PHARMACOGENETICS OF BREAST CANCER

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Outlines

- Breast cancer therapeutic situation
- Pharmacogenetics of antiestrogen therapy
  - Tamoxifen
- Pharmacogenetics of cytotoxic agents
  - Cyclophosphamide
  - 5-FU
  - Paclitaxel
- Trastuzumab pharmacogenetics
- Summary

Breast Cancer Facts

- The most common type of cancer and leading cause of cancer death in American women
- Two thirds of invasive breast cancer cases are ER-positive

Gonzales-Angulo et al 2007; Nicholson et al. 2003

BREAST CANCER: Statistics

Estimated New Breast Cancer Cases and Deaths Year 2006			
	Female	Male	Both
Estimated New Cases	212,920	1,720	214,640
Estimated Deaths	40,970	460	41,430

Jemal A, et al. 2006

Treatment Options

- Antihormonal treatment

  - Tamoxifen, aromatase inhibitors, fulvestrant

- Chemotherapy

  - Mono- poli-chemotherapy

  - Different schemes and schedules (CMF, taxanes, anthracyclines)

- Monoclonal antibodies

  - Herceptin

- Other transuctional pathways inhibitors

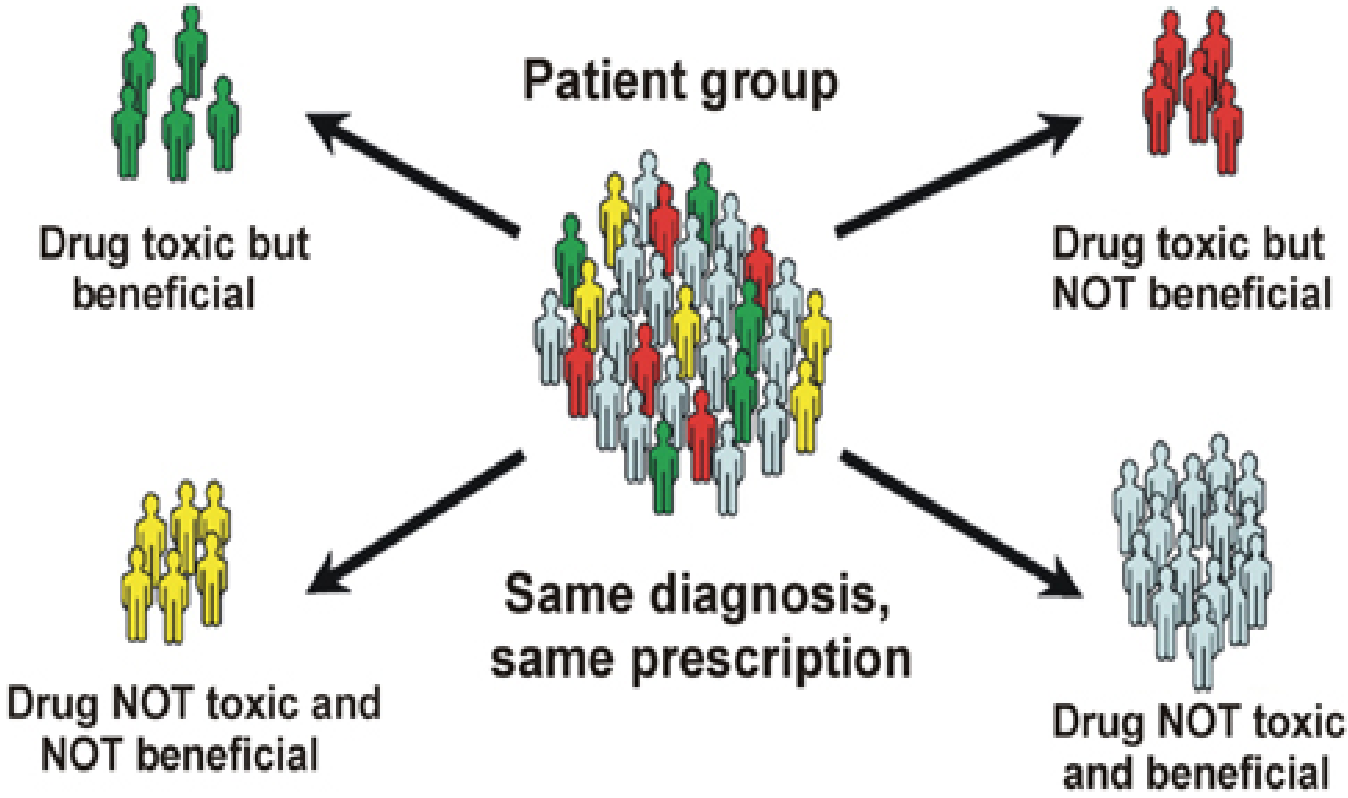
  tyrosine kinase inhibitors, mTOR inhibitors, VEGF inhibitors

How do we make treatment decisions currently?

- Tumor stage, lymph nodes involvement
- Patient's prior treatment history
- Concomitant illness
- General activity of the drugs and regimens
- ER status
- HER status

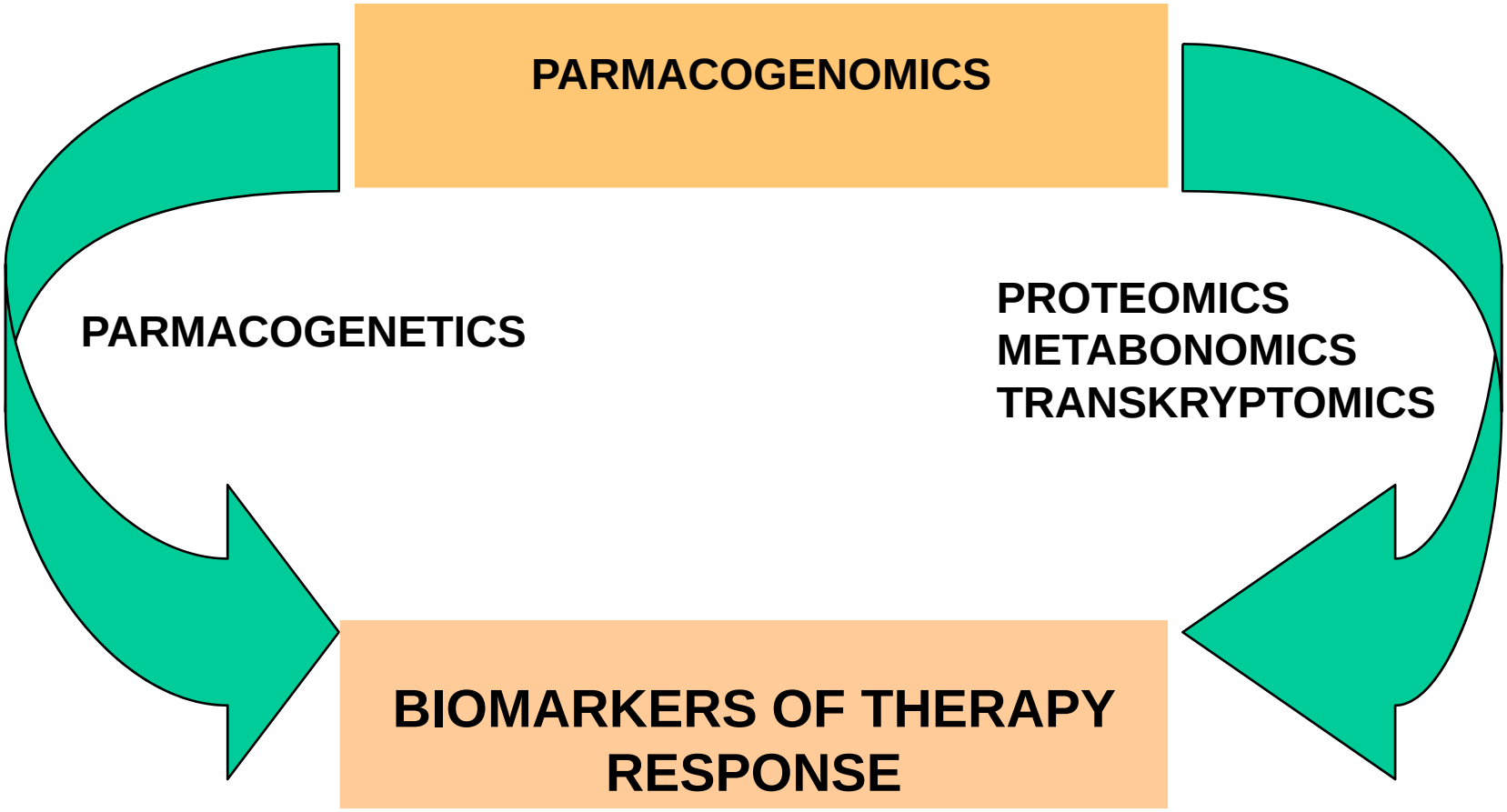
Selection of a particular treatment is not based on the molecular characteristic of the tumor.

