ESMO Preceptorship Programme

Breast Cancer

Multidisciplinary management, standards of care, therapeutic targets and future perspectives

Lisbon, Portugal

16-17 September 2016

BREAST CANCER CLASSIFICATION: TRADITIONAL PATHOLOGY AND MOLECULAR SUBTYPES

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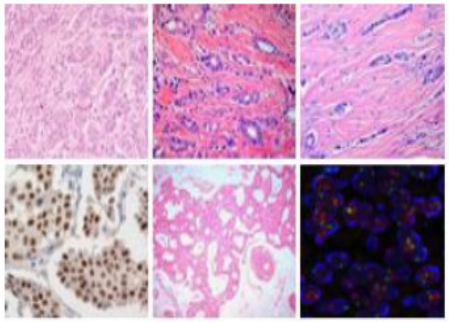
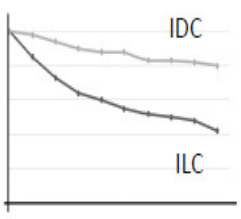
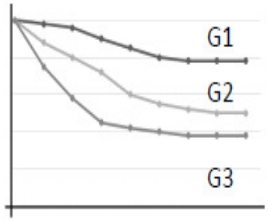
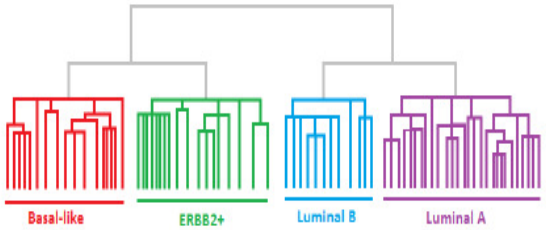
General-Secretary of the International Academy of Cytology

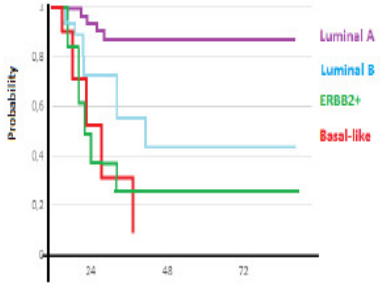




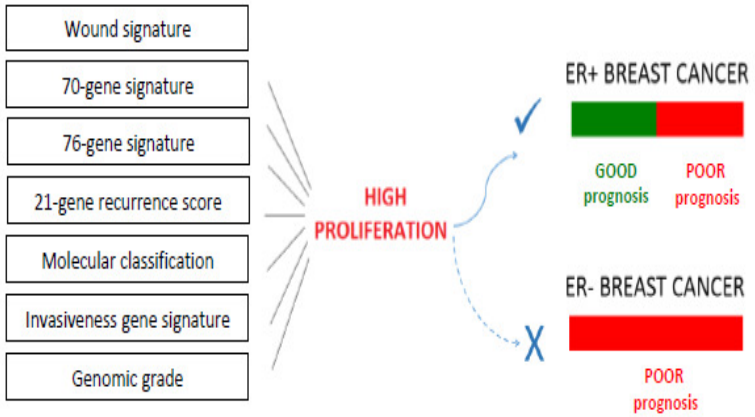


Histological type **Histological grade**





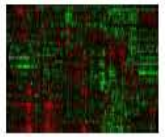
Molecular classification



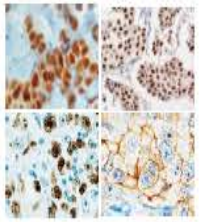
Microarray-based gene expression signatures

---1991---2000---2006---

12th St. Gallen International Breast Cancer Conference
Primary Therapy of Early Breast Cancer with Treatment Consensus Update
16-19 March 2011
St. Gallen, Switzerland



Approximately 500 GENES
Intrinsic gene list



4 IHC MARKERS
- ER
- PR
- HER2
- Ki67

Surrogate for molecular classification

13th St. Gallen International Breast Cancer Conference
Primary Therapy of Early Breast Cancer Evidence, Controversies, Consensus
13-16 March 2013
St.Gallen, Switzerland

Intrinsic subtype	Pathologic definition
Luminal A	ER >1% PR >1% Ki-67 <14%? <20%? HER2 negative
Luminal B	ER >1% PR >1% Ki-67 >20%? HER2 negative

MOLECULAR TESTS

Recurrence score(s)

Molecular tests for risk prediction

St Gallen 2015 Viena

Gene expression data
Copy number data
IntClusts

HER2 mutations

NGS ...

TCGA results: IntClust, mutation profile *et al.*

---2011---2013---(2012)-2014---20XX---

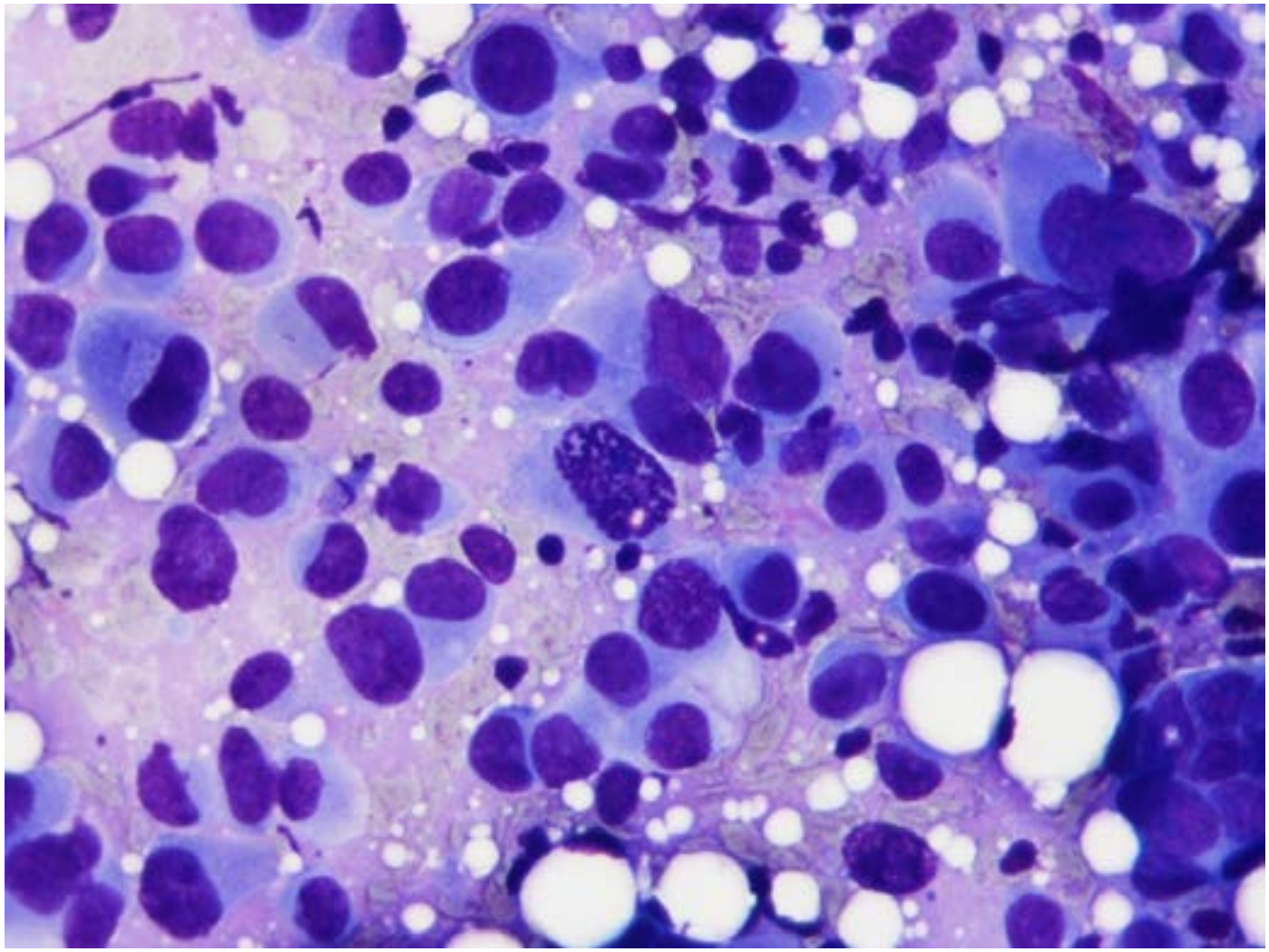
Why do we need a classification?

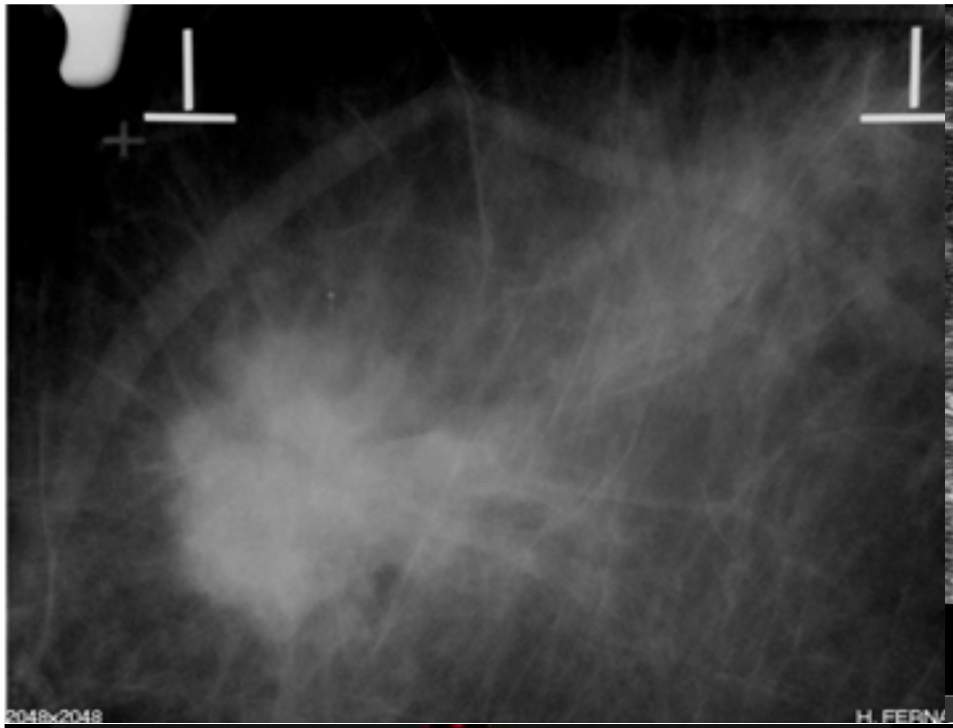
Aim 1: **Diagnosis**

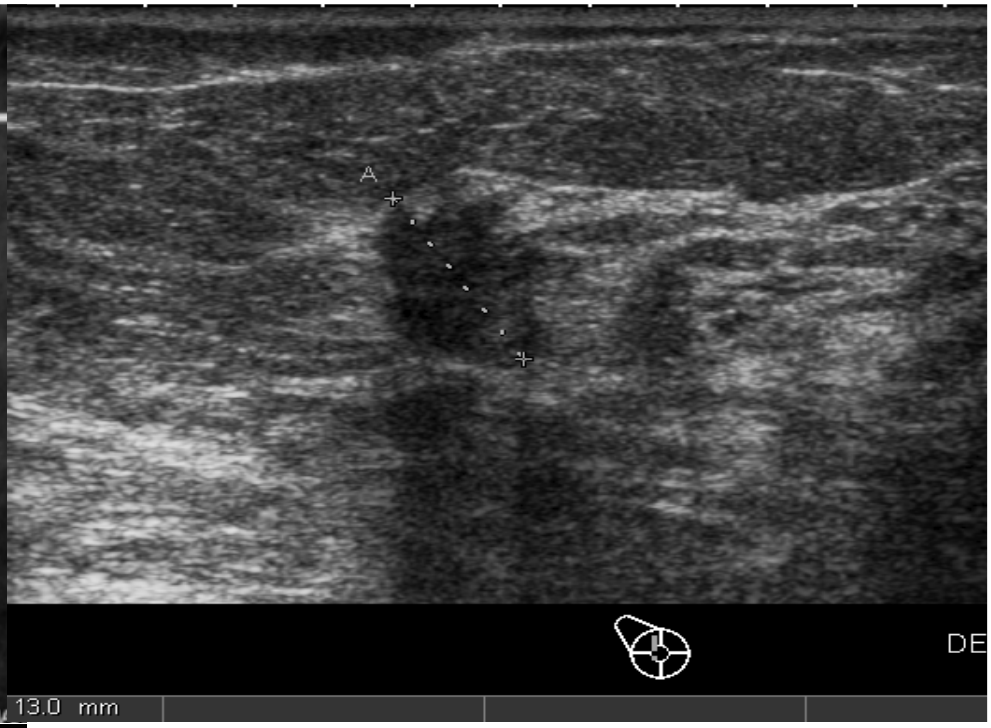
Aim 2: **Prognosis**

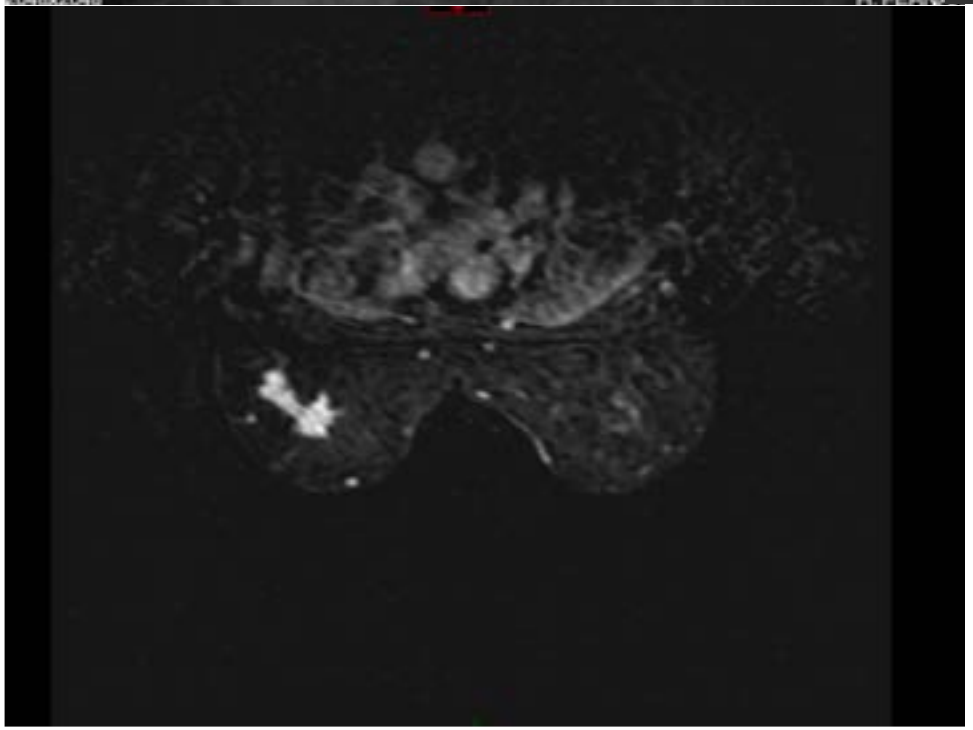
Aim 3: **Prediction**

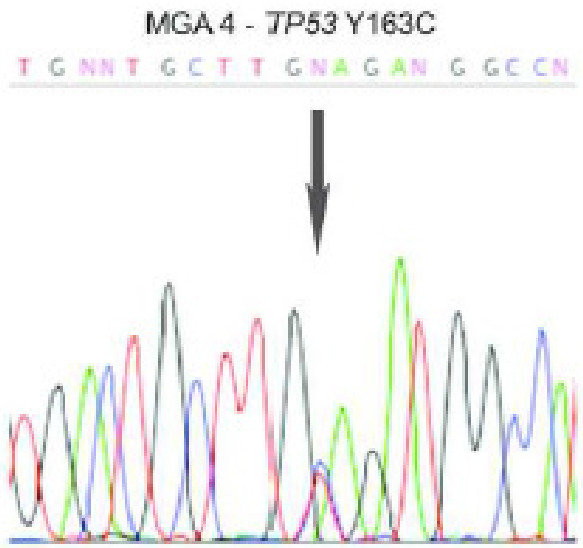
Breast cancer diagnosis is morphological

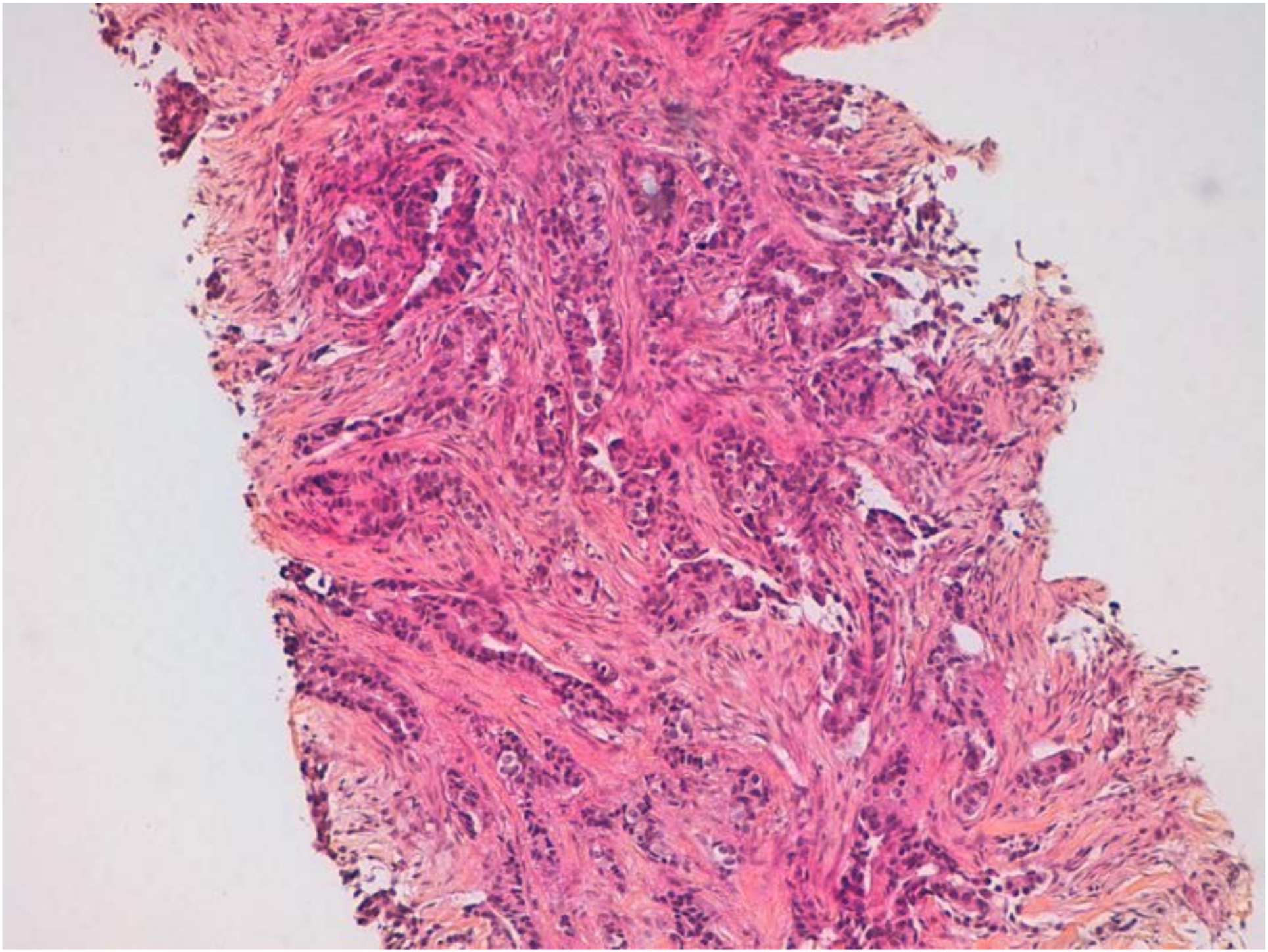


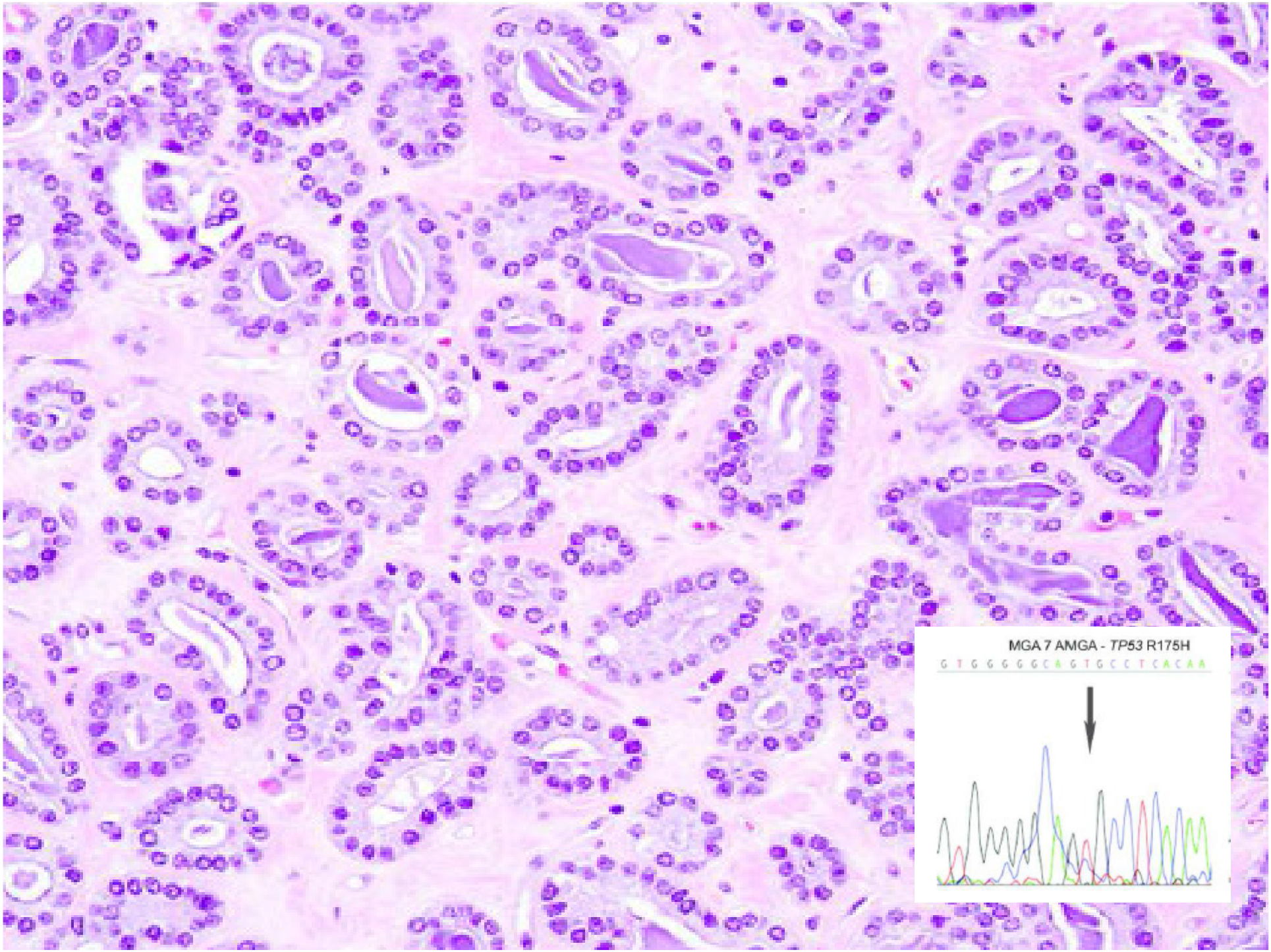

2048x2048


A
13.0 mm



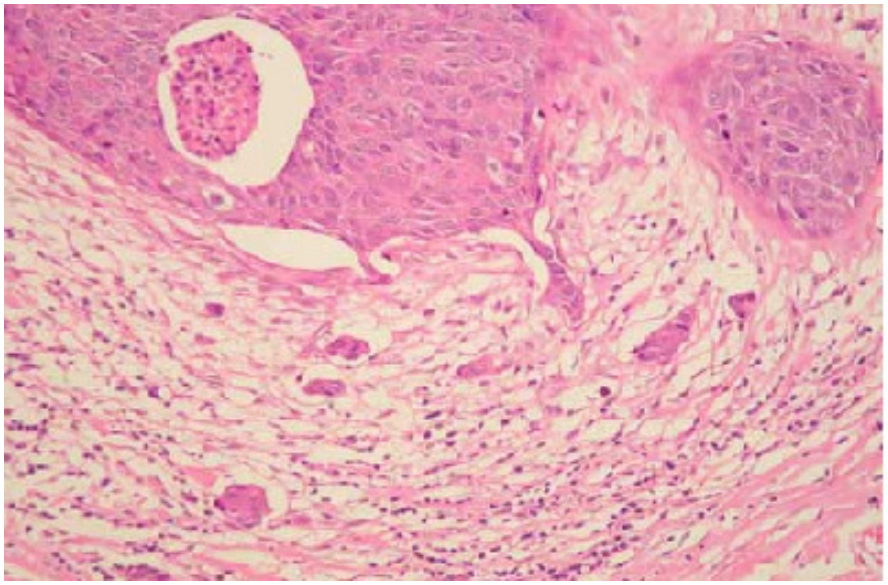

MGA 4 - TP53 Y163C
T G NN T G C T T G NA G AN G GC CN

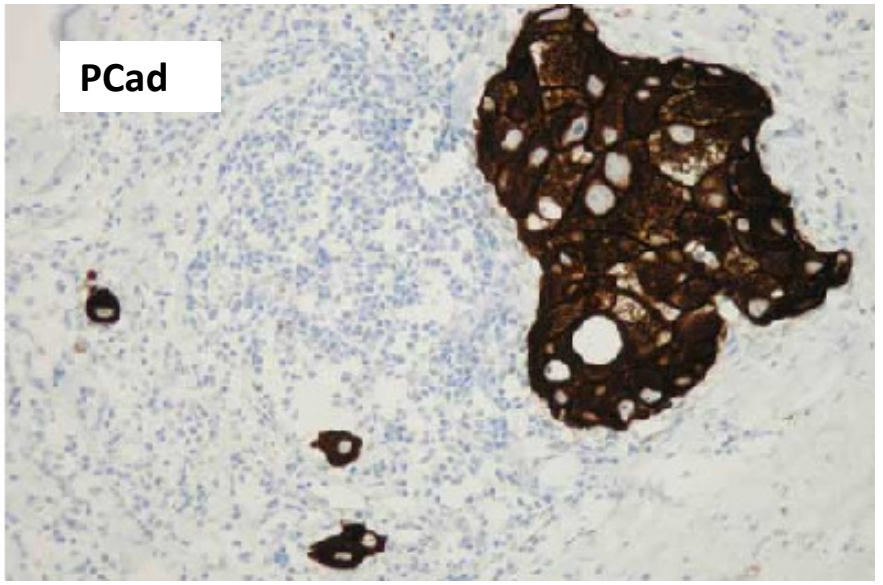



MGA 7 AMGA - *TP53* R175H
G T G G G G G C A G T G C C T C A C A A

Breast cancer diagnosis is morphological

Microinvasion

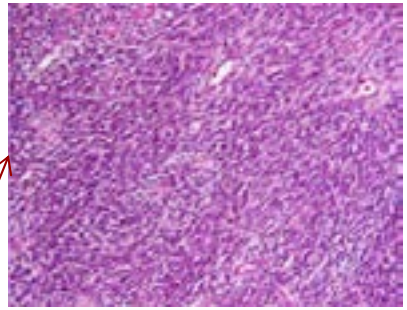




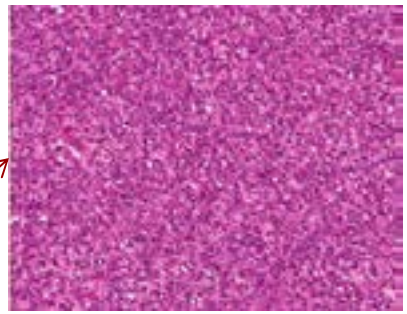
Molecular results without pathology can be messy...

