Breast cancer: IHC for diagnostic use

NordiQC Workshop in Diagnostic Immunohistochemistry 2021
Aalborg University Hospital
September 29nd – October 1st 2021

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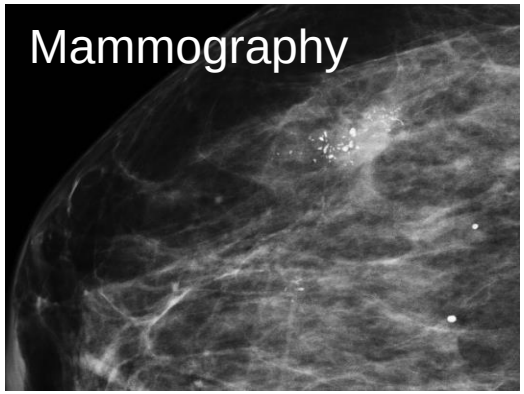
Agenda

- 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 and *ER low status*
    - Progesteron Receptor
    - HER2 and *HER2 low status*
    - Ki67
    - PD-L1
  - Molecular subtypes

Triple Test
Diagnostic approach – Breast Tumours



Physical breast exam/

Palpation

Radiology

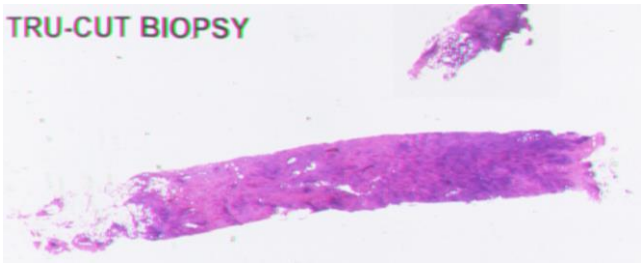
Mammography

Ultrasound

Pathology

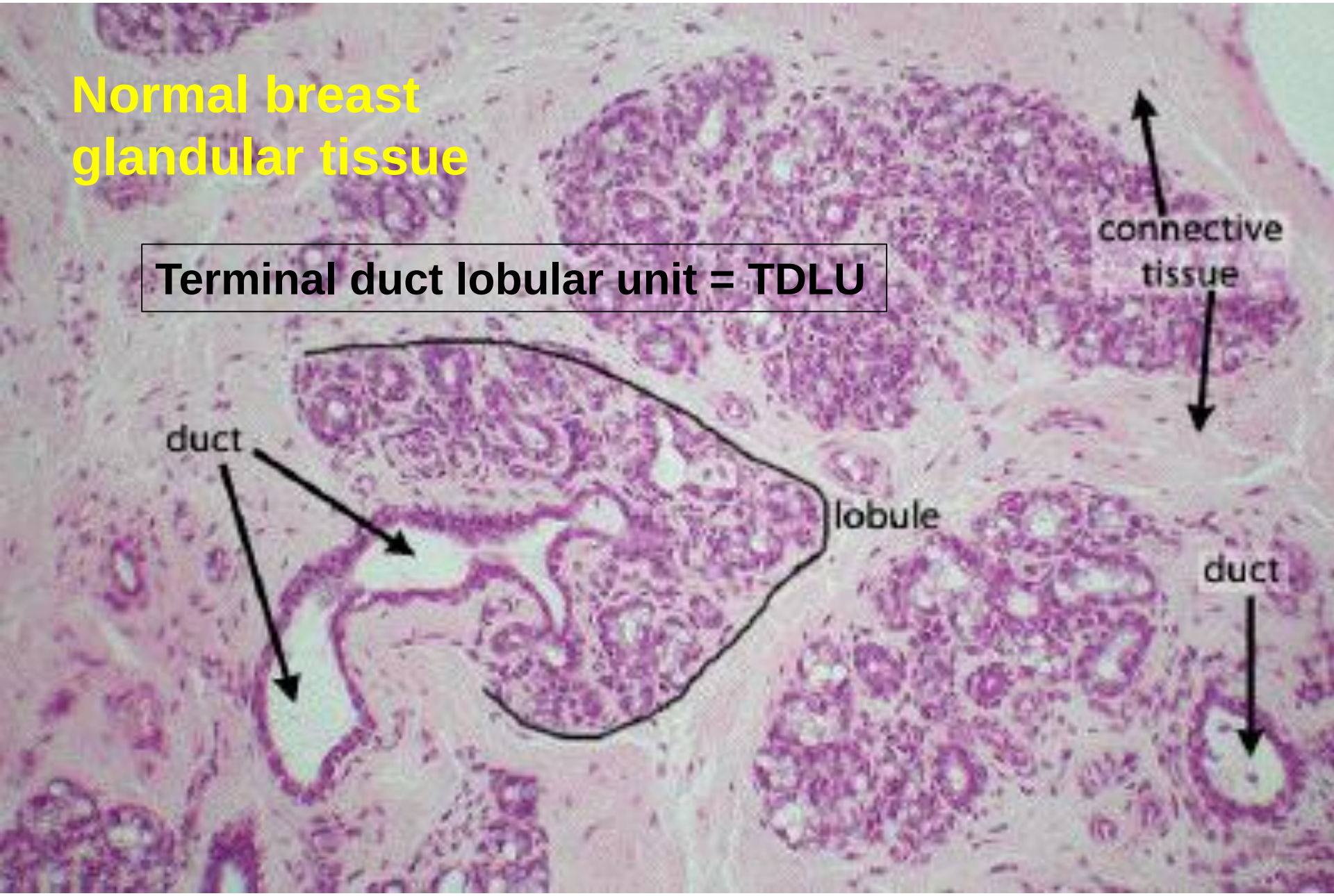
Core needle biopsy or Fine needle aspiration

Triple diagnostics



Normal breast glandular tissue

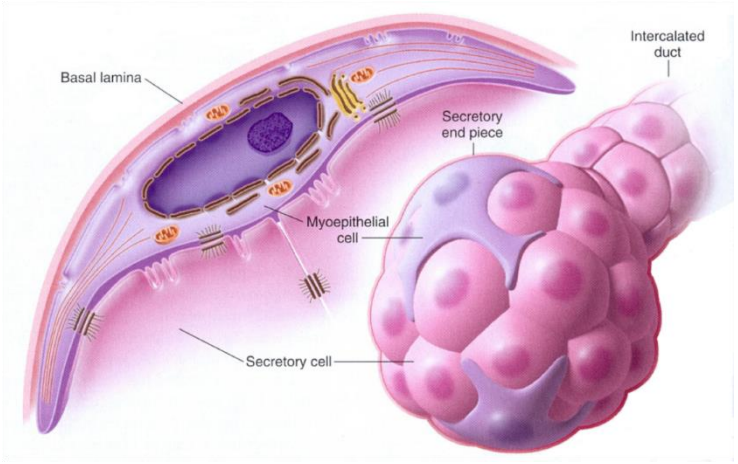
Terminal duct lobular unit = TDLU



Mammary gland epithelium
Two types of epithelial cells are present: Luminal cells and myoepithelial cells

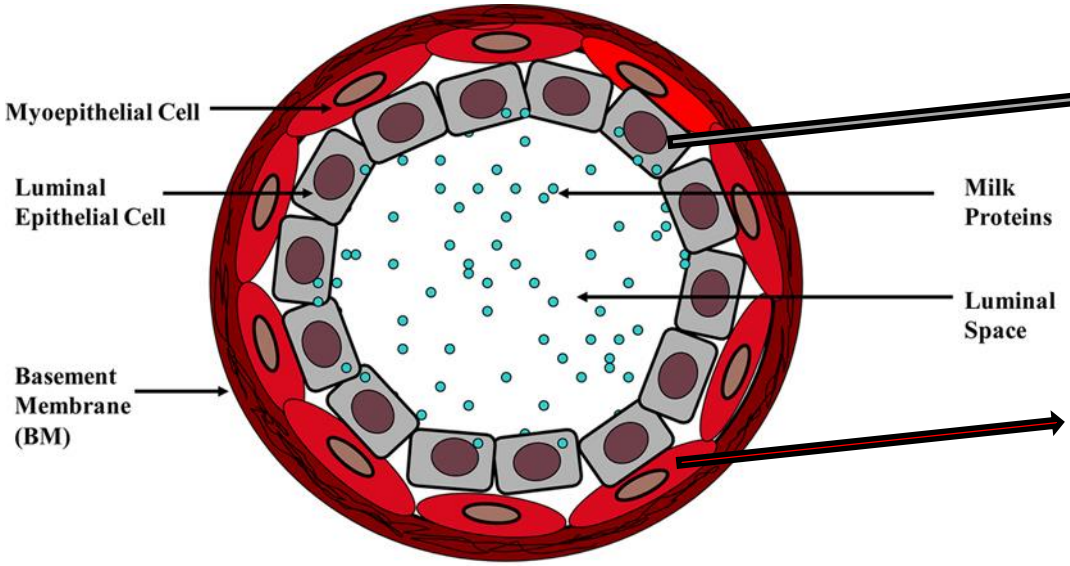
Luminal cell

Myoepithelial cell



Myoepithelial cells with contractile function forming a meshwork that does not cover the entire basement membrane nor the entire luminal cell

Epithelial cells with specific immunohistochemical phenotype

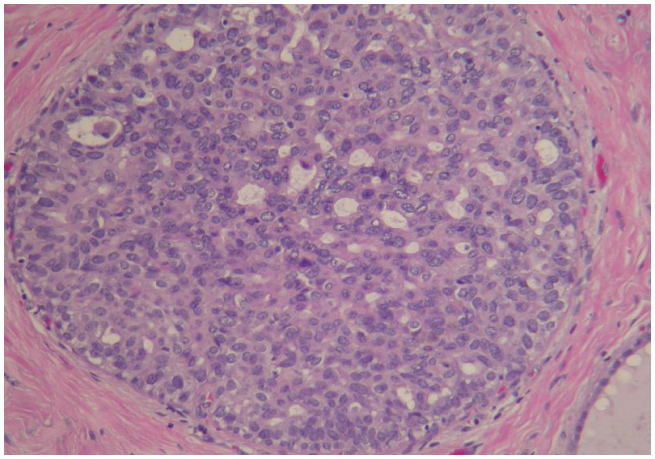


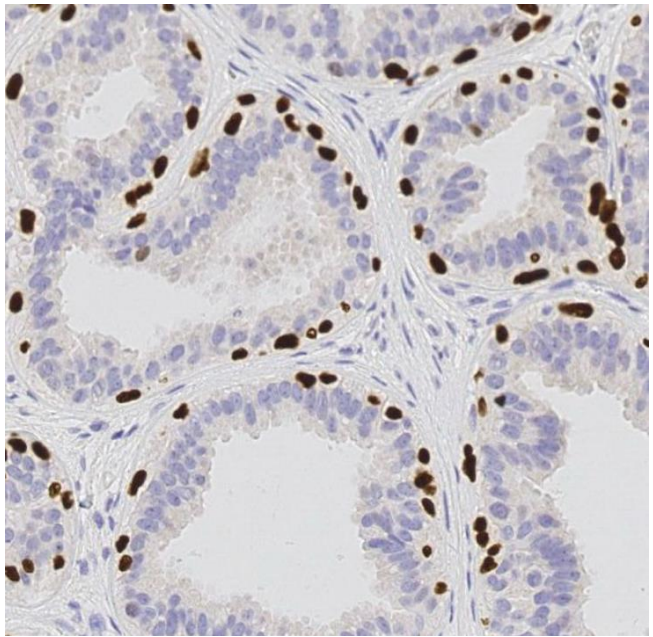
Luminal markers (LMW):
CK7, CK8, CK18, CK19

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

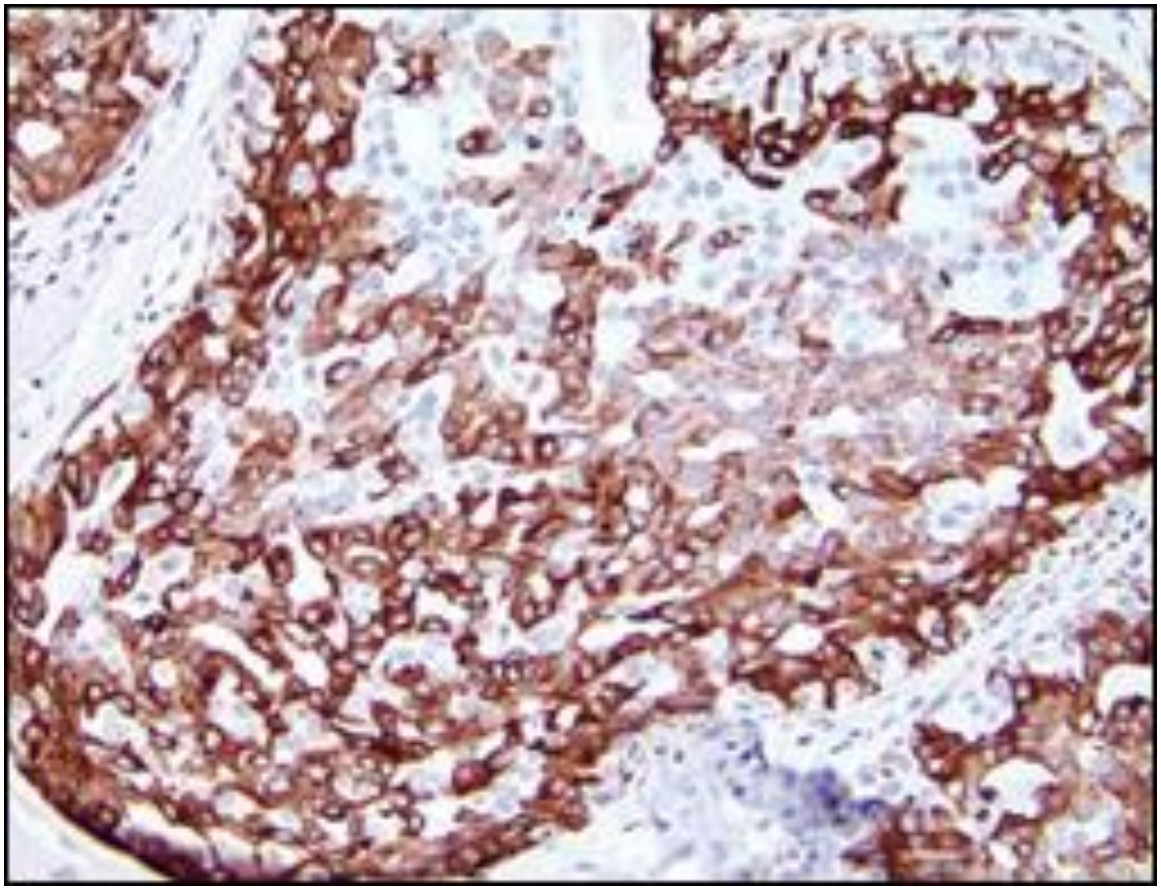
*Smooth muscle myosin heavy chain

Benign hyperplasia
Positive staining for myoepitelial cells





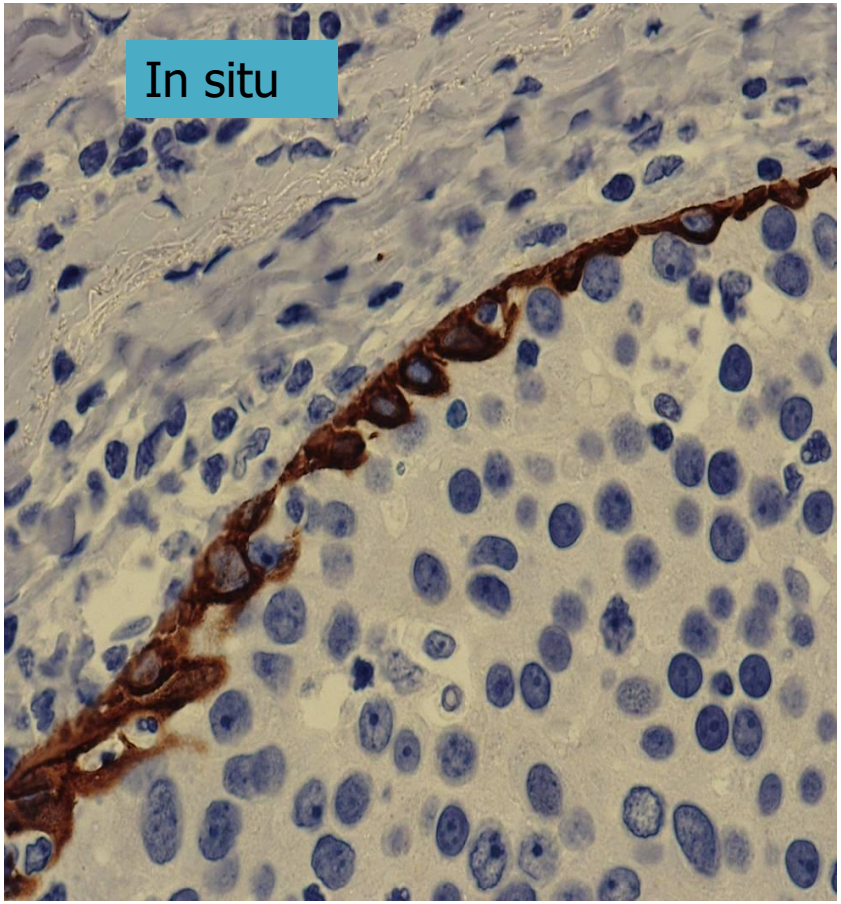
P63



CK5

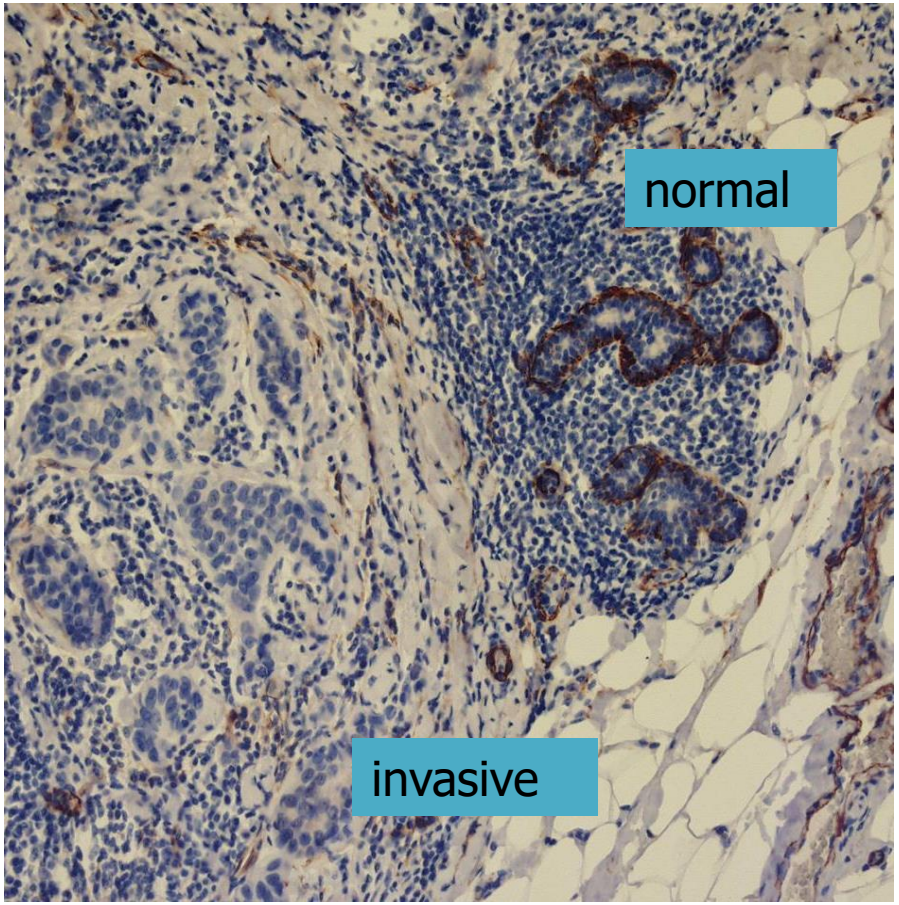
Differentiation between ductal carcinoma in situ and Invasive Carcinoma i.e. SMMHC*

