Drug Interactions, from bench to bedside

Candidate to Market, The Paterson Institute for Cancer Research, Manchester, UK

Michael Griffin PhD

Overview of presentation

- To understand the importance of drug-drug interactions
- Why study drug-drug interactions
- Summary of recommendations and guidance for drug-drug interactions
- Overview of, and experimental design of *in vitro* assays for drug-drug interactions focusing on inhibition, induction and transporters

© 2010 Cyprotex

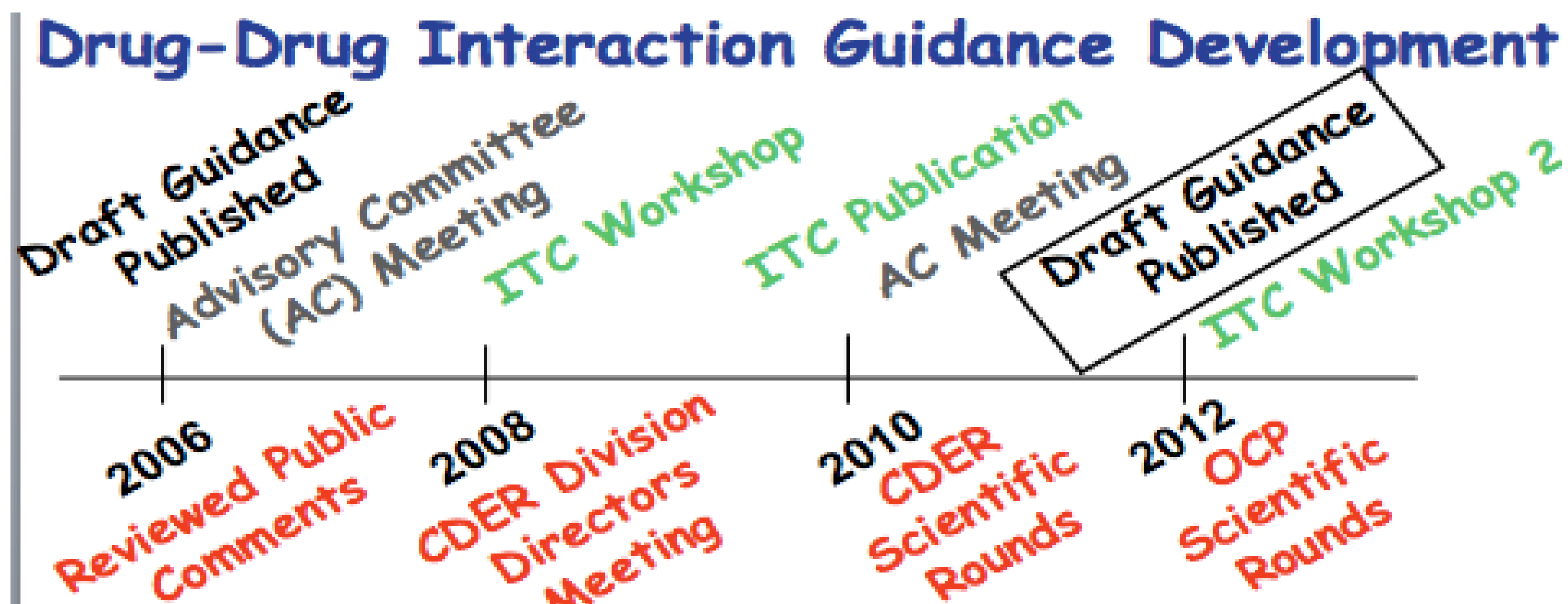
Why study drug-drug interactions?

- Drug-drug interactions are one of the primary concerns for co-administered drugs
    - Adverse effects, changes to AUC, decreased efficacy for drugs metabolised to active species
- Drug-drug interactions have led to number of drug withdrawals or black box warnings
    - E.g: Seldane and erythromycin: Seldane metabolised by CYP3A4 which erythromycin inhibits – fatal arrhythmias – withdrawn, replaced with fexofenadine
- DDIs cause 20% of Adverse Events leading to hospitalisation; 1.5-2% are serious AEs
- Most common victim drugs – verapamil, MTX, amiodarone, lithium, warfarin, CSA, aspirin, itraconazole, insulin, clopidogrel, digoxin
- Most common offender drugs – statins, NSAIDs, ACEI, beta-blockers, antiplatelet drugs, digoxin, diuretics, sulfa/trimethoprim

Br J Clin Pharmacol 70(2), 252-257 (2010), Amer J Geriatr Pharmacother 9(6), 364-377 (2011)

Evolution of FDA guidance

• FDA and EMEA now recommends potential for DDIs to be assessed early in drug development



http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf

http://www.emea.europa.eu/htms/human/humanguidelines/efficacy.htm

© 2010 Cyprotex

Overview of FDA DDI guidance 2012

- Latest draft guidance published Feb 2012
- Assess if a new drug is a substrate, inhibitor or inducer of metabolising enzymes
- Number of enzymes to be studied increased, not just CYP450
- Metabolites of parent drug (≥ 25% parent drug AUC) should also be considered
- Transporter studies as important as CYP450 studies
- Decision trees included to assist design of clinical DDI studies
- Increased use of modelling (static, mechanistic and PK/PD) to assist interpretation and design of DDI studies
- Consider potential for interactions by therapeutic proteins i.e. cytokines or cytokine modulators

