

## ARTICLE

# Identification and Evaluation of Clinical Substrates of Organic Anion Transporting Polypeptides 1B1 and 1B3

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Organic anion transporting polypeptides (OATPs) 1B1 and 1B3 facilitate the uptake of drugs and endogenous compounds into the liver. In recent years, the impact of these transporters on drug–drug interactions (DDIs) has become a focus of research, and the evaluation of their role in drug disposition is recommended by regulatory agencies worldwide.<sup>1–3</sup> Although sensitive substrates of OATP1B1/1B3 have been identified in the literature and probe drugs have been proposed by regulatory agencies, there is no general consensus on the ideal *in vivo* substrate for clinical DDI studies as analysis may be confounded by contribution from other metabolic and/or transport pathways.<sup>1–3</sup> A thorough analysis of the available *in vitro* and *in vivo* data regarding OATP1B1/1B3 substrates was performed using the *in vitro*, clinical, and pharmacogenetic modules in the University of Washington Drug Interaction Database. A total of 34 compounds were identified and further investigated as possible clinical substrates using a novel indexing system. By analyzing the compounds for *in vivo* characteristics, including sensitivity to inhibition by known OATP1B1/1B3 inhibitors, selectivity for OATP1B1/1B3 compared with other transport and metabolic pathways, and safety profiles, a total of six compounds were identified as potential clinical markers of OATP1B1/1B3 activity.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Currently, there are three recommended clinical substrates for the study of drug–drug interactions (DDIs) involving organic anion transporting polypeptides (OATP)1B1/1B3. Although these are sensitive substrates, they are also substrates of other metabolic and transport pathways, confounding data interpretation.

### WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Are there additional compounds that are more sensitive or more selective for OATP1B1/1B3 that can be identified using an objective, quantitative approach?

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ A novel indexing system was developed to rank clinical substrates of OATP1B1/1B3. Six substrates, including the current recommended clinical substrates, were identified and ranked as potential marker substrates of OATP1B1/1B3.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The indexing system developed provides an objective, reproducible method for OATP1B1/1B3 substrate selection using accessible literature data, whereas the marker compounds that were identified provide alternative substrates for use in studying OATP1B1/1B3-mediated DDIs.

Organic anion transporting polypeptides (OATPs) are uptake transporters in the solute carrier (SLC) transporter superfamily. The OATP family comprises 11 isoforms in 6 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. Proteomic analysis found that OATP1B1 accounts for 22% of total protein, whereas OATP1B3 is expressed at a significantly lower level, ~ 8%.<sup>4</sup> 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, although *SLCO1B3* variants have been identified, they are not as well studied, and the clinical impact of the variants is mostly unknown at this time.

OATP1B1 and 1B3 were first included in the 2012 US 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.<sup>1,2,5</sup> A recent review of new drug applications over the last 4 years highlights the relevance of OATP1B1/1B3, where <10 drugs were identified as OATP1B1/1B3 substrates; however, over 40 drugs were identified as inhibitors of OATP1B1/1B3, more than P-glycoprotein (P-gp; 37 drugs) or breast cancer resistance protein (BCRP; 34 drugs).<sup>6</sup>

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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, and those using medications likely to be coadministered 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, whereas the International Transporter Consortium also recommends the inclusion of atorvastatin, in DDI studies when the new molecular entity is an expected inhibitor of OATP1B1/1B3.<sup>7,8</sup> Although 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 twofold: first, to identify all clinical substrates of OATP1B1/1B3 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.

## METHODS

### Clinical substrate determination

Using the University of Washington Drug Interaction Database (DIDB; [www.druginteractioninfo.org](http://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 February 6, 2018). Filtering of these data sets was completed similar to previously published methods and a full description of the selection process is available in the **Supplemental Methods**.<sup>9,10</sup>

**Data refinement.** Following identification of potential substrates from all data sets, secondary queries of the DIDB were performed to ensure that all available data were considered. For compounds identified in the PGx or clinical DDI data sets, *in vitro* data were re-evaluated to ensure retention of all relevant data, even if below the initial cutoff criteria. Similarly, PGx data for compounds identified in the *in vitro* or clinical data sets were retained even if the results did not meet the initial criteria for inclusion. Finally, negative clinical DDI studies, those with an increase in area under the concentration-time curve (AUC) of < 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.

