HER2 Status in the Era of ADC and Artificial Intelligence

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The International Academy of Pathology
Hong Kong Division

Outlines of my talk

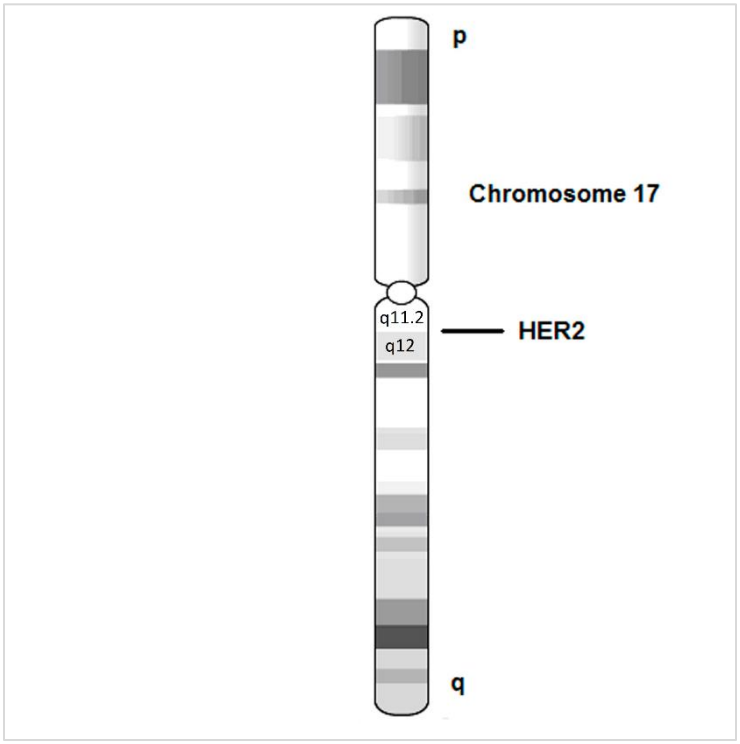
1. Introduction :
   a. *HER2* oncogene
   b. HER2 status assessment according to guidelines (ASCO / CAP 2018, 2023)
   c. HER2 positive tumors: are not a single entity

2. Definition and epidemiology of HER2 low and ultra-low breast cancers

3. AI assessment of HER2 status in breast cancers

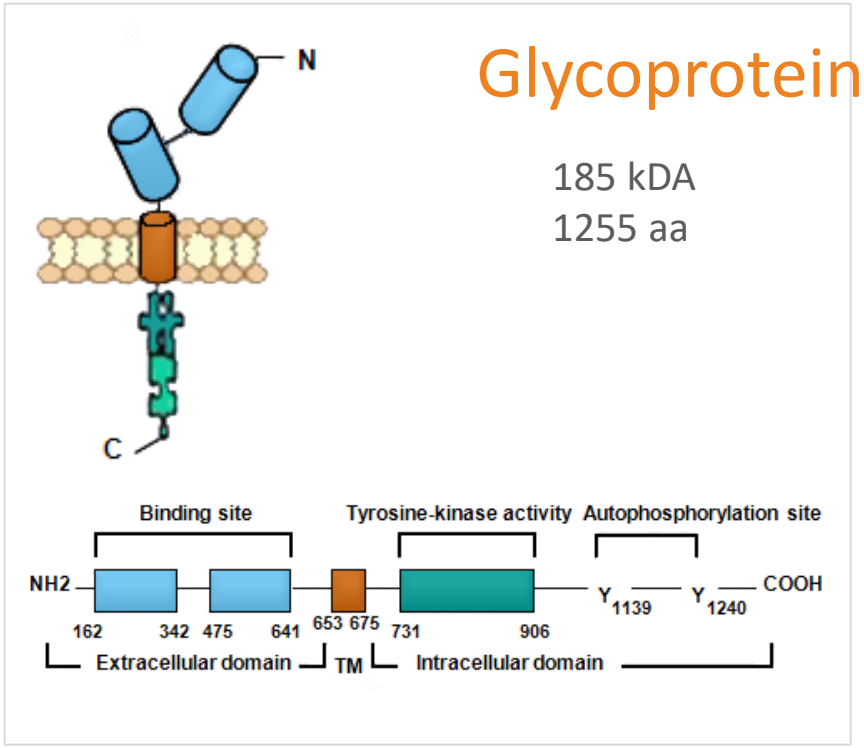
4. Take home messages

HER2



Gene

17q11.2-q12
30528 pb
27 exons



Glycoproteine

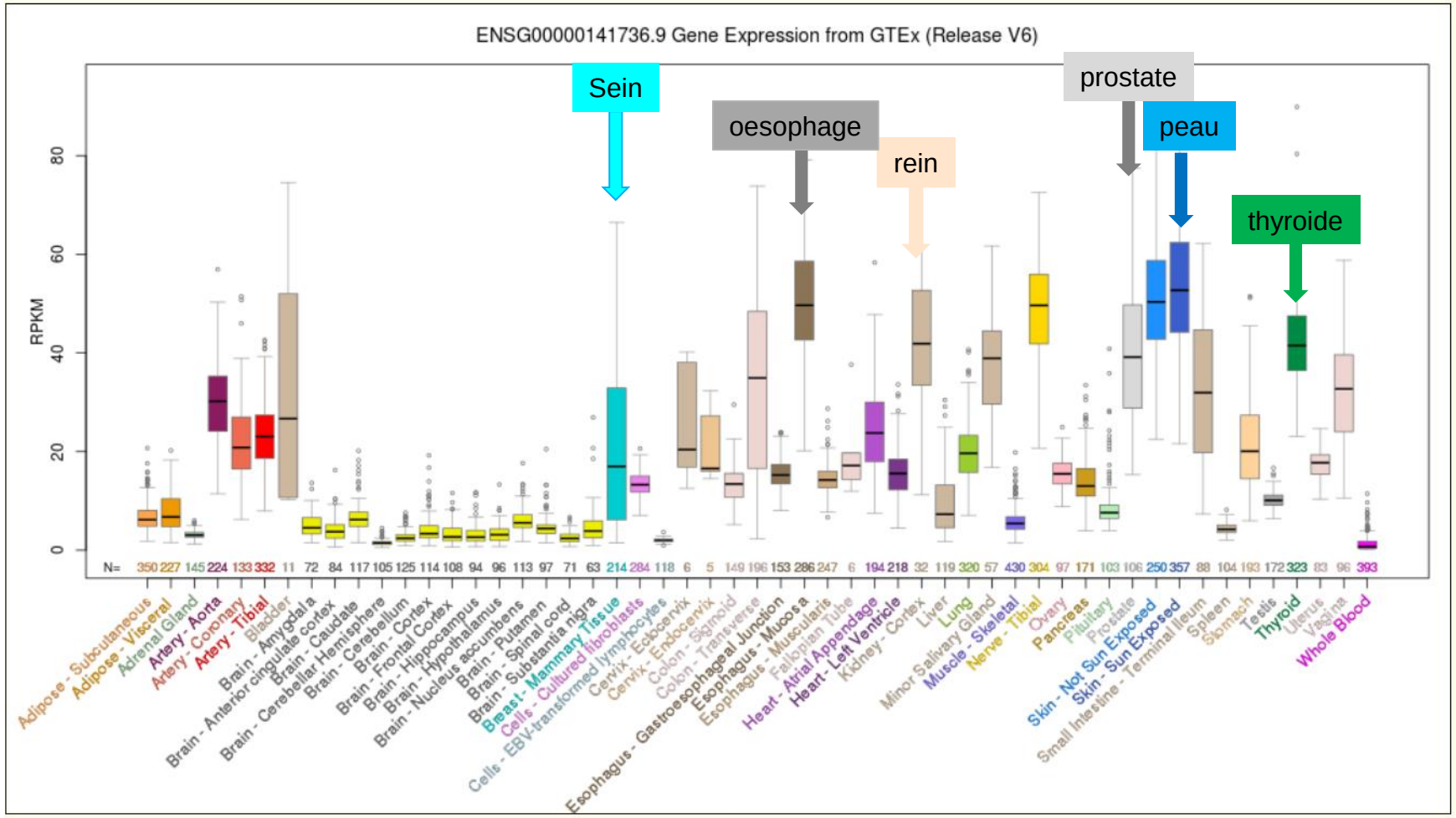
185 kDA
1255 aa

Oncogene

- expressed in the normal mammary gland
- necessary for the development of mammary gland ducts and lobules
- necessary for acini differentiation during lactation

Kokai et al, PNAS 1987; Natali et al, Int J of Cancer 1990; Jones et al, Oncogene 1999

HER2 mRNA Expression levels in normal tissues

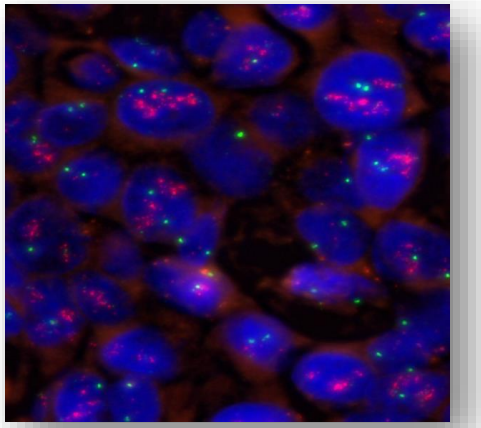


Genome Browser UCSC : RNA-Seq Expression Data from GTEx (53 Tissues, 570 Donors)

http://www.genome.ucsc.edu/cgibin/hgGene?hgsid=945615817_pP6BUmODfXGi1JASlAxzNbqn1mLL&hgg_section_microarray_close=0#microarray

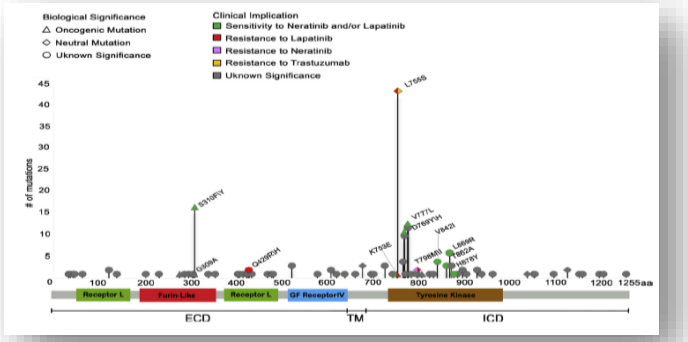
MECANISMS OF *HER2* ACTIVATION

1- Amplification



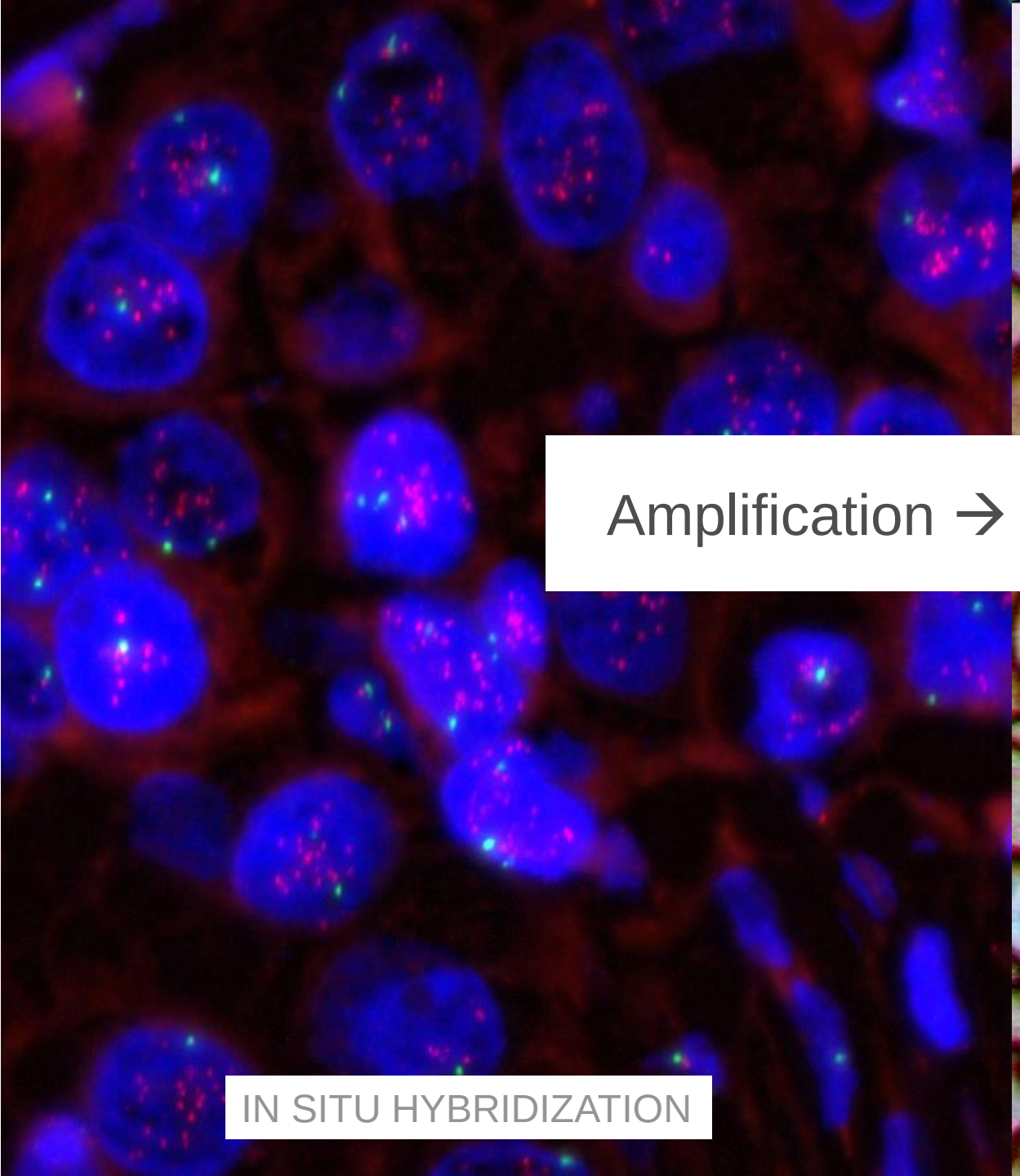
- ≥ 6 copies of *HER2/nucleus*
- Focal region of amplification in 17q12 (< 10Mb)
- Driver event → oncogenic addiction of the cells → cell proliferation, migration, invasion and survival
- **10 to 15% of breast cancers**

2- Mutations



- Observed in HER2 negative and HER2 low tumors
- In the tyrosine kinase domain (and extracellular domain)
- **2 % of IC-NST**
- **20% of Invasive lobular carcinomas grade 3**

Slamon et al. Science 1987, Slamon et al. Science 1989, Wolff et al. J Clin Oncol 2018, Ferrari et al Nat Com 2016; Marchio et al Sem Can Bio 2020;
Bose et al Cancer Discov 2013.; Deniziaut et al Oncotarget 2017; Kalleoniemi et al, Am J Pathol 2004; Staaf et al Br Can Res 2010; Condorelli et al Annals of Onc 2019
Ferrari *et al* Nat Com 2016; Marchio *et al* Sem Can Bio 2020; Bose *et al* Cancer Discov 2013; Deniziaut *et a*l Oncotarget 2017



Amplification → overexpression

IN SITU HYBRIDIZATION

IMMUNOHISTOCHEMISTRY

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   b. HER2 status assessment according to guidelines (ASCO / CAP 2018 & 2023)

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JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/ College of American Pathologists Clinical Practice Guideline Focused Update

Antonio C. Wolff, M. Elizabeth Hale Hammond, Kimberly H. Allison, Brittany E. Harvey, Pamela B. Mangu, John M.S. Bartlett, Michael Bilous, Ian O. Ellis, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, Lisa M. McShane, and Mitchell Dowsett

PRINCIPLES OF HER2 STATUS DETERMINATION

1- HER2 is determined by **immunohistochemistry (IHC)** for all invasive breast cancers and by In situ hybridization (ISH) for 2+ scores

2- Why IHC ?

