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PBPK modelling to predict drug-biologic interactions with cytokine modulators: Are these relevant and is IL-6 enough?

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Abstract

Drugs that modulate cytokine levels are often used for the treatment of cancer as well as inflammatory or immunological disorders. Pharmacokinetic drug-biologic interactions (DBI) may arise from suppression or elevation of cytochrome P450 (CYP) enzymes caused by the increase or decrease in cytokine levels following administration of these therapies. There is *in vitro* and *in vivo* evidence that demonstrates a clear link between raised interleukin (IL)-6 levels and CYP suppression, in particular CYP3A4. However despite this, the changes in IL-6 levels *in vivo* rarely lead to significant drug interactions (AUC and C_{max} ratios < 2-fold). The clinical significance of such interactions therefore remains questionable and is dependent on the therapeutic index of the small molecule therapy. Physiologically-based pharmacokinetic (PBPK) modelling has been used successfully to predict the impact of raised IL-6 on CYP activities. Beyond IL-6, published data show little evidence that IL-8, IL-10, and IL-17 suppress CYP enzymes. *In vitro* data suggest that IL-1β, IL-2, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ can cause suppression of CYP enzymes. Despite *in vivo* there being a link between IL-6 levels and CYP suppression, the evidence to support a direct effect of IL-2, IL-8, IL-10, IL-17, IFN-γ, TNF-α or vascular endothelial growth factor (VEGF) on CYP activity is inconclusive. This commentary will discuss the relevance of such drug-biologic interactions and whether current PBPK models considering only IL-6 are sufficient.

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Significance Statement

This commentary summarizes the current *in vitro* and *in vivo* literature regarding cytokine-mediated CYP suppression and compares the relative suppressive potential of different cytokines in reference to IL-6. It also discusses the relevance of drug-biologic interactions to therapeutic use of small molecule drugs and whether current PBPK models considering only IL-6 are sufficient to predict the extent of drug-biologic interactions.

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Introduction

Biologic drugs have been used or tested in combination with small molecule drugs for the treatment of immunological diseases, such as inflammatory bowel disease and rheumatoid arthritis (Paramsothy *et al.*, 2018; Dolinger *et al.*, 2021; Genovese *et al.*, 2008), and oncologic disorders, such as leukemia, breast cancer, gastrointestinal stromal tumors and colorectal cancer (Sharman *et al.*, 2019; Canoici *et al.*, 2019; Vallilas *et al.*, 2021; Poindessous *et al.*, 2011). Many of these biologics modulate cytokine levels. An increase or decrease in cytokines levels has been shown to suppress or elevate cytochrome P450 (CYP) enzymes (Abdel-Razzak *et al*., 1993, 1994; Aitken and Morgan, 2007; Dallas *et al*., 2012; Donato *et al*., 1993, 1997; Guillén *et al*., 1998; Sunman *et al*., 2004; Dickmann *et al*., 2012; Li *et al*., 2014; Mimura *et al*., 2015; Rubin *et al*., 2015; Klein *et al*., 2015). CYP enzymes are an important class of enzymes responsible for the metabolism of many small molecule drugs (Wienkers and Heath, 2005; Zanger and Schwab, 2013). Thus, pharmacokinetic drug-biologic interactions (DBIs) may arise following co-administration of small molecules and biologics. For therapeutic proteins acting as cytokine modulators, the U.S. Food and Drug Administration (FDA) requires language in the label to indicate whether there is a potential for drug-drug interactions (DDIs) (FDA Draft Guidance, 2020).

Despite the clear *in vitro* and *in vivo* links between interleukin (IL)-6 levels and CYP suppression and in particular CYP3A4, the changes in IL-6 levels *in vivo* rarely lead to significant drug interactions (i.e., < 2-fold; Morgan *et al*., 2008; Harvey and Morgan, 2014; Coutant & Hall, 2018). Physiologically-based pharmacokinetic (PBPK) modeling has been used successfully to predict the impact of raised IL-6 on CYP activities (Machavaram *et al.*, 2013, 2019; Jiang *et al.*, 2016; Xu *et al,*. 2015; Stader *et al.*, 2021; Lenoir *et al.*, 2021). Beyond IL-6,

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evidence to support a direct effect of other cytokines on CYP activity remains inconclusive. The underlying mechanisms of CYP modulation can be cytokine-specific (de Jong *et al*., 2020), potentially leading to synergistic effects. The effect of combining cytokines has been assessed *in vitro* (Dickmann *et al*., 2012; Xu *et al*., 2015), however, PBPK simulation to evaluate the impact of other cytokines on drug exposures has not been performed. This commentary summarizes the current literature regarding cytokine-mediated CYP suppression and compares the relative suppressive potential of different cytokines in reference to IL-6. Subsequently, the relevance of such DBIs and whether current PBPK models considering only IL-6 are sufficient are discussed. Cytokines can also affect other enzymes and transporters (Lévesque *et al.*, 1998; Richardson *et al*., 2006; Le Vée *et al*., 2009; Fardel and Le Vée, 2009), but this commentary will focus on their effect on CYPs.

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Evidence of Cytokine Suppression of CYP Enzymes *In Vitro*

In vitro CYP suppression by IL-6

The majority of the available *in vitro* data focus on IL-6. Of the cytokines tested *in vitro*, IL-6 generally causes the greatest CYP suppression, with CYP3A4 being the most sensitive isoform (Abdel-Razzak *et al*., 1993, 1994; Aitken and Morgan, 2007; Dallas *et al*., 2012; Donato *et al*., 1993, 1997; Guillén *et al*., 1998; Sunman *et al*., 2004; Dickmann *et al*., 2012; Li *et al*., 2014; Mimura *et al*., 2015; Rubin *et al*., 2015; Klein *et al*., 2015). Decreases in CYP1A2, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP2E1 and CYP3A4 activity/mRNA/protein abundance of up to 36, 78, 72, 54, 88, 39, 50 and 98% have been reported, although suppression of CYP1A2 often did not reach statistical significance (Abdel-Razzak *et al*., 1993, 1994; Aitken and Morgan, 2007; Dallas *et al*., 2012; Donato *et al*., 1993, 1997; Guillén *et al*., 1998; Sunman *et al*., 2004). These studies usually measure the effect of cytokines at a single concentration that is far in excess of those seen in diseases or following administration of immune modulators. For example, IL-6 concentrations of 0.5 – 200 ng/mL have been used *in vitro*, whereas mean values in rheumatoid arthritis (RA) patients and post-surgery are 54 and 229 pg/mL, respectively (Machavaram *et al*., 2013). Another meta-analysis comprising 11,583 cancer patients reported a median IL-6 serum level of 6.95 pg/mL (range: 0.23 – 78.5 pg/mL) compared to the control level of 1.31 pg/mL (range: 0 – 37 pg/mL) (Lippitz and Harris, 2016). A mean serum peak IL-6 concentration of 4400 pg/mL was observed after initiation of blinatumomab (up to 90 $\mu g/m^2$/day) in patients with acute lymphoblastic leukemia (Xu *et al*., 2015), which is still lower than IL-6 concentrations used in most *in vitro* assays.

