XENOTECH

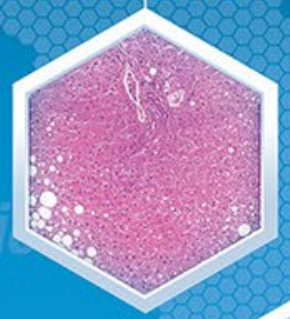
A BioIVT Company

25 Years

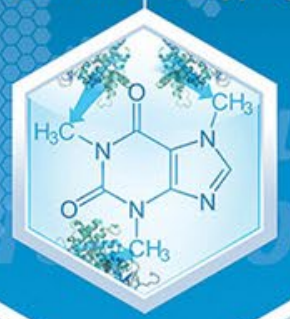
ADME | DRUG-DRUG INTERACTION | DMPK

CONTRACT RESEARCH & TEST SYSTEM EXPERTISE

Cell & Tissue-Based Products

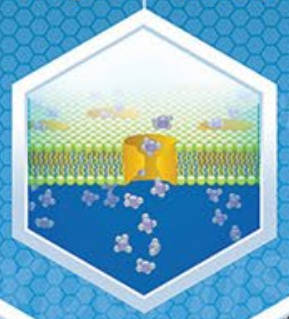


Reaction Phenotyping

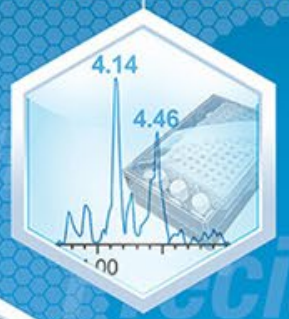


Enzyme Induction & Inhibition

Drug Transporters



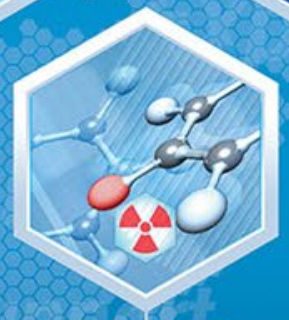
Metabolite ID & Production



Screening

Pharmacokinetics & QWBA

Radiolabeling



Bioanalytical

Drug Metabolism
Development & Risk Assessment
Seminars

2022 Seminar Series: Filing an IND and beyond: Development of CTD Section 2.6.4, Pharmacokinetics Written Summary

Griff Humphreys, Aranmore Pharma Consulting

Princeton, NJ

May 11, 2022

Drug Metabolism

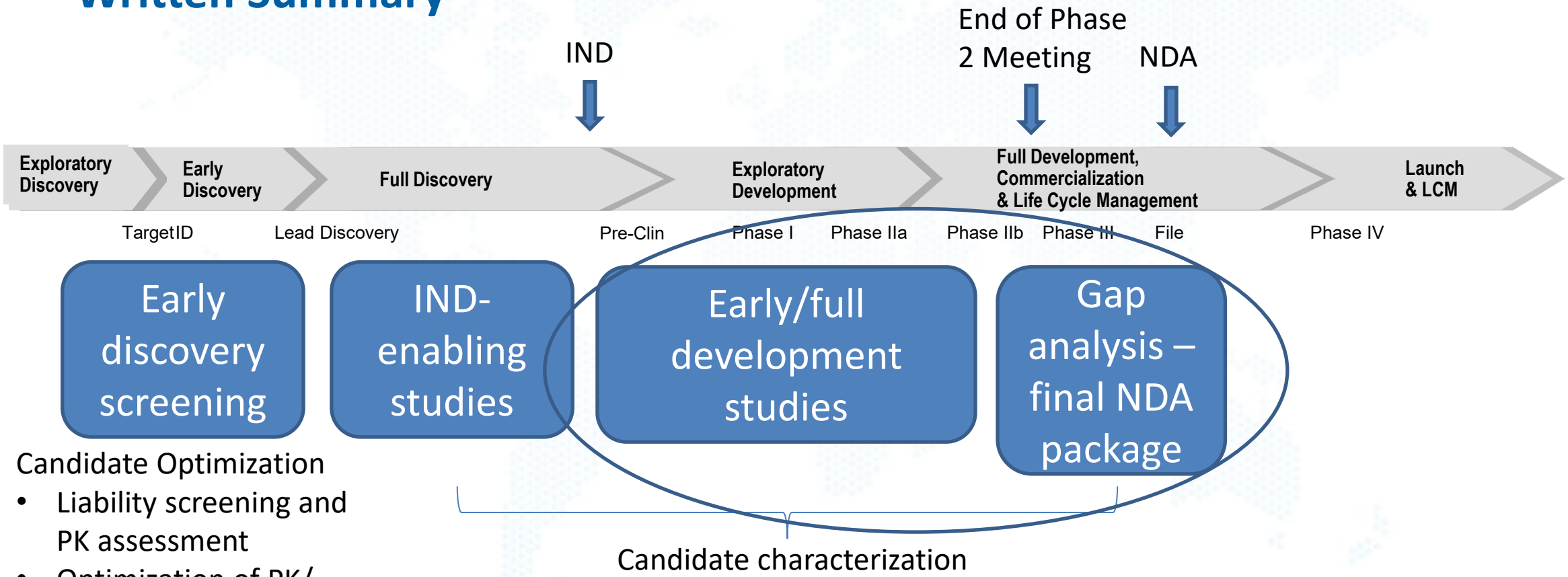
Development & Risk Assessment

Seminars

Areas covered:

- **Generation of ADME data for an NCE throughout development**
- **Contents of final filing package**
- **Considerations for what ADME data should be contained at the time of IND**
- **Types of ADME data typically found in IND**
- **Interdependencies with other non-clinical sections**
- **Additional detail on human dose projection and DILI liability prediction**

Key Milestones in Development of Section 2.6.4 Pharmacokinetic Written Summary



Candidate Optimization

- Liability screening and PK assessment
- Optimization of PK/ Minimization of liabilities

Section 2.6.4 Pharmacokinetic Written Summary - Methods

