Breast cancer: IHC for diagnostic use

NordiQC Workshop in Diagnostic Immunohistochemistry 2024
Aalborg University Hospital
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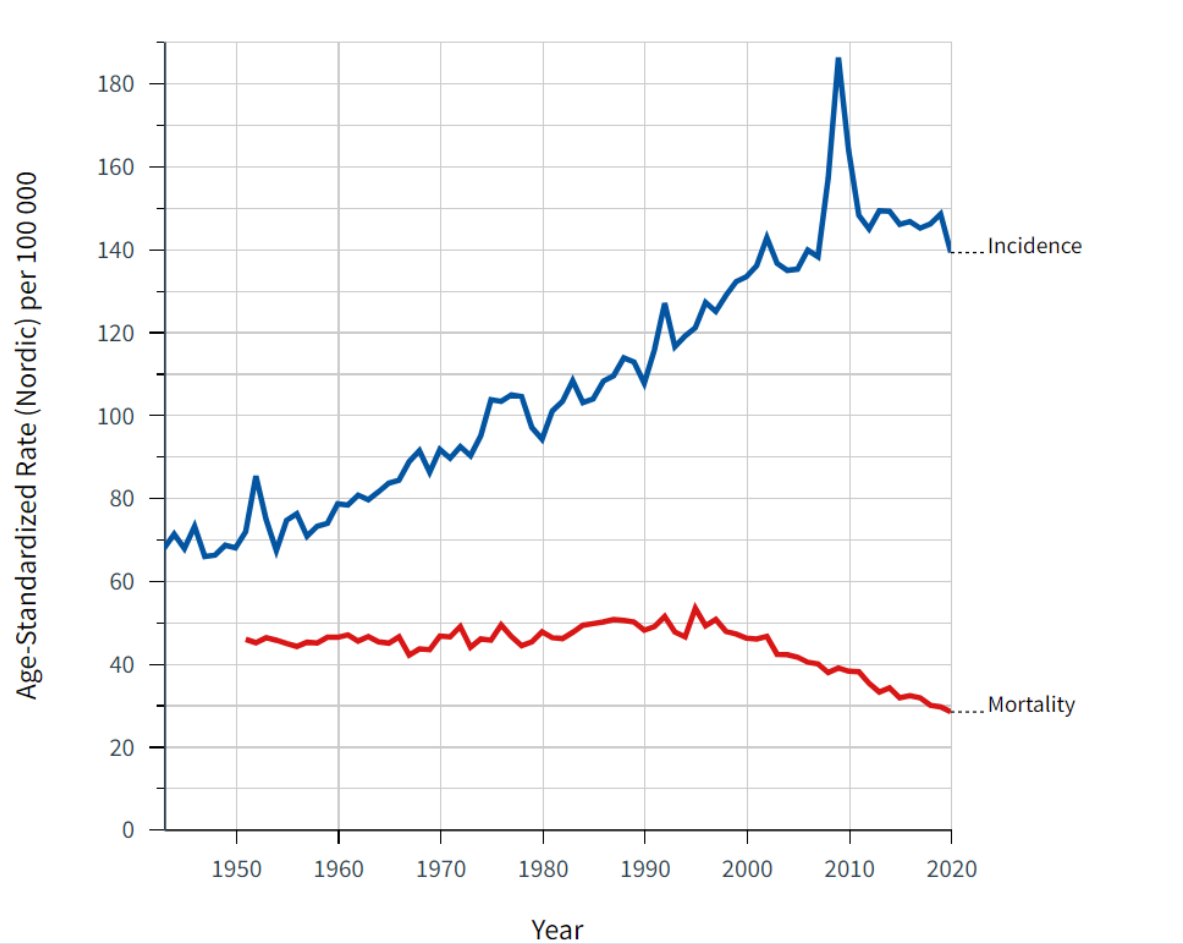
Breast cancer: incidence and mortality Denmark



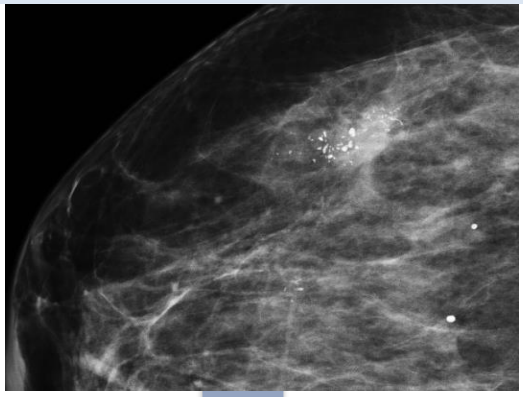
Annually app 4700-5000 new cases

https://nordcan.iarc.fr/

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
    - Molecular subtypes

Triple Test
Diagnostic approach – Breast Tumours



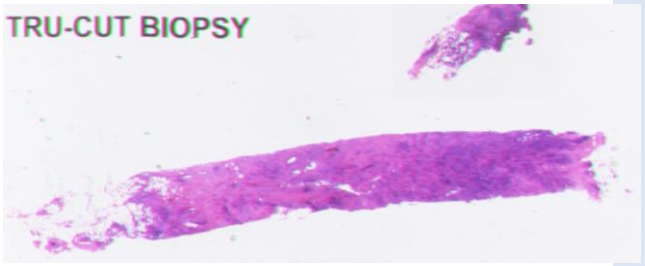
Physical breast exam/

Palpation

Pathology

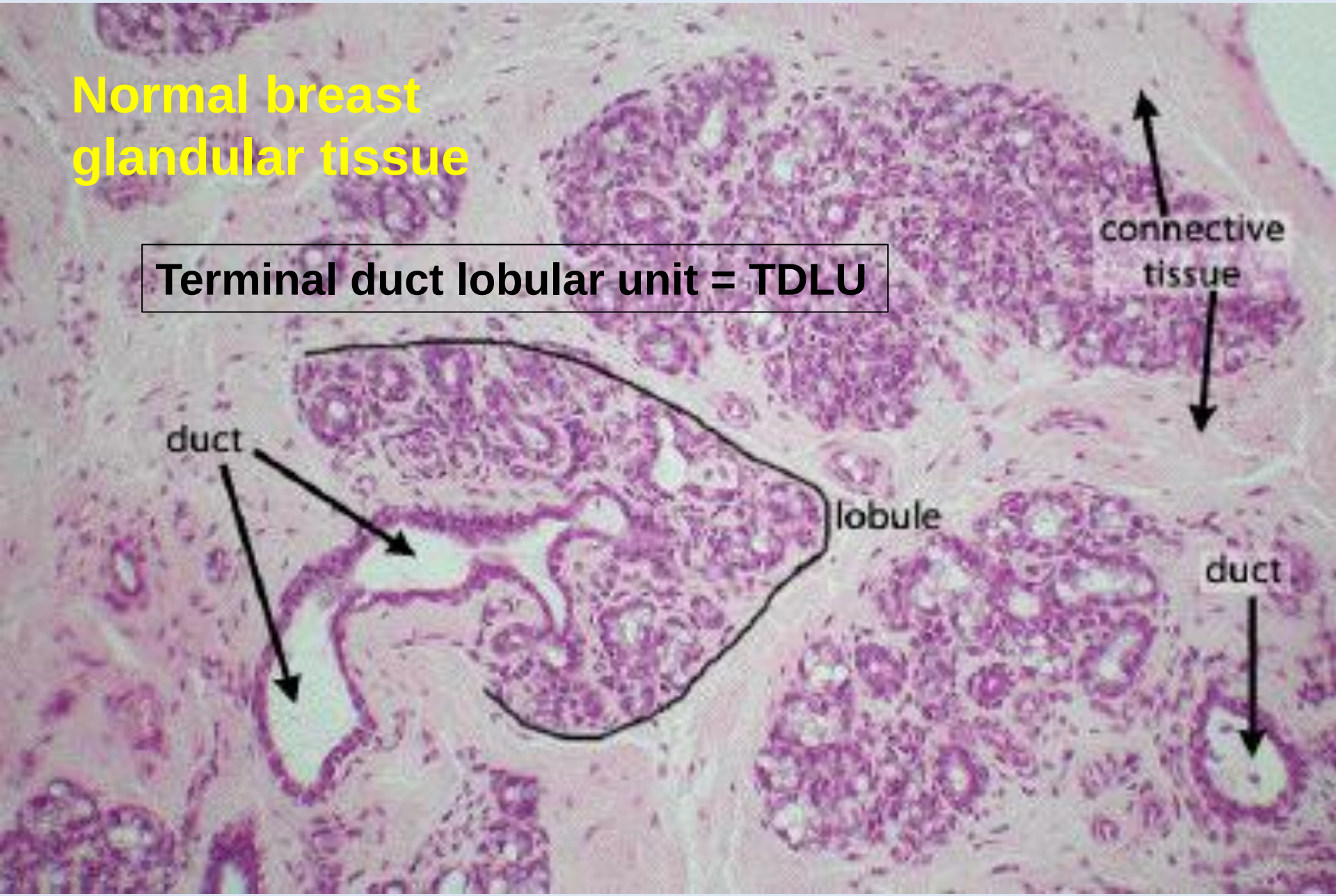
Core needle biopsy or Fine needle aspiration

Triple diagnostics

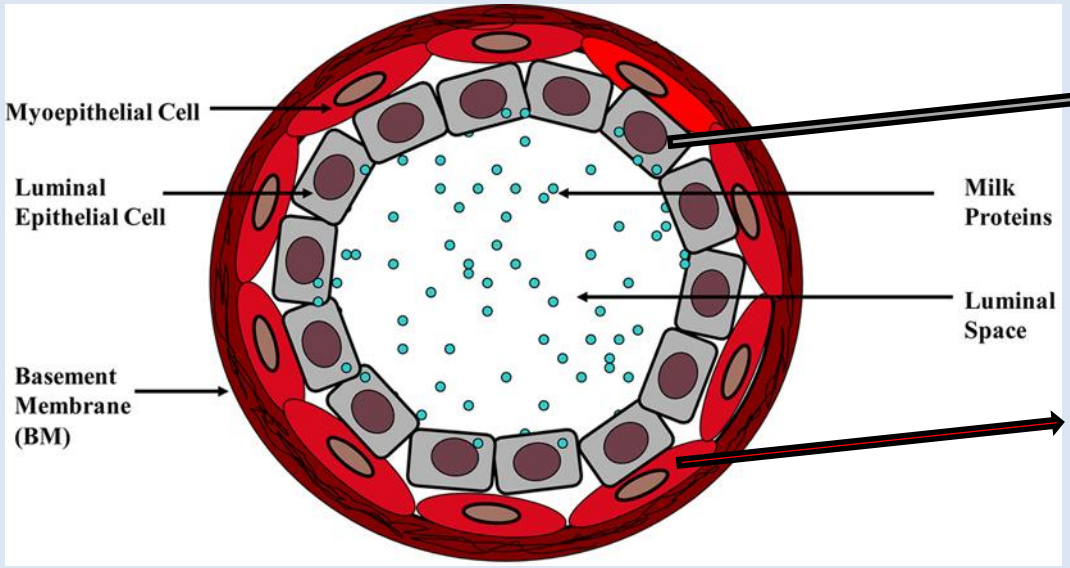


Normal breast glandular tissue

Terminal duct lobular unit = TDLU



Epithelial cells with specific immunohistochemical phenotype

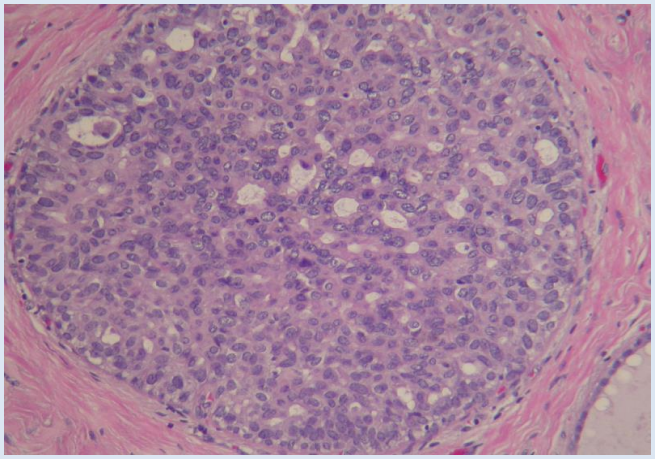


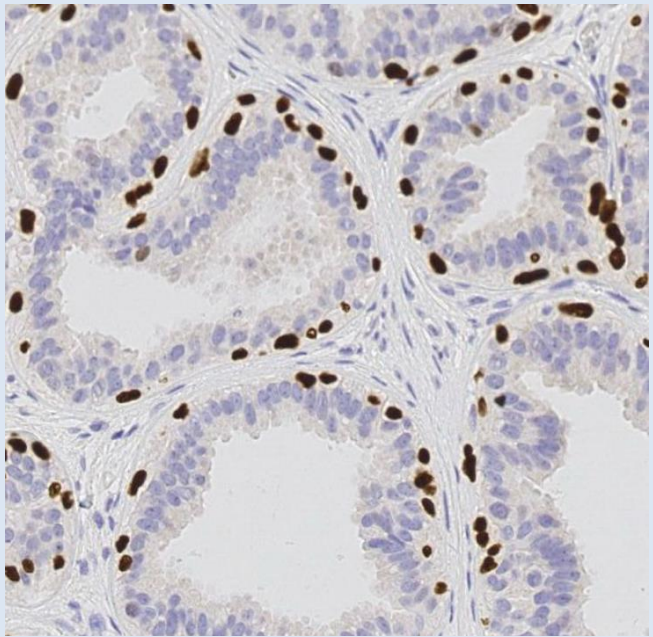
Luminal markers (LMW):
CK7, CK8, CK18, CK19

