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Emerging In Vitro Culture Technologies to Address Challenges in Hepatic Metabolism and Drug Interactions

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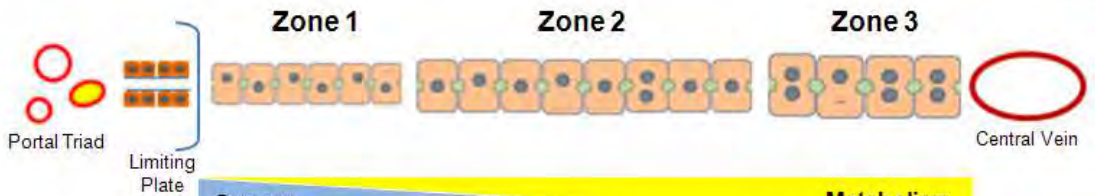
Need to capture key events and interactions with more complex culture models

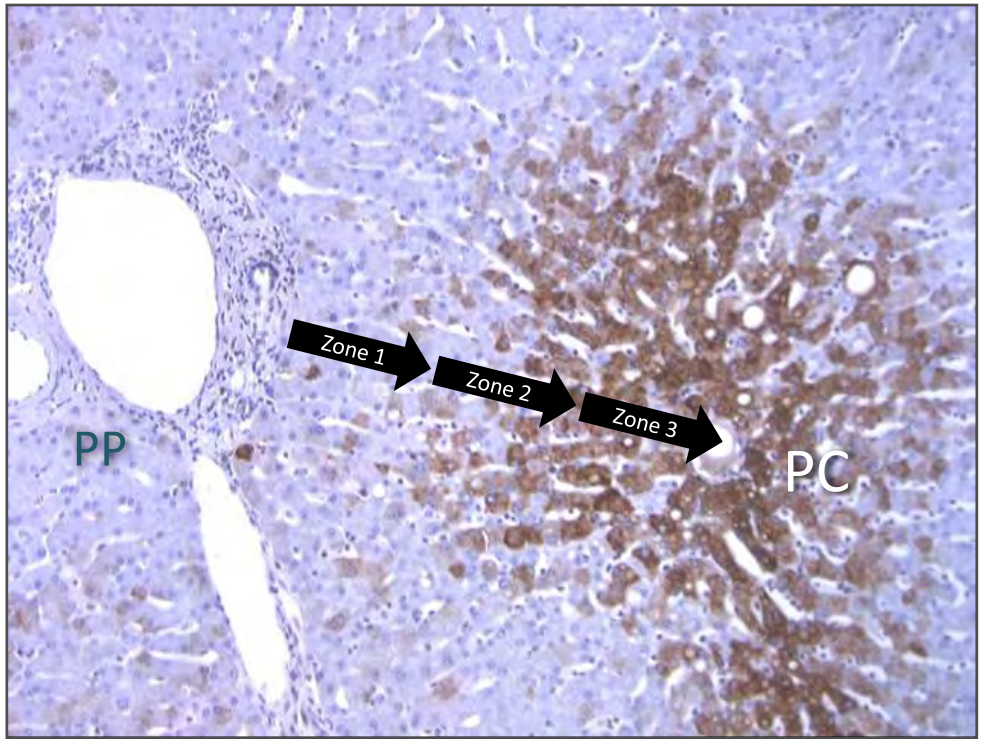
- Low CL_{in} and reactive metabolites
  - Phase 1 metabolism
  - Phase 2 (e.g. acyl glucuronides)
  - "Other" pathways
- Receptor-mediated events
  - Key receptors: AhR, RXR 'partners' (e.g. PXR, CAR, PPAR etc.)
  - DNA synthesis, lineage biology
  - Increases in ER, peroxisomes, endosomes etc.
- Cholestatic events
  - Key transporters leading to impaired biliary function: parent vs metabolites
  - Inhibition of uptake and/or efflux transport capacity (e.g. BSEP)
- Zonal differences in hepatic disposition
  - Statins vs APAP
  - Gradients in O_2, metabolism, uptake capacity etc.
- Inflammation
  - Role of Kupffer cells, HSCs and LSECs
  - Heterotypic interactions and pro- vs anti-inflammatory cytokine signaling
- Fatty liver (NAFLD)
  - Steatosis/NASH
  - Phospholipidosis
- Fibrosis
  - Role of hepatocellular injury
  - Stellate activation/regression
- Mitochondrial effects
  - Inhibition of the electron chain
  - Inhibition or uncoupling of oxidative phosphorylation
  - Induction of mitochondrial oxidative stress
  - Inhibition of DNA replication, transcription or translation

