

Hepatic Clearance Predictions from In Vitro–In Vivo Extrapolation and the Biopharmaceutics Drug Disposition Classification System[§]

Christine M. Bowman and Leslie Z. Benet

Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, California

Received May 11, 2016; accepted August 11, 2016

ABSTRACT

Predicting in vivo pharmacokinetic parameters such as clearance from in vitro data is a crucial part of the drug-development process. There is a commonly cited trend that drugs that are highly protein-bound and are substrates for hepatic uptake transporters often yield the worst predictions. Given this information, 11 different data sets using human microsomes and hepatocytes were evaluated to search for trends in accuracy, extent of protein binding, and drug classification based on the Biopharmaceutics Drug Disposition Classification System (BDDCS), which makes predictions about transporter effects. As previously reported, both in vitro systems (microsomes and hepatocytes) gave a large number of inaccurate results, defined as predictions falling more than 2-fold outside of

in vivo values. The weighted average of the percentage of inaccuracy was 66.5%. BDDCS class 2 drugs, which are subject to transporter effects in vivo unlike class 1 compounds, had a higher percentage of inaccurate predictions and often had slightly larger bias. However, since the weighted average of the percentage of inaccuracy was still high in both classes (81.9% for class 2 and 62.3% for class 1), it may be currently hard to use BDDCS class to predict potential accuracy. The results of this study emphasize the need for improved in vitro to in vivo extrapolation experimental methods, as using physiologically based scaling is still not accurate, and BDDCS cannot currently help predict accurate results.

Introduction

The current drug-development process is expensive, time-consuming, and inefficient due to compound attrition (Pammolli et al., 2011). Although failures due to pharmacokinetic parameters have decreased in recent years (Waring et al., 2015), continued improvement in pharmacokinetic predictions is crucial.

Metabolic stability studies are some of the earliest in vitro studies conducted during drug development to determine the rate and extent to which a molecule is metabolized, and can be useful for rank ordering candidates. After measuring in vitro metabolic turnover, or intrinsic clearance (CL_{int}), in vivo hepatic clearance can be predicted using in vitro–in vivo extrapolation (IVIVE) methods. A common approach is to apply physiologically based scaling factors to the raw in vitro data, such as hepatocellularity for studies using hepatocytes or a factor to account for incomplete microsomal recovery for microsomes, and to then apply a model of hepatic disposition, such as the well stirred model (Houston, 1994). Although the results are often used in the drug-development process, there is perhaps an overemphasis placed on their reliability.

The first part of the present study examined the overall accuracy of hepatic clearance predictions in the field at this time. Many groups have attempted IVIVE, tried to create new models to improve predictions from old in vitro values, or investigated different experimental setups. A study published 10 years ago collected and examined results from

85 compounds, concluding there was a paucity of literature data (Nagilla et al., 2006); however, much work has been done since then.

When examining the accuracy of these values, a prediction bias has been found that is unresolved from human variability and experimental uncertainty (Hallifax and Houston, 2009). There is also a commonly cited trend that substrates for hepatic uptake transporters and highly protein-bound compounds yield the poorest predictions (Soars et al., 2007). The Biopharmaceutics Drug Disposition Classification System (BDDCS), which categorizes transporter effects on drug disposition, says class 1 compounds exhibit minimal clinically relevant transporter effects, whereas class 2 compounds may be governed by transporter effects in the gut and liver (Wu and Benet, 2005). BDDCS has become an important part of early drug discovery for predicting routes of elimination, food effects, and potential drug interactions (Wu and Benet, 2005). Given this trend, the main objective of this study was to determine if BDDCS classification could be a determinant of accurate IVIVE results.

Materials and Methods

A literature search was conducted for previously described compounds for which both in vitro and in vivo clearance data were available. Studies using human microsomes as well as human hepatocytes were considered, as both systems are routinely used in the pharmaceutical industry. The terms used as keywords to help in the search included “in vitro–in vivo extrapolation,” “intrinsic clearance,” “microsomes,” “hepatocytes,” or a combination of these.

All of the studies considered here used the well stirred model in their predictions, and predictions were made using physiologically based scaling factors, not empirical or regression-based factors. The data sets were examined separately, excluding re-examination of previously published data, as different experimental setups (such as the inclusion of serum in incubations) and scaling (such as the inclusion of f_{ub} and f_{uinc} versus no binding terms) were used in each.

