Clinical Significance and Regulatory Framework for the Evaluation of Organic Anion Transporting Polypeptide 1B-Based Drug-Drug Interactions

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Abstract

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This dissertation research aims to conduct a comprehensive analysis of the clinical role of organic anion transporting polypeptides (OATPs) 1B1 and 1B3 and the regulatory approach for their evaluation. The work included here has identified the most relevant clinical substrates and inhibitors. Additionally, the contributing factors in the variability of in vitro inhibition constants as well as real-world implications for OATP1B1/1B3 drug-drug interactions are discussed.

In Chapter 2, six compounds were identified as potential clinical markers of OATP1B1/1B3 activity through the use of a novel indexing system. These drugs were identified from a list of 34 clinical substrates identified from a thorough analysis of the available in vitro and in vivo data. These findings also suggest that the risk for comedication interactions involve

drugs from multiple therapeutic areas showing a reliance on hepatic uptake via the OATP transporters.

Chapters 3 seeks to better understand the variability of in vitro inhibition data. By analyzing available OATP1B1/1B3 IC50 values, the primary contributing factors to in vitro variability were identified as the cell system used and inclusion of a preincubation with the inhibitor. The variability of the overall dataset (highest IC50 relative to the lowest) was reduced from 12.35 to 5.23 when both factors were considered. This variability, however, did not directly translate to clinical predictions and the calculated R-values did not show a significant shift relative to the FDA cut-off of 1.1 when these parameters were considered.

The goal of Chapter 4 was to identify clinical inhibitors of OATP1B1/1B3 and potential regulatory index drugs. Through the analysis of available clinical and preclinical data, 13 drugs and nine combination treatments were found to be clinical inhibitors. From these clinical inhibitors, two drugs were identified as potential clinical index inhibitors. While no novel index inhibitors were found, this work supports the inclusion of rifampin and cyclosporine as selective and broad-spectrum inhibitors, respectively, and again illustrates the broad range of therapeutic areas that should be considered for comedication studies.

The impact of OATP1B1/1B3 inhibition on patients, specifically those in low income countries, is discussed in Chapter 5. As the potent OATP1B1/1B3 inhibitor rifampin is part of the recommended treatment regimen for tuberculosis, this chapter evaluated the complexities of DDI management from pharmacological standpoints and illustrated the unique barriers to effective management of DDIs, such as the challenges of co- infection and treatment settings. The use of modeling and simulation techniques are discussed, as they can facilitate the implementation of optimal treatments for infectious diseases at the individual patient level.

This dissertation research has improved our understanding of the clinical relevance of OATP1B1/1B3. The implications of the broad-reaching OATP1B1/1B3 interaction profile was discussed using rifampin to illustrate the complications that can arise from comedication scenarios. Overall, this data can be used to improve the evaluation of OATP1B1/1B3 in drug disposition and potentially mitigate the risk of DDIs that could compromise patient safety and drug efficacy.

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DEDICATION

To my mom and dad-

As far back as I can remember you've always believed I could do anything and taught me that there are no limits to what you can dream and become, even when that dream was to be a pink dancing elephant. Your constant example of graceful resiliency and an unwillingness to accept that "good enough" is a solution brought me to where I am today. Thank you for teaching me that any dream is a good one.

Chapter 1. INTRODUCTION

(Part of this chapter was published as "Organic Anion Transporting Polypeptide 2B1 – More Than a Glass-Full of Drug Interactions." (2019) Pharmacology & Therapeutics, 196, 204–215.)

1.1 BACKGROUND

1.1.1 *Introduction*

Organic anion transporting polypeptides (OATPs) are uptake transporters in the solute carrier (SLC) transporter superfamily. The OATP family is comprised of 11 isoforms in six subfamilies (OATP1-6), including two liver-specific isoforms – OATP1B1 and 1B3. These transporters facilitate the entry of many drugs and endogenous compounds into tissues throughout the body and the high conservation across many species has been attributed to their central and critical role in distribution and detoxification processes.[1] Three OATPs are currently evaluated for their role in drug disposition, the aforementioned OATP1B1 and -1B3, as well as OATP2B1.

1.1.2 *Expression and Function*

OATP transporters facilitate the sodium-independent uptake of a variety of amphipathic organic compounds. The presumed physiological role is to assist in the distribution and elimination of regulatory compounds, as a majority of the endogenous substrates of the transporters are steroid hormones and bile acids. OATP2B1, along with -1B3, shows pH-dependent transport activity, although this has not been observed for OATP1B1.[2] Due to the high expression of OATP2B1 in the intestine, it is likely that this transporter plays a physiological

role in the uptake of drugs from the intestinal lumen where the microclimate pH is weakly acidic.[3]

In contrast to the liver-specific expression of OATP1B1 and -1B3, OATP2B1 has a wider expression profile, as it is expressed in most tissues throughout the body, and has been implicated in facilitating the intestinal absorption of many drugs.[4–6] Quantitative proteomics studies have determined the relative abundance of transporters in human liver tissue using liquid chromatography-tandem mass spectrometry (LC-MS/MS), including the abundance of the OATPs.[7–9] While initial experiments underestimated the abundance of the transporters, subsequent experiments using an optimized methodology have determined that OATP1B1 is the most prevalent hepatic transporter, accounting for approximately 22% of the total transporter protein quantified in the liver.[8] The abundance of OATP1B3 is approximately half that of OATP1B1 (8%), yet approximately equal in abundance to OATP2B1. (**Figure 1-1A**). The absolute abundance of OATP2B1 in human intestinal tissue has also been determined. Drozdzik *et al.* found that OATP2B1 was the primary OATP expressed in the intestine, accounting for approximately 6% and 12% of the total transporter protein quantified in the small intestine and colon, respectively (**Figure 1-1B, Figure 1-1C**) although this longitudinal variation in protein abundance was not significant ($P > 0.05$).[10]

A meta-analysis of transporter abundance data completed in 2017 using seven available studies confirmed the liver proteomics findings for healthy Caucasian subjects.[11] This analysis also found that there is no significant gender-related difference in abundance of OATP1B1/1B3, yet there is slightly higher abundance of OATP2B1 in males compared to females (1.3-fold, $P < 0.05$). Additionally, there is a weak correlation between abundance and age for OATP2B1 ($r_s = 0.268$, $P < 0.05$) that is not observed with the other OATPs.

1.1.3 *Substrate and Inhibitor Specificity*

While some selective substrates have been identified for each of the OATPs, there is significant overlap between the isoforms. Using the University of Washington Drug Interaction Database (DIDB), 256 *in vitro* substrates of OATP transporters were identified. Of these, 58% showed transport activity by two or more transporters (38% OATP1B1/-1B3, 15% for OATP1B1/-1B3/-2B1, 3% OATP1B1/-2B1, 2% OATP1B3/-2B1) (**Figure 1-2**). Substrates of all three transporters include many statins (including pitavastatin, pravastatin, and atorvastatin) as well as endogenous compounds such as coproporphyrin III. While the hepatic uptake of these compounds is most likely mediated primarily by OATP1B1, OATP2B1 likely shows some contribution to the hepatic uptake, as well as intestinal absorption and bioavailability of these drugs.

Many of the drugs that are transported by multiple OATP transporters show greater *in vitro* affinity for certain transporters. Some drugs, such as pitavastatin, have greater *in vitro* affinity for OATP1B1 compared to OATP1B3 and -2B1 (K_m = 0.81 µM, 2.6 µM, and 13.5 µM, respectively)[12] while fluvastatin shows greater affinity for OATP2B1 (K_m = 0.75 µM, OATP1B1 K_m = 2.45 µM).[13] Despite this significant overlap, compounds with isoform selectivity have been identified. For example, drugs such as aliskiren, celiprolol, and erolitinib are transported by OATP2B1 but not by OATP1B1/-1B3. Similarly, drugs such as eluxadoline and faldaprevir are substrates of only OATP1B1, and telmisartan is only transported by OATP1B3.[14–17]

1.1.4 *Pharmacogenetics*

OATP transporters are encoded by polymorphic genes, with some genetic variations showing an impact on drug exposure and efficacy. While variants of the *SLCO1B3* and *-2B1* genes have been identified, they are not as well studied, and the clinical impact of many variants remain unknown at this time. Conversely, the variants of the *SLCO1B1* gene have been extensively studied and the clinical effect of many of these variants are well understood.

To date, 21 *SLCO1B1* variant alleles have been identified with varying effects on transport efficiency relative to wild type (*SLCO1B1*1*). Of these, there are four haplotypes most commonly associated with altered activity – one resulting in increased function, *SLCO1B1*1B* (c.388A>G), and three showing various decreases in activity [*SLCO1B1*5* (c.521T>C), *SLCO1B1*15* (c.388A>G, c.521T>C), and *SLCO1B1*17* (c.388A>G, c.521T>C, -11187G>A)]. A gene-dose response has been observed for the *SLCO1B1*5* haplotype, with increases in exposure up to 136.8% and 536.8% for heterozygous and homozygous individuals, respectively, relative to wild type (*SLCO1B1*1*). The largest changes observed were in homozygous individuals in studies using simvastatin acid (536.8%)[18], pitavastatin (285.0%)[19], and pravastatin (281.5%)[20]. Conversely, decreases in exposure of up to 50.8% have been observed for those individuals homozygous for the SLCO*1B1*1B* variant relative to wild type (*SLCO1B1*1*).

One of the most studied OATP2B1 variants, *SLCO2B1**3 (rs2306168), describes the nonsynonymous mutation c.1457C>T, resulting in the amino acid change Ser486Phe.[21] While this variant has been frequently studied, the precise effect on function has yet to be fully elucidated. Studies conducted *in vitro* showed decreased transport activity for multiple substrates, however that has not been clearly reflected in clinical studies.[22,23] *In vivo*, significantly decreased intestinal absorption of fexofenadine (35.5% decrease in AUC) and

celiprolol (decrease in AUC of 49.9% and 29.1% for homozygous and heterozygous individuals, respectively) in *SLCO2B1*3* carriers compared to those with the *SLCO2B1*1/*1* has been observed.[24,25] However, in another report, *SLCO2B1*3* carriers (either *SLCO2B1*1/*3* or **3/*3*) exhibited a 51% increase in $AUC_{0\text{-}24}$ for *(S)*-fexofenadine ($P < 0.05$), but no significant change in C_{max}, suggesting predominately decreased hepatic uptake.[26] Additionally, there was no significant change in *(R)*-fexofenadine or montelukast exposure between those with *SLCO2B1*1* and *SLCO2B1*3* genotypes, indicating that the effect of this variant may be drug- and stereo-specific.[26,27]

1.1.5 *Drug-Drug and Food-Drug Interactions*

With high abundance in both the intestine and liver, OATP transporters play a critical role in the uptake of many compounds, making the transporters a likely site for drug-drug (DDIs) and food-drug interactions (FDIs). This has been demonstrated by multiple studies *in vivo* showing significant changes in exposure following treatment with known inhibitors. Considering the high degree of substrate and inhibitor overlap between OATP1B1/1B3 and OATP2B1, it is likely that a significant portion these interactions have intestinal as well as hepatic components. However, the inhibitory potency of the inhibitor for each transporter relative to the intestinal and hepatic concentrations will determine the predominant site of inhibition.

A review of clinical DDIs mediated by intestinal OATPs found that fruit juices are the most well-studied inhibitors of intestinal OATP2B1 activity *in vivo*, showing marked decreases in the plasma concentrations of several OATP2B1 substrates including fexofenadine, aliskiren, and celiprolol.[28] Apple juice decreased the AUC of fexofenadine by approximately 85% and that of aliskiren by 63%[25,29], while grapefruit juice decreased the AUC of fexofenadine up to 52%, aliskiren by 61%, and celiprolol by 84%.[30–32] Likewise, orange juice decreased the AUC of

aliskiren by approximately 62%.[29] In these studies, however, the typical volume of juice is much higher than normal consumption. While significant interactions can be observed with high volumes of juice, little to no effect is observed at low doses that are similar to a standard beverage volume.[33–35] This indicates that significant DFIs with juices can likely be avoided by reducing the volume of juice administered at a time. Additionally, it is likely that these interactions can also be avoided by staggering the administration of juices and affected drugs. Delaying administration of fexofenadine by two hours following ingestion of grapefruit juice resulted in a lower level of inhibition than concomitant dosing (38% versus 52% decrease in $AUC_{0\text{-}8}$) and no inhibition was observed when the drug was given four hours after.[35,36]

Hepatic interactions, attributable primarily to OATP1B1/1B3, show similar significant changes in substrate exposure to those identified for intestinal OATP2B1. Studies completed with selective substrates and recommended clinical inhibitors[37,38] show increases in exposure of 9.9- to 22.8-fold for cyclosporine/pravastatin[39,40] and 5.1- to 6.7-fold for rifampin/pitavastatin.[41,42] In fact, of the identified interactions with known sensitive clinical substrates of OATP1B1/1B3, a significant portion of the identified interactions had a change in exposure of ≥ 2-fold (43%), with 15% of interactions showing a change in exposure of 5-fold or more. More importantly, these interactions have been observed for precipitants in multiple therapeutic classes. Over 50% of identified OATP1B1/1B3 DDIs resulted from anti-infectives, with 78% of the observed changes in AUC ≥ 2-fold, highlighting the need to understand and mitigate the potential for these interactions.

Identifying the primary pathway contributing to transporter-based DDIs is challenging. For most drugs, multiple enzymes and/or transporters contribute to their *in vivo* disposition. For example, both the AUC and C_{max} of glyburide, a substrate of OATP2B1 as well as OATP1B1,

were significantly increased (125% and 81%, respectively) after a single intravenous infusion of rifampicin, a recommended OATP inhibitor.[43] While intravenous dosing removes the potential for intestinal inhibition, there is currently no way to determine the contribution of each hepatic transporter involved *in vivo* due to the lack of selective inhibitors. The calculation of the liver inlet concentration relative to the IC_{50} for each transporter can give some insight into the uptake pathway that is likely driving the interaction, but this method is not definitive as it is a static prediction based on estimated concentrations. Physiologically based pharmacokinetic (PBPK) modeling, which allows for improved predictions through the use of dynamic scenarios, is rapidly becoming a common tool for overcoming the limitations of static predictions, but requires sufficient data of both transport mechanisms and drug physiochemical properties to accurately portray clinical events.

Transporter-based DDIs are also complicated by cases where the interaction can occur at multiple organs and tissues in addition to involving multiple transporters. A study investigating the effect of asunaprevir on rosuvastatin exposure also highlights the difficulty in determining the mechanism of transport inhibition *in vivo*. Asunaprevir (C_{max} = 0.56 µM[44]) is a potent *in vitro* inhibitor of OATP2B1 (IC_{50} = 0.27 µM), OATP1B1 (0.3 µM), and OATP1B3 (3.0 µM); however, when asunaprevir was coadministered with rosuvastatin, only a modest increase in rosuvastatin exposure was observed (1.4-fold).[45] Although it appears that the main effect of asunaprevir is inhibition of hepatic uptake (affecting primarily OATP1B1 and OATP2B1) it is quite possible that there is incomplete inhibition or, since both drugs were administered orally, that the observed increase in exposure could be somewhat blunted by reduced absorption due to inhibition of intestinal OATP2B1.[45]

1.1.6 *Current Regulatory Perspective*

Since the early 2000s when these OATP transporters were first identified, the number of publications regarding their function and structure has steadily increased. Although OATP2B1 is not currently recommended for evaluation by the FDA, OATP1B1 and -1B3 were first included in the 2012 FDA drug-drug interaction (DDI) guidance as transporters recommended for evaluation for drugs under development.[46,47] Since then, the number of DDIs and food-drug interactions (FDIs) attributed to these transporters have continued to rise, with a 1,000% increase in publications for OATP1B1 and, despite the current lack of regulatory guidance surrounding the transporter, an 800% increase in publications evaluating the role of OATP2B1 in drug-drug interactions between 2006 and 2017 (**Figure 1-3**).[47]

As part of the guidance revisions in 2017, new molecular entities (NMEs) are to be evaluated as substrates of OATP1B1/1B3 when hepatic uptake is expected to meet or exceed 25% of total clearance. When uptake of the drug into cells over-expressing the transporter is ≥ 2 compared to vector-transfected cells, a clinical study is recommended for those drugs where coadministration with an OATP1B1/1B3 inhibitor is likely. The 2017 guidance also proposes that all NMEs are to be evaluated as inhibitors of OATP1B1/1B3. Experiments should first be completed with a sensitive substrate in cells overexpressing the transporter following a pre-incubation period of at least 30 minutes. For those compounds with a predicted clinical inhibition value ≥ 1.1 (R-value, calculated from the IC_{50} and expected maximum concentration at the liver inlet), subsequent clinical evaluation is recommended. This represents a significant change from the initial inhibitor evaluation guidelines proposed in 2012, shifting toward a more conservative approach, as a multistep process was originally used selecting first those drugs with an expected maximal plasma concentration 10-fold higher than the IC_{50} and an R-value ≥ 1.25.

This regulatory shift and growing recognition of the role of OATP1B1/1B3 in drug disposition and DDIs is highlighted in a review of new drug applications between 2013 and 2016. Few drugs were identified as *in vitro* substrates (less than 10 compared to over 40 for P-gp); however, as all drugs are required to be evaluated as substrates of P-gp while only those with significant hepatic clearance are required to be tested against OATP1B1/1B3, this resulted in fewer drugs tested (**Figure 1-4A**). In contrast, over 40 new drugs were identified as *in vitro* inhibitors of OATP1B1/-1B3: more than P-gp (37 drugs) or BCRP (34 drugs) (**Figure 1-4B**).[48]

Despite the growing number of new drugs that are inhibitors of OATP1B1/1B3 *in vitro*, little progress has been made in the identification of specific clinical substrates or inhibitors of these transporters since they were introduced in the guidance over six years ago. For the evaluation of a new drug as an inhibitor of OATP1B1/1B3 *in vivo*, the same three substrates – pitavastatin, pravastatin, and rosuvastatin – are recommended as marker compounds. While they are sensitive substrates for OATP1B1/1B3, other metabolic and transport pathways contribute to their *in vivo* disposition, which often creates ambiguity in the interpretation of clinical interactions. The same is true of the currently recommended *in vivo* inhibitors, rifampin and cyclosporine. While rifampin is more selective compared to cyclosporine, which is a broad inhibitor of metabolism and transport, induction of enzymes such as CYP3A following repeated doses of rifampin leads to difficulties in data interpretation.

1.2 Hypothesis and Specific Aims

It has been well established in recent years that OATP1B1/1B3 are clinically relevant transporters for drug-drug interactions and should be considered during development, yet the current regulatory guidance offers a limited choice of selective substrates. By analyzing clinical

and preclinical literature data, it is hypothesized that more sensitive and selective substrates and inhibitors of OATP1B1/1B3 can be identified, which can, in turn, be used to evaluate and improve the translatability of *in vitro* data to *in vivo* prediction.

The specific aims are:

Specific Aim 1 (Ch 2): Identify potential *in vivo* substrates of OATP1B1/1B3 and evaluate the identified compounds for clinical relevance using a novel indexing system

Specific Aim 2 (Ch 3): Evaluate the sources of variability in the *in vitro* evaluation of OATP1B/1B3 inhibitors and the effect on clinical interaction predictions

Specific Aim 3 (Ch 4): Identify potential inhibitors of OATP1B1/1B3 and evaluate the identified compounds for clinical relevance

Specific Aim 4 (Ch 5): Review the clinical impact of OATP inhibition using drug-drug interactions with anti-infectives in low income countries as a case study

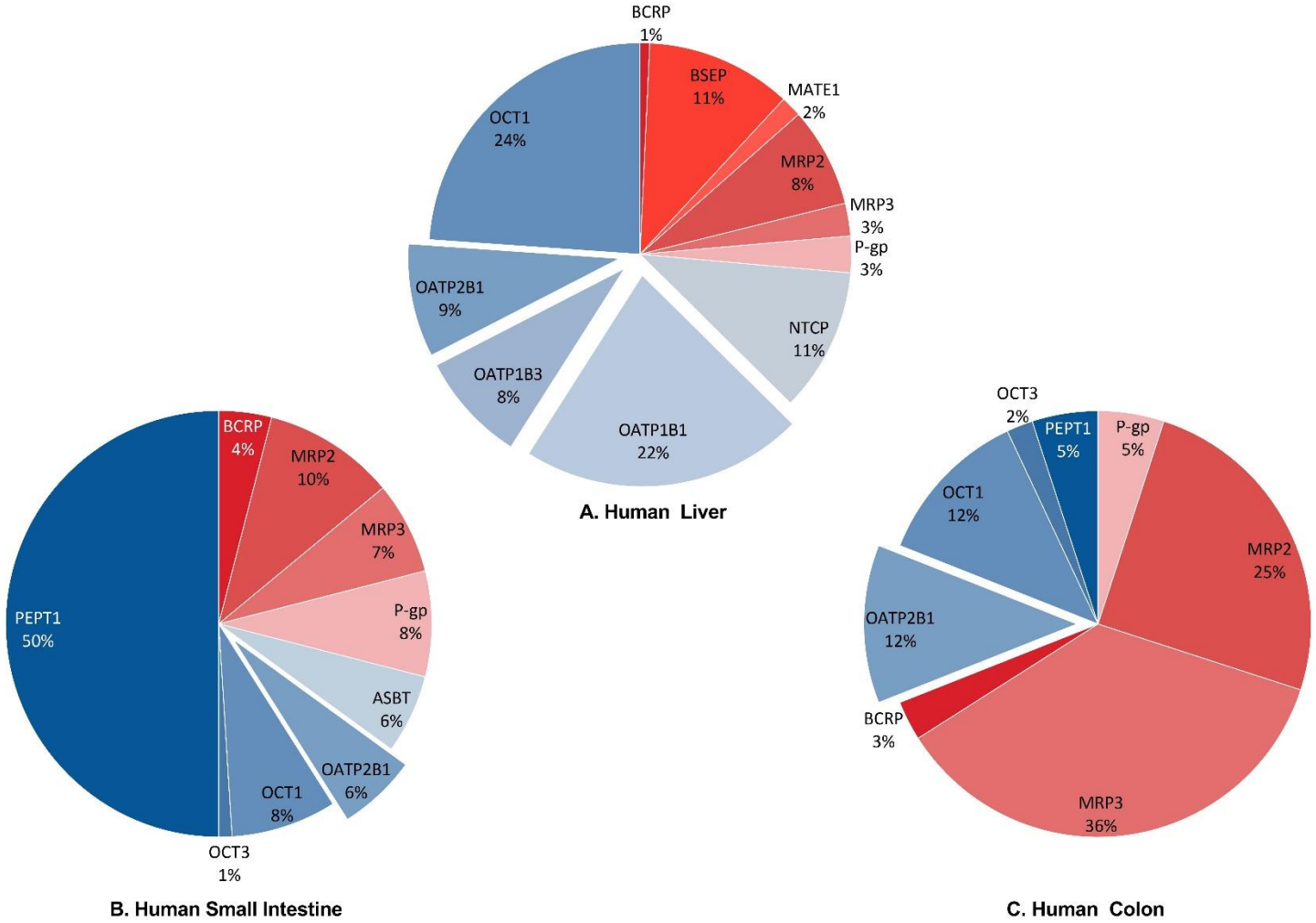

Figure 1-1. Abundance of the OATPs relative to other transporters in the human liver (A) and intestine (B, C).

Adapted from Wang et al., 2016 and Drozdzik et al., 2014. Blue- uptake transporters, red- efflux transporters. Expressed as percentage of total tissue transporter abundance.

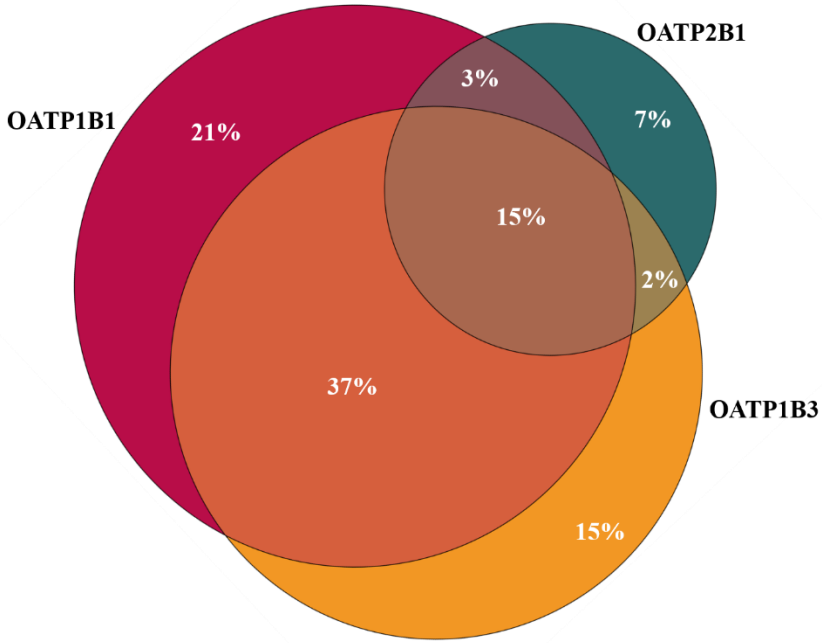

Figure 1-2. *In vitro* substrate overlap for OATP1B1, -1B3, and -2B1.

Of the identified *in vitro* substrates of OATP transporters, a majority are substrates of more than one isoform with 42% transported by two isoforms, and 15% are substrates of all three transporters evaluated.

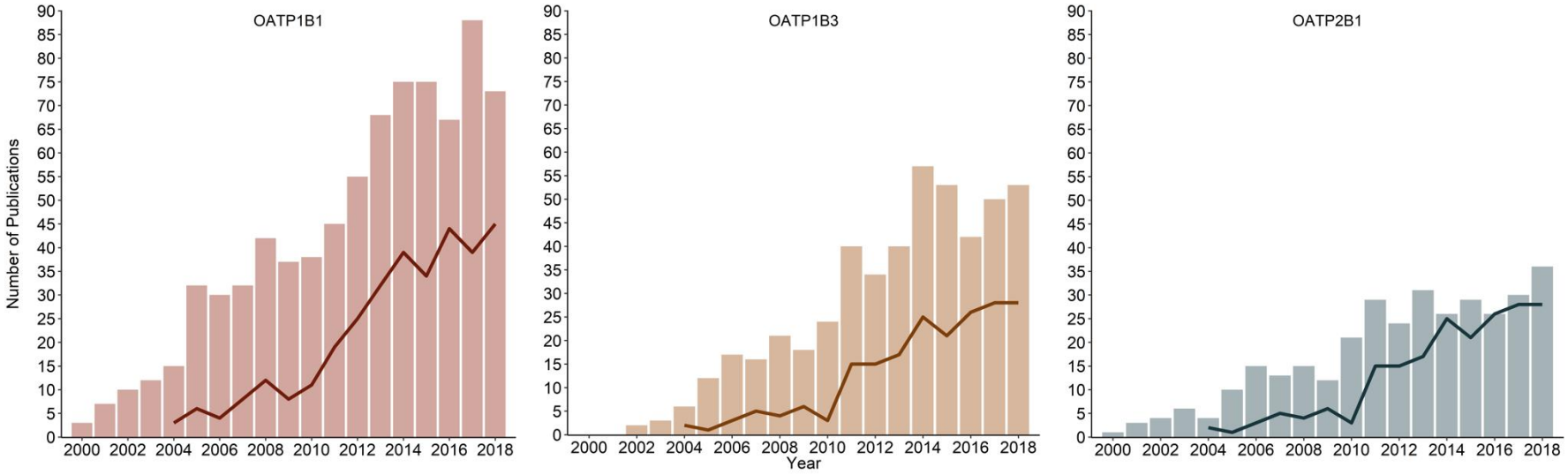

Figure 1-3. PubMed citations for OATP transporters (bars) and DDIs (line) per year.

PubMed was queried for “OATP1B1” or “OATP-C”, “OATP1B3" “OATP-8”, and “OATP2B1” or “OATP-C” for transporter and interaction data for studies relating to the three OATP transporters of interest, respectively.

