

# ***Critical Review of Preclinical Approaches to Investigate Cytochrome P450-mediated Therapeutic Protein Drug-Drug Interactions and Recommendations for Best Practices: A White Paper***

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**Running Title:** CYP-related Therapeutic Protein Drug Interaction White Paper

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**Text Pages:** 47

**Tables:** 4

**Figures:** 5

**References:** 66

**Abstract** (words): 246

**Introduction** (words): 465

**Discussion** (words): N/A

**Abbreviations:** AA, adjuvant arthritis; CRP, C-reactive protein; CYP, Cytochrome P450; DDI, drug-drug interaction; EMA, European Medicines Agency; FDA, Food and Drug Administration; IL, interleukin; LPS, lipopolysaccharide; PK, pharmacokinetics; PD, pharmacodynamics; SMD, small molecule drug; TP, therapeutic protein; WME, William's E Media.

## Abstract

Drug-drug interactions (DDI) between therapeutic proteins (TP) and small molecule drugs have recently drawn the attention of regulatory agencies, the pharmaceutical industry, and academia. TP-DDIs are mainly caused by proinflammatory cytokine or cytokine modulator-mediated effects on the expression of cytochrome P450 enzymes. To build consensus among industry and regulatory agencies on expectations and challenges in this area, a working group was initiated to review the preclinical state of the art. This White Paper represents the observations and recommendations of the Working Group on the value of *in vitro* human hepatocyte studies for the prediction of clinical TP-DDI. The White Paper was developed following a “*Workshop on Recent Advances in the Investigation of Therapeutic Protein Drug-Drug Interactions: Preclinical and Clinical Approaches*” held at the Food and Drug Administration White Oak Conference Center on June 4 and 5, 2012. Results of a workshop poll, cross laboratory comparisons and the overall recommendations of the *in vitro* working group are presented herein. The working group observed that evaluation of TP-DDI for anti-cytokine monoclonal antibodies is currently best accomplished with a clinical study in patients with inflammatory disease. Treatment-induced changes in appropriate biomarkers in Phase 2 and 3 studies may indicate the potential for a clinically measurable treatment effect on CYPs. Cytokine-mediated DDIs observed with anti-inflammatory TPs cannot currently be predicted using *in vitro* data. Future success in predicting clinical TP-DDIs will require an understanding of disease biology, physiologically-relevant *in vitro* systems, and more examples of well conducted clinical TP-DDI trials.

## 1. Introduction

The increased clinical use of therapeutic proteins (TPs) has raised awareness of drug-drug interactions (DDI) between TPs and small molecule drugs (SMD). Several recent workshops have addressed TP-DDIs, including the 2010 American Association of Pharmaceutical Scientists (AAPS) workshop on “Strategies to Address Therapeutic Protein–Drug Interactions during Clinical Development” (Girish et al., 2011), and related roundtables at the National Biotechnology Conference (NBC) in 2012 (Kenny et al., 2013). Recent publications from academia, industry and regulatory agencies have summarized the mechanisms involving TP-DDIs, evaluations of TP-DDIs included in Biologics License Application (BLA) submissions, and current strategies for the evaluation of TP-DDI potential during drug development (Morgan, 2009; Huang et al., 2010; Girish et al., 2011; Kraynov et al., 2011; Lee et al., 2010; Lloyd et al., 2011; Zhou and Mascelli, 2011; Dallas et al., 2013b; Slatter et al., 2013). As a result of discussions at several of these meetings, a collaboration among academia, industry and regulatory agencies was initiated under the leadership of the TP-DDI Steering Committee<sup>1</sup> to address knowledge gaps in the mechanisms of TP-DDI, the relevance of *in vitro* systems, current industry practices for assessing these interactions, and to develop a general framework of risk-based approaches for TP-DDI assessment during drug development. The efforts were focused on pharmacokinetic (PK) and metabolism-based DDIs of monoclonal antibodies (mAbs), fusion proteins, cytokines, and cytokine modulators. As such, TPs as perpetrators of effects on drugs metabolized by cytochrome P450 enzymes (CYPs) and as victims of other SMD or TPs were in scope. The Steering Committee subsequently formed two working subgroups as follows: The *In Vitro* TP-DDI Working Group<sup>2</sup>, focused on hepatocyte-based methodologies to assess TP-DDI and *in vitro-in vivo* correlation, and the Population Pharmacokinetics (Pop PK) Working Group<sup>3</sup>, focused on development of appropriate model-based TP-DDI evaluations, such as Population PK-based analyses. Both groups met by teleconference for more than one year, and organized a two day workshop at the Food and

Drug Administration (FDA) White Oak Conference Center on June 4 and 5, 2012. The workshop was co-sponsored by the FDA Office of Clinical Pharmacology and the Drug Metabolism and Clinical Pharmacology Leadership Groups of the International Consortium for Innovation and Quality in Pharmaceutical Development (The IQ Consortium<sup>4</sup>). The workshop was attended by more than seventy delegates, including scientists from two regulatory agencies, invited academic speakers and pharmaceutical industry scientists.

Following the workshop, both working groups agreed to publish independent White Papers. The white paper by the Pop PK group will appear elsewhere (Chow et al., manuscript in preparation). In this White Paper, we present cross laboratory comparisons of cytokine effects on CYPs in hepatocyte culture, the results of a current practices poll, and the observations and recommendations of the *In Vitro* Working Group regarding the use of *in vitro* models to study CYP-related TP-DDIs.

### **1.1. Overview of cytokine-mediated clinical drug interactions involving TPs**

TPs are often co-administered with SMDs. Initially, pharmacokinetic interactions between TPs and SMDs were viewed as unlikely, as mechanisms involved in the disposition of both modalities are fundamentally different. In the case of SMDs, various drug metabolizing enzymes and transporters are involved in absorption, distribution, metabolism and excretion, whereas clearance of TPs is typically mediated by non-specific catabolism or target-mediated elimination (Lobo et al., 2004; Deng et al., 2012). However, as more TPs progressed to the clinic, isolated examples of clinically measurable effects of cytokines or cytokine modulators on SMD PK have been described (Table 1). Clinical administration of pro-inflammatory cytokines such as IFN- $\alpha$  and IL-6 can reduce the activity of various CYPs, but in general these effects have been relatively small and variable (Lee et al., 2010). This class of TPs alters the expression of a

number of drug metabolizing enzymes and transporters *in vitro* (Aitken et al., 2006; Lee et al., 2010; Huang et al., 2010; Fardel and Le Vee, 2009). Several clinical studies have shown that SMD clearance can be impaired in individuals with inflammatory disease or cytokine-induced inflammation (Mahmood and Green, 2007; Schmitt et al., 2011; 2012a, b). Accordingly, there are now clinical studies showing that some pro-inflammatory cytokines (particularly IL-6), and cytokine modulators (particularly the anti-IL-6R mAb, tocilizumab) can affect pharmacokinetics of co-administered SMDs (Schmitt et al., 2011; 2012).

For the anti-IL6R mAb tocilizumab (8 mg/kg) in rheumatoid arthritis patients, a weak reduction (28%) was observed in the plasma exposure of omeprazole (a CYP2C19 substrate), and no effect was observed for dextromethorphan (a CYP2D6 substrate), or methotrexate (a substrate for OATs and OATPs) (Table 1; Schmitt et al., 2012a,b; Hoffmann-La Roche briefing document 2008; Zhang et al., 2009). Treatment of rheumatoid arthritis patients with a single dose of 10 mg/kg tocilizumab resulted in a 43% reduction in plasma exposure to the CYP3A4 substrate simvastatin (Schmitt et al., 2011). This reduction indicates reversal of IL-6 mediated CYP3A4 suppression in rheumatoid arthritis patients with tocilizumab exposure (Schmitt et al., 2011). In addition to the IL-6 mAb example, other cytokine modulators have been shown to affect drug metabolizing enzymes clinically. For the anti-IL2R mAb basiliximab, and anti-CD3 mAb muronomab, increased plasma levels of cyclosporine A have been observed, but these subsided over time (Vasquez and Pollack, 1997; Strehlau et al., 2000; Sifontis et al., 2002). These DDIs may occur as a result of changes in circulating pro-inflammatory cytokines that are induced by anti-cytokine mAb-mediated inflammatory disease mitigation, or can be caused by pro-inflammatory therapeutic cytokines used in infectious disease or cancer therapy (Morgan, 2009).

Overall, TP effects on SMD exposure observed so far have been modest (< 2-3 fold), and therefore may only be relevant for drugs like cyclosporine or warfarin that have a narrow therapeutic index and routinely undergo therapeutic monitoring. Furthermore, these effects are disease state- and/or drug target-dependent and are therefore different from typical pharmacokinetic DDIs between SMDs. As such, for inflammatory disease treatment, this is a drug-disease interaction, as opposed to a classical DDI.

## **1.2. Preclinical approaches to cytokine-mediated TP-DDI evaluation**

### **1.2.1 Cellular mechanisms of CYP suppression by cytokines and inflammatory mediators**

No single molecular mechanism can account for inflammation-mediated effects on various drug metabolizing enzymes and transcriptional effects may be coordinated by multiple different nuclear receptors and transcription factors (Zhou et al., 2009). NF- $\kappa$ B-mediated crosstalk with the pregnane X receptor (PXR, NR1I2) has been implicated (reviewed by Zordoky and El-Kadi (2009)), and Gu et al. (2006) demonstrated a direct interaction of NF- $\kappa$ B (p65) with the DNA binding domain of RXR $\alpha$ . Ghose et al., (2004) proposed that inflammation-induced effects on CYP could be related to the subcellular localization of RXR $\alpha$ , which is a partner in the heterodimeric nuclear receptors PXR, CAR, LXR, FXR, RAR, and PPAR $\alpha$ . Mechanisms like these may account for some of the general suppression effects that are observed across select enzymes involved in steroid, bile acid and lipid metabolism (Slatter et al., 2013).

In addition to effects on RXR, studies have implicated post-transcriptional mechanisms such as nitric oxide dependent ubiquitination and subsequent proteasomal degradation in the cytokine-mediated down-regulation of CYP enzymes (Ferrari et al., 2001; Lee et al., 2009). In rats, Lee et al. (2009) have shown that Cyp3a1 down-regulation by IL-1 $\beta$  occurs via two distinct modes; a

nitric oxide and proteasome dependent mechanism at early time points (< 9 hours) and a nitric oxide and proteasome independent mechanism at later time points (24 hours), with no decrease in Cyp3a1 mRNA at either time point. This study highlights the complexity of CYP regulation by cytokines and suggests that inflammation mediated suppression of metabolic enzymes can be a dynamic process and may involve several different measurable effects. For more in-depth reviews of cellular mechanisms of CYP suppression, the reader is referred to a number of review articles (Morgan, 2001; Morgan et al., 2002; Aitken et al., 2006; Jover et al., 2009; Zordoky and El-Kadi, 2009).

### **1.2.2. *In vitro* models to study CYP suppression by cytokines**

Unlike SMD metabolism and DDI, for which multiple *in vitro* methods are used routinely, there are only a few *in vitro* models that have been employed for predictive assessment of TP-DDIs (Abdel-Razzak et al., 1993; Aitken and Morgan, 2007; Dickmann et al., 2011; Dallas et al., 2013b). Primary human hepatocytes are the current *in vitro* model of choice for studies on the effects of individual pro-inflammatory cytokines on hepatic CYP expression and activity. Reports on the effect of cytokines on CYPs in primary hepatocytes culture date back to the early 1990s. Rat and human primary hepatocytes in sandwich culture were used to assess acute phase protein responses to cytokine stimulation (Bader et al., 1992). Donato et al. (1993) and Abdel-Razzak et al. (1993) showed that pro-inflammatory cytokines such as IL-6, TNF- $\alpha$ , and IFN- $\gamma$  suppressed the constitutive expression of CYP enzymes in human hepatocyte culture. The magnitude and direction of effects on CYP activity and expression depended on the particular cytokine and CYP isoenzyme measured (Nguyen et al., 2013). To maximize *in vitro* response, cytokine concentrations were based on or exceeded the maximum levels observed during the acute phase of inflammation (Muntane-Relat et al., 1995; Chen et al., 2011). Physiologically-relevant concentration thresholds for the *in vitro* effects of cytokines on hepatocytes are difficult to discern. Recent work in industry laboratories has attempted to benchmark the cytokine-



mediated suppression of CYPs to serum cytokine concentrations by measuring dose-response  $IC_{50}$  values at cytokine concentrations relevant to chronic inflammatory diseases such as rheumatoid arthritis (Dickmann et al., 2011, 2012).

Measurement of  $IC_{50}$  values for proinflammatory cytokine-mediated decreases in CYP activity requires that adequate basal CYP metabolism is available to quantify probe substrate metabolism both before and after suppression. Early hepatocyte studies suffered from a lack of highly sensitive and specific analytical assays and in the early 1990s were probably confounded by low basal CYP expression, most likely explained by non-optimal culture conditions. To overcome these limitations, hepatocytes were treated with CYP inducers, such as  $\beta$ -naphthoflavone (an AhR activator and CYP1A2 inducer) and rifampicin (a PXR activator and CYP3A4 inducer) in the presence and absence of different cytokines (Muntane-Relat et al., 1995). In these studies, TNF- $\alpha$  and IL-6 suppressed the induction of CYP1A2 and CYP3A4, respectively. These early studies showed that decreases in CYP expression correlated with CYP enzyme activities due to effects of the cytokines on transcriptional and post-transcriptional events, rather than on the rate of translation of CYP mRNA or the rate of CYP protein degradation (Muntane-Relat et al., 1995). Dickmann et al., (2011) recently measured the  $IC_{50}$  for the IL-6 mediated suppression of CYP3A4 in human hepatocytes in the presence and absence of the inducer rifampicin and showed that the  $IC_{50}$  value increased in induced hepatocytes. The degree to which induction ablates proinflammatory cytokine-mediated CYP suppression *in vivo* may depend on multiple factors such as the isoform involved, the inducer and dose, the cytokine involved, and the physiological concentrations of the cytokines evaluated.

Recent studies have employed cryopreserved human hepatocytes (Dickmann et al., 2011; Dallas et al., 2012), which offer the advantage of experiment scheduling, pre-selection and characterization of cells, and improved reproducibility in studies using the same donor. Dallas et

al. (2012) made a comprehensive analysis of the effects of IL-2, IL-6 and TNF- $\alpha$  on the gene expression and activities of multiple CYPs. This study reflects the findings of earlier studies using fresh human hepatocytes, and supports the use of cryopreserved hepatocytes as a model for cytokine mediated suppression of CYPs.

Although primary hepatocyte culture offers a useful model system to investigate the effect of a single cytokine on CYP expression, the model does not adequately reflect the complex interactions of multiple cytokines and cell types that are in play *in vivo* during inflammatory disease. For this reason, *in vitro* studies with a single cytokine and hepatocytes only may not accurately predict the magnitude of an effect *in vivo*.

Studies using the human hepatocyte-Kupffer cell co-culture model are currently rare due to the limited (commercial) availability of human Kupffer cells. In human hepatocytes co-cultured with hepatic Kupffer cells, the effects of three individual cytokines on CYP3A4 activities were measured by Sunman et al. (2004). The presence of Kupffer cells did not significantly perturb the suppression of CYP3A4 by IL-1 and IL-6. In contrast, IL-2 caused only a transient decrease in CYP3A when incubated with human hepatocytes alone, but in co-culture with Kupffer cells, caused a concentration-dependent and sustained suppression in CYP3A activity after 72 h.

