ICH M12 Drug Interaction Final Guidance – In Vitro DDI Assessments

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Outline

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- CYP enzymes
  - -- Investigational drug as substrate
  - -- Drug as inhibitor
  - -- Drug as inducer
- Transporters
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  - -- Drug as inhibitor
  - -- Drug as inducer
- DDI potential of Metabolites
- UGT-mediated DDIs
- Summary

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Updates in the ICH M12 DDI Guideline from FDA DDI Guidance

- Expanded the section on endogenous biomarkers (mainly for transporters) to inform DDI assessment (covered by next presentation on Clinical DDI assessment);
- Included expectations for plasma protein binding (PPB) determination to support use of experimentally measured F_u for very highly protein bound drugs (>99%) for projecting clinical DDI potential;
- Modified the simple criteria to evaluate in vivo DDI potential for drugs as time-dependent inhibitors, inducers, and inhibitors of MATE transporters);
- Modified the section on DDI liability assessment for metabolites as inhibitors or inducers of enzymes or transporters;
- Add new sections on the considerations for UGT-mediated DDIs;
- Provided *examples* of drugs used for in vitro or in vivo studies as substrates, inhibitors, or inducers of CYPs, UGTs, and transporters in Appendix.

Timing of In Vitro DDI evaluations

- Early during drug development
- Determine the need and timing of clinical DDI studies;
  Inform the decision on exclusion criteria/prohibited medications of clinical trial protocols
- In Vitro data generally recommended to be available before starting studies in patients.
- DDI potential of metabolites may be evaluated later in development when more knowledge about metabolites become available (e.g., from mass balance study in humans).

In Vitro Evaluation – As Substrate of Enzymes

- Metabolic phenotyping: CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4/5

- If the above CYP enzymes do not play a major role, consider other enzymes
  CYP2A6, CYP2J2, CYP4F2, and CYP2E1
  Other Phase I enzymes including aldehyde oxidase (AO), carboxylesterase (CES), monoamine oxidase (MAO), flavin monooxygenase (FMO), xanthine oxidase (XO), and alcohol/aldehyde dehydrogenase (ADH/ALDH)

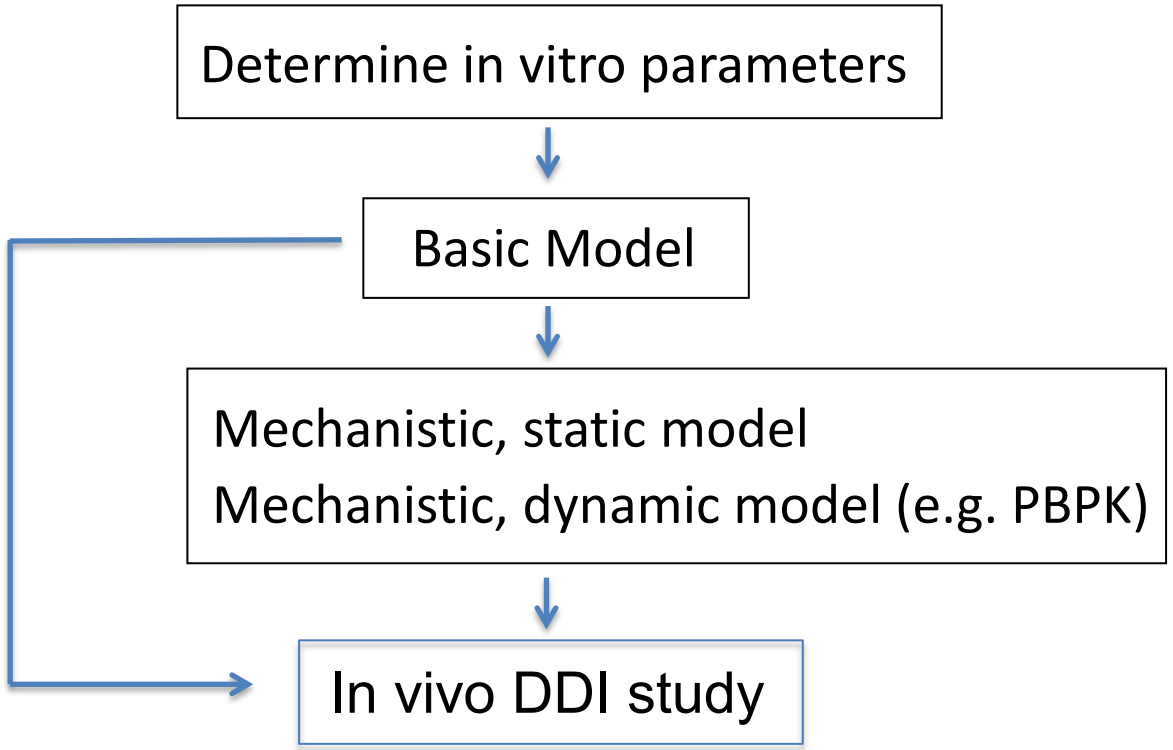
  Phase II enzymes, e.g., UDP glucuronosyl transferases (UGTs) and sulfotransferases (SULTs)

