Testing of CYP 450 in oncology treatment – one example Tamoxifen / Aromatase inhibitors

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Agenda of presentation

- **Pharmagogenetics / Pharmacogenomics**
- Role of Cytochrome P450 in drugs metabolism
- Technology
- Types of metabolisers
- One size or drug type does not fit all
- Breast cancer, Tamoxifen, Aromatase inhibitors
- Study design and preliminary results
- Summary

Pharmacogenetics vs. Pharmacogenomics

Pharmacogenetics

1. Study or clinical testing of genetic variation
2. Gives rise to differing response to drugs

Pharmacogenomics

1. Broader application of genomic technologies to new drug discovery
2. Further characterization of older drugs

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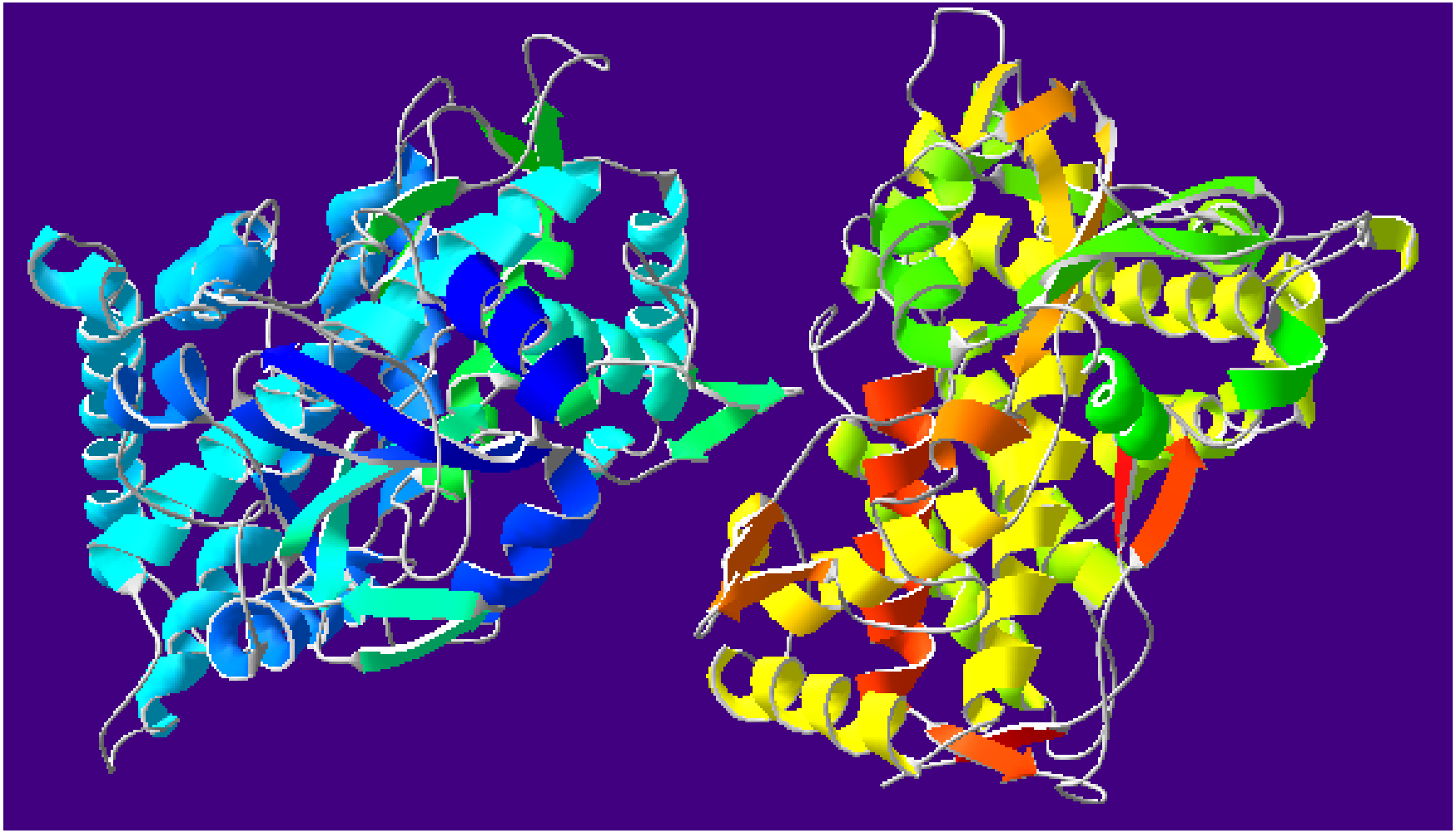
Cytochrome P 450



Cytochrome P 450

- Enzyme system
  - Group (superfamily) of hundreds similar proteins with catalytic functions
  - Present in animals, plants, fungi and bacteria
- Biosyntheisis of steroids, fatty acids
- Metabolism of endogenous and exogenous substrates
  - Metabolic activation
  - Detoxification

