while ensuring the quality needed to draw reliable research conclusions and (ii) replacing the prevailing view of practice and research as separate activities with a "learning health system" methodology that incorporates research into practice as a routine element of clinical care. These changes will require significant adjustments to the ethical frameworks that span the spectrum of learning activities, from quality improvement to interventional research involving new therapies.[10]

Conclusion

Selker and colleagues have articulated a vision that is consistent with our evolving understanding of therapeutic development. Before this vision can become a reality, numerous practical and conceptual barriers must first be overcome. However, revolutionary clinical research methods that are now being piloted have the potential to help make E2E a reality.

CONFLICT OF INTEREST

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In Vitro Prediction of Clinical Drug Interactions With CYP3A Substrates: We Are Not There Yet

DJ Greenblatt[1]

In 1973, Malcolm Rowland and associates described an approach to predicting clinical pharmacokinetic drug–drug interactions (DDIs) using an inhibition constant determined *in vitro* (K_i) together with anticipated inhibitor exposure *in vivo* ([I]). Despite numerous modifications and refinements of the core model over the following 40 years, we still have not achieved a predictive paradigm having accuracy sufficient to justify bypassing all, or even most, clinical DDI studies in the course of drug development.

The use of *in vitro* data to anticipate, predict, or explain clinical pharmacokinetic drug interactions was first described by Rowland and Matin in 1973, in the context of the inhibition of tolbutamide clearance by coadministration of sulfaphenazole.[1] The core of the model was what is now commonly termed "[I] over K_i"—the ratio of inhibitor exposure *in vivo* ([I]) divided by an *in vitro* inhibition constant (K_i) that reflects (in reciprocal fashion) the quantitative potency of the inhibitor. The more [I] exceeds K_i, the greater is the $[I]/K_i$ ratio, and the greater is the probability and/or magnitude of a clinical pharmacokinetic DDI caused by the perpetrator's (e.g., sulfaphenazole) inhibition of clearance of the victim (e.g., tolbutamide). Rowland and Matin at that time also pointed out the importance of f_m—the fraction of the dose metabolized via the target pathway—as a modulator of the predictive validity of the $[I]/K_i$ ratio.[1]

Clinical and scientific interest in DDIs intensified in the late 1980s and

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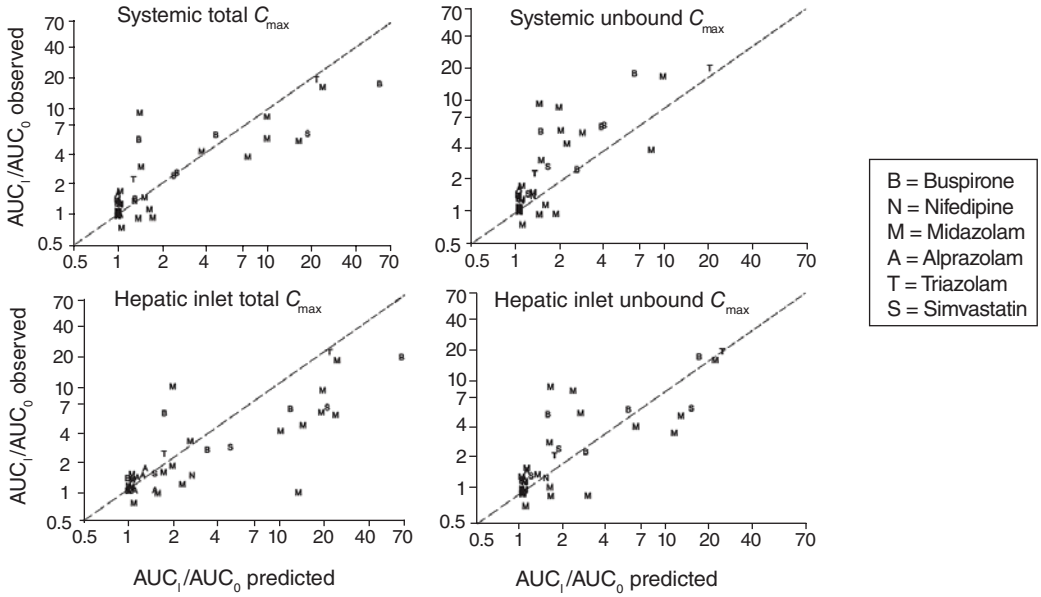

Figure 1 Observed values of area under the curve $(AUC)_I/AUC_0$ ratios (explained in the text) from clinical drug–drug-interaction studies of six CYP3A substrate drugs (*y*-axis) vs. values predicted from the *in vitro* paradigm (*x*-axis), as described by Obach and associates.[9] Four values of anticipated inhibitor exposure *in vivo* [I] are used in the prediction: systemic total maximum inhibitor concentration (C_{max}; upper left); systemic unbound C_{max} (upper right); hepatic inlet (portal) total C_{max} (lower left); and hepatic inlet (portal) unbound C_{max} (lower right). See text and **Table 1** for analysis of the data. Reprinted from ref. 8.

early 1990s, coincident with the regulatory and media attention attracted by the Seldane (terfenadine) affair. Predictive *in vitro–in vivo* DDI scaling models resurfaced,[2–4] again based on the $[I]/K_i$ concept from Rowland and Matin.