In studies using LPS, the importance of physical contact between Kupffer cells and hepatocytes *in vitro* was demonstrated for various inflammatory cytokines (Hoebe et al., 2001). In these studies, LPS (1 and 10  $\mu\text{g/ml}$ ) was incubated with a single cell type or in co-culture with or without direct physical contact, as provided by a semi-permeable membrane insert. Cultures with physical contact between cell types had 10-fold (TNF- $\alpha$ ), to 500-fold higher release of cytokines (IL-6). Phase 1 and 2 enzyme activities in the hepatocytes (e.g. CYP3A and UGT) were decreased to a greater extent relative to hepatocyte-only or non-contact co-cultures.

These *in vitro* data suggest that Kupffer cells have a significant role in priming hepatocytes to the suppressive effects of certain cytokines like IL-6 (Freudenberg et al., 1982). Hepatocyte co-culture with Kupffer cells, as currently employed, may not be able to predict TP-DDI quantitatively due to the complexity of the immune system. However, co-culture or other systems that recapitulate aspects of *in vivo* liver physiology may be worthy of further exploration for mechanistic studies on proinflammatory cytokine-mediated CYP suppression (Kenny et al., 2013).

Studies on CYP suppression by multiple cytokines in combination are rare and historically LPS was most commonly used to elicit a generalized acute phase response. A recent study extended single cytokine studies on IL-6 alone to examine the effect of combinations of IL-1 $\beta$ , IL-6 and TNF $\alpha$  on CYP in human hepatocytes (Dickmann et al., 2011). When hepatocytes were treated with IL-1 $\beta$  and IL-6 in combination at concentrations ranging from 1-100 pg/mL, IL-6 was the main determinant of increases in acute phase response marker mRNA and of decreases in CYP3A4 mRNA (Dickmann et al., 2012). There was no synergy between IL-1 $\beta$  and IL-6 in the regulation of CYP mRNA, although the effects of the two cytokines in combination were additive in certain instances. The preliminary study examined only a single donor and therefore awaits more detailed experiments that may shed light on inter-subject variability.

### **1.2.3. *In vivo* models to study suppression of CYPs by cytokines**

Yang and Lee (2008) have reviewed studies on LPS-endotoxin effects on CYP expression in rodent models. In general, LPS suppresses CYP probe substrate clearances by about 1-2 fold. Few studies have looked at disease treatment effects on CYP suppression. Piquette-Miller and Jamali (1995) examined the effect of ketoprofen on propranolol pharmacokinetics in a rodent model of severe adjuvant arthritis (AA). Plasma concentrations of propranolol enantiomers in AA were increased in the disease model and were related to the degree of inflammation and  $\alpha$ 1-

acid glycoprotein levels. Ketoprofen treatment decreased the disease-induced suppression of propranolol elimination. Ling and Jamali (2009) studied the effects of the anti-TNF $\alpha$  monoclonal antibody infliximab on CYPs in the resolution of early AA in rats. Hepatic Cyp1a and Cyp3a protein expression were decreased in rats with AA, and infliximab treatment increased Cyp1a and Cyp3a protein expression. Ashino et al. (2007) used a murine anti-IL-6 monoclonal antibody to treat arthritis in a HTLV-1 transgenic mouse arthritis model. IL-6 was significantly increased in the arthritic mice and Cyp3a protein was 60% of non-arthritic transgenic controls. The anti-IL-6 antibody normalized the Cyp3a response. More recently, the murine collagen antibody induced arthritis (CAIA) model was used to study Cyp suppression (Dickmann et al., 2012). Most hepatic Cyp mRNA levels were down-regulated, with the exception of Cyp2d9 and 3a13, which increased by 2.3 and 4.4-fold, respectively, relative to control mice. Cyp1a2, 2c29, 2b9, and 3a11 mRNA were decreased by 2-3-fold compared to controls, and the level of Cyp2e1 was unchanged. Intrinsic clearance data obtained with Cyp isoform selective probe substrates and hepatic S9 indicated the largest suppression of Cyp activity was ~2-fold. Strain- and isoform-dependent Cyp suppression has also been described in other *in vivo* models (reviewed in Dickmann et al., 2012). Preclinical *in vivo* models related to LPS mediated acute phase responses or animal models of chronic disease are labor intensive, time sensitive, and strain/species dependent and therefore do not appear to be robust enough for prediction of the probability or magnitude of a clinical TP-DDI.

#### 1.2.4. Hepatic Suppression of Drug Transporters

While the focus of TP-DDI studies has been around CYP enzymes, *in vitro* and preclinical *in vivo* studies have revealed evidence of pro-inflammatory cytokine-mediated effects on drug transporters. However, clinically relevant drug interactions involving inflammation-mediated suppression of transporter activity have not been reported. P-glycoprotein (P-gp) expression in the liver, intestine, and the blood brain barrier, is decreased in rodent models of inflammation

(LPS and turpentine), and increased in kidney (Kalitsky-Szirtes et al., 2004; Hartmann et al., 2005; Wang et al., 2005; Hartz et al., 2006; Matsumoto et al., 2012). In a mouse model of LPS-induced inflammation, biliary clearance of the P-gp substrate doxorubicin was decreased by 50%, and a 3-fold increase in renal clearance was observed. These changes were associated with reduced P-gp protein levels in the liver and increased levels in the kidney (Hartmann et al., 2005). Using radiolabeled P-gp substrate,  $^{99m}\text{Tc}$ -sestamibi, Wang et al., (2005) showed that LPS-induced systemic inflammation increased retention of  $^{99m}\text{Tc}$ -sestamibi in the brain, heart, liver, and fetal tissue and this correlated with reduction in Mdr1a mRNA levels in these organs. *In vitro* effects of pro-inflammatory cytokines on the expression of various transporters have also been reported (Petrovic et al., 2007). Three pro-inflammatory cytokines, IL-6, IL-1 and TNF- $\alpha$  down regulate hepatic transporter expression in hepatocyte sandwich cultures (Diao et al., 2010). IFN- $\gamma$  and Oncostatin M (a member of the interleukin IL-6 family) also influenced transporter levels in cultured hepatocytes (Donato et al., 1993; Chen et al., 2011; Guillen et al., 1998; Le Vee et al., 2011). IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 alter the expression profile of human hepatic transporters *in vitro* (Aitken et al., 2006; Fardel and Le Vee, 2009). The bile salt export pump as well as sinusoidal solute carrier uptake transporters are generally suppressed, whereas ATP-binding cassette drug efflux pumps are either unchanged, or up- or down-regulated depending on the transporter isoform. These changes are observed at mRNA levels for some transporters, and at protein and activity level for others (Diao et al., 2010). Currently, the clinical relevance of these changes in transporter levels is unclear.

### 1.2.5. Modeling, simulation and clinical prediction

*In vitro* and preclinical *in vivo* models of cytokine effects on drug metabolizing enzymes, may afford interesting data regarding mechanism of action, but unfortunately cannot currently provide much guidance on the likelihood of clinically relevant TP-DDI (Morgan, 2009; Dickmann et al., 2011). To enable TP-DDI clinical prediction and guide clinical development teams on the

need for a clinical study (the current gold standard) many factors need to be considered. These minimally include TP pharmacology and clearance pathways, a thorough understanding of relevant cytokines and their impact on CYPs in the disease setting, the patient population, and co-medications. Physiology based PK/PD modeling has been used in retrospective attempts to relate *in vitro* IL-6 effects on CYP3A4 to the clinical tocilizumab:simvastatin interaction and to SMD clearance in organ transplant and surgical trauma (Machavaram et al., 2013). Unfortunately, IL-6 is currently the only cytokine-mediated TP-DDI for which both *in vitro* and *in vivo* data are available. Advances in *in vitro-in vivo* extrapolation (IVIVE) have been limited by the subtle magnitude of the interaction, the challenges in generating physiologically relevant quantitative *in vitro* data and the paucity of positive clinical TP-DDI examples (Kenny et al., 2013). Disease-centric, rather than the current cytokine- and target-centric approaches have recently been proposed that may enable clinical risk assessment based primarily on the disease indication and treatment effectiveness (reviewed by Kenny et al., 2013 and Slatter et al., 2013).

#### **1.2.6. The Bio Survey**

The BioSafe Pharmacokinetics and Disposition Expert Working Group conducted a survey in January - March 2010 on TP-DDIs in drug development (Lloyd et al., 2011). The survey assessed practices of twenty-one BIO industry trade group member companies. The survey indicated that 1) most of the DDI studies for TPs have been undertaken in the oncology and immunology therapeutic areas; 2) most of the DDI studies in the oncology therapeutic area considered the TP as a potential victim; and 3) most of the DDI studies in the immunology therapeutic area considered the TP as a perpetrator. The survey was a snapshot-in-time that showed industry strategy in the early days of this issue. The survey prompted subsequent industry efforts to understand underlying mechanisms and clinical implications of TP-DDIs.

#### **1.3. Change in the Regulatory Landscape**

The number of TPs under development has steadily increased in recent years. For example, 46 BLAs were approved between 2000 and 2009, as compared to 25 BLAs in 1990-1999, and less than 10 BLAs in 1980-1989 (<http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/UCM333444.pdf>). Historically, drug interaction studies involving TPs were not routine, mainly because there were few examples of TP-DDI-related and clinically-relevant adverse events. The general lack of understanding of potential interaction mechanisms and of predictive tools became evident when clinically measurable TP-DDI started to appear. Reviews published in 2007 highlighted examples of drug interactions involving TPs and illustrated possible interaction mechanisms (Mahmood and Green, 2007; Seitz and Zhou, 2007). TP-DDIs (either PK- or PD-based) related information started to appear in product labeling, as summarized in a labeling overview published by the FDA in 2010 and more recently in 2013 (Huang et al., 2010; Lee et al., 2010; Zhao et al., 2013). In addition to clinical drug interaction studies, *in vitro* evaluation of TP-DDI started to influence drug labels and regulatory requests. For example, product labeling of tocilizumab and ustekinumab included results from *in vitro* studies on cytokine-mediated effects on CYPs (Tocilizumab Product Label, 2010; Ustekinumab Product Label, 2012).

### 1.3.1 Regulatory Guidelines

The 2007 European Medicines Agency (EMA) Guideline entitled “Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins” described DDI concerns about immunomodulators such as cytokines that have potential for inhibition or induction of CYP enzymes (European Medicines Agency, 2007). The Guideline suggested *in vitro* and/or *in vivo* studies should be considered on a case-by-case basis. The 2012 FDA draft Guidance on drug interactions expanded recommendations on TP-drug interaction assessment relative to the

previous 2006 draft (FDA Guidance for Industry, 2012). The new draft Guidance contained a decision tree which reflected the FDA's current view of TP-DDI as follows:

- If an investigational TP is a cytokine or cytokine modulator that has known effects on CYP enzymes or transporters, studies should be conducted to determine the magnitude of the TP's effects on drugs that are substrates of the affected CYP enzymes or transporters.
- *In vitro* or animal studies have limited value in the qualitative and quantitative projection of clinical interactions because translation of *in vitro* to *in vivo* and animal to human results to date has been inconsistent, necessitating clinical drug interaction studies. The *in vivo* evaluations of TPs in targeted patient populations can be conducted with individual substrates for specific CYP enzymes and transporters, or studies can be conducted using a "cocktail approach."
- If a TP will be used with other drug products (SM or TP) as a combination therapy, studies should evaluate the effect of each product on the other (PK or PD when appropriate). This evaluation is particularly important when the drug used in combination has a narrow therapeutic index (e.g. chemotherapeutic agents).
- If there are known mechanisms for interactions or prior experience suggests potential PK or PD interactions, appropriate evaluations for possible interactions should be conducted. Some interactions between drugs and TPs are based on mechanisms other than CYP enzyme or transporter modulation.

#### 1. ***In Vitro* TP-DDI Working Group Cross-Company Evaluation of Hepatocyte Studies**

Section 1 of this review summarized the body of literature that describes the effects of cytokines on CYP expression in isolated human hepatocytes. Overall, high inter-lab variability in CYP suppression by cytokines has been noted (Abdel-Razzak et al. (1993); Aitken and Morgan



(2007); Donato et al., (1997); Pascussi et al., (2000); Nguyen et al., 2013). Many factors could contribute to variability, including experimental design (e.g. plate format, seeding densities, and time-points), intrinsic donor attributes (age, disease state, and medical history), and sources of reagents (cytokines and culture media) (Nguyen et al., 2013; Kenny et al., 2013). Inter-laboratory variability is a concern from both a pharmaceutical industry and regulatory perspective, as it complicates the assessment of the validity of data and inter-laboratory data comparison.

Figure 1 illustrates variability in IL-6 suppression of CYP3A4 mRNA and activity in labs of several *In Vitro* TP-DDI Working Group members. Figure 1A shows how incubation time can influence potency assessments. Cryopreserved human hepatocytes from four donors studied by Company A revealed that IC<sub>50</sub> values for CYP3A4 mRNA suppression by IL-6 were generally higher following longer incubation times (72 hr vs. 48 hr). This may be due to IL-6 mediated suppression of IL-6 receptor expression over time (Dickmann et al., 2011). Intrinsic donor characteristics may also have affected experimental results as shown by IL-6 mediated down-regulation of CYP3A4 mRNA (Figure 1A), and CYP3A4 activity (Figure 1B) in different donors. These data indicate that experiment design and intrinsic donor attributes should be taken into account when comparing the effects of different cytokines on CYP enzymes. To better understand inter-lab experimental variability, six laboratories from the TP-DDI Working Group performed a cross-company *in vitro* study that assessed the effect of IL-6 on CYP3A4 in a single hepatocyte donor.

## 2.1. Experimental design

A single preselected human hepatocyte donor was used by all six laboratories (Table 2). The donor was pre-selected following initial studies to confirm that CYPs were induced by prototypical inducers ( $\beta$ -naphthoflavone, phenobarbital, and rifampicin), and suppressed by IL-6.

During the cross- company evaluation, cryopreserved cells were incubated for 48 hr with recombinant human IL-6. CYP3A enzyme activity was measured as testosterone metabolism to 6- $\beta$ -hydroxytestosterone, and CYP3A4 mRNA expression was evaluated with RT-PCR. CRP mRNA expression was measured as an indicator of the responsiveness of the experimental system to IL-6 (Dickmann et al., 2011). Rifampin (10  $\mu$ M) was included as a control that the cells were responsive to a prototypical inducer. The same hepatocyte donor, IL-6 source, IL-6 concentrations, and incubation time, were used and remaining experimental conditions were flexible to accommodate individual laboratory protocols. These included culture media (hepatocyte recovery, plating, and maintenance media), culture time prior to cytokine treatment, maintenance media supplements (with or without dexamethasone, fetal bovine serum, and other common tissue culture supplements), absence or presence of a matrigel overlay, and plate layout (24- vs. 96-well format). Table 2 summarizes experimental conditions across individual laboratories.

