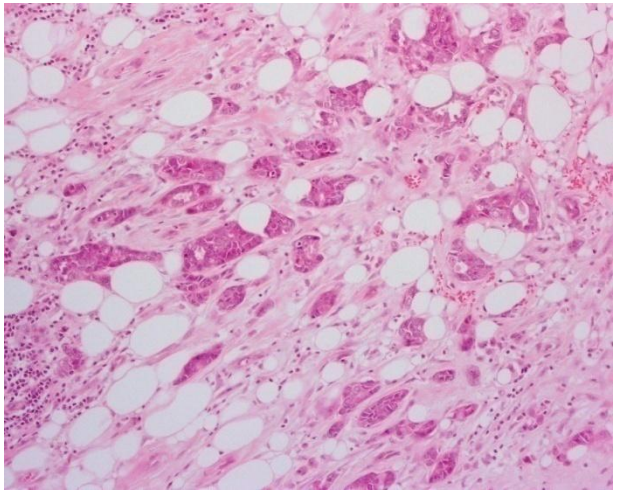
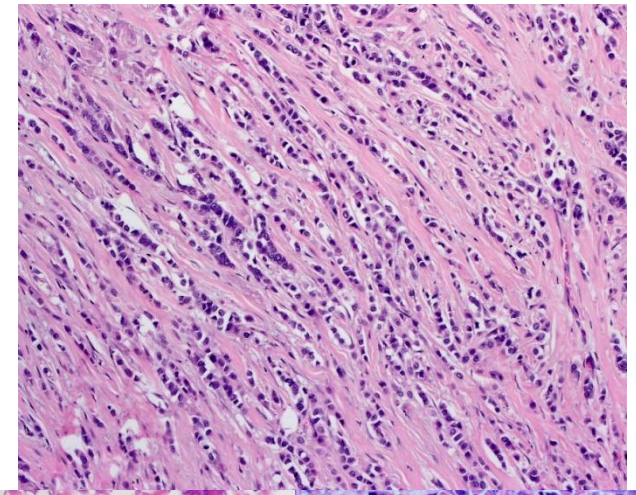
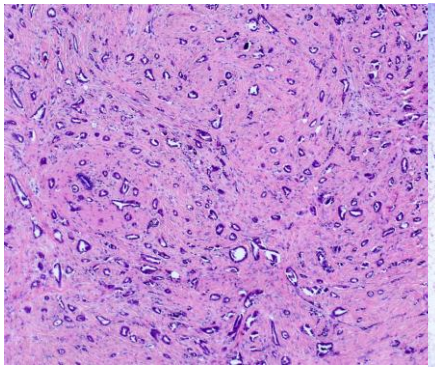
Breast Cancer:
Molecular Classification

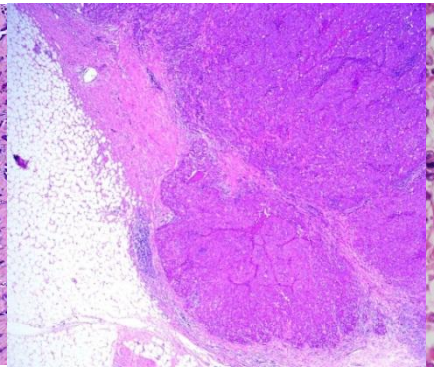
Prof. Fernando Schmitt
Medical Faculty of Porto University, Porto, Portugal
IPATIMUP
General Secretary of the International Academy of Cytology

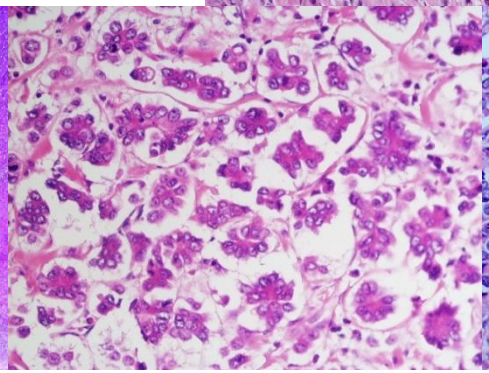
Heterogeneity of Breast Cancer

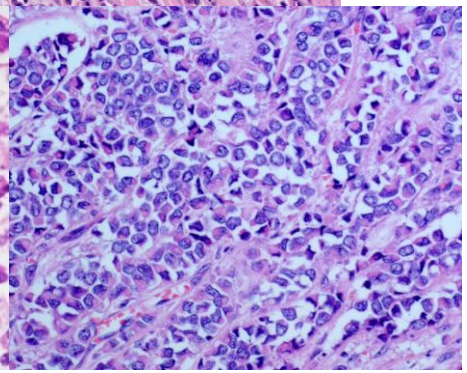


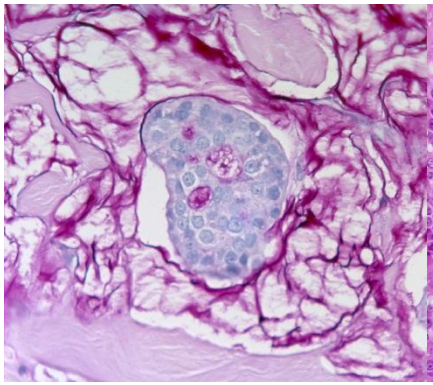


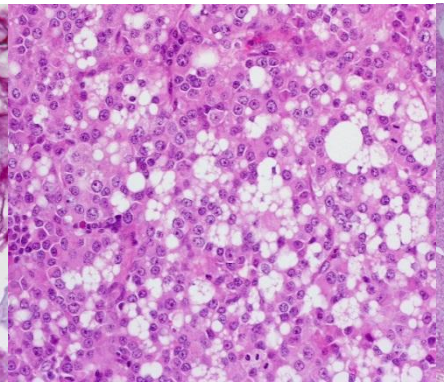


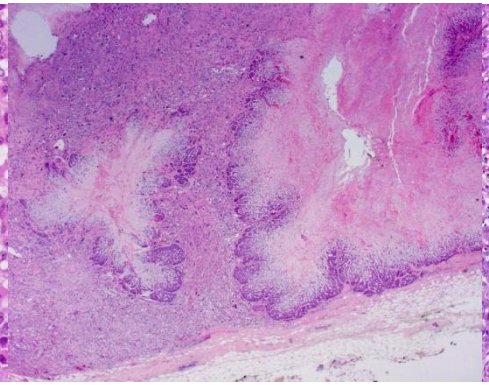


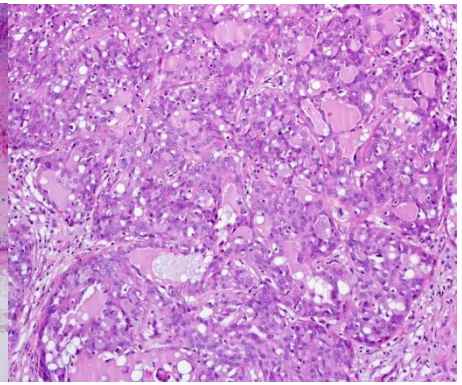








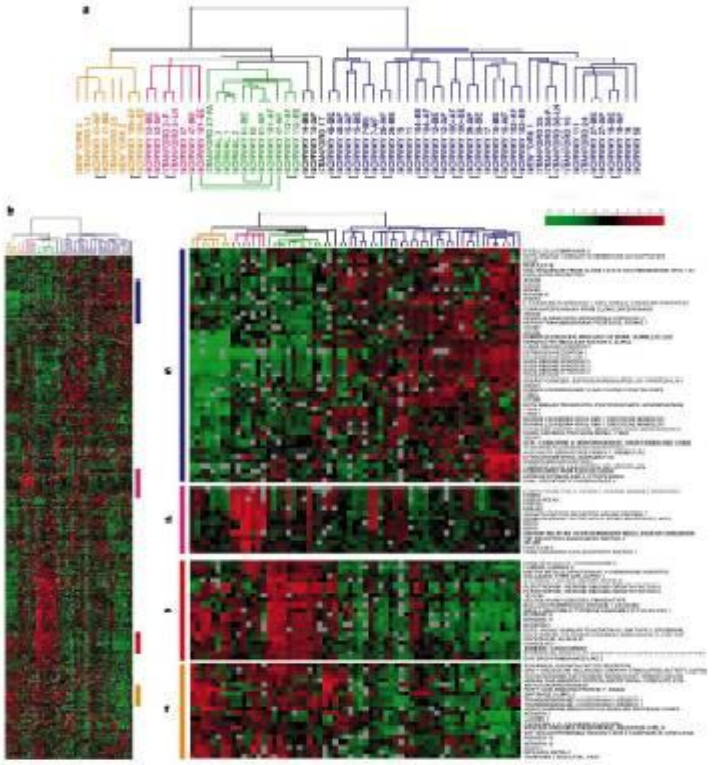




letters to nature

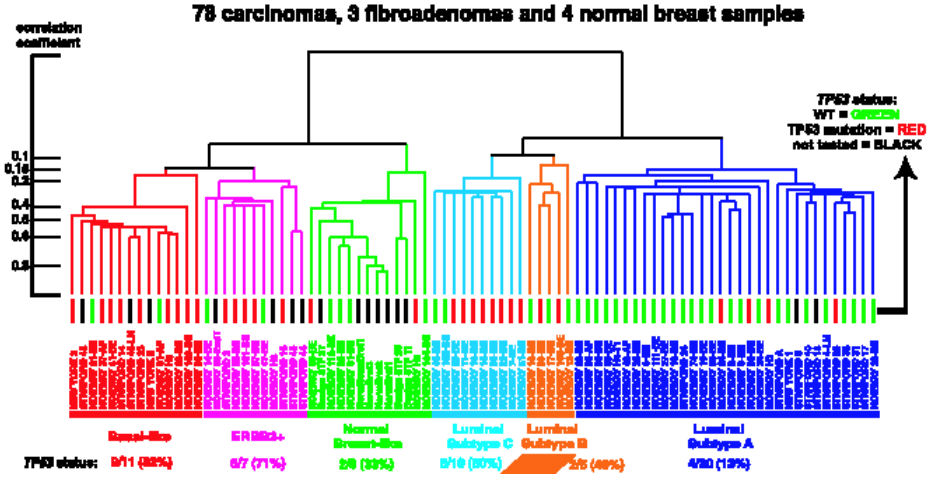
Molecular portraits of human breast tumours

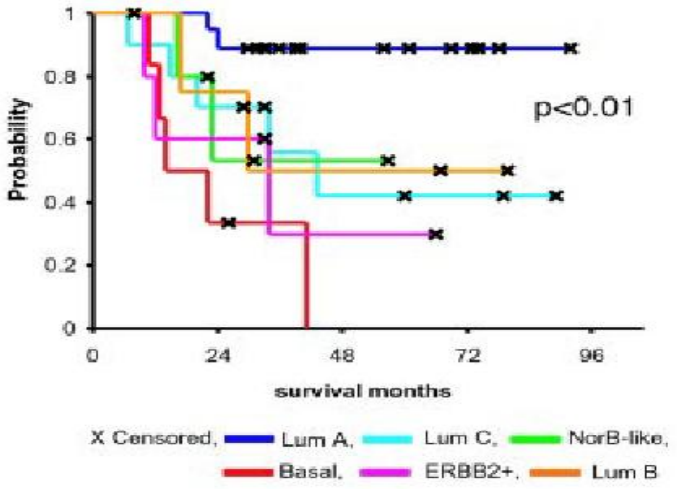
Charles M. Perou*†, Therese Sørlie†‡, Michael B. Eisen*, Matt van de Rijn§, Stefanie S. Jeffrey‖, Christian A. Rees*, Jonathan R. Pollack¶, Douglas T. Ross¶, Hilde Johnsen‡, Lars A. Akslen#, Øystein Fluge☆, Alexander Pergamenschikov*, Cheryl Williams*, Shirley X. Zhu§, Per E. Lønning**, Anne-Lise Børresen-Dale‡, Patrick O. Brown¶†† & David Botstein*



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

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





Molecular Classification of Breast Cancer

Prat *et al. Breast Cancer Research* 2010, **12**:R68
http://breast-cancer-research.com/content/12/5/R68

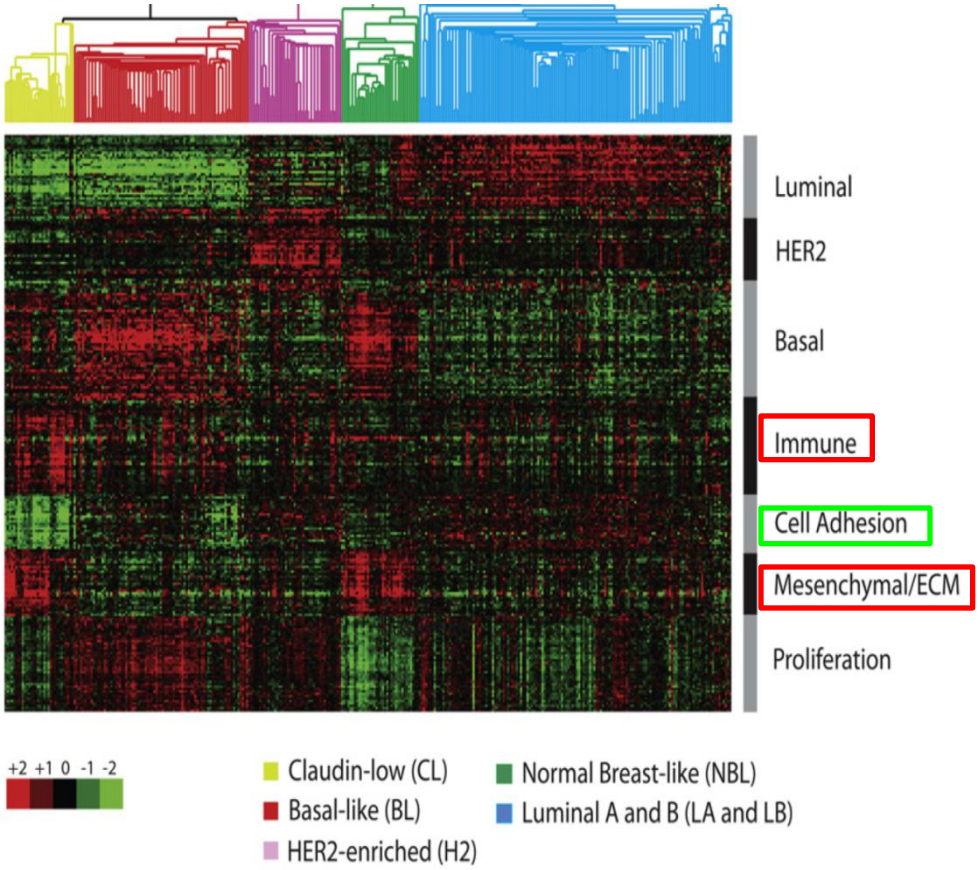
2010

Breast Cancer RESEARCH

RESEARCH ARTICLE Open Access

Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer

Aleix Prat[1,2,3], Joel S Parker[1,2†], Olga Karginova[1,2,3†], Cheng Fan[1], Chad Livasy[1,3], Jason I Herschkowitz[4], Xiaping He[1,2,3], Charles M Perou[1,2,3*]



LUMINAL A: ER+/PgR+/HER2-

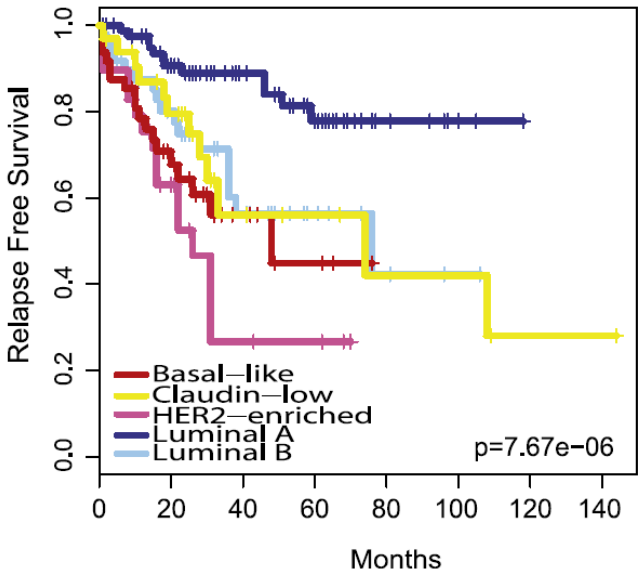
LUMINAL B: ER+/PgR+/HER2+and or Ki67+

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

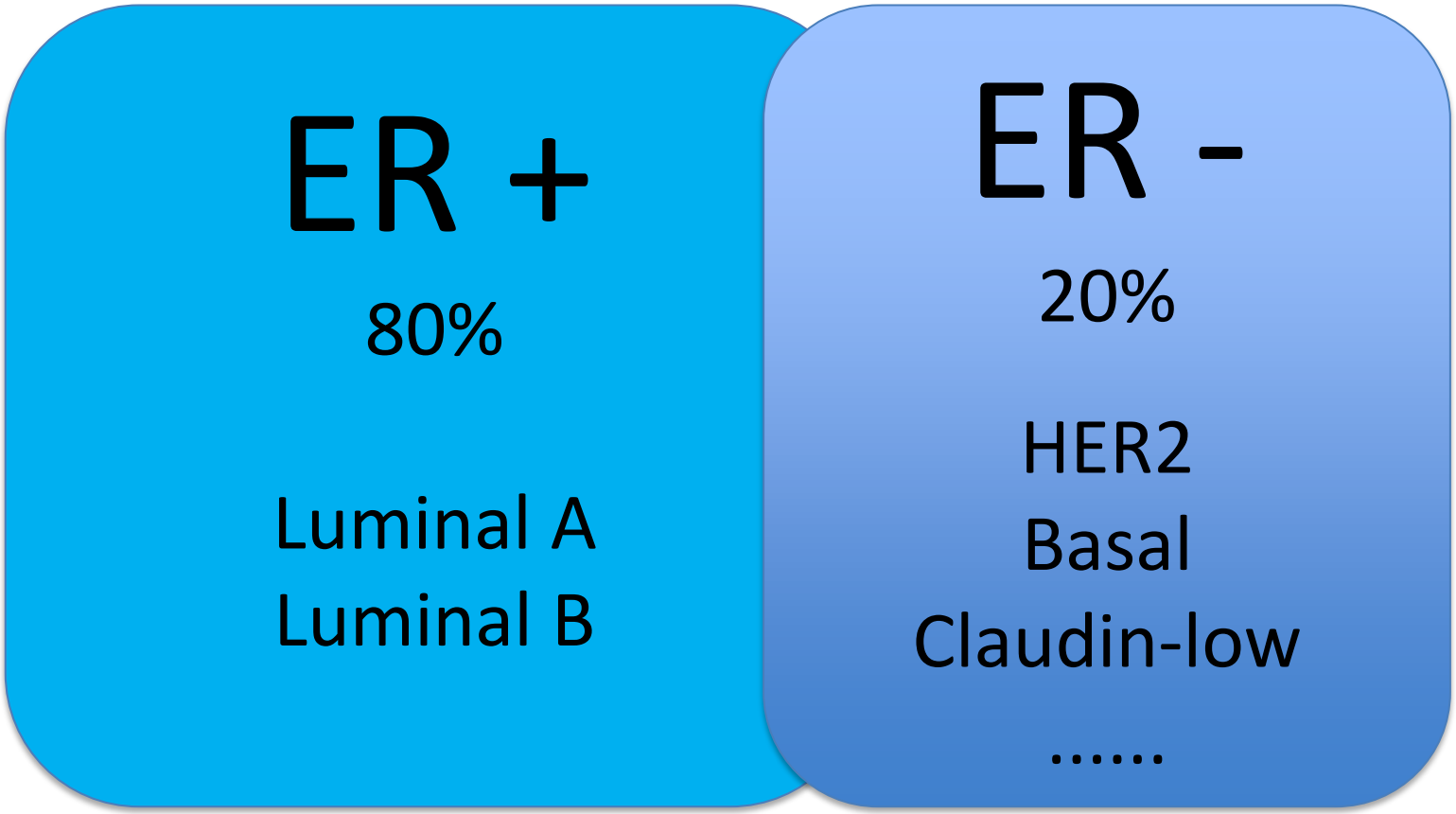
BASAL-LIKE: ER-/PgR-/HER2-/Basal Markers