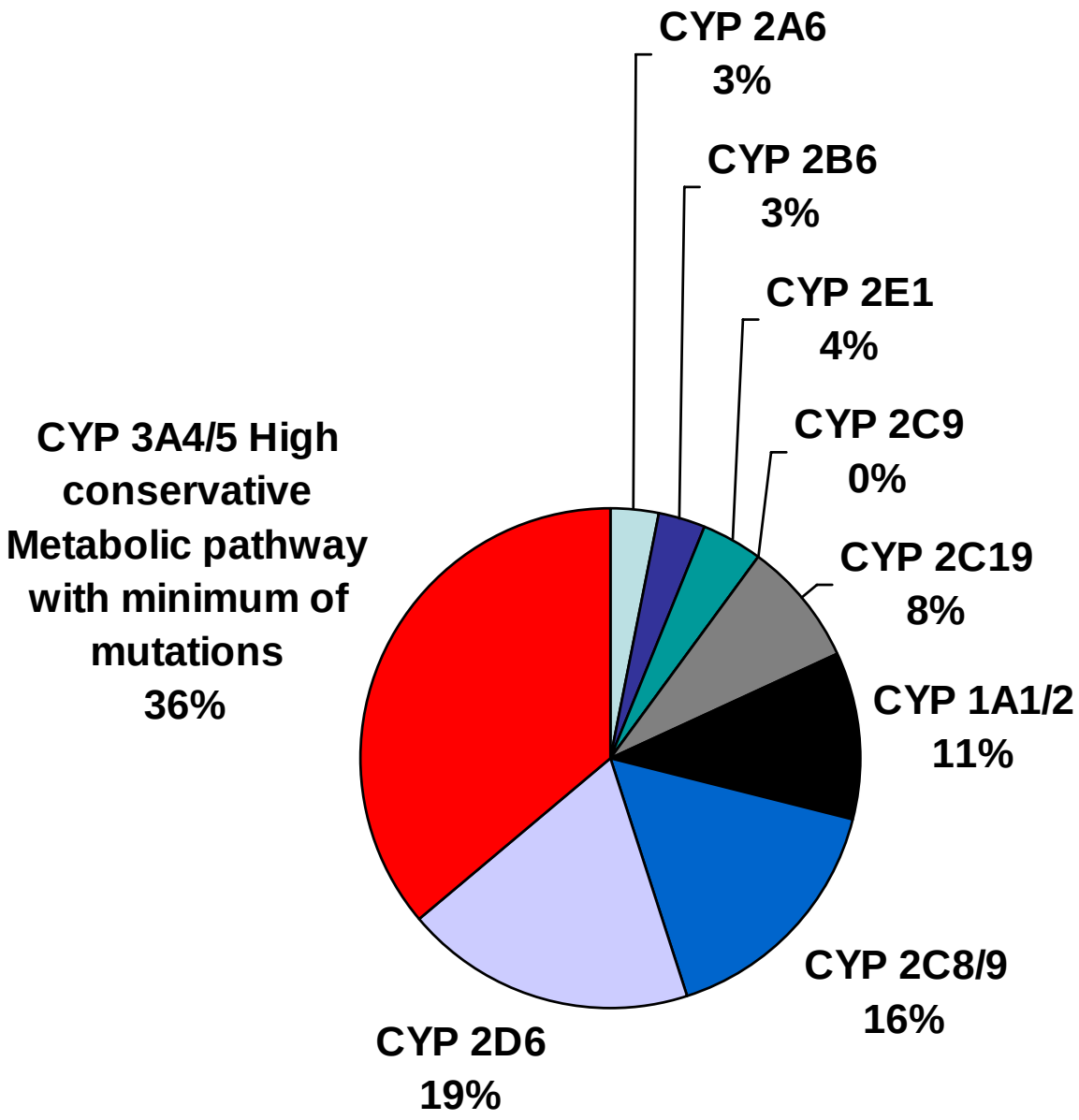
The CYP450 genes metabolize most therapeutic drugs

Examples

CYP450 Genes	Drugs Metabolized (%)
CYP 2A6	3
CYP 2B6	3
CYP 2E1	4
CYP 2C9	ND*
CYP 2C19	8
CYP 1A1/2	11
CYP 2C8/9	16
CYP 2D6	19
CYP 3A4/5	36

- Drugs can be metabolized by multiple enzymes
- 2D6 is the major metabolizer for its substrates
- 2D6 and 2C19 selected for AmpliChip CYP450 Test (CE-IVD)

CYP 450 Drugs Metabolized (%)



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AmpliChip CYP450 Test: combines gold standards in PCR and microarray technology

Sample (Whole Blood)

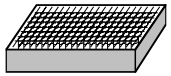
Sample Prep

QIAGEN (manual prep)

Amplification

ABI 9700

Fragmentation / Labeling



PCR Front-End

Array-based Detection

8 hrs

Hybridization

Scanning & Analysis

Report

Technology: Genotype prediction of Phenotype

Allele	1	2	3	4	5	6	7	8	9	10	11	14A	14B	15	17	19	20	25	26	29	30	31	35	36	40	41	1XN	2XN	4XN	10XN	17XN	35XN	41XN
1	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	E
2		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	E
3			P	P	P	P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
4				P	P	P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
5					P	P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
6						P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
7							P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
8								P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
9									I	I	I	I	N	I	I	I	I	N	N	I	N	N	E	I	I	I	E	E	I	I	I	E	I
10										I	I	I	N	I	I	I	I	N	N	I	N	N	E	I	I	I	E	E	I	I	I	E	I
11											P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
14A												P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
14B													N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
15														P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
17															I	I	I	N	N	I	N	N	E	I	I	I	E	E	I	I	I	E	I
19																P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
20																	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
25																		N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
26																			N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
29																				I	N	N	E	I	I	I	E	E	I	I	I	E	I
30																					N	N	E	N	N	N	N	N	N	N	N	N	N
31																						N	E	N	N	N	N	N	N	N	N	N	N
35																							E	E	E	E	U	U	E	E	E	U	E
36																								I	I	I	E	E	I	I	I	E	I
40																									P	I	E	E	P	I	I	E	I
41																										I	E	E	I	I	I	E	I

E	Extensive
I	Intermediate
P	Poor
U	Ultrarapid
N	Unknown

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- **Types of metabolisers**
- One size or drug type does not fit all
- Breast cancer, Tamoxifen, Aromatase inhibitors
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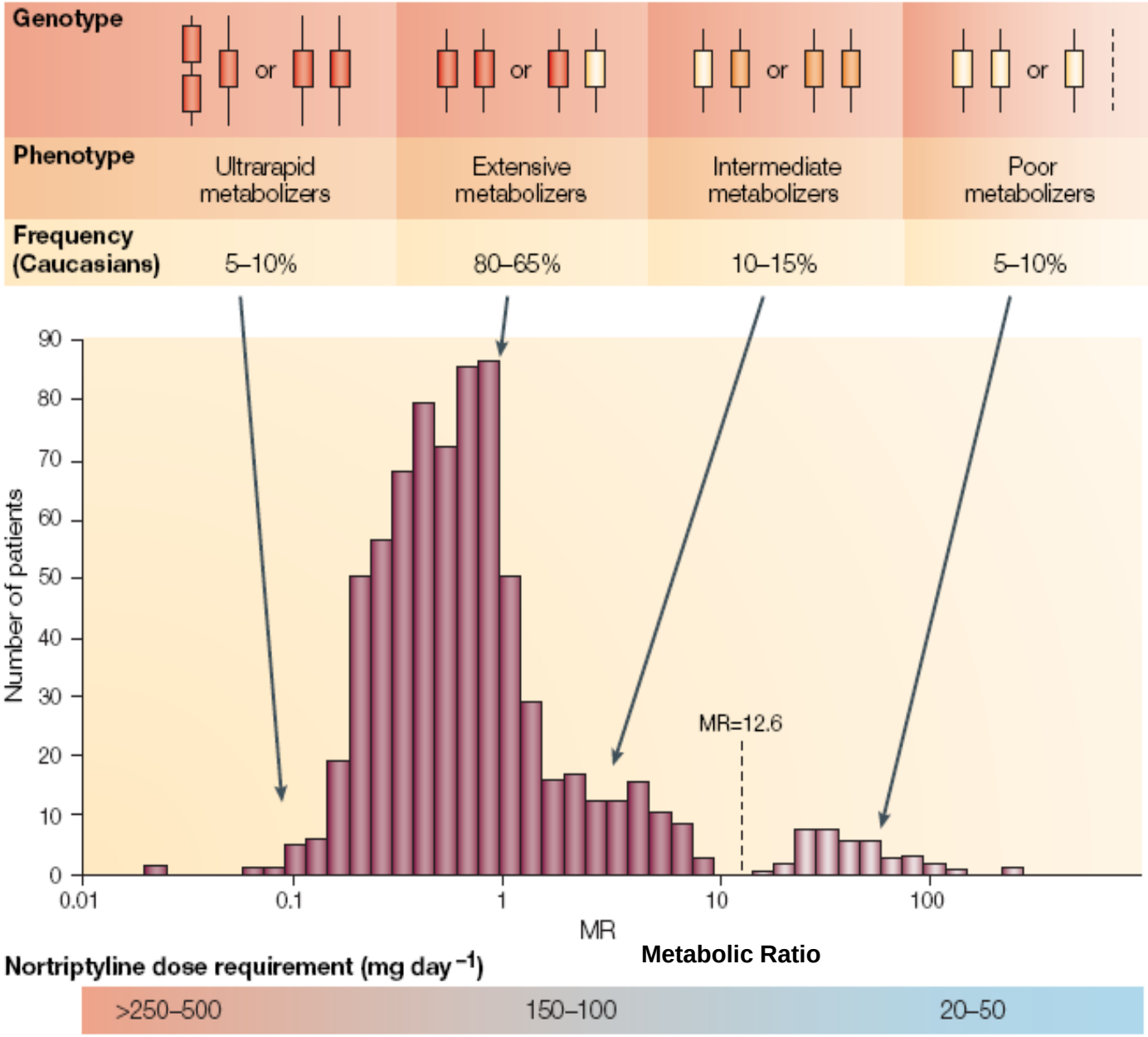
4 Types of Metabolizers (I)