C.M.B. was supported by the National Science Foundation Graduate Research Fellowship Program [Grant 1144247].

dx.doi.org/10.1124/dmd.116.071514.

[§]This article has supplemental material available at dmd.aspetjournals.org.

ABBREVIATIONS: AFE, average fold error; BDDCS, Biopharmaceutics Drug Disposition Classification System; CL_{int} , intrinsic clearance; IVIVE, in vitro to in vivo extrapolation; RMSE, root mean squared error.

Similarly, repeated drugs were not removed due to value differences among data sets. Overall evaluations were also tabulated. The data evaluated can be found in Supplemental Table 1.

The accuracy of predictions was determined based on whether the predictions fell within 2-fold of the true *in vivo* values, as has been a standard cutoff in previous studies (Zuegge et al., 2001; Blanchard et al., 2006; Fagerholm, 2007).

To measure bias, the average fold error (AFE) was calculated using the following equation (Obach et al., 1997):

$$AFE = 10^{\frac{1}{N} \sum \log \left(\frac{\text{observed}}{\text{predicted}} \right)}$$

AFE was recorded as the whole number reciprocal if less than 1.

The precision was also calculated with the root mean squared error (RMSE) using the following (Sheiner and Beal, 1981):

$$RMSE = \sqrt{\frac{1}{N} \sum (\text{predicted} - \text{observed})^2}$$

To divide the compounds based on their BDDCS classification, two publications categorizing over 900 drugs and over 175 drugs were consulted (Benet et al., 2011; Hosey et al., 2016). Five compounds were also classified here for the first time (class 1: amobarbital, bufuralol, levoprotidine, and triprolidine; class 2: tenidap). Trends in the accuracy of predictions compared with class 1 and class 2 drugs, where metabolism is the main route of elimination, were examined. Protein binding was also considered if the values used in the prediction calculations were available, as the interplay between protein binding, transporters, and enzymes is known to be important (Benet, 2009). Drugs with high protein binding were defined as having a free fraction less than or equal to 0.05.

Results

Seven different papers were examined that fit the criteria mentioned earlier (Obach, 1999; McGinnity et al., 2004; Ito and Houston, 2005; Riley et al., 2005; Brown et al., 2007; Hallifax et al., 2010; Sohlenius-Sternbeck et al., 2010). Hallifax et al. (2010) compiled a large database of predictions from many of the papers also examined here; however, not all drugs from the original papers were included, and different values of

$CL_{in\ vivo}$ were often compared, causing the same drugs to be accurately or inaccurately predicted based on the value choices. Furthermore, although it could be argued that the more recent Hallifax et al. (2010) paper provides refined values from the original papers, looking at the percentage of inaccuracy and AFE both overall and for class 1 and class 2 drugs reveals that the Hallifax et al. (2010) data often actually have a comparable or higher percentage of inaccuracy and AFE values compared with the original papers. All papers were therefore examined to try to obtain a fuller picture of the relationship to BDDCS. Five human microsome data sets, some with multiple scaling options, were included in this evaluation for a total of 332 values, and six human hepatocyte data sets were also included for a total of 332 values. The percentage of inaccurate predictions (more than 2-fold difference) for each data set as well as the AFE and RMSE are shown in Table 1. Every data set examined has 41.0% or greater inaccuracy, and AFE values are as high as 21.7. The paper by Sohlenius-Sternbeck et al. (2010) only provided individual prediction values using a regression model, so further analysis could not be conducted. However, since it is the most recent paper examined, the summary statistics using the well stirred model with protein binding that were given were still included in the table for comparison. The weighted average for the percentage of inaccurate results for microsomes is 66.8%, for hepatocytes is 66.2%, and overall is 66.5%.

