Impact of Age on the Biology of Breast Cancer

C. Benz, MD

Director, Cancer and Developmental Therapeutics Program
Buck Institute for Age Research

Adjunct Professor, University of California, San Francisco

NCI AYAO Biology Workshop
Bethesda, MD; June 9-10, 2009

Breast Cancer: a model disease of aging

- **Aging USA population = more cancers**

  ≥65y pop. = 4% in 1990, 12% in 1998, 20% in 2025

  12% pop. increase in 20y will bring a 60% cancer increase

- **Women are majority of elderly**

  55% of ≥60y; 65% of ≥80y

  Up to 80% of breast cancers occur after age 50y

- **Only ~6% of breast cancers occur before age 40**

  Up to 25% of these associated with BRCA1/2 mutations

- **Poor biological understanding of link with aging**

Altered cancer biology or host defenses?

Better Understanding = Better Treatment

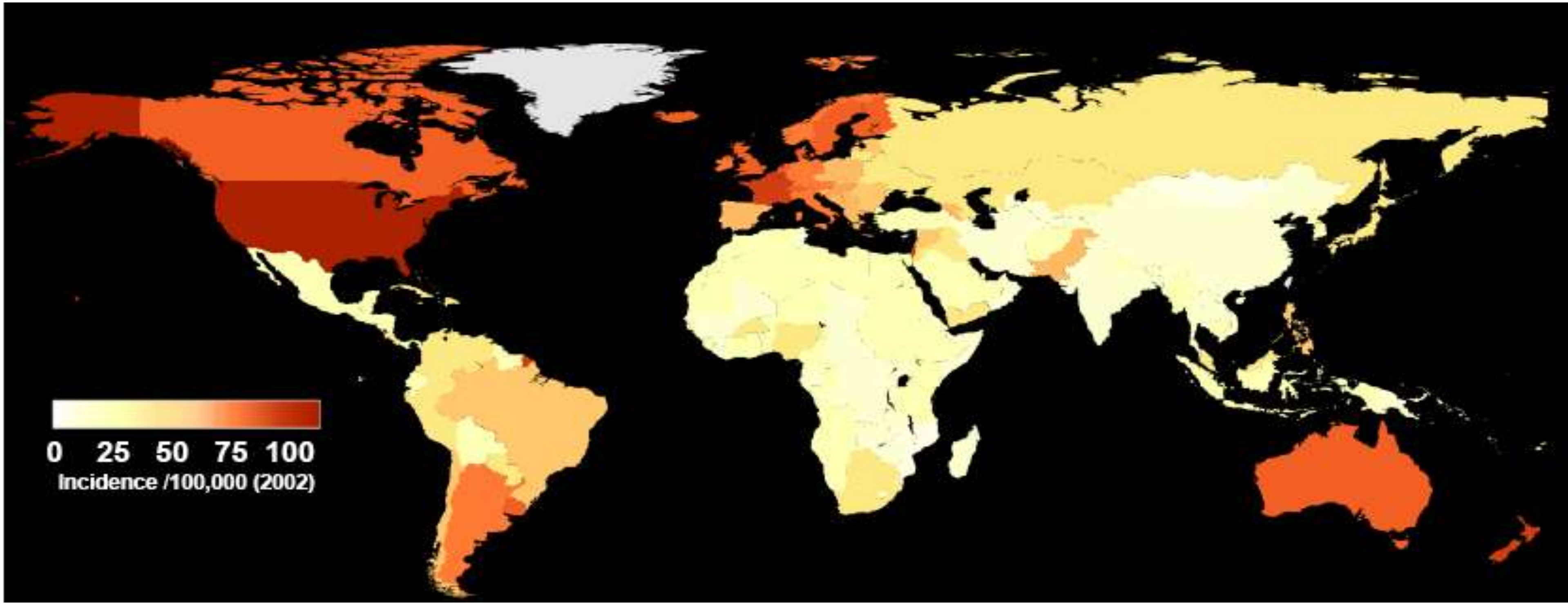
Breast Cancer Epidemiology

- Age and geographic variations in incidence?
- Age-dependent outcomes and risk factors?

10/15/07

Breast Cancer Incidence Worldwide

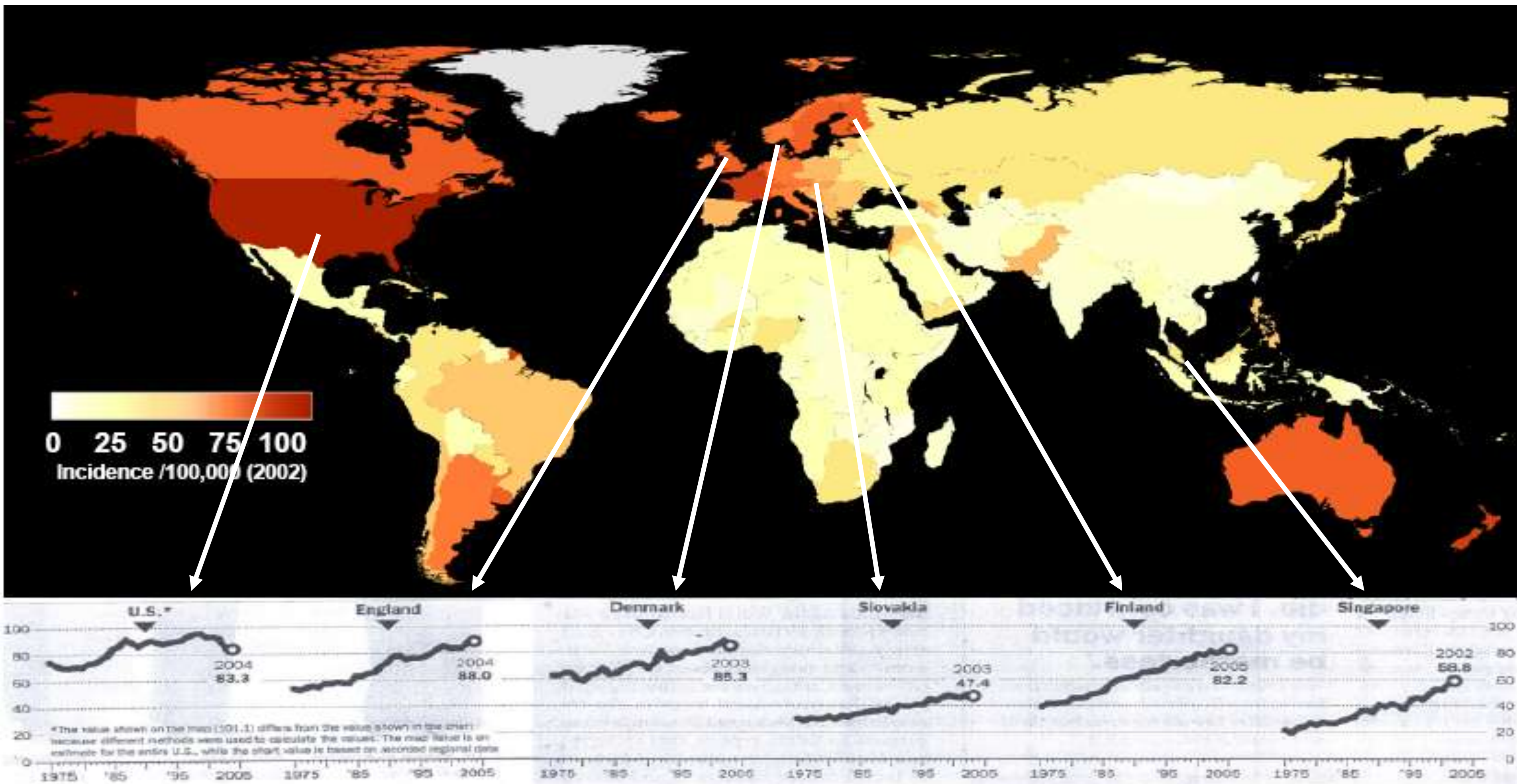
- Correlates with development and affluence.
- Adjusted for age, but not ethnicity.



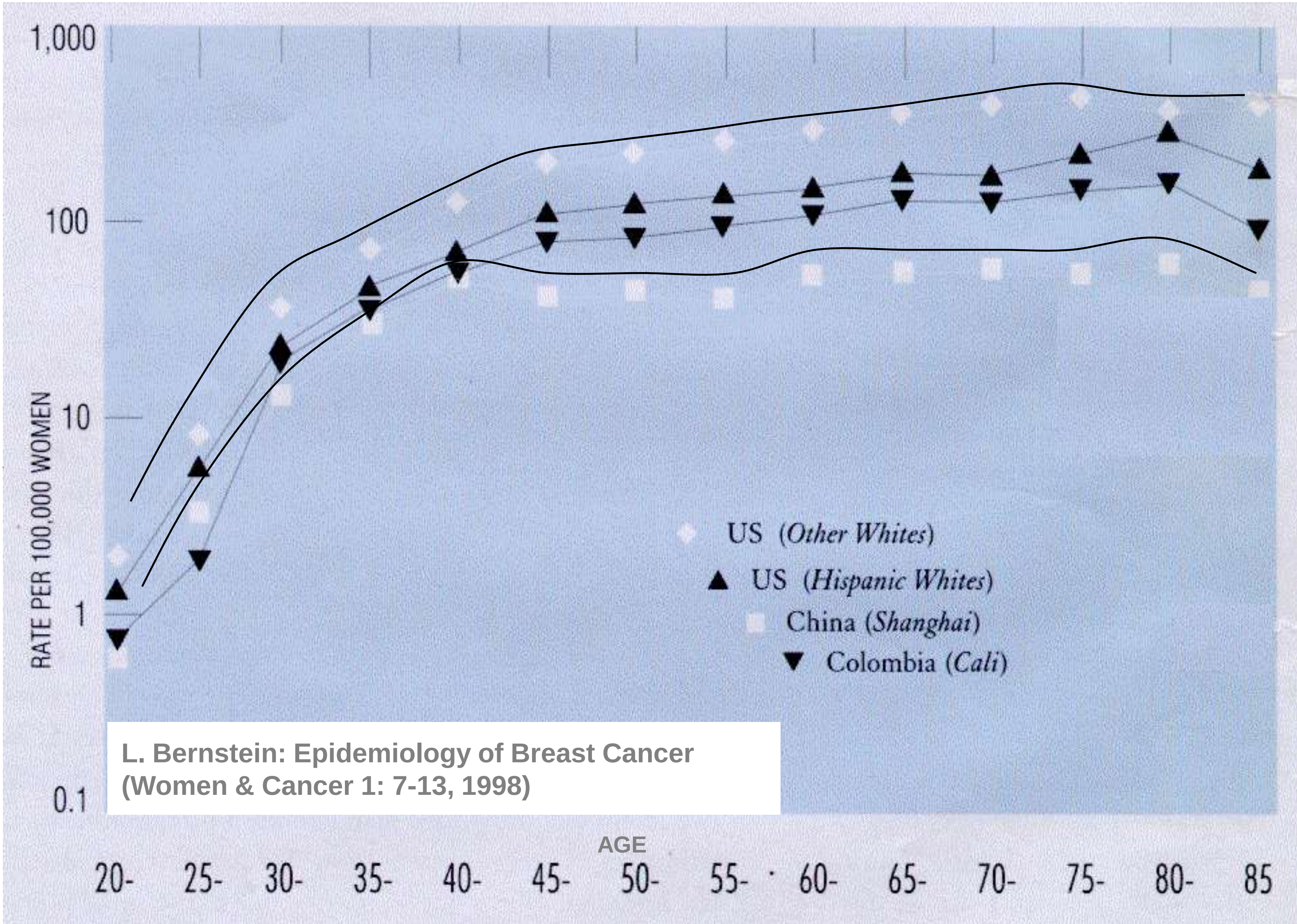
10/15/07

Breast Cancer Incidence Worldwide

- Correlates with development and affluence.
- Adjusted for age, but not ethnicity.
- *Generally increasing over past 30 years.*



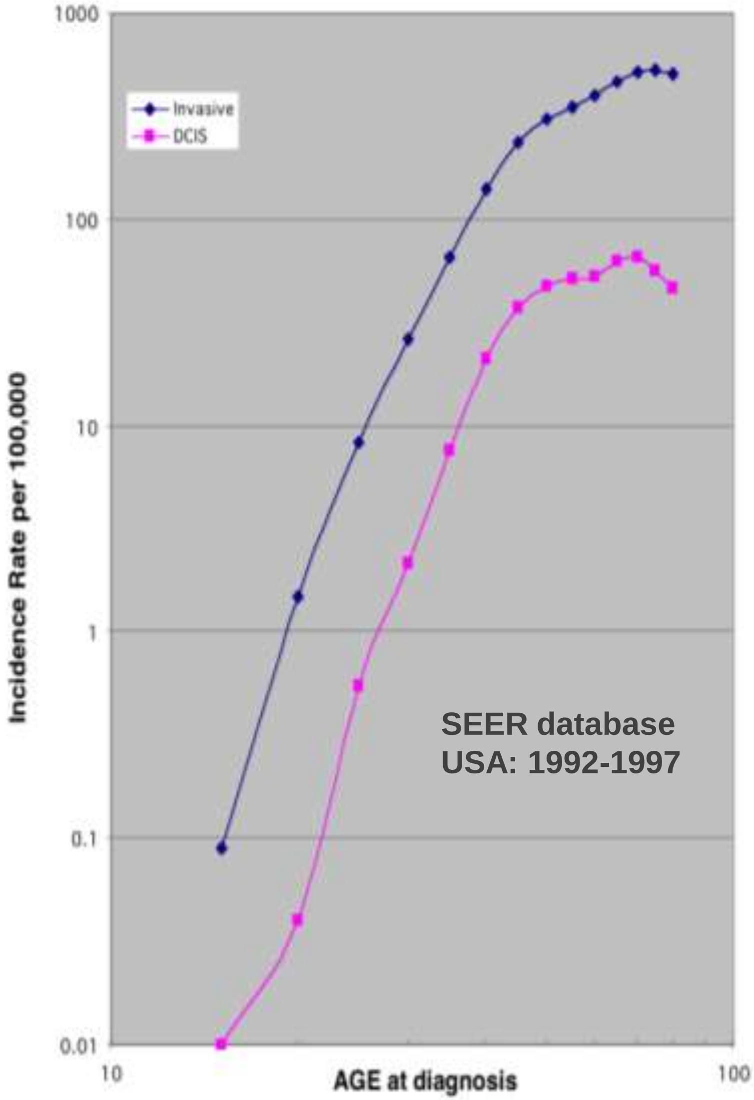
Geographic Variations in Breast Cancer Incidence Occur Primarily in Women Over Age 40