## **2.2. Cross-Company Hepatocyte Study Results**

Table 3 summarizes the cross-company assessment of IL-6 effects on CYP3A4 mRNA and activity. In general, suppression of CYP3A4 by IL-6 was consistently observed. Figure 2 depicts the concentration-effect profile of IL-6-mediated inhibition of CYP3A4 mRNA expression (Figure 2A) and activity (Figure 2B) from Company D.

The influence of glucocorticoids such as dexamethasone on CYP3A4 expression is well known (Hewitt et al., 2007). In general, there is a decline in CYP activity levels as hepatocytes de-differentiate in culture. As a consequence, dexamethasone is often used in hepatocyte culture protocols to decrease this effect on CYP. To evaluate the effect of dexamethasone on IL-6-mediated suppression of CYP3A4, several laboratories performed the cross-company study both in the presence and absence of dexamethasone. Table 3 shows that maximum

suppression of CYP3A4 activity by IL-6 across laboratories ranged between 20-38% and 68-91% in the absence or presence of 100 nM dexamethasone, respectively. For IL-6-mediated suppression of CYP3A4 mRNA expression, maximum suppression was between 55-95% and 93-99.6%, in the absence or presence of dexamethasone, respectively (Table 3). The level of CYP3A4 induction by rifampin was highly variable and did not correlate with either the expression of CRP or suppression of CYP3A4 mRNA by IL-6.

Hepatocytes from the single donor had low to moderate basal levels of 6 $\beta$ -testosterone hydroxylase activity, depending on the culture conditions used (Table 3, 2.7-13 pmol/min/10<sup>6</sup> cells (without dexamethasone) and 27-220 pmol/min/10<sup>6</sup> cells (with dexamethasone)) and this may have contributed to variability in study results. Despite variation in experimental conditions and low basal CYP levels, three out of six independent laboratories that reported IC<sub>50</sub> values for IL-6 mediated suppression of CYP3A4 mRNA expression provided results within 2.2-fold, and four out of six within 11.4-fold from each other (Table 3). However, a wide range of IC<sub>50</sub> values for suppression of CYP3A4 activity (24-1750 pg/ml) and mRNA expression (8.3-1600 pg/ml) were observed. These single donor data showed inter-laboratory variability in the measurement of IC<sub>50</sub> values for CYP3A4 suppression by IL-6 and do not support quantitative inter-laboratory comparisons of CYP-inhibition.

IL-6 resulted in dose-dependent upregulation of CRP mRNA (Figure 3), although the EC<sub>50</sub> values were variable across the laboratories (Table 3: 0.114-346 ng/mL (without dexamethasone), and 0.95-47.5 ng/ml (with dexamethasone)). The maximum fold increase in CRP expression over untreated controls ranged between 208-1000 and 572-1342-fold, in the absence or presence of dexamethasone, respectively (Table 3). These data indicated that the hepatocyte experimental system was responsive to IL-6.

### 2.3. Technical considerations

FBS is known to aid hepatocyte attachment and provide higher quality hepatocyte monolayers (Hewitt et al., 2007). In the cross company comparison, the impact of FBS on CYP suppression was minimal, since laboratories that incorporated or omitted FBS in recovery and plating media all reported good cell attachment at the beginning of the study period. Three out of six companies using 24-well collagen coated plates generally reported good cell monolayers throughout the entire study period. Three out of six companies used a 96-well format and well attached cells were generally reported for up to 48 hours. Culture time was also an important contributor to cell health, with better hepatocyte monolayers observed in the first 48 hr after cytokine dosing. Pre-selection and validation of donor performance in a given plate format is recommended prior to embarking on studies.

Three out of six laboratories that used dexamethasone supplementation reported generally higher basal levels of CYP activity. Without dexamethasone, incorporation of a matrigel overlay can sometimes mitigate the decline of CYP enzymes (Hewitt et al., 2007). However, the cross-company *in vitro* experiment did not conclusively demonstrate a benefit of a matrigel overlay.

Three out of six laboratories used the same commercially available recovery media and similar culture media (WME) supplemented with dexamethasone. Company F used a different commercial media for recovery, plating, and maintenance. The IC<sub>50</sub> of IL-6 mediated suppression of CYP3A4 mRNA reported by Company F was significantly higher than the other companies. Therefore, culture media may partially explain differences in the observed IC<sub>50</sub> values. The cross company studies highlight that pre-selection and validation of human donor lots should be performed prior to initiating TP-DDI studies in cryopreserved human hepatocytes. This ensures optimal cell attachment, growth, and response to positive control repressors such as IL-6 under experimental conditions that will ultimately be used by each laboratory.

In summary, the working group concludes, based on experience with primary hepatocyte culture studies on the well-studied CYP3A4 suppressor IL-6, that significant variability in IC<sub>50</sub> values across labs can be expected (Figure 1, Table 3). Experimental conditions that should be considered for mechanistic TP-DDI investigations are listed in Table 4. Future research should focus on achieving consensus on: (i) Optimal assay conditions; (ii) appropriate positive controls for various CYPs; (iii) the clinical relevance of effects on CYPs other than CYP3A4 and on non-CYP ADME enzymes and transporters; (iv) the effect of mixtures of cytokines on gene expression; (v) temporal changes in cytokine and cytokine receptor production and their ability to regulate each other *in vitro*; and (vi) mechanistic understanding of CYP suppression via multiple (additive or synergistic) pathways.

### 3. The TP-DDI Workshop

The objective of the workshop (for meeting agenda see supplementary Figure 1) was to facilitate better understanding of the current science, investigative approaches, and knowledge gaps in the study of TP-DDI. The audience was made up of industrial scientists (84%, from 20 companies) with invited academic speakers and regulatory scientists, representing 16% of attendees. Following lectures and panel discussions that covered the most recent state of the art data, the meeting culminated with presentation of the *In Vitro* Working Group recommendations as follows:

1. Evaluation of TP-DDI requires scientific diligence and is currently best accomplished with a clinical study. The need for a dedicated clinical TP-DDI study can be discerned with a four step assessment. (Kenny et al., 2013). First consider the disease indication and disease biology, then the TP class, and follow this with an analysis of risk related to concomitant medications, clearance mechanisms and patient factors (Figure 5).

2. A thorough understanding of relevant cytokines in the disease indication and their impact on CYPs, along with evidence that a novel TP will influence pro-inflammatory cytokines, will help determine whether a clinical study is necessary. When there is insufficient information for risk assessment, potential TP effects on important concomitant medications could be explored in Phase 2 studies using population PK approaches.
3. Treatment-induced changes in appropriate biomarkers in Phase 2 and 3 studies, possibly CRP or CYP3A markers, may indicate the potential for a clinically measurable treatment effect on CYPs.
4. Preclinical quantitative assessment of TP-DDI to predict a clinical interaction, is not recommended at this time.
5. In vitro studies on the mechanism of proinflammatory cytokine or cytokine modulator effects on CYP may help further elucidate the limitations and potential utility of current and future technologies.

### **3.1.2. Poll Results: *In Vitro* Evaluation of TP-DDI Potential**

To develop recommendations that would reflect a cumulative opinion/recommendation of the workshop participants, a live polling session was conducted after presentations and panel discussions. The polling session covered *in vitro* studies, clinical observations, Pop PK approaches, and regulatory interactions in the field of TP-DDIs. Questions and responses related to *in vitro* methods are listed in the appendix. The poll was comprised of 18 scientific questions and 2 questions on audience demographics. All responses were blind with respect to individual and company/institution. The poll was performed prior to presentation of the recommendations of the *In Vitro* Working Group, in order to gauge audience opinion without influence from the working group recommendations. All poll data are available as

supplementary material (supplementary Figure 2) and a summary of three key *in vitro* questions is provided in Figure 4.

The entire audience (industry and academic/regulatory) was polled with a 98% response rate. The majority of those polled were not clear about regulatory requirements on TP-DDI evaluation for cytokines and/or cytokine modulators. Forty five percent of respondents indicated they did not have full understanding, compared to 16% who were clear on regulatory requirements. A larger proportion of those polled were clear on clinical expectations (25%) compared to pre-clinical expectations (14%). Industrial attendees were asked if regulatory agencies had requested *in vitro* and/or clinical data on TP-DDI, and two thirds of the industry audience indicated they had received requests. Only one third of the industry members polled indicated their company had an internal guidance or strategy for addressing TP-DDI.

Approximately half of the industry audience (49%) had done *in vitro* work in human hepatocytes to investigate cytokine and/or cytokine modulators as perpetrators of TP-DDI (Figure 4). Where those studies were performed for a specific drug discovery program, there was an equal distribution of opinion on whether these studies were useful (21%) or not useful (19%) for decision making and/or project progression. Twenty three percent of the industry audience indicated their company had included *in vitro* data in regulatory submissions as justification that clinical studies were not needed. There was an equal distribution of opinion on whether *in vitro* data were accepted (13%) or were not accepted (13%) by regulatory agencies as justification that clinical studies were not needed.

When asked about the qualitative usefulness of *in vitro* hepatocyte studies for prediction of the clinical situation, nearly two thirds of the audience thought *in vitro* data could be useful with 12% answering “yes” and 53% answering “maybe”, compared to 33% who answered “no” (Figure 4).

In contrast, when asked about quantitative assessment, the majority of the audience (90%) thought *in vitro* hepatocyte data may not reliably predict the likelihood and magnitude of a clinical DDI. Of those polled on the need for alternative *in vitro* methods (e.g. hepatocytes in co-culture with Kupffer cells or other immune modulating cells), 56% indicated that alternative *in vitro* approaches should be investigated, 30% indicated “on a case by case basis”, 5% said “no” and 9% said “don’t know”.

#### **3.1.4. Application of the Working Group Recommendations to Address a TP-DDI**

Prior to initiation of preclinical evaluations, the disease impact on PK of a co-administered drug should be understood. The limited data currently available indicates that the absolute magnitude of chronic inflammatory disease effects on CYPs is modest and that only low therapeutic index CYP substrates present a risk of clinically significant TP-DDI. Additional factors include modality of the TP, clearance mechanisms of co-administered drugs, probability that a co-administered drug will alter clearance of the TP, and the ability of the TP to interact with endogenous proteins other than the target (Figure 5).

The need for a TP-DDI study may require a disease indication centered strategy in addition to “no effect” *in vitro* human hepatocyte data. “No effect” *in vitro* human hepatocyte data have recently been used to preclude the need for a clinical TP-DDI study (SD, personal communication). The anti-IL-12/IL-23p40 antibody ustekinumab is approved for the treatment of moderate to severe plaque psoriasis. In this case, a sufficient understanding of the cytokines in question (i.e., the absence of IL-12 and IL-23 receptors in hepatocytes) was available. However, the implications of these factors with respect to TP-DDI risk had not been generally established, so *in vitro* studies were conducted and supported the hypothesis that IL-12 or IL-23 would not directly suppress CYPs (Dallas et al., 2013a). Similar studies on diverse cytokines (both pro- and anti-inflammatory) and disease indications will help to elucidate molecular pathways that



are affected and determine which CYP isoforms may be suppressed. The clinical relevance of all these *in vitro* results remains to be fully elucidated.

#### **4. Conclusions and Future Directions**

Cytokine-mediated DDIs observed with anti-inflammatory TPs cannot currently be predicted using *in vitro* data and any pre-clinical systems employed to predict such interactions need to be capable of more closely mimicking the disease state. *In vitro* experimental conditions used in the cross-company evaluation described in Section 2 identified a number of important parameters for TP-DDI evaluations in hepatocytes. Future success in predicting clinical TP-DDIs will require an understanding of disease biology, physiologically-relevant *in vitro* systems, and additional clinically measurable effects to facilitate *in vitro-in vivo* extrapolations and mathematical modeling.

## **Acknowledgments**

We thank the members of the TP-DDI Steering Committee for their encouragement and support. We also acknowledge the following colleagues for their technical support in generating the *in vitro* cross-company hepatocyte data: Souvik Chattopadhyay and Carlo Sensenhauser (Janssen); Suzanne Tay and Susan Wong (Genentech); Sonal Patel (Amgen); and Xing Yang (Pfizer).

### **Authorship Contributions**

Wrote or contributed to writing of the manuscript: Evers, Dallas, Dickmann, Fahmi, Kenny, Kraynov, Nguyen, Patel, Slatter, Zhang.

## References

- Abdel-Razzak Z, Loyer P, Fautrel A, Gautier JC, Corcos L, Turlin B, Beaune P, and Guillouzo A (1993) Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. *Mol Pharmacol* **44**: 707-715.
- Aitken AE, Richardson TA, and Morgan ET (2006) Regulation of drug-metabolizing enzymes and transporters in inflammation. *Annu Rev Pharmacol Toxicol* **46**: 123-149.
- Aitken AE and Morgan ET (2007) Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. *Drug Metab Dispos* **35**: 1687-1693.
- Ashino T, Arima Y, Shioda S, Iwakura Y, Numazawa S, and Yoshida T (2007) Effect of interleukin-6 neutralization on CYP3A11 and metallothionein-1/2 expressions in arthritic mouse liver. *Eur J Pharmacol* **558**: 199-207
- Bader A, Borel Rinkes IH, Closs EI, Ryan CM, Toner M, Cunningham JM, Tompkins RG, and Yarmush ML (1992) A stable long-term hepatocyte culture system for studies of physiologic processes: cytokine stimulation of the acute phase response in rat and human hepatocytes. *Biotechnol Prog* **8**: 219-225
- Chen C, Han YH, Yang Z, and Rodrigues AD (2011) Effect of interferon- $\alpha$ 2b on the expression of various drug-metabolizing enzymes and transporters in co-cultures of freshly prepared human primary hepatocytes. *Xenobiotica* **41**: 476-485
- Dallas S, Sensenhauser C, Batheja A, Singer M, Markowska M, Zakszewski C, Mamidi RN, McMillian M, Han C, Zhou H, and Silva J (2012) De-Risking Bio-therapeutics for Possible Drug Interactions Using Cryopreserved Human Hepatocytes. *Curr Drug Metab* **13**: 923-929
- Dallas S, Chattopadhyay S, Sensenhauser S, Batheja A, Silva J (2013a) Interleukins-12 and -23 Do Not Alter Expression or Activity of Multiple CYP Enzymes in Cryopreserved Human Hepatocytes. *Drug Metab Dispos* **41**: 689-693

- Dallas S, Sensenhauser S, Chattopadhyay C, Silva J (2013b) Drug Interaction Assessment Strategies: Small Molecules versus Therapeutic Proteins. In: Drug-Drug Interactions for Therapeutic Proteins (Wiley): *in press*.
- Deng R, Jin F, Prabhu S, and Iyer S (2012) Monoclonal antibodies: what are the pharmacokinetic and pharmacodynamics considerations for drug development? *Expert Opin Metab Toxicol* **8**: 141-160
- Diao L, Li N, Brayman TG, Hotz KJ, and Lai Y (2010) Regulation of MRP2/ABCC2 and BSEP/ABCB11 expression in sandwich cultured human and rat hepatocytes exposed to inflammatory cytokines TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . *J Biol Chem* **285**: 31185-31192
- Dickmann LJ, Patel SK, Rock DA, Wienkers LC, and Slatter JG (2011) Effects of interleukin-6 (IL-6) and an anti-IL-6 monoclonal antibody on drug-metabolizing enzymes in human hepatocyte culture. *Drug Metab Dispos* **39**: 1415-1422.
- Dickmann LJ, Patel SK, Wienkers LC, and Slatter JG (2012a) Effects of interleukin-1B (IL-1B) and IL1B/interleukin 6 (IL6) combinations on drug metabolizing enzymes in human hepatocyte culture. *Curr Drug Metab* **13**: 930-937
- Dickmann LJ, McBride HJ, Patel SK, Miner K, Wienkers LC, and Slatter JG (2012b) Murine collagen antibody induced arthritis (CAIA) and primary mouse hepatocyte culture as models to study cytochrome P450 suppression. *Biochem Pharmacol* **83**: 1682-1689
- Donato MT, Herrero E, Gomez-Lechon MJ, and Castell JV (1993) Inhibition of monooxygenase activities in human hepatocytes by interferons. *Toxicol In Vitro* **7**: 481-485.
- Donato MT, Guillen MI, Jover R, Castell JV, Gomez-Lechon MJ (1997) Nitric oxide-mediated inhibition of cytochrome P450 by interferon- $\gamma$  in human hepatocytes. *J Pharmacol Exp Ther* **281**: 484-490
- European Medicines Agency's Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins, CHMP/EWP/89249/2004 (2007)

[http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2009/09/WC500003029.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003029.pdf).

