CYP2D6 Genotype and the Use of Tamoxifen in Breast Cancer

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Outline

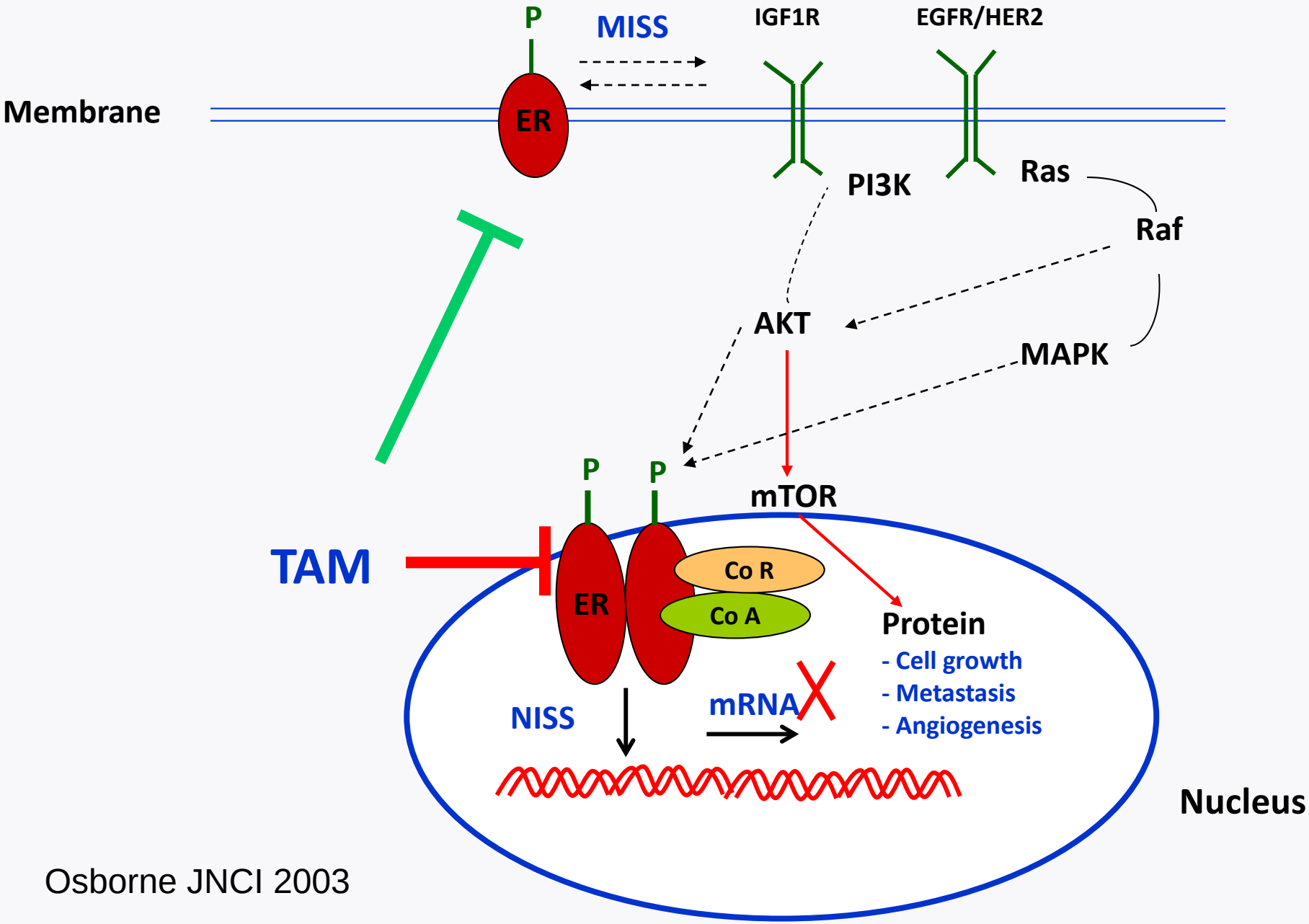
- ER+ breast cancer: brief review of the benefit of endocrine therapy
- Tamoxifen metabolism: Does variation in the hydroxylated metabolites matter?
  - Tamoxifen pk/pg
  - The effects of tamoxifen and metabolites on estrogen-induced proliferation and ER transcription
  - Clinical: CYP2D6 metabolism and Ki-67
  - Clinical: association between CYP2D6 genotype and recurrence: Reasons for discrepancy
- Steps forward for standardization

Breast Cancer
Endocrine Therapy

Therapeutic Strategies

- **Block ER – Tamoxifen**
- **Block estrogen synthesis – aromatase inhibitors**
- **Target the ER for degradation (Fulvestrant)**

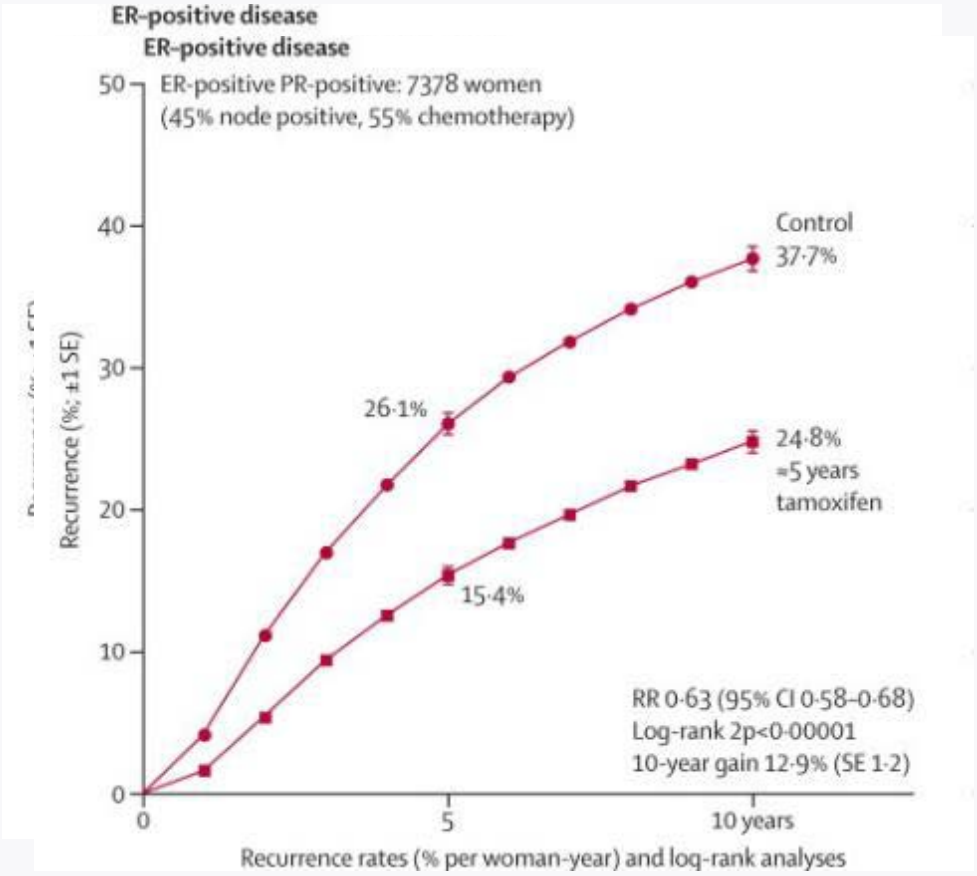
Tamoxifen Mechanism of Action



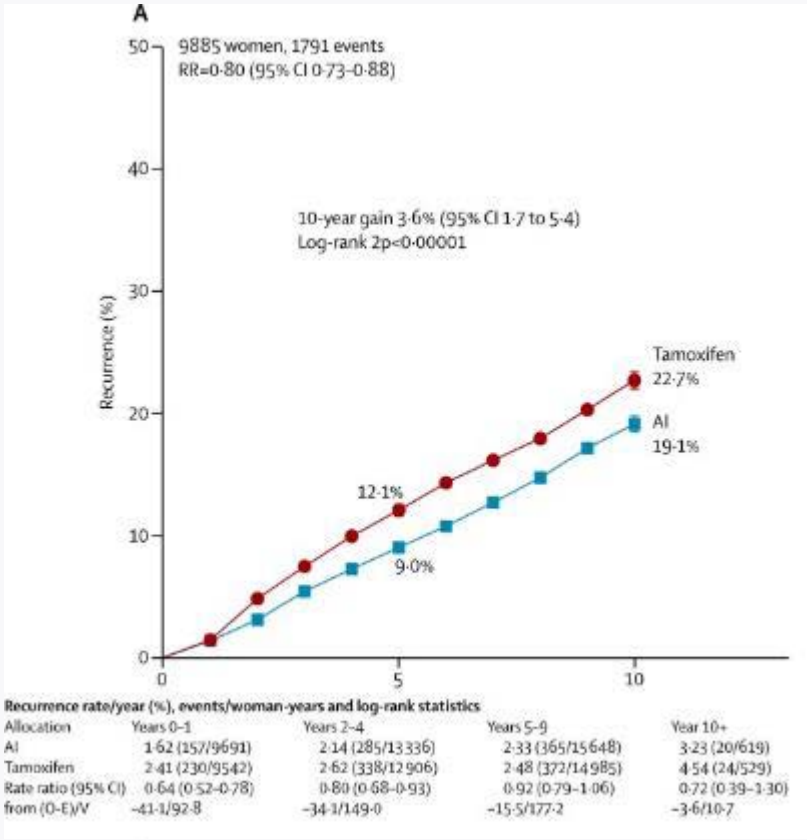
Osborne JNCI 2003

Tamoxifen and Aromatase Inhibitors: Adjuvant Treatment of Postmenopausal ER+ Breast Cancer

Tamoxifen vs Control



AI's vs Tamoxifen



Recurrence rate/year (%), events/woman-years and log-rank statistics

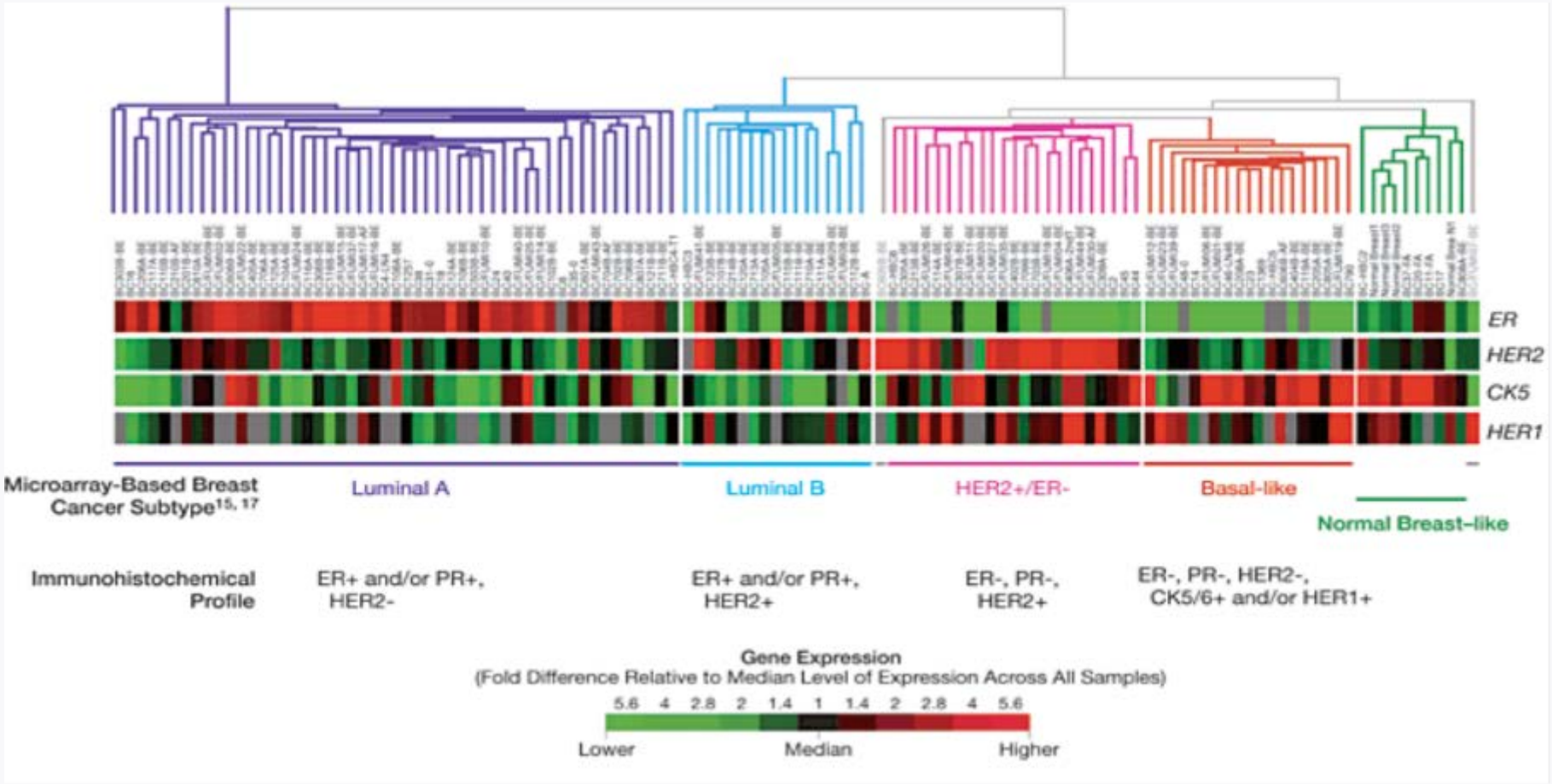
Allocation	Years 0–1	Years 2–4	Years 5–9	Year 10+
AI	1·62 (157/9691)	2·14 (285/13 336)	2·33 (365/15 648)	3·23 (20/619)
Tamoxifen	2·41 (230/9542)	2·62 (338/12 906)	2·48 (372/14 985)	4·54 (24/529)
Rate ratio (95% CI)	0·64 (0·52–0·78)	0·80 (0·68–0·93)	0·92 (0·79–1·06)	0·72 (0·39–1·30)
from (O–E)/V	−41·1/92·8	−34·1/149·0	−15·5/177·2	−3·6/10·7

Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Lancet 2011 and 2015

Summary

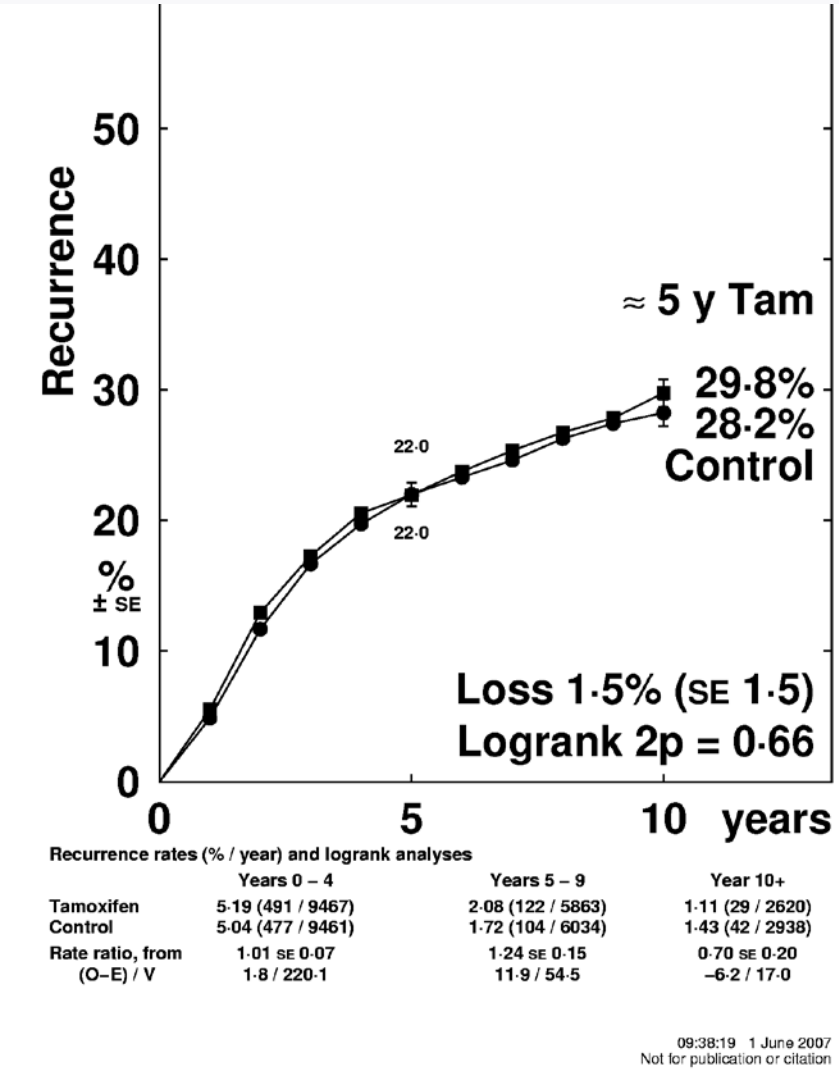
- Adjuvant hormonal therapy results in substantial improvements in recurrence and survival in women with ER+ breast cancer
- What are the accepted molecular markers which drive endocrine response?
- Does genetic variation in drug metabolism contribute to endocrine resistance?

