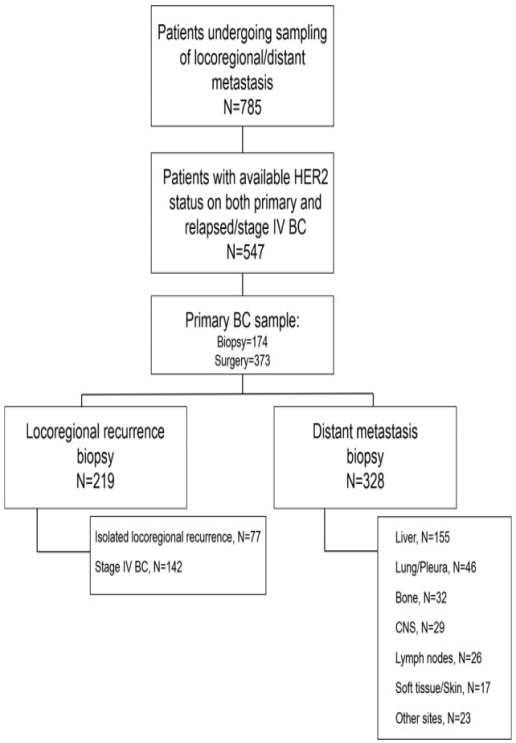
Images adapted from Schrijver W, et al. *Mod Pathol*. 2009;29:1460-1470.

Decalcifying agents containing EDTA are recommended to preserve HER2 antigenicity[1,2]

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; EDTA, ethylenediaminetetracetic acid; HER2, human epidermal growth factor receptor 2.

References: 1. Schrijver W, et al. *Mod Pathol.* 2009;29:1460-1470. 2. Van Es SC, et al. *Am J Surg Pathol*. 2019

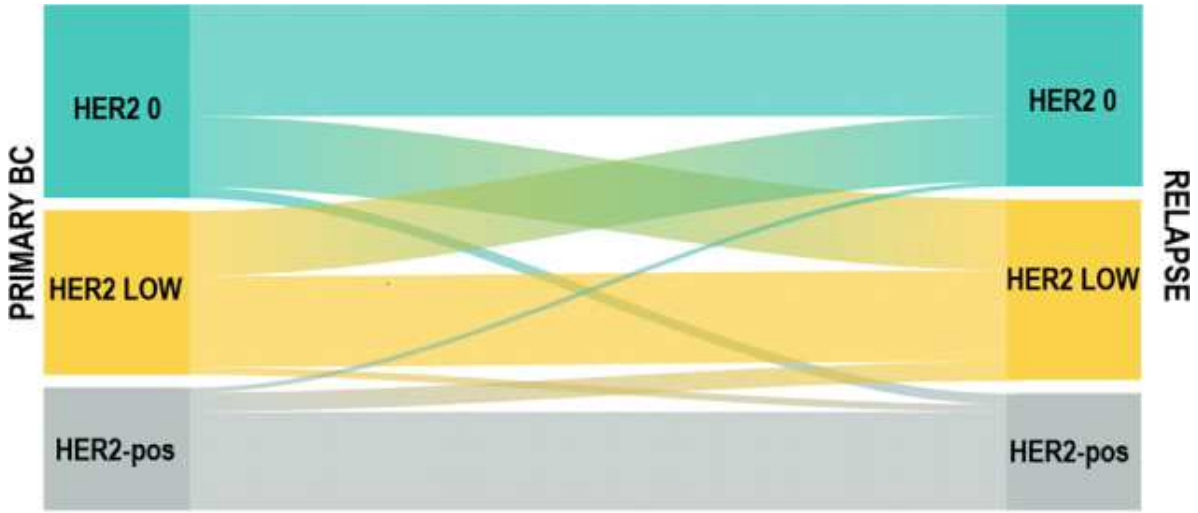
Evolution of HER2-low expression from primary to recurrent breast cancer



Miglietta et al. NPJ Breast Cancer. 2021; 7: 137.
Published online 2021 Oct 12. doi: 10.1038/s41523-021-00343-4

- **547 patienten met loco-regionaal herval of metastase op afstand**
- **HER2-low: 34.2% in de primaire tumoren, 37.3% in het herval**
- **HER2-low toegenomen in het herval**
- **Meer frequent in HR positive tumoren vs TNBC: 47.3% vs 35.4% in primaire tumoren en in herval 53.8% vs 36.2**
- **De overall rate van HER2 discordantie was 38.0%: voornamelijk door HER2-0 naar HER2-low (15%) en HER2-low naar HER2-0 (14%)**

Evolution of HER2-low expression from primary to recurrent breast cancer



		HER2 recurrence/metastasis N,%			Total
		0	Low	Positive	
HER2 primary BC N,%	0	132 (24.1)	83 (15.2)	13 (2.4)	228 (41.7)
	Low	77 (14.1)	101 (18.5)	9 (1.6)	187 (34.2)
	Positive	6 (1.1)	20 (3.7)	106 (19.4)	132 (24.1)
Total		215 (39.3)	204 (37.3)	128 (23.4)	547 (100)

Miglietta et al. NPJ Breast Cancer. 2021; 7: 137. Published online 2021 Oct 12. doi: 10.1038/s41523-021-00343-4

Cytologie ?

- Probeer te vermijden, indien mogelijk (maar soms is het het enige materiaal van metastatische lesies)
- Veel verschillende methoden in de literatuur, waarbij de grootste verschillen in performantie worden gezien tussen verschillende types van monsters (smears, liquid based cytology, cytospins en cell blocks)
- Cell blocks zijn beter !
- ASCO/CAP raadt formolfixatie aan
- Wanneer de fixatie of toereikendheid onzeker is, voorkeur voor een biopt en FISH testing

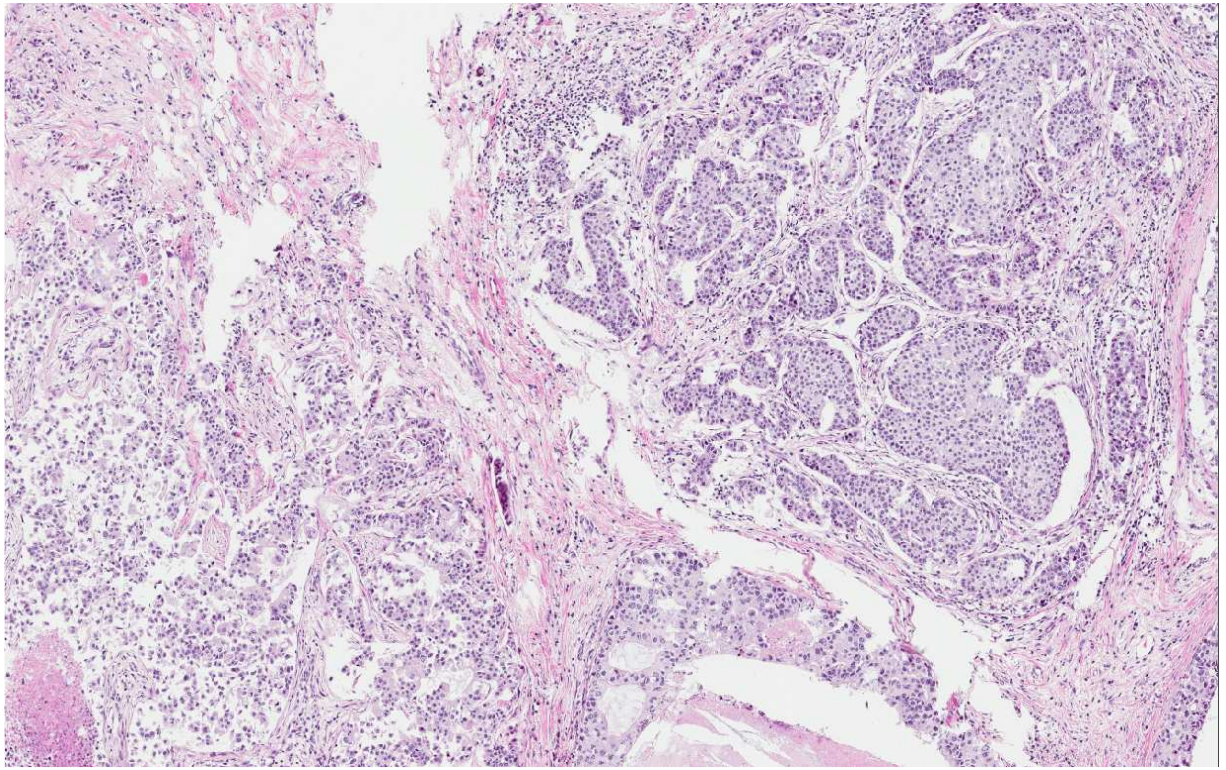
References: Rakha EA, et al. J Clin Pathol 2023;76:217–227; Wolff AC et al. Arch Pathol Lab Med. 2023 Sep 1;147(9):993-1000