In the Dickmann *et al.* (2011) *in vitro* study, cryopreserved and fresh human hepatocytes were used to determine the suppression of several CYPs by IL-6 over a range of concentrations

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(0.5 – 10,000 pg/mL) which are more relevant to those observed in disease states or following administration of immune modulators. The IL-6 concentration that supports half-maximal suppression (EC_{50}) and maximum fold suppression (E_{min}) values for CYP mRNA and for CYP1A2 and CYP3A4 activity were reported (Table 4). IL-6 EC_{50} values are generally in excess of IL-6 levels observed in RA patients, with the exception of CYP3A4 (EC_{50} = 3.23 pg/mL CYP3A4 for mRNA; mean EC_{50} (range) = 73.2 (4.23 – 176) pg/mL for CYP3A4 activity) (Dickmann *et al.*, 2011). Significant CYP suppression is unlikely if IL-6 concentration is below EC_{50} values, although IL-6 concentration can be transiently elevated to a much higher level following administration of cytokine modulators.

Evers *et al*. (2013) studied between-laboratory variability in IL-6 suppression of CYP3A4 by incubating hepatocytes from the same donor at 6 different laboratories for 48 h with 0.001 – 500 ng/mL IL-6. Despite high between-laboratory variability, the reported CYP3A4 activity E_{min} values (0.20 – 0.38 in the absence of dexamethasone) are consistent with those reported in Dickmann *et al.* (2011). Similarly, the CYP3A4 activity EC_{50} value reported by Evers *et al.* (2013) (217 pg/mL in the absence of dexamethasone) is close to the upper limit of the range of values for individual hepatocyte donors reported by Dickmann *et al*. (2011). Contrarily, the CYP3A4 mRNA EC_{50} value reported by Dickmann *et al*. (2011) is about 30-fold lower than the value reported by Evers *et al.* (2013) (94.7 pg/mL in the absence dexamethasone).

In vitro CYP suppression by other cytokines

Suppression of CYPs by IL-2 has been measured *in vitro* in two studies (Dallas *et al*., 2012; Sunman *et al*., 2004). Dallas *et al*. (2012) reported that IL-2 did not have a statistically significant effect on CYP1A2, CYP2C9 or CYP3A4 mRNA or activity in cryopreserved hepatocytes incubated with 10 ng/mL IL-2 for 48 h. Modest but significant suppression (< 25%)

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of CYP2B6 and CYP2C19 (activity only) were observed (Table 1). CYP2D6 mRNA was increased by 50%, whereas CYP2D6 activity was decreased by 22% (Table 1). Comparison to the CYP activity and mRNA suppression by IL-6 in the same *in vitro* assay as represented by the ratios of % decrease relative to IL-6, shows that IL-2 has less of a suppressive action when compared to IL-6 (Table 1). Sunman *et al*. (2004) reported no suppression of CYP3A activity by IL-2 (2 – 200 ng/mL) in hepatocyte cultures but a concentration-dependent 50 – 70% suppression in hepatocyte/Kupffer cell co-cultures. This suggests that IL-2 acts via an indirect mechanism, probably through stimulating Kupffer cells to produce other cytokines such as IL-6.

Suppression of CYPs by interferon (IFN)-γ has been measured *in vitro* in 6 studies (Abdel-Razzak *et al*., 1993, 1994; Aitken and Morgan *et al*., 2007; Donato *et al*., 1993, 1997; Guillén *et al*., 1998). Abdel-Razzak *et al*. (1993) reported that IFN-γ did not change CYP2C or CYP3A mRNA in fresh hepatocytes incubated with 50 U/mL IFN-γ for 72 h. In contrast, 21 to 55% decrease in nifedipine activity (CYP3A substrate) was found in 2 donors. A marked reduction in CYP1A2 and CYP2E1 mRNA was observed in 2 of 3 donors and ethoxyresorufin-*O*-deethylase (EROD) activity was reduced by 29 – 53% in 6 donors (Table 2). Similarly, in a follow up study, EROD activity was significantly reduced by 22 – 42% in 4 fresh hepatocyte donors incubated with 50 U/mL IFN-γ for 72 h (Table 2) (Abdel-Razzak *et al*., 1994). Aitken and Morgan (2007) reported that incubation of fresh hepatocytes with 10 ng/mL IFN-γ for 24 h led to a statistically significant decrease in CYP2C8, CYP3A4 and CYP2B6 mRNA and protein expression of CYP3A4 and CYP2B6 (Table 2). IFN-γ did not have a statistically significant effect on CYP2C9, CYP2C18 or CYP2C19 mRNA; however, a significant decrease in CYP2C9 protein expression was observed after 24 h in 1 donor (Aitken and Morgan, 2007). Guillén *et al*. (1998) reported that IFN-γ reduced CYP1A2, CYP2B6, CYP2A6 and CYP3A4 activity in fresh

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hepatocytes incubated with 300 U/mL IFN-γ for 48 h (Table 2). Comparison of the CYP activity, protein expression or mRNA suppression caused by IFN-γ to that caused by IL-6 in the corresponding studies shows that IFN-γ generally has a similar or reduced suppressive action when compared to IL-6, with the exception of CYP1A2 activity where IFN-γ is a more potent suppressor (Table 2).

There are no reported E_{min} and EC_{50} values describing the IFN-γ suppression of CYPs and the majority of the *in vitro* data have been measured at a single IFN-γ concentration. However, concentration dependent suppression of CYP1A2 activity has been reported, allowing the calculation of E_{min} and EC_{50} values for IFN-γ (Donato *et al.*, 1993, 1997; Guillén, 1998). The calculated IFN-γ E_{min} and EC_{50} values are 0.629 and 2460 pg/mL (measured at 500 – 150000 pg/mL in Donato *et al*., 1993), 0.572 and 4400 pg/mL (measured at 1500 – 50000 pg/mL in Donato *et al.*, 1997) and 0.601 and 4098 pg/mL (measured at 2500 – 50000 pg/mL in Guillén *et al.*, 1998), respectively (Table 4). Comparing these values to those for IL-6 (mean E_{min} (range) = 0.230 (0.0622 – 0.529) and mean EC_{50} (range) = 1251 (142 - 4070) pg/mL) (Table 4; Dickmann *et al*., 2011) suggests IFN-γ is not as potent a CYP1A2 suppressor as IL-6. It should be noted that the IFN-γ concentrations used *in vitro* are far in excess of physiological concentrations in healthy subjects (7.5 – 21.2 pg/mL or 0.3 ± 0.1 U/mL) and those with RA (17.9 – 32.6 pg/mL), acute respiratory infections (3.4 ± 1.3 U/mL) (Brockmeyer *et al*., 1992; Caris *et al*., 2020) or acute lymphoblastic leukemia following blinatumomab administration (peak ~ 440 pg/mL) (Xu *et al*., 2015).

Suppression of CYPs by tumor necrosis factor (TNF)-α has been measured *in vitro* in 5 studies (Abdel-Razzak *et al*., 1993; Aitken and Morgan, 2007; Dallas *et al*., 2012, Mimura *et al*., 2015; Klein *et al.*, 2015). Abdel-Razzak *et al*. (1993) reported that TNF-α decreased CYP1A2,

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CYP2C, CYP2E1, and CYP3A mRNA levels by 30% – 80% in all 3 donors after 72-hour incubation (Table 3). EROD and nifedipine oxidation activities were also decreased by 32 – 85% and 24 – 90%, suggesting reduced CYP1A2 and CYP3A4 activities. Similarly, Aitken and Morgan (2007) reported significant reduction in CYP2C8 (but not CYP2C9) and CYP3A4 mRNA levels (n = 9). Additionally, they quantified CYP2B6, CYP2C9, and CYP3A4 protein levels using western blotting and found that TNF-α treatment significantly reduced CYP2B6 and CYP2C9 proteins by 87% and 94%, respectively (Table 3). The CYP3A4 protein level decreased in a similar trend but statistical significance was not detected. Dallas *et al*. (2012) measured mRNA levels and enzyme activities for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Following TNF-α treatment, reduction in CYP1A2, CYP2D6, and CYP3A4 mRNA levels were detected (Table 3). TNF-α also reduced CYP3A4 mRNA by 51% in Hepatoma cell line FLC-4 over a 24-hour incubation, although TNF-α had no effect on CYP3A4 protein expression or activity (Mimura *et al*., 2015). Reduction in enzyme activities were significant in all CYP isoforms tested and generally comparable to reduction caused by IL-6, except for CYP1A2 where a greater reduction was observed following TNF-α treatment compared to IL-6 treatment (Table 3).