-Brief description of methods used for initial PK, in vitro studies, etc.
-Description of the GLP assays used for toxicology studies

Section 2.6.4 Pharmacokinetic Written Summary - Absorption

-Absorption data (BCS classification)
- -Animal PK/Bioavailability
- -Permeability models
- -Human BA (if available)

-Impact of efflux transporters

-Animal PK data
- -IV/PO studies
- -Ascending dose studies
- -Repeat dose PK studies
- -Animal food/pH effect studies
- -Formulation studies

Section 2.6.4 Pharmacokinetic Written Summary - Disposition

-Tissue distribution
  -Target organ information
  -Tissue retention/accumulation information
  -Transporter mediated distribution
-Plasma protein binding
  -Albumin/AGP
  -Impact of hepatic/renal insufficiency
  -Potential displacement interactions
-Blood:plasma ratio
-Placental transfer

Section 2.6.4 Pharmacokinetic Written Summary - Metabolism

-Complete understanding of circulating metabolite profile in human (*Safety Testing of Drug Metabolites - Guidance for Industry*):

- -Profiling of human plasma, determination of key metabolites
  - -Major metabolites, i.e., metabolites circulating at levels >10% of total drug related material
  - -Metabolites that may contribute to on- or off-target pharmacology/toxicology
- -Information on coverage of major metabolites in tox species
- -Profiling of key metabolites for on- and off-target pharmacology

-Data on enzymes responsible for metabolic clearance including fractional contribution of key enzymes

