Predicting DILI using multi-donor type human liver microtissues (hLiMTs), confocal high content screening (HCS) data and normalisation to therapeutically relevant tissue specific concentrations (tsC_{max})

Paul Walker, Stephanie Ryder, Caroline Bauch, Julie Eakins, Samantha Bevan, Katie Evans & Clive Dilworth

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INTRODUCTION

Drug-induced liver injury (DILI)

- Drug induced hepatotoxicity is a leading cause of attrition during drug development.
- *In vitro* three-dimensional (3D) cell cultures allow better recapitulation of the complex *in vivo* microenvironment than traditional 2D monolayer models.
- 3D models also permit long term compound exposures allowing a closer replication of clinical dosing strategies.
- Human liver microtissues (hLiMTs)
- Glutathione depletion, reactive oxygen species (ROS) formation, mitochondrial disruption and cellular ATP depletion are key responses of hepatocytes to drug induced toxicity.
- Confocal high content screening (HCS) allows the simultaneous detection of each cell health parameter within a 3D spheroid structure in combination with a measure of cellular ATP content.

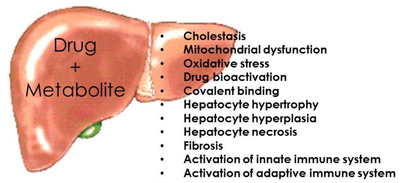

Figure 1. Mechanisms of drug induced liver injury (DILI)

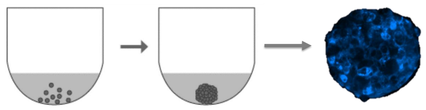

Figure 2. Microtissue formation in ultra-low attachment (ULA) plates

AIMS

- Develop and characterise multi-donor sourced human liver microtissues (hLiMTs) with either donor matched or unmatched non-parenchymal cells.
- Develop a three dimensional (3D) multiparameter high content screening (HCS) assay capable of predicting hepatotoxicity with improved *in vitro* to *in vivo* correlation and use this to assess the effect of hepatocyte patient cohort variability.

METHODS/RESULTS

High content screening (HCS) assay design

- Human liver microtissues (hLiMTs) were formed using scaffold free 96-well ultra low attachment round bottom plates (Corning®). Non-matched hLiMTs comprise cryopreserved primary human hepatocytes from different donors with cryopreserved human non-parenchymal cells from a different donor. Matched hLiMTs comprise cryopreserved primary human hepatocytes with cryopreserved human non-parenchymal cells from the same donor.
- Following exposure to hepatotoxins for 14 days microtissues were labelled with either Syto11 (DNA structure), monochlorobimane (mBCl) (GSH content), dihydroethidium (DHE) (ROS formation) and MitoTracker deep red (Mitochondrial function) by incubation for 30 minutes.
- Fluorescent images were acquired using the confocal mode of an ArrayScan™ XTI HCS reader (ThermoScientific) following with cellular ATP, which was measured using 3D CellTiter-Glo (Promega).

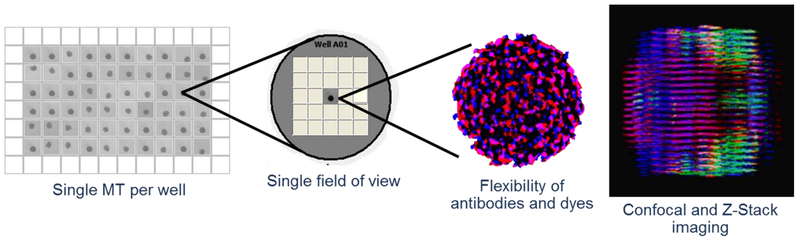

Figure 3. Principles of three dimensional (3D) confocal high content screening (HCS)

Cytochrome P450 activity varies between patient cohorts in 2D and 3D models

Table 1: Cytochrome P450 activity in monoculture 2D models (pmol/ min/ million cells)

Enzyme	Metabolite	Donor 1	Donor 2	Matched Donor
1A2	Acetaminophen	11.5	5.99	16.5
2B6	Hydroxybupropion	NA	10.9	46.1
2D6	Dextrorphan	0.42	52.6	31.7
3A4	1-hydroxymidazolam	NA	3.16	15.5

