Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart

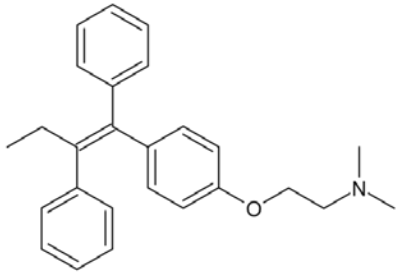
IKP STUTTGART

Pitfalls and shortcomings of pharmacogenomic association studies: The tamoxifen controversy

Hiltrud Brauch

20th International Symposium on Microsomes and Drug Oxidation
Stuttgart, Germany | 18- 22, 2014

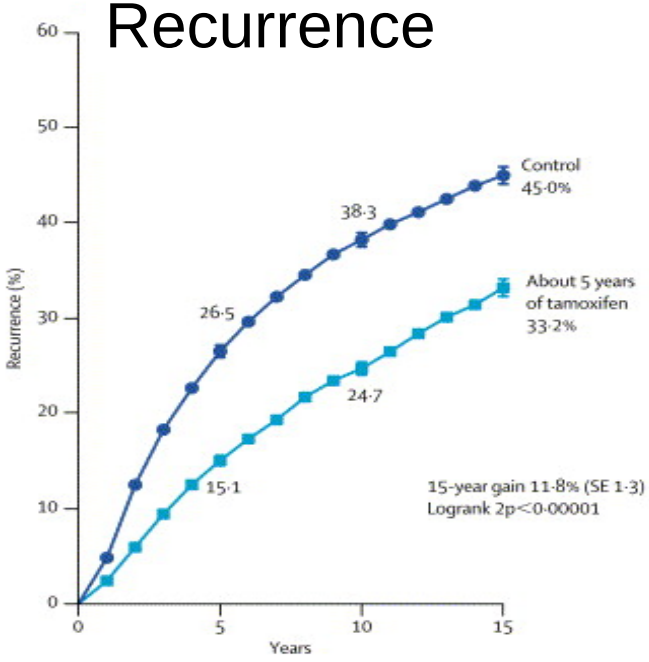
Tamoxifen in early breast cancer



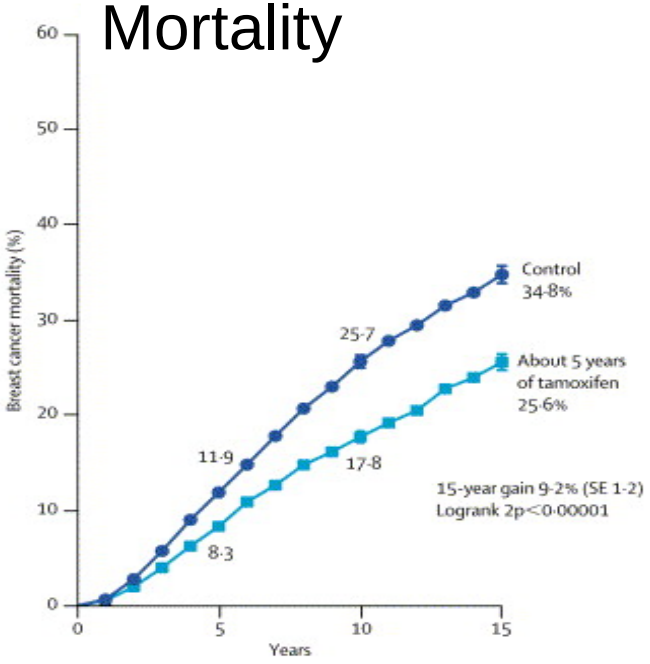
ICI 46,474 Novaldex®

- Non steroidal anti-estrogen
- Selective estrogen receptor modulator (SERM) first targeted and to date most successful therapy
- Four decades of experience
- Used in > 120 countries for all stages of breast cancer

Recurrence

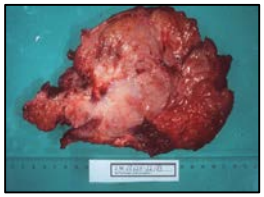


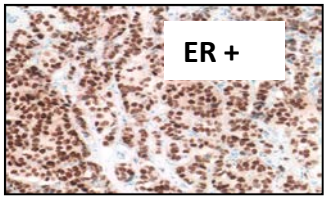
Mortality



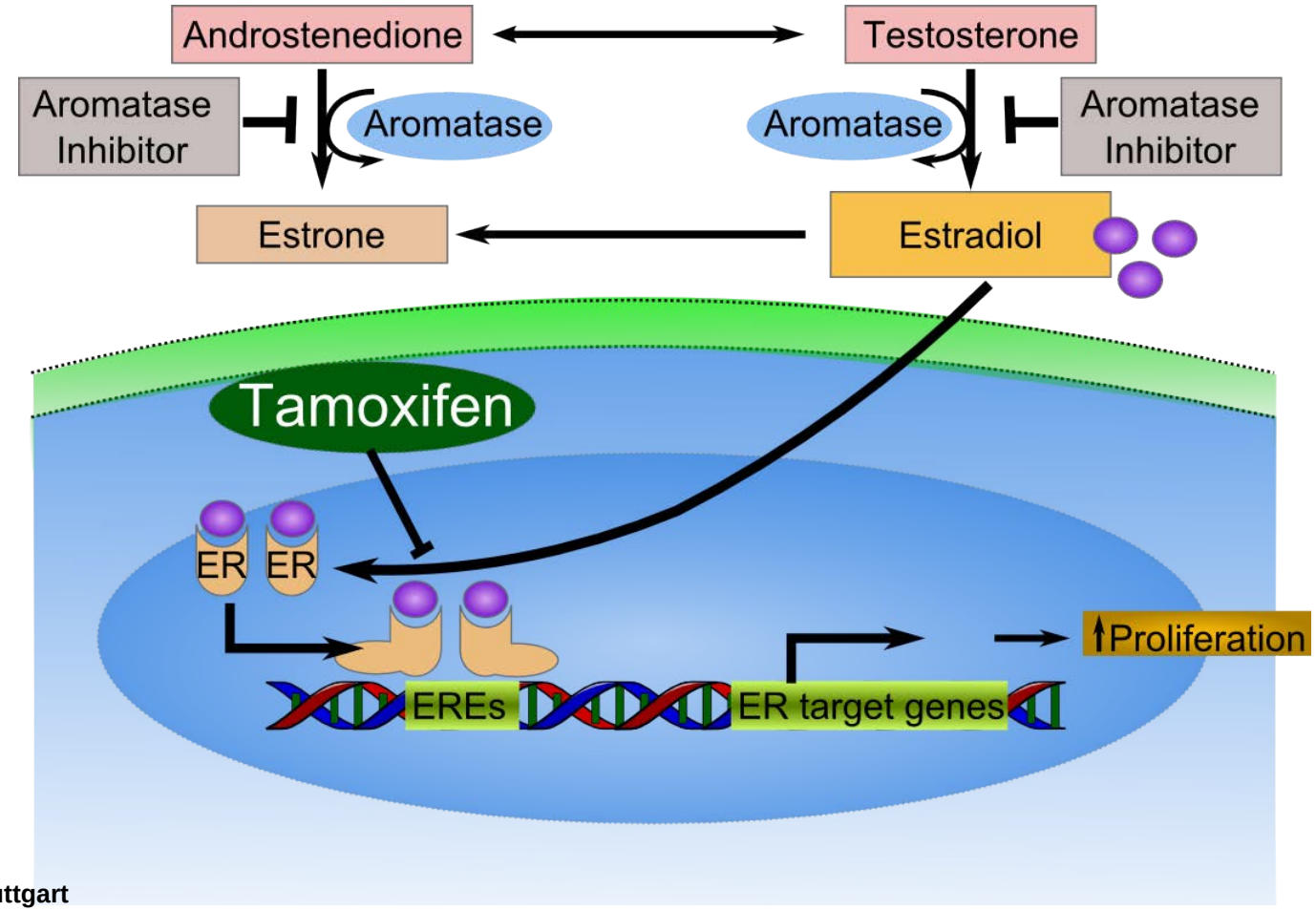
EBCTCG, Lancet 2005

Mechanisms of endocrine therapy in early BC





70% of patients are eligible



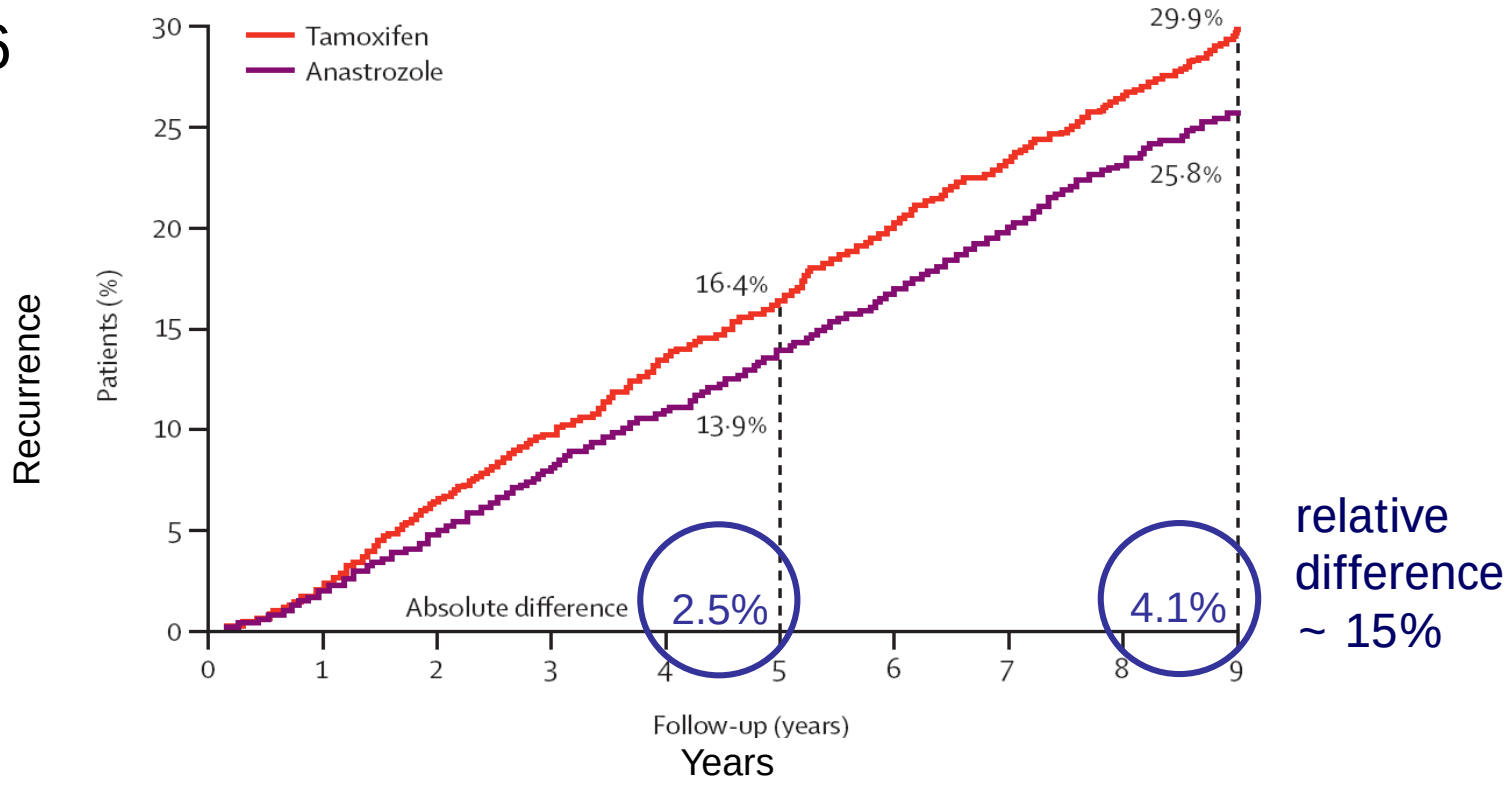
Aromatase inhibitor versus tamoxifen outcome

ATAC Trialists‘ Group
Lancet Oncol 9:45-53, 2008

ATAC: Anastrozole and Tamoxifen alone or in Combination

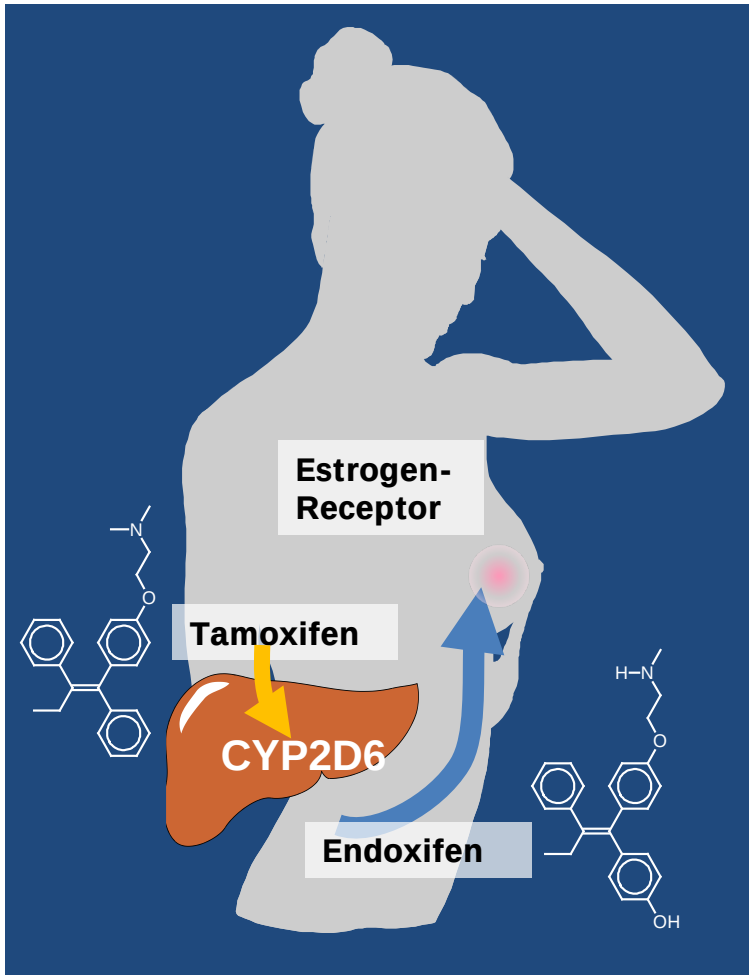
N=5216

5 years of tamoxifen versus anastrozole



relative difference ~ 15%

Tamoxifen is a prodrug



Tamoxifen (TAM)