Therapeutic Situation



Response Rates

- Up to 65% of breast cancer that initially respond to tamoxifen (TAM) acquire resistance during treatment
- CMF chemotherapy yield a 10-year disease-free survival rate of 28%
- Anthracycline based chemotherapy improves survival rate by 38% in pre- and 20% in post-menopausal patients


PARMACOGENOMICS
PARMACOGENETICS
PROTEOMICS
METABONOMICS
TRANSKRYPTOMICS
BIOMARKERS OF THERAPY RESPONSE

What is an SNP?

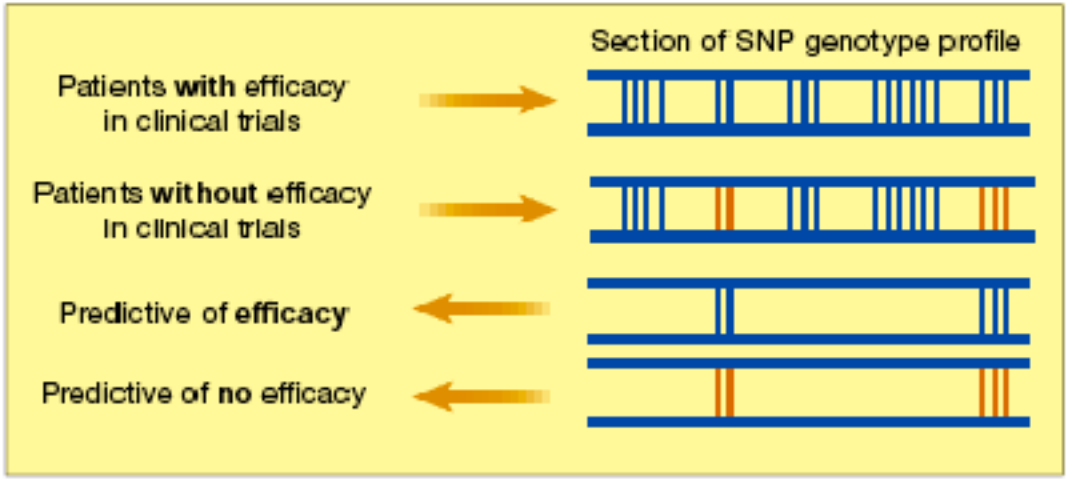
Different people can have a different nucleotide or base at a given location on a chromosome

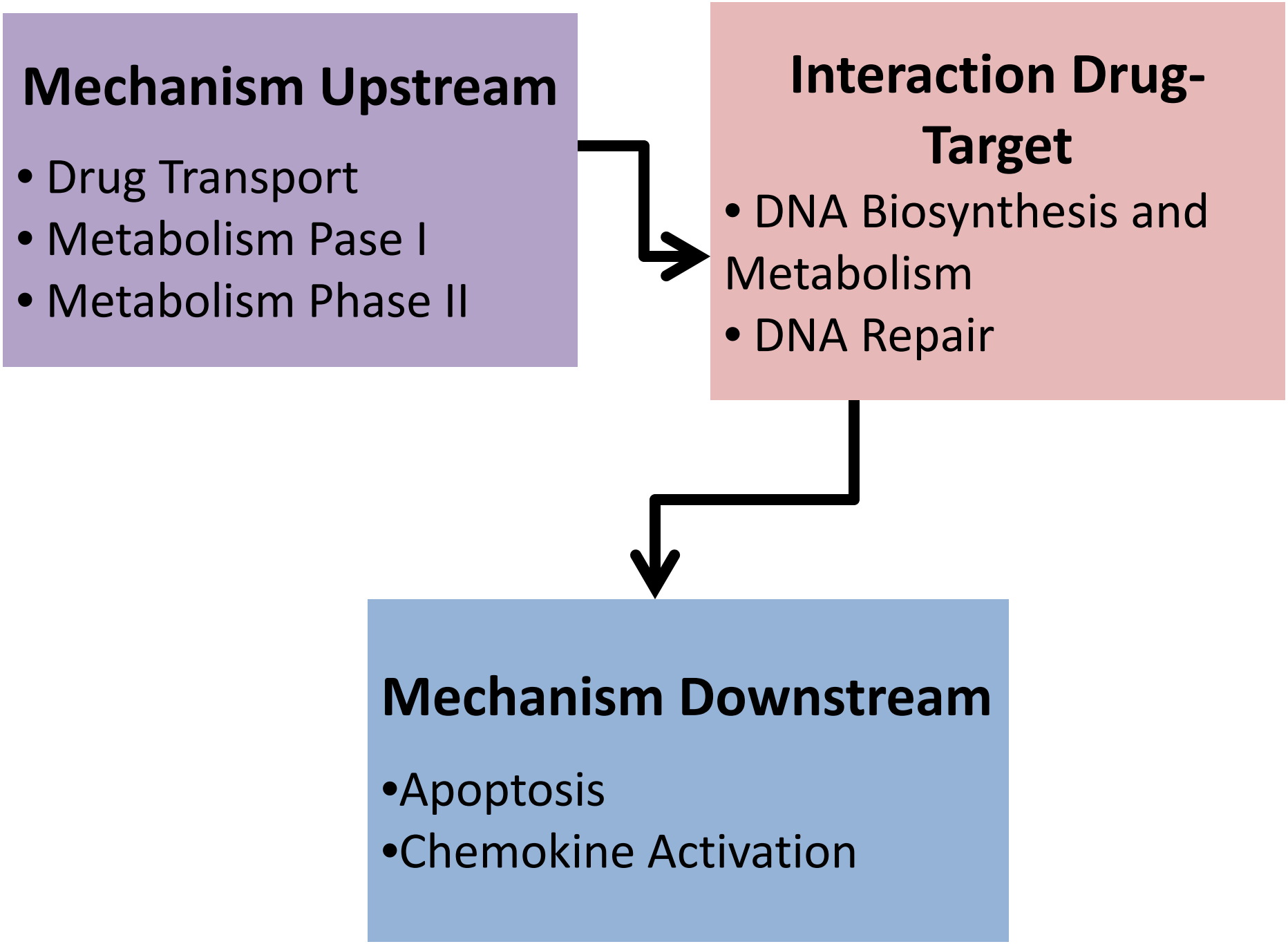
. . . G G **T** A A C T G . . .

. . . G G **C** A A C T G . . .

What is an SNP map?

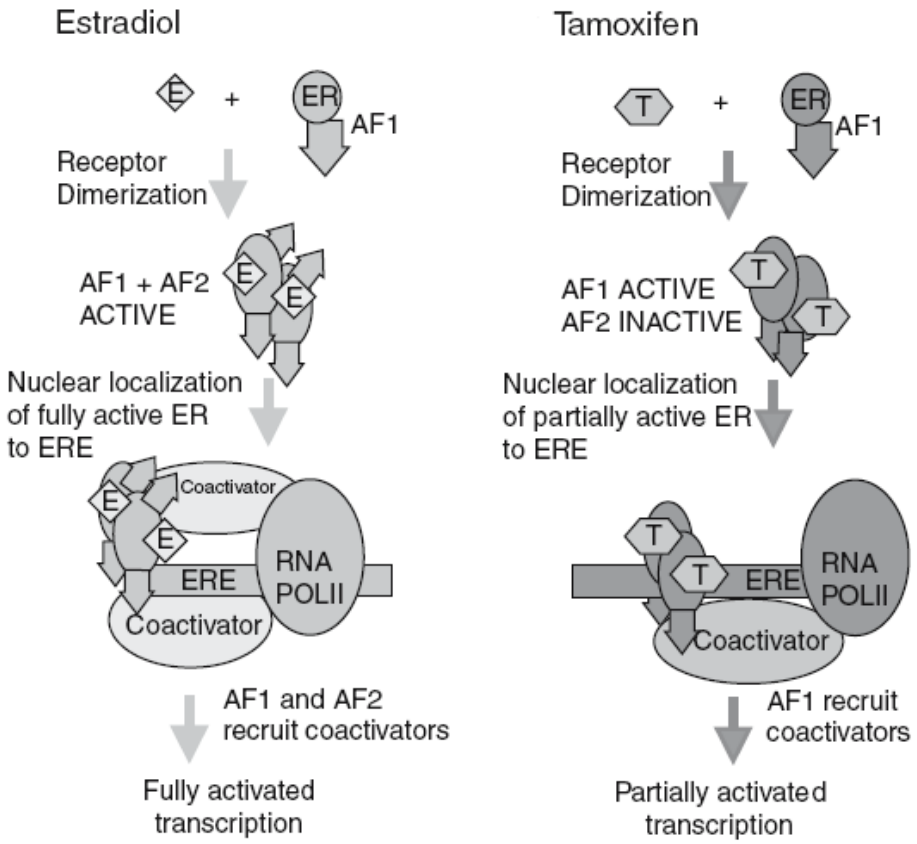
How can an SNP map be used to predict medicine response?




