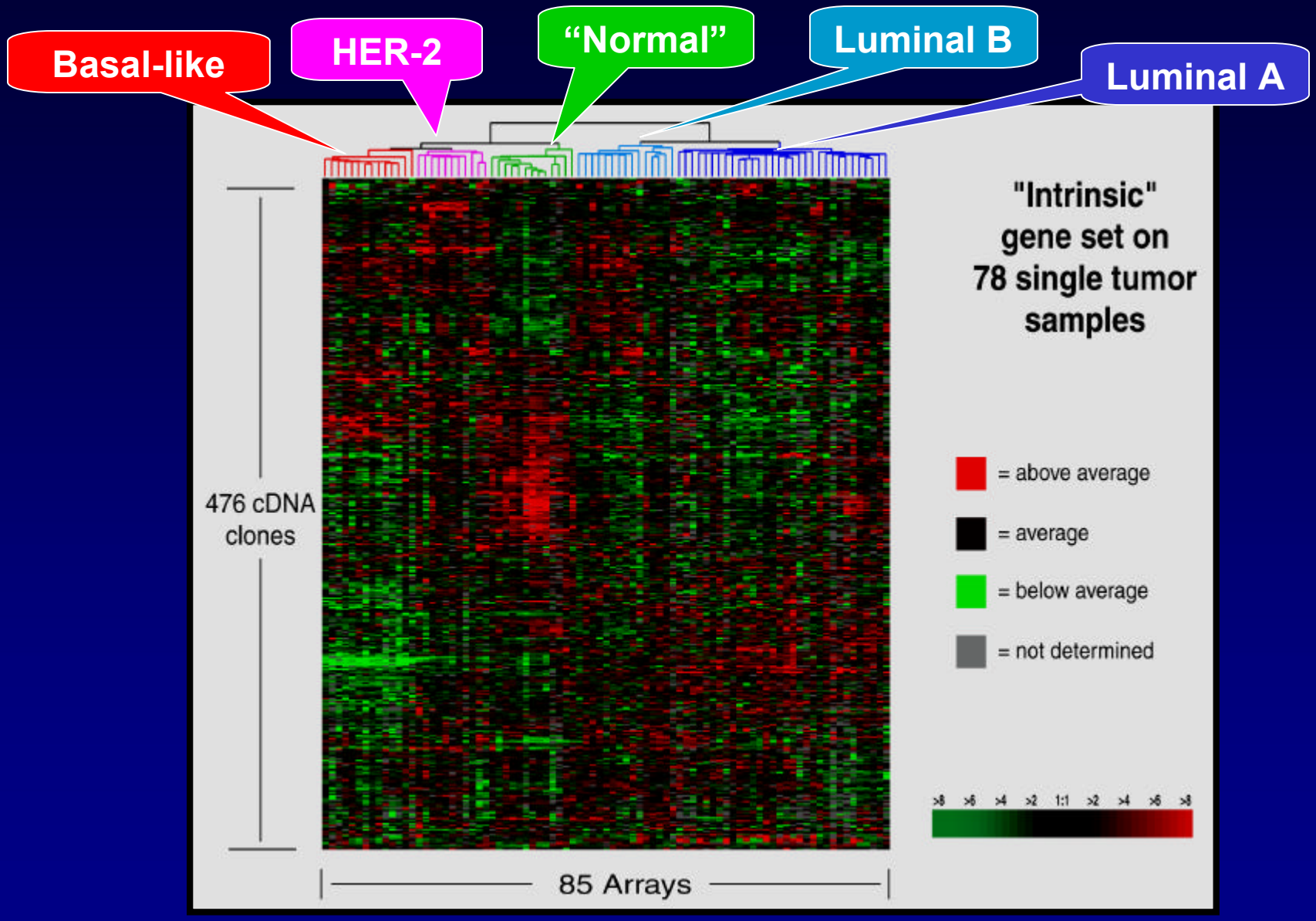
MOLECULAR PROFILING, PROGNOSTIC AND PREDICTIVE BIOMARKER AND GENOMIC STRATEGIES FOR RATIONAL COMBINATIONS OF TARGETED THERAPIES

Brian Leyland-Jones, M.D., Ph.D.

Molecular Portrait of Breast Cancers



Sorlie T et al, PNAS 2001

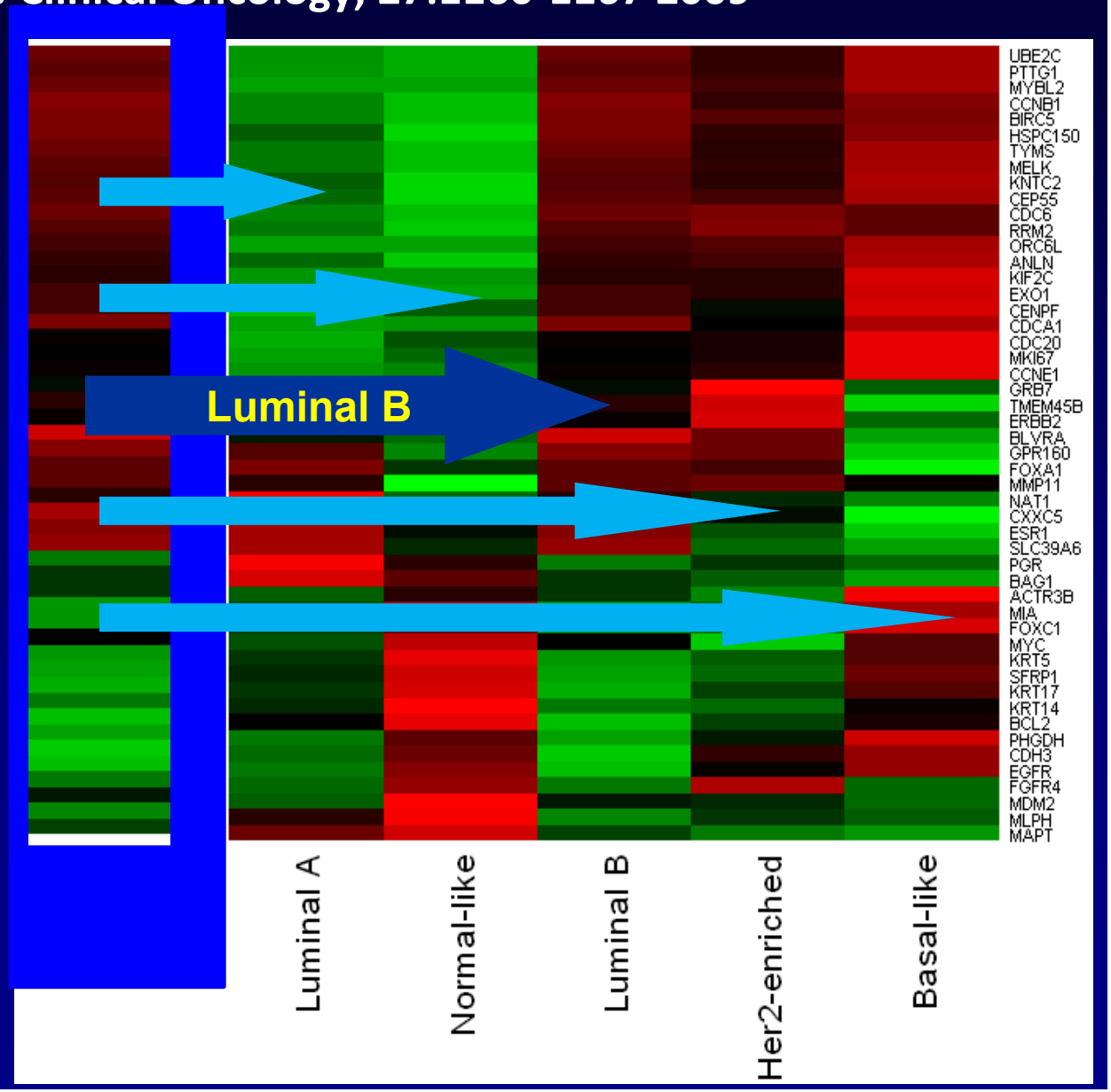
1. PROGRESSIVE MOVEMENT TO MOLECULAR SUBSETTING OF TUMOURS, AND, FROM "PATTERNS" TO INDIVIDUAL DRIVERS

Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes

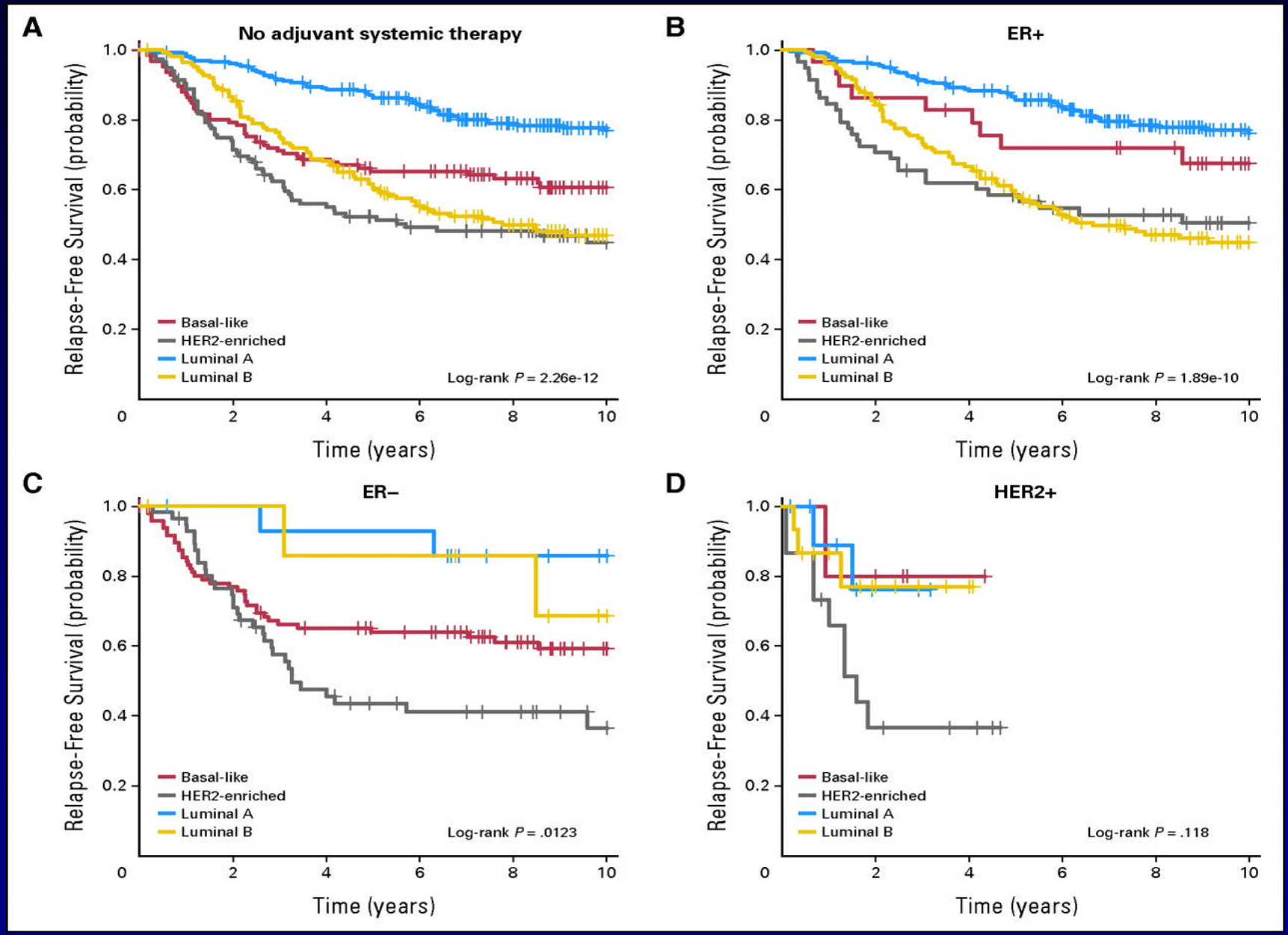
Parker et al., J Clinical Oncology; 27:1160-1167 2009

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



710 node-negative, no systemic adjuvant treatment patients



Parker et al. J Clin Oncol; 27:1160-1167 2009

JOURNAL OF CLINICAL ONCOLOGY

PAM 50 GENE LISTS

Luminal B

- BCL2*
- CXXC5
- EGFR*
- KIF2C*
- MDM2*
- MKI67*
- ORC6L*
- PGR*
- PHGDH

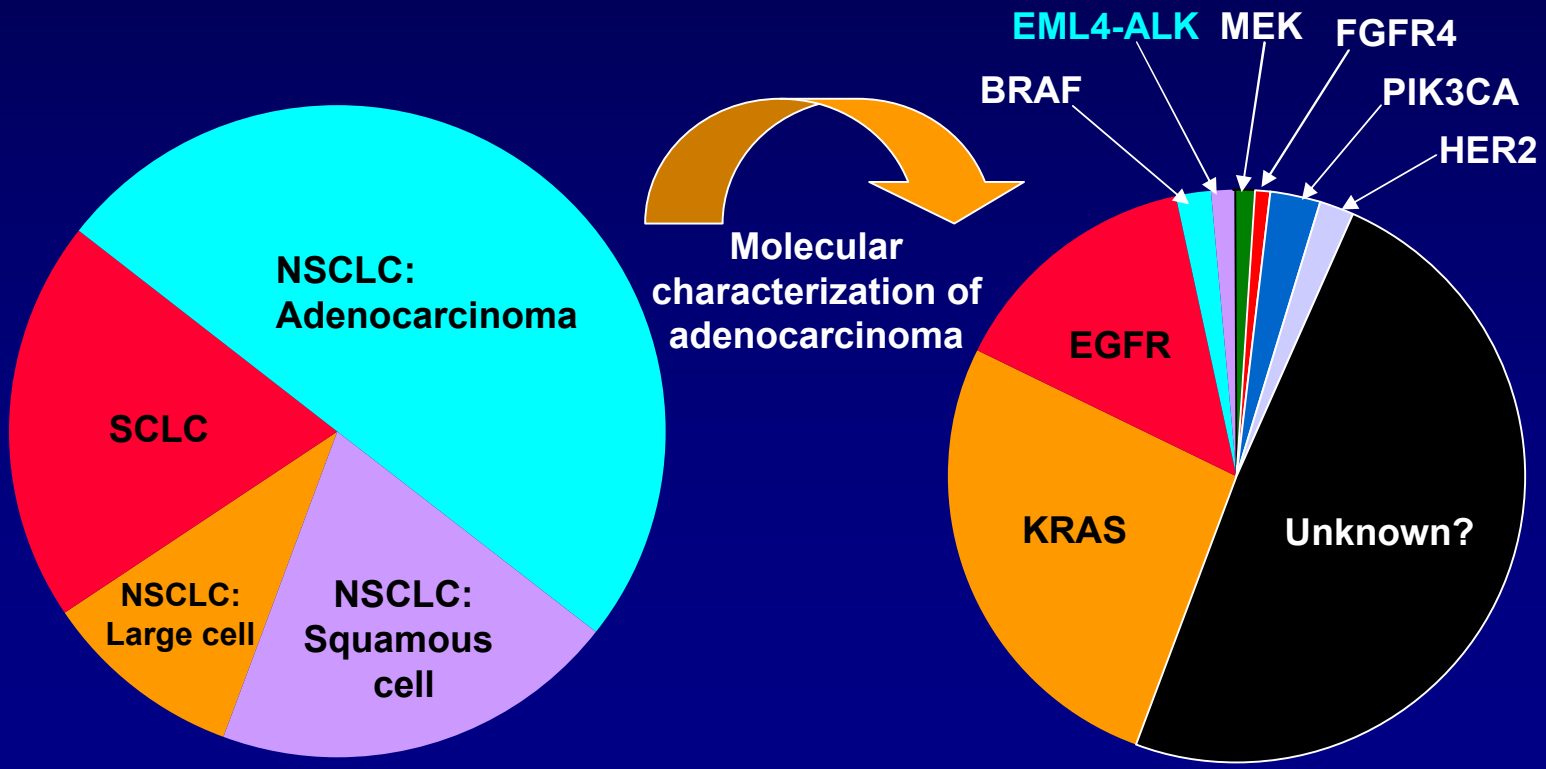
PAM 50 GENE LISTS

Basal-like

- ANLN
- CEP55
- ESR1*
- FOXA1*
- FOXC1*
- GPR160
- KNTC2*
- MELK*
- MIA
- TMEM45B

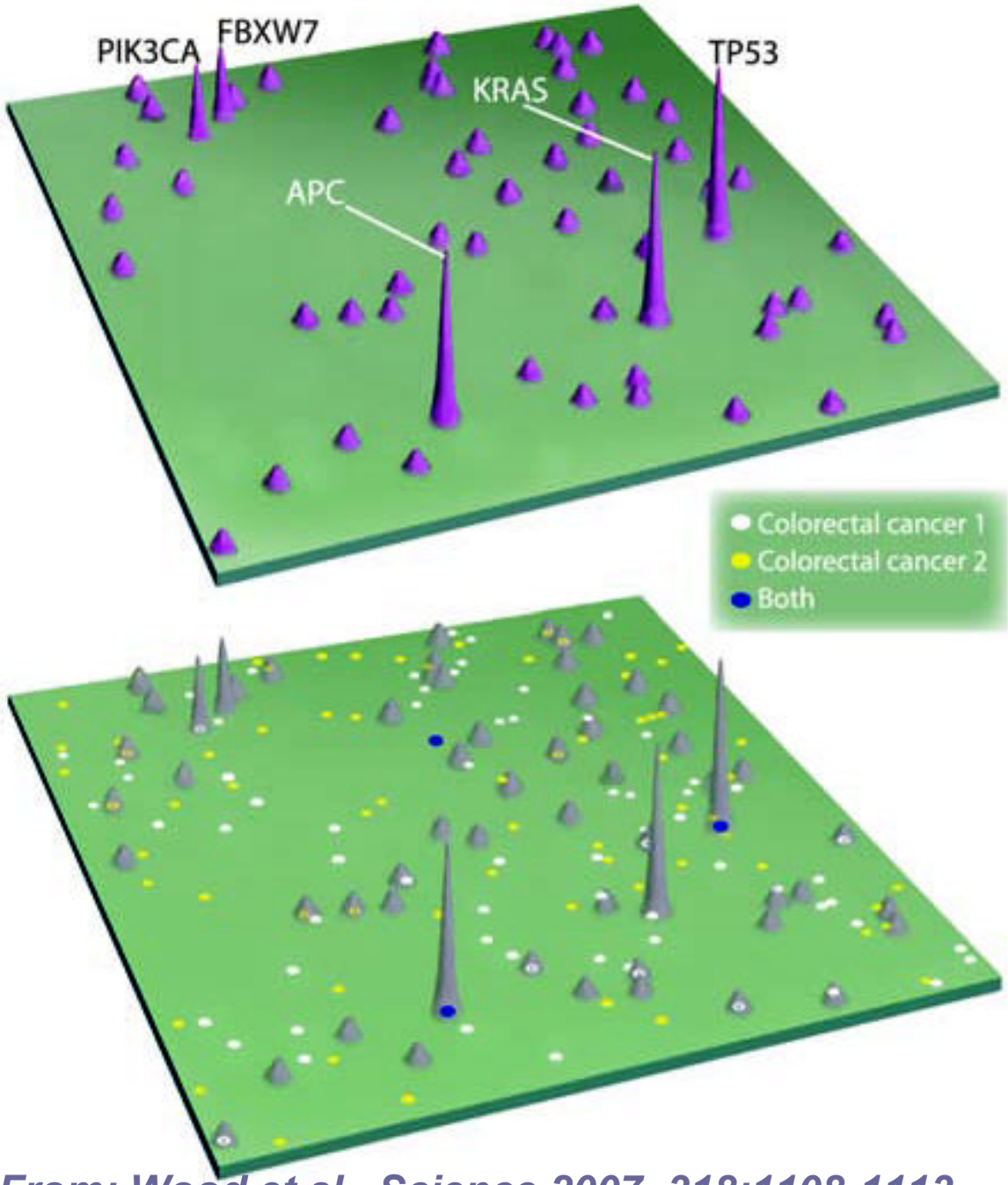
MOLECULAR SUBSETTING OF LUNG CANCER

- Insights into the complex biology of cancer is allowing identification of patients characterized by different presentation, natural history, and response to treatment



