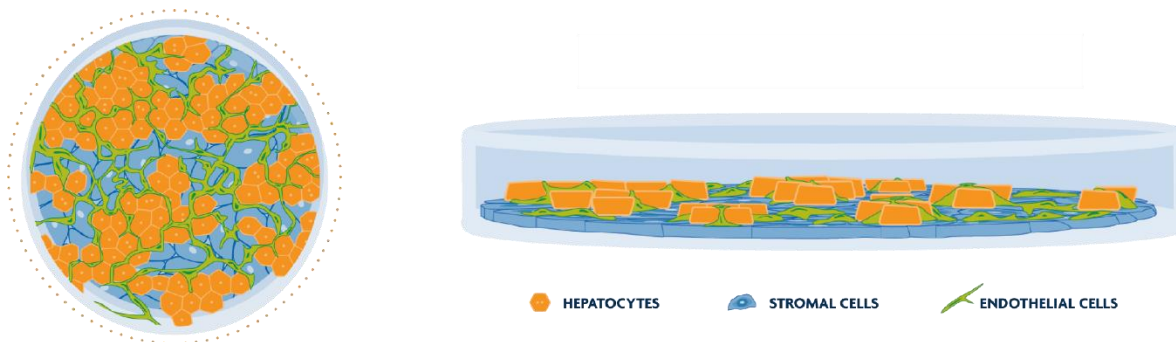# Pioneering Hepatic System Just Named Cell Biology Product of the Year

The First and Only 2D+ All-Human Hepatic System

Conventional *in vitro* models used to assess drug metabolism and toxicological effects often lack phenotypic and functional stability long term and can be inconvenient or difficult to use. TruVivo™, a novel all-human cell-based hepatic system, addresses these limitations by combining cryopreserved primary human hepatocytes with primary human feeder cells in a convenient, 2-dimensional platform. While the system provides the simplicity and flexibility of a 2D model, it also provides the relevance, longevity, architectural integrity, and robustness of a 3D model. TruVivo maintains hepatocyte morphology for up to 42 days, retains greater hepatocyte functionality compared to conventional sandwich culture, and generates stable phase I and II metabolites through at least 2 weeks.

BIOTECH BREAKTHROUGH AWARD 2023

CELL BIOLOGY PRODUCT OF THE YEAR

HEPATOCYTES   STROMAL CELLS   ENDOTHELIAL CELLS

Figure 1. Schematic representation of the TruVivo system depicting each cell type and self-assembled hepatocyte colonies

TruRELEVANCE

TruVivo mimics the microarchitecture of the human liver. Primary human hepatocytes, cultured at an optimized ratio with human endothelial and stromal cells, retain their native cuboidal morphology and self-assemble to form *in vivo*-like hepatocyte colonies with extensive cell-to-cell connections, including tight and gap junctions and bile canalicular networks. Albumin and urea production are within human physiologically relevant ranges, and Phase I and II metabolic pathways are sustained for at least 2 weeks.

TruRELIABILITY

TruVivo offers accurate, consistent results. Each batch of hepatocytes and feeder cells used in the system have been extensively characterized and pre-qualified to reduce experimental variability and aid in fit-for-purpose lot selection.

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Features of the hepatocyte lot that are available to the user include donor medical and social history, histopathological scoring and assessment, genotyping, phase I and II enzyme activity, and CYP enzyme induction.

TruSIMPLICITY

TruVivo is easy to use and adaptable to the user's timeline and workflow needs. The system is provided in ready-to-use kits with cryopreserved cells and optimized media sufficient for at least two weeks in culture. TruVivo requires only basic cell culture experience and standard equipment. Experiments can begin in as little as five days after culture setup. Flexibility in seeding densities, media changes, and more provide the user with the adaptability to meet unique assay needs.