- **Ultrarapid metabolizers (UM)**
  Carry multiple copies (3-13) of functional alleles and produce excess enzymatic activity
- **Extensive Metabolizers (EM)**
  Possess at least one normal functional allele
- **Intermediate metabolizers (IM)**
  Possess one reduced activity allele and one null allele
- **Poor metabolizers (PM)**
  Carry two mutant alleles which result in complete loss of enzyme activity

CYP2D6 – Genotypes – phenotype relationships of CYP2D6 polymorphisms



Meyer, U.A, J. Nature Reviews Genetics 2004; 5: 669

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Traditional dosage approach: One size fits all

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Dose and the type of drug are crucial

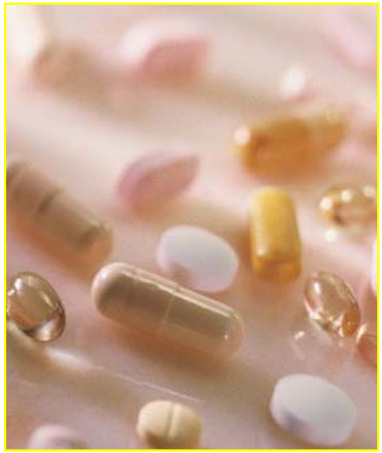
“ The dose makes the poison”

Paracelsus

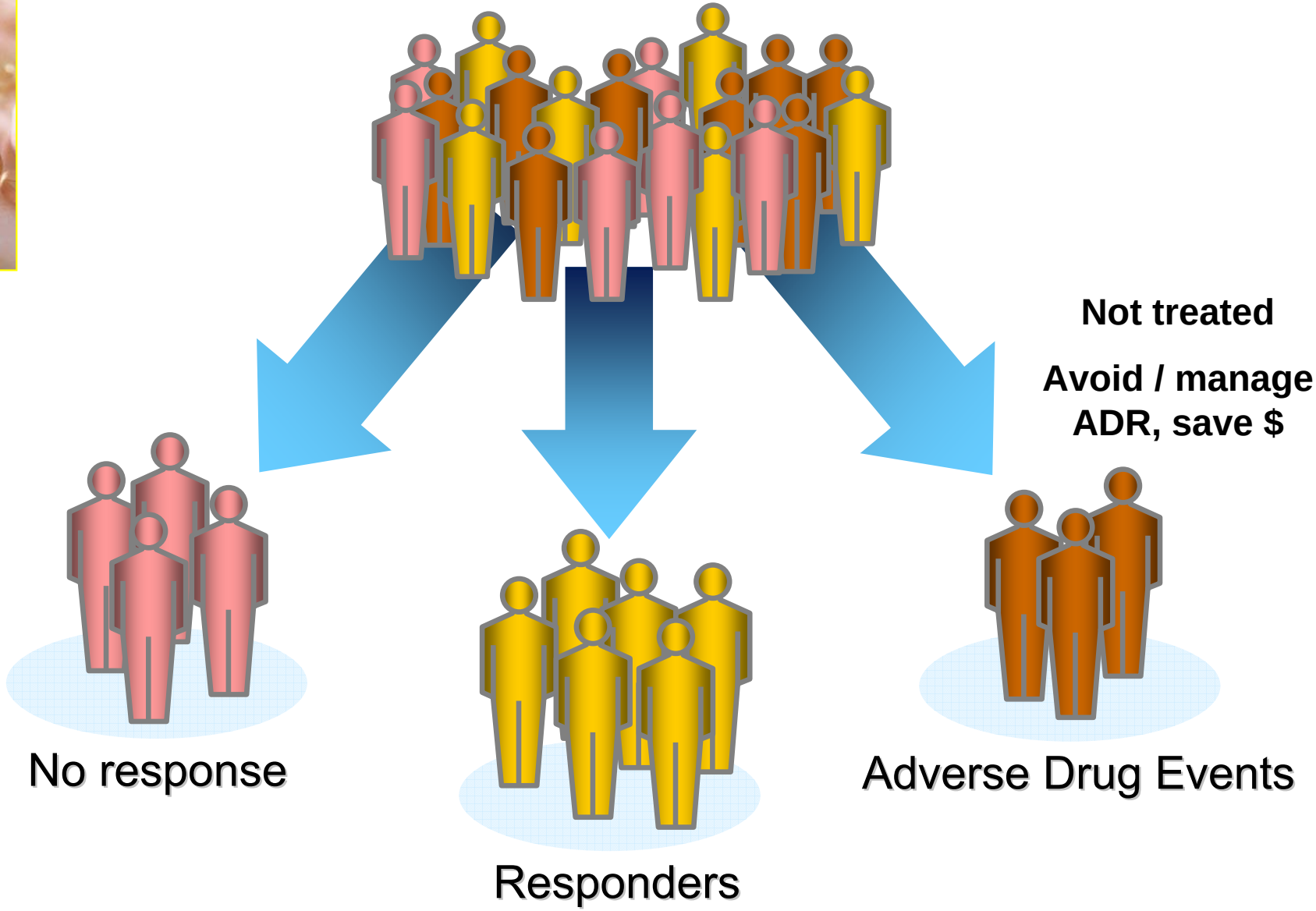
(1493-1541)

