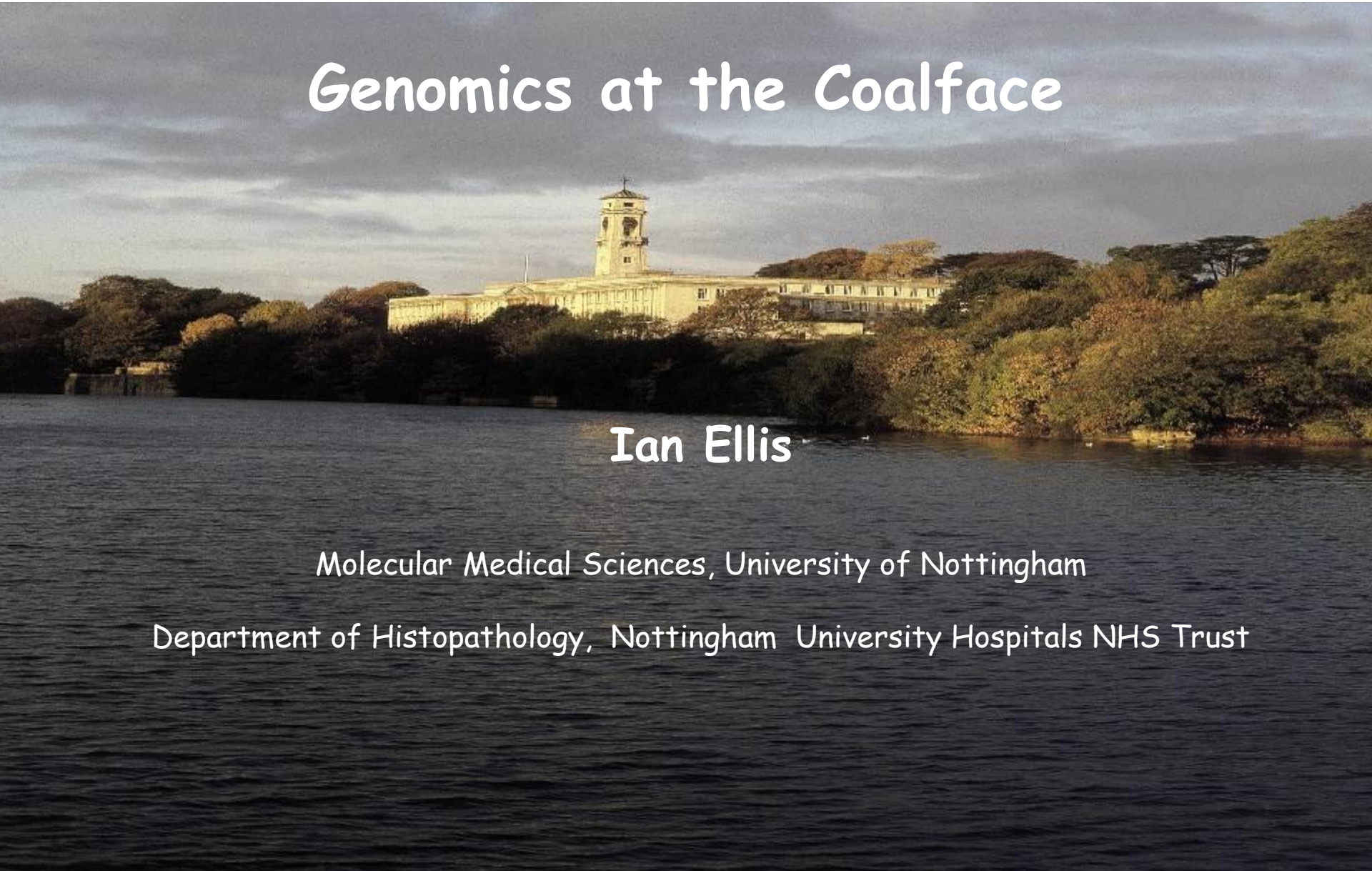
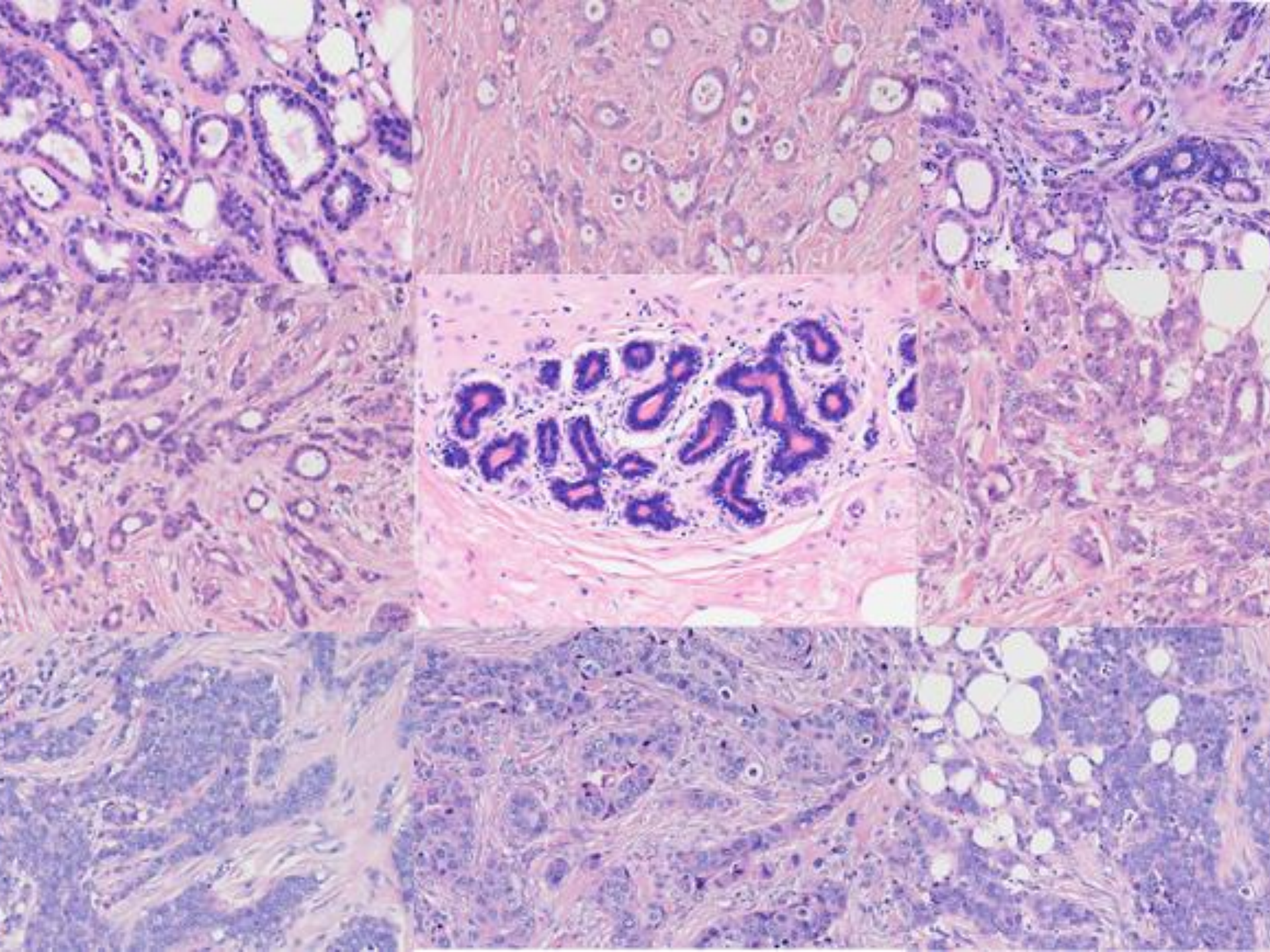


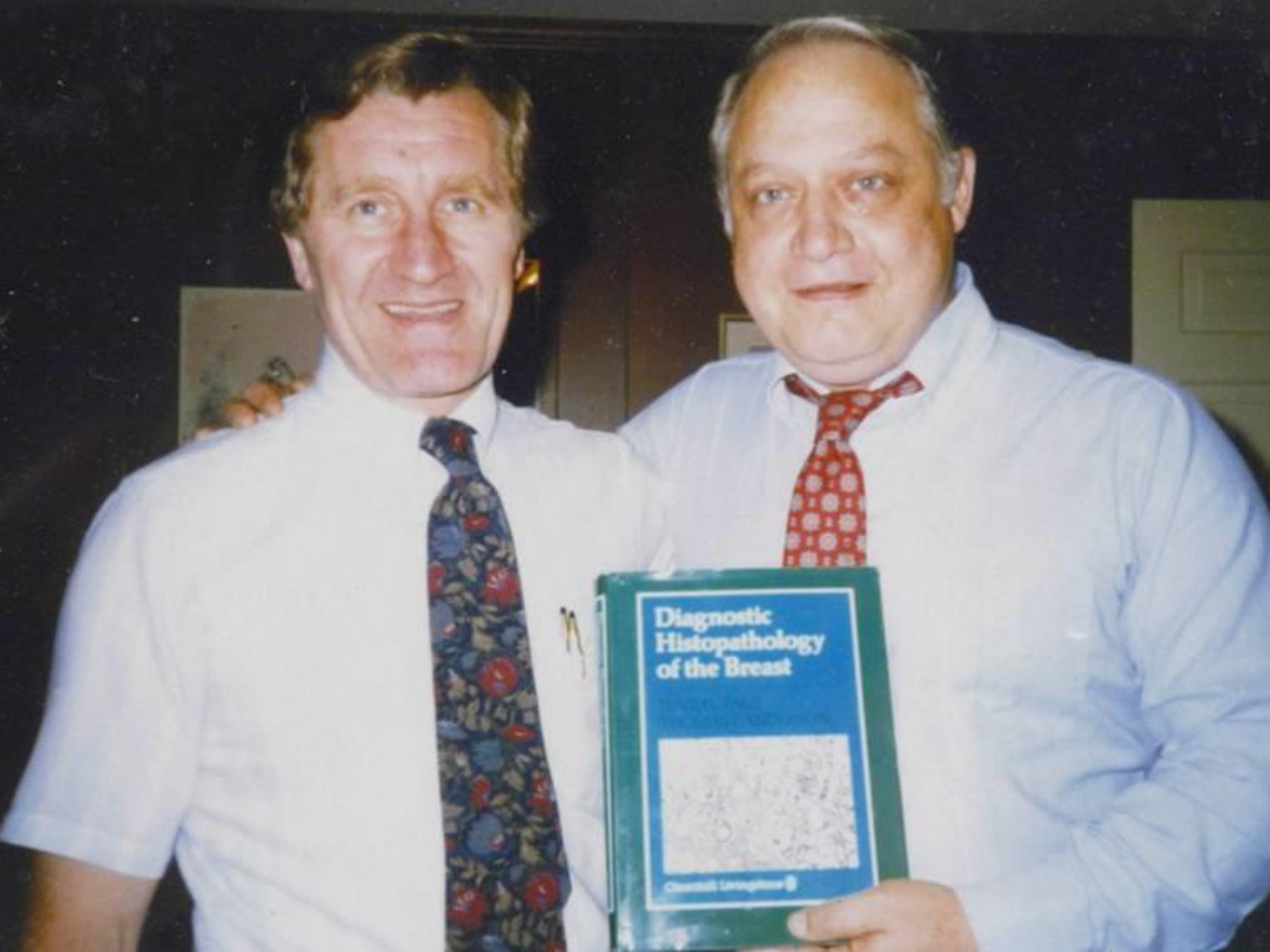
Genomics at the Coalface

Ian Ellis

Molecular Medical Sciences, University of Nottingham

Department of Histopathology, Nottingham University Hospitals NHS Trust



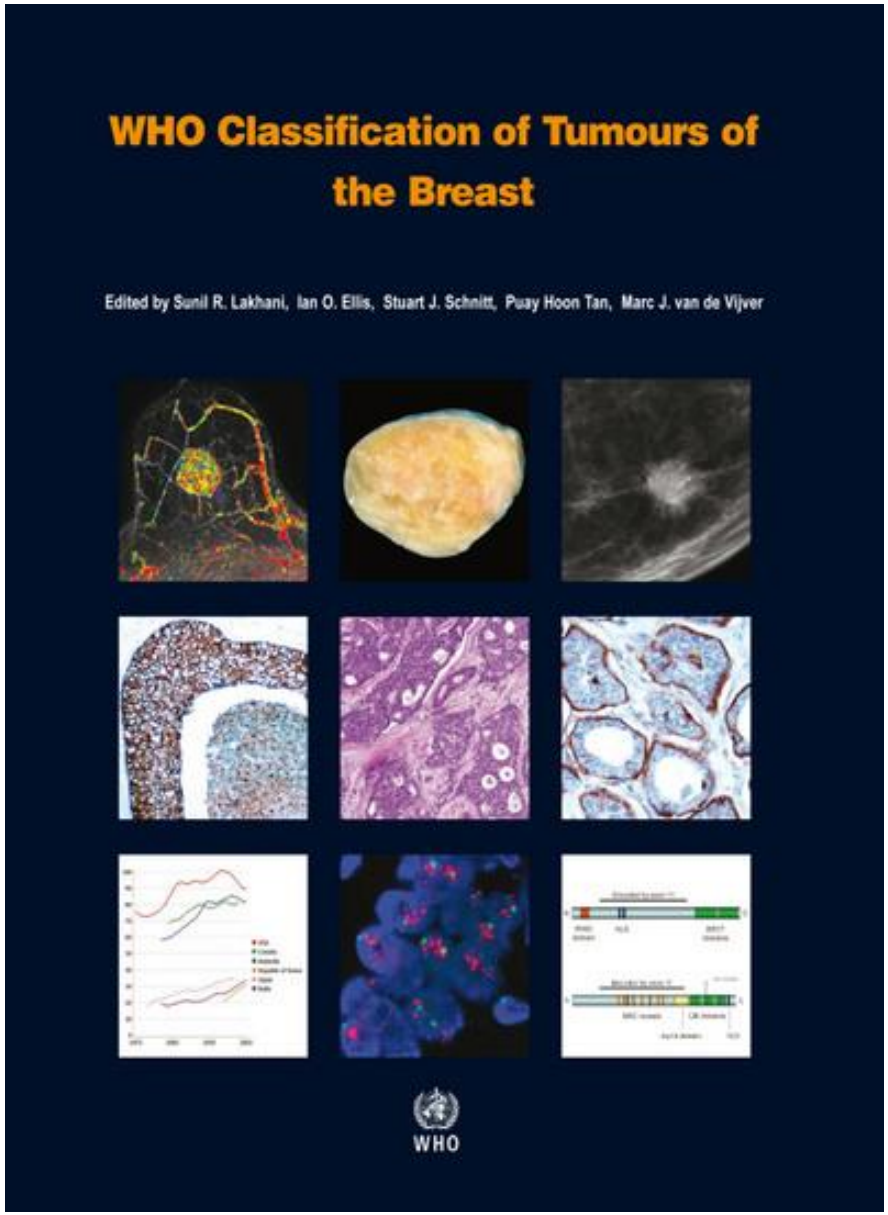

Diagnostic
Histopathology
of the Breast

Histological Type

Survival (%)

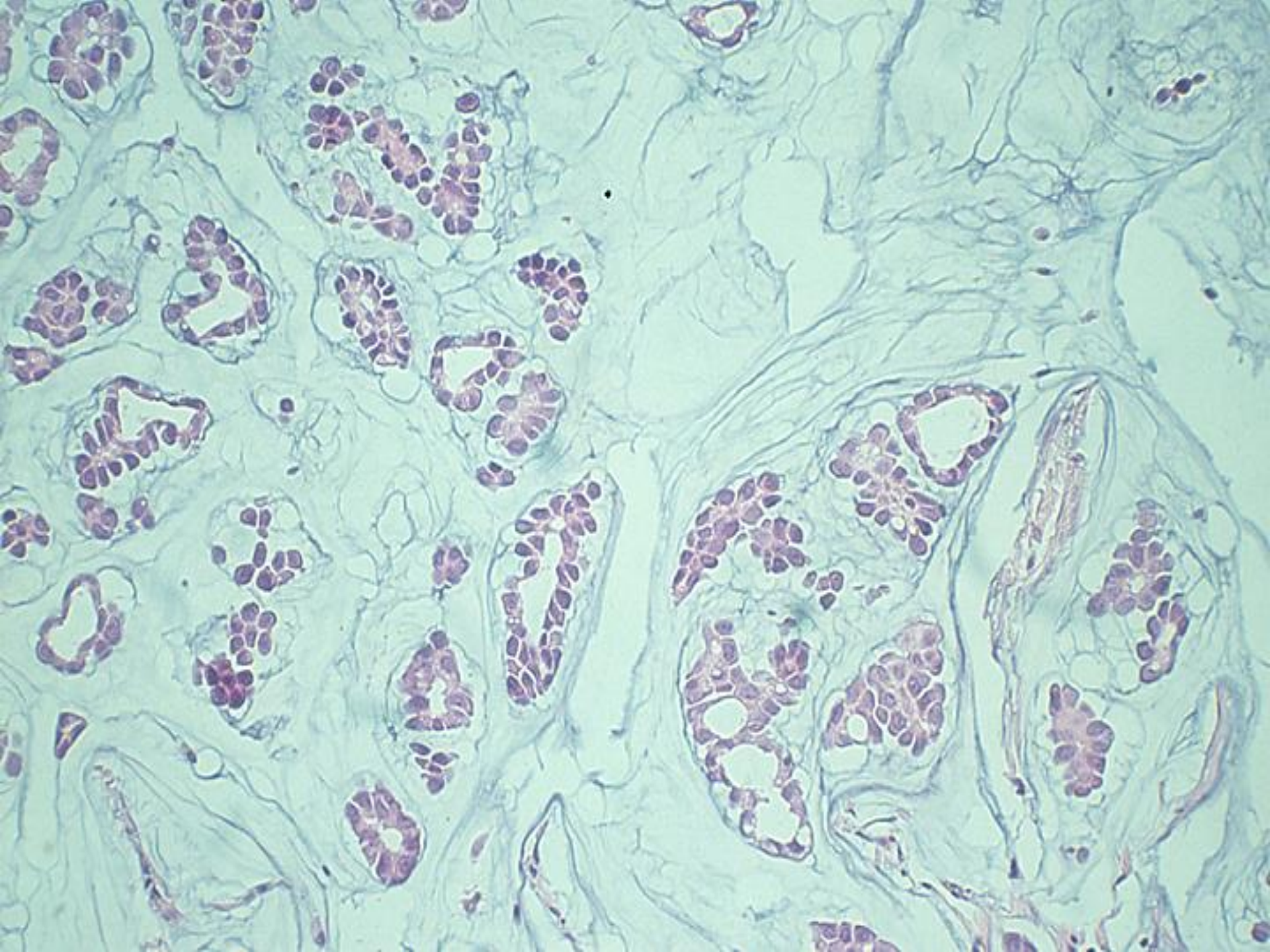
Histological type	Long term survivors n = 119	Consecutive series n = 1050	Short term survivors n = 200
Tubular	8	3	0
Tubular variant	7	5	0
Lobular	16	10	5
Cribriform	13	3	0
Papillary	5	1	0
Mucinous	2	2	0
Medullary	9	5	4
Ductal NST	30	67	83

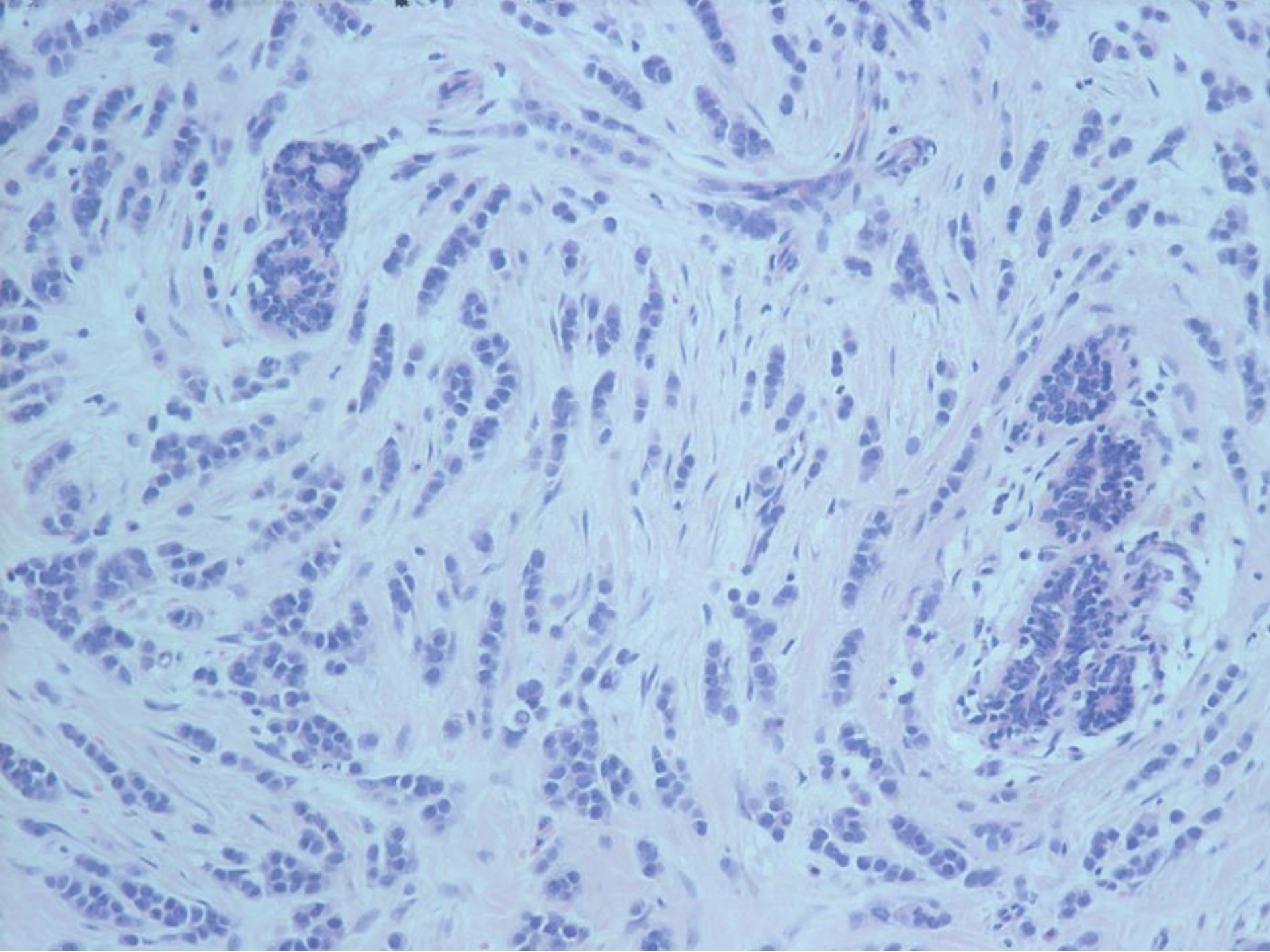
Dixon et al, Br J Surg 1985; 72: 445-448

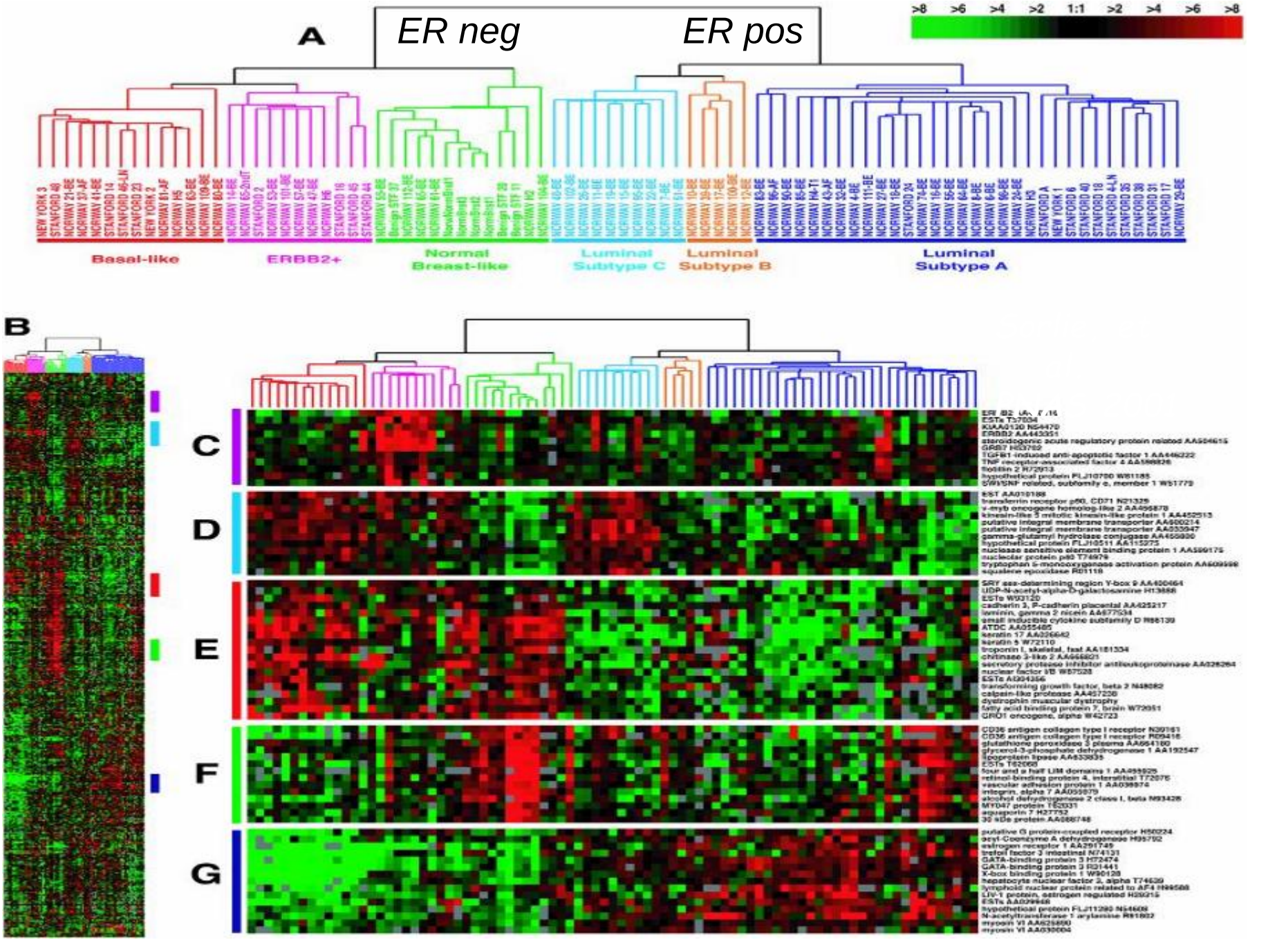


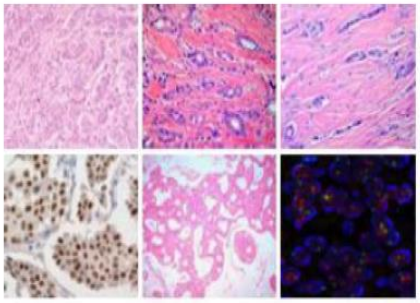
WHO classification of tumours of the breast

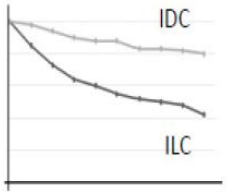
Table of contents

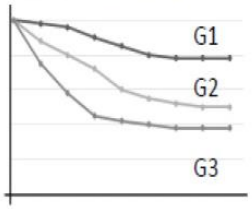





A
ER neg
ER pos
>8 >6 >4 >2 1:1 >2 >4 >6 >8
Basal-like
ERBB2+
Normal Breast-like
Luminal Subtype C
Luminal Subtype B
Luminal Subtype A
B
C
D
E
F
G

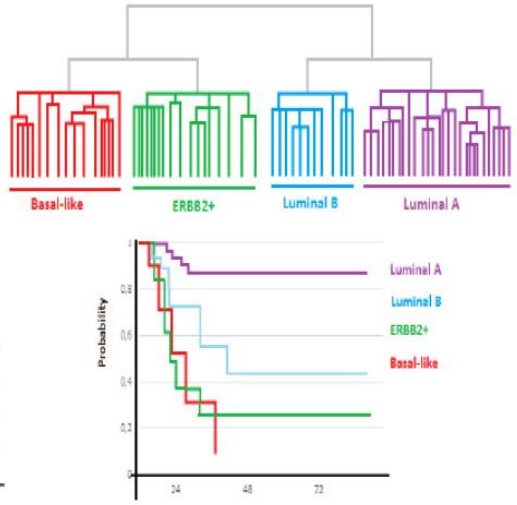




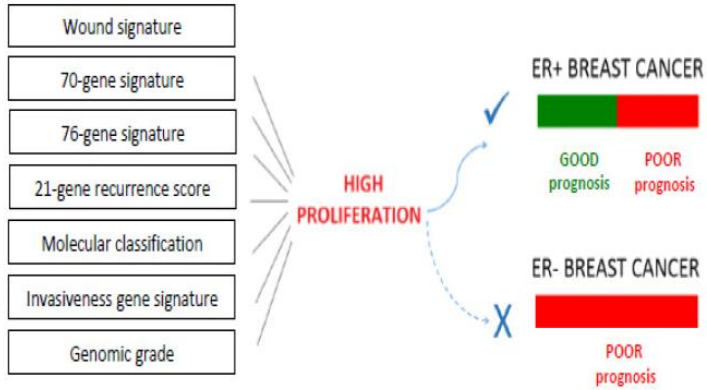


Histological type

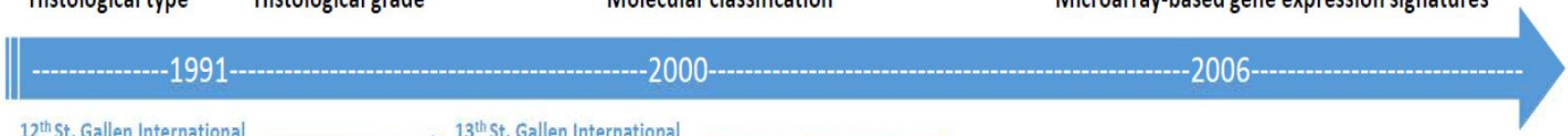
Histological grade



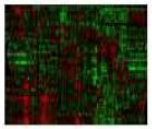
Molecular classification



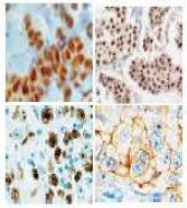
Microarray-based gene expression signatures



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)

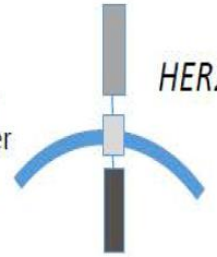
Molecular tests for risk prediction

St Gallen 2015 Viena

Gene expression data

Copy number data

IntClusts

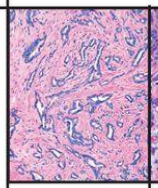
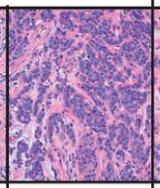
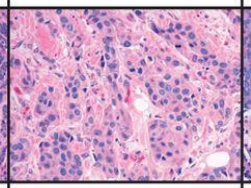
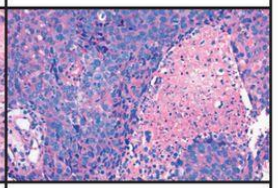
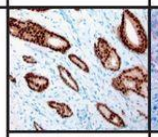
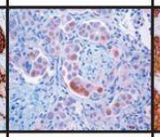
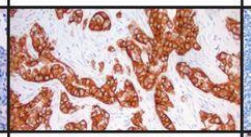
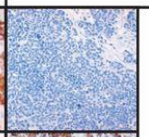
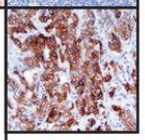


HER2 mutations

NGS ...

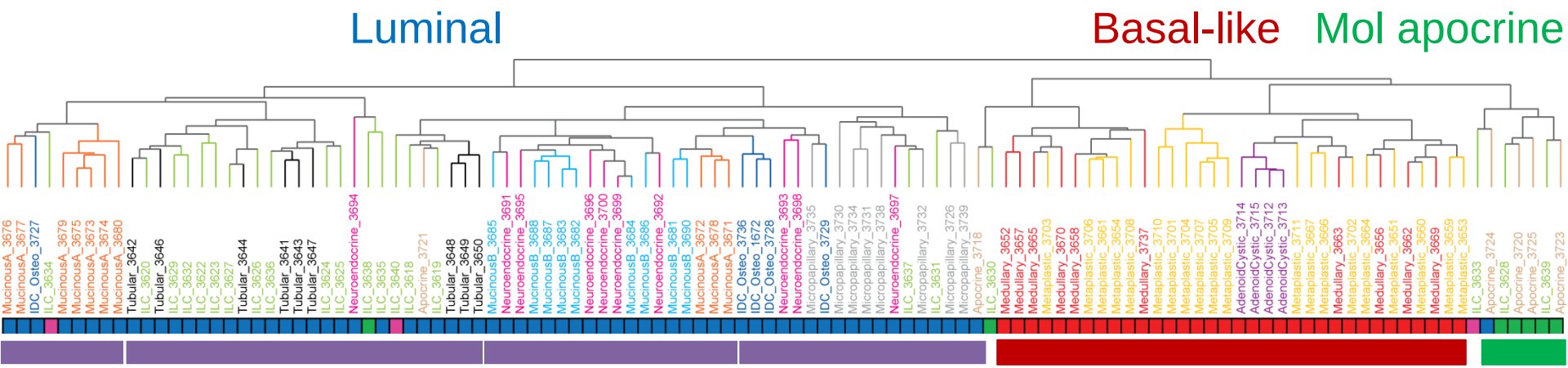
TCGA results: IntClust, mutation profile *et al.*

Summary of the features of the basic molecular / intrinsic breast cancer subtypes.

Molecular Subtype	Luminal (A and B)		HER2	Basal
Genetic profile	↑ Luminal CKs and ER-related genes (A>B) B↑ in proliferation-related genes		↑ HER2-related genes	↑ Basal CKs
Histologic correlates				
	A Lower-grade ER+	B Higher-grade ER+	High-grade, +/– apocrine features	High-grade, sheet-like, necrosis, inflammation *See exceptions
Surrogate markers				 ER/PR– HER2–
	A Strong ER+, PR+/–, HER2–, low Ki67	B Weaker ER+, PR+/–, HER2+/–, ↑ Ki67	HER2+, +/– ER/PR	 CK5/6+/– EGFR+/–
Prognosis	Good	Intermediate	Worse	Worse
Response to chemotherapy	Lower	Intermediate	Higher	Higher
Targeted therapies	Hormone therapies		HER2-targeted therapies	Currently investigational

American Journal of Clinical Pathology, Volume 138, Issue 6, December 2012, Pages 770–780, https://doi.org/10.1309/AJCPIV9IQ1MRQMOO

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

VOLUME 28 · NUMBER 1 · JANUARY 1 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Tubular Carcinoma of the Breast: Further Evidence to Support Its Excellent Prognosis

Emad A. Rakha, Andrew H.S. Lee, Andrew J. Evans, Sindhu Menon, Nancy Y. Assad, Zsolt Hodi, Douglas Macmillan, Roger W. Blamey, and Ian O. Ellis

Tubular carcinoma is known to have a favourable prognosis, but does this subtype represents a distinct type of breast carcinoma and does it behave like other low-grade luminal A-type breast carcinomas?