Zonal Expression of Hepatic Genes

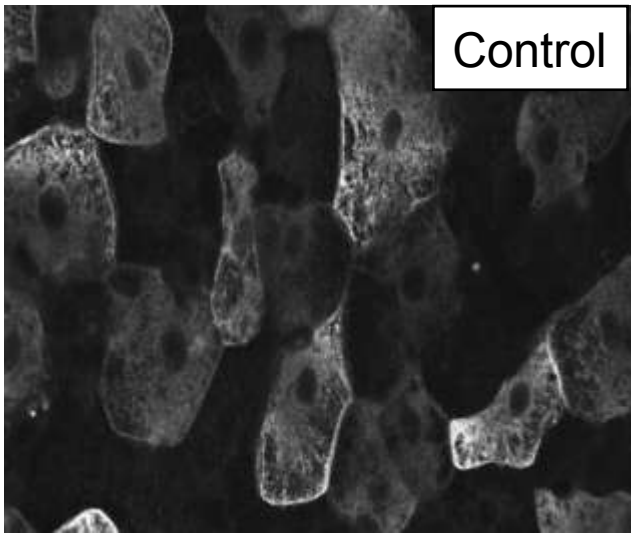


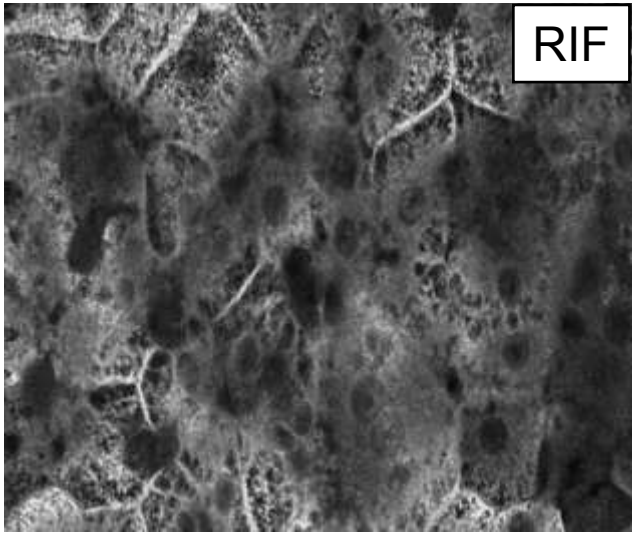
Oxygen ↓ Metabolism ↑

	Periportal (Zone 1)	Transitional (Zone 2)	Pericentral (Zone 3)
Primary Function	Oxidative metabolism, Gluconeogenesis, Ureagenesis	Mixture of Zone 1 and 2 functions	Glycolysis, Liponeogenesis, Xenobiotic metabolism