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

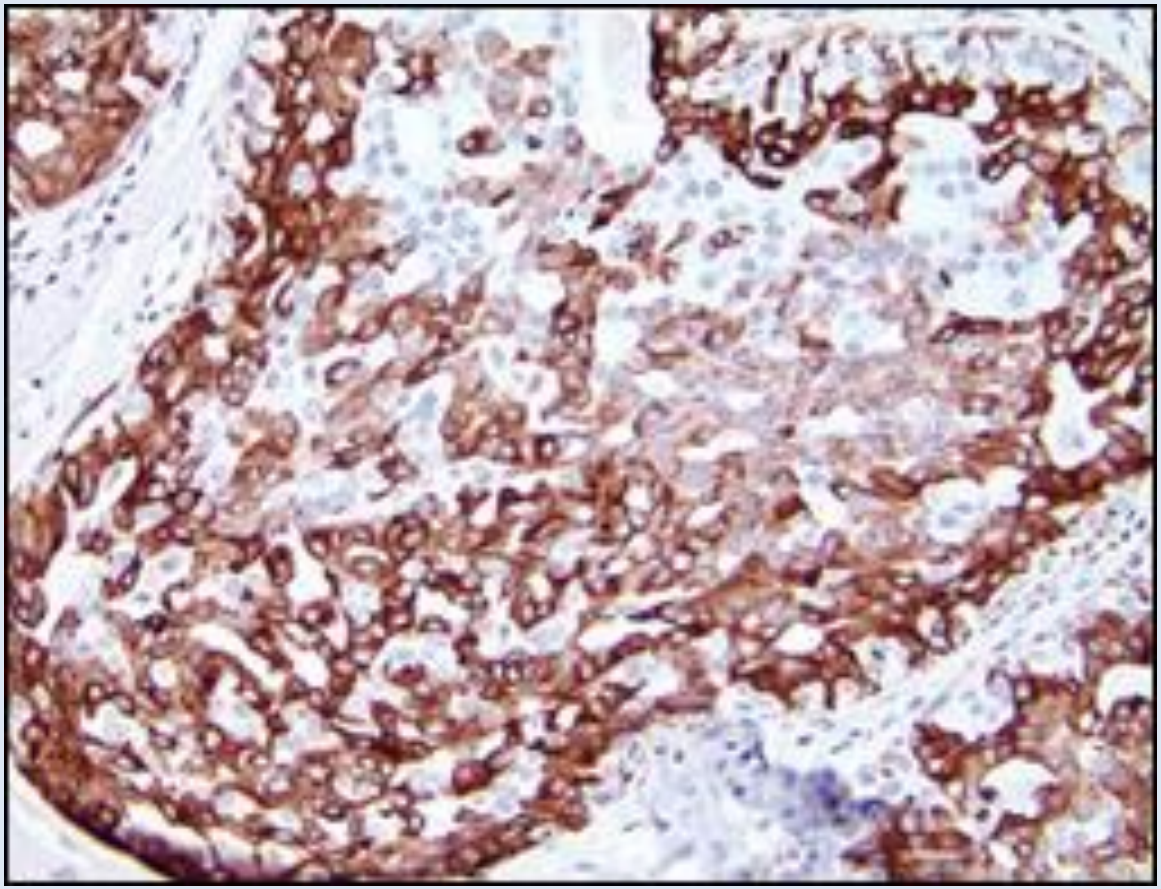
*Smooth muscle myosin heavy chain

Benign hyperplasia
Positive staining for myoepitelial cells





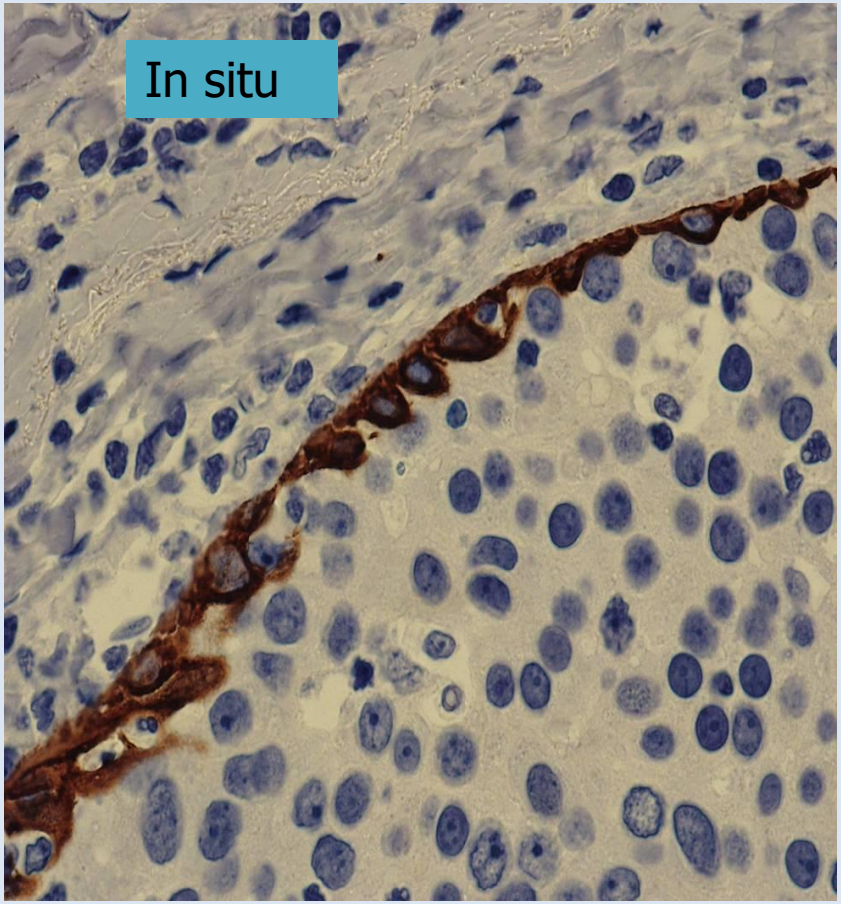
P63



CK5

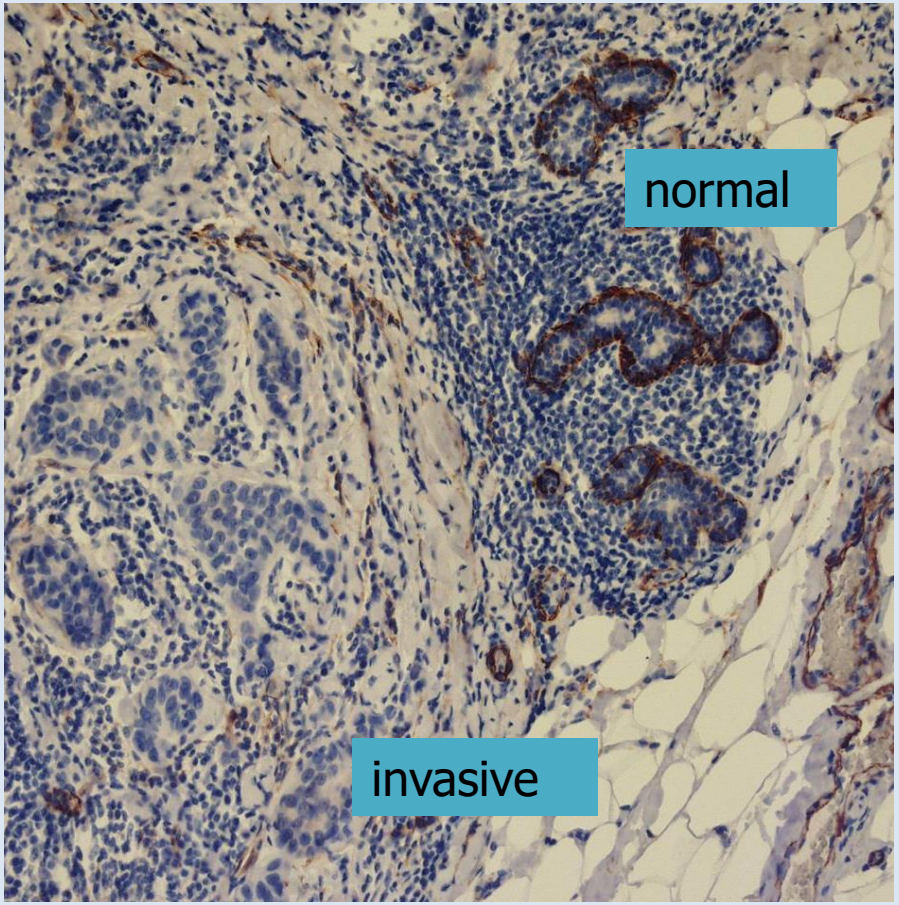
Differentiation between ductal carcinoma in situ and invasive carcinoma i.e. SMMHC*

present



Detecting ”presence”

Not present

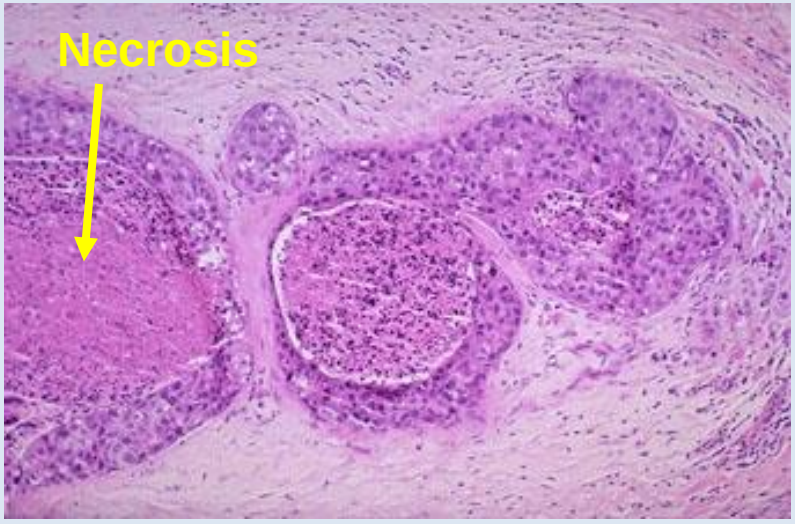


Detecting ”absence”

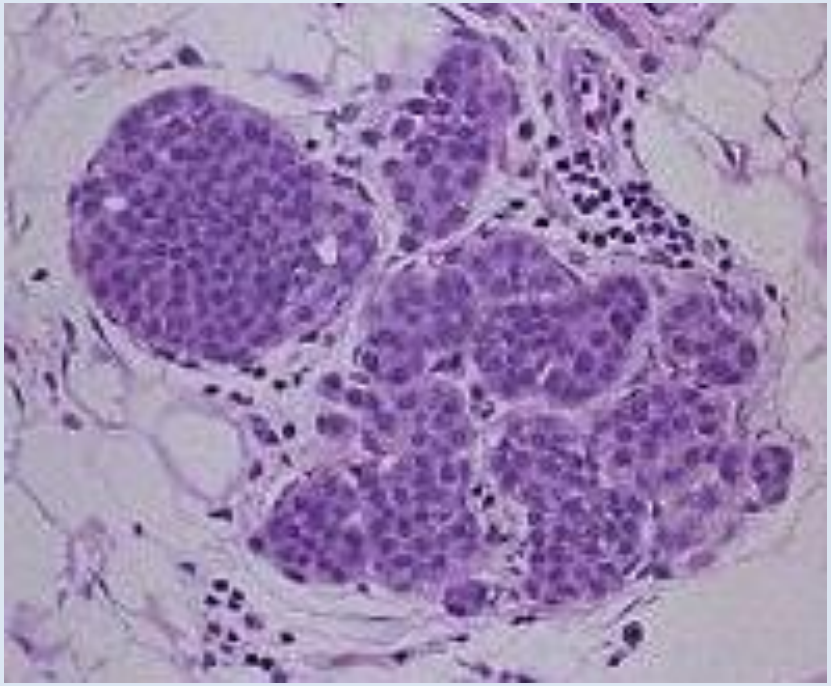
* Smooth muscle myosin heavy chain, as detected with clone SMMS-1