- If ≥ 25% elimination by an enzyme (in vitro phenotyping; human PK), consider further clinical evaluation, i.e., evaluate effect of inhibitor and inducer of the enzyme on the PK of the NME

Determine if an Investigational Drug is an Inhibitor or Inducer of Metabolic Enzymes

CYP inhibitor
(CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A)

CYP inducer
(CYP1A2, 2B6, 2C8, 2C9, 2C19, 3A)



Plasma Protein Binding Determination

- Unbound drug concentrations usually used in the In vitro-to-In vivo Extrapolation (IVIVE) criteria that we often use to predict in vivo DDI potential based on in vitro data for a drug as an inhibitor of enzymes or transporters

- In the past, the guidances recommended capping the free fraction of drug in plasma (fu,p) at 0.01 even if a compound has plasma protein binding (PPB) more than 99%.

- In the M12 guideline, flexibility is provided allowing companies to use experimentally measured PPB (i.e., fu,p can be below 0.01), if the accuracy and precision of PPB measurement has been demonstrated.
  If the reliability of fu,p measurements <0.01 cannot be demonstrated, a default value of 0.01 should be used.

Determine whether a drug inhibits CYP in humans - Basic model

The in vivo DDI risk for reversible enzyme inhibitor can be excluded based on *in vitro* data

- **Reversible inhibition**

$$K_{i,u} > 50 \times C_{max,u} \text{ (i.e., } \frac{C_{max,u}}{K_{i,u}} < 0.02)$$

Ki can be experimentally determined *or* calculated based on IC_{50} and substrate concentration using Cheng-Prusoff equation *or* roughly estimated by using $IC_{50}/2$.

Ki,u is unbound inhibition constant = Ki x fu,mic or fu,hep
fu,mic (unbound fraction of drug in human liver microsome, HLM) or fu,hep (unbound fraction in hepatocytes) can be measured experimentally or estimated *in silico*
[note: fu,mic or fu,hep is <u>not</u> same as fu,p in plasma]

- (Only) **for CYP3A inhibitors**, also need to consider intestinal CYP3A inhibition. The in vivo potential can be excluded if

$$K_{i,u} > 0.1 \times \frac{maximum\ clinical\ dose}{250\ mL} \text{ (i.e., } \frac{Dose/_{250\ mL}}{K_{i,u}} < 10)$$

Dose/250 mL is a rough estimate on intestinal inhibitor concentrations.

Determine whether a drug inhibits CYP in humans - Basic model

The in vivo DDI risk for a drug that is found as a time-dependent inhibitor (TDI) can be excluded based on *in vitro* data

$$\frac{(k_{obs}+k_{deg})}{k_{deg}} < 1.25$$

$$\text{where } k_{obs} = \frac{(k_{inact} \times 5 \times C_{max,u})}{(K_{I,u} + 5 \times C_{max,u})}$$

K_I and k_{inact} are inhibitory potency parameters of a compound determined experimentally.

k_{deg} is the apparent first-order degradation rate constant of the affected enzyme. Commonly used values for individual CYPs provided in the Appendix of the M12 guideline (for CYP3A, the kdeg values in liver and in intestine are different).

Note: the scaling factor is reduced from 50x in the FDA guidance to 5x in M12 guideline based on recent literature studies and reanalysis of data published previously which suggest no increase in false negative predictions when reducing the scaling factor.

Determine whether a drug induces CYP in humans

- Evaluate **CYP1A2, CYP2B6, and CYP3A4** initially.

- If no induction of CYP3A4 is observed, evaluating the induction potential of **CYP2Cs enzymes** (2C8/2C9/2C19) not needed because CYP3A and CYP2C enzymes are induced via activation of the pregnane X receptor (PXR) and CYP3A is more sensitive to inducer effect

- If the drug induces CYP3A4, evaluate the drug's potential to induce CYP2C enzymes.

- **Down-regulation.** Acknowledge the phenomenon. Clear recommendation not provided due to limited knowledge.