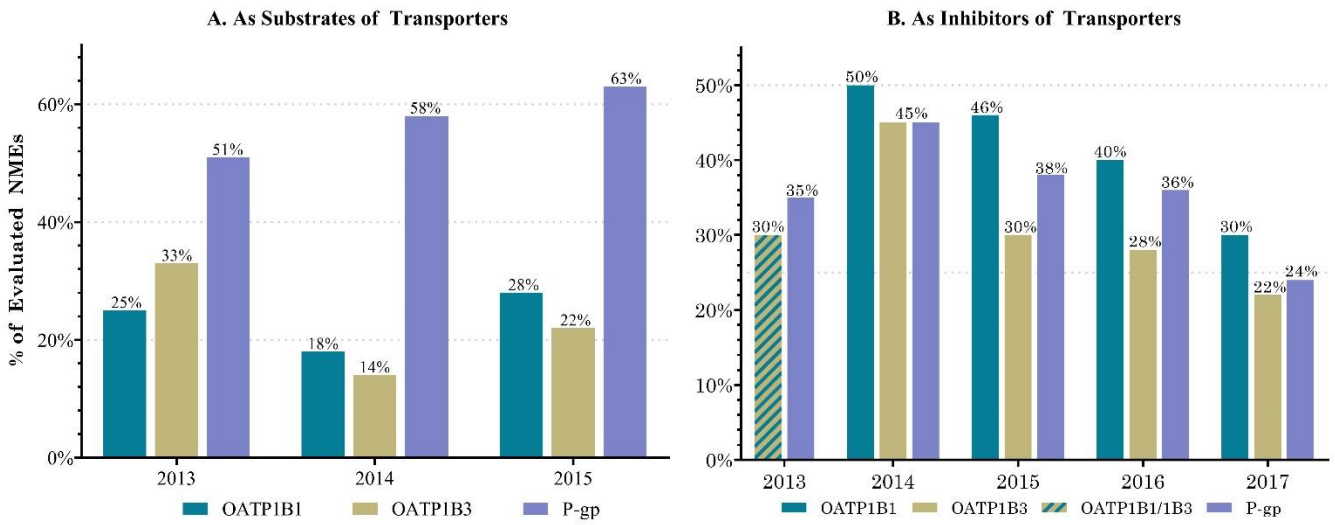

Figure 1-4. Percentage of approved drugs that are substrates (A) and *in vitro* inhibitors (B) of OATP1B1/1B3 since 2013.

Since OATP1B1/1B3 were included in the FDA interaction guidance, the number of studies evaluating the role of these transporters in drug disposition has increased, and subsequently increased the number of substrates and inhibitors identified. Since 2014, more compounds are inhibitors of OATP1B1/1B3 than any other transporter recommended for evaluation.

Chapter 2. IDENTIFICATION AND EVALUATION OF CLINICAL SUBSTRATES OF ORGANIC ANION TRANSPORTING POLYPEPTIDES 1B1 AND 1B3

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Organic anion transporting polypeptides (OATPs) are uptake transporters in the solute carrier (SLC) transporter superfamily. The OATP family comprises 11 isoforms in six subfamilies (OATP1-6), and OATP1B1 and 1B3 are the only liver-specific isoforms. These hepatic transporters facilitate the entry of many drugs and endogenous compounds into the liver. Of the transporters expressed in the liver, OATP1B1 is the most prevalent. Proteomics analysis found that OATP1B1 accounts for 22% of total protein while OATP1B3 is expressed at a significantly lower level, approximately 8%.[8] Both OATP1B1 and 1B3 are encoded by polymorphic genes (*SLCO1B1 and SLCO1B3*, respectively), with genetic variations showing an impact on drug exposure and efficacy. To date, 21 *SLCO1B1* variant alleles have been identified with varying effects on transport efficiency relative to wild type (*SLCO1B1*1*). In contrast, while *SLCO1B3* variants have been identified, they have not been as well studied and the clinical impact of the variants are mostly unknown at this time.

OATP1B1 and 1B3 were first included in the 2012 United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) drug-drug interaction (DDI) guidances, and since that time the number of reported *in vitro* interactions has steadily increased.[46,47,49] A recent review of new drug applications over the last four years highlights the relevance of OATP1B1/1B3, where < 10 drugs were identified as OATB1B substrates; however,

over 40 drugs were identified as inhibitors of OATP1B1/1B3, more than in the case of P-glycoprotein (P-gp; 37 drugs) or breast cancer resistance protein (BCRP; 34 drugs).[48]

For the evaluation of cytochrome P450 (CYP) enzymes, the FDA differentiates index studies, those using well-characterized substrates which can be extrapolated to other compounds, from concomitant use studies, those using medications likely to be co-administered in the target population. For transporters, however, it is evident that extrapolation from one substrate to another is difficult and that most studies performed will be based on concomitant use. Identification of index substrates for transporters, therefore, is less feasible using current methods and clinically relevant substrates are used for *in vivo* evaluation. The FDA currently recommends pitavastatin, pravastatin, or rosuvastatin as preferred clinical substrates, while the International Transporter Consortium (ITC) also recommends the inclusion of atorvastatin, in DDI studies when the new molecular entity (NME) is an expected inhibitor of OATP1B1/1B3.[38,50] While these drugs are sensitive substrates for OATP1B1/1B3, other metabolic and transport pathways contribute to their *in vivo* disposition, which creates ambiguity in the interpretation of clinical interactions.

The aim of the current investigation was two-fold. First, to identify as many clinical substrates of OATP1B1/1B3 as possible by conducting thorough analyses of all available *in vitro* and clinical data, including pharmacogenetic (PGx) and clinical DDI studies. And second, to propose potential index substrates using a new method of evaluating and ranking prospective OATP1B1/1B3 marker substrates.

2.1 METHODS

2.1.1 *Clinical Substrate Determination*

Using the University of Washington Drug Interaction Database (DIDB®, www.druginteractioninfo.org) potential substrates of OATP1B1/1B3 were identified from available *in vitro*, PGx, and clinical DDI studies (all queries of the DIDB were completed on or before 6 February 2018). Filtering of these datasets was completed similarly to previously published methods.[28,51]

2.1.1.1 *In Vitro Data*

To identify potential substrates of OATP1B1/1B3, multiple queries of the DIDB *in vitro* transport module were performed. The primary query identified *in vitro* studies using OATP1B1- or OATP1B3-transfected cell lines or *X. laevis* oocytes. Additional queries were also conducted for studies evaluating "OATP" transport, usually conducted in human hepatocytes, or *SLCO1B1/*SLCO1B3 genetic variants evaluated in transfected cell lines. The list of potential substrates identified through the database queries was filtered using previously established criteria - retaining those compounds with a reported $K_m \leq 10$ µM or uptake ratio ≥ 2.[28,51] These cut-off values served as a starting point for analysis and were chosen to be sufficiently broad so that the risk for false negatives would be low. A final list was determined by removing from consideration compounds that cannot be used clinically, such as experimental compounds or probe compounds that are not likely to be used for this purpose *in vivo* such as bromosulfophthalein.

2.1.1.2 *Clinical DDIs*

For clinical DDI data, OATP1B1 and 1B3 were evaluated together as they are indistinguishable *in vivo* due to the lack of specific substrates and inhibitors. To identify all relevant clinical studies regarding OATP1B1/1B3, multiple queries of the DIDB *in vivo* module were completed. First, studies were selected where the authors specifically attributed the

observed interaction to OATP1B1/1B3. Additional studies were identified through a query of inhibitors of OATP1B1/1B3 currently recommended by regulatory agencies – single dose or IV rifampin, cyclosporine, and gemfibrozil – or through identification of "OATP" as the primarily affected pathway. These studies were retained if there were *in vitro* or PGx data supporting a possible role of OATP1B1/1B3 in the disposition of the substrate. As previously established, the change in exposure of the substrate in the presence of an inhibitor (AUCR - ratio of inhibited AUC to control) was used to evaluate the clinical significance of the reported DDI.[28,51] For all studies, an AUCR ≥ 1.25 (or lower if statistically significant) was required to retain the study for analysis.

2.1.1.3 *PGx Studies*

Pharmacokinetic (PK) studies evaluating genetic variants of OATP1B1 and 1B3 were identified through a quantitative search for *SLCO1B1* and *SLCO1B3* using the e-PKgene module of the DIDB. Compounds were retained for further evaluation if over half of the studies reported a statistically significant overall effect of the variant on exposure of the substrate as determined by the authors. An AUCR-based cutoff was not feasible due to inherent population variability and the small number of subjects typically evaluated. Studies where the effects of more than one gene were investigated simultaneously (*i.e.* both *SLCO1B1* and *CYP3A5*) were removed from consideration.

2.1.2 *Data Refinement*

Following identification of potential substrates from all datasets, secondary queries of the DIDB were performed to ensure that all available data were considered. For compounds identified in the PGx or clinical DDI datasets, *in vitro* data were re-evaluated to ensure retention of all relevant data, even if below the initial cut-off criteria. Similarly, PGx data for compounds

identified in the *in vitro* or clinical datasets were retained even if the results did not meet the initial criteria for inclusion. Finally, negative clinical DDI studies, those with an increase in AUC of less than 25%, were searched for all identified compounds. These steps ensured that all published and relevant data were evaluated in the determination of the clinical significance of OATP1B1/1B3.

2.1.3 *Clinical Substrate Rank Ordering*

Following identification of clinically relevant substrates of OATP1B1/1B3, an indexing system was applied, and potential clinical marker substrates were proposed based on the following primary criteria – sensitivity, specificity, and single-dose safety (**Table 2-1**). Only drugs currently approved by the FDA and EMA were evaluated.

Sensitivity to OATP1B1/1B3 inhibition was assessed by identifying the largest increase in AUC following co-administration with a single oral or IV dose of rifampin as there is little confounding from the inhibition of other metabolic/transport pathways. When rifampin data was unavailable, drugs were evaluated based on results from studies completed with cyclosporine or gemfibrozil. When clinical DDI data were not available, the largest change in exposure for a genetic variant was used. Substrates were ranked on a scale of 0 – 6 according to whether the compound was weakly sensitive (1: $1.25 \leq AUCR < 2$), moderately sensitive (2 or 3: $2 \leq AUCR < 5$), sensitive (4 or 5: $5 \leq AUCR < 10$), or extremely sensitive (6: $AUCR \geq 10$) to inhibition of OATP1B1/1B3. Compounds with no clinical data available or with a change in $AUC < 1.25$ were given a score of zero.

Identified clinical substrates were also evaluated for specificity towards OATP1B1/1B3, determined by the magnitude of the contribution of metabolism or other transport to the drug's disposition. Similar to the assessment of sensitivity, the magnitude of change in AUC following

co-administration of a mechanistic inhibitor of CYP enzymes or other transporters was used to evaluate each substrate on a 0 – 6 scale. If a substrate showed changes in exposure for multiple pathways, the estimated cumulative effect was used in the ranking based on the assessment of the interactions [*i.e.* drugs that were sensitive to pathways other than OATP1B1/1B3 (AUCR $\geq$ 5) were ranked lower than those that showed moderate (2 $\leq$ AUCR <5) or weak (AUCR < 2) interactions]. Compounds that were substrates for only a single pathway were ranked higher than those with multiple contributing pathways as interpretation of the data is inherently less complex.

Finally, the safety profile following a single dose was evaluated for each compound. Compounds were reduced in the overall ranking if there was an unfavorable safety profile for a single dose given to healthy subjects or if no safety data was available. This included compounds with a narrow therapeutic range or those that are expected to have significant adverse events in the recommended therapeutic concentration range.

Final rankings were adjusted with additional positive and negative criteria regarding linear/atypical pharmacokinetics (PK), available formulations, and the availability of supporting data. Additional points were awarded to compounds with positive PGx data (statistically significant changes in exposure compared to control) as this adds confidence in the involvement of OATP1B1/1B3 in the disposition of the substrate. Scores were also increased for compounds with published and validated physiologically-based pharmacokinetic (PBPK) models, as expected changes in exposure can be accurately predicted prior to administration. Additionally, compounds for which microdosing has been validated were scored positively as this approach significantly increases clinical safety while maintaining measurable and informative changes in exposure.[52] Scores were reduced for compounds that are only marketed as combination therapies as the co-formulated agent can confound study results, as well as compounds that show non-

linear PK as observed changes in exposure cannot be correlated to inhibitor dose. Drugs with low bioavailability (F < 5%) received lower scores as the intraindividual variability is significantly higher and can confound inhibition study results. Finally, scores were decreased for compounds where $t_{1/2}$ > 24 h to prioritize those drugs where a shorter study can be designed, thereby decreasing clinic time.

Upon completion of compound ranking, compounds were classified as "good" or "poor" clinical marker compounds (highest and lowest 20%, respectively) based on their overall rank positioning. Compounds with scores falling between 20 – 80% were classified as "moderate". The maximum possible score, assuming the maximum of each category and all additional positive criteria, was 15.0 and the minimum was -5.5. To further validate the proposed probe indexing system, index scores were evaluated against the corresponding extended clearance classification system (ECCS) class for those compounds, as the ECCS has been shown to accurately classify compounds based on sensitivity to hepatic uptake.[53]

2.2 RESULTS

2.2.1 *In Vitro Substrates of OATP1B1/1B3*

Queries of the *in vitro* data module of the DIDB identified 140 compounds evaluated as substrates of OATP1B1 and/or 1B3. These compounds were first filtered to select those with an uptake ratio ≥ 2 and/or $K_m \leq 10$ µM, resulting in retention of 86 substrates. Of these, 31 (36%) were identified as substrates of OATP1B1, 19 (22%) of OATP1B3, and 36 (42%) were substrates of both isoforms. A final list of 56 compounds was identified after separating compounds that could not be used clinically. Interestingly, 53% of the initial 140 compounds identified did not have clinical data available despite some showing strong affinity for the

transporters, while 29 of the 86 compounds (34%) were retained after filtering compounds that did not have corresponding *in vivo* data (**Table A-1**).

2.2.2 *Clinical DDIs Potentially Attributable to OATP1B1/1B3*

From the transporter-specific and supplemental queries of available clinical DDI studies, 51 compounds were identified involving 128 studies. Of these, 41 compounds met the retention criteria with interactions primarily attributable to inhibition of OATP1B1/1B3 and a change in AUC ≥ 25%. A majority of the selected compounds (35 of 41, 85%) were also identified in the *in vitro* and/or PGx queries. All 41 compounds were retained for further evaluation and determination of the clinical relevance of OATP1B1/1B3. Observed increases in exposure ranged from 1.1- to 22.8-fold (atrasentan/rifampin and pravastatin/cyclosporine, respectively) with an overall median AUCR of 2.13. While a majority (71 of 128, 55%) of the interactions were minor, with observed increases in AUC less than 2-fold, approximately 30% of the identified interactions were moderate (2- to 5-fold increase in exposure), and 22% had increases greater than 5-fold.

2.2.3 *SLCO1B1 and SLCO1B3 PGx studies*

A quantitative search of the e-PKgene module of the DIDB identified 17 and 85 drugs evaluated for *SLCO1B3* and *SLCO1B1* polymorphisms, respectively. Selecting for those with a statistically significant change in drug exposure, 33 drugs involving 71 studies were retained for further evaluation. Very few studies involving *SLCO1B3* variants, 8 of 71 studies (11%), showed statistically significant changes in exposure and no substrate showed a majority of significant effects; therefore only 3 drugs which had supporting in *vitro* or clinical DDI data were retained from the *SLCO1B3* dataset. Of the identified compounds from the PGx data set, 6 (18%) did not

reach significance in the other datasets evaluated, 11 (33%) were identified in one of the other datasets, and 16 (48%) were identified in all datasets.

2.2.4 *Clinical Impact of OATP1B1/1B3 Inhibition*

A total of 83 compounds were identified from the three datasets, which was trimmed to a final list of 50 potential clinical substrates of OATP1B1/1B3 by removing drugs with minimal or no *in vivo* data. The evaluated list of 50 potential substrates was composed of 47 drugs, two endogenous compounds, and one imaging probe. Only the 47 drugs were subsequently evaluated for clinical relevance of OATP1B1/1B3 inhibition, as the remaining three compounds were outside the scope of the current work. Evaluation was based on the depth of available data, prioritizing those with data from multiple sources over those with single-source data. This first group included 16 drugs with data from all three datasets – clinical DDI, PGx, and *in vitro* – which were therefore given priority for further analysis of clinical relevance of OATP1B1/1B3 inhibition. An additional 24 drugs that had data from two of the three sources (22 drugs with *in vitro* and DDI or PGx data and 2 drugs with DDI and PGx data) were selected based on the number of studies showing an impact and the magnitude of the observed change in exposure, while only one drug with only PGx data was retained for evaluation. The remaining 6 drugs only had clinical DDI studies available and were not evaluated further due to a lack of confirmatory data (**Figure 2-1a**).

A thorough search of the available clinical and *in vitro* data was completed to determine the clinical relevance of OATP1B1/1B3 inhibition for each of the remaining 41 drugs identified (**Figure 2-1b**). There was sufficient data for 34 drugs to support a clinically significant role of OATP1B1/1B3 (**Table 2-2** and **Table A-2**). Significance was assessed through statistically significant changes in exposure following inhibition or due to genetic variants, or from a

potential change in patient safety, as determined by the safety profile of the drug and documented adverse events following inhibition of OATP1B1/1B3. Positive and negative data regarding the involvement of other transport and/or metabolic pathways were also considered to determine the specificity of the observed interactions. Of the identified substrates, there were 21 for which inhibition of OATP1B1/1B3 was likely to impact patient safety. Among these 21 drugs, 16 had label recommendations regarding OATP1B1/1B3 inhibition (**Table A-3**).

Among the 34 identified clinical substrates, an appreciable fraction (41%) have a site of action in the liver. Multiple therapeutic areas were represented. The largest contributors were statins (8 drugs, 24% of total) and anti-infective agents, including six hepatitis C virus (HCV) treatments (18%). Other represented classes were antidiabetics (12%), cardiovascular treatments [18% - including angiotensin II inhibitors (9%) and endothelin receptor antagonists (9%)], human immunodeficiency virus treatments (9%), and oncology therapies (6%).

Full evaluation of the available data, both positive and negative, resulted in the determination that OATP1B1/1B3 does not or is unlikely to play a significant role in the *in vivo* disposition of the remaining 7 drugs (**Table A-4**). These drugs were initially identified from clinical studies with one of the recommended inhibitors (single dose or IV rifampin, cyclosporine, or gemfibrozil) resulting in a change in exposure ≥ 25%, or PGx studies with a statistically significant effect of variants of *SLCO1B1* and/or *SLCO1B3*. However, on further evaluation, available corroborating data were insufficient to accurately determine the clinical role of OATP1B1/1B3 for six compounds, while one (digoxin) was found not to be a substrate of OATP1B1/1B3. To illustrate, simeprevir was initially identified from *in vitro* uptake ratios > 2, yet there was insufficient clinical data to determine the *in vivo* role of OATP1B1/1B3. Simeprevir is a sensitive substrate and inhibitor of multiple CYP enzymes and transporters which

confounded clinical data interpretation of the available studies that utilized broad inhibitors (such as cyclosporine) or multiple doses of rifampin where the observed decrease in AUC is likely attributable to the induction of CYP3A, whereas the increase in C_{max} is possibly due to inhibition of hepatic uptake by OATP1B1/1B3. This suggests that OATP inhibition may mask the full effect of CYP induction. Similarly, digoxin was initially selected from *in vitro* data showing an uptake ratio > 2.0 and an AUCR > 1.25 following rifampin co-administration (600 mg, multiple dose). When a comprehensive review of the available clinical and *in vitro* data was completed, a lack of change in AUC found in PGx studies, as well as multiple studies showing significant decreases in exposure following multiple-dose rifampin, supported the decision that digoxin is not a clinical OATP1B1/1B3 substrate and observed increases in exposure are likely due to combined inhibition and induction of P-gp at the liver and intestine, respectively.

2.2.5 *Clinical Marker Substrate Identification*

As a secondary analysis, the identified clinical substrates of OATP1B1/1B3 were evaluated for possible utility as a clinical marker substrate using the newly proposed indexing system. Of the identified drugs, only those currently approved by the FDA/EMA were scored (30 drugs). All remaining drugs were assessed regardless of observed AUCR to ensure a range of high and low scores to evaluate the indexing criteria. Upon scoring all compounds, the median score was 6.0 out of 15.0, with scores ranging from 1.5 to 12.0 (**Table A-5**). Using a selection criterion of the top 20% of scores to define "good" classification, 6 drugs scoring 7.6 points or higher were proposed as potential marker substrates. These drugs showed high sensitivity towards OATP1B1/1B3 inhibition, a low or manageable contribution of other metabolic and transport pathways, and a favorable clinical safety profile (**Table 2-3**). It is important to note that this approach identified the four clinical substrates currently recommended by the FDA and/or

ITC – atorvastatin, pravastatin, pitavastatin, and rosuvastatin, supporting the appropriateness of the selection parameters used.[38] The six proposed substrates are primarily statins and HCV treatments, consistent with the site of action for these drugs in the liver and the relatively low contribution of other metabolic and transport pathways for these drugs.

2.2.6 *Comparison of Clinical Substrates to ECCS Classification*

The ECCS classification evaluates drugs based on a combination of permeability, ionization state, molecular weight, and the separation of metabolic and transport rate-determining steps.[53–55] According to the ECCS classification, drugs in the 1B and 3B classes should be the most promising markers of OATP1B activity since hepatic uptake is the rate-limiting step. Indeed, a correlation was found between ECCS class and the maximum observed AUC with a selective OATP1B inhibitor (**Figure 2-2a**). Drugs in the 1B class had the highest proportion of AUC changes greater than 5-fold (3 of 8, 38%) followed by the 3B class (1 of 6, 17%). These classes also represented the highest proportion of moderate sensitive substrates (AUCR $\geq$ 2) with 75% and 83% of compounds for class 1B and 3B falling above the threshold, respectively. Additionally, dividing the compounds by ECCS classifications showed that the proposed indexing system accurately predicted those compounds expected to be sensitive clinical substrates (**Figure 2-2b**). It was found that drugs in ECCS 1B and 3B classes contained the highest scores, with median scores of 7.5 and 7.25 for the 1B and 3B classes, respectively (range 5.0 – 10.5 for class 1B and 5.0 – 12.0 for 3B).

2.3 DISCUSSION

Recognition of the clinical importance of OATP1B1/1B3 is continually increasing, consistent with an increased awareness of their role in research and their inclusion in regulatory

guidances. This analysis utilized a multi-pronged approach to identify new compounds where OATP1B1/1B3 contribute highly to the *in vivo* drug disposition based on data from multiple sources. The breadth of data available, both clinical and *in vitro*, allowed an in-depth analysis of each compound to accurately determine the *in vivo* significance of OATP1B-mediated hepatic uptake. Those compounds with data from three sources – clinical DDI, PGx, and *in vitro* – were prioritized for further analysis based on the breadth and strength of the confirmatory data. When data from all three sources were not found, available data were analyzed based on the strength of the source. That is, those with PGx data were prioritized because of the inherent specificity of genetic studies. Studies confirmed with *in vitro* data were also prioritized over those with only clinical DDI data. When only DDI studies were available, those compounds were not evaluated due to a lack of confirmatory studies. As many of those identified compounds are substrates of multiple enzymes and transporters and the inhibitors are also not specific for OATP1B1/1B3, it is likely that the observed interactions are hybrids of the various pathways/mechanisms involved.

The proposed index system was able to differentiate between possible "good" and "poor" marker substrates with a high degree of accuracy as confirmed by comparison to the assigned ECCS classification. All drugs scoring 7.6 points or higher were either class 1B or 3B, with the exception of letermovir, which does not currently have a published classification. In the ECCS classifications, these two categories are defined as having hepatic uptake (and/or renal clearance for class 3B) as the rate-determining step. Overall, most of the evaluated drugs, regardless of index ranking, were found in these categories due to the initial selection of those drugs where OATP1B1/1B3 play a significant role in the *in vivo* disposition (14/22, 64%, of drugs with available ECCS classifications). Interestingly, six drugs from class 2 (simvastatin; score 5.0), 3A (ambrisentan, nateglinide, and torsemide; median score: 6.0), or 4 (empagliflozin and

erythromycin; median score: 6.5) also showed scores in the moderate range. PGx studies evaluating OATP1B1 variants for drugs identified as moderate marker substrates all showed minor changes in exposure (range 1.2- to 2.1-fold increase[56–58]) with the exception of simvastatin, which showed an increase of 3.2-fold.[59] These findings indicate that while not the rate-determining step, hepatic uptake via OATP1B1/1B3 plays a role in the disposition of these drugs. Additionally, all identified drugs have *in vitro* data supporting contributions from other transporters, such as organic anion transporters 1 and 3 (OAT1/3), P-gp, and BCRP, which may contribute to the observed interactions. These overlapping contributions make data interpretation difficult and highlight the remaining challenges in identifying selective substrates for OATP1B1/1B3.

Multiple weighting schemes were tested for the proposed index, but ultimately no categorical weighting was applied. Although selectivity is highly desirable for a marker substrate, there were insufficient data available at this time to justify a higher assigned weight. While one drug, empagliflozin, appears to have a limited *in vivo* contribution by other metabolic enzymes and transporters, almost all other drugs have at least one other pathway contributing to their disposition. Because of this, any weighting turned out to be arbitrary in the assignment and did not significantly change the rank-order of the evaluated compounds (data not presented). As new drugs that are more selective for OATP1B1/1B3 are approved in the future, this aspect can be re-evaluated and an accurate weight determined for each category. Additionally, the availability of *in vitro* data supporting the role of OATP1B1/1B3 was not included in the current index. In the dataset used, all except one drug had published *in vitro* data and inclusion of this category did not assist in the ranking order.

This analysis resulted in the identification of six possible clinical marker substrates that could be used in the *in vivo* evaluation of OATP1B1/1B3 inhibition, allowing for selection of a fit-for-purpose substrate. For example, eluxadoline seems to have little to no contribution of other metabolic and transport pathways and changes in exposure for these six substrates are likely due to changes in OATP1B1/1B3 activity. However, eluxadoline shows lower sensitivity towards OATP1B inhibition compared to some of the other compounds, with a maximum observed AUCR of 4.2 for eluxadoline/cyclosporine.[14] Conversely, other compounds, such as atorvastatin and pravastatin, can provide information on "worst-case-scenario" inhibition, as they are substrates for multiple metabolic and transport pathways, such as CYP3A, BCRP, and P-gp, in addition to being sensitive OATP1B1/1B3 substrates. These drugs tend to show higher sensitivity to OATP1B1/1B3 inhibition (such as a 12.0-fold increase for asunaprevir/rifampin[45]), as well as significant changes with a broad-spectrum inhibitor such as a 22.8-fold increase for pravastatin/cyclosporine (BCRP, P-gp).[60,61] Identification of these substrates shows that the application of an indexing system, such as the one proposed, could have broad utility in the identification and selection of additional clinical marker substrates. This approach could be refined for application to other transporters and metabolic enzymes, following rigorous testing and validation, and could serve as a single criterion for selection of a clinical marker substrate for any pathway.

It is important to note that these evaluations were made based on the available, published data for each compound. It is highly likely that for some compounds with missing data, such as results of *in vitro* screens, those results are not publicly available at this time. This additional information may alter the conclusions made based on currently available data, and these assessments should be updated when more data become available. In addition, it is well known

that *in vitro* parameters for OATP1B1/1B3 show high variability between cell lines and between laboratories. For example, the *in vitro* probe bromosulfophthalein shows higher uptake in OATP1B3-injected *X. laevis* oocytes relative to OATP1B1 (uptake ratio = 8.9 and 5.0, respectively[62]), whereas the opposite is true for HEK293 cells expressing OATP1B1 and OATP1B3 (uptake ratio = 7.7 and 3.5, respectively[63]). Additionally, there are substrate differences in affinity for both isoforms. The conclusions reached from *in vitro* data, however, provide a valuable starting point for further investigation into the identification of specific markers for each OATP1B isoform *in vivo*.

In summary, we have identified and ranked 34 prospective clinically relevant substrates of OATP1B1/1B3, with six showing promise as potential marker substrates. Identification of compounds with diverse metabolic and transport profiles can allow for selection of fit-for-purpose marker substrates and could reduce the impact of confounding factors in the interpretation of interaction data involving OATP1B1/1B3.

