Optimization of *In vitro* -*In vivo* Extrapolation (IVIVE) Approaches for Human Hepatic Clearance Prediction : Application to EZH1/2 Dual Inhibitor HM97662

Yunju Kang, Seokhyun Hong, Sunyoung Lim, Taehun Song, Seung Hyun Jung, Sang Hyun Lee, Young Gil Ahn and In Young Choi
Hanmi Pharmaceutical Co., Ltd., Seoul, Republic of Korea

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Introduction

The prediction of human pharmacokinetics (PK) at the preclinical stage is a critical step in drug development, as it enables the efficient design of first-in-human (FIH) studies and reduces the risk of clinical failures. In particular, the estimation of human hepatic clearance (CL) is essential because hepatic metabolism represents the major elimination pathway for a wide range of small-molecule drugs.

In vitro–in vivo extrapolation (IVIVE) is a widely applied framework that integrates *in vitro* experimental data with *in vivo* scaling approaches to quantitatively predict human pharmacokinetics. This strategy not only provides mechanistic insights into drug metabolism and disposition but also reduces reliance on animal studies and enhances the translational relevance of preclinical findings. As such, IVIVE has become an indispensable tool in modern drug development, particularly for predicting human clearance.

Although various IVIVE approaches have been proposed to improve predictive accuracy, the strategy for selection of adequate methods remains unclear. Here, we systematically review and compare these IVIVE approaches.

Objectives

The objective of this study is to evaluate the predictive accuracy of various IVIVE methods using reference compounds and to develop an integrated IVIVE flowchart. To demonstrate this flowchart, we used HM97662, an EZH1/2 (Enhancer of Zeste Homolog 1/2) inhibitor currently in Phase I clinical trials by Hanmi Pharma. HM97662 targets the catalytic subunit of the polycomb repressive complex 2 (PRC2), which regulates gene expression through trimethylation of lysine 27 on histone H3 (H3K27me3). We aimed to apply preclinical data of HM97662 to the IVIVE flowchart to predict human clearance (CL) and to verify its predictive performance by comparing the results with those obtained from the first-in-human (FIH) study.

Methods

The basic well-stirred model (unbound fraction-corrected, basic-WSM) has been reported to systemically underestimate the actual *in vivo* clearance. To overcome this limitation, various IVIVE methods have been proposed, including the application of additional correction factors, empirical scalars and scaling factors (Figure 1). In this study, we developed an IVIVE flowchart incorporating these approaches and evaluated its predictive performance in prediction of human CL using actual dataset with animal and human.

Figure 1. IVIVE methods for Human CL Prediction

Basic Well-stirred model

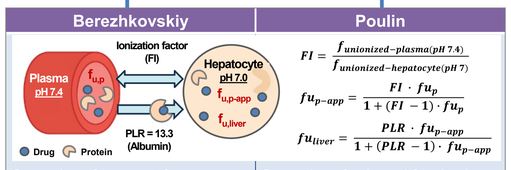
Unbound fraction correction ($f_{u,p}$, $f_{u,inc}$)

$$CL_h = \frac{Q_h \cdot \frac{fu_p}{R_{bp}} \cdot \frac{CL_{int}}{fu_{inc}}}{Q_h + \frac{fu_p}{R_{bp}} \cdot \frac{CL_{int}}{fu_{inc}}}$$

Correction Factor (CF)

Ionization | Ionization & PLR

Berezhkovskiy | Poulin



$$FI = \frac{f_{unionized-plasma(pH\,7.4)}}{f_{unionized-hepatocyte(pH\,7)}}$$

$$fu_{p-app} = \frac{FI \cdot fu_p}{1 + (FI - 1) \cdot fu_p}$$

$$fu_{liver} = \frac{PLR \cdot fu_{p-app}}{1 + (PLR - 1) \cdot fu_{p-app}}$$

Correction of apparent f_{up} ($f_{up\text{-}app}$) considering differences in drug ionization between plasma and liver cells (ionization factor = FI)

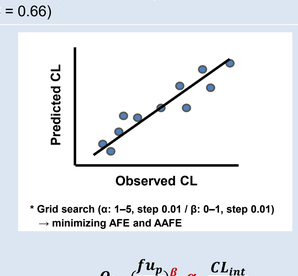
$$CL_h = \frac{Q_h \cdot \frac{fu_{p-app}}{R_{bp}} \cdot \frac{CL_{int}}{fu_{inc}}}{Q_h + \frac{fu_{p-app}}{R_{bp}} \cdot \frac{CL_{int}}{fu_{inc}}}$$

