Electronic Supplementary Material (ESI) for Lab on a Chip. This journal is © The Royal Society of Chemistry 2020

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	View MPS Models										
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			View	Edit	Hepatocyte Suspension	University of Pittsburgh Drug Discovery Institute			Liver	Eppendorf Tube 1.5 mL	None
			• View	Edit	Mimetas liver	University of Pittsburgh Drug Discovery Institute		Liver (Mimetas)	Liver	Mimetas OrganoPlate	None
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			() View	Edit	SQL-SAL 1.5	University of Pittsburgh Drug Discovery Institute		Liver (UPDDI)	Liver	Nortis Single Chamber	None

Supplemental Figure S1: Selecting the appropriate MPS experimental model. The MPS database contains detailed bench ready protocols the user can print to assemble and test compounds in various liver models. In this example, the traditional 2D monolayer culture for toxicity and metabolism testing, the gold standard hepatocyte suspension culture for metabolism, a 4 cell organoid type 3D microfluidic liver system in a 96 well Mimetas® plate suitable for high throughput screening, the a 4 cell supervised/self assembly 3D microfluidic Liver Acinus MicroPhysiology (LAMPS) and the earlier version of the LAMPS called the Sequentially Layered, Self Assembly Liver (SQL-SAL) models, and the Vascularized Liver Acinus MicroPhysiology (vLAMPS) model are choices available to meet user needs. The models vary by cell number, types, organization and complexity for the user to select one appropriate to answer the experimental hypothesis.

		*MPS	Studies - Analysis	 Models - Compounds - 	Cell Samples	- Help Feedb	ack		mes234 -
	(Sp)	Home / Adver	rse Events						
	Compounds -			Adver	rse Events	S			
	Compound Data View Chemical Data	Show M	IPS Show	EPA Show TCTC	✓ She	ow Unassigned			
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	View Adverse Events	View	Compound	Event 0	Number of Reports Ø	Normalized # of Reports	Estimated Usage 😧	Organ 🔶	Black Box Warning
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	Compare Adverse Events Compound Report	View	TOLCAPONE	ASPARTATE AMINOTRANSFERASE INCREASED	5	4.90	10,195	Liver	0
very Institute Microph	Requires Permission	View	TOLCAPONE	BLOOD BILIRUBIN INCREASED	4	3.92	10,195	Liver	0
iences Tissue Chips	Add Compound	View	TOLCAPONE	LIVER FUNCTION TEST ABNORMAL	4	3.92	10,195	Liver	0
		View	ENTACAPONE	ASPARTATE AMINOTRANSFERASE INCREASED	40	2.29	174,449	Liver	
		View	ENTACAPONE	ALANINE AMINOTRANSFERASE INCREASED	34	1.95	174,449	Liver	
		View	ENTACAPONE	HEPATIC FUNCTION ABNORMAL	25	1.43	174,449	Liver	
		View	ENTACAPONE	GAMMA- GLUTAMYLTRANSFERASE INCREASED	20	1.15	174,449	Liver	
		View	ENTACAPONE	LIVER DISORDER	17	0.97	174,449	Liver	

Supplemental Figure S2: Selecting the appropriate compounds for testing. Clinical adverse events reporting records are contained within the database. In this example, filters are used to identify compounds with black box warning (!) for liver toxicity, increased incidents of liver enzyme elevations and suitable compounds without liver effects from all reported adverse events found in the FDA Adverse Events Reporting System (FAERS) to test in the LAMPS model. The incidents of adverse events found in the FAERS database are 'normalized' to estimated drug use from the CDC database.