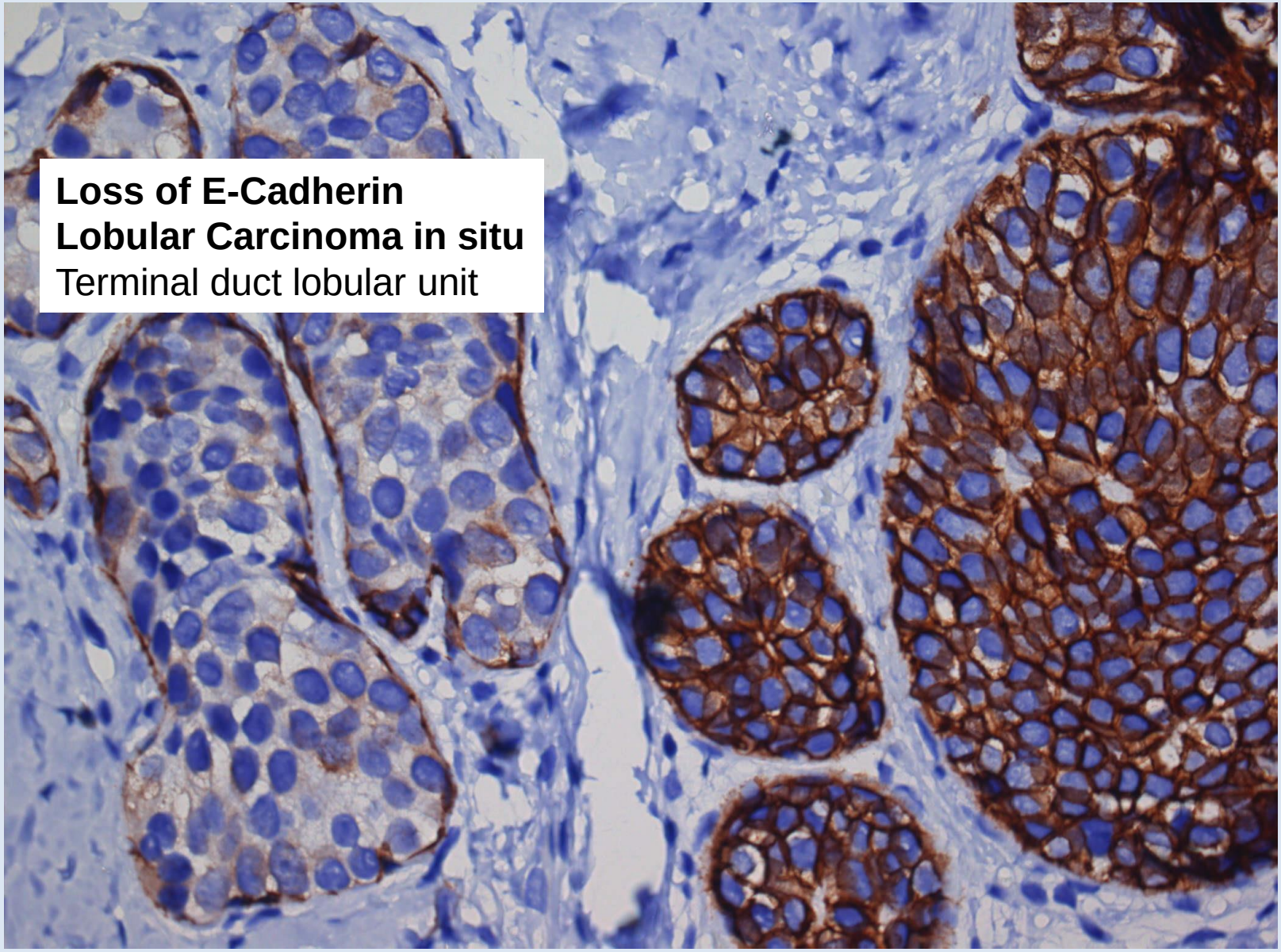
Carcinoma in situ

- 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

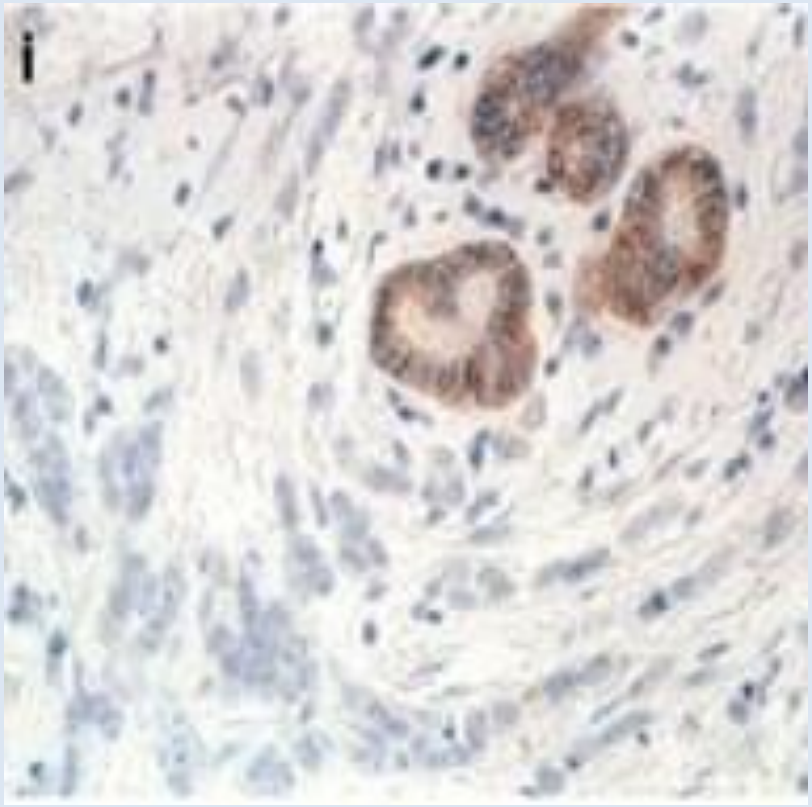
WHO blue books

Histological subtypes (>20)

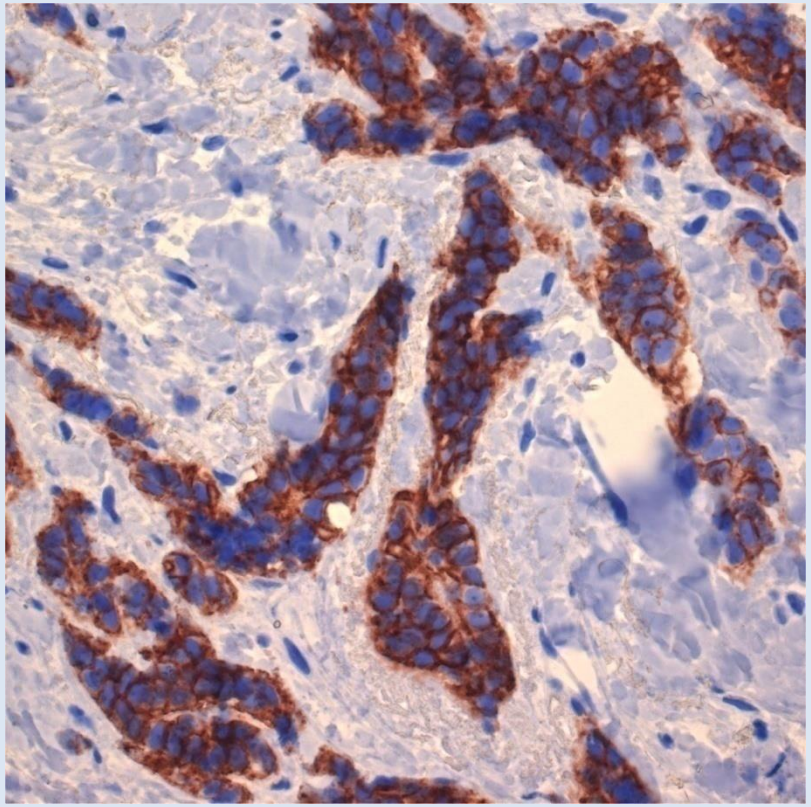
- Ductal : up to 80%
- Lobular: 5 - 14%
- Tubular: 2 - 8%
- Mucinous: 2 - 4 %
- Apocrine: 1 – 4%
- Papillary 1 – 2%
- Other

E-Cadherin
Cell adhesion molecule

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

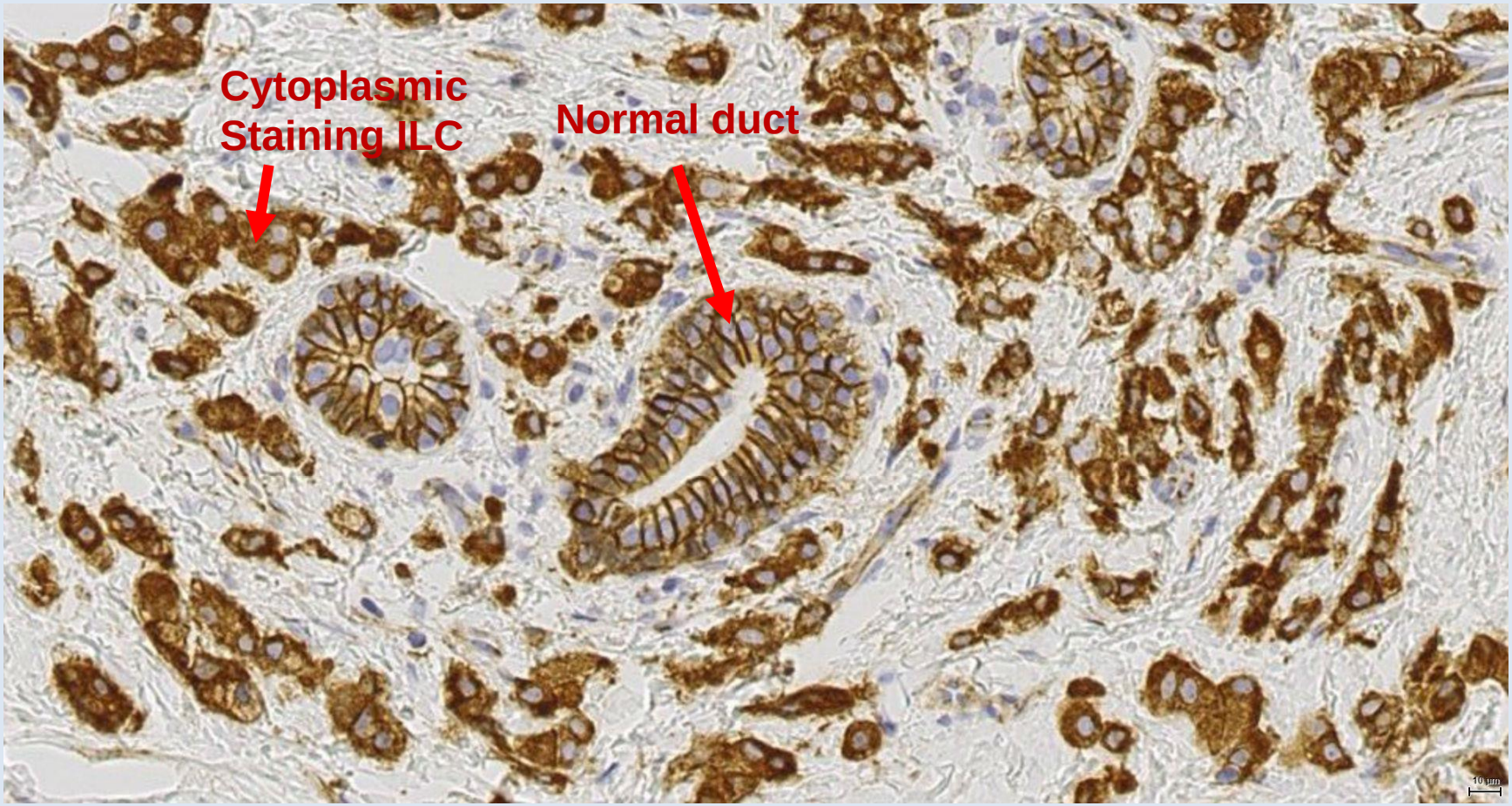


E-Cadherin positive Invasive Ductal Carcinoma



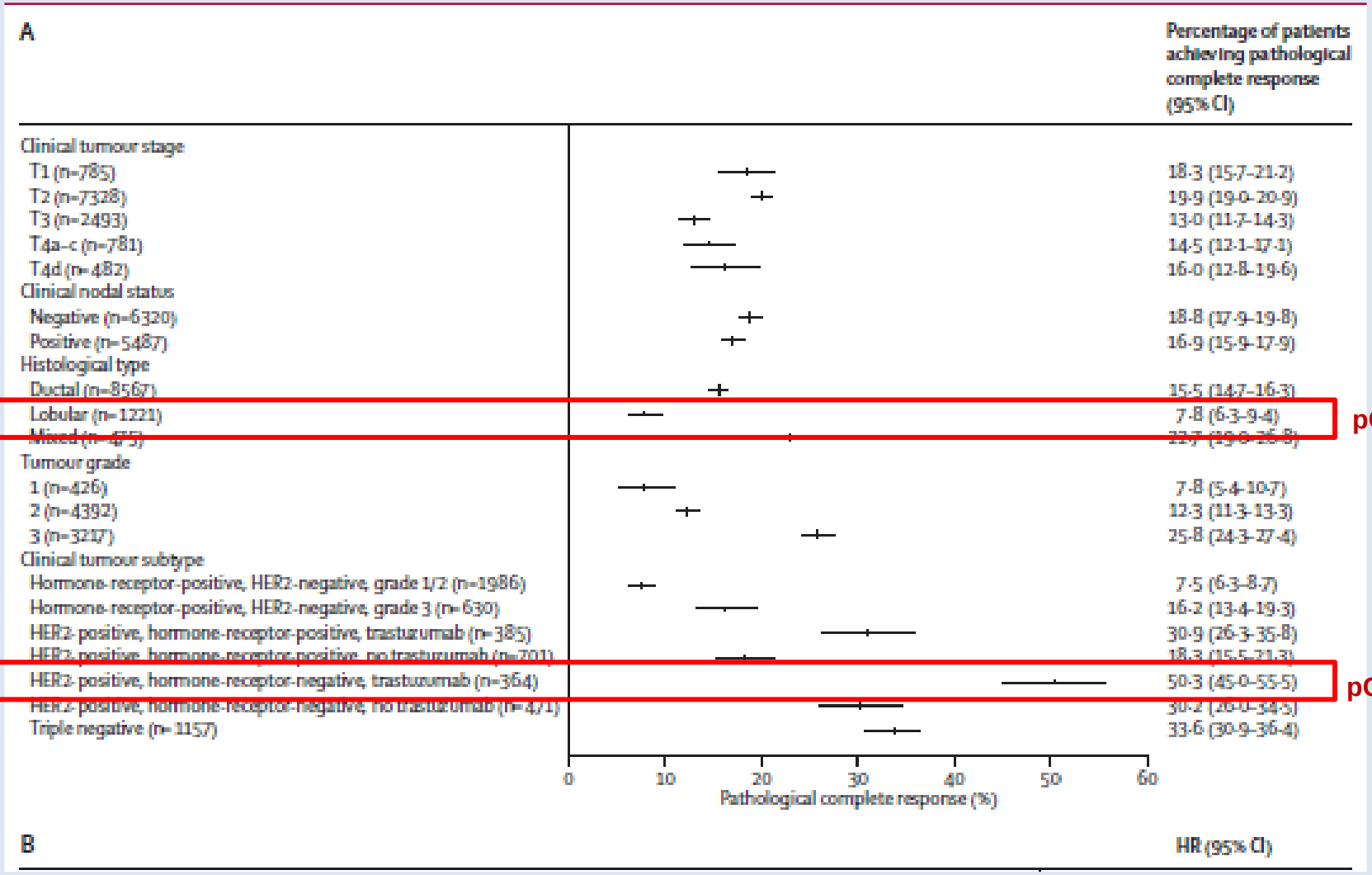
CDH1 (16q22.1) loss of function mutation or deletion resulting in loss of the adhesion molecule E-cadherin

