Utilizing a long-term all-human Tri-Culture system to assess hepatic clearance of low turnover drugs

Alexander Byer-Alcorace[1] Cody Thomas[1], Stephanie Piekos[1], Jessica Weaver[2], Justin J. Odanga[2], Kristina K. Wolf[3], Jingsong Chen[2], Jung Bok Lee[2], Edward L. LeCluyse[3], and Mitchell E. Taub[1]

[1]Nonclinical DMPK, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, Ridgefield, CT 06877

[2]Institute of Regenerative Medicine, LifeNet Health, 1864 Concert Drive, Virginia Beach, VA 23453

[3]Research and Development, LifeSciences, LifeNet Health, 6 Davis Drive, Research Triangle Park, NC, 27709

INTRODUCTION

Predicting human hepatic clearance ($CL_{hepatic}$) is often challenging in early drug development, particularly when low-clearance compounds are involved. To assess the metabolic stability of such drugs, suspended primary human hepatocytes (PHH) are commonly used; however, their ability to accurately predict in vivo CL, particularly of slowly cleared drugs, can be limited by short incubation times and rapid loss of enzymatic activity. To mitigate these issues, long-term in vitro models, like the Tri-Culture System (TCS) developed by LifeNet Health, have been implemented in an effort to improve the characterization of hepatic metabolism and clearance in vitro by lengthening the amount of time hepatocytes can be cultured successfully. The TCS is an all-human cell-based in vitro model comprised of PHH and two different types of primary feeder cells (FC) that are plated together, shown in (Figure 1), on either a 24-well or 96-well collagen-I coated plate. In this study, the TCS (in both 24- and 96-well formats) was assessed for its functionality and its ability to predict human $CL_{hepatic}$ over the course of a 7-day incubation with no media change.

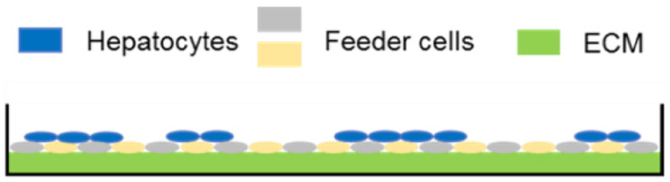

Figure 1: Cross sectional illustration of the TCS depicting hepatocyte chords and the FC monolayer attached to extracellular matrix (ECM). FCs consist of endothelial and stromal cells in a 1:1 mixture. Image provided by LifeNet Health.

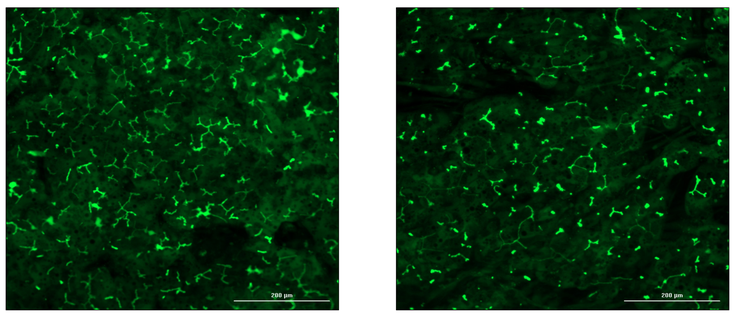

Figure 2: Hepatocyte polarity and functionality. 5-(and-6)-carboxy-2′,7′ – dichlorofluorescein (CDFDA) staining of bile canaliculi in polarized human hepatocytes on days 9 (left) and 15 (right) of incubations with no media change in the 24-well format indicative of MRP-2 transporter function.

EXPERIMENTAL METHODS

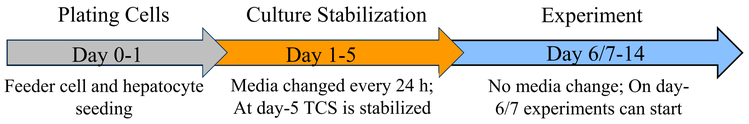

Figure 3: TCS plating and incubation timeline.

Culture Model	Culture Format	Incubation duration	Seeding Density (Hepatocytes) cells/well	Number of attached (Hepatocytes) cells/well
TCS	**24-well**	7-days	300,000	124,000
	96-well		20,000	9,837
HepatoPac	**24-well**	7-days	Proprietary	21,000
	96-well		Proprietary	3,100
Suspended	**96-well**	6 hours	50,000	N/A

Table 1: Incubation conditions. Number of cells seeded, cells attached, and incubation duration in both 24- and 96-well formats. (N/A; not applicable as cells were in suspension).

