Assessing trends in cytokine-CYP drug interactions and relevance to drug dosing

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Running Title: Trends in cytokine-CYP drug interactions and clinical relevance

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Text pages: 14
Number of tables: 1
Number of Figures: 3
Number of References: 56

Abstract: 250
Introduction: 688
Discussion: 1971

Abbreviations: 1C, one compartment; ARDS, Acute respiratory distress syndrome; AUC_{ss}, area under the curve at steady state; BW, Body weight; C_{avg}, Average concentration achieved in plasma; CD4+ Th1, complement-dependent T-helper cells subtype 1; CD4+ Th2, complement dependent T-helper cells subtype 2; CL, clearance; COVID-19, Coronavirus Disease of 2019; CRP, C-reactive protein; CSG, colony stimulating growth factor; CYP, cytochrome P450; CYP3A, Cytochrome P450 family 3 subfamily A; CYP1A2, Cytochrome P450 subfamily 1A2; CYP2C19, Cytochrome P450 subfamily 2C19; CYP2D6, Cytochrome P450 subfamily 2D6; FAAH, fatty acid amide hydrolase; FDA, Food and Drug administration; FIH, first-in-human; IL, interleukin; IL-x, inter-leukin subtype X; IQ, International consortium of quality and innovation; IV, intravenous; mAb, monoclonal antibody; mRNA, messenger ribonucleic acid; PBMC, peripheral blood mononuclear cells; PK, Pharmacokinetics; SC,
subcutaneous; TGF, tumor growth factor; TNF, tumor necrosis factor; UW DIDB, University of Washington Drug Interaction Database; V, volume of distribution.
ABSTRACT

The regulation of drug-metabolizing enzymes and transporters by cytokines has been extensively studied, in vitro and in clinic. Cytokine-mediated suppression of CYPs or drug transporters may increase or decrease the systemic clearance of drug substrates that are primarily cleared via these pathways; neutralization of cytokines by therapeutic proteins may thereby alter systemic exposures of such drug substrates. The FDA recommends evaluating such clinical drug interactions during clinical development and has provided labeling recommendations for therapeutic proteins. To determine the clinical relevance of these drug interactions to dose adjustments, trends in steady-state exposures (AUC\textsubscript{ss}) of CYP-sensitive substrates co-administered with cytokine modulators as reported in the UW DIDB were extracted and examined for each of the CYPs. Co-administration of CYP3A (midazolam/simvastatin), CYP2C19 (omeprazole), or CYP1A2 (caffeine/tizanidine) substrates with anti-IL-6 and with anti-IL-23 therapeutics led to changes in systemic exposures of CYP substrates ranging from ~ -58% to ~35%; no significant trends were observed for CYP2D6 (dextromethorphan) and CYP2C9 (warfarin) substrates. Although none of these changes in systemic exposures have been reported as clinically meaningful, dose adjustment of midazolam for optimal sedation in acute care settings has been reported. Simulated concentration-time profiles of midazolam under conditions of elevated cytokine levels when co-administered with tocilizumab, suggest a ~6-7 fold increase in midazolam clearance suggesting potential implications of cytokine-CYP drug interactions on dose adjustments of sensitive CYP3A substrates in acute care settings. Additionally, this article also provides a brief overview of non-clinical and clinical assessments of cytokine-CYP drug interactions, in drug discovery and development.

Significance statement: There has been significant progress in understanding cytokine-mediated drug interactions for CYP-sensitive substrates. This article provides an overview of
the progress in this field, including a trend analysis of systemic exposures of CYP-sensitive substrates co-administered with anti-IL-x therapeutics. In addition, the review also provides a perspective of current methods used to assess these drug interactions during drug development, and a focus on individualized medicine, particularly in acute care settings.
INTRODUCTION

