

# Breast cancer: IHC for diagnostic use

**NordiQC: Seminar in Diagnostic Immunohistochemistry 2026**  
**Aalborg University Hospital**  
**September 23-25**

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# Breast cancer mortality at ages 35-69

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

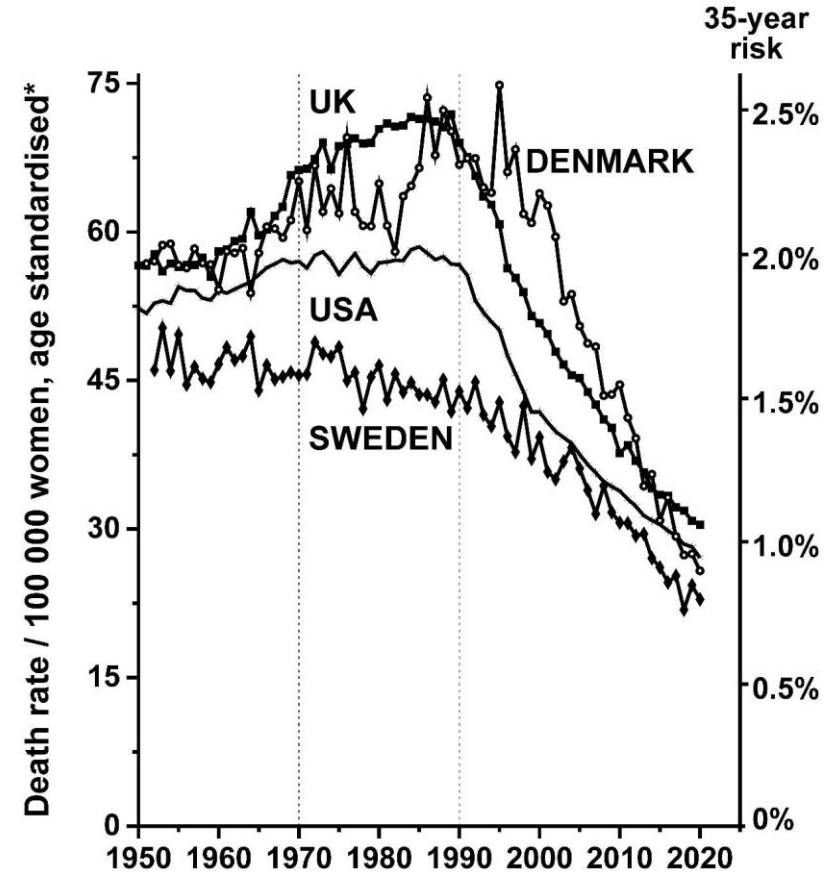
Population based data reveals markedly  
improved breast cancer outcomes in UK,  
USA, Sweden and Denmark *Slide courtesy  
Jonas Berg*

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

# Current risk stratification markers in early breast cancer (Denmark)

- Age
- 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

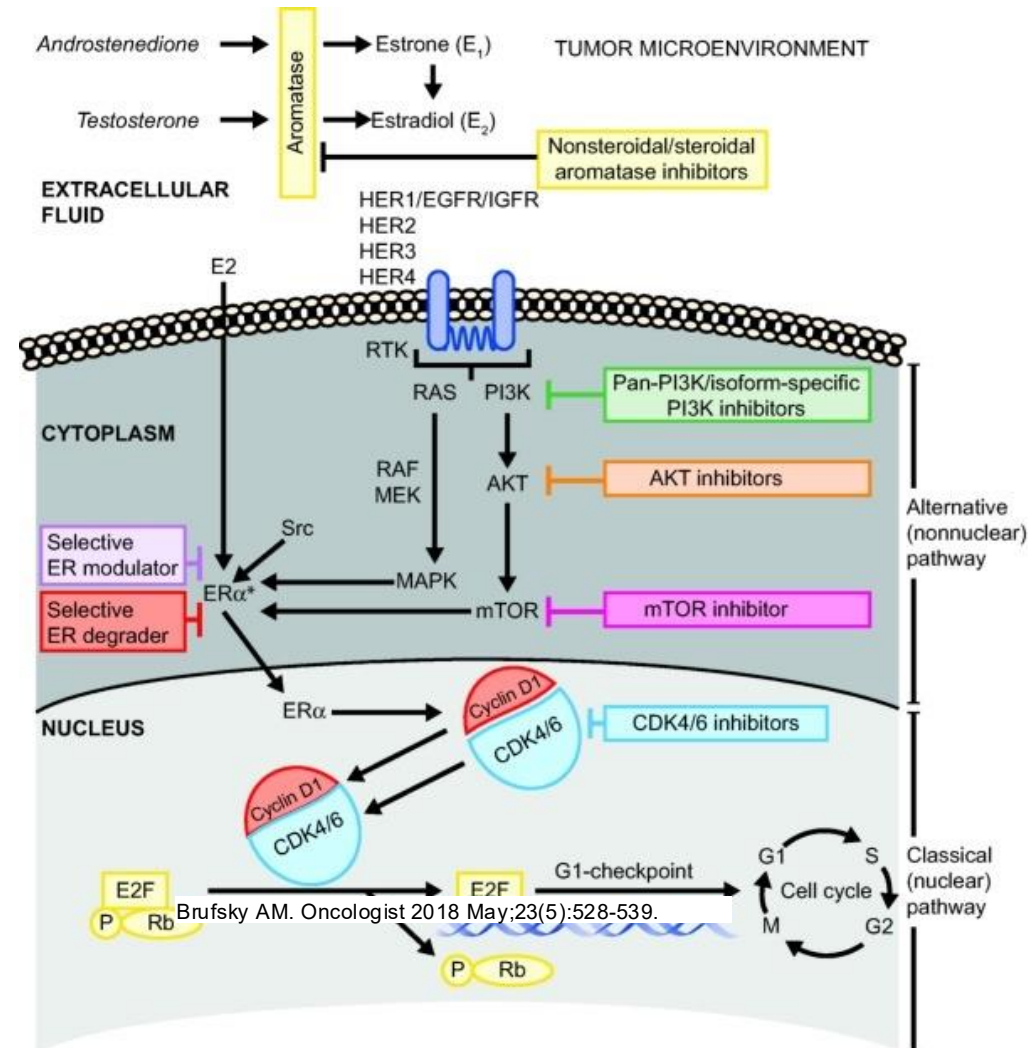
- ER,
- HER2
- PD-L1

*Additional biomarkers for treatment allocation with i.e.*

*Inavolisib (PIK3CA mutation)*

*Elacestrant (ESR1 mutation)*

*Capivasertib (PIK3CA, AKT, PTEN)*

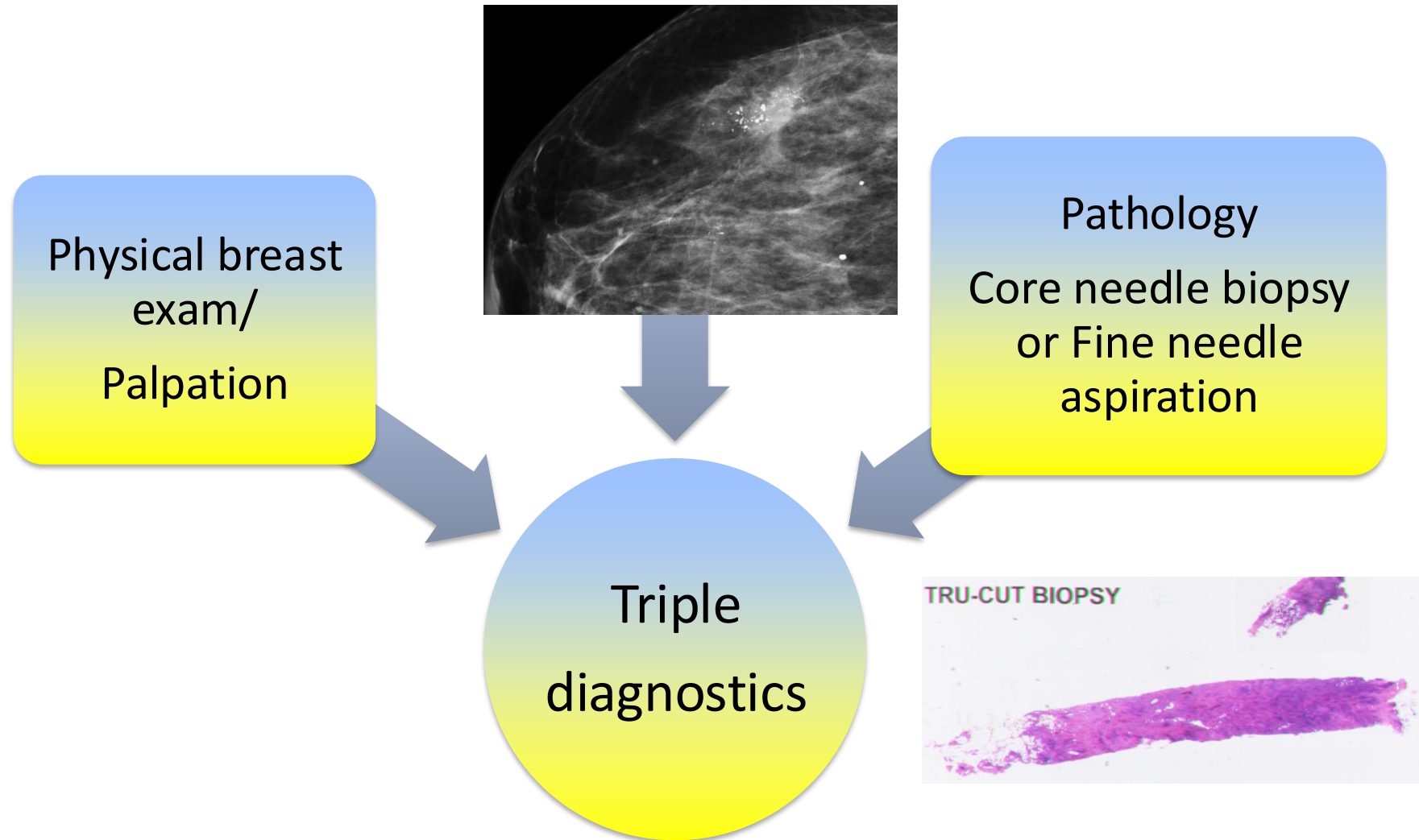


# Agenda

- 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)