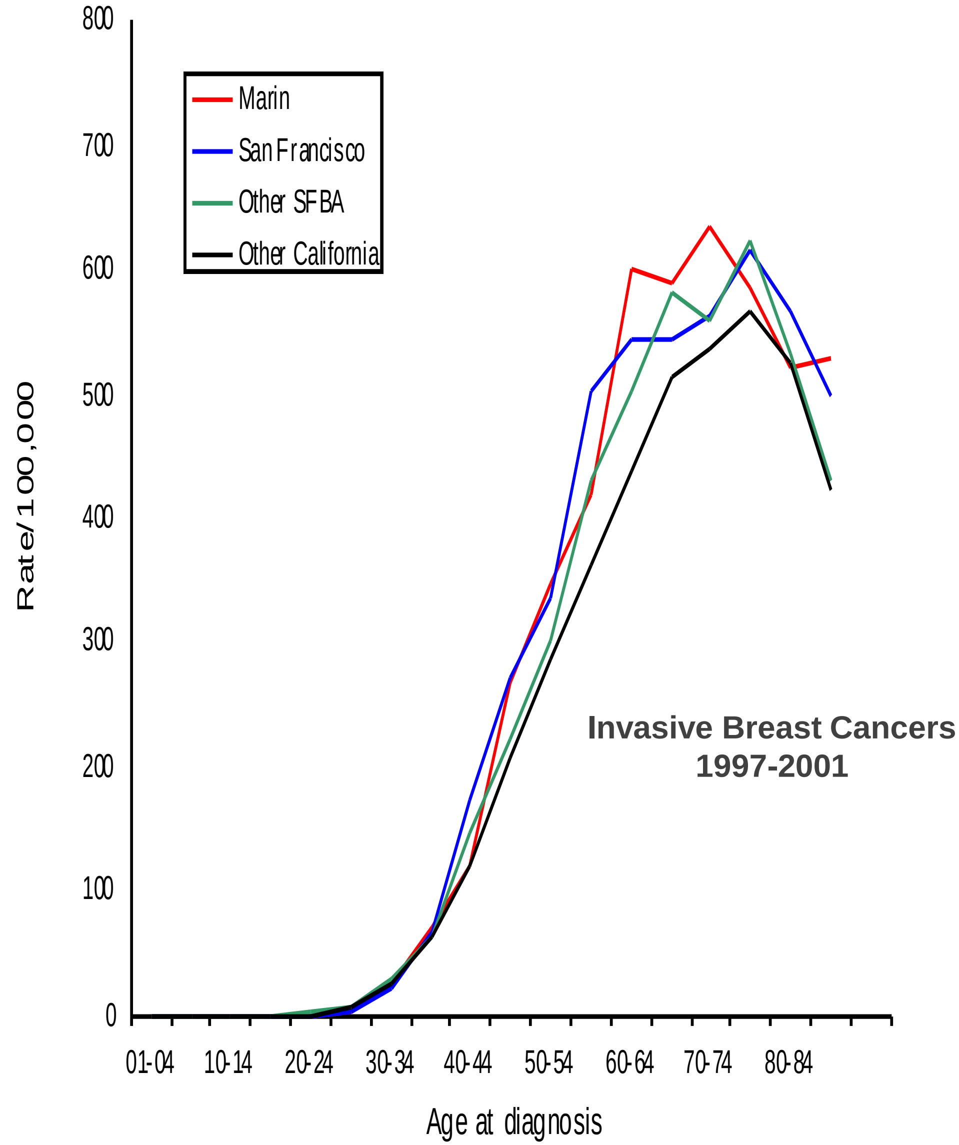


L. Bernstein: Epidemiology of Breast Cancer (Women & Cancer 1: 7-13, 1998)

Age-dependent Breast Cancer Incidence Rates

Younger Onset Incidence More Geographically Stable Than Older Onset Rates



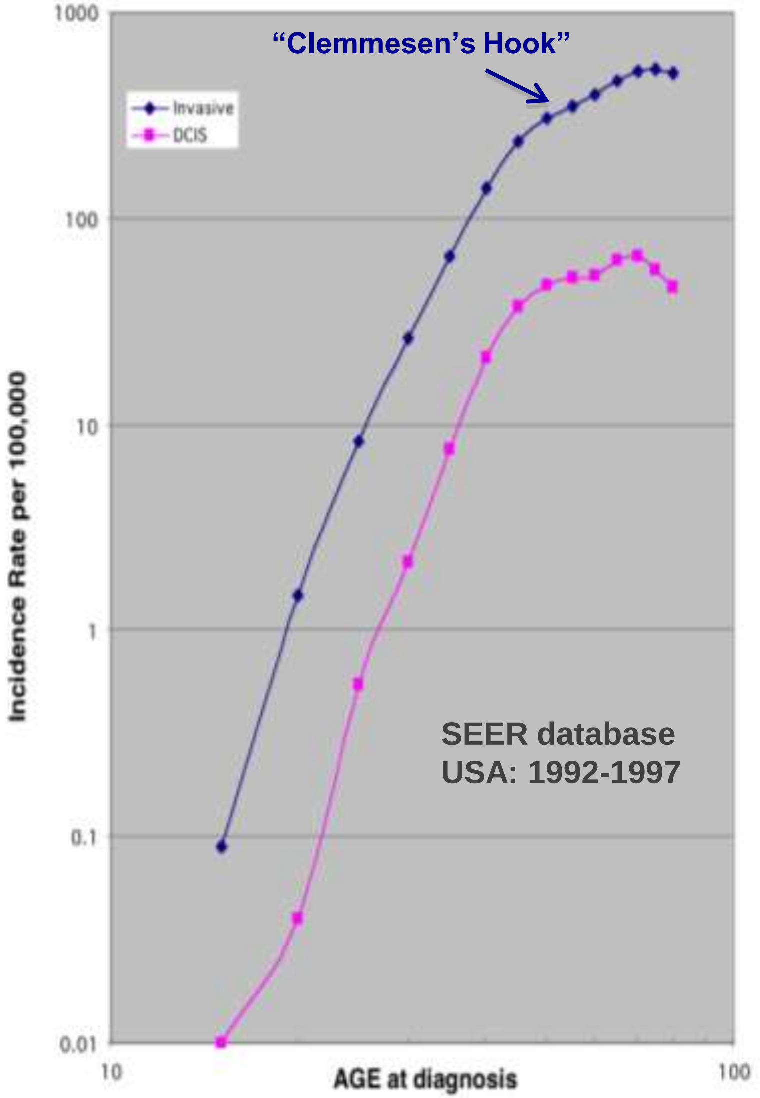


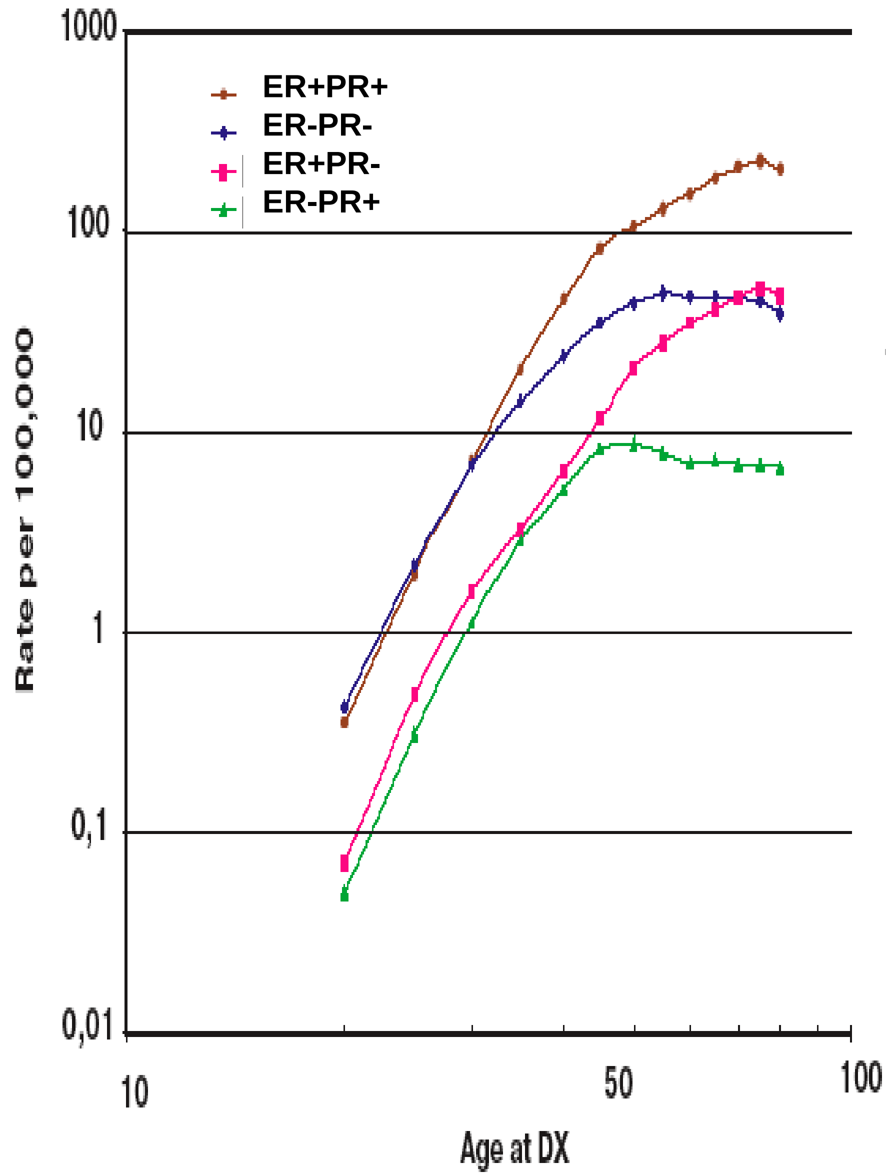
(Benz; Crit Rev Oncol/Hemat, 2008)

(Phipps, Clarke, Ereman; BCR, 2005)

Age-dependent Breast Cancer Incidence Rates

Younger Onset Breast Cancer: Less age-dependent ER/PR variability

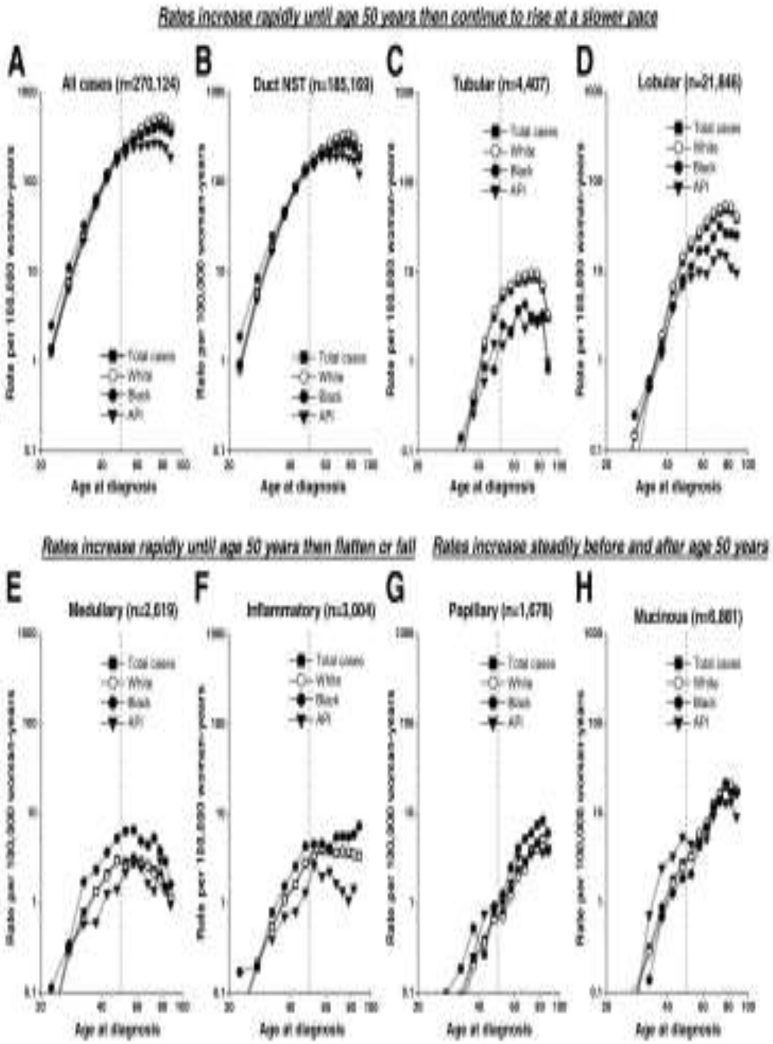




(Benz; Crit Rev Oncol/Hemat, 2008)

Age-dependent Breast Cancer Incidence Rates

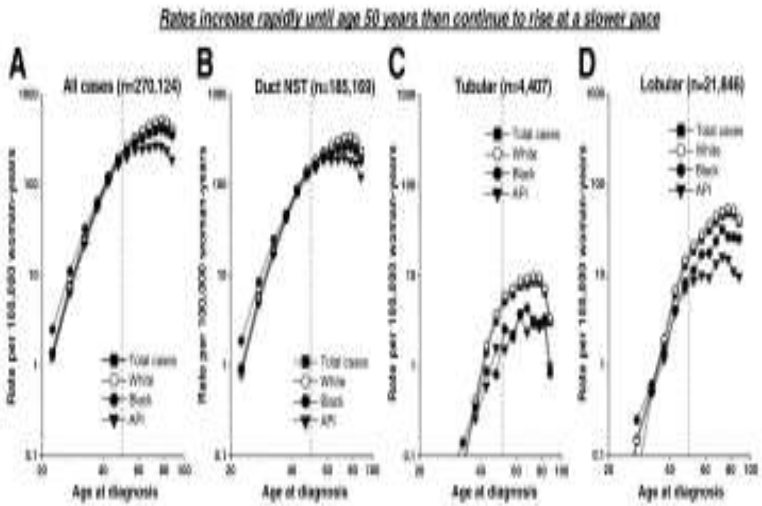
Younger Onset Breast Cancer: Less age-dependent histologic & ethnic variability

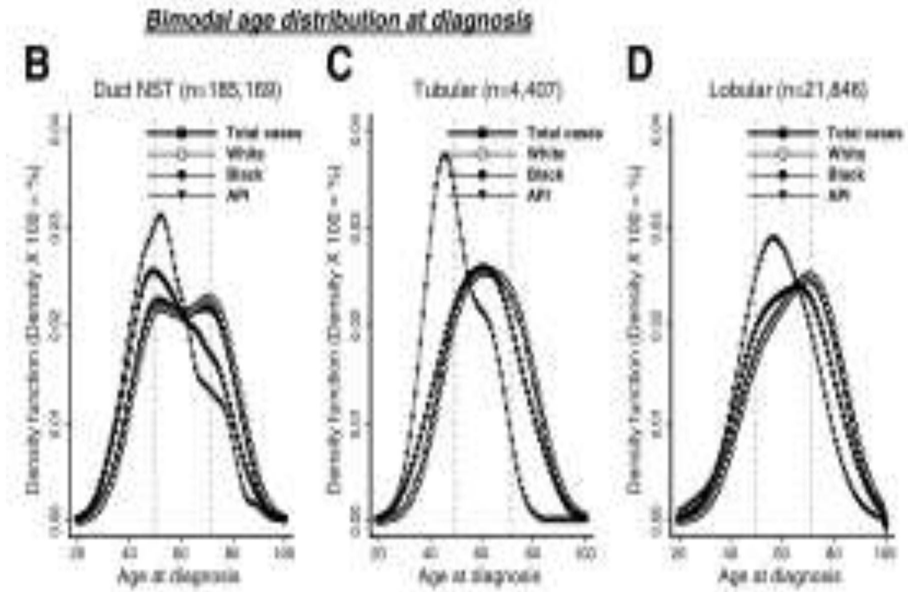


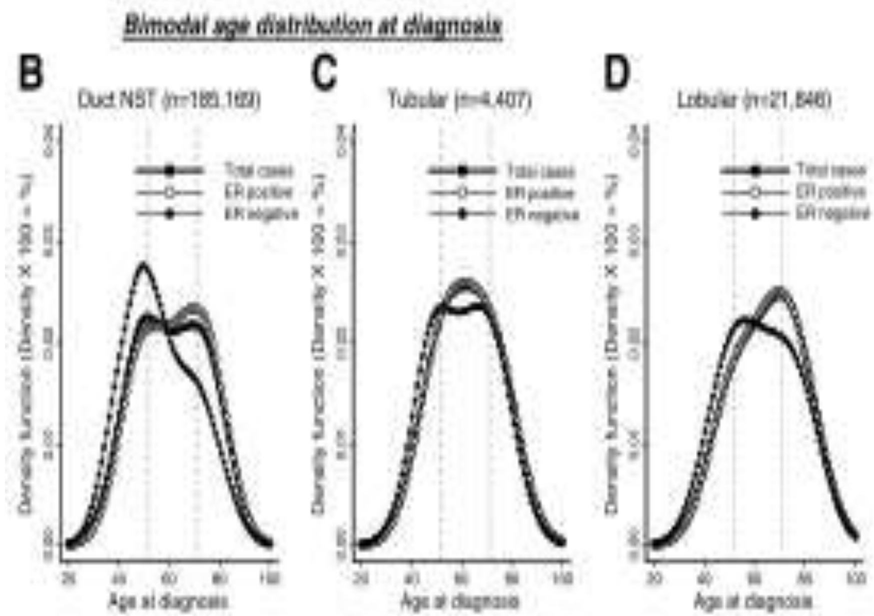
(Anderson et al.; CEBP, 2006)

Age-dependent Breast Cancer Incidence Rates

"Clemmesen's Hook" = superimposition of two different incidence rate curves







Bimodal Age-density Distributions

Early onset breast cancer

Inherited or early-life initiating events?

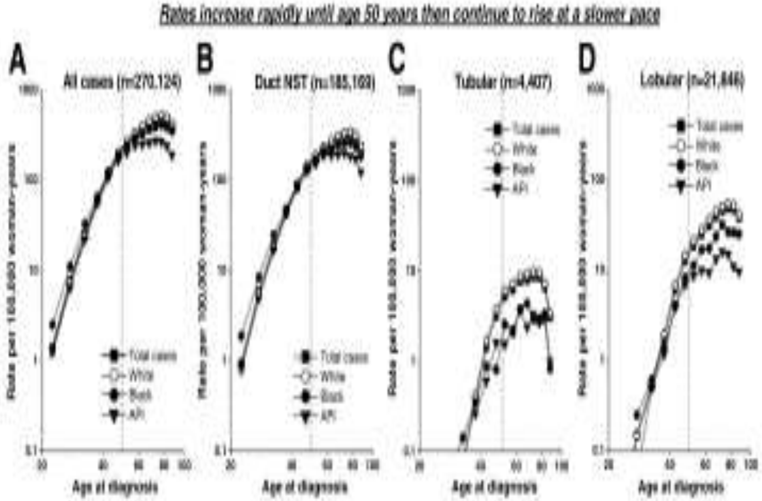
Late onset breast cancer

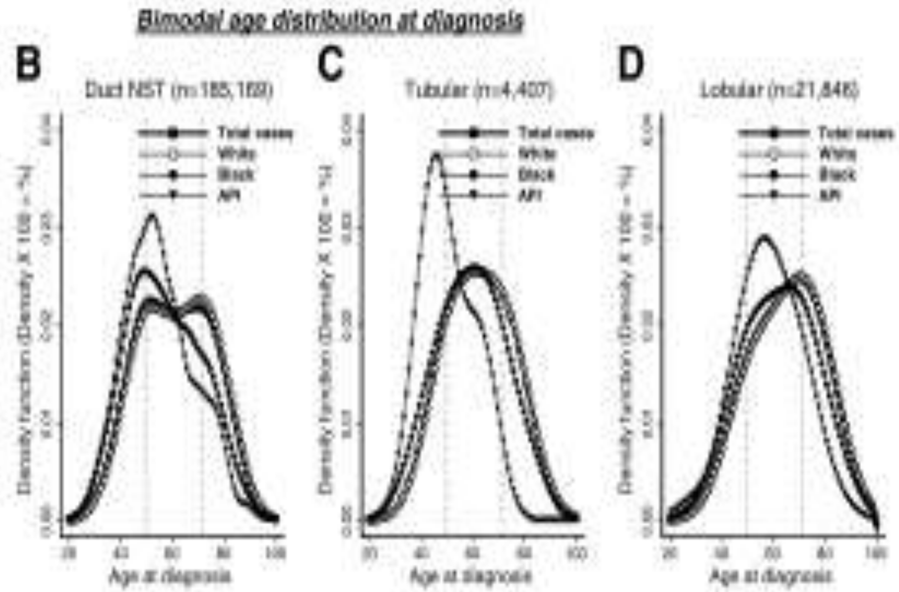
Later-life promoting events?

