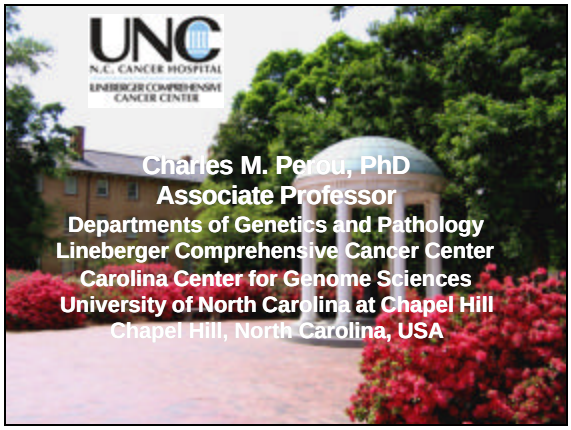
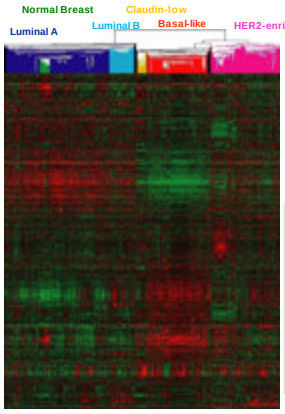
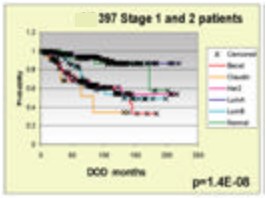




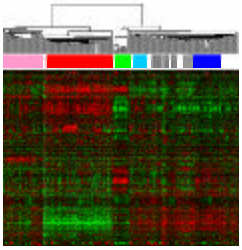
Intrinsic Subtypes

Perou et al., Nature, 2000
Sorlie et al., PNAS, 2001
Sorlie et al., PNAS, 2003
Hu et al., BMC Genomics, 2006
Parker et al., JCO, Feb 2009



Array/PCR Training Set

1. Simultaneously assay subjects with
   A. microarray on frozen samples (20k genes)
   B. qRT-PCR on FFPE samples (160 genes)
2. Objectively identify gold standard sample classifications using microarray
   A. Cluster analysis of intrinsic genes
   B. SigClust to identify significant clusters

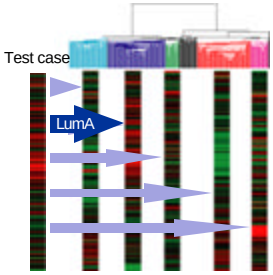


SigClust: Liu et al., JASA 2008

Array/PCR Training Set

3. Construct classification model in qRT-PCR (FFPE) data using gold standard labels
   A. Calculate each subtype centroid
   B. Compare a test case to each centroid
   C. Assign the label of the most similar centroid
   D. Repeatedly test with cross-validation over decreasing number of genes

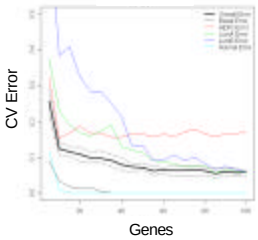
3 methods for ranking genes
3 methods for constructing centroids



Dudoit & Fridlyand JASA 2002
Storey et al. Bioinformatics 2006
Tibshirani et a. PNAS 2002

Array/PCR Training Set

3. Construct classification model in qRT-PCR (FFPE) data using gold standard labels
   A. Calculate each subtype centroid
   B. Compare a test case to each centroid
   C. Assign the label of the most similar centroid
   D. Repeatedly test with cross-validation over decreasing number of genes
   E. Select reasonable gene set based on cross-validated accuracy

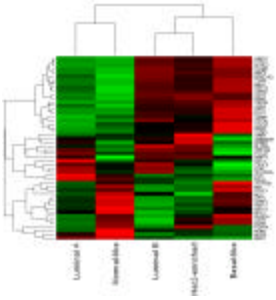


Final classifier consists of 50 gene centroids from gold standard samples

Accuracy estimate of 93% (91.7-93.6%)

Array/PCR Training Set

3. Construct classification model in qRT-PCR (FFPE) data using gold standard labels
   A. Calculate each subtype centroid
   B. Compare a test case to each centroid
   C. Assign the label of the most similar centroid
   D. Repeatedly test with cross-validation over decreasing number of genes
   E. Select reasonable gene set based on cross-validated accuracy



Final classifier consists of 50 gene centroids from gold standard samples

Accuracy estimate of 93% (91.7-93.6%)