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

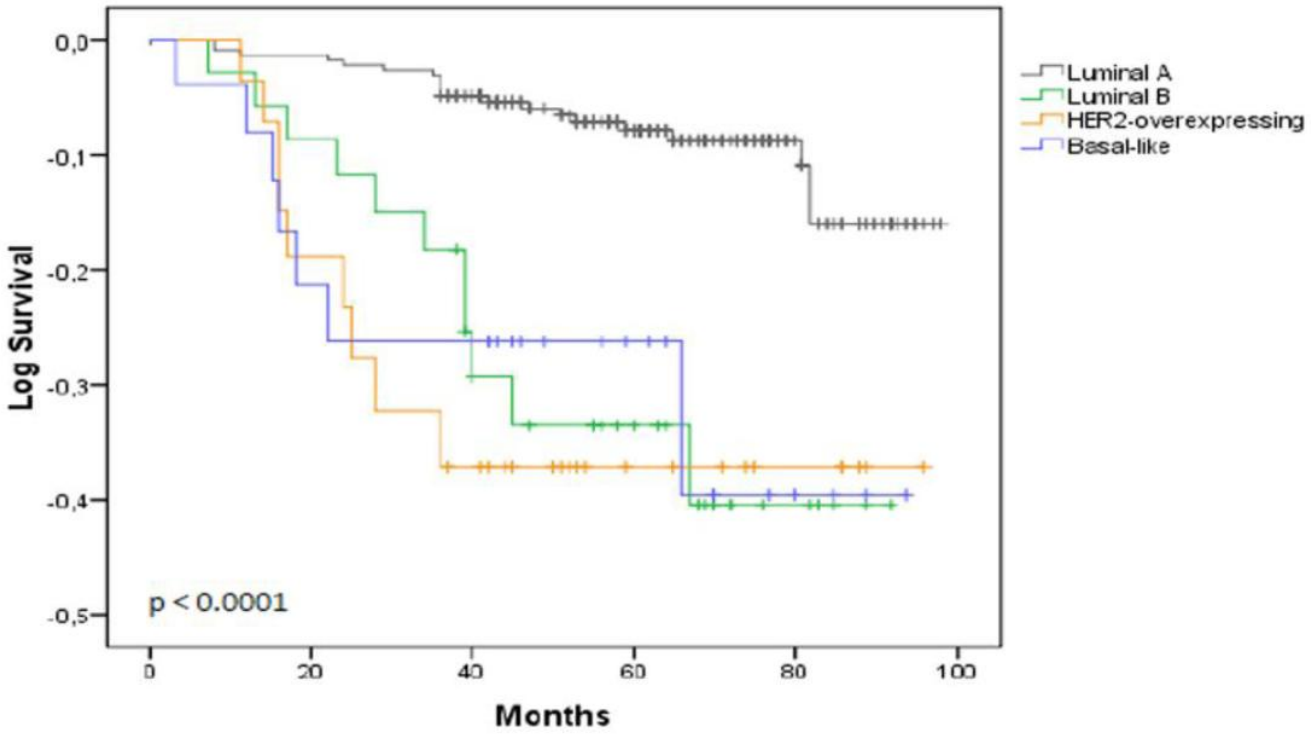
CSC features



Molecular Classification of Breast Cancer

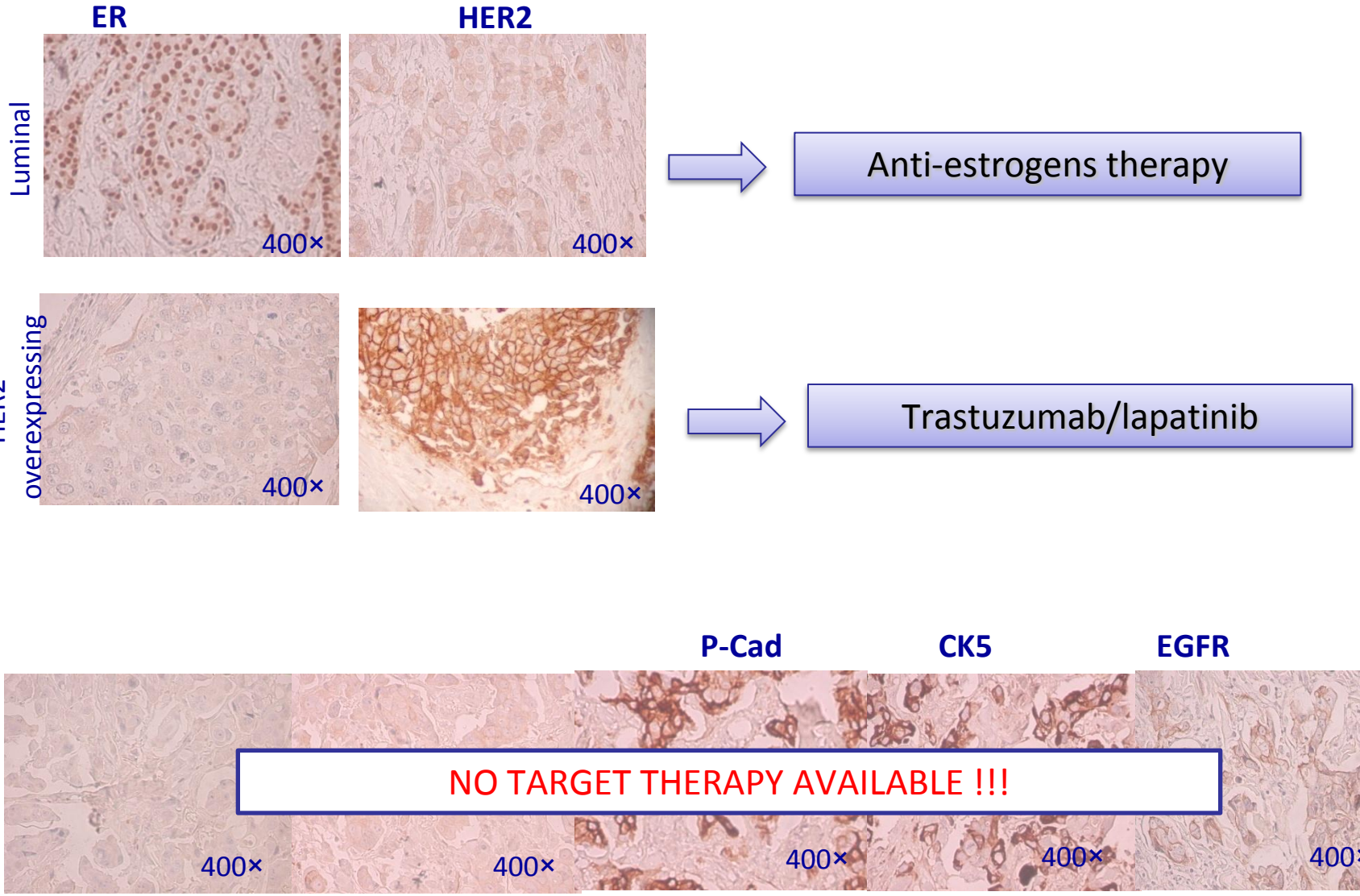


BREAST CANCER SURVIVAL ACCORDING MOLECULAR SUBTYPES

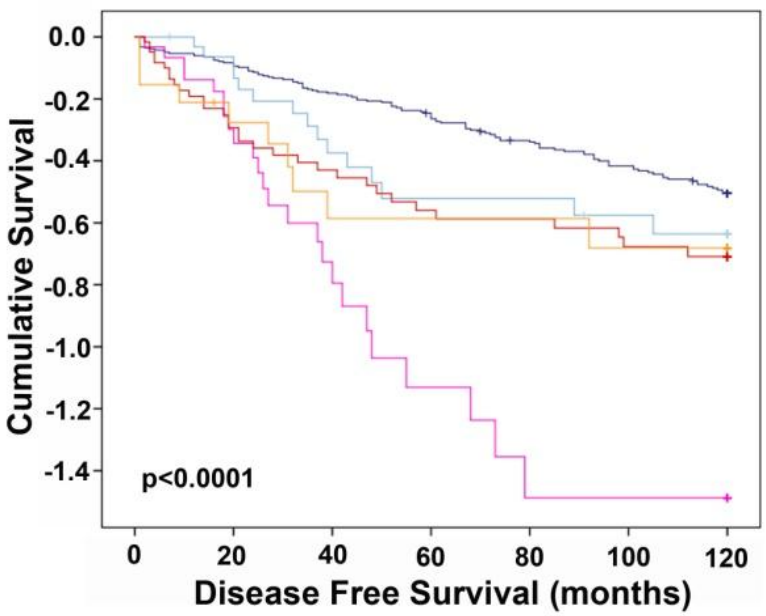


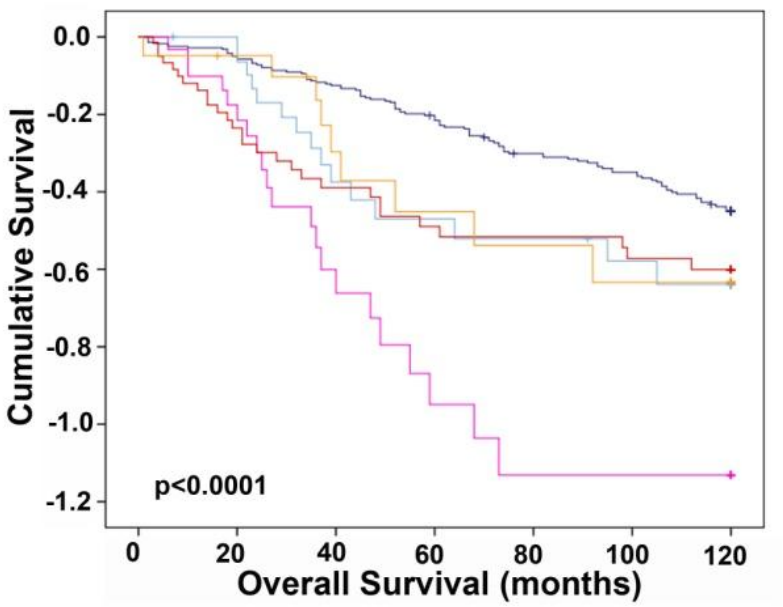
362 cases

THERAPEUTIC STRATEGIES IN BREAST CANCER



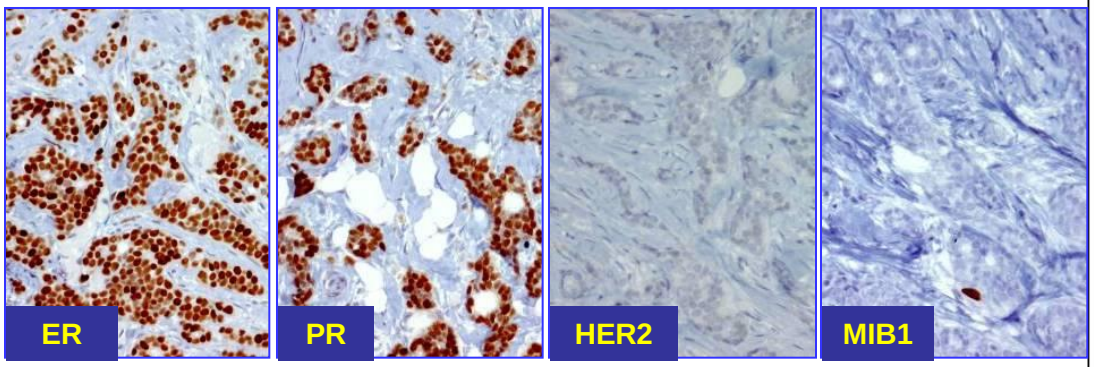
DFS OF BREAST CANCER CASES FROM IPATIMUP TUMOUR BANK



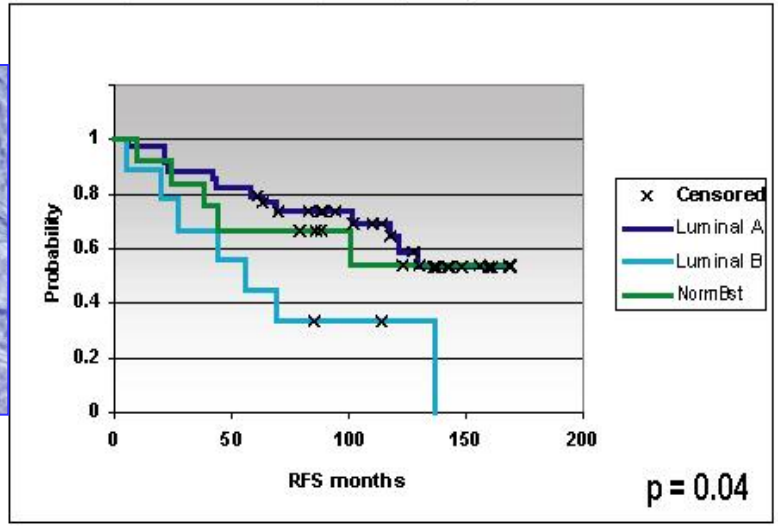


Variable	Disease-free survival			Overall survival		
	HR	(95% CI)	*P*	HR	(95% CI)	*p*
Molecular Subtype						
Luminal A (ref)	1			1		
Luminal B	1.21	0.72-2.01	**0.474**	1.38	0.83-2.31	**0.216**
HER2-OE	3.14	2.02-4.90	**<0.001**	2.89	1.80-4.63	**<0.001**
Basal-like	1.54	1.03-2.29	**0.035**	1.57	1.03-2.39	**0.035**
Unclassified	1.57	0.82-3.01	**0.171**	1.46	0.74-2.88	**0.280**

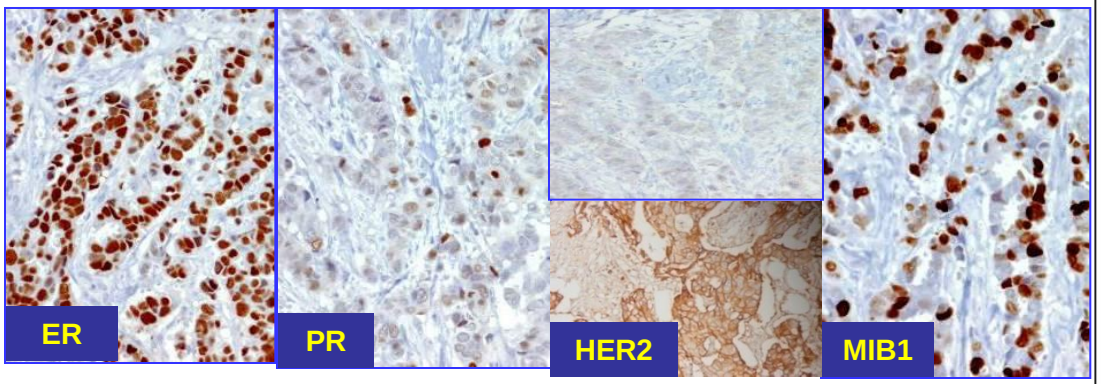
Luminal A

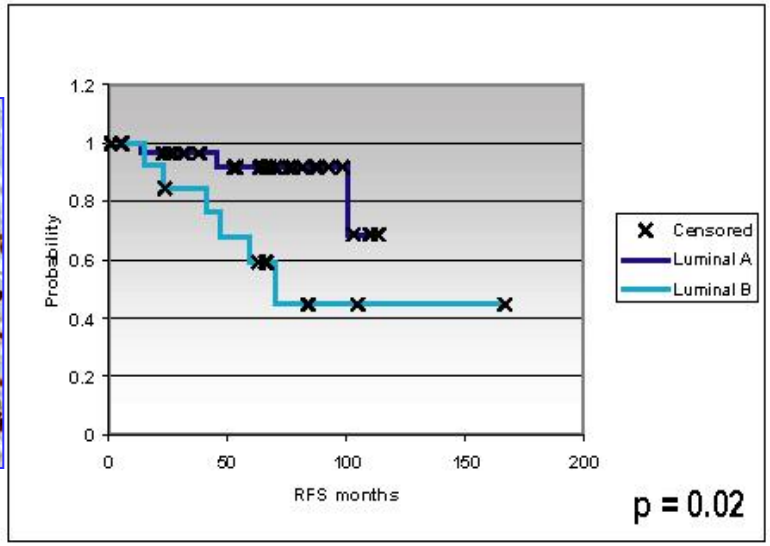


60 Sample ER+ Tamoxifen-Treated Test Set
Ma et al., Cancer Cell 5, 1-10 (2004).



Luminal B

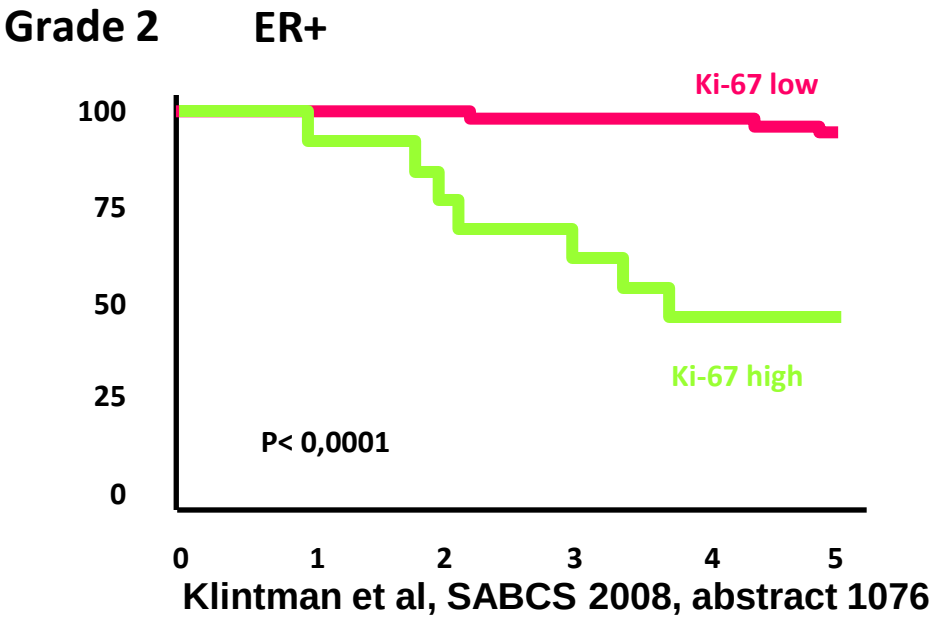




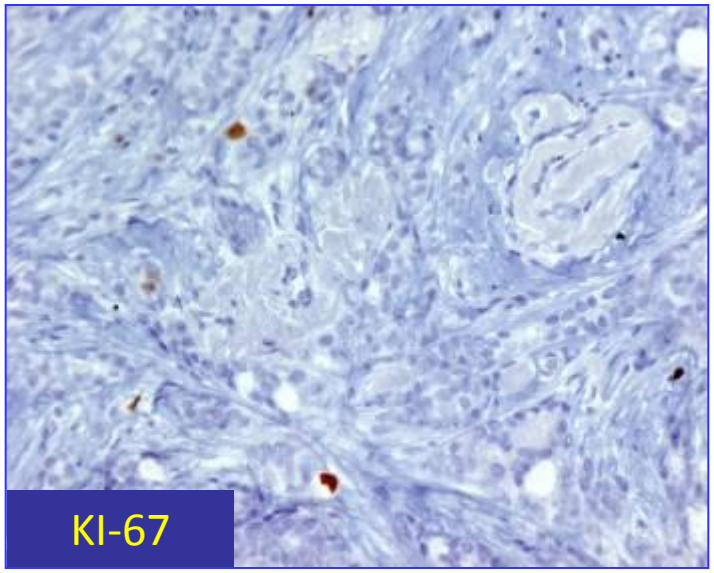
45 Tamoxifen Treated Test Set #2
Chang et al., PNAS 102, 3738-43 (2005) + UNC