HER2-low – Praktische Aspecten

- Keuze van het monster
- **Pre-analyse**
- IHC assay
- Controles
- Aflezen van de assay
- Rapportering

Quality of staining – cold ischemic time and fixation

Staining gradient and shrinkage artifacts indicators of poor fixation



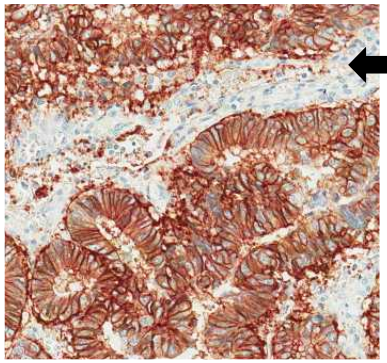
References: 1. Wolff AC, et al. *J Clin Oncol.* 2018;36:2105-2122

Quality of staining - cold ischemic time and fixation

Inadequate formalin fixation

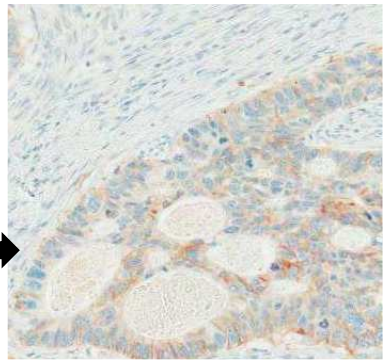
"false" heterogeneity in IHC – BUT homogeneous amplification in ISH!

HER2 4B5
20x



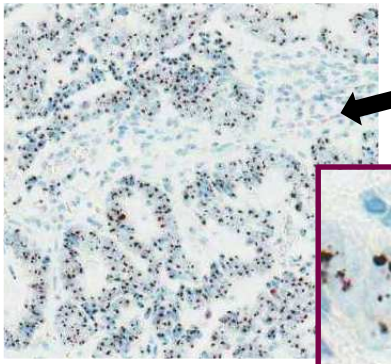
Strong staining at periphery

Moderate but heterogeneous staining in inner parts of the specimen

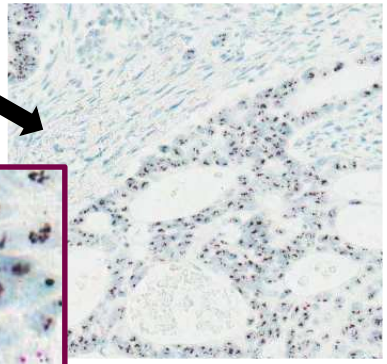


HER2 4B5,
20x

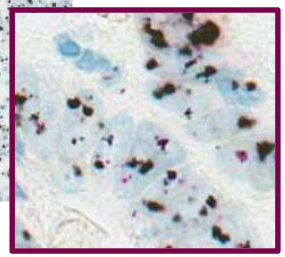
HER2
DDISH, 20x
and 40x

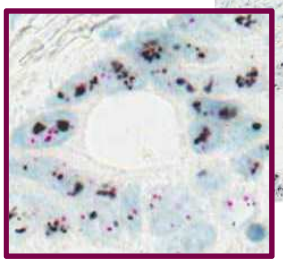


Homogeneous high level amplification in all parts of the specimen



HER2
DDISH, 20x
and 40x



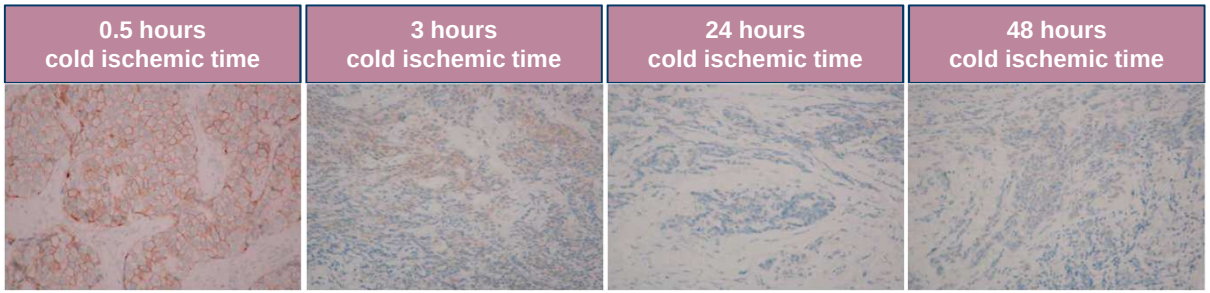


DDISH, dual-colour dual-hapten in situ hybridisaiton; HER2, human epidermal growth factor receptor 2; IHC, Immunohistochemistry; ISH, In situ hybridization. Images: Copyright & Ownership by Discovery Biomarker Academy™

Quality of staining - cold ischemic time and fixation

Cold ischemic times and fixation methods could influence[1]

- Percentage of HER2 positive cells
- Intensity of the stain



Adapted from Yildiz-Aktas IZ, et al. *Mod Pathol.* 2013;25:1098-1105.

- **Cold ischaemic time should be as short as possible, ideally within 1 hour[2]**
- **Fixation time in 10% NBF should be between 6 and 72 hours to minimise the occurrence of false-positive or -negative results[2]**
- **Surgical specimens should be grossly cut in 5-10 mm slices and fixate for preferably 24 hours to ensure uniform fixation and antigenicity of the sample[2]**

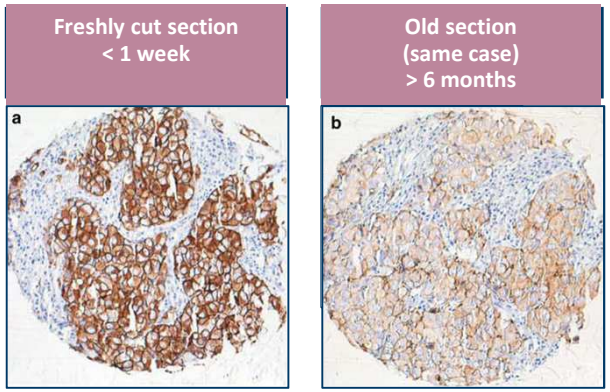
ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; NBF, neutral buffered formalin.

References: 1. Hicks DG, Schiffhauer L. *Lab Med.* 2011;42:459-467. 2. Wolff AC, et al. *J Clin Oncol.* 2018;36:2105-2122.

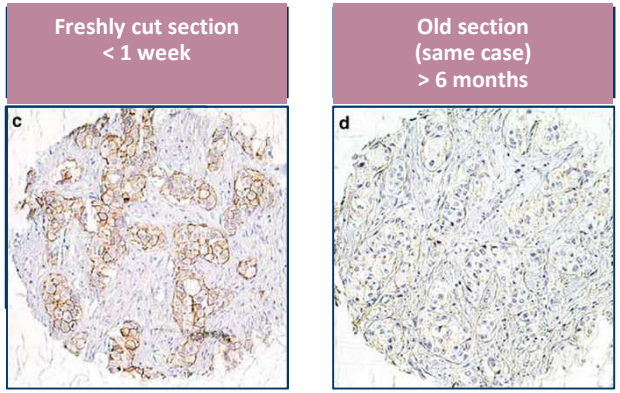
Quality of specimen – Sample age

HER2 signal intensity decreases over time[1]

HER2 (case #1)



HER2 (case #2)



Adapted from Mirlacher M, et al. *Mod Pathol.* 2004;17:1414-1420.

Sections stored for more than 6 weeks should not be used for HER2 testing[2]

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2.

References: 1. Mirlacher M, et al. *Mod Pathol.* 2004;17:1414-1420. 2. Wolff AC, et al. *J Clin Oncol.* 2018;36:2105-2122.

