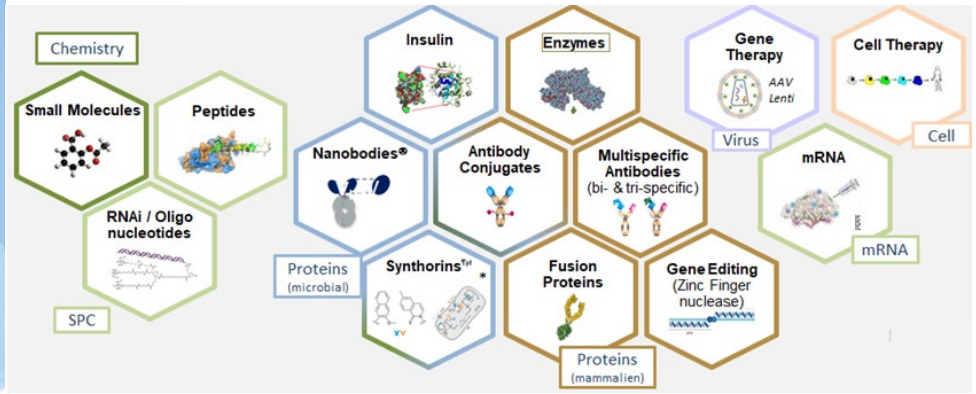

Micro-physiological models and other innovative in vitro systems for toxicity evaluation of new drugs - a Pharma Industry perspective -

Philippe Detilleux, DVM, PhD, DACVP
Global Head Preclinical Safety, Danofi R&D

With the help of Piyush Bajaj, Bruno Biton, Nicolas Redon and Karissa Adkins

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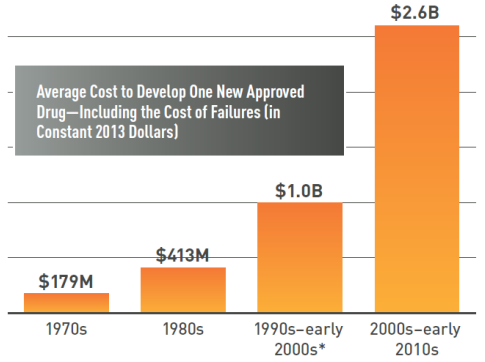
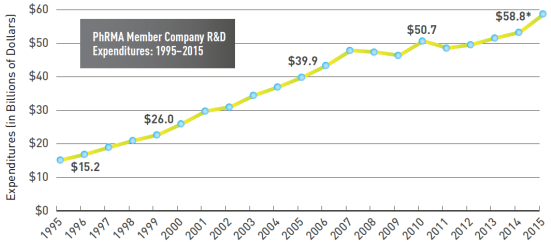
Key metrics about drug discovery and challenges for the pharma industry

- **Average cost to develop one new drug - $2.6B**
- **Time needed to develop a new drug - 13.5 years**
- **[1]9-11 NMEs are needed in Phase I to have one successful launch**
- **Only 2 of 10 marketed drugs return revenue that exceeds its R&D costs**
- **[1]Attrition rates:**
  - Phase I – **46%**
  - Phase II – **66%**
  - Phase III – **30%**
- **Current models do not successfully recapitulate human biology**
  - Animal models – systemic but cannot predict human
  - Conventional 2D models – neither systemic nor organotypic
- **Better models are needed!**





2016 PhRMA profile

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[1]Paul et al., *Nat. Rev. Drug Discov.* 2010

Major reasons for failure in the pharma industry: Safety and Efficacy

Table 1 | **Populations of the primary cause of failure categories for terminated compounds***

	Termination reason	Overall	Period		Phase		
			2000–2005	2006–2010	Candidate nomination	Phase I	Phase II
2	Clinical safety	68 (11%)	48 (13%)	20 (8%)	5 (1%)	40 (25%)	22 (25%)
	Commercial	40 (7%)	23 (6%)	17 (7%)	26 (7%)	10 (6%)	4 (4%)
3	Efficacy	55 (9%)	45 (11%)	10 (4%)	10 (3%)	14 (9%)	31 (35%)
	Formulation	9 (1%)	4 (1%)	5 (2%)	8 (2%)	1 (0.6%)	0
1	Non-clinical toxicology	240 (40%)	144 (40%)	96 (40%)	211 (59%)	21 (13%)	7 (8%)
	Patent issue	1 (0.2%)	0	1 (0.4%)	1 (0.3%)	0	0
	Pharmacokinetics or bioavailability	29 (5%)	19 (5%)	10 (4%)	3 (0.8%)	25 (16%)	1 (1%)
	Rationalization of company portfolio	124 (21%)	46 (13%)	78 (32%)	75 (21%)	29 (18%)	19 (21%)
	Regulatory	2 (0.3%)	2 (0.6%)	0	1 (0.3%)	1 (0.6%)	0
	Scientific	33 (5%)	28 (8%)	5 (2%)	13 (4%)	15 (10%)	5 (6%)
	Technical	3 (1%)	3 (1%)	0	2 (0.6%)	1 (0.6%)	0
	Other	1 (0.2%)	0	1 (0.4%)	1 (0.3%)	0	0
	Total	605	362	243	356	157	89

*Table entries for each column indicate the total number and the percentage in parentheses.

