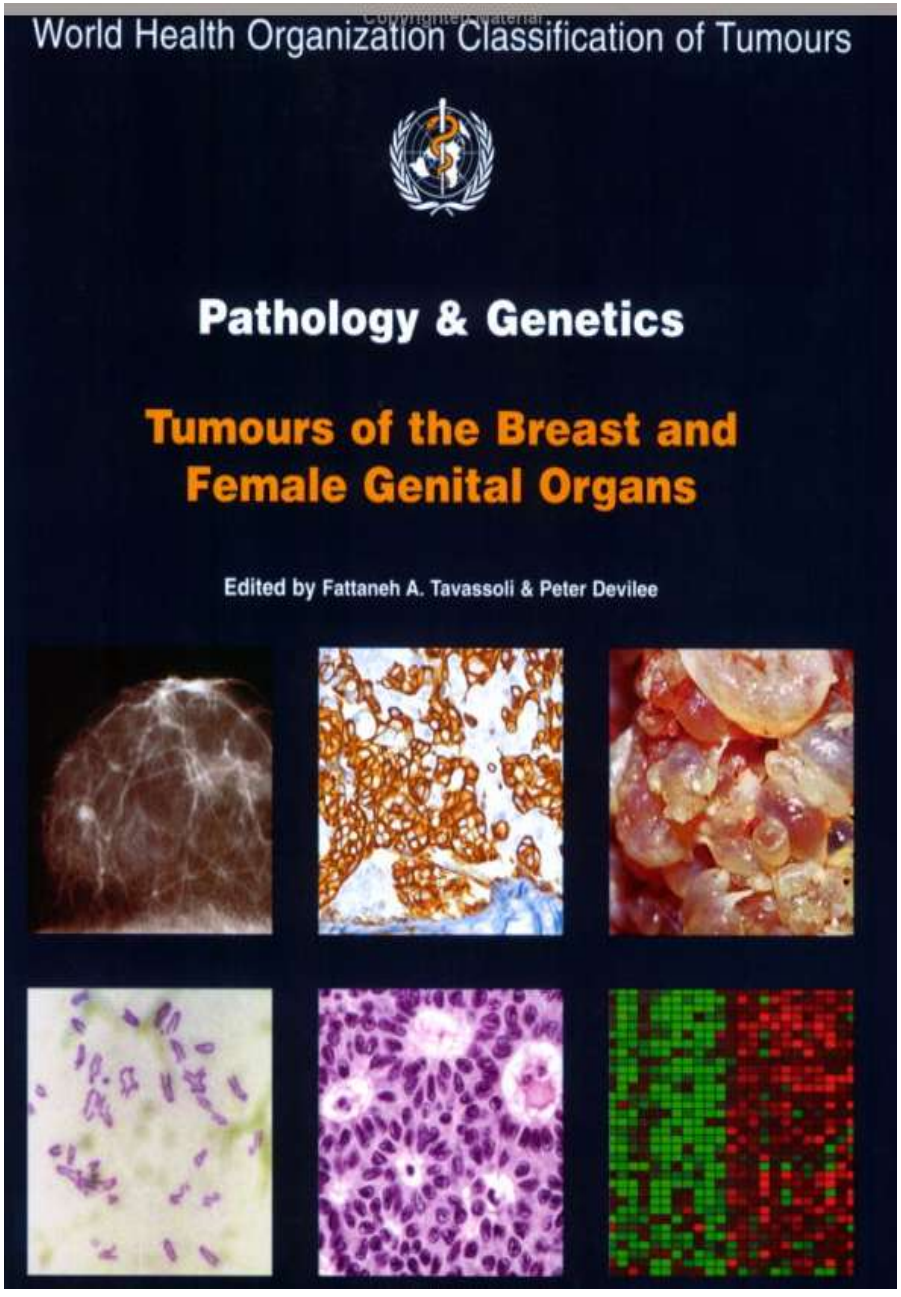
XXV Congreso de la Sociedad Española de Anatomía Patológica y División Española de la *International Academy of Pathology*

NUEVOS FENOTIPOS DEL CÁNCER DE MAMA: ¿NUEVOS PROBLEMAS PARA EL PATÓLOGO?

¿Tienen actualmente utilidad el grado y el tipo histológico?

José Palacios
Hospital Universitario Virgen del Rocío.
Sevilla

WHO histological classification of tumours of the breast



Epithelial tumours	
Invasive ductal carcinoma, not otherwise specified	8500/3
Mixed type carcinoma	
Pleomorphic carcinoma	8022/3
Carcinoma with osteoclastic giant cells	8035/3
Carcinoma with choriocarcinomatous features	
Carcinoma with melanotic features	
Invasive lobular carcinoma	8520/3
Tubular carcinoma	8211/3
Invasive cribriform carcinoma	8201/3
Medullary carcinoma	8510/3
Mucinous carcinoma and other tumours with abundant mucin	
Mucinous carcinoma	8480/3
Cystadenocarcinoma and columnar cell mucinous carcinoma	8480/3
Signet ring cell carcinoma	8490/3
Neuroendocrine tumours	
Solid neuroendocrine carcinoma	
Atypical carcinoid tumour	8249/3
Small cell / oat cell carcinoma	8041/3
Large cell neuroendocrine carcinoma	8013/3
Invasive papillary carcinoma	8503/3
Invasive micropapillary carcinoma	8507/3
Apocrine carcinoma	8401/3
Metaplastic carcinomas	8575/3
Pure epithelial metaplastic carcinomas	8575/3
Squamous cell carcinoma	8070/3
Adenocarcinoma with spindle cell metaplasia	8572/3
Adenosquamous carcinoma	8560/3
Mucoepidermoid carcinoma	8430/3
Mixed epithelial/mesenchymal metaplastic carcinomas	8575/3
Lipid-rich carcinoma	8314/3
Secretory carcinoma	8502/3
Oncocytic carcinoma	8290/3
Adenoid cystic carcinoma	8200/3
Acinic cell carcinoma	8550/3
Glycogen-rich clear cell carcinoma	8315/3
Sebaceous carcinoma	8410/3
Inflammatory carcinoma	8530/3

Rakha *et al. Breast Cancer Research* 2010, **12**:207
http://breast-cancer-research.com/content/12/4/207

Breast Cancer RESEARCH

REVIEW

Breast cancer prognostic classification in the molecular era: the role of histological grade

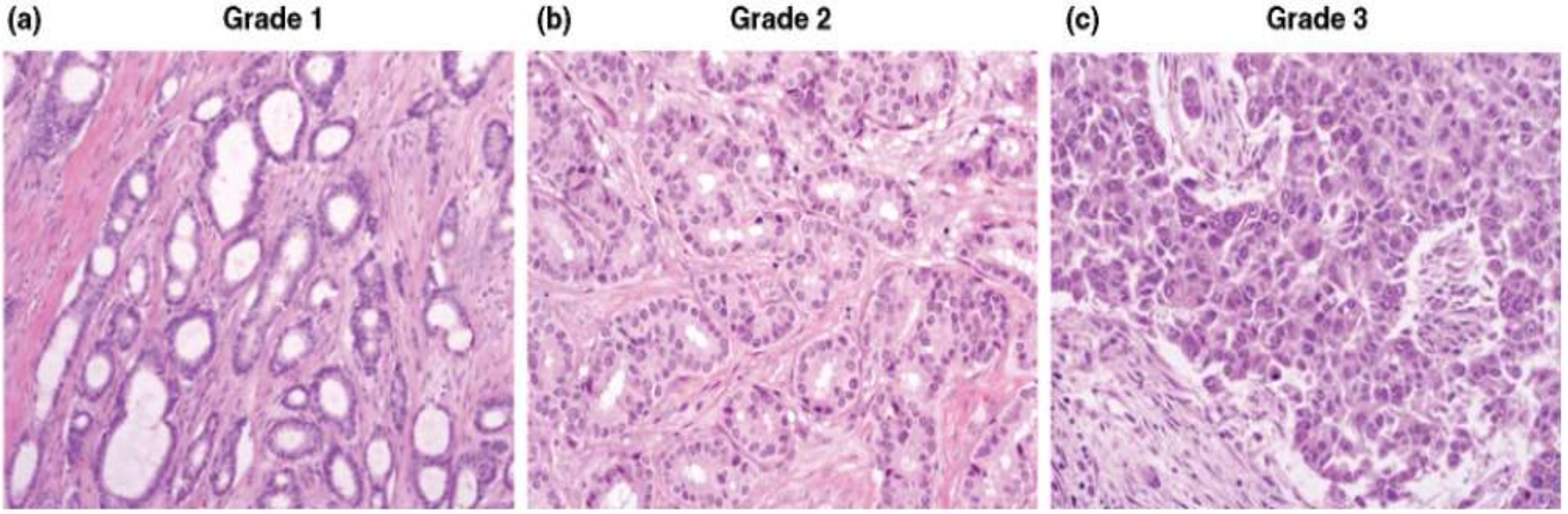
Emad A Rakha[1], Jorge S Reis-Filho[2], Frederick Baehner[3], David J Dabbs[4], Thomas Decker[5], Vincenzo Eusebi[6], Stephen B Fox[7], Shu Ichihara[8], Jocelyne Jacquemier[9], Sunil R Lakhani[10], José Palacios[11], Andrea L Richardson[12], Stuart J Schnitt[13], Fernando C Schmitt[14], Puay-Hoon Tan[15],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

HISTOLOGICAL TUMOR GRADE

Histological tumor grade is based on the degree of differentiation of the tumor tissue.

In breast cancer, it refers to the semi-quantitative evaluation of morphological characteristics (Nottingham Grade System-NGS-):

(a) degree of tubule or gland formation
(b) nuclear pleomorphism
(c) mitotic count.



Nuclear atypia/pleomorphism

Only about 5% of symptomatic cancers score 1 for nuclear atypia; about 50% score 3.