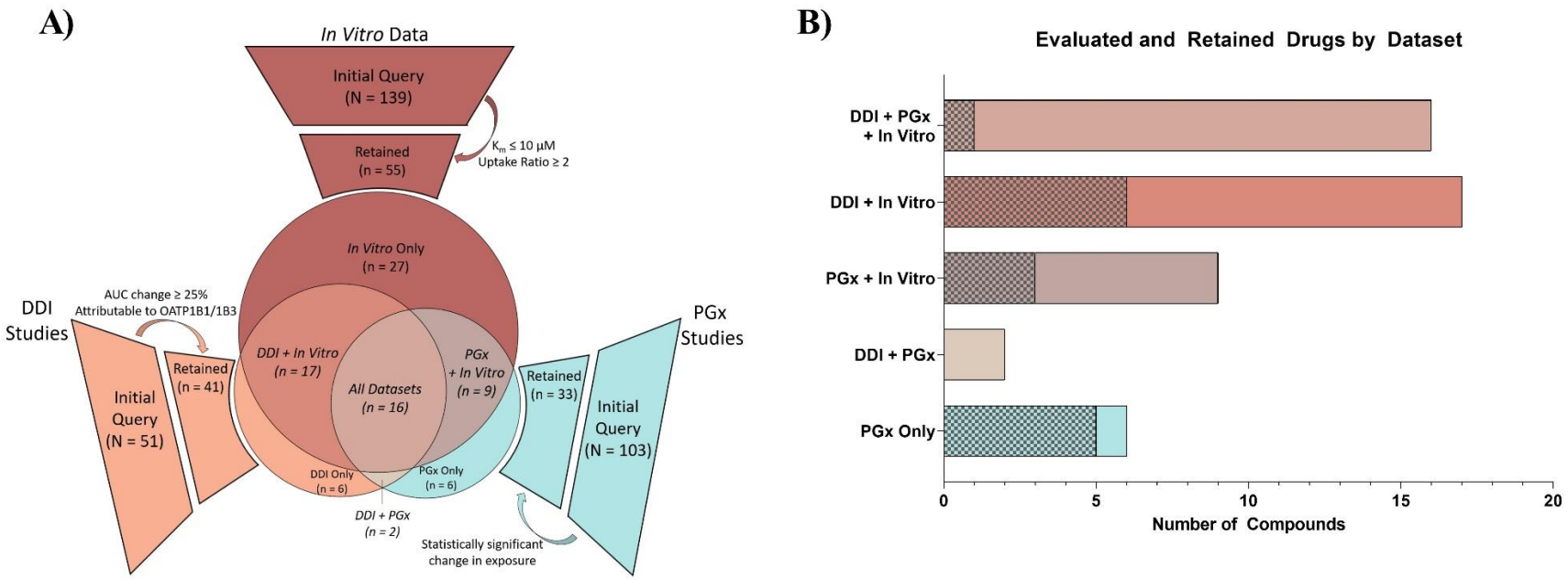

Figure 2-1. Selection process for potential OATP1B1/1B3 substrates from the in vitro, PGx, and clinical DDI datasets.

A) The substrate list generated from the initial queries was filtered for relevance to define a list of compounds to evaluate. The overlap in the generated substrate lists between the datasets was determined to assess strength of substrate association.

B) Those compounds with data from multiple sources (DDI, PGx, and *in vitro*) were given priority over those with single data sources. The number of compounds removed from consideration are indicated by a checkered pattern while those retained are in solid color.

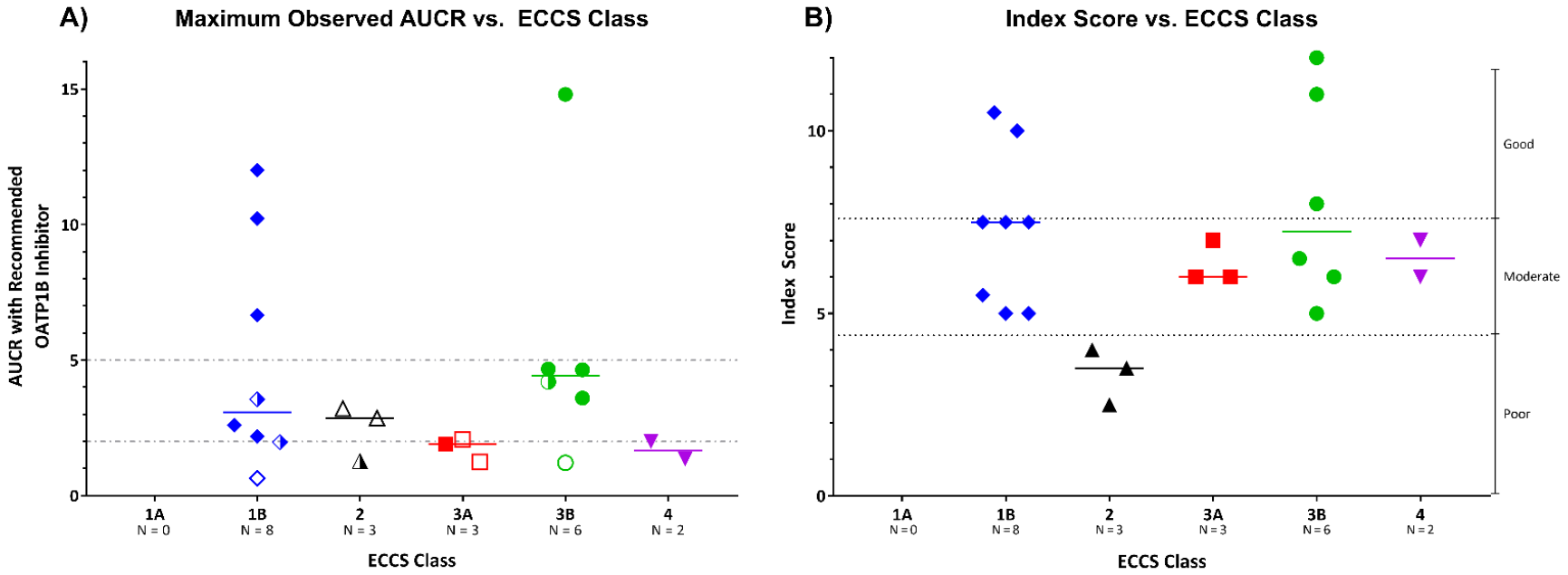

Figure 2-2. Comparison of ECCS to observed AUCR and Index Score for OATP1B Substrates.

(A) Maximum observed AUCR with a recommended OATP1B inhibitor relative to the ECCS class. Each point is a specific drug, and horizontal line indicates the median AUCR for the class. Dotted gray lines show 2-fold and 5-fold change in AUC. Filled shapes are changes observed from rifampin studies (single oral dose or IV), hollow shapes are PGx study data, half-filled shapes are from cyclosporine or gemfibrozil studies.

(B) Probe index score by ECCS classification. Horizontal line indicates median score for the class and dotted grey lines indicate the cut-off values for each index classification (poor < 4.4, good ≥ 7.6). ECCS classification determined by Varma, et al.[53,54] ECCS class 1A: high permeability, low molecular weight acids/zwitterions primarily cleared by metabolism; 1B: high permeability, high molecular weight acids/zwitterions primarily cleared by hepatic uptake; 2: high permeability bases/neutral compounds with high metabolic clearance; 3A: low permeability, low molecular weight acids/zwitterions with primarily renal clearance; 3B: low permeability, high molecular weight acids/zwitterions primarily cleared by hepatic uptake or renal elimination; 4: low permeability bases/neutral compounds primarily cleared through renal elimination.

Table 2-1. Clinical substrate index for the evaluation of drugs as sensitive clinical index substrates for OATP1B1/1B3.

TOTAL SCORE	15	(top of each category + all positive criteria)
Sensitivity to OATP1B1/1B3 inhibition[a]	0	No PGX data or clinical studies with a specific inhibitor for OATP1B1/1B3 -or- AUC Ratio < 1.25
	1	1.25 ≤ AUCR < 2
	2	2 ≤ AUCR < 3.5
	3	3.5 ≤ AUCR < 5
	4	5 ≤ AUCR < 7.5
	5	7.5 ≤ AUCR < 10
	6	AUCR ≥ 10
Specificity[b]	0	Sensitive substrate for at least 2 metabolic enzymes or transporters (AUCR ≥ 5 for each pathway)[c,d]
	1	Moderate sensitive substrate for at least 2 metabolic enzymes or transporters (2 ≤ AUCR < 5 for each pathway)[c,d]
	2	Sensitive substrate of one metabolic enzyme or transporter (AUCR ≥ 5)
	3	Weak substrate for at least 2 metabolic enzymes or transporters (AUCR < 2 for each pathway)[c,d]
	4	Moderate sensitive substrate of one metabolic enzyme or transporter (2 ≤ AUCR < 5)
	5	Weak substrate of one metabolic enzyme or transporter (AUCR < 2)
	6	Only OATP1B1/1B3 contributes to the disposition of the compound
Safety Profile	-2	Unfavorable safety profile for a single dose (narrow therapeutic range or expected significant side effects) or clinical safety has not been fully evaluated at this time
	1	Can be administered as a single, low dose with a low risk of adverse events in a healthy population or is well tolerated over a wide dose range, no concerns administering to a healthy population
Additional Criteria:		
Positives	1	PGx studies completed showing an impact of *SLCO1B1* or *1B3* variants
	0.5	Microdosing validated
	0.5	Published and validated PBPK model
Negatives	-2	Only available as a combination therapy
	-0.5	Non-linear pharmacokinetics
	-0.5	Half-life longer than 24 h
	-0.5	Very low bioavailability (F < 5%)

[a]Assessed primarily on the AUCR observed following single oral dose or IV rifampin. Studies with gemfibrozil/cyclosporin or PGx data used when rifampin data was unavailable.

[b]Score assigned from DDI studies with mechanistic inhibitors or PGx data

[c]If there is a difference in sensitivity between the two involved pathways (i.e. one moderate and one sensitive) score as follows: sensitive substrate + weak substrate = 1.5; sensitive substrate + moderate sensitive substrate = 0.5; moderate sensitive substrate + weak substrate = 3.5.

[d]If there is no clinical evidence but strong *in vitro* support for the involvement of a pathway (i.e. data reported in three or more cell systems or studies) subtract one point (-1.0) from the score assigned based on the *in vivo* data from the single enzyme/transporter category. If there is only minimal *in vitro* evidence (i.e. single study or cell system) subtract one half point (-0.5) from the score assigned based on the *in vivo* data from the single enzyme/transporter category. That is, if clinical data supports the substrate is a moderate sensitive substrate of CYP3A ($2 \leq$ AUC Ratio <5 with ketoconazole) yet there is strong *in vitro* evidence that CYP2C9 also contributes to the disposition, sensitivity score would be $4 – 1 = 3.0$.

Table 2-2. Compounds identified as *in vivo* substrates of OATP1B1/1B3.

Substrate	Possible Significant Clinical Issues Associated with OATP1B1/1B3 Inhibition	Data Available for Analysis		
		DDI	PGx	*In Vitro*
Ambrisentan	yes	✓	✓	--
Asunaprevir	yes	✓	--	✓
Atorvastatin	yes	✓	✓	✓
Atrasentan	no	✓	✓	✓[a]
Bosentan	yes	✓	--	✓
Caspofungin	yes	✓	--	✓[a]
Cerivastatin	yes [discontinued]	✓	--	✓
Danoprevir	yes	✓	--	✓[a]
Docetaxel	yes	✓	✓	✓
Eluxadoline	yes	✓	✓	✓
Empagliflozin	no	✓	--	✓[a]
Erythromycin	unclear [insufficient data]	✓	✓	✓[a]
Fexofenadine	unclear [confounding by P-gp]	✓	✓	✓[a]
Fimasartan	unclear [insufficient safety data]	✓	--	✓[a]
Fluvastatin	yes	✓	✓	✓
Glecaprevir	yes	✓	✓	✓
Glyburide	unclear [insufficient data]	✓	✓	✓
Grazoprevir	yes	✓	--	✓[a]
Letermovir	yes	✓	✓	✓[a]
Lopinavir	unclear [confounding by CYP3A]	--	✓	✓
Lovastatin	yes	✓	✓	✓[a]
Nateglinide	unclear [insufficient data]	--	✓	✓[a]
Nelfinavir	unclear [confounding by CYP3A]	✓	--	✓[a]
Olmesartan	no	--	✓	✓
Paritaprevir	yes	✓	--	✓
Pitavastatin	yes	✓	✓	✓

Pravastatin	yes	✓	✓	✓
Repaglinide	yes	✓	✓	✓
Rosuvastatin	yes	✓	✓	✓
Simvastatin	yes	✓	✓	✓[a]
SN-38	yes	--	✓	✓
Telmisartan	unclear [insufficient data]	--	✓	✓
Torsemide	no	--	✓	✓
Voxilaprevir	yes	✓	--	✓[a]

Clinical significance of OATP1B1/1B3 was assessed based on documented safety concerns associated with increased exposure of the drug. A "✓" indicates data was available for the specified dataset, "--" indicates no data available. Full data for each drug is available in **Table A-2**.

[a]Data is outside the cut-off value ($K_m \leq 10$ µM and/or uptake ratio ≥ 2) and was identified in the secondary evaluation after identification of substrates from clinical data.

Table 2-3. Potential clinical marker compounds of OATP1B1/1B3 as identified by the indexing system – those in the 80th percentile, with a score of 7.6 or higher.

Drug	Rank	Index Score	ECCS Classification	Therapeutic Area	Highest Reported AUC Ratio[a]	Highest Observed PGX Effect	Other Metabolism / Transport[b]
Pravastatin*	1	12.0	3B	statin	4.64	3.81	BCRP/OATP2B1/P-gp
Rosuvastatin*	2	11.0	3B	statin	4.67	2.18	CYP2C9 BCRP/OATP2B1/P-gp
Pitavastatin*	3	10.5	1B	statin	6.67	3.85	BCRP/OATP2B1/P-gp
Atorvastatin*	4	10.0	1B	statin	12.0	2.51	CYP3A BCRP/P-gp
Eluxadoline	5	8.0	3B	GI agent	4.20 (CsA)	2.01	N/A[c]
Letermovir	5	8.0	--[d]	antiviral	2.10 (CsA)	1.40	N/A[c]

* - compounds are currently recommended probe compounds by the FDA and/or ITC

[a]Rifampin studies were used when available due to the lower confounding from other pathways. When no rifampin study data was available, cyclosporine or gemfibrozil were used and selected to ensure the lowest contribution of other pathways possible.

[b]Listed alphabetically

[c]No other enzymes or transporters are currently identified as contributing to the disposition of the drug

[d]ECCS classification has not been assigned

CsA – cyclosporine; "—" no data available.

Chapter 3. VARIABILITY IN *IN VITRO* OATP1B1/1B3 INHIBITION DATA: IMPACT OF INCUBATION CONDITIONS ON VARIABILITY AND SUBSEQUENT DRUG INTERACTION PREDICTIONS

3.1 INTRODUCTION

Organic anion transporting polypeptides (OATP) 1B1 and 1B3 are the major hepatic uptake transporters involved in the distribution and disposition of many drugs. As such, the US Food and Drug Administration (FDA) recommends that all new drug entities are tested as inhibitors of both OATP1B1 and OATP1B3 *in vitro* in order to predict the risk of *in vivo* drug interactions. The most recent FDA guidance on clinical drug interaction studies, released in October 2017, recommends the determination of the inhibitory potency (IC_{50} or K_i) of the compound in cells overexpressing the relevant transporter, with a preincubation period of at least 30 minutes with the inhibitor.[64] As there are many other experimental variables, such as cell type, culture conditions, and probe substrate used, a thorough descriptive analysis of the literature data was completed to determine which factors may significantly contribute to the observed interlaboratory variability in inhibition potency, which may subsequently affect drug interaction predictions based on those values.

3.2 METHODS

Literature data were retrieved from the University of Washington Drug Interaction Database (DIDB®, www.druginteractioninfo.org). All studies showing *in vitro* inhibition of OATP1B1 and/or OATP1B3 were collected, and those studies providing K_i and/or IC_{50} data

were retained. Experiments with K_i values were analyzed separately from those with IC_{50} values for consistency of data. Data was collated by substrate/inhibitor pair and, to allow for adequate data comparison, those with a minimum of three experimental results were analyzed by calculating variability ratios (VR - highest value for a given dataset relative to lowest value). VR were calculated for all data for each substrate/inhibitor pair as well as after considering key experimental factors to evaluate sources of variability. To determine the effect of *in vitro* variability on DDI predictions, R-values were calculated, and ranges were determined for the full datasets as well as for individual experimental conditions. Due to the small dataset available for evaluation, only a descriptive analysis was able to be completed.

3.3 RESULTS

A total of 128 studies from 44 publications were examined in the final dataset, published between 2001 and 2018. For OATP1B1, 21 inhibitor/substrate pairs were identified with ≥ 3 IC_{50} values available and 7 inhibitor/substrate pairs were identified with ≥ 3 K_i values (5 inhibitor/substrate pairs had IC_{50} and K_i values available while 2 were unique to the K_i dataset). For OATP1B3, only 2 substrate/inhibitor pairs were identified with ≥ 3 IC_{50} values, and none of the inhibitor/substrate pairs had K_i data reaching the required number of studies. For both transporters the most commonly used substrate was estradiol-17-β-glucuronide ($E_2$17βG, 62% of all studies), while the top three inhibitors used were rifampin (27%), cyclosporine (25%), and gemfibrozil (18%) and the most commonly used cell type was HEK293 cells (79%).

3.3.1 *IC_{50} Variability*

The largest IC_{50} VRs calculated for OATP1B1 were cyclosporine/$E_2$17βG (86.4; n = 11), and rifampin/bromosulfophthalein (BSP, 43.6; n = 3), while the highest for OATP1B3 was

rifampin/$E_2$17βG (58.2; n = 7). Lower variability was observed in OATP1B1 K_i values, where the highest VR was for gemfibrozil/$E_2$17βG (7.2; n = 3). Two experimental factors were found to contribute the most to inhibition constant variability – cell type and preincubation versus co-incubation with the inhibitor. The overall VR of the entire dataset was reduced from 12.4 to 5.2 filtering by these two factors. For OATP1B1 IC_{50} values, the mean VR for all 21 substrate/inhibitor pairs was 11.7, which was reduced to 8.5 and 7.3 when only HEK293 cells and co-incubation were considered, respectively, and further reduced to 4.2 when both factors were considered together (**Figure 3-1A**, **Table 3-1**). Regarding K_i data, none of the experiments that were analyzed involved preincubation, therefore only the effect of cell type could be analyzed, and the average VR was reduced from 3.8 to 2.0 when only HEK293 cells were considered (**Figure 3-1B**). For OATP1B3, only two substrate/inhibitor pairs were analyzed, and a similar effect was observed regarding preincubation (**Figure 3-1C**). Because a majority of the experiments identified for OATP1B3 were conducted in HEK293 cells, the contribution of cell type to the variability was unable to be evaluated.

In order to determine the contribution of the substrate used to inhibitory constant variability, inhibitors that were tested with the largest array of substrates were analyzed. Of the inhibitors evaluated, rifampin and cyclosporine were studied with the largest array of substrates in OATP1B1 overexpressing cells, and interestingly, the highest variability in IC_{50} values was observed with non-clinically relevant substrates, namely $E_2$17βG, estrone-3-sulfate (E3S), and bromosulfophthalein (BSP). When cyclosporine was used as the inhibitor, the VR for atorvastatin, $E_2$17βG, and pitavastatin were 3.4, 86.3, and 12.6 for all data and 3.4, 12.6, and 3.0 when only HEK293 cells and co-incubation was considered, respectively. With rifampin as inhibitor, the VR for atorvastatin, BSP, $E_2$17βG, E3S, and pitavastatin were 3.9, 43.6, 15.8, 11.

9, and 3.9 for all data and 3.9, 4.3, 6.9, 7.9, and 3.9 when only HEK293 cells and co-incubation was considered, respectively (**Table 3-1**). These data indicate that the variability observed is not entirely due to cell type and/or coincubation and was at least partially due to the substrate used.

3.3.2 *R-Value Variability*

To determine the effect of the observed variability on clinical predictions, R-values, the predicted AUC ratio of a substrate in the presence and absence of the inhibitor as described in the 2017 FDA *in vitro* guidance[64], were calculated for each constant and the range and fold-change was determined for each inhibitor/substrate pair, as well as each inhibitor overall. Despite significant changes in VR when incubation conditions were accounted for, the resulting R-values did not show a significant shift with respect to the FDA cut-off value for prompting a clinical evaluation ($R \geq 1.1$, **Figure 3-2**). For the recommended index inhibitors cyclosporine and rifampin, all calculated R-values were ≥ 1.1 regardless of the *in vitro* conditions. Similar to VR, R-values showed substrate-dependent variability, with less variability observed for the statins compared to the *in vitro* only probes. For cyclosporine, the fold-change ranged from 2.3 with atorvastatin to 51.1 with $E_2$17βG, while the same substrates showed a 3.1 and 12.8- fold change, respectively, when rifampin was the inhibitor (**Table 3-2**, **Table 3-3**). This variability, both within the pairs and for the inhibitors overall, was decreased when the two primary sources of variability were accounted for, resulting in R-values ranging from 2.3 – 8.6 for cyclosporine and 2.8 – 5.7 for rifampin. In contrast, for gemfibrozil, a known *in vivo* inhibitor, only 5/14 (36%) of the R-values met the FDA cut-off, however this is likely due to the major circulating metabolite, gemfibrozil-1-O-β-glucuronide, which is also an OATP inhibitor and therefore contributes to *in vivo* inhibition.[65]

Similarly, for lopinavir, rifamycin, saquinavir, and troglitazone all R-values calculated were greater than the cut-off value, regardless of the *in vitro* conditions, while in contrast, ritonavir and verapamil had R-values on both sides of the FDA cut-off, even using the most uniform dataset (HEK293 and co-incubation). Interestingly, very few of these drugs had clinical data with a sensitive OATP1B1/1B3 substrate available, with no supporting clinical data identified for rifamycin or troglitazone. Ritonavir did not show significant inhibition of OATP1B1/1B3 (maximum observed AUCR of 1.31-fold with pravastatin[66]) when administered alone; however, when ritonavir was administered as part of a combination therapy with lopinavir or saquinavir, significant clinical inhibition was observed (maximum observed AUCR of 2.08 for lopinavir + ritonavir/rosuvastatin[67] and 3.93 saquinavir + ritonavir/atorvastatin[68]). Only one study with a sensitive OATP1B1/1B3 substrate was identified for verapamil, conducted with pravastatin. The observed change in exposure was minimal (1.32-fold) and could be attributable, at least in part, to inhibition of P-gp.[69] In the case of ketoconazole, by narrowing the dataset to the most uniform (HEK293 cells and coincubation only) all of the resulting R-values were below the FDA cut-off, although the sample size was reduced to n = 2, which is supportive of ketoconazole not being an OATP1B inhibitor *in vivo*.[70]

3.4 DISCUSSION

Interlaboratory variability involving inhibition of transporters, specifically P-glycoprotein (P-gp), has been discussed and addressed in previous years, however a similar analysis has not yet been performed for OATPs.[71,72] With the importance of OATP1B1/1B3 in drug disposition becoming increasingly apparent, addressing this variability, and the subsequent effect on *in vivo* predictions, is prudent. The descriptive analysis performed herein evaluated a broad dataset, identifying two main areas of experimental design that significantly contributed to this variability

– cell system and preincubation with the inhibitor. By removing these factors, the variability in the overall dataset dropped substantially. In addition, the choice of substrate influenced inhibitor variability.

The latest revision to the FDA guidance on *in vitro* assessment of DDIs addresses the topic of preincubation, adding in the requirement that studies should be completed with a 30-minute preincubation with inhibitor before the addition of substrate. The preincubation data analyzed within the overall data set tended towards lower IC_{50} values than co-incubation, which represents more of a worse-case scenario for *in vivo* predictions. It is likely that this experimental design will be reflected in the literature in coming years as this approach is implemented. Similarly, it appears that while there is no current recommendation for cell system, there is a trend towards a singular preferred cell system for the determination of inhibition constants, HEK293 cells. In the overall analyzed dataset (2001-2018), HEK293 cells were used in 68% of assays, whereas they were used in approximately 80% of experiments performed in the last five years (**Figure 3-3**). Aside from cell type and preincubation with inhibitor, another experimental condition that seemed to contribute to variability was the choice of probe substrate. Although there is known substrate dependence for inhibition of OATP transporters, that alone does not explain the variability observed within a single substrate/inhibitor pair.[73] In general, there were two classes of compounds used, *in vitro* probes ($E_2$17βG, E3S, and BSP), and statins (atorvastatin, rosuvastatin, pravastatin, and pitavastatin). When the *in vitro* probes were used, the variability was higher than when statins were used. Many experimental factors could contribute to this, including substrate permeability, dynamic range of uptake for an individual substrate within the cell line, and analytical detection method of substrate, as the *in vitro* probes tend to be radiolabeled while the statins require mass spectrometry for analysis.[74] These factors lend

credence to the *in vitro* use of more clinically relevant substrates, such as statins, which may lead to improved *in vitro* to *in vivo* predictions based on not just inhibitor potency, but also reproducibility and the reduced variability for a substrate/inhibitor pair.

While the most uniform experimental system appears to be HEK293 cells and accounting for preincubation, this analysis does not identify an experimental procedure that outperforms the others. For strong inhibitors the observed *in vitro* variability does not appear to have an effect on clinical predictions relative to the FDA cut-off. Even when these two factors were accounted for, moderate and weak inhibitors showed values above and below 1.1 which was, for those with clinical data available, reflected in the low level of *in vivo* inhibition observed.

It is important to note, however, that this descriptive analysis was limited by the availability of literature data regarding *in vitro* OATP1B1/1B3 inhibition. As many compounds are tested as inhibitors only during the drug development stage, requiring at least three experimental values significantly decreased the number of inhibitors that could be evaluated in this analysis. Due to this criterion, it is possible that the conclusions reached here may underestimate the variability in OATP1B1/1B3 inhibitory constants, and subsequently R-values, as the drugs with the most studies available for analysis are marker inhibitors as recommended by the FDA and therefore are likely to show more consistent results.[64] Furthermore, negative data is rarely available in literature and failure to take such data into account could bias the calculated variability to be lower than the true range of inhibitor constants observed. This inherent complication from the limited scope of published data highlights the need for a prospective study to fully evaluate laboratory practices for the *in vitro* evaluation of OATP1B1/1B3 inhibition. It should also be noted that although significant variability was observed in the IC_{50} determination for OATP1B1 (approximately 12-fold for the dataset overall,

reduced to 5-fold when controlling for cell type and preincubation), this is much lower than what has been observed for P-gp interactions with a range of IC_{50} values of over 700-fold being seen for a single inhibitor/substrate pair.[71] Despite the lower variability observed for OATP1B1/1B3 IC_{50} values, it remains prudent to further evaluate the potential sources of inter-laboratory variability so as to ensure accurate and reproducible data is being generated.

In summary, accounting for cell type and inclusion of a preincubation period significantly reduced the observed variability in IC_{50} values for the dataset overall as well as for specific inhibitor/substrate pairs. Additionally, the choice of substrate also contributed to the variability with clinical substrate, such as statins, showing lower variability overall compared to *in vitro* probe substrates. The preclinical variability did not appear to affect *in vitro* to *in vivo* predictions for the inhibitors evaluated, as almost all calculated R-values were above the FDA cut-off value. As more data become available, evaluating the relationship between the extent of variability and inhibitory potency and its impact on clinical predictions would be valuable to confirm these preliminary results.