A TWO-DIMENSIONAL MAP OF GENES MUTATED IN COLO-RECTAL CANCERS

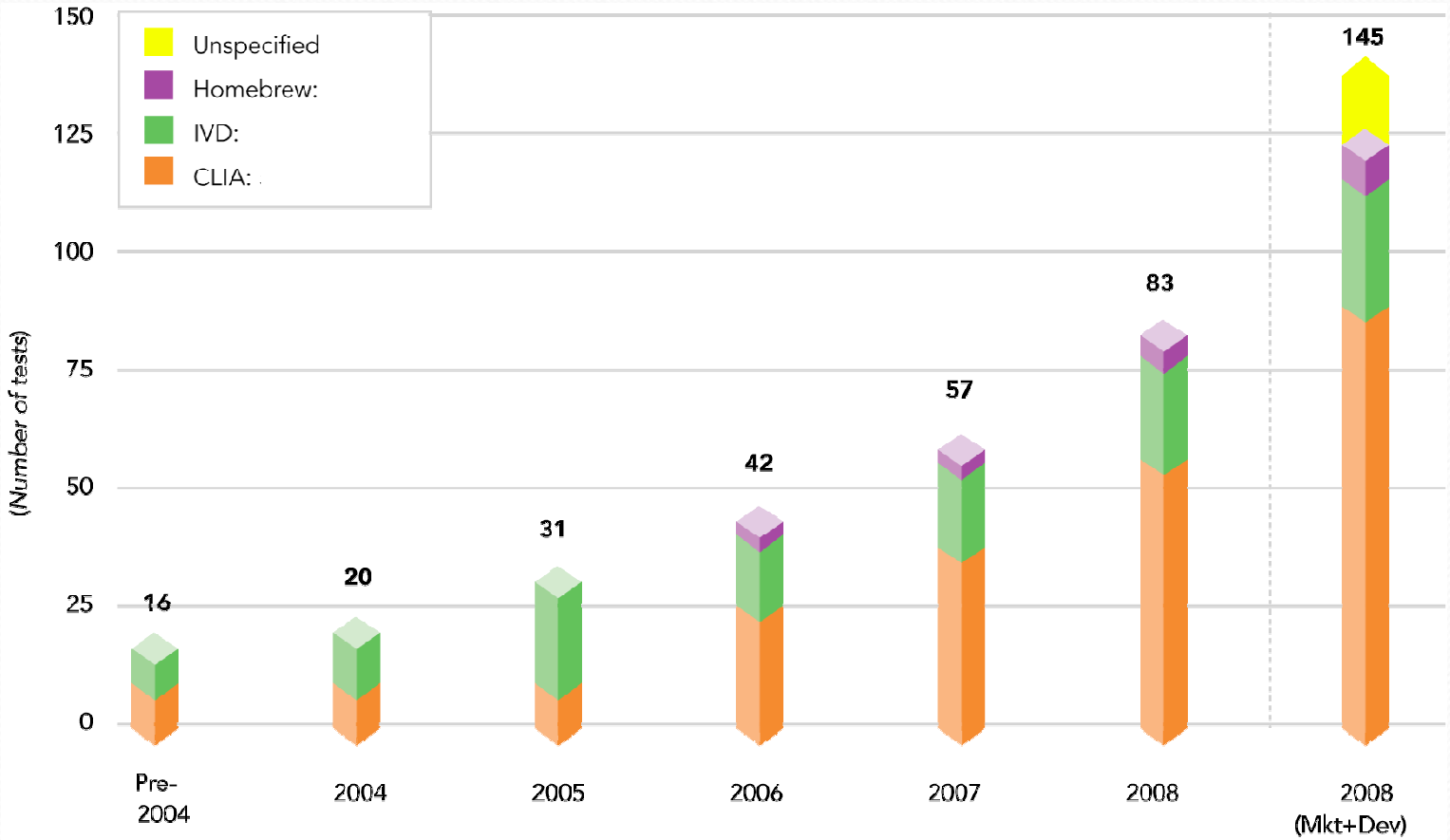
- gene "mountains" are mutated in a large proportion of tumors
- most "hills" are mutated infrequently
- mutations in two individual tumors are indicated on the lower map
- most mutations are outside hills or mountains and may be harmless



From: Wood et al., Science 2007, 318:1108-1113

Companion Diagnostics Leverage CLIA Lab Models

Cumulative Number of Companion Dx Tests by Business Model



* CAGR for Homebrew: 2006–2008 ** LDT Laboratory Developed Tests

***IVDMIA: In Vitro Diagnostic Multivariate Integrating Assay

Source: Company websites, SEC filings, Capital IQ, L.E.K analysis

Sample Pipeline

Pipeline supported by Barcoding System

Samples (e.g. blood, Saliva, FFPE Tissue) assigned barcode

DNA/RNA extraction using /Thermofisher FLEX

Quantitation using Thermofisher Nanodrop

Liquid handling - (e.g. DNA normalization & plate preparation of quantitated DNA with Beckman FX robotics)

Tecan EVO Liquid Handling

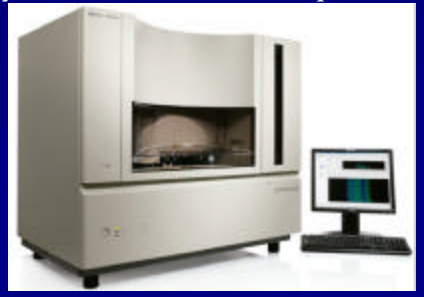
Agilent Mx3005P Realtime PCR System

ANALYSIS

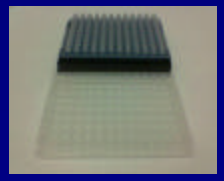
Luminex XMap System

Illumina BeadXpress

Illumina HiScanSQ

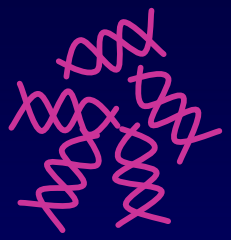


ABI 3730XL Sequencer



Daughter Microtiter plates

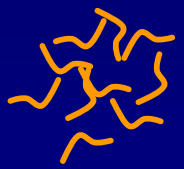
Molecular profiling of tumors

DNA  Genomic aberrations
Epigenomic alterations

Array-CGH
SNP analysis
Methylation profiling

RNA Expression changes

mRNA profiling

miRNA  Expression changes

miRNA profiling

NUCLEIC ACIDS → ARRAY PLATFORMS
DATA ANALYSIS → PROGNOSTIC BIOMARKERS
PREDICTIVE BIOMARKERS
THERAPEUTIC TARGETS

Initial Genomics and Epigenomics Profile

- Routine Biomarker Profiling Combination for Clinical Trials and Tumour Banks comprising:
  - Nucleic Acid Extractions and QC
  - 1. SNP and Copy Number Variation assays utilizing the Omni Product line. E.g. Omni -1 Quad Chip includes:
    - 22K ADME SNPs representing a core set of 300 genes for PGx
    - 20K MHC/HLA SNPs
    - SNPs in gene regions
    - Resolution to 1.2kb for CNV (especially useful for cancer studies)

    2. Whole Genome Methylation profiling using the Human Methylation27 BeadChip or new Array that includes 450K sites

    3. Whole Genome Expression using Human Expression BeadChip

Currently under Analysis

- TRANSHERA (with Mitch Dowsett); TRASTUZUMAB
- E2100 (with Bryan Schneider and George Sledge); BEVACIZUMAB
- E2197 (with Joe Sparano); TAXANES
- IBCSG VIII and IX (with Beppe Viale and Aron Goldhirsch): TAMOXIFEN – CMF -GOSERELIN
- BIG 1-98 (with Jimmy Rae); TAMOXIFEN
- SUNNYBROOK PROSTATE TUMOUR BANK (with Arun Seth)
- EMORY PROSTATE TUMOUR BANK (with John Petros, Leland Chung and Carlos Moreno)
- BAYLOR NEOADJUVANT AC vs TAXOTERE (through DOD COE-1A with Jenny Chang and George Sledge)
- POLAND CAPECITABINE METASTATIC DATA SET (through DOD COE-05 with George Sledge)
- GRADY AA ADJUVANT DATA SET (with Gabriella Oprea and Ruth O'Regan)

2. IN PARALLEL…

MOUSE MODELS TO DETERMINE OPTIMAL COMBINATIONS OF 2-4 TARGETED AGENTS

Hormone Receptor Positive Disease:

Preclinical Evaluation of a VEGF Receptor Tyrosine Kinase Inhibitor, in Combination with an Aromatase Inhibitor, and an IGFIR monoclonal antibody in Post-Menopausal Breast Cancer Model

HORMONE POSITIVE: in vivo study (MCF-7 Ca (Ac1):

Group 1: Control, 4-androstenedione and carboxymethylcellulose
Group 2: Aromasin (100 µg/day) (21)
Group 3: Aromasin (250 µg/day) (21)
Group 4: Sutent (20 mg/kg) (22, 23)
Group 5: Sutent (40 mg/kg) (24)
Group 6: IGFIR mAb (single injection i.p. 62.5 µg) (25)
Group 7: IGFIR mAb (single injection i.p. 125 µg) (25)
Group 8: Aromasin (100 µg/day) + Sutent (20 mg/kg)
Group 9: Aromasin (250 µg/day) + Sutent (20 mg/kg)
Group 10: Aromasin (100 µg/day) + Sutent (40 mg/kg)
Group 11: Aromasin (250 µg/day) + Sutent (40 mg/kg)
Group 12: Aromasin (100 µg/day) + IGFIR mAb (62.5 µg)
Group 13: Aromasin (250 µg/day) + IGFIR mAb (125 µg)
Group 14: Aromasin (100 µg/day) + Sutent (20 mg/kg) + IGFIR mAb (62.5 µg)
Group 15: Aromasin (250 µg/day) + Sutent (40 mg/kg) + IGFIR mAb (125 µg)

Her2 OverExpressing Disease:

Preclinical Evaluation of different combination strategies targeting HER2, VEGF, PI3 K, mTOR and HDAC in Her-2 Overexpressing Breast Cancer Models: BT474 and Bt474 HerR

HER2 POSITIVE: in vivo(BT474 HR):

Group1: Control Saline, 100µl, i.p., twice weekly for 8 weeks.
Group2: Control IgG: 3mg/kg, i.p., twice weekly for 8 weeks.
Group3: Herceptin 15mg/kg, i.p., twice weekly for 8 weeks.
Group4: TDM1 15mg/kg, i.v., every 3 week (total 3 injections).
Group5: IGF-1R mAb (R1507), 6mg/kg, i.p. once weekly for 8 weeks
Group6: Pertuzumab, 15mg/kg (after first treatment), i.p., once weekly for 8 weeks.
Group7: rhuMAb IGF-1R (R1507) + Herceptin
Group8: rhuMAb IGF-1R (R1507) + TDM1
Group9: rhuMAb IGF-1R (R1507) + Pertuzumab
Group10: Herceptin + Pertuzumab
Group11: TDM1 + Pertuzumab
Group12: rhuMAb IGF-1R (R1507) + Herceptin + Pertuzumab
Group13: rhuMAb IGF-1R (R1507) + TDM1 + Pertuzumab

Triple Negative Disease:

Preclinical evaluation of the anti-tumor activity of a PI3k inhibitor and anti-angiogenic agent in combination with a PARP inhibitor and carboplatin in triple negative subset of breast cancer

TRIPLE NEGATIVE; IN VIVO (MDA-MB-486 +231):

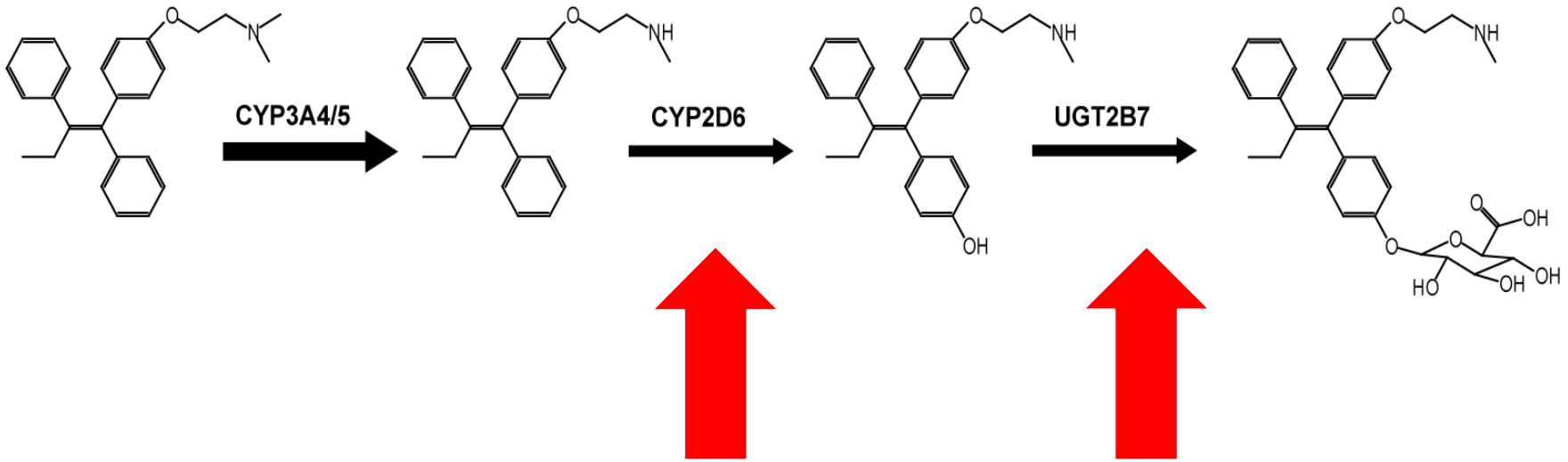
Arm 1: Vehicle Control group: Vehicle for Carboplatin, saline
Arm 2: Vehicle Control group: Vehicle for BEZ235 and RAF265, 10% NMP-90%PEG, p.o. once daily for 3 weeks
Arm 3: Carboplatin alone: 40mg/Kg, i.p. daily for 3 weeks
Arm 4: BEZ235 alone: 25-50mg/kg, in10% NMP-90%PEG, p.o. once daily for 3 weeks
Arm 5: RAF265 alone: 25-50mg/kg, in10% NMP-90%PEG, p.o. once daily for 3 weeks
Arm 6: Carboplatin + BEZ235: Carboplatin (40mg/Kg, i.p. daily) + BEZ235 (25-50mg/kg, in10% NMP-90%PEG, p.o. once daily) for 3 weeks
Arm 7: Carboplatin + RAF265: Carboplatin (40mg/Kg, i.p. daily) + RAF265 (25-50mg/kg, in10% NMP-90%PEG, p.o. once daily) for 3 weeks
Arm 8: BEZ235 + RAF265: BEZ235 (25-50mg/kg, in10% NMP-90%PEG, p.o. once daily) + RAF265 (25-50mg/kg, in10% NMP-90%PEG, p.o. once daily) for 3 weeks
Arm 9: Carboplatin + BEZ235 + RAF265: Carboplatin (40mg/Kg, i.p. daily) + BEZ235 (25-50mg/kg, in10% NMP-90%PEG, p.o. once daily) + RAF265 (25-50mg/kg, in10% NMP-90%PEG, p.o. once daily) for 3 weeks

3. LARGE TRIALS ARE CRITICAL

Enzymes with Known Genetic Variants are Responsible for Endoxifen Production and Elimination