Breast Cancer Molecular Subtypes



TCGA. Nature. 2012.

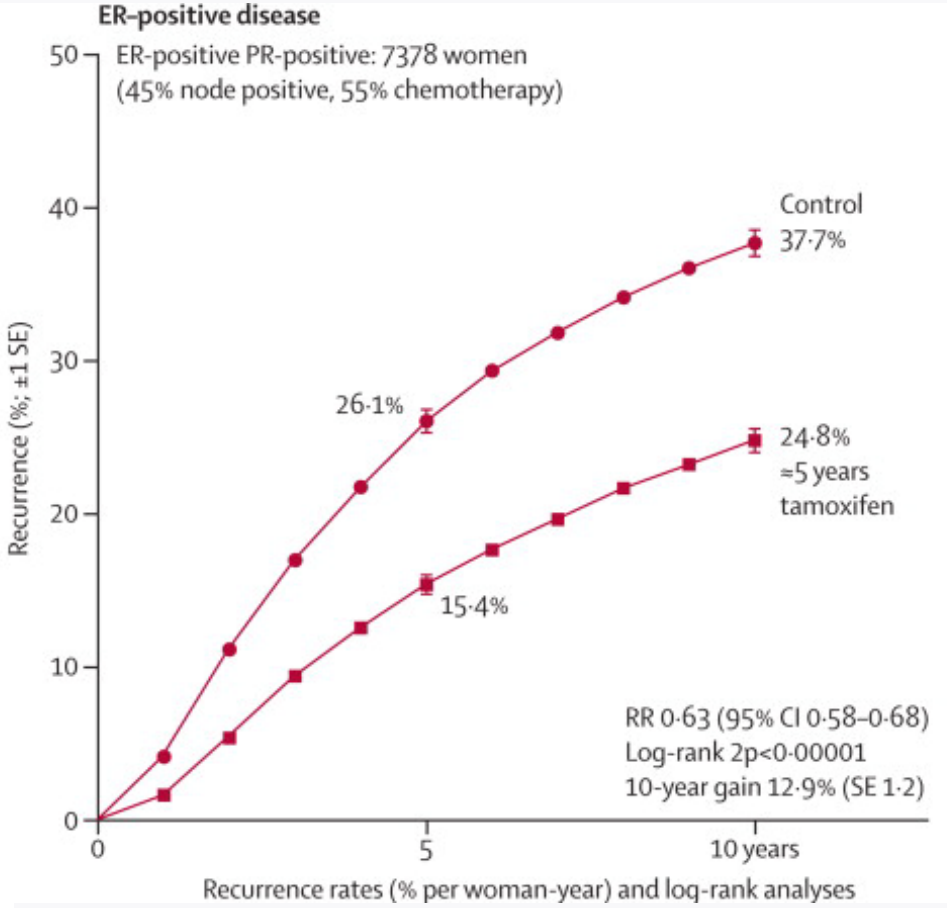
Importance of ER in Tamoxifen-treated Breast Cancer



Recurrence rates (% / year) and logrank analyses

	Years 0 – 4	Years 5 – 9	Year 10+
Tamoxifen	5·19 (491 / 9467)	2·08 (122 / 5863)	1·11 (29 / 2620)
Control	5·04 (477 / 9461)	1·72 (104 / 6034)	1·43 (42 / 2938)
Rate ratio, from (O–E) / V	1·01 SE 0·07 1·8 / 220·1	1·24 SE 0·15 11·9 / 54·5	0·70 SE 0·20 –6·2 / 17·0

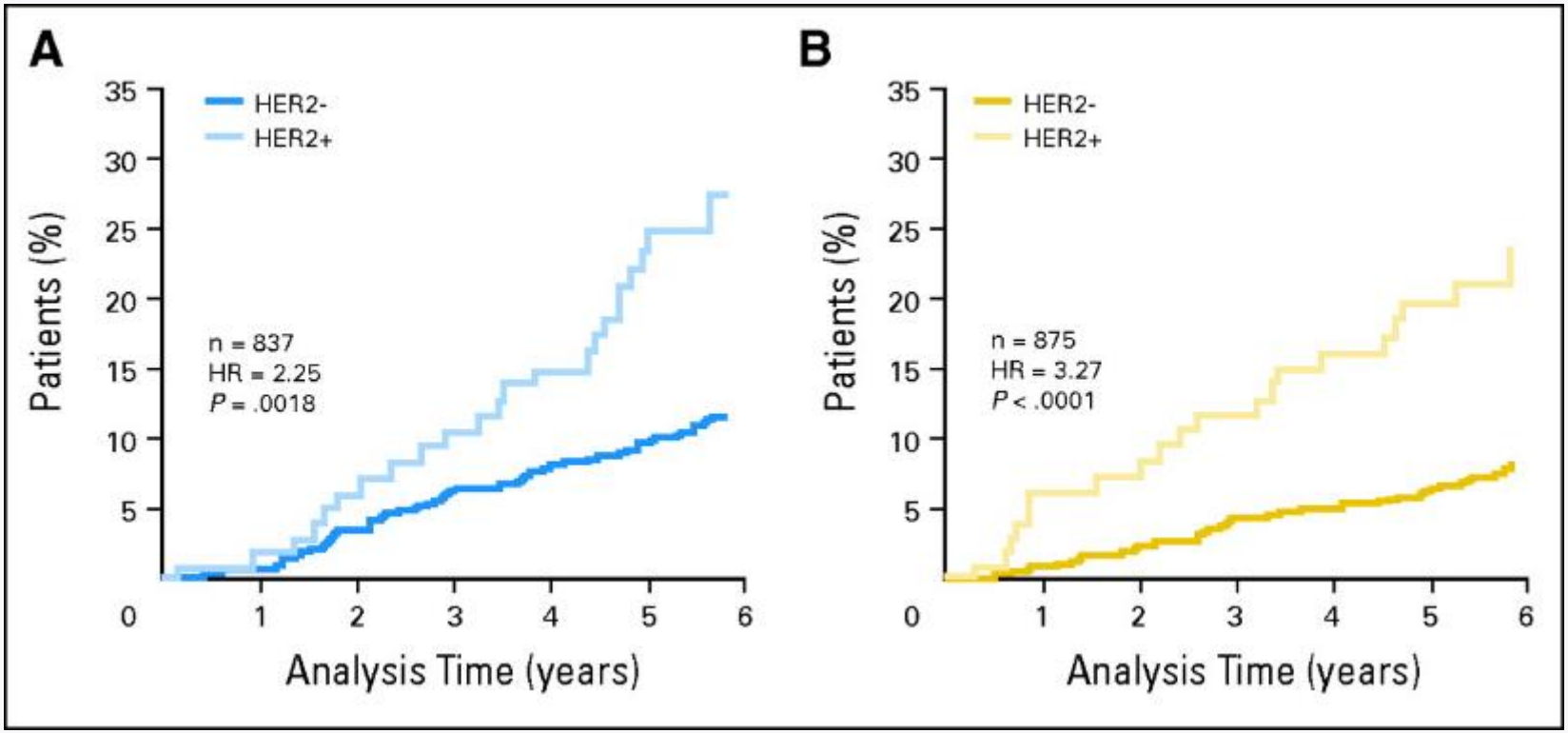
09:38:19 1 June 2007
Not for publication or citation



Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Lancet 2011 and 2015

CP1280197-8

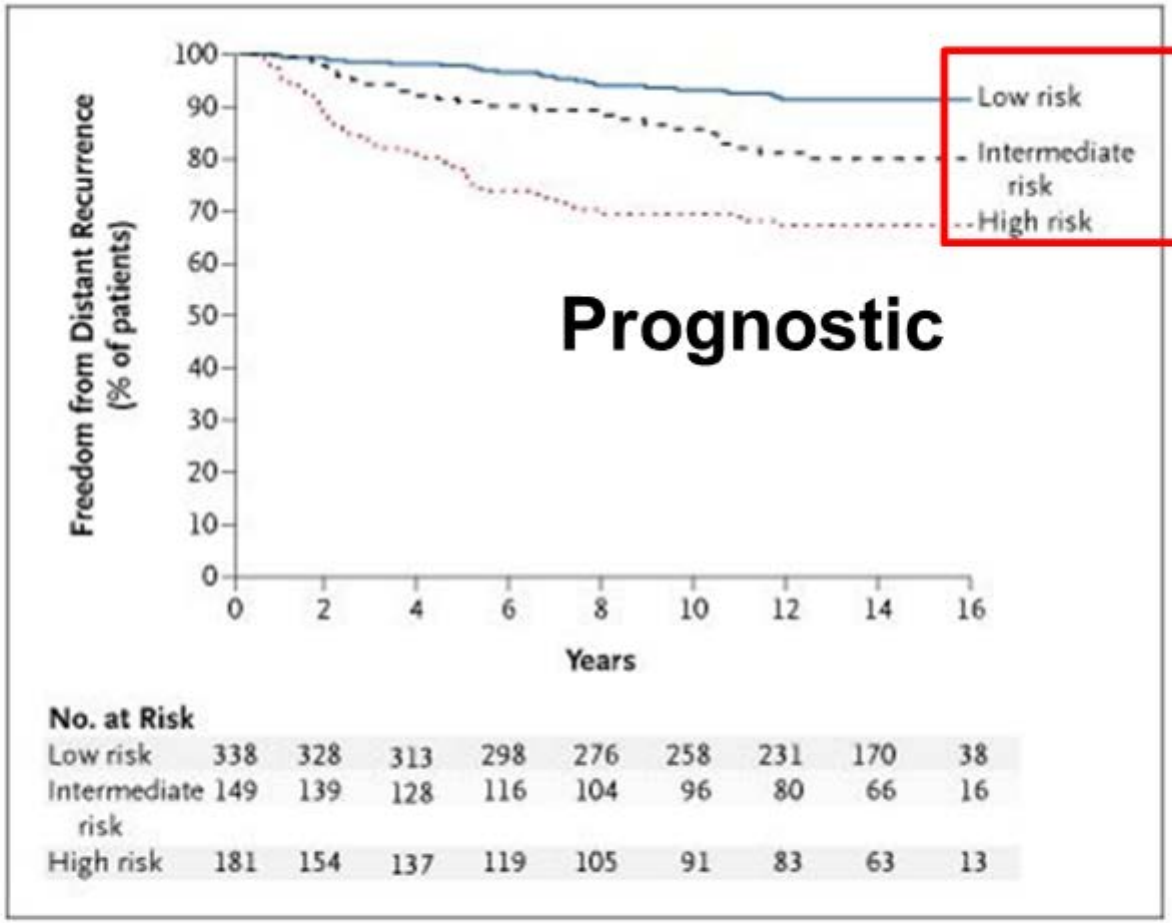
Time to Recurrence in ATAC according to HER2 status



Dowsett M et al. JCO 2008;26:1059-1065

JOURNAL OF CLINICAL ONCOLOGY

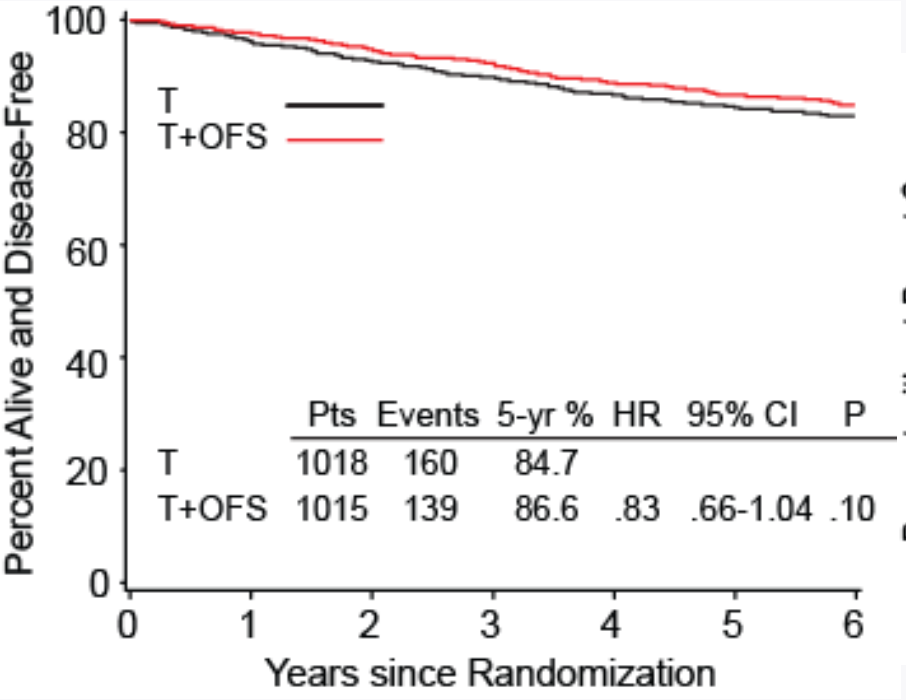
21 Gene Recurrence Score: Distant Recurrence in NSABP B14

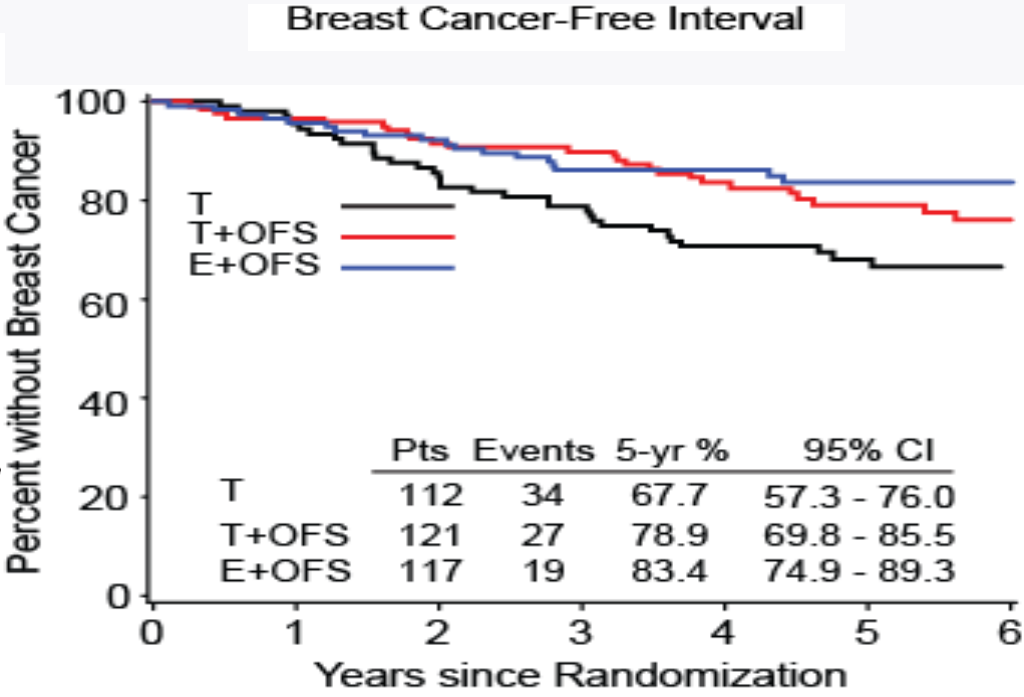


The difference among the three recurrence score (RS) groups is significant (P<0.001)

Paik et al. N Engl J Med 2004.