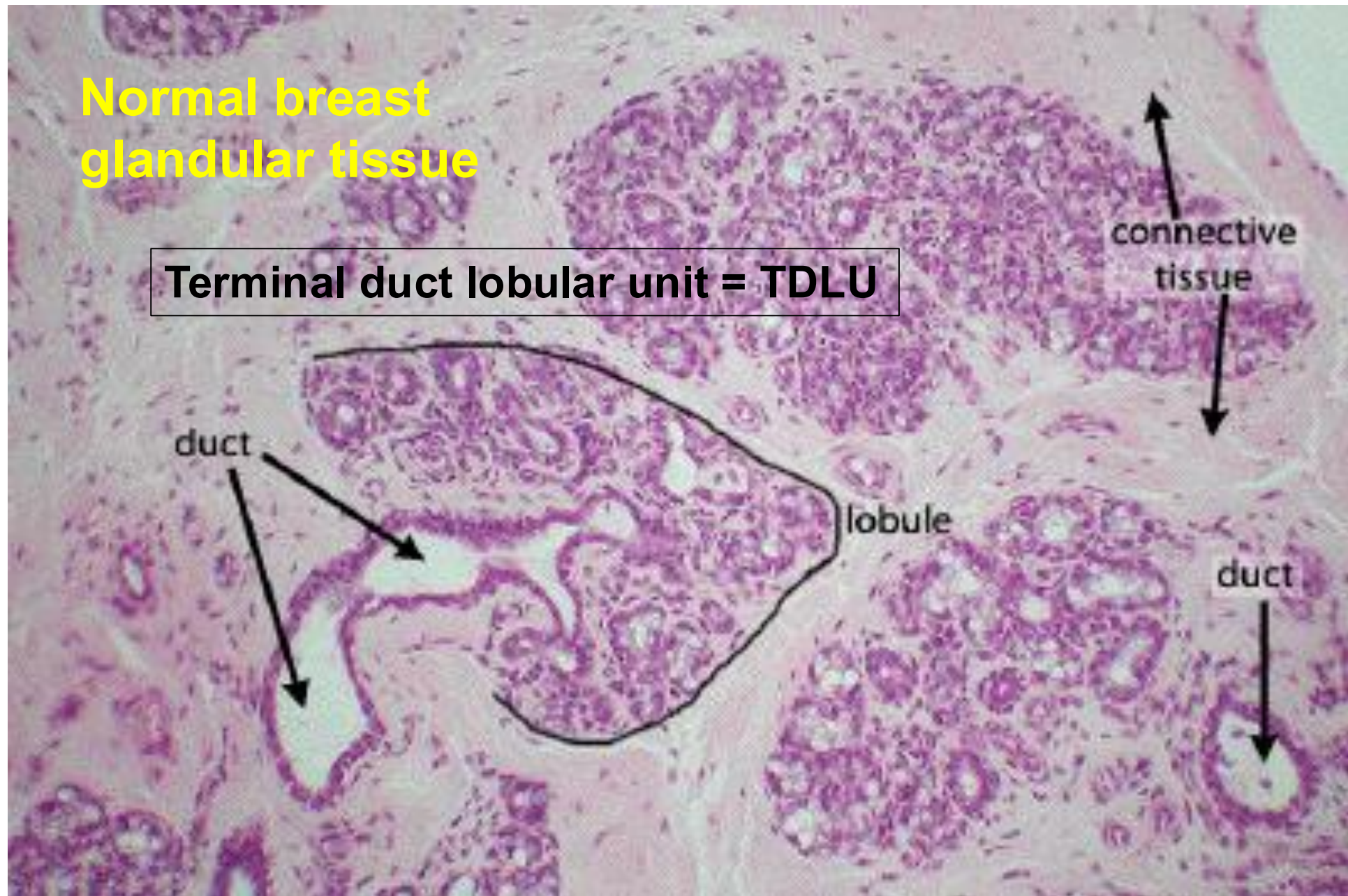
# Triple Test

## Diagnostic approach – Breast Tumours

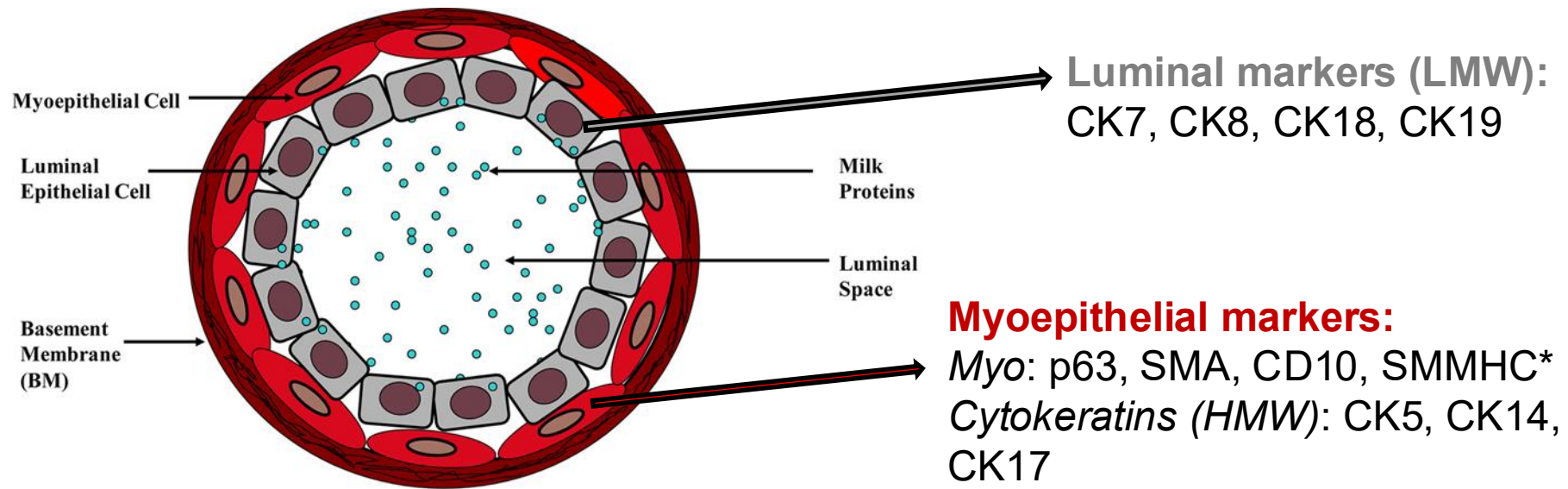


## Normal breast glandular tissue

Terminal duct lobular unit = TDLU



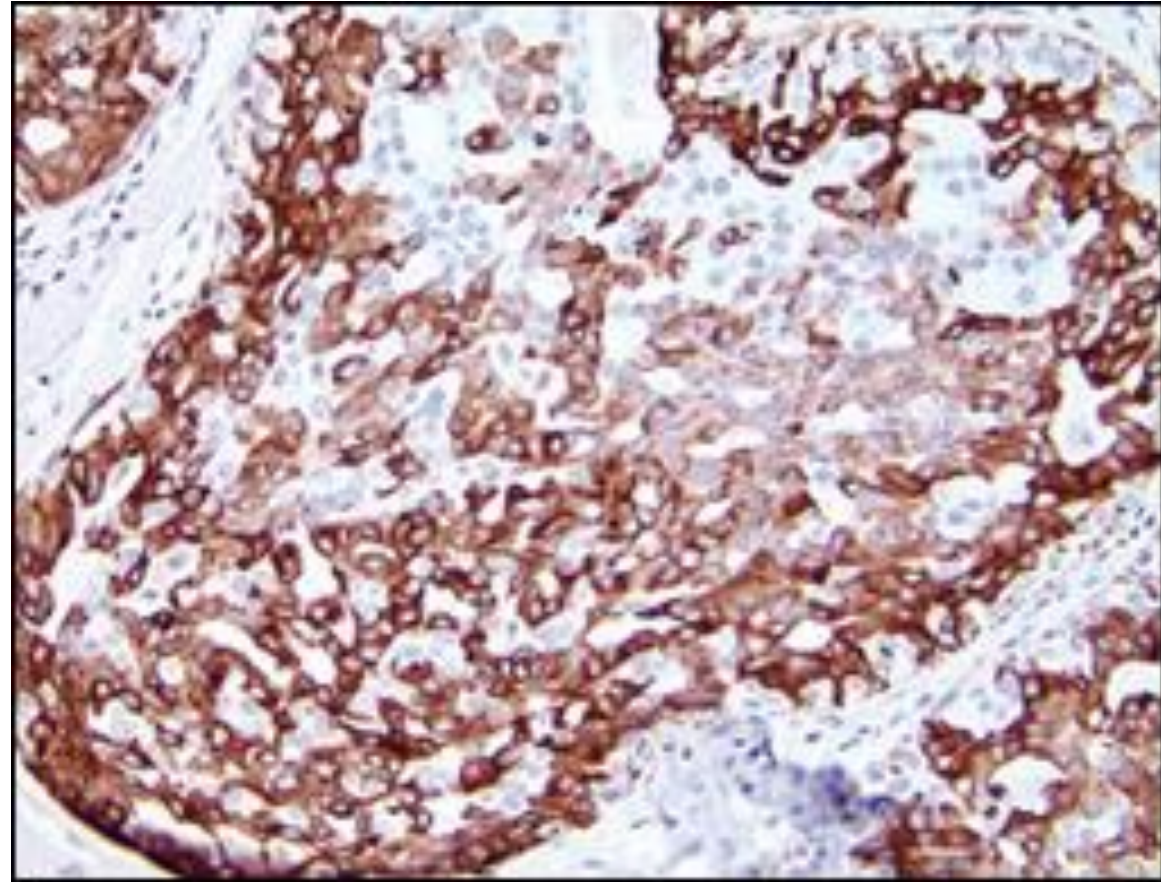
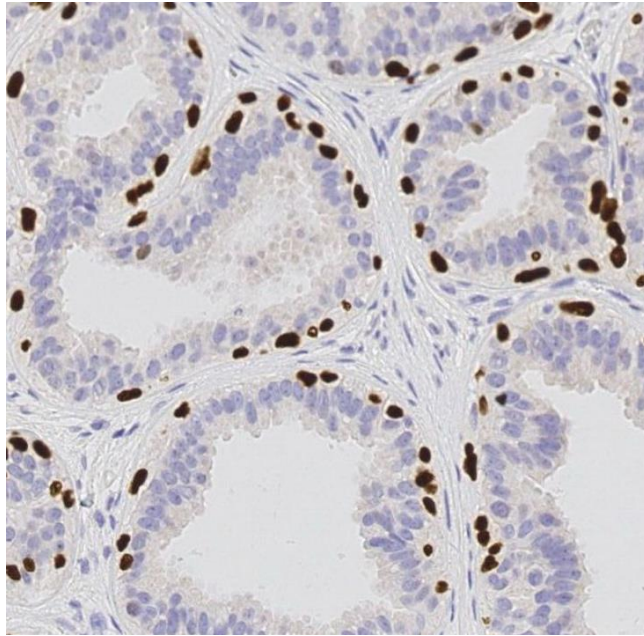
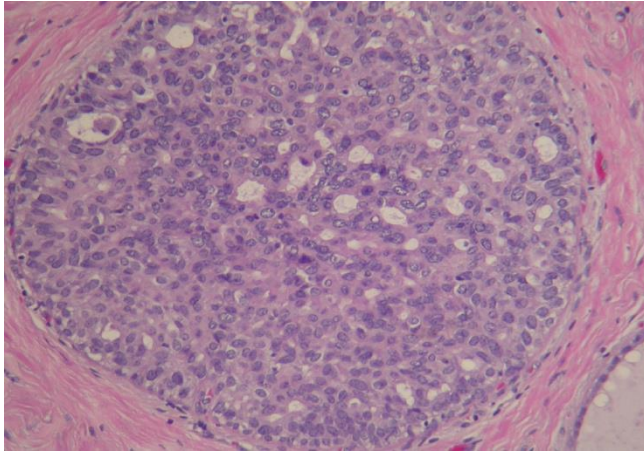
# Epithelial cells with specific immunohistochemical phenotype



\*Smooth muscle myosin heavy chain

# Benign hyperplasia

## Positive staining for myoepithelial cells

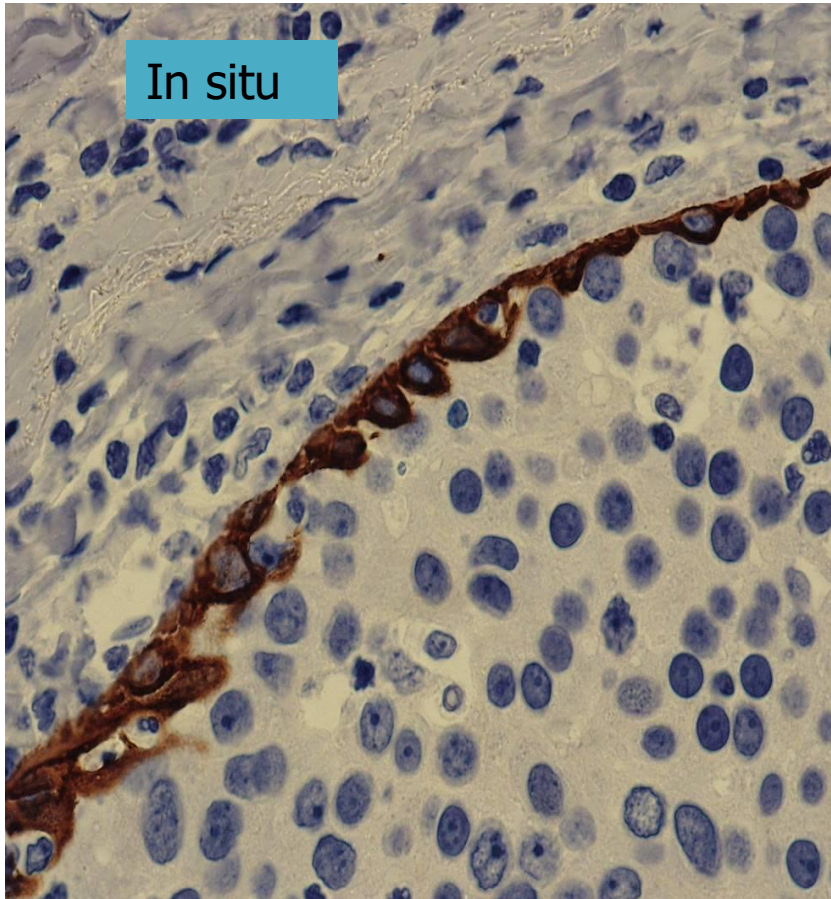


P63

CK5

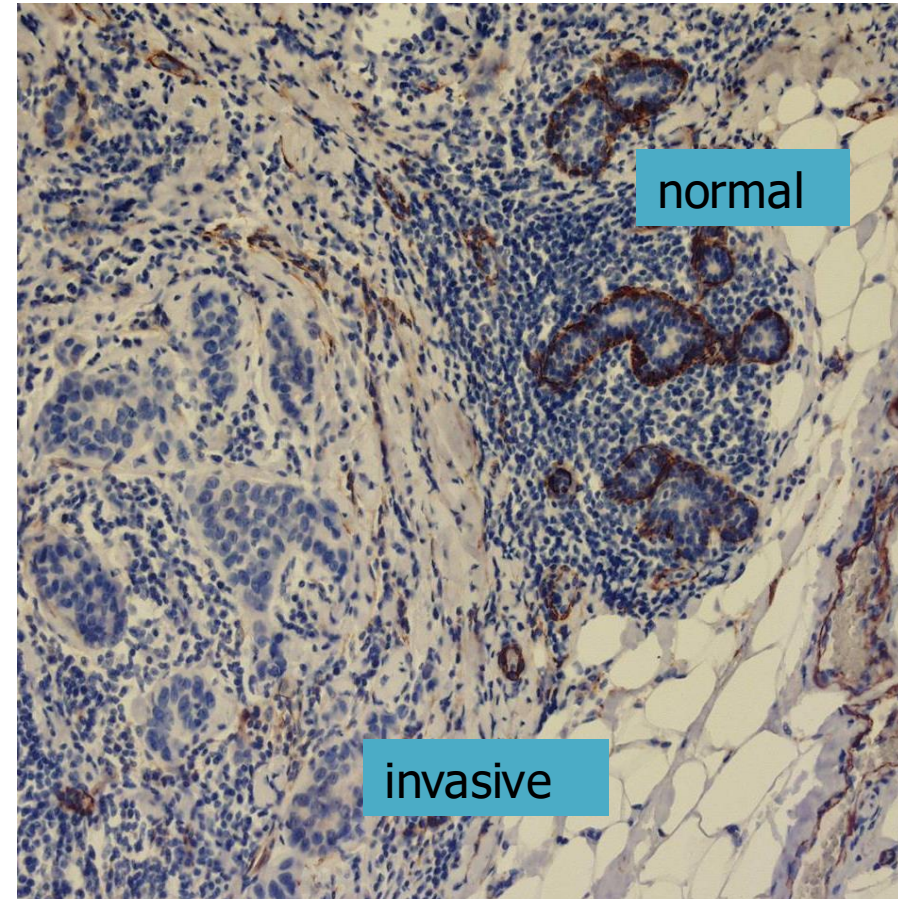
# Differentiation between ductal carcinoma in situ and invasive carcinoma i.e. SMMHC\*

present



Detecting "presence"

Not present

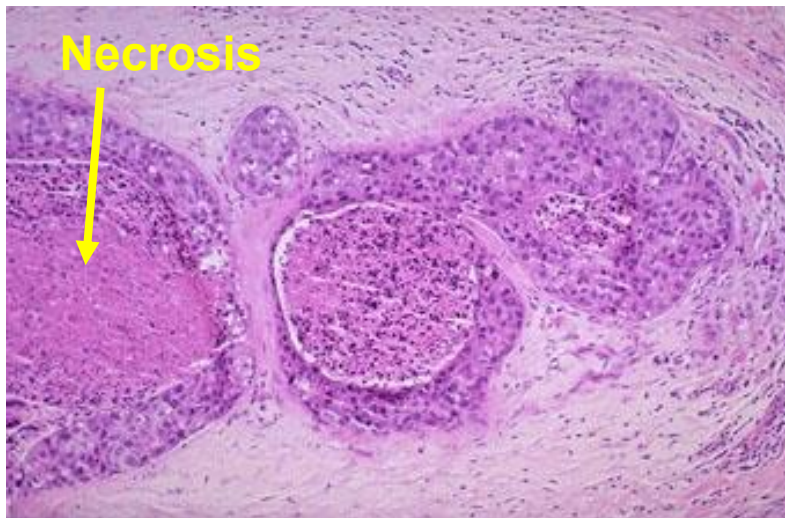


Detecting "absence"