Fardel O and Le Vee M (2009) Regulation of human hepatic drug transporter expression by pro-inflammatory cytokines. *Expert Opin Drug Metab Toxicol* **5**: 1469-1481

FDA Guidance for Industry: Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (2012)  
<http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm>.

Ferrari L, Peng N, Halpert JR, and Morgan ET (2001) Role of nitric oxide in down-regulation of CYP2B1 protein, but not RNA, in primary cultures of rat hepatocytes. *Mol Pharmacol* **60**: 209-216

Freudenberg MA, Freudenberg N, and Galanos C (1982) Time course of cellular distribution of endotoxin in liver, lungs and kidneys of rats. *Br J Exp Pathol* **63**: 56-65.

Ghose R, Zimmerman TL, Thevananther S, and Karpen SJ (2004) Endotoxin leads to rapid subcellular re-localization of hepatic RXRalpha: A novel mechanism for reduced hepatic gene expression in inflammation. *Nucl Recept* **2**: 4

Girish S, Martin SW, Peterson MC, Zhang LK, Zhao H, Balthasar J, Evers R, Zhou H, Zhu M, Klunk L, Han C, Berglund EG, Huang SM, and Joshi A (2011) AAPS workshop report: Strategies to address therapeutic protein-drug interactions during clinical development. *AAPS J.* **13**: 405-416

Guillen MI, Donato MT, Jover R, Castell JV, Fabra R, Trullenque R, and Gomez-Lechon MJ (1998) Oncostatin M down-regulates basal and induced cytochromes P450 in human hepatocytes. *J Pharmacol Exp Ther* **285**: 127-134

Hartmann G, Vassileva V, and Piquette-Miller M (2005) Impact of endotoxin-induced changes in P-glycoprotein expression on disposition of doxorubicin in mice. *Drug Metab Dispos* **33**: 820-828.

- Hartz AM, Bauer B, Fricker G, and Miller DS (2006) Rapid Modulation of P-glycoprotein-mediated transport at the blood brain barrier by tumor necrosis factor-alpha and lipopolysaccharide. *Mol Pharmacol* **69**: 462-470
- Hewitt NJ, Lechón MJ, Houston JB, Hallifax D, Brown HS, Maurel P, Kenna JG, Gustavsson L, Lohmann C, Skonberg C, Guillouzo A, Tuschl G, Li AP, LeCluyse E, Groothuis GM, and Hengstler JG (2007) Primary hepatocytes: current understanding of the regulation of metabolic enzymes and transporter proteins, and pharmaceutical practice for the use of hepatocytes in metabolism, enzyme induction, transporter, clearance, and hepatotoxicity studies. *Drug Metab Rev* **39**: 159-234
- Hoebe KH, Witkamp RF, Fink-Gremmels J, Van Miert AS, and Monshouwer M (2001) Direct cell-to-cell contact between Kupffer cells and hepatocytes augments endotoxin-induced hepatic injury. *Am J Physiol Gastrointest Liver Physiol* **280**: G720-728
- Hoffmann-La Roche (2008) Tocilizumab Briefing document (BLA 125276). <http://www.fda.gov/ohrms/dockets/ac/08/briefing/2008-4371b1-01-FDA.pdf>
- Huang SM, Zhao H, Lee JI, Reynolds K, Zhang L, Temple R, and Lesko LJ (2010) Therapeutic protein-drug interactions and implications for drug development. *Clin Pharmacol Ther* **87**: 497-503
- Jover R, Moya M, and Gomez-Lechon MJ (2009) Transcriptional regulation of cytochrome p450 genes by the nuclear receptor hepatocyte nuclear factor 4-alpha. *Curr Drug Metab* **10**: 508-519
- Kalitsky-Szirtes J, Shayeganpour A, Brocks DR, and Piquette-Miller M (2004) Suppression of drug-metabolizing enzymes and efflux transporters in the intestine of endotoxin-treated rats. *Drug Metab Dispos* **32**: 20-27
- Kenny J, Liu MM, Chow AT, Earp JC, Evers R, Slatter JG, Wang DD, Zhang L, and Zhou H (2013) Therapeutic protein drug-drug interactions: Navigating the knowledge gaps.

Highlights from the 2012 NBC AAPS Roundtable and IQ Consortium/FDA Workshop,  
*AAPS J, In press*

Kraynov E, Martin SW, Hurst S, Fahmi OA, Dowty M, Cronenberger C, Loi CM, Kuang B, Fields O, Fountain S, Awwad M, and Wang D (2011) How current understanding of clearance mechanisms and pharmacodynamics of therapeutic proteins can be applied for evaluation of their drug-drug interaction potential. *Drug Metab Dispos* **39**: 1779-1783.

Lee CM, Pohl J, and Morgan ET (2009) Dual mechanisms of CYP3A protein regulation by proinflammatory cytokine stimulation in primary hepatocyte cultures. *Drug Metab Dispos* **37**: 865-872

Lee JI, Zhang L, Men AY, Kenna LA, and Huang SM (2010) CYP-mediated therapeutic protein-drug interactions: clinical findings, proposed mechanisms and regulatory implications. *Clin Pharmacokinet* **49**: 295-310.

Le Vee M, Lecureur V, Stieger B, Fardel O (2009) Regulation of drug transporter expression in human hepatocytes exposed to the proinflammatory cytokines tumor necrosis factor- $\alpha$  or interleukin-6. *Drug Metab Dispos* **37**: 685-693

Le Vee M, Jouan E, Stieger B, Lecureur V, and Fardel O (2011) Regulation of drug transporter expression by oncostatin M in human hepatocytes. *Biochem Pharmacol* **82**: 304-311.

Ling S and Jamali F (2009) The effect of infliximab on hepatic cytochrome P450 and pharmacokinetics of verapamil in rats with pre-adjuvant arthritis: a drug-disease and drug-drug interaction. *Basic Clin Pharmacol Toxicol* **105**: 24-29

Lloyd P, Zhou H, Theil HP, Kakkar T, Nestorov I, and Roberts SA (2012) Highlights from a recent BIO survey on therapeutic-drug interactions. *J Clin Pharmacol* **52**: 1755-1763

Lobo ED, Hansen RJ, and Balthasar JP (2004) Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci* **93**: 2645-2668.

Machavaram KK, Almond LM, Rostami-Hodjegan A, Gardner I, Jamei M, Tay S, Wong S, Joshi A, and Kenny JR (2013) A physiologically-based pharmacokinetic modeling approach to



- predict disease-drug interactions: Suppression of CYP3A by IL-6. *Clin Pharmacol Ther*  
doi: 10.1038/clpt.2013.79
- Mahmood I and Green MD (2007) Drug interaction studies of therapeutic proteins or monoclonal antibodies. *Clin Pharmacol* **47**: 1540-1554.
- Matsumoto J, Dohqu S, Takata F, Mishioku T, Sumi N, Machida T, Takahashi H, Yamauchi A, Kataoka Y (2012) Lipopolysaccharide-activated microglia lower P-glycoprotein function in brain microvascular endothelial cells. *Neurosci Lett* **524** :45-48.
- Morgan ET, Li-Masters T, and Cheng PY (2002) Mechanisms of cytochrome P450 regulation by inflammatory mediators. *Toxicology* **181-182**: 207-210.
- Morgan ET (2009) Impact of infectious and inflammatory disease on cytochrome P450-mediated drug metabolism and pharmacokinetics. *Clin Pharmacol Ther* **85**: 434-438.
- Morgan ET (2001) Regulation of cytochrome p450 by inflammatory mediators: why and how? *Drug Metab Dispos* **29**: 207-212.
- Muntane-Relat J, Ourlin JC, Domergue J, and Maurel P (1995) Differential effects of cytokines on the inducible expression of CYP1A1, CYP1A2, and CYP3A4 in human hepatocytes in primary culture. *Hepatology* **22**: 1143-1153.
- Nguyen T, Kishnani N, and Evers R (2013) Utility of in vitro methods in drug-drug interaction assessments and prediction for therapeutic biologics. (*In*: Meibohm B and Zhou H, Editors, Drug interactions of therapeutic proteins: Chapter 7, Wiley, New York). *In press*
- Pascussi JM, Gerbal-Chaloin S, Pichard-Garcia L, Daujat M, Fabre JM, Maurel P, Vilarem MJ (2000) Interleukin-6 negatively regulates the expression of pregnane X receptor and constitutively activated receptor in primary human hepatocytes. *Biochem Biophys Res Commun* **274**: 707-713
- Petrovic V, Teng S, Piquette-Miller M (2007) Regulation of drug transporters during infection and inflammation. *Mol Interv* **7**: 99-111

- Piquette-Miller M and Jamali F (1995) Influence of severity of inflammation on the disposition kinetics of propranolol enantiomers in ketoprofen-treated and untreated adjuvant arthritis. *Drug Metab Dispos* **23**: 240-245.
- Schmitt C, Kuhn B, Zhang X, Kivitz A, and Grange S (2012a) Disease drug-drug-interaction involving tocilizumab and simvastatin in patients with rheumatoid arthritis. *Clin Pharmacol Ther* **89**: 735-740.
- Schmitt C, Kuhn B, Zhang X, Kivitz A, and Grange S (2012b) Tocilizumab has no clinically relevant effect on methotrexate pharmacokinetics in patients with rheumatoid arthritis. *Int J Clin Pharmacol* **50**: 218-223
- Seitz K and Zhou H (2007) Pharmacokinetic drug-drug interaction potential for therapeutic monoclonal antibodies: reality check. *J Clin Pharmacol* **47**: 1104-1118
- Sifontis NM, Benedetti E and Vasquez EM (2002) Clinically significant drug interaction between basiliximab and tacrolimus in renal transplant patients. *Transplant Proc* **34**: 1730-1732.
- Slatter JG, Wienkers LC, and Dickmann LJ (2013) Drug interactions of cytokines and anticytokine therapeutic proteins (*In*: Meibohm B and Zhou H, Editors, Drug interactions of therapeutic proteins: Chapter 13, Wiley, New York). *In press*.
- Strehlau J, Pape L, Offner G, Nashan B, and Ehrich JH (2000) Interleukin-2 receptor antibody-induced alterations of ciclosporin dose requirements in paediatric transplant recipients. *Lancet* **356**: 1327-1328
- Sunman JA, Hawke RL, LeCluyse EL, and Kashuba AD (2004) Kupffer cell-mediated IL-2 suppression of CYP3A activity in human hepatocytes. *Drug Metab Dispos* **32**: 359-363.
- Tocilizumab Product Label, Supplement 0000, FDA@Drugs.  
[http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2010/125276lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/125276lbl.pdf).
- Ustekinumab Product Label, Supplement 0059, FDA@Drugs.  
[http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2012/125261s0059lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125261s0059lbl.pdf).

- Vasquez EM and Pollak R (1997) OKT3 therapy increases cyclosporine blood levels. *Clin Transplant* **11**: 38-41.
- Wang JH, Scollard DA, Teng S, Reilly RM, and Piquette-Miller M (2005) Detection of P-glycoprotein activity in endotoxemic rats by 99mTc-sestamibi imaging. *J Nucl Med* **46**: 1537-1545.
- Yang KH and Lee MG (2008) Effects of endotoxin derived from *Escherichia coli* lipopolysaccharide on the pharmacokinetics of drugs. *Arch Pharm Res* **31**: 1073-1086.
- Zhang X, Schmitt C, Grange S, Terao K, Miya K, Kivitz A, and Marino M (2009) Disease-Drug Interaction studies of Tocilizumab with cytochrome P450 substrates in vitro and in vivo. *Clin Pharmacol Ther* **85** (Supplement 1): S37-S64 [Abstract PII-73].
- Zhao H, Zhang L, Reynolds KS, and Huang S-M. FDA Perspectives on Therapeutic Protein Drug-Drug Interaction Assessments. In: *Drug-Drug Interactions for Therapeutic Proteins* (Wiley), *in press*.
- Verma S, and Blumberg B (2009) The steroid and xenobiotic receptor (SXR), beyond xenobiotic metabolism. *Nucl Recept Signal* **7**: e001.
- Zhou H and Mascelli MA (2011) Mechanism of monoclonal antibody-drug interactions. *Annu Rev Pharmacol Toxicol* **51**: 359-372.
- Zordoky BN and El-Kadi AO (2009) Role of NF-kappaB in the regulation of cytochrome P450 enzymes. *Curr Drug Metab* **10**: 164-178

## Footnotes

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<sup>1</sup> **Members of the BIO TP-DDI Steering Committee are:** Honghui Zhou (Janssen; Co-Chair), Joseph Balthazar (SUNY-Buffalo), Raymond Evers (Merck), Shiew Mei Huang (FDA, Co-Chair), Lei Zhang (FDA), Amita Joshi (Genentech), Andrew Chow (Amgen), Lewis Klunk (Biogen Idec).

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<sup>4</sup> **The International Consortium for Innovation and Quality in Pharmaceutical Development** (The IQ Consortium) is an international association of pharmaceutical and biotechnology companies that aims to advance innovation and quality in the development of pharmaceutical products through scientifically-driven best practices and standards. Their ultimate goal is to improve safety and efficacy of medical products for patient benefit. (<http://iqconsortium.com/>)

## Figure Legends

**Figure 1.** IC<sub>50</sub> values for IL-6 suppression of CYP3A4 mRNA expression (A) and activity (B). Results compiled from internal data generated by Company A to D within the TP-DDI Working Group.

**Figure 2.** Representative profiles of IL-6 inhibition of CYP3A4 mRNA expression (A) and activity (B). Increasing concentration of IL-6 generally produced a corresponding decrease in mRNA levels and CYP3A4 activity. IL-6 was studied at concentrations of 0, 0.001, 0.01, 0.1, 1, 10, 100, and 500 ng/mL.

**Figure 3.** Representative profile of IL-6 induction of CRP mRNA. Increasing concentrations of IL-6 generally produced a corresponding increase in CRP mRNA expression. IL-6 was studied at concentrations of concentrations of 0, 0.001, 0.01, 0.1, 1, 10, 100, and 500 ng/mL.

**Figure 4.** Poll results on the application of in vitro hepatocyte assays in drug development in pharmaceutical industry.

