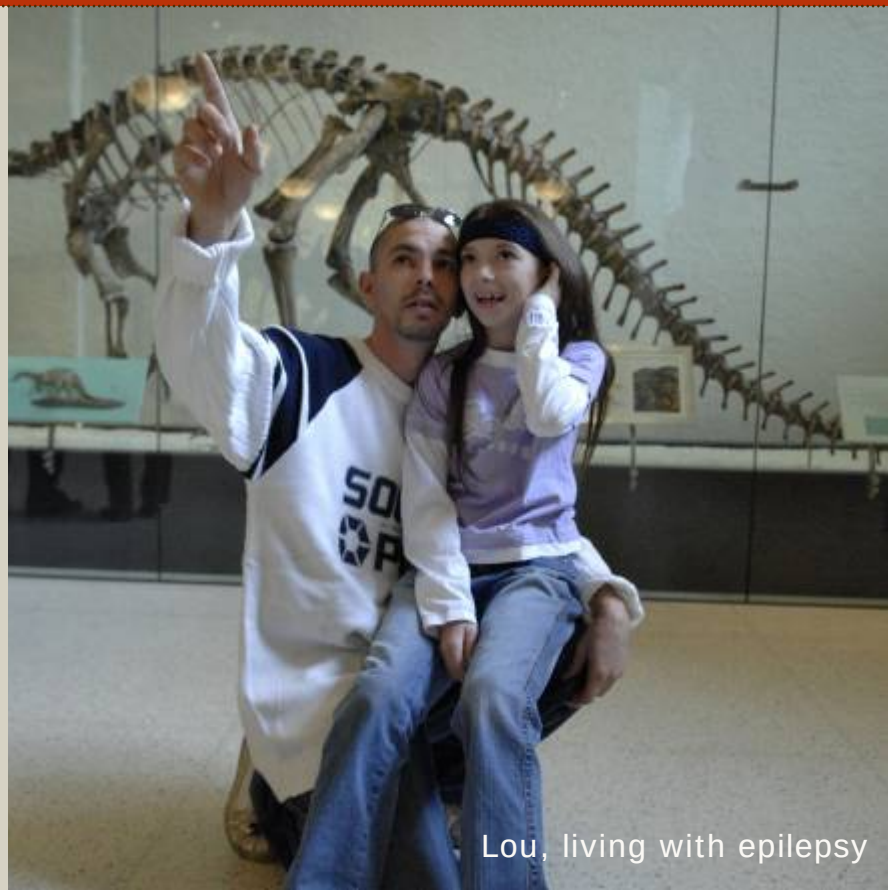
Which models to use in Early Toxicology Assessment to detect Human Hepatotoxic Drugs ?

Franck Atienzar

Associate Director

Head in Vitro Toxicology Unit

UCB Pharma



N.B.: slides 16, 23, and 34-40

ADME & Pred Tox meeting,

Barcelona, 11-12 April 2013

Plan of the talk

1. Drug withdrawal due to toxicology and idiosyncratic DILI
2. Idiosyncrasy: a case study with tolcapone & entacapone
3. Key publications in the field of hepatotoxicity
4. UCB investigations
5. Conclusions

1) Drug withdrawal due to Toxicology and idiosyncratic DILI

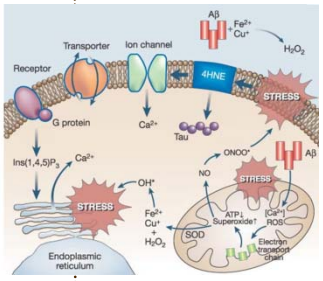
• Many drugs have either been discontinued from clinical trials or withdrawn from the market after being approved because of **hepatic** & **cardiac** adverse effects

• Most Drug Induced Liver injuries (DILIs) are linked to patient-specific susceptibility (idiosyncratic)

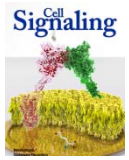
• Idiosyncratic DILIs: likely to be "**multi-hit**", including **environmental** and **genetic** factors

• **Idiosyncratic** toxicities: not dose dependent, not easily detected in clinical studies as around 1 in 10,000 patients is affected