Optimizing Treatment …

Responder identification – better drug efficacy

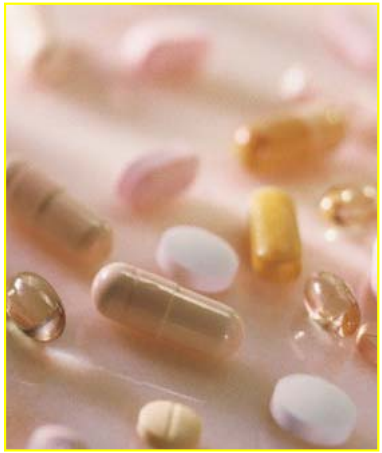


Optimal treatment

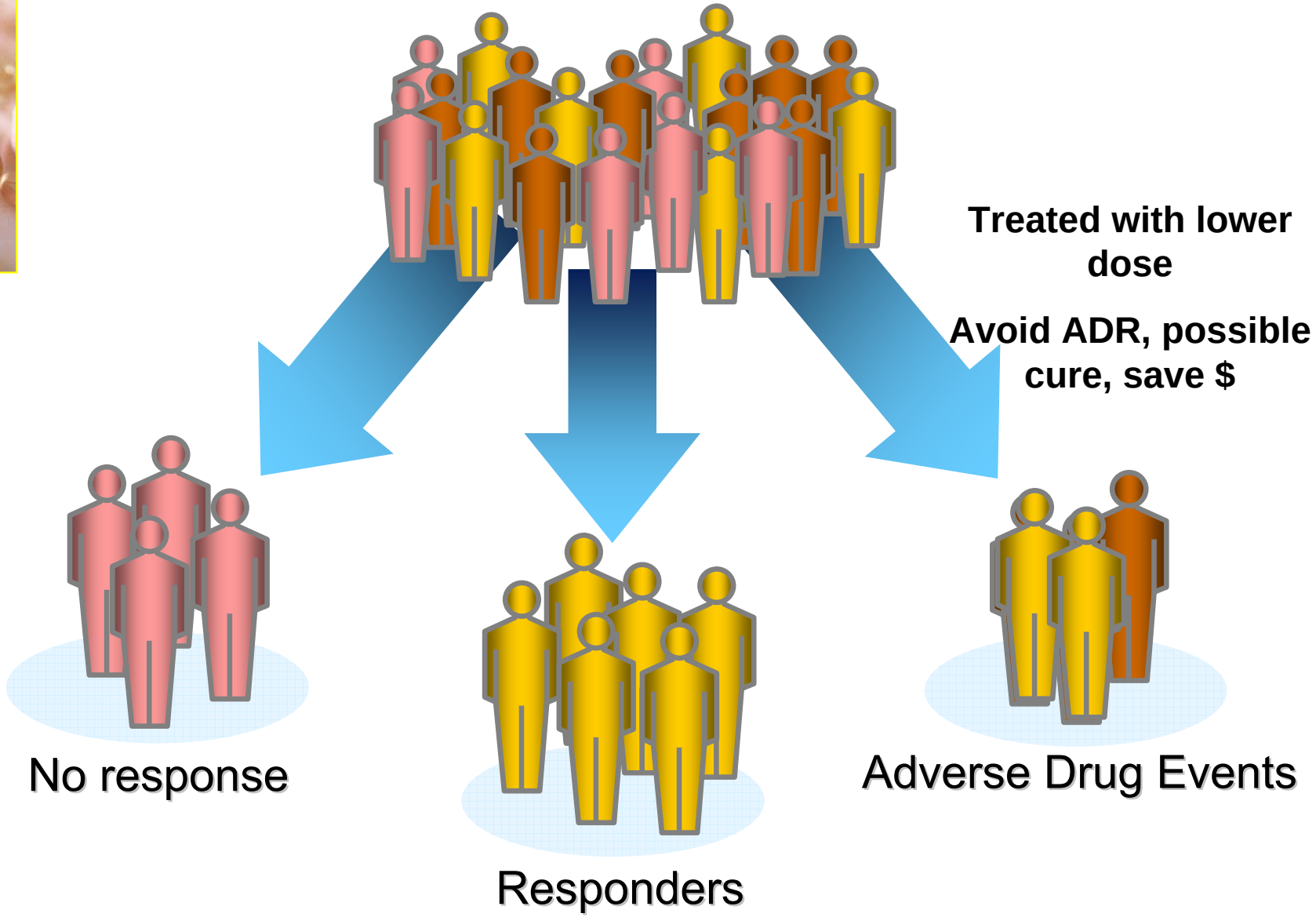


Optimizing Treatment …

Dosage alteration for better patient outcomes



Optimal treatment



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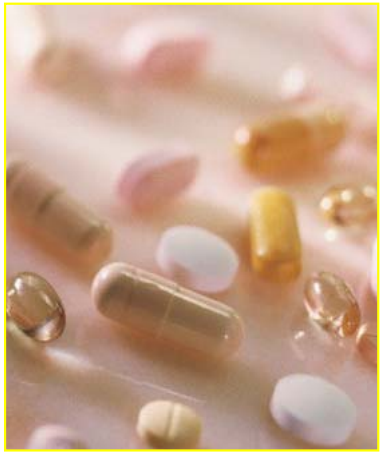
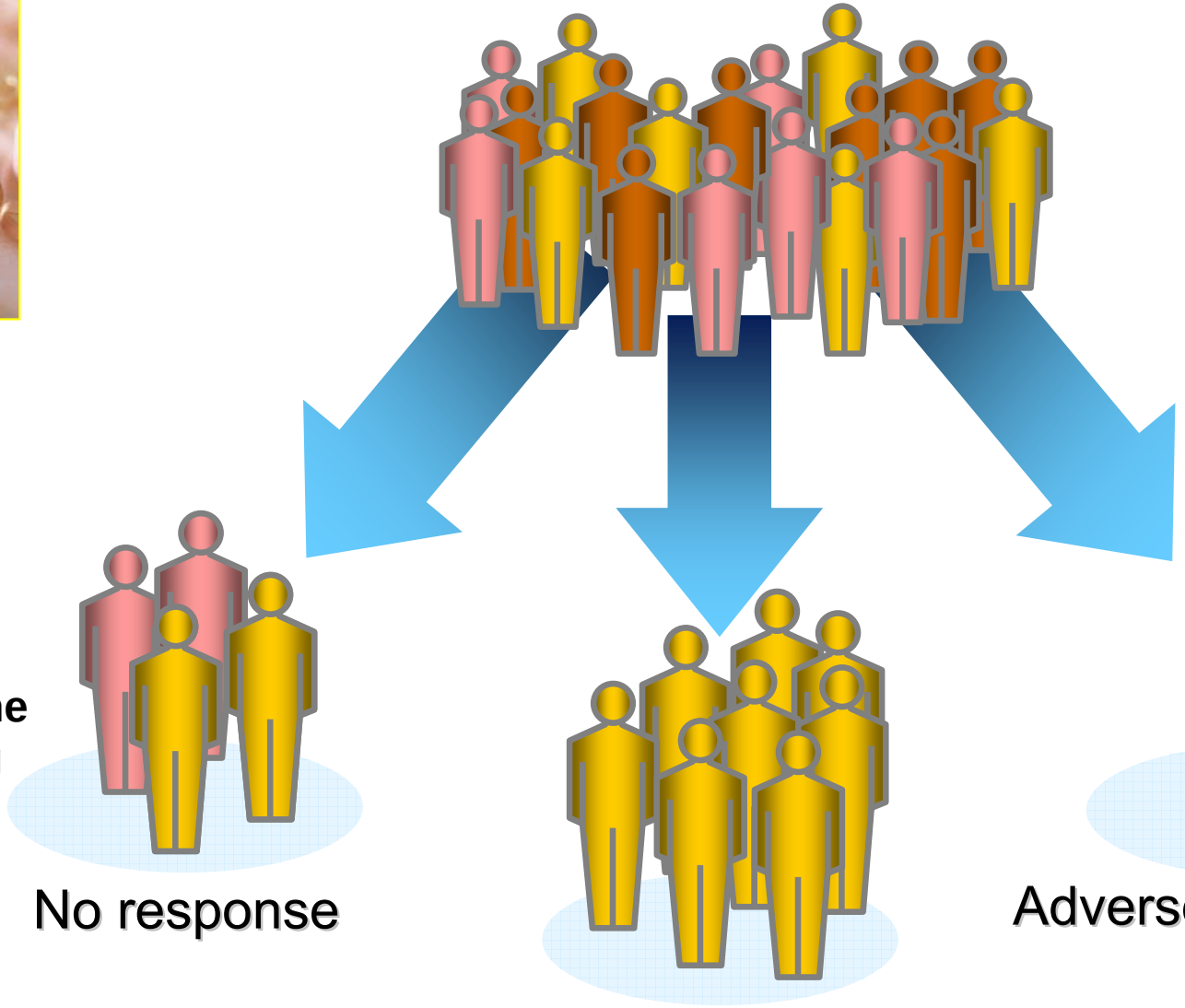


Optimal treatment

Treated with higher dosage taking into consideration the increased drug metabolism

Possible cure



Treated with lower dose

Avoid ADR, possible cure, save £

Examples: Dose recommendations for antidepressants

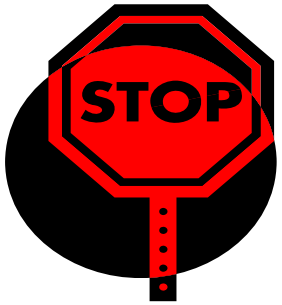
Drug	Usual Dose (mg)	Dose Adjustment (based on metabolizer type)			
		Poor	Intermediate	Extensive	Ultra-rapid
Tricyclics					
Amitriptyline	150 (50-150)	50 %	(90 %)	120 %	
Clomipramine	150 (100-200)	60 %	(90 %)	120 %	
Desipramine	150 (10-100)	30 %	30 %	130 %	260 %*
Fluvoxamine	100 (100)	90 %	(100 %)	110 %	
Imipramine	150 (25-100)	30 %	(80 %)	130 %	
Nortriptyline*	50 (25-150)	50 %	70 %	140 %	
SSRI					
Fluoxetine*	20 (20-60)	70 %	(90 %)	110 %	
Paroxetine	20 (30)	70 %	(90 %)	110 %	
Mixed-Function					
Venlafaxine	150 (20-225)	20 %	(80 %)	130 %	

* single dose recommendations; recommendations in brackets are estimations and require clinical confirmation