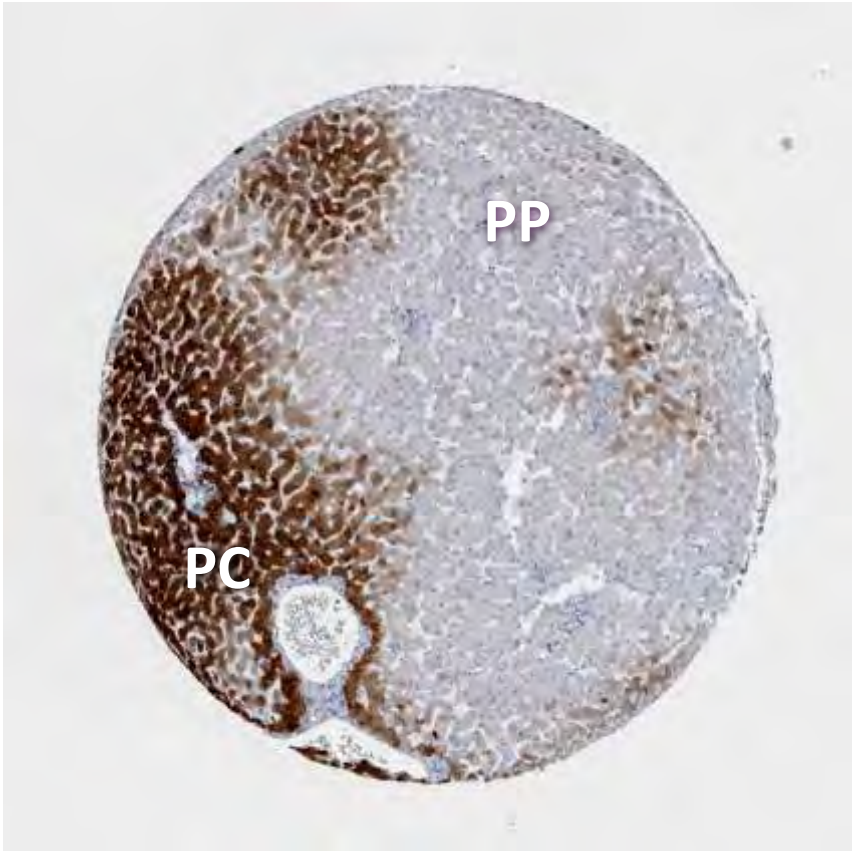


In Vitro

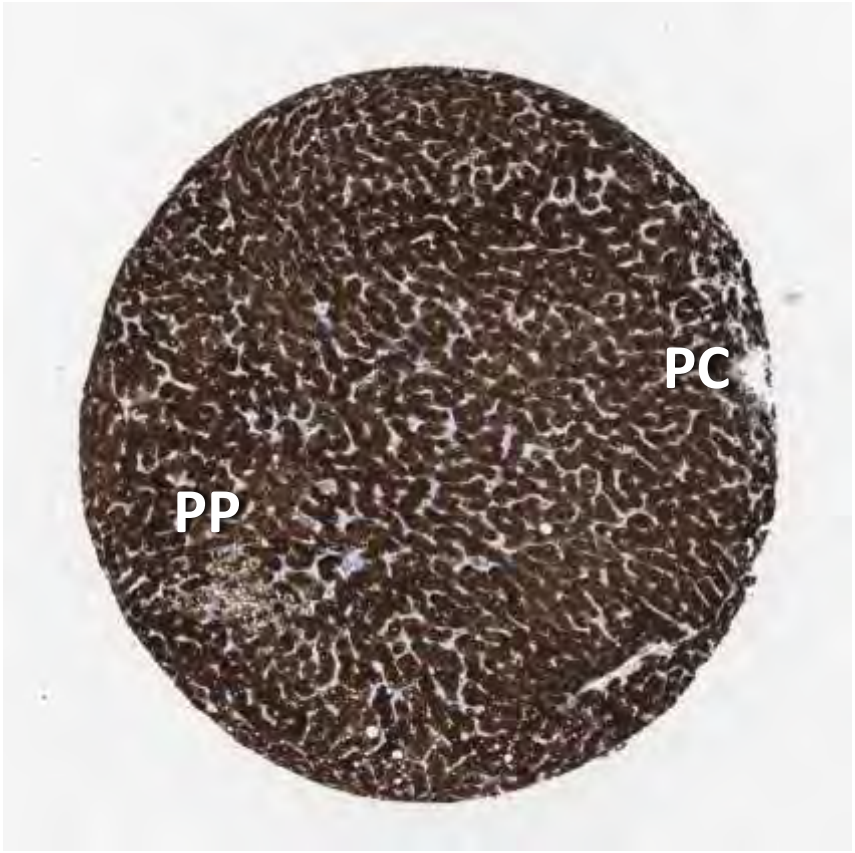




Zonal Disposition of CYP's and Membrane Transporter Proteins



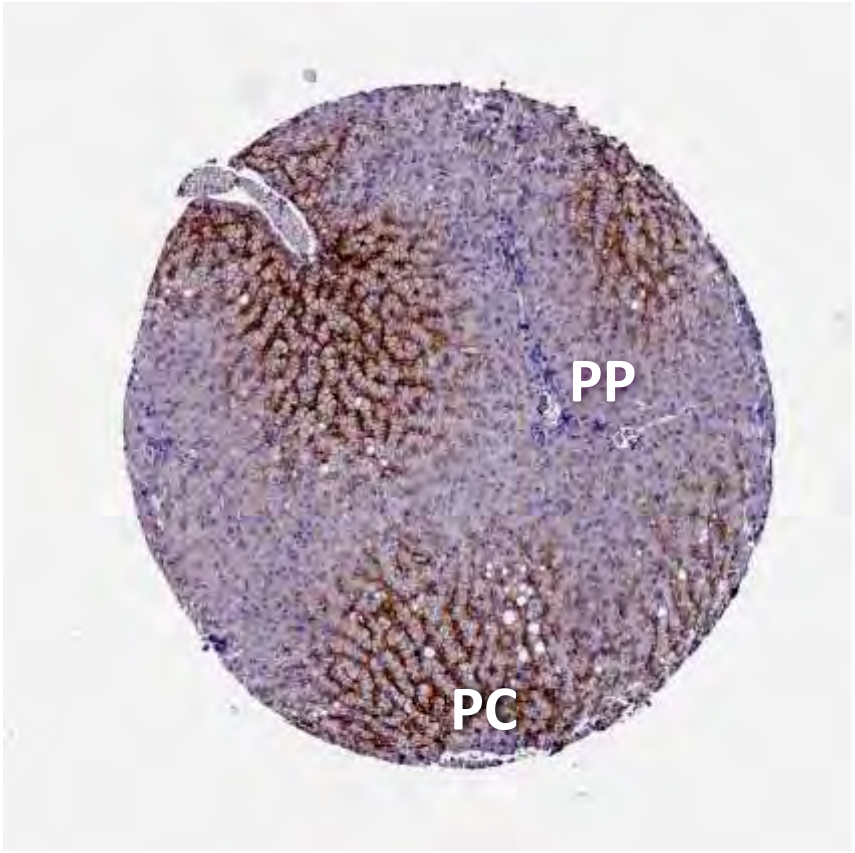
CYP3A4



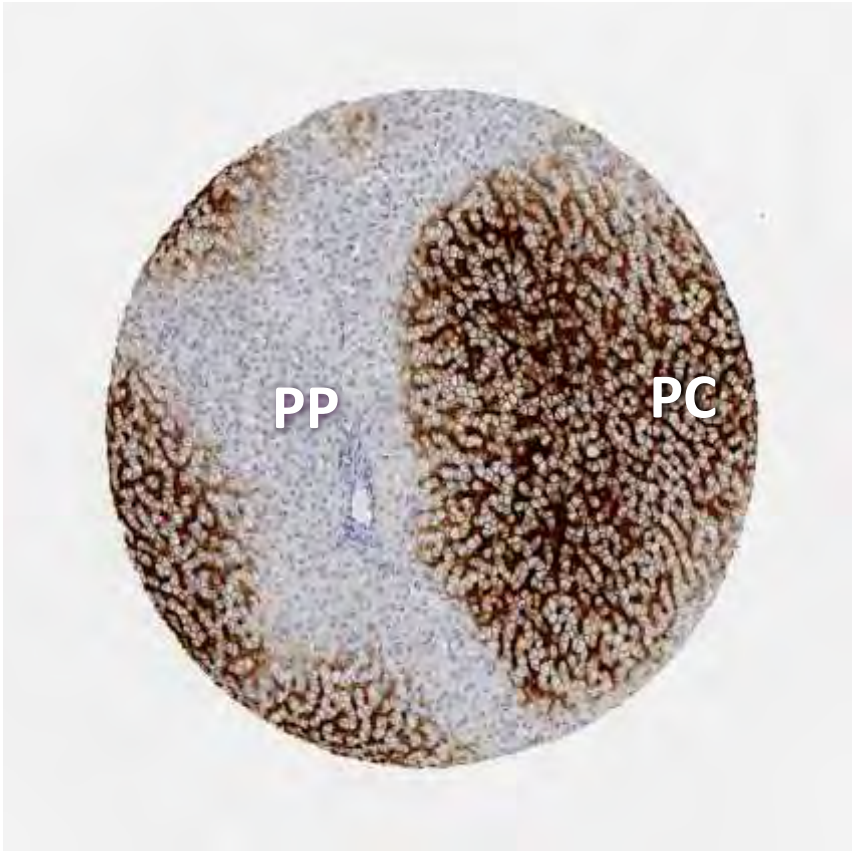
CYP2C9

Human Protein Atlas Project, www.proteinatlas.org

Zonal Disposition of CYP's and Membrane Transporter Proteins



OATP1B1

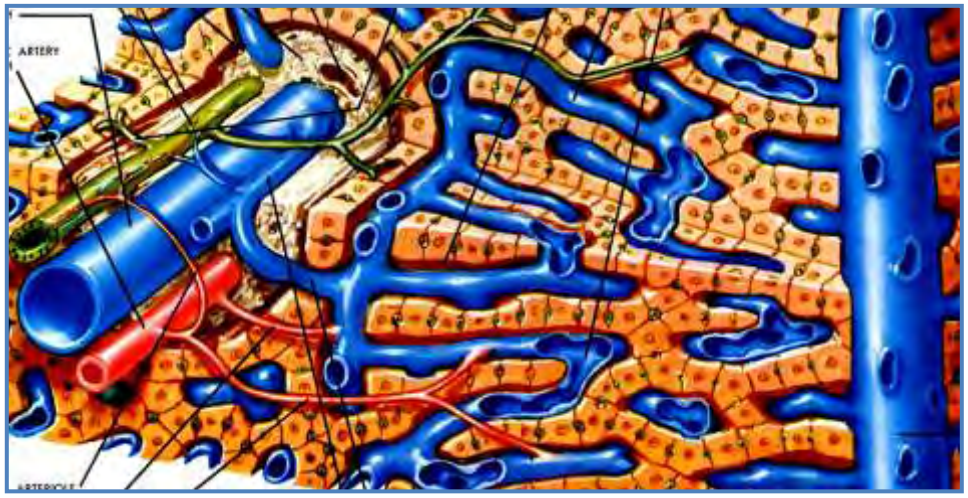


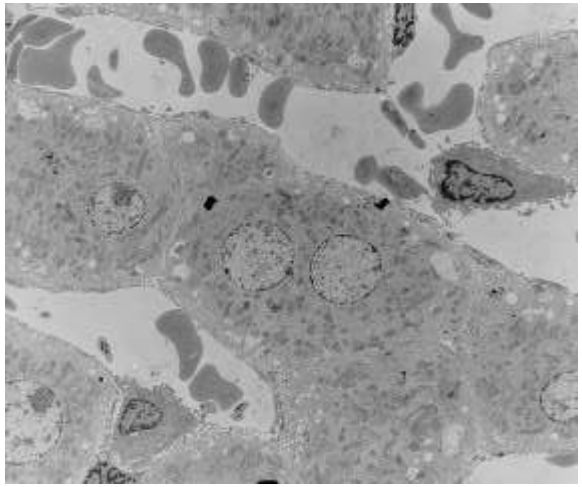
OATP1B3

Human Protein Atlas Project, www.proteinatlas.org

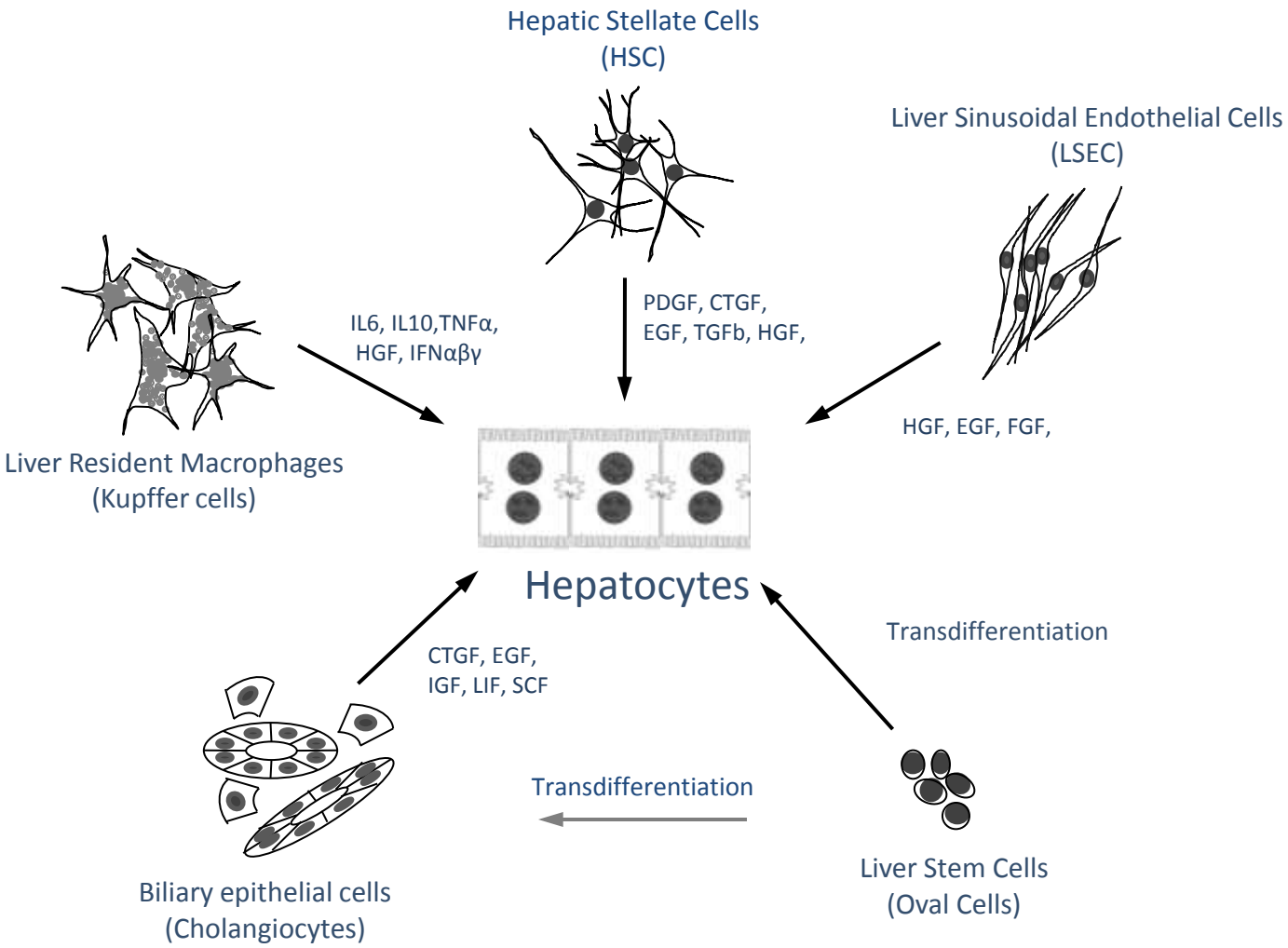
The Situation and the Challenge

- Most of our traditional *in vitro* hepatic culture models are too simple and non-physiologic to recapitulate some of the more complex metabolic and toxic events that occur *in vivo*.
- The relationship between the unique cell types within the sinusoids of the liver often dictate the uptake, metabolism and pharmaco/toxicokinetics of a compound.
- As such, one of the biggest challenges for DMPK and safety assessment today is to develop more complex, multicellular *in vitro* models of the liver that integrate the architectural and cellular complexities of the organ *in vivo*.