**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 1**). Only drugs that are currently approved by the FDA and EMA were evaluated.

Sensitivity to OATP1B1/1B3 inhibition was assessed by identifying the largest increase in AUC following coadministration with a single oral or i.v. dose of rifampin as there is little confounding from the inhibition of other metabolic/transport pathways. When rifampin data were 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:  $1.25 \leq \text{area under the concentration-time curve ratio (AUCR)} < 2$ ), moderately sensitive (2 or 3:  $2 \leq \text{AUCR} < 5$ ), sensitive (4 or 5:  $5 \leq \text{AUCR} < 10$ ), or extremely sensitive (6:  $\text{AUCR} \geq 10$ ) 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 toward 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 coadministration 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 ( $\text{AUCR} \geq 5$ ) were ranked lower than those that showed moderate ( $2 \leq \text{AUCR} < 5$ ) or weak ( $\text{AUCR} < 2$ ) 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 were available. These 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 (PKs), available formulations, and the availability of supporting data. Additional points were awarded to compounds with positive PGx data (statistically significant changes in exposure compared with control) as this adds high 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 models; as expected, changes in exposure can be accurately predicted prior to administration. Additionally, compounds for which microdosing had been validated were scored positively, as this approach significantly increases clinical safety while maintaining measurable and informative changes in exposure.<sup>11</sup> Scores were reduced for compounds that are only marketed as combination therapies because the coformulated agent can confound study results, as well as compounds that show nonlinear PKs, as observed changes in exposure cannot be correlated to inhibitor dose. Drugs with low bioavailability ( $F < 5\%$ ) received lower scores as

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

Total score	15	(Top of each category + all positive criteria)	
Sensitivity to OATP1B1/1B3 inhibition <sup>a</sup>	0	No PGx data or clinical studies with a specific inhibitor for OATP1B1/1B3 -or- AUCR < 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 <sup>b</sup>	0	Sensitive substrate for at least two metabolic enzymes or transporters (AUCR ≥ 5 for each pathway) <sup>c,d</sup>	
	1	Moderate sensitive substrate for at least two metabolic enzymes or transporters (2 ≤ AUCR < 5 for each pathway) <sup>c,d</sup>	
	2	Sensitive substrate of one metabolic enzyme or transporter (AUCR ≥ 5)	
	3	Weak substrate for at least two metabolic enzymes or transporters (AUCR < 2 for each pathway) <sup>c,d</sup>	
	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 <i>SLCO1B1</i> or <i>1B3</i> variants
		0.5	Microdosing validated
		0.5	Published and validated PBPK model
	Negatives	-2	Only available as a combination therapy
		-0.5	Nonlinear PKs
		-0.5	Half-life longer than 24 hours
		-0.5	Very low bioavailability (F < 5%)

AUCR, area under the concentration-time curve ratio; OATP, organic anion-transporting polypeptide; PBPK, physiologically based pharmacokinetic; PGx, pharmacogenetic; PKs, pharmacokinetics; SLC, solute carrier.

<sup>a</sup>Assessed primarily on the AUCR observed following single oral dose or i.v. rifampin. Studies with gemfibrozil/cyclosporin or PGx data used when rifampin data were unavailable. <sup>b</sup>Score assigned from drug–drug interaction studies with mechanistic inhibitors or PGx data. <sup>c</sup>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. <sup>d</sup>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 support the substrate, it is a moderate sensitive substrate of cytochrome P450 (CYP)3A (2 ≤ AUCR < 5 with ketoconazole) yet there is strong *in vitro* evidence that CYP2C9 also contributes to the disposition, the sensitivity score would be 4-1 = 3.0.

the intra individual variability is significantly higher and can confound inhibition study results. Finally, scores were decreased for compounds where terminal half-life > 24 hours 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% and 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 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.<sup>12</sup>

## RESULTS

### *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  $K_m \leq 10 \mu\text{M}$ , 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, whereas 29 of the 86 compounds (34%) remained after filtering compounds that did not have corresponding *in vivo* data (Table S1).