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

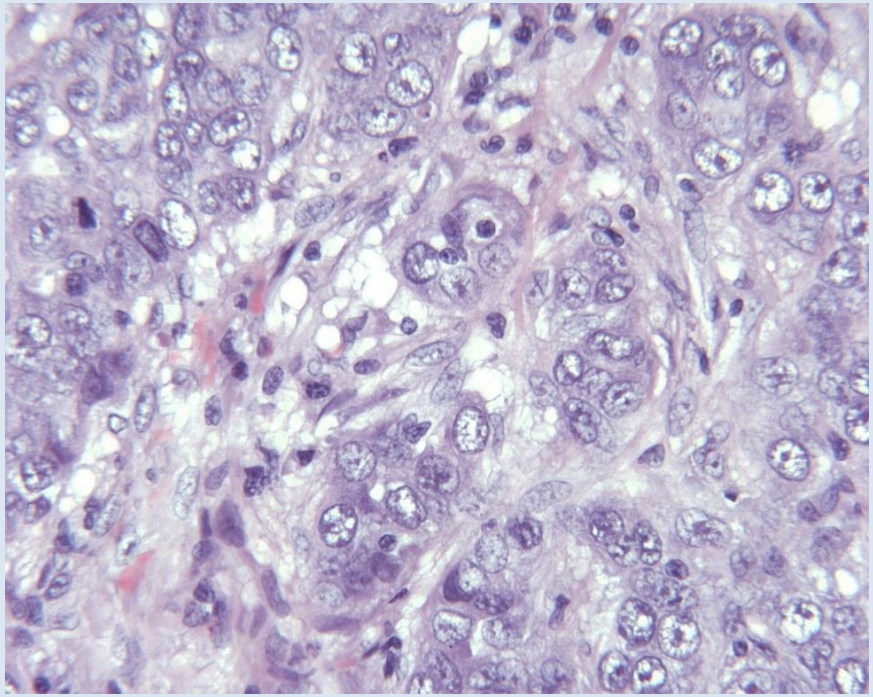
Tumor characteristics and association with pCR
Neoadjuvant chemotherapy (NACT)



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

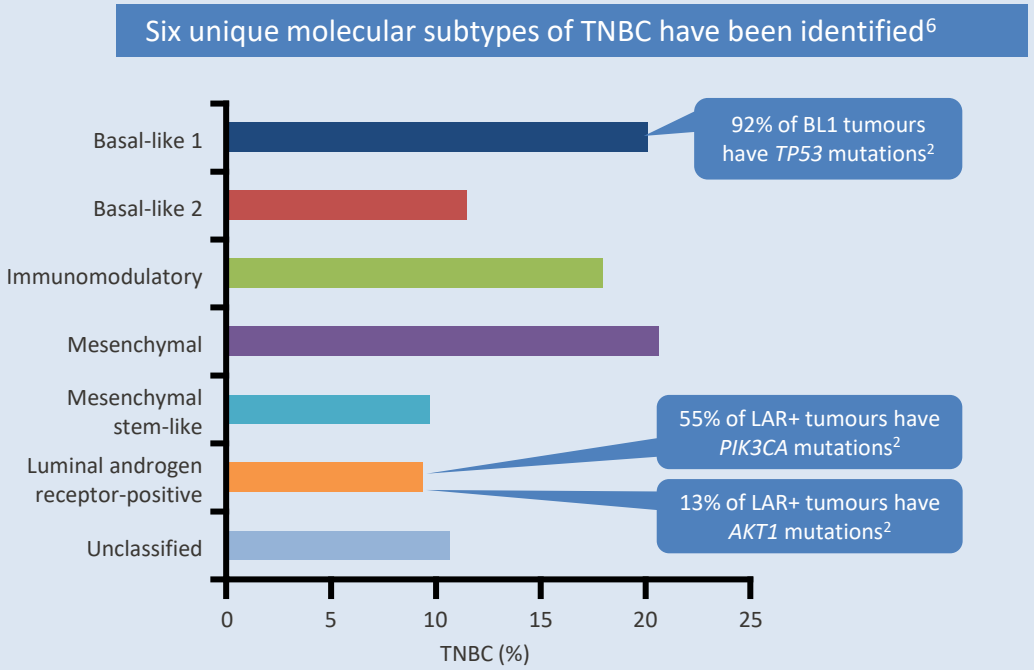
TNBC : 8-10% of primary breast cancers

- 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]

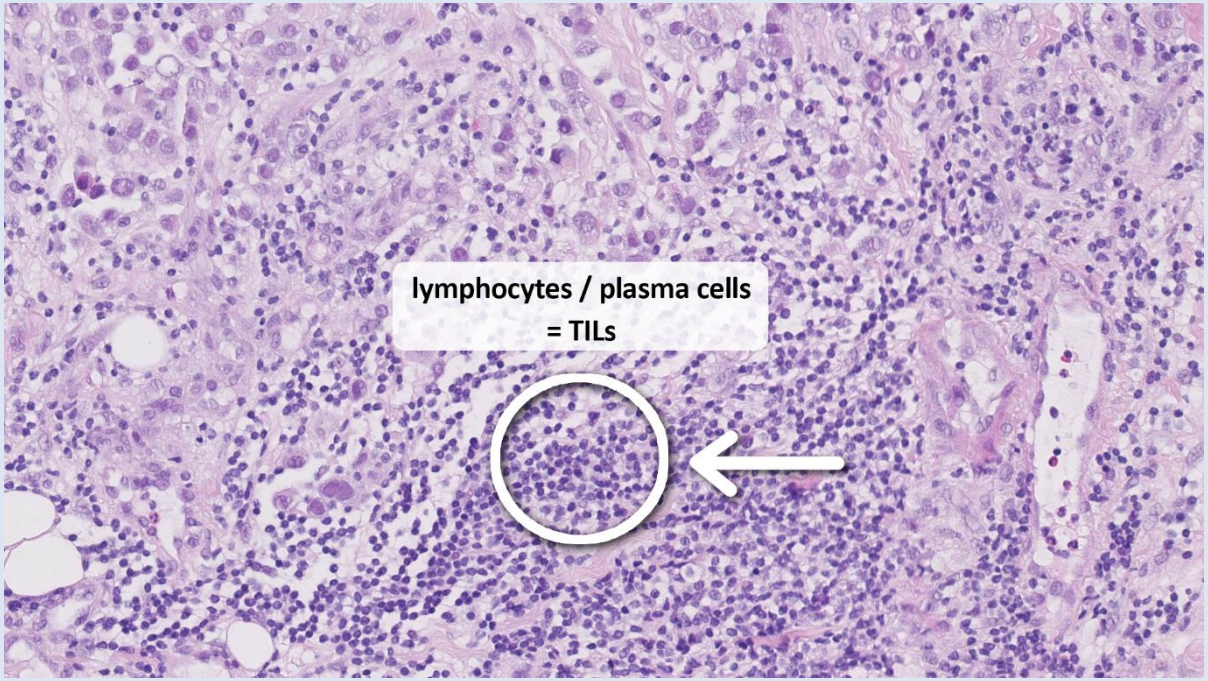


1. Lehmann, et al. J Clin Investig 2011; 2. Bareche, et al. Ann Oncol 2018

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

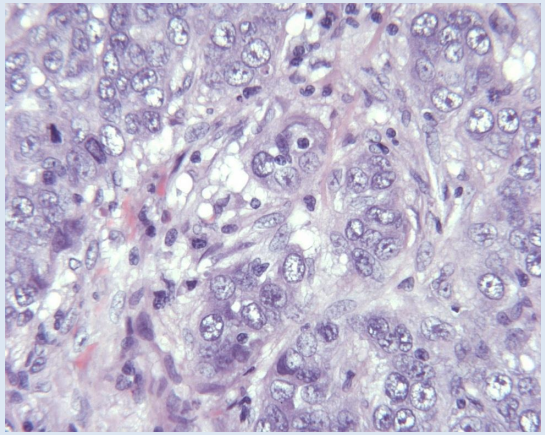


Figure 1
High grade IDC

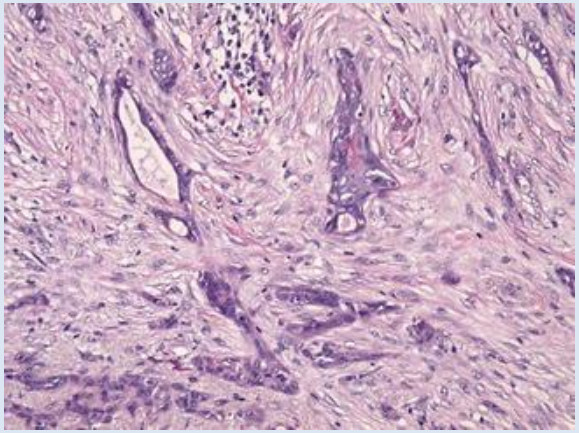


Figure 2
Low grade adenosquamous carcinoma (subtype of metaplastic carcinoma)
luminal (CK7, CK8) and basal (CK5, CK14) CKs and squamous (myoepithelial) markers p63 and p40.

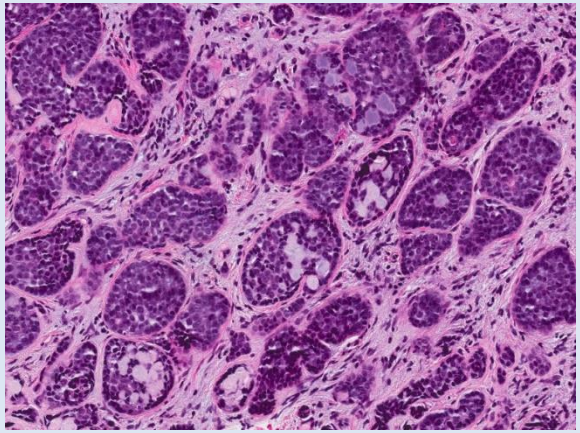
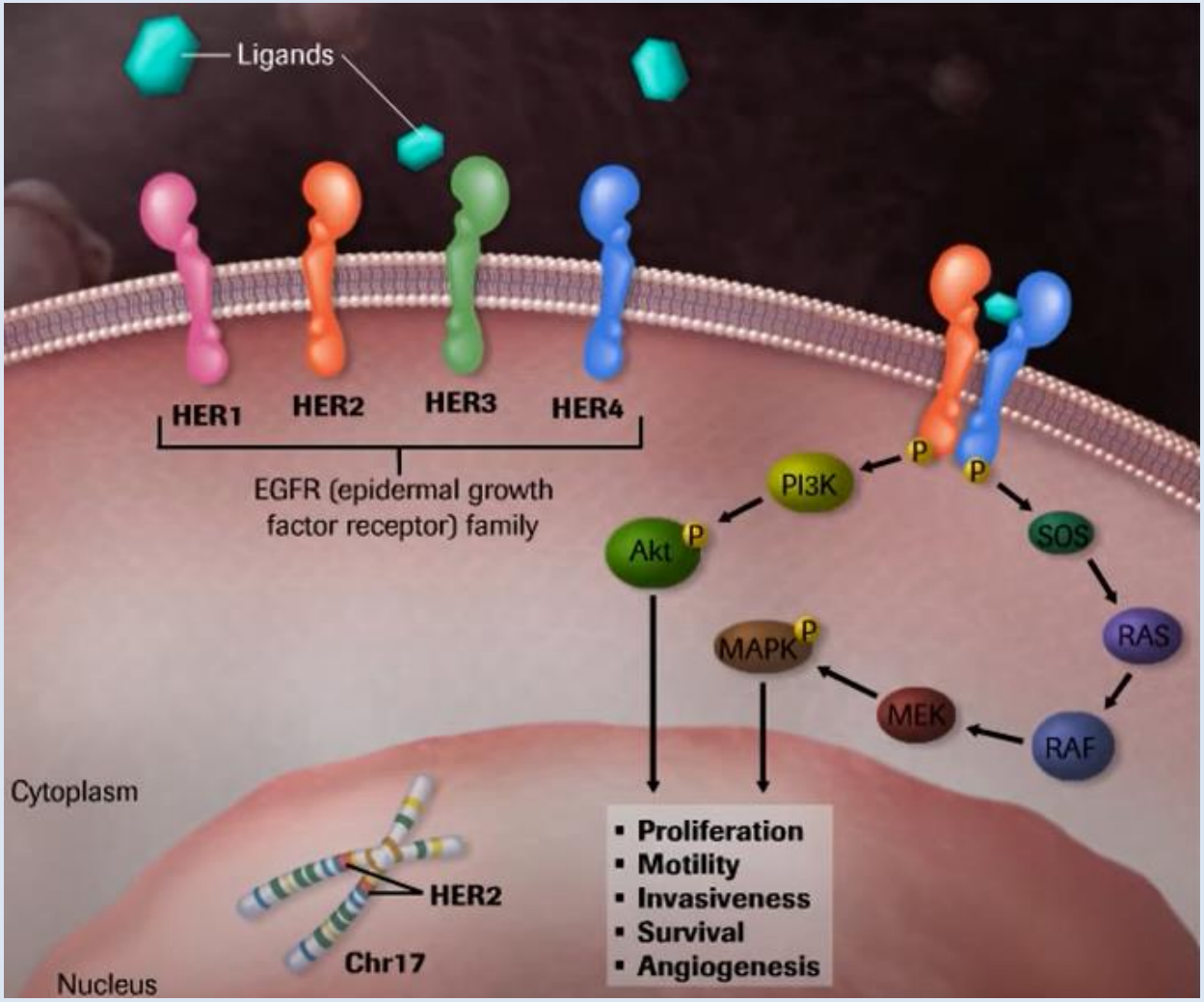


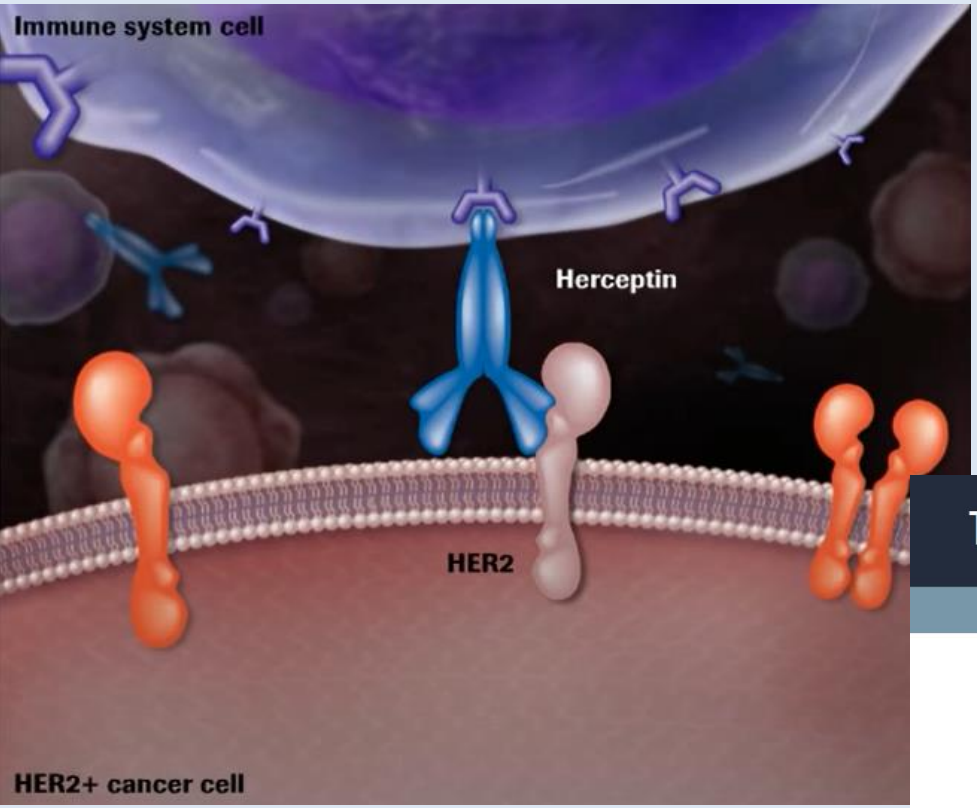
Figure 3
Adenoid cystic carcinoma of the breast. The cells of the epithelial component are positive for CK7, CK5/6, CK 8/18 and CD117. The myoepithelial /abluminal cells express p63, smooth muscle actin and basal CKs: CK5/6, CK14, CK17.