present



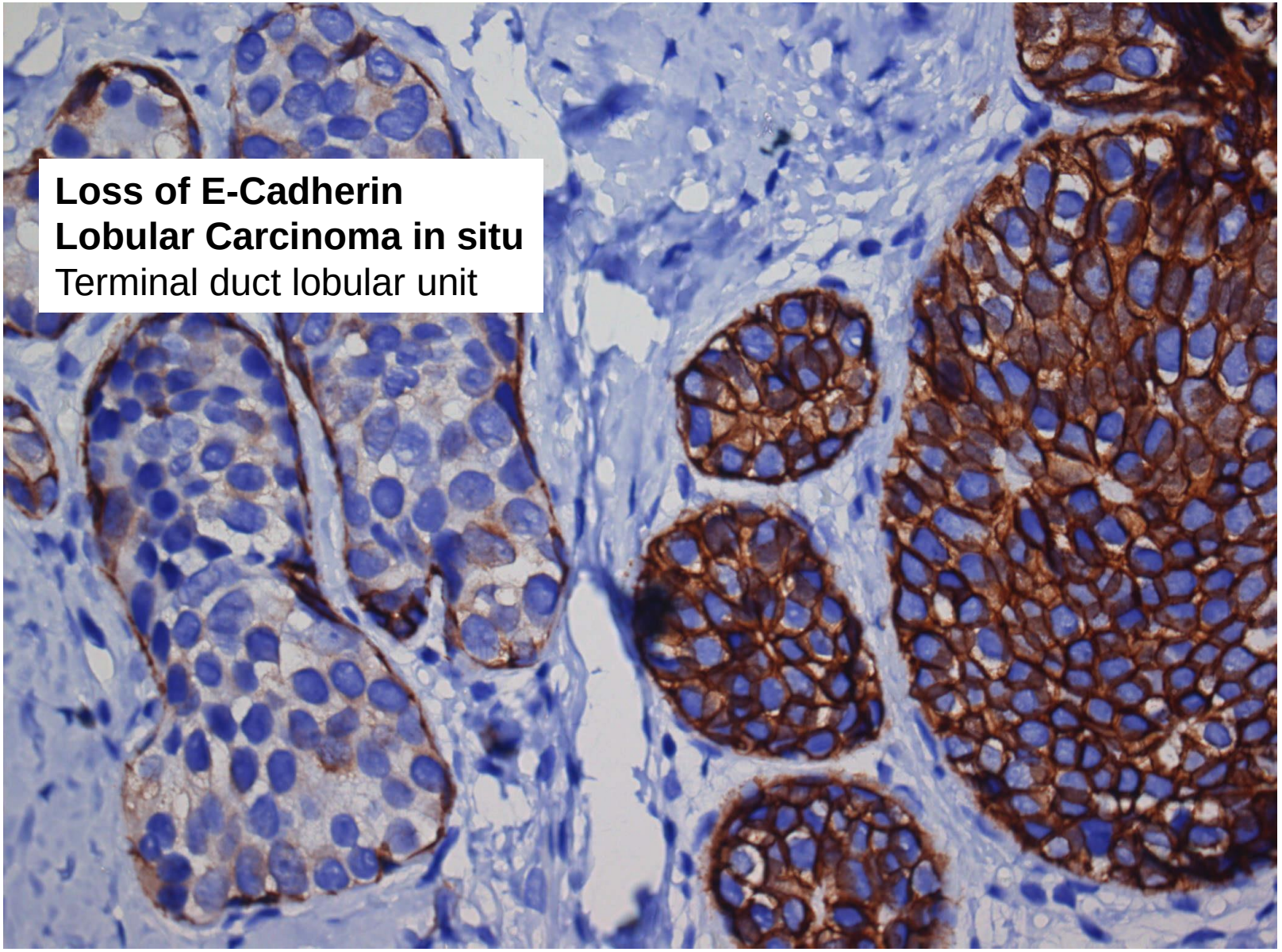
Detecting ”presence”

Not present



Detecting ”absence”

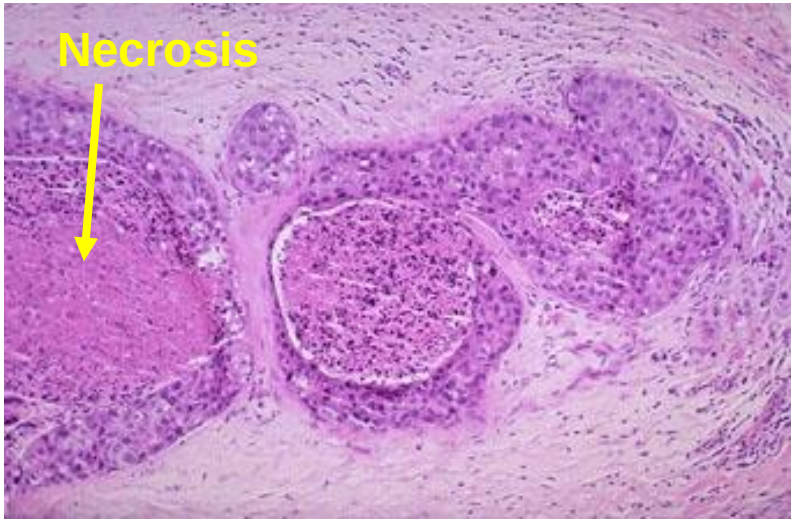
* Smooth muscle myosin heavy chain, as detected with clone SMMS-1



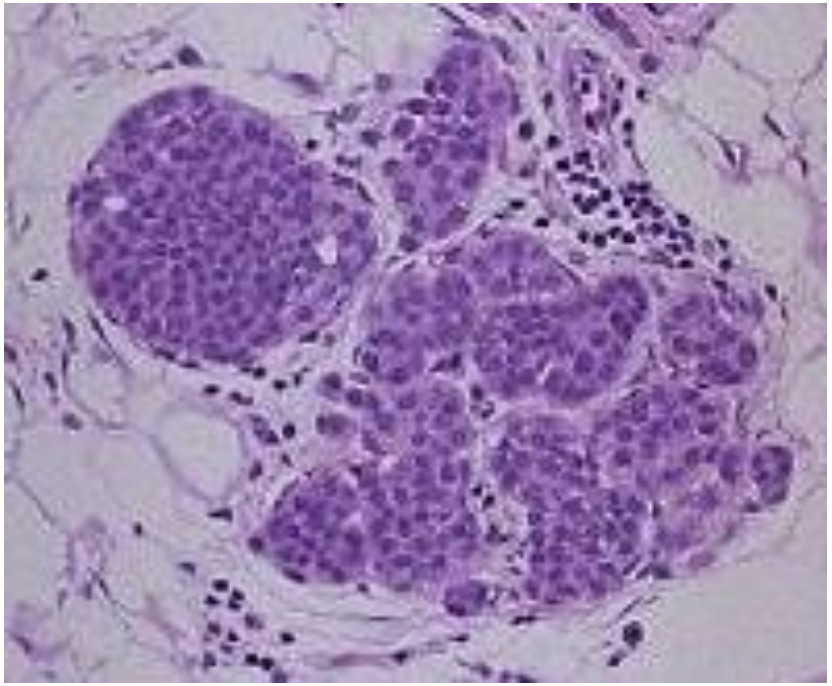
E-cadherin: Cell Adhesion Molecule

Carcinoma in situ

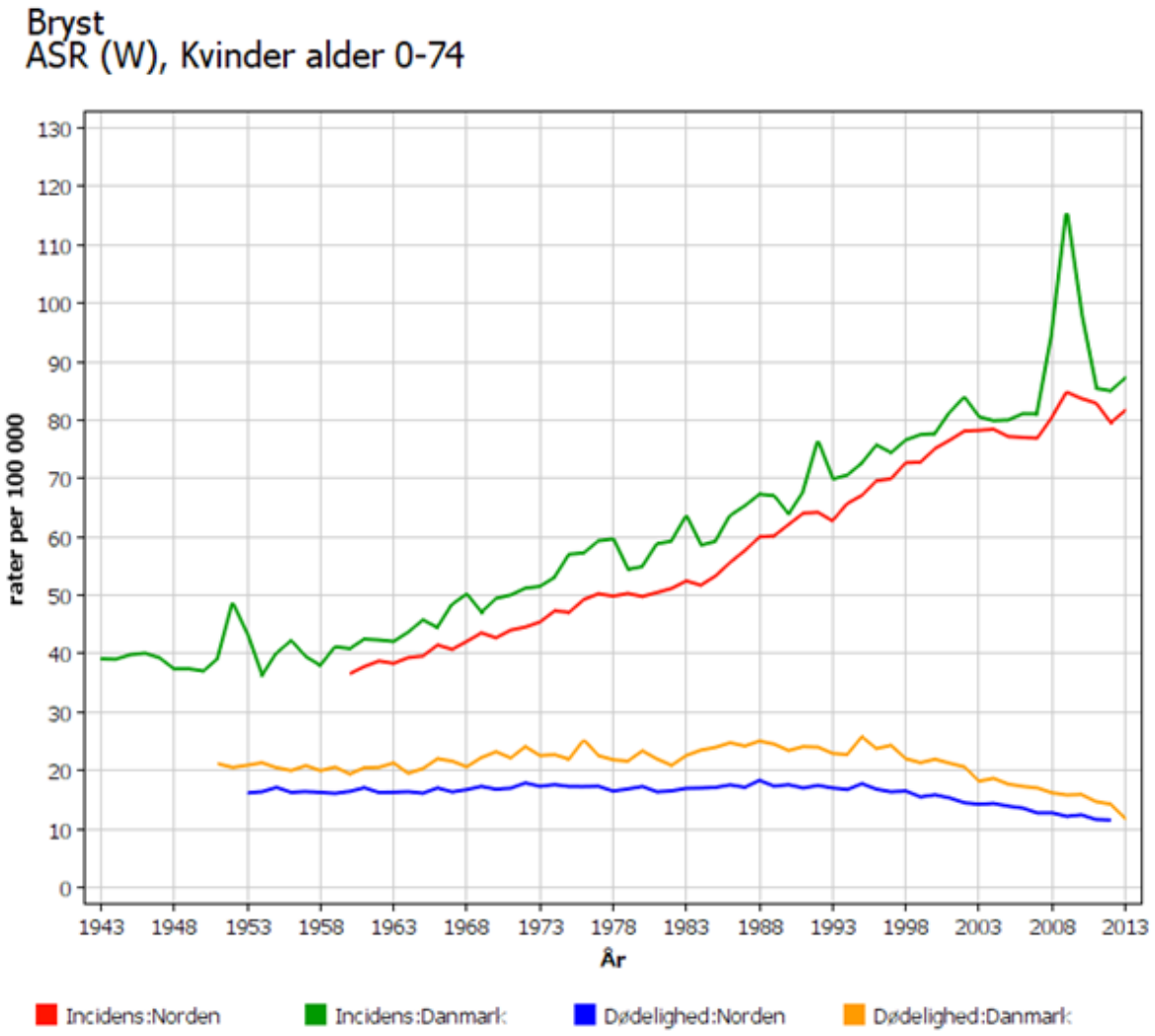
- Ductal carcinoma in situ
  - 12-15% of malignant lesions in the Danish screening population
  - Microcalcifications
  - Risk of progression to invasive carcinoma
  - Surgery with free margins (2 mm)
  - Radiation therapy after breast conserving surgery



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



Breast cancer: Incidence and mortality Denmark



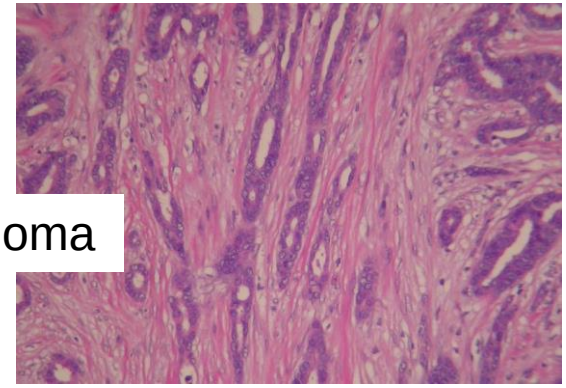
Annually app 4700-5000 new cases

Classification of malignant tumors of the breast

WHO blue books

Histological subtypes

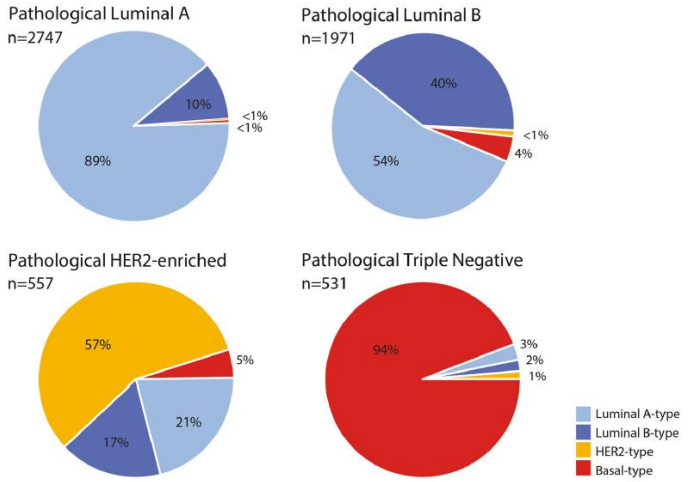
- Ductal : up to 80%
- Lobular: 5 - 14%
- Tubular: 2 - 8%
- Mucinous: 2 - 4 %
- Apocrine: 1 – 4%
- Papillary 1 – 2%
- Other



Tubular Carcinoma

Intrinsic molecular subtypes

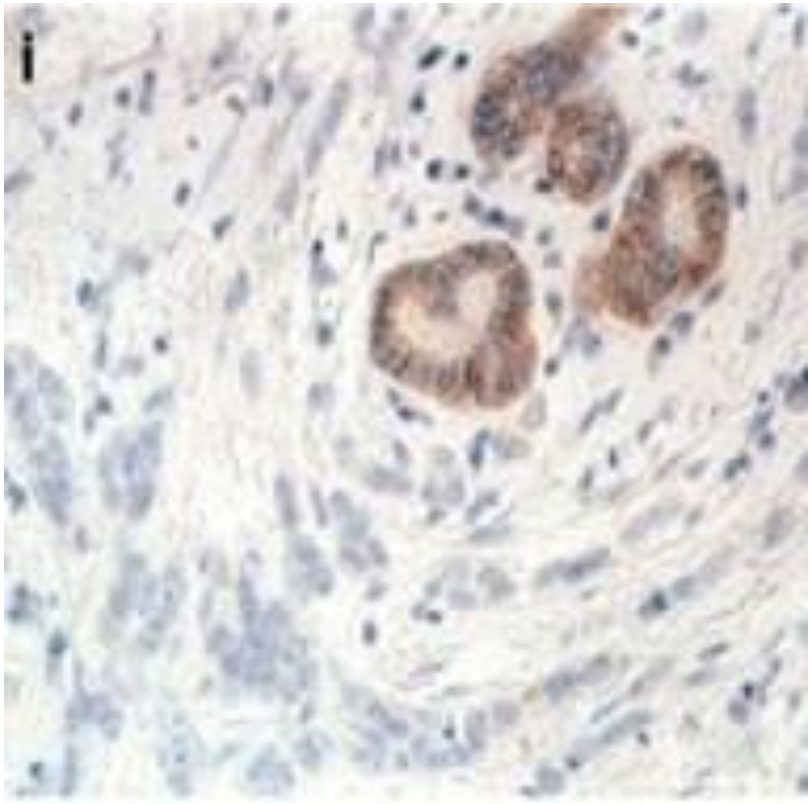
- **Luminal A**: ER+, low proliferative
- **Luminal B**: ER+, high(er) proliferative, (HER2+)
- **HER2 Enriched:** (HER2 positive)
- **Basallike:** (ER-, PR- HER2-)



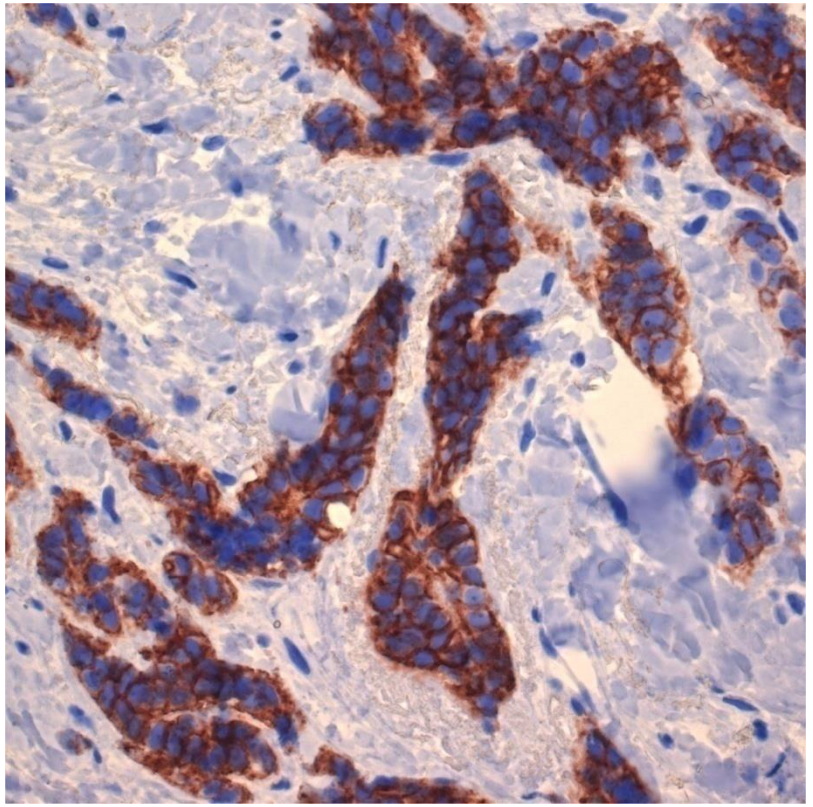
Lack of correlation between IHC subtype and molecular subtype