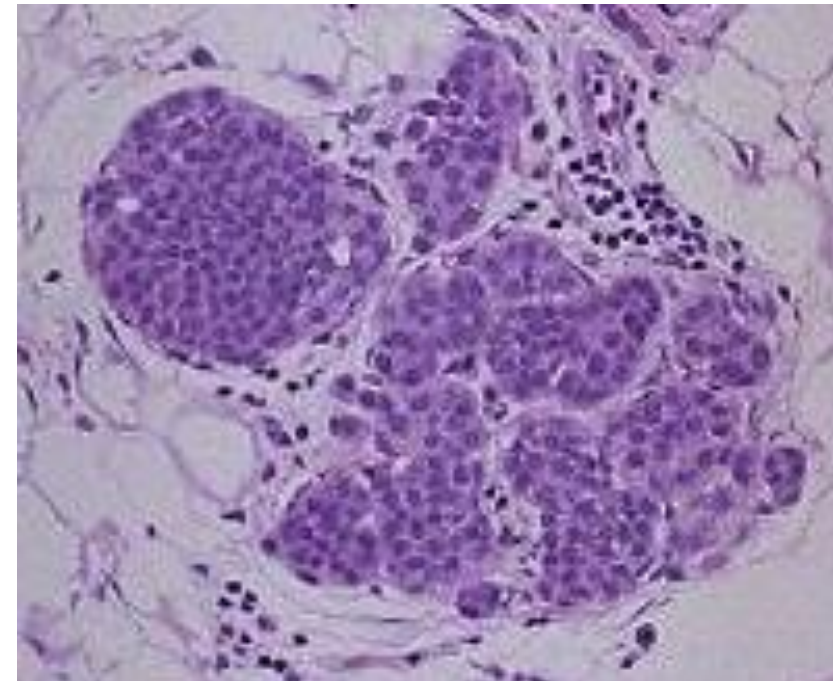
\* Smooth muscle myosin heavy chain, as detected with clone SMMS-1

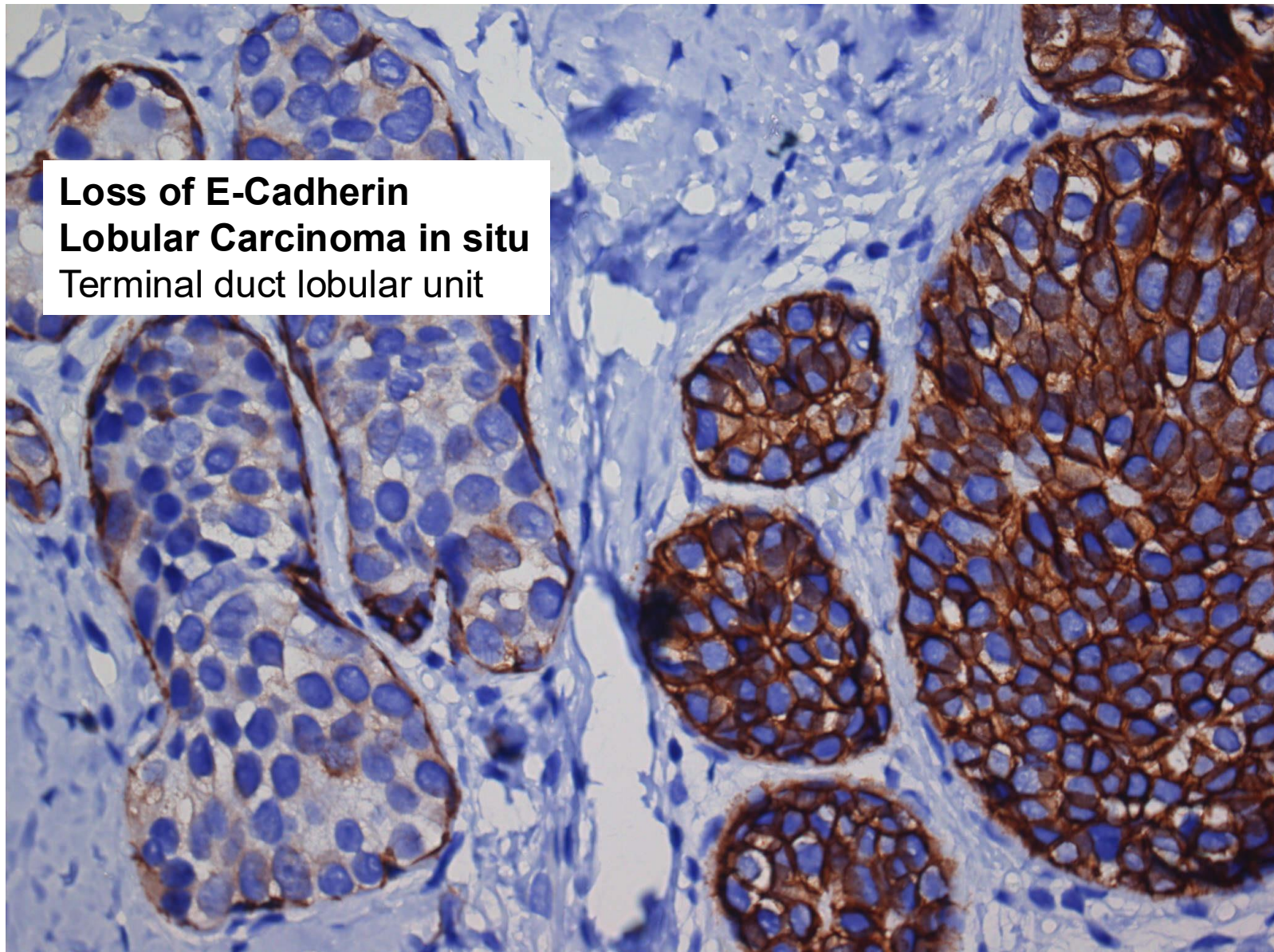
# Carcinoma in situ

- 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





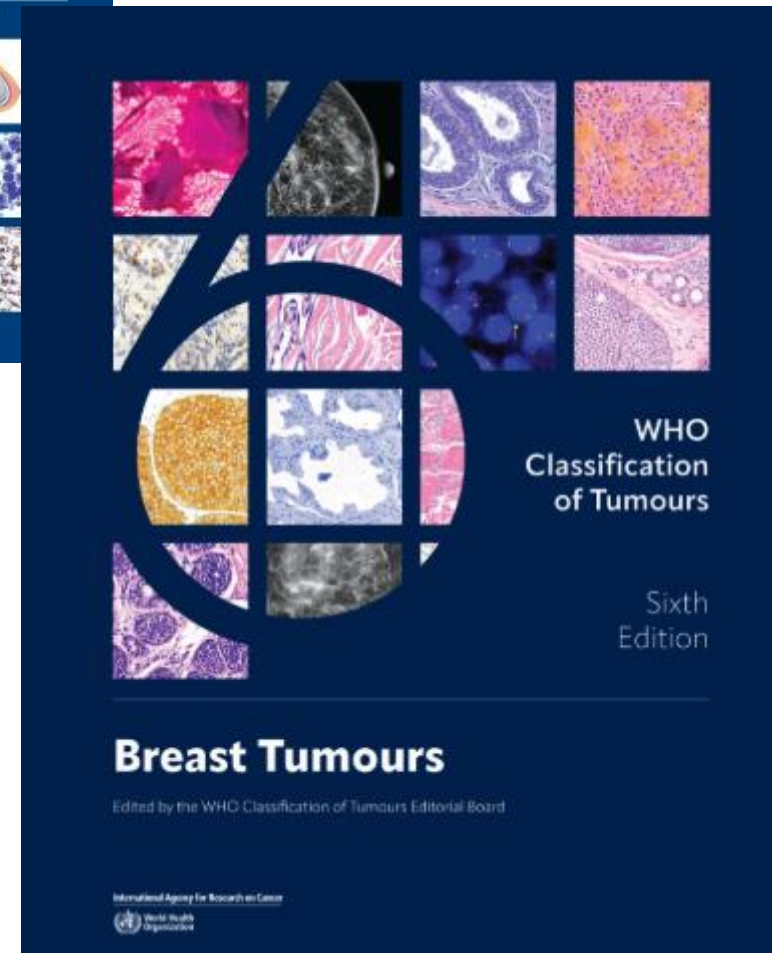
**E-cadherin: Cell Adhesion Molecule**

# Classification of malignant tumors of the breast

## WHO blue books

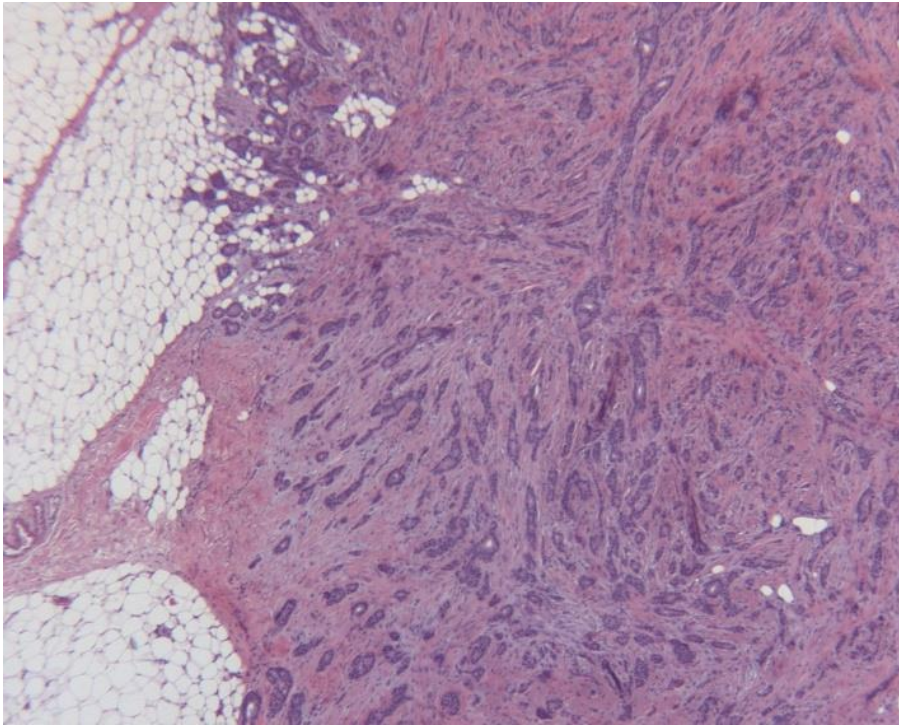
### Histological subtypes (>20)