~40% in 1990s

Waring et al., *Nat. Rev. Drug Discov.* 2015

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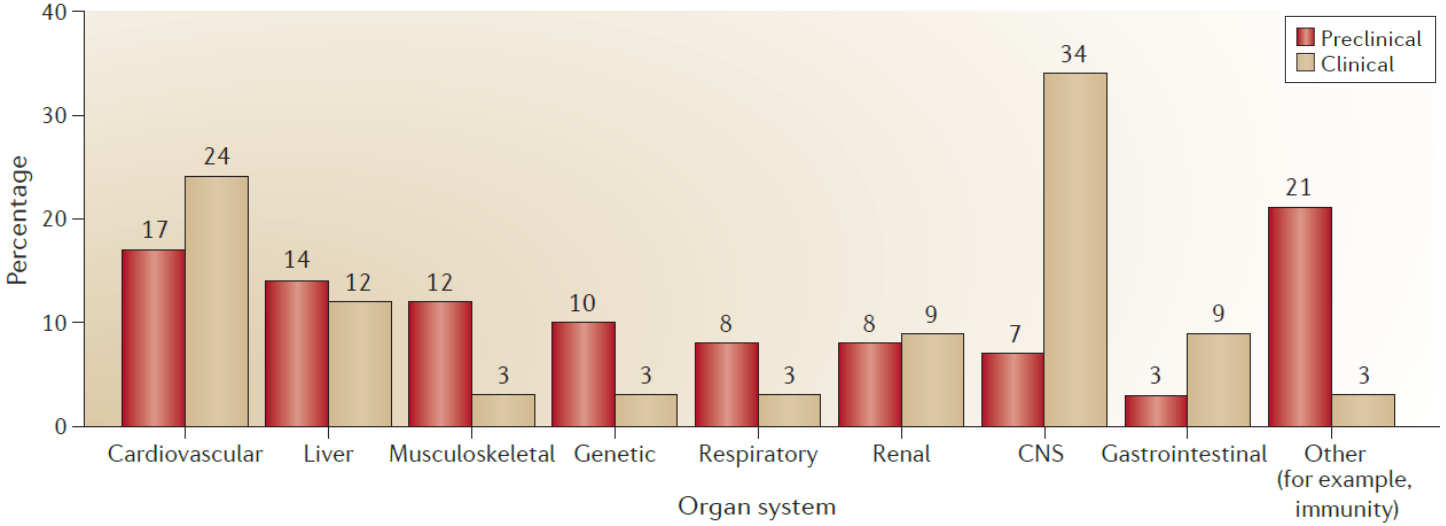
Organ systems often involved with safety related failures



Cook et al., *Nat. Rev. Drug Discov.* 2014

Above chart is AZ specific, but similar trend in the pharma industry

ADVANCED PHYSIOLOGICALLY-RELEVANT MODELS/MPS

- **Organ-on-a-chip**: Microfluidic devices (2D/3D) with physiologically relevant perfusion
- **Organoid/spheroid**: 3D single or multi-cell aggregates of stem cells/primary cells
- **Co-culture or engineered**: 2D co-cultured with ECM proteins or cellular microenvironments

Zhang et al., *Lab Chip* 2017

Partnerships between pharma industry and MPS companies


COLLABORATIONS, PRE-CLINICAL, R&D

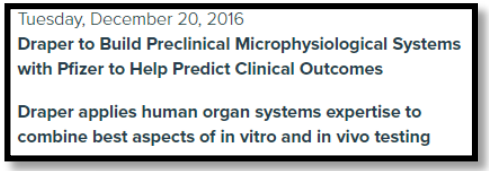
Roche and Takeda try on Emulate's 'organ chip' tech for R&D

by BRITTANY MEILING — on February 20, 2018 07:00 AM EDT

Updated: 10:25 AM


1.6 million USD pharma funding for kidney-on-a-chip nephrotoxicity model

MIMETAS, in a consortium with Radboudumc (Nijmegen) and FHNW (Basel), has received 1.6 million USD funding for development of a kidney-on-a-chip for toxicological applications. A panel of experts from GlaxoSmithKline, Pfizer, Roche, NC3Rs and renowned academic institutions selected MIMETAS' solution from a strong line-up of competing technologies.


Tuesday, December 20, 2016

Draper to Build Preclinical Microphysiological Systems with Pfizer to Help Predict Clinical Outcomes

Draper applies human organ systems expertise to combine best aspects of in vitro and in vivo testing


Takeda And HemoShear Therapeutics Enter Into Exclusive Drug Discovery Partnership In Liver Diseases

Takeda HEMOSHEAR THERAPEUTICS

Takeda and HemoShear Therapeutics Enter into Exclusive Drug Discovery Partnership in Liver Diseases

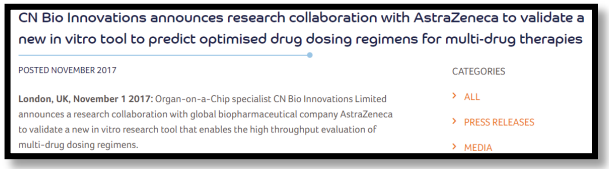
Deal combines HemoShear's REVEAL-Tx™ platform with Takeda's drug discovery and development capabilities

Osaka, Japan and Charlottesville, Va. – Oct. 17, 2017 – Takeda Pharmaceutical Company Limited (TSE: 4502) and HemoShear Therapeutics, LLC, a privately held biotechnology company, today announced a partnership to discover and develop novel therapeutics for liver diseases, including nonalcoholic steatohepatitis (NASH).