E-Cadherin
Cell adhesion molecule

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

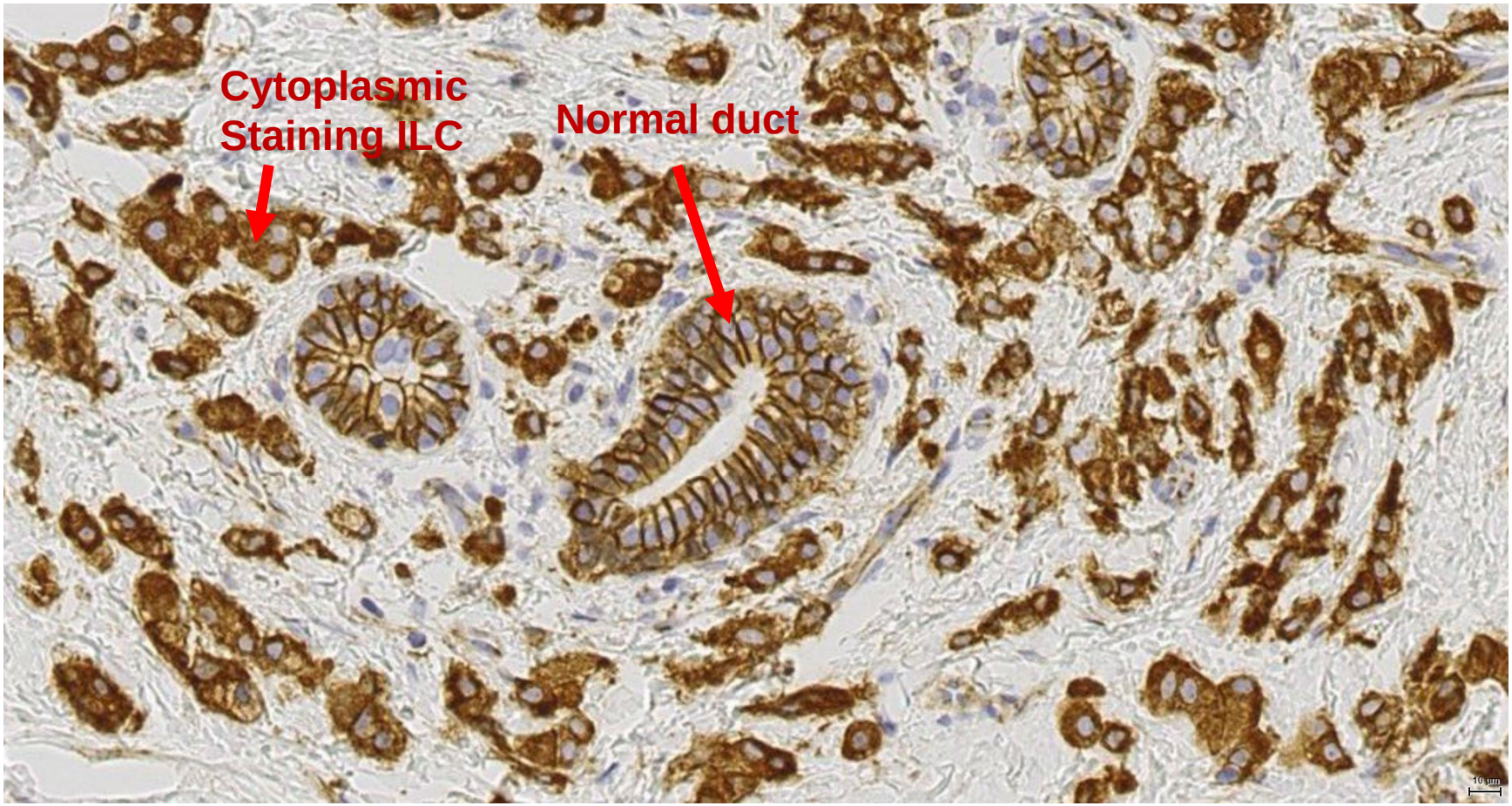


E-Cadherin positive Invasive Ductal Carcinoma



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

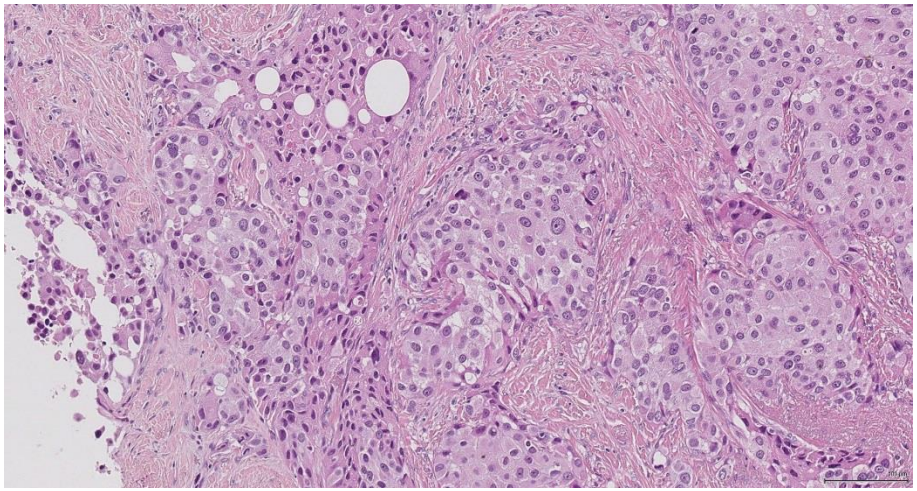
P120 catenin dislocated to the cytoplam in lobular carcinoma (ILC)
A supplement for classification of lobular neoplasia



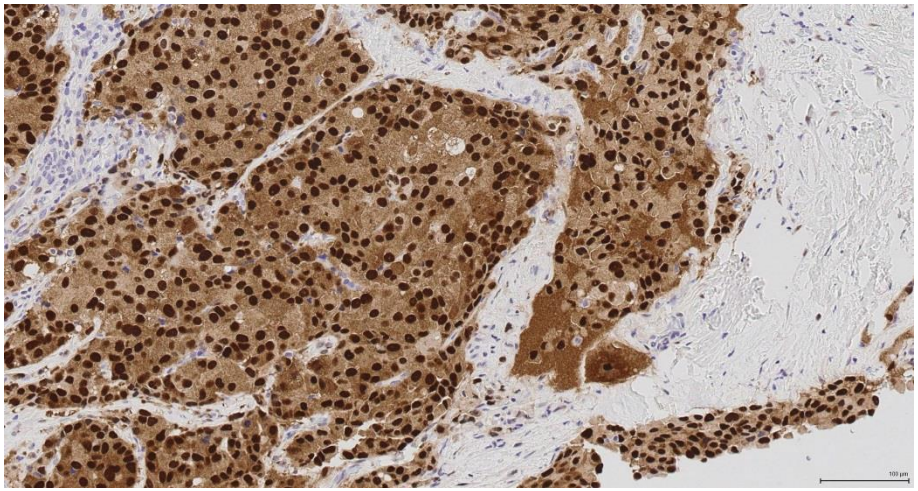
Lobular cancer - not candidate for neoadjuvant chemotherapy
Low proliferating tumors, often luminal A molecular subtype

Apocrine carcinoma classification

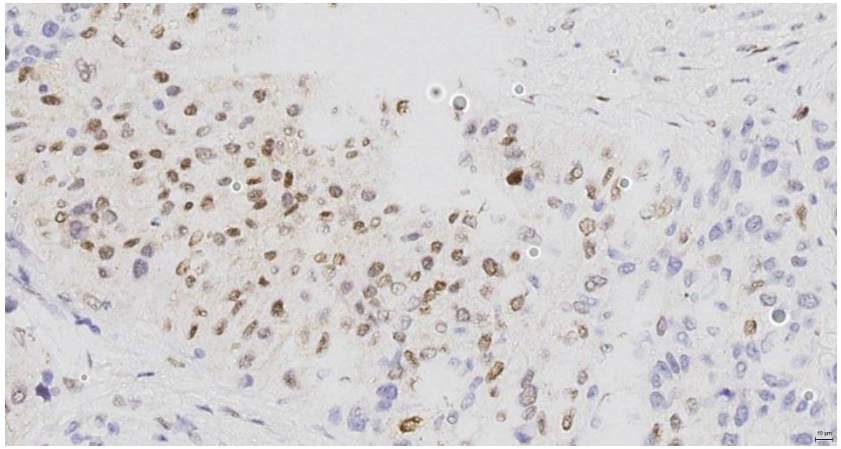
HE



Androgen Receptor



AR staining in IHC-basallike breast cancer as potential marker for AR targeted treatment



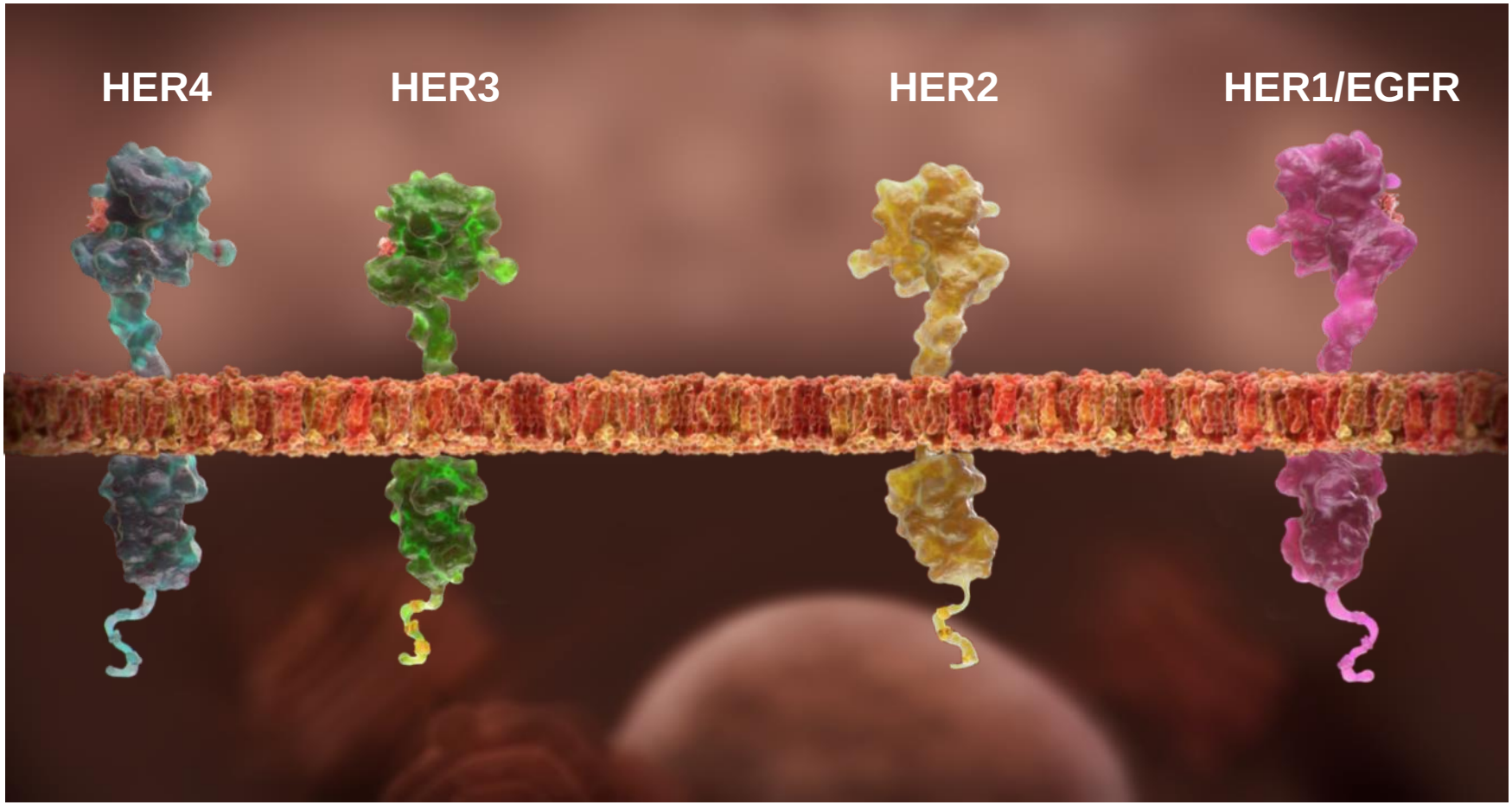
Prognostic and predictive biomarkers

HER2 positive breast cancer: 12%

Family of four receptors in the HER family

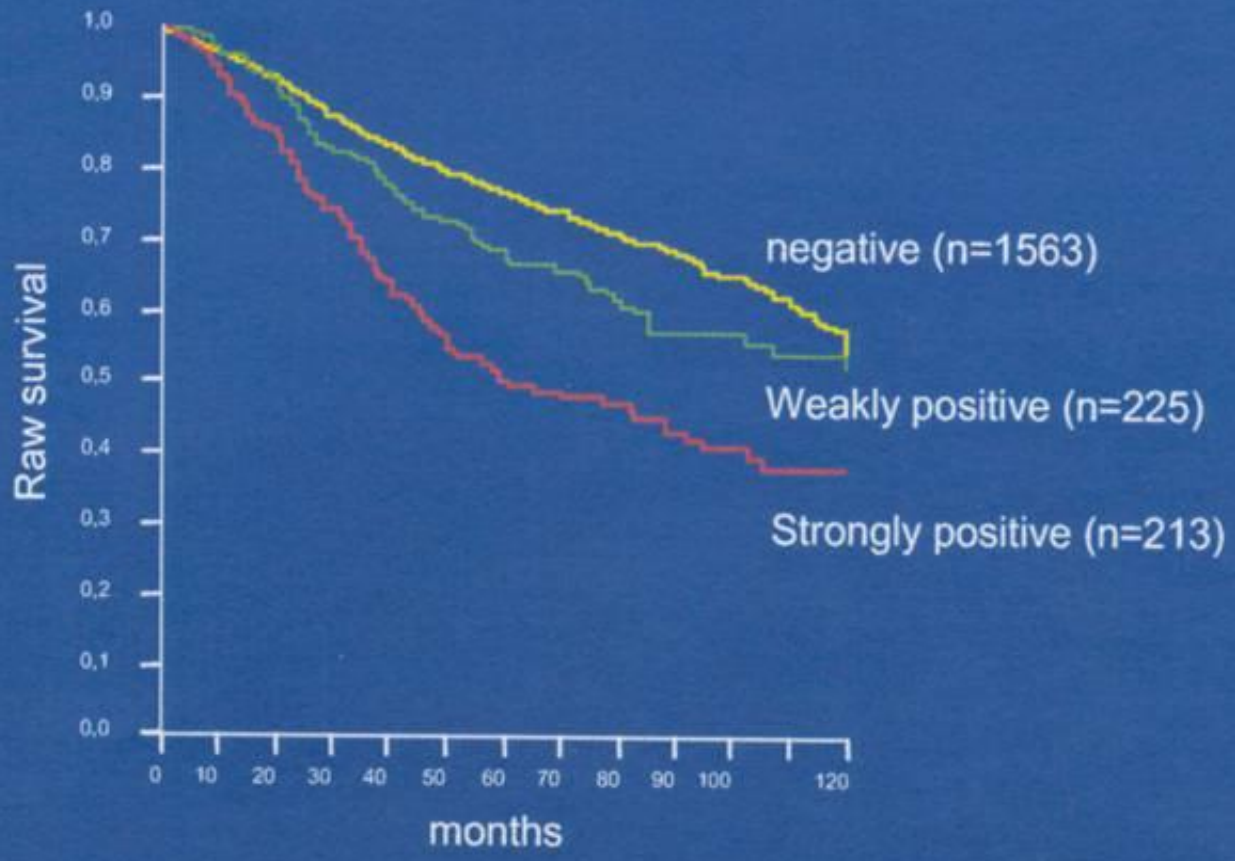
HER2: Growth factor tyrosine kinase receptor