Score 1: nuclei only slightly larger than benign breast epithelium (< 1.5 × normal area); minor variation in size, shape and chromatin pattern

Score 2: nuclei distinctly enlarged (1.5–2 × normal area), often vesicular, nucleoli visible; may be distinctly variable in size and shape but not always

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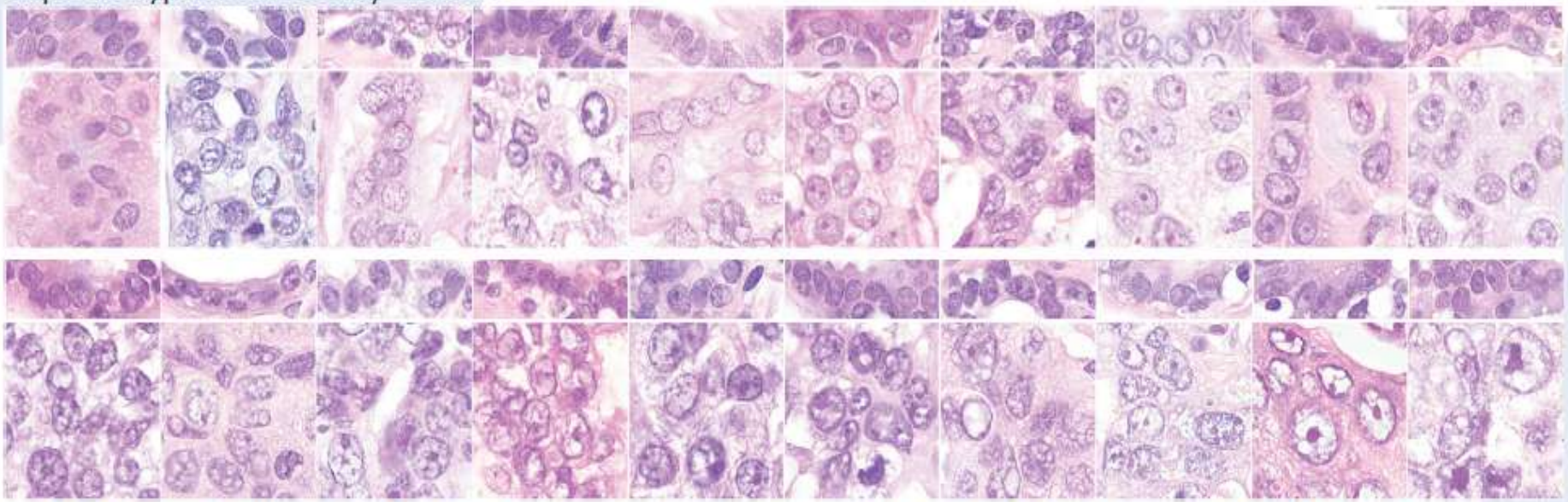


Nuclei of 20 consecutive breast cancers by increasing mean nuclear area (left to right, top to bottom). Paired non-neoplastic breast epithelium is shown above each case for comparison. Only one cancer (top left) has nuclei which score 1. The others in the top row score 2. All 10 in the bottom row score 3

http://www.cancerscreening.nhs.uk/breastscreen/publications/nhsbsp58-poster.pdf

MITOTIC COUNT

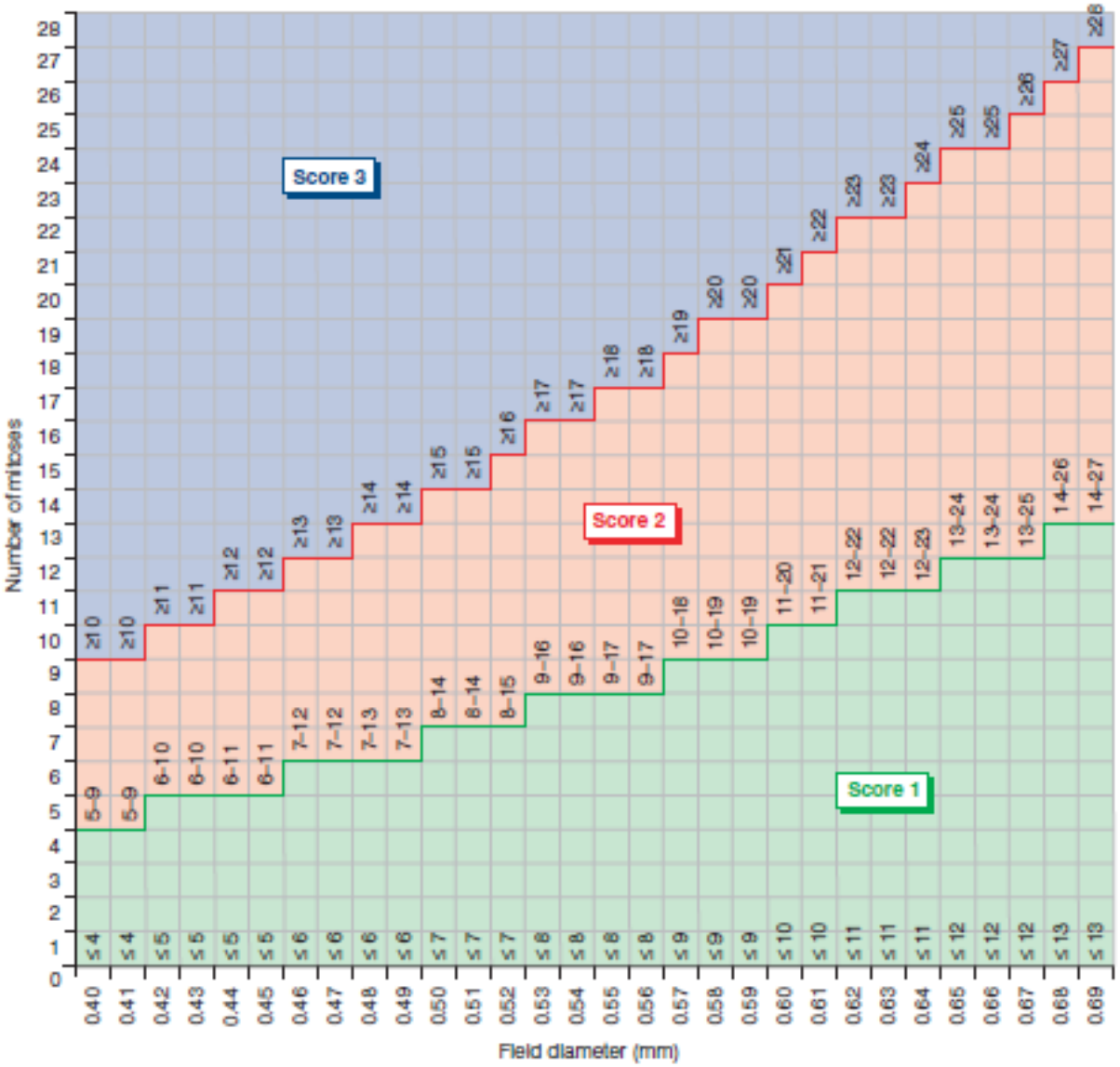
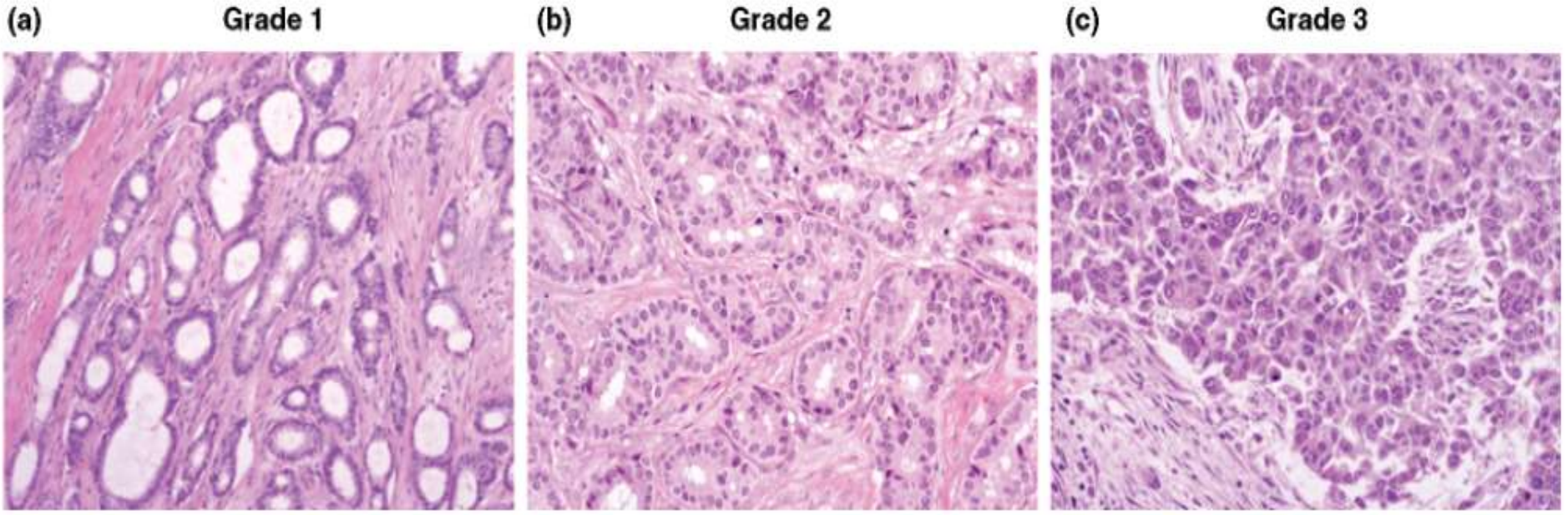


Figure 49 Aide-memoire to assist calibration of microscope field diameter with mitotic frequency count grading cut off points (see also Table 4).

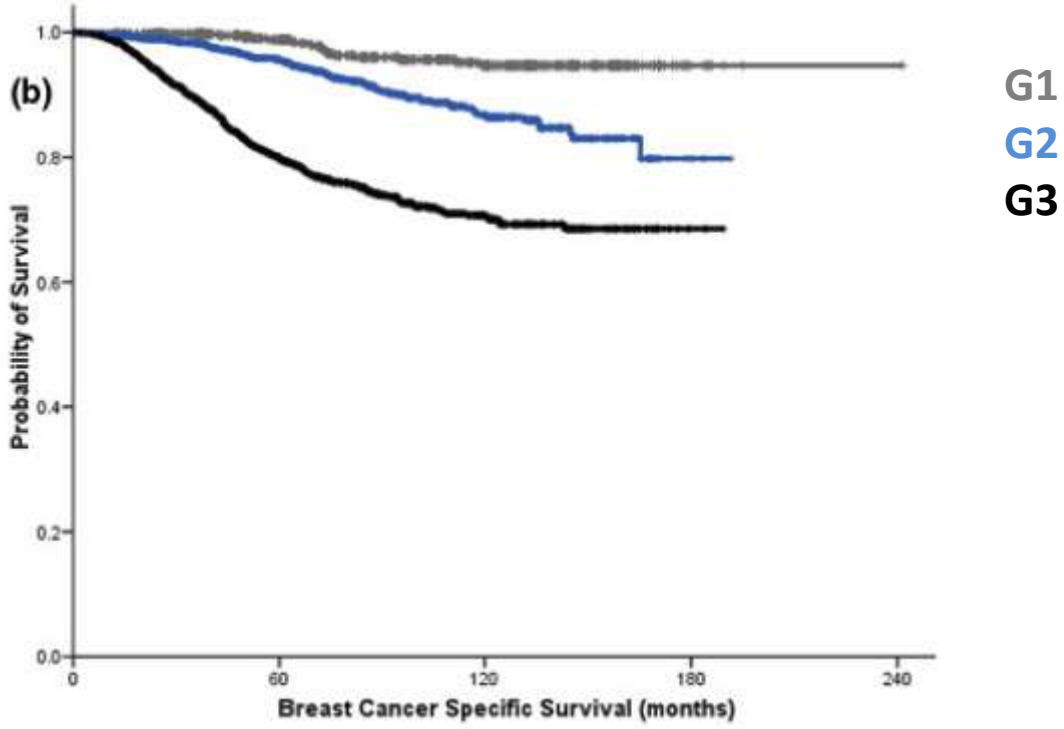
HISTOLOGICAL TUMOR GRADE

NGS is a relatively simple and low-cost method, requiring only adequately prepared hematoxylin-eosin-stained tumor tissue sections to be assessed by an appropriately trained pathologist using a standard protocol.