Biopsy

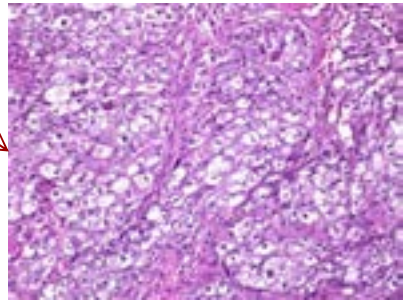
ETV6-NTRK3



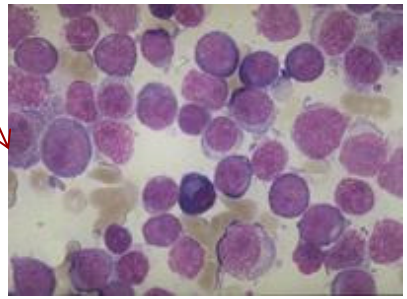
Congenital fibrosarcoma



Cellular mesoblastic nephroma

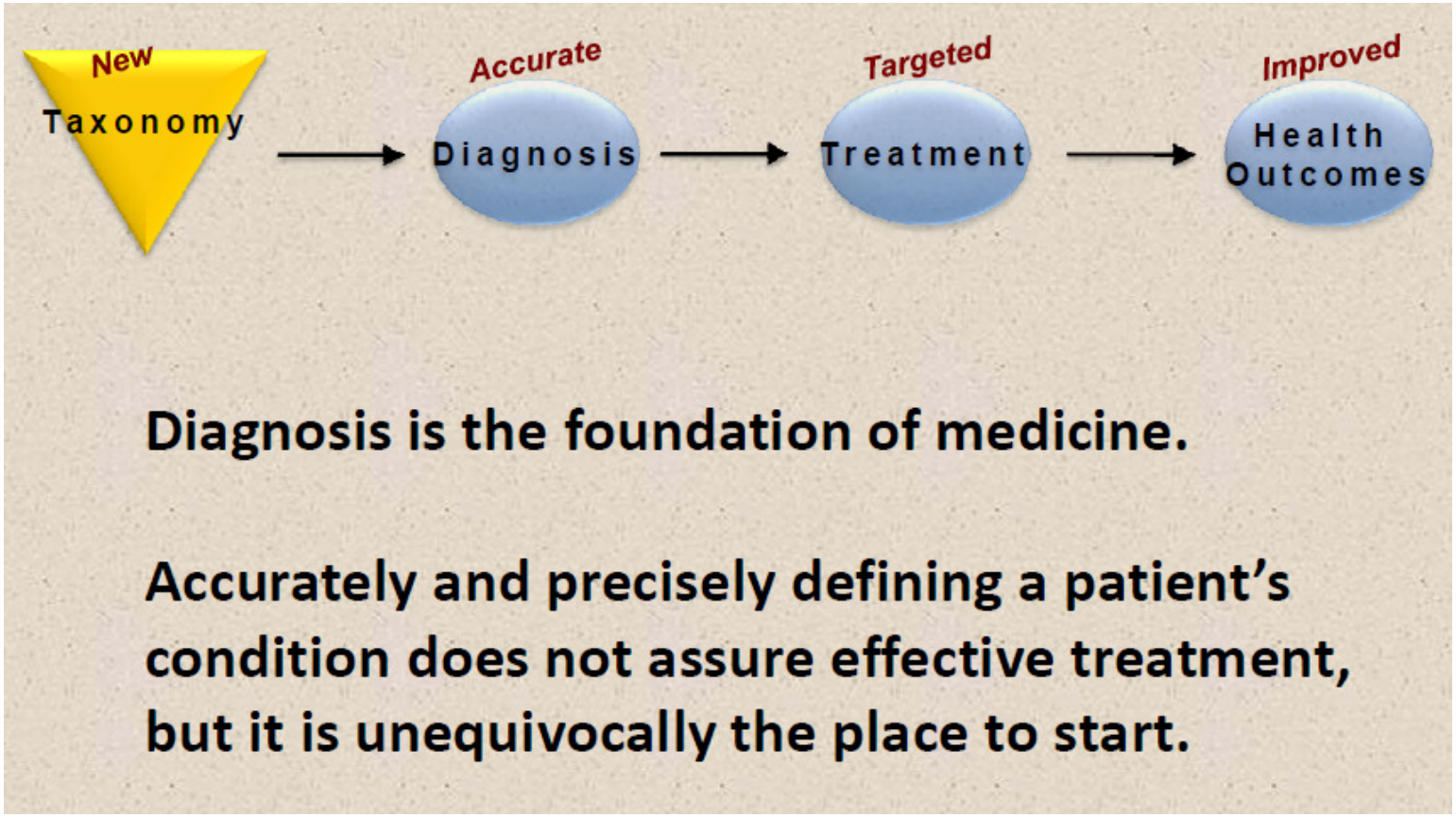


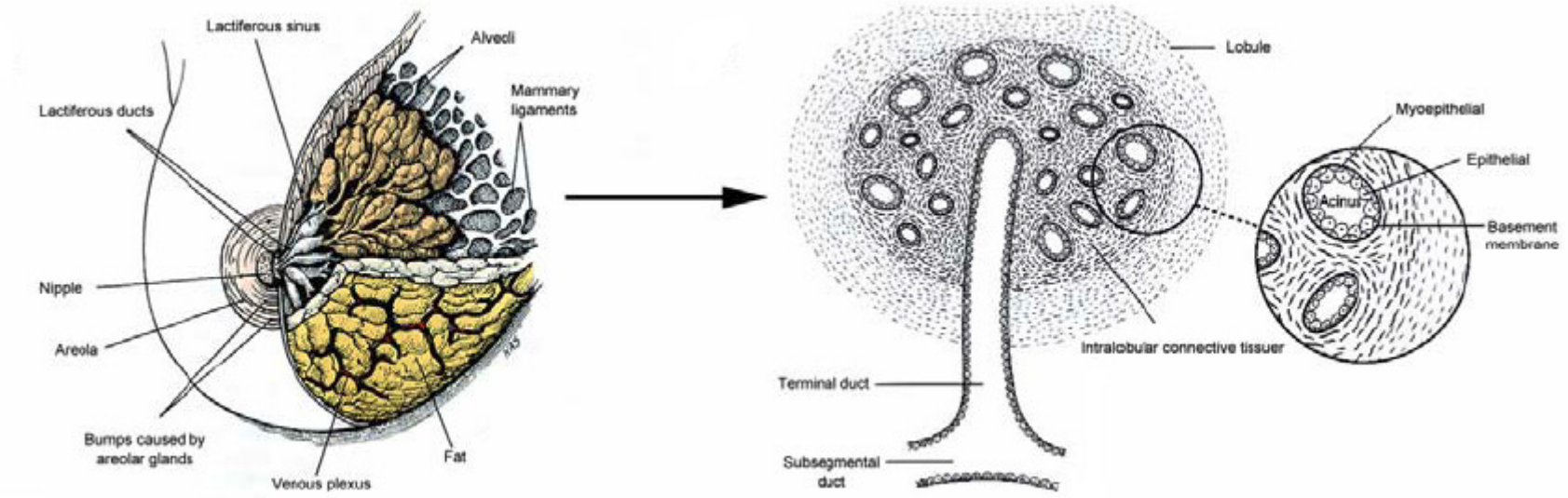
Secretory carcinoma

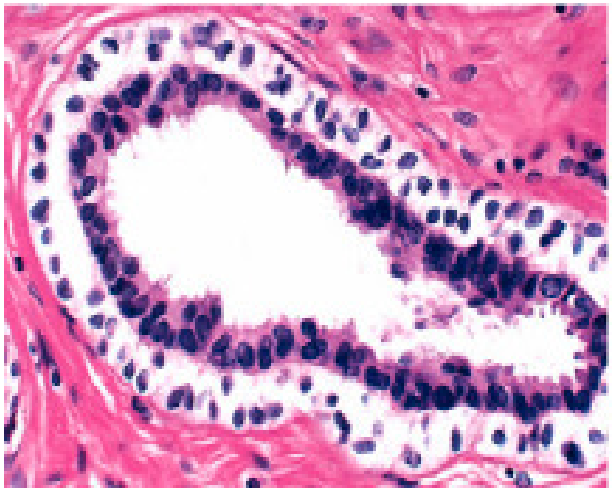


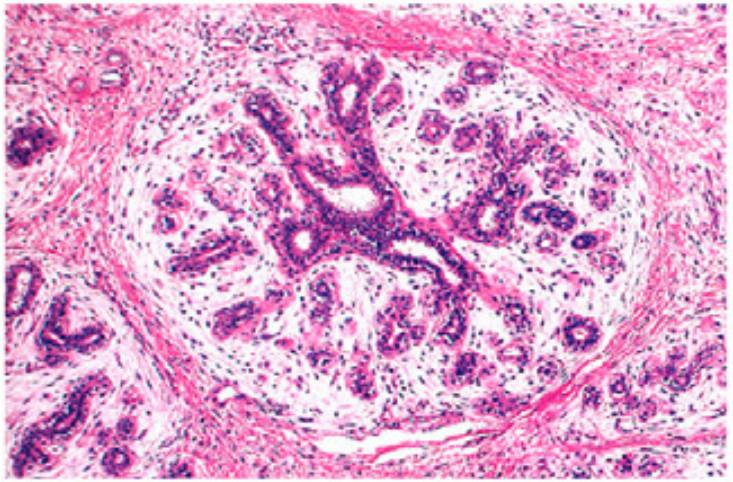
Acute myeloid leukaemia

Precision Medicine




Lactiferous sinus
Alveoli
Mammary ligaments
Lactiferous ducts
Nipple
Areola
Bumps caused by areolar glands
Venous plexus
Fat
Lobule
Myoepithelial
Epithelial
Acinus
Basement membrane
Intralobular connective tissue
Terminal duct
Subsegmental duct





Putative Model to explain Breast Cancer Molecular Signatures

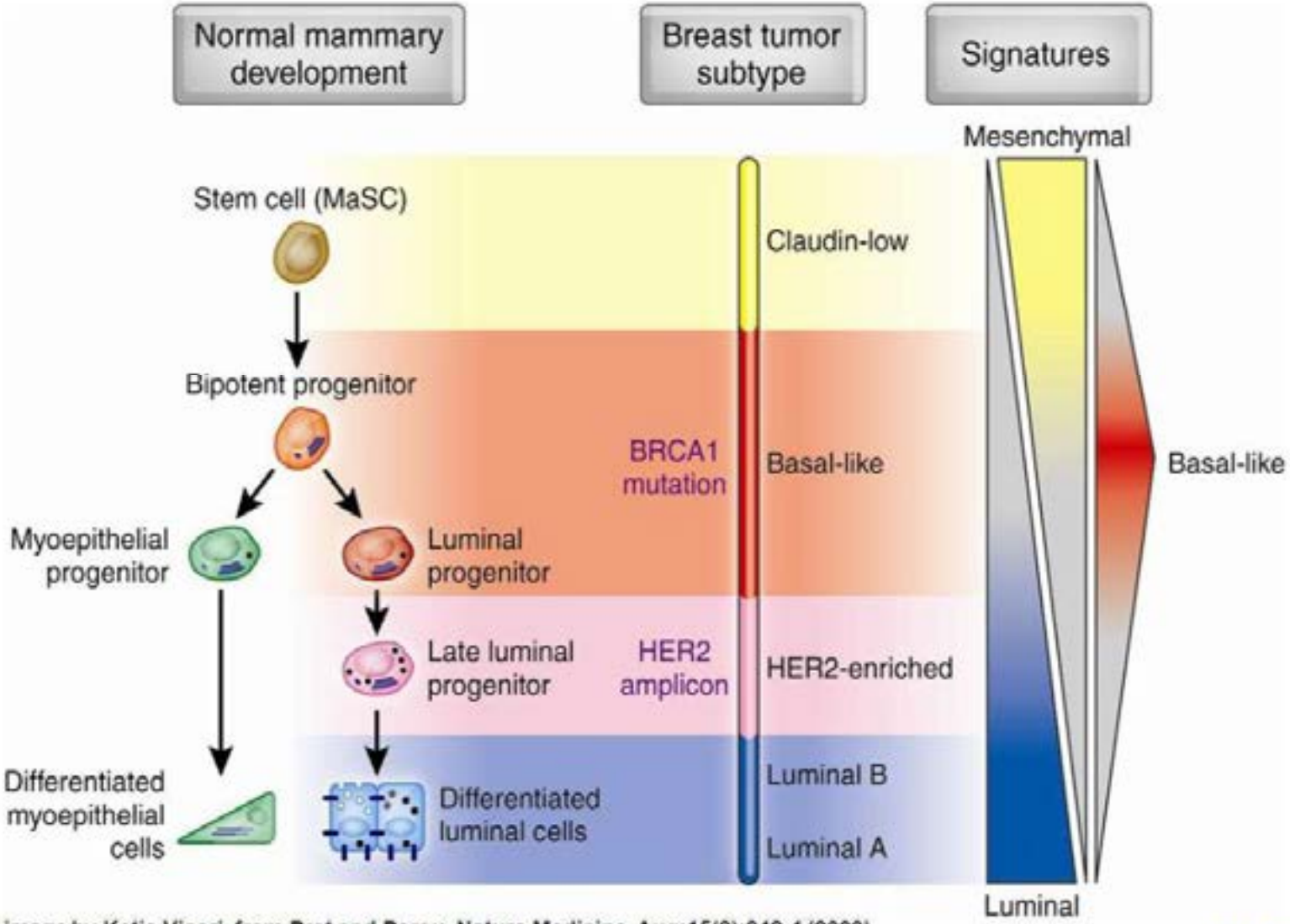
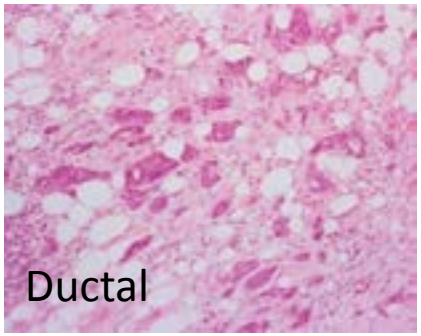
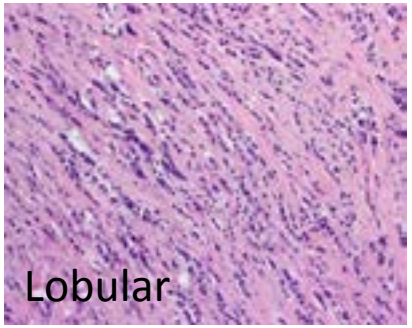


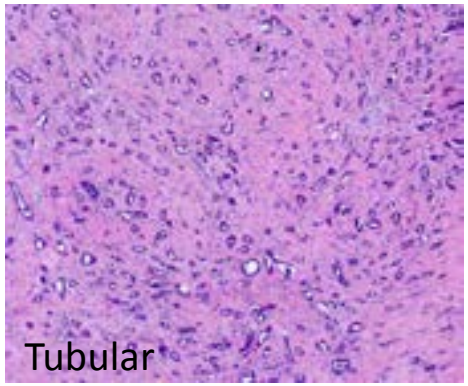
image by Katie Vicari, from Prat and Perou, Nature Medicine, Aug;15(8):842-4 (2009)

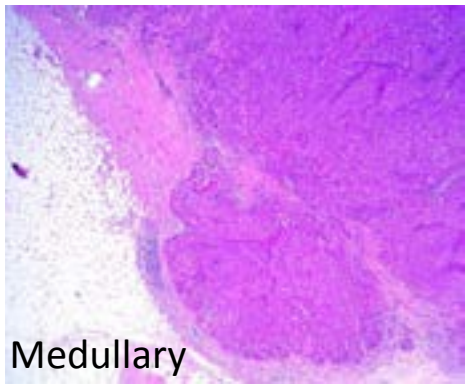
IPATIMUP

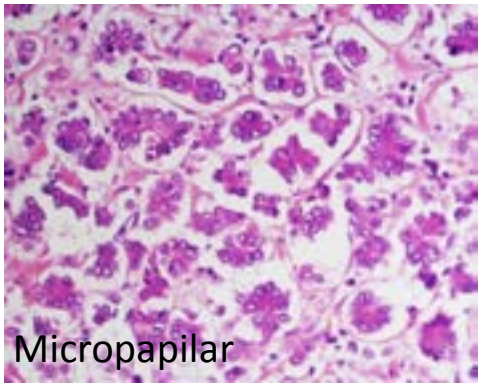
Histological types of breast carcinoma

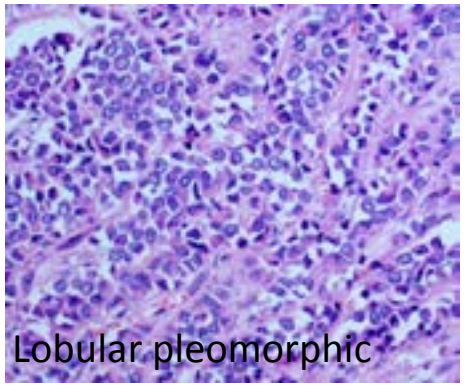


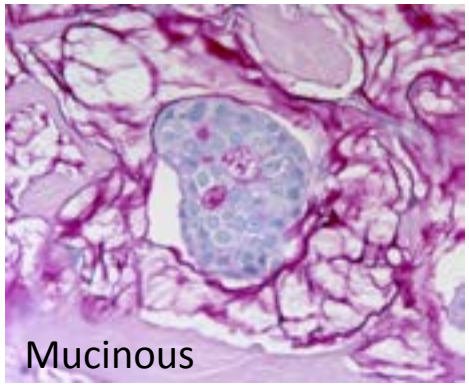


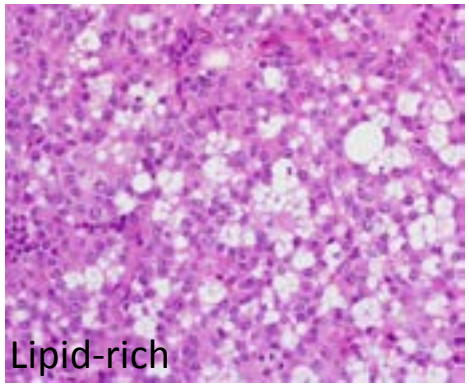


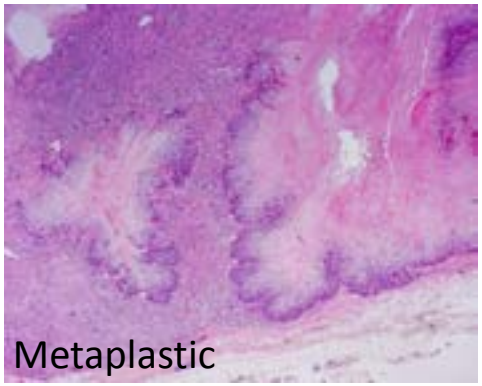












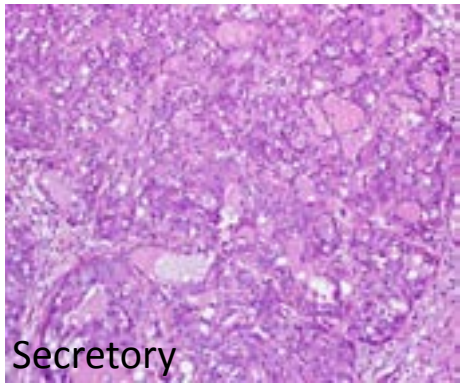
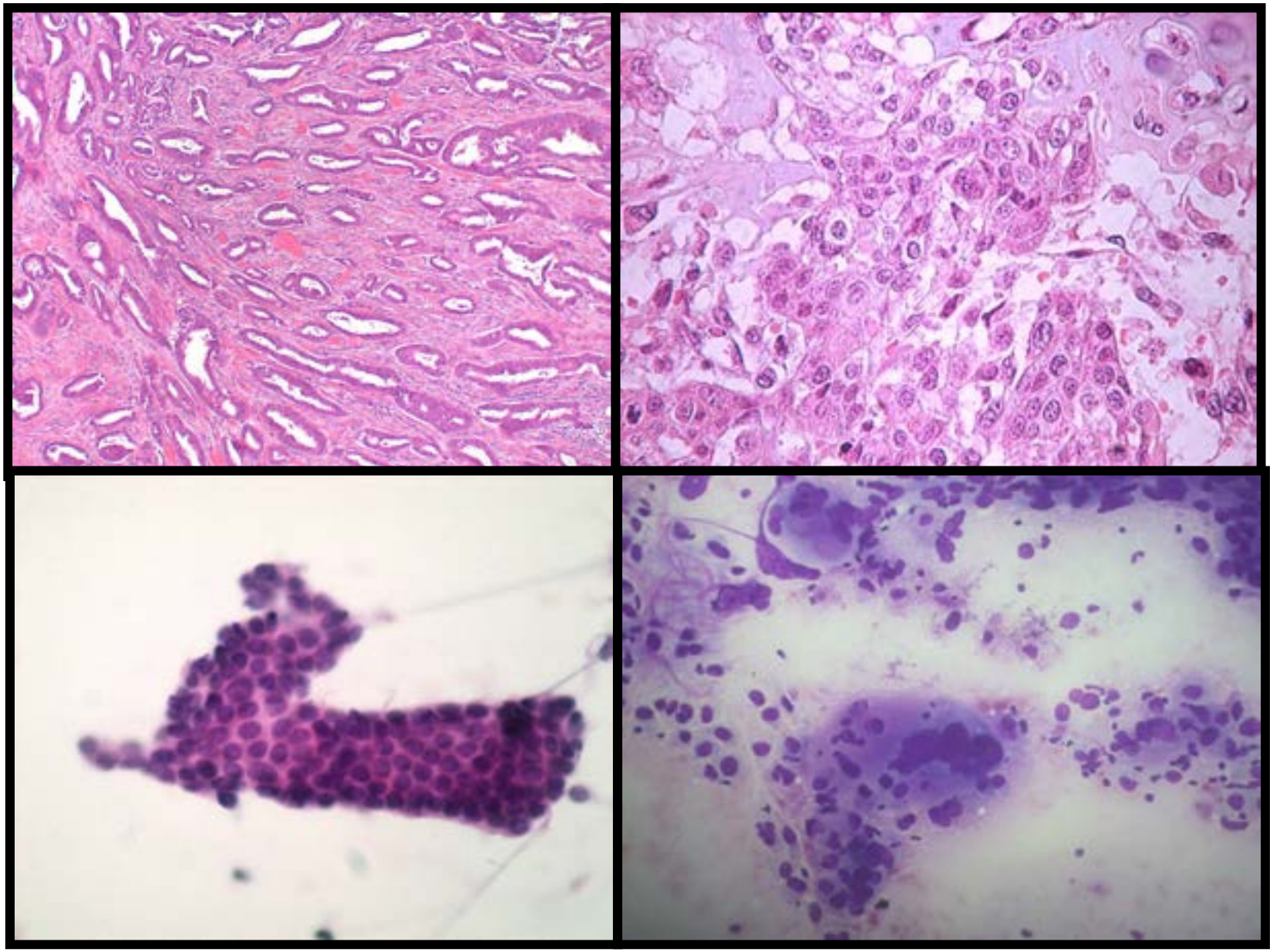


Table 1. Invasive breast carcinomas (without microinvasive carcinoma and invasive papillary lesions)

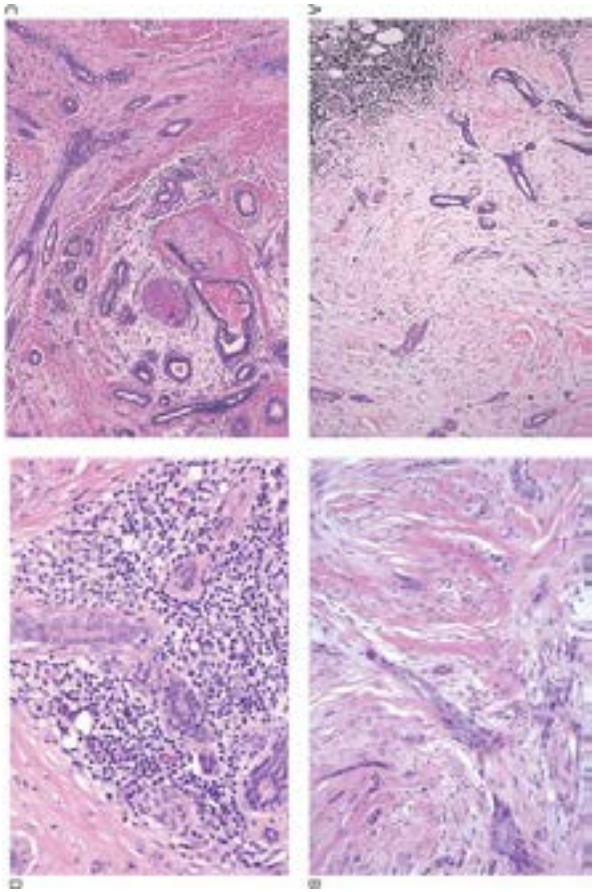
Type	Classification
Invasive carcinoma of no special type (NST)	8500/3
Pleomorphic carcinoma	8522/3
Carcinoma with osteoclast-like stromal giant cells	8035/3
Carcinoma with choriocarcinomatous features	
Carcinoma with melanotic features	
Invasive lobular carcinoma	8520/3
Classic lobular carcinoma	
Solid lobular carcinoma	
Alveolar lobular carcinoma	
Pleomorphic lobular carcinoma	
Tubulolobular carcinoma	
Mixed lobular carcinoma	
Tubular carcinoma	8211/3
Cribriform carcinoma	8201/3
Mucinous carcinoma	8480/3
Carcinoma with medullary features	
Medullary carcinoma	8510/3
Atypical medullary carcinoma	8513/3
Invasive carcinoma NST with medullary features	8500/3
Carcinoma with apocrine differentiation	
Carcinoma with signet-ring-cell differentiation	
Invasive micropapillary carcinoma	8507/3
Metaplastic carcinoma of no special type	8575/3
Low-grade adenosquamous carcinoma	8570/3
Fibromatosis-like metaplastic carcinoma	8572/3
Squamous cell carcinoma	8070/3
Spindle cell carcinoma	8032/3
Metaplastic carcinoma with mesenchymal differentiation	
Chondroid differentiation	8571/3
Osseous differentiation	8571/3
Other types of mesenchymal differentiation	8575/3
Mixed metaplastic carcinoma	8575/3
Myoepithelial carcinoma	8982/3
Epithelial-myoepithelial tumors	
Adenomyoepithelioma with carcinoma	8983/3
Adenoid cystic carcinoma	8200/3
Rare types	
Carcinoma with neuroendocrine features	
Neuroendocrine tumor, well-differentiated	8246/3
Neuroendocrine carcinoma poorly differentiated (small cell carcinoma)	8041/3
Carcinoma with neuroendocrine differentiation	8574/3
Secretory carcinoma	8502/3
Invasive papillary carcinoma	8503/3
Acinic cell carcinoma	8550/3
Mucoepidermoid carcinoma	8430/3
Polymorphous carcinoma	8525/3
Oncocytic carcinoma	8290/3
Lipid-rich carcinoma	8314/3
Glycogen-rich clear cell carcinoma	8315/3
Sebaceous carcinoma	8410/3

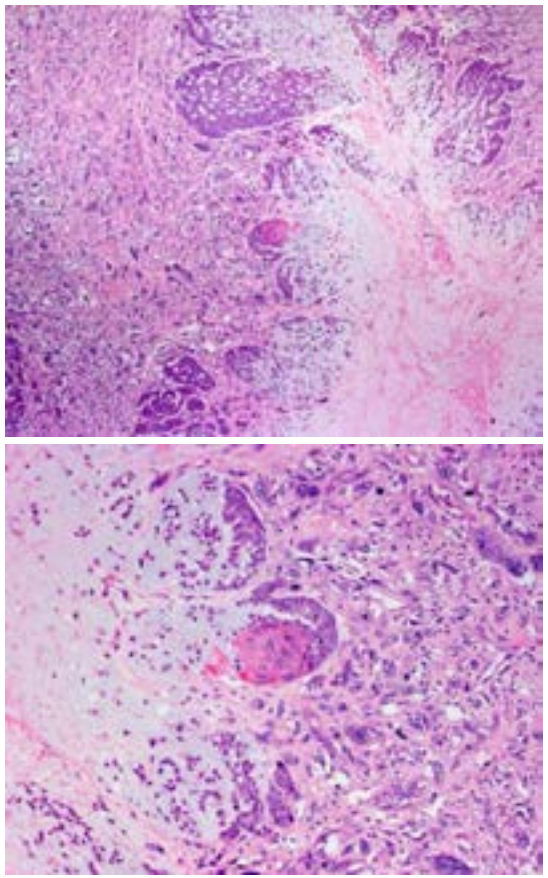
Type	Classification
Precursor lesions	
Ductal carcinoma in situ	8500/2
Lobular neoplasia	
Lobular carcinoma in situ	
Classic lobular carcinoma in situ	8520/2
Pleomorphic lobular carcinoma in situ	8519/2*
Atypical lobular hyperplasia	
Intraductal proliferative lesions	
Usual ductal hyperplasia	
Columnar cell lesions including flat epithelial atypia	
Atypical ductal hyperplasia	
Papillary lesions	
Intraductal papilloma	8503/0
Intraductal papilloma with atypical hyperplasia	8503/0
Intraductal papilloma with ductal carcinoma in situ	8503/2*
Intraductal papilloma with lobular carcinoma in situ	8520/2
Intraductal papillary carcinoma	8503/2
Encapsulated papillary carcinoma	8504/2
Encapsulated papillary carcinoma with invasion	8504/3
Solid papillary carcinoma	
In situ	8509/2
Invasive	8509/3