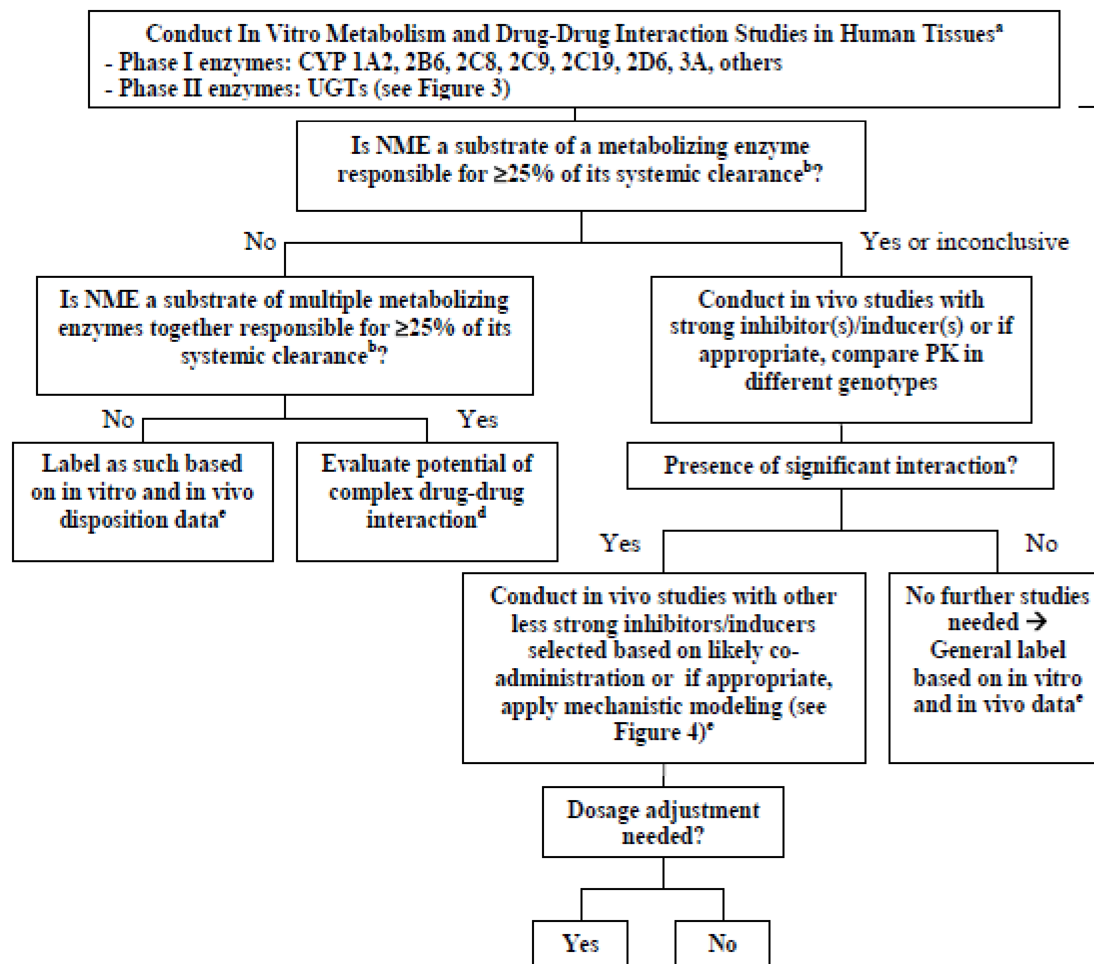
© 2010 Cyprotex

Metabolism studies

- Identify metabolic pathways that represent ≥ 25% of clearance
- Identify secondary routes of metabolism/elimination
- No specific recommendations on methods for the conduct of *in vitro* studies. Test systems mentioned: HLM, rCYPs and hepatocytes.
- More than just the big 7 CYP enzymes – UGTs, MAO, FMO, XO, CR, ADH etc. Others such as AO not discussed

© 2010 Cyprotex

Metabolism Based DDI Studies – Decision Tree



© 2010 Cyprotex

Metabolism Based DDI Studies – Decision Tree

Is glucuronidation a major metabolic pathway (≥ 25% of total metabolism)[1]?

Yes ↓

In vitro study with UGT recombinant enzymes (to identify which UGT isoforms[2] are responsible for)

- **Mainly by UGT1A1**
  - Either of the following
    - In vivo human comparative PK study based on genotype
    - In vivo human inhibition study with a UGT 1A1 inhibitor (e.g., atazanavir)
- **Mainly by other UGTs or multiple UGTs**
  - Either of the following
    - In vivo human comparative PK study based on genotype, if appropriate
    - In vivo human inhibition study with a general inhibitor (probenecid or valproic acid)

[1] In an in vitro system capable of informing contribution by UGT and non-UGT enzymes (e.g., hepatocytes or microsomes supplemented with appropriate co-factors).

[2] Main UGTs recommended to be studied: UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15.

In vitro studies used to assess rate and route of metabolism

- Human liver Microsomes
  - NADPH (Phase I metabolism)
  - UDPGA (alamethicin) (Phase II metabolism)
  - Both NADPH and UDPGA (Phase I and II metabolism)
  - Chemical Inhibition/Antibody studies, Correlation analysis
- Hepatocytes – Phase I and II metabolism
- Recombinant Enzymes – CYP, UGTs, Non CYP such as MAO

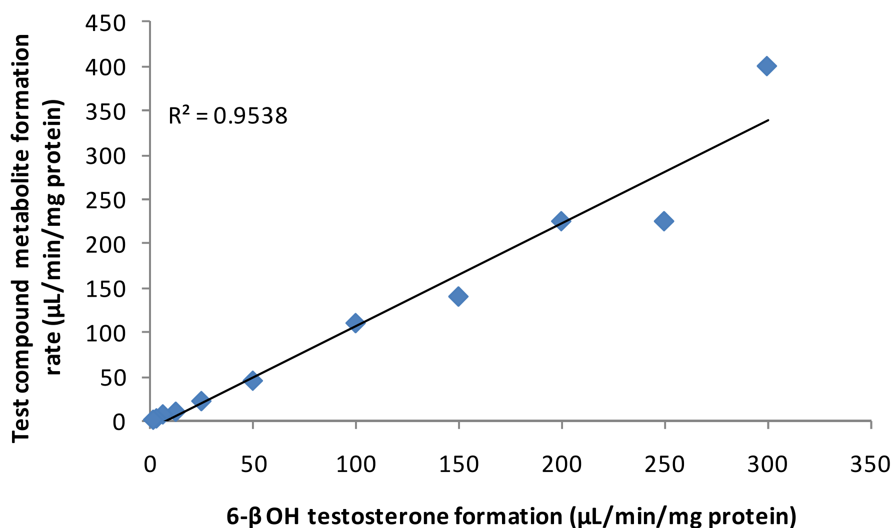
Individually Expressed Recombinant Enzymes

