Human liver patient-derived organoids are a promising tool to study drug-induced liver injury and drug metabolism for improved preclinical toxicology assessment

HUB ORGANOIDS

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Introduction

Drug toxicology studies are essential to assess **drug safety** and represent a pivotal step in preclinical drug development. Advances in drug discovery are often hampered by the **lack of suitable preclinical models** that recapitulate the *in vivo* physiology of the tissue, its gene expression and drug metabolism. Liver toxicity is one of the leading causes of new drugs not reaching the market or failing in clinical trials, thus, there is a compelling need for advanced *in vitro* preclinical models to address drug toxicity and to **predict Drug-Induced Liver Injury** (DILI).

HUB Organoids® are innovative "mini-organs in a dish" derived from adult stem cells within epithelial organs which form **3D** structures resembling the architecture and physiology of the tissue of origin. They are **genetically and phenotypically stable** in culture and can be **expanded** for screening purposes, providing a unique **platform for ADME studies** (Absorption, Distribution, Metabolism and Excretion). Liver patient-derived organoids (PDO) can be enriched with hepatocyte-like cells showing a more mature phenotype than hepatocytes derived from human induced pluripotent stem cells. This study aimed at assessing hepatotoxicity of a list of compounds using differentiated human liver organoids and providing insights into their capacity of predicting DILI.

Advantages of using HUB Organoids in DILI studies

HUB Organoids are genetically **stable PDOs**, representative of **patient's heterogeneity** and can be used in **long-term** culture. Liver organoids can be derived from healthy liver but also from diseased liver tissue such as liver from patients with Wilson's disease and Crigler-Najjar.

	Genetic Stability	Representative Of patient	Expansion	Cost	Ethical Concerns	Drug metabolism
Cell lines (e.g., HepG2	✓	✓	unlimited	$	none	±
iPSC-derived hepatocytes	✓✓	✓✓	long-term	$$	minor	++
Primary human hepatocytes	✓✓✓	✓✓✓	not expandable	$$$	minor	+++
Animal models	✓✓✓	✓	not expandable	$$$	major	±
Liver organoids	**✓✓✓**	**✓✓✓**	**long-term**	**$$**	**minor***	**+++**

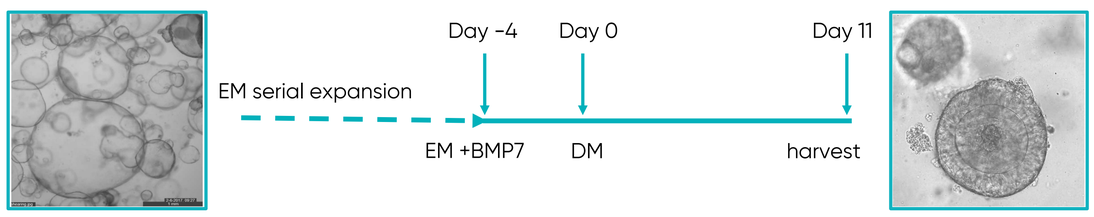
* Pharma drug development consent in place