The same papers and data sets were used to examine BDDCS trends. Class 1 and class 2 drugs were compiled from each set, and the inaccuracy of the predictions, AFE, and RMSE for each class was determined (Table 2). As expected, class 2 drugs have a higher percentage of inaccurate predictions than class 1 drugs in every case except one, where all predictions were inaccurate. The AFE was either slightly higher or almost identical for class 2 drugs compared with class 1 drugs. Considering a total of 305 class 1 drug values, the weighted average of the percentage of inaccurate predictions is 62.3%. For a total of 155 class 2 drug values, the weighted average of the percentage of inaccuracy is 81.9%. [The total number of class 1 and class 2 drugs is

TABLE 1
Percentage of inaccuracy, AFE, and RMSE of IVIVE predictions for 11 data sets

System	Number of Compounds Evaluated	Number of Inaccurate Predictions (%)	AFE	RMSE
Brown et al. (2007)				
Hepatocytes	37	26 (70.3)	4.5	6460.2
Hallifax et al. (2010)				
Microsomes	68	53 (77.9)	5.2	3708.6
Hepatocytes	89	60 (67.4)	3.9	3137.7
Ito and Houston, (2005)				
Microsomes	52	45 (86.5)	7.9	1337.0
McGinnity et al. (2004) ^a				
Hepatocytes	44	22 (50.0)	1.4	94.1
Obach (1999)				
Microsomes (f_{ub} and f_{inc})	29	13 (44.8)	2.3	4.9
Microsomes (f_{ub})	29	22 (75.9)	4.3	6.8
Microsomes (no binding)	29	13 (44.8)	1.5	4.3
Riley et al. (2005) ^b				
Microsomes	37	27 (73.0)	3.3	2314.2
Hepatocytes	56	38 (67.9)	3.1	1356.5
Hepatocytes (with serum)	14	14 (100.0)	21.7	2124.3
Sohlenius-Sternbeck et al. (2010) ^c				
Microsomes (f_{ub} and f_{inc})	44	70.0	3.8	5.8
Hepatocytes (f_{ub} and f_{inc})	46	89.0	5.9	8.0
Microsomes (no binding)	44	41.0	0.5	6.1
Hepatocytes (no binding)	46	41.0	0.8	5.4

^a CL_{int} data were evaluated.

^b CL_{int} , ub , $in\ vivo$ data were evaluated.

^cIndividual values for predictions with well stirred model were not presented, only summary statistics.

TABLE 2
Percentage of inaccuracy, AFE, and RMSE of IVIVE predictions for BDDCS class 1 and class 2 drugs

System	Number of Class 1 Drugs	Number of Inaccurate Class 1 Predictions	AFE	RMSE	Number of Class 2 Drugs	Number of Inaccurate Class 2 Predictions	AFE	RMSE
		%				(%)		
Brown et al. (2007)								
Hepatocytes	24	14 (58.3)	3.0	294.5	12	11 (91.7)	7.4	11,335.9
Hallifax et al. (2010)								
Microsomes	42	30 (71.4)	5.2	4521.7	22	20 (91.0)	4.7	1834.4
Hepatocytes	55	36 (65.5)	4.0	3976.5	30	22 (73.3)	3.7	466.1
Ito and Houston (2005)								
Microsomes	32	27 (84.4)	6.8	390.8	16	15 (93.8)	11.2	2312.3
McGinnity et al. (2004)								
Hepatocytes	32	16 (50.0)	1.1	99.3	9	5 (55.6)	3.0	90.9
Obach (1999)								
Microsomes (fu _b and fu _{inc})	20	7 (35.0)	1.9	4.6	9	6 (66.6)	3.2	5.4
Microsomes (fu _b)	20	13 (65.0)	3.7	6.9	9	9 (100.0)	6.0	6.7
Microsomes (no binding)	20	7 (35.0)	1.2	4.0	9	6 (66.7)	2.5	4.8
Riley et al. (2005)								
Microsomes	24	16 (66.7)	2.7	2399.1	11	9 (81.8)	6.0	2298.5
Hepatocytes	28	16 (57.1)	2.4	175.7	22	18 (81.8)	3.8	2125.8
Hepatocytes (serum)	8	8 (100.0)	9.6	251.0	6	6 (100.0)	64.2	3232.0

less than 644, since individual drugs are not enumerated in Sohlenius-Sternbeck et al. (2010) and some unapproved proprietary compounds are included in other data sets.] For class 1 drugs, studies done in microsomes have a weighted average of 63.3% inaccuracy, whereas studies in hepatocytes are 66.2% inaccurate. For class 2 drugs, studies in microsomes have a weighted average of prediction inaccuracy of 85.6%, whereas studies in hepatocytes have a 78.4% average.