Correction of unbound fraction in the liver ($f_{u,liver}$) for ionization and plasma-to-liver ratio (PLR) of plasma binding proteins

$$CL_h = \frac{Q_h \cdot \frac{fu_{liver}}{R_{bp}} \cdot \frac{CL_{int}}{fu_{inc}}}{Q_h + \frac{fu_{liver}}{R_{bp}} \cdot \frac{CL_{int}}{fu_{inc}}}$$

Empirical scalar

Empirical scalars for human hepatocyte intrinsic clearance (α = 1.74) and plasma unbound fraction (β = 0.66)

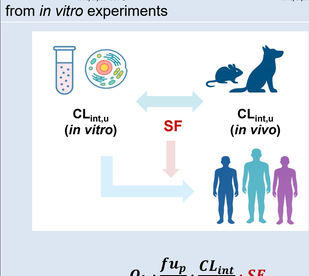


* Grid search (α: 1–5, step 0.01 / β: 0–1, step 0.01) → minimizing AFE and AAFE

$$CL_h = \frac{Q_h \cdot (\frac{fu_p}{R_{bp}})^{\beta} \cdot \alpha \cdot \frac{CL_{int}}{fu_{hep}}}{Q_h + (\frac{fu_p}{R_{bp}})^{\beta} \cdot \alpha \cdot \frac{CL_{int}}{fu_{hep}}}$$

Scaling factor (SF)

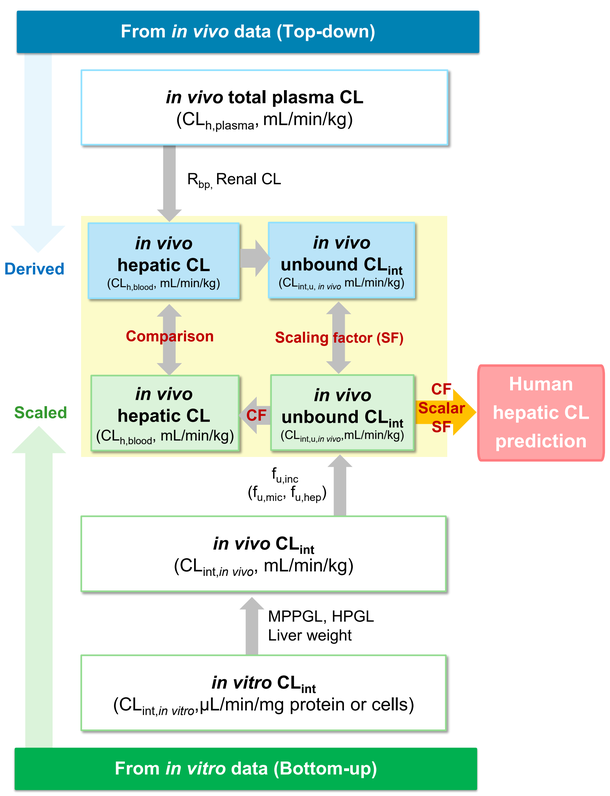
Correction of animal SF (difference between derived $CL_{int,u,\textit{in vivo}}$ from *in vivo* PK and $CL_{int,u,\textit{in vivo}}$ from *in vitro* experiments



$$CL_h = \frac{Q_h \cdot \frac{fu_p}{R_{bp}} \cdot \frac{CL_{int}}{fu_{inc}} \cdot SF}{Q_h + \frac{fu_p}{R_{bp}} \cdot \frac{CL_{int}}{fu_{inc}} \cdot SF}$$

Conceptual Approach for IVIVE

Figure 2. IVIVE flowchart for human CL prediction



- MPPGL = Microsomal protein per gram of liver
- HPGL = Hepatocellularity number per gram of liver

▪ Dataset selection

The *in vitro* CL_{int} and *in vivo* CL dataset are obtained from the literatures (Poulin *et al.*, 2012, 2013; Wood *et al.*, 2017). To apply the animal scaling factor (SF), drugs for which both animal (rat and/or dog) and human data were available were selected, except for hepatocytes (only human data, Poulin *et al.*, 2013). In the Poulin dataset (2013), a randomly selected subset of data were utilized for minimizing bias across datasets.