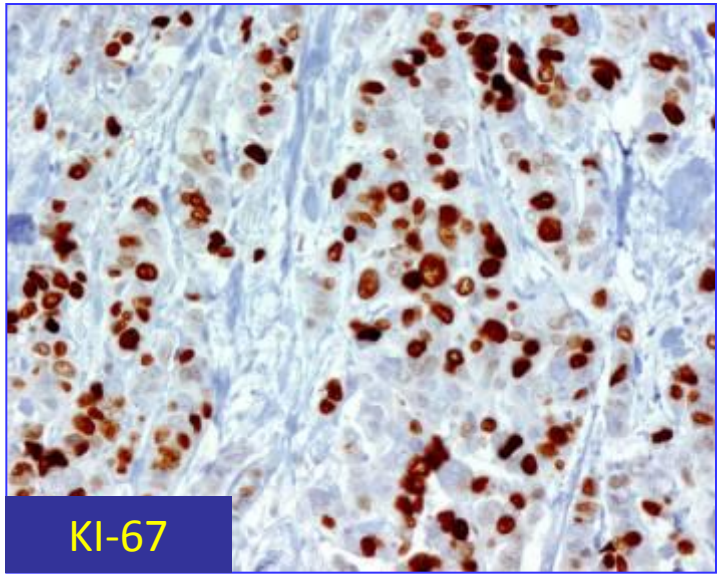
Importance of proliferation markers in ER positive breast cancer

Ki-67 is important to assess in Grade II, ER positive breast cancers, but not in ER negative or Grade III tumors