Merck 'bio-inks' deal to use Organovo's 3D printed liver for preclinical studies

By Dan Stanton

22-Apr-2015 - Last updated on 23-Apr-2015 at 10:03 GMT


CN Bio Innovations announces research collaboration with AstraZeneca to validate a new in vitro tool to predict optimised drug dosing regimens for multi-drug therapies

POSTED NOVEMBER 2017

London, UK, November 1 2017: Organ-on-a-Chip specialist CN Bio Innovations Limited announces a research collaboration with global biopharmaceutical company AstraZeneca to validate a new in vitro research tool that enables the high throughput evaluation of multi-drug dosing regimens.

CATEGORIES

› ALL

› PRESS RELEASES

› MEDIA


InSphero Announces Agreement with Pfizer to Develop Novel Assays for Predicting Drug-induced Liver Injury

Research effort will use InSphero 3D InSight™ Human Liver Microtissues to develop novel assays to predict and evaluate certain mechanisms that cause drug-induced liver injury (DILI). PDF Vesion

Schlieren, Switzerland – April 27, 2017

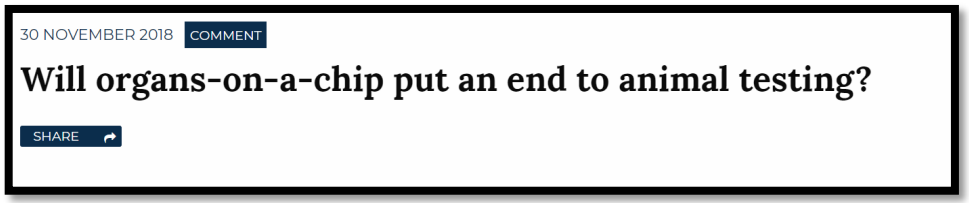
What advanced models cannot do, anytime soon …


Living organ-on-a-chip could soon replace animal testing

By Sebastian Anthony on June 22, 2012 at 8:03 am | 24 Comments


The FDA just struck a deal that could replace animal testing with a tiny chip

Lydia Ramsey Apr. 11, 2017, 1:07 PM

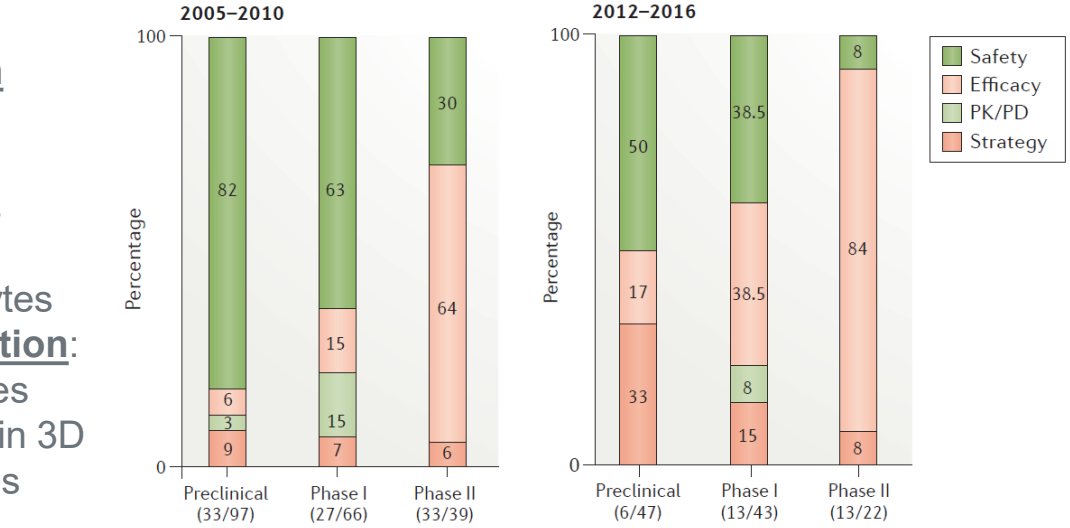

30 NOVEMBER 2018 COMMENT

Will organs-on-a-chip put an end to animal testing?

SHARE

What advanced models can do: improvement in safety!

- **Lead generation stage**:
  - 3D liver microtissues
  - iPS-cardiomyocytes
- **Candidate selection**:
  - Cross species comparison in 3D gut organoids



Morgan et al., *Nat. Rev. Drug Discov.*, 2018

Use of Advanced Models in Pharmaceutical Safety Assessment

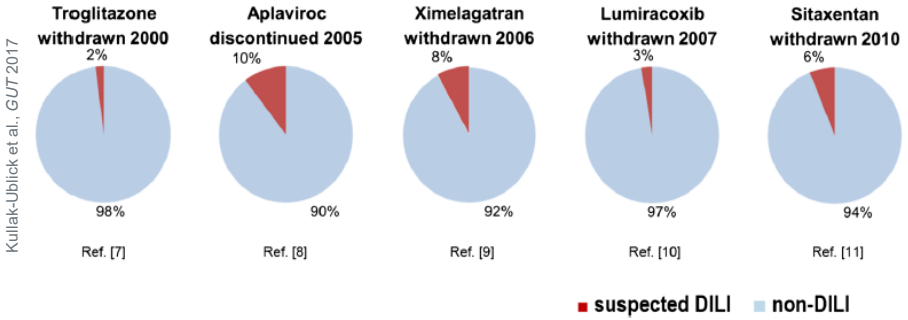
- **More in research than in Development**
  - More for Internal decision making than for Regulatory submission
  - More to increase mechanistic understanding than for compound screening
  - Occasionally submitted in regulatory dossier to complement
- **Points to be considered when developing an advanced model**
  - Context of use (stage, throughput)
  - End-point analysis (Imaging, molecular, analytical, biomarker)
  - Ease of use and Cost
  - Performance characterization (validation set, number of publications)
  - Robustness and reproducibility
  - Ability to run multi-species studies
  - Translation potential
  - Allometric scaling