Mechanism Upstream
• Drug Transport
• Metabolism Pase I
• Metabolism Phase II
Interaction Drug-Target
• DNA Biosynthesis and Metabolism
• DNA Repair
Mechanism Downstream
•Apoptosis
•Chemokine Activation

I. Pharmacogenetics of Antiestrogen Treatments

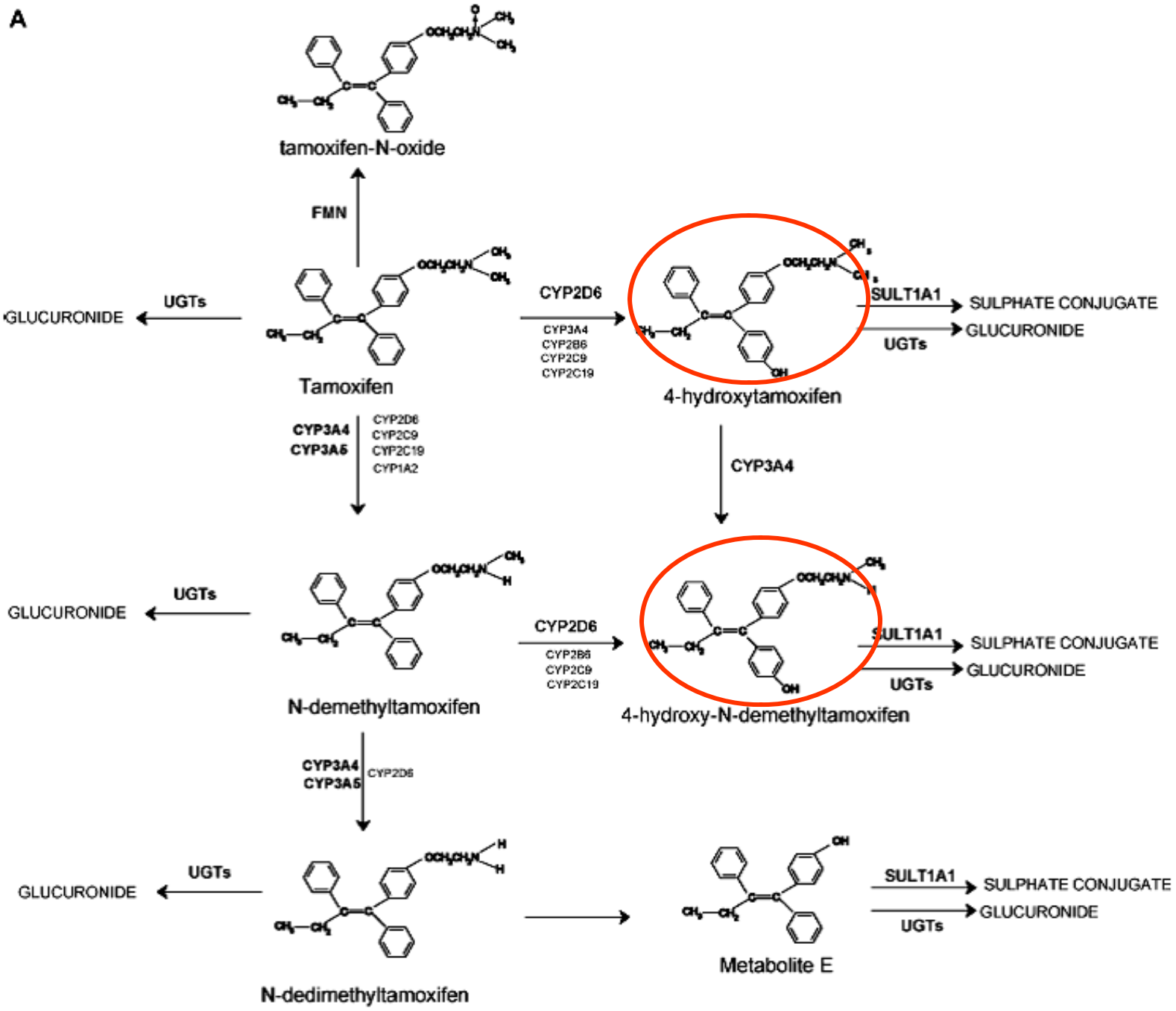
Tamoxifen



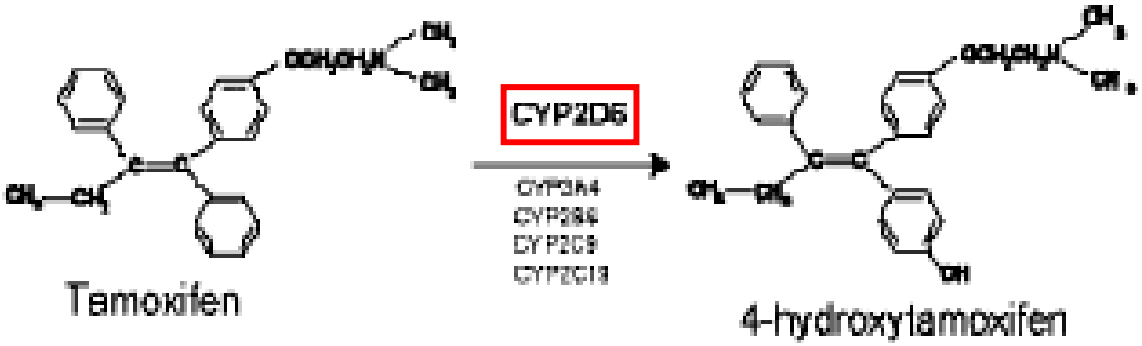
- SERM
- Use by pre- and post-menopausal breast cancer patients
- Prodrug- requires activation

Tamoxifen- Clinical Assessment

Indications	Year of Approval
Metastatic Breast Cancer (postmenopausal)	1977
Adjuvant Breast Cancer (postmenopausal node +)	1986
Metastatic Breast Cancer (premenopausal)	1989
Adjuvant Breast Cancer (postmenopausal node -)	1990
Metastatic Breast Cancer (male)	1993
Reduction in Breast Cancer Incidence	1998
Ductal Carcinoma in Situ (DCIS)	2000


A
tamoxifen-N-oxide
FMN
GLUCURONIDE
UGTs
Tamoxifen
CYP2D6
CYP3A4
CYP2B6
CYP2C9
CYP2C19
4-hydroxytamoxifen
SULT1A1
SULPHATE CONJUGATE
UGTs
GLUCURONIDE
CYP3A4
CYP3A5
CYP2D6
CYP2C9
CYP2C19
CYP1A2
CYP3A4
GLUCURONIDE
UGTs
N-demethyltamoxifen
CYP2D6
CYP2B6
CYP2C9
CYP2C19
4-hydroxy-N-demethyltamoxifen
SULT1A1
SULPHATE CONJUGATE
UGTs
GLUCURONIDE
CYP3A4
CYP3A5
CYP2D6
GLUCURONIDE
UGTs
N-dedimethyltamoxifen
Metabolite E
SULT1A1
SULPHATE CONJUGATE
UGTs
GLUCURONIDE