Rakha et al. J Clin Oncol 28:99-104

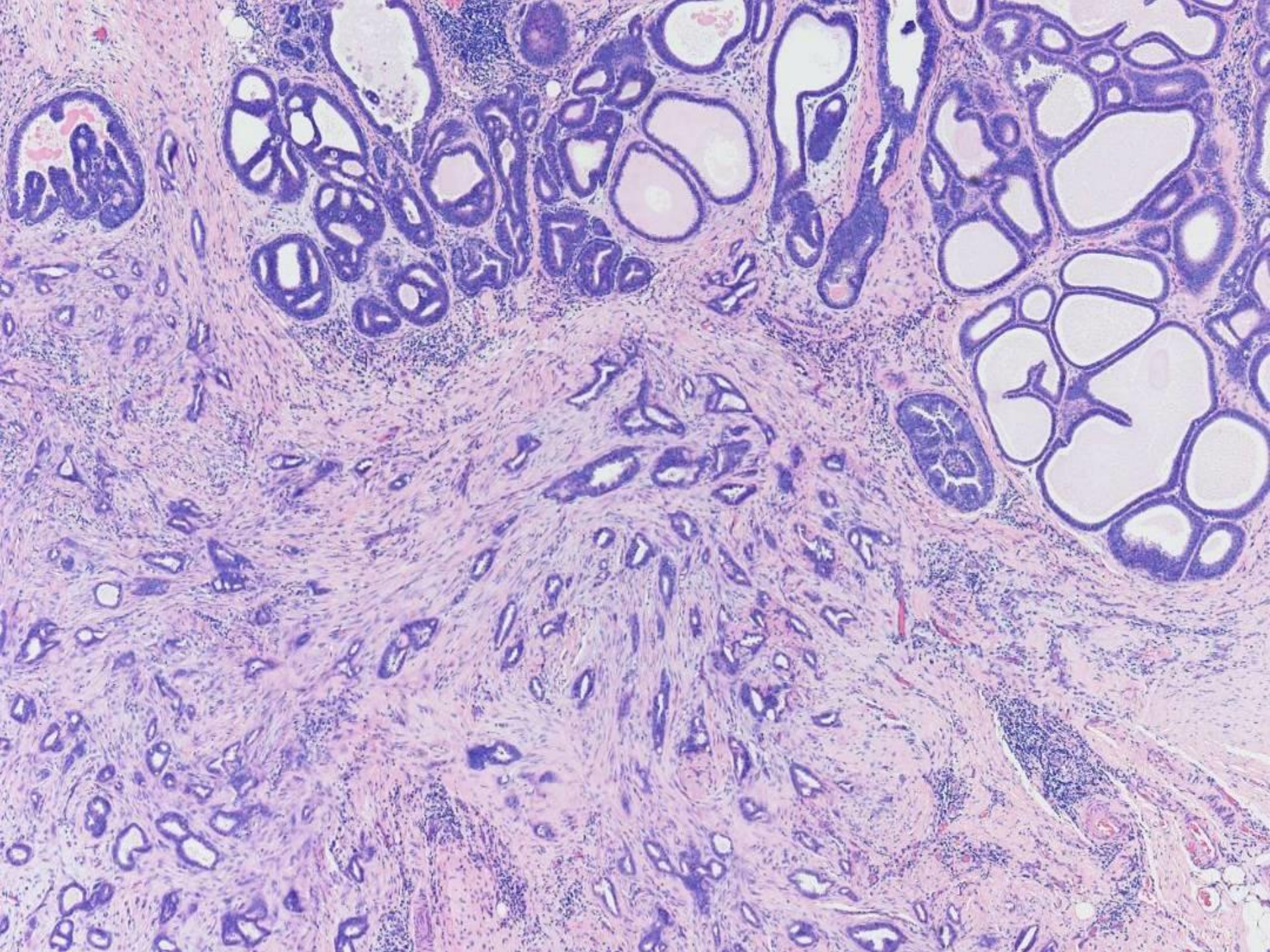
Conclusion

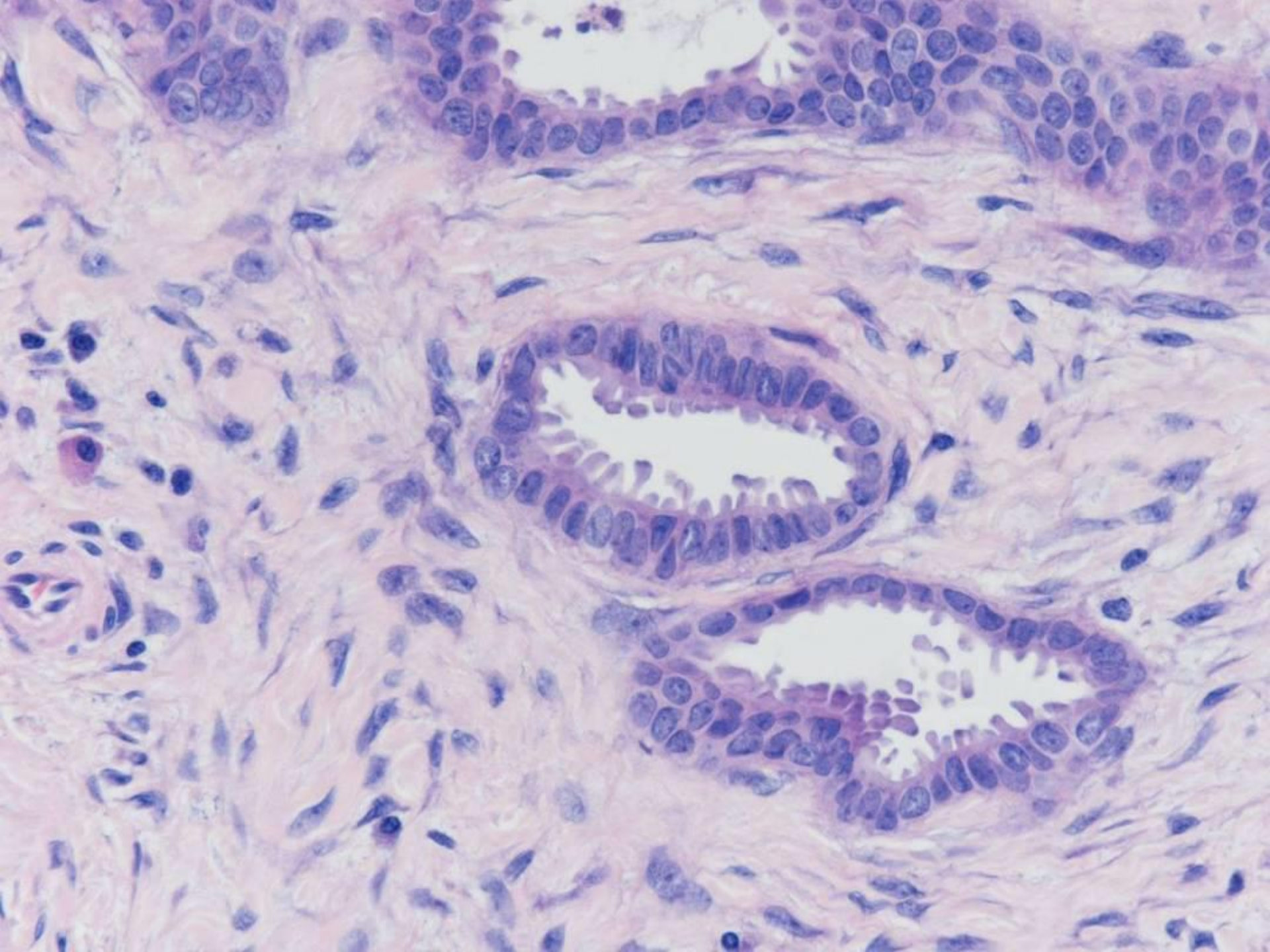
The biologic behaviour of TC is excellent and is more favourable than that of grade 1 ductal carcinoma.

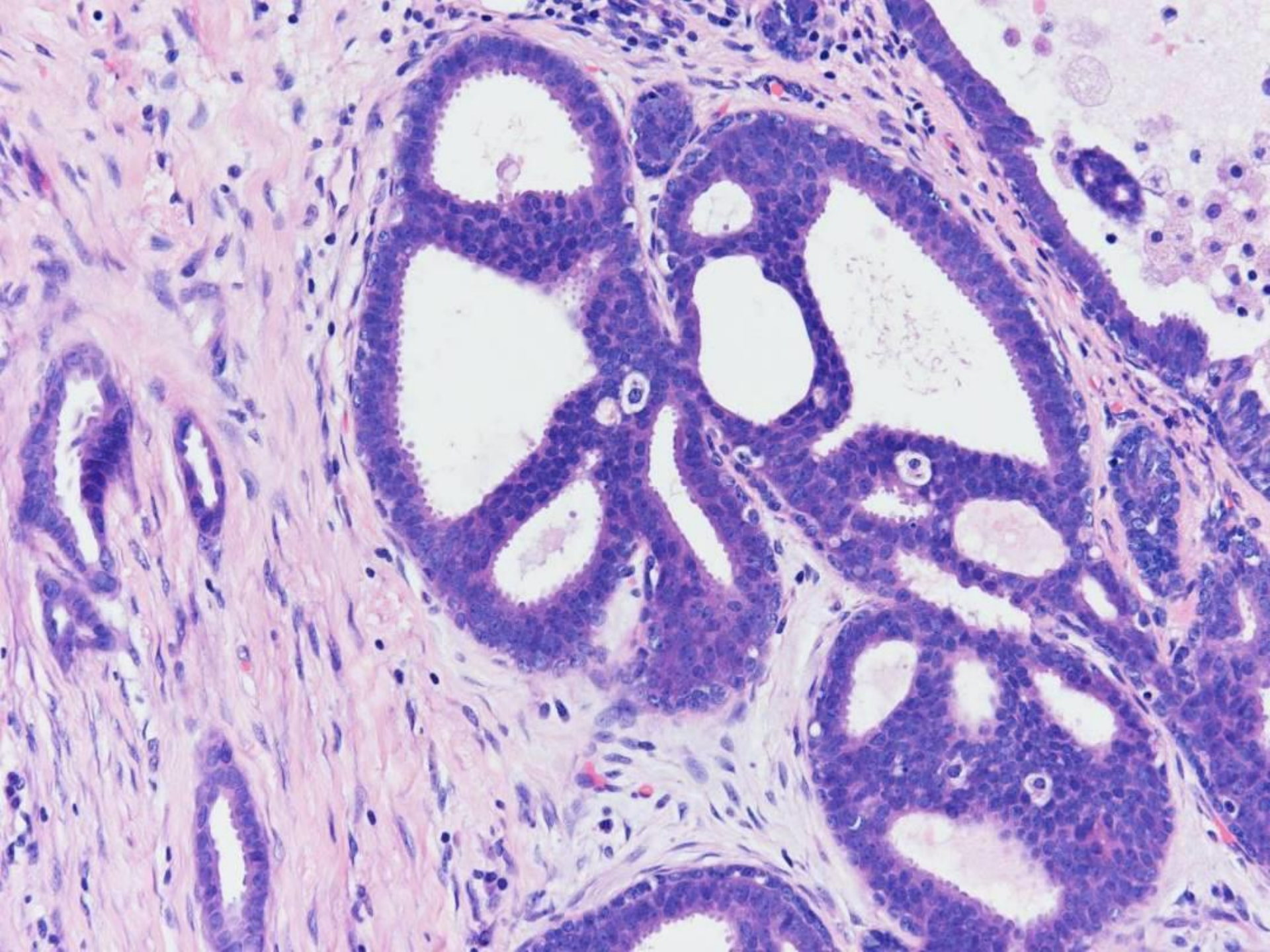
Patients with TC may be at risk of developing second primary carcinomas in the contralateral breast, which may be of higher grade and poorer potential prognostic outcome.

Patients with TC have a close to normal life expectancy, and as a consequence, adjuvant systemic therapy may not be justified in their routine management.

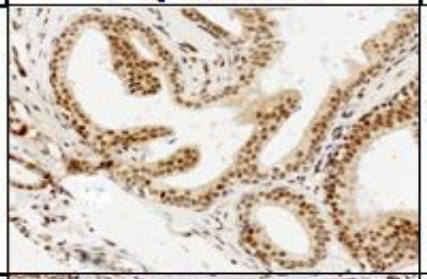
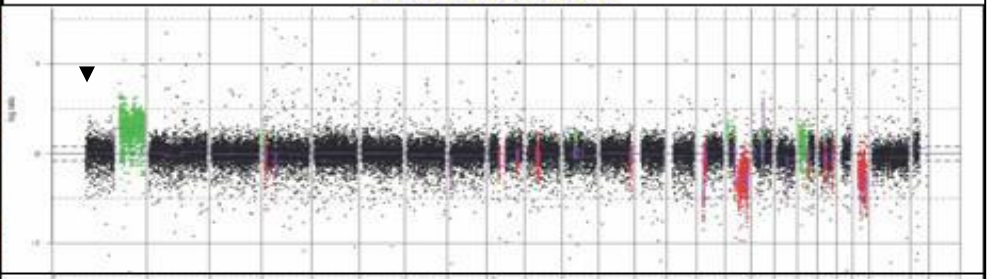
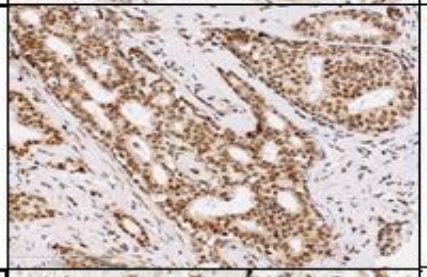
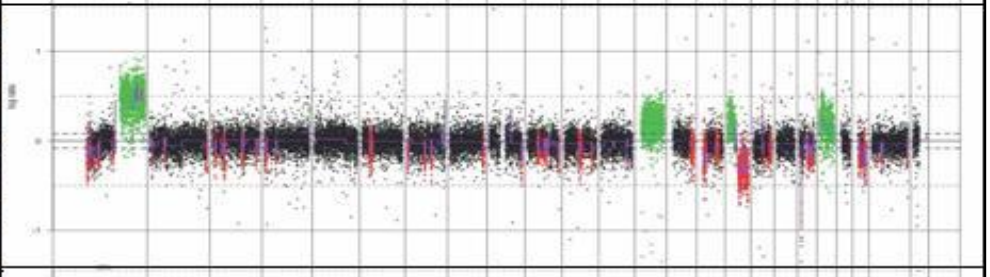
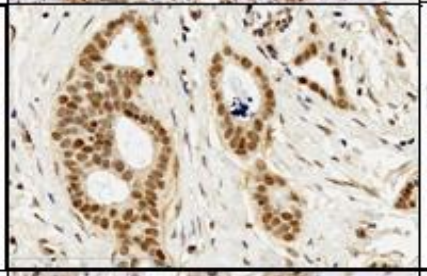
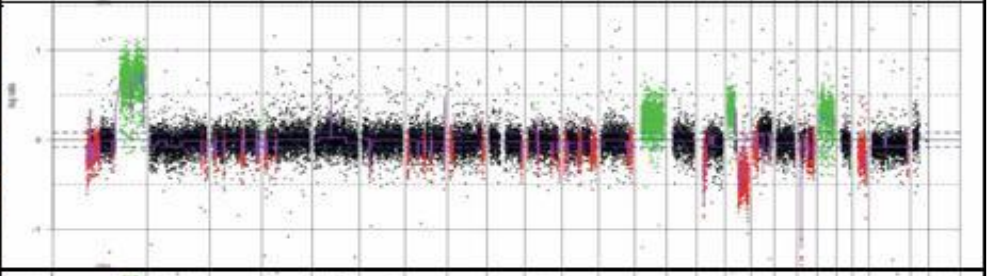
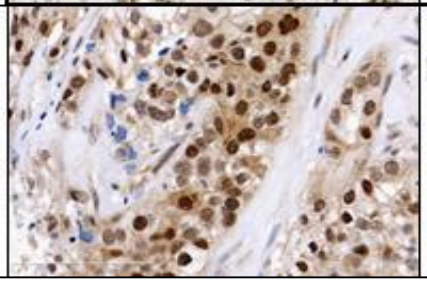
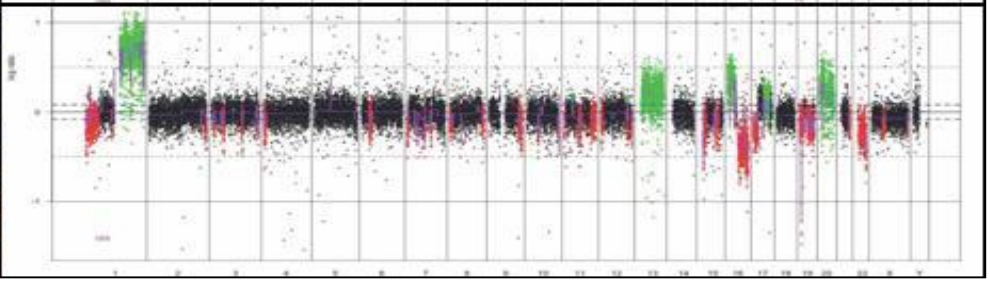
Rakha et al. J Clin Oncol 28:99-104







A case of tubular carcinoma

Microdissected Lesions	MDM4 Expression	Genome Plot
Columnar Cell Lesion		
Ductal Carcinoma In Situ		
Invasive Tubular Carcinoma (at centre)		
Invasive Tubular Carcinoma (at periphery -solid)		

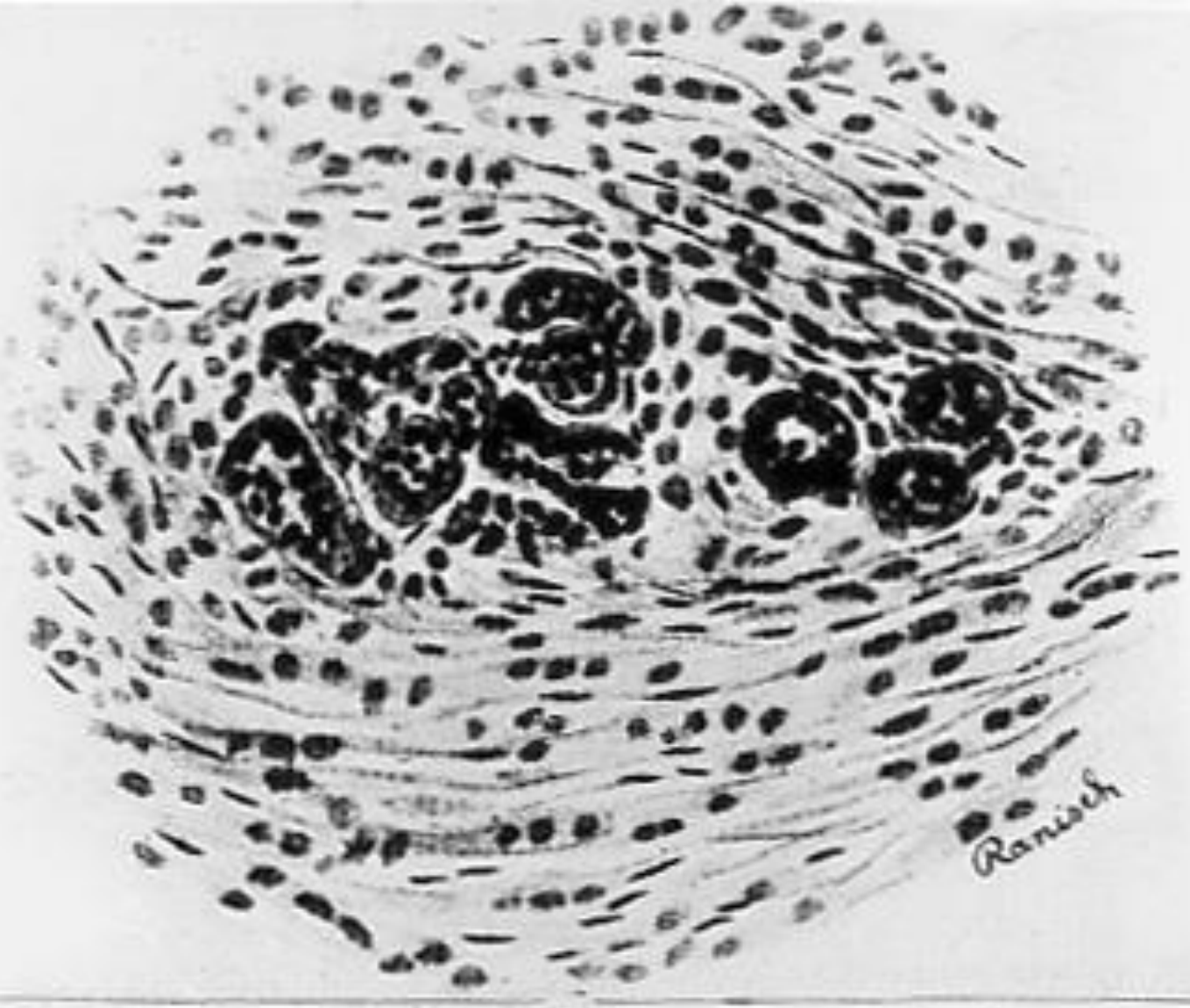


Fig
det
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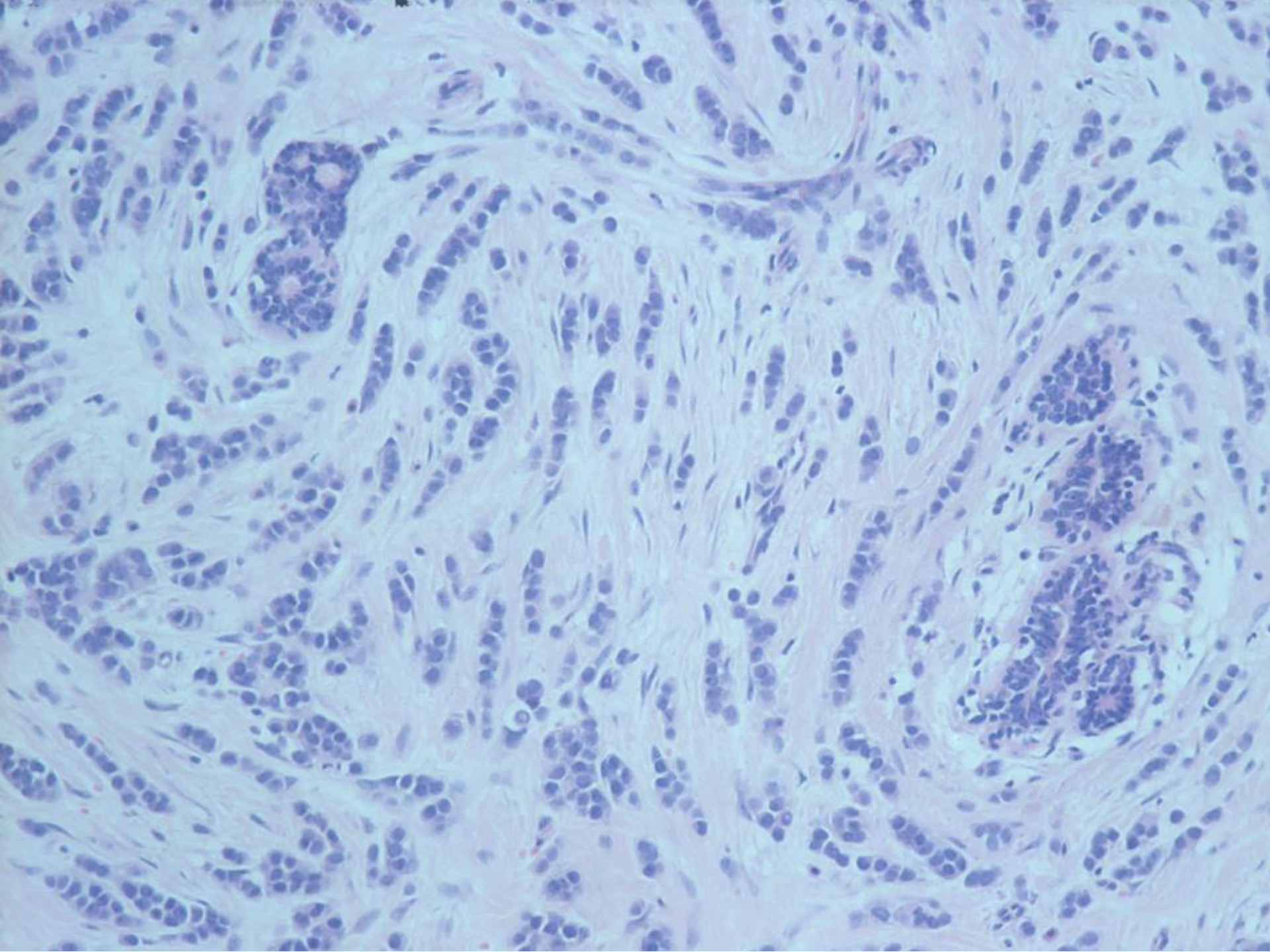
SALOMON, A.: Beiträge zur Pathologie und Klinik der Mammacarcinome.
Arch.Klin.Chir. 101, 573-668, 1913

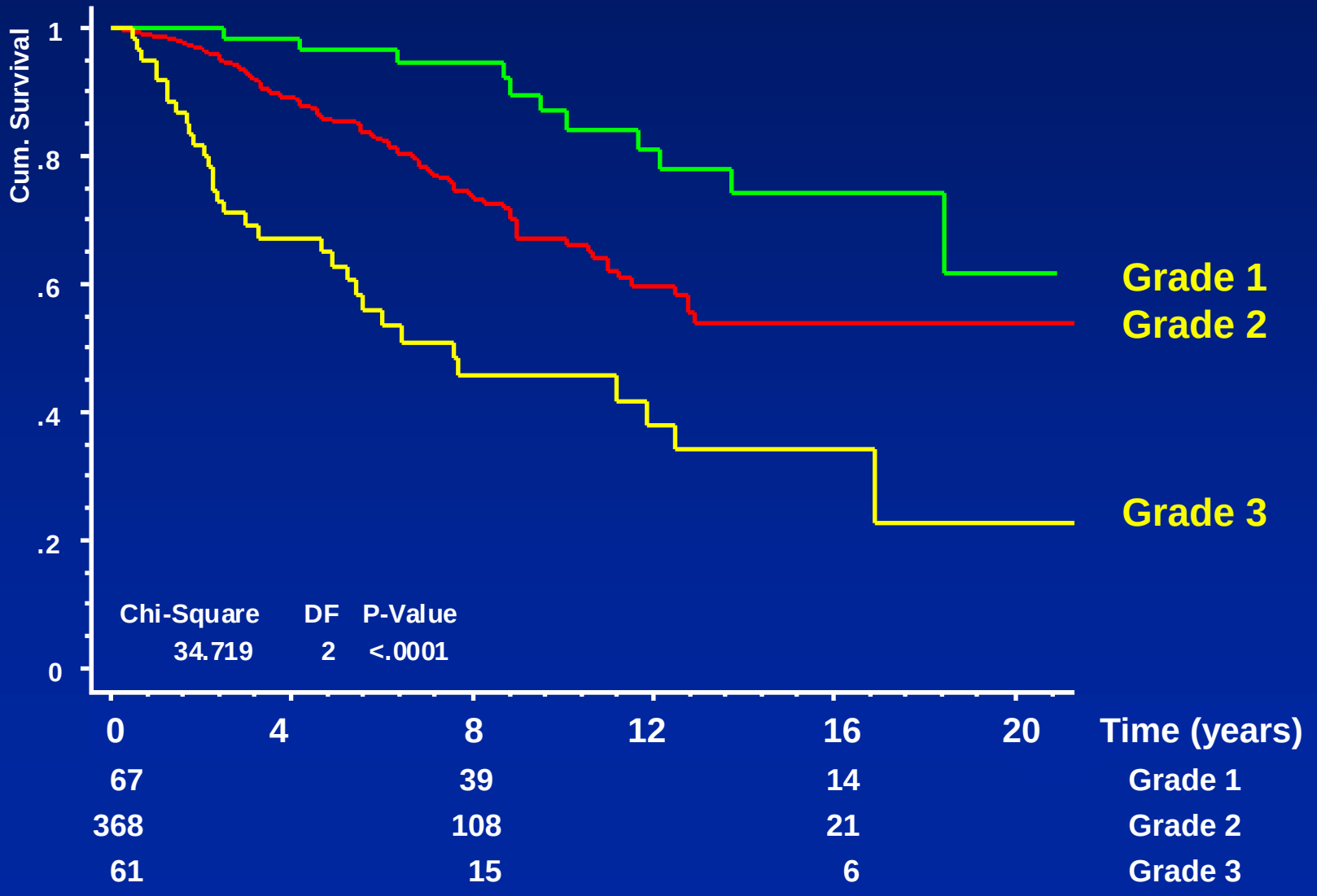


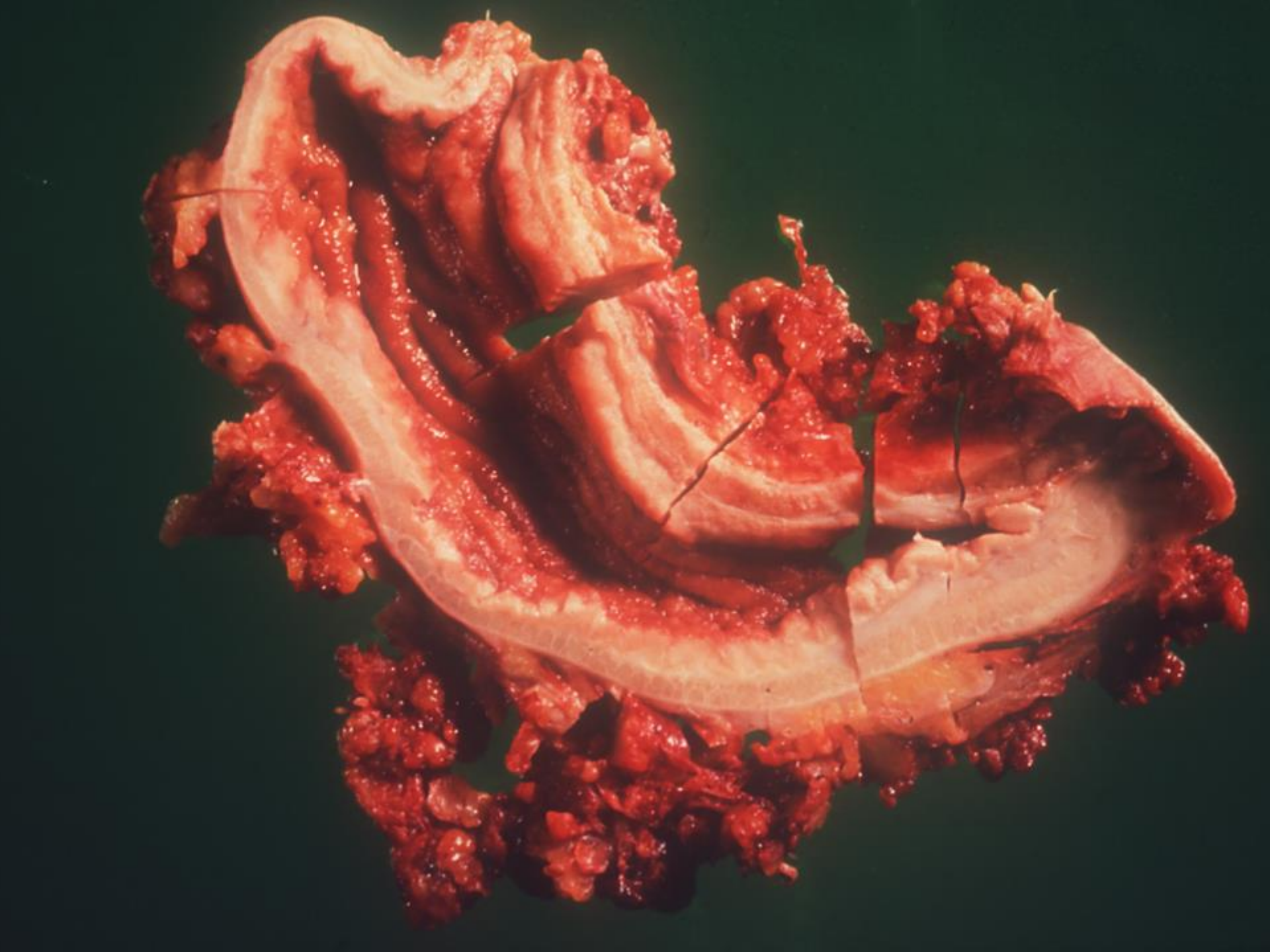
Invasive Lobular Carcinoma

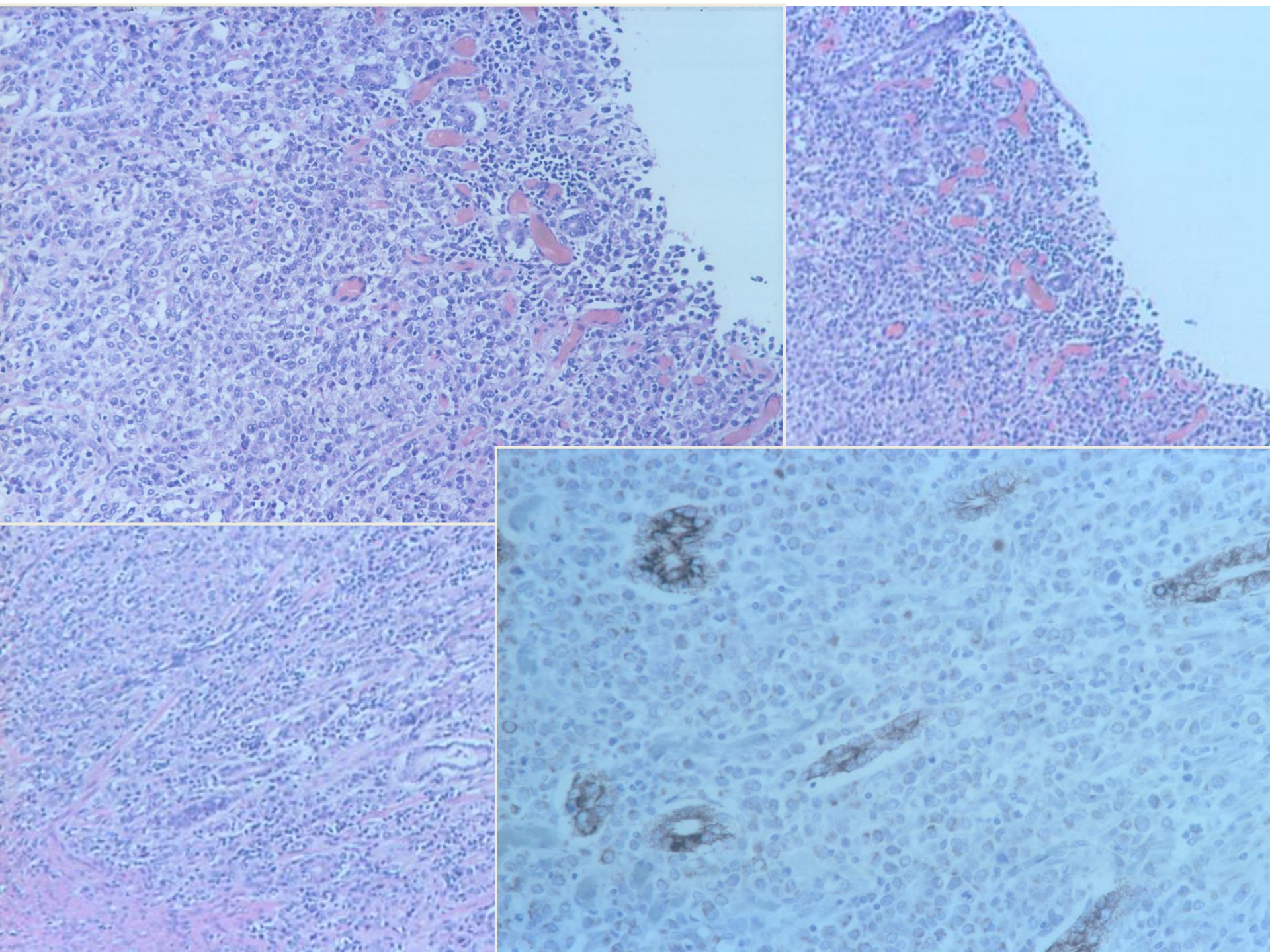
- Commonest special type cancer – 5-15% of breast cancers in women
- Predominantly Western disease – rare in Asia, Africa and Middle East
- Increase in incidence related to use of HRT
- Majority sporadic – rare secondary tumour in families with hereditary diffuse gastric ca syndrome linked to germline *CDH1* mutations
- Extremely rare in men (<1%)

Nottingham Tenovus Primary Breast Cancer Study

Infiltrating Lobular Carcinoma by Grade



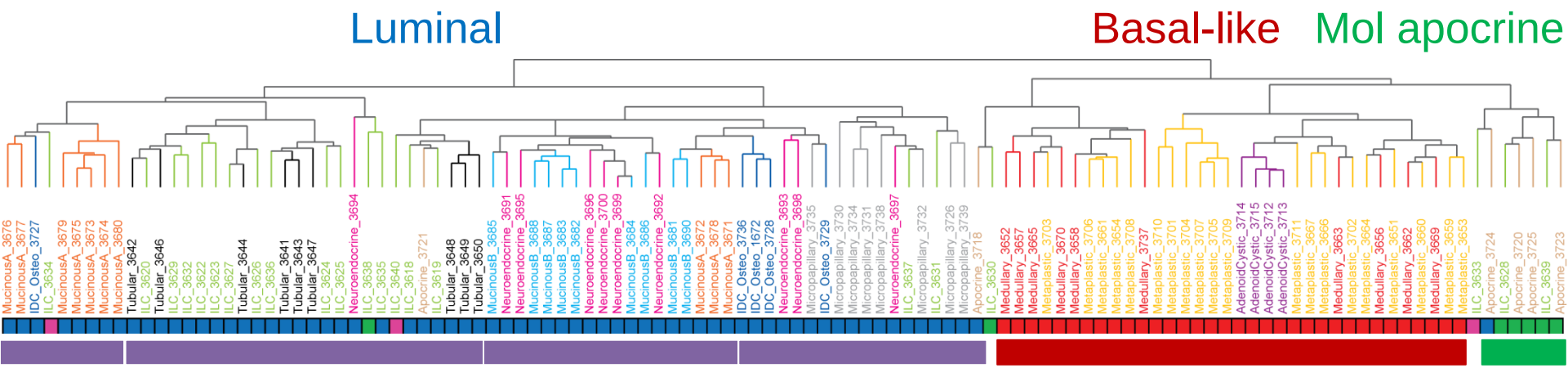




ILC – patterns of metastasis

- Tendency to spread to peritoneum, leptomeninges, gynaecological and GI tract cf NST
- GI tract uncommon site of metastasis - <1% of all breast cancers, 7% ILC.
- Can be primary presentation or many years after breast cancer
- Clinical appearances at endoscopy can mimic IBD or primary GI malignancy including linitus plastica
- Poor prognosis – often in context of widespread metastatic disease with average survival < 2 years