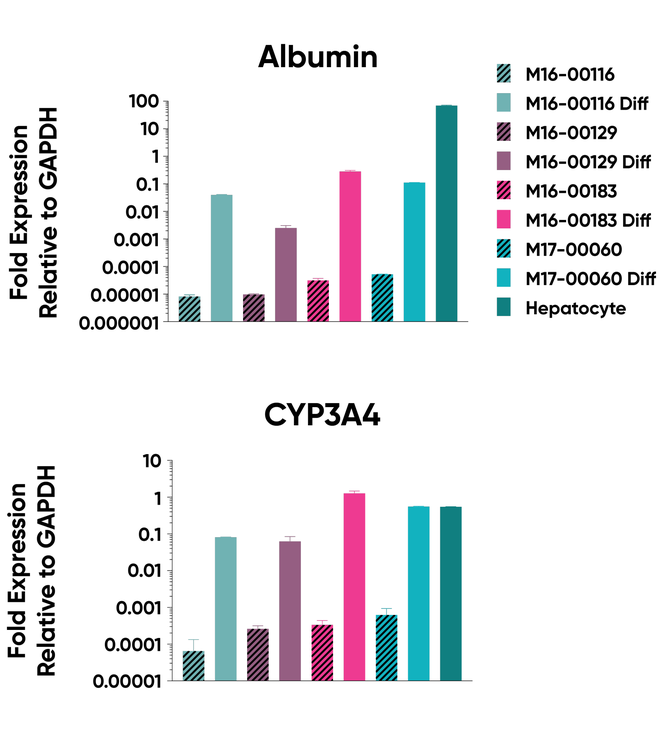
Study outline

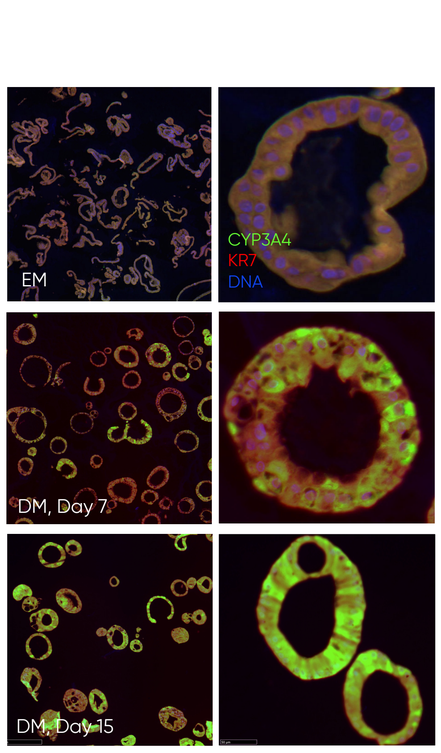
- ✓ Human liver organoid differentiation to enrich for cells with hepatocyte-like features
- ✓ Characterization of differentiation markers in hepatocyte-enriched organoids
- ✓ Assessment of liver metabolic activity by luminescent assays and LC-MS
- ✓ Organoid viability assay after short- and long-term treatment with hepatotoxic drugs

Liver organoid differentiation

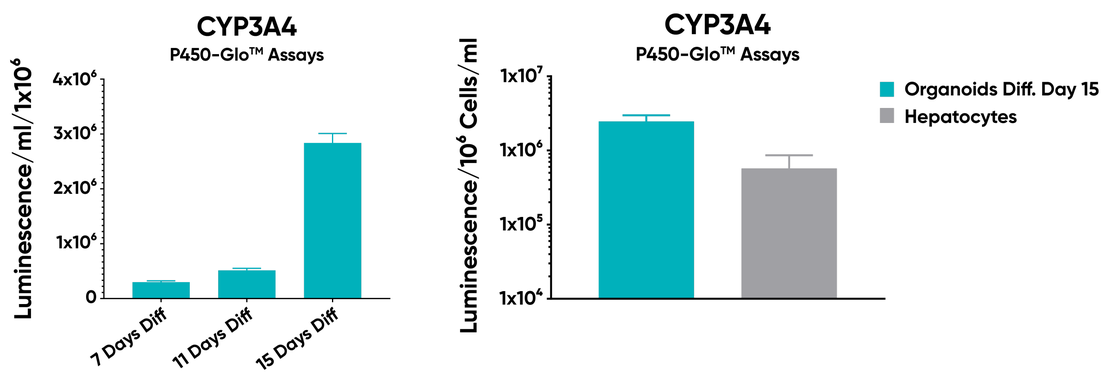
Liver HUB Organoids can differentiate towards cells displaying **hepatocyte-like features**

