Breast cancer: IHC for diagnostic use

NordiQC: Seminar in Diagnostic Immunohistochemistry 2025
Aalborg University Hospital
September 17-19h

Anne-Vibeke Lænkholm
Department of Pathology
Herlev-Gentofte Hospital
Denmark

Breast cancer mortality at ages 35-69

DENMARK 1951-2020, SWEDEN 1952-2020, UK and USA 1950-2020

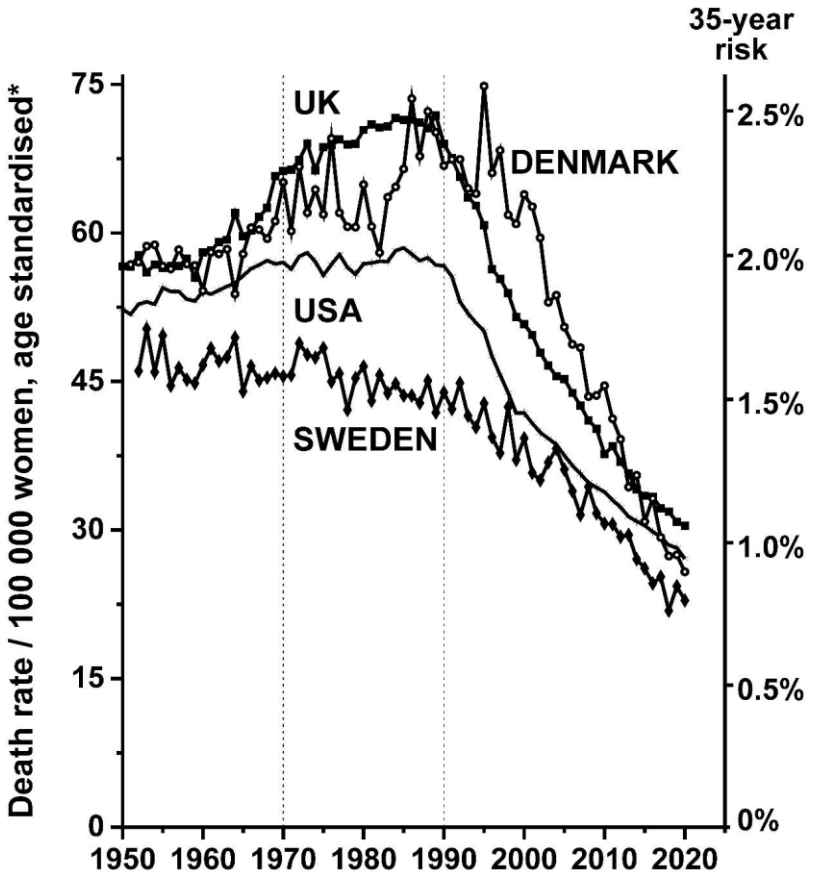
Population based data reveals markedly improved breast cancer outcomes in UK, USA, Sweden and Denmark *Slide courtesy Jonas Berg*

Female breast cancer is the second leading cause of global cancer incidence in 2022, with an estimated 2.1-2.3 million new cases.

The disease is the fourth leading cause of cancer mortality worldwide, with 666,000 deaths

Bray F, et al. CA Cancer J Clin. 2024 MayJun;74(3):229-263).

markedly improved breast cancer outcomes



* Mean of annual rates in the 7 component 5-year age groups Source: WHO mortality & UN population estimates

Slide from Lecturer Hongchao Pan, PhD and Prof, Sir Richard Peto, EBCTCG June 27, 2024.

Current risk stratification markers in early breast cancer (Denmark)

- Anatomic features
  - Size, Nodal Status
- Molecular features
  - ER, HER2,
  - Grade,
  - histological subtype
- Genomic features
  - PAM50
- Functional markers
  - pCR, Residual Cancer Burden

Less chemotherapy for ER+ and small HER2+ cancers

Escalating therapy when needed for TN and HER2+ cancers with residual disease

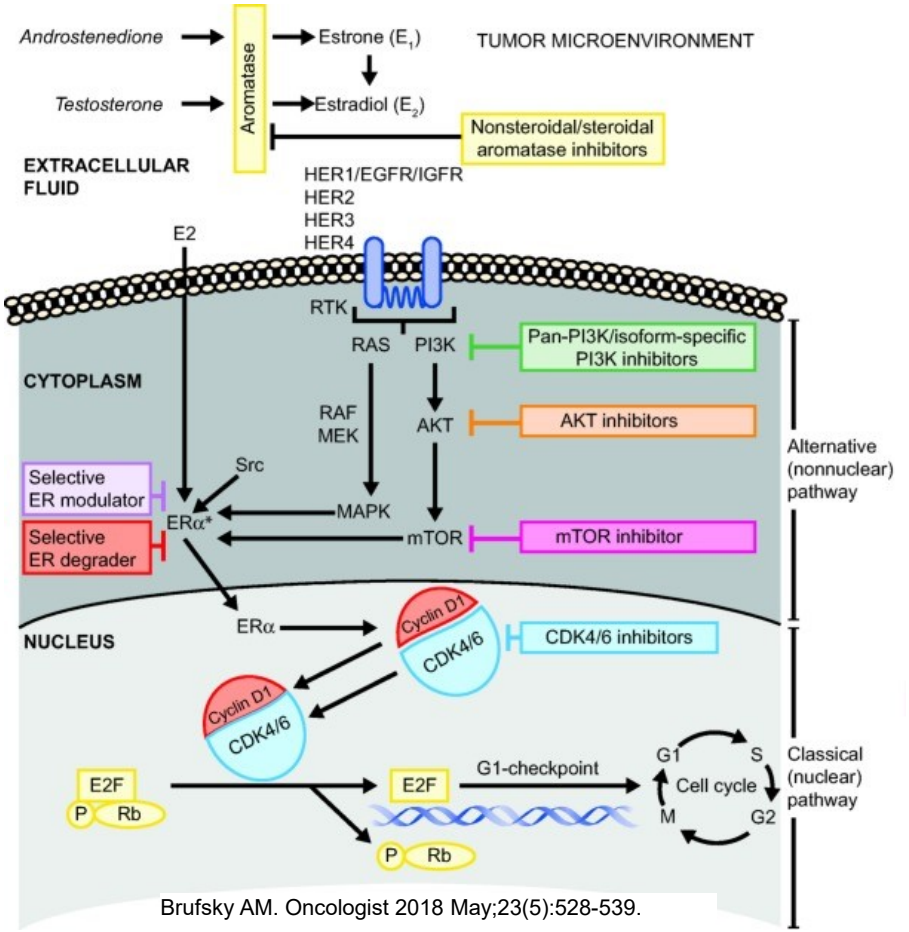
Multiparameter gene expression tests to aid prognostic classification/treatment decisions in HR+HER2- breast cancer

These tests may help the clinicians in decisions on adjuvant treatment – but the availability and use differs markedly across countries and health care systems

Treatment stratification biomarkers in the metastatic setting (Denmark)

- Molecular features
  - ER,
  - HER2
  - PD-L1

Additional biomarkers for treatment allocation with i.e.
Inavolisib (PIK3CA mutation)
Elacestrant (ESR1 mutation)
Capivasertib (PIK3CA, AKT, PTEN)



Brufsky AM. Oncologist 2018 May;23(5):528-539.

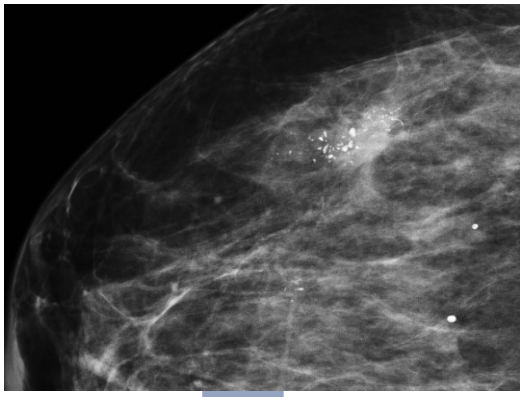
Agenda

- Immunohistochemical biomarkers for
  - Diagnostics
    - Benign Hyperplasia and Ductal Carcinoma in Situ
    - Ductal Carcinoma in Situ and Lobular Carcinoma in Situ
    - Carcinoma In Situ and Invasive Carcinoma
  - Histological subtype classification
    - Malignant breast tumors
  - Predictive/Prognostic markers
    - Estrogen Receptor
    - Progesteron Receptor
    - HER2 and *HER2 low status*
    - (Ki67)
    - PD-L1/sTILs
    - Additional molecular analyses (prognostic gene profiling/mutational analyses)

Triple Test
Diagnostic approach – Breast Tumours

Physical breast exam/

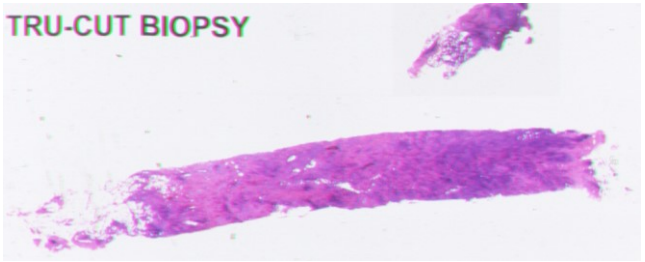
Palpation



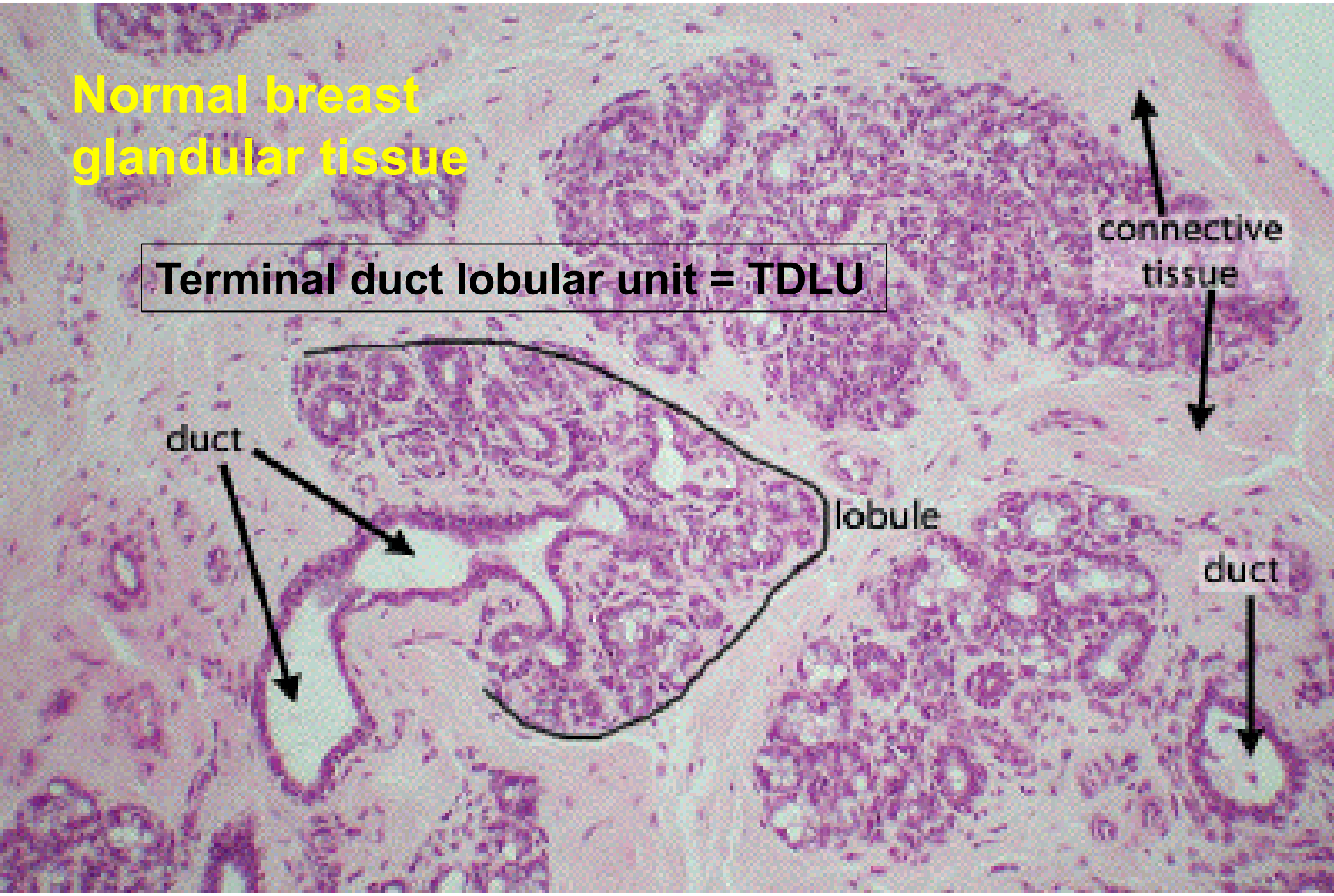
Pathology

Core needle biopsy or Fine needle aspiration

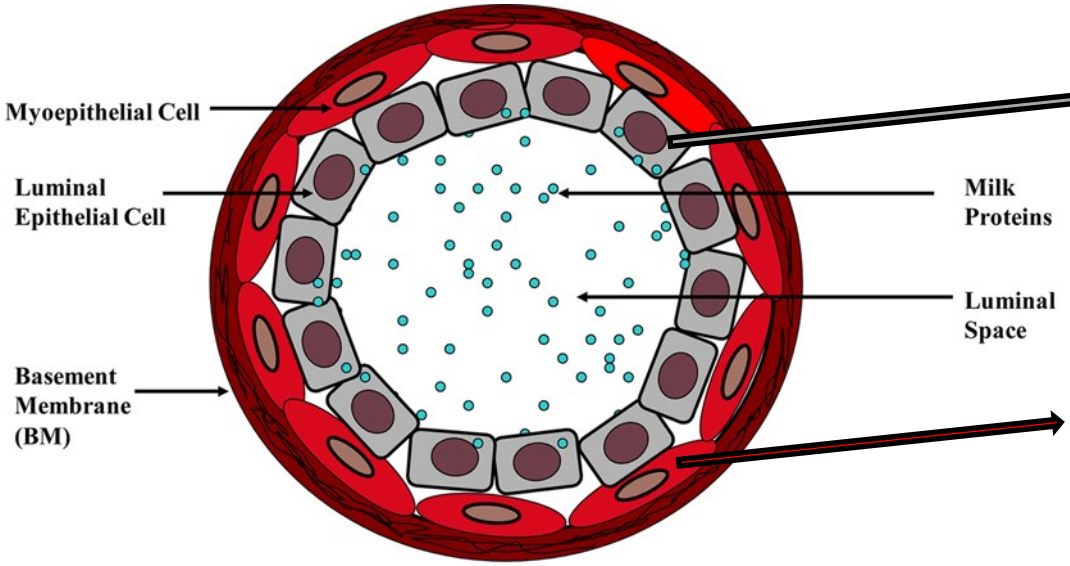
Triple diagnostics



Normal breast glandular tissue



Epithelial cells with specific immunohistochemical phenotype

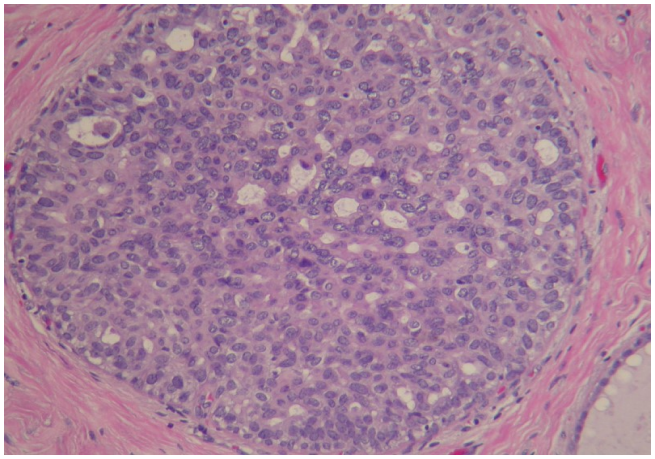


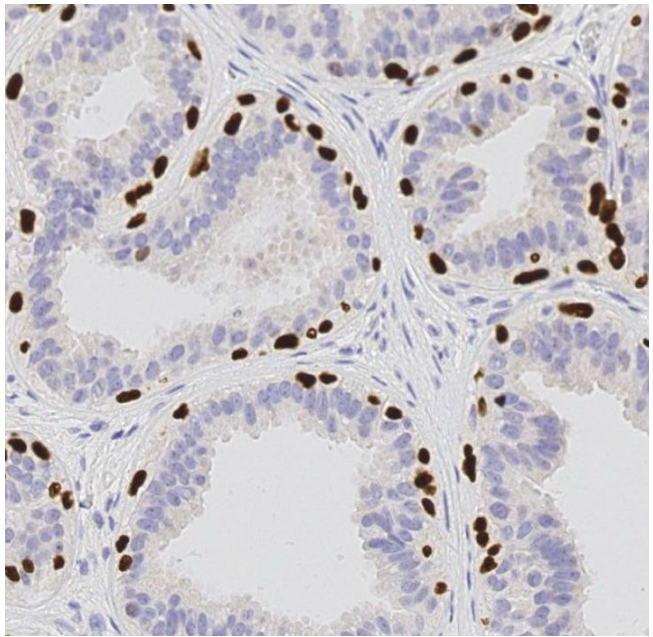
Luminal markers (LMW):
CK7, CK8, CK18, CK19

