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

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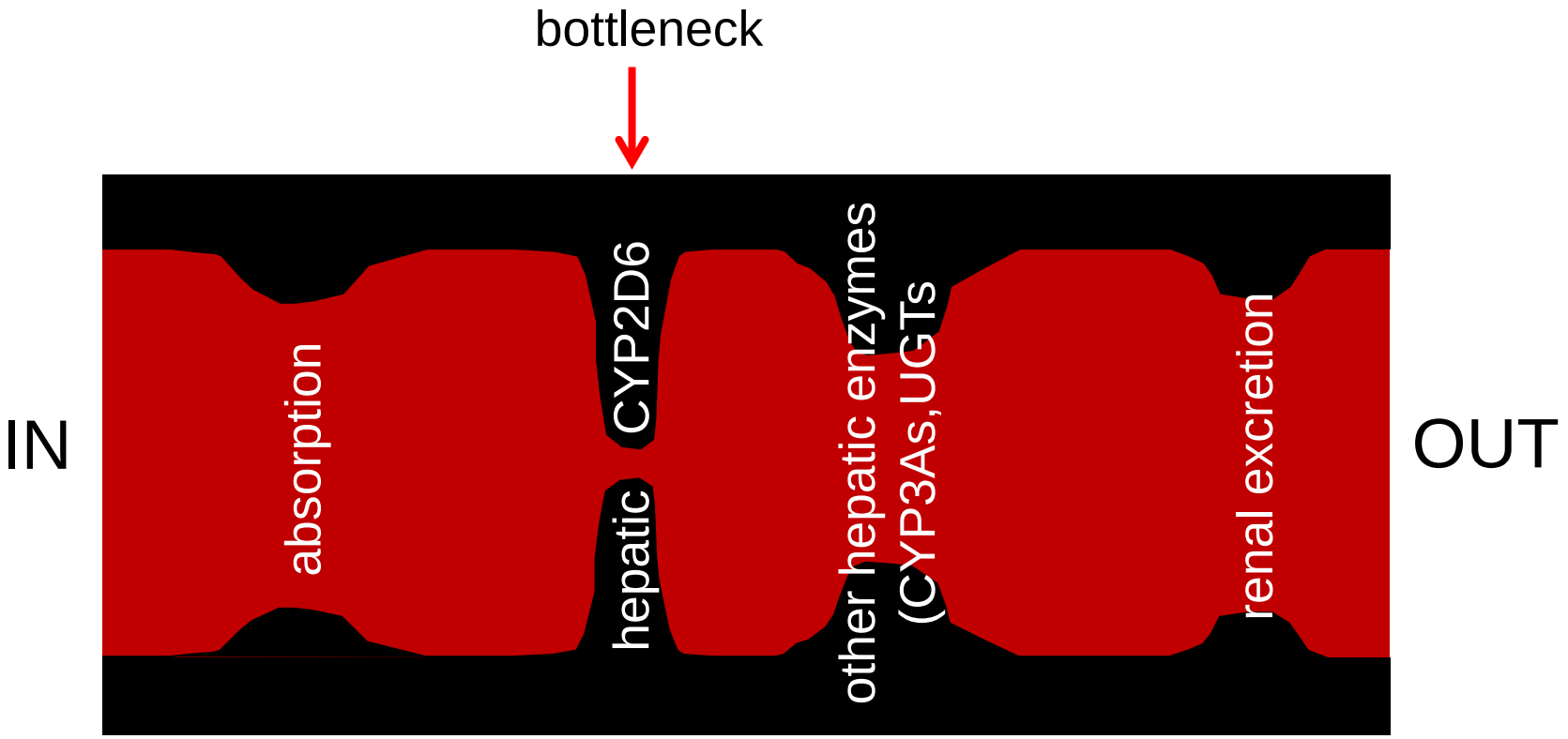
Definition

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:	**Examples:**
amount of metabolite formed	nmol
per unit of time	minute
per amount of enzyme	nmol of enzyme, g of liver, cm of jejunum, entire liver…
at a given substrate concentration	Km, infinity (=Vmax), XX µmol/L

What we optimally get is clearance:	**Examples:**
volume of plasma cleared	mL
per unit of time	minute
per amount of enzyme	entire person
at changing substrate concentrations (which are unknown at the binding site)	?