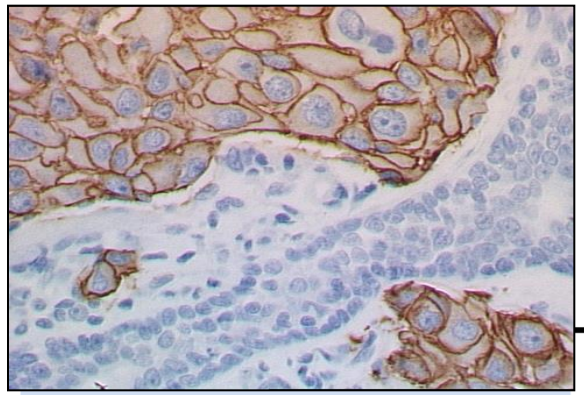
- Level of protein expression correlated to the gene copy number
- HER2 protein is the target of the anti-HER2 therapies

3- How ?

- By calibration of IHC on *HER2* gene status to detect overexpression in relation to gene amplification
- With:
  - Negative internal controls (normal glands)
  - External controls with known HER2 gene copy number in each batch and positive control on each slide
  - External quality control (AFAQAP; NordiQC, UKNEQAS…).

Wolff et al JCO 2018; Franchet et al, Annales de Pathologie 2021

HER2 STATUS DETERMINATION FOR THERAPEUTIC DECISIONS



HER2 testing (invasive component) by validated IHC assay

Batch controls and on-slide controls show appropriate staining

Circumferential membrane staining that is complete, intense, and in > 10% of tumor cells*

IHC 3+
POSITIVE

Weak to moderate complete membrane staining observed in > 10% of tumor cells

IHC 2+
equivocal

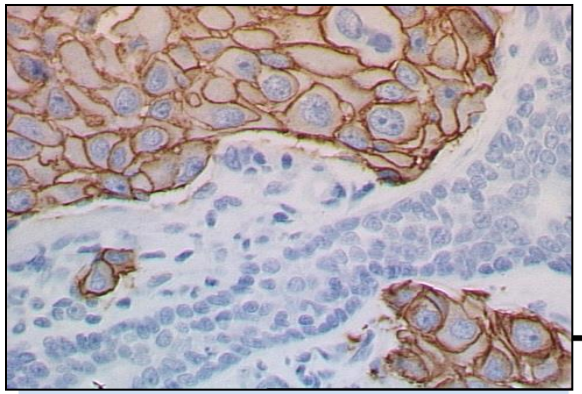
Incomplete membrane staining that is faint/barely perceptible and in > 10% of tumor cells

IHC 1+
negative

No staining is observed
or
Membrane staining that is incomplete and is faint/barely perceptible and in ≤ 10% of tumor cells

IHC 0
negative

HER2 STATUS DETERMINATION FOR THERAPEUTIC DECISIONS



HER2 testing (invasive compo

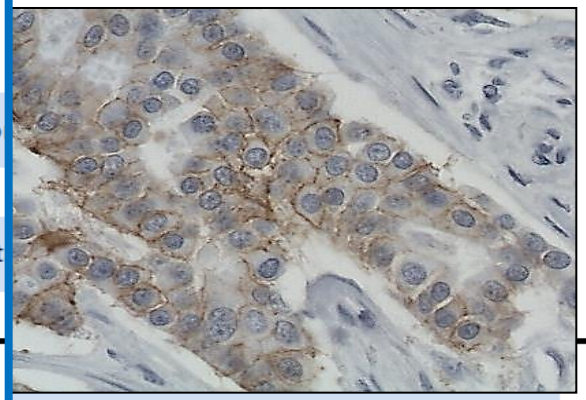
Batch controls and on-slide cont

Circumferential membrane staining that is complete, intense, and in > 10% of tumor cells*

IHC 3+ positive

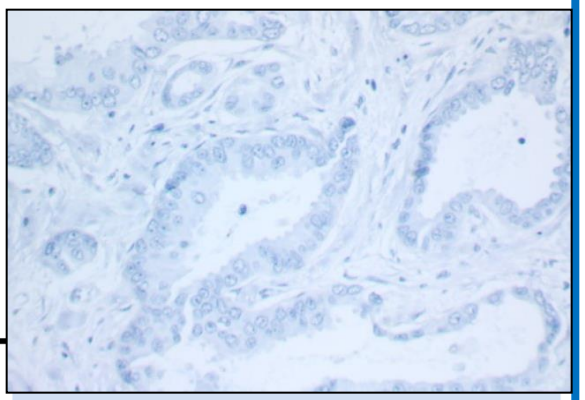
Weak to moderate complete membrane staining observed in > 10% of tumor cells

IHC 2+ equivocal



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

IHC 1+



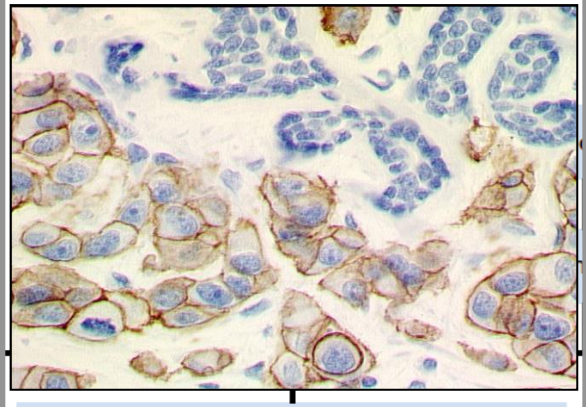
No staining is observed
or
Membrane staining that is incomplete and is faint/barely perceptible and in ≤ 10% of tumor cells

IHC 0

Negative

The 2023 ASCO/CAP update of HER2 testing guidelines provides best practice recommendations for the distinction of HER2 0 from 1+ including evaluation of HER2 IHC at **high-power magnification** and seeking consensus review when needed.

Wolff et al JCO 2023



onent) by validated IHC assay

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intense, and in > 10% of tumor cells*

Weak to moderate complete membrane staining observed in > 10% of tumor cells

and in > 10% of tumor cells

No staining is observed

incomplete and is faint/barely perceptible and in ≤ 10% of tumor cells

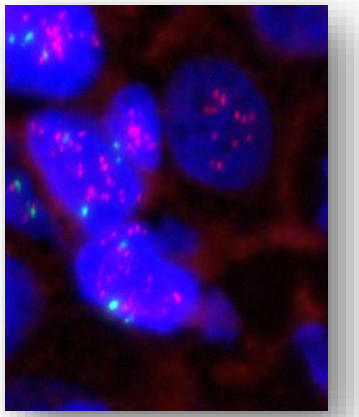
IHC 3+ positive

IHC 2+

IHC 1+ negative

IHC 0 negative

Positive 10-15%

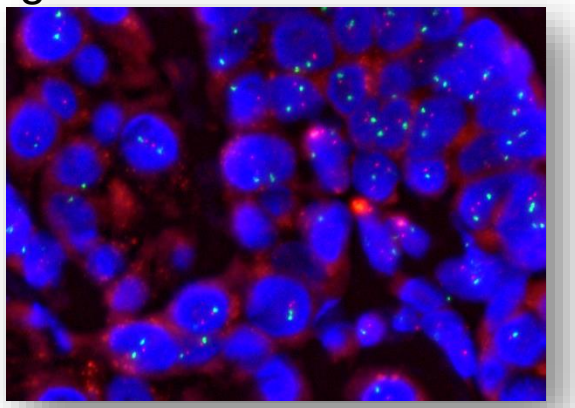


ISH assay

FISH amplified
15-30%
HER2 POSITIVE

FISH not amplified
70%
HER2 NEGATIVE

Negative : 70%



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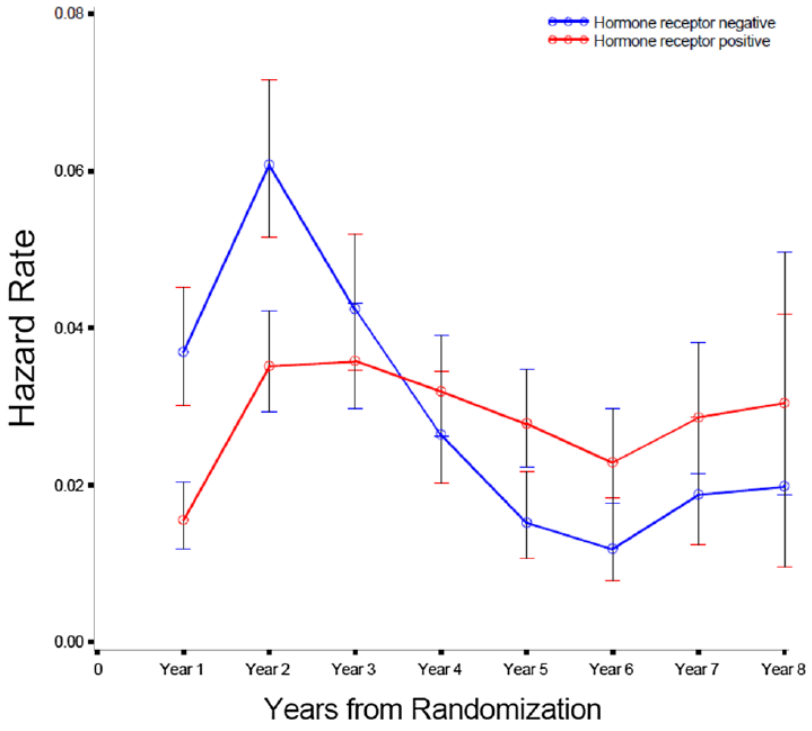
HER2 POSITIVE ER+ TUMORS ARE DIFFERENT FROM HER2 POSITIVE ER- TUMORS

- 50% of all HER2 positive cases are ER+
- Have a different natural history and different metastatic spreading than HER2+ER-



Mean annual hazards of reccurence

- In the ALTTO trial HER2 positive and ER+ are:
  - Younger & Premenopausal
  - N -
  - Lower level of *HER2* amplification
  - Better survival outcomes in the first 5 years
  - Same prognosis at 8 years than ER- HER2+
  - Different Pattern of events:
    - more bone metastasis (31.7% vs 18.7%; $p < 0.001$)
    - more liver metastasis (21% vs 16.3%)

Lambertini et al Br Can Res TTT 2019

HER2 POSITIVE ER+ TUMORS RESPONSE TO NEOADJUVANT CHEMOTHERAPY

- ER status and *PIK3CA* mutation status influence HER2 + breast cancer pCR rates to neoadjuvant anti HER2 therapies

	pCR rates
HER2+ ER+	**30.9%**
HER2+ ER+ *PIK3CA* mutation	**16.2%**
HER2+ ER-	**60 – 75%**

- But screening for *PIK3CA* mutations in HER2 positive breast cancer **is not yet of clinical relevance.**

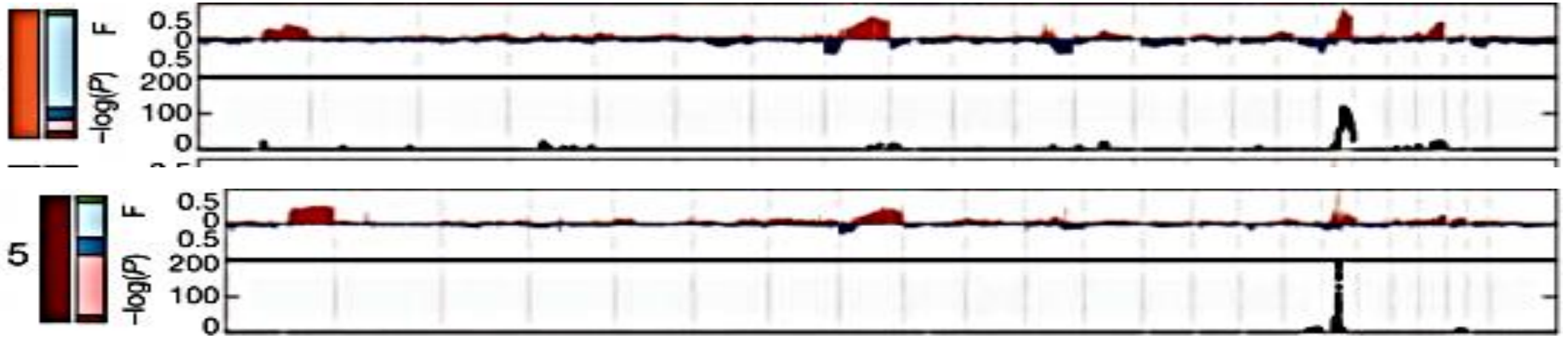
Houssami, EJC 2012; Von Minckwitz, JCO 2012; Cortazar et al, Lancet 2014
Loibl et al Annals of Oncol 2016; Igniatidis et al JNCI 2019