Myoepithelial markers:
Myo: p63, SMA, CD10, SMMHC*
Cytokeratins (HMW): CK5, CK14, CK17

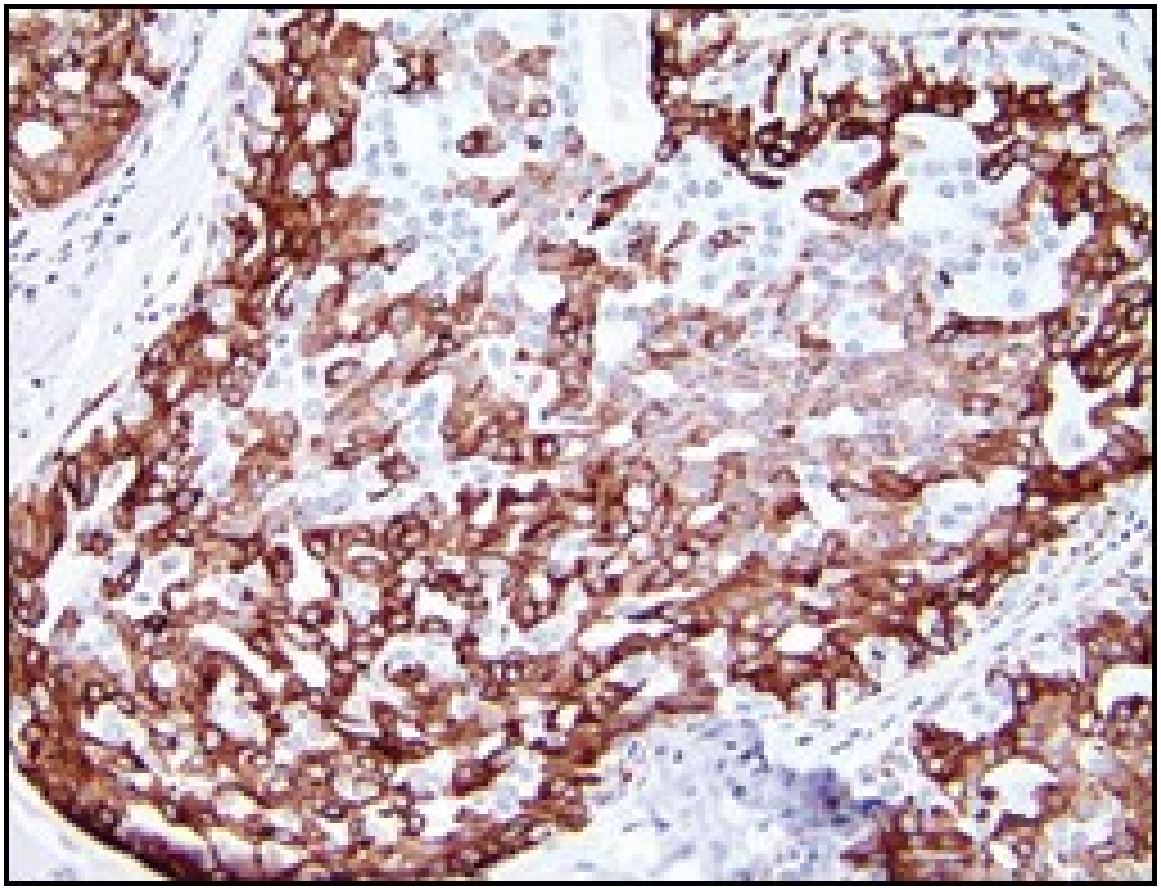
*Smooth muscle myosin heavy chain

Benign hyperplasia
Positive staining for myoepitelial cells





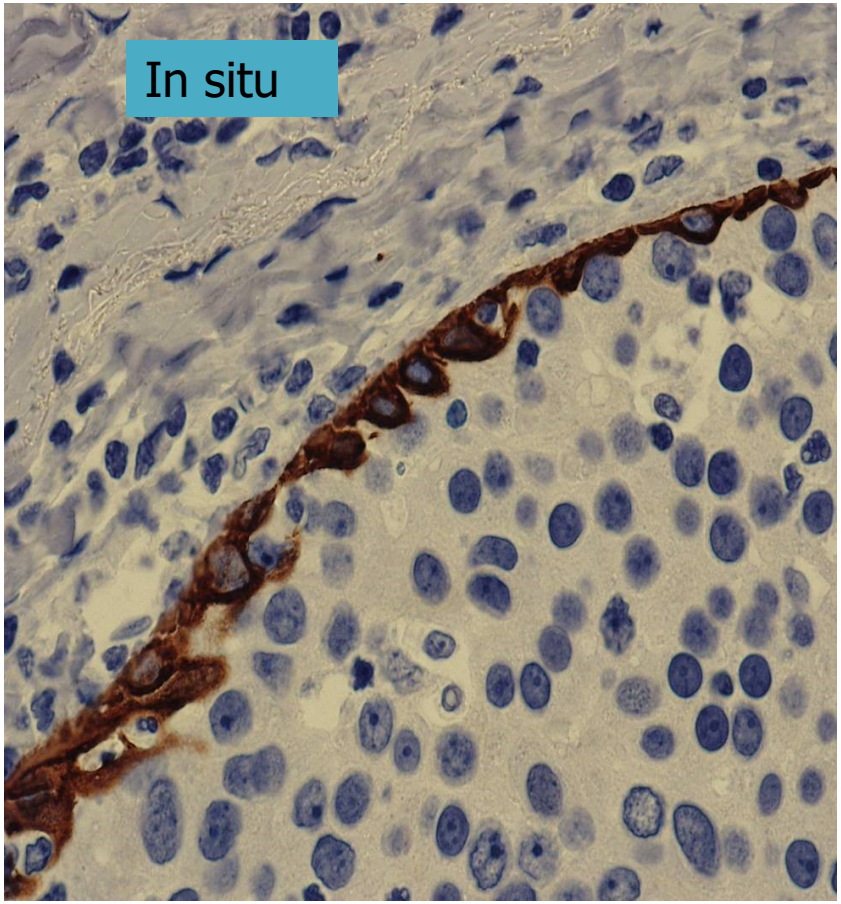
P63



CK5

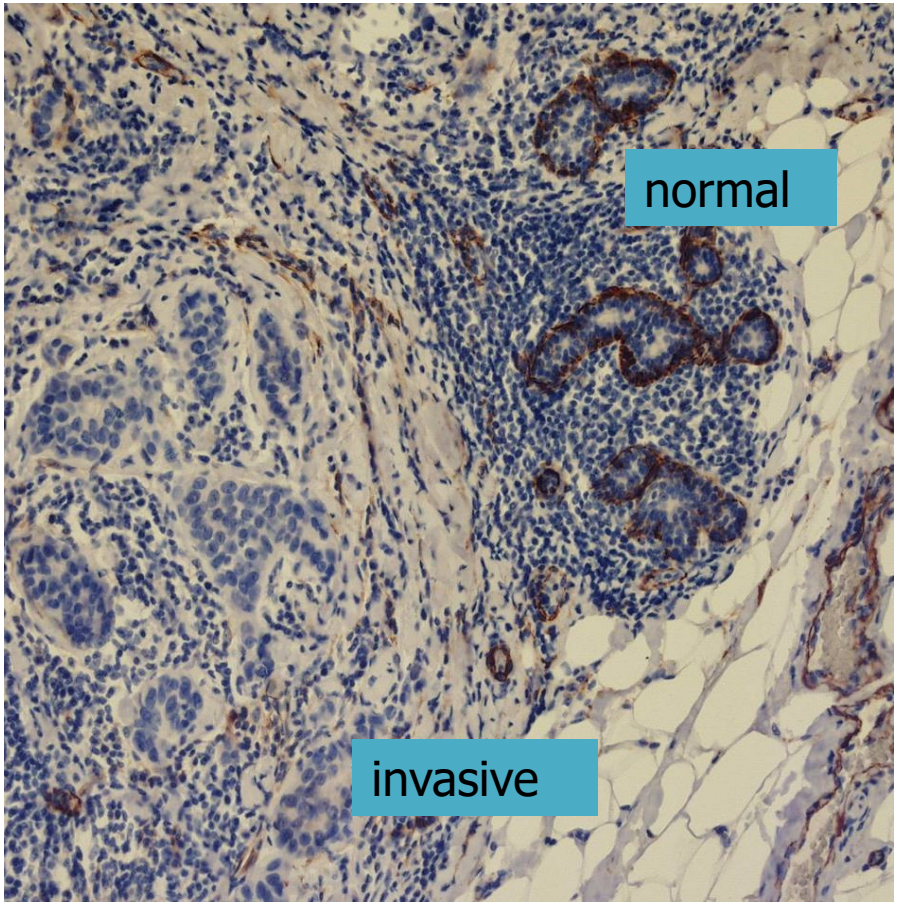
Differentiation between ductal carcinoma in situ and invasive carcinoma i.e. SMMHC*

present



Detecting ”presence”

Not present

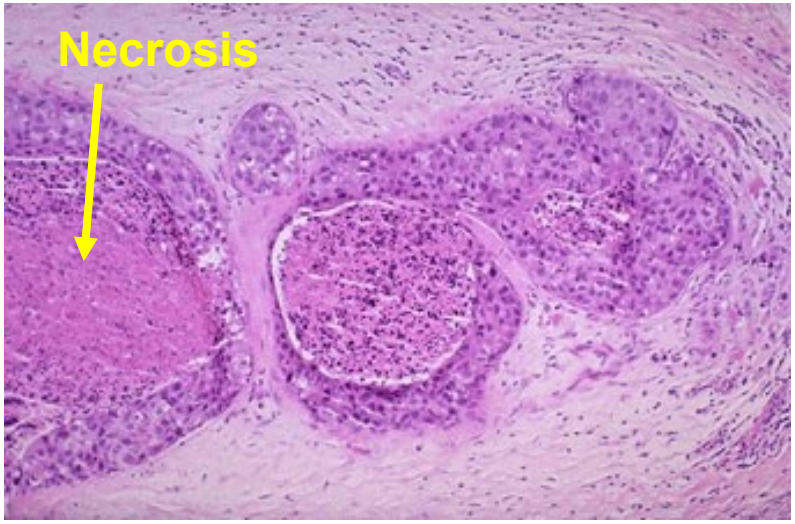


Detecting ”absence”

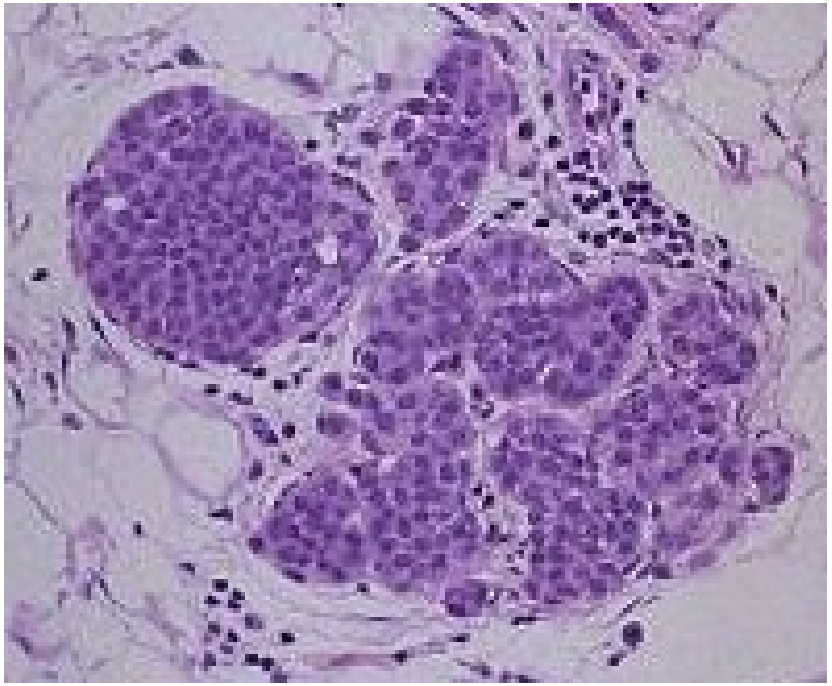
* Smooth muscle myosin heavy chain, as detected with clone SMMS-1

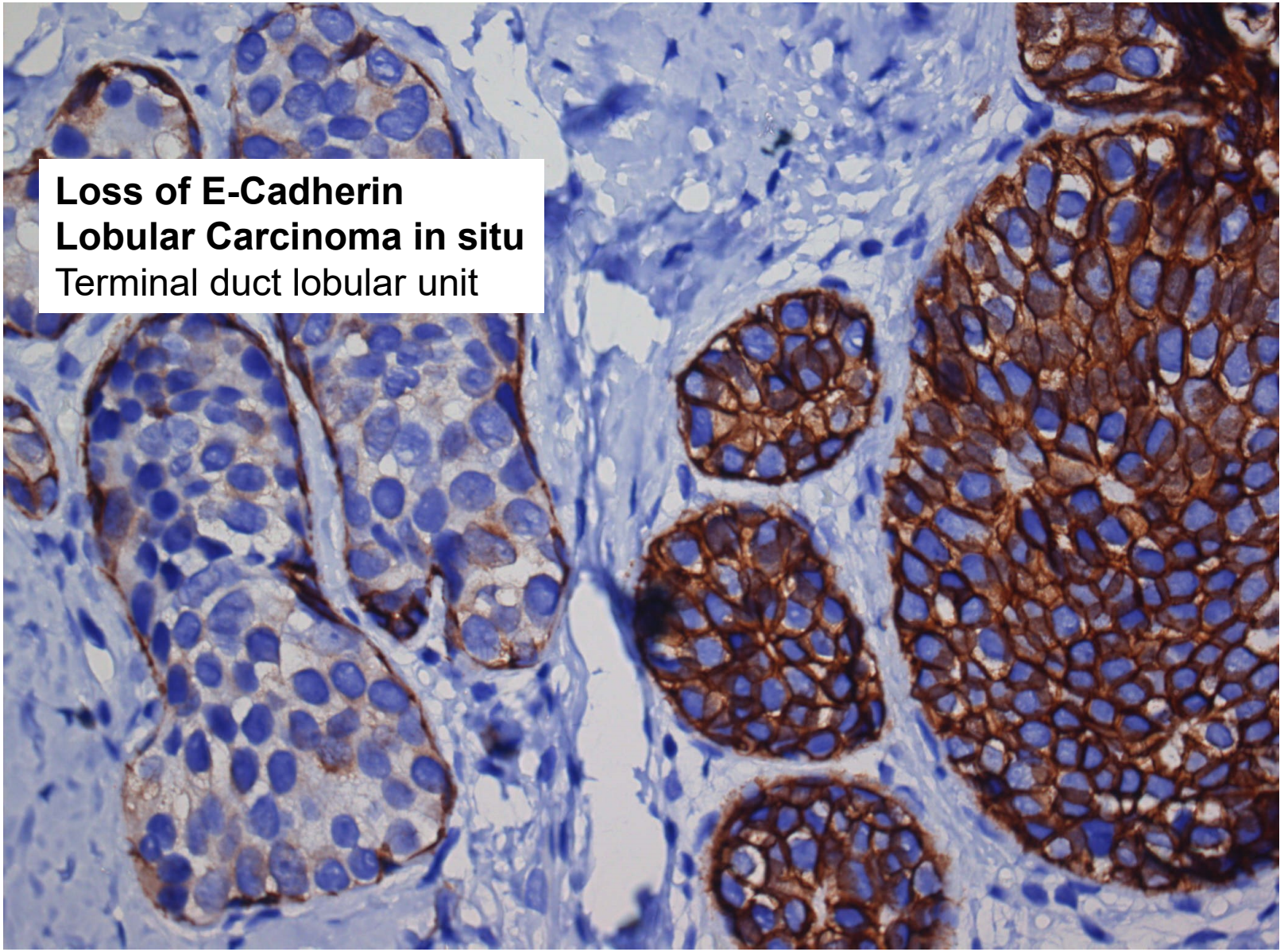
Carcinoma in situ

- Ductal carcinoma in situ
  - 12-15% of malignant lesions in the Danish screening population
  - Microcalcifications
  - Risk of progression to invasive carcinoma
  - Surgery with free margins (2 mm)
  - Radiation therapy after breast conserving surgery