SOFT: Estrogen Suppression in Addition to Tamoxifen: Overall and Age < 35



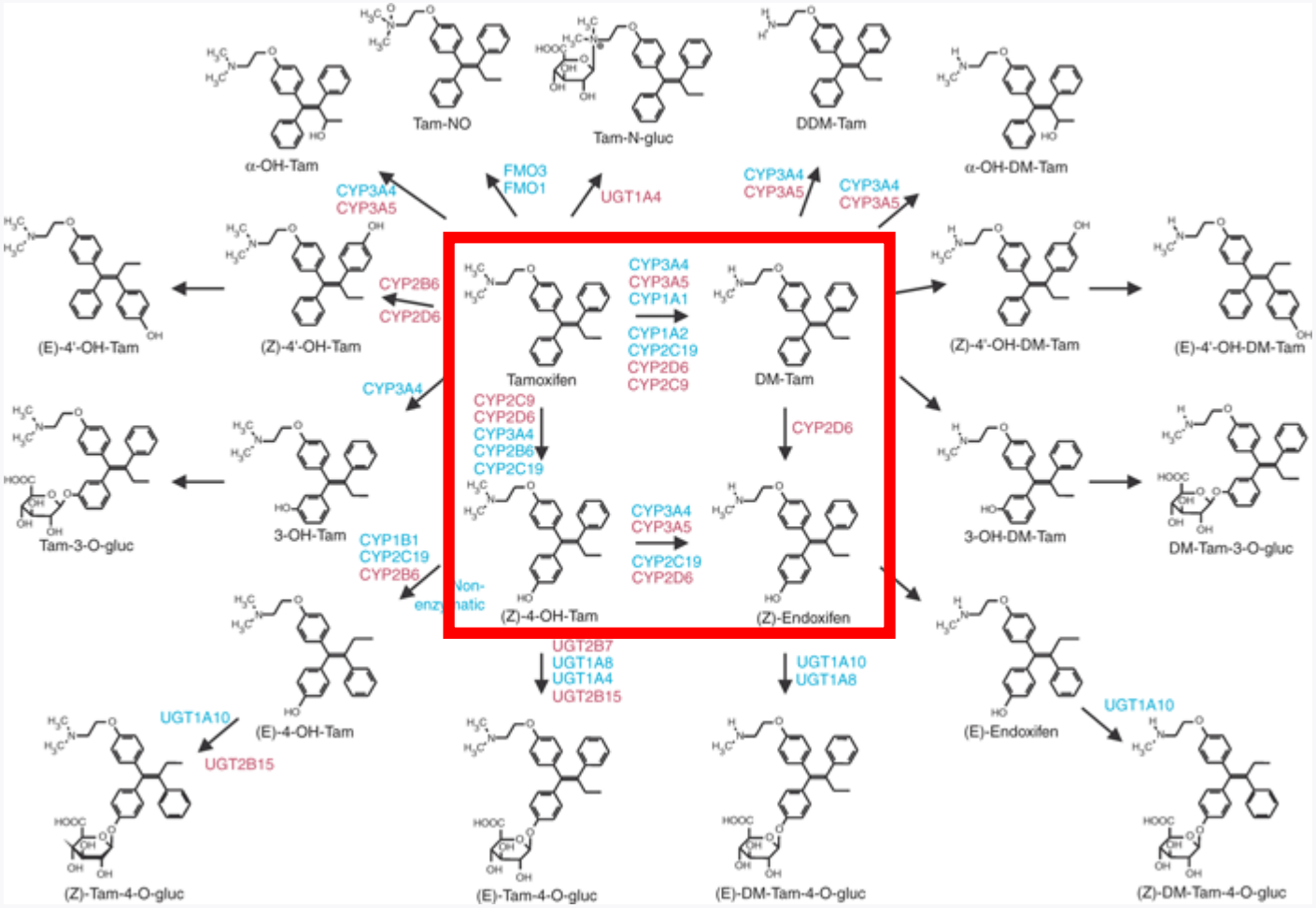


Francis et al. NEJM 2014

Endocrine Resistance

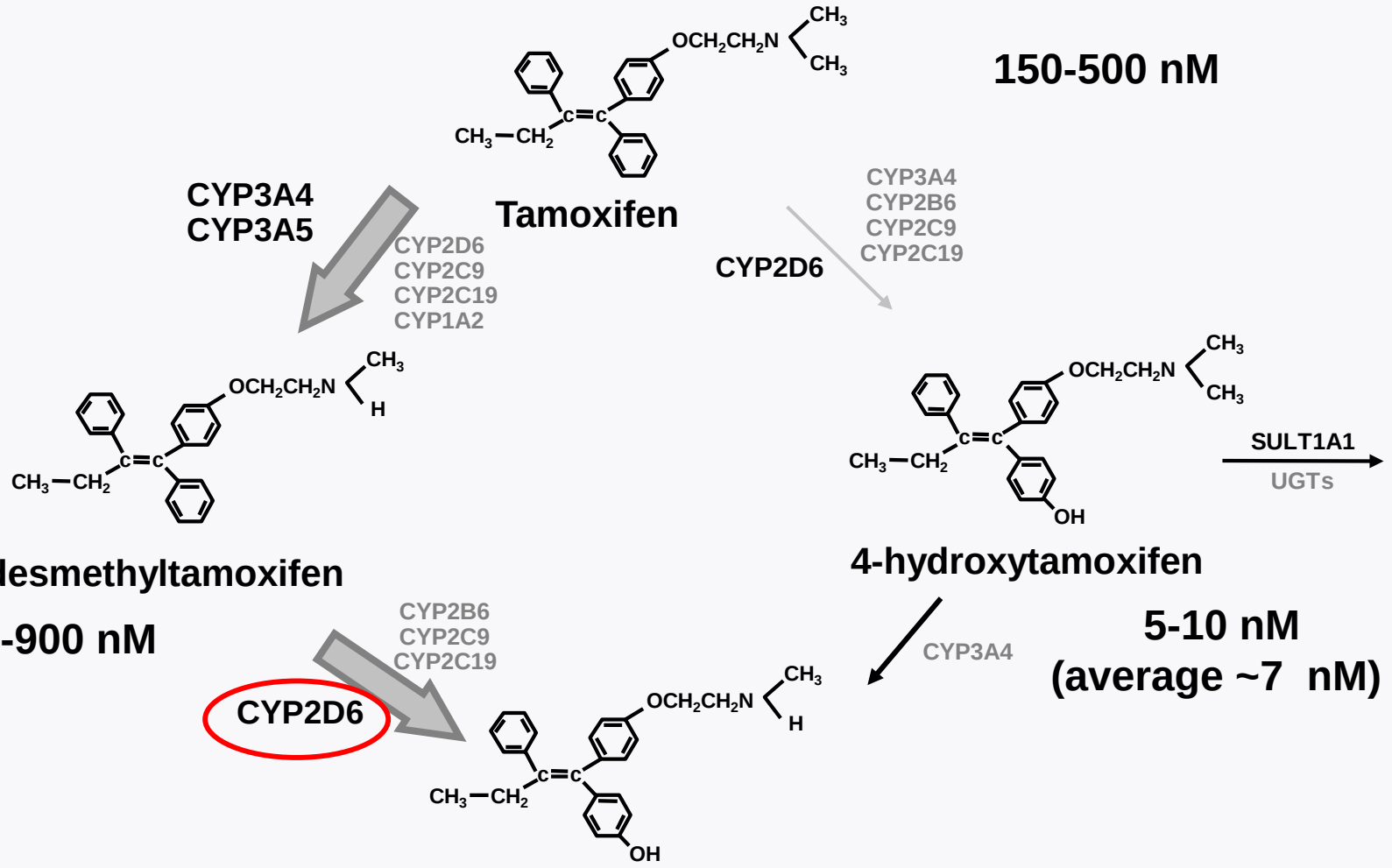
- **Partial List of genes/pathways associated with primary endocrine resistance**
  - Low or absent ER
  - ER+/HER2+ (luminal HER2) (effects abrogated in the setting of trastuzumab)
  - Luminal B (heterogeneous)
    - Activation of Growth Factor Pathways (e.g. EGFR1)
    - Activation of proliferation genes
  - Gene expression assays encompass many of these genes/pathways (Oncotype Dx)
  - Host “estrogen” levels
  - ? Does tamoxifen metabolism provide independent “prediction” of treatment benefit?

Tamoxifen Biotransformation



Murdter et al. Clin Phar Ther 2011

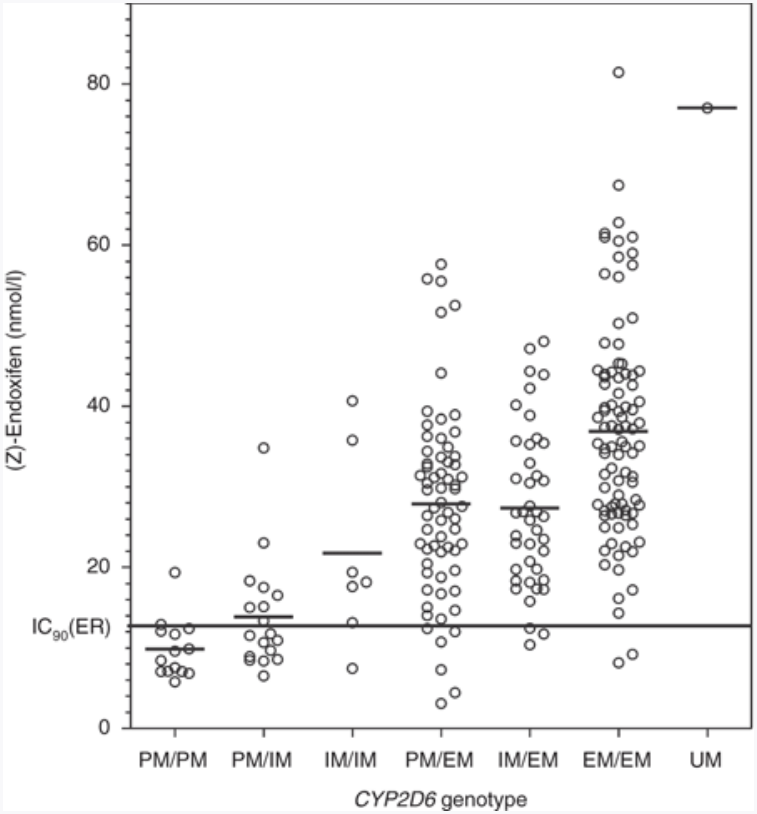
Tamoxifen Biotransformation



Drug and metabolite	EMs	IMs	PMs
Tamoxifen	383 nM	413 nM	459 nM
NDMTAM	558 nM	776 nM	952 nM
4OHT	6.3 nM	5.3 nM	3.1 nM
Endoxifen	35.6 nM	24.7 nM	9.0 nM

Endoxifen Concentrations and *CYP2D6* Genotype

*Genotype	SNPs detected by xTAG CYP2D6 v3 Kit	Frequency in the U.S. Caucasian population[2]	Frequency in the African American population[2]	Predicted Enzyme Activity
*1	None	37 to 40%	29 to 35%	Normal
*2	-1584C>G, 1661G>C, 2850C>T, 4180G>C	26 to 33%	18 to 27%	Normal
*3	2549A>del	1%	0.2 to 0.6%	None
*4	100C>T, 1661G>C, 1846G>A, 4180G>C,2850C>T	18 to 20%	6 to 9%	None
*5	deletion	2 to 4%	6 to 7%	None
*6	1707T>del, 4180G>C	1%	0.50%	None
*7	2935A>C	Not known	Not known	None
*8	1661G>C, 1758G>T, 2850C>T, 4180G>C	Not known	Not known	None
*9	2613delAGA	2 to 3%	0.30%	Reduced
*10	100C>T, 1661G>C, 4180G>C	2 to 8%	0.3% to 0.40%	Reduced
*11	883G>C, 1661G>C, 2850C>T, 4180G>C	Not known	Not known	None
*15	138insT	Not known	Not known	None
*17	1023C>T, 1661G>C, 2850C>T, 4180G>C	0.2 to 0.3%	15 to 26%	Reduced
*29	1659G>A, 1661G>C, 2850C>T, 3183G>A, 4180G>C	Not known[3]	Not known[3]	Reduced
*35	-1584C>G, 31G>A, 1661G>C, 2850C>T, 4180G>C	7.4%[4]	1%[4]	Normal
*41	1661G>C, 2850C>T, 2988G>A, 4180G>C	9%[4]	11%[4]	Reduced
DUP	duplication			