HISTOLOGICAL TUMOR GRADE AND PROGNOSIS

The prognostic relevance of NGS in breast cancer was initially demonstrated in 1991 and has been validated subsequently in multiple independent studies.



HISTOLOGICAL TUMOR GRADE AND PROGNOSIS

- NGS has independent prognostic value in breast cancer, it has been combined with LN stage and tumor size to form prognostic indices: the Nottingham Prognostic Index (NPI), which includes NGS and LN stage with equal weighting.

- NSG is the grading system recommended by WHO, AJCC, EU, and UK RCPath.

- NGS has also been incorporated in algorithms (for example, Adjuvant! Online) and guidelines (for example, the St. Gallen guidelines) to determine the use of adjuvant chemotherapy.

HISTOLOGICAL TUMOR GRADE AND PROGNOSIS

Histological grade can provide important prognostic information for clinically relevant subgroups in which the benefit of chemotherapy is less certain (ER+/HER2-)

(a) 10-year risk of relapse for the LN-negative/ER-positive subgroup, who received only adjuvant hormone therapy (n = 797):

7% for grade 1
14% for grade 2
31% for grade 3

(b) 10-year risk of relapse for ER-positive tumors with small-volume LN metastasis (pN1) (n = 316):

5% for grade 1,
24% for grade 2
43% for grade 3.

HISTOLOGICAL TUMOR GRADE REPRODUCIBILITY

Table 2. Proportion of grades among different studies.

Study	Number of cases	Grade 1	Grade 2	Grade 3
Elston, 1984 [77]	625	17%	37%	46%
Davis *et al.*, 1986 [78]	1,537	22%	49%	29%
Hopton *et al.*, 1989 [59]	874	29%	46%	25%
Le Doussal *et al.*, 1989 [79]	1,262	11%	45%	46%
Balslev *et al.*, 1994 [80]	9,149	32%	49%	19%
Saimura *et al.*, 1999 [5]	741	19	37%	44%
Reed *et al.*, 2000 [32]	613	25%	41%	35%
Simpson *et al.*, 2000 [7]	368	22%	45%	33%
Lundin *et al.*, 2001 [6]	1,554	26%	47%	27%
Frkovic-Grazio and Bracko, 2002 [9]	270	38%	38%	24%
Warwick *et al.*, 2004 [10]	1,988	23%	37%	40%
Williams *et al.*, 2006 [26]	1,058	20%	46%	34%
Rakha *et al.*, 2008 [11]	2,219	18%	36%	46%
Thomas *et al.*, 2009 [81]	1,650	26%	45%	29%
Blamey *et al.*, 2009 [12]	16,944	29%	41%	30%

HISTOLOGICAL TUMOR GRADE REPRODUCIBILITY

- Differences in patient cohorts, including age distribution, symptomatic versus screening population, early versus advanced breast cancer groups.

	SCREENING-DETECTED	***SYMPTOMATIC***
IDC	70-74%	71-75%
ILC	12-17%	16-21%
Others	9-14%	8-9%
Grade 1	31-34%	16-23%
Grade 3	13-15%	22-32%

HISTOLOGICAL TUMOR GRADE REPRODUCIBILITY

- Grading is dependent on a high quality of tissue preservation. Suboptimal levels of tissue fixation lead to disruption and loss of visibility of mitotic figures, one of the three variables assessed in NGS. Assessment of grade in poorly fixed tissue will therefore introduce a bias leading to a reduction in the proportion of cases classified as grade 3.

- Guidelines for standardization of pre-analytical parameters, including tissue handling, fixation, and preparation.

HISTOLOGICAL TUMOR GRADE REPRODUCIBILITY

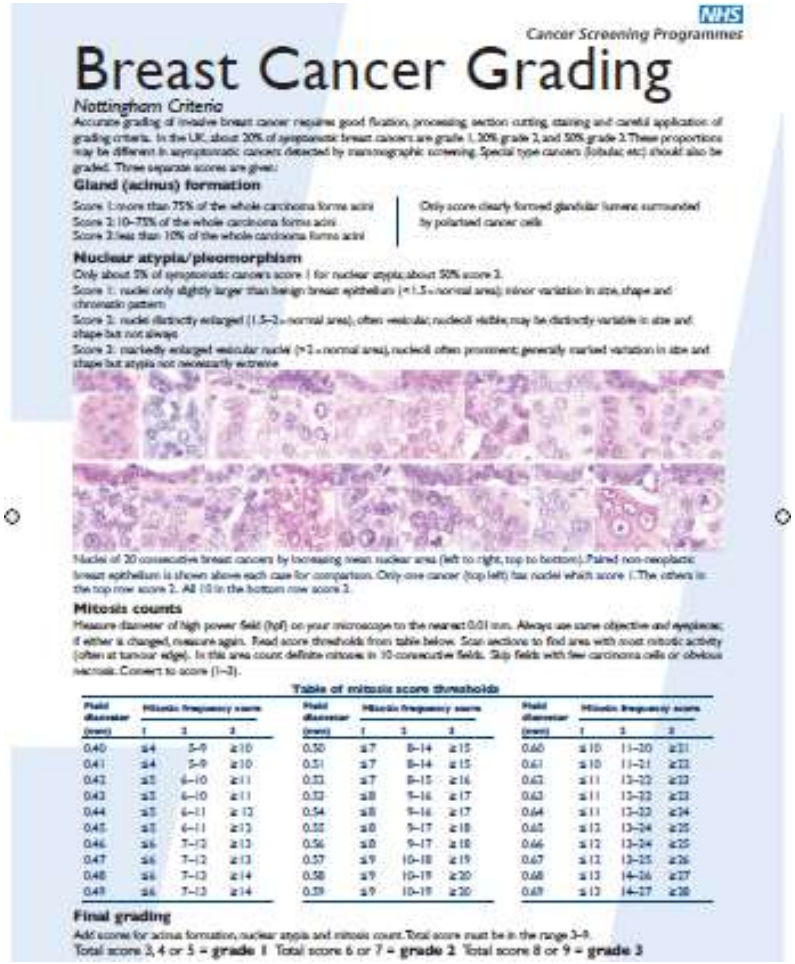
Table 1. Inter-observer and intra-observer agreement of breast cancer histological grade.

Study	Number of cases	Number of readers	Grade	Inter-observer
[32]	613	2	NGS	Kappa 0.69
[8]	52	2	NGS	Kappa 0.54
[55]	425	2	NGS	Complete agreement 76%
[50]	75	6	NGS	Kappa 0.43 to 0.74
[51]	12	600	NGS	Kappa 0.45 to 0.53 (figures after application of guidelines)
[52]		3	NGS	Complete agreement 72.3%; kappa 0.57
[53]	24	21	NGS	Complete agreement 69%; kappa 0.53
[54]	50	5	NGS	Mean polychoric correlation 0.8
[56]	35	13	NGS	Kappa 0.5 to 0.7
[57]	93	7	NGS	Kappa 0.54
[58]	40	3	NGS	Kappa 0.68 to 0.83
[59]	874	2	WHO criteria	Complete agreement 78.1%; kappa 0.66
[61]	50	5	NGS	Complete agreement 83.3%; kappa 0.73

NGS, Nottingham Grading System; WHO, World Health Organization.

HISTOLOGICAL TUMOR GRADE REPRODUCIBILITY

- Strict adherence to guidelines for tumor grading.



NHS
Cancer Screening Programmes

Breast Cancer Grading

Nottingham Criteria

Accurate grading of invasive breast cancer requires good fixation, processing, section cutting, staining and careful application of grading criteria. In the UK, about 20% of symptomatic breast cancers are grade 1, 30% grade 2, and 50% grade 3. These proportions may be different in asymptomatic cancers detected by mammographic screening. Special type cancers (tubular, etc) should also be graded. Three separate scores are given:

Gland (acinus) formation

Score 1: more than 75% of the whole carcinoma forms acini
Score 2: 10–75% of the whole carcinoma forms acini
Score 3: less than 10% of the whole carcinoma forms acini

Only score clearly formed glandular lumens surrounded by polarised cancer cells

Nuclear atypia/pleomorphism

Only about 5% of symptomatic cancers score 1 for nuclear atypia; about 50% score 3.

Score 1: nuclei only slightly larger than benign breast epithelium (<1.5× normal area); minor variation in size, shape and chromatin pattern

Score 2: nuclei distinctly enlarged (1.5–2× normal area); often vesicular; nucleoli visible; may be distinctly variable in size and shape but not always

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Nuclei of 20 consecutive breast cancers by increasing mean nuclear area (left to right, top to bottom). Paired non-neoplastic breast epithelium is shown above each case for comparison. Only one cancer (top left) has nuclei which score 1. The others in the top row score 2. All 10 in the bottom row score 3.

Mitosis counts

Measure diameter of high power field (hpf) on your microscope to the nearest 0.01 mm. Always use same objective and eyepieces; if either is changed, measure again. Read score thresholds from table below. Scan sections to find area with most mitotic activity (often at tumour edge). In this area count definite mitoses in 10 consecutive fields. Skip fields with few carcinoma cells or obvious necrosis. Convert to score (1–3).

Table of mitosis score thresholds

Field diameter (mm)	Mitotic frequency score 1	2	3	Field diameter (mm)	Mitotic frequency score 1	2	3	Field diameter (mm)	Mitotic frequency score 1	2	3
0.40	≤4	5–9	≥10	0.50	≤7	8–14	≥15	0.60	≤10	11–20	≥21
0.41	≤4	5–9	≥10	0.51	≤7	8–14	≥15	0.61	≤10	11–21	≥22
0.42	≤5	6–10	≥11	0.52	≤7	8–15	≥16	0.62	≤11	12–22	≥23
0.43	≤5	6–10	≥11	0.53	≤8	9–16	≥17	0.63	≤11	12–23	≥23
0.44	≤5	6–11	≥12	0.54	≤8	9–16	≥17	0.64	≤11	12–23	≥24
0.45	≤5	6–11	≥12	0.55	≤8	9–17	≥18	0.65	≤12	13–24	≥25
0.46	≤6	7–12	≥13	0.56	≤8	9–17	≥18	0.66	≤12	13–24	≥25
0.47	≤6	7–12	≥13	0.57	≤9	10–18	≥19	0.67	≤12	13–25	≥26
0.48	≤6	7–13	≥14	0.58	≤9	10–19	≥20	0.68	≤13	14–26	≥27
0.49	≤6	7–13	≥14	0.59	≤9	10–19	≥20	0.69	≤13	14–27	≥28

Final grading

Add scores for acinus formation, nuclear atypia and mitosis count. Total score must be in the range 3–9.
Total score 3, 4 or 5 = **grade 1** Total score 6 or 7 = **grade 2** Total score 8 or 9 = **grade 3**

http://www.cancerscreening.nhs.uk/breastscreen/publications/nhsbsp58-poster.pdf

HISTOLOGICAL TUMOR GRADE REPRODUCIBILITY

- St. Gallen Consensus (2009) recommended that grade 1 and grade 3 be taken into consideration for the assessment of indications of adjuvant chemotherapy. **Grade 2** was regarded as being similar to other parameters, of **intermediate-risk significance**, such as tumor size of between 2 and 5 cm, low numbers (one to three) of involved LNs, and intermediate scores on multigene assays.