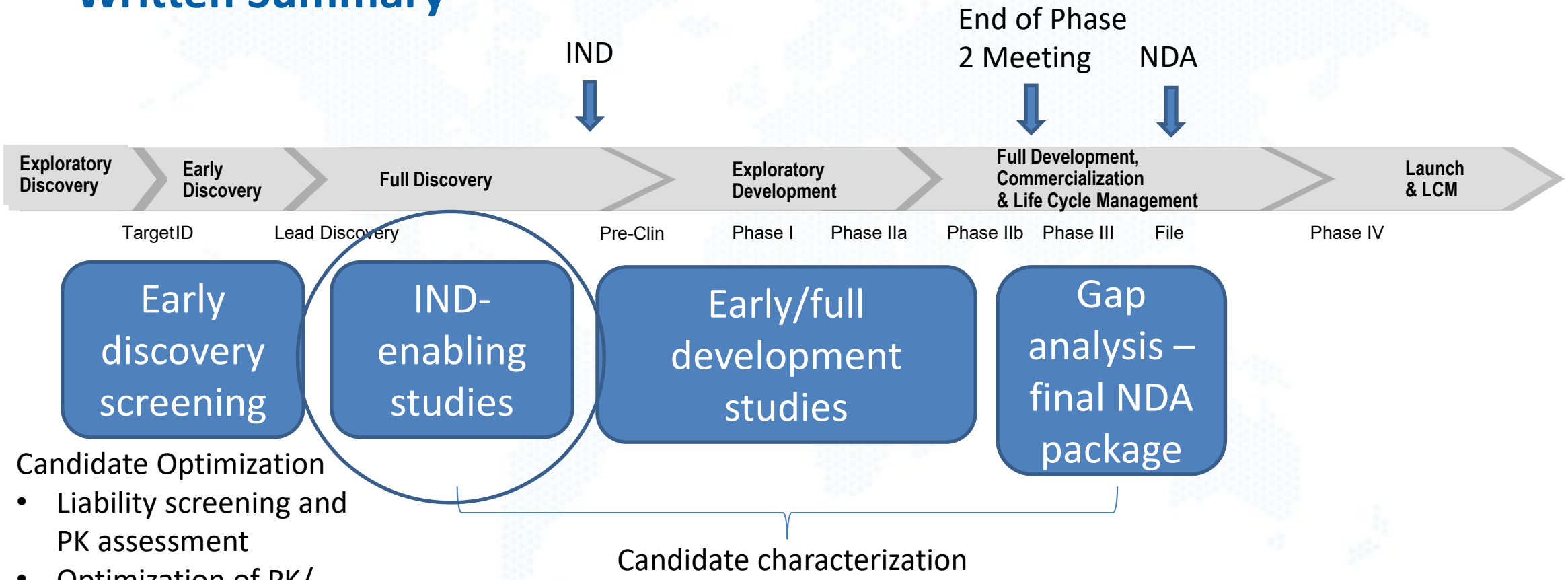
Section 2.6.4 Pharmacokinetic Written Summary - Excretion

-Excretion data from mass balance study
  -parent vs. metabolite
  -urine vs. feces
-Information on transporter-mediated excretion
-Secretion into milk

Section 2.6.4 Pharmacokinetic Written Summary – Drug-drug Interactions

-NCE/major metabolites as victim
  -CYP, UGT, other enzymes involved in CL (fractional CL >25%)
  -Transporters involved in tissue uptake/excretion
-NCE/major metabolites as perpetrator
  -Inhibition/Induction of:
    -CYP, UGT, other enzymes
    -Transporters
-Information on key co-medications, cross-references to clinical DDI study results
-Covered in *In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry*

Key Milestones in Development of Section 2.6.4 Pharmacokinetic Written Summary



Section 2.6.4 Pharmacokinetic Written Summary – How to determine what studies need to be included in IND?

- What are major drivers for conduct of these studies at the IND stage?:
  - To support tox studies
    - Species selection
    - Interpretation of tox multiples
  - **To understand risk of drug attrition due to unacceptable ADME properties (sponsor and/or partners)**
    - Potential impact of DDIs, polymorphic metabolism
    - Human dose projection, does the projected dose meet expected target product profile?, Drug expected to have low therapeutic index?
  - To allow early patient studies/early use of key co-medications

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- Key question to address: Is there sufficient ADME data to support administration in Phase 1 studies?
  - Some key considerations:
    - Normal healthy volunteers vs patients?
    - Plan to include patients as part of Phase1 studies?
    - Are there any drug interactions that need to be studied within Phase1 protocol to allow co-administrations in patient studies?
    - Is the drug targeted for an indication with high unmet medical need?
    - Are there other drugs in late development/already marketed for the same target/indication?