Klein *et al.* (2015) proposed HepaRG cells as a surrogate for primary human hepatocytes and, using HepaRG cells, characterized dose-response curves for CYP1A2, CYP2B6, CYP2C9, and CYP3A4 by measuring mRNA levels in a range of TNF-α concentrations (0.1 – 50000 pg/mL). Using data from this study, the E_{min} and EC_{50} values of TNF-α for CYP1A2 (0.0337 and 817 pg/mL), CYP2B6 (0.639 and 86.6 pg/mL), CYP2C9 (0.289 and 153 pg/mL) and CYP3A4 (0.107 and 133 pg/mL) were determined (Table 4), suggesting weaker suppression for CYPs compared to IL-6 except CYP1A2. Despite a stronger effect in CYP1A2 suppression compared

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to IL-6 (Table 4), it should be noted that TNF-α EC_{50} values were in excess of concentrations observed in patients with immunological diseases (e.g., 30.5 pg/mL in RA patients with chronic periodontitis vs. 5.5 pg/mL in control (Thilagar *et al.*, 2018) and 25.7 pg/mL in psoriatic patients vs. 11.2 pg/mL in control (Arican *et al.,* 2005)), although within the range of TNF-α concentrations observed following administration of cytokine modulators (e.g., peak ~ 200 pg/mL in acute lymphoblastic leukemia patients given blinatumomab (Xu *et al*., 2015)).

In vitro data for other cytokines are limited. Currently there are no *in vitro* data available in the public domain regarding CYP suppression by IL-8, IL-17 and vascular endothelial growth factor (VEGF). Xu *et al.* (2015) indicate that *in vitro* studies in human hepatocytes revealed no effect of IL-10 on CYP enzymes even at 5000 pg/mL. The studies were conducted internally by Amgen Inc.; however, the data were not reported in the publication (Xu *et al.*, 2015).

Although multiple cytokines are raised simultaneously in disease states and following dosing of certain cytokine modulator biologic drugs *in vivo*, the majority of *in vitro* studies have assessed the CYP suppression caused by one cytokine alone rather than the effect of a combination of cytokines. Underlying mechanisms of CYP suppression are cytokine-specific, hence synergistic effects on CYP modulation are possible. The pre- and post-transcriptional mechanistic pathways, such as transcriptional factor regulation and nitric oxide stimulation, were previously discussed (de Jong *et al*., 2020). However, *in vitro* data to confirm or refute cytokine synergism are limited. In the Dickmann *et al.* (2012) *in vitro* study, cryopreserved and fresh human hepatocytes from 1 donor were used to determine the suppression of several CYPs by IL-1β alone or in combination with IL-6 over a range of physiologically relevant concentrations (10 and 100 pg/mL IL-1β and IL-6). Dickmann *et al.* reported IL-1β alone was 6-fold less potent than IL-6 (based upon EC_{50} values) for suppression of CYP3A4. The combination of IL-1β and

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IL-6 did not increase the CYP suppression caused by IL-6 alone for CYP1A2 and CYP2C9. The combination of 100 pg/mL IL-1β with IL-6 reduced the suppression of CYP2B6 caused by 100 pg/mL IL-6 alone. In contrast, the combination of 100 pg/mL IL-1β and IL-6 had an additive down regulation on CYP3A4 mRNA and activity compared to IL-6 alone, reducing CYP3A4 mRNA/activity to 26% *versus* 37% of control values, respectively.

Xu *et al*. (2015) also studied the suppression of multiple CYPs in 3 hepatocyte donors when incubated with a cocktail of cytokines (IL-2, IL-6, IL-10, IFN-γ and TNF-α). Three concentrations of cytokines were used, based on the low (125 pg/mL for all cytokines), mid (2000 pg/mL IL-6, IL-10 and IFN-γ with 500 pg/mL for IL-2 and TNF-α) and high (20000 pg/mL IL-6, IL-10 and IFN-γ with 1000 pg/mL for IL-2 and TNF-α) levels of cytokines observed following dosing of blinatumomab (0.5 to 90 $\mu g/m^2$/day) to non-Hodgkin Lymphoma patients. Similar CYP suppression was observed with the mid and high concentration cytokine cocktails, indicating the suppression is maximized by the mid strength cytokine levels (Xu *et al*., 2015). Limited suppression was observed in most donors for CYP2C19 and CYP2D6 activity, even with high cytokine levels, whereas > 50% suppression of CYP1A2, CYP2C9 and CYP3A4 activity was observed in 2 or 3 donors with the highest cytokine levels. The level of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 suppression observed with mid or high strength cytokine cocktails by Xu *et al*. (2015) was within the range of values reported by other *in vitro* studies using IL-6, TNF-α or IFN-γ alone (Abdel-Razzak *et al*., 1993, 1994; Aitken and Morgan, 2007; Dallas *et al*., 2012; Donato *et al*., 1993, 1997; Sunman *et al*., 2004; Dickmann *et al*., 2012). This suggests that suppression by combinations of cytokines is not likely to increase the extent of CYP suppression compared to incubation with a single cytokine.

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Evidence of Cytokine Suppression of CYP Enzymes *In Vivo*

Changes in circulating cytokine levels have been linked to alterations in drug metabolism *in vivo* (Harvey and Morgan, 2014; Morgan *et al*., 2008). There are several reports of alteration of CYP substrate PK in disease/infection/following vaccination where there is an inflammatory response and hence increase in circulating cytokine levels. In addition, DDIs between CYP substrates and biologic drugs that are themselves cytokines or cytokine modulators have been reported (Evers *et al*., 2013; Huang *et al*., 2010; Lee *et al*., 2010).

In vivo CYP suppression by IL-6

The correlations between raised IL-6 concentrations and CYP activity have been reviewed previously (Morgan *et al*., 2008; Harvey and Morgan, 2014; Coutant & Hall, 2018). A few examples are detailed here. Raised IL-6 concentrations have been significantly correlated with decreased CYP1A2, CYP2C9, CYP2C19 and CYP3A4 activity in congestive heart failure, cancer, bone marrow transplant, COVID-19 infection and surgery patients (Frye *et al*., 2002; Lenoir *et al*., 2021a; Sato *et al*., 2016; Trousil *et al*., 2019; Chen *et al.*, 1994; Lenoir *et al*., 2021b). In contrast, raised IL-6 concentrations were associated with increased CYP2E1 activity in ovarian cancer patients and CYP2C9 activity in hip surgery patients (Trousil *et al*., 2019; Lenoir *et al*., 2021b). However, CYP2D6 activity did not change in hip surgery patients within 3 days of surgery or in COVID-19 infected patients (Lenoir *et al*., 2021a; Lenoir *et al*., 2021b).