- Lobular carcinoma in situ
  - Non obligate precursor
  - Incidence 0.5 – 3.6%
  - Often incidental finding
  - Multifocal and often bilateral
  - Slowly proliferating lesions
  - Observation / screening





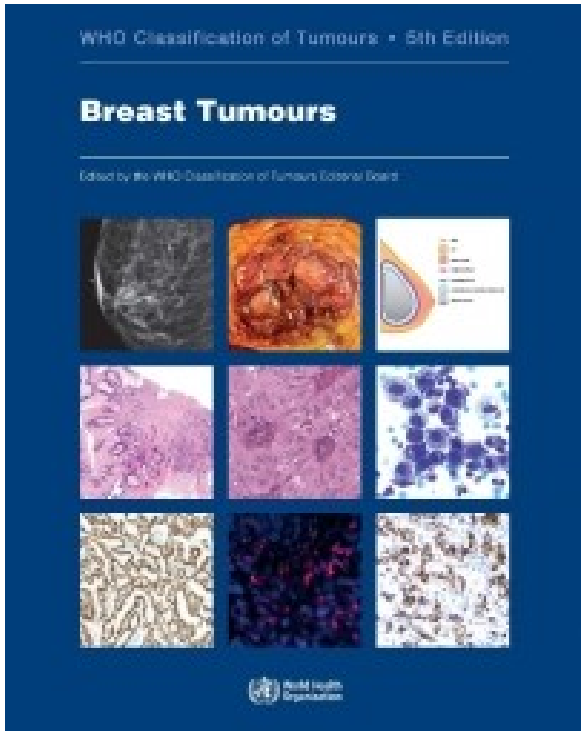
E-cadherin: Cell Adhesion Molecule

Classification of malignant tumors of the breast

WHO blue books

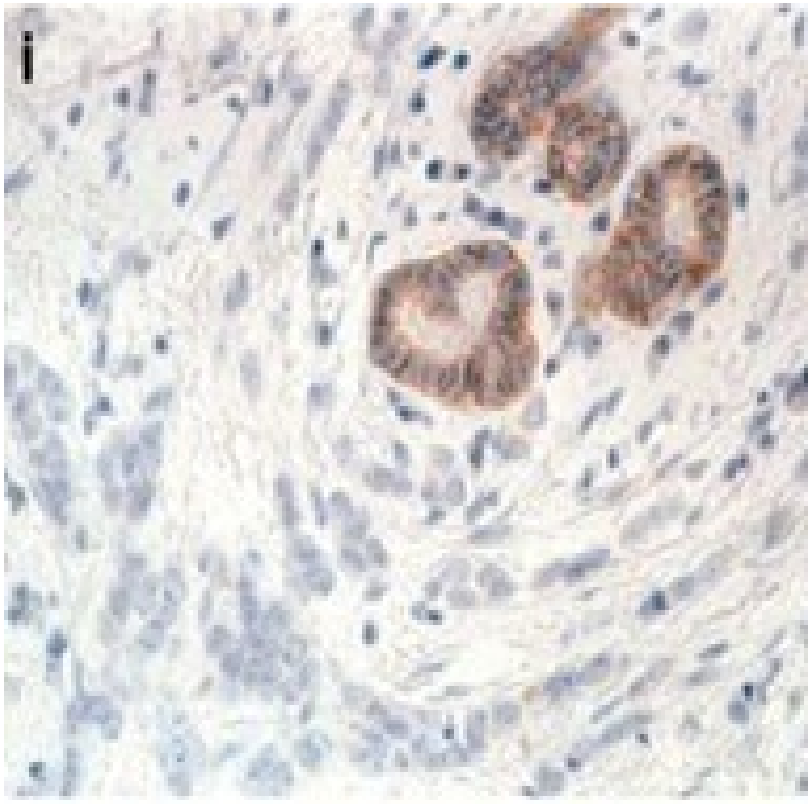
Histological subtypes (>20)

- Ductal : up to 80%
- Lobular: 5 - 14%
- Tubular: 2 - 8%
- Mucinous: 2 - 4 %
- Apocrine: 1 – 4%
- Papillary 1 – 2%
- Other

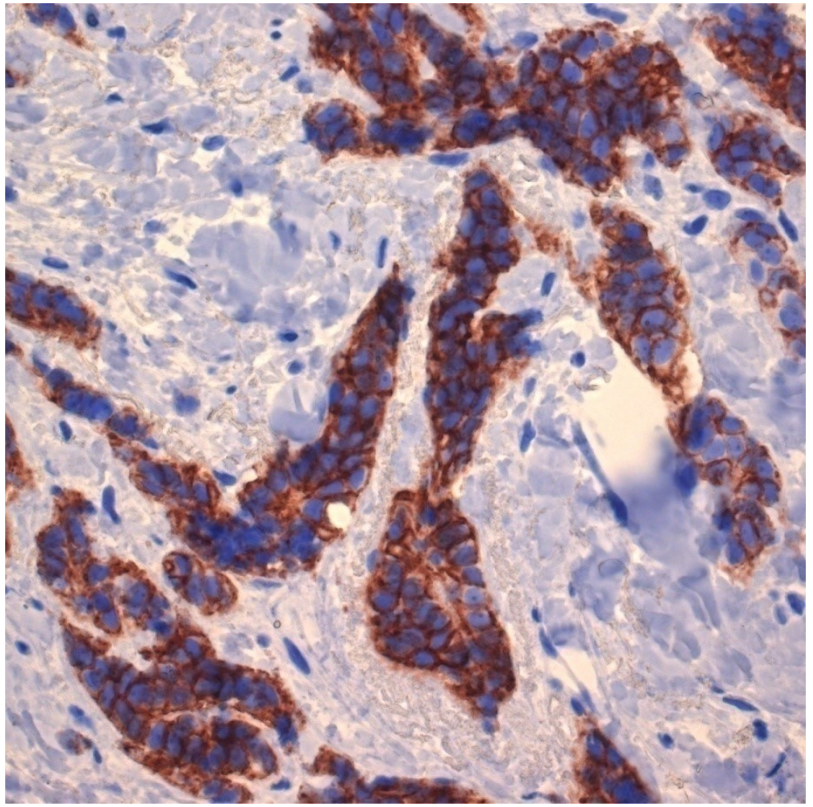


E-Cadherin
Cell adhesion molecule

Loss of E-Cadherin in 90% of Invasive lobular Carcinoma

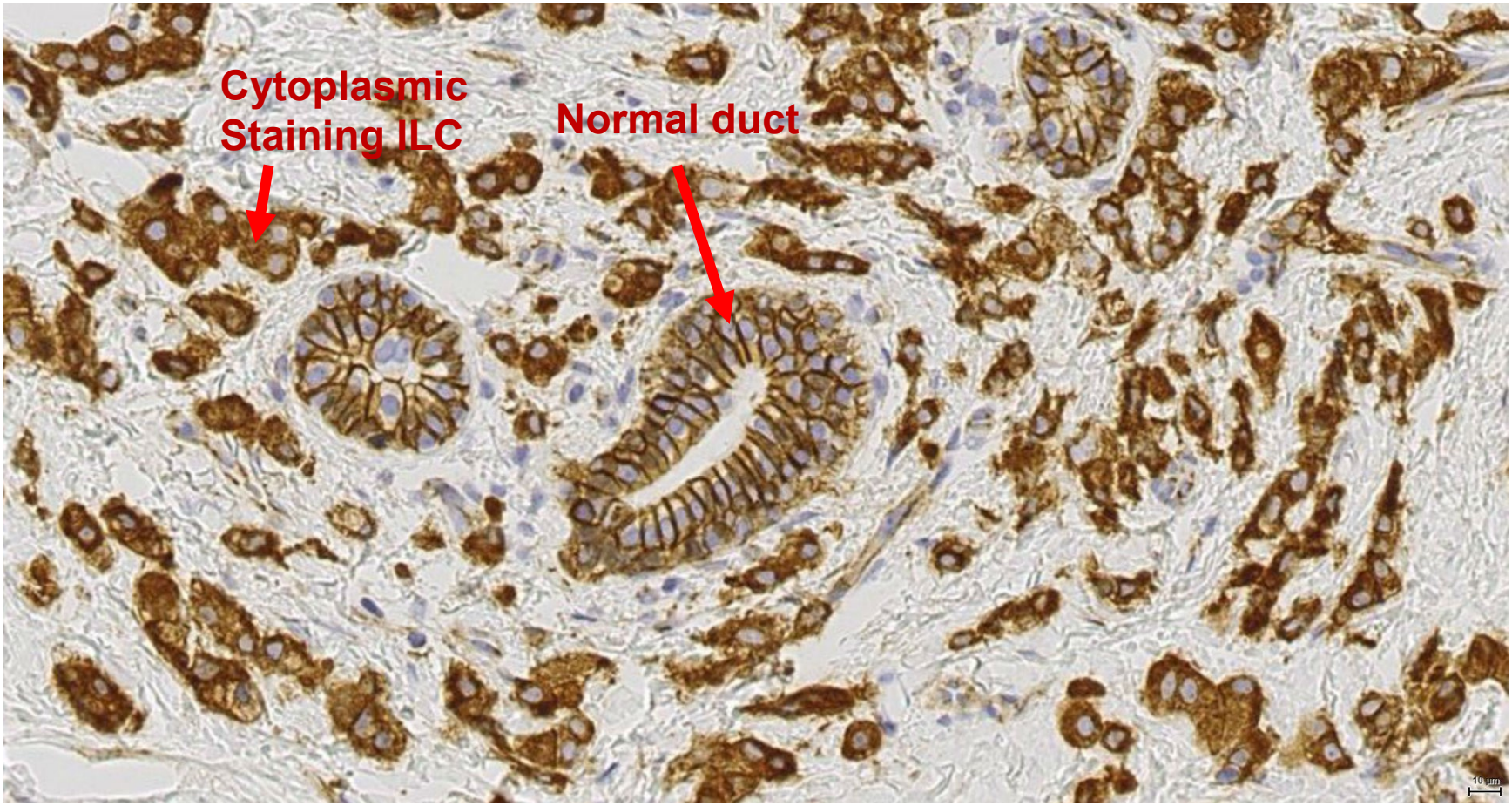


E-Cadherin positive Invasive Ductal Carcinoma



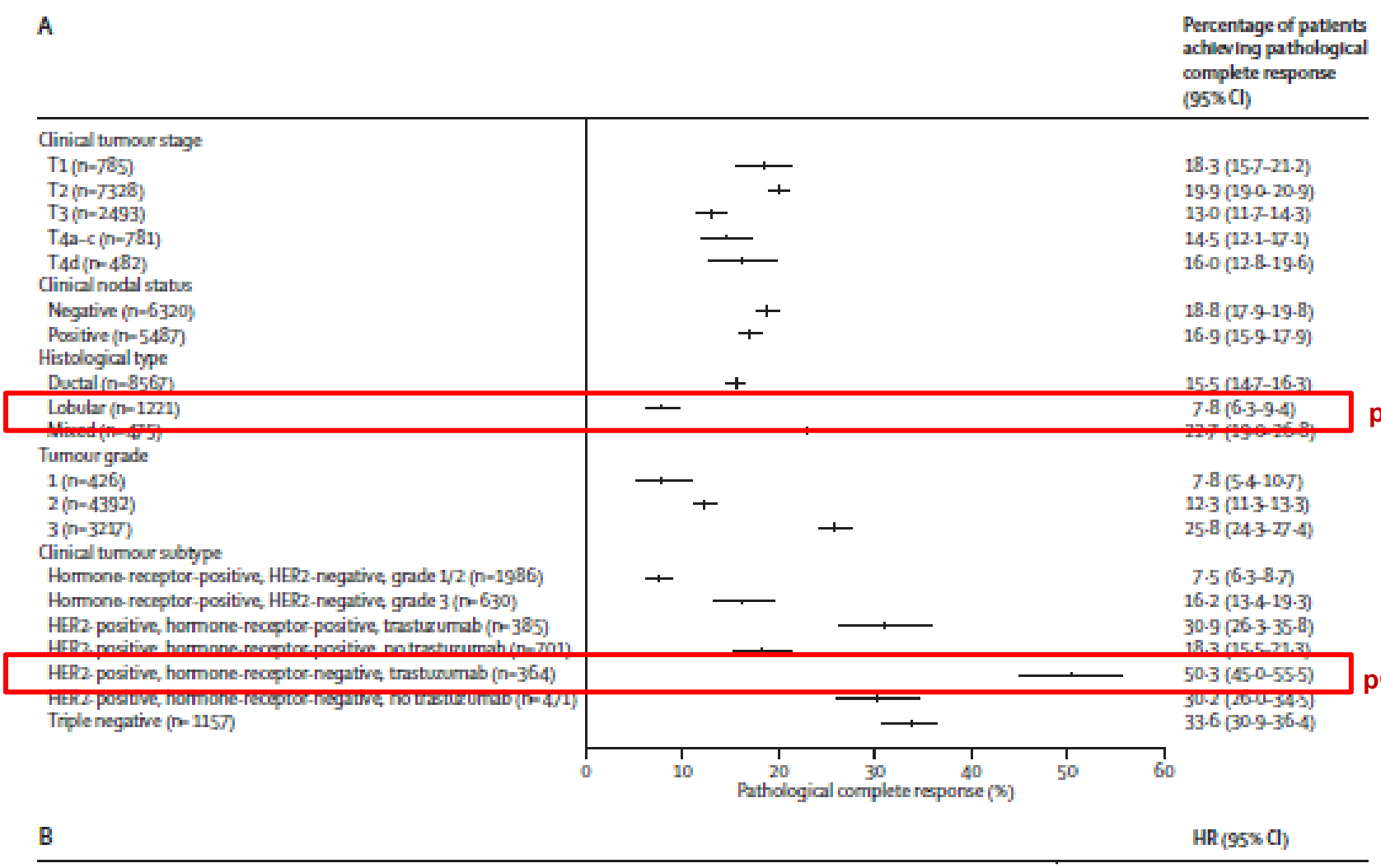
CDH1 (16q22.1) loss of function mutation or deletion resulting in loss of the adhesion molecule E-cadherin

P120 catenin dislocated to the cytoplam in lobular carcinoma (ILC)
A supplement for classification of lobular neoplasia



Lobular cancer - not candidate for neoadjuvant chemotherapy
Low proliferating tumors, often luminal A molecular subtype

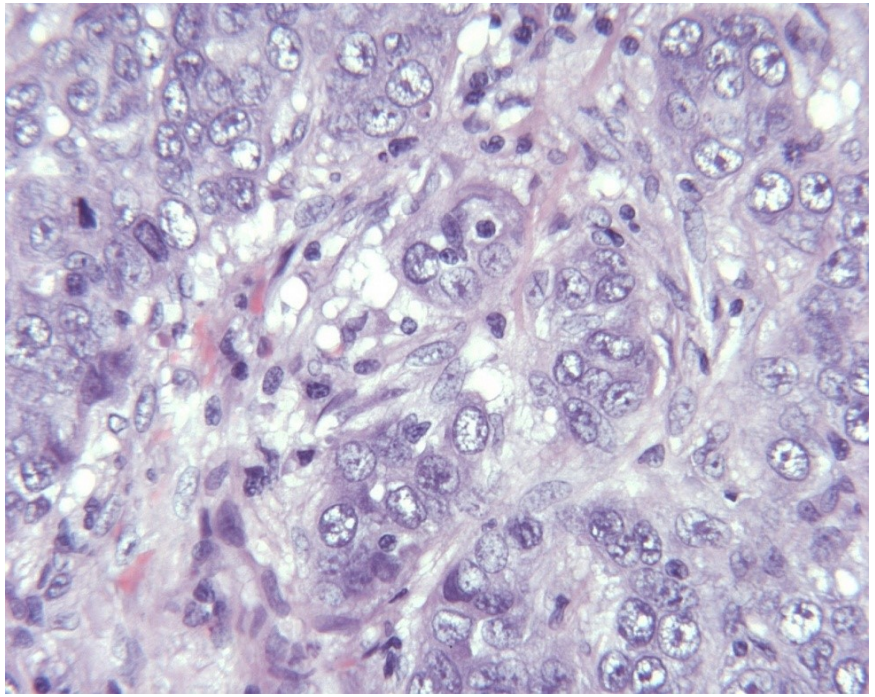
Tumor characteristics and association with pCR
Neoadjuvant chemotherapy (NACT)