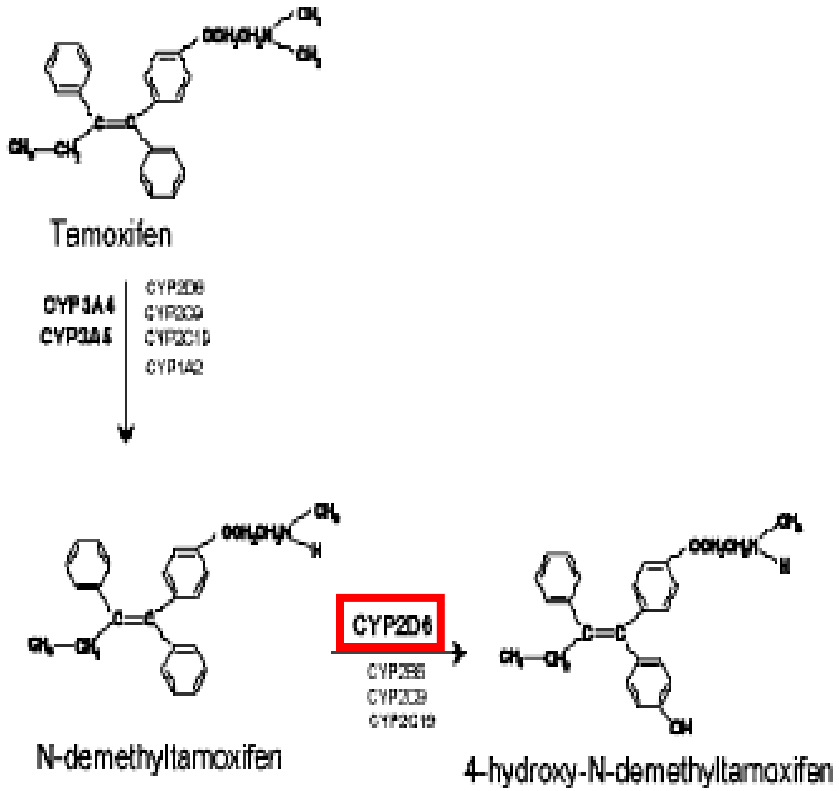
4-Hydroxytamoxifen



- 100- fold greater affinity than TAM towards ER
- 30- to 100-fold more potent inhibitor than TAM

4-Hydroxy-N-Demethyltamoxifen (Endoxifen)

- Equivalent potency to 4-OH-TAM, but 10-fold higher concentration in plasma
- Impact of CYP2D6 enzyme variant and inhibitors of CYP2D6 on endoxifen concentration.



CYP2D6 Gene

- Encodes enzyme involved in biotransformation of many drugs
- Highly polymorphic: 63 allelic variants

  www.cypalleles.ki.se
- Wide range of metabolic phenotypes: UM, EM, IM and PM
- *CYP2D6*4* the most frequent null allele in Caucasians (20-25%), causes 70-90% of all PM

Metabolic Phenotype

- **Extensive Metabolizers (EM)** have two copies of functional alleles. They have a normal response to the standard dose of a particular drug.
- **Intermediate Metabolizers (IM)** may showed impaired metabolism, by missing one functional allele.
- **Poor Metabolizers (PM)** have problems processing the standard dose of a drug, because they miss two functional alleles. Require different dose.
- **Ultra Metabolizers (UM)** have one or more extra copy of the gene that produce the 2D6 enzyme, so they will require different dose.

CYP2D6 Alleles

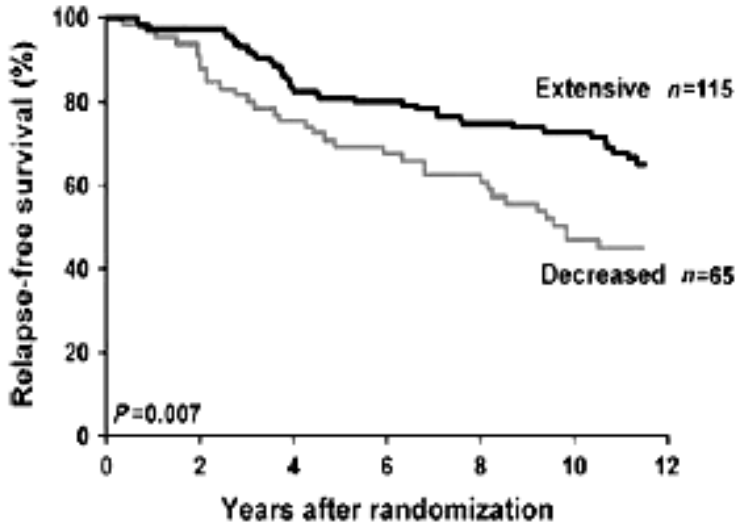
Table 1. Frequencies of the Most Prevalent Alleles Across the Broad Ethnic Groups of Caucasians, Black Africans, and Asians

Allele Type	CYP2D6 Allele	Caucasian (%)	Black African (%)	Asian (%)
Functional	*1	33–40	28–50	23–42
	*2	22–34	11–78	9–20
Reduced function	*9	0–2.9	0	3.3
	*10	1.9–8	3.1–8.6	38–70
	*17	0.1–0.3	9–34	0.5
	*41[18]	8	—	—
Nonfunctional	*3	1–3.9	0–0.5	0.8–1
	*4	12–23	1.2–7	0–2.8
	*5	1.6–7.3	0.6–6.1	4.5–6.1
	*6	0.7–1	0	—
Duplication	*1 × 2	0.2–0.5	3.3	0.5
	*2 × 2	0.7–1.6	1.6–2.5	0–1
	*4 × 2	0.1–0.2	0.9	—

Impact of *CYP2D6*4* Allele

Study of 180 postmenopausal patients with 5 years of TAM treatment *(Goetz et al. 2007)*

- Patients with decreased CYP2D6 metabolism
  - Shorter time to recurrence
    p=0.034 HR=1.91
  - Worse RFS
    p=0.017 HR=1.74



Replication Study for C*YP2D6*4*

Study of 206 postmenopausal patients treated with TAM *(Schroth et al. 2007)*

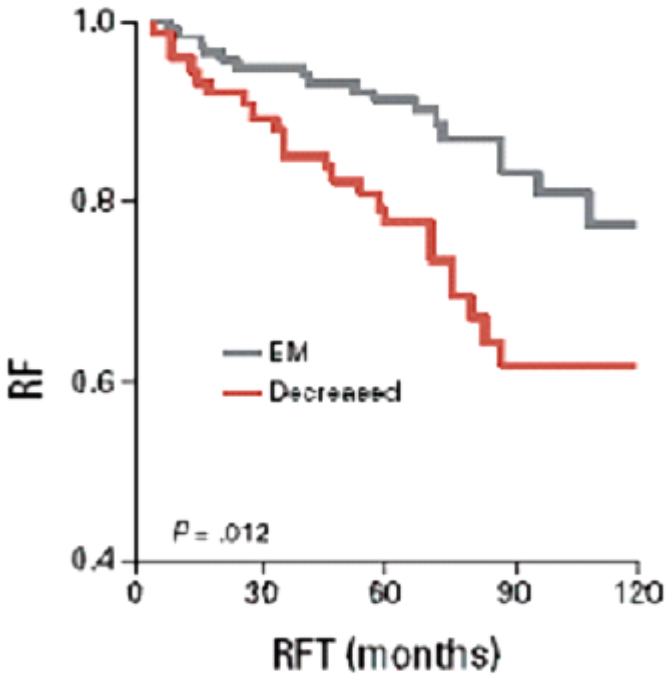
- Patients with decreased CYP2D6 metabolism

  -shorter RFT

  p=0.012 HR=2.24

  -worse EFS

  p=0.02 HR=1.89



How could CYP2D6 testing change clinical practice?

- Alter therapy course for postmenopausal patients, who are carriers of *CYP2D6*4* allele
- Development of treatment algorithms for carriers of CYP2D6 alleles variants
- Avoiding co-administration of certain antidepressants with TAM, since they may act as CYP2D6 inhibitors

Tamoxifen and an FDA Perspective

FDA Recommends Relabeling Tamoxifen

On October 18, an FDA panel unanimously agreed that the 2D6 gene is a predictor of tamoxifen efficacy. They recommend relabeling tamoxifen to say that 2D6 poor metabolizers who take tamoxifen have a higher risk for breast cancer recurrence, and that testing is available.