HER2-low – Praktische Aspecten

- Keuze van het monster
- Pre-analyse
- **IHC assay**
- Controles
- Aflezen van de assay
- Rapportering

Quality of staining – Type of assay

Several IHC assays are available to test for HER2 expression in breast cancer[1]

- These assays use distinct antibodies that may display different specificity and sensitivity[2]

HER2 IHC assay*	Antibody/clone
Bond™ Oracle™ HER2 IHC system (Leica Biosystems)[3]	Mouse CB11
HercepTest™ (Dako/Agilent)[4]	Rabbit A0485 (historically) DG44 mAb (current IVD)
InSite® HER-2/neu (BioGenex Laboratories)[5]	Mouse CB11
PATHWAY® anti-HER2/neu (Roche Tissue Diagnostics)[6]	Rabbit monoclonal 4B5

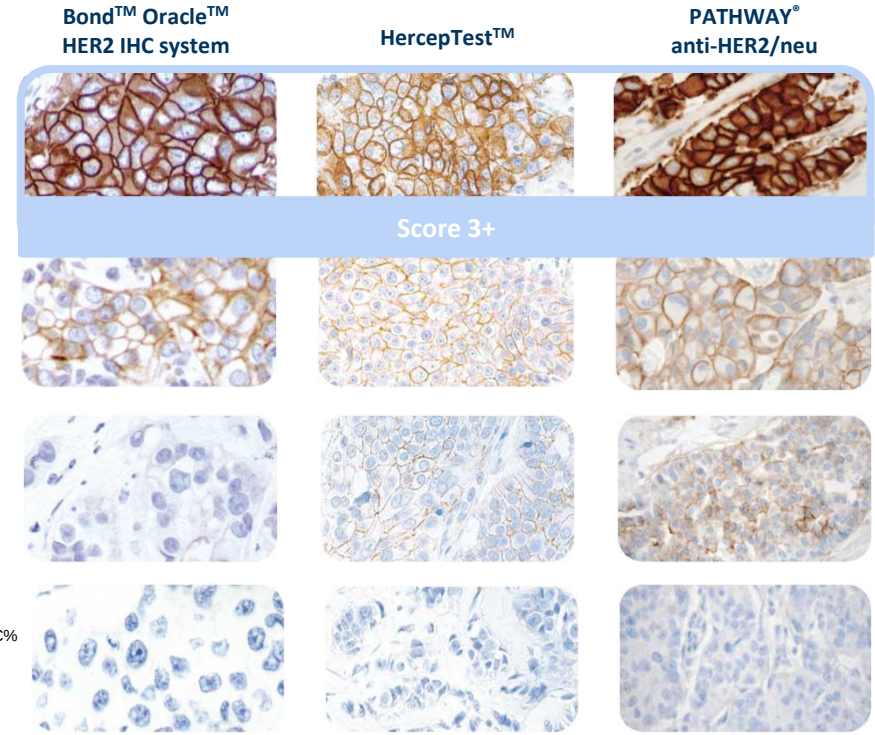
* Alphabetical order.

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

References: 1. Furrer D, et al. *Am J Clin Pathol.* 2015;144:686-703. 2. Dekker TJ, et al. *Breast Cancer Res*. 2012. 3. Bond™ Oracle™ HER2 IHC system. https://drp8p5tqcb2p5.cloudfront.net/fileadmin/downloads_lbs/Leica%20Oracle%20HER2%20Bond%20IHC%20System%20%28USA%20Breast%20Only%29/Brochures/Oracle_HER2_Interpretation_Guide_USA.pdf. Accessed January 15, 2022; 4. Dako. HerepTest™ Interpretation Manual Breast Cancer. https://www.agilent.com/cs/library/usemanuals/public/28630_herceptest_interpretation_manual-breast_ihc_row.pdf. Accessed January 15, 2022. 5. InSite® HER-2/neu. https://www.technologynetworks.com/genomics/news/fda-approves-biogenex-insite-her2neu-cb11-monoclonal-antibody-194349. Accessed January 15, 2022. 6. Interpretation Guide for VENTANA anti-HER2/neu (4B5). http://www.hsl-ad.com/newsletters/HER2_4B5_Interpretation_Guide.pdf. Accessed January 15, 2022. 7. Wolff AC, et al. *J Clin Oncol.* 2018;36:2105-2122.

ASCO/CAP HER2 Testing Guideline Recommendations for Pre-analytics and Analysis

- Circumferential membrane staining that is complete, intense, and in >10% of tumor cells[1]



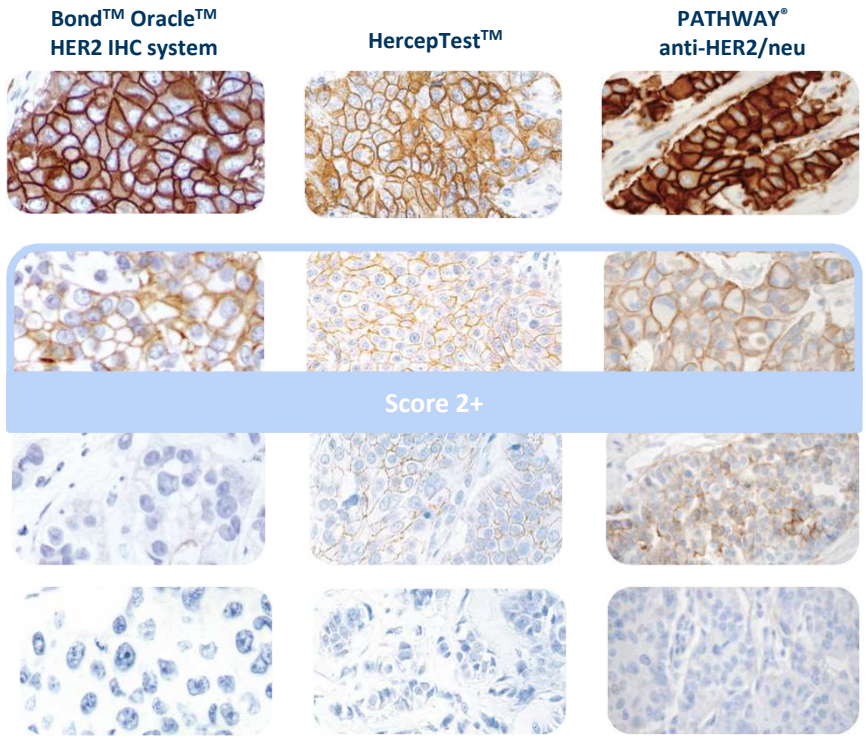
Images adapted from:
Bond™ Oracle™ HER2 IHC system.
https://drp8p5tqcb2p5.cloudfront.net/fileadmin/downloads_lbs/Leica%20Oracle%20HER2%20Bond%20IHC%20System%20%28USA%20Breast%20Only%29/Brochures/Oracle_HER2_Interpretation_Guide_USA.pdf.
Dako. HercepTest™ Interpretation Manual Breast Cancer.
https://www.agilent.com/cs/library/usemanuals/public/28630_herceptest_interpretation_manual-breast_ihc_row.pdf.
Interpretation Guide for VENTANA anti-HER2/neu (4B5).
http://www.hsl-ad.com/newsletters/HER2_4B5_Interpretation_Guide.pdf.

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; ISH, immunohistochemistry.

Reference: 1. Wolff AC, et al. *J Clin Oncol.* 2018;36:2105-2122.