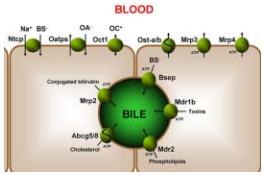
Liver toxicity: many mechanisms, tools & models

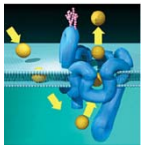


Ca⁺⁺ homeostasis disruption

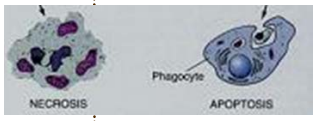


Cholestasis

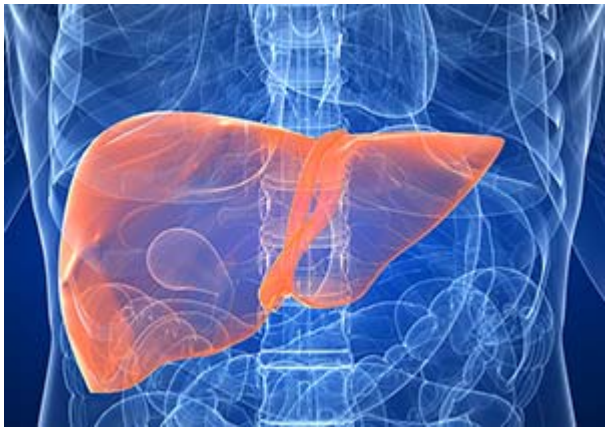


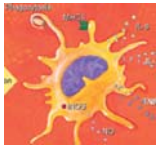


Transport pump inhibition



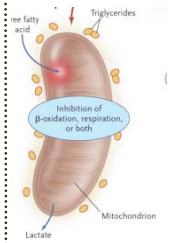
Apoptosis



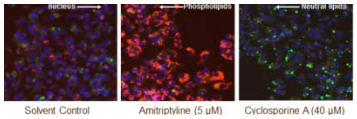


Immune reactions

Necrosis

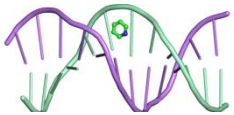


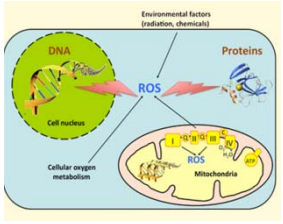
Steatosis

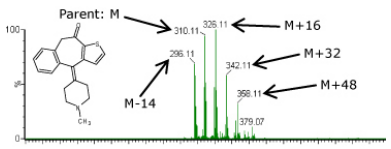


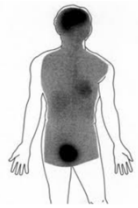
Mitochondrial toxicity

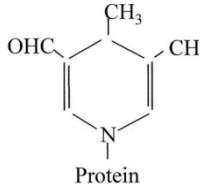
CYP 450 mediated toxicity

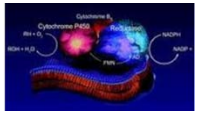


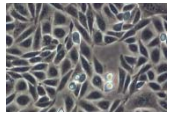


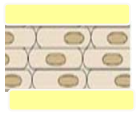


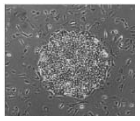


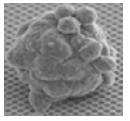


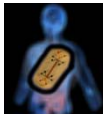


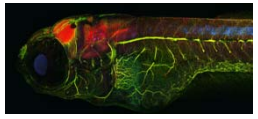












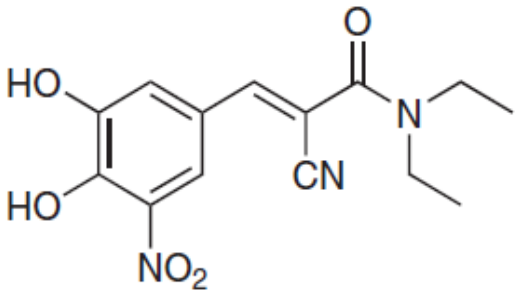
Model complexity (screen)