Several therapeutic protein-drug interactions relating to IL-6 have also been reported. Administration of sarilumab or tocilizumab to RA patients led to a decrease in simvastatin, midazolam, omeprazole and S-warfarin area under the curve (AUC), a slight increase in CYP1A2 AUC and no effect on CYP2D6 activity (Lee *et al*., 2017; Schmitt *et al.*, 2011; Zhuang *et al*., 2015; Zhang *et al*., 2009; Terao *et al*., 2010). Similarly, COVID-19 infected patients who

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received tocilizumab > 12 hours prior to lopinavir administration had significantly lower lopinavir (CYP3A4 substrate) exposure (Marzolini *et al.*, 2020). Sarilumab and tocilizumab are monoclonal antibodies (mAbs) that block IL-6 from binding to its receptor and hence remove the suppressive effect of raised IL-6 on CYPs in RA and COVID-19 patients, leading to increased CYP activity and co-administered drug clearance.

In vivo CYP suppression by other cytokines

CYP1A2, CYP2C, CYP2E1 and CYP3A protein expression was reduced to 37, 45, 60 and 39% of control values in hepatic microsomes isolated from surgical samples from hepatectomy patients receiving high doses of IL-2 (9 or 12 x 10^6 U/m^2) prior to surgery (Elkahwaji *et al*., 1999). Similarly, methoxyresorufin and erythromycin activity was significantly reduced following IL-2 administration (Elkahwaji *et al*., 1999). Indinavir trough concentrations and AUC significantly increased with a corresponding significant decrease in oral clearance (CL_{po}) following administration of IL-2 (Proleukin) in human immunodeficiency virus (HIV) patients (Piscitelli *et al*., 1998). However, significant increases in IL-6 concentration were also observed over the 5-day IL-2 infusion, with mean IL-6 concentrations of ~ 80 pg/mL by Day 5 (Piscitelli *et al*., 1998). These levels of IL-6 are similar to those observed in RA patients (Machavaram *et al*., 2013), where administration of IL-6R antagonists tocilizumab and sirukumab led to a 2.4- and 1.5-fold decrease in AUC of CYP3A4 substrates simvastatin and midazolam (Schmitt *et al*., 2011; Zhuang *et al*., 2015). Thus, the similar level of interaction (AUC ratio 1.9-fold) between Proleukin and indinavir (CYP3A4 substrate) suggests that IL-2 does not cause an increased suppression of CYP3A4 compared to that caused by IL-6 alone.

Basiliximab and daclizumab are monoclonal antibodies (mAb) that act as IL-2 receptor α (IL-2Rα) antagonists. Following administration of IL-2Rα antagonists (mainly basiliximab) to

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renal transplant patients, tacrolimus trough concentrations significantly increased (Sifontis *et al*., 2002; Lin *et al*., 2015). Similarly, significantly increased cyclosporine trough concentrations, early cyclosporine toxicity and a lower dose requirement were found in pediatric renal transplant patients following dosing with basiliximab when compared to controls (Strehlau *et al*., 2000). The reduction in tacrolimus and cyclosporine clearance is thought to be due to basiliximab blocking the binding of circulating IL-2 to IL-2Rα on T cells and instead IL-2 binds to the IL-2R on hepatic and intestinal cells leading to suppression of CYP3A4 (Sifontis *et al*., 2002). In contrast, administration of daclizumab to multiple sclerosis patients had no effect on the exposure of midazolam, S-warfarin, omeprazole, caffeine or dextromethorphan (Tran *et al*., 2016). IL-6 concentrations were not monitored in these studies.

There are few reports linking IL-8 to suppression of CYP metabolism *in vivo* and no data following direct dosing of IL-8 or IL-8 antagonists. In psoriasis patients, IL-8 concentrations were significantly increased (~ 10 pg/mL) when compared to healthy subjects; however, no correlation was found between raised IL-8 levels and venlafaxine (CYP2D6 and P-glycoprotein (P-gp) substrate) metabolic ratios (Godoy *et al*., 2016). In contrast, raised IL-8 levels in ovarian cancer patients (71.6 pg/mL) were significantly associated with increased CYP2E1 activity (3-fold) and reduced CYP3A4 activity (42%) when compared to healthy volunteers (Trousil *et al*., 2019). However, the changes in enzyme activity were also significantly associated with IL-6 and TNF-α levels. In fact, the IL-6 concentration (37.3 pg/mL) and the extent of reduction in CYP3A4 activity in ovarian cancer patients were comparable to those in RA patients before sirukumab treatment (Zhuang *et al*., 2015). Since IL-8, TNF-α and IL-6 could all contribute to effects in CYP2E1 and CYP3A4 activity, a direct role of IL-8 in a CYP2E1 or CYP3A4-mediated DDIs is inconclusive.

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A double-blind crossover study where 8 μg/kg of IL-10 and placebo were administered to healthy volunteers once-daily for 6 days has been published (Gorski *et al.*, 2000). On Days 4 and 5, tolbutamide (CYP2C9), caffeine (CYP1A2), dextromethorphan (CYP2D6), and midazolam (CYP3A4) were co-administered. The study showed that administration of IL-10 did not alter CYP1A2, CYP2C9, and CYP2D6 activities, and the CYP3A activity was reduced by only 12% ± 17%. The IL-10 concentrations following dosing of 8 μg/kg are likely to be much higher than those observed in patients with immune disorders (89.5 pg/mL in psoriasis, 58.7 pg/mL in RA and 12.6 pg/mL in systemic lupus erythematosus) (Sobhan *et al.*, 2016; Lacki *et al.*, 1995; Godsell *et al.*, 2016).

There are few reports linking IFN-γ to suppression of CYP metabolism *in vivo* and there are no data following direct dosing of IFN-γ or IFN-γ antagonists. In healthy subjects suffering with an acute viral respiratory infection, IFN-α and IFN-γ concentrations were significantly increased (2.7- and 11.3-fold, respectively), and antipyrine clearance significantly decreased (1.3-fold) compared to controls (Brockmeyer *et al*., 1992). IFN-α and IFN-γ concentrations are also markedly higher in HIV patients with severe disease. When these patients were treated with zidovudine, the IFN-α and IFN-γ concentrations significantly decreased (60 and 59%, respectively) and antipyrine clearance significantly increased (1.2-fold) (Brockmeyer *et al*., 1992, 1998). Decreases in theophylline, antipyrine, caffeine, mephenytoin, debrisoquine, chlorzoxazone, and erythromycin metabolism have been reported following direct administration of INF-α to hepatitis and melanoma patients, as reviewed by Lee *et al*. (2010). Therefore, the CYP suppression observed in respiratory infection and HIV patients may be due to increased IFN-α rather than IFN-γ. Other cytokines such as IL-6 may also be increased in these diseases. Changes in antipyrine clearance in subjects with respiratory infection or in HIV patients treated

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with zidovudine are generally more limited than the changes in CYP substrate clearance reported upon administration of IL-6R antagonists to RA patients (Schmitt *et al*., 2011; Zhuang *et al*., 2015).