Validation in Independent Datasets

Table 1. Clinical Characteristics by Cohort

Characteristic		Training set	No adjuvant systemic therapy*	Neoadjuvant chemotherapy^
Samples		189	761	133
Median Followup (yrs)		4	9	NA
Mean Age		58+/-15	53+/-13	52+/-11
ER	+	114	544	82
	-	77	195	51
N	+	96	35	93
	-	100	710	40
HER2	+	NA	66	33
	-	NA	192	99
Tumor Size	<= 2 cm	63	409	13
	> 2 cm	136	339	120
Grade	Low	12	133	2
	Med	56	218	54
	High	127	286	75
Subtype	Luminal A	23	269	37
	Luminal B	12	168	27
	HER2-enriched	31	120	29
	Basal-like	56	128	27
	Normal-like	12	76	13

*compiled from Ivshina et al., 2006; Loi et al., 2007; van de Vijver et al., 2002; Wang et al., 2005; https://genome.unc.edu/pubsup/breastGEO/
^Hess et al., 2006

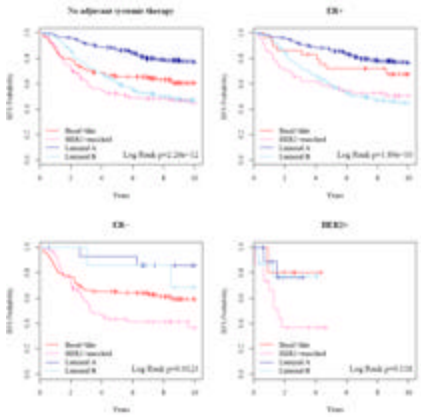
Validation using Test Datasets

Table 1. Clinical Characteristics by Cohort

Characteristic		Training set	No adjuvant systemic therapy*	Neoadjuvant chemotherapy^
Samples		189	761	133
Median Followup (yrs)		4	9	NA
Mean Age		58+/-15	53+/-13	52+/-11
ER	+	114	544	82
	-	77	195	51
N	+	96	35	93
	-	100	710	40
HER2	+	NA	66	33
	-	NA	192	99
Tumor Size	<= 2 cm	63	409	13
	> 2 cm	136	339	120
Grade	Low	12	133	2
	Med	56	218	54
	High	127	286	75
Subtype	Luminal A	23	269	37
	Luminal B	12	168	27
	HER2-enriched	31	120	29
	Basal-like	56	128	27
	Normal-like	12	76	13

*compiled from Ivshina et al., 2006; Loi et al., 2007; van de Vijver et al., 2002; Wang et al., 2005; https://genome.unc.edu/pubsup/breastGEO/
^Hess et al., 2006

Parker et al., JCO, In Press



Parker et al., JCO, In Press

Prognostic Model Evaluation

Table 2. Models of Relapse Free Survival (untreated)

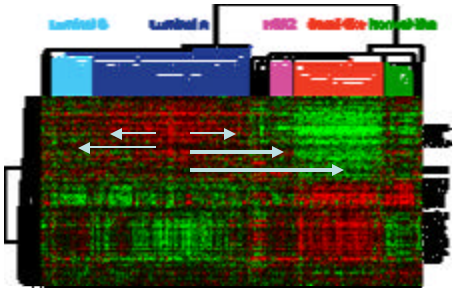
Model	A		B		C	
Variable	Hazard Ratio	p-value	Hazard Ratio	p-value	Hazard Ratio	p-value
Basal-like*	1.33	0.330	***1.79***	***0.030***	1.58	0.066
HER2-enriched*	***2.53***	***0.00012***	***3.25***	***<0.0001***	***2.90***	***<0.0001***
Luminal B*	***2.43***	***<0.0001***	***2.88***	***<0.0001***	***2.54***	***<0.0001***
ER Status~	0.83	0.38	0.83	0.34	0.83	0.32
Tumor Size†	***1.36***	***0.034***	***1.43***	***0.012***	***1.57***	***0.001***
Node Status‡	***1.75***	***0.035***	***1.72***	***0.041***	-	-
Histologic Grade^	***1.40***	***0.0042***	-	-	-	-
Full vs Subtype¨		***<0.0001***		***<0.0001***		***<0.0001***
Full vs Clinical¥		***<0.0001***		***<0.0001***		***<0.0001***

*Luminal A class used as reference state in multivariate
~Hazard ratios for ER using positive marker in the numerator
†Size <= 2cm versus >2cm
‡Any positive node
^Grade encoded as an ordinal variable with three levels
¨Significant p-values indicate improved prediction relative to subtype alone
¥Significant p-values indicate improved prediction relative to clinical data alone

