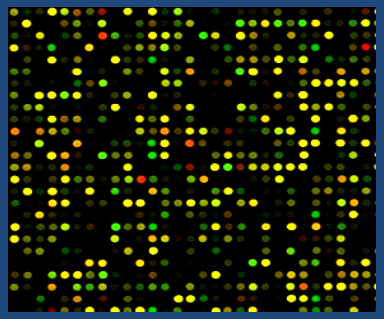
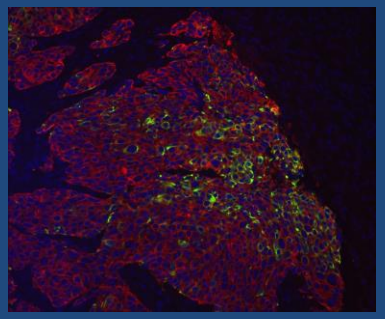
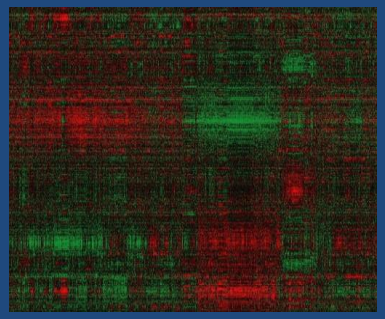
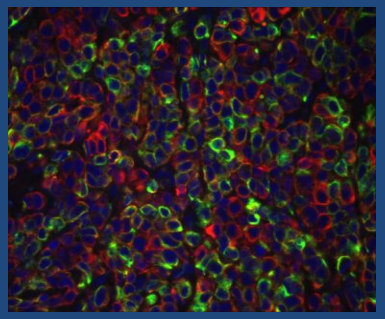





Characterization of one disease in different subtypes

Aleix Prat, MD

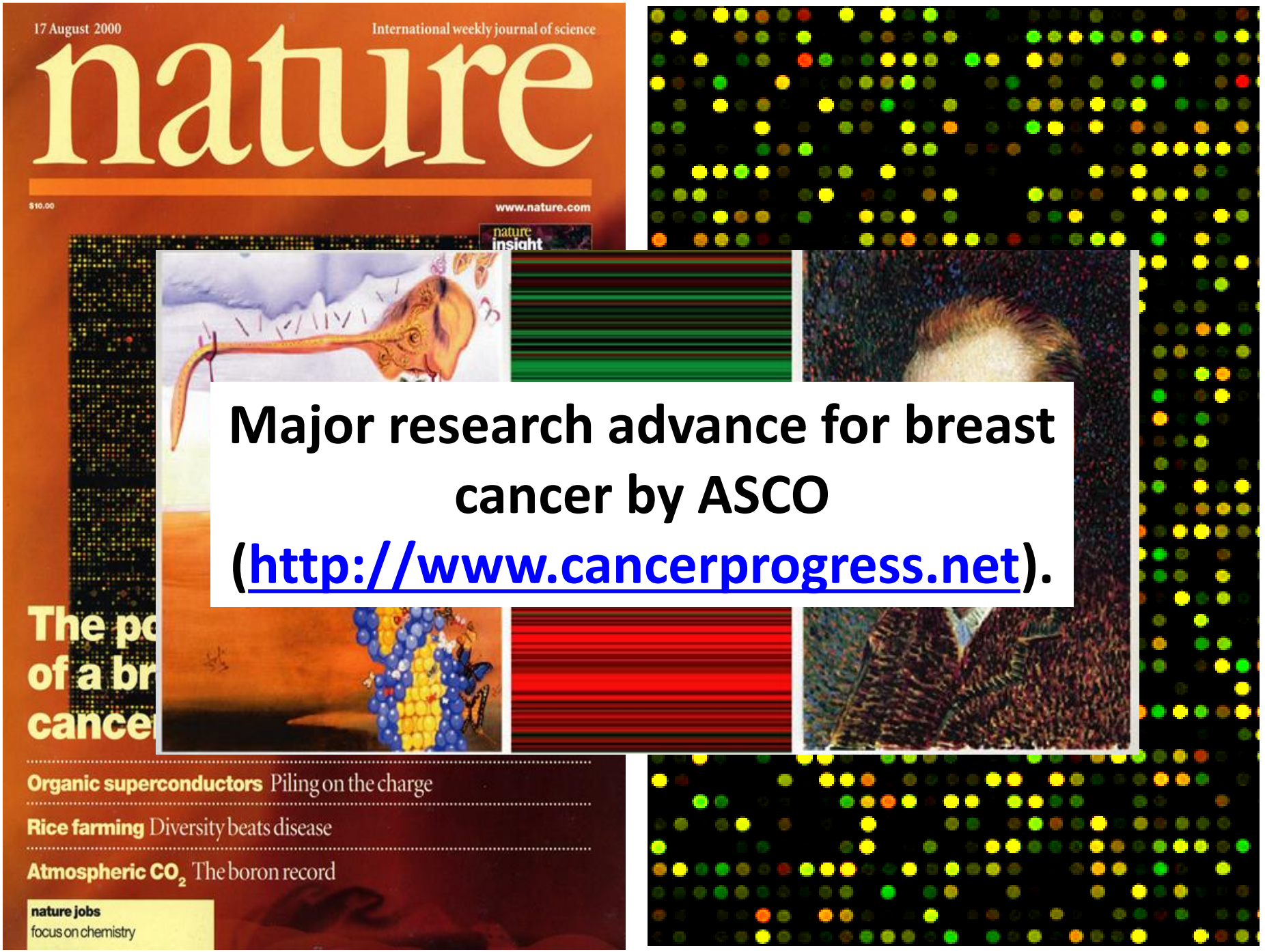
Translational Genomics Unit

Vall d'Hebron Institute of Oncology (VHIO)