### 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  $\geq 25\%$ . 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-fold to 22.8-fold (atrasentan/rifampin and pravastatin/cyclosporine, respectively) with a median AUCR of 2.13. Although a majority (71 of 128; 55%) of the interactions was minor, with observed increases in AUC less than twofold, ~30% of the identified interactions were moderate (twofold to fivefold increase in exposure), and 22% had increases greater than fivefold.

### SLCO1B1 and SLCO1B3 PGx studies

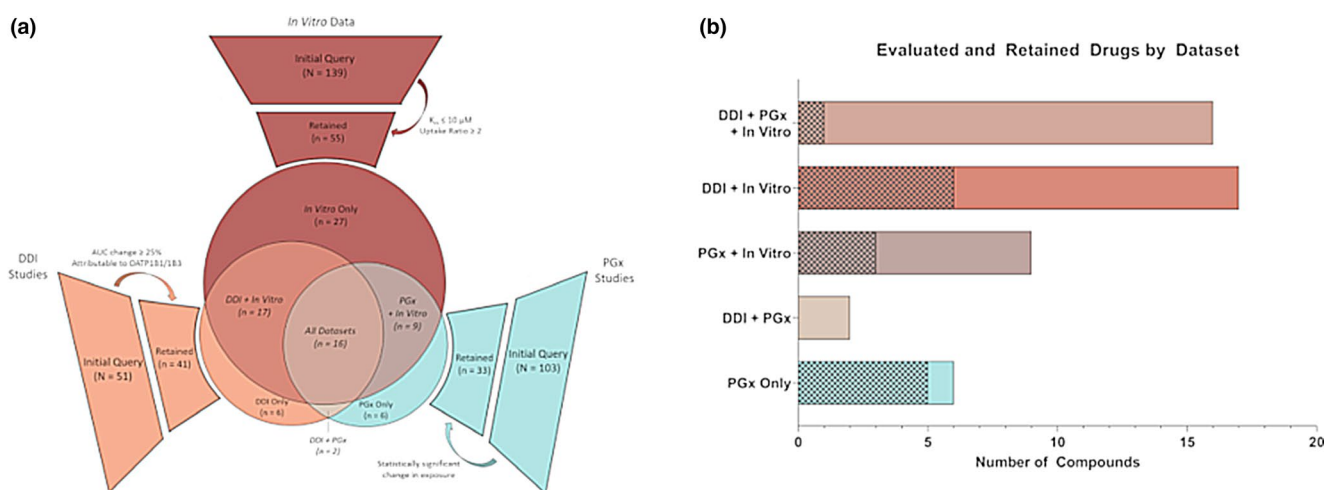
A quantitative search of the e-PKgene module of the DIB identified 17 and 85 drugs evaluated for *SLCO1B3* and *SLCO1B1* polymorphisms, respectively. Selecting those with a statistically significant change in drug exposure, 33 drugs involving 71 studies were retained for further evaluation. Very few 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* data set. Of the identified compounds from the PGx data

set, 6 (18%) did not reach significance in the other data sets evaluated, 11 (33%) were identified in one of the other data sets, and 16 (48%) were identified in all data sets.

### Clinical impact of OATP1B1/1B3 inhibition

A total of 83 compounds were identified from the 3 data sets, 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 was composed of 47 drugs, 2 endogenous compounds, and 1 imaging probe. Only the 47 drugs were subsequently evaluated for clinical relevance of OATP1B1/1B3 inhibition, as the remaining 3 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 included 16 drugs with data from all 3 data sets—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 2 of the 3 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, whereas only 1 drug with only PGx data was retained for evaluation. The remaining six drugs only had clinical DDI studies available and were not evaluated further due to a lack of confirmatory data (Figure 1a,b).