Kirchheiner et al, (2001) Acta Psychiat. Scand. 104: 173

Important message of Genotyping:
Genotype Predicts Phenotype

- POOR METABOLIZER DOES NOT NECESSARILY MEAN



Taking medication

- It means, **adjust dose according to genotype**:

⇧ Drug Efficiency and Patient Care

AND

⇩ Adverse Drug Reactions

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Breast Cancer

- The American Cancer Society estimated that 32% of all female cancers in 2004 were breast cancer, and that there is a 1 in 7 chance of developing breast cancer over a woman's lifetime.

- US: The second most common cause of cancer-related death in women accounting for 15% of all female cancer deaths.

- UK: 30% of all cancer incidences and 17% of all cancer deaths in women

AMERICAN CANCER SOCIETY:
Staistics for 2006.

Breast Cancer

- The majority of breast tumors express estrogen receptors.

- Several studies have shown that 5 years of Tamoxifen therapy in breast cancer patients with receptor positive tumors reduces the risk of recurrence and mortality.

- 30% of patients however acquire Tamoxifen resistance and relapse in the disease

Breast Cancer treatment

- **Aromatase inhibitors in post-menopausal women**
- Block conversion of circulating androgens to estrogen
- (CYP1A2, CYP2C9, CYP 3A5)

  - Anastrazole (Arimidex)
  - Letrozole (Femara)
  - Exemestane (Aromasin)