There have been several reports of vaccine-drug interactions, which have been attributed to increases in IFN-γ concentrations following vaccination (Pellegrino *et al*., 2015). The data for warfarin (CYP2C9) are conflicting between studies, which may reflect the limited effect of IFN-γ on CYP2C9 *in vitro* (Aitken and Morgan, 2007). Reports for theophylline (CYP1A2) are also conflicting (Pellegrino *et al*., 2015; Jonkman and Upton, 1984), potentially due to inappropriate timing of some studies, whereby the maximum CYP suppression and effect on theophylline PK were missed due to the sparse sampling used. There are limited reports of vaccine interactions with anticonvulsants (e.g. carbamazepine, phenytoin, phenobarbital) showing an increase in anticonvulsant exposure following vaccination, although the extent and duration of the drug-vaccine interaction differs widely between reports (Pellegrino *et al*., 2015). Vaccination also causes a significant transient increase in IL-6 and other cytokine concentrations (Herrin *et al*., 2014; Kuhlman *et al*., 2018; Tsai *et al*., 2005; Sharpley *et al*., 2016; Brydon *et al*., 2008; Harrison *et al*., 2009; Wright *et al*., 2005). Therefore, the role of IFN-γ in drug-vaccine interactions is not clear.

Frye *et al.* (2002) reported that, in congestive heart failure patients given a metabolic probe cocktail consisting of caffeine (CYP1A2), mephenytoin (CYP2C19), dextromethorphan (CYP2D6), and chlorzoxazone (CYP2E1), a significant inverse relationship was found between both TNF-α and IL-6 plasma concentrations and the activity of CYP2C19. Since both TNF-α and IL-6 could contribute to suppression of CYP2C19 activity, the role of TNF-α in a CYP2C19-mediated DDI is inconclusive. In another study, HIV patients had lower CYP3A4 and CYP2D6

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activity when compared to age and sex matched healthy volunteers (18% and 90%, respectively) but no significant difference for CYP1A2 (Jones *et al*., 2010). Higher TNF-α concentrations in HIV patients were significantly correlated with the reduced CYP3A4 activity but not CYP2D6 activity (Jones *et al*., 2010). Raised TNF-α levels in ovarian cancer patients (45.4 pg/mL) were significantly associated with increased CYP2E1 activity (3-fold) and reduced CYP3A4 activity (42%) when compared to healthy volunteers (Trousil *et al*., 2019). However, the changes in enzyme activity were also significantly associated with IL-6 and IL-8 levels. Since IL-8, TNF-α and IL-6 could all contribute to effects on CYP2E1 and CYP3A4 activity, the role of TNF-α in a CYP2E1 or CYP3A4-mediated DDI is inconclusive. In psoriasis patients, TNF-α concentrations were also significantly increased (~ 12 pg/mL) when compared to healthy subjects; however, no correlation was found between raised TNF-α levels and venlafaxine (CYP2D6 and P-gp substrate) metabolic ratios (Godoy *et al*., 2016). Similarly, TNF-α levels in patients infected with COVID-19 or following hip surgery did not correlate with the decreased CYP1A2, CYP2C19 or CYP3A4 activity observed in these patients, which is likely due to the increased IL-6 levels (Lenoir *et al*., 2021a; Lenoir *et al*., 2021b).

A few therapeutic protein-drug interactions relating to TNF-α have also been reported. Etanercept is a fusion protein that blocks TNF-α from binding to its receptor and hence would remove any suppressive effect of raised TNF-α on CYPs in patients. Administration of etanercept to healthy volunteers had no effect on digoxin (P-gp substrate) or warfarin (CYP2C9 substrate) exposure (Zhou *et al*., 2004a; Zhou *et al*., 2004b); however, healthy volunteers would be expected to have low circulating TNF-α levels and hence any potential CYP suppression prior to etanercept administration would be minimal. Wen *et al*. (2020) reported a case study of a patient with ankylosing spondylitis, hypertension, diabetes mellitus and IgA nephropathy who

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was receiving etanercept and cyclosporine. Use of etanercept was correlated with increased cyclosporine (CYP3A4 substrate) clearance; however the authors suggest this was due to the large decrease in circulating IL-2 concentrations following administration of etanercept, rather than a direct effect of TNF-α on CYP3A4 (Wen *et al*., 2020).

In vivo data regarding CYP suppression by VEGF and IL-17 are extremely limited. In one Phase I/II clinical trial, bevacizumab (anti-VEGF mAb) was administered to non-small-cell lung cancer patients with erlotinib (CYP3A4 substrate) and exposure of both drugs was compared to that in patients receiving each drug alone (Herbst *et al*., 2005). No differences in erlotinib PK were found upon co-administration of bevacizumab, suggesting that VEGF does not have a suppressive effect on CYP3A4. In psoriasis patients, IL-17 concentrations were significantly increased (~ 4 pg/mL) when compared to healthy subjects; however, no correlation was found between raised IL-17 levels and venlafaxine (CYP2D6 substrate and P-gp) metabolic ratios (Godoy *et al*., 2016).

Finally, it is worth noting the cytokine modulatory effects of some small molecule drugs e.g. toll-like receptor (TLR)-7 agonists. Jones *et al*. (2012) hypothesized that the time dependent PK observed for PF-04878691 was as a result of CYP suppression caused by TLR-7 agonism causing elevation of cytokine levels. This example illustrates that cytokine-mediated DDIs are not limited to biologics only, as small molecules that alter cytokine levels can also lead to modulation of CYP expression.

PBPK Modeling of Cytokine Suppression of CYP Enzymes

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Data for the *in vitro* suppression of CYP activity or mRNA from Dickmann *et al.* (2011) have been used to successfully describe the clinical consequences of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5 suppression by IL-6 in PBPK models (Machavaram *et al.,* 2013, 2019; Jiang *et al*., 2016; Xu *et al*,. 2015; Stader *et al*., 2021; Lenoir *et al.*, 2021c). The former three models are designed to capture the therapeutic protein-drug interactions in patients who have chronically raised IL-6 levels (Machavaram *et al.,* 2013, 2019; Jiang *et al*., 2016). Even at the highest IL-6 concentration tested (100 pg/mL) by Machavaram *et al*. (2019), only small suppression of CYP2C9 and CYP2D6 (37.7 and 26.8% reduction in enzyme levels, respectively) and weak DDIs with S-warfarin and dextromethorphan (AUC ratios = 1.33 and 1.36, respectively) were predicted. No interaction was predicted for the CYP1A2 substrate caffeine. Suppression of CYP2C19 and CYP3A4 was moderate (46 and 43% reduction in enzyme levels, respectively) and moderate DDIs were predicted with CYP3A4 substrates (AUC ratios = 2.30 and 2.07 for simvastatin and midazolam, respectively). The predicted and observed AUC and peak concentration (C_{max}) ratios in the presence and absence of chronically elevated IL-6 are summarized in Figure 1.

In contrast, the models published in Xu *et al.* (2015), Stader *et al*. (2021) and Lenoir *et al.* (2021) focus on transiently raised IL-6 levels. IL-6 levels increased rapidly to a mean peak of ~ 1600 pg/mL (60,000 pg/mL in the subject with the highest levels) at 6 h post blinatumomab administration and then decreased to baseline by 48 h (Xu *et al*., 2015). The maximum predicted suppression of CYP3A4, CYP1A2 and CYP2C9 was 28, 9 and 17%, occurring at 48, 48 and 70 h post blinatumomab administration, respectively (Xu *et al*., 2015). Predicted CYP3A4 and CYP1A2 levels had returned to baseline by 1 week and CYP2C9 by 9 days post blinatumomab administration (Xu *et al*., 2015). Weak interactions were predicted for CYP3A4 substrates

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simvastatin and midazolam (AUC ratios = 1.9 and 1.7, respectively), whereas no interaction (AUC ratio < 1.25-fold) was predicted for CYP1A2 substrates theophylline and caffeine or CYP2C9 substrate S-warfarin when dosed 48 h after blinatumomab (Xu *et al*., 2015). IL-2, IL-10, IFN-γ and TNF-α were also significantly raised following blinatumomab dosing (peak ~ 170, 2400, 440 and 200 pg/mL, respectively, at the highest blinatumomab dose level). However, these cytokines were not included within the PBPK model due to the lack of *in vivo* data showing a meaningful effect of IL-10 or a direct effect of IL-2, IFN-γ and TNF-α on CYP activity. The predicted AUC and C_{max} ratios in the presence and absence of transiently elevated IL-6 are also summarized in Figure 1.