Prognostic and predictive biomarkers

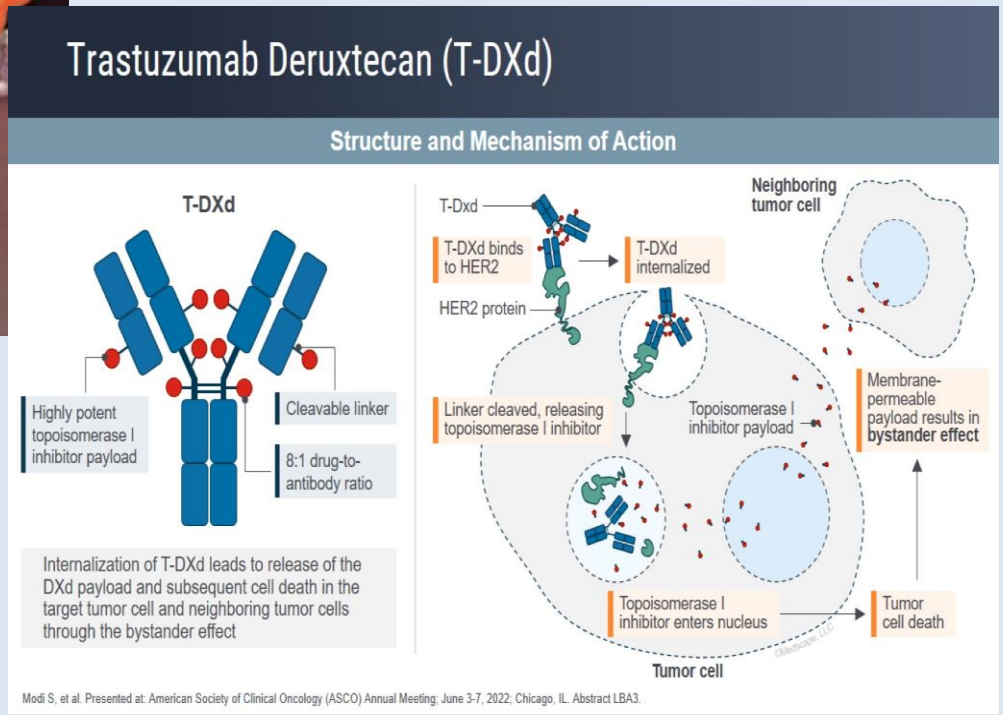
The HER2 gene is located on 17q21. and encodes the human epidermal growth factor receptor 2 (HER2), a transmembrane tyrosine kinase receptor



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

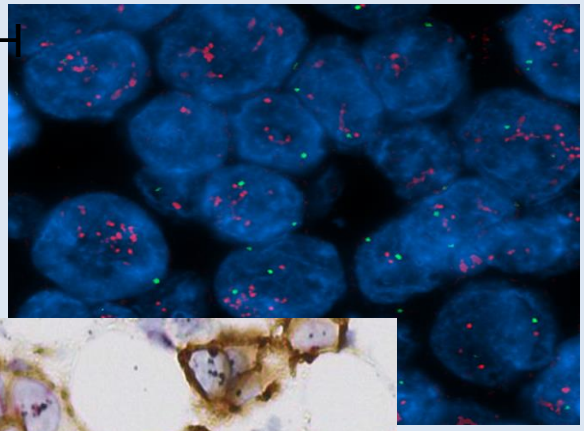


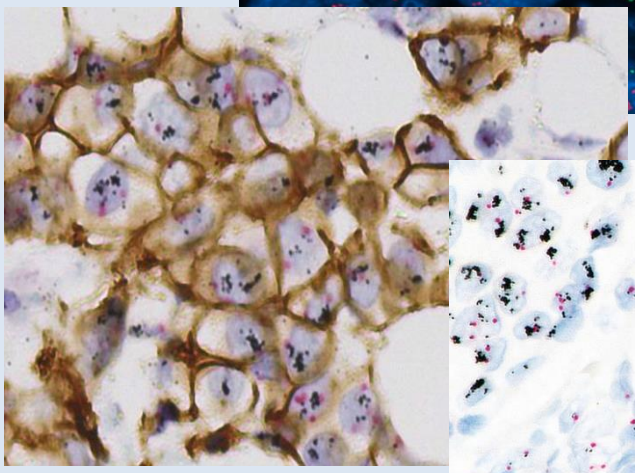
ASCO CAP guidelines 2007, 2013, 2018, Update 2023

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.

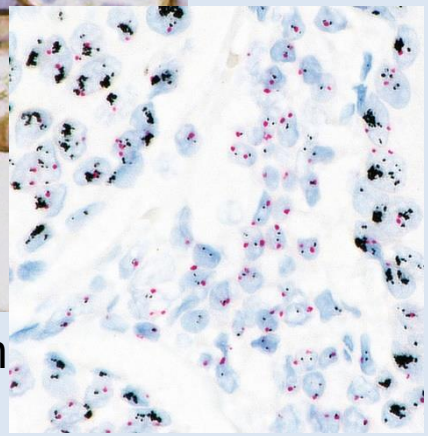
HER2 interpretation BC

FISH



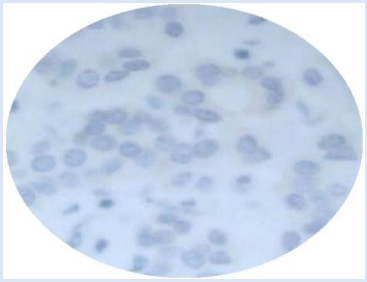


HER2 Gene/Protein

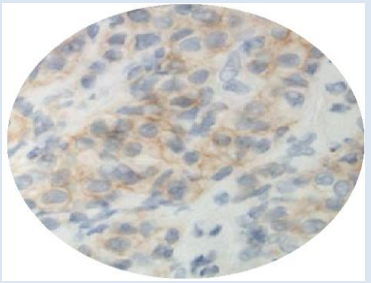


Dual ISH

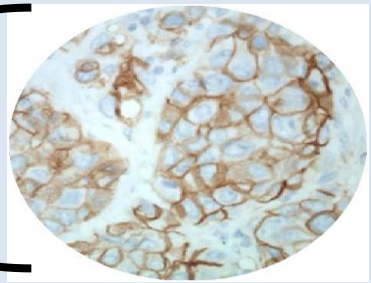
0



1+



2+

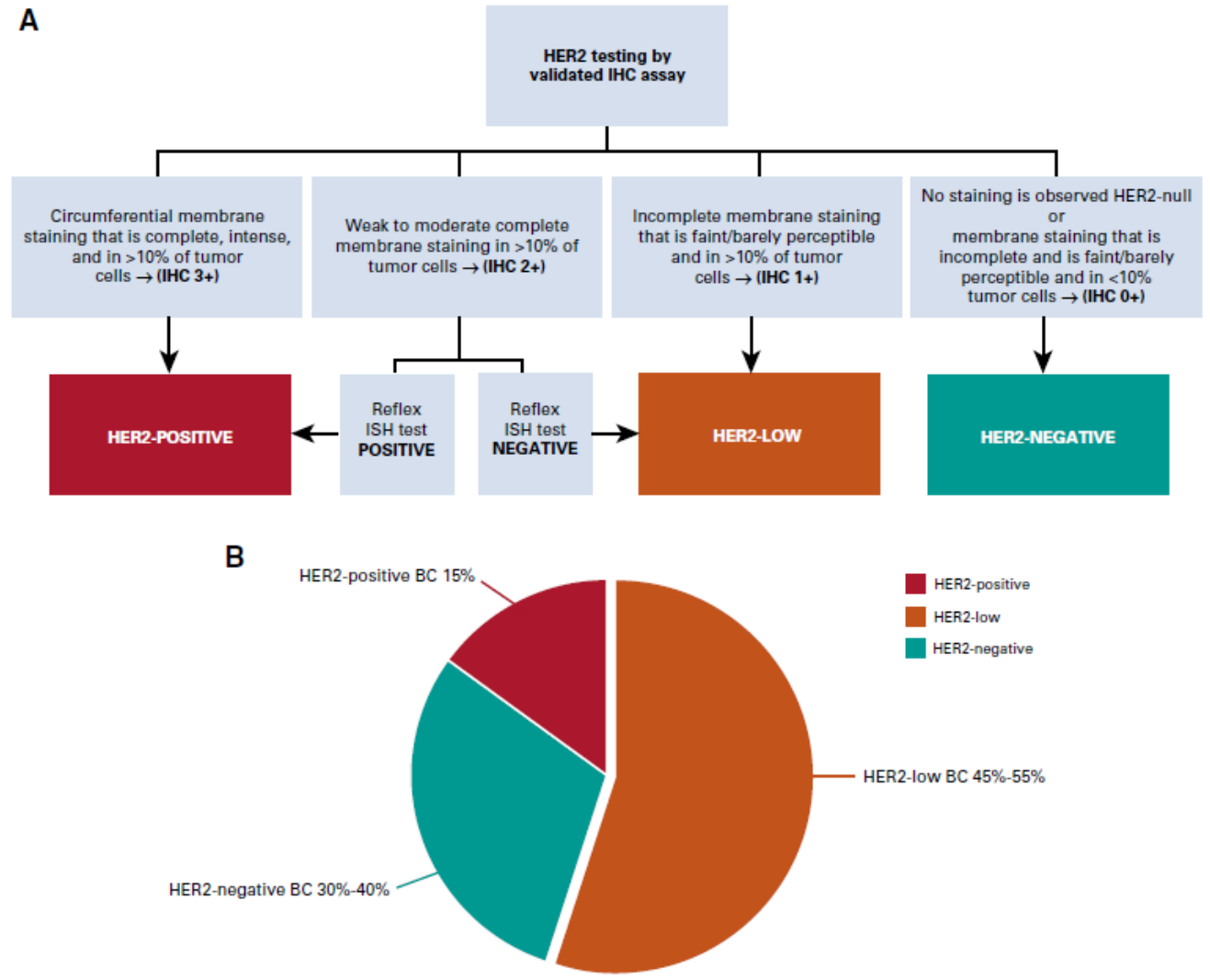


3+

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

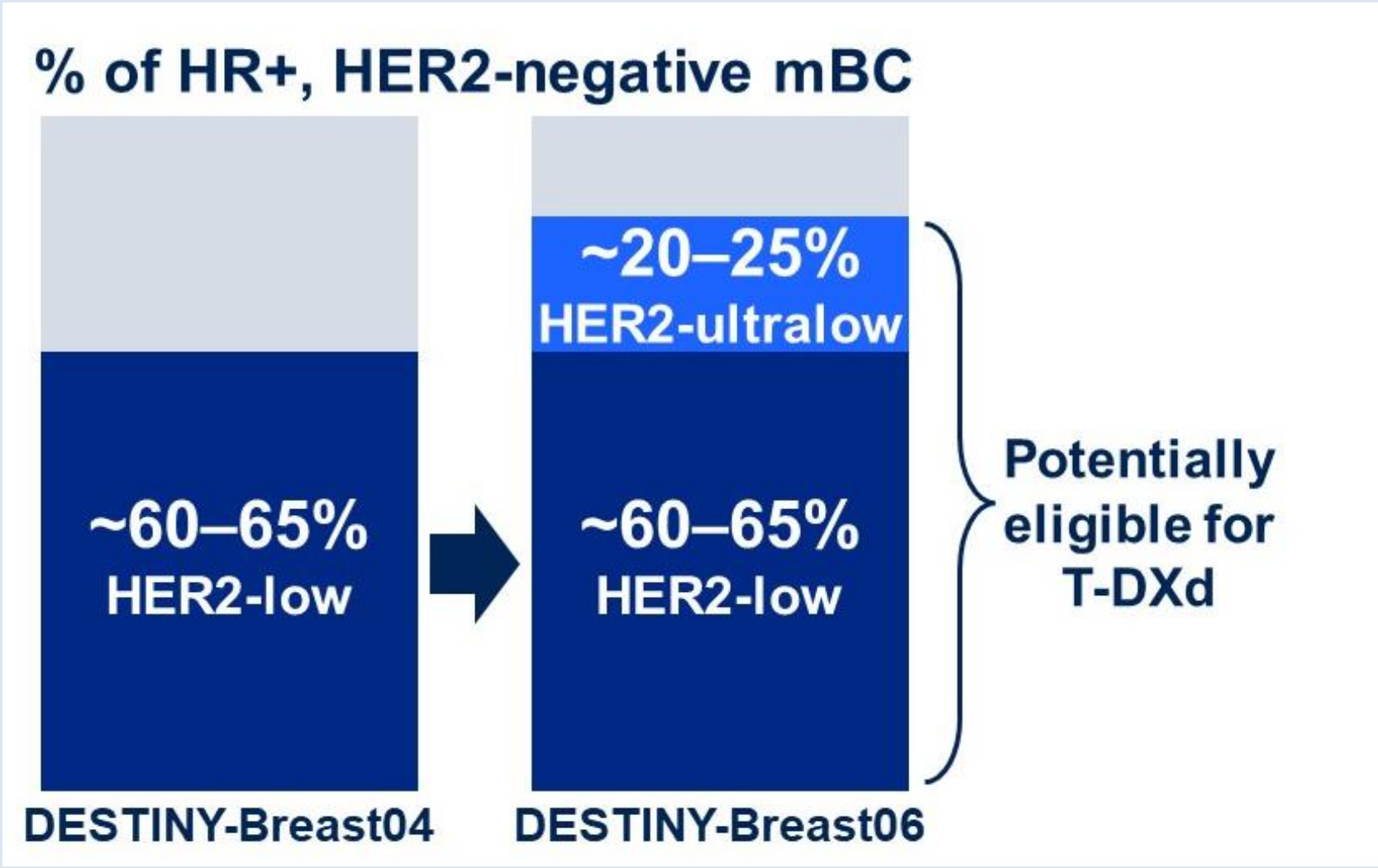
N Engl J Med. 2022 PMID: 35665782
N Engl J Med. 2022 PMID: 35665782