ASCO/CAP HER2 Testing Guideline Recommendations for Pre-analytics and Analysis

- Weak to moderate complete membrane staining observed in >10% of tumor cells. For equivocal cases, must order reflex ISH[1]



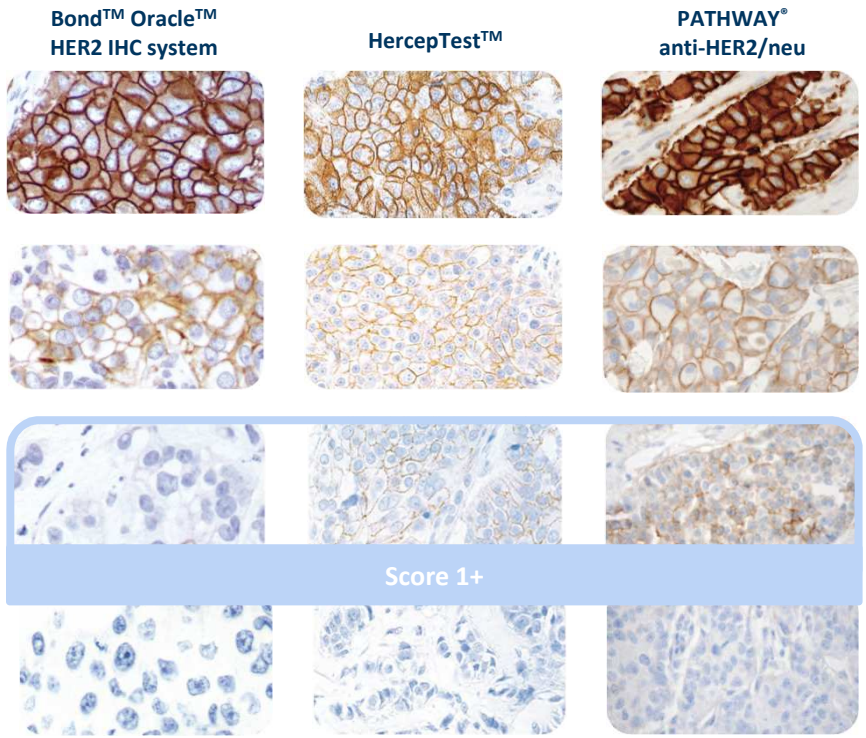
Images adapted from:
Bond™ Oracle™ HER2 IHC system.
https://drp8p5tqcb2p5.cloudfront.net/fileadmin/downloads_lbs/Leica%20Oracle%20HER2%20Bond%20IHC%20System%20%28USA%20Breast%20Only%29/Brochures/Oracle_HER2_Interpretation_Guide_USA.pdf.
Dako. HercepTest™ Interpretation Manual Breast Cancer.
https://www.agilent.com/cs/library/usemanuals/public/28630_herceptest_interpretation_manual-breast_ihc_row.pdf.
Interpretation Guide for VENTANA anti-HER2/neu (4B5).
http://www.hsl-ad.com/newsletters/HER2_4B5_Interpretation_Guide.pdf.

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; ISH, in situ hybridisation; ISH, immunohistochemistry.

Reference: 1. Wolff AC, et al. *J Clin Oncol.* 2018;36:2105-2122.

ASCO/CAP HER2 Testing Guideline Recommendations for Pre-analytics and Analysis

- Incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells[1]



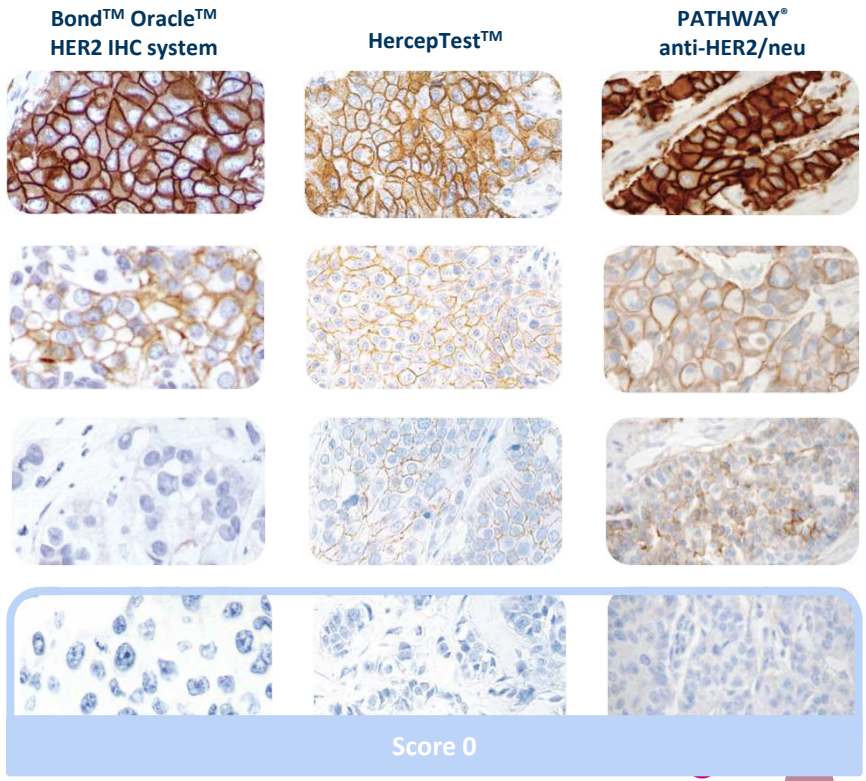
Images adapted from:
Bond™ Oracle™ HER2 IHC system.
https://drp8p5tqcb2p5.cloudfront.net/fileadmin/downloads_lbs/Leica%20Oracle%20HER2%20Bond%20IHC%20System%20%28USA%20Breast%20Only%29/Brochures/Oracle_HER2_Interpretation_Guide_USA.pdf.
Dako. HercepTest™ Interpretation Manual Breast Cancer.
https://www.agilent.com/cs/library/usemanuals/public/28630_herceptest_interpretation_manual-breast_ihc_row.pdf.
Interpretation Guide for VENTANA anti-HER2/neu (4B5).
http://www.hsl-ad.com/newsletters/HER2_4B5_Interpretation_Guide.pdf.

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

Reference: 1. Wolff AC, et al. *J Clin Oncol.* 2018;36:2105-2122.

ASCO/CAP HER2 Testing Guideline Recommendations for Pre-analytics and Analysis

- No staining observed
- Incomplete membrane staining that is faint or barely perceptible and within ≤10% of the invasive tumor cells[1]



Images adapted from:
Bond™ Oracle™ HER2 IHC system.
https://drp8p5tqcb2p5.cloudfront.net/fileadmin/downloads_lbs/Leica%20Oracle%20HER2%20Bond%20IHC%20System%20%28USA%20Breast%20Only%29/Brochures/Oracle_HER2_Interpretation_Guide_USA.pdf.
Dako. HercepTest™ Interpretation Manual Breast Cancer.
https://www.agilent.com/cs/library/usemanuals/public/28630_herceptest_interpretation_manual-breast_ihc_row.pdf.
Interpretation Guide for VENTANA anti-HER2/neu (4B5).
http://www.hsl-ad.com/newsletters/HER2_4B5_Interpretation_Guide.pdf.

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

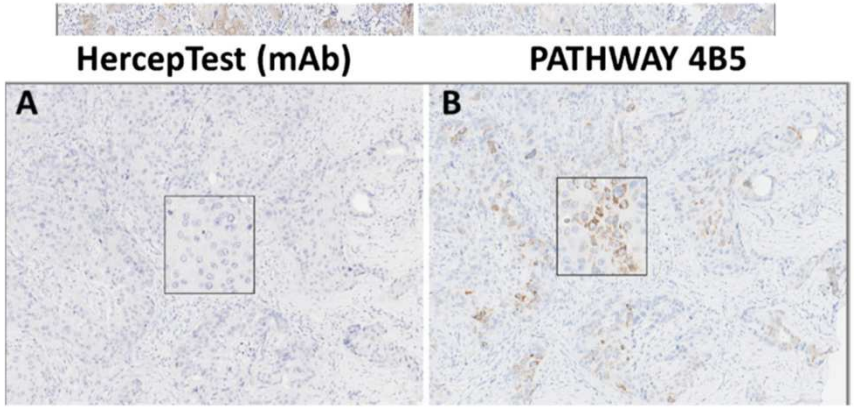
Reference: 1. Wolff AC, et al. *J Clin Oncol.* 2018;36:2105-2122.