Cortazar et al. Lancet 2014; 384: 164-72

TNBC : 8-10% of primary breast cancers

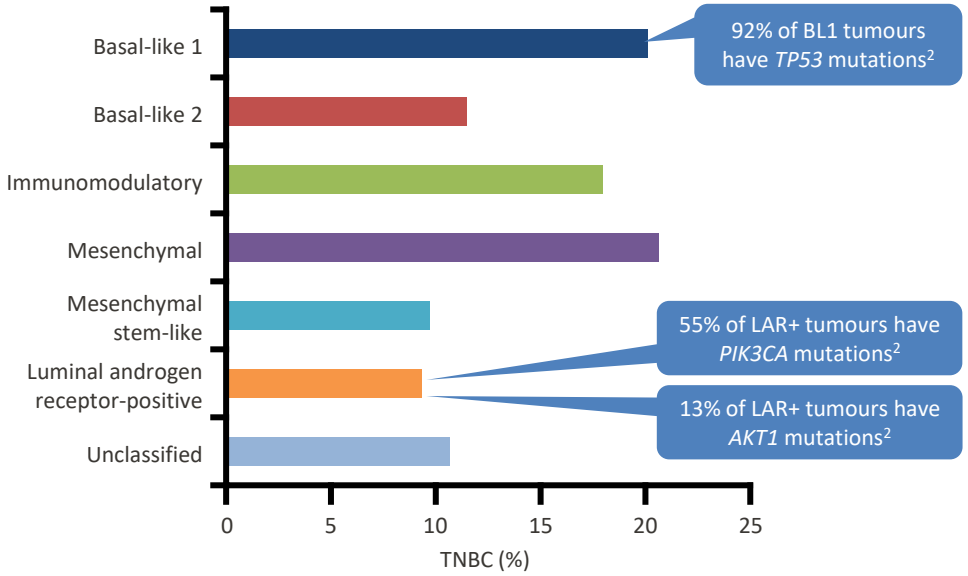
- ER, PR and HER2 negative
- Heterogeneous group of tumours
- High grade
- Younger age at diagnosis
- Poor prognosis
- Risk of *gBRCA* mutation



Heterogeneity of TNBC

- TNBC is a combination of many disease entities that have been grouped together for ease of clinical categorization.
- But studies reveal a high level of heterogeneity[1–3]
  - High levels of genetic instability versus other BC subtypes
  - Complex patterns of copy number alterations and structural rearrangements
- *PIK3CA/AKT1/PTEN* alterations are seen in ~24%[4]
- *BRCA1/2* mutations are seen in ~20%[5]

Six unique molecular subtypes of TNBC have been identified[6]

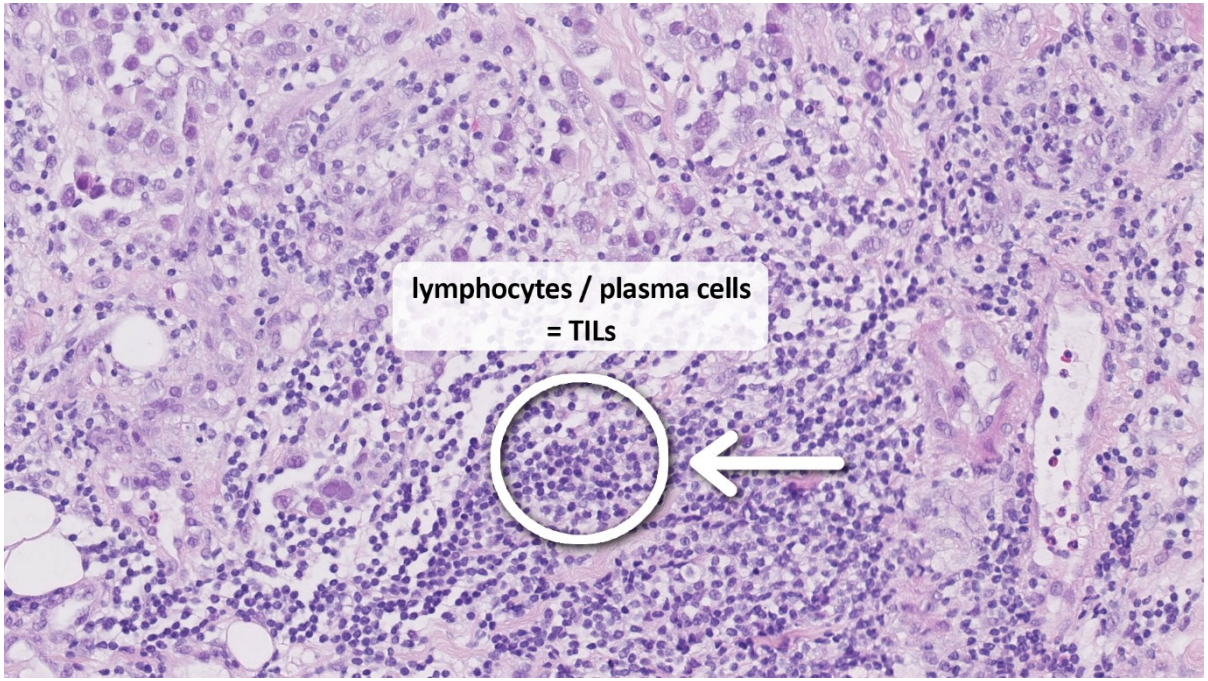


1. Lehmann, et al. J Clin Investig 2011; 2. Bareche, et al. Ann Oncol 2018

3. TCGA, Nature 2012; 4. Schmid, et al. ASCO 2015
5. Gonzalez-Angulo, et al. Clin Cancer Res 2011; 6. Abramson et al. Cancer 2015

Figure reproduced from Abramson 2015[6]

Tumor infiltrating lymphocytes and TNBC



TNBC is considered to be the most immunogenic breast cancer subtype, with a higher median number of tumor-infiltrating lymphocytes (TILs), PD-L1 expression, both markers associated with tumor microenvironment (TME) immune activity.

Level 1B evidence / prognostic marker

Loi, S., et al., *Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers.* J Clin Oncol, 2019. **37**(7): p. 559-569.

Triple-Negative Breast Cancer Histological Subtypes with a Favourable Prognosis

The majority of TNBC are invasive ductal carcinomas (IDC) – Figure 1
Rare special histological subtypes are low proliferative tumours with good prognosis allthough being triple negative (Figure 2 and 3).
Cserni G et al. Cancers 2021, PMID: 34830849

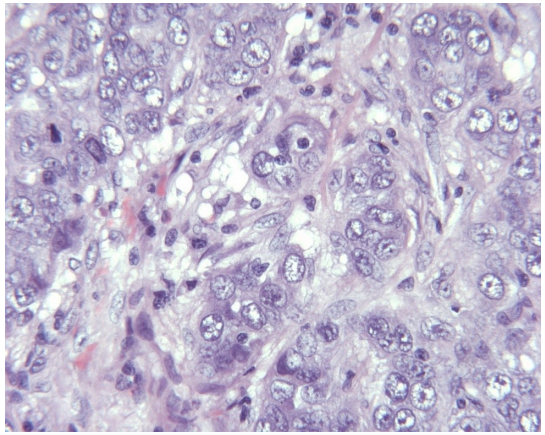


Figure 1
High grade IDC

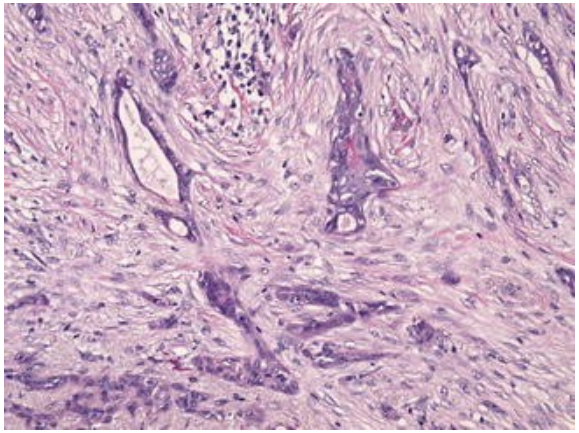


Figure 2
Low grade adenosquamous carcinoma (subtype of metaplastic carcinoma)
luminal (CK7, CK8) and basal (CK5, CK14) CKs and squamous (myoepithelial) markers p63 and p40.

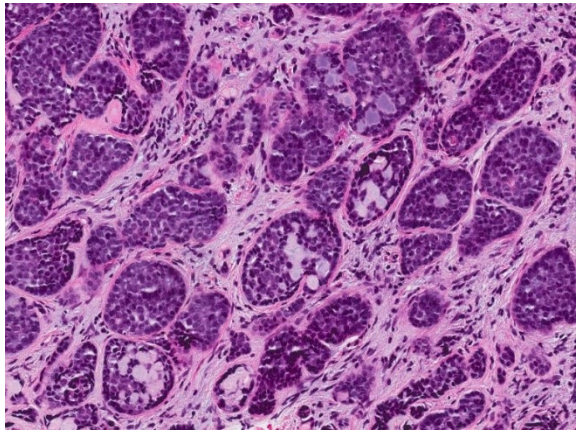
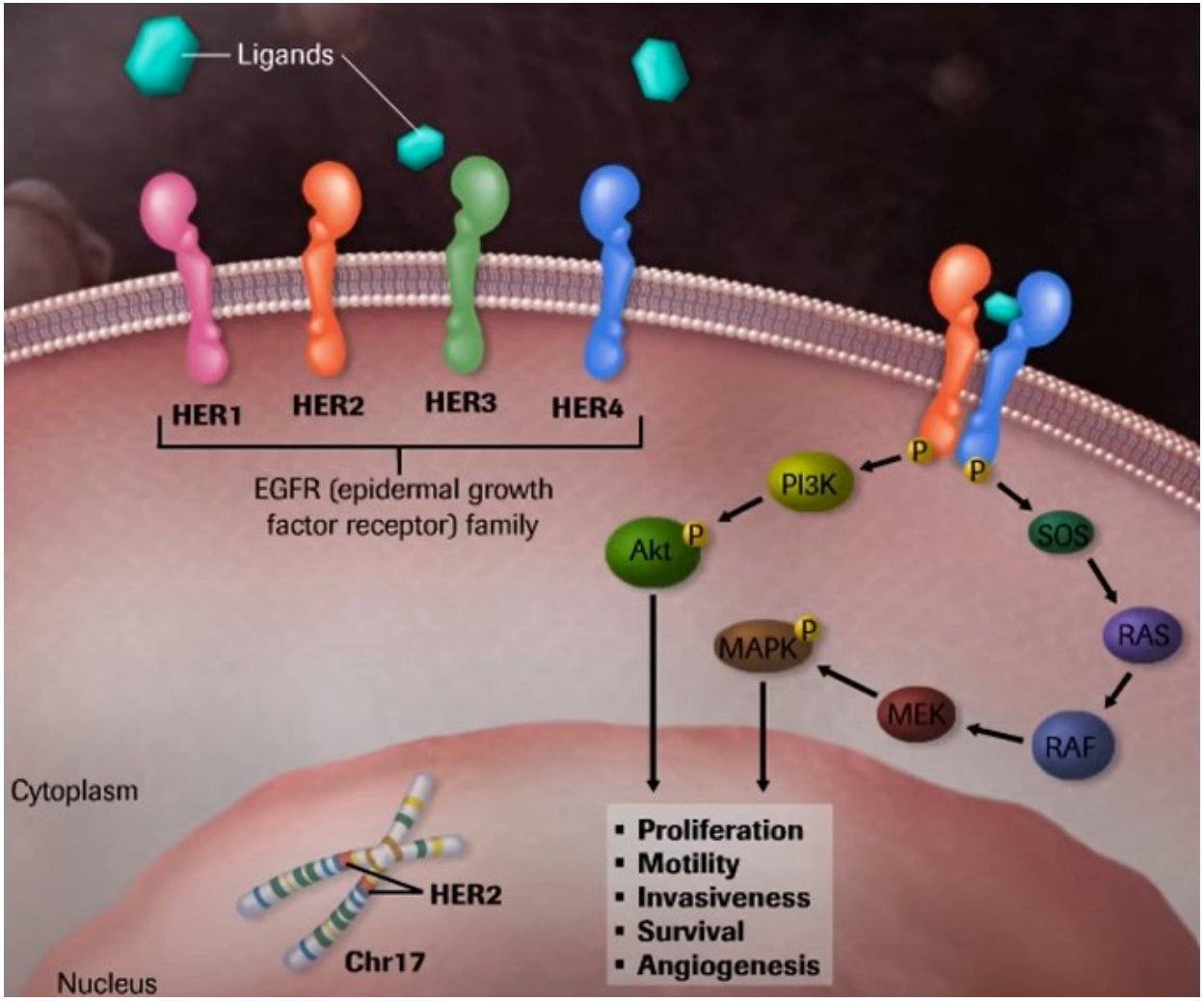


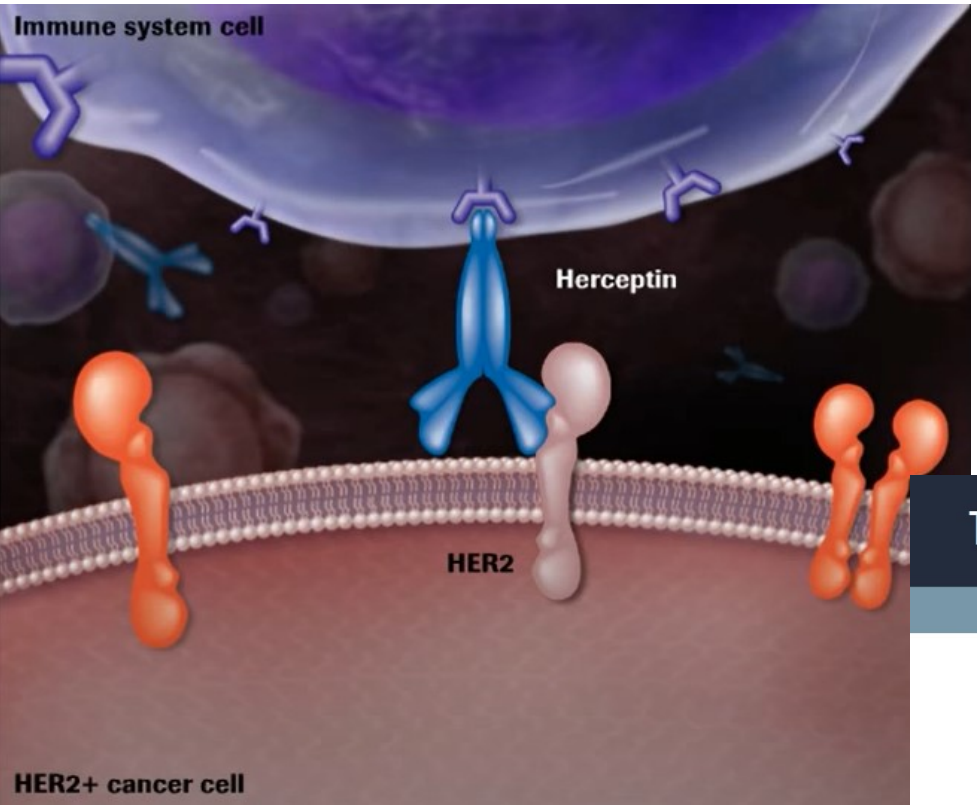
Figure 3
Adenoid cystic carcinoma of the breast. The cells of the epithelial component are positive for CK7, CK5/6, CK 8/18 and CD117. The myoepithelial /abluminal cells express p63, smooth muscle actin and basal CKs: CK5/6, CK14, CK17.

