



# Current Approaches and Applications of Phenotyping Methods for Drug Metabolizing Enzymes and Transporters

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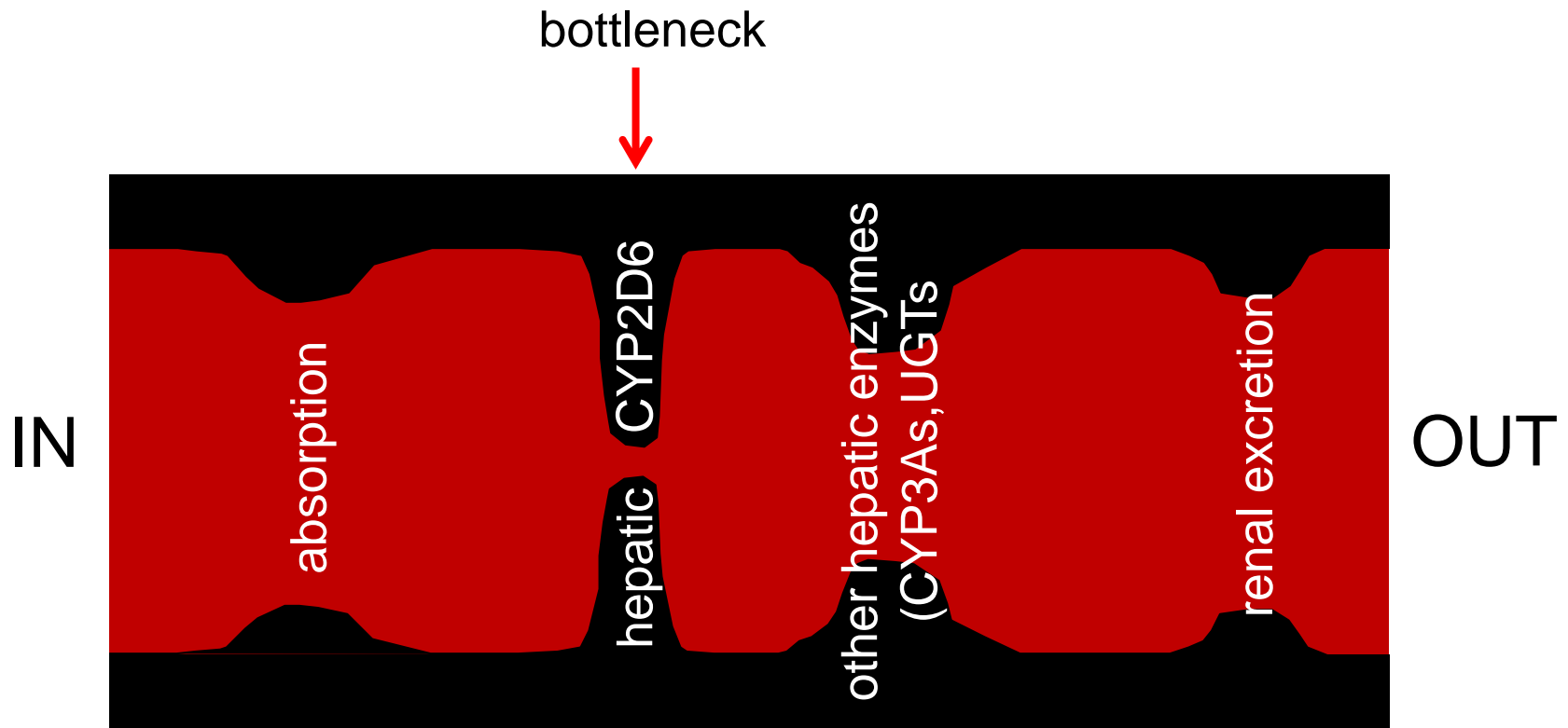


## Definition

Phenotyping is  
quantifying the *in vivo activity*  
of an enzyme or a transporter (E/T)  
mediating a *rate-limiting process* in pharmacokinetics

(probably to be added:  
at a *specific site of expression*)

## Dextromethorphan AUC as a Metric of Hepatic CYP2D6 Activity



(marginally simplified model of human body)



## E/T Activity vs. PK Parameters

### Reasonable unit for activity:

amount of metabolite formed  
per unit of time  
per amount of enzyme  
at a given substrate concentration

### Examples:

nmol  
minute  
nmol of enzyme, g of liver, cm of jejunum, entire liver...  
Km, infinity (=Vmax), XX  $\mu\text{mol/L}$

### What we optimally get is clearance:

volume of plasma cleared  
per unit of time  
per amount of enzyme  
at changing substrate concentrations  
(which are unknown at the binding site)

### Examples:

mL  
minute  
entire person  
?