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Copy CSV	Print	Column visibility						
Search: cmax	clinical						Show 5	0 v entries
View	Drug Trial A ID	Treatment	Species #	Trial Type	¢ Finding ¢ Descri	ptor	Value 🛊	Value Units
View	187	ACETAMINOPHEN	Human	Clinical	Blood :: PK :: Cmax	Pos	21.0	µg/mL
View	174	ENTACAPONE	Human	Clinical	Blood :: PK :: Cmax	Pos	1.22	µg/mL
View	184	NIMESULIDE	Human	Clinical	Blood :: PK :: Cmax	Pos	6.5	µg/mL
View	178	TOLCAPONE	Human	Clinical	Blood :: PK :: Cmax	Pos	7.2	µg/mL
View	172	TROGLITAZONE	Human	Clinical	Blood :: PK :: Cmax	Pos	2.82	µg/mL
5 View	178	TOLCAPONE	Human	Clinical	Blood :: PK :: Cmax	Pos	7.2	µg/mL
View	172	TROGLITAZONE	Human	Clinical	Blood :: PK :: Cmax	Pos	2.82	µg/mL
Funded by the N	ational Cent	ter for Advancing Trans	ational Salana	es Tissue Chi	ine Program		Contr	bute on Github



Supplemental Figure S5. Albumin data from 14 compounds. Data are measured as ng/ml and normalized to percent of the control response in efflux media collected on Days 5, 11 and 17. The MPS experimental models in duplicate or triplicate devices were treated 18 consecutive days by continuous perfusion flow to entacapone (40 μ M); tolcapone (88 μ M), tolcapone (220 μ M); caffeine (600 μ M); Valproic Acid (1500 μ M); Warfarin (90 μ M); Buspirone (0.5 μ M); Methotrexate (0.03 μ M); Rifampicin (12 μ M); Erythromycin (54 μ M); Famotidine (0.5 μ M); Levofloxacin (600 μ M); Rosiglitazone (30 μ M) or Trovafloxacin (200 μ M).



Supplemental Figure S6. BUN data from 14 compounds. Data are measured as ng/ml and normalized to percent of the control response in efflux media collected on Days 5, 11 and 17. The MPS experimental models in duplicate or triplicate devices were treated 18 consecutive days by continuous perfusion flow to entacapone (40 μ M); tolcapone (88 μ M), tolcapone (220 μ M); caffeine (600 μ M); Valproic Acid (1500 μ M); Warfarin (90 μ M); Buspirone (0.5 μ M); Methotrexate (0.03 μ M); Rifampicin (12 μ M); Erythromycin (54 μ M); Famotidine (0.5 μ M); Levofloxacin (600 μ M); Rosiglitazone (30 μ M) or Trovafloxacin (200 μ M).



Supplemental Figure S7. LDH data from 14 compounds. Data are measured as ng/ml and normalized to percent of the control response in efflux media collected on Days 1 - 18. The MPS experimental models in duplicate or triplicate devices were treated 18 consecutive days by continuous perfusion flow to entacapone (40 μ M); tolcapone (88 μ M), tolcapone (220 μ M); caffeine (600 μ M); Valproic Acid (1500 μ M); Warfarin (90 μ M); Buspirone (0.5 μ M); Methotrexate (0.03 μ M); Rifampicin (12 μ M); Erythromycin (54 μ M); Famotidine (0.5 μ M); Levofloxacin (600 μ M); Rosiglitazone (30 μ M) or Trovafloxacin (200 μ M).



Supplemental Figure S8. Cytochrome C data from 14 compounds. A High Content Analysis instrument was used to measure fluorescent intensity on Days 5 and 18 of the mitochondria located Cytochrome C GFP biosensor. The data was normalized to control levels. The MPS experimental models in duplicate or triplicate devices were treated 18 consecutive days by continuous perfusion flow to entacapone (40 μ M); tolcapone (88 μ M), tolcapone (220 μ M); caffeine (600 μ M); Valproic Acid (1500 μ M); Warfarin (90 μ M); Buspirone (0.5 μ M); Methotrexate (0.03 μ M); Rifampicin (12 μ M); Erythromycin (54 μ M); Famotidine (0.5 μ M); Levofloxacin (600 μ M); Rosiglitazone (30 μ M) or Trovafloxacin (200 μ M).



Supplemental Figure S9. Increasing Incidents of Adverse Responses in LAMPS and Tracked FAERS Data by Clinical Hepatotoxicity in the MPS-Db. Pink designates hepatotoxic compounds, yellow designates DILI compounds and green designate non liver toxic compounds. Although the absolute order varies slightly between the in vitro and clinical assessments of liver toxicity, the overall concordance can be accurately categorized.