- Attempts to classify grade 2 tumors into two distinct subclasses:

  grade 1-like subgroup, which has an excellent outcome
  grade 3-like subgroup, tumors that behave like high-grade cancers.

¿GGI, KI67?

HISTOLOGICAL TUMOR GRADE IN CORE BIOPSY

- Some cases may be upgraded in the excision specimen: grade I in the core biopsy and grade II in the excision specimen (30% to 40%).
- A diagnosis of NGS grade III in a core biopsy is not commonly changed when the excision specimen is graded (5% to 8%).
- Changes from grade I in the core to grade III in the excision specimen and vice versa are very rare (0% to 1%).

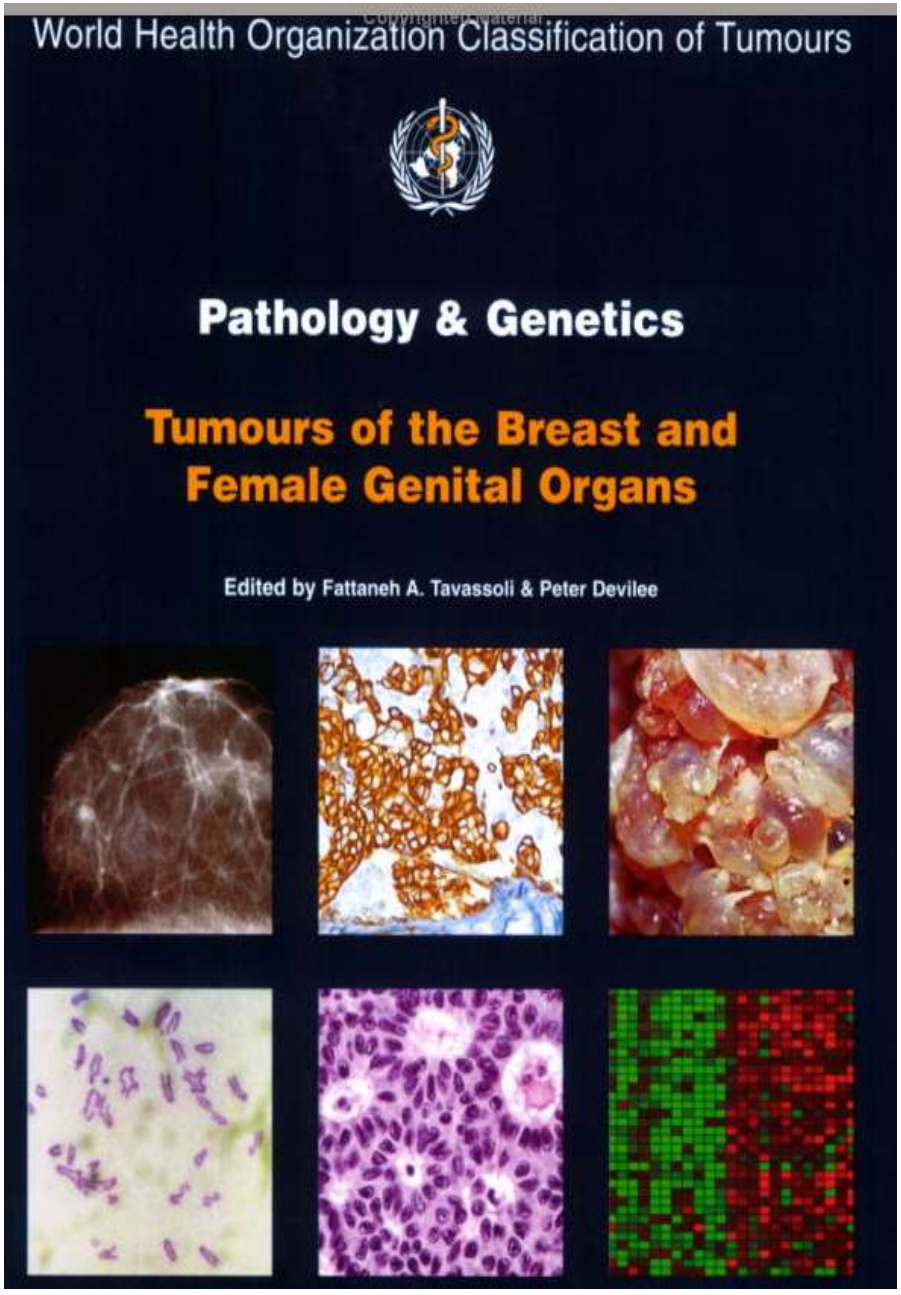
HISTOLOGICAL TUMOR GRADE

- The Nottingham Grading System, when adequately carried out, provides a simple, inexpensive, accurate, and validated method for assessing patient prognosis.

- Assessment of histological grade is an important determinant of breast cancer prognostication and should be incorporated in algorithms to define therapy for patients with breast cancer.

- Consensus criteria for histological grading and recommendations for good practice should be followed.

WHO histological classification of tumours of the breast



Epithelial tumours

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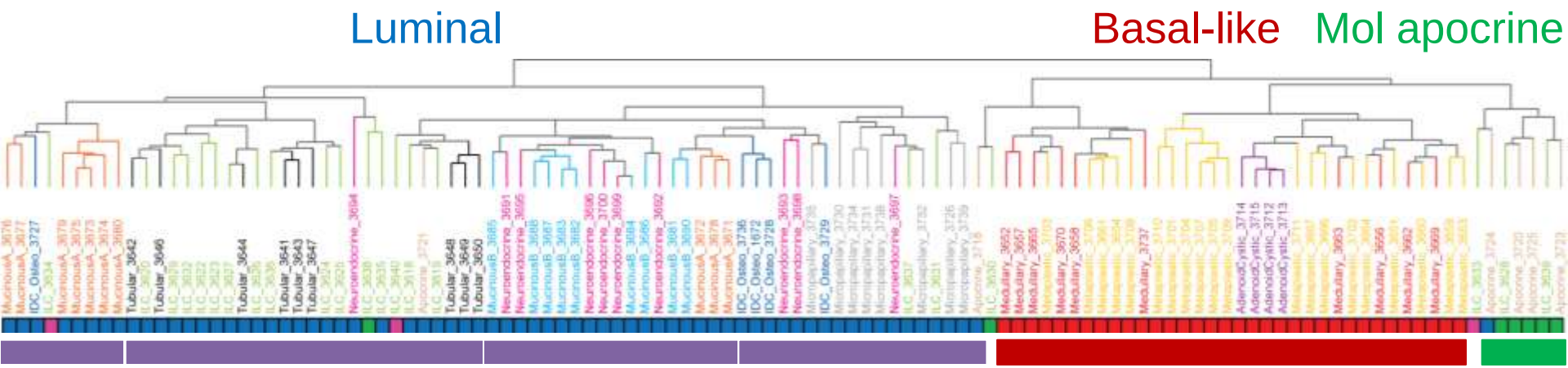
Mixed type carcinoma	
Pleomorphic carcinoma	8022/3
Carcinoma with osteoclastic giant cells	8035/3
Carcinoma with choriocarcinomatous features	
Carcinoma with melanotic features	
Invasive lobular carcinoma	8520/3
Tubular carcinoma	8211/3

- 17 morphological special types
- 25-30% of all breast carcinomas
- Significant prognostic/ clinical implications
- Different biological characteristics

Mixed epithelial/mesenchymal metaplastic carcinomas	8575/3
Lipid-rich carcinoma	8314/3
Secretory carcinoma	8502/3
Oncocytic carcinoma	8290/3
Adenoid cystic carcinoma	8200/3
Acinic cell carcinoma	8550/3
Glycogen-rich clear cell carcinoma	8315/3
Sebaceous carcinoma	8410/3
Inflammatory carcinoma	8530/3