Definition of HER2 Low

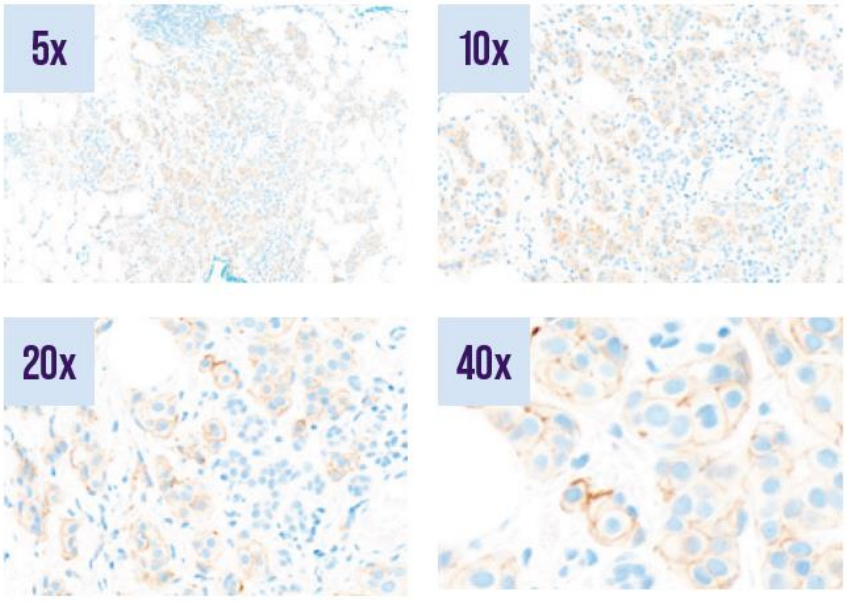


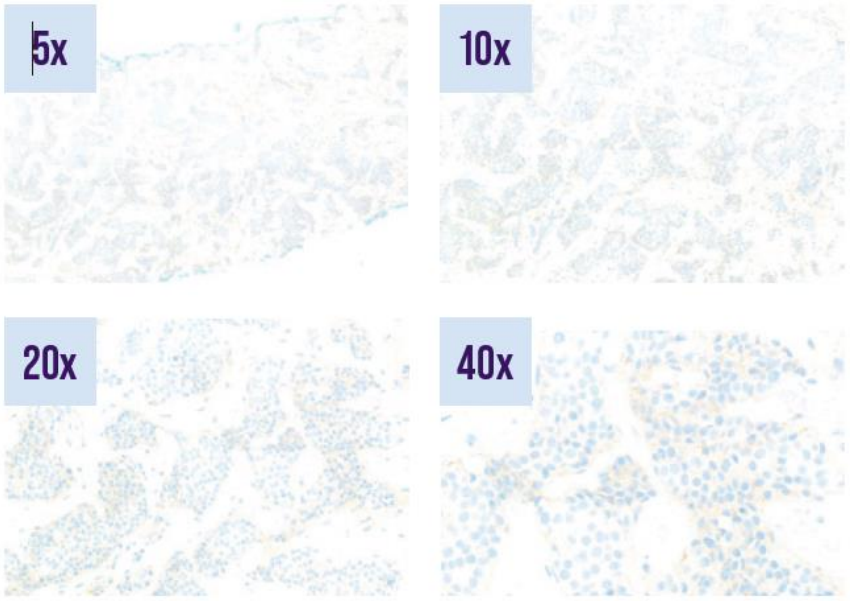
Ultra-Low: HER2>0% and ≤ 10% Destiny Breast 06

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
Potentially eligible for T-DXd
DESTINY-Breast04
DESTINY-Breast06

Example of HER2-low and HER2 0 (null)





CASE 3:

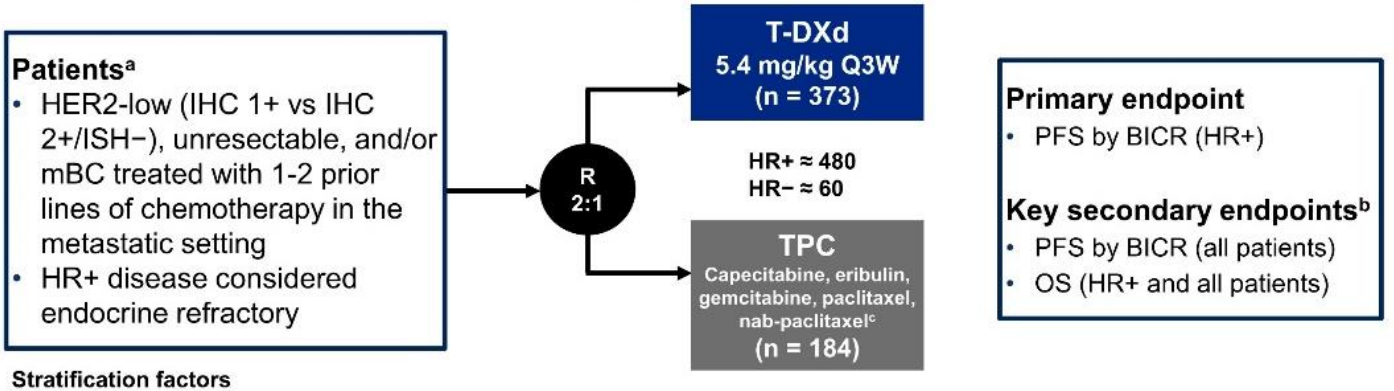
- At 5x there is vague staining that cannot be localised to the membrane. At 10x there are a few cells with discontinuous membrane staining (moderate staining intensity). At 20x more cells show membrane staining; however, this remains discontinuous. Observation at 40x confirms that membrane staining is not circumferential
- **IHC score:** 1+
- **HER2 classification:** HER2-low

CASE 4:

- Even with 40x, only some weak cytoplasmic membrane staining visible
- **IHC score:** 0
- **HER2 classification:** HER2-null

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

DESTINY-Breast06

5

Study design

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

PATIENT POPULATION

- HR+ mBC
- HER2-low (IHC 1+ or IHC 2+/ISH−) or HER2-ultralow (IHC 0 with membrane staining)*
- **Chemotherapy naïve in the mBC setting**

Prior lines of therapy

- ≥2 lines of ET ± targeted therapy for mBC
  OR
- 1 line for mBC **AND**
  - Progression ≤6 months of starting first-line ET + CDK4/6i
    OR
  - Recurrence ≤24 months of starting adjuvant ET

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)

R 1:1

T-DXd 5.4 mg/kg Q3W (n=436)

HER2-low = 713
HER2-ultralow = 153†

TPC (n=430)

Options: capecitabine, nab-paclitaxel, paclitaxel

ENDPOINTS

Primary

- PFS (BICR) in HER2-low

Key secondary

- PFS (BICR) in ITT (HER2-low + ultralow)
- OS in HER2-low
- OS in ITT (HER2-low + ultralow)

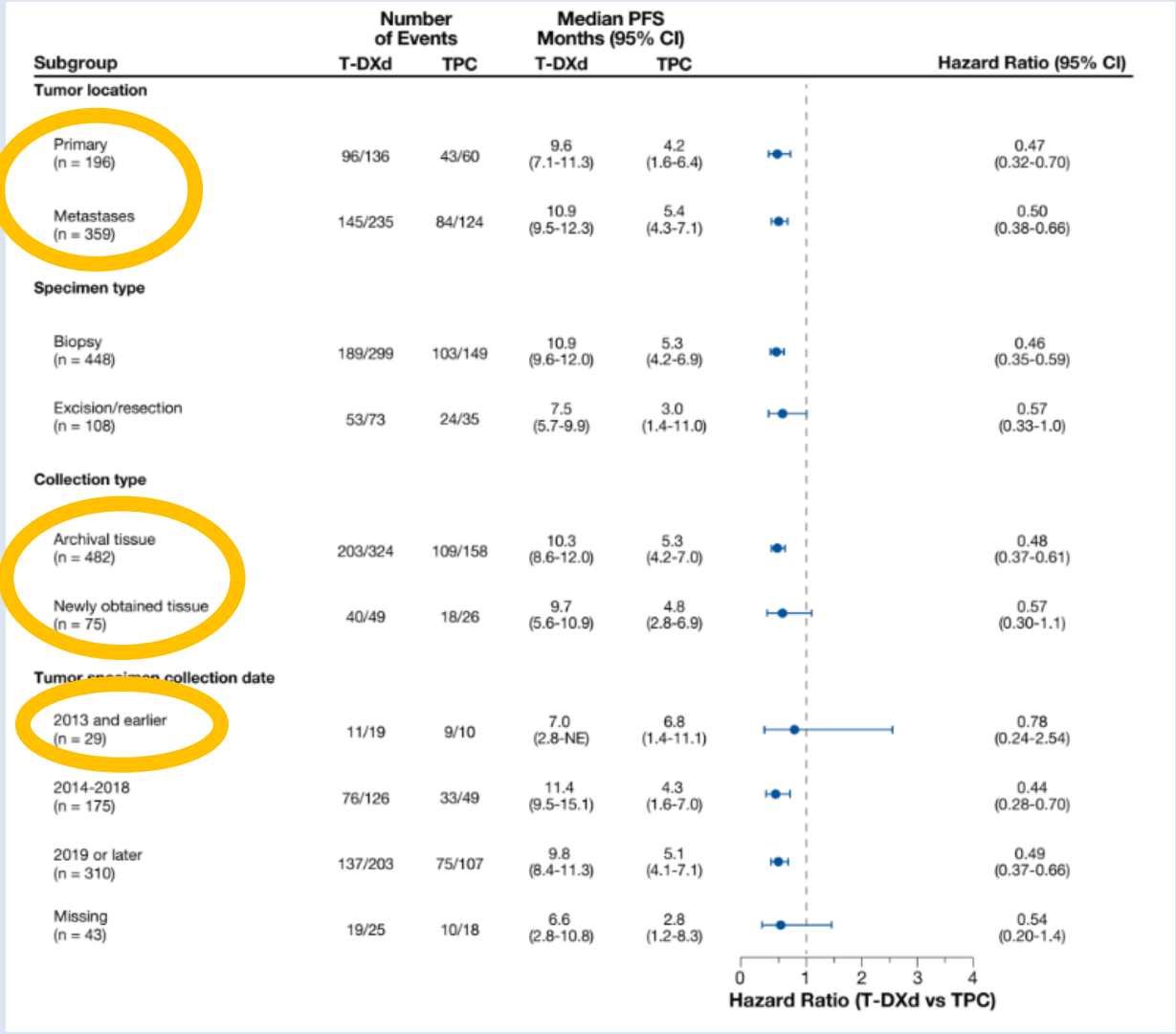
Other secondary

- PFS (INV) in HER2-low
- ORR (BICR/INV) and DOR (BICR/INV) in HER2-low and ITT (HER2-low + ultralow)
- Safety and tolerability
- Patient-reported outcomes‡

Tumor sample characteristics Destiny Breast04

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

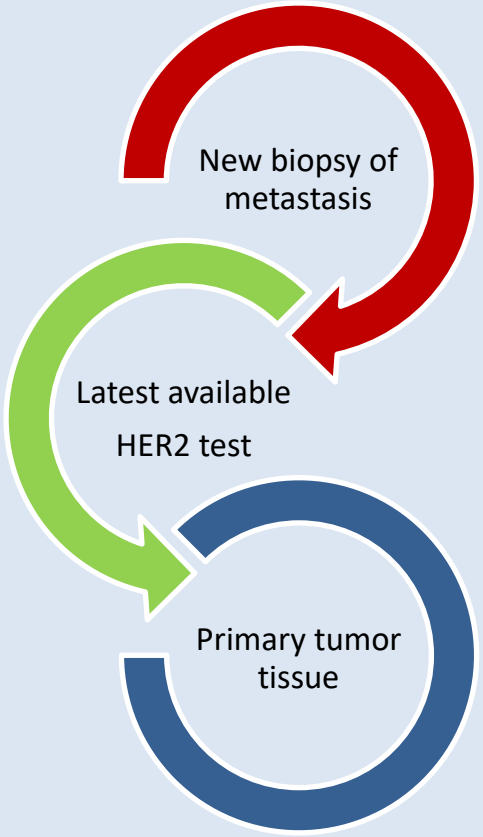
For patients enrolled in DESTINY-Breast 04, efficacy of T-DXd compared with TPC was consistent regardless of tumor sample characteristics



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)