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 1b). There were sufficient data for 34 drugs to support a clinically significant role of OATP1B1/1B3 (Table 2 and Table S2). 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



**Figure 1** Selection process for potential organic anion-transporting polypeptide (OATP)1B1/1B3 substrates from the *in vitro*, pharmacogenetic (PGx), and clinical drug–drug interaction (DDI) data sets. **(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 data sets 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 numbers of compounds removed from consideration are indicated by a checked pattern while those retained are in solid color.

Table 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	<i>In vitro</i>
Ambrisentan	Yes	✓	✓	–
Asunaprevir	Yes	✓	–	✓
Atorvastatin	Yes	✓	✓	✓
Atrasentan	No	✓	✓	✓ <sup>a</sup>
Bosentan	Yes	✓	–	✓
Caspofungin	Yes	✓	–	✓ <sup>a</sup>
Cerivastatin	Yes (discontinued)	✓	–	✓
Danoprevir	Yes	✓	–	✓ <sup>a</sup>
Docetaxel	Yes	✓	✓	✓
Eluxadoline	Yes	✓	✓	✓
Empagliflozin	No	✓	–	✓ <sup>a</sup>
Erythromycin	Unclear (insufficient data)	✓	✓	✓ <sup>a</sup>
Fexofenadine	Unclear (confounding by P-gp)	✓	✓	✓ <sup>a</sup>
Fimasartan	Unclear (insufficient safety data)	✓	–	✓ <sup>a</sup>
Fluvastatin	Yes	✓	✓	✓
Glecaprevir	Yes	✓	✓	✓
Glyburide	Unclear (insufficient data)	✓	✓	✓
Grazoprevir	Yes	✓	–	✓ <sup>a</sup>
Letermovir	Yes	✓	✓	✓ <sup>a</sup>
Lopinavir	Unclear (confounding by CYP3A)	–	✓	✓
Lovastatin	Yes	✓	✓	✓ <sup>a</sup>
Nateglinide	Unclear (insufficient data)	–	✓	✓ <sup>a</sup>
Nelfinavir	Unclear (confounding by CYP3A)	✓	–	✓ <sup>a</sup>
Olmesartan	No	–	✓	✓
Paritaprevir	Yes	✓	–	✓
Pitavastatin	Yes	✓	✓	✓
Pravastatin	Yes	✓	✓	✓
Repaglinide	Yes	✓	✓	✓
Rosuvastatin	Yes	✓	✓	✓
Simvastatin	Yes	✓	✓	✓ <sup>a</sup>
SN-38	Yes	–	✓	✓
Telmisartan	Unclear (insufficient data)	–	✓	✓
Torseamide	No	–	✓	✓
Voxilaprevir	Yes	✓	–	✓ <sup>a</sup>

<sup>a</sup>Data are outside the cutoff value ( $K_m \leq 10 \mu\text{M}$  and/or uptake ratio  $\geq 2$ ) and were identified in the secondary evaluation after identification of substrates from clinical data.

CYP, cytochrome P450; DDI, drug–drug interaction; OATP, organic anion-transporting polypeptide; PGx, pharmacogenetic; P-gp, P-glycoprotein. Clinical significance of OATP1B1/1B3 was assessed based on documented safety concerns associated with increased exposure of the drug. A “✓” indicates data were available for the specified data set, “–” indicates no data available. Full data for each drug are available in Table S2.

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 S3).

Multiple therapeutic areas were represented among the 34 identified clinical substrates, with an appreciable fraction having a site of action in the liver (41%). The largest contributors were statins (8 drugs; 24% of total) and anti-infective



agents, including six hepatitis C virus 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 seven drugs (**Table S4**). These drugs were initially identified from clinical studies with one of the recommended inhibitors (single dose or i.v. rifampin, cyclosporine, or gemfibrozil) resulting in a change in exposure  $\geq 25\%$  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, whereas one (digoxin) was found to not be a substrate of OATP1B1/1B3. To illustrate, simeprevir was initially identified from *in vitro* uptake ratios  $> 2$ , yet there were 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 maximum plasma concentration ( $C_{max}$ ) 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 coadministration (600 mg, multiple doses). 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.