Comparison of basic cellular function and $CL_{hepatic}$ in both 24- and 96-well formats:

- Hepatic health was evaluated by measuring albumin and lactate dehydrogenase (LDH) at each timepoint normalized to 24h for up to 9-days in culture with no media change.
  - Albumin secretion was assessed using the Abcam Human Albumin SimpleStep 90min ELISA® kit.
  - LDH release was measured using Promega's LDH-Glo Cytotoxicity® kit.
- TCS $CL_{hepatic}$ was assessed by administering test compound and monitoring parent depletion over a 7-day incubation with no media change.
  - Predicted $CL_{hepatic}$ was compared to well established models such as HepatoPac (multidonor lot) and suspended hepatocytes (TCS donor lot).
  - Depletion was quantitated by LC-MS/MS.

Substrates	Concentration, µM	Major metabolic pathway
Alprazolam	**0.1**	**CYP3A**
Tolbutamide		**CYP2C9**
Theophylline		**CYP1A2**

Table 2: Incubation substrates and clearance pathways.

RESULTS

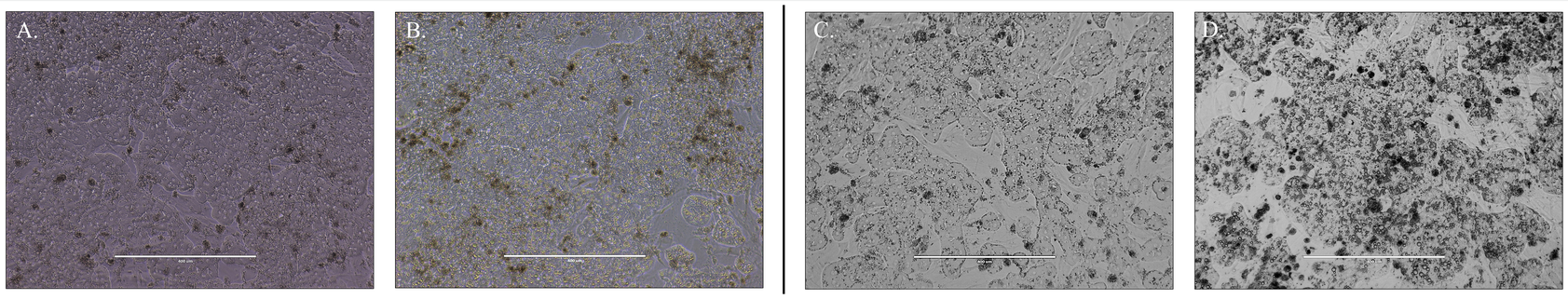

Figure 4: Hepatocyte morphology after 7-days without media change. Microscopy images of 24-well (**A** & **B**) and 96-well (**C** & **D**) depicting incubations on days 7 (immediately following media change) and 14 (7 days without a media change) at 10X.

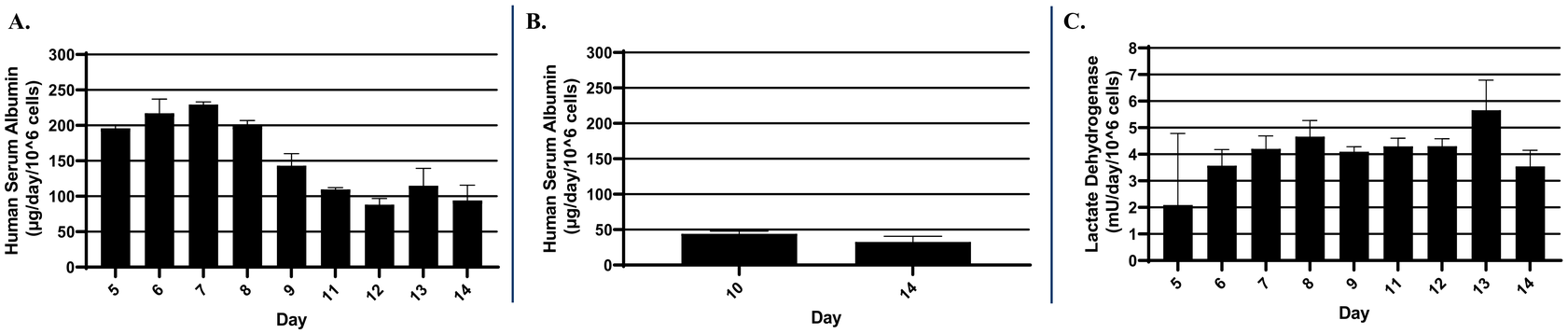

Figure 5: Long-term hepatocyte function and viability. Figure 5A (96-well) & 5B (24-well) depict human serum albumin (HSA) secretion from days 5 to 14. Average HSA secretion for both 96- and 24-well formats with daily media changes are (159 and 59 µg/day/10^6 cells), respectively (data not shown). Figure 5C shows LDH release for the 96-well format from days 5 to 14. Average LDH release in the 96-well format with daily media changes is (3.3 mU/day/10^6 cells, data not shown).