Barcelona, Spain

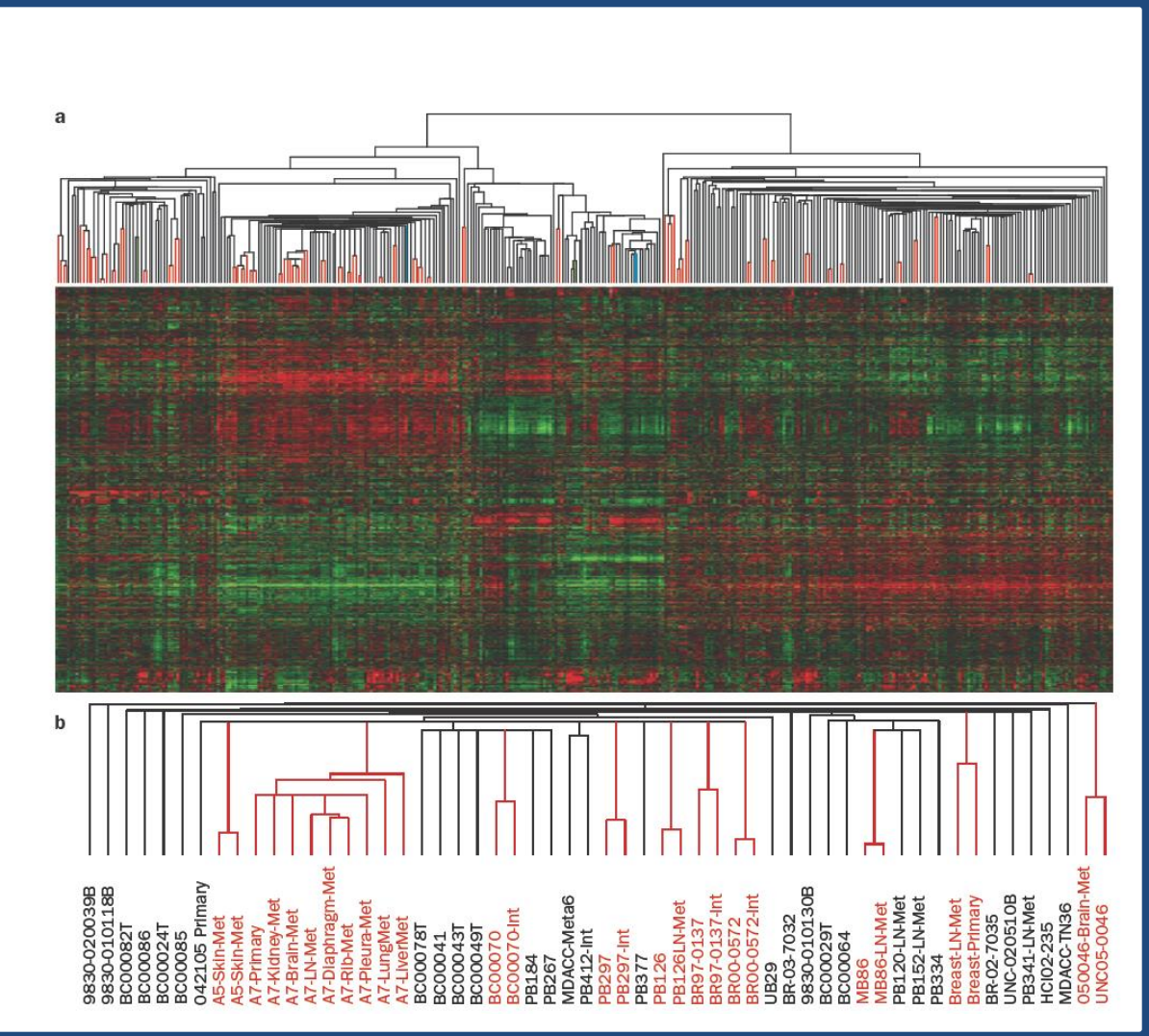
Summary of topics

- Introduction and characterization.
- Clinical implementation.
- Improving the current IHC-based subtype definitions.



Major research advance for breast cancer by ASCO (http://www.cancerprogress.net).

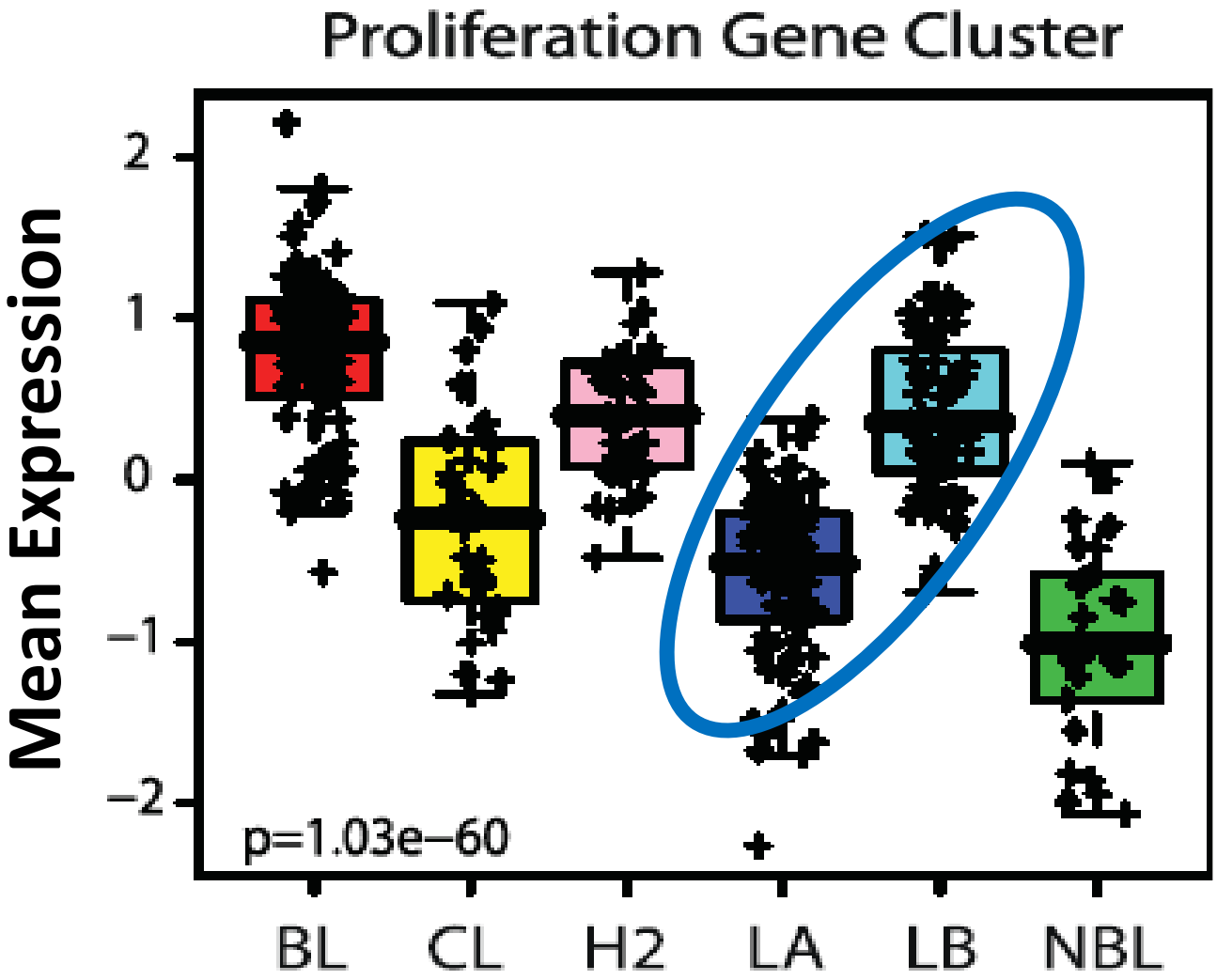
Identification of tumor individuality using global gene-expression analyses



