Caveat: Validation and Limitations of Phenotyping Methods for Drug Metabolizing Enzymes and Transporters

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How to Safeguard that Metrics Reflect E/T Activity?

- in healthy volunteers?
- in (any) patients?
- in the presence of co-medications?

Obviously, this applies primarily for mechanistics metrics but it also important for metrics focussing on exposure
Empirical Validation Criteria for Phenotyping (I)

(originally described with respect to hepatic CYP3A)

Metric should:

1. Correlate with the activity of the target enzyme determined in liver biopsies
2. Correlate with the fractional clearance of the probe mediated by the enzyme
3. Correlate with the fractional clearance of other substrates of the enzyme
4. Be reduced when subjects are treated with other substrates of the enzyme (?)
5. Be reduced dramatically when subjects are treated with potent inhibitors of the target enzyme
6. Increase when subjects are treated with known inducers of the target enzyme
7. Be reduced in patients with severe liver disease
8. Be markedly reduced during the anhepatic phase of a liver transplant operation

Empirical Validation Criteria for Phenotyping (II)

Additional criteria:

Test measurement should:

a) Correlate with the content of the target E/T determined in liver biopsies
b) Have high specificity in *in vitro* tests for the metabolic step used
c) Be reproducible (= should have a low coefficient of variation for repeated tests)
d) Reflect respective genetic polymorphisms (if there are any)
e) Be independent of other factors which do not change enzyme activity (e.g. urinary flow, glomerular filtration, hepatic blood flow, other E/Ts…)
f) Analytical assays should adhere to general validation criteria
g) Probe drugs should be registered and readily available
Mechanistic Validation (I):
Know Your Probe Drug and Your Target E/T!

Probe drug: Experimental assessment of
- absolute bioavailability
- intestinal extraction, intestinal secretion
- hepatic extraction, biliary excretion
- relevant transporters and enzymes involved in any possibly relevant pathway
- renal secretion

(would need studies in surgical patients with access to respective blood vessel locations)

Target enzyme / transporter:
- expression sites
- regulation in disease
- genetic polymorphisms
- Splice variants…
EMA 2012: Suggested Probe Drugs for CYPs

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>PROBE DRUG</th>
</tr>
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<tbody>
<tr>
<td>CYP1A2</td>
<td>theophylline, caffeine</td>
</tr>
<tr>
<td>CYP2B6*</td>
<td>efavirenz, S-bupropion (hydroxylation)</td>
</tr>
<tr>
<td>CYP2C8*</td>
<td>amodiaquine (N-deethylation), repaglinide</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>S-warfarin, tolbutamide</td>
</tr>
<tr>
<td>CYP2C19*</td>
<td>omeprazole (single dose)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>metoprolol, desipramine</td>
</tr>
<tr>
<td>CYP3A</td>
<td>midazolam**</td>
</tr>
</tbody>
</table>

*no well-documented probe drug available
**obligatory
Omeprazole: Preparation Related Problems

![Graph showing omeprazole plasma concentrations over time after dosing of 20 mg omeprazole](image)

- **Omeprazole**:
  - **Preparation Related Problems**
  - After dosing of 20 mg omeprazole (hrs):
    - Plasma concentrations (nM)
Effect of Ciprofloxacin (500 mg bid) on Selective CYP1A2 Probes

Caffeine (not sensitive):
mean increase from 1.5 to 3.1
corresponds to 2.1-fold change

Tizanidine (sensitive):
mean increase from 3.4 to 33.1 ng/mL*h
corresponds to 9.8-fold change

Granfors et al., CPT 2004, 76: 598-606
Effect of Ketoconazole (400 mg daily) on Selective CYP3A Probes

### Table: Effect of Ketoconazole on Alprazolam and Midazolam

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alprazolam</th>
<th>Midazolam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral dose</td>
<td>0.5 mg</td>
<td>2 mg</td>
</tr>
<tr>
<td>F</td>
<td>0.9</td>
<td>0.3-0.5</td>
</tr>
<tr>
<td>Fm&lt;sub&gt;CYP3A4&lt;/sub&gt;</td>
<td>about 0.9</td>
<td>about 0.9</td>
</tr>
<tr>
<td>Cmax change (mean)</td>
<td>1.2-fold</td>
<td>4.3-fold</td>
</tr>
<tr>
<td>AUC change (mean)</td>
<td>2.7-fold</td>
<td>16.3-fold</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2z&lt;/sub&gt; change (mean)</td>
<td>2.4-fold</td>
<td>3.7-fold</td>
</tr>
</tbody>
</table>

Boulenc X et al., Eur J Drug Metab Pharmacokinet. 2016;41:45-54.
Time Course of Covariate Effect in DDIs

e.g., inflammation

e.g., gut wall concentration of oral inhibitor at steady state

e.g., plasma concentration of oral inhibitor at steady state
Remember: the (cocktail) phenotyping approach is an established procedure …