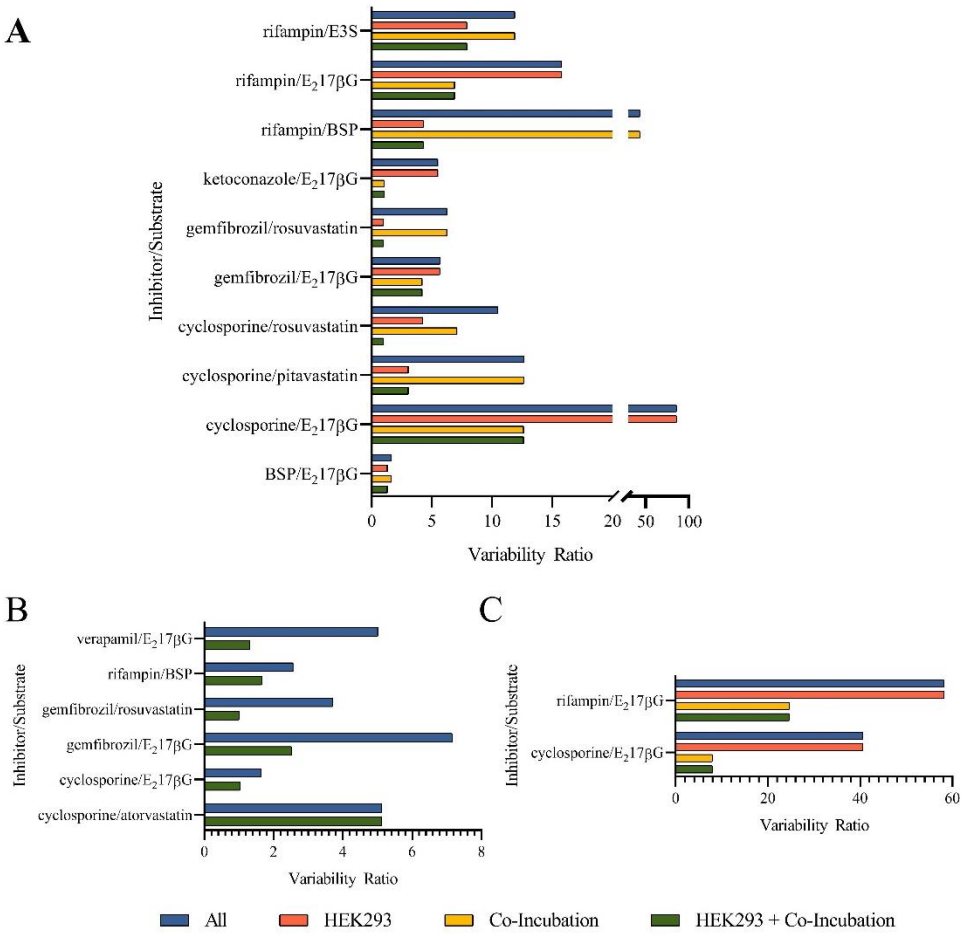

Figure 3-1. Effect of experimental conditions on variability ratio.

Only those inhibitor/substrate pairs where the VR changed are shown for clarity. A) OATP1B1 IC_{50} variability ratio, B) OATP1B1 K_i variability ratio, C) OATP1B3 IC_{50} variability ratio. Blue bars are all collected data, orange bars are experiments performed in HEK293 cells only, gold bars are only co-incubation with inhibitors, and green bars HEK293/co-incubation only.

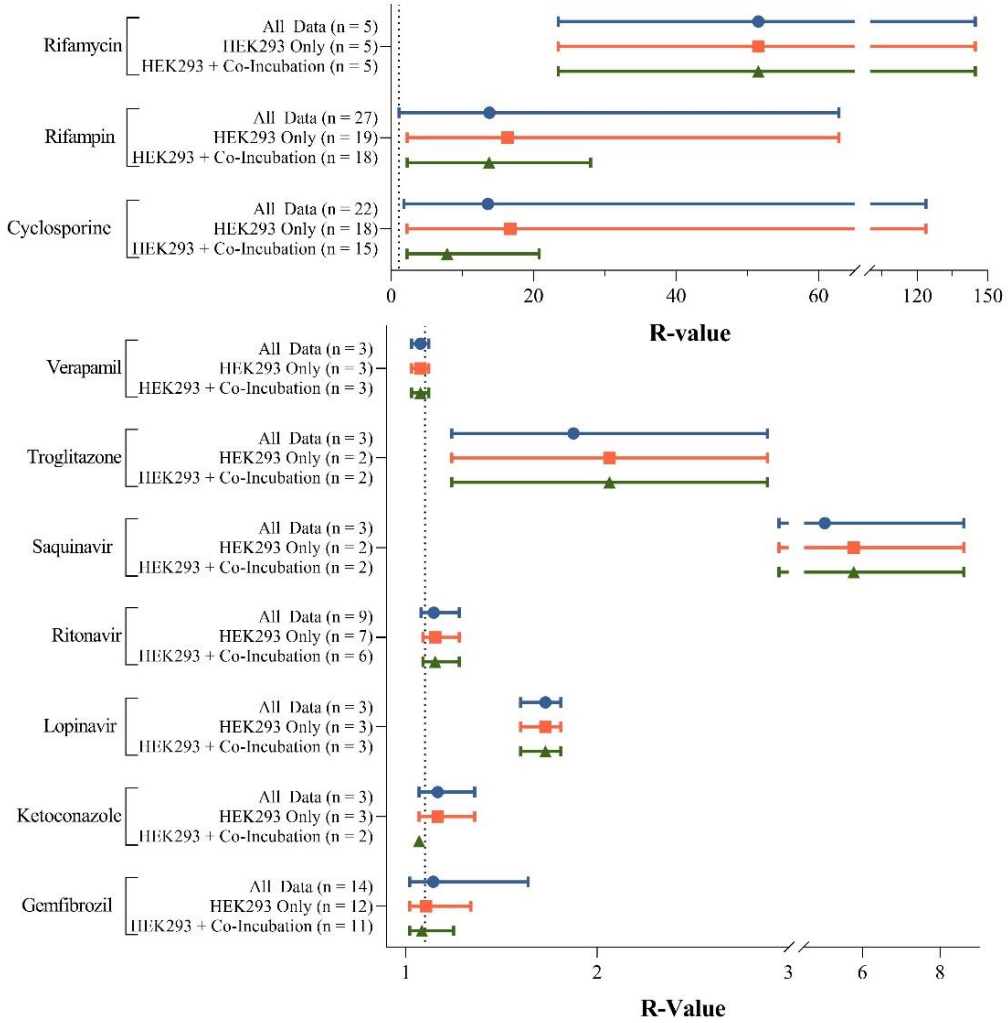

Figure 3-2. Effect of experimental conditions on R-value.

All inhibitors are included regardless of if there was a change in the R-value between subsets. For each inhibitor, the mean R-value and range for each subset is presented, with the number of studies (N) in parenthesis. Rifamycin, rifampin, and cyclosporine are

presented separately due to the significantly higher R-values compared to the remaining inhibitors. The vertical dashed line indicates the FDA cut-off value of 1.1.

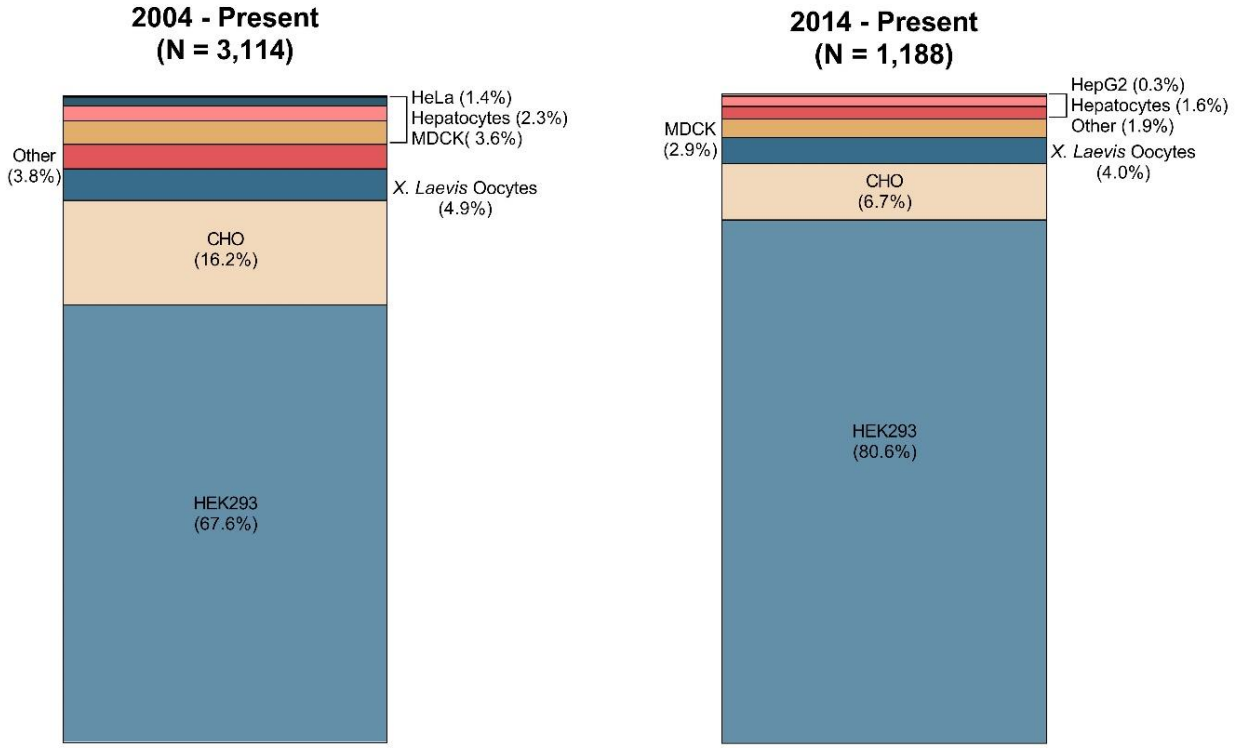

Figure 3-3. Cell types used in the *in vitro* evaluation of OATP1B1/1B3 inhibitors.

Overall, most published experiments use HEK293 cells in the *in vitro* evaluation of OATP1B1/1B3 inhibition (67.6% overall), which has dramatically increased in the last five years (80.6%). The rank-order of cell types has not changed significantly with increased research, with HEK293, CHO, and *X. laevis* oocytes comprising the top three most commonly used cell types and contributing to approximately 90% of experiments for both time periods.

Table 3-1. IC_{50}/K_i values and variability ratios for identified *in vitro* OATP1B1/1B3 inhibitors.

Inhibitor	Substrate	Transporter	Cell System	IC_{50} (µM)	K_i (µM)	PI[a]	Variability Ratio: All Data	Variability Ratio: HEK293 Only	Variability Ratio: Co-Incubation Only	Variability Ratio: HEK293 + Co-Incubation	Reference
BSP	E_2-17β-g	OATP1B1	HEK293	0.10			1.64	1.31	1.64	1.31	75
				0.13							73
			HeLa	0.08				[c]		[c]	76
Cyclosporine	Atorvastatin	OATP1B1	HEK293	0.55			3.42	NC[b]	NC[b]	NC[b]	77
				1.50							78
				1.88							79
			HEK293		0.16		5.13	NC[b]	NC[b]	NC[b]	80
					0.66						78
					0.82						79
	E_2-17β-g	OATP1B1	HEK293	0.12			86.32	NC[b]	12.62	12.62	73
				0.13							81
				0.20							82
				0.20							83
				0.87							84
				0.90							85
				1.40							86
				1.64							87
				0.02		Y			[c]	[c]	82
				0.05		Y			[c]	[c]	88
			HeLa	0.37				[c]		[c]	76
			HEK293		0.19		1.64	1.03	NC[b]	1.03	89
					0.20						75
			HepRG		0.32			[c]		[c]	89
		OATP1B3	HEK293	0.16			40.63	NC[b]	8.02	8.02	82
				1.23							82
				1.30							90

				0.032	Y			[c]	[c]	86
	Pitavastatin	OATP1B1	HEK293	0.23		12.65	3.04	12.65	3.04	91
				0.23						92
				0.70						85
			X. laevis oocytes	2.91			[c]		[c]	93
	Rosuvastatin	OATP1B1	HEK293	0.89		10.48	4.24	7.10	--[d]	87
				0.21	Y			[c]	[c]	94
			HeLa	0.31			[c]		[c]	95
			X. laevis oocytes	2.20			[c]		[c]	96
Gemfibrozil	Atorvastatin	OATP1B1	HEK293	32.0		4.88	NC[b]	NC[b]	NC[b]	97
				75.0						77
				130.0						78
				156.2						79
			HEK293		46.0	1.48	NC[b]	NC[b]	NC[b]	80
					58.0					78
					68.05					79
	E_2-17β-g	OATP1B1	HEK293	10.0		5.68	NC[b]	4.20	4.20	83
				26.4						73
				27.0						98
				27.5						85
				41.4						84
				42.0						97
				7.4	Y			[c]	[c]	88
			HEK293		12.5	7.16	2.52	2.52	2.52	83
					31.5					89
			HepRG		89.5					89
	Pravastatin		HEK293		9.65	3.71	--[d]	NC[b]	--[d]	80
			X. laevis oocytes		15.5					99
			Hepatocytes		35.8					99
	Rosuvastatin	OATP1B1	HEK293	19.0		6.25	--[d]	NC[b]	--[d]	97

			HeLa	25.0			[c]		[c]	95
			X. laevis oocytes	4.0			[c]		[c]	100
Ketoconazole	E_2-17β-g	OATP1B1	HEK293	9.5		5.50	NC[b]	1.04	1.04	101
				9.9						73
				1.8	Y			[c]	[c]	102
Lopinavir	Atorvastatin	OATP1B1	HEK293	0.74		1.35	NC[b]	NC[b]	NC[b]	79
				0.77						78
				1.00						77
Pravastatin	E_2-17β-g	OATP1B1	HEK293		31.90	2.56	1.66	NC[b]	1.66	89
					53.00					103
			CHO		21.80		[c]		[c]	104
			HepRG		20.70		[c]		[c]	89
Rifampin	Atorvastatin	OATP1B1	HEK293	1.3		3.85	NC[b]	NC[b]	NC[b]	77
				5.0						78
			Hepatocytes	3.0						105
	BSP	OATP1B1	HEK293	2.8		43.64	4.33	NC[b]	4.33	73
				11.9						106
			MDCK cells	120.0			[c]		[c]	107
	E_2-17β-g	OATP1B1	HEK293	0.55		15.79	NC[b]	6.89	6.89	84
				0.59						73
				0.59						81
				0.60						85
				0.74						108
				1.20						86
				3.49						87
				3.79						109
				0.24	Y			[c]	[c]	108
			CHO	1.50			[c]		[c]	110
			HeLa	0.94			[c]		[c]	76
		OATP1B3	HEK293	0.26						108

				1.50					86
				1.40					111
				6.40					112
				1.63					109
				0.11	Y		[c]	[c]	108
			CHO	2.60			[c]	[c]	110
	E3S	OATP1B1	HEK293	0.88		11.89	7.91	NC[b]	7.91	113
				1.90						112
				2.65						85
				6.96						73
			CHO	10.46			[c]		[c]	114
			X. laevis oocytes	5.16			[c]		[c]	115
	Pitavastatin	OATP1B1	HEK293	0.56		3.93	NC[b]	NC[b]	NC[b]	111
				2.20						85
			Hepatocytes	1.50			[c]		[c]	105
			MDCK cells	1.60			[c]		[c]	42
Rifamycin	E_2-17β-g	OATP1B1	HEK293	0.05		6.40	NC[b]	NC[b]	NC[b]	81
				0.23						116
				0.23						98
				0.30						75
				0.32						97
Ritonavir	E_2-17β-g	OATP1B1	HEK293	0.397		3.27	NC[b]	NC[b]	NC[b]	73
				0.85						85
				1.30						86
				1.30						84
				0.68	Y		[c]	[c]	102
			HeLa	0.71			[c]	[c]	76
	Pitavastatin	OATP1B1	HEK293	0.5		2.80	2.00	NC[b]	2.00	92
				1.0						85
			Hepatocytes	1.4			[c]		[c]	105

Saquinavir	E_2-17β-g	OATP1B1	HEK293	0.41		3.90	NC[b]	NC[b]	NC[b]	81
				1.60						84
			HeLa	1.23			[c]		[c]	76
			HEK293		0.91	5.01	1.32	NC[b]	1.32	89
					1.20					75
			HepRG		4.54		[c]		[c]	89
Troglitazone	E_2-17β-g	OATP1B1	HEK293	0.32		7.81	NC[b]	NC[b]	NC[b]	111
				2.50						87
			CHO	1.20			[c]		[c]	110
Verapamil	E_2-17β-g	OATP1B1	HEK293	14.8		3.48	NC[b]	NC[b]	NC[b]	84
				22.3						73
				51.5						85

[a]Preincubation

[b]No change in VR relative to the complete dataset

[c]Shaded cells indicate that the value was not included in the VR calculation for the specified subset

[d]Ranges were not calculated when N = 1 and are indicated with a dash (--)

E_2-17β-g, estradiol-17-beta-glucuronide; E3S, estrone-3-sulfate

Table 3-2. R-value ranges calculated for OATP1B1/1B3 inhibition data using IC_{50} values.

		All Data			HEK293 Only			Co-Incubation Only			HEK293 + Co-Incubation			
Inhibitor	Substrate	Range	Fold-Change	N	Range	Fold-Change	N	Range	Fold-Change	N	Range	Fold-Change	N	In Vivo Inhibitor?[a]
cyclosporine	atorvastatin	2.24 - 5.24	2.3	3	NC[b]			NC			NC			Y
	$E_2$17βG	2.42 - 123.78	51.1	11	2.42 - 123.78	51.1	10	2.42 - 20.77	8.6	9	2.42 - 20.77	8.6	8	
	$E_2$17βG (1B3)	2.79 - 73.9	26.4	4	NC			2.79 - 15.4	5.5	3	NC			
	pitavastatin	1.80 - 11.14	6.2	4	4.33 - 11.14	2.6	3	NC			4.33 - 11.14	2.6	3	
	rosuvastatin	2.06 - 12.11	5.9	4	3.62 - 12.11	3.3	2	2.06 - 8.53	4.1	3	3.62	--[c]	1	
gemfibrozil	atorvastatin	**1.02**[d] - **1.08**[d]	1.1	4	NC			NC			NC			Y
	$E_2$17βG	**1.06**[d] - 1.34	1.3	7	NC			**1.06**[d] - 1.25	1.2	6	NC			
	rosuvastatin	**1.10**[d] - 1.64	1.5	3	1.13	--	1	NC			1.13	--	1	
ketoconazole	$E_2$17βG	**1.07**[d] - 1.36	1.3	3	NC			**1.07**[d] – **1.07**[d]	1.0	2	NC			N
lopinavir	atorvastatin	1.60 - 1.81	1.1	3	NC			NC			NC			Y
rifampin	atorvastatin	3.97 - 12.42	3.1	3	3.97 - 12.42	3.1	2	NC			3.97 - 12.42	3.1	2	Y
	BSP	1.12 - 6.4	5.7	3	2.25 - 6.4	2.8	2	NC			2.25 - 6.40	2.8	2	
	$E_2$17βG	4.92 - 62.85	12.8	11	4.92 - 62.85	12.8	9	4.92 - 27.99	5.7	10	4.92 - 27.99	5.7	8	
	$E_2$17βG (1B3)	3.32 - 135.95	41.0	7	3.32 - 135.95	41.0	6	3.32 - 58.1	17.5	6	3.32 - 58.1	17.5	5	
	E3S	2.42 - 17.87	7.4	6	3.13 - 17.87	5.7	4	NC			3.13 - 17.87	5.7	4	
	pitavastatin	7.75 - 27.51	3.6	4	7.75 - 27.51	3.6	2	NC			7.75 - 27.51	3.6	2	
rifamycin	$E_2$17βG	23.46 - 144.75	6.2	5	NC			NC			NC			Y
ritonavir	$E_2$17βG	**1.09**[d] - 1.28	1.2	6	**1.09**[d] - 1.28	1.2	5	**1.09**[d] - 1.28	1.2	5	**1.09**[d] - 1.28	1.2	4	Y/N[e]
	pitavastatin	**1.08**[d] - 1.22	1.1	3	1.11 - 1.22	1.1	2	NC			1.11 - 1.22	1.1	2	
saquinavir	$E_2$17βG	2.95 - 8.61	2.9	3	2.95 - 8.61	2.9	2	NC			2.95 - 8.61	2.9	2	Y
troglitazone	$E_2$17βG	1.24 - 2.89	2.3	3	1.24 - 2.89	2.3	2	NC			1.24 - 2.89	2.3	2	ND[f]
verapamil	$E_2$17βG	**1.03**[d] - 1.12	1.1	3	NC			NC			NC			ND[f]

All values are for inhibition of OATP1B1 unless otherwise specified.

[a]Supporting clinical data is presented in **Table 3-4** and **Table 3-5**.

[b]no change from full data set (All Data).

[c]Ranges were not calculated when N = 1 and are indicated with a dash (--).

[d]Values in **bold** indicate R < 1.1.

[e]When used as monotherapy, no clinical inhibition was observed. However, significant inhibition has been observed for ritonavir-containing treatments.

[f]No data available.

Table 3-3. Calculated R-values for all available IC_{50} values of *in vitro* OATP1B1/1B3 inhibitors.

Precipitant	MW (g/mol)	$f_{u,p}$	Single / Multiple Dose	Dose (µM)	C_{max} (µM)	$I_{in,max}$ (µM)	IC_{50} (µM)	R-value
cyclosporine	1202.61	0.1	single	332.61	1.15	23.33	0.019	123.78
							0.05	47.66
							0.12	20.77
							0.13	18.94
							0.20	12.78
							0.20	12.66
							0.21	12.11
							0.23	11.14
							0.23	11.14
							0.31	8.53
							0.37	7.30
							0.55	5.24
							0.70	4.33
							0.87	3.68
							0.89	3.62
							0.90	3.59
							1.40	2.67
							1.50	2.56
							1.64	2.42
							1.88	2.24
							2.20	2.06
							2.91	1.80
gemfibrozil	250.35	0.01	multiple	2396.64	94.41	254.18	4.0	1.64
							7.4	1.34
							10.0	1.25
							19.0	1.13
							25.0	1.10

							26.4	1.10
							27.0	**1.09**
							27.5	**1.09**
							32.0	**1.08**
							41.4	**1.06**
							42.0	**1.06**
							75.0	**1.03**
							130.0	**1.02**
							156.2	**1.02**
ketoconazole	531.44	0.01	single	752.67	14.69	64.87	1.8	1.36
							9.5	**1.07**
							9.9	**1.07**
lopinavir	628.8	0.01	multiple	636.13	17.63	60.04	0.74	1.81
							0.77	1.78
							1.00	1.60
rifampin	822.95	0.2	multiple	729.08	25.62	74.22	0.24	62.85
							0.55	27.99
							0.56	27.51
							0.59	26.38
							0.59	26.16
							0.60	25.74
							0.74	21.06
							0.88	17.87
							0.94	16.79
							1.20	13.37
							1.30	12.42
							1.50	10.90
							1.50	10.90
							1.60	10.28
							1.90	8.81

							2.20	7.75
							2.65	6.60
							2.75	6.40
							3.00	5.95
							3.49	5.25
							3.79	4.92
							5.00	3.97
							5.16	3.88
							6.96	3.13
							10.46	2.42
							11.90	2.25
							120.00	1.12
rifamycin	720	0.2	multiple	538.89	0.01	35.94	0.05	144.75
							0.23	32.25
							0.23	32.25
							0.30	24.96
							0.32	23.46
ritonavir	720.95	0.01	multiple	138.71	1.82	11.07	0.397	1.28
							0.50	1.22
							0.68	1.16
							0.71	1.16
							0.85	1.13
							1.00	1.11
							1.30	**1.09**
							1.30	**1.09**
							1.40	**1.08**
saquinavir	670.86	0.03	multiple	1490.62	4.68	104.05	0.41	8.61
							1.23	3.54
							1.60	2.95
troglitazone	441.55	0.01	multiple	905.90	0.00	60.40	0.32	2.89

							1.2	1.50
							2.5	1.24
							14.8	1.12
verapamil	454.607	0.1	single	263.96	0.24	17.83	22.3	**1.08**
							51.5	**1.03**

Table 3-4. Clinical data for identified *in vitro* OATP1B1/1B3 inhibitors

Inhibitor	Substrate	Inhibitor Dose (mg)	Single/Multiple Dose	% Change in AUC	Reference
cyclosporine	atorvastatin	variable[a]	multiple	644.7	83
	atorvastatin acid	2.5 mg/kg		1431.3	117
		not provided	not provided	769.2	118
	eluxadoline	600	single	318.3	119
				319.6	14
	letermovir	50	single	89.5	120
				130.7	120
		200	single	110.4	121
				237.1	120
	pitavastatin	2 mg/kg	single	351.5	122
		variable[a]	multiple	1077.0	123
				892.9	40
			not provided	2183.5	39
		not provided	not provided	2183.1	61
	rosuvastatin	variable[a]	multiple	608.2	96
gemfibrozil	atorvastatin	600	multiple	23.9	124
	atorvastatin acid			34.7	125
	pitavastatin	600	multiple	25.0	122
	pravastatin	600	multiple	101.9	126
	rosuvastatin	600	single	**6.5**[b]	127
			multiple	88.0	100
ketoconazole	rosuvastatin	200	multiple	**1.6**[b]	70
rifampin	atorvastatin	300	single	259.7	128
		600	single	361.5	129
				407.2	128
				410.8	129
				751.5	129

				757.1	[130]
				1100.0	[131]
	atorvastatin acid			625.3	[132]
	pitavastatin	300	single	112.5	[128]
		600	single	153.9	[128]
				323.0	[130]
				405.3	[41]
				428.4	[133]
				441.4	[42]
				565.5	[42]
			multiple	35.4	[122]
	pravastatin	600	single	126.6	[134]
				364.0	[131]
	rosuvastatin	300	single	110.3	[128]
		600	single	126.2	[128]
				203.4	[42]
				221.3	[135]
				237.0	[135]
				308.9	[42]
				359.1	[130]
				362.5	[136]
				367.0	[137]
ritonavir	pravastatin	100	single	**31.3**[b]	[66]
		20	single	**19.2**[b]	[66]

[a]Variable dose indicates that the provided information only stated that the dose was sufficient to reach the target plasma concentration.

[b]No significant inhibition, as determined by the authors, for the values presented in **bold**.

Table 3-5. Clinical data for combination treatments identified as *in vitro* inhibitors

Identified Inhibitor(s)	Formulated With[a]	Substrate	Inhibitor Dose	Single/Multiple Dose	% Change in AUC[b]	Reference
lopinavir/ritonavir		pitavastatin	400 mg/100 mg	multiple	**-16.7**	138
		rosuvastatin	400 mg/100 mg	multiple	107.6	67
ritonavir	darunavir	atorvastatin	100 mg/300 mg	multiple	240	139
		pravastatin	100 mg/600 mg	multiple	90.2	139
					7.9	140
					18.2	140
					18.7	140
					23	140
		rosuvastatin	100 mg/600 mg	multiple	50.8	141
	fosamprenavir	rosuvastatin	100 mg/700 mg	multiple	**7.9**	142
	ombitasvir/paritaprevir	pravastatin	100 mg/150 mg/25 mg	multiple	74.1	143
					76.2	144
		rosuvastatin	100 mg/150 mg/25 mg	multiple	33.5	143
					33.5	144
	ombitasvir/paritaprevir/dasabuvir	pravastatin	100 mg/150 mg/25 mg/400 mg	multiple	82	145
					82.1	144
		rosuvastatin	100 mg/150 mg/25 mg/400 mg	multiple	159	145
					159.4	144
	tipranavir	atorvastatin	200 mg/500 mg	multiple	836.2	146
		rosuvastatin	200 mg/500 mg	multiple	36.9	146
saquinavir/ritonavir		atorvastatin	400 mg/400 mg	multiple	293.1	68

[a]If no coformulated drugs are listed, all components of the combination therapy were evaluated in the *in vitro* data.

[b]No significant inhibition, as determined by the authors, for the values presented in **bold**.