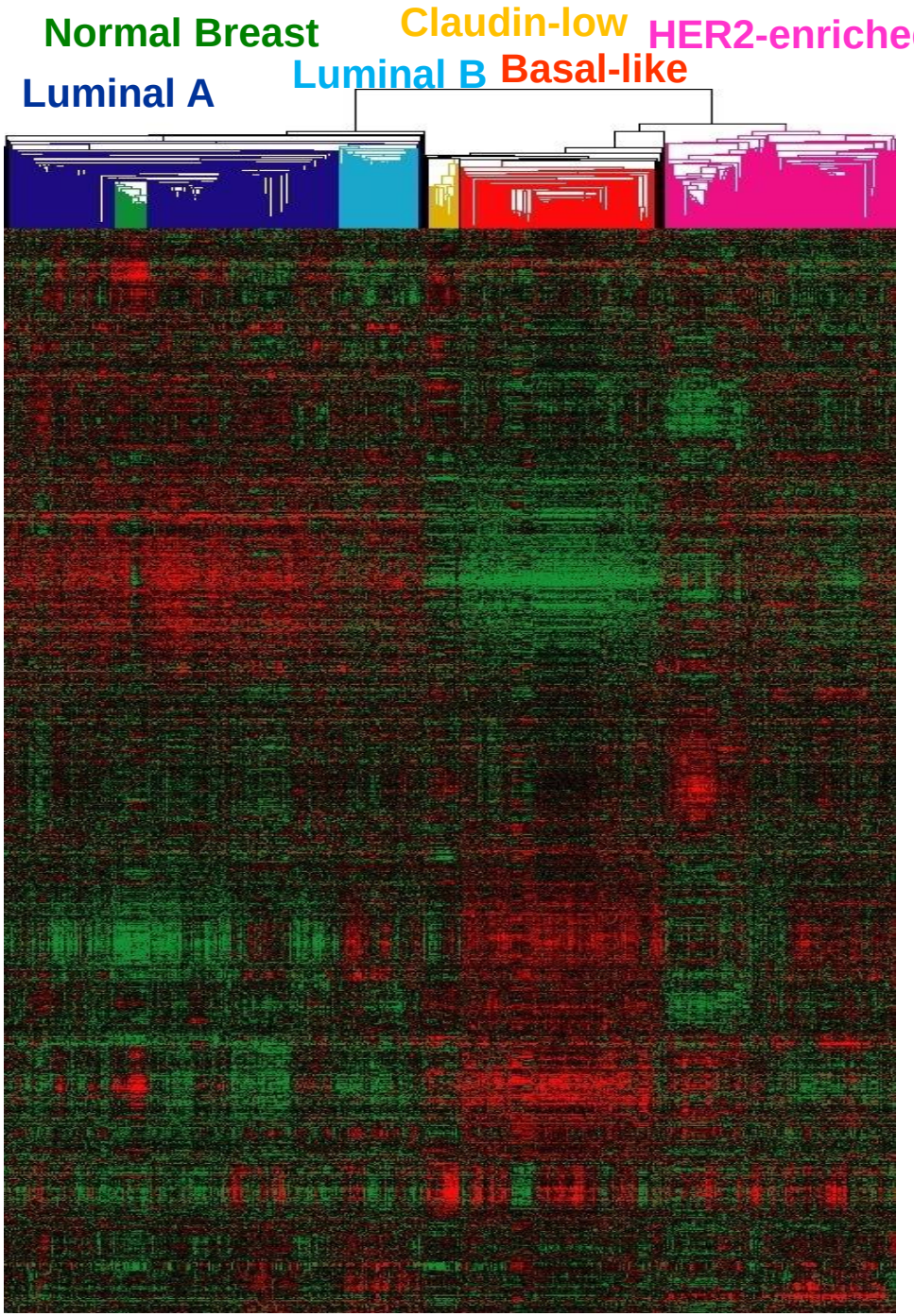
- Supervised hierarchical clustering of breast cancer data of 367 breast samples using 1,900 intrinsic genes.
- Paired tumor samples are highlighted by the red lines in the array tree, with **41 out of 43 (95%) possible pairs being paired**.