Breast cancer classification and prognosis

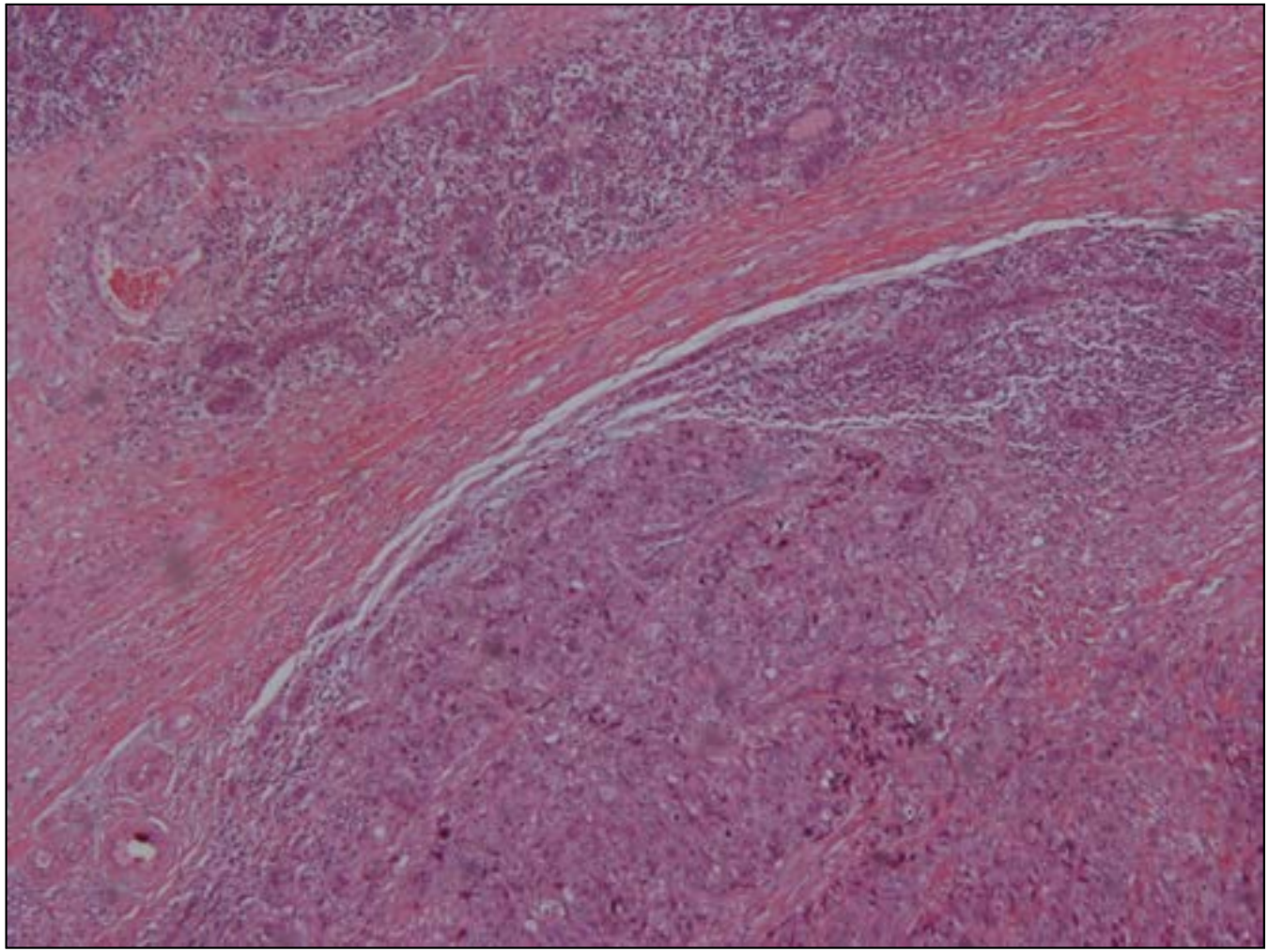


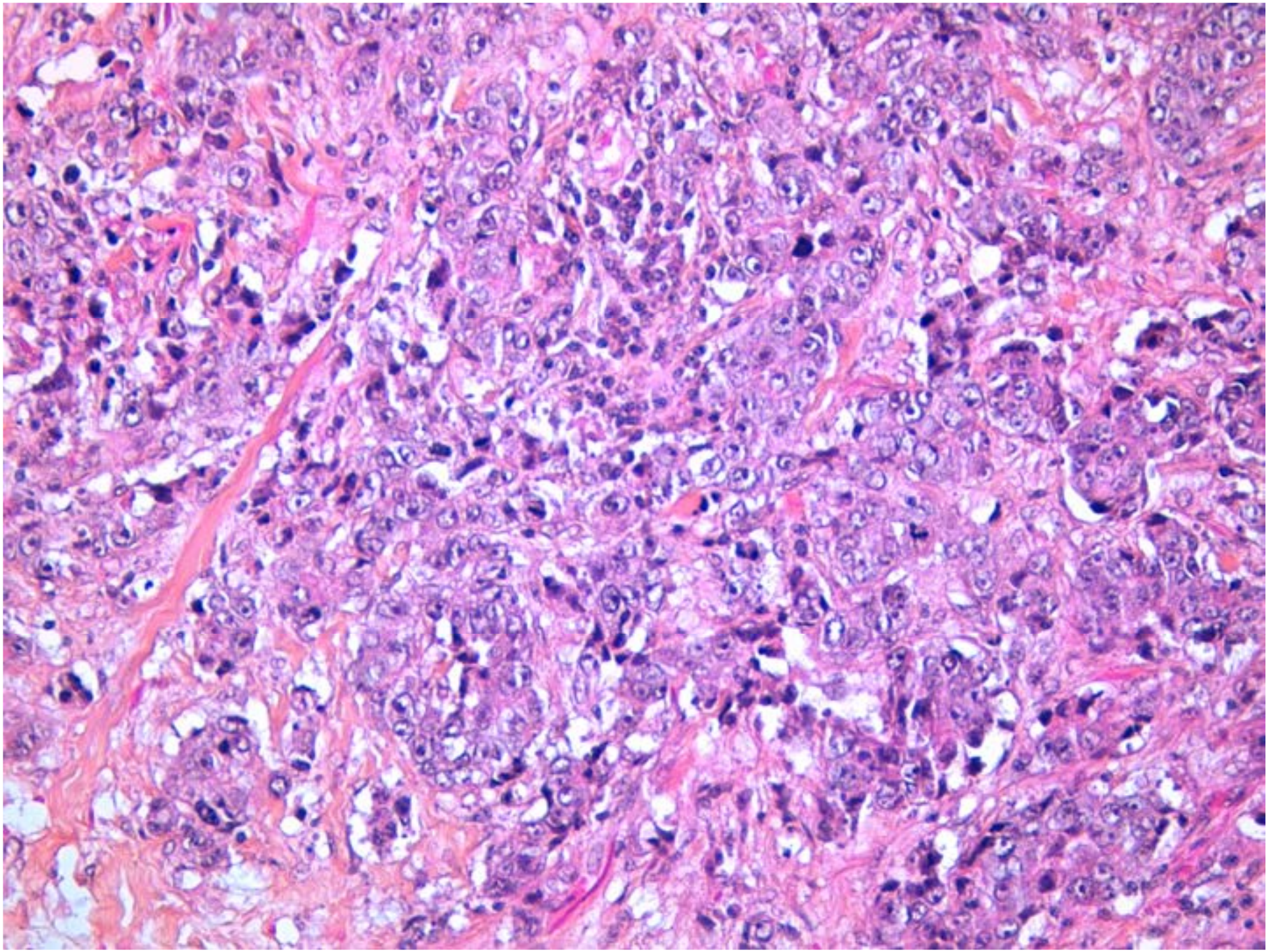
Breast cancer classification and prognosis



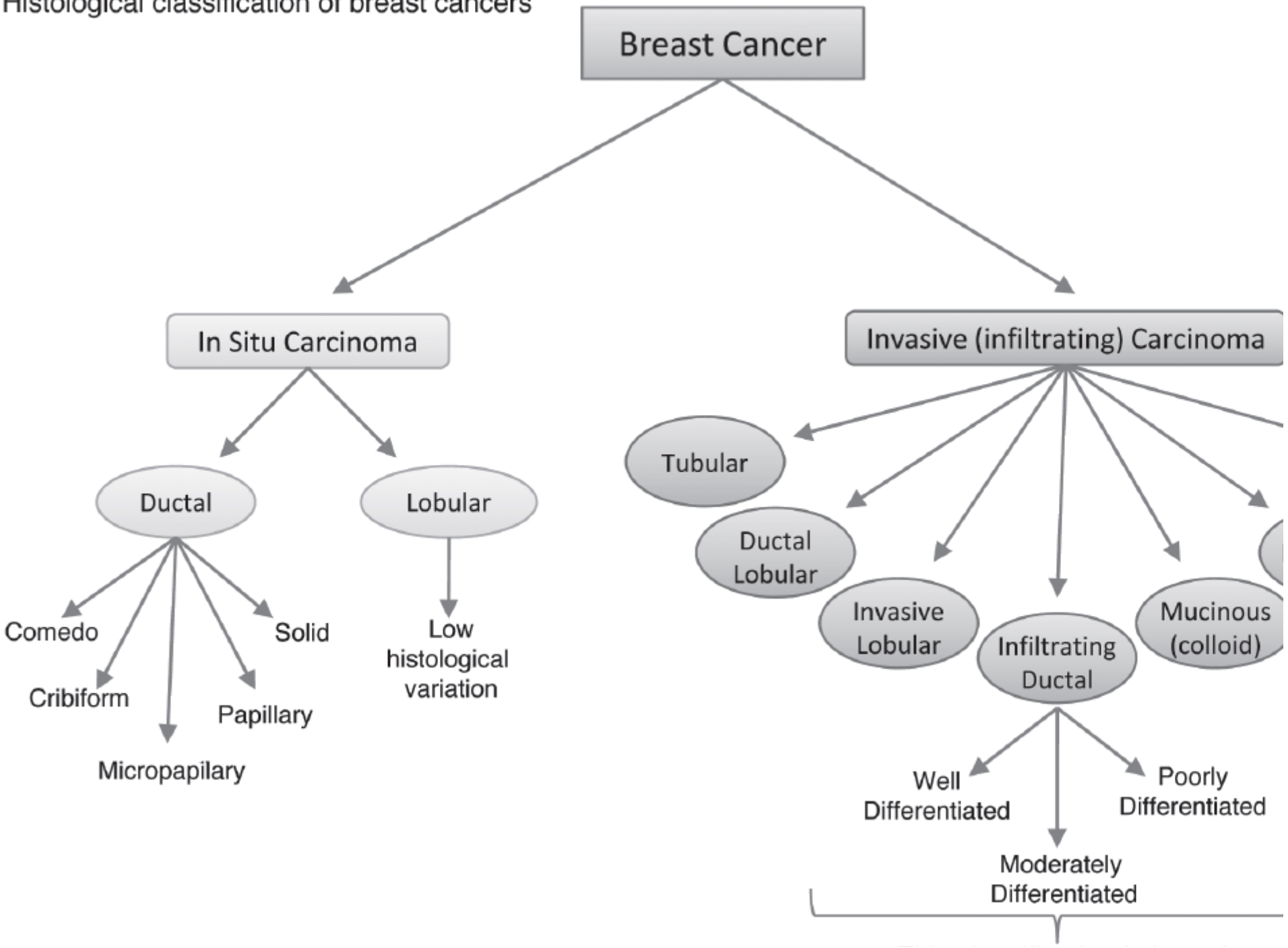


Breast cancer classification and prognosis

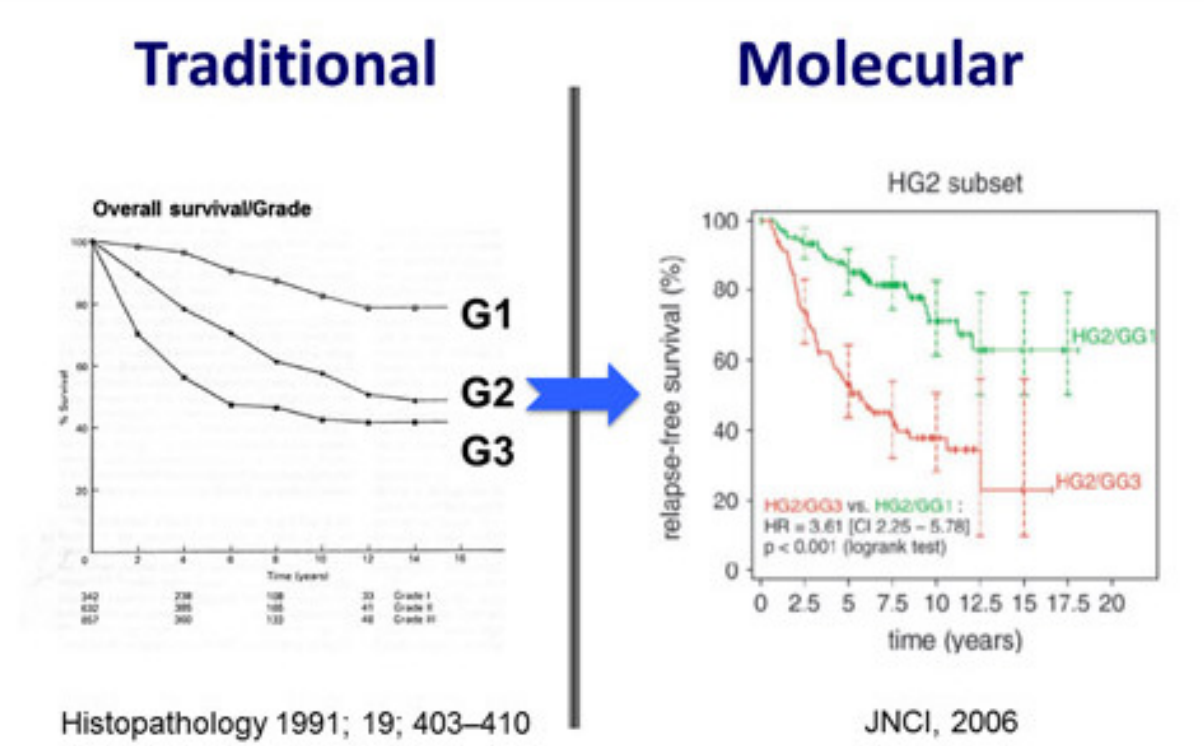




Histological classification of breast cancers

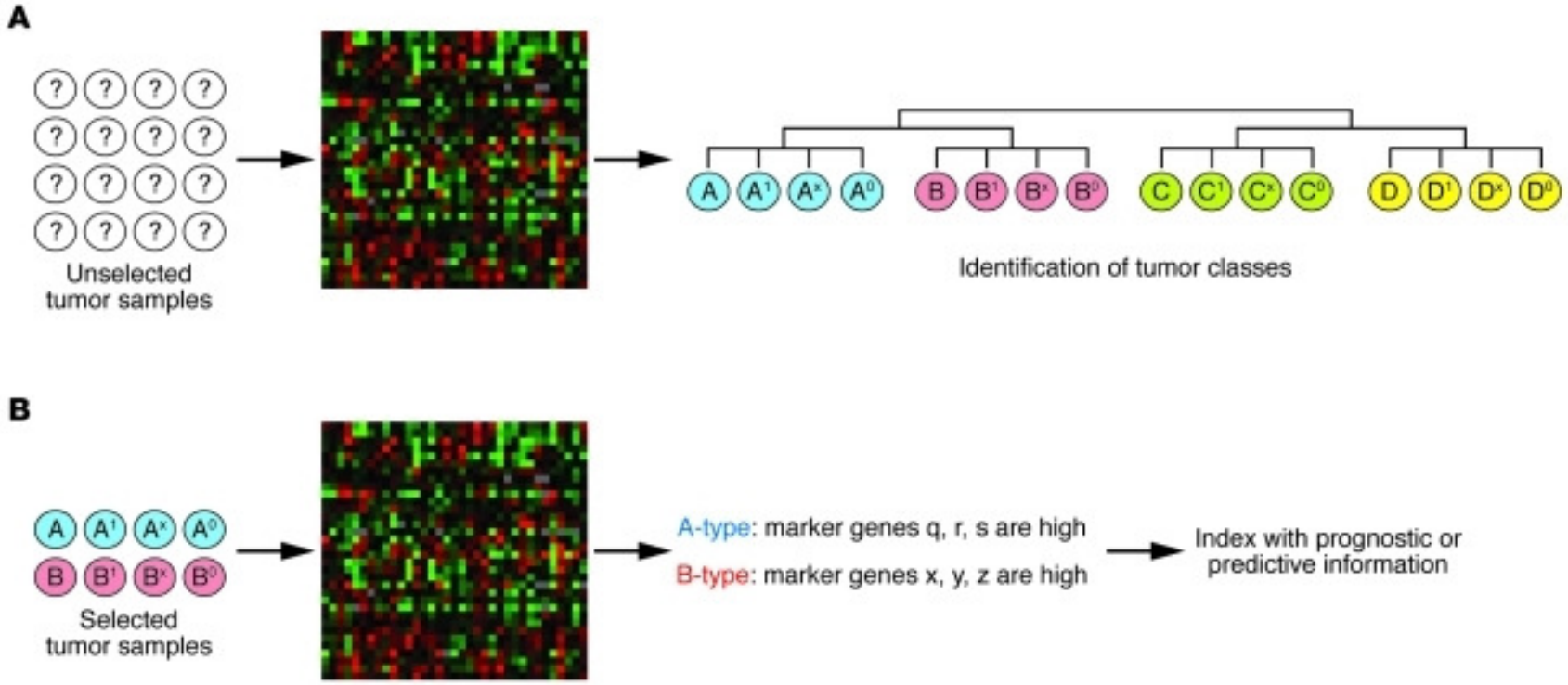


Breast cancer classification and prognosis



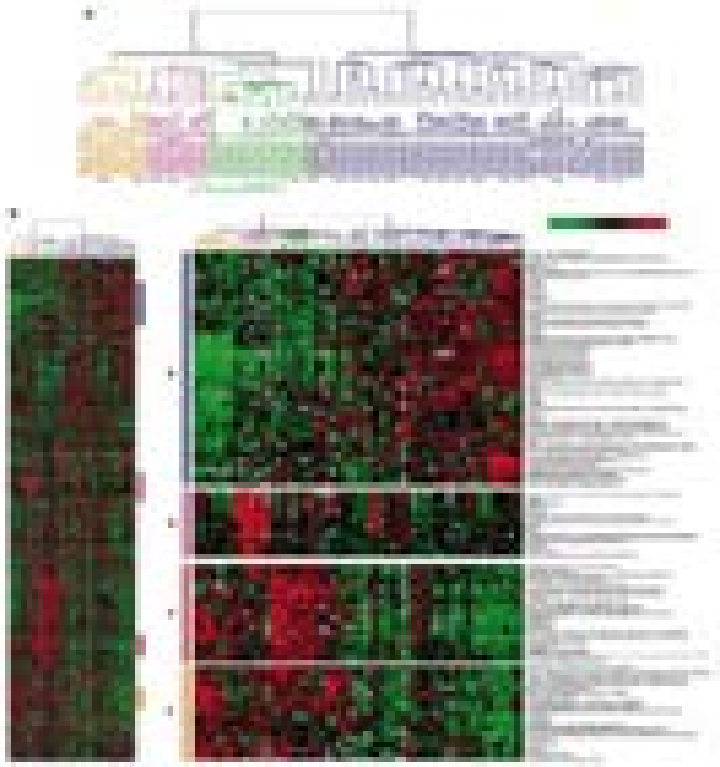
Oncotype DX and PAM 50 approximately split this group in half when classified as low risk RS (56%) and Luminal A (63%) approximately.

Gene-expression profiling (microarray-based)



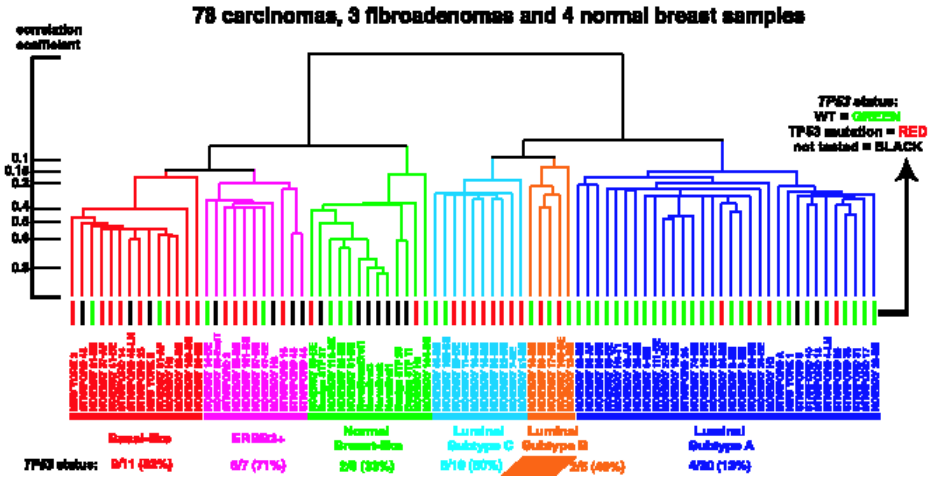
letters to nature

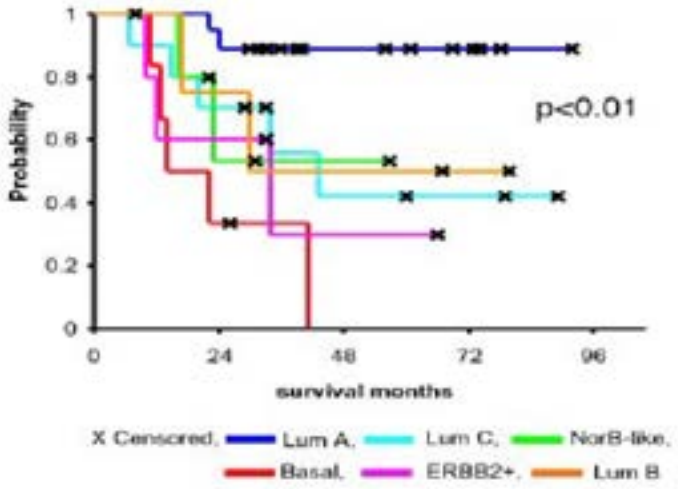
Molecular portraits of human breast tumours



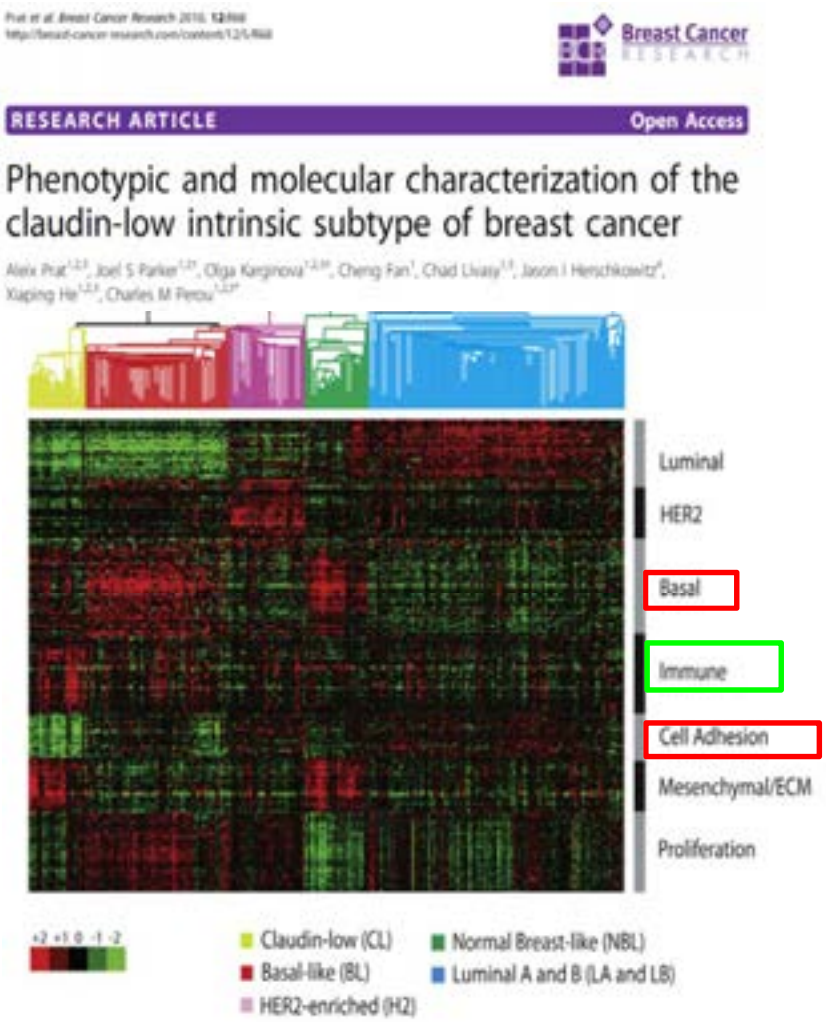
Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications

Therese Sorlie[a,b,*], Charles M. Perou[a,d], Robert Tibshirani[e], Turid Aas[f], Stephanie Geisler[g], Hilde Johnsen[g], Trevor Hastie[e], Michael B. Eisen[h], Matt van de Rijn[i], Stefanie S. Jeffrey[j], Thor Thorsen[k], Hanne Quist[g], John C. Matese[c], Patrick O. Brown[m], David Botstein[c], Per Eystein Lonning[g], and Anne-Lise Borresen-Dale[a,n]