Prognostic and predictive biomarkers

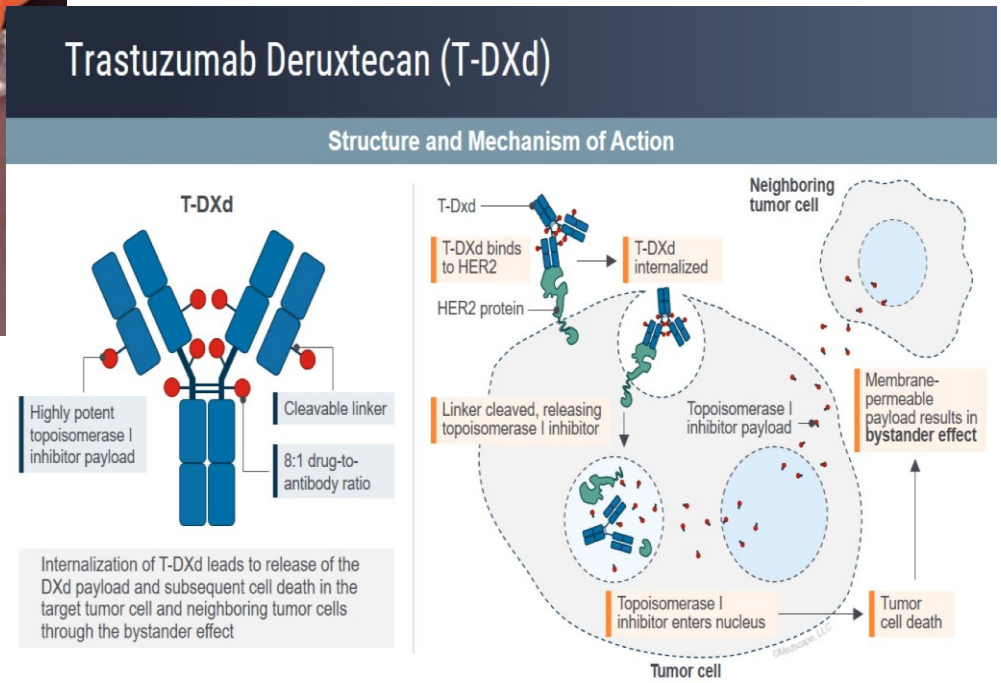
The HER2 gene is located on 17q21. and encodes the human epidermal growth factor receptor 2 (HER2), a transmembrane tyrosine kinase receptor



Targeting the HER2 receptor



a) Herceptin targets the extracellular part of the HER2 receptor
b) Pertuzumab inhibits the potent HER2–HER3 interaction in the presence of heregulin, which activates the PI3k/Akt signaling pathway.
c) ADCs: ex: T-DXd



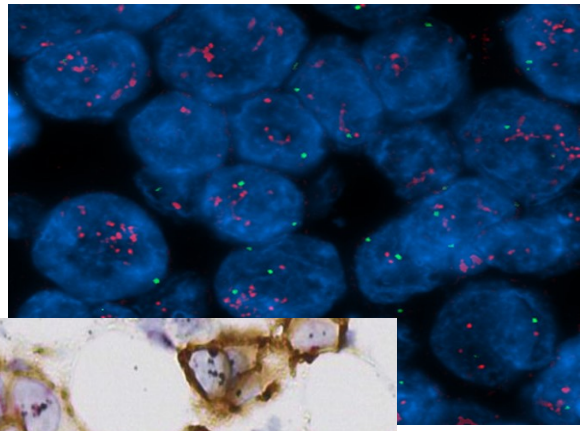
Modi S, et al. Presented at: American Society of Clinical Oncology (ASCO) Annual Meeting; June 3-7, 2022; Chicago, IL. Abstract LBA3.

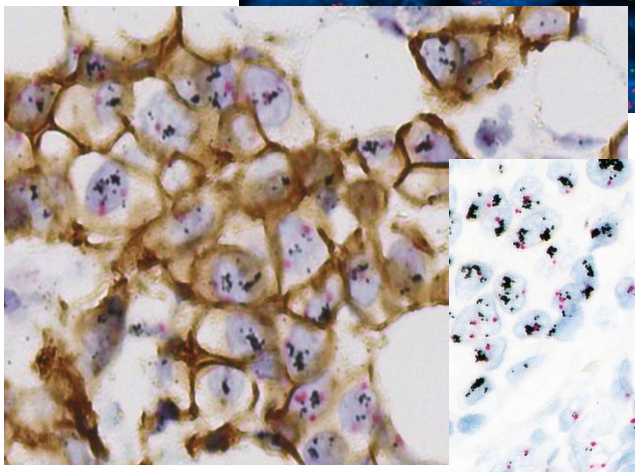
ASCO CAP guidelines
2007, 2013, 2018, Update 2023

HER2 SCORE	ASCO/CAP 2007	ASCO/CAP 2013	ASCO/CAP 2018
0	No staining	No staining or ≤10% of tumor cells with incomplete, faint or barely perceptible staining.	No staining or ≤10% of tumor cells with incomplete, faint or barely perceptible staining.
1+	Weak, incomplete membrane staining in any proportion of tumor cells.	>10% of tumor cells with incomplete, faint membrane staining.	>10% of tumor cells with incomplete, faint membrane staining.
2+ (equivocal)	>10% of tumor cells with non-uniform or weak, circumferential staining or intense membranous staining in ≤30% of tumor cells.	>10% of tumor cells with circumferential, incomplete and/or weak to moderate membranous staining or ≤10% of tumor cells with circumferential, intense membranous staining.	>10% of tumor cells with complete, membranous staining.
3+	>30% of tumor cells with uniform, intense membranous staining.	>10% of tumor cells with circumferential, intense membranous staining.	>10% of tumor cells with circumferential, intense membranous staining.

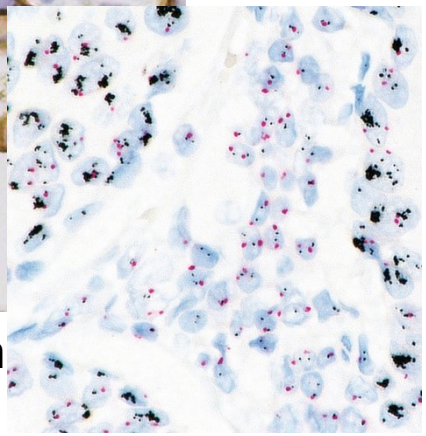
HER2 interpretation BC

FISH

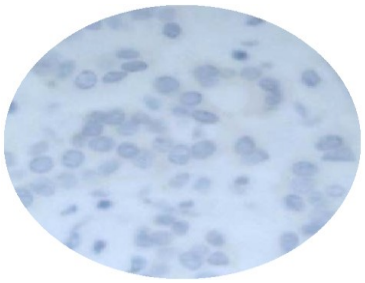


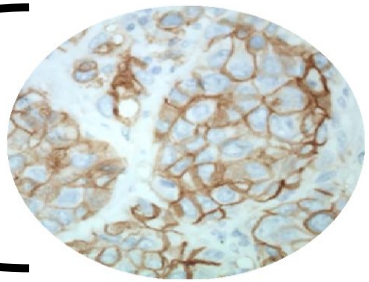


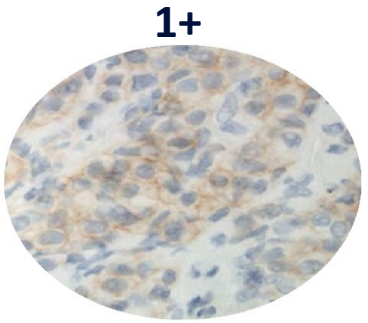
HER2 Gene/Protein

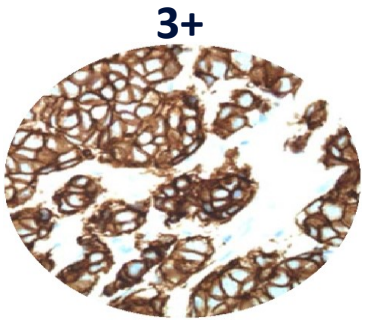


Dual ISH





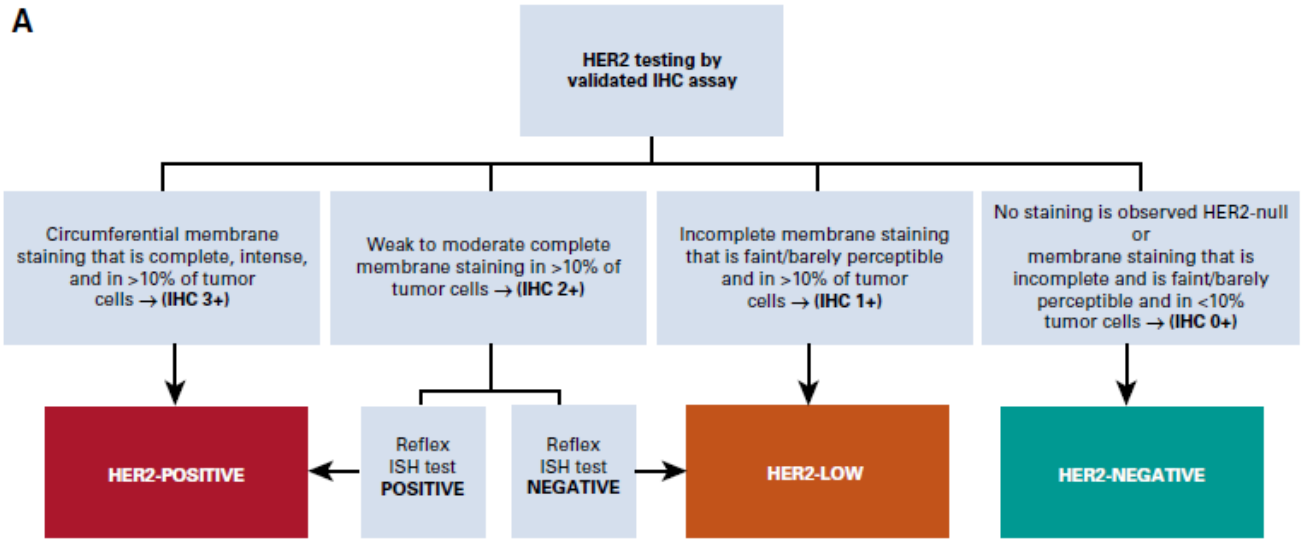




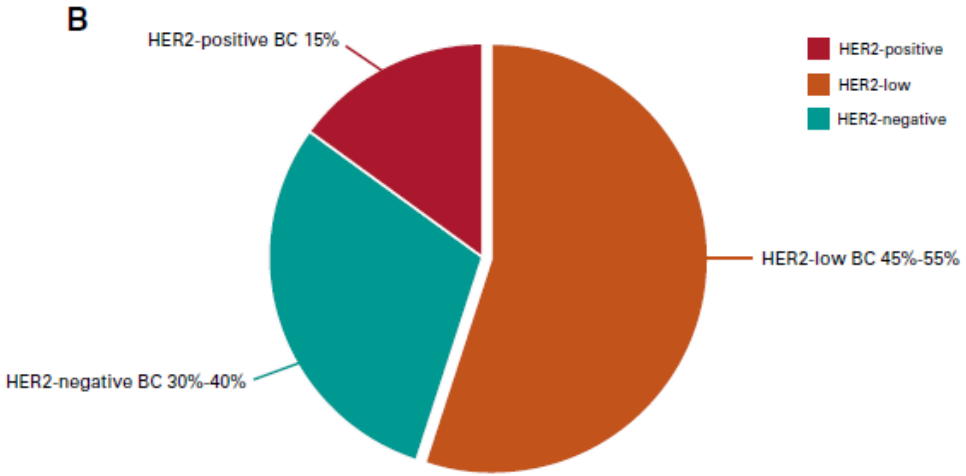
ASCO CAP guidelines 2018
https://pubmed.ncbi.nlm.nih.gov/29846122/

N Engl J Med. 2022 PMID: 35665782

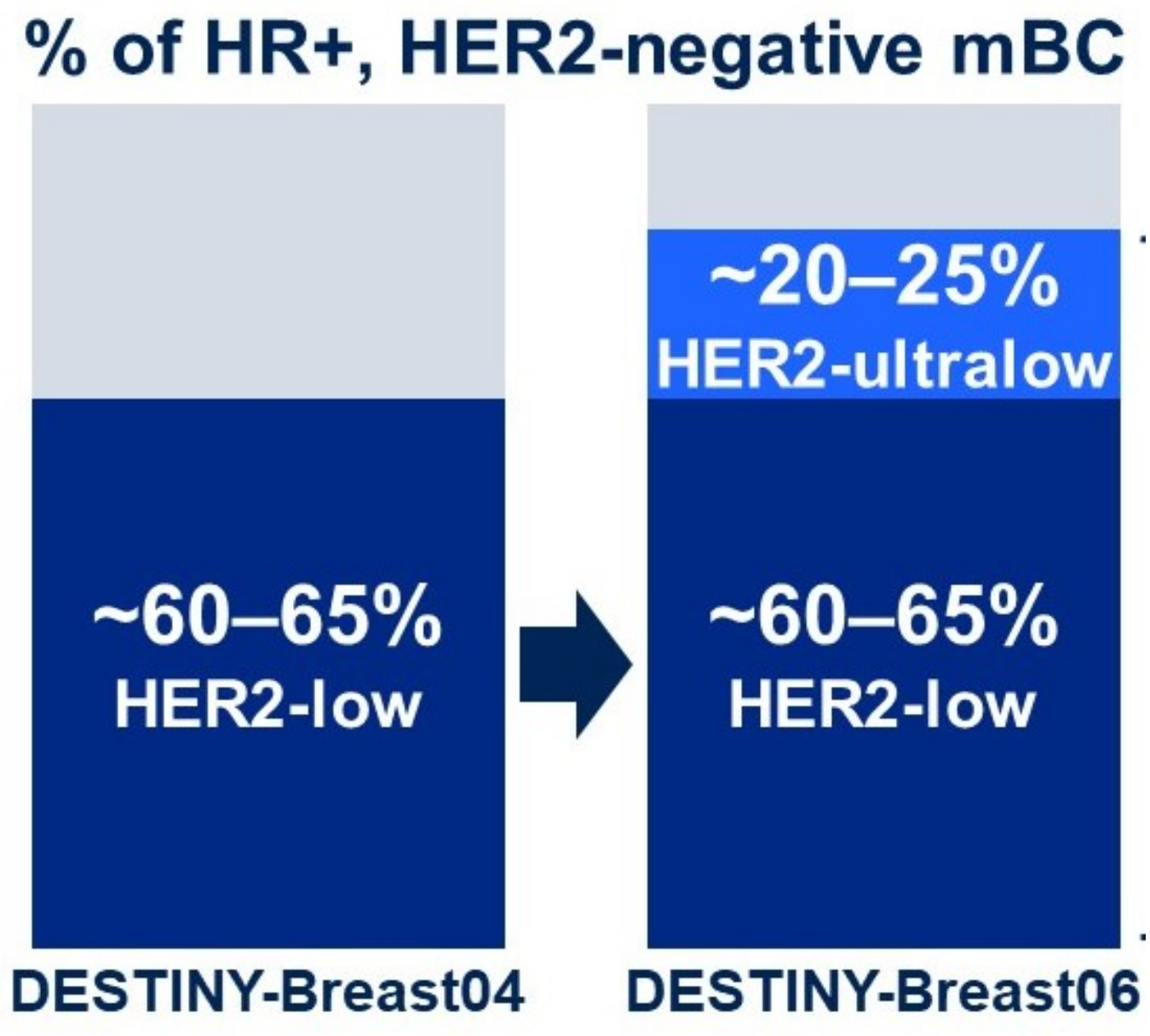
Definition of HER2 Low



Ultra-Low: HER2>0% and ≤ 10%
Destiny Breast 06



Tarantino et al.
JCO, 2020
https://doi.org/10.1200/JCO.19.02488


% of HR+, HER2-negative mBC
~20–25%
HER2-ultralow
~60–65%
HER2-low
~60–65%
HER2-low
DESTINY-Breast04
DESTINY-Breast06