A New Alternative for Drug Discovery

Enzyme and transporter activity remains relatively high and stable in TruVivo for at least two weeks. CYP3A4 induction response is also robust and stable for over 2 weeks.
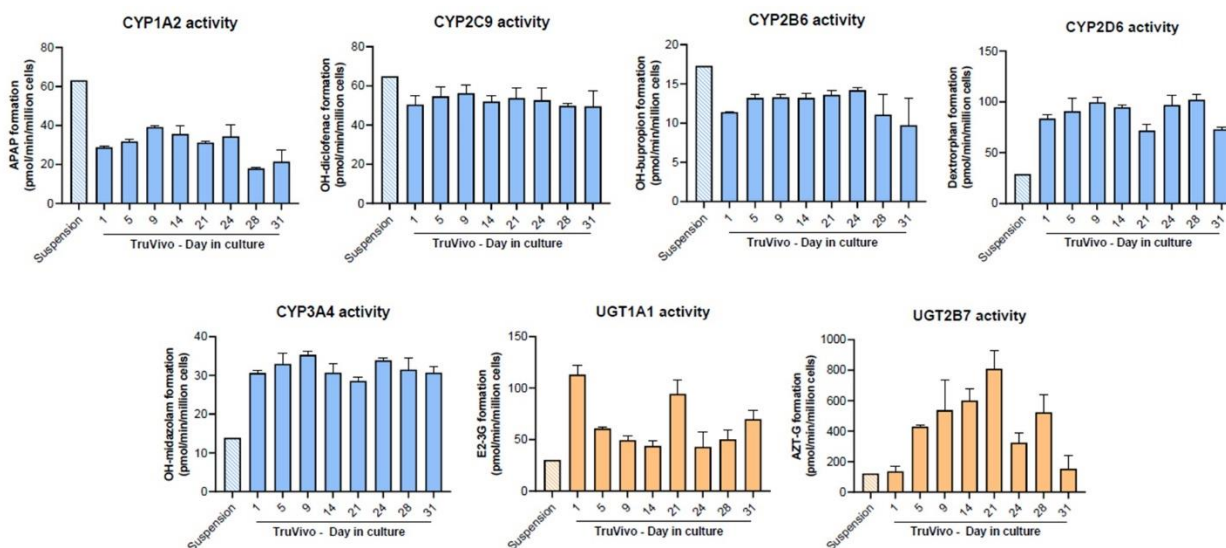

Fig 2. Enzyme activity levels of one hepatocyte lot in TruVivo compared to suspension culture. Stable phenotype was established in TruVivo at about day five and remained stable through the next two weeks. Data generated and provided by Piekos *et al.*, Boehringer Ingelheim, Nonclinical DMPK. Shared with permission.
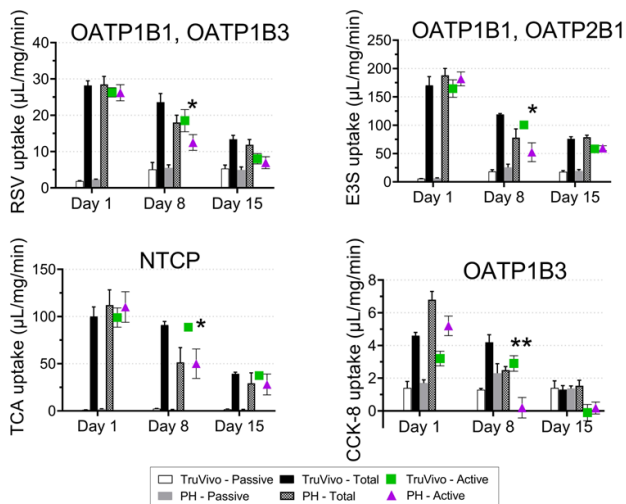
Figure 3. Uptake transporter activities in TruVivo compared to plated hepatocytes (PH) over time. Activities changed with culture time in both formats; however, on day 8, TruVivo retained 61 to 100% of initial transporter activities, whereas plated hepatocytes retained 4 to 70%. OATP1B1 and/or OATP2B1, and NTCP activities were 1.5 to 4.7-fold higher in TruVivo compared to PH. OATP1B3 activity was retained in TruVivo but not measurable in PH. Data generated and provided by Mitra *et al.*, Boehringer Ingelheim, Nonclinical DMPK. Shared with permission.