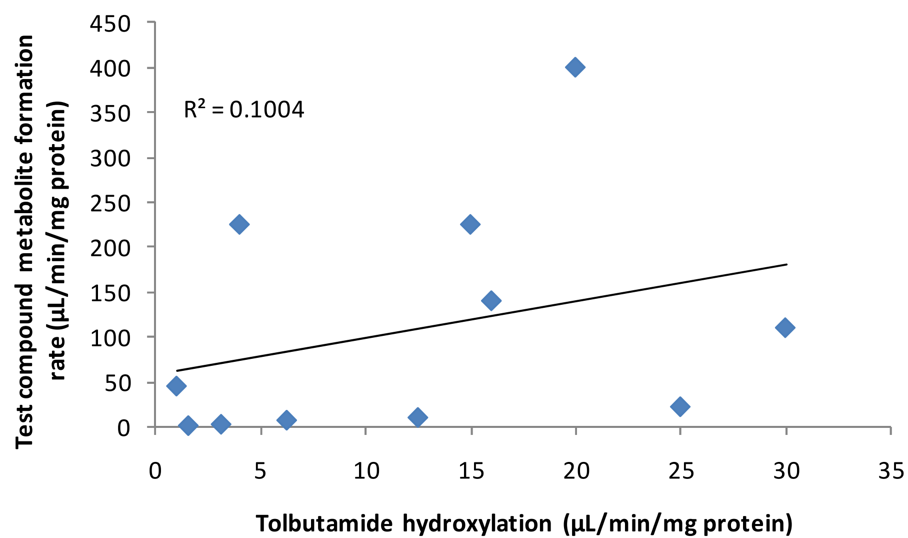
	Test Compound CLint (µL/min/mg protein)
CYP1A2	0
CYP2C9	10
CYP2C19	0
CYP2D6	0
CYP3A4	40

Data can be scaled using relative activity factors to account for abundance of CYPs

© 2010 Cyprotex

Correlation analysis





© 2010 Cyprotex

Inhibition and Induction – Why?

Inhibition

- Reversible
  - Cisapride withdrawn in the US due to interactions with CYP3A4 inhibitors including Ketoconazole, increase in cardiac arrythmias and death
- Mechanism based interaction
  - Mibefradil withdrawn from market due to inhibition of CYP3A4 metabolism of Simvastatin↑ caused Rhabdomyolysis

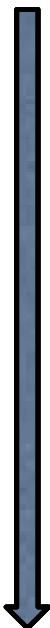
Induction

- Induction of CYP3A4 enzyme by Rifampicin reduces effectiveness of oral contraceptives (CYP3A4 substrates)

© 2010 Cyprotex

Systems for assessing CYP450 inhibition

Range of *in vitro* systems available to study CYP inhibition

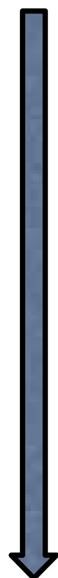


- Expressed purified enzymes
  - Cheap easy to use, issues with correlation to in vivo
- Fluorescent and LC-MS/MS CYP probe substrates
  - False negatives, fluorescent compounds
  - Recommend to use MS based probe substrates
- <u>Human liver microsomes</u>
  - Full set of human CYP enzymes, easy to use, cofactor required
- Human hepatocytes
  - No cofactor required, more expensive, incorporates membrane and transporter effect

© 2010 Cyprotex

Reversible CYP450 inhibition - methodologies

Range of *in vitro* assays available to study reversible CYP450 inhibition



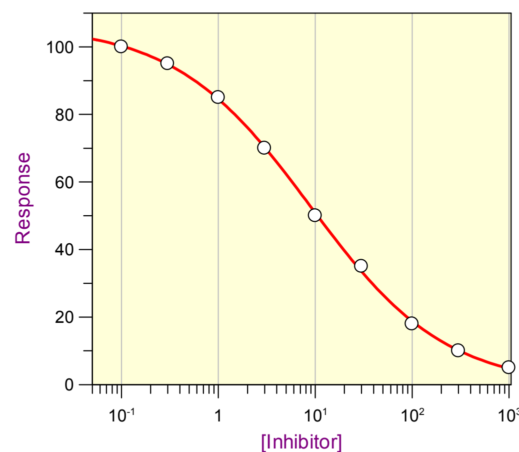
- Single point inhibition assay
  - % inhibition at 10 uM, prediction of IC_{50} – fast, cheap
- IC_{50} determination
  - Range of test compound at single [S]
- K_i determination
  - Multiple test compound concentrations, multiple [S] concentrations

© 2010 Cyprotex

Reversible IC_{50} determination assay

Incubate HLM with [S] and range of [I]

- Incubate each CYP450 substrate separately under linear conditions with respect to time and protein with low substrate depletion
- Isoform specific conditions – concerns about cassette incubation interactions
- Measure specific metabolite formation using LC-MS/MS – improvements with analytical conditions (e.g RapidFire) allows fast turnaround moving assay earlier into discovery
- Generate IC_{50} values based upon inhibition of specific CYP metabolite formation
- Include CYP specific positive control inhibitor



© 2010 Cyprotex

Determination of K_i values

K_i determination allows mechanism/type of inhibition to be determined:

- Competitive: inhibitor binds to prevent substrate binding
- Non competitive inhibition: inhibitor and substrate bind reversible and independently, bound I renders enzyme inactive
- Uncompetitive binding: inhibitor only binds to enzyme that has substrate bound, very rare!
- Linear mixed: form of non competitive, substrate and inhibitor bind independently but binding of inhibitor to enzyme effects binding of substrate (and v.v)

© 2010 Cyprotex

Experimental determination of K_i values

- Matrix of inhibitor concentrations (assume 0, $1/3K_i$, K_i, $3K_i$, $10K_i$) and substrate concentrations ($1/3K_m$, K_m, $3K_m$, $10K_m$), in duplicate=40 samples, hence IC_{50} popularity even with its limitations!
- Use Eadie-Hofstee analysis for mechanism (v vs v/S) to visualise date
- Use Dixon (1/v vs [I]) plot to sanity check data
- Analyse all data using non linear regression (GraFit, WinNonLin) which uses all velocities to simultaneously fit data to chosen model and use goodness of fit (e.g. Akaike Criteria AIC) to determine best model(s)

© 2010 Cyprotex

Irreversible time dependent inhibition of CYP450

- Mechanism based inhibitors bind to CYP450 and becomes catalytically active (also known as time-dependent inhibitors or suicide inhibitors)
- The active species subsequently alters the enzyme irreversibly through covalent bonding thereby removing it permanently from the pool of enzyme activity
- Enzyme activity is not restored until the synthesis of new enzyme
- Increasing concern as to the impact of time dependent inhibition (TDI) on potential drug-drug interactions – onset in vivo much slower than with reversible inhibition
- Viewed as potentially more concern than reversible inhibition as the inhibitory effect remains after elimination of parent drug