Molecular Classification of Breast Cancer



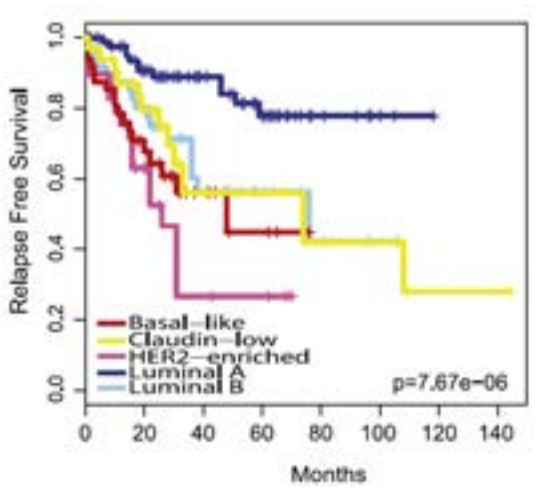
LUMINAL A: ER+/PgR+/HER2-

LUMINAL B: ER+/PgR+/HER2+and or Ki67+

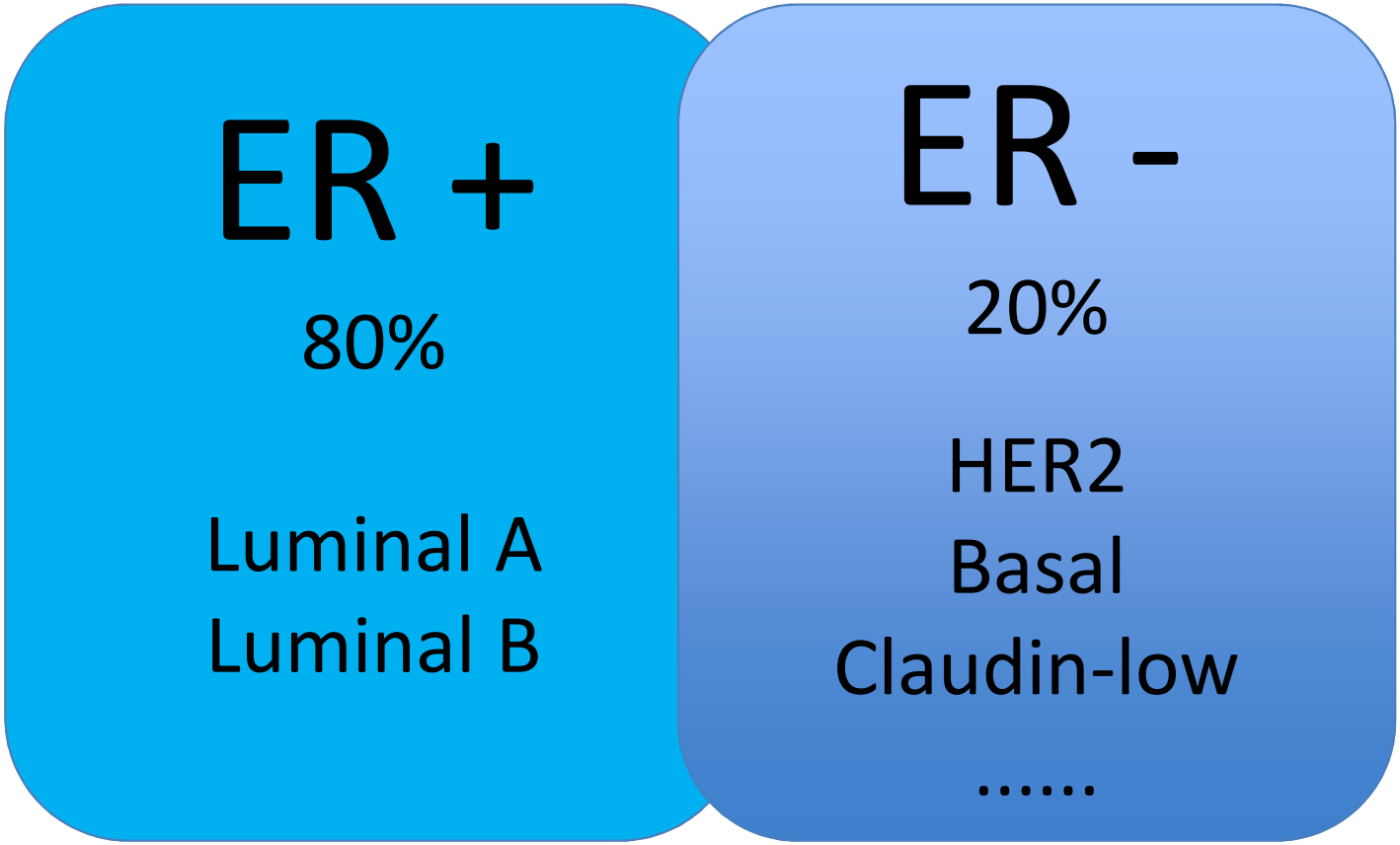
HER-OE: ER-/PgR-/HER2+

BASAL-LIKE:ER-/PgR-/HER2-/Basal Markers

CLAUDIN-LOW:ER-/Pg-/HER2-/Claudin^{low}



Molecular Classification of Breast Cancer



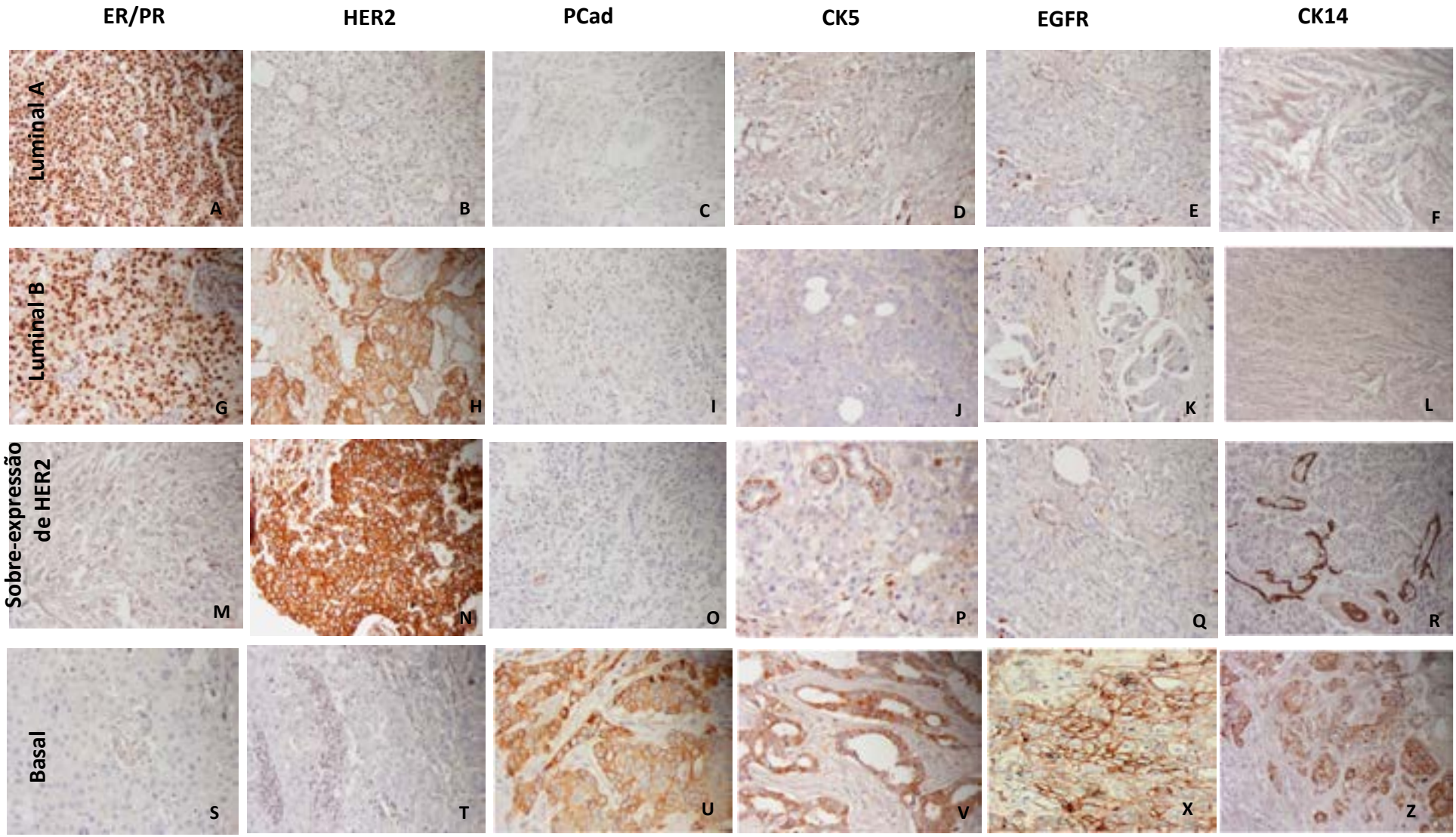
Virchows Arch (2005) 447: 688–694
DOI 10.1007/s00428-005-0019-7

ORIGINAL ARTICLE

Irina Matos · Rozany Dufloth · Marcelo Alvarenga · Luiz Carlos Zeferino · Fernando Schmitt

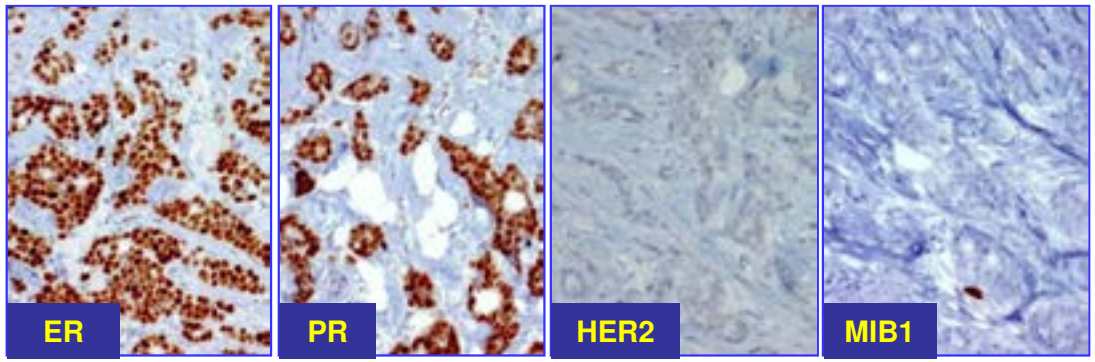
p63, cytokeratin 5, and P-cadherin: three molecular markers to distinguish basal phenotype in breast carcinomas

IHC TRANSLATION OF MOLECULAR CLASSIFICATION

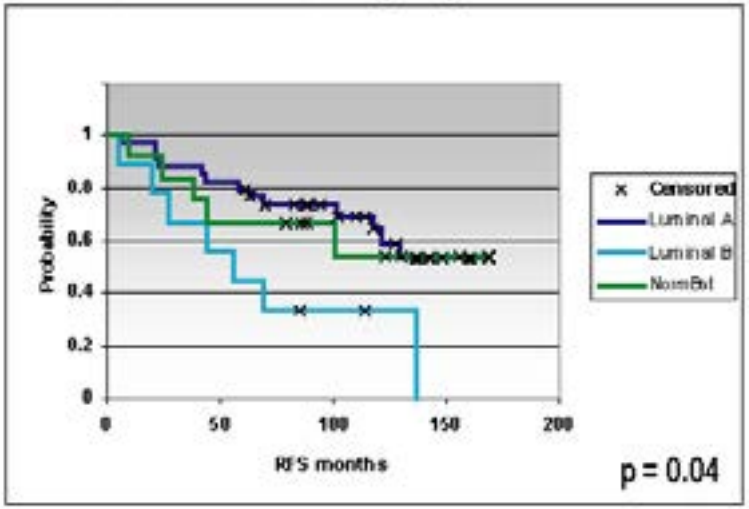


ER Positive Breast Cancer

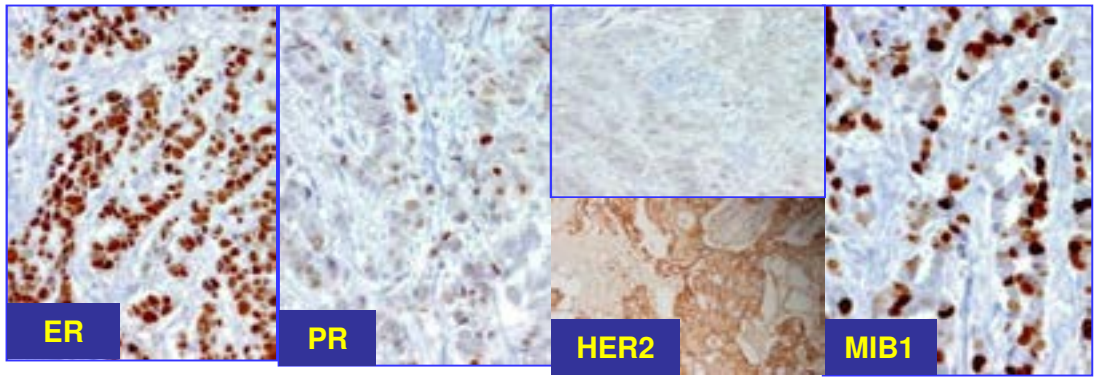
Luminal A

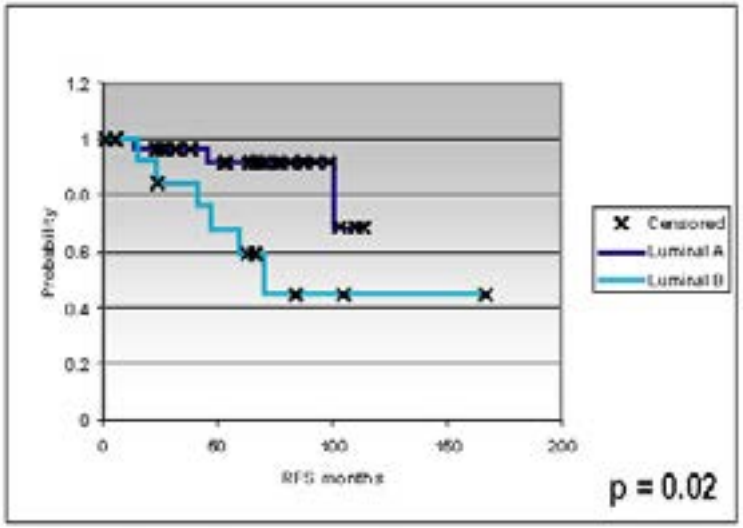


60 Sample ER+ Tamoxifen-Treated Test Set
Ma et al., Cancer Cell 5, 1-10 (2004).



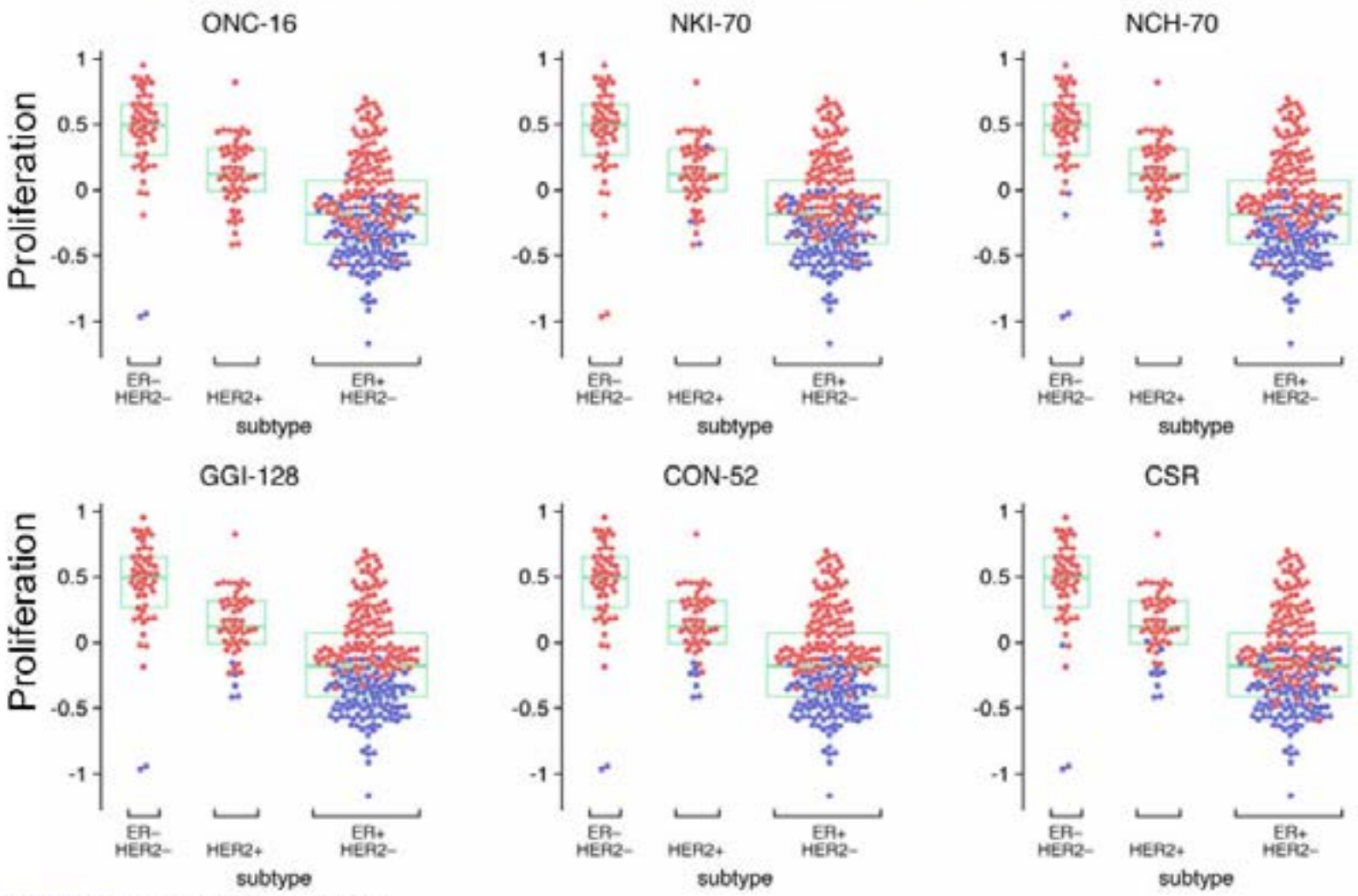
Luminal B





45 Tamoxifen Treated Test Set #2
Chang et al., PNAS 102, 3738-43 (2005) + UNC

Meta-Analysis – Gene signatures



Blue dots: good prognosis

Red dots: poor prognosis

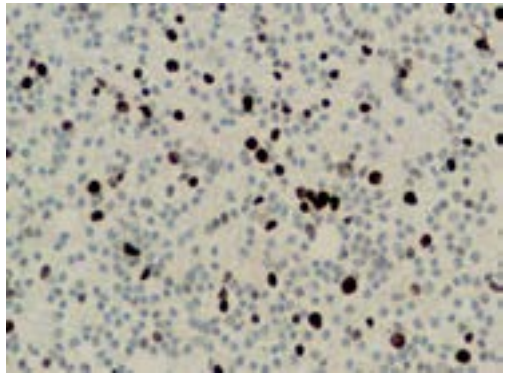
Wirapati et al. Breast Cancer Res 2008;10:R65

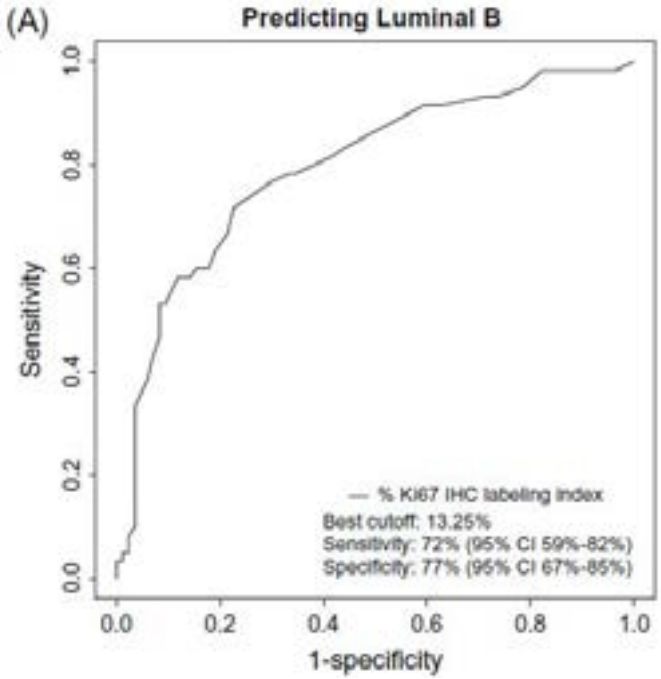
Intrinsic subtype	Clinico-pathologic surrogate definition	Notes
Luminal A	**'Luminal A-like'** *all of:* ER and PgR positive HER2 negative Ki-67 'low'[a] Recurrence risk 'low' based on multi-gene-expression assay (if available)[b]	The cut-point between 'high' and 'low' values for Ki-67 varies between 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 (HER2 positive)'** ER positive HER2 over-expressed or amplified Any Ki-67 Any PgR	'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)'.
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.

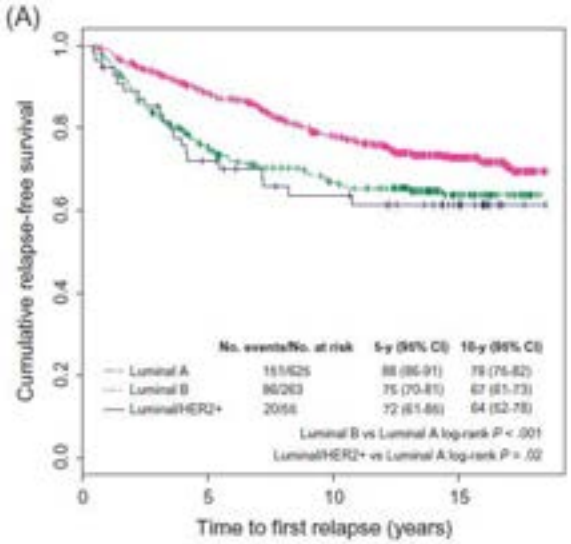
Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer

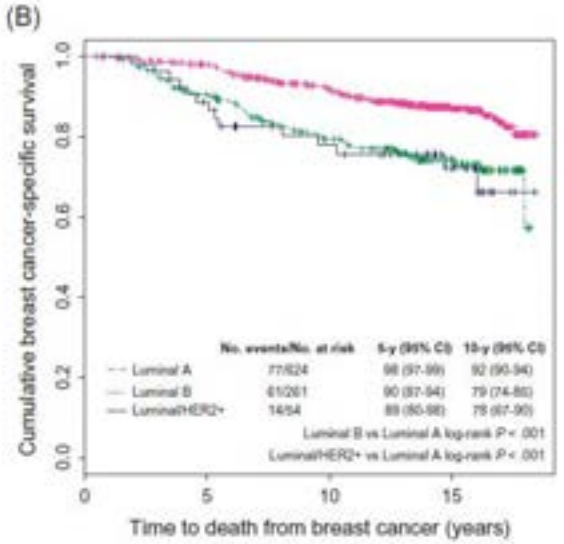
Maggie C. U. Cheang, Stephen K. Chia, David Voduc, Dongxia Gao, Samuel Leung, Jacqueline Snider, Mark Watson, Sherri Davies, Philip S. Bernard, Joel S. Parker, Charles M. Perou, Matthew J. Ellis, Torsten O. Nielsen

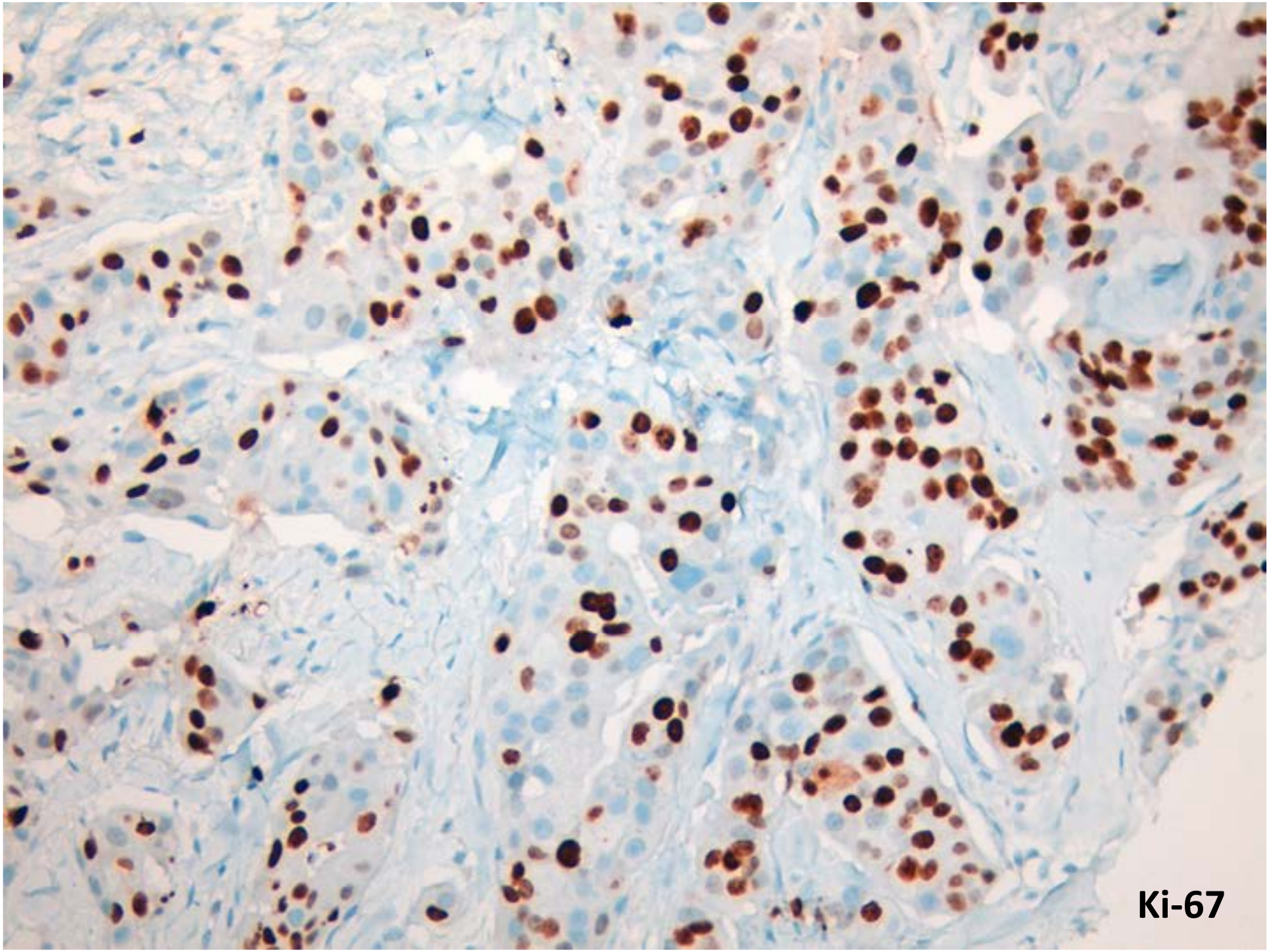
J Natl Cancer Inst 2009;101:736–750







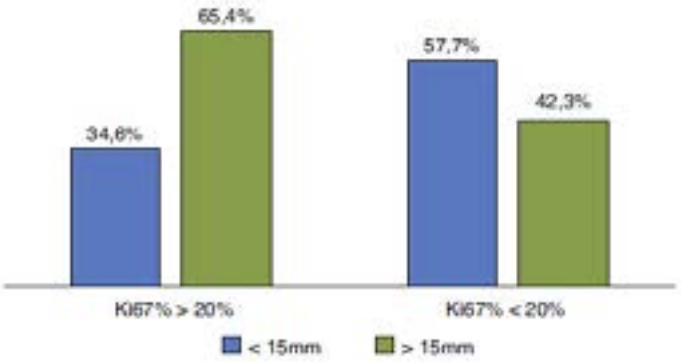


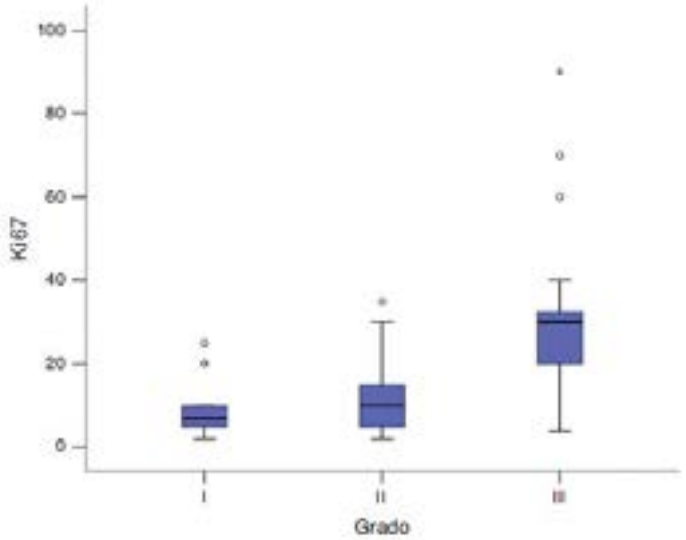

Ki-67

St Gallen Conference 2015

Distinction between Luminal A-like and Luminal B-like (HER2 neg) can be:

- Der
- If us



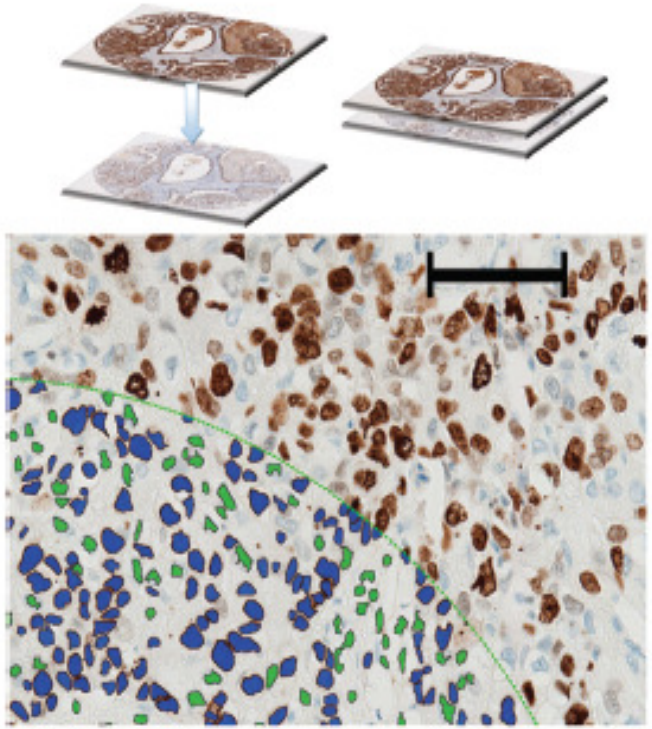


- Only appropriately determined by multi-gene classifiers? No 66.7%
- Subtype need not be determined since it can be replaced by risk socres derived from multi-gene tests: No 59.5%

MODERN PATHOLOGY (2016), 1–12

Digital image analysis outperforms manual biomarker assessment in breast cancer

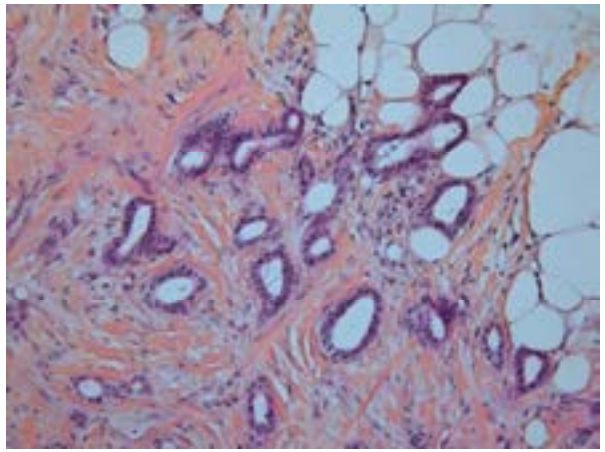
Gustav Stålhammar[1,2], Nelson Fuentes Martinez[1,3], Michael Lippert[4], Nicholas P Tobin[5], Ida Mølholm[4,6], Lorand Kis[7], Gustaf Rosin[1], Mattias Rantalainen[8], Lars Pedersen[4], Jonas Bergh[1,5,9], Michael Grunkin[4] and Johan Hartman[1,5,7]



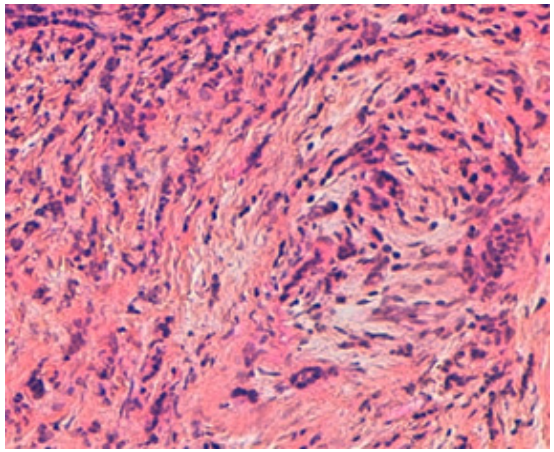
Ki67 scoring method	*Sensitivity for PAM50 Luminal B vs A*	*Specificity for PAM50 Luminal B vs A*
DIA invasive margin		
Cutoff ≥ 20%	84%	78%
Cutoff ≥ 20.2%*	82%	79%
DIA hot spot		
Cutoff ≥ 20%	90%	65%
Cutoff ≥ 25.2%*	86%	77%
DIA average		
Cutoff ≥ 20%	60%	90%
Cutoff ≥ 15.5%*	80%	83%
Manual		
Cutoff ≥ 20%	75%	70%
Cutoff ≥ 22.5%*	74%	75%

Do we still need a morphological classification?

