

Classification of Breast Cancer in the Molecular Era

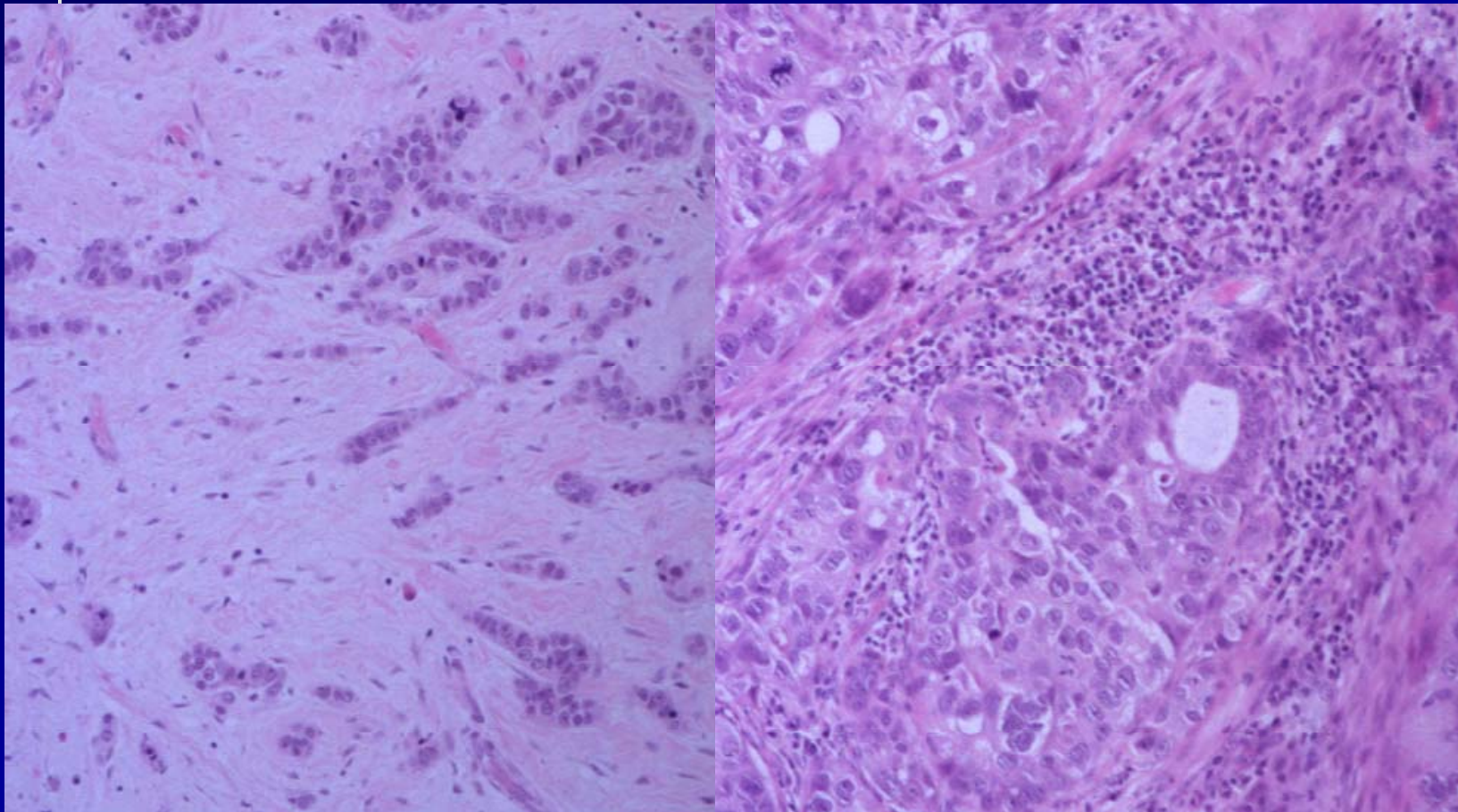
Susan J. Done

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Why classify?