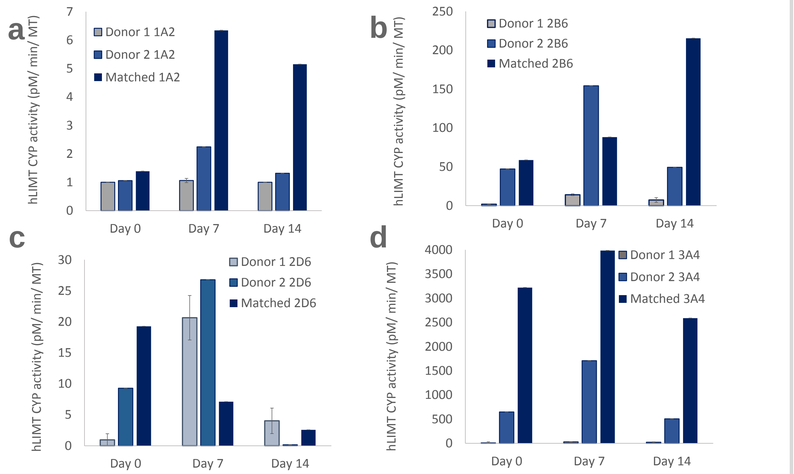

Figure 4. Characterisation of Cytochrome P450 (CYP) activity in hLiMTs from different patient cohorts over 14 day compound treatment period. (a) CYP1A2 activity, (b) CYP2B6 activity, (c) CYP2D6 activity and (d) CYP3A4 activity.

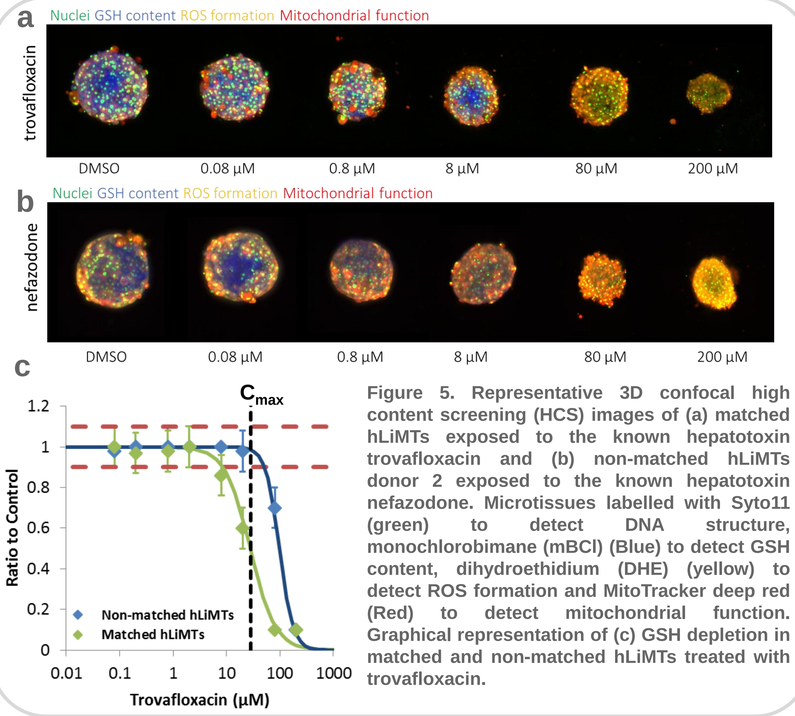

Figure 5. Representative 3D confocal high content screening (HCS) images of (a) matched hLiMTs exposed to the known hepatotoxin trovafloxacin and (b) non-matched hLiMTs donor 2 exposed to the known hepatotoxin nefazodone. Microtissues labelled with Syto11 (green) to detect DNA structure, monochlorobimane (mBCl) (Blue) to detect GSH content, dihydroethidium (DHE) (yellow) to detect ROS formation and MitoTracker deep red (Red) to detect mitochondrial function. Graphical representation of (c) GSH depletion in matched and non-matched hLiMTs treated with trovafloxacin.

Chronic exposure combined with high content screening (HCS) and tsC_{max} normalization improves the *in vitro* to *in vivo* correlation and minimizes the effect of patient cohort variability on overall hepatotoxicity prediction accuracy

Table 2: Hepatotoxicity prediction of 22 reference compounds categorised according to literature data