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



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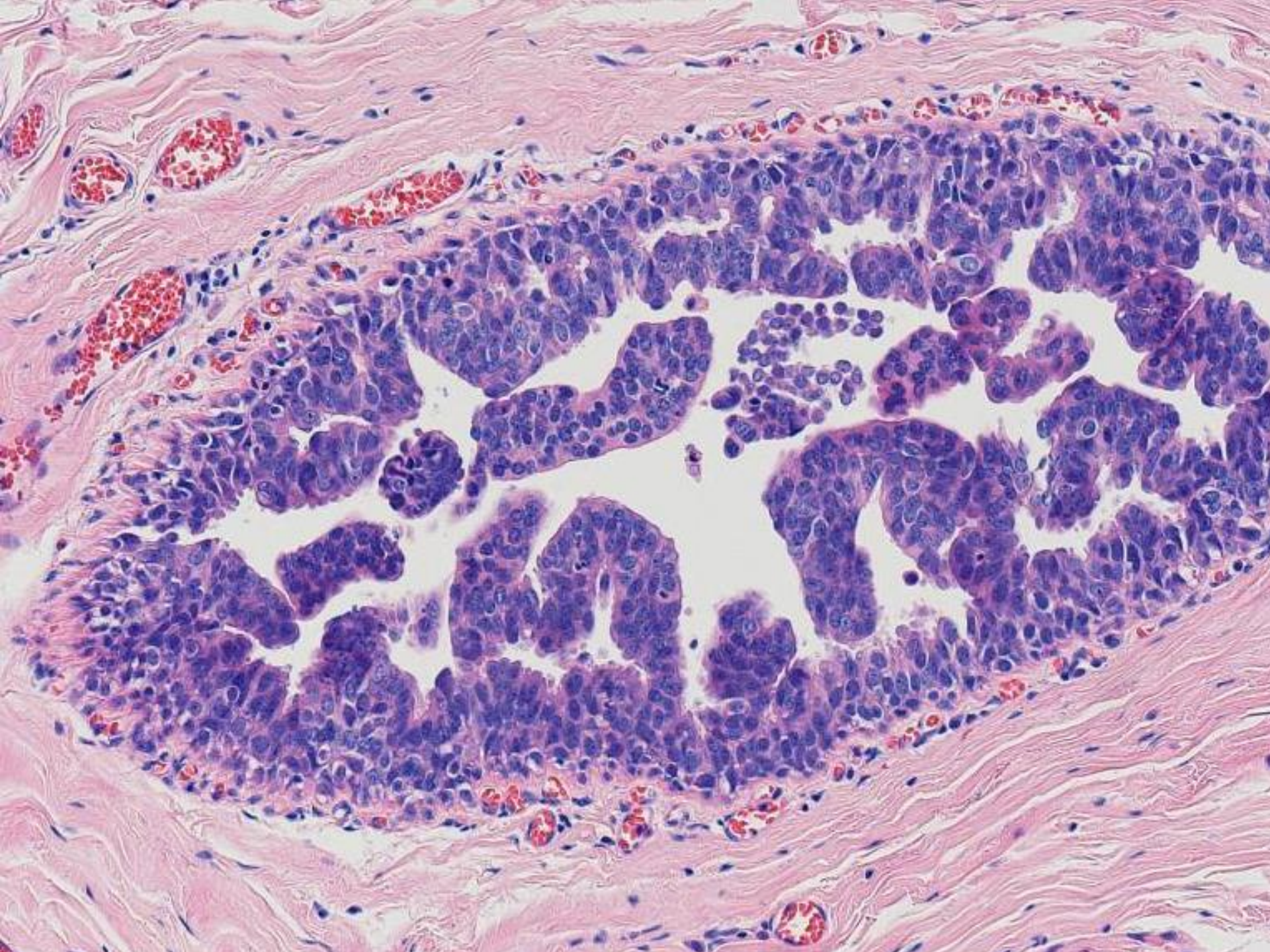
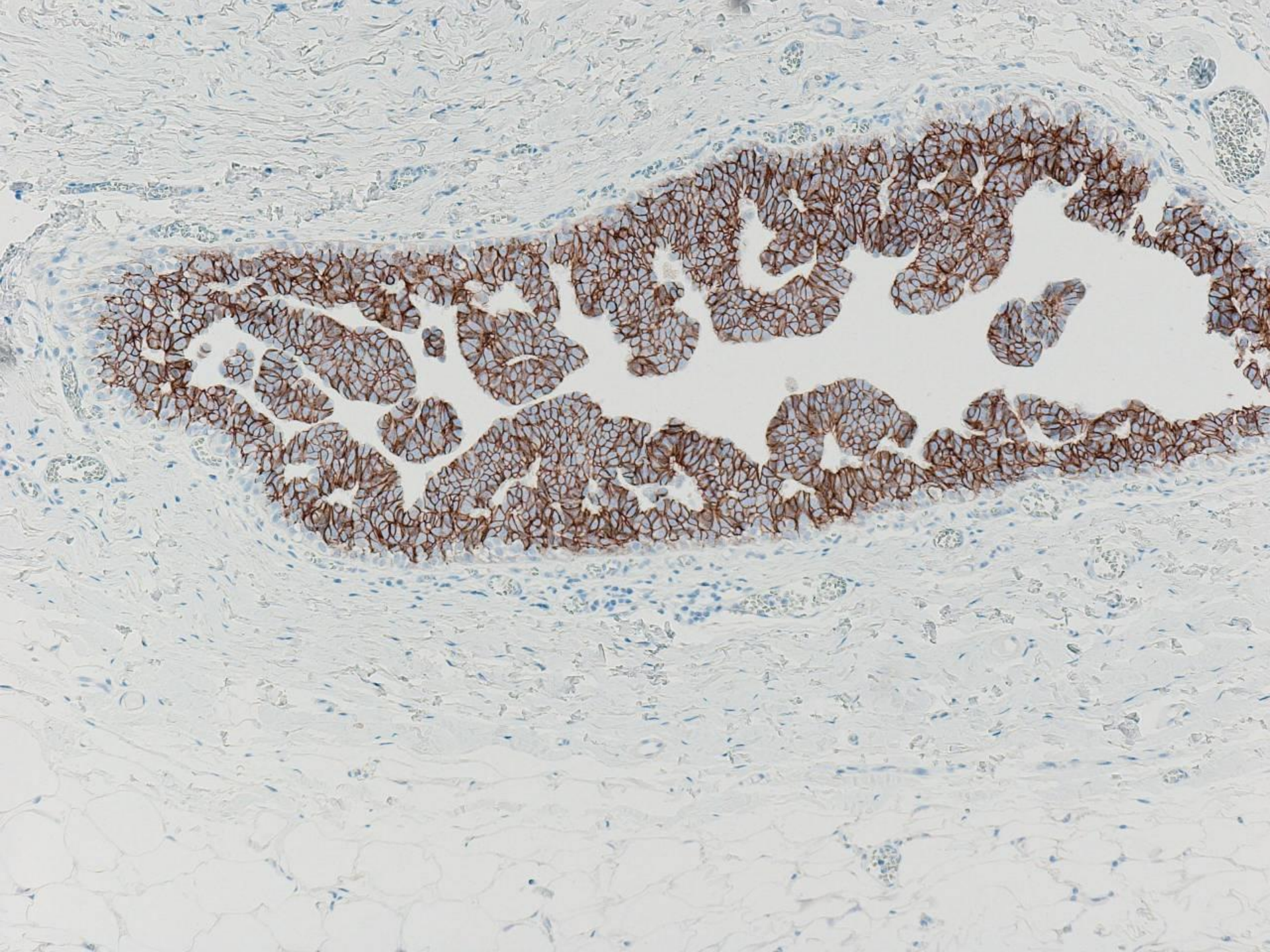
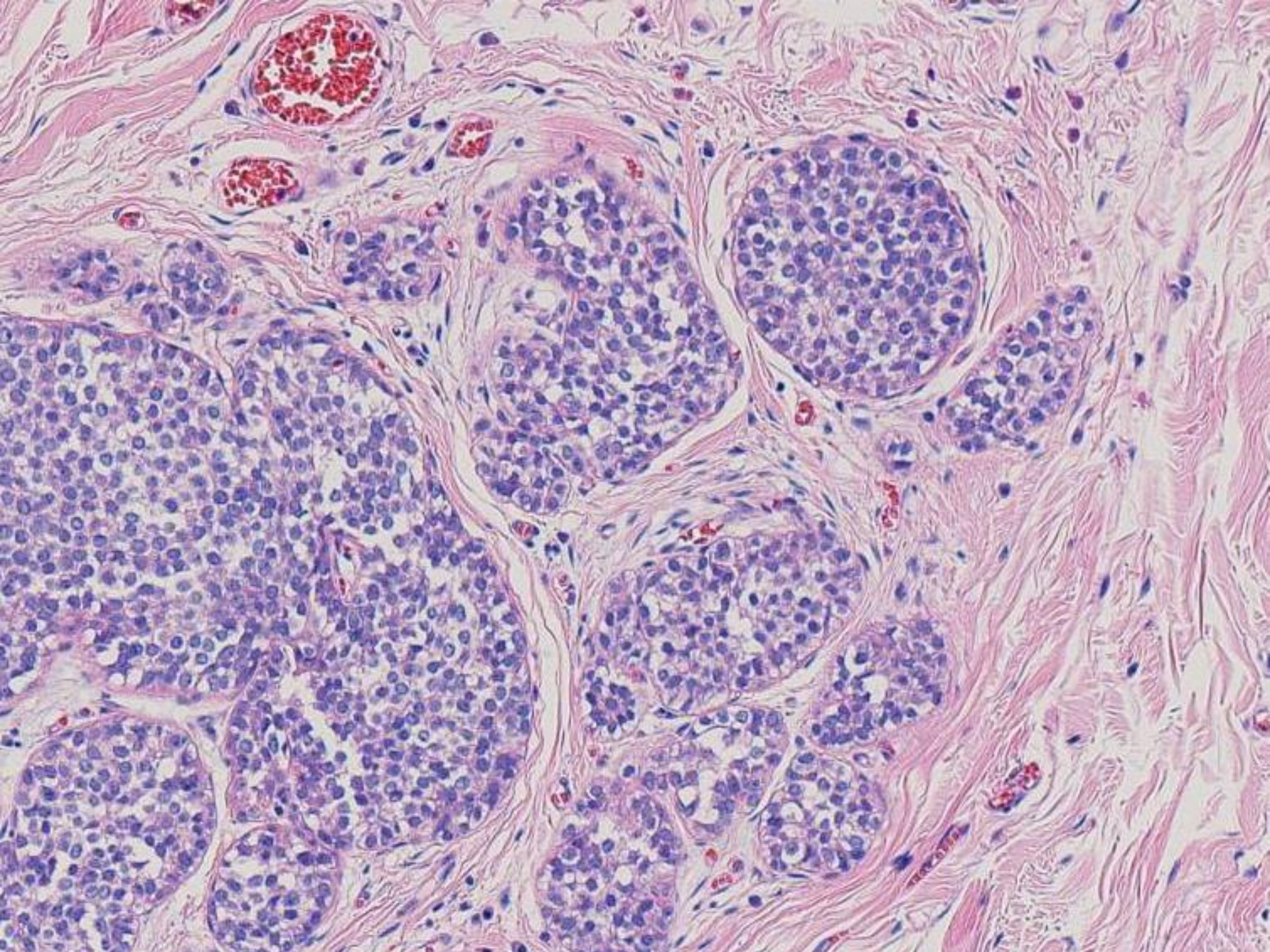
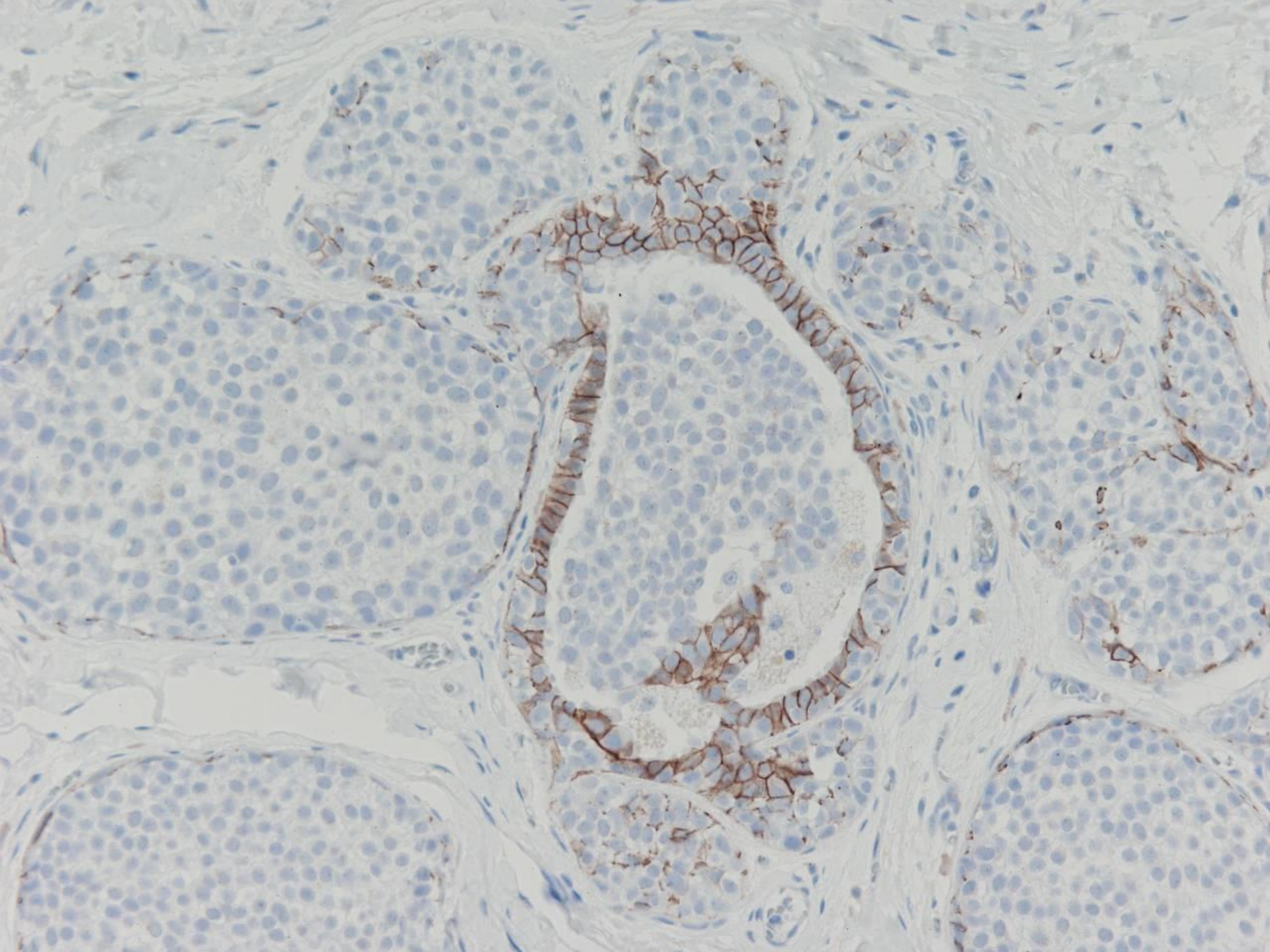
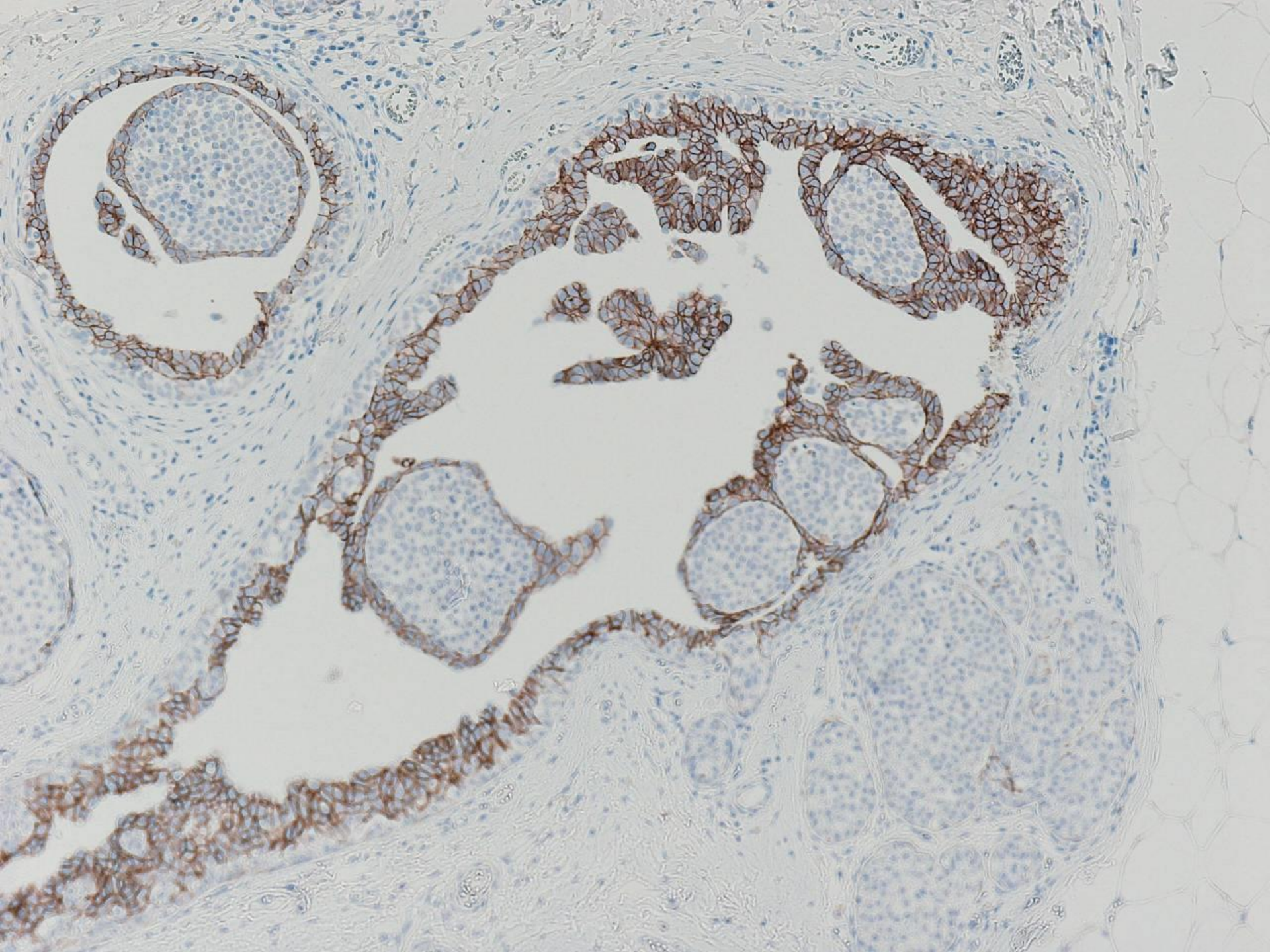


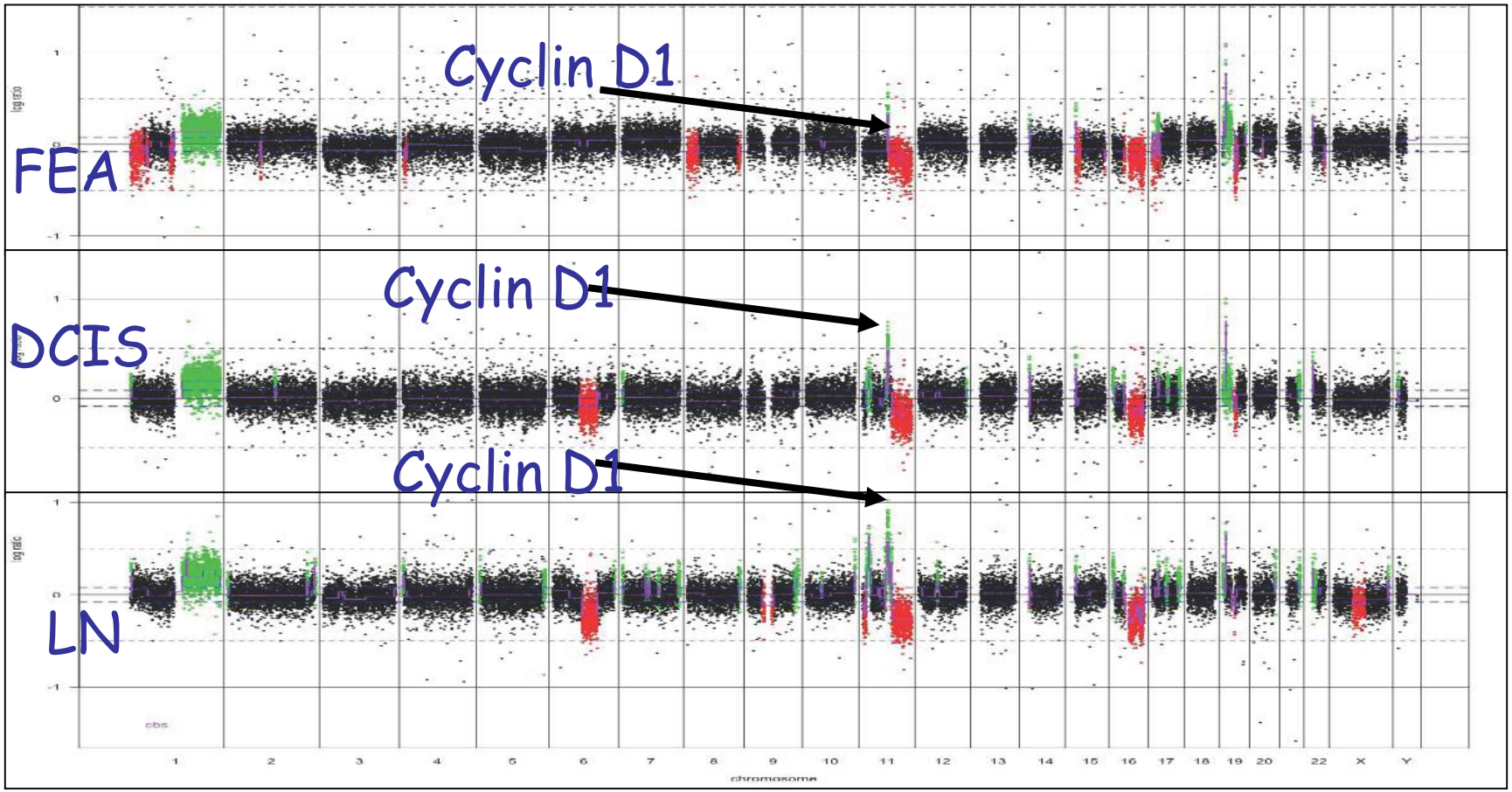








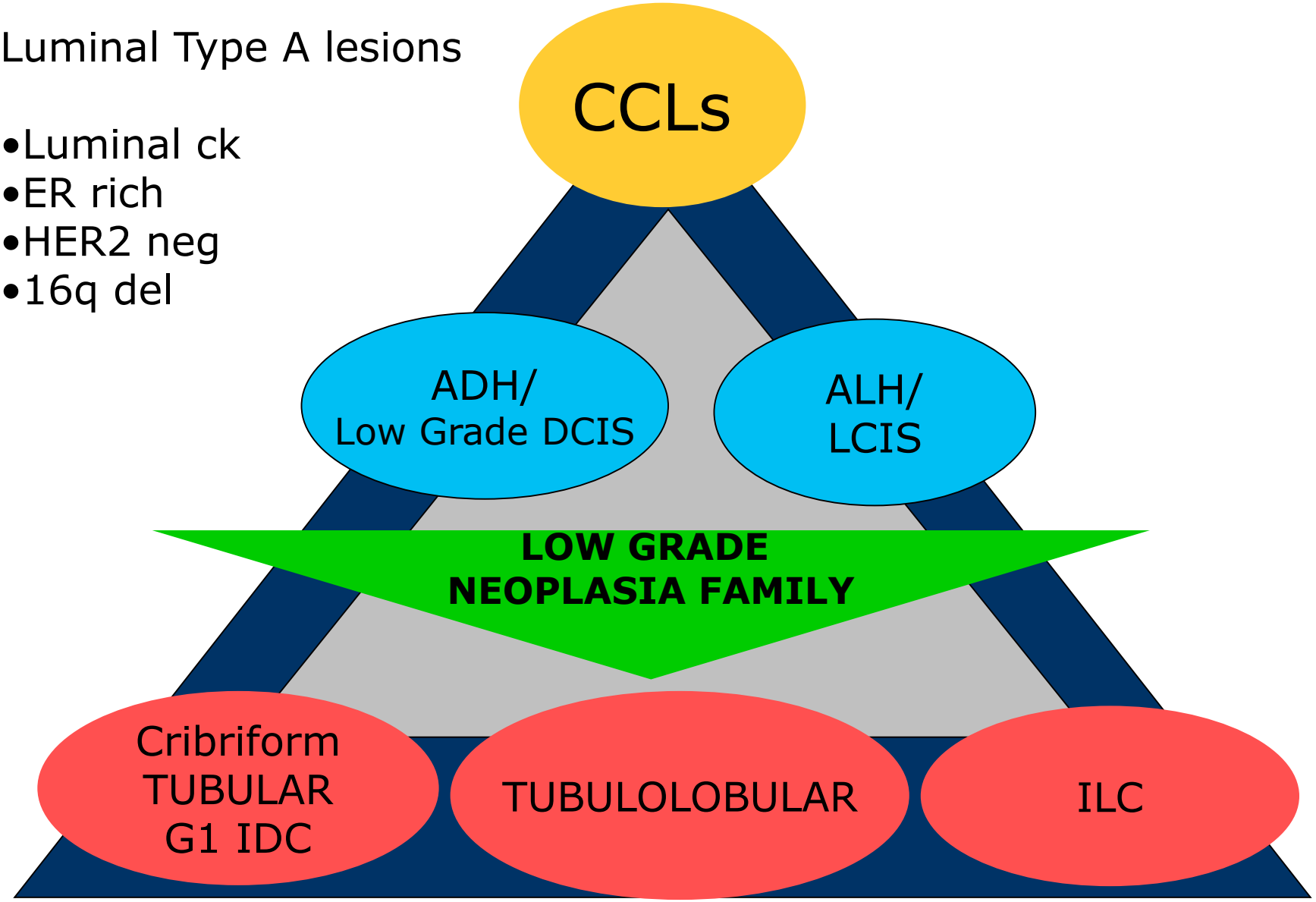
Genome plots of the previous case



Conclusion

Luminal Type A lesions

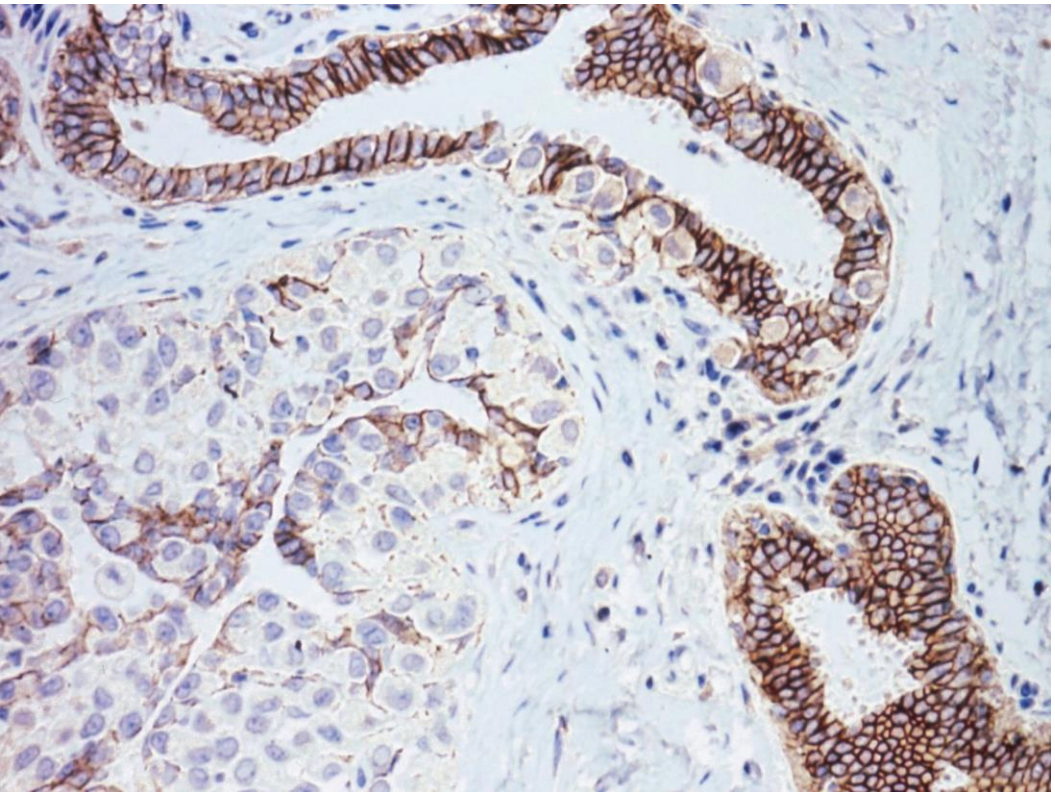
- Luminal ck
- ER rich
- HER2 neg
- 16q del

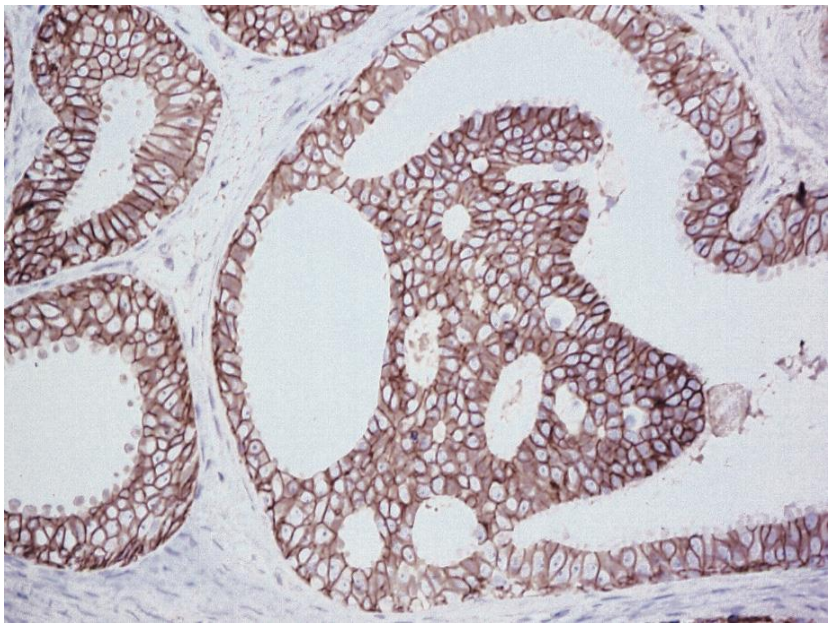


Ductal v lobular

Moll R, et al.
Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. Am J Pathol 143: 1731-1742, 1993.

De Leeu et al.
Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma in situ. J.Pathol. 183:404-411,1997

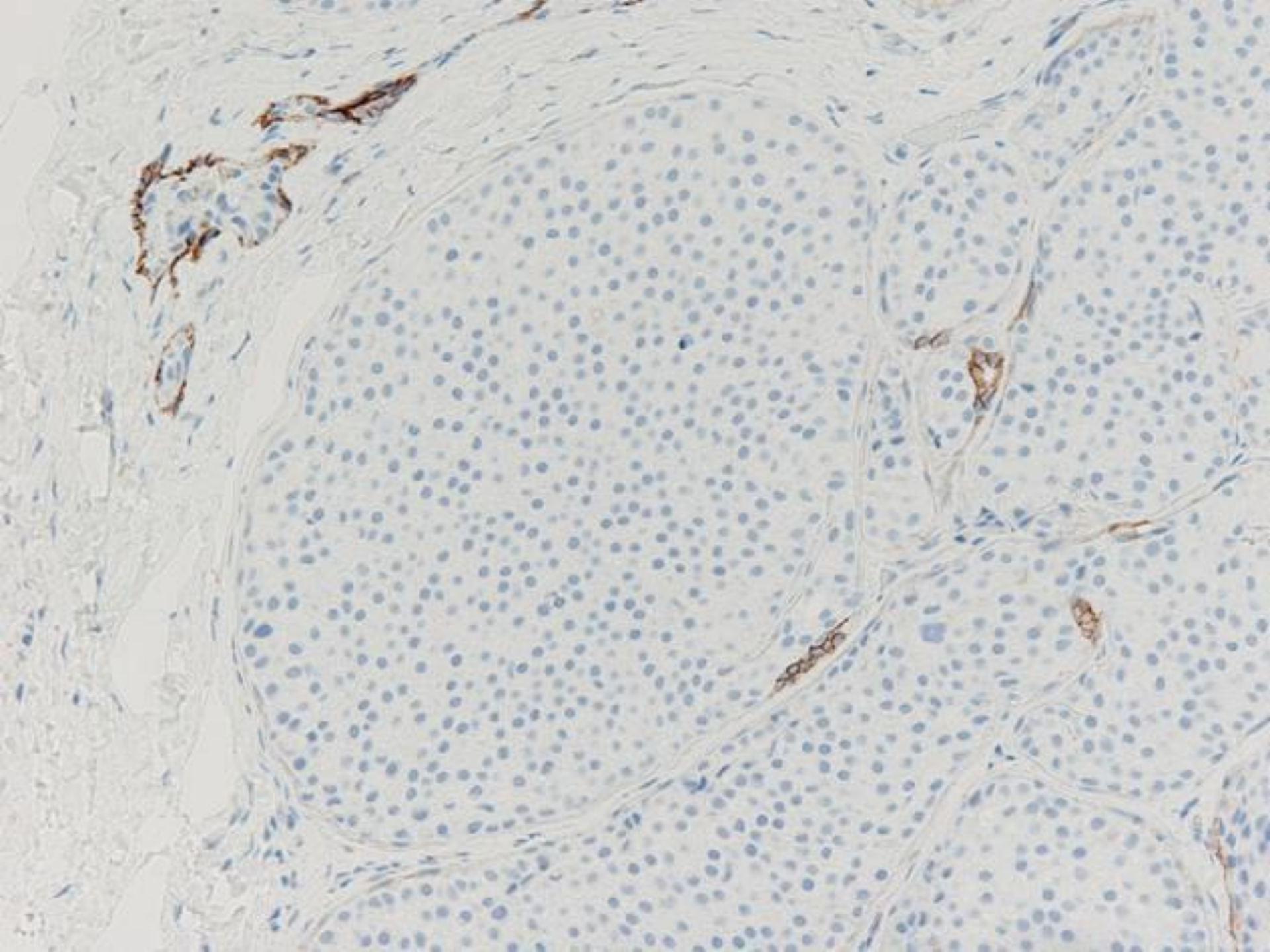




Ancillary markers
E-cadherin

- Encoded by CDH1 gene (16q22.1)
- Adhesion molecule

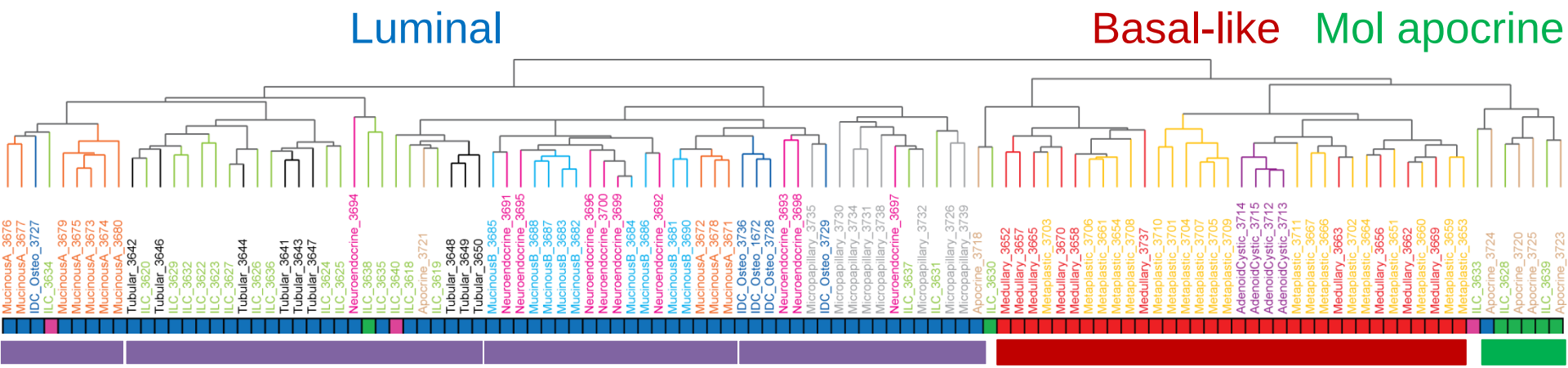
- E-cadherin innactivation
  - ALH(?)
  - LCIS
  - Invasive LCs
- LOH on 16q in ALH and LCIS