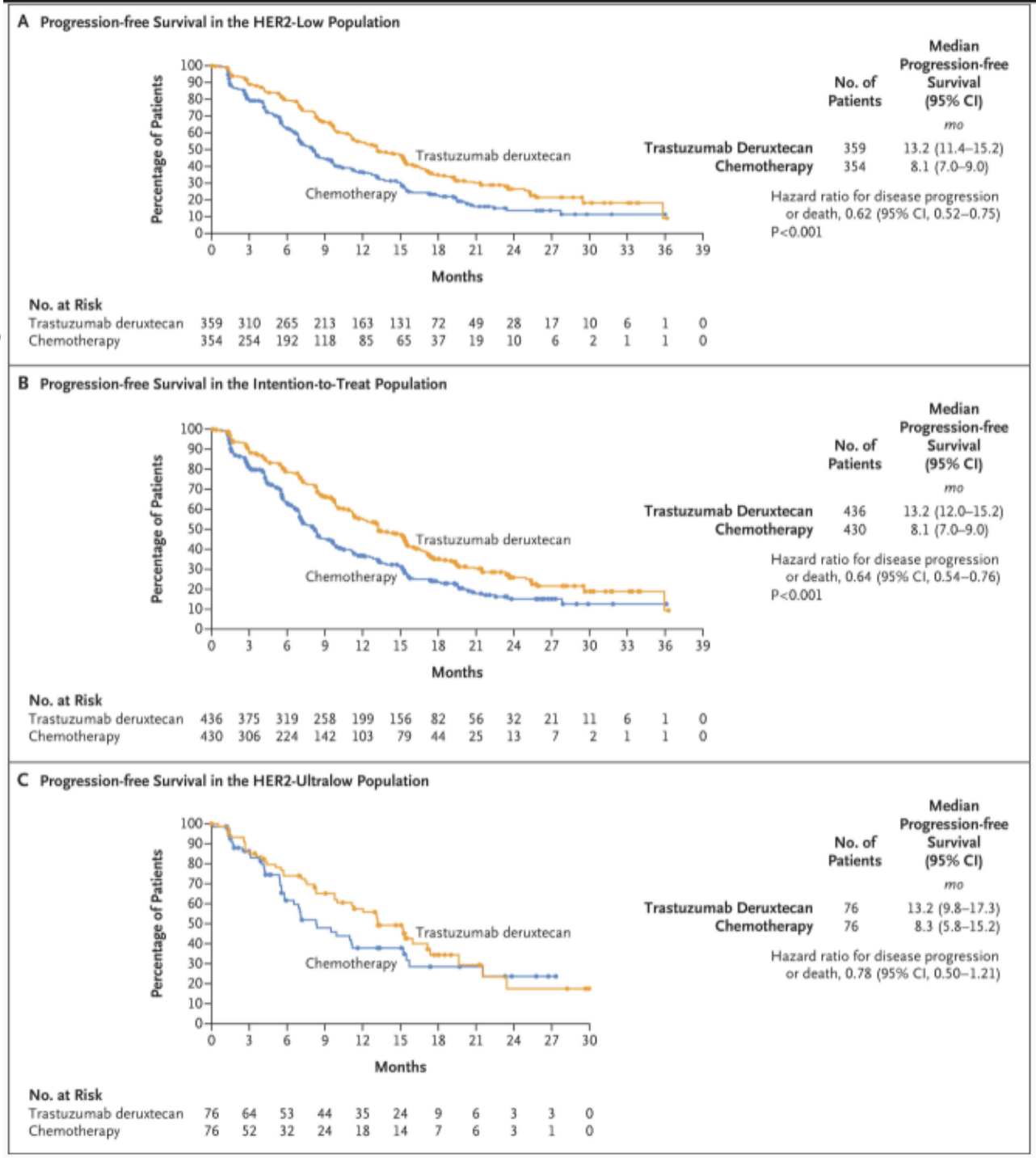
Destiny Breast 06

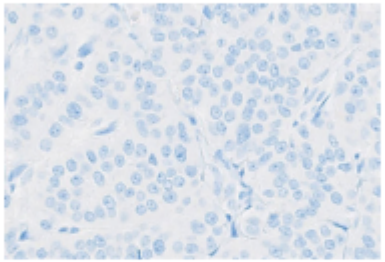
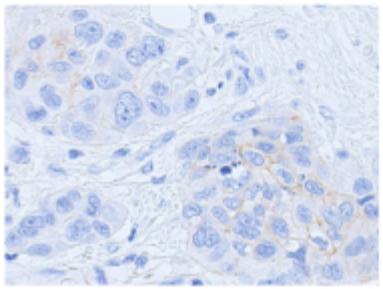
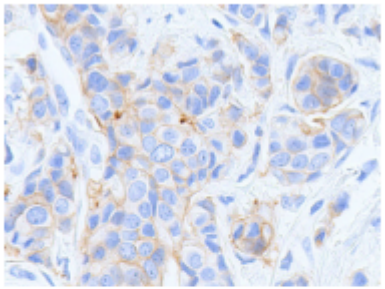
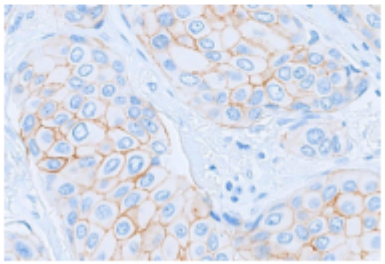
DOI: 10.1056/NEJMoa2407086

FDA approval of T-DXd for HER2 ultralow in january 2025

EMA approval april 2025

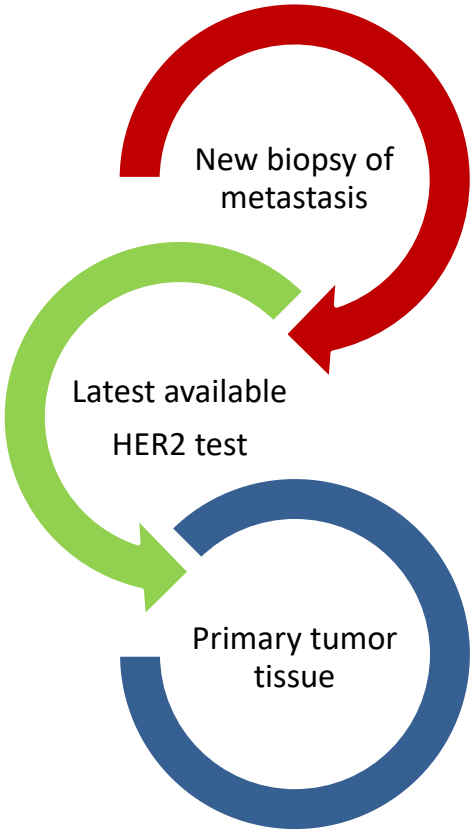
For those with HER2-ultralow disease given T-DXd, (n = 76) the median PFS was 13.2 months vs 8.3 months in the chemotherapy arm (n = 76; HR, 0.78; 95% CI, 0.50-1.21



IHC staining pattern	HER2 (4B5) score	Recommended reporting status	Representative images
No membrane staining is observed[a]	IHC 0 *absent* membrane staining	HER2-null	
Any staining of the membrane in >0 and ≤10% of the cancer cells[a,b,c]	IHC 0 *with* membrane staining	HER2-ultralow expression	
Faint, partial staining of the membrane in >10% of the cancer cells[a]	IHC 1+	HER2-low expression	
Weak to moderate complete staining of the membrane in >10% of the cancer cells[d]	IHC 2+ *Reflex test: HER2 non-amplified*	HER2-low expression	
	IHC 2+ *Reflex test: HER2 amplified*[e]	HER2-positive/ overexpression	

2025 Jun;10(6):105310. doi: 10.1016/j.esmoop.2025.105310. Epub 2025 Jun 9.

Suggested flow for analysis



Re-evaluation of HER2 staining is adviced on archival material if HER2 score 0

2025 Jun;10(6):105310. doi: 10.1016/j.esmoop.2025.105310. Epub 2025 Jun 9.

Assay sensitivity

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**

* 2 amplified

	% cases categorised as HER2-Low (n=119 cases)
HercepTest	35%
4B5	19%

Rueshoff J, et al. Virchows Arch 2022

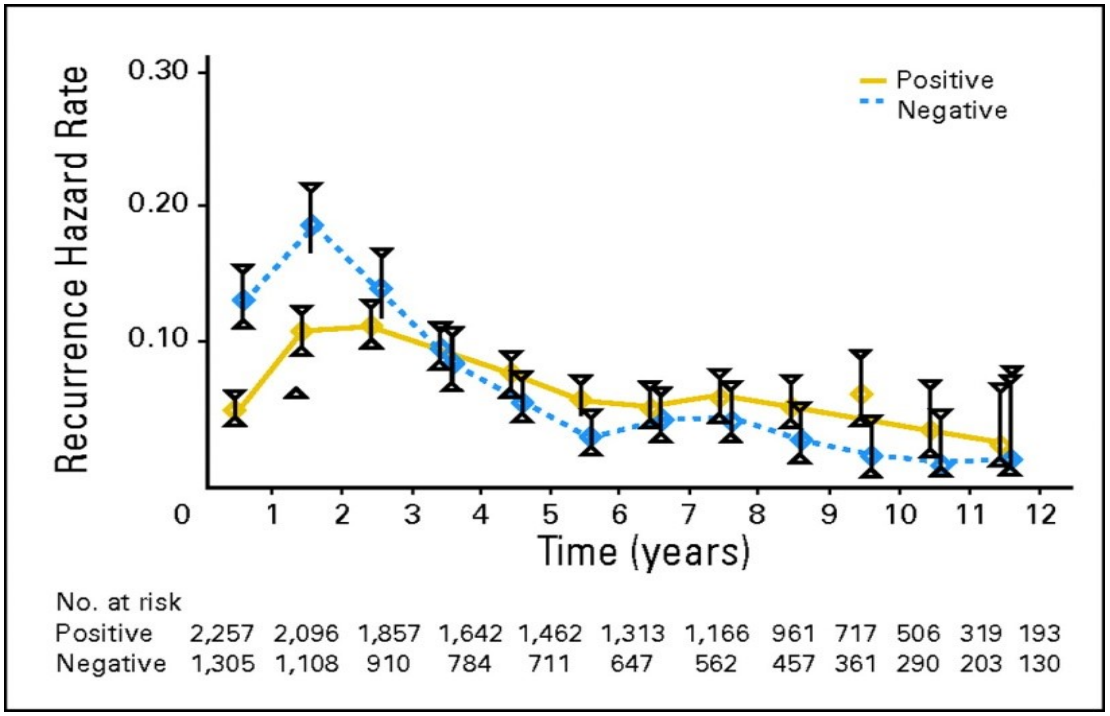
Summary: HER2-low BC

- Common and usually found in HR-positive disease
- At present, main role is to predict response to ADC (T-DXd) in mBC
- Variable assay sensitivity, concordance, spatial and temporal heterogeneity for HER2-Low category with existing assays
- For now, use of existing assays is advised with focused training on the full spectrum of low HER2 expression

ASCO CAP HER2 guidelines 2023 Update https://doi.org/10.1200/JCO.22.02864
ESMO consensus 2023 https://doi.org/10.1016/j.annonc.2023.05.008

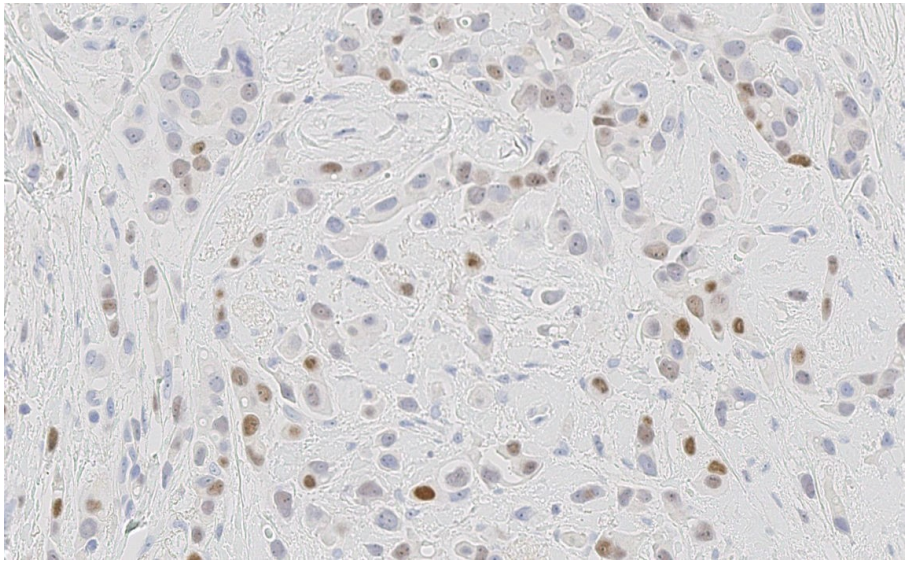
The Estrogen receptor as a prognostic/predictive marker

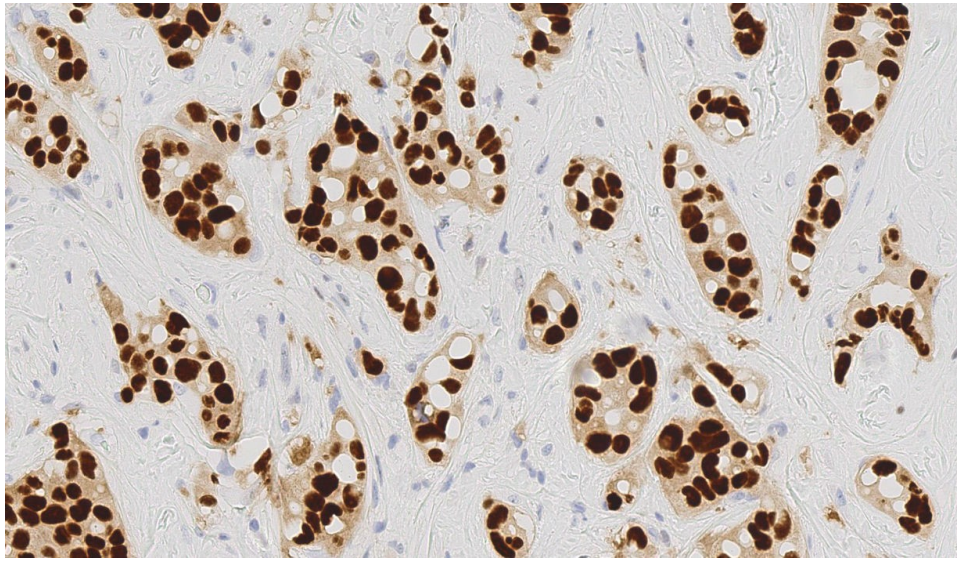
Risk of recurrence pr. year
N = 3,562 patients



Lin, N. U. et al. J Clin Oncol; 26:798-805 2008

2020 – ASCO CAP Update
Hormone receptors

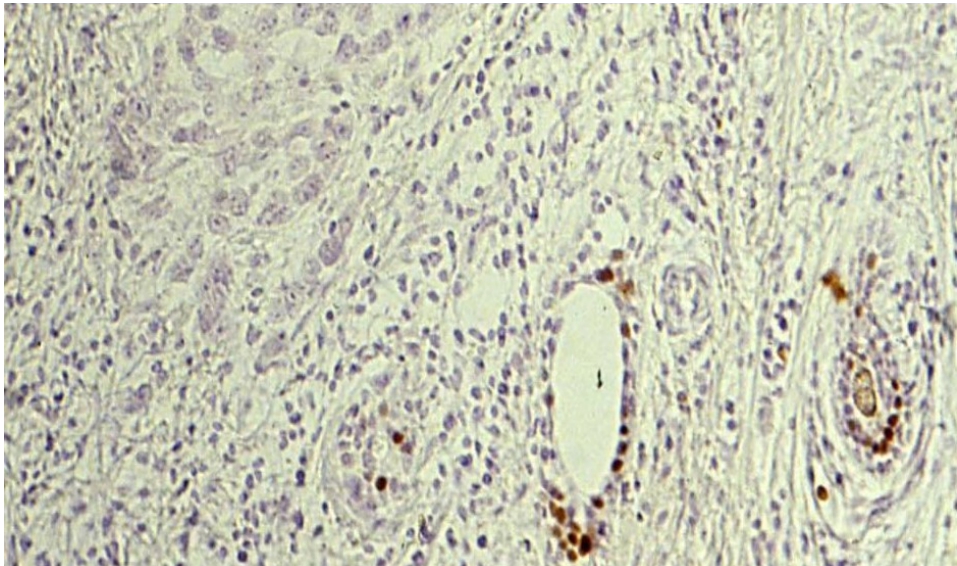




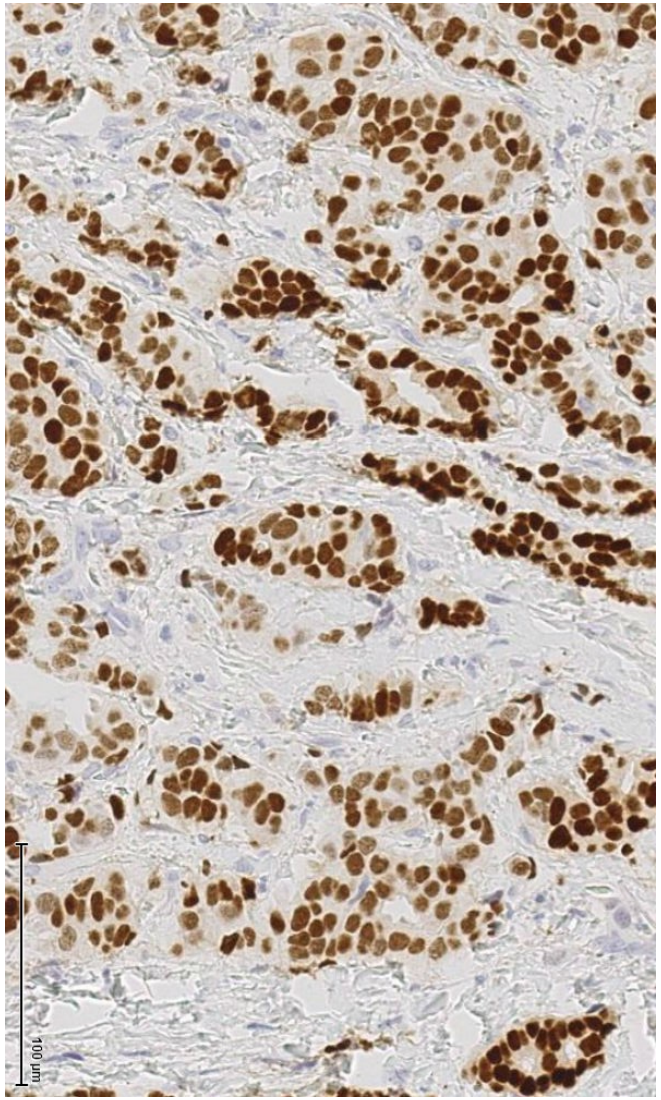
ER positive 86% of breast carcinomas (DK)