**Figure 5.** Approach to assess TP-DDI risk for cytokine or cytokine modulator and effects on CYP enzymes ( $f_m$ ; fraction metabolized by CYP; NTI, narrow therapeutic index).

**Table 1. Clinical Examples of Interactions between Cytokine Modulators and Small Molecule Drugs**

Generic Name Cytokine Modulator	Mechanism of Action	CYP Enzyme Substrate	Type of Study	Clinical Findings	Labeling	Reference
Basiliximab	Binding to IL-2R on activated T cells	Cyclosporine	Retrospective	In pediatric transplant patients, higher whole-blood levels of cyclosporine within first 10 days post-transplant: at day 28-50 20% higher doses required to maintain adequate trough concentrations	No dose adjustments recommended	Strehlau et al., 2000
Basiliximab	Binding to IL-2R on activated T cells	Tacrolimus	Retrospective	In adult transplant patients, 63% increase in tacrolimus blood trough levels on day 3 compared to controls. Decrease in drug levels by day 30.	No dose adjustments recommended	Sifontis et al., 2002
Muronomab	CD3 T cell blocker	Cyclosporine	Retrospective	On day 5, cyclosporine trough levels 48% higher in treated patients compared to the control group. No differences observed on days 7 and 10	No dose adjustments recommended	Vasquez and Pollak, 1997
Tocilizumab	Anti-IL6R	Omeprazole (CYP2C19) Dextromethorphan (CYP2D6) Simvastatin (CYP3A4) Methotrexate (excreted mainly as parent drug via renal transporters)	Prospective	28% reduction in AUC  No change  57% reduction in AUC  No change	Therapeutic monitoring of CYP substrates with a narrow therapeutic range should be performed and dose adjustments made as needed	Schmitt et al., 2011, 2012; Hoffmann-La Roche briefing document 2008; Zhang et al., 2009

**Table 2. Cross-company experimental design of *in vitro* study to assess effects of IL-6 on CYP3A4**

	Variable Experimental Conditions Used by Different Laboratories <sup>a</sup>			
	Cell Culture Media	Dex in MM	Matrigel Overlay	Plate Layout
Company A	RM: CHRM PM: InVitroGRO CP +Torpedo antibiotics MM: InVitroGRO HI +Torpedo antibiotics	-	+/-	CC, 96-well
Company B	RM: CHRM PM: InvitroGRO CP +Torpedo antibiotics, +FBS Maint: WME +Invitrogen Hepatocyte Maintenance Supplement Pack (containing Pen/Strep, ITS, BSA, linoleic acid, glutamine, and HEPES)	+/-	+	CC, 96-well
Company C	RM: CHRM PM: WME +Dex +FBS +Invitrogen Hepatocyte Thawing/Plating Supplement Pack (Pen/strep + insulin + Glutamine + HEPES) MM: WME + Invitrogen Hepatocyte Maintenance Supplement Pack (containing Pen/Strep, ITS, BSA, linoleic acid, glutamine, and HEPES)	+/-	+	CC, 24-well
Company D	RM: In VitroGRO HT PM: In VitroGRO CP MM: WME + ITS, NEAA, Pen/Strep	+/-	-	CC, 96-well
Company E	RM: CHRM PM: CHPM MM: WME +Pen/Strep, insulin, HEPES	+/-	-	CC, 24-well
Company F	RM: In VitroGRO HT PM: InvitroGRO CP + Torpedo Antibiotics MM: In VitroGRO HI + Torpedo Antibiotics	-	-	CC, 24-well

<sup>a</sup>Similar experimental conditions used by all labs: Hepatocytes were obtained from a 35 year old Caucasian female donor (Lot NON, Celsis IVT, Baltimore, MD) and the cultures were incubated for 48 hr with recombinant human IL-6 from a single source (Lot 742250I, Invitrogen, Federick, MD) and at the same concentrations (0, 0.001, 0.01, 0.1, 1, 10, 100, and 500 ng/mL). CYP3A4 enzyme activity was assessed through measurement of metabolite formation of 6- $\beta$ -hydroxytestosterone. CYP3A4 and CRP mRNA expression were evaluated using RT-PCR or branched DNA techniques. Additional experimental conditions were variable among the different labs as described below. BSA, bovine serum albumin; CC, collagen-coated; Dex, dexamethasone; FBS, Fetal bovine serum; ITS, insulin-transferrin-selenium; MM, maintenance media, NEAA, non-essential amino acids; Pen/Strep, penicillin/streptomycin; PM, plating media; RM, Recovery media; WME, William's Medium E.

**Table 3. Effects of IL-6 on CYP3A4 in hepatocytes**

	Basal Activity	Maximum CYP3A4 Suppression		IC <sub>50</sub> for CYP3A4 Suppression		CRP mRNA Expression		Max CYP3A4 Induction
	CYP3A4 Activity (pmol/min/10 <sup>6</sup> cells)	CYP3A4 Activity (% Suppression)	CYP3A4 mRNA (% Suppression)	CYP3A4 Activity <sup>a</sup> (pg/mL)	CYP3A4 mRNA <sup>b</sup> (pg/mL)	Maximum fold increase	EC <sub>50</sub> <sup>a</sup> (ng/mL)	CYP3A4 mRNA (fold Induction)
Company A	5.0 (-dex)	20 <sup>c</sup> (-dex)	ND	NM <sup>d</sup> (-dex)	ND	ND	ND	ND
Company B	7.0 (-dex) 27 (+dex)	38 <sup>e</sup> (-dex) 74 <sup>e</sup> (+dex)	78 <sup>e</sup> (-dex) 99.6 <sup>e</sup> (+dex)	217 (-dex) 24 (+dex)	18.5 (+dex)	208 (-dex) 572 (+dex)	0.114 (-dex) 0.950 (+dex)	2130
Company C	NM <sup>f</sup>	ND	93 <sup>e</sup> (+dex)	ND	11.6 (+dex)	ND	ND	7
Company D	5.9 (-dex) 13 (-dex) 220 (+dex) 93 (+dex)	ND (-dex) 91 <sup>e</sup> (+dex) 68 <sup>e</sup> (+dex)	95 <sup>e</sup> (-dex) 55 <sup>e</sup> (-dex) 99 <sup>e</sup> (+dex) 99 <sup>e</sup> (+dex)	ND (-dex) 1750 (+dex) 600 (+dex)	ND (-dex) 1600 (+dex)	1000 (-dex) 1342 (+dex)	346 (-dex) 48 (+dex)	4
Company E	ND	NM (-dex) NM (+dex)	56 <sup>c</sup> (-dex) 94 <sup>c</sup> (+dex)	NM <sup>d</sup> (-dex) NM <sup>d</sup> (+dex)	8.3 (+dex)	>21 <sup>g</sup> (-dex) >35 <sup>g</sup> (+dex)	>500 <sup>h</sup> (-dex) >500 <sup>h</sup> (+dex)	369
Company F	2.7 (-dex)	39 <sup>e</sup> (-dex)	94 <sup>e</sup> (-dex)	NM <sup>d</sup> (-dex)	94.7(-dex)	ND	ND	7
Summary	2.7-13 (-dex) 27-220 (+dex)	20-38 (-dex) 68-91 (+dex)	55-95 (-dex) 93-99.6 (+dex)	217 (-dex) 24-1750 (+dex)	94.7 (-dex) 8.3-1600 (+dex)	208-1000 (-dex) 572-1342 (+dex)	0.114-346 (-dex) 0.950-47.5 (+dex)	ND

ND: Not determined

NM: Not measurable

a: Reported IC<sub>50</sub> and EC<sub>50</sub> values were calculated uniquely by each lab that generated the respective data setb: Reported absolute IC<sub>50</sub> values were calculated with data collated across companies and uniformly analyzed using GraFit® v.5.0.8 (Erithacus Software Ltd., Surrey, U.K.) and 4-parameter equation fitting

c: Maximum suppression achieved at concentrations ≥10 ng/mL

d: Not measureable due to less than 50% CYP3A4 suppression

e: Maximum suppression achieved at concentrations ≥100 ng/mL

f: Not measurable due to low turnover rates in control

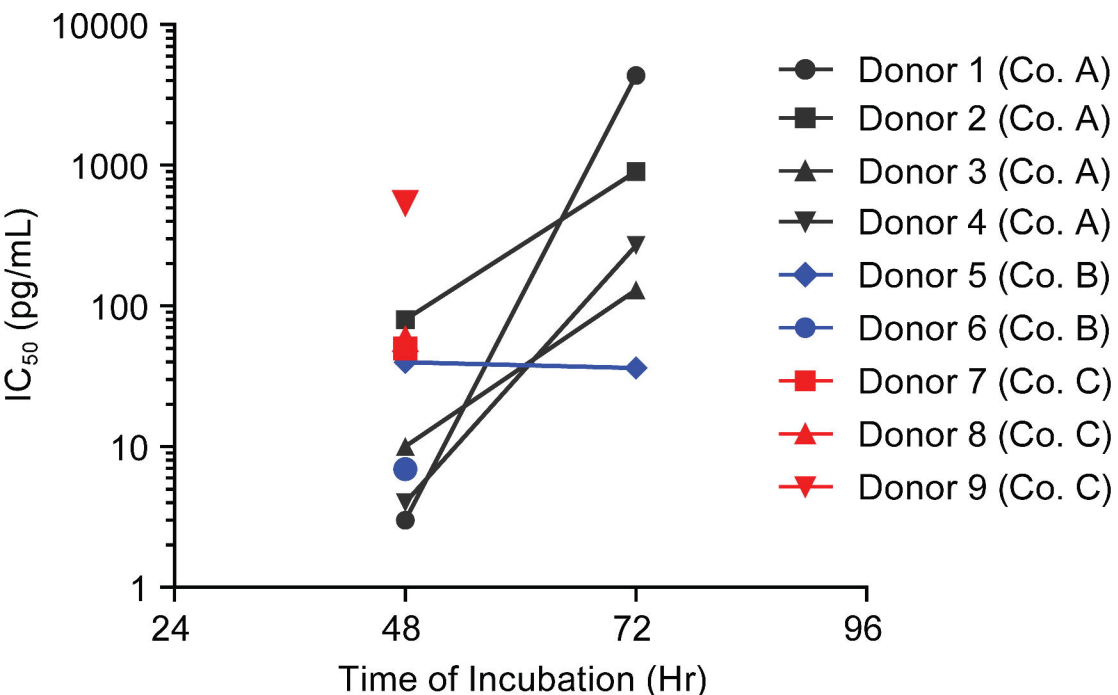
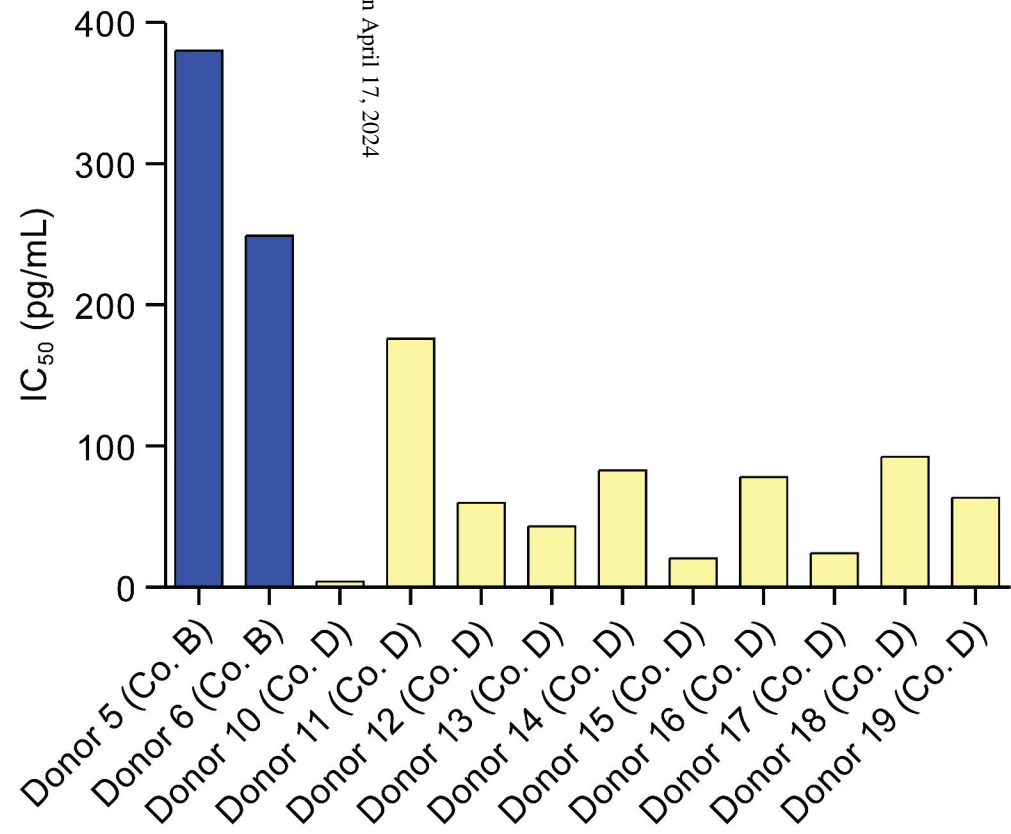
g: Observed highest fold increase in CRP; maximum fold increase in CRP was not achieved within the concentration range studied

h: EC<sub>50</sub> assumed to be greater than the highest concentration studied



**Table 4: In vitro experimental conditions that can be considered for hepatocyte studies**

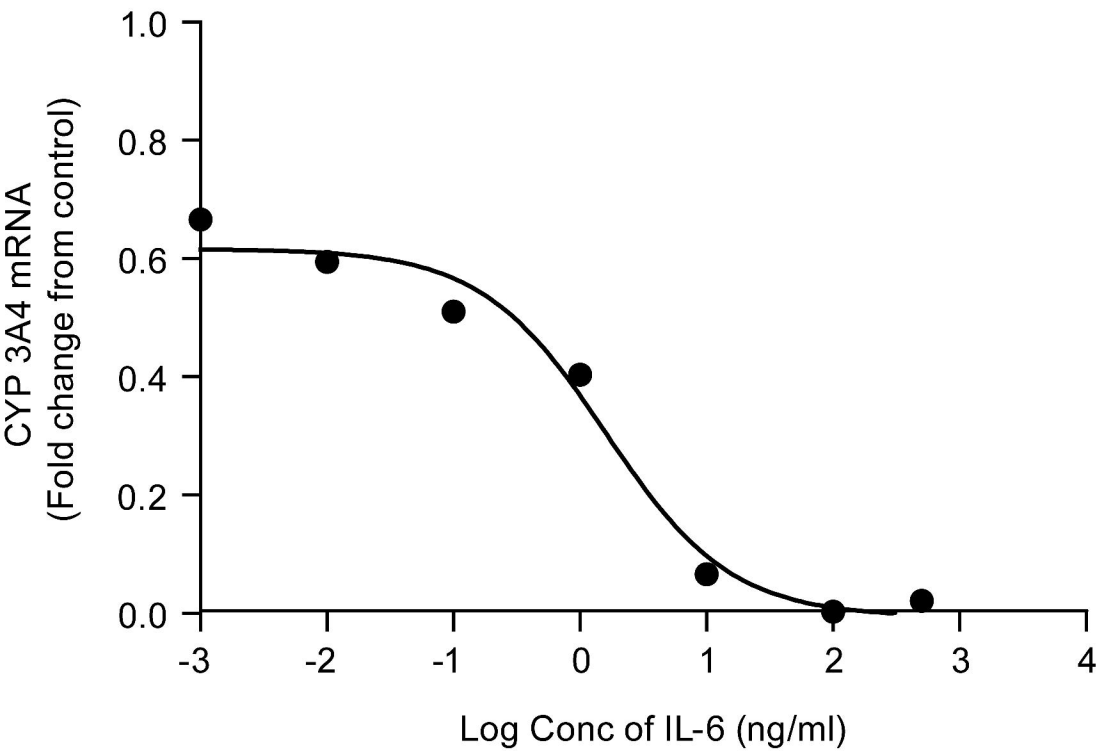
	General Optimal Conditions
Cell culture media	Use of commercial media to recover and plate hepatocytes from cryopreservation and supplemented WME media to maintain cell culture was adequate.
Media supplementation	Supplementation of culture media with dexamethasone produced higher basal CYP3A4 activity levels. (The additional benefit of other standard media supplements such as non-essential amino acids or glutamine will depend on base culture media used)
Experimental layout	24- or 96 well collagen-coated plate well format (pre validate donors for ability to grow on selected surface)
Cell culture supplementation	Collagen coated plates with or without matrigel are acceptable. (Inclusion of a matrigel overlay did not conclusively show benefits to experimental design or outcome)
Culture/experimental time window	Measureable effects of IL-6 on CYP mRNA levels can be detected as early as 24 hr following cytokine incubation; however 48 hr exposure is often necessary to see substantial effects on CYP enzyme activity. It is recommended to hold the maximum dosing period to 48 or 72 hr (5 day total culture time).
Positive controls	Measure effects of IL-6 on CYP3A4 and CRP to indicate the overall responsiveness of the experimental system for cytokine mediated repression