E cadherin - IHC

- Note: expression of Ecadherin protein is preserved in around 12-16% of ILC – *CDH1* mutations identified resulting in non-functional protein with abnormal catenin complex formation
- Note: 25-50% of Invasive Ca NST (ductal) show reduced or absent expression of Ecadherin, especially high grade basal-like cancers
- Ecadherin IHC is a useful diagnostic adjunct for LCIS v DCIS and for invasive cancers with a single file growth pattern where you are not sure if it is NST or lobular
- Note: Tumours are primarily classified as lobular or NST/ ductal on the basis of their morphology NOT Ecadherin staining

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

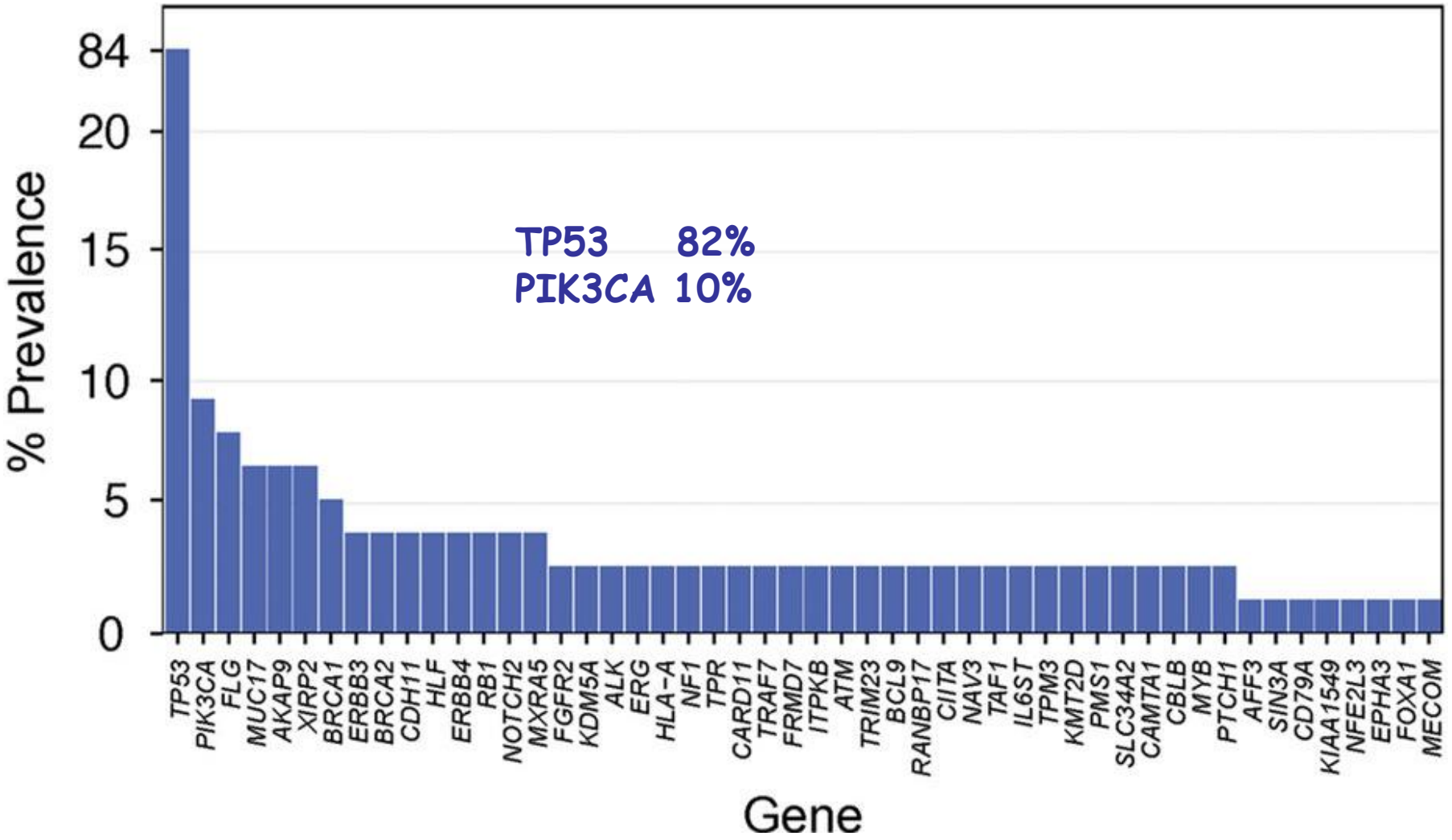
Molecular Types of TNBC

Transcriptomic classification of TNBCs revised to four subtypes:

- Basal-like/immune-suppressed (BLIS),
- Basal-like/immune activated (BLIA),
- Luminal (AR)
- Mesenchymal (MES)

Burstein MD et al. *Clin Cancer Res* 2015;**21**;1688-1698

Somatic Mutations in TNBC (Cacer Genome Atlas)



High Grade Special Histological Types of TNBC

1. *Carcinoma with Medullary Features*

TP53, BRCA1 (germline) mutation

2. *Metaplastic Breast Carcinomas*

Chondroid & spindle cell preferentially MES subtypes. No MBC classified as IM or LAR

MBCs display enrichment for mutations affecting members of PI3K and Wnt pathways

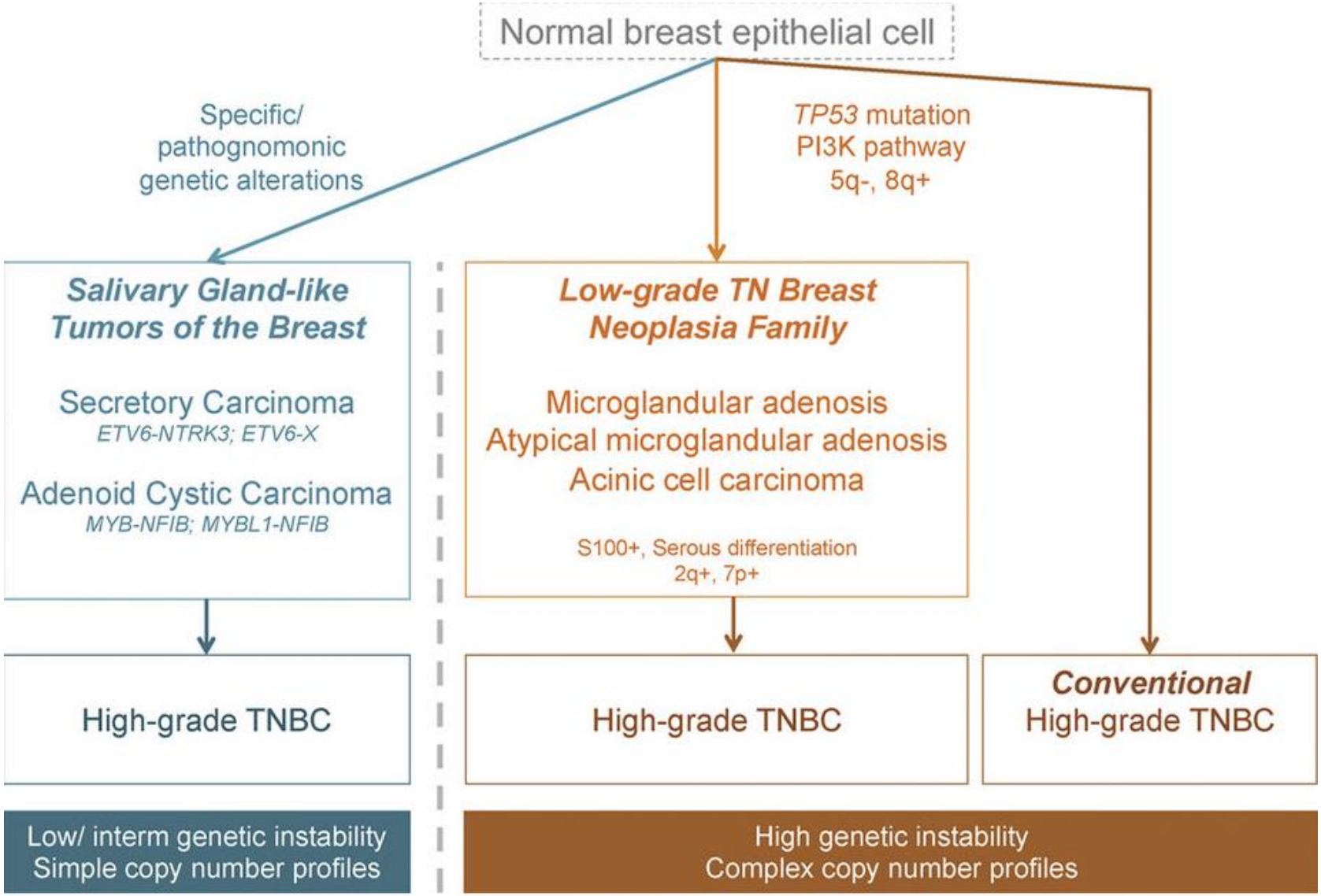
2. *Carcinoma with Apocrine Differentiation*

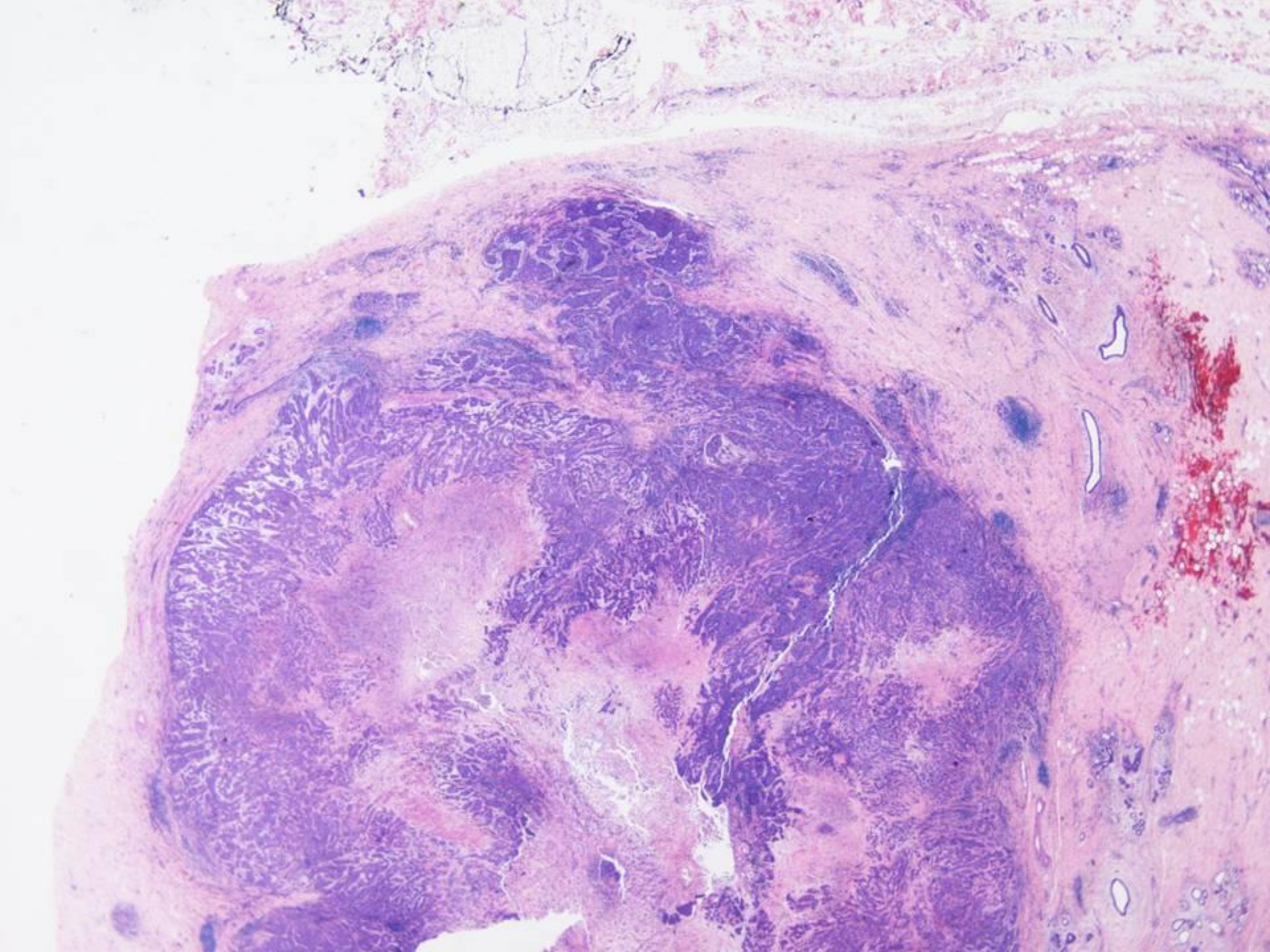
Higher frequency of mutations in *PIK3CA* and other PI3K pathway genes

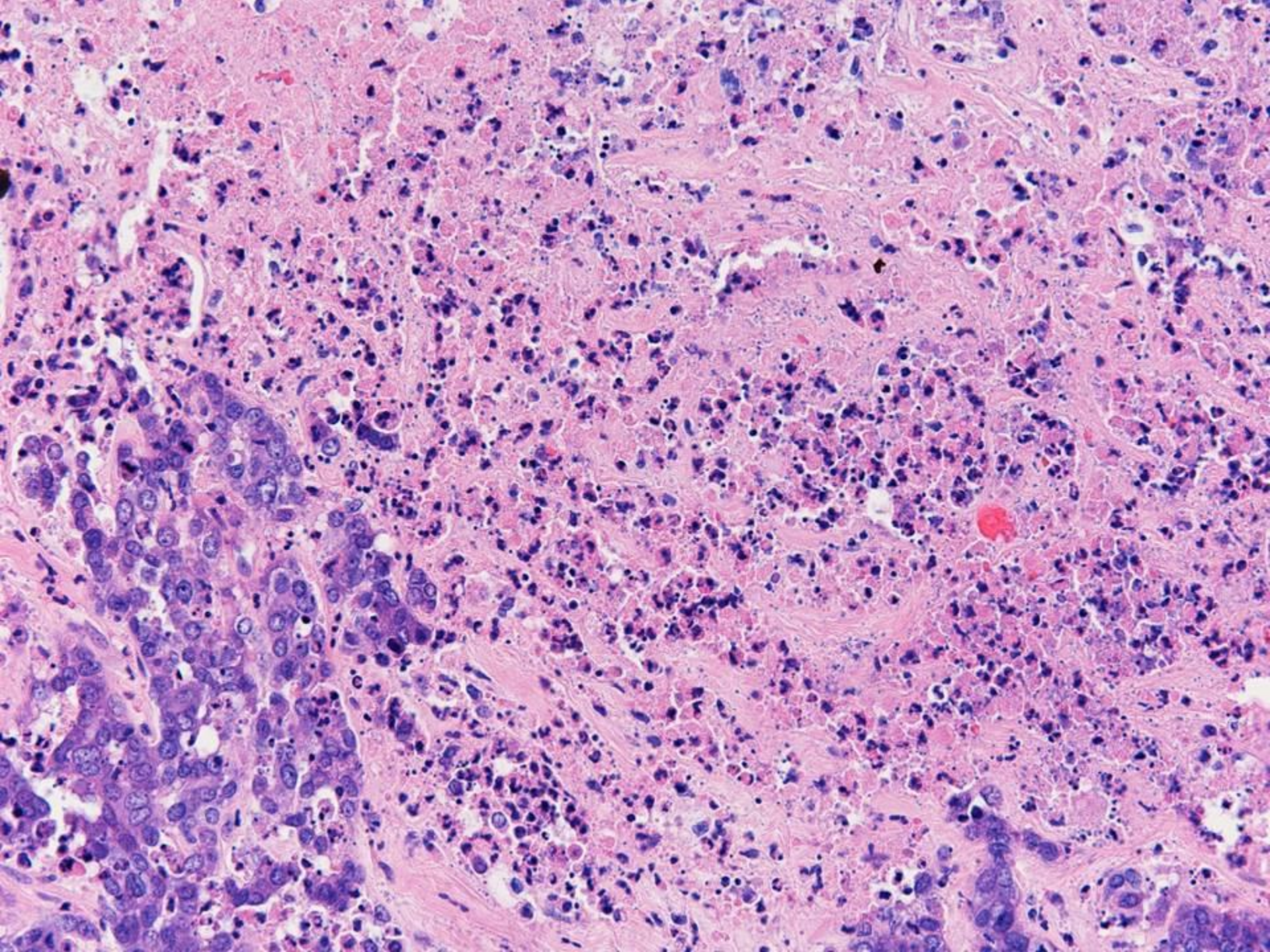
lower rate of *TP53* mutations and *MYC* gains

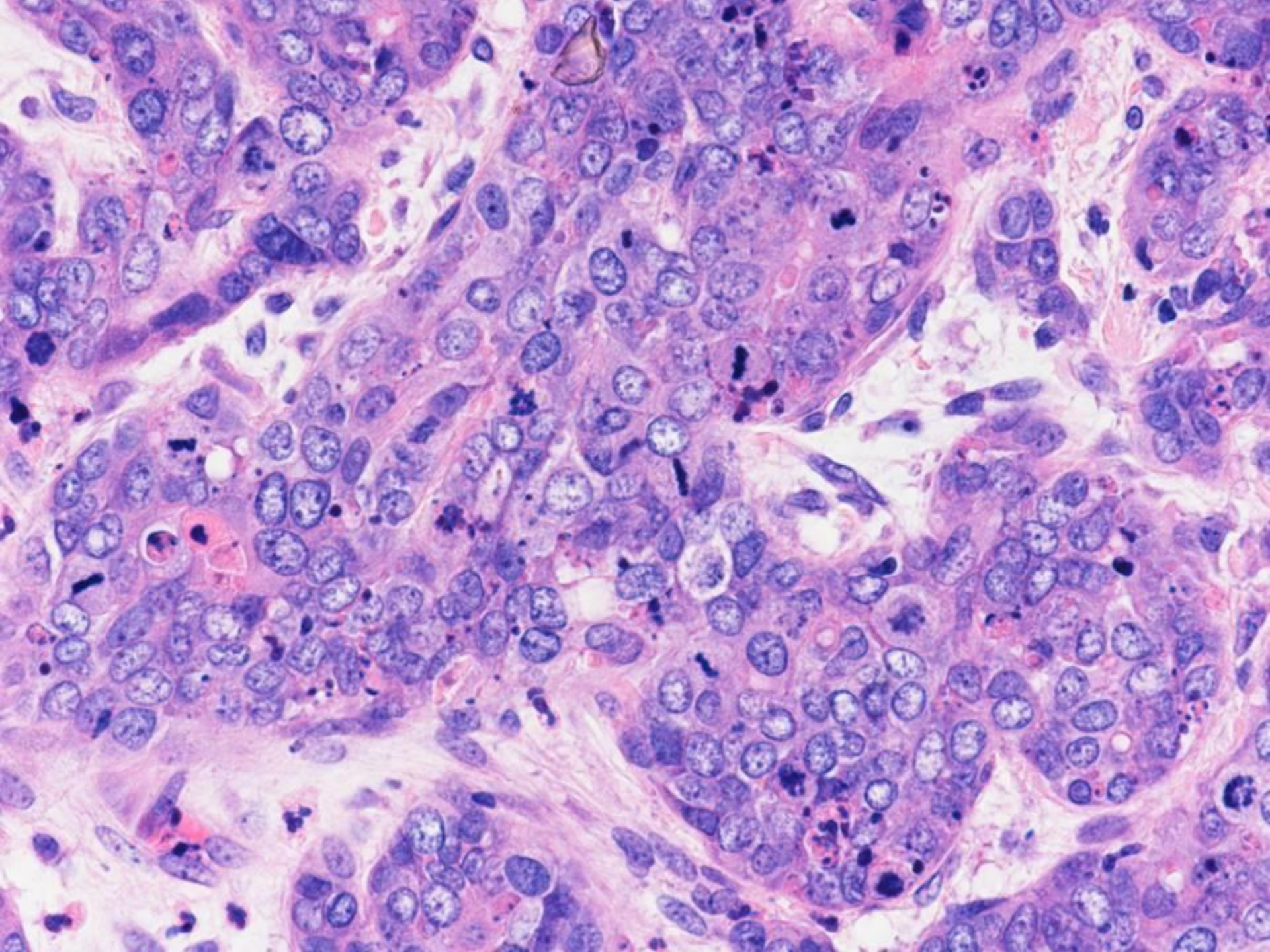
Geyer FC et al in preparation

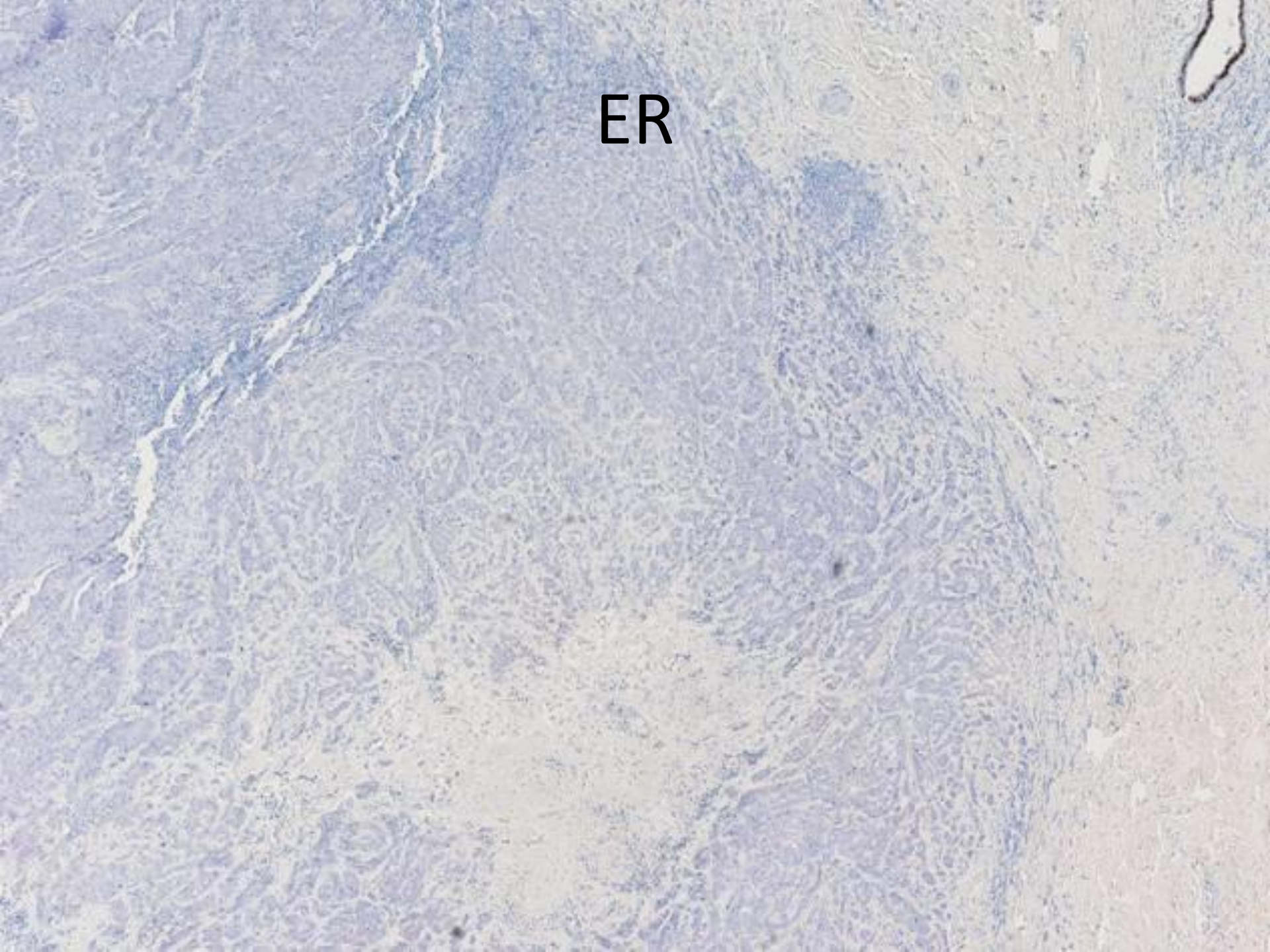
Triple Negative Breast Cancer

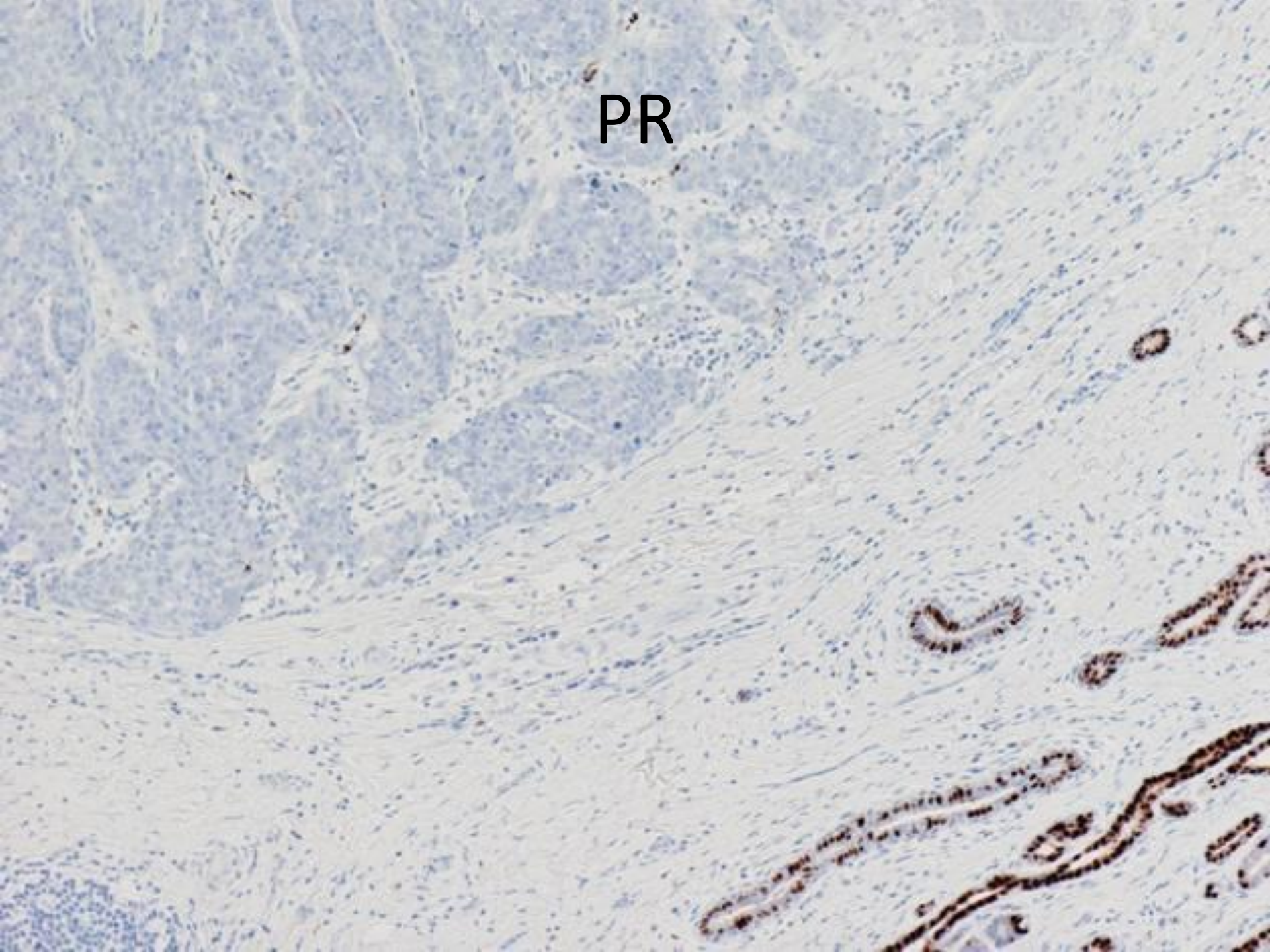


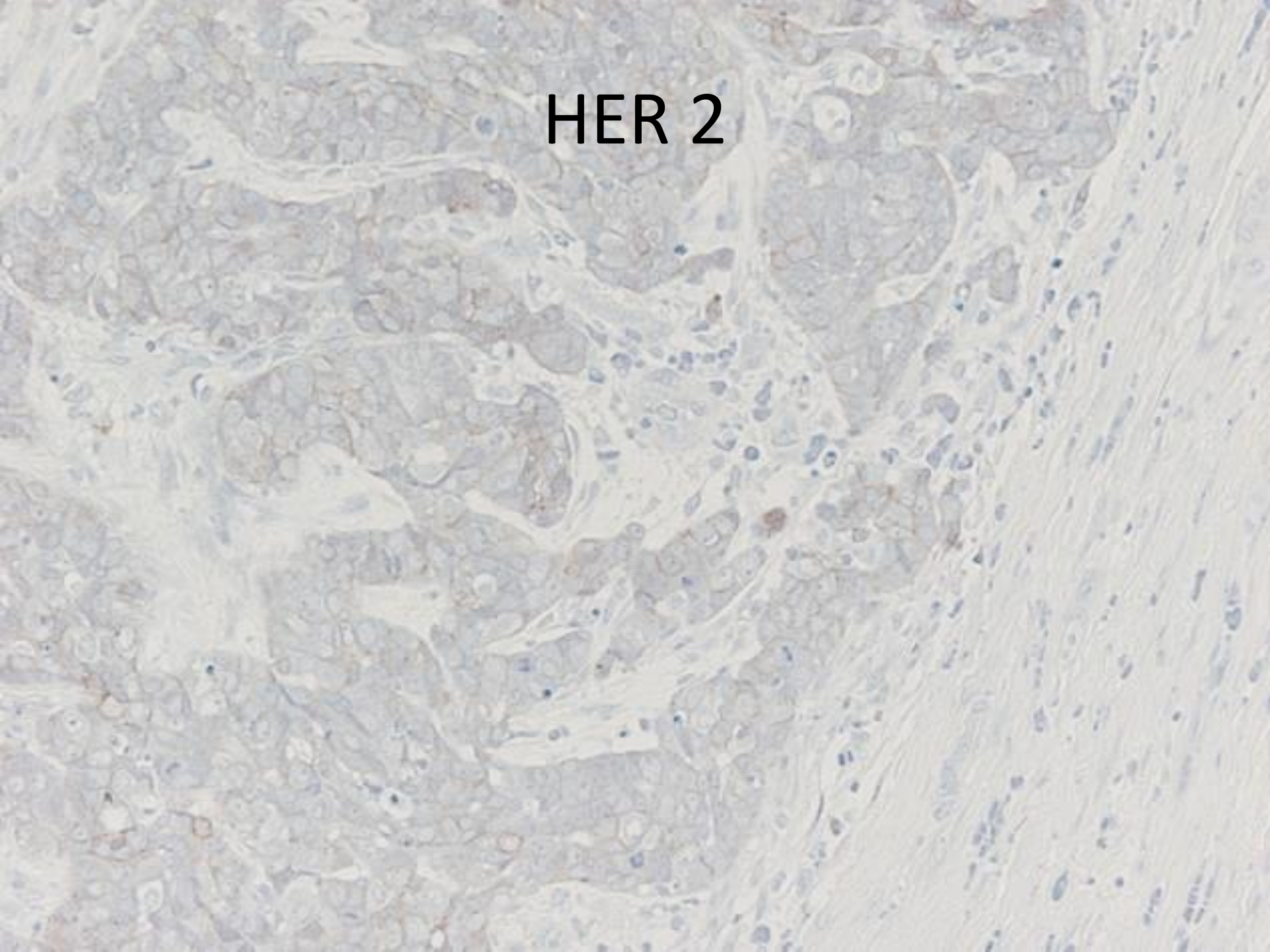




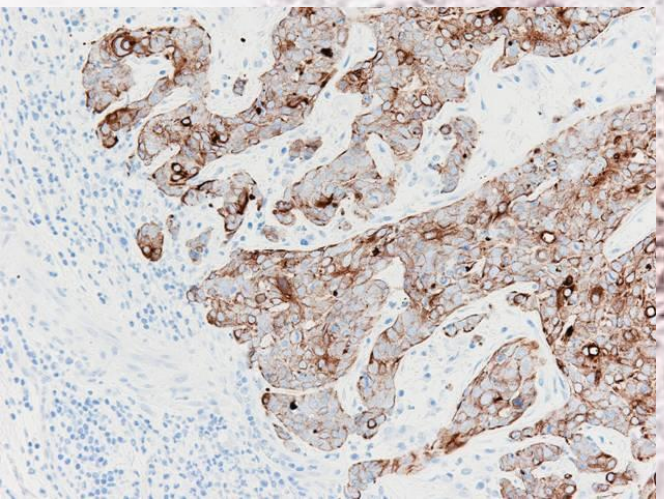


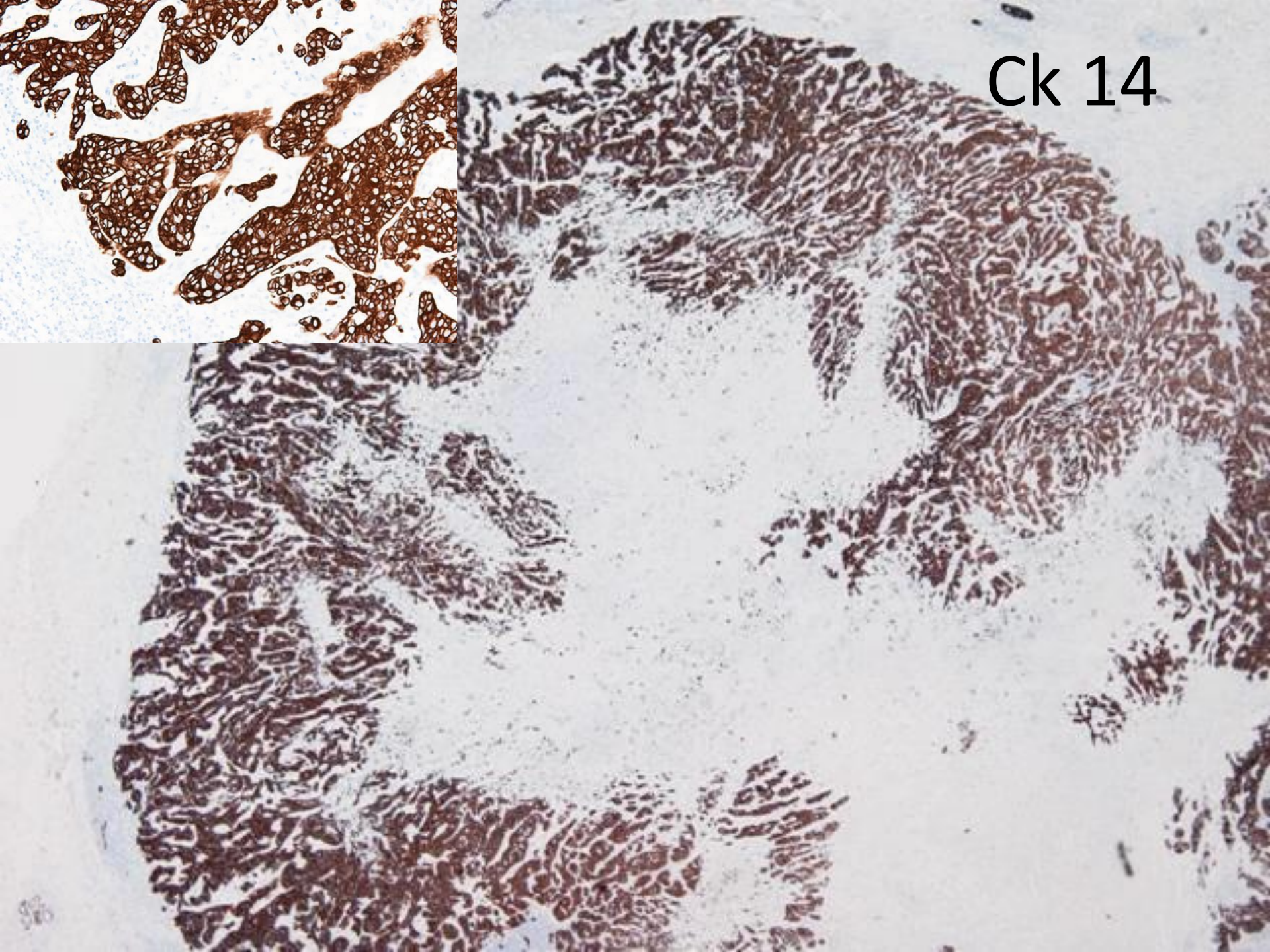

ER


PR


HER 2

Ck 5 6

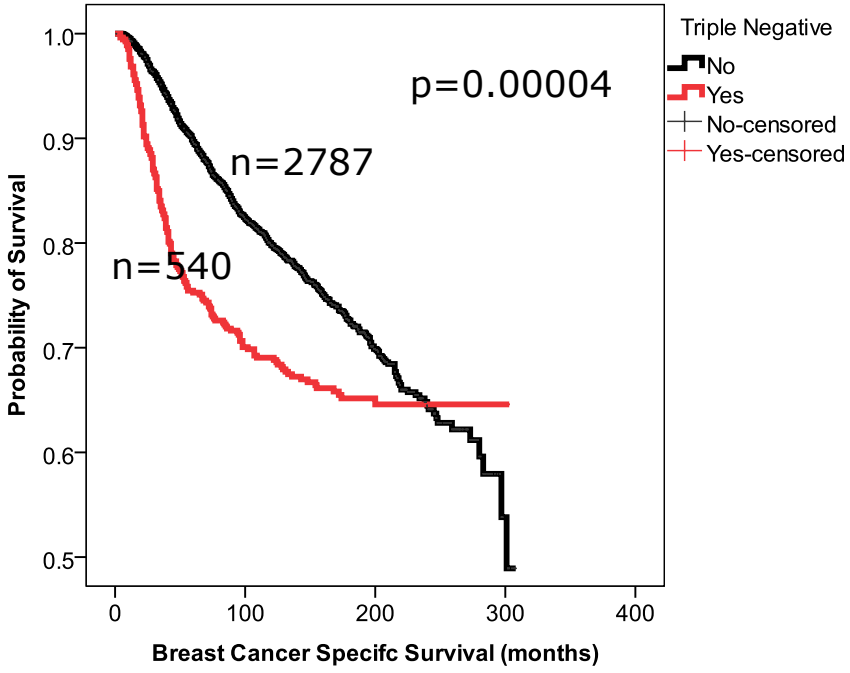



Ck 14

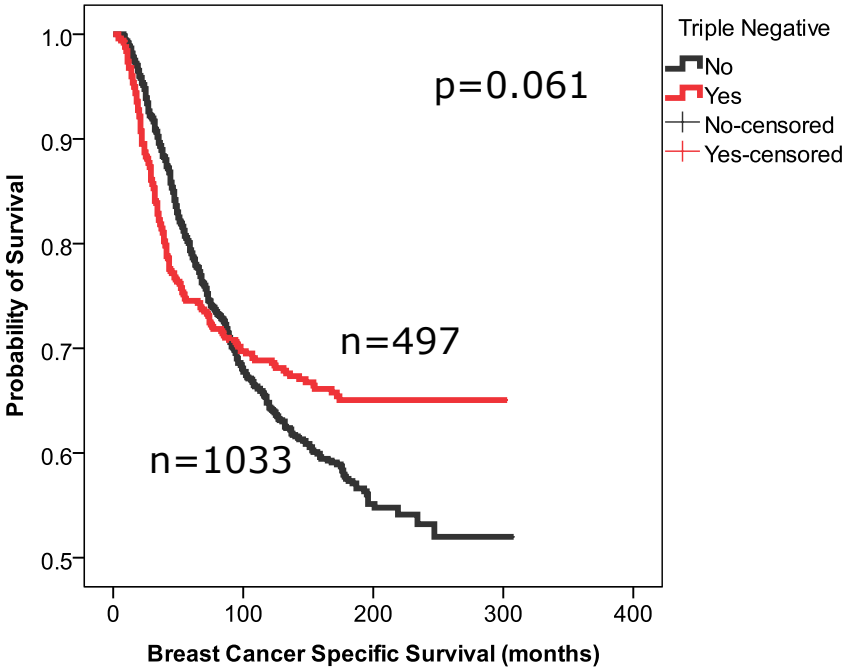
Basal / Classic TNBC Phenotype

- Grade 3
- Duct/NST, Medullary like carcinoma
- High mitotic count, lack of tubule formation, geographic necrosis
- Larger size, LN disease, poorer NPI, DM and recurrence
- High rate of liver, lung, and brain mets, less bone mets
- Not with VI or with age