Evaluate Induction Potential of a Drug on CYPs

Based on mRNA data

mRNA data is the primary endpoint,

1) activity measurement can be confounded by inhibition if a drug is also an inhibitor;

2) mRNA usually has large changes than activity for CYP3A. More suitable for characterizing Emax and EC_{50}.

- **mRNA fold-of-change method**
- **Correlation method** (e.g., relative induction score (RIS))
- **Basic kinetic model**

$$R = 1 / [1 + (d \times E_{max} \times 10 \times I_{max,u}) / (EC_{50} + 10 \times I_{max,u})]$$

Determine In Vivo Induction Potential of a Drug on CYPs

mRNA fold-of –change method

In vivo induction potential cannot be excluded if the drug in hepatocytes from *at least one* donor meets both of the following criteria

(1) concentration-dependent ↑ in mRNA expression of a CYP enzyme
(2) the fold-change of CYP mRNA expression relative to the vehicle control is **≥ 2-fold** at the 50x Cmax,u or lower concentrations.

Note: the scaling factor in the FDA guidance is 30x and 15x in the draft version of M12 guideline. This is changed to 50x in the final M12 guideline, based on the data from a literature study (Kenny et al., 2018) and internal analysis of data from NDAs approved by EMA and FDA in recent years.

The increase of scaling factor is intended to mitigate false negative predictions when using fu,p below 0.01 for certain inducer drugs that have PPB >99%.

Determine In Vivo Induction Potential of a Drug on CYPs

mRNA fold-of –change method

- The response to positive control is expected usually ≥6-fold increase in mRNA.
- When the response to a positive control is <6-fold, induction potential cannot be ruled out for an investigational drug that increases CYP mRNA <2-fold of the vehicle control (e.g., DMSO) *but* >20% of the response of the positive control. Further evaluation is recommended.

$$\%\ of\ positive\ control = \frac{(mRNA\ fold\ increase\ of\ test\ drug\ treated\ cells - 1)}{(mRNA\ fold\ increase\ of\ positive\ control - 1)} \times 100$$

- It is not uncommon that even positive controls have limited induction effects on CYP2C8, 2C9, or 2C19, making it difficult to interpret the in vitro data.

  An approach is to leverage in vivo DDI study results with a sensitive CYP3A substrate, since CYP2Cs share regulation pathways with CYP3A but are less inducible.

Evaluate Induction Potential of a Drug on CYPs

Enzyme Activity

No clear recommendation on how to evaluate activity data at this moment. Need further evaluation.

Note: for **CYP2C19**, somehow its mRNA is not sensitive to inducers, even positive control, e.g., rifampin, may not be able to induce CYP2C19 mRNA more than 2-fold.
Thus, it is hard to rule out induction potential of a drug even if it induces CYP2C19 <2-fold. In M12 guideline, it recommends measurement of CYP2C19 activity.

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Drug as a Substrate of Transporters – In Vitro Experiments

P-gp or BCRP

- Often conducted for orally administered drugs since P-gp or BCRP may limit drug absorption
- When biliary excretion or renal active secretion is likely a major elimination pathway of a drug
- Pharmacological target of the drug is in brain

OATP1B1/3

- Conducted if hepatic metabolism or biliary excretion accounts for ≥25% of elimination of a drug or the pharmacological target of a drug is in liver

OAT1, OAT3, OCT2 (Uptake transporters), **MATE1, MATE2/K** (Efflux transporters)

- Conducted for drugs having significant active renal secretion (≥ 25% of *systemic clearance* of the drug)