Cytokines are a group of glycoproteins predominantly produced by T-cells, macrophages, and B-cells. In some instances, mast cells, fibroblasts, and endothelial cells may also produce cytokines. These are key mediators of inflammation and may be triggered by pathogens, cancers, autoimmune conditions, or in some cases drug therapies. Cytokines have been classified into tumor necrosis factors (TNFs), interleukins (ILs), lymphokines, interferons (IFNs), colony-stimulating factors (CSFs), and transforming growth factors (TGFs) and are numbered based on the cell type producing them: Type 1 cytokine produced by CD4+ T<sub>h</sub>1 (e.g. IL-2, IL-12, IL-23, IFN-γ, TNF-α and TNF-β), Type 2 cytokine produced by CD4+ T<sub>h</sub>2 (e.g. IL-4, IL-5, IL-6, IL-10, and IL-13), IL-9 and IL-10 produced by T<sub>h</sub>9 and IL-17 subtypes produced by T<sub>h</sub>17. Cytokines may be pro-inflammatory (IL-6, IL-12, IL-17, IL-23, TNF-α, IFN-γ etc.) or anti-inflammatory (e.g. IL-1, IL-9, IL-10) (Tang et al., 2012; Sallusto, 2016; Fajgenbaum and June, 2020; Liu et al., 2021). Endogenous cytokine release has been reported under several disease conditions including a broad range of inflammatory disorders (Megha et al., 2021), infections (Kim et al., 2021; Tang et al., 2021), cancer (Grivennikov et al., 2010), and organ impairment (Albillos et al., 2014; Chen et al., 2022). Exogenous cytokine release caused by administration of novel investigational drugs gained significant attention after healthy volunteers (N=6) dosed with CD28 superagonist TGN1412, resulted in a systemic inflammatory response and elevation of pro-inflammatory cytokines accompanied by clinical manifestations and hospitalization (Suntharalingam et al., 2006). A fatty acid amide hydroxylase (FAAH) inhibitor, BIA10-2474 caused a similar cadence of events during FIH/dose escalation studies that led to neurological events and the death of one subject on the trial (Rocha et al., 2022). Under disease conditions, the production of pro-inflammatory cytokines may result in persistent inflammation and tissue damage (Floege et al., 2012). In addition, uncontrolled release of pro-inflammatory cytokines can be life-threatening
(Fajgenbaum and June, 2020) and can cause organ infiltration followed by tissue damage, multi-organ failure, and eventually sepsis. To minimize the likelihood of such events, cytokine release is often monitored during early stages of development of immunomodulators (Frey and Porter, 2019; Cosenza et al., 2021). Apart from safety concerns associated with cytokine release, regulation of drug-metabolizing enzymes and transporters has received significant attention based on the seminal work conducted by (Morgan, 1993); early reports by the authors have shown suppression of multiple CYP mRNAs in female rats treated with endotoxins. This initial work led to an extensive study of the mechanisms underlying CYP regulation and clinical impact on molecules that were primarily cleared by CYPs, particularly under conditions of inflammation and disease (Morgan, 1997b). During these years, rapid advances have been made in the development of immunotherapeutics to treat inflammation and cancer (McCune, 2018). This has led to an increased interest in understanding the clinical relevance of cytokine-mediated drug interactions and their implications on the safety and efficacy of CYP substrates. During the COVID-19 pandemic, these drug-disease interactions, and their impact on PK of COVID-19 therapies gained significant attention. This was especially important in critical care settings where patients may be at a risk for developing a cytokine storm, and such interactions could impact the effectiveness or safety of concomitantly administered therapeutics (Deb and Arrighi, 2021; Pilla Reddy et al., 2023).

This current mini-review summarizes the historical perspectives from the work in this field conducted by Morgan and colleagues and advances made by consortia and working groups that have culminated into guidances for industry and sponsors. In addition, a trend analysis conducted using the UW DIDB, provides a snapshot of the changes in systemic exposures because of such interactions, reported to date. Clinical relevance of the observed trends has been contextualized to critical care settings using recent case reports, to assess the potential impact of such drug interactions on dose adjustments of CYP substrates. Finally, we discuss
the potential drug interactions that may occur when CYP substrates may be co-administered alongside cell therapies that may carry a risk of cytokine release. In addition, authors provide insights into how cytokine-mediated PK drug interactions can be assessed during drug development, using an integrated approach of clinical studies and model-informed approaches.

