TEMPORAL ACTIVITY ASSESSMENTS OF MAJOR METABOLIC PATHWAYS IN HUMAN/HUMAN™ HEPATIC AND STROMAL CO-CULTURE MODEL

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INTRODUCTION

- Primary human hepatocytes are used routinely during drug development for metabolism, transport, toxicity, and drug-drug interaction studies.
- Despite being the "gold standard", hepatocyte cultures can vary tremendously in quality, functional capabilities, and longevity.
- An advanced multi-cellular model consisting of only human-derived cells may be better capable of maintaining physiologically relevant morphologic and biochemical hepatocellular characteristics, as well as mirror *in vivo* responses, for extended periods of time.
- We have developed and optimized the Human/Human™ hepatic co-culture model, consisting of primary human hepatocytes and stromal cells.

The objective of this study was to characterize the temporal activities of phase I and phase II metabolic pathways in the Human/Human™ hepatic co-culture model.

METHODS

Culture Model

- Cryopreserved primary human hepatocytes from individual donors (***TABLE 1***) were thawed in Cryopreservation Recovery Medium™. Cryopreserved growth-arrested stromal cells, not donor matched, were thawed in Hepatocyte Plating Medium (***TABLE 1***).
- The co-cultures were created by seeding hepatocytes and stromal cells together at a 1:1 ratio in Hepatocyte Plating Medium in collagen I-coated 24-well and 96-well plates. Cells were seeded at 0.3 x 10^6 and 0.48 x 10^6 cells/well for 24- and 96-well plates, respectively. Hepatocyte only and stromal cell only cultures also were seeded at the same densities.
- Cultures were maintained in Hepatocyte Culture Medium with daily medium changes.

TABLE 1. *Characteristics of hepatocyte and stromal cell (LMSC-1006) donors.*

Donor	Characteristics								
	Gender	Race	Age	BMI	Tobacco History	Alcohol History	Drug History	Medication	Cause of Death
QHum13035	Male	Caucasian	30	20.9	Yes	2/day	No	None	Stroke
QHuf15028	Female	Caucasian	21	25	1-1½ ppd	Socially	Marijuana	Tylenol	Head Trauma
LHuf15908B	Female	Caucasian	27	30	½ ppd	Socially	No	Unknown	Pulmonary embolism
LHum16081	Male	African American	67	28	No	No	No	Unknown	Stroke
NL-1001	Male	African American	2 mo	N/A	N/A	N/A	N/A	Unknown	Anoxia
LMSC-1006	Female	Caucasian	11	Unknown	N/A	N/A	N/A	Unknown	N/A

N/A = not applicable

Metabolic Activity

- For the initial studies, the CYP3A4 P450-Glo® assay with Luciferin-IPA (Promega) was used.
- For the CYP and phase II profiling study, cultures were incubated with probe substrates (***TABLE 2***) for 30 min on Days 0 (3 hr), 3, 5, 7, 14, 21 and 28. After sample removal, the wells were washed and fresh medium was added for continued culturing. Samples were analyzed by LC-MS/MS.

TABLE 2. *Probe substrates for Phase I and Phase II metabolic pathways.*

Isoform	Probe Substrate	Metabolite(s)
CYP1A2	Phenacetin	Acetaminophen
CYP2B6	Bupropion	Hydroxybupropion
CYP2C9	Diclofenac	4'-Hydroxydiclofenac
CYP2D6	Dextromethorphan	Dextrorphan
CYP3A4/5	Midazolam	1'-Hydroxymidazolam
CYP3A4/5	Testosterone	6β-Hydroxytestosterone
UGT and SULT	7-Hydroxycoumarin	7-Hydroxycoumarin Glucuronide/Sulfate

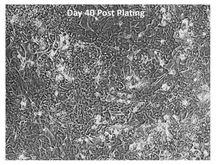
Imaging

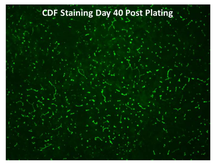
- Cultures were incubated with 5(6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFDA; 5 µM) for 20 minutes on Days 7, 14, 21, 28, 35 and 43 to determine bile canaliculi formation.
- Images were captured on either a fluorescent microscope or a Celígo® imaging cytometer.