Tamoxifen **N-desmethyl- TM** **ENDOXIFEN** **Endox-O- Glu**

Weak anti-E2 **Weak anti-E2** **Potent anti-E2** **Inactive**



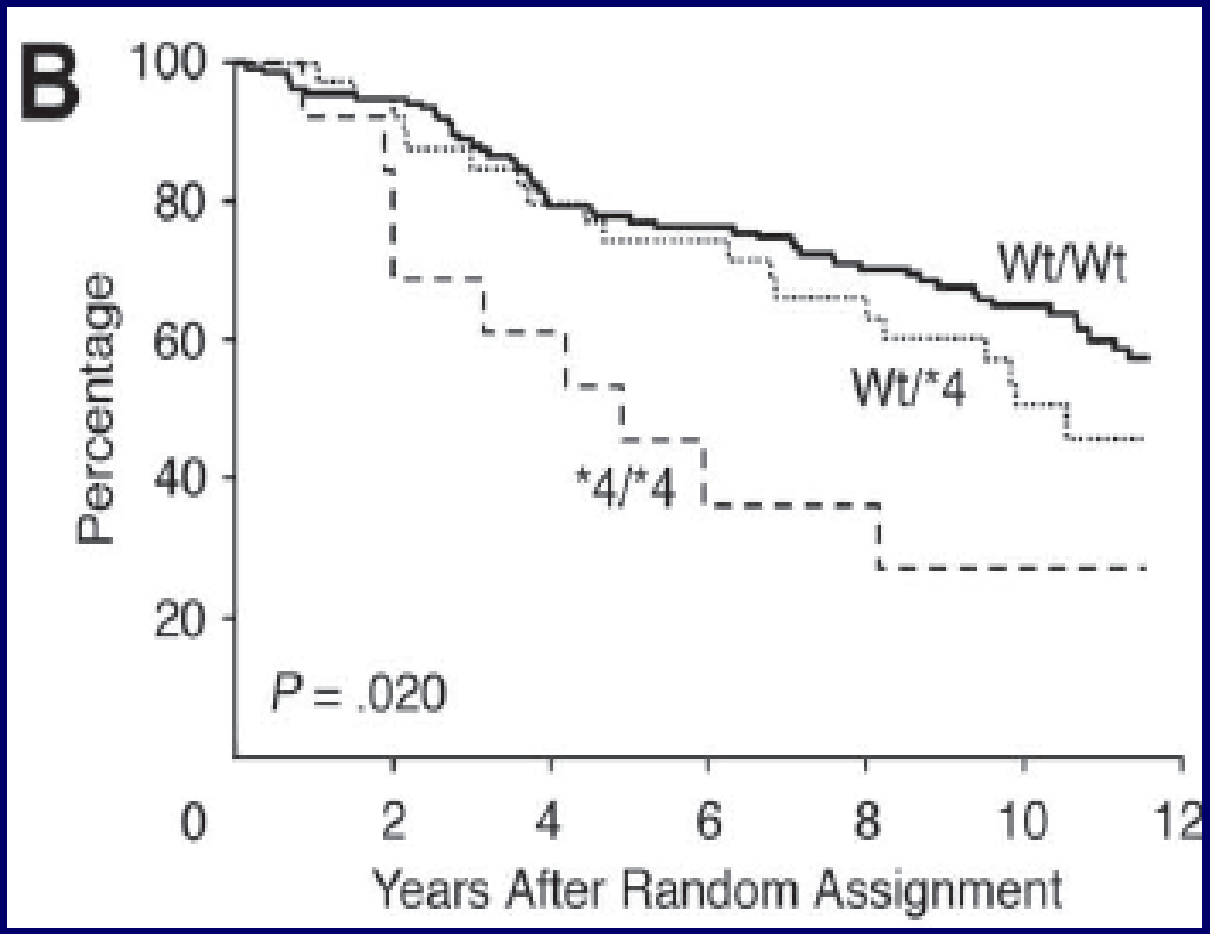
Known Genetic Variants

Rae, J.M

University of Michigan Comprehensive Cancer Center

North Central Cancer Treatment Group Adjuvant Breast Cancer Trial 89-30-52

Tam alone - 257 eligible pts, 228 FFPE, 190



- High Endoxifen
- Med Endoxifen
- Low Endoxifen

Goetz, MP, et al. J Clin Oncol. 23:9312, 2005

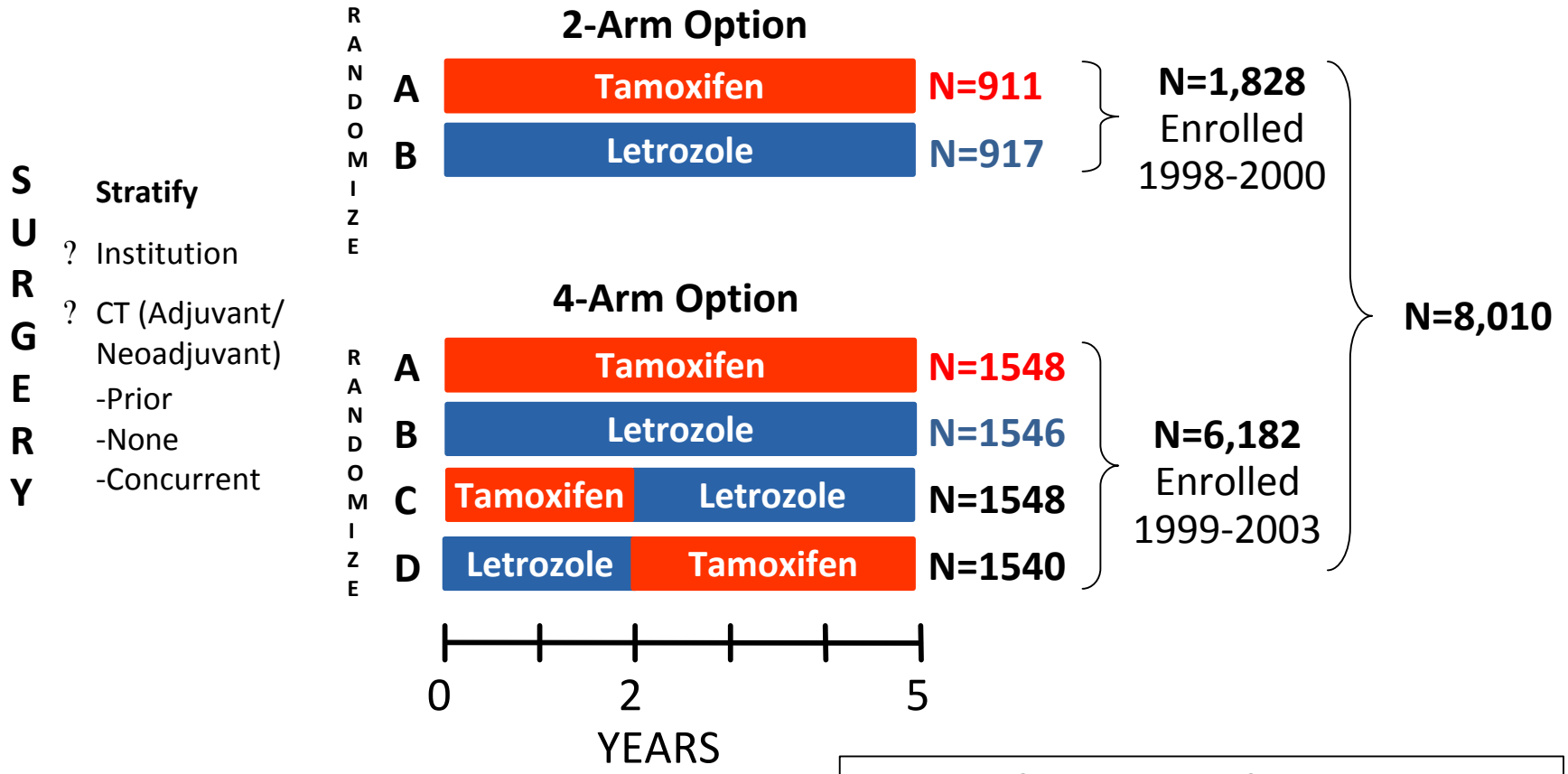
Rae, J.M

University of Michigan Comprehensive Cancer Center

Controversy: CYP2D6 enzyme activity and tamoxifen clinical outcome

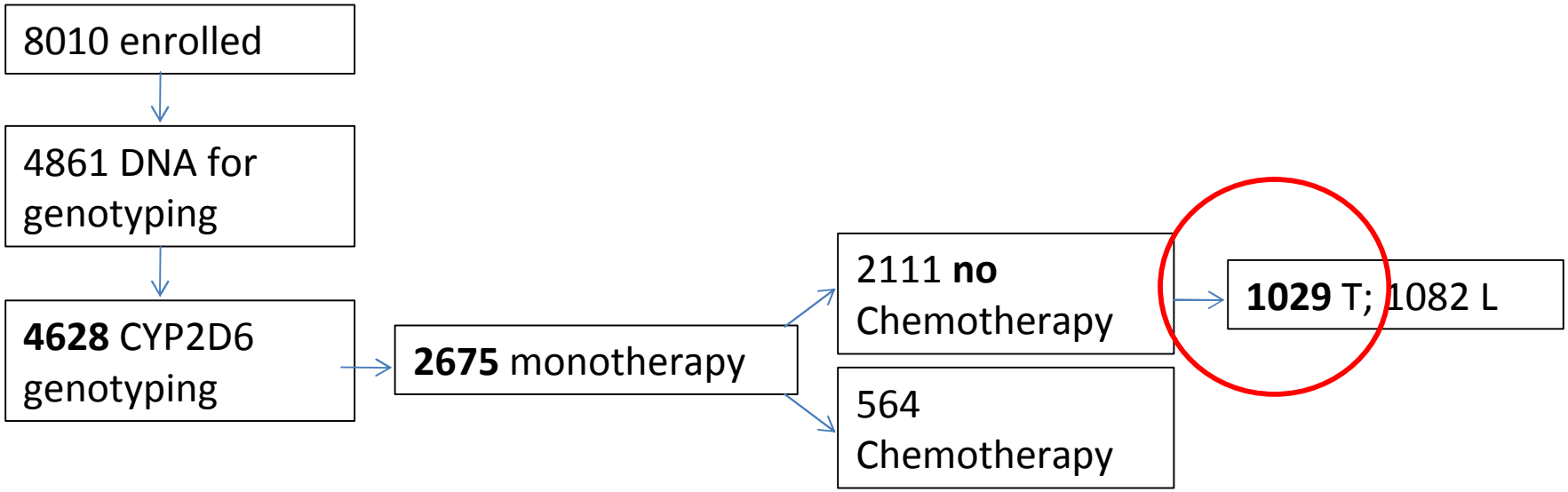
- **Between-study variability in**
  - **definition of metabolism; spectrum of CYP2D6 alleles tested, determination of inhibitor use, determination of duration of inhibitor use**
  - **Patient populations, study design (case studies, clinical trials, endpoints (DFS, DDFS, BR)**
- **Resulted in inconsistent findings**
- **Since, 2003, 178 publications indexed in PubMed**
  - **Positive association between CYP2D6 genotype or inhibitor use with clinical outcome (14 separate studies)**
  - **No association between CYP2D6 genotype or drug inhibitor use and breast cancer outcome (15 studies)**

BIG 1-98 Design



Monotherapy Population N=4,922
(2459 T; 2463 L)
76 Months Median Follow-Up

BIG 1-98 Analytic Cohort



- 98% Caucasian
- Worldwide collaborative group
- 57% node-negative
- ER+ (=1% immunostained cells by central review)
- 77% no chemotherapy

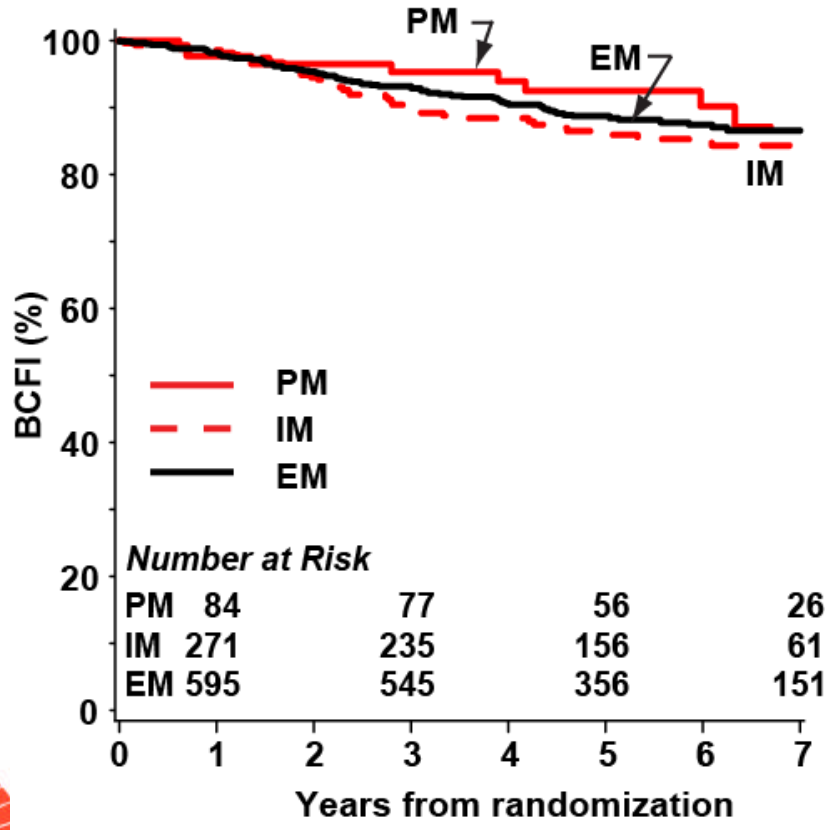
Genotyping Methods

CYP2D6 Allele	N Patients	Allele Frequency
Null Function		
*3	3012	1.3 %
*4	3828	18.9 %
*6	2707	1.9 %
*7	2767	0.0 %
Reduced Function		
*10	0	
*17	0	
*41	3842	8.4 %

- DNA isolated from FFPE in IBCSG and Danish central labs (n=4861)
- Genotyping at CLIA Accredited Lab (Emory)
- CYP2D6 SNPs genotyped on ABI 7900 TaqMan {*4, *41} or Beckman SNPstream
- 4628 patients had some CYP2D6 genotyping, after excluding 54 patients with centrally-assessed ER-absent tumors

Outcome by CYP2D6

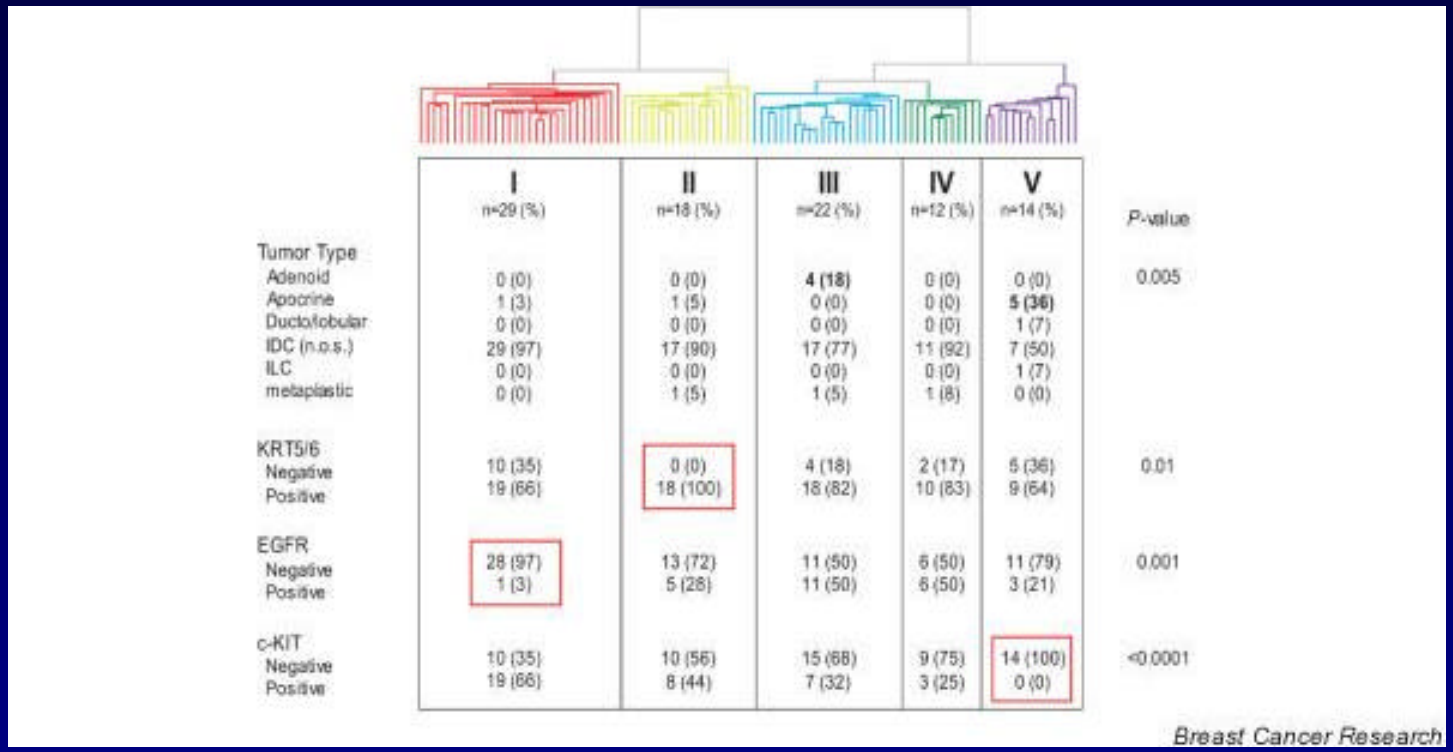
TAMOXIFEN
No Chemotherapy



	N	Events	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
PM	86	8	.71 (.34-1.46)	.58 (.28-1.21)
IM	277	40	1.22 (.83-1.79)	.95 (.50-1.40)
EM	610	75	1	1
			P=0.32	P=0.35

4. WHERE ARE WE NOW?

5 subclasses of the TN breast cancer subgroup have been thus far identified



	I n=29 (%)	II n=18 (%)	III n=22 (%)	IV n=12 (%)	V n=14 (%)	P-value
Tumor Type						
Adenoid	0 (0)	0 (0)	**4 (18)**	0 (0)	0 (0)	0.005
Apocrine	1 (3)	1 (5)	0 (0)	0 (0)	**5 (36)**	
Ducto/lobular	0 (0)	0 (0)	0 (0)	0 (0)	1 (7)	
IDC (n.o.s.)	29 (97)	17 (90)	17 (77)	11 (92)	7 (50)	
ILC	0 (0)	0 (0)	0 (0)	0 (0)	1 (7)	
metaplastic	0 (0)	1 (5)	1 (5)	1 (8)	0 (0)	
KRT5/6						
Negative	10 (35)	0 (0)	4 (18)	2 (17)	5 (36)	0.01
Positive	19 (66)	18 (100)	18 (82)	10 (83)	9 (64)	
EGFR						
Negative	28 (97)	13 (72)	11 (50)	6 (50)	11 (79)	0.001
Positive	1 (3)	5 (28)	11 (50)	6 (50)	3 (21)	
c-KIT						
Negative	10 (35)	10 (56)	15 (68)	9 (75)	14 (100)	<0.0001
Positive	19 (66)	8 (44)	7 (32)	3 (25)	0 (0)	

Breast Cancer Research

The majority of TN tumors do not express EGFR by IHC (at least 1% of cells staining positive). Only ~27% (26/97) expressed the EGFR protein in this study.

The majority do express KRT5/6. Only ~22% did not express KRT5/6 by IHC.

Kreike et al, Breast Cancer Res. 9: R65, 2007

BERGH: HER2

Immune response and tumor invasion have been suggested as the main molecular processes associated with prognosis in HER2-positive BC. This is supported by the HDPP signature, based on the coherent expression of immune response–associated genes selected from various gene signatures and overrepresentation of immune system processes in gene ontology analysis.

HDPP also includes several genes implicated in tumor invasion and metastasis (eg, *CXCR4, PLAU, CX3CR1, TGFBR3, and STAT5A)*

Lymphocytic infiltration has been reported to be an indication of good prognosis in early-stage HER2-positive tumors. In two independent data sets, we found that cluster 2 tumors showed higher percentage of invasive tumor cells and more frequently lower lymphocytic infiltration, suggesting that HDPP distinguishes a subgroup of aggressive HER2-positive tumors characterized by high invasive ability and low immune response, a feature that may have therapeutic implications.

BERGH: HER2

Although the mechanisms behind resistance to trastuzumab treatment still remains elusive, increased PI3K signaling and *IGF1R levels and observations* of lower lymphocytic infiltration have been reported in poor responders.

Furthermore, the low immune response phenotype of some HER2-positive tumors, predominantly classified as cluster 2 in different data sets, may indicate a reduced ability to invoke an antibody-dependent cellular cytotoxicity, suggested as one of the main mechanisms of trastuzumab activity.

Interestingly, these observations correlated with cluster 2 in the HDPP classification of a small HER2-positive data set preoperatively treated with trastuzumab and vinorelbine

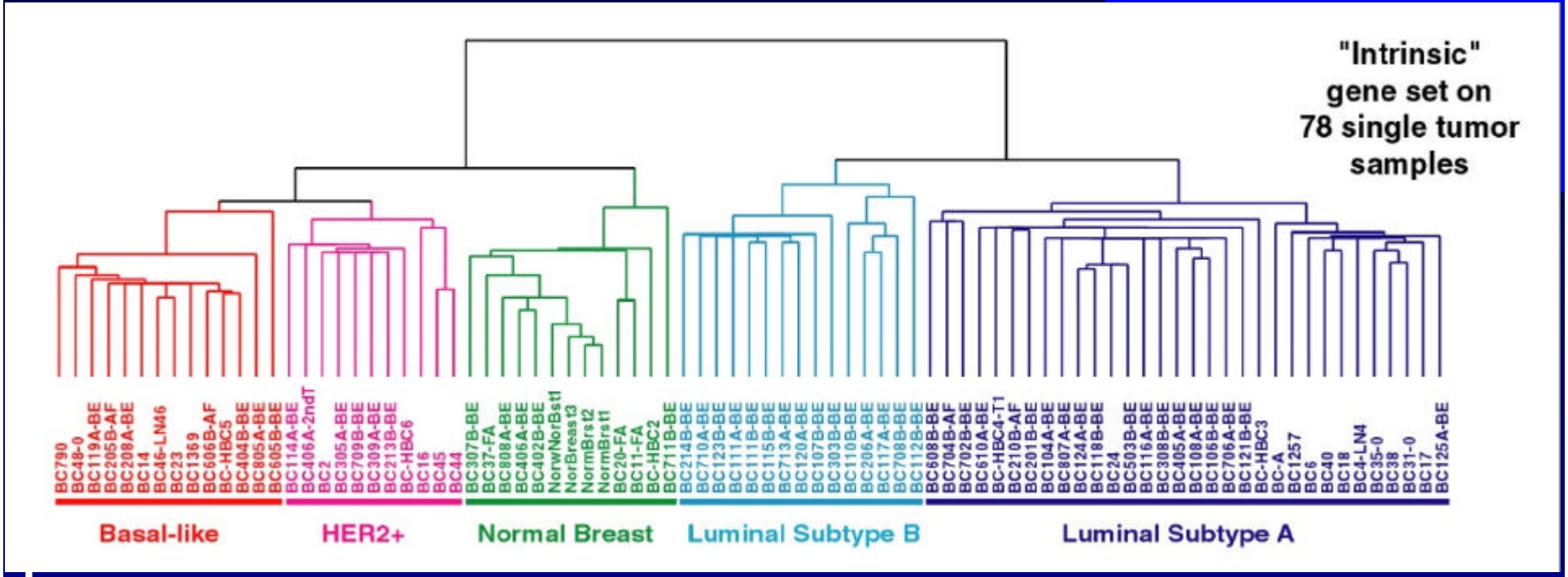
letters to nature

Molecular portraits of human breast tumours

Charles M. Perou*†, Therese Sørlie†‡, Michael B. Eisen*, Matt van de Rijn§, Stefanie S. Jeffrey‖, Christian A. Rees*, Jonathan R. Pollack¶, Douglas T. Ross¶, Hilde Johnsen‡, Lars A. Akslen#, Øystein Fluge☆, Alexander Pergamenschikov*, Cheryl Williams*, Shirley X. Zhu§, Per E. Lønning**, Anne-Lise Børresen-Dale‡, Patrick O. Brown¶†† & David Botstein*

Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications

Therese Sørlie[a,b,c], Charles M. Perou[a,d], Robert Tibshirani[e], Turid Aas[f], Stephanie Geisler[g], Hilde Johnsen[b], Trevor Hastie[e], Michael B. Eisen[h], Matt van de Rijn[i], Stefanie S. Jeffrey[i], Thor Thorsen[k], Hanne Quist[l], John C. Matese[c], Patrick O. Brown[m], David Botstein[c], Per Eystein Lønning[g], and Anne-Lise Børresen-Dale[b,n]



c.TNT

?PARPi

Herceptin, other anti-HER2

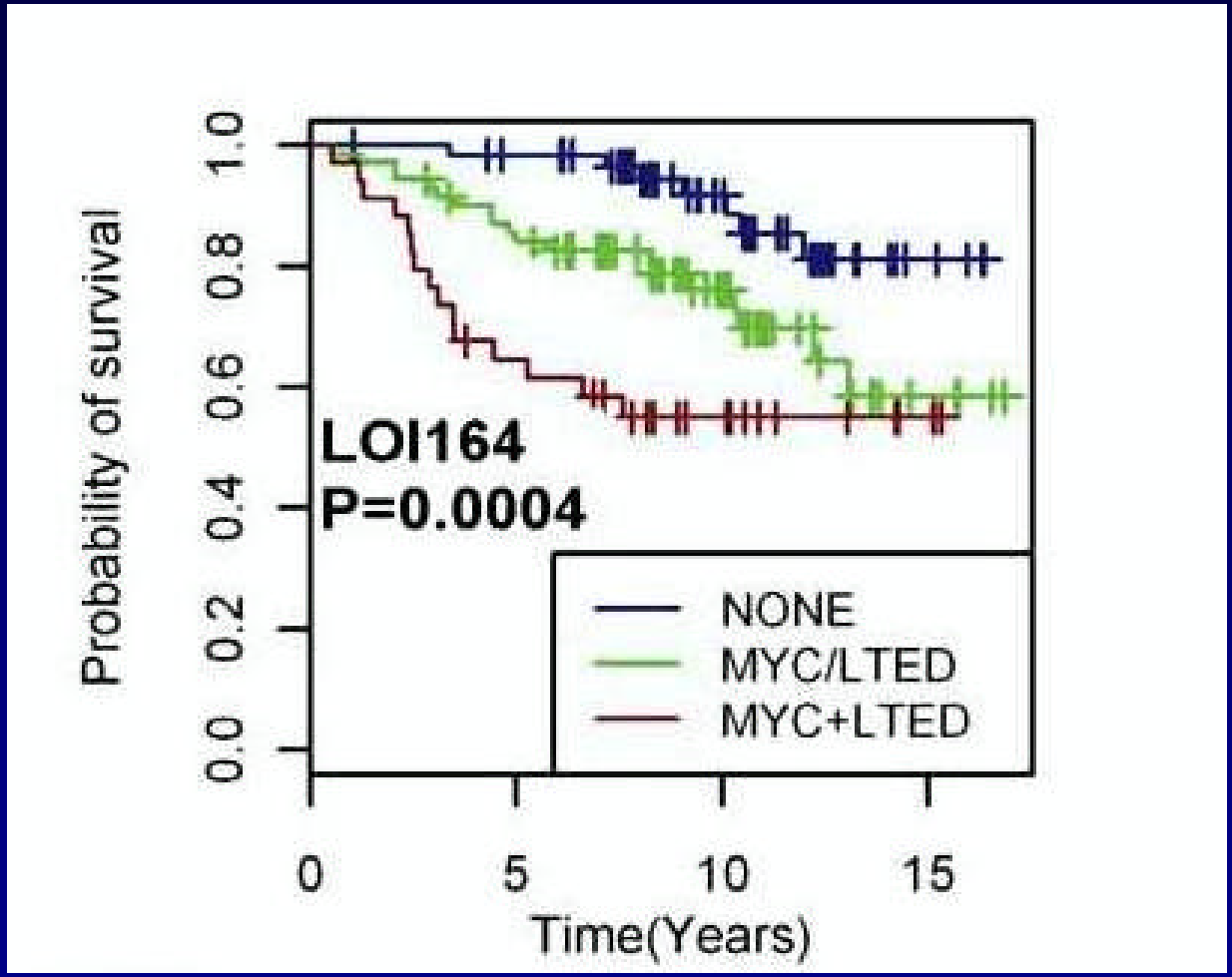
High proliferation

Endocrine + ?

Low proliferation

Endocrine

Coexpression of patterns of MYC activation and estrogen independence predicts poor outcome following adjuvant tamoxifen

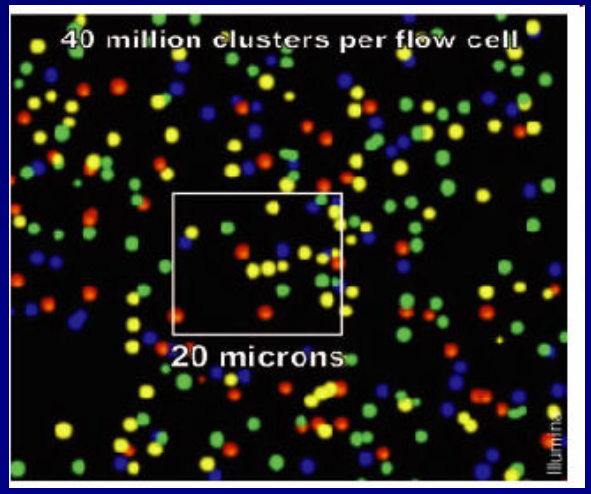


Vol 464|15 April 2010|doi:10.1038/nature08822 nature

Genome remodelling in a basal-like breast cancer metastasis and xenograft

Li Ding[1,2]*, Matthew J. Ellis[3,4]*, Shunqiang Li[3], David E. Larson[1], Ken Chen[1], John W. Wallis[1,2], Christopher C. Harris[1], Michael D. McLellan[1], Robert S. Fulton[1,2], Lucinda L. Fulton[1,2], Rachel M. Abbott[1], Jeremy Hoog[3], David J. Dooling[1,2], Daniel C. Koboldt[1], Heather Schmidt[1], Joelle Kalicki[1], Qunyuan Zhang[2,5], Lei Chen[1], Ling Lin[1], Michael C. Wendl[1,2], Joshua F. McMichael[1], Vincent J. Magrini[1,2], Lisa Cook[1], Sean D. McGrath[1], Tammi L. Vickery[1], Elizabeth Appelbaum[1], Katherine DeSchryver[3], Sherri Davies[3], Therese Guintoli[3], Li Lin[3], Robert Crowder[3], Yu Tao[6], Jacqueline E. Snider[3], Scott M. Smith[1], Adam F. Dukes[1], Gabriel E. Sanderson[1], Craig S. Pohl[1], Kim D. Delehaunty[1], Catrina C. Fronick[1], Kimberley A. Pape[1], Jerry S. Reed[1], Jody S. Robinson[1], Jennifer S. Hodges[1], William Schierding[1], Nathan D. Dees[1], Dong Shen[1], Devin P. Locke[1], Madeline E. Wiechert[1], James M. Eldred[1], Josh B. Peck[1], Benjamin J. Oberkfell[1], Justin T. Lolofie[1], Feiyu Du[1], Amy E. Hawkins[1], Michelle D. O'Laughlin[1], Kelly E. Bernard[1], Mark Cunningham[1], Glendoria Elliott[1], Mark D. Mason[1], Dominic M. Thompson Jr[7], Jennifer L. Ivanovich[7], Paul J. Goodfellow[7], Charles M. Perou[8], George M. Weinstock[1,2], Rebecca Aft[7], Mark Watson[9], Timothy J. Ley[1,2,3,4], Richard K. Wilson[1,2,4] & Elaine R. Mardis[1,2,4]

Massively Parallel Sequencing (next-generation sequencing)



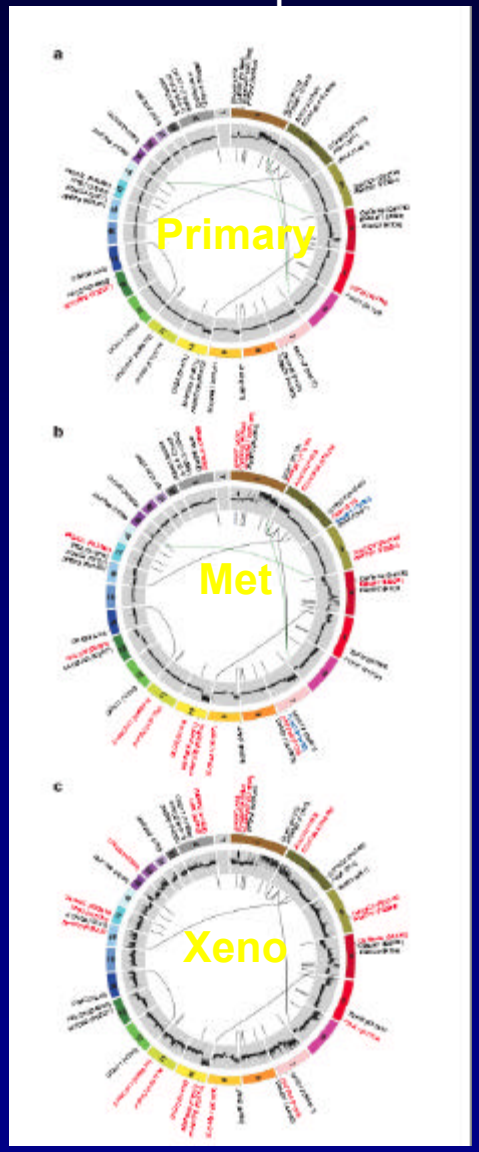
- **Sequenced blood, primary basal tumor, brain metastasis, and mouse xenograft from the primary AA**
- **First complete sequence of a basal tumor**

Mutation Spectrum

- 40 coding point mutations detected— 55% (C-G to A-T) — similar to complete sequence of a lobular tumor
- Several previously mutated genes were detected (JAK2, PTCH2, CSMD1, NRK)
- Several genes known to be important in signal transduction (MAPK3K8, PTPR, WWTR1)

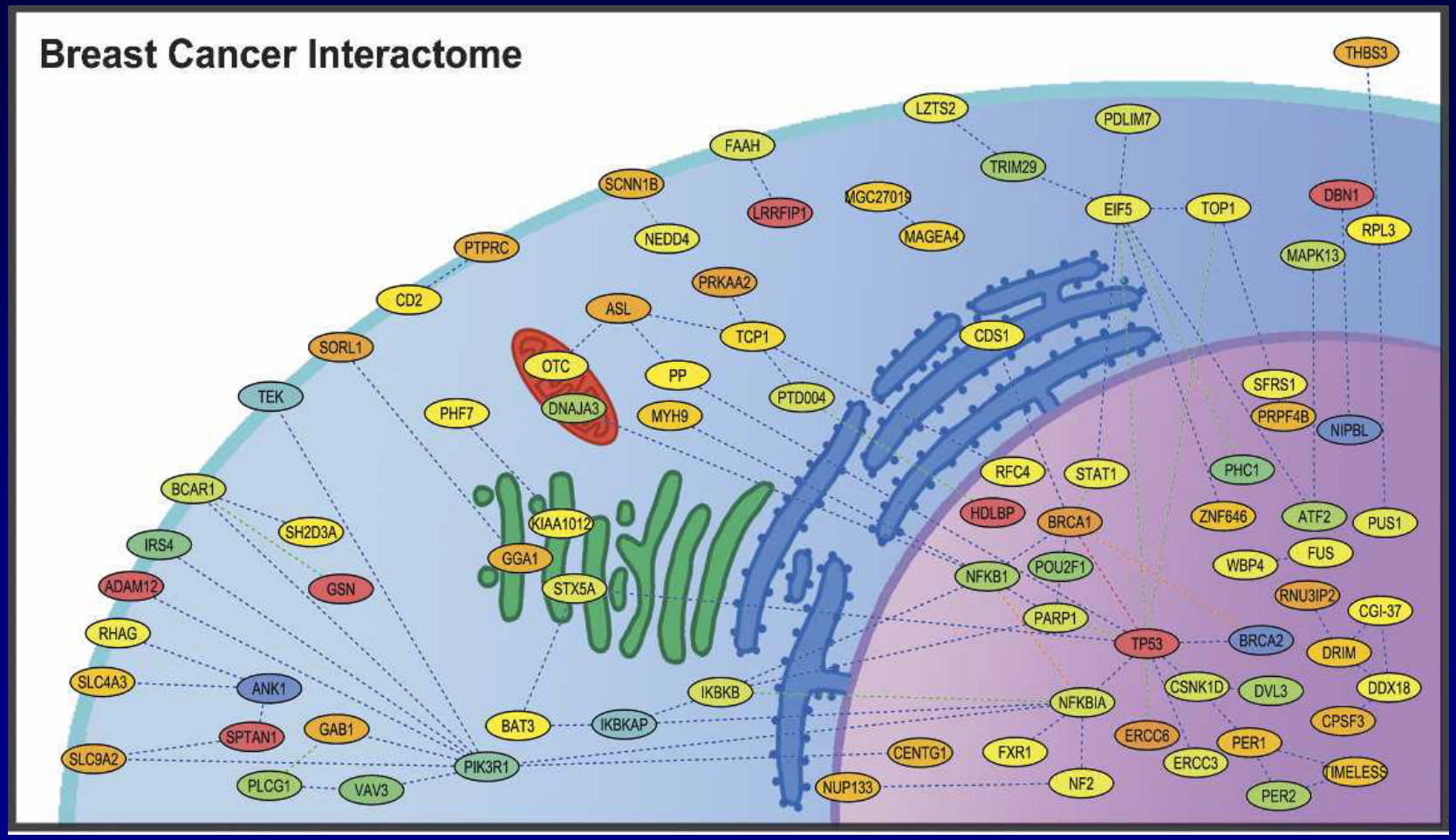
Considerable Heterogeneity in the Primary: Clonal Selection?

Circos plots



- Met shared 20 mutations with primary.
- Met contained 2 *de novo* mutations and large deletion not in primary.
- Xenograft from primary shared mutation spectrum with Met not primary.
- Suggests cellular selection during xenograft formation is similar to that during metastasis!

A multidimensional analysis of genes mutated in breast and colorectal cancers (Velculescu : GR 2007)



"Here's my sequence..."

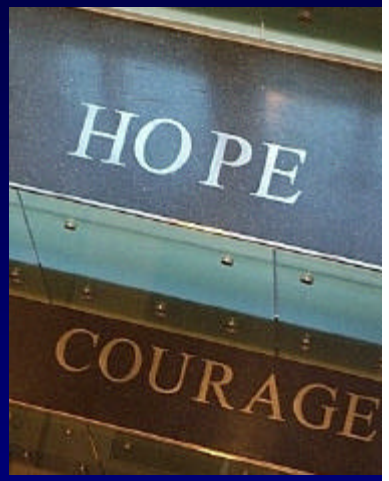
New Yorker

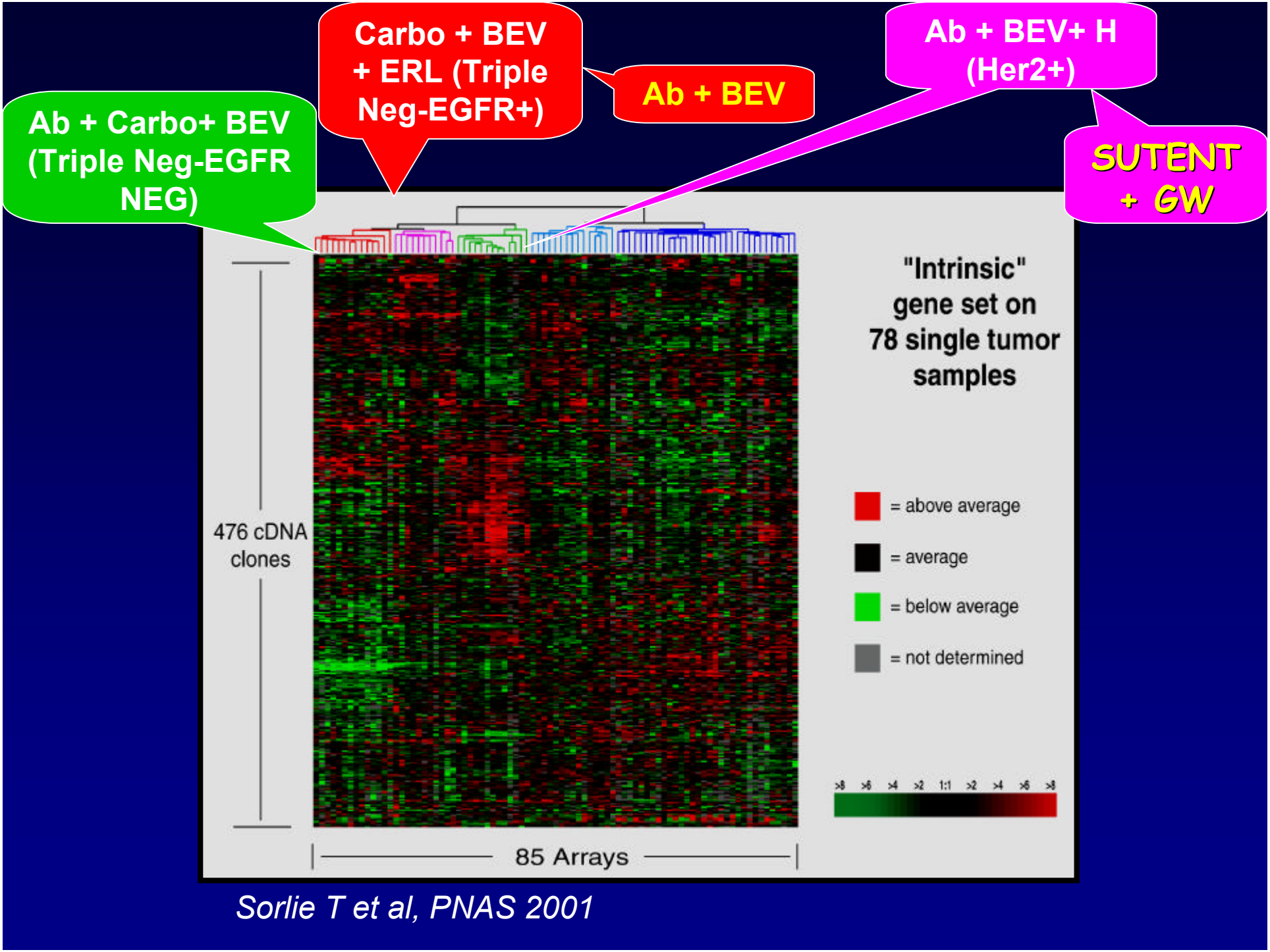
Optimal Combinations of Biologics

EMORY | WINSHIP CANCER INSTITUTE

Acknowledgements

- Emory Biomarker Core
- Georgia Research Alliance
- Georgia Cancer Coalition
- Winship Breast Translational Group
- Bostick Lab
  - Robin Bostick, Vaunita Cohen, Katerina Marks
- Winship Cell Imaging & Microscopy Core
- Winship Pathology Core
  - Dan Brat, Diane Alexis
- Yuliang Sun
- CCDx Core: Franklin Salisbury, Michael Wang, Sreekumar Kaimal, Kwok Leung





Sorlie T et al, PNAS 2001

Asian ethnicity and breast cancer subtypes: a study from the California Cancer Registry

Melinda L. Telli • Ellen T. Chang • Allison W. Kurian •
Theresa H. M. Keegan • Laura A. McClure • Daphne Lichtensztajn •
James M. Ford • Scarlett L. Gomez

8,140 Asian women:

Compared to non-Hispanic White women,

1. Korean (OR = 1.8, 95% CI = 1.5–2.2],
2. Filipina (OR = 1.3, 95% CI = 1.2–1.5),
3. Vietnamese (OR = 1.3, 95% CI = 1.1–1.6), and,
4. Chinese (OR = 1.1, 95% CI = 1.0–1.3)

…..women had a significantly increased risk of being diagnosed with HER2- positive breast cancer subtypes (after adjusting for age, stage, grade, socioeconomic status, histology, diagnosis year, nativity, and hospital ownership status.)

odds ratio (OR) confidence interval (CI)

PAM 50 GENE LISTS

Luminal A

- BIRC5*
- CDCA1
- CDH3*
- CENPF
- EXO1
- MAPT*
- MYBL2*
- NAT1*
- PTTG1
- SLC39A6*
- UBE2C

PAM 50 GENE LISTS

HER2-enriched

- ACTR3B
- BAG1*
- BLVRA
- CCNE1*
- CDC20*
- ERBB2*
- FGFR4*
- GRB7*
- MYC*
- SFRP*1

Histological Grade and Breast Cancer Biology
PATTERN of TUMOR MARKERS

Low Grade

Positively correlated to ER + status:

PGR
TFF1
CDH1
DSP
MDM2
NME1
CCND1
TJP1...