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
A Case for Focusing on Mechanistic Approaches

- (Semi)empirical approach: worst case scenario, attempt to assess maximal effect on exposure (vs.)
- Mechanistic approach: attempt to achieve the most accurate understanding of a factor to modify activity of an enzyme / transporter

- If effect on E/T activity is quantified correctly, PBPK can be used to predict effects of this factor on any drug depending on and E/T pathway
- Worst case scenarios generated by PBPK are expected to be more reliable and more specific; they may even be worse because situations can be simulated which were not addressed in the „worst case scenario“ (e.g., other timing of intake; missing additional pathways; impaired liver function...)
Inhibitor with Effect on 1st Pass Metabolism and on Systemic Clearance

Red: Inhibitor

Blue: substrate

Cmax/Ki = 0.1, rapid inh. absorp.

Cmax/Ki = 10, rapid inh. absorp.

Cmax/Ki = 0.1, slow inh. absorp.
Mechanistic Validation (II): Compare Predictions by PBPK with Available Experimental Data

Virtual assessment using:

- Sensitive probe drugs (i.e. with high 1st pass metabolism) and less sensitive probe drugs for E/T
- Potent and less potent inhibitors / inducers of E/T
- E/T genotype effects
- Patients with organ dysfunction and/or multiple interacting drugs

- If no experimental data available, at least sensitivity analyses should be done
Validation Issues for Phenotyping Cocktails

- The “cocktail” should be composed of specific probe drugs (see also appendix VIII) for each of the enzymes to be studied. The specificity of the probe drugs should have been demonstrated in DDI studies with selective inhibitors of the specific enzyme and/or in pharmacogenetic studies.

- It should have been demonstrated in vivo that the probe drugs combined in the “cocktail” do not interact with each other. The doses used should preferably be the doses used in this validation. Deviations from this should be justified.

- Full characterisation of the plasma concentration-time curves of the probe drug is recommended, estimating the effect on (oral) clearance or AUC.

EMA DDI guideline 2012.
Mutual Interaction of Cocktail Components (I)

Mean ± SD

„no significant differences“ (ANOVA)
N=14

caffeine, chlorzoxazone, dapsone, debrisoquin, and mephenytoin

CYP1A2, paraxanthine/caffeine in plasma 8 hours after 100 mg caffeine;

CYP2C19, urinary excretion of 4’-OH-mephenytoin after 100 mg mephenytoin;

CYP2D6, urinary molar ratio 4-OH-debrisoquine /(debrisoquine+ 4-OH-debrisoiline) 0-8 hours after 10 mg debrisoquine;

CYP2E1, 6-OH-chlorzoxazone/ chlorzoxazone in plasma 4 hours after 250 mg chlorzoxazone;

CYP3A, urinary molar ratio dapsone/(dapsone + dapsone) 0-8 hours after 100 mg dapsone

Mutual Interaction of Cocktail Components (II)

CYP1A2 (100 mg caffeine),
CYP2C9 (10 mg warfarin),
CYP2C19 (20 mg omeprazole),
CYP2D6 (100 mg metoprolol),
CYP3A (0.03 mg/kg midazolam)
administered orally alone and as a cocktail, N=30

$C_{\text{max}}$, AUC(last) and AUC 90% ratio CIs were within the prespecified bioequivalence limits of 0.80 - 1.25.

Cocktail Tolerability

- Drugs with very long experience and excellent individual tolerability
- Low doses
- No mutual interaction

- Excellent tolerability also as a cocktail
Surprise!

- Cocktail with 50 mg tramadol (CYP2D6), 20 mg omeprazole (CYP2C19), 25 mg losartan (CYP2C9) and 200 mg caffeine (CYP1A2)
- Pilot study in 12 healthy males: no AEs
- Subsequent study in 15 males & 7 females: 4 females had unacceptable moderate and severe adverse reactions including headache, dizziness, nausea, vomiting, blue fingers, nails and lips and difficulties in urinating

Reason: unknown, tramadol is a “dirty” drug with many active metabolites binding to a large range of targets

Take-Home Considerations

+ Phenotyping (including phenotyping cocktails) for drug metabolizing enzymes is a very helpful, partially validated, and accepted tool for the assessment of drug-drug interactions.

+ Phenotyping typically has excellent tolerability.

- Phenotyping (including phenotyping cocktails) for drug transporters is still exploratory; while selective probes are sparse, at least better metrics can be used.

- Selection of phenotyping metrics should be mechanism-focused rather than exposure-focused.

- Validation of individual probe drugs needs to be extended. This means detailed understanding of pharmacokinetic properties in addition to fulfillment of defined empirical validation criteria, and the application of PBPK for mechanistic validation.

- PBPK modeling is also essential to extrapolate results to other substrates.

- Registration of probe drugs as diagnostic drugs would be desirable to avoid losing a wealth of knowledge once a therapeutic drug used as probe is withdrawn from the market.
Thank you!