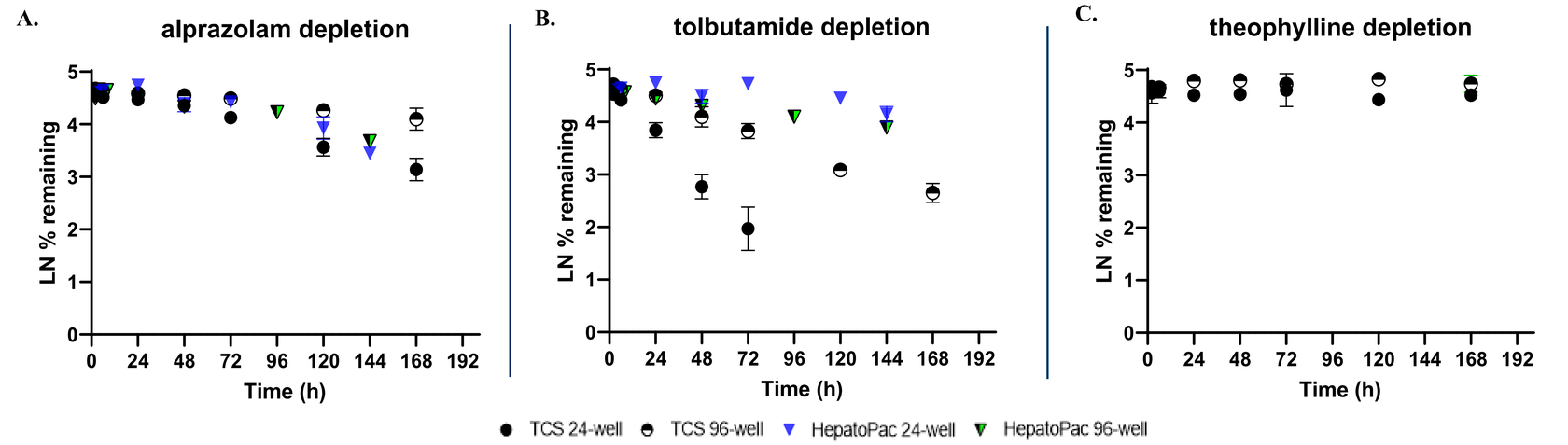

Figure 6: Utilizing the TCS to predict hepatic clearance of low turnover compounds. Representative plots of parent compound depletion for alprazolam, tolbutamide, and theophylline**.** (Each point represents the average of three replicates). Suspension incubations resulted in no measurable depletion for all substrates evaluated (data not shown).

		Predicted $CL_{hepatic}$ (mL/min/kg)		
Substrate	**Culture format**	**TCS**	**HepatoPac**	**Actual in vivo CLnon-renal (mL/min/kg)[1]**
Alprazolam	**24-well**	0.503	1.783	0.61
	96-well	0.478	2.5	
Tolbutamide	**24-well**	0.438	0.144	0.31
	96-well	0.376	0.488	
Theophylline	**24-well**	NC	N/A	1.13
	96-well	NC	N/A	

Table 3: Comparison of predicted in vitro $CL_{hepatic}$ values for alprazolam, tolbutamide, and theophylline to actual in vivo non-renal clearance. Appropriate scaling parameters were applied to determine the predicted $CL_{hepatic}$ and all formats were normalized to 10^6 cells. (N/A) not applicable as it was not assessed; (NC) not calculated, no detectable depletion.

CONCLUSIONS

- Hepatocytes in both the 24-well and 96-well formats appeared healthy morphologically and functionally demonstrated high levels of albumin secretion and relatively low levels of LDH release with no media change for up to 7 days when compared to values observed with daily media changes.
- The TCS predicted $CL_{hepatic}$ that is within 2-fold of actual human in vivo non-renal CL values in both the 24-well and 96-well formats for 2 of 3 compounds tested. Theophylline $CL_{hepatic}$ could not be calculated as no depletion was observed, potentially due to low CYP1A2 activity in this donor. The TCS shows that it performs equally to that of current established hepatocyte systems.
- Overall, the TCS can be used to assess and characterize potential drug candidates for their metabolic stability and clearance in vitro while maintaining hepatic function in long duration incubations.

REFERENCES

1. Chan TS, Yu H, Moore A, Khetani SR, Tweedie D. Meeting the challenge of predicting hepatic clearance of compounds slowly metabolized by cytochrome P450 using a novel hepatocyte model, HepatoPac. Drug Metab Dispos. 2013 Dec;41(12):2024-32. doi: 10.1124/dmd.113.053397. Epub 2013 Aug 19. Erratum in: Drug Metab Dispos. 2014 Jan;42(1):200. Kehtani, Salman R [corrected to Khetani, Salman R]. Erratum in: Drug Metab Dispos. 2019 Jan;47(1):58-66. Corrected and republished in: Drug Metab Dispos. 2019 Jan;47(1):58-66. PMID: 23959596.