DDI regulatory guidance
An easy to follow guide.
3rd Edition
Background

This review has been put together by popular request from our clients and provides a summary of the guidance documents currently used for making regulatory decisions on whether to proceed with clinical drug drug interaction (DDI) studies.

It reviews and summarises the following two documents and focuses predominantly on in vitro assessment;


This guide should be only used in conjunction with the regulatory guidance as these provide more detailed information on the test system requirements and data analysis techniques.
## Reaction Phenotyping

Reaction phenotyping studies identify which enzymes are catalysing the main elimination pathways.

### Enzyme Systems
- Human liver subcellular fractions (e.g., microsomes, S9 and cytosol).
- Freshly isolated or cryopreserved human hepatocytes.
- Human recombinant enzymes.

### Main Enzymes of Interest
- CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A.

### Significance of Data
If a metabolic pathway constitutes ≥25% of a drug's overall elimination, in vivo studies using appropriate inhibitors/inducers are warranted (or PBPK modelling can be considered).

### Special Considerations
- Investigation of other CYP pathways (e.g., CYP2A6, CYP2J2, CYP4F2, CYP2E1), non-CYP Phase I metabolism (e.g., AO, CES, MAO, FMO, XO and ADH/ALDH), or Phase II enzymes (e.g., UGTs and SULTs).
- Genetic polymorphic enzymes and special populations.
- Pharmacologically active metabolites.
- Two methods recommended (specific enzyme inhibition and human recombinant enzymes).

### US FDA Guidance 2020
- Human liver subcellular fractions (e.g., microsomes, S9 and cytosol).
- Freshly isolated or cryopreserved human hepatocytes.
- Human recombinant enzymes.

### EMA Guidance, adopted 2012
- Human liver microsomes or S9 fraction.
- Human hepatocytes.
- Human recombinant enzymes.

### Enzymes involving metabolic pathways estimated to contribute ≥25% of drug elimination should be identified if possible and the in vivo contribution quantified.

- Genetic polymorphic enzymes and special populations.
- Pharmacologically active metabolites / prodrugs.
- Metabolites with off target activity.
- Mass balance studies to confirm and quantify relevant metabolic pathways.
# Enzyme Inhibition

Enzyme inhibition studies identify the potential of an investigational drug to inhibit the metabolism of other co-administered drugs.

<table>
<thead>
<tr>
<th>Description</th>
<th>US FDA Guidance 2020</th>
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</table>
| **Enzyme System** | • Human liver microsomes.  
• Human hepatocytes.  
• Human recombinant enzymes. | • Human liver microsomes.  
• Human hepatocytes.  
• Cells expressing the enzyme of interest. |
| **Main Enzymes of Interest** | • CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 (2 substrates). | • CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A (2 substrates).  
• UGT's including UGT1A1 and UBT2B7 if major elimination pathway is direct glucuronidation. |
| **Type of Inhibition** | • Reversible.  
• Time dependent. | • Reversible.  
• Time dependent. |
| **Significance of Data** | **Basic model**  
Clinical study recommended if;  
\[ R_1 \geq 1.02 \] (also for oral CYP3A4 inhibitors, \( R_{1,gut} \geq 11 \)) and  
\[ R_2 \geq 1.25 \] where,  
For reversible inhibition:  
\[ R_1 = 1 + \left( \frac{I_{\text{max},u}}{K_{I,u}} \right) \]  
\[ R_{1,gut} = 1 + \left( \frac{I_{\text{gut}}}{K_{I,u}} \right) \]  
For time dependent inhibition:  
\[ R_2 = \left( \frac{k_{\text{obs}} + k_{\text{deg}}}{k_{\text{deg}}} \right) \]  
Mechanistic and dynamic models can also be used.  
A lower fu than 1% should not be used in the calculations due to uncertainty in the determination, and a figure of 1% should be used in these circumstances. | **Clinical study recommended if;**  
For reversible inhibition:  
**Hepatic (and renal exposure);**  
\[ [I]/K_i \geq 0.02 \] where \([I]\) is the mean unbound \( C_{\text{max}} \) at the highest dose.  
A lower fu than 1% should not be used in the calculations due to uncertainty in the determination, and a figure of 1% should be used in these circumstances.  
**Orally administered drugs which inhibit enzymes with pronounced intestinal expression (e.g., CYP3A4);**  
\[ [I]/K_i \geq 10 \] where \([I]\) is maximum dose taken on one occasion/250mL.  
For time dependent inhibition:  
Ratio of predicted CL in presence and absence of inhibitor \( (R) = \left( \frac{k_{\text{obs}} + k_{\text{deg}}}{k_{\text{deg}}} \right) \)  
and \( k_{\text{obs}} = \left( \frac{\text{kinact} \times [I]}{K_{I} + [I]} \right) \)  
If \( \geq 20\% \) inhibition (i.e., \( R \geq 1.25 \)) then multiple dose in vivo interaction study recommended.  
Mechanistic and dynamic models can also be used. |
| **Special Considerations** | \( IC_{50}/2 \) can be used as estimate of \( K_i \) if substrate concentration equal to \( K_m \)  
Evaluation of enzyme inhibition of major metabolites under certain circumstances. | Evaluation of enzyme inhibition of any single Phase I metabolites present at \( \geq 25\% \) of the parent drug AUC and \( \geq 10\% \) of drug-related exposure (determined in mass balance study).  
The free fraction in the incubate (\( f_{\text{incubate}} \)) should be determined and used in the calculation of the inhibition constant (especially if no subsequent in vivo studies planned or if it is suspected there could be non-specific binding issues). |