Similarly, Stader *et al*. (2021) considered the effects of higher IL-6 concentrations (1 – 50,000 pg/mL) observed in COVID-19 patients; however, only a weak interaction with CYP3A4 substrate midazolam (AUC ratio = 1.33) was predicted even at the highest IL-6 concentration. Lenoir *et al*. (2022) recovered the concentrations of omeprazole and 5-OH-omeprazole in subjects before and after hip-surgery by incorporating the combinatory effects of elevated IL-6 (peak ~ 50 pg/mL at 24 h post-surgery) and co-administration of esomeprazole (a mechanism-based inhibitor for CYP2C19). It is likely that reduction in CYP2C19 activity was due mostly to inhibition by esomeprazole and minimally to suppression by IL-6, but this was not confirmed using the model in the paper.

In comparison to the PBPK models described above, a recent publication has used a top down fitting approach with a PBPK model to predict the effect of inflammation on CYP2C19 and CYP3A4 suppression (Simon *et al*., 2021). Instead of modeling IL-6, C-reactive protein (CRP) concentrations were related to CYP2C19 and CYP3A4 activity using an empirical model fitted to clinical data, and the resultant *in vivo* parameters for downregulation of CYP activities

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were integrated into a PBPK model (Simon *et al*., 2021). The recovery of midazolam, voriconazole and omeprazole concentrations in patients with a mean CRP of 25.3 mg/L vs. 0.5 mg/L suggest that the activity of CYP2C19 and CYP3A4 can be predicted using CRP concentration. The production of CRP is stimulated by IL-6, thus they are highly correlated in diseases (Del Giudice and Gangestad, 2018).

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Conclusions and current knowledge gaps

Raised cytokine levels have been linked to suppression of CYP enzymes both *in vitro* and *in vivo* (Evers *et al*., 2013; Huang *et al*., 2010; Lee *et al*., 2010; Harvey and Morgan, 2014; Morgan *et al*., 2008, Gorski *et al.*, 2000; Aitken and Morgan, 2007; Klein *et al.*, 2015; Dallas *et al*., 2012). *In vitro* data suggest that IL-6 is the most potent suppressor of the majority of CYPs and CYP3A4 is the most sensitive CYP enzyme (Tables 1-3). Compared to IL-6, IFN-γ and TNF-α appear to have a reduced or similar suppressive effect, although they may cause greater suppression of CYP1A2. IL-2 and IL-1β CYP suppression is minor and IL-10 does not cause CYP suppression even at high concentrations. Incubation data are lacking for other cytokines. The limited data for incubation of cytokine cocktails with hepatocytes suggest similar extents of CYP suppression for combined cytokines compared to IL-6 alone. *In vivo* data supporting a direct effect of IL-2, IL-8, IL-10, IL-17, IFN-γ, TNF-α or VEGF on CYP activity are inconclusive, and the reported interactions could be driven by increases in a range of cytokines, including IL-6. Although raised levels of IL-6, IFN-γ and/or TNF-α could lead to CYP suppression, the cytokine levels observed in common immune disorders are generally lower than the *in vitro* EC_{50} values (Table 4), suggesting minimal CYP suppression in most patients. However, transiently elevated concentrations following dosing of cytokine modulators (e.g., blinatumomab) may be close to or above EC_{50} values.

By incorporating *in vitro* CYP mRNA E_{min} and EC_{50} values of IL-6 (Dickmann *et al.*, 2011), current PBPK models are able to predict concentrations of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 substrates in the presence of chronically raised IL-6 (Machavaram *et al.,* 2013, 2019; Jiang *et al*., 2016) and concentrations of CYP2C19 and CYP3A4 substrates in the presence of transiently raised IL-6 (Xu *et al*,. 2015; Stader *et al*., 2021; Lenoir *et al.*, 2021c) with

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reasonable performance (i.e., predicted-to-observed ratios of mean AUC or C_{max} < 2; Figure 1). There are no E_{min} and EC_{50} values reported for most other cytokines, hindering their inclusion in PBPK models. Here we have calculated CYP1A2 values for IFN-γ from reported *in vitro* hepatocyte data. E_{min} and EC_{50} values of TNF-α for CYP1A2, CYP2B6, CYP2C9, and CYP3A4 mRNA were also estimated, using data measured in HepaRG cells (Klein *et al*., 2015) but should be confirmed in human hepatocytes in future experiments.

When evaluating DBI liability, one should consider two opposite directions for the interaction. Cytokine levels can decrease due to down-regulation of the activity of proinflammatory cytokines (e.g, RA treatment), or contrarily, transiently increase due to rapid release of cytokines into the blood from immune cells (e.g., cytokine release syndrome). The former case is similar to PBPK simulation scenarios used in Machavaram *et al.* (2013 and 2019) and Jiang *et al*. (2016), whereas the latter case would be analogous to PBPK simulation scenarios in Xu *et al*. (2015) and Stader *et al*. (2021). In both cases, the risk of DBIs is generally reported to be moderate or weak in most patients (Coutant & Hall 2018) and can likely be predicted with PBPK models considering IL-6 alone, given that other cytokines are less potent CYP suppressors and circulating at concentrations much lower in diseases than those used in most *in vitro* incubations. While cytokines can markedly increase after cytokine modulator dosing, significant DBI is still unlikely as the elevation is transient and enzyme levels return to baseline quickly as demonstrated in the PBPK simulations. Despite the low risks, caution needs to be taken in DBI assessment for drugs with a narrow therapeutic index/low safety margins.

In conclusion, following dosing of cytokine modulating drugs the levels of multiple cytokines are likely to be increased or decreased simultaneously. The *in vitro* and *in vivo* data suggest that IL-6 is the most important cytokine when considering the effect of cytokine

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modulators on small molecule drug PK, although the available data are very limited in some cases. Published PBPK models assessing the effect of IL-6 on small molecule PK can adequately predict DBIs in a range of disease states. Hence, it is likely that inclusion of other cytokines into such PBPK models is not warranted and that any DBI interactions will generally be weak.

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Authorship contributions

Participated in research design: Chen, K-F, Gill, K.L., and Jones, H.M.

Conducted experiments: Chen, K-F and Gill, K.L.

Contributed new reagents or analytic tools: Chen, K-F and Gill, K.L.

Performed data analysis: Chen, K-F and Gill, K.L.

Wrote or contributed to the writing of the manuscript: Chen, K-F, Gill, K.L., and Jones, H.M.