Roche AmpliChip™ CYP450

- FDA & EU cleared for diagnostic analysis of two critical drug metabolism genes: **CYP2D6** and **CYP2C19**
- Pharmacogenetics microarray- based test
- Recommended for cyclophosphamide (CYP2C19) and tamoxifen (CYP2D6)

Tag-It™ Mutation Detection Kit for

P450-2D6 v2

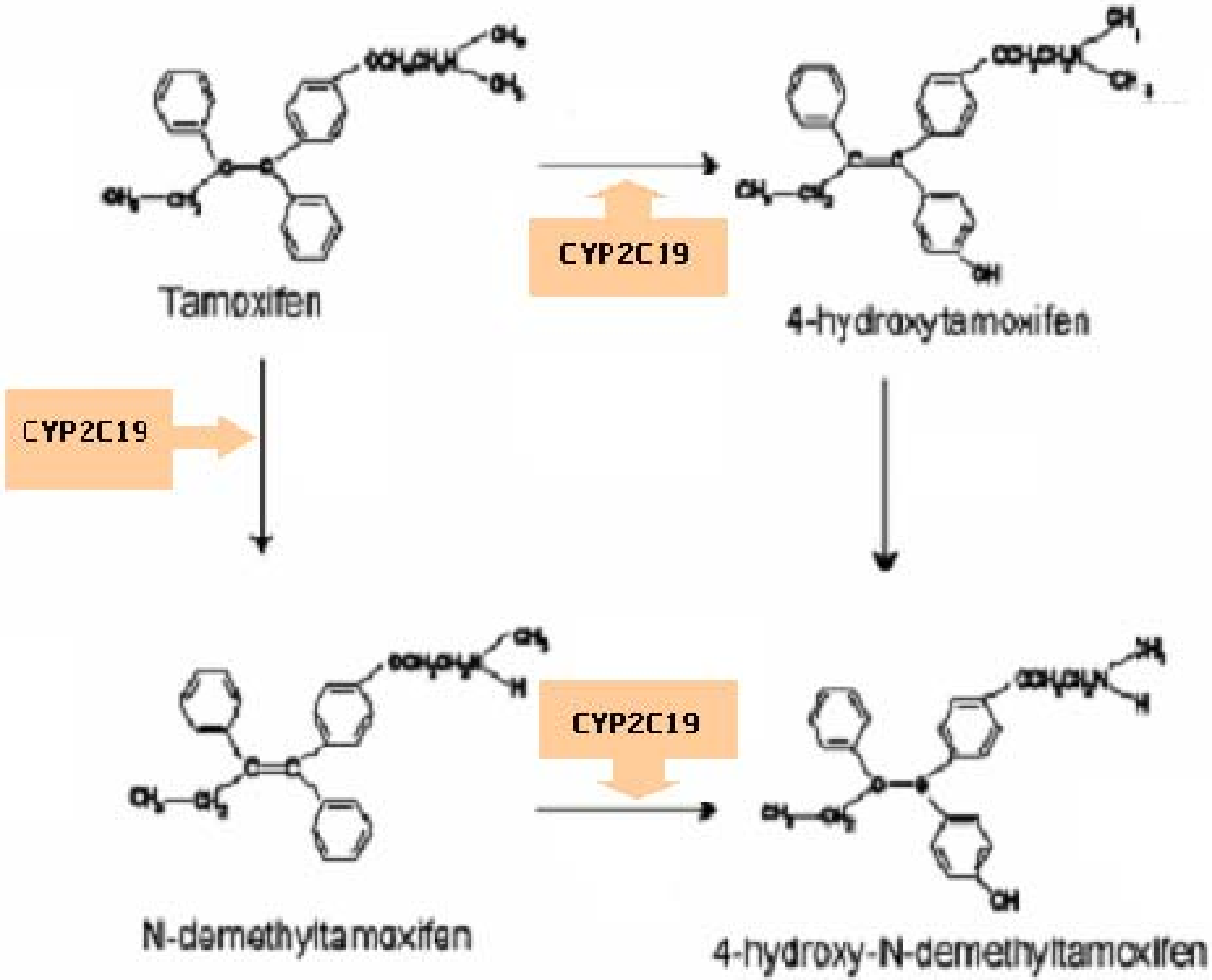
For use with Luminex® 100 or 200 xMAP® System

Variant*	* Designation	Effect
/	Gene Duplication	↑ Activity
/	*5 Gene Deletion	No Gene
-1584C>G	*2A Promoter	Expression
100C>T	*4 (A-L) [a] *10 (A,B)	P34S
124G>A	*12	G42R
138InsT	*15	Frameshift
883G>C	*11	Splicing
1023C>T	*17	T107I
1661G>C [b]	*2	Silent
1707T>del	*6 (A-D)	Frameshift
1758G>T	*8	Stop Codon
1758G>A	*14	G169R
1846G>A	*4 (A-L) [a]	Splicing
2549A>del	*3 (A,B)	Frameshift
2613-2615 delAGA	*9	K281Δ
2850C>T [b]	*2,*17	R296C
2935A>C	*7	H324P
2988G>A	*41	mRNA Stability
4180G>C [b]	*2	S486T

* Nomenclature derived from CYP2D6 Allele Nomenclature Database[3]

[a] For *4, no *4I designation exists

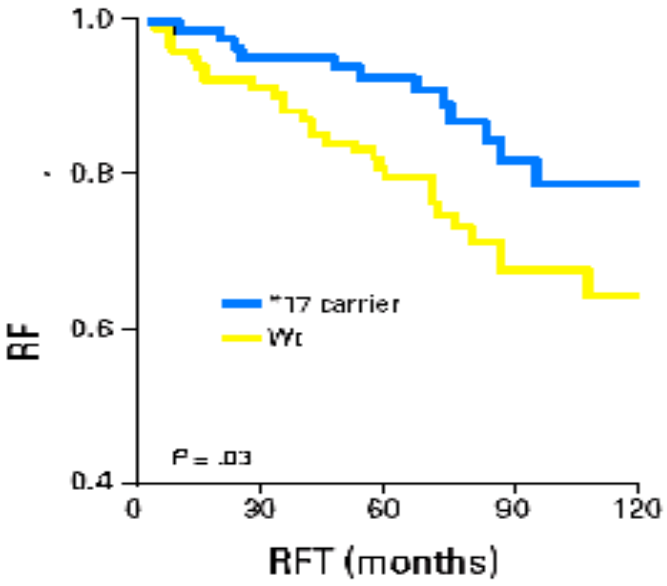
[b] 1661G>C, 2850C>T, and 4180G>C are found in a wide range of alleles


Tamoxifen
CYP2C19
4-hydroxytamoxifen
CYP2C19
CYP2C19
N-demethyltamoxifen
4-hydroxy-N-demethyltamoxifen

Impact of *CYP2C19*17* Allele

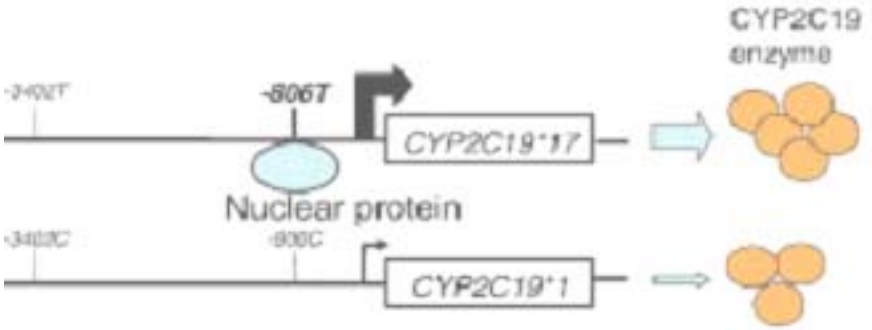
Carriers of *CYP2C19*17* allele showed *(Schroth et al. 2007)*

- Decreased risk of relapse p=0.02 OR=0.43
- Favorable RFT p=0.03 HR=0.45



*CYP2C19*17*

- Associated with increased CYP2C19 activity
- UM phenotype
- Carriers of this allele include 18% of Swedes and Ethiopians, but only 4% Chinese



Sim et al. 2006

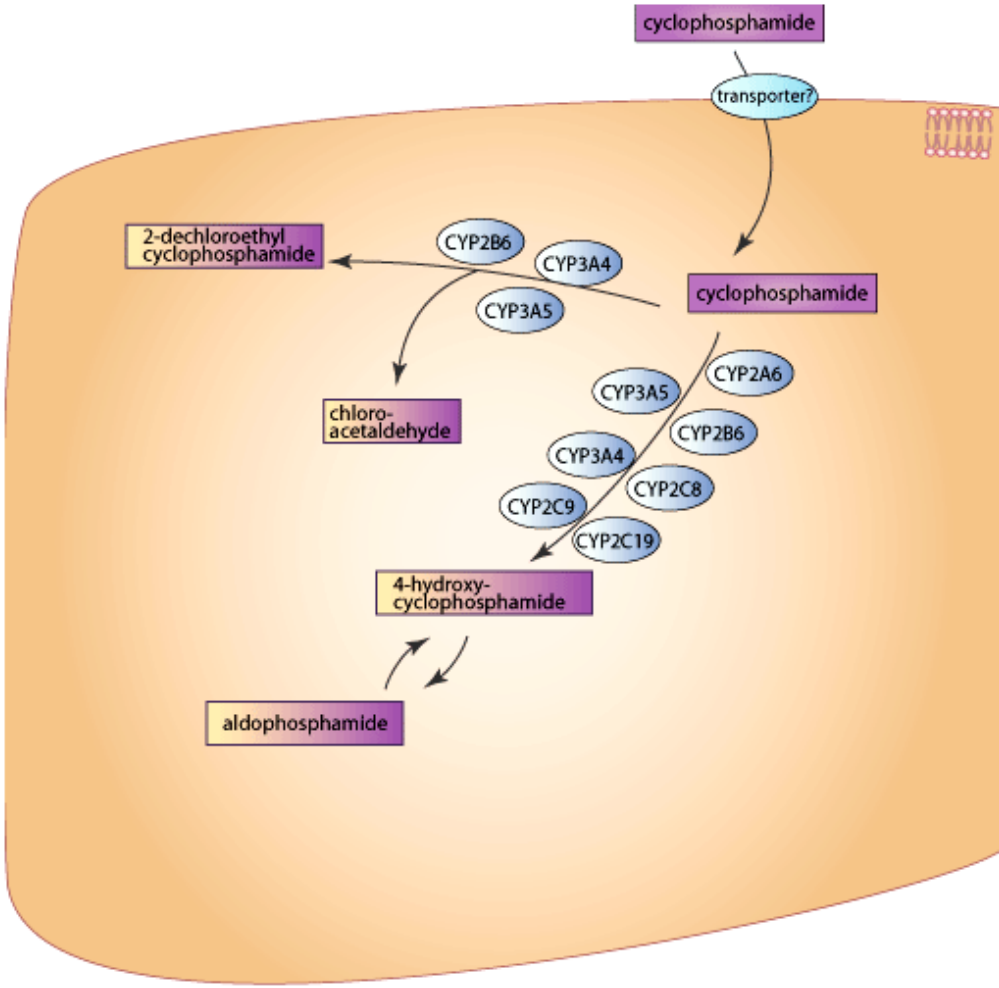
II. Pharmacogenetics of Cytotoxic Agents