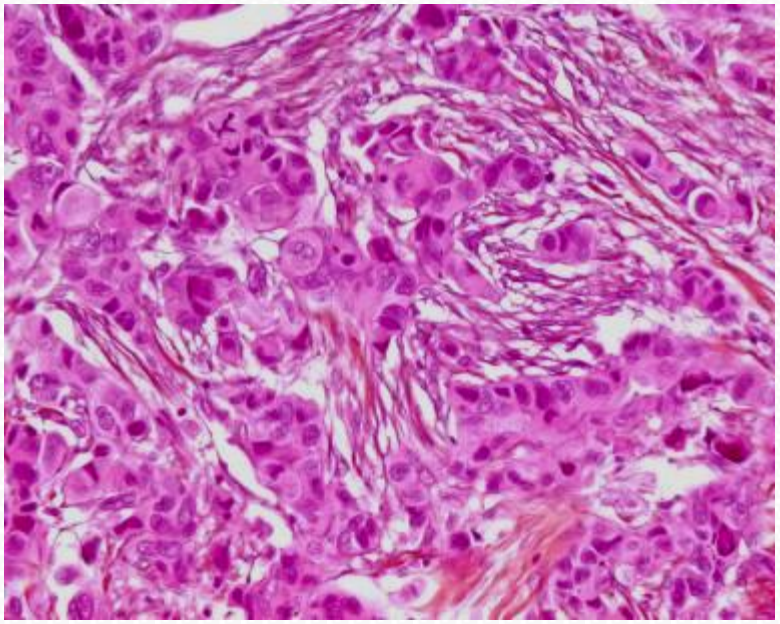


200 premenopausal LN negative patients

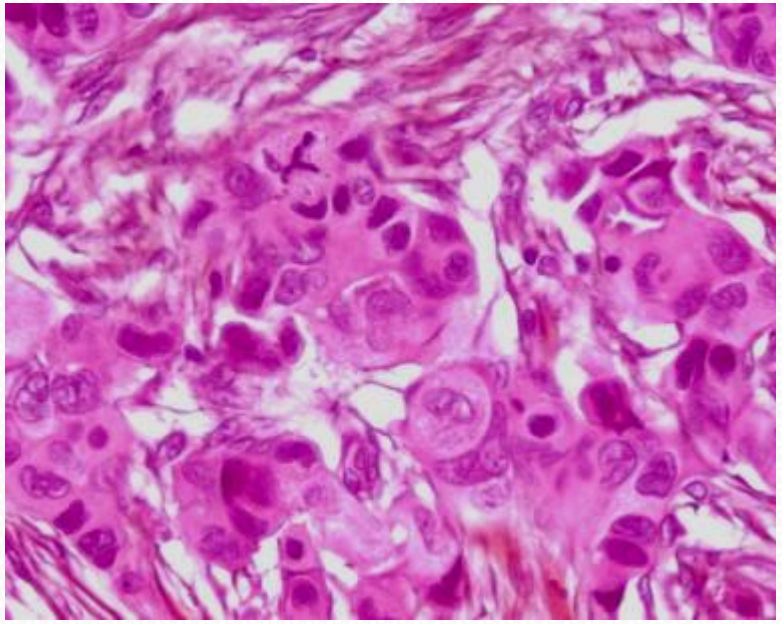


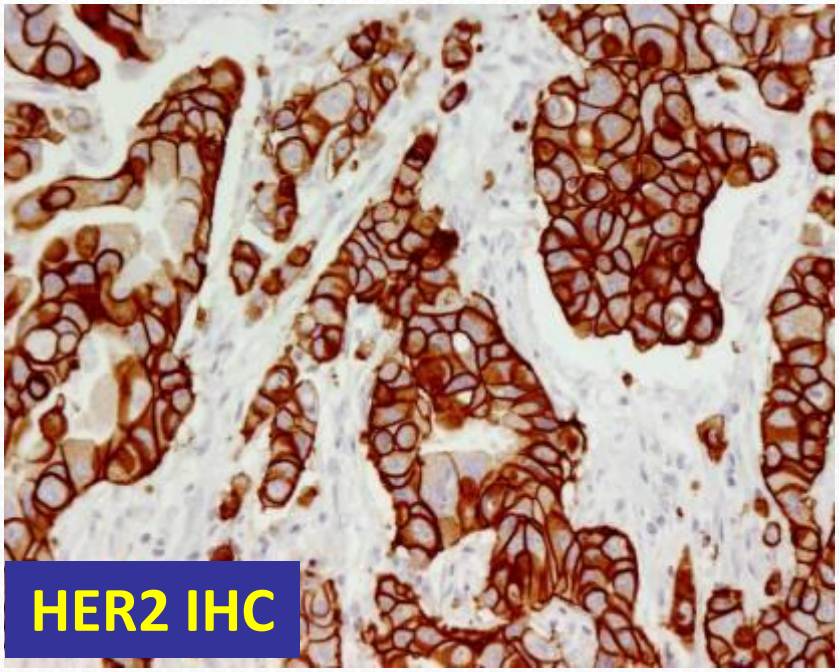


IMUNO-HISTOQUÍMICA E CLASSIFICAÇÃO MOLECULAR

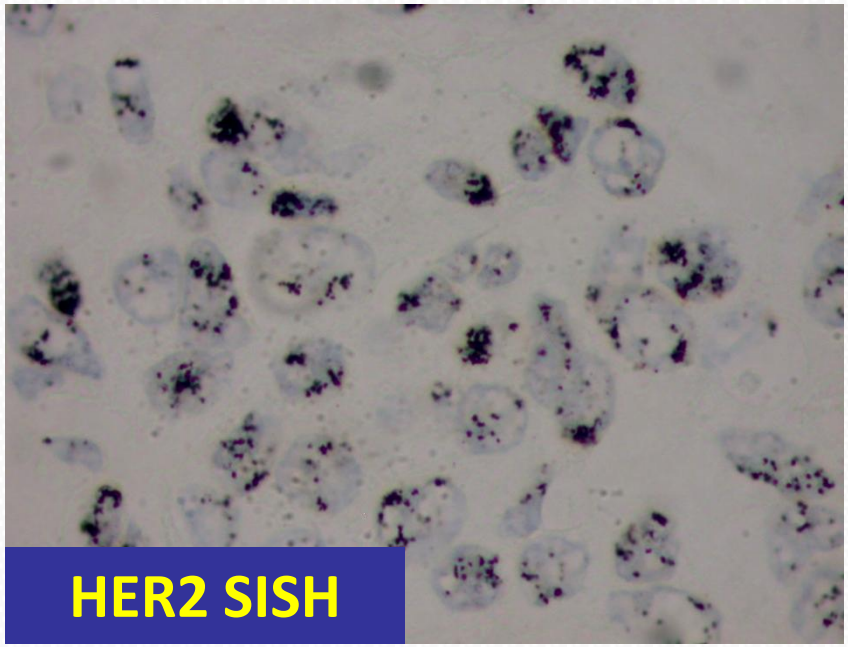


HER 2 +



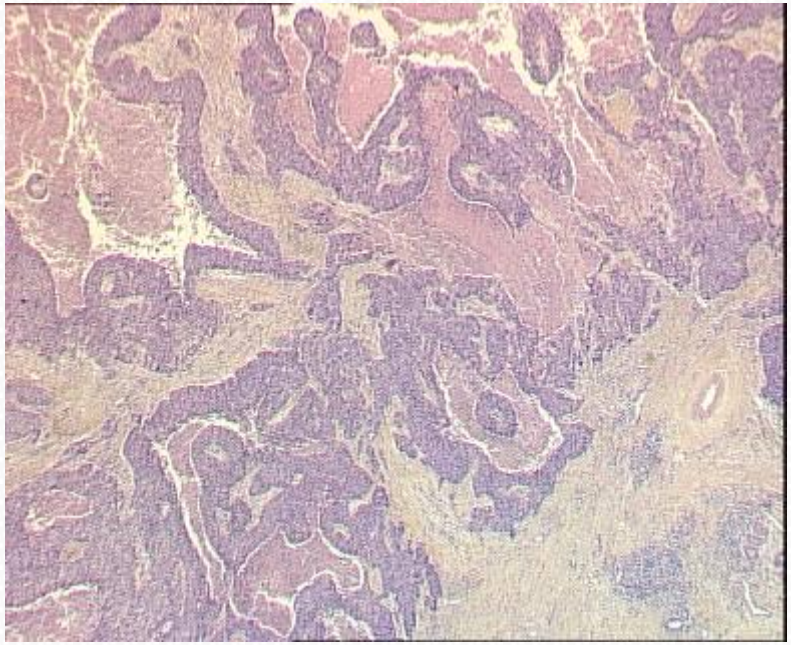


HER2 IHC

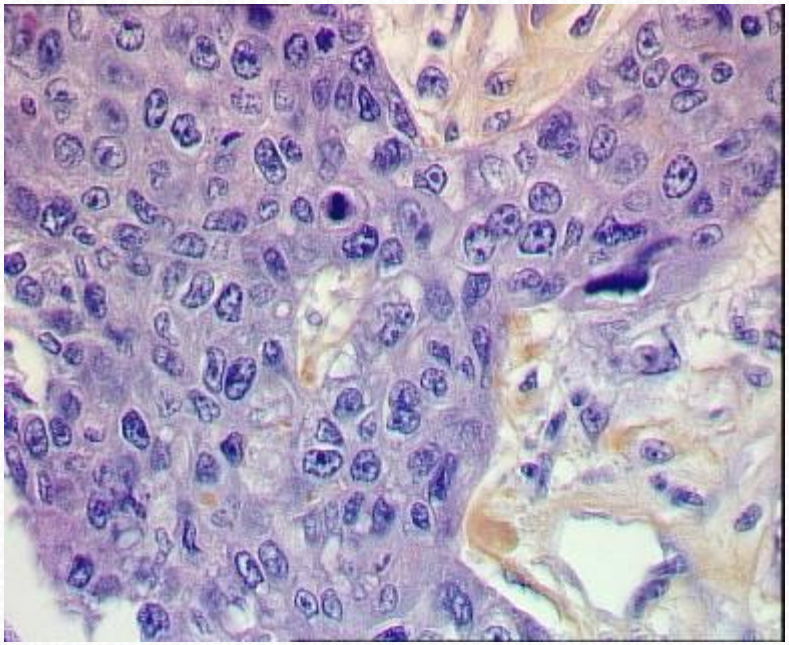


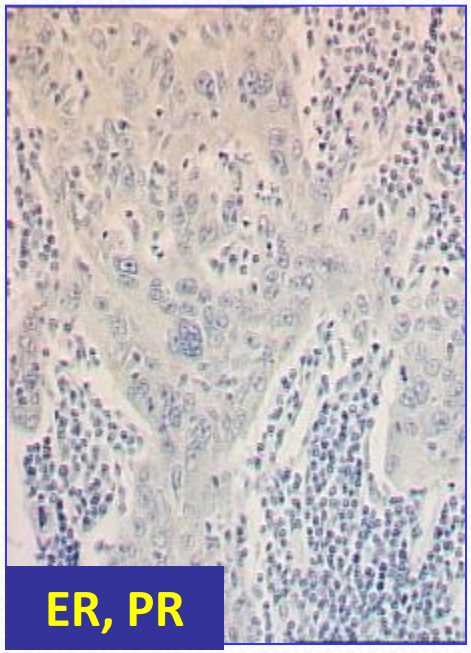
HER2 SISH

IMUNO-HISTOQUÍMICA E CLASSIFICAÇÃO MOLECULAR

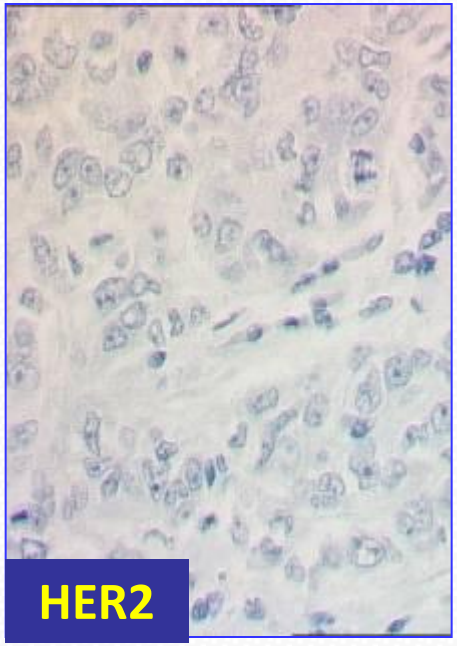


Basal/TN

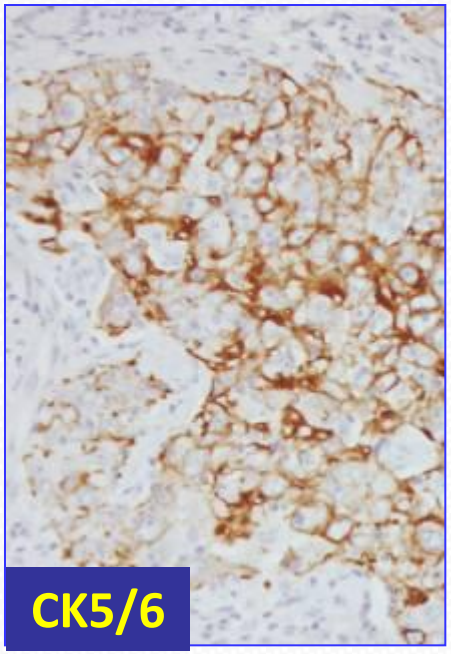




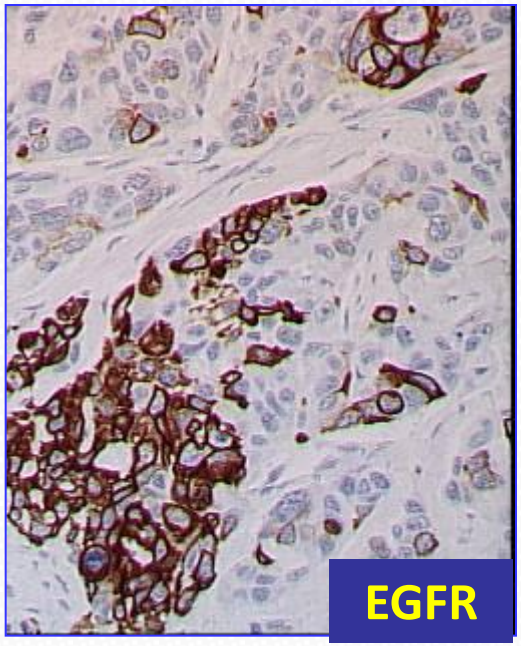
ER, PR



HER2



CK5/6

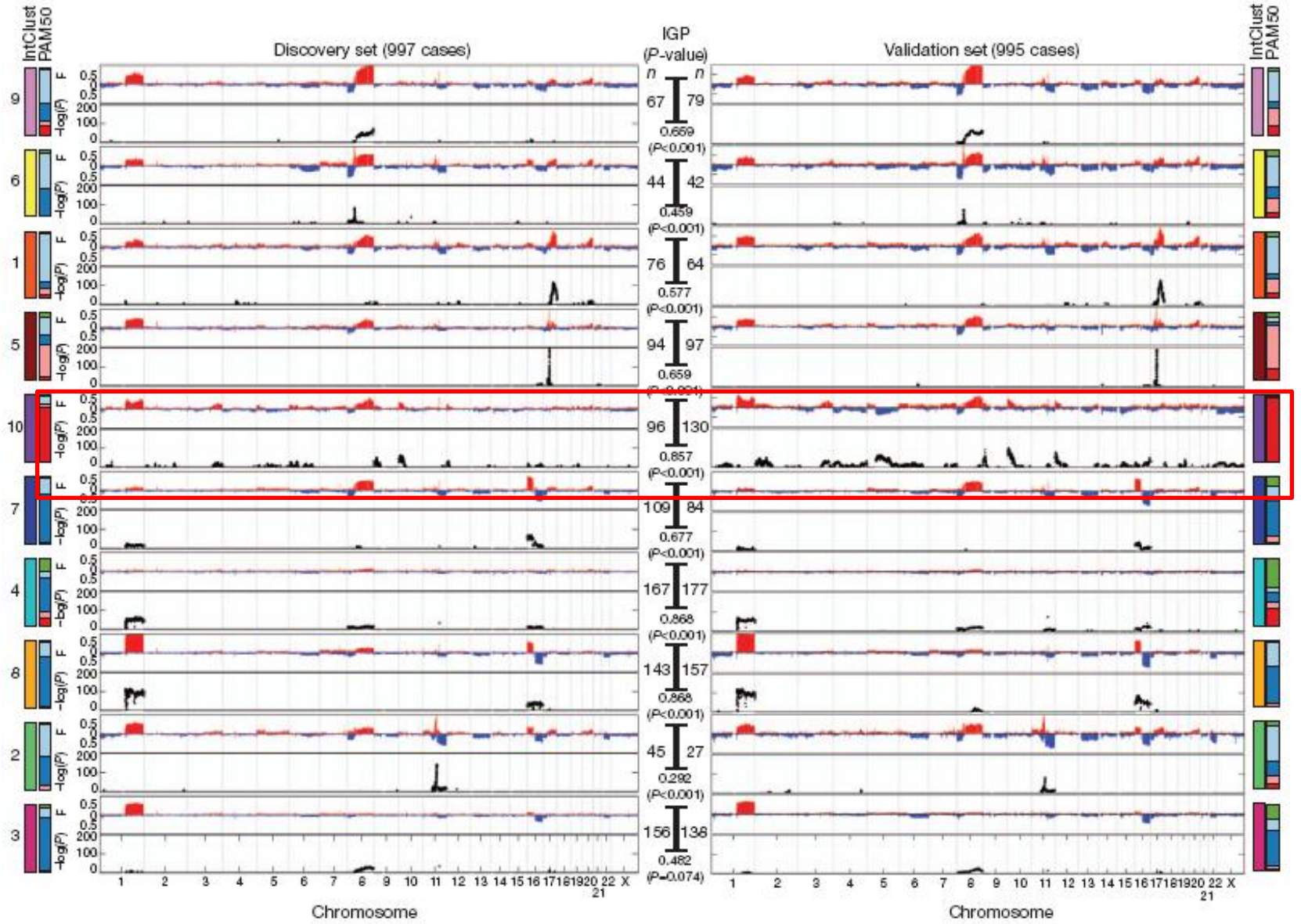


EGFR

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.

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

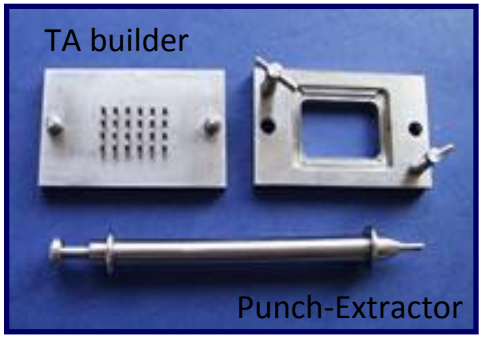


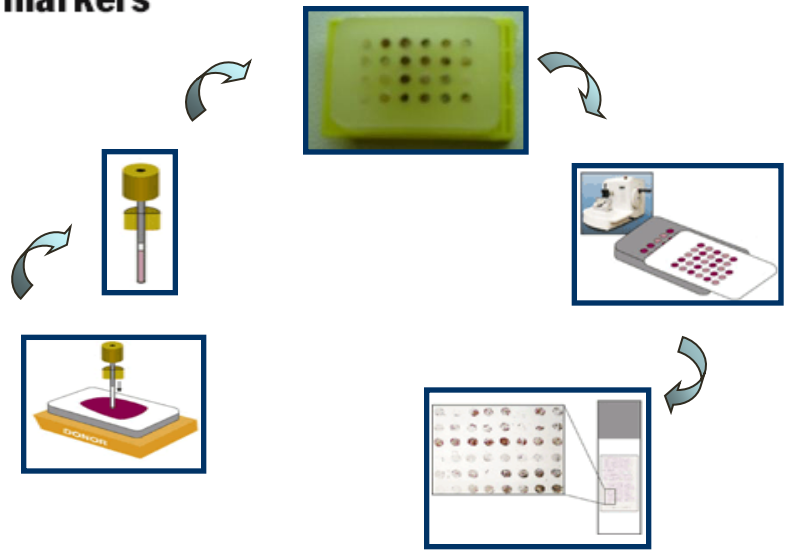
Virchows Arch (2005) 447: 688–694
DOI 10.1007/s00428-005-0010-7

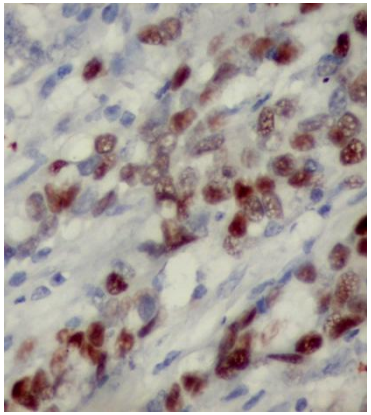
ORIGINAL ARTICLE

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

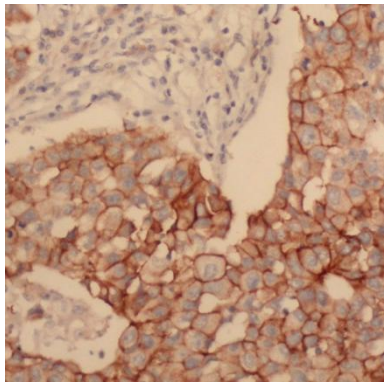
p63, cytokeratin 5, and P-cadherin: three molecular markers to distinguish basal phenotype in breast carcinomas



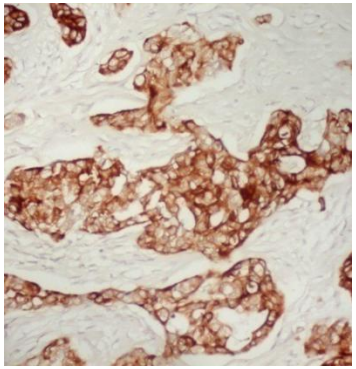




p63



P-Cad



CK 5

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

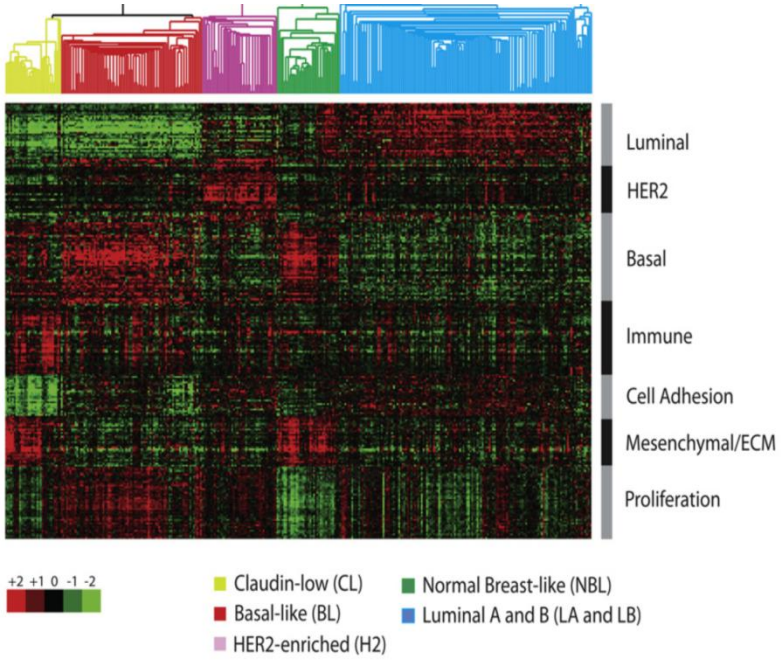
Prat *et al. Breast Cancer Research* 2010, **12**:R68
http://breast-cancer-research.com/content/12/5/R68

2010 Breast Cancer RESEARCH

RESEARCH ARTICLE Open Access

Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer

Aleix Prat[1,2,3], Joel S Parker[1,2†], Olga Karginova[1,2,3†], Cheng Fan[1], Chad Livasy[1,3], Jason I Herschkowitz[4], Xiaping He[1,2,3], Charles M Perou[1,2,3*]



- 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

Contents lists available at SciVerse ScienceDirect

The Breast

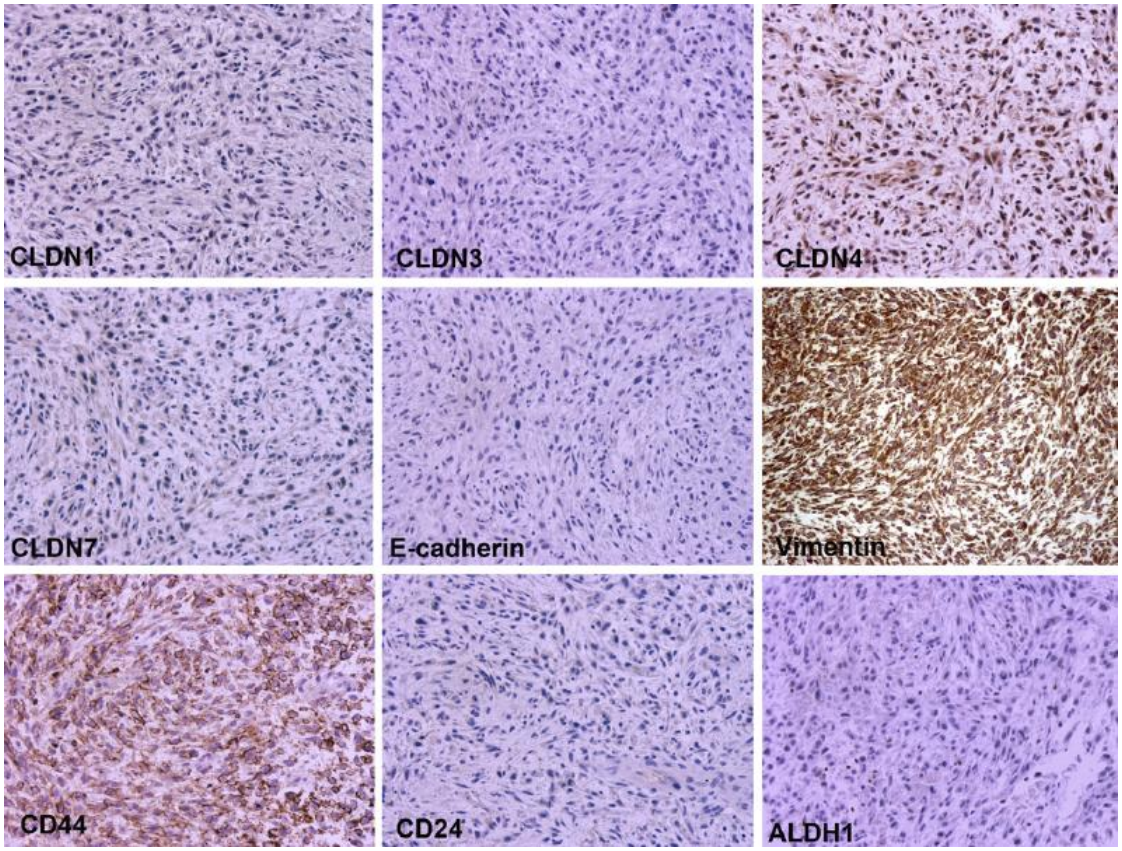
journal homepage: www.elsevier.com/brst

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

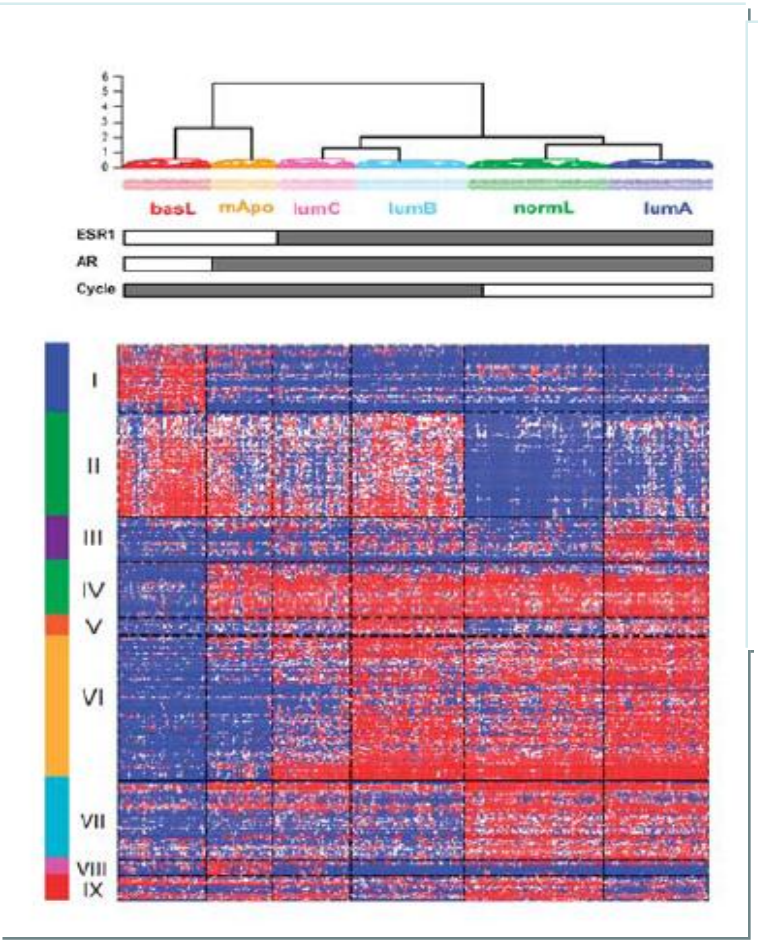


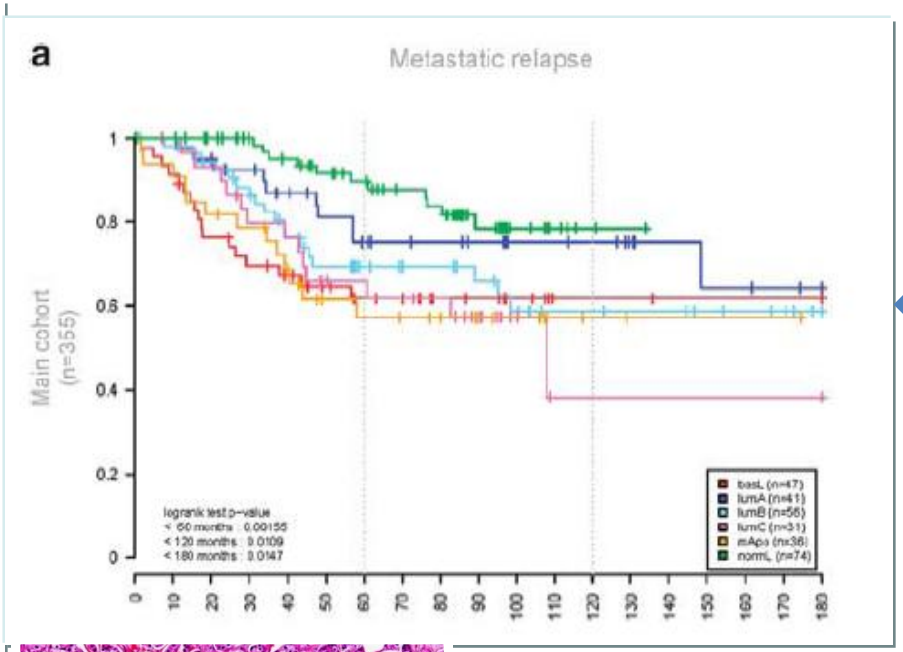
Molecular Apocrine

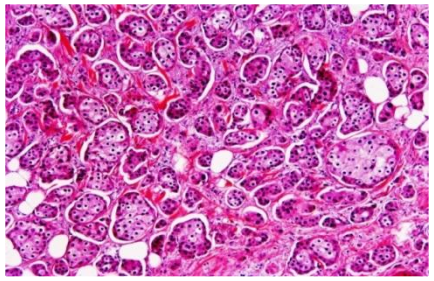
Benign and malignant apocrine lesions of the breast

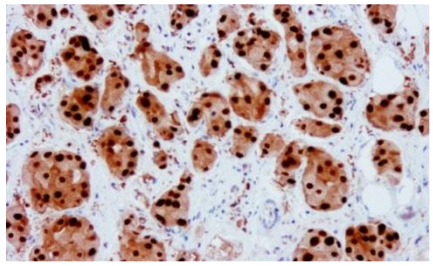
Expert Rev. Anticancer Ther. 12(2), 215–221 (2012)

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









Basal-like and TNBC
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- What is a triple-negative breast cancer?
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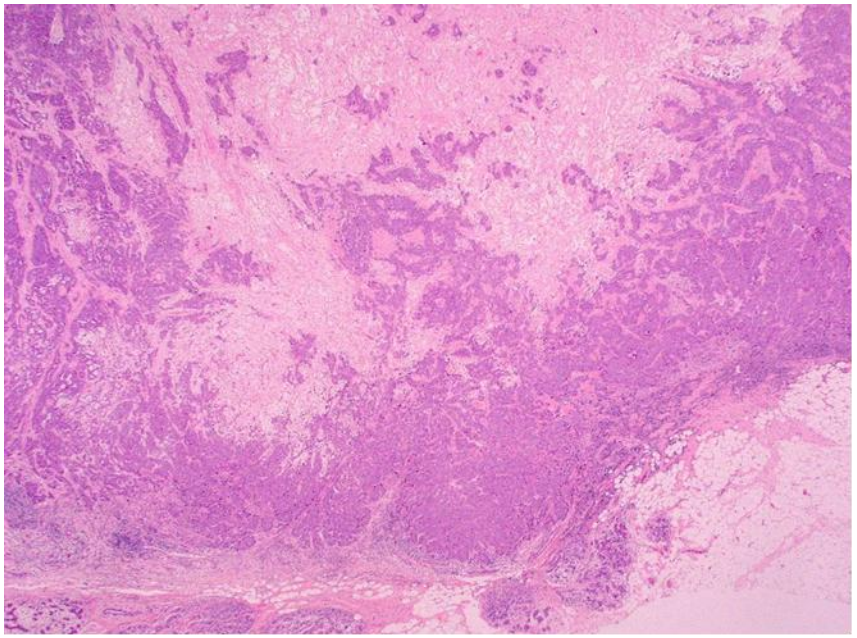

