**In vitro ADME & PK**

Cytochrome P450 Induction

**Background Information**

- Induction of cytochrome P450 enzymes is associated with an increased prevalence of clinical drug-drug interactions.
- Cyprotex's Cytochrome P450 induction assay identifies the potential of test compounds to induce CYP1A2, CYP2B6 or CYP3A4 in cultured human hepatocytes by evaluating mRNA levels and/or catalytic activity. Assays are designed to meet FDA and EMA guidelines.
- Test drug concentrations should be based on the expected human plasma drug concentrations and dose. Solubility, cytotoxicity and plasma protein binding should also be taken into consideration.
- Cyprotex's Cytochrome P450 induction assay delivers fold-induction data normalised to vehicle control which can be compared to positive control responses. If appropriate, data is fit using non-linear regression analysis to four-parameter sigmoidal equation to produce $E_{\text{max}}$ and $EC_{50}$ values.
- The clinical consequences of induction may be therapeutic failure caused by a decreased systemic exposure of the drug itself or a co-administered therapy, or toxicity as a result of increased bioactivation.

**Supplementary Assays**

- Preliminary aqueous solubility assessment
- Cytotoxicity assessment in primary human hepatocytes or HepaRG (e.g. MTT)
- Assessment of non-specific binding
- Measurement of parent drug on final day of dosing

**Protocol**

**Test System**
- Cryopreserved or fresh human hepatocytes (3 donors recommended)
- HepaRG cells are available on request

**Test Article Concentration**
- 1, 3 or 6 concentrations (dependent upon unbound $C_{\text{max}}$, dose, solubility and cytotoxicity) plus vehicle control, in triplicate

**CYP Isoforms**
- CYP1A2, CYP2B6 and CYP3A4
- For CYP2C, UGT or transporter studies, please contact directly for information

**Controls**
- Omeprazole (CYP1A2 positive control)
- Phenobarbital (CYP2B6 positive control)
- Rifampicin (CYP3A4 positive control)
- Negative control (non-inducer)

**Test Article Requirements**
- Dependent on top concentration (recommend 0.1% DMSO in incubation)

**Exposure Period**
- 72 hr (media changed every 24 hours)

**Probe Substrates for Catalytic Activity**
- Phenacetin (CYP1A2)
- Bupropion (CYP2B6)
- Midazolam (CYP3A4)

**Analysis Method**
- LC-MS/MS quantification of acetaminophen (CYP1A2), hydroxybupropion (CYP2B6) and 1-hydroxymidazolam (CYP3A4)
- qRT-PCR for relative mRNA expression levels (CYP1A2, CYP2B6 and CYP3A4)

**Data Delivery**
- Report detailing methodology, donor demographics, mRNA levels, fold induction relative to vehicle control, concentration of metabolite of probe substrate, $E_{\text{max}}$, $EC_{50}$ and $F2$ (concentration which leads to a 2-fold increase above $E_{\text{min}}$) if appropriate.
‘The evaluation of CYP enzyme induction should begin with studies of CYP1A2, CYP2B6, CYP3A4 \textit{in vitro}. If the \textit{in vitro} induction results are positive according to predefined thresholds using basic models, the investigational drug is considered an enzyme inducer and further \textit{in vivo} evaluation may be warranted.’

\textbf{Figure 1}  
Induction of CYP1A2 mRNA levels by omeprazole in cryopreserved human hepatocytes.

\textbf{Figure 2}  
Induction of CYP2B6 mRNA levels by phenobarbital in cryopreserved human hepatocytes.

\textbf{Figure 3}  
Induction of CYP3A4 mRNA levels by rifampicin in cryopreserved human hepatocytes.

References