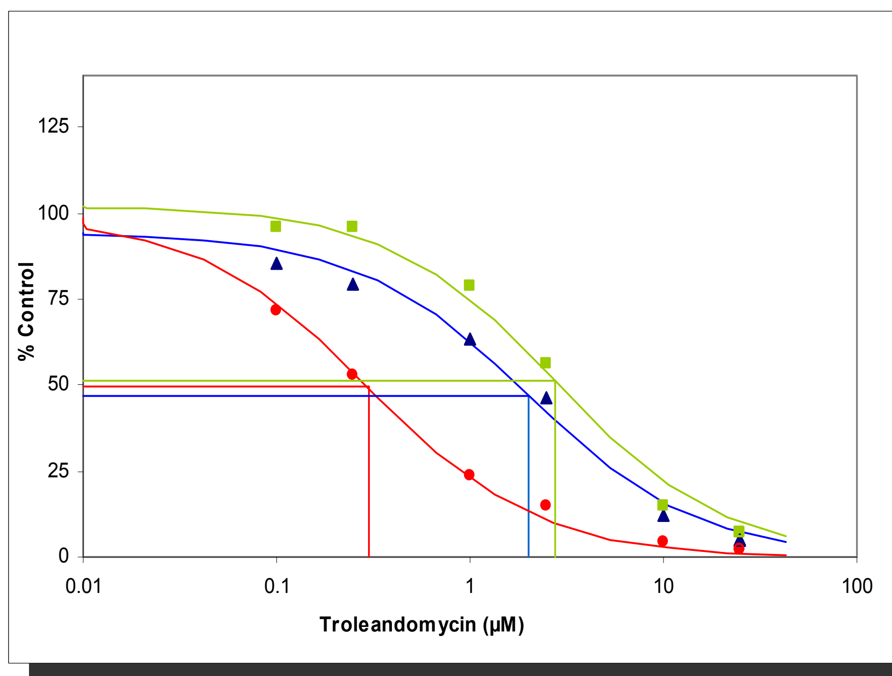
© 2010 Cyprotex

TDI CYP450 inhibition methodologies

Range of *in vitro* systems available to study mechanism based CYP inhibition

- Single point inhibition assay
  - 30 minute pre-incubation, 10-fold dilution, [S] at $5K_m$ to minimise reversible inhibition
- IC_{50} shift determination
  - Range of test compound at single [S], 30 minute pre-incubation, no dilution step to detect reversible and TDI, [S] at K_m
- K_I and K_{inact} determination
  - Multiple test compound concentrations, multiple pre-incubation times, 10-fold dilution, [S] at $5K_m$

CYP3A4 TDI IC_{50} shift data

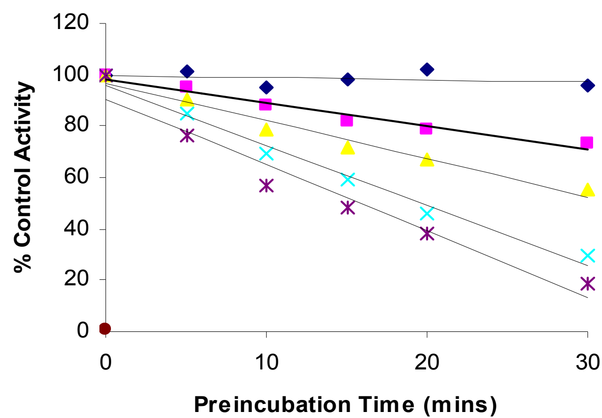


- Avoid dilution step: want to determine reversible and TDI effects. Concerns also about Fu_{mic} in pre-incubation
- IC_{50} shift > 1.5 fold: characterise further

	Predicted IC_{50} (µM)	SD	% Inhibition
0 Minute	2.003	0.318	95.08
30 Minute Minus NADPH	2.766	0.385	92.97
30 Minute Plus NADPH	0.298	0.025	97.91

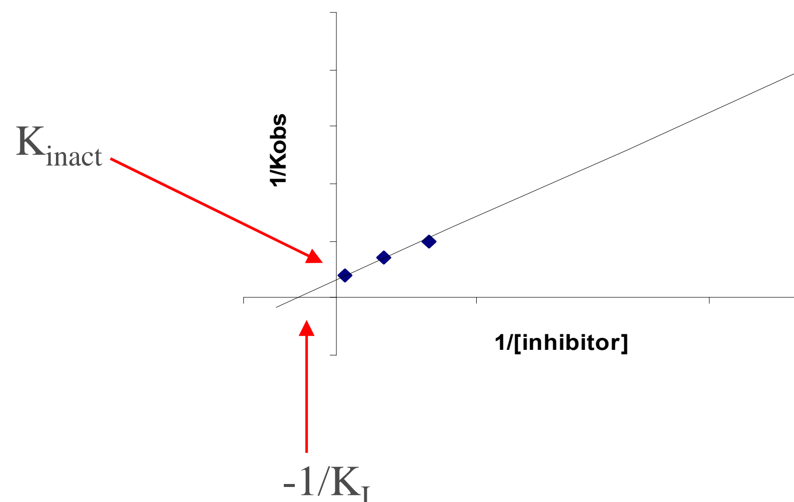
© 2010 Cyprotex

Experimental determination of K_I and K_{inact} values



- •Screen multiple test compound concentrations against multiple pre-incubation times
- •Plot residual activity against pre-incubation time – slope of line = K_{obs}

- •Kitz-Wilson plot of $1/K_{obs}$ against 1/[I]
- •K_{inact} and K_I determined from X and Y intercepts
- •K_{inact} = rate of enzyme inactivation
- •K_I = concentration of half-max inactivation



Enzyme Induction

An increase in the drug metabolizing capacity of an enzyme

Mainly focused on CYP450 enzymes but other enzymes are inducible:

- UGT's
- GST's

Various mechanisms involved in enzyme induction:

- Increased transcription
- Protein stabilisation
- mRNA stabilisation

© 2010 Cyprotex

Induction of Cytochrome P450

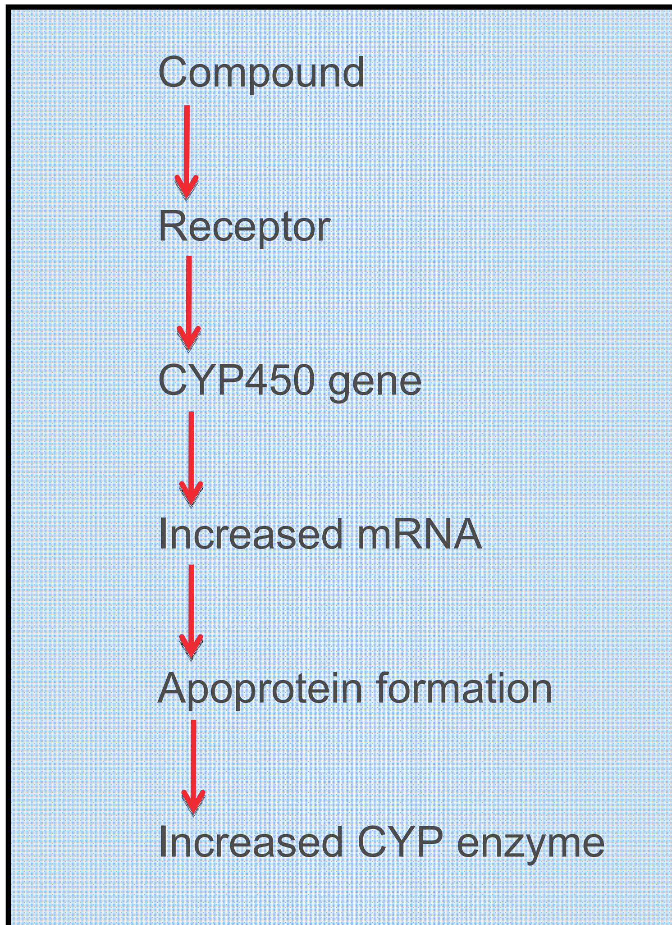
A number of human CYPs are inducible:

- CYP1A Inducer: cigarette smoke, omeprazole
- CYP2B6 Inducer: rifampicin
- CYP2C9 Inducer: rifampicin, PB
- CYP2C19 Inducer: rifampicin
- CYP2E1 Inducer: ethanol
- CYP3A4 Inducer: rifampicin, PB, phenytoin, St Johns Wort

- **Induction is primarily by increased transcription, CYP2E1 via mRNA and protein stabilization**

© 2010 Cyprotex

Overview and assays available for CYP450 induction



Assay

Ligand binding assay, transfection/reporter assays

qRT-PCR, Northern blots

Western blots

Catalytic activity assays

© 2010 Cyprotex

Nuclear receptors and CYP450 induction

Major mechanism for CYP induction is via increased transcription activated via receptor dependent mechanisms

- **CYP1A: Ahr receptor**
  - Induction of CYP1A1, 1A2 but also certain GST, UGT isoforms
- **CYP3A: Pregnane X receptor (PXR)**
  - Induction of CYP3A4
- **CYP2B: Constitutive androstane receptor (CAR)**
  - Induction of CYP2B6
- **CYP4A: PPAR alpha**
  - Endogenous fatty acid metabolism
- **PXR appears to be main regulator: cross talk with 2B, 2C induction and CAR receptor also regulates P-gp, MRP2**

© 2010 Cyprotex

Assays to investigate human CYP450 induction

Primary human hepatocytes

- Good *in vitro* model for human CYP induction
- Considerable inter-individual variation
- Issues with supply of cells, increasing acceptance of cryopreserved hepatocytes
- mRNA, protein and catalytic activity can be assessed using qRT-PCR, Western blots and substrate metabolism studies
- Due to supply issues other cells have been developed:
  - Immortalised hepatocytes with SV40 antigen: Fa2N-4 cells
  - Human hepatoma cell line: HepaRG

© 2010 Cyprotex

Regulatory guidance for human CYP450 induction

- **FDA requires definitive assessment for all NCE's**
- ***In vitro* data recommendations:**
  - 3 donors
  - 6 isoforms (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A4)
  - List of recommended positive control inducers and negative control
  - FDA recommend mRNA as primary endpoint, EMEA still recommend catalytic and mRNA as endpoints
  - Emax and EC_{50} should be determined to allow for modelling of data

© 2010 Cyprotex

CYP450 induction in human hepatocytes- overview

Freshly isolated human hepatocytes

Seed 24 well collagen coated plate (0.15 x 10^6 cells/cm^2)

Following attachment, replace media and incubate for ~40 hr.

Test compound in DMSO

DMSO

Dilution in culture media

10μm 1μm 0.1μm

Addition of test compound dilutions and vehicle

Incubate for 72 hr, replacing every 24 hr.

Isoform specific probe substrate.

Incubate with isoform specific probe substrate.

qRT-PCR

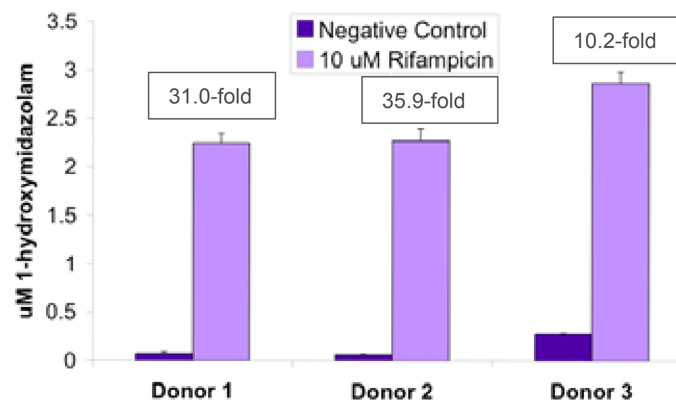
LC-MS/MS analysis (CYP3A4)

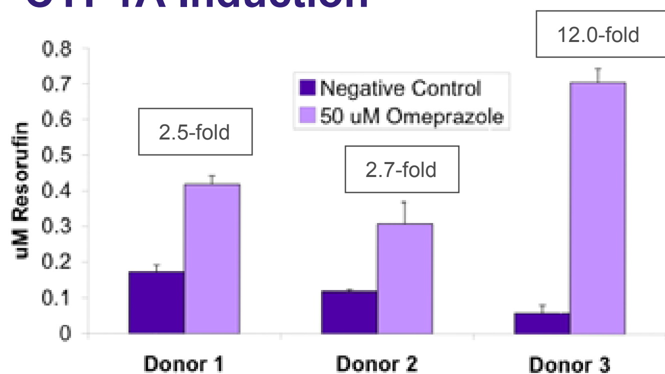
Fluorescence (CYP1A)

Quantification of isoform-specific metabolite.