Mediate cell growth differentiation and survival



EGFR, epidermal growth factor receptor; HER, human epidermal growth factor

HER2 and Breast Cancer Progression



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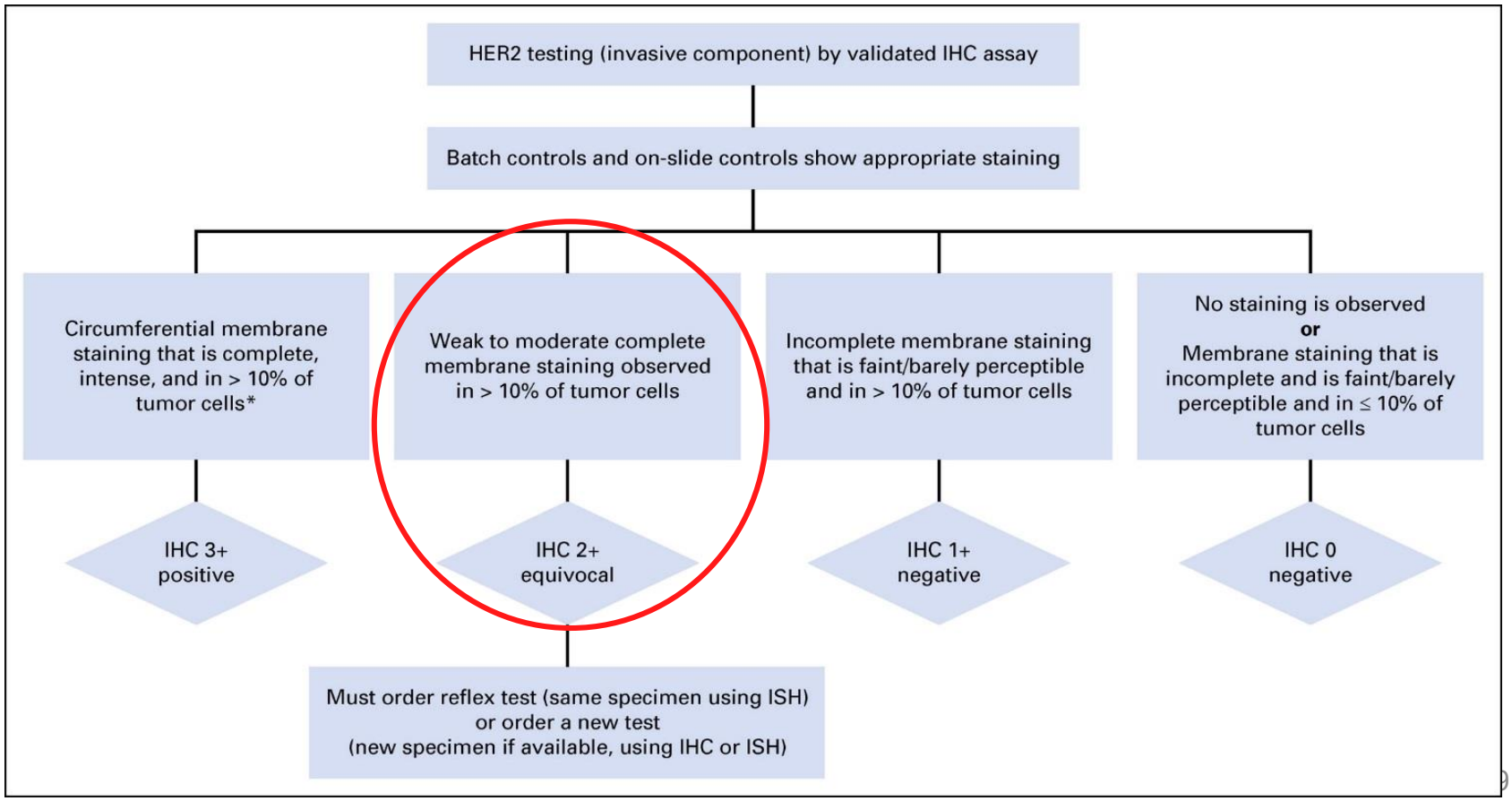
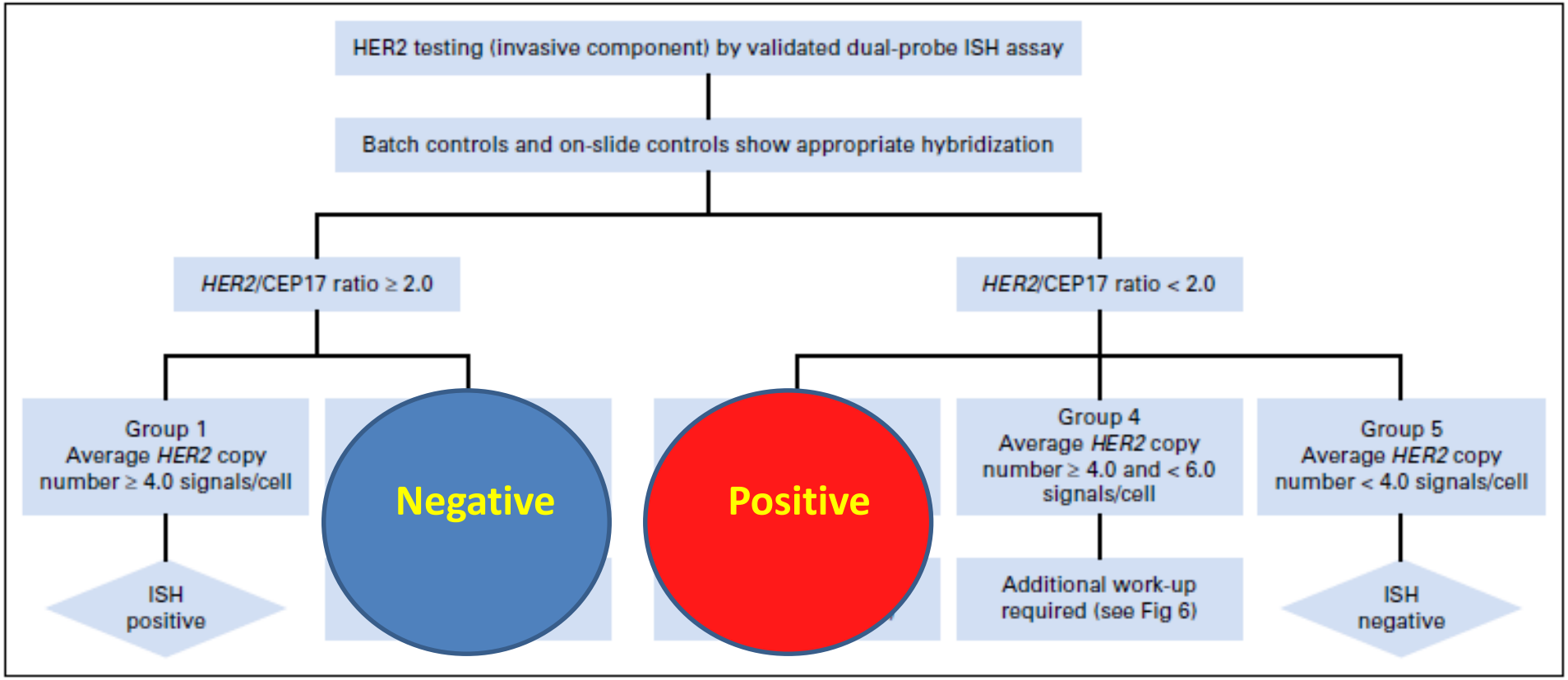
JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

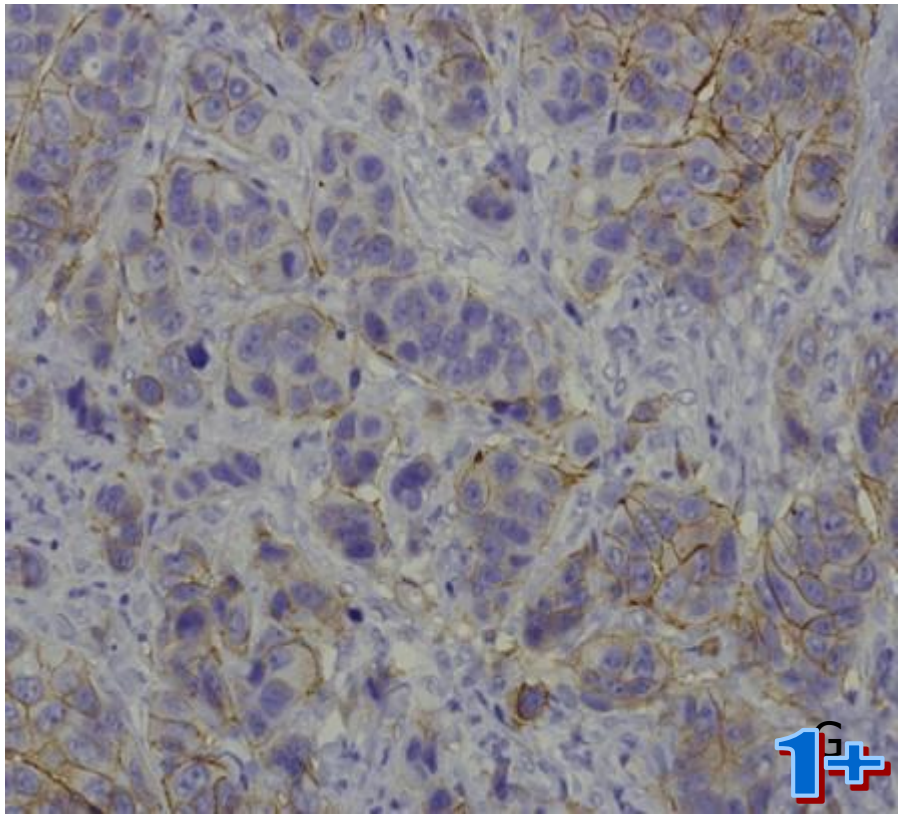
Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/ College of American Pathologists Clinical Practice Guideline Focused Update

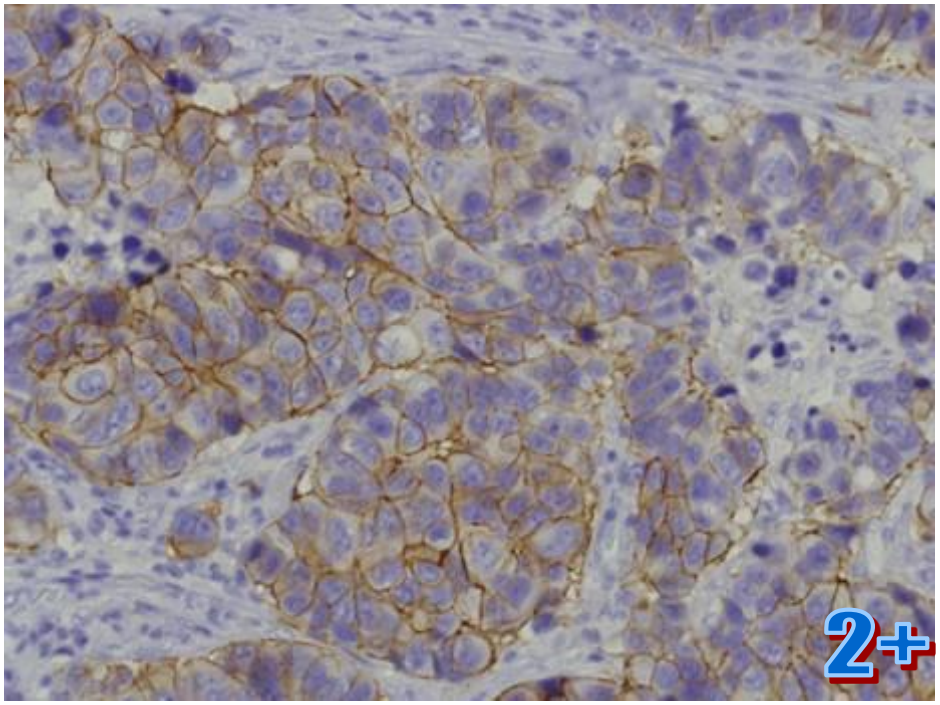


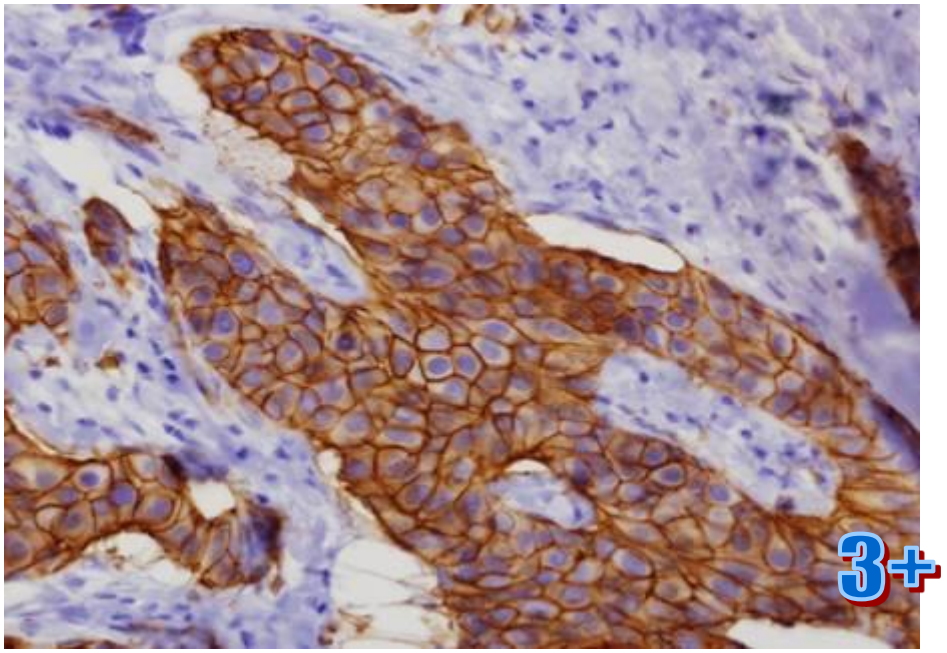
HER2 testing by validated dual-probe ISH assay



HER2 IHC



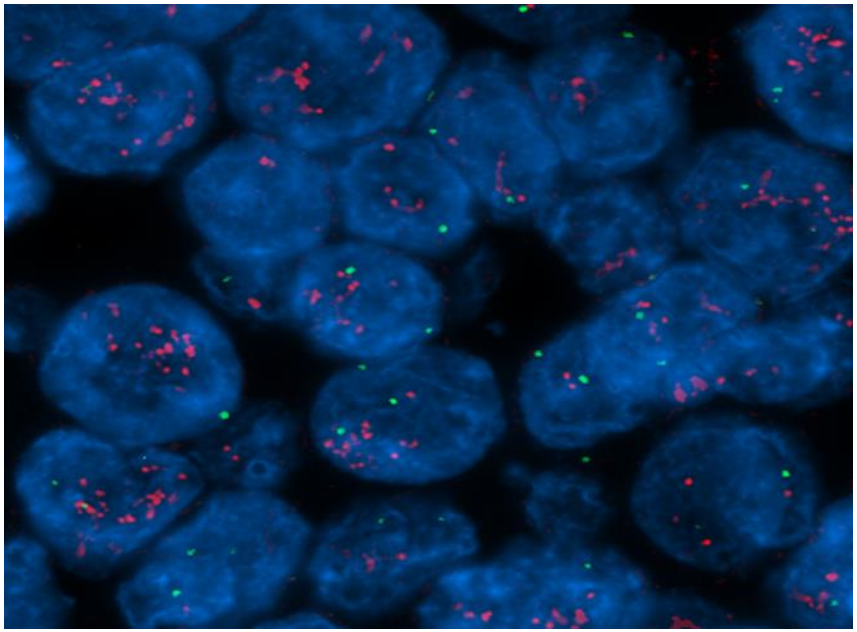




HER2 3+ and ISH + : 12 % (DK)

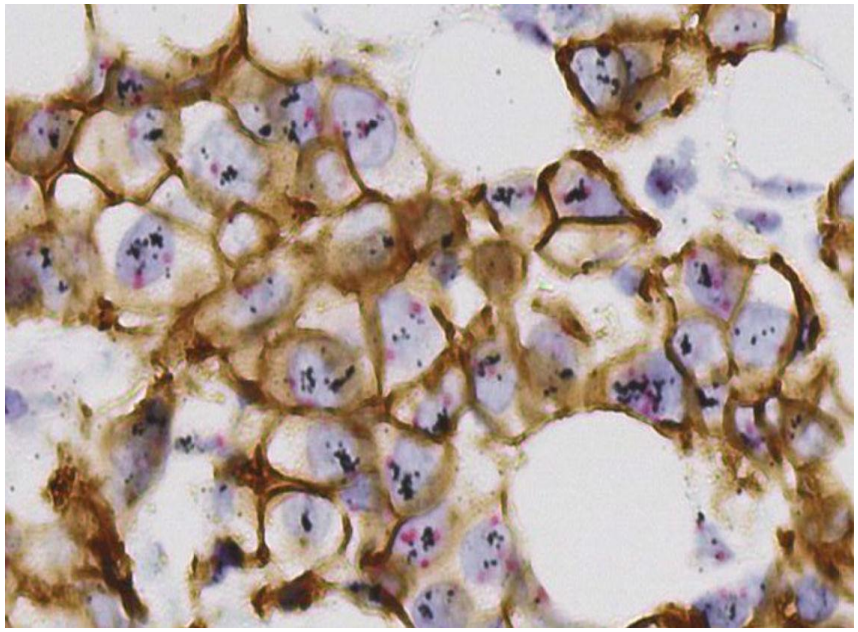
HER2 dual probe (F)ISH assay

FISH



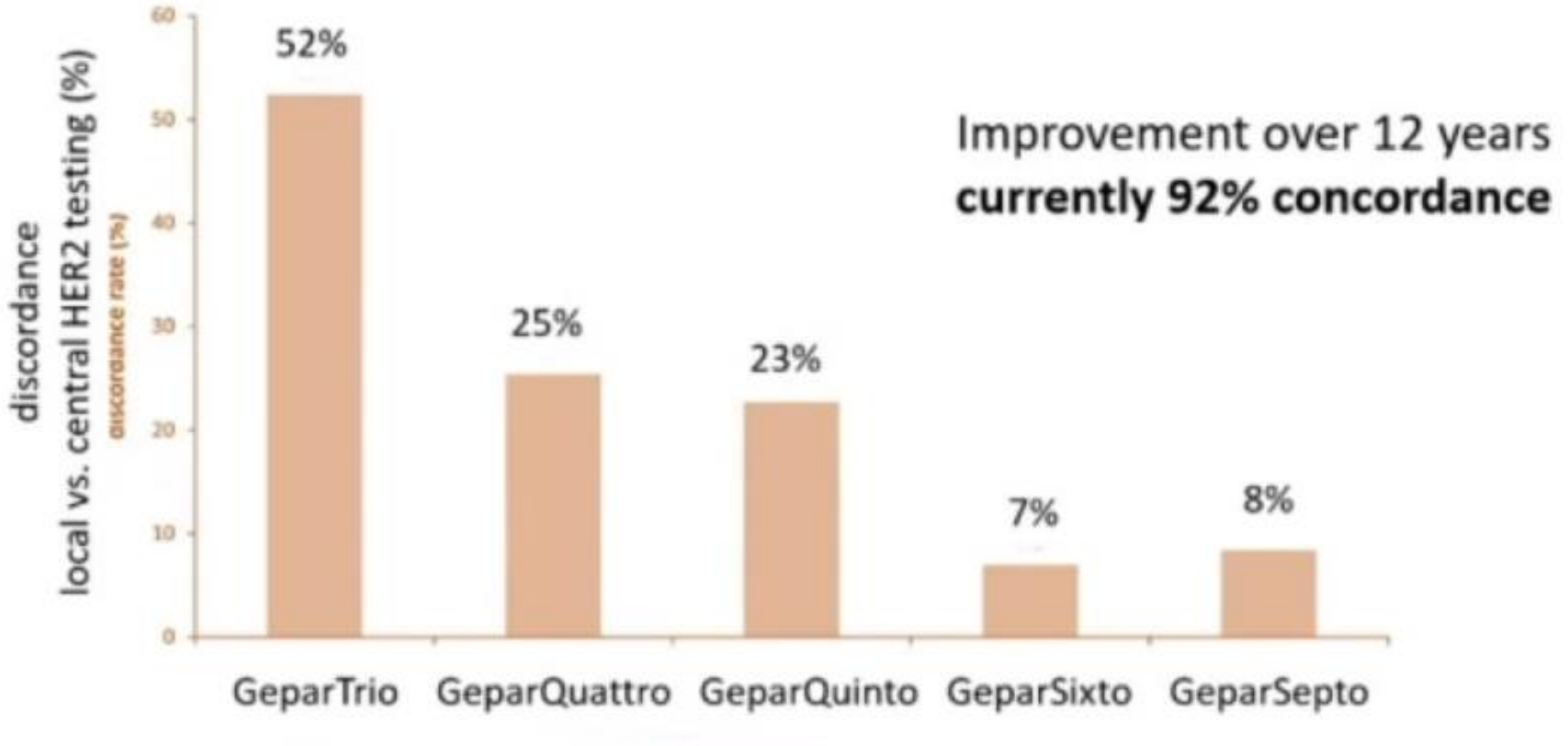
Red: HER2 gene
Green: Centromere region/chromosome 17
HER2 amplified ratio > 2

HER2 Gene/Protein Assay



Black: HER2 gene
Red: Centromere region/chromosome 17
HER2 amplified ratio > 2 and HER2 IHC 3+