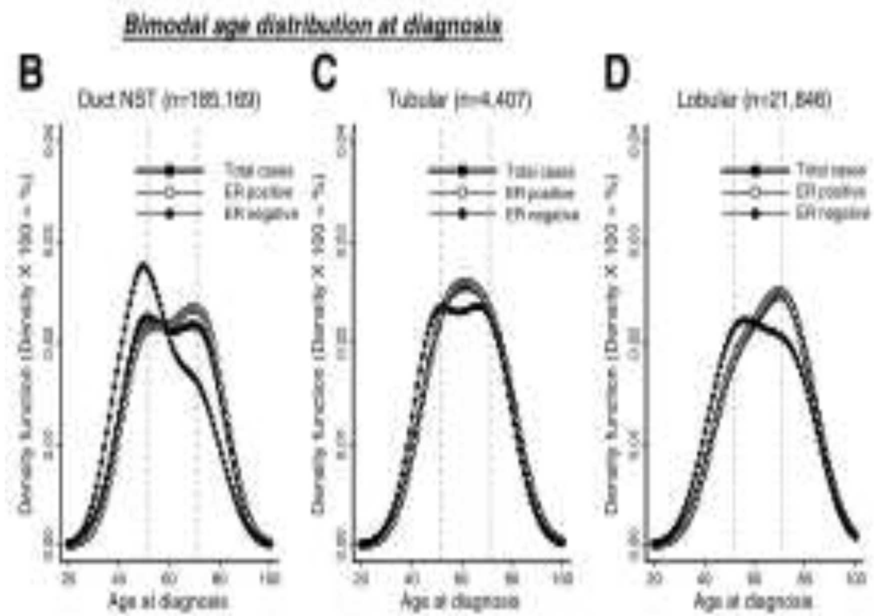
(Anderson et al.; CEBP, 2006)

Age-dependent Breast Cancer Incidence Rates

Are There Early vs. Late Onset Differences in Breast Cancer Outcome?







Bimodal Age-density Distributions

Early onset breast cancer

Inherited or early-life initiating events?

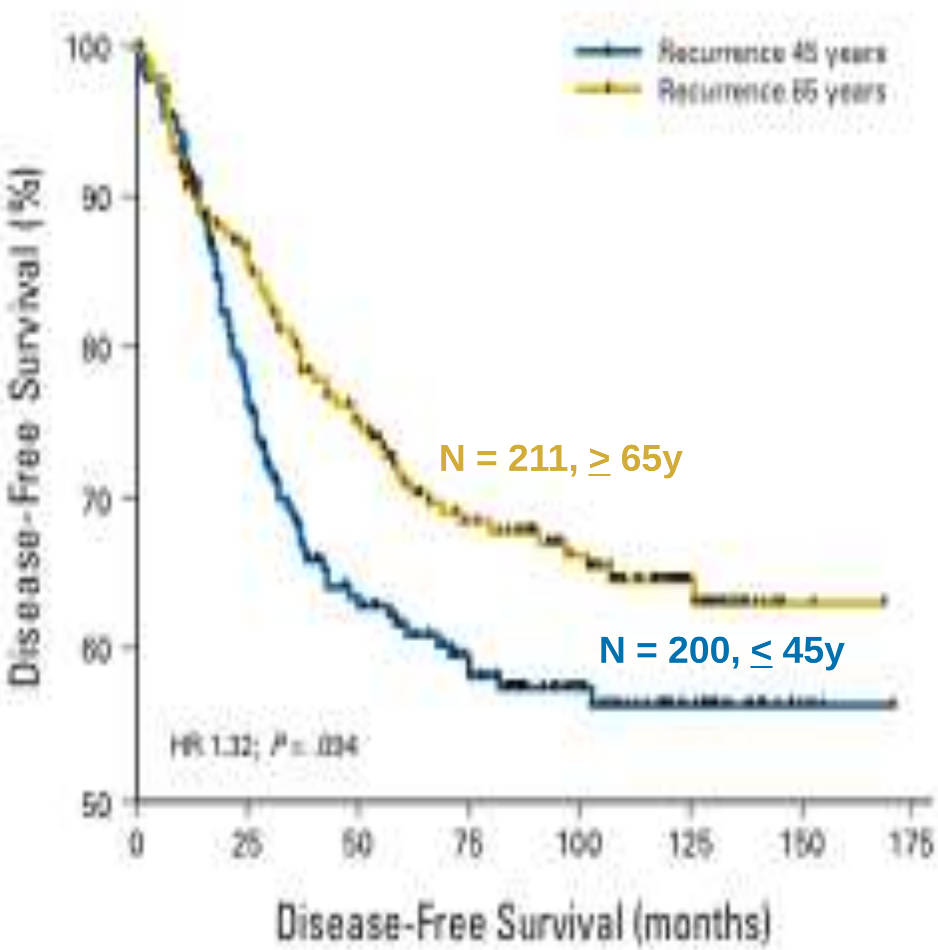
Late onset breast cancer

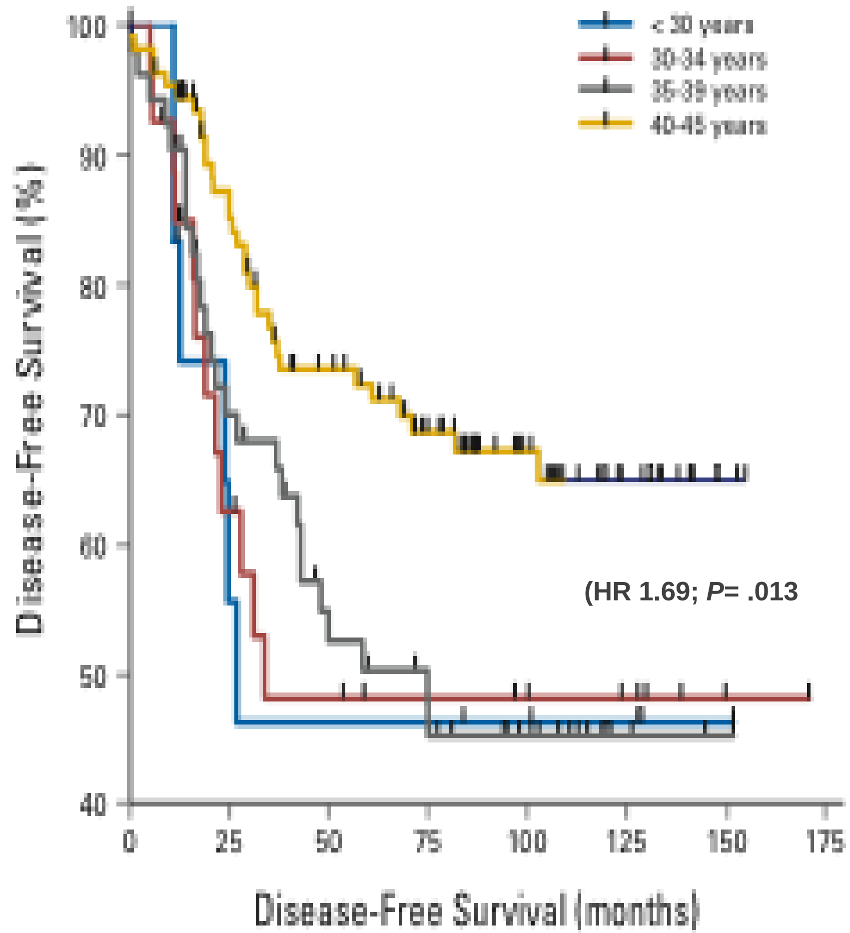
Later-life promoting events?

(Anderson et al.; CEBP, 2006)

Early Onset Breast Cancer = Worse Outcome

Age cohorts selected from four public data sets and 784 clinically annotated breast tumor samples, heterogeneous with regard to stage, grade, ER status, and adjuvant therapy





(Anders et al., J Clin Oncol 26: 3324-3330, 2008)

What Are the Known Risk Factors?

"Not modifiable"

- Gender
- Age *
- Family history (1st degree relatives) *
- Age at menarche *
- Age at natural menopause
- Race/ethnicity
- Prior benign biopsies *

"Modifiable"

- Parity/Age 1st live birth *
- Mammographic density
- Breastfeeding
- Obesity/weight gain
- Hormone therapy (E+P)
- Radiation exposure
- Alcohol consumption
- Physical activity
- Diet

*Incorporated into Breast Cancer Risk Assessment Tool (BCRAT)/Gail Model

What Are the Known Risk Factors?

"Not modifiable"

- Gender
- Age*
- Family history (1st degree relatives)*
- Age at menarche*
- Age at natural menopause
- Race/ethnicity
- Prior benign biopsies*

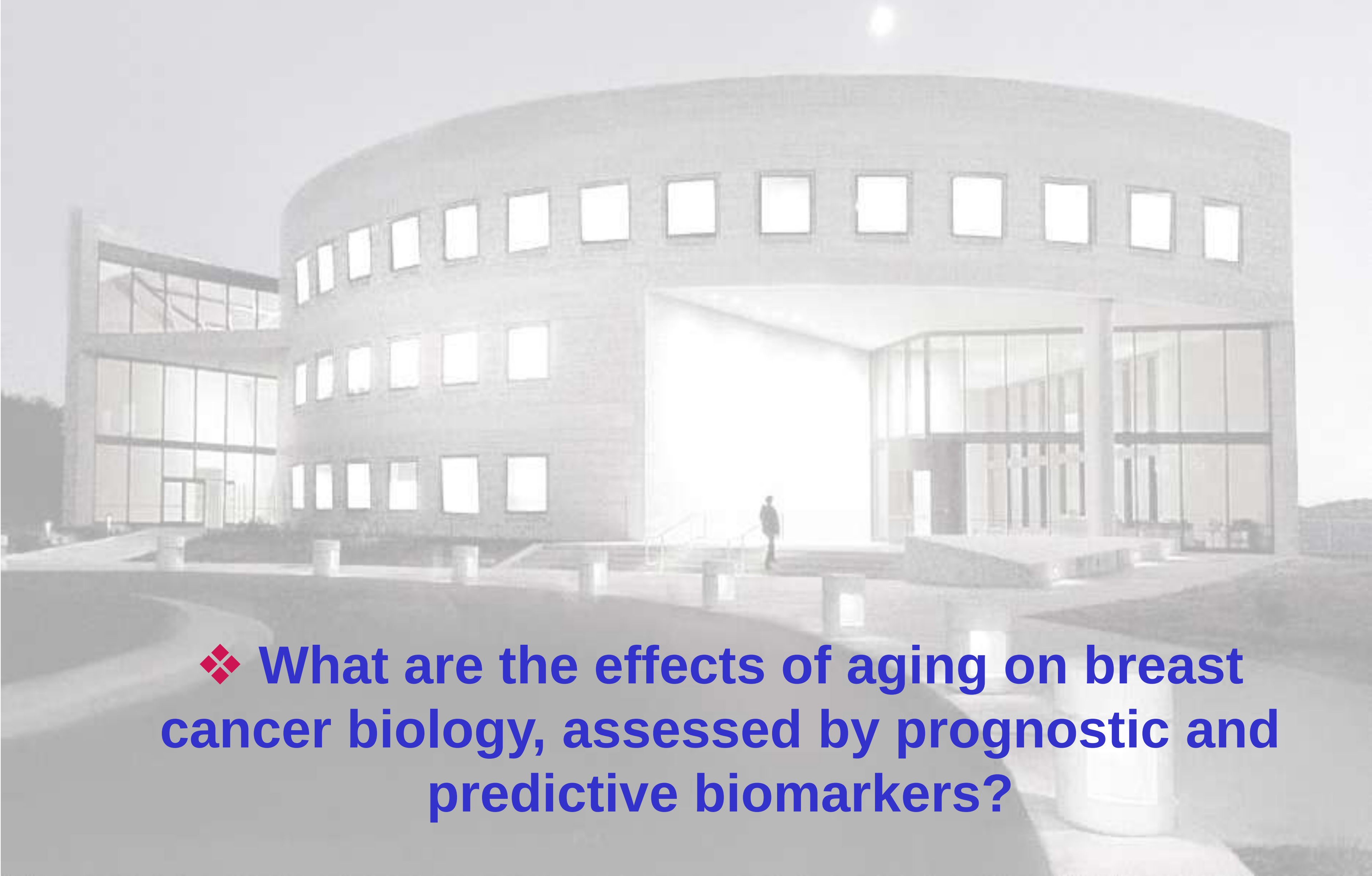
"Modifiable"

- Parity/Age 1st live birth*
- Mammographic density
- Breastfeeding
- Obesity/weight gain
- Hormone therapy (E+P)
- Radiation exposure
- Alcohol consumption
- Physical activity
- Diet

*Incorporated into Breast Cancer Risk Assessment Tool (BCRAT)/Gail Model

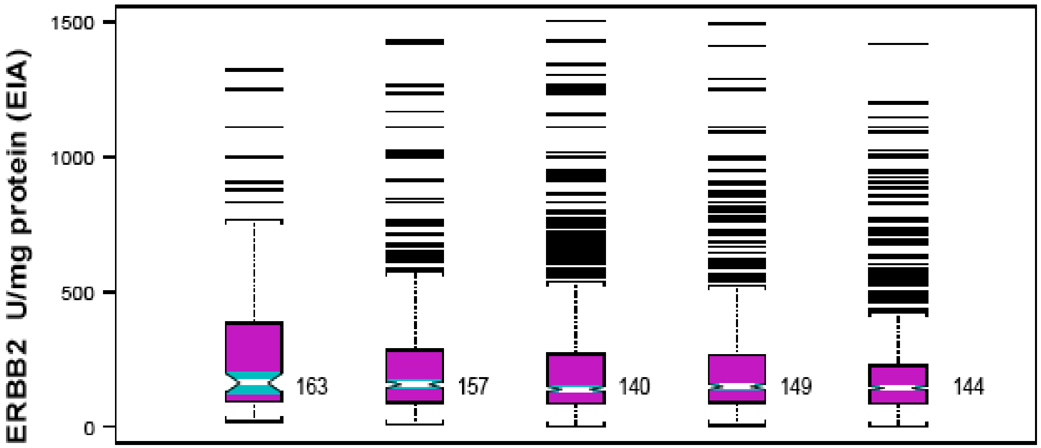
Strong associations with early onset breast cancer

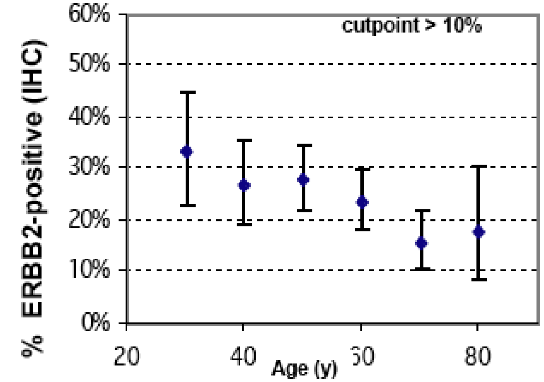
Breast Cancer & Aging: Questions



- What are the effects of aging on breast cancer biology, assessed by prognostic and predictive biomarkers?