- Prognosis
- Prediction of response to therapy
- Pathogenesis

Invasive breast cancer can have many different appearances

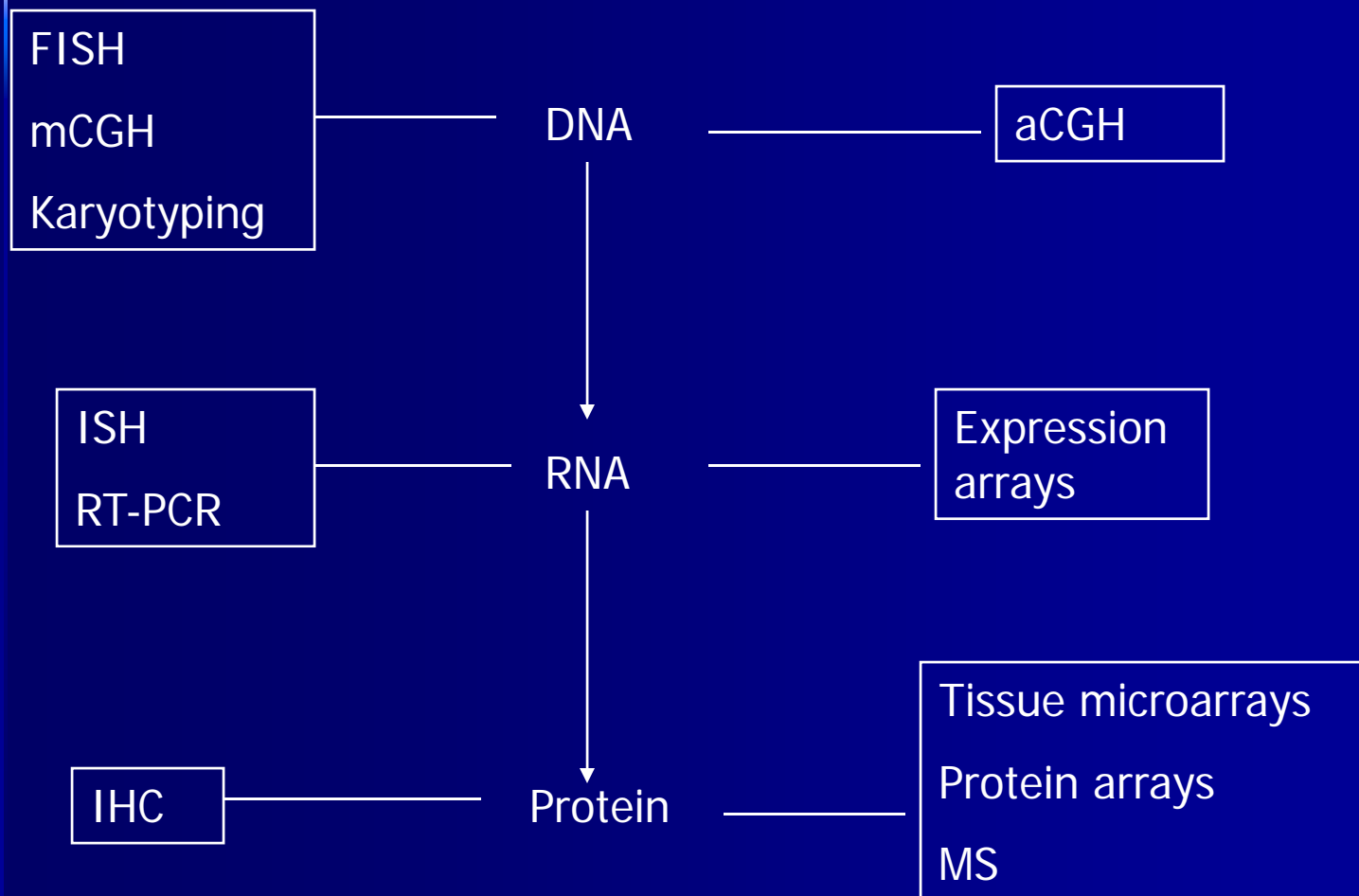


Current Pathological Classification