Chapter 4. INHIBITORS OF ORGANIC ANION TRANSPORTING POLYPEPTIDES 1B1 AND 1B3 – CLINICAL RELEVANCE AND REGULATORY PERSPECTIVE

4.1 INTRODUCTION

Organic anion transporting polypeptides 1B1 and 1B3 (OATP1B1/1B3) are the primary hepatic uptake transporters for the distribution and disposition of many drugs, including some of the widely used HMG-CoA reductase inhibitors that are known sensitive clinical substrates of OATP1B transporters. Because of this, both transporters are recommended for evaluation as a target of inhibition by all new molecular entities (NMEs) during development by the United States Food and Drug Administration (FDA).[38,46,64] Since their initial inclusion in the regulatory drug-drug interaction (DDI) guidance documents in 2012, the number of reported interactions has been continuously increasing with over 70 publications referencing interactions of OATP1B1/1B3 in 2018 alone.[47]

In addition to understanding the risk in a comedication scenario, identifying clinical inhibitors allows for mechanistic evaluation of NMEs, typically during drug development. Currently the FDA recommends two drugs – rifampin and cyclosporine – as inhibitors for the clinical evaluation of OATP1B1/1B3 substrates. However, these drugs are not without their limitations as cyclosporine is a broad metabolism and transport inhibitor which can lead to difficulties in identifying the driving force of those *in vivo* interactions, and rifampin shows inductive effects following repeated administration. The purpose of the current work, therefore, was to investigate clinical inhibitors of OATP1B1/1B3 from both clinical and regulatory perspectives by first identifying clinically relevant inhibitors of OATP1B1/1B3 through a thorough analysis of *in vitro* and clinical data and second, by presenting the most potent and

selective drugs as alternate clinical index inhibitors to improve the interpretation of mechanistic DDI studies.

4.2 METHODS

Potential clinical inhibitors of OATP1B1/1B3 were identified using the University of Washington Drug Interaction Database (DIDB®, www.druginteractioninfo.org) queries of *in vitro* and clinical DDI studies (all queries completed on or before 23 January 2019). Data filtering was completed by a method similar to previously published methods for both datasets.[28,37,51]

4.2.1 *In Vitro Data*

The *in vitro* query module was utilized to identify compounds as inhibitors of OATP1B1/1B3 using transfected cell lines or *X. laevis* oocytes. The list of potential inhibitors was filtered to only include those studies with published IC_{50} values to ensure equal comparisons of potency between compounds. This list was further refined to include only those compounds with a reported $IC_{50} \leq 10$ μM for either isoform. This cut-off served as a starting point for analysis and was selected to identify those compounds (parent and metabolites) most likely to be potent inhibitors *in vivo*. A final working list of candidate compounds was determined by removing from consideration any natural products and endogenous compounds, as clinical use of these compounds is limited.

4.2.2 *Clinical DDI Data*

As OATP1B1 and 1B3 are clinically indistinguishable due to overlap in both substrates and inhibitors, these transporters were evaluated together in the clinical DDI queries. Clinical

inhibitors were identified through two parallel queries. First, studies were selected where the authors explicitly state that the observed interaction was primarily attributable to inhibition of OATP1B1/1B3. Next, additional inhibitors were identified through queries of studies performed with previously identified substrates – pravastatin, rosuvastatin, pitavastatin, atorvastatin, eluxadoline, and letermovir.[37,38,64] Any duplicate studies when the two query results were combined were removed. For all queries, a change in AUC ≥ 1.25-fold in the presence of the inhbitor was required for the study to be retained. Similar to the *in vitro* queries, any non-drug compounds were excluded from further analysis. Due to their complex nature, combination treatments were evaluated separately. Drugs that are part of combination therapies but had clinical data for each component available were evaluated as the individual drug components.

4.2.3 *In Vitro to In Vivo Predictions*

The identified *in vitro* inhibitors were evaluated for predicted clinical potency. The R-value (predicted victim AUC ratio in the presence and absence of the inhibitor) was calculated for all identified *in vitro* inhibitors. To represent a "worst-case-scenario" as defined by the FDA, the parameters of F_aF_g and k_a were set to 1 and 0.1, respectively, for all compounds and $f_u = 0.1$ and equal partitioning ($R_B = 1$) were used when values were not available in literature. For each compound, C_{max} was determined from the average of all studies at the most commonly reported dose. Equations and parameters used in determining the R-values are presented in **Appendix B**. Those compounds with R ≥ 1.1 were identified as likely clinical inhibitors.

4.2.4 *Clinical Data Refinement*

Following completion of queries for both *in vitro* and clinical DDI data, secondary queries were completed to ensure that all available data were considered for the identified

compounds. For those compounds identified *in vitro* with $R \geq 1.1$ but without identified clinical data, a search of negative clinical DDI studies (change in AUC following administration of the inhibitor (AUCR) < 1.25) was conducted. Also, to confirm the role of OATP1B in the observed clinical interactions where *in vitro* data meeting the selection criteria were not available, a secondary query was performed to identify (i) any reported K_i values ≤ 10 μM when an IC_{50} was not available or (ii) K_i or IC_{50} values > 10 μM. Following these secondary queries, those drugs with clinical and supporting *in vitro* data and an AUCR ≥ 2, indicative of moderate inhibition or higher, were retained for further evaluation into the clinical relevance of the interaction. Although the current FDA guidance utilizes an AUCR ≥ 1.25 to indicate a clinical DDI, only moderate and strong inhibition was evaluated in this study to increase the likelihood that identified inhibitors will show clinically significant effects on sensitive OATP1B1/1B3 substrates.

4.2.5 *Identification of Clinical Index Inhibitors*

Following the identification of clinical inhibitors of OATP1B1/1B3, the identified drugs were subsequently evaluated as potential index inhibitors. Using labeling information, *in vitro*, and clinical data, the drugs with the lowest likelihood of contribution from other pathways were identified. These compounds were then evaluated as fit-for-purpose inhibitors, ranking those that are most selective for OATP1B1/1B3 and those that are broad inhibitors and results offer a determination of the worst-case scenario effect.

4.3 RESULTS

4.3.1 *In Vitro Inhibitors of OATP1B1/1B3*

Using the *in vitro* query function of the DIDB, 632 compounds from 2,258 studies were identified as potential inhibitors of OATP1B1 and/or OATP1B3. Only studies with reported IC_{50} values were evaluated further, resulting in a list of 321 compounds. When studies testing non-drug compounds, such as natural products and *in vitro* probes, and studies without at least one IC_{50} value $\leq 10\mu M$ were removed, 113 drugs remained for further evaluation. No metabolites were identified that met the selection criteria. Most of the identified compounds, 55%, had data for both isoforms while 36% and 9% had data for only OATP1B1 and 1B3, respectively (**Figure 4-1A**). To represent a worst-case scenario, only the lowest reported IC_{50} value was used in subsequent clinical predictions.

Following identification of the most potent *in vitro* inhibitors, R-values were calculated for 102 drugs with clinical data available. As mentioned above, C_{max} was determined from the average of all reported concentrations at the dose with the most studies available. Full calculations for R-value determination are available in **Table B-1**. Most drugs evaluated had R-values above the FDA cut-off value of 1.1 (68%). Among these drugs, 35% had clinical data supporting an interaction (AUCR $\geq$ 1.25 for a sensitive OATP1B1/1B3 substrate in the presence of the inhibitor) and 8% were not found to be clinical inhibitors (AUCR < 1.25). Interestingly, 57% of the drugs with R $\geq$ 1.1 did not have clinical data, positive or negative, available. For drugs that did not meet the FDA criteria for clinical evaluation, 35% had clinical data available, with 14% showing significant *in vivo* inhibition of OATP1B1/1B3 (**Figure 4-1B, Table B-2**). These five drugs – clarithromycin, daclatasvir, erythromycin, telmisartan, and velpatasvir – all

have at least one study conducted with a sensitive substrate available, with AUCR values between 1.25 and 4.45 (clarithromycin/pitavastatin and clarithromycin/atorvastatin, respectively). While clarithromycin does show inhibition of CYP3A and P-gp in addition to OATP1B1/1B3, these interactions are primarily attributable to the transporters of interest. It is unclear why the effect was underpredicted based on *in vitro* data, however it is possible that the IC_{50} value use in the calculations may be higher than what is observed *in vivo* due to substrate-specific effects.

4.3.2 *Identification of Clinical Inhibitors of OATP1B1/1B3*

Using the transporter-specific *in vivo* queries and queries of interactions involving known sensitive substrates of OATP1B1/1B3, 292 studies involving 70 compounds with an AUCR ≥ 1.25 were identified. When only drug products, excluding combination therapies which were evaluated separately, were considered, 46 drugs were retained. Finally, those most likely to result in a clinically significant interaction, defined as an AUCR ≥ 2 with a sensitive substrate, were identified which resulted in 13 drugs evaluated as clinical inhibitors (**Table 3-1**). Following identification of the clinical inhibitors, negative data were queried to ensure that a complete profile for each drug was considered. All identified clinical inhibitors had IC_{50} values ≤ 10 μM for at least one isoform and 10/13 drugs had calculated R-values ≥ 1.1. For the three that did not reach the FDA cut-off – clarithromycin, erythromycin, and velpatasvir - two showed borderline significance (clarithromycin and erythromycin; R = 1.097 and 1.096, respectively). Over half of the identified interactions were greater than 2-fold (61/90 studies, 68%), with 19% showing an increase in exposure greater than 5-fold. The highest change in exposure attributable primarily to OATP1B1/1B3 inhibition was observed for cyclosporine/pravastatin, 22.83-fold[39,61], followed by rifampin/atorvastatin, 12.0-fold.[131] It is important to note that four of the identified clinical

inhibitors are not currently approved by the FDA and therefore were not evaluated further. Additionally, two drugs – grazoprevir and velpatasvir – are only available as combination treatments and the co-formulated drugs are also potent OATP1B1/1B3 inhibitors (grazoprevir with elbasvir (OATP1B3 IC_{50} = 0.1 μM[60]; velpatasvir with voxilaprevir (OATP1B1 IC_{50} = 0.18 μM[147]) and sofosbuvir (OATP1B1 IC_{50} = 16.5 μM[148])), however no data were available for independent administration of the co-formulated drugs.

Additionally, 16 combination treatments have been evaluated as clinical inhibitors of OATP1B1/1B3. After selecting for those where studies used sensitive substrates and resulted in an AUCR ≥ 2, nine combination treatments were identified as clinically relevant inhibitors of OATP1B1/1B3 (**Table 4-2**). For these therapies, observed changes in exposure ranged from 1.19- to 9.36-fold. With the exception of three drugs, each component in these combination treatments was identified as potential clinical inhibitor based on an R-value ≥ 1.1. Since the components of these treatments did not have clinical data available from independent evaluation it is not possible to determine the nature of the combined interaction (synergistic, additive, etc) based on the data available. These treatments were exclusively approved as treatment for hepatitis C viral infection (HCV, 44%) or human immunodeficiency virus (HIV, 56%). Similar to the individual drugs identified, these combinations showed significant inhibition potency toward CYP3A and/or P-gp when studied with substrates of these pathways. Combination therapies were evaluated separately from stand-alone treatments due to the complex nature of these treatments, no further analysis to determine their utility as regulatory index inhibitors was performed.

Interestingly, very few of the identified clinical inhibitors had statements in the labeling reflecting the potential for OATP1B1/1B3-mediated interactions (**Table 4-3**, **Table B-3**). Of the

9 marketed drugs identified, only three – gemfibrozil, letermovir, and velpatasvir – had warnings surrounding OATP1B1/1B3 inhibition. The statements varied and were substrate-dependent, ranging from dose monitoring and reduction to contraindication. Supporting the lack of specificity for most inhibitors and the potential for confusion, five drugs had warnings for sensitive OATP1B1/1B3 substrates with regard to CYP3A inhibition or induction (rifampin), and no comments on possible OATP1B1/1B3-mediated interactions.

4.3.3 *Clinical Index Inhibitors of OATP1B1/1B3*

The 13 identified clinical inhibitors were subsequently evaluated as possible clinical index inhibitors. Ideal index inhibitors are selective for the transporter of interest and result in a consistent and measurable change in substrate AUC. Of the identified interactions (substrate/inhibitor pairs), 22/38 (58%) were unlikely to have significant contributions of other pathways to the observed interactions. For the 16 interactions where other pathways are likely involved, contributions from both CYP3A and P-gp was most common (44% of all multi-pathway interactions) with 19% and 13% showing only CYP3A or P-gp inhibition, respectively. Possible contribution from other transporters, such as BCRP and MRP2, accounted for the remaining 31% of multi-pathway interactions. When labeling recommendations for each inhibitor were considered, 5/13 (38%) had language regarding interaction with CYP3A and/or P-gp, typically in the context of compounds that are also sensitive OATP1B1/1B3 substrates such as atorvastatin (**Table 4-3**). From these data, rifampin appears to show the most utility as a clinical index inhibitor due to the highest proportion of interactions with no contribution of other pathways and is, in fact, one of two drugs currently recommended for use in clinical evaluations by the FDA.[38] While induction of CYP3A and P-gp is observed following multiple dose administration, this is unlikely to confound inhibition study results as most were completed using

a single rifampin dose (77% of identified studies). Conversely, cyclosporine, the other inhibitor currently recommended by the FDA, shows utility when the study goal is to identify the worst-case scenario outcome for a substrate where CYP3A and/or P-gp also contribute to the overall disposition of the drug.[38]

4.4 DISCUSSION

The impact of OATP1B1/1B3 inhibition on *in vivo* drug disposition continues to be a critical topic in current research on drug interactions involving transporters. Here, both preclinical and clinical data were evaluated to identify those drugs likely to significantly increase systemic exposure of sensitive substrates and from those, identify promising index inhibitors. By supporting clinical findings with *in vitro* data and labeling recommendations for these drugs, a complete and accurate presentation of the role that the drug plays in *in vivo* inhibition of OATP1B1/1B3 was achieved. As almost all inhibitors are not selective for OATP1B1/1B3, only studies involving previously identified selective substrates were retained to limit confounding contributions from other pathways. Despite these efforts, many interactions did show likely contributions from inhibition of CYP3A and P-gp. In this analysis, priority was given to those drugs where coadministration with a sensitive substrate resulted in an AUCR $\geq$ 2 and where *in vitro* data showed inhibition of OATP1B1 and/or OATP1B3. Again, these criteria were used to reduce the number of studies retained where the driving interaction mechanism did not include the transporters of interest. A significant number of the clinical inhibitors identified were parts of combination products. Due to the complex nature of combination therapies, these treatments were evaluated separately from single drug entities and not considered for subsequent evaluation as clinical index inhibitors. Overall, it is noteworthy that a majority of the identified clinical inhibitors, both single entity and combination therapies, are approved for the treatment of

infections: hepatitis C viral infection (10/22, 45%), human immunodeficiency virus (HIV) treatments and anti-infectives (23% each).

While this analysis did not identify novel possible index inhibitors, the data evaluated support the regulatory use of rifampin and cyclosporine. As cyclosporine is a broad metabolism and transport inhibitor, it shows utility for studies where inhibition of multiple metabolizing enzymes and transporters is desired. Rifampin, however, is more suited for those studies where only inhibition of OATP1B1/1B3 is desired. Despite the long-standing use of rifampin to evaluate the clinical role of OATP1B1/1B3, many aspects of its disposition and use as an index inhibitor, such as variability and proper dosing, have not been fully evaluated. When a single 600 mg oral dose of rifampin is administered, observed changes in AUC exhibit up to 62% variability with a specific substrate (45% - 62% for rosuvastatin/rifampin and pitavastatin/rifampin, respectively) with a 5-fold difference in AUCR across all studies. For the studies available, route of administration, intravenous versus oral, does not appear to significantly affect the interaction with the reported AUCRs for IV and oral rifampin being similar for all substrates. Because almost all studies were completed with a similar study design in healthy volunteers, the cause for this variability is not immediately clear. However, as no formulation data is available, it is possible that choice of formulation could be contributing to the observed differences.

Currently, 68% of inhibition studies are conducted using a single 600 mg oral dose of rifampin, the recommended clinical dose for treatment of tuberculosis. At this time, only nine studies are published using different doses – with 300 mg being the lowest tested. However, there is little evidence that the most commonly used dose is optimal for evaluating the clinical role of OATP1B1/1B3 for studies of NMEs. When static predictions are completed for doses available in literature (300 mg, 450 mg, and 600 mg) with the sensitive substrate pravastatin,

there is little difference in outcome between those doses (range 2.53 – 2.67, calculations presented in **Table B-4**). This lack of change is likely due to the fact that the resulting plasma concentrations are significantly higher than the lowest reported Ki for OATP1B1 ($I_{in,max,u}$ 3.58 µM – 8.72 µM, K_i = 0.278 µM[89]). Only two studies have used lower doses of rifampin, as low as 2 mg, however no plasma concentration data are available and the effect on OATP1B inhibition was not evaluated.[149,150] It is likely that lower doses of rifampin can be used in OATP1B inhibition studies, reducing the risk to patients while still providing maximal inhibition.

It should be noted that this evaluation was limited by the data available in the literature, both clinical and *in vitro*, for the drugs of interest. In some cases, potent *in vitro* inhibitors (identified as $R \geq 1.1$) are only marketed as a combination treatment. This results in limited information regarding the clinical effect of the single drug entity. It is likely that more clinical inhibitors could be identified if and when such data become available. Furthermore, this approach does not account for *in vitro* inhibitors that are either in early development and do not have clinical studies conducted, or for drugs that have been voluntarily discontinued by the sponsor. These data are also heavily influenced by substrate selection, as the OATP1B transporters are known to show substrate-dependent transport and no selective substrates have currently been identified. This can be observed in the variable potency of each clinical inhibitor identified, due in part to contributions from other pathways in the observed interactions. Finally, while no metabolites were identified as clinical inhibitors in this work, they cannot be discounted as contributors to observed DDIs. Many known inhibitors of OATP1B1/1B3, such as gemfibrozil and cyclosporine, have inhibitory metabolites which are likely contributing to the magnitude of the observed interactions. Particularly in cases where metabolites circulate at equal or higher concentrations than the parent compound, potential for metabolite-based interactions should be

considered. This study serves as a starting point for the evaluation of relevant clinical inhibitors and as support for the currently recommended index inhibitors used in the regulatory evaluation of OATP1B1/1B3.

In sum, 13 drugs and nine combination therapies were identified as clinical inhibitors of OATP1B1/1B3. While no novel potential index inhibitors were identified, this work confirms that rifampin and cyclosporine remain the best options for targeted and worst-case evaluations of clinical OATP1B1/1B3 inhibition, respectively.

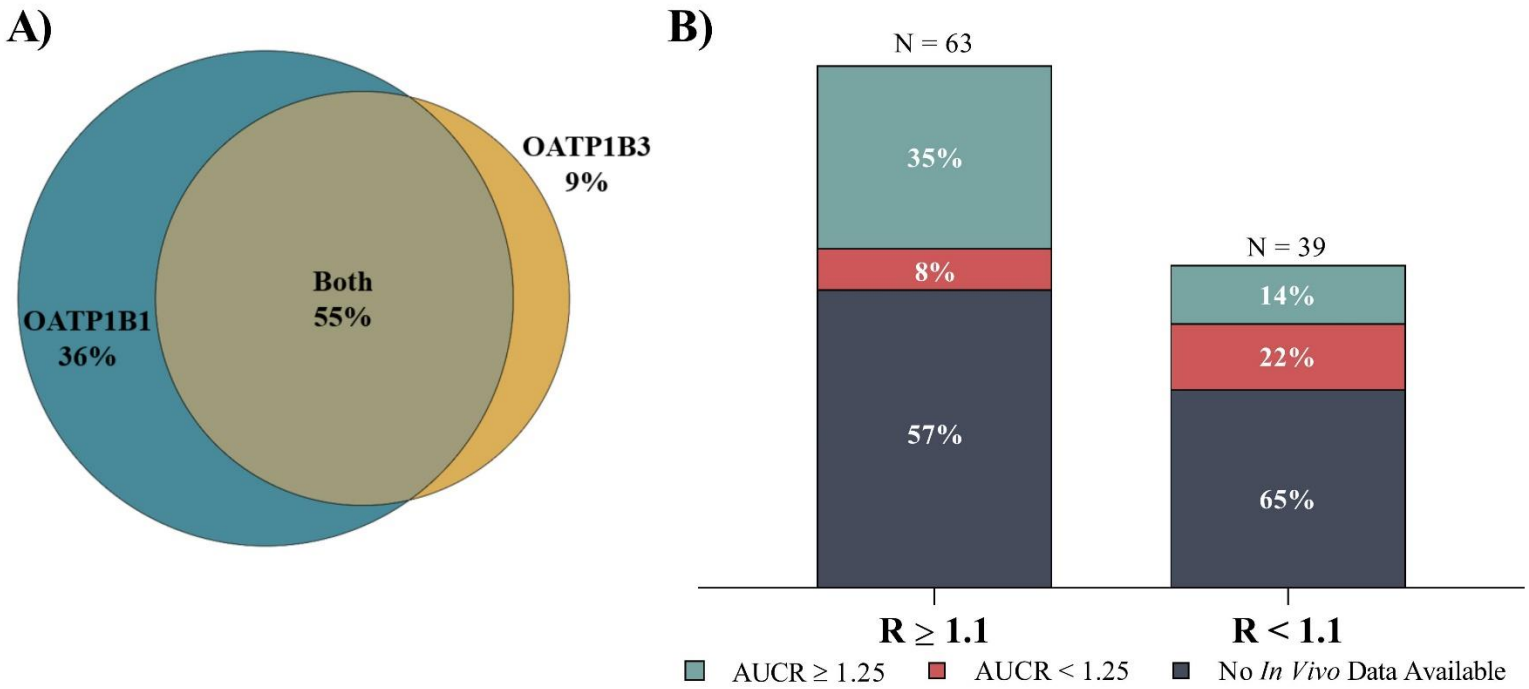

Figure 4-1. *In vitro* overlap between isoforms (A) and clinical data availability by R-value (B).

While a majority of compounds were evaluated for both isoforms, inhibitors potentially selective for OATP1B1 and 1B3 were identified. For those drugs with a calculated R-value above the FDA cut-off value, R ≥ 1.1, 43% had clinical data available (35% showing an interaction) while 36% of drugs below the FDA cut-off had clinical data available. Regardless of the R-value significance, most drugs did not have clinical data available (57% and 65% for R ≥ 1.1 and < 1.1, respectively).

Table 4-1. Identified clinical inhibitors of OATP1B1/1B3 with an AUCR ≥ 2.

				Inhibitor Dosing					
Inhibitor	**OATP1B1/1B3 IC_{50} (μM)[a]**	**R-Value**	**Substrate**	**Route of Administration**	**Dose**	**Single / Multiple**	**N[b]**	**AUCR[c]**	**Other Pathways Likely Contributing to Observed AUCR[d]**
Boceprevir	6.3 / 4.9	5.2	Atorvastatin	oral	800 mg	multiple	1	2.25	CYP3A, P-gp
			Pravastatin	oral	800 mg	multiple	1	1.46	--[e]
Clarithromycin	5.1 / 9.8	1.097	Atorvastatin	oral	500 mg	multiple	5	1.82 – 3.05	CYP3A, P-gp
			Pitavastatin	oral	500 mg	multiple	1	1.24	--[e]
			Pravastatin	oral	500 mg	multiple	1	2.11	--[e]
			Rosuvastatin	oral	500 mg	multiple	1	1.56	--[e]
Cyclosporine	0.019 / 0.032	123.78	Atorvastatin	oral	variable[f]	multiple	3	7.45 – 15.31	CYP3A, P-gp
			Eluxadoline	oral	600 mg	single	2	4.18, 4.20	--[e]
			Letermovir	oral	200 mg	single	2	2.10, 3.37	--[e]
					50 mg	single	2	1.90, 2.31	
			Pitavastatin	oral	variable[f]	single	1	4.52	--[e]
			Pravastatin	oral	variable[f]	multiple	4	9.93 - 22.84	MRP2
			Rosuvastatin	oral	variable[f]	multiple	1	7.08	BCRP
Erythromycin	4.88 / 27.0	1.096	Atorvastatin	oral	500 mg	multiple	1	1.33	CYP3A, P-gp
			Pitavastatin	oral	500 mg	multiple	1	2.79	--[e]
			Rosuvastatin	oral	500 mg	multiple	1	0.72	--[e]
Faldaprevir	0.57 / 0.18	2.07	Atorvastatin	oral	240 mg	multiple	1	9.42	CYP3A
			Rosuvastatin	oral	240 mg	multiple	1	14.66	BCRP, P-gp, MRP2
Gemfibrozil	4.0 / 10.0	1.64	Atorvastatin	oral	600 mg	multiple	2	1.24, 1.35	--[e]
			Pitavastatin	oral	600 mg	multiple	1	1.25	--[e]
			Pravastatin	oral	600 mg	multiple	1	2.02	--[e]
			Rosuvastatin	oral	600 mg	multiple	2	1.88	--[e]
				other[g]	600 mg	single	1	1.07	
Grazoprevir	0.7 / 1.1	1.31	Atorvastatin	oral	200 mg	multiple	1	3.00	CYP3A
			Pitavastatin	oral	200 mg	multiple	1	1.11	--[e]

			Rosuvastatin	oral	200 mg	single	1	1.59	BCRP
Itraconazole	< 0.19 / 0.71	1.28[h]	Atorvastatin	IV	200 mg	multiple	1	1.09	CYP3A, P-gp
				oral	200 mg	multiple	4	1.47 – 5.58	
			Pitavastatin	oral	200 mg	multiple	3	0.79, 0.80	--[e]
						single	1	0.96	
			Pravastatin	IV	200 mg	multiple	1	1.47	--[e]
				oral	200 mg	multiple	3	1.12 – 1.72	
			Rosuvastatin	oral	200 mg	multiple	3	1.26 – 1.78	--[e]
Letermovir	2.9 / 1.1	1.28	Atorvastatin	oral	480 mg	single	1	3.28	CYP3A
Rifampin	0.39 / 0.22	52.19	Atorvastatin	oral	300 mg	single	1	3.60	P-gp

[a]Lowest reported literature value for each isoform

[b]Number of studies identified for each substrate/inhibitor/dose.

[c]AUCR range presented for $N \geq 3$, individual study results presented for $N \leq 2$.

[d]P-gp has been identified to have a minor role in the disposition of pravastatin and pitavastatin. However, the contribution is minimal and unlikely to contribute to the interactions presented.

[e]No other pathways expected to significantly contribute to the observed interaction

[f]No dose stated, patients were treated to maintain the desired plasma concentration of the drug.

[g]Intrajejunal dose

[h]The IC_{50} value for OATP1B3 was used to calculate the R-value

Table 4-2. Combination therapies identified as clinical inhibitors of OATP1B1/1B3

Precipitant	Object	Precipitant Dose	N[a]	AUCR[b]	Other Pathways Likely Contributing to Observed AUCR[c]	Reference
Atazanavir/Ritonavir	Rosuvastatin	300 mg/100 mg	1	3.13	--[d]	142
Darunavir/Ritonavir	Atorvastatin	300 mg/100 mg	1	3.40	CYP3A, P-gp	139
	Pravastatin	600 mg/100 mg	5	***1.08*** - 1.23	--[d]	139
	Rosuvastatin	600 mg/100 mg	1	1.51	--[d]	141
Elbasvir/Grazoprevir	Atorvastatin	50 mg/200 mg	1	1.95	CYP3A	60
	Pravastatin	50 mg/200 mg	1	1.33	--[d]	60
	Rosuvastatin	50 mg/200 mg	1	2.25	BCRP	60
Glecaprevir/Pibrentasvir	Atorvastatin	400 mg/120 mg	1	8.28	CYP3A, P-gp	151
	Pravastatin	400 mg/120 mg	1	2.30	--[d]	151
	Rosuvastatin	400 mg/120 mg	1	2.15	BCRP	151
Lopinavir/Ritonavir	Pitavastatin	400 mg/100 mg	1	***0.83***	--[d]	138
	Rosuvastatin	400 mg/100 mg	1	2.08	--[d]	67
Paritaprevir/Ritonavir/Ombitasvir/ Dasabuvir	Pravastatin	150 mg/100 mg/ 25 mg/400 mg	1	1.82	--[d]	144
	Rosuvastatin	150 mg/100 mg/25 mg/400 mg	1	2.59	BCRP	145
Saquinavir/Ritonavir	Atorvastatin	400 mg/400 mg	1	3.93	CYP3A, P-gp	68
Sofosbuvir/Velpatasvir/ Voxilaprevir	Pravastatin	400 mg/100 mg/ 200 mg	1	2.13	--[d]	147
	Rosuvastatin	400 mg/100 mg/ 200 mg	1	7.35	BCRP	147
Tipranavir/Ritonavir	Atorvastatin	500 mg/200 mg	1	9.36	CYP3A, P-gp	146
	Rosuvastatin	501 mg/200 mg	1	1.37	--[d]	146

[a]Number of studies identified for each substrate/inhibitor/dose.