Prat et al. Nat Rev Clin Oncol 2012

Deconstructing the molecular portraits of breast cancer

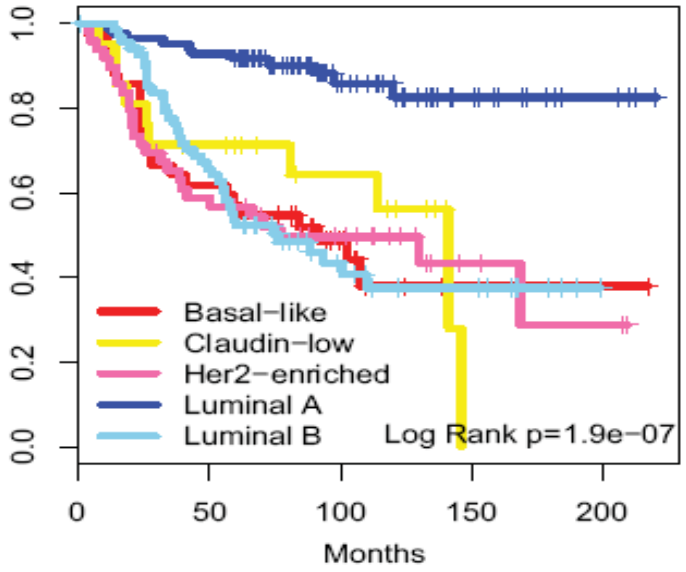


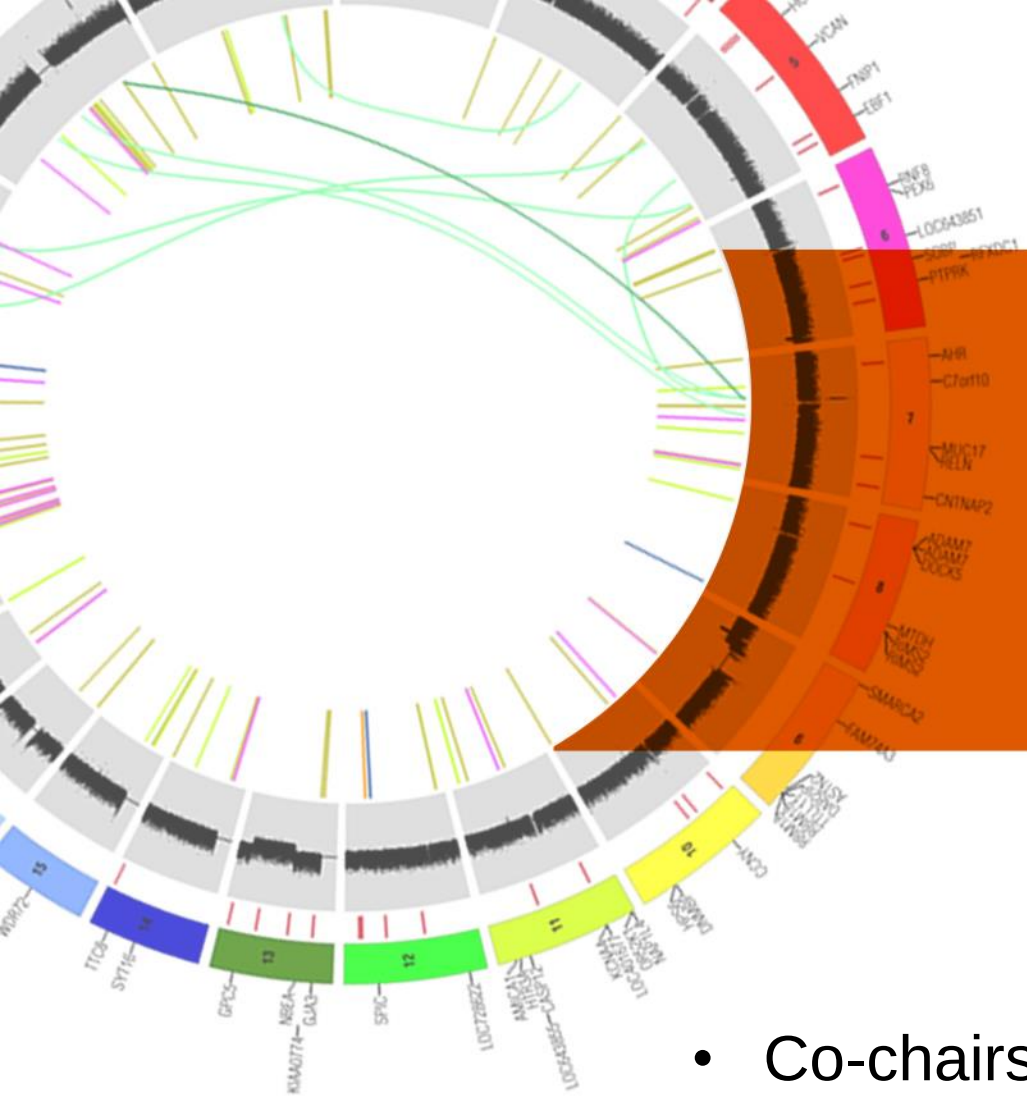
Prat & Perou Mol Oncol 2011; Prat et al. BCR 2010



Intrinsic Subtypes

Perou et al., Nature 2000
Sorlie et al., PNAS 2001
Sorlie et al., PNAS 2003
Hu et al., BMC Genomics 2006
Herschkowitz et al., GB 2007
Cheang et al. JNCI 2008
Parker et al., JCO, Feb 2009
Prat et al., BCR 2010
Nielsen et al., CCR 2010
Cheang et al., CCR 2012
Chia et al., CCR 2012
Prat et al. BCRT 2012
TCGA Nature 2012
Prat et al. JCO 2013
Prat et al. Oncologist 2013





The Cancer Genome Atlas

Breast Cancer Project

- Co-chairs: Chuck Perou and Matthew Ell

http://cancergenome.nih.gov/

Analyses Performed

825 patients

↓

463 patients

- Gene Expression
- DNA Copy Number
- miRNA Expression
- DNA Methylation
- Exome Sequencing