**HISTORICAL PERSPECTIVES**

In human hepatocytes, pro-inflammatory cytokines including interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-α), and Interferon gamma (IFN-γ) have been found to downregulate CYP3A, CYP2C, and CYP2B6 mRNA levels (Morgan, 1997a; Aitken and Morgan, 2007); similarly, TGFβ has been found to downregulate CYP2C and CYP2B6 while no effect of cytokines has been reported on the mRNA or protein levels of CYP2D6. At a transcriptional level, this regulation occurs via nuclear and hormone receptors (Wu and Lin, 2019). The clinical relevance of this phenomenon on PK of CYP sensitive substrates has been discussed (Reiss and Piscitelli, 1998; Stipp and Acco, 2021) previously. Changes in systemic exposures under different levels of inflammation have been illustrated by (Harvey and Morgan, 2014), using computational approaches. These simulations predicted an increase in systemic exposures of CYP substrates under disease conditions, with a further increase in exposures following treatment with immunomodulators when compared to healthy individuals. Additionally, these simulations predicted a subsequent reduction in exposure of CYP substrates due to neutralized or reduced levels of pro-inflammatory cytokines. These *in silico* predictions were eventually validated in the clinic by interactions reported between tocilizumab, an anti-IL-6 mAb, and simvastatin, a sensitive CYP3A substrate (Schmitt et al., 2011). Similarly, (Coutant and Hall, 2018) have extensively reviewed the disease states that have led to non-stationary kinetics of CYP-sensitive substrates under disease conditions. With significant progress made in immunotherapies and immune checkpoint inhibitors, much
attention has been drawn towards the release or neutralization of cytokines during such drug therapies and its clinical relevance on PK and pharmacodynamics of CYP substrates due to changing inflammatory tone. During drug development, such complex interactions may also impact the design of combination regimens, where an immunomodulator may be combined with a CYP substrate to maximize efficacy or minimize safety concerns of the combination partners, or both. To assess the likelihood of such interactions recommendations for best practices are in place (Kraynov et al., 2011; Evers et al., 2013; Yu et al., 2023). In 2013, IQ and FDA developed a risk assessment to determine if a dedicated clinical drug interaction study was needed for investigational therapeutic proteins, during drug development (Kenny et al., 2013b). FDA released their first draft guidance on considerations for conducting clinical DDI studies to assess therapeutic protein-drug interactions and labeling recommendations (Huang et al., 2010; Kenny et al., 2013a). Over the years, through clinical trial observations or using population-based approaches an understanding of cytokine-mediated PK DDIs has evolved (Machavaram et al., 2013; Khatri et al., 2019; Sathe et al., 2021).