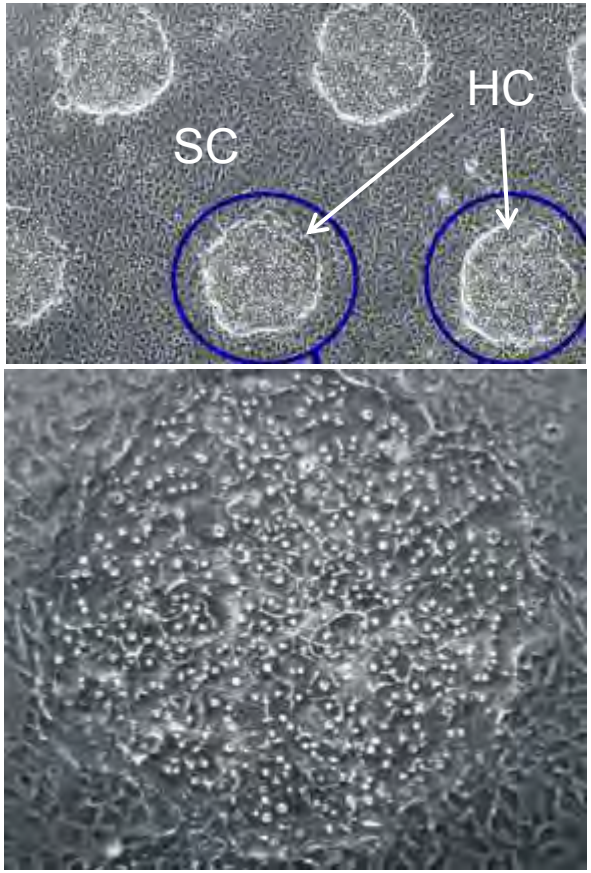
Interdependence of Hepatic Cell Types



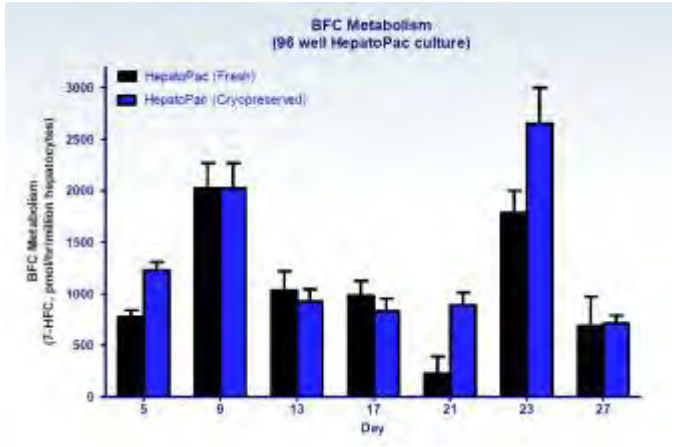
Advanced Models for DMPK/Tox Testing

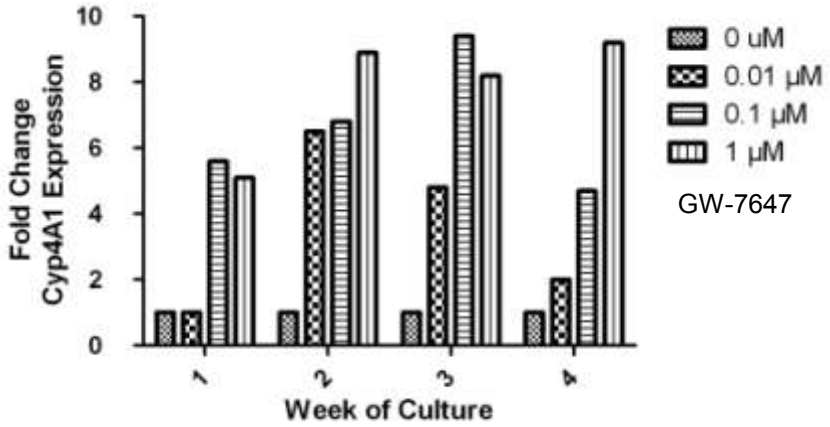
CO-CULTURE SYSTEMS FOR DMPK/TOX PROFILING