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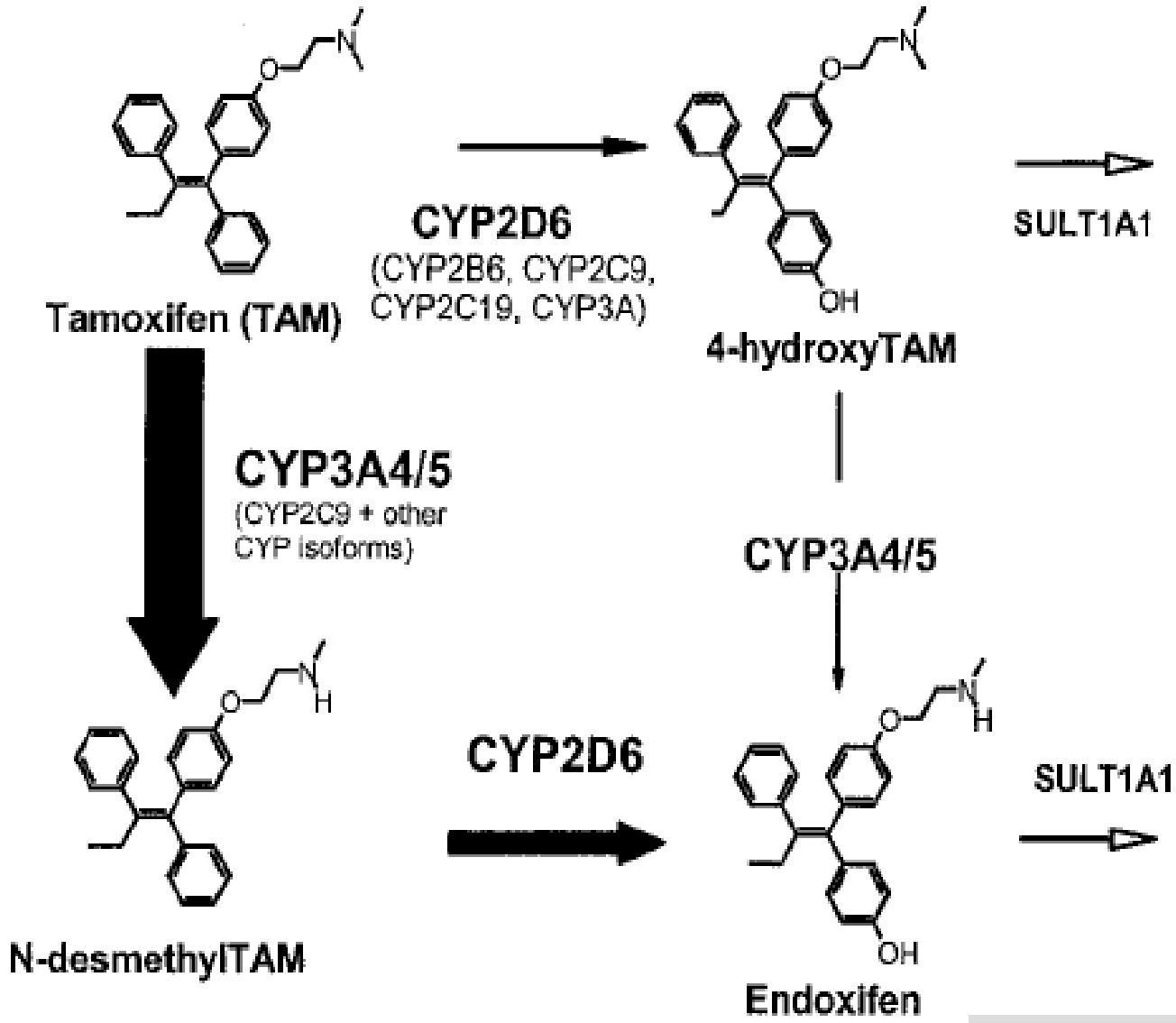
Breast Cancer treatment

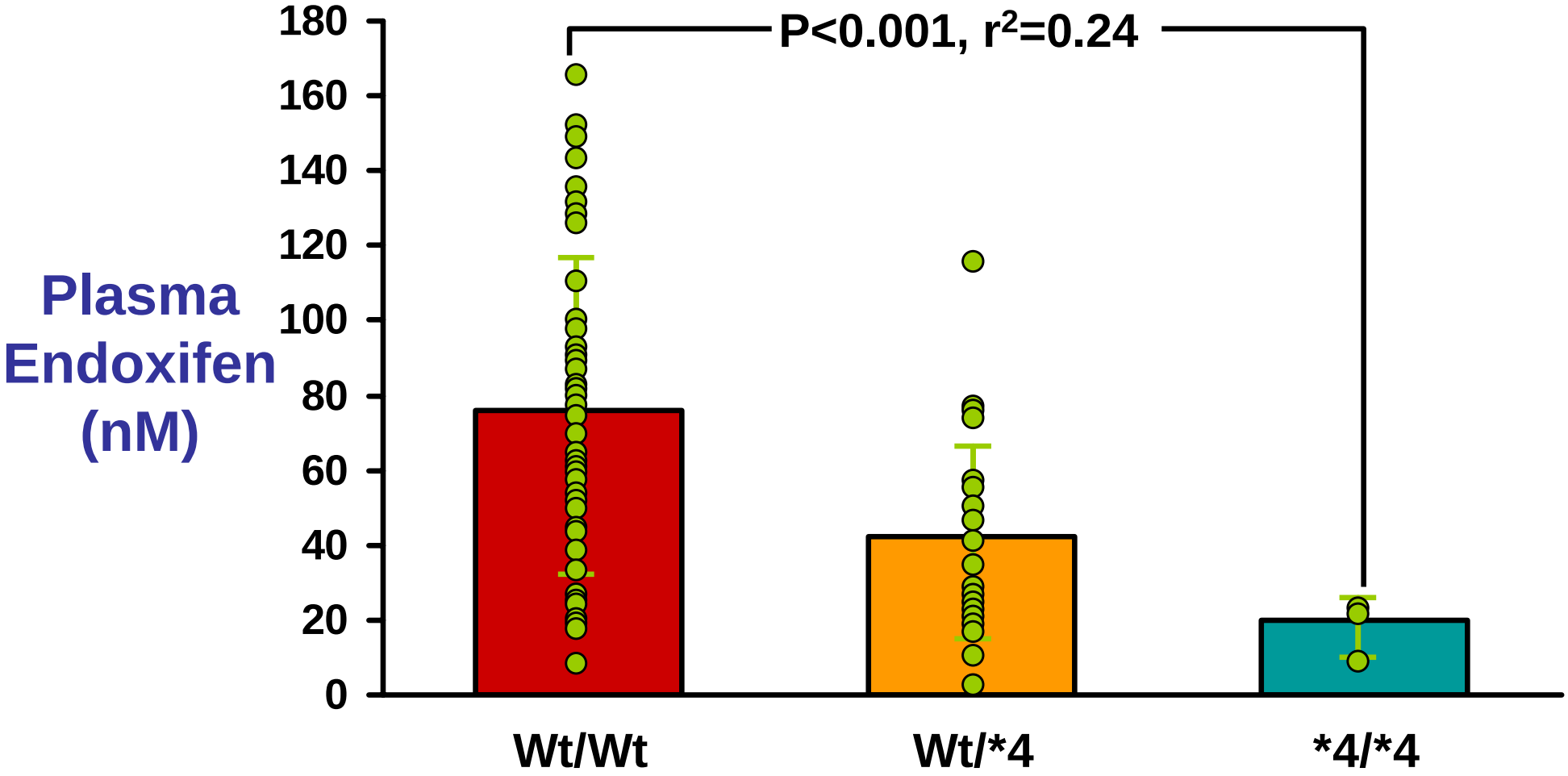
- **Tamoxifen in pre- and post-menopausal women**
- Blocks estrogen receptors and controls tumor growth
- (Activating enzymes: **CYP 2D6**, CYP3A5, CYP2C9, CYP2C19, SULT1A)