4-OH-TAM

2D6

2C19
2B6, 2C9, 3A4

3A4/5
2C19

2D6

2D6

3A4/5
2C19

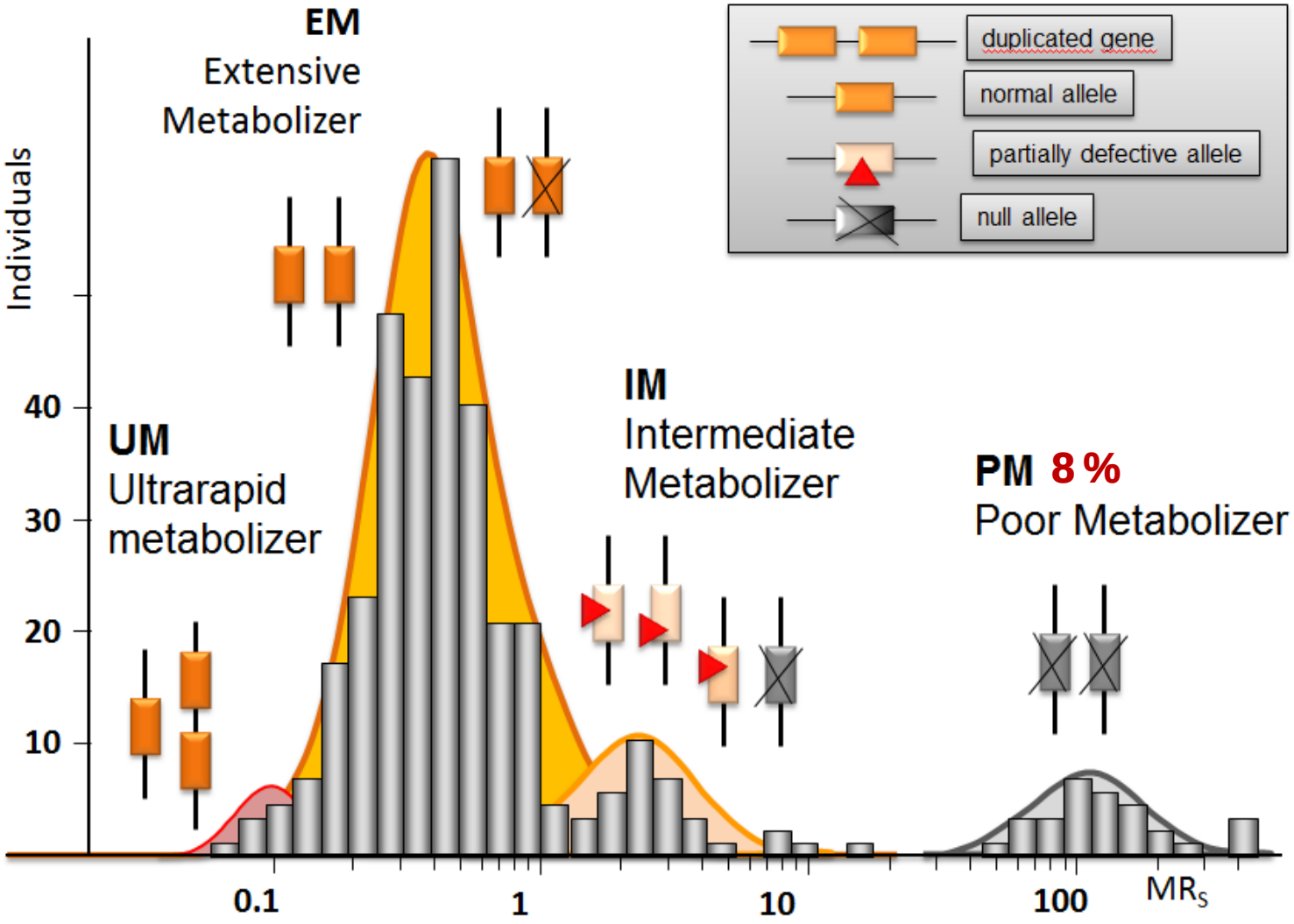
2D6

nDM-TAM

Endoxifen

100x more effective

Sparteine oxidation phenotypes and distribution in a German population

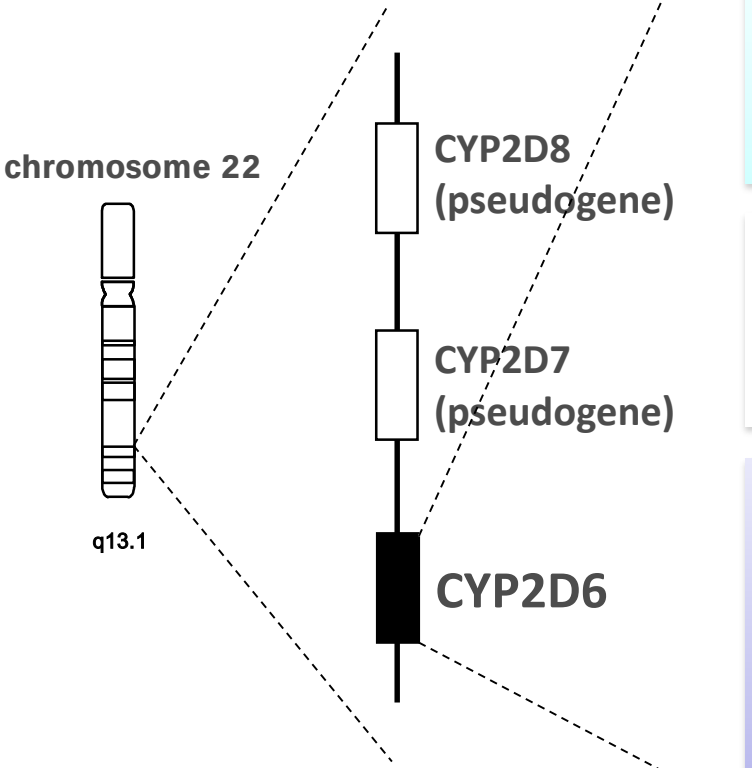


Zanger & Schwab, Pharmacol Ther 138:103-141, 2013

Molecular Basis of the *CYP2D6* Polymorphism

>100 genetic variants define phenotypes and their rate of drug metabolism

CYP2D gene locus



UM **1.2 %**

duplicated alleles
*1x2, *2x2, *35x2

AmpliChip CYP450

EM **80.7 %**

functional alleles
*1, *2, *35

37.2 % EM
53.2 % hetEM / IM

Schroth *et al* , Clin Cancer Res 2010

IM **9.7 %**

low function alleles
*9, *10, *17, *41, *59

PM **8.3 %**

nonfunctional (null, *0)

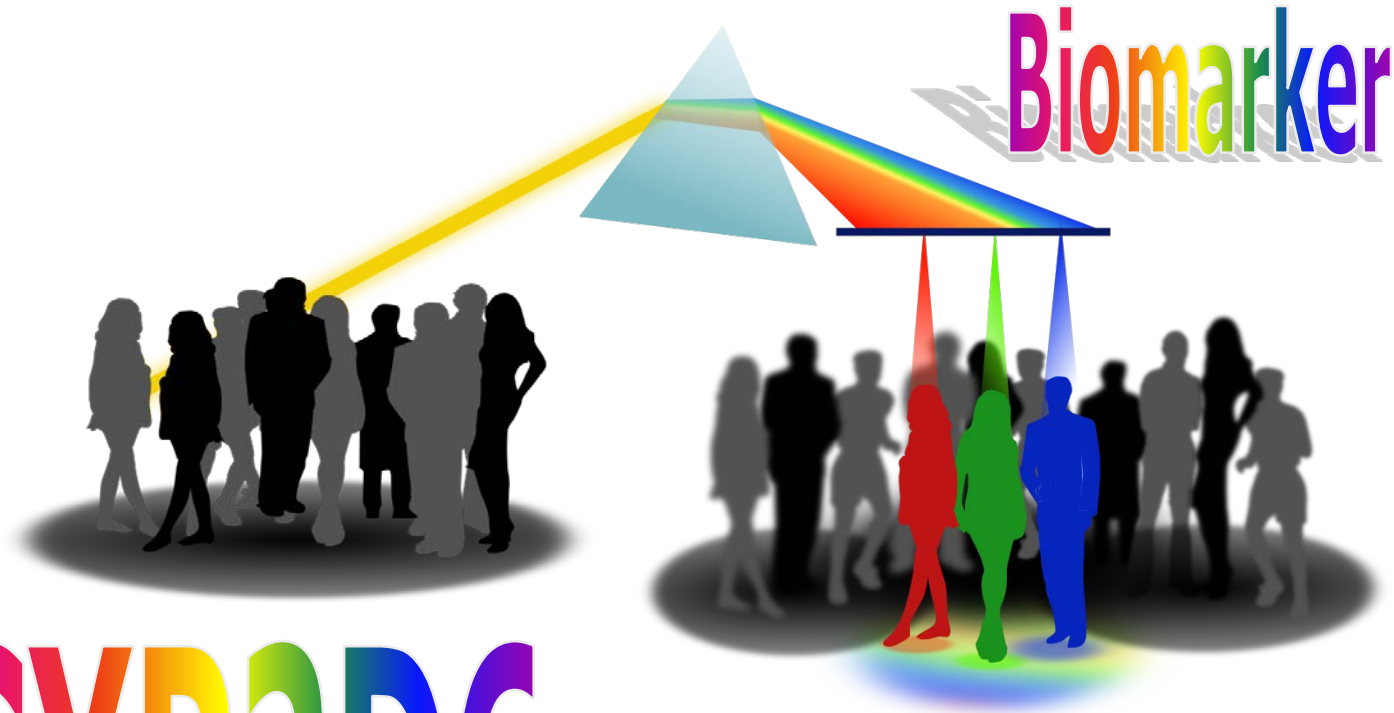
*3, *4, *5, *6, *7, *8,
*12, *13, *14, *15, *16,
*18, *19, *20, *21, *38 ..

UM ultrarapid
EM extensive
IM intermediate
PM poor metabolizer

Human CYP Allele Nomenclature Home Page: http://www.cypalleles.ki.se/

Pharmacogenetics / Pharmacogenomics

Personalized cancer treatment and patient stratification

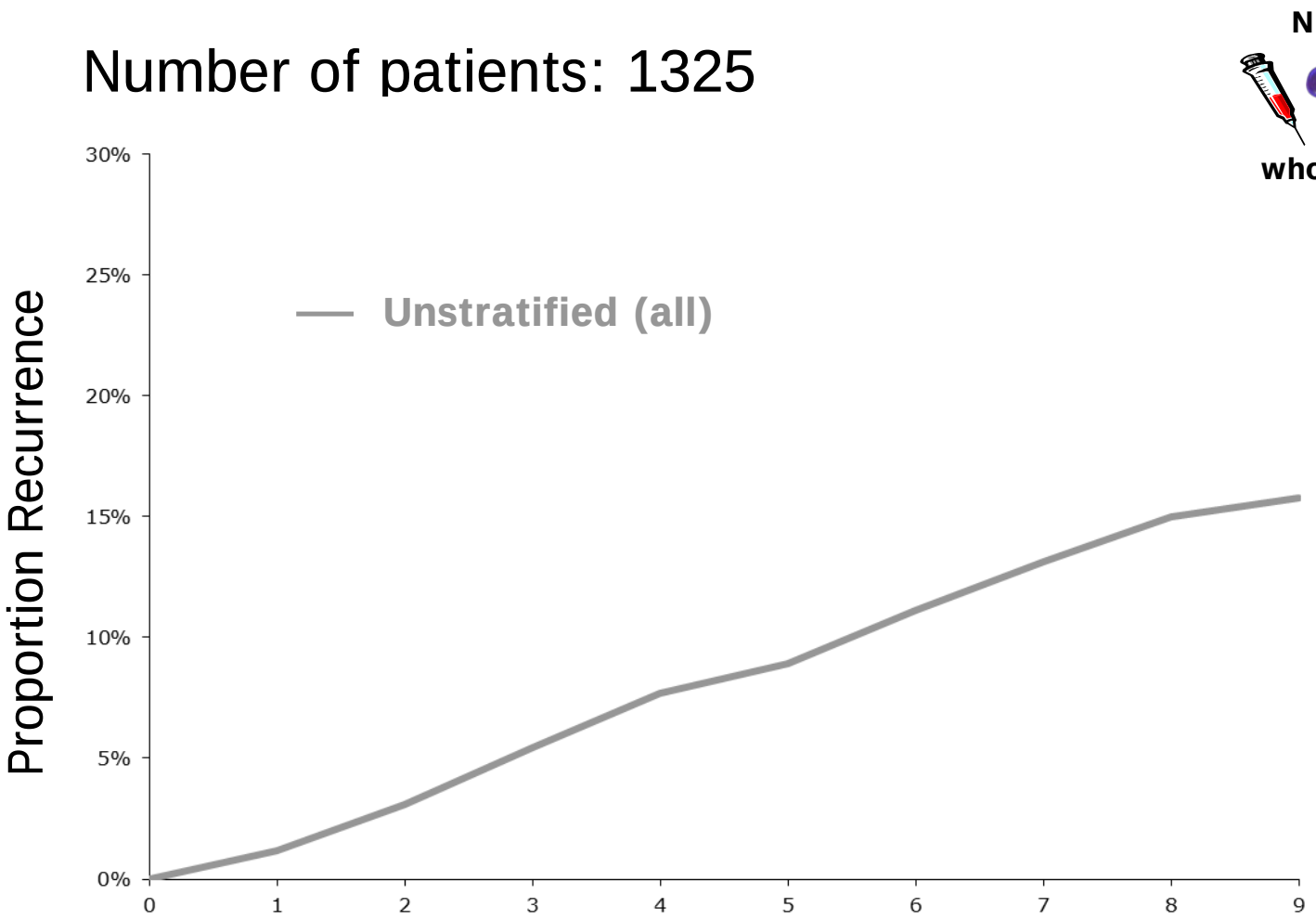


CYP2D6

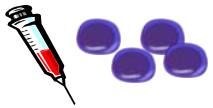
- the right drug
- to the right patient
- at the right time
- in the right dose