↓

348 patients

- Reverse Phase Protein Arrays

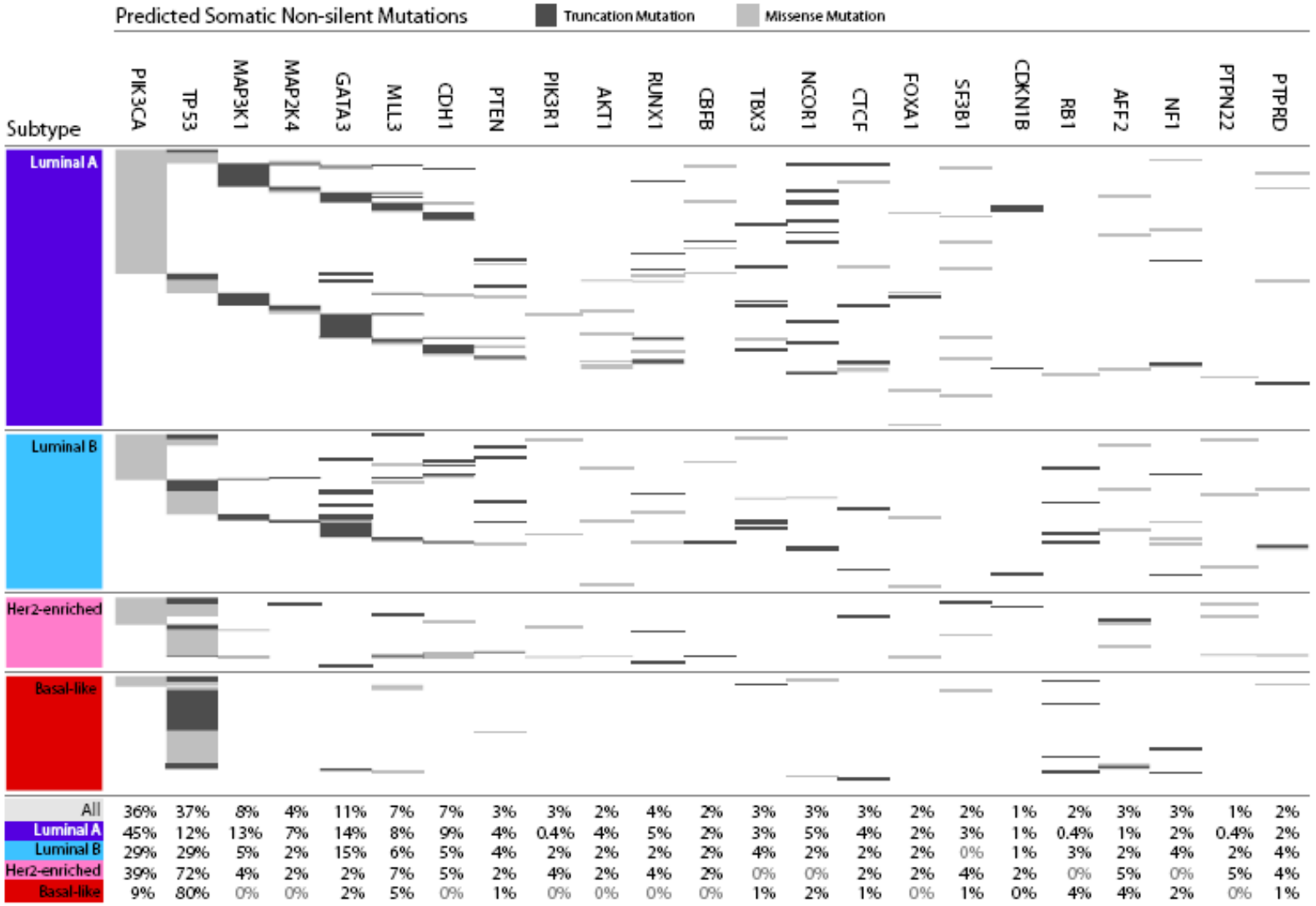
http://cancergenome.nih.gov/

Significantly Mutated Genes

SMG	All Tumors (n=507)	
	Mutated	FDR
TP53	187 (37%)	0.00E+00
PIK3CA	180 (36%)	0.00E+00
GATA3	54 (11%)	0.00E+00
MAP3K1	39 (8%)	0.00E+00
MLL3	37 (7%)	0.00E+00
CDH1	33 (6.5%)	0.00E+00
MAP2K4	21 (4%)	0.00E+00
RUNX1	18 (3.5%)	0.00E+00
PTEN	17 (3.4%)	0.00E+00
TBX3	13 (2.5%)	6.72E-13

TCGA Nature 2012

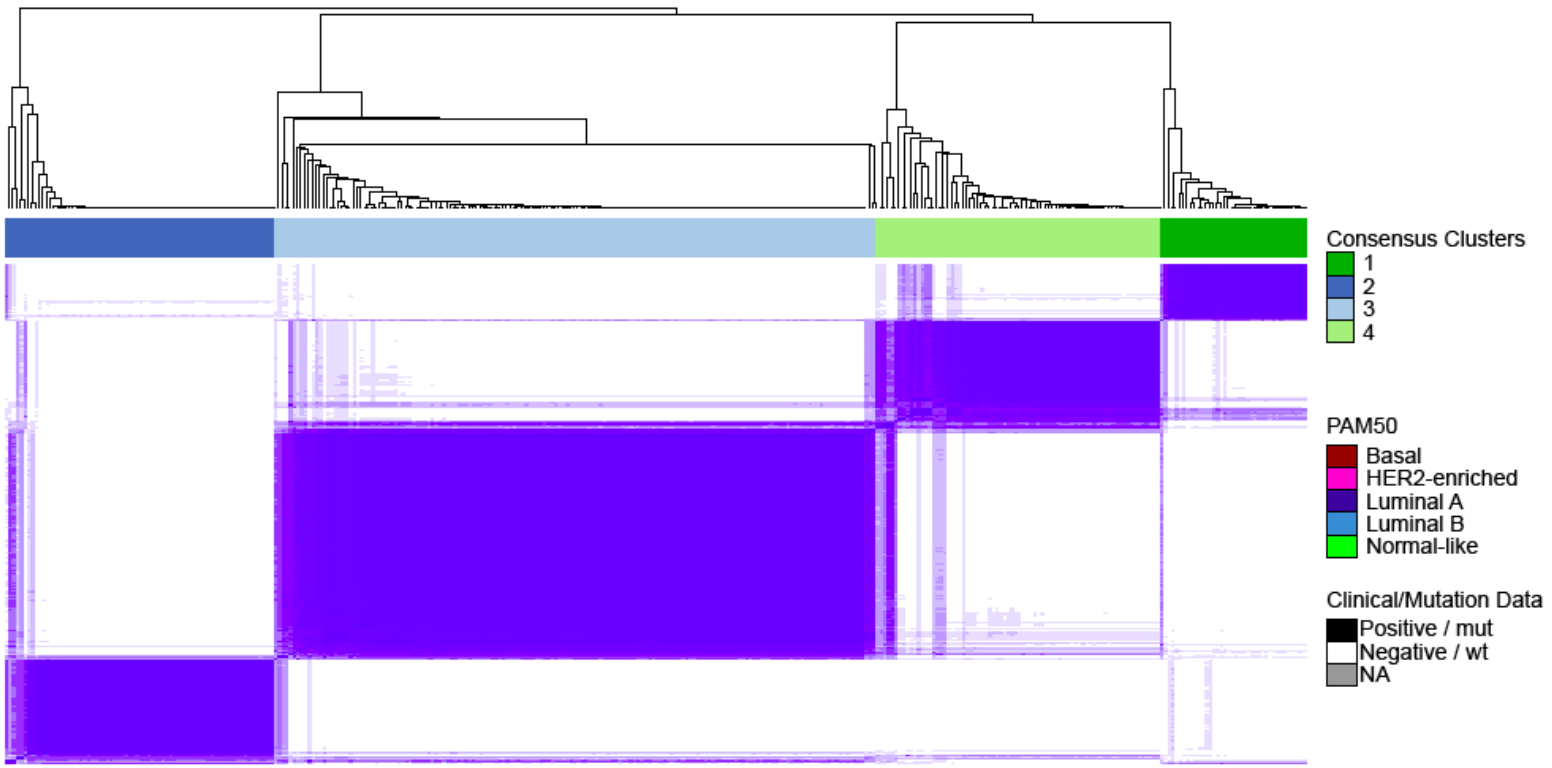
Significantly Mutated Genes



	PIK3CA	TP53	MAP3K1	MAP2K4	GATA3	MLL3	CDH1	PTEN	PIK3R1	AKT1	RUNX1	CBFB	TBX3	NCOR1	CTCF	FOXA1	SF3B1	CDKN1B	RB1	AFF2	NF1	PTPN22	PTPRD
All	36%	37%	8%	4%	11%	7%	7%	3%	3%	2%	4%	2%	3%	3%	3%	2%	2%	1%	2%	3%	3%	1%	2%
Luminal A	45%	12%	13%	7%	14%	8%	9%	4%	0.4%	4%	5%	2%	3%	5%	4%	2%	3%	1%	0.4%	1%	2%	0.4%	2%
Luminal B	29%	29%	5%	2%	15%	6%	5%	4%	2%	2%	2%	2%	4%	2%	2%	2%	0%	1%	3%	2%	4%	2%	4%
Her2-enriched	39%	72%	4%	2%	2%	7%	5%	2%	4%	2%	4%	2%	0%	0%	2%	2%	4%	2%	0%	5%	0%	5%	4%
Basal-like	9%	80%	0%	0%	2%	5%	0%	1%	0%	0%	0%	0%	1%	2%	1%	0%	1%	0%	4%	4%	2%	0%	1%