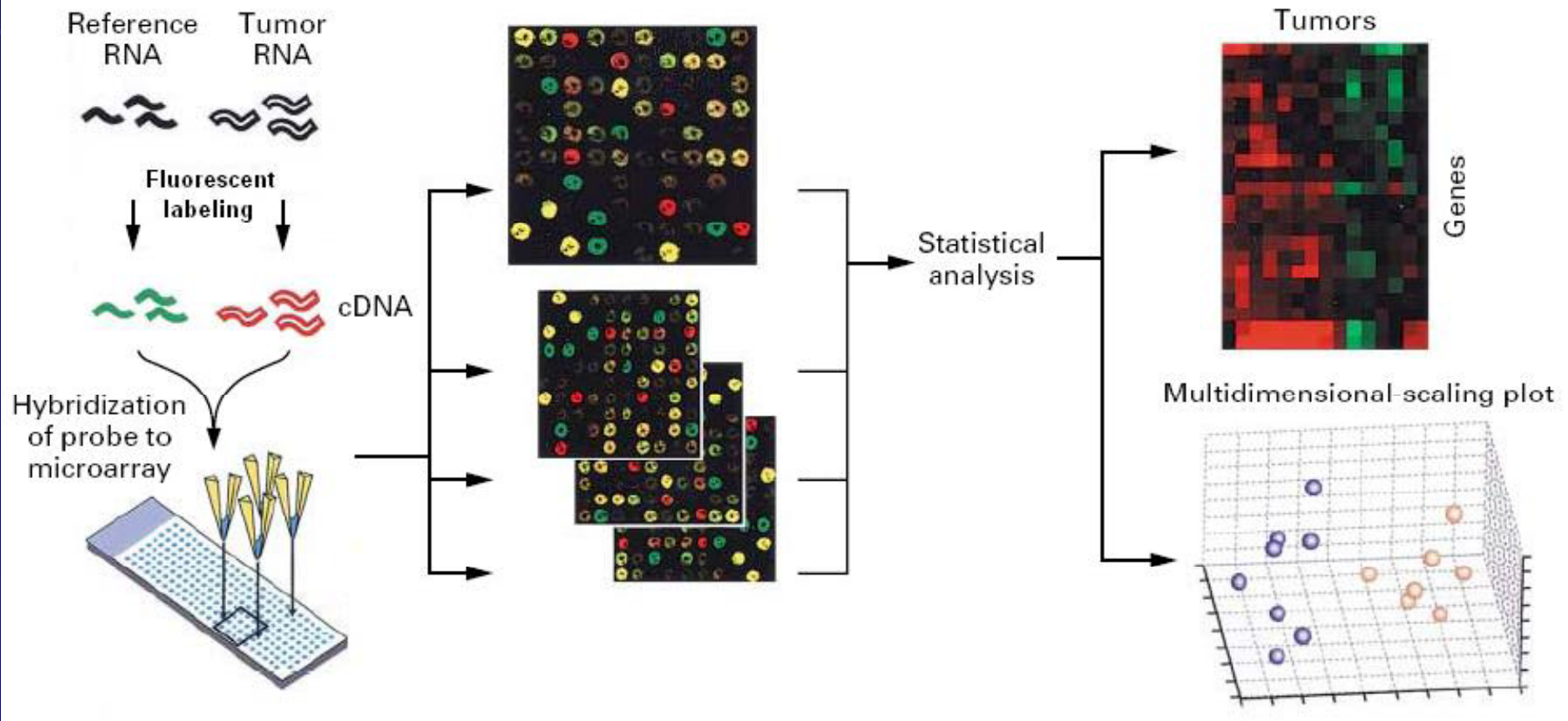
- Lymph node +/-
- Grade; tubules, nuclear pleomorphism, mitoses
- Histological type

- ER/PR
- Her 2

Molecular profiling



What are microarrays?