**RECENT ADVANCES**

Most recently, an IQ white paper has summarized various mechanisms of therapeutic protein-drug interactions of clinical concern and has suggested that clinical drug interaction studies are not required for all therapeutic proteins or in patient populations with low inflammatory burden (Coutant et al., 2023). In the current landscape of drug development, cell therapies such as bi-specifics and Chimeric Antigen Receptor T-cell (CAR-T) therapies are emerging. These therapies have the potential to release pro-inflammatory cytokines, the levels of which may vary depending on disease state, especially in critical care settings. Predicting the changes in the PK and thereby the optimal dose of concomitant medications or supportive therapies that are sensitive CYP substrates (e.g.: corticosteroids, pain agents, sedatives etc.) could be challenging under such settings.
To investigate the trends in cytokine-mediated CYP drug interactions, we queried the UW DIDB for sensitive substrates (FDA, 2020) of CYP3A (simvastatin, midazolam), CYP2D6 (dextromethorphan), CYP2C19 (omeprazole), CYP2C9 (warfarin), and CYP1A2 (caffeine, tizanidine) when co-administered with broad class of immunomodulators. The sensitive CYP substrates were queried as “objects” and the therapeutic classes of “Anti-Inflammatory” and “Immunomodulators” were queried as “precipitants”, “non-precipitants”, and “Other” mechanisms within the UW DIDB (Figure 1). All “precipitants”, “non-precipitants”, and “Other” therapeutic drug classes that were non-cytokine modulators were filtered out. The remaining pairs were merged. The % change in AUC (Figure 2) for each of the pairs was plotted, by CYP substrates. Overall, % change in AUC ranged from approximately -58% to up to 35%. Minimal changes were observed for CY1A2 substrates with the largest observed % change in AUC of caffeine (Figure 2, Panel 2A) in the presence of the anti-IL-23 antibody, risankizumab (Aitken and Morgan, 2007). Similarly, co-administration of omeprazole, 20 mg, single dose with sirukumab, 300 mg, single dose (Figure 2, Panel 2B) resulted in up to 48% decrease in AUC of omeprazole (Zhuang et al., 2015). As observed in Figure 2, Panel 2C co-administration of anti-IL-6 mAbs (tocilizumab, sirukumab, sarilumab), anti-IL-17A mAb (brodalumumab), and anti-IL-23 mAb (risakizumab) with sensitive CYP substrate/s resulted in significant changes in AUC of these substrates; highest magnitude of % change in AUC was reported for simvastatin, 40 mg, single dose (decrease in AUC by 58%) when co-administered with tocilizumab, 800 mg, IV (Schmitt et al., 2011); in contrast, the anti-IL-17A antibody broadalumab, 210 mg, single dose, SC, increased the AUC of midazolam, 2 mg, administered orally, by ~30%. This small magnitude of change in systemic exposures of midazolam with broadalumab is not anticipated to cause sedation in these participants; interestingly its directionally opposite to the in vitro observations (Aitken and Morgan, 2007), and is attributed to increased serum IL-17A levels due to administration of
broadalumab (Roman and Chiu, 2017). There are no known drug interactions of cytokine modulators with CYP2D6 sensitive substrates in the UW DIDB, which is consistent with in vitro assessments made by Morgan and colleagues. Based on the collective analysis, clinically meaningful changes in systemic exposures of sensitive CYP substrates in the presence of cytokine modulators appear to be of low incidence; no exposure-response or significant changes to the overall safety and efficacy of these CYP substrates have been reported, to date.

While the current analysis suggests no clinically meaningful drug interactions of cytokines with sensitive CYP substrates, recent case reports suggest that potential interactions and implications to dose adjustments of sensitive CYP substrates cannot be ruled out. (Mefford et al., 2022) have recently reported that five critically ill COVID-19 patients with acute respiratory distress syndrome (ARDS) required higher doses of midazolam, IV to maintain sedation goals. These patients were administered tocilizumab 800 mg, SC (1-2 doses administered over 3-15 days) to manage cytokine release. The optimal sedation dose range of midazolam, IV in healthy volunteers is 0.02-0.1 mg/kg/hr (Barr et al., 2013). However, in this case study patients required a dose of 0.15-0.68 mg/kg/hr of midazolam, IV for optimal sedation. Assuming that the higher dose of midazolam required for optimal sedation was due to increased systemic clearance of midazolam resulting from restored CYP3A activity due to neutralization of IL-6 by tocilizumab, we investigated the magnitude of drug interaction using simulation approach. To assess this drug interaction between tocilizumab and midazolam under conditions of cytokine release, we simulated concentration-time profiles of midazolam, IV at varying systemic clearance and at fixed volume of distribution (USPI). Changes in midazolam clearance in presence of tocilizumab, 800 mg, SC, were assessed as summarized in Figure 3 and Table 1, using midazolam, IV 0.1 mg/kg/h PK parameters (USPI) in healthy, adult populations (BW= 65 kg, 1C PK model, CL= 0.49 L/kg/h; V=1.44 L). Assuming no
change in volume of distribution, the 1C PK model predicted an increase in systemic clearance of midazolam to account for the dose of 0.68 mg/kg/hr, ($C_{avg}$ of ~204 ng/mL) that was required, to achieve optimal sedation in these patients. Overall, these simulations suggested a ~6-7 fold increase in systemic clearance of midazolam to explain the ~6-fold dose adjustment reported in these patients to achieve sedation (Table 1). Similarly, Gunes and colleagues (Güneş et al., 2020), have recently reported potential for drug interaction between warfarin and tocilizumab, with no changes in coagulation parameters or any increases in bleeding risks. Neutralization of IL-6 by tocilizumab may restore CYP2C9 levels, increasing the systemic clearance of warfarin. Such interactions may be consequential to dosing decisions for warfarin due to the narrow therapeutic range required for maintenance of the international normalized ratio (INR). As no pharmacokinetic data on warfarin was available in this case report, authors are unable to assess the magnitude of drug interaction.