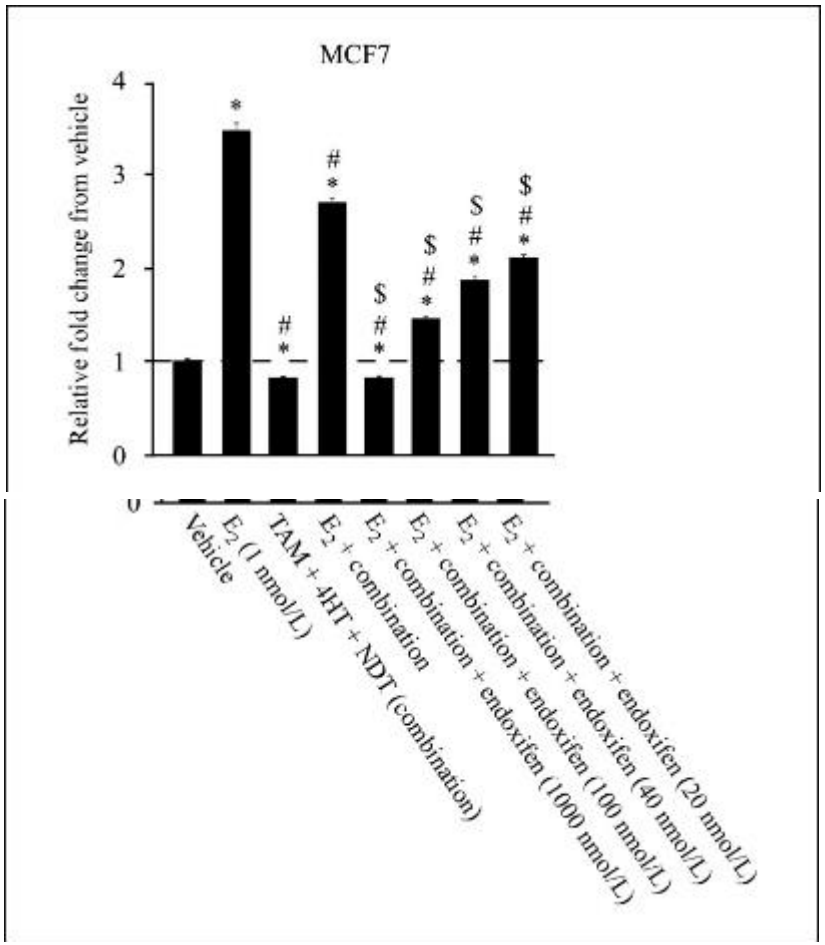


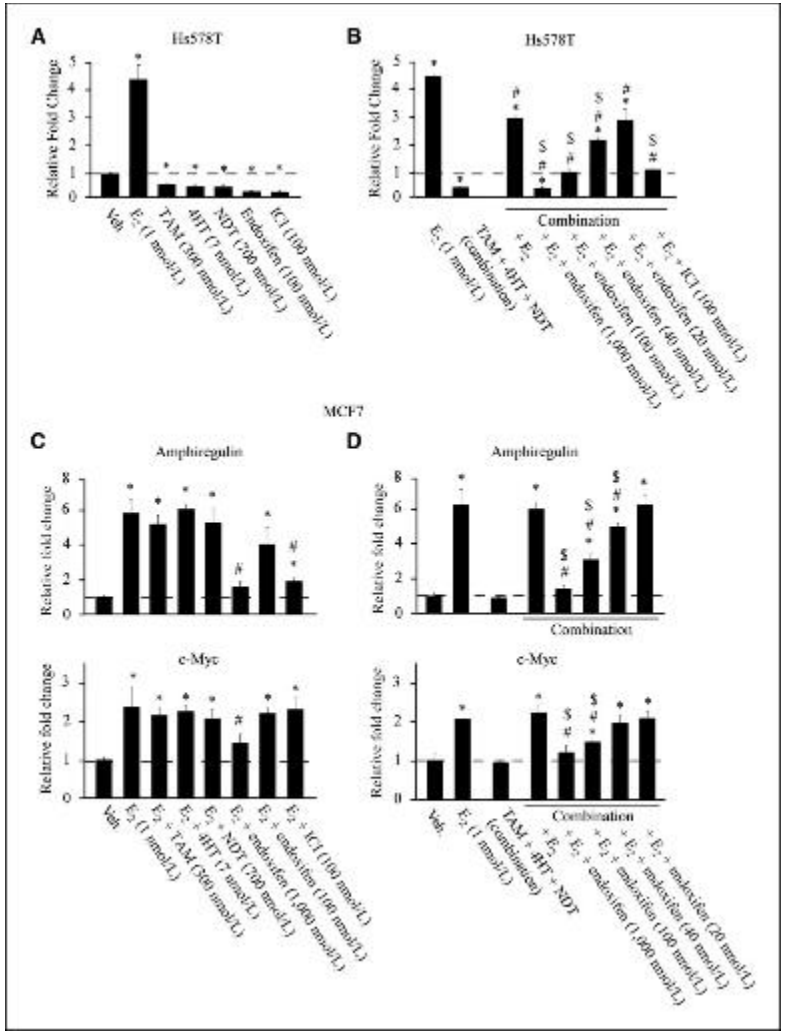
Murdter et al. Clin Phar Ther 2011

Summary

- Extensive variability in the concentration of tamoxifen and its metabolites
- CYP2D6 is responsible for the hydroxylation of N-desmethyl tamoxifen
- *CYP2D6* genetic variation accounts for approximately 30-50% of the variation in endoxifen concentrations in tamoxifen treated patients
- Does variability in the concentrations of tamoxifen and its metabolites affect estrogen induced stimulation/transcription?

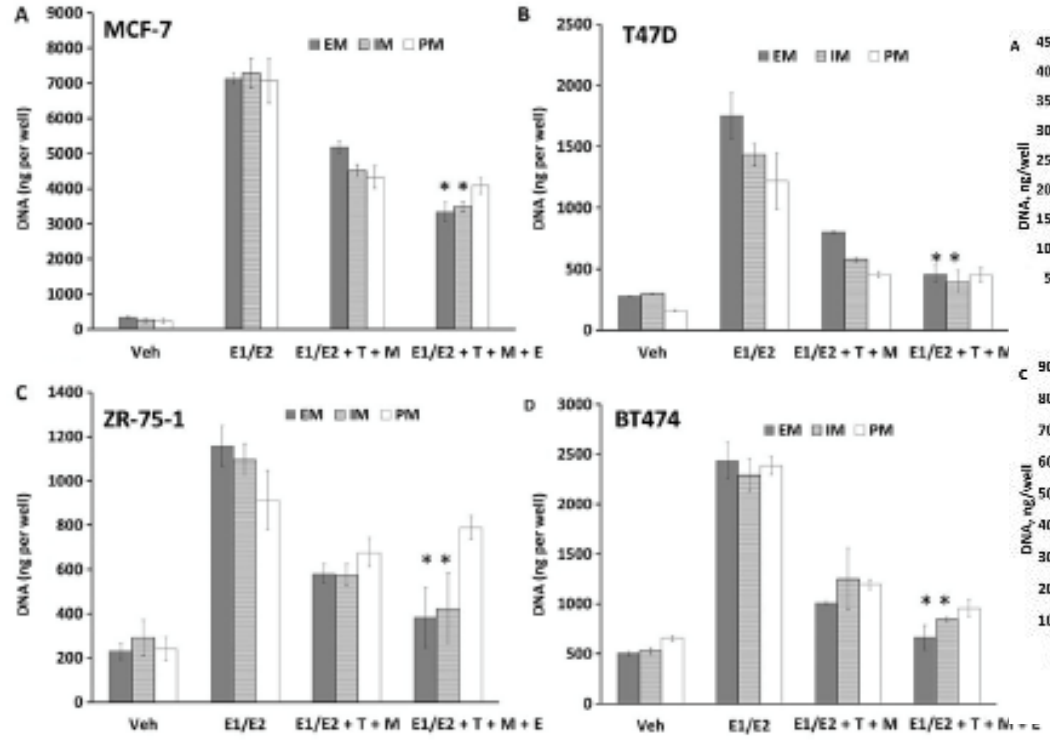
Effects of tamoxifen and metabolites on ER stimulated proliferation and transcription

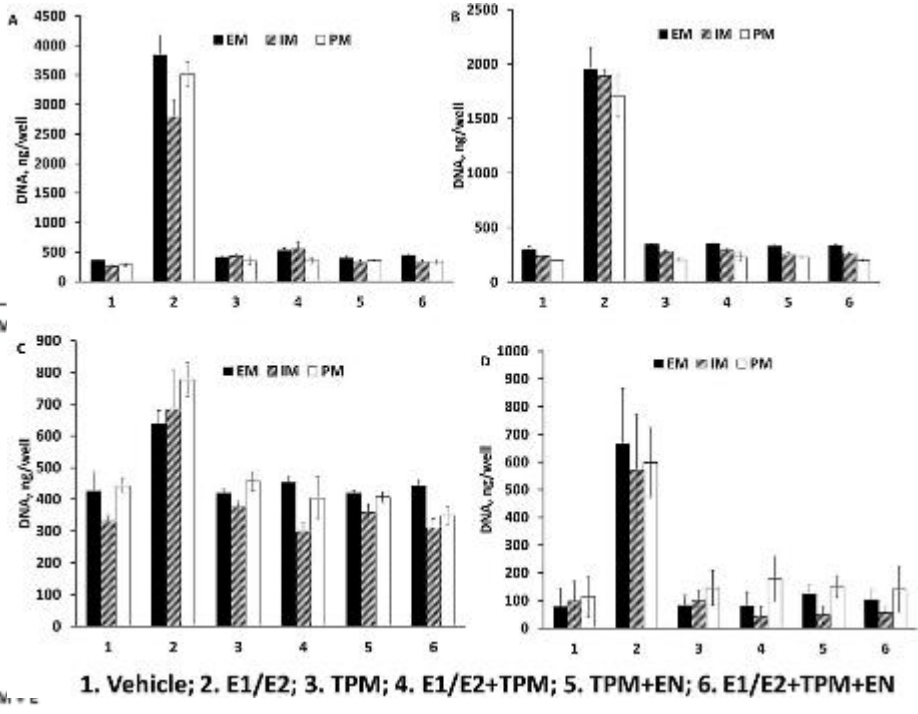




Xianglin Wu et al. Cancer Res 2009;69:1722-1727

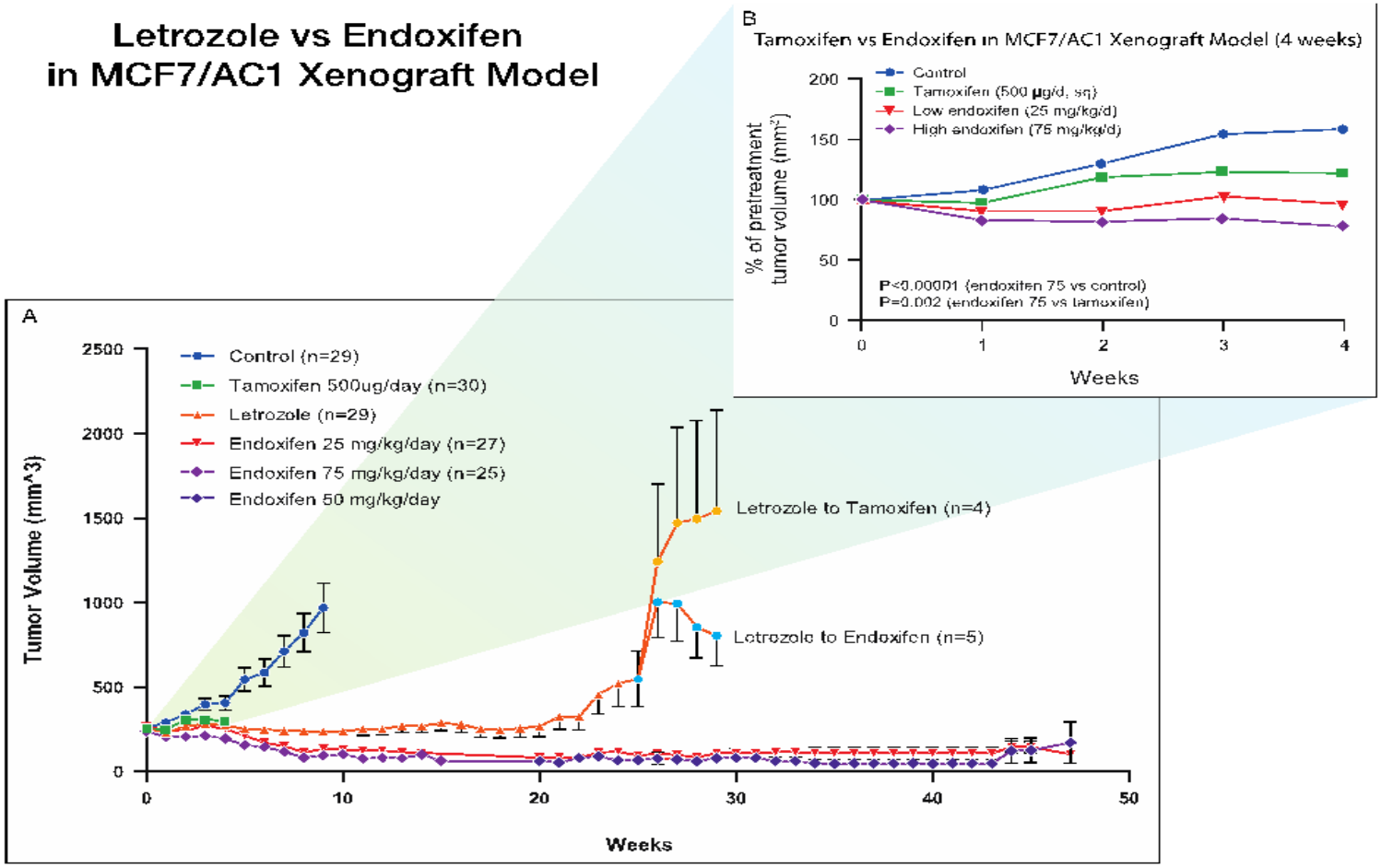
Effects of “pre-menopausal” estrogen levels on ER-stimulated proliferation in the presence of tamoxifen and metabolites





Maximov et al. British Journal of Pharmacology 2014
Maximov et al. JNCI 2014

Endoxifen and Letrozole in MCF7/AC1 Xenografts

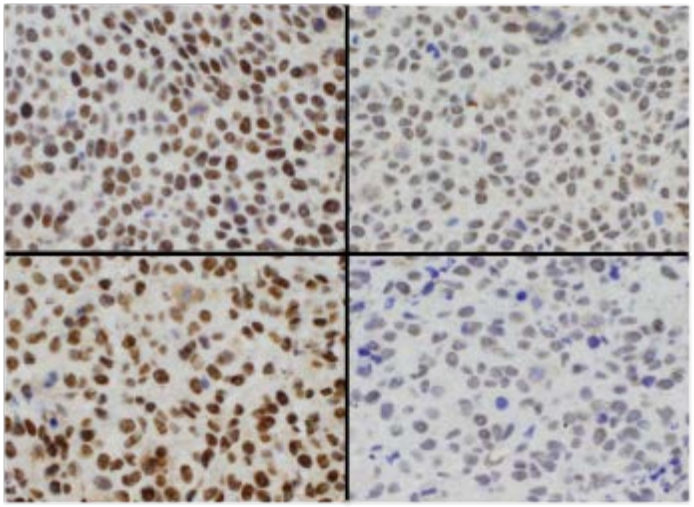


Goetz et al.

Effects of SERMs on AKT on letrozole sensitive and resistant MCF7 cells in vivo and in vitro (1 hour)

Letrozole sensitive treated with AND

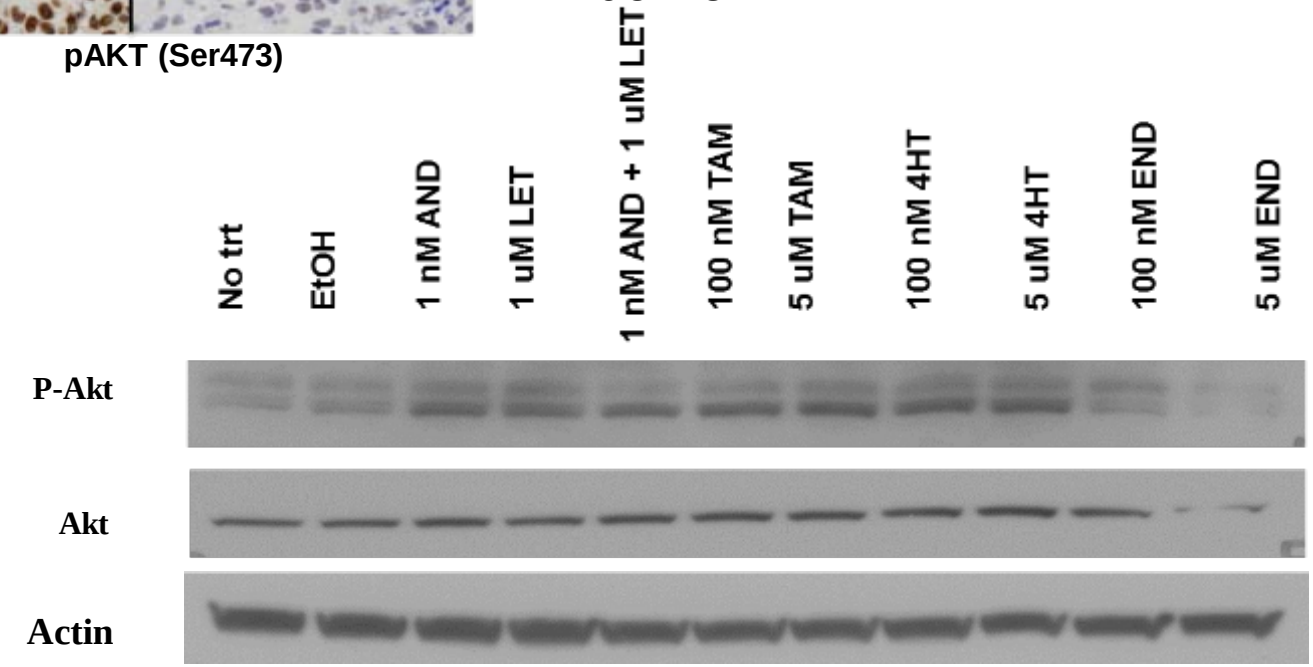
LR –treated with tamoxifen



pAKT (Ser473)

Letrozole resistant (LR)

LR treated with Endoxifen



Letrozole resistant MCF7: effects of SERMs on AKT

Goetz et al.