© 2010 Cyprotex

Inter-individual CYP450 induction responses

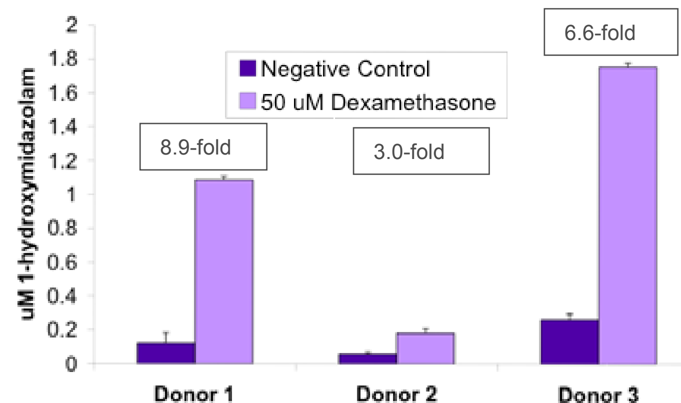




Smoker

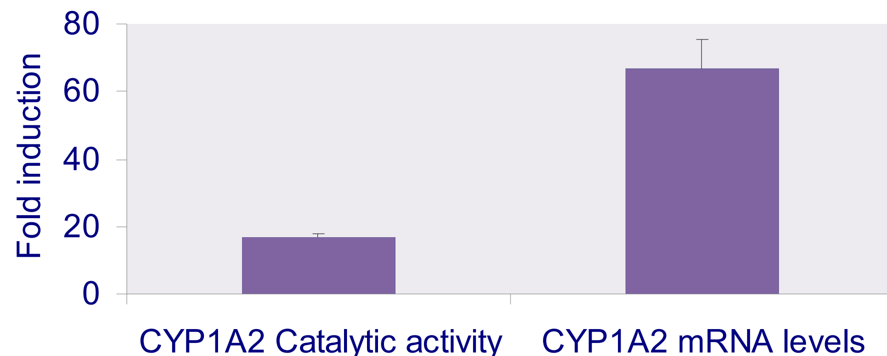
Smoker

Non-Smoker

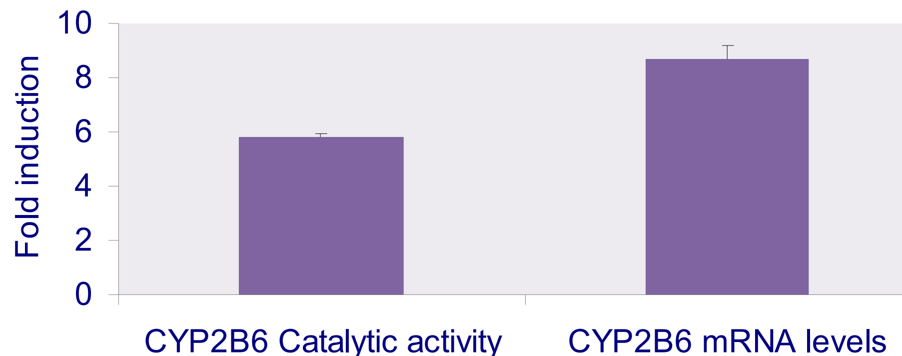


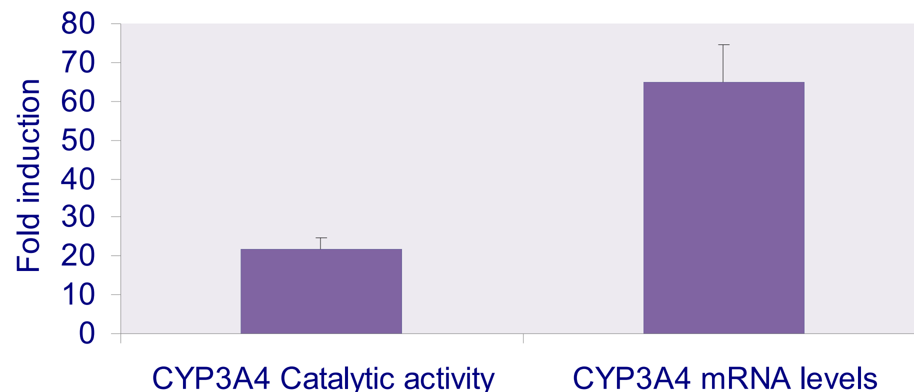
© 2010 Cyprotex

Positive control responses in CYP450 induction – comparison of mRNA and enzyme activity



Positive control induction of CYP1A2 (omeprazole), CYP2B6 (phenobarbital) and CYP3A4 (rifampicin): mRNA and activity





© 2010 Cyprotex

Inhibition and Induction Decision Tree

CYP inhibition
(reversible and time-dependent inhibition, TDI)

- Measure enzyme activity in human liver microsomes
- Estimate DDI parameters

CYP induction

- Measure mRNA change by investigational drug in cultured human hepatocytes from ≥3 donors [a]
- Estimate DDI parameters

Basic models

Is the calculated R value >1.1 (also, for CYP3A inhibitors given orally, is alternate R value>11)[b]?

- *Reversible inhibitor,* $R_1 = 1 + [I]/K_i$
- *TDI,* $R_2 = (K_{obs}+K_{deg})/K_{deg}$ *and* $K_{obs}=k_{inact}\times[I]/(K_I+[I])$

Is increase in mRNA > a predefined threshold[a]? Or, is the calculated R value<1/1.1 (i.e., 0.9)?
$R_3=1/(1+d\times E_{max}\times[I]/(EC_{50}+[I]))$[c]

No → Label as non inhibitor or non inducer based on in vitro data

Yes → Investigational drug likely a CYP inhibitor

Yes → Investigational drug likely a CYP inducer

Mechanistic models

Is AUCR >1.25 (inhibition) or AUCR <0.8 (induction)? [d]

Estimate AUCR of a sensitive probe substrate using

- a mechanistic static model[e]

$$AUCR = \left(\frac{1}{[A_g \times B_g \times C_g] \times (1-F_g) + F_g}\right) \times \left(\frac{1}{[A_h \times B_h \times C_h] \times f_m + (1-f_m)}\right)$$

- or a dynamic model, including PBPK[f]