CYP2D6 polymorphism and recurrence probabilities upon tamoxifen treatment

Number of patients: 1325



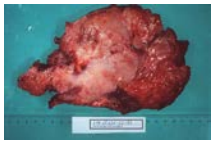
N = 601



whole blood

N = 659

N = 601

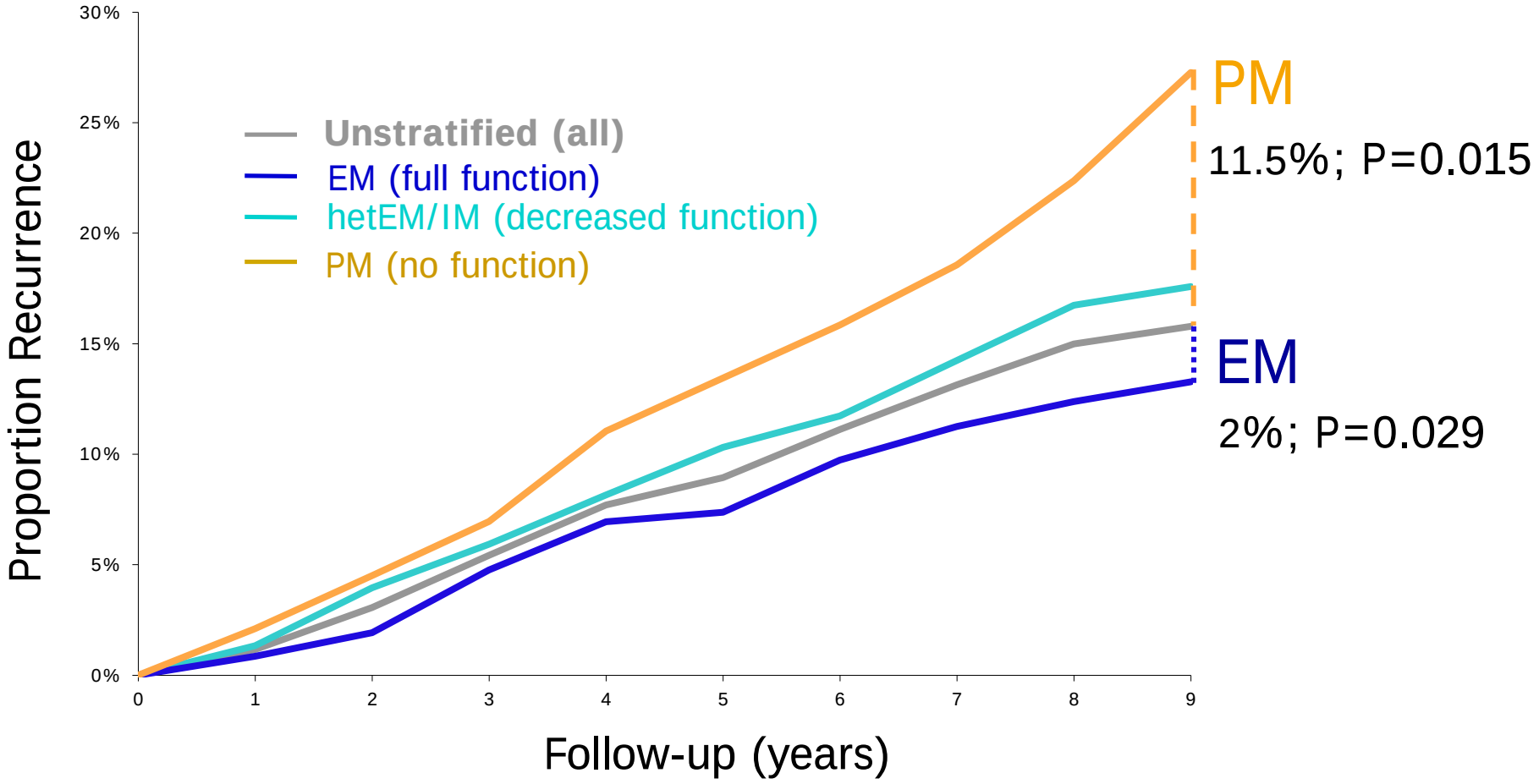


frozen tissue

Schroth et al. JAMA 302:1429-1436, 2009

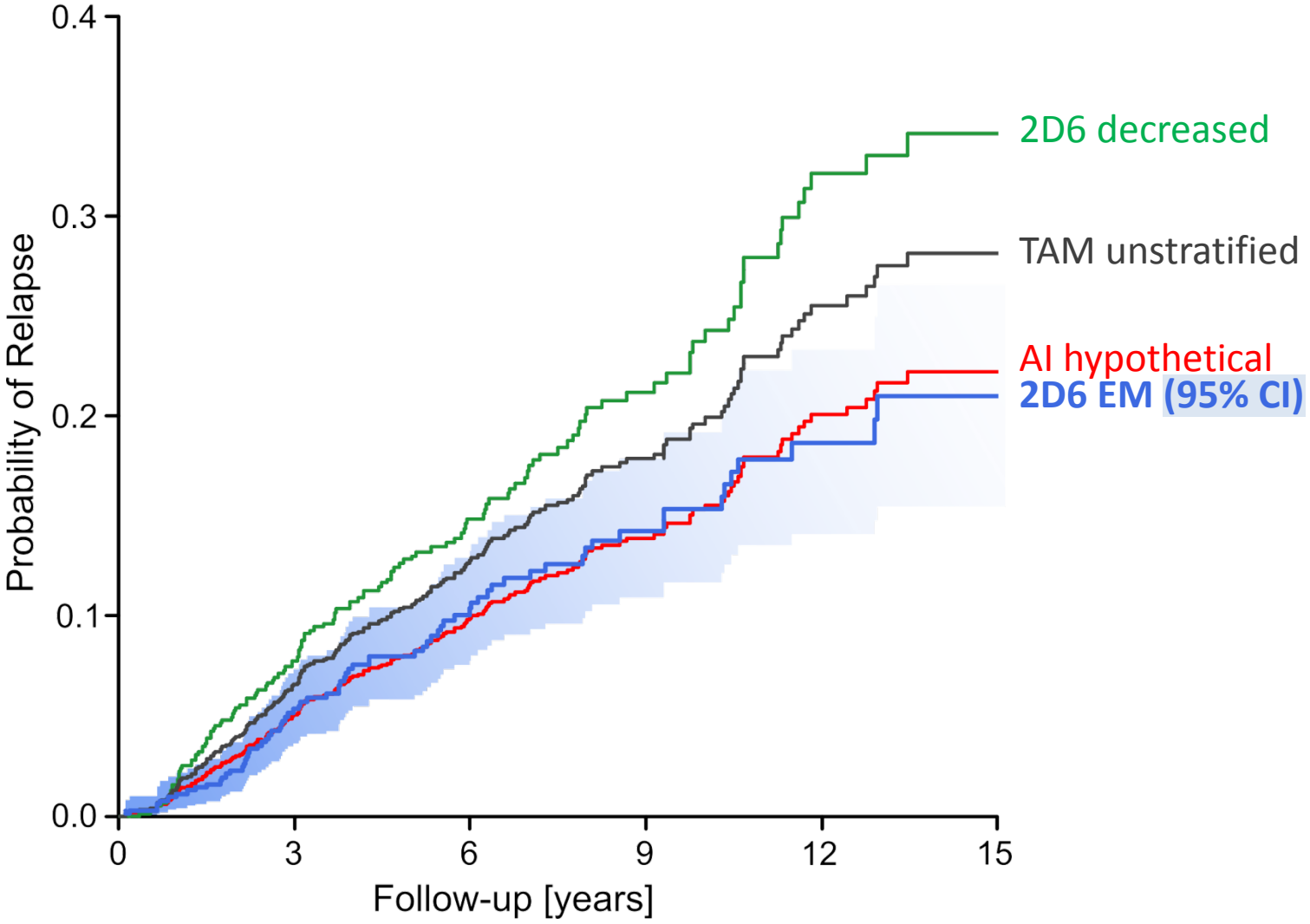
CYP2D6 polymorphism and recurrence probabilities upon tamoxifen treatment

Number of patients: 1325



Schroth et al. JAMA 302:1429-1436, 2009

The Tamoxifen benefit for patients with fully functional CYP2D6 is similar as that of AI



Schroth et al. JAMA 302:1429-1436, 2009

The Stuttgart – Mayo study cohort

Retrospective analysis

Total

- 1580 patients (German and US origin)

Inclusion criteria

- Histologically proven breast cancer
- No previous chemotherapy or endocrine treatment other than **adjuvant tamoxifen**
- No metastatic disease at diagnosis
- Steroid receptor positivity (ER^+ and/or PgR^+)

Pharmacogenetic analysis with 1361 patients meeting criteria

- median follow-up of 6.3 years
- 95.4% **postmenopausal**

Schroth et al. JAMA 302:1429-1436, 2009

What level of evidence is needed?

Simon RM, Paik S, Hayes DF J Natl Cancer Inst 101:1446-1452, 2009
Use of archived specimens in evaluation of prognostic and predictive markers

Studies with the following characteristics should have the potential to provide valid data:

- prospective clinical trials (gold standard, but costly)
- use of archived specimen (source of genetic material)
- sample size calculation
- determined subject eligibility
- marker cut point specification (phenotype definition)
- analytical plan
- confirmation

Highest level evidence was expected from prospective clinical trials

ATAC

Rae *et al.* **Lack of correlation between gene variants in tamoxifen metabolizing enzymes with primary endpoints** in the ATAC trial.
J Natl. Cancer Inst 104:452-460; 2012

BIG 1-98

Leyland-Jones *et al.* **Outcome according to CYP2D6 genotype among postmenopausal women with endocrine-responsive early invasive breast cancer** randomized in the BIG 1-98 Trial.
J Natl. Cancer Inst 104:441-451; 2012

No association

between CYP2D6 polymorphism and outcome

Characteristics of Tamoxifen-CYP2D6-pharmacogenetics studies and pitfalls

Study	Total number TAM	Subjects genotyped
Stuttgart Mayo	1580 (mono)	1325 (84%)
ATAC	3116 (+/- chemo)	588 (19%)
BIG1-98	2459 (mono)	1243 (48%)

Brauch et al. J Clin Oncol 31:176-180, 2013

Characteristics of Tamoxifen-CYP2D6-pharmacogenetics studies and pitfalls

Study	Total number TAM	Subjects genotyped	End-points	TAM dose [mg]	Sample size calc.	DNA source
Stuttgart Mayo	1580 (mono)	1325 (84%)	TTR, EFS, DFS	20 (88%) >20 (12%)	yes	PBMC (44.5 %) tumor section (55.5%)
ATAC	3116 (+/- chemo)	588 (19%)	TTDR	20	no	tumor section
BIG1-98	2459 (mono)	1243 (48%)	TTR	20	no	tumor core biopsy

Brauch et al. J Clin Oncol 31:176-180, 2013

Characteristics of Tamoxifen-CYP2D6-pharmacogenetics studies and pitfalls

Study	Total number TAM	Subjects genotyped	End-points	TAM dose [mg]	Sample size calc.	DNA source	Alleles genotyped	Violation of HWE for CYP2D6*4	Use of CYP2D6 inhibitors	TAM adherence
Stuttgart Mayo	1580 (mono)	1325 (84%)	TTR, EFS, DFS	20 (88%) >20 (12%)	yes	PBMC (44.5 %) tumor section (55.5%)	*3, *4, *5, *10, *41	no yes p=0.015	unkown	unknown
ATAC	3116 (+/- chemo)	588 (19%)	TTDR	20	no	tumor section	*3, *4, *6, *10, *41	yes* p=0.00002	provided	not provided
BIG1-98	2459 (mono)	1243 (48%)	TTR	20	no	tumor core biopsy	*3, *4, *6, *7, *10, *41, *17	yes* $p=2.5x10^{-92}$	not provided	not provided