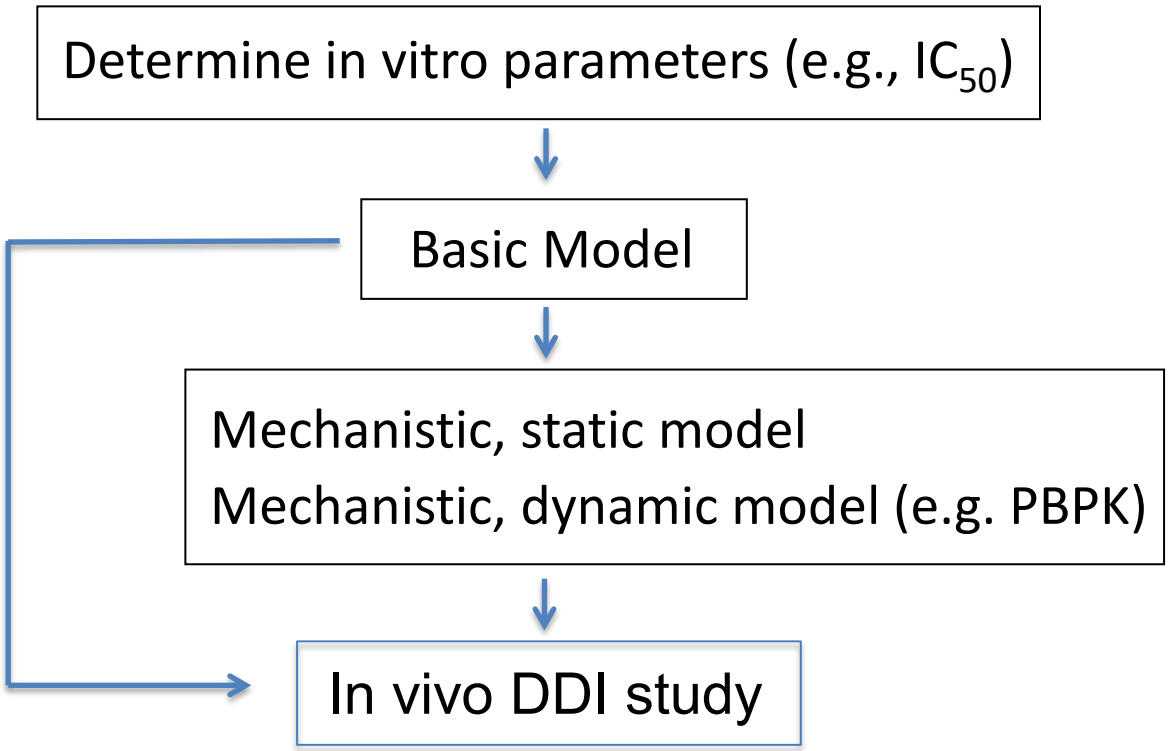
$$CL_{renal\ active\ secretion} = CL_{renal} - GFR \times f_{u,p}$$

assuming that passive reabsorption is equal to passive secretion and there is no active reabsorption

Additional transporters considered case-by-case (e.g., **MRP2, OATP2B1, OCT1**)

Drug as an Inhibitor of Transporters

P-gp, BCRP
OATP1B1, OATP1B3
OAT1, OAT3, OCT2,
MATE1, MATE-2K



Drug as an Inhibitor of Transporters – In Vitro Experiments

In vitro studies usually conducted to evaluate the effect of a drug on the following transporters. The in vivo DDI risk of an inhibitor can be excluded if

P-gp or BCRP	$IC_{50,u}^{*} > 0.1 \times$ (Dose/250 mL) (i.e., (Dose/250 mL)/ $IC_{50,u} < 10$) for orally administered drugs
OATP1B1 or OATP1B3	$IC_{50,u} > 10 \times C_{max,\ inlet,u}^{\#}$ (i.e., $C_{max,inlet,u} / IC_{50,u} < 0.1$)
OAT1, OAT3, OCT2	$IC_{50,u} > 10 \times C_{max,u}$ (i.e., $C_{max,u}/ IC_{50,u} < 0.1$)
MATE1/MATE2-K	$IC_{50,u} > 50 \times C_{max,u}$ (i.e., $C_{max,u}/ IC_{50,u} < 0.02$)

Note: the criteria are same as those in the FDA final guidance except that the cut-off value for MATE transporters is reduced from 0.1 to 0.02.

For a drug administered parenterally or if it is a metabolite formed post-absorption that inhibits P-gp or BCRP, $IC_{50,u} > 50 \times C_{max,u}$ (i.e., $C_{max,u} / IC_{50,u} < 0.02$) can be used.

Other cutoff values can be proposed if justified based on IVIVE and a calibration of in vitro systems with known inhibitors and non-inhibitors of these transporter systems.

Drug as an Inducer of Transporters

- In vitro methods to evaluate the induction of P-gp and other transporters are not well established at this time. Therefore, recommendations for the in vitro evaluation of investigational drugs as transporter inducers are not provided.

- An approach is to leverage in vivo DDI study results with a sensitive CYP3A substrate, since P-gp shares regulation pathways with CYP3A but are less inducible (see next presentation on Clinical DDI assessment for more details).