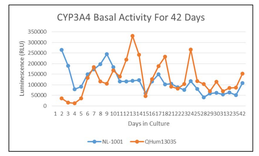
Human Human Hepatic Model

RESULTS

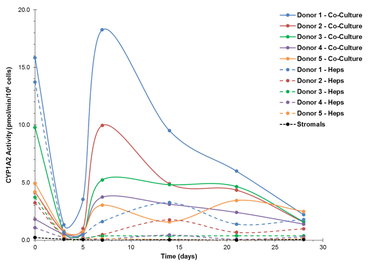
HUMAN/HUMAN™ CO-CULTURE OVER 42 DAYS IN CULTURE



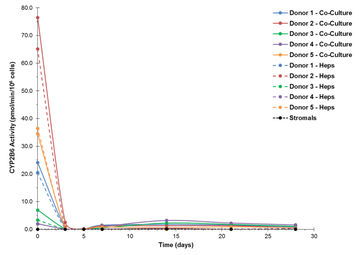




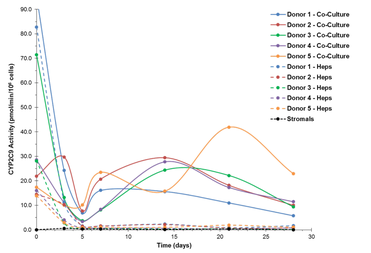
CYP1A2 ACTIVITY



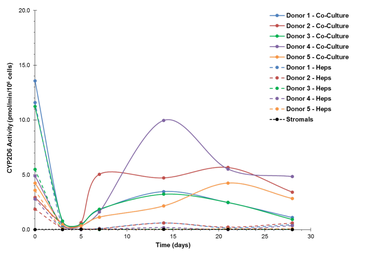
CYP2B6 ACTIVITY



CYP2C9 ACTIVITY

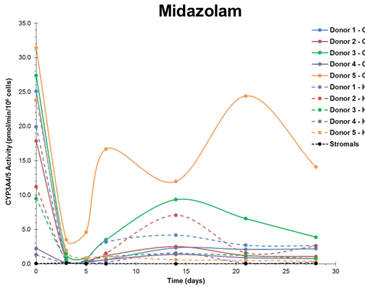


CYP2D6 ACTIVITY

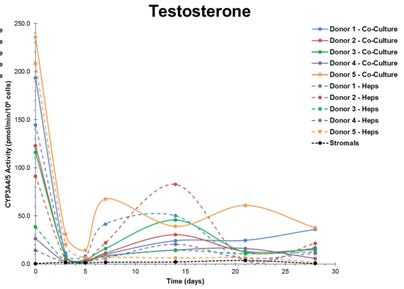


CYP3A4/5 ACTIVITY

Midazolam

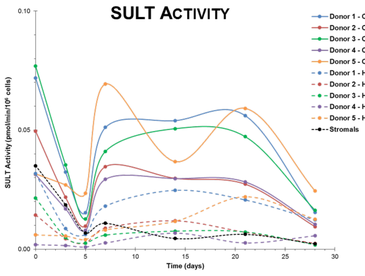


Testosterone

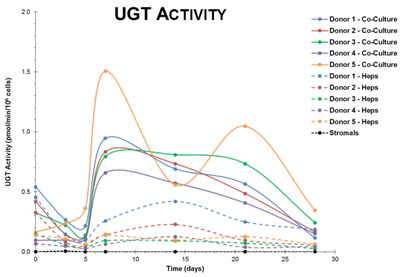


PHASE II METABOLISM

SULT ACTIVITY

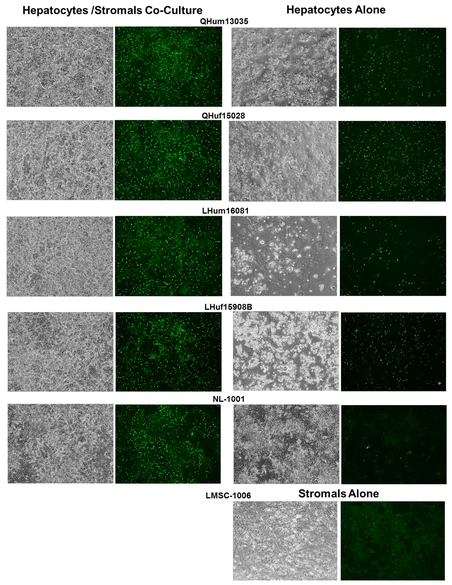


UGT ACTIVITY

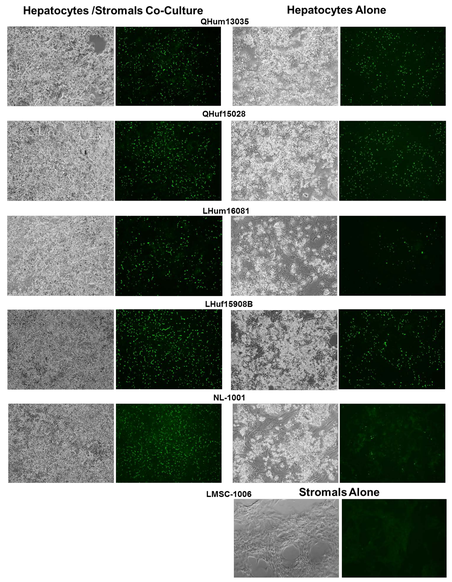


Donor 1 = QHum13035 Donor 2 = QHuf15028 Donor 3 = LHum16081 Donor 4 = LHuf15908B
Donor 5 = NL-1001 Stromal = LMSC-1006

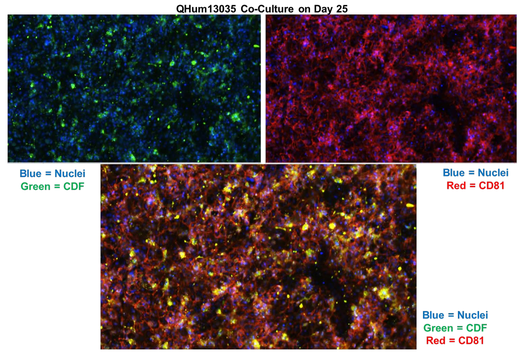
