Le “ultime novità” in termini di ANATOMIA PATOLOGICA

Ki-67

TNBC

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Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013

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Identification of intrinsic subtypes is most precise using molecular technologies [22]. Where such assays are unavailable, surrogate definitions of subtype can be obtained by IHC measurements of ER, PgR, Ki-67 and HER2 with *in situ* hybridization confirmation, where appropriate [23]. Moderate or strong expression of PgR has been proposed as an additional restriction in the surrogate definition of 'Luminal A-like' disease [24]. Ki-67 level as a marker of proliferation is also important for this distinction [23]. Both of these markers require quality control. In particular, Ki-67 measurement is not currently standardized among laboratories [25–27] (see panel deliberations below).

Table 2. Surrogate definitions of intrinsic subtypes of breast cancer

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Intrinsic subtype	Clinico-pathologic surrogate definition	Notes
Luminal A	**'Luminal A-like'** *all of:* ER and PgR positive HER2 negative Ki-67 'low'[a] Recurrence risk 'low' based on multi-gene-expression assay (if available)[b]	The cut-point between 'high' and 'low' values for Ki-67 varies between laboratories.[a] A level of <14% best correlated with the gene-expression definition of Luminal A based on the results in a single reference laboratory [23]. Similarly, the added value of PgR in distinguishing between 'Luminal A-like' and 'Luminal B-like' subtypes derives from the work of Prat et al. which used a PgR cut-point of ≥20% to best correspond to Luminal A subtype [24]. Quality assurance programmes are essential for laboratories reporting these results.
Luminal B	**'Luminal B-like (HER2 negative)'** ER positive HER2 negative and *at least one of:* Ki-67 'high' PgR 'negative or low' Recurrence risk 'high' based on multi-gene-expression assay (if available)[b]	'Luminal B-like' disease comprises those luminal cases which lack the characteristics noted above for 'Luminal A-like' disease. Thus, either a high Ki-67[a] value or a low PgR value (see above) may be used to distinguish between 'Luminal A-like' and 'Luminal B-like (HER2 negative)'.
	'Luminal B-like (HER2 positive)' ER positive HER2 over-expressed or amplified Any Ki-67 Any PgR	
Erb-B2 overexpression	**'HER2 positive (non-luminal)'** HER2 over-expressed or amplified ER and PgR absent	
'Basal-like'	**'Triple negative (ductal)'** ER and PgR absent HER2 negative	There is an 80% overlap between 'triple-negative' and intrinsic 'basal-like' subtype. Some cases with low-positive ER staining may cluster with non-luminal subtypes on gene-expression analysis. 'Triple negative' also includes some special histological types such as adenoid cystic carcinoma.

- **< 14% ??**
- **>= 20% ??**
- **local laboratory specific cut- point ??**

[a]A majority of the Panel voted that a threshold of ≥20% was indicative of 'high' Ki-67 status. Others, concerned about the high degree of inter-laboratory variation in Ki-67 measurement [26] and the possibility for undertreatment of patients with luminal disease who might benefit from chemotherapy, would use a lower (local laboratory specific) cut-point to define Ki-67 'high' or use multi-gene-expression assay results, if available.

[b]This factor was added during Panel deliberations after circulation of the first draft of the manuscript, to reflect a strong minority view. Although neither the 21-gene RS nor the 70-gene signature was designed to define intrinsic subtypes, a concordance study noted that over 90% of cases with a low RS and almost 80% of those with a 70-gene low-risk signature were classified as Luminal A [95].

Table 3. Systemic treatment recommendations

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'Subtype'	Type of therapy	Notes on therapy
'Luminal A-like'	Endocrine therapy is the most critical intervention and is often used alone.	Cytotoxics may be added in selected patients. Relative indications for the addition of cytotoxics accepted by a majority of the Panel included: (i) high 21-gene RS (i.e. >25), if available; (ii) 70-gene high risk status, if available; (iii) grade 3 disease; (iv) involvement of four or more lymph nodes (a minority required only one node). The Panel was almost equally divided as to whether young age (<35 years) *per se* was an indication to add cytotoxics. Studies suggest a wide geographical divergence in the threshold indications for the inclusion of cytotoxics for the treatment of patients with luminal disease [96].
'Luminal B-like (HER2 negative)'	Endocrine therapy for all patients, cytotoxic therapy for most.	
'Luminal B-like (HER2 positive)'	Cytotoxics + anti-HER2 + endocrine therapy	No data are available to support the omission of cytotoxics in this group.
'HER2 positive (non-luminal)'	Cytotoxics + anti-HER2	Threshold for use of anti-HER2 therapy was defined as pT1b or larger tumour or node-positivity.
'Triple negative (ductal)'	Cytotoxics	
'Special histological types'[a]		
A. Endocrine responsive	Endocrine therapy	
B. Endocrine non-responsive	Cytotoxics	Adenoid cystic carcinomas may not require any adjuvant cytotoxics (if node negative).

[a]Special histological types: endocrine responsive (cribriform, tubular and mucinous); endocrine non-responsive (apocrine, medullary, adenoid cystic and metaplastic).

For practical purposes, distinction between 'Luminal A' and 'Luminal B' (Her2 Neg) tumors can be:

•made by ER, PR alone?

Yes	No	Abstain
6.1%	91.8%	2.0%

• made by ER, PR and Ki-67?

Yes	No	Abstain
72.9%	27.1%	0.0%

•made with grade 3 as a substitute for high Ki-67?

Yes	No	Abstain
36.0%	64.0%	0.0%

•only safely determined by molecular diagnostics?

Yes	No	Abstain
34.0%	60.0%	6.0%

•only safely determined by laboratories participating in quality assurance programs?