Percentages of Cases with Mutation by Expression Subtype

Integration of all data types



- Integration of information across 5 platforms demonstrated the existence of 4 main breast cancer classes….

Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes (Parker J Clin Oncol 2009)

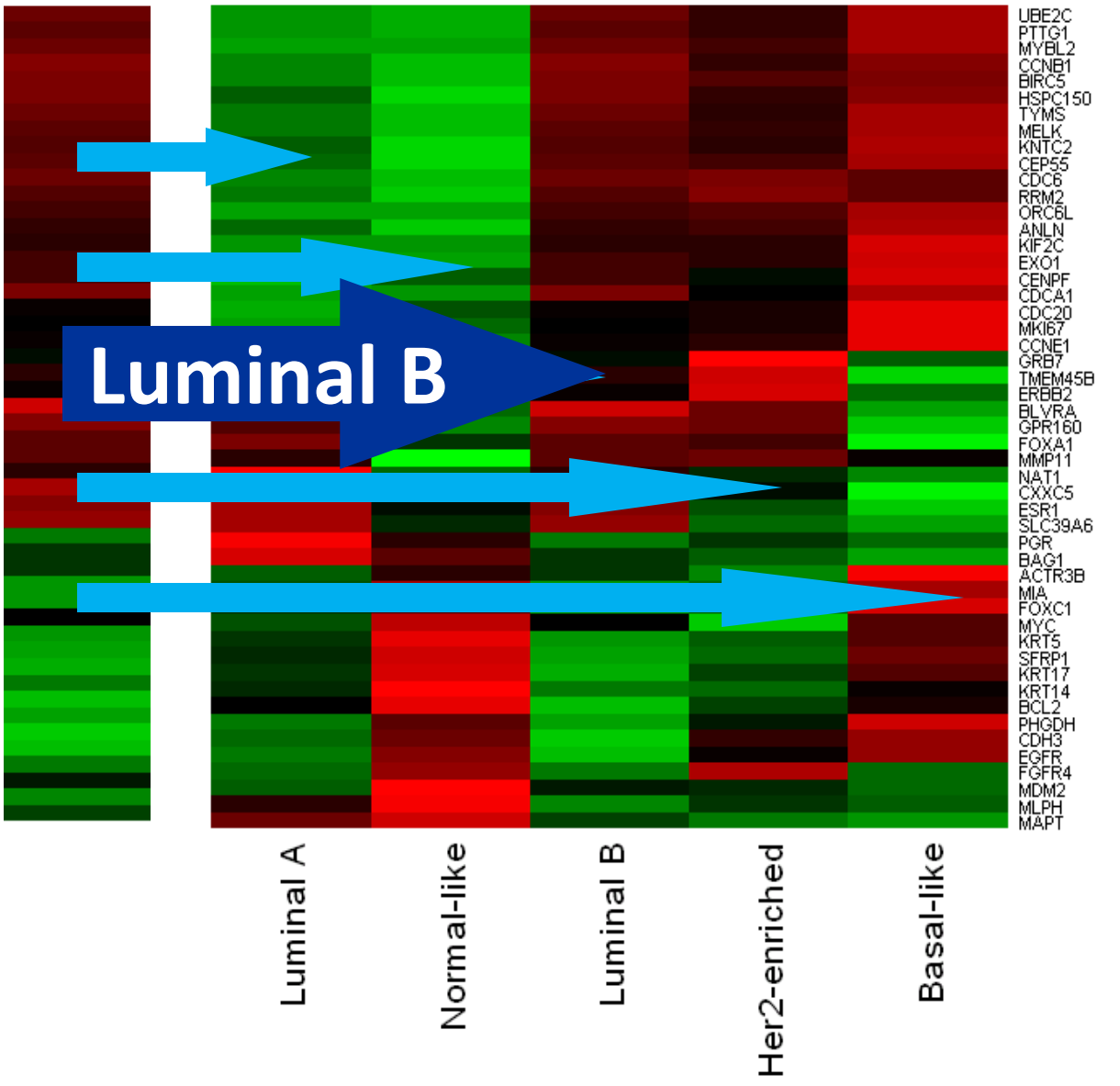
1. The qRT-PCR assay consists of 50 genes and 5 centroids (provided at https://genome.unc.ed)

2. The CV classification accuracy of the 50 genes by qRT-PCR versus 2000 genes by microarray was 93%

3. The assay works using RNA from FFPE materials or fresh frozen tissues.

4. The assay is called the

"PAM50"



2011 St Gallen International Expert Consensus

Intrinsic Subtype (1)	Clinico-pathologic definition
Luminal A	**'Luminal A'** ER and/or PgR positive(76) HER2 negative (77) Ki-67 low (<14%)*
Luminal B**	**'Luminal B (HER2 negative)'** ER and/or PgR positive HER2 negative Ki-67 high
	'Luminal B (HER2 positive)' ER and/or PgR positive Any Ki-67 HER2 over-expressed or amplified
Erb-B2 overexpression	**'HER2 positive (non luminal)'** HER2 over-expressed or amplified ER and PgR absent
'Basal-like'	**'Triple negative (ductal)'** ER and PgR absent HER2 negative

→ **Endocrine Therapy**

→ **Endocrine +/- Chemo**

→ **Endocrine+ Chemo + anti-HER2**

→ **Chemo + anti-HER2**

→ **Chemo**

Quote: "The endorsed clinicopathological criteria are likely to be refined in the future".

Goldhirsch et al. Ann Oncol 2011

Cheang et al. JNCI 2008

IHC-based versus PAM50 subtype definitions

- Whereas a large majority (81-85%) of Luminal A tumors was identified as IHC-Luminal A, 35-52% of Luminal B tumors were identified as IHC-Luminal A.