Hepatotoxicity and drug induced liver injury (DILI)

- **DILI is one of the most frequently cited reasons of drug attrition in the pharmaceutical industry**
  - Black-box warning
  - Post-market drug withdrawals
    - ~18% or 81 of 462 cases from 1953 – 2013[1]

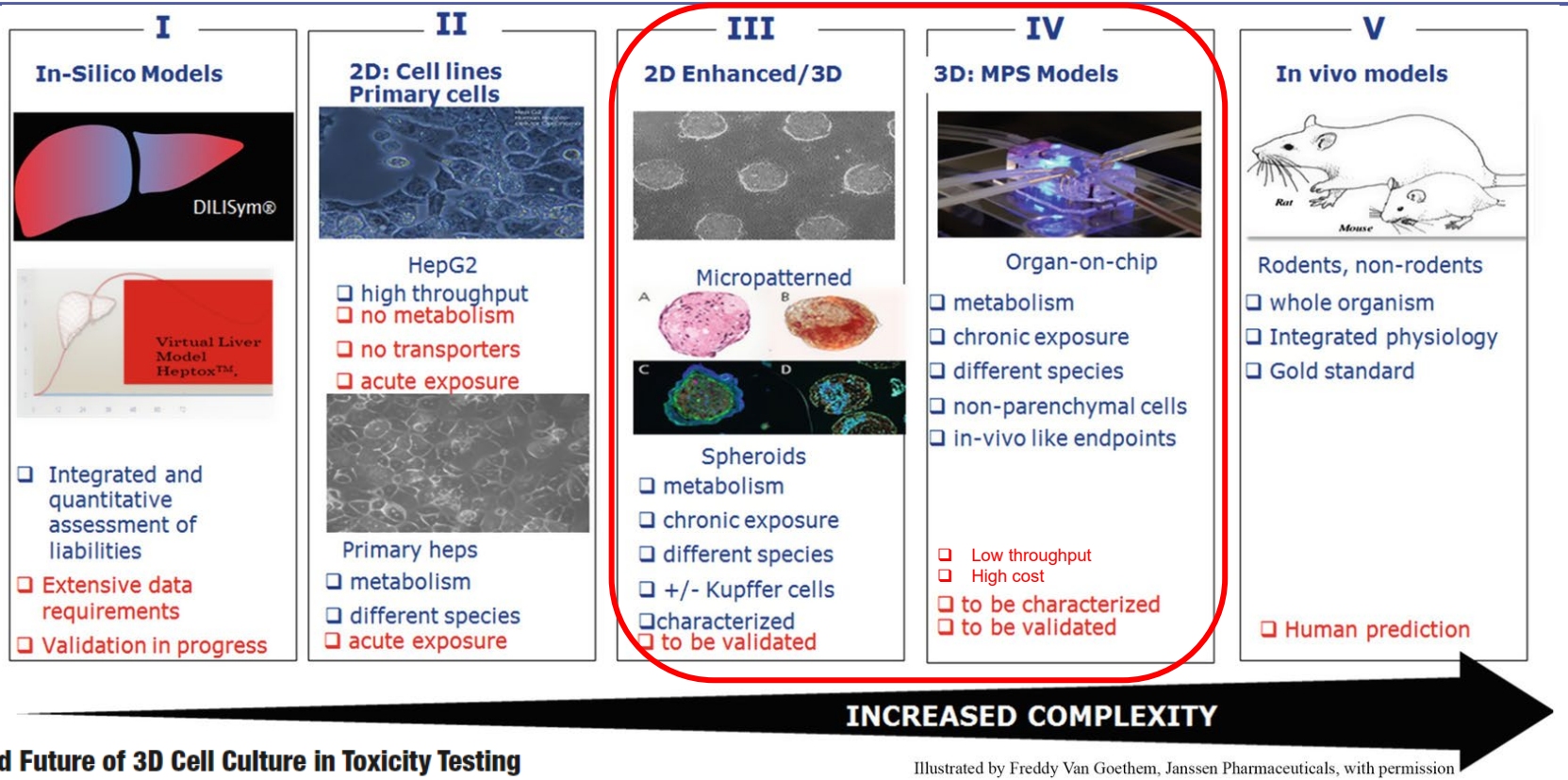
- **Differences in drug handling between the pre-clinical species and humans**
  - Two human toxicities with the poorest correlation to animal studies are hepatic (<60%) and hypersensitivity (<40%)[2]

- **2 types of DILI**
  - **Intrinsic (Type A)** – dose dependent, often reproducible in animals, innate immune system involved (APAP)
    - *Dose is the "poison"*
  - **Idiosyncratic (Type B)** – may not be dose dependent, often not reproduced in animals, innate and adaptive immune system involved, occurs in 1/1000 – 1/10,000
    - *Host is the "poison"*



[1]Onakpoya et al., *BMC Medicine* 2016
[2]Olson et al., *Reg. Toxicol. Pharmacol.* 2000