HER2 IHC assay concordance

Comparison of HercepTest™ mAb pharmDx (Dako Omnis, GE001) with Ventana PATHWAY anti-HER-2/neu (4B5) in breast cancer: correlation with *HER2* amplification and HER2 low status

Josef Rüschoff[1] · Michael Friedrich[1] · Iris Nagelmeier[2] · Matthias Kirchner[2] · Lena M. Andresen[3] · Karin Salomon[3] · Bryce Portier[4] · Simone T. Sredni[4] · Hans Ulrich Schildhaus[1,2] · Bharat Jasani[1] · Marius Grzelinski[1] · Giuseppe Viale[5]

		PATHWAY 4B5				
		0	**1+**	**2+**	**3+**	**Total**
HercepTest (mAb)	**0**	35	0	0	0	**35**
	1+	17	8	0	0	**25**
	2+	4	12	13	1	**30**
	3+	0	0	2	27	**29**
	Total	**56**	**20**	**15**	**28**	**119**



Rüschoff et al. *Virchows Archiv* 2022;481:685-694

o Results

Overall inter-reader agreement was 84% (100/119) for the HercepTest (mAb) and 89.1% (106/119) for the PATHWAY 4B5 assay. Study IRR was recorded as 89.4% and 92.7%, respectively.

Most disagreements (68.8%) between pathologists' scores were observed within the HER2-low range (later consented as IHC score 0 or 1 +), especially near the cut-off for HER2 ultra-low category exhibiting a HER2 score of 0 with incomplete and faint staining in ≤ 10% of tumor cells.

Complete concordance between both assays was reached in 83 of 119 tumors (69.7%).

The concordance of both assays was found to be 83.7% (87/104 cases) for HER2- negative (IHC 0/1+) versus HER2-positive (IHC 3 +).

HER2 assay concordance – Prevalence of Her2 low in breast cancer subtypes using the VENTANA anti-Her2/neu (4B5) assay

- 500 BC samples
- 28.0% were IHC 1+/2+ using the 4B5 assay vs. 11.6% using HercepTest (mAb pharmDx; Dako Omnis)
- 4B5 assay classed several patients as IHC 1+/2+ that are IHC 0 by HercepTest, but almost all patients IHC 0 by 4B5 were also IHC 0 by HercepTest

References: Scot M et al. Presented at ASCO virtual Congress 2021

ESMO expert consensus on HER2-low

Can all Her2 assays be used to identify HER2 0, 1+ or Her2+/ISH- breast cancer?

Het is de verantwoordelijkheid van de patholoog om te bepalen welke assay/test de meest geschikte is

Aanvaardbare HER2 assays zijn oa de ‘Pathway 4B5 assay’ en eender welke andere gevalideerde assay die door de patholoog als aanvaardbaar wordt beschouwd.

Controles of referentiemateriaal gecalibreerd met aankleuring met lage intensiteit zullen helpen om de assay te harmoniseren.

Reference: Tarantino P, et al.. Ann Oncol. 2023. 34 (8): 645-659

HER2-low – Praktische Aspecten

- Keuze van het monster
- Pre-analyse
- IHC assay
- **Controles**
- Aflezen van de assay
- Rapportering

Gebruik van controles is zeer belangrijk om Her2-low aankleuring te kunnen identificeren

- Positieve en Negatieve controles
- Best ‘on slide’ controle
- Controles niet te lang (na het snijden) bewaren
- Ook 1+ en 2+ controles
- Weefsel en/of cellijncontroles, eventueel TMA
- Cave: te sterke antigen retrieval >> aankleuring normale borst

HER2-low – Praktische Aspecten

- Keuze van het monster
- Pre-analyse
- IHC assay
- Controles
- **Aflezen van de assay**
- Rapportering

Challenges for interpreting tumours with varying levels of HER2 expression

Definition not established
No formally established clear parameters defining low HER2 expression levels

Limited tissue or sample exhaustion[3]
Core needle biopsy is preferred for metastatic samples

Intratumoural heterogeneity[1]
Intratumoural heterogeneity affecting result interpretation

Tumour quality[2]
Tissue begins to degrade when biopsied, which can impact results

Subjective analysis and IHC limitations[1]
Accurate scoring is key for determining accurate levels of HER2 expression across the spectrum

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry

1. Tarantino P, et al. J Clin Oncol. 2020;38(17):1951–63; 2. Compton C, et al. Arch Pathol Lab Med. 2019;143(11):1346–63; 3. Rakha EA & Ellis IO. J Clin Pathol 2007;60(12):1300–6

Significant Advances in Clinical Practice and Standardisation of HER2 Testing Reduced Discordance Rate of HER2 Status[1]

- A comprehensive analysis of five neoadjuvant breast cancer studies comparing central and local HER2 IHC status showed a decrease of the discordance rate over 12 years in a total of 1581 tumour samples[1]

Discordance rate between the centrally and locally assessed HER2 IHC status over 12 years

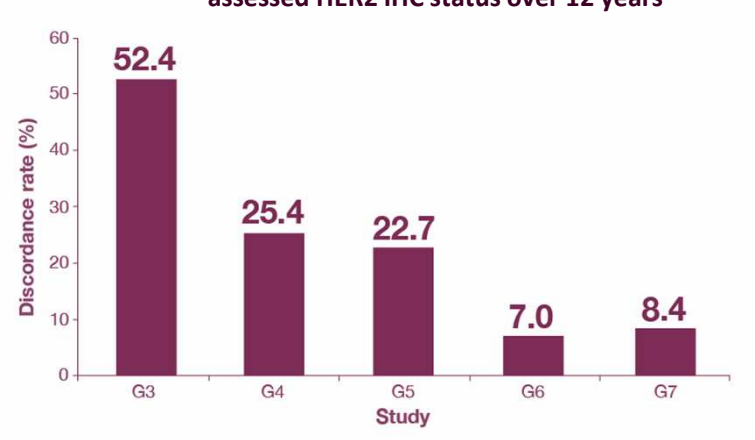


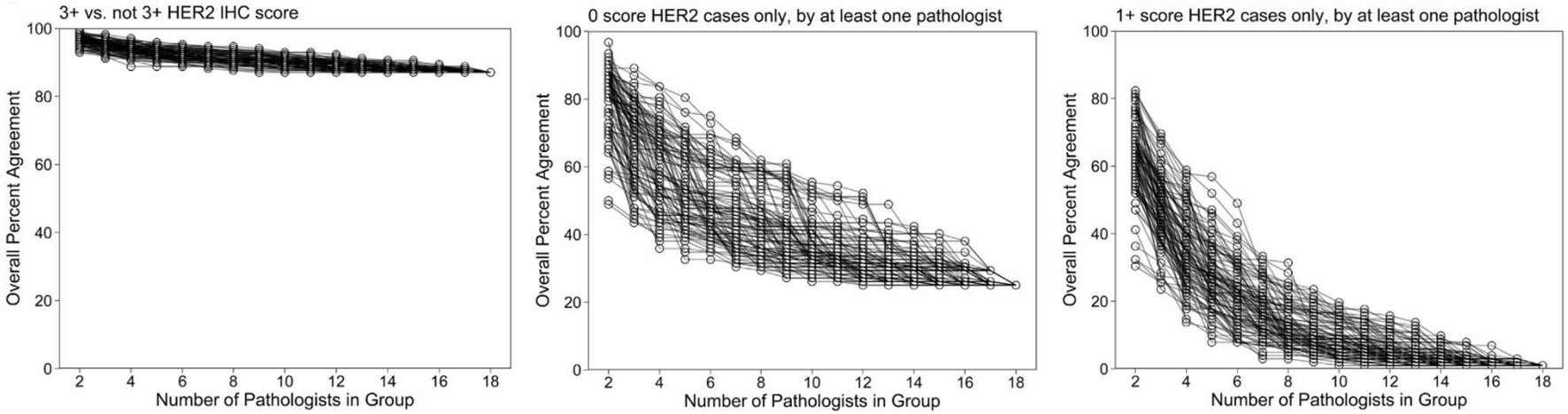
Image adapted from Pfitzner BM, et al. *Modern Pathol.* 2018;31:607-615.

Over the last two decades, consensus for the binary classification of HER2 expression has markedly improved with the evolution of test standardisation processes and interpretation criteria[1]

G3, GeparTrio; G4, GeparQuattro; G5, GeparQuinto; G6 GeparSixto; G7, GeparSepto; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

Reference: 1. Pfitzner BM, et al. Modern Pathol. 2018;31:607-615.