Yes	No	Abstain
88.9%	8.9%	2.2%

Ki-67: guidelines and quality assurance programs

St. Gallen 2013

Annals of Oncology 24: 2206–2223, 2013

Ki67

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

J Natl Cancer Inst 2011;103:1–9

Table 1. Factors that may affect Ki67 immunohistochemistry*

Setting	Factor	Variables	Important?	Comments
Preanalytical	Type of biopsy	Core vs whole section	No	Both are suitable. Some data suggest that whole section may give higher scores than core biopsy.
	Type of fixative	Previously frozen, or EtOH or EDTA fixative, or previous acid decalcification vs neutral buffered formalin	Yes	Avoid all but neutral buffered formalin. Others reduce Ki67 staining compared with neutral buffered formalin.
	Time to fixation	Integrity of nuclei	Yes	For visual analysis, has little impact unless extreme. Important for image analysis.
	Means of storage	Tissue in paraffin block vs cut section	Yes	Prolonged storage of formalin-fixed paraffin-embedded tissue block at room temperature has little effect on Ki67. Avoid prolonged exposure to air of cut sections on glass slides.

whole section has HIGHER SCORE than core biopsy

Ki67

Table 1. Factors that may affect Ki67 immunohistochemistry*

Setting	Factor	Variables	Important?	Comments
Interpretation and scoring	Method of reading	Cellular component, staining intensity	Yes	1) Count all positive cells within region in which all nuclei have been stained. 2) Scoring requires determination of percentage cells positive. 3) No interpretation of intensity.
	Area of slide read	Edge vs central; hot spots vs area without hot spots vs all areas	Yes	Controversial: currently recommend average score across the section.
	Image	Visual vs automated analysis	Unknown	Unknown whether either method is superior.
Data analysis	Cut point	Any vs no staining; arbitrary vs data-derived cut point; or continuous variable	Controversial	It is controversial because there is no recommended consensus cut point at this time.

- Edge vs Central ?
- Hot spots vs area without hot spots ?
- All areas ?

J Natl Cancer Inst 2011;103:1–9

Controversial:
currently average score across the section

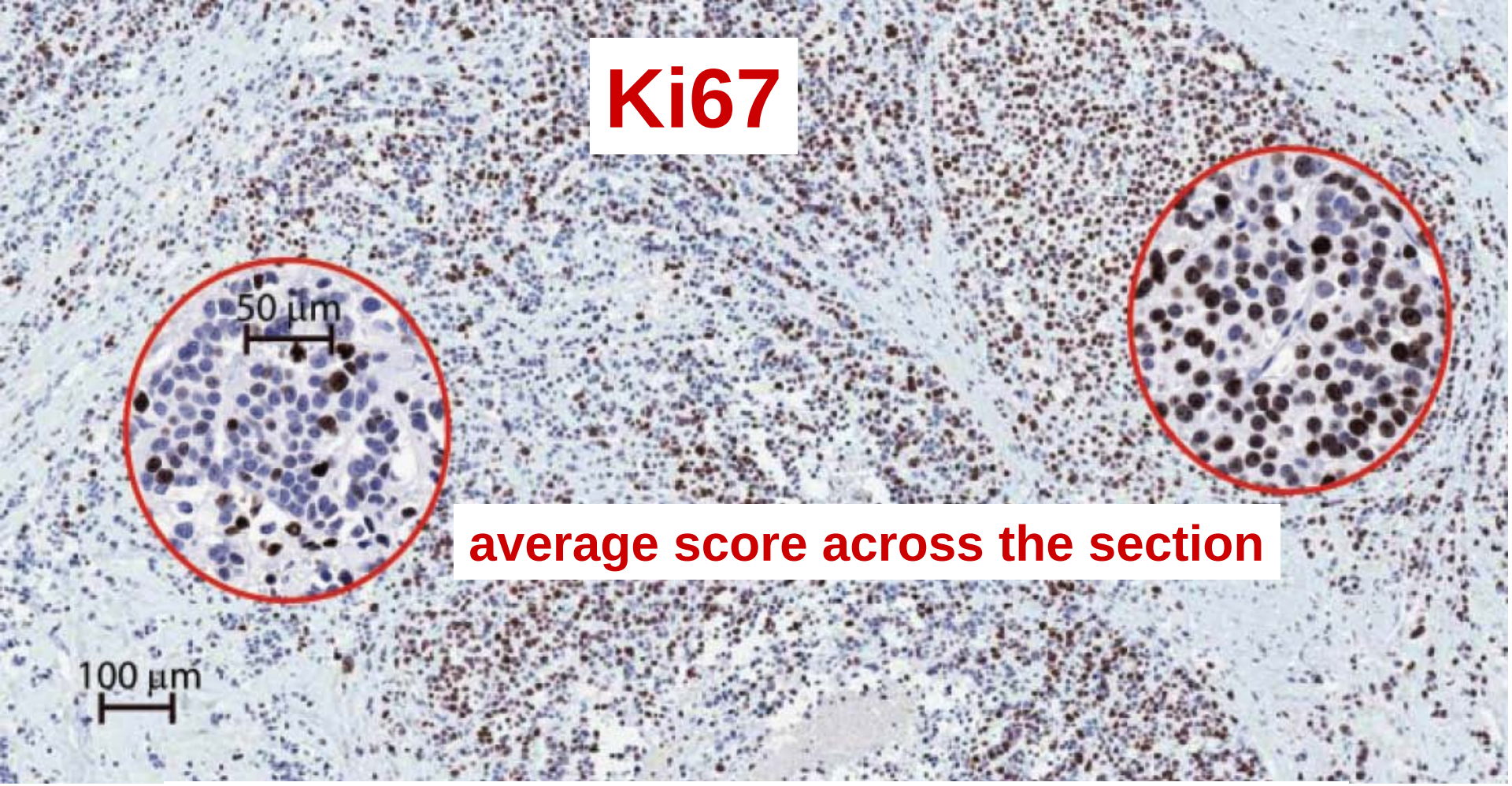


Figure 3. Variable levels of Ki67 staining in breast cancer. Tumor biopsies were fixed in neutral buffered formalin and sections stained for Ki67 with MIB1 antibody (**brown stain**) and counterstained with Mayer's hematoxylin (**blue stain**). The two areas **circled in red** are shown at higher magnification to illustrate the differences in scores that can occur in different high-power fields. The average score across the whole section should be taken.