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

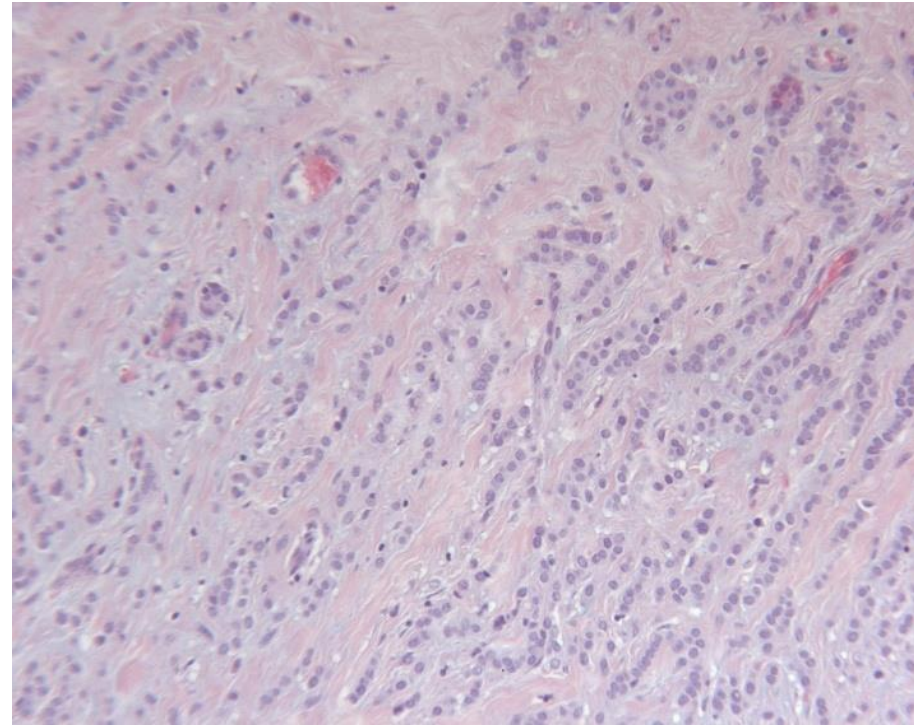


# Histological Subtypes

**Ductal Carcinoma (Invasive carcinoma of no special type)**



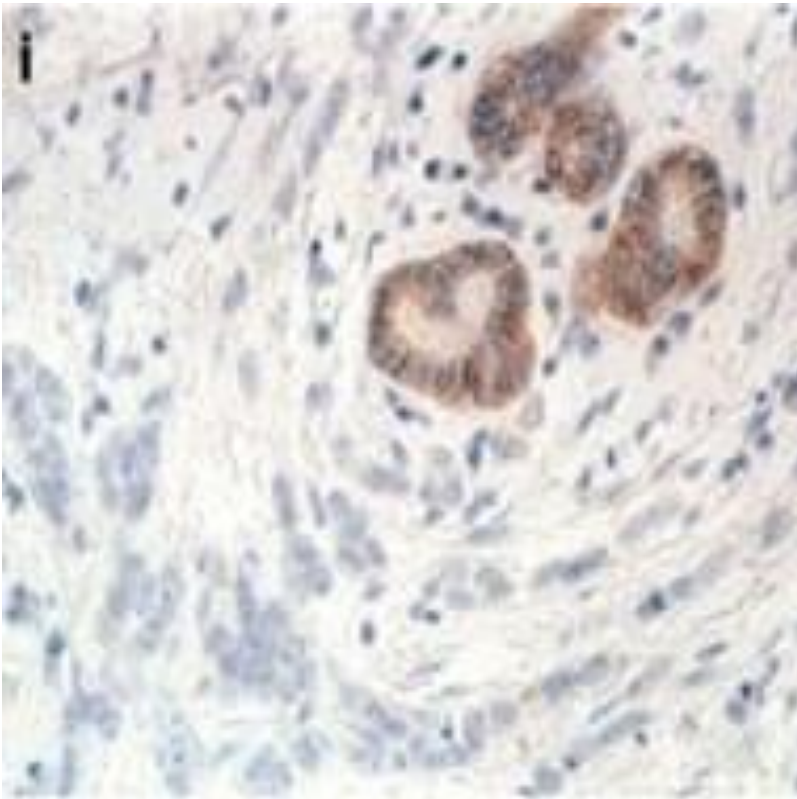
**Lobular Carcinoma**



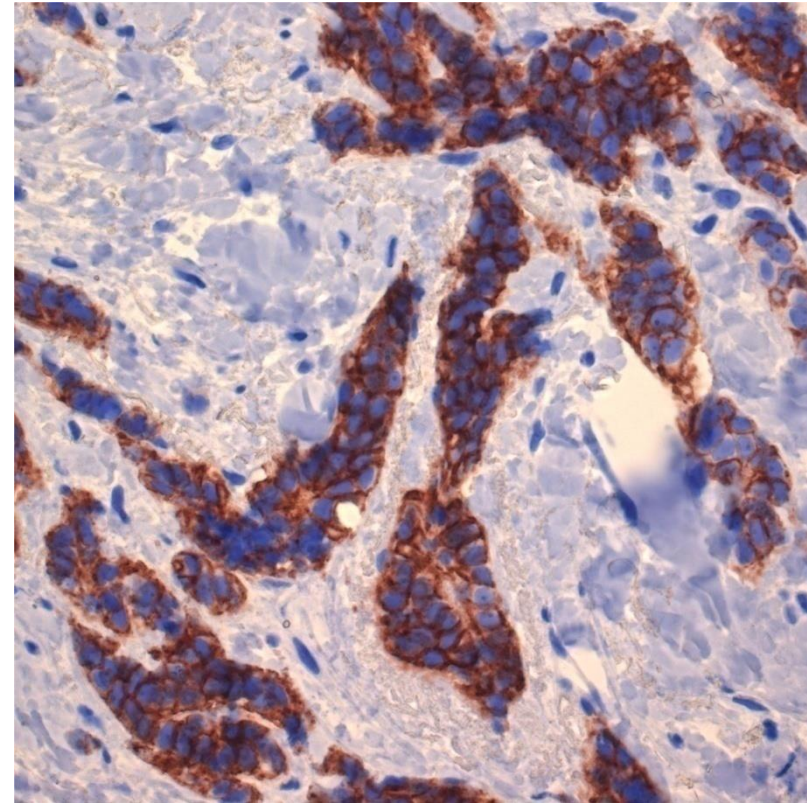
# E-Cadherin

## Cell adhesion molecule

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

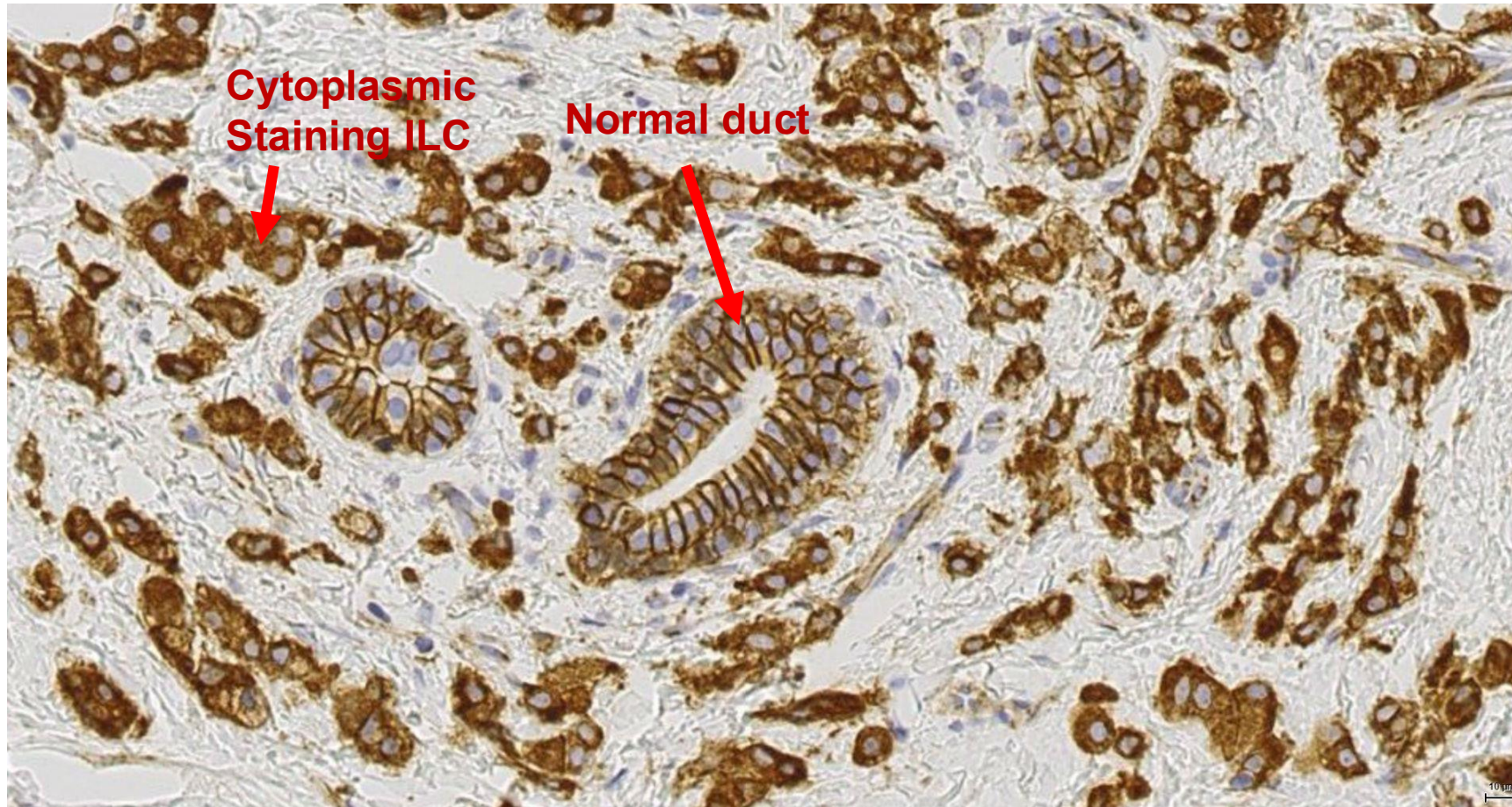


E-Cadherin positive  
Invasive Ductal Carcinoma



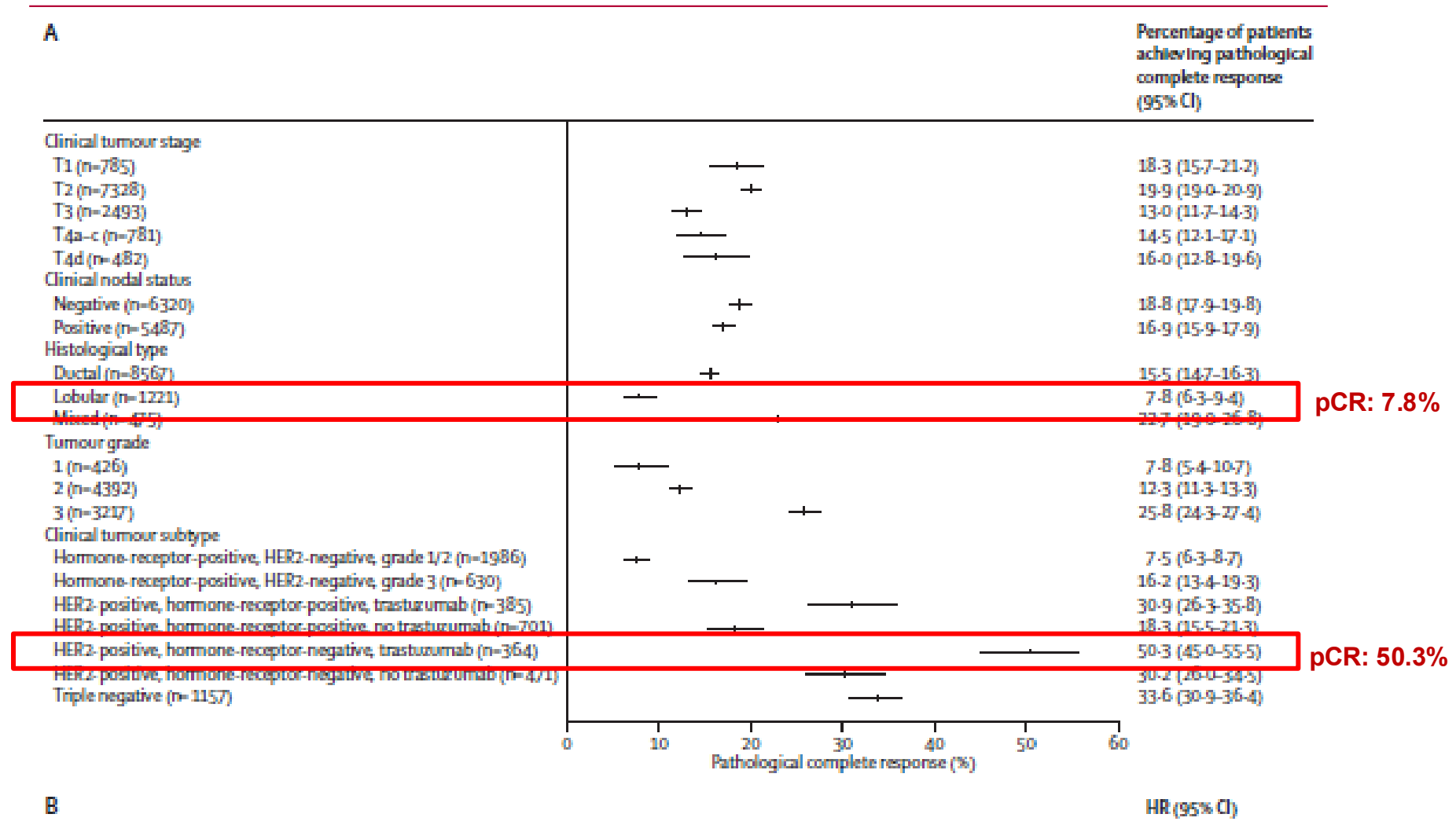
*CDH1* (16q22.1) loss of function mutation or deletion resulting in loss of the adhesion molecule E-cadherin

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



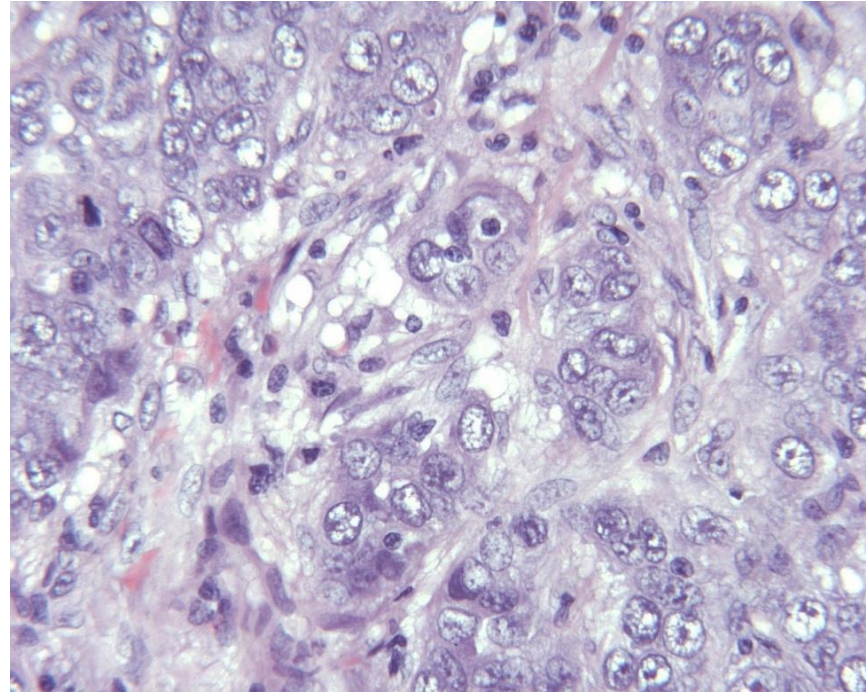
Lobular cancer - not candidate for neoadjuvant chemotherapy  
Low proliferating tumors, often luminal A molecular subtype