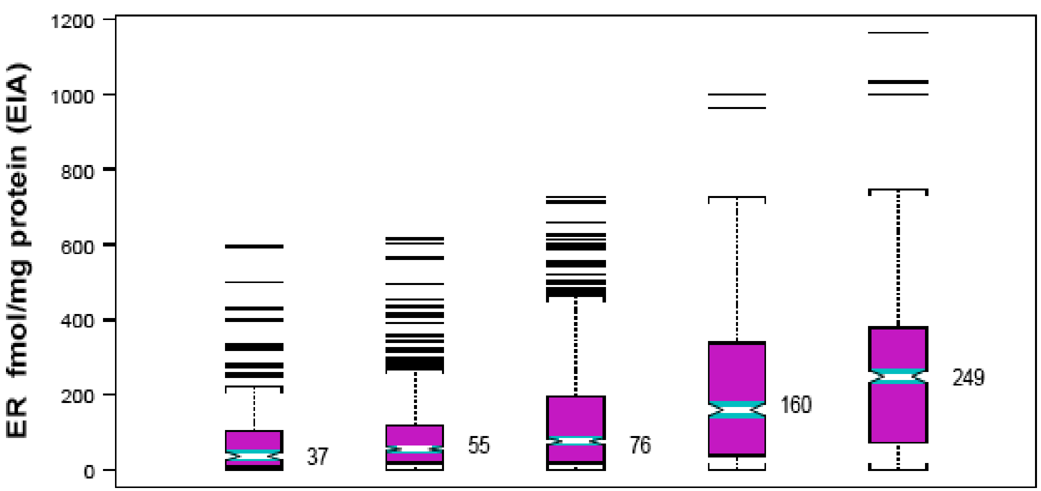
◆ Growth receptors ERBB2/HER2 & ER
Inverse relationships

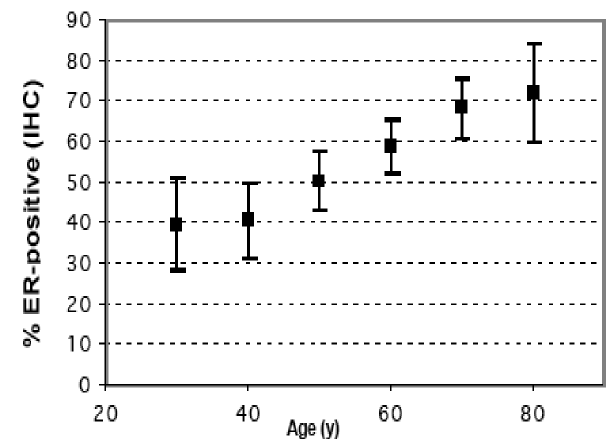




(N = 820; r = -0.136, p < 0.0001)

age (y):	22-39.9	40-49.9	50-59.9	60-69.9	70-85	
N:	151	541	782	702	813	(total = 2989)





(N = 813; r = 0.213, p < 0.0001)

(Benz; Crit Rev Oncol/Hemat, 2008)

- **Markers of invasiveness & metastatic potential**

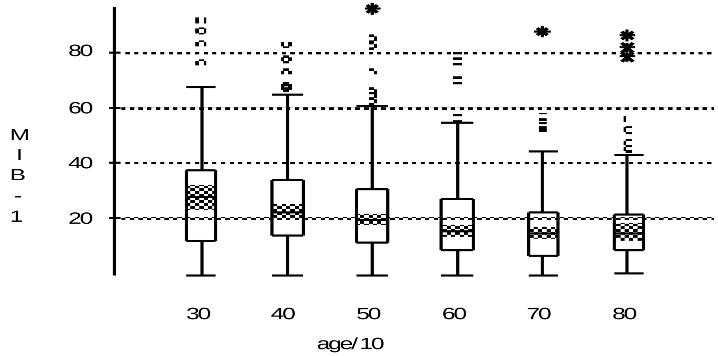
  angiogenic factors: ***VEGF, bFGF***

  proteases: ***Cath. D, uPA, uPAR, PAI-1***

No association with age after 40 y

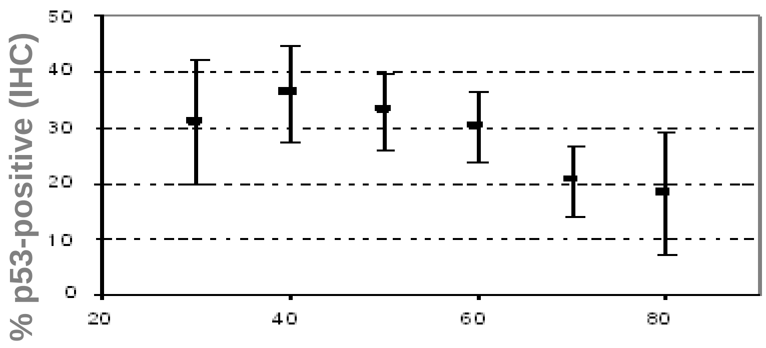
- **Markers of proliferation & genetic instability**

Ki-67/MIB-1



(N = 802; r = -0.216, p < 0.0001)

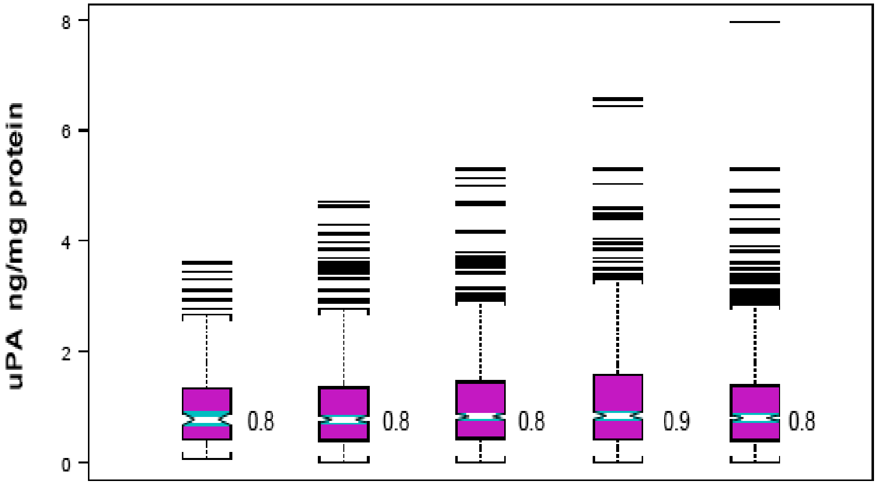
p53-positvity



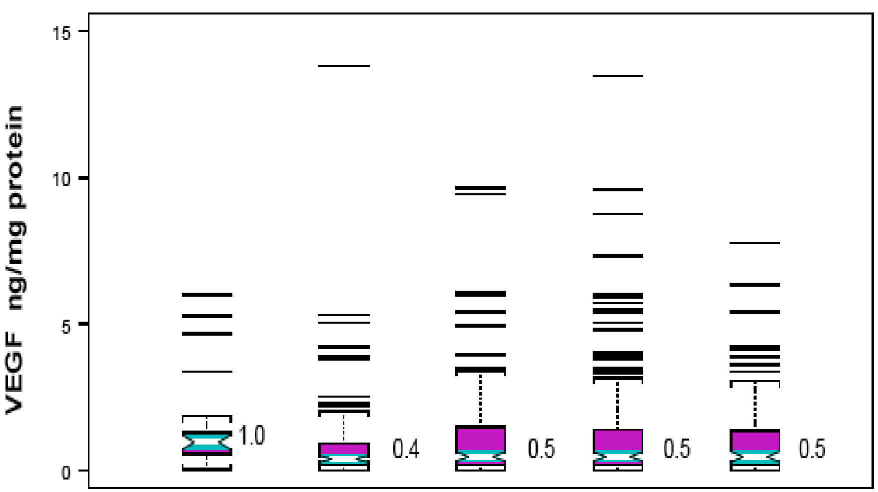
(N = 823; r = -0.111, p = 0.0014)

Decline significantly with age after 40 y

Absent age-expression relationship does not preclude age-dependent prognostic effect

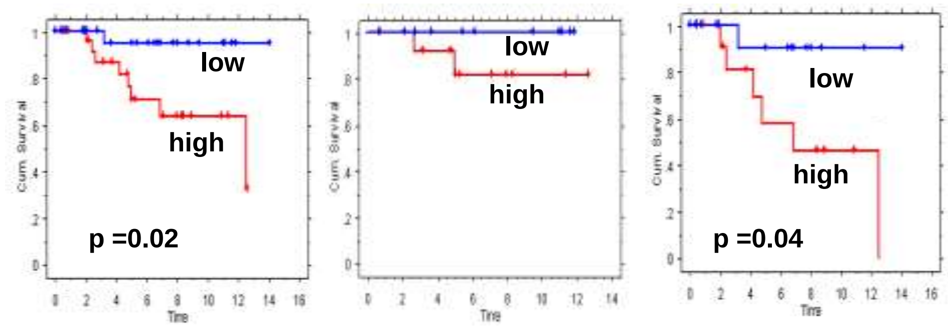


N (uPA):	151	541	782	702	813
age (y):	22-39.9	40-49.9	50-59.9	60-69.9	70-85
N (VEGF):	21	107	178	170	103



All (n=54) | **Age ≥70 (n=25)** | **Age ≤ 45 (n=29)**

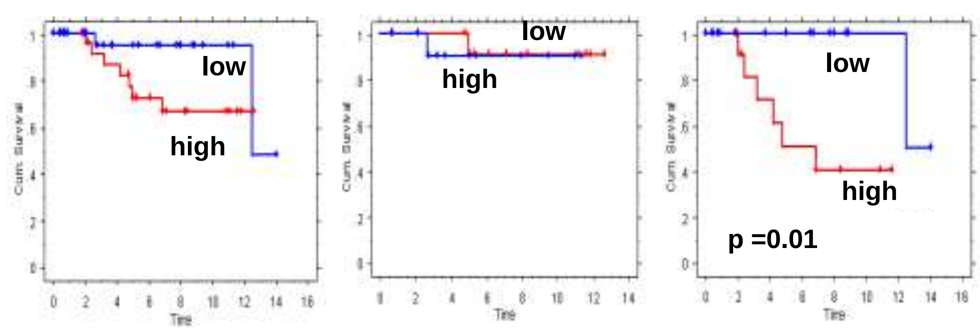
uPA



uPA & VEGF

prognostic only in early onset breast cancer

VEGF



(Benz; Crit Rev Oncol/Hemat, 2008)

Biomarker results from retrospective analysis of ~4,000 breast cancer cases...

- **Most show no association between age and level**
  - ***PR, pS2, Bcl-2, VEGF, uPA, uPAR, PAI-1, Cath-D***
- **Some are strongly associated with age**
  - **Negative:** ***grade, MI/Ki67, AI, p53, ErbB1&2***
  - **Positive:** ***ER positivity & content***

cf. Benz et al., Age-associated biomarker profiles of human breast cancer. Int. J. Biochem. Cell Biol., 2002
Quong et al., Age-dependent changes in breast cancer hormone receptors and oxidant stress markers. Breast Cancer Res. Treat., 2002

- ***Demonstrate that aging affects breast cancer biology and its clinical behavior.***

- ***Since ER-positivity correlates inversely with other biomarkers, what is more important… Aging or ER status?***

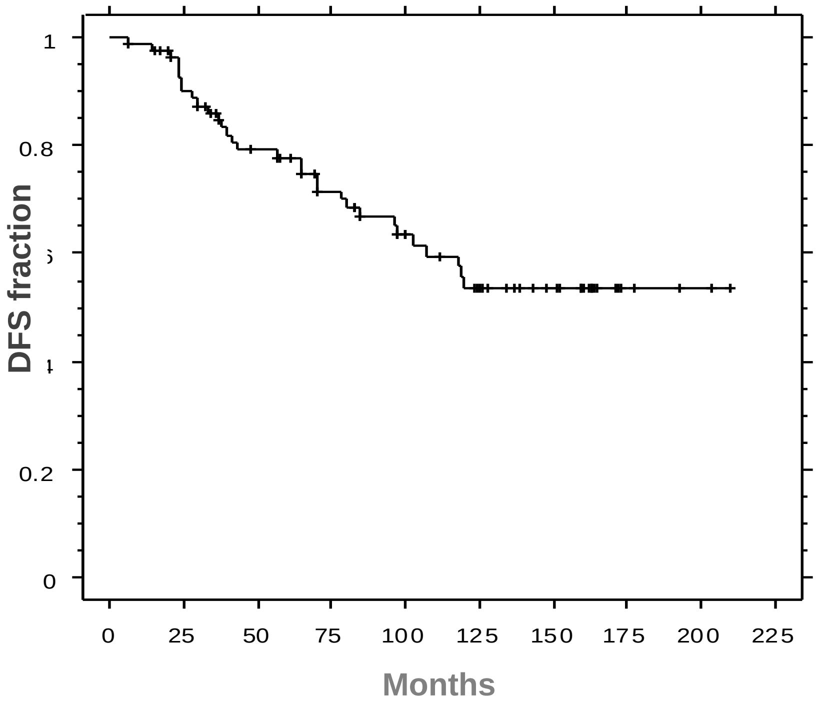
Breast Cancer & Aging: Questions

- Among the more prevalent forms of ER+ breast cancer, are there age-associated biological differences?

Pilot Retrospective Outcome Analysis: *Impact of Aging*

ER-positive, $T_{1/2}$ N_0, ductal BrCa: n = 83; Older (≥ 70 y) vs. Younger (< 45 y) cases

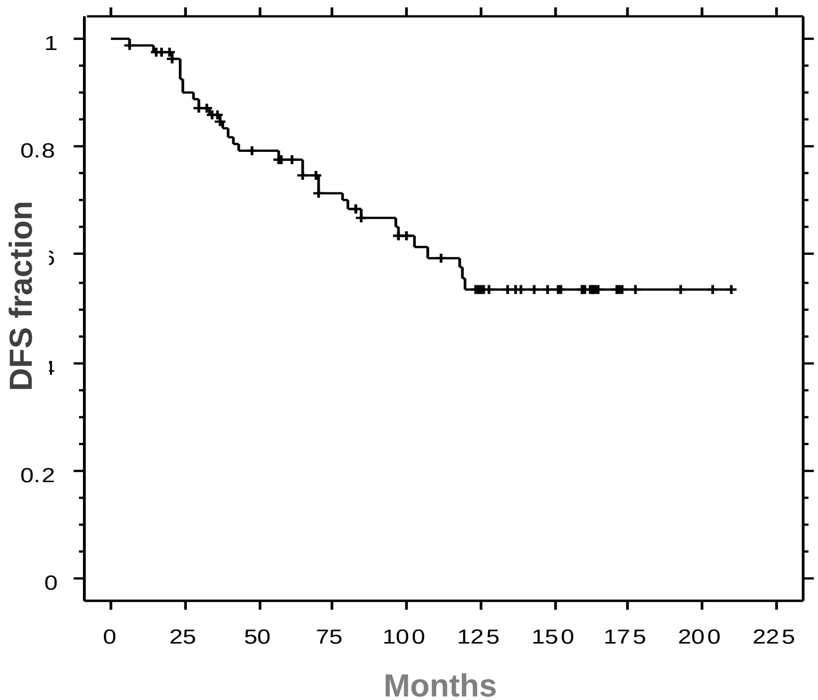
[A. Thor FFPE archive of 828 breast cancers; >16y follow-up; no adj. tx]

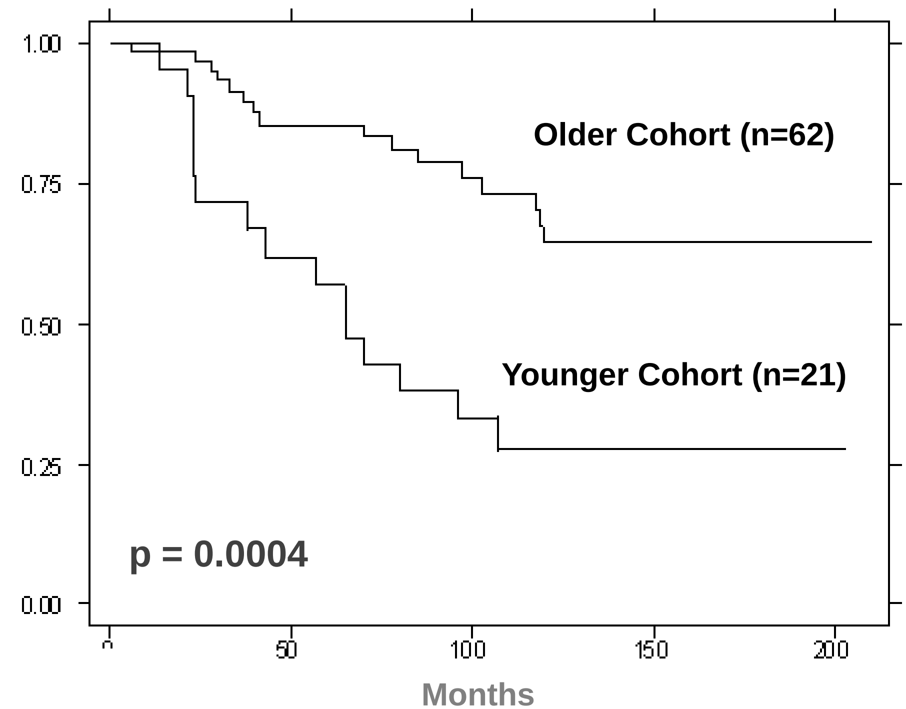


Pilot Retrospective Outcome Analysis: *Impact of Aging*

ER-positive, $T_{1/2}$ N_0, ductal BrCa: n = 83; Older (≥ 70 y) vs. Younger (< 45 y) cases

[A. Thor FFPE archive of 828 breast cancers; >16y follow-up; no adj. tx]





Even for ER+ breast cancers, age is a significant breast cancer risk factor

(Benz; Crit Rev Oncol/Hemat, 2008)

R01-AG020521 (2003-2009)
“Biology of Breast Cancers Arising in Older Women”

Study Design: ER-positive, early-stage (T1/2, N0) ductal breast cancers

- Cohort comparison: YOUNGER (≤ age 45) vs. OLDER (≥ age 70) age-at-diagnosis Cauc. cases
- Cryobanked tumor samples for DNA and RNA (± protein fractions); sample sources from:
  - -- UCSF/BOP; n = 83 (Y = 21, O = 62) for DNA, 68 for RNA; 54 with RFS (Y<<O; p < 0.04)
  - -- NCI-Bari, Italy; n = 70 (Y = 27, O = 43) for DNA, 30 for RNA; no RFS data