### 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 established 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 of 15.0, with scores ranging from 1.5–12.0 (**Table S5**). 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 toward OATP1B1/1B3 inhibition, a low or manageable contribution of other metabolic and transport pathways, and a favorable clinical safety profile (**Table 3**). It is important to note that this approach identified the four clinical substrates currently recommended by the FDA and/or International Transporter Consortium—atorvastatin, pravastatin, pitavastatin, and rosuvastatin, supporting the appropriateness of the selection parameters used.<sup>8</sup> The six proposed substrates are primarily statins and hepatitis C virus 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.

### Comparison of clinical substrates to ECCS

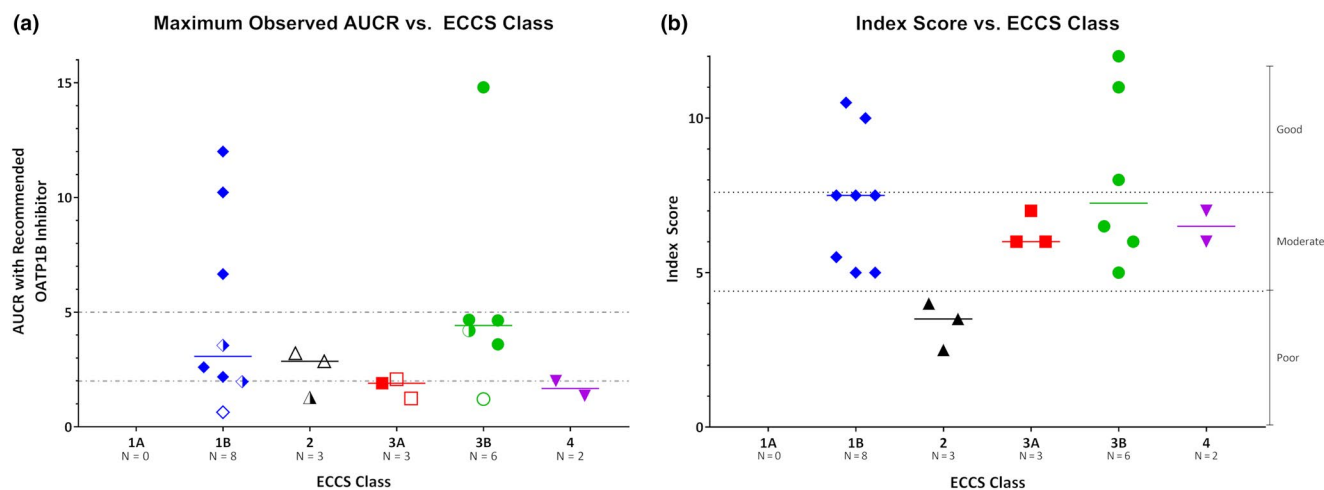
The ECCS evaluates drugs based on a combination of permeability, ionization state, molecular weight, and the separation of metabolic and transport rate-determining steps.<sup>12–14</sup> According to the ECCS, drugs in the 1B and 3B classes should be the most promising markers of OATP1B activity, as these are compounds where 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 2a**). Drugs in the 1B class had the highest proportion of AUC changes greater

**Table 3** Potential clinical marker compounds 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 AUCR <sup>a</sup>	Highest observed PGx effect	Other metabolism/transport <sup>b</sup>
Pravastatin <sup>c</sup>	1	12.0	3B	Statin	4.64	3.81	BCRP/OATP2B1/P-gp
Rosuvastatin <sup>c</sup>	2	11.0	3B	Statin	4.67	2.18	CYP2C9 BCRP/OATP2B1/P-gp
Pitavastatin <sup>c</sup>	3	10.5	1B	Statin	6.67	3.85	BCRP/OATP2B1/P-gp
Atorvastatin <sup>c</sup>	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 <sup>d</sup>
Letermovir	5	8.0	— <sup>e</sup>	Antiviral	2.10 (CsA)	1.40	N/A <sup>d</sup>

AUCR, area under the concentration-time curve ratio; BCRP, breast cancer resistance protein; CsA, cyclosporine A; CYP, cytochrome P450; ECCS, extended clearance classification system; GI, gastrointestinal; N/A, not applicable; OATP, organic anion-transporting polypeptide; P-gp, P-glycoprotein; PGx, pharmacogenomics.