Hepregen Micropatterned Co-culture System



HepatoPac - Day 21

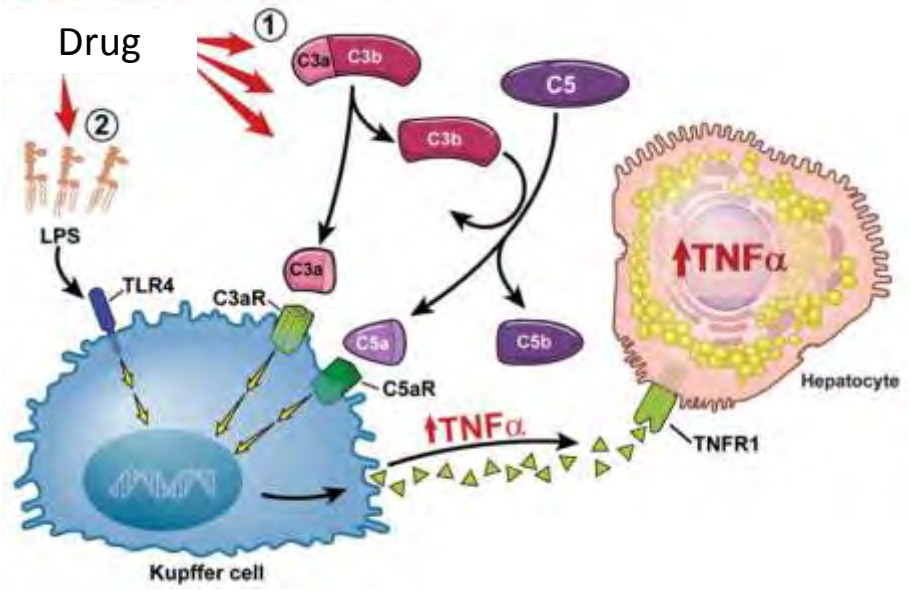




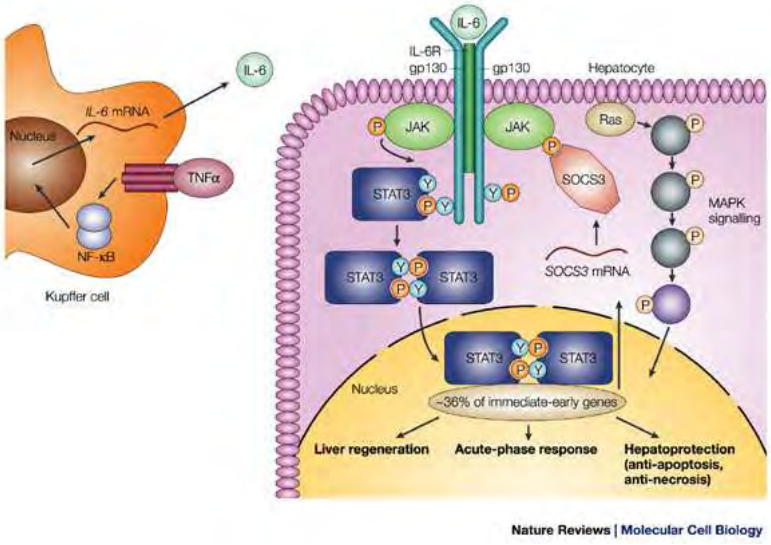
- Enhanced hepatocellular morphology and phenotype for >3 weeks
- Maintenance of nuclear receptor response for several weeks (e.g. PPARα)
- Very amenable to high-content screening strategies

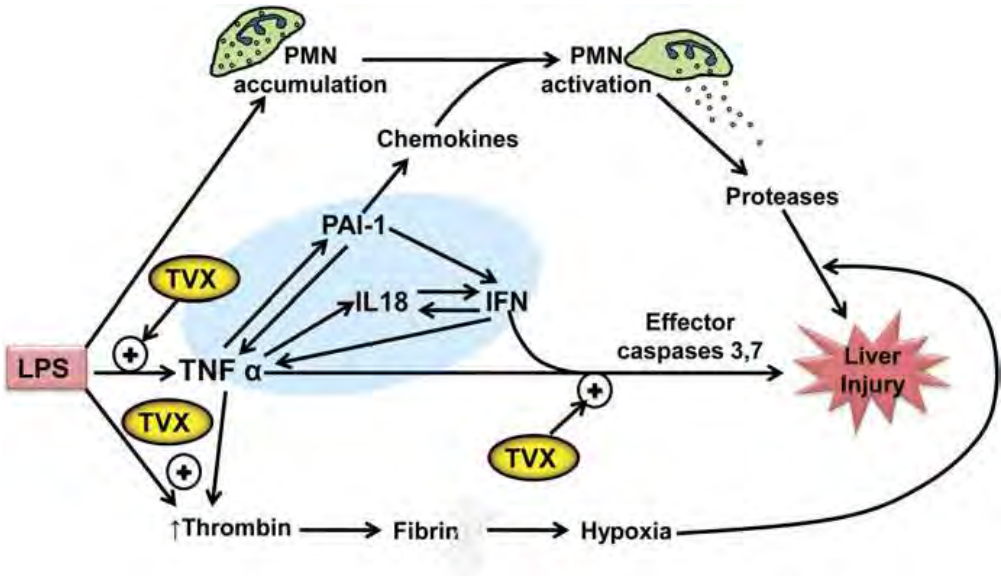
Ukairo O, et al. J Biochem Mol Toxicol. 2013 Oct;27(10):471-8

Immune-mediated Acute and Adaptive Hepatic Effects



- Drug- or LPS-induced activation or sensitization of KC or HC
- Subsequent effects on KC/HC paracrine and gene expression profiles.
- Pathway perturbations & toxicity exacerbated by inflammation (e.g. TVX)