Table 1. Literature dataset

Test systems	Liver microsomes			Hepatocytes	
References	Rat	Dog	Human	Rat	Human
Poulin *et al.*, 2012	✓	✓	✓	-	-
Poulin *et al.*, 2013	-	-	-	-	✓
Wood *et al.*, 2017	✓	-	✓	✓	✓

Figure 3. Drug characteristic in dataset

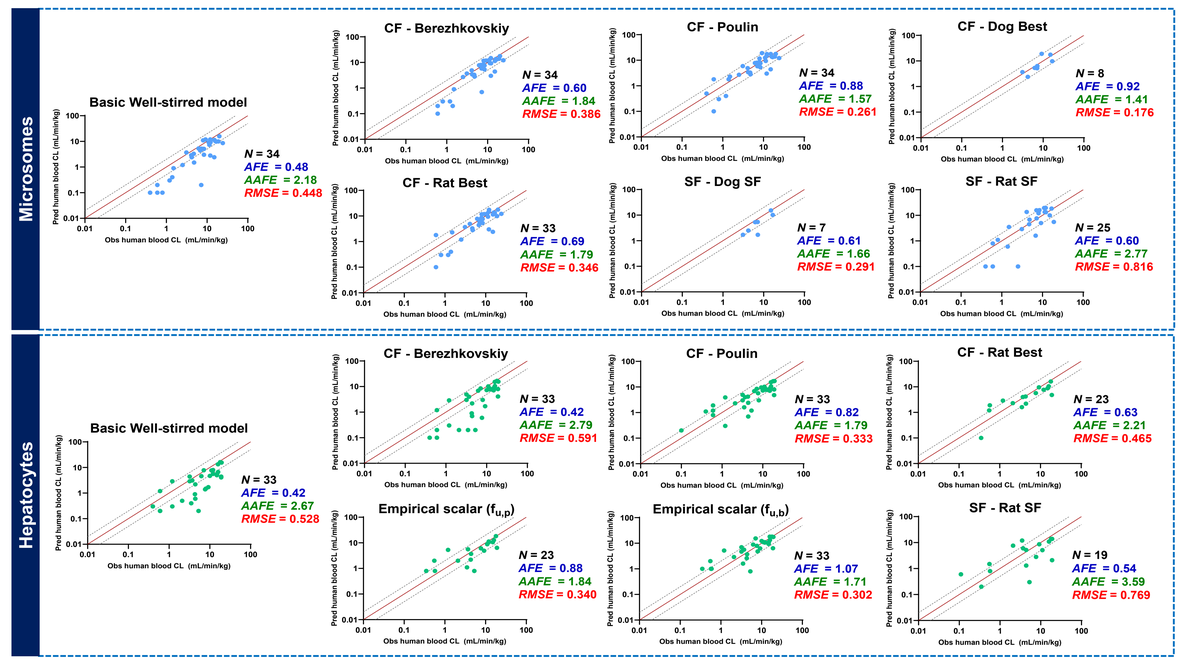
Table 2. Data analysis for predictive performance

Categories	Equations	Criteria
AFE	$10^{(1/n \cdot \sum(log(Fold-error))}$	≈ 1
AAFE	$10^{(1/n \cdot \sum(log(Fold-error))}$	≤ 2.0
RMSE	$\sqrt{(1/n \cdot \sum(log(Obs.CL_i) - log(Pred.CL_i))^2}$	≤ 0.4

- AFE = average fold-error; AAFE = absolute average fold-error; RMSE = root mean square error
- Fold-error = predicted CL / observed CL

Comparative Evaluation

Figure 4. Comparison between observed and predicted human clearance



- In both microsomes and hepatocytes, the basic well-stirred model (basic-WSM) underestimated human clearance (AFE = 0.42–0.48), which is consistent with the literature. The other methods generally improved prediction accuracy compared with the basic-WSM, and Poulin method (ionization and PLR correction) and empirical scalar (scalars for CL_{int} and unbound fraction) demonstrated the highest predictive performance in microsomes and hepatocytes dataset, respectively.
- The empirical scalar approach in hepatocytes showed comparable predictive performance with $f_{u,p}$ (assuming R_{bp} = 1, original method) and $f_{u,b}$ (with R_{bp} correction), indicating that empirical scalar method could be applied regardless of R_{bp} correction.