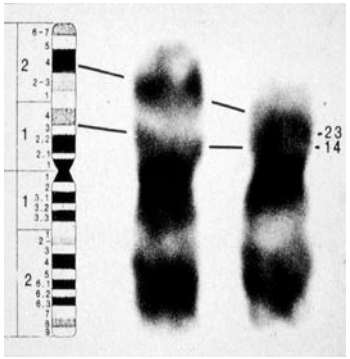
*Stanton V Jr.; JNCI 104: 1265-1266, 2012
Nakamura et al , JNCI 104:1264; 2012

Brauch et al. J Clin Oncol 31:176-180, 2013

Loss of heterozygosity (LOH) is a hallmark of many cancers

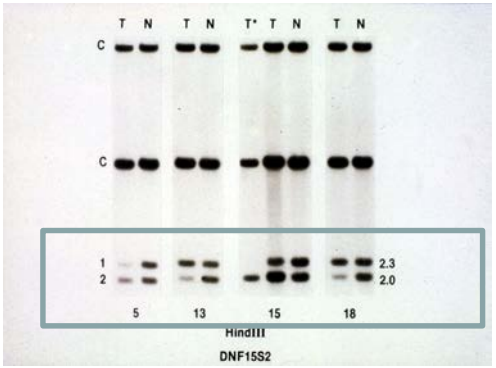
The 1980ies

Deletion



Chromosom 3p

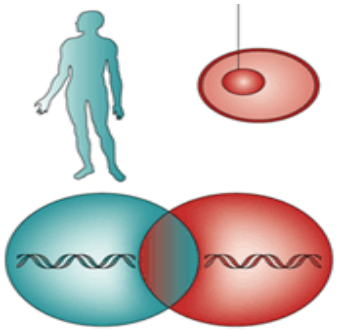
LOH



3p Locus

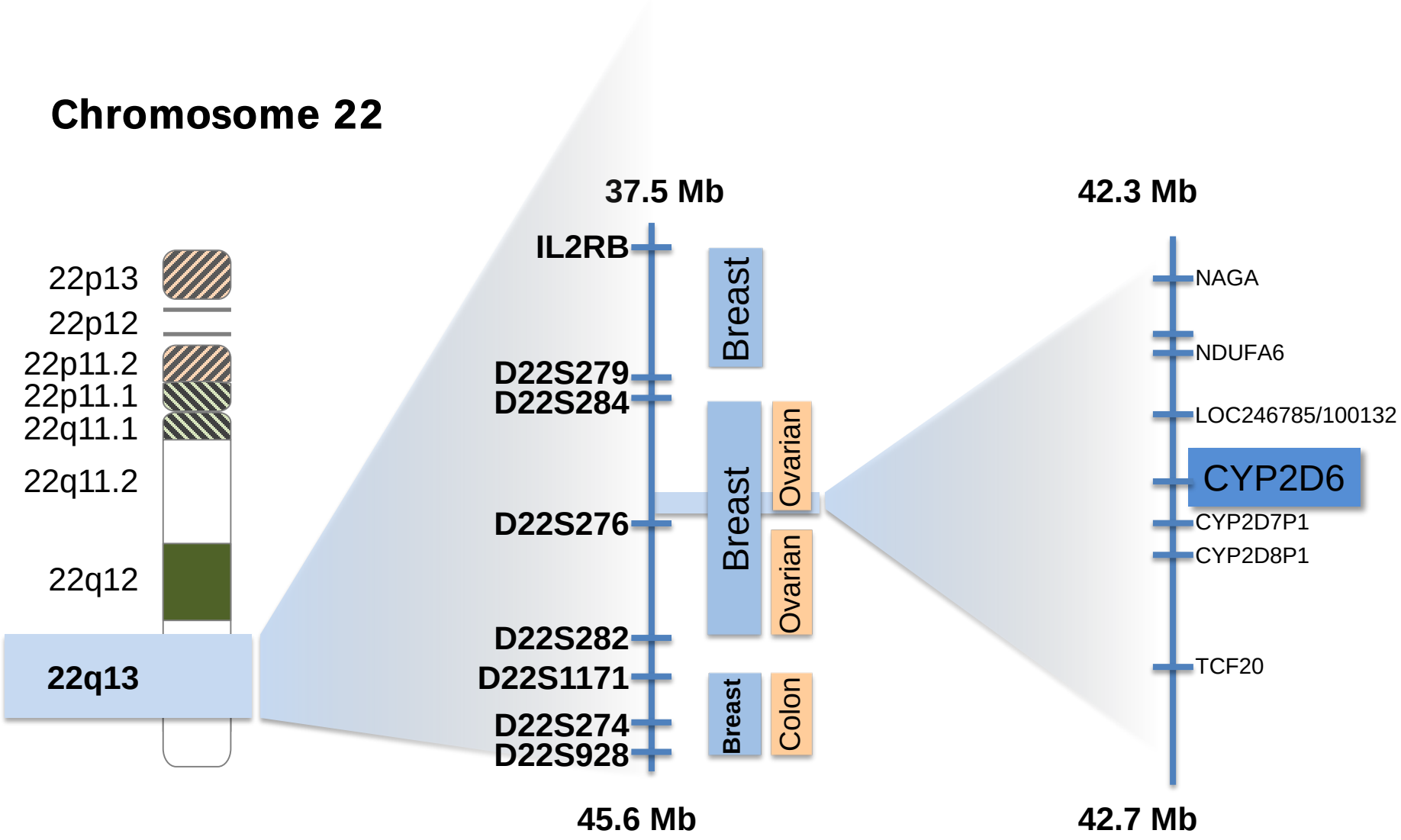
Wang-Peng et al. Cancer Genet Cytogenet 1982
Zbar et al Nature 1987
Brauch et al NEJM, 1987

PATIENT genome + **TUMOR genome**



Tumor tissue contains normal cells (stroma, infiltrating lymphocytes)

22q13 LOH is common in breast cancer



J Natl Cancer Inst. 2013 Sep4;105(17):1332-1334

BRIEF COMMUNICATION

Concordance Between *CYP2D6* Genotypes Obtained From Tumor-Derived and Germline DNA

James M. Rae, Meredith M. Regan, Jacklyn N. Thibert, Christina Gersch, Dafydd Thomas, Brian Leyland-Jones, Giuseppe Viale, Lajos Pusztai, Daniel F. Hayes, Todd Skaar, Catherine Van Poznak

CYP2D6 score from WBCs	From FFPETs							From FFPELNs						
		PM	IM	IM	IM	EM			PM	IM	IM	IM	EM	
	ND	0	0.5	1	1.5	2	Total	ND	0	0.5	1	1.5	2	Total
0	0	**4**	0	0	0	0	4	0	**3**	0	0	1	0	4
0.5	0	0	**7**	0	0	0	7	0	0	**7**	0	0	0	7
1	1	0	1	**42**	0	0	44	2	0	1	**41**	0	0	44
1.5	0	0	0	0	**15**	0	15	1	0	0	0	**14**	0	15
2	0	0	0	0	1	**51**	52	3	0	0	0	0	**49**	52
Total	1	4	8	42	16	51	122	6	3	8	41	15	49	122

CYP2D6 score

Concordance **119/121 98.3: [95%; CI 94.2-99.8%]** **114/117 97.4: [95% CI 92.7-99.5%]**

Agreement **K = 0.98 (95% CI: 0.94-1.00)** **K = 0.96 (95% CI: 0.92-1.00)**

CYP2D6 phenotype

Concordance **120/121 99.2: [95%; CI 95.5-1.0%]** **115/117 98.3: [95% CI 94.0-99.8%]**

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white blood cells / FFPE / FFPE of non affected lymphnodes

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CYP2D6 score from WBCs	From FFPETs							From FFPELNs						
	ND	PM 0	IM 0.5	IM 1	IM 1.5	EM 2	Total	ND	PM 0	IM 0.5	IM 1	IM 1.5	EM 2	Total
0	0	**4**	0	0	0	0	4	0	**3**	0	0	1	0	4
0.5	0	0	**7**	0	0	0	7	0	0	**7**	0	0	0	7
1	1	0	1	**42**	0	0	44	2	0	1	**41**	0	0	44
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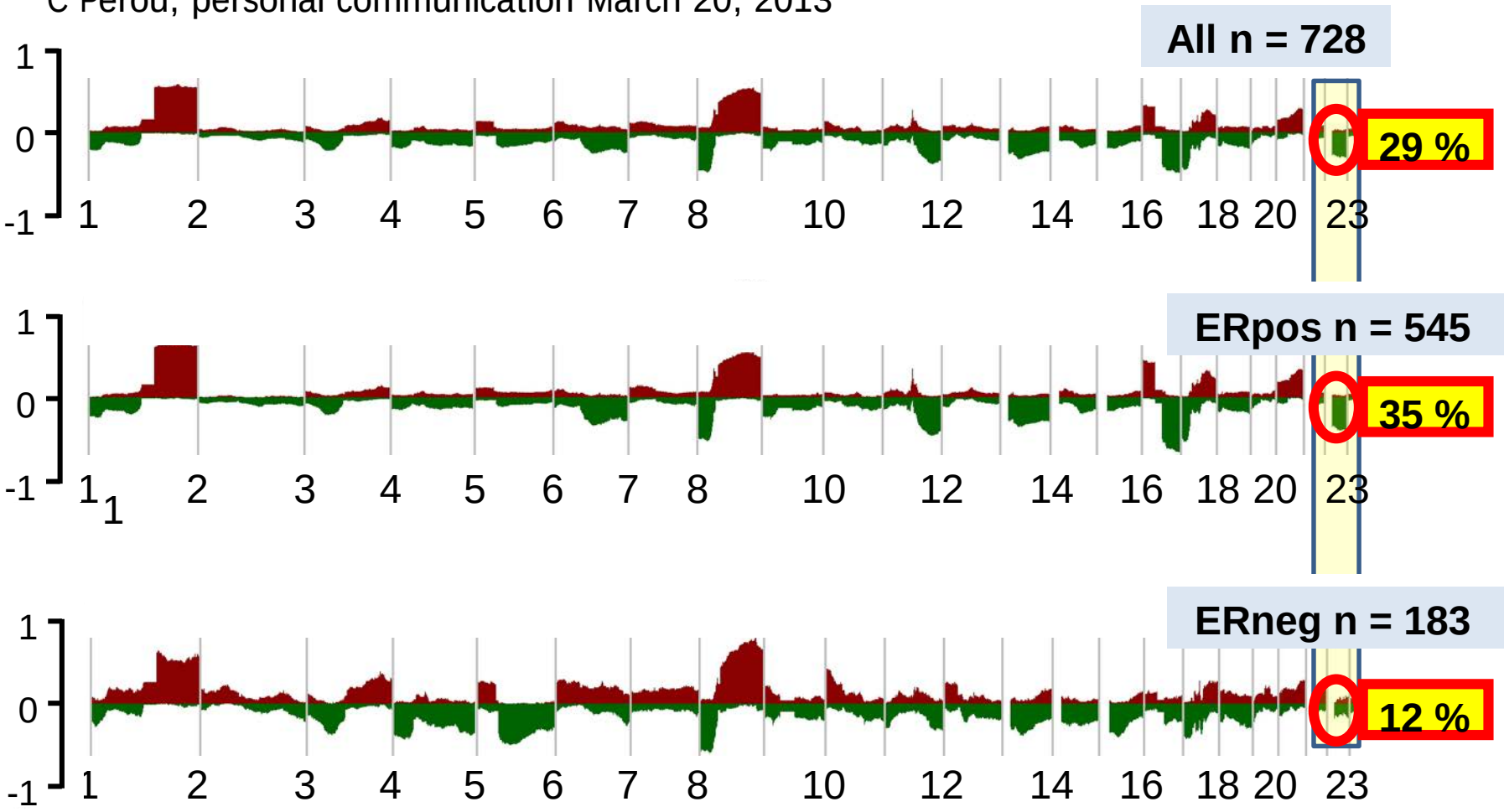
K = 0.97 (95% CI: 0.92-1.00)