Special types of breast cancer are more homogeneous at the transcriptome level



Mucinous A

Classic ILC/ Tubular

Mucinous B
Neuroendocrine

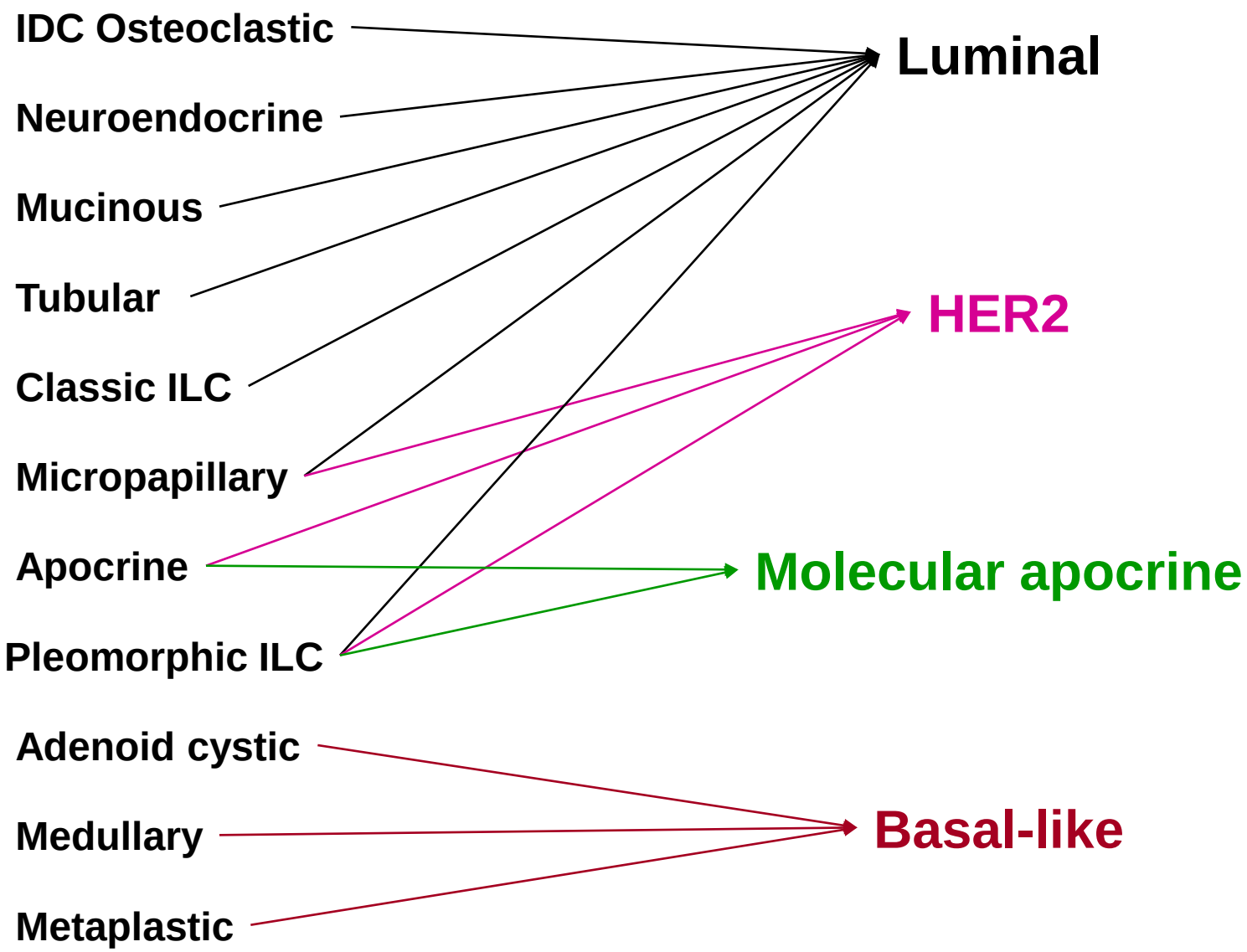
Micropapillary

Adenoid cystic
Medullary
Metaplastic

Pleomorphic ILC
Apocrine

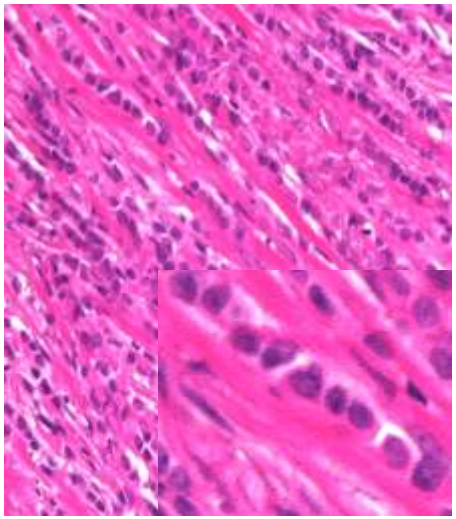
Weigelt et al. J Pathol 2008

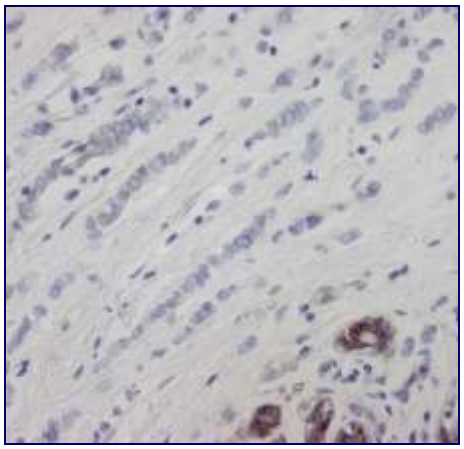
SPECIAL TYPES VERSUS MOLECULAR SUBTYPES



LOBULAR BREAST CANCER

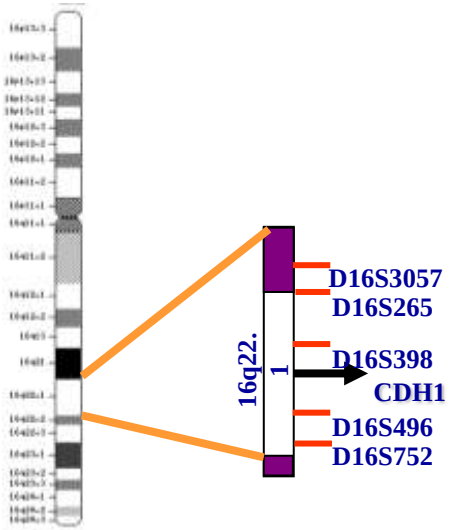
- **Invasive breast carcinoma composed by non-cohesive cells individually dispersed or arranged in single-file linear pattern in a fibrous stroma.**

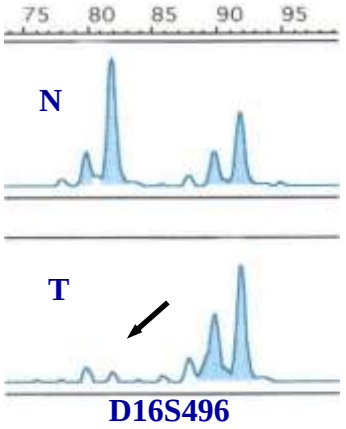




Absence of E-cadherin >80%

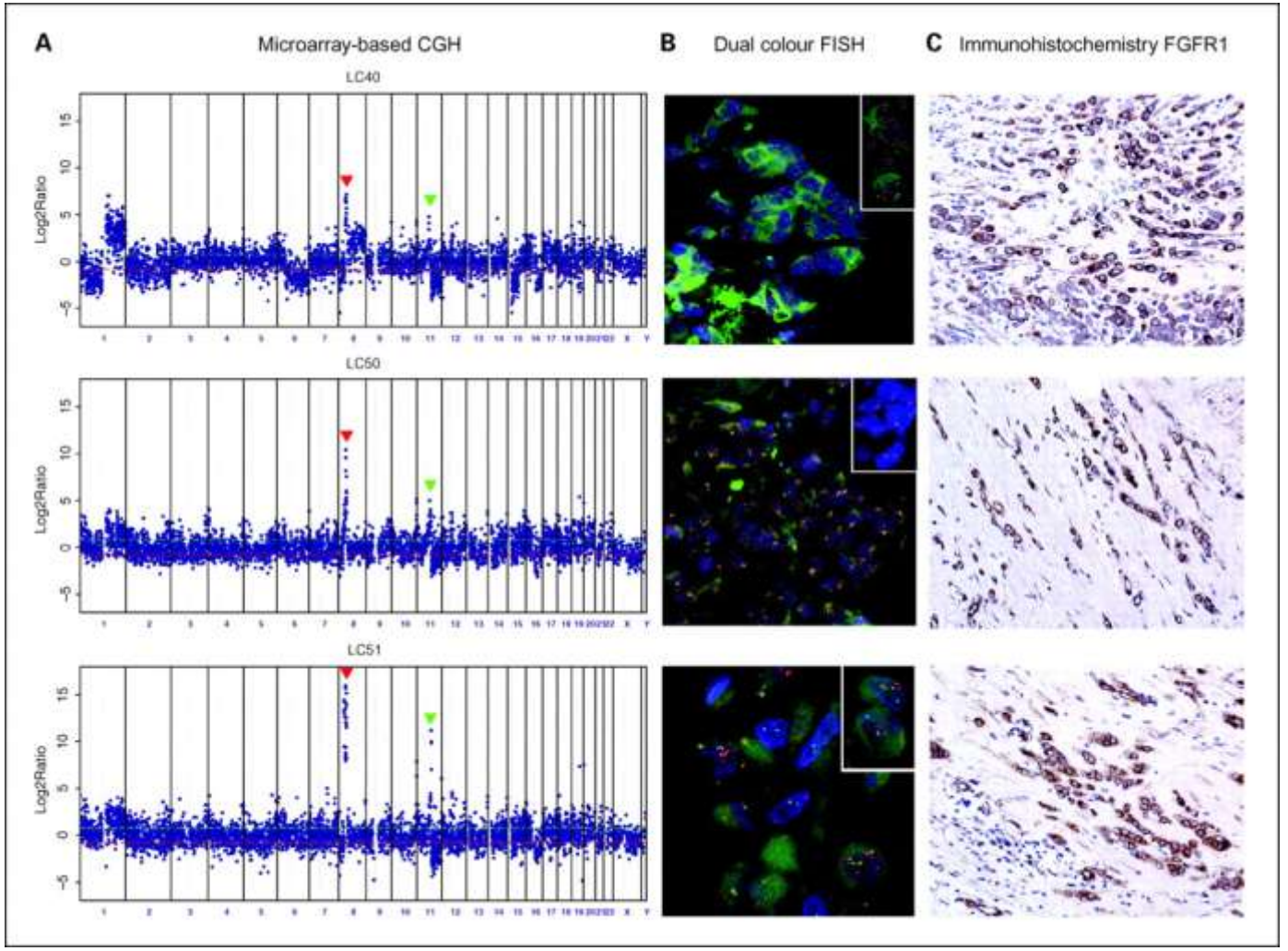
- ✓INCREASING INCIDENCE (HRT?)
- ✓HIGHER AGE AT DIAGNOSIS
- ✓HIGHER SIZE AT DIAGNOSIS
- ✓LOWER SENSITIVITY OF RX TO DETECT ILC
- ✓ DIFFUSE GROWTH PATERN
- ✓POOR RESPONSE TO CHEMOTHERAPY
- ✓METASTATIC PATTERN





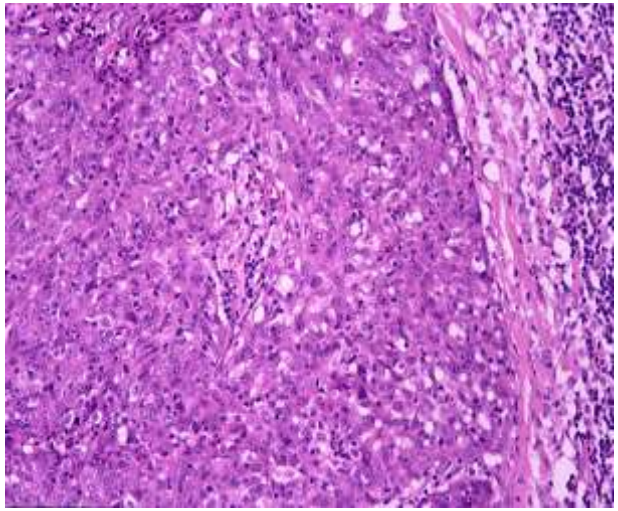
FGFR1 Emerges as a Potential Therapeutic Target for Lobular Breast Carcinomas

Jorge Sergio Reis-Filho,[1,2] Pete T. Simpson,[3] Nicholas C. Turner,[1] Maryou Ballo Lambros,[1] Chris Jones,[4] Alan Mackay,[1] Anita Grigoriadis,[1] David Sarrio,[6] Kay Savage,[1] Tim Dexter,[1] Marjan Iravani,[1] Kerry Fenwick,[1] Barbara Weber,[5] David Hardisson,[7] Fernando Carlos Schmitt,[2] Jose Palacios,[6] Sunil R. Lakhani,[3] and Alan Ashworth[1]

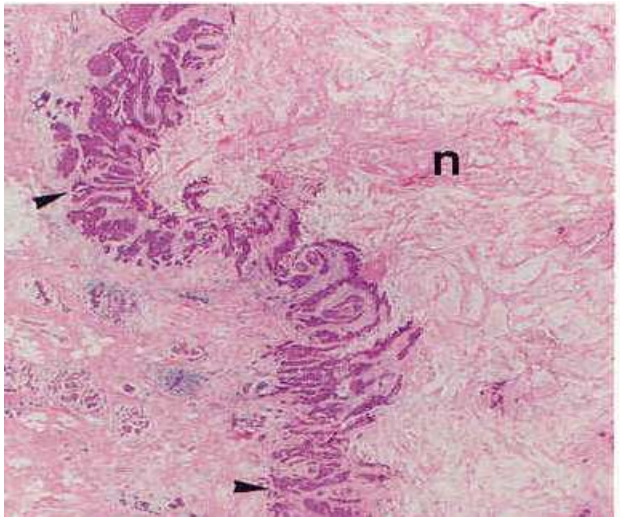


Reis-Filho, J. S. et al. Clin Cancer Res 2006;12:6652-6662

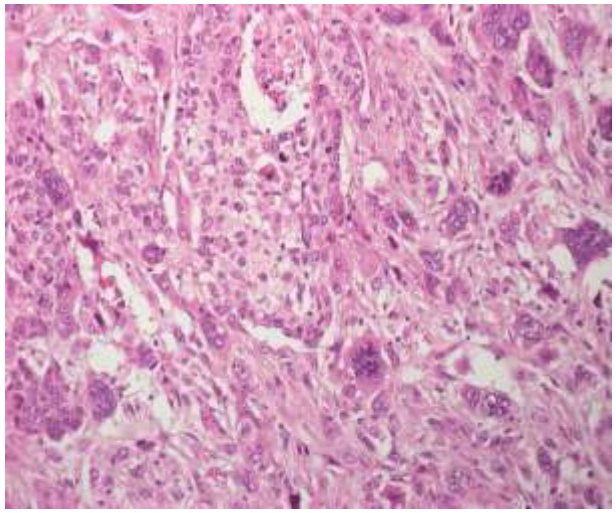
MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL HETEROGENEITY OF BREAST CARCINOMAS WITH BASAL-LIKE PHENOTYPE