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- While there are no hard and fast answers to the question of what is required for an IND, below presents a classification scheme to help determine the level of impact for each study type:
  - Category 1: Studies needed to judge the quality of toxicity studies and aid in assessment of tox findings; characterization of animal distribution/excretion
  - Category 2: Studies that have reasonable translation from non-clinical to human and: a) predict high impact liabilities (*e.g*. CYP 3A4 inhibition/induction, OATP1B1 inhibition, prediction of human PK) and b) predict the lower impact liabilities (*e.g*. inhibition/substrate of OAT3)
  - Category 3: High impact liabilities with lower fidelity of translation (*e.g*. GSH adduct formation, BSEP inhibition)

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- What is needed in Section 2.6.4 for an IND to support toxicology studies?
  Category 1 Studies:
  - Species selection
    - PK to support species/dose levels/dose frequency/formulation
    - Interspecies metabolite ID to ensure all major human metabolites are covered by at least one tox species
  - Interpretation of tox multiples, interspecies protein binding to ensure no major differences in free fraction

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- What is needed in Section 2.6.4 for an IND to support toxicology studies?
  Category 1 Studies:
  - Tissue distribution
    - Drug distribution to target tissue, to aid PK/PD understanding
    - Drug distribution to off-target tissues, to aid liability assessment
  - Excretion studies
    - Typical study conducted in BDC rat to understand role of urinary vs biliary excretion and parent vs metabolite excretion

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- What is needed in Section 2.6.4 for an IND to do a reasonable liability assessment?
  Category 2a Studies:
  - Prediction of DDI potential, perpetrator and victim, with high impact enzymes, transporters (*e.g*. CYP3A4, OATP1B1, P-gp)
    - Covered in DDI Guidance (*In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry*
  - Prediction of DDI with any key co-administered drugs

These studies are standard offerings at many CROs, however, often require customization of protocol or special attention to interpretation of results. Will be topic of second presentation.

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- What is needed in Section 2.6.4 for an IND to do a reasonable liability assessment?
  Category 2a Studies:
  - Prediction of human PK/relationship to efficacy models/human dose projection
    - Prediction of effect of polymorphic enzymes on metabolic clearance/probability of highly variable PK
    - PK prediction based on animal PK and in vitro metabolism studies; does it meet target product profile?
    - Best information on target plasma profile based on PK/PD in efficacy models
    - Projected dose level and frequency to reach target plasma levels
    - *Although this assessment is not completely necessary, it adds considerable weight to overall IND, interpretation of toxicity studies, early clinical plan, etc.*

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- What is needed in Section 2.6.4 for an IND to do a reasonable liability assessment? Category 2b Studies:
  - Prediction of DDI potential, perpetrator and victim, with lower impact enzymes, transporters (*e.g*. OAT3, UGT1A9)
    - *Very reasonable to defer these assays to post-IND*
    - Covered in DDI Guidance (*In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry*

These studies are standard offerings at many CROs, however, often require customization of protocol or special attention to interpretation of results. Will be topic of second presentation.