Tamoxifen –
Biotransformation of tamoxifen and its metabolites



Jin, Y. et al, J. National Cancer Institute 2005; 97: 30-39

CYP2D6 Genotype and Endoxifen Plasma Levels



CYP2D6*4 (most common genetic variant associated with the CYP2D6 poor metabolizer state)

Jin Y et al., Journal of National Cancer Institute **97**:30, 2005

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Study of Tamoxifen efficiency
Methods

- Retrospective study
- Primary hormon independent non – metastatic breast cancer
- No differentiation between pre and post menopausal women

  (21,6% vs 78.4% in our pilot group)
- **Group of 125 patients**, **<u>primary HORT TMX treatment in 108 women</u>**

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Study of Tamoxifen efficiency Methods II.

- AmpliChip CYP 450 (Roche) has been used for diagnostics of metabolic profile

- 2 ways of results evaluation:
  1. Manufacturter (CE mark evaluation
  2. Internal type of evaluation (even one mutated alele is IM)

- Detailed investigation of co-medication during TMX therapy

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Aim of the project

- Describe the risk of possible relaps of breast cancer during TMX treatment in the connection with CYP 2D6 activity

  - genotype

  - possible inhibition of CYP 2D6 metabolic pathway by co-mediaction

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Stratification of basic patient group by phenotype

Phenotype	AmpliChip-evaluation		Internal evaluation		
UM	1	0.8 %	1	0.8 %	
EM	103	82.4%	58	46.4 %	**75% - 80%**
IM	10	8.0%	55	44.0%	**10% - 15%**
PM	11	8.8%	11	8.8%	**5 % - 10%**
together	125		125		

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Tamoxifen in adjuvance

Median of TMX therapy: **27 months**

Phenotype	AmpliChip evaluation			Internal evaluation		
	No	%	median	No	%	median
UM EM	90	83.3%	25	49	45.4%	23
IM	8	7.4%	20	49	45.3%	25
PM	10	4.3%	27	10	4.3 %	27
Together	108			108		

Tamoxifen progression

	AmpliChip evaluation			Internal evaluation			
	No	%	DFS	No	%	DFS	
UM+EM	13	14.4	37,8	9	18,4	36.5	**90 / 49**
IM	3	37.5	22,7	7	14.3	33.0	**8 / 49**
PM	1	10.0	8	1	10.0	8	**10**
together	17			17			

Preliminary conclusions I

- **Deficiency of CYP 2D6 activity probably increases risk of relaps of breast cancer during Tamoxifen therapy**
- **Diagnostics of CYP 2D6 genotype before breast cancer treatment could improve the decision making process between Tamoxifen and Aromatase inhibitors**

- **„Proper therapy for individual patient in the right time“**

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Tamoxifen – vlastní výsledky

- 160 žen s karcinomem prsu
- hormonálně dependentní primárně nemetastazující karcinom
- délka sledování 5 let
- Primární parametr: progrese onemocnění

- **Cíle projektu :** popsat riziko relapsu nemoci při léčbě tamoxifenem v závislosti na aktivitě CYP2D6 a glykoproteinu P (MDR1

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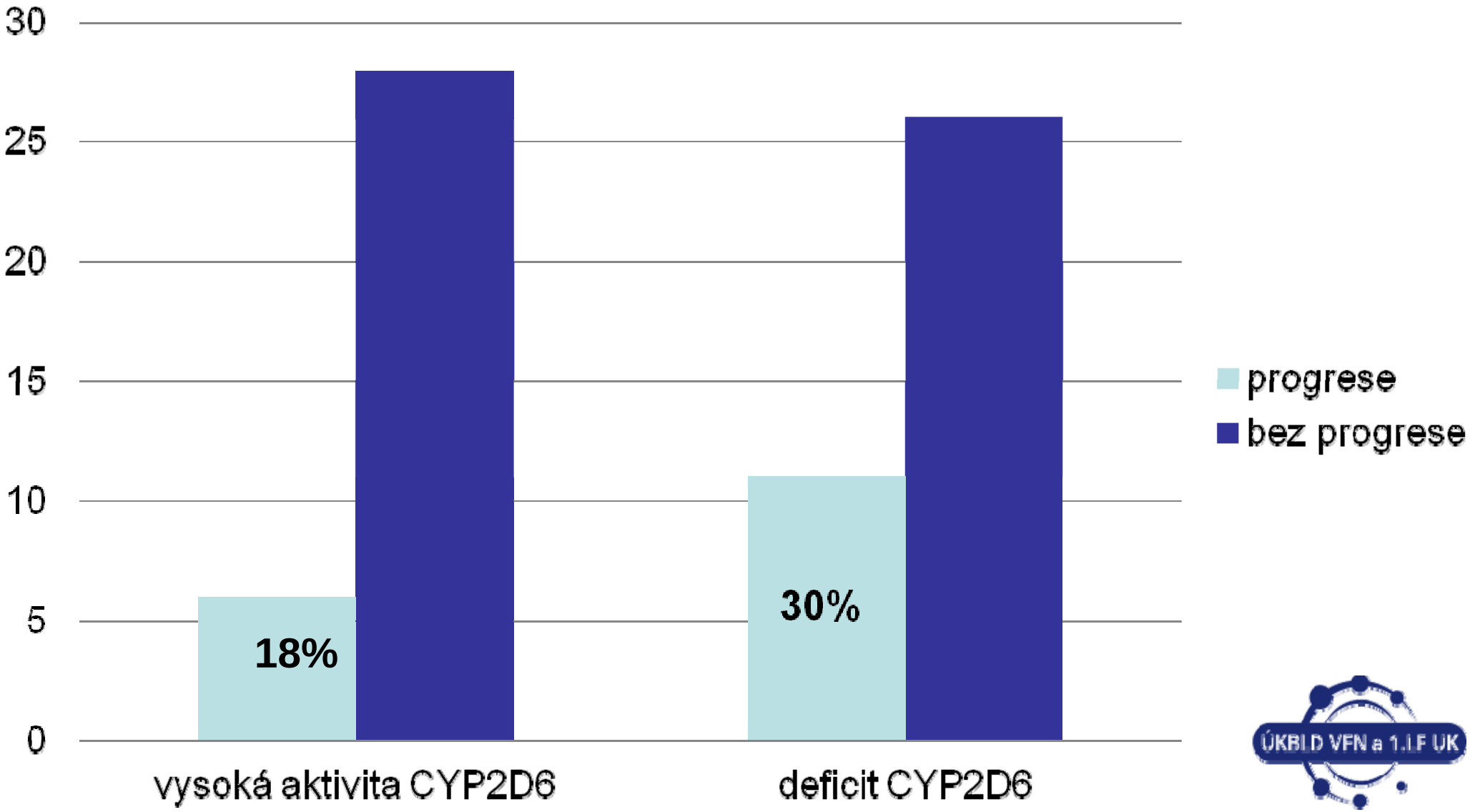
Předběžné výsledky (n=84) CYP2D6