white blood cells / FFPE / FFPE of non affected lymphnodes

The Cancer Genome Atlas (TCGA) Breast Data

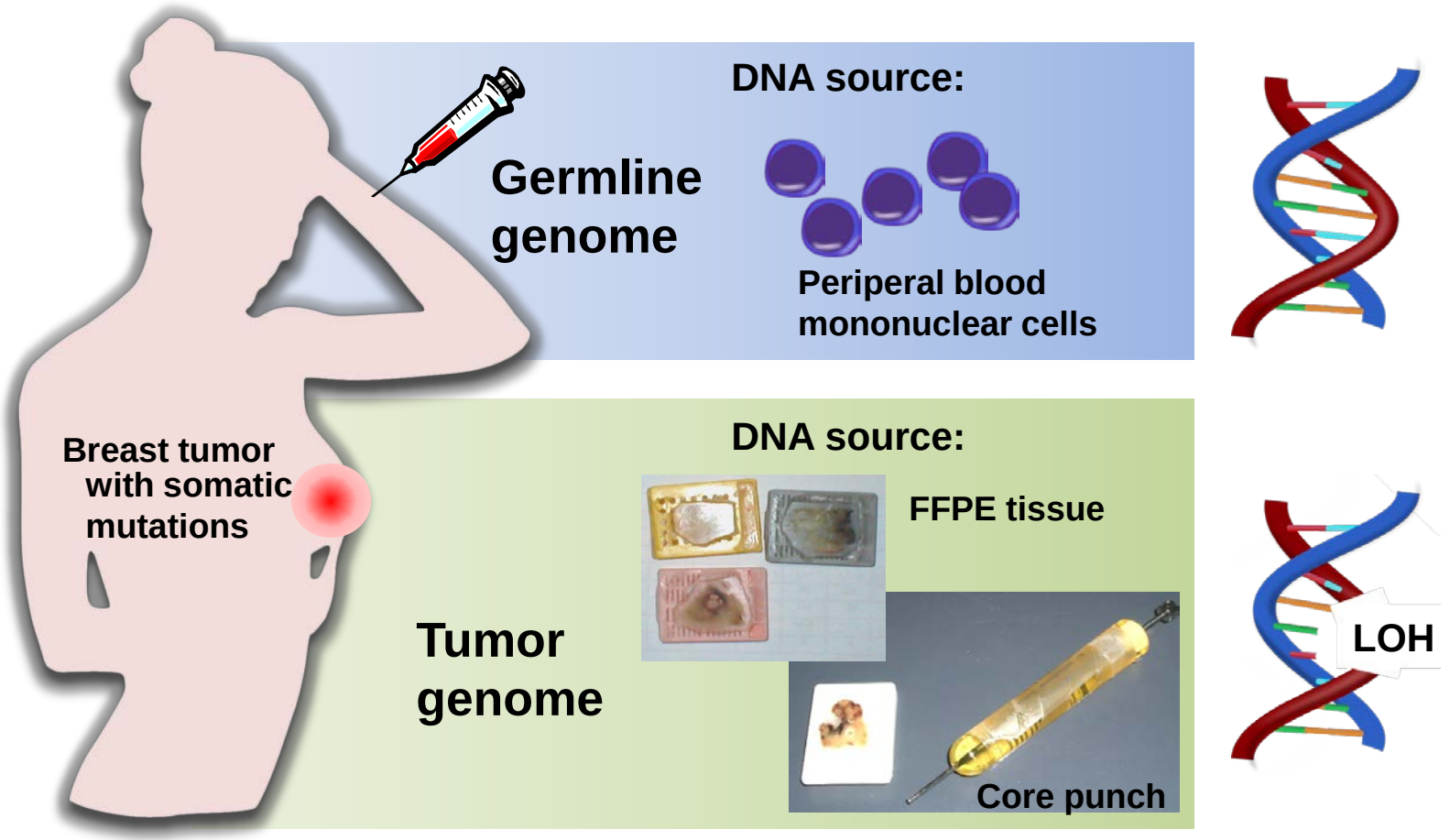
CYP2D6 Chr. 22: 42,522,501-42,525,911
Switchplots indicating CYP2D6 LOH in breast tumor

C Perou, personal communication March 20, 2013



Brauch & Schwab, Br J Clin Pharmacol 77: 695-703, 2013

Attention must be paid when genomic DNA is isolated for pharmacogenetic investigations



Brauch & Schwab, Br J Clin Pharmacol 77: 695-703, 2013

Austrian ABCSG 8 Trial: Reduced CYP2D6 metabolism and higher risk for recurrence

Goetz et al *Clin Cancer Res*. **2013;** 19: 500 – 507

	Tamoxifen only (Arm A)		**Tamoxifen followed by anastrozole (Arm B)**	
	112 cases		**102 cases**	
	OR (95% CI)	***P***	**OR (95% CI)**	***P***
PM/PM relative to EM/EM	2.45 (1.05 - 5.73)	0.04	0.60 (0.15 - 2.37)	0.47
EM/PM and PM/IM relative to EM/EM	1.67 (0.95 - 2.93)	0.07	0.76 (0.43 - 1.31)	0.32
EM/IM and IM/IM relative to EM/EM	1.23 (0.58 - 2.61)	0.60	1.02 (0.52 - 2.01)	0.96

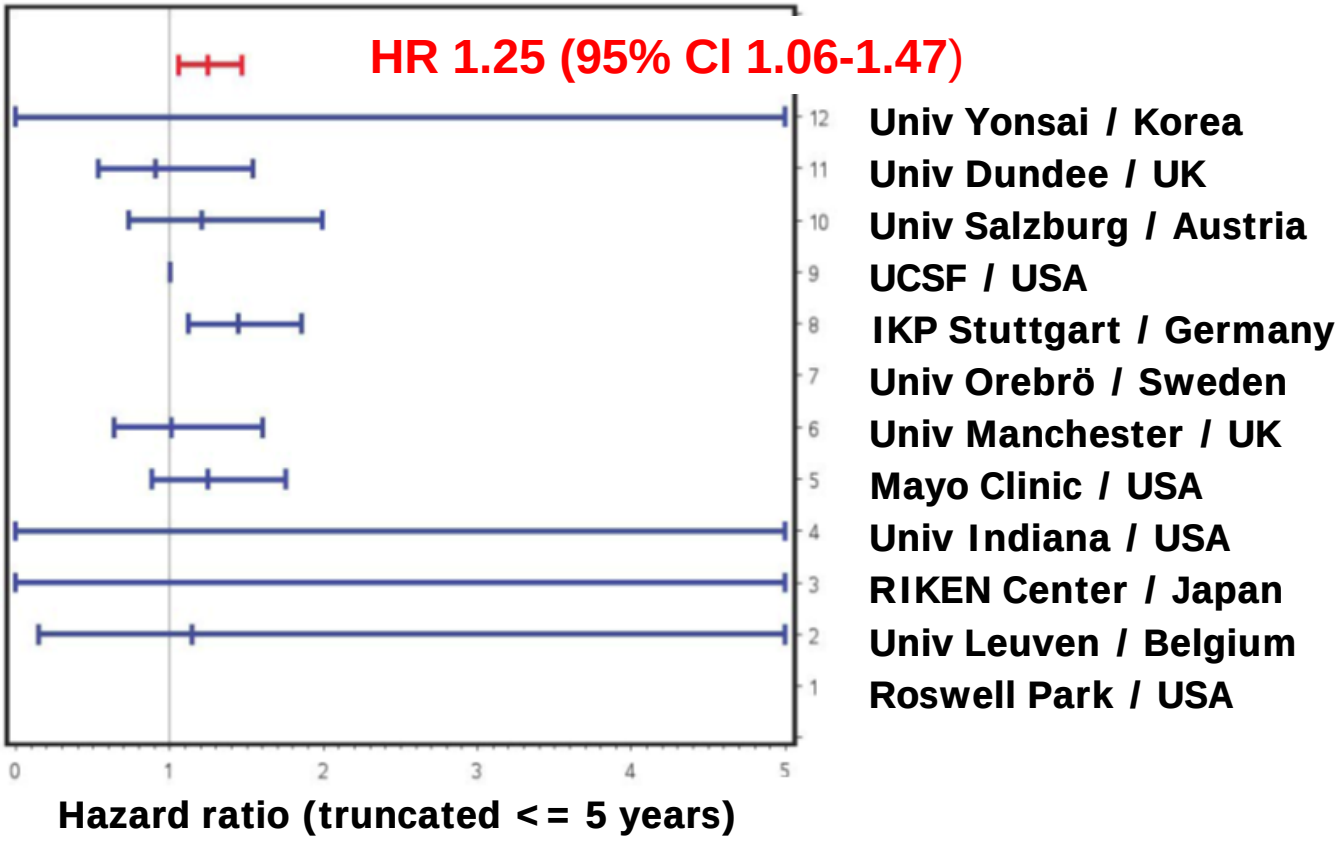
Case-control study: 5 year treatment

CYP2D6 genotype is associated with a higher risk of recurrence

International Tamoxifen Pharmacogenetics Consortium (ITPC)
Province et al ***Clin Pharmacol Ther*** 95:216-27, 2014

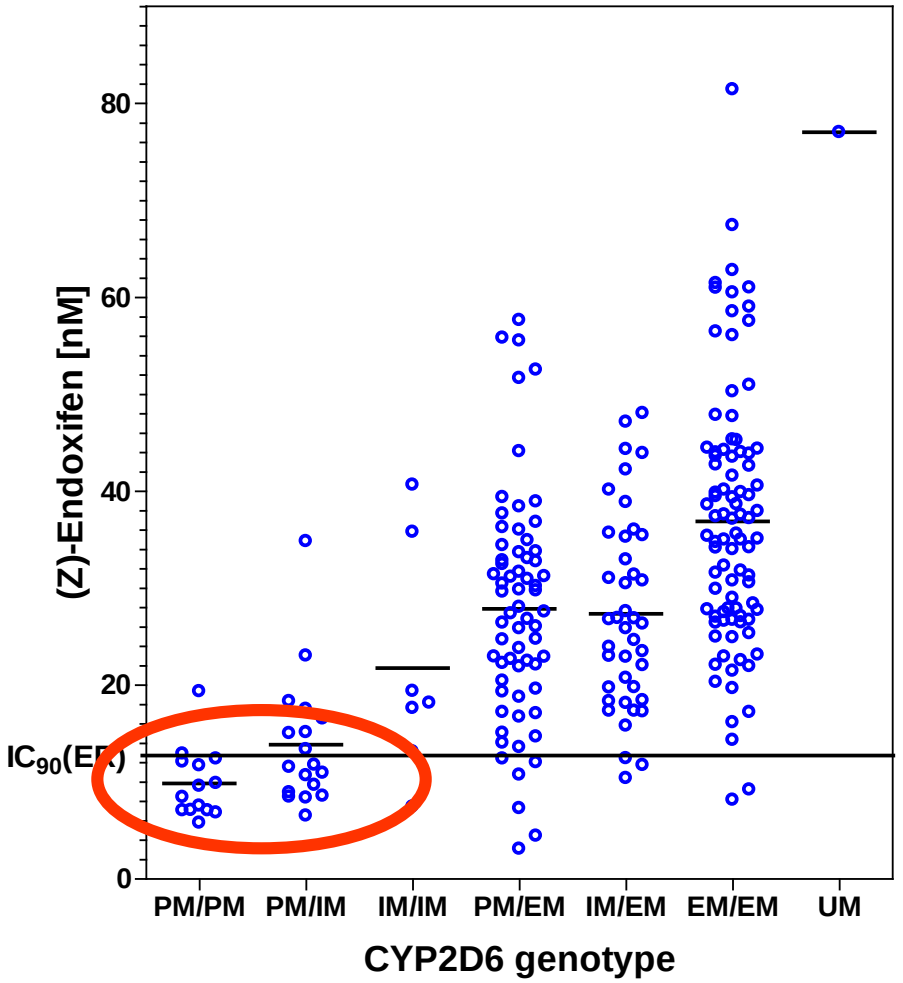
Metaanalysis of 1996 patients

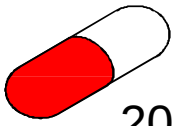
Invasive Disease-free Survival



Variability of the (Z)-endoxifen plasma levels

N=236



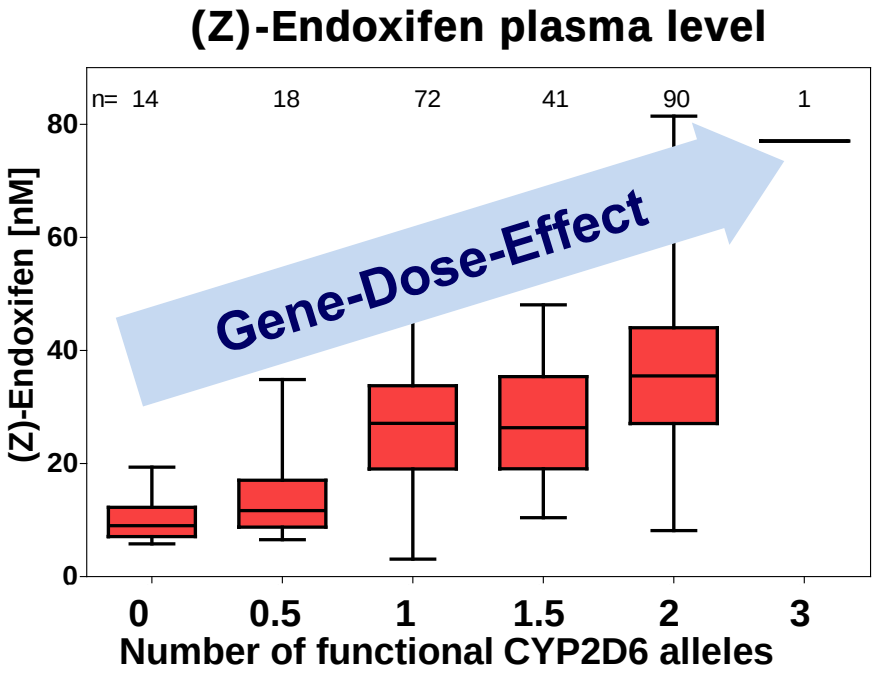


20 mg/day

IC_{90} Endoxifen

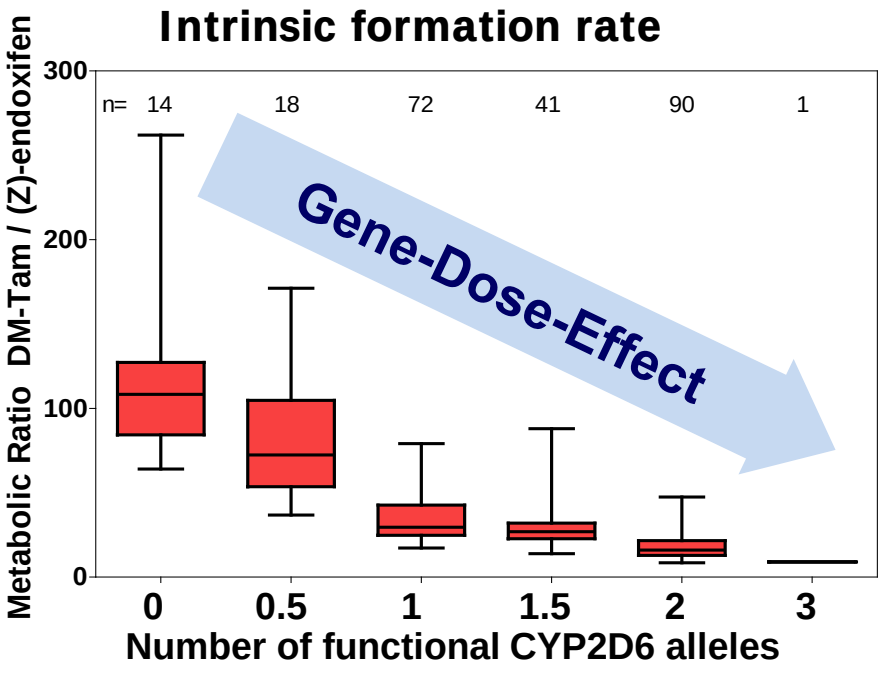
Mürdter et al. Clin Pharmacol Ther 89:708-717, 2011