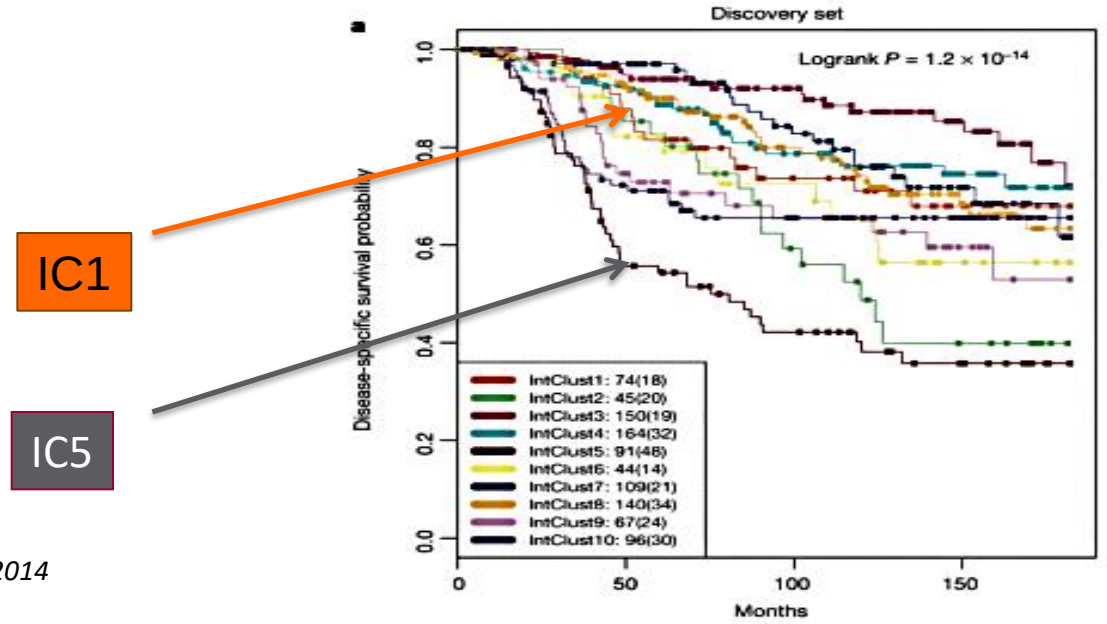
ER+ HER2 positive tumors have different molecular profiles and prognosis compared to ER- HER2 enriched cancers

METABRIC consortium: Integrated transcriptomic and genomic classification:

Lum B /HER2 **IC1**

HER2 **IC5**





IC1

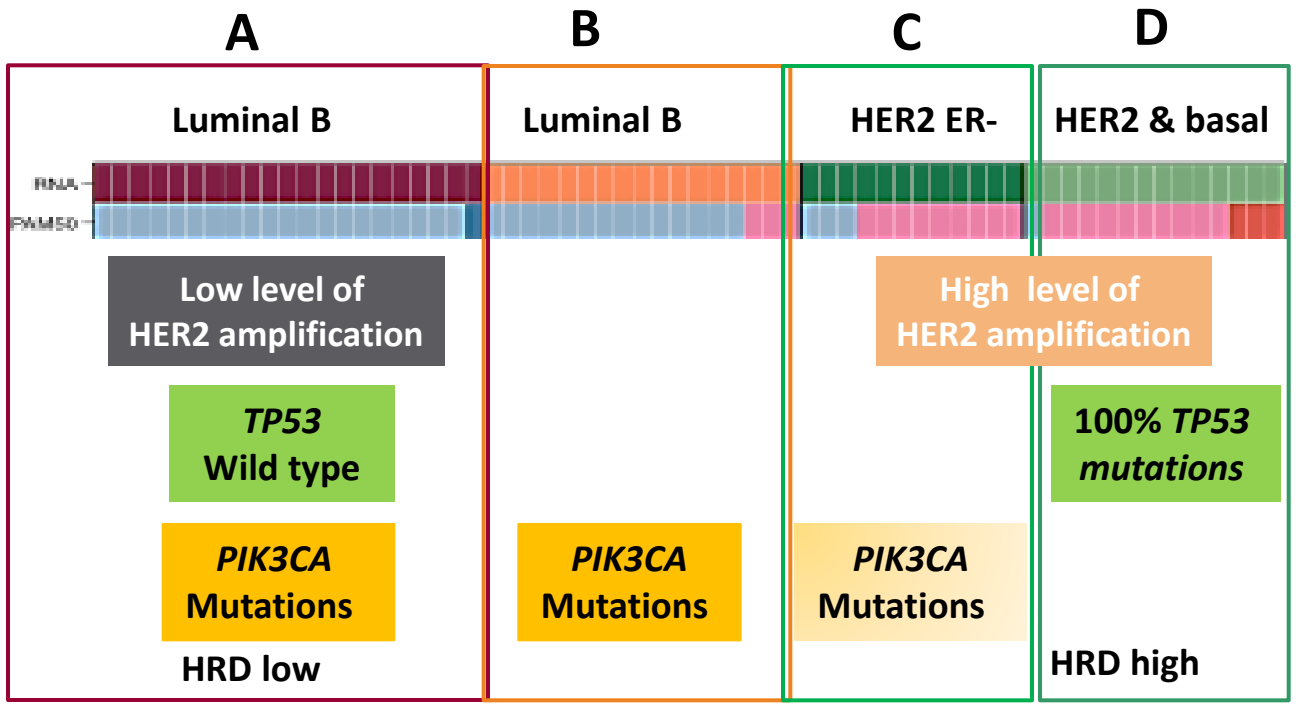
IC5

Curtis et al. Nature 2012; Ali et al Genome Bio, 2014

INTER-TUMOR MOLECULAR HETEROGENEITY OF HER2 TUMORS

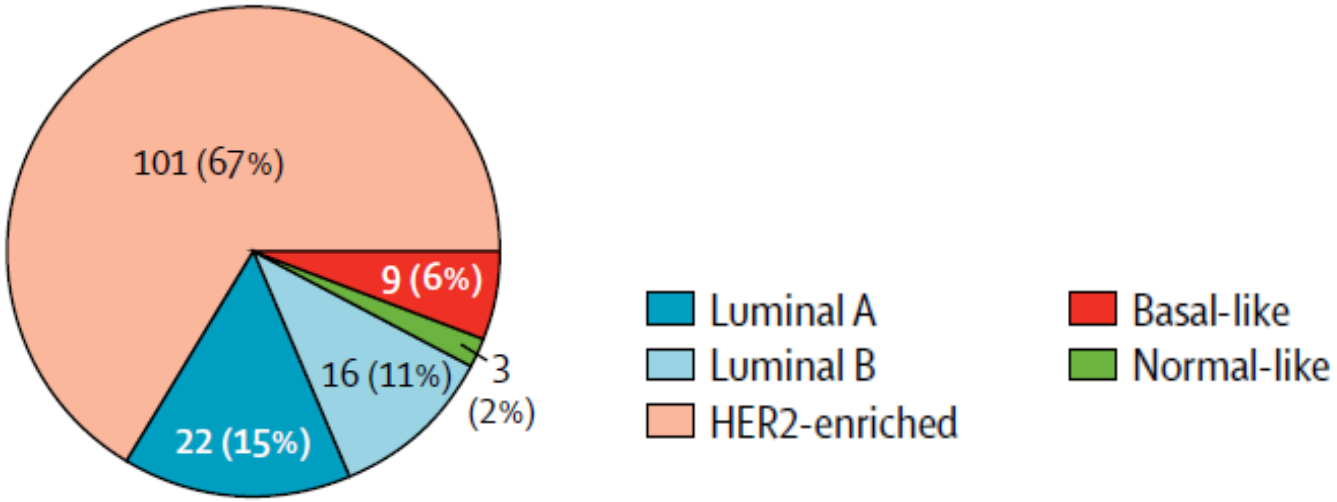
- 84 HER2+ breast carcinomas analyzed by WGS and RNA seq **(ICGC-France)**

RNAseq
WGS
WES



- Pamela trial
- 151 patients HER2 +

PAM50

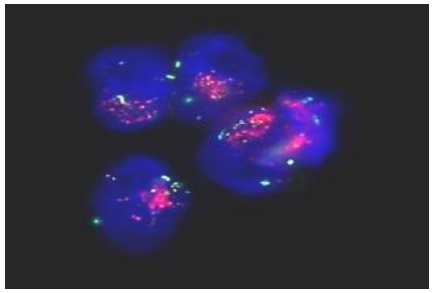


Ferrari et al, Nature Com 2016
Llombart-Cussac et al Lancet Oncol 2017

LEVELS OF EXPRESSION AND AMPLIFICATION:

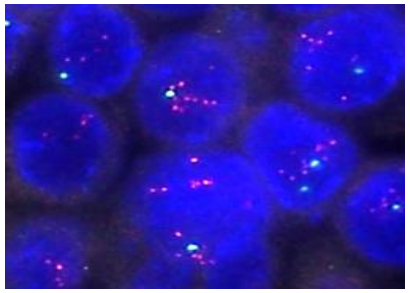
predictive markers of response to anti-Her2 therapies

- Retrospectively:



> 10 copies

56% of pCR



Low level of amplification: 6-10 copies

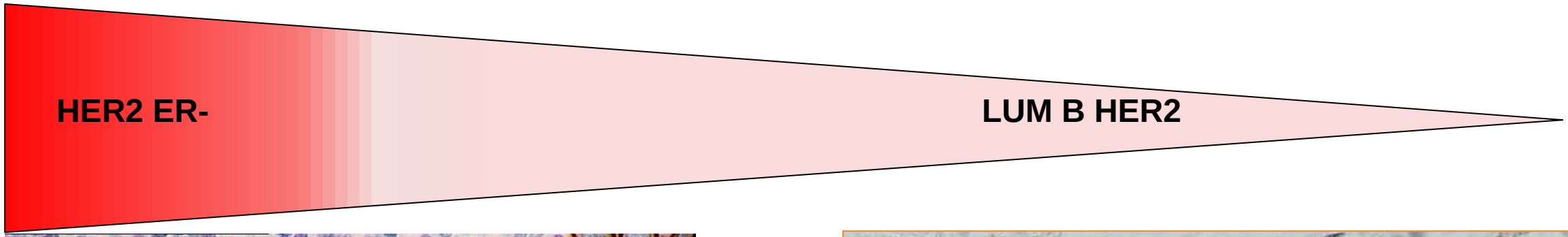
22% of pCR

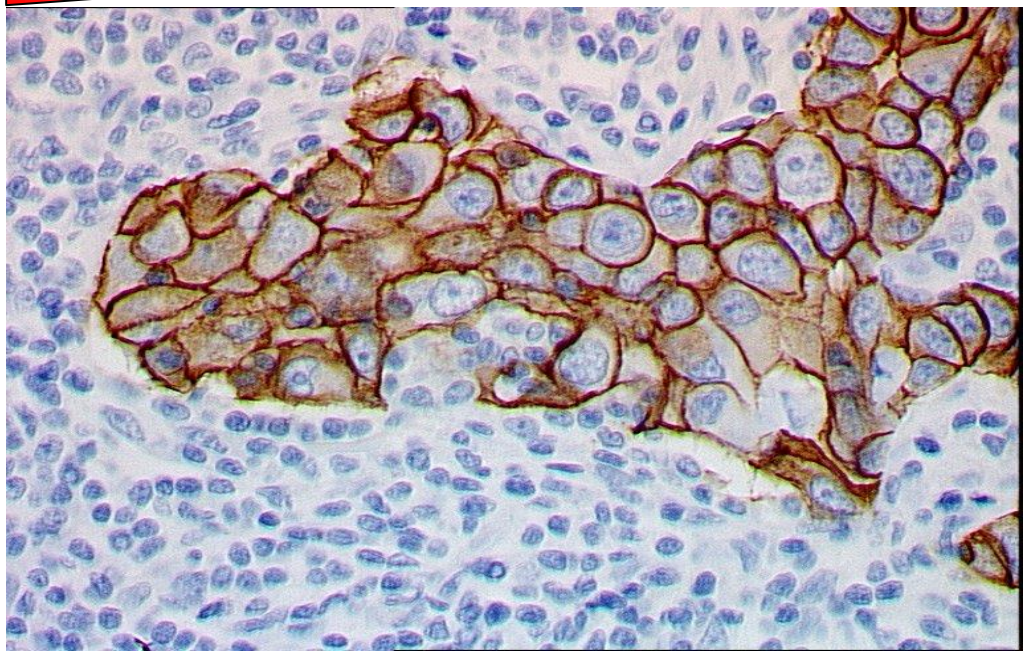
- Prospectively

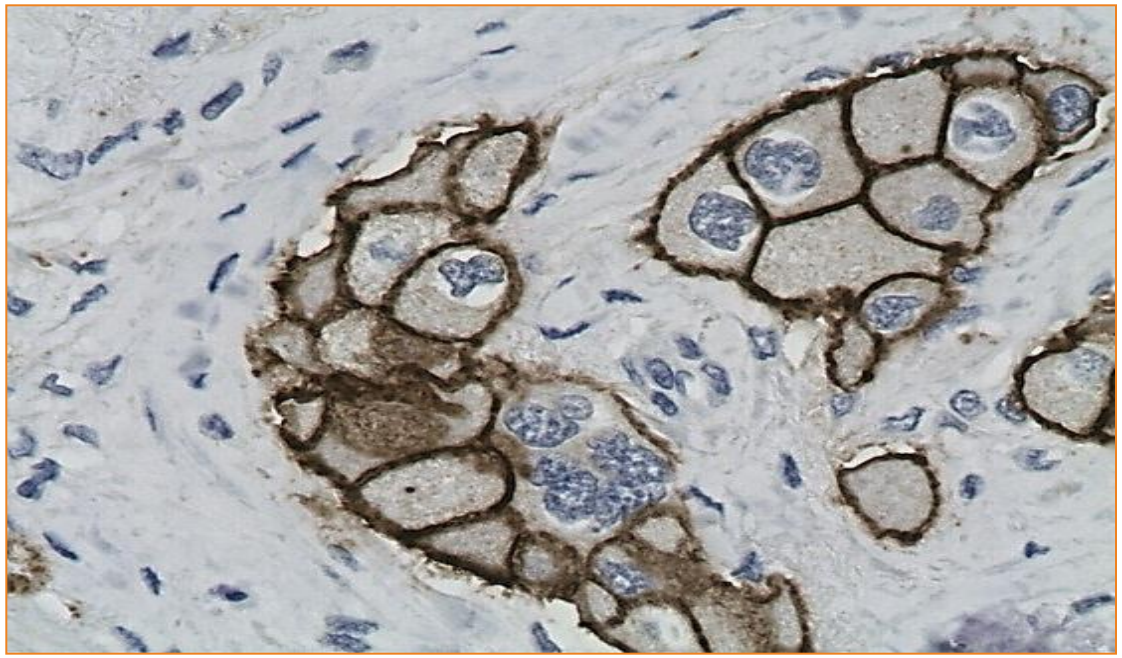
Neoadjuvant setting				
TAXHERO1 and GETNA01 (REF. 31)	93	DH or DCbH	FISH	Degree of *HER2* amplification by FISH is correlated with pCR (high versus low amplification: 56% versus 22%; $P<0.005$)
TRYPHAENA[28]	106	FECHP→DHP versus FEC→DHP TCbHP	IHC, mRNA	High HER2 mRNA and IHC score associated with a higher pCR rate (73.6% versus 51%, $P=0.0006$ and 72.3% versus 36.1%, $P=0.00002$)
GeparQuattro[29]	217	ECT→DH ECT→DXH	mRNA	High HER2 mRNA level associated with higher pCR rate, but only in ESR1+ tumours
BrUOG BR-211B[24]	27	Nab-T CbH	AQUA (HER2 prot)	Higher levels of HER2 protein predict pCR; phosphorylated HER2 not predictive of pCR
NeoALTTO[30]	324	L→LT H→HT LH→LHT	Protein expression (HERmark)	High HER2 protein expression associated with higher pCR rate and greater benefit from dual anti-HER2 therapy (OR 2.02; 1.42–2.87)