Current Approaches

Standard approach:

1. systemic (mostly oral) administration of a specific substrate of the E/T (often as a ocktail),
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

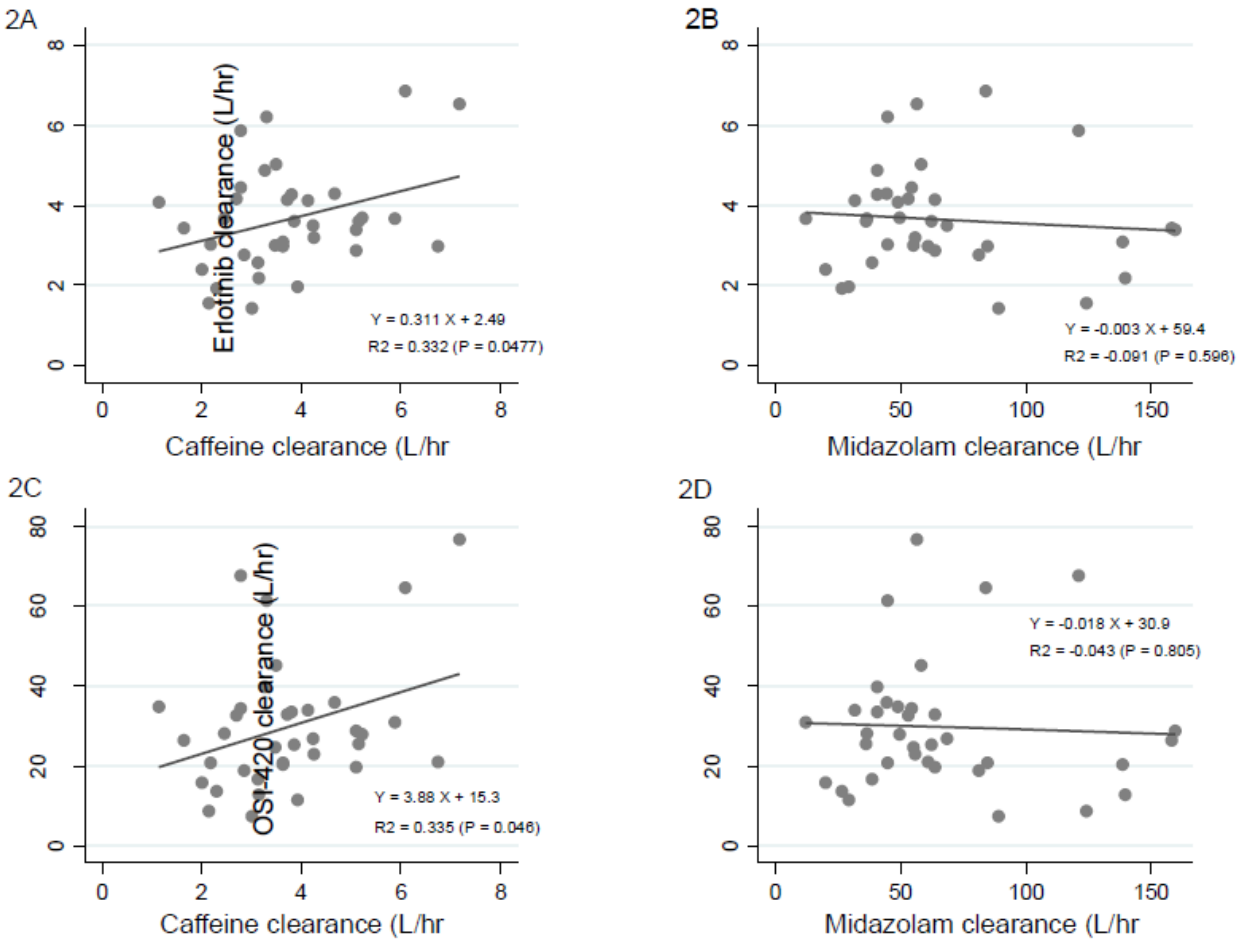
Search terms: drug interaction + …			
(none)		1137	
Cocktail		18	
Individual CYP probes		Individual Transporter probes	
Midazolam	39 (CYP3A4/5)	Digoxin	13 (P-gp)
Caffeine	19 (CYP1A2)	Dabigatran etexilate	3 (intestinal P-gp)
Tizanidine	0 (CYP1A2)	Fexofenadin	2 (P-gp)
Dextromethorphan	10 (CYP2D6)	Rosuvastatin	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