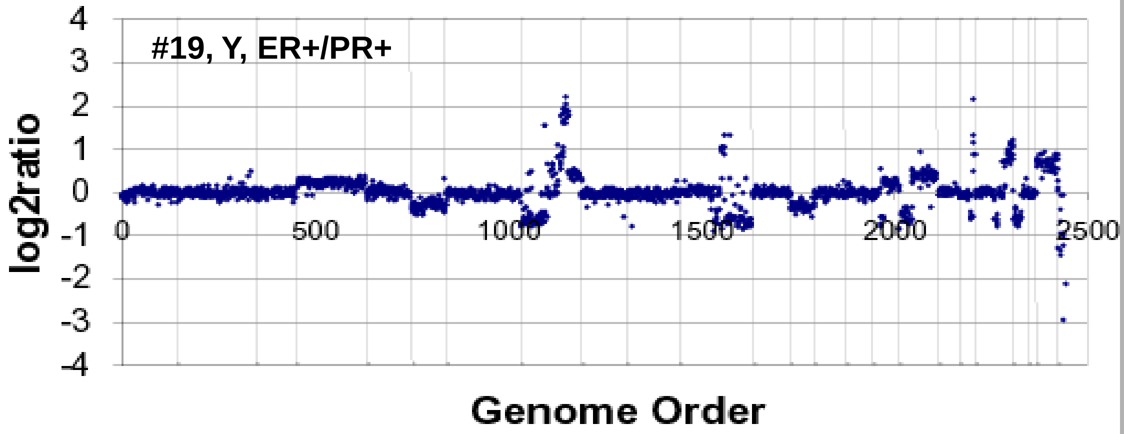
    [from larger collective of ER+ & ER- cases with matching blood sample]

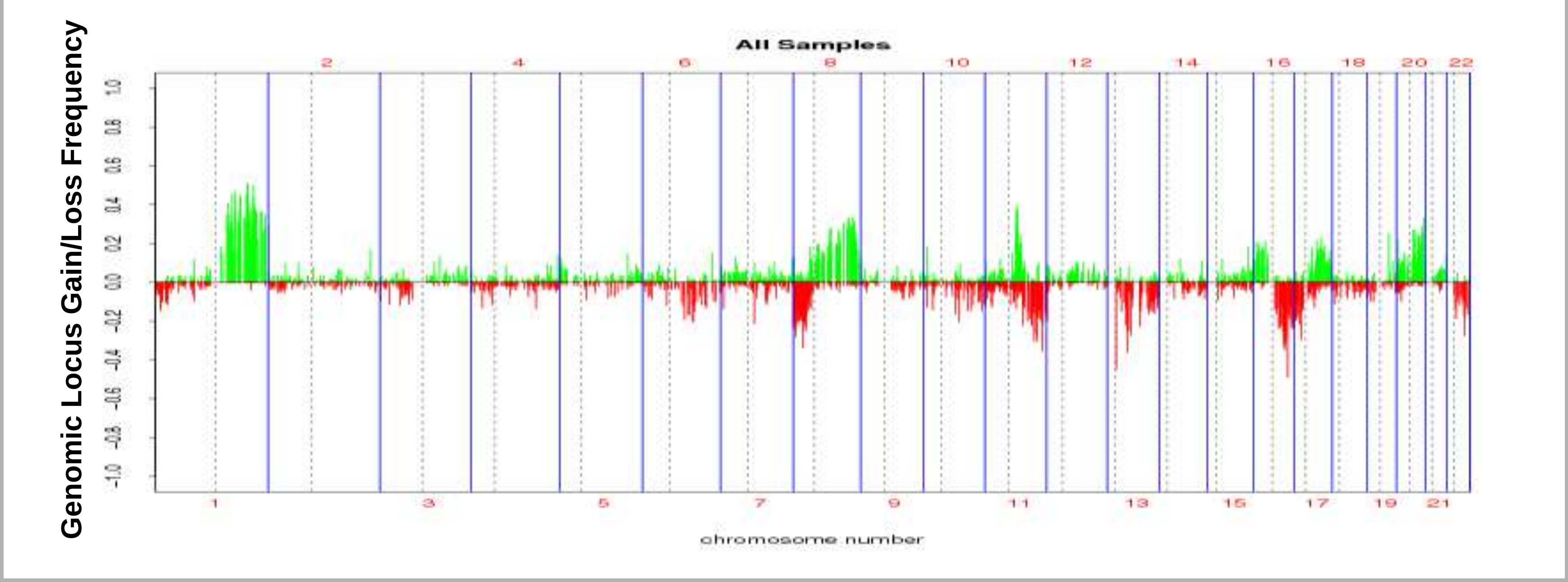
Specific Aims:

- Identify genomic differences between Older and Younger ER+ cohorts using DNA samples.
  - - Genome copy number phenotypes (2.5 K BAC CGH arrays)
  - - p53 mutations in DNA core (microsequence exons 5-8)
- Identify gene expression differences between Older and Younger ER+ cohorts using RNA samples.
  - - Expression array signatures & phenotypes (Affy arrays)

(Yau and Benz, BCR, 2007)

Array CGH Analysis of Breast Cancers

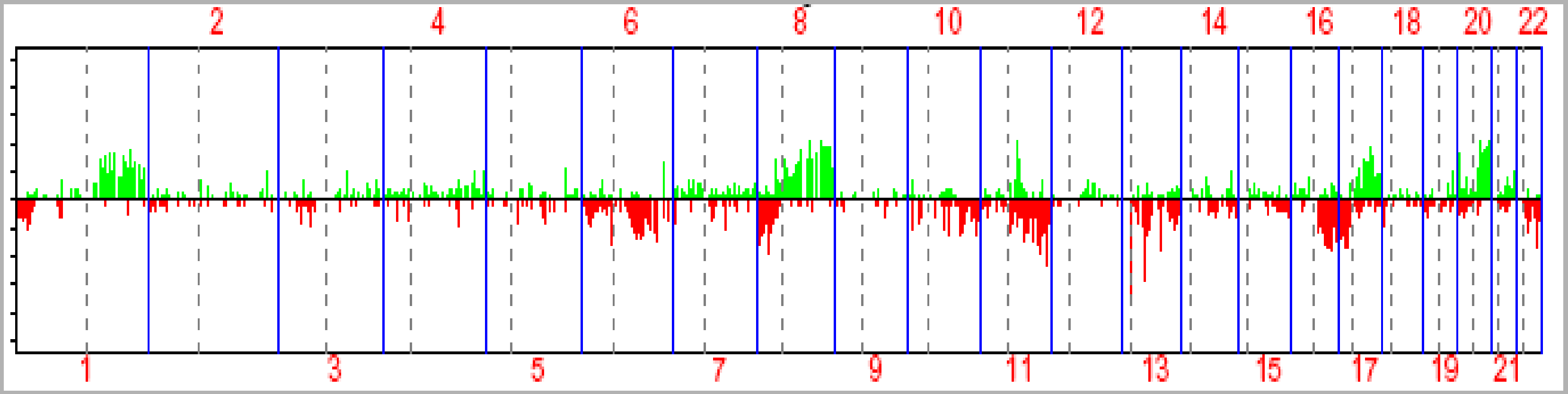




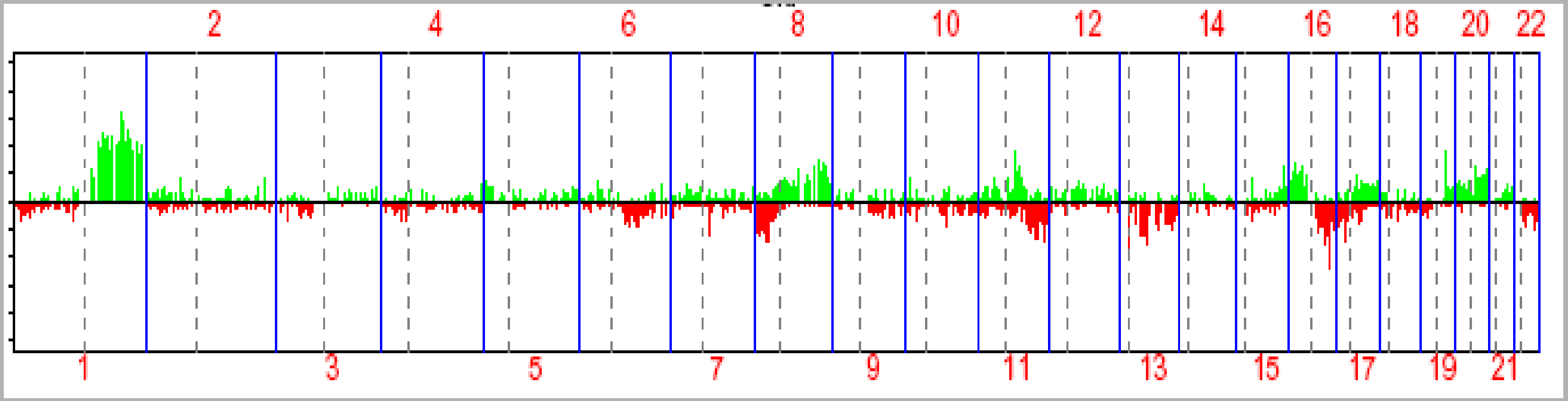
No Age Associated Differences in Genomic Locus Aberration Frequencies

Frequency gained or lost

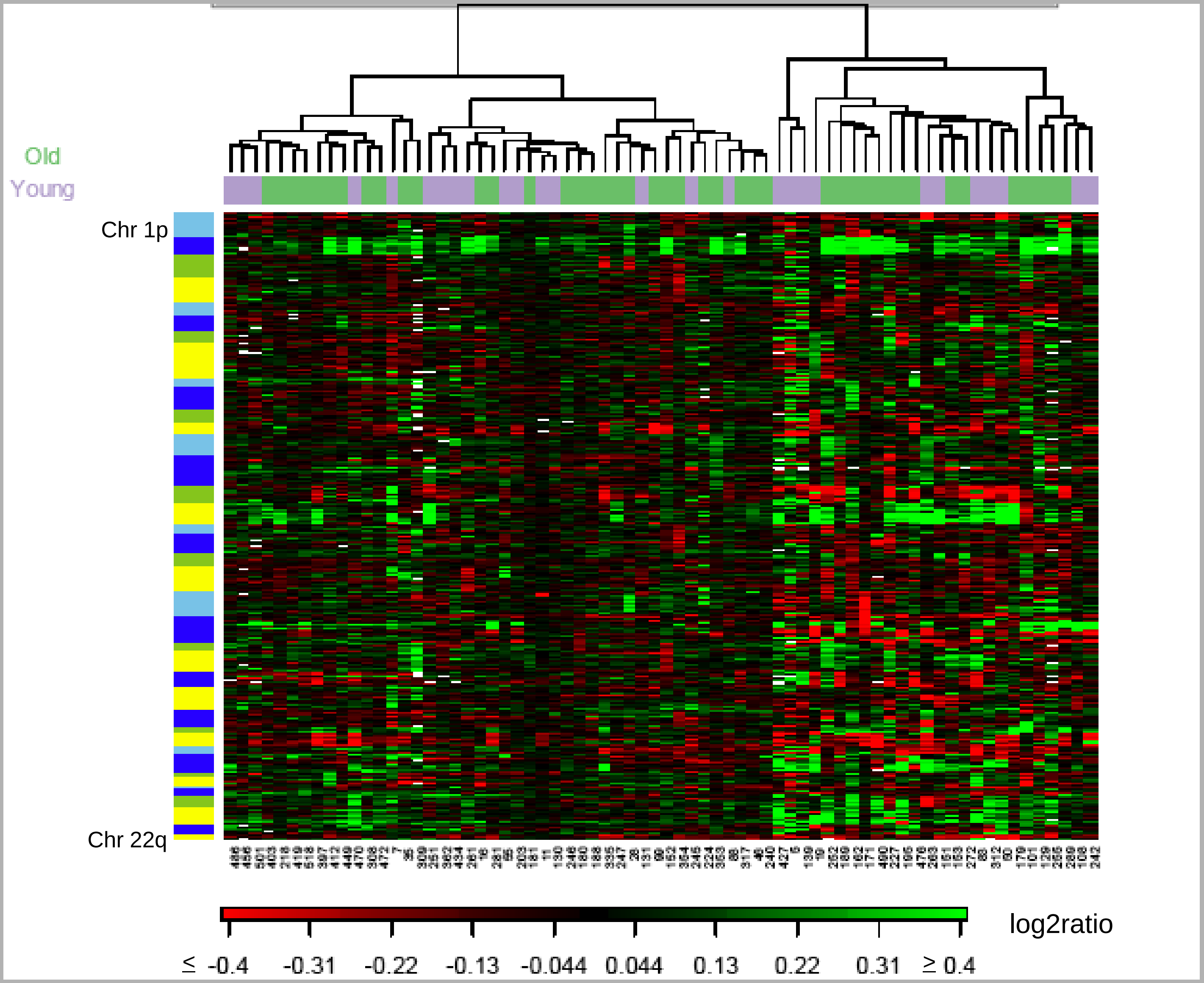
Young



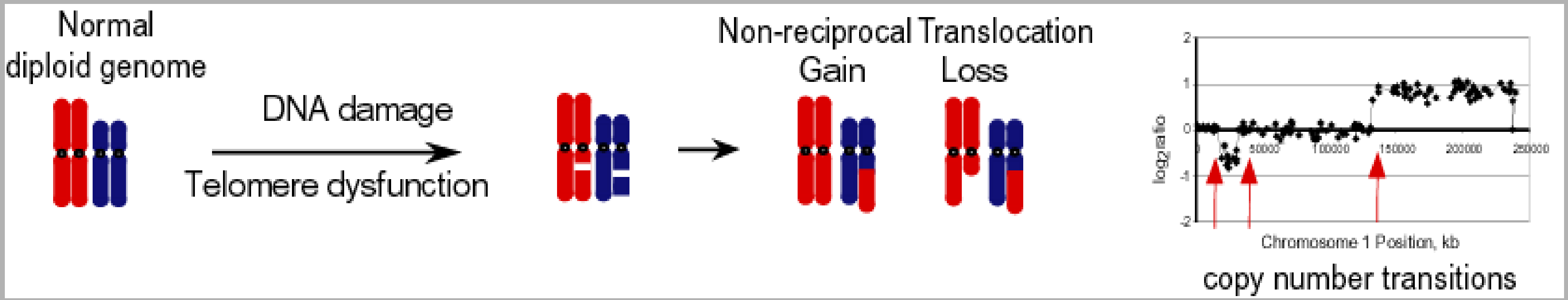
Old

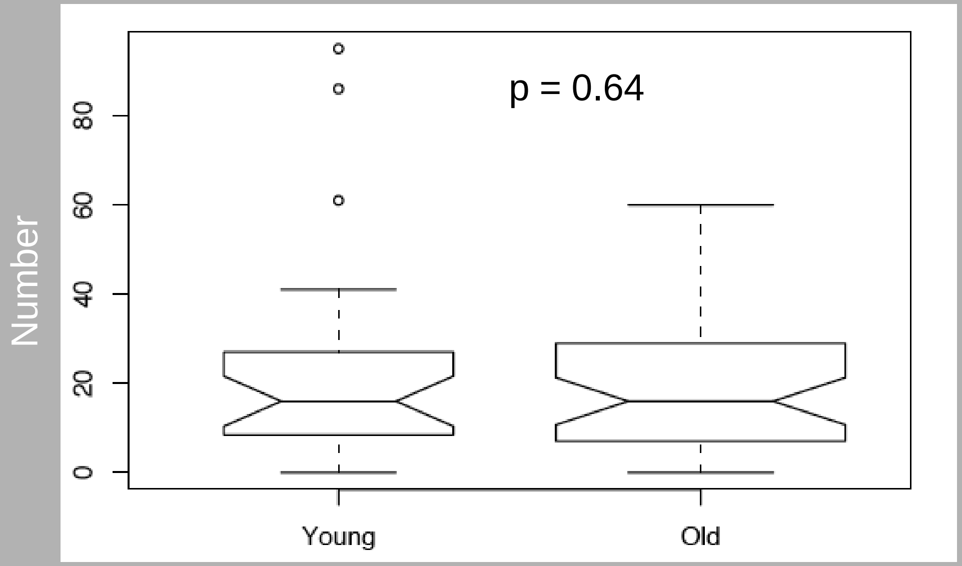


Unsupervised Hierarchical Clustering of 70 ER+ IDC Shows no Age Association with Subgroups