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DDI Liability of Metabolites as Substrates

Metabolite as substrate for CYPs – situations where in vitro experiments are needed to characterize how a metabolite is further metabolized (i.e., metabolic phenotyping)

- Pharmacologically active metabolite, e.g., contributing to efficacy more than or similarly as its parent drug. Contribution to efficacy estimated based on

  -- unbound AUC **(in molar units)**
  -- pharmacological potency (e.g., receptor binding affinity, enzyme inhibitory potency)
  -- data related to target tissue distribution, if available

- Metabolite suspected to cause significant AEs based on nonclinical and/or clinical information
- Above considerations generally also applied to metabolites as transporter substrates.

DDI Liability of Metabolites as Inhibitors

Metabolite as inhibitor for CYPs – situations where in vitro experiments are needed to characterize whether a metabolite inhibits major CYPs and transporters.

FDA 2020 Guidance	ICH M12 Guideline
• For metabolites more polar than parent: AUCmetabolite ≥ AUCparent **(in molar units)** • For metabolites less polar than parent: AUCmetabolite ≥ 25% x AUCparent **(in molar units)** • For metabolite that acts as time-dependent inhibitor (TDI), consider a lower exposure than parent	As a pragmatic rule, it is recommended to investigate the enzyme inhibitory potential of metabolites that have total AUCmetabolite ≥25% of AUCparent AND also account for at least 10% of drug-related material in circulation (i.e., considered as a major metabolite)

Based on the results of in vitro studies of a metabolite, whether to conduct a clinical DDI study will be determined with the same approaches as those for parent drug.

Metabolites may not be relevant for intestinal CYP/transporter inhibition unless metabolites are formed substantially in the gut or intestinal cells.

DDI Liability of Metabolites as Inhibitors

- When a parent drug is found to inhibit a CYP in vitro and predicted to have such potential in humans, if a clinical study is already decided to be conducted, then no need to perform in vitro experiments to evaluate the inhibitory effect of a metabolite on the CYP affected by parent drug, since the clinical DDI study will cover the effects of both parent and metabolite,

  unless clinically relevant exposures of the metabolite cannot be adequately represented in the clinical DDI study (i.e., the study duration does not allow the metabolite to accumulate).

- When a model (mechanistic static or PBPK model) is used to predict the inhibitory effect of a drug on a CYP in lieu of clinical DDI study, the contribution of metabolite needs to be incorporated.

DDI Liability of Metabolites as Inducers

While metabolites may be able to induce CYP enzymes, it is *assumed* that generally the effect of a parent drug in in vitro experiment may reflect the effect of metabolites since metabolites may be generated during incubation of the parent drug with hepatocytes (usually 2-3 days).

However, when the drug is a prodrug or when a metabolite is mainly formed extra-hepatically, in vitro evaluation of a metabolite's induction potential on CYP enzymes is recommended for metabolite that accounts for at least 10% of drug-related material in circulation AND has $AUC_{metabolite}/AUC_{parent}$ ≥25%.

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UGTs-mediated DDIs – In Vitro Experiments

Investigational Drug as a UGT Substrate

When a drug is *mainly* eliminated by direct glucuronidation in humans, recommended to characterize which UGT isoforms are responsible.

Investigational Drug as a UGT inhibitor

While a drug can be an inhibitor of an enzyme even if it is not a substrate, routine evaluation of any drug to inhibit UGTs may not be warranted, considering overall limited magnitudes of DDIs mediated by UGT inhibition and challenge with predicting in vivo DDI potential from in vitro data.

Recommend in vitro studies for a drug whose major elimination pathway is direct glucuronidation to evaluate its inhibition potential towards UGT (including 1A1, 1A4, 1A9, 2B7, and 2B15).

IVIVE criteria have not been established for UGTs due to limited available data. Further research is warranted. Companies may consider using the same criteria as the one for CYPs or proposing an alternative with justification.

In addition, when an investigational drug will be commonly administered with a drug that is mainly metabolized by direct glucuronidation, recommend to evaluate in vitro the potential inhibitory effect of the investigational drug on the UGT isoform(s) responsible for the elimination of the other drug.

In Vitro Experiments and Bioanalytical Assays

- In vitro experiments and bioanalytical methods are not necessarily GLP-standard.
- Bioanalytical assays should meet general requirements to ensure reliable measurements.
- Several factors may cause actual drug concentrations in the in vitro assays to deviate from nominal concentrations, including poor aqueous solubility, non-specific binding (e.g., with apparatus), and instability. Correction for binding or stability or solubility issues should be considered when interpreting the data.

Summary

- Tremendous advancements have been made enhancing our understanding of DDIs. Collective effort from industry, academia, and regulatory agencies led to a globally harmonized DDI guideline.

- While we have reached consensus on a number of important topics, there are still areas that warrant further research which will improve the efficiency of DDI assessments during drug development.

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