Arnould et al, Clin Can Res 2007; Jankowski, Br Can Res TTT 2017

TILS DENSITY: HIGHER IN ER- HER2 ENRICHED CARCINOMAS







Lal et al, Am J Clin Pathol 2005; Koneckny et al, JNCI 2003; Taucher et al, Cancer 2003
Huang et al, Annals of Oncology , 2005; Bartlett et al, JCO 2007; Vaz-Luis et al Annals of Oncol 2013; Toullec et al , EMBO Mol Medicine 2010; Staaf et al JCO 2010

HER2 POSITIVE BREAST CANCER WITH HIGH TILS: BETTER RESPONSE TO THERAPY

TILs

Immune transcriptomic signatures

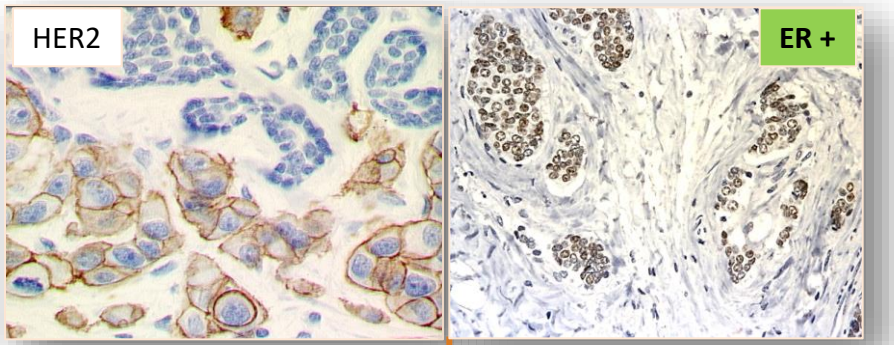
Table 4 | **Exploration of immune-related biomarkers in randomized trials in HER2-positive breast cancer**

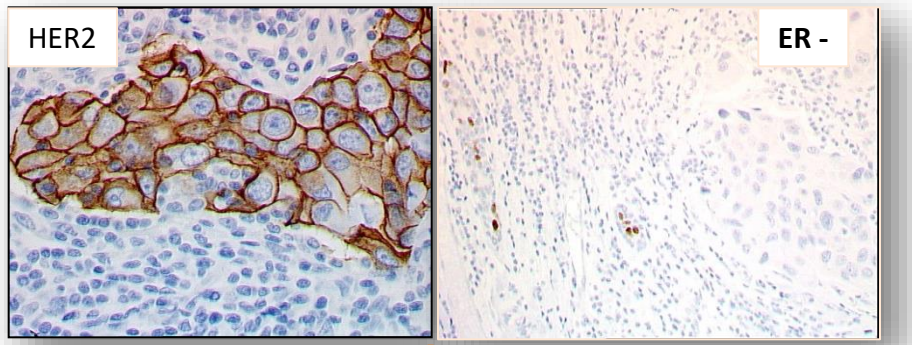
Trial	n	Biomarker	Conclusions
TILs			
GeparSixto (Neoadjuvant)[97]	580	TILs	• Higher TILs associated with increased pCR rate • Interaction with benefit from carboplatin
NeoSphere (Neoadjuvant)[98]	243	TILs	TILs not significantly associated with pCR in the breast
NeoALTTO (Neoadjuvant)[99]	387	TILs	• TILs >5% associated with increased pCR • Prognostic: every 1% increase in TILs associated with a 3% decrease in the rate of an event (HR 0.97, 95% CI 0.95–0.99, $P = 0.002$)
FinHER (Adjuvant)[95]	934	TILs	• Prognostic: higher TILs associated with better distant disease-free survival • Predictive: patients with LPBC benefit the most from trastuzumab
N9831 (Adjuvant)[100]	945	TILs	• Prognostic: higher TILs associated with better RFS, but only in the chemotherapy_alone group • Predictive: (P interaction 0.03) Patients with LPBC do not benefit from trastuzumab (HR 2.43, 95% CI 0.58–10.22)
CLEOPATRA (metastatic)[101]	678	TILs	Prognostic: strongly associated with overall survival
Gene-expression signatures			
Pooled analysis neoadjuvant studies[102]	996	Immune GES	Immune-gene enrichment associated with increased pCR probability
GeparSixto[97]	580	Immune GES	Higher expression of immune markers associated with increased pCR rate
NeoSphere[98]		Expression of *IFNG*, *PD1*, *PDL1*, *PDL2*, *CTLA4* or immune metagenes related to plasma cells, T cells, antigen-presenting cells 1 and 2, STAT1, IF-I	In the dual anti-HER2 therapy arm, low expression of PD1, STAT1, PDL1, CTLA4, MHC1 and IF-1 associated with higher pCR rate in the breast. In the other arms, high expression of PD1, MHC2, STAT1, but low expression of PDL1, MHC1 and IF-I associated with higher pCR rate in the breast
N9831 (REF. 103)	1,282	Immune GES	• Prognostic: immune-gene enrichment associated with better RFS • Predictive: patient without immune-gene enrichment did not benefit from trastuzumab
NSABP-B31 (REF. 104)	731	TILs-associated GES	Predictive: high expression of TILs-associated genes correlated with more benefit from trastuzumab (HR 0.06 versus 0.57, $P_{interaction} = 0.03$)
FinHER (REF. 105)	202	pSTAT3 GES	Predictive: high expression associated with lack of benefit from trastuzumab in ER-negative patients
FinHER (REF. 106)	232	ANXA1 metagene	Predictive: high expression associated with lack of benefit from trastuzumab

Abbreviations: ER, oestrogen receptor; GES, gene-expression signatures; HR, hazard ratio; LPBC, lymphocyte-predominant breast cancer; MHC1, major histocompatibility complex class 1; pCR, pathological complete response; RFS, relapse-free survival; TILs, tumour-infiltrating lymphocytes.

Lal et al, Am J Clin Pathol 2005; Koneckny et al, JNCI 2003; Taucher et al, Cancer 2003; Huang et al, Annals of Oncology , 2005; Bartlett et al, JCO 2007; Vaz-Luis et al Annals of Oncol 2013; Toullec et al , EMBO Mol Medicine 2010; Staaf et al JCO 2010

HER2 TUMORS: DIFFERENT MOLECULAR ENTITIES





ER+ LumB
HER2 low level of amplification

PIK3CA mutation
TP53 WT

PIK3CA
WT

ER –
HER2 high level of amplification

PIK3CA
mutation

TP53 Mutation
HRD

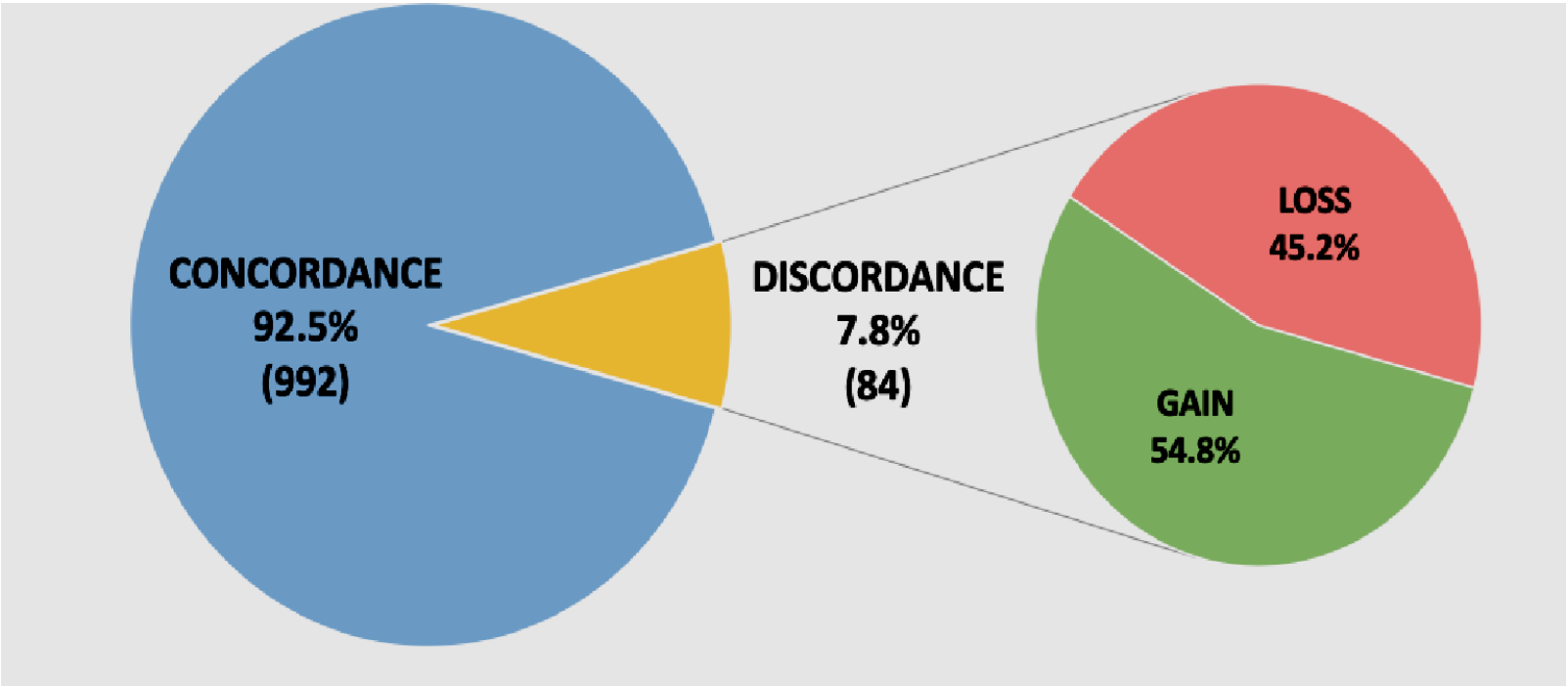
Epithelial cells

TILS
Cancer Associated Fibroblasts (CAF S4)

Stroma

Costa et al, Cancer Cell 2018
Pelon et al Nat Com 2020

HER2 POSITIVE STATUS CHANGE FROM PRIMARY TUMOR TO METASTASIS



Grinda et al NPJ Breast cancer 2021

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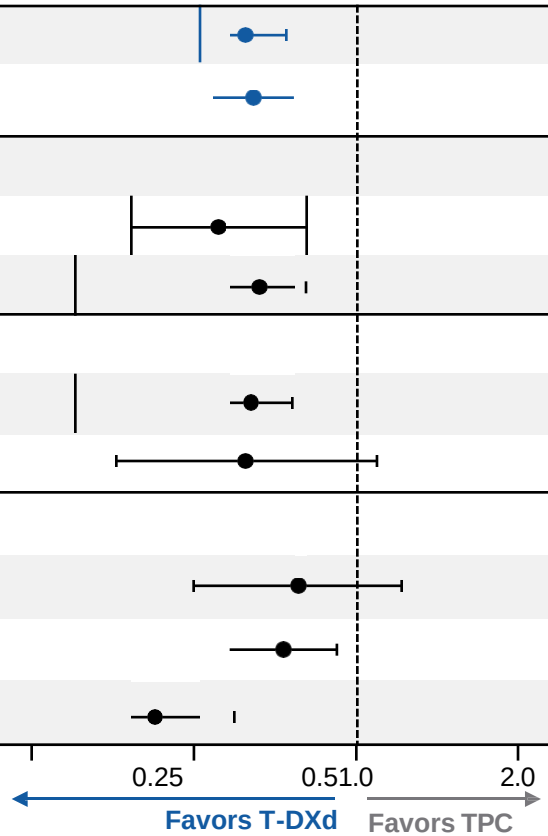
Antibodies drug-conjugated for HER2-low breast cancer

Trials In metastatic phase with visceral and cerebral meta	Patients	Overal survival TDxD	Median survival TDM1	Median overall survival Treatment of clinician's choice
Destiny 03	HER2 positive (11% HER2 low)	28,8 months IC (22,4-37,9) (PFS)	6,8 months IC (5,6-8,2) (PFS)	
Destiny 04	HER2 low, majority of HR+	23,4 months (overall survival)		16.8 months (Overall survival)

Cortes *et al* NEJM 2022; Hurvitz *et al* ,Lancet 2023; 401: 105–17; Tarantino *et al,* Annals of Oncology, 2023, Mosele *et al* Nature Medicine 2023

DESTINY-Breast06: PFS (BICR) in ITT by tumor sample characteristics and IHC score

Subgroup	Number of events / patients (%)		Median, months (95% CI)		Hazard ratio (95% CI)
	T-DXd	TPC	T-DXd	TPC	
HER2-low (primary endpoint)	225/359 (62.7)	232/354 (65.5)	13.2 (11.4, 15.2)	8.1 (7.0, 9.0)	0.62 (0.51, 0.74)
ITT (ie HER2-low and HER2-ultralow) (secondary endpoint)	269/436 (61.7)	271/430 (63.0)	13.2 (12.0, 15.2)	8.1 (7.0, 9.0)	0.63 (0.53, 0.75)
Tumor location*					
Primary	55/93 (59.1)	63/99 (63.6)	14.9 (9.8, 19.4)	7.9 (5.8, 9.7)	0.55 (0.38, 0.80)
Metastatic	214/343 (62.4)	208/331 (62.8)	13.2 (12.0, 15.2)	8.1 (7.0, 9.5)	0.66 (0.55, 0.80)
Specimen collection type					
Biopsy	232/375 (61.9)	249/389 (64.0)	13.1 (11.3, 15.2)	8.1 (6.9, 9.3)	0.63 (0.53, 0.76)
Excision/resection	37/61 (60.7)	22/41 (53.7)	16.4 (9.7, 19.5)	8.3 (6.9, 18.1)	0.62 (0.36, 1.08)
HER2 IHC expression					
IHC 0 with membrane staining	44/76 (57.9)	39/76 (51.3)	13.2 (9.8, 17.3)	8.3 (5.8, 15.2)	0.78 (0.50, 1.21)
IHC 1+	157/239 (65.7)	150/234 (64.1)	13.1 (11.0, 15.2)	8.2 (7.1, 9.8)	0.73 (0.59, 0.92)
IHC 2+/ISH−	65/117 (55.6)	80/118 (67.8)	15.2 (12.2, 21.4)	7.0 (6.2, 8.4)	0.43 (0.31, 0.60)



*Primary tumor samples were breast or regional lymph node samples obtained from patients who had confirmed metastatic disease (ie in the metastatic setting)
BICR, blinded independent central review; CI, confidence interval; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH−, in situ hybridization–negative; ITT, intent-to-treat; PFS, progression-free survival; T-DXd, trastuzumab deruxtecan; TPC, chemotherapy treatment of physician's choice

Adapted from Giuseppe Viale, ESMO 2024

Now, HER2 status interpretation

The 2023 ASCO/CAP update of HER2 testing guidelines provides best practice recommendations for the distinction of HER2 0 from 1+ including evaluation of HER2 IHC at **high-power magnification +** seeking consensus review when needed.

