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ARTICLE IN REVIEW:

Predicting Human Clearance: A Head-to-Head Comparison of Four Hepatocyte Models

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TITLE: Clearance prediction with three novel plated human hepatocyte models compared to conventional suspension assays: Assessment with 50 compounds and multiple donors[1]

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STUDY DESIGN: Preclinical (*in vitro*)

SUMMARY: This study benchmarked three plated hepatocyte systems - preload monoculture ("preload assay"), coculture, and a triculture model (TruVivo®) - against traditional suspension hepatocytes (SH) using 50 compounds spanning diverse physiochemical properties, Lipinski classifications, and clearance rates. The goal was to assess each model's ability to measure unbound intrinsic clearance ($CL_{int,u}$) and predict human *in vivo* blood clearance (CL_b).

SH showed limited metabolic stability, with ~40% of compounds exhibiting insufficient turnover for $CL_{int,u}$ estimation. All plated systems improved metabolic turnover, with insufficient turnover dropping to 18% (preload), 8% (coculture), and 4% (TruVivo). Prediction accuracy improved accordingly, with coculture and TruVivo yielding the highest proportions of compounds within 3-fold of observed $CL_{int,u}$ and CL_b and the strongest correlations with *in vivo* data. TruVivo showed the greatest metabolic stability, consistent performance across donors, and the strongest *in vitro–in vivo* correlation.

Plated hepatocyte models also reduced inter-experimental and donor-to-donor variability compared with SH, with the coculture and TruVivo exhibiting the most consistent performance despite inherent differences in enzyme activity. Across compound subgroups – including Rule of 5 (Ro5), beyond-Ro5 (bRo5), clearance ranges, and Extended Clearance Classification System (ECCS) classes—the plated models consistently outperformed SH, with the most substantial improvements observed for low-clearance and bRo5 compounds.

Extended Metabolic Stability Supports Low-Clearance Compounds Assessment

Across a diverse set of 50 test compounds, TruVivo's extended incubation capability and preserved metabolic activity allowed for the quantification of low-turnover drugs that conventional 4-hour suspension hepatocyte (SH) assays failed to assess reliably. Among all plated hepatocyte models evaluated, TruVivo demonstrated the lowest rates of insufficient turnover, reinforcing its value in evaluating low-clearance drugs.

TruVivo Delivers High-Fidelity Predictions of Human Hepatic Clearance

TruVivo consistently produced a high proportion of clearance estimates within 3-fold of observed *in vivo* values and demonstrated improved accuracy relative to suspension and preload assays. Prediction performance was comparable to coculture and extended across Ro5 compliant, beyond-Ro5, $CL_b \leq 1$, $CL_b \leq 6$, $CL_b > 6$, demonstrating broad applicability and robust metabolic fidelity.

Plated Hepatocyte Models Minimize Donor Noise, Maximize Predictive Accuracy

Plated models demonstrated markedly reduced donor-to-donor variability and TruVivo produced consistent clearance estimates, even across donors with divergent enzyme activity profiles. This drug metabolizing enzyme stability highlights their robustness for low-clearance drug assessment.

	$CL_{int,u}$				CL_b			
	SH	Preload	Cocultures	Tricultures	SH	Preload	Cocultures	Tricultures
Insufficient Turnover[a]	41%	18%	8%	4%	40%	18%	8%	4%
<2-fold[b]	16% (8/29)	20% (10/40)	40% (20/45)	34% (17/47)	20% (10/30)	34% (17/41)	48% (24/46)	38% (19/48)
<3-fold[b]	20% (10/29)	41% (19/40)	53% (26/45)	49% (24/47)	32% (16/30)	52% (26/41)	62% (31/46)	60% (30/48)
3–10-fold[b]	22% (11/29)	24% (13/40)	27% (13/45)	39% (19/47)	16% (8/30)	18% (9/41)	16% (8/46)	30% (15/48)
>10-fold[2]	16% (8/29)	16% (8/40)	12% (12/45)	8% (4/47)	12% (6/30)	12% (6/41)	14% (7/46)	6% (3/48)
MAFE[c]	4.7	3.2	2.4	3.0	2.9	2.3	2.0	2.3
R^2	.135	.423	.667	.754	.0138	.116	.392	.507