Finally, given that substrates of transporters and highly bound drugs often have the poorest clearance predictions (Soars et al., 2007), protein-binding differences were examined between the two BDDCS classes. First, the percentage of drugs with inaccurate predictions that are also highly protein-bound in both classes was determined (Table 3). There are more inaccurate class 2 drugs that are highly protein-bound than class 1 drugs in every case examined. The weighted average of inaccurate class 1 drugs with high protein binding is 19.8%, whereas the weighted average for class 2 is 67.3%. Since class 2 drugs in general are often highly protein-bound (Broccatelli et al., 2012), the numbers of highly bound drugs in both classes that have inaccurate predictions were also determined (Table 4). These results agree with several other conclusions that highly protein-bound compounds are often poorly predicted. Class 1 highly protein-bound drugs were inaccurately predicted 81.3% of the time, and class 2 highly bound drugs had an 85.7% average inaccuracy

rate. In four data sets, highly bound class 2 drugs had a higher percentage of inaccuracy than class 1 drugs; in one data set, the opposite was true; and in the last set, all highly bound drugs were inaccurate.

Looking at the bias between the high and low protein-binding drugs in the two classes (Table 5), it is difficult to see trends between the two classes; however, the bias is always higher for the high protein-binding drugs, except in the case of the data from Obach (1999), using fu_b and fu_{inc}, and Brown et al. (2007), where there are only two class 1 high protein-binding drugs and four class 2 low protein-binding drugs, perhaps skewing the results.

Discussion

Being able to accurately predict pharmacokinetic parameters, especially clearance, early in the drug-development process is a key part of lead optimization. However, although some studies have claimed to find success in predicting in vivo clearance from in vitro data, others have questioned the reliability (Masimirembwa et al., 2003). Underpredicting in vivo clearance may result in inefficiency in the drug-discovery pipeline or an ineffective therapeutic dosing regimen, whereas overpredicting in vivo clearance may lead to missed opportunities that were rejected early in the development process (Clarke and Jeffrey, 2001).

TABLE 3
Percentage inaccuracy of BDDCS class 1 and class 2 drugs that are highly protein-bound

System	Number of Inaccurate Class 1 Predictions	Number of Inaccurate Highly Protein-Bound Class 1 Predictions	Number of Inaccurate Class 2 Predictions	Number of Inaccurate Highly Protein-Bound Class 2 Predictions
		(%)		(%)
Brown et al. (2007)				
Hepatocytes	14	1 (7.1)	11	7 (63.6)
Hallifax et al. (2010)				
Microsomes	30	6 (20.0)	20	9 (45.0)
Hepatocytes	36	9 (25.0)	22	15 (68.2)
Obach (1999)				
Microsomes (fu _b and fu _{inc})	7	1 (14.3)	6	4 (66.6)
Microsomes (fu _b)	13	1 (7.7)	9	4 (44.4)
Microsomes (no binding)	7	2 (28.6)	6	4 (66.7)
Riley et al. (2005)				
Hepatocytes	16	4 (25.0)	18	17 (94.4)
Hepatocytes (serum)	8	2 (25.0)	6	6 (100.0)

TABLE 4
Percentage of highly protein-bound BDDCS class 1 and class 2 drugs that are inaccurate

System	Number of Highly Protein-Bound Class 1 Drugs	Number of Inaccurate Highly Protein-Bound Class 1 Predictions (%)	Number of Highly Protein-Bound Class 2 Drugs	Number of Inaccurate Highly Protein-Bound Class 2 Predictions (%)
Brown et al. (2007)				
Hepatocytes	2	1 (50.0)	8	7 (87.5)
Hallifax et al. (2010)				
Microsomes	8	6 (75.0)	10	9 (90.0)
Hepatocytes	9	9 (100.0)	20	15 (75.0)
Obach (1999)				
Microsomes (f_{ub} and f_{inc})	2	1 (50.0)	4	4 (100.0)
Microsomes (f_{ub})	2	1 (50.0)	4	4 (100.0)
Microsomes (no binding)	2	2 (100.0)	4	4 (100.0)
Riley et al. (2005)				
Hepatocytes	5	4 (80.0)	21	17 (81.0)
Hepatocytes (serum)	2	2 (100.0)	6	6 (100.0)