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

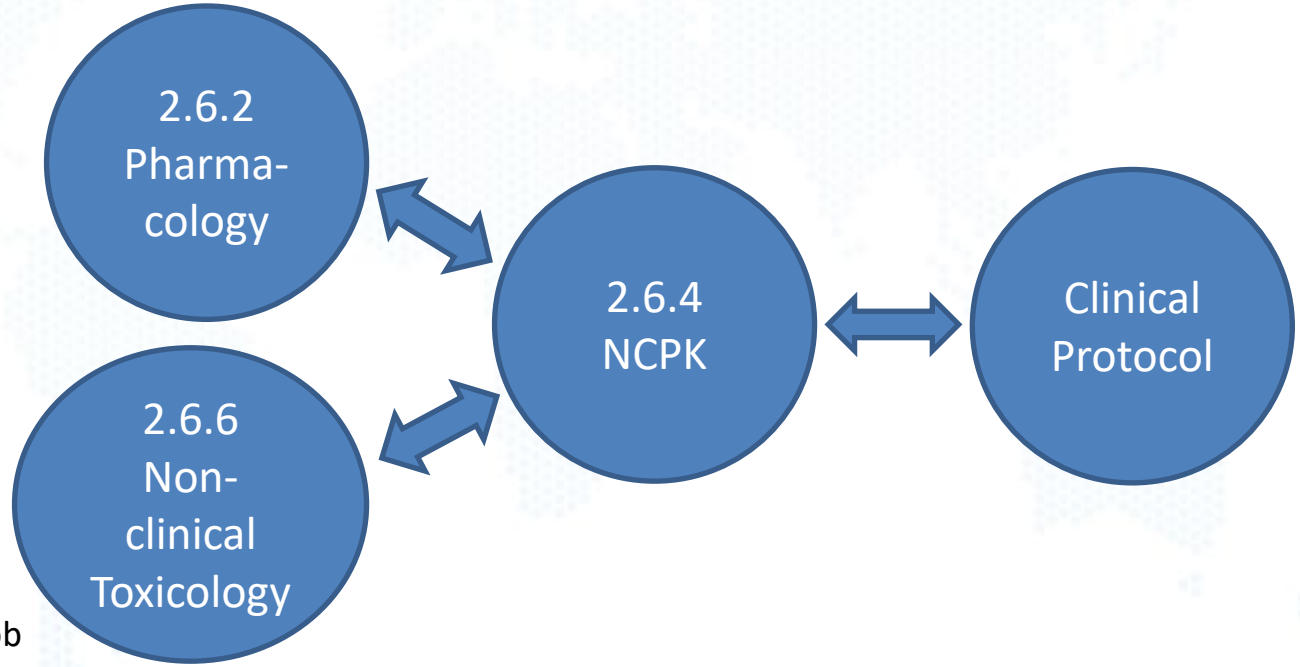
- What is needed in Section 2.6.4 for an IND to do additional (value added) liability assessment? Category 3 Studies:
    - Assessment of drug induced liver injury (DILI)/hypersensitivity risk

Section 2.6.4 Pharmacokinetic Written Summary

- Key points of contact with other IND sections:

- What is known about PK/PD relationship?
- Safety pharmacology section.
  - What are predicted plasma levels in human, relationship to safety pharmacology findings

- What are predicted efficacious exposures in human, comparison to exposures in GLP toxicology studies
- Any differences in protein binding between tox species and human that require adjustment of margin calculations?
- Do the toxicology species do a reasonable job in representing human metabolite profile?



- Dose escalation scheme

Section 2.6.4 Pharmacokinetic Written Summary

Several Key Activities at IND stage and in Early Development:

1) **Human Dose Projection**
2) Methods Used to Inform DILI Risk Assessment

Section 2.6.4 Pharmacokinetic Written Summary

Step 1

- Prediction of human PK
  - In silico methods
  - In vitro to in vivo correlations (IVIVC)
    - Use interspecies in vitro rates of metabolism (intrinsic clearance, CLint) in liver tissue (liver microsomes, hepatocytes) to derive hepatic clearance values
  - Scaling of animal PK
    - Simple allometry from IV data for prediction of CL and steady state volume of distribution (Vd,ss)
    - Wajima allometric relationship, other methods
  - PBPK