2) Case study with tolcapone and entacapone

- **Background:**

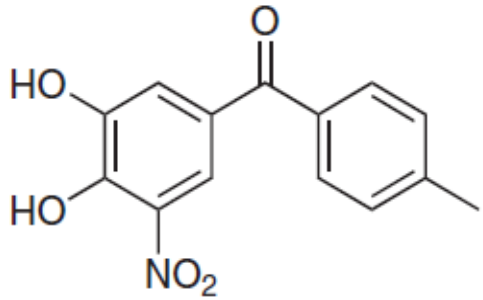
- Tolcapone (Roche) and Entacapone (Orion) are two structurally related Catechol O Methyl Transferase (COMT) inhibitors which do not present the same risk in terms of liver toxicity

- Both drugs are given to PD patients with L-DOPA



Entacapone

Safe drug



Tolcapone

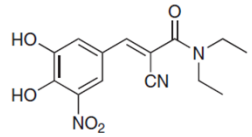
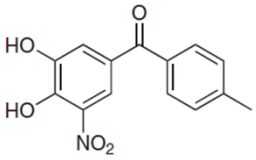
Idiosyncratic hepatotoxicity (1/20.000)

- **Objective:**

- Understand the reasons? What are the most common Hypothesis? How different/common are the drugs in term of doses, exposures, DMPK profiles, efficacy, …

Toxicity assessment (tolcapone, entacapone)

- **KO COMT mice:** viable, no liver effect reported (Haasio et al., 2003)
- **Animal and Human data:**

Structure	COMT inhibitor	Non Clinical Studies		Clinical studies*	Market*
		In vitro Toxicity	in vivo Toxicity	Serum transaminases activity	Observation/conclusion
	**Entacapone**	No	No abnormalities detected	No abnormalities detected (4 cases independent of E treatment)	Considered as non hepatotoxic
	**Tolcapone**	Yes	Necrosis Hepatitis	Increased (e.g. in **5.7%** of patients, N=3848, 200 mg of T 3x)	**3 instances of acute liver failure with death** after 60,000 patients had received T (**hepatitis**)
		Considered as safe in animal studies		Considered as safe in clinical studies (low incidence, reversibility)	**Withdrawn** in 1998 (EU + Canada), **reintroduced with liver function monitoring test**

*: PD patients treated with L-DOPA + COMT inhibitor; References: Haasio, 2010; Gasser and Smit, 2001; Watkins, 2000

Pharmacokinetic and Efficacy (tolcapone, entacapone)

(Kaakkola, 2010)

- Pharmacokinetic properties of oral (200mg) Entacapone & Tolcapone in healthy volunteers:

Parameters	Entacapone	Tolcapone	Ratio (T/E)
Cmax (mg/l)	1.8	6.3	3.5
AUC 0-->∞ (h mg /l)	1.6	18.5	11.6
T1/2 (h)	3.4	2.1	0.6
F (%)	36	60	1.7

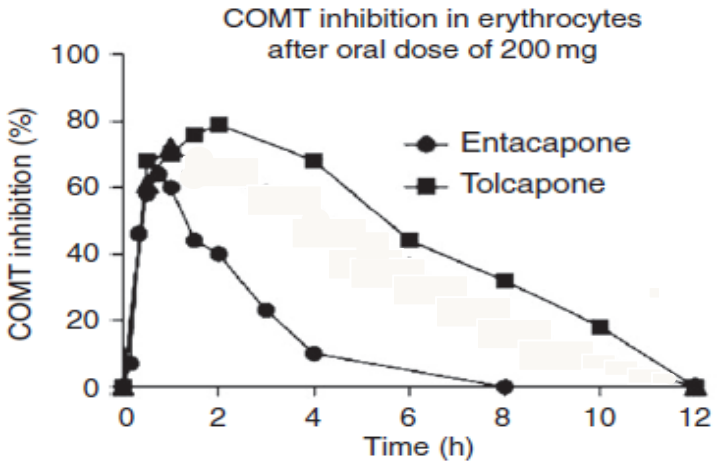
Total clearance (ml/min/kg) in healthy volunteers (IV):

- Tolcapone (50 mg): 1.7
- Entacapone (20 mg): 11.7

→ **Exposition** after **tolcapone** administration is **higher** due to **lower clearance**

- Efficacy: COMT inhibition in erythrocytes after oral dose (200 mg)