No → Label as non-inhibitor or non-inducer

Yes → **Conduct a clinical study using an appropriate probe substrate [g]**

© 2010 Cyprotex

Transporter mediated drug-drug interactions

- Example 1 Digoxin-Ketoconazole
- Digoxin undergoes minimal metabolism (16%)
- Ketoconazole P-gp inhibitor
- Increase in plasma concentrations (164%) when co-administered with ketoconazole

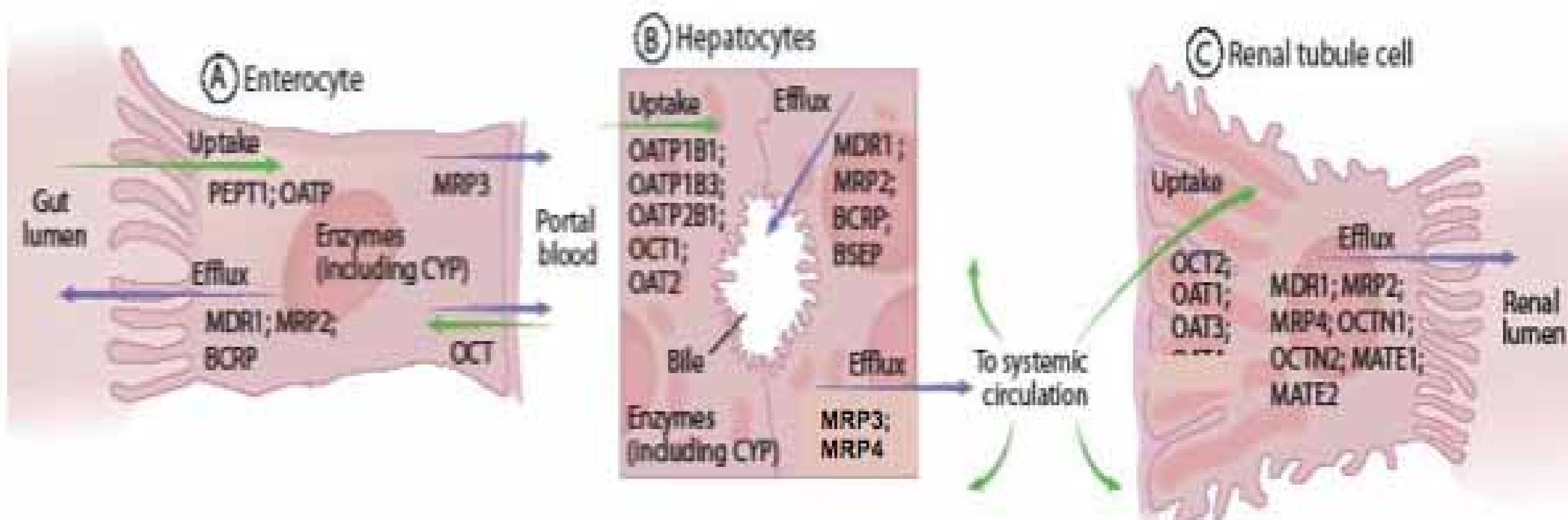
Benet, LZ and Salphati, L. (1998) *Pharmacology*, **56**:308-313

- Example 2 Digoxin-Rifampicin
- Digoxin undergoes minimal metabolism (16%)
- Rifampicin P-gp inducer
- Decrease in digoxin plasma concentration (58%) and oral bioavailability (30%) when co-administered with rifampicin

Greiner *et al* (1999), *The Journal of Clinical Investigation,* **104**(2); 147-153

© 2010 Cyprotex

Transporter location



- FDA Recommend 7 different transporters for study
- MDR1 (P-gp), BCRP (enterocyte efflux)
- OATP1B1, OATP1B3 (hepatocyte uptake)
- OCT2, OAT1, OAT3 (renal tubule uptake)
- EMA also recommend BSEP and OCT1

- Coming soon...... MATE1 and MATE2

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/Development Resources/DrugInteractionsLabeling/ucm080499.htm

Same seven transporters and workflow to those presented in the 2010 ITC White Paper (*Giacomini et al., Nat Rev Drug Discov. 9:215-236, 2010)*

Evaluation of transporter substrates

- P-gp and BCRP (all drug candidates)
  - Bi-directional transport assays (Caco-2, MDCK, LLC-PK, etc.)
  - Net flux ratio > 2 indicates a substrate
  - Efflux in the presence of specific P-gp and/or BCRP inhibitors
  - DDI in vivo with P-gp inhibitors (e.g. verapamil, itraconazole)
- OATP1B1 and OATP1B3 (> 25% hepatic clearance)
  - Knowledge of drug's physiochemical properties (e.g. permeability and charge)
  - Hepatocytes and/or transfected cells (typically > 2-fold vs. control)
  - DDI in vivo with rifampin or cyclosporin or in various OATP1B1 genotypes
- OAT1, OAT3 and OCT2 (> 25% renal clearance or CLr > GFR)
  - Uptake into transporter over-expressing cell lines (typically > 2-fold vs. control)
  - DDI in vivo with probenecid (OAT) or cimetidine (OCT)

© 2010 Cyprotex

Evaluation of transporter inhibitors

- P-gp and BCRP (all drug candidates)
- Bi-directional transporter assays (Caco-2, MDCK, LLC-PK, etc.)
- Net flux ratio of probe substrate (digoxin or loperamide) decreases indicates an inhibitor
- Determine Ki or IC50 (cell lines or vesicles)
- If $[I]_1/IC50 \geq 0.1$ or $[I]_2/IC50 \geq 10$ in vivo study required
  - $[I]_1$ = Cmax (total) OR portal vein unbound (fub)
  - $[I]_2$ = Dose/250 mL (often mM concentrations)

© 2010 Cyprotex

Caco-2 – a valuable *in vitro* model of drug permeability

- Caco-2 cells derived from human colon carcinoma.
- Cells have characteristics which resemble intestinal epithelial cells (e.g. polarised monolayer, well defined apical brush border, intercellular junctions)
- Caco-2 assay is an established and valuable *in vitro* model for drug permeability which is recommended by FDA
- Contains multiple transporter proteins (Pgp, BCRP)

© 2010 Cyprotex

Caco-2 Permeability Protocol Overview

Day 1

Cells Seeded

Caco-2 cells
(passage 40-60)