Section 2.6.4 Pharmacokinetic Written Summary

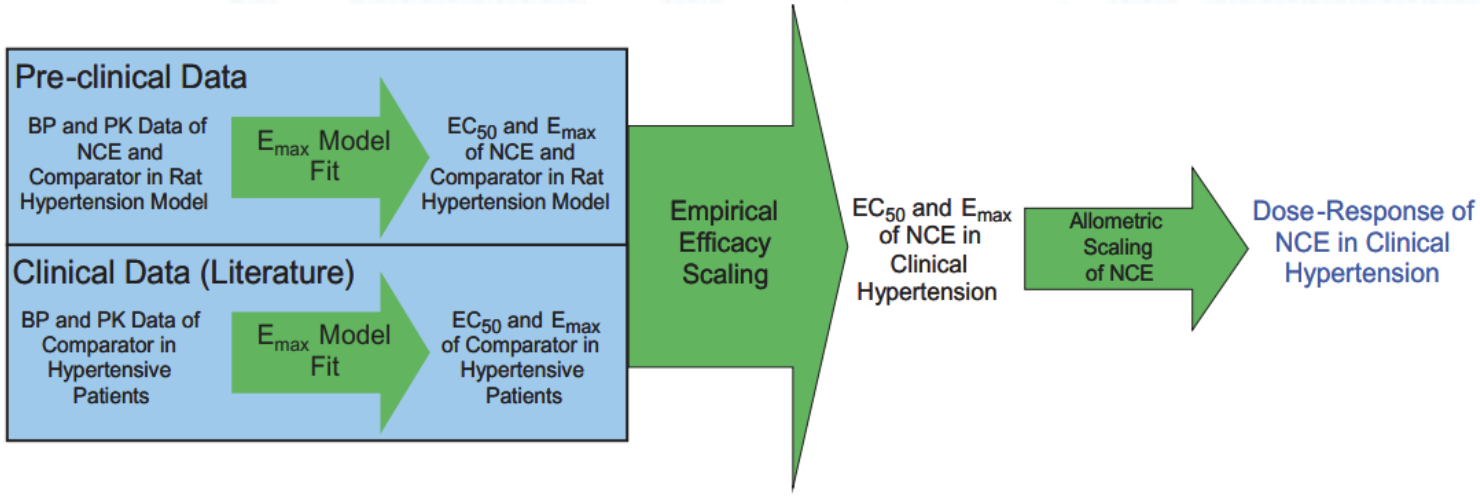
Step 2

- Prediction of human efficacious plasma concentration
  - Requires analysis of plasma drug levels in efficacy models or closely related satellite animals
  - Ideal to develop understanding of PK-PD relationship during optimization efforts – helps to drive a better understanding of key PK parameters that are most closely linked to efficacy
    - What PK profile best describes observed PD (e.g. AUC, time over IC50, trough coverage of IC50)