→ It takes time ++++ to read

- Intensity **and** the exact % of labelled cells
- Complete or incomplete membranous staining
- (Positive cells scattered or clustered)

Wolff et al ASCO/CAP 2023

2023 ESMO Consensus for HER2 status assessment

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

SPECIAL ARTICLE

ESMO expert consensus statements (ECS) on the definition, diagnosis, and management of HER2-low breast cancer

P. Tarantino[1,2,3], G. Viale[4], M. F. Press[5], X. Hu[6], F. Penault-Llorca[7], A. Bardia[2,8], A. Batistatou[9], H. J. Burstein[1,2], L. A. Carey[10], J. Cortes[11,12], C. Denkert[13], V. Diéras[14], W. Jacot[15], A. K. Koutras[16], A. Lebeau[17], S. Loibl[18,19], S. Modi[20], M. F. Mosele[21], E. Provenzano[22], G. Pruneri[3,23], J. S. Reis-Filho[24], F. Rojo[25], R. Salgado[26,27], P. Schmid[28], S. J. Schnitt[2,29], S. M. Tolaney[1,2], D. Trapani[3,30], A. Vincent-Salomon[31], A. C. Wolff[32], G. Pentheroudakis[33], F. André[34] & G. Curigliano[3,30]*

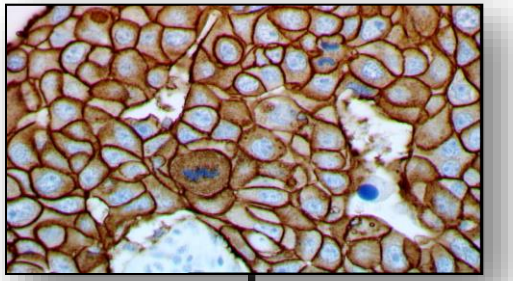
Table 1. Interpretation by the ASCO/CAP 2018 Guidelines and by the 2023 ESMO Consensus on HER2-low breast cancer regarding each pattern of HER2 staining

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**

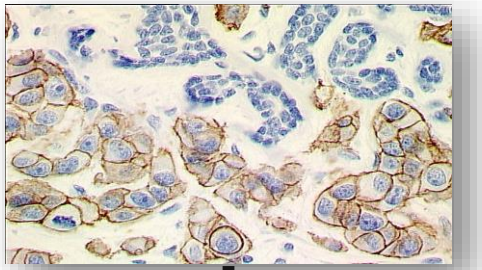
ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; ISH, *in situ* hybridization.
Bold are the actual definitions. In italics are potential future sub-definitions within the HER2-0 category.
[a]The decision to establish the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

HER2: predictive marker for anti–HER2 therapies Trastuzumab, and ADC

Score 3+

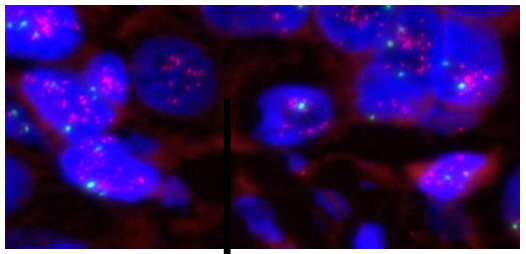


Score 2+

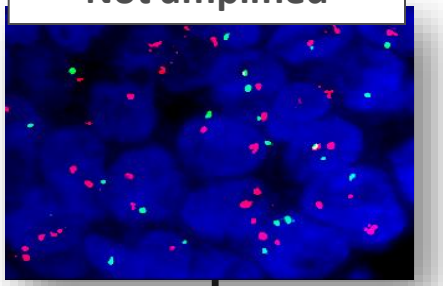


In situ hybridization

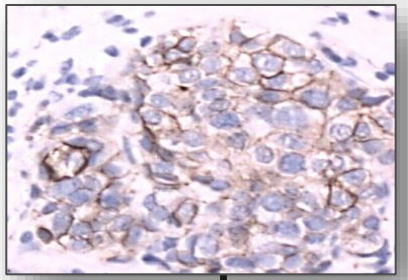
Amplified



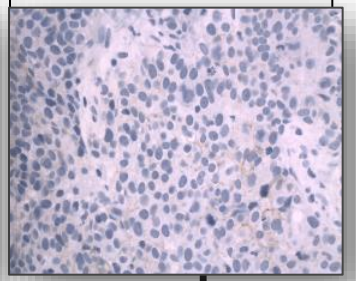
Not amplified



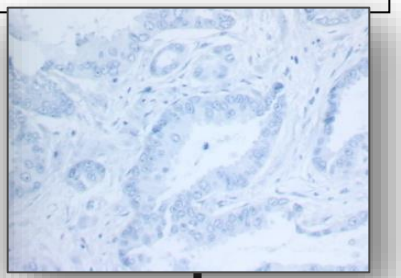
Score 1+



Score 0
ultra-low
1-10% cells +



Score 0 NULL
0% of membranous staining



Signalling pathway activated by *HER2 gene* amplification

Detection of HER2 expression on the surface of tumour cells

HER2 positive carcinomas

HER2 low carcinomas

HER2 Ultra-low carcinomas

HER2 null carcinomas

Identifying ULTRA-LOW scores: DESTINY06 experience

- Of samples scored as HER2-low locally, 94% met DESTINY-Breast06 inclusion criteria
  (were either HER2-low or HER2-ultralow by central testing)

- Overall percent agreement was 77.8% for HER2-low*

- Of samples scored as IHC 0 locally, central testing found
  - ❖ 35% were IHC 0 (absent membrane staining; 0% of stained cells)
  - ❖ 40% were HER2-ultralow
  - ❖ 24% were HER2-low

*Agreement was assessed between central and local laboratories determining if samples were 'HER2-low' or 'not HER2-low' and overall percent agreement was calculated as the total number of samples that agreed divided by the total number of tests.

Frequency of low and ultra-low HER2 tumors

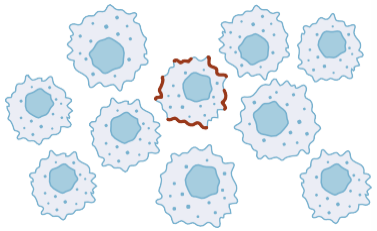
HER2 Low
Score 2+ non-amplified & Score 1+

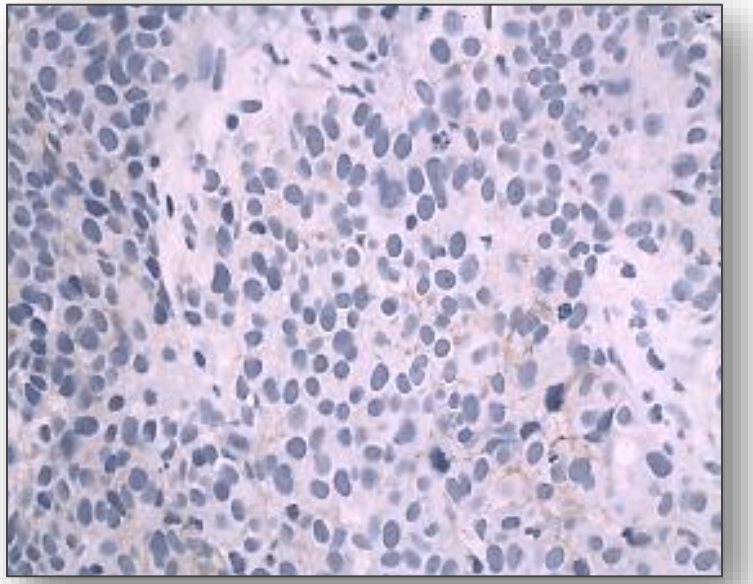
60-65% of all breast carcinomas

- Most HER2 low are HER2 score 1+
- HER2 low are: 64% HR+ 36% HR -

HER2 ULTRA- low
Score 0 with 1-10% + cells

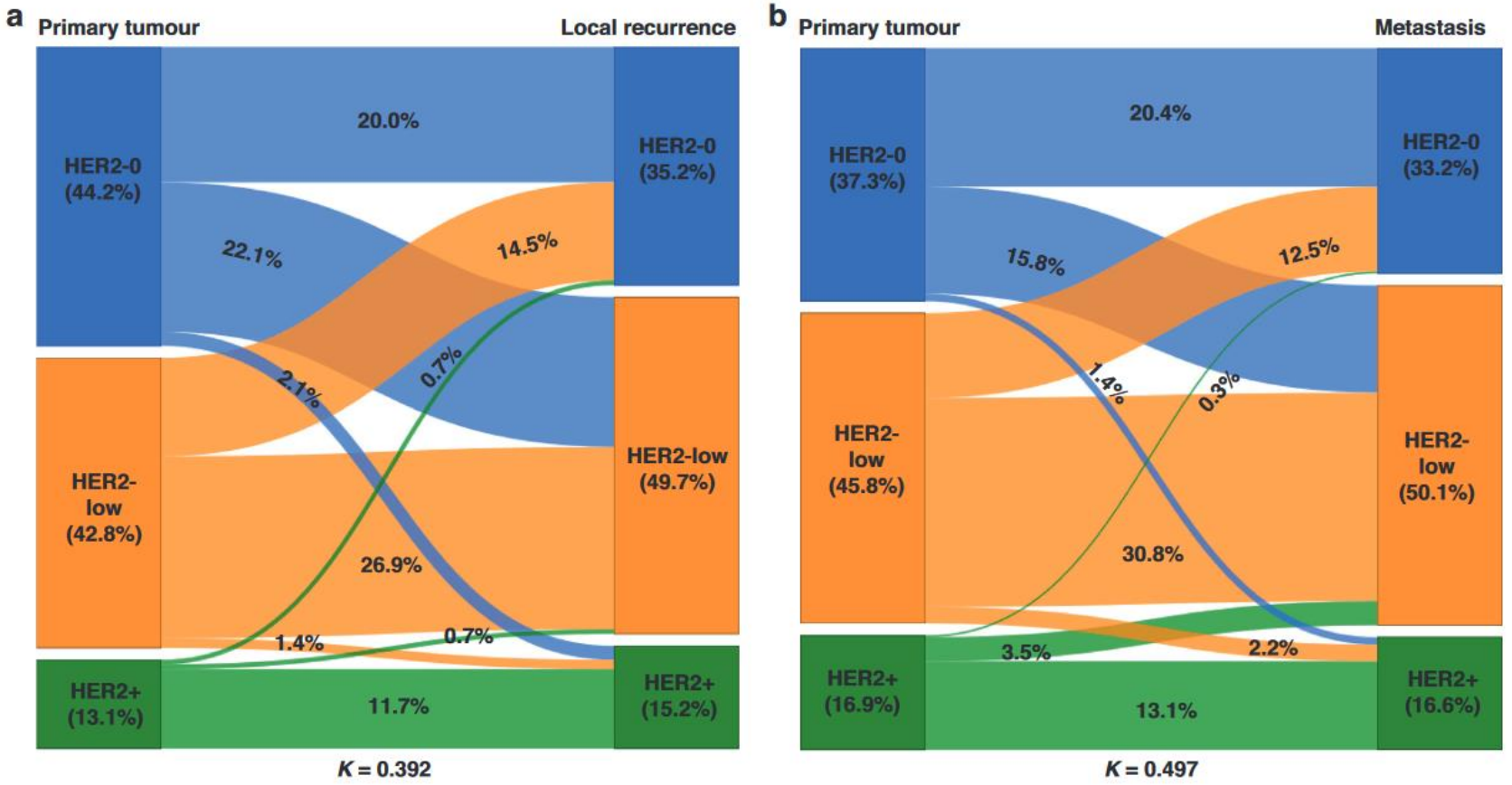
20-25% of all breast carcinomas





Tarantino et al JCO 2020; Denkert et al Lancet Oncol 2021; Modi et al NEJM 2020 Destiny 01; Viale ESMO 2024

HER2 status varies between primary tumour and relapse: local relapse & metastatic disease and between different metastatic sites of the same patient