Ki67

Determinazione dell'indice di proliferazione (Ki67)

Ki67 va determinato in ogni carcinoma primitivo invasivo della mammella.

La valutazione della frazione di cellule proliferanti dovrebbe essere espressa come percentuale di cellule positive per Ki67 indipendentemente dalla intensità di colorazione e deve essere effettuata alla periferia della neoplasia su più campi non selezionati.

Raccomandazioni AIOM-SIAPEC-IAP Catania 2010

SIAPEC - IAP

Breast Cancer Res Treat (2013) 139:539–552
DOI 10.1007/s10549-013-2560-8

EPIDEMIOLOGY

Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry

E. C. Inwald · M. Klinkhammer-Schalke · F. Hofstädter · F. Zeman · M. Koller · M. Gerstenhauer · O. Ortmann

Ki67

was associated with common histopathological parameters, but was shown to be an independent prognostic parameter for DFS and OS in breast cancer patients. These findings underline the importance of Ki-67 as a prognostic parameter. Therefore, future work in this field is called for; it should focus on the standardization of Ki-67 assessment in routine clinical settings and on the role of Ki-67 in treatment decisions.

Molecular subclasses of breast cancer: how do we define them? The IMPAKT 2012 Working Group Statement†

Intrinsic subtypes (GEP)	IHC classification (St Gallen)	Agreement IHC/GEP
Luminal A	'Luminal A' ER and/or PR positive HER2 negative Ki-67<14%	73%–100%
Luminal B	'Luminal B (HER2 negative)' ER and/or PR positive HER2 negative Ki-67 ≥14% 'Luminal B (HER2 positive)' ER and/or PR positive Any Ki-67 HER2 over-expressed or amplified	73%–100%
HER2-enriched	'HER2 positive (non-luminal)' HER2 over-expressed or amplified ER and PR absent	41%–69%
Basal-like	'Triple negative' ER and PR absent HER2 negative	80%

GEP, gene expression profiling; IHC, immuno-histochemical; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