The models did not work well, even after numerous refinements and modifications described by many authors in the late 1990s and up to the late 2000s (see **Supplementary References** online). The determination of K_i *in vitro*—even for a specific inhibitor vs. a specific substrate—was subject to technical and interpretive bias and inaccuracy[5] and did not necessarily reflect the susceptibility of the metabolic enzyme to chemical inhibition *in vivo*. Most importantly, the value of [I] in the $[I]/K_i$ ratio—still the cornerstone of all scaling models—should reflect the concentration of inhibitor at the site of metabolic enzyme activity *in vivo*. We can *measure* the total or unbound inhibitor levels in the systemic circulation, and we can *guess at* what might be more relevant concentrations (e.g., intra-enteric, total portal, or unbound portal concentrations), but we cannot actually measure the quantitative exposure of the enzyme

We previously evaluated[8] the validity of a predictive model reported in 2006 by Obach and associates[9] for a series of 42 observed-vs.-predicted DDI pairs for six different CYP3A substrates. The model was based (as in 1973) on the $[I]/K_i$ concept along with f_m, but with additional assumptions: bioavailability of the substrate across the gastrointestinal tract mucosa (F_g), intestinal-wall inhibitor concentration ($[I]_g$), apparent first-order absorption rate constant (k_a), fraction of the inhibitor passing through the intestine unchanged (F_a), enteric blood flow (Q_g), and hepatic blood flow (Q_h). IC_{50} was used as a surrogate for K_i. The observed quantitative DDI *in vivo* was expressed as area under the plasma concentration curve (AUC) for the substrate (victim) during coadministration of the inhibitor (AUC_I) divided by the corresponding AUC in the control state (AUC_0).[8] The predicted quantitative DDI was calculated from the model, using four possibilities for [I]: total systemic plasma C_{max}, unbound systemic C_{max}, total portal (hepatic inlet) C_{max}, and unbound portal C_{max}. Observed and predicted AUC ratios were plotted using logarithmic axes for clarity (**Figure 1**).

Based on linear regression analysis of log-transformed values, all four [I] options yielded r^2 values in a similar range, with the most variability explained using the total systemic C_{max}

Table 1 Observed vs. predicted drug interactions for CYP3A substrates

	Maximum inhibitor concentration			
Observed vs. predicted interaction	Systemic total	Systemic unbound	Portal total	Portal unbound
Overall r^2	0.75	0.66	0.67	0.67
Observed > predicted	57%	76%	38%	57%
Predicted > observed	38%	24%	60%	38%
Percent differing by >50%	19%	24%	36%	19%

option (r^2 = 0.75) (**Table 1**). Systemic unbound C_{max} yielded a high fraction of underpredicted values, while total portal C_{max} yielded a high fraction of overpredicted values. Unbound portal C_{max} was no better than total systemic C_{max}, either in overall r^2, the frequency of under- and overprediction, or the percentage of pairs for which observed and predicted values differed by more than 50% (**Table 1**, **Figure 1**). Our conclusion at the time[8] was that reasonable predictive accuracy was not achieved, and that no other estimate of [I] improved on that based simply on total systemic C_{max}.

The most recent iteration of CYP3A DDI prediction is described by Vieira and associates in this issue.[10] Some data points from the 2006 paper[9] are shared, and other data points were added (some of which come from regulatory submissions, with perpetrators not identified, and data not available to the public). The predictive model is more complex and refined, and it includes additional parameters that are measured or assumed: the intraluminal gastrointestinal concentration ($[I]_{gut}$), the unbound *in vitro* inhibition constant ($K_{i,u}$), the unbound inhibitor concentration causing half-maximal inactivation ($K_{I,u}$), the maximal inactivation rate constant (k_{inact}), and the enzyme degradation rate constant (k_{deg}). The last three named parameters are connected to perpetrators presumed to cause time-dependent (mechanism-based) inhibition. When induction is coincident with inhibition, the induction component is accounted for with an approach similar to that of Einolf and associates (described in this issue).[11]

Figures 1–3 in the paper by Vieira and associates[10] are disheartening, especially when the $y = x$ lines (not drawn by the authors) are drawn in. The deviation of observed from predicted is extensive, and major overprediction is the rule. Model validity does not look improved since 2006. We are not there yet.

From a regulatory standpoint, it could be argued that the principal objective of DDI prediction should be the avoidance of false negatives—real clinical DDIs not predicted by the model. If so, major overprediction by the model protects the public, and the model is a "success." But this is balanced by the high prevalence of negative clinical DDI studies, with the associated low, but still nonzero, risk to DDI study participants, as well as the cost burden to the drug development process which is passed on to the health-care system. From a scientific standpoint, we seem to be going in the wrong direction; model validity is not matching model complexity. Because more complex approaches are not leading to improved predictive capacity, we should look back to the core components of the scaling paradigm—the same [I] and K_i that Rowland's group identified 40 years ago—rather than pursue increasingly complex models as proposed in current regulatory guidance. Improved validity of prediction may well be achieved through molecular physiology approaches to determining the inhibitor concentration that the enzyme actually "sees," and a K_i value that reflects the effect of the inhibitor on the metabolic enzyme as it functions *in vivo*.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

CONFLICT OF INTEREST

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