N=710 local therapy only test cases

Parker et al., JCO, In Press

Diversity Within Subtypes



Distance to each centroid for a genomic summary

Parker et al., JCO, In Press

Risk Classification

- Similarity to the subtypes are used as variables in the prognostic model where the outcome is risk of recurrence (ROR):

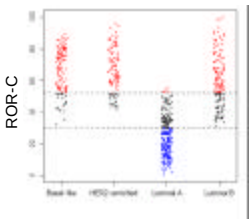
(1) $ROR_1 = \beta_1 \cdot Basal + \beta_2 \cdot HER2 + \beta_3 \cdot LumA + \beta_4 \cdot LumB$

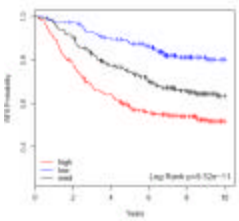
(2) $ROR_2 = \beta_1 \cdot Basal + \beta_2 \cdot HER2 + \beta_3 \cdot LumA + \beta_4 \cdot LumB + \beta_5 \cdot T$

- Weights for each term are learned from training data using a Cox model with Ridge Regression
- The weighted sum is assigned as the risk score for a test case and a threshold may be applied for class assignment

Ridge regression with Cox model: Tibshirani, Statistics in Medicine 1997
Comparative study: Bovelstad et al. Bioinformatics 2007

Risk Classification

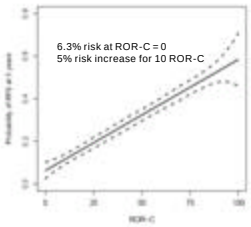


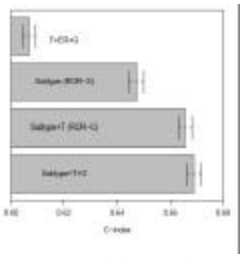


N=558 untreated test cases

ROR-C threshold determined from training data

Risk Classification





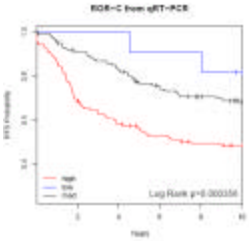
N= 710 untreated

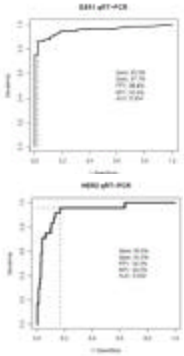
100 re-sampled test sets from random split 2/3 train 1/3 test

SiZer analysis to identify linear relationship
Marron et al. Annals of Statistics, 2000

C-index: Harrell FE, Jr. et al. JAMA 1982

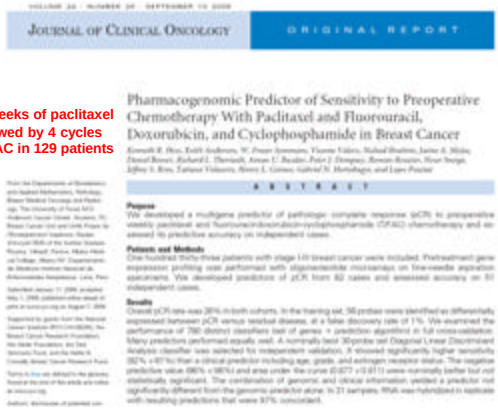
Performance of qRT-PCR Assay





N=279 test cases

Parker et al.,
JCO, In Press



12 weeks of paclitaxel followed by 4 cycles of FAC in 129 patients

T/FAC Neoadjuvant Response By PAM50 Subtype

The overall pCR rate in this study was 22%

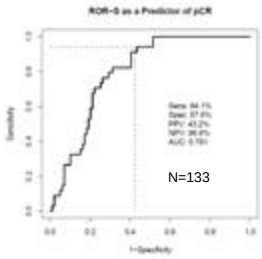
T/FAC pathological complete response rates for PAM50 subtypes and the triple-negative classification

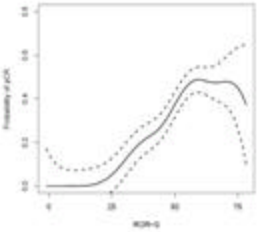
Classification	RD	pCR
Basal-like	11 (41%)	16 (59%)
HER2-enriched	17 (59%)	12 (41%)
LumA	36 (100%)	0 (0%)
LumB	22 (82%)	5 (18%)
Normal-like	13 (93%)	1 (7%)
Triple Negative	13 (50%)	13 (50%)
Any Positive	82 (80%)	20 (20%)
Triple Negative/Basal	6 (35%)	11 (65%)
Triple Negative/Non-Basal	7 (78%)	2 (22%)
Non-Triple Negative/Basal	4 (50%)	4 (50%)
Non-Triple Negative/Non-Basal	78 (83%)	16 (17%)