Pathologist participation in EQA schemes has resulted in High Concordance Rates in Assessing HER2 Positive vs Negative BCs, While Individual IHC Scores at the Low End of the HER2 Spectrum May Likely Vary[1]



Retrospective data on population distributions or comparisons with other biomarkers is compromised by the lack of pathologist distinction of IHC 0 vs IHC 1+ (which was not clinically relevant until now)

1. Robbins et al., Modern Pathol. 2023

Peer-led Education and New Standards May Be Needed to Ensure Scoring Reproducibility of varying HER2 expression across the spectrum[1]

These **RESULTS** **are not a good indicator** of the ability to identify breast cancers expressing HER2 IHC 1+

Discriminating between HER2 IHC 0 and 1+ cases **will require** **OPTIMISATION of technical variables and interpretation criteria to improve consistency**[1]

Clinical validation and adoption of **NOVEL HER2 ASSAYS** **and methodologies** are an area of continued interest and exploration[1-3]

Pathologists may need more time and careful examination of staining patterns to discern between HER2 IHC 0 and 1+ than they do for discerning between HER2 positive and HER2 negative classifications

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

References: 1. Schettini F, et al. *NPJ Breast Cancer*. 2021. 2. Fernandez A, et al. *JAMA Oncol*. Published online February 3, 2022. 3. Allison KH, Wolff AC. *JAMA Oncol.* Published online February 3, 2022.

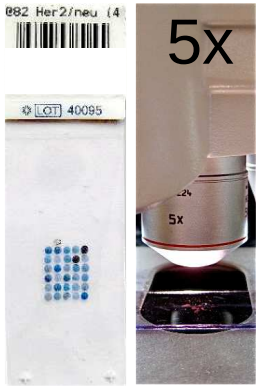
HER2 IHC evaluation according to 2018 ASCO-CAP guidelines

		HER2-Positive	Spectrum of Lower HER2 Expressing Carcinomas		HER2-0
	HER2 IHC	**3+**	**2+**	**1+**	**0**
Intensity	Magnification rules	Intense/strong (5x obj)	Weak/moderate (10-20x obj)	Faint (40x obj)	
Staining **patterns**	Membrane staining	Complete		Incomplete	
Heterogeneity	% stained tumour cells		>10%		≤10% or none (focal staining)

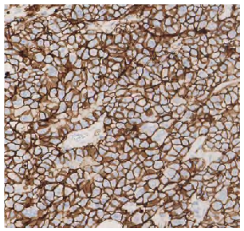
HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry
1. Courtesy: adapted from Prof. F. Penault-Llorca, France; ECP 2021

SCORING PARAMETERS - STAINING INTENSITY
Magnification or Microscope rule

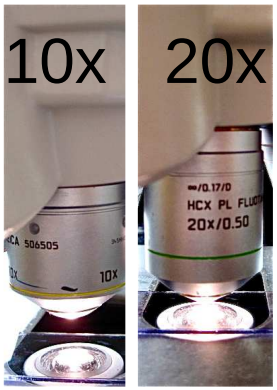
3+



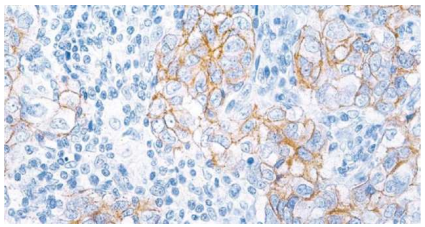
Membranous staining visible at low mag (2.5-5x)

5x 

2+

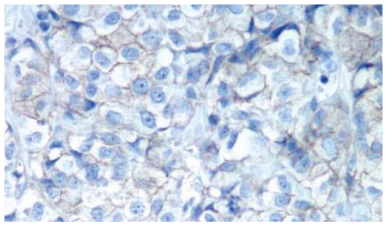


Needs intermediate magnification (10-20x)

20x 

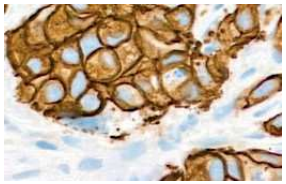
1+

Needs high magnification (40x)

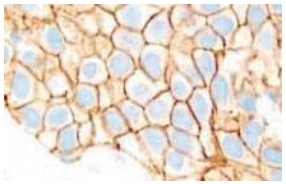
40x 

1. Rüschoff J, et al. Virchows Arch. 2010;457(3): 299-307. 2. Rüschoff J, et al. Mod Pathol. 2012;25(5):637-50

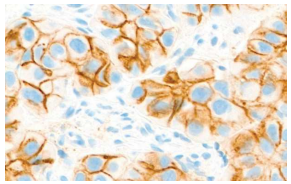
SCORING PARAMETERS - STAINING PATTERN



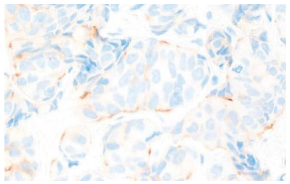
Complete (circumferential)



Complete (circumferential)

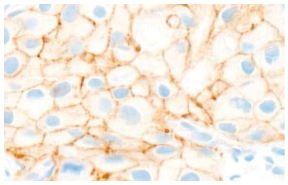


Basolateral

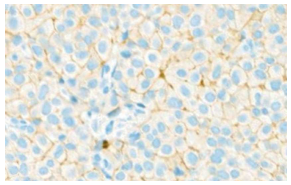


Basal membrane-like staining

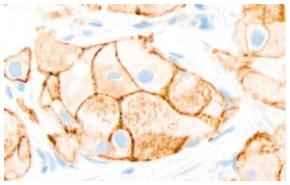
Chickenwire



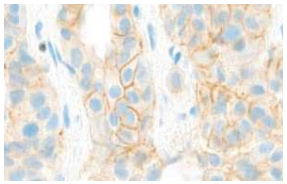
Beads on a string



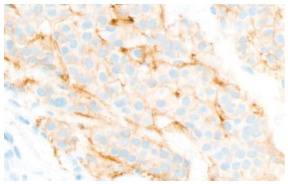
Crow feet (dotted/patchy)



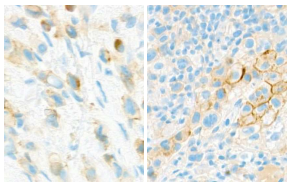
Diffuse granular



Incomplete (partial)

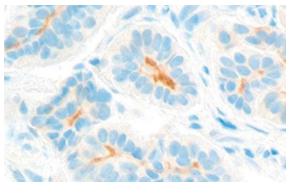


Incomplete (partial)



Cytoplasmic (focal)

Cytoplasmic (diffuse)