CYP2D6 and (Z)-endoxifen formation (N=236)



$P < 10^{-16}$

~40 % of variability explained by CYP2D6 genotype



$P < 10^{-16}$

~68 % of variability explained by CYP2D6 genotype

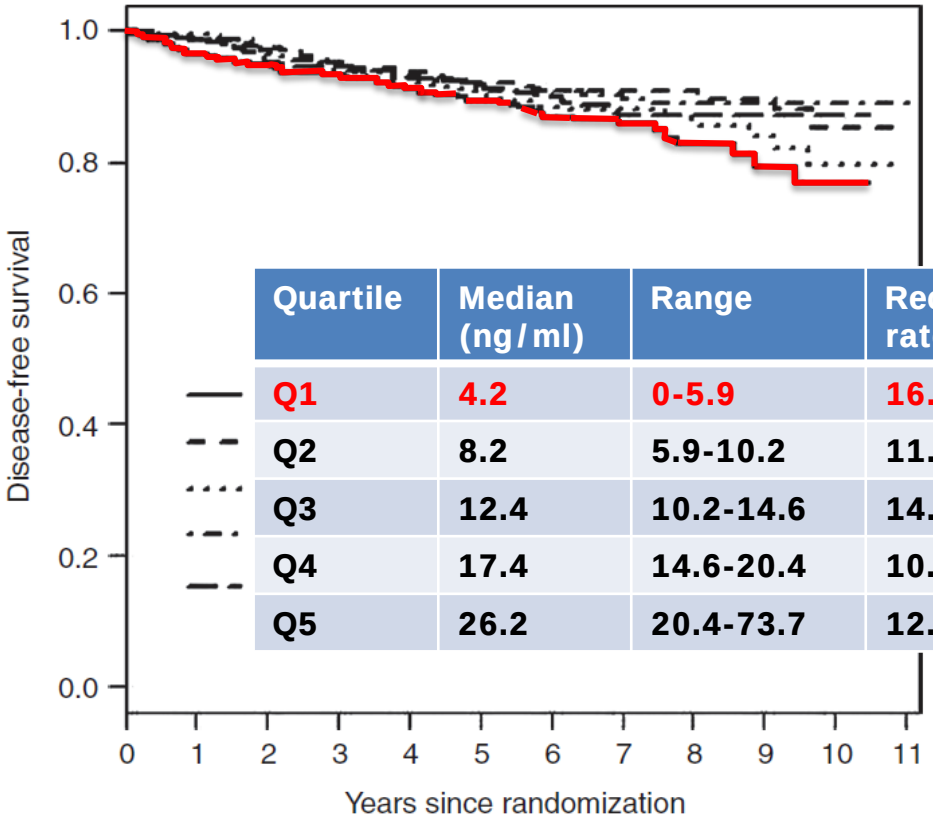
PM:	*3, *4, *5, *6, *7, *8	: 0
IM:	*9,*10, *41	: 0.5
EM:	*1, *2	: 1
UM:	2x*1, 2x*2	: 2

Mürdter et al. Clin Pharmacol Ther 89:708-717, 2011

Breast cancer outcomes in the WHEL study

Madlensky et al. *Clin Pharmacol Ther* 89:718-25, 2011

N = 1,370 patients



Quartile	Median (ng/ml)	Range	Recurrence rate (%)
Q1	4.2	0-5.9	16.0
Q2	8.2	5.9-10.2	11.7
Q3	12.4	10.2-14.6	14.7
Q4	17.4	14.6-20.4	10.1
Q5	26.2	20.4-73.7	12.4

Q1: Women with low endoxifen concentrations are at higher risk to develop recurrences

HR 0.74
95% CI 0.55-1.00

Volume 31 Number 12 January 10 2013

JOURNAL OF CLINICAL ONCOLOGY

COMMENTS AND CONTROVERSIES

Tamoxifen Use in Postmenopausal Breast Cancer: CYP2D6 Matters

Hiltrud Brauch and Werner Schroth, *Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart; and University Tübingen, Tübingen, Germany*
Matthew P. Goetz, *Mayo Clinic, Rochester, MN*
Thomas E. Mürdter and Stefan Winter, *Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart; and University Tübingen, Tübingen, Germany*
James N. Ingle, *Mayo Clinic, Rochester, MN*
Matthias Schwab, *Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart; University Tübingen; and Institute of Experimental and Clinical Pharmacology and Toxicology, University Hospital Tübingen, Tübingen, Germany*
Michel Eichelbaum, *Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart; and University Tübingen, Tübingen, Germany*

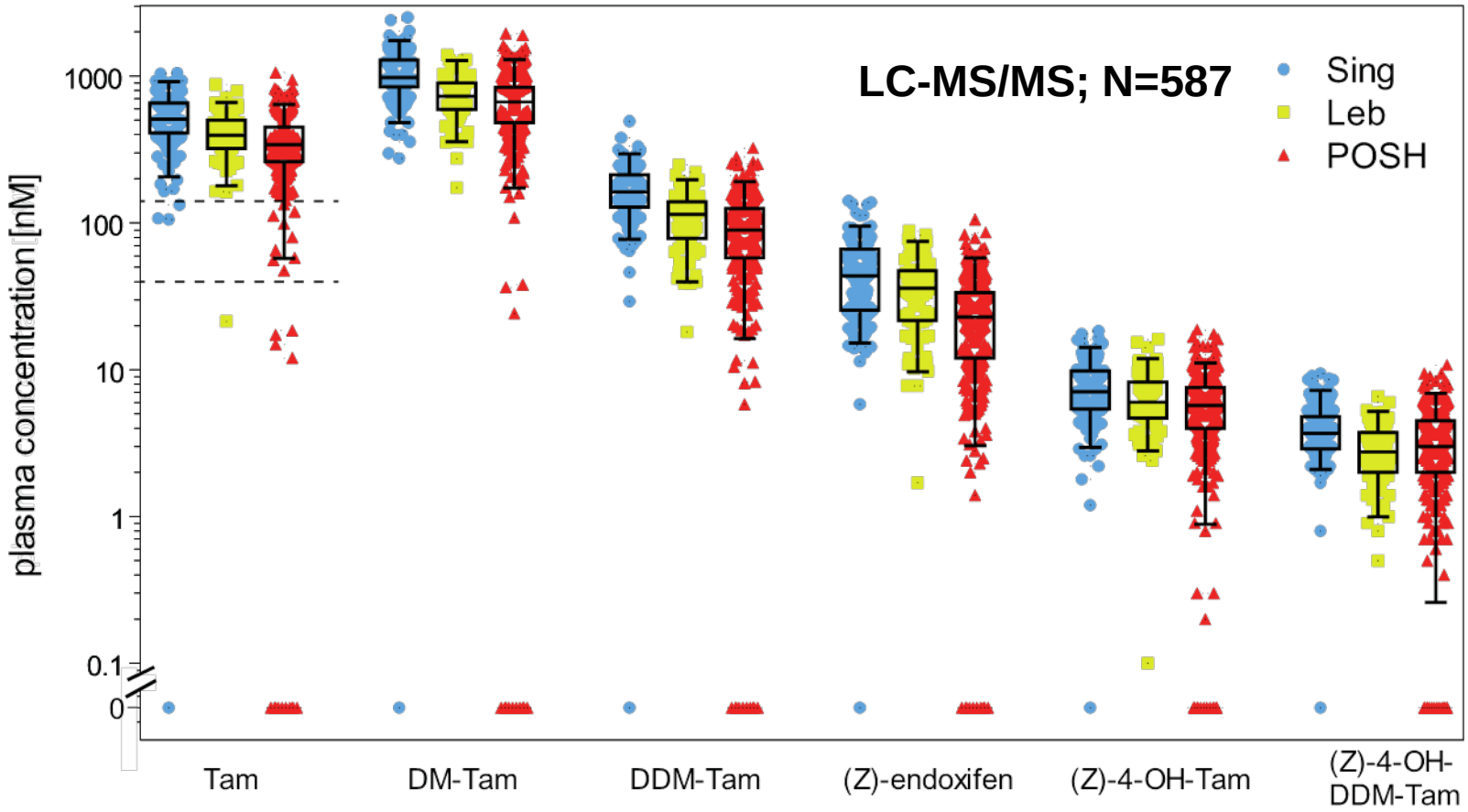
Modeling the pharmacological importance of endoxifen for the treatment of ER positive breast cancer in premenopausal women

Maximov *et al.* AACR 2013, Washington DC

Long term adjuvant tamoxifen therapy for five years is the antiestrogenic standard of care for ER positive breast cancer in premenopausal patients. The metabolic activation of tamoxifen by CYP2D6 to endoxifen remains controversial to plan the treatment of patients with breast cancer. However, all retrospective studies focus entirely on postmenopausal patients and no studies have been undertaken in the relevant premenopausal treatment population. We have addressed the issue of the pharmacological importance of endoxifen to control the estrogen-stimulated growth of four ER positive breast cancer cell lines. We have modeled the actual estrogen environment in the laboratory (estradiol plus estrone) based on previous data from premenopausal patients taking tamoxifen [1]. Our strategy was to evaluate the anti-proliferative actions of actual concentrations of tamoxifen, N-desmethyltamoxifen and 4-hydroxytamoxifen combined, based on the actual measurements reported for those metabolites in patients who were extensive metabolizers (EM), intermediate metabolizers (IM) or poor metabolizers (PM) [2]. The results demonstrate the essential requirement with the appropriate concentration of endoxifen necessary to block estrogen-mediated cell replication.

The premenopausal situation

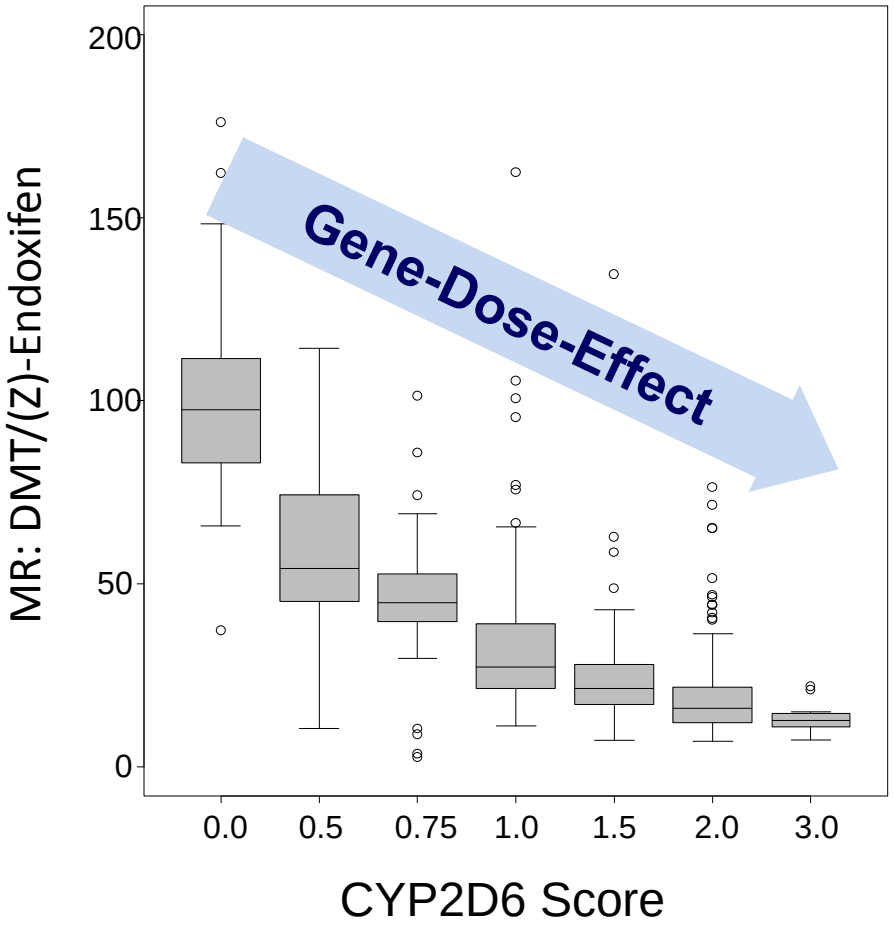
Tamoxifen metabolite levels are similar between ethnic groups



Network for the investigation of Tamoxifen Outcome in Premenopausal Breast Cancer