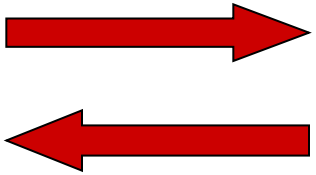
Annals of Oncology 23: 2997–3006, 2012

Molecular subtypes of BC

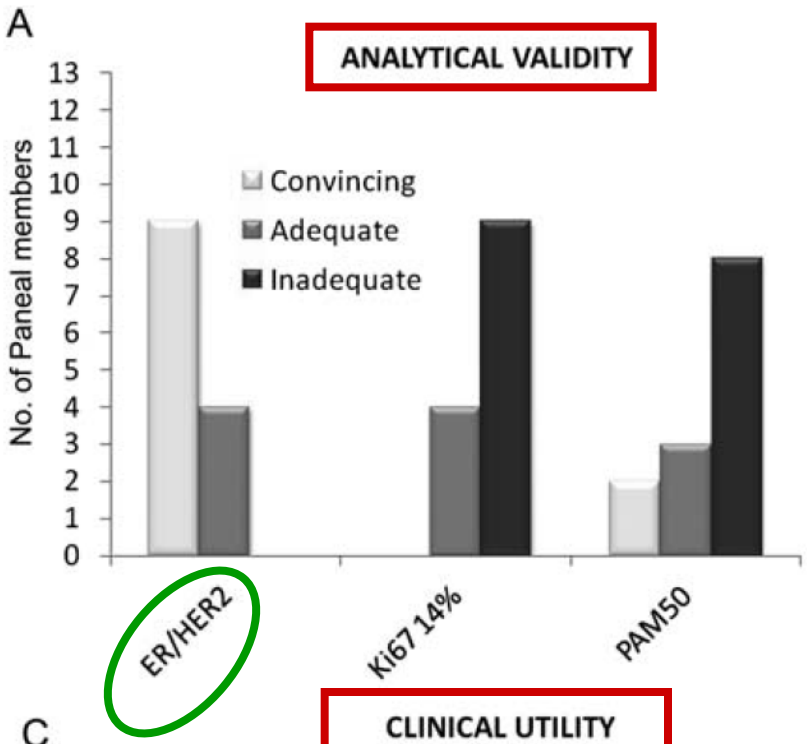
IHC

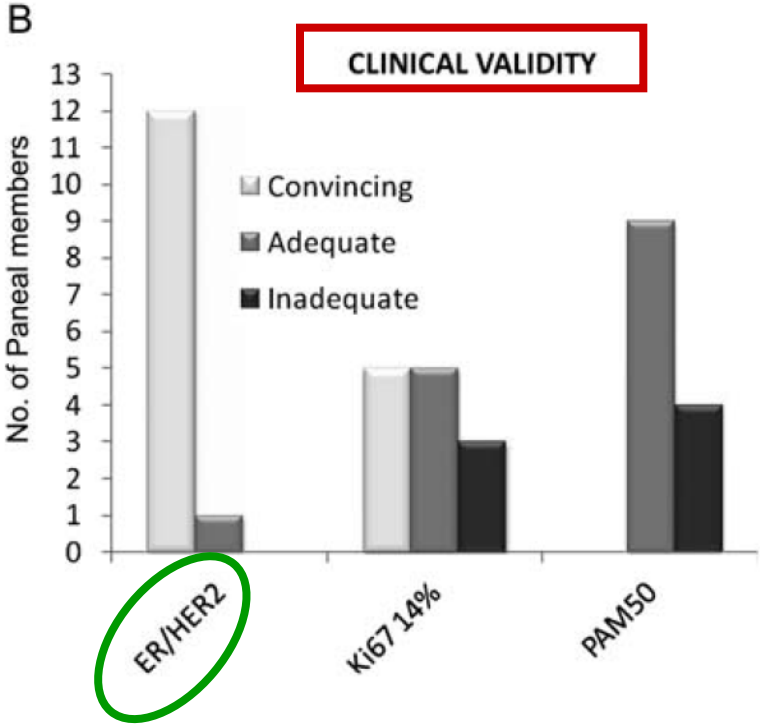
GEP

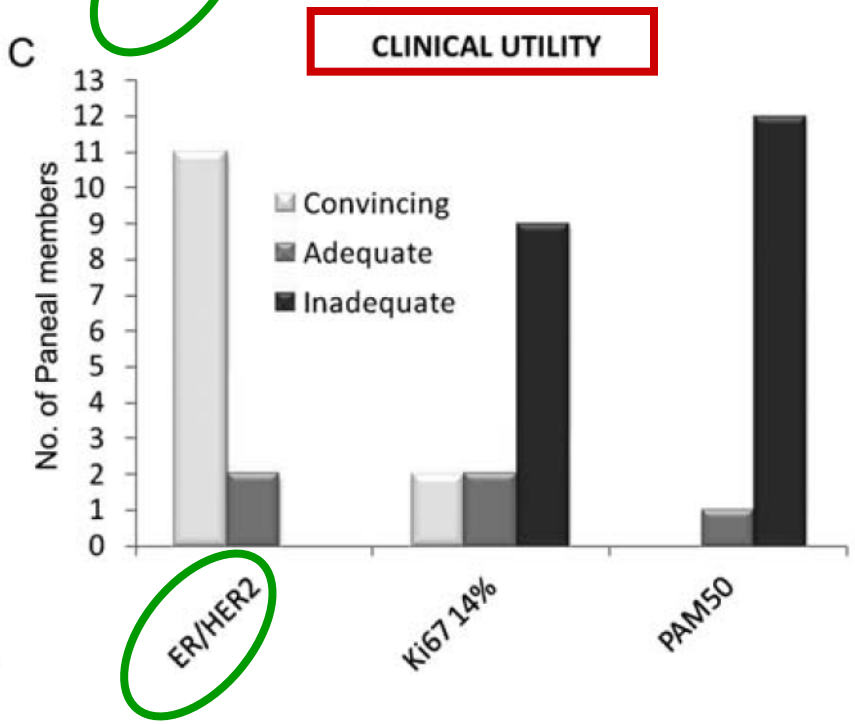
ER
HER2
Ki67 (14% cut-off)



PAM50







Annals of Oncology 23: 2997–3006, 2012

BC classification into molecular subtypes based on the IHC assessment of ER, HER2 and ki67 with a 14% cut-off and on PAM50 test does not provide sufficiently robust information to modify systemic treatment decisions.

The use of IHC for **ER** and **HER2** for the identification of clinical relevant subtypes of BC is recommended.

Currently tumor size, nodal status, histological grade, ER, PR, HER2 status remain the current “gold standard” for systemic theraphy decision making.