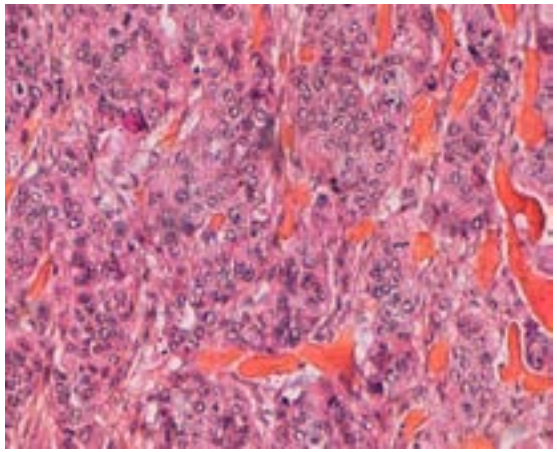
"ER-positive" breast carcinomas



Tubular carcinoma

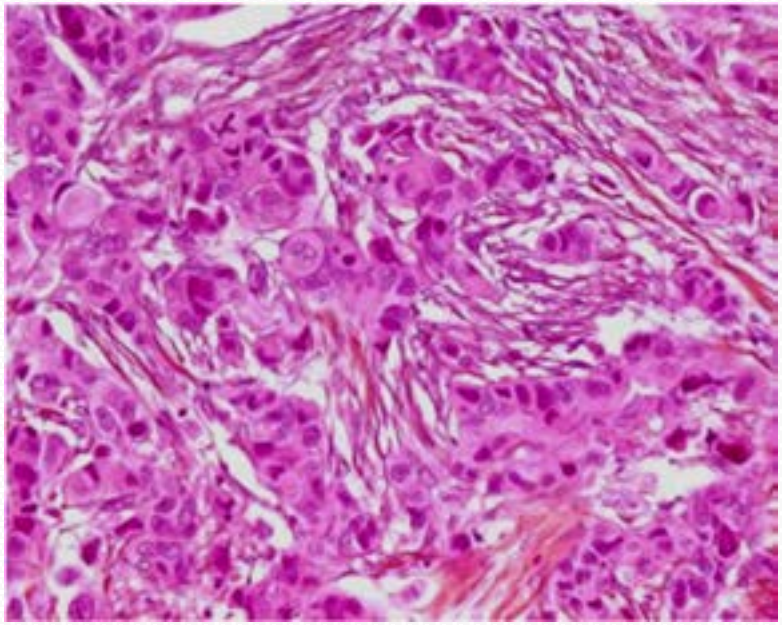


Lobular carcinoma

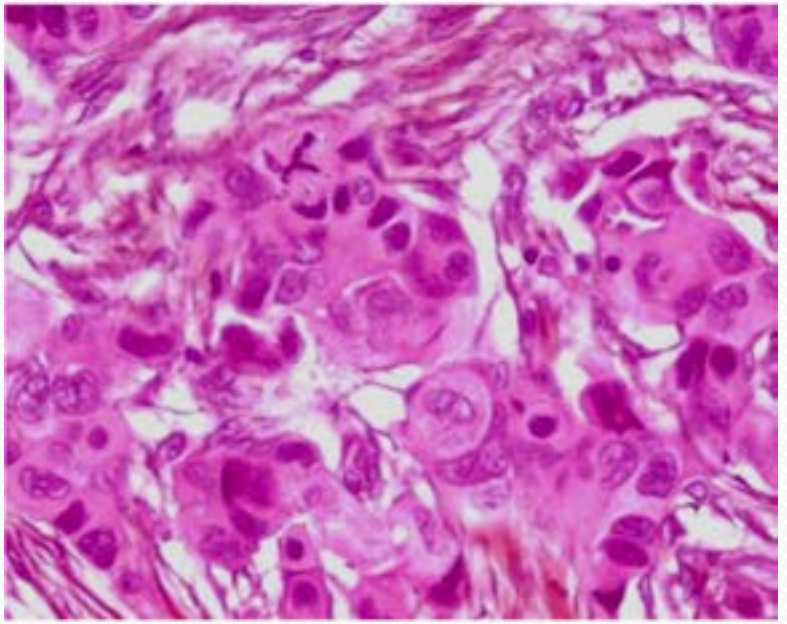


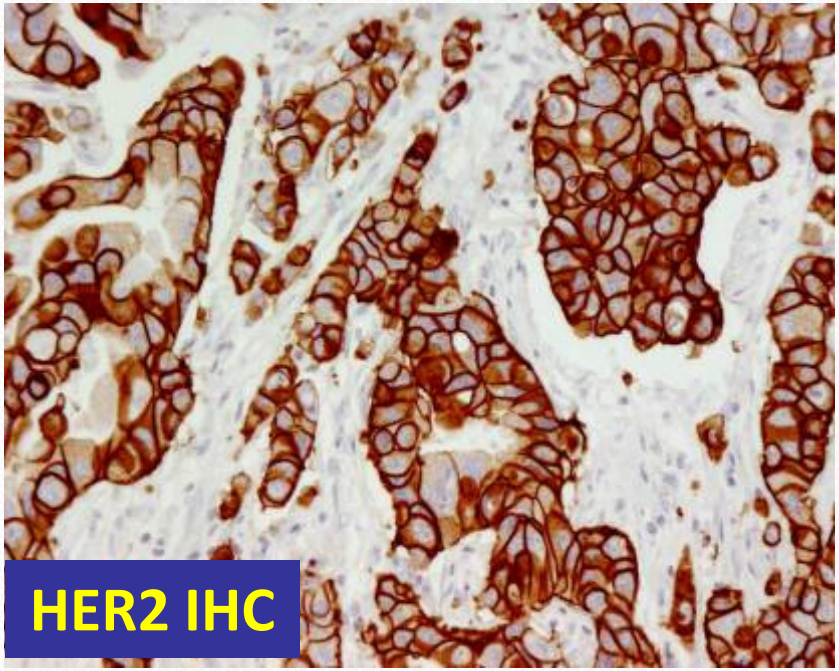
IDC Grade III

HER 2- OE BREAST CANCER

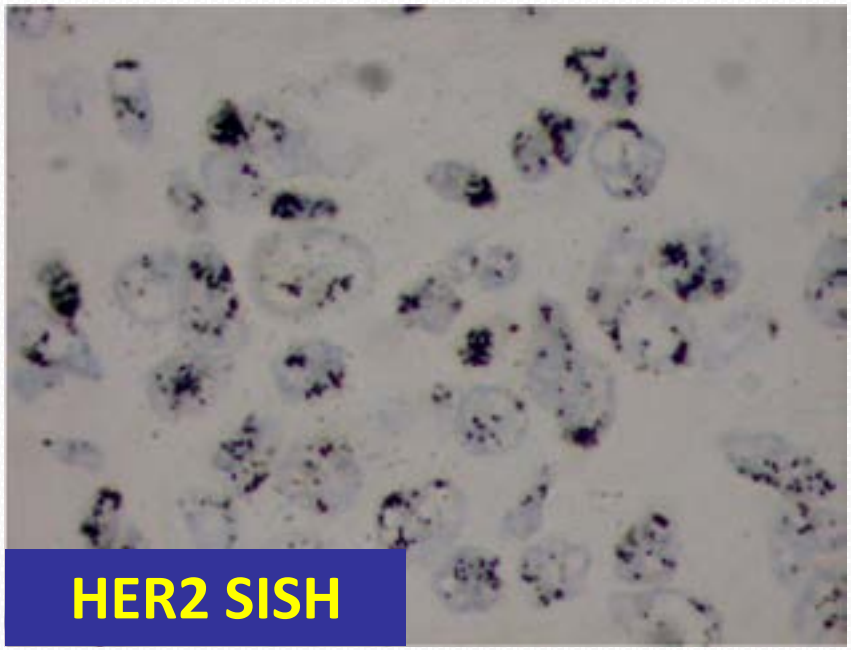


HER 2 +



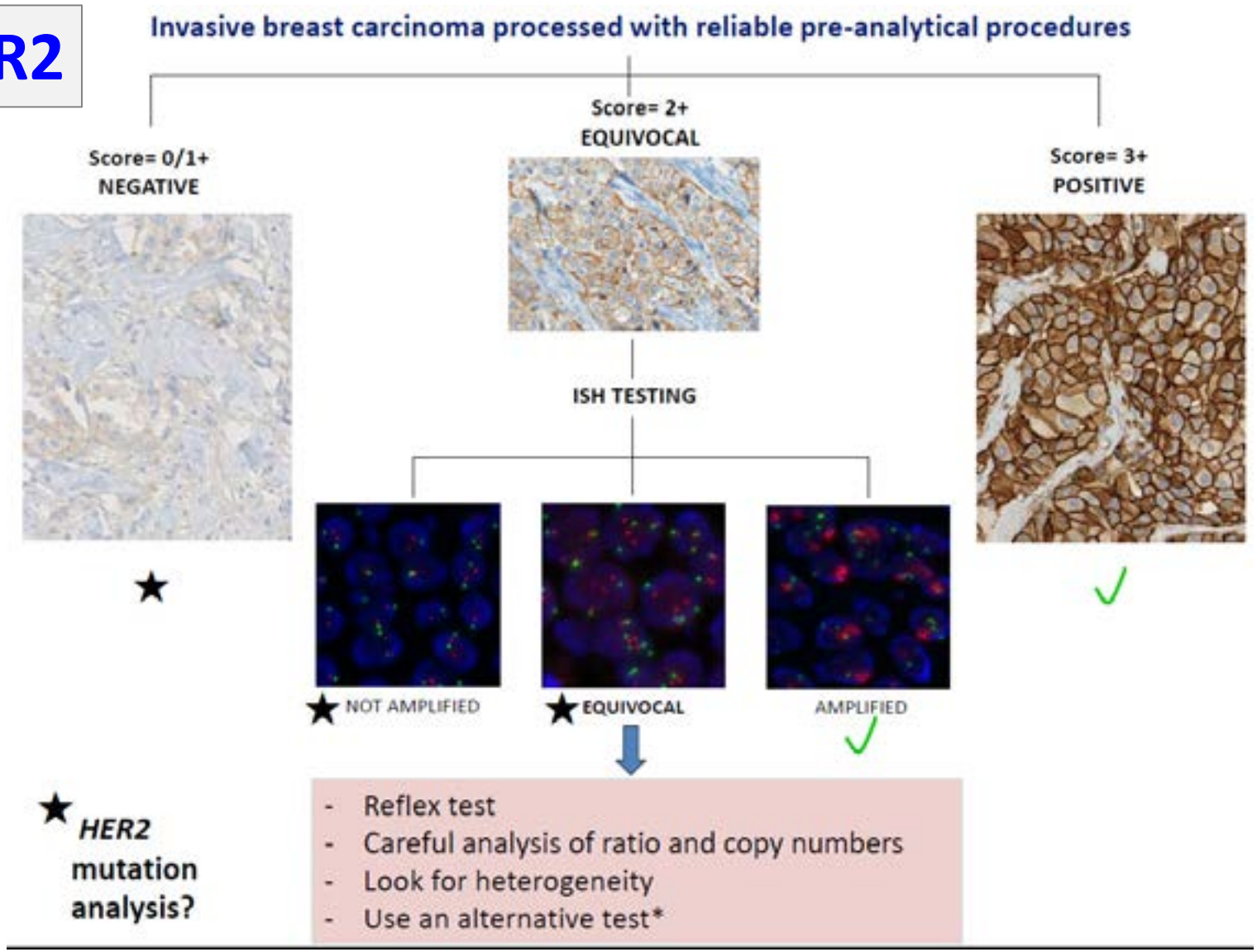


HER2 IHC

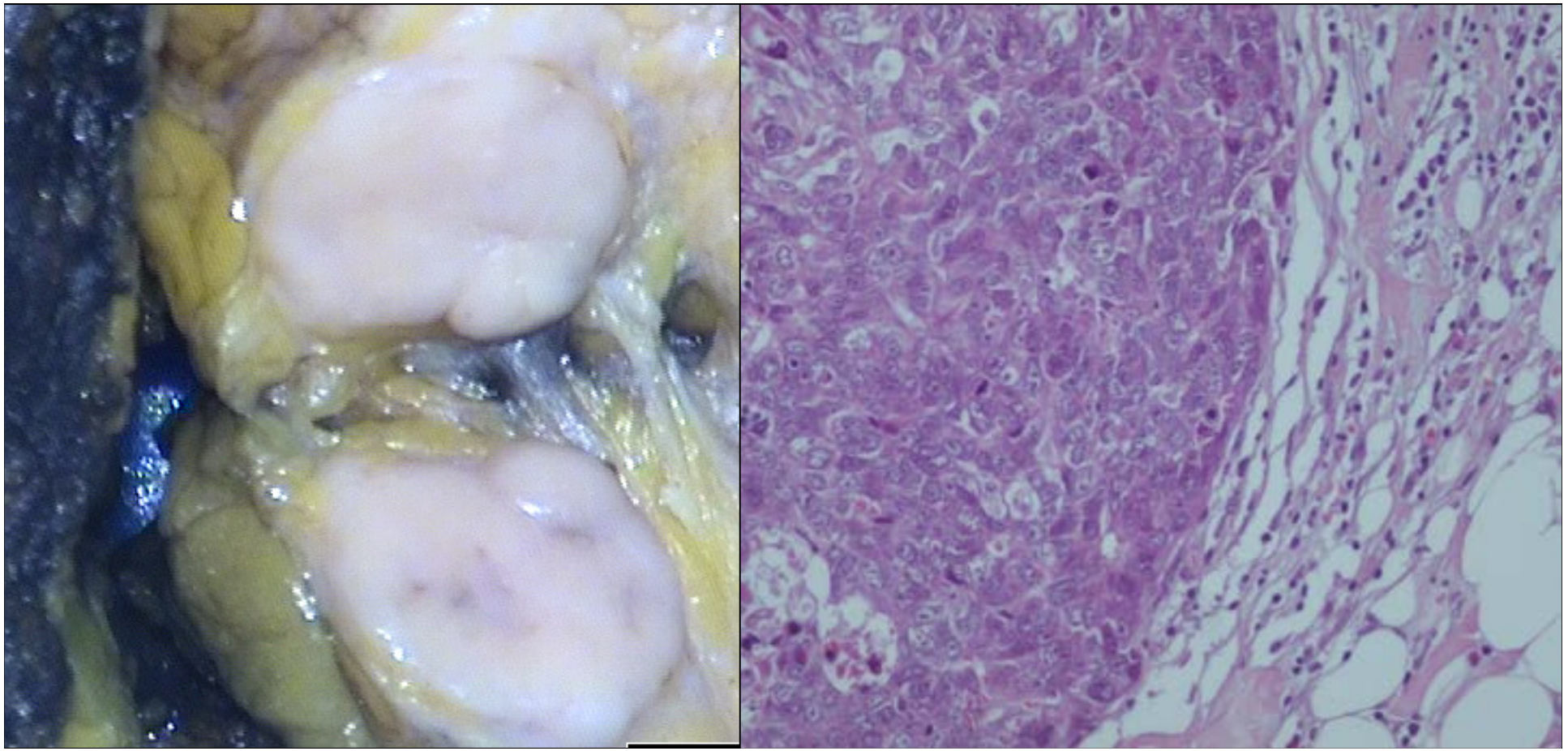


HER2 SISH

HER2


Invasive breast carcinoma processed with reliable pre-analytical procedures
Score= 0/1+
NEGATIVE
Score= 2+
EQUIVOCAL
Score= 3+
POSITIVE
ISH TESTING
NOT AMPLIFIED
EQUIVOCAL
AMPLIFIED
HER2 mutation analysis?
- Reflex test
- Careful analysis of ratio and copy numbers
- Look for heterogeneity
- Use an alternative test*

1994



The American Journal of Surgical Pathology 24(2): 197–202, 2000

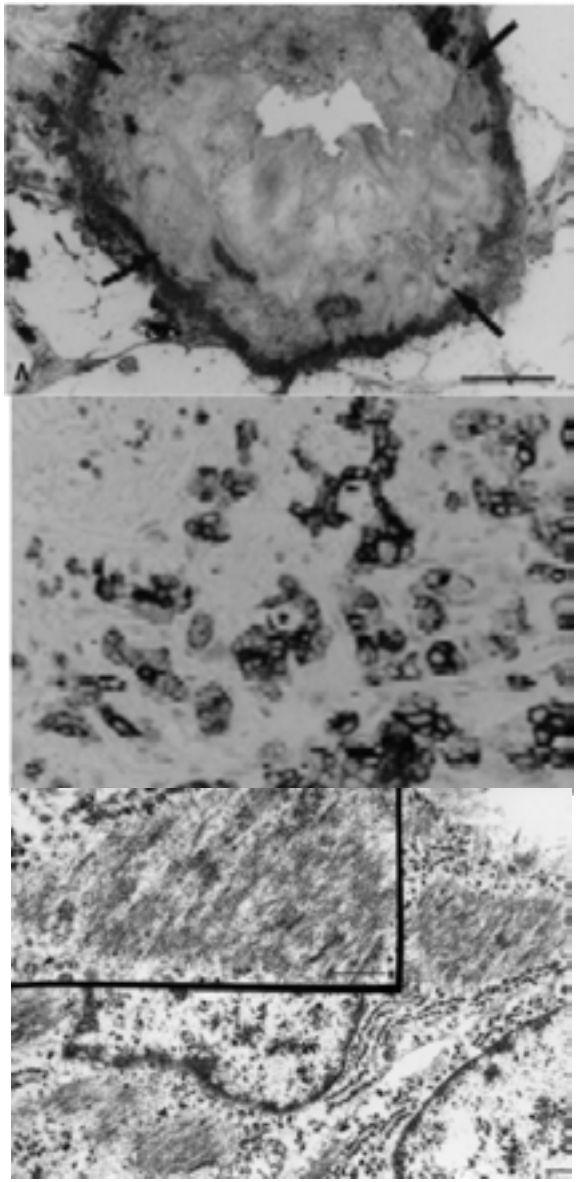
Large, Central Acellular Zones Indicating Myoepithelial Tumor Differentiation in High-Grade Invasive Ductal Carcinomas as Markers of Predisposition to Lung and Brain Metastases

Hitoshi Tsuda, M.D., Teruko Takarabe, C.T., Fumio Hasegawa, M.T., Takashi Fukutomi, M.D., and Setsuo Hirohashi, M.D.

TABLE 3. *Effect on patient prognosis and preferential metastasis sites of IDCs with large central acellular zones by Cox's univariate analysis model*

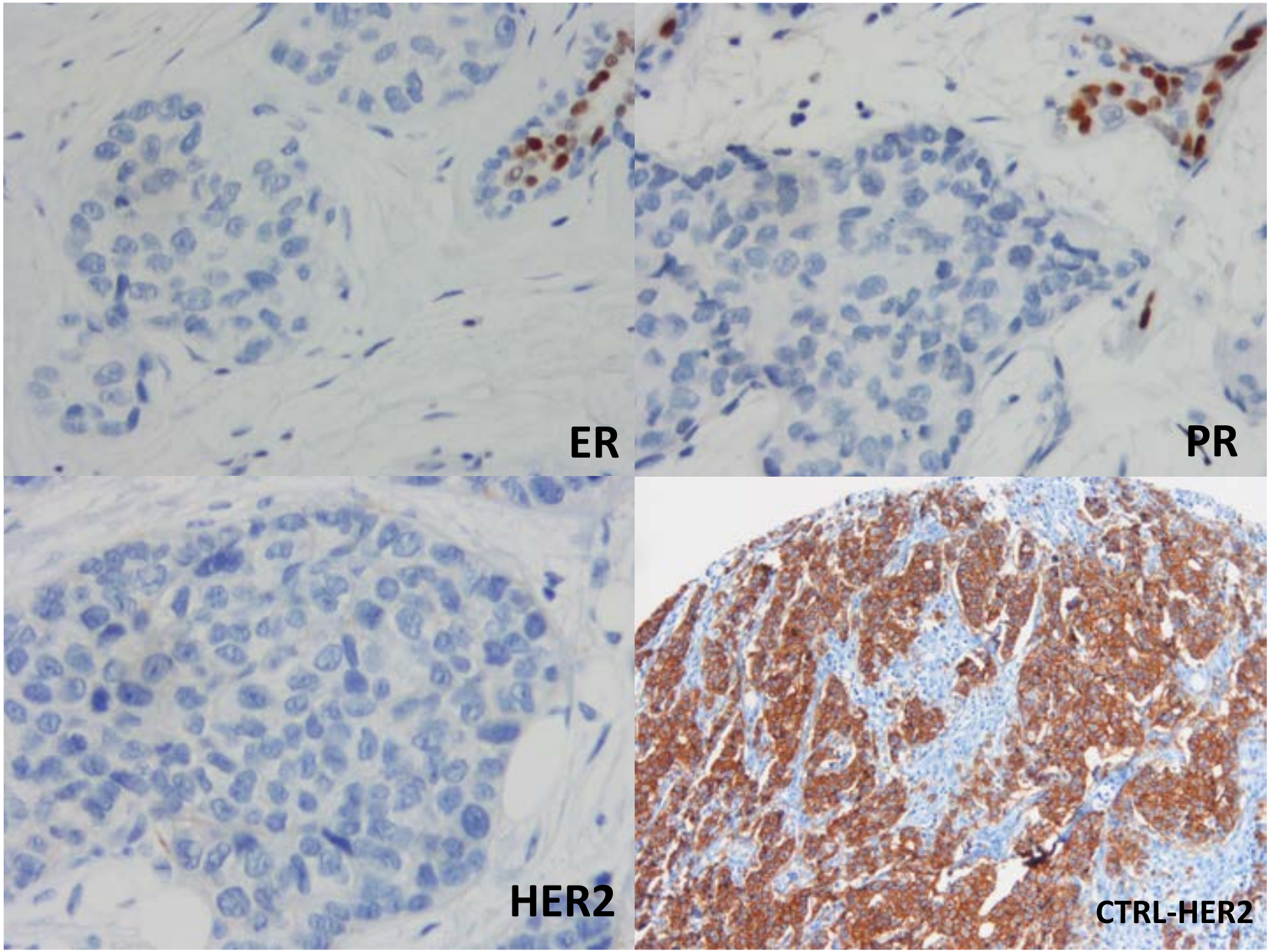
	No. of tumors with metastasis (%)	Risk ratio	95% confidence interval	p value*
A. Metastasis				
1. Metastasis to any organ				
Cases (n = 20)	13 (65)	2.74	1.28–5.86	0.0096
Control subjects (n = 40)	14 (35)			
2. Brain metastasis				
Cases (n = 20)	6 (30)	3.77	1.14–12.45	0.030
Control subjects (n = 40)	5 (13)			
3. Lung metastasis				
Cases (n = 20)	9 (45)	3.67	1.40–9.61	0.008
Control subjects (n = 40)	8 (20)			
4. Bone metastasis				
Cases (n = 20)	4 (20)	1.18	0.36–3.86	NS
Control subjects (n = 40)	5 (13)			
5. Locoregional recurrence				
Cases (n = 20)	4 (20)	1.41	0.42–4.74	NS
Control subjects (n = 40)	8 (20)			
6. Liver metastasis				
Cases (n = 20)	1 (5)	0.78	0.087–7.08	NS
Control subjects (n = 40)	4 (10)			
B. Death by cancer				
Cases (n = 20)	10 (50)	3.78	1.48–9.63	0.0054
Control subjects (n = 40)	8 (20)			

IDC, invasive ductal carcinoma



Triple-negative breast cancer

- Tumour cells negative for ER,PR and HER2
- 10 to 15% of sporadic breast cancer cases
- Characteristics include:
    - higher prevalence among premenopausal African-American patients
    - high nuclear grade and proliferative indices
    - frequently abnormalities on p53 and BRCA 1 genes
    - chemosensitive but poor prognosis
    - peak risk of recurrence is between first and third years and the majority of deaths occur in the first 5 years following therapy.


ER
PR
HER2
CTRL-HER2

The NEW ENGLAND JOURNAL of MEDICINE

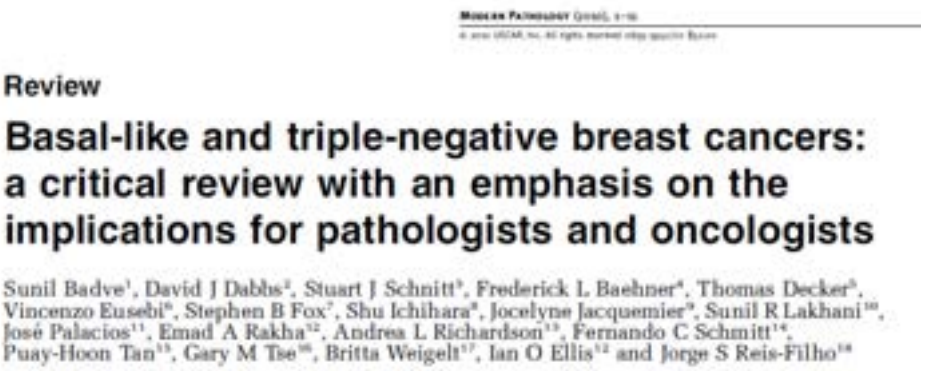
REVIEW ARTICLE

CURRENT CONCEPTS

Triple-Negative Breast Cancer

William D. Foulkes, M.B., B.S., Ph.D., Ian E. Smith, M.D., and Jorge S. Reis-Filho, M.D., Ph.D.

N ENGL J MED 363;20 NEJM.ORG NOVEMBER 11, 2010

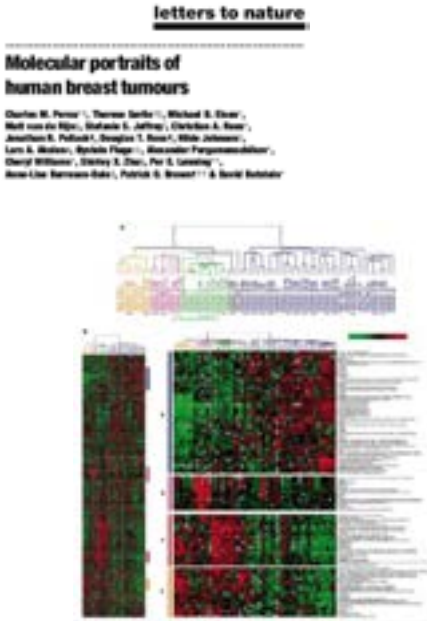


Review

Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists

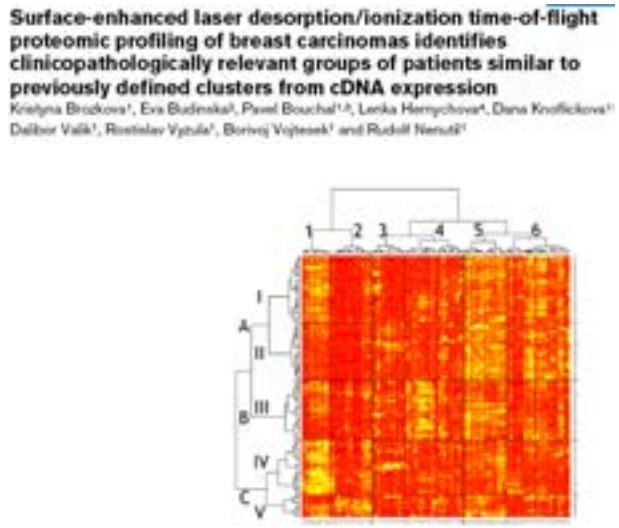
Sunil Badve[1], David J Dabbs[2], Stuart J Schnitt[3], Frederick L Baehner[4], Thomas Decker[5], Vincenzo Eusebi[6], Stephen B Fox[7], Shu Ichihara[8], Jocelyne Jacquemier[9], Sunil R Lakhani[10], José Palacios[11], Emad A Rakha[12], Andrea L Richardson[13], Fernando C Schmitt[14], Puay-Hoon Tan[15], Gary M Tse[16], Britta Weigelt[17], Ian O Ellis[12] and Jorge S Reis-Filho[18]

- There is still no internationally accepted definition for basal-like breast cancers and how best to define these tumours is a matter of controversy and ongoing debate.