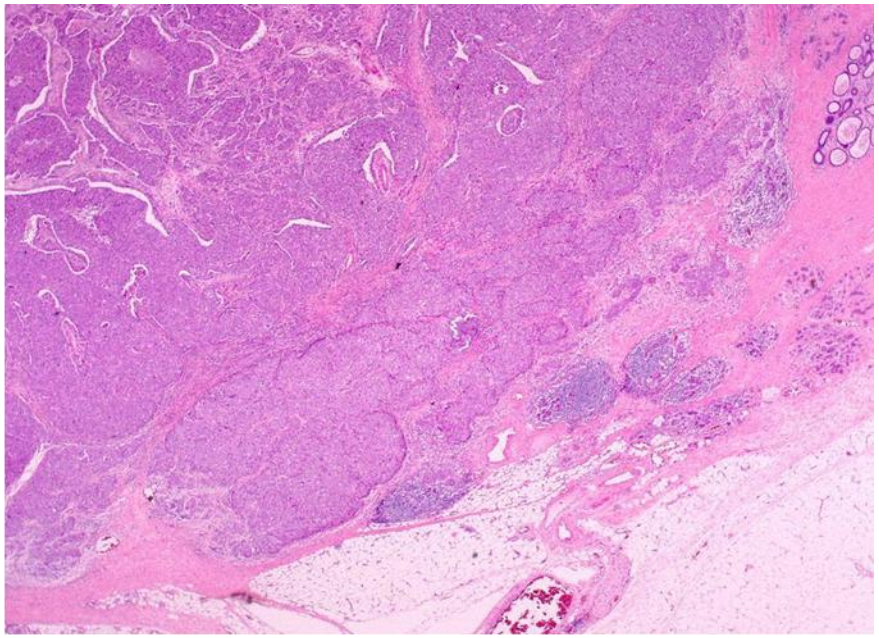

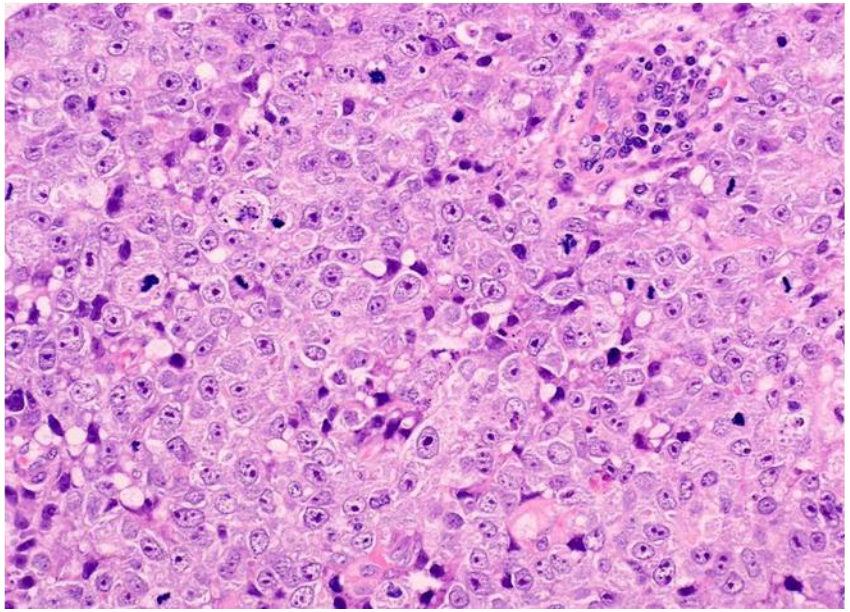

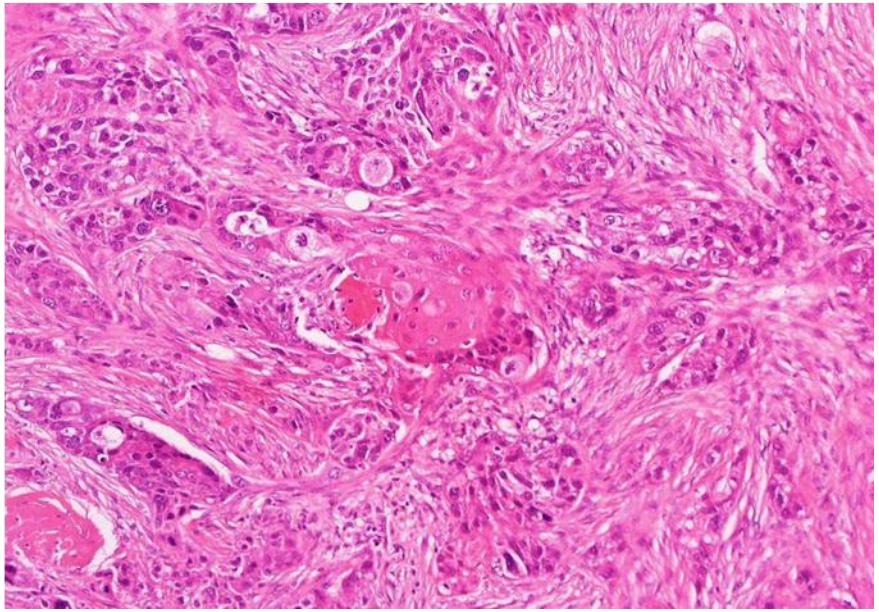

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- Therapeutic targets

Basal-like breast cancer: origin in luminal progenitors

nature medicine

Aberrant luminal progenitors as the candidate target population for basal tumor development in *BRCA1* mutation carriers

Elgene Lim[1,2,9], François Vaillant[1,9], Di Wu[1,2], Natasha C Forrest[1], Bhupinder Pal[1], Adam H Hart[3], Marie-Liesse Asselin-Labat[1], David E Gyorki[1,2], Teresa Ward[1], Audrey Partanen[4], Frank Feleppa[4], Lily I Huschtscha[5], Heather J Thorne[6], kConFab[7], Stephen B Fox[6], Max Yan[6], Juliet D French[8], Melissa A Brown[8], Gordon K Smyth[1], Jane E Visvader[1,9] & Geoffrey J Lindeman[1,2,4,9]

Cell Stem Cell
Article
Cell PRESS

***BRCA1* Basal-like Breast Cancers Originate from Luminal Epithelial Progenitors and Not from Basal Stem Cells**

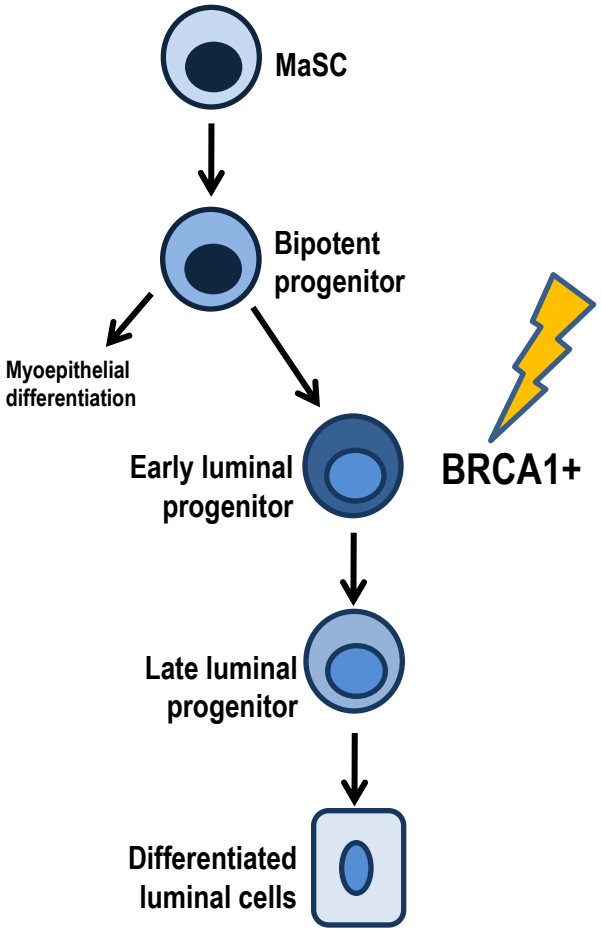
Gemma Molyneux,[1] Felipe C. Geyer,[1] Fiona-Ann Magnay,[1] Afshan McCarthy,[1] Howard Kendrick,[1] Rachael Natrajan,[1] Alan MacKay,[1] Anita Grigoriadis,[2] Andrew Tutt,[2] Alan Ashworth,[1] Jorge S. Reis-Filho,[1] and Matthew J. Smalley[1,*]
[1]The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK
[2]Breakthrough Breast Cancer Research Unit, Guy's Hospital, King's Health Partners AHSC, London SE1 9RT, UK
*Correspondence: matthew.smalley@icr.ac.uk
DOI 10.1016/j.stem.2010.07.010

Breast Cancer Res Treat
DOI 10.1007/s10549-009-0565-0

PRECLINICAL STUDY

BRCA1 transcriptionally regulates genes associated with the basal-like phenotype in breast cancer

Julia J. Gorski · Colin R. James · Jennifer E. Quinn · Gail E. Stewart · Kieran Crosbie Staunton · Niamh E. Buckley · Fionnuala A. McDyer · Richard D. Kennedy · Richard H. Wilson · Paul B. Mullan · D. Paul Harkin



Oncogene (2006), 1–7

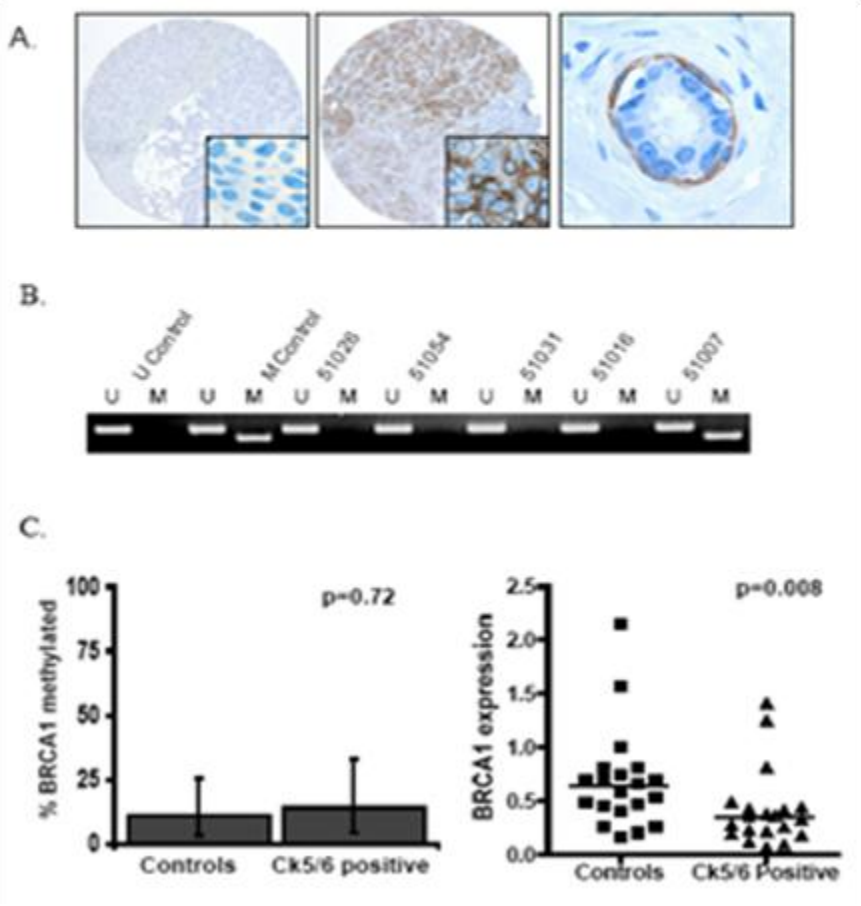
www.nature.com/onc

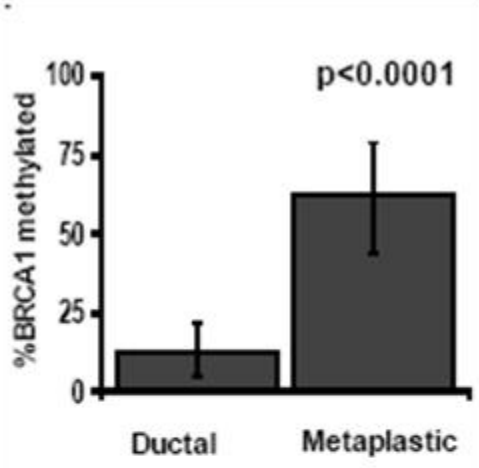
SHORT COMMUNICATION

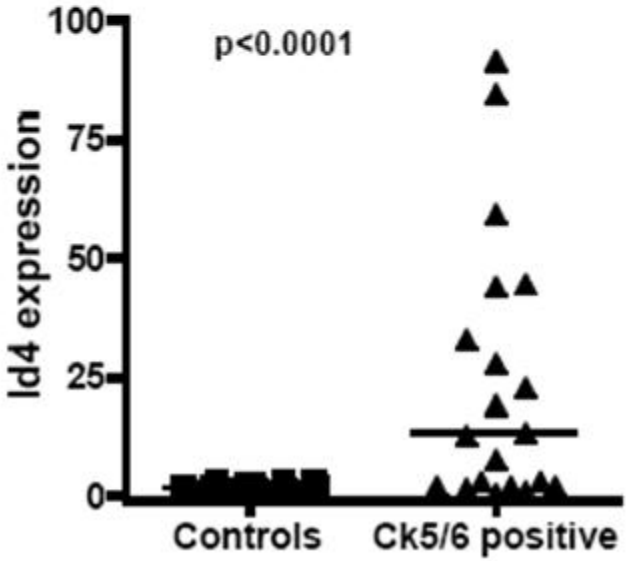
BRCA1 dysfunction in sporadic basal-like breast cancer

NC Turner[1], JS Reis-Filho[1,2], AM Russell[1], RJ Springall[3], K Ryder[3], D Steele[1], K Savage[1], CE Gillett[3], FC Schmitt[2], A Ashworth[1] and AN Tutt[1,3]

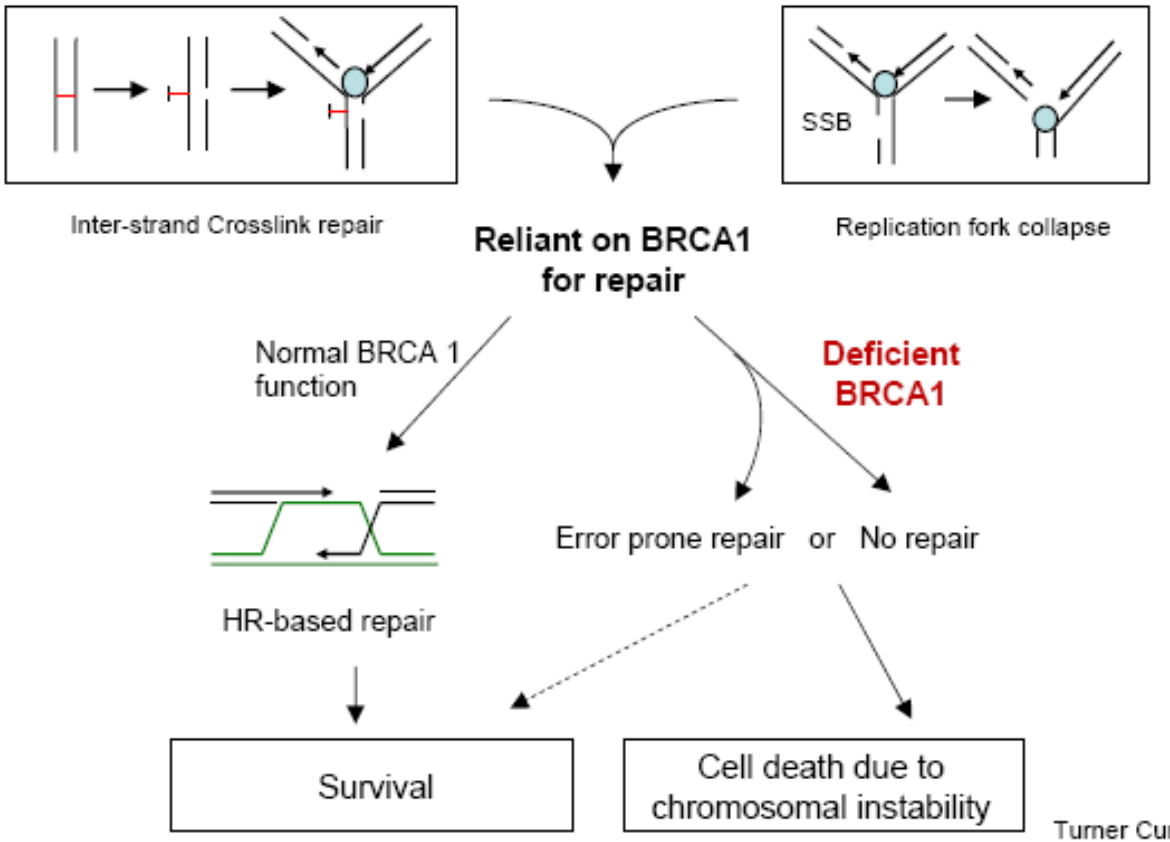
[1]*Chester Beatty Laboratories, The Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, London, UK;* [2]*Medical Faculty and IPATIMUP Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal and* [3]*Breast Pathology Laboratory, Guy's Hospital, London, UK*







BRCA1 dysfunction as a therapeutic target in triple negative and basal-like cancers