[b]AUCR range presented for N ≥ 3, individual study results presented for N ≤ 2. Values in bold are not significant interactions as stated by the authors.

[c]P-gp has been identified to have a minor role in the disposition of pravastatin and pitavastatin. However, the contribution is minimal and unlikely to contribute to the interactions presented.

[d]No other pathways expected to significantly contribute to the observed interaction

Table 4-3. Labeling recommendations for clinical inhibitors of OATP1B1/1B3

Clinical Impact of OATP1B1/1B3 Inhibition

Inhibitor	R ≥ 1.1	AUC ≥ 1.25	Initial FDA Approval Date	Combination Only	Type of Labeling Recommendation	Product/Formulation Evaluated (revision date)
Gemfibrozil	X	X	1981		Dose Reduction with OATP1B1/1B3 substrates Contraindication with simvastatin and repaglinide	LOPID tablet (April 2018)
Letermovir	X	X	2017		Dose reduction for atorvastatin Coadministration not recommended for pitavastatin, simvastatin	PREVYMIS tablet (Nov 2017)
Velpatasvir		X	2016/2017	X	Dose adjustment for pravastatin, atorvastatin, fluvastatin, lovastatin, simvastatin Coadministration is not recommended for rosuvastatin, pitavastatin	VOSEVI tablet (Nov 2017)

Effect on OATP1B1/1B3 Substrates by CYP3A Inhibition

Inhibitor	R ≥ 1.1	AUC ≥ 1.25	Initial FDA Approval Date	Combination Only	Type of Labeling Recommendation	Product/Formulation Evaluated (revision date)
Clarithromycin		X	1991		Contraindicated with simvastatin	BIAXIN suspension (Dec 2018)
Cyclosporine	X	X	1990		Statin dose should be reduced	SANDIMMUNE capsule (March 2015)
Erythromycin		X	1965		Do not use lovastatin, atorvastatin, or simvastatin	E.E.S. granule (April 2018)
Itraconazole	X	X	1992		Contraindicated with lovastatin/simvastatin Monitoring and/or dose reduction required for coadministration with atorvastatin	TOLSURA capsule (Dec 2018)
Rifampin[a]	X	X	1971		CYP3A-mediated metabolism of some statins may be accelerated	RIFADIN capsule (Nov 2010) RIFAMPIN injectable (Dec 2018)

The following clinical inhibitors did not have any language regarding OATP1B1/1B3 or substrates of the transporters in the labels evaluated: boceprevir, faldaprevir, grazoprevir, simeprevir, telaprevir, velpatasvir.

[a]Inductive effects of rifampin as discussed in the label only apply to multiple dose regimens.

Chapter 5. DRUG-DRUG INTERACTIONS OF INFECTIOUS DISEASE TREATMENTS IN LOW INCOME COUNTRIES: A NEGLECTED TOPIC?

(A version of this chapter was published in Clinical Pharmacology and Therapeutics 105 (6), 2019.)

5.1 INTRODUCTION

Contrary to the situation in developed countries, major infectious diseases such as human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), malaria, and tuberculosis (TB) continue to cause the majority of deaths in low income countries (LICs) worldwide.[152,153] Even when effective treatment options exist, a limited understanding of what constitutes safe and effective use of these medications may lead to adverse drug reactions or loss of efficacy, with the later contributing to drug resistance. An overarching risk factor is ineffective management of drug-drug interactions (DDIs) that can lead to changed systemic exposure, resulting in variations in drug response of the co-administered drugs.[154] Recognizing the significance of DDIs, leading regulators in the world require assessment and management of DDIs as an integral part of the development of a new drug prior to its approval, and strategies to manage these DDIs are routinely included in prescribing information.

Patients with infectious diseases in LICs are often predisposed to potential DDIs. Today, effective treatment of HIV, TB, or malaria frequently includes two or more drug molecules with diverse mechanisms of actions. Co-infection (e.g., TB in HIV patients) and concomitant non-infectious disease, particularly with an aging population, requires the use of additional drugs, increasing the potential for DDIs. Despite recognition of DDIs by drug developers and

regulators, management of DDIs and education of health care providers to ensure safe and effective use of anti-infectives in LICs has not gained much attention. While this is an area requiring significant consideration, there is currently a paucity of data available regarding optimal anti-infective use in these patients and there are significant delays between revisions of dosage guidelines adding to the need for a better understanding of these drugs. Using TB infection to illustrate these problems, three central aspects regarding the identification and management of DDIs in LICs will be reviewed – (i) the DDI potential of anti-infectives from pharmacological standpoints, (ii) the potential barriers to effective management of DDIs in the LIC setting, including challenges with co-infection and co-medication, and (iii) areas for future research so that optimal treatment at the individual patient level can be achieved.

5.2 Pharmacokinetic DDIs – Determination and Current Regulatory Expectations

There are two main categories of DDIs – pharmacodynamic (PD) and pharmacokinetic (PK). In general, PD-DDIs occur when the clinical effect of the victim drug is changed by the perpetrator, while PK-DDIs result from modulation of a process such as absorption, metabolism, or elimination of the victim drug by a perpetrator drug. Once characterized, PK-DDIs can often be managed effectively through methods such as changes in dosage or timing of administration. Only PK-DDIs will be discussed here as they relate to management in the LIC setting.

Current regulatory guidances require testing for possible DDIs in early drug development for any new molecular entity (NME).[38,64] The testing is typically achieved through a combination of *in vitro*, *in vivo*, and *in silico* studies to identify the metabolic and/or transport pathways susceptible to inhibition or induction, and to quantify the magnitude of interaction. Index substrates and inhibitors/inducers are used in clinical DDI studies (for evaluation of the NME as

an inhibitor/inducer and substrate, respectively) to prospectively determine mechanistic interactions as these compounds have a predictable change in exposure and the metabolic/transport pathways involved are well documented. Clinical studies can also be completed with medications commonly co-administered in the target population to determine DDI potential between co-medications and the NME. Besides these standalone and prospective DDI studies, one can assess DDI potential by collecting sparse samples from a nested study within a large trial (Phase II or Phase III) and employ population PK (popPK) modeling to analyze data obtained from the study. If adequately designed, popPK analyses can help "characterize the clinical DDI and determine recommendations for dose modifications when investigational drug is a substrate".[38]

Because clinical DDI studies may have limitations to inform untested clinical scenarios, such as the effect of dose regimens or of an inhibitor/inducer with different interaction potency, major regulators recommend the use of *in silico* methods such as physiologically-based pharmacokinetic (PBPK) modeling and simulations to complement the overall DDI assessment.[38] A PBPK model combines physiological knowledge of the target population and drug characteristics (e.g., PK, physiochemical, absorption, and disposition properties) to define the PK of the drug.[155,156] The development of sophisticated models allows for the simulation of PK changes under various clinical scenarios by incorporating multiple interaction mechanisms and effects of several patient factors. [157]

Depending on the confidence of use, predictions using PBPK can be used in lieu of additional clinical DDI studies to support product labeling.[158] For example, prediction of the effect of moderate or weak perpetrators may replace dedicated clinical DDI studies, provided that the PBPK model is verified with clinical PK data and information from dedicated DDI

studies that used strong index inhibitors/inducers (drugs that increase or decrease the AUC of a sensitive substrate ≥ 5-fold).[38] PBPK models can also be used to research into DDI risk for previously understudied populations such as pregnant women and children. Development of physiological models representative of these populations is critical for quantitative assessment of DDIs in these populations.[159–161] It has to be recognized that application of PBPK is limited by the availability of data for both the drug (such as permeability and transporter involvement) and the population.

5.3 Hurdles to Effective Management of DDIs in LICs

Effectively mitigating the risk of PK-DDIs in LICs has unique challenges that are not applicable in other regions, and there are important caveats to directly translating the findings of clinical studies to practical execution in these regions. In all regions, combination therapy is increasingly the mainstream strategy for anti-infectives and treatments for HIV/AIDS (differentiated here from anti-infective drugs for descriptive and comparative purposes). One advantage is to use more than two drugs with different mechanisms of action to synergistically combat a pathogen. However, many of these drugs are designed and evaluated from a monotherapy standpoint, as opposed to combination therapy as they will be used clinically. Furthermore, unlike the development of monotherapy, whose safety and efficacy in humans can be tested at various dose levels, development of combinations may be limited by the permutation of different doses for each partner drug in clinical trials, making it challenging to adequately determine full PK- and PD-DDI potentials and to select optimal doses.

Beyond the issues that are faced in managing DDIs in all regions, the interpretation of PK-DDIs in LICs is complicated by the current lack of understanding of the effects of comorbidity and other intrinsic factors. For example, malnutrition may affect the PK of test

drug(s) and a patient with co-infection may take medications in addition to those for one target infection.[162,163] In conventional drug development, the understanding of PK-DDIs is often based on studies completed in healthy adult volunteers. While this is typically the most straight-forward approach, as the contribution from other factors is minimal, these results may not be applicable in determining DDIs in target populations in LICs. Compared to healthy subjects, patients can display changes in drug disposition that are unexpected due to factors associated with infection, such as dehydration or changes in gastrointestinal motility from diarrhea, and disease progression dependent drug disposition (e.g. altered metabolism and therefore clearance). Subsequently, the response to DDIs can differ.

5.4 Current Understanding of DDIs Associated with the World Health Organization (WHO)-Recommended Treatment for Tuberculosis

As mentioned earlier, the use of multiple medications is commonplace for the treatment of major infectious diseases and co-infection requires that medications indicated for different infections be used concomitantly. Metabolism- and transport-based DDIs among concomitant medications may lead to increased or decreased drug exposure, putting patients at risks of adverse events, loss of efficacy, and drug resistance. This section reviews the groups of drugs recommended for treatment of TB in LICs and potential PK-DDIs among partner drugs in cases of common co-infections, such as with HIV, according to known or suspected mechanisms. We investigated the latter using the University of Washington Metabolism and Transport Drug Interaction Database (DIDB®, www.druginteractioninfo.org).

5.4.1 *DDI Potentials of TB Drugs*

With over 10 million new cases in 2017, most occurring in LICs, TB is one of the leading causes of death worldwide.[164] For treatment of drug-susceptible TB, the WHO recommends a 6-month course of antibiotics – two months of daily isoniazid (H), rifampin (R), pyrazinamide (Z), and ethambutol (E), followed by four months of isoniazid and rifampin, referred to as 2HRZE/4HR. A fixed-dose combination of these anti-TB drugs is recommended since compliance is higher than with separate drugs.[165] Dosing strategies and PK properties pertaining to metabolism- and transport-mediated DDI potential for these anti-TB partner drugs are summarized in Table 1. From a delivery standpoint, current treatment guidelines from the WHO seem to focus on convenience and compliance. Obviously, the one-dose-fits-all approach offers a simple solution for delivery, and the use of fixed-dose combinations allows all medications to be taken on schedule to ensure compliance. However, these may not fit the needs of the individual patient and can limit individualized dose adjustments needed to minimize the risk of PK-DDIs.

Except for rifampin, the recommended anti-TB drugs considered as victim drugs are primarily renally cleared with metabolism occurring by non-CYP enzymes; therefore, DDI potential due to CYP modulation is not a primary concern. However, some of these drugs are substrates of polymorphic non-CYP enzymes and/or transporters, and therefore the PK of these drugs is likely to be altered in patients with impaired enzyme/transporter functions. For example, *in vitro* studies suggest that rifampin is a substrate of the major hepatic uptake transporters organic anion transporting peptides (OATPs), with K_m values of 1.5 μM[76] and 2.3 μM[166,167] for OATP1B1 and OATP1B3, respectively. With chronic treatment of rifampin in TB patients (median daily dose of 15.8 mg/kg) the exposure to rifampin was 3.16- and 2.88-fold higher in

patients homozygous (N = 77) and heterozygous (N = 34) for the 388A>G mutation in *SLCO1B1*, the gene encoding the OATP1B1 transporter, respectively, compared to the reference group (*SLCO1B1* 388A/388A, N = 2).[168]

Similarly, isoniazid is mainly metabolized by the polymorphic enzyme N-acetyltransferase 2 (NAT2). After administration of a 300 mg single dose of isoniazid in healthy subjects, the AUC of isoniazid was more than 4-fold higher in NAT2 poor metabolizers (PM, *NAT2**5D/*7A and *6B/*6B) compared to normal metabolizers (NM, *NAT2**4/*4).[169] A similar exposure increase was observed in NAT2 poor metabolizer TB patients compared to NM TB patients who were on chronic treatment (5 mg/kg daily).[170] With a significant fraction of individuals in LICs showing a PM phenotype (approximately 33% of individuals in Sub-Saharan Africa[171] and 48% of Senegalese[172], for example) significant increases in exposure are a common treatment concern for TB in these regions.

The first-line TB partner drugs are inhibitors and inducers of many CYP enzymes and transporters, making them common perpetrators of DDIs (Table 1). To illustrate, rifampin is a known perpetrator of many interactions and is a recommended *in vitro* and as a clinical index inducer of multiple CYPs and as an inhibitor of OATP1B1/1B3 by the US Food and Drug Administration (FDA).[173] As an inducer, rifampin affects many CYP enzymes (e.g., CYPs 1A2, 2B6, 2C8, 2C9, 2C19, and 3A4), phase II enzymes such as uridine 5'-diphospho-glucuronosyltransferases (UGTs), as well as transporters such as P-glycoprotein (P-gp). As an inhibitor, rifampin primarily affects OATP1B1/1B3.[38,64,173] With this broad scope of potential interactions, rifampin has been extensively studied and a wealth of *in vitro* and clinical DDI data is available. This prompted us to use rifampin to investigate the current state of DDI evaluation in LICs.

A search of the DIDB yielded over 1,600 DDI studies (*in vitro* and clinical) involving rifampin. *In vitro*, there were 664 studies using rifampin as the perpetrator (inhibition or induction), with 85% concluding in a positive DDI. Similarly, rifampin has been predominantly studied clinically as a perpetrator, with 96.3% of the 1,007 clinical DDI studies using rifampin as an inhibitor, and 87.9% concluding in a positive DDI (defined by the FDA as changes in victim exposure $\geq 25\%$).[38] While study design (i.e., frequency and magnitude of dose) can affect the observed changes in victim exposure and subsequent conclusions, the studies identified in the query almost exclusively utilized a 600 mg oral dose (single administration to evaluate inhibition and multiple doses for induction).

Clinical DDI studies using rifampin as the perpetrator were completed for a broader range of compounds than *in vitro* (113 substrates were evaluated *in vitro*, and only 37 of those compounds were evaluated both *in vitro* and *in vivo*) and included over 300 different compounds as victims. These include 294 (83%) victims of induction, 24 (7%) for inhibition, and 35 (10%) for both. Identified interactions ranged from a 99.7% decrease in victim AUC (induction, rifampin – budesonide[174]) to an increase in victim AUC of almost 1,400% (inhibition, rifampin – asunaprevir[45]). In addition to the wide range in the magnitude of interactions, these studies were performed for drugs in 90 classes from 24 diverse therapeutic areas (**Figure 5-1a**) illustrating the high potential for DDIs during TB treatment. Two therapeutic areas were investigated in more detail, anti-infectives and HIV/AIDS treatments, as co-infection is common in LICs. Investigation of potential DDIs between rifampin and these common treatment classes for co-infection account for 19% of identified studies – 10.7% between rifampin and anti-infectives and 7.2% with treatments for HIV/AIDS (**Figure 5-1b** and **Figure 5-1c**).

5.4.2 *DDI Under Co-Infection*

In individuals living with HIV, co-infection with TB is approximately 20 times more likely compared to those without HIV. In fact, just over half of the reported cases of TB in 2017 were in people living with HIV, most of whom were already on antiretroviral therapy.[164] Of the four preferred first-line treatments for HIV – efavirenz, emtricitabine, lamivudine, and tenofovir disoproxil fumarate - all are substrates of multiple enzymes and transporters that are inhibited and/or induced by rifampin. These drugs are not the exception, as the alternative first-line treatments such as dolutegravir and nevirapine are also substrates of enzymes and transporters that can be affected by rifampin. In fact, higher dosages of dolutegravir are recommended in those taking concomitant rifampin to ensure adequate plasma concentrations.[175] Among the first-line HIV medications, efavirenz is most susceptible to interactions because it is a substrate of CYP2B6, an enzyme that is not only polymorphic but also inducible by rifampin. When studied in healthy volunteers, repeated dosing of rifampin (450 mg/day for 7 days) significantly decreased the AUC of efavirenz (38.6%).[176] This result has also been observed in multiple other studies completed in healthy volunteers, with a significant decrease in efavirenz exposure by co-administration with rifampin (range: 17.8% - 61.0% decrease)[177] and in all cases the decreased exposure is attributed to induction of CYP2B6 by rifampin (EC_{50} = 0.127 µM)[178]. With the observed decreases in exposure, it is recommended to increase efavirenz dose in adult patients who receive concurrent rifampin to ensure that adequate plasma concentrations are achieved.[179] Low-dose (400 mg) efavirenz has been conditionally approved as treatment by the WHO to reduce the occurrence of adverse events, however the PK and efficacy in patients also receiving TB treatment has yet to be determined.[175]

However, interpretation of the results from a healthy volunteer study for efavirenz is highly complicated. First, CYP2B6 is polymorphic, and efavirenz itself can induce multiple drug metabolizing enzymes (CYPs and UGTs). Indeed, more than 20 clinical studies with chronic efavirenz treatment (600 mg daily) in patients have shown substantial increases of 2- to 4-fold in the exposure of efavirenz in CYP2B6 PM or intermediate metabolizers compared to patients with normal CYP2B6 function, with one study showing a 44-fold increase in the AUC of efavirenz in CYP2B6 PMs (*CYP2B6* 516T/516T, N = 2) compared to NMs.[180] Without genotyping prior to initiating treatment, it is possible that patients may have significantly higher exposure to efavirenz than expected, which further increases their risk for adverse events – both as a victim of interactions and as a perpetrator of comedications. Second, decreases in efavirenz exposure induced by coadministration with rifampin in healthy subjects do not manifest in patients co-infected with TB and HIV. In one study, efavirenz clearance decreased by 29.8% in patients co-infected with TB and HIV receiving dual treatment, as compared to those only receiving HIV treatment.[181] In other studies, non-significant but variable changes in efavirenz AUC, ranging from a 6.7% decrease to a 1.9% increase, have been reported.[182] This highlights that co-infection, co-medication and other intrinsic factors unique to patients, together can change the PK of both the perpetrator and victim drugs, leading to novel interactions or magnitudes of interactions that cannot be readily extrapolated from findings in healthy subjects.

Similar to HIV, co-infection of TB with malaria is common in LICs and the recommended treatments for malaria show a high susceptibility to DDIs.[183] In fact, studies have shown that induction of metabolic pathways by rifampin causes significant decreases in drug exposure for the primary therapies including artemether, lumefantrine, mefloquine, and quinine.[177,183] These changes can cause treatment failure as the systemic concentrations are

below the level needed to fully combat the infection. To illustrate, in healthy volunteers, concomitant administration of rifampin significantly increased mefloquine metabolism (281% increase in clearance), reducing plasma concentrations of mefloquine by 67.9%.[184] In patients with uncomplicated falciparum malaria treated with quinine, the addition of rifampin resulted in a 75.4% decrease in quinine AUC and 18.1% decrease in C_{max}. This decrease in quinine concentrations was associated with a 5-fold increase in re-infection compared to those patients only receiving quinine. These significant changes in exposure and subsequent treatment failures can be attributed to induction of CYP3A, evidenced by significantly higher metabolite exposure (5-fold increase) in those taking rifampin.[185]

As another example, rifampin caused significant decreases in exposure for partner drugs in Coartem® (a fixed dose combination including artemether and lumefantrine) administered in a HIV-positive Ugandan population without comorbid malaria. In these patients, the AUC of both artemether and lumefantrine were significantly and appreciably decreased in the presence of rifampin (89% and 68% decreases, respectively).[186] This is again consistent with induction of enzymes such as CYP2B6 and CYP3A4, and possibly the induction of intestinal efflux transporters such as P-gp, resulting in lower absorption of the drugs.[186] These findings imply that patients with TB and malaria co-infection may require higher doses of antimalarial drugs that are susceptible for DDIs resulting from induction of P-gp and/or metabolizing enzymes.

5.4.3 *Disease Effect on Drug PK*

Concerns in co-infected populations are not solely limited to interactions between treatments for the infections. It cannot be assumed that the PK of either the victim or perpetrator compounds are consistent among healthy subjects, singly infected, and co-infected patients. A study conducted in TB/HIV co-infected patients in Burkina Faso found that while rifampin

exposure was increased when it was used in a combined therapy, with the exact mechanism for the increase still unknown but likely due to increased absorption and/or decreased clearance due to liver toxicity caused by drugs such as nevirapine, systemic concentrations of rifampin still remained markedly lower than in other populations.[187] In fact, no subjects in the study had sustained plasma levels of rifampin above the accepted therapeutic threshold of 8 µg/mL after ten weeks of standard dosing (10 mg/kg/day).

This unexplained decrease in exposure is not exclusive to rifampin. A similar decrease in exposure in co-infected patients compared to those with only TB infection has also been observed for isoniazid (C_{max} = 11 µg/mL in TB patients compared to 7.0 µg/mL in TB/HIV patients).[188] For many anti-infectives, threshold concentrations must be reached for effective treatment, and significant decreases in exposure such as these may lead to an increased risk for treatment failure and more importantly, an increased risk of development of drug resistance. Conversely, quinine also shows disease-dependent changes in PK, with clearance in malaria patients being significantly decreased compared to healthy subjects.[189] While the exact mechanisms for these disease-related changes are unknown, it is likely that changes in absorption, protein binding, and altered hepatic function can all contribute to changes in systemic concentrations.[189,190] These changes in exposure are still unable to be accurately predicted due to the number of covariates present, resulting in a unique challenge not only in ensuring effective treatment, but also in accurately determining risk and developing strategies to mitigate potential DDIs.

5.4.4 *Target Global Health Populations*

Beyond the inherent complexities of treatment of co-infections, the understanding and evaluation of DDIs in LICs is further complicated by the occurrence of infection and co-infection

in specific populations, such as children, pregnant women, and women on oral contraceptives. Conventionally considered as special populations in mainstream drug development, these populations in fact are target populations of product development in global health. Due to the paucity of data on both the expected PK and expected severity of DDIs in these patients from the inherent difficulties in conducting clinical research to collect such data, the potential risk almost always has to be extrapolated from healthy, non-pregnant adults in order to optimize dose selection. Such extrapolation is not straightforward and is challenged by the lack of quantitative understanding of the unique physiology of these patients that may impact the PK and PD characteristics of both victim and perpetrator drugs. For example, a recent study comparing the AUC fold-change of 24 drug pairs in adult and pediatric patients showed that more often than not, there was a significantly different magnitude of effect between the two groups (69.7% of pediatric studies were > 1.25-fold or < 0.8-fold of the adult values).[191] Research is needed to systematically understand such age-related differences.

Treatment of TB in pregnant women is also not immune from this imbalance in research. Currently, the WHO does not recommend any changes in treatment protocol for pregnant women.[165] While first line treatments are currently considered safe for both mother and fetus, there is little research supporting this and at least three medications – isoniazid, rifampin, and ethambutol – are able to cross the placental barrier and are known to have an increased risk for adverse events such as hepatotoxicity.[192] Research on TB treatment in children also lags behind that of adults in LICs. Historically, a combined approach of 2HRZE/4HR, the same as what is preferred with adults, has been used to treat TB in children without appreciating developmental and ontogeny changes. While the 2014 WHO guidance on treatment of TB in children did propose updated daily doses, these changes are based primarily on observational studies and

"moderate-quality" evidence.[193,194] Although dose modification could help to ensure that sufficient concentrations of drug are reached, there is little evidence on the safety of these doses in children and limited understanding of the potential for hepatotoxicity. These populations become more complex when co-infection exists. It is estimated that almost half of adult HIV-related TB deaths in 2017 were in women of childbearing age.[195] Pregnant women with concurrent TB/HIV infection face higher risk of poor delivery outcomes and higher mortality rates.[196,197] Co-infection and subsequent comedication increase the potential for drug interactions far beyond what is predicted for non-pregnant adults due to the physiological changes during pregnancy that can dramatically affect drug exposures in both pregnant women and fetus.

Children also bear the burden of co-infection with over 50,000 TB-related deaths in 2016 occurring in children living with HIV.[197] In children, the relationships between exposure and toxicity as well as effective dosing for anti-HIV treatments, especially when combined with TB medications such as rifampin, are still unknown. To illustrate, in children under the age of 3 years old who were cotreated for HIV/TB infection, nevirapine exposure was 41% lower compared to those without TB.[198] Additionally, nevirapine concentrations are more variable than those seen in adults, which makes prediction of interactions much more difficult. Similar changes in exposure have also been found for rifabutin, a rifampin alternative. In a clinical study evaluating the PK and safety of rifabutin in children also on anti-HIV treatment, severe neutropenia was observed for all subjects resulting in the early termination of the study.[199] It was found that concentrations of rifabutin were more than 2-fold higher than those found in adult studies, which could be attributed to decreased CYP3A metabolism due to immature enzyme function and inhibition by ritonavir (HIV treatment).

Similarly, DDIs with oral contraceptives has been a growing topic of research in recent years as oral contraceptives rely on a minimum concentration for efficacy and changes in exposure can result in treatment failure.[200] For example, co-administration of rifampin and dienogest (DNG)/estradiol valerate resulted in an 83% decrease in DNG AUC and 44% decrease in the AUC of estradiol. This resulted in concentrations falling below the minimum effective concentrations leading to an increased risk for unplanned pregnancies.[201]

5.5 Future Directions

The challenges and hurdles described in the previous sections call for the need to establish a quantitative understanding of relevant population-specific attributes and drug-related properties for effective assessment of DDIs in target populations of LICs. Encouragingly, research into drug disposition and interactions in special populations has increased in recent years, which has led to enhanced understanding of the physiological component of a PBPK model for these populations (virtual populations).[159,202–204] For example, a dedicated guidance on diagnosis and management of TB in children was released by the WHO in 2014, marking the first departure from a uniform treatment approach for adults and children. Here, dosage recommendations could be updated to reflect age-specific predictions for exposure based on enzyme ontogeny and accumulated clinical data in those under the age of 10 years old.[194] Despite such advances, large gaps still remain in the fundamental understanding of many population specific variables, especially for those in LICs.

To fully understand the current landscape, existing DDI studies with co-medications for relevant anti-infective and HIV/AIDs drugs first need to be evaluated in detail to identify specific areas requiring further investigation. A panel of outcome measures, such as PK parameters for drug and metabolite, major PD endpoints, and safety observations, should be evaluated and

compiled. Furthermore, information on the inherent changes in patient physiology from these diseases that cannot be captured from healthy volunteers should be collected when available. These observations can be further refined using *in vitro* techniques when appropriate to determine the mechanism(s) behind the changes. The understanding of relevant population- and drug-related properties, through the compilation of clinical data and *in vitro* research, enables the mechanistic prediction of potential DDIs through predictive models such as PBPK. Through modeling, preliminary predictions can be made for a specific population under various clinical scenarios such as potential drug combinations, allowing for clinical studies to be prioritized so that resources are allocated to the most needed studies. This knowledge will also allow for the design of appropriate protocols that best fit the needs of the community– ideally reducing the duration of the study and optimizing patient follow-up requirements. Depending on the confidence level of PBPK models, simulations can be used to support dosing recommendations in scenarios that cannot be informed by the conduct of clinical DDI studies, due to either ethical reasons or feasibility considerations. Understanding these DDIs is only part of the solution, however. The knowledge gained on these topics will then need to be translated to strategies that can be implemented in LIC communities. Cooperative efforts in manufacturing additional dosage options and updated training for health care providers will also need to be undertaken to ensure that the benefits from the ability to understand and predict DDIs are available to those who are at risk.