Annals of Oncology 23: 2997–3006, 2012

TNBC

heterogeneities in the nomenclature and classification

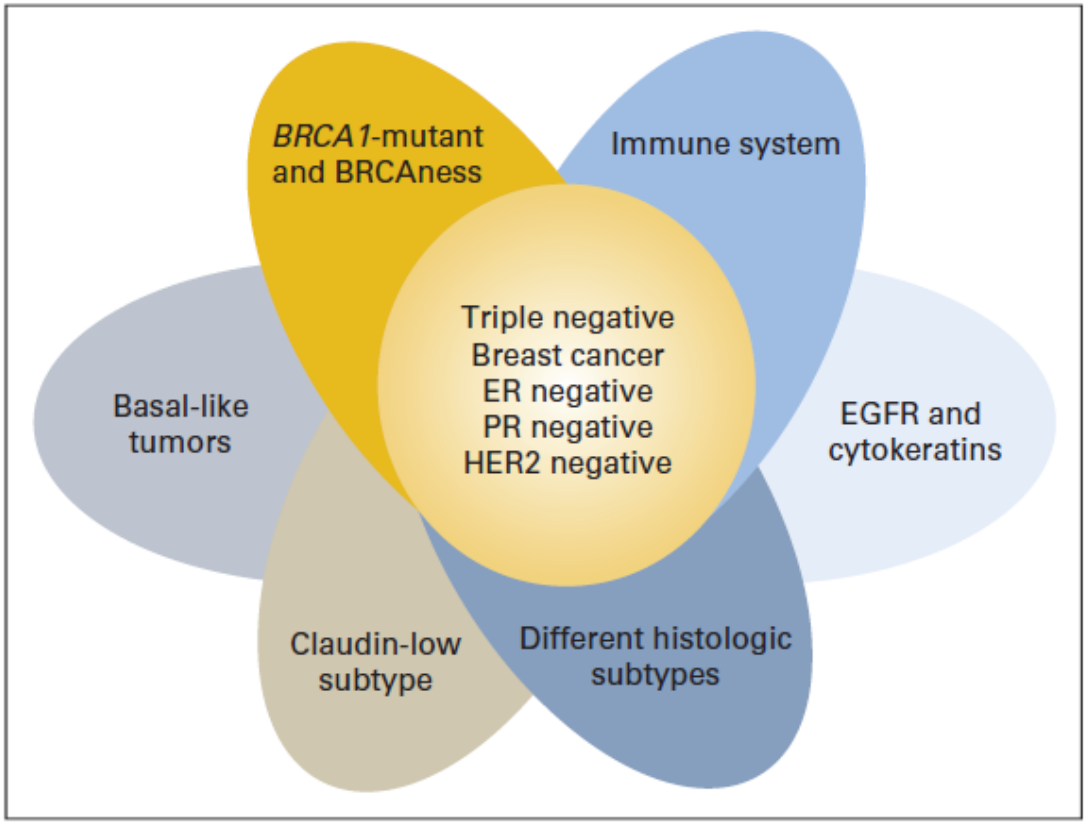


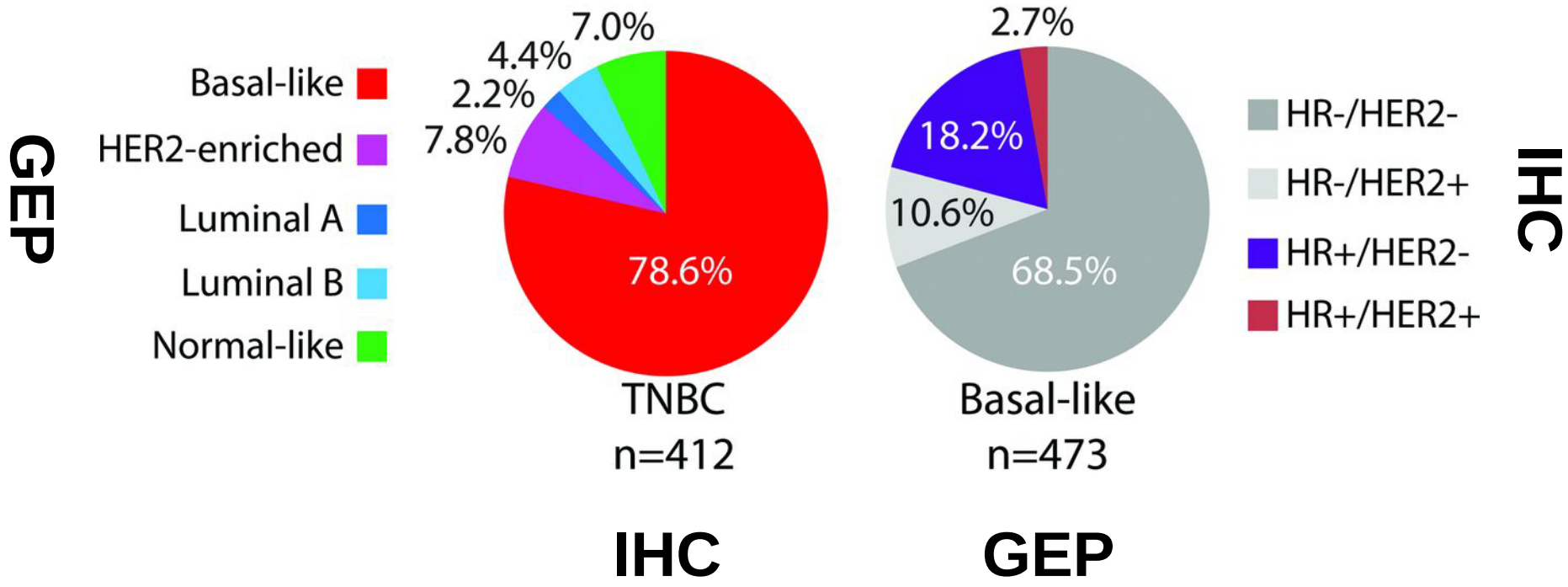
Fig 1. Heterogeneities in the nomenclature and classification of triple-negative breast cancer. EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

- **histologic subtypes**
- **presence of biomarkers**
- **gene signatures**
- **BRCA1 mutation**
- there is significant overlap between these categories; TN is a heterogeneous entity with 70% of basal-like

J Clin Oncol 30:1879-1887. © 2012

Distribution of the intrinsic molecular and pathology-based subtypes within triple-negative and basal-like tumors

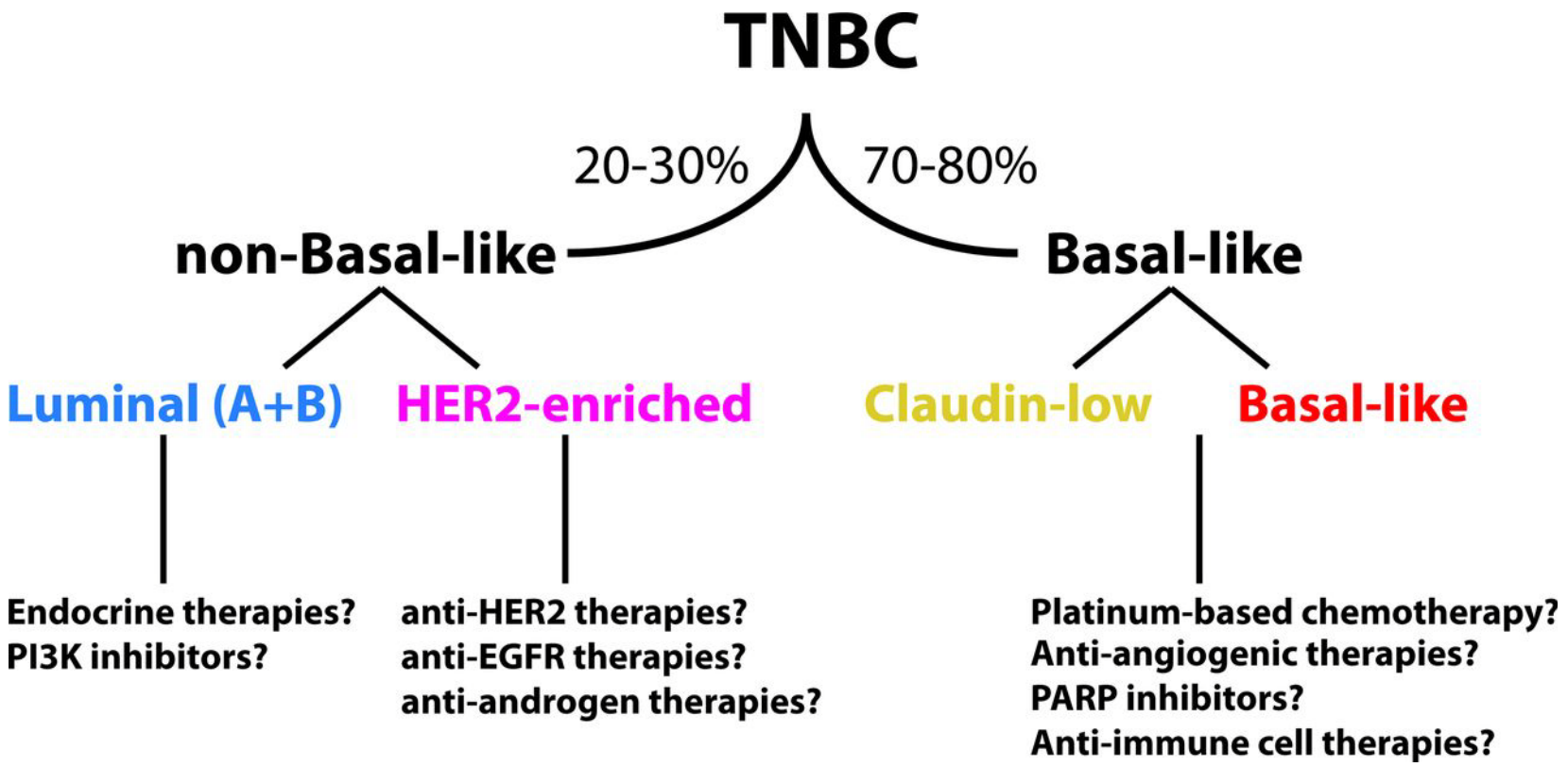
TN and BL are not synonymous terms
20%-30% of discordance rate:
not all TN are BL tumors by GEP and not all BL by GEP are TN



