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;

US FDA Guidance for Industry – In Vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (Jan 2020)

European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted by the CHMP in Jun 2012. Date of effect Jan 2013)

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.

Description	US FDA Guidance 2020	EMA Guidance, adopted 2012
Enzyme Systems	• Human liver subcellular fractions (e.g., microsomes, S9 and cytosol). • Freshly isolated or cryopreserved human hepatocytes. • Human recombinant enzymes.	• Human liver microsomes or S9 fraction. • Human hepatocytes. • Human recombinant enzymes.
Main Enzymes of Interest	• CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A.	• CYP and non-CYP enzymes.
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).	Enzymes involving metabolic pathways estimated to contribute ≥25% of drug elimination should be identified if possible and the *in vivo* contribution quantified.
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)	• 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.

Description	US FDA Guidance 2020	EMA Guidance, adopted 2012
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: $R_1 = 1+(I_{max,u} / K_{i,u})$ $R_{1,gut} = 1+(I_{gut} / K_{i,u})$ For time dependent inhibition: $R_2 = (k_{obs} + k_{deg})/k_{deg}$ and $k_{obs} = (k_{inact} \times 50 \times I_{max,u}) / (K_{i,u} + 50 \times I_{max,u})$ 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_{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) = $(k_{obs} + k_{deg})/ k_{deg}$ and $k_{obs} = k_{inact} \times [I] / (K_i + [I])$ If $\geq 20\%$ inhibition (i.e., R ≥ 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 exposured (determined in mass balance study). The free fraction in the incubate (fu_{inc}) 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).

$I_{max,u}$ = maximal unbound plasma concentration of interacting drug at steady state
I_{gut} = intestinal luminal concentration of interacting drug = molar dose/250mL
R = predicted ratio of CL_{int} of probe substrate in absence and presence of inhibitor

$K_{i,u}$ = unbound reversible inhibition constant
k_{deg} = apparent first order degradation rate constant of affected enzyme
k_{inact} = maximal inactivation rate constant
$K_{i,u}$ = [unbound inhibitor] at ½ maximal inactivation
k_{obs} = apparent inactivation rate

K_i = reversible inhibition constant
k_{deg} = degradation constant of the enzyme
$[I]$ = inhibitor concentration
k_{inact} = maximum inactivation rate constant
K_i = inhibitor concentration producing half maximal rate of inactivation
k_{obs} = apparent inactivation rate constant

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.

Description	US FDA Guidance 2020	EMA Guidance, adopted 2012
Enzyme System	• Plateable, cryopreserved or freshly isolated, human hepatocytes (at least 3 donors). • Immortalised hepatic cell lines (with justification). • Cell receptor assays only considered as supportive data.	• Cultured fresh or cryopreserved human hepatocytes (at least 3 donors). • Cell lines, nuclear receptor binding or reporter gene assays only considered as supportive data.
Main Enzymes of Interest	• CYP1A2, CYP2B6 and CYP3A4 initially. • CYP2C8, CYP2C9, CYP2C19 if CYP3A4 induction observed.	• CYP1A2, CYP2B6 and CYP3A
Controls	• Vehicle control. • Positive control (known strong inducer). • Negative control (known non inducer).	• Vehicle control • Positive control (known strong inducer)
Endpoint	• mRNA and/or catalytic activity. • Measuring concentration of parent drug in the medium at several time points on the last day of the incubation is encouraged. • The concentration of unbound test drug should be measured.	• mRNA recommended. • Catalytic activity recommended if induction due to protein stabilisation suspected. • 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. • The degree of protein binding in the medium and non-specific binding should be considered and unbound concentrations used for the evaluation. • Cell viability/morphology should be determined at the beginning and end of the incubation period at the highest concentration.
Significance of Data	A drug has the potential to induce *in vivo* if in any 1 donor; • Fold change in CYP mRNA is ≥2 and concentration dependent at expected hepatic concentrations of drug. OR • Increase in mRNA is >20% of positive control response. OR • $R_3 \leq 0.8$ where, $R_3 = 1/[1 + d \times ((E_{max} \times 10 \times I_{max,u}) / (EC_{50} + (10 \times I_{max,u})))]$ OR • Exceeds positive criteria defined using correlation methods.	Induction considered positive if; • The drug gives rise to more than a 100% increase in mRNA which is concentration dependent. • Increase in mRNA is greater than 20% of response of positive control. OR • Exceeds positive criteria defined using correlation methods.
Special Considerations	The unbound fraction in plasma should be set to 1% if <1%.	Enzyme down-regulation should also be considered. 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.

EC_{50} = concentration causing half maximal effect $\quad$ $I_{max,u}$ = maximal unbound plasma concentration of the interacting drug
E_{max} = maximum induction effect
d $\quad$ = 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.