The premenopausal situation

Endoxifen concentration depends on CYP2D6 activity score



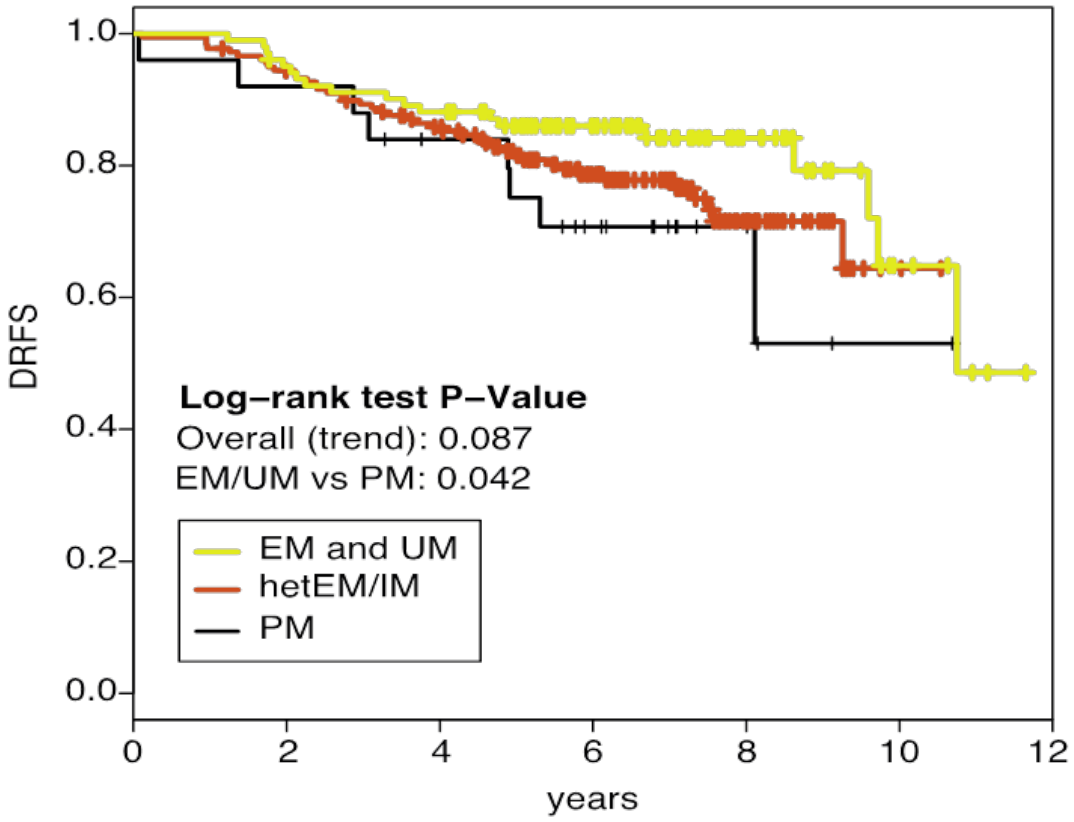
N=548
median age: 39.1 yrs
[range 22-59 yrs]

R^2=53%; P < 10^{-77}

Network for the investigation of Tamoxifen Outcome in Premenopausal Breast Cancer

CYP2D6 predicts outcome in POSH

Prospective Study of Outcomes in Sporadic versus Hereditary Breast Cancer



women diagnosed with invasive breast cancer **aged 40 years or younger at diagnosis**

women **aged 41-50 years with *BRCA1* or *BRCA2* mutation** diagnosed with invasive cancer

N = 306

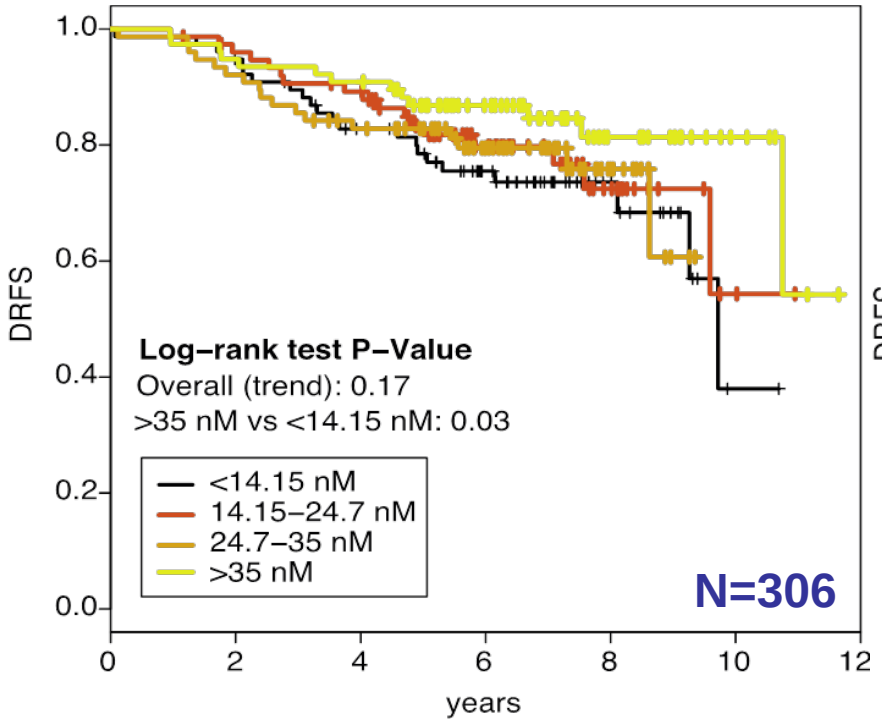
The Premenopausal Breast Cancer Study Group

Endoxifen predicts outcome in POSH

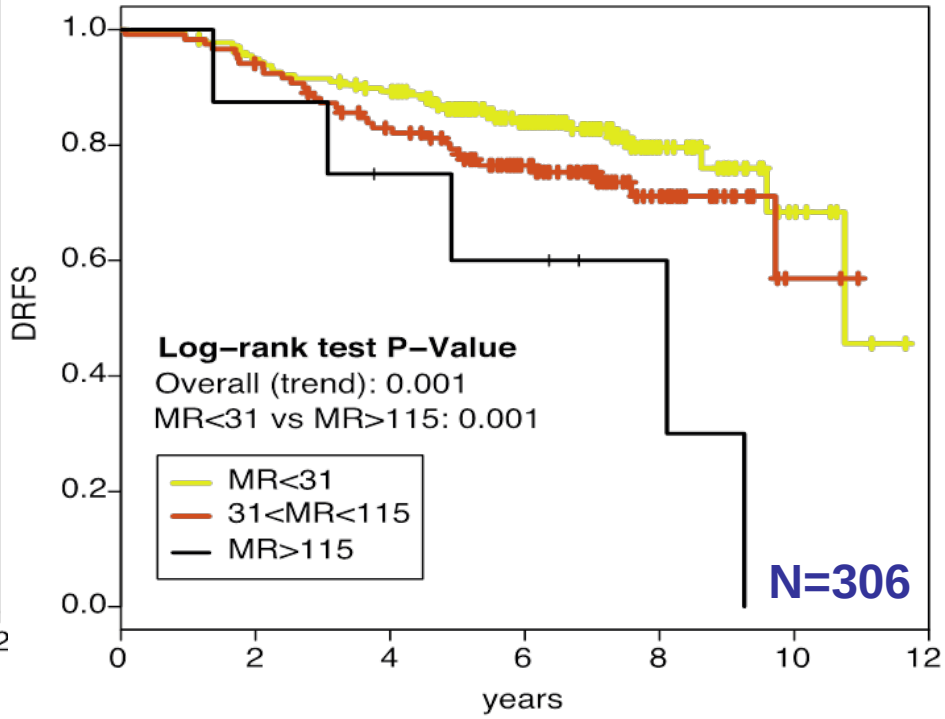
Prospective Study of Outcomes in Sporadic versus Hereditary Breast Cancer

Patients aged 40 years or younger at diagnosis & *BRCA* carriers

Endoxifen



MR: DMT/(Z)-Endoxifen



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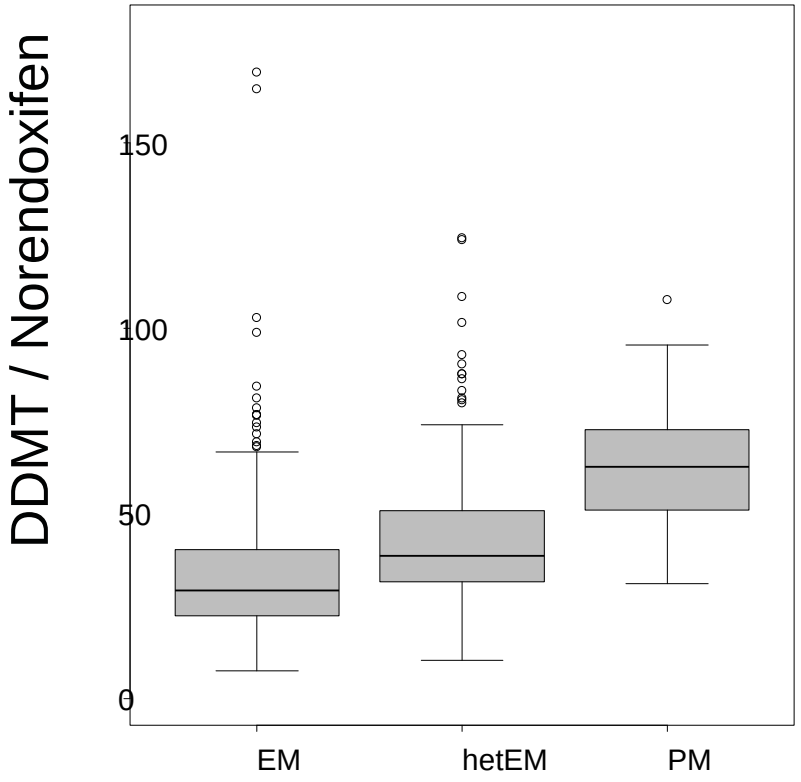
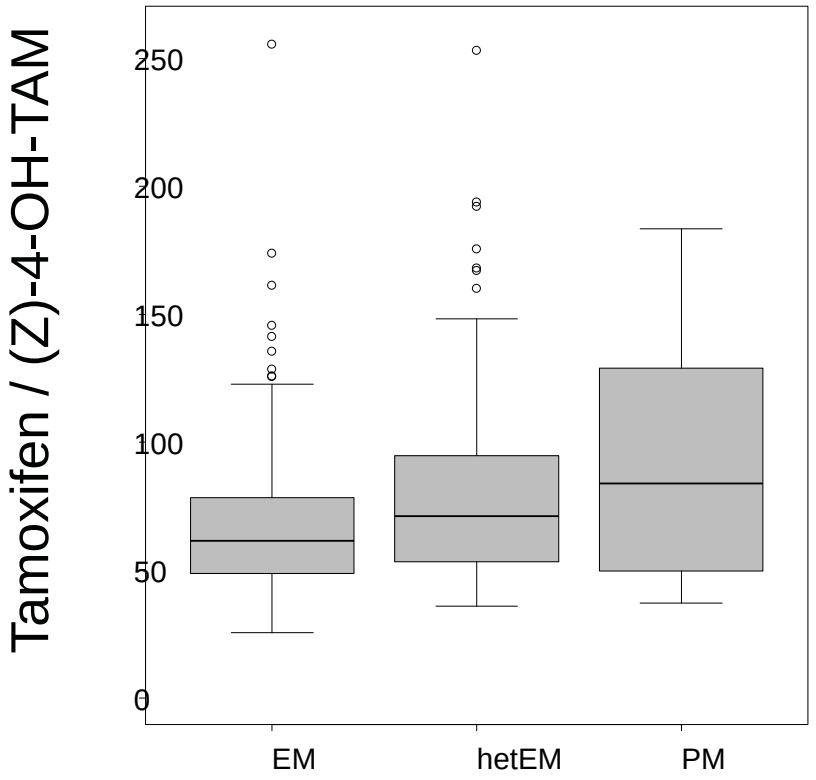
Impact of CYP2C19 and CYP2C9 on tamoxifen metabolite ratios



CYP2C19 phenotype
(loss of function alleles *2/*3)