## Tumor characteristics and association with pCR Neoadjuvant chemotherapy (NACT)

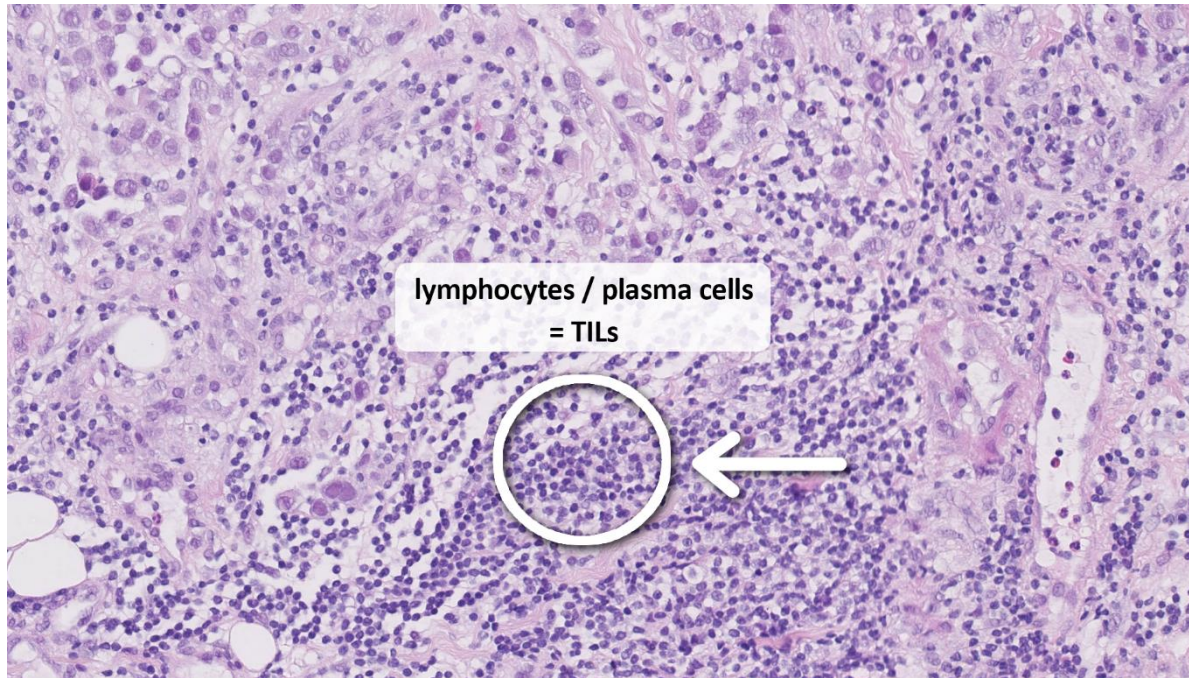


# TNBC : 8-10% of primary breast cancers

- ER, PR and HER2 negative
- Heterogeneous group of tumours
- High grade
- Younger age at diagnosis
- Poor prognosis
- Risk of germline mutation (ie BRCA)



# 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 although being triple negative (Figure 2 and 3).

Cserni G et al. Cancers 2021, PMID: 34830849

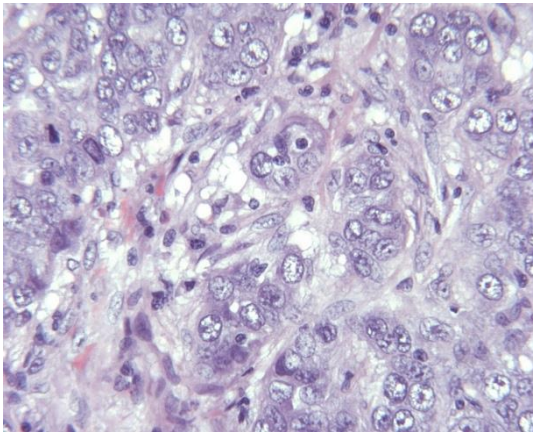


Figure 1  
High grade IDC

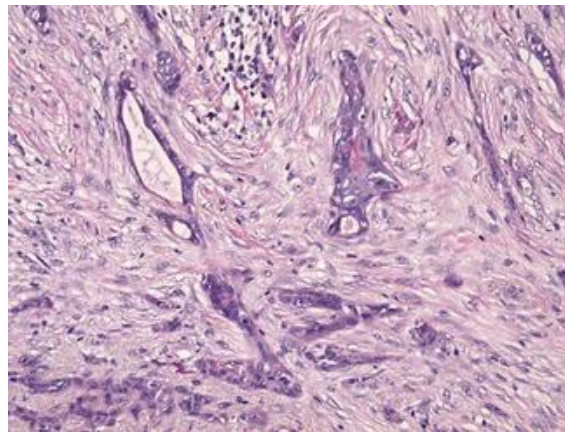


Figure 2  
Low grade adenosquamous carcinoma (subtype of metaplastic carcinoma)  
luminal (CK7, CK8) and basal (CK5, CK14) CKs and squamous (myoepithelial) markers p63 and p40.

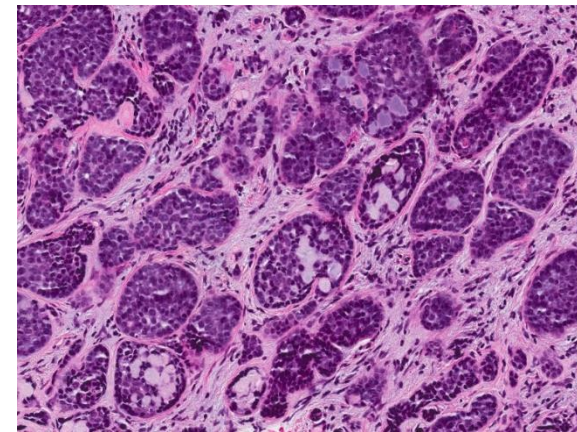
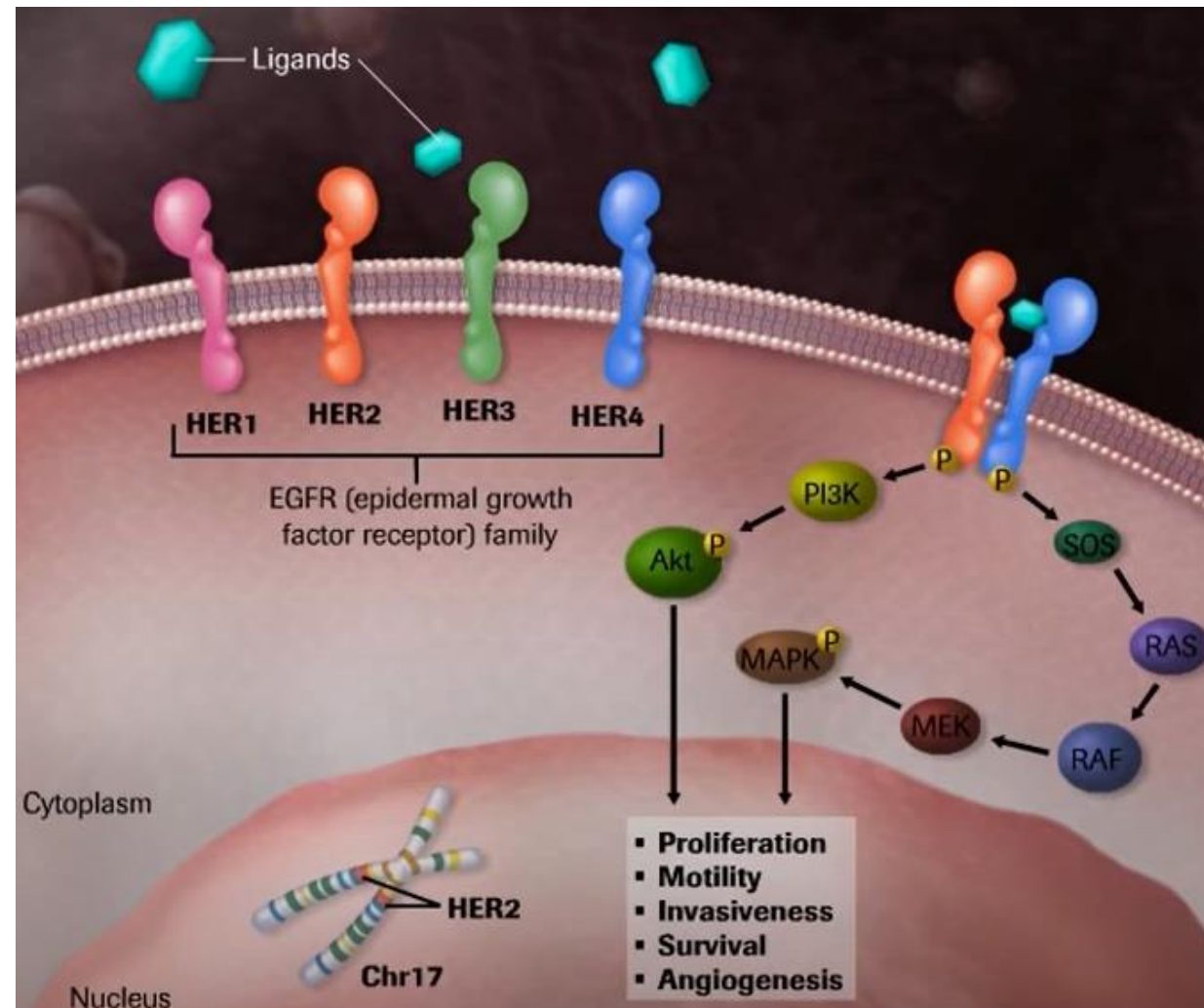


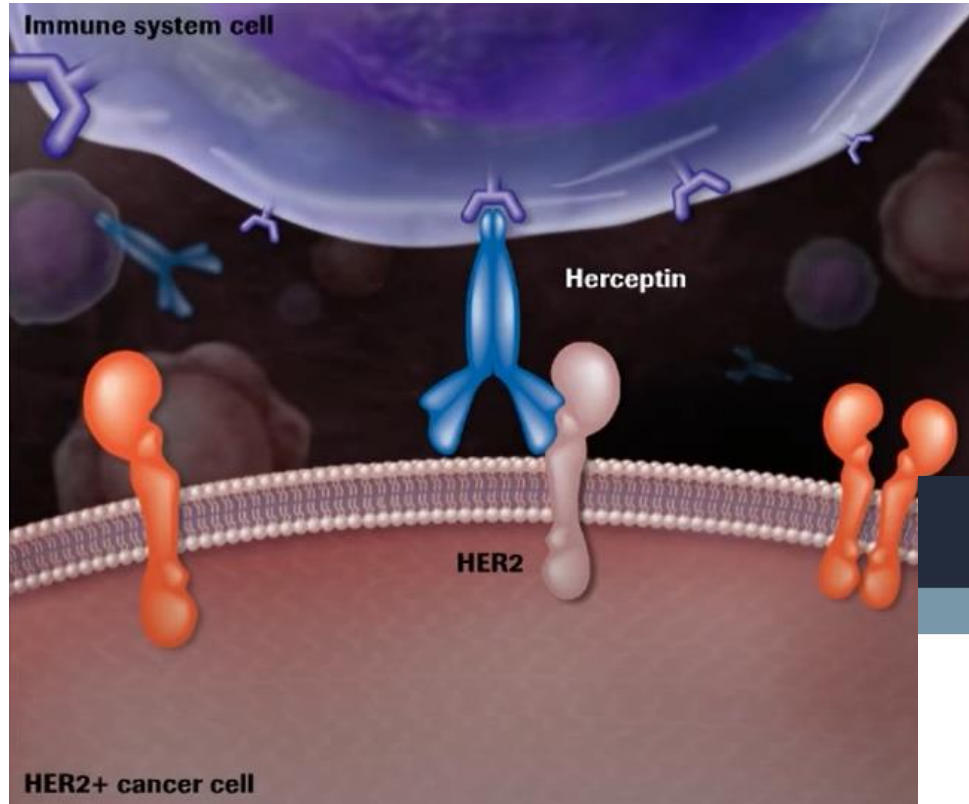
Figure 3  
Adenoid cystic carcinoma of the breast. The cells of the epithelial component are positive for CK7, CK5/6, CK 8/18 and CD117. The myoepithelial /abluminal cells express p63, smooth muscle actin and basal CKs: CK5/6, CK14, CK17.

# 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



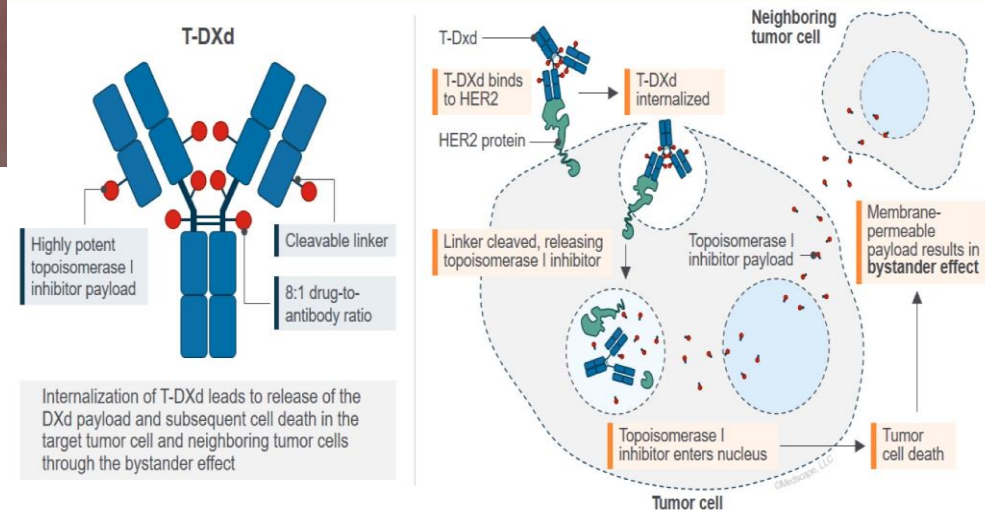
# Targeting the HER2 receptor



- 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

## Trastuzumab Deruxtecan (T-DXd)

### Structure and Mechanism of Action



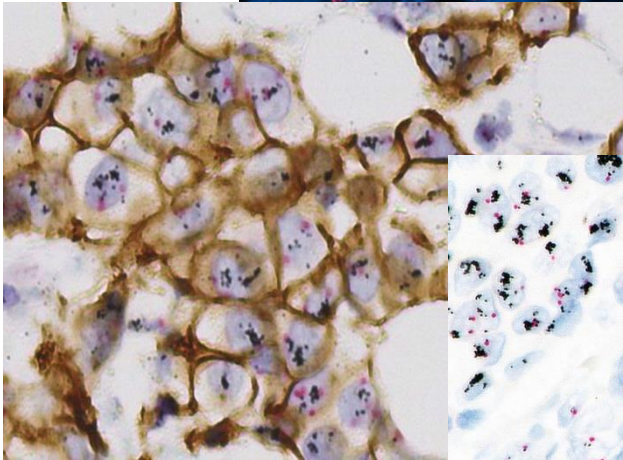
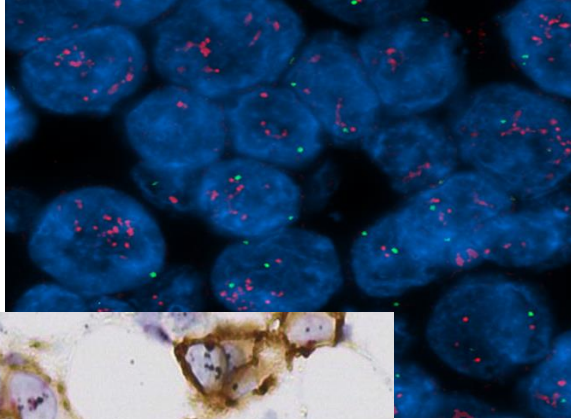
# ASCO CAP guidelines