PERSPECTIVES ON FUTURE DIRECTIONS AND KEY CHALLENGES

As there is emerging interest in the development of cell therapies like bispecifics and CAR-Ts which have the potential to release cytokines during drug therapy (Supplemental Table S1), cytokine-mediated drug interactions and their impact on safety, efficacy, and dose adjustment of concomitantly administered sensitive CYP substrates will continue to be of interest, especially in critical care settings. Currently, in vitro assays in whole blood or peripheral blood mononuclear cells (PBMC) provide a limited, qualitative assessment of an investigational agent to release cytokines (Finco et al., 2014); for cell therapies, prior to start of FIH studies, assessment of cytokine release is conducted in non-human primates, as they closely mimic the human innate immune system (Taraseviciute et al., 2016; Blincyto, 2020). The initial indication of cytokine release in these non-clinical models could be used as a guide to generate a preliminary assessment of cytokine-mediated drug interactions, using human hepatocyte models that have been previously developed (Aitken and Morgan, 2007).
However, these in vitro and non-clinical models are not designed to quantitatively predict clinical outcomes or to advise dose adjustments in the clinic. Therefore, this non-clinical assessment of cytokine-CYP drug interactions requires exploration during early clinical development. During FIH dose escalation studies of immunomodulators and cell therapies, the time of onset, magnitude, and duration of cytokine release can be measured; doses may be optimized using step-up dosing (Hosseini et al., 2020) as exemplified by mosentuzumab (Budde et al., 2022) and glofitamab (Carlo-Stella et al., 2021). Cytokine release following these therapeutic modalities is often transient, occurring during the first 24-72 hours post-first dose of the drug; thereby it is important to collect the duration and magnitude of cytokine release for purposes of subsequent in vitro or computational assessments. Once an optimal dose or dose range of the therapeutic modality has been identified, all available information on the dose range/exposures, and cytokine release profile (magnitude and duration) over the intended dose range should be collected. Using this information, the cytokine mediated drug interaction with concomitantly administered standard-of-care supportive therapies that may be sensitive CYP substrates could be evaluated using PBPK approach (ex: mosentuzumab) (Chen et al., 2023). This may enable the prediction of cytokine-mediated drug interactions with CYP substrates/inhibitors/inducers typically administered in intended patient population, especially during later stages of drug development. The recently updated FDA guidance provides a risk-based approach to assess the need for clinical drug-drug interaction studies of therapeutic proteins under investigation (FDA, 2023). While population-based variability associated with cytokine release may not allow for an accurate prediction of cytokine-mediated drug interactions, recently such approaches have been used successfully to design clinical DDI studies (Xu et al., 2015; Jiang et al., 2016; Sathe et al., 2021) and could aid in labeling recommendations. Most recently a systems-based approach has been described for midazolam, where a population-based PK model of midazolam in critically ill patients
(neonates, infants, children, and adults), has been developed using baseline C-reactive protein (CRP) levels and using organ failure as covariates on systemic clearance (Vet et al., 2016; Brussee et al., 2018). In acute care settings, a similar systems-based approach could be used to evaluate dose adjustments of CYP substrates and minimize any impact on efficacy and safety, towards personalized dosing (Mefford et al., 2022).