Suggested flow for analysis



Re-evaluation of HER2 staining is mandatory if the staining is performed before 2023/2024
and representing HER2 score 0/1+

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

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		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
- Newer quantitative technologies e.g. quantitative IF, RT-qPCR; digital imaging and machine learning may be more accurate and predictive value will have to be determined
- 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/JC
ESMO consensus 2023 https://doi.org/10.1016/j.annonc.2023.05.00

2023 ESMO Consensus

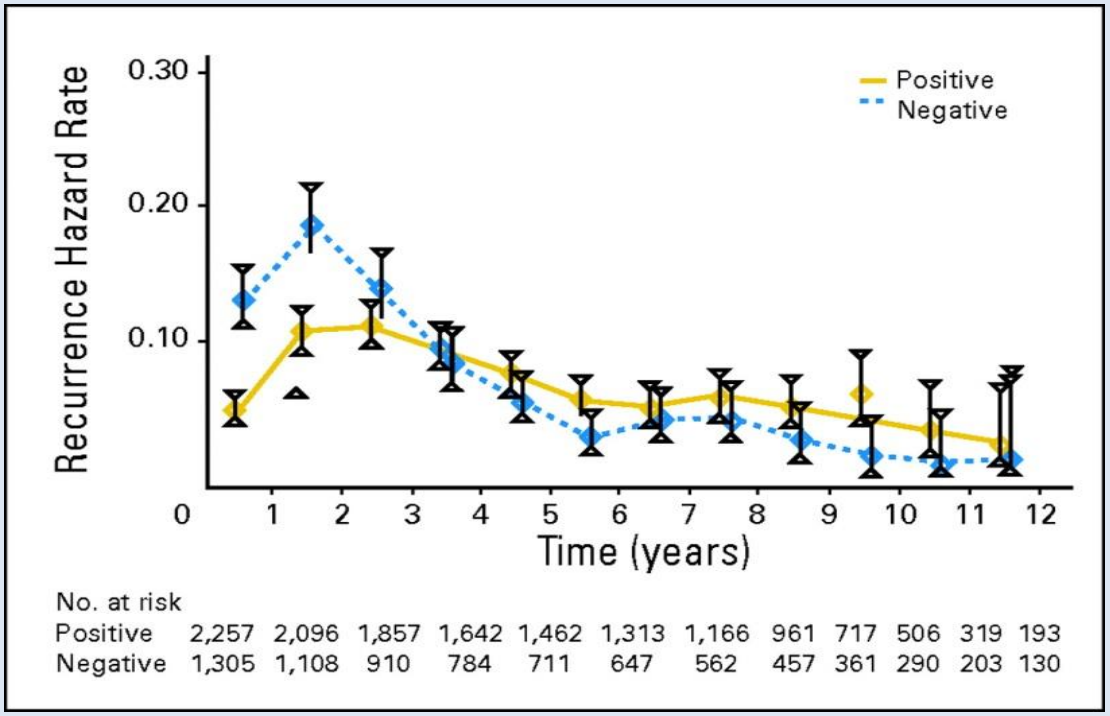
Table 1. Interpretation by the ASCO/CAP 2018 Guidelines and by the 2023 ESMO Consensus on HER2-low breast cancer regarding each pattern of HER2 staining

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**

https://doi.org/10.1016/j.annonc.2023.05.008

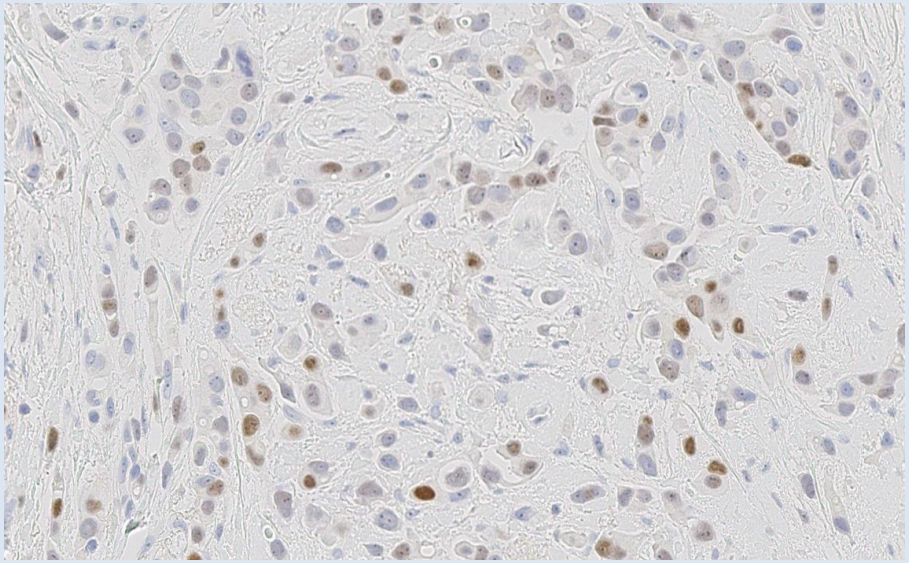
The Estrogen receptor as a prognostic/predictive marker

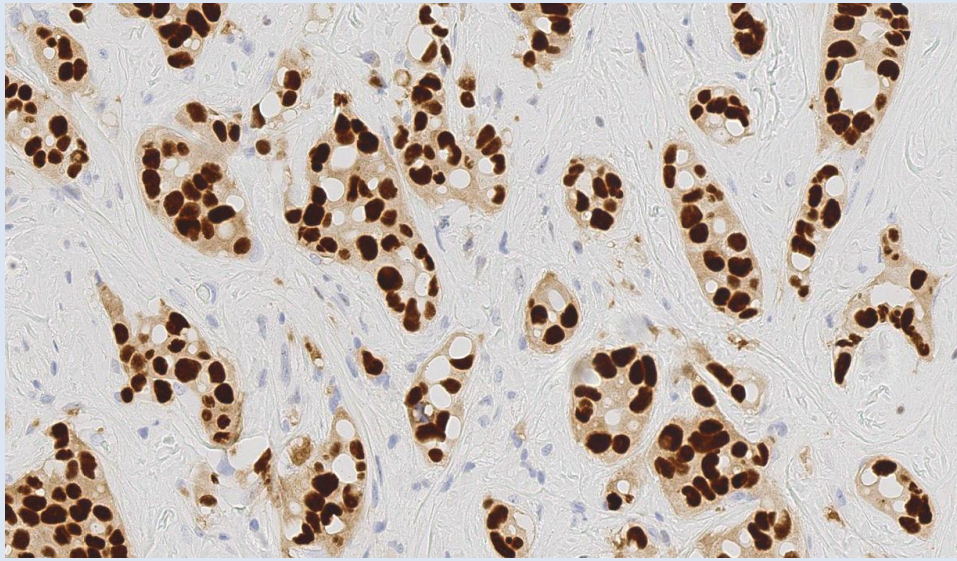
Risk of recurrence pr. year
N = 3,562 patients



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

2020 – ASCO CAP Update
Hormone receptors

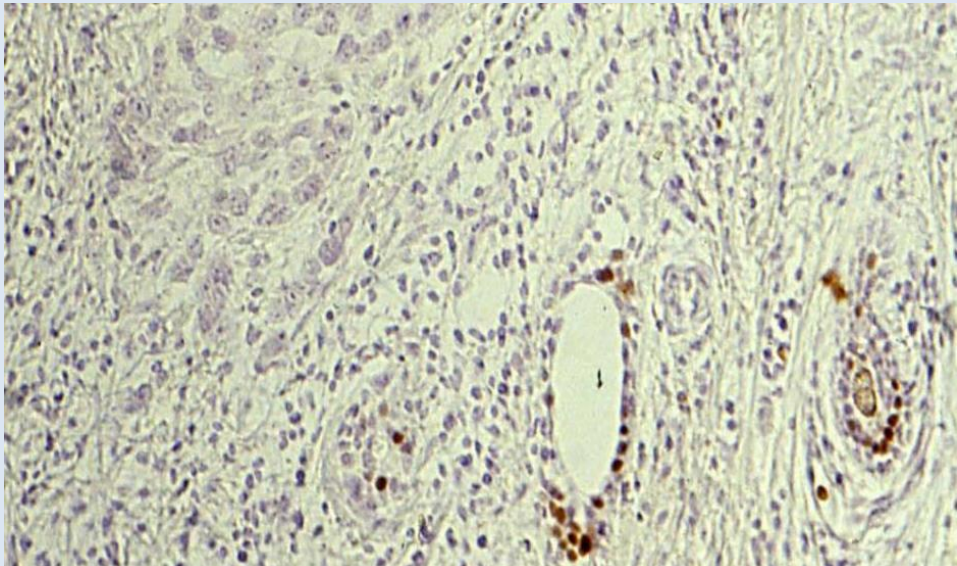




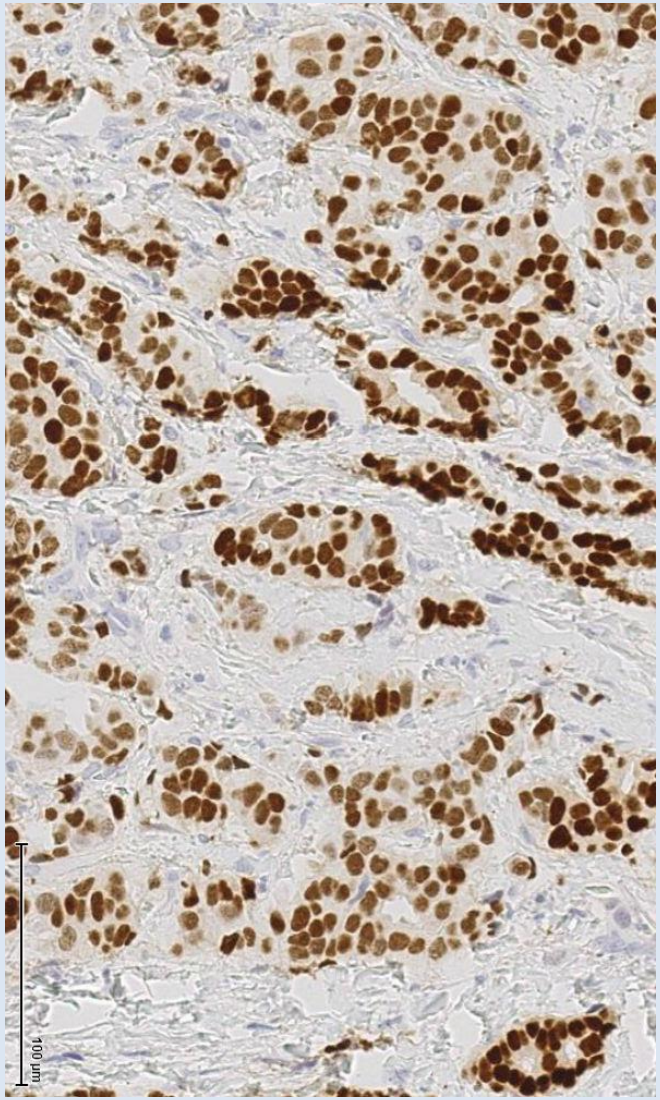
ER positive 86% of breast carcinomas (DK)

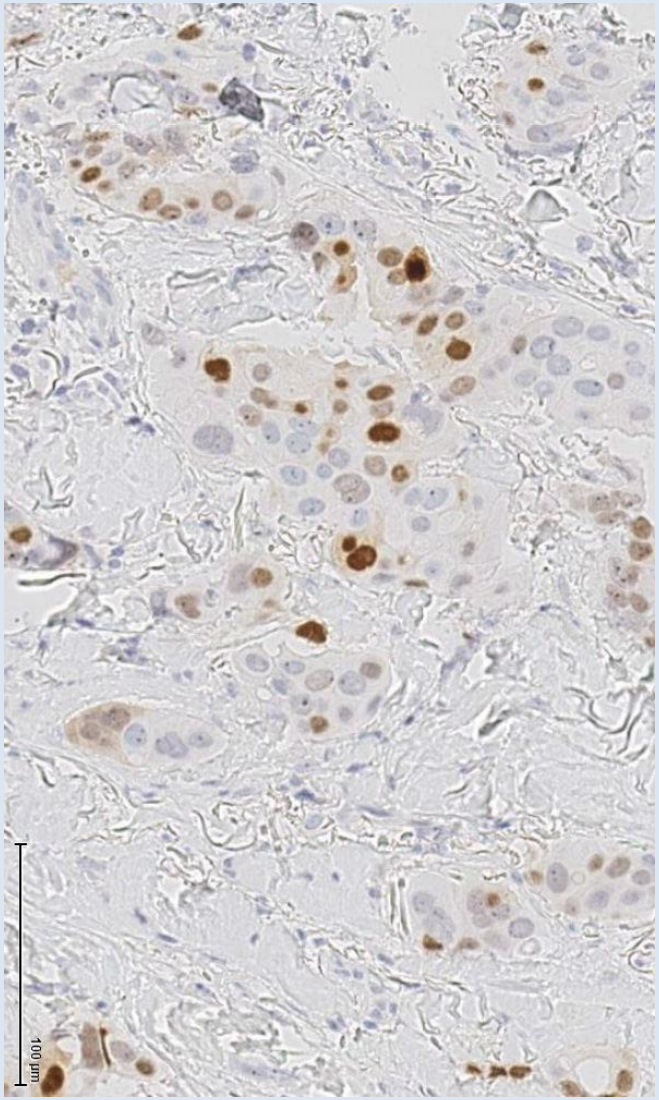
Cut off ≥ 1%
A sample is reported negative for ER or PgR if < 1% or 0% of tumor cell nuclei are immunoreactive.