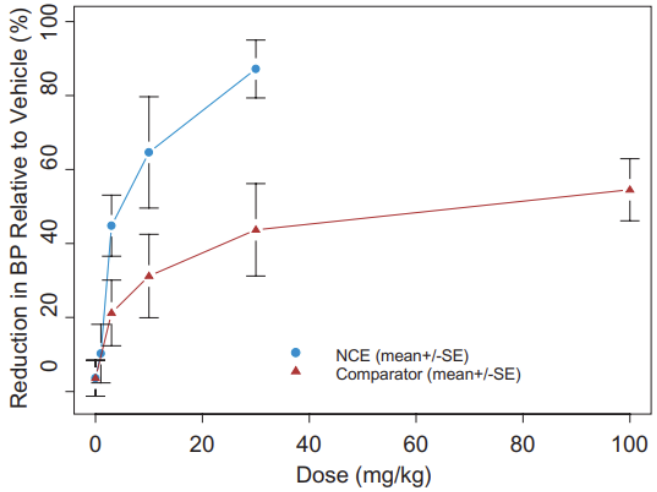
Step 3

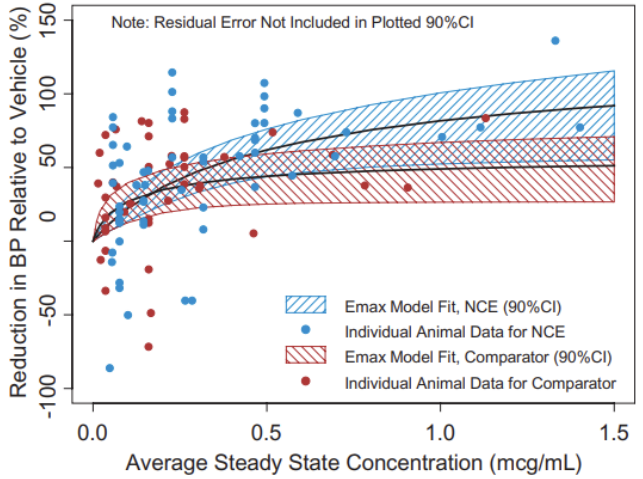
- Derive dose predictions using human PK parameters that provide target plasma profile

Section 2.6.4 Pharmacokinetic Written Summary



Chien J., et al. AAPS J. 2005, 7(3): E544–E559.





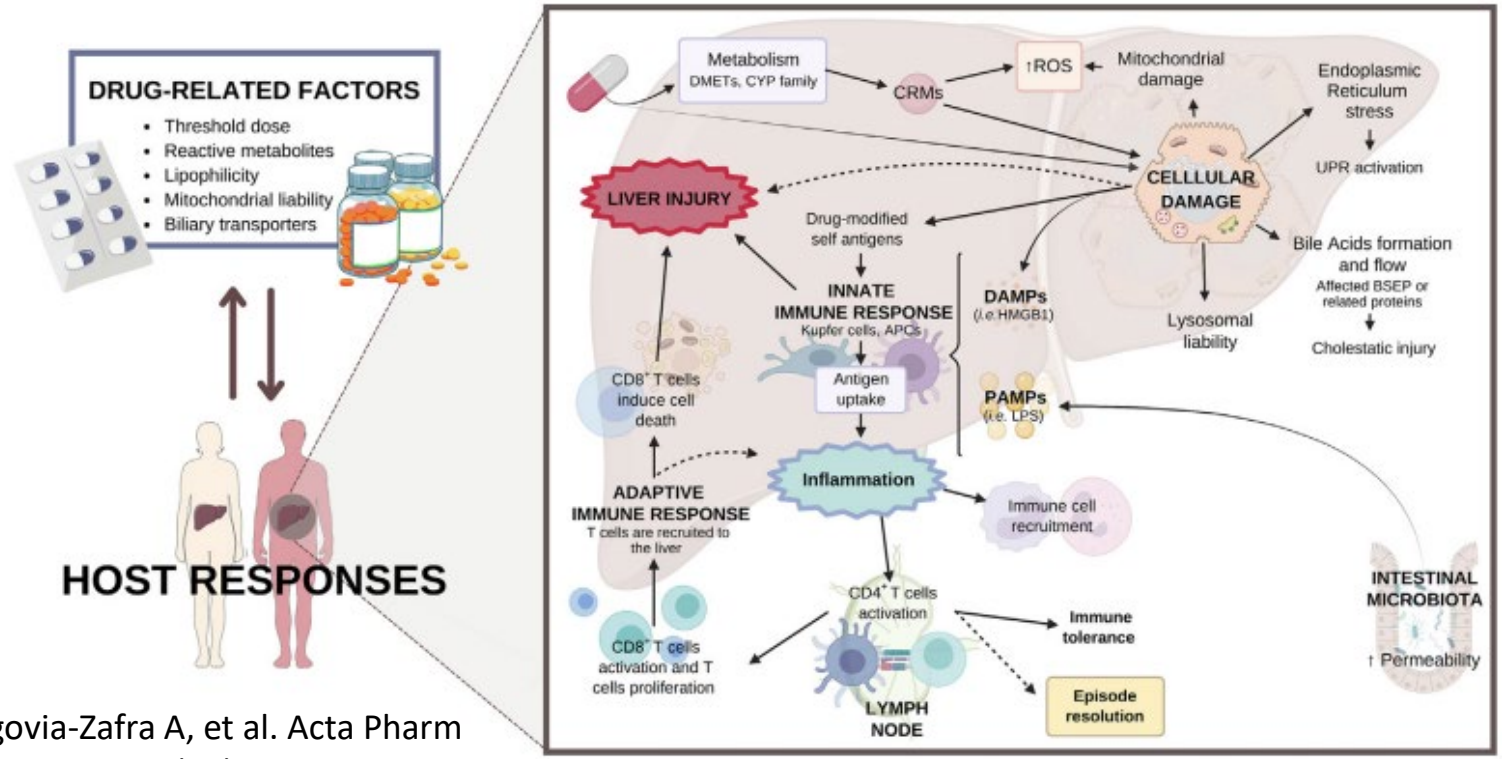
Section 2.6.4 Pharmacokinetic Written Summary

