Molecular Pathology of Breast Cancer

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Biology of breast cancer

- Breast cancer is not a single disease but a collection of diseases with different molecular characteristics and clinical outcomes

Why do we need molecular characterisation of breast cancer

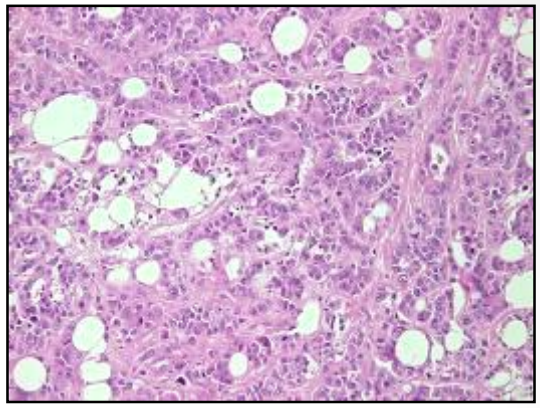
- To identify patients whose prognosis is so good that adjuvant therapy after local surgery would not be beneficial
- To identify patients whose prognosis is so poor that a more aggressive adjuvant approach would be warranted
- To identify patients likely to be responsive or resistant to particular forms of therapy (= predictive factors)
- => Individualised patient management

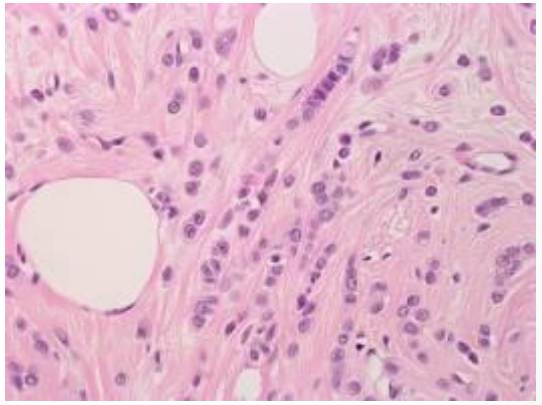
Traditional classification of breast cancer

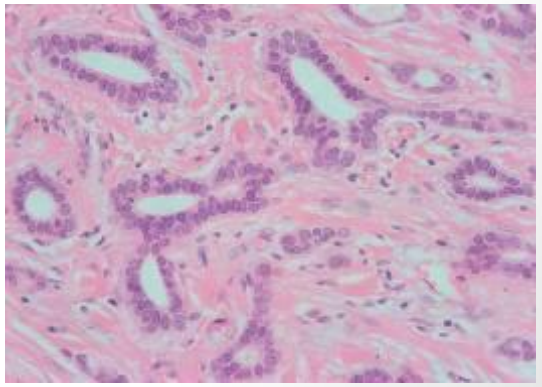
- Assessment of the extent to which the appearance of a carcinoma resembles normal breast glandular tissue
- Tumour type
- Histological Grade

Traditional classification of breast cancer

- Histological tumour type
- Invasive carcinoma NST/ ductal
- Special types:
  - Invasive lobular carcinoma
  - Invasive tubular carcinoma
  - Invasive mucinous carcinoma
  - Medullary-like carcinoma
  - Metaplastic carcinoma
  - Salivary type triple negative tumours

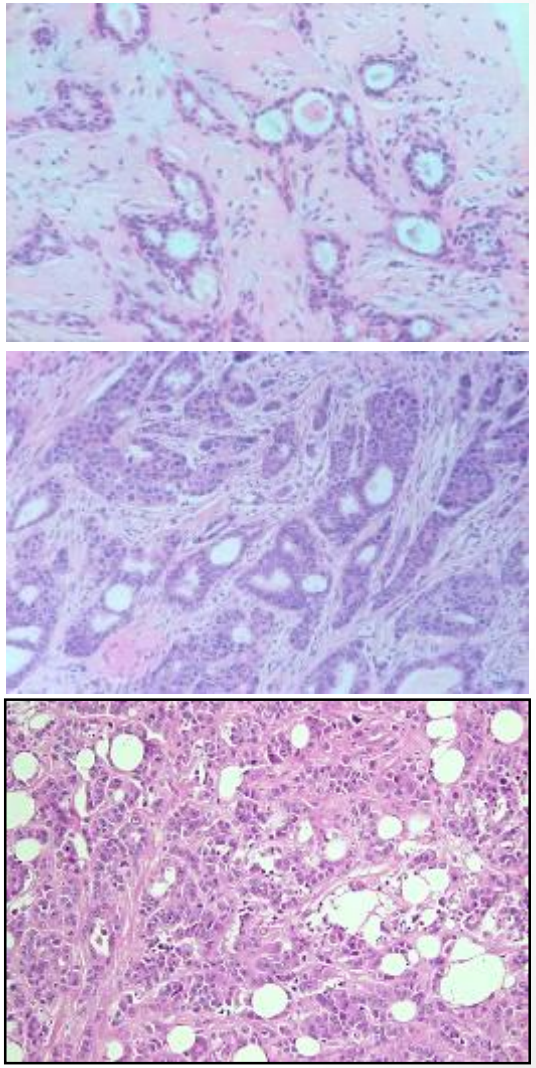




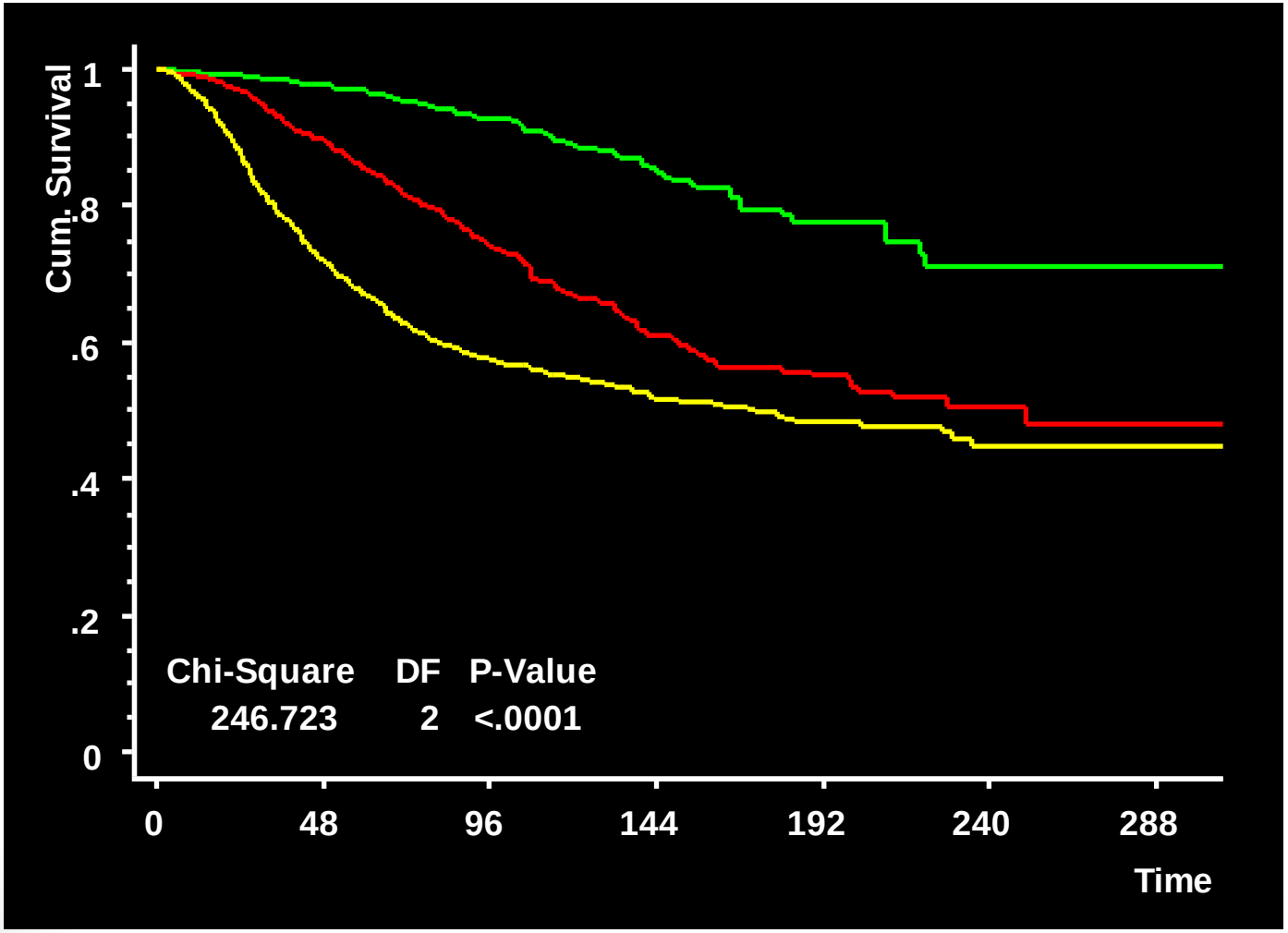


Traditional classification of breast cancer

- Histological grade
- Assess 3 variables:
  - Tubule formation (1-3)
  - Nuclear pleomorphism (1-3)
  - Mitotic count (1-3)
- Overall grade
  - Low grade (score 3-5)
  - Intermediate grade (score 6-7)
  - High grade (score 8-9)

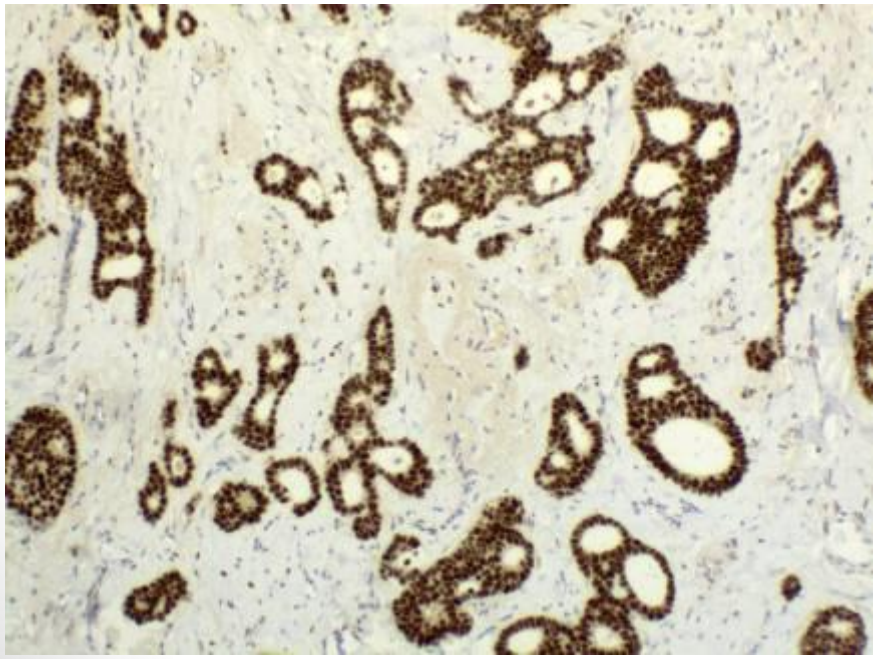


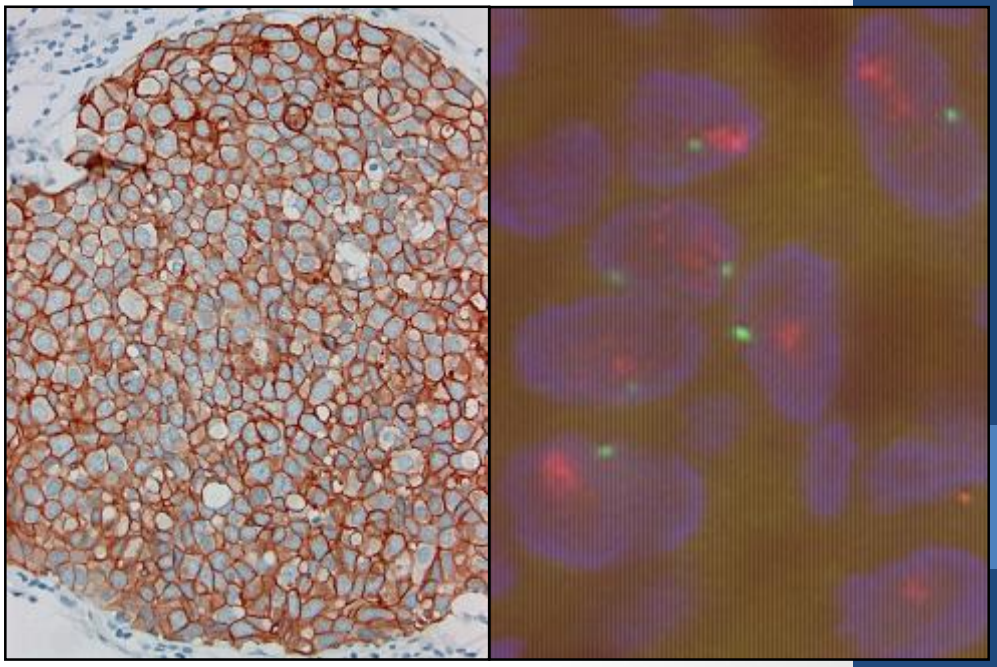
Traditional classification of breast cancer



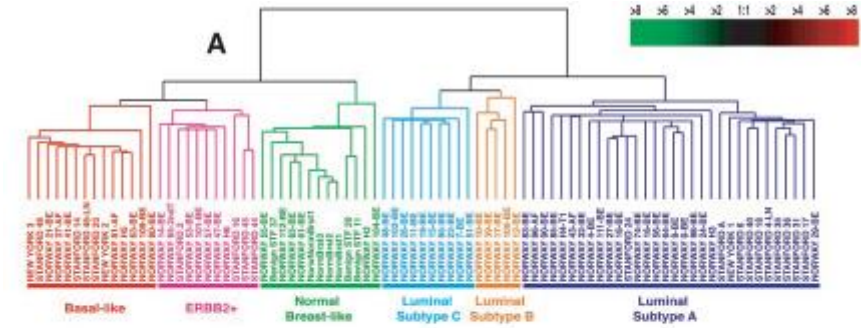
Hormone receptors and HER2

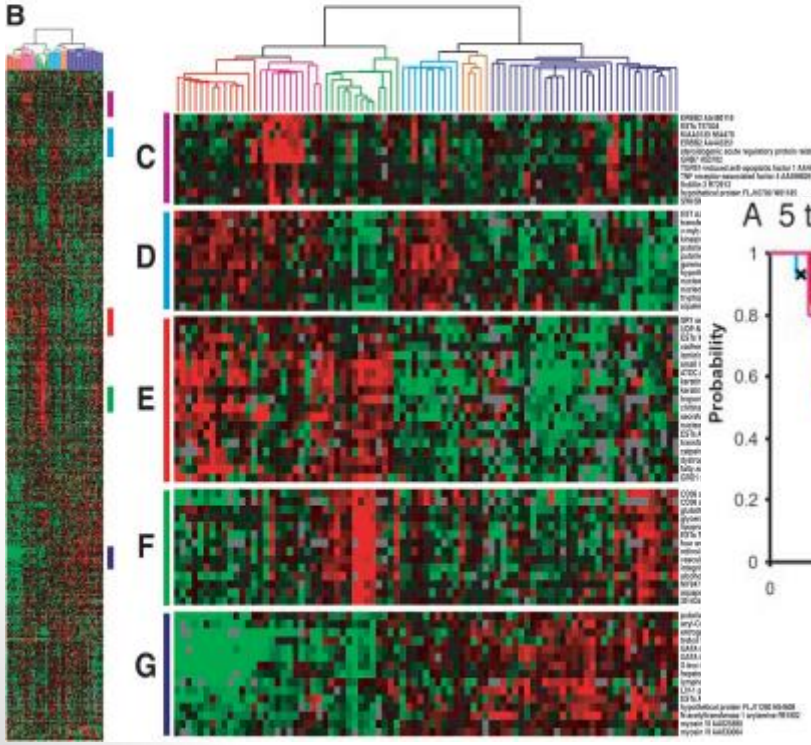
- Oestrogen receptor
- (Progesterone receptor)
- HER2