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Figure Legends

Figure 1. Summary of observed and simulated effects of chronic and transient IL-6 elevation using PBPK modeling. Data extracted from Machavaram et al. (2019) (steady-state IL-6 = 50 or 100 pg/mL), Jiang et al. (2016) (steady-state IL-6 = 50 pg/mL), Xu et al. (2015) (peak IL-6 = 1600 pg/mL) and Stader et al. (2021) (peak IL-6 = 50000 pg/mL). Black and blue bars represent predicted and observed data, respectively. White, yellow, pink and red areas represent insignificant, weak, moderate and strong interaction, respectively. The observed data included in the Machavaram and Jiang papers were from the same sources (Schmitt et al., 2011, Zhang et al., 2009 and Zhuang et al., 2015).

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Tables

Table 1. CYP mRNA or activity percent decrease measured in cryopreserved human hepatocytes incubated with IL-2 or IL-6.

	% decrease by IL-2	% decrease by IL-6	% decrease by IL-2 / % decrease by IL-6 [a]
CYP2B6 activity	21	30	0.70
CYP2C19 activity	22	65	0.34
CYP2D6 activity	22	39	0.56
CYP2D6 mRNA	1.5	2.4	0.63

Data extracted from Dallas et al., 2012; n = 3 donors, treated with 10 ng/mL IL-2 or IL-6 for 48 h. [a] *The ratios compare the extent of suppression by IL-2 relative to IL-6.*

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Table 2. CYP mRNA, protein expression or activity percent decrease measured in primary human hepatocytes treated with IFN-γ or IL-6.

Measure	% decrease by IFN-γ	% decrease by IL-6	% decrease by INF-γ / % decrease by IL-6 [a]	Study
EROD activity (mainly CYP1A2)	40	28	1.44	Abdel-Razzak et al., 1993
	35	10	3.41	Abdel-Razzak et al., 1994
CYP1A2 activity	53	36	1.47	Guillén et al., 1998
CYP1A2 mRNA	18	24	0.75	Abdel-Razzak et al., 1993
CYP2C8 mRNA	48	54	0.89	Aitken and Morgan, 2007
CYP2C9 mRNA	NS	34	-	Aitken and Morgan, 2007
CYP2C9 protein	67	88	0.76	Aitken and Morgan, 2007
CYP2C18 mRNA	NS	26	-	Aitken and Morgan, 2007
CYP2C19 mRNA	NS	37	-	Aitken and Morgan, 2007
CYP2B6 activity	48	47	1.03	Guillén et al., 1998
CYP2B6 mRNA	74	78	0.95	Aitken and Morgan, 2007
CYP2B6 protein	40	62	0.65	Aitken and Morgan, 2007
CYP3A4 activity	34	19	1.80	Guillén et al., 1998
	38	38	1.00	Abdel-Razzak et al., 1993
CYP3A4 mRNA	77	95	0.80	Aitken and Morgan, 2007
CYP3A mRNA	NS	53	-	Abdel-Razzak et al., 1993
CYP3A4 protein	54	45	1.19	Aitken and Morgan, 2007
CYP2E1 mRNA	39	53	0.74	Abdel-Razzak et al., 1993

n = 1 - 11 donor. In Adbel-Razzak et al. (1993) and Adbel-Razzak et al. (1994), primary human hepatocytes were treated with 50 U/mL IFN-γ or 50 U/mL IL-6 for 72 h. In Guillén et al. (1998), primary human hepatocytes were treated with 300 U/mL IFN-γ or 100 U/mL IL-6 for 24 h. In Aitken and Morgan (2007), primary human hepatocytes were treated with 10 ng/mL IFN-γ or 10 ng/mL IL-6 for 24 h. NS = no significant change. [a] The ratios compare the extent of suppression by INF-γ relative to IL-6.

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Table 3. CYP mRNA, protein expression or activity percent decrease measured in primary human hepatocyte cultures treated with TNF-α or IL-6.

Measure	% decrease by TNF-α	% decrease by IL-6	% decrease by TNF-α / % decrease by IL-6 [a]	Study
CYP1A2 activity	72	23	3.13	Dallas *et al.*, 2012
CYP1A2 activity	57	28	2.04	Abdel-Razzak *et al.*, 1993
CYP1A2 mRNA	73	24	3.09	Abdel-Razzak *et al.*, 1993
CYP1A2 mRNA	45	15	3.00	Dallas *et al.*, 2012
CYP2B6 activity	35	30	1.17	Dallas *et al.*, 2012
CYP2B6 mRNA	NS	78	-	Aitken & Morgan, 2007
CYP2B6 mRNA	NS	63	-	Dallas *et al.*, 2012
CYP2B6 protein	59	62	0.95	Aitken & Morgan, 2007
CYP2C8 mRNA	64	54	1.17	Aitken & Morgan, 2007
CYP2C9 activity	16	35	0.47	Dallas *et al.*, 2012
CYP2C9 mRNA	NS	34	-	Aitken & Morgan, 2007
CYP2C9 mRNA	NS	63	-	Dallas *et al.*, 2012
CYP2C9 protein	94	88	1.07	Aitken & Morgan, 2007
CYP2C19 activity	84	65	1.29	Dallas *et al.*, 2012
CYP2C19 mRNA	NS	37	-	Aitken & Morgan, 2007
CYP2C19 mRNA	NS	72	-	Dallas *et al.*, 2012
CYP2D6 activity	45	39	1.15	Dallas *et al.*, 2012
CYP2D6 mRNA	46	-240	opposite direction	Dallas *et al.*, 2012
CYP2E1 mRNA	44	52	0.84	Abdel-Razzak *et al.*, 1993
CYP3A4 activity	70	76	0.92	Dallas *et al.*, 2012
CYP3A4 activity	60	38	1.58	Abdel-Razzak *et al.*, 1993
CYP3A4 activity	NS	69	-	Mimura *et al.*, 2015
CYP3A4 mRNA	58	53	1.10	Abdel-Razzak *et al.*, 1993
CYP3A4 mRNA	81	95	0.85	Aitken & Morgan, 2007
CYP3A4 mRNA	85	98	0.87	Dallas *et al.*, 2012
CYP3A4 mRNA	61	78	0.78	Mimura *et al.*, 2015
CYP3A4 protein	NS	45	-	Aitken & Morgan, 2007