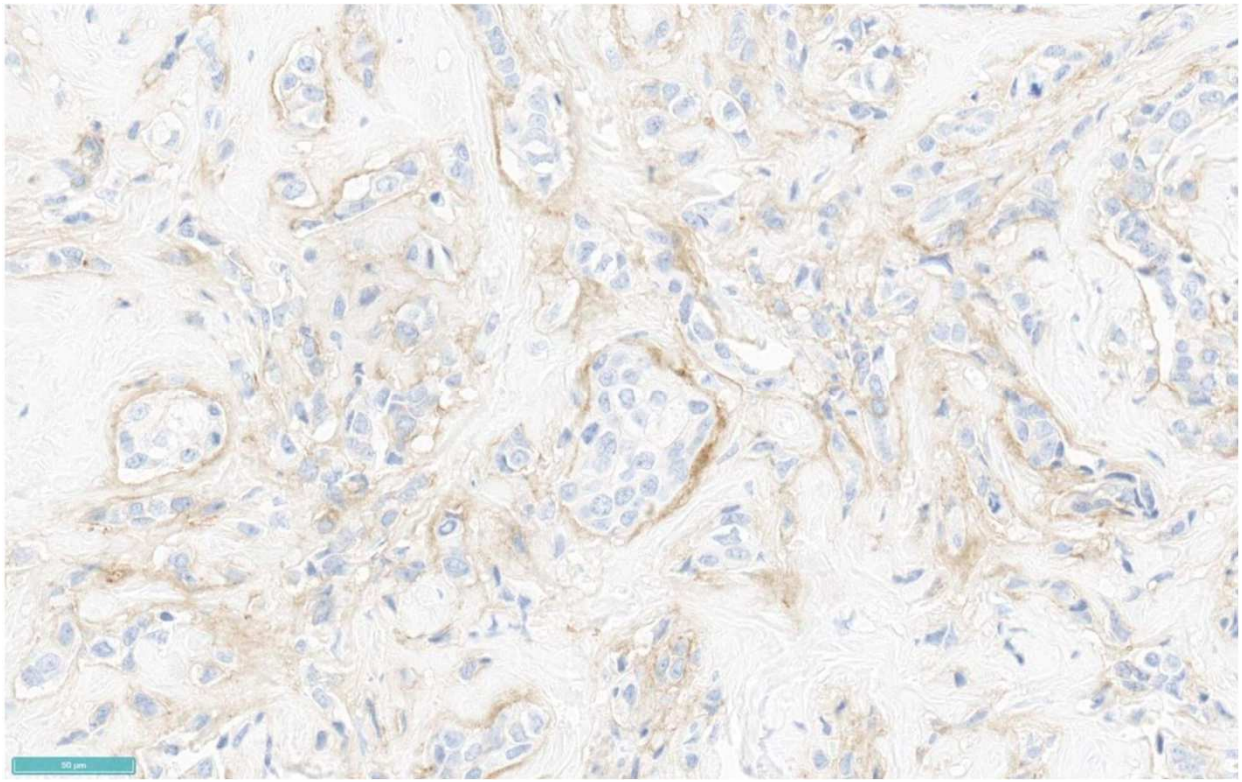


Luminal extracellular staining

Acceptable staining patterns: linear complete (basolateral), linear incomplete, beads on a string, crow feet.[1-2] **Unacceptable staining patterns:** diffuse cytoplasmic, granular cytoplasmic, basal membrane-like staining, luminal or extracellular only.[4] **Interpret with caution:** basolateral, diffuse granular[4]

SCORING PARAMETERS - STAINING PATTERN
Risk of false positive scoring

Basal membrane staining – to be excluded from scoring



IHC 0

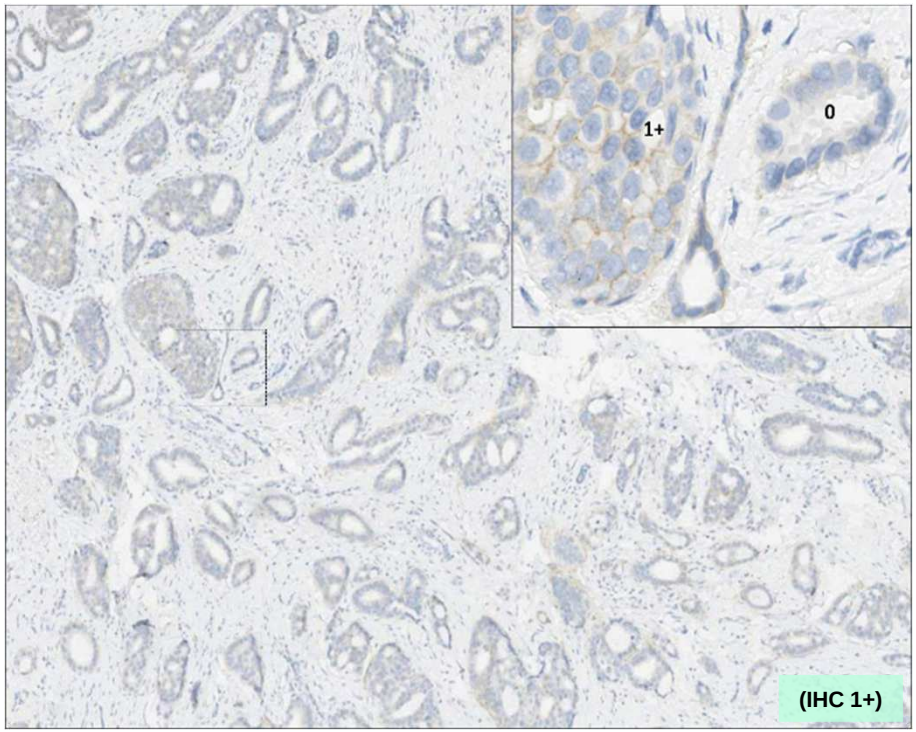
- **<10%** cells with membrane staining
- Mostly basal membrane pattern

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry

SCORING PARAMETERS

lower her2 expression vs HER2-zero

Risk of false negative scoring



Heterogeneity & Assessment at 10% cut-off

At 10% cut-off: cell counting is of use

Objective	~Cells / Visual Field	24” Monitor
4x	60.00	36.000
10x	10.000	6.000
20x	2.500	1.600
40x	600	360

(Mean cell diameter ~20µm; cells densely packed)

HER2, human epidermal growth factor receptor 2

Meuten D, et al. Vet Pathol. 2016;53(1):7-9
Schöniger S., Bänfer G., Diezko R., Jasani B. Importance of the size of the field of view for standardized biomarker evaluations. Poster abstract (PS-25-010), Virchows Archiv 2020; 477 (Suppl 1) S205.

ESMO expert consensus on HER2-low

How should we handle cases that are on the borderline between 0 and 1+?

Er is op dit moment onvoldoende evidentie om een uitspraak te doen over hoe men borderline casussen moet categoriseren, maar volgende zaken kunnen helpen bij de interpretatie ervan

- Bekijk de Her2 IHC op grote vergroting !
- Gebruik controles met een range van eiwitexpressie die ook 1+ bevat !
- Overweeg om een herevaluatie te vragen aan een collega patholoog!
- Besteed voldoende aandacht aan pre-analytische factoren !

Reference: Tarantino P, et al.. Ann Oncol. 2023. 34 (8): 645-659

HER2-low – Praktische Aspecten

- Keuze van het monster
- Pre-analyse
- IHC assay
- Controles
- Aflezen van de assay
- **Rapportering**

HER2 reporting – open suggestions

Today's Report

- HER2 – negative
- Score 2+ (20% of tumour cells)
- ISH: not amplified

- What about the remaining 80% tumour cells?
- Important to know if they (and how many of them) are 1+?
- Should we report on the % of tumour cells without any staining (null)?
- Should we adopt the 'HER2-low' terminology in the report?

References: based on Her2 masterclass Daiichi Sankyo

ESMO expert consensus statements

Table 1. Interpretation by the ASCO/CAP 2018 Guidelines and by the 2023 ESMO Consensus on HER2-low breast cancer regarding each pattern of HER2 staining

Description of staining	Denomination by 2018 ASCO/CAP Guidelines	Conclusion by 2018 ASCO/CAP Guidelines	Conclusion by 2023 ESMO clinical practice recommendations
- No staining	HER2-0	HER2-negative	**HER2-0** *HER2-null*[a]
- Incomplete or faint staining in ≤10% of invasive tumor cells	HER2-0	HER2-negative	*HER2-ultralow (or >no staining <1+)*[a]
- Incomplete or faint staining in >10% of invasive tumor cells	HER2 1+	HER2-negative	**HER2-low**
- Weak to moderate complete membrane staining in >10% of invasive tumor cells (ISH-negative)	HER2 2+ nonamplified	HER2-negative	**HER2-low**
- Weak to moderate complete membrane staining in >10% of invasive tumor cells (ISH-positive)	HER2 2+ amplified	HER2-positive	**HER2-positive**
- Intense complete membrane staining in >10% of invasive tumor cells	HER2 3+	HER2-positive	**HER2-positive**

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; ISH, *in situ* hybridization.
Bold are the actual definitions. In italics are potential future sub-definitions within the HER2-0 category.
[a]The decision to establish the HER2-null and HER2-ultralow (or >no staining <1+) categories will be dependent on the results of the DB-06 trial.

Tarantino P, et al. Ann Oncol. 2023;34:645-659

Current recommendations - Reporting

ESMO Expert Consensus Statements[1]

- In reporting HER2 testing results, pathologists should maintain a nomenclature consistent with the ASCO/CAP 2018 algorithm. The HER2 IHC score (0,1+, 2+ or 3+) should always be included in the report. This in turn allows clinicians to determine whether the case can be considered eligible for T-Dxd, or for trials of other agents targeting HER2-low expression. **The use of the term "HER2-low" is not preferable in the pathology report**, whereas its use is justified in clinical practice as an interpretation of the HER2 status of the disease.