High Grade

Positively correlated to ER - status:

CDKN2A
CCNE
EGFR
ERBB2
SERPINE1
PLAU
HXB
CDH3...

Lacroix et al, ERC 2004

LUNG CANCER

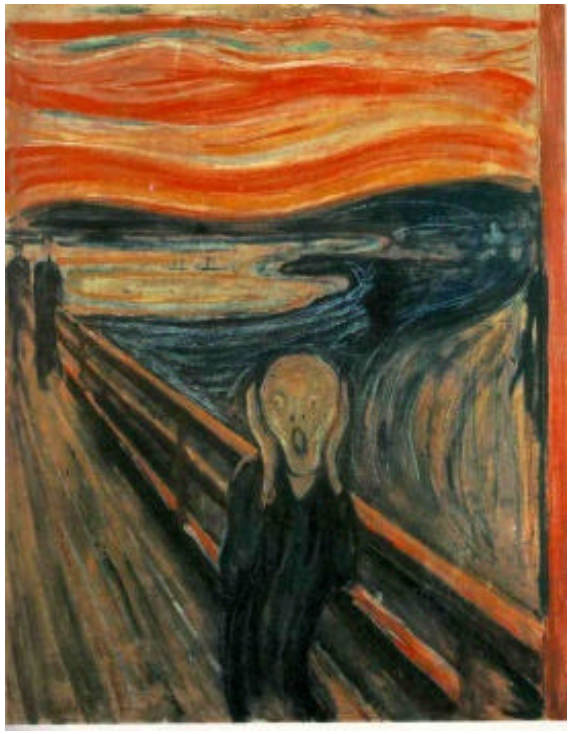
- EGFR MUTATION: Gefitinib
  EGFR W/T: Chemo

- Low ERCC 1: Platinum
  Low RRM1: Gemcitabine
  Low TS: Pemetrexate

- RAS: ADENO
  Myc: Sq

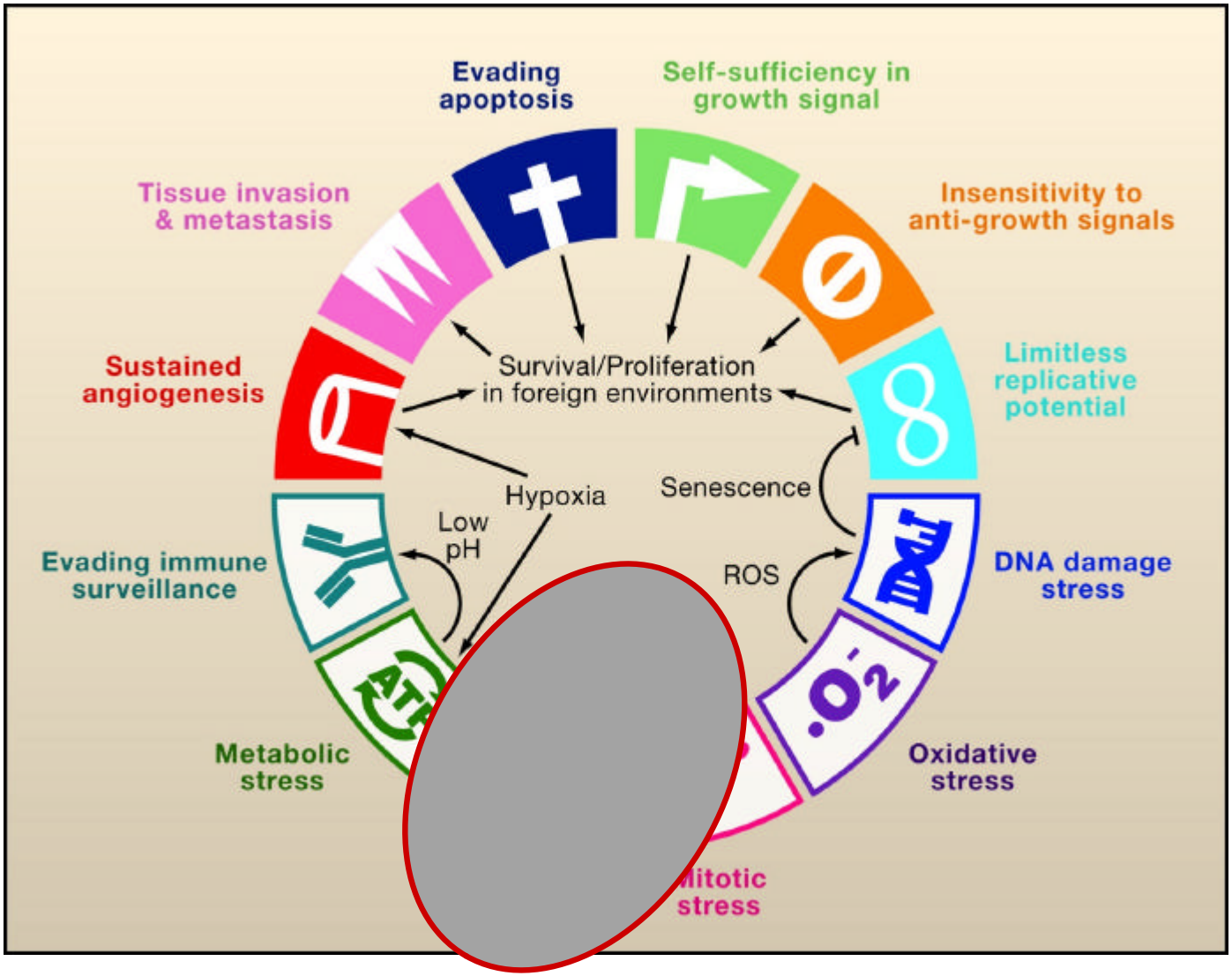
The malignant lifestyle causes stress

- Genomic abnormalities
- Overexpressed and mutated oncogenes
- Deregulated biochemical pathways
- Microenvironmental pressures, eg
  - Nutrient deprivation
  - Hypoxia



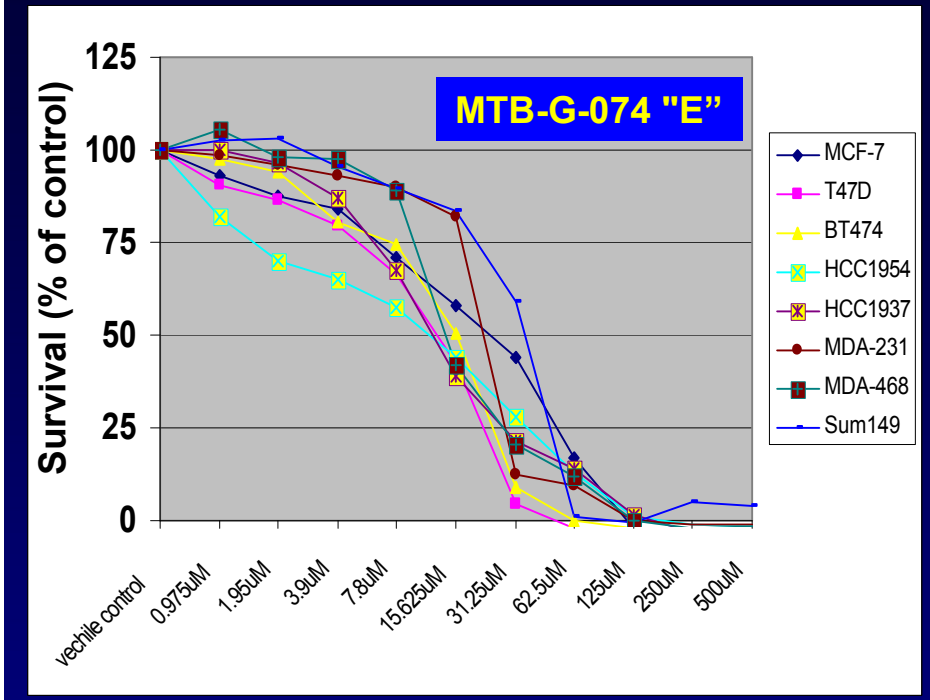
Workman, Burrows, Neckers, Rosen Ann NY Acad Sci 1113 202-216. 2007

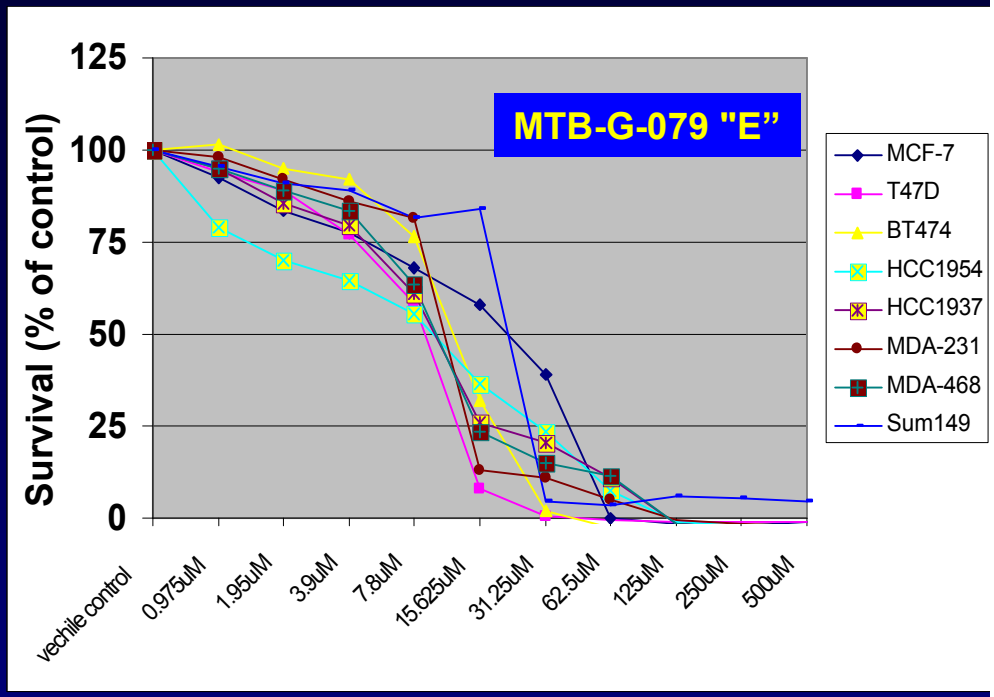
Stress is now included in extended hallmarks of cancer



Luo, Solimini and Elledge Cell 136 823-37 2009

EC50s of PKi, MTB-G-074 "E" [IUPAC: (E)-methyl 3-chloro-2-((dimethoxyphosphoryl)oxy)acrylate)] & MTB-G-079 "E" [IUPAC: (E)-methyl 3-bromo-2-((dimethoxyphosphoryl)oxy)acrylate] in different BT cell lines by MTT assay

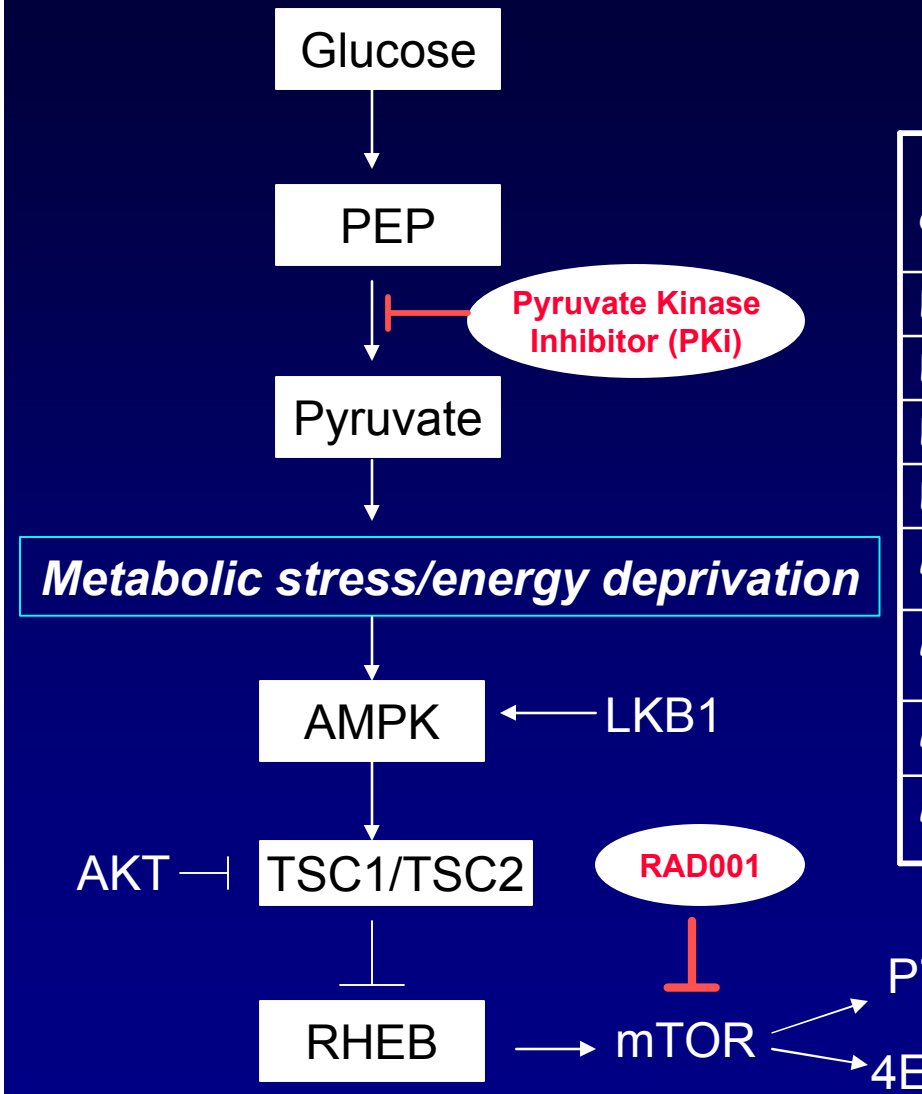




EC50 of PKi in BT cell lines

List of PKIs	MCF7 (μM)	T47D (μM)	BT474 (μM)	HCC1954 (μM)	HCC1937 (μM)	MDA-MB231 (μM)	MDA-MB468 (μM)	SUM149 (μM)
3Br-Py	45	40	70	23/20	80	100 uM	20	18
74E	20	10	15	12/6	10	20/ 20	12	33
79E	20	8	10	10/4	9	10/10	9	22
74Z	45	25	25	15/4	17	20/ 25	28	46
79Z	30	20	17	15/10	10	20/ 20	20	40
79Br2	31	14	30	35/12	23	35/45	20	40

Tumor cell metabolism meets survival pathway: Rationale for the combination of PKi and mTORi in BT cells

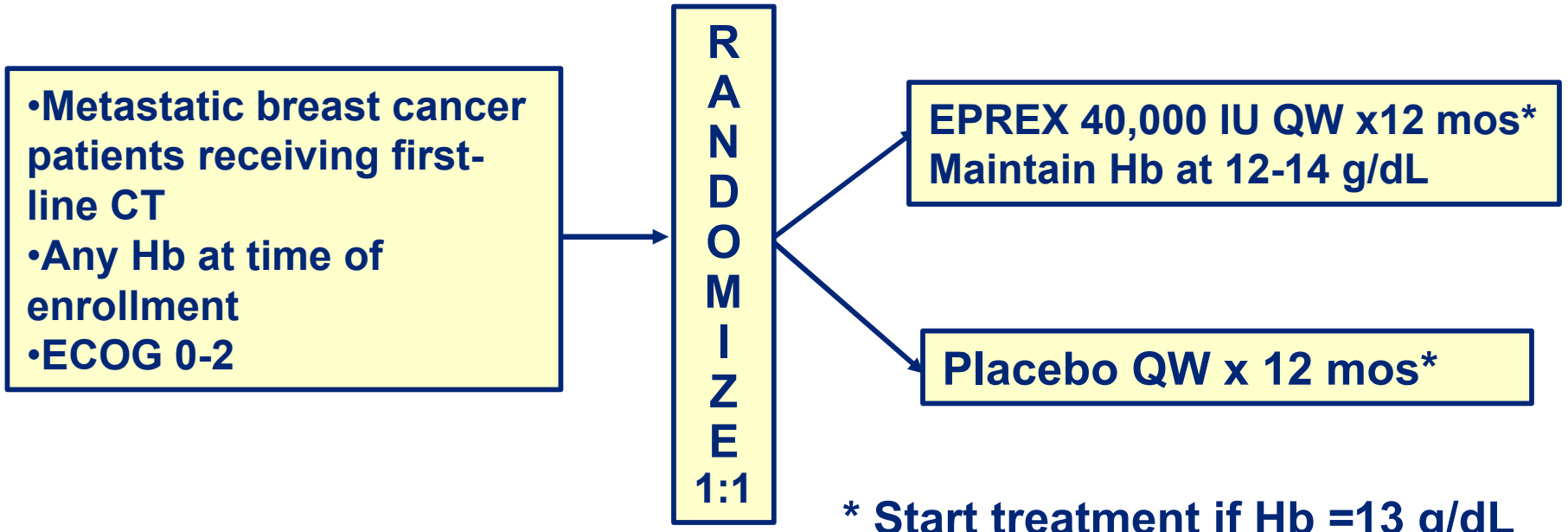


Effect of combination of PKi and with RAD001 in BT cell lines

BT Cell lines	Treatments: PKi alone & PKi plus RAD001	EC50s (μM)
HCC1937	74E	10
HCC1937	74E plus 2.5nM RAD001	3
HCC1937	79E	9
HCC1937	79Eplus 2.5nM RAD001	2
MDA-MB468	74E	12
MDA-MB468	74E plus 2.5nM RAD001	0.4
MDA-MB468	79E	9
MDA-MB468	79Eplus 2.5nM RAD001	0.5

EPO-INT-76 (BEST): Study Design

Objective: Effect of EPO on maintaining Hb in range of 12-14 g/dL on Overall Survival
1º Endpoint: 12-month Overall Survival
2º Endpoints: Hb, transfusion rates, tumor response to chemo



Leyland-Jones B, et al. *J Clin Oncol* 2005;23:5960-72.