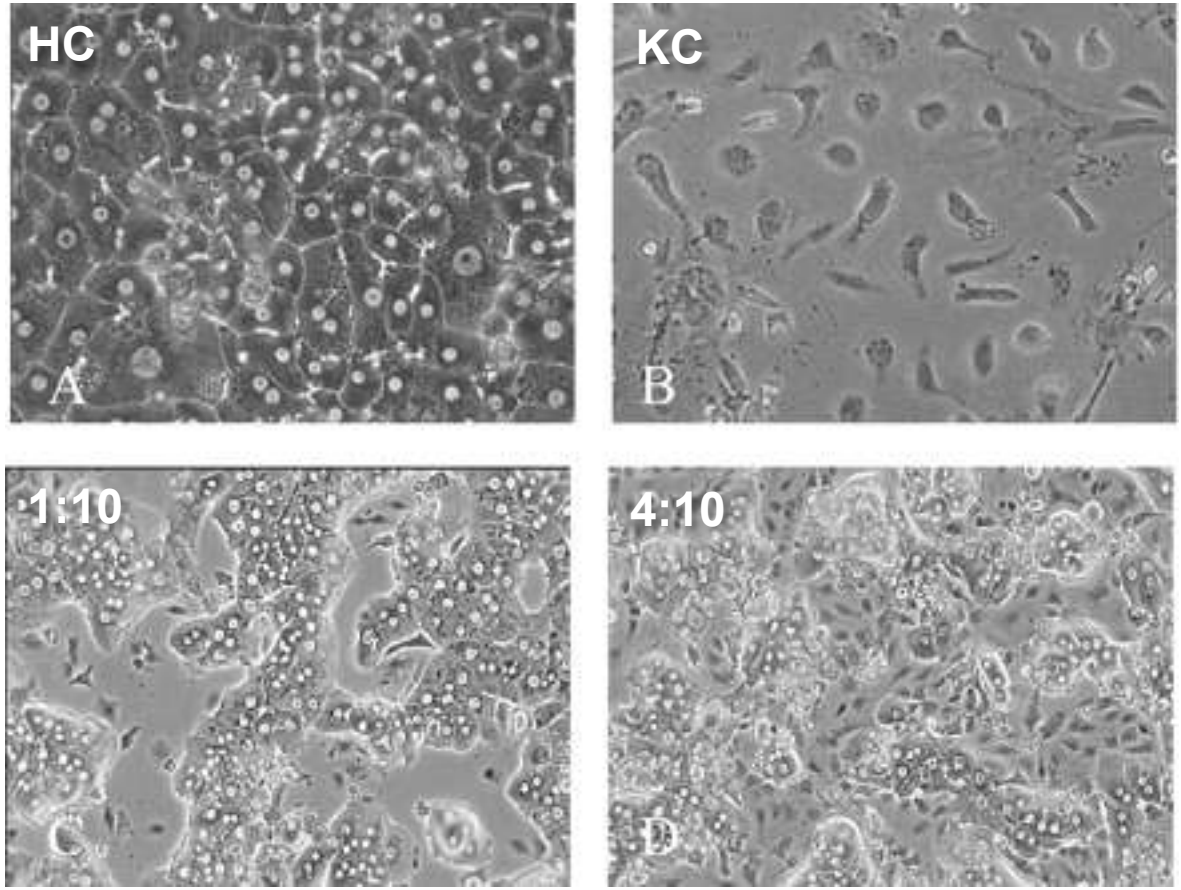




FDA/EMA Guidance Considerations

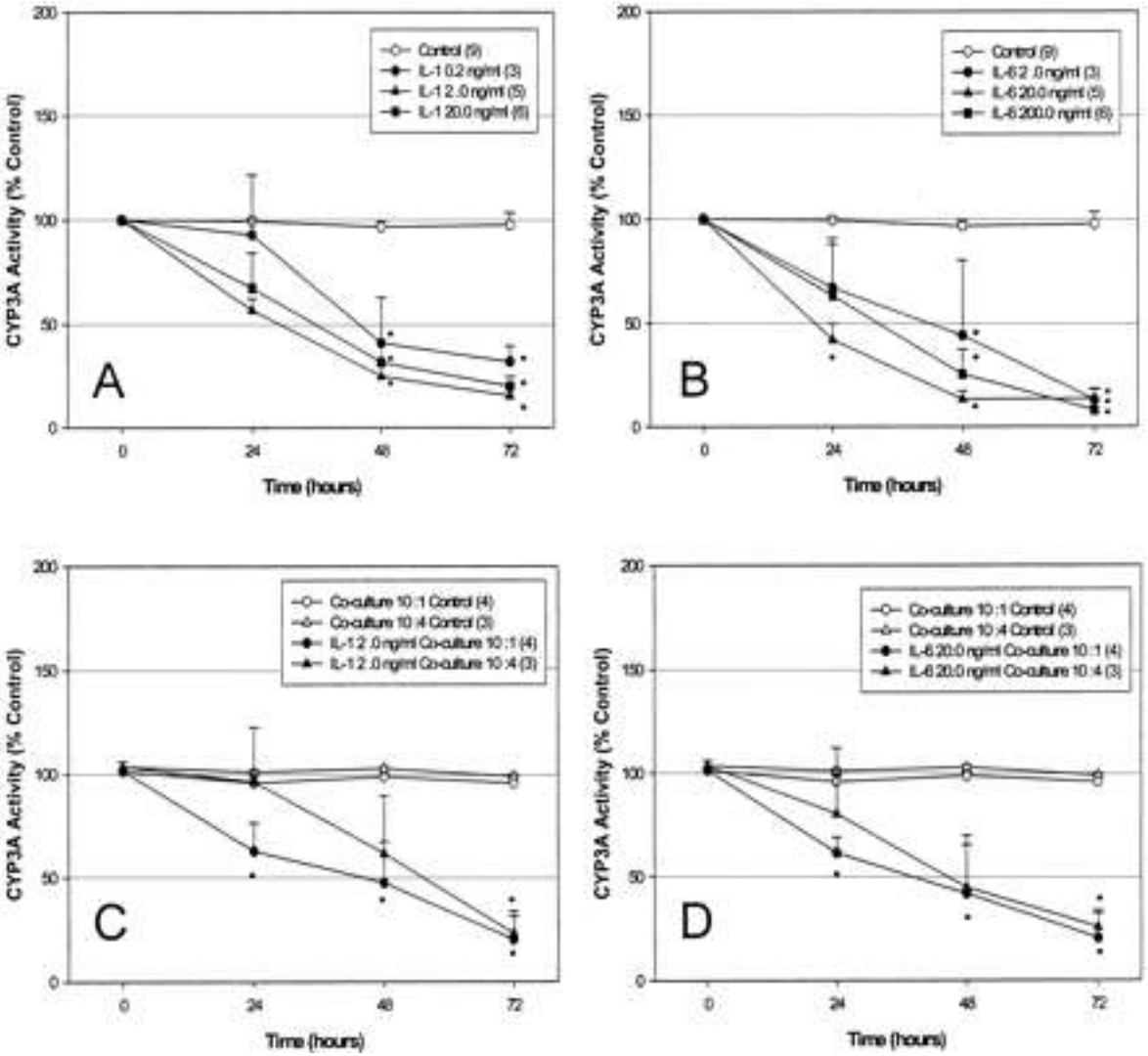
- If a concentration-dependent **down regulation** (i.e. in CYP expression) is observed *in vitro*, additional *in vitro* (or *in vivo*) studies of the effect on other drug metabolizing enzymes are recommended to investigate which enzymes are affected unless this may be predicted.
- ….If a **50% decrease** in mRNA is observed which may not be attributable to cell toxicity, this may indicate down-regulation of the enzyme and an in vivo study investigating this time-dependent phenomenon is recommended.
- The potential for interactions with drug products should be considered for certain classes of therapeutic proteins (TPs).
- If an investigational TP is a cytokine or cytokine modulator, studies should be conducted to determine the TP's effects on CYP enzymes or transporters.
- When there are known mechanisms or prior experience with certain PK or PD interactions for other similar TPs, appropriate in vitro or in vivo assessments for possible interactions should be conducted.