Microarrays - Platforms

Planar

- cDNA
- BAC
- Oligo

Immobilised bead – randomly arranged optically encoded beads

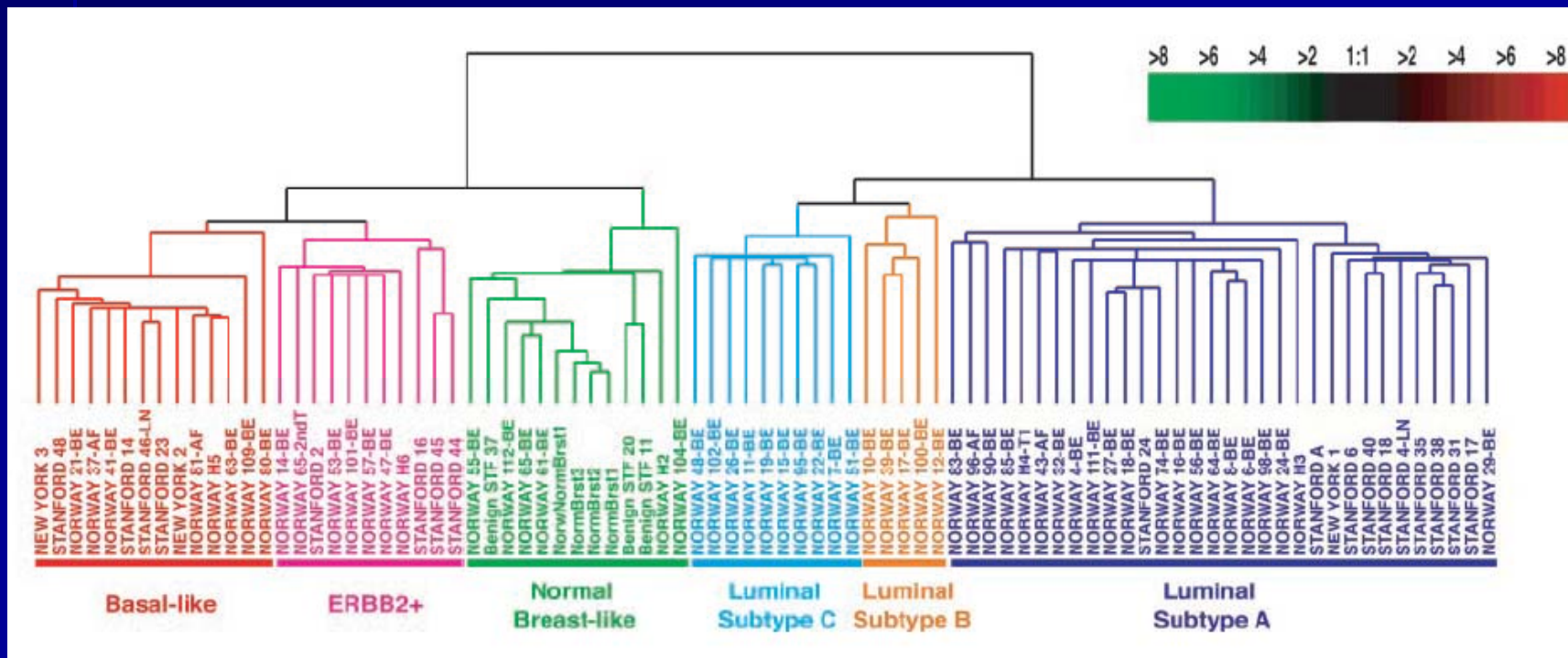
Liquid bead arrays – optically encoded by colour or size, can be simultaneously decoded and analysed by flow cytometry

Barcoded nanoparticles or quantum dots

**Suddenly able to look at
whole transcriptome**

Subsets of breast cancer

- Luminal A and luminal B, basal, ErbB2 and normal
- Tumours cluster into distinct groups reflected in different patterns of cytokeratin expression