## Current Approaches

### Standard approach:

1. systemic (mostly oral) administration of a specific substrate of the E/T (often as a cocktail),
2. blood/plasma sampling for a **complete PK profile**,
3. calculation of a metric reflecting systemic (or organ-specific?) E/T **activity and** at the same time **substrate exposure** (**AUC in most cases**).

Alternative substrates: endogenous/dietary compounds

Alternative sampling: less data points, different matrices including DBS

Alternative metrics: systemic clearance, partial clearance, intestinal extraction, renal clearance, renal secretion, metabolic ratios (in plasma, saliva, urine), single point concentrations...



## Number of DDI Studies in clinicaltrials.gov Received in the Last 2 Years

Search terms: drug interaction + ...			
(none)		1137	
Cocktail		18	
Individual CYP probes		Individual Transporter probes	
Midazolam	39 (CYP3A4/5)	Digoxin	13 (P-gp)
Caffeine	19 (CYP1A2)	Dabigatran etexilate	3 (intestinal P-gp)
Tizanidine	0 (CYP1A2)	Fexofenadin	2 (P-gp)
Dextromethorphan	10 (CYP2D6)	Rosuvastatin	12 (OATP1B1&3)
Metoprolol	5 (CYP2D6)	Pitavastatin	2 (OATP1B1)
Desipramine	0 (CYP2D6)	Metformin	11 (OCT2, MATE1, MATE2-K)

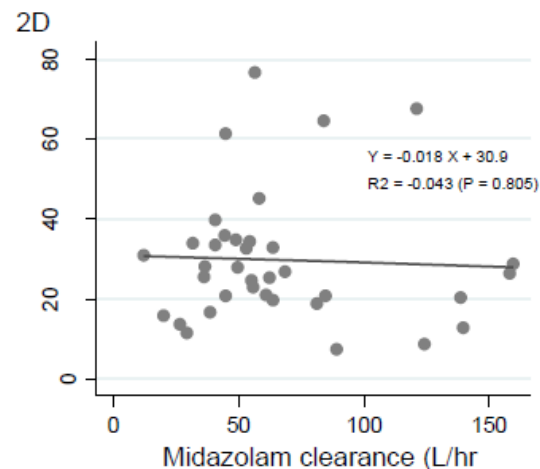
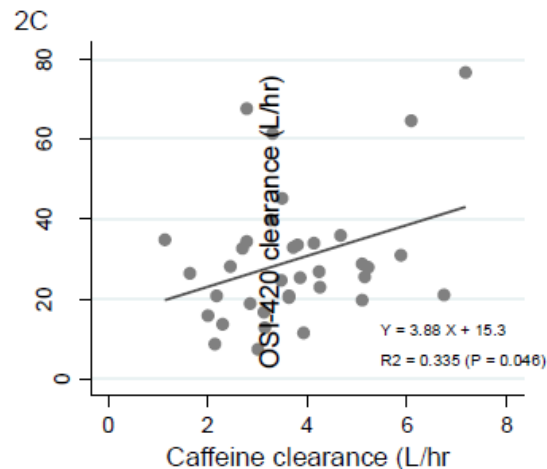
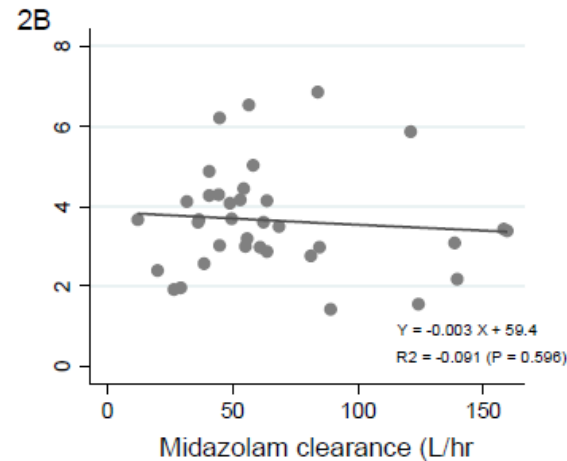
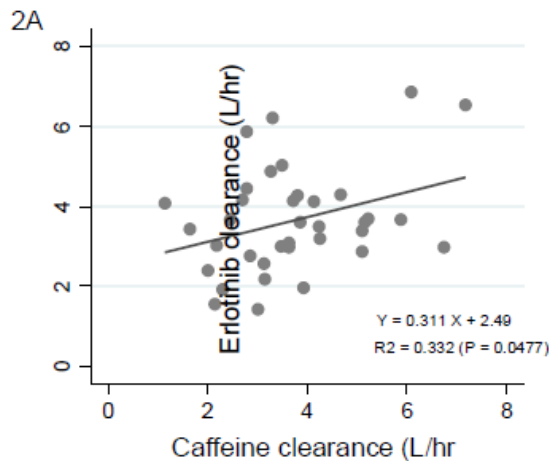