Turner Curr Opin Pharmacol 2000

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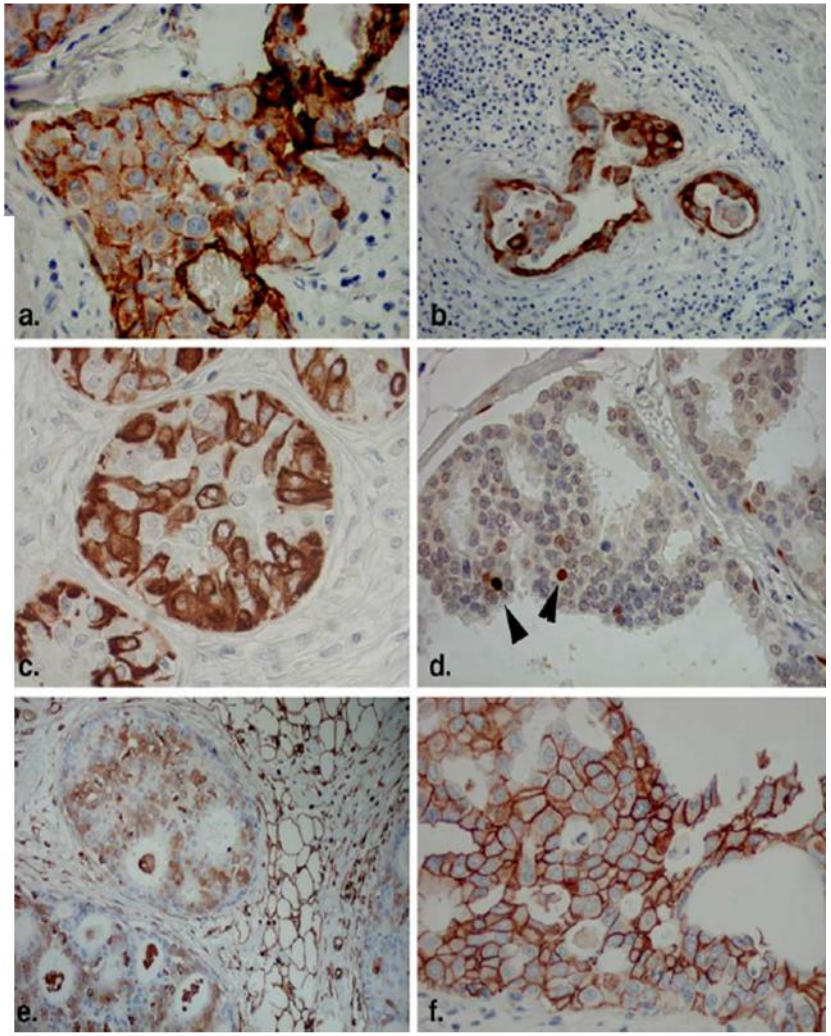
Virchows Arch (2007) 450:73–80
DOI 10.1007/s00428-006-0334-y

ORIGINAL ARTICLE

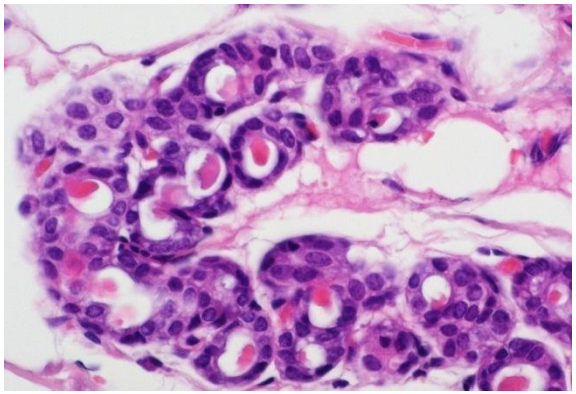
P-cadherin and cytokeratin 5: useful adjunct markers to distinguish basal-like ductal carcinomas in situ

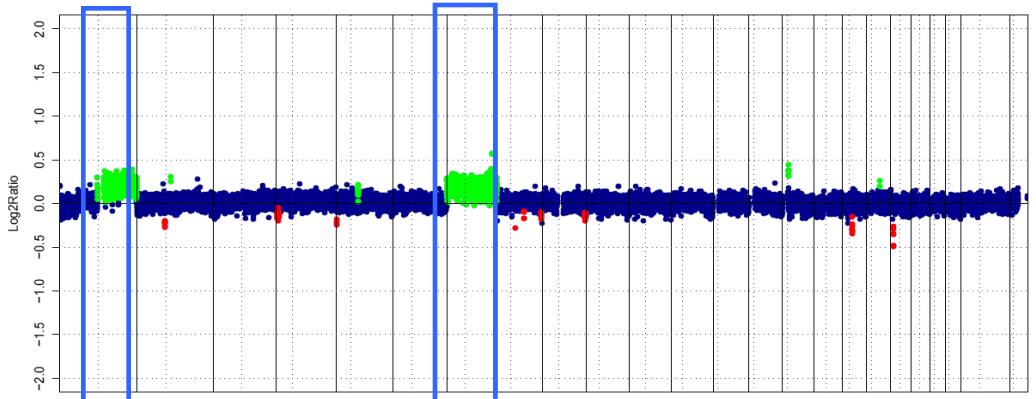
Joana Paredes · Nair Lopes · Fernanda Milanezi · Fernando C. Schmitt

	Luminal A [*n* (%)]	Luminal B [*n* (%)]	HER-2 overexpressing [*n* (%)]	Basal [*n* (%)]	*p* value
Nuclear grade					
I	11 (27.5%)	0 (0%)	0 (0%)	0 (0%)	0.0006
II	13 (32.5%)	0 (0%)	3 (15%)	1 (12.5%)	
III	16 (40.0%)	11 (100%)	17 (85%)	7 (87.5%)	
Comedo-necrosis					
Present	22 (55%)	10 (90.9%)	19 (95%)	7 (87.5%)	0.0026
Absent	18 (45%)	1 (9.1%)	1 (5%)	1 (12.5%)	
P-CD					
Positive	3 (8.1%)	1 (9.1%)	9 (45%)	6 (75%)	<0.0001
Negative	34 (91.9%)	10 (90.9%)	11 (55%)	2 (25%)	
CK5					
Positive	1 (2.6%)	0 (0%)	2 (10%)	2 (33.3%)	0.0319
Negative	37 (97.4%)	10 (100%)	18 (90%)	4 (66.7%)	
CK14					
Positive	8 (22.2%)	1 (11.1%)	2 (10%)	3 (42.9%)	0.2442
Negative	28 (77.8%)	8 (88.9%)	18 (90%)	4 (57.1%)	
P63					
Positive	1 (2.6%)	0 (0%)	1 (5.3%)	1 (12.5%)	0.5486
Negative	37 (97.4%)	9 (100%)	18 (94.7%)	7 (87.5%)	
Vimentin					
Positive	3 (7.9%)	1 (10%)	0 (0%)	0 (0%)	0.5013
Negative	35 (92.1%)	9 (90%)	18 (100%)	8 (100%)	
EGFR					
Positive	3 (8.1%)	0 (0%)	1 (5.3%)	2 (25%)	0.2601
Negative	34 (91.9%)	9 (100%)	18 (94.7%)	6 (75%)	

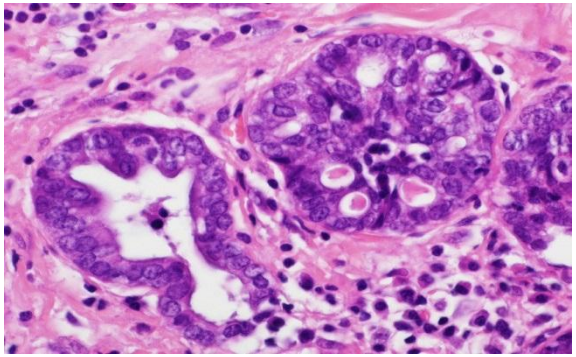


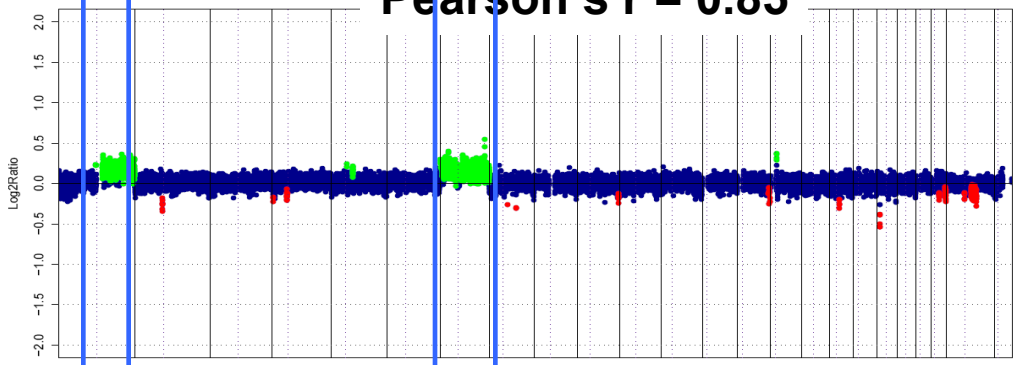
MGA



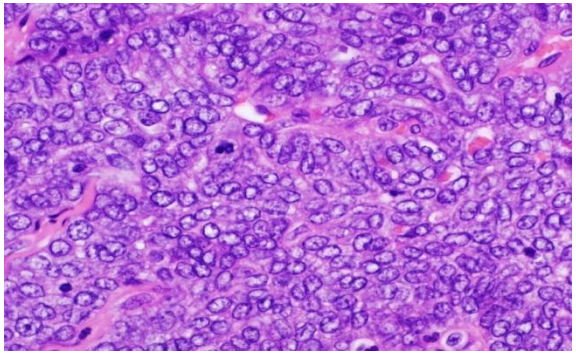


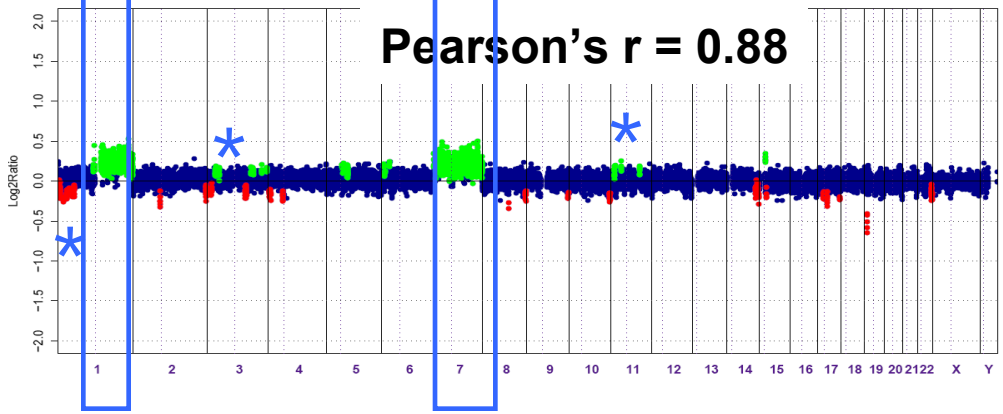
AMGA





IDC-NST





MGA is a non-obligate precursor of invasive breast cancer

Histopathology

Histopathology 2012, 60, E115–E130. DOI: 10.1111/j.1365-2559.2012.04207.x

Molecular evidence in support of the neoplastic and precursor nature of microglandular adenosis

Felipe C Geyer,[1,*] Magali Lacroix-Triki,[2] Pierre-Emmanuel Colombo,[3] Neill Patani,[1] Arnaud Gauthier,[4] Rachael Natrajan,[1] Maryou B K Lambros,[1] Ibrahim Khalifeh,[5] Constance Albarracin,[6] Sandra Orru,[7] Caterina Marchiò,[8] Anna Sapino,[8] Alan Mackay,[1] Britta Weigelt,[9] Fernando C Schmitt,[10] Jelle Wesseling,[11] Nour Sneige[4] & Jorge S Reis-Filho[1]

Molecular Evidence for Progression of Microglandular Adenosis (MGA) to Invasive Carcinoma

Sandra J. Shin, MD, Peter T. Simpson, PhD,†‡ Leonard Da Silva, MD,†‡ Janani Jayanthan, BSc,†‡§ Lynne Reid, BSc,†‡ Sunil R. Lakhani, FRCPA,†‡‖ and Paul Peter Rosen, MD**

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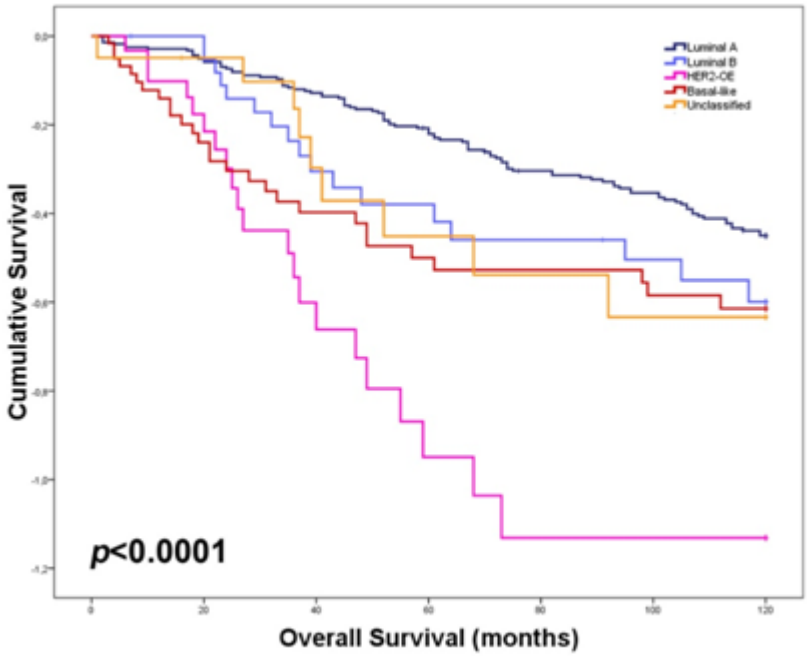
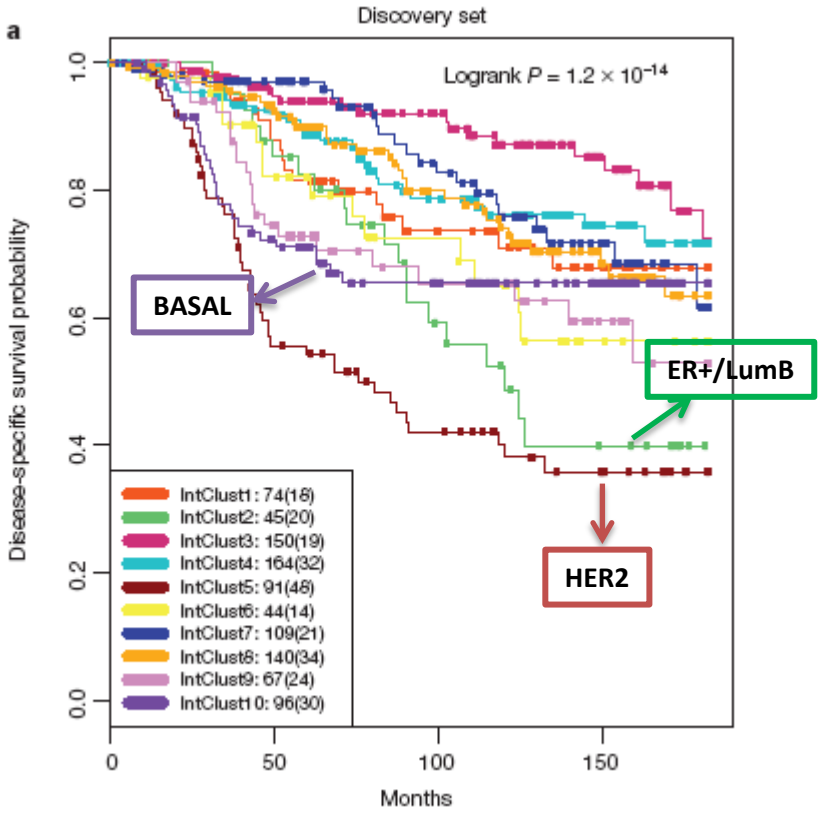
“Triple-Negative” breast carcinomas

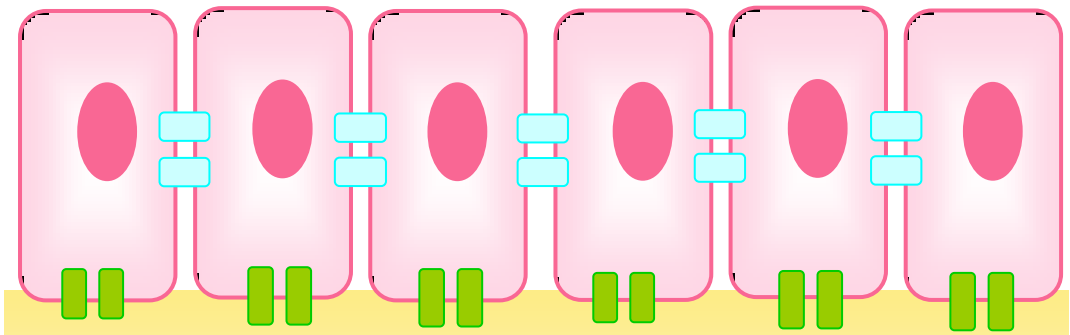


Ricardo S, JCP 2011



Aparício S, Nature 2012

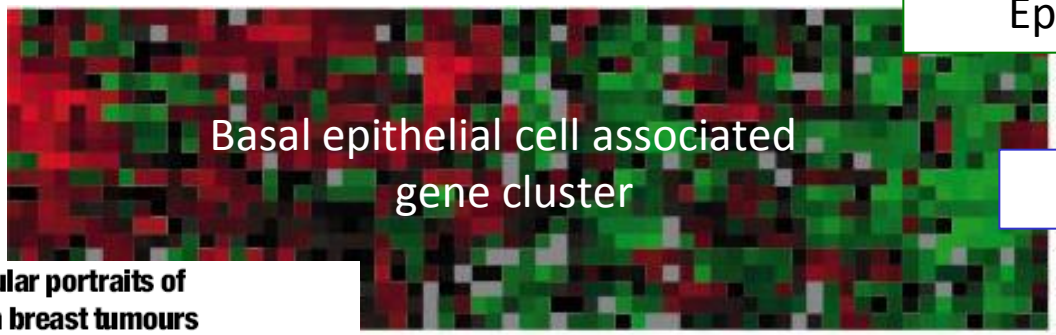
Adherens Junctions



Focal Adhesions

Integrins (cten)

Epidermal growth factor receptor



P-cadherin

HUMAN DNA-BINDING PROTEIN ABP/ZF MRNA, COMPLETE CDS
ANTILEUKOPROTEINASE
FATTY ACID BINDING PROTEIN 7, BRAIN
CHITINASE 3-LIKE 2
TRANSMEMBRANE 4 SUPERFAMILY MEMBER 1
SRY SEX-DETERMINING REGION Y-BOX 9 CAMPOMELIC DYSPLASIA
KERATIN 13
KERATIN 13
2255917
INTEGRIN, BETA 4
TROPONIN I, SKELETAL, FAST