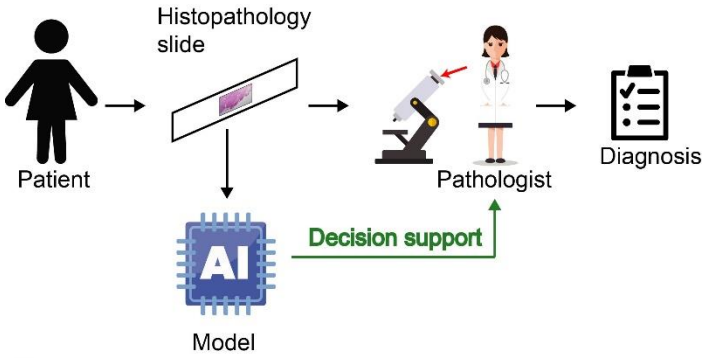
Concordance in HER2 (IHC) testing

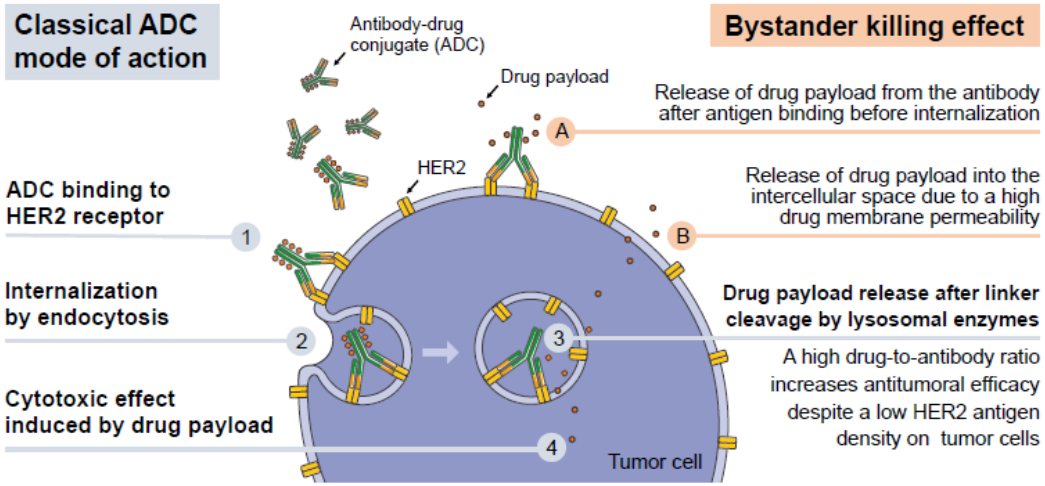


Modern Pathology (2018) 31, 607–615

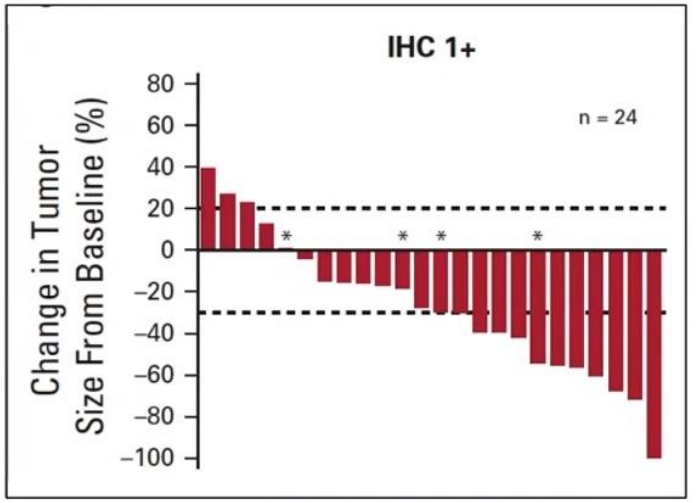
HER2 Low – a new entity for targeted treatment (metastatic disease)

- Tumors with HER2 IHC score of 1+ or 2+/ISH-negative are classified as “HER2-low” and represent ~ 55% of breast tumors





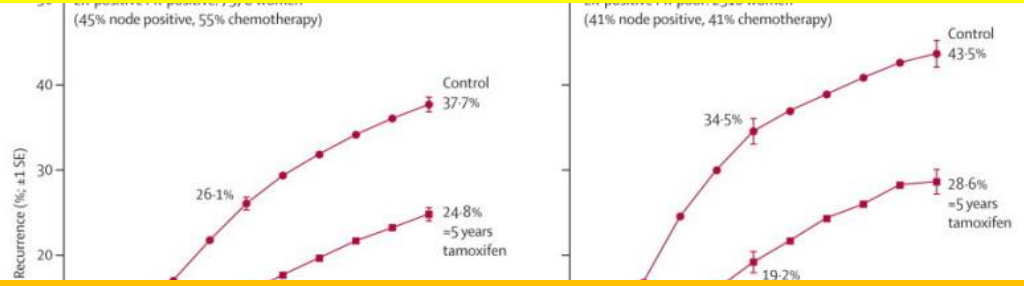
Int. J. Mol. Sci. **2019**, *20*, 1115



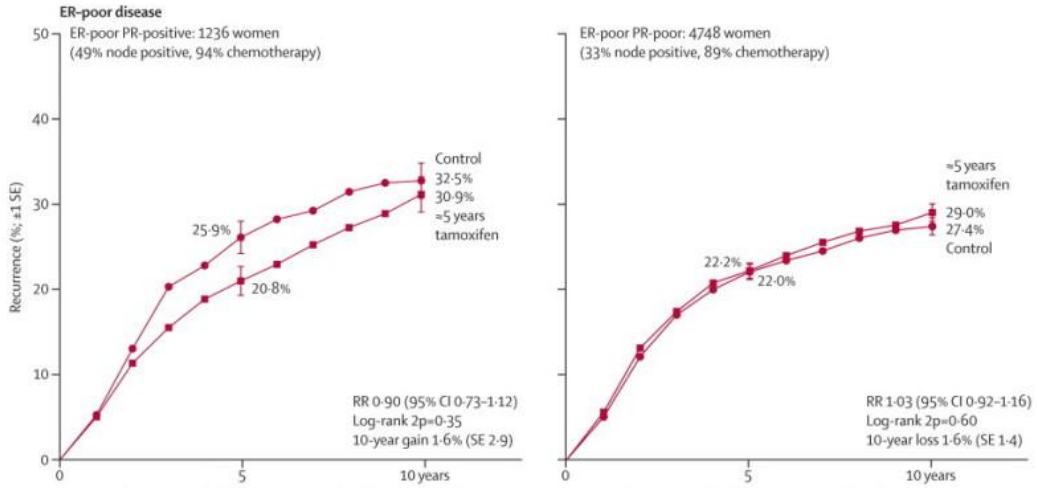
Modi et al. JCO 2020

ADC= Anti-body-drug conjungates

Estrogen Receptor: a prognostic and predictive factor



ER predictive of response to endocrine treatment



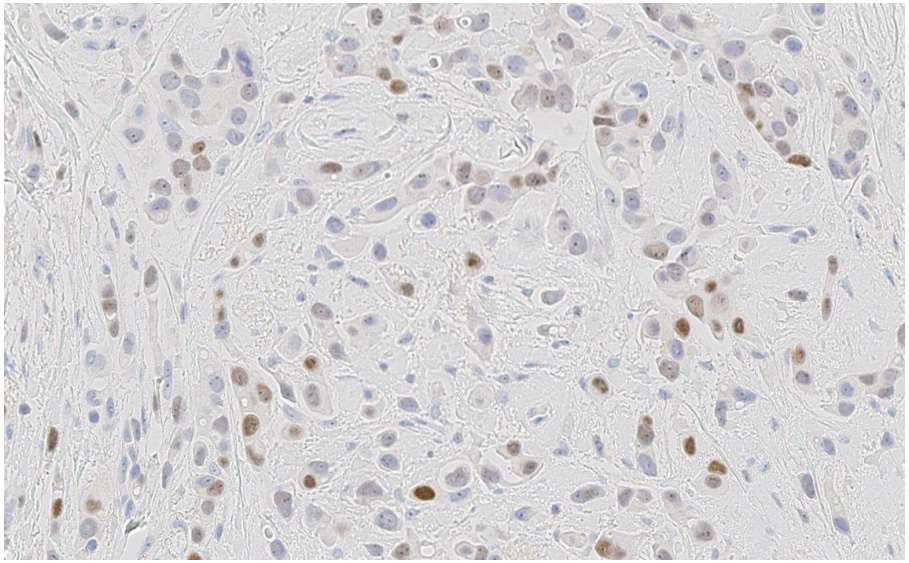
Recurrence rates (% per woman-year) and log-rank analyses

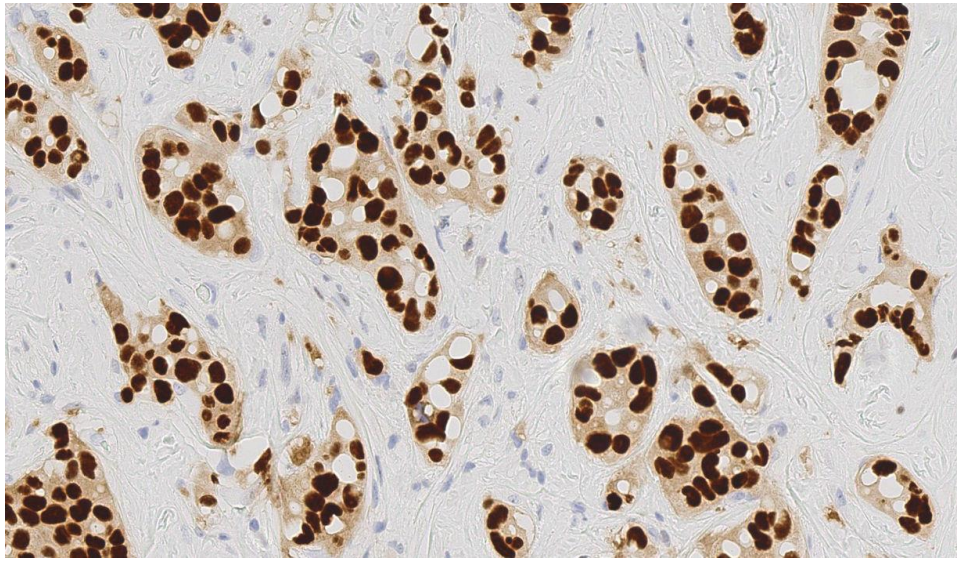
	Years 0–4	Years 5–9	Year 10+
Tamoxifen	4·66 (122/2616)	2·74 (46/1677)	1·88 (12/640)
Control	6·23 (158/2538)	1·93 (31/1603)	1·04 (7/675)
Rate ratio	0·78 (SE 0·11)	1·27 (SE 0·28)	2·03 (SE 0·69)

Recurrence rates (% per woman-year) and log-rank analyses

	Years 0–4	Years 5–9	Years 10+
≈5 years tamoxifen	5·26 (519/9870)	1·86 (113/6081)	1·09 (29/2652)
Control	5·05 (493/9754)	1·50 (93/6183)	1·45 (43/2961)
Rate ratio	1·02 (SE 0·07)	1·27 (SE 0·16)	0·70 (SE 0·20)

2020 – ASCO CAP Update
Hormone receptors

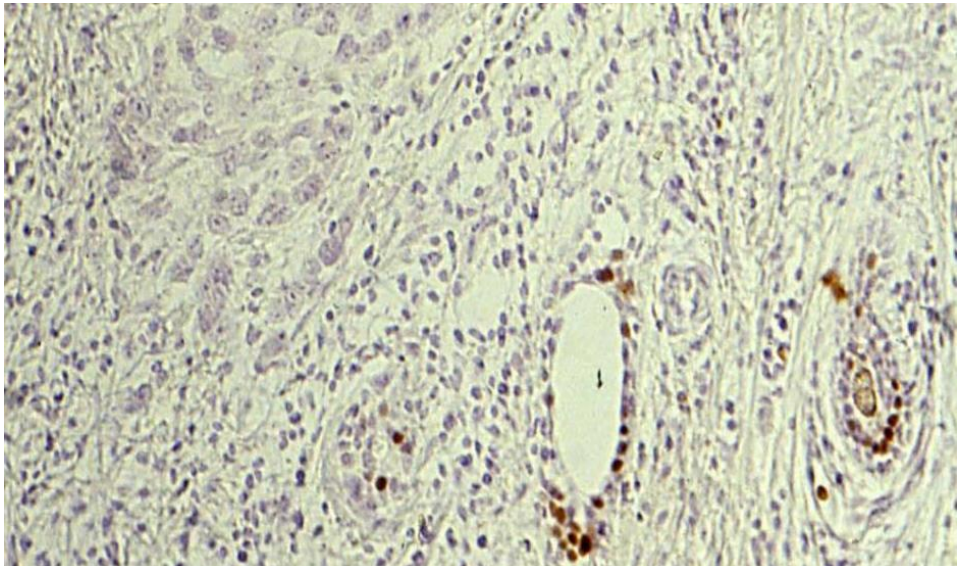




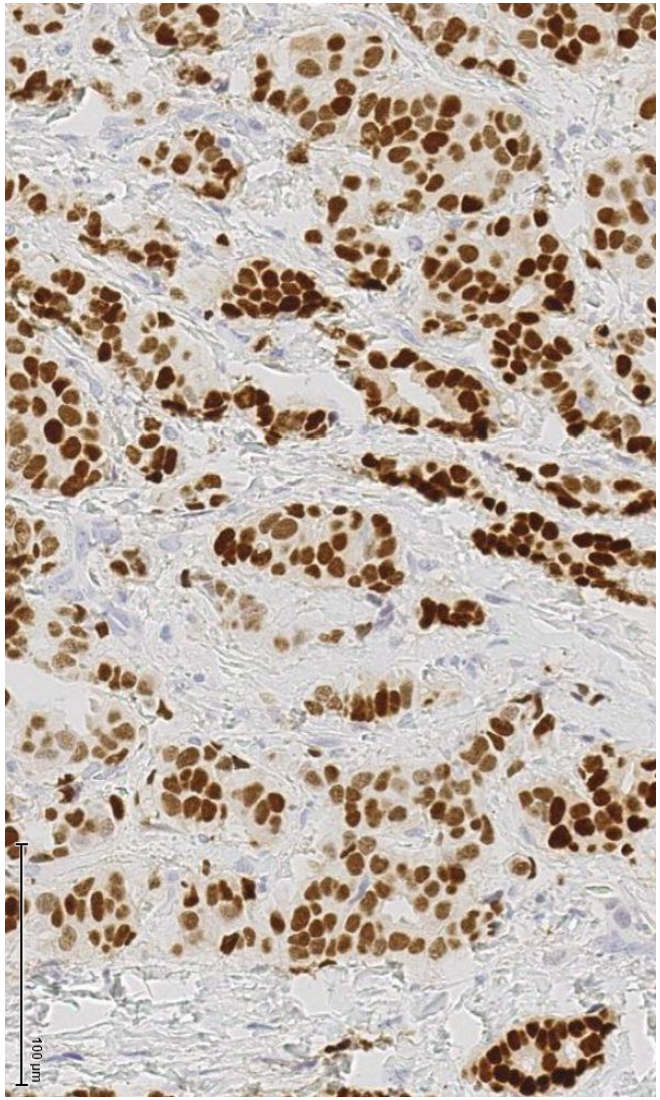
ER positive 86% of breast carcinomas (DK)

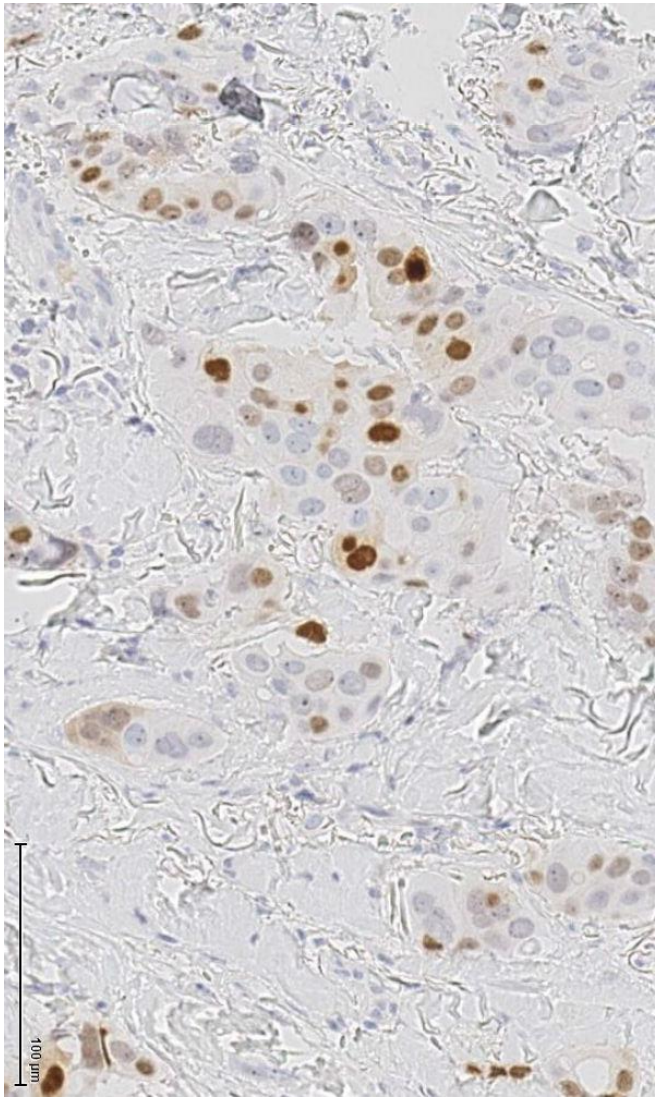
Cut off ≥ 1%
A sample is reported negative for ER or PgR if < 1% or 0% of tumor cell nuclei are immunoreactive.