→ **COMT inhibition** after **tolcapone** administration last for a **longer period of time**



Reference: Kaakkola, 2010

Main hypothesis to explain tolcapone idiosyncratic hepatotoxicity

- Mitochondrial toxicity: uncoupling of oxidative phosphorylation (Haasio et al., 2002)

- AKT cell survival pathway (Sei et al., 2010)

- Oxidative stress (Smith et al., 2003)

- Other off-target effects (rat Omics analysis: liver, plasma & urine, McBurney et al., 2012)

- Genetic (polymorphism): UDP-glucoronosyl transferase (Ferrari et al., 2012), mitochondrial complex 1 deficiency (Schapira, 1994)

- Higher exposure to Tolcapone and/or to Tolcapone metabolite (Smith et al., 2003)

- Inhibition of soluble-COMT in the periphery has been proposed to contribute to tolcapone linked hepatotoxicity (Chen et al., 2011)

→ Likely to be due to **multiple parameters** (combination of effects):

E.g. ***nitro-catechol + threshold concentration (parent and/or metabolite) + mitochondrial toxicity + UDP-glucoronosyl transferase (reduced activity) + mitochondrial complex 1 deficiency***

3) Key publications in the field of hepatotoxicity

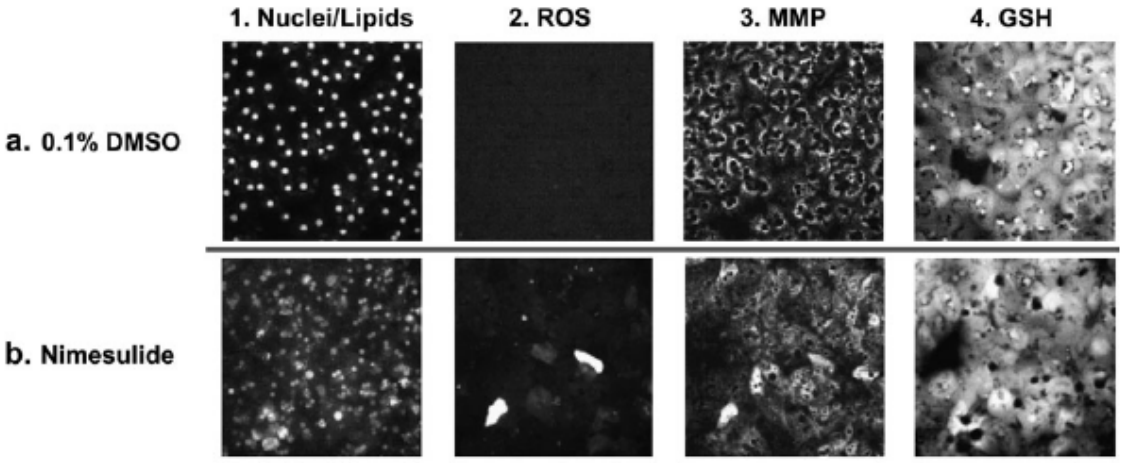
Hepatotoxicity predictivity with different models:

- *Primary Human Hepatocytes*: Xu et al., 2008
- *HepG2 cells*: O'Brien et al., 2006; Hill et al., 2012; Tolosa et al., 2012
- *Specific organ toxicity*: Lin and Will, 2012
- *Micropatterned co-cultures*: Khetani et al., 2013; Wang et al., 2010
- *Hurel microscale culture device:* Chao et al., 2009; Novik et al., 2009
- *Mitochondrial Toxicity*: Marroquin et al., 2007
- *Zebrafish*: Jones et al., 2009; Hill et al., 2012; Aleo et al., 2010
- *Integration of multiple assays:* Thompson et al., 2012
- *Liver Toxicity Knowledge Base:* Chen et al., 2011

Primary human hepatocytes

- Fresh primary hepatocytes are the gold standard but problem of availability, price and predictivity
- Platable cryopreserved hepatocytes can be used in screening
- **Xu et al., 2008:** freshly isolated and/or cryopreserved human hepatocytes exposed to 300 drugs and chemicals (liver tox)

  Concentrations tested up to 100 Cmax, HCS, 1 day exposure



sensitivity = 50-60% (n=200)

specificity = 95-100% (n=100)