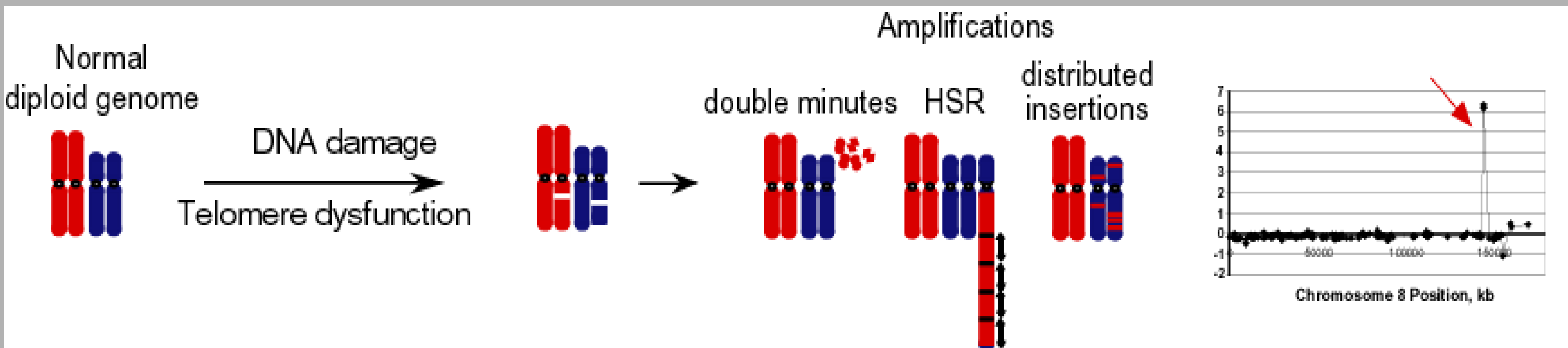
Copy Number Transitions
Old = Young

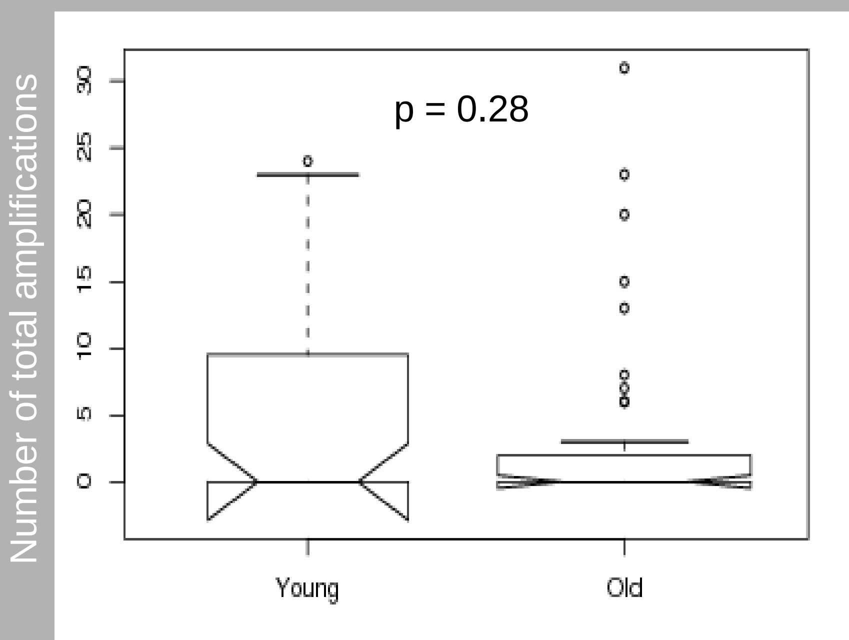




Number of Amplifications: Old ~ Young

(*ERBB2, MYC, CCND1, MDM2, EGFR, AIB1, TOPO2, ZNF217*, etc.)



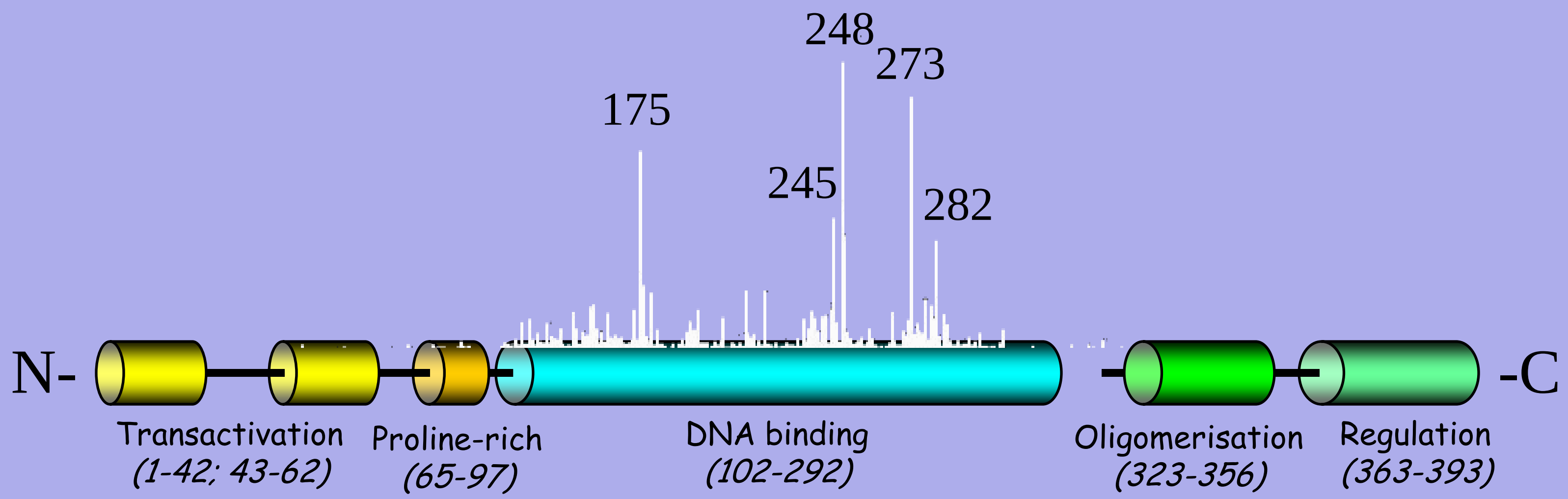


ERBB2 amplifications:

Young = 11%

Old = 9%

Age, ER status & p53 Mutations?



www-p53.iarc.fr

Lacroix et al., Endo-Rel Ca, 2006

~ 20% p53mut frequency reported among all breast cancers

~ 90% missense mutations, >90% in DNA-binding core (exons 5-8, aa 126-306)

N = 289	ER-/p53wt	ER+/p53wt	ER-/p53mut	ER+/p53mut
Early onset ($\leq$ 45 y) n=135	49 (36.3%)	64 (47.4%)	14 (10.4%)	8 (5.9%)
Late onset ($\geq$ 70 y) n=154	25 (16.2%)	107 (69.5%)	12 (7.8%)	10 (6.5%)

P = 0.004, Fisher Exact

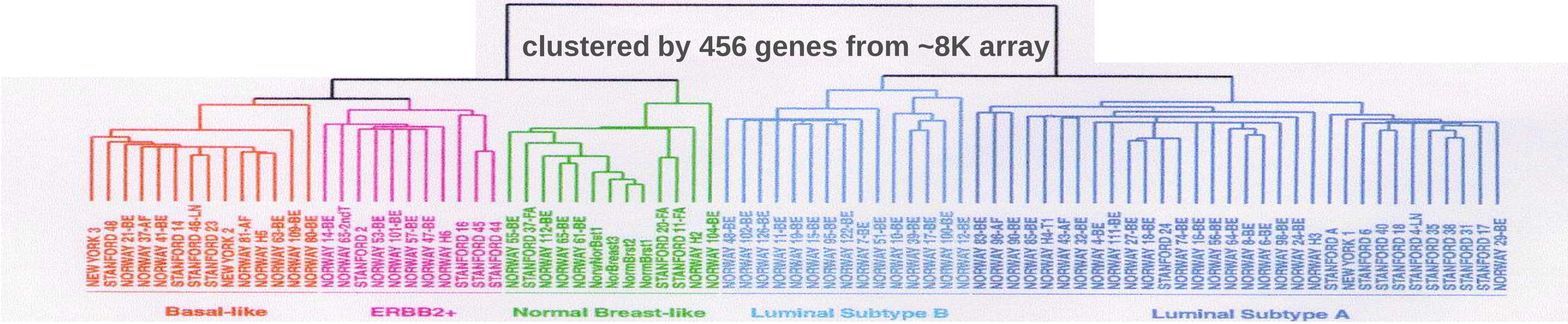
No age link with p53mut when ER status considered

Microarrays Identify Multiple Breast Cancer Subsets

(Sørlie et al., PNAS 98: 10869-10874, 2001)

78 breast cancers (+7 benign breast samples)

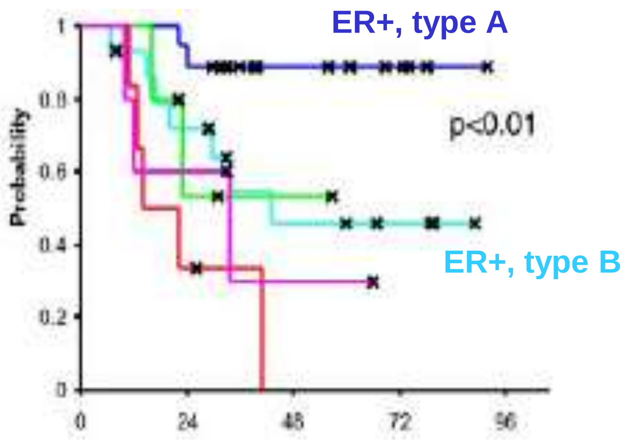
clustered by 456 genes from ~8K array



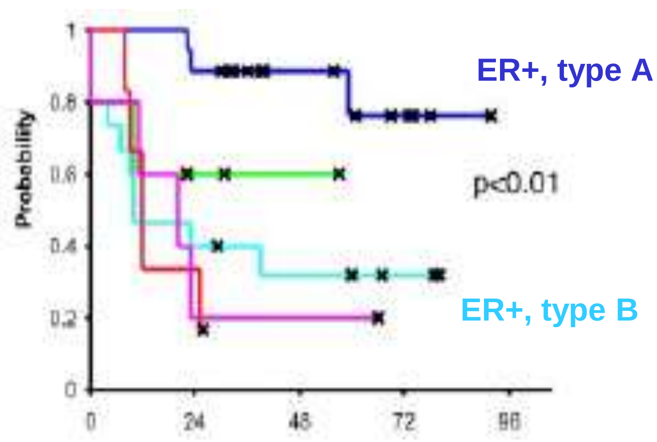
ER+, type B

ER+, type A

82%	71%	33%	67%	13%	%mutated p53



Overall Survival (Mo.)



Relapse-freel Survival (Mo.)

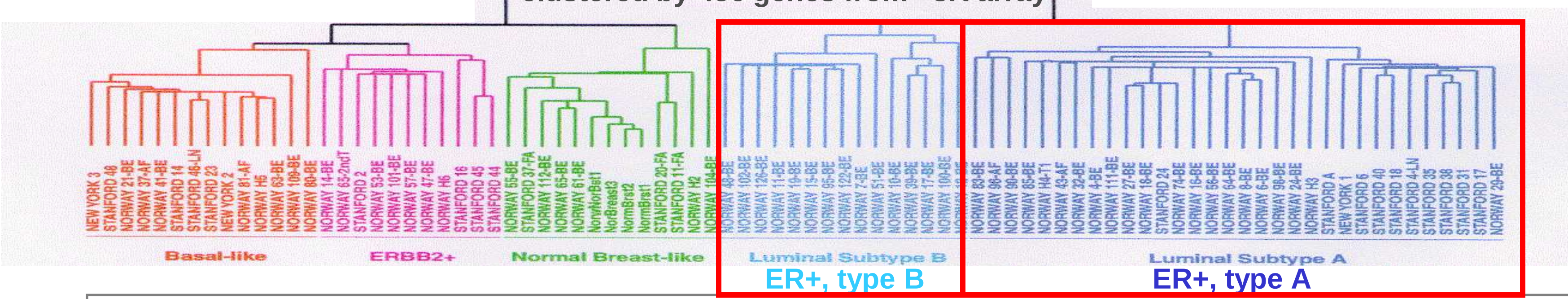
N = 49 breast cancer patients (Stage II/III, uniform adj. treatment)

Microarrays Identify Several ER+ Br Ca Subsets

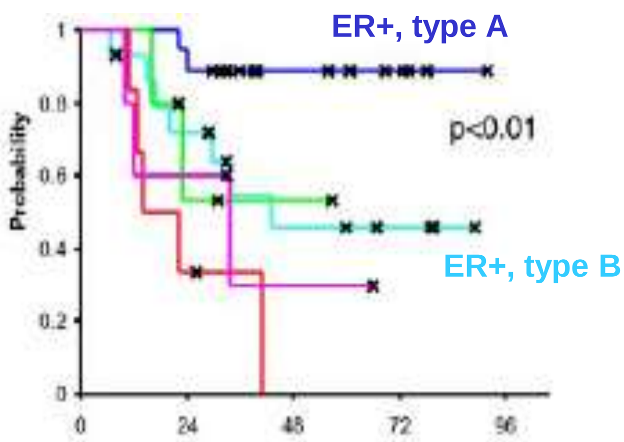
(Sørlie et al., PNAS 98: 10869-10874, 2001)

78 breast cancers (+7 benign breast samples)

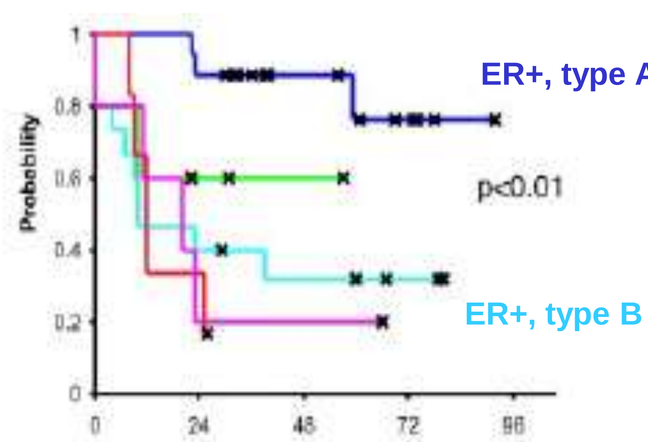
clustered by 456 genes from ~8K array



82%	71%	33%	67%	13%	%mutated p53



Overall Survival (Mo.)

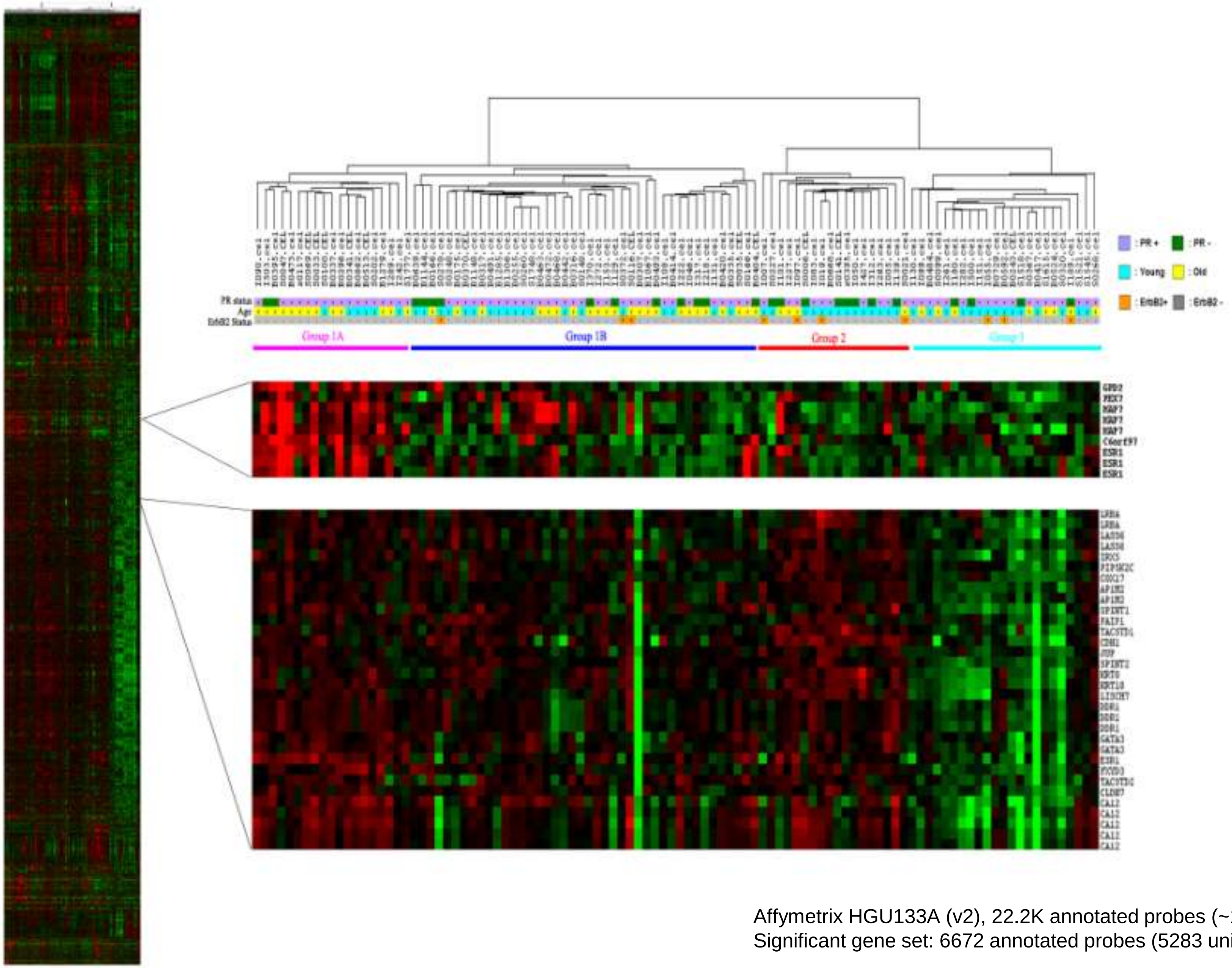


Relapse-freel Survival (Mo.)