Medullary carcinoma



Poorly differentiated carcinoma with central acellular zones



Metaplastic carcinoma

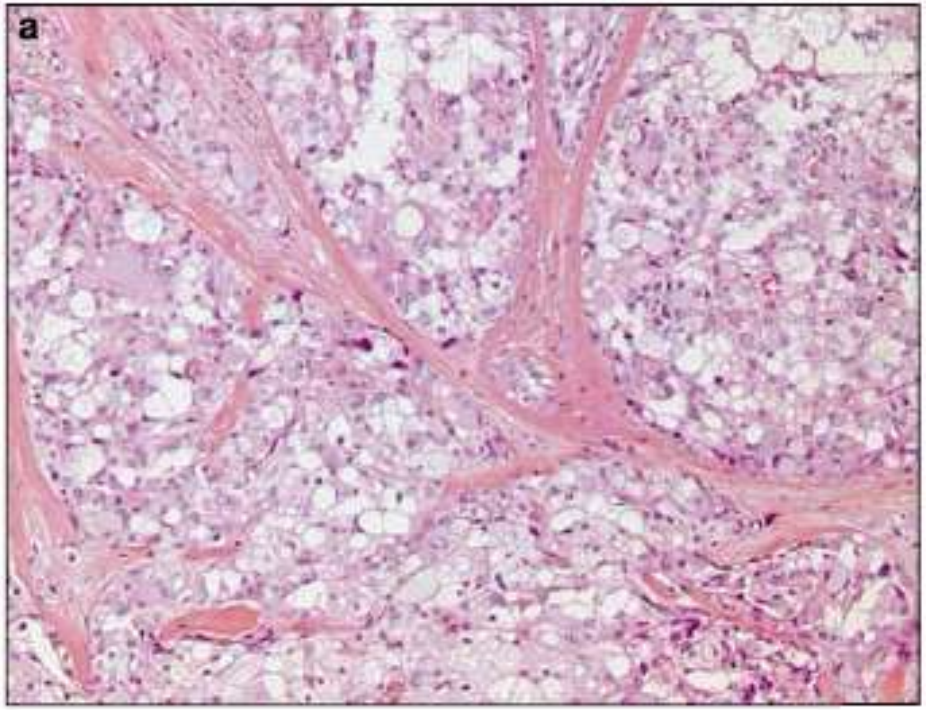
- **Lack of expression of ER, PR, and HER2 in conjunction with expression of CK5/6 and/or EGFR.**

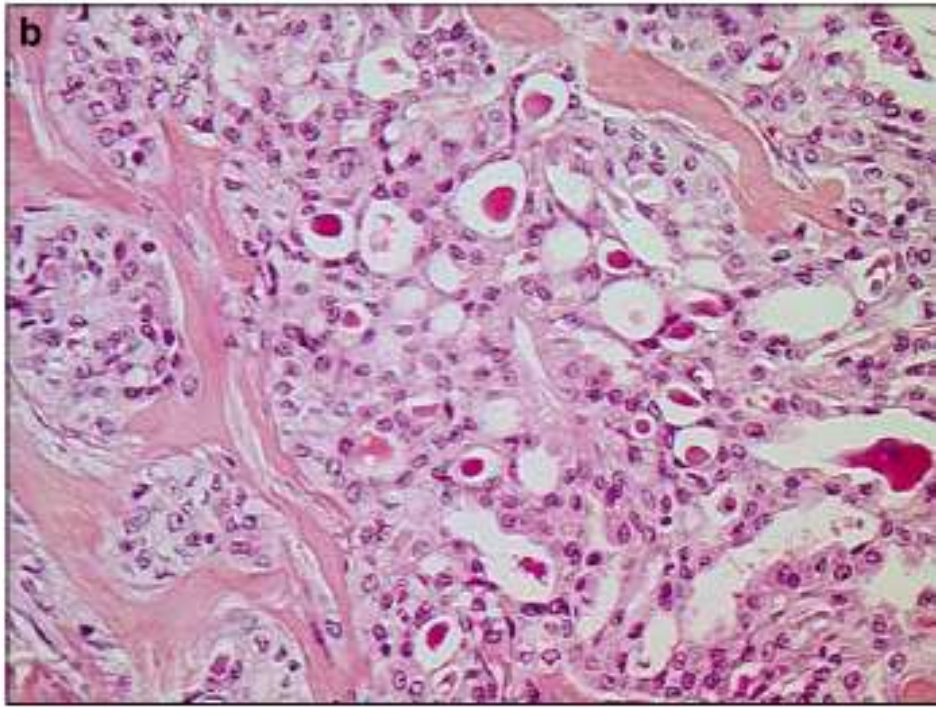
- **Vimentin, P-cadherin, caveolins 1 and 2, CD10, OSTEONECTIN, SMA, p16, Cyclin E, etc.**

Secretory breast carcinomas with *ETV6-NTRK3* fusion gene belong to the basal-like carcinoma spectrum

Marick Laé, Paul Fréneaux, Xavier Sastre-Garau, Olfa Chouchane, Brigitte Sigal-Zafrani and Anne Vincent-Salomon

Service de Pathologie, Section Médicale, Institut Curie, Paris Cedex, France





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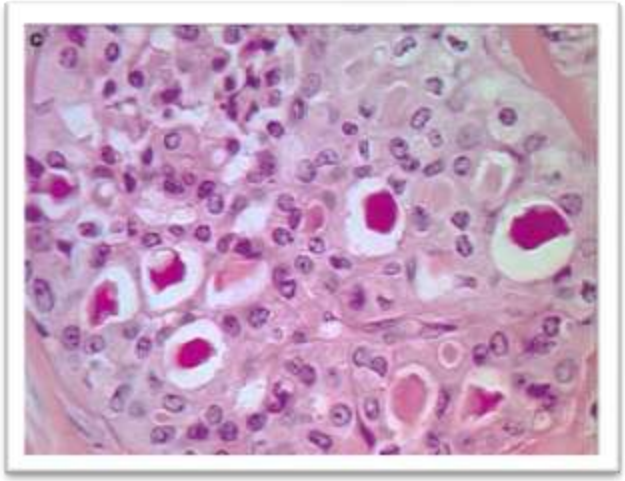
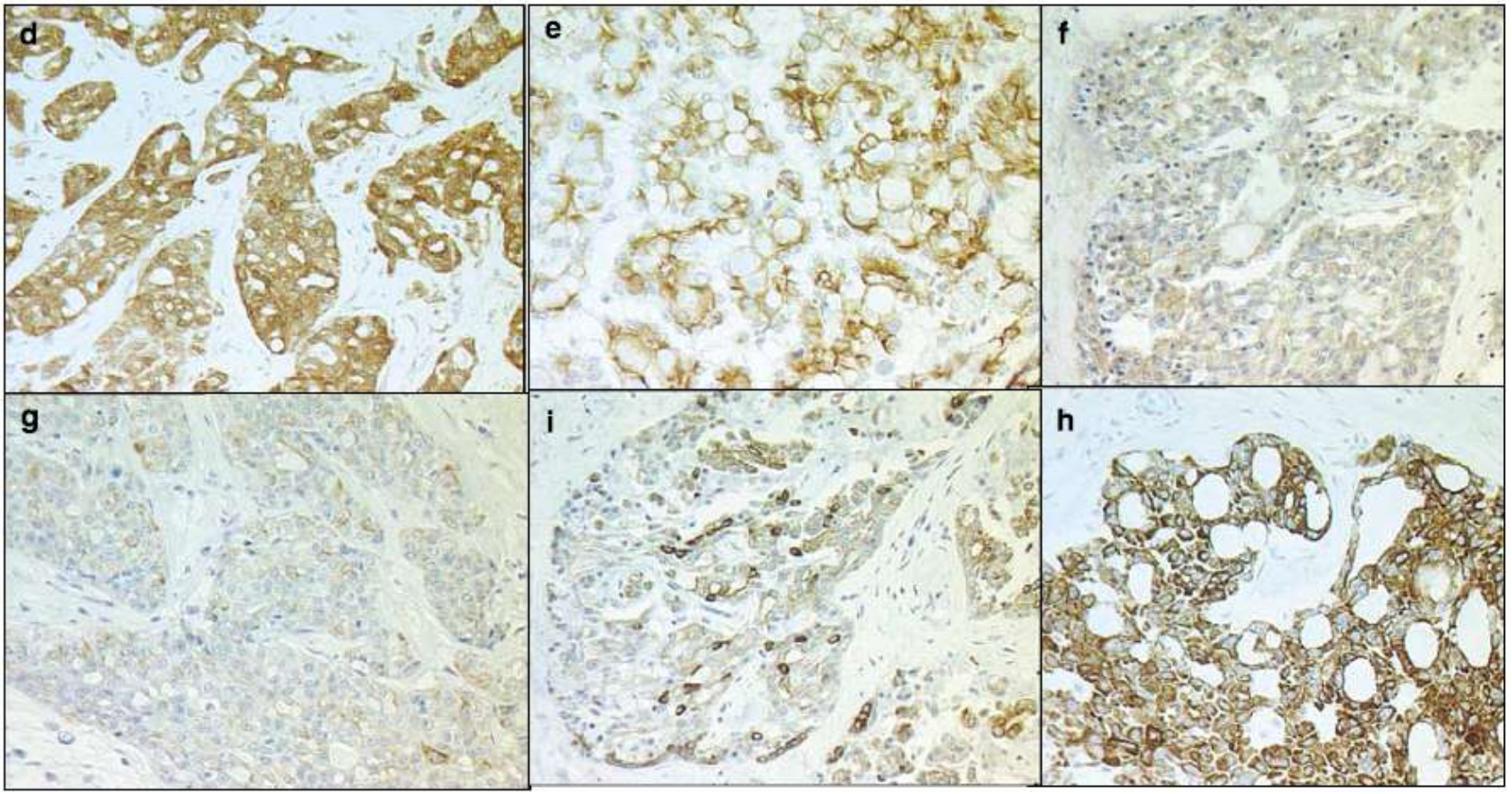
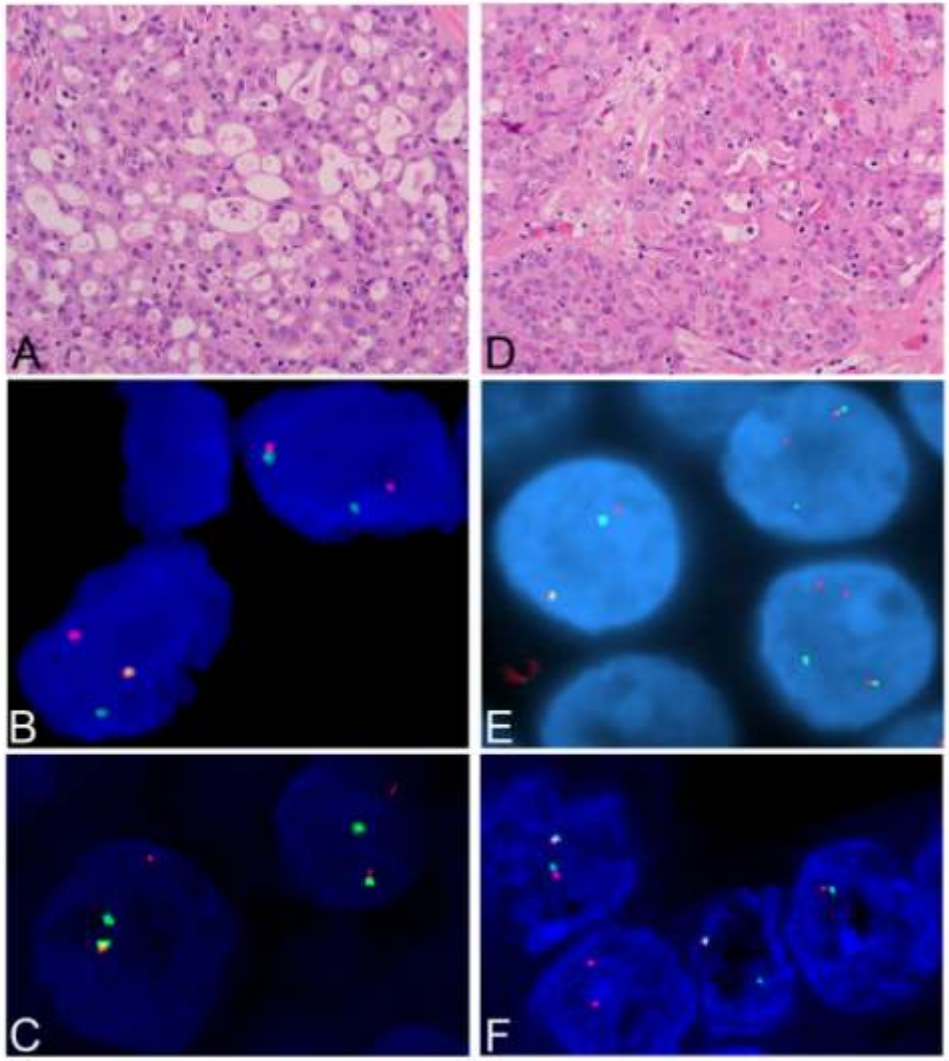




