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pixlbio wild-type pixHep™ models tell hepatotoxic drugs from their liver-safe analogues

One of the most stringent challenges for any liver-safety model is not the detection of overt hepatotoxicity, but the ability to discriminate between a hepatotoxic compound and a structurally related analogue with an established clinical safety profile. Many drugs associated with drug-induced liver injury have closely related compounds, often within the same chemical class, that are well tolerated in patients; a genuinely predictive platform must therefore resolve these subtle but clinically meaningful differences.

As part of a broader initiative led by the 3Rs Collaboration and the FDA's Center for Drug Evaluation and Research, eight commercially available microphysiological systems were evaluated using the same blinded compound panel. Unbeknown to the participating laboratories, the panel comprised four matched pairs, each containing a High-DILI compound and a structurally related Low-DILI analogue, specifically designed to assess the discriminatory performance of each platform. At pixlbio, we subjected our lead wild-type, iPSC-derived pixHep™ platform to this rigorous benchmark.

Wild-type pixHep™ mature into functional human hepatocytes

pixlbio differentiated its wild-type iPSC line into mature pixHep™ hepatocytes displaying the characteristic cobble-stone morphology and high expression of hepatocyte-specific markers (albumin 95.5 ± 0.4%; HNF4A 89.9 ± 0.3% positive), exceeding internal quality-control criteria. Each blinded compound was then applied across 0.01x, 0.1x, 1x and 10x Cmax for 10 days, refreshed every 48 hours, and assessed on five complementary endpoints - morphology, albumin secretion, AST release, ATP content and CYP3A4 activity. Compound identities and pairings were revealed only after analysis.

pixHep™ resolves all four matched pairs correctly

Presented below as matched pairs, the contrast within each is stark. The High-DILI drug resulted in convergent damage across multiple endpoints, while the cells exposed to its Low-DILI analogue remained largely unaffected - often demonstrating only CYP3A4 induction, a sign of metabolism rather than injury.

Pair 1: Troglitazone vs Pioglitazone

The classic thiazolidinedione benchmark: troglitazone, a drug originally intended for managing type 2 diabetes, was withdrawn for fatal liver injury, while pioglitazone remains widely used. Pioglitazone is not DILI-free; in fact, the FDA classifies it as Less-DILI-Concern and it carries liver-related cautions in its label. This was mirrored by the mild AST rise and albumin reduction in treated pixHep™. The severity gap was unmistakable: troglitazone ablated the pixHep™ monolayer and abolished albumin, while pioglitazone's effects remained mild and confined to the highest dose.

Table 1. Pair 1: endpoint responses at 10x Cmax (10-day exposure unless noted).

Endpoint	Troglitazone · HIGH DILI	Pioglitazone · LOW DILI
Morphology	Monolayer destroyed	Mild change at 10× only
Albumin secretion	Abolished (-83% by day 2)	Reduced (-61% at 10×)
AST release	3x increase	2x increase
ATP content	Below detection	No change
CYP3A4 activity	↓ from 0.01x Cmax	Dose-dependent ↓
Verdict	**High DILI** ✓	**Low DILI** ✓

Pair 2: Tolcapone vs Entacapone

Two COMT inhibitors for the treatment of Parkinson's disease. Tolcapone carries a boxed warning for hepatotoxicity, while entacapone does not. Exposure of pixHep™ to these drugs produced the panel's most extreme signal for tol-capone, including a 24-fold AST increase, but no differences were observed in entacapone-exposed pixHep™.

Table 2. Pair 2: endpoint responses at 10x Cmax (10-day exposure unless noted).

Endpoint	Tolcapone · HIGH DILI	Entacapone · LOW DILI
Morphology	Cell death by day 2	No change
Albumin secretion	-90%	No change
AST release	24x increase	No change
ATP content	Below detection	No change
CYP3A4 activity	Abolished at 10x	Induced (+62–77%)
Verdict	**High DILI** ✓	**Low DILI** ✓

Pair 3: Lumiracoxib vs Celecoxib

Two COX-2 inhibitors indicated for pain and inflammation management. Lumiracoxib was withdrawn in several markets for liver failure, while celecoxib remains on the market. Exposure of pixHep™ resulted in monolayer destruc tion and a 54% ATP loss for lumiracoxib, only, while no alterations were observed upon celecoxib exposure.

Table 3. Pair 3: endpoint responses at 10x Cmax (10-day exposure unless noted).

Endpoint	Lumiracoxib · HIGH DILI	Celecoxib · LOW DILI
Morphology	Monolayer destroyed	Minor vesicles at 10x
Albumin secretion	Abolished (-71% by day 2)	No change
AST release	No change	No change
ATP content	-54%	No change
CYP3A4 activity	Dose-dependent ↓	Induced (+75% at 10x)
Verdict	**High DILI** ✓	**Low DILI** ✓

Pair 4: Trovafloxacin vs Levofloxacin
Two broad-spectrum fluoroquinolone antibiotics. Trovafloxacin was severely restricted due to hepatotoxicity, while levofloxacin is a widely used, liver-safe agent. pixHep™ flagged albumin loss and morphological damage for trova floxacin, while levofloxacin treatment resulted in no adverse effects in pixHep™.

Table 4. Pair 4: endpoint responses at 10x Cmax (10-day exposure unless noted).

Endpoint	Trovafloxacin · HIGH DILI	Levofloxacin · LOW DILI
Morphology	Mild disruption + vacuoles	No change
Albumin secretion	-88%	No change
AST release	2x increase	No change
ATP content	-14%	No change
CYP3A4 activity	No clear effect	Induced at 10x
Verdict	**High DILI** ✓	**Low DILI** ✓

Why matched-pair discrimination matters

Resolving matched blind pairs is a far more demanding test than ranking unrelated compounds - and pixHep™ passed it cleanly in all four. It separated each hepatotoxic drug from its closest safe analogue: the exact discrimin ation that matters when choosing between candidates in a series or backing a lead with a safer follow-on. Differ- ent endpoints did not always move together - lumiracoxib hit ATP and morphology but not AST, while the safe ana logues often showed CYP3A4 induction without injury - therefore, this five-endpoint panel did not only reveal whether a compound was toxic or not, but also captured the mechanistic fingerprint of each drug.

Conclusions

Blind to compound identity, pixHep™ correctly distinguished the hepatotoxic drug from its closely related, liver-safe analogue in all four matched pairs - including well-established benchmarks, such as troglitazone vs pioglitazone and tolcapone vs enta-capone. Effects were resolved across five complementary endpoints, therefore providing a mechanistic profile rather than a single readout. Built on iPSC-derived hu-man hepatocytes, pixHep™ gives drug-development teams a predictive, human-rel-evant and animal-free platform to separate toxic candidates from safe analogues - de-risking selection early, when it matters most.

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