All triple negative versus All breast cancers



Triple negative grade 3 versus all other grade 3 cancers



Basal-like Breast Cancer and Chemotherapy (MDACC)

Gene expression array subtyping and pathologic complete response to neoadjuvant chemotherapy with T-FAC (n=83)

Molecular classification	Residual Disease	Pathologic complete response
Luminal	93% [78-99]	7% [1-22]
Normal breast	100% [29-100]	0% [0-31]
HER2+	55% [32-77]	45% [23 -68]
Basal subtype	55% [32-76]	45% [24-68]

Chi square: P<0.001

Rouzier R et al, SABCS 2004

Response to Neoadjuvant Therapy and Long-Term Survival in Patients With Triple-Negative Breast Cancer

Patients with TNBC have increased pCR rates compared with non-TNBC (22% *v* 11%; *P* = .034)

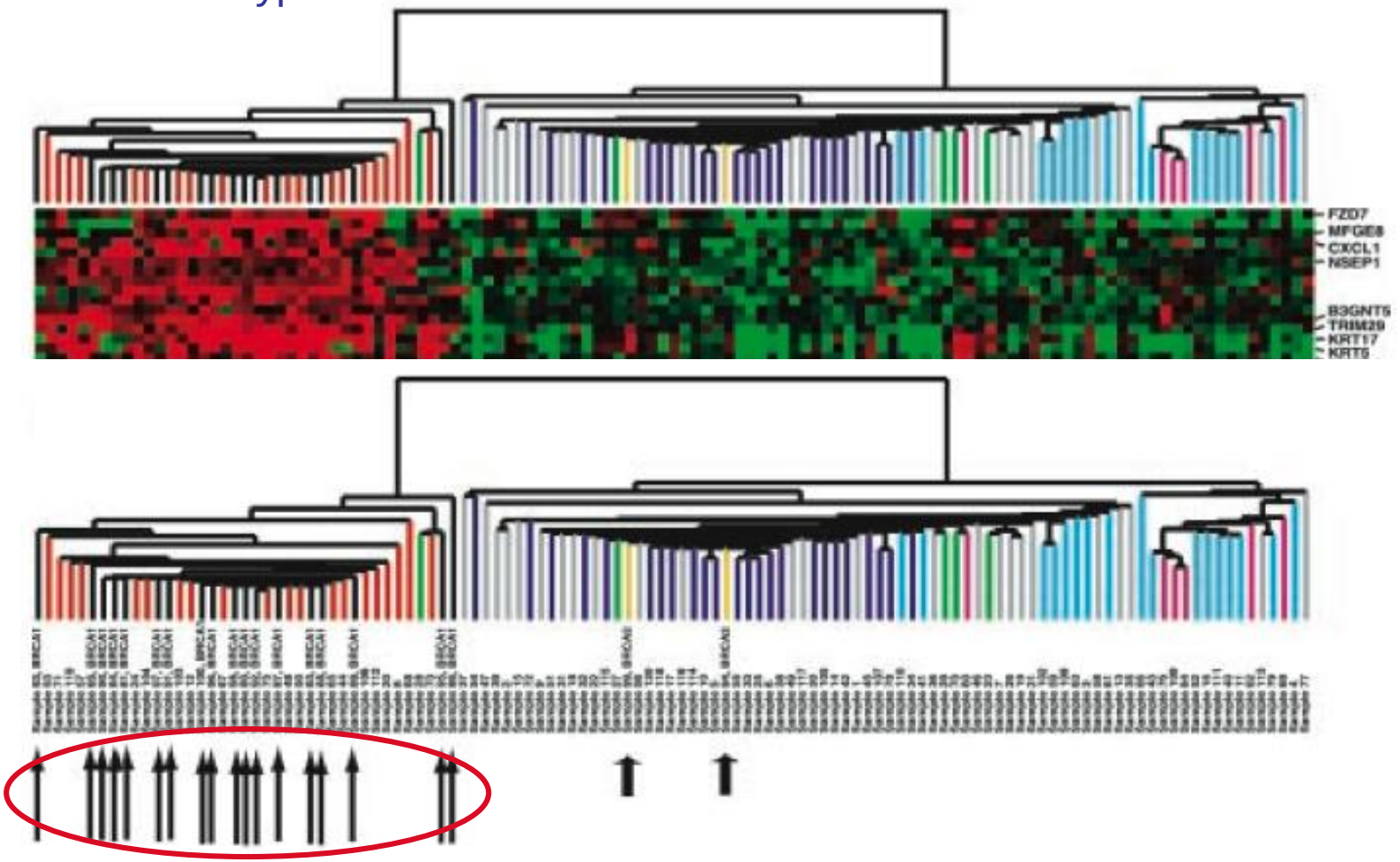
Those with pCR have excellent survival.

However, patients with RD after neoadjuvant chemotherapy have significantly worse survival if they have TNBC compared with non-TNBC, particularly in the first 3 years.

Liedtke C at al J C O 2007

Overlap of BRCA1 and Basal-like Genotypes

Basal Sub-Type



Sorlie PNAS 2003

BRCA1 downregulation

- High histological grade
- Medullary histological type
- Basal-like immunophenotype

Int. J. Cancer: **116,** 340–350 (2005)

FAST TRACK

High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses

Dalia M. Abd El-Rehim[1], Graham Ball[2], Sarah E. Pinder[1], Emad Rakha[1], Claire Paish[1], John F.R. Robertson[1], Douglas Macmillan[1], Roger W. Blamey[1] and Ian O. Ellis[1]*

Journal of Pathology
J Pathol 2003; **200**: 207–213.
Published online 17 March 2003 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1348

Original Paper

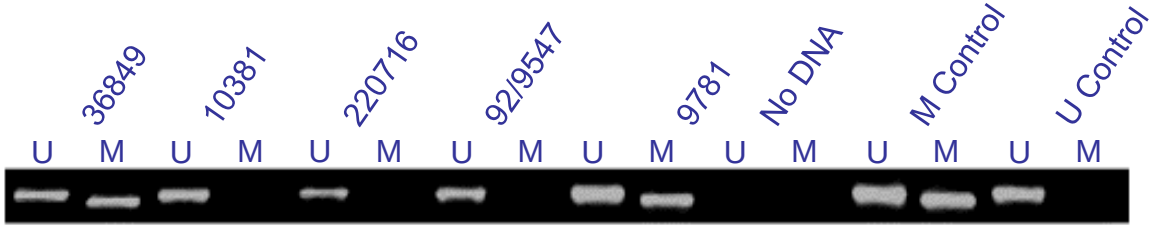
Prognostic significance of BRCA1 expression in sporadic breast carcinomas

H Lambie,[1] A Miremadi,[1] SE Pinder,[1] JA Bell,[1] P Wencyk,[1] EC Paish,[1] RD Macmillan[2] and IO Ellis[1]*

Hypothesis

- BRCA1 inactivation in Basal-Like cancers
  - Gene promoter methylation
  - Transcriptional inactivation

BRCA1 methylation in metaplastic breast carcinomas



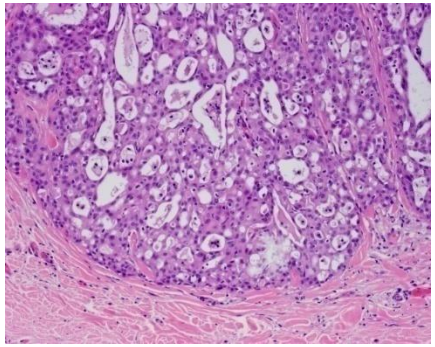
- *BRCA1* gene promoter methylation
  - 17/ 27 (63%)

Type	BRCA1 M	BRCA1 U
Metaplastic	17	10
IDC-basal	4	25

$p<0.0005$

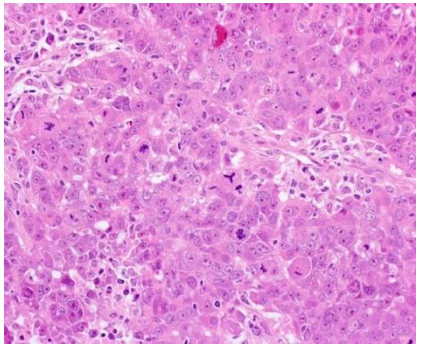
Basal-like carcinomas

?



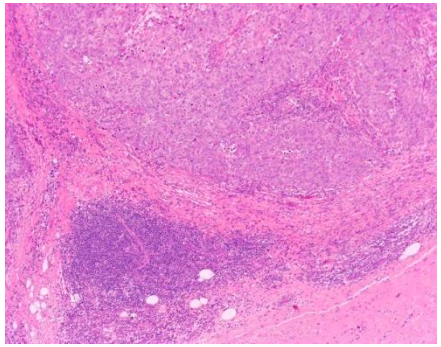
Salivary gland-like tumours

BRCA1 downregulation

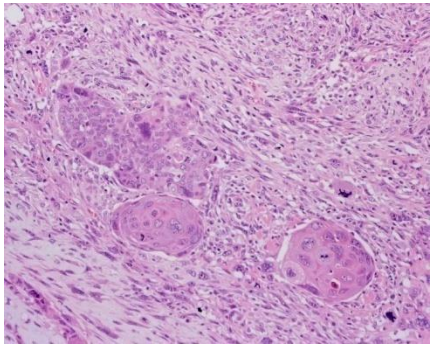


IDC
Basal-like

BRCA1 methylation



Medullary



Metaplastic

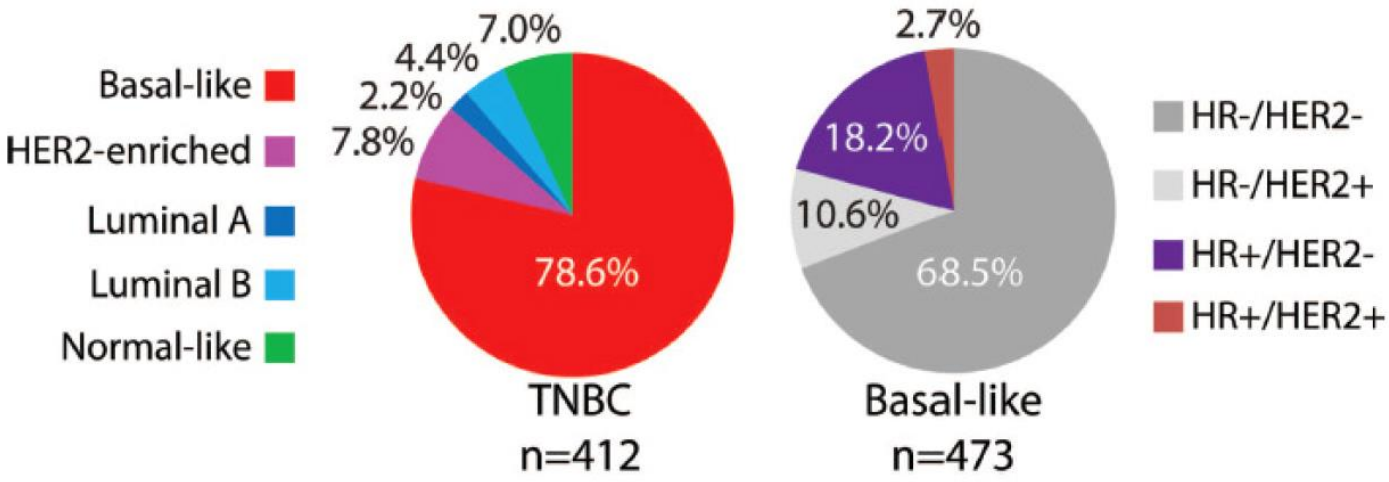
The Oncologist®

Breast Cancer

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

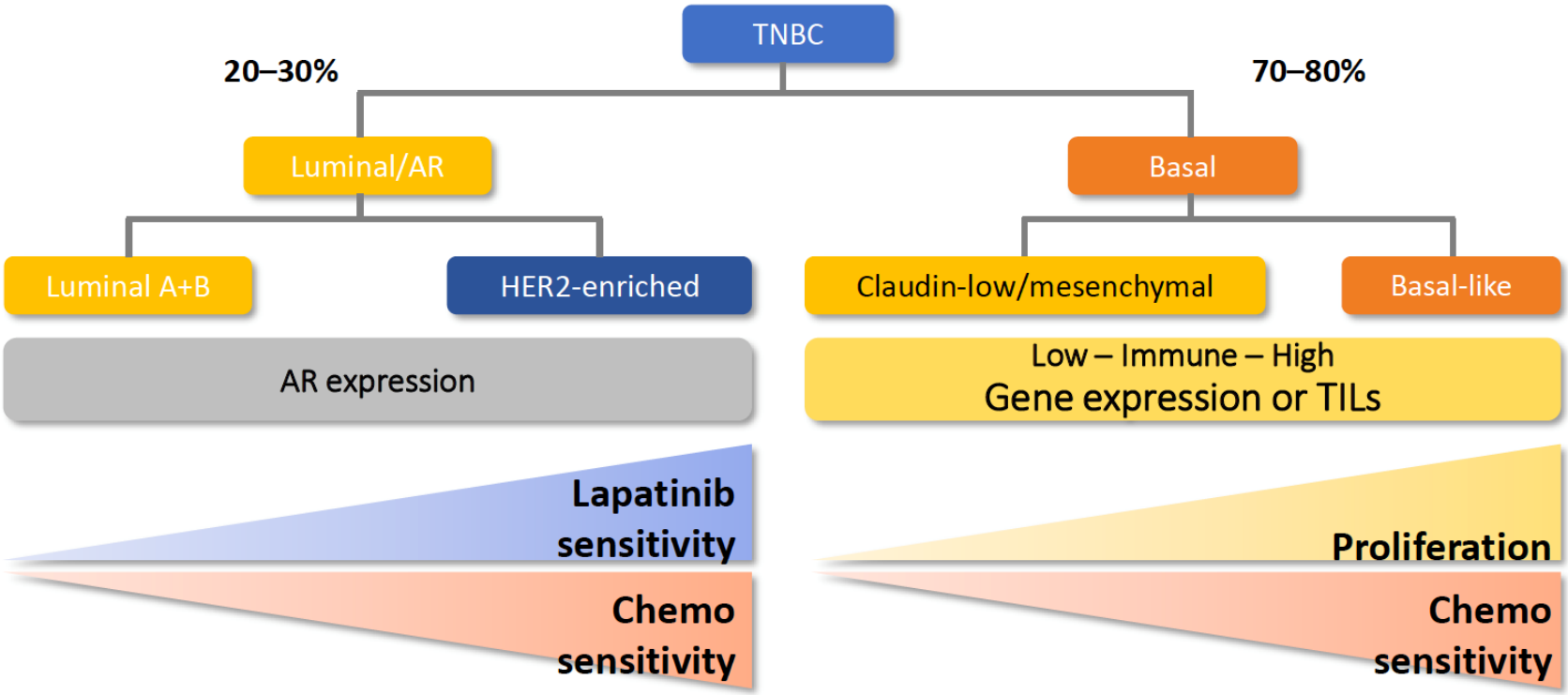
ALEIX PRAT,[a,b,c] BARBARA ADAMO,[b,c] MAGGIE C.U. CHEANG,[d] CAREY K. ANDERS,[d] LISA A. CAREY,[d] CHARLES M. PEROU[d,e,f]

The Oncologist 2013;18:123–133



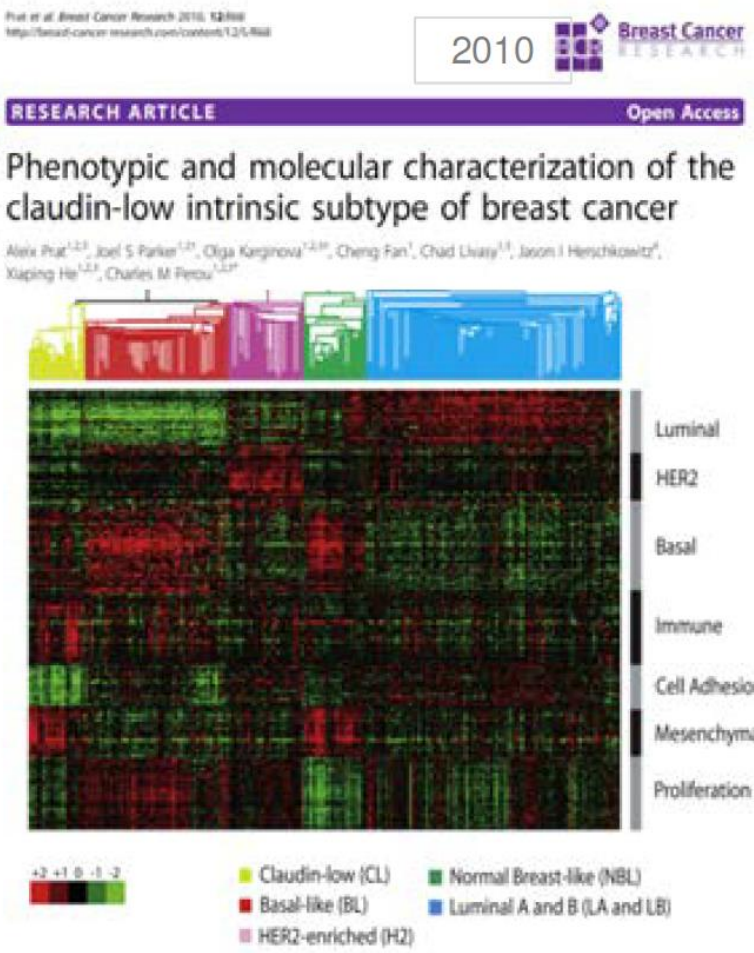
There are limitations to use IHC for Receptors as Surrogates for Molecular Subtype

Stratification of TNBC



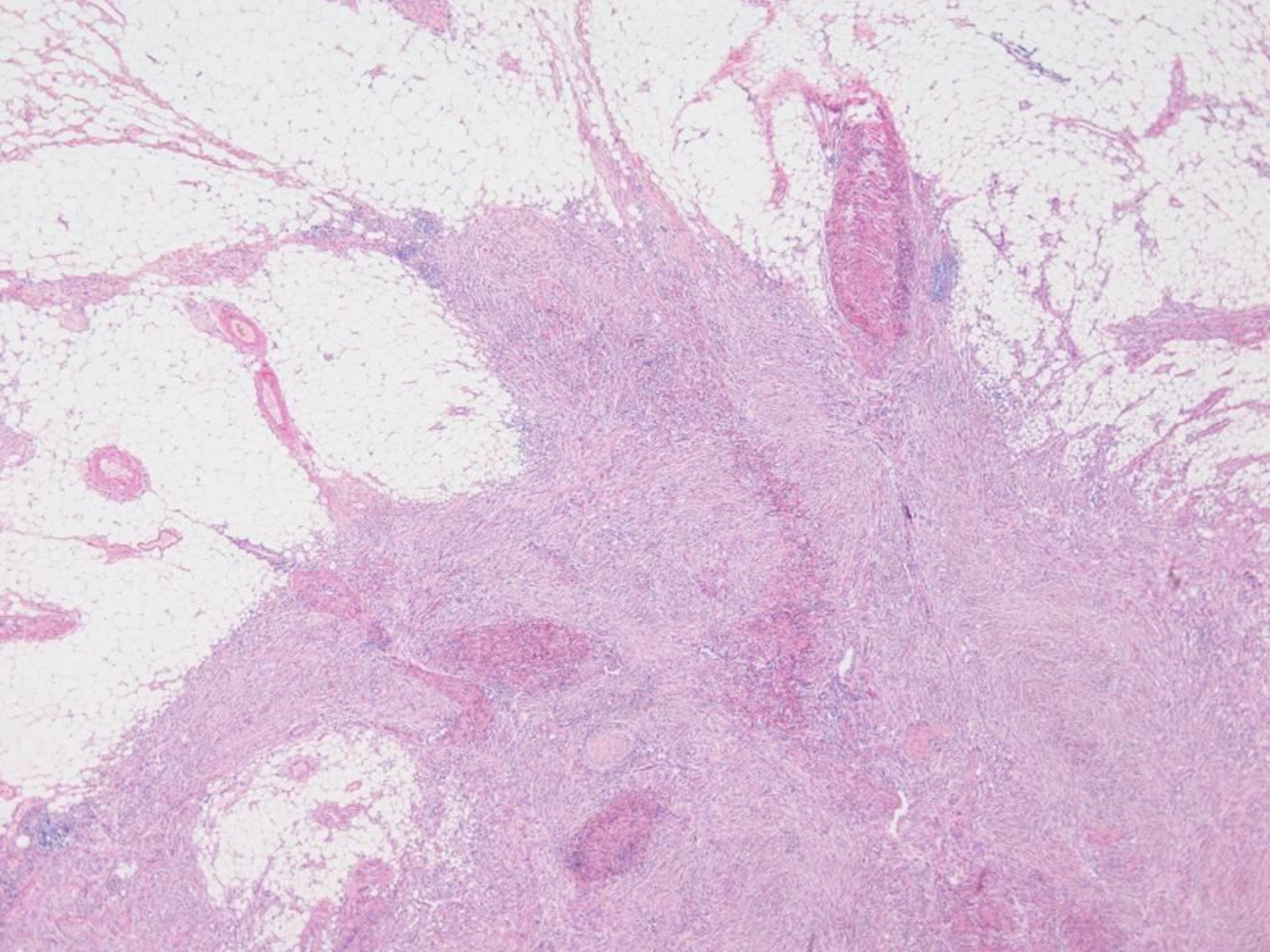
Claudin-low carcinomas

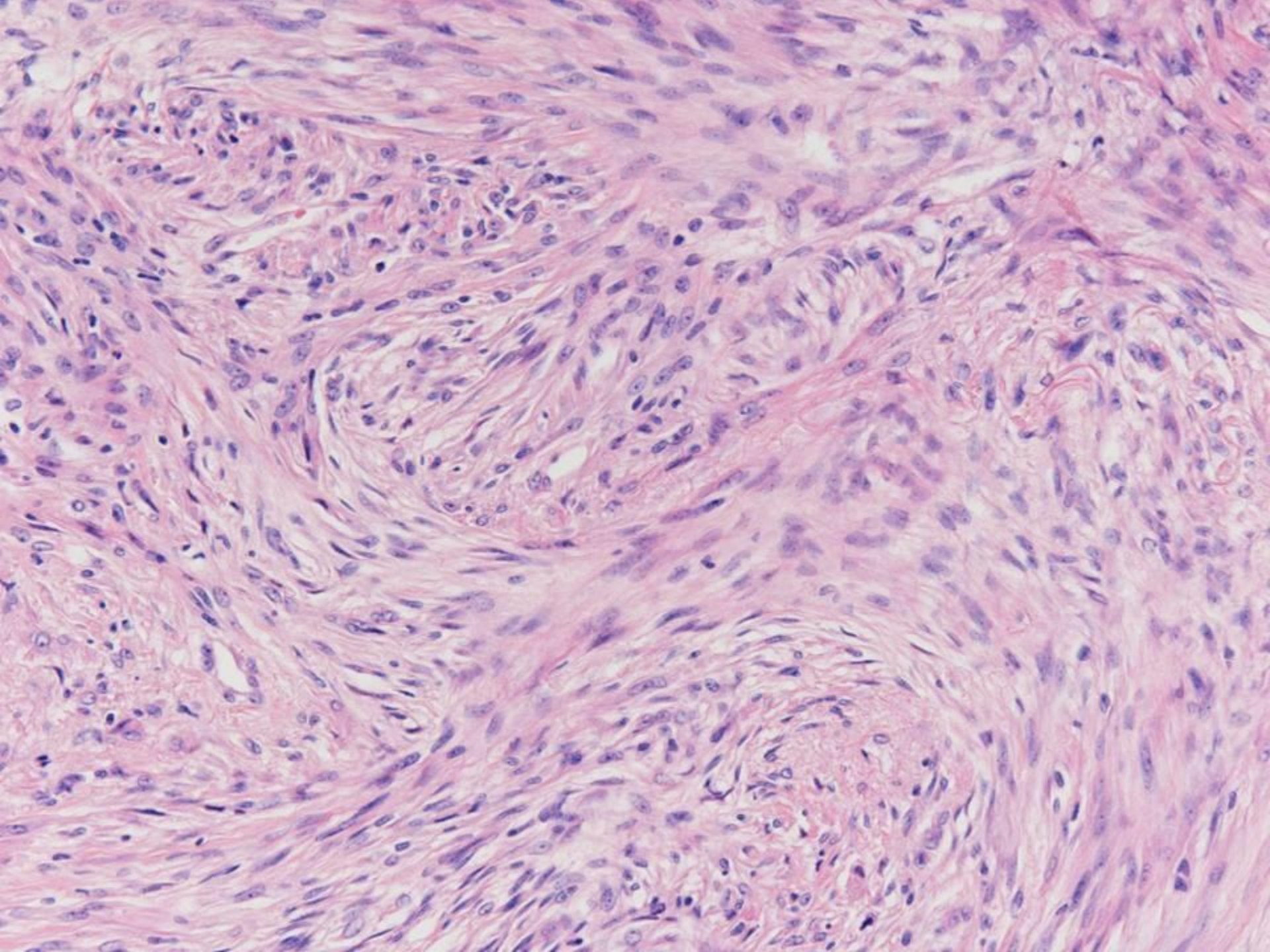
New molecular subgroup, sorted from the triple negative breast cancer group

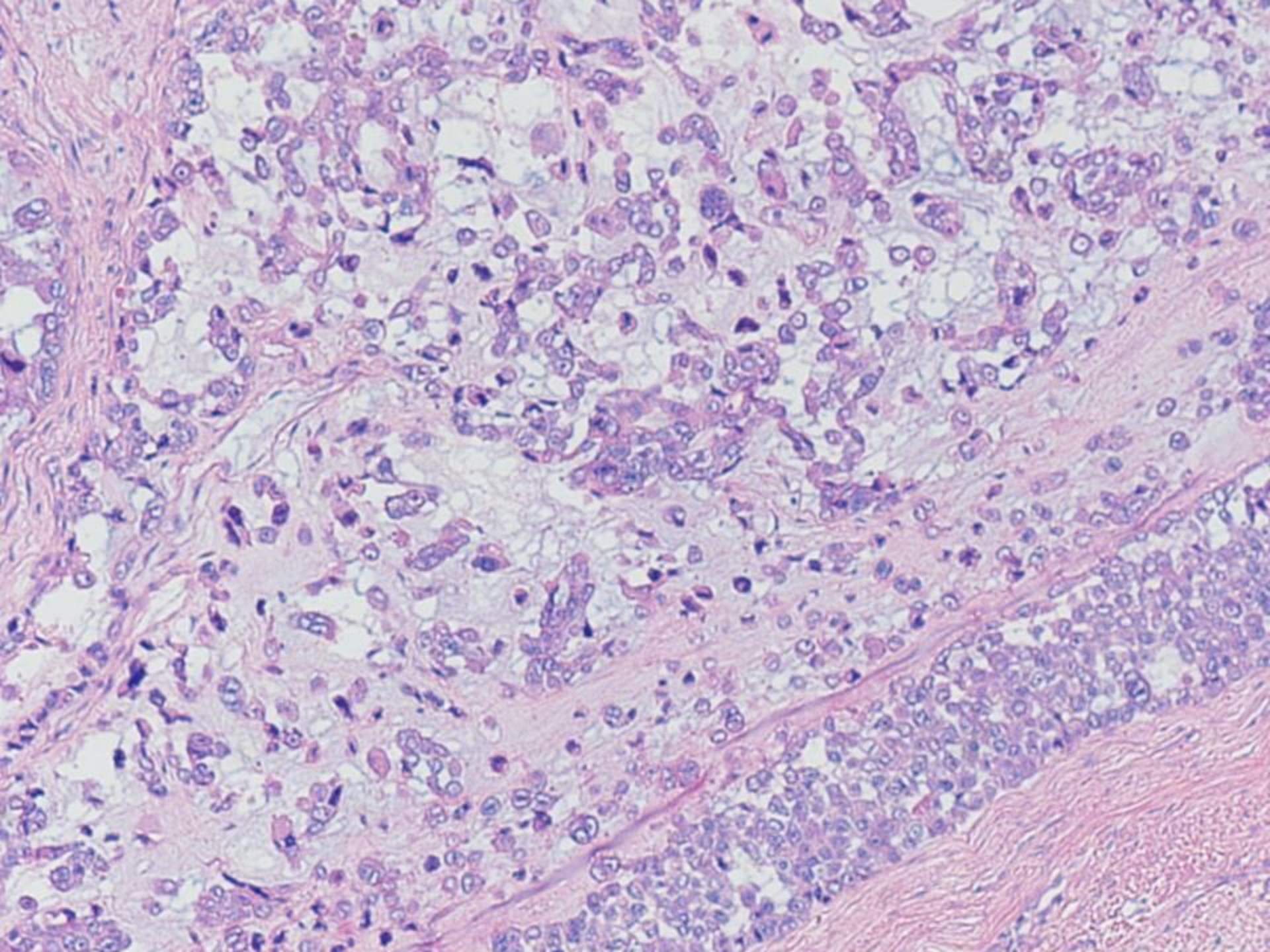


- Low expression of genes involved in tight junctions and cell-cell adhesion:
  - *Claudins 3, 4, 7,*
  - *Occludin*
  - *Ecadherin*

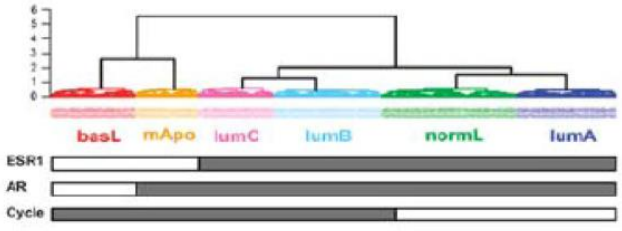
- Low expression of luminal genes,
- Inconsistent basal gene expression
- High expression of lymphocyte and endothelial cell markers

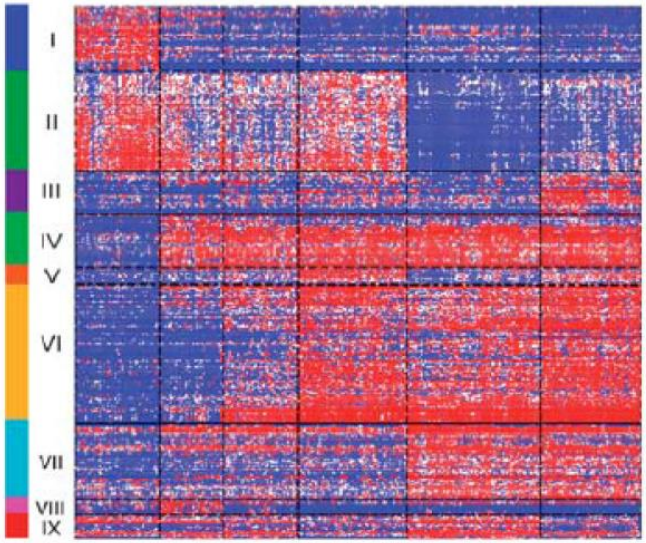






Molecular Apocrine

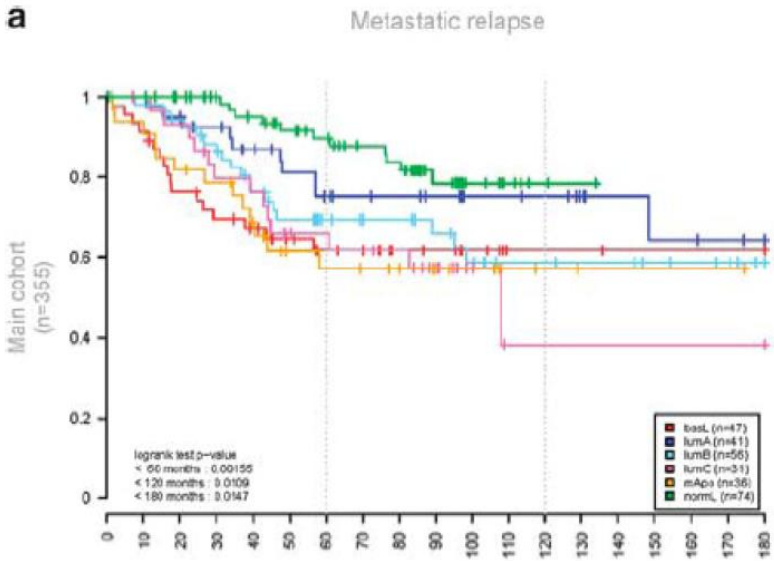


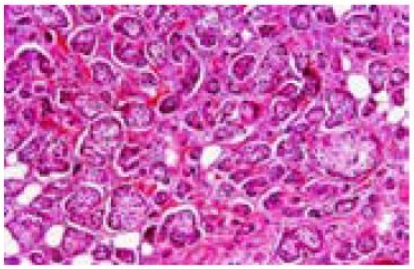


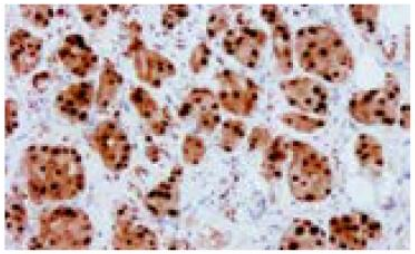
Benign and malignant apocrine lesions of the breast

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

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







Invasive Apocrine Carcinoma

- Rare subtype in pure form– 0.3 – 4.0%
- Focal apocrine differentiation common –

  60% NST on morphology

  72% express GCDFP
- Outcome comparable to that of conventional IDC-NSTs.
- When compared to non-apocrine TNBCs, TN apocrine carcinomas are less likely to be of grade 3, occur in older patients, and display a favorable prognosis.