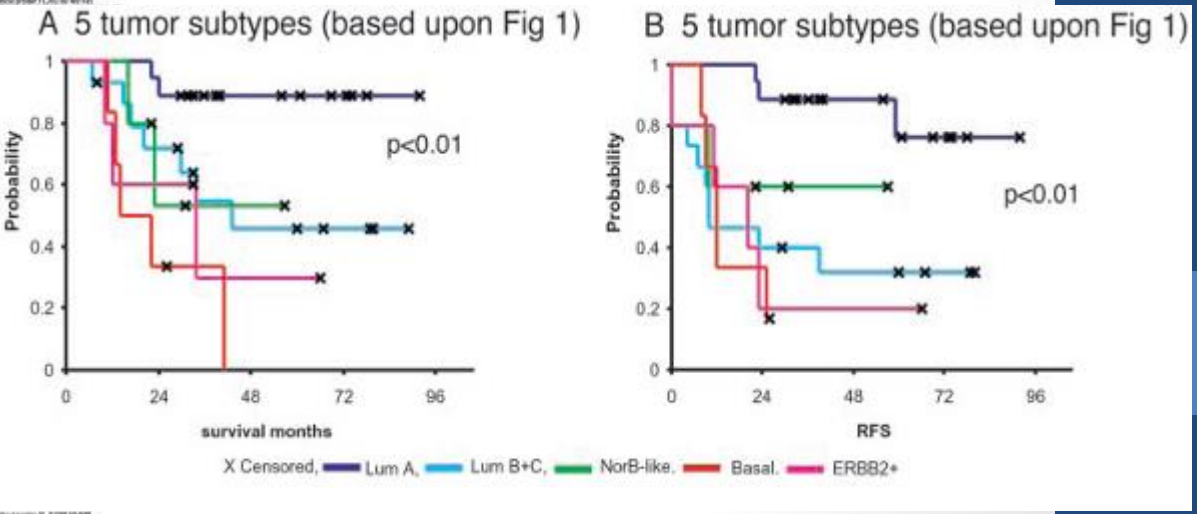


Newer classification systems for invasive breast cancer – Intrinsic Subtypes





- Gene expression patterns of breast carcinomas distinguish tumour subclasses with clinical implications
- 'Intrinsic subtypes'
- Sørlie, Perou et al.
- PNAS, 2001; 98(19):10869–74



Newer classification systems for invasive breast cancer – Intrinsic Subtypes

- Intrinsic subtypes are reproducible across platforms, however assignment of individual cancers to a molecular subtype shows only moderate reproducibility
- Dependent upon platform used, expression thresholds, and composition of the population
- Basal-like group most reproducible, luminal B and HER2 enriched least reproducible

Newer classification systems for invasive breast cancer – Intrinsic Subtypes

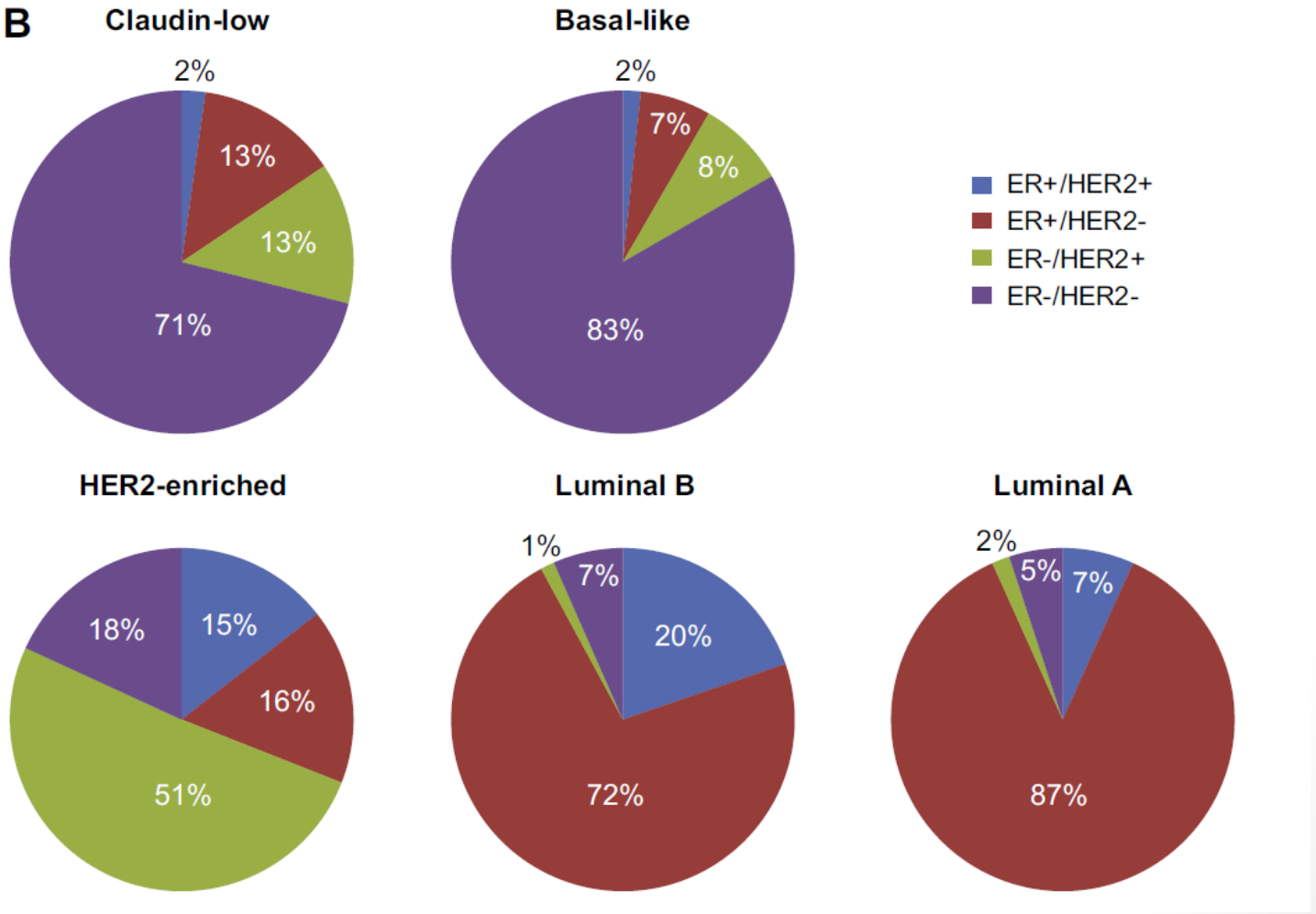
- Immunohistochemical correlates
- **ER positive - Luminal breast cancers**
- type A – PgR +, HER2 -, low proliferation
- type B – PgR +/-, HER2 +/-, high proliferation

- **ER negative**
- HER2 positive
- Basal breast cancers – ER/PgR/HER2 -, CK5/ CK14 / EGFR +

Newer classification systems for invasive breast cancer – Intrinsic Subtypes

- Concordance between gene expression and IHC defined subtypes modest at best
- Luminal A versus Luminal B – Ki67 cut point of 14% [Cheang et al., JNCI 2009]. Sensitivity 72%, specificity 77%
- Follow up study looking at 2 large clinical series [Prat et al., JCO 2013]:
- 81-85% of luminal A correctly identified
- 35-52% of luminal B misclassified as Luminal A
- Improvement if include PR with cut off of 20%

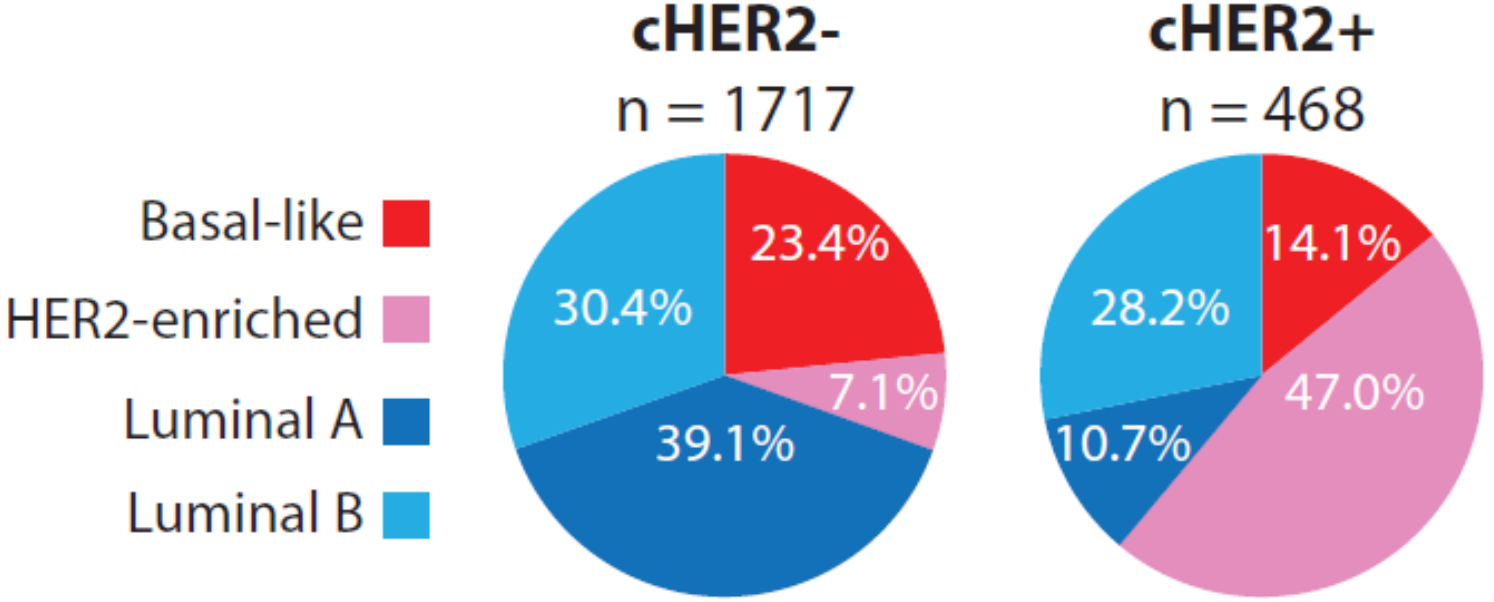
Newer classification systems for invasive breast cancer – Intrinsic Subtypes



Prat A and Perou C, Mol Oncol 2011

Newer classification systems for invasive breast cancer – Intrinsic Subtypes

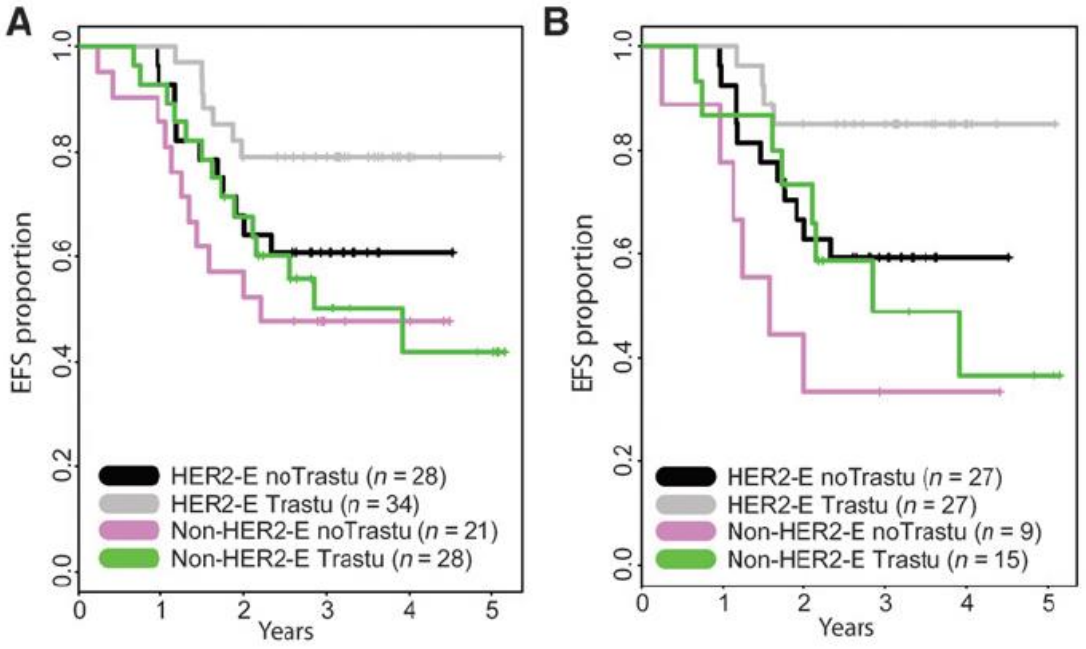
- Heterogeneity within HER2 positive disease, largely driven by ER status
- Clinically HER2 + and – tumours within each intrinsic subtype differ only in expression of genes in or near the HER2 amplicon on 17q
- Highest levels of HER2 pathway activation in cHER2+ HER2 enriched tumours



Prat et al., JNCI 2014;106(8).

Newer classification systems for invasive breast cancer – Intrinsic Subtypes

- Retrospective analysis of NOAH study looking at PAM50 subtypes
- Only 55% of HER2+ tumours HER2-E subtype; 21% luminal, 7% basal-like, 18% normal-like
- Better pCR rates in HER2-E vs luminal HER2+ tumours (53% v 29%) with larger improvement in EFS with addition of Trastuzumab



Prat et al., Clin Cancer Res 2014;20(2):511-21.