The goal of this study was to compile data to examine the accuracy of the prediction methods for in vivo clearance and relate this accuracy to BDDCS classification. For the 11 data sets considered, there is a large percentage of inaccuracy. To have a true understanding of the accuracy of in vitro methods, physiologically scaled in vitro estimations and observed in vivo clearance were directly compared, since incorporating established physiologic scaling factors as well as unbound fractions in the blood and possibly in vitro matrix should, in theory, give accurate predictions. This is in contrast to some groups creating linear regression equations from reference compound data and then applying an empirical scaling factor to try to further improve predictions (Sohlenius-Sternbeck et al., 2012). The fact that 66.5% of predictions overall are inaccurate emphasizes the idea that a mechanistic understanding of this inaccuracy still needs to be determined before IVIVE predictions can be completely trusted.

BDDCS classification and protein binding were then examined to evaluate if accurate results could be separated from inaccurate results to help determine whether predictions can be trusted in the future or not. Class 1 drugs, or those that are extensively metabolized and highly soluble, appear to overwhelm transporter effects, whereas class 2 drugs, also extensively metabolized but poorly soluble, can be affected by efflux transporters in the gut and both uptake and efflux transporters in the liver (Shugarts and Benet, 2009). Given the trend that poorly predicted compounds are often substrates for transporters (Soars et al., 2007), it was expected that class 1 drugs that have no clinically relevant transporter effects would yield better predictions than class 2 drugs. The other part of the trend is that poorly predicted compounds are also often highly protein-bound, which is why protein binding was considered when data were available (Ring et al., 2011). Overall, the hypothesis was that class 2 drugs would be more poorly predicted due to the fact that they are substrates for transporters, and these poorly predicted class 2 drugs would also be highly protein-bound.

As expected, class 2 drugs yielded poorer predictions in every case examined; however, there was still large inaccuracy for both class 1 and class 2 drugs. Class 2 drugs also often had a higher AFE, but the difference was not enough (or sometimes did not exist at all) to indicate bias. However, AFE provides a better measure of bias than RMSE, which is highly influenced by the marked differences in CL_{int} values from study to study. For example, the values reported by Brown et al. (2007) for predicted and measured CL_{int} for propofol were 2773 and 5052 ml/min/kg, respectively, whereas for the same drug McGinnity et al. (2004) reported 283 and 24 ml/min/kg. At this point in time, with the current methodology, relying on BDDCS class cannot confidently provide information about whether predictions will be accurate or not. This agrees with previous findings from Poulin et al. (2012), who found

that predictivity was similar between classes for a human microsome data set of 42 drugs. It is interesting to note that microsomes and hepatocytes gave similar prediction accuracies in both class 1 and class 2 drugs. A bigger difference between the two systems would have been expected for class 2 drugs where transporters play a role, since necessary uptake transporters are not present in microsomes. This again emphasizes that there are likely major missing determinants when trying to mimic the interplay between protein binding, uptake, and metabolism in vitro.

Poulin et al. (2012) also suggested that an approach involving determination of effective fraction unbound in plasma based on albumin-facilitated hepatic uptake of acidic/neutral drugs improved the prediction accuracy and precision for 25 high protein-binding drugs. Hallifax and Houston (2012) examined this approach for 107 drugs studied in hepatocytes and microsomes, also finding an increase in prediction accuracy but no change

TABLE 5
AFE and RMSE of high and low protein-binding BDDCS class 1 and class 2 drugs

Protein Binding	Class 1		Class 2	
	AFE	RMSE	AFE	RMSE
Brown et al. (2007)				
Hepatocytes				
High	2.0	56.4	6.3	13,882.7
Low	3.1	307.1	10.3	229.6
Hallifax et al. (2010)				
Microsomes				
High	7.8	10,335.3	5.3	2671.0
Low	4.8	349.9	4.2	473.3
Hepatocytes				
High	12.1	9814.8	4.2	479.9
Low	3.3	242.7	2.9	437.0
Obach (1999)				
Microsomes (f_{ub} and f_{inc})				
High	1.7	0.3	4.7	3.1
Low	2.0	4.9	2.3	6.7
Microsomes (f_{ub})				
High	4.7	0.4	7.3	3.1
Low	3.6	7.3	5.2	8.6
Microsomes (no binding)				
High	13.7	1.5	7.7	6.8
Low	1.1	17.7	1.0	2.2
Riley et al. (2005)				
Hepatocytes				
High	3.1	175.2	3.9	2175.6
Low	2.2	173.3	2.8	136.5
Hepatocytes (serum)				
High	17.0	406.3	64.2	3232.0
Low	8.0	170.2	—	—