## 2007, 2013, 2018, Update 2023

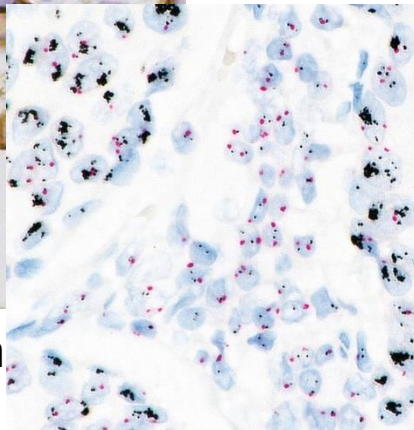
| HER2 SCORE            | ASCO/CAP 2007                                                                                                                           | ASCO/CAP 2013                                                                                                                           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

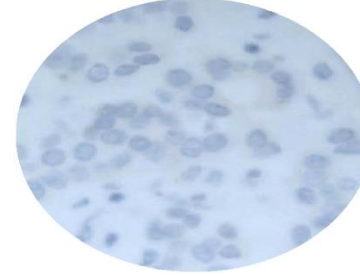


HER2 Gene/Protein

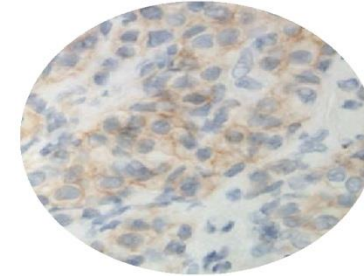


Dual ISH

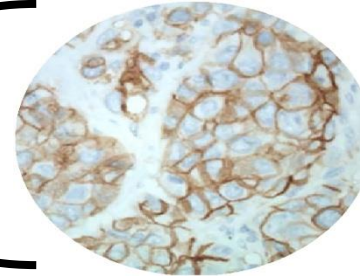
0



1+



2+

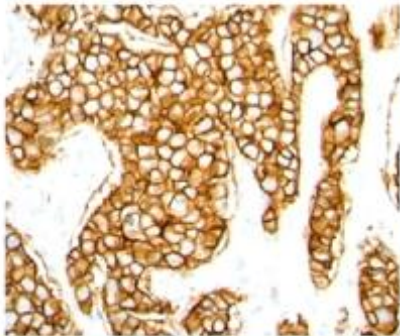
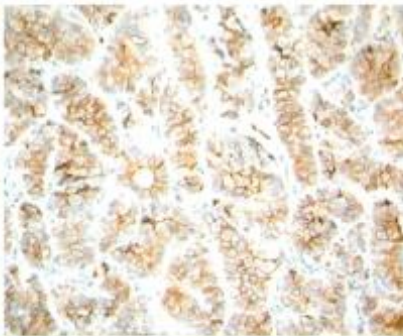
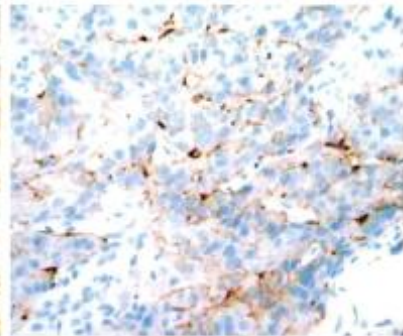
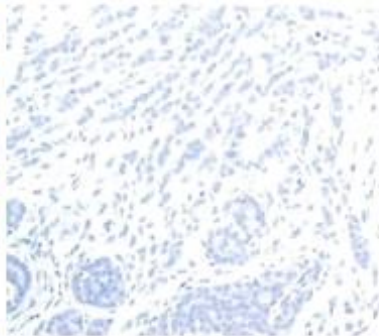


3+

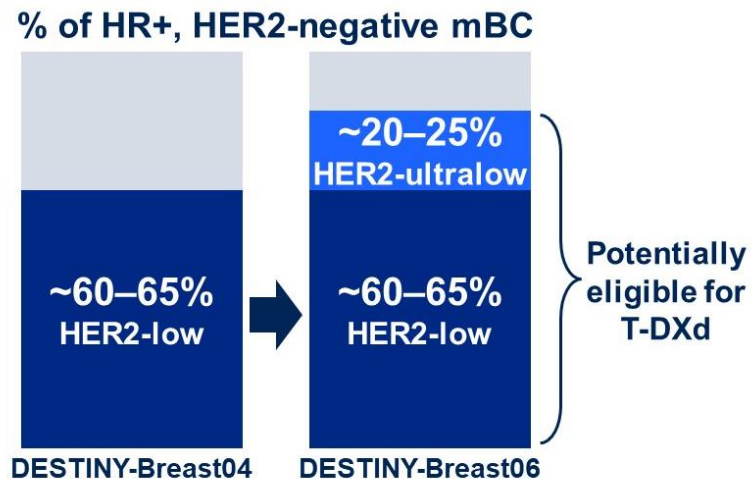


ASCO CAP guidelines 2018  
<https://pubmed.ncbi.nlm.nih.gov/29846122/>

# HER2 low and ultralow

|                          |                                                                                   |                                                                                    |                                                                                     |                                                                                     |                                                       |
|--------------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------|
| HER2 IHC Stain           |  |  |  |  |                                                       |
| IHC Stain Score Criteria | Circumferential and intense in > 10% (visible at 2-5x)                            | Weak to moderate, complete in > 10% (visible at 10-20x) (+ uncommon patterns*)     | Incomplete, faint/barely perceptible (visible at 40x)                               | No membrane staining detected (at 40x)                                              |                                                       |
|                          |                                                                                   |                                                                                    | In > 10%                                                                            | In ≤ 10%                                                                            |                                                       |
| IHC Result               | <u>Positive</u><br>(Score 3+)                                                     | <u>Equivocal</u><br>(Score 2+)                                                     | <u>Negative</u><br>(Score 1+)                                                       | <u>Negative</u><br>(Score 0+/<br>with membrane staining)                            | <u>Negative</u><br>(Score 0/absent membrane staining) |
| ISH Result               |                                                                                   | Amplified                                                                          | Not Amplified                                                                       |                                                                                     |                                                       |
| Clinical Status          | <u>HER2 Positive</u>                                                              |                                                                                    | <u>HER2 Low</u>                                                                     | <u>HER2 Ultralow</u>                                                                | <u>HER2 NULL</u>                                      |
| HER2 Rx:                 | Eligible for various HER2 targeted therapy regimens                               |                                                                                    | May be eligible for trastuzumab-deruxtecan in the metastatic setting                |                                                                                     | Not currently eligible for HER2 therapies             |

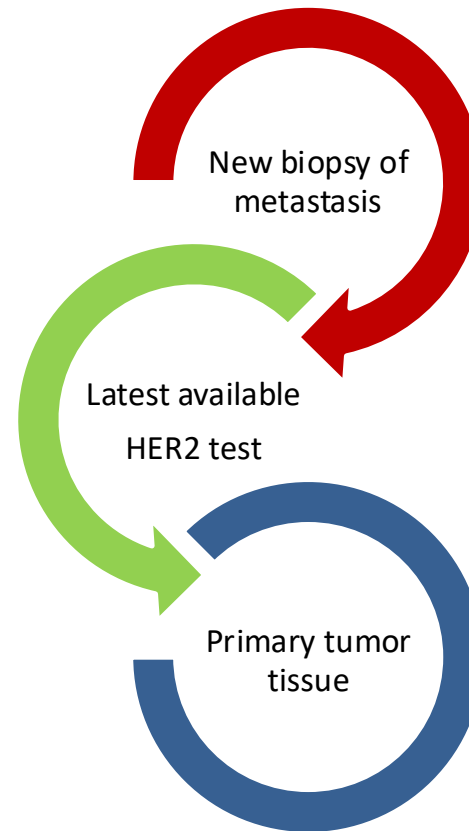
# DESTINY-Breast06



- T-DXd demonstrated efficacy in **HER2-low mBC** in an **earlier line of treatment** to DESTINY-Breast04
- Including HER2-ultralow, the proportion of patients who could benefit from T-DXd is **~85% of HR+, HER2-negative mBC** after DESTINY-Breast06

In DESTINY-Breast06, T-DXd demonstrated a statistically significant and clinically meaningful PFS benefit vs TPC (chemotherapy) in HR+, HER2-low mBC after  $\geq 1$  endocrine-based therapy, with consistent results in HER2-ultralow mBC

# Suggested flow for analysis



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

# 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<sup>1</sup> · Michael Friedrich<sup>1</sup> · Iris Nagelmeier<sup>2</sup> · Matthias Kirchner<sup>2</sup> · Lena M. Andresen<sup>3</sup> · Karin Salomon<sup>3</sup> ·  
Bryce Portier<sup>4</sup> · Simone T. Sredni<sup>4</sup> · Hans Ulrich Schildhaus<sup>1,2</sup> · Bharat Jasani<sup>1</sup> · Marius Grzelinski<sup>1</sup> · Giuseppe Viale<sup>5</sup>

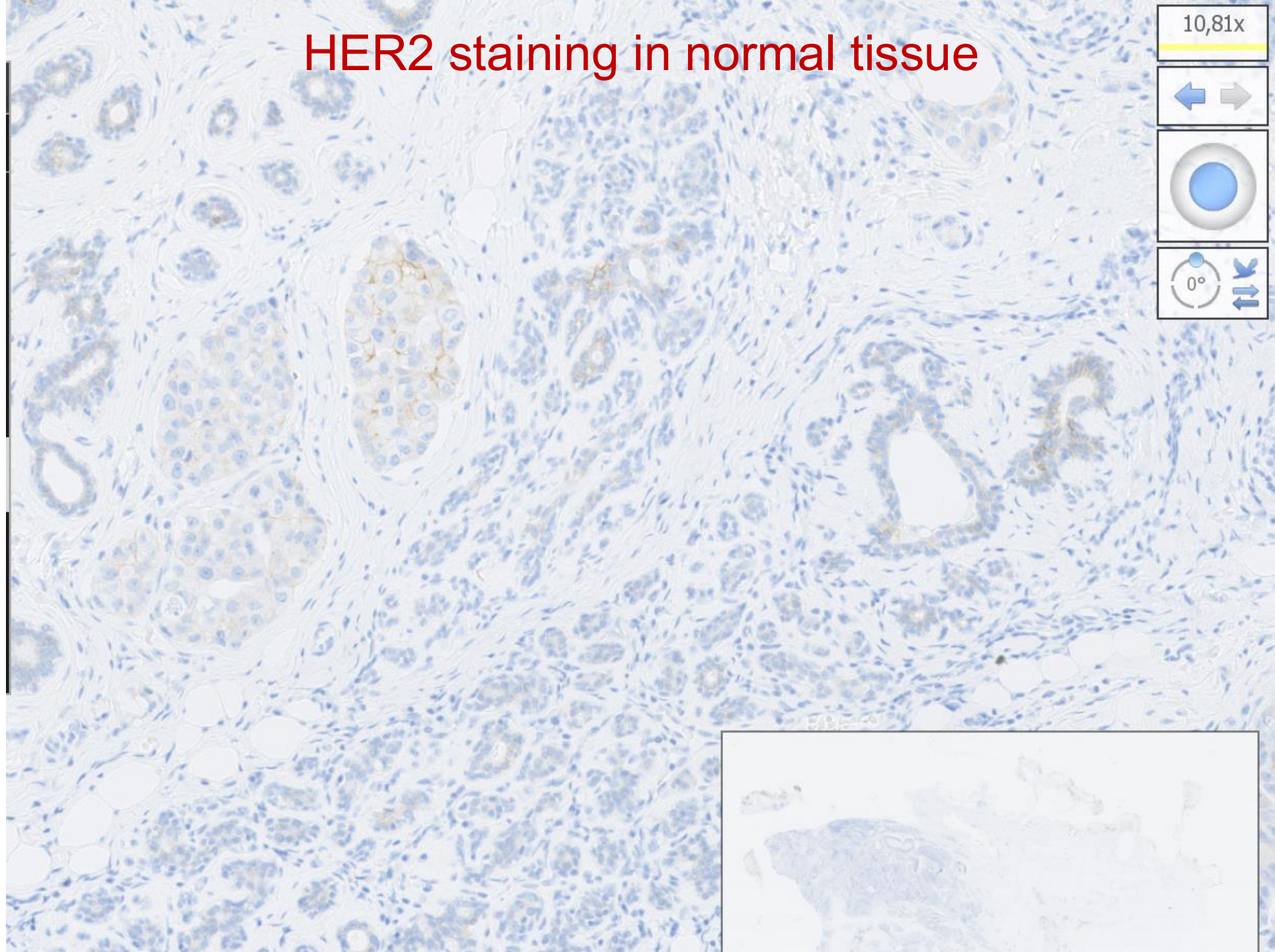
|                     |       | PATHWAY 4B5 |      |    |    |       |
|---------------------|-------|-------------|------|----|----|-------|
|                     |       | 0           | 1+   | 2+ | 3+ | Total |
| HercepTest<br>(mAb) | 0     | 35          | 0    | 0  | 0  | 35    |
|               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