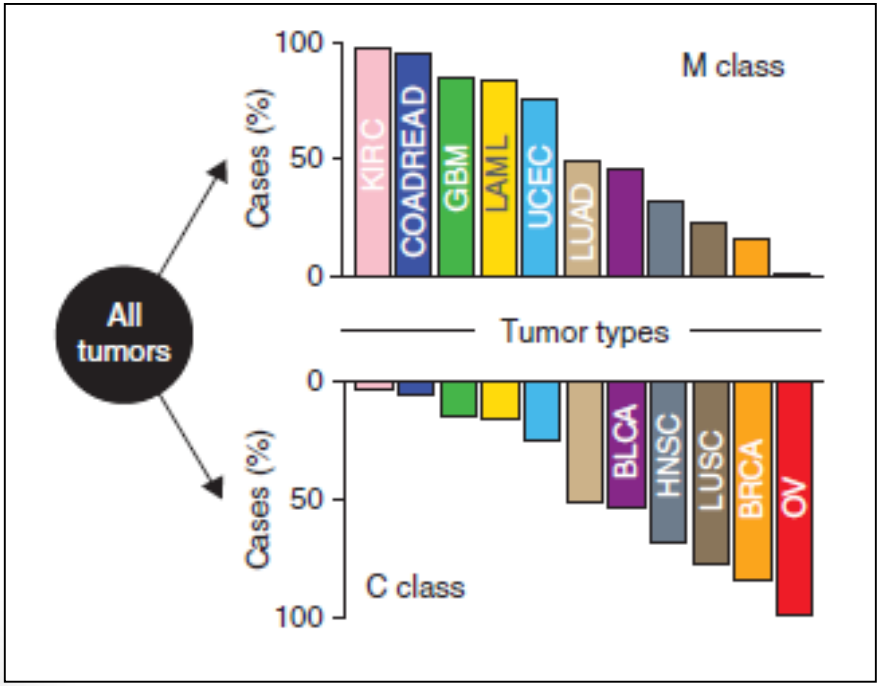
ANALYSIS

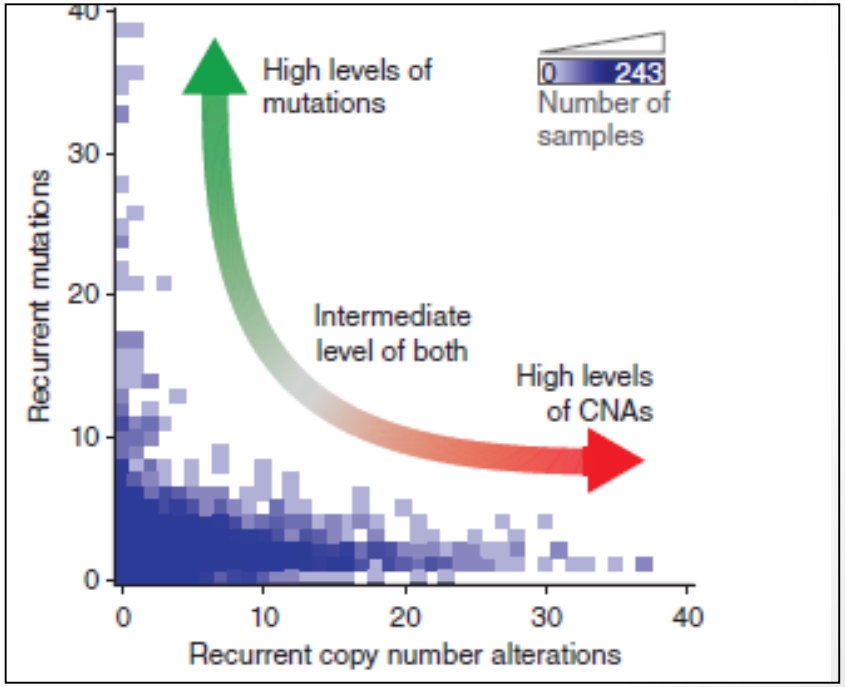
nature genetics

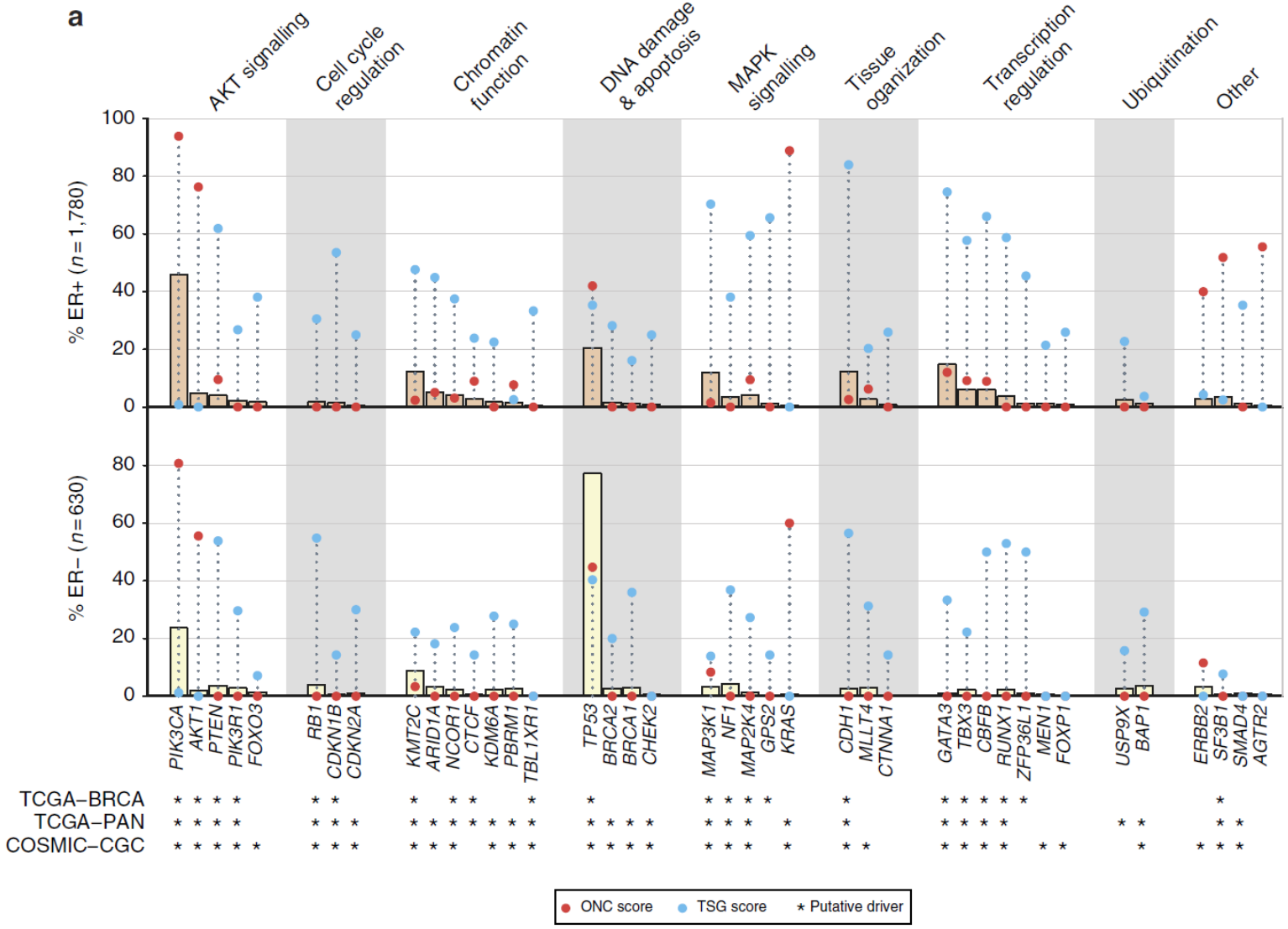
OPEN

Emerging landscape of oncogenic signatures across human cancers

Giovanni Ciriello, Martin L Miller, Bülent Arman Aksoy, Yasin Senbabaoglu, Nikolaus Schultz & Chris Sander

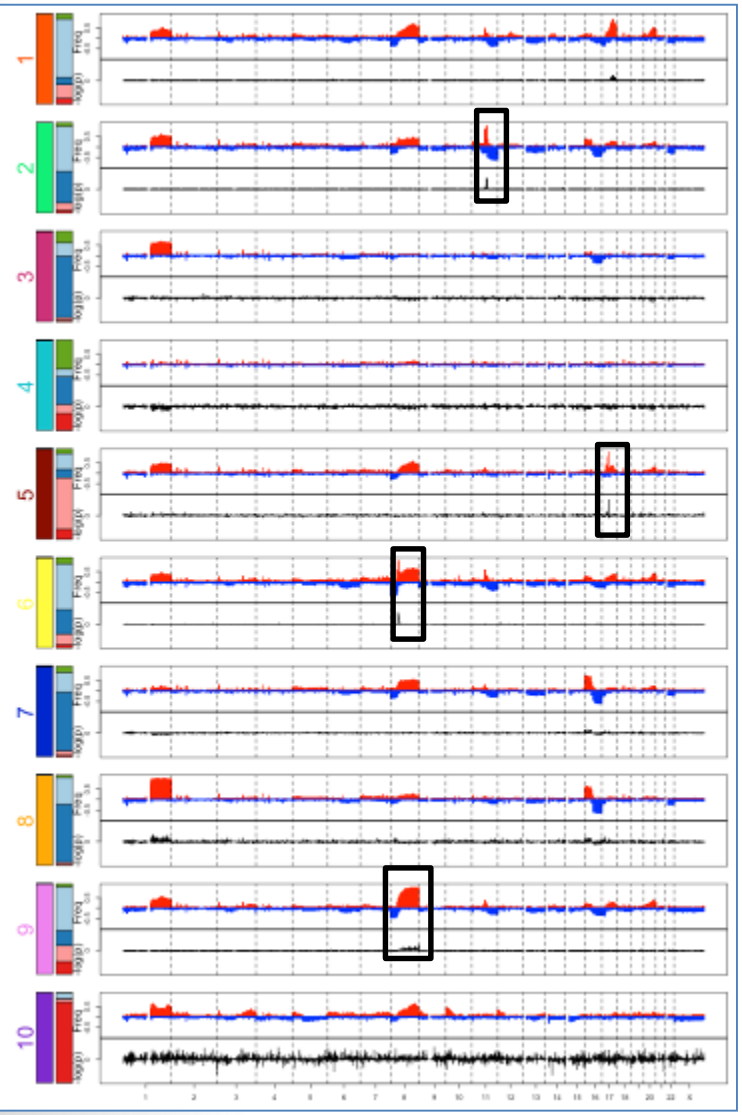






Pereira et al., Nature Comms 2016.

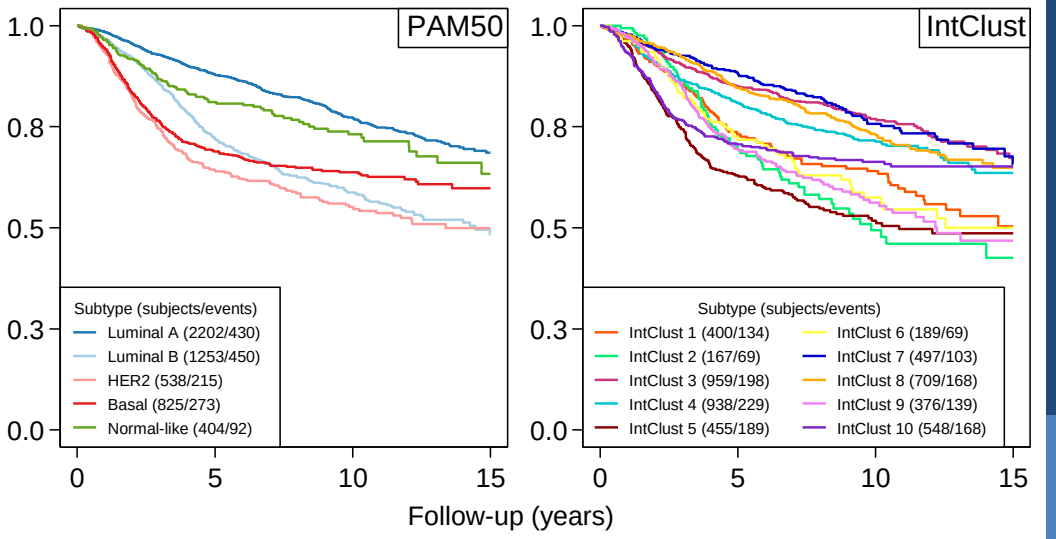
A new molecular taxonomy of breast cancer