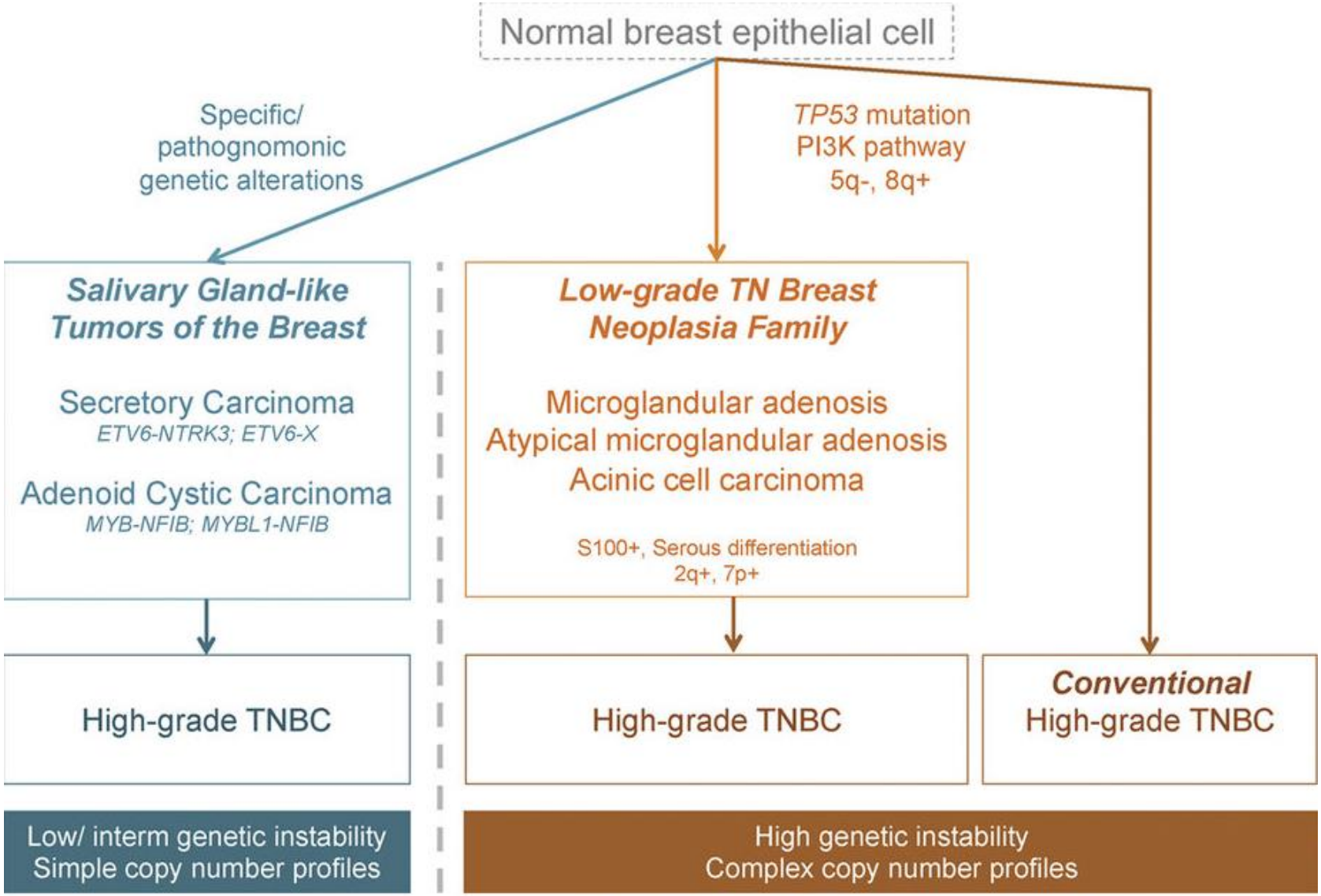
Invasive Apocrine Carcinoma

- Presentation, prognostic characteristics and behaviour similar to NST

- Immunophenotype
  - ER neg
  - PR neg
  - AR pos
  - HER 2 +/-

- Lack of robust criteria

Triple Negative Breast Cancer



Low Grade TN BC

Salivary gland-like tumors of the breast

- Adenoid cystic carcinoma (AdCC)
  - *MYB-NFIB fusion gene*
- Secretory
  - *ETV6-NTRK3* fusion-gene
- Vare rare subtypes:
  - Polymorphous carcinoma
  - Mucoepidermoid carcinoma
  - Adenomyoepithelioma
  - Acinic like???

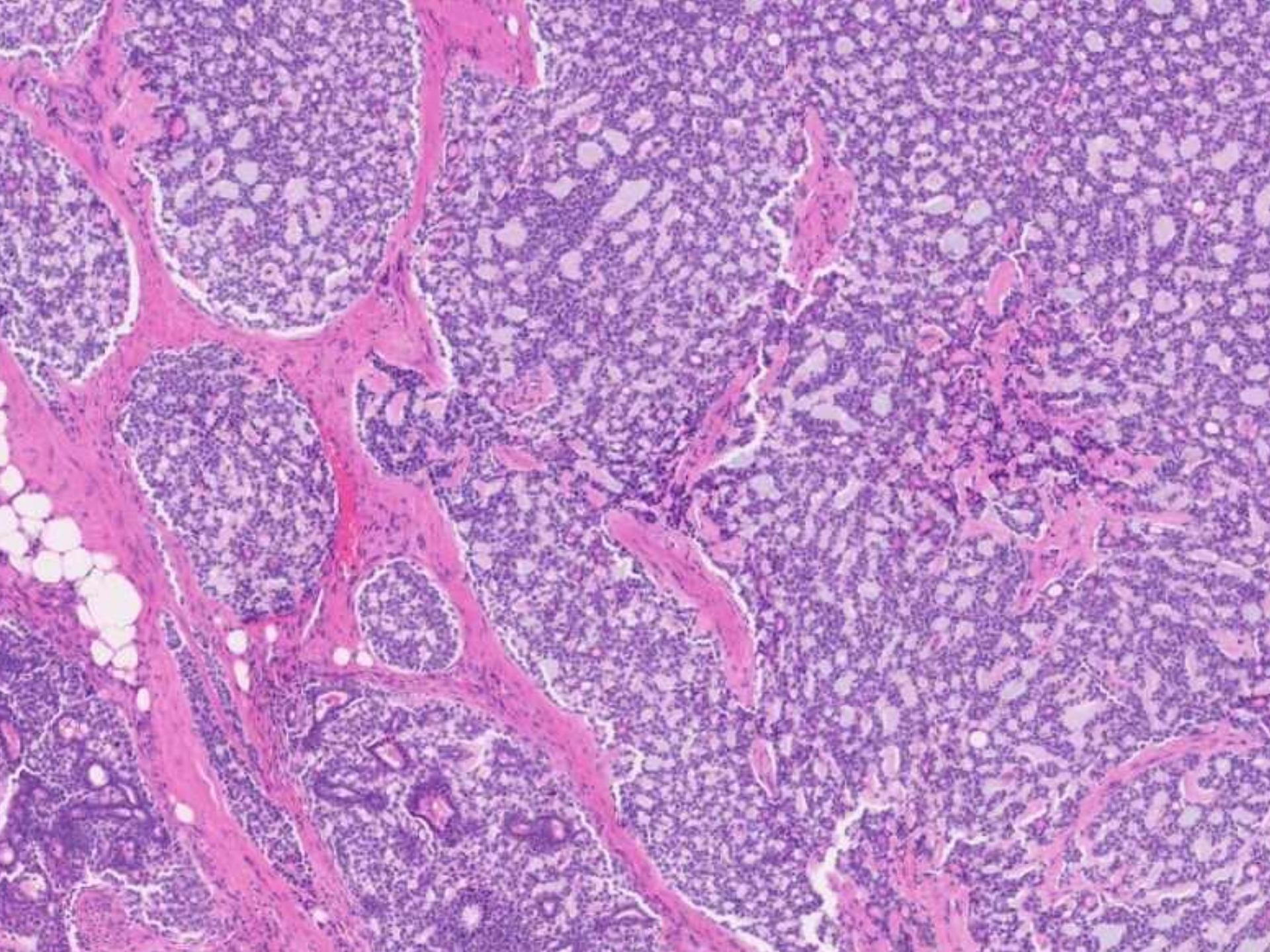
Salivary gland like tumours of the breast

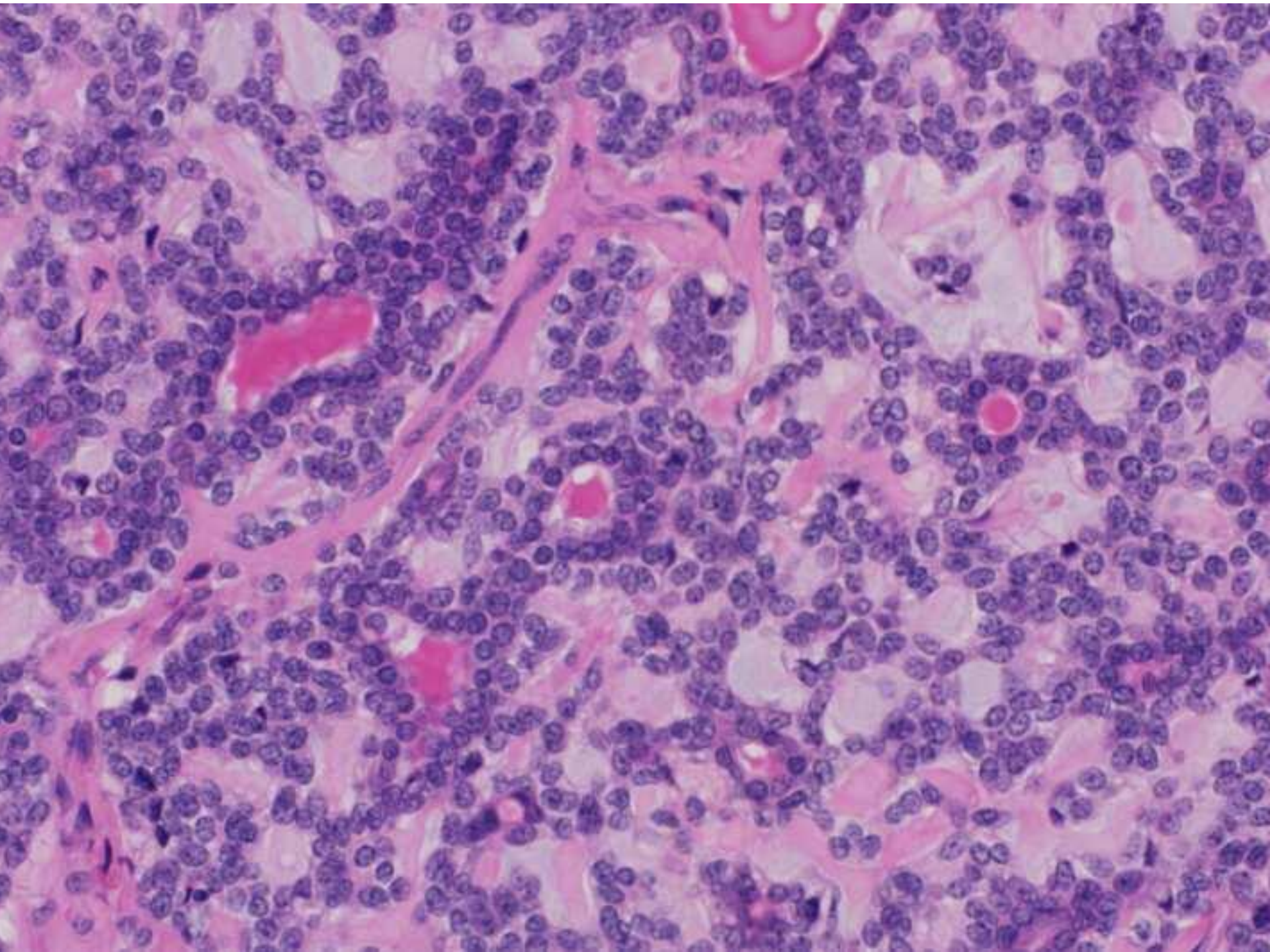
Benign

- Mixed tumour
- Adenomyoepithelioma
- Benign myoepithelioma

Malignant

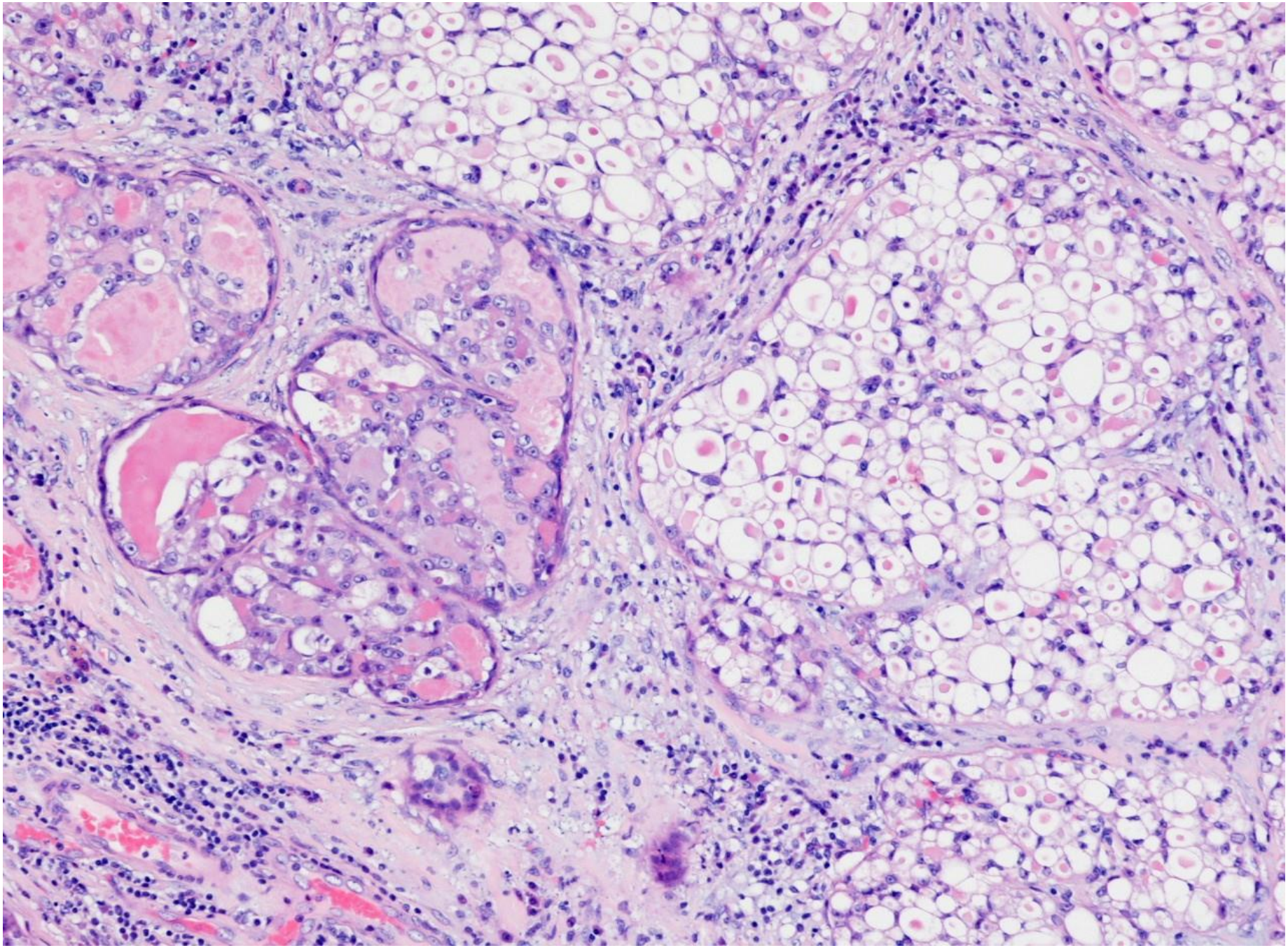
- Acinic cell carcinoma
- Adenoid cystic carcinoma
- Low grade adenosquamous carcinoma
- Oncocytic carcinoma
- Mucoepidermoid carcinoma
- Malignant myoepithelioma





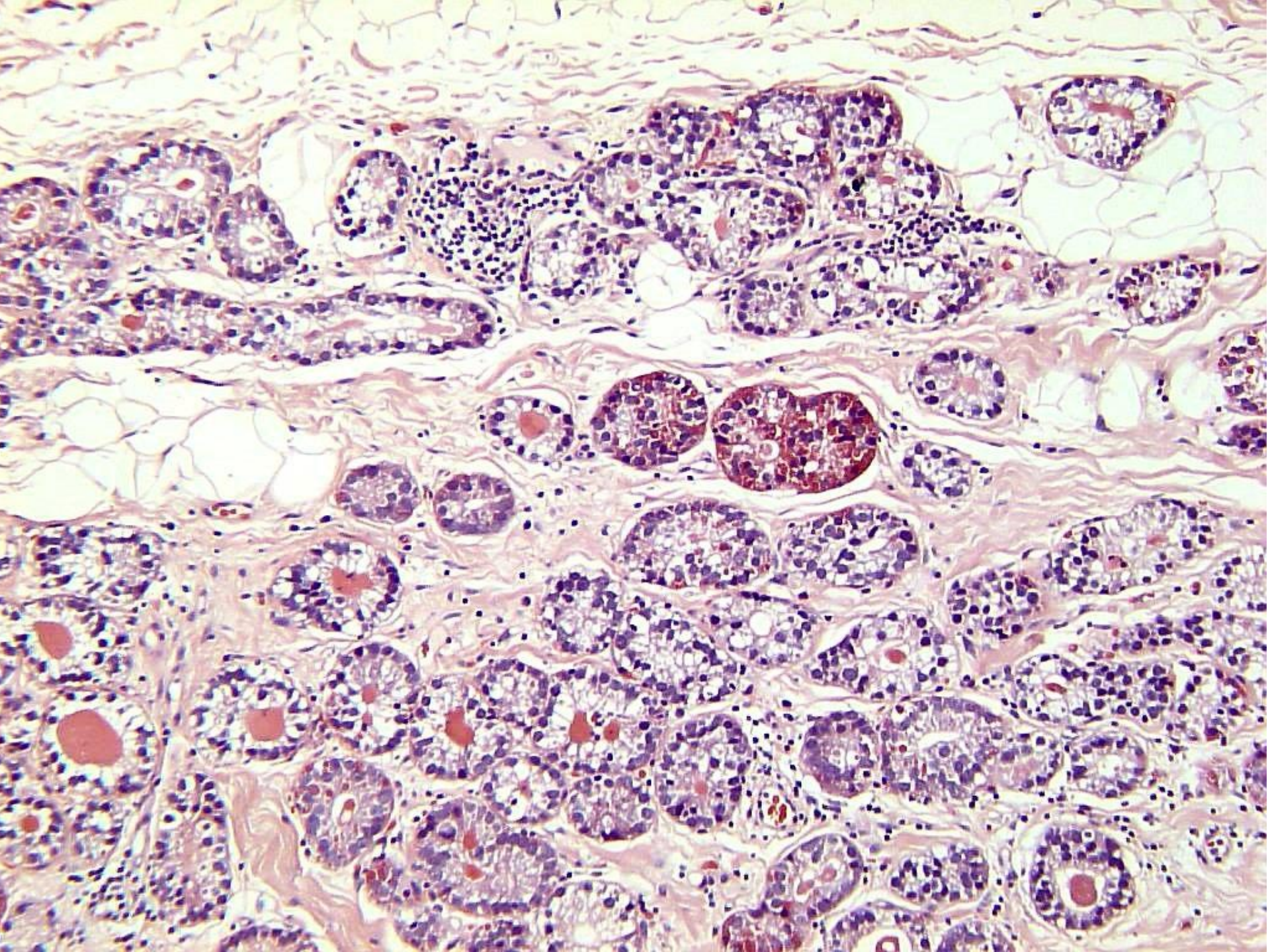
Adenoid cystic carcinoma- molecular pathology

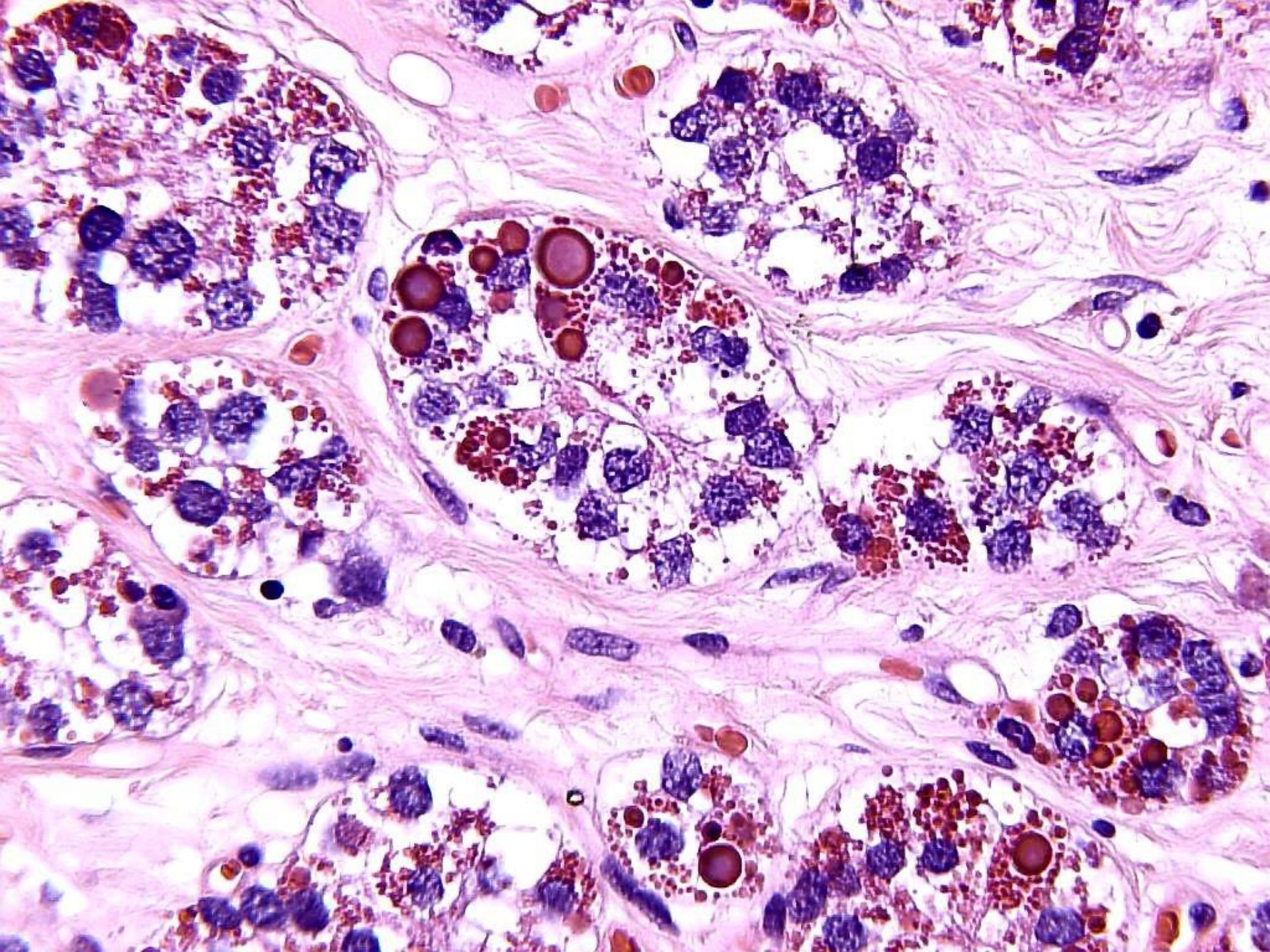
- Clusters with metaplastic and medullary carcinomas – triple negative
- Translocation t (6;9) (q22-23; p23-24) – similar to salivary and other adenoid cystic carcinomas

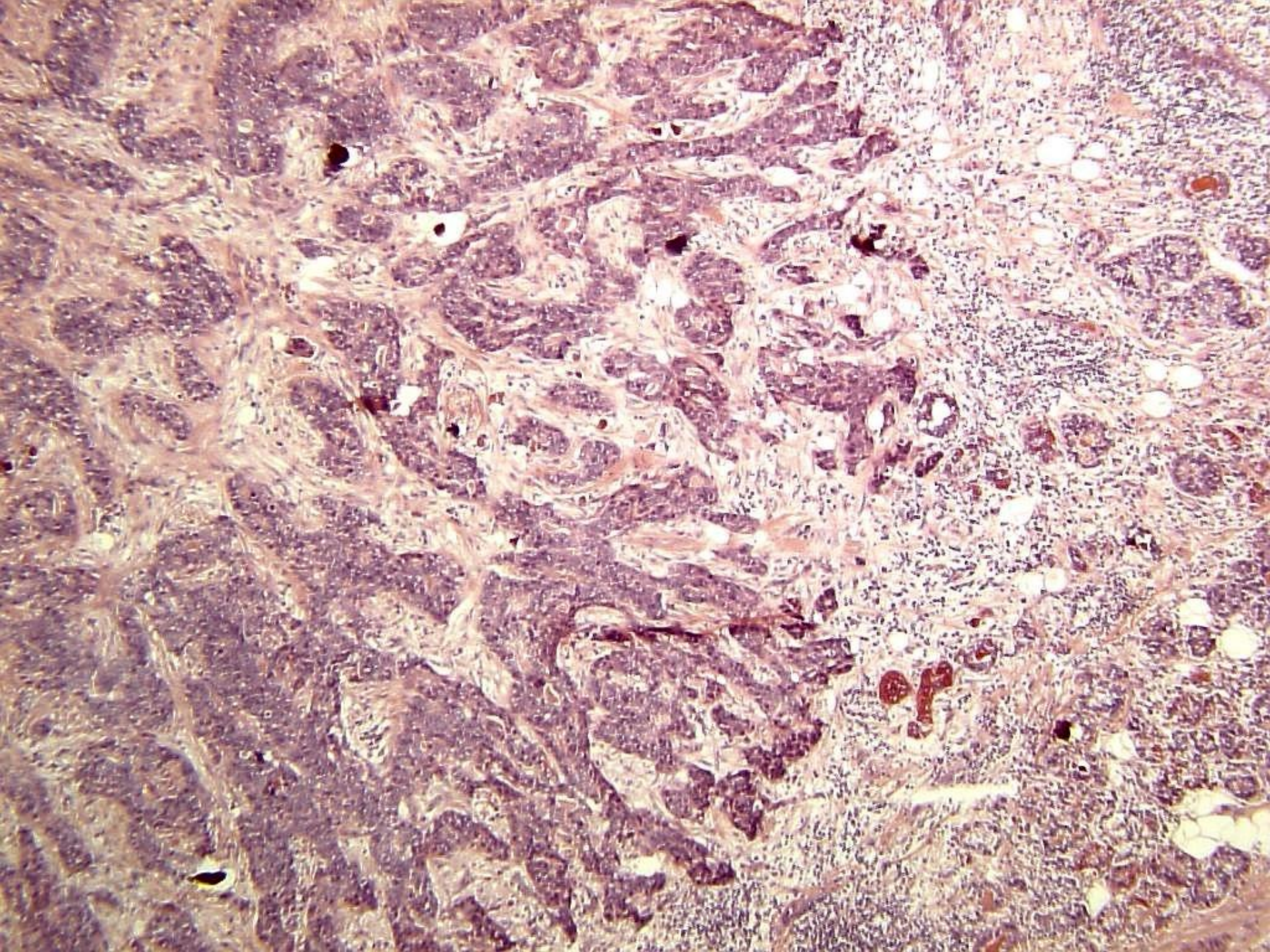


Secretory carcinoma

- Low nuclear grade with vacuolated cytoplasm which may contain eosinophilic secretion arranged in cribriform patterns with the spaces containing eosinophilic secretions
- Typically, they show strong reactivity with S100
- They are mostly triple negative
- Express basal cytokeratins, and belong to the basal-like molecular group of breast cancers
- Genetically they are characterised by the presence of a chromosomal translocation t(12;15)(p13;q25) which results in the formation of ETV6-NTRK3 fusion gene







Acinic cell carcinoma

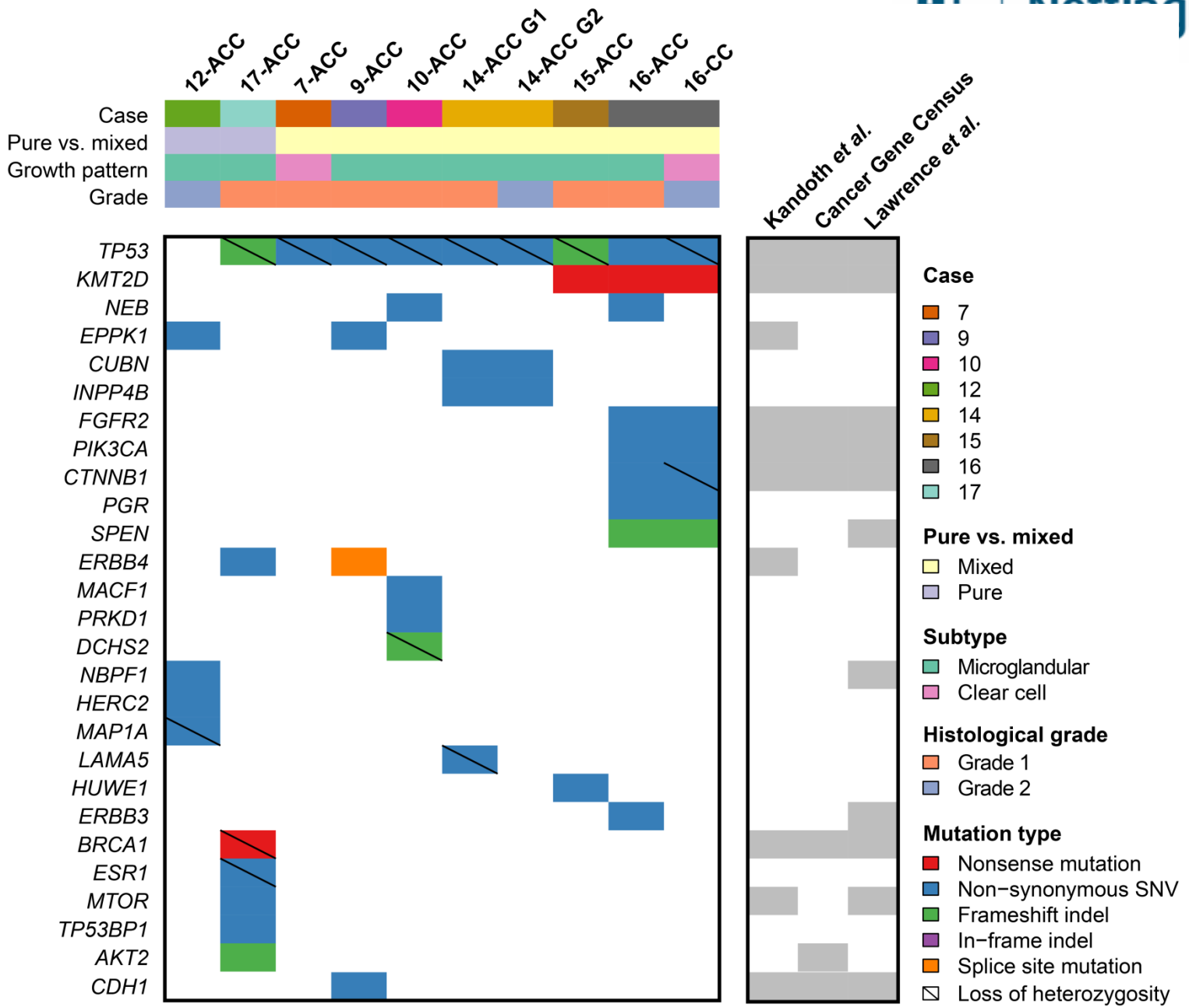
Journal of Pathology
J Pathol 2015; **237:** 166–178
Published online 29 July 2015 in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.4566

ORIGINAL PAPER

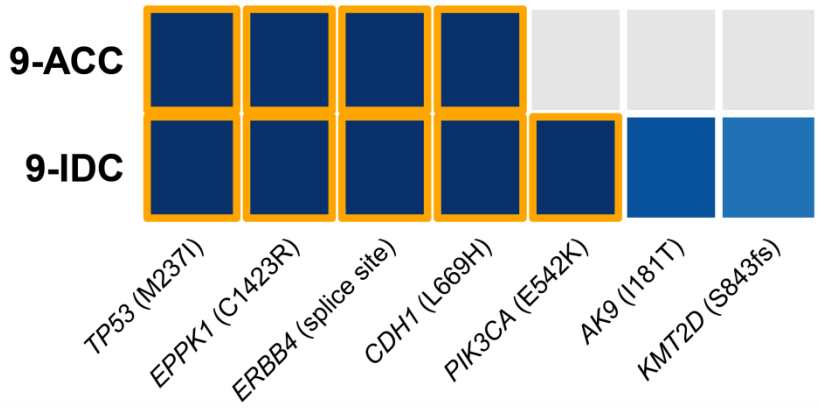
The repertoire of somatic genetic alterations of acinic cell carcinomas of the breast: an exploratory, hypothesis-generating study

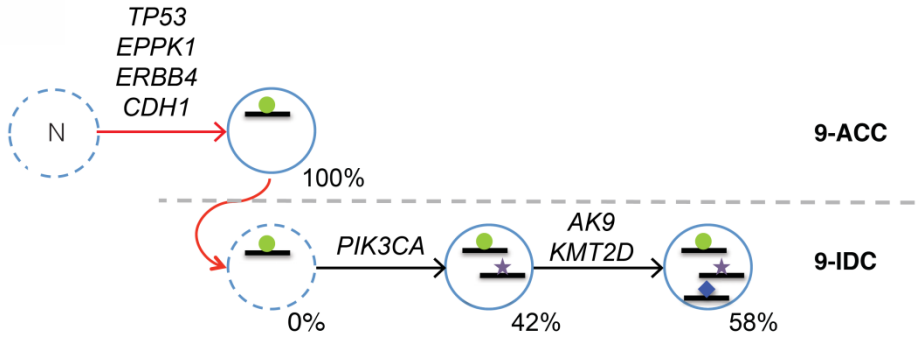
Elena Guerini-Rocco,[1,2†] Zsolt Hodi,[3†] Salvatore Piscuoglio,[1†] Charlotte KY Ng,[1†] Emad A Rakha,[3] Anne M Schultheis,[1] Caterina Marchiò,[1,4] Arnaud da Cruz Paula,[1] Maria R De Filippo,[1] Luciano G Martelotto,[1] Leticia De Mattos-Arruda,[1,5] Marcia Edelweiss,[1] Achim A Jungbluth,[1] Nicola Fusco,[1,2] Larry Norton,[6] Britta Weigelt,[1*] Ian O Ellis[3*] and Jorge S Reis-Filho[1*]

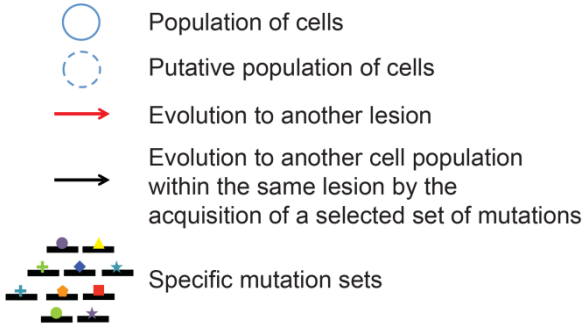
Landscape of somatic genetic alterations