Figure 4. Three different hepatocyte lots were cultured in TruVivo and uninduced (light shaded bars) and induced (dark shaded bars) CYP3A4 activity levels were measured on days 4, 10, and 16. *** p≤0.001 versus uninduced samples at same time point. Mean ± SD. These results indicate retention of metabolic activity and nuclear receptor response in hepatocytes cultured in TruVivo that is stable over time and significantly greater than in sandwich cultures. Weaver, J. R., *et al.* 2023.
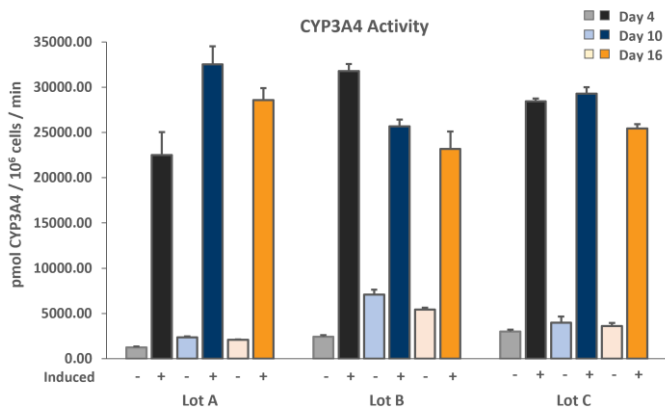
TruVivo is an ideal platform for applications that require prolonged or repeated compound exposures, including the clearance of low-turnover compounds. Internal data produced at LifeNet Health, as well as validation work by Boehringer Ingelheim, has demonstrated the potential for TruVivo to predict *in vivo* clearance. Below is data from an internal metabolic clearance study.
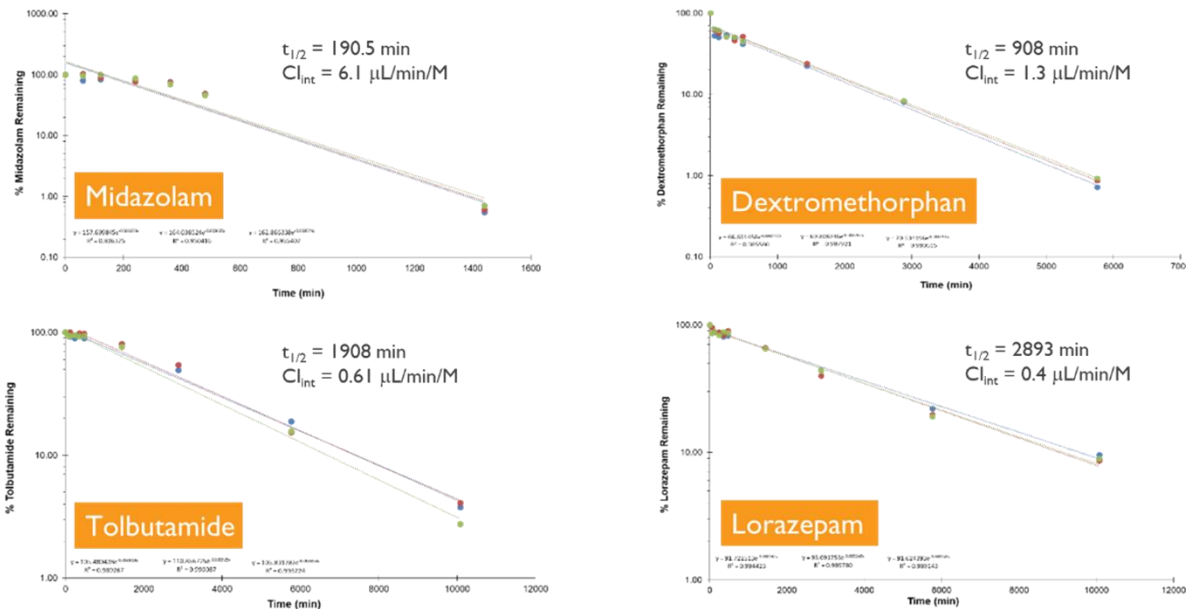
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SUBSTRATE	$T_{1/2}$ (min)	Range	Clinical *In Vivo* CL_{sys}* (mL/min/kg)	TruVivo Calc CL_{sys}
Midazolam	114	108-384	12	15.3
Dextromethorphan	250**	180-360	8.6**	3.2**
Tolbutamide	870	240-1500	1.6	1.5
Lorazepam	12,840	ND	1.1	1.0

** PM: 1770 ± 504   ** PM: 3.9 ± 1.4
EM: 204 ± 30   EM: 1575 ± 658

Table 1. Four substrates representing different metabolic clearance rates were administered in the TruVivo system. CYP450-based midazolam, dextromethorphan, and tolbutamide represent high-, mid, and low-turnover compounds respectively. Lorazepam is a substrate representing mainly Phase II metabolism. *Known *in vivo* systemic clearance values as reported by Goodman and Gilman 11th ed. (2006). Calculated systemic clearance rates in TruVivo were compared to reported *in vivo* values and were predictive of *in vivo* clearance rates, except notably for dextromethorphan. The systemic clearance value calculated in TruVivo for dextromethorphan is representative of a poor metabolizer, which upon further investigation, correlates to the donor's genotype that included alleles indicative of a poor metabolizer phenotype.

Figure 5. Corresponding profiles of percent substrate remaining over time in TruVivo. The profiles were also calculated in feeder cell only cultures (data not shown). No Phase I metabolism was detected in feeder cell only cultures. The contribution of feeder cells to Phase II metabolism was seen for lorazepam but was less than 10%.

Conclusion

TruVivo is the first and only 2D+ hepatic system to provide human-relevant, reliable results in an easy-to-use format. Users can expect the simplicity and flexibility of traditional 2D models, along with the relevance, longevity, and robustness of more advanced 3D models. TruVivo mimics the microarchitecture and basic functionality of the human liver. Hepatocytes self-assemble to form *in vivo*-like colonies with extensive cell-cell connections and bile canalicular networks. Albumin and urea production, phase I and II metabolic pathways, and transporter activity are maintained for at least two weeks. The platform has been tested extensively by leading pharmaceutical and agriscience companies for various applications, including hepatic clearance of low-turnover drugs and drug-drug interactions. Findings from these studies, which demonstrate TruVivo's superior performance compared to sandwich cultures, have been featured in multiple scientific poster presentations.