Table 1. Summary of $CL_{int,u}$ and CL_b prediction accuracy across four assay formats (SH, Preload, Cocultures, and Tricultures). Insufficient turnover[a] indicates the percentage of compounds with <10% parent loss. Prediction performance[b] reflects the percentage of compounds predicted within 2-, 3-, or 10-fold of observed *in vivo* values, rounded to one decimal, with parentheses indicating accuracy based only on compounds exhibiting sufficient turnover. Median absolute fold error (MAFE)[c] was calculated for each assay to assess overall prediction accuracy. Reproduced from Table 1 with permission under an open-access license[1].

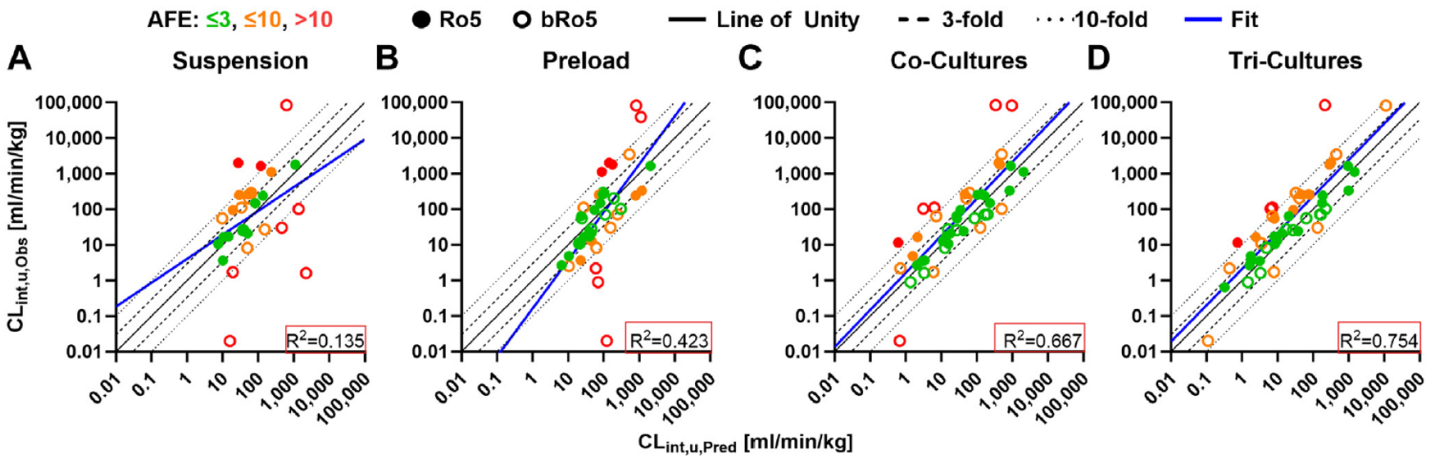

Figure 1. *In vitro–in vivo* correlation of intrinsic clearance ($CL_{int,u}$). Hepatic clearance prediction accuracy was evaluated by comparing observed *in vivo* $CL_{int,u}$ values with $CL_{int,u}$ predicted from (A) suspension hepatocytes, (B) the preload assay, (C) cocultures, and (D) tricultures for Ro5 (closed circles) and bRo5 (open circles) compounds. Data points predicted within 3-fold, within 10-fold, or exceeding 10-fold of *in vivo* values are shown in green, orange, and red, respectively. The solid, dashed, and dotted lines represent the line of unity, 3-fold deviation, and 10-fold deviation, respectively. The blue line indicates the linear regression fit. Figure reproduced from portions of Figure 3 with permissions under an open access license[1].

References

1. **Kukla, D. A., Schulz Pauly, J. A., Lesniak, P. R., Sande, E., Wang, Y.-T., Kalvass, J. C., & Stresser, D. M. (2024).** Clearance prediction with three novel plated human hepatocyte models compared to conventional suspension assays: Assessment with 50 compounds and multiple donors. *Drug Metabolism and Disposition, 53*(2), 100032. https://doi.org/10.1016/j.dmd.2024.100032