\( I_{\text{max},u} = \) maximal unbound plasma concentration of interacting drug at steady state  
\( I_{\text{gut}} = \) intestinal luminal concentration of interacting drug = molar dose/250mL  
\( R = \) predicted ratio of CLint of probe substrate in absence and presence of inhibitor  
\( K_{I,u} = \) unbound reversible inhibition constant  
\( k_{\text{deg}} = \) degradation constant of affected enzyme  
\( k_{\text{inact}} = \) maximal inactivation rate constant  
\( k_{\text{obs}} = \) apparent inactivation rate  
\( K_i = \) reversible inhibition constant  
\( K_m = \) inhibition constant producing half maximal rate of inactivation  
\( K_{I} = \) maximum inactivation rate constant  
\( I = \) inhibitor concentration  
\( f_{\text{incubate}} = \) free fraction in the incubate  
\( \text{kinact} = \) maximal inactivation rate constant  
\( \text{mass balance study} = \) study that determines the exposure of a drug and its active metabolites.
# Enzyme Induction

Enzyme induction studies identify the potential of an investigational drug to induce the metabolism of other co-administered drugs through upregulation of enzyme expression.

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<tr>
<td><strong>Enzyme System</strong></td>
<td>• Plateable, cryopreserved or freshly isolated, human hepatocytes (at least 3 donors). &lt;br&gt; • Immortalised hepatic cell lines (with justification). &lt;br&gt; • Cell receptor assays only considered as supportive data.</td>
<td>• Cultured fresh or cryopreserved human hepatocytes (at least 3 donors). &lt;br&gt; • Cell lines, nuclear receptor binding or reporter gene assays only considered as supportive data.</td>
</tr>
<tr>
<td><strong>Main Enzymes of Interest</strong></td>
<td>• CYP1A2, CYP2B6 and CYP3A4 initially. &lt;br&gt; • CYP2C8, CYP2C9, CYP2C19 if CYP3A4 induction observed.</td>
<td>• CYP1A2, CYP2B6 and CYP3A</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>• Vehicle control. &lt;br&gt; • Positive control (known strong inducer). &lt;br&gt; • Negative control (known non inducer).</td>
<td>• Vehicle control &lt;br&gt; • Positive control (known strong inducer)</td>
</tr>
<tr>
<td><strong>Endpoint</strong></td>
<td>• mRNA and/or catalytic activity. &lt;br&gt; • Measuring concentration of parent drug in the medium at several time points on the last day of the incubation is encouraged. &lt;br&gt; • The concentration of unbound test drug should be measured.</td>
<td>• mRNA recommended. &lt;br&gt; • Catalytic activity recommended if induction due to protein stabilisation suspected. &lt;br&gt; • Concentration of parent drug in the medium should be measured at several time points on the last day of the incubation unless shown previously that loss is negligible or medium change interval compensates for loss. &lt;br&gt; • The degree of protein binding in the medium and non-specific binding should be considered and unbound concentrations used for the evaluation. &lt;br&gt; • Cell viability/morphology should be determined at the beginning and end of the incubation period at the highest concentration.</td>
</tr>
<tr>
<td><strong>Significance of Data</strong></td>
<td>A drug has the potential to induce in vivo if in any 1 donor; &lt;br&gt; • Fold change in CYP mRNA is ≥2 and concentration dependent at expected hepatic concentrations of drug. &lt;br&gt; OR &lt;br&gt; • Increase in mRNA is &gt;20% of positive control response. &lt;br&gt; OR &lt;br&gt; • ( R_3 \leq 0.8 ) where, &lt;br&gt; [ R_3 = \frac{1}{1 + d \times \left( \frac{E_{\text{max},i} \times 10 \times I_{\text{max,u}}}{E_{\text{IC50}} + (10 \times I_{\text{max,u}})} \right)} ] &lt;br&gt; OR &lt;br&gt; • Exceeds positive criteria defined using correlation methods.</td>
<td>Induction considered positive if; &lt;br&gt; • The drug gives rise to more than a 100% increase in mRNA which is concentration dependent. &lt;br&gt; • Increase in mRNA is greater than 20% of response of positive control. &lt;br&gt; OR &lt;br&gt; • Exceeds positive criteria defined using correlation methods.</td>
</tr>
<tr>
<td><strong>Special Considerations</strong></td>
<td>The unbound fraction in plasma should be set to 1% if &lt;1%.</td>
<td>Enzyme down-regulation should also be considered. &lt;br&gt; Potential human teratogens need to be studied in vivo for effects on contraceptive steroids if intended for use in fertile women, regardless of in vitro induction data.</td>
</tr>
</tbody>
</table>

- \( E_{\text{IC50}} \) = concentration causing half maximal effect  
- \( E_{\text{max},i} \) = maximal unbound plasma concentration of the interacting drug  
- \( I_{\text{max,u}} \) = maximum induction effect  
- \( d \) = scaling factor that is assumed as 1 for the basic model
## Transport Substrate Identification

Transport substrate identification studies identify the potential of an investigational drug to be a substrate of a clinically relevant transport.