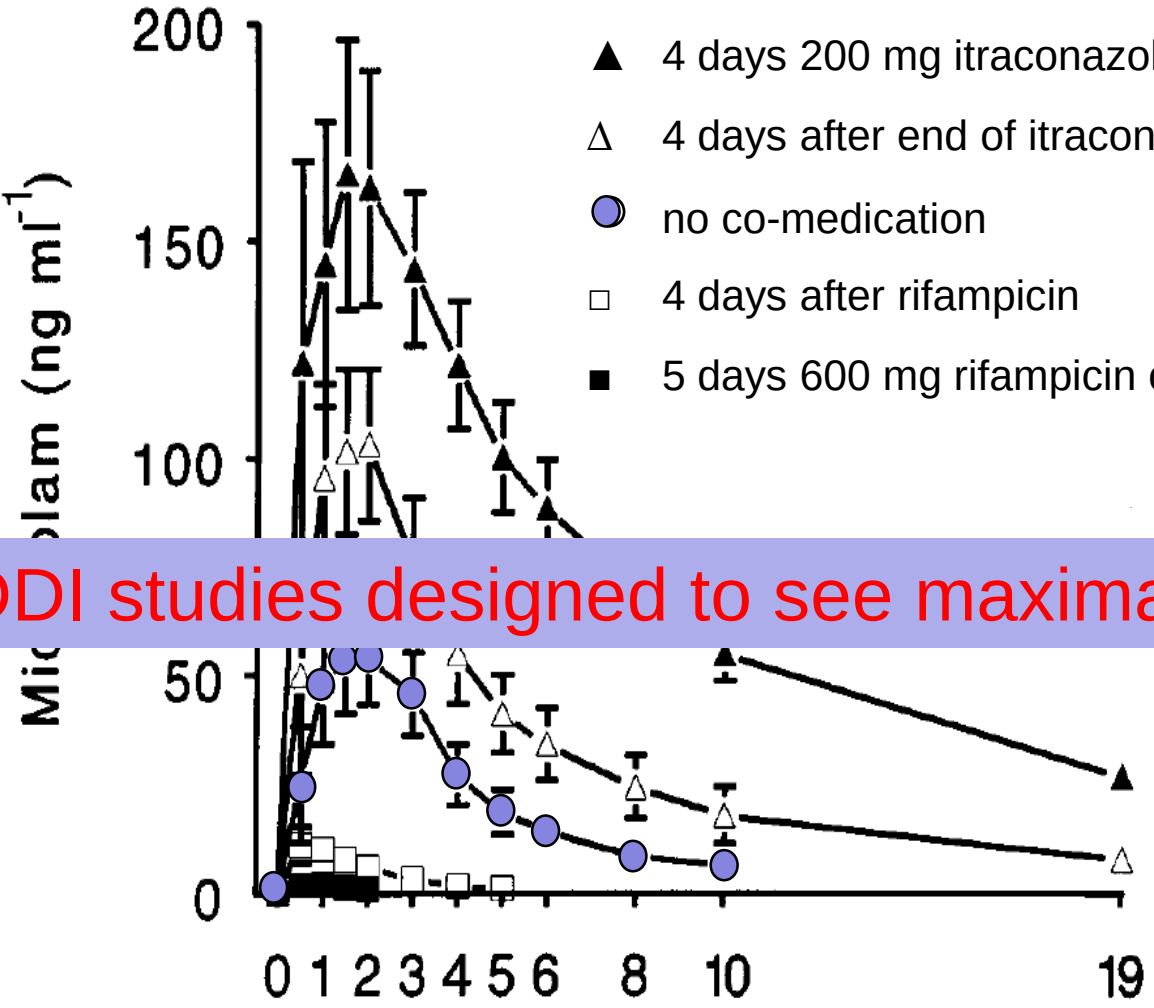
Phenotyping 2

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

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.

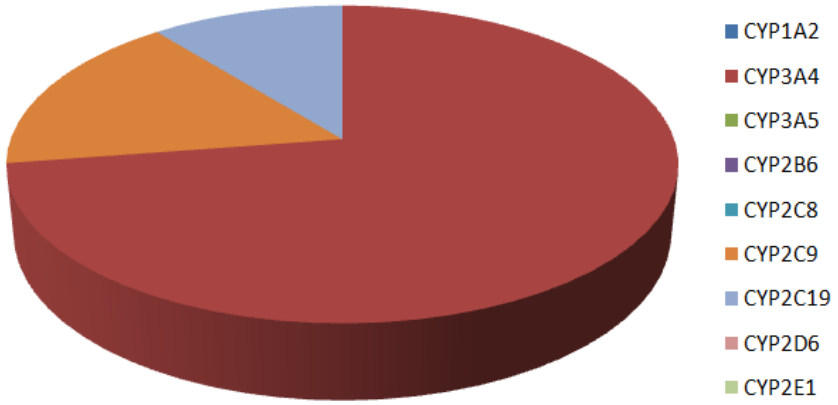
Table 1: Cytochrome P450 (CYP) model substrates and the fraction of the dose metabolized by the enzyme that they mark (fm)

CYP	**Substrate**	**fm**
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

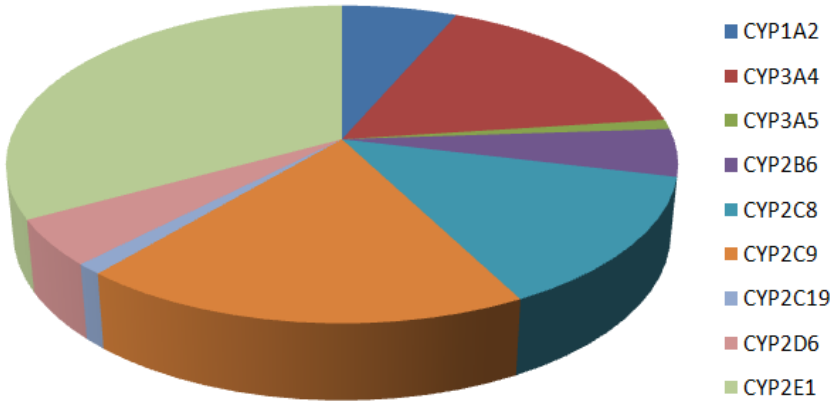
Barter ZE et al., Clinical Pharmacokinetics 2013, 52: 1085–1100

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[a,b]
- Key metric: AUC after oral administration[a,b]
- clearance through a specific pathway recommended if no specific substrate available[a]
- if renal secretion is affected, renal clearance should be considered (e.g., digoxin for P-gp)[a]

but….

[a]EMA: Guideline on the investigation of drug interactions. 21 June 2012, CPMP/EWP/560/95/Rev. 1 Corr. 2**; [b]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)

Universität zu Köln

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