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

\* 2 amplified

Rueschoff J, et al. Virchows Arch 2022

# HER2 staining in normal tissue



# cytoplasmic staining

Score=0

23,08x



## Summary: HER2-low BC

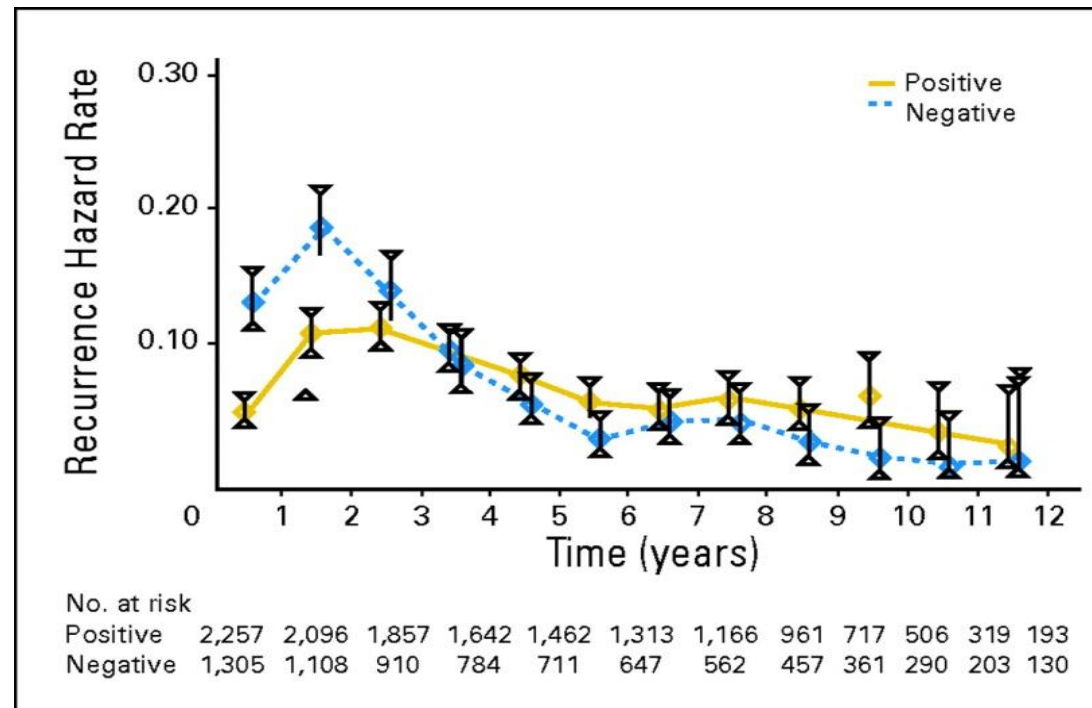
- Common and usually found in HR-positive disease
- At present, main role is to predict response to ADC (T-DXd) in mBC
- Variable assay sensitivity, concordance, spatial and temporal heterogeneity for HER2-Low category with existing assays

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

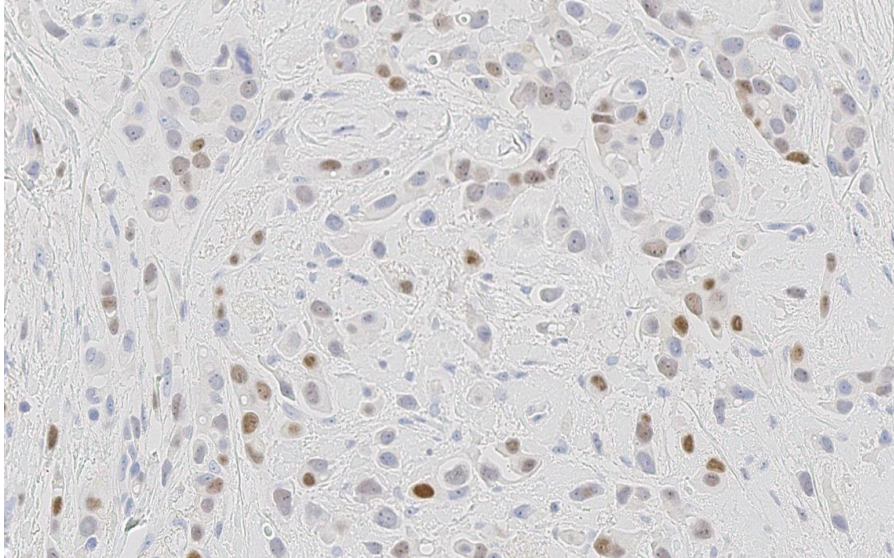
Risk of recurrence pr. year  
N = 3,562 patients



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

# 2020 – ASCO CAP Update

## Hormone receptors

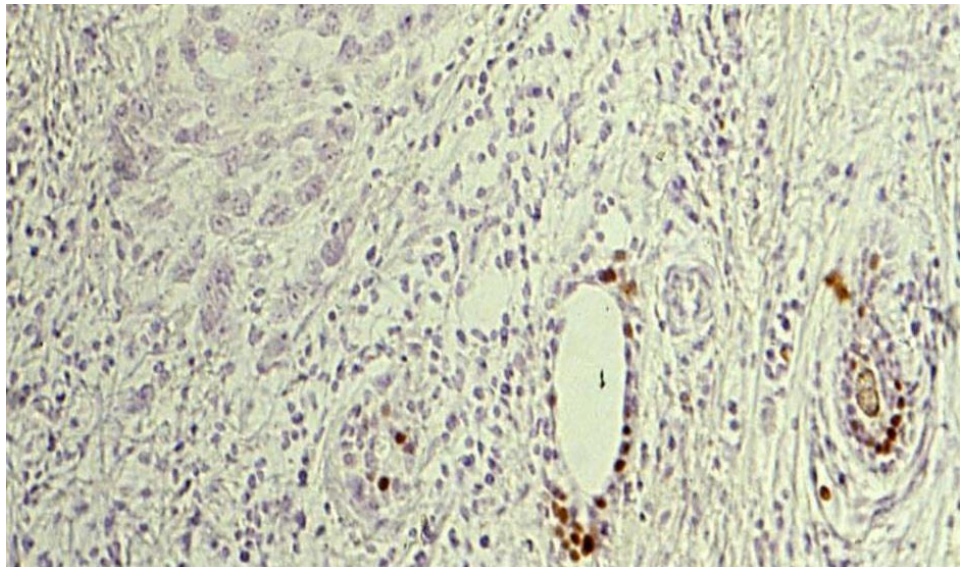
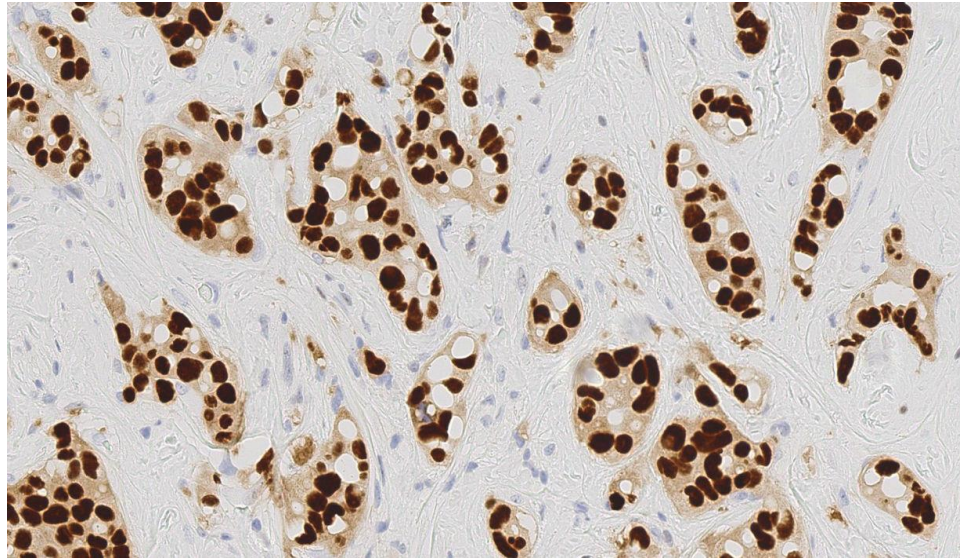


ER positive 86% of breast carcinomas (DK)

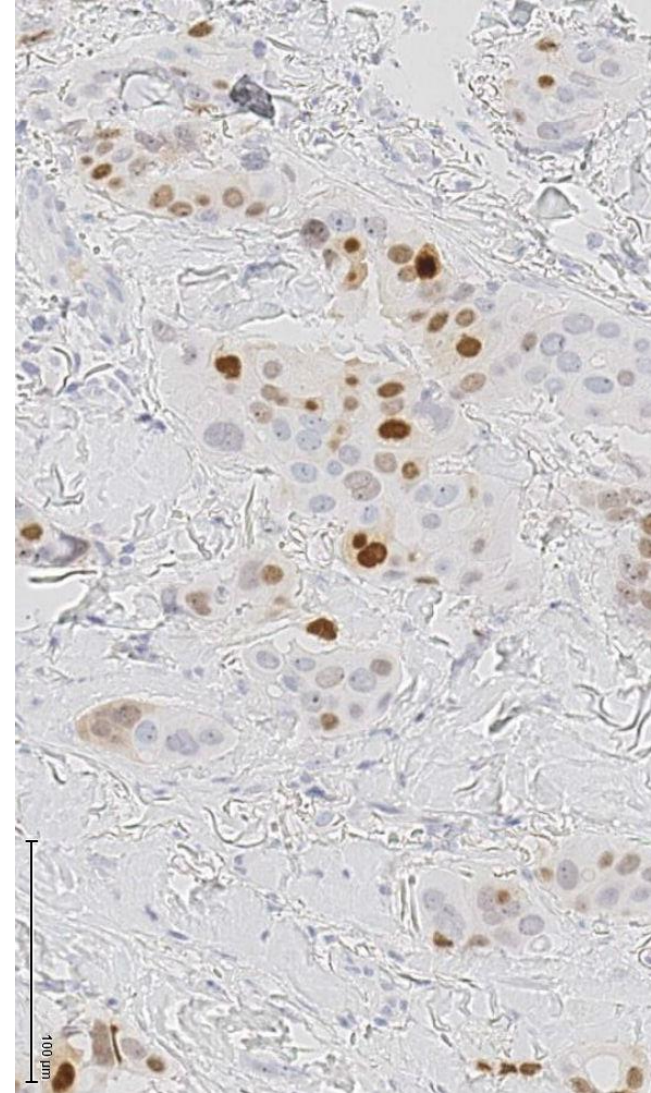
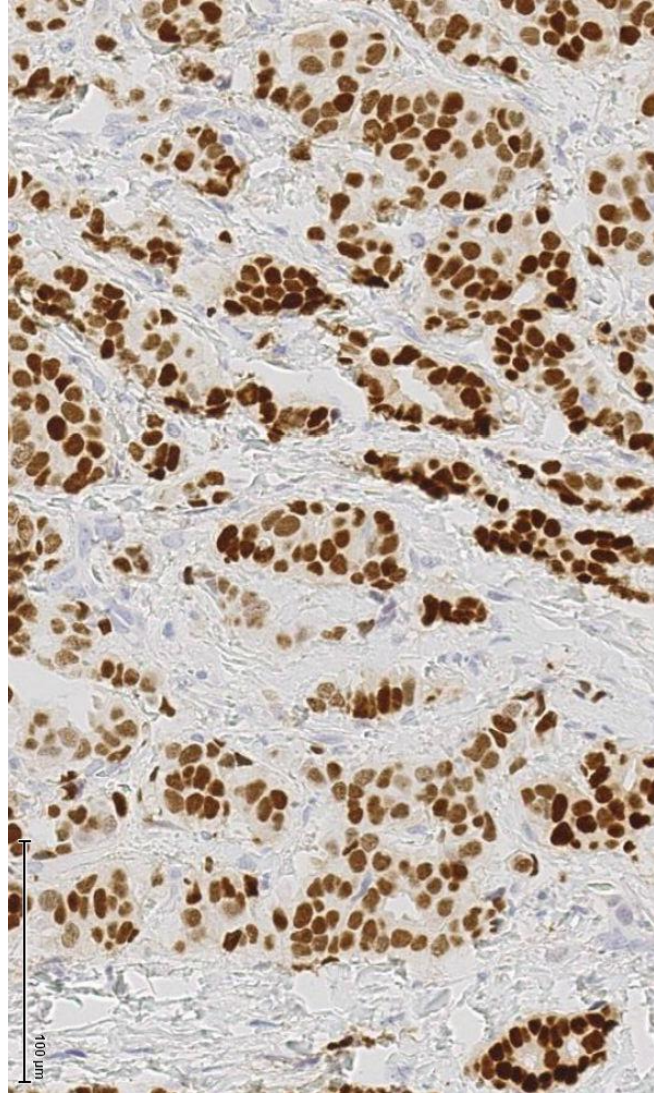
Cut off  $\geq 1\%$

A sample is reported negative for ER or PgR if  $< 1\%$  of tumor cell nuclei are immunoreactive.