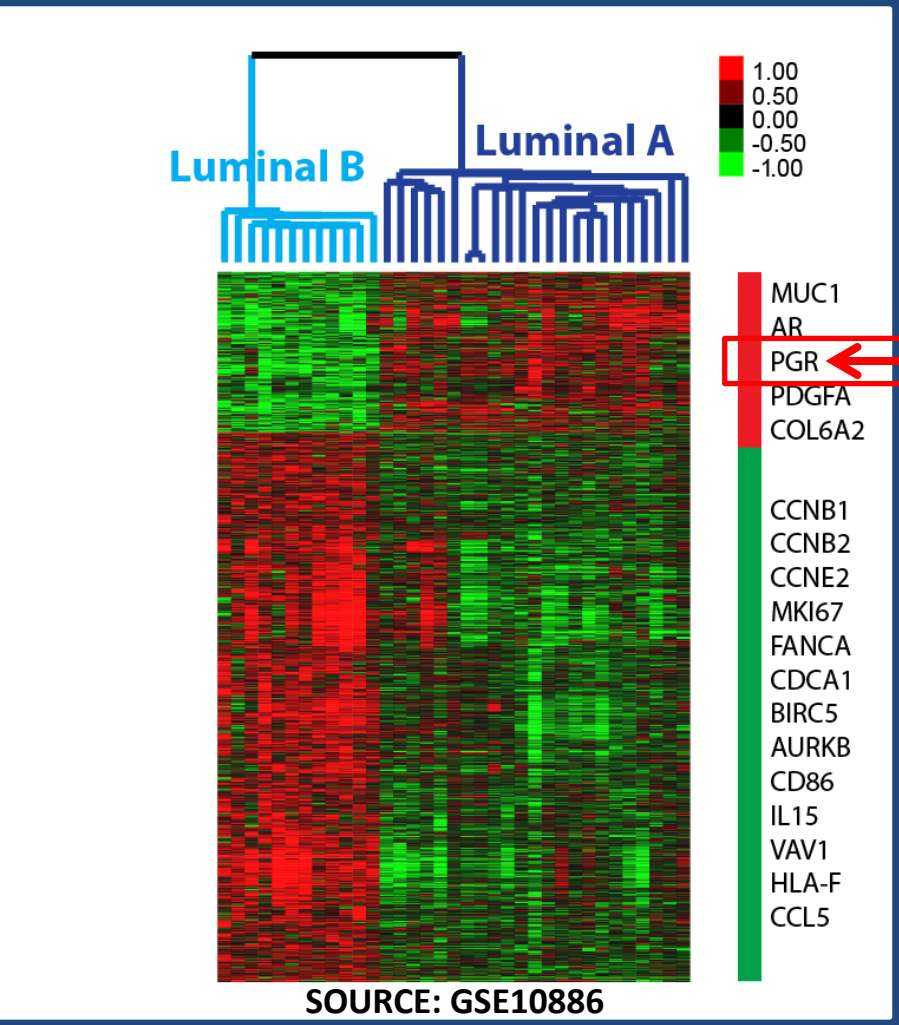
	IHC-based subtypes									
	IHC-Luminal A	%	IHC-Luminal B /HER2-	%	IHC-Luminal B /HER2+	%	HER2+	%	Triple-negative	%
BCCA-tamoxifen										
Luminal A	286	81.5	50	14.2	15	4.3	-	-	-	-
Luminal B	109	35.4	169	54.9	30	9.7	-	-	-	-
GEICAM/9906										
Luminal A	231	85.2	32	11.8	4	1.5	0	0	4	1.5
Luminal B	134	51.9	77	29.8	30	11.6	7	2.7	10	3.9

- Within HR+/HER2- disease, the concordance kappa value between the PAM50 and IHC-based Luminal A and B definitions was 0.196 and 0.407 (slight to fair agreement) in the GEICAM/9906 cohorts and BCCA-tamoxifen and, respectively.

Prat et al. J Clin Oncol 2013

Gene expression differences between the prototypical Luminal A and B tumors

- A total of 1,539 genes (348 up-regulated and 1,191 down-regulated in Luminal A tumors) were found differentially expressed (False Discovery Rate [FDR] <1%).



SOURCE: GSE10886

GO:0030154	cell differentiation
GO:0007584	response to nutrient
GO:0009991	response to extracellular stimulus
GO:0048869	cellular developmental process
GO:0042221	response to chemical stimulus
GO:0032501	multicellular organismal process
GO:0045444	fat cell differentiation
GO:0007155	cell adhesion
GO:0030198	extracellular matrix organization
GO:0008544	epidermis development

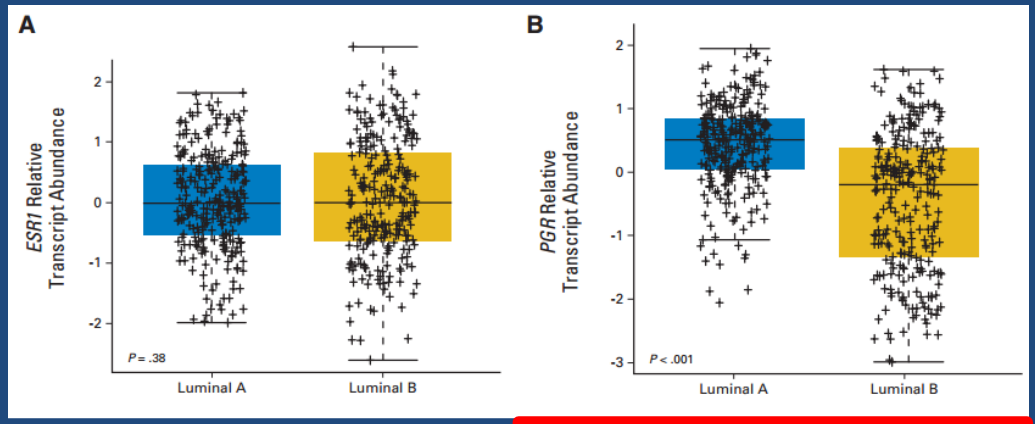
GO:0000278	mitotic cell cycle
GO:0007049	cell cycle
GO:0000279	M phase
GO:0031396	regulation of protein ubiquitination
GO:0002376	immune system process
GO:0006974	response to DNA damage stimulus
GO:0007051	spindle organization
GO:0006281	DNA repair
GO:0042110	T cell activation
GO:0046649	lymphocyte activation

Prat et al. J Clin Oncol 2013

Gene and protein expression differences between Luminal A and B tumors in GEICAM/9906 cohort

- PR gene or protein, but not ER gene or protein, was found significantly up-regulated in Luminal A tumors.