HepG2

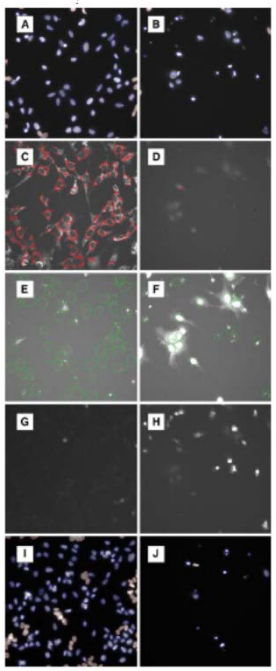
- $HepG_2$: a perpetual cell line derived from the liver tissue of a 15 year old Caucasian American male with a well differentiated hepatocellular carcinoma

  - Widely used in toxicology but many publications reveal the lack of metabolisation

  - Good tool to study the toxicity of parent compounds

- **O'Brien et al., 2006**: $HepG_2$ cells exposed to 243 compounds for 3 days and multiple endpoints determined by cell imaging (HCS)

Control **0.1 mM furazolidone**



►Nuclear area

►Mitochondrial Mbne Pot.

►Calcium

►Membrane permeability

►Cell Membrane

sensitivity = 88% (n=102)

specificity = 98% (n=40)

NB: drugs toxic to other organs and positive controls have been excluded.

Improvement due to:
- Cmax approach (up to 30 Cmax)
- Multiplexing
- Length of experiments (3 days)
- Kinetic measurements

HepG2

- **Hill et al., 2012:** Comparison HCS versus ZF

  J&J validation, 67 ref cpds, HepG2 (cell number, nuclear size, MMP, …),

  HCS, LC_{20}, cut off: 30µM, 3 day exposure, top concentration: 100 µM

  - sensitivity: 53% (n=49)
  - specificity: 100% (n= 18)

- **Tolosa et al., 2012:** HCS in HepG2

  HepG2 exposed to 78 compounds for 3 and 24 hours, 1-10-100-1000 µM

  HCS (cell number, nuclear morphology, MMP, calcium, OS)

  - sensitivity: 94% (n=66, at least one endpoint, cut off: 1000µM)
    79% (n=66, at least one endpoint, cut off: 100µM)
  - specificity: 92% (n= 12, cut off: 1000µM)

Specific organ toxicity

- **Lin and Will, 2012**: Pfizer investigation

  - Testing of 273 hepatotoxic, 191 cardiotoxic, and 85 nephrotoxic compounds in HepG2 (hepatocellular carcinoma), H9c2 (embryonic myocardium), and NRK-52E (kidney proximal tubule) cells for their cytotoxicity, 3 day exposure, ATP.

  → Cut off:100 Cmax (ie neg if LC_{50}>100 Cmax, pos if LC_{50}< 100Cmax)

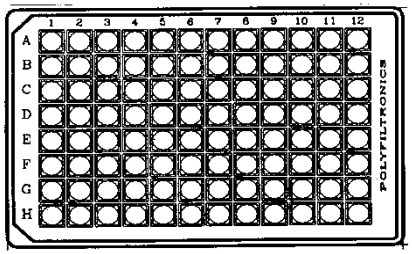
Hepatotox prediction (HepG2):	sensitivity: 68% (n=109)
	specificity: 75% (n=72)
Cardiotoxicity prediction (H9c2):	sensitivity: 55% (n=62)
	specificity: 81% (n=72)
Nephrotoxicity prediction (NRK-52E):	sensitivity: 73% (n=41)
	specificity: 78% (n=72)

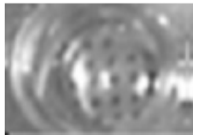
  → *The majority of compounds, regardless of their designated organ toxicities, had similar effects in all three cell lines*

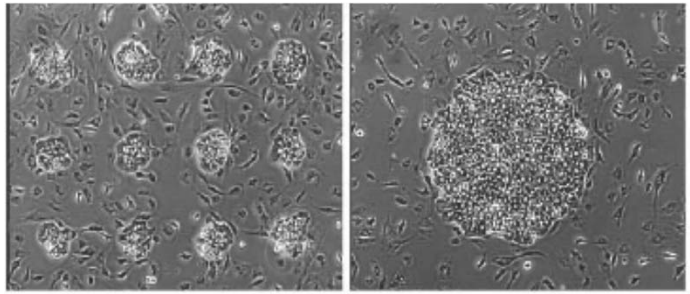
  → *Organ toxicity cannot be accurately predicted using such simple approach*