In conclusion, this analysis suggests that the overall incidences of PK drug interactions under clinical conditions of cytokine release are relatively low; while the magnitude of anti-IL-6 and anti-IL-23 with CYP3A and CYP2C19 appears to be ~40-60% (decrease in AUC of sensitive CYP substrates), the clinical relevance to overall safety, efficacy, and dose adjustments appears low. In clinical settings where high levels of IL-6 or IL-23 or high interindividual variability in these cytokines may be prevalent, leveraging model-informed approaches to support a precision dosing approach may be required for optimal dosing.

ACKNOWLEDGEMENTS

The authors would like to sincerely thank Eddie Morgan for his expertise and discussions during the development of the work.

FUNDING

This work received no external funding.

CONFLICT OF INTEREST

No author has an actual or perceived conflict of interest with the contents of this article.

DATA AVAILABILITY STATEMENT

The data extracted for this study are openly available in the University of Washington Drug Interaction Database or UW DIDB. All other data presented are contained within the manuscript/supplemental data.
AUTHOR CONTRIBUTIONS

Participated in research design: Sawant-Basak A., Olabode D.

Conducted experiments and performed data analysis: Sawant-Basak A., Olabode D.

Wrote or contributed to the writing of the manuscript: Sawant-Basak A., Olabode D., Dai D., Vishwanathan K., Phipps, A.
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Blincyto (2020) Drugs@FDA.


FIGURE LEGENDS

**Figure 1.** Flow diagram to extract PK drug interaction dataset of sensitive CYP substrates co-administered with cytokine modulators, using the UW DIDB.

**Figure 2:** %Change in AUC of substrates of CYP1A2 (caffeine, CAFF) in Panel 2A, CYP2C19 (omeprazole, OMP) in Panel 2B, and CYP3A (simvastatin, SIMVA or midazolam, MDZ) in Panel 2C, when co-administered with cytokine modulators.

**Figure 3:** Simulations of concentration-time profile of midazolam, IV infusion with or without tocilizumab, to predict systemic clearance of midazolam at different dose levels. MDZ (IV CL, 0.1 mg/kg/hr, red): predicted concentration-time profile of midazolam at 0.1 mg/kg/hr using 1C PK model, and IV CL of 0.49 L/kg/hr; MDZ (IV CL, 0.68 mg/kg/hr, blue): predicted concentration-time profile of midazolam at 0.68 mg/kg/hr using 1C PK model, and IV CL of 0.49 L/kg/hr; TOCI+MDZ (IV CLadj, 0.68 mg/kg/hr, orange): predicted concentration-time profile of midazolam at 0.68 mg/kg/hr, by simulating its systemic clearance to achieve exposures comparable to those at 0.1 mg/kg/hr; a 6.8-x higher systemic clearance of midazolam was required to achieve this exposure equivalence.
**TABLE LEGEND**

**Table 1.** Summary of PK parameters of midazolam, IV to predict steady state plasma concentration as shown in Figure 3, at 0.1 mg/kg/hr and 0.68 mg/kg/hr, assuming BW=65 kg of an average individual.

<table>
<thead>
<tr>
<th>Dose (mg/kg/hr)</th>
<th>CL (L/kg/hr)</th>
<th>$C_{ss}^a$ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDZ, IV 0.1</td>
<td>0.49</td>
<td>204</td>
</tr>
<tr>
<td>MDZ, IV 0.68</td>
<td>0.49</td>
<td>1388</td>
</tr>
<tr>
<td>MDZ, IV + Toci, SC 0.68</td>
<td>3.33</td>
<td>204</td>
</tr>
</tbody>
</table>
CYP450 Sensitive substrates as objects AND “Anti-inflammatory/immunomodulators” as “precipitant”, OR “non-precipitants” OR “Other mechanisms”

- CYP3A: “Midazolam”/“Simvastatin”
- CYP2D6: “Dextromethorphan”/“desipramine”
- CYP2C9: “Warfarin”
- CYP2C19: “Omeprazole”
- CYP1A2: “Caffeine”/“Tizanidine”

- Filter out non-cytokine modulator compounds

- Datasets merged
  - Summary of %Change in AUC
Figure 2
Figure 3