Day 20

Compound Addition

Lucifer Yellow

Compound 10µM

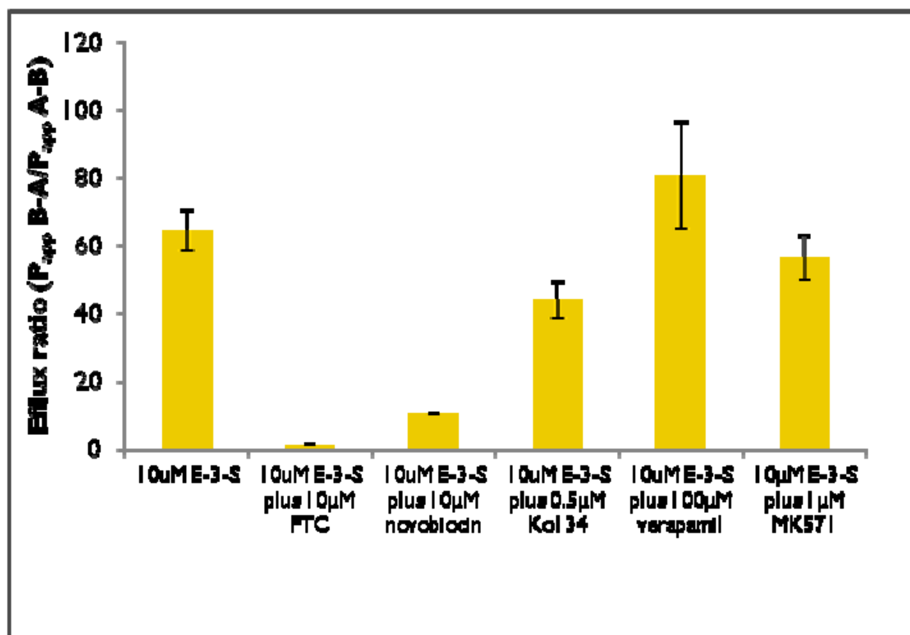
A-B

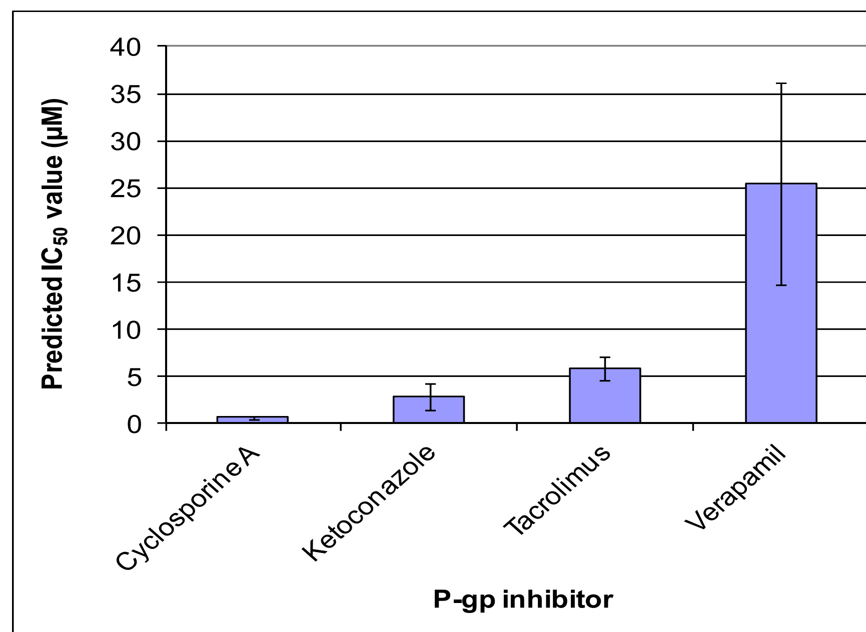
Fluorimeter

Permeation, Q, over 2hr measured by LC-MS/MS

$$\frac{dQ}{dt} = P_{app} A C_o$$

BCRP and P-glycoprotein data





© 2010 Cyprotex

Typical design of *in vivo* study

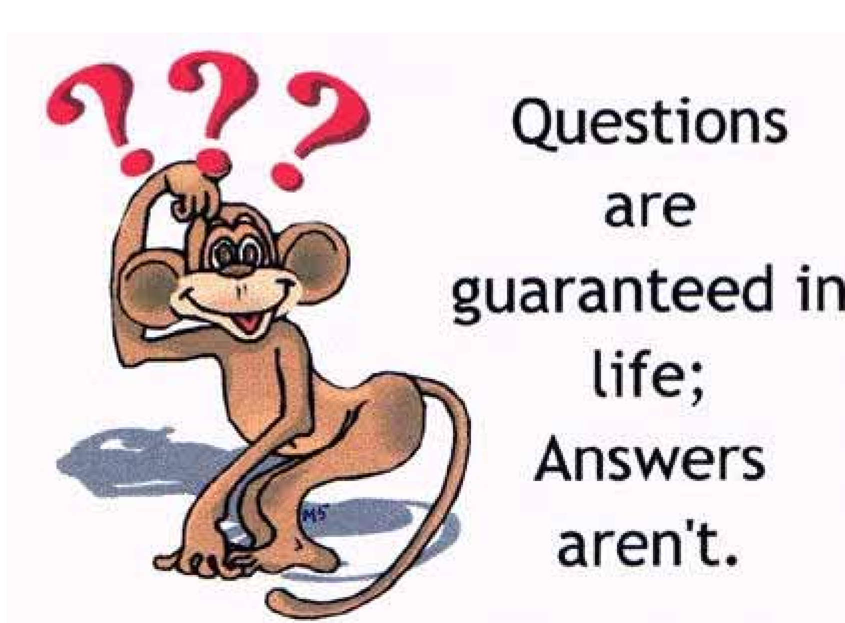
- Dependent on prior knowledge
  - Randomised crossover, one sequence crossover or a parallel design
  - Removal of dietary supplements, tobacco, alcohol and juices that may affect enzymes and transporters 1 week prior to study
  - Genotyping where appropriate
  - Timing of administration
  - Choice of interacting drugs
  - Route of administration
  - Dose selection (maximum planned dose and shortest dosing interval recommended)
- Labelling considerations

© 2010 Cyprotex

Summary

- Drug-drug interactions extremely important in drug development
- Be aware of guidance documents
- Minimise inhibition, transporter and induction potential where possible
- If unavoidable be prepared to invest time and money in PBPK modelling and appropriate clinical studies

Thanks for your Time and Attention!



m.griffin@cyprotex.com