Primary human hepatocyte and Kupffer cell cultures

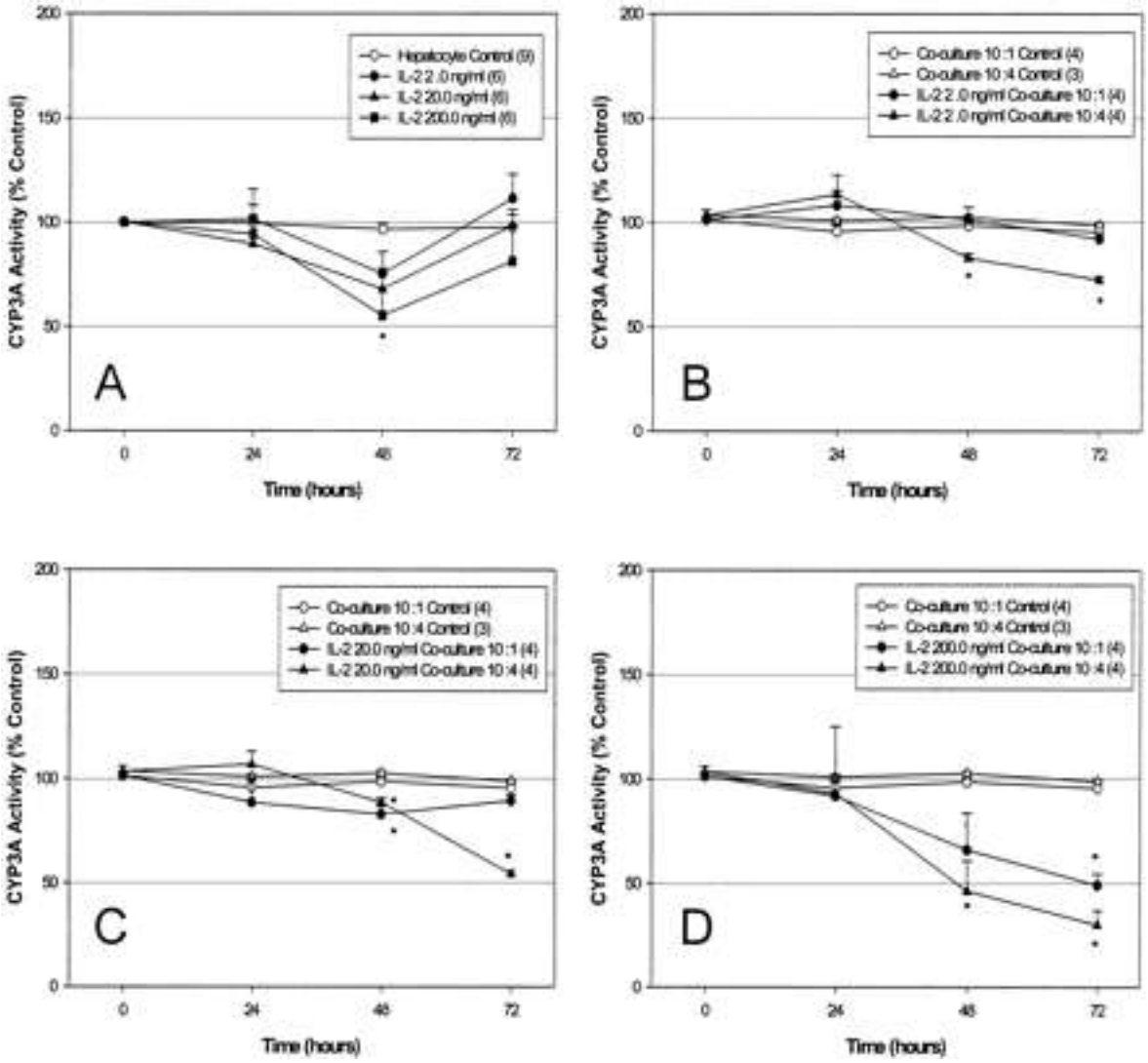


Sunberg et al, DMD, 3:359-363, 2004

Direct suppression of CYP3A activity by IL-1 and IL-6



Kupffer cell-mediated IL-2 suppression of CYP3A activity



Summary and Conclusions

- Incorporating basic biological ‘principles’ of tissue architecture and cellular interactions into the design of new cell culture platforms is leading to a new generation of cell culture models that more closely recapitulate the *in vivo* situation.
- It is clearly evident that ‘3D’ architecture and heterotypic cell interactions affect the responses of target cells and can alter the corresponding responses during compound testing.
- These emerging technologies enable us to ask new questions and distinguish new mechanisms of drug action and disposition previously not observed in short-term monocultures of hepatocytes or cell lines.
- As a result, new and exciting opportunities exist in the use of these novel culture systems for predicting drug efficacy and liability under various physiological and pathological conditions.

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THANK YOU!

Questions?