ARTICLE

doi:10.1038/nature10983

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

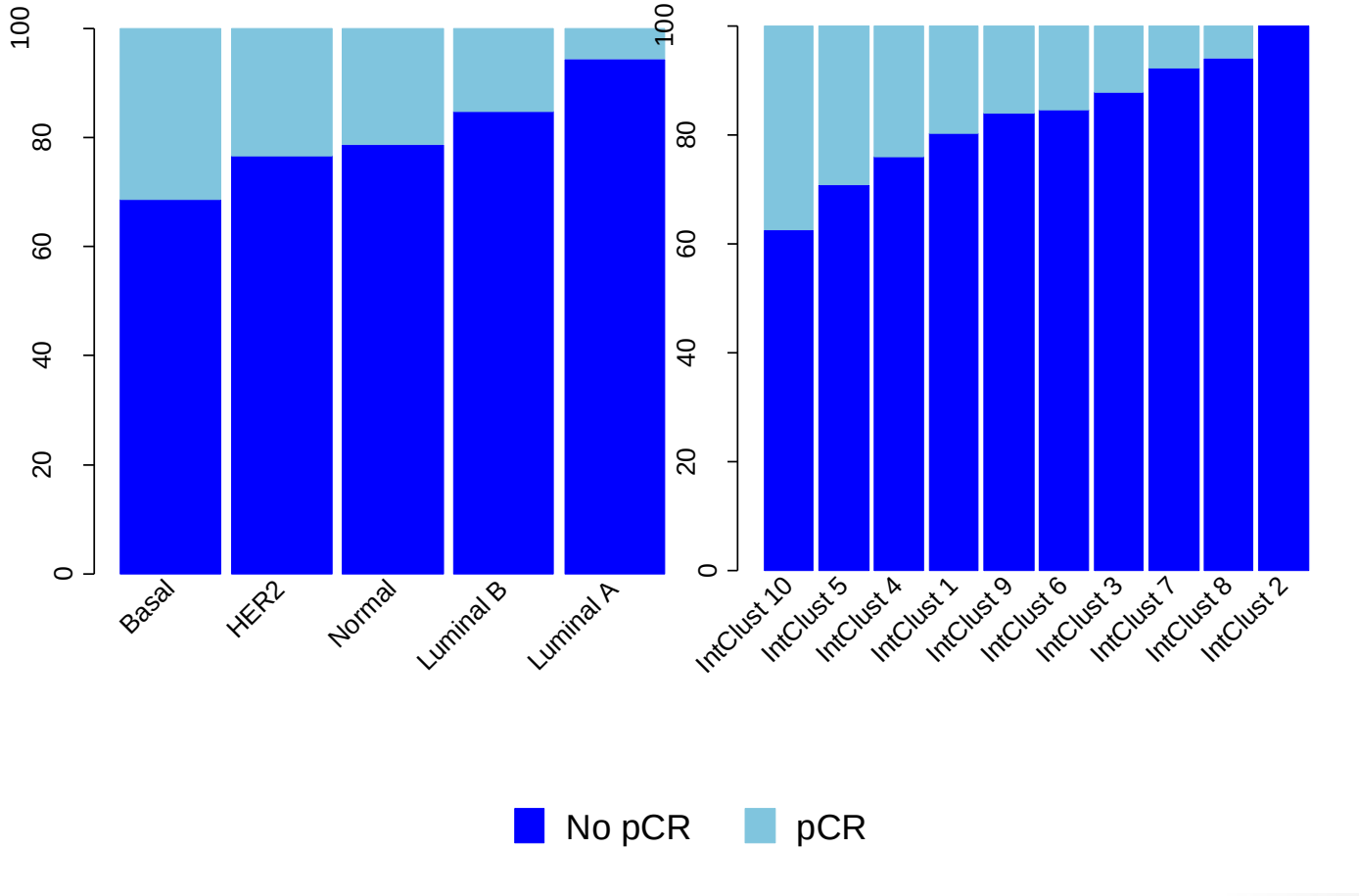


Different therapeutic targets

IntClust	Frequency (*n*, %)	Defining molecular features	Expression (*n*, %)	PAM50 (*n*, %)	Clinical features	Prognosis (5-year, 10-year DSS)	Genomic instability	
1	139 (7%)	17q23 amplification	ER +: 123 (88.49%) PR +: 60 (43.17%) HER2 +: 20 (14.39%)	Basal: 9 (6.47%) HER2: 21 (15.11%) LumA: 11 (7.91%) LumB: 90 (64.75%) Normal: 8 (5.76%)	High grade	Intermediate 0.80, 0.69	High	RPS6KB1; PPM1D
2	72 (4%)	11q13/14 amplification	ER +: 69 (95.83%) PR +: 51 (70.83%) HER2 +: 3 (4.17%)	Basal: 2 (2.78%) HER2: 6 (8.33%) LumA: 25 (34.72%) LumB: 36 (50%) Normal: 3 (4.17%)	No distinct clinical features	Poor 0.78, 0.51	High	CCND1; PAK1
3	290 (15%)	Paucity of copy number changes	ER +: 278 (95.86%) PR +: 211 (72.76%) HER2 +: 1 (0.34%)	Basal: 4 (1.39%) HER2: 9 (3.14%) LumA: 195 (67.94%) LumB: 43 (14.98%) Normal: 36 (12.54%)	Low grade Low LN+	Good 0.93, 0.88	Low	PIK3CA
4	343 (17%)	CNA devoid	ER +: 238 (69.39%) PR +: 155 (45.19%) HER2 +: 20 (5.83%)	Basal: 64 (18.71%) HER2: 34 (9.94%) LumA: 106 (30.99%) LumB: 29 (8.48%) Normal: 109 (31.87%)	Low grade	Good 0.89, 0.76	Low	Immune response
5	190 (10%)	ERBB2 amplification	ER +: 79 (41.58%) PR +: 40 (21.05%) HER2 +: 181 (95.26%)	Basal: 21 (11.05%) HER2: 108 (56.84%) LumA: 18 (9.47%) LumB: 33 (17.37%) Normal: 10 (5.26%)	Younger age at diagnosis High grade High LN +	Poor 0.62, 0.45	Intermediate	HER2
6	85 (4%)	8p12 amplification	ER +: 85 (100%) PR +: 36 (45.88%) HER2 +: 3 (3.53%)	Basal: 3 (3.53%) HER2: 10 (11.76%) LumA: 23 (27.06%) LumB: 43 (50.59%) Normal: 6 (7.06%)	No distinct clinical features	Intermediate 0.83, 0.59	High	HDACs
7	190 (10%)	16p gain, 16q loss, 8q amplification	ER +: 187 (98.42%) PR +: 150 (78.95%) HER2 +: 2 (1.05%)	Basal: 3 (1.59%) HER2: 9 (4.76%) LumA: 123 (65.08%) LumB: 41 (21.69%) Normal: 13 (6.88%)	Older age at diagnosis Low grade	Good 0.94, 0.81	Intermediate	
8	299 (15%)	1q gain, 16q loss	ER +: 297 (99.3%) PR +: 236 (78.93%) HER2 +: 1 (0.33%)	Basal: 1 (0.33%) HER2: 9 (3.01%) LumA: 192 (64.21%) LumB: 89 (29.77%) Normal: 8 (2.68%)	Older age at diagnosis Low grade	Good 0.88, 0.78	Intermediate	
9	146 (7%)	8q gain, 20q amplification	ER +: 125 (85.62%) PR +: 79 (54.11%) HER2 +: 10 (6.85%)	Basal: 20 (13.79%) HER2: 26 (17.93%) LumA: 24 (16.55%) LumB: 70 (48.28%) Normal: 5 (3.45%)	High grade	Intermediate 0.78, 0.62	High	TP53
10	226 (11%)	5q loss, 8q gain, 10p gain, 12p gain	ER +: 25 (11.06%) PR +: 19 (8.41%) HER2 +: 6 (2.65%)	Basal: 202 (89.38%) HER2: 8 (3.54%) LumA: 1 (0.44%) LumB: 14 (6.19%) Normal: 1 (0.44%)	Younger age at diagnosis High grade Large tumours	Poor 0.71, 0.68	Intermediate	Mitotic regulators; BRCA1

Dawson et al, EMBO J 2013

Different clinical behaviour

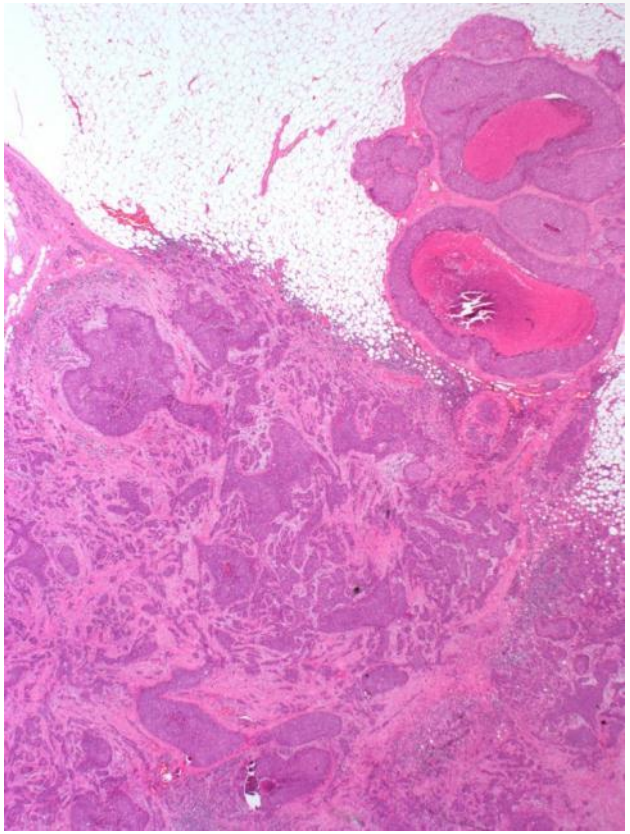


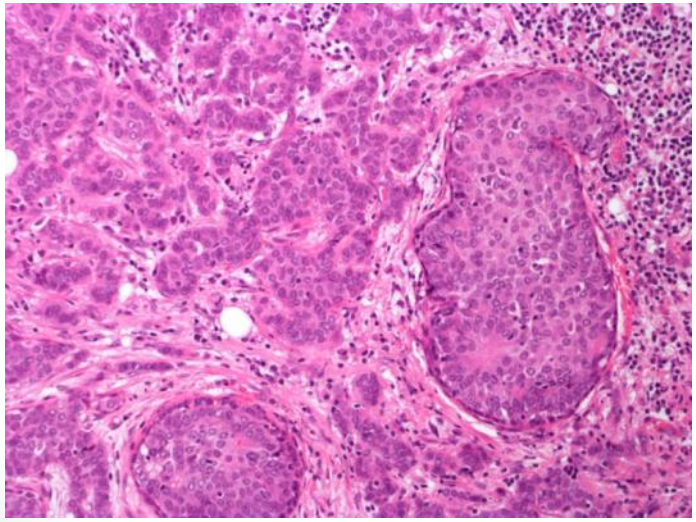
Case 1

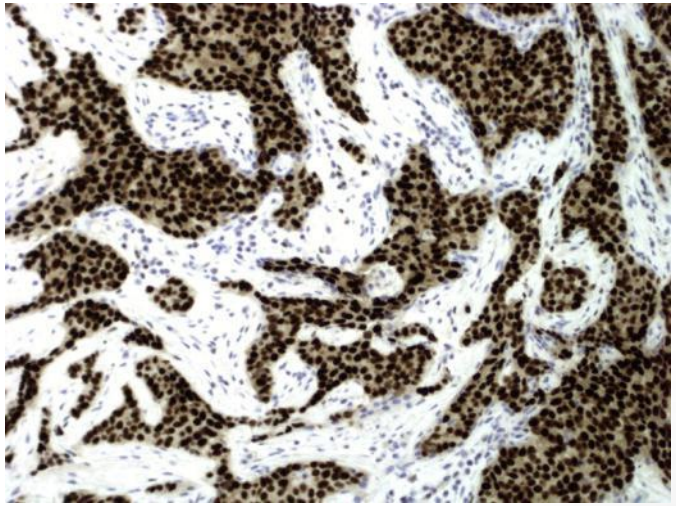
- 39 year old female presenting with a lump and tenderness in the left breast
- On clinical examination there was a discrete lump palpable in the left breast
- Core biopsy – Invasive carcinoma NST, grade 2, ER/ PR positive, HER2 negative
- Clinical management - Mastectomy and immediate reconstruction with Sentinel Lymph Node Biopsy

Final Histology:

- Left: Multifocal grade 2 invasive carcinoma NST, largest focus 30 mm
- Background DCIS, total lesion size 80 mm
- ER/ PR 8, HER2 negative
- SLN – 0/1



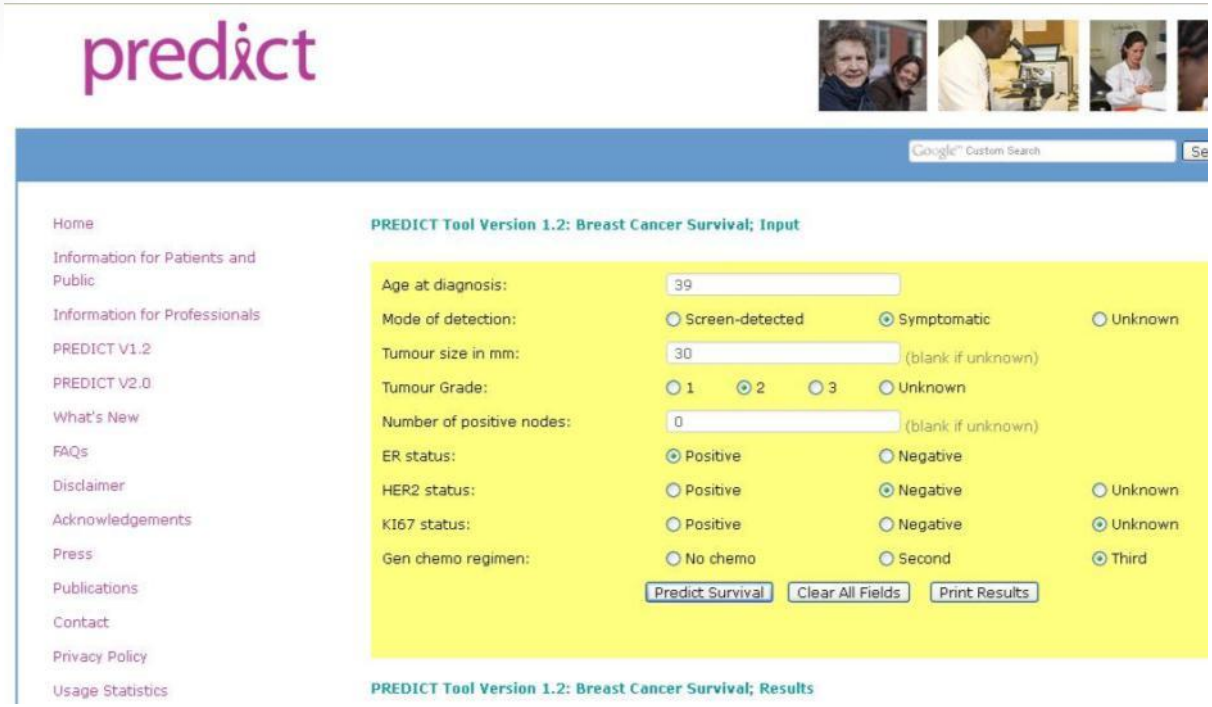


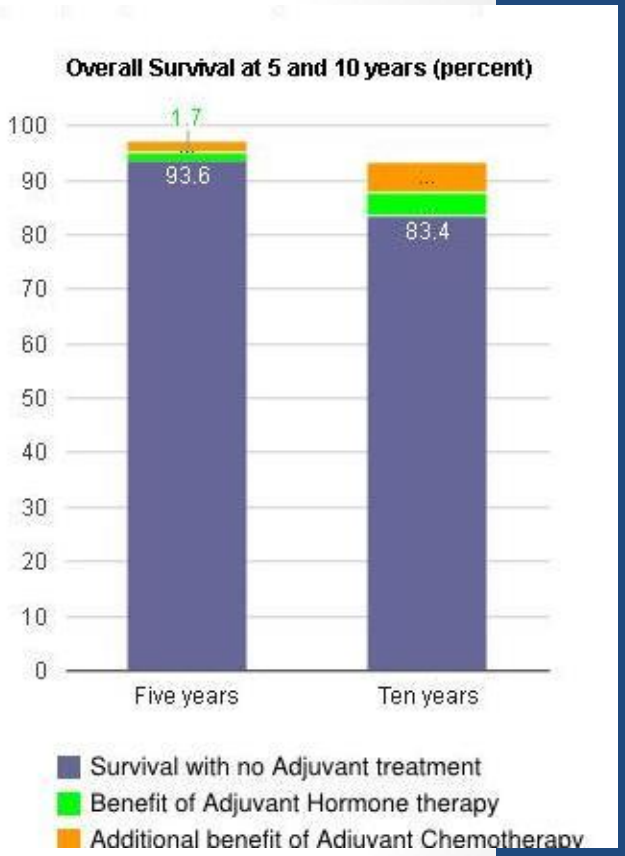


Clinical management

- Chest wall radiotherapy
- Systemic adjuvant therapy
- Endocrine Rx alone versus endocrine Rx + chemotherapy