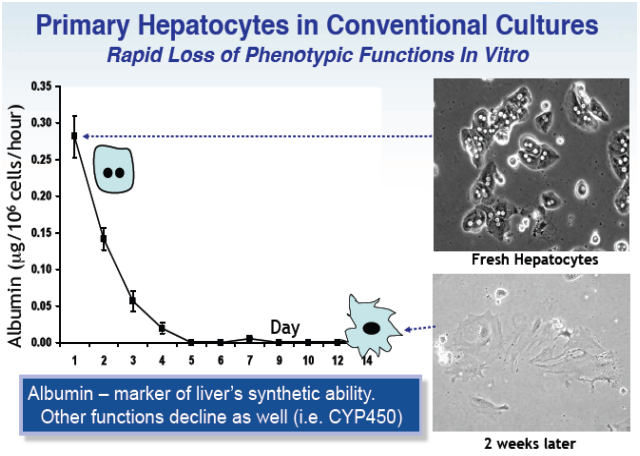
Micropatterned co-cultures of hepatocytes (Hepregen)

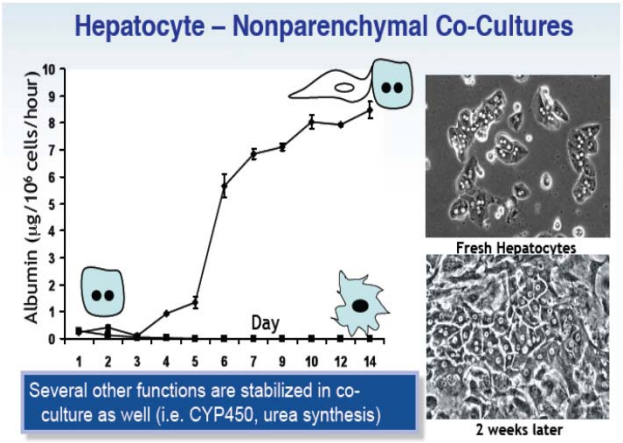
- Hepregen technology (Khetani and Bhatia, 2008)











Main advantages: non random distribution (hepatocytes and fibroblast), long term, bile caniculi formation, model has started to be validated, rat and Human model available

Micropatterned co-cultures of hepatocytes (Hepregen)

- **Khetani et al., 2013** (in press): Hepregen and Pfizer

Rat and Human co-culture models exposed to 35 human hepatotoxic cpds and 10 non-hepatotoxic cpds: multiple of cmax (1, 30, 60, 100), 9 day exposure.
Endpoints: GSH, ATP, Urea, albumin.
Cpd classified as pos if LC_{50} for at least one endpoint < 100Cmax

Human co-culture:	sensitivity: 66% (n=35)
	specificity: 90% (n=10)
Rat co-culture:	sensitivity: 49% (n=35)
	specificity: 80% (n=10)

- **Wang et al., 2010**: Human co-culture model more relevant to generate in vivo Human metabolites than liver microsomes, liver S9 fraction and hepatocytes suspension with a set of 27 cpds

Hurel microscale culture device

Chao *et al*., 2009:

- Patented HμREL® microdevice: a microfluidic in vitro Human culture system to predict hepatic clearance

  →The obtained clearance rates are comparable to in vivo data (literature)

Novik *et al*., 2009:

- Combine HμREL® microdevice with a hepatic Human co-culture system
- To study clearance and metabolite generation

  → System is capable of clearing cpds with higher resolution and predictive value

  → When combining co-culture and flow → superior metabolite generation and better in vitro in vivo correlation prediction