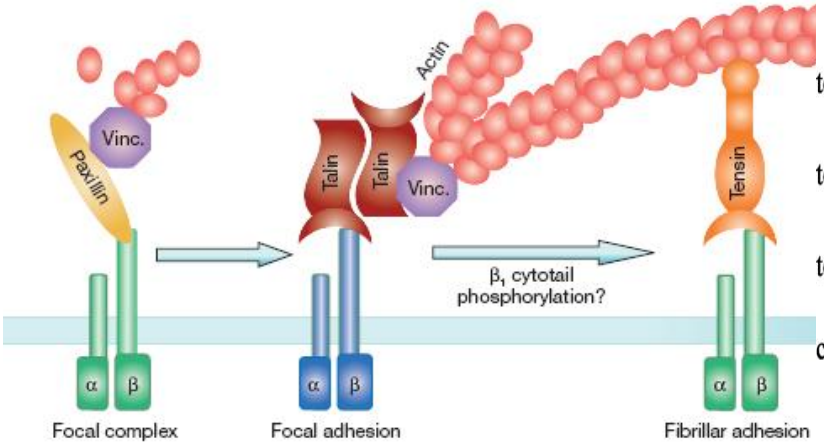
Molecular portraits of human breast tumours

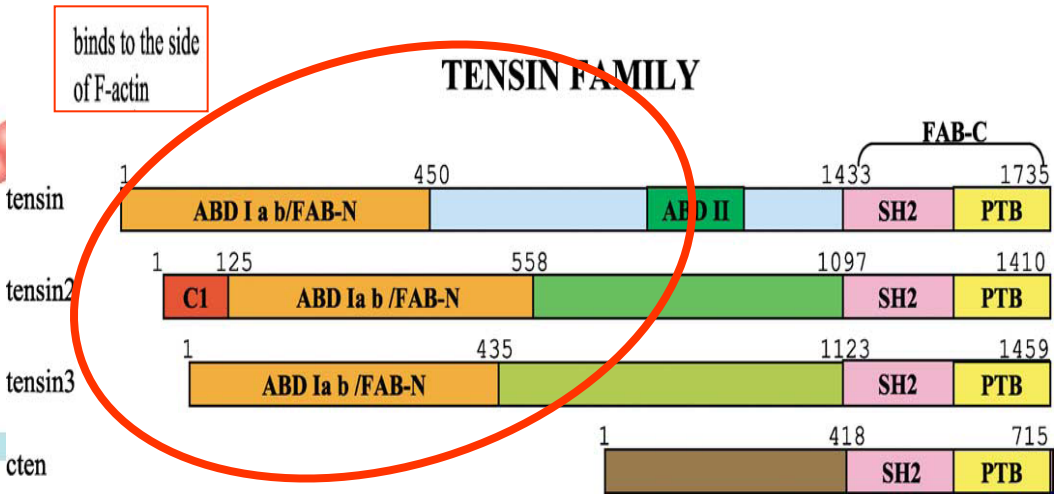
Charles M. Perou*†, Therese Sørlie†‡, Michael B. Eisen*, Matt van de Rijn§, Stefanie S. Jeffrey‖, Christian A. Rees*, Jonathan R. Pollack¶, Douglas T. Ross¶, Hilde Johnsen‡, Lars A. Akslen#, Øystein Fluge☆, Alexander Pergamenschikov*, Cheryl Williams*, Shirley X. Zhu§, Per E. Lønning**, Anne-Lise Børresen-Dale‡, Patrick O. Brown¶†† & David Botstein*

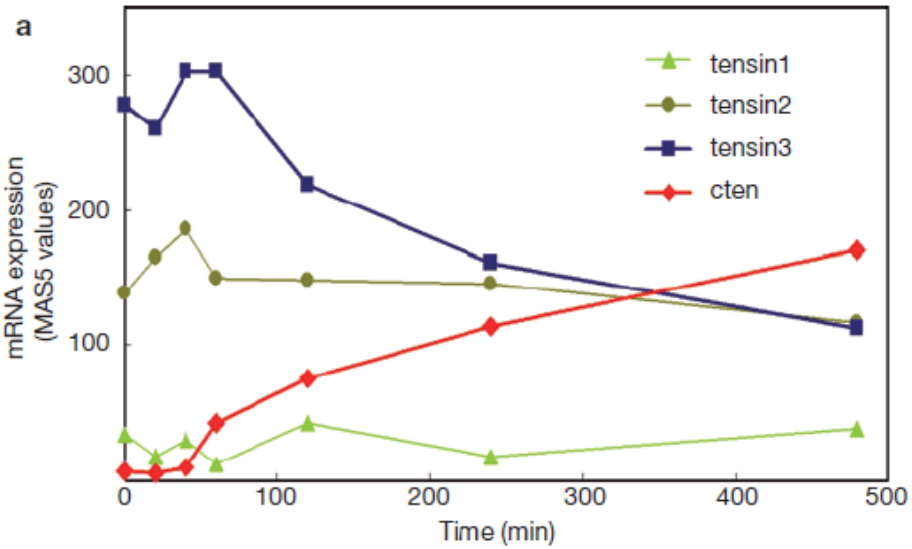
Tensin relief facilitates migration

Yuliya Pylayeva and Filippo G. Giancotti

NATURE CELL BIOLOGY VOLUME 9 | NUMBER 8 | AUGUST 2007





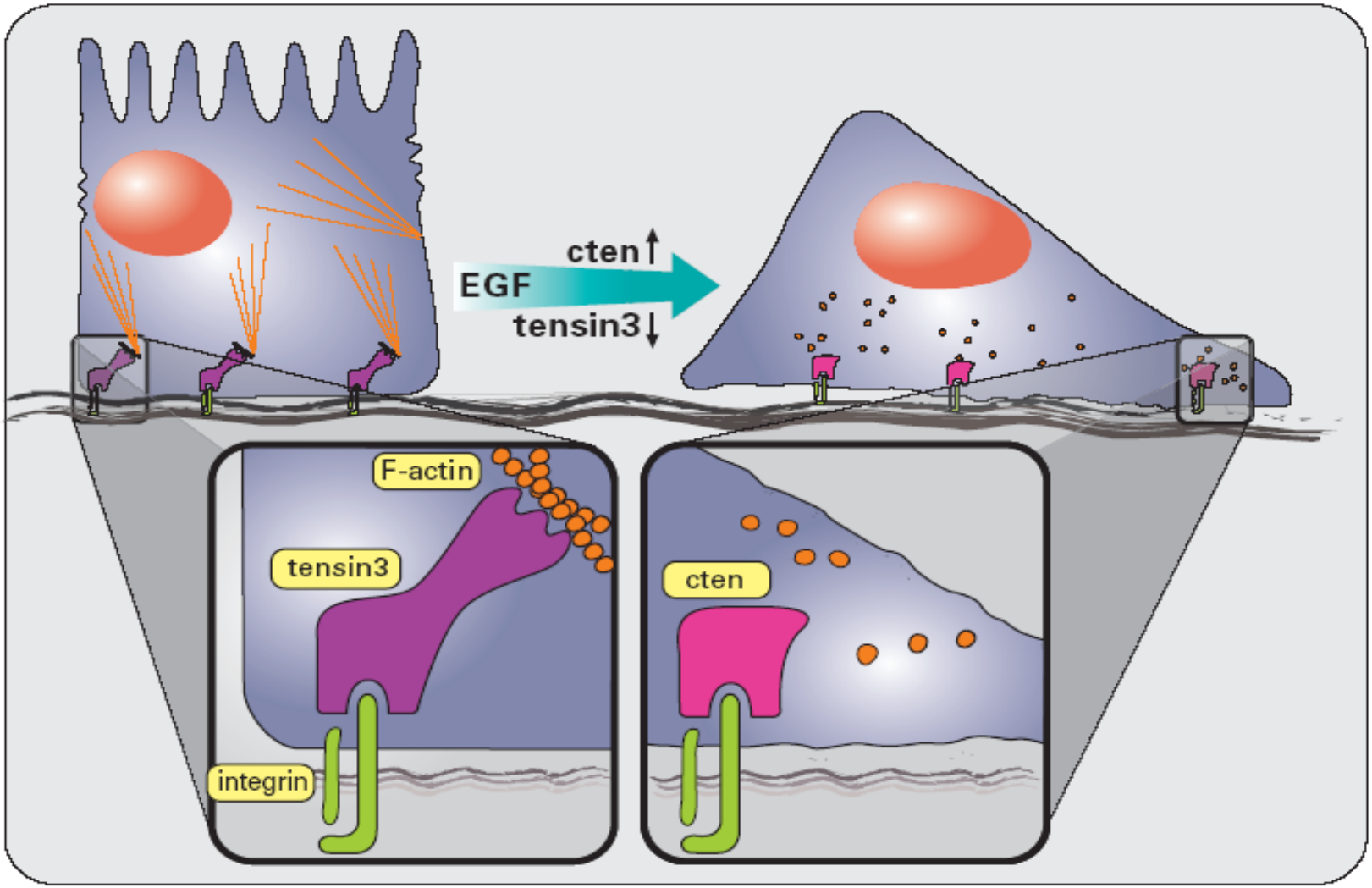


Nature Cell Biology (2007) 9: 961-969

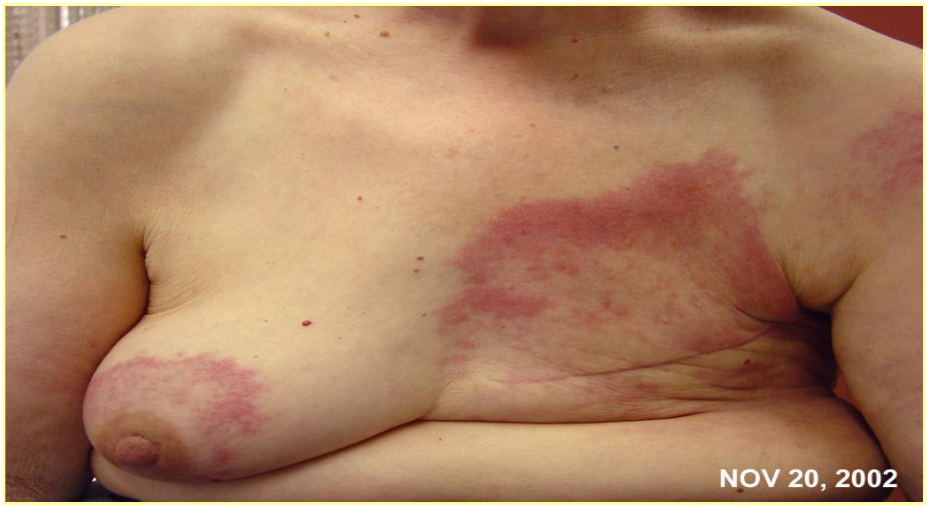
nature cell biology

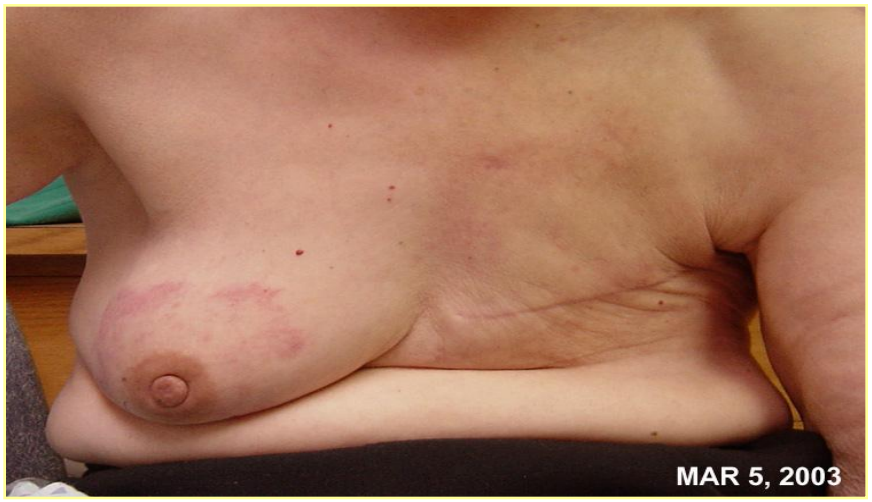
A reciprocal tensin-3–cten switch mediates EGF-driven mammary cell migration

Menachem Katz[1], Ido Amit[1], Ami Citri[1], Tal Shay[2], Silvia Carvalho[3], Sara Lavi[1], Fernanda Milanezi[3], Ljuba Lyass[4], Ninette Amariglio[5], Jasmine Jacob-Hirsch[5], Nir Ben-Chetrit[1], Gabi Tarcic[1], Moshit Lindzen[1], Roi Avraham[1], Yi-Chun Liao[6], Patricia Trusk[4], Asya Lyass[7], Gideon Rechavi[5], Neil L. Spector[8], Su Hao Lo[6], Fernando Schmitt[3,9], Sarah S. Bacus[4] and Yosef Yarden[1]



Nature Cell Biology (2007) 9: 961-969

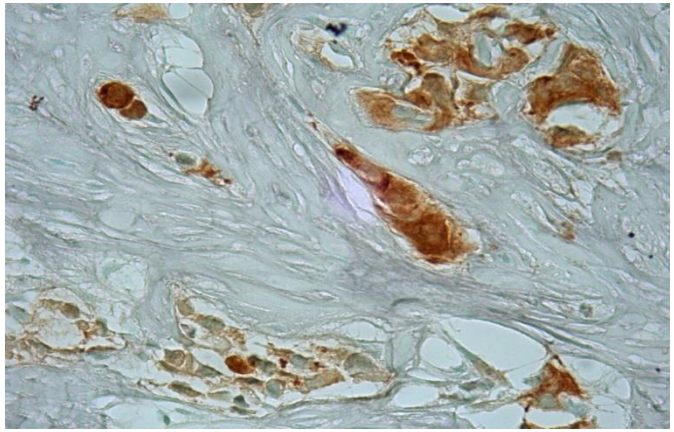


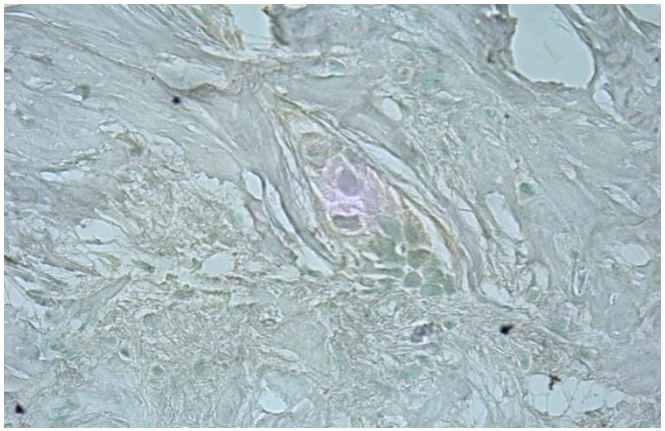


Before TKI treatment

After TKI treatment

IHC Ab:
Cten

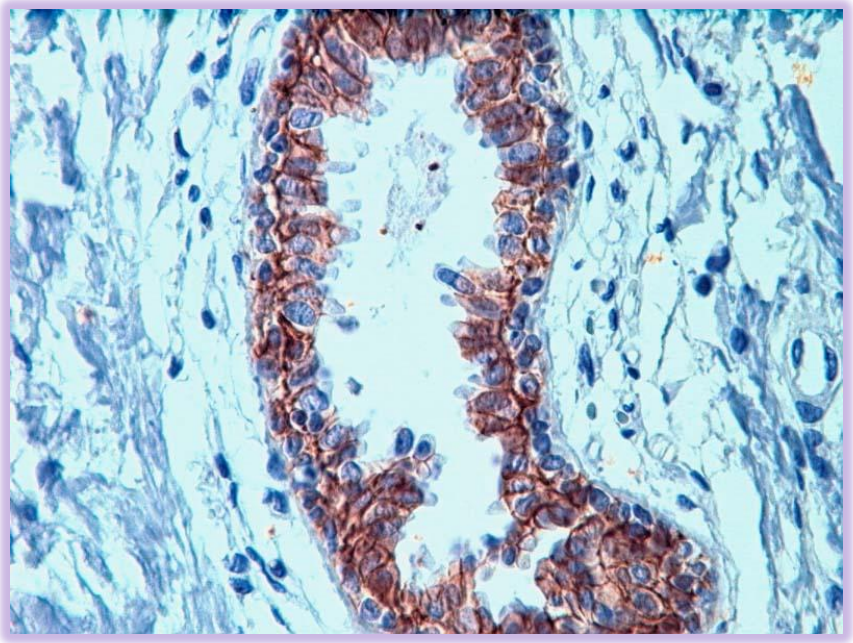




Breast Cancer Patients: cten undergoes down-regulation upon treatment with an EGFR Kinase Inhibitor

E- AND P-CADHERIN EXPRESSION IN NORMAL BREAST

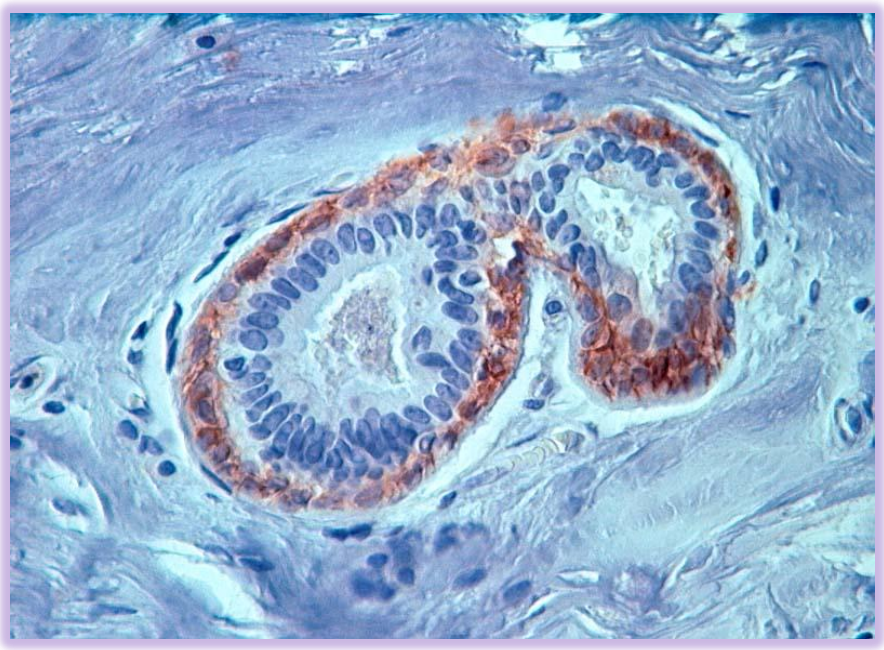
E-cadherin (CDH1)



Luminal / Epithelial Cells

Myoepithelial Cells

P-cadherin (CDH3)



Myoepithelial cells

Lactating mammary tissue (epithelial cells)