PREDICT





- PREDICT – 3.1% benefit chemotherapy
  -> discuss

Gene based prognostic tests

- Gene expression profiling
- cDNA arrray or RT-PCR based
- Several gene signatures have been proposed
- 21 gene – Oncotype Dx® - TAILORx
- 70 gene – Mammaprint® - MINDACT
- PAM50 – uses intrinsic subtypes
- 12 gene - EndoPredict
- Little overlap in specific genes that make up the signatures, but all include genes involved in proliferation and ER signalling

Oncotype DX

HER2 GROUP
HER2 GRB7

ER GROUP
ER PgR
Bcl2 SCUBE2

INVASION GROUP
Cathepsin L2
Stromelysin 3

REF GROUP
Beta-actin GAPDH
RPLPO GUS
TFRC

PROLIFERATION GROUP
KI67
STK-15
SURVIVIN
CYCLIN B1
MYBL2

Combine results in an algorithm to get the recurrence score:

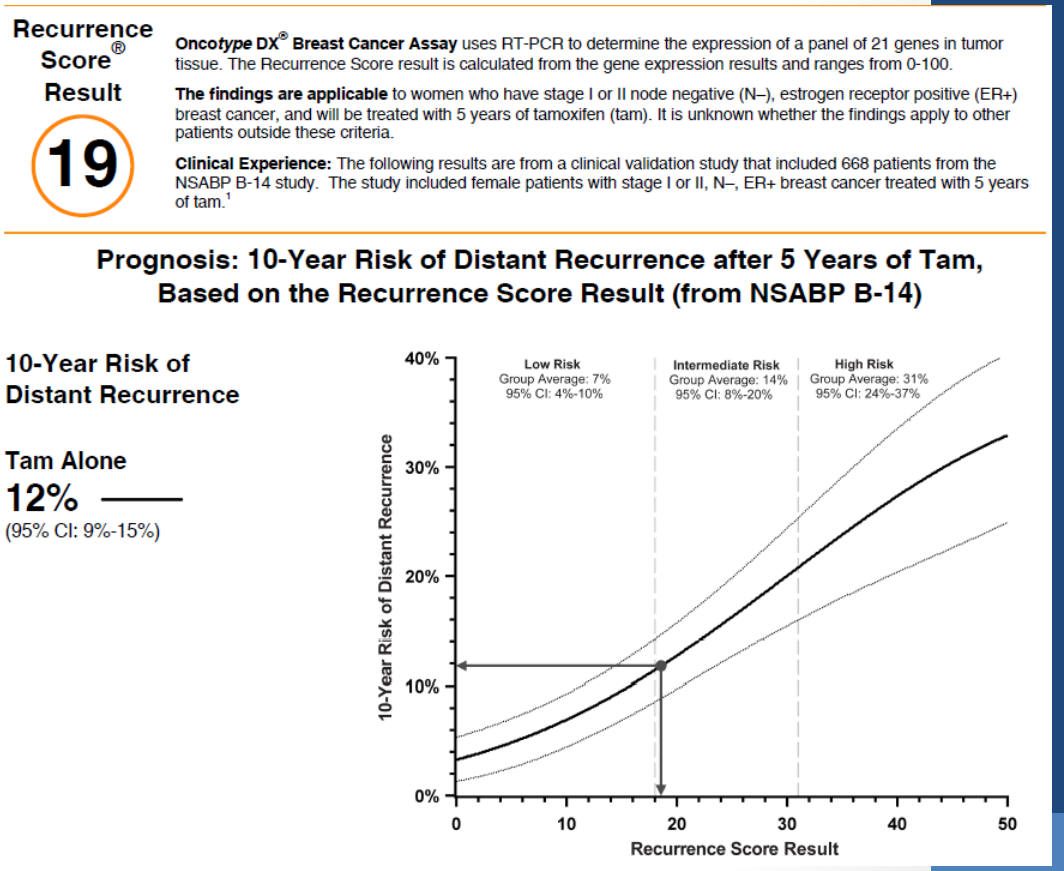
<18	6.8%	
18-30	14.3%	10yr distant recurrence rate
>30	30.5%	

Clinical management

- ONCOTYPE DX RESULT

  Recurrence score = 19 (Intermediate risk). This equates to an estimated 10 year risk of distant recurrence of 12% on Tamoxifen alone.

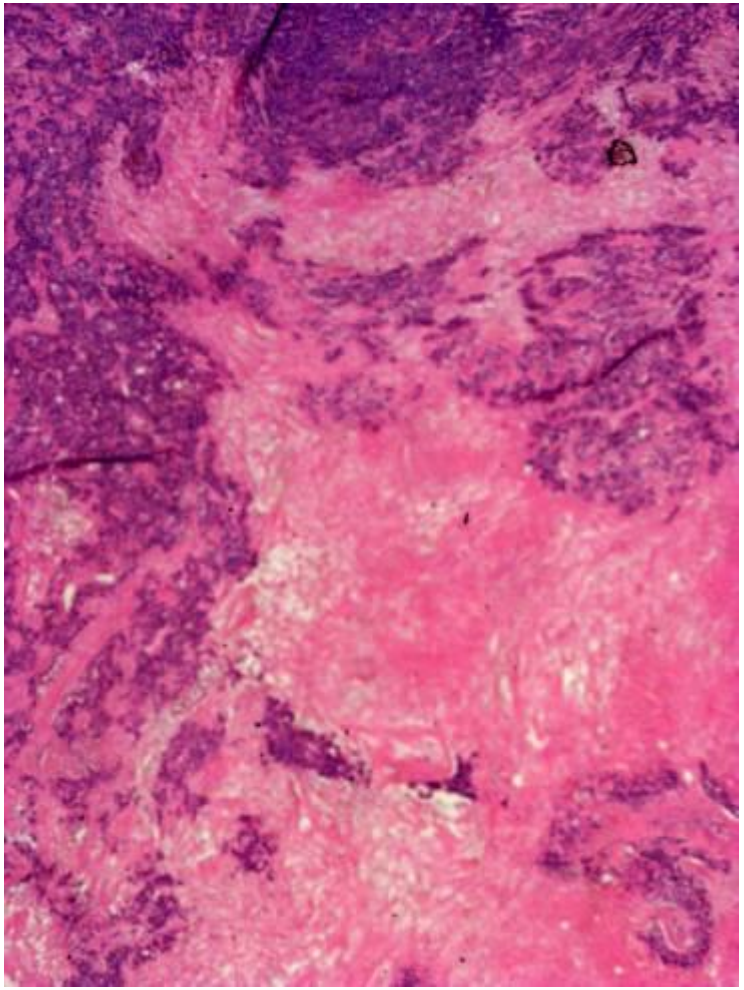
- Decision for extended endocrine therapy alone (no chemotherapy)

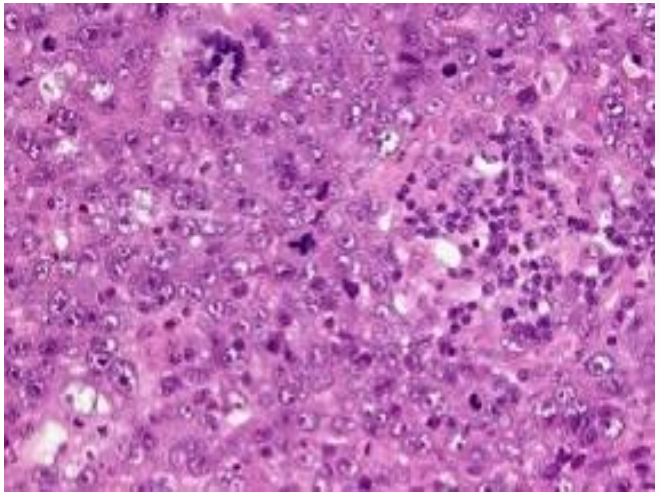


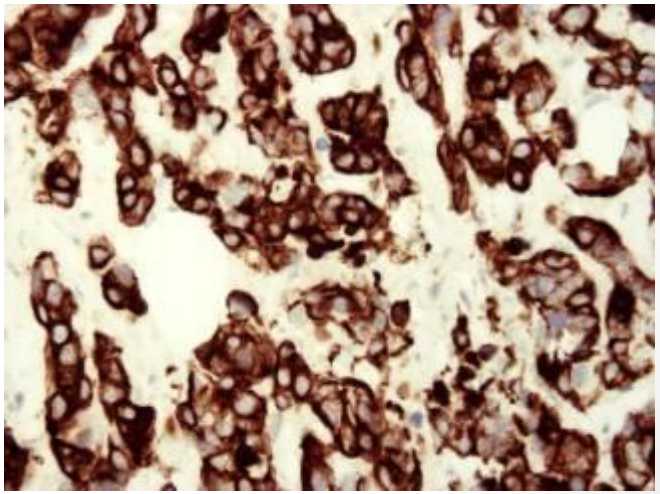
Basal Breast Cancers

- ‘Triple negative’ – ER, PgR and HER2 negative
- Express basal cytokeratins – CK 5/6, CK14
- Express EGFR
- Distinct morphology – high grade, central acellular zones, necrosis, high mitotic count
- Heterogeneous group – medullary-like, metaplastic, adenoid cystic carcinoma
- Associated with BRCA1 mutations in young women
- Associated with worse prognosis and distant metastasis, particularly visceral metastases