Summary

- **Endoxifen is a critical metabolite that necessary for full inhibition of proliferation and ER transcription in ER+ cells**
- **The pre-clinical effects of endoxifen may vary depending on the amount of estrogen present and the endocrine sensitivity of the tumor cells**
- **The importance of endoxifen (and thus *CYP2D6* genotype) in tamoxifen treated women may greatest in the premenopausal setting**

Outline

- ER+ breast cancer: brief review of the benefit of endocrine therapy
- Tamoxifen metabolism: Does variation in the hydroxylated metabolites matter?
  - Tamoxifen pk/pg
  - The effects of tamoxifen and metabolites on estrogen-induced proliferation and ER transcription
  - **Clinical: CYP2D6 metabolism and Ki-67**
  - Clinical: association between CYP2D6 genotype and recurrence: Reasons for discrepancy
- Steps forward for standardization

Ki-67 Change is Correlated with Clinical Outcome after Tamoxifen Therapy

Prognostic significance of Ki-67 labeling index after short-term presurgical tamoxifen in women with ER-positive breast cancer

A. DeCensi[1,*], A. Guerrieri-Gonzaga[1], S. Gandini[1], D. Serrano[1], M. Cazzaniga[1], S. Mora[1], H. Johansson[1], E. A. Lien[1,†], G. Pruneri[5,‡], G. Viale[5,‡] & B. Bonanni[1]

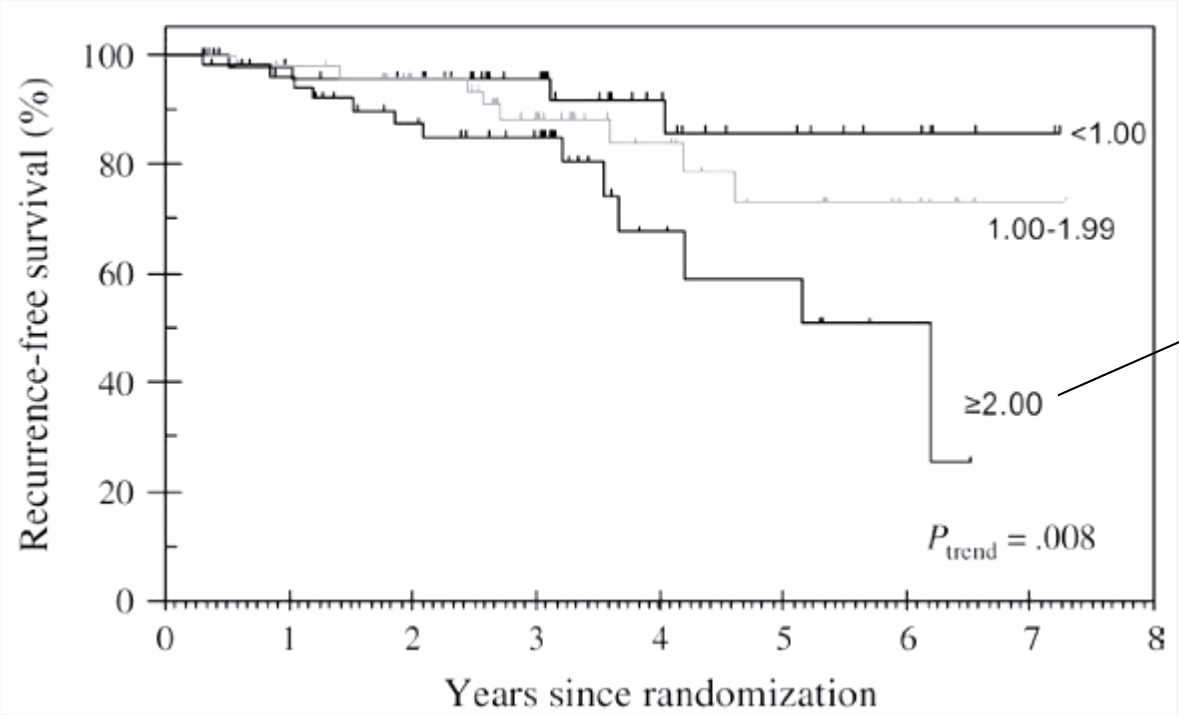
Annals of Oncology 22- 582-587, 2011

Prognostic Value of Ki67 Expression After Short-Term Presurgical Endocrine Therapy for Primary Breast Cancer

Mitch Dowsett, Ian E. Smith, Stephen R. Ebbs, J. Michael Dixon, Anthony Skene, Roger A'Hern, Janine Salter, Simone Detre, Margaret Hills, Geraldine Walsh

On behalf of the IMPACT Trialists Group

JNCI Vol. 99, Issue 2 | January 17, 2007



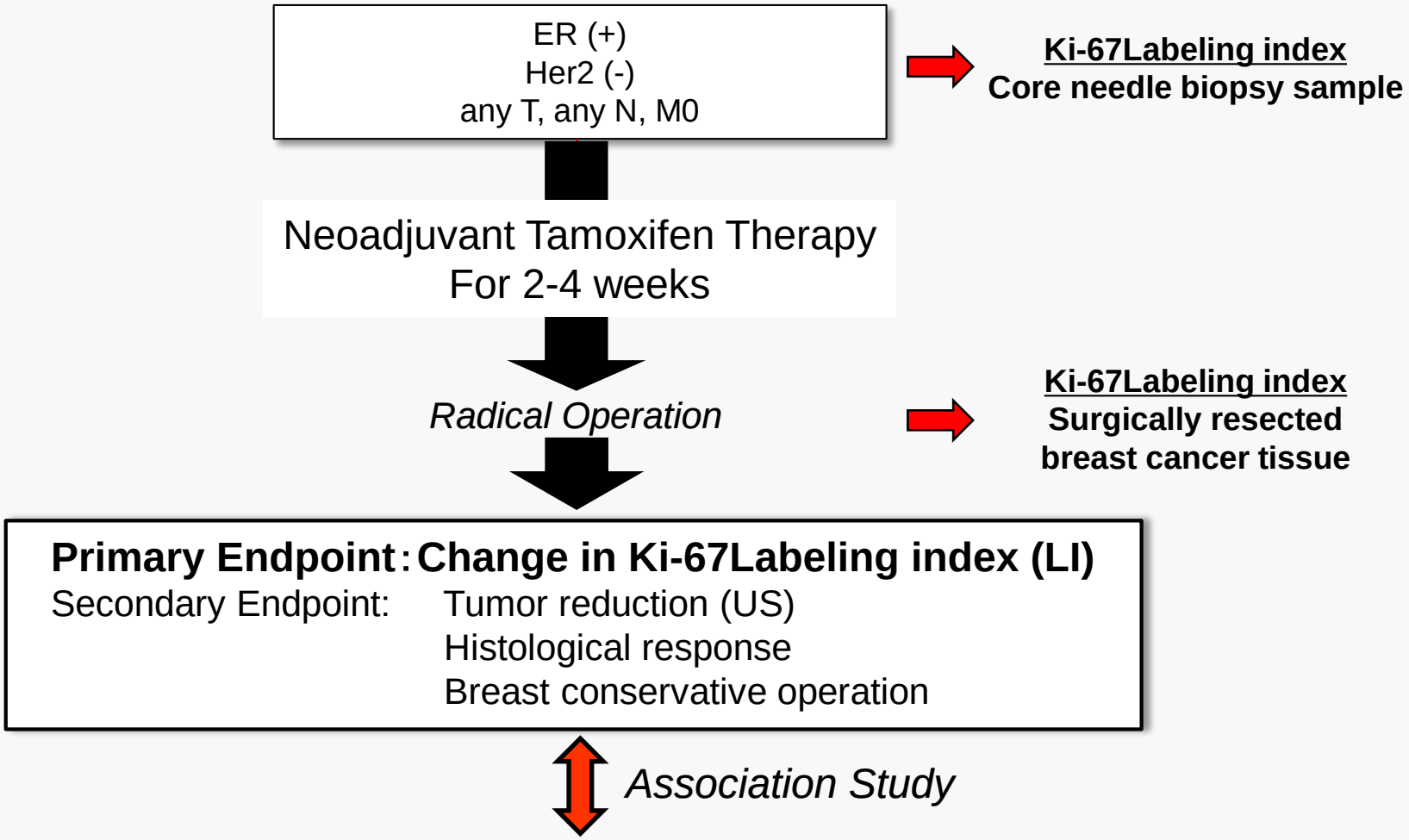
Ki-67 change after TAM

Higher Ki-67 expression after 2 weeks of endocrine therapy was significantly associated with lower recurrence-free survival (*P=0.004*)

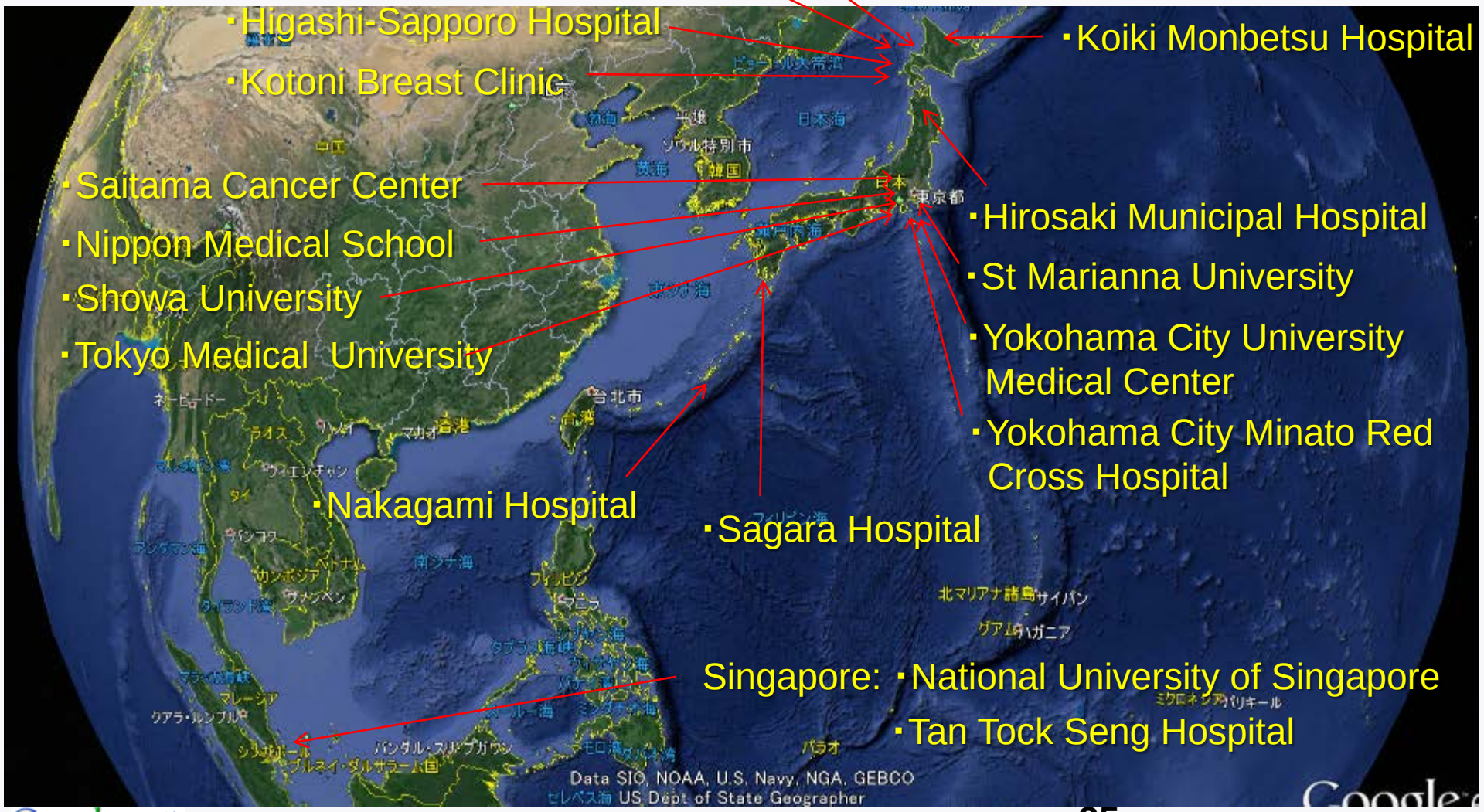
➡ Ki-67 is a surrogate marker for the response to tamoxifen therapy

C-GENT study

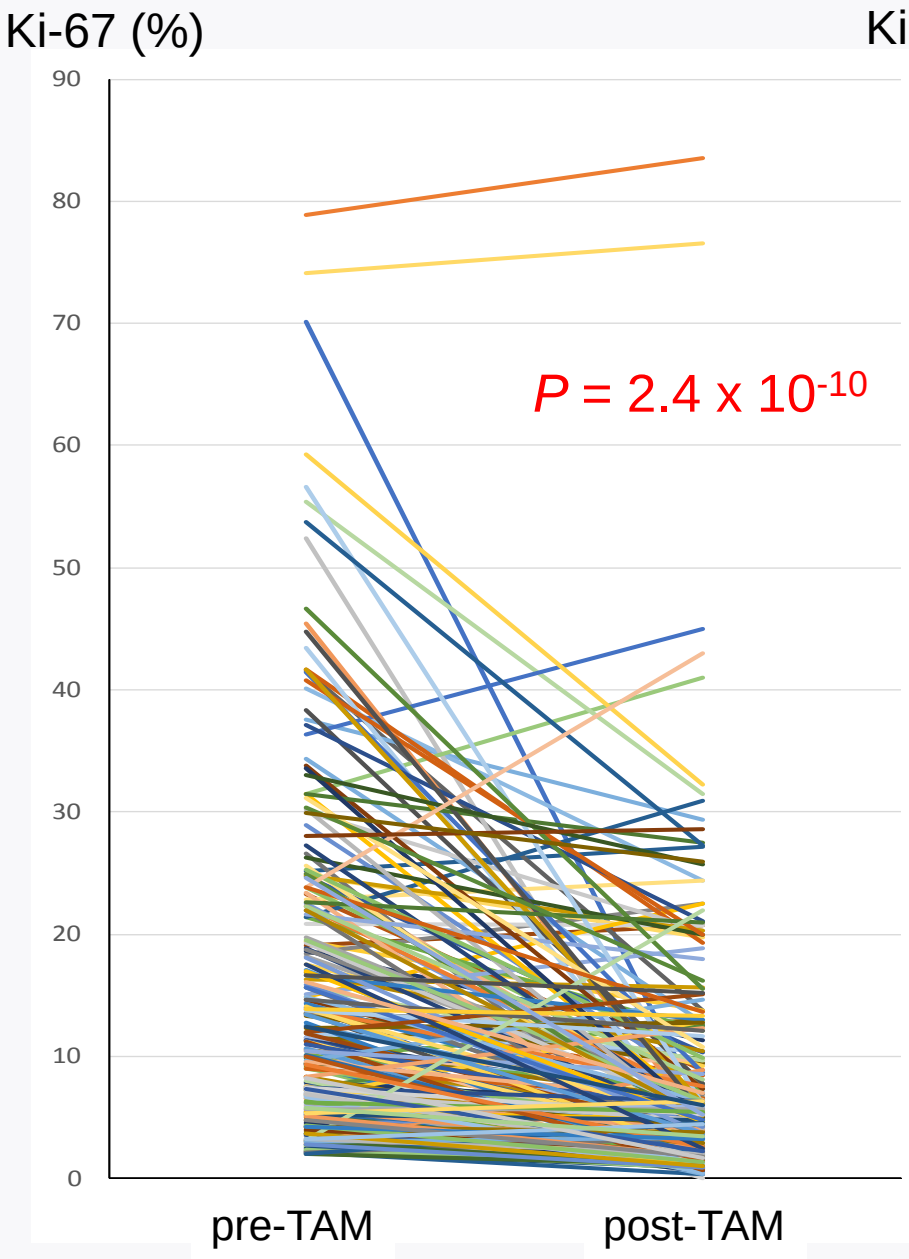
- Prospective Clinical Study to Clarify the Relationship between *CYP2D6* Genotype and the Therapeutic Effects of Preoperative Tamoxifen Therapy-