**Figure 1****A****B**

**Figure 2**

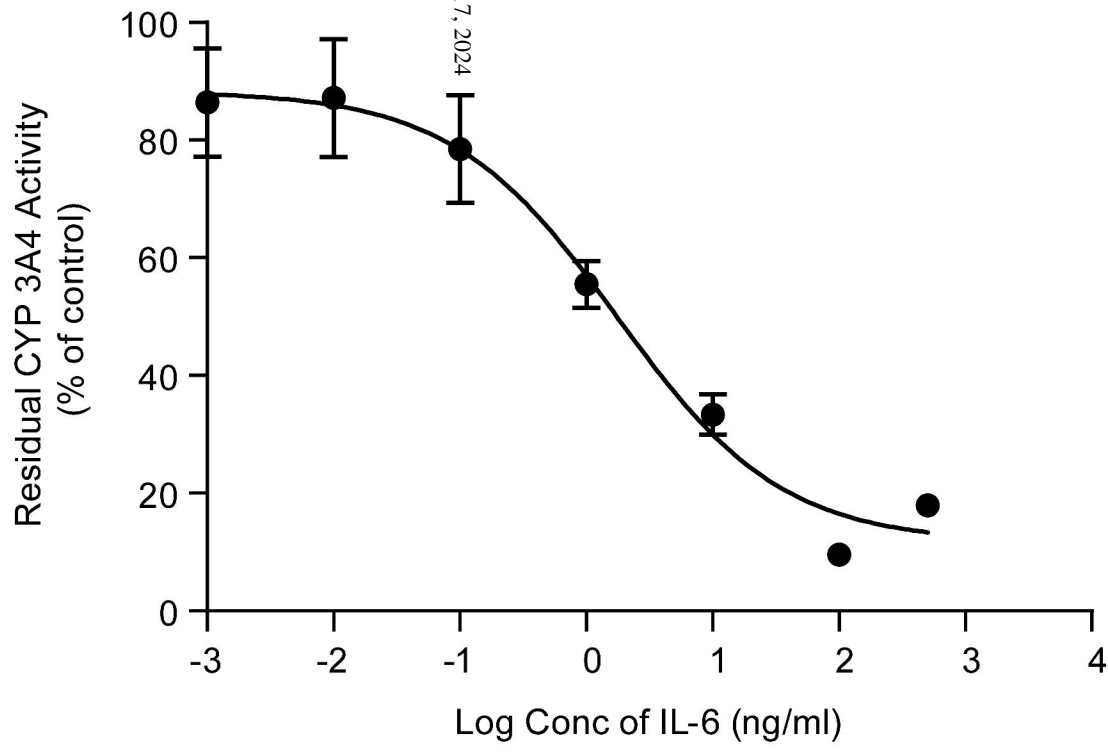
**A**

**Inhibition of CYP 3A4 mRNA by IL-6**



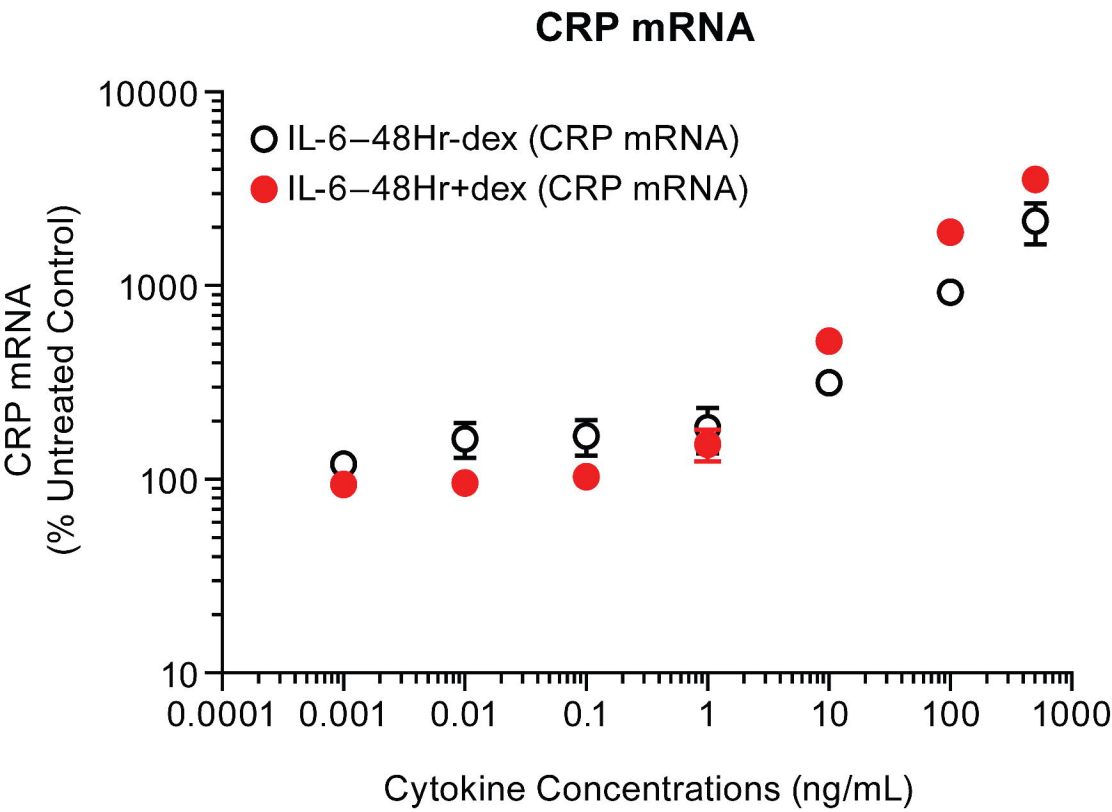
**B**

**Inhibition of CYP 3A4 Activity by IL-6**



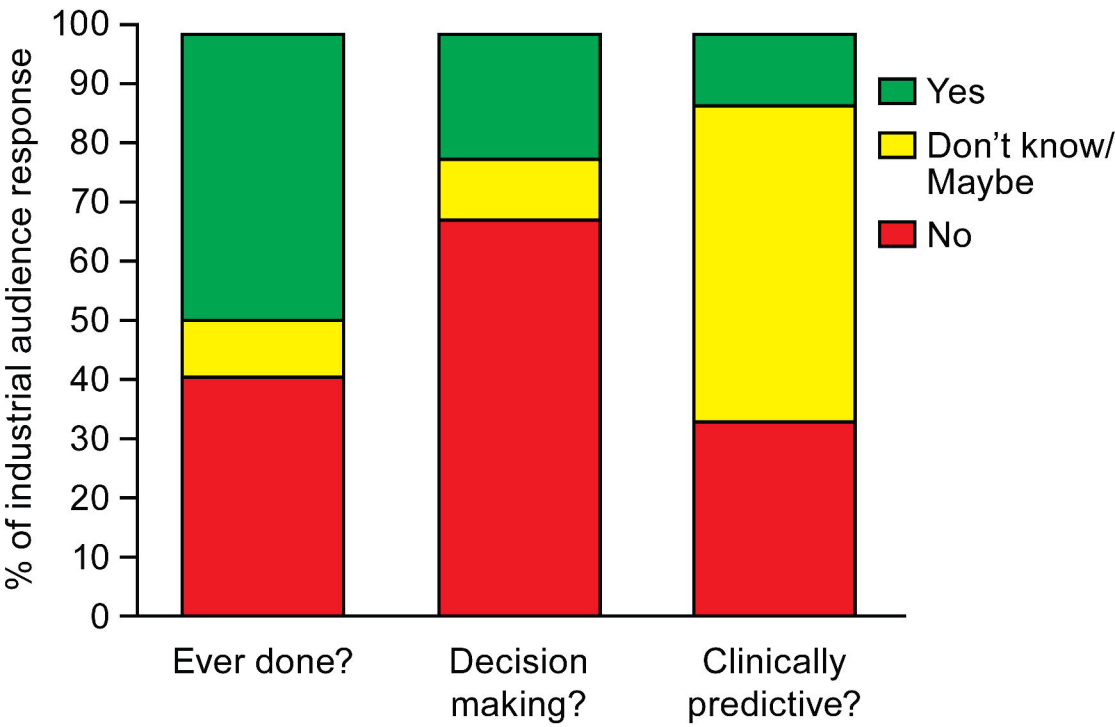
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**Figure 3**



**Figure 4**

**Were in vitro TP-DDI studies for a cytokine and/or cytokine-modulating TP as a DDI perpetrator in human hepatocytes...**



# Figure 5

## Consider the disease

- What are serum or liver cytokine levels in disease compared to healthy individuals?
- Any evidence of CYP suppression in the disease indication?

## Consider the therapeutic protein

- Proinflammatory cytokine or cytokine modulator?
- Hypothesis for mechanistic impact on CYP?

**Integrated approach to assess TP-DDI risk for cytokine or cytokine modulator and effects on CYP enzymes**

## Perform risk assessment

- Consider common small molecule (SM) co-medications ( $f_{m_{CYP}}$ , therapeutic index etc.)
- What are clearance mechanisms of TP and SM co-meds?
- Is there a potential CYP interaction point in disease between co-meds and TP?

## Determine the clinical strategy

Consider the following:

- POP PK approach?
- Monitor CYP biomarkers?
- Need to monitor NTI?
- Need for dedicated TP-DDI study?
- Monitor cytokine levels and acute phase response markers?

**Supplementary Figure 1**

**Meeting agenda of the TP-DDI Workshop organized workshop at the Food and Drug Administration (FDA) White Oak Conference Center on June 4 and 5, 2012.**

**Manuscript title:**

Critical Review of Preclinical Approaches to Investigate Cytochrome P450-mediated Therapeutic Protein Drug-Drug Interactions and Recommendations for Best Practices: A White Paper

**Authors:**

Raymond Evers, Shannon Dallas, Leslie J. Dickmann, Odette A. Fahmi, Jane R. Kenny, Eugenia Kraynov, Theresa Nguyen, Aarti H. Patel, J. Greg Slatter, Lei Zhang

**Journal Title:**

Drug Metabolism and Disposition

***Workshop on Recent Advances in the Investigation of Therapeutic Protein Drug-Drug Interactions: Preclinical and Clinical Approaches***

***Co-sponsored by the FDA Office of Clinical Pharmacology and the Drug Metabolism and Clinical Pharmacology Leadership Group of the IQ Consortium***

***Hosted by the FDA Office of Clinical Pharmacology (OCP)***

***June 4-5, 2012***

*FDA White Oak Campus  
Building 31, Room 1503A  
10903 New Hampshire Ave, Silver Spring, MD 20993*

A significant number of knowledge gaps still remain in the area of therapeutic protein-drug-drug interactions (TP-DDI). This workshop aims to facilitate better understanding of the current science, investigative approaches, and gaps in knowledge in relation to TP-DDI evaluation through the following objectives:

- Provide an overview of the current scientific knowledge regarding in vitro, in vivo and clinical approaches to the prediction and clinical assessment of TP-DDI
- Highlight limitations and considerations of current methodologies (in vitro/in vivo and clinical) used for DDI analysis and study design
- Discuss the utility of population PK methodology as an exploratory and confirmative approach for DDI data analysis
- Highlight operational and logistical challenges in conducting clinical DDI studies with therapeutic proteins
- Gather input from academics, industry and regulatory leaders on the value of in vitro/in vivo or population PK approach to TP-DDI assessment, and their potential impact on labeling and dosing guidance selection for biologics
- Develop industry best practices/recommendations based on currently available in vitro approaches on the prediction and assessment of TP-DDI, with emphasis on cytokines and cytokine modulators
- Outline components of White Paper(s)

This workshop will cover a readout from the two active TP-DDI IQ working groups (in vitro and clinical) with participants across industry and FDA that have been actively collaborating in exploring various in vitro and clinical approaches for the assessment of TP-DDI. The proceedings from this workshop are intended to be compiled in white paper(s) that will be submitted to a peer reviewed scientific journal. This workshop will provide an opportunity for experts in TP-DDI area to share their science, insights, and experience in order to achieve a



consensus on what is the best science to be applied to this important topic in drug development at this time.