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<tr>
<td><strong>Transporter System</strong></td>
<td>Caco-2 cells, overexpressed cell lines or membrane vesicles.</td>
<td>Caco-2 cells or other in vitro systems (vector systems overexpressing the transporter, gene knockout systems or silencing mRNA systems etc).</td>
</tr>
<tr>
<td><strong>Recommended Transporters</strong></td>
<td>P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K</td>
<td>OATPs and clinically relevant renal, biliary and intestinal transporters.</td>
</tr>
<tr>
<td><strong>Specific Recommendations for in vitro Substrate Identification Studies</strong></td>
<td>• All drugs should be evaluated for P-gp and BCRP unless BCS Class I.</td>
<td>• Drugs with estimated hepatic elimination (total hepatic metabolism and biliary secretion) of ≥25% need to be evaluated for OATP1B1 and OATP1B3.</td>
</tr>
<tr>
<td></td>
<td>• Drugs need to be evaluated for OATP1B1 and OATP1B3 if hepatic or biliary secretion is ≥25% of total clearance or if hepatic uptake is clinically important.</td>
<td>• If renal or biliary/gut wall secretion separately is estimated to account for &gt;25% of drug elimination then transporter involved in the active secretion should be identified.</td>
</tr>
<tr>
<td></td>
<td>• Drugs need to be evaluated for OAT1, OAT3, OCT2, MATE1 and MATE2-K if active renal secretion* is ≥25% of total clearance.</td>
<td></td>
</tr>
<tr>
<td><strong>Significance of Data</strong></td>
<td>• A drug is considered to be a substrate for P-gp or BCRP if the net flux ratio (or efflux ratio) is ≥2 and is inhibitable by known inhibitor(s).</td>
<td>• In Caco-2 cells, if efflux ratio &lt;0.5 or &gt;2, it is concluded that active uptake or efflux is occurring respectively. This is confirmed using selective inhibitors.</td>
</tr>
<tr>
<td></td>
<td>• A drug is considered to be a substrate for OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K if uptake in transfected cells is ≥2 fold that of empty vector cells and is inhibitable by known inhibitor.</td>
<td>• An in vivo study with a strong inhibitor is recommended for any transporter which is identified.</td>
</tr>
<tr>
<td><strong>Special Considerations</strong></td>
<td>• Multiple concentrations of the test drug should be evaluated to cover the range of clinically relevant concentrations.</td>
<td>• Genetic polymorphic transporters.</td>
</tr>
<tr>
<td></td>
<td>• Factors such as recovery, stability, solubility, cytotoxicity, and nonspecific binding of the the test drug should be considered.</td>
<td>• Special populations.</td>
</tr>
<tr>
<td></td>
<td>• Evaluation of more polar (e.g., Phase II) metabolites should be assessed on a case-by-case basis.</td>
<td>• Metabolites with pharmacological activity or off-target effects.</td>
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<tr>
<td></td>
<td></td>