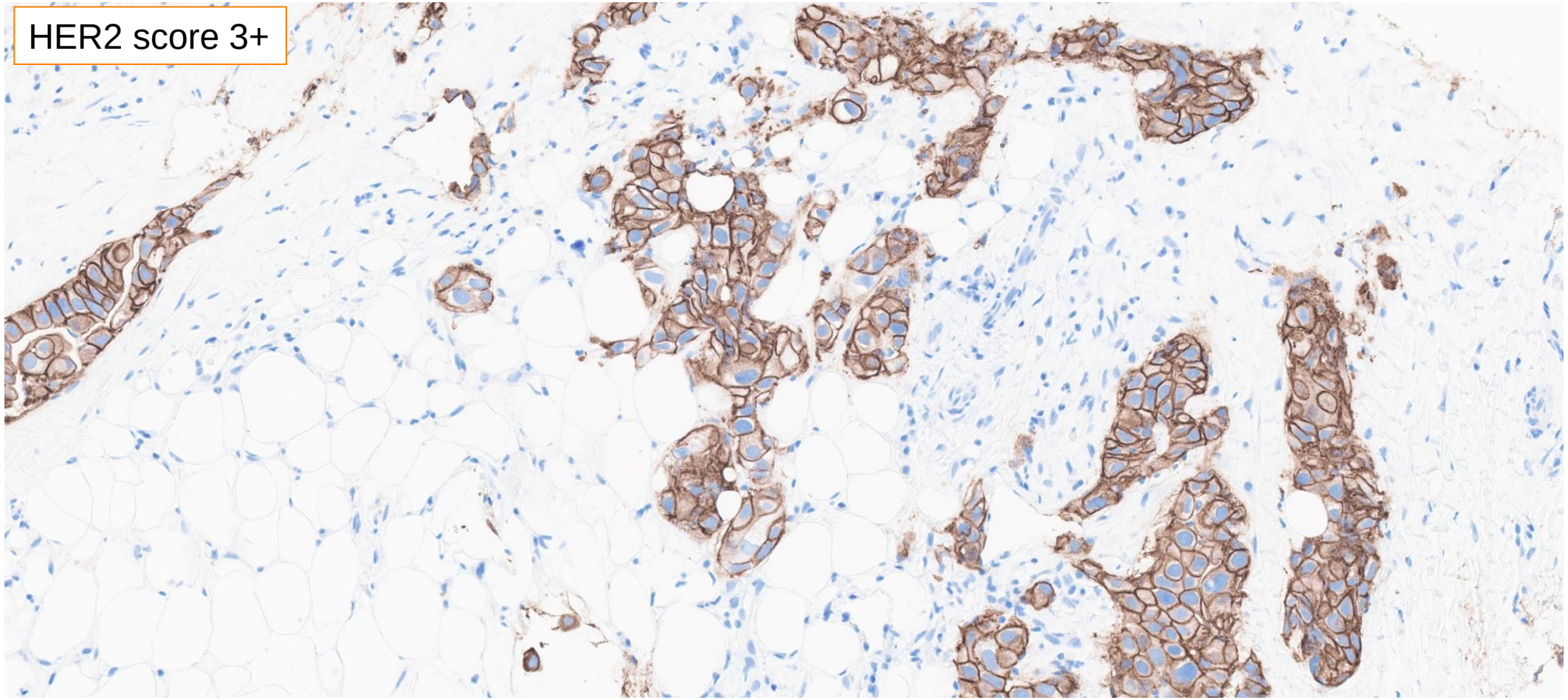


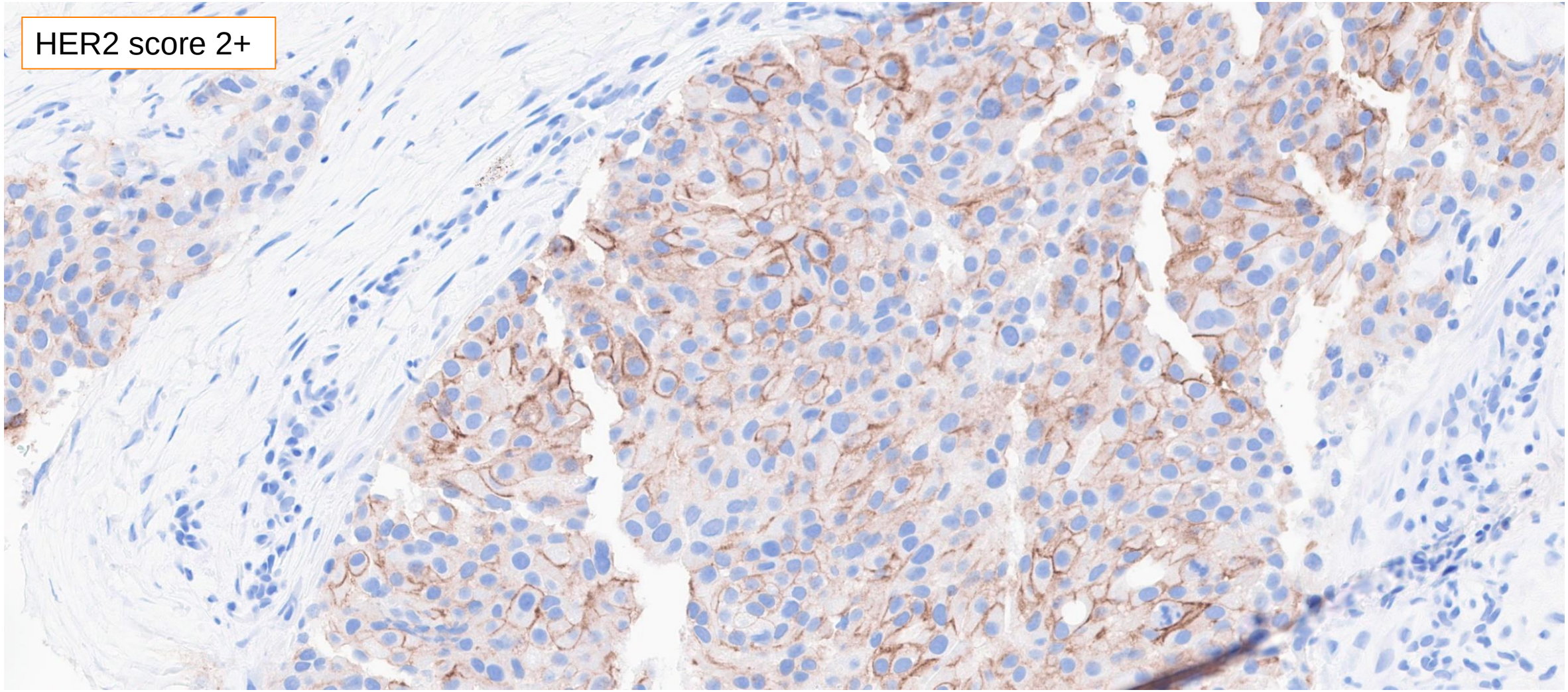
37.5% change in status

41.4% to 47 % change in status

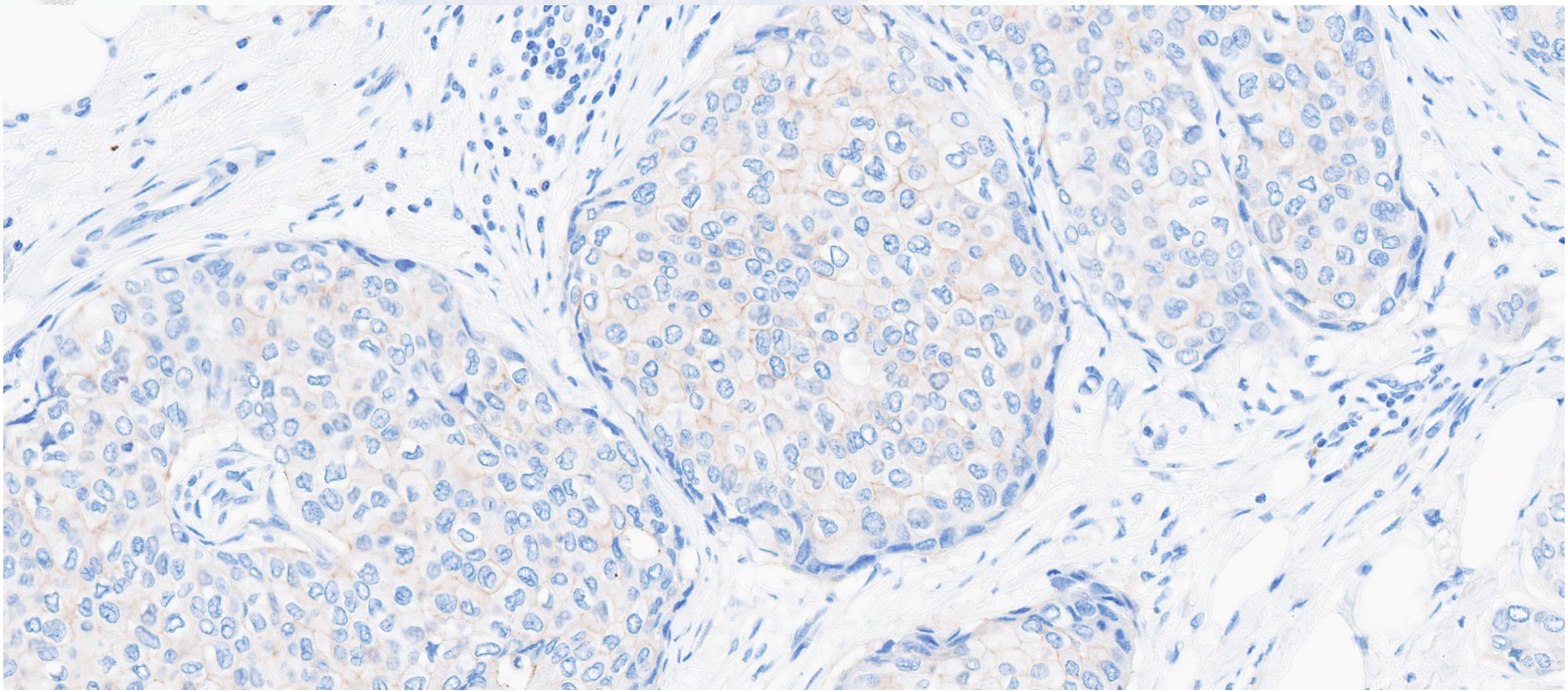
"Among different metastatic sites from the same breast primary, HER2 status was **discordant between distant metastatic sites in 53% of patients"**

Bergeron *et al* BJ cancer 2023; Yang *et al* Histopathology 2024


HER2 score 3+


HER2 score 2+

HER2 score 1+



Outlines of my talk

1. Introduction :
   a. *HER2* oncogene
   b. HER2 status assessment according to guidelines (ASCO / CAP 2018)
   c. HER2 positive tumors: are not a single entity

2. Definition and epidemiology of HER2 low and ultra-low breast cancers

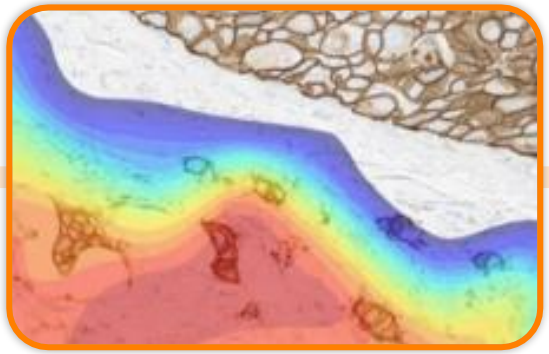
3. AI assessment of HER2 status in breast cancers

4. Take home messages

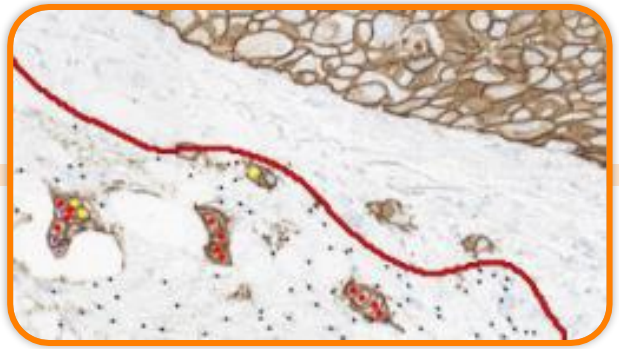
Multi-Site European Study of a HER2 AI Solution as Clinical Decision-Support Tool in Breast Cancer

AI Solution for Evaluation of HER2 Immunostain: IBEX Breast HER2

AI to detect areas of invasive carcinoma



AI to analyze cell by cell staining



Detection of intensity and quality (complete / incomplete) of the staining

2018 ASCO/CAP guidelines

HER2 score

Globerson *et al.* SABCS 2022

Heatmaps

Cells
- Intense incomplete
- Intense complete
- Moderate incomplete
- Moderate complete
- Faint incomplete
- Not stained
- Not invasive
- Artifact / Unclassified

Area Contours
- Invasive AOI
- Control


Heatmaps
Cells
Intense incomplete
Intense complete
Moderate incomplete
Moderate complete
Faint incomplete
Not stained
Not invasive
Artifact / Unclassified
Area Contours
Invasive AOI
Control
16.0x
Active Heatmap: HER2 Score

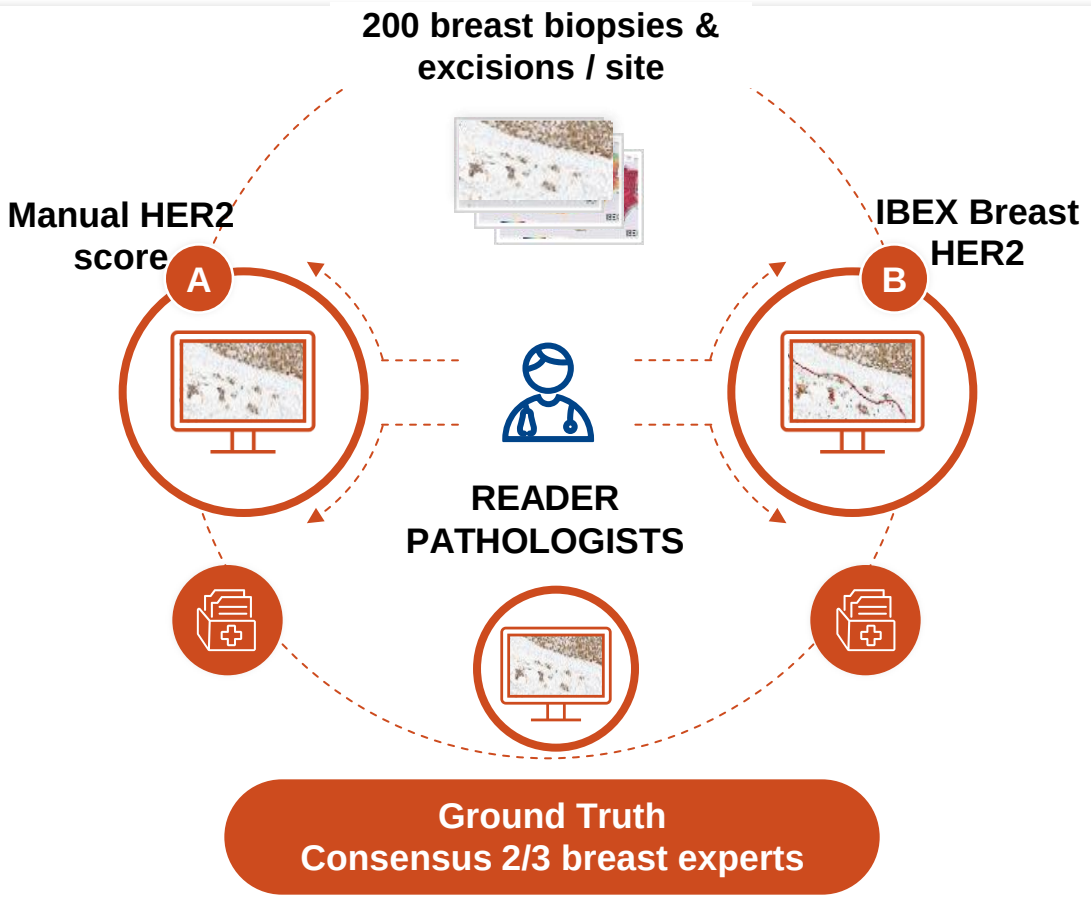
Study Cohort

- 969 consecutive invasive carcinoma cases from **6 European labs**
  - IDC - 700 slides (72.2%)
  - ILC - 120 slides (12.4%),
  - IDC+ILC - 17 slides (1.8%)
  - Other invasive - 132 slides (13.6%)
- Age: 63.4 years (± 14.8)
- 83% biopsies, 17% excisions
- Typical distribution of HER2 scores (15-20% HER2 positive, 80-85% HER2 negative)