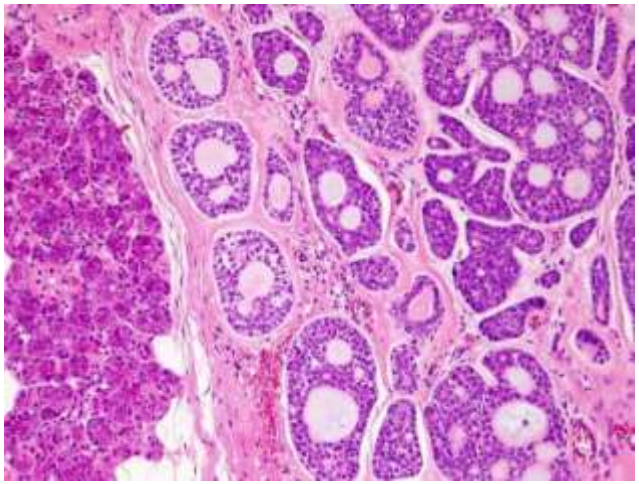
Secretory breast carcinomas harbour the t(12;15)(p13;q25) translocation

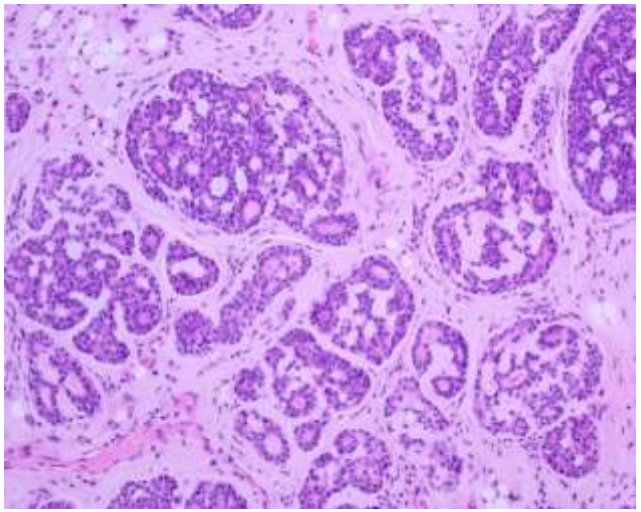
ETV6 split apart

ETV6-NTRK3 fusion

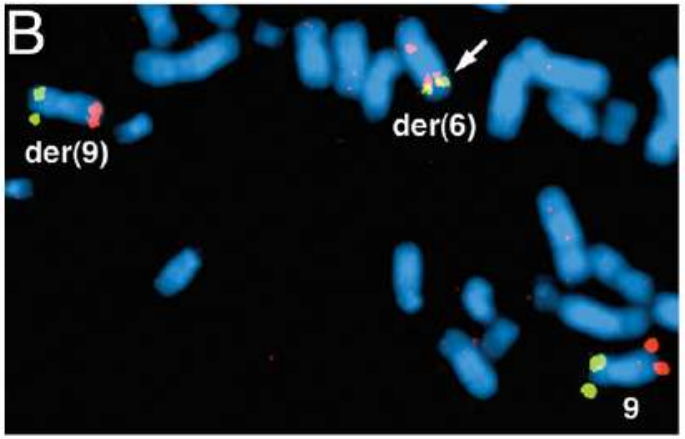


Adenoid Cystic Carcinoma

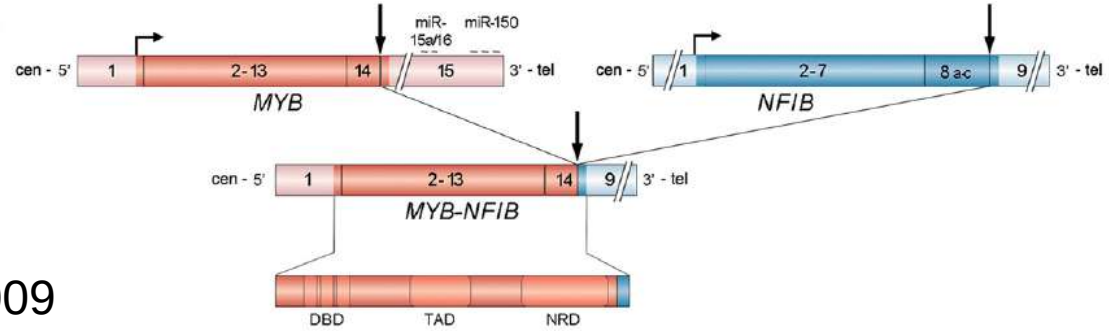




t(6;9)(q22–23;p23–24)