DAY 14– PHASE & CDF PHOTOMICROGRAPHS



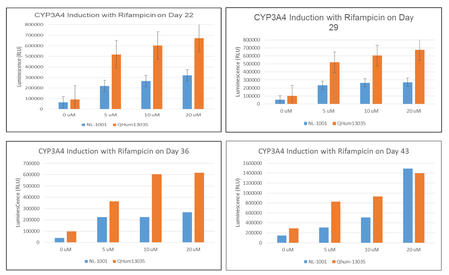
DAY 28 – PHASE & CDF PHOTOMICROGRAPHS



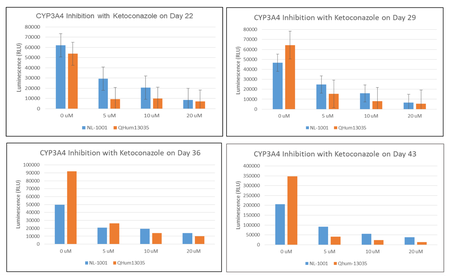
BILE CANALICULI FORMATION IN CO-CULTURE



CYP3A4 INDUCTION WITH RIFAMPICIN



CYP3A4 INHIBITION WITH KETOCONAZOLE



CONCLUSIONS

- **In the Human/Human™ hepatic co-culture model, stromal cells provide the hepatocytes with extracellular matrix, soluble factors, and cell-cell interactions.**
- **Hepatocytes in the co-culture model maintain relevant morphological and physiological characteristics, including polarity and metabolic function.**
- **Further testing with additional stromal cell lots is required to validate these findings.**
- **Future directions include examining the capabilities of the model for enzyme induction and the metabolism of slow turnover compounds.**