ORTHO BIOTECH ONCOLOGY RESEARCH & DEVELOPMENT

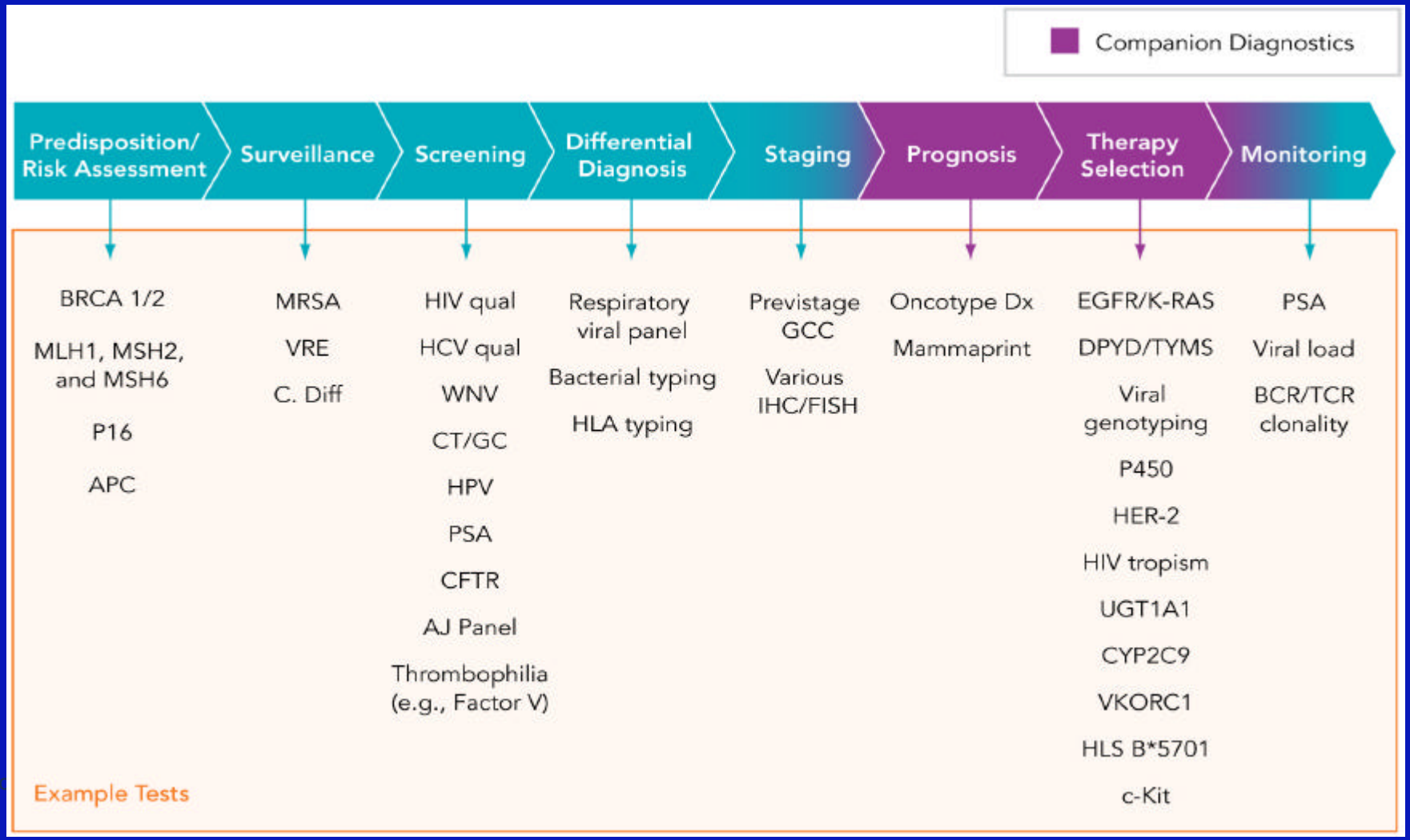
2. THE EXPLORATION OF RETROSPECTIVE CLINICAL TRIALS AND TUMOUR BANKS USING 2-5 DIFFERENT PLATFORMS AT CLIA LEVEL

Pairing Drugs and Genes

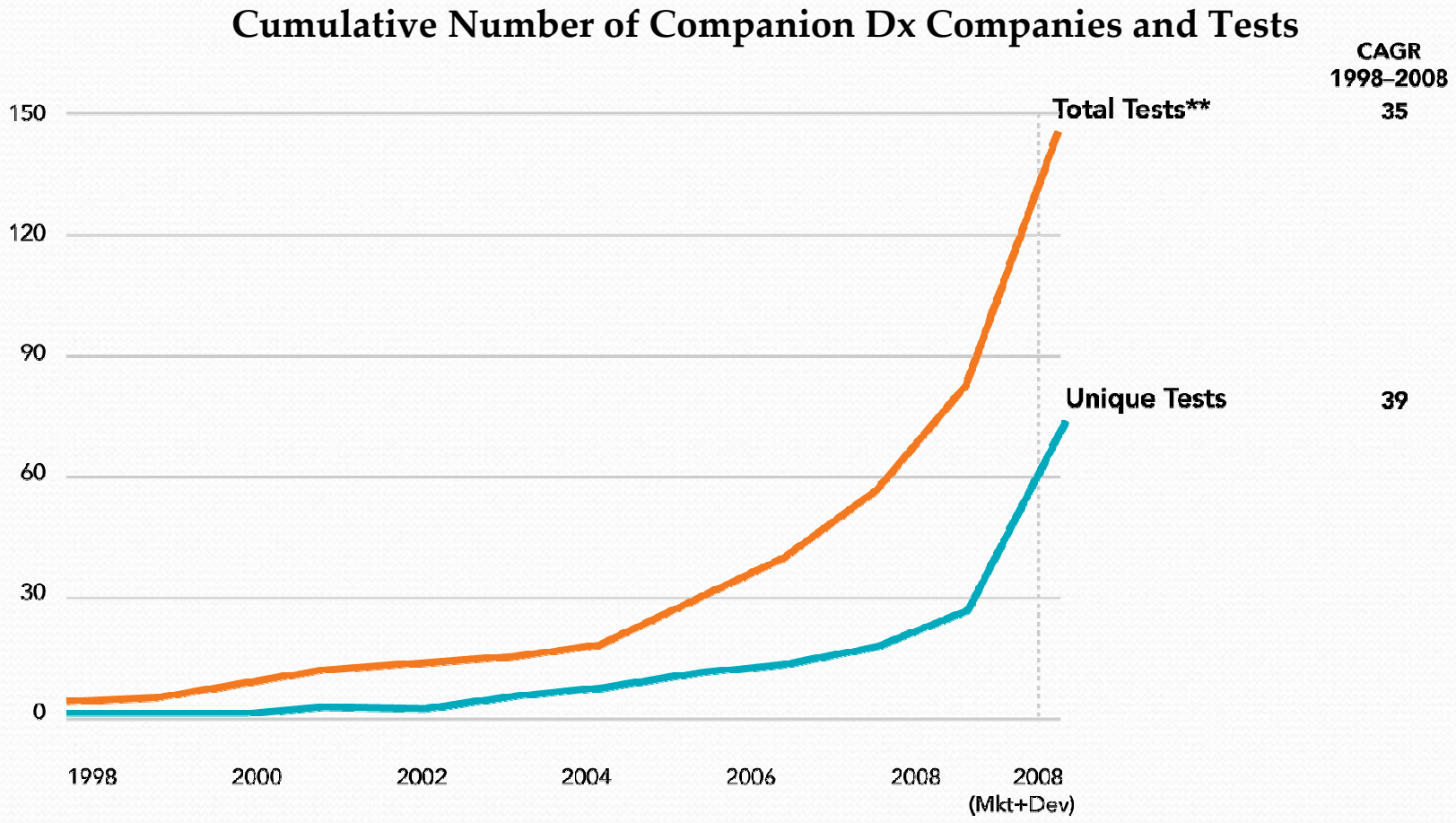
Drug	Use	Genetic Marker
Identifying suitable patients		
Herceptin (trastuzamab)	Breast Cancer	Her2 in tumor
Erbitux (cetuximab)	Colon Cancer	KRAS
Tamoxifen	Breast Cancer	CYP2D6
Avoiding Side Effects		
Ziagen (abacavir)	HIV	HLA-B*5701
Campostar (irinotecan)	Colon Cancer	UGT1A1*28
Tegretol (carbamazepine)	Epilepsy	HLA-B*1502
Coumadin (warfarin)	Blood clot prevention	CYP2C9 and VKORC1
Celebrex (celecoxib)	Pain and arthritis	CYP2C9
Prospective Markers		
Plavix (clopidogrel)	Blood clot prevention	CYP2C19
Iressa (gefitinib) and Tarceva (erlotinib)	Lung cancer	EGFR in tumor
Bucindolol	Heart failure	Alpha-2c & beta-1
Prozac (fluoxetine)	Depression	CYP2D6, CYP2C19, et al.
Avastin (bevacizumab)	Various cancers	VEGF

Companion Diagnostics

Diagnostic Testing Overview



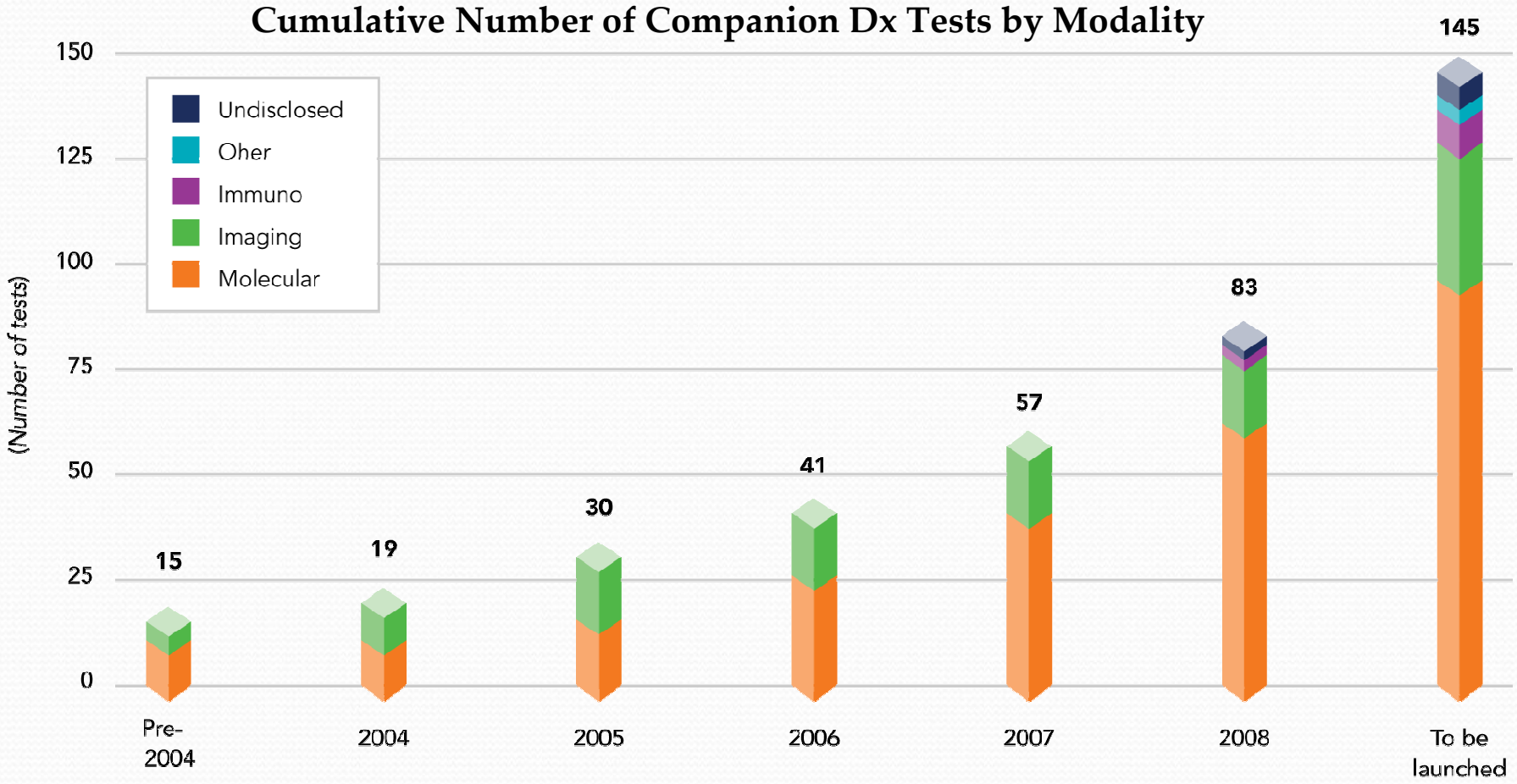
Companion diagnostics tests have surged



* The launch date for 16 tests (out of 161) could not be identified **Includes duplicates

Source: Company websites, SEC filings, Capital IQ, L.E.K analysis

A Majority of Companion Tests are Molecular



Other includes Mass Spec

* TBL: Assumed 2009 launch; Immuno growth driven by expansion of VeraTag technology

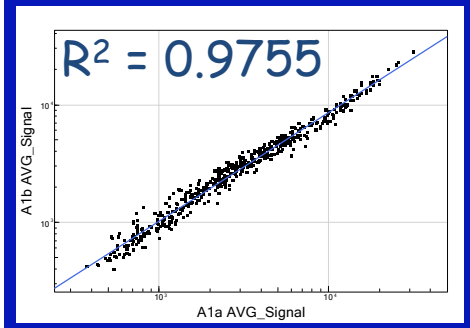
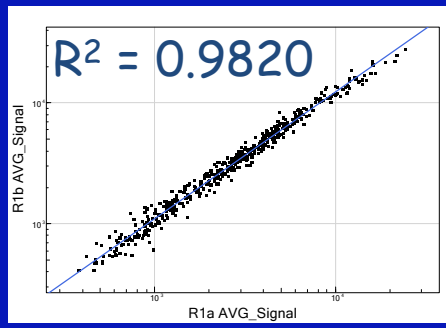
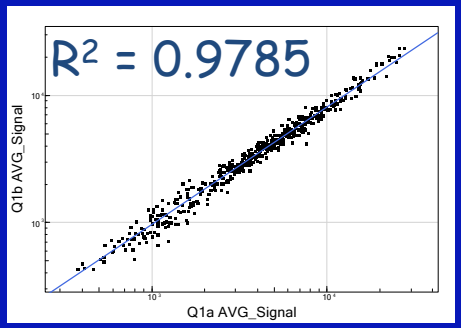
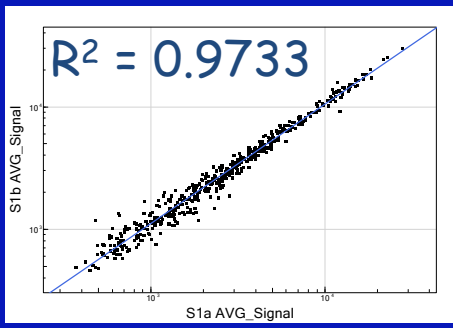
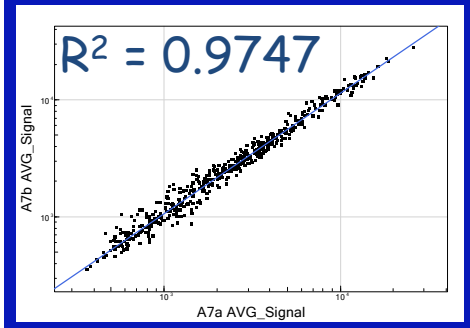
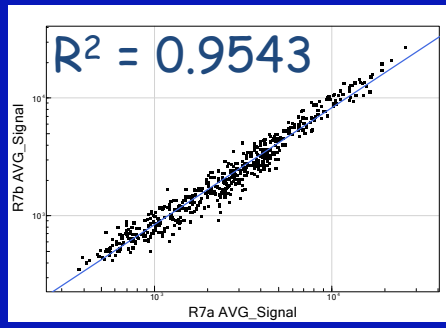
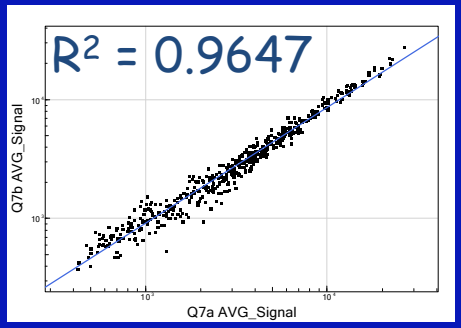
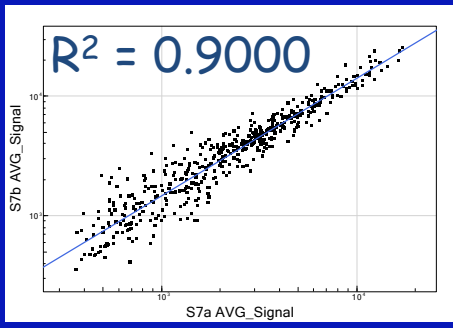
Source: Company websites, SEC filings, Capital IQ, L.E.K analysis

Genomic Analyses: What is feasible

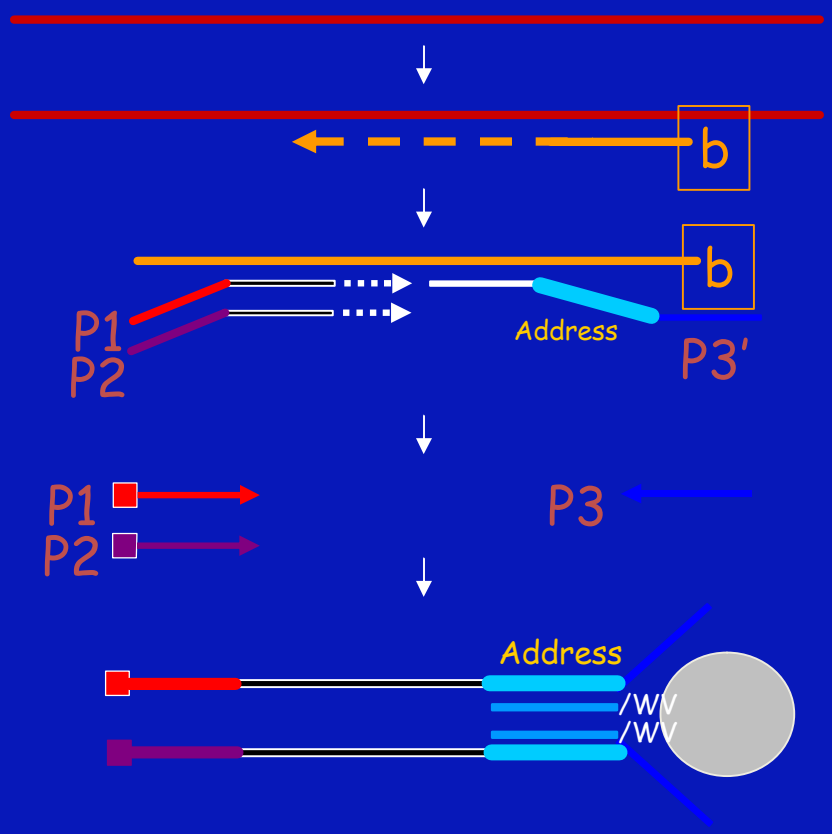
- How much DNA or RNA would you expect to retrieve from various starting materials?