Description	US FDA Guidance 2020	EMA Guidance, adopted 2012
Transporter System	Caco-2 cells, overexpressed cell lines or membrane vesicles.	Caco-2 cells or other *in vitro* systems (vector systems overexpressing the transporter, gene knockout systems or silencing mRNA systems etc).
Recommended Transporters	P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K	OATPs and clinically relevant renal, biliary and intestinal transporters.
Specific Recommendations for *in vitro* Substrate Identification Studies	• All drugs should be evaluated for P-gp and BCRP unless BCS Class I. • 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. • Drugs need to be evaluated for OAT1, OAT3, OCT2, MATE1 and MATE2-K if active renal secretion* is ≥25% of total clearance.	• Drugs with estimated hepatic elimination (total hepatic metabolism and biliary secretion) of ≥25% need to be evaluated for OATP1B1 and OATP1B3. • If renal or biliary/gut wall secretion separately is estimated to account for >25% of drug elimination then transporter involved in the active secretion should be identified.
Significance of Data	• 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). • A drug is considered to be a substrate for OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 or MATE2-K if uptake in transfected cells is ≥2 fold that of empty vector cells and is inhibitable by known inhibitor.	• In Caco-2 cells, if efflux ratio <0.5 or >2, it is concluded that active uptake or efflux is occurring respectively. This is confirmed using selective inhibitors. • An *in vivo* study with a strong inhibitor is recommended for any transporter which is identified.
Special Considerations	• Multiple concentrations of the test drug should be evaluated to cover the range of clinically relevant concentrations. • Factors such as recovery, stability, solubility, cytotoxicity, and nonspecific binding of the the test drug should be considered. • Evaluation of more polar (e.g., Phase II) metabolites should be assessed on a case-by-case basis.	• Genetic polymorphic transporters. • Special populations. • Metabolites with pharmacological activity or off-target effects. • 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.

*Active secretion = $CL_r - (f_{u,p} \times GFR)$, where
CL_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.

Description	US FDA Guidance 2020	EMA Guidance, adopted 2012
Transporter System	Caco-2 cells, overexpressed cell lines or membrane vesicles.	Suitable *in vitro* system where human transporter function preserved.
Recommended Transporters	P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K.	P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, OAT3. BSEP also preferably investigated. OCT1, MATE1 and MATE2 could also be considered.
Specific Recommendations for Transporter Inhibition Studies	• All drugs should be evaluated as inhibitors for P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE-2K.	• All drugs should be evaluated as inhibitors for P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, OAT3, and preferably BSEP.
Significance of Data	A drug has the potential to inhibit *in vivo* if; *P-gp and BCRP inhibition* I_{gut}/IC_{50} or $K_i \geq 10$ (oral route) or I_1/IC_{50} or $K_i \geq 0.1$ (parenteral route or metabolite) Where I_{gut} = Dose of inhibitor (in mol)/250mL (if IC_{50} is molar unit) $I_1 = C_{max}$ of inhibitor or metabolite *OATP1B1 and OATP1B3 inhibition* $R = 1 + ((f_{u,p} \times I_{in,max})/IC_{50}) \geq 1.1$ *OCT2, OAT1, OAT3, MATE1 and MATE2-K inhibition* $I_{max,u} / IC_{50} \geq 0.1$ Unbound fraction should be set to 1% if determined to be <1%.	Clinical study recommended if; *Intestinal transporters (eg P-gp)* $K_i \leq 0.1$ 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* $K_i \leq 25$-fold the unbound maximum hepatic inlet concentration. *Renal uptake and efflux, hepatic efflux and hepatic uptake (after i.v. administration) transporters* $K_i \leq 50$-fold the unbound C_{max}. Unbound fraction should be set to 1% if determined to be <1%.
Special Considerations	• 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.	• The use of 2 separate systems is recommended for P-gp due to the current high inter-laboratory variability.

R   = predicted ratio of victim drug's AUC in presence and absence of the investigational drug
$f_{u,p}$ = 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:
$I_{in,max} = (I_{max} + ((F_a \times F_g \times k_a \times dose)/Q_h))/R_B$
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

Description	US FDA Guidance 2020	EMA Guidance, adopted 2012
Transporter Induction	*In vitro* methods to evaluate transporter induction are not well defined and as such it is not currently recommended to evaluate investigational drugs as transporter inducers *in vitro*. However, information from CYP3A4 induction studies can inform P-gp induction due to similarites in induction mechanism (i.e., through PXR activation).	If PXR and/or CAR mediated induction is observed *in vivo*, a study investigating the *in vivo* induction of P-gp mediated transport is recommended.
Pharmacodynamic Interactions	Clinical pharmacodynamic endpoints can be monitored to support *in vitro* evidence of drug interaction potential. For example, this may occur if transport interactions alter tissue distribution and in turn affect efficacy or toxicity.	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.
pH Dependent Solubility	-	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 *in vivo*.
Plasma Protein Binding Interactions	-	The risk of clinically relevant interactions caused by displacement from plasma protein binding sites is considered low. Highly bound drugs (arbitrary fu<1%) with: • a narrow therapeutic window, or • a high hepatic extraction ratio (if administered i.v.), or • a high renal extraction ratio should have the risk of interaction identified *in vitro* and if a clinically relevant interaction is suspected, an *in vivo* study could be performed. Unbound concentrations should be determined in such a study.
Gastric Emptying or Intestinal Motility	-	Effect of drug on gastric emptying or intestinal motility and the impact on other co-administered drugs may need to be considered.
Active Metabolites	Active metabolites should be evaluated in DDI studies if they have the potential to affect safety or efficacy based on *in vitro* pharmacology and toxicology assessments.	If active metabolites contribute to the efficacy and safety of the drug, exposure of these should be evaluated in interaction studies.

Cyprotex Europe
Tel (UK): +44 (0) 1625 505 100
No. 24 Mereside Alderley Park
Macclesfield, Cheshire, SK10 4TG UK

Cyprotex North America
Tel (USA): +1 888 297 7683
313 Pleasant Street
Watertown MA 02472 USA

Email: eduguide@cyprotex.com
Website: www.cyprotex.com