Score	# slides	%
0	316	33%
1+	314	32%
2+	203	21%
3+	136	14%

Multi-Reader Study Design

- Ground truth (GT) set by **18 breast expert pathologists**
- Two parallel arms with a crossover design
- **12 reader pathologists from 6 different labs** interpreting HER2 IHCs :
  - without AI (Arm A)
  - And with AI (Arm B)



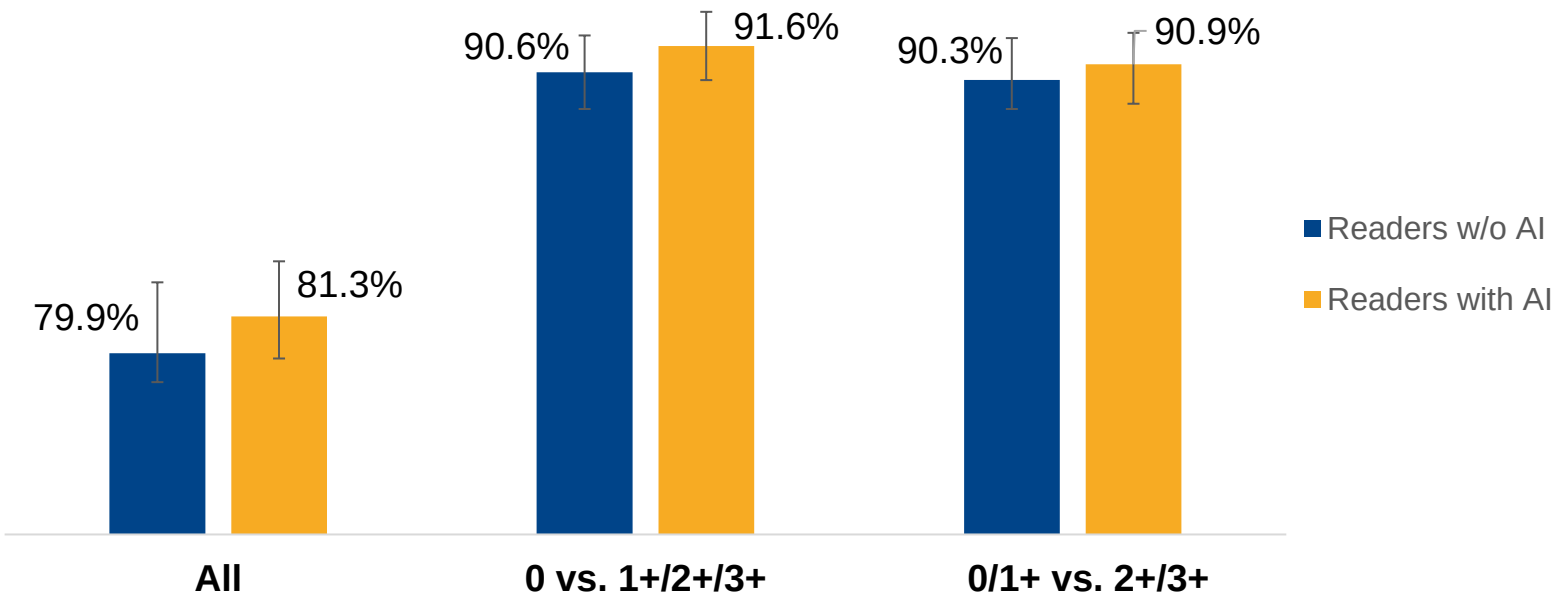
Pathologists' Agreement per HER2 score

% agreements (Weighted Mean Agreement)

Score	N slides	Experts' Agreement	Readers' Agreements
0	267	**85.6% (82.5%, 88.2%)**	**88.0% (83.6%, 88.2%)**
1+	359	72.5% (69.3%, 75.4%)	65.7% (60.7%, 70.5%)
2+	169	66.3% (61.3%, 71%)	65.7% (58.2%, 72.4%)
3+	128	**95.2% (92%, 97.2%)**	**89.8% (83.4%, 94%)**

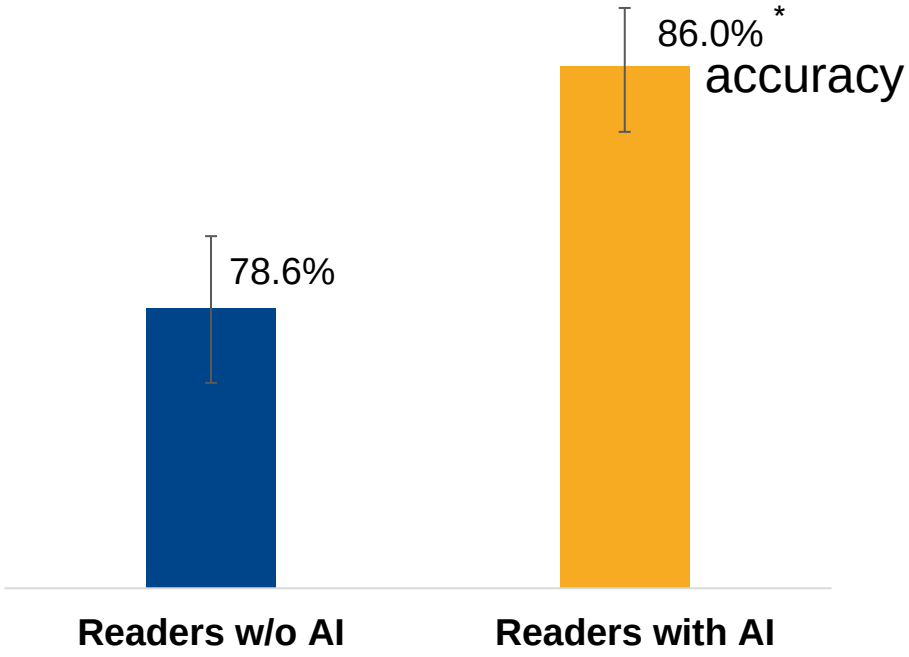
Reader Pathologists Accuracy without / with AI for all HER2 cut-offs

Readers agreement with experts' GT was higher with AI, for all HER2 cut-offs



Reader Pathologists Accuracy for HER2 0/1+ slides

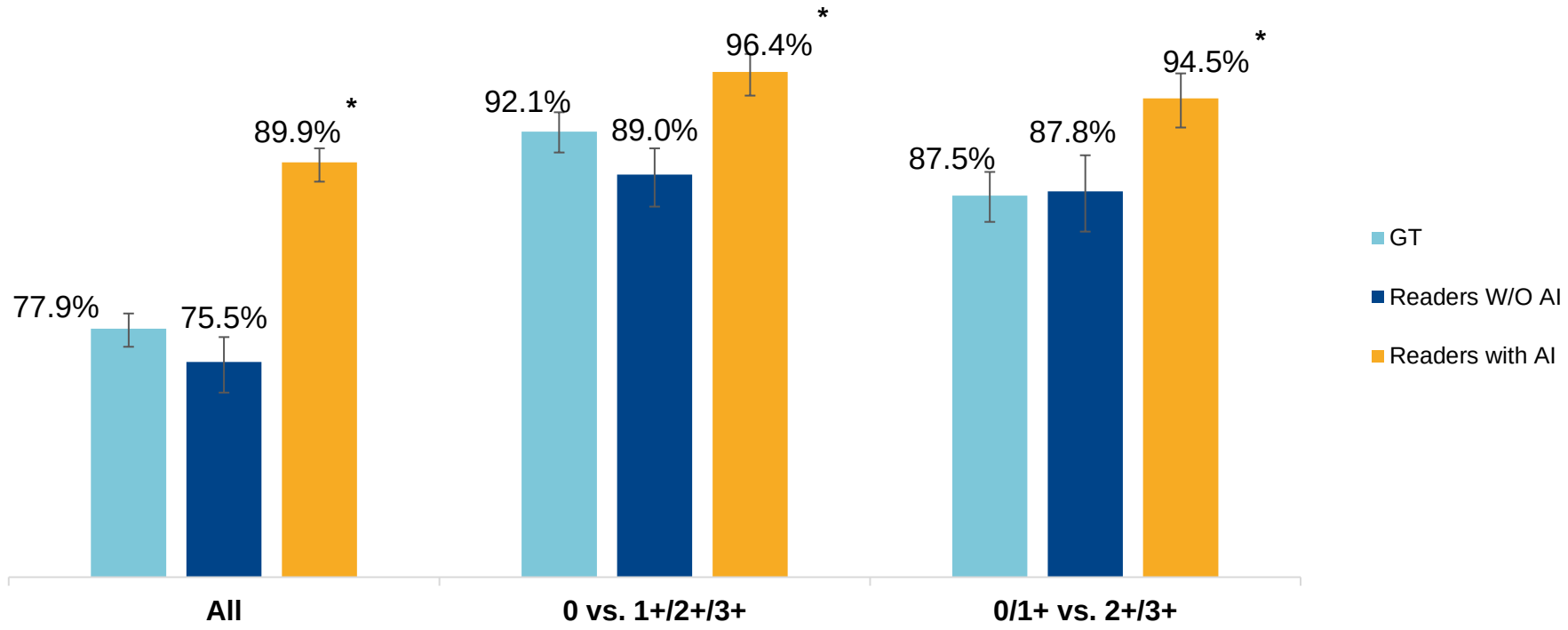
Readers with AI showed **7.4% improvement** in agreement with experts' GT for HER2 0/1+ slides



N=641 slides **p-value*<.05

Readers' Inter-Observer Agreement with and without AI

Readers with AI showed significantly higher inter-observer agreement



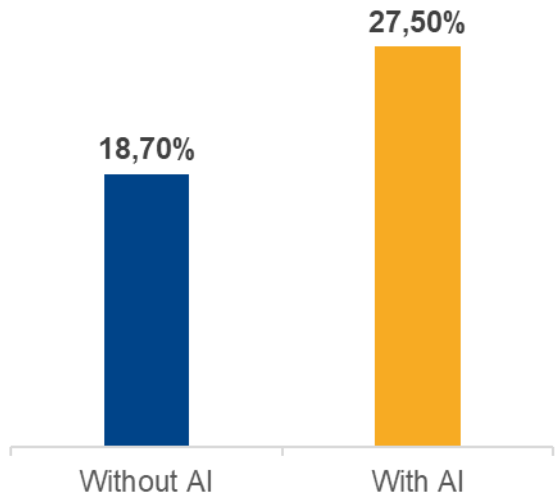
**p-value*<.05

HER2 2+ cases and FISH Tests

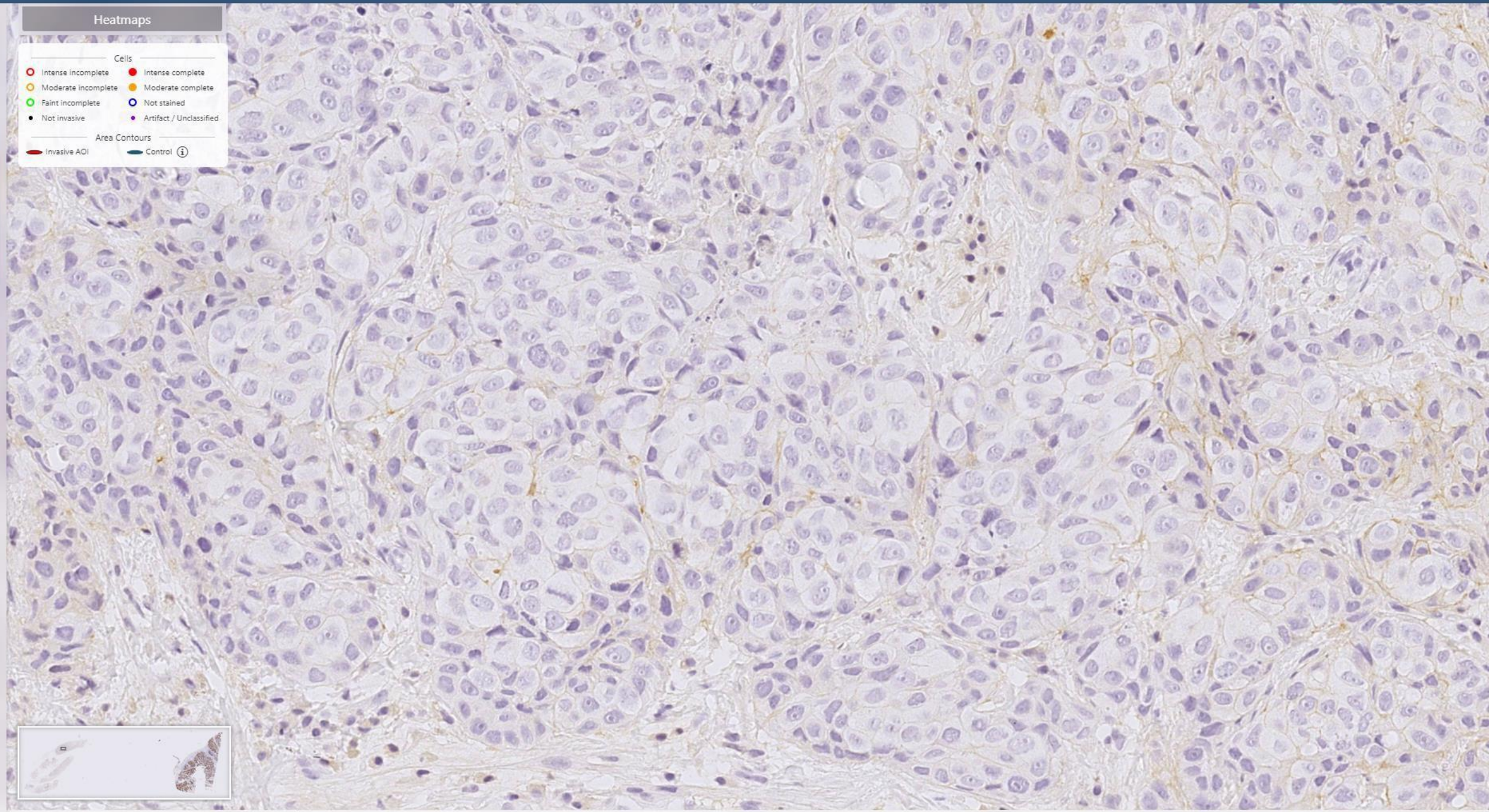
Rate of HER2 1+ and 2+ scored by readers without and with AI