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## Agenda

### Monday, June 4

7:45-9:00 –Check-In/Security (**Building 1**) and escort to meeting room (*please come at least 45 minutes before 9 AM to allow time for security clearance*)

### **9:00-11:45 –Morning Session – Industry and Academia Perspectives on Clinical TP-DDI**

Moderator: *Manish Gupta (BMS)*

9:00-9:15: Introduction and Workshop Goals (*Andrew Chow, Amgen*)

9:15-10:00: Mechanistic Basis for Potential Drug-drug Interactions with Therapeutic Proteins (*Bernd Meibohm, University of Tennessee*)

10:00-10:30: Use of Population PK-based Approach in TP-DI Assessment: Merits, Pitfalls, and Future Perspective (*Honghui Zhou, J&J*)

#### **10:30-10:45: Break**

10:45-11:45: Population PK-based TP-DI Assessment: Methodological Considerations (*Diane Wang, Pfizer, and Chuanpu Hu, J&J*)

**11:45-12:45 – Lunch Break in Great Room lobby or FDA cafeteria**

### **12:45-5:00 – Afternoon Session– Clinical Panel Discussion and Regulatory Perspectives on TP-DDI**

12:45-1:15: Therapeutic Protein DDIs: Recent Regulatory Interactions & Clinical Experience

(*Graham Jang, Amgen*)

1:15-2:30: Clinical TP-DDI panel discussion (*Host: Manish Gupta, BMS*)

2:30-3:15: Challenges in Assessing Pharmacokinetic Drug-drug Interactions from Late Phase Clinical Trials (*Yaning Wang, FDA*)

#### **3:15-3:30: Break**

3:30-4:15: Drug Interactions with Therapeutic Proteins - a Swedish MPA Perspective (*Eva Gil Berglund, MPA/EMA*)

4:15-5:00: Evaluation of Therapeutic Protein-Drug Interactions: an FDA Perspective (*Lei Zhang, FDA*)

### Tuesday, June 5

7:45-9:00 –Check-In/Security (**Building 1**) and escort to meeting room (*please come at least 45 minutes before 9 AM to allow time for security clearance*)

### **9:00-11:45 – Morning Session – Industry and Academia Perspectives On In Vitro TP-DDI**

Moderator: *Greg Slatter (Amgen)*

9:00-9:15: Introduction (*Raymond Evers, Merck*)

9:15-9:45: Disease-drug-drug Interaction: Example of Tocilizumab in Rheumatoid Arthritis Patients (*Christophe Schmitt, Roche*)

9:45-10:30: Disease-dependent Drug-drug Interactions: What Can the Basic Science Tell Us? (*Eddie Morgan, Emory University*)

**10:30-10:45: Break**

10:45-11:15: Assessment of Human Hepatocyte Culture for the Prediction of Cytokine and Anti-cytokine Effects on Cytochromes P450 In Vivo (*Leslie Dickmann, Amgen*)

11:15-11:45: Impact of IL-6 on CYP3A4: In Vitro Data Interpretation and Introducing a Physiologically-Based Pharmacokinetic Approach to Predicting Disease-Drug Interactions (*Jane Kenny, Genentech*)

**11:45-12:45 – Lunch Break in Great Room lobby or FDA cafeteria**

**12:45-5:00 – Afternoon Session– In Vitro Panel Discussion and Wrap Up Sessions**

12:45-1:15: The In Vitro TP-DDI Working Group: Recommendations and Future Opportunities (*Raymond Evers, Merck*)

1:15-1:45: Polling session: Current Thinking and Experience in the Industry (*Jane Kenny, Genentech, Eugenia Kraynov, Pfizer*)

1:45-2:45: In Vitro TP-DDI Panel Discussion (*Host: Greg Slatter, Amgen*)

**2:45-3:00: Break**

3:00-4:00: Preclinical and Clinical Working Group individual wrap up session (White paper assignments)

4:00-5:00: Joint Working Group session to discuss path forward

## **Supplementary Figure 2**

### **Results from the poll conducted at the TP-DDI Workshop**

#### **Manuscript title:**

Critical Review of Preclinical Approaches to Investigate Cytochrome P450-mediated Therapeutic Protein Drug-Drug Interactions and Recommendations for Best Practices: A White Paper

#### **Authors:**

Raymond Evers, Shannon Dallas, Leslie J. Dickmann, Odette A. Fahmi, Jane R. Kenny, Eugenia Kraynov, Theresa Nguyen, Aarti H. Patel, J. Greg Slatter, Lei Zhang

#### **Journal Title:**

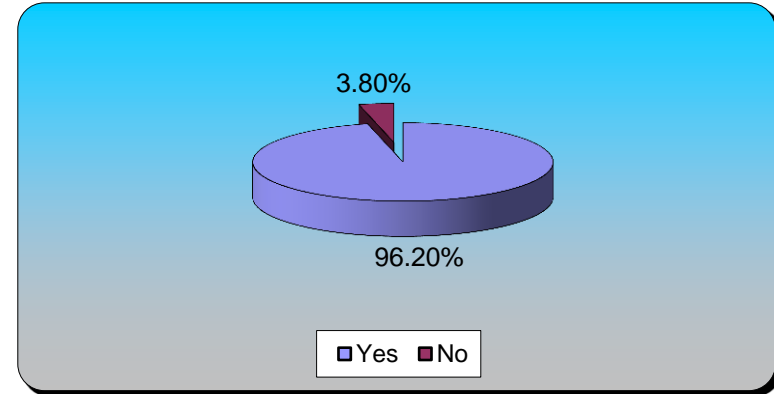
Drug Metabolism and Disposition

## Turning Graphical Results by Question

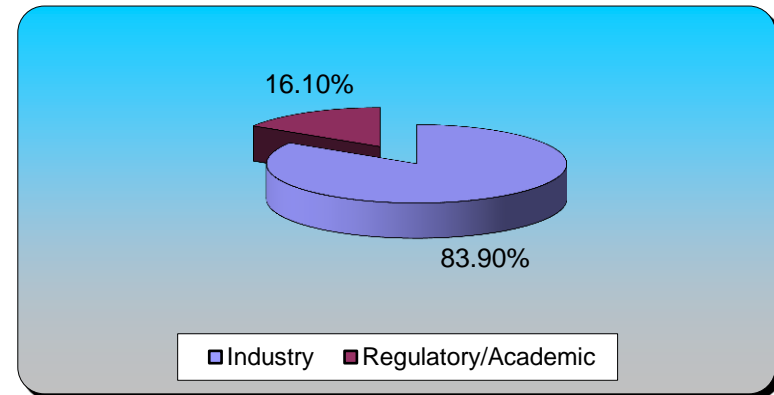
Session Name: TP-DDI\_Poll

Created: 6/5/2012 1:35 PM

<u>1.) I know how to use the polling device</u>	<u>Responses</u>	
Yes	51	96.2%
No	2	3.8%
<b>Totals</b>	<b>53</b>	<b>100.0%</b>

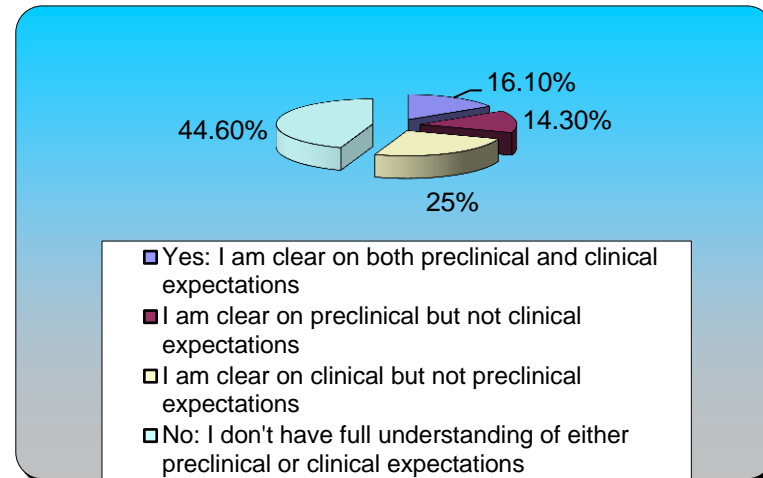


<u>2.) What is your affiliation?</u>	<u>Responses</u>	
Industry	47	83.9%
Regulatory/Academic	9	16.1%
<b>Totals</b>	<b>56</b>	<b>100.0%</b>



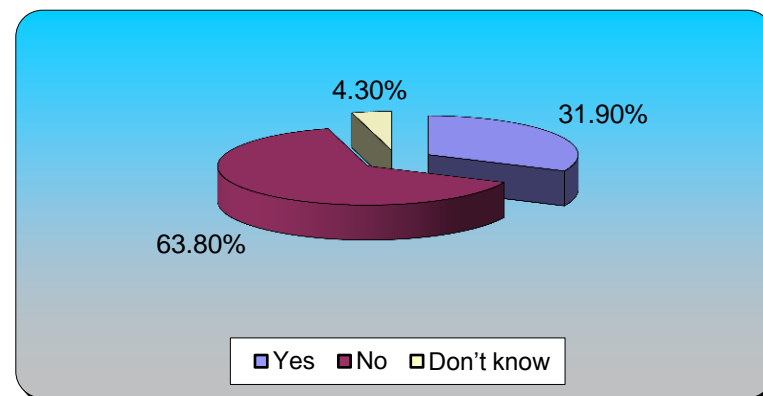
**3.) Do you have a clear understanding of what studies are required for regulatory submission around TP-DDI for cytokines and/or cytokine modulating TPs?**

	Responses	
Yes: I am clear on both preclinical and clinical	9	16.1%
I am clear on preclinical but not clinical expectations	8	14.3%
I am clear on clinical but not preclinical expectations	14	25.0%
No: I don't have full understanding of either preclinical or	25	44.6%
<b>Totals</b>	<b>56</b>	<b>100.0%</b>



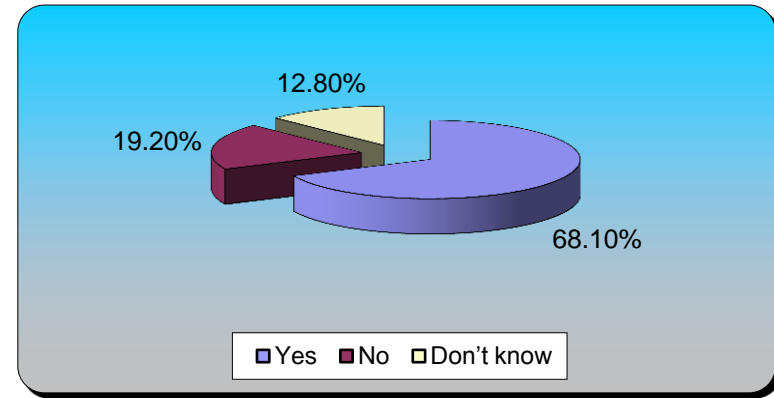
**4.) Does your company have an internal guidance regarding the strategy for addressing TP-DDIs?**

	Responses	
Yes	15	31.9%
No	30	63.8%
Don't know	2	4.3%
<b>Totals</b>	<b>47</b>	<b>100.0%</b>



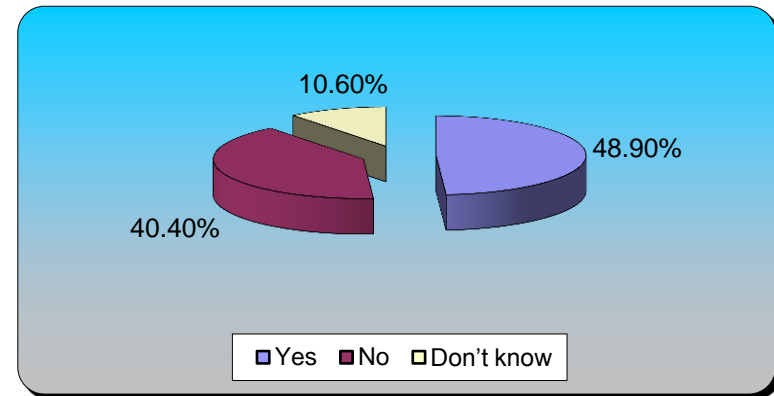
**5.) Has your company received requests around TP-DDI studies (in vitro and/or clinical) from a regulatory agency?**

	<b>Responses</b>	
Yes	32	68.1%
No	9	19.2%
Don't know	6	12.8%
<b>Totals</b>	<b>47</b>	<b>100.0%</b>



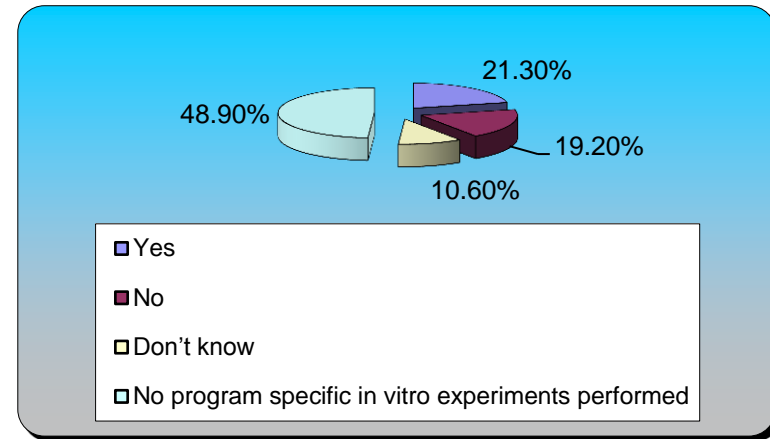
**6.) Has your company ever performed in vitro TP-DDI studies for a cytokine and/or cytokine modulating TP as a DDI perpetrator in human hepatocytes?**

	<b>Responses</b>	
Yes	23	48.9%
No	19	40.4%
Don't know	5	10.6%
<b>Totals</b>	<b>47</b>	<b>100.0%</b>



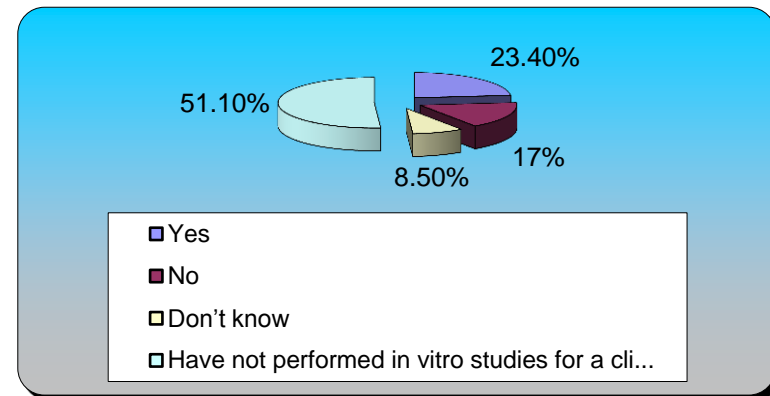
**7.) Were in vitro TP-DDI studies in human hepatocytes for a cytokine and/or cytokine modulating TP as a DDI perpetrator useful for internal decision making and/or project progression within your company?**

	Responses	
Yes	10	21.3%
No	9	19.2%
Don't know	5	10.6%
No program specific in vitro experiments performed	23	48.9%
<b>Totals</b>	<b>47</b>	<b>100.0%</b>



**8.) Has your company ever used in vitro TP-DDI human hepatocyte data for a cytokine and/or cytokine modulating TP as a DDI perpetrator as justification to a regulatory agency that a clinical DDI study would not be needed?**

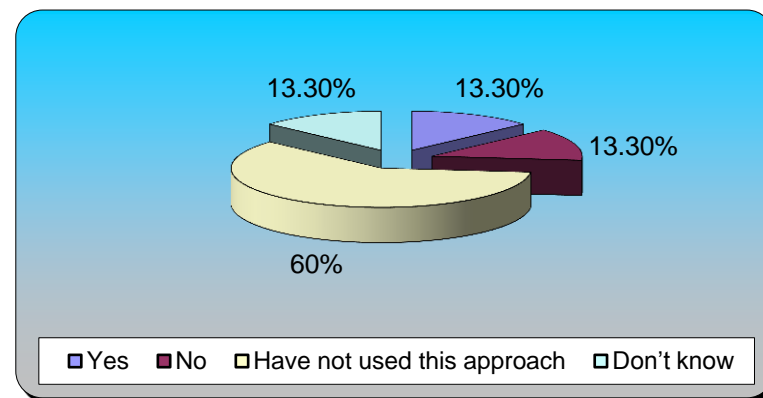
	Responses	
Yes	11	23.4%
No	8	17.0%
Don't know	4	8.5%
Have not performed in vitro studies for a cli...	24	51.1%
<b>Totals</b>	<b>47</b>	<b>100.0%</b>