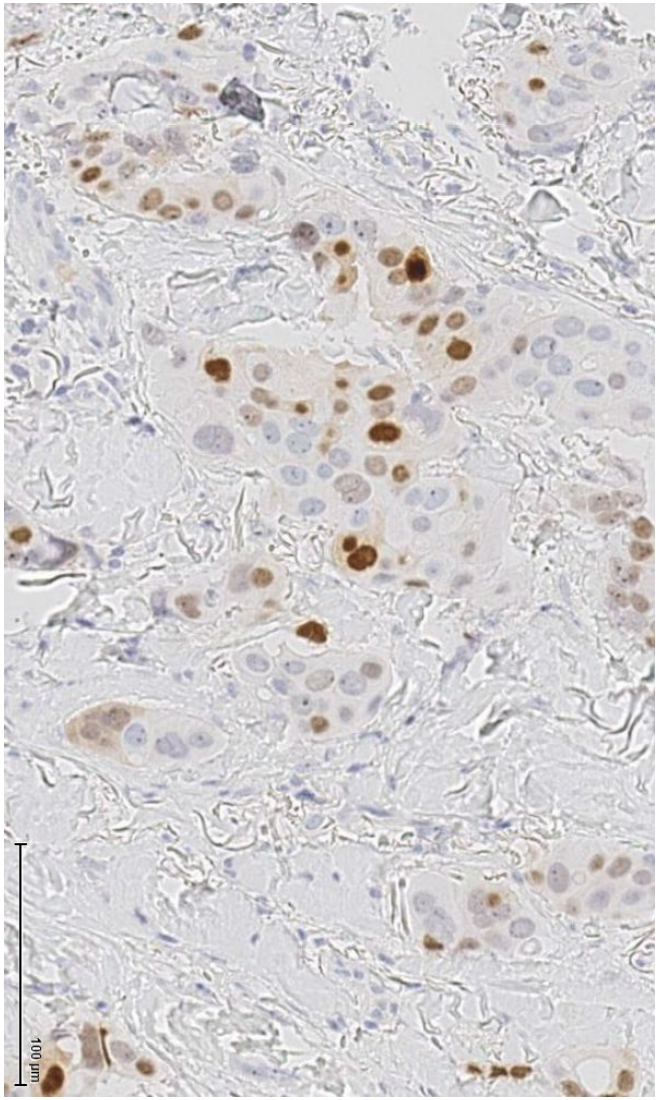
Cut off ≥ 1%
A sample is reported negative for ER or PgR if < 1% or 0% of tumor cell nuclei are immunoreactive.

Limited data on the overall benefit of endocrine therapies for patients with low level (1-10%) ER expression.



Interpretation of PgR

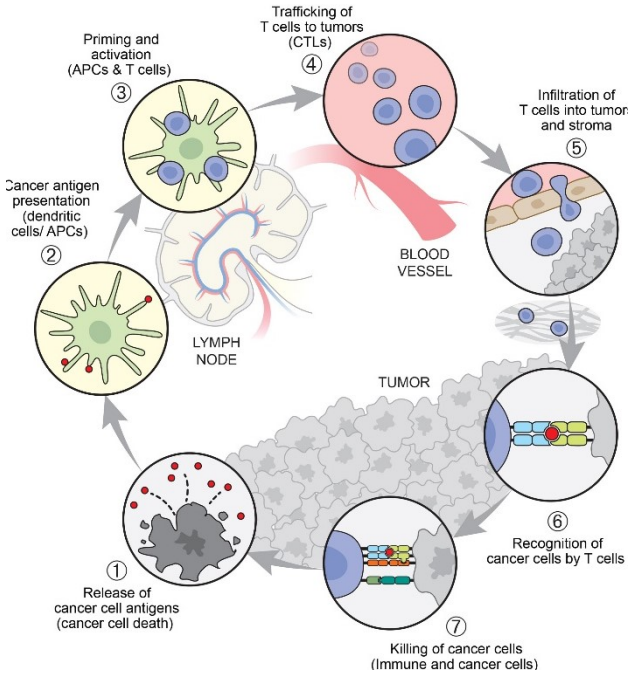




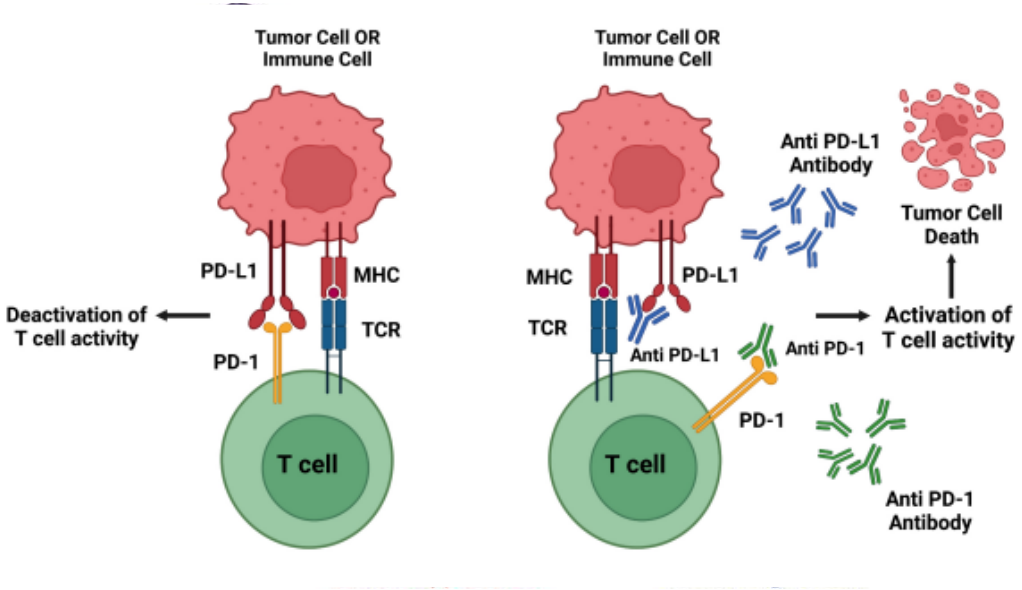
Heterogeneous expression

PD-L1
in metastatic TNBC

PD-L1 in breast cancer

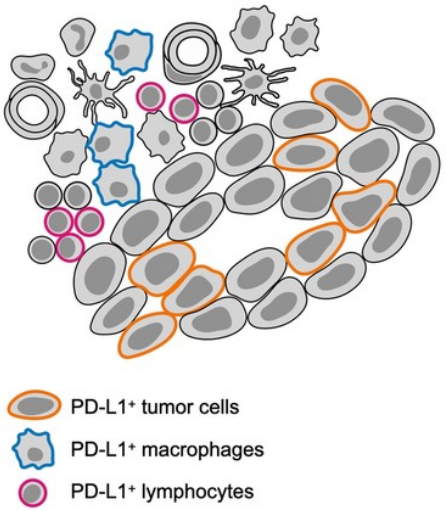


Mellman et al, Immunity, Volume 56, Issue 10, 10 October 2023, Pages 2188-2205



Salgado R et al, Annals of Oncology 2014 Sep 11;26(2):259–271.
Parvez et al, Front. Immunol. 14:1296341. doi: 10.3389/fimmu.2023.1296341

PD-L1 analysis and reporting



$$CPS = \frac{\text{\# PD-L1 positive cells (tumor cells, lymphocytes, macrophages)}}{\text{\# viable tumor cells}} \bullet 100$$

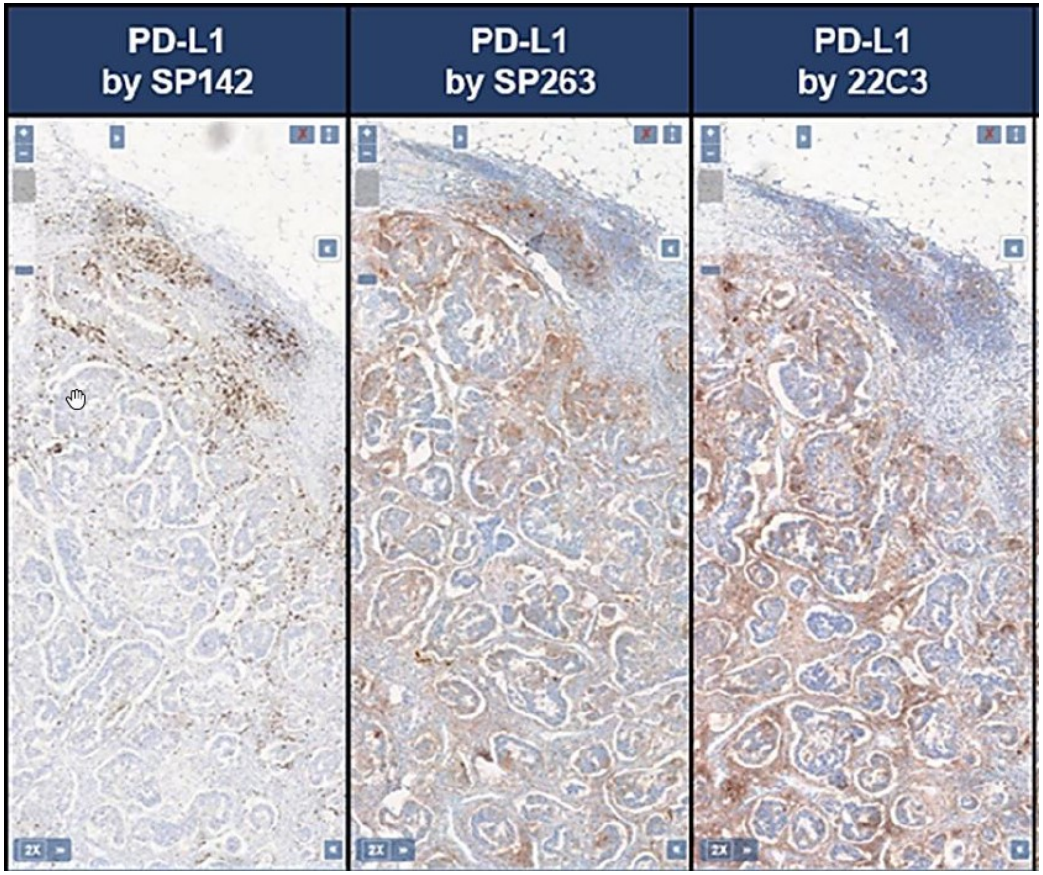
Pembrolizumab (anti-PD-1)
Keynote-355
Assay 22C3

$$IC = \frac{\text{\# PD-L1 positive immune cells}}{\text{\# viable tumor cells}} \text{ X } 100$$

Atezolizumab (anti-PD-L1)
ImPassion130
Assay SP142

PD-L1 is expressed on lymphocytes, macrophages, fibroblasts, tumour cells

Cortes et al. NEJM 2022; Schmid P et al: NEJM 2018 and Lancet Oncol 2020;



Challenges PD-L1

- Reproducebility
- Assay sensitivity
- Assay Interchangeable ?
- Various interpretation guidelines
- Different cut-off levels

DOI: 10.1093/jnci/djr393
Advance Access publication on September 29, 2011.

COMMENTARY

Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group

Mitch Dowsett, Torsten O. Nielsen, Roger A'Hern, John Bartlett, R. Charles Coombes, Jack Cuzick, Matthew Ellis, N. Lynn Henry, Judith C. Hugh, Tracy Lively, Lisa McShane, Soon Paik, Frederique Penault-Llorca, Ljudmila Prudkin, Meredith Regan, Janine Salter, Christos Sotiriou, Ian E. Smith, Giuseppe Viale, Jo Anne Zujewski, Daniel F. Hayes

OXFORD

JNCI J Natl Cancer Inst (2021) 113(7): djaa201

doi: 10.1093/jnci/djaa201
First published online December 28, 2020
Commentary

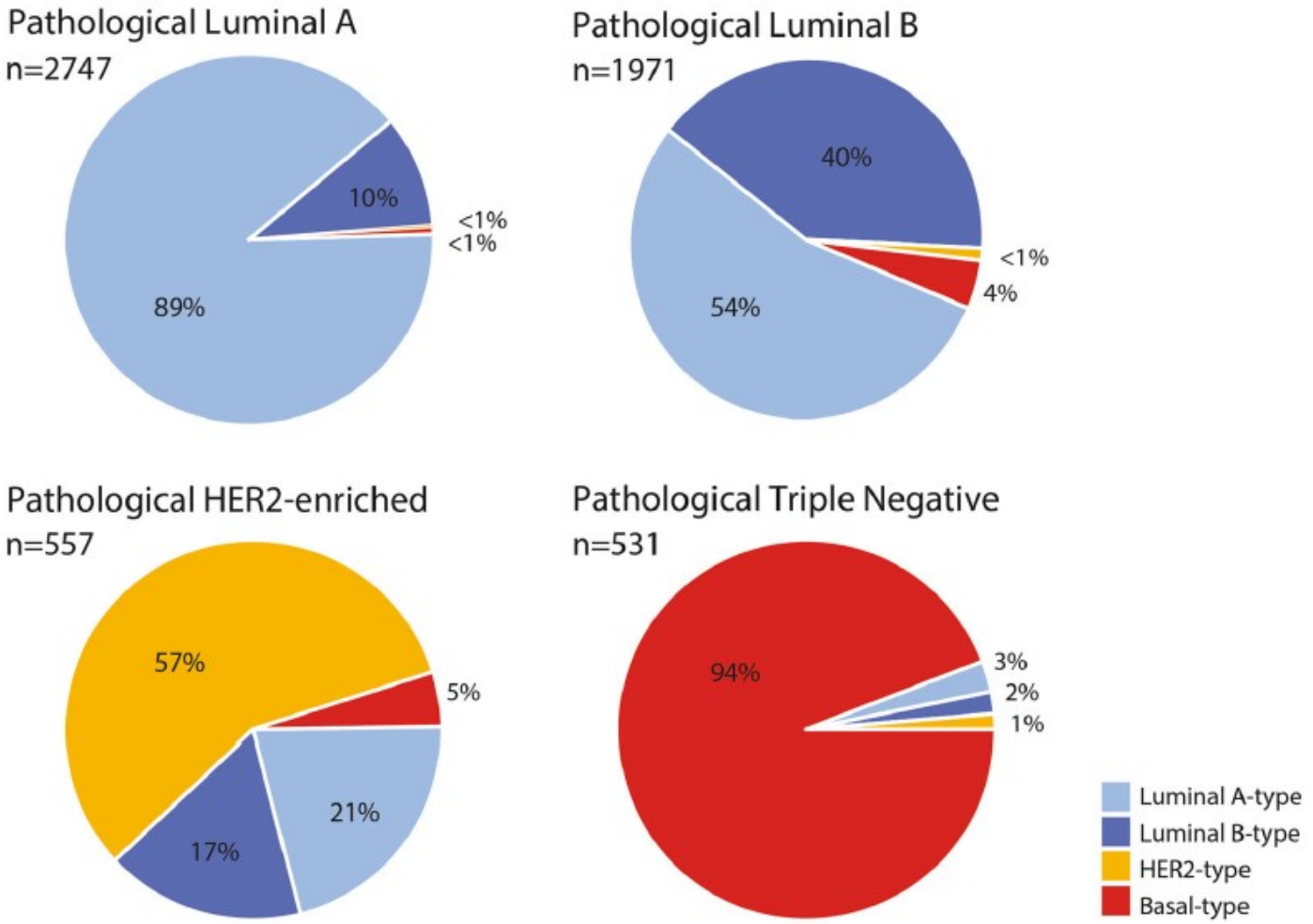
Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group