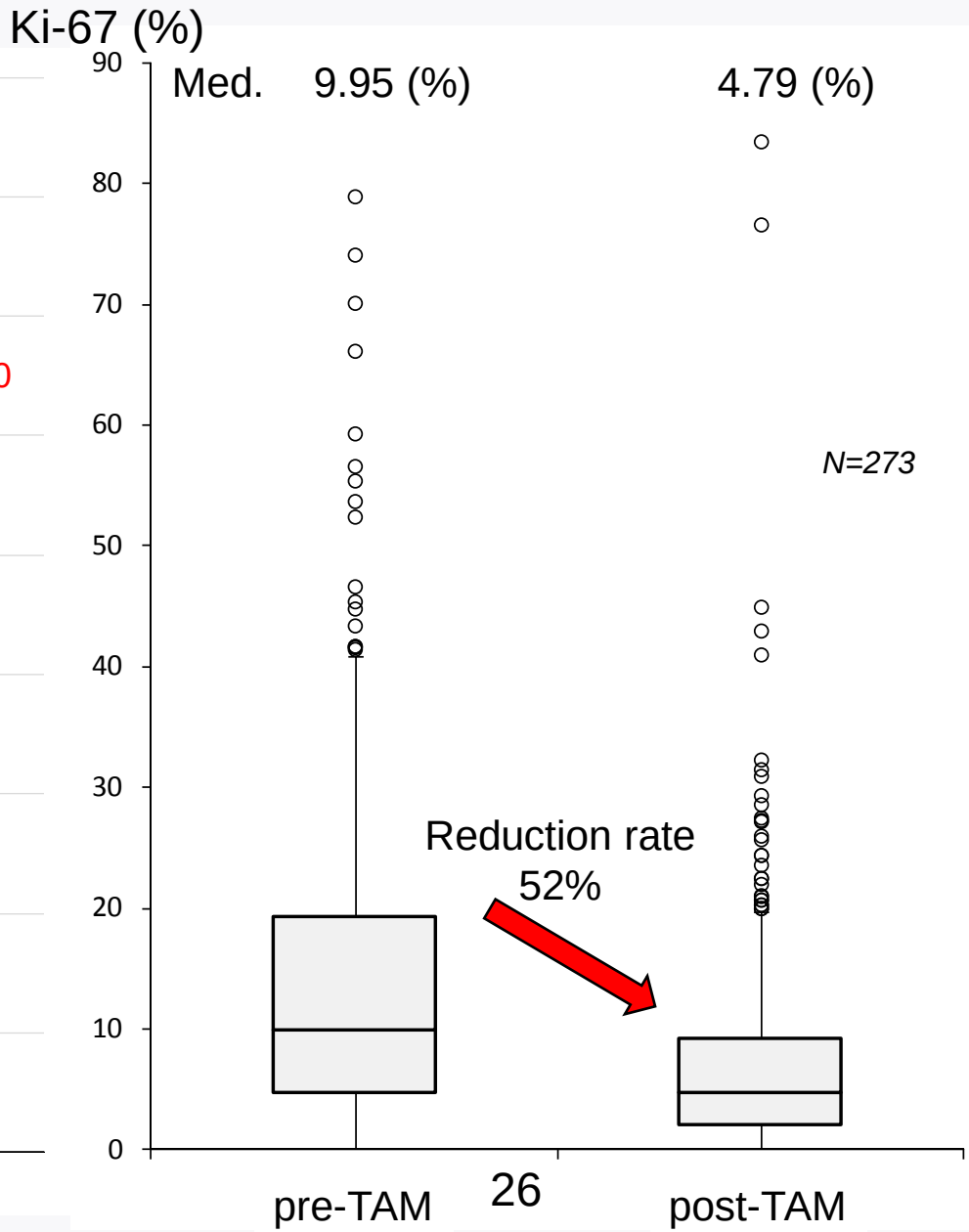


C-GENT collaborative study group (17 sites)



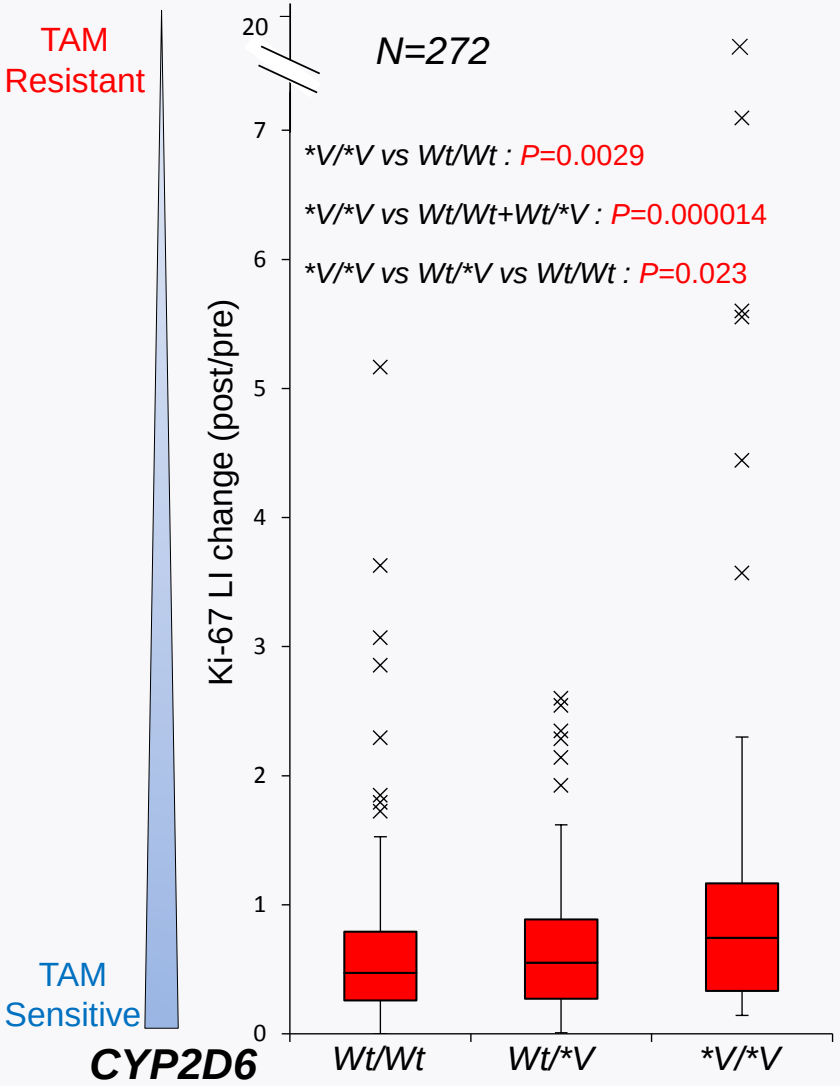
Ki-67 change after neoadjuvant tamoxifen therapy

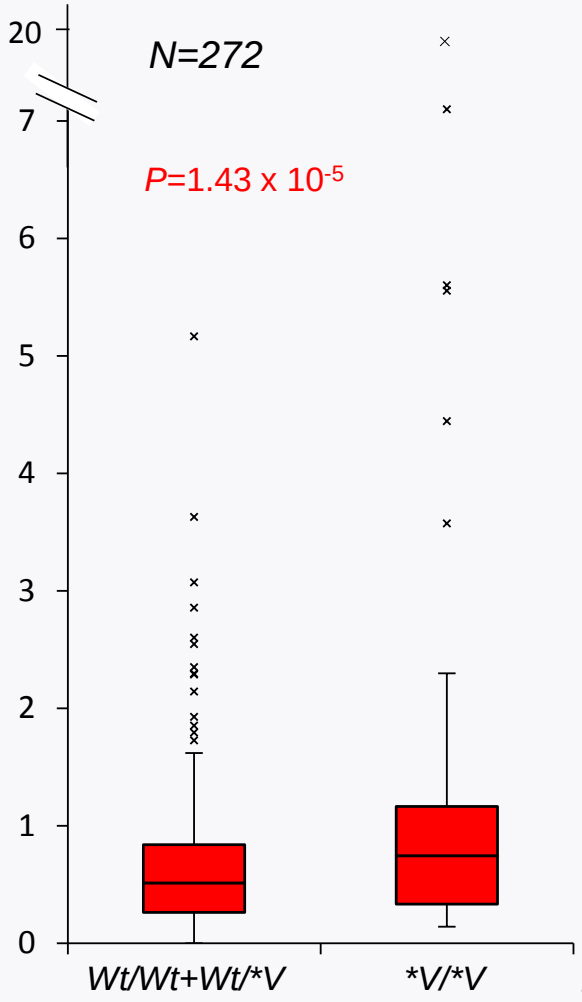




Primary Endpoint

(CYP2D6 genotype and Ki-67 LI change after tamoxifen therapy)





Summary

- Level 1 data that…..
- *CYP2D6* genetic variation is associated with endoxifen concentrations
- *CYP2D6* genotype affects Ki-67 response in a 2-week window study
- What is the level of evidence for the use of *CYP2D6* genotyping for patients with ER+ breast cancer?

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  - Tamoxifen pk/pg
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  - Clinical: CYP2D6 metabolism and Ki-67
  - **Clinical: association between *CYP2D6* genotype and recurrence**
- Steps forward for standardization

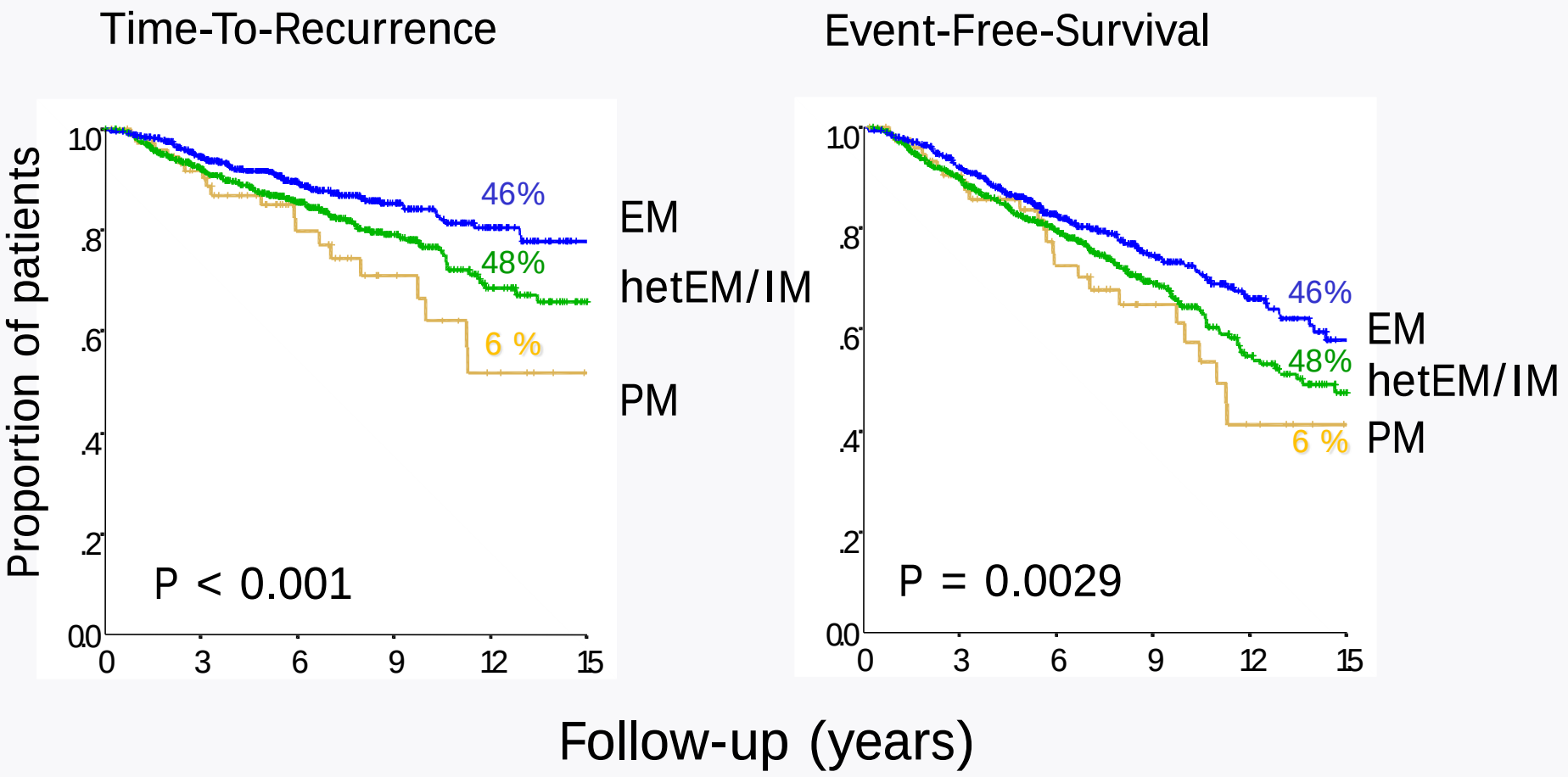
Simon/Hayes/Paik Criteria for Biomarker Studies

- **Category A: Prospective Clinical Trial (PCT) designed to address tumor biomarker**
- **Category B: PCT not designed to address biomarker, but design accommodates tumor marker utility**
- **Category C: Prospective Observational Registry, treatment and follow-up not dictated**
- **Category D: No prospective aspect to study**

1. **Simon**, R. M., S. Paik, Hayes et al. (2009). Journal of the National Cancer Institute **101**(21): 1446-1452.

CYP2D6 Polymorphism is a Determinant of Tamoxifen Response in Early Breast Cancer

Number of patients: 1325



Schroth, Goetz *et al.,* JAMA 2009

ITPC: Pooled Analysis of Category C Studies

- International Tamoxifen Pharmacogenomics Consortium. 4,973 tamoxifen-treated patients
- Using clinical trial eligibility (Criterion 1: n=1,996) (postmenopausal ER+-positive, 20 mg/day tamoxifen for 5 years, standard follow-up), CYP2D6 PM associated with IDFS and BCFI: HR 1.25; (1.06, 1.47; p = 0.009).