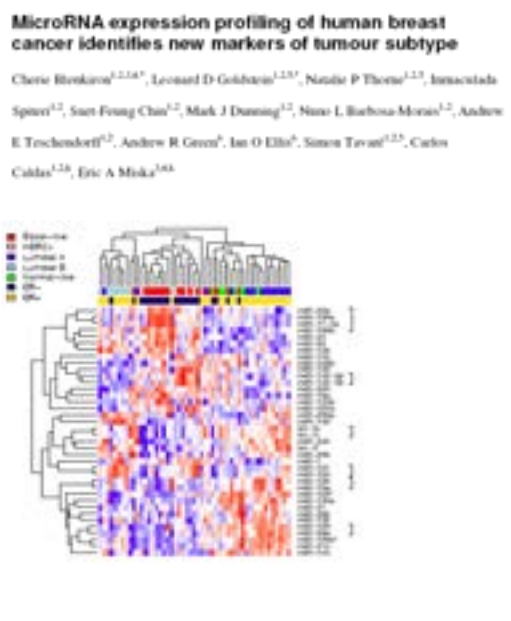
letters to nature

Molecular portraits of human breast tumours



Surface-enhanced laser desorption/ionization time-of-flight proteomic profiling of breast carcinomas identifies clinicopathologically relevant groups of patients similar to previously defined clusters from cDNA expression

Kristyna Brozkova[1], Eva Budinska[2], Pavel Bouchal[1,3], Lenka Hernychova[4], Dana Knoflickova[1], Dalibor Valik[1], Rostislav Vyzula[1], Borivoj Vojtesek[1] and Rudolf Nenutil[1]



MicroRNA expression profiling of human breast cancer identifies new markers of tumour subtype

Cheryl Blenkiron[1,2,3,4,*], Leonard D Goldstein[1,2,5,*], Natalie P Thorne[1,2,3], Inmaculada Spiteri[1,2], Suet-Feung Chin[1,2], Mark J Dunning[1,2], Nuno L Barbosa-Morais[1,2], Andrew E Teschendorff[1,2], Andrew R Green[6], Ian O Ellis[6], Simon Tavaré[1,2,3], Carlos Caldas[1,2,6], Eric A Miska[3,4,5]

Basal-like breast carcinomas

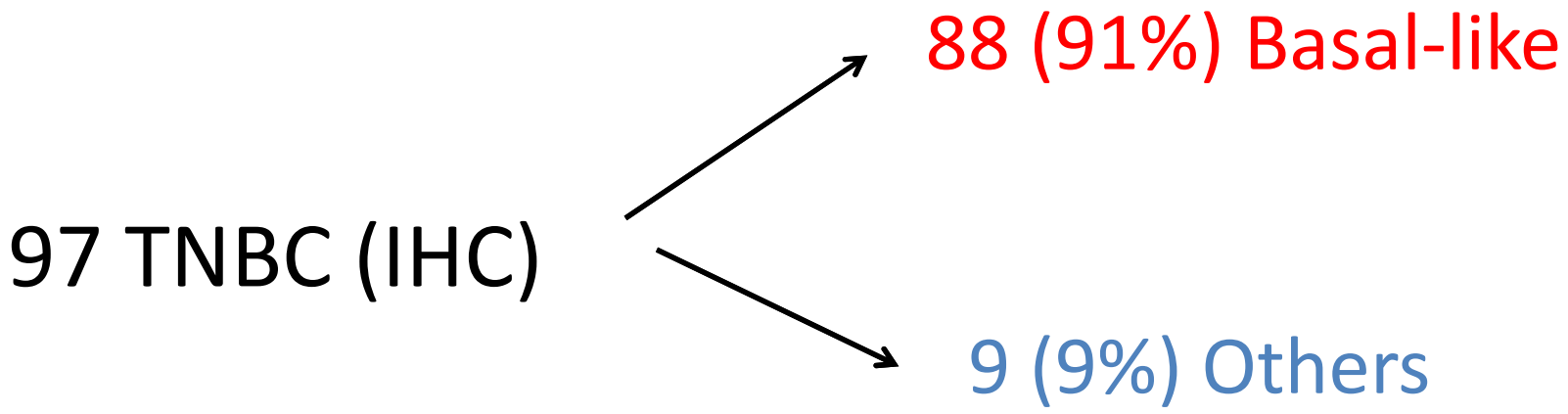
Table 1 | Highlights of genomic, clinical and proteomic features of subtypes

Subtype	Luminal A	Luminal B	Basal-like	HER2E
$ER^+/HER2^-$ (%)	87	82	10	20
$HER2^+$ (%)	7	15	2	68
TNBCs (%)	2	1	80	9
TP53 pathway	*TP53* mut (12%); gain of *MDM2* (14%)	*TP53* mut (32%); gain of *MDM2* (31%)	*TP53* mut (84%); gain of *MDM2* (14%)	*TP53* mut (75%); gain of *MDM2* (30%)
PIK3CA/PTEN pathway	*PIK3CA* mut (49%); *PTEN* mut/loss (13%); *INPP4B* loss (9%)	PIK3CA mut (32%) PTEN mut/loss (24%) INPP4B loss (16%)	PIK3CA mut (7%); PTEN mut/loss (35%); INPP4B loss (30%)	PIK3CA mut (42%); PTEN mut/loss (19%); INPP4B loss (30%)
RB1 pathway	Cyclin D1 amp (29%); *CDK4* gain (14%); low expression of *CDKN2C*; high expression of *RB1*	Cyclin D1 amp (58%); *CDK4* gain (25%)	*RB1* mut/loss (20%); cyclin E1 amp (9%); high expression of *CDKN2A*; low expression of *RB1*	Cyclin D1 amp (38%); *CDK4* gain (24%)
mRNA expression	High ER cluster; low proliferation	Lower ER cluster; high proliferation	Basal signature; high proliferation	HER2 amplicon signature; high proliferation
Copy number	Most diploid; many with quiet genomes; 1q, 8q, 8p11 gain; 8p, 16q loss; 11q13.3 amp (24%)	Most aneuploid; many with focal amp; 1q, 8q, 8p11 gain; 8p, 16q loss; 11q13.3 amp (51%); 8p11.23 amp (28%)	Most aneuploid; high genomic instability; 1q, 10p gain; 8p, 5q loss; *MYC* focal gain (40%)	Most aneuploid; high genomic instability; 1q, 8q gain; 8p loss; 17q12 focal *ERRB2* amp (71%)
DNA mutations	*PIK3CA* (49%); *TP53* (12%); *GATA3* (14%); *MAP3K1* (14%)	*TP53* (32%); *PIK3CA* (32%); *MAP3K1* (5%)	*TP53* (84%); *PIK3CA* (7%)	*TP53* (75%); *PIK3CA* (42%); *PIK3R1* (8%)
DNA methylation	–	Hypermethylated phenotype for subset	Hypomethylated	–
Protein expression	High oestrogen signalling; high MYB; RPPA reactive subtypes	Less oestrogen signalling; high FOXM1 and MYC; RPPA reactive subtypes	High expression of DNA repair proteins, PTEN and INPP4B loss signature (pAKT)	High protein and phospho-protein expression of EGFR and HER2

Percentages are based on 466 tumour overlap list. Amp, amplification; mut, mutation.

COMPREHENSIVE MOLECULAR PORTRAITS OF HUMAN BREAST TUMOURS
Nature 2012

Not all TN are basal-like!



Kreike B et al. et al. BCR 2007

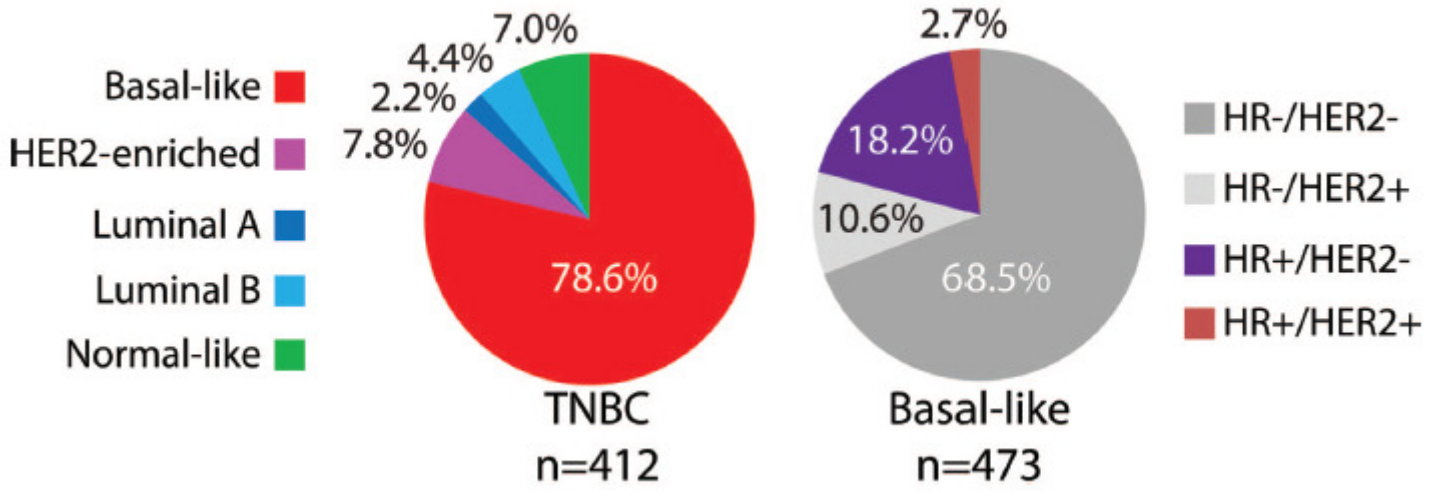
The Oncologist®

Breast Cancer

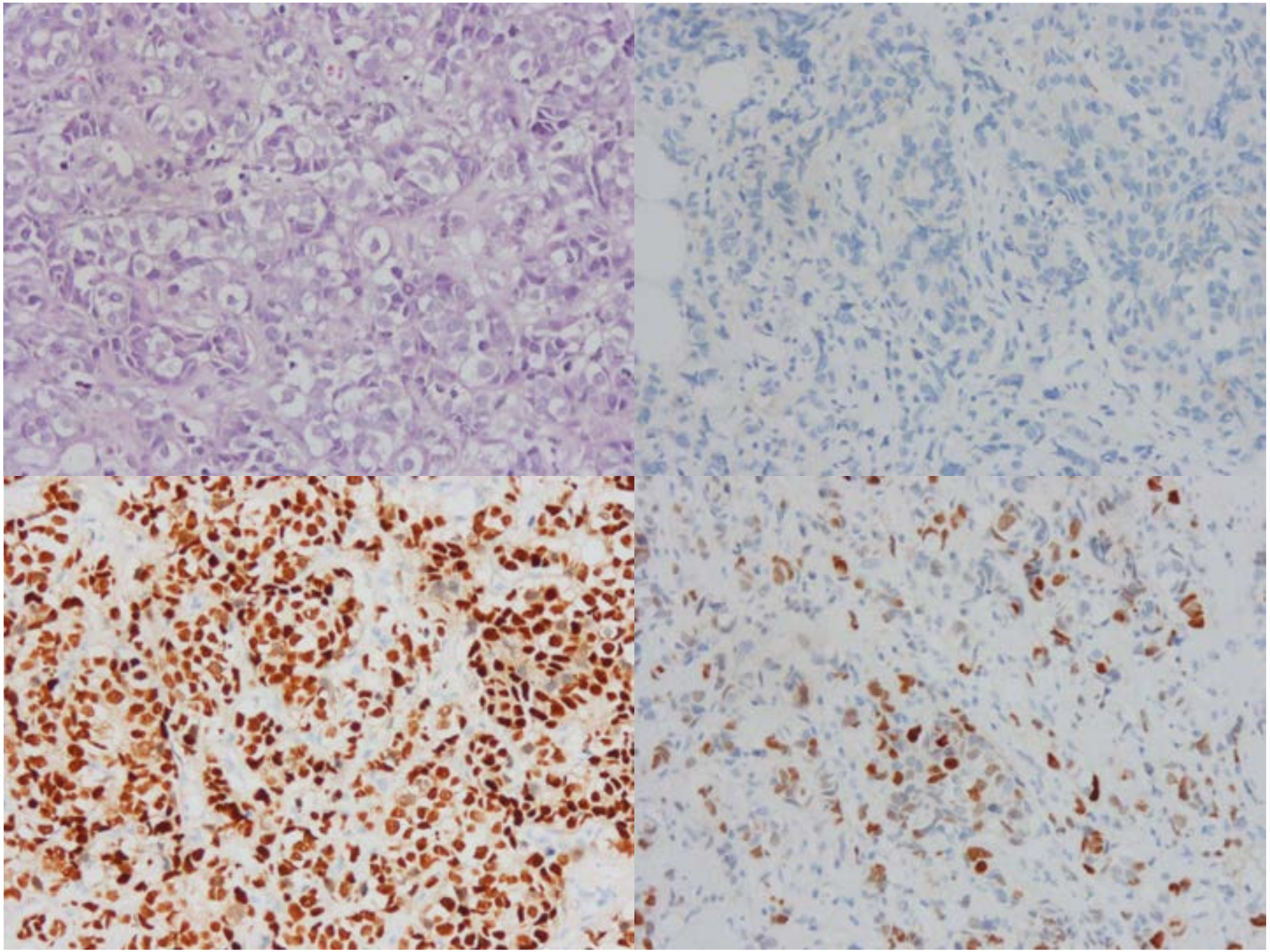
Molecular Characterization of Basal-Like and Non-Basal-Like Triple-Negative Breast Cancer

ALEIX PRAT,[a,b,c] BARBARA ADAMO,[b,c] MAGGIE C.U. CHEANG,[d] CAREY K. ANDERS,[d] LISA A. CAREY,[d] CHARLES M. PEROU[d,e,f]

The Oncologist 2013;18:123–133



There are limitations to use IHC for Receptors as Surrogates for Molecular Subtype

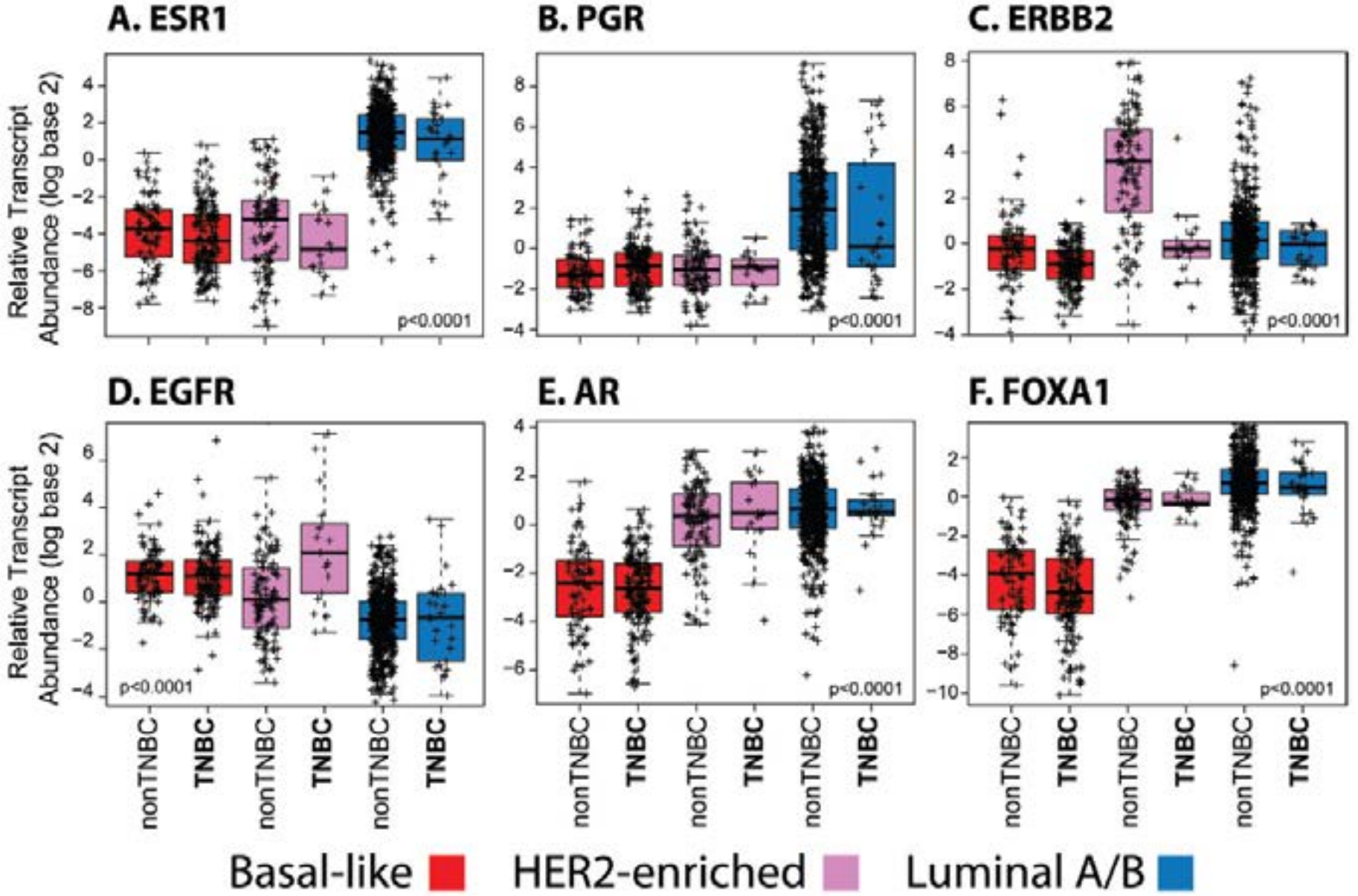


TN and basal-like definitions should not be considering synonymous because considerable discordance exists (~25%)

- False-positivity or false-negativity of the IHC-based assays for determining HR and HER2 status, because these tests are challenged by interlaboratory and intermethod discordance rates.

- Assessment in different areas of the tumour ? Unlikely that two different subtypes coexist in the same tumour enough to explain the discordance rate.

- Gene expression measures a large number of related genes, compared with the 3 individual biomarkers used to define TN disease. For example, a TN tumour that has low levels of ESR1 and PGR might be luminal due to the expression of other luminal-related genes (GATA3 and/or FOX1A).


A. ESR1
B. PGR
C. ERBB2
D. EGFR
E. AR
F. FOXA1
Relative Transcript Abundance (log base 2)
p<0.0001
nonTNBC
TNBC
Basal-like
HER2-enriched
Luminal A/B

Research article

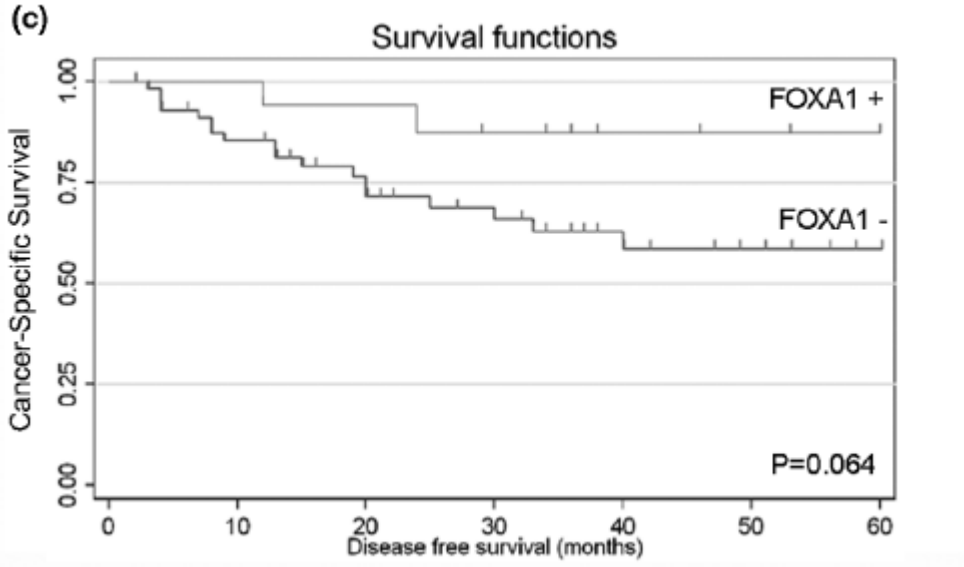
Open Access

Expression of FOXA1 and GATA-3 in breast cancer: the prognostic significance in hormone receptor-negative tumours

André Albergaria[1,2], Joana Paredes[2], Bárbara Sousa[2], Fernanda Milanezi[2], Vítor Carneiro[3], Joana Bastos[4,5], Sandra Costa[1], Daniella Vieira[6], Nair Lopes[2], Eric W Lam[7], Nuno Lunet[4,5] and Fernando Schmitt[2,8]

Breast Cancer Research 2009, **11**:R40 (doi:10.1186/bcr2327)

ER NEGATIVE TUMOURS

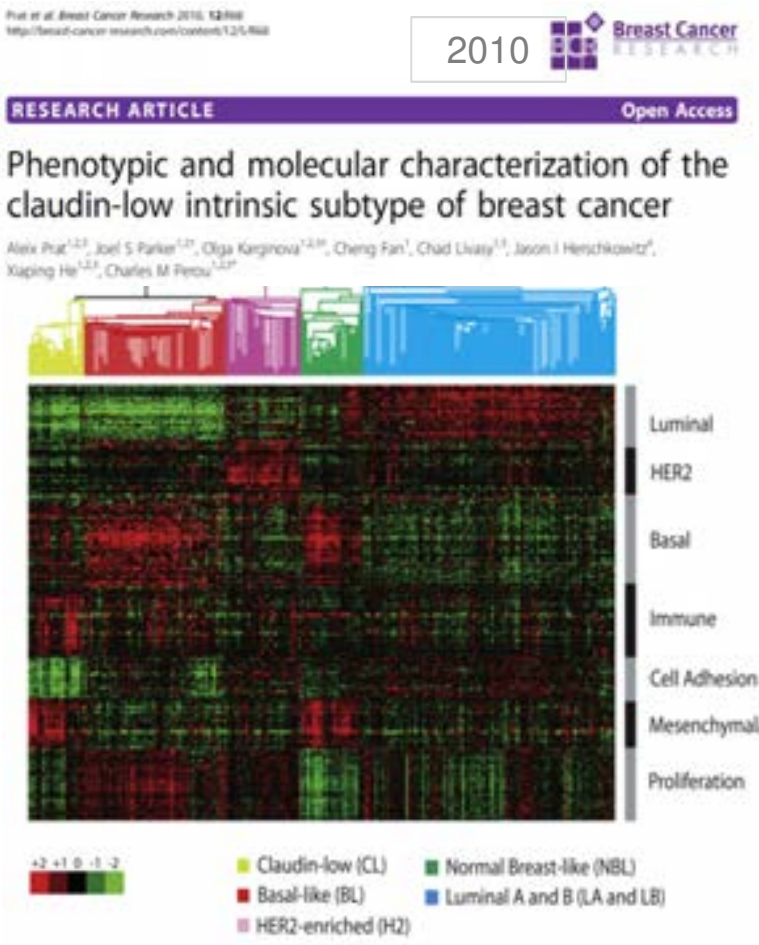


Triple-negative breast cancer is a heterogeneous clinical entity

- Gene expression profile classification revealed an heterogeneous group of breast malignancies:
  - Basal-like (EGFR and/or CK5/6 and /or CK14 and/or PCad)
  - Claudin-low (low/absent expression of adhesion molecules)
  - Molecular apocrine
  - Other intrinsic molecular subtypes
  - Normal-breast like (normal adipose tissue and other non epithelial and basal epithelial) ???

Claudin-low carcinomas

New molecular subgroup, sorted from the triple negative breast cancer group



- Low expression of genes involved in tight junctions and cell-cell adhesion:
  - *Claudins 3, 4, 7,*
  - *Occludin*
  - *Ecadherin*

- Low expression of luminal genes,
- Inconsistent basal gene expression
- High expression of lymphocyte and endothelial cell markers

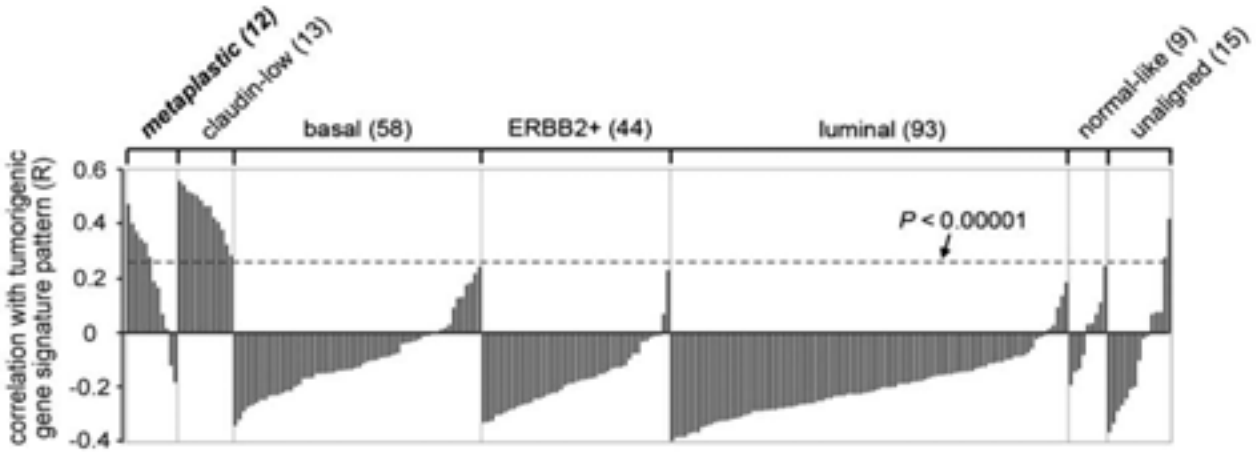
Research Article

Characterization of a Naturally Occurring Breast Cancer Subset Enriched in Epithelial-to-Mesenchymal Transition and Stem Cell Characteristics

Bryan T. Hennessy, Ana-Maria Gonzalez-Angulo, Katherine Stemke-Hale, Michael Z. Gilcrease, Savitri Krishnamurthy, Ju Seog Lee, Jane Fridlyand, Aysegul Sahin, Roshan Agarwal, Corwin Joy, Wenbin Liu, David Stivers, Keith Baggerly, Mark Carey, Ana Lluch, Carlos Monteagudo, Xiaping He, Victor Weigman, Cheng Fan, Juan Palazzo, Gabriel N. Hortobagyi, Laura K. Nolden, Nicholas J. Wang, Vicente Valero, Joe W. Gray, Charles M. Perou, and Gordon B. Mills

Departments of Gynecologic Medical Oncology, Systems Biology, Breast Medical Oncology, Pathology, and Bioinformatics and Computational Biology and Kleberg Center for Molecular Markers, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; Lawrence Berkeley National Laboratory, Berkeley, California; Vall d'Hebron Hospital and University of Valencia, Valencia, Spain; Lineberger Comprehensive Cancer Center, Chapel Hill, North Carolina; and Department of Pathology, Thomas Jefferson University, Philadelphia, Pennsylvania

CD44+/CD24-/low phenotype



MBCs and Claudin-low tumors present similar transcriptional profiles and are enriched in stem cell characteristics

Contents lists available at SciVerse ScienceDirect

The Breast

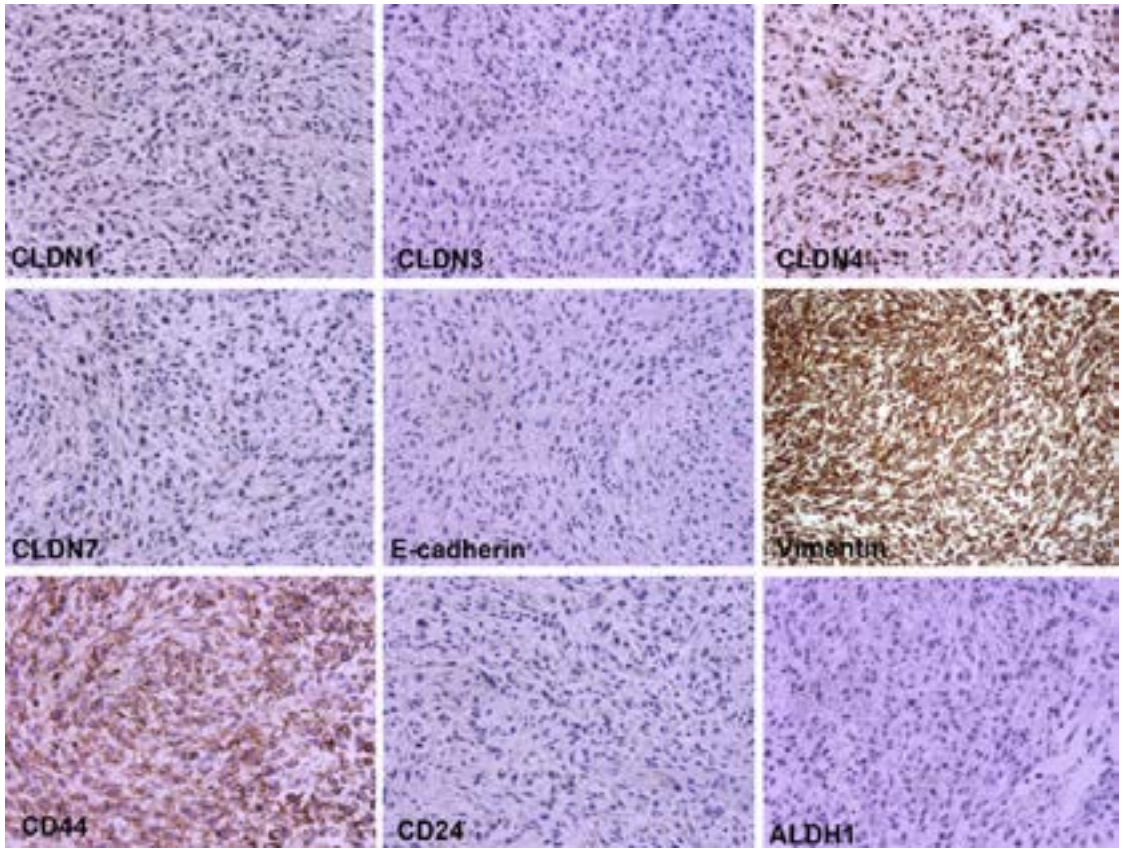
journal homepage: www.elsevier.com/brst

Original article

Immunohistochemical features of claudin-low intrinsic subtype in metaplastic breast carcinomas