	DNA	RNA
Whole blood (10ml)	100 - 300ug	30ug
Buffy Coat	100 - 300ug	N/A
Serum (1ml)	Nanograms	Nanograms
Tissue (1mg)	0.2 - 3ug	1 – 6ug
Urine (1ml)	Nano - ug	Nanograms
Saliva (2ml) -Oragene	100ug	20ug
Cell Line ($5x10^6$)	15 - 20ug	50 – 150ug
FFPE (5x 5u sections)	1 - 3ug	1 - 3ug

Technical Replicate Reproducibility RNA extraction methods from FFPE samples: CLIA

Ambion	Roche	Qiagen	SuperArray
			
O/N	O/N	3 hr	O/N
			
3 hr	3 hr	15 min	3 hr

DASL assay (cDNA-mediated Annealing, Selection, extension and Ligation)



Total RNA isolated from FFPE tissue

cDNA synthesis

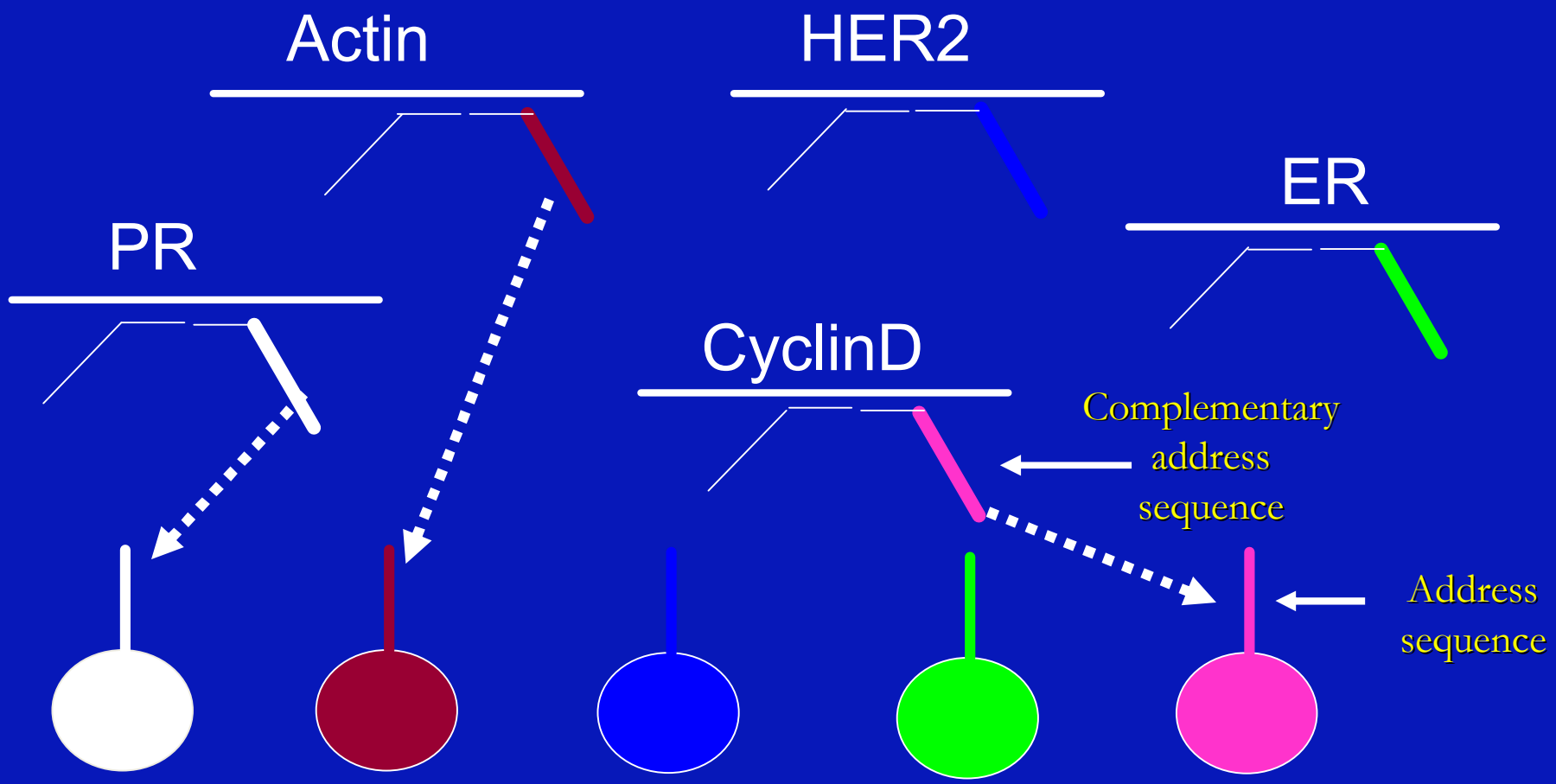
Query oligo annealing, extension, and ligation

PCR with common primers

Product capture by hybridization to the BeadArray

Yeakley et al. Nature Biotechnology. (2002)
Fan et al. Genome Research. (2004)

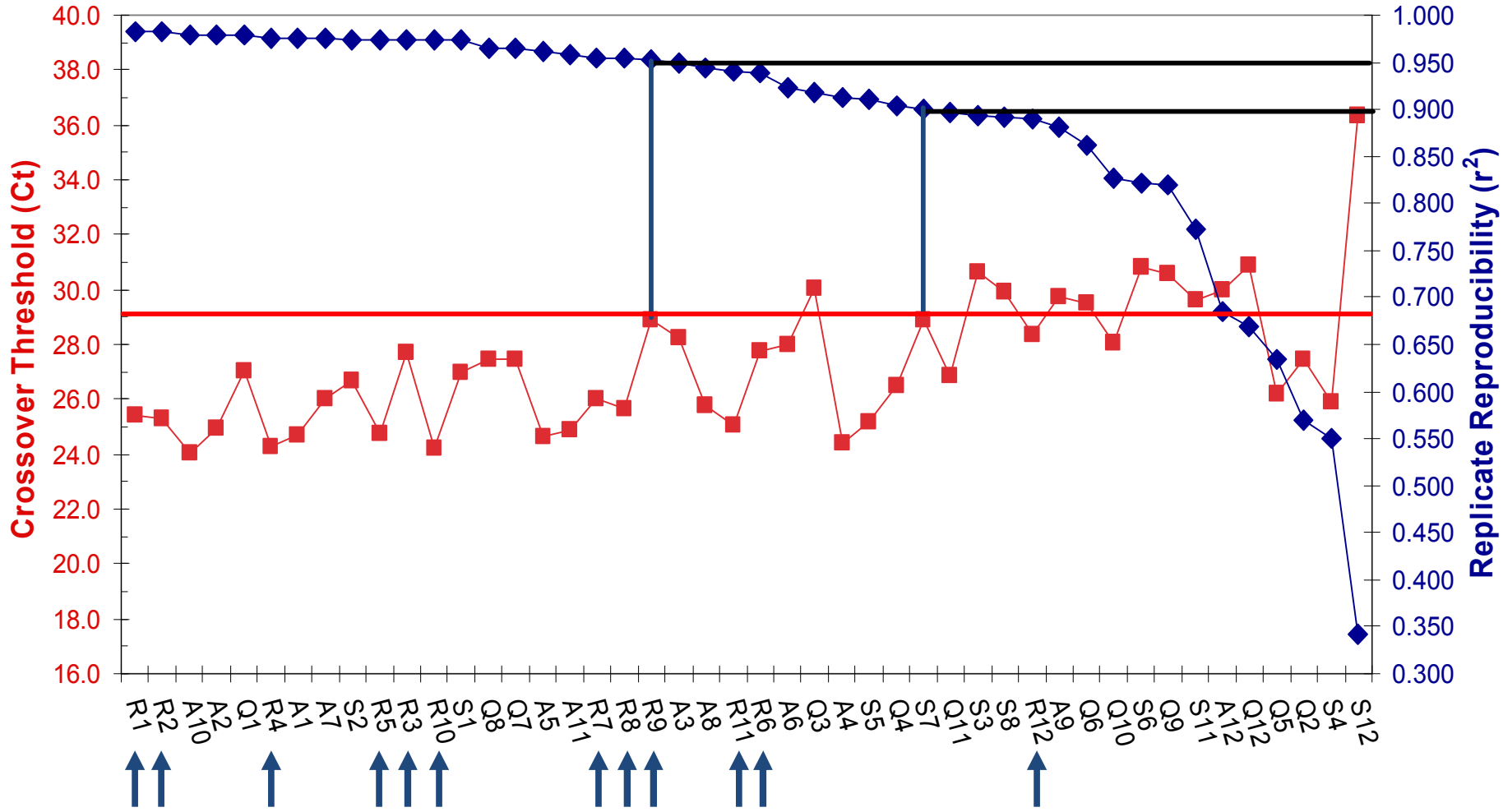
Labeled DASL Products Hybridize to the Bead Array Through Unique Address Complementarity



Array of microbeads with address sequences (n=1536)

Slide from S. Paik

Replicate reproducibility (r^2) vs Crossover threshold (Ct)



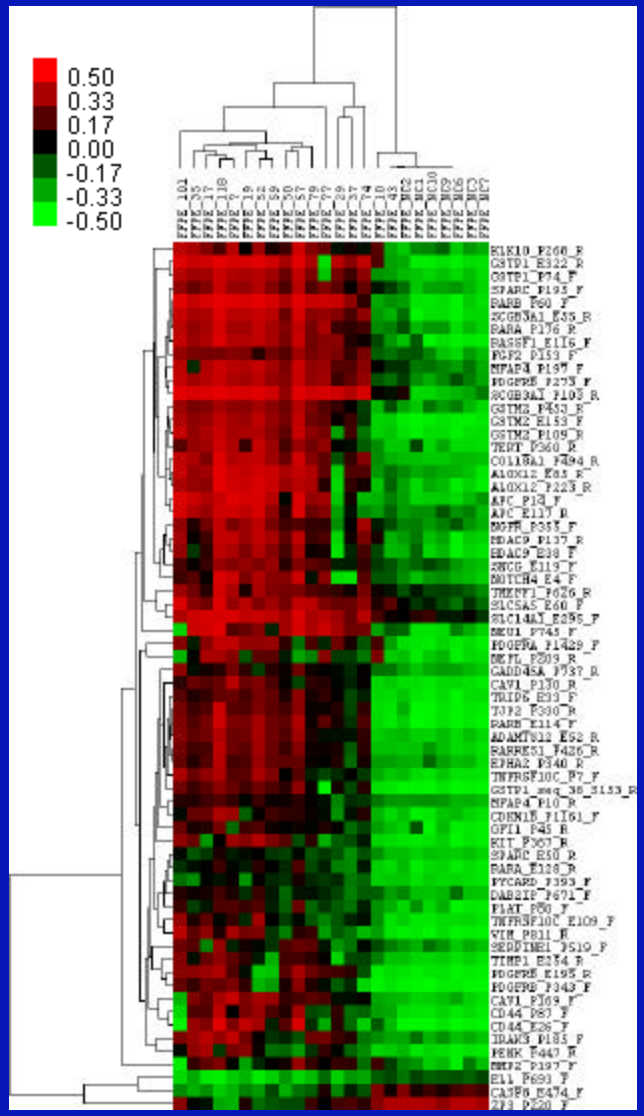
Samples were sorted by correlation (r^2) between replicates in the DASL assay and compared to the crossover threshold (Ct) in qPCR.

Make-up of our Breast Cancer Specific DASL Panel of 512 Genes

- Subtype selective signature genes (Sorlie)
- Mammaprint
- Oncotype DX
- Tamoxifen signature
- Docetaxel signature
- pCR predictive signature
- p53 signature

- Angiogenesis-related genes
- Apoptosis-related genes
- Breast cancer stem cell gene
- Cell cycle genes
- DNA repair genes
- Immune response genes
- Metastasis-related genes
- Oncogenes
- Proliferation genes
- Senescence-related genes
- Telomerase-related genes
- Thrombosis-related genes

METHYLATION PROFILING OF FFPE PROSTATE TISSUES



- **Illumina standard cancer methylation panel assayed (807 genes; 1505 CpG sites)**
- **Prostate cancer highlighted in red across top**
- **Hypermethylated genes in prostate cancer indicated in this study and previous ones:**
  - Glutathione S-Transferases –
    - GSTP1 – 3/3 probes
    - GSTM2 – 3/3 probes
  - APC - 2/3 probes
  - Retinoic Acid Receptors
    - RARA – 2/3 probes
    - RARB – 2/2 probes
  - SPARC - 2/2 probes
  - CD44 - 2/2 probes
  - SCBG3A1 - 2/2 probes
  - RASSF1 - 1/2 probes

17 prostate cancer tissues
7 normal prostate tissues

49 genes differentially Methylated

(r2) = 0.9954

SELECTIVE SILENCING OF THE HYPOXIA-INDUCIBLE FACTOR 1 TARGET GENE BNIP3 BY HISTONE DEACETYLATION AND METHYLATION IN COLORECTAL CANCER

A L Bacon[1], S Fox[1], H Turley[1] and A L Harris[1]

[1]Laboratory of Molecular Oncology, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Cancer Research UK, Oxford, UK

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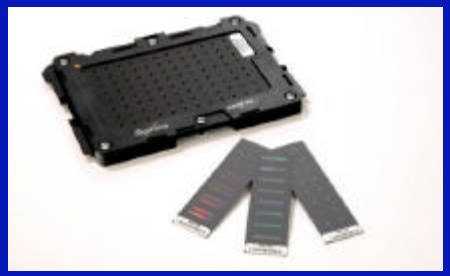
ILLUMINA BEAD STATION 500GX AND iSCAN



- Full range of genotyping products
  - Custom genotyping up to 1536plex (GoldenGate Assay) including FFPE
  - Whole Genome Genotyping (Infinium Assay)
  - Both assays used for Methylation
  - Infinium assay used for CNV
- Full range of gene expression products
  - Whole Genome Gene Expression
  - Focused Gene Sets including DASL Assay for FFPE samples
  - DASL Assay for whole genome from FFPE

3. OUR CURRENT RESULTS

Background: CYP2D6 & Hot Flushes

Table 4. Incidence of Moderate (grade 2) or Severe (grade 3) Hot Flashes Within Genotype Subgroups

	Patients Who Developed Moderate or Severe Hot Flashes		
Genotype	No. of Patients	Total Patients	%
CYP2D6 (*4), n = 190			
*4/*4	0	13	0
*4/wt	9	40	23
wt/wt	27	137	20

One-sided P=0.064

Goetz et al. JCO 2005

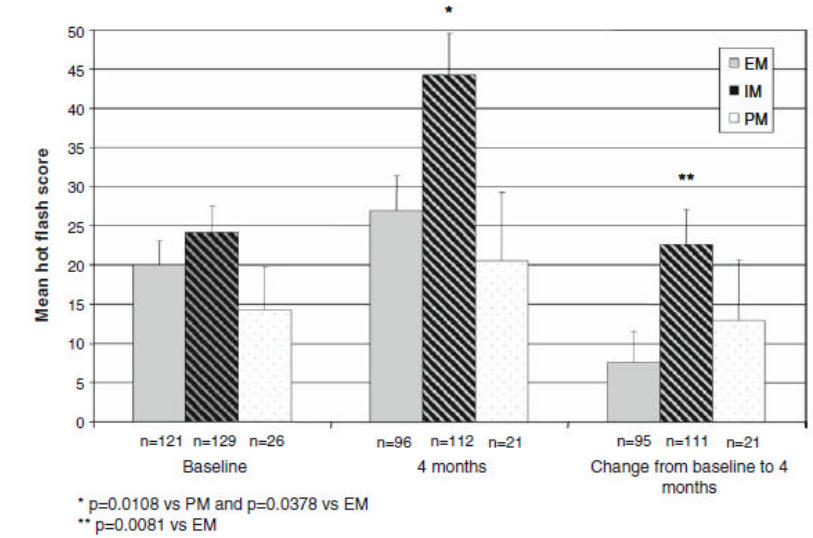


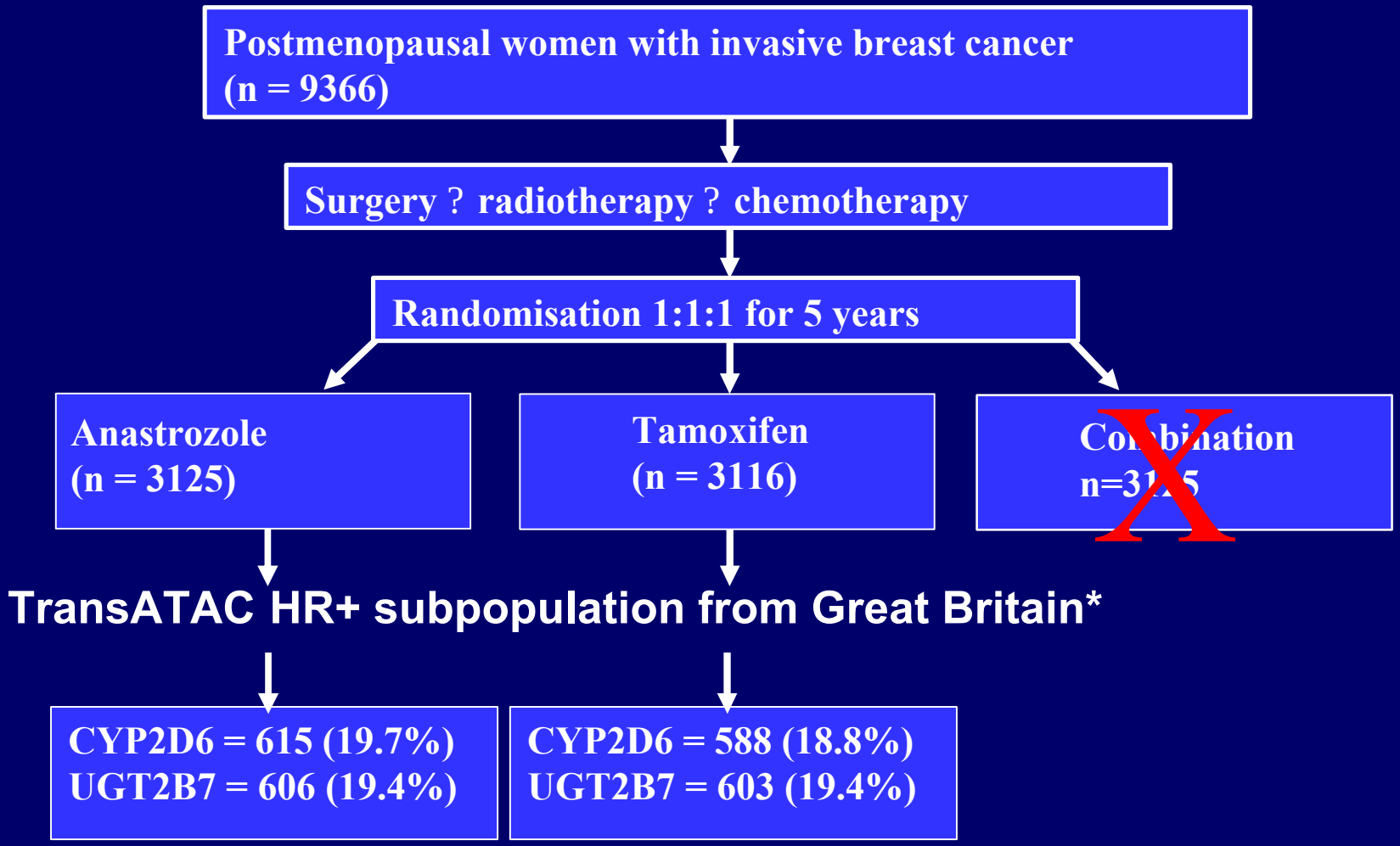
Fig. 1 Mean weekly hot flash score at baseline, 4 months after initiating tamoxifen, and change in hot flash score from baseline to 4 months by CYP2D6 metabolizer group derived from *CYP2D6* genotype for all study participants. Number of subjects evaluated at each time point listed below the *bars*. *Error bars* signify standard error. *EM* extensive metabolizer (*solid bars*), *IM* intermediate metabolizer (*diagonal bars*), *PM* poor metabolizer (*dotted bars*)

Henry et al. BCRT 2009

Goetz (NCCTG), 2005: Tamoxifen-treated patients with CYP2D6 *4/*4 genotype (PM) tended to have fewer moderate-severe hot flushes

Henry (COBRA), 2009: IM had greater mean hot flush scores after 4 months of tamoxifen