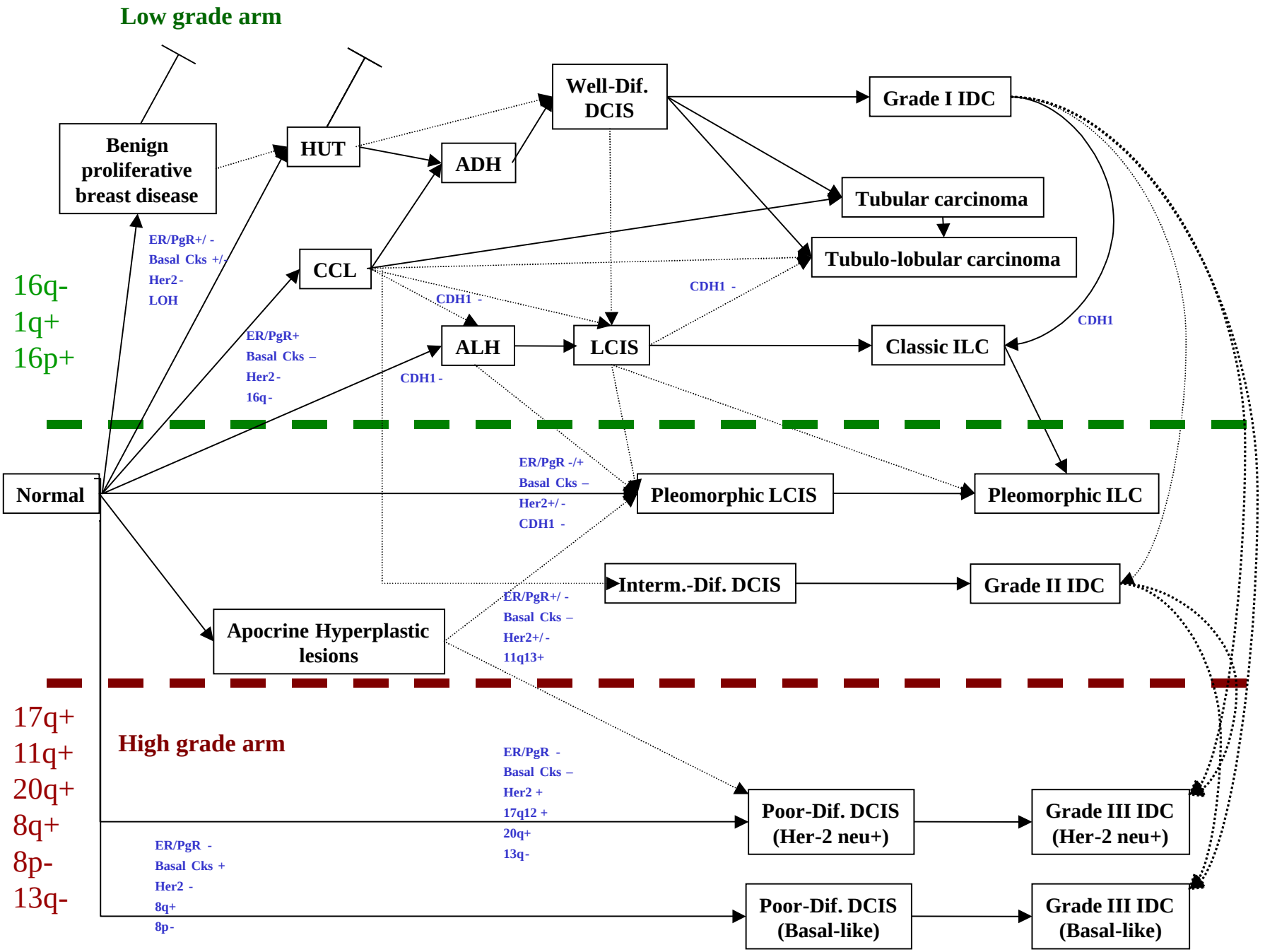


Progression from ACC to high-grade TNBC







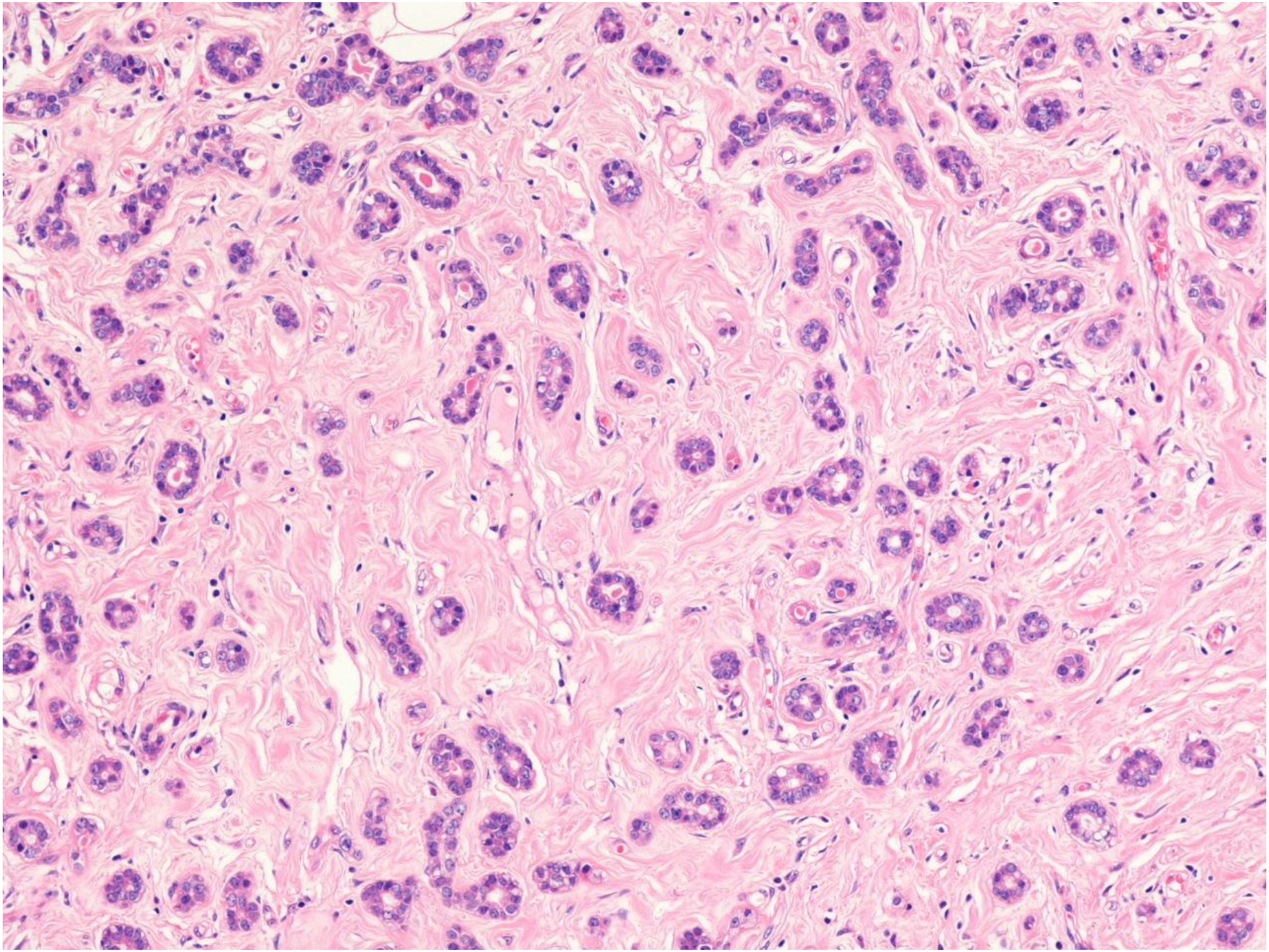


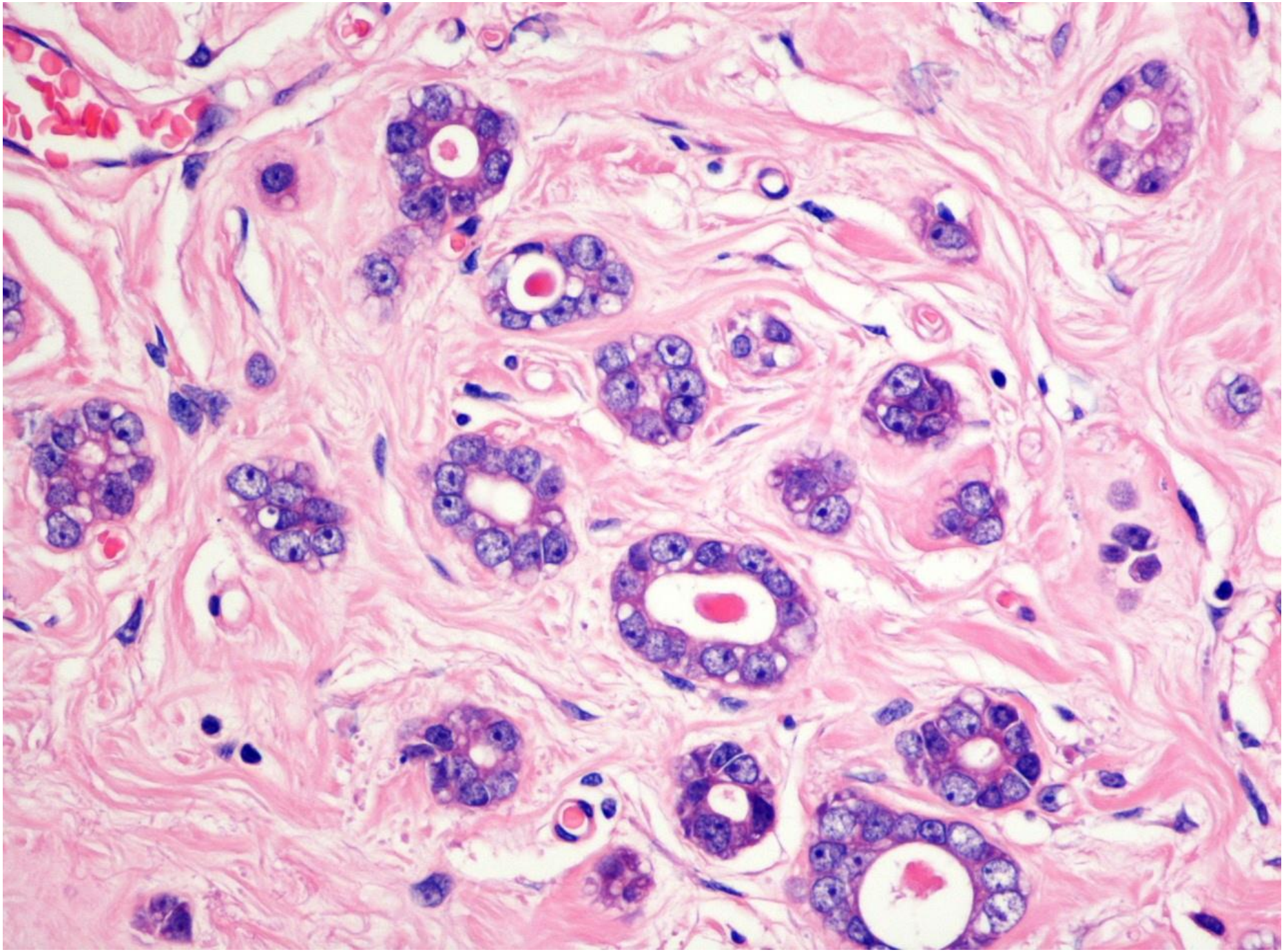
Simpson P, et al. J Pathol. 2005 Jan;205(2):248-54.

Low Grade TN BC

Low-grade TN breast neoplasia family

- Microglandular adenosis (MGA)
- Atypical MGA (AMGA)
- Acinic cell like carcinoma (ACC)





Microglandular adenosis

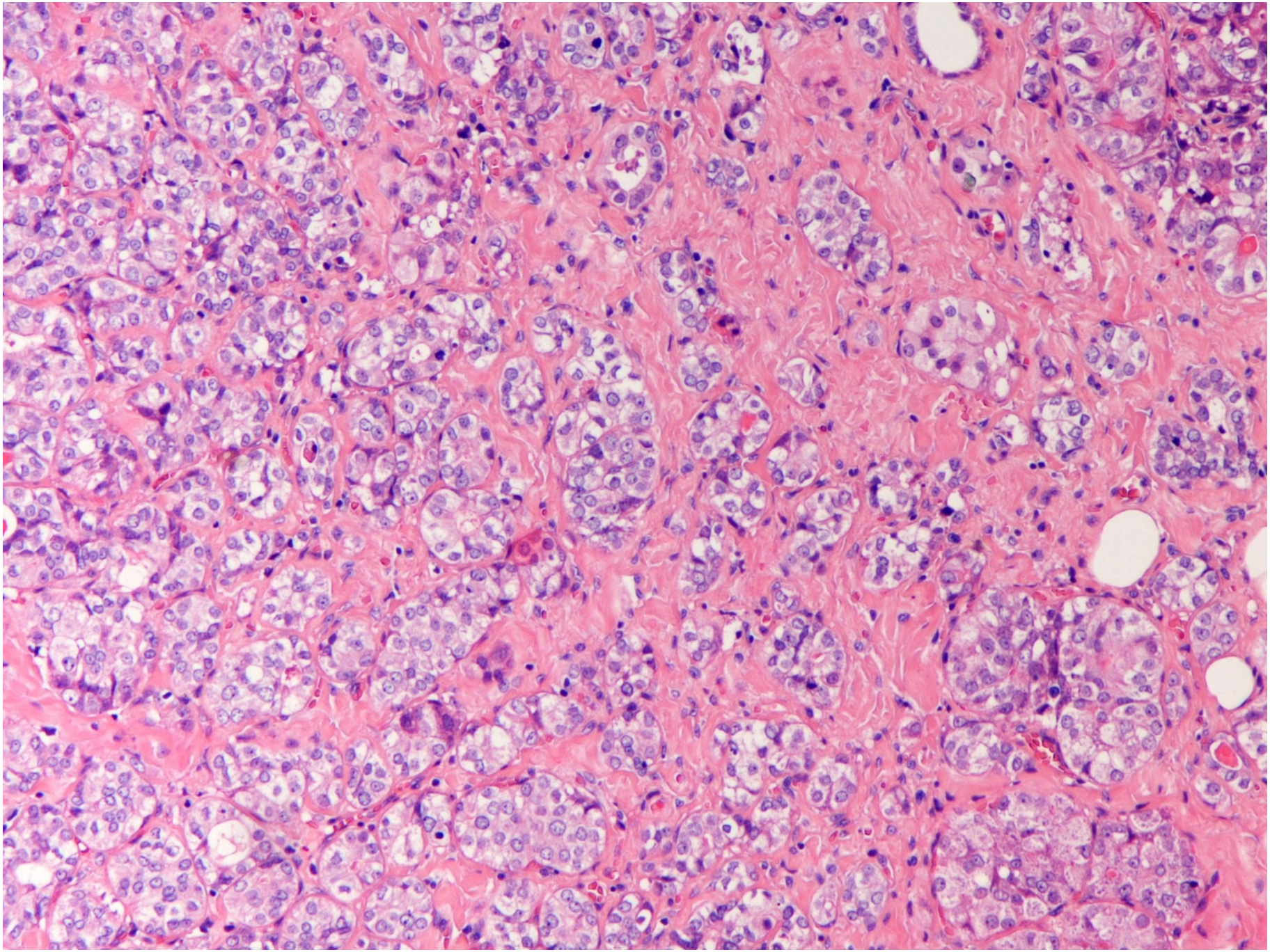
Prognostic implications

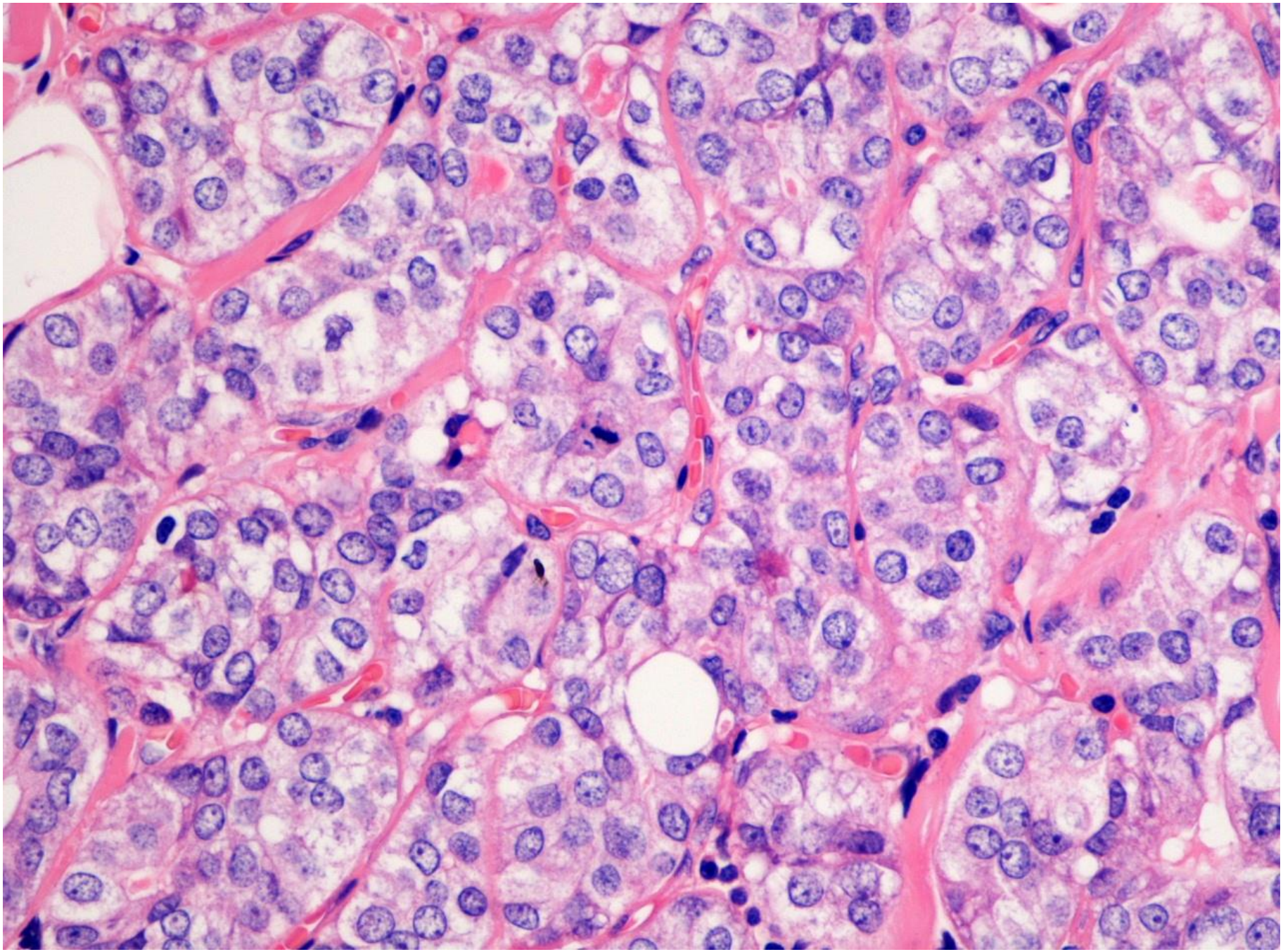
- Probably indolent in its uncomplicated form

BUT:

- Rosen (1) reported 14 carcinomas among 60 MGA
- Page (2) reported 17 cases of ACC associated with MGA
- Tavassoli (3) reported 20 cases of in situ and invasive carcinoma associated with MGA
- Atypical MGA

(1) Carcinoma of the breast arising in Microglandular Adenosis. Am.J.Clin. Path. 1993; 100:507-13
(2) Microglandular Adenosis with transition into Adenoid Cystic Carcinoma of the breast. Am.J.Surg.Path. 27(8) 1052-60 2003
(3) Carcinoma arising in MGA Int.J.Surg.Path. 2000;8 303-15





Journal of Pathology
J Pathol 2016; **238:** 677–688
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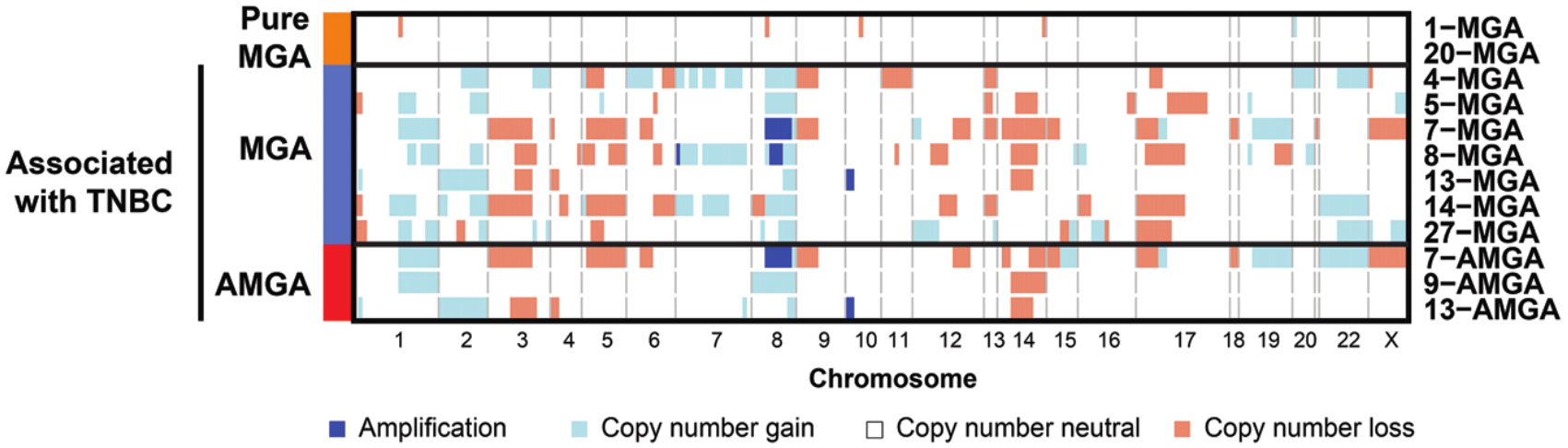
ORIGINAL PAPER

Microglandular adenosis associated with triple-negative breast cancer is a neoplastic lesion of triple-negative phenotype harbouring *TP53* somatic mutations

Elena Guerini-Rocco,[1,2,†] Salvatore Piscuoglio,[1,†] Charlotte KY Ng,[1,†] Felipe C Geyer,[1,3] Maria R De Filippo,[1] Carey A Eberle,[1] Muzaffar Akram,[1] Nicola Fusco,[1,4] Shu Ichihara,[5] Rita A Sakr,[6] Yasushi Yatabe,[7] Anne Vincent-Salomon,[8] Emad A Rakha,[9] Ian O Ellis,[9] Y Hannah Wen,[1] Britta Weigelt,[1,*] Stuart J Schnitt[10] and Jorge S Reis-Filho[1,*]



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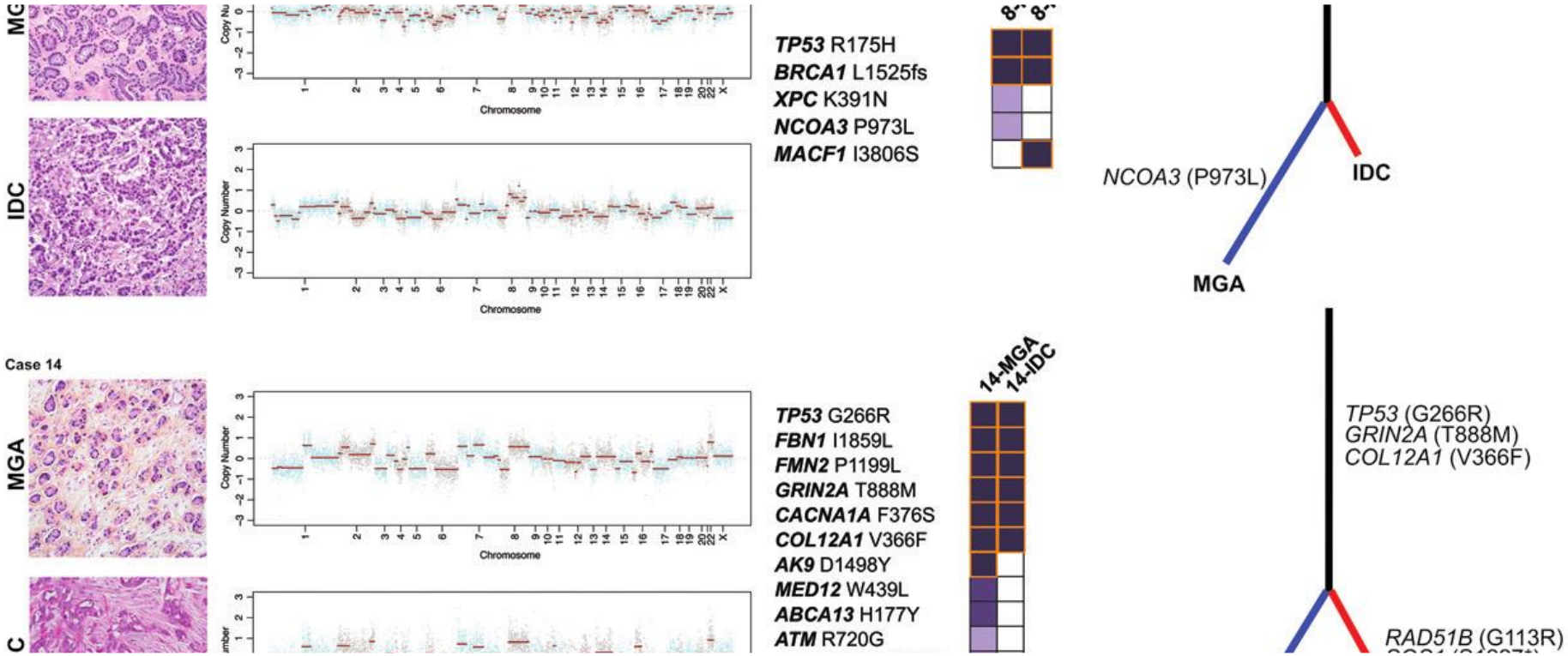
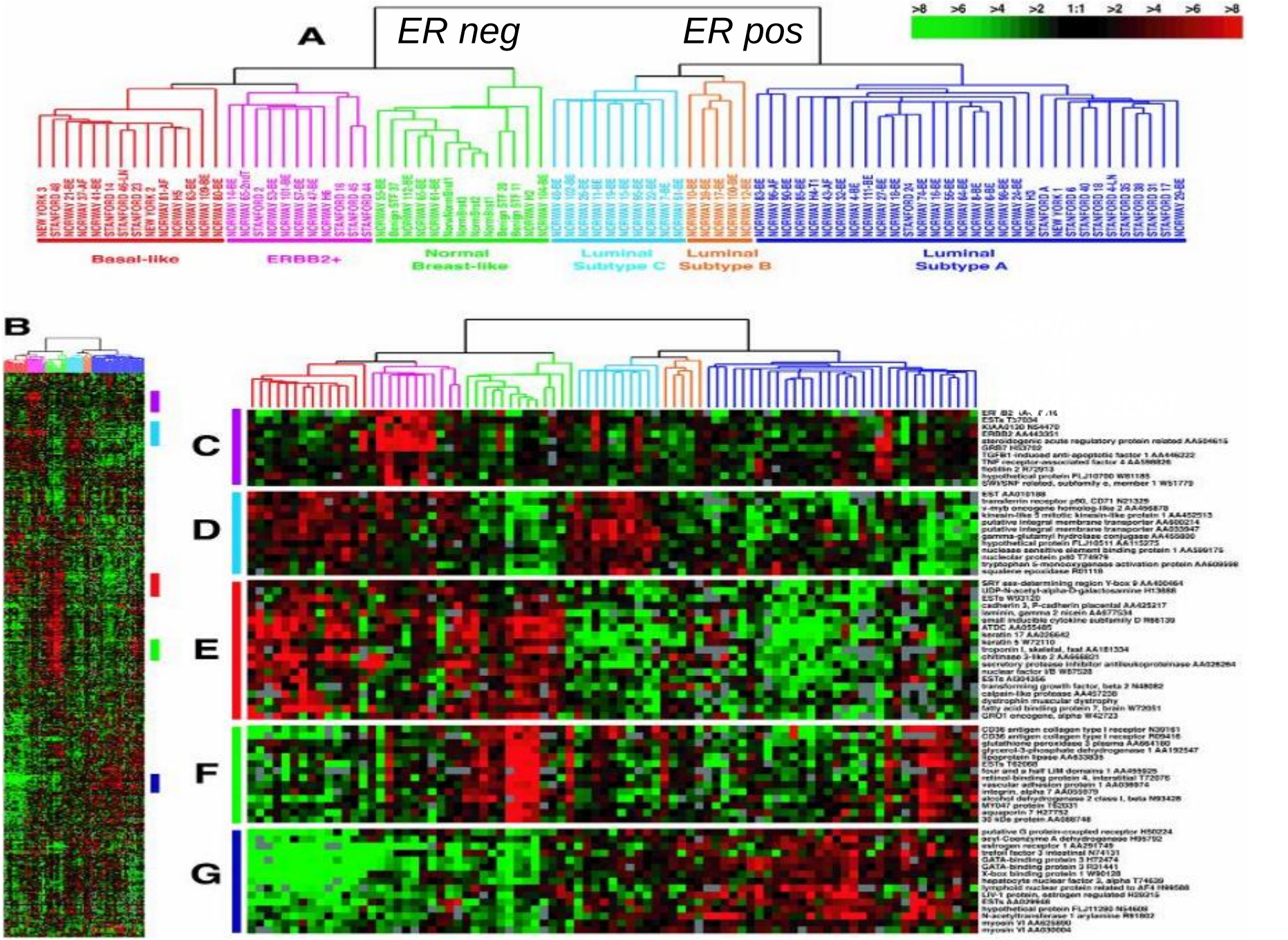
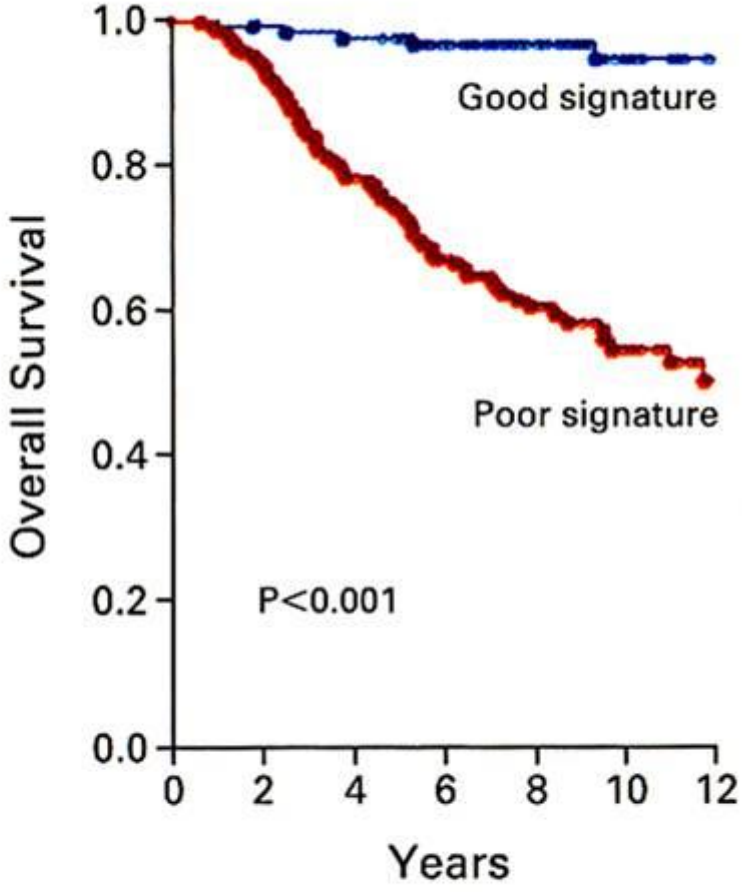



A
ER neg
ER pos
>8 >6 >4 >2 1:1 >2 >4 >6 >8
Basal-like
ERBB2+
Normal Breast-like
Luminal Subtype C
Luminal Subtype B
Luminal Subtype A
B
C
D
E
F
G

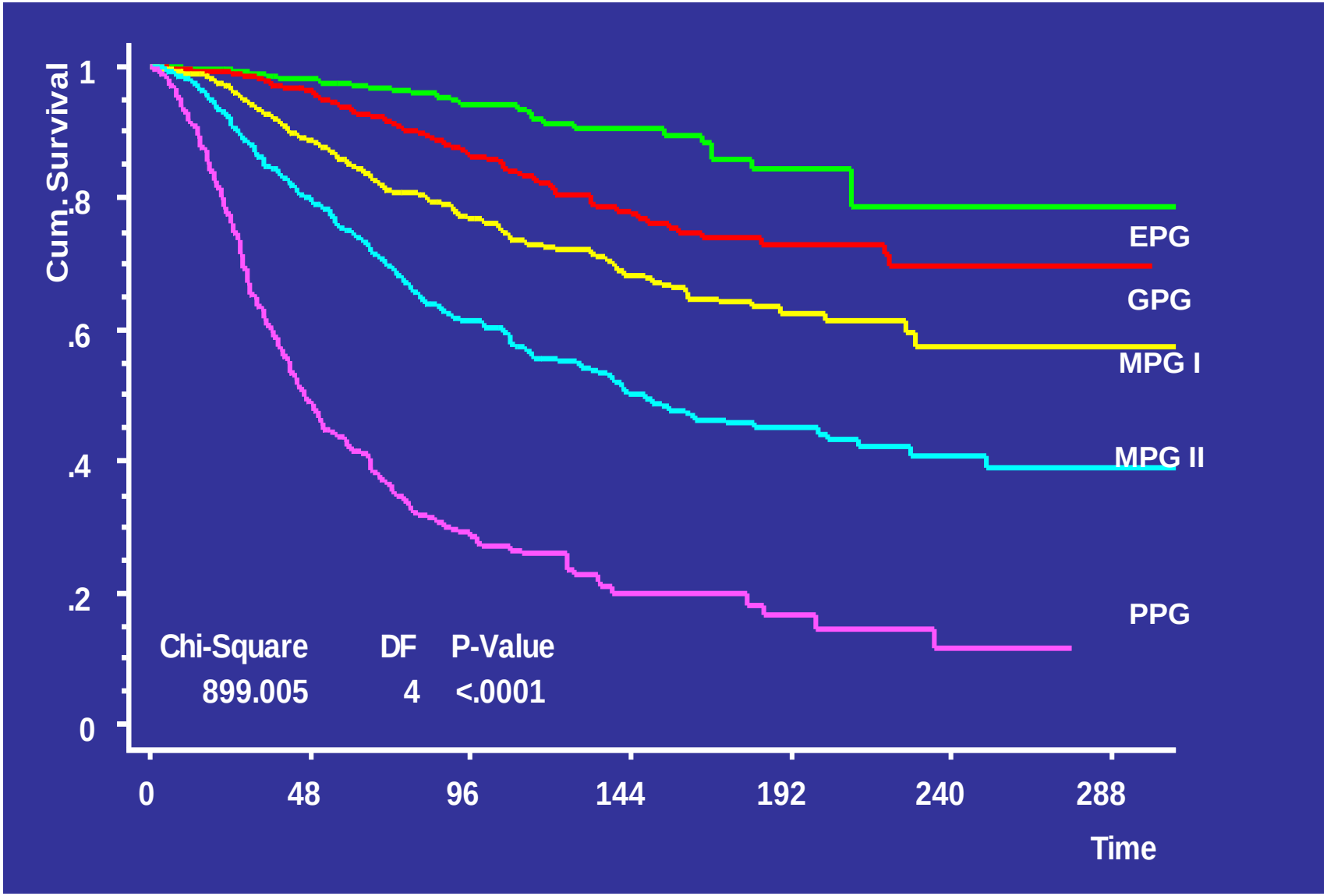
All Patients



No. at Risk							
Low risk	115	114	112	91	65	43	23
High risk	180	167	134	100	62	40	19

N P I

The University of Nottingham



Oncotype DX™ 21-Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

PROLIFERATION
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN
ER
PR
Bcl2
SCUBE2

GSTM1

BAG1

INVASION
Stromelysin 3
Cathepsin L2

CD68

HER2
GRB7
HER2

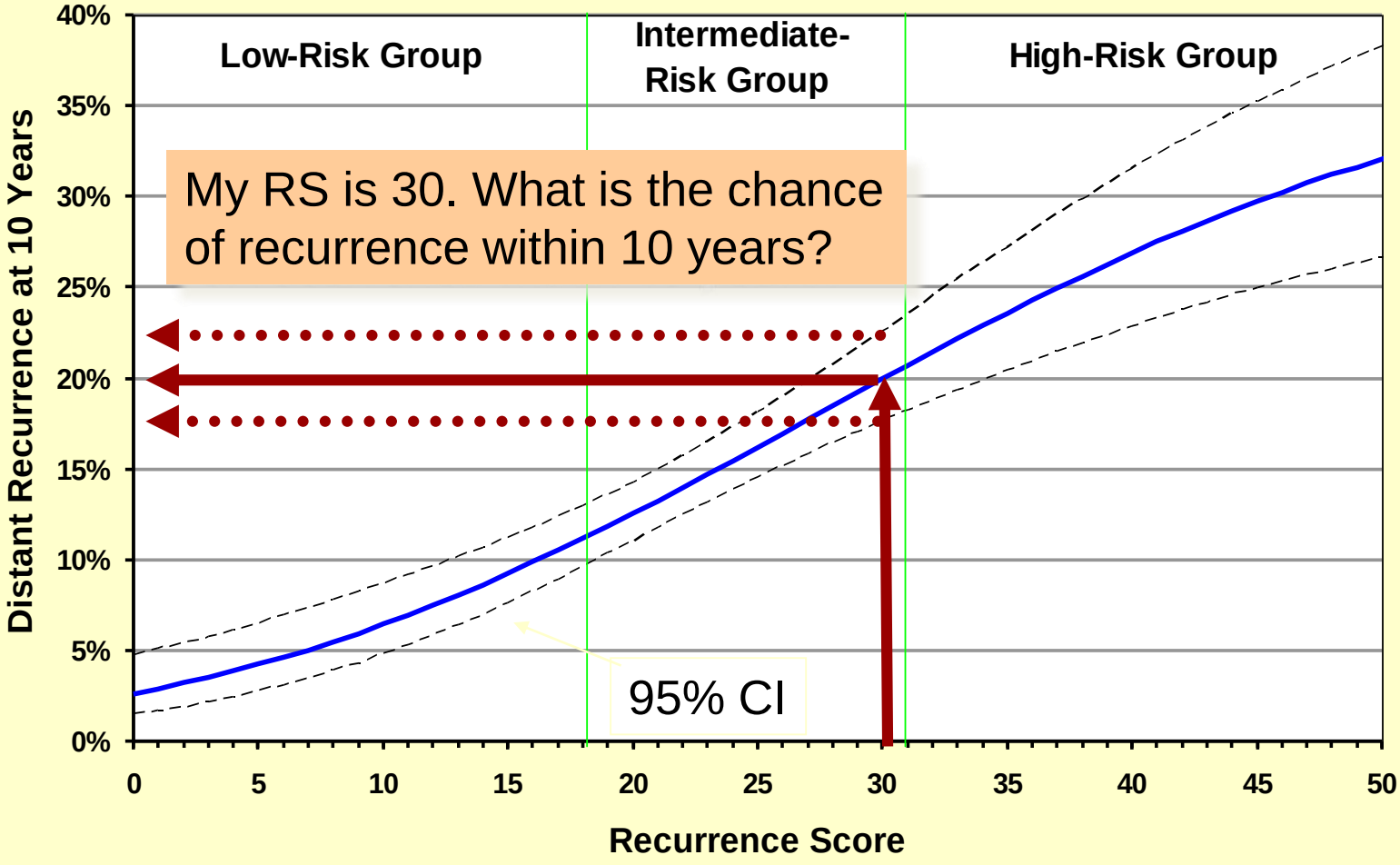
REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

RS = + 0.47 x HER2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

Category	RS (0-100)
Low risk	RS <18
Int risk	RS ≥18 and <31
High risk	RS ≥31

Paik et al. *N Engl J Med.* 2004;351:2817-2826.