## Applications / Objectives of Phenotyping

- (Improve the understanding of pharmacokinetic processes)
- (Quantify the absolute activity of an E/T in an individual to personalize dosing)
- Assess the effect of potential covariates on E/T activity (mainly DDIs) to predict the effect on pharmacokinetics of E/T substrates



# CYP3A and CYP1A2 Phenotyping to Individualize Erlotinib Treatment in NSCLC?



Day 1 (oral drugs):

- erlotinib 150 mg
- midazolam 2 mg
- caffeine 100 mg
- 6 samples until 6 hours postdose

For erlotinib and metabolite, 4 additional trough values during 10 weeks

→ „phenotyping not suitable to substitute therapeutic drug monitoring”

Parra-Guillen ZP et al., Basic Clin Pharmacol Toxicol. 2017 Apr 26. [Epub ahead of print]





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## Frequent Study Design in DDI Studies

perpetrator drug at highest chronic dose until steady state is reached

Phenotyping 1

about 24 healthy volunteers

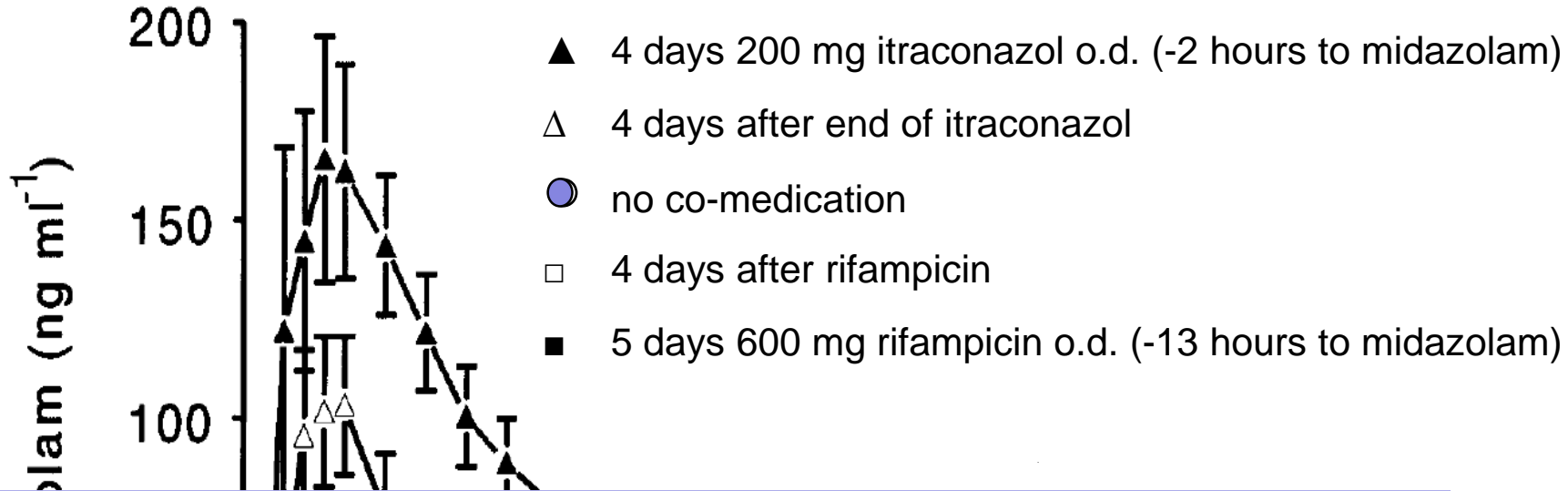
Average bioequivalence approach to estimate effect

Weak effect:	1.25-fold to 2-fold
Moderate effect:	2-fold to 5-fold
Strong effect:	more than 5-fold