Prat A et al. The Oncologist 2013;18:123-133

Proposed algorithm of stratification of triple-negative tumors

(using GEP or IHC for EGFR, basal CKs)



EGFR: epidermal growth factor receptor
PARP: poly (ADP-ribose) polymerase

Prat A et al. The Oncologist 2013;18:123-133

Gene expression profiles from 21 BC data sets
597 TNBC
6 TNBC subtypes
genetic heterogeneity

- Basal - like 1 (BL1)
- Basal - like 2 (BL2)

high levels of genes involved in cell proliferation and DNA damage response: antimitotic and DNA-damaging agents (cisplatin) PARP inibitors in BRCA1/2 mutant tumors

- Immunomodulatory (IM)

immune response gene signatures; stromal components including immune cell infiltrate?

- Mesenchimal (M)
- Mesenchimal stem - like (MSL)

share similar GEP involving TGF-ß, mTOR, Rac1/Rho Wnt/ß-catenin, FGFR,PDGFR VEGF, PI3K signaling pathways; PI3K/mTOR inibitors

- Luminal androgen receptor (LAR)

AR gene signature, luminal CKs expression; PI3K/mTOR patwhay; anti AR therapy (PI3K/mTOR inibitors?)

Lehmann BD et al

J Clin Invest doi:10.1172/JCI45014. **2011**

MODERN PATHOLOGY (2013) 26, 955–966

Comparison of basal-like triple-negative breast cancer defined by morphology, immunohistochemistry and transcriptional profiles

Patrycja Gazinska[1,2,5], Anita Grigoriadis[1,5], John P Brown[2], Rosemary R Millis[2], Anca Mera[3,4], Cheryl E Gillett[3], Lars H Holmberg[3,4], Andrew N Tutt[1] and Sarah E Pinder[2]

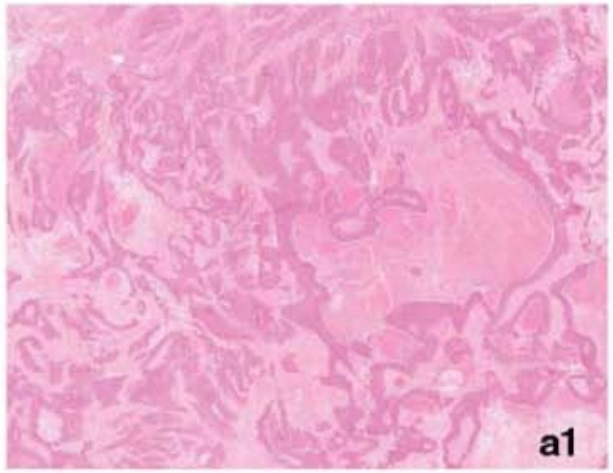
142 TNBC

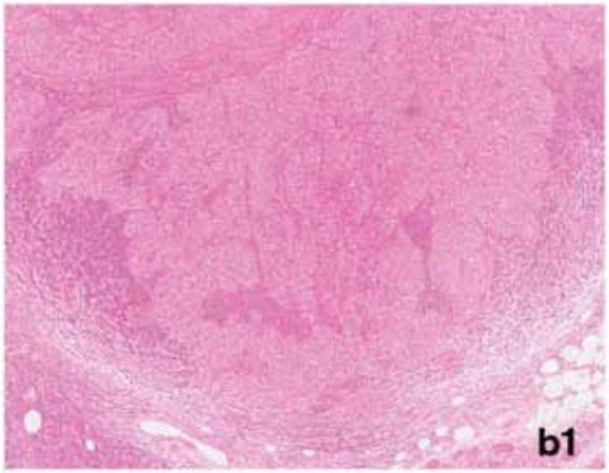
- Morphology: **Path Basal**
- IHC: **Core Basal**
- GEP: **PAM50 Basal**