Sørli et al. Proc. Natl. Acad. Sci. USA 2001 98, 10869-74

At least three groups

- Her 2
- Luminal
- Basal

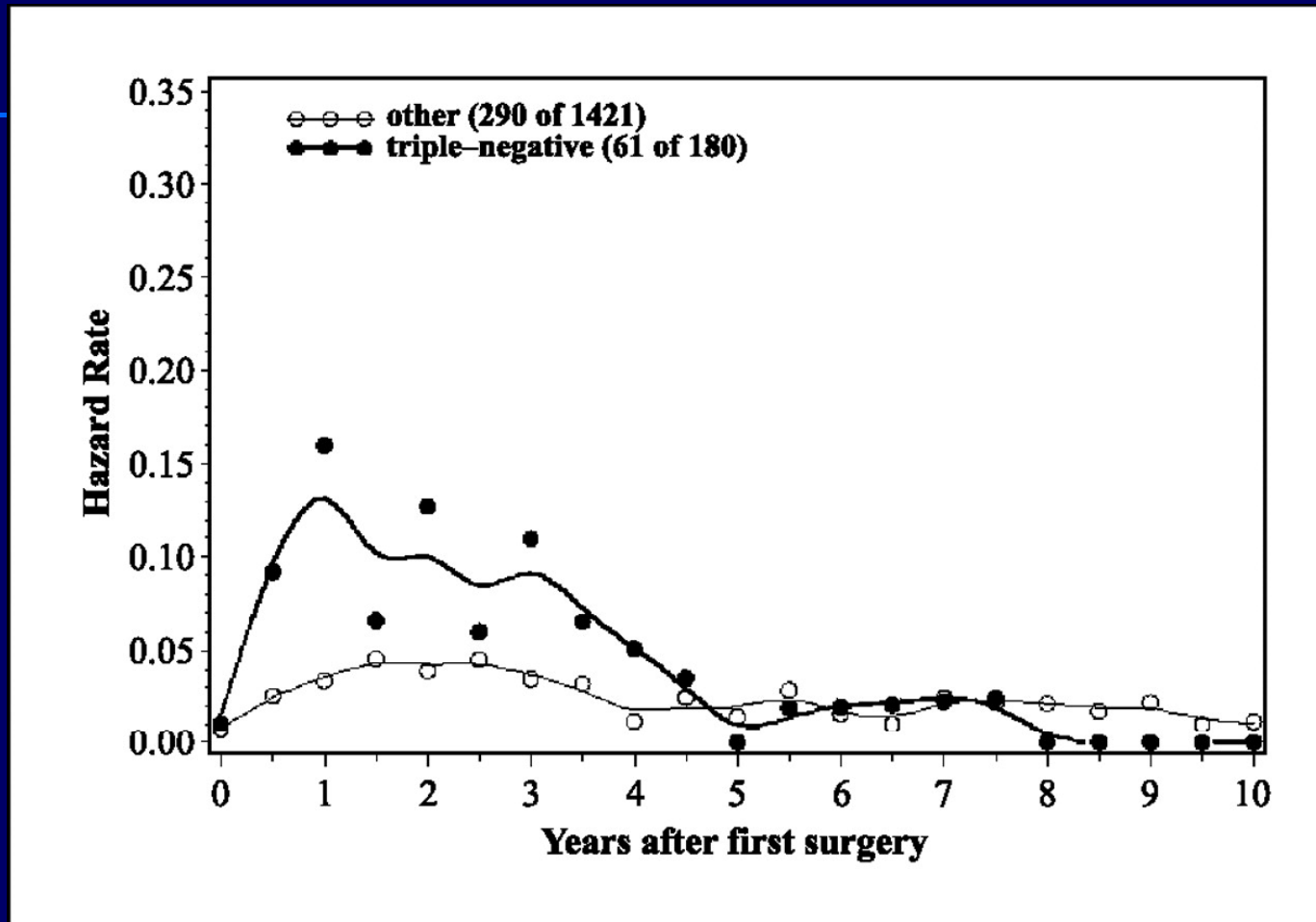
Luminal

- Usually ER positive
- Low or intermediate grade
- CK8/18
- A/B/C ?
- Less sensitive to chemotherapy than basal or HER+