Further research into these areas will also serve to complement related, ongoing efforts within the scientific community. For example, Lesko *et al*. recently proposed the collaboration of multidisciplinary research to evaluate oral contraceptive-based DDIs.[205] Indeed, the combination of PBPK modeling and model-based meta-analysis allows for an integrated

approach to identifying, and subsequently bridging, existing knowledge gaps within this field. Additionally, collaborative efforts to develop PBPK models for antimalarial drugs and anti-TB drugs are currently underway between The Bill & Melinda Gates Foundation and organizations such as Medicines for Malaria Venture and the Critical Path Institute. Together, these research activities enable researchers to capitalize on the existing and emerging knowledge in this field and allow the utilization of modeling and simulation methods to assess and manage complex DDIs in target populations of LICs.

While significant progress has been made to better understand PK-DDIs in LICs, there is still work to be done. Better understanding the underlying conditions and the resultant changes in drug disposition in these populations will allow for the development of effective risk mitigation strategies when co-medication is required. Acquiring a mechanistic understanding of the unique confounding factors in these patients, as well as the application of popPK techniques to identify critical covariates, is essential for effective information and implementation of predictive models to evaluate these interactions, and for progression to the implementation of population-specific treatment strategies.

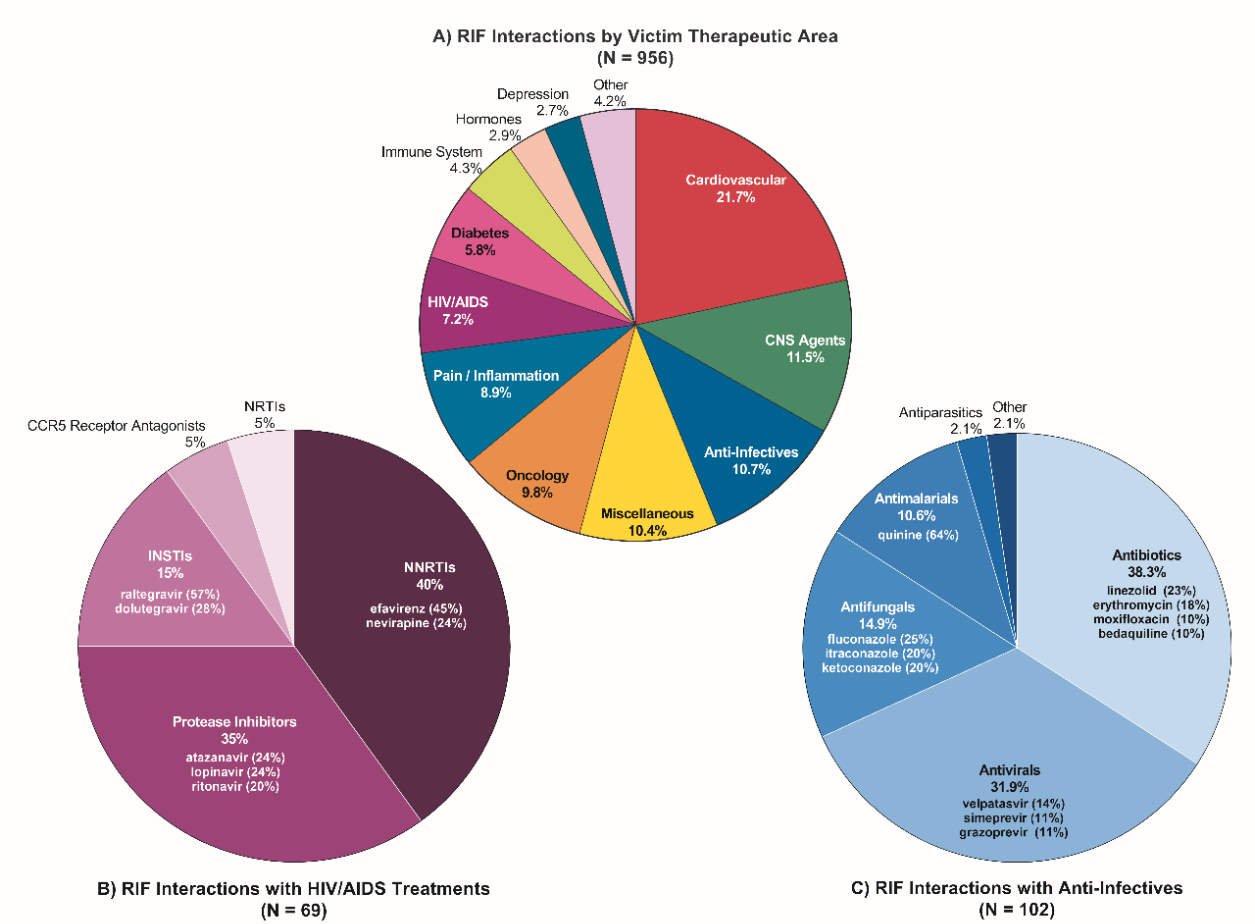

Figure 5-1. Therapeutic areas for reported DDIs with rifampin.

Distribution of therapeutic areas with reported interactions (≥ 25% change in victim AUC) with rifampin as the perpetrator for both induction and inhibition studies. Data was retrieved from the UW DIDB on or before 18 December 2018.

(A). The “miscellaneous” group includes drug categories such as endogenous compounds and cannabinoids; “other” is a summation of the following therapeutic areas with < 5 compounds tested (% of total): respiratory agents (1.9%), skin agents (1.2%), erectile dysfunction treatments (1.2%), dietary supplements and vitamins (0.6%), Parkinson’s disease treatments (0.6%), antigout and uricosuric agents (0.5%), drug addiction treatments (0.5%), beta3-adrenoreceptor agonist (0.5%), osteoporosis treatment (0.5%), muscle relaxants (0.5%), and migraine treatments. (0.5%).

(B, C). Interactions with rifampin by selected therapeutic area. Percentage indicates the relative contribution by that class to the overall number of observed interactions, the contribution from the primary drugs evaluated is included in parenthesis for the fractional contribution to interactions for that class.

CCR5: C-C chemokine receptor type 5, CNS: central nervous system, INSTI: HIV-integrase strand transfer inhbitor, NRTI: nucleoside reverse-transcriptase inhibitor, NNRTI: Non-nucleoside reverse-transcriptase inhibitor, RIF: rifampin.

Table 5-1. Summary of dosing strategies and PK properties pertaining to metabolism and transport-mediated DDI potential for WHO-recommended treatments for TB infection.

				In Vivo Metabolism / Transport[a]		
Drug	**Dose and Range (mg/kg body weight)**[165]	**Dosing Order/Duration**	**Primary Elimination Route**	**Substrate**	**Inhibitor**	**Inducer**
ethambutol (E)	15 (15-20)	1st, 2 months	renal	--	--	not available
isoniazid (H)	5 (4-6)	1st, 2 months 2nd, 4 months	renal	NAT2	CYPs 3A4, 2C19, 2E1, 1A2	CYP2E1
pyrazinamide (Z)	25 (20-30)	1st, 2 months	renal	pyrazinamidase	-	not available
rifampicin (R)	10 (8-12)	1st, 2 months 2nd, 4 months	biliary	arylacetamide deacetylase OATP1B1/1B3, P-gp	OATP1B/3, P-gp	CYPs 3A, 2B6, 2C9, 2C19, 2C8, 1A2 P-gp

[a]Listed from most to least sensitive substrate (K_m), potent inhibitor (K_i or IC_{50}), or inducer (fold-increase enzyme activity).

Chapter 6. CONCLUSIONS AND FUTURE DIRECTIONS

The goal of this dissertation research was to determine the clinical role of OATP1B1/1B3 transporters and evaluate the regulatory approach for their evaluation during drug development through a thorough analysis of literature data. From this, novel marker substrates as well as clinically relevant substrates and inhibitors have been identified which can serve to inform future clinical evaluations.

In Chapter 2, 34 drugs were identified as clinically relevant substrates of OATP1B1/1B3, with six showing promise as potential marker compounds. The novel indexing system used to identify the potential marker compounds proposes an integrated approach to objectively evaluate known substrates for use in regulatory studies. The findings from this analysis are supported by the ECCS classifications, with most drugs showing a high contribution of hepatic uptake to their disposition. As this analysis is limited by the availably of data in literature, it is likely that additional substrates and promising marker compounds can be identified as data becomes available.

The descriptive analysis performed in Chapter 3 evaluated a broad dataset, identifying two main areas of experimental design that significantly contributed to *in vitro* inhibitor variability – cell system and preincubation with the inhibitor. By controlling these factors, the variability in the overall dataset dropped substantially. In addition, the choice of substrate influenced inhibitor variability. It should also be noted that although significant variability was observed in the IC_{50} determination for OATP1B1 (approximately 5-fold when controlling for cell type and preincubation), this is much lower than what has been observed for P-gp interactions (700-fold being seen for a single inhibitor/substrate pair[71]). Despite the lower variability

observed for OATP1B1/1B3 IC_{50} values, it remains prudent to further evaluate the potential sources of inter-laboratory variability so as to ensure accurate and reproducible data is being generated.

When the inhibitors were evaluated in Chapter 4, both preclinical and clinical data were considered to identify those drugs likely to be clinically relevant perpetrators of OATP1B1/1B3-based DDIs and from those, identify promising index inhibitors. Most of the identified compounds also inhibit CYP3A and P-gp, accounting for 42% of all interactions (by substrate/inhibitor pairs). There were no novel clinical index inhibitors identified from this work; only rifampin and cyclosporine, both currently recommended by the FDA, had data supporting their utility. Rifampin allows for the most selective inhibition of OATP1B1/1B3, however many aspects of the current study design show room for further research. Investigation of a lower, more appropriate dose as well as dosing interval could allow for increased patient safety as well as tailored inhibition (ie. dose levels able to show moderate and strong inhibition) for the study goal.

The clinical impact of OATP1B1/1B3-mediated DDIs in the context of anti-infective use in low income countries was discussed in Chapter 5. As many common treatments are substrates and/or inhibitors of OATP1B1/1B3 the identification and mediation of such interactions is of utmost importance, yet there are significant gaps in the current understanding and mitigation strategies. The understanding of relevant population- and drug-related properties, through the compilation of clinical data and *in vitro* research, will enable the mechanistic prediction of potential DDIs through predictive models such as PBPK. This knowledge will also allow for the design of appropriate protocols that best fit the needs of the community– ideally reducing the

duration of the study, optimizing patient follow-up requirements, and allowing for multiple dose combinations for co-formulated treatments.

To conclude, this dissertation research has furthered our understanding of the clinical role of OATP1B1/1B3 through identification of relevant substrates and inhibitors, and the potential interactions between them. Additional drugs are proposed as potential index substrates which allows for a fit-for-purpose approach to evaluating the role of OATP1B1/1B3 in DDIs, either as a targeted interaction study or as a worst-case scenario that considers multiple metabolism and transport pathways. While no novel index inhibitors were identified, this work supports the inclusion of rifampin and cyclosporine in the FDA guidance documents due to their selectivity and broad-spectrum potency, respectively. This work also looked at the clinical use of rifampin as a tuberculosis treatment in low income countries and evaluated the current challenges to the optimal management of OATP1B-based DDIs.

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Appendix A

Supplemental Data – Identification and Evaluation of Clinical Substrates of Organic Anion Transporting Polypeptides 1B1 and 1B3

Table A-1. Kinetic and uptake data for those *in vitro* substrates that do not have corresponding clinical data.

Object	Cell System	Object Concentration	K_m (µM)		Uptake Ratio		Reference
			OATP1B1	OATP1B3	OATP1B1	OATP1B3	
4-aminosalicylic acid (PAS)	HEK293	1-400 µM	50	--	3	--	1
Amanitin	MDCK	0.7µM	--	3.7	--	--	2
Atazanavir	CHO	1 µM	--	--	3.1	1.6	3
Atorvastatin Acid	HEK293	0-10 µM	0.93	--	--	--	4
Beraprost	HEK293	0.012 and 0.12 µM	--	--	19.9	16	5
Dioscin	HEK293	0.2-25 µM	--	2.08	--	--	6
Eprosartan	MDCK	1 µM	--	--	3	--	7
Faldaprevir	HEK293	1 µM	--	--	3	--	8
Gemfibrozil	Hepatocytes	1 µM	--	--	1.5	--	9
Gimatecan	MDCK	1 µM	--	--	2.5	--	10
Irbesartan	HEK293	0.1-50 µM	0.695	11.9	-	-	11
Indinavir	CHO	2.5 µM	--	--	1.6	2.2	3
Lesinurad	MDCK	not specified	--	--	2.25	--	12
Nilotinib	CHO	0.01-50 µM	10.14	7.84	--	--	13
Ouabagenin	HEK293	10 µM	--	--	--	3.12	14
Ouabain	HEK293	10 µM	--	--	--	12.4	14
Penicillin G	MDCK	1.3 µM	--	--	--	1.7	2
Pitavastatin Acid	*X. laevis* oocytes	1-100 µM	3.6	3.8	--		15
Quercetin	HEK293	0.1 µM	--	--	1.9	1.3	16
Rifampin	HeLa	0.5-10 µM	1.5	--	--	--	17
	X. laevis oocytes	0-25 µM	--	2.3	--	--	18
Ritonavir	CHO	0.25 µM	--	--	1.4	1.8	3
Saquinavir	CHO	0.25 µM	--	--	2.1	1.7	3
Tenofovir AF	CHO	10 µM	--	--	1.3	2.68	19
Thyroxine	*X. laevis* oocytes	10 nM	--	--	2	1.7	20
Tipranavir	CHO	0.25 µM	--	--	2.4	3	3
Vandetanib	CHO	0.01-50 µM	2.72	4.37	--	--	13

— : data not available

Table A-2. Compounds identified with sufficient data to determine clinical relevance of OATP1B1/1B3.

Substrate	Possible Significant Clinical Issues Associated with OATP1B1/1B3 Inhibition	Maximum Fold-Increase in AUC Attributable to OATP1B1/1B3 Inhibition	Maximum Fold-Increase in AUC from PGx Data[a]	*In Vitro* Data[b]	Other Significant *In Vivo* Metabolism/Transport (Fold-Increase AUC)[c]
Ambrisentan	Yes	1.86 (RIF)[21]	1.3[22]	-- [d]	CYP3A, OATP1B1/1B3: 2.2 (CsA)[23]
Asunaprevir	Yes	14.8 (RIF)[24]	-- [d]	Hepatocytes K_m: 0.69 µM[24] 1B1 Uptake Ratio: 2.0[24] 1B3 not a substrate[24]	CYP3A, P-gp: 9.6 (keto), ↓ 1.26 (RIF)[25]
Atorvastatin	Yes	12.0 (RIF)[26]	2.5[27]	K_m 1B1: 0.62 µM[28] 1B3: 0.73 µM[29] Uptake Ratio 1B1: 18.5[30] 1B3: 1.9[31]	CYP3A, P-gp: 5.5 (itra)[32], ↓5.2(RIF)[33] CYP3A, OATP1B1/1B3, P-gp, BCRP: 9.4 (TPV/RTV)[34]
Atrasentan	No	1.06 (RIF)[35]	1.7[36]	Uptake Ratio[36] 1B1: 1.25 1B3: 1.36	CYP3A: 1.9 (keto)[37]
Bosentan	Yes	1.97 (CsA)[38]	-- [d]	K_m 1B1: 4.27 µM[39] 1B3: 141 µM[40]	CYP3A, P-gp: 2.2 (keto)[41] CYP3A, P-gp: 5.2 (LPV/RTV)
Caspofungin	Yes	1.61 (RIF)[42]	-- [d]	1B1 Uptake Observed[43] 1B3 not a substrate[43]	-- [d]
Cerivastatin	Yes [discontinued]	4.75 (CsA)[44]	-- [d]	1B1 Uptake Ratio: 2.7[45] 1B3 CL_{int}: 8.6 µL/min/mg[46]	CYP2C8, OATP1B1/1B3: 4.4 (GEM)[47]

Danoprevir	Yes	Unclear [nonspecific inhibitor]	-- [d]	Uptake Observed[48] 1B1/1B3	CYP3A, P-gp: 1.4 (keto)[49] CYP3A, P-gp, OATP1B1/1B3: 15.6 (CsA)[48]
Docetaxel	Yes	Unclear [nonspecific inhibitor]	1.6 (1B3)[50]	K_m 1B1: 0.43 µM[51] 1B3: 0.33 µM[52] Uptake Ratio[53] 1B1: 2.5 1B3: 5.1	CYP3A, P-gp: 2.6 (keto)[54] CYP3A, P-gp, OATP1B1/1B3: 7.3 (CsA)[55]
Eluxadoline	Yes	4.20 (CsA)[56]	2.0[57]	1B1 Uptake Ratio: 4.3[57] 1B3 not a substrate[57]	MRP2, OAT3: 1.3 (probenecid)[56]
Empagliflozin	No	1.35[58]	-- [d]	Uptake Observed[59] 1B1/1B3	OAT3: 1.5 (probenecid)[58]
Erythromycin	Unclear [insufficient data]	-0.44[60] (14C met/h)	2.6[61] (CL)	1B1 K_m: 13.2 µM[61] 1B3 Uptake Observed[62]	CYP3A, OATP1A2: 1.5 (GFJ)[63]
Fexofenadine	Unclear [confounding by P-gp]	3.6 (RIF)[64]	-59.4% (CL_H)[65]	K_m 1B1: 61.6 µM[66] 1B3: 108 µM[67]	OATP2B1: ↓6.7 (apple juice)[68] P-gp: 2.7 (keto)[69]
Fimasartan	Unclear [insufficient safety data]	4.3 (RIF)[70]	-- [d]	Transport Observed[71] 1B1 ND 1B3	CYP3A: 1.9 (keto)[70]
Fluvastatin	Yes	3.6 (CsA)[72]	no effect[73]	K_m 1B1: 2.5 µM[74] Uptake Ratio 1B1: 9.2[30] 1B3: 1.7[74]	OATP1B1/1B3, P-gp, BCRP: 3.6 (CsA)[72]
Glecaprevir	Yes	8.5 (RIF)[75]	no effect[75]	K_m[75] 1B1: 0.098 µM 1B3: 0.19 µM	P-gp: ↓3.0 (carbamazepine)[75], ↓9.1(RIF)[75] OATP1B1/1B3, BCRP, P-gp: 5.1 s(CsA)[75]

Glyburide	Unclear [insufficient data]	2.2 (RIF)[76]	no effect[77]	1B1[78] K_m: 2.0 µM Uptake Ratio: 3.5 1B3 not a substrate[31]	CYP3A: 1.3 (clarithromycin)[79] CYP2C9: 3.0 (PGx)[80]
Grazoprevir	Yes	10.2 (RIF)[81]	-- [d]	Uptake Observed[81] 1B1/1B3	CYP3A, P-gp: 3.0 (keto)[81] OATP1B1/1B3, P-gp, BCRP: 15.3 (CsA)[81] CYP3A, OATP1B1/1B3, P-gp: 12.8 (LPV/RTV)[81]
Letermovir	Yes	2.1 (CsA)[82]	1.4[82]	Uptake Observed[82] 1B1/1B3	-- [d]
Lopinavir	Unclear [confounding by CYP3A]	-- [d]	1.5 (C_{min})[83]	1B1 Uptake Ratio: 3.2[3] 1B3 NS[3]	CYP3A: ↓4.0 (RIF)[84]
Lovastatin	Yes	5.0 (CsA)[85]	2.9[86]	1B1 Uptake Observed[46] 1B3 not a substrate[46]	CYP3A, P-gp: 36.0 (itra)[87]
Nateglinide	Unclear [insufficient data]	-- [d]	2.1[88]	K_m 1B1: 36.4 µM[66] Uptake Ratio[89] 1B1: 1.4 1B3: 1.2	CYP3A: 1.5 (GEM/itra)[90], ↓1.3 (RIF)[91]
Nelfinavir	Unclear [confounding by CYP3A]	Unclear [nonspecific inhibitor]	-- [d]	Uptake Ratio[3] 1B1: 1.3 1B3: 1.5	CYP3A, P-gp: 1.4 (keto)[92], ↓5.9 (RIF)[92] CYP3A, OATP1B1/1B3, P-gp: 1.3 (CsA)[93]
Olmesartan	No	-- [d]	1.2[94]	K_m[95] 1B1: 12.8 µM 1B3: 44.2 µM Uptake Ratio[96] 1B1: 2.9 1B3: 15.3	-- [d]

Paritaprevir	Yes	1.4 (GEM)[97]	-- [d]	K_m[97] 1B1: 0.18 µM 1B3: 0.09 µM	CYP3A/P-gp: 47.4 (RTV)[97]
Pitavastatin	Yes	6.7 (RIF)[98]	3.9[99]	K_m[100] 1B1: 0.81 µM 1B3: 2.6 µM Uptake Ratio[30] 1B1: 45.7	P-gp, OATP1B1/1B3, BCRP: 4.5 (CsA)[101]
Pravastatin	Yes	4.6 (RIF)[26]	3.8[73]	1B1[102] K_m: 13.7 µM Uptake Ratio: 5.9 1B3 Uptake Ratio: 10.3[103]	P-gp, OATP1B1/1B3, BCRP: 22.8 (CsA)[104]
Repaglinide	Yes	2.6 (RIF)[105]	2.9[106]	Hepatocytes K_m: 12.8 µM[107] 1B1 K_m: 1.36 µM[39] 1B3 ND	CYP2C8: 8.3 (GEM)[108], 5.0 (clopidogrel)[109] CYP3A, CYP2C8, OATP1B1/1B3: 19.3 (GEM/itra)[110]
Rosuvastatin	Yes	4.1 (RIF)[98]	2.2[111,112]	K_m 1B1: 0.80 µM[113] 1B3: 9.80 µM[114] Uptake Ratio 1B1: 35.3[30] 1B3: 5.9[115]	BCRP, OATP1B1/1B3, P-gp: 7.1 (CsA)[116] P-gp, OATP1B1/1B3: 4.1 (RIF)[98] BCRP: 3.2 (PGx)[117]
Simvastatin	Yes	1.4 (GEM)[118]	3.2[119]	1B1 Uptake Ratio: 1.8[30] CL_{int}: 14.4 µL/min/mg[46] 1B3 CL_{int}: 14.3 µL/min/mg[46]	CYP3A, P-gp: 12.6 (keto)[120]
SN-38	Yes	-- [d]	2.1[121]	K_m[122] 1B1: 5.0 µM 1B3: 0.0177 µM	UGT1A1

Telmisartan	Unclear [insufficient data]	-- [d]	no effect (1B1)[123,124] ↓1.6 (1B3)[124]	1B3 K_m: 0.81 µM[125] 1B1 not a substrate[126]	-- [d]
Torsemide	No	-- [d]	1.2[127]	1B1[128] K_m: 6.2 µM Uptake Ratio: 2.2	--[d]
Voxilaprevir	Yes	9.7 (CsA)	-- [d]	1B3 ND Transport observed[129] 1B1/1B3	CYP3A, P-gp, OATP1B1/1B3: 9.7 (CsA) CYP3A, P-gp: ↓3.5 (RIF)

[a]*SLCO1B1* unless otherwise stated.

[b]Values are the lowest reported K_m and highest uptake ratio.

[c]Significant inhibition classified as those interactions resulting in ≥ 2-fold change in AUC or significant clinical effect.

[d]No reported studies or interactions.

Abbreviations: CsA- cyclosporine, GEM- gemfibrozil, GFJ- grapefruit juice, itra- itraconazole, keto- ketoconazole, RIF- rifampin.

All *in vitro* and clinical data retrieved from the DIDB on or before 6 February 2018.

Table A-3. Summary of the current labeling recommendations for drugs identified as clinical OATP1B1/1B3 substrates.

Substrate	FDA Recommendation Summary	Label Revision Date
Ambrisentan	Reduce ambrisentan dose when it is co-administered with CsA	October 2015
Asunaprevir	Caution when used with strong inhibitors of OATP1B1/1B3	January 2017
Atorvastatin	Reduce atorvastatin dose when it is co-administered with CsA	June 2009
Bosentan	Contraindication with co-administered CsA[a]	October 2003
Eluxadoline	Reduce eluxadoline dose when it is co-administered with OATP1B1 inhibitors	May 2015
Fluvastatin	Reduce fluvastatin dose when it is co-administered with CsA; Contraindication with co-administered GEM	August 2017
Glecaprevir	Contraindication with co-administered atazanavir; Co-administration of glecaprevir with CsA or LPV/RTV is not recommended	August 2017
Grazoprevir	Contraindication with OATP1B1/1B3 inhibitors known or expected to increase grazoprevir concentrations	January 2016
Letermovir	Reduce letermovir dose when it is co-administered with CsA; Contraindication with pitavastatin and simvastatin when co-administered with CsA	November 2017
Paritaprevir	Caution for increased plasma concentrations of paritaprevir when co-administered with inhibitors of OATP1B1/1B3	December 2014
Pitavastatin	Reduce pitavastatin dose when it is co-administered with RIF; Contraindication with co-administration of CsA; Avoid co-administration of GEM with pitavastatin	February 2012
Pravastatin	Reduce pravastatin dose when it is co-administered with CsA; Avoid concomitant administration of GEM	July 2016
Repaglinide	Contraindication with GEM[b]; Caution for increased plasma concentrations of repaglinide when co-administered with OATP1B1 inhibitors	July 2008
Rosuvastatin	Reduce rosuvastatin dose when it is administered with CsA or GEM	February 2010
Simvastatin	Contraindication with co-administered CsA or GEM	March 2015
Voxilaprevir	Contraindication with co-administered RIF	July 2017

[a]Interaction attributed in the prescribing information to CYP3A, but unlikely given the weak inhibition of the enzyme by CsA.

[b]Interaction attributed to inhibition of CYP2C8, however GEM is also an OATP1B1/1B3 inhibitor.

Abbreviations: CsA- cyclosporine, GEM- gemfibrozil, LPV- lopinavir, RIF- rifampin, RTV- ritonavir.

Table A-4. Drugs identified in the queries but found to not be *in vivo* substrates or data is insufficient to determine the *in vivo* role of OATP1B1/1B3.

Substrate	Confirmed as Clinical Substrate	Maximum Fold-Increase in AUC Attributable to OATP1B1/1B3 Inhibition	Maximum Fold-Increase in AUC from PGx Data[a]	*In Vitro* Data[b]	Other Significant *In Vivo* Metabolism/Transport (Fold-Increase AUC)[c]
Digoxin	No	1.46 (RIF)[130]	no effect (1B3)[131]	1B1 not a substrate[20] 1B3 Uptake Ratio: 2.4[20]	P-gp: 3.1 (valspodar)[132]
Levothyroxine	Unclear [insufficient data]	1.25 (RIF)[133]	-- [d]	1B1 K_m: 3.0 µM[134] Uptake Ratio: 2.0[20] 1B3 Uptake Ratio: 1.7[20]	-- [d]
Mercaptopurine	Unclear [insufficient data]	-- [d]	0.66[135] (% of dose)	ND	-- [d]
Methotrexate	Unclear [insufficient data]	-- [d]	no effect[135]	1B1 Uptake Observed[136] 1B3 K_m: 4.5 µM[137]	-- [d]
Mitoxantrone	Unclear [nonspecific inhibitor]	Unclear [nonspecific inhibitor]	-- [d]	1B1 Uptake Ratio: 2.0[138] 1B3 Uptake Observed[139]	P-gp, OATP1B1/1B3: 1.1 (CsA)[140]
Simeprevir	Unclear [insufficient data]	Unclear [confounding by CYP3A]	-- [d]	Uptake Ratio[141] 1B1: 4.3 1B3: 6.5	CYP3A, P-gp: 7.2 (ritonavir)[142]

Substrate	Confirmed as Clinical Substrate	Maximum Fold-Increase in AUC Attributable to OATP1B1/1B3 Inhibition	Maximum Fold-Increase in AUC from PGx Data[a]	*In Vitro* Data[b]	Other Significant *In Vivo* Metabolism/Transport (Fold-Increase AUC)[c]
Valsartan	Unclear [insufficient data]	-- [d]	↓2.0[143] (effect unknown)	K_m[144] 1B1: 1.4 1B3: 18.2 Uptake Ratio[145] 1B1: 6.2	-- [d]

[a]*SLCO1B1* unless otherwise stated.