*Percentages are calculated by the total per classification

Parker et al., JCO, In Press and Presented at ASCO 2008, Parker et al., Abstract 11008
Data from 83 patients was earlier published as Rouzier et al., Clinical Cancer Research 11, 5678-85 (2005)

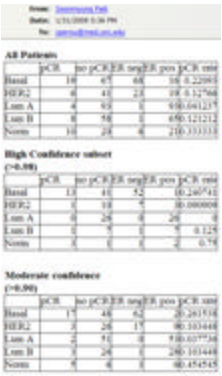
T/FAC Response By ROR-S





Hess et al., 2006 (n=51)
Sens: 92% PPV: 52%
Spec: 71% NPV: 96%

All patients, All Arms results



All Patients

	pCR	no pCR	ER neg	ER pos	pCR rate
Basal	18	67	68	18	0.2209
HER2	6	41	23	18	0.12766
Lum A	4	93	1	96	0.041237
Lum B	8	58	1	65	0.121212
Norm	10	24	6	28	0.333333

High Confidence subset (>0.90)

	pCR	no pCR	ER neg	ER pos	pCR rate
Basal	13	41	52	0	0.2407[illegible]
HER2	1	13	7	0	0.0000[illegible]
Lum A	0	24	0	24	0
Lum B	1	7	1	7	0.125
Norm	1	1	1	1	0.5[illegible]

Moderate confidence (>0.90)

	pCR	no pCR	ER neg	ER pos	pCR rate
Basal	17	48	62	2	0.2615[illegible]
HER2	3	24	17	0	0.1034[illegible]
Lum A	2	51	0	53	0.037736
Lum B	3	24	1	26	0.1034[illegible]
Norm	5	6	1	10	0.454545

ACT vs. AC results

All patients according to ER by central IHC (Duke PharmDx)

	ACT	AC
ER neg	1/131 (0.19)	10/88 (0.13)
ER pos	1/165 (0.16)	9/146 (0.06)

over 95% confidence data only

	ACT	AC
Basal	9/24 (0.38)	0/41 (0.00)
HER2	1/7 (0.14)	2/22 (0.09)
LumA	1/18 (0.06)	1/37 (0.03)
LumB	1/9 (0.11)	2/20 (0.1)
Normal	6/6 (0.67)	3/5 (0.6)

All patients

	ACT	AC
Basal	9/28 (0.32)	10/58 (0.17)
HER2	3/12 (0.25)	2/35 (0.06)
LumA	3/36 (0.1)	2/87 (0.02)
LumB	4/12 (0.33)	4/45 (0.09)
Normal	6/12 (0.5)	4/18 (0.22)

Multiagent Chemotherapy Response

Subtype	Pathologic Complete Response Rate				Overall Prevalence
	NSABP B-27	ISPY	Hess et al	All Studies	
Luminal A	0/26 (0%)	2/39 (5%)	0/36 (0%)	2/101 (2%)	27%
Luminal B	1/7 (13%)	4/26 (13%)	5/22 (19%)	10/55 (15%)	17%
Her2-enriched	1/10 (9%)	12/10 (55%)	12/17 (41%)	25/37 (40%)	16%
Basal-like	13/41 (24%)	15/29 (34%)	16/11 (59%)	44/81 (35%)	33%
Normal-like	3/1 (75%)	3/3 (50%)	1/13 (7%)	7/17 (29%)	6%
All Subjects	18/85 (17%)	36/107 (25%)	34/99 (26%)	88/291 (23%)	

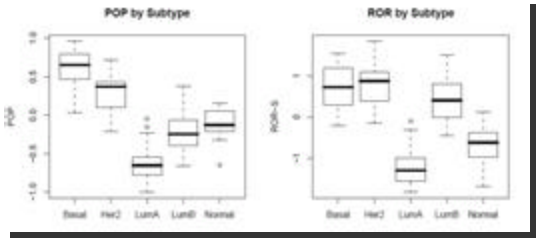
Different multiagent regimens
- NSABP B-27: AC or AC/T
- Hess et al: T/FAC
- ISPY:

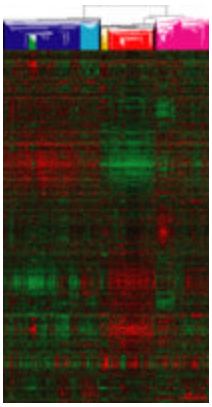
Hess et al., JCO 2006
Beat et al., JCO 2003