Current approaches to predict/de-risking DILI



Status and Future of 3D Cell Culture in Toxicity Testing

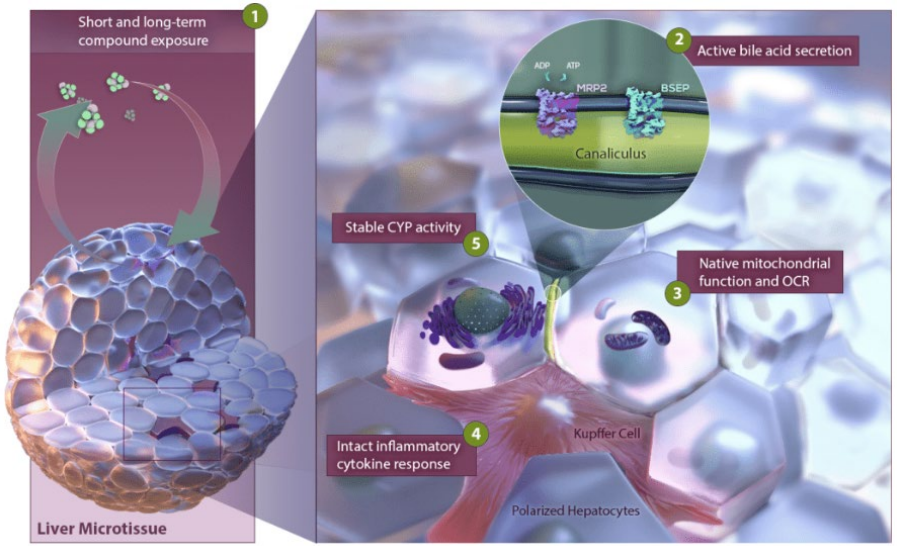
Illustrated by Freddy Van Goethem, Janssen Pharmaceuticals, with permission

Monicah A. Otieno, Jinping Gan, and William Proctor

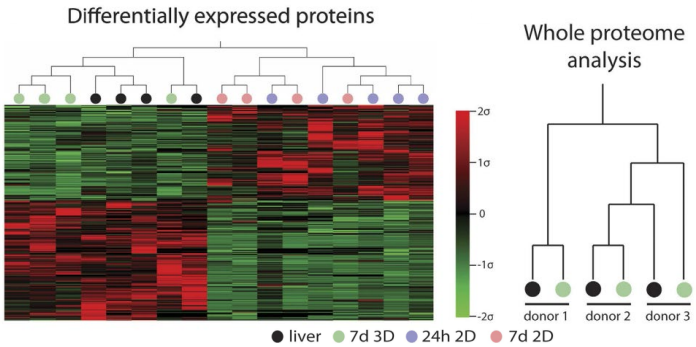
Minjun Chen and Yvonne Will (eds.), *Drug-Induced Liver Toxicity*, Methods in Pharmacology and Toxicology,
https://doi.org/10.1007/978-1-4939-7677-5_12, © Springer Science+Business Media, LLC, part of Springer Nature 2018

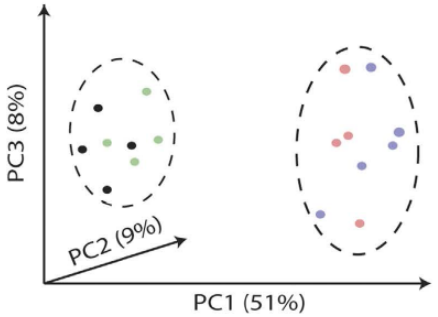
3D liver spheroids as an in vitro tool for testing of hepatotoxicity liabilities

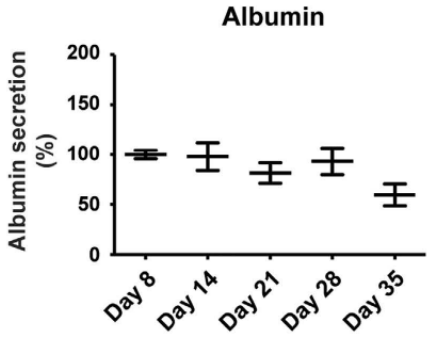
- Liver spheroids are 3D balls of hepatocytes with non-parenchymal cells (Kupffer cells, LSECs) composed of about 1200 - 1500 cells and about 250 -300 µm in diameter
- Stable and long term (28-days) CYP activity and presence of relevant transporters (OATPs, BSEP, MRPs, etc.)
- **Ability to do other mechanistic endpoints such as GSH depletion, ROS generation, bile acid modulation, etc.**
- Responsive to inflammatory cytokines – LPS
- Studies with short (7-day) and long (≥14-day) term compound exposures possible
- **Cross-species possible as well (rat, dog, cyno)**



3D liver spheroids are “closer” to the in vivo liver





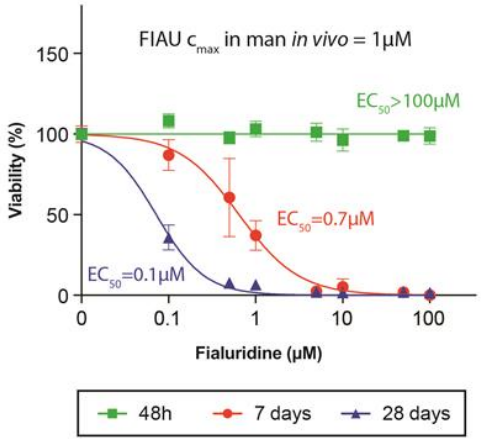


Compound	*In vivo* $C_{max}^{(ref.)}$	EC50 (µM) 48h	7d	28d
Amiodarone	0.8[21]	>100	6.5	1.6
Bosentan	7.4[22]	>250	69.5	41.8
Diclofenac	8[23]	190.8	56.8	45.9
Fialuridine	1[24]	>100	0.7	0.1
Tolcapone	22[25]	18.8	9.4	5.6