Chemotherapy

- Cyclophosphamide-methotrexate-fluorouracil (CMF)- “golden standard”
- Other agents i.e. taxanes, anthracyclines (FEC, FAC).
- Adjuvant chemotherapy is considered for more than 70% of BC, but is not beneficial for all of them
- Response rates
- Toxicity

Cyclophosphamide

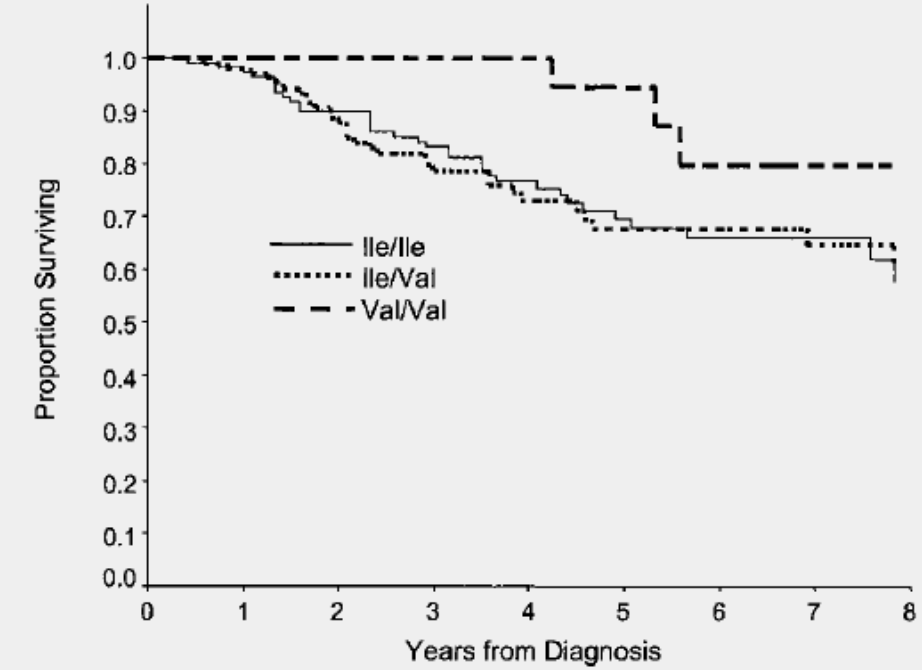
- Alkylating agents
- Prodrug- requires activation by P-450 enzymatic complex
- Eliminated by glutathione S-transferases (GST) A1 and P1



Cyclophosphamide and GSTP1

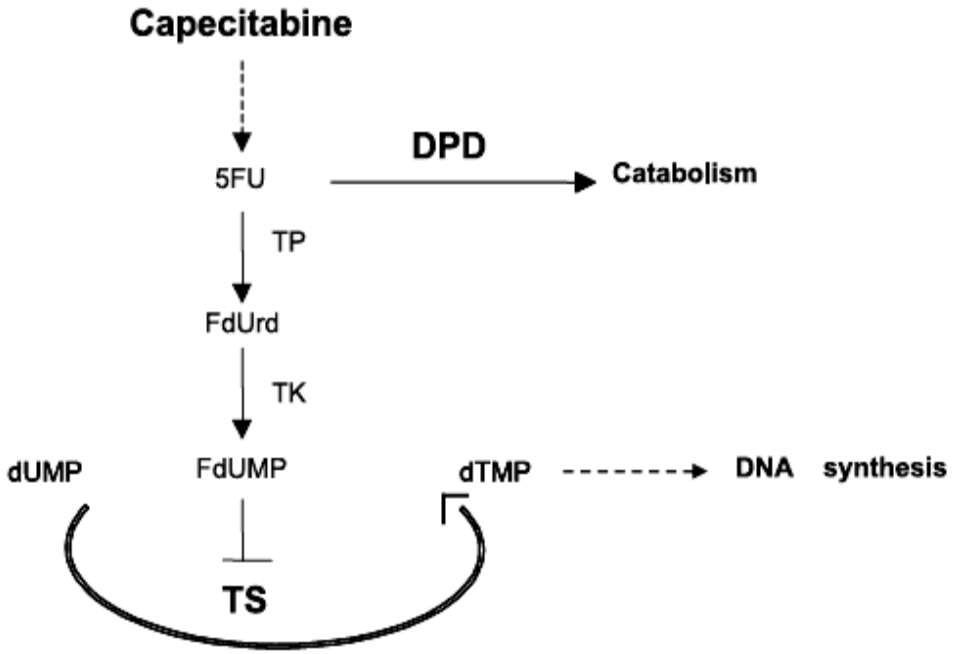
Study of 240 patients treated with cyclophosphamide *(Sweeney et al. 2000)*

- Low- activity Val/Val GSTP1 genotype showed better survival HR=0.3



5-Fluorouracil

- Uracil analogue
- Prodrug- active form FdUMP
- TS is a main target
- 85% inactivated by DPD
- Toxicity caused by DPYD polymorphisms

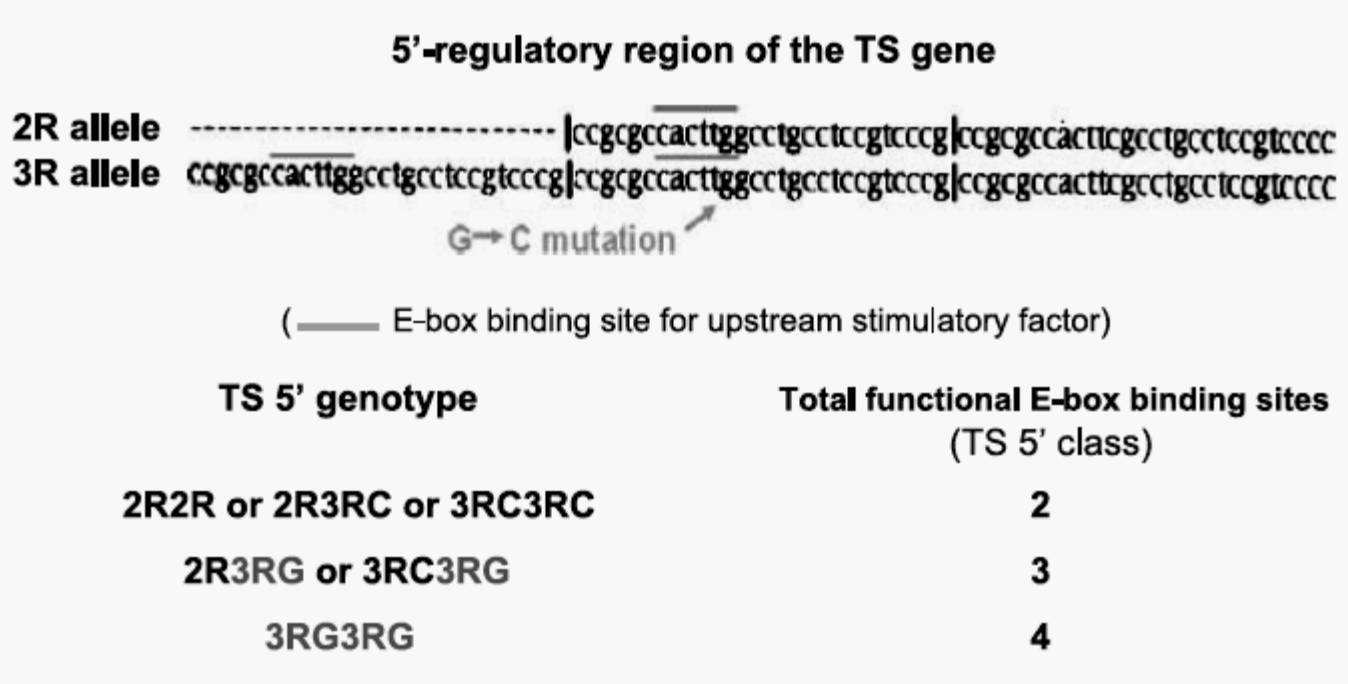


5-FU Toxicity and DPYD Polymorphisms

- Wide variation of enzyme activity among individuals
- At least 20 polymorphic loci are associated with reduced DPD activity, and 3-5% of population are heterozygous carriers of deficient allele
- *DPYD*2A* allele accounts for ~ 50% of known nonfunctional DPYD alleles
- Reduced DPD activity leads to 5-FU toxicity