N = 49 breast cancer patients (Stage II/III, uniform adj. treatment)

Microarray Unsupervised Clustering of ER+ BrCa

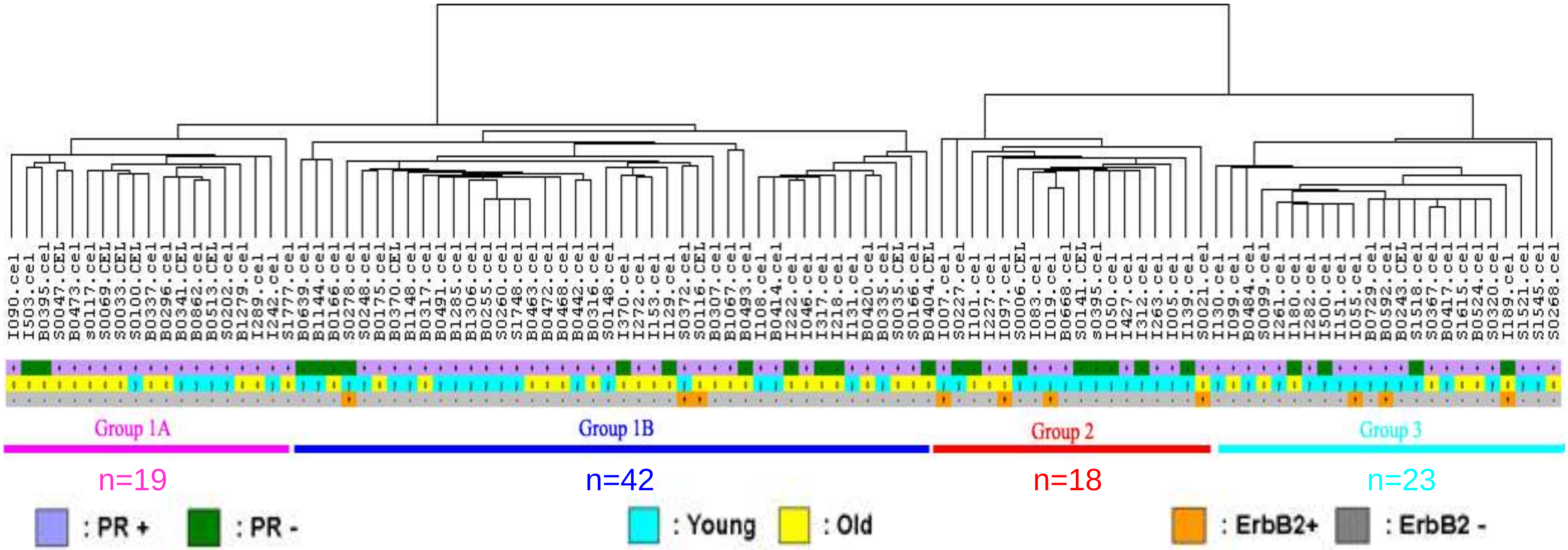
N = 102 RNA samples from O + Y age cohorts of node-neg ER-pos ductal BrCa



Two ER-associated Gene Groups

Affymetrix HGU133A (v2), 22.2K annotated probes (~13K unigenes)
Significant gene set: 6672 annotated probes (5283 unique genes).

Unsupervised Analysis of ER+ Ductal BrCa



Group 1A: older patients (68%)

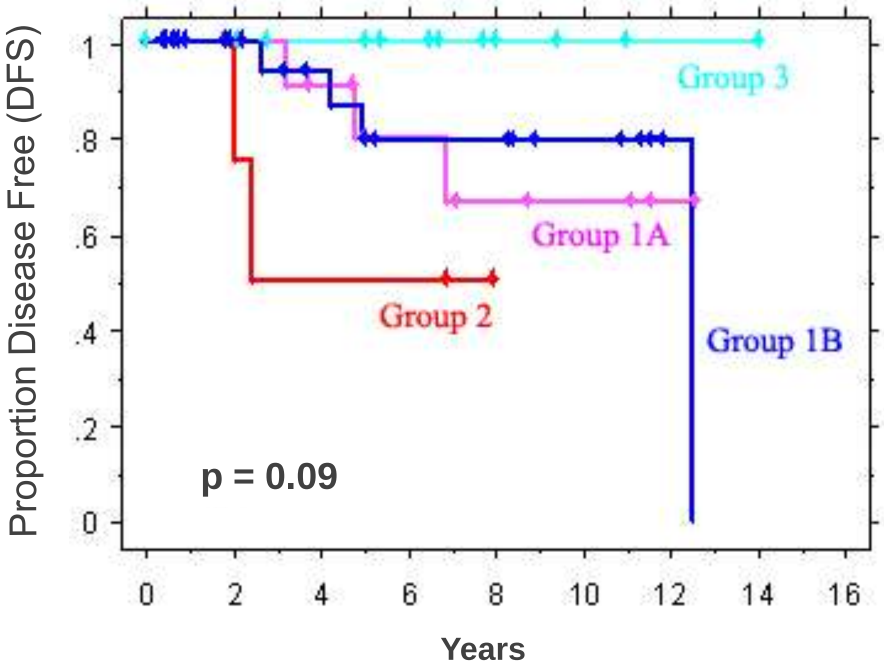
Group 1B: older patients (55%)
74% PR+

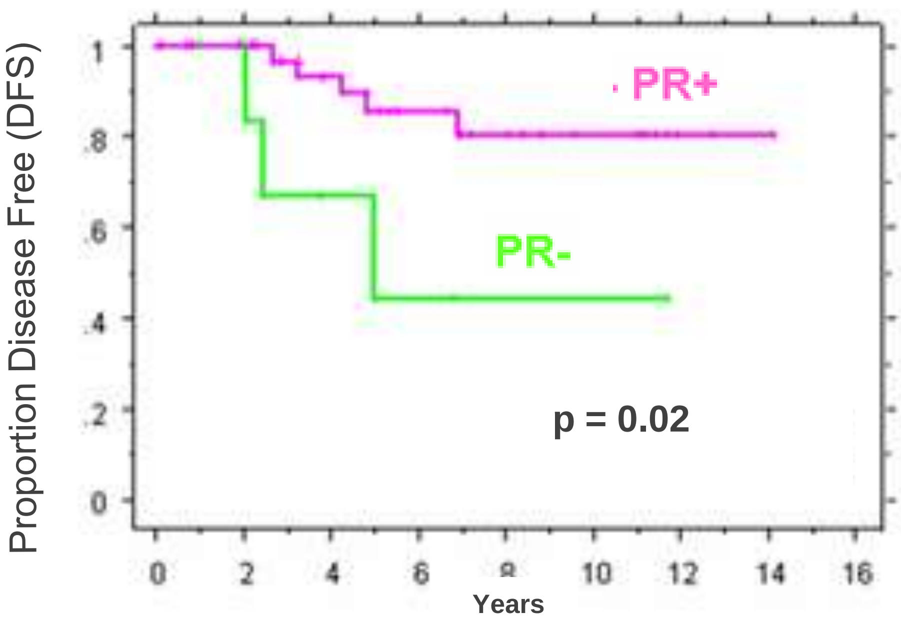
Group 2: younger patients (77%)
80% PR+
56% PR+

Group 3: younger patients (65%)
83% PR+

p<0.05 for age cohort difference between Group 1A/B & 2

Unsupervised ER+ Clusters: *Not as prognostic as PR status*





Adjuvant tamoxifen use (>60%) balanced in all comparison groups

Supervised Analysis: *Differentially expressed genes*

59 unique genes, including ER, are significantly up-regulated in the Older Age cohort (FDR, p<0.05)

Gene Symbol	Gene Name	Average Fold Cha
PIP	prolactin-induced protein	2.897171237
HOXB6	homeo box B6	2.427071015
TMC5	transmembrane channel-like 5	2.348352694
MUC1	mucin 1, transmembrane	2.15628676
ST6GALNAC5	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 5	2.015265567
HOXB2	homeo box B2	1.98017106
AZGP1	alpha-2-glycoprotein 1, zinc	1.842570564
PYGL	phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	1.83660205
KIAA1102	KIAA1102 protein	1.807412067
TNFSF10	tumor necrosis factor (ligand) superfamily, member 10 /	1.784881661
GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	1.747515938
RNASE4	ribonuclease, RNase A family, 4	1.743136055
GLRX	glutaredoxin (thioltransferase)	1.74154014
FLJ20152	hypothetical protein FLJ20152	1.703679702
* ESR1	estrogen receptor 1	1.703505228
ENTPD5	ectonucleoside triphosphate diphosphohydrolase 5	1.698208431
DSPG3	dermatan sulfate proteoglycan 3	1.696934665
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	1.694442334
SH3BGRL	SH3 domain binding glutamic acid-rich protein like	1.691287951
ITPR1	inositol 1,4,5-triphosphate receptor, type 1	1.684080092
SASH1	SAM and SH3 domain containing 1	1.661930964
ANG	angiogenin, ribonuclease, RNase A family, 5	1.65247741
HOXB5	homeo box B5	1.651947336
MANSC1	MANSC domain containing 1	1.640397581
IQGAP2	IQ motif containing GTPase activating protein 2	1.626663156
ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	1.599498019
FAH	fumarylacetoacetate hydrolase (fumarylacetoacetase)	1.588779873
WWOX	WW domain containing oxidoreductase	1.575998923
COBLL1	COBL-like 1	1.573396023
C20orf35	chromosome 20 open reading frame 35	1.570715981
EFNA1	ephrin-A1	1.566894511
CLMN	calmin (calponin-like, transmembrane)	1.544615024
CLEC5A	C-type lectin domain family 5, member A	1.526354974
P8	p8 protein (candidate of metastasis 1)	1.524997885
PDE4A	phosphodiesterase 4A, cAMP-specific	1.522622035
C21orf25	chromosome 21 open reading frame 25	1.518977895
SEP6	septin 6	1.518389257
RHOB	ras homolog gene family, member B	1.517922747
SC5DL	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, fungal)-like	1.511043372
F13A1	coagulation factor XIII, A1 polypeptide	1.494782742
TAPBPL	TAP binding protein-like	1.493899325
PPFIBP2	PTPRF interacting protein, binding protein 2 (liprin beta 2)	1.481709389
CCDC28A	coiled-coil domain containing 28A	1.479526238
CPM	carboxypeptidase M	1.456362458
CALM3	calmodulin 3 (phosphorylase kinase, delta)	1.45286054
SLC25A12	solute carrier family 25 (mitochondrial carrier, Aralar), member 12	1.45071349
CHRD	chordin	1.435989772
MARCH8	membrane-associated ring finger (C3HC4) 8	1.434202973
HOXB7	homeo box B7	1.427637221
FLJ20298	FLJ20298 protein	1.415049658
PEX3	peroxisomal biogenesis factor 3	1.407988318
SLC12A8	solute carrier family 12 (potassium/chloride transporters), member 8	1.396215292
SLC7A8	solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	1.388462864
DBI	diazepam binding inhibitor	1.383572929
PREPL	prolyl endopeptidase-like	1.375064955
PIGT	phosphatidylinositol glycan, class T	1.36827487
LOC57146	promethin	1.366922322
RANBP2	RAN binding protein 2	1.357316172
TGOLN2	trans-golgi network protein 2	1.318716433

26 unique genes are significantly up-regulated in the Younger Age cohort

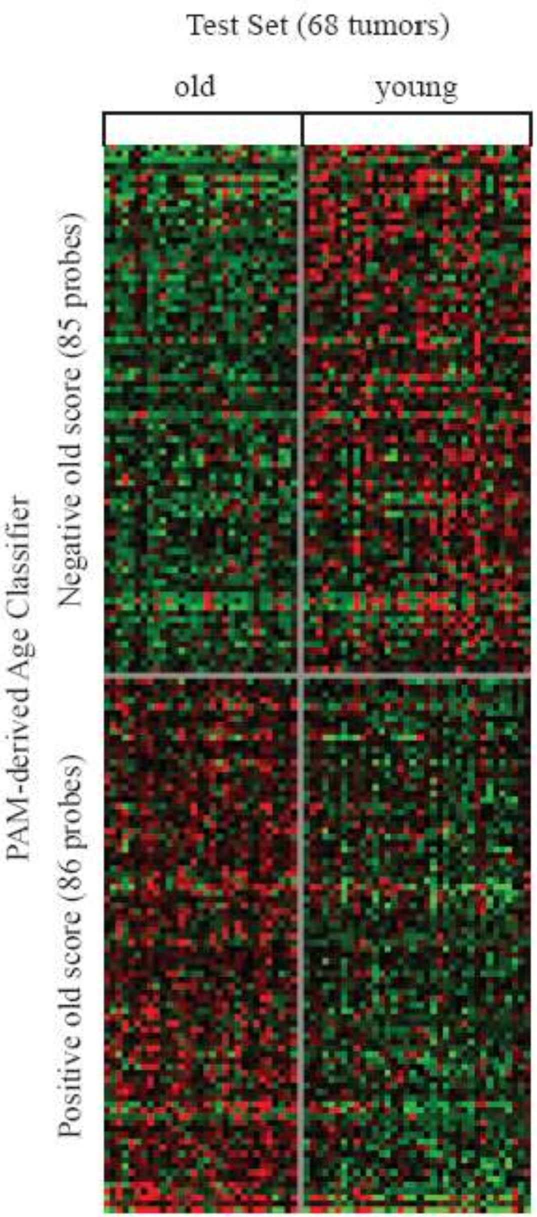
Gene Symbol	Gene Name	Average Fold Change
* AREG	amphiregulin (schwannoma-derived growth factor)	3.050486212
ATP6V1B1	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B, isoform 1	1.786519486
C20orf59	chromosome 20 open reading frame 59	1.55845922
CDC14A	CDC14 cell division cycle 14 homolog A (S. cerevisiae)	1.403284819
CSE1L	CSE1 chromosome segregation 1-like (yeast)	1.289236819
DIO2	deiodinase, iodothyronine, type II	1.766684077
DLG7	discs, large homolog 7 (Drosophila)	1.573429521
ELL3	elongation factor RNA polymerase II-like 3	1.422051733
ELOVL2	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2	1.525456138
ETV1	ets variant gene 1	1.382182688
FGFR1	fibroblast growth factor receptor 1	1.63878109
GREB1	GREB1 protein	2.254459871
HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	1.959432805
KIF2C	kinesin family member 2C	1.520640169
LAMA3	laminin, alpha 3	1.799657592
PRSS1	protease, serine, 1 (trypsin 1)	1.752316415
PRSS2	protease, serine, 2 (trypsin 2)	2.632877101
RRM2	ribonucleotide reductase M2 polypeptide	1.663875164
S100A2	S100 calcium binding protein A2	1.749919232
SLC27A6	solute carrier family 27 (fatty acid transporter), member 6	1.401931319
SPANXA1	sperm protein associated with the nucleus, X-linked, family member A1	1.894803442
SPANXC	SPANX family, member C	1.588554967
STK6	serine/threonine kinase 6	1.547568059
TP73L	tumor protein p73-like	1.562168298
UST	uronyl-2-sulfotransferase	1.448189019
WNT4	wingless-type MMTV integration site family, member 4	1.56355306

Note: Highlighted in red are genes with implied or established roles in cancer

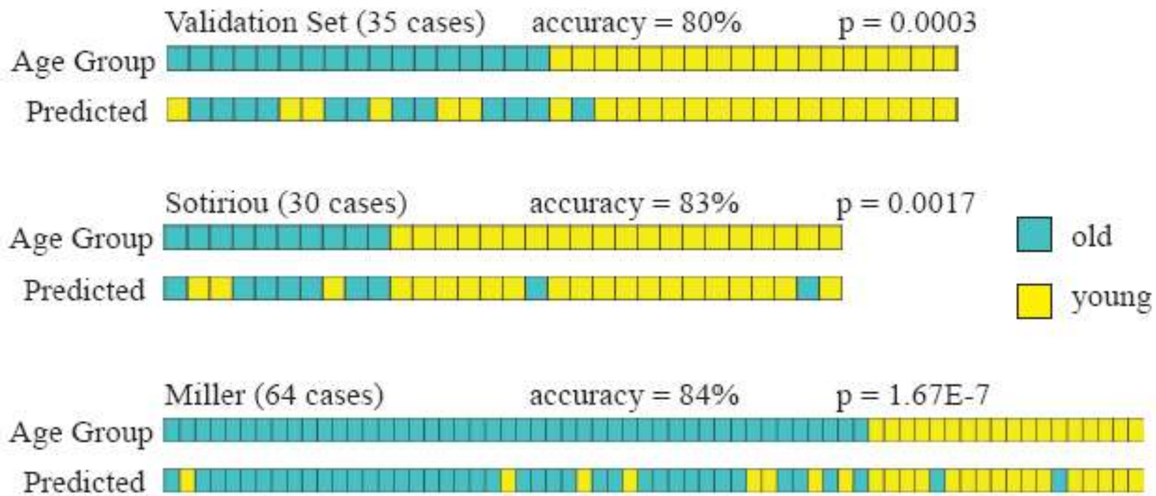
(Yau and Benz, BCR, 2007)

Predictive Analysis: *Is there an ER+ age signature?*

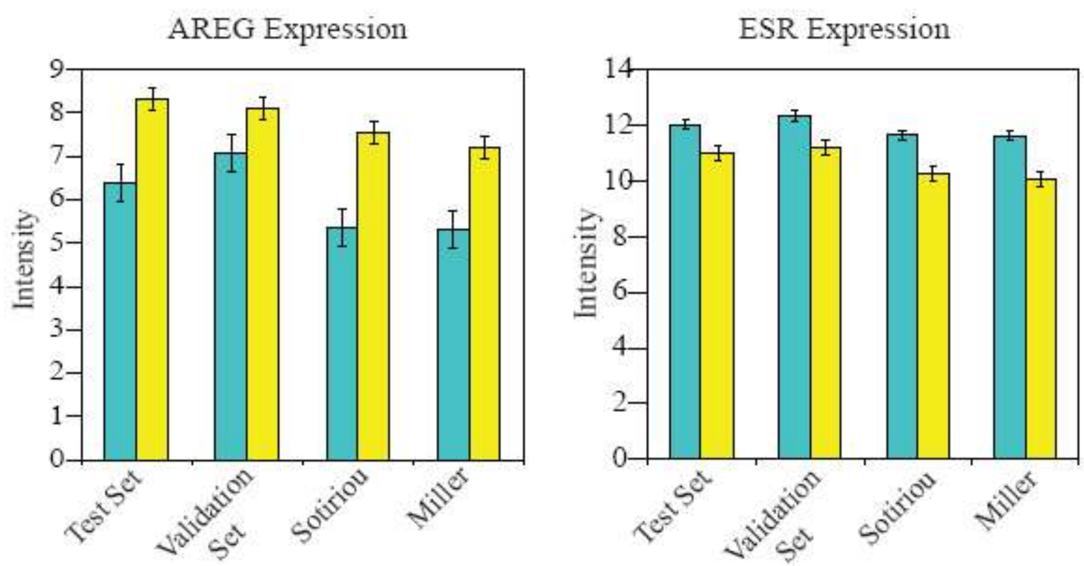
A. ER+ test set:



B. ER+ validation sets:



C. Two genes correlating (-,+) most strongly with age:



(Yau and Benz, BCR, 2007)

Age & ER+ Gene Expression Profiles

Unsupervised Analysis

- ER+ breast cancers are heterogeneous (4 subtypes)
- PR status not reflected in ER+ transcriptional subtypes
- Subset of early onset cases have worse prognosis (RFS).