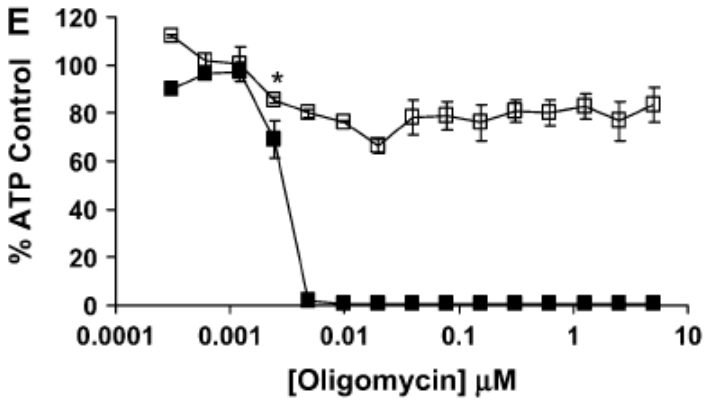
Mitochondrial toxicity: Crabtree effect

- Many, but not all, drugs with organ toxicity have a mitochondrial liability.

  Screen of > 550 drugs reveals 34% have mitochondrial liabilities (Dr Dykens pres.)

- But high glucose concentration is used during in vitro culturing conditions:
  - ATP is produced through glycolisis (Crabtree effect)
  - mitochondria are inactivated
  - cpds affecting mitochondria are not detected

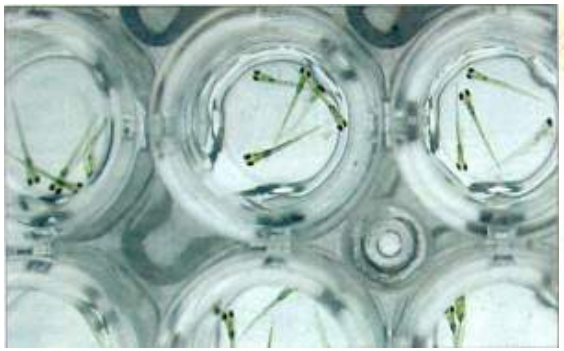
- **<u>Marroquin et al., 2007</u>:** When glucose is replaced by galactose:
  - mitochondria are activated: O_2→ATP
  - drugs affecting mitochondria are detected

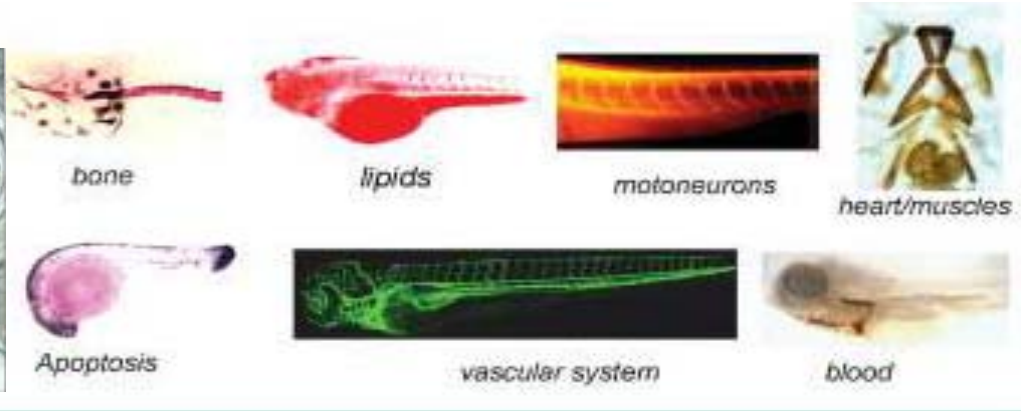


Zebrafish (ZF)

In brief:

- A complex vertebrate model, transparent, high fecundity
- mg of cpd required, rapid analysis, easy to use in screening
- Basic metabolic machinery similar to mammals (even in larvae)
- ZF mutants exhibit phenotypes similar to human disease states
- Multiple toxicity endpoints can be measured





Zebrafish (ZF)

- **Jones et al., 2009** (abstract): Evotec/J&J investigation
  → 50 cpds tested in ZF: sensitivity: 86% (n=37)
  specificity: 77% (n=13)

- **Hill et al., 2012:** comparison of HCS performed on HepG2 (J&J), primary hepatocytes (Pfizer) and ZF assay using 33 cpds (mainly hepatotoxic drugs)
  → *Added value to use ZF assay (but not alone)*
  → *I.e. some cpds neg in HCS are pos in ZF*