Application to EZH1/2 inhibitor HM97662

Table 3. Prediction of HM97662 in human hepatic CL

IVIVE	Test systems		Microsomes				Hepatocytes			
	Species		Human	Dog	Rat	Mouse	Human	Dog	Rat	Mouse
In vivo	Observed blood CL_h (mL/min/kg)		-	31.7	50.0	33.3	-	31.7	50.0	33.3
	Derived $CL_{int,u,\textit{in vivo}}$ (mL/min/kg)		-	2481	1944	963	-	2481	1944	963
In vitro	Scaled $CL_{int,u,\textit{in vivo}}$ (mL/min/kg)		27	76	30	114	35	98	53	80
IVIVE Pred. CL	Basic-WSM ($f_{u,b}$, $f_{u,inc}$)		2.3	7.9	2.6	5.9	2.9	9.5	4.4	4.2
	Correction factor (CF)	Berezhkovskiy	4.4	13.1	5.3	12.3	5.4	15.2	9.0	9.0
		Poulin	10.3	22.5	17.1	40.9	11.6	24.5	25.5	33.2
	Species		Human				Human			
	Empirical scalar		-				7.9			
	Scaling factor (SF)		-	16.2	17.9	10.5	-	16.3	17.3	13.4

- Assumption : Total CL = hepatic CL (very low renal CL in rats) & R_{bp} = 1
- Scaled to *in vivo* condition using a scaling factors (MPPGL and HPGL) and liver weight (g liver/ kg body weight)

Figure 5. Obs. *vs.* Pred. CL in animals

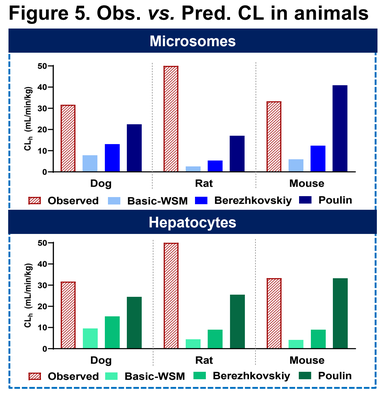
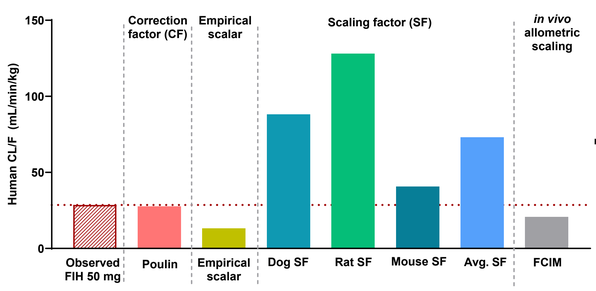


Figure 6. Obs. *vs.* Pred. CL/F in human



- Assumption : F = F_h = 1 – CL_h / Q_h
- FCIM = f_u-corrected intercept method

- Application of IVIVE flowchart, we predicted the human CL of HM97662, which has major clearance pathway via hepatic metabolism, using *in vitro* and *in vivo* preclinical data. The scaled CL_{int} values from microsomes and hepatocytes were comparable, indicating CYP-mediated metabolism is predominant metabolic pathway of HM97662. In IVIVE approaches using CF, the Poulin method exhibited highest predictive accuracy in both microsomes and hepatocytes within 2-fold (except, microsomes in rats) compared to observed *in vivo* CL in animal species (Figure 5).
- A comparison between the observed human CL/F (28.6 mL/min/kg) and predicted CL/F using the Poulin, Empirical scalar, and Scaling factor (across species) approaches, the Poulin method (best prediction method in animals) showed comparable predicted value of 27.6 mL/min/kg (Obs/Pred ratio=1.04), indicating the highest predictive accuracy. Moreover, the predicted CL/F by Poulin method was similar to CL/F via allometry-based method (FCIM), supporting reliable prediction in both *in vitro* and *in vivo*-based approach.

Concluding remarks & Discussions

1. The developed IVIVE flowchart, integrating 'bottom-up' and 'top-down' approaches, had the high prediction accuracy across species using reference compounds.
2. Application of IVIVE flowchart to HM97662 (EZH1/2 inhibitor in clinical development), it showed the highest predictive accuracy with Poulin method compared to observed *in vivo* CL in animals. The predicted human CL/F using Poulin method and FCIM of allometry-based method were comparable, and these results ensured robust accuracy for predicted CL in the preclinical stage.
3. Additionally, when the dataset used in the IVIVE flowchart was applied to IVIVE approach in the recent reference (David Tess *et al.*, 2023), basic drugs showed high prediction accuracy, whereas acidic and neutral drugs exhibited poor accuracy with some limitations.
4. Further research will be required to optimize IVIVE predictive performance by incorporating a wide range of compounds and apply this to drug development to support study design and dose selection in First-in-Human (FIH) studies.

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