Basal and 'Triple negative' breast cancer

- Lack of expression of ER, PR, Her2
- Approximately 15%, higher in African and African American women
- Overlap with basal subgroup (CK5/6/14, EGFR, P53 mt)
- Similarities to BRCA1 associated breast cancers
- Metaplastic

In patients with triple negative breast cancers the risk of recurrence peaks at approximately 3 years and then declines



Dent, R. et al. Clin Cancer Res 2007;13:4429-4434

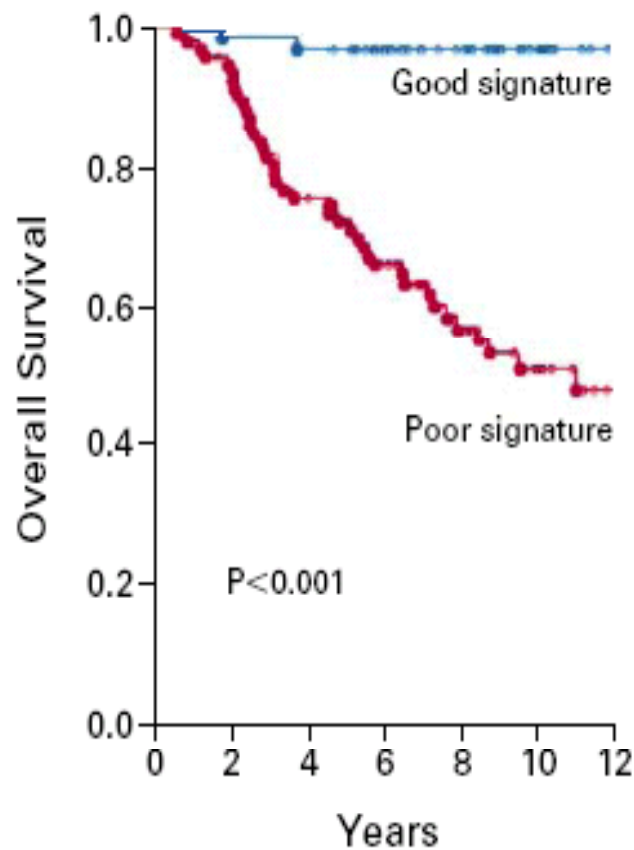
Significance of gene expression patterns

- Clusters of genes associated with
 - ER
(Bertucci *et al. Hum Mol Gen* 2002, **11**;863-72)
 - Tumour stage
(van de Vijer *et al. NEJM* 2002, **347**;1999-2006)
 - Tumour size
(Martin *et al. Cancer Res* 2000, **60**;2232-8)
 - BRCA1 or BRCA2 mutations
(Hedenfalk *et al. NEJM* 2001, **344**;539-48)
 - Basal vs. Luminal cancers
(Sørliie *et al. Proc. Natl. Acad. Sci. USA* 2001 **98**;10869-74)