ESR1

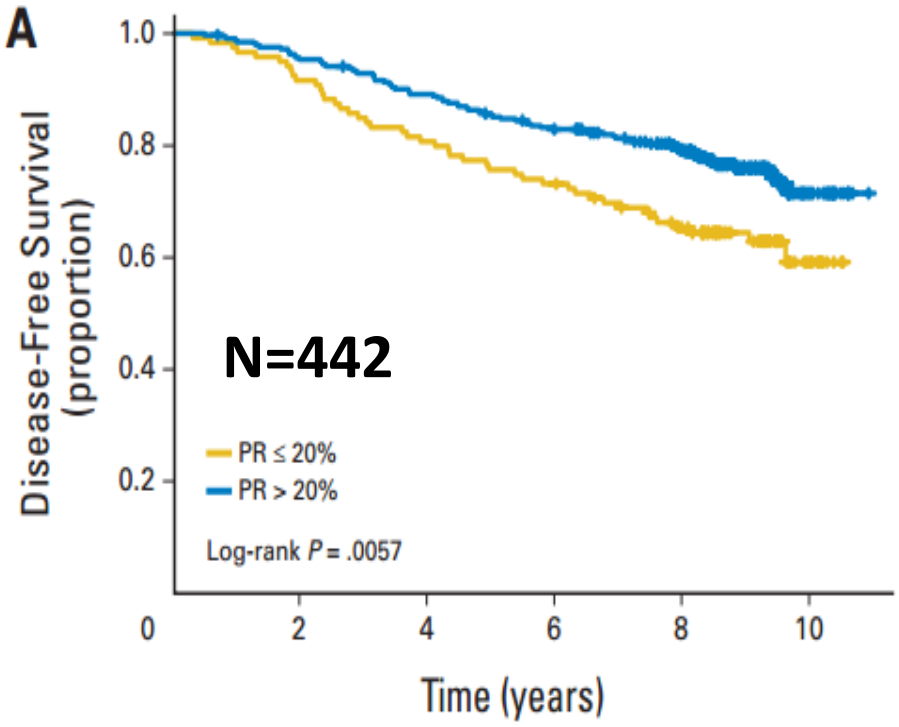


PGR

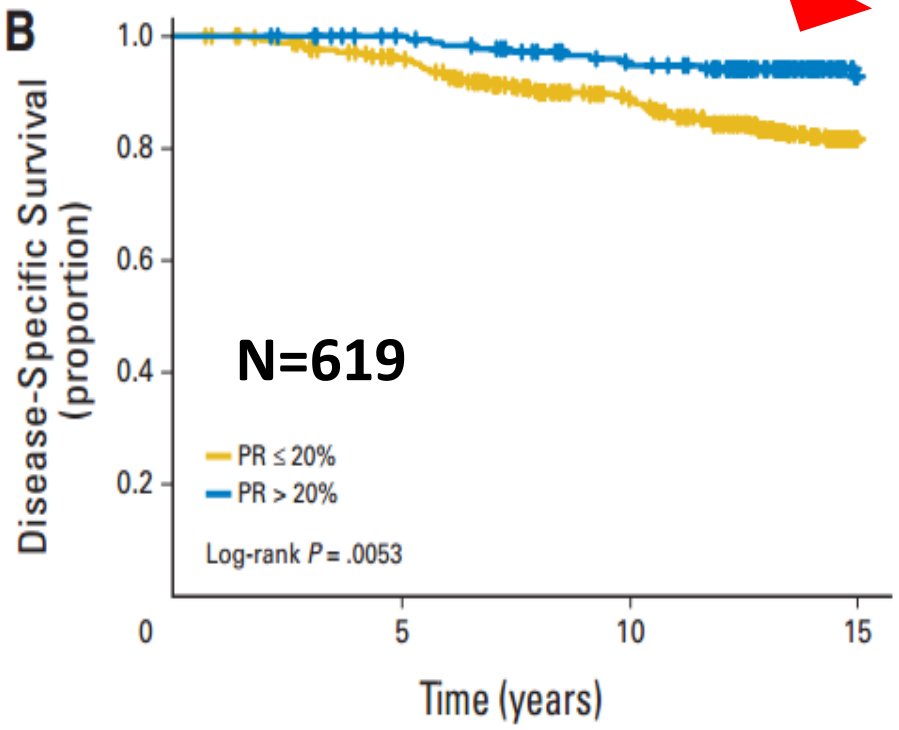
Prat et al. J Clin Oncol 2013

Survival outcomes within IHC-Luminal A based on the % of PR+ cells in 2 testing sets

GEICAM/9906
(Testing set#1)



BCCA-no AST
(Testing set#2 – independently test[illegible])



Prat et al. J Clin Oncol 2013

- Therefore, the new proposed IHC-based definition of Luminal A tumors is

HR+/HER2-/Ki67<14% and PR>20%

Conclusions

- Breast cancer is an heterogeneous disease in terms of gene, protein and miRNA expression and DNA somatic mutations, copy-number and methylation…, all of which converge into 4 main intrinsic molecular subtypes (Luminal A and B, HER2-enriched and Basal-like).

- Identification of these molecular entities in the clinical setting will be possible with the PAM50 subtype predictor.

- Surrogate definitions of the intrinsic subtypes using a combination of 3-4 biomarkers (IHC and/or gene-based) are suboptimal.

- Including percentage of PR+ tumors cells can improve the current St. Gallen IHC-based definition of the Luminal A subtype.

- To improve patient outcome and treatment efficacy, biomarkers of prognosis and response **within each subtype** will be needed and clinical trials focusing on a particular subtype are currently underway.

Acknowledgements

Patricia Galván
Maria Vidal
Ana Vivancos
Barbara Adamo
Javier Cortés
Josep Tabernero

University of North Carolina, NC, USA

Chuck Perou
Lisa Carey
Maggie Cheang
Joel S. Parker
Barbara Adamo

University of British Columbia, Canada

Torsten Nielsen
Stephen K. Chia
David Voduc
Samuel Leung

Washington University in St Louis, MO, USA

Matthew Ellis
Elaine Mardis
Sherri Davies
Jackie Snider
Tammi Vickery
Mark Watson

GEICAM, Spain

Miguel Martín
Eva Carrasco
Rosalía Caballero
Maribel Casas

University of Utah, UT, USA

Phil Bernard
Mark Ebbert
Inje Stijleman
Roy Bastien