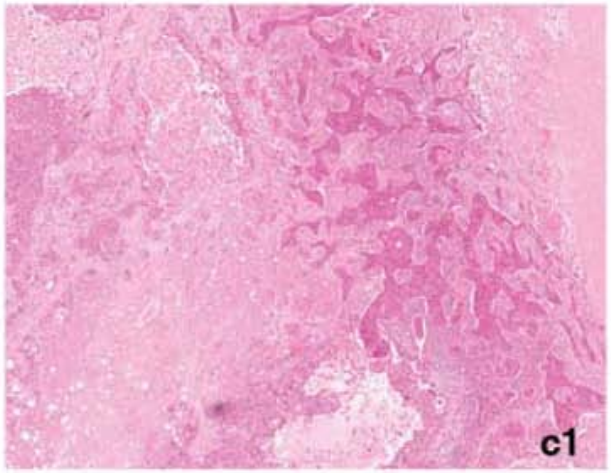
geographical necrosis

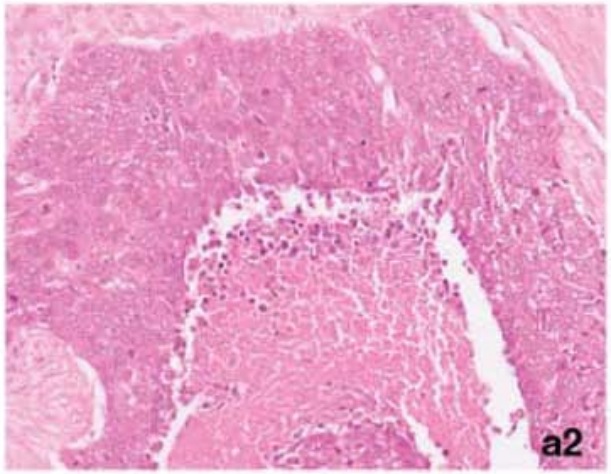
pushing edges

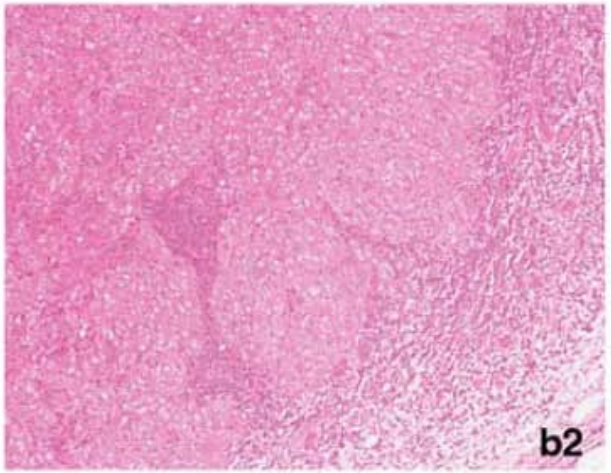
metaplastic tumor

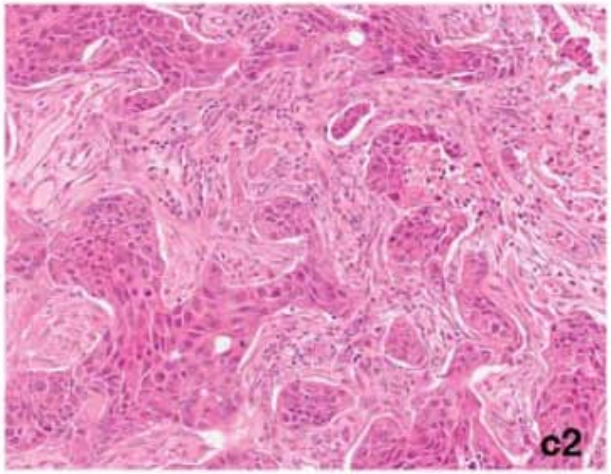












high mitotic rate, necrosis apoptotic cells

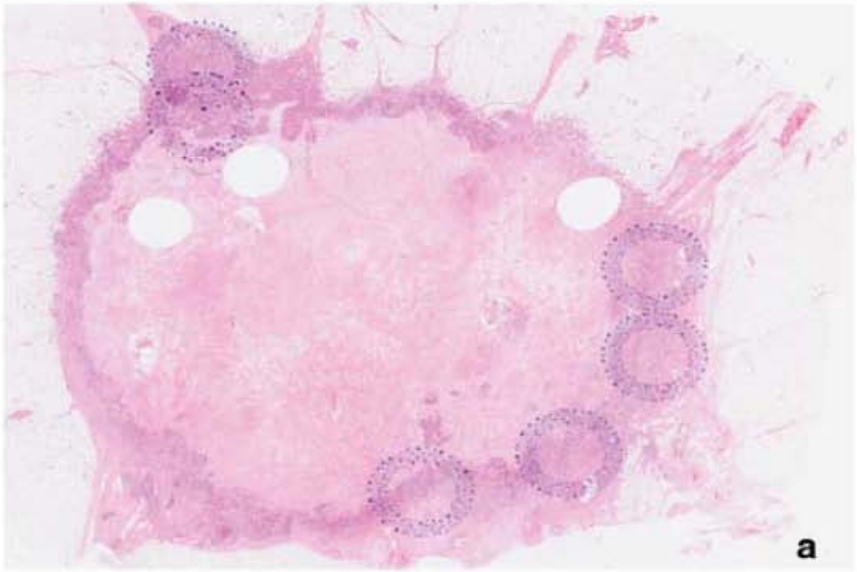
lymphoplasmacytic stromal infiltration

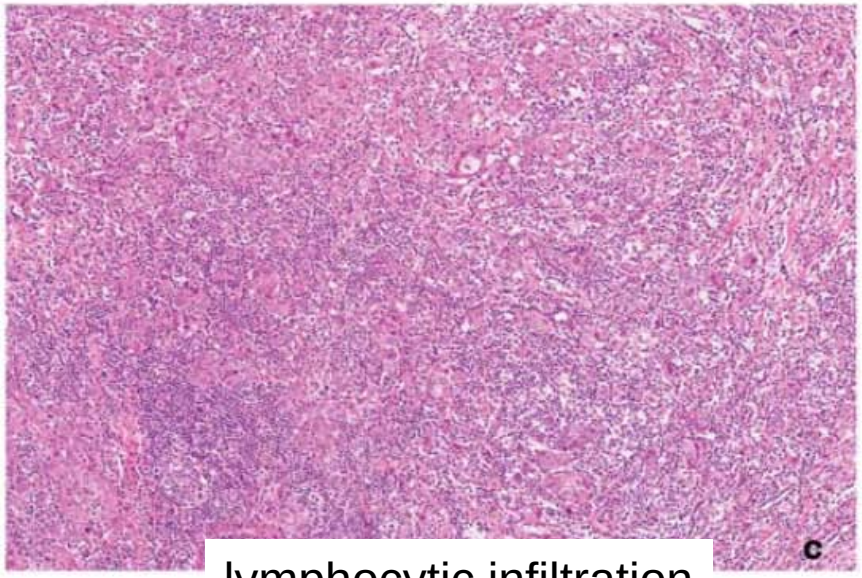
metaplastic tumor

Path Basal

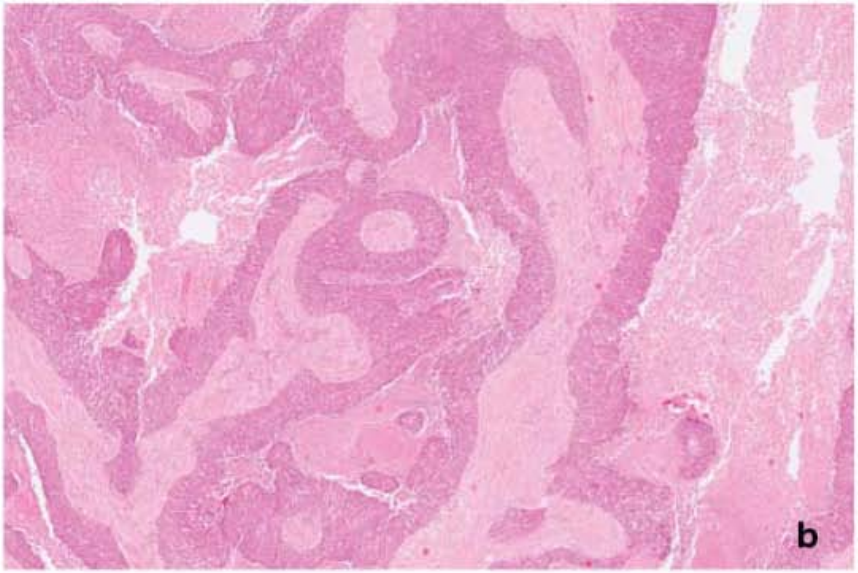
MODERN PATHOLOGY (2013) 26, 955–966

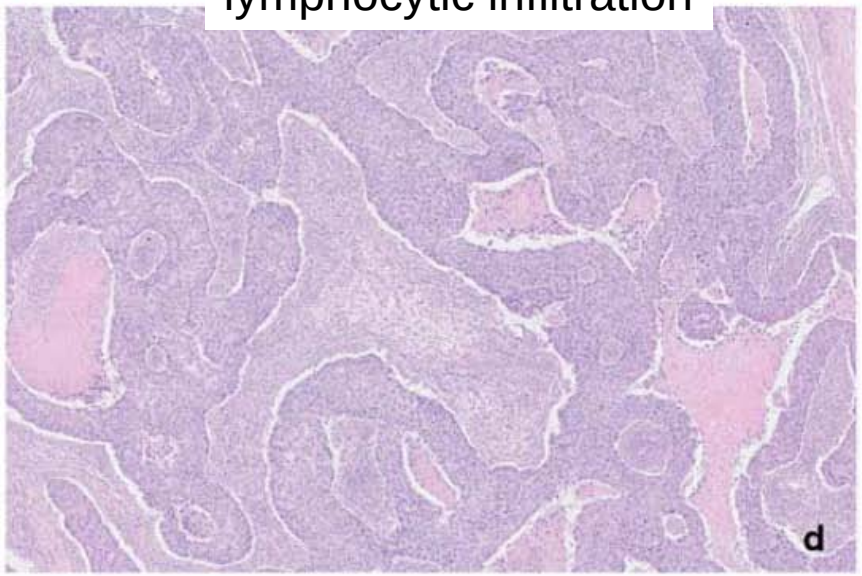
central fibrosis





lymphocytic infiltration





fibrosis/necrosis

Path Basal

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Core Basal

- ER
- PR
- HER2
- CK5/6
- CK14
- EGFR

Er, PR, HER2 negative