Subtype Frequencies Across 13 Studies

	Luminal A	Luminal B	Her2-enriched	Basal-like	Normal-like	Total	Outcome	Platform
CALGB9840	17	36	21	30	8	112	OS, RFS	DASL
CALGB9342	24	12	12	20	1	69	OS, RFS	DASL
NSABP B-27	97	66	47	86	30	326	pCR, OS	Affymetrix U133+2
XENA	44	25	25	45	15	154	pCR	Agilent1x44k
US Oncology	28	17	17	29	7	98	pCR	Affymetrix U133A
ISPY	34	21	22	38	9	124	pCR, OS, RFS	Agilent1x44k
TAM	370	346	66	8	16	806	OS, DSS, RFS	qRT-PCR
van de Vijver et al.	89	70	53	53	30	295	OS, RFS	Agilent
Ivshina et al.	91	67	36	30	21	245	RFS	Affymetrix U133A
Hess et al.	39	22	29	31	12	133	pCR	Affymetrix U133A
Wang et al	78	70	45	60	33	286	RFS	Affymetrix U133A
Loi et al.	145	135	44	37	33	394	RFS	Affymetrix U133A & U133+2
UNC	91	65	61	117	44	378	OS, RFS	Agilent
Total	1147	952	478	584	259	3420		
Total	33%	27%	14%	18%	8%			

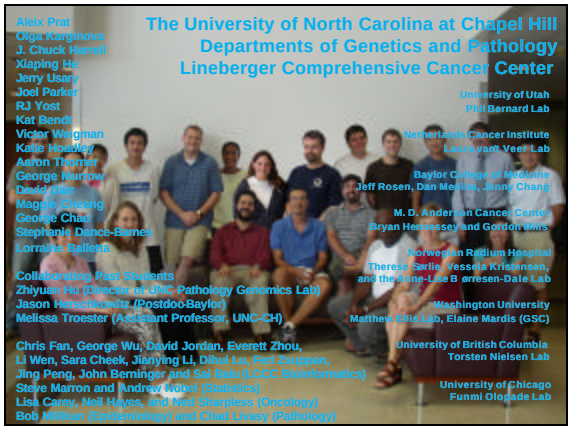
Subtype Distribution of POP Differs from ROR-S





Genomic Assay Conclusions

1. The subgroup of ER+ and node-negative patients are the group for whom prognostic gene expression assays are of the most value.
2. Genomic assays like the PAM50 Intrinsic Subtypes, OncotypeDX, Mammaprint, and the Theros Breast Cancer Index, are providing new and valuable information that is not provided by the standard clinical variables.
3. The best treatment plan is guided by a combination of conventional clinical assays (ER status, node status and tumor size), and genomic biomarkers, and the two are complementary.
4. Genetic biomarkers (like CYP2D6 for tamoxifen responsiveness) are important and may also come into standard use.


The University of North Carolina at Chapel Hill
Departments of Genetics and Pathology
Lineberger Comprehensive Cancer Center
Aleix Prat
Olga Kurginova
J. Chuck Hansell
Xiaping He
Jerry Usary
Joel Parker
RJ Yost
Kat Bendt
Victor Weigman
Katie Hoadley
Aaron Thorner
George Murrow
David Parker
Magdie Cheong
George Chao
Stephanie Dance-Barnes
Lorraine Balletta
Collaborating Past Students
Zhiyuan Hu (Director of UNC Pathology Genomics Lab)
Jason Herschkowitz (Postdoc-Baylor)
Melissa Troester (Assistant Professor, UNC-CH)
Chris Fan, George Wu, David Jordan, Everett Zhou,
Li Wen, Sara Cheek, Jianying Li, Dihui Lu, Fei Zhaoyan,
Jing Peng, John Bernlinger and Sol Rohm (LCCC Bioinformatics)
Steve Marron and Andrew Nobel (Statistics)
Lisa Carey, Neil Hayes, and Ned Sharpless (Oncology)
Bob Millikan (Epidemiology) and Chad Livasy (Pathology)
University of Utah
Phil Bernard Lab
Netherlands Cancer Institute
Laura van Veer Lab
Baylor College of Medicine
Jeff Rosen, Dan Medina, Jenny Chang
M. D. Anderson Cancer Center
Bryan Hennessey and Gordon Mills
Norwegian Radium Hospital
Therese Sørlie, Vessela Kristensen,
and the Anne-Lise Børresen-Dale Lab
Washington University
Matthew Ellis Lab, Elaine Mardis (GSC)
University of British Columbia
Torsten Nielsen Lab
University of Chicago
Funmi Olopade Lab