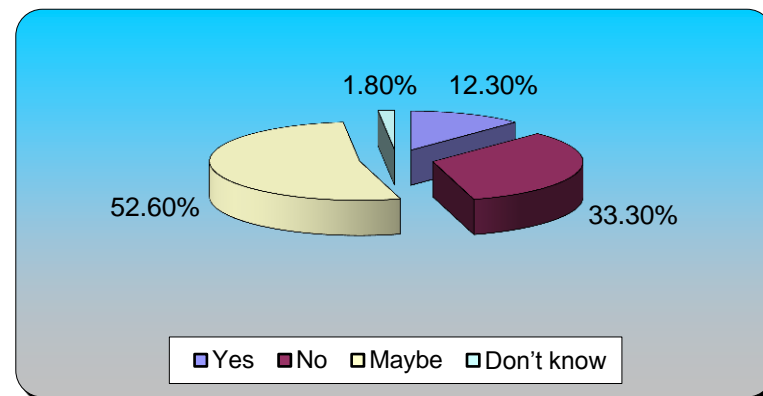
9.) If your company has included in vitro human hepatocyte data for a cytokine and/or cytokine modulating TP as a DDI perpetrator in a regulatory submission to justify that a clinical DDI study would not be needed, was this justification accepted by a

	Responses	
Yes	6	13.3%
No	6	13.3%
Have not used this approach	27	60.0%
Don't know	6	13.3%
<b>Totals</b>	<b>45</b>	<b>100.0%</b>



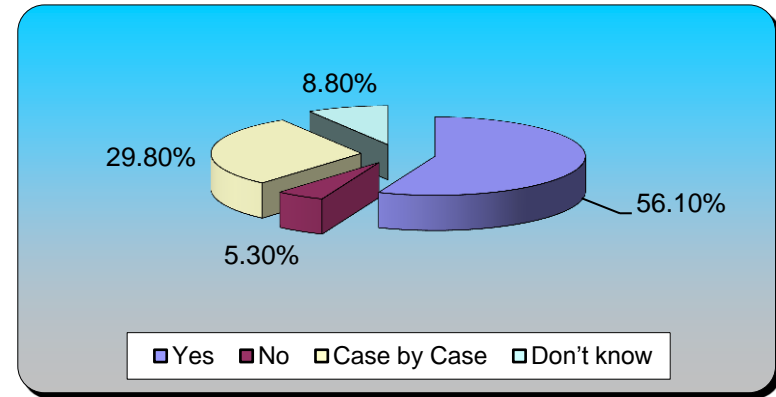
10.) Do you believe in vitro TP-DDI human hepatocyte studies can be predictive of the clinical situation for a cytokine and/or cytokine modulating TP as a DDI perpetrator?

	Responses	
Yes	7	12.3%
No	19	33.3%
Maybe	30	52.6%
Don't know	1	1.8%
<b>Totals</b>	<b>57</b>	<b>100.0%</b>



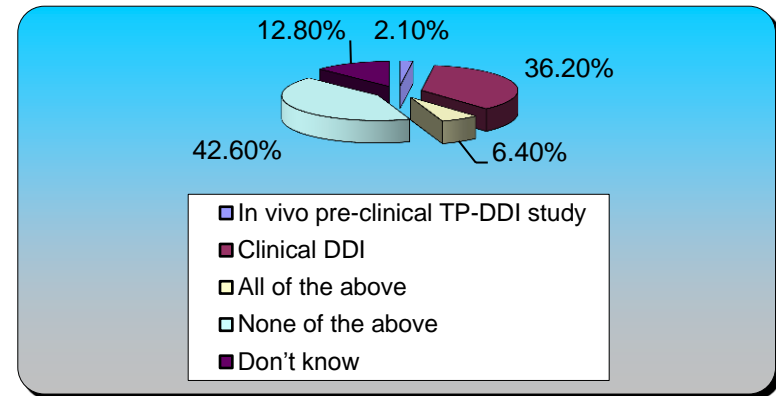
11.) Should alternative in vitro approaches beyond hepatocyte monolayers be investigated for cytokines and/or cytokine modulating TPs as DDI perpetrators, for instance including Kupffer cells or other immune modulating cells in co-culture?

	Responses	
Yes	32	56.1%
No	3	5.3%
Case by Case	17	29.8%
Don't know	5	8.8%
<b>Totals</b>	<b>57</b>	<b>100.0%</b>



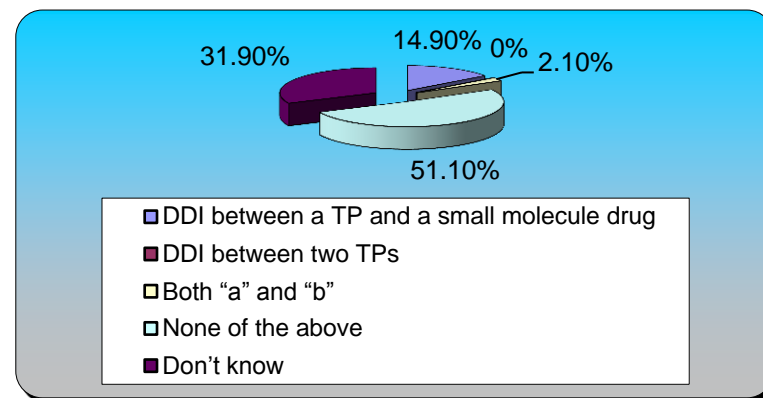
12.) What type of in vivo TP-DDI study has your company conducted in the past for cytokine and/or cytokine modulating TPs as a DDI perpetrator?

	Responses	
In vivo pre-clinical TP-DDI study	1	2.1%
Clinical DDI	17	36.2%
All of the above	3	6.4%
None of the above	20	42.6%
Don't know	6	12.8%
<b>Totals</b>	<b>47</b>	<b>100.0%</b>



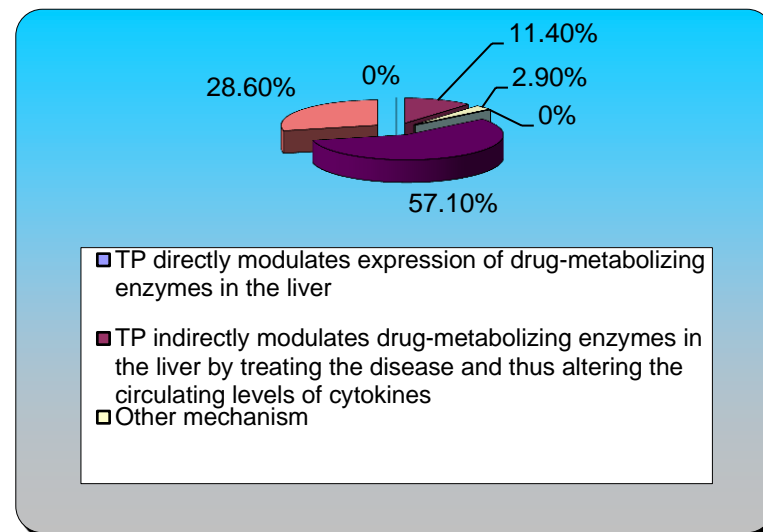
**13.) What type of TP-DDI has your company observed in the past?**

	Responses	
DDI between a TP and a small molecule drug	7	14.9%
DDI between two TPs	0	0.0%
Both "a" and "b"	1	2.1%
None of the above	24	51.1%
Don't know	15	31.9%
<b>Totals</b>	<b>47</b>	<b>100.0%</b>



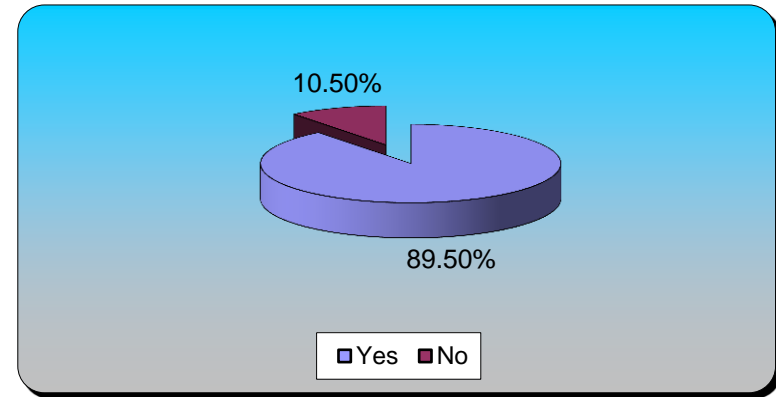
**14.) What type of TP-DDI related to drug metabolizing enzymes have you observed with TP that are not cytokines/cytokine modulators?**

	Responses	
TP directly modulates expression of drug-metabolizing enzymes in the liver	0	0.0%
TP indirectly modulates drug-metabolizing enzymes in the liver by treating the disease and thus altering the circulating levels of cytokines	4	11.4%
Other mechanism	1	2.9%
All of the above	0	0.0%
None of the above	20	57.1%
Don't know	10	28.6%
<b>Totals</b>	<b>35</b>	<b>100.0%</b>



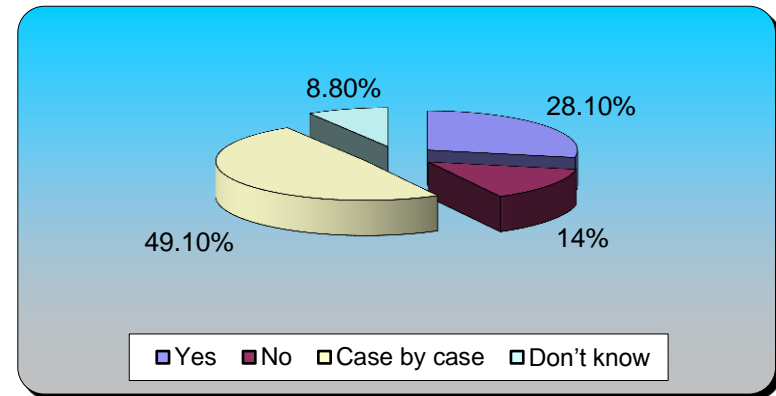
15.) Do you think that the in vitro data obtained with currently employed hepatocyte systems may not reliably predict the likelihood of a clinical DDI for a novel cytokine or cytokine modulator as a DDI perpetrator?

	Responses	
Yes	51	89.5%
No	6	10.5%
<b>Totals</b>	<b>57</b>	<b>100.0%</b>



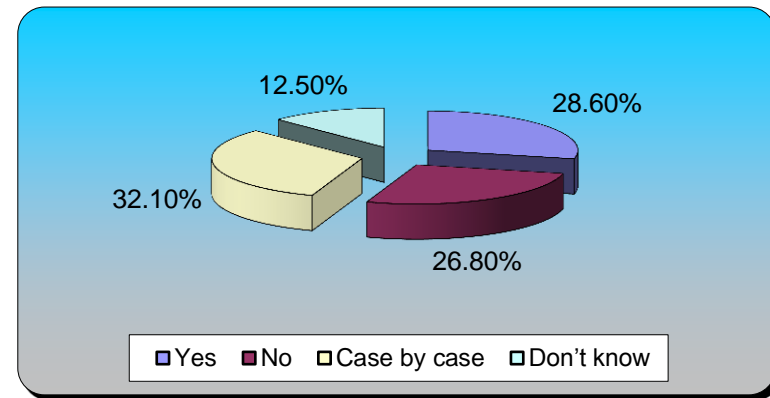
16.) Do you think a population PK approach can be useful for quantifying the extent of TP-DDI (vs. use as a screening tool)?

	Responses	
Yes	16	28.1%
No	8	14.0%
Case by case	28	49.1%
Don't know	5	8.8%
<b>Totals</b>	<b>57</b>	<b>100.0%</b>



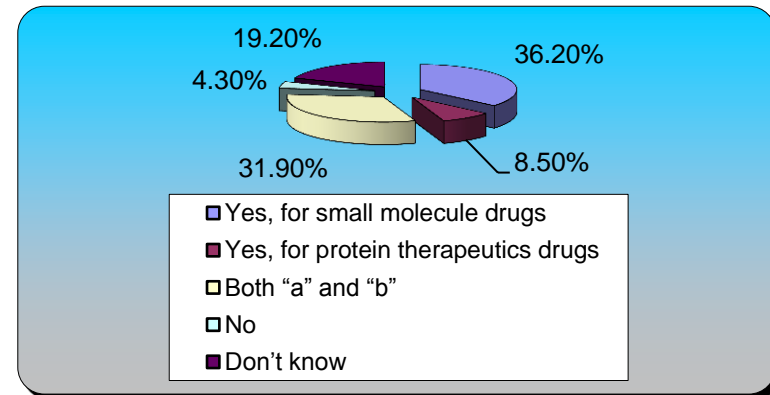
**17.) If a population PK approach is used in assessing TP-DDI, do you think the framework should be consistent with the standard analysis (90% confidence interval falling in e.g., 80-125) in principle, and provide valid p-values?**

	Responses	
Yes	16	28.6%
No	15	26.8%
Case by case	18	32.1%
Don't know	7	12.5%
<b>Totals</b>	<b>56</b>	<b>100.0%</b>



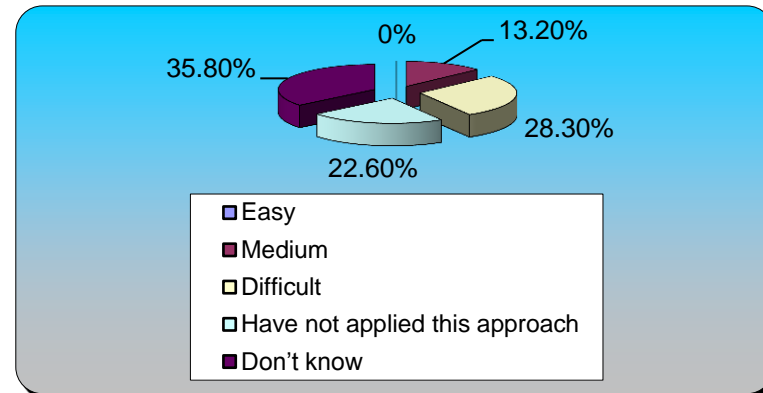
**18.) Has your company utilized the population PK approach to assess DDI issues?**

	Responses	
Yes, for small molecule drugs	17	36.2%
Yes, for protein therapeutics drugs	4	8.5%
Both "a" and "b"	15	31.9%
No	2	4.3%
Don't know	9	19.2%
<b>Totals</b>	<b>47</b>	<b>100.0%</b>



**19.) How challenging do you think is the application of the confirmatory modeling approach for TP-DDI evaluation?**

	Responses	
Easy	0	0.0%
Medium	7	13.2%
Difficult	15	28.3%
Have not applied this approach	12	22.6%
Don't know	19	35.9%
<b>Totals</b>	<b>53</b>	<b>100.0%</b>



**20.) How important is it in your opinion to establish a rational population PK based TP-DDI assessment framework?**

	Responses	
Not Important	5	8.8%
Somewhat important	26	45.6%
Very Important	22	38.6%
Don't know	4	7.0%
<b>Totals</b>	<b>57</b>	<b>100.0%</b>