in precision, and reported that there was no evidence that prediction bias was associated with measured fraction unbound in plasma. These latter authors emphasized the need for further “mechanistic elucidation to improve prediction methodology rather than empirical correction of bias” (Hallifax and Houston, 2012).

Last, protein binding was considered along with BDDCS. Given current trends, class 2 drugs with high protein binding would have been expected to yield the poorest results. There were more inaccurate class 2 drugs that had higher protein binding than class 1, but this may be because class 2 drugs generally have higher protein binding than class 1 (Broccatelli et al., 2012). This coupled to the fact that there may be a slight dependency of bias on protein binding, both here and as found previously with hepatocytes by Hallifax et al. (2010), could explain some of the difference seen between the inaccuracies in class 1 and class 2 drugs. However, on average, highly bound drugs in both classes had similar high percentages of inaccuracy, and there were no clear trends in the bias or precision of highly bound drugs between classes.

This study emphasizes the fact that the *in vitro* to *in vivo* extrapolation of hepatic clearance needs to be improved through a better understanding of clearance mechanisms, as *in vitro* methods on their own are often not accurate, and looking at BDDCS class cannot separate out which compounds will have accurate predictions.

Authorship Contributions

Participated in research design: Bowman, Benet.

Conducted experiments: Bowman.

Performed data analysis: Bowman.

Wrote or contributed to the writing of the manuscript: Bowman, Benet.