> 30 x C_{max}

1-30 x C_{max}

< 1x C_{max}



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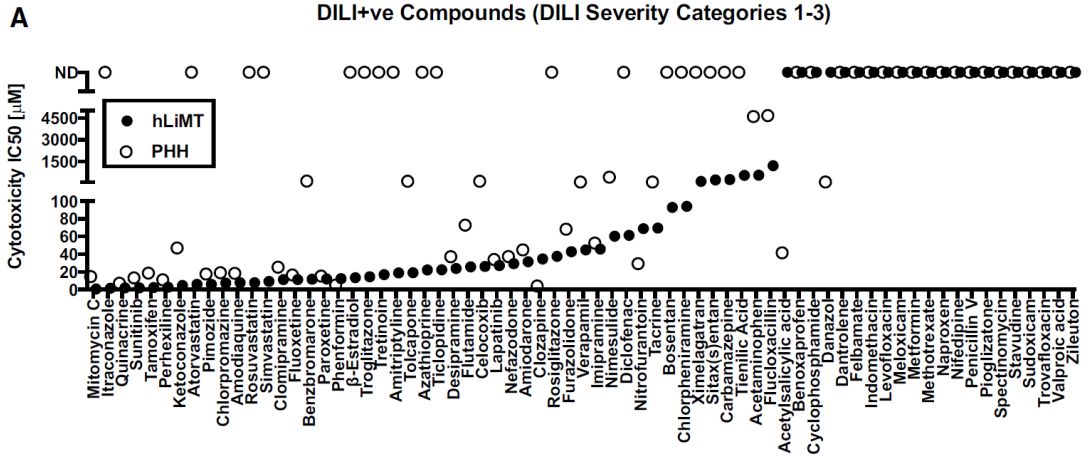
Bell C., et al., *Sci. Rep.* (2016)

3D liver spheroid models for testing hepatic liability of drugs



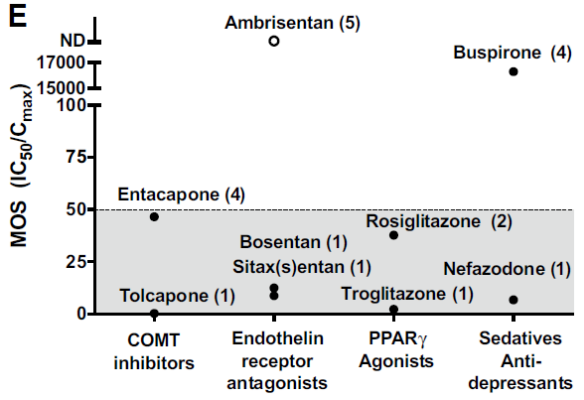


Table 2 Assay performance for PHH and hLiMT described based on pre-defined cytotoxicity IC_{50} thresholds

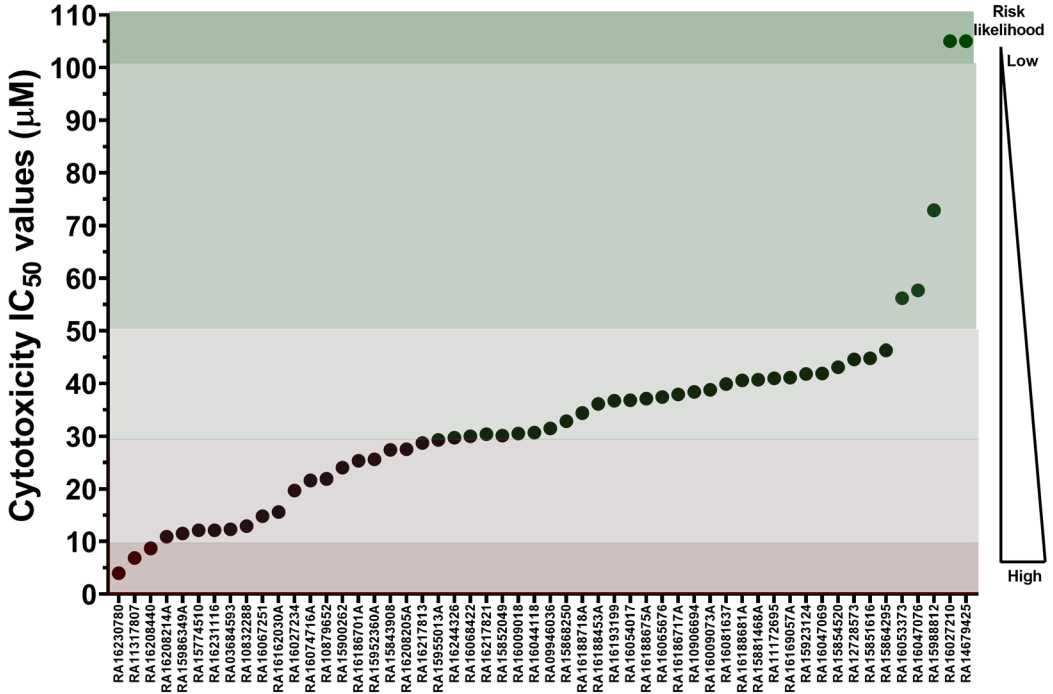
Assay	Number DILI+ve	Number DILI−ve	TP	TN	FP	FN	Threshold (μM)	Sensitivity (%)	Specificity (%)	PLR	NLR	Kappa	*P* value
2D PHH IC_{50} [μM]	69	41	3	40	1	66	10	4.3	97.6	1.78	0.98	0.014	0.61
	69	41	12	38	3	57	25	17.4	92.7	2.38	0.89	0.080	0.004
	69	41	20	37	4	49	50	29.0	90.2	2.97	0.79	0.177	0.007
	69	41	23	35	6	46	100	33.3	85.4	2.28	0.78	0.176	0.014
3D hLiMT IC_{50} [μM]	69	41	13	38	3	56	10	18.8	92.7	2.57	0.88	0.091	0.097
	69	41	26	36	5	43	25	37.7	87.8	3.1	0.71	0.215	0.004
	69	41	36	35	6	33	50	52.2	85.4	3.57	0.56	0.331	0.0001
	69	41	42	35	6	27	100	60.9	85.4	4.16	0.46	0.419	<0.0001

