NoAb BioDiscoveries

# CYTOCHROME P450 INDUCTION STUDIES IN HUMAN CRYOPRESERVED HEPATOCYTES

## *Background*

Many drug-drug interactions are metabolism based and mediated primarily via the Cytochrome P450 (CYP) family of enzymes. Ten CYP isoforms are expressed in a typical human liver (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1, and CYP3A4). Some of these enzymes may be induced following drug exposure (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/19, CYP2E1, and CYP3A4) and may have important clinical consequences. The induction of these CYP enzymes are regulated by three main nuclear receptors, aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), and pregnane X receptor (PXR). Therefore, an effective strategy to investigate the inductive effects of new chemical entities (NCEs) is to evaluate changes in the expression and function of the most sensitive genes regulated by the three respective nuclear receptors – CYP1A2 (AhR), CYP2B6 (CAR), and CYP3A4 (PXR). As detailed in the FDA's Draft Guidance document for Drug-Drug Interactions (2006)[1], the FDA has placed emphasis on evaluating the induction potential of a new chemical entity (NCE) at an earlier stage in drug-development, to identify and avoid potential adverse drug interactions.

## *Assay Outline*

In accordance with the FDA Draft Guidance for Drug-Drug Interactions CYP1A2, CYP2B6, and CYP3A4 induction studies are carried out as follows:

- Cryopreserved human hepatocyte cultures from three individual donors are used
- Cells are treated with three concentrations (0.1, 1, and 10x $C_{max}$) of test compound(s) in triplicates
- As a positive control, cells are treated with FDA accepted prototypical inducers for CYP1A2, CYP2B6, and CYP3A4 (table below)
- As a negative control, cells are treated with vehicle
- Following 48 hours of treatment, CYP1A2, CYP2B6, and CYP3A4 activity is assessed by measuring the conversion of FDA-recommended substrates to metabolites quantified using validated LC/MS-MS methods. Gene expression can also be measured by qRT-PCR.
- The degree of induction by the test compound(s) is determined by comparing the inductive effect of the test compound to the inductive effect of the known prototypical inducer for the corresponding CYP isoenzyme
- 48-well plating of cells, substrate cocktail dosing approach, and cassette analysis of marker metabolite quantification by LC-MS/MS ensures a high quality data output with a rapid turn around time.

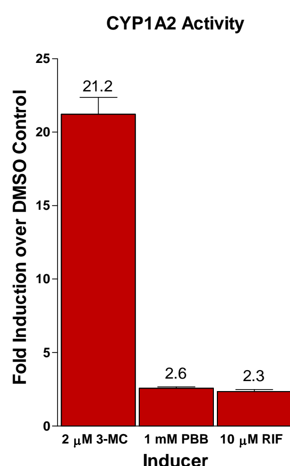
## *Experimental Results*

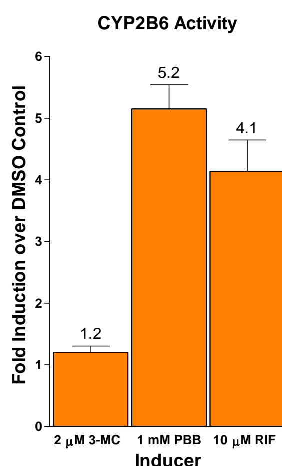
As an example, cryopreserved human hepatocytes were treated with prototypical CYP1A2, CYP2B6, and CYP3A4 inducers, 3-MC, Phenobarbital, and rifampicin, respectively, for 48 hours. The fold induction in CYP1A2, CYP2B6, and CYP3A4 activity was determined as a measure of specific metabolite formation (acetaminophen, hyroxybupropion, and 6-β-OH-testosterone, respectively) relative to vehicle control cells (un-induced). The fold induction in mRNA expression of treated cells over control cells was quantified by qRT-PCR. The data (mean ± S.D) are depicted graphically in the figure below.

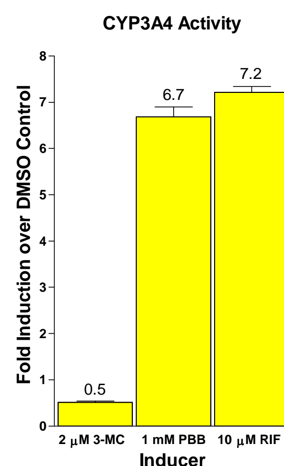

***References:***

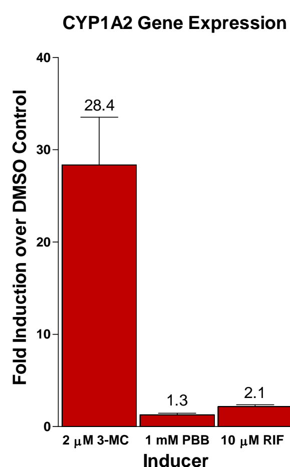
1. USFDA (2006) Draft Guidance for Industry: Drug Interaction Studies-Study Design, Data Analysis, and Implications for Dosing and Labeling, U.S. Food and Drug Administration Publication
2. Roymans et al., Drug Metabolism and Disposition, Vol. 33 No.7 (2005)

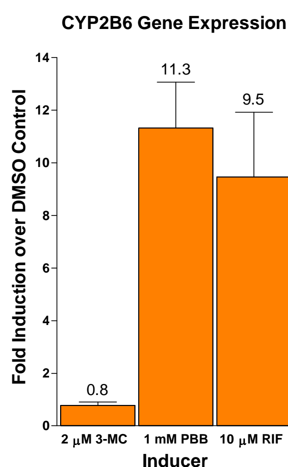
## Assessment of the Induction of CYP1A2, CYP2B6, and CYP3A4 Activity in Human Cryopreserved Hepatocytes following 48 hours of Treatment with 3-MC, Phenobarbital, and Rifampicin

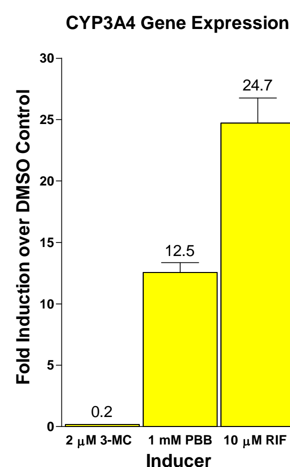



NoAb's CYP450 induction assay allows the evaluation of the induction potential of a compound, which helps to identify and avoid potential adverse drug interactions. NoAb also offers a complementary activity based CYP450 inhibition assay, to evaluate the inhibitory potential of NCEs. These services are examples of NoAb's commitment to providing the best drug discovery tools for our clients, helping to shape drug discovery.

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