<td>• If transporter is present at multiple sites and exposed to markedly different concentrations (e.g., P-gp in intestine and kidney/liver/BBB) at least 4 concentrations should be studied to cover an approx. 100 fold range.</td>
</tr>
</tbody>
</table>

*Active secretion = \( \text{CL} \text{r} - (f_{u,p} \times \text{GFR}) \), where
- \( \text{CL} \text{r} \) = renal clearance
- \( f_{u,p} \) = fraction unbound in plasma
- GFR = glomerular filtration rate = 125 mL/min
## Transport Inhibition

Transport inhibition studies identify the potential of an investigational drug to inhibit clinically relevant transport of other co-administered drugs.

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<tr>
<td>Transporter System</td>
<td>Caco-2 cells, overexpressed cell lines or membrane vesicles.</td>
<td>Suitable in vitro system where human transporter function preserved.</td>
</tr>
<tr>
<td>Recommended Transporters</td>
<td>P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K.</td>
<td>P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, OAT3. BSEP also preferably investigated. OCT1, MATE1 and MATE2 could also be considered.</td>
</tr>
<tr>
<td>Specific Recommendations for Transporter Inhibition Studies</td>
<td>• All drugs should be evaluated as inhibitors for P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K.</td>
<td>• All drugs should be evaluated as inhibitors for P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, OAT3, and preferably BSEP.</td>
</tr>
<tr>
<td>Significance of Data</td>
<td>A drug has the potential to inhibit in vivo if; P-gp and BCRP inhibition I_{gt}/IC_{50} or K_{i} ≥ 10 (oral route) or I_{i}/IC_{50} or K_{i} ≥ 0.1 (parenteral route or metabolite) Where I_{gt} = Dose of inhibitor (in mol)/250mL (if IC_{50} is molar unit) I_{i} = C_{max} of inhibitor or metabolite OATP1B1 and OATP1B3 inhibition R = 1 + ((fu,p x I_{in,max})/IC_{50}) ≥ 1.1 OCT2, OAT1, OAT3, MATE1 and MATE2-K inhibition I_{max,u} / IC_{50} ≥ 0.1 Unbound fraction should be set to 1% if determined to be &lt;1%.</td>
<td>Clinical study recommended if; Intestinal transporters (eq P-gp) K_{i} ≤ 0.1 fold the maximum dose on one occasion/250mL (or, if low solubility, the maximum possible concentration range at the pH range of the GI tract). Hepatic uptake (after oral administration) transporters K_{i} ≤ 25-fold the unbound maximum hepatic inlet concentration. Renal uptake and efflux, hepatic efflux and hepatic uptake (after i.v. administration) transporters K_{i} ≤ 50-fold the unbound C_{max}. Unbound fraction should be set to 1% if determined to be &lt;1%.</td>
</tr>
<tr>
<td>Special Considerations</td>
<td>• Preincubation step with the test drug is suggested for OATP1B1 and OATP1B3 inhibition. • Evaluation of more polar (e.g., Phase II) metabolites should be assessed on a case-by-case basis. • Clinically relevant concentrations of test drug based on transporter location should be considered.</td>
<td>• The use of 2 separate systems is recommended for P-gp due to the current high inter-laboratory variability.</td>
</tr>
</tbody>
</table>