Supervised and Predictive Analyses

- Early onset ER+ breast cancer associated with:

  - reduced expression of ER and some tumor suppressors (ARHGDIB, SASHI), development regulators (HOXB6/B7), & apoptosis inducer (TNFSF10)

  - increased expression of growth factor (AREG) & receptor (FGFR1), ER-inducible growth regulator (GREB1), mitotic factors (CDC14A, STK6), & serine proteases (PRSS1/2)

- Early onset ER+ cases enriched in poor prognostic signatures:

  - proliferation

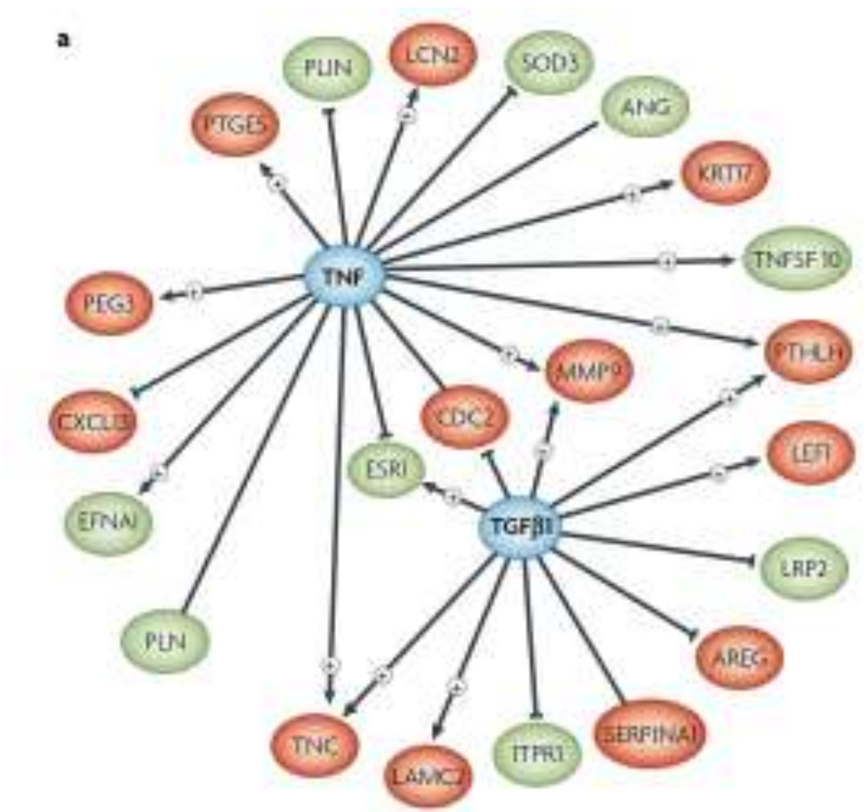
  - oxidative stress

Oxidative Stress & Early Onset ER+ Breast Cancer

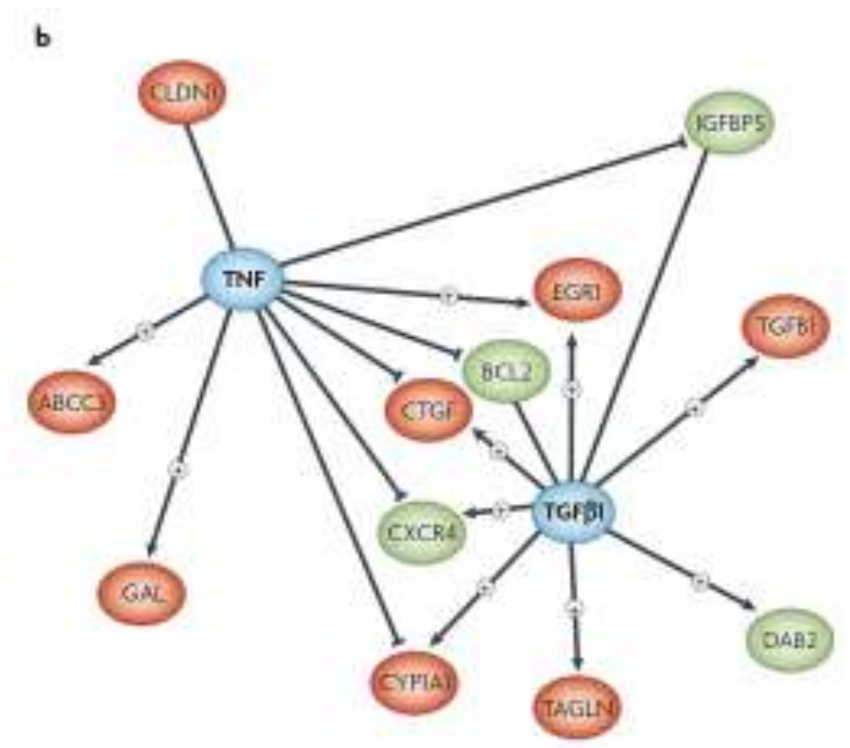
- Oxidative stress signature (Ox-E/ER) linked to poor-outcome ER+ breast cancers (Yau et al., BCR 2008)
- Early onset ER+ breast cancers enriched with both proliferation and Ox-E/ER gene signatures
- Gene pathways shared by early onset and Ox-E/ER enriched tumors share upstream TNF & TGFβ nodes
- At least 75% of signature genes regulated by TNF & TGFβ contain NFκB and/or AP-1 promoter elements

Pathway Comparisons Between ER+ Age Signature and Ox-E/ER Signature

From ER+ age signature (Yau et al., BCR 9:R59, 2007):

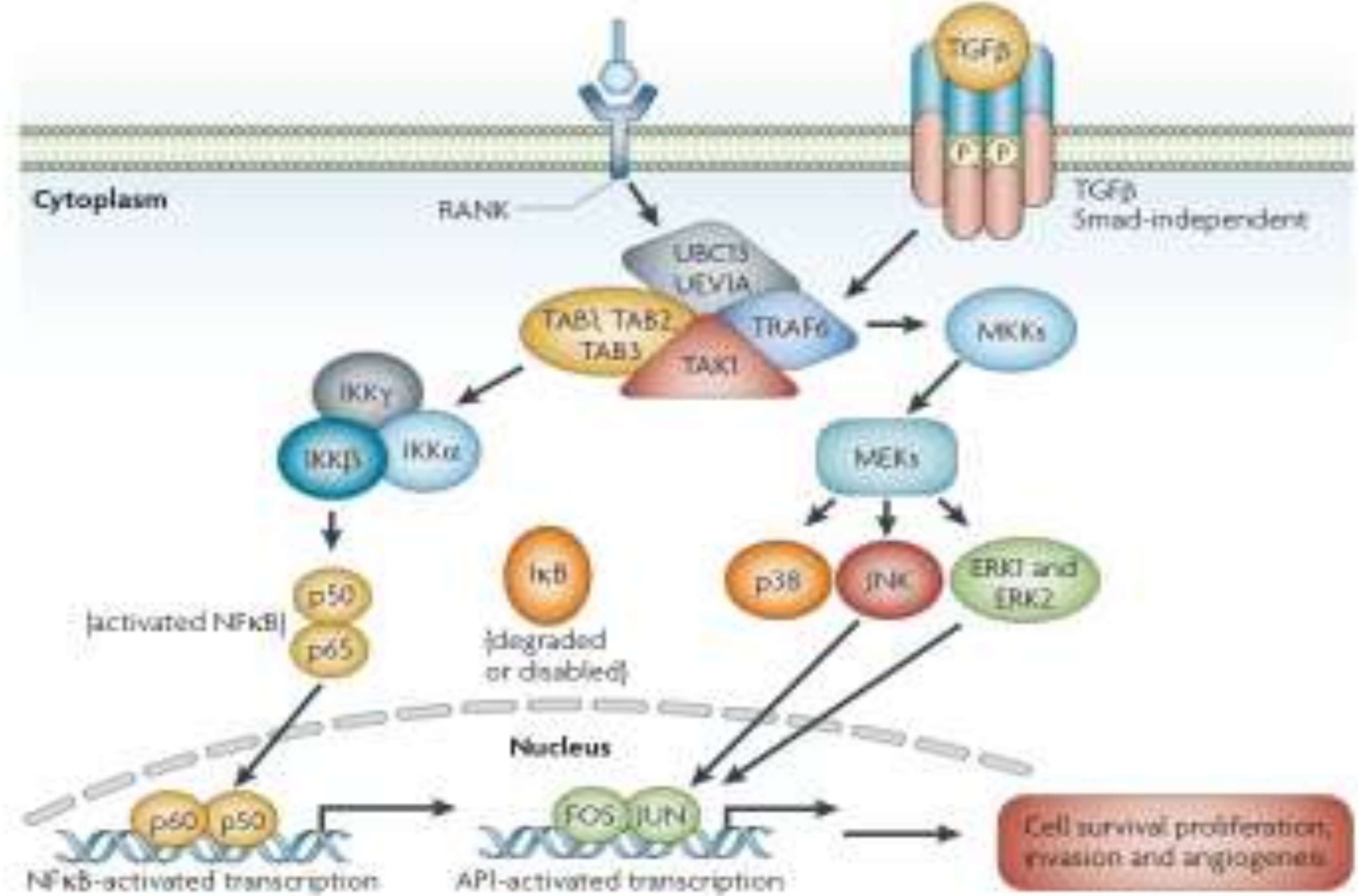


From Ox-E/ER signature (Yau & Benz, BCR 10:R61, 2008):



Signaling Pathways Shared by Oxidatively Stressed and Early Onset ER+ Breast Cancers

Opportunites for Therapeutic Intervention?



Benz & Yau, Nature Rev. Cancer , 2009

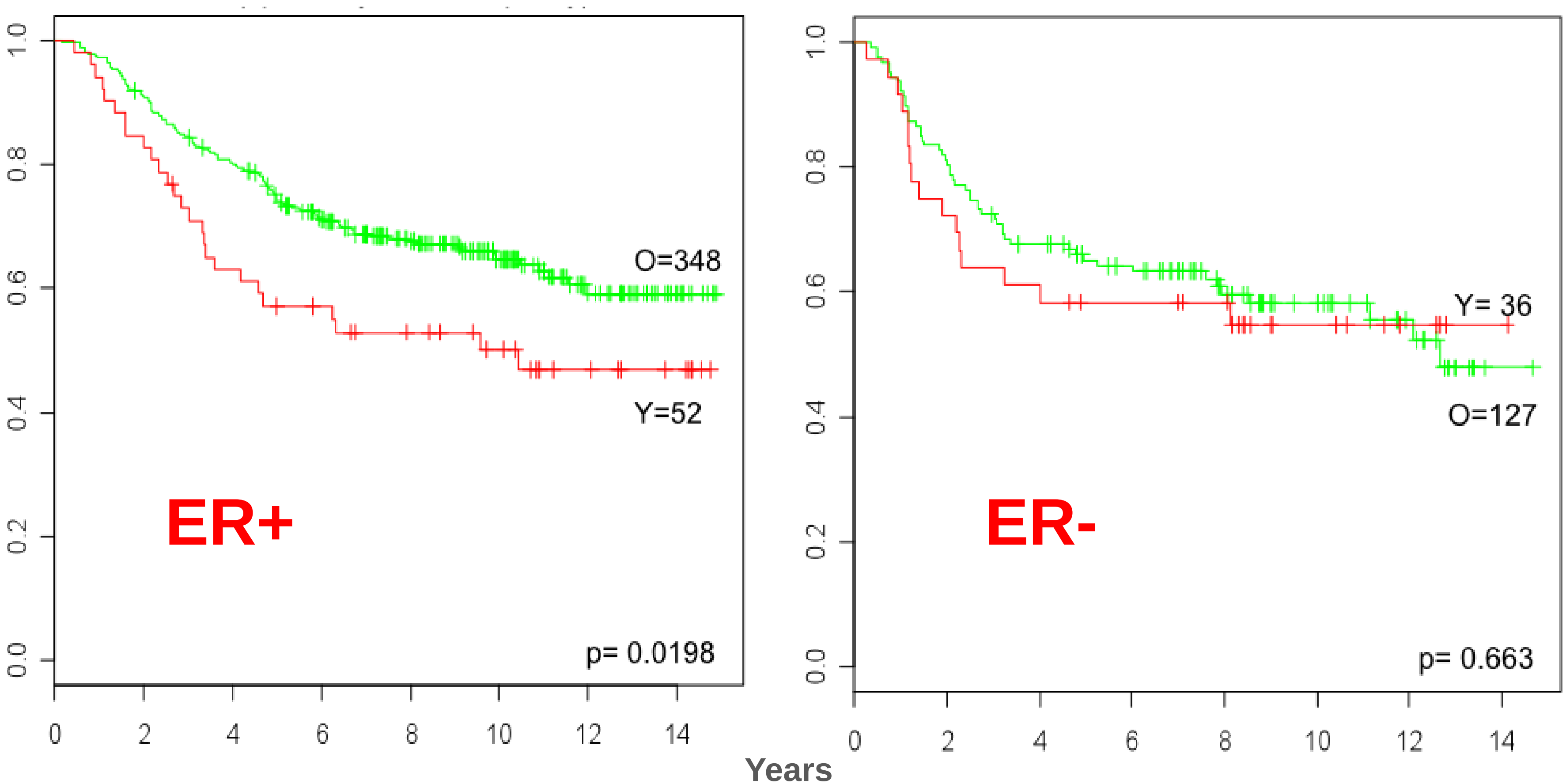
Breast Cancer & Aging: Questions

- Do ER- breast cancers show age-associated outcome and biology differences?

Maybe not…

Metastasis-free Survival

Pooled outcome analyses comparing ER+ vs. ER- untreated N_0 cases from age-annotated data sets (Y ≤ 39 years; O ≥ 40 years)



(GSE2034: Wang et al., Lancet 2005; GSE7390: Desmedt et al., Clin Cancer Res. 2007; NKI: Van de Vijver et al. NEJM 2002)

Conclusions

- Breast cancer is a heterogeneous disease with early and late onset forms, even within known clinical subtypes (e.g. ER+ vs. ER-).
- Inverse age relationship between ER and biomarkers of breast cancer growth (e.g. Ki-67, ERBB2/HER2) and genomic stability (nuclear grade, p53).
- Among sporadic ER+ breast cancers, age has little effect on cancer genome but predictably alters breast cancer gene expression (epigenome).
- Sporadic, early onset ER+ breast cancer is clinically and biologically more aggressive, with features indicating enhanced NFκB and AP-1 activated gene programs that correlate with endocrine resistance.

& Collaborators

- UCSF Breast SPORE
- NCI-Bari, Italy
- STB, Basel, Switz.

BUCK INSTITUTE

- Gary Scott, PhD
- David Britton PhD
- Christina Yau, BS
- Corina Marx, PhD
- Christian Atsriku, PhD
- Yamei Zhou, PhD
- Crystal Berger, BS
- Chris Benz, MD