TP true positive, *TN* true negative, *FP* false positive, *FN* false negative, *PLR* positive likelihood ratio, *NLR* negative likelihood ratio, *Kappa* Cohen's kappa concordance value

3D spheroids shows ~2X sensitivity and same specificity to 2D models

Proctor et al., *Arch. Toxicol.*, 2017

3D liver spheroids can be applied as a ranking tool for Sanofi programs



Screening performed in media containing 1% BSA to improve solubility

- Compound soluble up 100 µM
  - Strong positives
  - Positives
  - Negatives
- Chemical diversity
- Compounds were dosed 3X (day 0, 2, 5) and cell viability (CellTiter-Glo®3D) was run on day 7 and compared to vehicle control which was set to 100% viability

We are also working with IDD chemists to further "qualify" the model with internal Sanofi and marketed compounds

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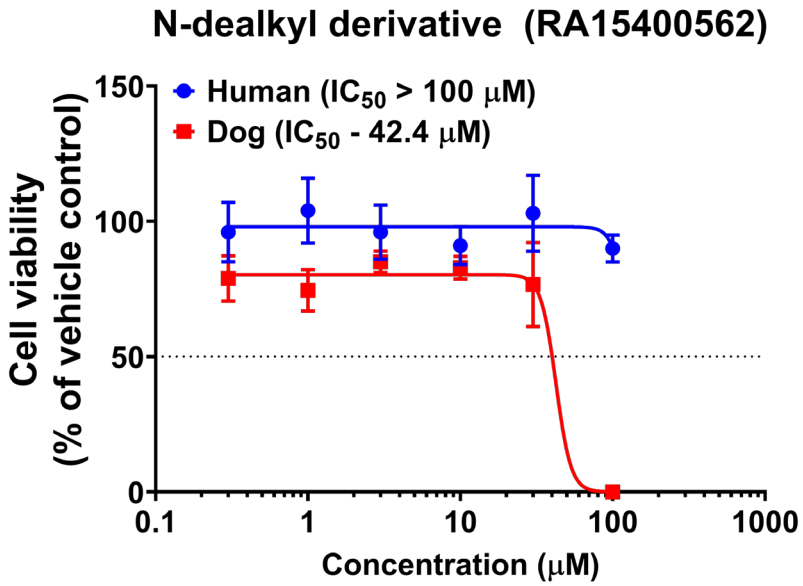
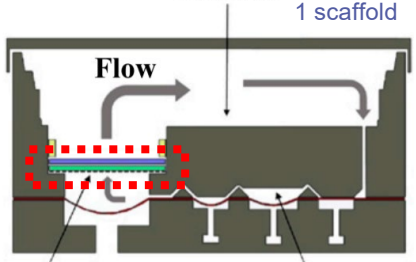
Cross-species testing using 3D liver spheroids



Dog liver spheroids are more sensitive to the lead as well as the N-dealkyl metabolite compared to human liver spheroids

In vitro liver model with re-circulating media – ability to introduce adaptive immune cells (PBMCs)

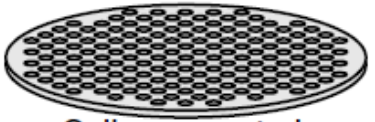
12-wells/plate with 3 plates/controller



One well of the plate with 1 scaffold

Sarkar et al., *DMD* 2015

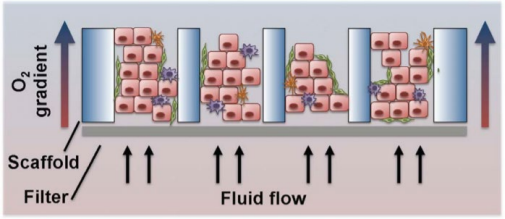
Each scaffold contains 301 micro-channels with 300 µm diameter and 250 µm height