	IDFS				BCFI			
	Meta-estimates		*P* value		Meta-estimates		*P* value	
	HR	95% CI	Homog[a]	Association[b]	HR	95% CI	Homog[a]	Association[b]
Criterion 1	1.25	(1.06,1.47)	0.899	0.009	1.27	(1.01,1.61)	0.858	0.041
Criterion 2	1.17	(0.90,1.52)	0.055	0.249	1.21	(0.889,1.65)	0.130	0.224
Criterion 3	1.07	(0.92,1.26)	0.099	0.382	1.10	(0.868,1.35)	0.114	0.352

BCFI, breast cancer–free interval; Homog, homogeneity; HR, hazard ratio; IDFS, invasive disease–free survival; ITPC, International Tamoxifen Pharmacogenomics Consortium.

[a]The homogeneity *P* value tests the hypothesis that the individual ITPC site estimates meet the statistical random-effects modeling assumptions of the meta-analysis. A significant value indicates that there is significant heterogeneity among the sites, which casts doubt on the "combinability" of the studies for that parameter and on the validity of the corresponding association test. [b]The association *P* value tests the hypothesis that the combined meta-analysis estimate of the HR is significantly different from the null hypothesis value of HR = 1.

Province, M; Goetz MP et al. Clin Pharmacol Ther. 2014 Feb; 95(2): 216–227

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1. **Simon**, R. M., S. Paik, Hayes et al. (2009). Journal of the National Cancer Institute **101**(21): 1446-1452.

Paradigm for Biomarker Study Design

- **Secondary analysis of a phase III trial[1]**
  - "prospective-retrospective" designs using archived specimens
  - adequate amounts of archived tissue must be available for statistical power
  - patients studied must be representative of patients in the trial
  - test should be analytically and pre-analytically validated for use with archived tissue
- **Did ATAC, BIG 1-98, and ABCSG8 fulfill each of these criteria?**

1. **Simon**, R. M., S. Paik, Hayes et al. (2009). Journal of the National Cancer Institute **101**(21): 1446-1452.

Prospective 5 year Tamoxifen Trials in Postmenopausal Women Evaluating CYP2D6

- **Adjuvant treatment of invasive ER+ breast ca**
  - ATAC: Tam or anastrozole for 5 years (Rae et al JNCI 2012)
  - BIG 1-98 Tam or letrozole for 5 years (Regan, Leyland-Jones: JNCI 2012
  - ABCSG 8: Tam for 5 yrs or Tam for 2 yrs followed by anastrozole for 3 yrs (Goetz: Clin Canc Research 2013)

CYP2D6 Genotype: Results from the 5 year Adjuvant Tamoxifen and AI Trials (Category B)

Postmenopausal Women with ER+ Breast Cancer:

- **BIG 1-98: Tam or letrozole (5 yrs) (Negative Study)**
  - **DNA derived from FFPE Tumor Cores**
- **ATAC: Tam or anastrozole (5 yrs) (Negative Study)**
  - **DNA derived from FFPE Tumor Cores**
  - **Analyzed less than 20% of the patients**
- **ABCSG 8: Tam (5 yrs) or Tam (2 yrs) followed by anastrozole (3 yrs) (Positive Study)**
  - **DNA derived from FFPE enriched for normal tissue**

1. Regan et al. J National Canc Institute 2012
2. Rae et al J National Canc Institute 2012
3. Goetz et al. Clin Canc Research 2013

Controversy: *CYP2D6* and Tamoxifen

- **The first two studies:**
  - BIG 1-98 and ATAC demonstrated no association between CYP2D6 genotype and outcomes in either study
  - CYP2D6 Genotyping has “no value in practice:

- **<u>Editorial:</u>**
- “the fact that these two studies confirm each other suggests that this matter has likely been laid to rest. Why has such a good hypothesis gone wrong?”

Kelly, CM Pritchard K JNCI 2012

BIG 1-98: Observed vs Expected CYP2D6 Alleles---excess homozygotes and deficiency of heterozygotes

CYP2D6 Phenotype	N	Observed (%)	Expected (%)
Poor (PM)	236	9	5-7
Intermediate (IM)	716	27	55-65
Extensive (EM)	1585	59	30-35

***Hardy Weinberg equilibrium:
CYP2D6 *4: X^2 test P value= 1×10^{-92}
*CYP2D6*41*: X^2 test P value= $2 \times 10^{-174;}$

Regan et al. J Natl Cancer Inst 2012
Stanton V Jr: J Natl Cancer Inst 104:1265-1266; author reply
Nakamura Y, Ratain MJ, Cox NJ, et al. J Natl Cancer Inst 104:1264; author reply Pharoah PD, Abraham J, Caldas C: J Natl Cancer Inst 104:1263-1264; author

CYP2D6 LOH and Genotyping Error

OXFORD

JNCI J Natl Cancer Inst, 2015, 1–8

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Article

ARTICLE

Loss of Heterozygosity at the *CYP2D6* Locus in Breast Cancer: Implications for Germline Pharmacogenetic Studies

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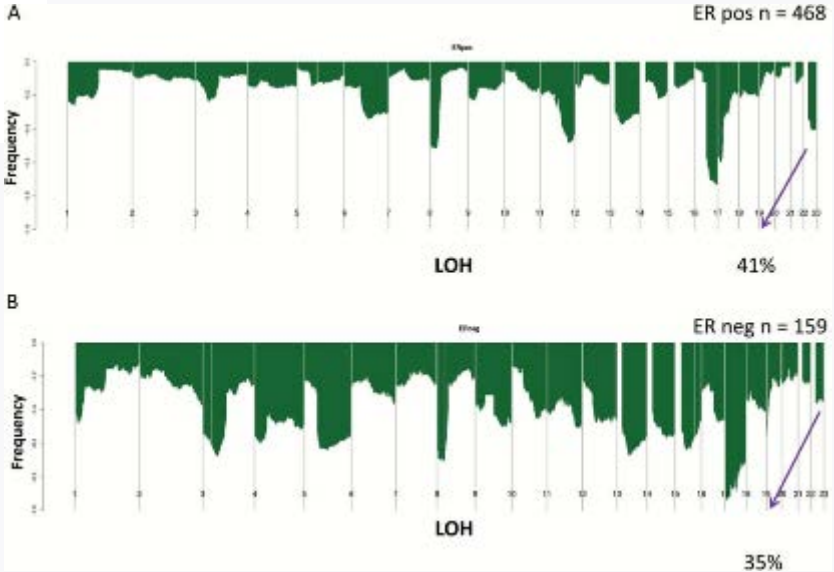
Department of Oncology (MPG, MMA, JNI), Department of Health Sciences Research (VJS, KRK), and Department



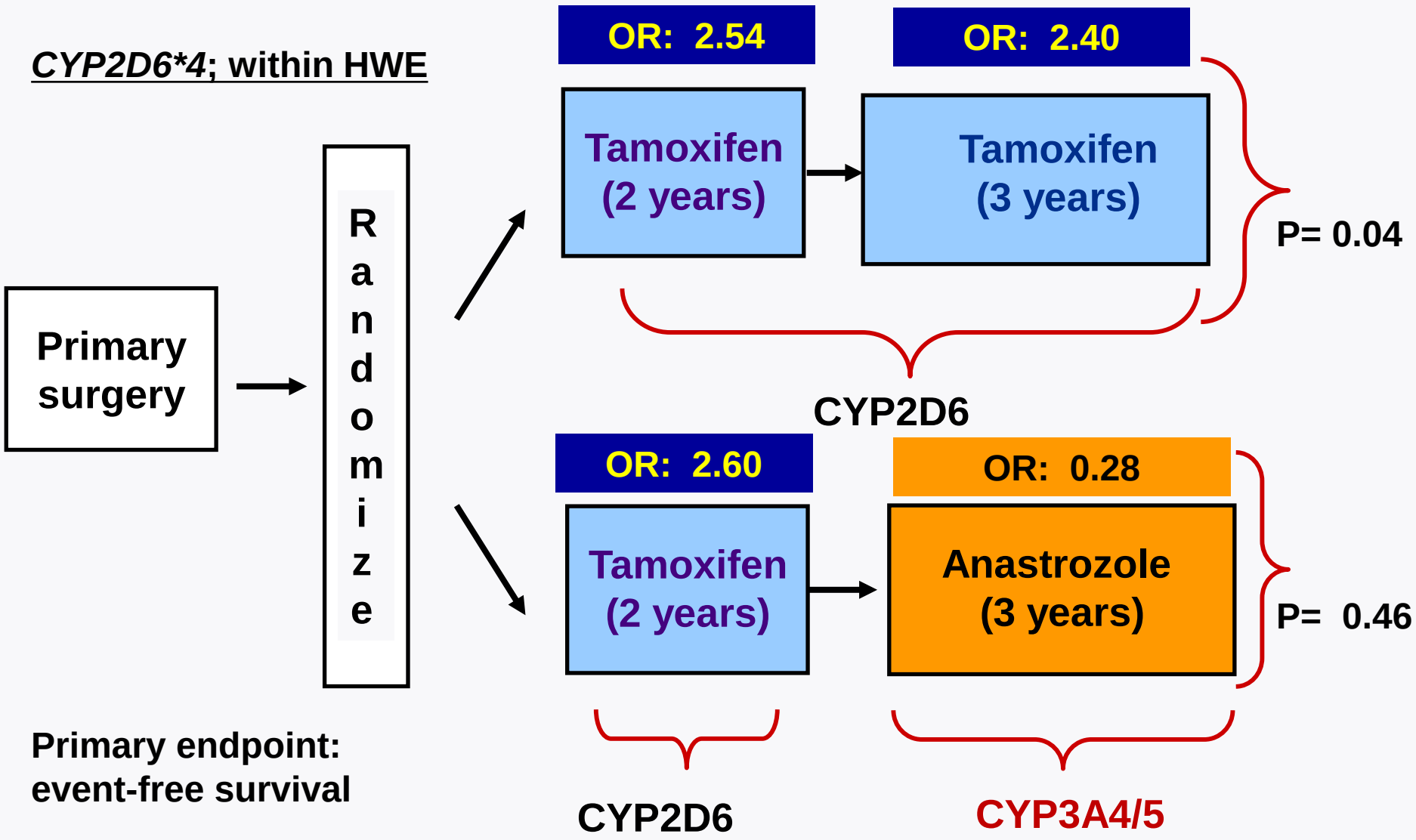
Goetz JNCI 2015

CYP2D6 LOH and Genotyping Error

- TCGA: Loss of Heterozygosity (LOH) at the CYP2D6 Locus (chromosome 22q13.1) > 40% of ER+ BC
- Heterozygotes mislabeled as homozygotes
- Comparing purified ER+ tumor DNA (FFPE tumor cores) vs germline (buccal cells), *CYP2D6*4* genotype discordant in 6/31(19.4%)
- Conclusion: Tumor DNA should not be used to determine germline CYP2D6 genotype without sensitive techniques to detect low frequency alleles and quality control procedures appropriate for somatic DNA.

Goetz et al. J Natl Cancer Inst. 2014

ABCSG Trial 8: CYP2D6 PM/PM vs EM/EM



Goetz, M. P. et al. (2013). Clinical cancer research : **19**(2): 500-507.

Summary

- Three (Category B) studies have been reported regarding *CYP2D6* and tamoxifen
- Only ABCSG 8 *CYP2D6* analysis demonstrated a positive association
- Significant methodology issues in BIG -98 and ATAC preclude their use in formulating guidelines
- When should patients be tested for CYP2D6? ASCO and NCCN Guidelines reference BIG 1-98 and ATAC and recommend against *CYP2D6* genotyping

CPIC Tamoxifen Guidelines

- Focusing only on studies evaluating the use of tamoxifen in the adjuvant setting for ER+ breast cancer
- Excluded studies which focused on single *CYP2D6* allele (e.g. *10 or *4)
- Nearly 50 studies extensively curated for all clinical and PG phenotypes
- Group of experts have classified the literature findings regarding the effects of *CYP2D6* genotype on PK, PD, side-effects, recurrence, DFS, and OS

CPIC Tamoxifen Guidelines (Summary)

- Strong consensus that *CYP2D6* genotype consistently alters endoxifen concentrations and pharmacodynamic effects (e.g. Ki-67)
- Moderate consensus that the literature demonstrates evidence for an effect of CYP2D6 genotype on recurrence and event free survival
- Next steps:
  - Crafting of clinical recommendations for each CYP2D6 phenotype (AS)
  - Publication

Ways to overcome CYP2D6 metabolism in Tamoxifen treated patients

- Substitute AI for tamoxifen[1]
- Increase the dose of tamoxifen (40-60 mg/day)[2-4]
- Substitute another SERM (toremifene)
- Directly administer endoxifen

1. Schroth, Goetz et al., JAMA 2009
2. Hertz et al. Oncologist 2016
3. Fox et al. Clin Canc Res 2016
4. Dezentje Breast Cancer Res Treat 2015

Final results of a First-in-human Phase I Study of the Tamoxifen (TAM) Metabolite, Z-Endoxifen Hydrochloride (Z-Endx) in Women with Aromatase Inhibitor (AI) Refractory Metastatic Breast Cancer (MBC) (NCT01327781)

Matthew P. Goetz; Vera J Suman; Joel M. Reid; Don W. Northfelt; Michael A. Mahr; Travis Dockter; Mary Kuffel; Andrew T. Ralya; Sarah Burhow; Stephanie Safgren; Renee McGovern; Jerry Collins; Howard Streicher; John R. Hawse; Tufia Haddad; Charles Erlichman; Matthew M. Ames; and James N. Ingle

Mayo Clinic, Rochester, MN and Scottsdale, AZ; NCI

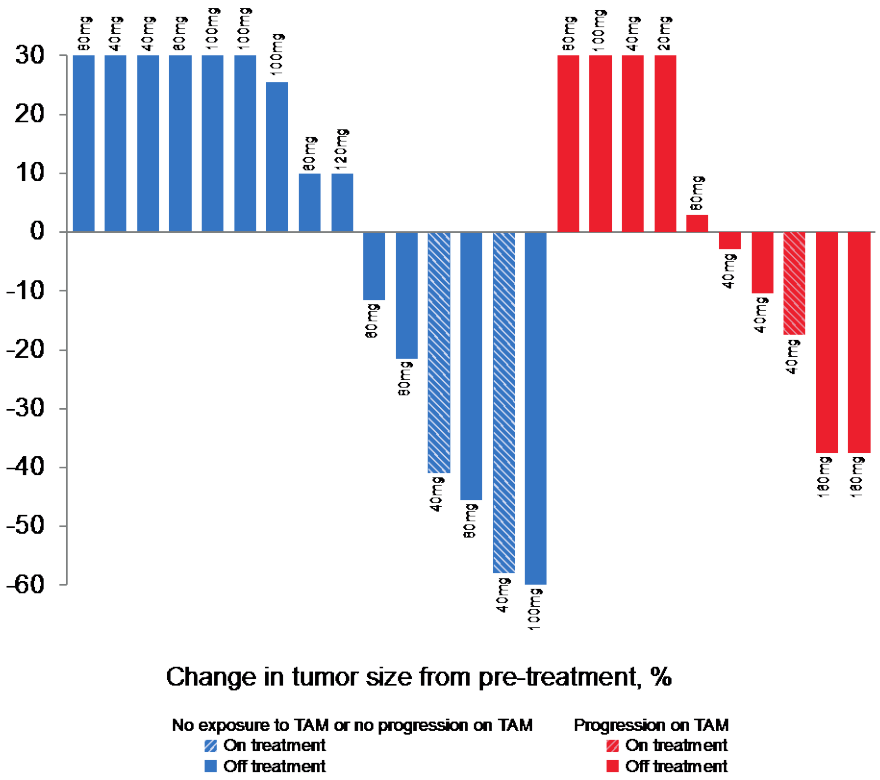
Goetz SABC 2015 and submitted 2017

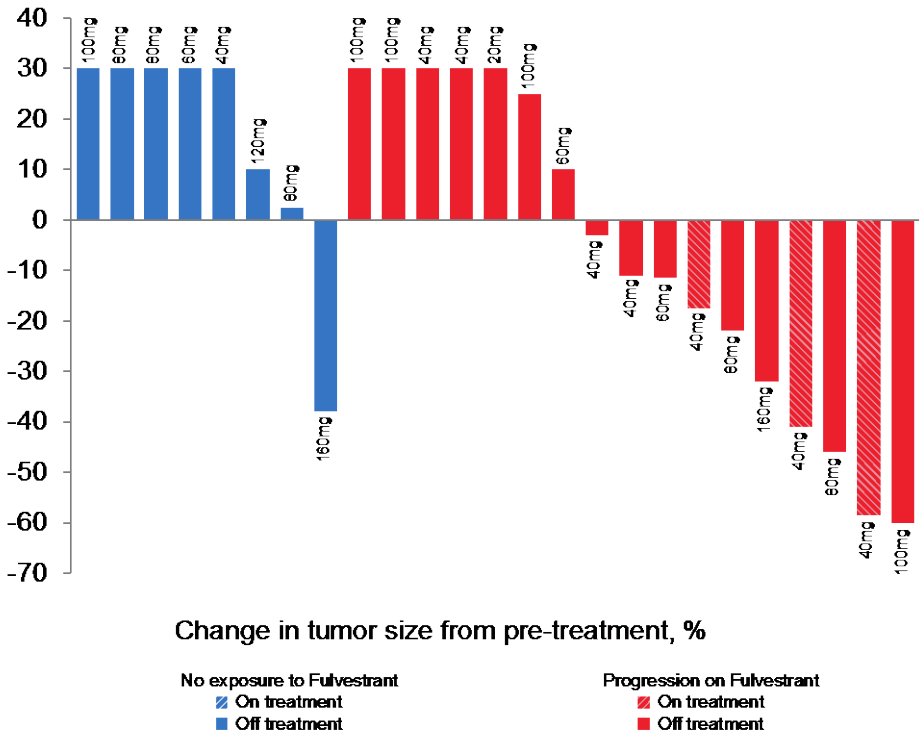
Endoxifen PK Summary

- 40 and 80 mg/day: C_{min}: 248 and 602 ng/ml respectively
- Compare to tamoxifen 20 mg/day: endoxifen conc: range 2-25 ng/ml
- $T_{1/2}$ of 50 hours
- 3 fold accumulation over 28 days, but no further accumulation at 2 and 6 months