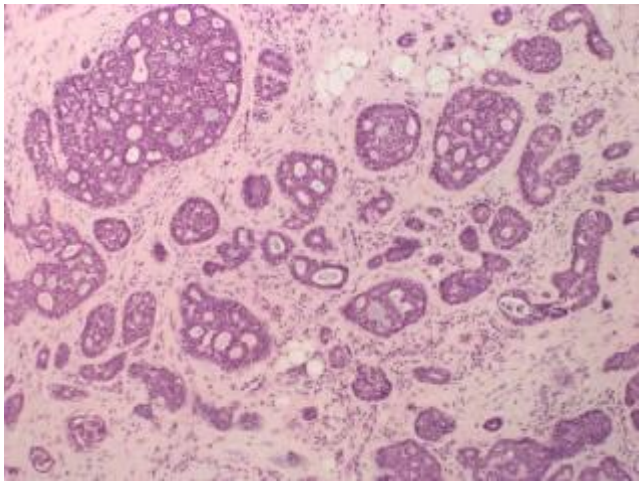
Basal Breast Cancers

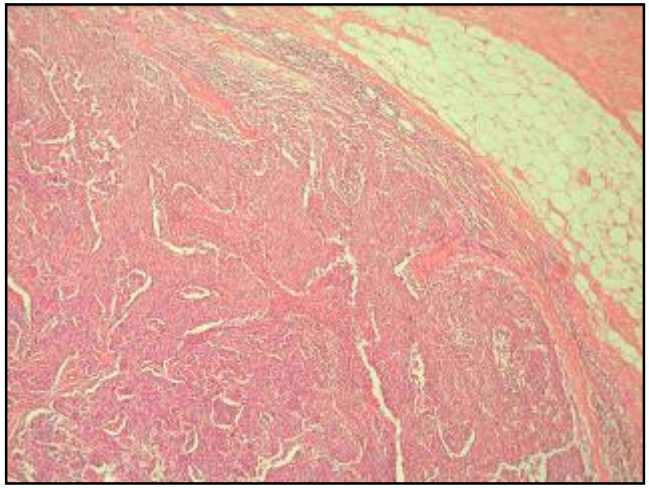


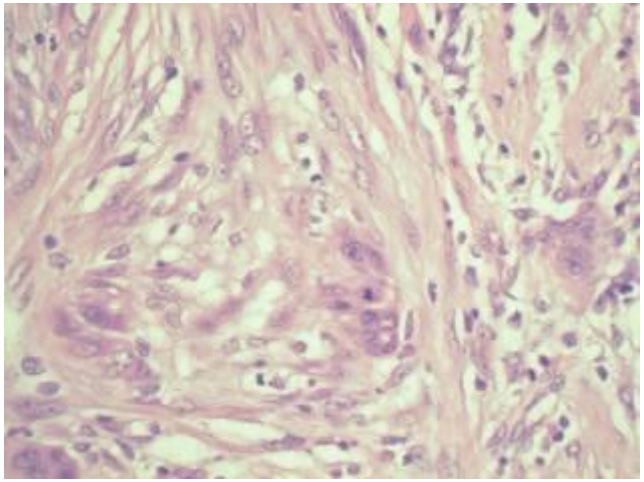


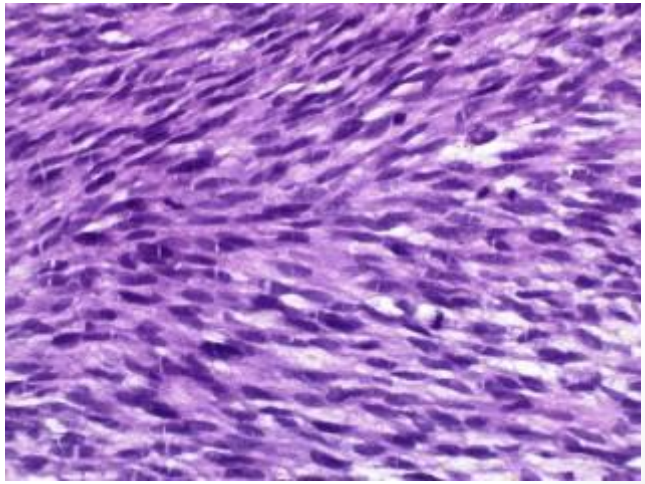


Basal Breast Cancers



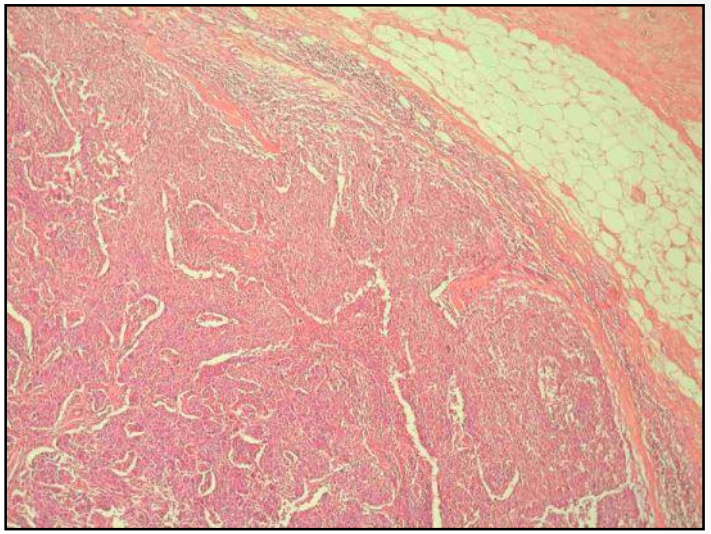


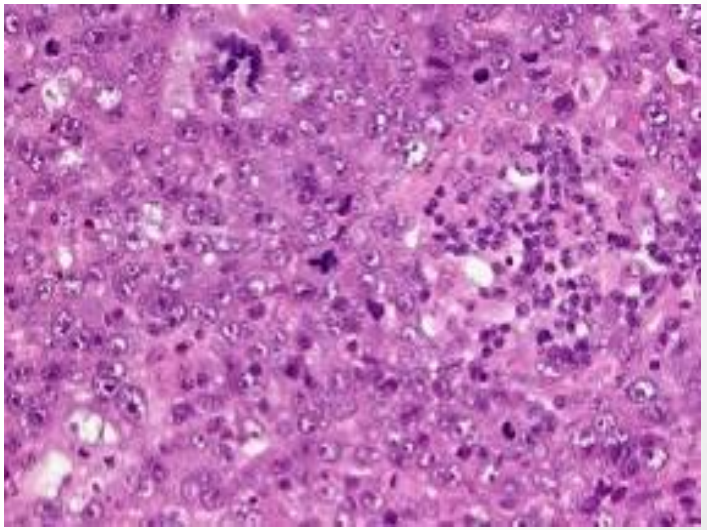




Carcinoma with medullary features

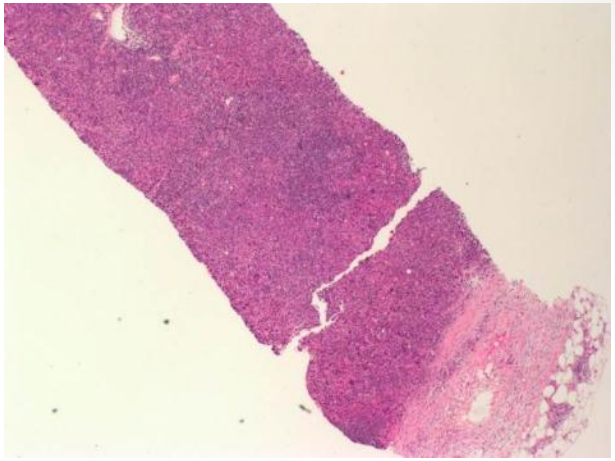
- Circumscribed tumour with pushing rather than infiltrating margin
- Interconnecting sheets of large, bizarre and pleomorphic carcinoma cells forming a syncytial network
- Prominent lymphocytic inflammatory cell infiltrate
- Usually ER/ HER2 negative
- Association with BRCA1 mutations

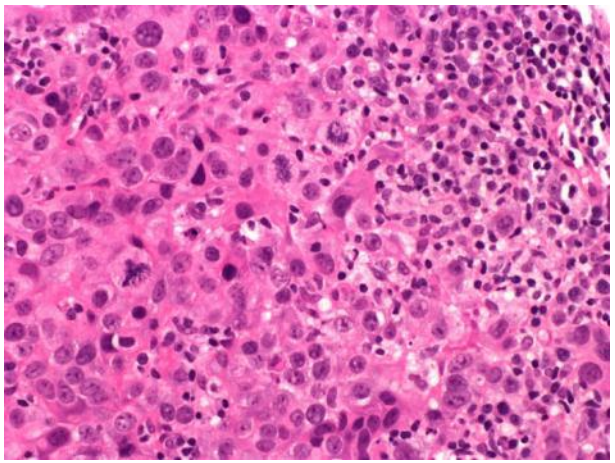


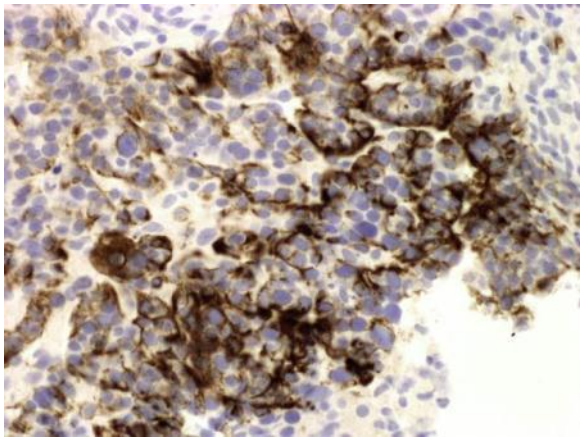


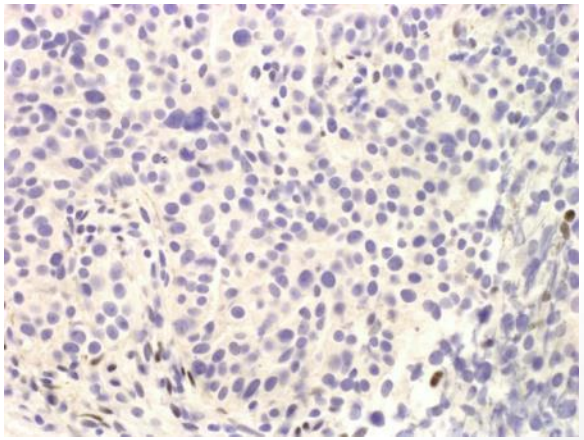
Case 2

- 35 year old female presenting with palpable masses in the right breast and right axilla
- Core biopsy: Grade 3 invasive carcinoma NST, ER/ PR/ HER2 negative
- Axillary biopsy: Metastatic carcinoma





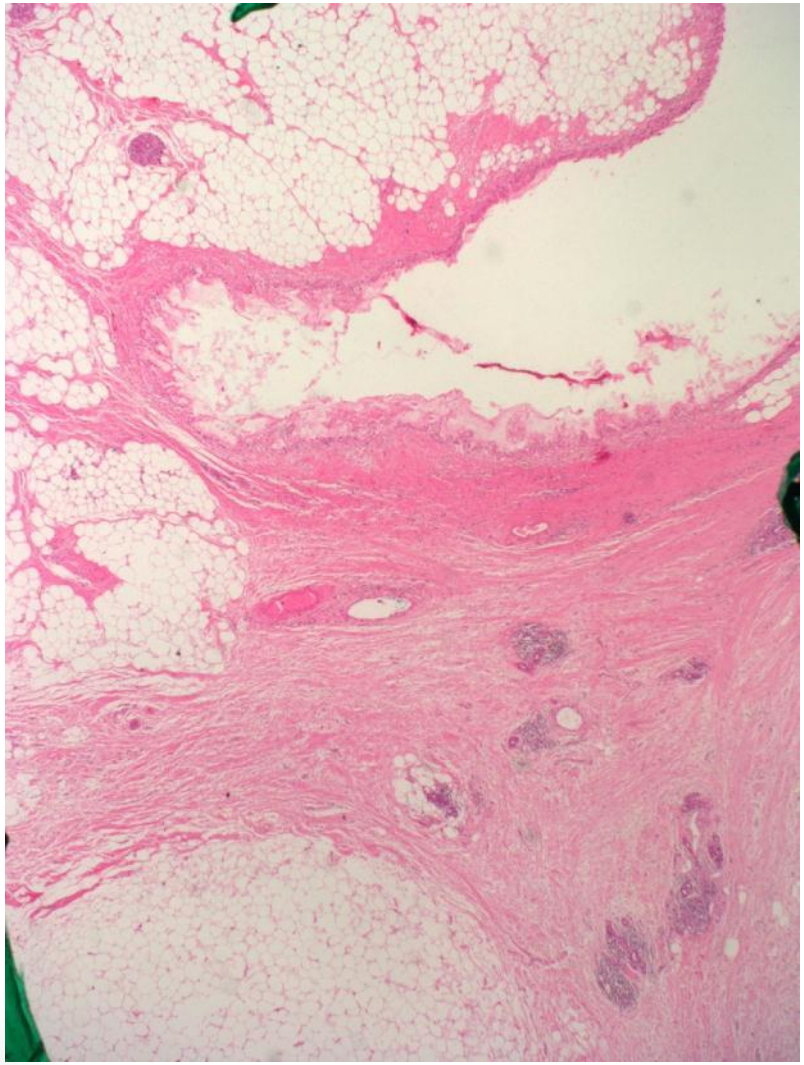


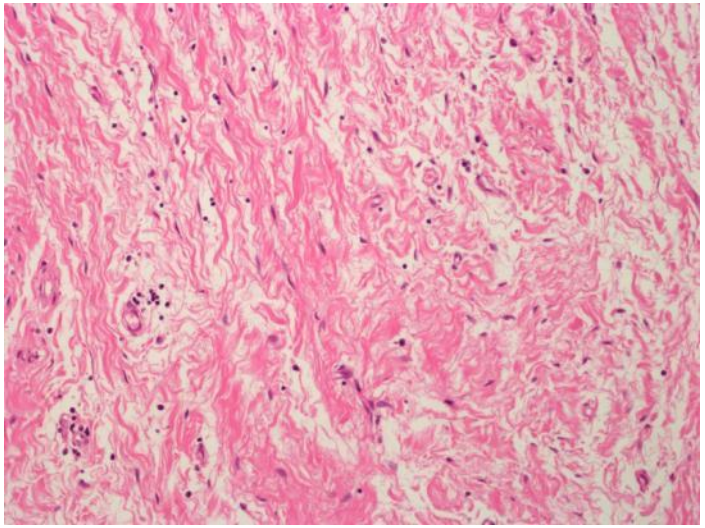


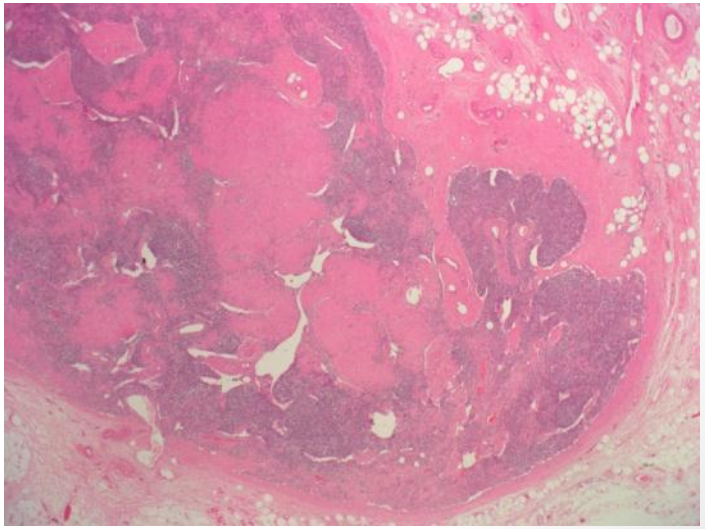
Clinical management

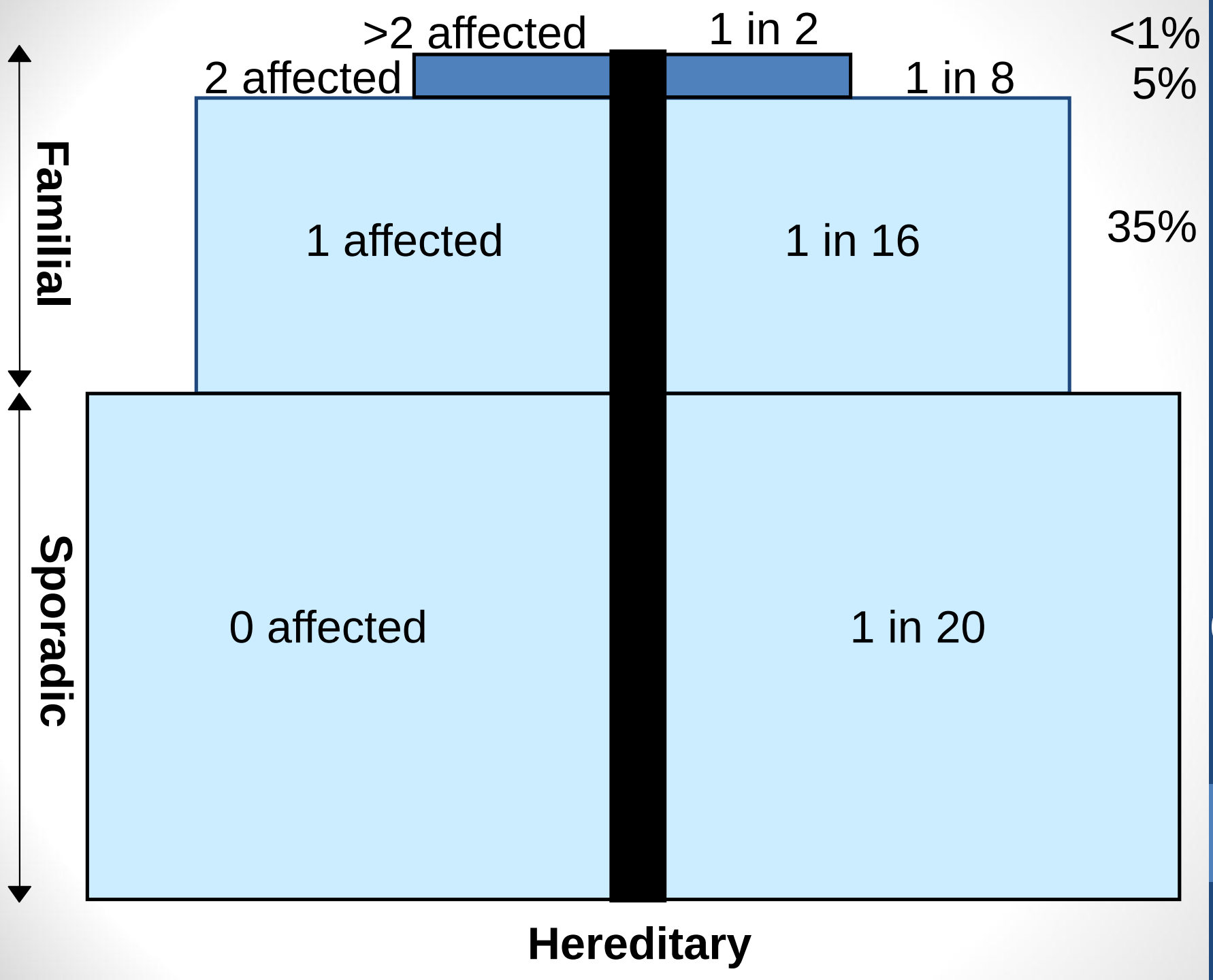
- Referral to clinical genetics – positive family history of breast cancer
- Neoadjuvant chemotherapy including carboplatin
- Right wide local excision and axillary clearance
- Final histology:
- Breast: Clip site and tumour bed identified. No residual invasive malignancy (pCR)
- Axilla: No metastatic carcinoma. Four nodes with fibrosis indicating previous involvement with regression.