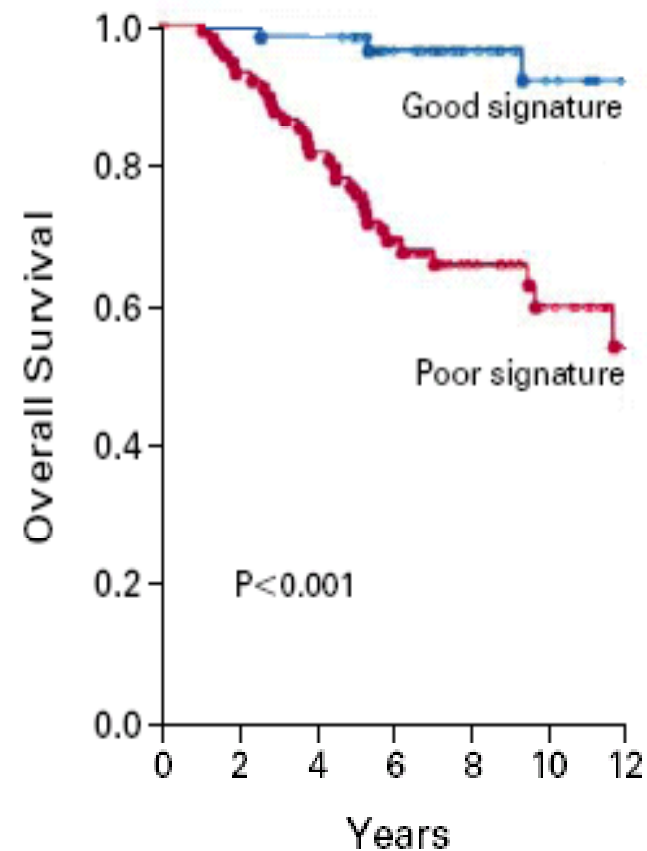
Gene profile predicts outcomes

117 small cancers from Netherlands Cancer Institute
70-gene profile independently predicted distant spread and overall survival.
Better than standard predictive models and nodal status.

Lymph-Node-Negative Patients



Lymph-Node-Positive Patients



70 gene prognosis profile

- Further tested in 295 patients (< 53 years, stage I or II, 144 LN positive) van de Vijer *et al. NEJM* 2002, 347;1999-2006
- Found to be an independent prognostic indicator
- Approved by FDA Feb 2007
- For use in Stage I and II disease
- Patients < 61 years

Debate about size of prognostic group of genes

- 70 (van de Vijer *et al. NEJM* 2002, 347;1999-2006)
- 23 (Bertucci *et al. Hum Mol Gen* 2002, 11;863-72)
- ? More or less

- Too early to say

Gene expression profiles as predictors

- Response to docetaxel
- 92 gene set
- Primary breast tumours from 24 patients before treatment with neoadjuvant docetaxel
- Monitored tumour size

Clinical trials in Breast Cancer using Gene Microarrays

- MINDACT comparing NKI prognostic signature to Adjuvant!-Online
- Massachusetts General Hosp. validating NKI prognostic signature (5000 patients)
- M.D. Anderson validating own signature for predicting drug (paclitaxel-FAC) response (210 patients)
- Baylor College validating own 90-gene prognostic signature (100 patients)
- Oncotype DX 18-75 years LNN ER+/or PR pos (10,046)

Are Gene Microarrays Ready for Routine Clinical Use?

- Requirement for fresh tissue - RNA may be degraded during routine specimen handling
- Standardised prognostic markers considered
- Need to standardise the technology
- Technology expensive – cost coming down as we move to more focused arrays
- Need to conduct larger studies
- MIAME (Minimal Information About a Microarray Experiment) guidelines www.mged.org

Lack of overlap of gene expression signatures

- van Vliet et al. BMC Genomics 2008
- Six breast cancer datasets, Affymetrix HG U133A arrays
- Different platforms and references
- Different supervised protocols
- Same set of pathways
- Different clinical composition
- * Sample size problems

RT-PCR panel

- Reverse transcriptase PCR
- RNA in formalin-fixed paraffin-embedded tissue blocks
- Panel of 21 genes (5 control) selected based on literature
- Devised model using cases from NSABP-B20 and two other groups and validated using NSAPB-B14
- ER-positive, node-negative, stage I or II
- Low (< 10%), intermediate (10-30%) and high risk of recurrence
- FDA approved