Renê Gerhard [a,g], Sara Ricardo [a,b,g], André Albergaria [a], Madalena Gomes [a], Alfredo Ribeiro Silva [c], Ângela Flavia Logullo [d], Jorge F. Cameselle-Teijeiro [e], Joana Paredes [a,f], Fernando Schmitt [a,f,*]

[a] IPATIMUP – Institute of Molecular Pathology and Immunology of Porto University, Porto, Portugal
[b] ICBAS – Abel Salazar Biomedical Science Institute, Porto, Portugal
[c] Department of Pathology, Medical Faculty, University of São Paulo, Ribeirão Preto, Brazil
[d] Department of Pathology, School of Medicine, Federal University of São Paulo, São Paulo, Brazil
[e] Complexo Hospitalar Universitario de Vigo (CHUVI), Vigo, Spain
[f] Medical Faculty of Porto University, Porto, Portugal

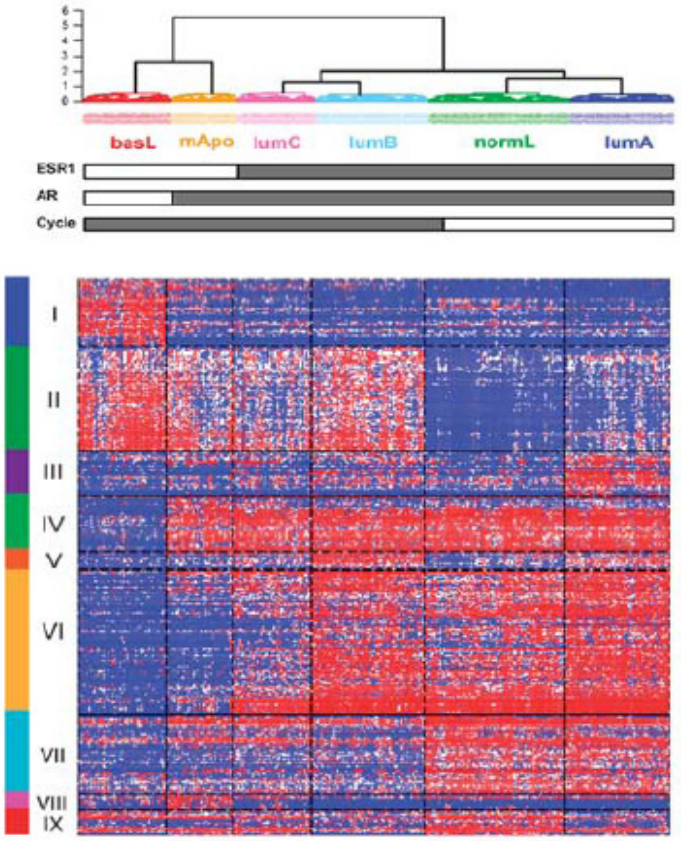


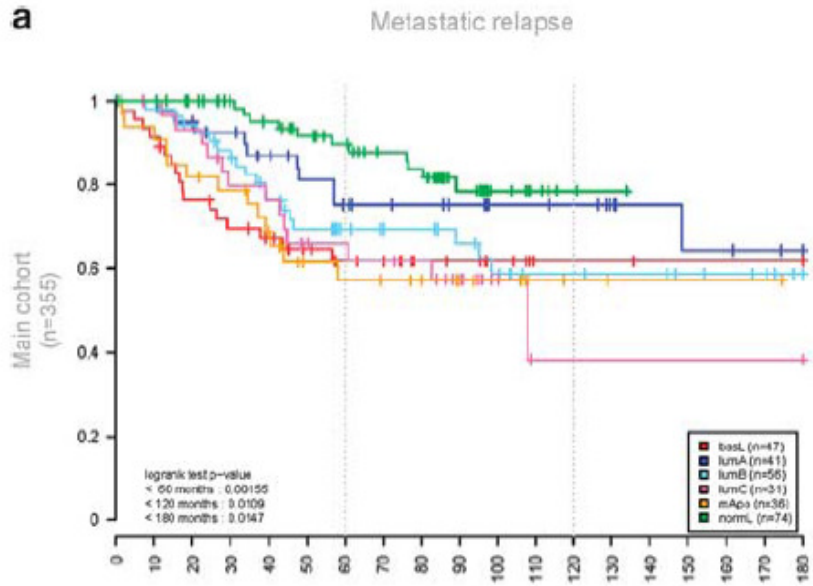
Molecular Apocrine

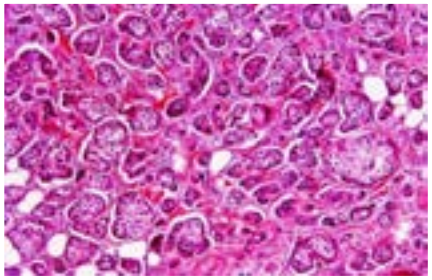
Benign and malignant apocrine lesions of the breast

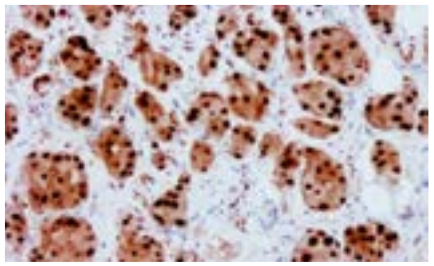
Expert Rev. Anticancer Ther. 12(2), 215–221 (2012)

Renê Gerhard‡1, José Luis Costa‡1 and Fernando Schmitt*1,2





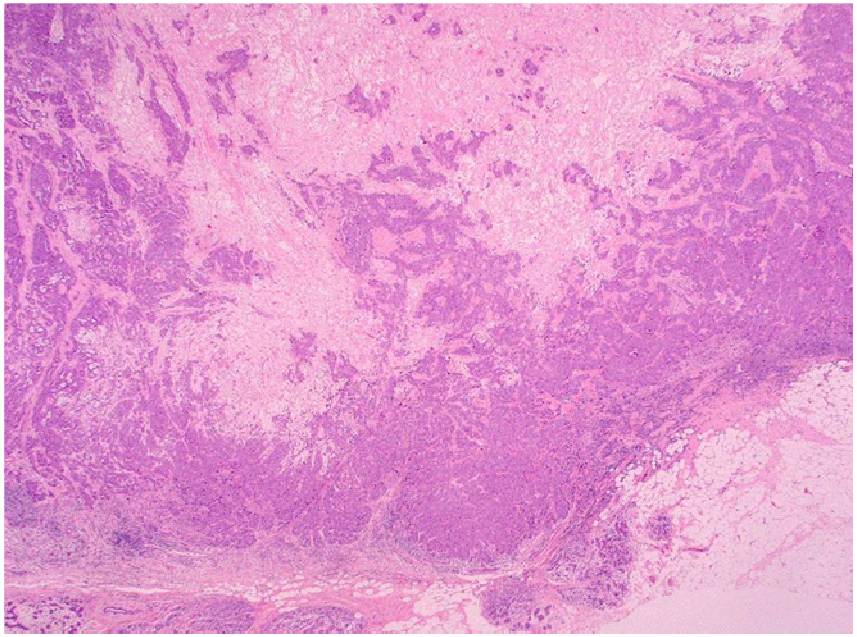


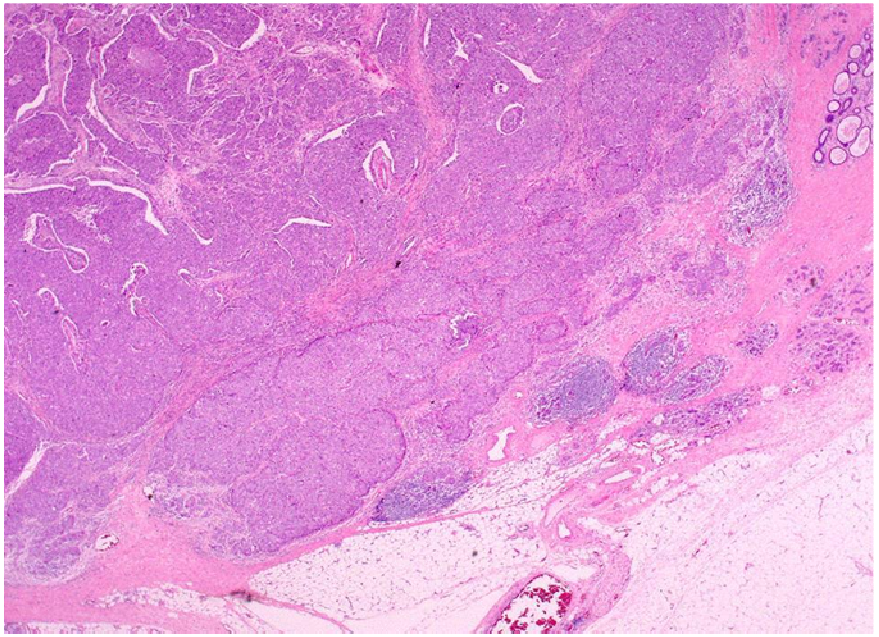


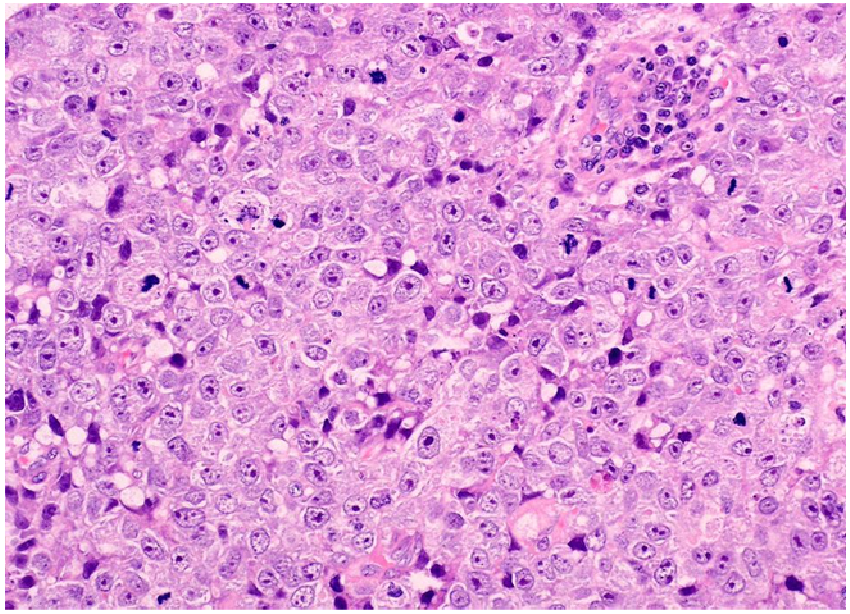
Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma

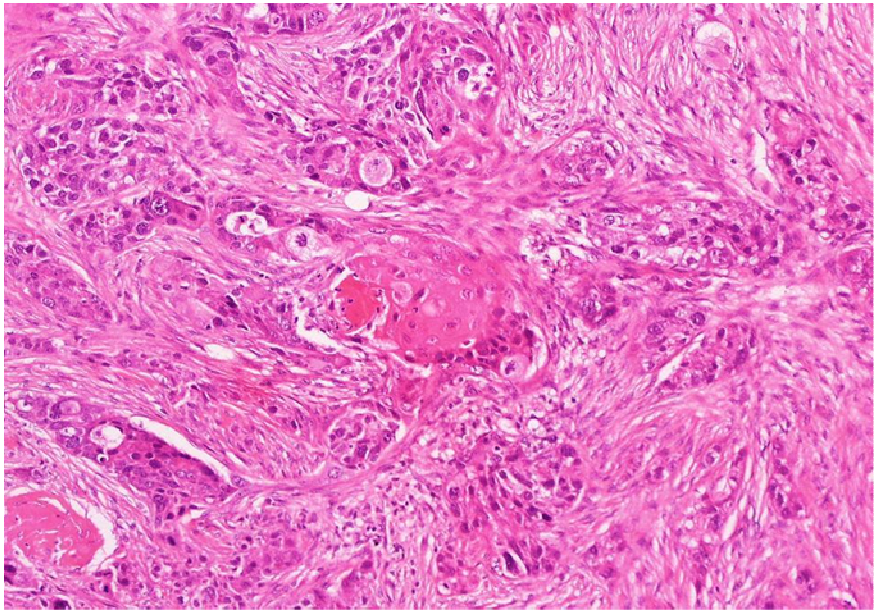
Histology of Basal-Like Cancers Identified By Expression Profiling

- Histologic grade 3 (100%)
- Solid architecture
- No tubule formation, high density of cells with no intervening stroma
- Pushing border (61%)
- Stromal lymphocytic infiltrate (56%)
- High mitotic rate (100%)
- Geographic zones of necrosis (74%)
- Medullary-like features
- *(Central fibrotic/acellular zone)*
- *(Little or no associated DCIS)*



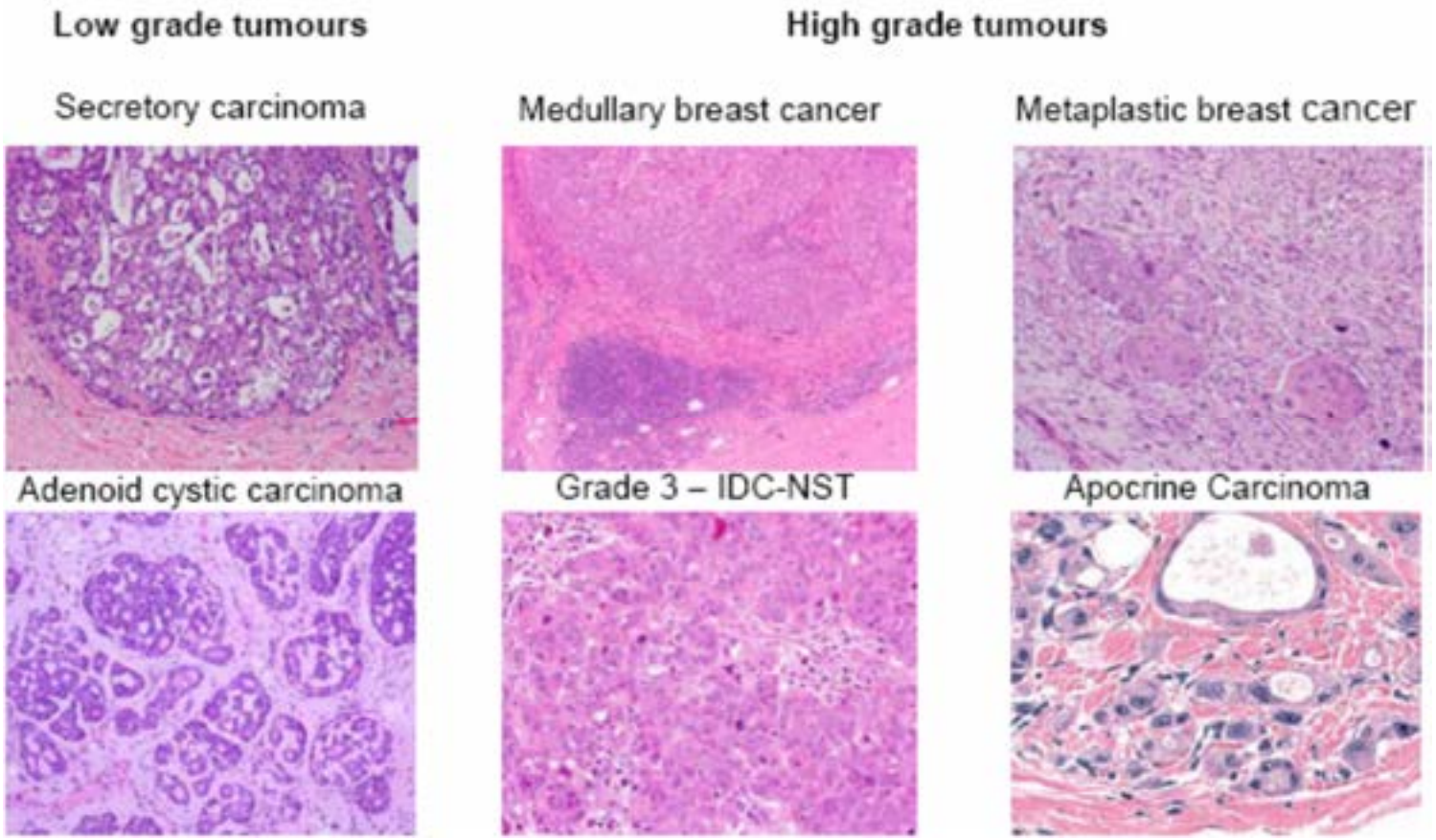




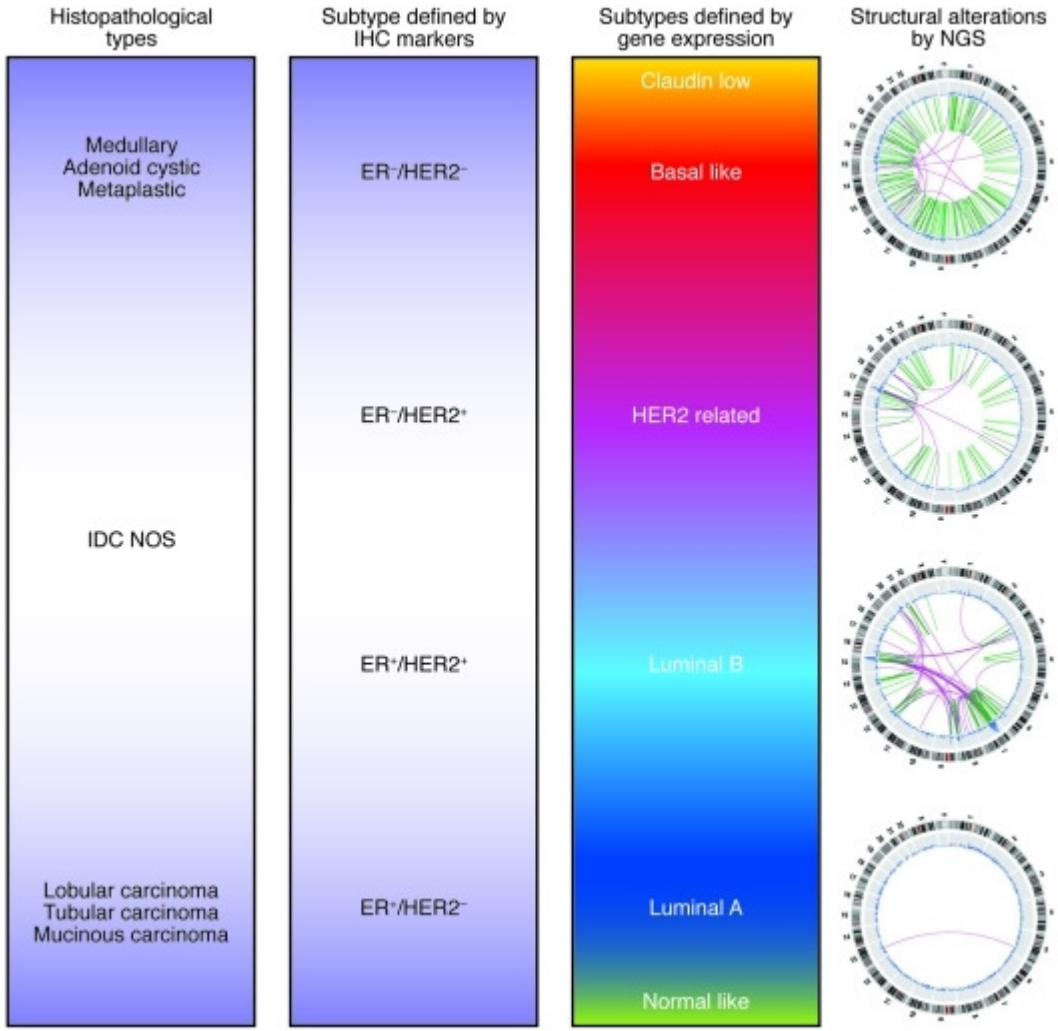


Do we still need a morphological classification?