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



Interpretation of PgR IHC



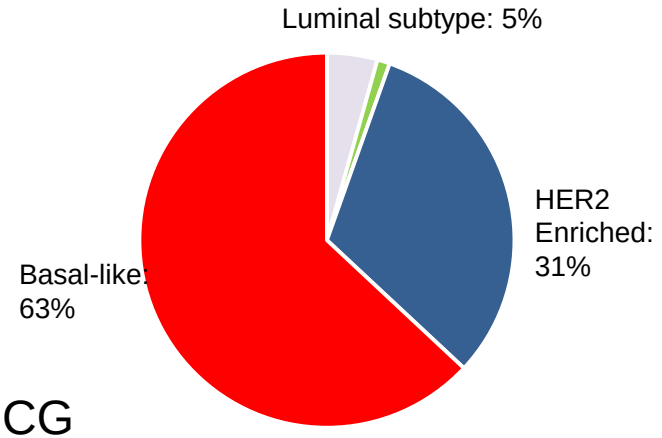


Heterogeneous expression

ER low status

< 2.0% of breast tumors in DK are characterized by ER low expression:

1-9% (IHC)



DBCG Molecular intrinsic subtypes 2019/2020, ER low (1-9%)

Biology and gene expression profiles more similar to ER negative tumors

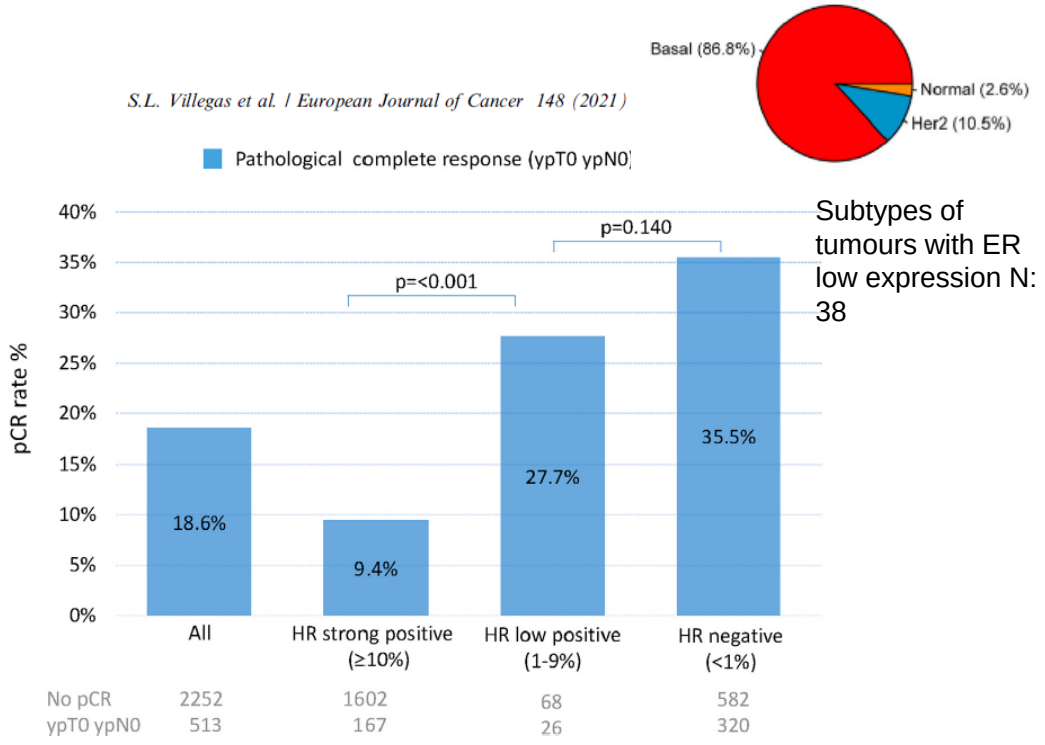
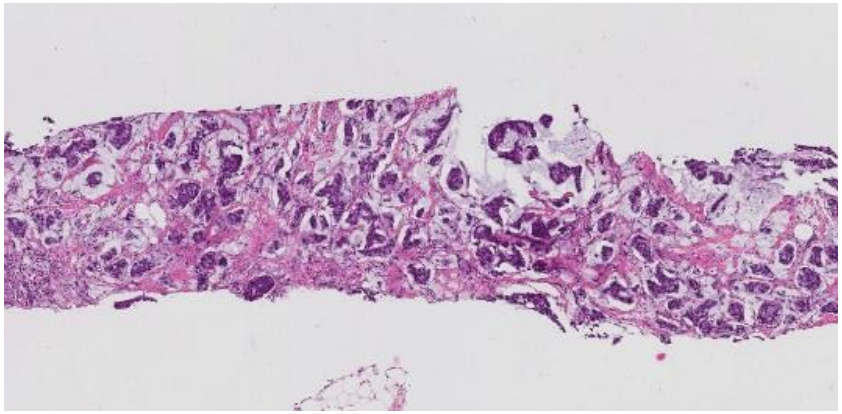


Fig. 2. Pathological complete response (pCR; ypT0 ypN0) across hormone receptor (HR) subgroups from GBG/AGO-B neoadjuvant clinical trials. N = 2765.

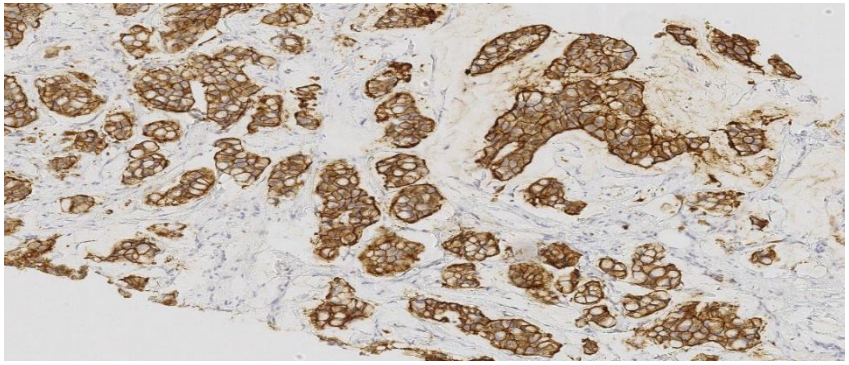
Subtypes of tumours with ER low expression N: 38

Expression of ER and HER2 predictive of pCR Neoadjuvant treatment

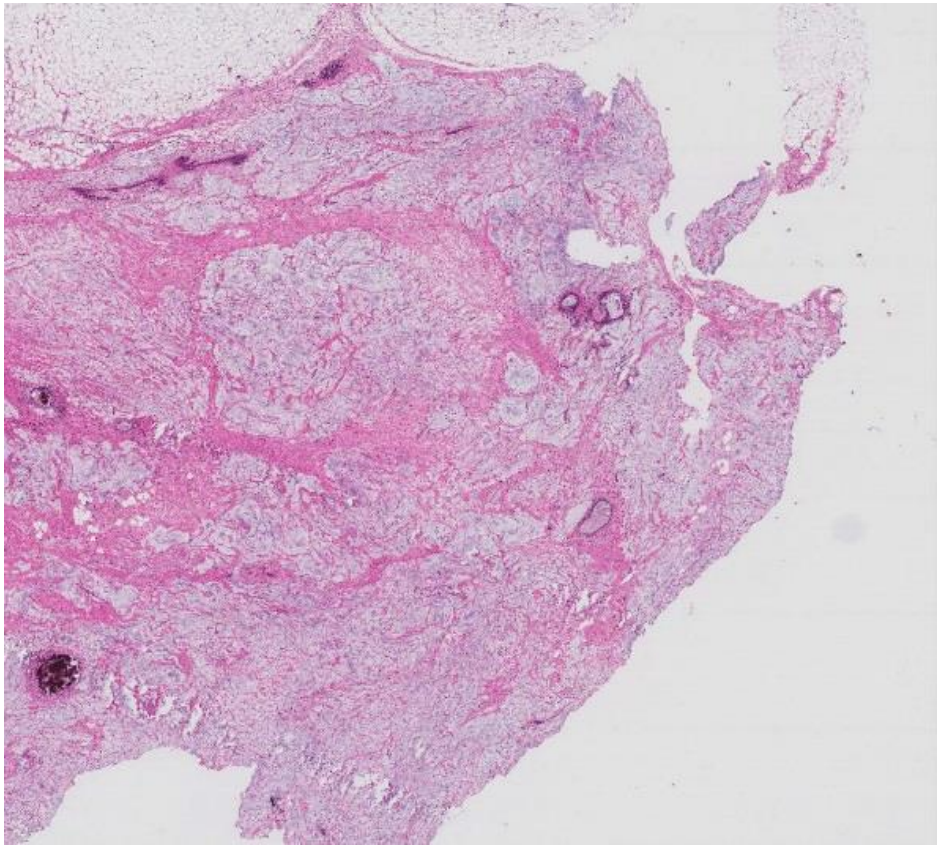
- Neoadjuvant systemic therapy for early breast cancer.
  - pCR (pathological complete response) is a valuable end point for determining the efficacy of the treatment.
    - Prognostic information



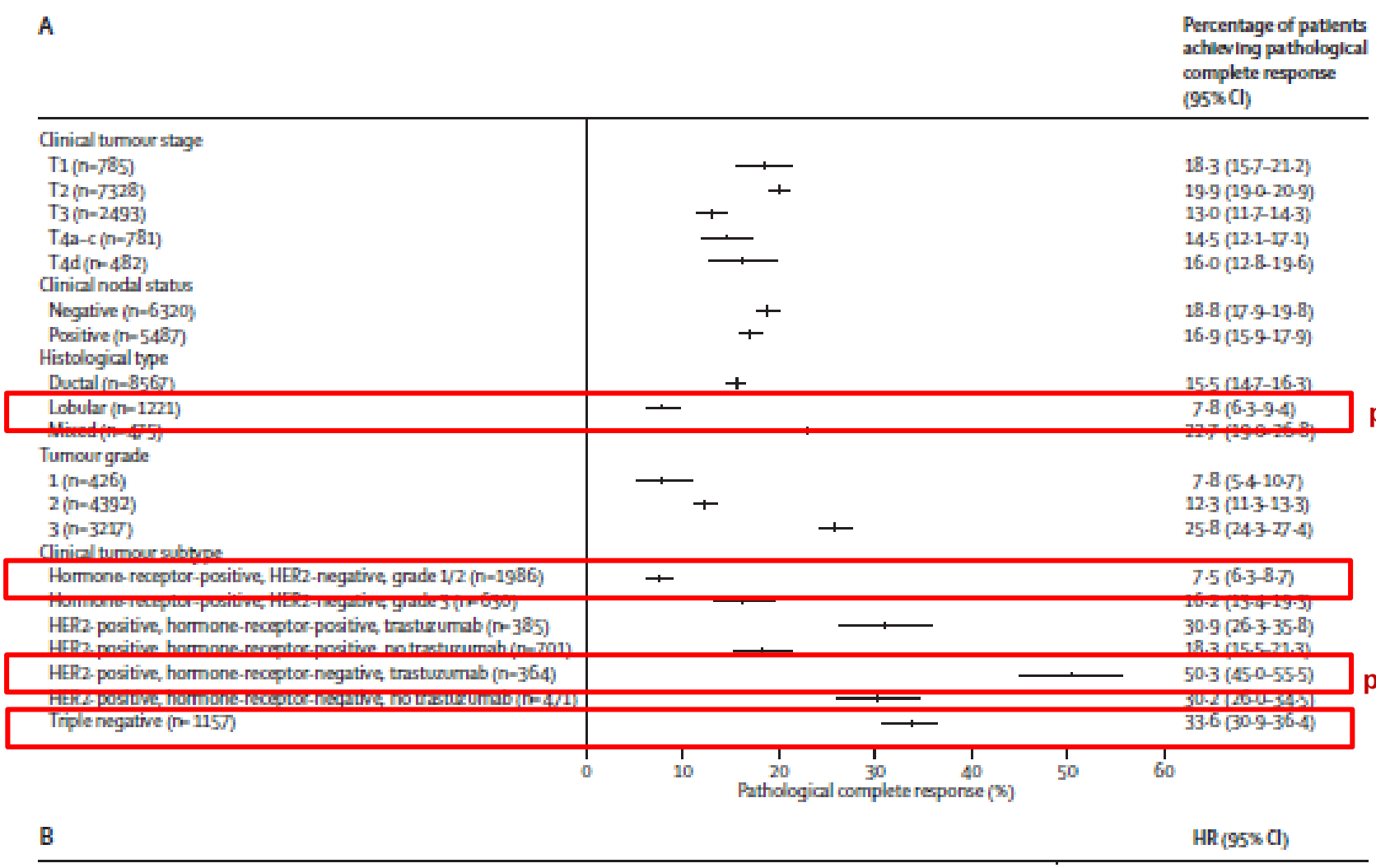
HER2 IHC



Post treatment - surgery



Tumor characteristics and association with pCR
Lobular carcinoma not recommended for neoadjuvant chemotherapy (NACT)



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

Histopathological subtype classification important - not all TNBC´s are candidates for NACT

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.)
Consensus statement in preparation by the European Working Group for Breast Screening Pathology (EWGBSP).

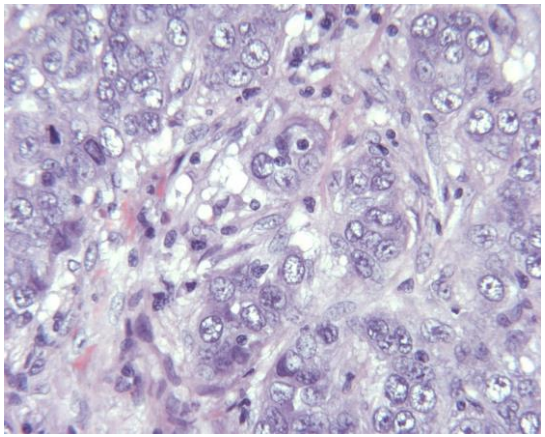


Figure 1
High grade
IDC

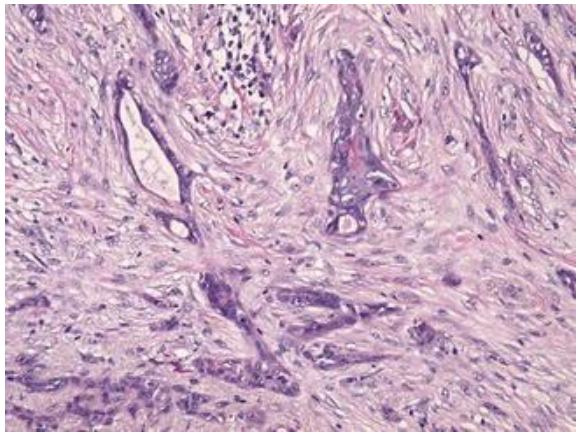


Figure 2
Low grade adenosquamous carcinoma (subtype of metaplastic carcinoma)
luminal (CK7, CK8) and basal (CK5, CK14) CKs and squamous (myoepithelial) markers p63 and p40.

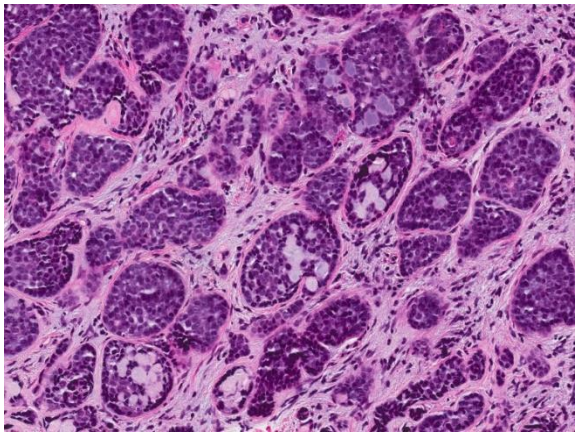
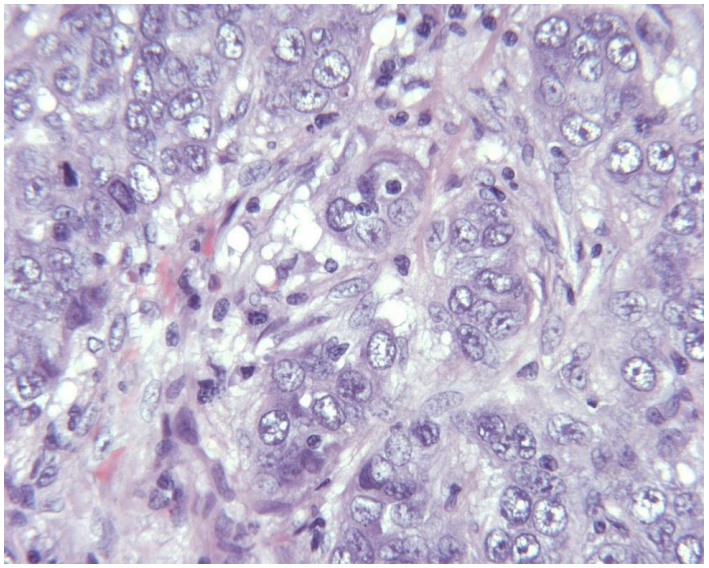


Figure 3
Adenoid cystic carcinoma of the breast. The cells of the epithelial component are positive for CK7, CK5/6, CK 8/18 and CD117. The myoepithelial /abluminal cells express p63, smooth muscle actin and basal CKs: CK5/6, CK14, CK17.

TNBC : 8-10% of primary breast cancers

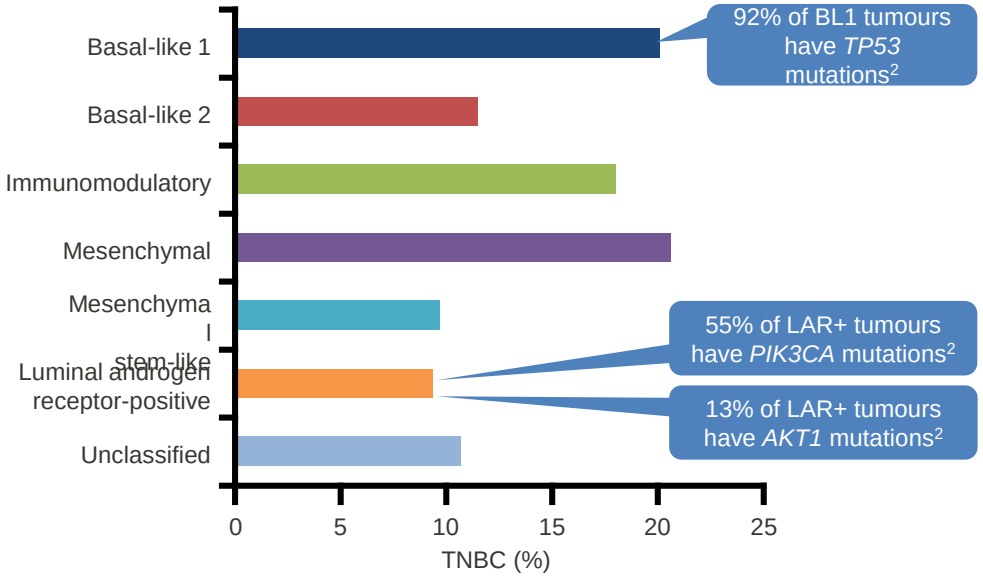
- ER, PR and HER2 negative
- Heterogeneous group of tumours,
- High grade,
- Younger age at diagnosis,
- Poor prognosis
- Risk of *gBRCA* mutation



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]

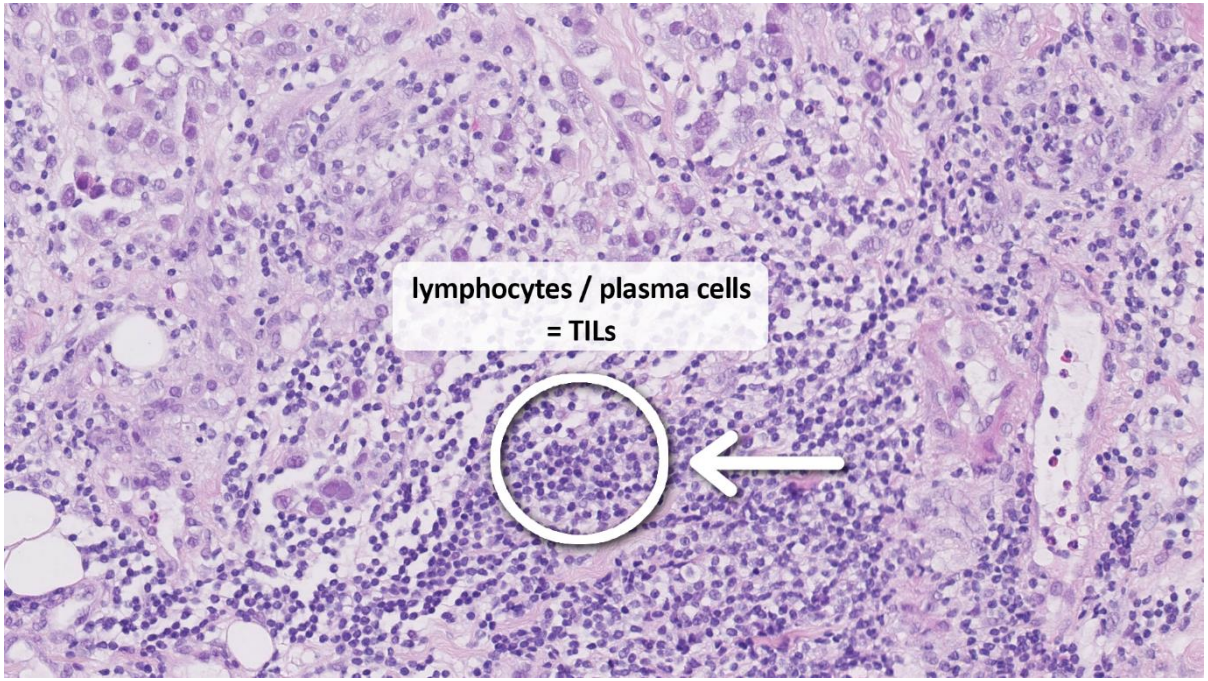


1. Lehmann, et al. J Clin Investig 2011; 2. Bareche, et al. Ann Oncol 2018

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



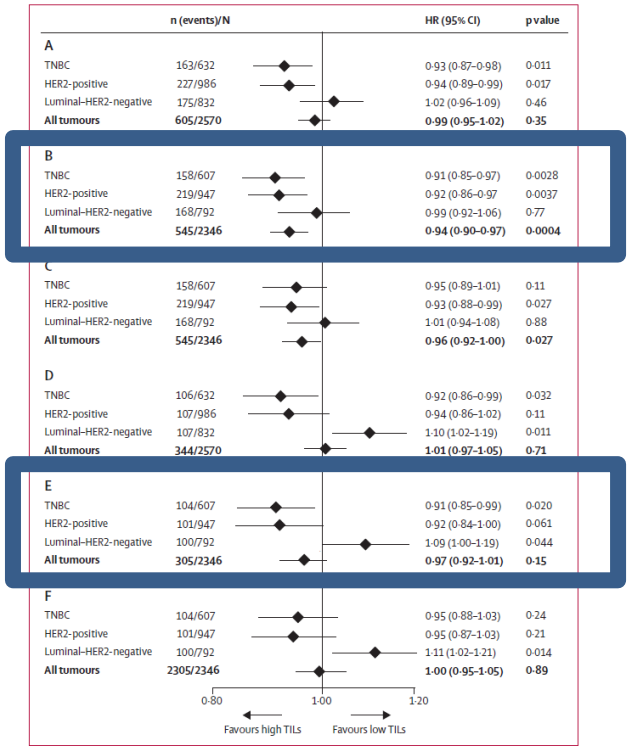
TNBC is considered to be the most immunogenic breast cancer subtype, with a higher median number of tumor-infiltrating lymphocytes (TILs), PD-L1 expression, both markers associated with tumor microenvironment (TME) immune activity.