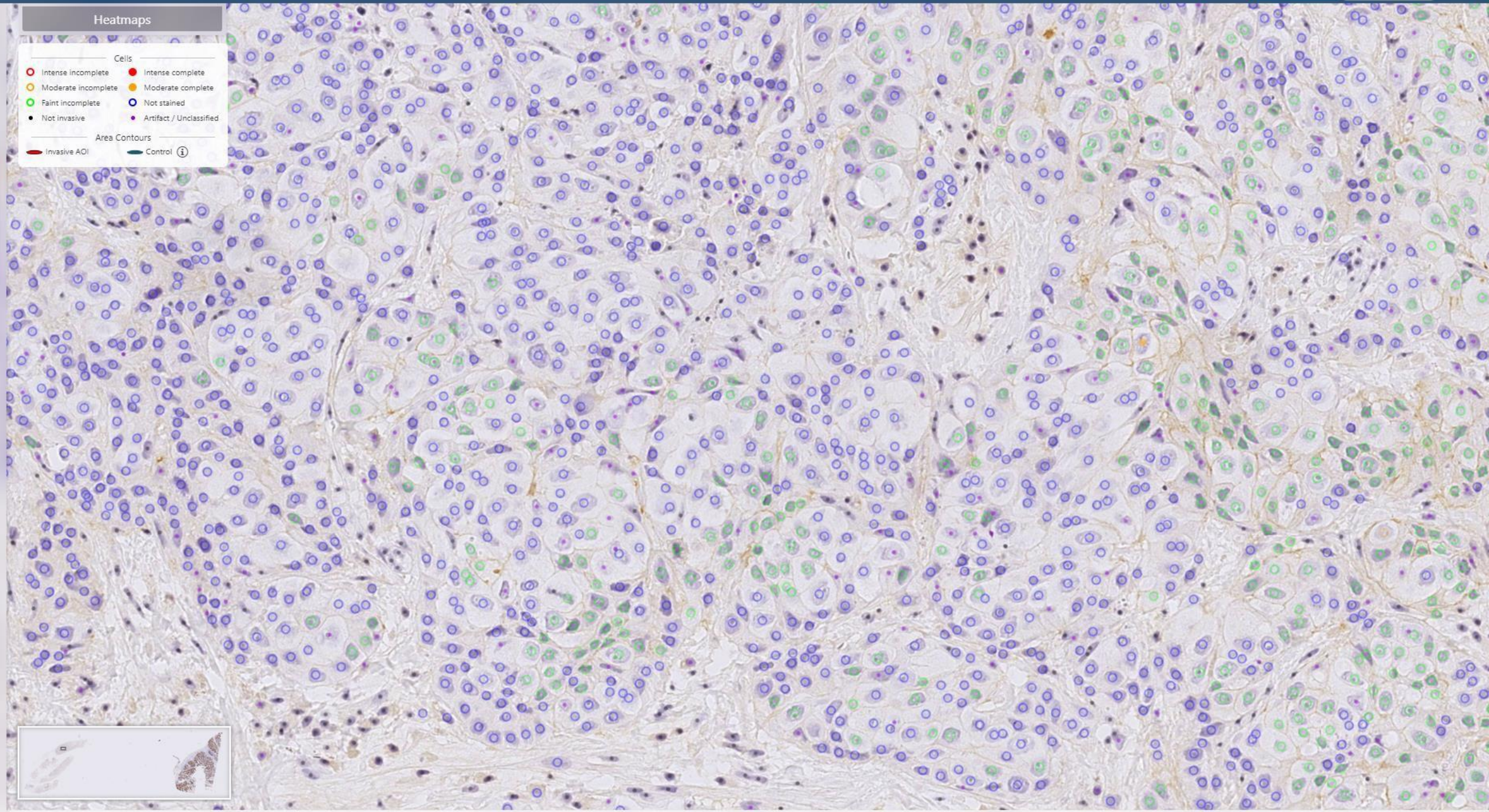
	1+ cases	2+ cases
Without AI	36.2%	17.2%
With AI	44.8%	10.1%
Change	**+8.6%**	**-7.1%**

Percentage of FISH positive cases from cases scored by readers as 2+



A decrease of 41% in the required number of FISH tests


Heatmaps
Cells
Intense incomplete
Intense complete
Moderate incomplete
Moderate complete
Faint incomplete
Not stained
Not invasive
Artifact / Unclassified
Area Contours
Invasive AOI
Control
20.0x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Active Heatmap: HER2 Score

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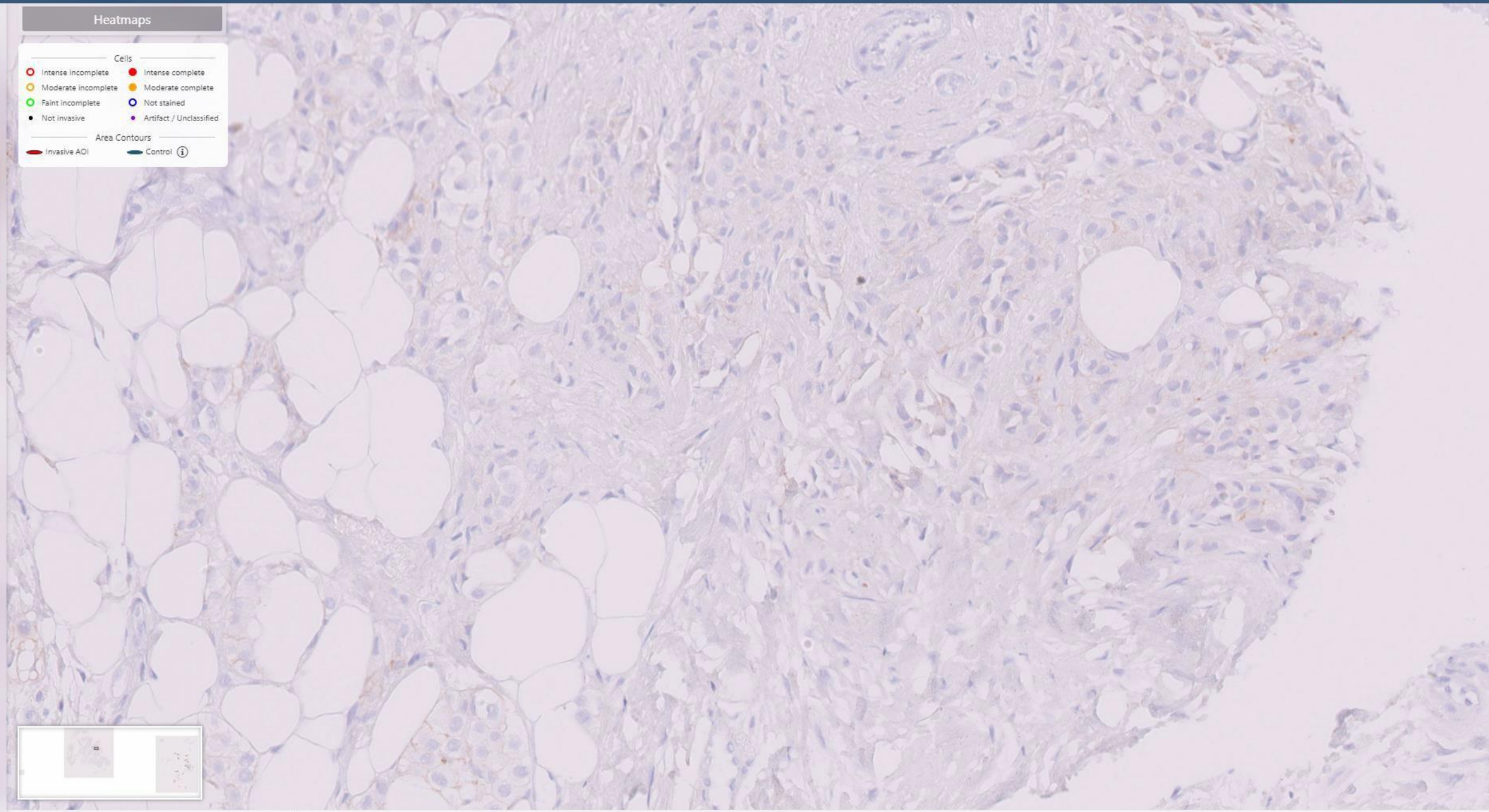

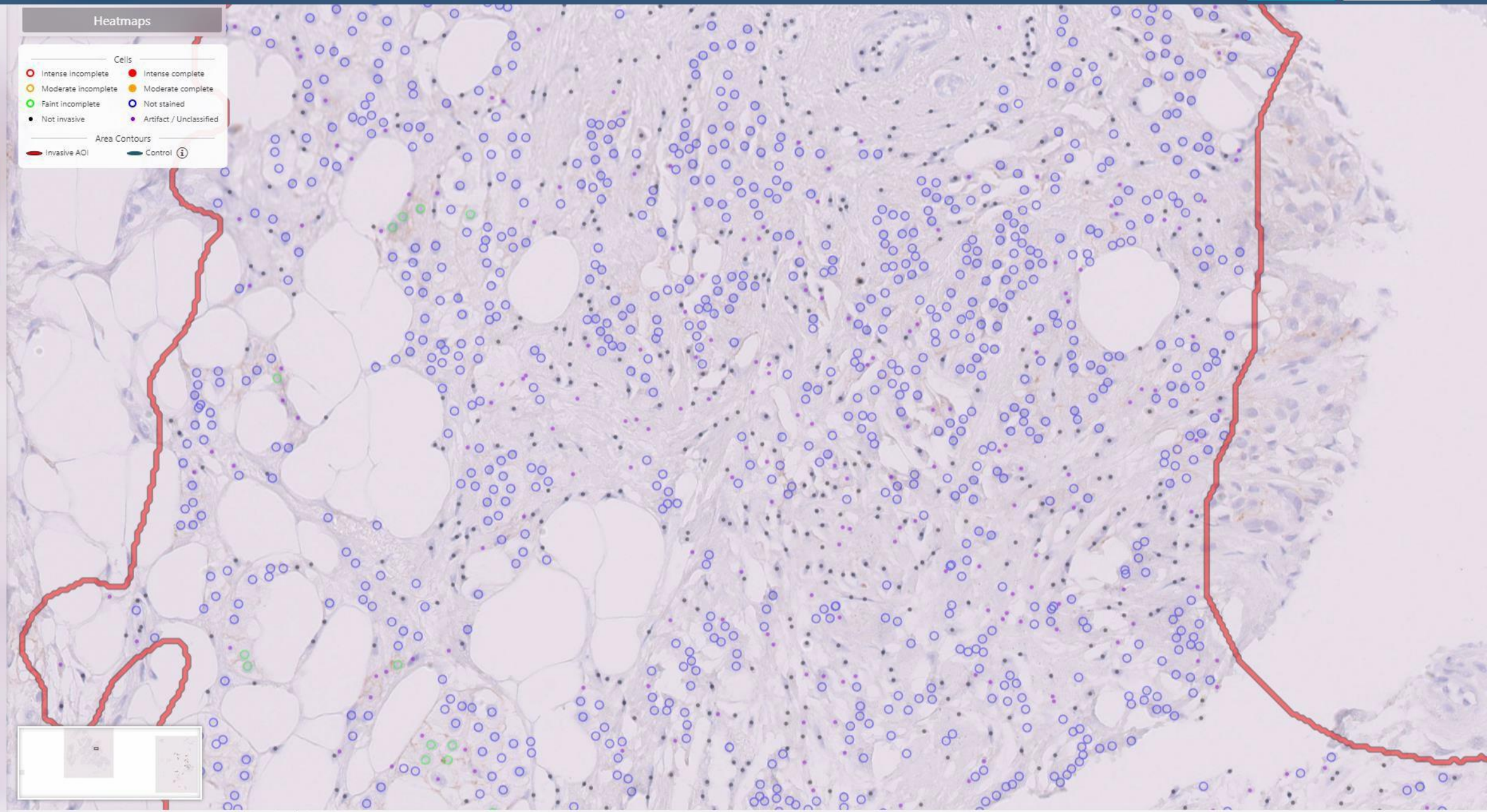
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Area Contours
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20.0x
Active Heatmap: HER2 Score

Conclusions

- This study reports an independent multi-site validation of a fully automated AI solution for HER2 scoring in breast cancer

Pathologists supported by AI showed:

- **Improved HER2 scoring consistency and accuracy, specifically for differentiating between 0 and 1+ cases**
- A decrease in the required number of FISH tests that can potentially lead to reduced TAT
- AI solutions could be used as decision-support tools for pathologists in routine clinical practice

TAKE HOME MESSAGES

1- **HER2 status assessment is key** for patients clinical management in early and advanced/metastatic breast cancers.

2- **HER2 positive (score 3+ and 2+ amplified) cancers are not a single entity** (ER status, TILs, *PIK3CA* and *TP53* mutations, Level of *HER2* amplification…)

3- **It takes time to properly determine %, intensity and membranous staining (complete or incomplete) to correctly assess the HER2 low and ultra-low**

4- **AI will certainly help** to increase standardization of HER2 low and ultra-low status

Thank you

Institut des Cancers des Femmes

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