Milk (80kD form)

Paredes J et al. Virchows Archiv 440: 16-21, 2002

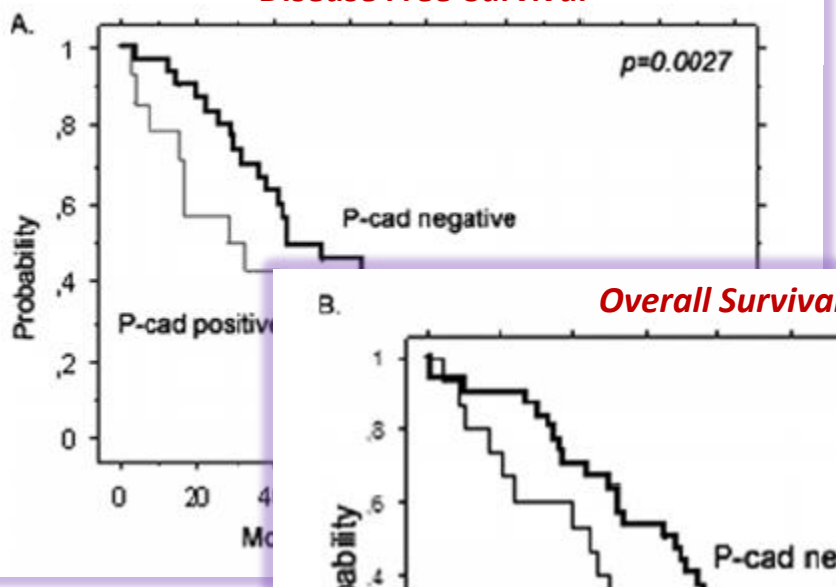
Paredes J et al. Pathology Research and Practice 198: 795-801, 2002

P-Cadherin Overexpression Is an Indicator of Clinical Outcome in Invasive Breast Carcinomas and Is Associated with *CDH3* Promoter Hypomethylation

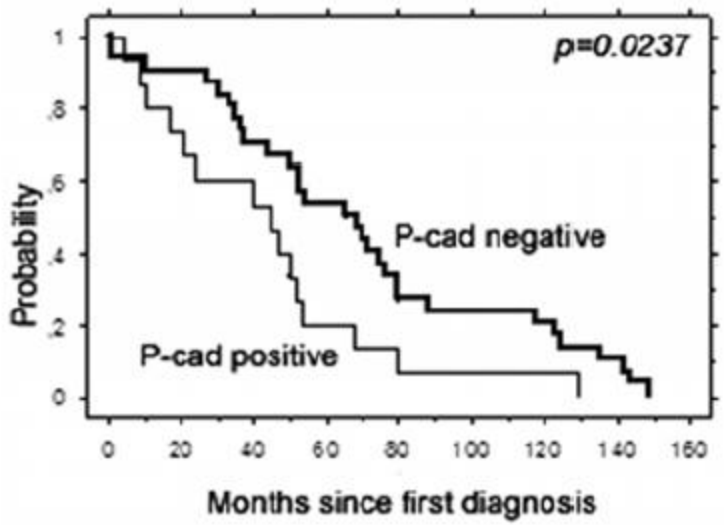
Joana Paredes,[1] André Albergaria,[1] João T. Oliveira,[1] Carmen Jerónimo,[2,3] Fernanda Milanezi,[1,5] and Fernando C. Schmitt[1,4]

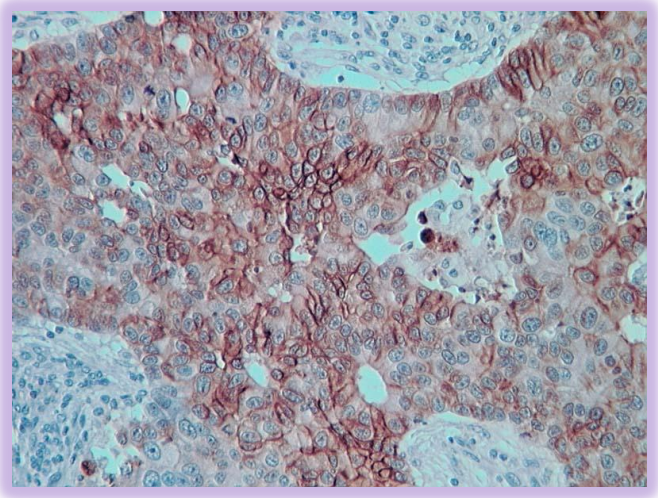
Clin Cancer Res 2005;11 (16) August 15, 2005

Disease Free-Survival



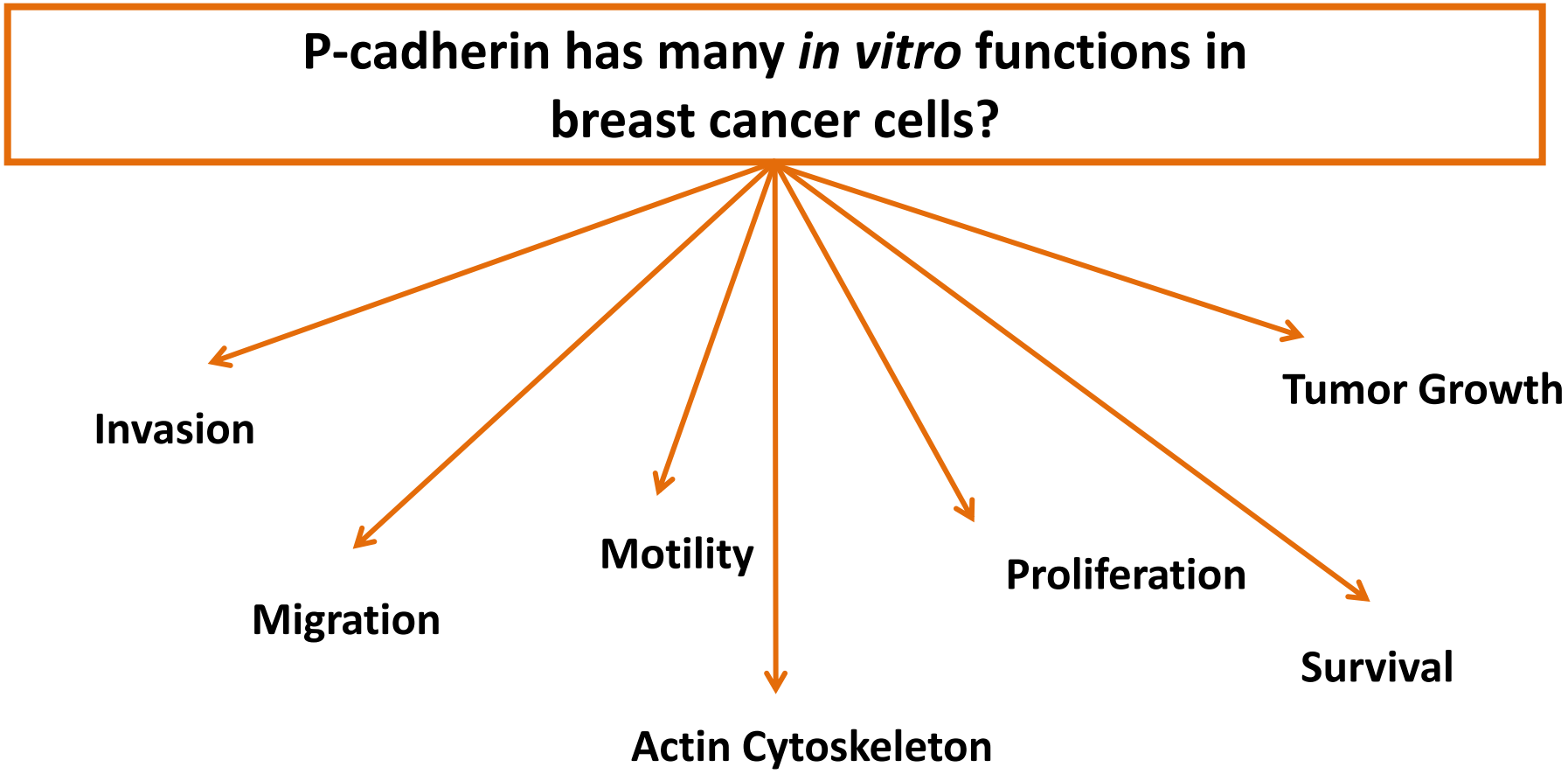
Overall Survival



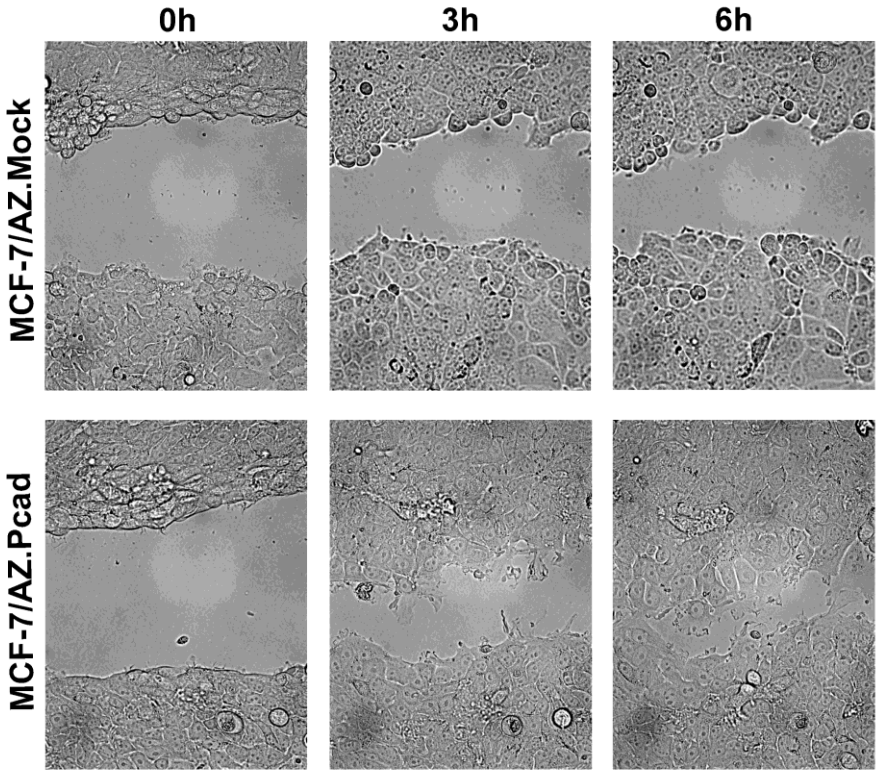


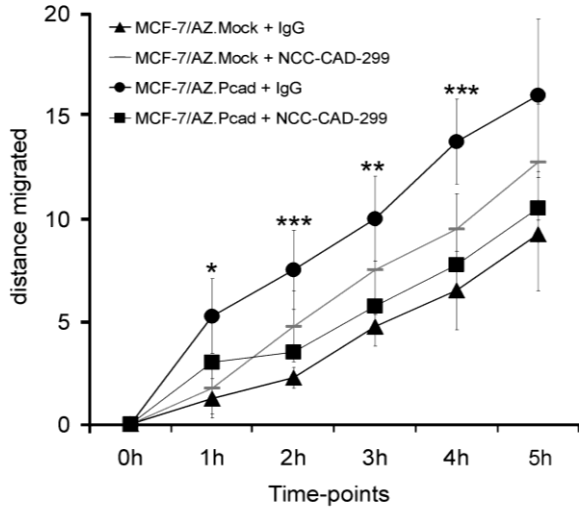
P-cadherin expression is significantly associated with decreased survival in a short-term follow-up (≈ 5 years after diagnosis)

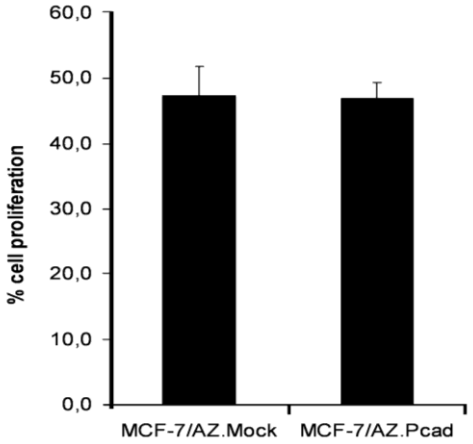
WHY IS P-CADHERIN ASSOCIATED WITH WORSE PROGNOSIS IN BREAST CANCER?



P-Cadherin modulation increase cell migration



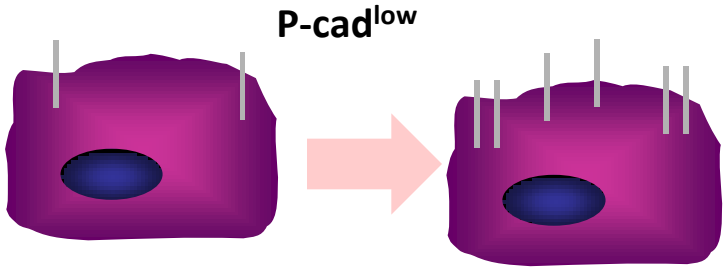




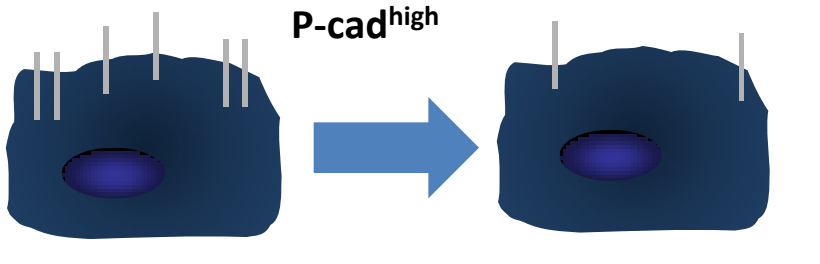
P-cadherin expression induces cancer cell migration

Ribeiro AS et al. Oncogene 29:392-402, 2010

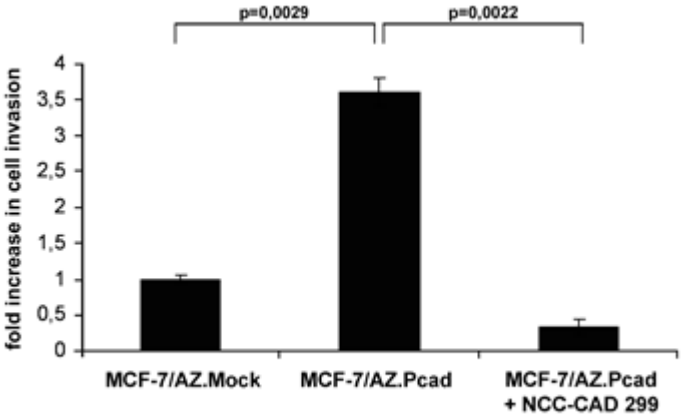
P-Cadherin modulation increase cell invasion

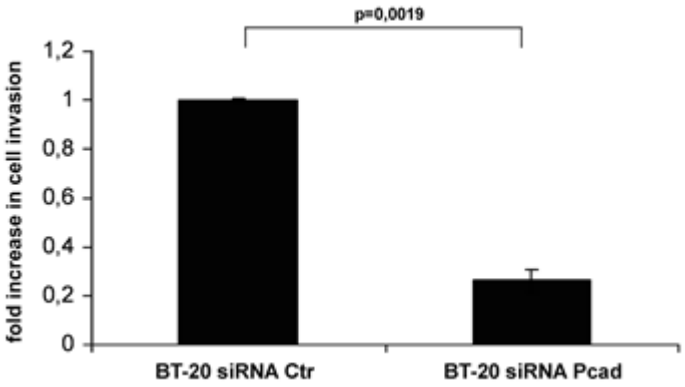


Non-invasive



Invasive





Paredes J et al. Cancer Res 2004

Ribeiro AS et al. Oncogene 29:392-402, 2010

Basal-like and TNBC
Outline

- What is a triple-negative breast cancer?
- What is a basal-like breast cancer?
- Are basal-like and TNBC synonymous?
- Morphological findings
- Relationship with BRCA1 mutations
- Precursor lesions
- Biological behaviour and prognostic factors
- Therapeutic targets

Weigelt and Reis-Filho *Breast Cancer Research* 2010, **12**(Suppl 4):S5
http://breast-cancer-research.com/supplements/12/S4/S5

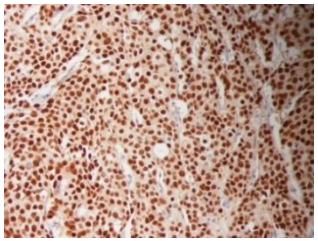
Breast Cancer RESEARCH

SECTION INTRODUCTION

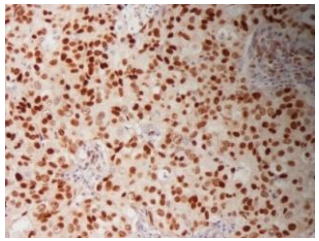
Molecular profiling currently offers no more than tumour morphology and basic immunohistochemistry

➢ Despite the huge amount of resources allocated to translational research, only three predictive markers are used to define the therapy of breast cancer patients:

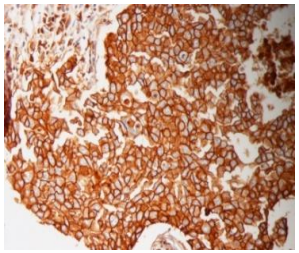
ER



PR



HER2



research article

Annals of Oncology 23 (Supplement 6): vi13–vi18, 2012
doi:10.1093/annonc/mds188

Understanding the biology of triple-negative breast cancer

C. Criscitiello[1*,†], H. A. Azim, Jr[1,†], P. C. Schouten[2], S. C. Linn[2,‡] & C. Sotiriou[1,‡]

Histology	Molecular hallmarks	Proportion TNBC (%)	Prognosis
IDC-NOS	HER1+and/or CK5/6+	12–17	Reference group: 5-year DFS: 60%–65% 10-year DFS: 55%–60%
Metaplastic carcinoma	Squamous epithelium differentiation; mesenchymal elements; EGFR+, CK5/6+, CK14+, p63+	90	Adverse in comparison with IDC-NOS
Medullary carcinoma	Lymphoplasmacytic infiltrate; *P53* mutation; *BRCA1* mutation	95	Favourable in comparison with IDC-NOS
Adenoid cystic carcinoma	Low grade; resembles tumours found in salivary glands; c-KIT+; fusion gene *MYB-NFIB*+; MYB overexpression	90–100	Favourable in comparison with IDC-NOS; 10-year OS: >90%
Apocrine carcinoma	Androgen receptor overexpression	40–60	Favourable in comparison with IDC-NOS

PUTATIVE TARGETS

ANGIO
MAK- KINASE
PCAD

EGFR

BRCA1

AR

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.

ACKNOWLEDGEMENTS