<sup>a</sup>Rifampin studies were used when available due to the lower confounding from other pathways. When no rifampin study data were available, cyclosporine or gemfibrozil were used and selected to ensure the lowest contribution of other pathways possible. <sup>b</sup>Listed alphabetically. <sup>c</sup>Compounds are currently recommended probe compounds by the US Food and Drug Administration and/or International Transporter Consortium. <sup>d</sup>No other enzymes or transporters are currently identified as contributing to the disposition of the drug. <sup>e</sup>ECCS has not been assigned.



**Figure 2** Comparison of extended clearance classification system (ECCS) to observed area under the concentration-time curve ratio (AUCR) and index score for organic anion-transporting polypeptide (OATP)1B substrates. (a) Maximum observed AUCR with a recommended OATP1B inhibitor relative to the ECCS class. Each point is a specific drug, and the horizontal line indicates the median AUCR for the class. Dotted gray lines show twofold and fivefold changes in AUC. Filled shapes are changes observed from rifampin studies (single oral dose or i.v.), hollow shapes are pharmacogenetic study data, and half-filled shapes are from cyclosporine or gemfibrozil studies. (b) Probe index score by ECCS. Solid horizontal line indicates median score for the class and dotted gray lines indicate the cutoff values for each index classification (poor < 4.4, good  $\geq 7.6$ ). ECCS determined by Varma *et al.*<sup>12,13</sup> 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.

than fivefold (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 showed that the proposed indexing system accurately predicted those compounds expected to be sensitive clinical substrates (Figure 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).

## DISCUSSION

Recognition of the clinical importance of OATP1B1/1B3 is continually increasing, consistent with an increased awareness of their role in both research and regulatory guidelines. This analysis utilized a multipronged 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 all 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 composites of the various pathways 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. 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, 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 plays a significant role in the *in vivo* disposition (14/22; 64%; of drugs with available ECCS). 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-fold to 2.1-fold increase<sup>15–17</sup>) with the exception of simvastatin, which showed an increase of 3.2-fold.<sup>18</sup> These findings indicate that, although 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, 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. Even though one drug, empagliflozin, seems 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 was 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 can be reevaluated 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 data set 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 substrates are likely due to changes in OATP1B1/1B3 activity. However, eluxadoline shows lower sensitivity toward OATP1B inhibition compared with some of the other compounds, with the maximum observed AUCR of 4.2 for eluxadoline/cyclosporine.<sup>19</sup> 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<sup>20</sup>), as well as significant changes with a broad-spectrum inhibitor, such as a 22.8-fold increase for pravastatin/cyclosporine (BCRP and P-gp).<sup>21,22</sup> 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 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 simply 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<sup>23</sup>), whereas the opposite is true for human embryonic kidney (HEK) 293 cells expressing OATP1B1 and OATP1B3 (uptake ratio = 7.7 and 3.5, respectively<sup>24</sup>). 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 6 showing promise as potential marker substrates. Identification of compounds with diverse metabolic and transport profiles can allow for selection of fit-for-purpose marker substrate selection and could reduce the impact of confounding factors in the interpretation of interaction data.

**Supporting Information.** Supplementary information accompanies this paper on the *Clinical and Translational Science* website ([www.cts-journal.com](http://www.cts-journal.com)).

**Table S1.** *In vitro* substrates of OATP1B1/1B3.

**Table S2.** Compounds identified with sufficient data to determine clinical relevance of OATP1B1/1B3.

**Table S3.** Summary of the current labeling recommendations for drugs identified as clinical OATP1B1/1B3 substrates.

**Table S4.** Drugs identified in the queries but found to not be *in vivo* substrates or data are insufficient to determine the *in vivo* role of OATP1B1/1B3.

**Table S5.** Calculation of index score for evaluated drugs.

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