MYB-NFIB

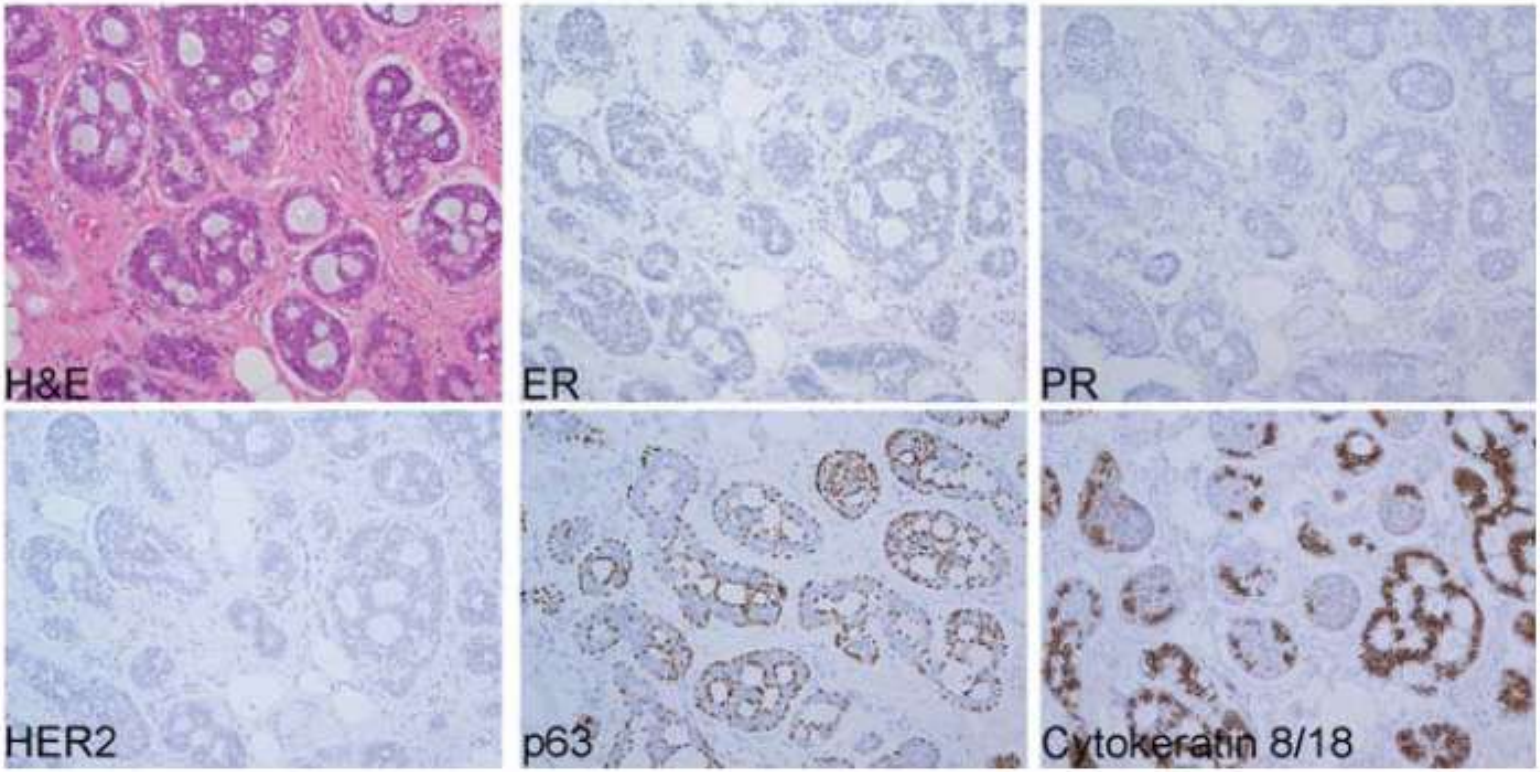


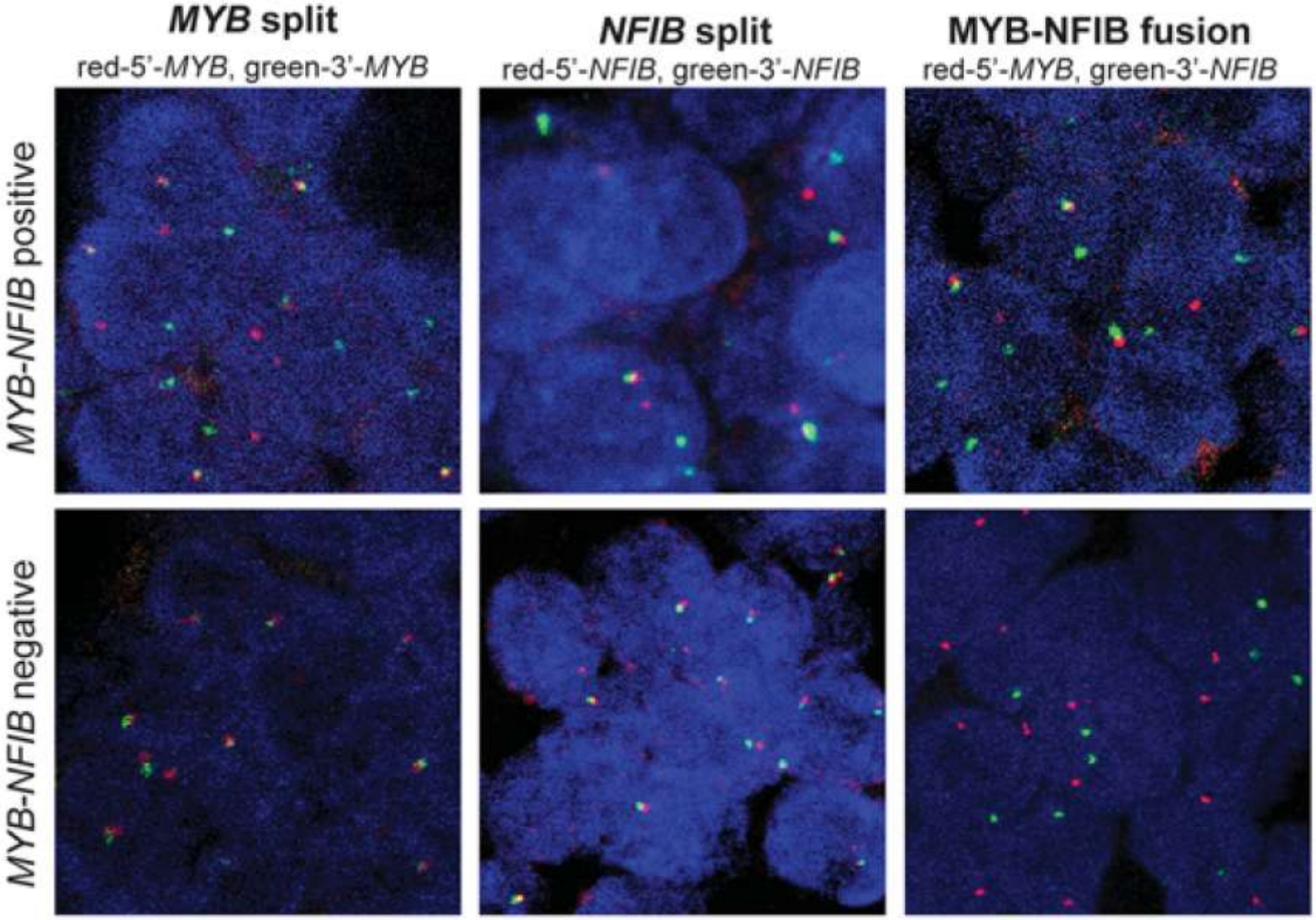
Persson et al. PNAS November 2009

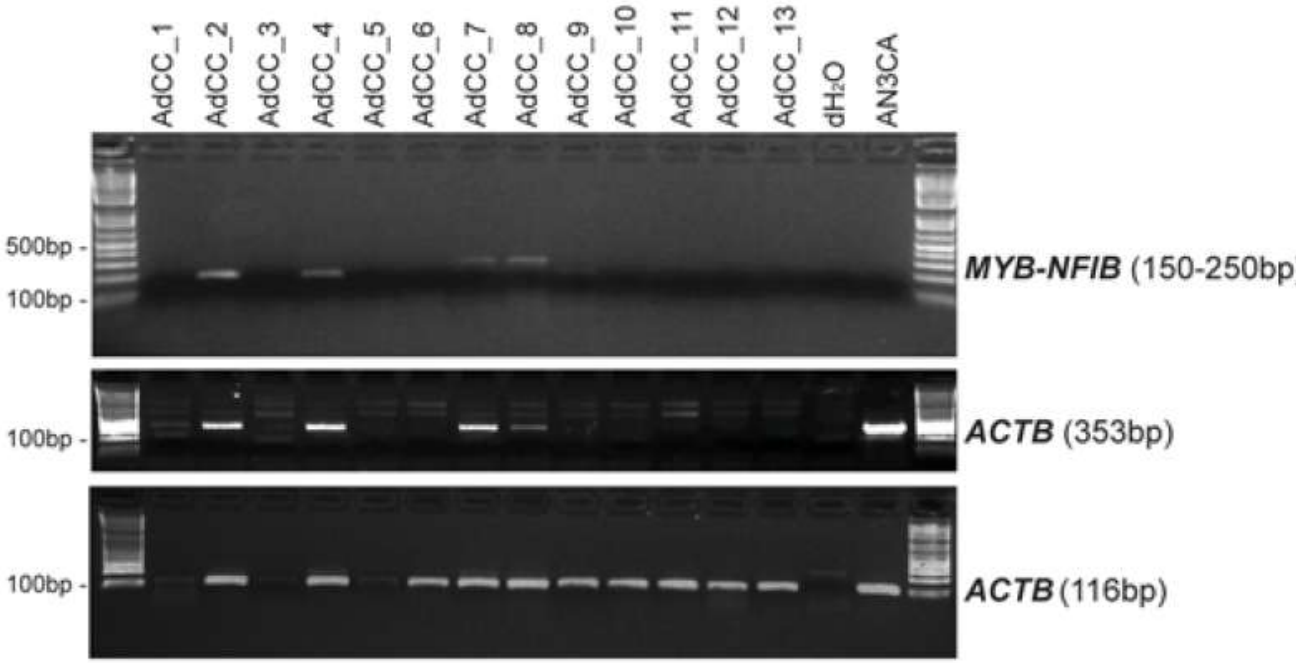
Breast Adenoid Cystic Carcinomas Constitute a Genomically Distinct Subgroup of Triple-negative and Basal-like Breast Cancers

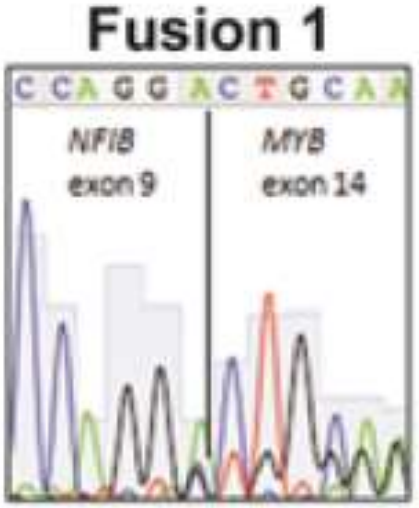
Daniel Wetterskog[1*], Maria-Angeles Lopez-Garcia[1,2*], Maryou B Lambros[1], Felipe C Geyer[1], Fernanda Milanezi[1], Maria C Cabral[1], Rachael Natrajan[1], Kai-Keen Shiu[1], Sami Shousha[3], Zoran Gatalica[4], Alan Mackay[1], Jose Palacios[2], Jorge S Reis-Filho[1*] & Britta Weigelt[5*]

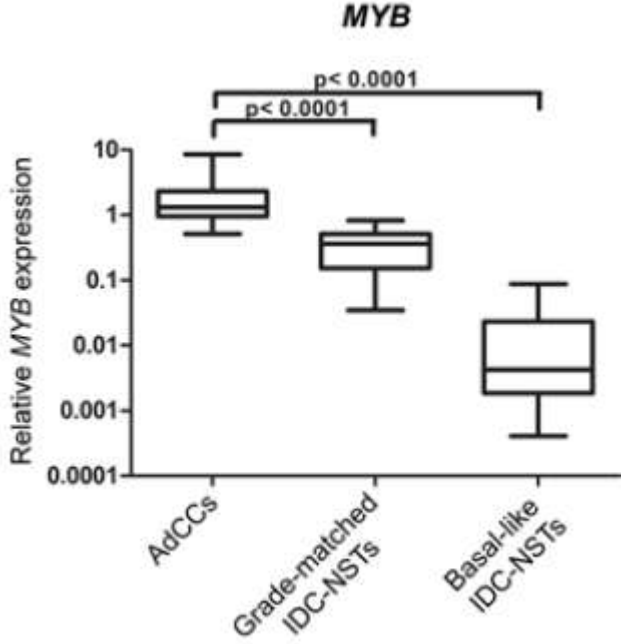
[1]The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, UK; [2]Hospital Universitario Virgen del Rocio, Seville, Spain; [3]Department of

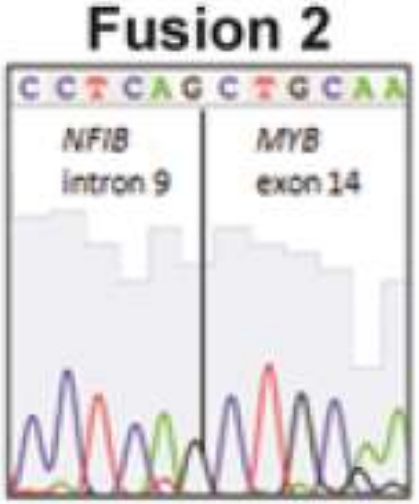



MYB split
red-5'-*MYB*, green-3'-*MYB*
NFIB split
red-5'-*NFIB*, green-3'-*NFIB*
MYB-NFIB fusion
red-5'-*MYB*, green-3'-*NFIB*
MYB-NFIB positive
MYB-NFIB negative


AdCC_1
AdCC_2
AdCC_3
AdCC_4
AdCC_5
AdCC_6
AdCC_7
AdCC_8
AdCC_9
AdCC_10
AdCC_11
AdCC_12
AdCC_13
dH2O
AN3CA
500bp -
100bp -
MYB-NFIB (150-250bp
100bp -
ACTB (353bp)
100bp -
ACTB (116bp)


Fusion 1
NFIB
exon 9
MYB
exon 14


MYB
p< 0.0001
p< 0.0001
Relative MYB expression
10
1
0.1
0.01
0.001
0.0001
AdCCs
Grade-matched IDC-NSTs
Basal-like IDC-NSTs


Fusion 2
NFIB
intron 9
MYB
exon 14

SPECIAL TYPES OF BREAST CANCER

- Special types of breast cancer account for up to one quarter of all invasive breast malignancies and their importance should not be disregarded.
- Studies focusing on specific subtypes of carcinomas have recently identified pathognomonic mutations and specific fusion genes that can be used not only for diagnostic purposes, but also therapeutically.
- Understanding the biological drivers of these entities may lead to a better understanding of the biology of breast cancer.