References

- Benet LZ (2009) The drug transporter-metabolism alliance: uncovering and defining the interplay. *Mol Pharm* **6**:1631–1643.
- Benet LZ, Broccatelli F, and Oprea TI (2011) BDDCS applied to over 900 drugs. *AAPS J* **13**: 519–547.
- Blanchard N, Hewitt NJ, Silber P, Jones H, Coassolo P, and Lavé T (2006) Prediction of hepatic clearance using cryopreserved human hepatocytes: a comparison of serum and serum-free incubations. *J Pharm Pharmacol* **58**:633–641.
- Broccatelli F, Cruciani G, Benet LZ, and Oprea TI (2012) BDDCS class prediction for new molecular entities. *Mol Pharm* **9**:570–580.
- Brown HS, Griffin M, and Houston JB (2007) Evaluation of cryopreserved human hepatocytes as an alternative *in vitro* system to microsomes for the prediction of metabolic clearance. *Drug Metab Dispos* **35**:293–301.
- Clarke SE and Jeffrey P (2001) Utility of metabolic stability screening: comparison of *in vitro* and *in vivo* clearance. *Xenobiotica* **31**:591–598.
- Fagerholm U (2007) Prediction of human pharmacokinetics—improving microsome-based predictions of hepatic metabolic clearance. *J Pharm Pharmacol* **59**:1427–1431.
- Hallifax D, Foster JA, and Houston JB (2010) Prediction of human metabolic clearance from *in vitro* systems: retrospective analysis and prospective view. *Pharm Res* **27**:2150–2161.
- Hallifax D and Houston JB (2009) Methodological uncertainty in quantitative prediction of human hepatic clearance from *in vitro* experimental systems. *Curr Drug Metab* **10**: 307–321.
- Hallifax D and Houston JB (2012) Evaluation of hepatic clearance prediction using *in vitro* data: emphasis on fraction unbound in plasma and drug ionisation using a database of 107 drugs. *J Pharm Sci* **101**:2645–2652.
- Hosey CM, Chan R, and Benet LZ (2016) BDDCS predictions, self-correcting aspects of BDDCS assignments, BDDCS assignment corrections, and classification for more than 175 additional drugs. *AAPS J* **18**:251–260.
- Houston JB (1994) Utility of *in vitro* drug metabolism data in predicting *in vivo* metabolic clearance. *Biochem Pharmacol* **47**:1469–1479.
- Ito K and Houston JB (2005) Prediction of human drug clearance from *in vitro* and preclinical data using physiologically based and empirical approaches. *Pharm Res* **22**:103–112.
- Masimirembwa CM, Bredberg U, and Andersson TB (2003) Metabolic stability for drug discovery and development: pharmacokinetic and biochemical challenges. *Clin Pharmacokinet* **42**:515–528.
- McGinnity DF, Soars MG, Urbanowicz RA, and Riley RJ (2004) Evaluation of fresh and cryopreserved hepatocytes as *in vitro* drug metabolism tools for the prediction of metabolic clearance. *Drug Metab Dispos* **32**:1247–1253.
- Nagilla R, Frank KA, Jolivet LJ, and Ward KW (2006) Investigation of the utility of published *in vitro* intrinsic clearance data for prediction of *in vivo* clearance. *J Pharmacol Toxicol Methods* **53**:106–116.
- Obach RS (1999) Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of *in vitro* half-life approach and nonspecific binding to microsomes. *Drug Metab Dispos* **27**:1350–1359.
- Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, MacIntyre F, Rance DJ, and Wastall P (1997) The prediction of human pharmacokinetic parameters from preclinical and *in vitro* metabolism data. *J Pharmacol Exp Ther* **283**:46–58.
- Pammolli F, Magazzini L, and Riccaboni M (2011) The productivity crisis in pharmaceutical R&D. *Nat Rev Drug Discov* **10**:428–438.
- Poulin P, Hop CE, Ho Q, Halladay JS, Haddad S, and Kenny JR (2012) Comparative assessment of *In Vitro-In Vivo* extrapolation methods used for predicting hepatic metabolic clearance of drugs. *J Pharm Sci* **101**:4308–4326.
- Riley RJ, McGinnity DF, and Austin RP (2005) A unified model for predicting human hepatic, metabolic clearance from *in vitro* intrinsic clearance data in hepatocytes and microsomes. *Drug Metab Dispos* **33**:1304–1311.
- Ring BJ, Chien JY, Adkison KK, Jones HM, Rowland M, Jones RD, Yates JWT, Ku MS, Gibson CR, He H, et al. (2011) PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 3: comparative assessment of prediction methods of human clearance. *J Pharm Sci* **100**:4090–4110.
- Sheiner LB and Beal SL (1981) Some suggestions for measuring predictive performance. *J Pharmacokinetic Biopharm* **9**:503–512.
- Shugarts S and Benet LZ (2009) The role of transporters in the pharmacokinetics of orally administered drugs. *Pharm Res* **26**:2039–2054.
- Soars MG, McGinnity DF, Grime K, and Riley RJ (2007) The pivotal role of hepatocytes in drug discovery. *Chem Biol Interact* **168**:2–15.
- Sohlenius-Stembeck AK, Afzelius L, Prusis P, Neelissen J, Hoogstraate J, Johansson J, Floby E, Bengtsson A, Gissberg O, Stembeck J, et al. (2010) Evaluation of the human prediction of clearance from hepatocyte and microsome intrinsic clearance for 52 drug compounds. *Xenobiotica* **40**:637–649.
- Sohlenius-Stembeck AK, Jones C, Ferguson D, Middleton BJ, Projean D, Floby E, Bylund J, and Afzelius L (2012) Practical use of the regression offset approach for the prediction of *in vivo* intrinsic clearance from hepatocytes. *Xenobiotica* **42**:841–853.
- Waring MJ, Arrowsmith J, Leach AR, Leeson PD, Mandrell S, Owen RM, Paireudeau G, Pennie WD, Pickett SD, Wang J, et al. (2015) An analysis of the attrition of drug candidates from four major pharmaceutical companies. *Nat Rev Drug Discov* **14**:475–486.
- Wu C-Y and Benet LZ (2005) Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm Res* **22**:11–23.
- Zuegge J, Schneider G, Coassolo P, and Lavé T (2001) Prediction of hepatic metabolic clearance: comparison and assessment of prediction models. *Clin Pharmacokinet* **40**:553–563.

Address correspondence to: Leslie Z. Benet, Department of Bioengineering and Therapeutic Sciences, University of California, 533 Parnassus Avenue, Room U-68, San Francisco, California 94143-0912. E-mail: leslie.benet@ucsf.edu