Supplemental Figure S10: Detailed analysis of inter-study reproducibility assessment. The detailed analysis shows the data used to calculated the inter-study reproducibility with links to the individual items (with same treatment) and the studies being compared. The intra-study reproducibility status is given for the samples in each of the studies being compared. The graphs show the individual data points for each of the samples (Items), the average value of the samples and a trimmed version of the average graph showing only the time points that overlapped between the studies. A) Albumin study to study reproducibility; and B) Cytochrome C study to study reproducibility.



Supplemental Figure S11. Additional information generated to assess the human MPS experimental model by Power Analysis. In this example, the effect of warfarin on albumin secretion is being compared with the no compound control. The user selects the treatments to be analyzed (A) and a graph of the experimental data is generated (B). The user then selects the desired method of calculating the effect size (C, see Methods and Materials), the desired significance level (D), and runs the analysis. The p-values and the power values for the difference between the samples is plotted for each point on the data curve (E and F, respectively). Finally, a power curve is generated showing the required sample size to achieve different statistical power values for the given dataset (G). See Figure 6 for selecting the Target/Analyte to analyze, power estimates for different sample sizes and estimates for different sample sizes.

		Disease Overview	Disease Biology	Clinical Data	Disease M	odels & Studies	
letastatic Brea	st Cancer Di	sease Biology					
treast cancer is the lei the mammary gland. idermal growth factor ubtype), hormone rece promone receptor posit thiways, and stimulat otein have been confi aly three genes (TPS3 eference: KEGG Brea	ading cause of can The molecular sub receptor-2 (HER2) ptor negative and H ive breast cancers c cell growth, surviv rmed. In the case of , PIK3CA and GAT, st Cancer	ter death among women types of breast cancer, v i include: hormone recey IER2 positive (HER2 po are largely driven by the al and differentiation. In f breast cancer only 8% A3) occurred at >10% in	worldwide. The vast m which are based on the ptor positive and HER2 sitive), and hormone re estrogen/ER pathway, patients suffering from of all cancers are here cidence across all brea	ajority of breast ci presence or abser negative (luminal ceptor negative ar In HER2 positive i TNBC, the deregu ditary, a phenomer st cancers."	incers are ca ice of hormor A subtype), h d HER2 neg oreast tumou lation of vario non linked to	rcinomas that originate f le receptors (estrogen a ormone receptor positiv stive (basal-like or triple s, HER2 activates the F us signalling pathways genetic changes in BRC	rom cells lining the milk-forming duct nd progesterone subtypes) and huma e and HER2 positive (luminal B negative breast cancers (TNBCs)), 13K/AKT and the RAS/RAF/MAPK Notch and Wnt/beta-catenin), EGFR A1 or BRCA2. Somatic mutations in
Genomic Databas	st Cancer Ge	enomic Resource	es			KECC: Motor	tatia Braact Canaar Disaas
Name	Description					REGG. Metas	Entry
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Phenotype Relationship DISEASES.org	phenotypes with disease biology DISEASES is a associations fro data, and genor that displays dis	 full-text, referenced over portal delievers a curate weekly updated web resim automatic text mining, ne-wide association studie ease relevant search residuation 	erviews of all known Me ed query of the most rel- source that integrates er, manually curated litera fies. The disease biolog sults.	ndelian disorders. evant genes. vidence on diseas ature, cancer muta gy portal provides :	The s-gene tion a query	Click to view an ir	teractive pathway map for Metastatio Breast Cancer.
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ProteomicsDB by SA and chromosomes of	P is a proteomic da interest.	tabase that allows you t	o browse proteins	Metabolom metabolom	icsworkbench ics data.	n serves as a national ar	d international repository for
PharmaGKB				DrugBa	ık		

Supplemental Figure S12. Disease Biology portal. The <u>Disease Biology</u> portal allows the user to link to various genomic, proteomics, metabolomics, and pharmacogenomic databases. The links on this page are automatically pre-queried for the disease of interest.