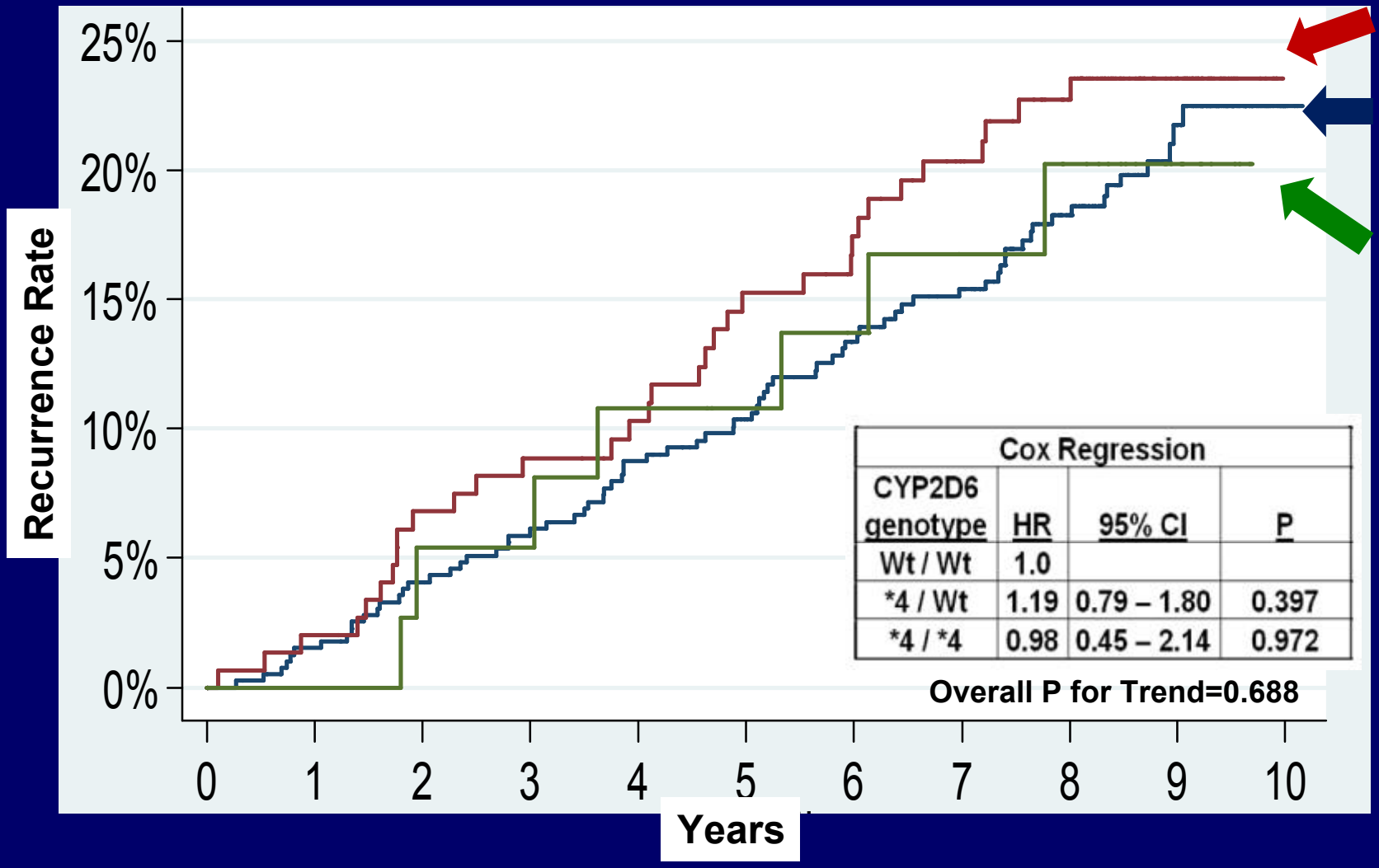
Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trial Design



**Dowsett M, et al. J Clin Oncol. 26(7):1059-65, 2008*

University of Michigan
Comprehensive Cancer Center

CYP2D6*4 Does Not Predict Recurrence in Tamoxifen Arm

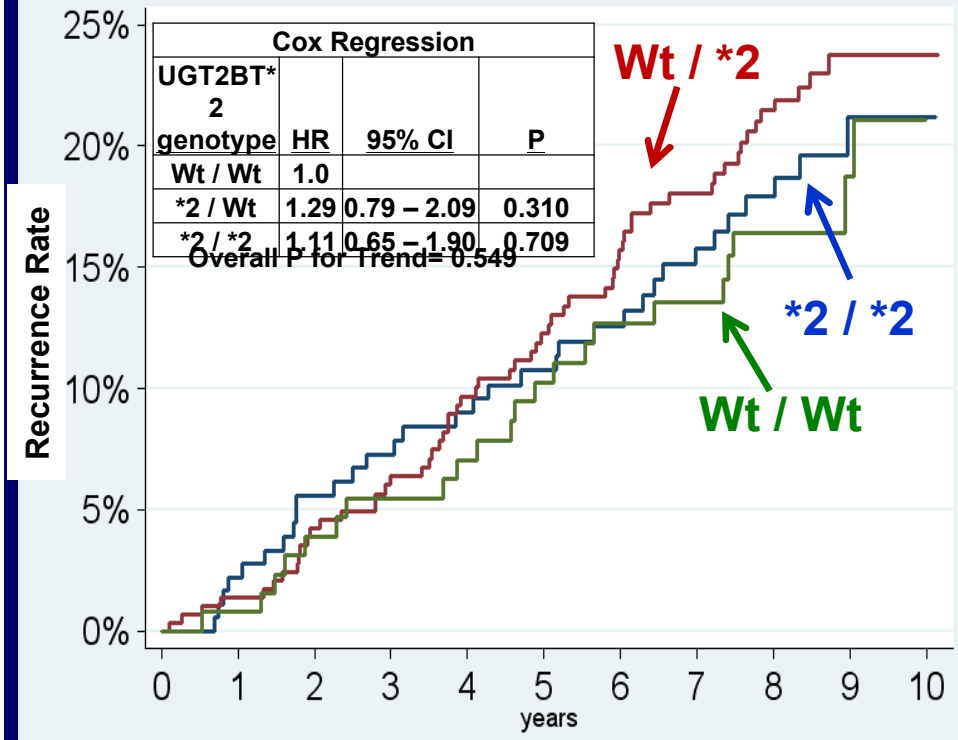


Rae, J.M

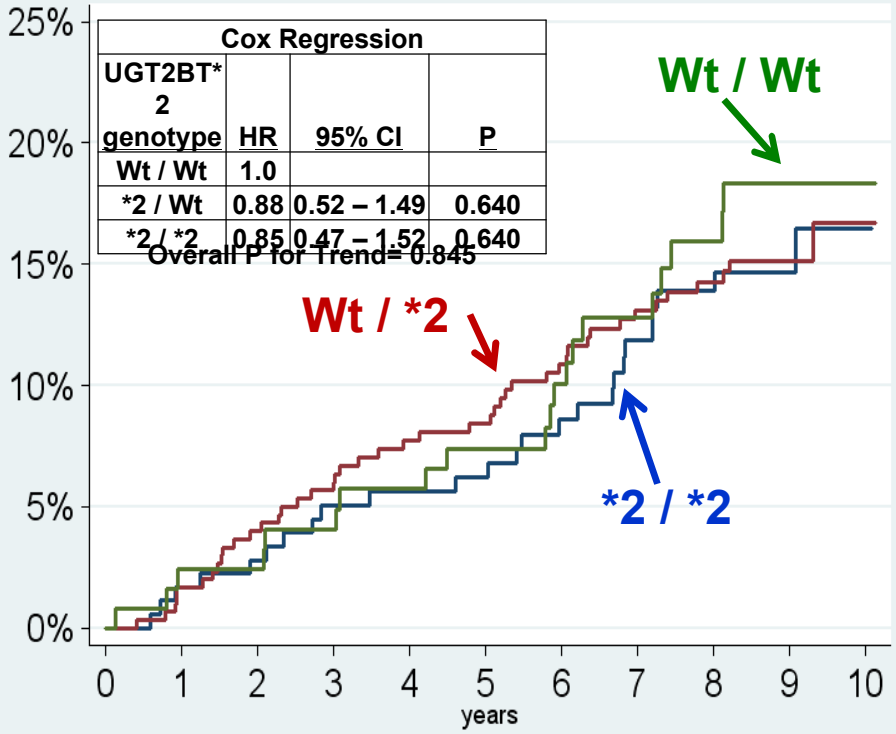
University of Michigan Comprehensive Cancer Center

UGT2B7*2 in Does Not Predict Recurrence

Tamoxifen



Anastrozole



Rae, J.M.

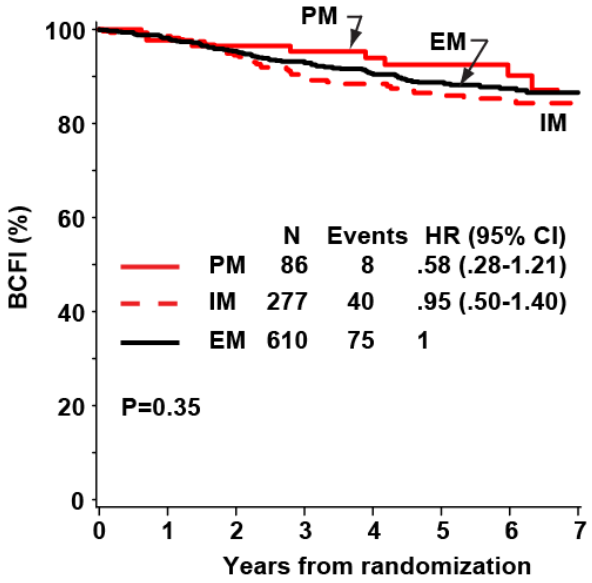
University of Michigan Medical School

CYP2D6 Phenotype
BIG 1-98 Monotherapy Cohort

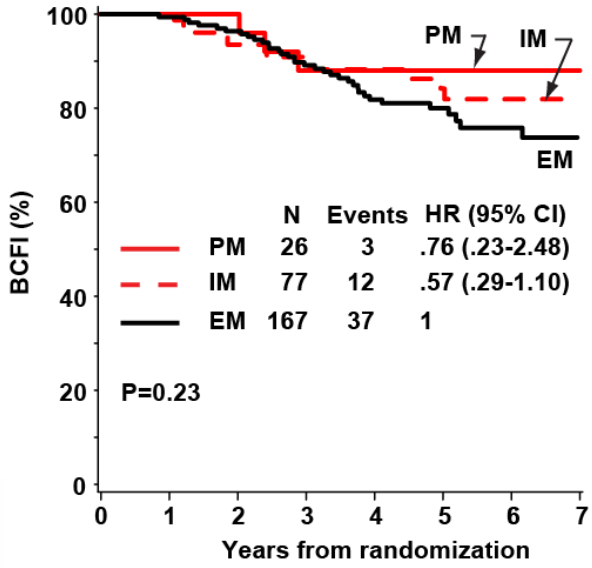
CYP2D6 Phenotype	N	(%)	Enzyme activity
Poor (PM)	236	(9)	absent
Intermediate (IM)	716	(27)	low
Extensive (EM)	1585	(59)	
Unclear	138	(5)	
TOTAL	2675		

TAMOXIFEN

No Chemotherapy

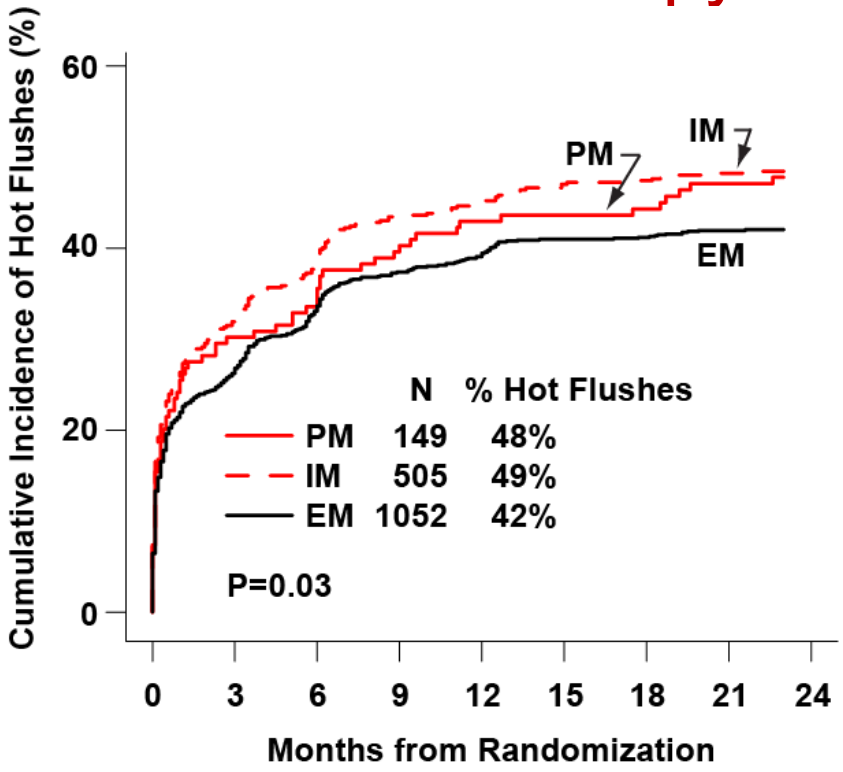


Chemotherapy



BIG 1-98: Hot Flushes

TAMOXIFEN
No Chemotherapy



Hot flushes and night sweats were targeted AEs, collected every 6 months with date of onset recorded.

BIG 1-98 and ATAC Summary

- No association between either *CYP2D6 *4* genotype or CYP2D6 haplotype with breast cancer recurrence, either in tamoxifen or AI arm.
- No association between *UGT2B7*2* polymorphisms and breast cancer recurrence
- No association between CYP2D6 genotype and hot flashes (BIG 1-98)

LARGE DEFINITIVE TRIALS, LIKE ANE 3010, ARE CRITICALLY IMPORTANT

“ROUNDED UP THE USUAL SUSPECTS”:

ER, PgR, HER2, Ki67, EGFR, IGF1R - PI3K - AKT – TORC1-TORC2, VEGFR, HIF-1a, RAS - RAF – MEK - MAPK, c-Myc, Cyclin D1,SRC, WNT, PDGFR, KDR, Kit, Flt-3, MMPs, HDAC,……

Biological Markers:

- Identify the pattern of biological markers of response from different tumor samples by examining the levels of ER, IGFIR, VEGFR, PDGFR, KDR, Kit, Flt-3 and their phosphorylation status as well as the downstream signaling pathways: MAPK and AKT.

- Identify surrogate angiogenic markers from different tumor samples: CD31, CD34, CD105, matrix metalloproteinases (MMPs 2, 7 & 9) and PCNA using Western blot analysis and/or immunohistochemistry (IHC).

Biological Markers would be used:

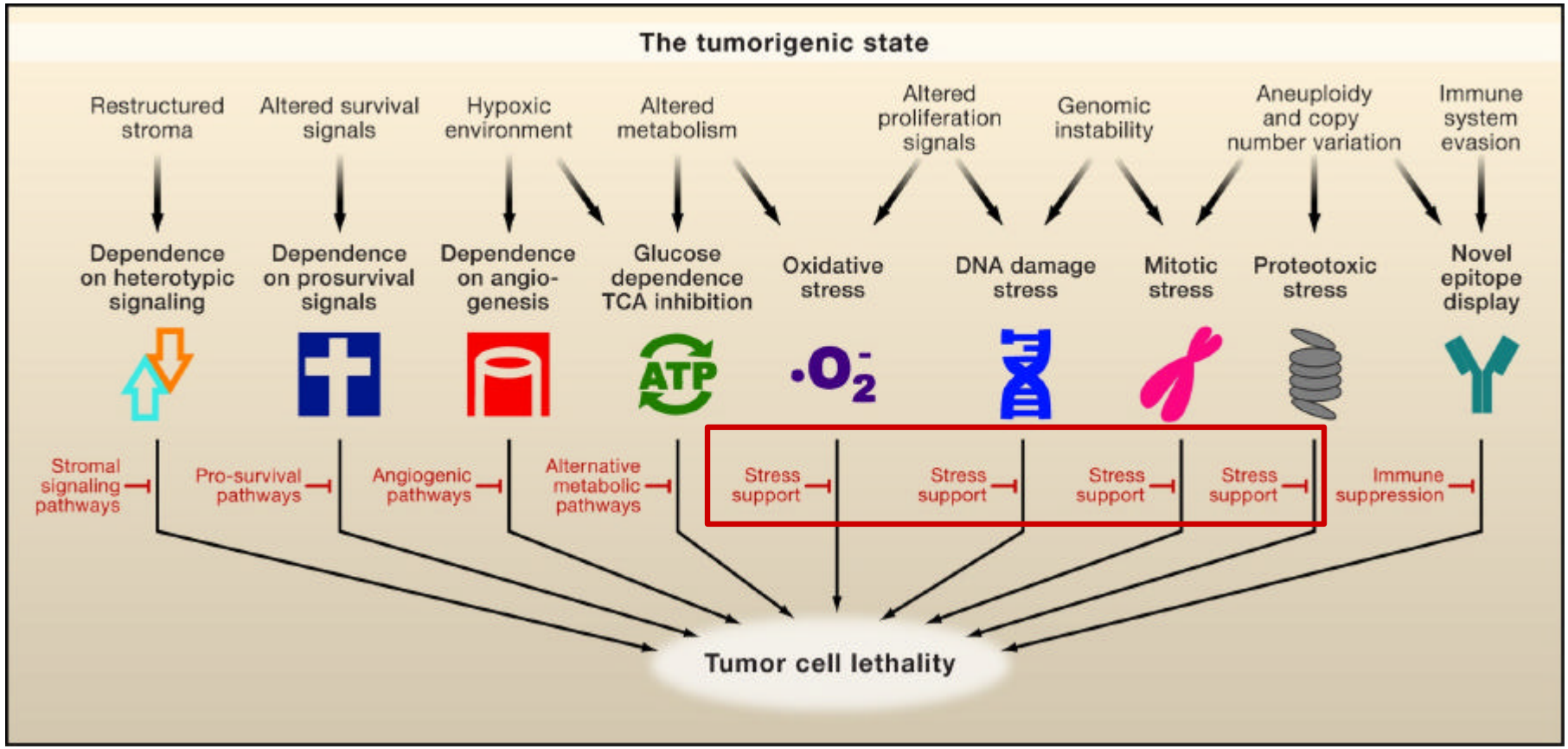
- HER2, p-HER2, IGF-1R, p-IGF-1R, AKT, p-AKT, mTOR, p-mTOR, ERK1/2, p-ERK1/2, RAS-GTP (RAS activation status)
- Hypoxia-induced HIF-1a (Western blot), CD31, CD34, CD105 (IHC), MMPs 2, 7 & 9 (WB) and proliferation marker, Ki67 (IHC).

Biological Markers used:

- Signature markers for Proliferation (PCNA, Ki67, c-Myc, Survivin)

- Signature markers for Angio-genesis / Hypoxia related genes (VEGF)

Exploiting the stressed tumorigenic state for therapy



Stress support systems are targets for tumor-selective therapy

Luo, Solimni and Elledge Cell 136 823-837 2009

EPO-ANE-3010: Original Study Schema*

Metastatic Breast Cancer

- **Hb ? 11 g/dL**
- **Receiving 1st or 2nd line Chemotherapy**
- **Measurable disease**
- **ECOG: 0 or 1**

EPO 40,000 IU QW
Until 4 weeks post chemo
Hb Target: 12.0 g/dL
(n=500)

Non-ESA Control
(n=500)

1º Endpoint: PFS from randomization (Central Review)
2º Endpoints: RR, TTP, OS, TVE
Stratification: HER2 / DFI / Adj. anthracycline

*Final Protocol (Dec-04)

ORTHO BIOTECH ONCOLOGY RESEARCH & DEVELOPMENT

CCDx

- The Consortium for Clinical Diagnostics (CCDx) is a unique consortium focusing on biomarker research and clinical diagnostics, designed to foster scientific collaboration and diagnostic innovation.
- Its goal is to be the not-for-profit consortium for the biomarker analysis of data and trials, as a service centre for performing GLP, CLIA, or highest research standard genomic, transcriptional and proteomic analyses and as an interface for key opinion leaders in the clinical diagnostic and biomarker fields.

CCDx BENEFITS FROM:

- Being an integral member of the NFCR research network with special close affiliations with the NFCR Personalised Medicine clinic, the consortium of tumour banks that NFCR is building and Dan Haber's centre at the Mass General.
- As a not-for-profit Institute, preserving and honouring any IP that an investigator brings with a sample set to be analysed.
- Contracting assay work to the best global centres at academic operating rates and minimal overhead.
- Close affiliations with Bioinformatics expertise and a mandate for transparent discussions and advocacy with NIH and FDA.