Level 1B evidence / prognostic marker (adjuvant setting).

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.

Tumor infiltrating lymphocytes and TNBC

- Increased TILs concentrations are associated with increased frequency of response to neoadjuvant treatment (in all breast cancer subtypes).
- Increased TILs concentration is associated with longer survival for patients with TNBC and HER2 positive breast cancer (after neoadjuvant treatment)



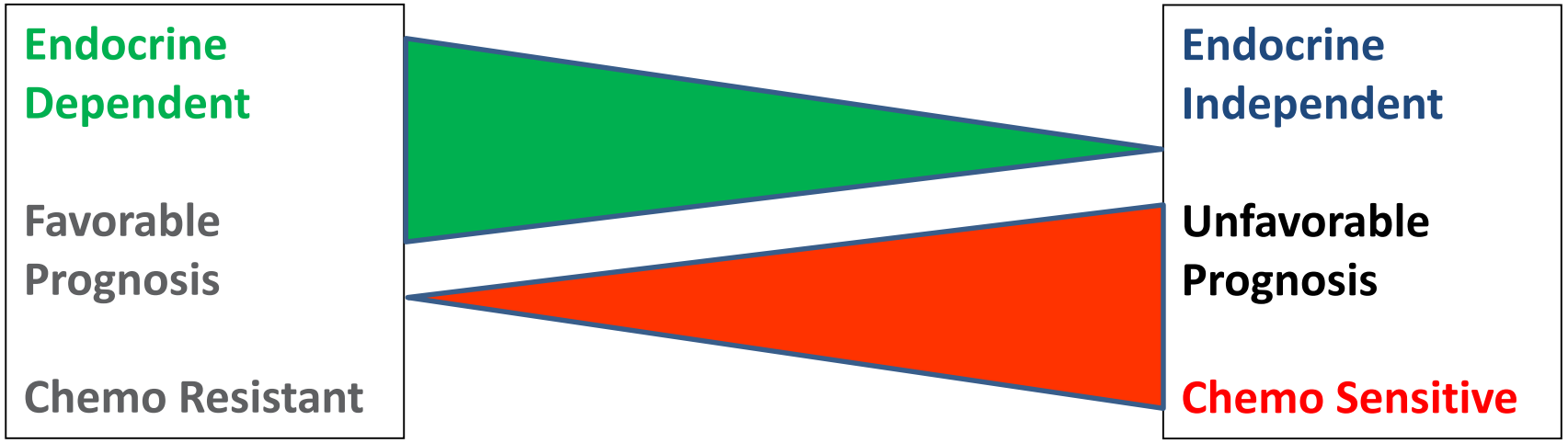
DFS Multivariate

OS Multivariate

Figure 4: **Continuous TIL concentration as a prognostic marker for disease-free survival and overall survival for all tumour subtypes**
p values have been obtained from a logistic regression analysis. Disease-free survival by univariable analysis (A), multivariable analysis including all baseline parameters (B), and multivariable analysis including all baseline parameters and pCR (C). Overall survival by univariable analysis (D), multivariable analysis including all baseline parameters (E), and multivariable analysis including all baseline parameters and pCR (F). TIL=tumour-infiltrating lymphocyte. TNBC=triple-negative breast cancer. pCR=pathological complete response.

Molecular subtypes

Breast cancer – Molecular intrinsic subtypes prognostic information



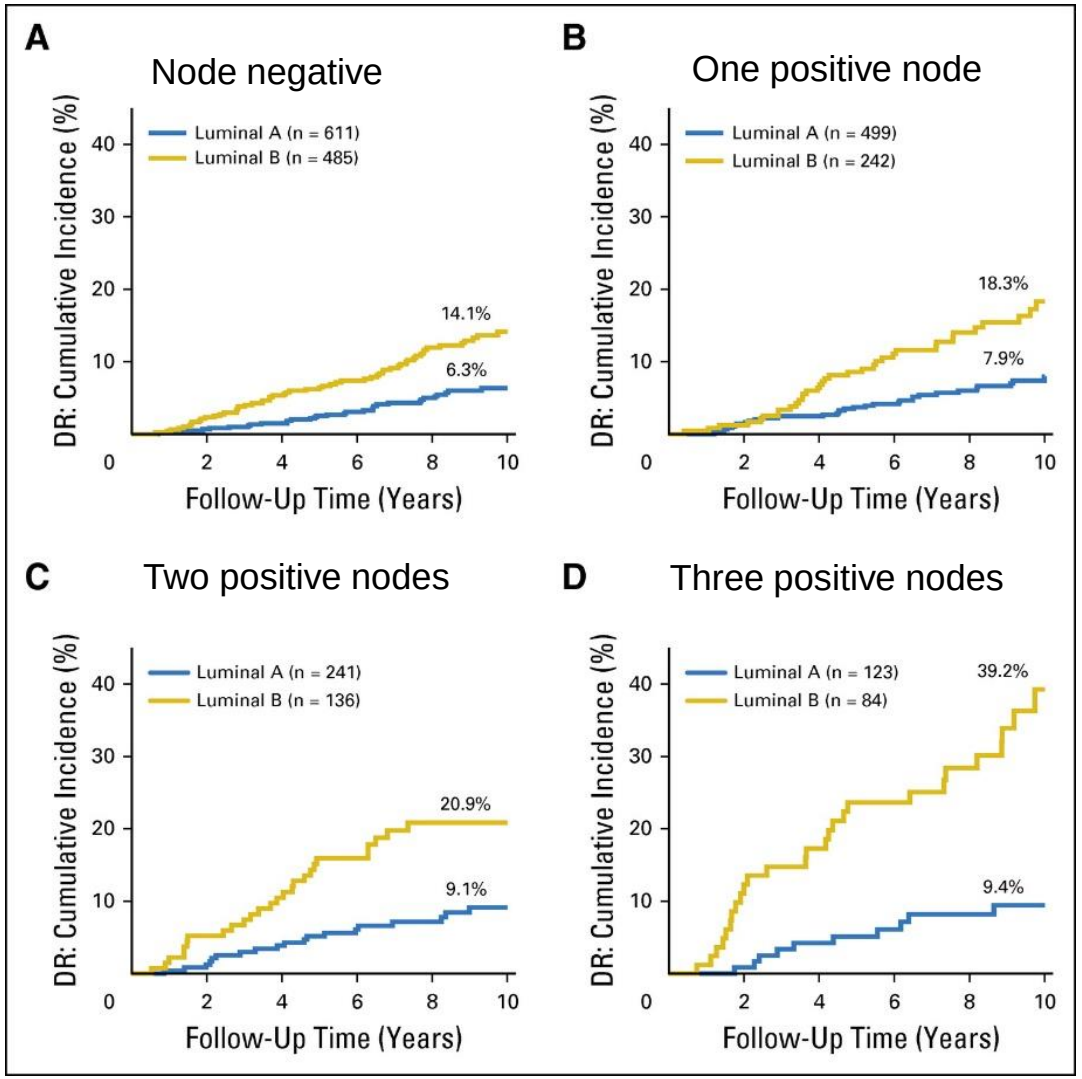
Lum A

Lum B

HER2-enriched

Basal-like

De-escalation of treatment
More patients can be spared chemotherapy



Luminal A; and Luminal B

JCO 2018 Laenkholm et al.

PAM50 implemented in the Danish guidelines

Immunohistochemical surrogate markers for the molecular intrinsic subtypes

- Limitations
  - No uniform cut off value for Ki67
  - Lack of analytical validity - reproducebility
  - Lack of correlation between molecular subtypes and surrogate IHC subtypes

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

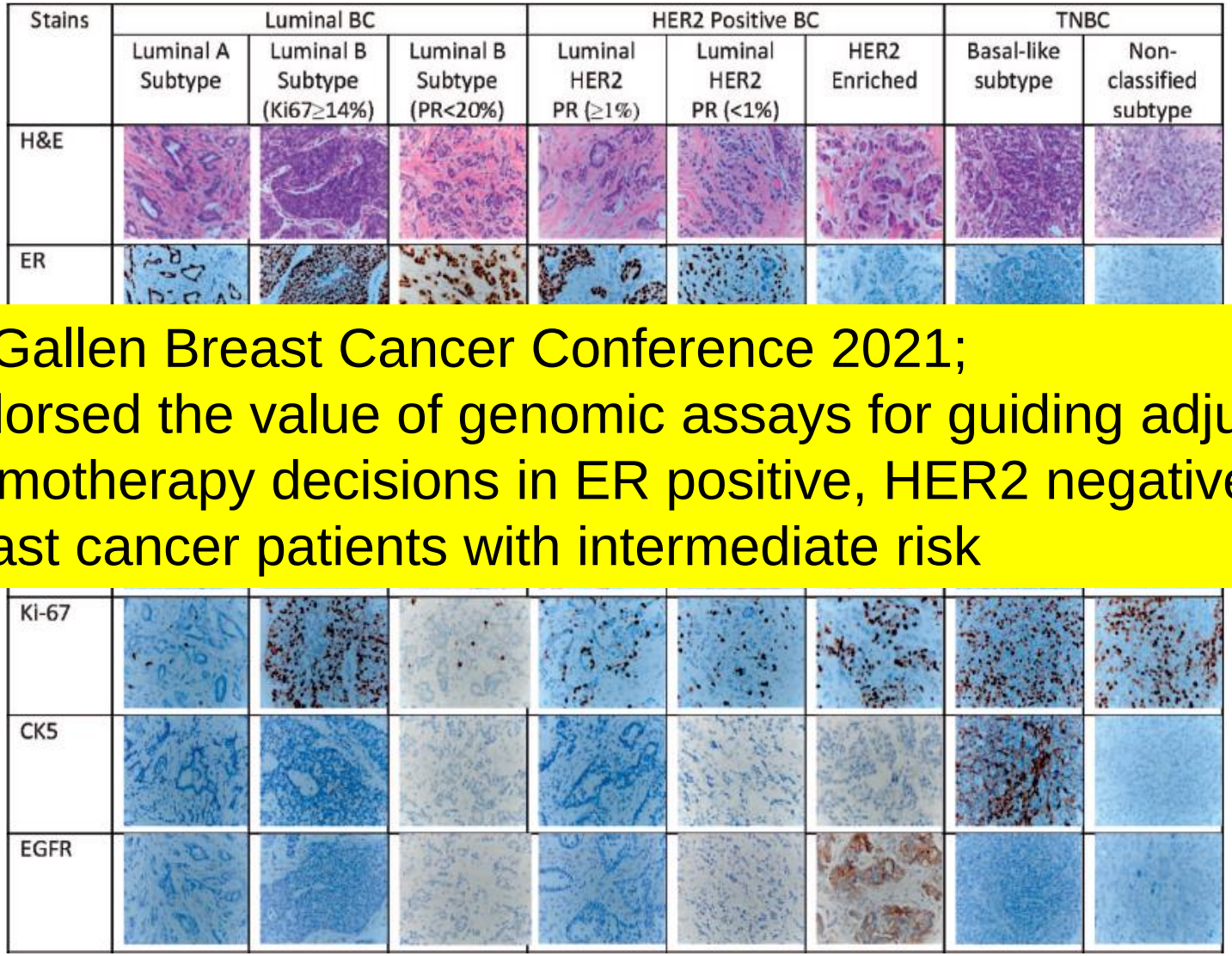
doi: 10.1093/jnci/djaa201
First published online December 28, 2020
Commentary

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,]

Immunohistochemical surrogate markers for the molecular intrinsic subtypes

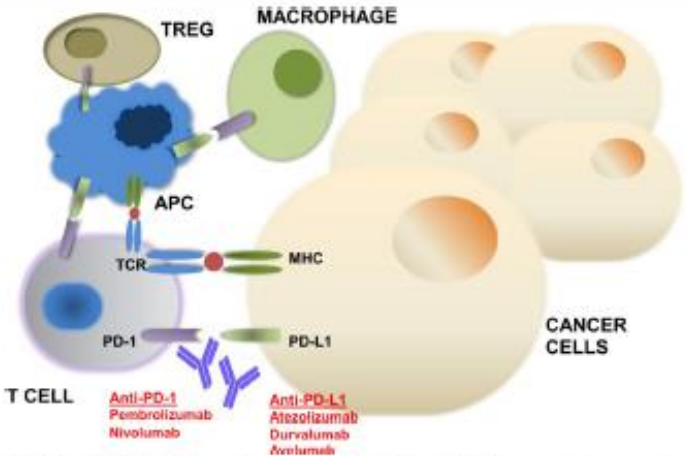
Arch Pathol Lab Med—Vol 140, August 2016



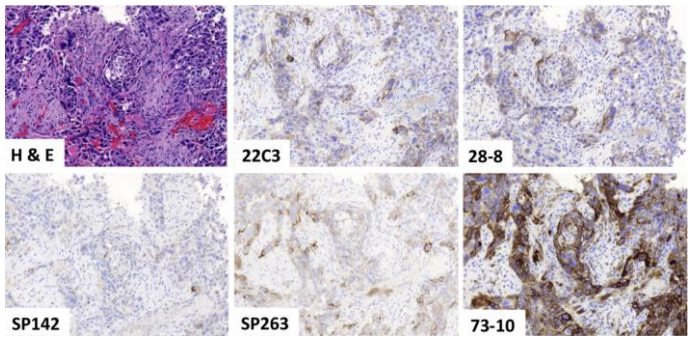
St. Gallen Breast Cancer Conference 2021;
Endorsed the value of genomic assays for guiding adjuvant chemotherapy decisions in ER positive, HER2 negative breast cancer patients with intermediate risk

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 immunohistochemistry – new biomarker in TNBC

- PD-L1 is a biomarker for metastatic TNBC
- currently only for atezolizumab, but other trials ongoing
- pathologists know PD-L1 from other tumor types (extensive existing training material, currently adapted to TNBC)
- Typical questions:
  - Which material to apply for analysis? (primary tumor/metastasis)
  - Which antibody to use?
  - **Which scoring system?**
  - Which cell type?
    - (tumor cell, immune cell (which type of immune cell?)
  - Which cutpoint? – depends on clinical setting
  - Reproducebility?