Copy CS	V Print C	Column visibility							Show 50	 entries
View	Drug Trial ID	Compound	Species	Trial Type ♦	Finding	Descriptor	\$ +/ - \$	Frequency \$	Value \$	Value Units
View	225	EVEROLIMUS 10.0 mg Exemestane 25.0 mg	Human	Clinical	All :: Other :: No Toxicity	Progression Free Survival	Pos			
View	225	Exemestane 25.0 mg	Human	Clinical	All :: Other :: No Toxicity	Progression Free Survival	Pos			
View	227	Fulvestrant 500.0 mg	Human	Clinical	All :: Other :: No Toxicity	Progression Free Survival	Pos			
View	226	LETROZOLE 2.5 mg	Human	Clinical	All :: Other :: No Toxicity	Progression Free Survival	Pos			
View	226	LETROZOLE 2.5 mg	Human	Clinical	All :: Other :: No Toxicity	Progression Free Survival	Pos			
View	227	Palbociclib 125.0 Fulvestrant 500.0 mg	Human	Clinical	All :: Other :: No Toxicity	Progression Free Survival	Pos			
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Show <u>50</u> ▼ entries Group ¢ Review ¢ Taylor_MPS Taylor_MPS	Creator Dillon Gavlock Dillon Gavlock	0	0 58	PS MCF7 static Breast er Model PS MCF7 static Breast er Model	LAMP C Meta Cano LAMP M Meta Cano	Feb 25, 2019 Jul 10, 2018	Effect of CF7 Y537S	Relationship Between He Ind MCF7 Cells IPDDI-DM-2018-07-10-1 IZD8496 on growth of M Autant Cells	View/Edit R av View/Edit A M

Supplemental Figure S14. Disease Models & Studies portal. The <u>Disease Models & Studies</u> portal provides a list of all in vitro experimental models and studies in the MPS-Db for the disease of interest. All of the information for the experimental models and studies is easily accessible through the View and View/Edit links.

		Tumor Cells (mCherry RED)
as the sund the		Note: This image may have been altered to assist with viewing. To perform analysis, please download the unaltered image.
	Chip ID	N0341
at a long the second the second second	Assay Plate ID	none
I have she was a first in the same	Assay Well ID	none
	Time	D9 H0 M0
	Method/Kit	Protein Fluorescence (mCherry)
and the second	Target-Stain Pairings	Tumor Cells (mCherry, red)
	Target/Analyte	Tumor Cells
as Street and a	Sample Location	Chamber
and the second sec	Notes	This is one in a time series of images showing cell proliferation.
the second s	Image File Name	Y537S +E2 D9 B.tif
and the second	Image Field	20
and the second sec	Image Magnification	1.0x
	Image Resolution	2.2 µm
and the second	Image Sample Label	mCherry
AND A THE R. L.	Image Wavelength (ex/em nm)	587/610
and the second s	Image Color Mapping	red
	Image Setting Note	
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Supplemental Figure 15: Images and video data are also supported in the MPS-Db. Day 9 growth of mCherry containing MCF7 Y537S cells in the MPS device. The metadata contains the information on device number, day of exposure, magnification and fluorescent wavelengths. Images can also be downloaded as tif files for additional analysis.

Supplemental Table S1. List of methods used to generate data for a variety of targets in different MPS organ models, which have been uploaded into the MPS-Db.