Torsten O. Nielsen, MD, PhD, FRCPC,[1,*] Samuel C. Y. Leung, MSc,[1] David L. Rimm, MD, PhD,[2] Andrew Dodson, MPhil, FIBMS, CSci,[3] Balazs Acs, MD, PhD,[4,5] Sunil Badve, MBBS, MD, FRCPath,[6] Carsten Denkert, MD,[7] Matthew J. Ellis, MB, BChir, BSc, PhD, FRCP,[8] Susan Fineberg, MD,[9] Margaret Flowers, PhD,[10] Hans H. Kreipe, MD,[11] Anne-Vibeke Laenkholm, MD,[12] Hongchao Pan, PhD,[13] Frédérique M. Penault-Llorca, MD, PhD,[14] Mei-Yin Polley, PhD,[15] Roberto Salgado, MD, PhD,[16,17] Ian E. Smith, MD, FRCP, FRCPE,[18] Tomoharu Sugie, MD, PhD,[19] John M. S. Bartlett, BSc, PhD, FRCPath,[20,21] Lisa M. McShane, PhD,[22] Mitch Dowsett, BSc, PhD[23,] Daniel F. Hayes MD[24,]

Table 4. Recommendations for Ki67 in postmenopausal ER+ HER2 normal BC since St. Gallen 2009

Year	Recommendations for decision making regarding adjuvant chemotherapy	Ref.
2009	3 categories low <15%, intermediate 16–30% and high >30%.	[188]
2011	Approximation of molecular subtypes with Ki67 cut off: 14%.	[24]
2013	Classification of subtypes with Luminal A: ER+, PR ≥20% and Ki67 <20%, HER2-. Luminal B: ER+ and PR<20% and/or Ki67≥20%, HER2-.	[25]
2015	Threshold value of Ki-67 within the range of 20%–29% to distinguish 'luminal B-like` subtype.	[222]
2017	"low" ki67 versus "high" ki67.	[223]
2019	Recommendation of genomic testing. Caution when applying surrogate markers due to lack of validity.	[224]
2021	Ki67 ≤5% do not receive chemotherapy, whereas tumors with Ki67 ≥30% receive chemotherapy. Genomic testing is advised for the Ki67 interval > 5% to < 30%.	[79]
2023	Genomic signatures can define chemotherapy benefit in ER+, HER2 normal patients where the indication for chemotherapy is uncertain.	[225]

Correlation between IHC subtype and molecular subtype



Additional analyses

- Metastatic lesions
  - i.e. CK7, CK8/18 (if TNBC)
  - GCDFP
  - Mammaglobin
  - GATA-3 (obs, only app. 60% of TNBC positive)
  - TRPS-1 (higher positivity rate in TNBC)
  - ER
  - HER2 (re-analysis)
  - If indicated NGS panel

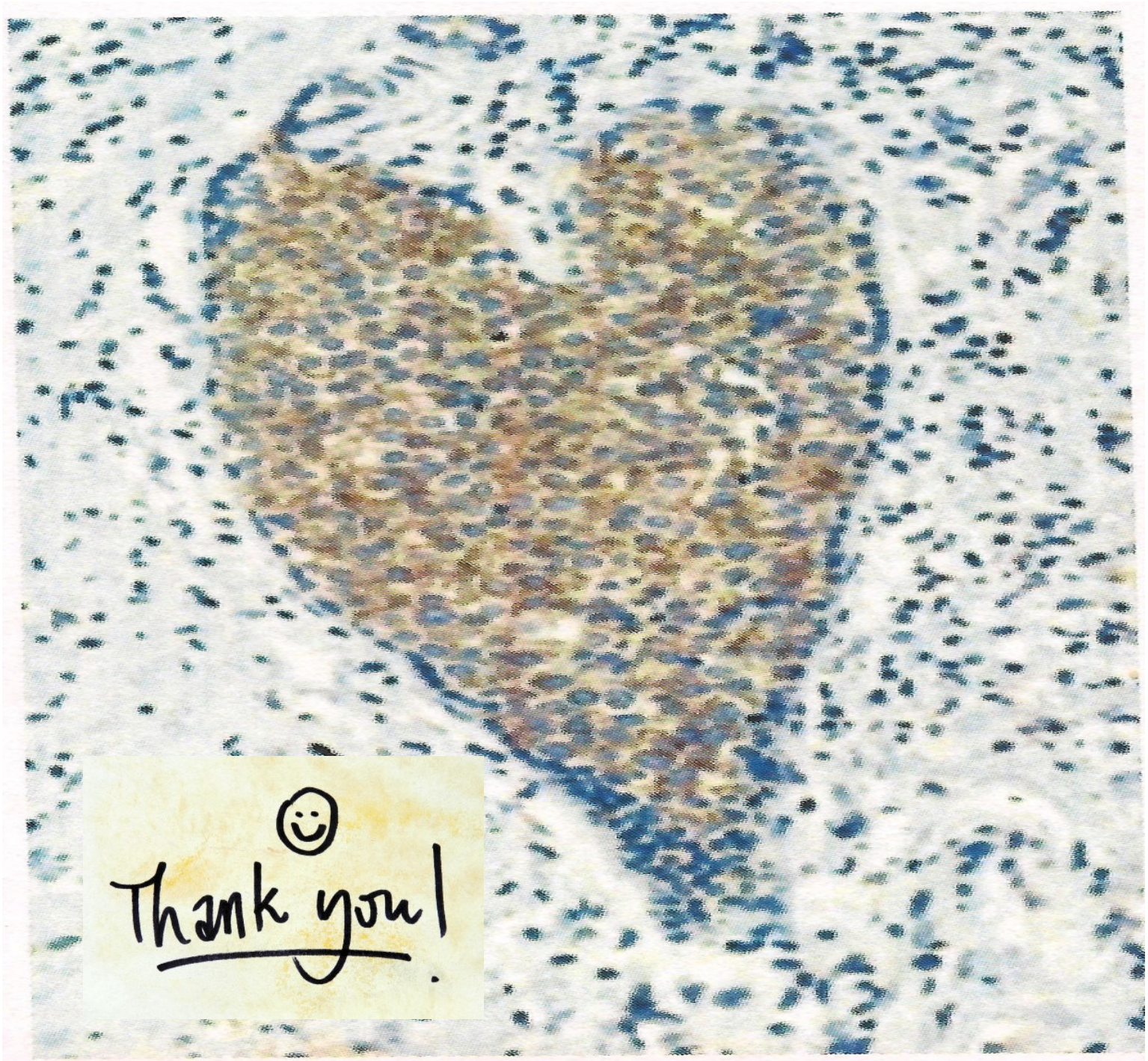
- Diagnostic additional biomarkers
  - Androgen Receptor (diagnostic for aprocrine carcinoma – potential target for treatment in the metastatic setting)
  - Synaptophysin + other neroendocrine markers (neuroendocrine differentiation – no treatment implication)

In conclusion
IHC for diagnostic use in breast tumors

- *A valuable supplement for the diagnosis of ”benign versus in situ” and ”in situ versus invasive”*

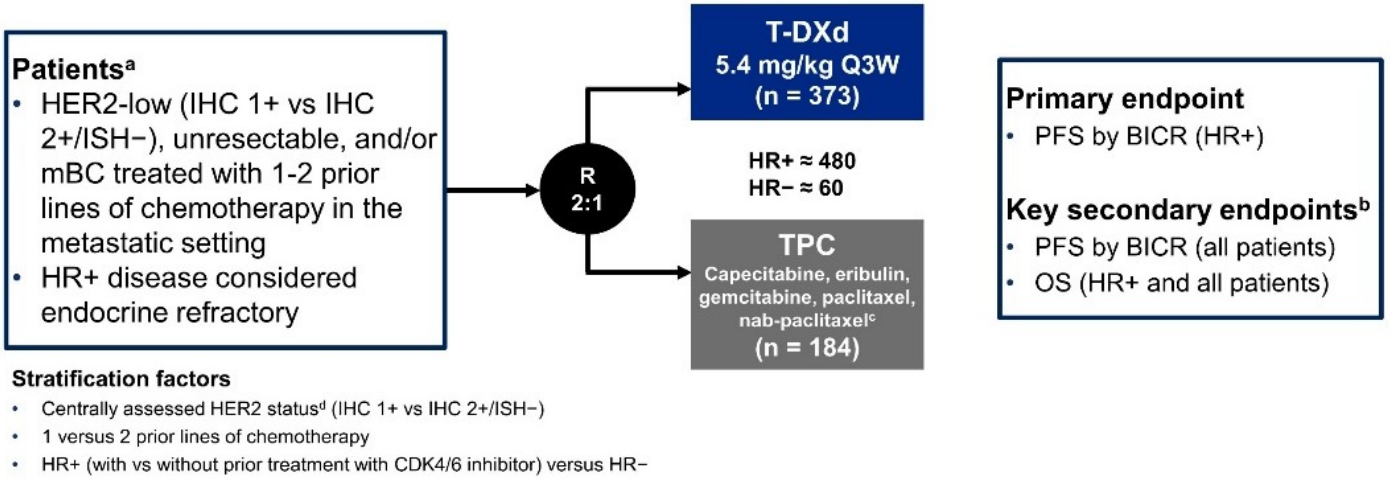
- *Histopathological classification of malignant breast tumors*
  - Treatment allocation (IDC vs ILC)

- *Prognostic and predictive factors*
  - Assay, interpretation and treatment
  - Repeat analysis on metastatic lesions


Thank you!

DESTINY-Breast04: First Randomized Phase 3 Study of T-DXd for HER2-low mBC

An open-label, multicenter study (NCT03734029)



Stratification factors

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

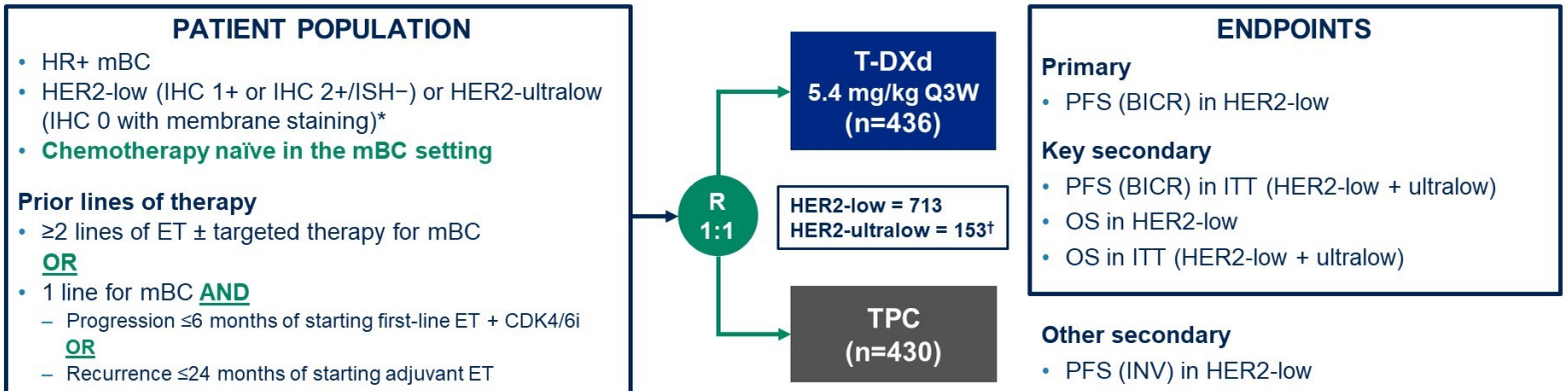
N Engl J Med. 2022 PMID: 35665782

DESTINY-Breast06

5

Study design

DESTINY-Breast06: a Phase 3, randomized, multicenter, open-label study (NCT04494425)



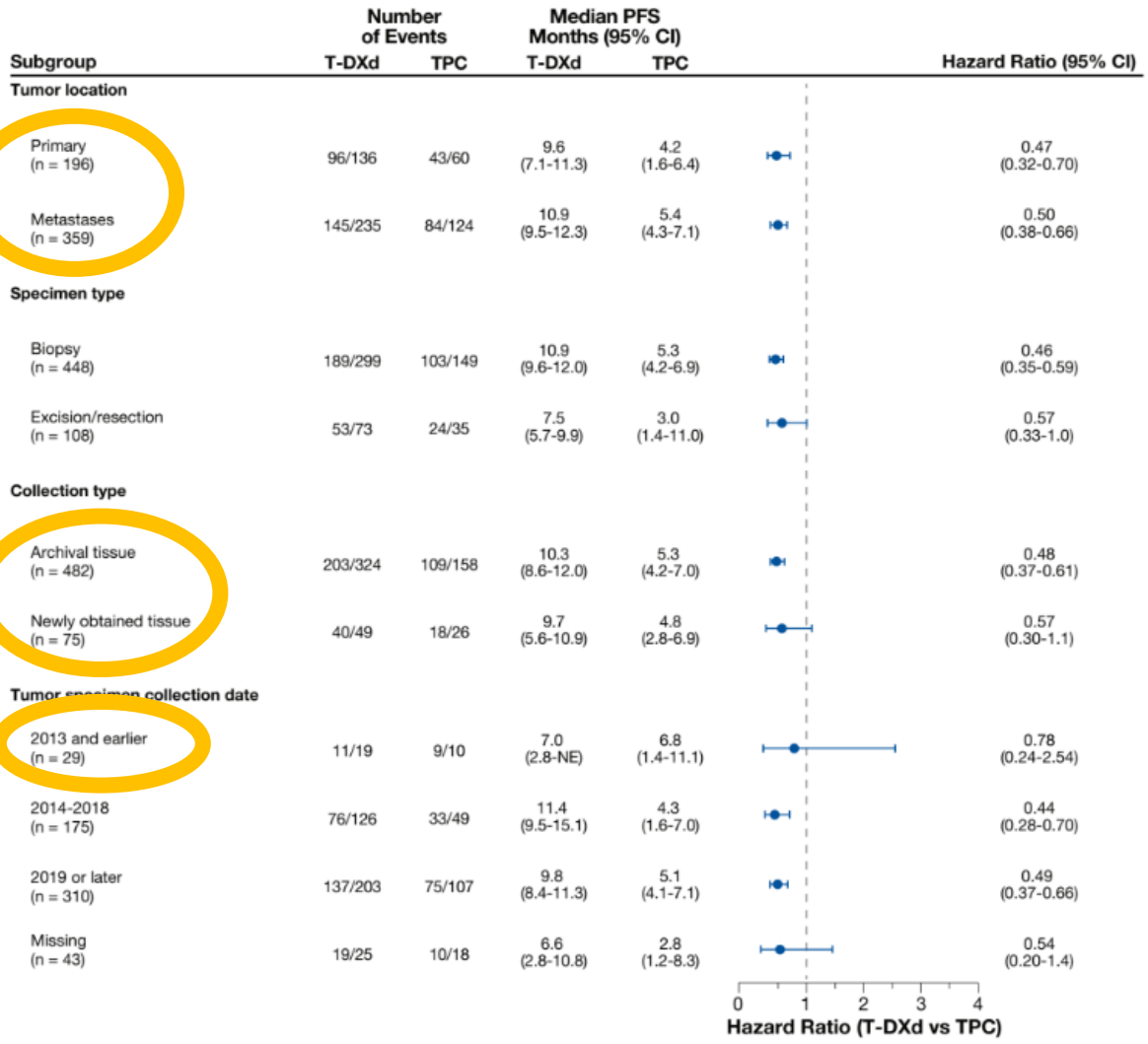
Stratification factors

- Prior CDK4/6i use (yes vs no)
- HER2 expression (IHC 1+ vs IHC 2+/ISH− vs IHC 0 with membrane staining)
- Prior taxane in the non-metastatic setting (yes vs no)

Tumor sample characteristics Destiny Breast04

35% primary tumors,
65% metastatic lesions
10% new biopsy

For patients enrolled in DESTINY-Breast 04, efficacy of T-DXd compared with TPC was consistent regardless of tumor sample characteristics



Cancer Res (2023) 83 (5_Supplement)
Virchows Archiv https://doi.org/10.1007/s00428-023-03671-x

DESTINY-Breast04 Clinical Trial | ENHERTU® (fam-trastuzumab deruxtecan-nxki) | For HCPs (scoreher2low.com)