EGFR and/or CK5/6 positive

ER, PR, HER2, CK5/6 and EGFR negative

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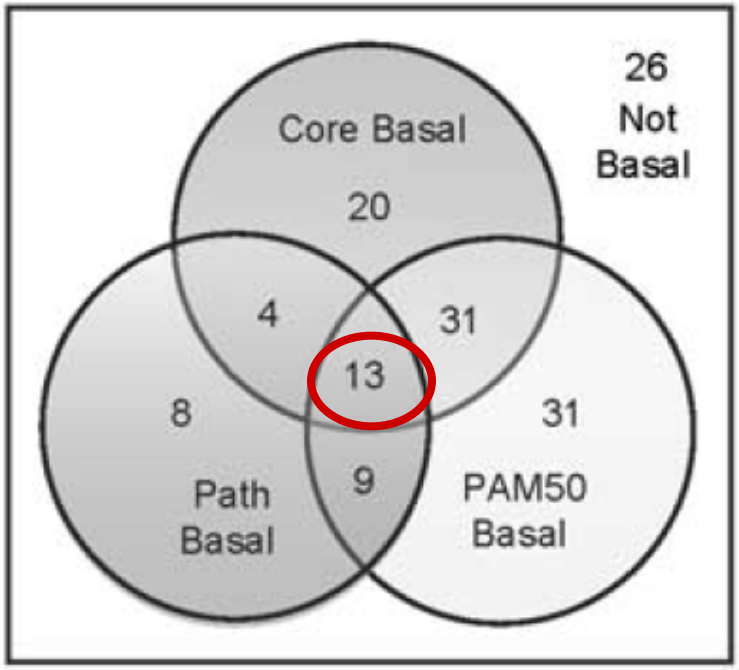

- the definition of basal-like BC based on different methodologies varies significantly and does not identify the same lesions

- the incomplete overlap of cases emphasizes the need for consistent or new approaches to improve precise identification

- the highest risk of death was seen for the **Core Basal** group (EGFR and basal CKs as biomarkers to predict prognosis?)

MODERN PATHOLOGY (2013) 26, 955–966

CONCLUSION

- Ki-67
  - standardization of assessment in routine clinical settings
  - cut-off
  - role in treatment decisions
- Better characterization of TNBC
- Consistent identification of basal-like tumor