$R$ = predicted ratio of victim drug's AUC in presence and absence of the investigational drug

$f_{up}$ = unbound fraction in plasma

$IC_{50}$ = half-maximal inhibitory concentration

$I_{in,max}$ = estimated maximum plasma inhibitor concentration at the inlet to the liver calculated as:

$I_{in,max} = \left( I_{max} \times (F_{a} \times F_{g} \times k_{a} \times dose/Q_{h}) \right) / R_{b}$

where $F_{a}$ = fraction absorbed; $F_{g}$ = intestinal availability (if unknown $F_{a} = 1$ and $F_{g} = 1$ as worst case estimate) $k_{a}$ = absorption rate constant (if unknown $= 0.1\ min^{-1}$ as worst case estimate) $Q_{h}$ = hepatic blood flow rate; $R_{b}$ = blood to plasma concentration ratio
## Other Recommendations

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<tr>
<td>Transporter Induction</td>
<td><em>In vitro</em> methods to evaluate transporter induction are not well defined and as such it is not currently recommended to evaluate investigational drugs as transporter inducers <em>in vitro</em>. However, information from CYP3A4 induction studies can inform P-gp induction due to similarities in induction mechanism (i.e., through PXR activation).</td>
<td>If PXR and/or CAR mediated induction is observed <em>in vivo</em>, a study investigating the <em>in vivo</em> induction of P-gp mediated transport is recommended.</td>
</tr>
<tr>
<td>Pharmacodynamic Interactions</td>
<td>Clinical pharmacodynamic endpoints can be monitored to support <em>in vitro</em> evidence of drug interaction potential. For example, this may occur if transport interactions alter tissue distribution and in turn affect efficacy or toxicity.</td>
<td>Pharmacodynamic interactions study needs to be considered for drugs which compete with each other at the pharmacological target and / or have similar or opposing pharmacodynamic (therapeutic or adverse) effect.</td>
</tr>
<tr>
<td>pH Dependent Solubility</td>
<td></td>
<td>If solubility of the drug or dissolution of the formulation is markedly pH dependent in the physiological pH range then the effect of drugs which increase gastric pH (e.g., proton pump inhibitors, H2-receptor antagonists or antacids) should be evaluated <em>in vivo</em>.</td>
</tr>
<tr>
<td>Plasma Protein Binding Interactions</td>
<td></td>
<td>The risk of clinically relevant interactions caused by displacement from plasma protein binding sites is considered low. Highly bound drugs (arbitrary fu&lt;1%) with: <em>a narrow therapeutic window, or</em> <em>a high hepatic extraction ratio (if administered i.v.), or</em> <em>a high renal extraction ratio</em> should have the risk of interaction identified <em>in vitro</em> and if a clinically relevant interaction is suspected, an <em>in vivo</em> study could be performed. Unbound concentrations should be determined in such a study.</td>
</tr>
<tr>
<td>Gastric Emptying or Intestinal Motility</td>
<td></td>
<td>Effect of drug on gastric emptying or intestinal motility and the impact on other co-administered drugs may need to be considered.</td>
</tr>
<tr>
<td>Active Metabolites</td>
<td>Active metabolites should be evaluated in DDI studies if they have the potential to affect safety or efficacy based on <em>in vitro</em> pharmacology and toxicology assessments.</td>
<td>If active metabolites contribute to the efficacy and safety of the drug, exposure of these should be evaluated in interaction studies.</td>
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</tbody>
</table>