Collagen-coated polystyrene scaffold

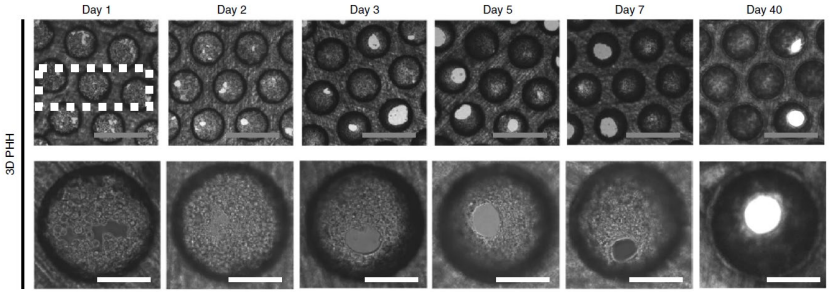
Oretga-Prieto et al., *Nat. Comm.* 2018

Microfluidic 3D *ex vivo* liver cultures



Hepatocytes
Kupffer cells
Hepatic stellate cells
Sinusoidal endothelial cells

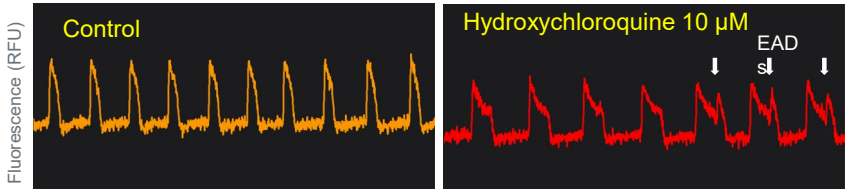
Beckwitt et al., *Exp. Cell Res.* 2018



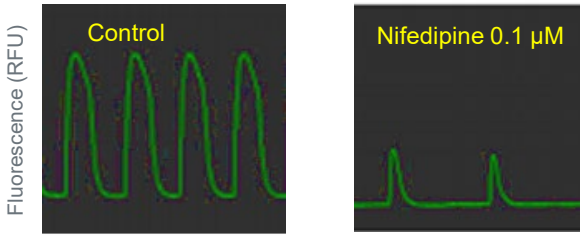
Oretga-Prieto et al., *Nat. Comm.* 2018

Human IPSC-derived cardiomyocytes for early cardiosafety assessment

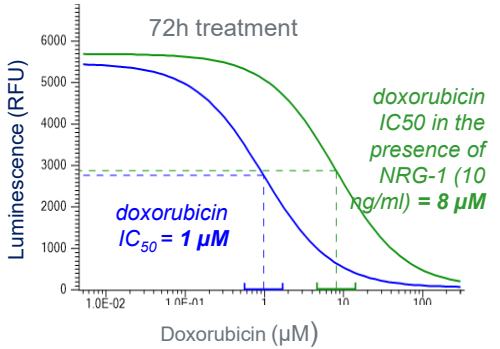
- **Detection of torsadogenic/arrhythmogenic effects:**
  - Action potential recording
  - Voltage-sensitive dye FluoVolt™/Pluricyte™ cardiomyocytes/384 well plate format.
  - Assay sensitivity/specificity comparable to Purkinje assay



- **Detection of calcium homeostasis perturbation**
  - Calcium transient recording
  - Calcium-sensitive dye Cal520/Pluricyte™ cardiomyocytes/384 well plate format.
  - Detection of compounds perturbating calcium entry, sequestration and release.



- **Detection of cardiotoxic effects**
  - Cellular ATP content measurement
  - CellTiter Glo™ luminescent assay/Axol cardiomyocytes/384 well plate format. Repeated treatment, typically, 48/72h.
  - Detection of non specific cellular toxicity with involvement of protective pathway such as HER2 signaling.



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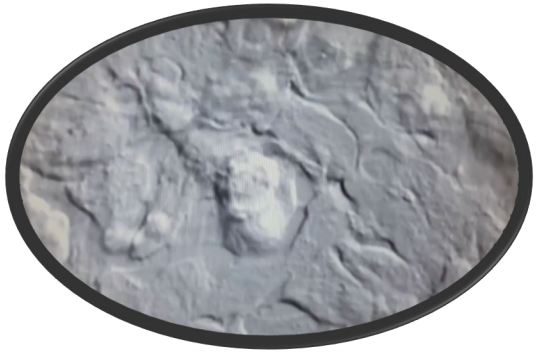
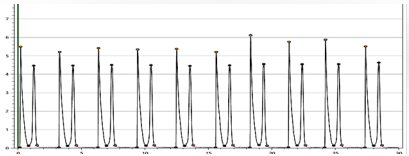
hIPSC-derived CMs: 2D and 3D models for contractility assessment

hiPSC-CM (Pluricyte™) monolayer 2D



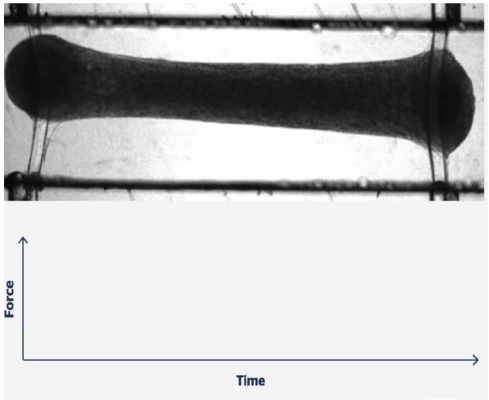


Cell Motion Imaging System SI8000 (SONY)

Contractility is the most integrated parameter indicating a potential cardiotoxicity.

- 2D model give access to higher throughput and high selectivity.
- Inactive compounds confirmed in a 3D model, with a better detection of positive inotropes

Engineered Heart Tissue (3D) Cardiotype/Biowire™ II platform. Tara Bisosytems



https://tarabiosystems.com/

TARA

THANK YOU!

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