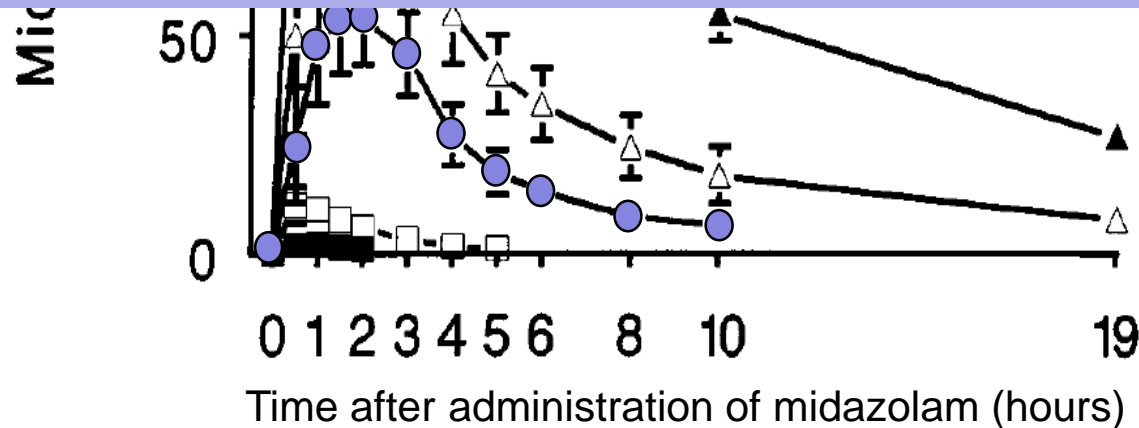
Phenotyping 2



# 400-fold Mean AUC Range of Midazolam 15 mg Single Oral Dose



DDI studies designed to see maximal effect size



Backman et al. Eur J  
Clin Pharmacol 1998  
Mar;54(1):53-8



## Some Established and/or Reasonable Phenotyping Methods

Enzyme/ transporter	target site of expression	Substrate	Dose	Route of ad- ministration	metric
CYP1A2	liver	caffeine	50 - 150 mg	oral	AUC (F=1)
CYP2B6	liver	bupropion	20 mg	oral	AUC (F<0.9)
CYP2C9	liver	tolbutamide	125-500 mg	oral	AUC (F?)
CYP2C9	liver	flurbiprofen	10 mg	oral	AUC (F=0.8)
CYP2C19	liver	omeprazole	10 - 20 mg	oral	AUC (F=0.4)
CYP2D6	liver	dextromethorphan	7 – 22 mg	oral	AUC (F = 0.8 in PMs, 0.01-0.2 in EMs?)*
CYP3A4/5	liver	midazolam	3 µg – 7.5 mg	i.v.	AUC
CYP3A4/5	gut	midazolam	3 µg – 7.5 mg	oral (plus i.v.)	intestinal extraction
CYP3A4/5	gut + liver	midazolam	3 µg – 7.5 mg	oral	AUC (F=0.3)
P-gp	kidney	digoxin	0.25-0.5 mg	oral	renal secretion
P-gp	gut + liver + kidney	digoxin	0.25-0.5 mg	oral	AUC (F= 0.7)
P-gp	gut + liver + kidney	fexofenadin	0.1-125 mg	oral	AUC (F = 0.3)
OATP1B1&3	liver	rosuvastatin	25 µg-10 mg	oral	AUC (F=0.2)
OCT2, MATE1, MATE2-K	kidney	metformin	500 mg	oral	AUC (F=0.55)

\*Capon DA et al., Clin Pharmacol Ther. 1996 Sep;60(3):295-307; Duedahl TH et al., Pain. 2005 Feb;113(3):360-8.



## „Oral Clearance“ – a Confusing Misnomer

Example:

(text) The main CYP enzyme and its contribution to the clearance of each model substrate is listed in Table 1.

Table 1: Cytochrome P450 (CYP) model substrates and the fraction of the dose metabolized by the enzyme that they mark (fm)

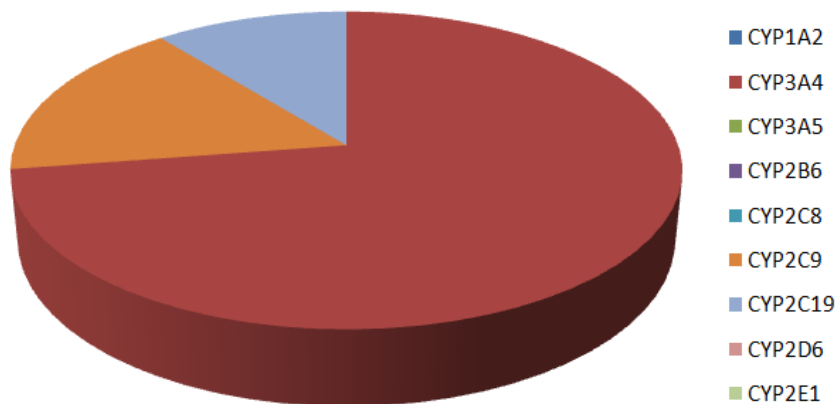
<b>CYP</b>	<b>Substrate</b>	<b>fm</b>
1A2	Phenacetin	0.71
2B6	Bupropion	0.60
2C9	Tolbutamide	1.00
2C19	Omeprazole	0.76
2D6	Desipramine	0.91
3A4/5	Midazolam	0.87/0.1
3A4/5	Alprazolam	0.95/0.05