Final Histology








>2 affected
1 in 2
<1%
2 affected
1 in 8
5%
Familial
1 affected
1 in 16
35%
0 affected
1 in 20
60%
Sporadic
Hereditary

BRCA1 Phenotype - Predictive Value

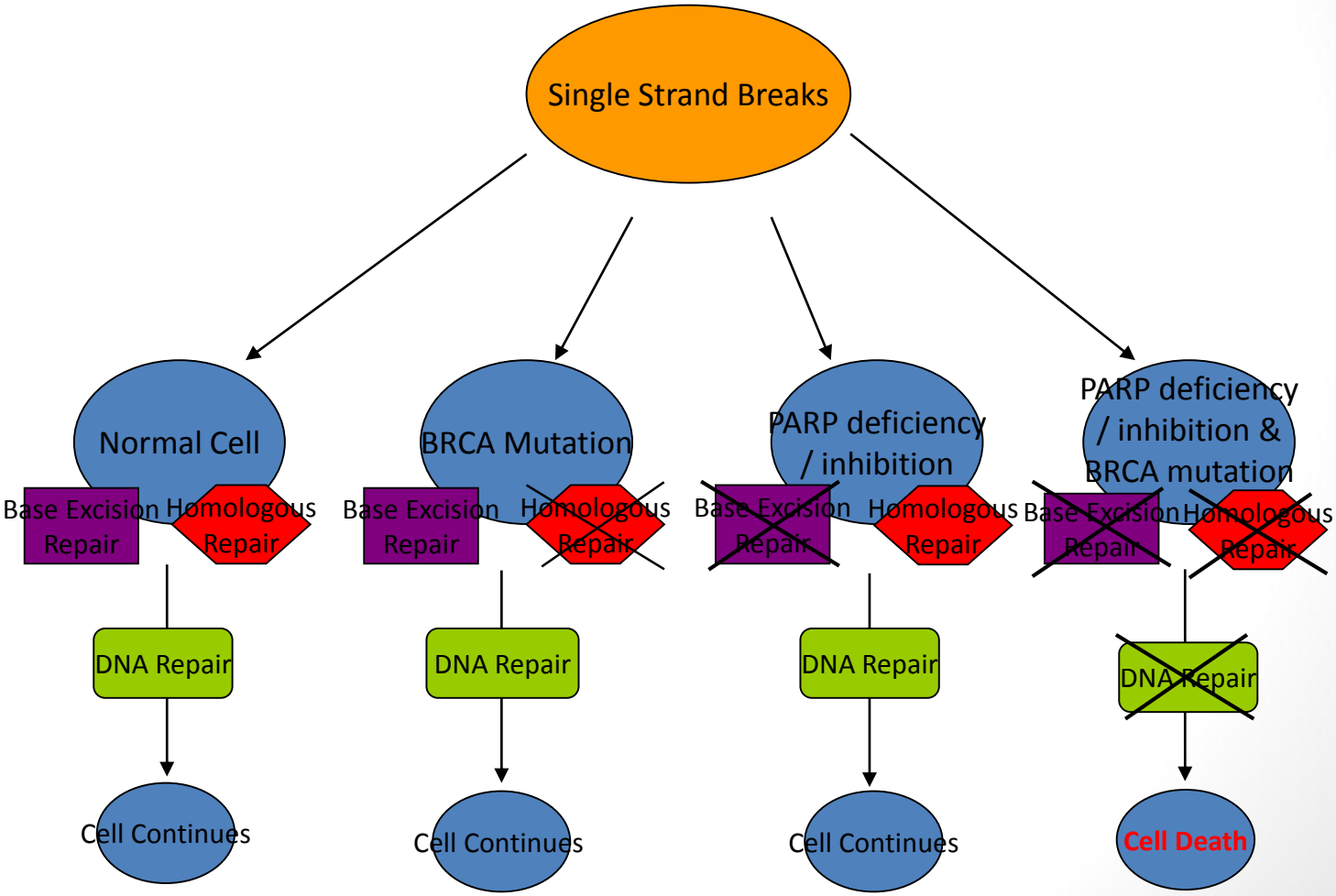
- ER negativity strongest predictor of BRCA1 mutation

- Patients <35 – 5% chance of BRCA1 mutation
- <35, ER negative and grade 3 – increases to 29%
- Add CK 5/6 positivity – 56% chance

- Phenotype better predictor of BRCA1 mutation status than family history

Personalised Medicine

- Concept of ‘synthetic lethality’



Triple negative breast cancer

- 6 triple negative subtypes
- 2 basal-like groups: cell cycle and damage repair signatures – platinum and PARP
- Immunomodulatory group
- Mesenchymal and mesenchymal stem-like groups
- Luminal AR group: androgen receptor inhibitors

Lehmann et al. J Clin Inv 2011;121(7):2750-67.

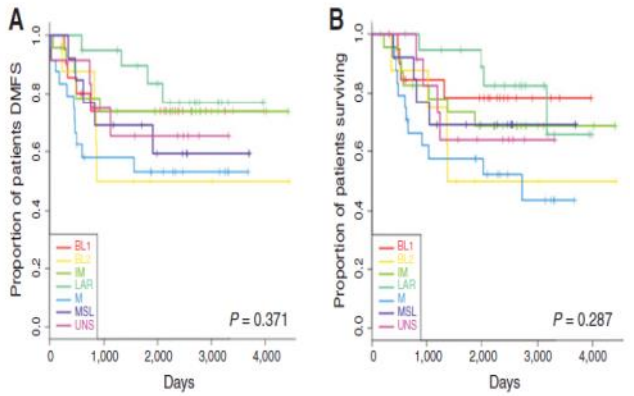
TNBC – LAR Subtype

- Gene expression array analysis of TNBC identified 6 subtypes, now refined to 4 (immunomodulatory group reflects TILs and MSL reflects stromal contamination)
- Basal like 1 – elevated cell cycle and damage response genes
- Basal like 2 – growth factor signalling and myoepithelial genes
- Mesenchymal – epithelial-mesenchymal transition and growth factor pathways
- Luminal Androgen Receptor
  – luminal gene expression driven by AR
  - lower grade, higher incidence of lymph node and bone metastasis
  - high incidence PIK3CA mutations (40%)

LAR – response to NACT

- Masuda et al., Clin Ca Res 2013;19(19):5533-40
- 6 subtypes of triple negative breast cancer
- Different rates of pCR between subtypes
- No difference in OS – LAR group had low pCR rate but best survival at 3 years

	pCR	Non-pCR	pCR rate
BL1	11	10	0.52
BL2	0	8	0.00
M	8	18	0.31
IM	8	19	0.30
MSL	3	10	0.23
LAR	2	18	0.10
UNS	5	10	0.33

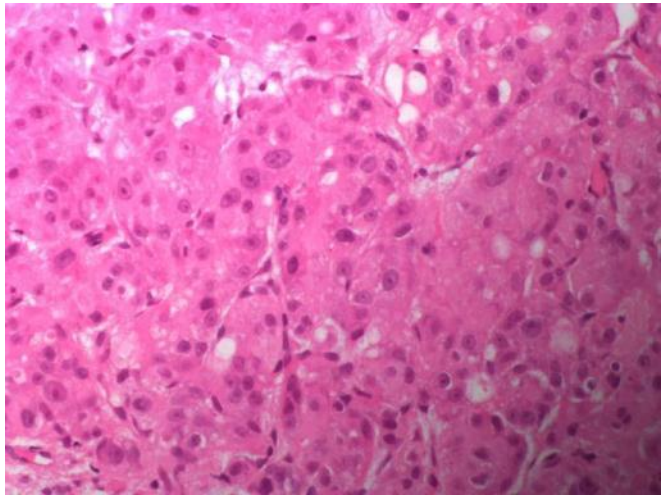


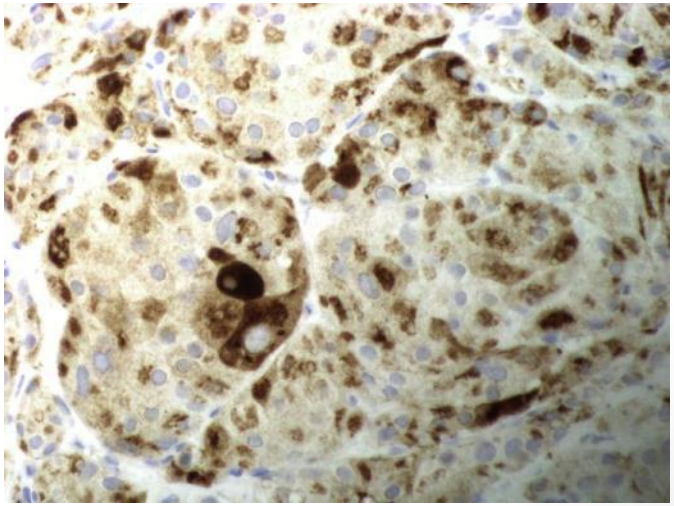
Molecular Apocrine Subtype

- Farmer et al., Oncogene2005
- Gene expression analysis of 49 breast cancers
- 3 groups; luminal (ER+), basal and an ‘intermediate’ group -> ER- but with a luminal keratin expression pattern
- 50% HER2 positive
- Androgen receptor positive with expression of metabolism related signatures and increased androgen signalling
- Review of histology – apocrine features but not classical apocrine carcinomas

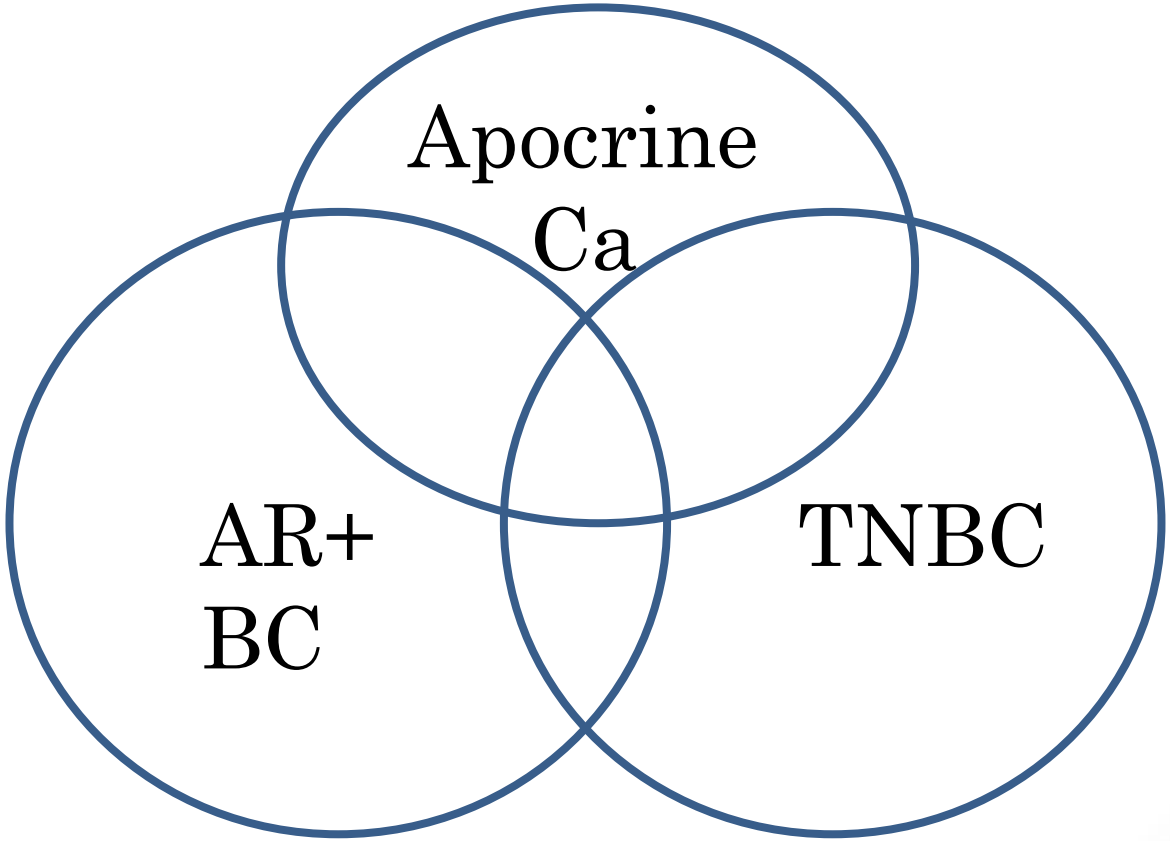
Carcinoma with Apocrine Features

- Large cells with abundant granular eosinophilic cytoplasm
- Round nuclei with prominent nucleoli
- Pure apocrine carcinoma incidence 1-5%
- Older women
- AR and GCDFP15 positive
- ER/ PR negative
- 10-60% HER2 positive





Apocrine Carcinoma and AR

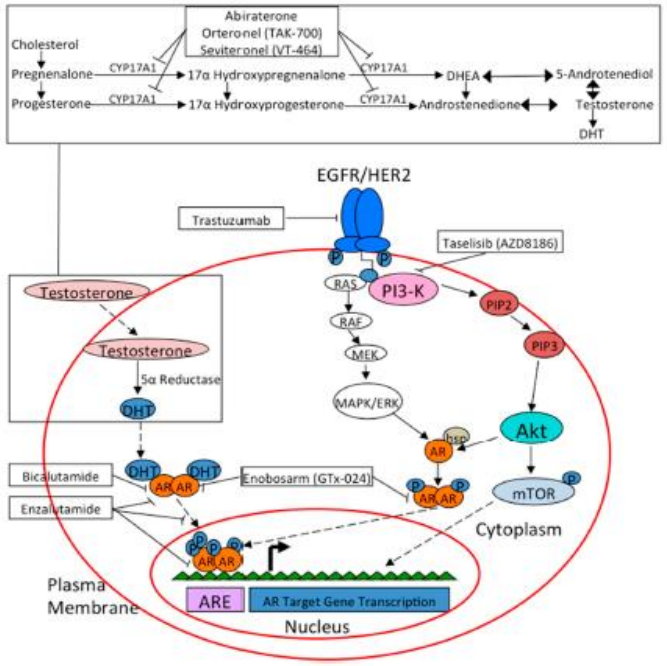


Androgen Receptor

- AR is the most commonly expressed hormone receptor in breast cancer
- Up to 90% of breast cancers are positive depending upon methods and cut offs used (literature 60-90%)
- ER + - 85-95% AR+ (LumA – 91%, LumB – 68%)
- ER - - 15-70% AR+ depending on series
- ER-/ HER2+ - 50-66% AR+
- TNBC – 32% AR+ (20-55%)

Androgen Receptor and Therapy

- ER negative cells – role of AR is complex
- Interaction with other signalling pathways including HER2, EGFR, PI3K, MAPK and AKT/mTOR
- HER2 positive tumours – cross regulation of genes
- AR activates HER2/HER3 signalling with a positive feedback loop acting via myc and the ERK pathway that increases expression of AR and ARE related genes