Předběžné závěry

- Deficit aktivity CYP2D6 a vysoká aktivita glykoproteinu P pravděpodobně zvyšují riziko relapsu karcinomu prsu při léčbě tamoxifenem
- Stanovení genotypu před zahájením léčby by mohlo přispět k volbě terapie mezi tamoxifenem nebo inhibitory aromatáz

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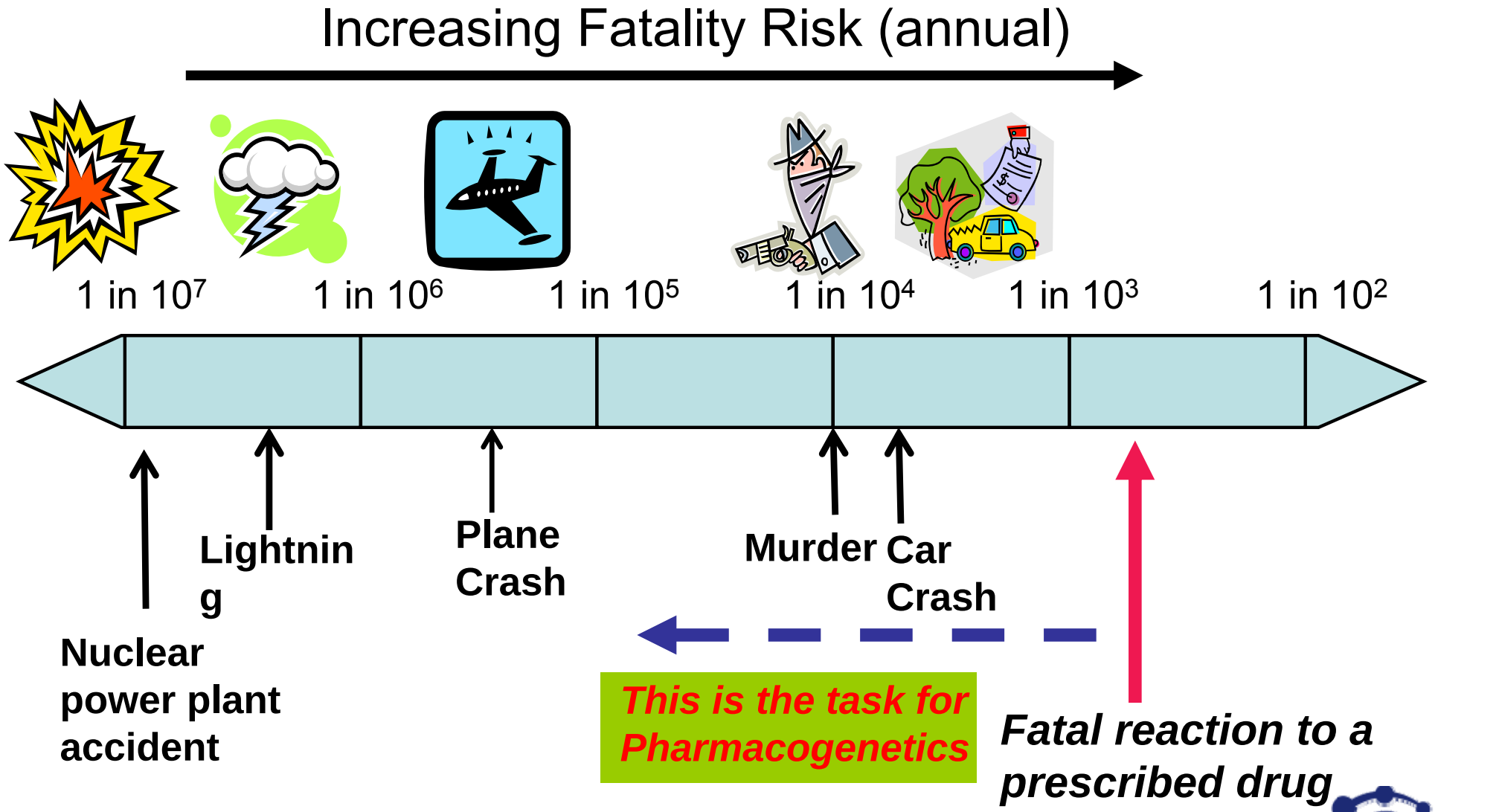
Pharmacogenetic testing will be able to reduce risks



Summary – take home messages

- Individual patients have different capacity of therapeutic drugs metabolization
- Adverse drugs effects or non efficient treatment are serious complicating factors and may lead up to fatalities
- Diagnostics of metabolic profile is valid through the whole life time (genotype does not change)
- World trend to presonalized health care has already started and is irreversible

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Portfolio of other pharmacogenomics tests performed in our hospital

TPMT - Thiopurine S-methyltransferase – azathioprin
UGT1A1 – irinotekan - before starting therapy

CYP2C9 +VKOCR1 (Vitamin K epoxide reductase) – warfarin
CYP2C19 – klopidogrel
CYP2D6 – tamoxifen - before starting therapy

TPMT, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5
- complication, non- compliance

Research – debrisoquine (CYP2D6), MDR1 (P-glycoprotein) – opiates

Methodology

- Microarray – Amplichip (CYP2D6, CYP2C19)
- FRET-PCR (Förster Resonance Energy Transfer)
- multiplex PCR- hybridization
- PCR-RFLP

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Acknowledgement

- Oncology Department
  - Prof. Petruželka, Dr. Argalácsová
- Laboratory
  - Dr. Dražďáková
- Genotype evaluation
  - Dr. Slanař
- AmpliChip CYP 450 kits
  - Roche Diagnostics Prague

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Future?

Here is my sequence…