Several Key Activities at IND stage and in Early Development:

1) Human Dose Projection
2) **Methods Used to Inform DILI Risk Assessment**

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- Prediction of drug induced liver injury (DILI)/hypersensitivity risk



Segovia-Zafra A, et al. Acta Pharm Sin B. 2021, 11(12):3685-3726

Current hypothesis for the underlying causes of DILI:

- Multiple factors lead to the combination of Innate and Adaptive Immune Responses
  - Mechanisms of innate immune response are largely downstream effects of reactive metabolite formation or perturbation of bile acid homeostasis

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- Prediction of DILI/hypersensitivity risk – Assays used to predict
  - Inhibition of bile acid transport
    - BSEP inhibition (vesicle assay)
    - MRP2/3/4 (uptake assay in transfected cells) and MDR3 (??) inhibition assays
  - Reactive metabolite generation
    - Glutathione trapping (need some quantitative estimate for risk assessment)
    - Acyl glucuronide stability (rate of acyl gluc degradation, rate of acyl migration)

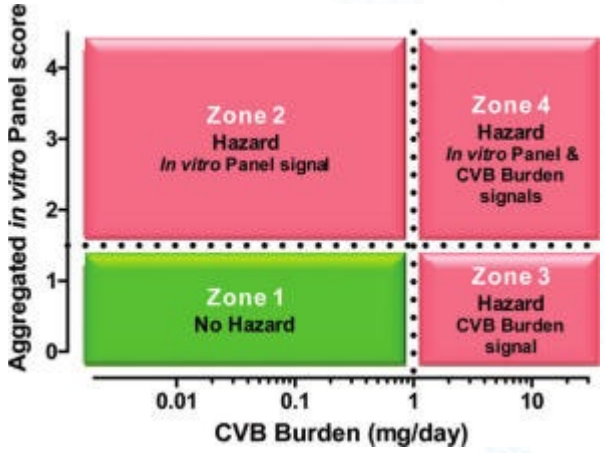
- Final determination is through some combination of the above results along with other factors such as predicted dose, non-metabolism clearance pathways, etc.

Section 2.6.4 Pharmacokinetic Written Summary – What to include in IND?

- Prediction of drug induced liver injury (DILI)/hypersensitivity risk
  - Example of a published decision framework for preclinical DILI risk evaluation

Thompson RA, et al. In Vitro Approach to Assess the Potential for Risk of Idiosyncratic Adverse Reactions Caused by Candidate Drugs.

Chem Res Toxicol. 2012. 25(8):1616-32.



The cellular effects were tested in a Panel of five assays:
(1) toxicity to THLE cells (SV40 T-antigen-immortalized human liver epithelial cells), no P450s,
(2) toxicity to a THLE cell line with P450 3A4,
(3) cytotoxicity in HepG2 cells in glucose and galactose media, which is indicative of mitochondrial injury,
(4) inhibition of the human bile salt export pump, BSEP,
(5) inhibition of rat Mrp2.
CVB Burden was estimated by determining the CVB in human hepatocytes and factoring in both the maximum prescribed daily dose and the fraction of metabolism leading to CVB.