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



Interpretation of PgR

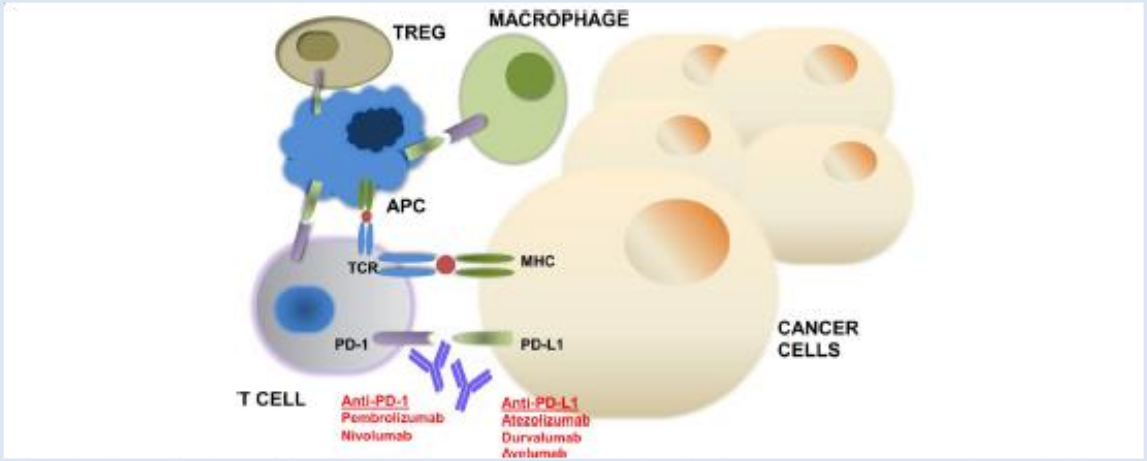




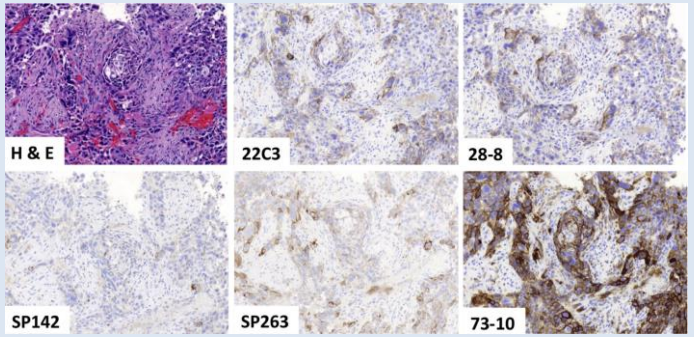
Heterogeneous expression

PD-L1
in TNBC

Mechanism of action of PD-1 and PD-L1 inhibitors



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



Binding of PD-1 to its ligand PD-L1 results in suppression of proliferation and immune response of T cells. Activation of PD-1/PD-L1 signaling serves as a principal mechanism by which tumors evade antigen-specific T-cell immunologic responses.
Antibody blockade of PD-1 or PD-L1 reverses the process and enhances antitumor immune activity

Gong et al. Journal for ImmunoTherapy of Cancer (2018) 6:8

PD-L1 scoring system

Which scoring system should be used for PD-L1 staining?

	IVD diagnostic antibodies used in clinical trials	
Drug	Pembro-lizumab **(MSD)**	Atezo-lizumab **(Roche)**
AB clone	22C3 Dako	SP-142 Ventana
Score	CPS	IC_A
cell type	Tumor Immune	Immune
Breast cancer trial	KN-012 KN-522	**Impassion -130**

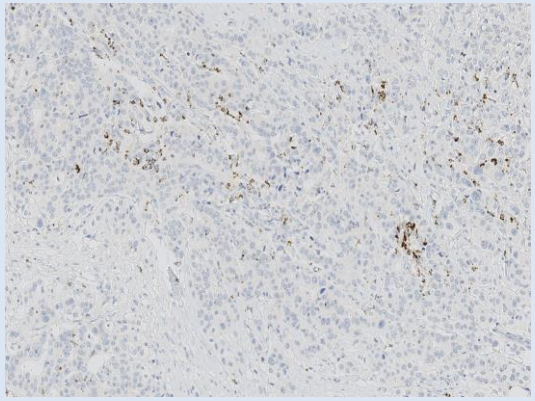
IC_A score: percentage of tumor area covered by PD-L1 positive immune cells (designed for Atezolizumab)

CPS score: positive tumor or immune cells as percentage of all tumor cells (designed for Pembrolizumab)

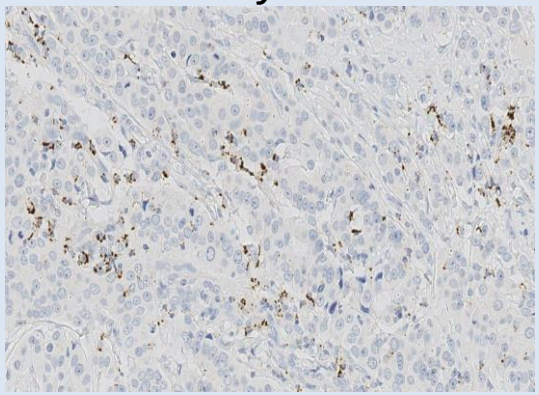
Keynote-355

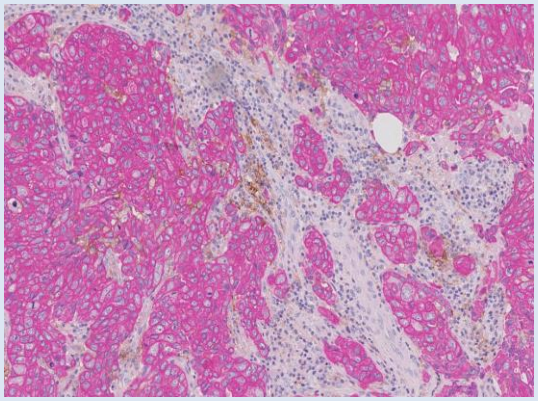
Impassion130

PD-L1 immunohistochemistry

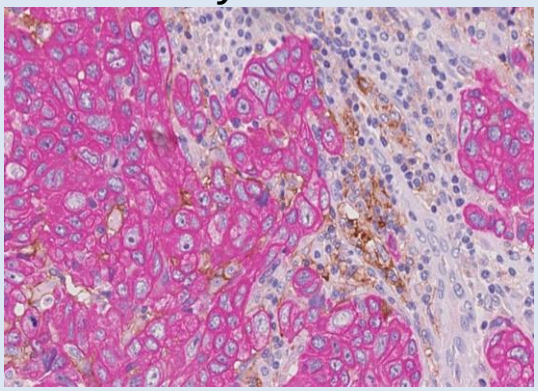


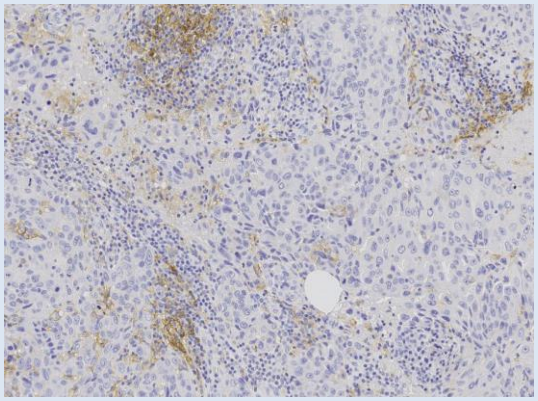
Assay SP142



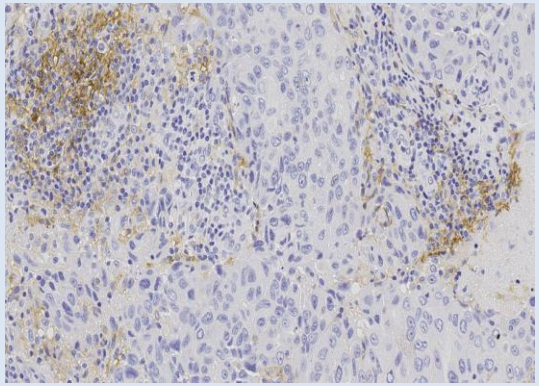


Assay 22C3+CK8



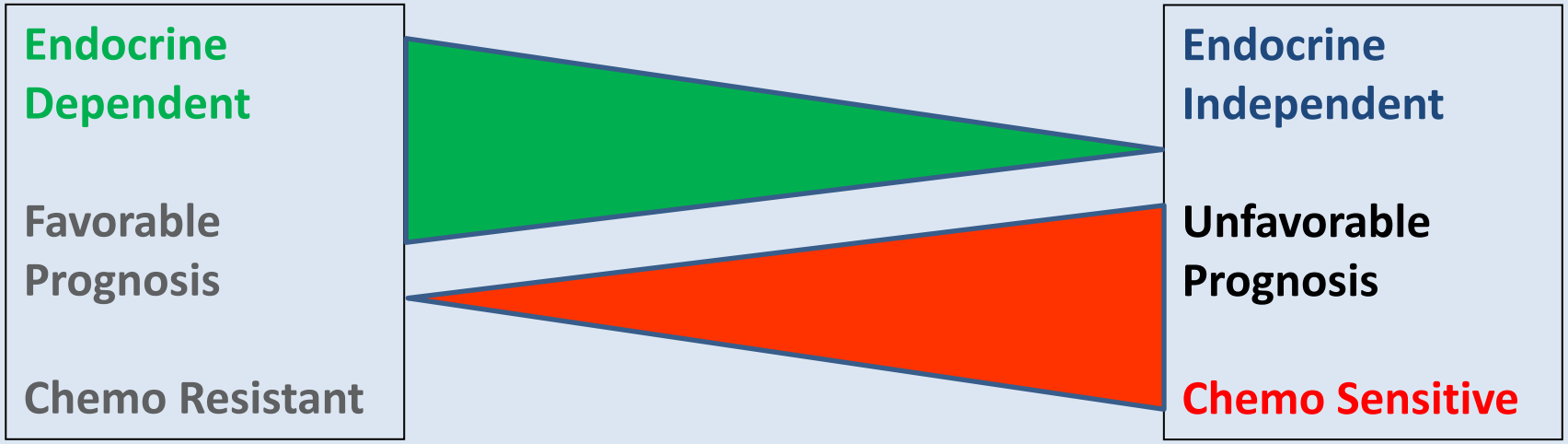


Assay 22C3



courtesy of Ulriche Thiim Pedersen

Breast cancer – Molecular intrinsic subtypes prognostic information



Lum A | Lum B | HER2-enriched | Basal-like

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

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Commentary

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

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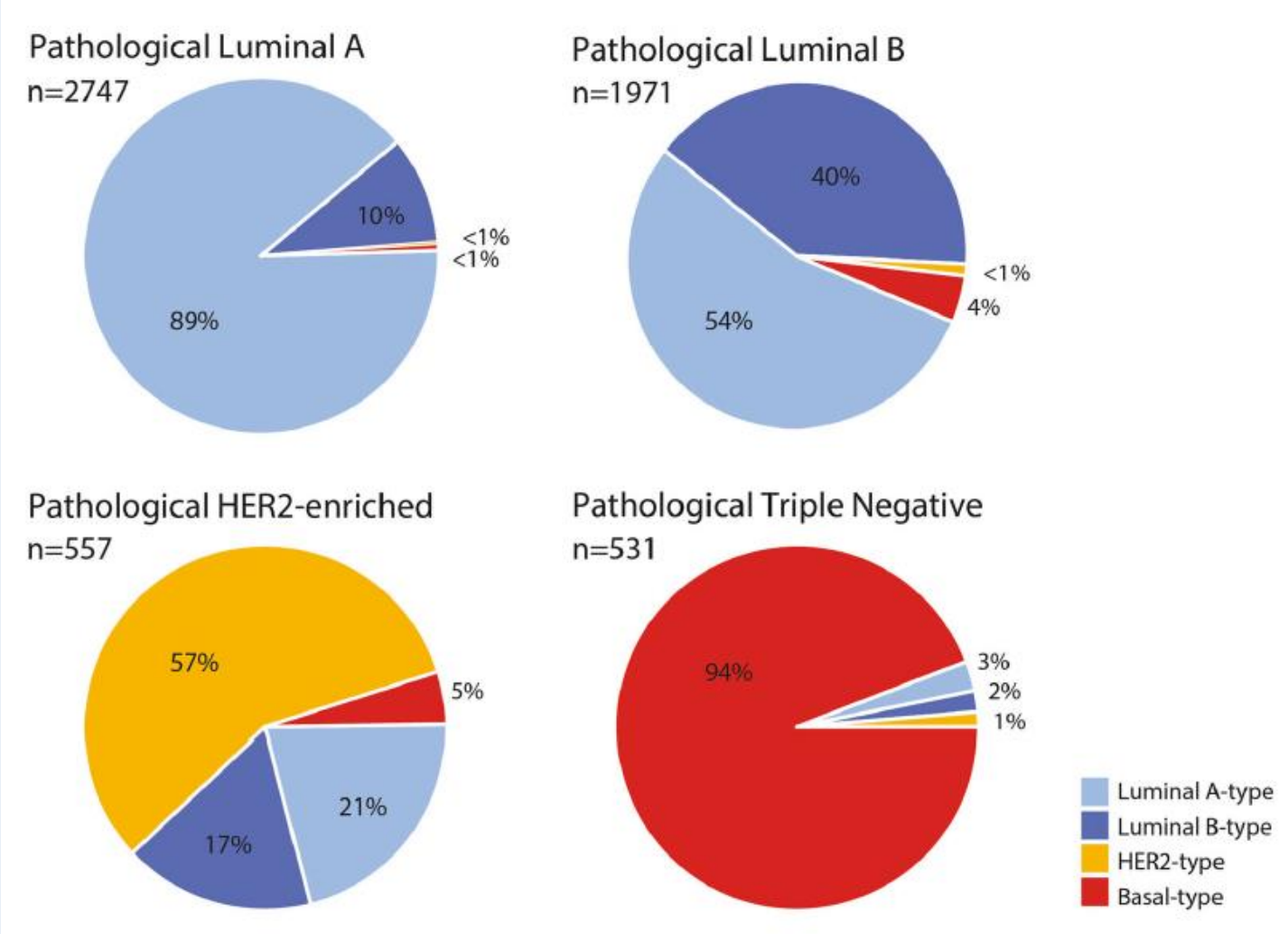
Table 4. Recommendations for Ki67 in postmenopausal ER+ HER2 normal BC since St. Gallen 2009

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
- *Intrinsic molecular subtype / gene expression profile*
  - Identification of patients who can be spared chemotherapy


Thank you!