Drug	DILI category	non-matched hLiMTs Donor 1 MEC (µM)	non-matched hLiMTs Donor 2 MEC (µM)	Matched hLiMTs MEC (µM)	C_{max} (µM)	non-matched hLiMTs Donor 1 MEC (µM)	non-matched hLiMTs Donor 2 MEC (µM)	Matched hLiMTs MEC (µM)	Liver_kP exposure C_{max} (µM)
Amiodarone	Hepatotoxic	6.51	3.03	2.66	5.3	6.51	3.03	2.66	49.5
Diclofenac	Hepatotoxic	51	21.4	17.2	10.1	51	21.4	17.2	2.7
Troglitazone	Hepatotoxic	0.99	42.40	34.60	6.29	0.99	42.40	34.60	37.5
Nefazodone	Hepatotoxic	13.7	4.5	11.6	4.3	13.7	4.5	11.6	22.4
Perhexiline	Hepatotoxic	1.03	0.967	1.4	2.16	1.03	0.967	1.4	53.6
Tolcapone	Hepatotoxic	21.9	<1.56	113	21.96	21.9	<1.56	113	16.8
Acetaminophen	Hepatotoxic	302	1020	292	165.4	302	1020	292	360.8
Bosentan	Hepatotoxic	12.3	26.3	38.2	4.7	12.3	26.3	38.2	4.3
Trovafloxacin	Hepatotoxic	45.2	64.5	11.2	19.7	45.2	64.5	11.2	28.3
Flutamide	Hepatotoxic	3.63	23.4	12.3	5.4	3.63	23.4	12.3	9.7
Nitrofurantoin	Hepatotoxic	24.7	19.4	3.21	21	24.7	19.4	3.21	13.0
Carbamazepine	Hepatotoxic	49.2	111	79.1	50.8	49.2	111	79.1	41.3
Sunitinib	Hepatotoxic	0.24	0.157	<0.1	0.25	0.24	0.157	<0.1	1.7
Ticlopidine	Hepatotoxic	17.5	81.2	0.957	8.1	17.5	81.2	0.957	38.4
Azathioprine	Hepatotoxic	2.48	0.636	0.175	2.22	2.48	0.636	0.175	3.0
Chlorpromazine	Hepatotoxic	0.34	2.47	<0.2	0.94	0.34	2.47	<0.2	19.3
Fialuridine	Hepatotoxic	11.5	<1.56	0.932	1	11.5	<1.56	0.932	0.9
Tamoxifen	Hepatotoxic	1.54	1.97	7.77	1.18	1.54	1.97	7.77	20.8
Buspirone	Non-hepatotoxic	3.12	47.8	NR	0.01	3.12	47.8	NR	0.057
Entacapone	Non-hepatotoxic	40.2	15.5	88.8	3.276	40.2	15.5	88.8	4.14
Pioglitazone	Non-hepatotoxic	3.13	18.2	5.62	3	3.13	18.2	5.62	6.36
Metformin	Non-hepatotoxic	28.1	NR	129	7.74	28.1	NR	129	8

≤ C_{max}
≤ 3x C_{max}
> 3x C_{max}

MEC = minimum effective concentration
DILI = Drug induced liver injury

Table 3: Hepatotoxicity prediction accuracy of two non-matched hLiMT's donors and one matched hLiMT donor normalized to either plasma C_{max} or tissue specific (ts) C_{max}.

Using MEC (µM)	Non-matched hLiMTs Donor 1	Non-matched hLiMTs Donor 2	Matched hLiMTs
Correctly predicted under 3x C_{max}	82%	73%	77%
Correctly predicted under 3x tsC_{max}	86%	86%	82%
Correctly predicted under C_{max}	41%	36%	55%
Correctly predicted under tsC_{max}	55%	46%	73%

SUMMARY/CONCLUSIONS

- Human hepatocyte cytochrome P450 (CYP) activity varies between patient cohorts, table 1 shows the initial CYP activity of the matched donor hepatocytes is greater than donors 1 & 2 when cultured alone in 2D and continues when co-cultured as 3D microtissues (figure 4).
- Using a 3x C_{max} cut off variability in hLiMT toxicity prediction (table 3), whether matched or non-matched donors, is not as significant as the variations in CYP activity displayed (table 1 & figure 4).
- Using a tissue specific C_{max} (tsC_{max}) cut off (1x or 3x C_{max}) we see improved *in vitro* to *in vivo* correlation with all hLiMTs. Matched hLiMTs show a higher degree of hepatotoxicity prediction accuracy as we approach therapeutic C_{max}.
- We show that overall IVIVE DILI prediction is not significantly altered by variations in hepatocyte phenotypes (CYP-activity and matched vs non matched donors), when utilized within a 3D chronic exposure multi-parametric HCS assay (normalized to tissue specific C_{max}).

REFERENCES

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