Barter ZE et al., Clinical Pharmacokinetics 2013, 52: 1085–1100

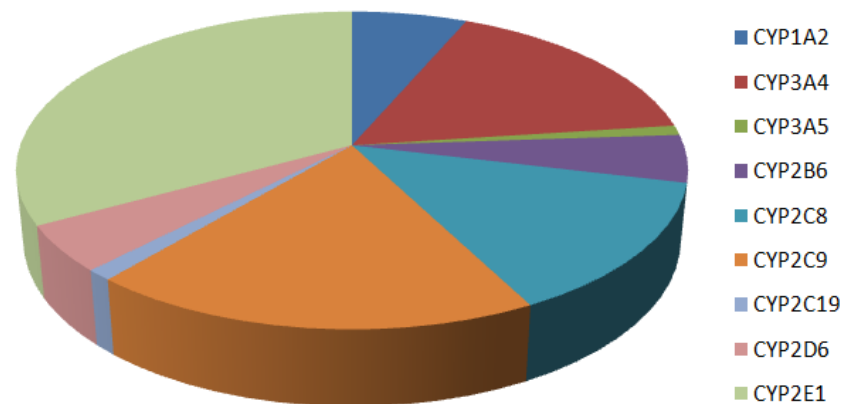


## The Average Human Small Intestine and Hepatic CYP Pies (enzymes quantified by LC-MS/MS)

intestine



liver



Gröer C et al. J Pharm Biomed Anal. 2014 Nov;100:393-401.



## Regulatory Phenotyping Approach for DDIs

- Sensitive probe drugs should be used<sup>a,b</sup>
- Key metric: AUC after oral administration<sup>a,b</sup>
- clearance through a specific pathway recommended if no specific substrate available<sup>a</sup>
- if renal secretion is affected, renal clearance should be considered (e.g., digoxin for P-gp)<sup>a</sup>

but....

<sup>a</sup>EMA: Guideline on the investigation of drug interactions. 21 June 2012, CPMP/EWP/560/95/Rev. 1 Corr. 2\*\*; <sup>b</sup>FDA: Draft Guidance for Industry. Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations February 2012



## Regulatory Phenotyping Approach for DDIs

- Sensitive probe drugs may not be representative for all respective drugs depending on E/T and contribution of first pass metabolism
- AUC depends on bioavailability (F); for many phenotyping drugs, F is low, highly variable or even unknown
- AUC reflects exposure but often is not very helpful for mechanistic considerations
- For transporter substrates, AUC typically reflects activity of multiple transporters at multiple sites
- Renal clearance consists of glomerular filtration plus renal secretion – it appears that using renal secretion would be more informative to assess transporter activity



## Phenotyping Cocktails

Combined administration of phenotyping drugs with no (?) mutual interaction at low doses

Emphasis on CYPs (well established, more mechanism-related):

- Cologne: caffeine + tolbutamide + omeprazole + dextromethorphan + midazolam + digoxin (all orally) + midazolam (i.v.) (see poster Gazzaz et al. on ethanol effect)
- Basel: caffeine + efavirenz + losartan + omeprazole + metoprolol + midazolam (all orally)
- Geneva: caffeine + bupropion + flurbiprofen + omeprazole + dextromethorphan + midazolam + fexofenadine (all orally)
- CIME, Pittsburgh, Karolinska...

Emphasis on transporters (exploratory, more exposure-related):

- (Boehringer): digoxin + furosemide + metformin + rosuvastatin.
- (Merck): midazolam + dabigatran etexilate + pitavastatin + rosuvastatin + atorvastatin (all orally)
- (Cologne): pitavastatin + metformin + adefovir dipivoxil + sitagliptin + digoxin (all orally) (see poster Stoffel et al. for potential mutual interactions)



In summary, the (cocktail) phenotyping approach is an established procedure only in the area of DDIs,

but with a number of limitations to be addressed, including:

- 1: Lack of important PK information for many probe substrates
- 2: (extent of) intestinal metabolism
- 3: extent of hepatic first pass metabolism
- 4: „bottleneck“ property / specificity in all situations
- 5: temporal variability of covariates (mainly co-medication)
- 6: focused on exposure instead of E/T activity, thus results have limited predictive precision for other substrates