Oncotype DX™ Clinical Validation: RS as Continuous Predictor



Multigene signatures

Microarray and RT-PCR based assays

- 21 gene signature (Oncotype Dx)
- 70 gene signature (MammaPrint)
- 76 gene signature (Rotterdam)
- 50 genes: Risk of Recurrence (ROR) score (Prosigna)
- 8 genes (Endopredict) & Epclin
- 5 genes (Molecular grade index)
- 2 gene ratio (H/I™)

} 7 gene assay (THEROS The Breast Cancer Index)

- 97 gene: Genomic grade index (MapQuant Dx)
- 14 genes (BreastOncPx)
- 14 gene signature (*Celera Metastasis Score™*)
- 186 gene signature (*Invasiveness Gene Signature*)

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-186 gene signature (*Invasiveness Gene Signature*)

Three Elements of Prosigna Breast Cancer Assay

Hardware:
nCounter Analysis System

Prep Station

Digital Analyzer

Consumable:
Prosigna Kits

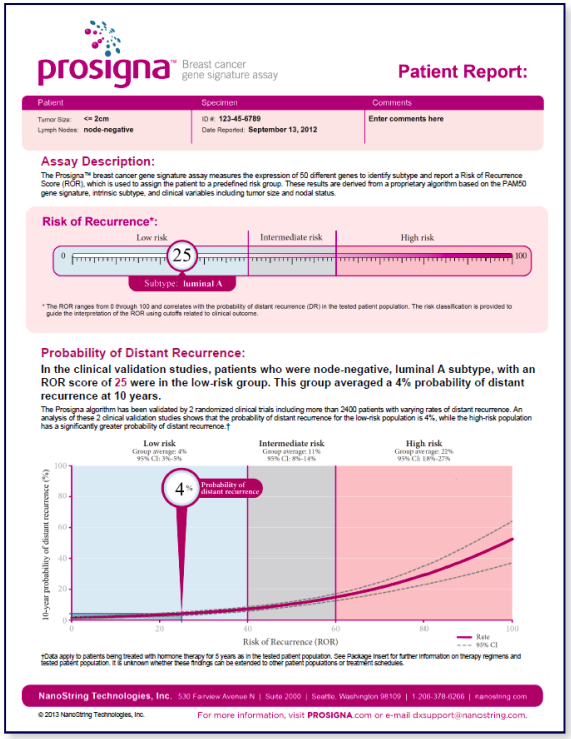
Includes:

50 gene-based CodeSet with 8 controls

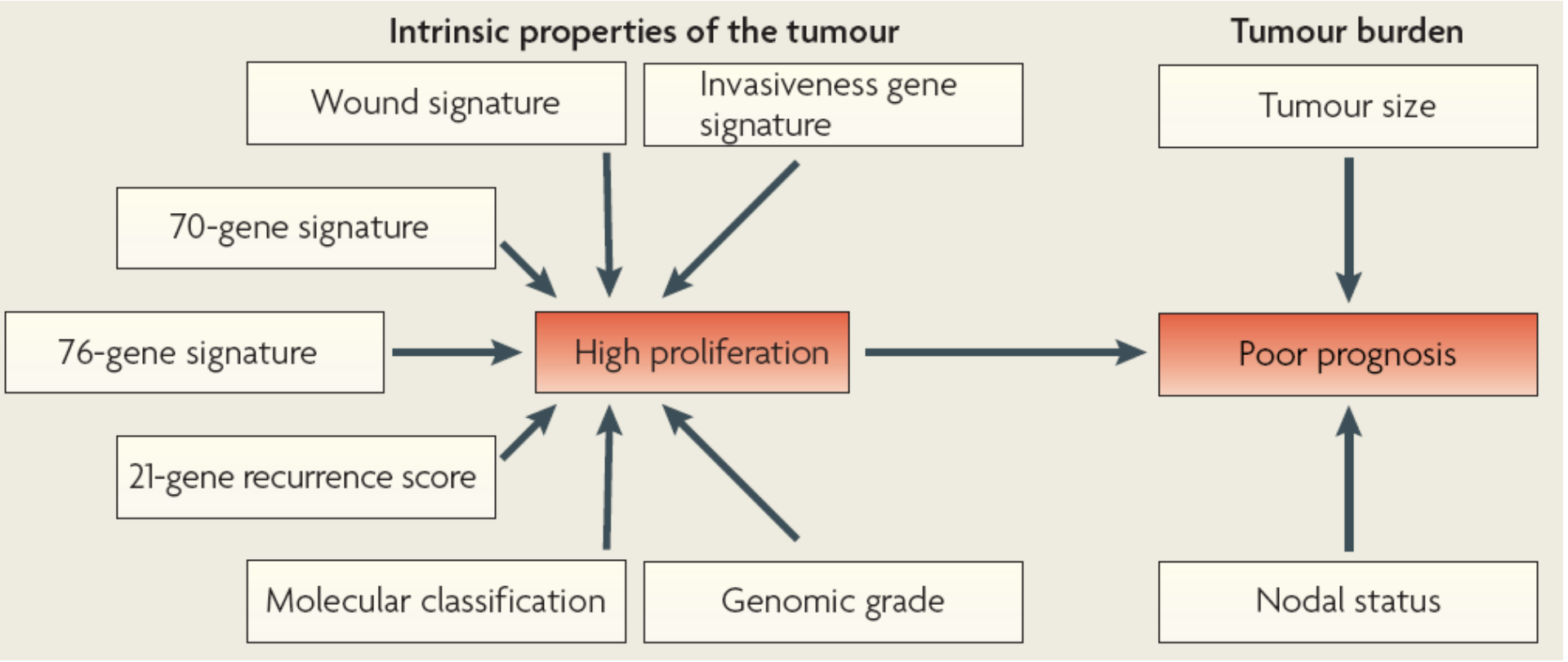
Other consumables required for assay

GMP RNA isolation kit

Software:
Prosigna Report

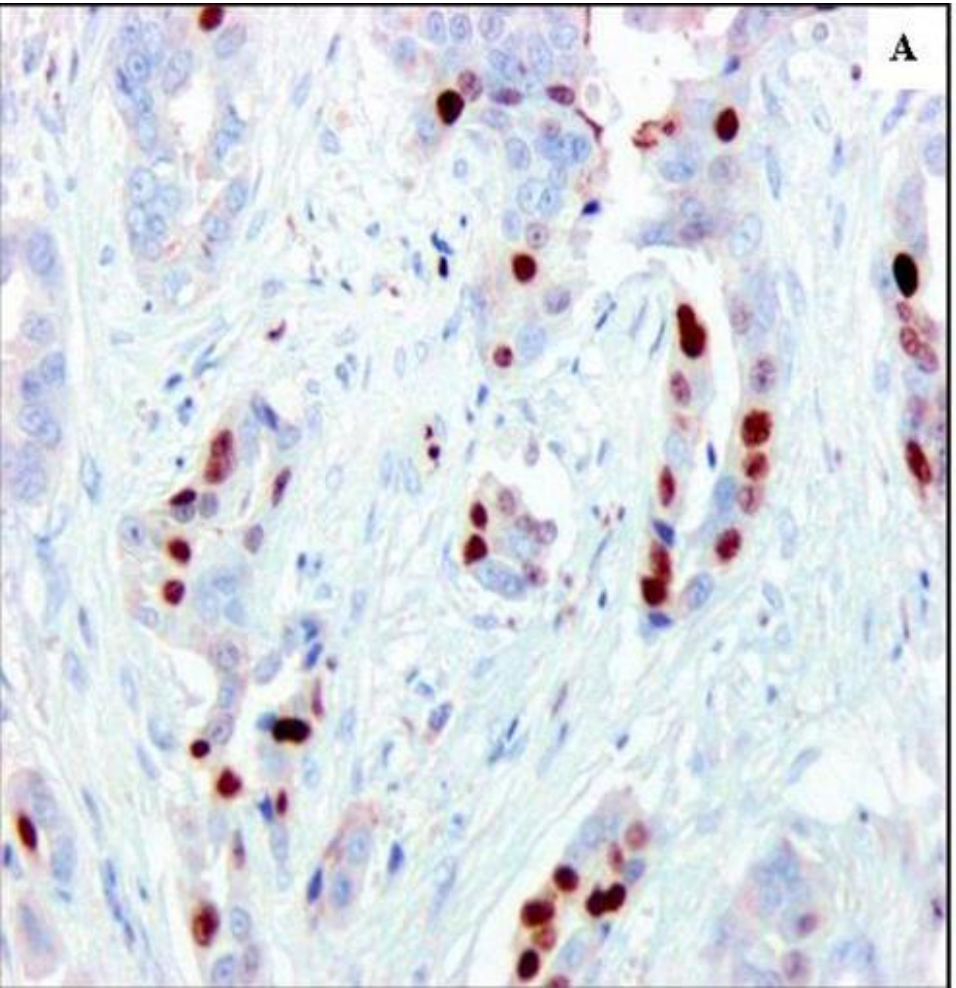


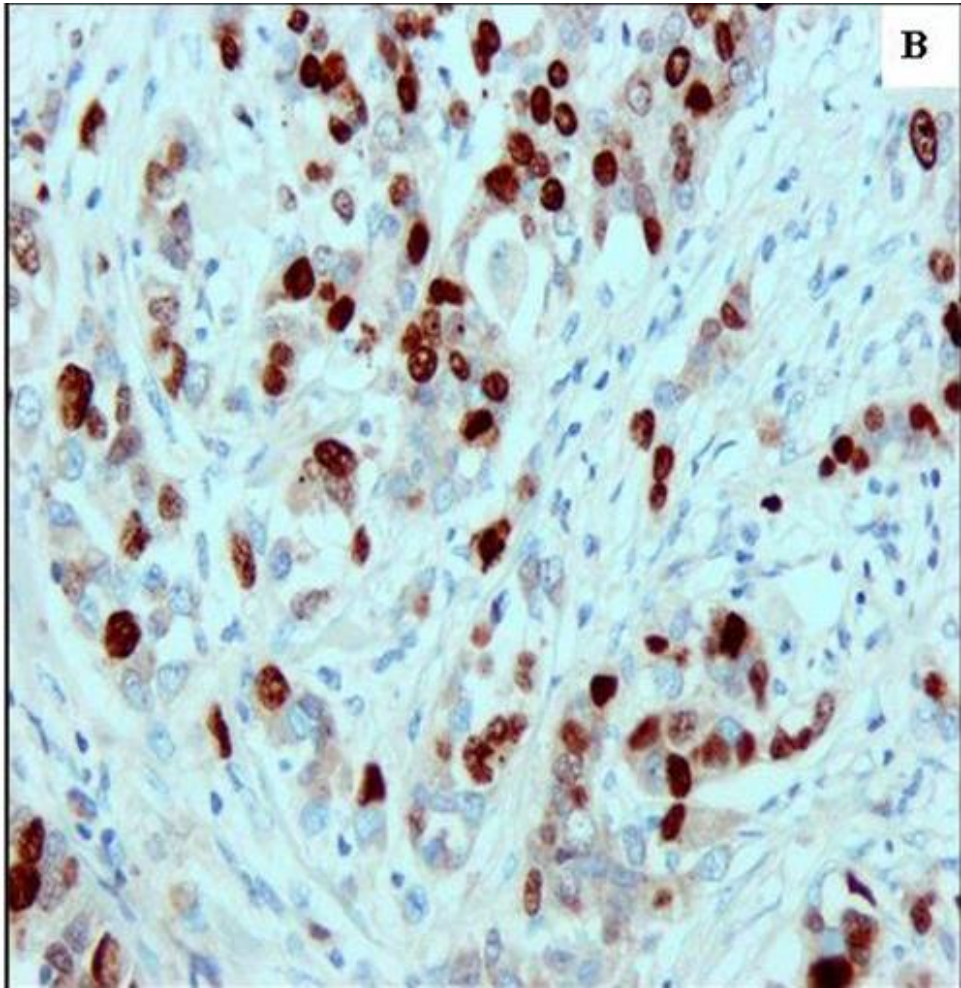
A signature to rule them all?

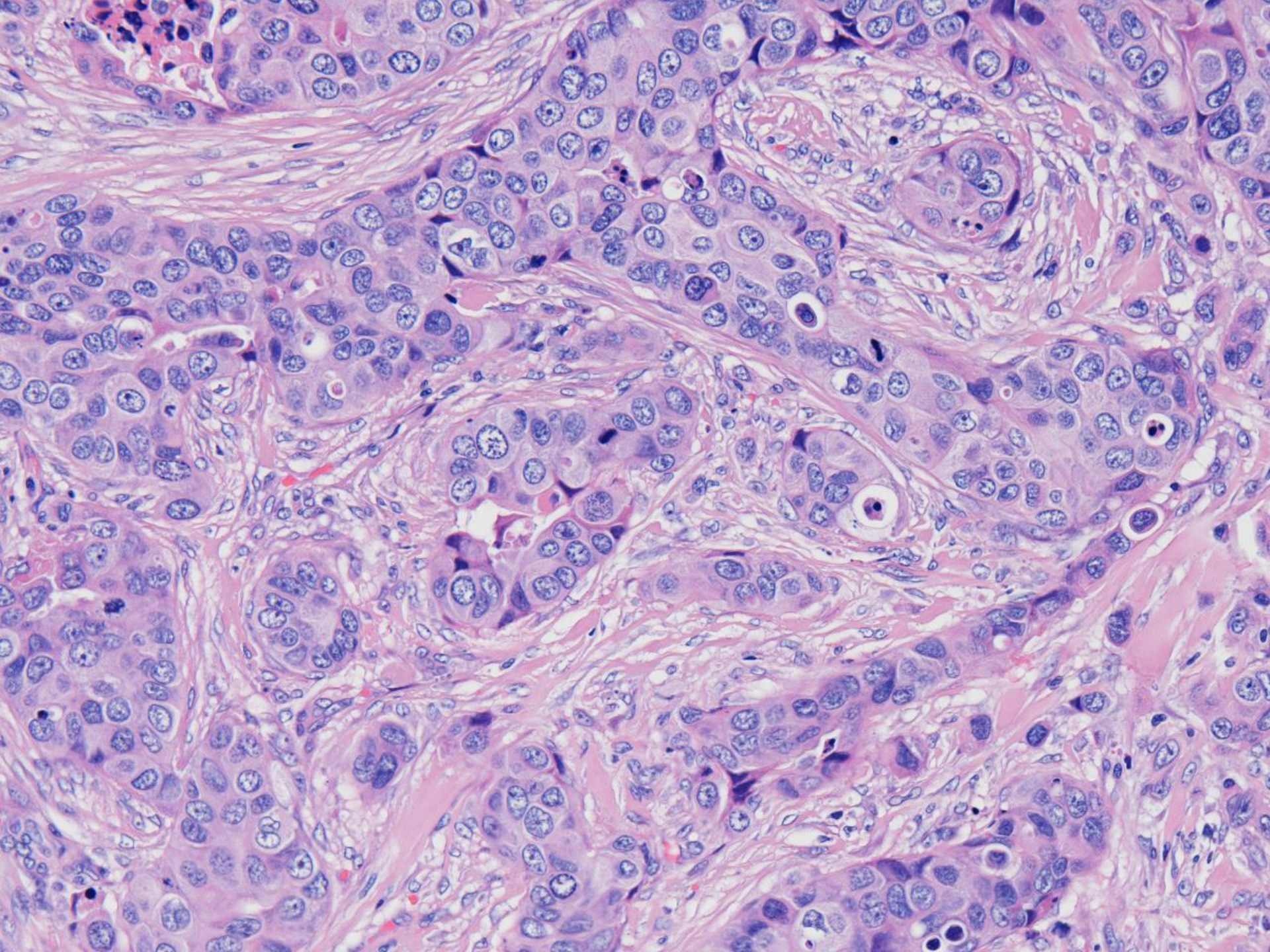


Fan et al. NEJM 2006; Sotiriou et al. JNCI 2006

MIB1 growth fraction in breast cancer





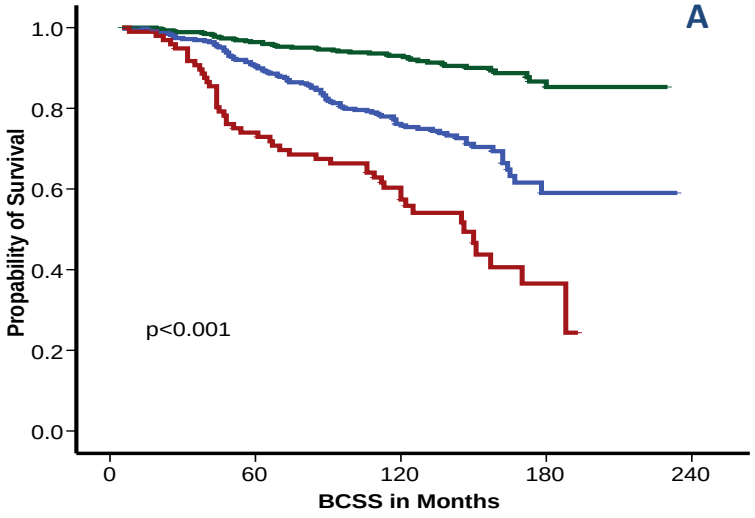


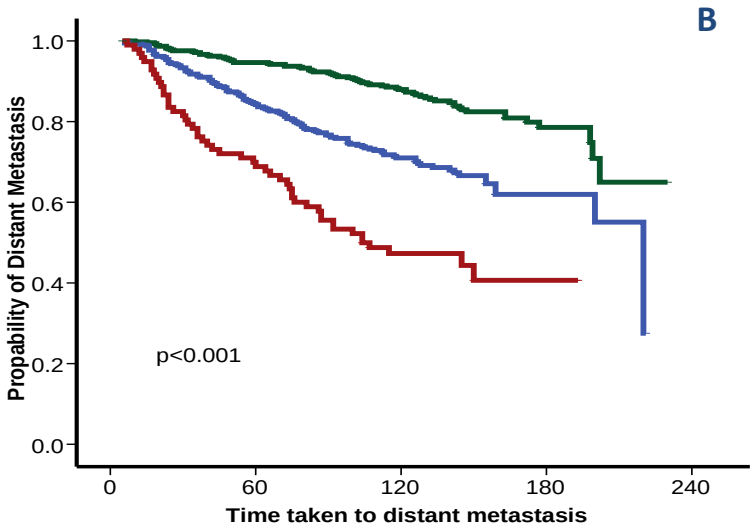
Kaplan-Meier survival plot for luminal BC using Ki67LI and Mitotic Index

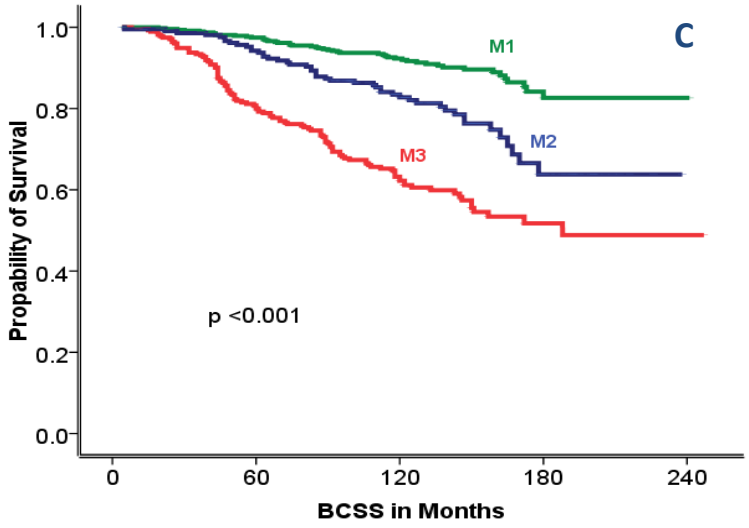
(A) Breast cancer specific survival (BCSS) at 10 and 70% Ki67LI

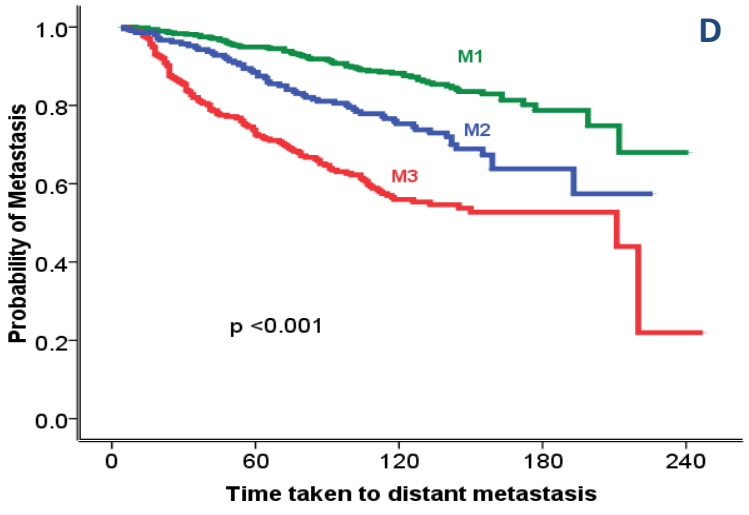
(B) Metastasis-free survival at 10 and 70% Ki67LI

(C) & (D) BCSS and DMFS for mitosis frequency scores.









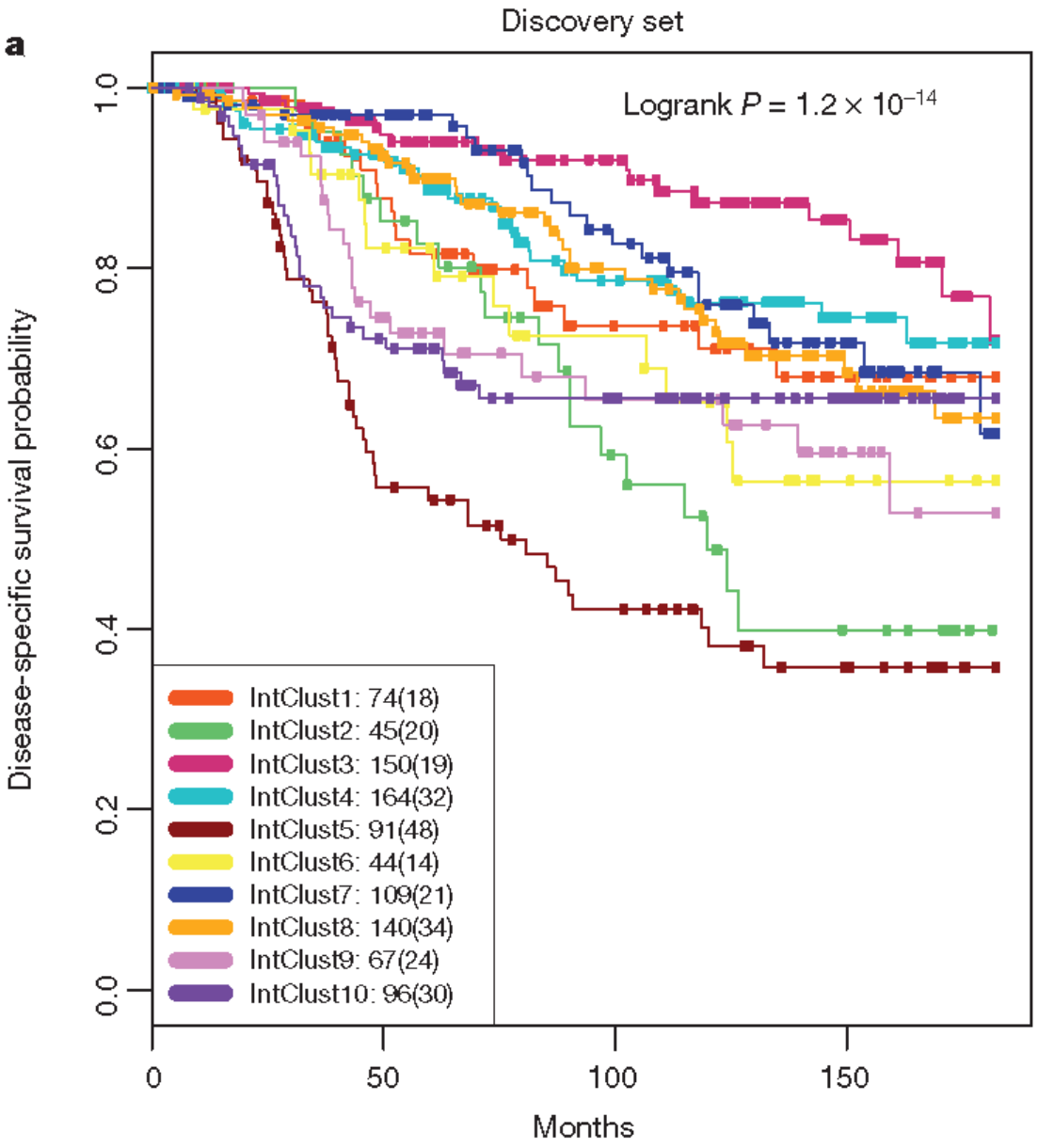
METABRIC

ARTICLE

doi:10.1038/nature10983

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

Christina Curtis[1,2]†*, Sohrab P. Shah[3,4]*, Suet-Feung Chin[1,2]*, Gulisa Turashvili[3,4]*, Oscar M. Rueda[1,2], Mark J. Dunning[2], Doug Speed[2,5]†, Andy G. Lynch[1,2], Shamith Samarajiwa[1,2], Yinyin Yuan[1,2], Stefan Gräf[1,2], Gavin Ha[3], Gholamreza Haffari[3], Ali Bashashati[3], Roslin Russell[2], Steven McKinney[3,4], METABRIC Group‡, Anita Langerød[6], Andrew Green[7], Elena Provenzano[8], Gordon Wishart[8], Sarah Pinder[9], Peter Watson[3,4,10], Florian Markowetz[1,2], Leigh Murphy[10], Ian Ellis[7], Arnie Purushotham[9,11], Anne-Lise Børresen-Dale[6,12], James D. Brenton[2,13], Simon Tavaré[1,2,5,14], Carlos Caldas[1,2,8,13] & Samuel Aparicio[3,4]



The integrative subgroups have distinct clinical outcomes

IntClust 3

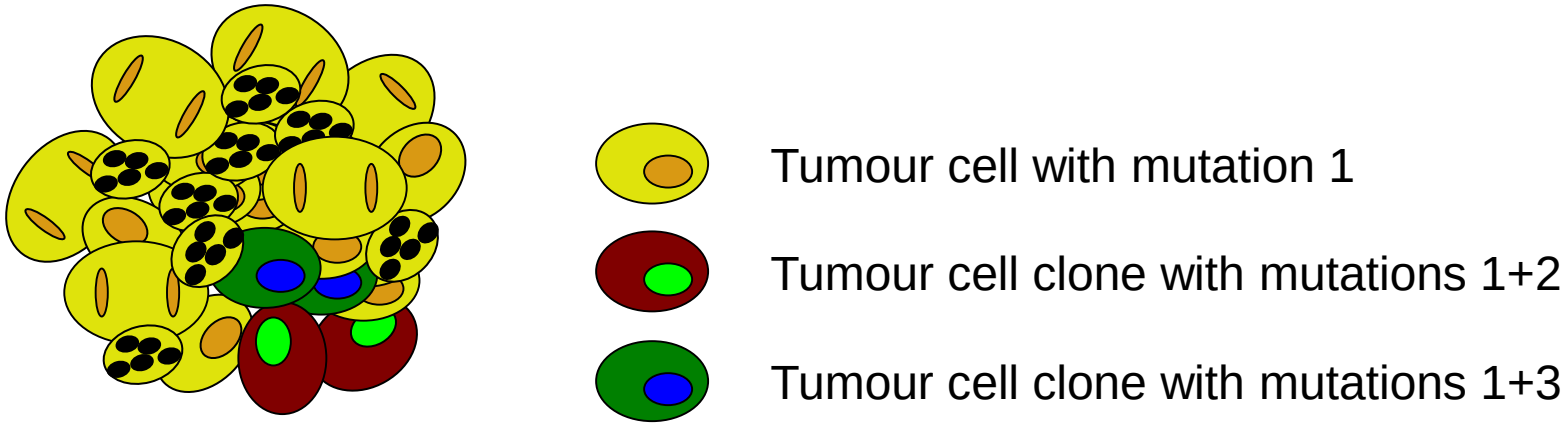
- Low genetic instability
- Luminal A predominant
- Good prognoses types (tubular, lobular)

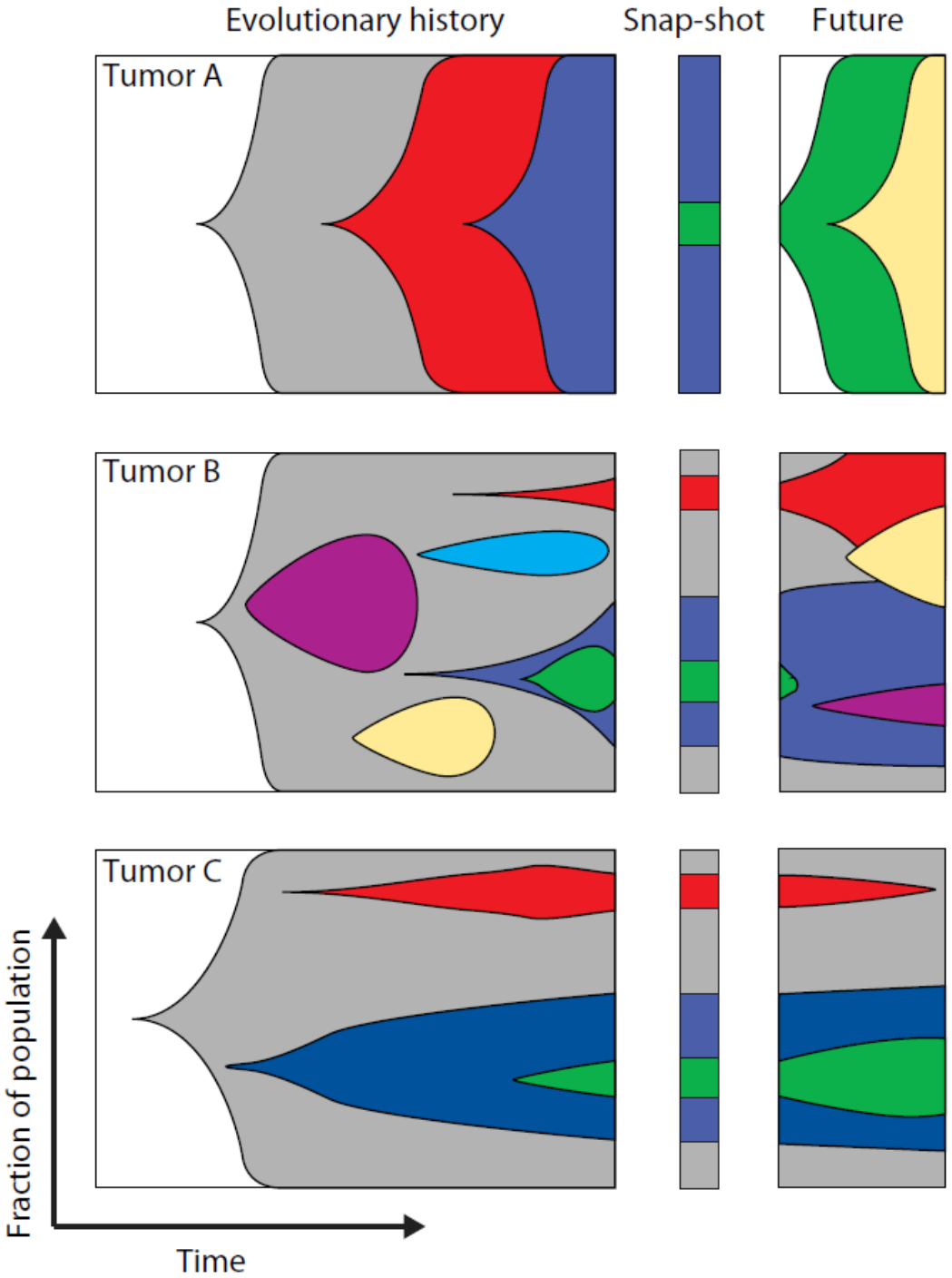
IntClust 2

- ER positive, poor prognosis
- 11q13/14 *cis*-acting tumours
- CCND1 (11q13.3), EMSY (11q113.5), PAK1 (11q14.1), RSF1 (11q14.1)
- 11q13/14 amplicon(s)

Intra-tumour genetic heterogeneity

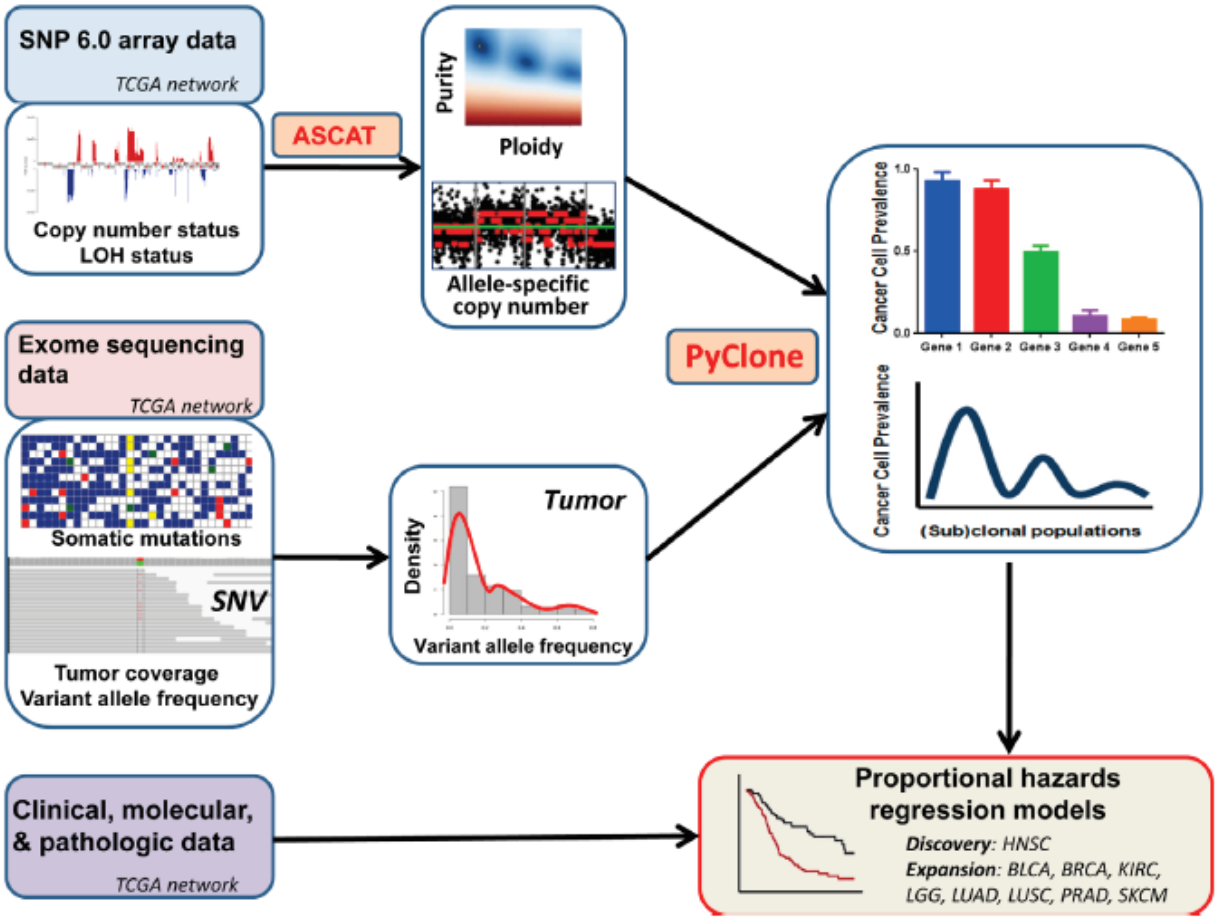
- Tumours are composed of tumours cells
  - Diverse phenotype
  - Distinct genetic aberrations





Heterogeneity

Analysis of intratumor heterogeneity



Oncotarget, 2016 Vol. 7, No. 9, 10051