"Triple-Negative" breast carcinomas

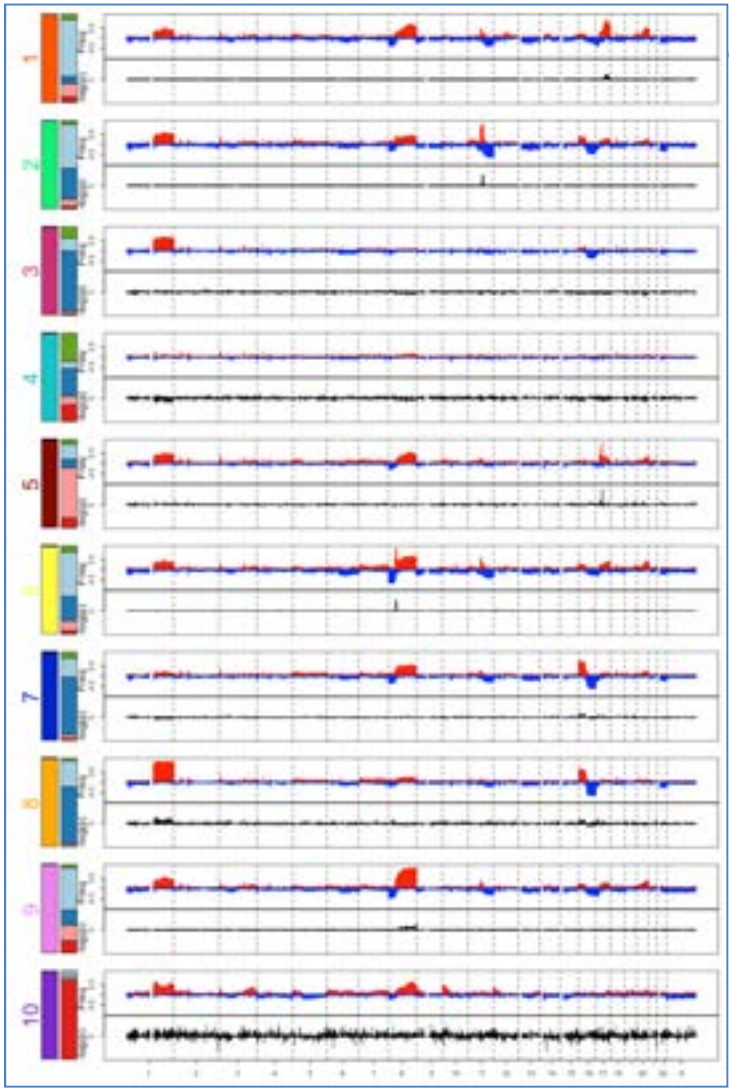


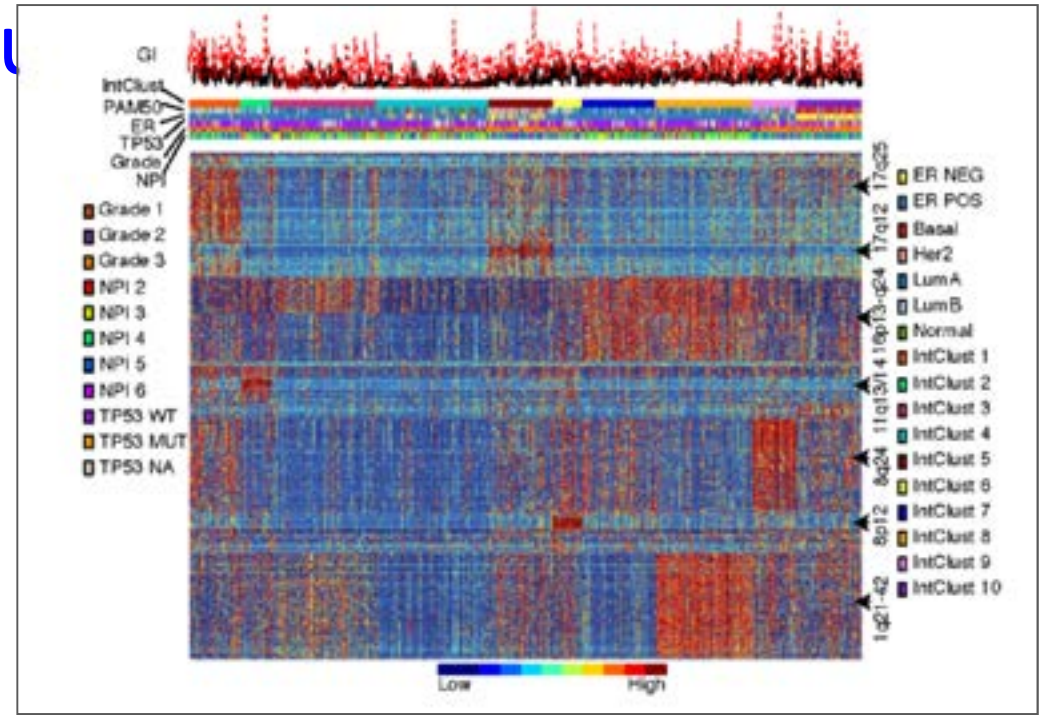
Breast cancer classification

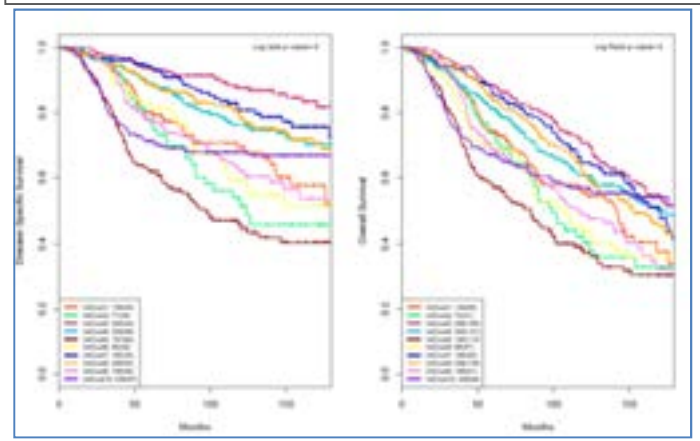


Russnes et al. JCI 2011

The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel

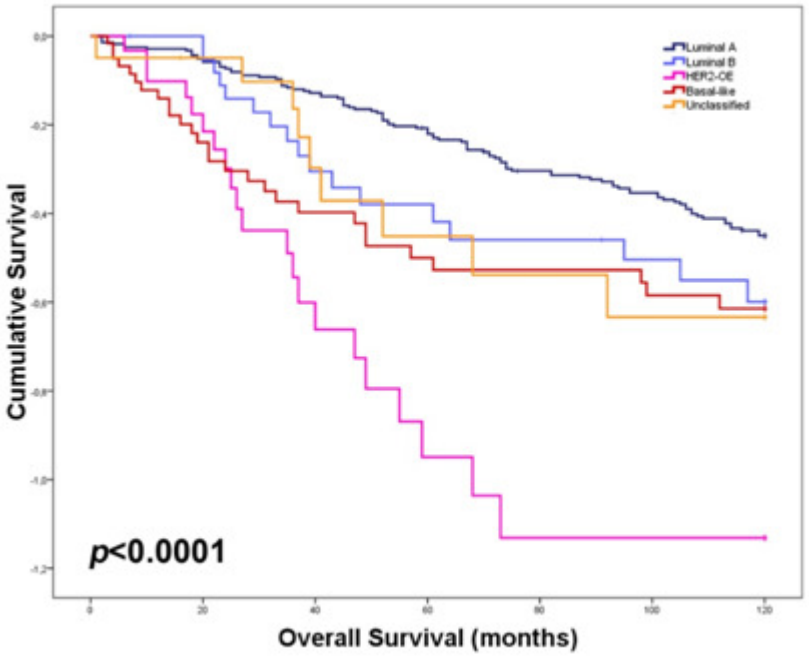


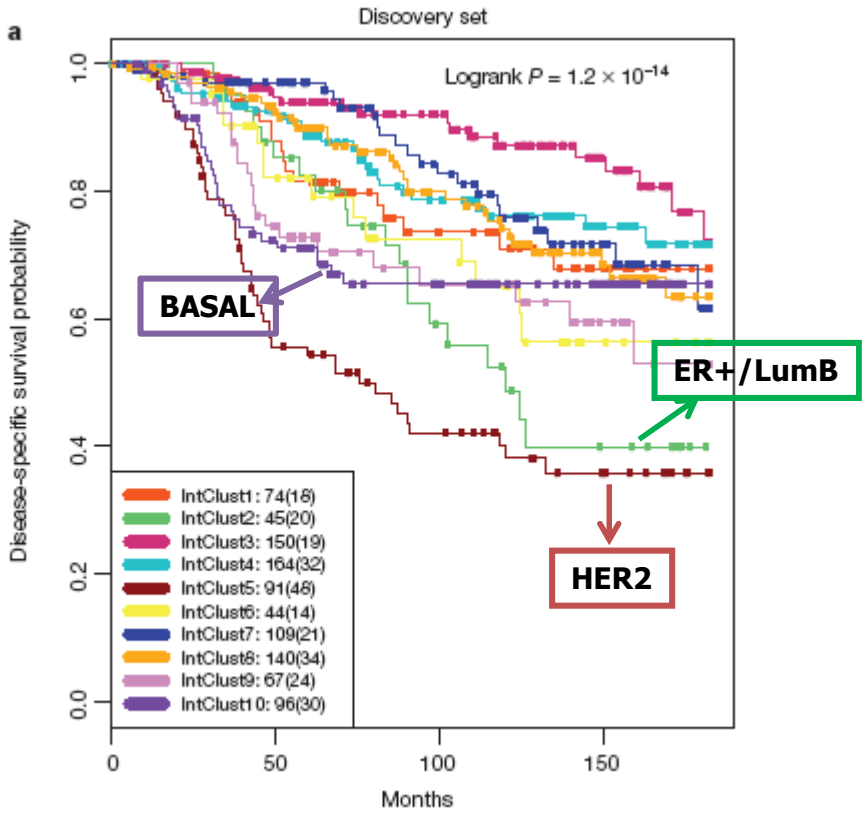




Curtis C et al. Nature

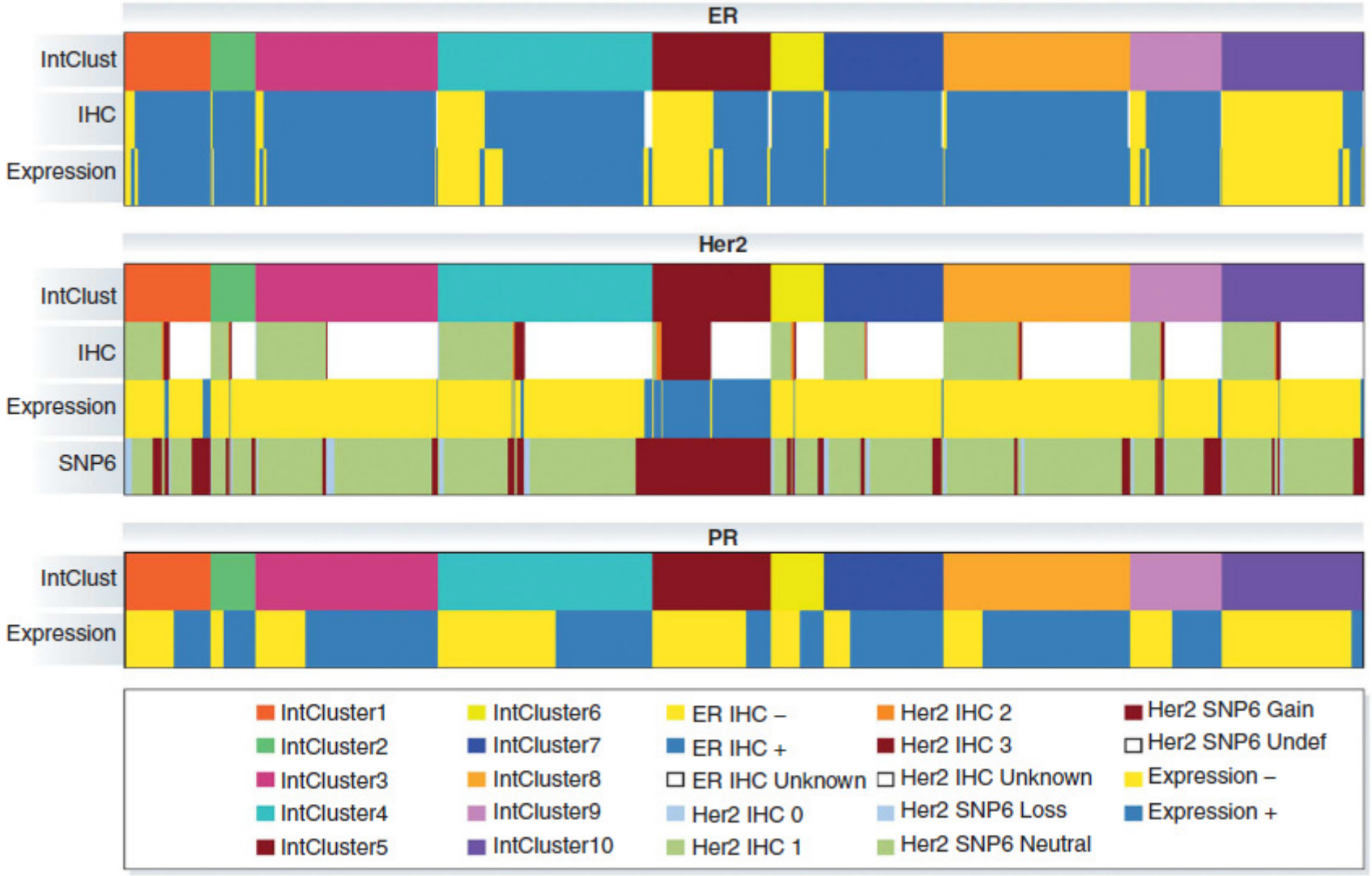
Integrative clusters and survival





The open question:
How can we integrate these subtypes into daily clinical work?

A new genome-driven integrated classification of breast cancer and its implications



The EMBO Journal (2013) 32, 617–628

High-throughput DNA sequencing

DNA

Are the batteries included?

Overview of all genomic variation

ARTICLE

doi:10.1038/nature17676

Landscape of somatic mutations in 560 breast cancer whole-genome sequences

Serena Nik-Zainal[1,2], Helen Davies[1], Johan Staaf[3], Manasa Ramakrishna[1], Dominik Glodzik[1], Xueqing Zou[1], Inigo Martincorena[1], Ludmil B. Alexandrov[1,4,5], Sancha Martin[1], David C. Wedge[1], Peter Van Loo[1,6], Young Seok Ju[1], Marcel Smid[7], Arie B. Brinkman[8], Sandro Morganella[9], Miriam R. Aure[10,11], Ole Christian Lingjærde[11,12], Anita Langerød[10,11], Markus Ringnér[3], Sung-Min Ahn[13], Sandrine Boyault[14], Jane E. Brock[15], Annegien Broeks[16], Adam Butler[1], Christine Desmedt[17], Luc Dirix[18], Serge Dronov[1], Aquila Fatima[19], John A. Foekens[7], Moritz Gerstung[1], Gerrit K. J. Hooijer[20], Se Jin Jang[21], David R. Jones[1], Hyung-Yong Kim[22], Tari A. King[23], Savitri Krishnamurthy[24], Hee Jin Lee[21], Jeong-Yeon Lee[25], Yilong Li[1], Stuart McLaren[1], Andrew Menzies[1], Ville Mustonen[1], Sarah O'Meara[1], Iris Pauporté[26], Xavier Pivot[27], Colin A. Purdie[28], Keiran Raine[1], Kamna Ramakrishnan[1], F. Germán Rodríguez-González[7], Gilles Romieu[29], Anieta M. Sieuwerts[7], Peter T. Simpson[30], Rebecca Shepherd[1], Lucy Stebbings[1], Olafur A. Stefansson[31], Jon Teague[1], Stefania Tommasi[32], Isabelle Treilleux[33], Gert G. Van den Eynden[18,34], Peter Vermeulen[18,34], Anne Vincent-Salomon[35], Lucy Yates[1], Carlos Caldas[36], Laura van't Veer[16], Andrew Tutt[37,38], Stian Knappskog[39,40], Benita Kiat Tee Tan[41,42], Jos Jonkers[16], Åke Borg[3], Naoto T. Ueno[24], Christos Sotiriou[17], Alain Viari[43,44], P. Andrew Futreal[1,45], Peter J. Campbell[1], Paul N. Span[46], Steven Van Laere[18], Sunil R. Lakhani[30,47], Jorunn E. Eyfjord[31], Alastair M. Thompson[28,48], Ewan Birney[9], Hendrik G. Stunnenberg[8], Marc J. van de Vijver[20], John W. M. Martens[7], Anne-Lise Børresen-Dale[10,11], Andrea L. Richardson[15,19], Gu Kong[22], Gilles Thomas[44] & Michael R. Stratton[1]

Minions Language

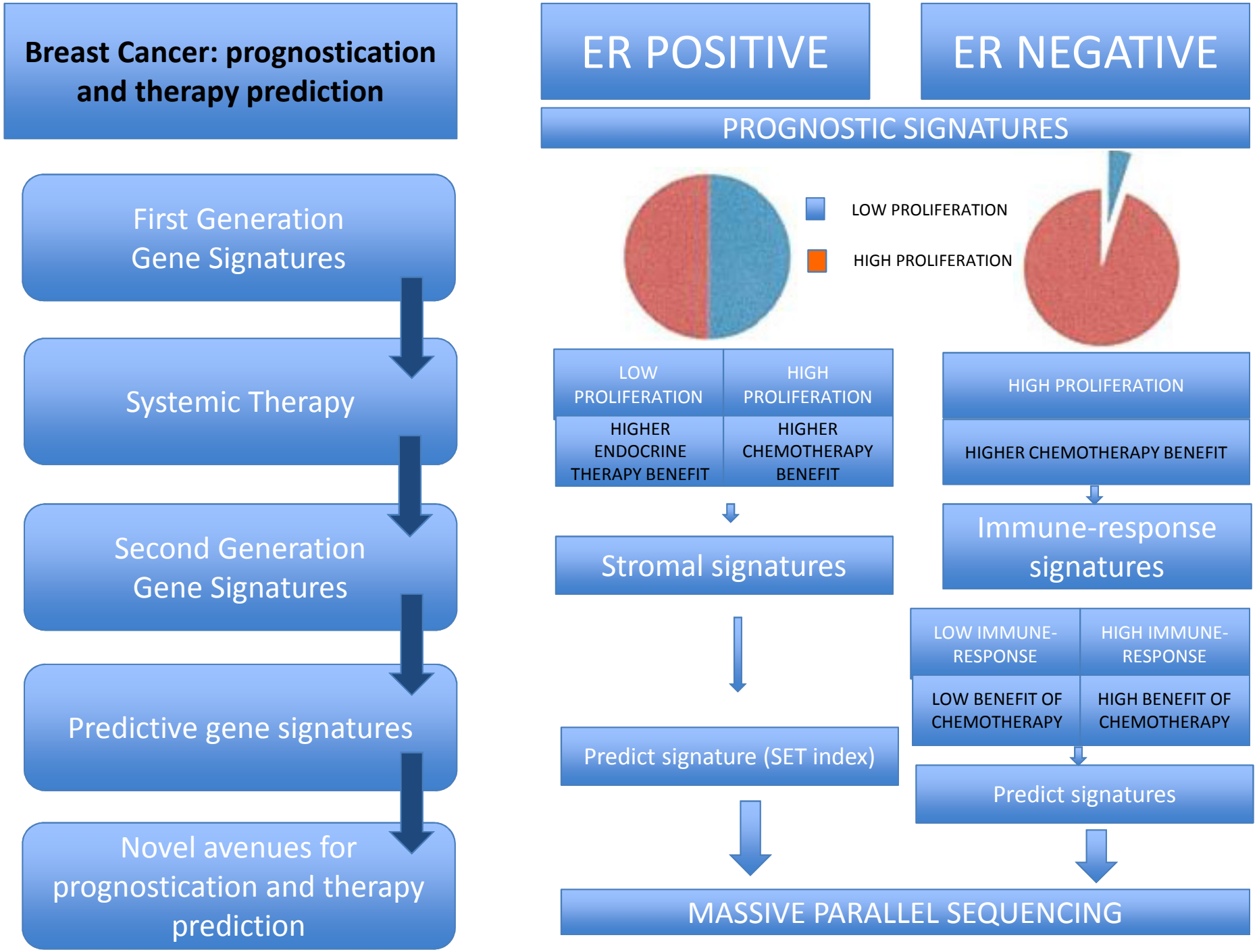
English:	Minions:
Hello!	Bello!
Goodbye!	Poopaye!
Thank you!	Tank yu!
For you	Para tu
Marriage	La boda
Apples	Papples
Ice-cream	Gelato
I'm sorry	Bi-do
I'm hungry	We want banana!
Ugly!	Bananonina!
I swear...	Underwear...
Fire	Bee-do-bee-do-bee-do
We love you	Tulaliloo ti amo
I hate you!	Tatata-bala-tu
What	Po-ka
Cheers	Kampai
Kiss kiss	Muak muak muak

Massively Parallel Sequencing-based studies of Breast Cancer

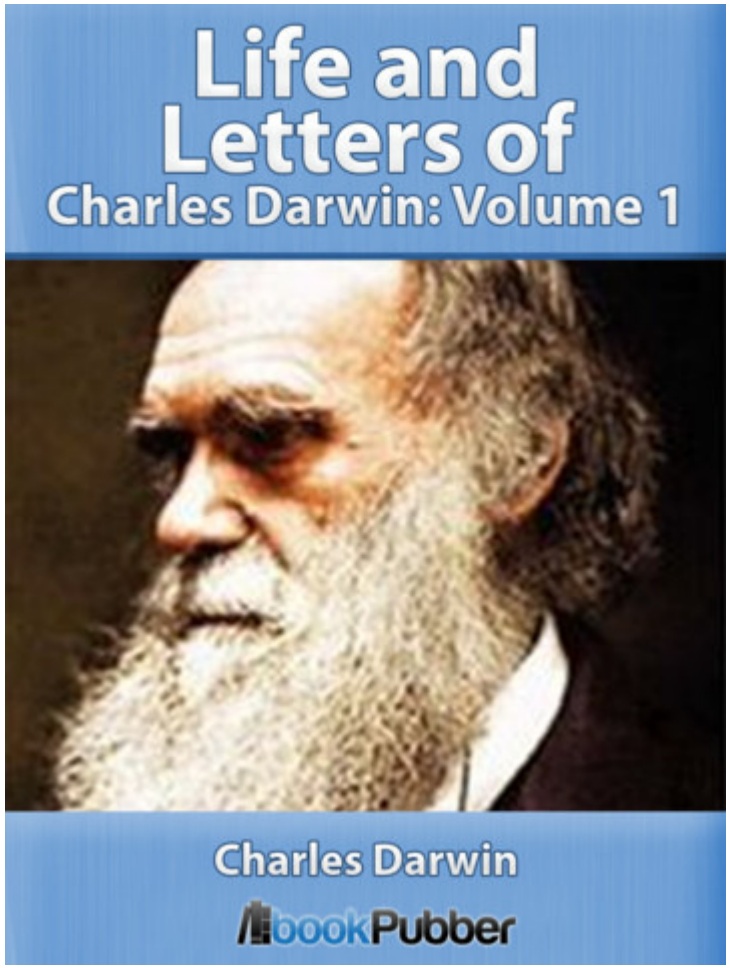
- The collection of genetic aberrations found in breast cancer is complex with a limited number of genes that are frequently mutated in unselected cases.
- The number of genes mutated in small minorities of breast cancer is vast.
- The repertoire of mutations in luminal and basal-like breast cancer is rather different.
- There is no gene or mutation that defines a subtype of breast cancer.
- These studies led to the identification of novel driver genes and that genes that encodes ER alpha (ESR1) and HER2 can be targeted by activating mutations.

Molecular Classification Conclusions

- GEP studies have provided significant advances in the molecular classification and prognostication of breast cancer, and has given new insights regarding therapeutic prediction.
- The clinical management of patients is still based on the assessment of morphology, ER,PR, HER2 and Ki67.
- New avenues for discovering and validating prognostic and predictive biomarkers are being developed through NGS approaches.


Breast Cancer: prognostication and therapy prediction
First Generation Gene Signatures
Systemic Therapy
Second Generation Gene Signatures
Predictive gene signatures
Novel avenues for prognostication and therapy prediction
ER POSITIVE
ER NEGATIVE
PROGNOSTIC SIGNATURES
LOW PROLIFERATION
HIGH PROLIFERATION
LOW PROLIFERATION
HIGH PROLIFERATION
HIGHER ENDOCRINE THERAPY BENEFIT
HIGHER CHEMOTHERAPY BENEFIT
HIGH PROLIFERATION
HIGHER CHEMOTHERAPY BENEFIT
Stromal signatures
Immune-response signatures
LOW IMMUNE-RESPONSE
HIGH IMMUNE-RESPONSE
LOW BENEFIT OF CHEMOTHERAPY
HIGH BENEFIT OF CHEMOTHERAPY
Predict signature (SET index)
Predict signatures
MASSIVE PARALLEL SEQUENCING

Taxonomy dilemma: *lumpers vs splitters*



- “Those who make many species are splitters, and those who make few are the lumpers”.
- In Medicine, this divide is exacerbated when a clear mechanistic understanding of a disease entity is incomplete.

Taxonomy of breast cancer based on normal cell phenotype predicts outcome

Sandro Santagata,[1] Ankita Thakkar,[2] Ayse Ergonul,[2]
Bin Wang,[2] Terri Woo,[1] Rong Hu,[3,4] J. Chuck Harrell,[5] George McNamara,[2]
Matthew Schwede,[6] Aedin C. Culhane,[6] David Kindelberger,[1] Scott Rodig,[1]
Andrea Richardson,[1] Stuart J. Schnitt,[7] Rulla M. Tamimi,[3,4] and Tan A. Ince[2]

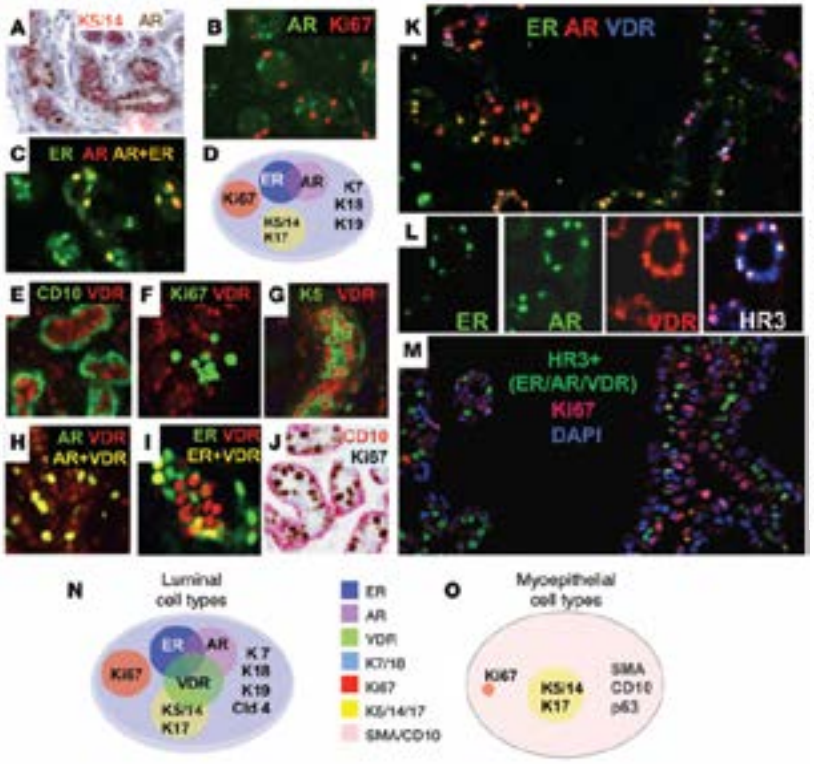
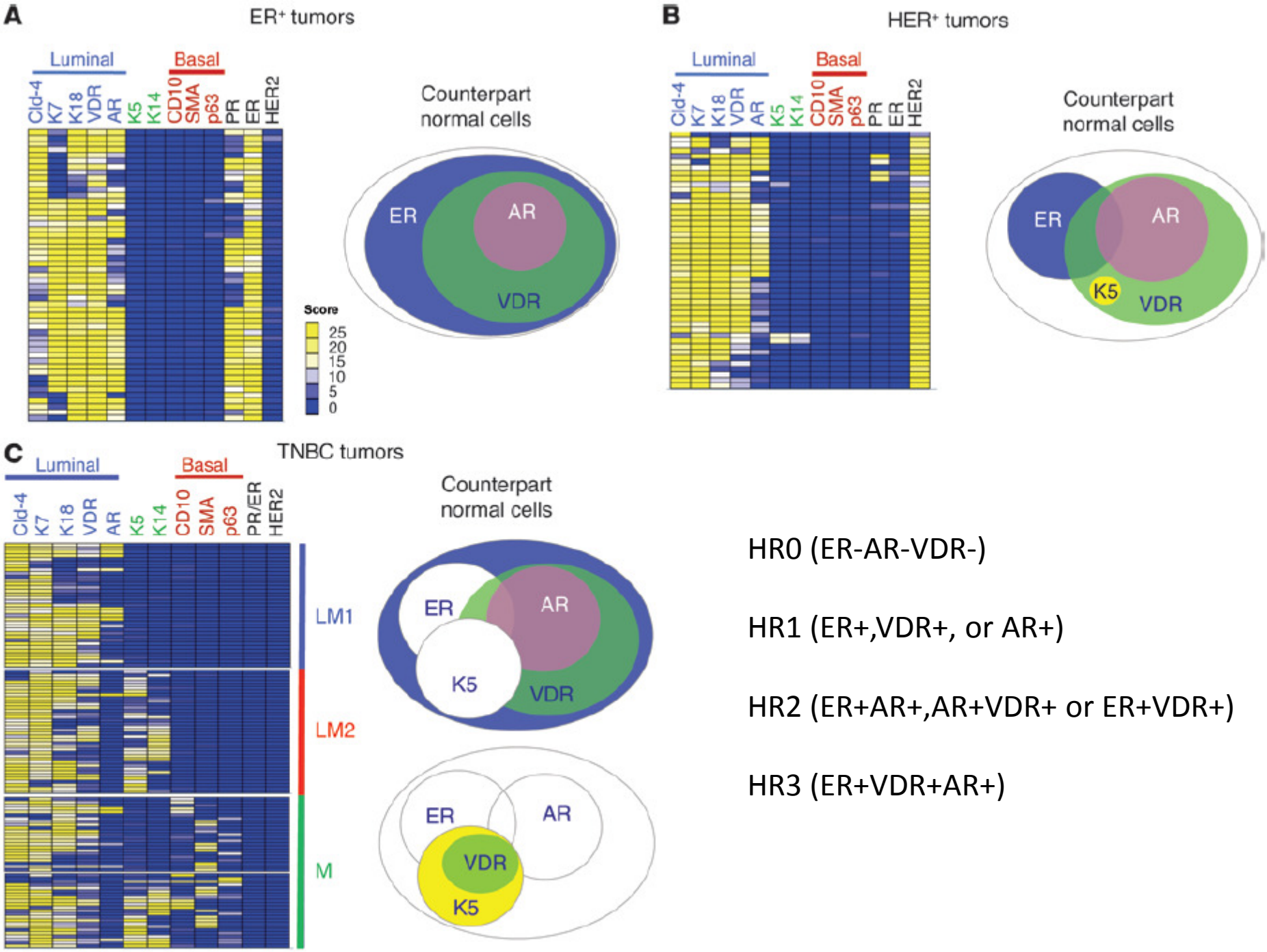


Table 1
Cellular differentiation states in normal human breast lobules

Cell type		ER	AR	VDR	K5/14/17	Ki67	Cld-4	K7/8/18	CD10/SMA/p63
Luminal									
L1 (HR0)	Ki67$^+$	–	–	–	–	+	+	+	–
L2 (HR0)	K18$^+$	–	–	–	–	–	+	+	–
L3 (HR0)	K5$^+$	–	–	–	+	–	+	+	–
L4 (HR1)	ER$^+$	+	–	–	–	–	+	+	–
L5 (HR1)	AR$^+$	–	+	–	–	–	+	+	–
L6 (HR1)	VDR$^+$	–	–	+	–	–	+	+	–
L7 (HR1)	K5$^+$VDR$^+$	–	–	+	+	–	+	+	–
L8 (HR2)	ER$^+$AR$^+$	+	+	–	–	–	+	+	–
L9 (HR2)	ER$^+$VDR$^+$	+	–	+	–	–	+	+	–
L10 (HR2)	AR$^+$VDR$^+$	–	+	+	–	–	+	+	–
L11 (HR3)	ER$^+$AR$^+$VDR$^+$	+	+	+	–	–	+	+	–
Myoepithelial									
My1	CD10$^+$	–	–	–	–	–	–	–	+
My2	K5$^+$	–	–	–	+	–	–	–	+

J Clin Invest. 2014;124(2):859–870.

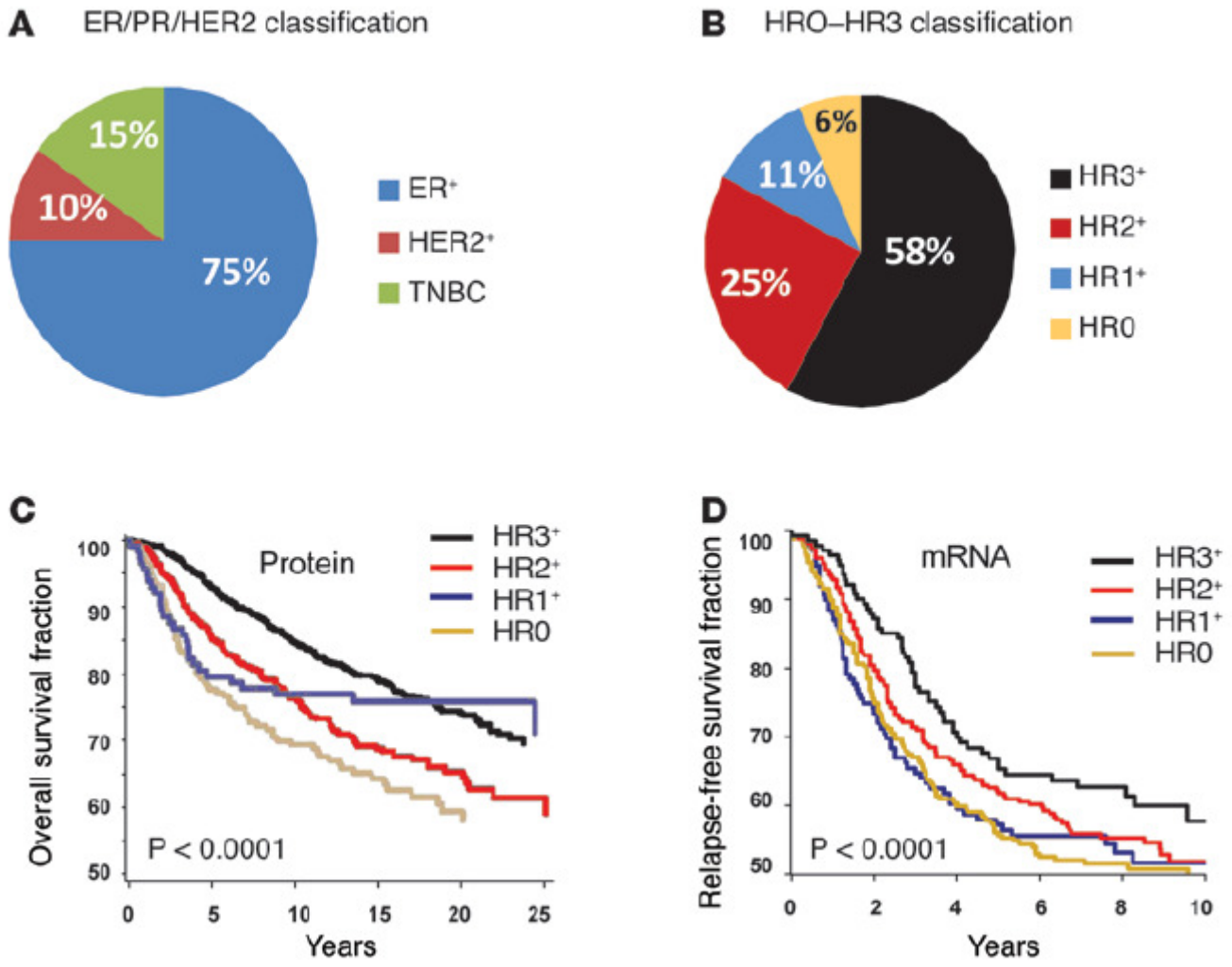


HR0 (ER-AR-VDR-)

HR1 (ER+,VDR+, or AR+)

HR2 (ER+AR+,AR+VDR+ or ER+VDR+)

HR3 (ER+VDR+AR+)



Examination of 3,157 human breast tumors revealed that these HR subtypes were distinct from the current classification scheme, which is based on ER,PR and HER2. Patient outcomes were best when tumors expressed all three hormone receptors (HR3) and worst when they expressed none (HR0)

Balancing between classic morphology and molecular classification

- There will be no morphology versus molecular but personalized medicine is based on a combined morphological-molecular pathology report including classical morphology (HE/IHC/ISH) and diverse molecular analyses.

Where are we today (at least at our Institution)?

- ER, PR and HER2 status are the major drivers of clinical decision making regarding the type of systemic therapy.
- These 3 biomarkers in conjunction with histologic grade/mitotic count could be used to infer luminal, HER2 and TN subtypes .
- But given current options for systemic therapy, need to sub-classify beyond ER,PR and HER2 in clinical practice is debatable.
- Clinicians are increasingly thinking about breast cancers by their molecular subtype.