Goetz SABC 2015 and submitted 2017

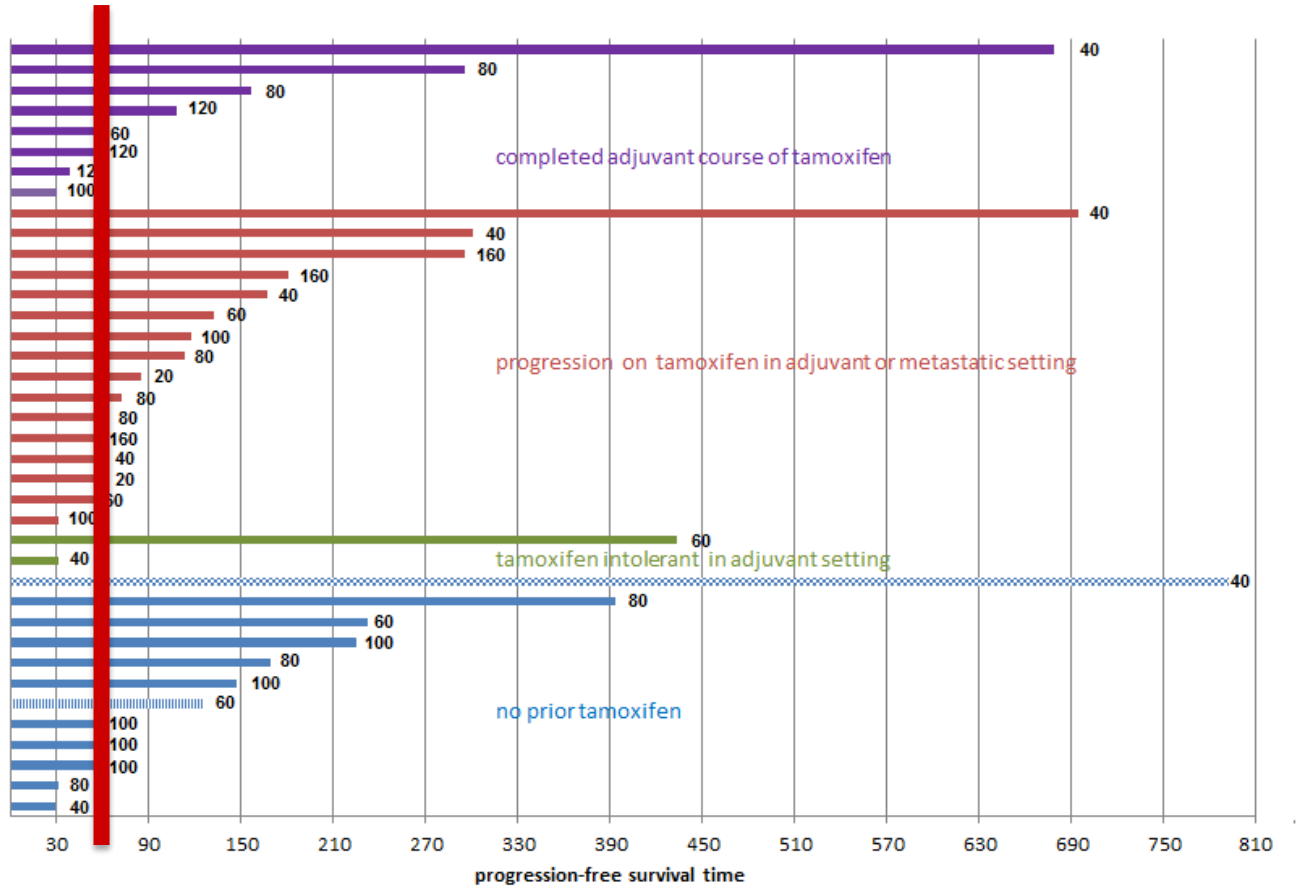
AI Refractory Breast Cancer: Endoxifen Responses according to progression on Tamoxifen (A) and Fulvestrant (B)





Goetz SABC 2015 and submitted 2017

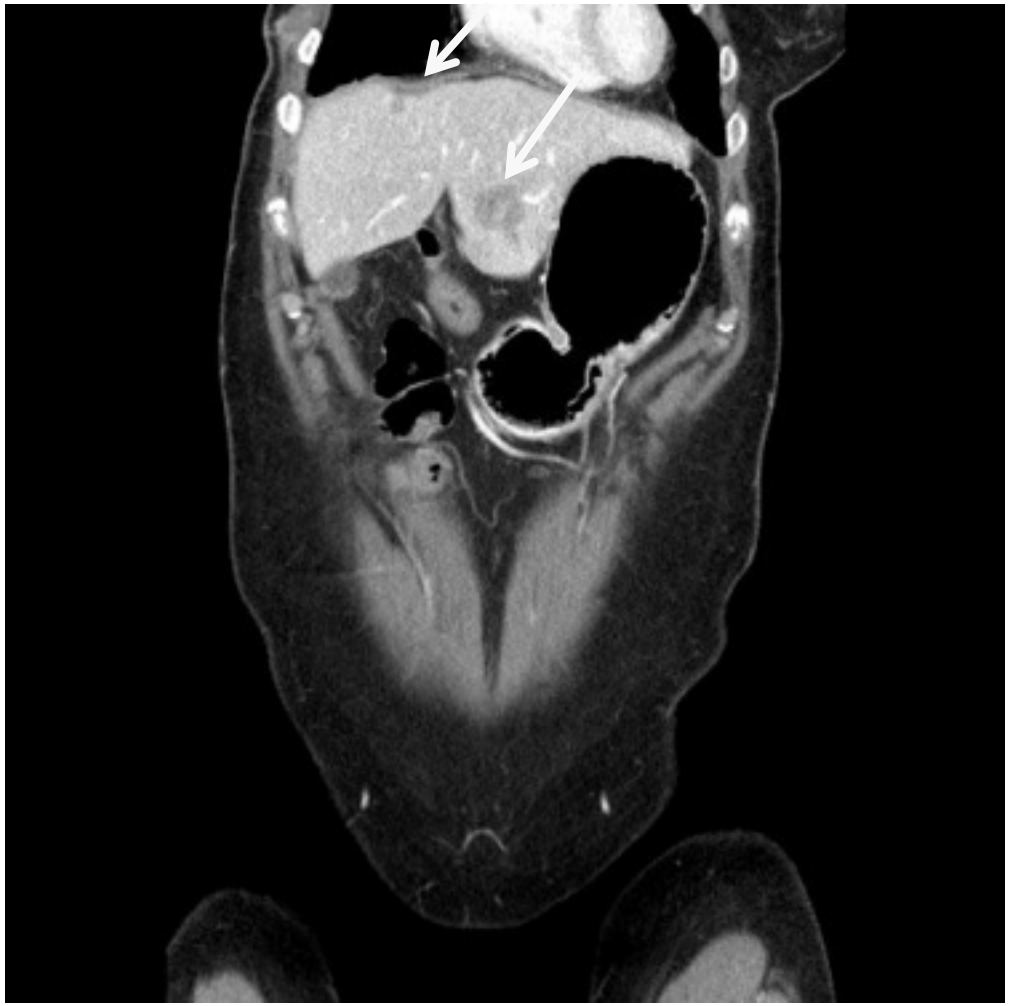
Endoxifen Responses according to Prior Tamoxifen

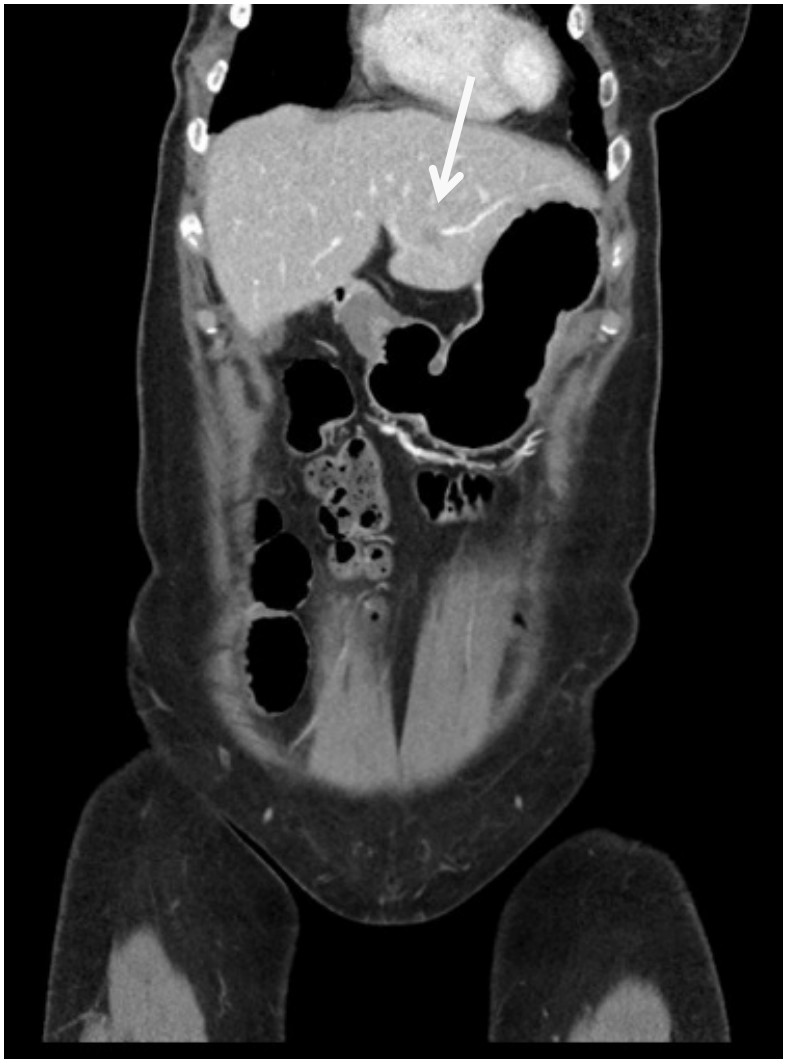


Expected median PFS of 60-90 days:

Goetz SABC 2015 and submitted 2017

Endoxifen activity in tamoxifen, AI, fulvestrant and Everolimus refractory breast cancer





Goetz SABC 2015

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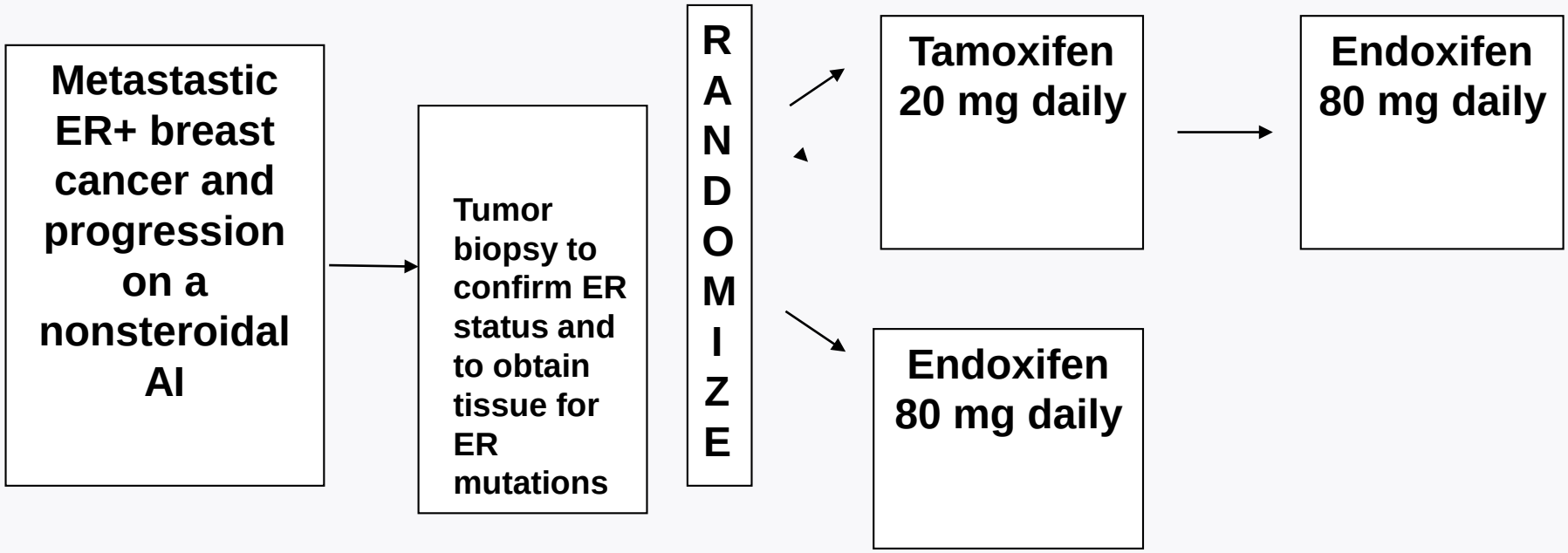

Stratify: endocrine resistance (primary/secondary) and prior everolimus/palbociclib (yes/no)

Summary

- Genetic Variation in tamoxifen metabolism alters
  - Endoxifen concentrations
  - The effects of tamoxifen metabolism on clinical outcomes is controversial
  - A substantial proportion of the unresolved controversy can be explained by poorly conducted studies.
- Recommendations:
  - Prospective Clinical Trials are key
  - Secondary analyses of prospective clinical trials are still forthcoming (SOFT/TEXT)