[b]Values are the lowest reported K_m and highest uptake ratio.

[c]Significant inhibition classified as those interactions resulting in ≥ 2-fold change in AUC or significant clinical effect.

[d]No reported studies or interactions.

Abbreviations: CsA- cyclosporine, GEM- gemfibrozil, GFJ- grapefruit juice, itra- itraconazole, keto- ketoconazole, RIF- rifampin.

All *in vitro* and clinical data retrieved from the DIDB on or before 6 February 2018.

Table A-5. Calculation of index score for evaluated drugs.

			Core Criteria			Additional Positive Criteria			Additional Negative Criteria				
Substrate	**Therapeutic Class**	**ECCS Class**	**Sensitivity[a]**	**Specificity[b,c]**	**Safety**	**Positive PGx Data**	**Microdosing Validated**	**Published PBPK Model**	**Only Co-Formulated**	**Non-linear PK**	**$t_{1/2}$ > 24h**	**F < 5%**	**Overall Score**
Pravastatin	statin	3B	4	5	1	1	0.5	0.5	0	0	0	0	**12**
Rosuvastatin	statin	3B	4	4	1	1	0.5	0.5	0	0	0	0	**11**
Pitavastatin	statin	1B	4	3.5	1	1	0.5	0.5	0	0	0	0	**10.5**
Atorvastatin	statin	1B	6	1.5	1	1	0.5	0.5	0	-0.5	0	0	**10**
Eluxadoline	GI agent	3B	3	3	1	1	0	0	0	0	0	0	**8**
Letermovir	antiviral	--	2	4.5	1	1	0	0	0	-0.5	0	0	**8**
Fluvastatin	statin	1B	3	3.5	1	0	0	0.5	0	-0.5	0	0	**7.5**
Glyburide	diabetes treatment	1B	2	3.5	1	0	0.5	0.5	0	0	0	0	**7.5**
Grazoprevir	antiviral	1B	6	3.5	1	0	0	0	-2	-0.5	-0.5	0	**7.5**
Empagliflozin	diabetes treatment	4	1	5	1	0	0	0	0	0	0	0	**7**
Nateglinide	diabetes treatment	3A	2	3	1	1	0	0	0	0	0	0	**7**
Voxilaprevir	antiviral	--	5	4	1	0	0	0	-2	-0.5	-0.5	0	**7**
Fexofenadine	H-1 receptor antagonist	3B	3	1	1	1	0.5	0	0	0	0	0	**6.5**
Ambrisentan	ERA	3A	1	3	1	1	0	0	0	0	0	0	**6**
Asunaprevir	antiviral	3B	6	1.5	1	0	0	0	-2	-0.5	0	0	**6**
Erythromycin	antibiotic	4	1	3	1	1	0	0	0	0	0	0	**6**
Torsemide	diuretic	3A	0	4	1	1	0	0	0	0	0	0	**6**
Repaglinide	diabetes treatment	1B	2	1	1	1	0	0.5	0	0	0	0	**5.5**
Bosentan	ERA	1B	1	3.5	1	0	0	0	0	-0.5	0	0	**5**
Glecaprevir	HIV/AIDS	--	5	0.5	1	1	0	0	-2	-0.5	0	0	**5**
Olmesartan	ARB	3B	0	3	1	1	0	0	0	0	0	0	**5**
Simvastatin	statin	2	2	1	1	1	0	0.5	0	0	0	-0.5	**5**
Telmisartan	ARB	1B	0	4	1	1	0	0	0	-0.5	-0.5	0	**5**
Caspofungin	antifungal	--	1	3	1	0	0	0	0	0	-0.5	0	**4.5**
Lopinavir	HIV/AIDS	--	1	3	1	1	0	0	-2	0	0	0	**4**

			Core Criteria			Additional Positive Criteria			Additional Negative Criteria				
Substrate	**Therapeutic Class**	**ECCS Class**	**Sensitivity[a]**	**Specificity[b,c]**	**Safety**	**Positive PGx Data**	**Microdosing Validated**	**Published PBPK Model**	**Only Co-Formulated**	**Non-linear PK**	**$t_{1/2}$ > 24h**	**F < 5%**	**Overall Score**
SN-38	cancer treatment	--	2	3.5	-2	1	0	0	0	-0.5	0	0	**4**
Lovastatin	statin	2	2	0	1	1	0	0	0	0	0	-0.5	**3.5**
Nelfinavir	HIV/AIDS	2	1	1	1	0	0	0	0	-0.5	0	0	**2.5**
Paritaprevir	antiviral	--	1	3	1	0	0	0	-2	-0.5	0	0	**2.5**
Docetaxel	cancer treatment	--	1	1	-2	1	0.5	0	0	0	0	0	**1.5**

Double horizontal lines indicate the good (≥ 7.6), moderate, and poor (< 4.4) compound classifications.

[a]Score was assigned based on the highest observed AUCR with single oral dose or IV rifampin. When a rifampin study was not available, the best alternative study (PGx data or cyclosporine/gemfibrozil) was used.

[b]If there is a difference in sensitivity between the two involved pathways (i.e. one moderate and one sensitive) score as follows: sensitive substrate + weak substrate = 1.5; sensitive substrate + moderate substrate = 0.5; moderate substrate + weak substrate = 3.5.

[c]If there is no clinical evidence but strong *in vitro* support for the involvement of a pathway (i.e. data reported in three or more cell systems or studies) subtract one point (-1.0) from the score assigned based on the *in vivo* data from the single enzyme/transporter category. If there is only minimal *in vitro* evidence (i.e. single study or cell system) subtract one half point (-0.5) from the score assigned based on the *in vivo* data from the single enzyme/transporter category. That is, if clinical data supports the substrate is moderately metabolized by CYP3A (2 ≤ AUC Ratio <5 with ketoconazole) yet there is strong *in vitro* evidence that CYP2C9 also contributes to the disposition, sensitivity score would be 4 – 1 = 3.0.

[d]Highest observed change with cyclosporine.

[e]Highest observed change from PGx study data.

[f]Highest observed change with gemfibrozil.

ARB – Angiotensin II inhibitor, ERA – endothelin receptor antagonist.

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Appendix B

Supplemental Data – Inhibitors of Organic Anion Transporting Polypeptides 1B1 and 1B3: Clinical Relevance and Regulatory Perspective

Table B-1. R-value calculations for *in vitro* inhibitors with an $IC_{50} \leq 10$ µM.

Inhibitor	MW	f_u	IC_{50} (µM)	Single / Multiple	Dose (mg)	Dose (µM)	C_{max} (µM)	$C_{max,u}$ (µM)	$I_{in,max}$ (µM)	R-value[a]
Amprenavir	505.64	0.10	7.50	multiple	1200	2373.2	14.91	1.49	173.12	**3.31**
Asunaprevir	748.29	0.01	0.30	multiple	200	267.3	109.58	1.10	127.40	**5.25**
Atazanavir	704.90	0.14	0.37	multiple	300	425.6	6.48	0.91	34.85	**14.19**
Atorvastatin	558.64	0.02	0.03	single	40	71.6	0.024	0.00	4.80	**4.43**
Avatrombopag	649.65	0.04	2.80[b]	single	20	30.8	0.16	0.01	2.21	1.03
Baloxavir	483.49	0.06	6.81	single	40	82.7	0.28	0.02	5.80	1.05
Boceprevir	519.70	0.25	6.30	multiple	800	1539.35	3.23	0.81	105.85	**5.20**
Bosentan	569.64	0.02	5.00	multiple	125	219.44	2.50	0.05	17.13	1.07
Brexpiprazole	433.57	0.01	8.39	single	2	4.61	0.057	0.00	0.36	1.00
Brilanestrant	446.90	0.01[c]	0.30	multiple	600	1342.58	49.23	0.49	138.73	**5.62**
Bromocriptine	654.60	0.10	0.12	single	5	7.64	0.00	0.00	0.51	**1.42**
Carfilzomib	719.90	0.03	3.60	single	45.9[d]	63.76	7.99	0.24	12.24	**1.10**
Cerivastatin	481.50	0.01	1.70	single	0.3	0.62	0.006	0.00	0.05	1.00
Clarithromycin	747.96	0.01[c]	5.10	multiple	500	668.48	5.01	0.05	49.58	1.10
Clopidogrel	419.90	0.01[c]	3.95	single	300	714.46	0.02	0.00	47.66	**1.12**
Cobicistat	776.03	0.03	3.50	multiple	150	193.29	1.93	0.06	14.81	**1.13**
Cyclosporine	1202.61	0.10	0.02	single	400	332.61	1.15	0.12	23.33	**123.78**
Dabrafenib	615.68	0.01	1.40	multiple	75	121.82	1.72	0.02	9.84	1.07
Daclatasvir	738.88	0.01	1.50	multiple	60	81.20	1.95	0.02	7.36	1.05
Danoprevir	731.84	0.01[c]	4.00	single	100	136.64	0.0368	0.00	9.15	1.02
Dapagliflozin	502.98	0.09	8.00[b]	single	10	19.88	0.28	0.02	1.60	1.02
Darunavir	593.73	0.05	3.50	multiple	600	1010.56	11.753	0.59	79.12	**2.13**
Dasabuvir	493.58	0.01	0.90	multiple	250	506.50	2.26	0.02	36.03	**1.40**
Dasatinib	488.01	0.04	4.81	single	100	204.91	0.22	0.01	13.88	**1.12**
Degarelix[1]	1632.30	0.10	10.00	multiple	480	294.06	0.06	0.01	19.66	**1.20**
Digoxin	780.95	0.75	7.90	single	0.5	0.64	0.00	0.00	0.05	1.00
Elbasvir	882.02	0.01	0.10[b]	multiple	50	56.69	0.19	0.00	3.97	**1.40**

Eltrombopag	442.50	0.01	0.09	single	200	451.98	42.94	0.43	73.07	**9.12**
Elvitegravir	447.90	0.01	2.00	multiple	200	446.53	6.837	0.07	36.61	**1.18**
Encorafenib	540.00	0.14	5.35	single	450	833.33	12.05	1.69	67.60	**2.77**
Erlotinib	429.90	0.07	1.19[b]	multiple	100	232.61	3.71	0.26	19.22	**2.13**
Erythromycin	733.93	0.01[c]	4.88	single	500	681.26	1.19	0.01	46.61	1.10
Etoposide	588.56	0.03	4.18[b]	multiple	127.5[d]	216.63	21.15	0.63	35.60	**1.26**
Everolimus	958.20	0.01[c]	4.10	multiple	5	5.22	0.049	0.01	0.40	1.03
Ezetimibe	409.40	0.10	8.90[b]	single	10	24.43	0.08	0.01	1.71	1.02
Fasiglifam	524.63	0.01	2.28	single	50	95.31	4.38	0.04	10.73	1.05
Gemfibrozil	250.35	0.01[c]	4.00	multiple	600	2396.64	94.41	0.94	254.18	**1.64**
Glecaprevir	838.87	0.03	0.02	multiple	700	834.45	14.92	0.37	70.55	**104.75**
Glimepiride	490.62	0.01[c]	3.55	multiple	4	8.15	0.38	0.004	0.92	1.00
Glyburide	493.99	0.01[c]	0.26	single	5	10.12	0.24	0.002	0.91	1.04
Grazoprevir	766.90	0.01	0.70	multiple	200	260.79	0.91	0.01	18.29	**1.31**
Idelalisib	415.42	0.16	10.00	single	400	962.88	8.55	1.37	72.74	**2.16**
Indinavir	711.88	0.40	4.10	multiple	800	1123.78	11.755	4.70	86.67	**9.46**
Irbesartan	428.50	0.10	4.65	multiple	300	700.12	7.509	0.75	54.18	**2.17**
Itraconazole	705.64	0.01	0.71[b]	multiple	200	283.43	0.84	0.01	19.74	**1.28**
Ivosidenib	583.00	0.08	9.56	single	250	428.82	3.79	0.30	32.38	**1.27**
Ketoconazole	531.44	0.01	1.80	single	400	752.67	14.69	0.15	64.87	**1.36**
Lapatinib	943.50	0.01	4.00	multiple	1250	1324.85	2.23	0.02	90.56	**1.23**
Ledipasvir	889.00	0.01	3.50	multiple	90	101.24	0.68	0.01	7.43	1.02
Lenvatinib	522.96	0.02	1.86	single	24	45.89	0.56	0.01	3.62	1.04
Lesinurad	404.28	0.02	9.30	multiple	600	1484.12	35.35	0.71	134.29	**1.29**
Letermovir	572.55	0.01	2.90	multiple	480	838.35	24.03	0.24	79.92	**1.28**
Lobeglitazone	480.54	0.01[c]	2.44	multiple	0.5	1.04	0.104	0.001	0.17	1.00
Lopinavir	628.80	0.01	0.50	multiple	400	636.13	17.63	0.18	60.04	**2.20**
Lovastatin	404.55	0.05	6.10	single	80	197.75	0.027	0.001	13.21	**1.11**
Macitentan	588.57	0.01	2.00	multiple	10	16.99	0.54	0.01	1.67	1.01
Midostaurin	570.65	0.01	0.30	single	50	87.62	2.81	0.03	8.65	**1.29**

Mifepristone	429.60	0.02	3.30	multiple	600	1396.65	8.03	0.16	101.14	**1.61**
Mitoxantrone	571.41	0.22	3.39[a]	single	22.36	39.13	0.54	0.12	3.15	**1.20**
Montelukast	608.18	0.01	1.40	single	10	16.44	0.70	0.01	1.80	1.01
Moracizine	427.52	0.05	8.10	multiple	250	584.77	1.32	0.07	40.30	**1.25**
Nelfinavir	567.79	0.02	0.93	multiple	1250	2201.52	9.98	0.20	156.75	**4.37**
Neratinib	673.11	0.01	0.65[b]	single	240	356.55	0.090	0.001	23.86	**1.37**
Nilotinib	565.98	0.02	2.78	single	400	706.74	0.770	0.02	47.89	**1.34**
Nisoldipine	388.40	0.01	7.40	single	20	51.49	0.005	0.0001	3.44	1.00
Paclitaxel	853.90	0.11	0.03	single	301[d]	352.50	4.99	0.55	28.49	**105.45**
Paritaprevir	801.91	0.03	0.03	single	150	187.05	1.03	0.03	13.50	**14.50**
Pazopanib	473.99	0.01	0.79	multiple	400	843.90	83.9	0.84	140.20	**2.77**
Pibrentasvir	1113.18	0.01	0.30	multiple	120	107.80	0.211	0.002	7.40	**1.25**
Pioglitazone	392.90	0.01	5.09	single	15	38.18	1.33	0.01	3.88	1.01
Pradigastat	455.48	0.01[c]	1.66	multiple	40	87.82	2.94	0.03	8.79	1.05
Pravastatin	446.52	0.50	3.60	single	4	8.96	0.13	0.07	0.73	**1.10**
Ramipril	416.50	0.27	4.00	multiple	5	12.00	0.028	0.01	0.83	1.06
Repaglinide	452.60	0.02	0.32	single	0.25	0.55	0.0094	0.0002	0.05	1.00
Rifampin	822.95	0.20	0.29	multiple	600	729.08	25.62	5.12	74.22	**52.19**
Rifamycin	720.00	0.20	0.05	multiple	388	538.89	0.012[e]	0.002	35.94	**144.75**
Rilpivirine	402.88	0.01	4.10	multiple	150	372.32	2.72	0.03	27.54	1.07
Ritonavir	720.95	0.01	0.40	multiple	100	138.71	1.82	0.02	11.07	**1.28**
Rofecoxib	314.36	0.13	1.17	multiple	12.5	39.76	0.621	0.08	3.27	**1.36**
Rosiglitazone	357.44	0.01	4.27	single	8	22.38	1.74	0.02	3.23	1.01
Rosuvastatin	1001.14	0.12	0.05	single	10	9.99	0.0061	0.001	0.67	**2.72**
Sacubitril	411.49	0.06	1.91	multiple	97	235.73	4.96	0.30	20.67	**1.65**
Saquinavir	670.86	0.03	0.41	multiple	1000	1490.62	4.68	0.14	104.05	**8.61**
Selexipag	496.62	0.01	2.40	single	0.4	0.81	0.011	0.0001	0.06	1.00
Sildenafil	666.70	0.04	1.50	single	50	75.00	0.329	0.01	5.33	**1.14**
Simeprevir	749.94	0.01	0.06	multiple	150	200.02	3.60	0.04	16.93	**3.82**
Simvastatin	418.57	0.05	5.00	single	40	95.56	0.025	0.001	6.40	1.06

Sirolimus	914.20	0.03	1.10	single	2	2.19	0.011	0.0003	0.16	1.00
Sorafenib	637.00	0.01	0.07	multiple	400	627.94	10.45	0.10	52.31	**8.52**
Sulfasalazine	398.39	0.10	0.56	single	500	1255.04	12.29	1.23	95.96	**18.14**
Tacrolimus	822.03	0.01	0.67	single	5	6.08	0.028	0.0003	0.43	1.01
Telaprevir	679.85	0.41	1.36	multiple	750	1103.18	4.52	1.85	78.07	**24.54**
Telmisartan	514.63	0.01	7.41	multiple	80	155.45	1.96	0.02	12.32	1.02
Telotristat[f]	575.00	0.01	9.61	multiple	500	869.57	1.65	0.02	59.62	1.06
Teriflunomide	270.20	0.01	7.14	single	70	259.07	37.38	0.37	54.65	1.08
Tezacaftor	520.50	0.01	3.24	multiple	50	96.06	12.66	0.13	19.07	1.06
Tipranavir	602.70	0.01	0.70	multiple	500	829.60	87.93	0.88	143.23	**3.05**
Trametinib	693.53	0.03	1.30	multiple	2	2.88	0.037	0.001	0.23	1.00
Troglitazone	441.55	0.01	0.32	multiple	400	905.90	0.0036	0.00004	60.40	**2.89**
Velpatasvir	883.00	0.01	1.50	multiple	100	113.25	0.816	0.01	8.37	1.06
Voxilaprevir	868.90	0.01	0.18	multiple	200	230.18	0.971	0.01	16.32	**1.91**
Faldaprevir	869.82	0.01[c]	0.57	multiple	240	275.92	42.767	0.43	61.16	**2.07**
Verapamil	454.61	0.10	14.80	single	120	263.96	0.237	0.02	17.83	**1.12**

Drug parameters (MW, f_u) are taken from the NDA unless otherwise noted. C_{max} was calculated from the average of the available studies at the most commonly administered dose. R-value is defined as $R = 1 + \frac{f_{u,p} \; x \; I_{in,max}}{IC_{50}}$ where $f_{u,p}$ is the fraction unbound in plasma and $I_{in,max}$ is the estimate of the maximum inhibitor concentration in plasma at the liver inlet. $I_{in,max} = \frac{C_{max} + (F_a F_g \; x \; k_a \; x \; Dose)}{Q_h / R_B}$ with F_a = fraction absorbed, F_g = intestinal bioavailability, k_a = absorption rate constant, Q_h = hepatic blood flow (1.5 L/min) and R_B is the blood-to-plasma ratio.

[a]Values in bold are above the FDA cut-off value of 1.1

[b]OATP1B3 IC_{50}, no value available for OATP1B1

[c]No literature value available, FDA worst-case-scenario value used

[d]Dose calculated based on normal body surface area = 1.7 m^2

[e]Concentration 6 hr post-dose

[f]Dosed as telotristat ethyl

Table B-2. Drugs showing a clinical interaction with a sensitive OATP1B1/1B3 substrate (AUCR ≥ 1.25) and R < 1.1

Precipitant	R-value	Object	AUCR	Reference
clarithromycin	1.097	atorvastatin	1.82	[2]
			3.45	[3]
			4.45	[4]
			2.52	[5]
			3.05	[5]
		pitavastatin	1.24	[3]
		pravastatin	2.11	[4]
		rosuvastatin	1.56	[3]
daclatasvir	1.05	rosuvastatin	1.47	[6]
erythromycin	1.096	atorvastatin	1.33	[7]
		pitavastatin	2.79	[8]
telmisartan	1.02	rosuvastatin	1.25	[9]
			1.27	[9]
velpatasvir	1.06	pravastatin	1.35	[10]
			1.36	[11]
		rosuvastatin	2.60	[11]
			2.69	[10]

Table B-3. Labeling recommendations for drugs with an R-Value ≥ 1.1 and/or AUCR ≥ 1.25.

Clinical Impact of OATP1B1/1B3 Inhibition

Inhibitor	R ≥ 1.1	AUC ≥ 1.25	Initial FDA Approval	Combination Only	Type of Labeling Recommendation	Product/Formulation Evaluated (revision date)
Cobicistat	X	X	2014		Inhibitor of OATP1B1/1B3 May increase systemic exposure of OATP1B1/1B3 substrates	EVOTAZ tablet (March 2019)
Daclatasvir		X	2015		Inhibitor of OATP1B1/1B3 May increase systemic exposure of OATP1B1/1B3 substrates	DAKLINZA tablet (Nov. 2017)
Eltrombopag	X	X	2008		Use caution	PROMACTA tablet (Nov 2018)
Encorafenib	X		2018		*In vitro* Inhibition Reference	BRAFTOVI capsule (Jan 2019)
Gemfibrozil	X	X	1981		Dose Reduction with OATP1B1/1B3 substrates Contraindication with simvastatin and repaglinide	LOPID tablet (April 2018)
Glecaprevir	X		2017		Inhibitor of OATP1B1/1B3 May increase systemic exposure of OATP1B1/1B3 substrates	MAVYRET tablet (August 2018)
Lapatinib	X		2007		*In vitro* Inhibition Reference	TYKERB tablet (Dec 2018)
Letermovir	X	X	2017		Dose reduction for atorvastatin Coadministration not recommended for pitavastatin/ simvastatin	PREVYMIS tablet (Nov 2017)
Paritaprevir	X		2014	X	Do not exceed 40 mg/day pravastatin Do not exceed 10 mg/day rosuvastatin	VIEKIRA PAK tablet (Dec 2014)
Pazopanib	X		2009		*In vitro* Inhibition Reference	VOTRIENT tablet (Oct 2009)
Pibrentasvir	X	X	2017	X	Coadministration of statins is not recommended	MAVYRET tablet (August 2018)
Sacubitril	X		2015	X	*In vitro* Inhibition Reference	ENTRESTO tablet (Nov 2017)
Velpatasvir		X	2016/2017	X	Dose adjustment for pravastatin, atorvastatin, fluvastatin, lovastatin, simvastatin Coadministration is not recommended for rosuvastatin, pitavastatin	VOSEVI tablet (Nov 2017)
Voxilaprevir	X		2017	X	Dose adjustment for pravastatin, atorvastatin, fluvastatin, lovastatin, simvastatin Coadministration is not recommended for rosuvastatin, pitavastatin	VOSEVI tablet (Nov 2017)

No Clinical Impact of OATP1B1/1B3 Inhibition

Inhibitor	R ≥ 1.1	AUC ≥ 1.25	Initial FDA Approval	Combination Only	Type of Labeling Recommendation	Product/Formulation Evaluated (revision date)
Idelalisib	X		2014		No changes in exposure observed	ZYDELIG tablet (Oct 2018)
Ivosidenib	X		2018		No *in vitro* inhibition	TIBSOVO tablet (July 2018)

Inhibitor	R ≥ 1.1	AUC ≥ 1.25	Initial FDA Approval	Combination Only	Type of Labeling Recommendation	Product/Formulation Evaluated (revision date)
Ledipasvir		X	2014	X	No significant interaction observed with pravastatin	HARVONI tablet (Oct 2014)
Lopinavir	X		2005		Pravastatin/fluvastatin listed as safe alternatives to simvastatin/lovastatin/atorvastatin	KALETRA solution (Dec 2005)
Ritonavir	X	X	1996		Pravastatin/fluvastatin listed as safe alternatives to atorvastatin/rosuvastatin	NORVIR tablet (Feb 2010)
Telmisartan		X	1998		No significant interaction observed with simvastatin	MICARDIS tablet (Feb 2018)

Effect on OATP1B1/1B3 Substrates by CYP3A Inhibition

Inhibitor	R ≥ 1.1	AUC ≥ 1.25	Initial FDA Approval	Combination Only	Type of Labeling Recommendation	Product/Formulation Evaluated (revision date)
Clarithromycin		X	1991		Contraindicated with simvastatin	BIAXIN suspension (Dec 2018)
Cyclosporine	X	X	1990		Statin dose should be reduced	SANDIMMUNE capsule (March 2015)
Erythromycin		X	1965		Do not use lovastatin, atorvastatin, or simvastatin	E.E.S. granule (April 2018)
Itraconazole	X	X	1992		Contraindicated with lovastatin/simvastatin Monitoring and/or dose reduction required for coadministration with atorvastatin	TOLSURA capsule (Dec 2018)
Ketoconazole	X		1981		Contraindicated with simvastatin/lovastatin	NIZORAL tablet (Feb 2014)[a]
Lopinavir	X		2005		Concomitant use with lovastatin/simvastatin is not recommended Use lowest possible dose of atorvastatin	KALETRA solution (Dec 2005)
Nelfinavir	X	X	1997		Do not exceed 40 mg/day atorvastatin Potential for serious reaction with lovastatin/simvastatin	VIRACEPT tablet (Sept 2016)
Rifampin	X	X	1971		May accelerate metabolism and reduce activity of statins metabolized by CYP3A	RIFAMPIN injection (Dec 2018)
Saquinavir[b]	X		2004	X	Contraindicated with lovastatin/simvastatin Do not exceed 20 mg/day atorvastatin	INVIRASE tablet (March 2019)
Tipranavir[b]	X		2005	X	Contraindicated with lovastatin/simvastatin	APTIVUS capsule (June 2018)
Lesinurad	X		2015		Could affect HMG-CoA reductase inhibitors that are CYP3A substrates	ZURAMPIC tablet (Dec 2015)

The drugs not listed either did not have any language regarding OATP1B1/1B3 inhibition or sensitive substrates of the transporters in the labeling evaluated or are not currently FDA approved.

[a]Labeling evaluated is for a discontinued formulation

[b]Required to be administered with ritonavir

Supplemental Methods – Static Predictions

To estimate the magnitude of clinical DDIs observed with lower doses of rifampin, static predictions using pravastatin, an index substrate of OATP1B1/1B3, were conducted. Using Equation 1, the average AUCR for each dose was calculated. The unbound liver inlet concentration ($I_{in,max,u}$) was calculated from Equation 2, with C_{max} being the average reported value for each dose. The fraction transported by OATP1B1 for pravastatin was set to 0.65 for all calculations.[12]

$$(1) \quad AUCR = \frac{1}{\frac{f_{t,OATP}}{1+\frac{I_{in,max,u}}{K_i}} + (1 - f_{t,OATP})}$$

$$(2) \quad I_{in,max,u} = f_u * C_{max} + \frac{F_a F_g * k_a * D}{\frac{Q_h}{R_B}}$$

Table B-4. Static predictions for pravastatin/rifampin

Inhibitor Dose	Route of Administration	Single / Multiple Dose	Mean $I_{in,max,u}$ (μM)	Predicted AUCR	Observed AUCR
10 mg/kg	Oral	multiple	6.75	**2.66**	--
300 mg	Oral	single	3.75	**2.53**	--
		multiple	3.58	**2.52**	--
450 mg	Oral	multiple	4.99	**2.60**	--
600 mg	IV	single	2.57[a]	**2.42**	--
	Oral	single	8.72	**2.70**	2.26[13], 4.64[14]
		multiple	7.16	**2.67**	--
720 mg	Oral	multiple	8.17	**2.69**	--
900 mg	Oral	multiple	9.47	**2.71**	--

[a] $C_{max,u}$

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