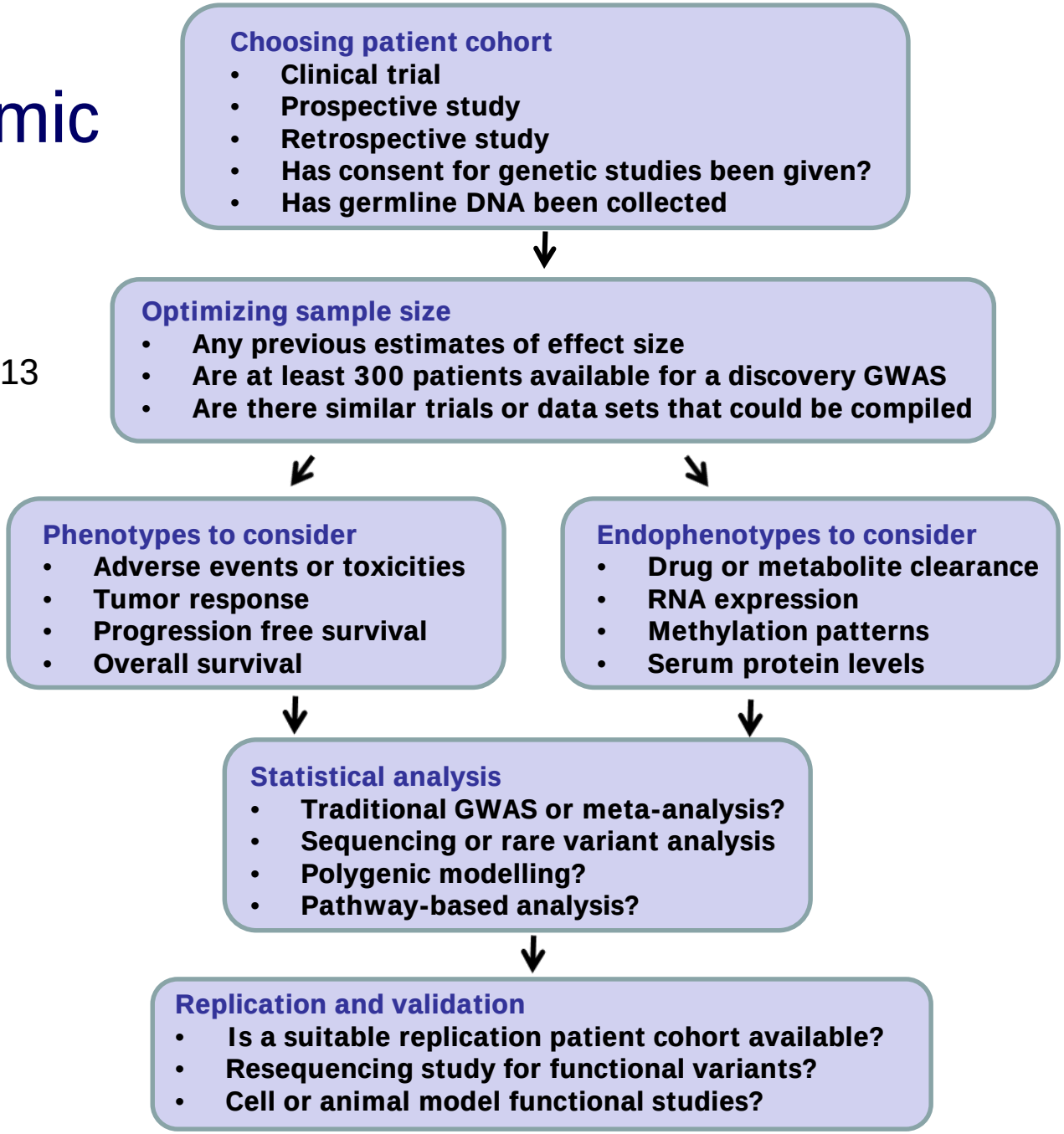
CYP2C9 phenotype
(reduced activity alleles *2/*3)

Tamoxifen Pharmacogenetics

- **Postmenopausal patients**: CYP2D6 predicts outcome
- The mechanism of tamoxifen metabolism is the same in pre- and postmenopausal women
- **Premenopausal patients**: first evidence that endoxifen concentrations and CYP2D6 activity predict outcome
- Regular and continuous drug intake is necessary for treatment success (no pain no gain)
- CYP2D6 inhibitors must be avoided
  ASCO guidelines July 2010 (JCO)

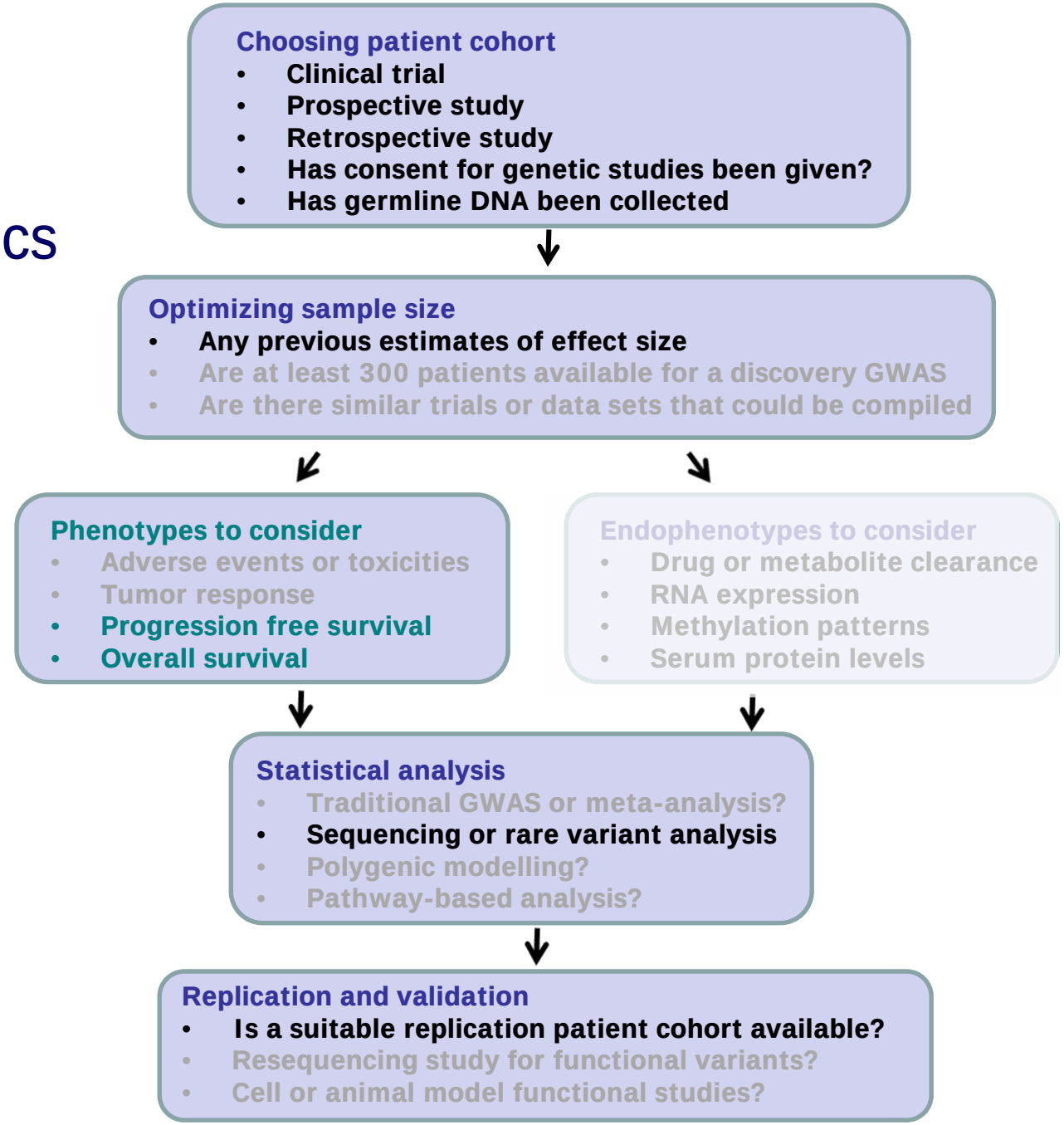
Cancer Pharmacogenomic Study Design

Wheeler H *et al*
Nat Rev Genet 14:23-34; 2013

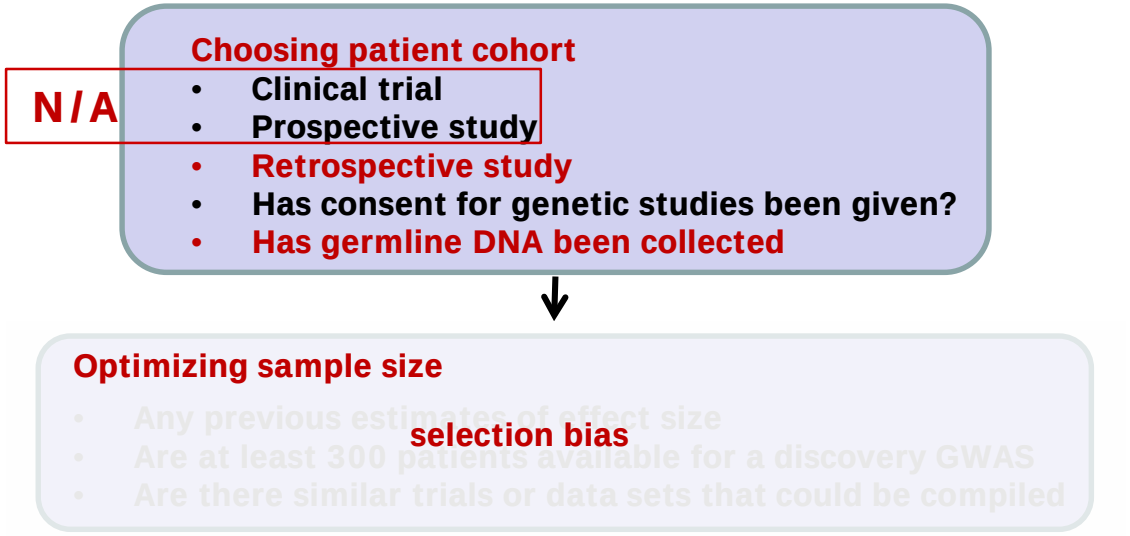


Lessons learned from pitfalls in tamoxifen pharmacogenomics

CYP2D6 allele coverage sufficient



Lessons learned from pitfalls in tamoxifen pharmacogenomics



Lessons learned from pitfalls in tamoxifen pharmacogenomics

Choosing patient cohort
- Clinical trial
- Prospective study
- Retrospective study
- Has consent for genetic studies been given?
- Has germline DNA been collected

N/A

Optimizing sample size

selection bias
- Any previous estimates of effect size
- Are at least 300 patients available for a discovery GWAS
- Are there similar trials or data sets that could be compiled

Phenotypes to consider
- Adverse events or toxicities
- Tumor response
- Progression free survival
- Overall survival

Endophenotypes to consider
- Drug or metabolite clearance
- RNA expression
- Methylation patterns
- Serum protein levels

Statistical analysis

Lack of quality control
- Traditional GWAS or meta-analysis?
- Sequencing data analysis
- Polygenic modelling?
- Pathway-based analysis?

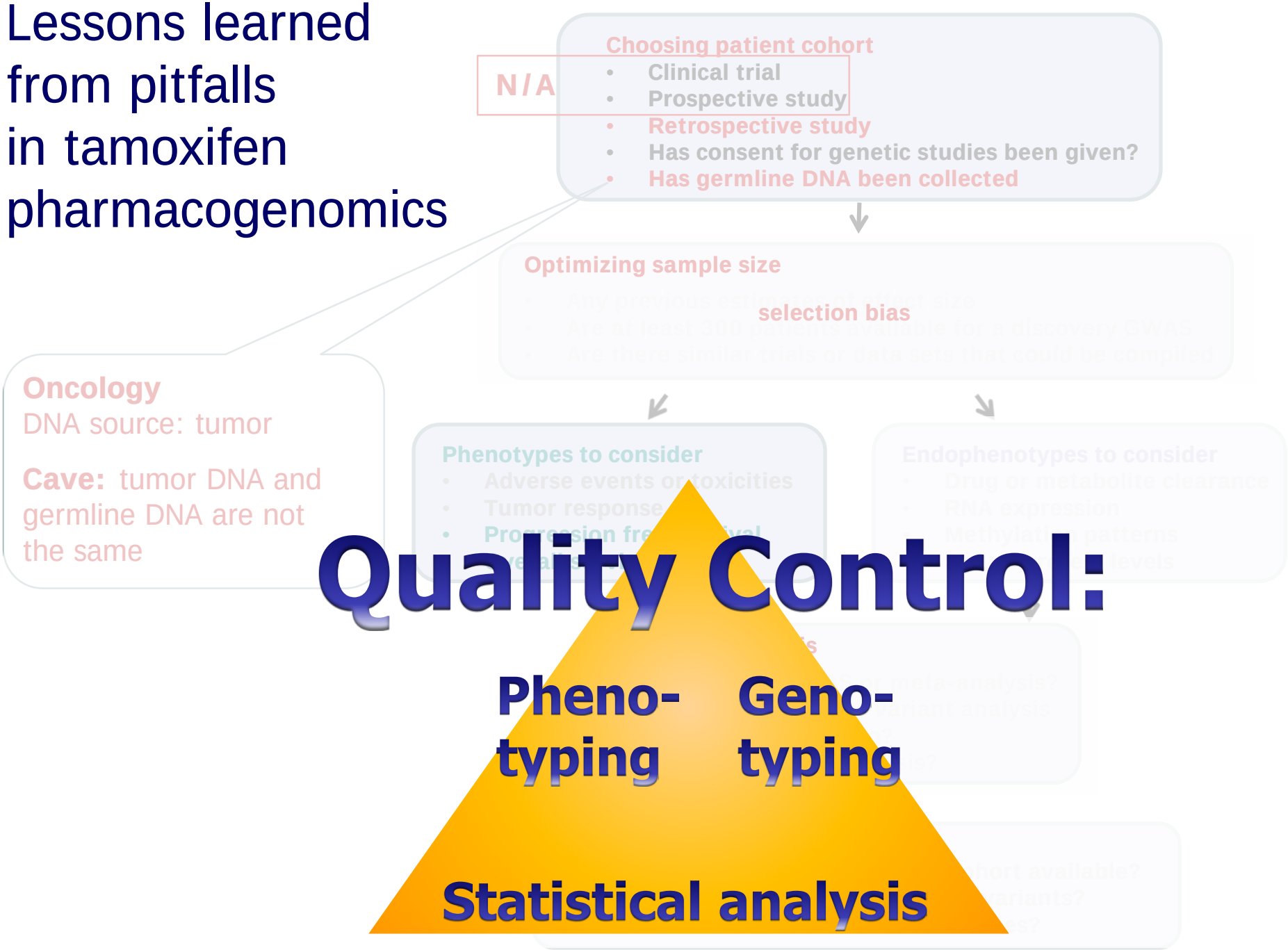
Replication and validation

invalid analysis
- Is a suitable replication patient cohort available?
- Resequencing study for functional variants?
- Cell or animal model functional studies?

Oncology
DNA source: tumor

Cave: tumor DNA and germline DNA are not the same

Lessons learned from pitfalls in tamoxifen pharmacogenomics



Thank you

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SEVENTH FRAMEWORK PROGRAMME

MARIE CURIE ACTIONS

Fighting Drug Failure!