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



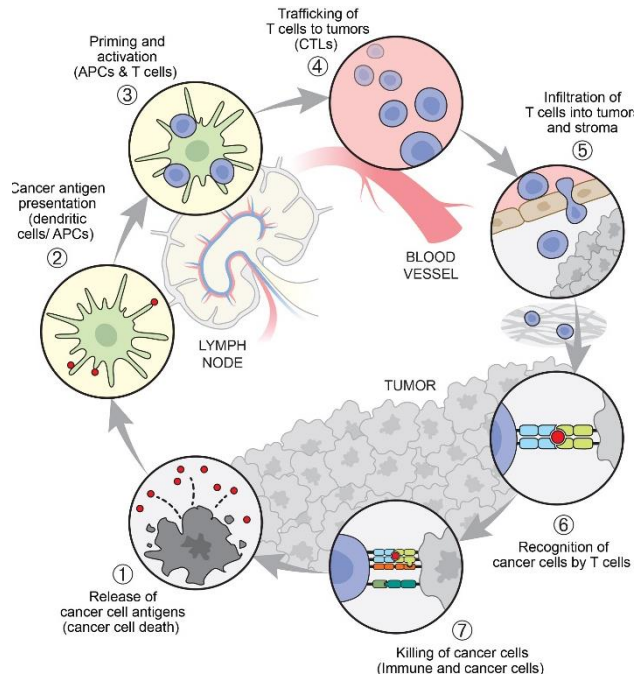
# Interpretation of PgR



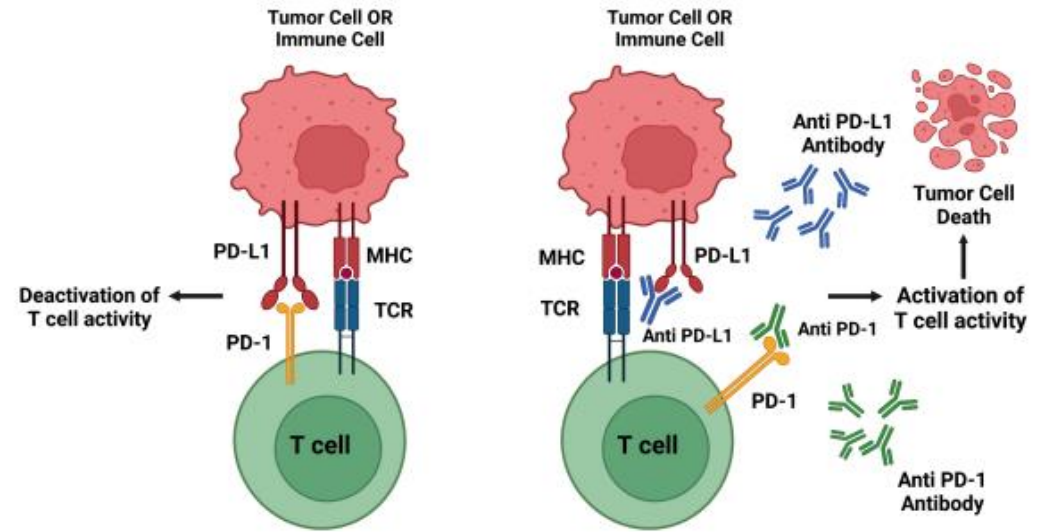
Heterogeneous expression

# PD-L1 in metastatic TNBC

# PD-L1 in breast cancer



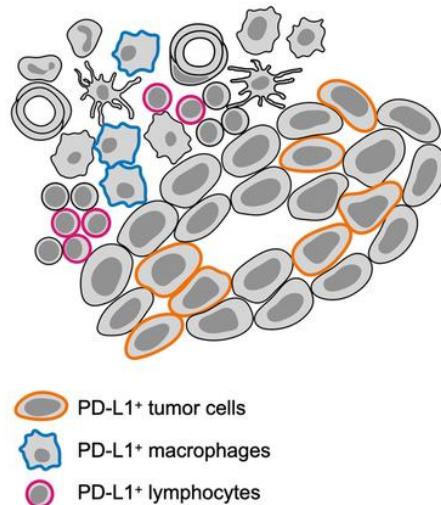
Mellman et al, Immunity, Volume 56, Issue 10, 10 October 2023, Pages 2188-2205



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



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

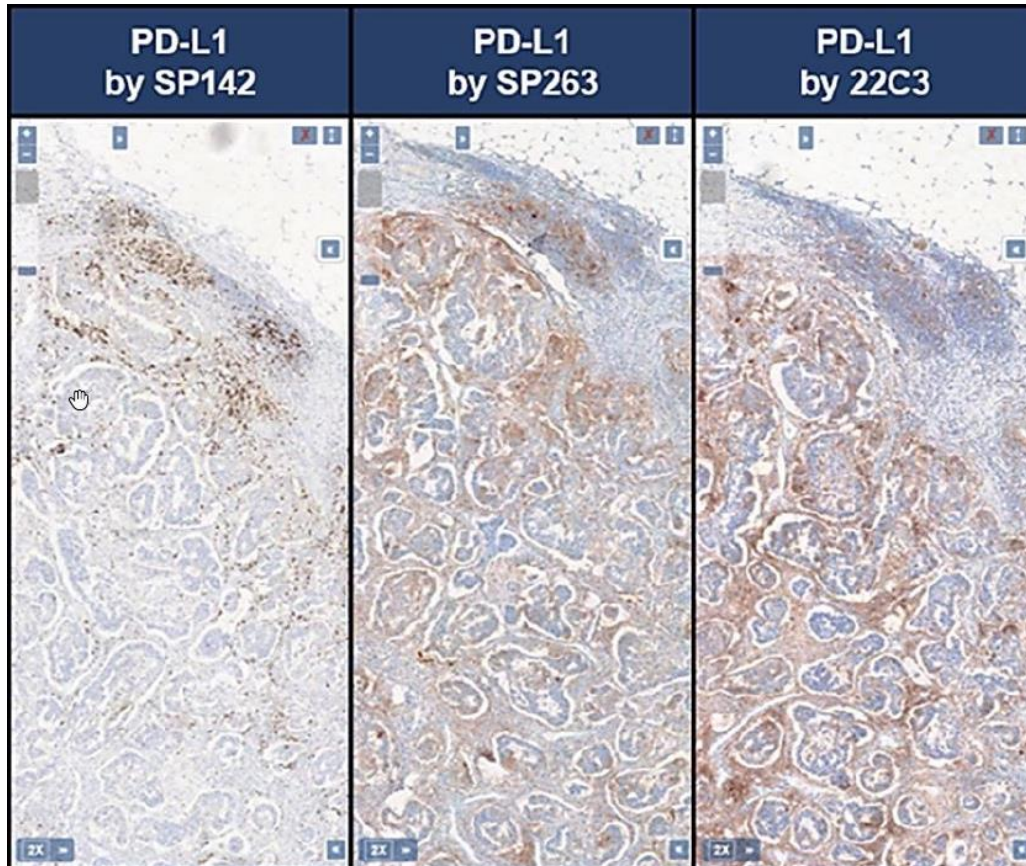
$$\text{CPS} = \frac{\# \text{ PD-L1 positive cells (tumor cells, lymphocytes, macrophages)}}{\# \text{ viable tumor cells}} \bullet 100$$

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

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

Atezolizumab (anti-PD-L1)  
ImPassion130  
Assay SP142

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



## Challenges PD-L1

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

# 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 apocrine carcinoma – potential target for treatment in the metastatic setting)
  - Synaptophysin + other neuroendocrine 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!

## 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

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doi: 10.1093/jnci/djaa201

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Commentary

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

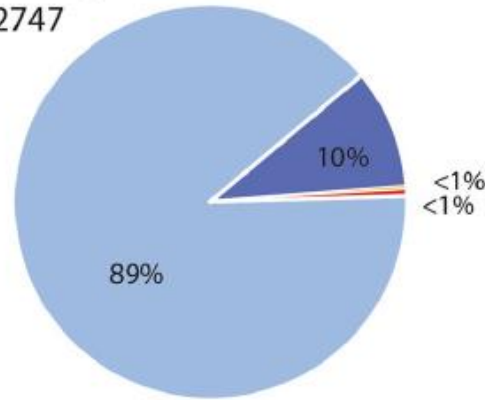
Torsten O. Nielsen , MD, PhD, FRCPC,<sup>1,\*</sup> Samuel C. Y. Leung , MSc,<sup>1</sup> David L. Rimm , MD, PhD,<sup>2</sup> Andrew Dodson , MPhil, FIBMS, CSci,<sup>3</sup> Balazs Acs , MD, PhD,<sup>4,5</sup> Sunil Badve , MBBS, MD, FRCPath,<sup>6</sup> Carsten Denkert , MD,<sup>7</sup> Matthew J.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

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

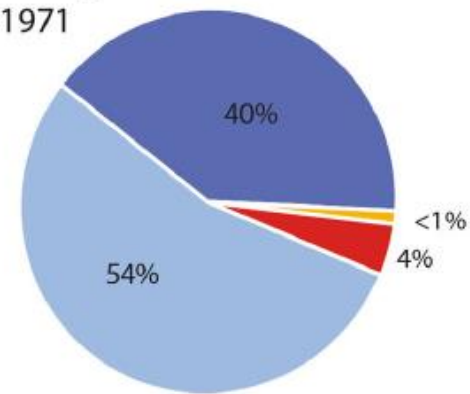
| <b>Year</b> | <b>Recommendations for decision making regarding adjuvant chemotherapy</b>                                                                                            | <b>Ref.</b> |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 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 $\geq$ 20% and Ki67 <20%, HER2-.<br>Luminal B: ER+ and PR<20% and/or Ki67 $\geq$ 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 $\leq$ 5% do not receive chemotherapy, whereas tumors with Ki67 $\geq$ 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

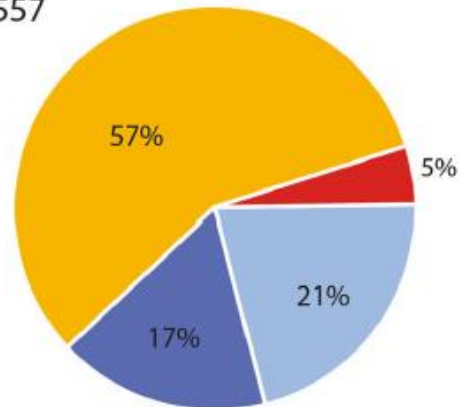
Pathological Luminal A  
n=2747



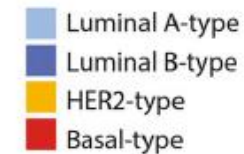
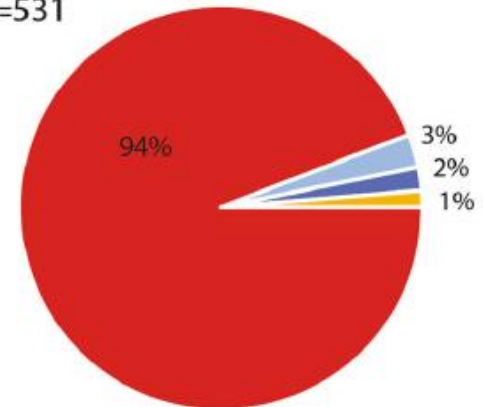
Pathological Luminal B  
n=1971



Pathological HER2-enriched  
n=557

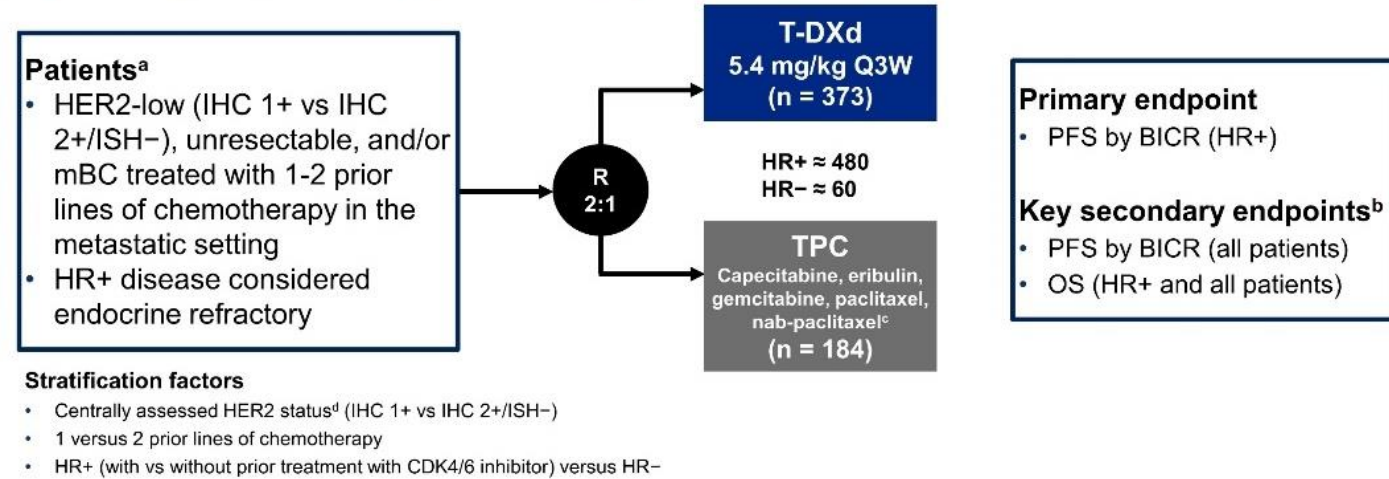


Pathological Triple Negative  
n=531



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

An open-label, multicenter study (NCT03734029)

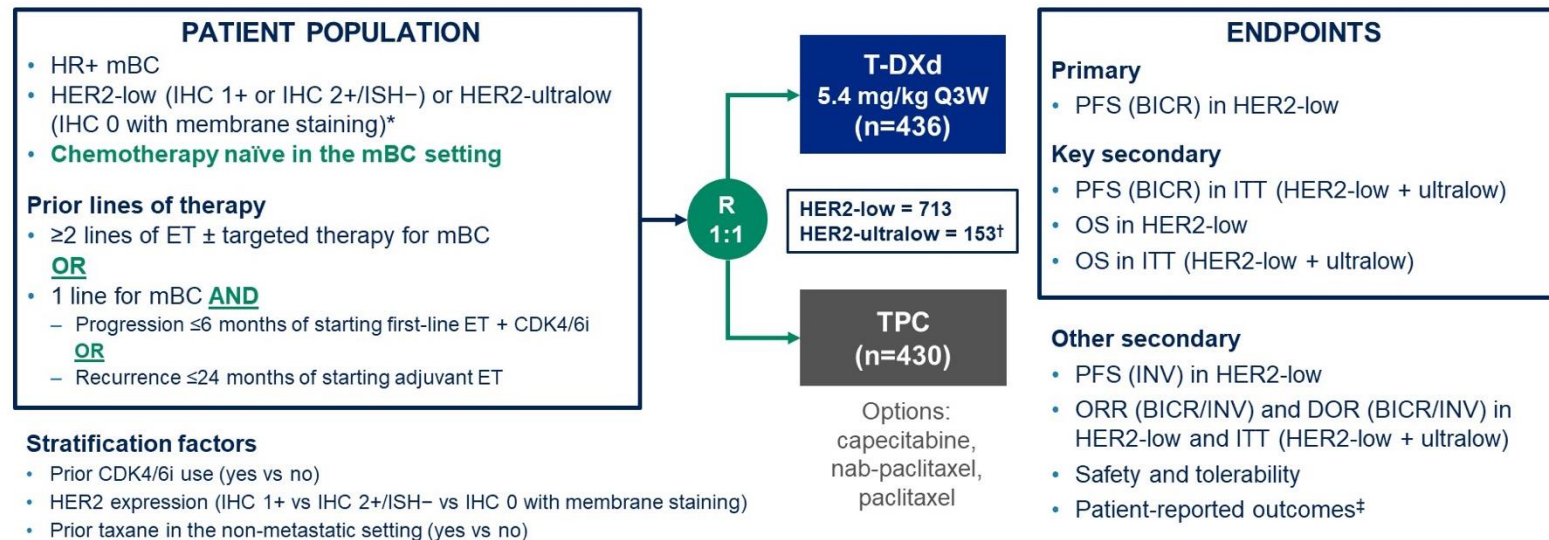


N Engl J Med. 2022 PMID: 35665782



## Study design

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



## 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>