TS and 5-FU

- Inhibition of TS by 5-FU ⟶ cell death
- Polymorphisms G/C in 28bp tandem repeat sequence of TS increases gene expression



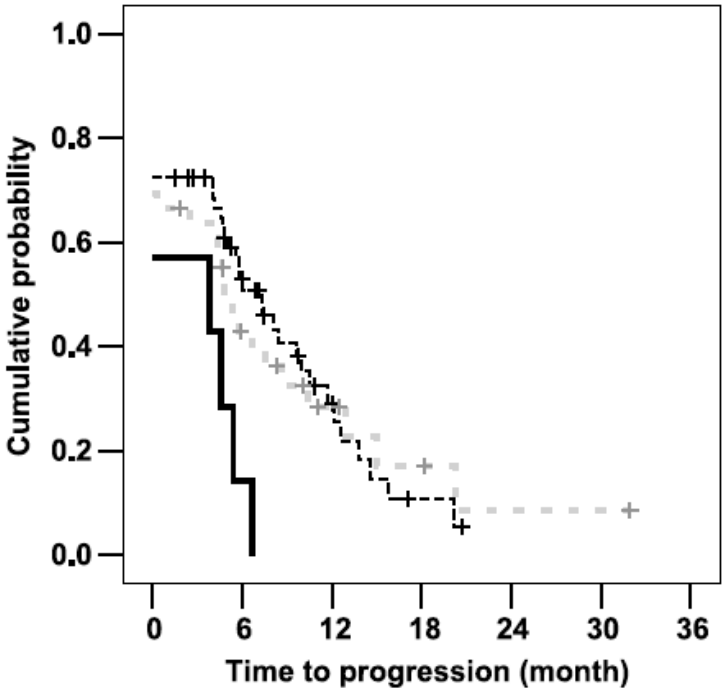
TS 5' genotype	Total functional E-box binding sites (TS 5' class)
2R2R or 2R3RC or 3RC3RC	2
2R3RG or 3RC3RG	3
3RG3RG	4

TS and 5-FU

Carriers of TS 5'genotype class 4 (3RG3RG) showed

(Largillier et al. 2006)

- rapid disease progression (p=0.037)
- trend toward a higher global toxicity grade 3 and 4

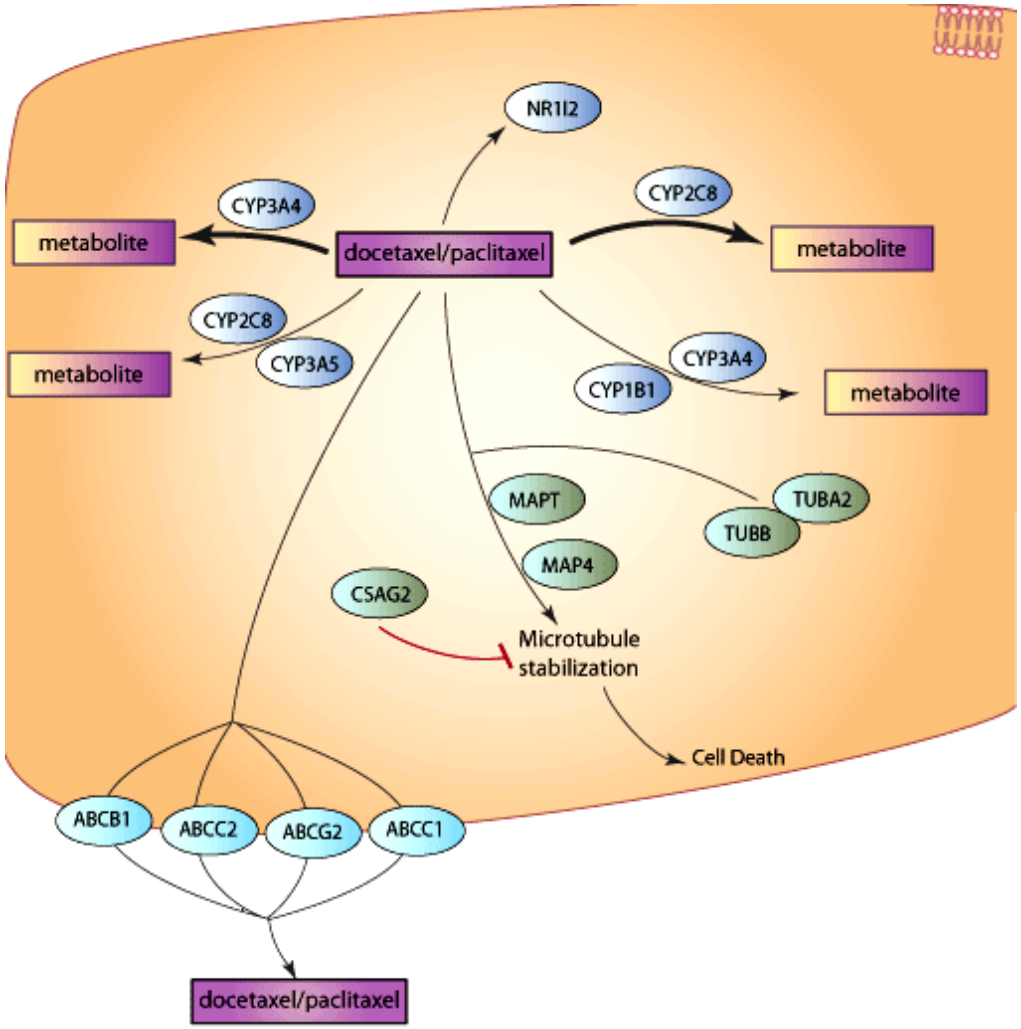


Commercial Testing

TherapID™: 5-FU test

- is a simple test that analyzes the genetic variations in the **DPYD gene** in order to predict the risk for serious side effects in patient treated by 5-FU.

Paclitaxel



- Block cell cycle progression
- Broad spectrum of activity against solid tumors
- Dose dependent induced toxicity

Paclitaxel Resistance

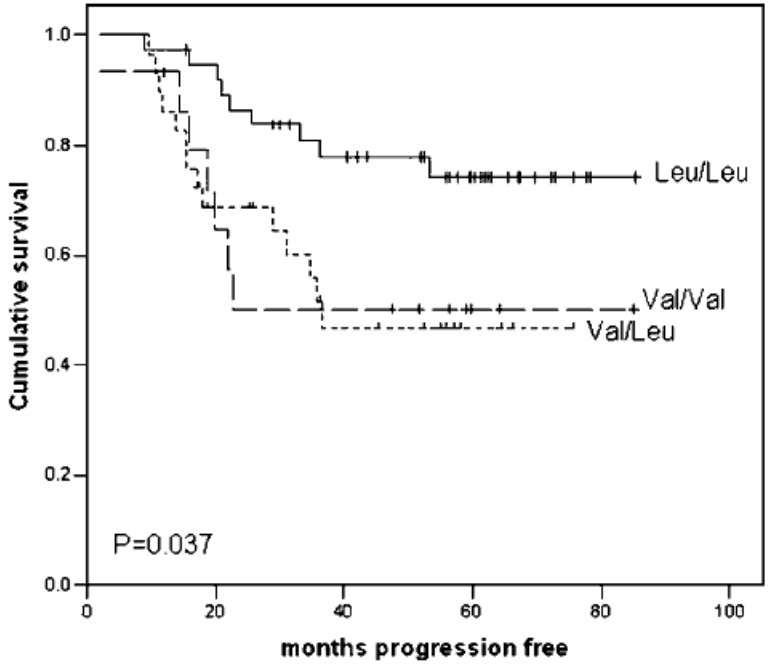
Molecular Mechanism	Implicated in paclitaxel resistance
P-glycoprotein [17–19]	yes
Tubulin mutations and isoforms [24, 28, 1]	yes
Impaired apoptotic response (bcl-2, bax) [49, 50]	yes
Alterations in microtubule-binding proteins (stathmin, tau) [39, 42, 51]	yes