Androgen Receptor and Therapy

- Bicalutamide – Metastatic breast cancer -> clinical benefit in form of prolonged progression free survival in 19%; no complete or partial responses
- Enzalutamide – Metastatic breast cancer -> clinical benefit in 35% with 2 complete responses, 5 partial responses and improved progression free survival
    - Identification of gene expression signature associated with response – now phase III trial using Dx test
- Trials of dual inhibition with either CDK4/6 or PI3K/mTOR inhibitors

Personalised medicine

Personalised Breast Cancer Programme – whole genome sequencing looking for mutations and copy number alterations

Actionable mutations:

Highly Actionable (Tier 1) - Robust evidence

- Genomic alteration validated in clinical trials

– Clinical evidence of association with response to therapy

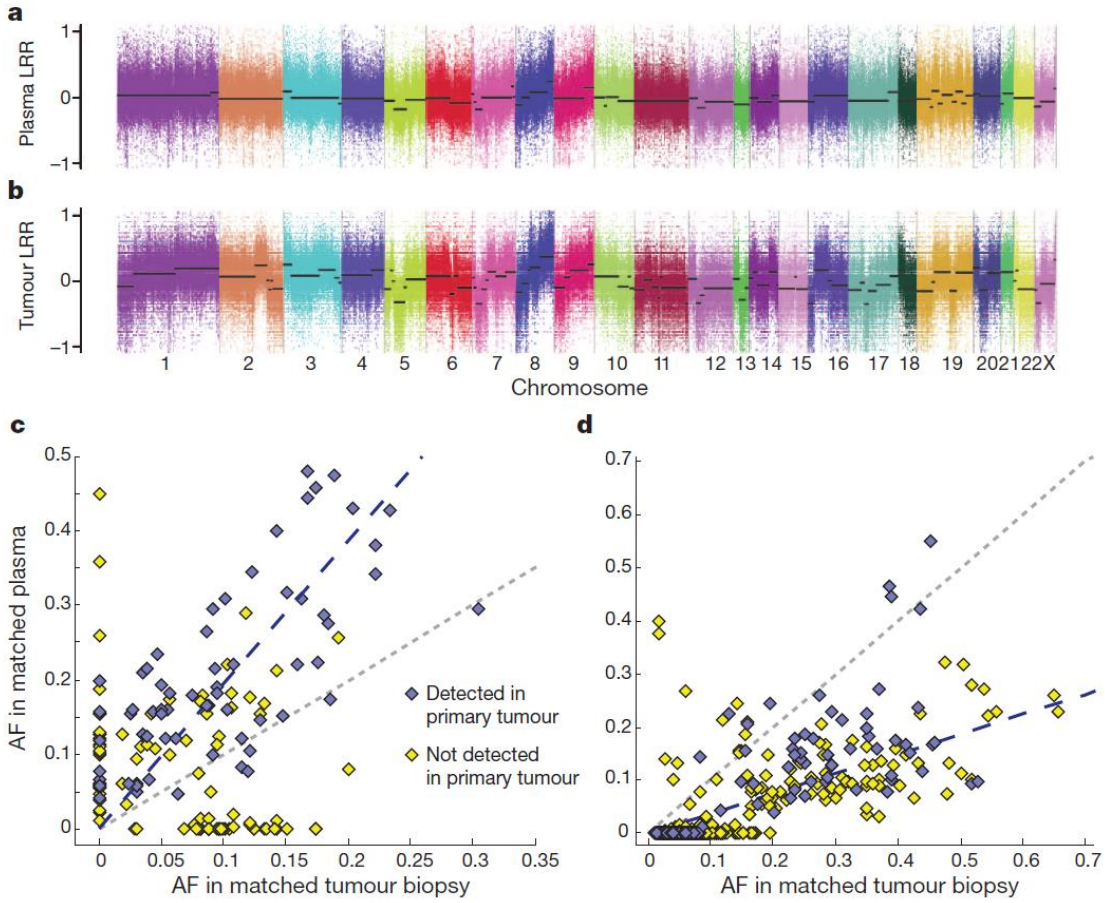
Potentially Actionable (Tier 2)

- Evidence mutation is activating (oncogene) or non-activating (tumour suppressor gene) in models

- Pre clinical evidence of association with response to treatment but clinical evidence lacking/ insufficient

ctDNA in breast cancer

- Genetic changes present in the tumour can also be identified in circulating DNA ->Liquid biopsy

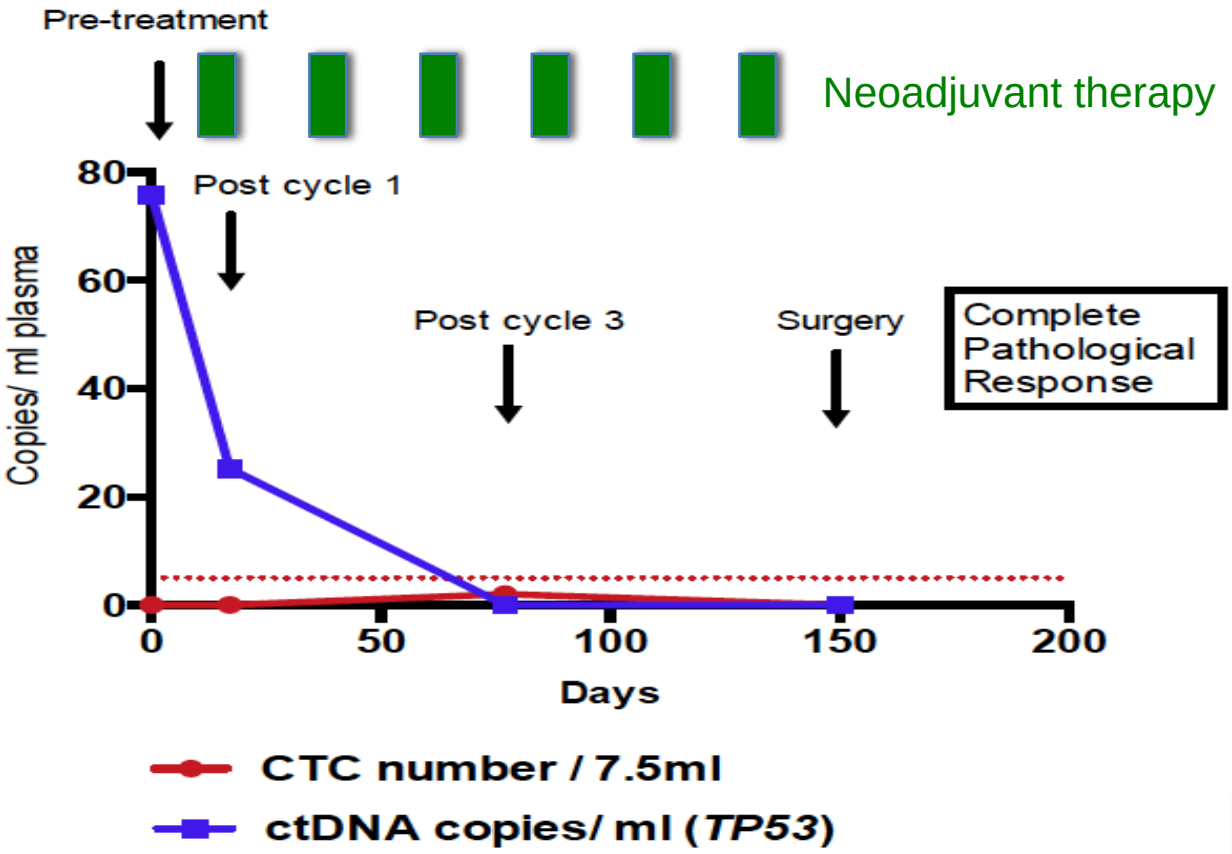


ctDNA: Liquid biopsy

- Single biopsy only gives a snapshot of tumour biology– need multiple or repeat biopsies to reflect spatial and temporal heterogeneity
- ctDNA = circulating tumour DNA. Small fragments of DNA in plasma arising from the tumour
- ctDNA is shed from all sites of tumour – represents complete repertoire of mutations present across entire tumour
- Identifiable in plasma so potential for monitoring with serial blood tests:
    - total tumour burden
    - can monitor several mutations simultaneously
    - clonal response with resistant subclones
    - can detect new mutations – resistance to therapy

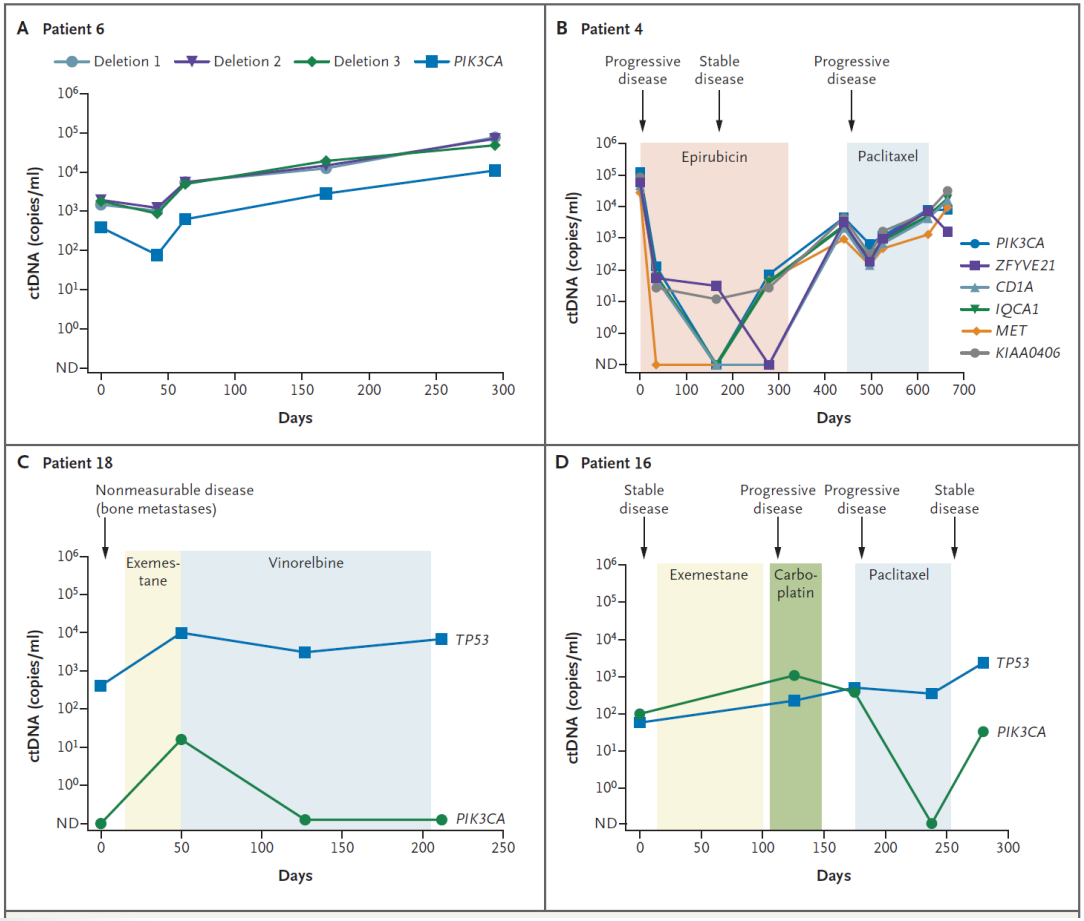
ctDNA in breast cancer

- Tumor monitoring (and clonal tracking) in the metastatic and neo-adjuvant settings



ctDNA in breast cancer

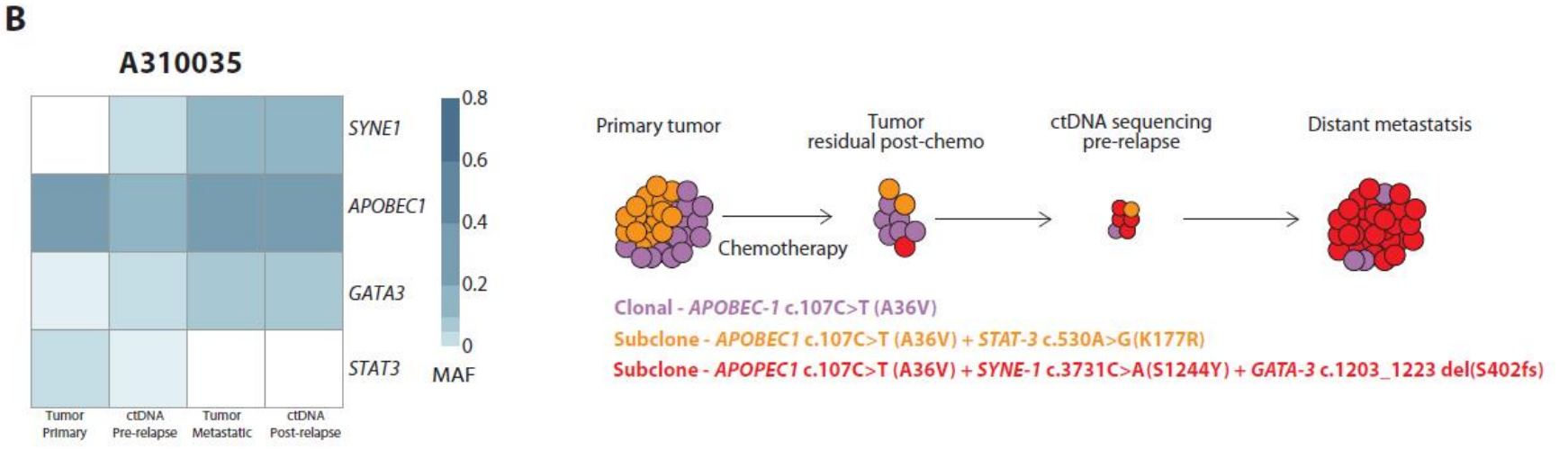
- Tumour monitoring (and clonal tracking) in the metastatic and neo-adjuvant settings



Dawson, *New Eng J Med*, 2013

ctDNA: Liquid biopsy

- Study looking at 55 women treated with NACT
- Mutations identified in 78% of primary biopsies -> personalised digital PCR assays performed post surgery then 6 monthly
- 19% had detectable ctDNA post op -> marker of early relapse
- High depth MPS of plasma DNA revealed divergent genetic changes
- Enrichment of subclones present in residual disease indicating clonal response also detectable in plasma samples
- Identification of mutation loss or emergence of new mutations with development of resistance



Garcia-Murillas et al., Sci Trans Med 2015:302(7).

ctDNA: Summary

- ctDNA can be used as a ‘liquid biopsy’
- ctDNA to monitor tumor burden is superior to CTCs and has a greater dynamic range (ctDNA/CTCs= median133)
- ctDNA often provides the earliest measure of treatment response and relapse (compared with radiology RECIST)
- ctDNA allows clonal tracking and detection of tumor evolution
- Analysis of cancer exomes in ctDNA has the potential to unravel acquired resistance to cancer therapy

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