						Or	gan	Mod	els i	n MPS-I	Db		
Assay Category	Target	Method/Kit	Adipose	Bone	Brain	Heart	Intestine	Kidney	Liver	Skeletal Muscle	Skin	Vasculature	Liver Metastasis
	Beat Interval Microscopy Video Quantification (MotionGUI)												
	Beat Rate	EarlyTox Cardiotoxicity Kit, Microscopy Video Quantification (Manual, MotionGUI)				х							
	Bile Efflux	Bile Efflux, Image Algorithm (UPDDI)							х				
	Collagen	Picrosirius Red Stain Kit (Polysciences: 24901-250)	х										
	Contractile Force	Force Transducer (Thorlabs)								х			
	Contraction Velocity	Microscopy Video Quantification (MotionGUI)				х							
	СҮРЗА4	P450-Glo CYP3A4 Assays (Promega: V9001)							х				
	Decay/Rise Ratio	EarlyTox Cardiotoxicity Kit (Molecular Devices: R8211)				х							
	Dextran-FITC (10kDa)	Fluorescence (490/525) (Sigma-Aldrich: FD10S)			х		х						
	E-Cadherin	anti-E-Cadherin							х				
Cell morphology/function	Fatty Acid	BODIPY 500/510 C1, C12 (ThermoFisher: D3823)	х										
	Glucose Uptake	Flow-through Biosensor B.LV5							х				
	Image	ICC/IFC (DAPI, FITC, Cy5), Live imaging, Phase Contrast							х				
	Lipid Droplets, Lipid to Nuclei Ratio	Lonza AdipoRed Assay Reagent (Lonza: PT-7009), Hoechst 33342	х										
	Maximum Elongation	Stimulation and Microscopy Video Quantification (1, 5, 10, or 20 Hz)								х			
	Neutral lipids	BODIPY 493/503 (ThermoFisher: D3922)	х										
	Relaxation Velocity	Microscopy Video Quantification (MotionGUI)				х							
	Steatosis (macro and micro)	HCS LipidTOX Red Neutral Lipid Stain (Thermo: H34476)							х				
	Transepithelial Electrical Resistance	EVOM2 Volt/Ohm Meter (WPI: 300523)					х						
	Tumor Area, Intensity	Protein Fluorescence (DAPI, Texas Red) with Quantification (ImageJ, AngioTool)										х	
	Tumor Integrated Intensity	mCherry 587/610	_										х
	Vessel Area, Length, Junctions	Protein Fluorescence (DAPI, Texas Red) with Quantification (ImageJ, AngioTool)										х	
	Cell Viability (Quantitative)	CellTiter-Glo Luminescent Cell Viability Assay (Promega: G7573)	_				х		х			х	
	Cellular Metabolism	MTT Assay Kits	_						х		х		
	Lactate Dehydrogenase	Multiple commercial kits	х	х	х			х	х		х		
Cell viability/proliferaton/toxicity	Live Cells / Dead Cells	LIVE/DEAD Cell Imaging Kits	х					х	х				
	Mitochondria	Fluorescence (490/525)	_						х				
	PrestoBlue	PrestoBlue Cell Viability (ThermoFisher: A13261, A13262)	х	х	х			х	х		х		
	Tumor Growth	Cell Proliferation Kit II (XTT) (Sigma: 11465015001)	_									х	
	WST-1	WST-1 Assay Reagent - Cell Proliferation (ready to use) (ab155902)											х
Compound Level	User define compound(s) of interest	RapidFire-MS, HPLC-MS, LC-MS/MS, ICP-MS, IM/MS		х	х	х	х	х	х		х		
Device Characterization	Flowrate	Flowrate (by setting, volume, or weight)						x	х				
Gene Expression	User defined gene(s) of interest	RT-PCR (Applied Biosystems: StepOnePlus and SYBR Green Reaction Mix)		х									
	Cytochrome C	CytC Biosensor, Image Algorithm							х				
Intracellular Biosensor	GCaMP6	Live Imaging Algorithm (FITC)								х			
	Luciferase Expression	ONE-Glo Luciferase Assay System (Promega: E6110, E6120, E6130)		х									
Protein Binding	User defined compound of interest	Single-Use RED Plate with Inserts (Thermo Scientific: 2034.6) and ICP-MS		х				х	х				
	Alpha-fetoprotein	Human alpha-Fetoprotein DuoSet (R&D Systems: DY1369)							х				
	Ammonium	Ammonia Assay Kit (abcam: ab83360)						х					
	Blood Urea Nitrogen	BUN Liquid Reagent (Stanbio Laboratory: SB-0580-250)							х				
Secreted Protein/Compound	Glucose	Amplex Red Glucose/Glucose Oxidase Assay Kit, Flow-through Biosensor B.LV5							х				
Secreteu Protein/Compound	Insulin Secretion	Insulin ELISA							х				
	L-lactate	Flow-through Biosensor B.LV5							х				
	User define protein(s) of interest	Human ELISA Kits from various vendors	х	х	х		х	х	х				
	User define protein(s) of interest	Meso Scale Discovery Assay kits and V-PLEX panels		x	х	x		х					