ASCO/CAP Guideline Update[2]

- While **it is premature to change reporting terminology for lower levels of HER2 IHC expression (e.g. HER2-low)**, pathology labs should include a footnote in their HER2 testing reports with the following recommended comment:

- *"… Patients with breast cancers that are HER2 IHC 1+ or IHC2+/ISH not-amplified may be eligible for a treatment that targets non-amplified/non-overexpressed levels of HER2 expression for cytotoxic drug delivery (IHC 0 results do not result in eligibility currently)."*

References: 1. Tarantino P, et al. Ann Oncol. 2023;34:645-659; 2. Wolff AC, JCO 2023:41-22, 3867-3872.

ASCO-CAP HER2 testing guideline update 2023

- The 2023 Guideline reaffirms the 2018 “HER2 Breast Testing Guideline Focused update”
- This update acknowledges a new indication for Trastuzumab deruxtecan when HER2 is not overexpressed or amplified but is immunohistochemistry (IHC) 1+ or 2+ without amplification by in situ hybridization
- Although creating new result categories of HER2 expression (eg HER2-Low, HER2-Ultra-Low)is premature, distinguishing IHC 0 from 1+ is now clinically relevant
- The guideline update was published on June 7 in Archives of Pathology & Laboratory Medicine (CAP) and Journal of Clinical Oncology (ASCO)
- Editorials by Dr Shabnam Jaffer and Drs Stuart Schnitt, Paolo Tarantino & Laura Collins

Wolff AC, JCO 2023:41-22, 3867-3872

ASCO-CAP 2023 guideline – best practice

- Although it is **premature to create new result categories** of HER2 expression (eg, HER2-Low, HER2-Ultra-Low), **best practices to distinguish IHC 0 from 1+ are now clinically relevant**.

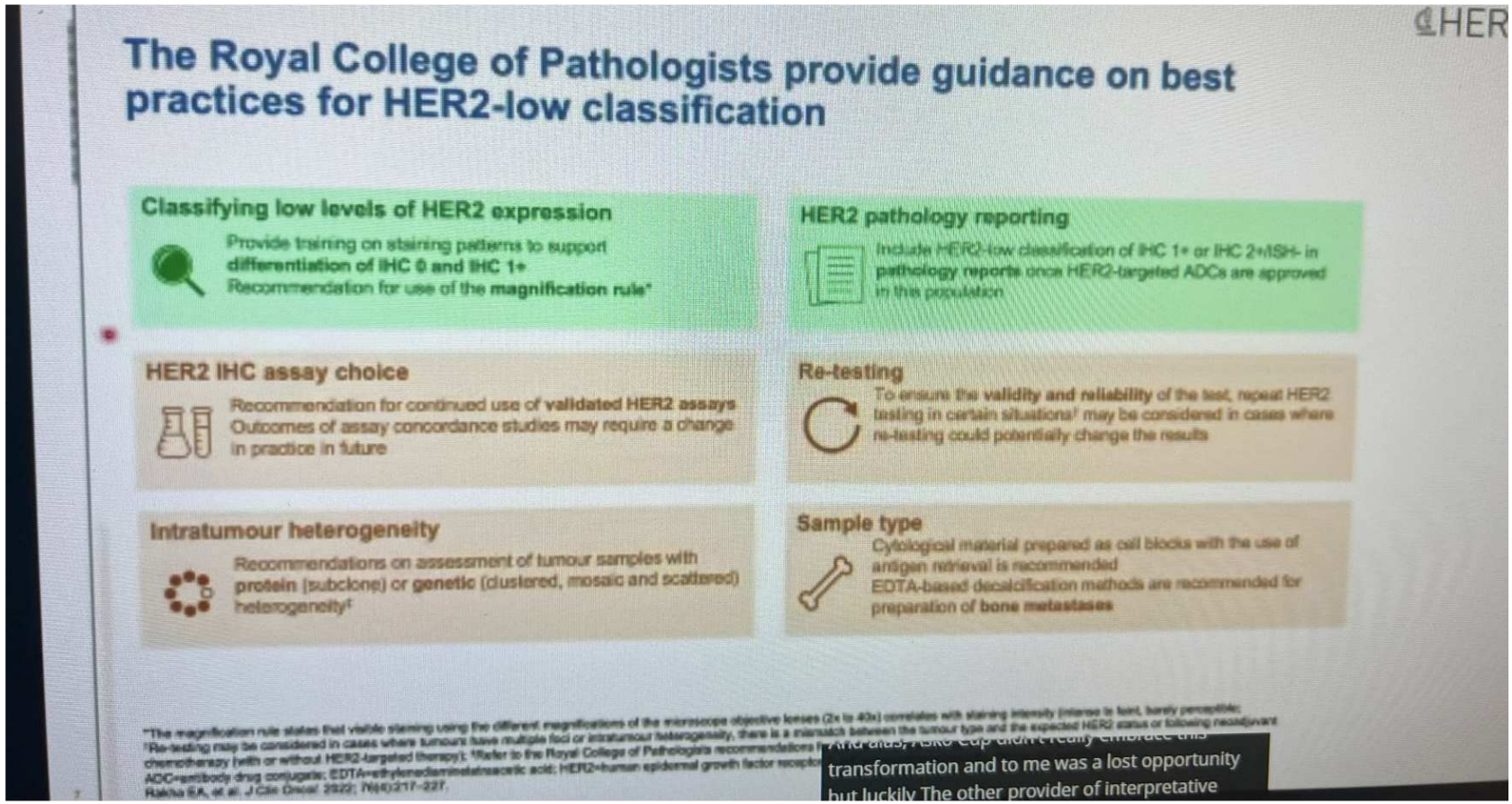
- Best practice efforts:
  - Examining HER2 IHC-stained slides using standardized ASCO-CAP guidelines scoring criteria (see the Figure for interpretation).
  - Examining HER2 IHC at high power (40x) when discriminating 0 from 1+ staining.
  - Considering second pathologist review when results are close to the 0 versus 1+ interpretive threshold (>10% of cells with incomplete membrane staining that is faint/barely perceptible).
  - Using controls with a range of protein expression (including 1+) to help ensure the assay has an appropriate limit of detection.
  - Careful attention to pre-analytic conditions of breast cancer tissue samples from both primary and metastatic sites.
  - The semiquantitative IHC score must always be reported as well to ensure patients who meet eligibility criteria for trastuzumab deruxtecan can be identified. Example: HER2-negative for protein overexpression (1+ staining present).
- Consider HER2 IHC results on prior or concurrent primary samples (or other metastatic sites)

Wolff AC, JCO 2023:41-22, 3867-3872.

Her2 low
let’s prepare

Conclusions

- IHC blijft de gouden standaard voor Her2 testing
- Her2- low borstkanker patienten kunnen behandeld worden met anti-Her2 therapieën. Hiervoor is de specifieke IHC score nodig
- Drempel voor re-assessment moet laag zijn omdat het en andere therapie optie voor de patiënt kan betekenen
- We moeten onze assays goed in de vingers hebben (sensitiviteit,factoren die resultaat beïnvloeden) en ze ook correct valideren


HER
The Royal College of Pathologists provide guidance on best practices for HER2-low classification
Classifying low levels of HER2 expression
Provide training on staining patterns to support differentiation of IHC 0 and IHC 1+
Recommendation for use of the magnification rule*
HER2 pathology reporting
Include HER2-low classification of IHC 1+ or IHC 2+/ISH- in pathology reports once HER2-targeted ADCs are approved in this population
HER2 IHC assay choice
Recommendation for continued use of validated HER2 assays
Outcomes of assay concordance studies may require a change in practice in future
Re-testing
To ensure the validity and reliability of the test, repeat HER2 testing in certain situations† may be considered in cases where re-testing could potentially change the results
Intratumour heterogeneity
Recommendations on assessment of tumour samples with protein (subclone) or genetic (clustered, mosaic and scattered) heterogeneity‡
Sample type
Cytological material prepared as cell blocks with the use of antigen retrieval is recommended
EDTA-based decalcification methods are recommended for preparation of bone metastases
transformation and to me was a lost opportunity
but luckily The other provider of interpretative

EXTERNAL QUALITY ASSURANCE (EQA) program links

- Belgium https://www.eqascheme.org/
- UK NEQAS https://ukneqas.org.uk/
- CAP https://www.cap.org/
- Pathology Australia https://www.australianpathology.com/
- European Society of Pathology https://www.esp-pathology.org/
- NordiQC https://www.nordiqc.org/
- Germany https://www.quip.eu/de_DE/
- France https://www.afaqap.fr/