ESMO 2019

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**

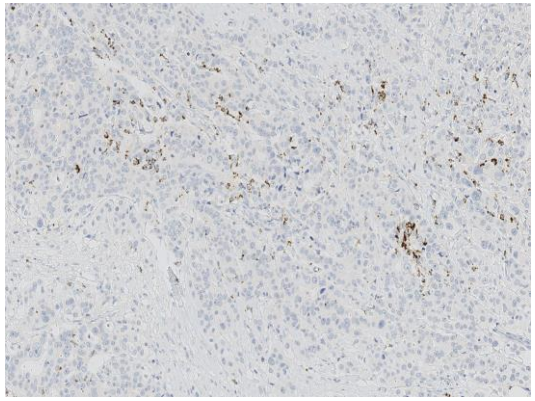
IC_A score: percentage of tumor area covered by PD-L1 positive immune cells (designed for Atezolizumab)

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

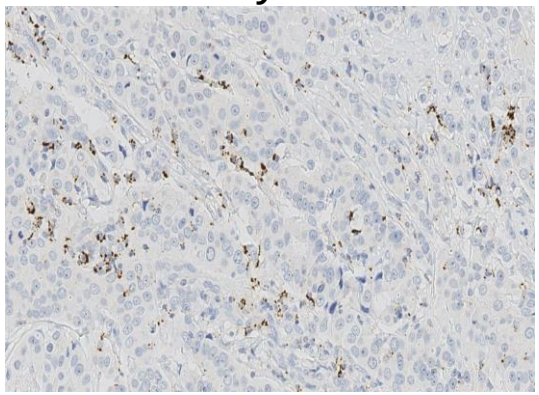
Keynote-355

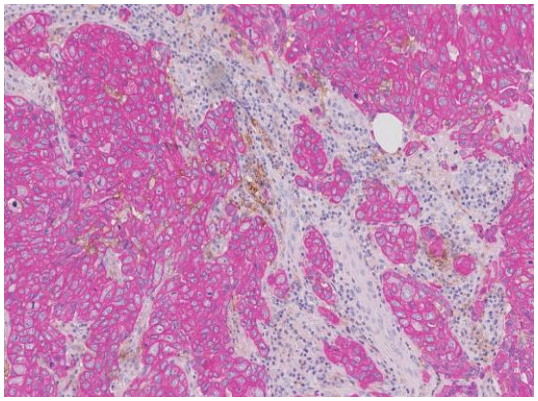
Impassion130

PD-L1 immunohistochemistry

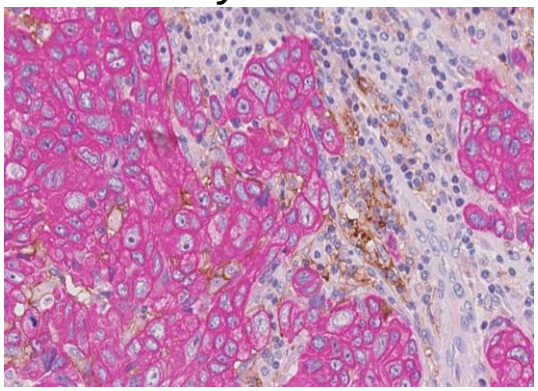


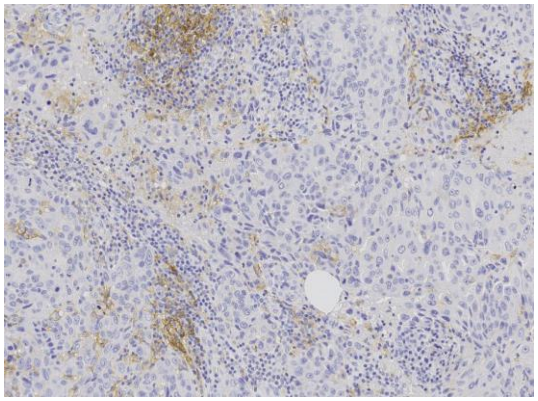
Assay SP142



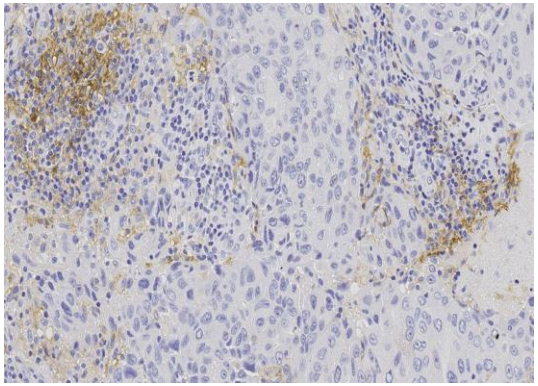


Assay 22C3+CK8





Assay 22C3



courtesy of Ulriche Thiim Pedersen

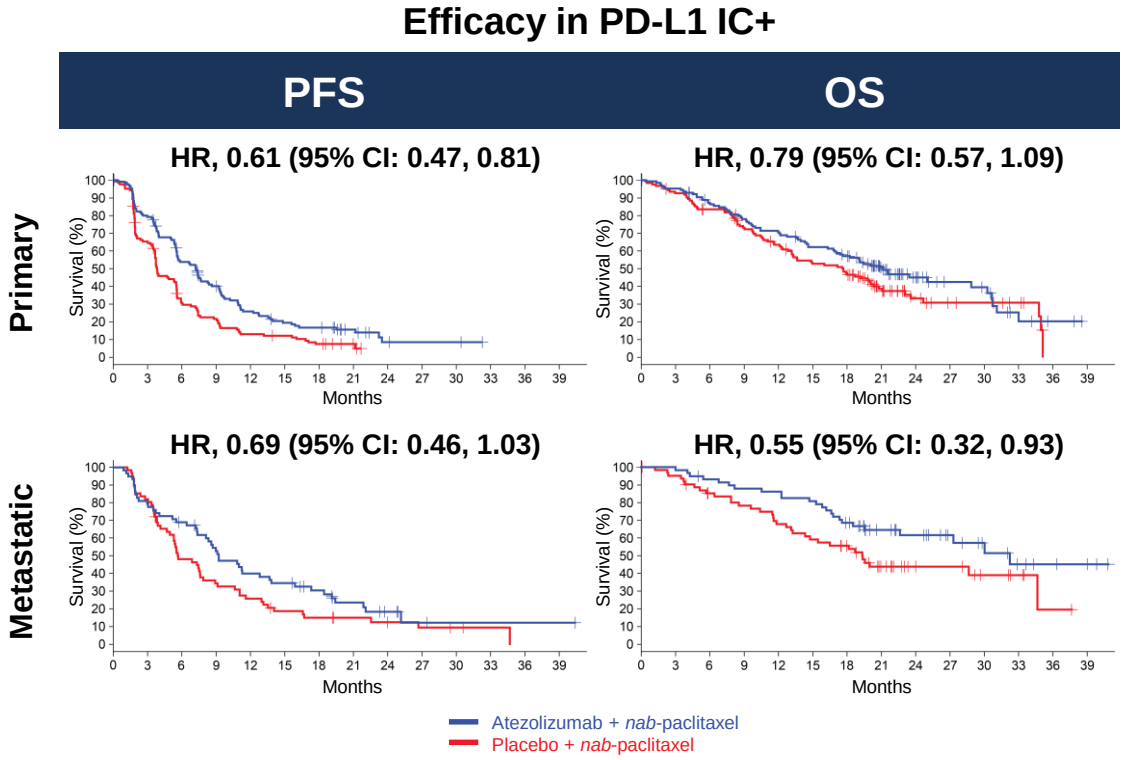
BARCELONA 2019 ESMO congress

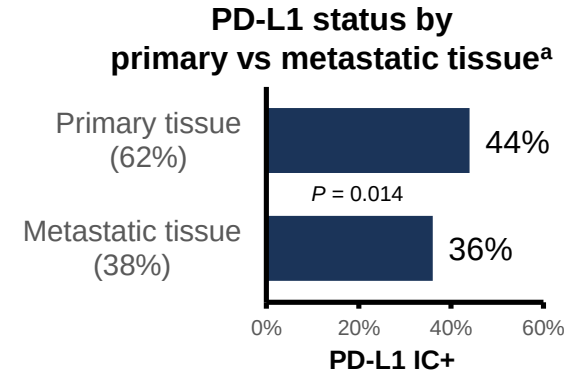
Performance of PD-L1 immunohistochemistry assays in unresectable locally advanced or metastatic triple-negative breast cancer: post hoc analysis of IMpassion130

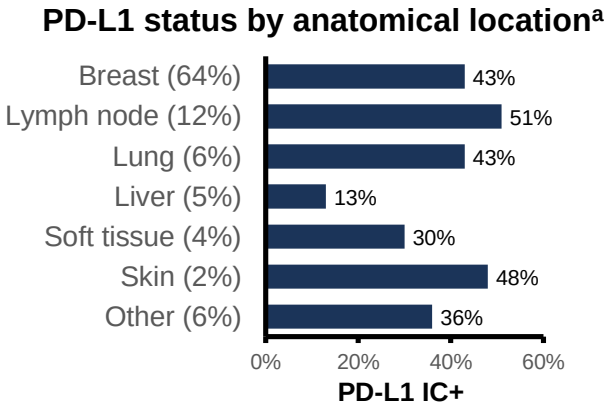
Hope S. Rugo,[1] Sherene Loi,[2] Sylvia Adams,[3] Peter Schmid,[4] Andreas Schneeweiss,[5] Carlos H. Barrios,[6] Hiroji Iwata,[7] Véronique Diéras,[8] Eric P. Winer,[9] Mark M. Kockx,[10] Dieter Peeters,[10] Stephen Y. Chui,[11] Jennifer C. Lin,[11] Anh Nguyen Duc,[11] Giuseppe Viale,[12] Luciana Molinero,[11] Leisha A. Emens[13]

[1]University of California San Francisco Comprehensive Cancer Center, San Francisco, CA, USA; [2]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; [3]NYU Langone Medical Center, New York, NY, USA; [4]Barts Cancer Institute, Queen Mary University London, London, UK; [5]University Hospital and German Cancer Research Center Heidelberg, Heidelberg, Germany; [6]Centro de Pesquisa Clínica, HSL, PUCRS, Porto Alegre, Brazil; [7]Aichi Cancer Center Hospital, Nagoya, Japan; [8]Department of Medical Oncology, Centre Eugène Marquis, Rennes, France; [9]Dana-Farber Cancer Institute, Boston, MA, USA; [10]HistoGeneX NV, Antwerp, Belgium; [11]Genentech, Inc., South San Francisco, CA, USA; [12]University of Milan, European Institute of Oncology IRCCS, Milan, Italy; [13]University of Pittsburgh Medical Center Hillman Cancer Center, Pittsburgh, PA, USA

PD-L1 status in primary vs metastatic tissues







- Median time of sample collection to randomization: 61 days

[a] Evaluable population (n = 901). PD-L1 IC+: PD-L1 in ≥ 1% of IC as percentage of tumour area assessed with the VENTANA SP142 assay.
HRs adjusted for prior taxanes, presence of liver metastases, age and ECOG PS. No major differences were observed for clinical benefit in samples collected within 61 days of randomization or beyond that period (Emens, et al, manuscript in preparation).

Clinical activity was observed in the SP142 PD-L1 IC+ subgroup, regardless of whether the sample was from the primary tumour or metastatic tissue

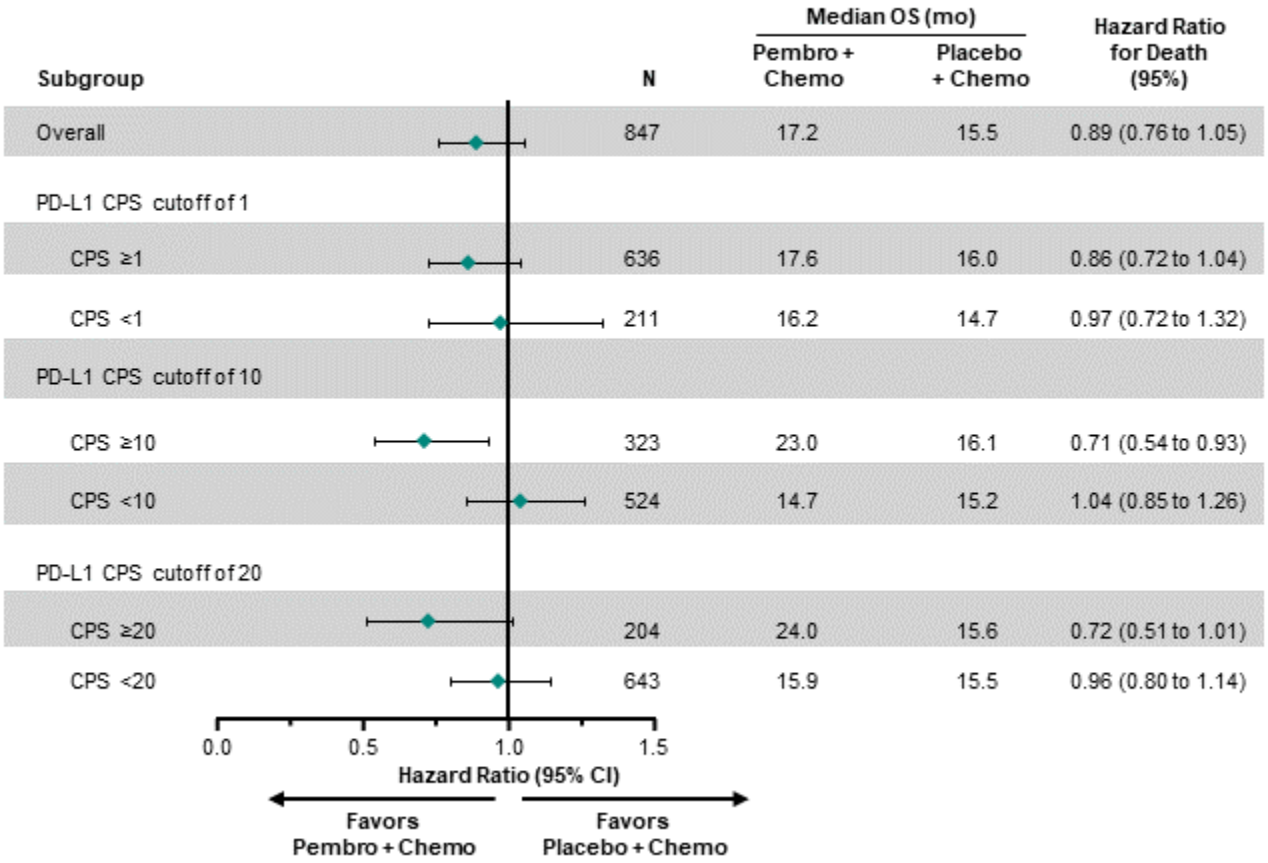
ESMO 2021
Keynote-355

KEYNOTE-355: Final Results from a Randomized, Double-blind, Phase 3 Study of First-line Pembrolizumab + Chemotherapy versus Placebo + Chemotherapy for Metastatic Triple-Negative Breast Cancer

Hope S. Rugo[1], Javier Cortes[2], David W. Cescon[3], Seock-Ah Im[4], Mastura Md Yusof[5], Carlos Gallardo[6], Oleg Lipatov[7], Carlos Henrique Barrios[8], Jose Perez-Garcia[9], Hiroji Iwata[10], Norikazu Masuda[11], Marco Torregroza Otero[12], Erhan Gokmen[13], Sherene Loi[14], Zifang Guo[15], Xuan Zhou[15], Vassiliki Karantza[15], Wilbur Pan[15], Peter Schmid[16]

1. Department of Medicine, University of California San Francisco Comprehensive Cancer Center, San Francisco, CA, USA; 2. International Breast Cancer Center (IBCC), Quiron Group, Madrid and Barcelona, Spain; Vall d'Hebron Institute of Oncology, Barcelona, Spain; Universidad Europea de Madrid, Faculty of Biomedical and Health Sciences, Department of Medicine, Madrid, Spain. 3. Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada; 4. Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea; 5. Cancer Center at Pantai Hospital, Kuala Lumpur, Malaysia; 6. Oncology Institute, Arturo Lopez Perez Foundation, Santiago, Chile; 7. Department of Oncology, Republican Clinical Oncology Dispensary, Republic of Bashkortostan, Russian Federation; 8. Oncology Research Unit, Hospital São Lucas, PUCRS, Porto Alegre, Brazil; 9. International Breast Cancer Center (IBCC), Quiron Group, Barcelona, Spain; 10. Department of Breast Oncology, Aichi Cancer Center Hospital, Nagoya, Japan; 11. Department of Surgery, Breast Oncology, National Hospital Organization Osaka National Hospital, Osaka, Japan; 12. Hematology & Oncology, Oncomedica S.A., Monteria, Colombia; 13. Medical Faculty, Ege University Medical School, Izmir, Turkey; 14. Division of Cancer Research, Peter MacCallum Cancer Centre, Melbourne, Australia; The Sir Peter MacCallum Department of Medical Oncology, University of Melbourne, Parkville, Australia; 15. Merck Research Laboratories, Merck & Co., Inc., Kenilworth, NJ, USA; 16. Barts Cancer Institute, Centre for Experimental Cancer Medicine, London, UK

Overall Survival in PD-L1 CPS Subgroups

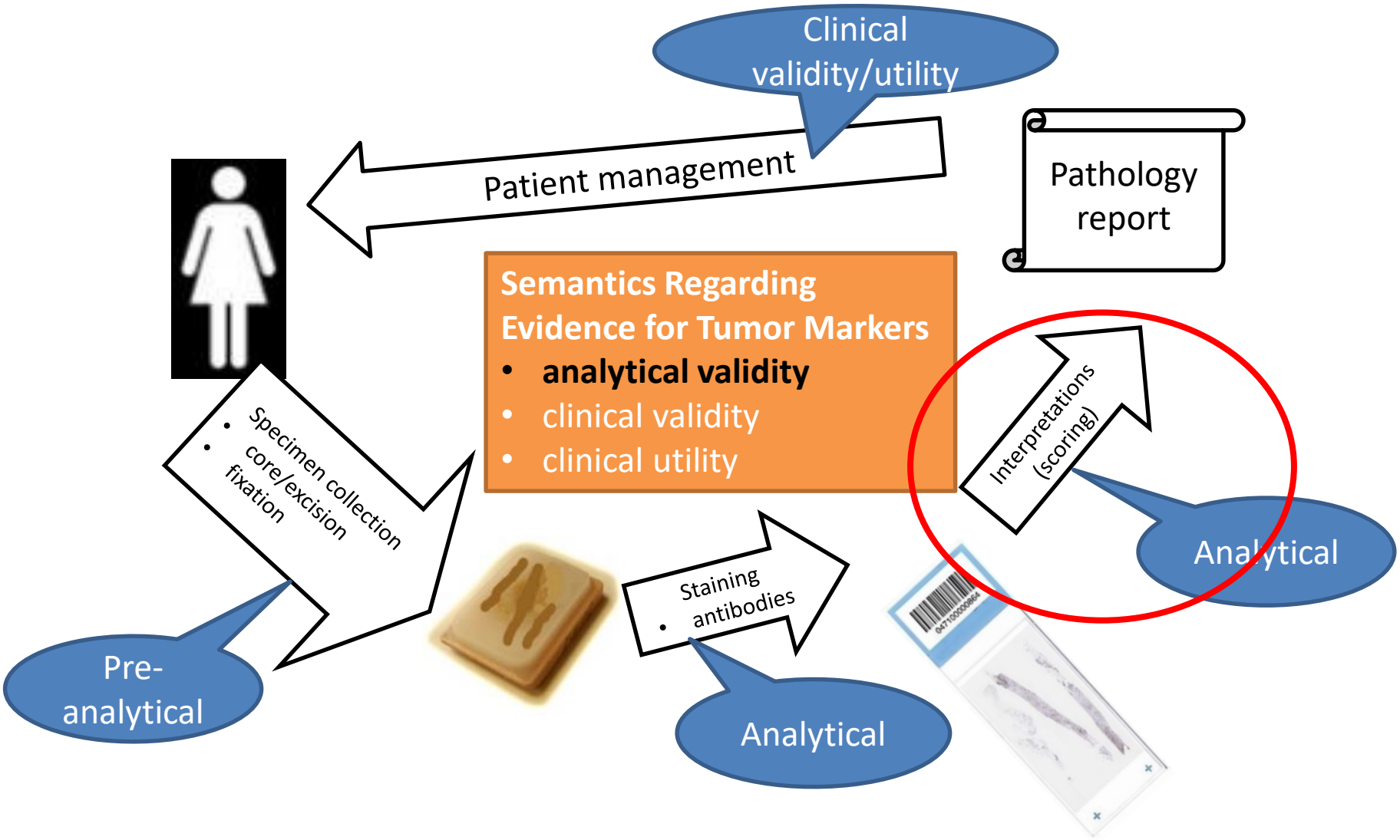


Analysis (HR and 95% CI) in the overall population is based on the stratified Cox regression model; analysis in the subgroups is based on the unstratified Cox model. OS in the CPS ≥1 and CPS ≥10 populations were primary endpoints; OS in the CPS ≥20 population was an exploratory endpoint. Data cutoff: June 15, 2021.

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
  - Prognostic and predictive factors
- Intrinsic molecular subtype / gene expression profile
  - Identification of patients who can be spared chemotherapy
- PD-L1 in TNBC
  - Assay preference and treatment
  - Tumor heterogeneity
- Always keep focus on analytical validity

Evidence for Tumor Markers




Thank you!