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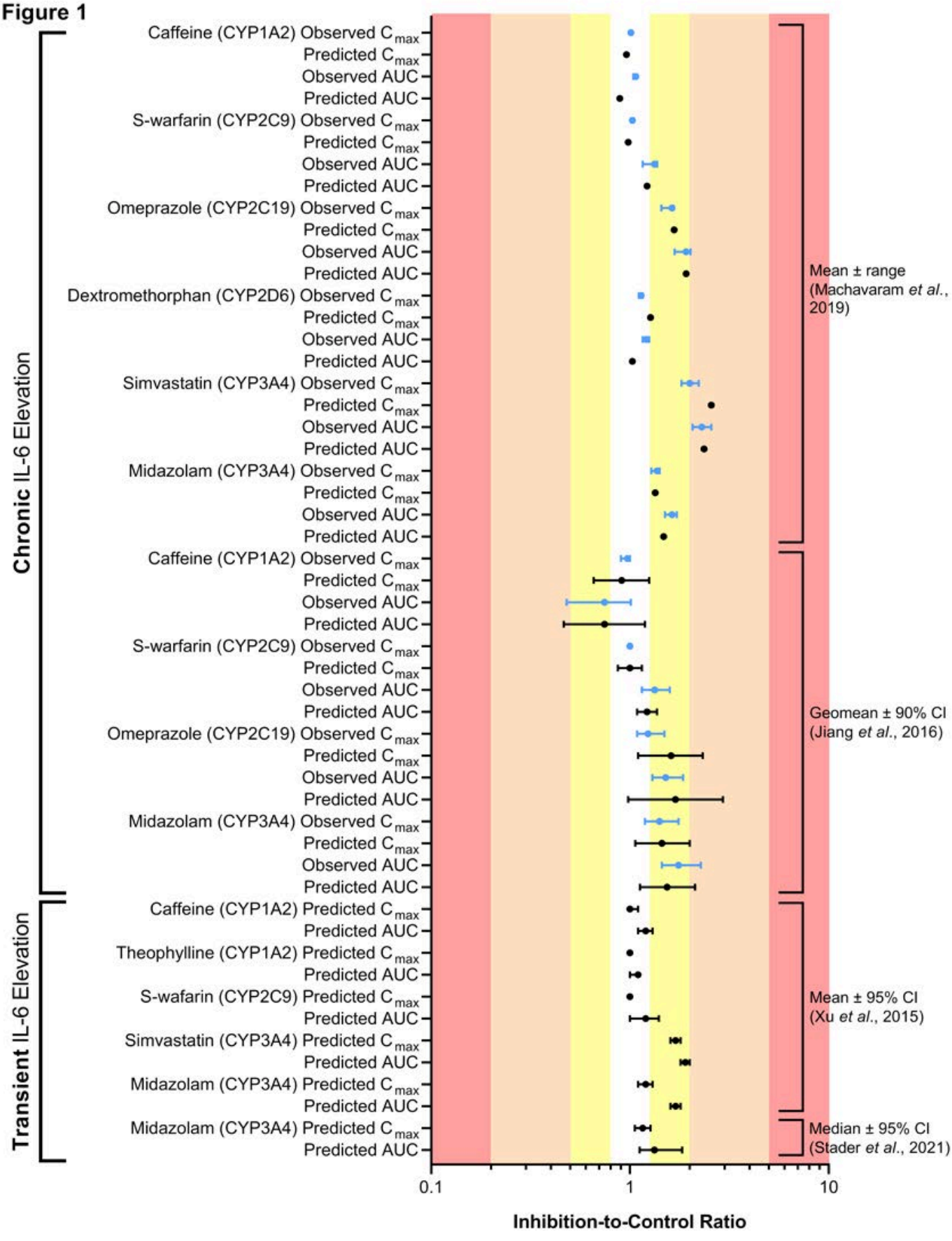
n = 1 - 9 donors. In Dallas et al. (2012), cryopreserved human hepatocytes were treated with 10 ng/mL TNF-α or 10 ng/mL IL-6 for 48 h. In Abdel-Razzak et al. (1993), primary human hepatocytes were treated with 50 U/mL TNF-α or 50 U/mL IL-6 for 72 h. In Aitken and Morgan (2007), primary human hepatocytes were treated with 10 ng/mL TNF-α or 10 ng/mL IL-6 for 24 h. In Mimura et al. (2015), primary human hepatocytes were treated with 10 ng/mL TNF-α or 10 ng/mL IL-6 for 48 h. NS = no significant change. [a] The ratios compare the extent of suppression by TNF-α relative to IL-6.

Table 4. Calculated and reported E_{min} and EC_{50} values of IL-6, IFN- γ and TNF-α for different CYP isoforms

Measure	System	Cytokine	Incubation concentration (pg/mL)	Incubation time (h)	E_{min} (fold)	EC_{50} (pg/mL)	$E_{min} \times EC_{50}$	Study
CYP1A2 mRNA	Human hepatocyte	IL-6	5 - 50000	72	0.157	271	42.5	Dickman *et al.*, 2011
CYP1A2 mRNA	HepaRG cells	TNF-α	0.1 – 50000	24	0.0337	817	27.5	Klein *et al.*, 2015
CYP2B6 mRNA	Human hepatocyte	IL-6	5 - 50000	72	0.0311	70	2.18	Dickman *et al.*, 2011
CYP2B6 mRNA	HepaRG cells	TNF-α	0.1 – 50000	24	0.639	86.6	55.3	Klein *et al.*, 2015
CYP2C19 mRNA	Human hepatocyte	IL-6	5 - 50000	72	0.214	71.3	15.3	Dickman *et al.*, 2011
CYP2C8 mRNA	Human hepatocyte	IL-6	5 - 50000	72	0.0386	153	5.91	Dickman *et al.*, 2011
CYP2C9 mRNA	Human hepatocyte	IL-6	5 - 50000	72	0.0525	121	6.35	Dickman *et al.*, 2011
CYP2C9 mRNA	HepaRG cells	TNF-α	0.1 – 50000	24	0.289	153	44.3	Klein *et al.*, 2015
CYP2D6 mRNA	Human hepatocyte	IL-6	5 - 50000	72	0.302	151	45.6	Dickman *et al.*, 2011
CYP3A4 mRNA	Human hepatocyte	IL-6	5 - 50000	72	0.00	3.23	0.00	Dickman *et al.*, 2011
CYP3A4 mRNA	HepaRG cells	TNF-α	0.1 – 50000	24	0.107	133	14.2	Klein *et al.*, 2015
CYP3A5 mRNA	Human hepatocyte	IL-6	5 - 50000	72	0.0343	51	1.75	Dickman *et al.*, 2011
CYP1A2 activity	Human hepatocyte	IL-6	5 - 50000	72	0.241	73.2	17.6	Dickman *et al.*, 2011
CYP1A2 activity	Human hepatocyte	IFN-γ	500 - 150000	24	0.629	2460	1547	Donato *et al.*, 1993
CYP1A2 activity	Human hepatocyte	IFN-γ	1500 - 50000	24	0.572	4400	2517	Donato *et al.*, 1997
CYP1A2 activity	Human hepatocyte	IFN-γ	2500 - 50000	24	0.601	4098	2463	Guillén *et al.*, 1998
CYP3A4 activity	Human hepatocyte	IL-6	5 - 50000	72	0.23	1251	288	Dickman *et al.*, 2011

IFN-γ and TNF-α data were digitalized from the figures in the publications; subsequently, E_{min} and EC_{50} values were estimated using a 4-parameter fit.

Figure 1


Chronic IL-6 Elevation
Caffeine (CYP1A2) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
S-warfarin (CYP2C9) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
Omeprazole (CYP2C19) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
Dextromethorphan (CYP2D6) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
Simvastatin (CYP3A4) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
Midazolam (CYP3A4) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
Caffeine (CYP1A2) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
S-warfarin (CYP2C9) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
Omeprazole (CYP2C19) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
Midazolam (CYP3A4) Observed C_{max}
Predicted C_{max}
Observed AUC
Predicted AUC
Transient IL-6 Elevation
Caffeine (CYP1A2) Predicted C_{max}
Predicted AUC
Theophylline (CYP1A2) Predicted C_{max}
Predicted AUC
S-wafarin (CYP2C9) Predicted C_{max}
Predicted AUC
Simvastatin (CYP3A4) Predicted C_{max}
Predicted AUC
Midazolam (CYP3A4) Predicted C_{max}
Predicted AUC
Midazolam (CYP3A4) Predicted C_{max}
Predicted AUC
Mean ± range (Machavaram et al., 2019)
Geomean ± 90% CI (Jiang et al., 2016)
Mean ± 95% CI (Xu et al., 2015)
Median ± 95% CI (Stader et al., 2021)
0.1
1
10
Inhibition-to-Control Ratio