Paclitaxel and *CYP1B1*3* Allele

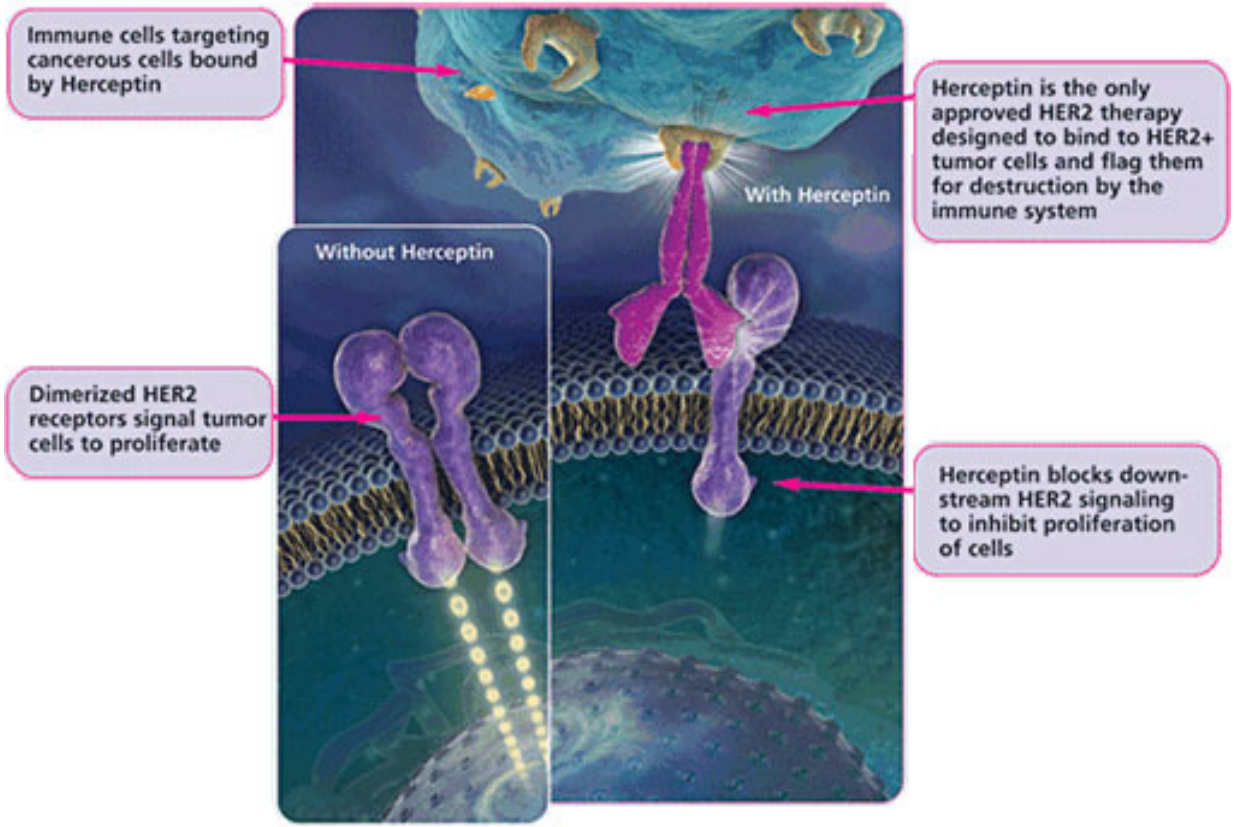
Study of 93 patients with primary breast cancer treated by paclitaxel (*Marsh et al.2007)*

- *CYP1B1*3* (L432V) allele significantly associated with progression- free survival

p= 0.037



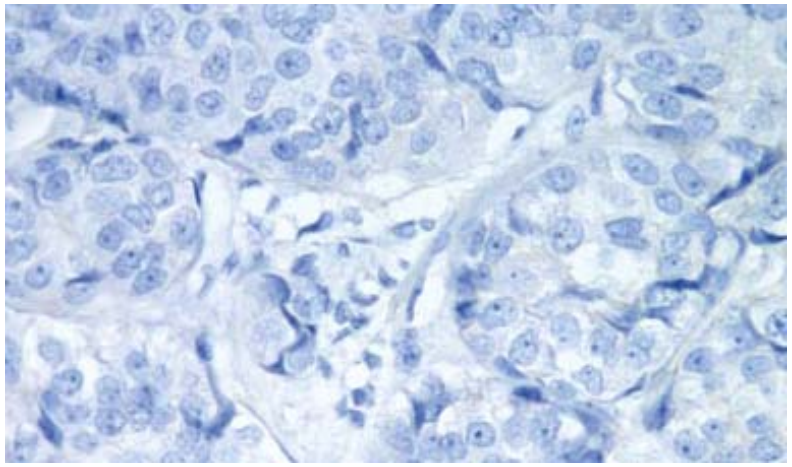
Herceptin

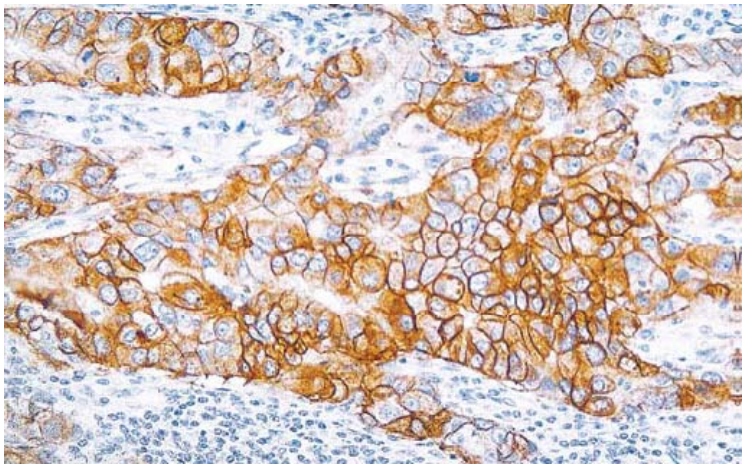


Monoclonal antibody targets an extracellular domain of the HER-2 receptor

HercepTestTM

- The only FDA-approved diagnostic method to aid in the selection of patients for Herceptin therapy is immunohistochemistry (IHC).



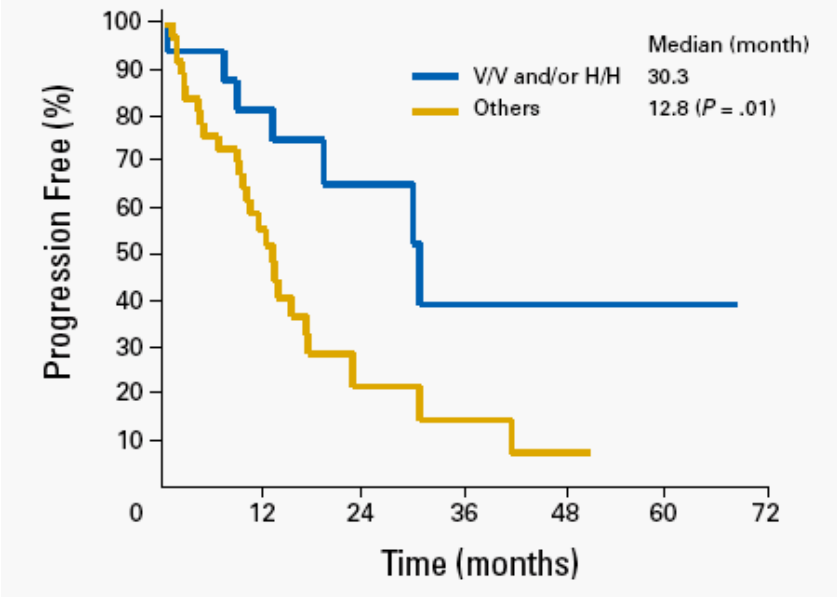


Trastuzumab

FcγR polymorphisms associated with PFS

(Musolino et al. 2008)

- FcRIIIa 158 V/V and FcRIIa 131 H/H genotype p=0.01



Strategies to Overcome Therapy Resistance