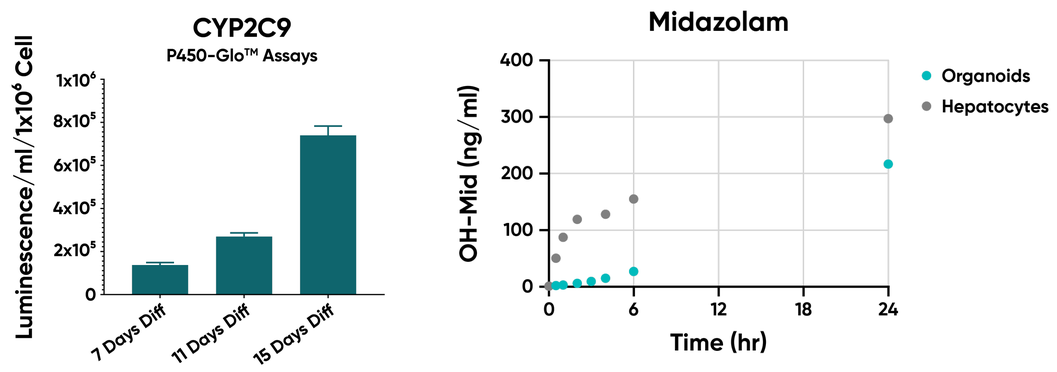






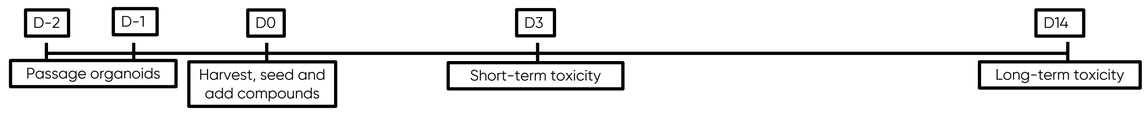
Liver organoid metabolic activity





Liver HUB Organoids reflect **cytochrome P450** enzymatic activity

Liver organoid toxicity screen



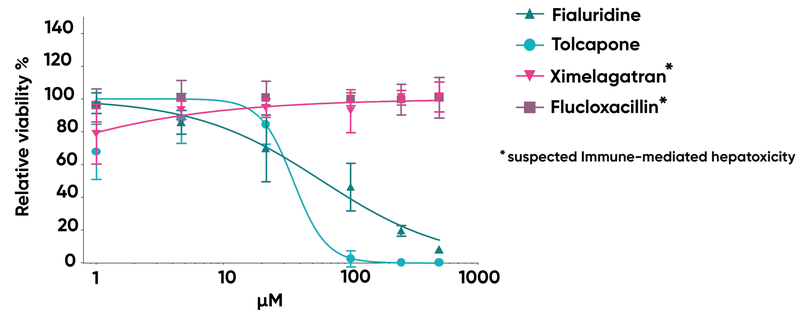
Short-term exposure

More Toxic — IC_{50} ranking (mM) — Less Toxic

	Perhexiline	Nefazodone	Amiodarone	Tolcapone	Troglitazone	Entacapone*	Diclofenac	Buspirone*	Bosentan	Pioglitazone*	Metformin*	Acetaminophen
PDO-1	0.014	0.040	0.140	0.330	0.140	0.175	0.420	undefined#	undefined#	undefined#	2.898	18.520
PDO-2	0.026	0.070	0.140	0.235	0.345	0.255	0.642	1.170	0.580	undefined#	2.760	15.250
Hepatocytes	0.010	0.013	0.018	0.010	0.013	0.035	0.068	0.026	0.090	1.000	0.680	1.343
HepG2 (+5%BME)	0.004	0.015	0.004	0.060	0.110	0.070	0.920	0.170	0.290	2.210	2.580	2.570

*Compounds with negative DILI
#IC50 > than max. tested dose

Long-term exposure



Liver HUB Organoids are better at **predicting DILI** after **long-term exposure.** Animal models failed to detect Fialuridine toxicity that is instead evident in PDO models.

Summary

Liver HUB Organoids:

- ✓ Differentiate towards cells displaying **hepatocyte-like features**
- ✓ Reflect **cytochrome P450** enzymatic activity
- ✓ Better **predict DILI** after **long-term exposure** compared to hepatocytes and HepG2

Conclusion

HUB liver PDOs can be enriched with cells having similar gene expression and functionality of primary human hepatocytes in culture.
HUB liver PDOs are suitable for **measuring metabolic activity** and **susceptibility to hepatotoxic compounds** at a large scale, becoming a valuable tool in preclinical DILI studies for the identification of hepatotoxic compounds and characterization of drug metabolism.

References

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