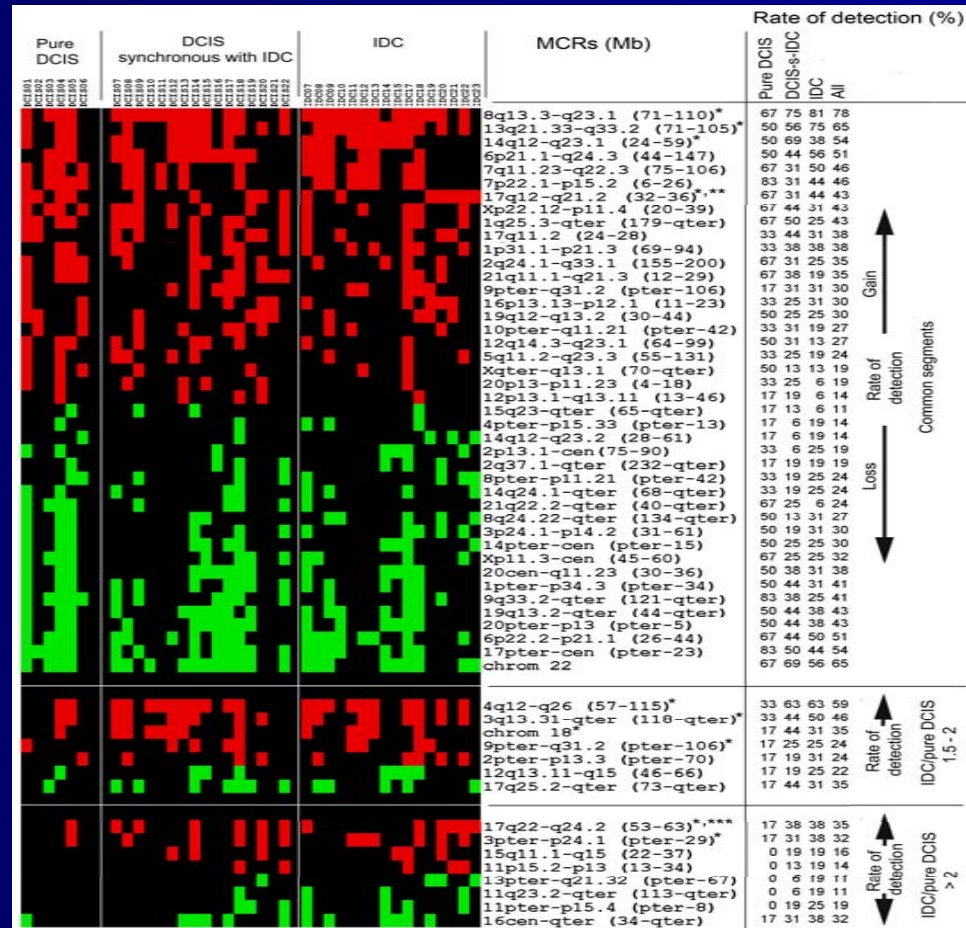
What about 'special types'?

- Lobular – E-cadherin loss
- Secretory - t(12;15) triple neg, ETV6-NTRK3
- Weigelt et al. JPathol 2008 profiled 113 cases of 11 special types
- Most, except apocrine, belong to one molecular subtype
- Some like micropapillary have distinct profile
- Adenoid cystic, metaplastic and medullary were basal-like
- Mucinous, tubular and neuroendocrine were luminal
- Role of prognostic signatures

What about DCIS?

- Hanneman et al. Br C Res 2008
- 18K cDNA NKI arrays
- 35 genes differed between DCIS (duct carcinoma in situ) and invasive breast cancer
- 43 genes separated well and poorly differentiated DCIS

Pure DCIS may be different from DCIS a/w IDC



Selection of some of the many high-throughput breast profiling studies ongoing at PMH

■ DNA

- DCIS – clinical features (CBCRA) - Done
- DCIS – genomic heterogeneity (WWBCIF) – Done, Miller, Youngson, Leong, Crystal
- SLN – Done, McCready
- IDC – Mak, McCready, Done
- CTCs (CBCF-O)- Done, Clemons, Fitzgerald

■ RNA

- FNA's - (Genome Canada/CRP) Leong, Done, McCready
- miRNA - Liu, Miller

■ Proteomics

- LN status, atypia; tissue, serum (Genome Canada/CRP) – Done, Leong, McCready

Impact of molecular classification in breast cancer

- New disease subtypes
- New prognostic/predictive panels
- New therapeutic targets
- Personalised medicine

Questions?