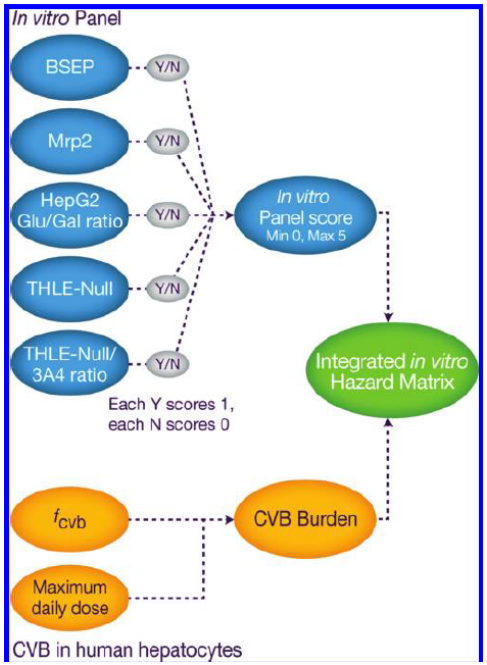
- **Aleo et al., 2010** (abstract):
  - 18 cpds tested in ZF: overall predictive value: 85%
    → *Correlation between grade of severity in ZF and Human*

Integration of multiple assays

- **<u>Thompson et al., 2012</u>:** AZ investigation

  - *Multiple in vitro approaches* [cytotox in control THLE, 3A4-THLE, HepG2 glu/gal, inhibition of human bile salt export pump (BSEP) and Multiresistance protein (Mrp2)] + *covalent binding (CVB*) in human hep to assess Idiosyncratic Adverse Drug Reactions (IADRs)

  - 36 cpds tested: sensitivity: 100% (n= 27 severe + marked IADRs concern)
    specificity: 78% (n=9 low IADRs concern)



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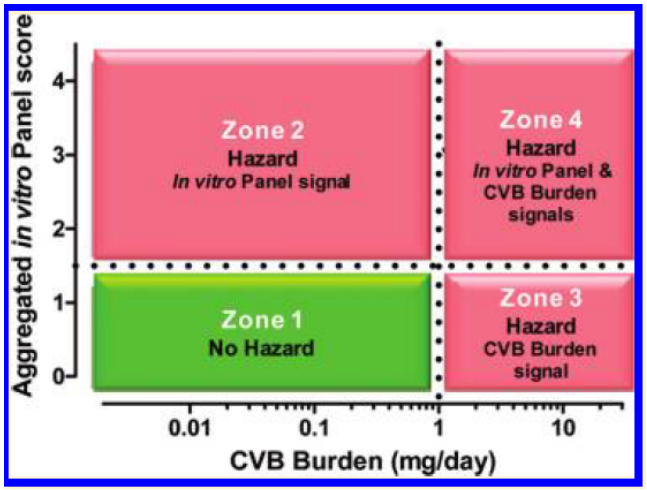


Liver Toxicity Knowledge Base (LTKB)

Chen et al., 2011: FDA-approved drug labeling for the study of DILI

- Assessing the DILI potential of a drug is a challenge with no existing consensus methods
- FDA proposed a systematic classification scheme using FDA-approved drug labeling to assess the DILI potential of drugs: 287 drugs
- Classification is based on the drug labels: Keywords for text-mining
- DILI SCORE: 0: No DILI concern (not hepatotoxic), 1-6: less DILI concern, 7-8: most DILI concern, -1: withdrawn (no score)

TABLE 1

DILI severity categories based on the DILI descriptions in the drug labeling

Severity level	DILI category	Specification and keywords	Examples of labeling language
8	Fatal hepatotoxicity	Death; fatal liver failure; or needed liver transplantation	When used orally, ketoconazole has been associated with hepatic toxicity, including some fatalities
7	Acute liver failure	Liver/hepatic failure; fulminant hepatic necrosis	Elevations of liver aminotransferases (ALT, AST) and liver failure have been reported with Tracleer®
6	Liver necrosis	Histologically confirmed liver necrosis caused by drug	Rare instances of severe liver injury, including hepatic necrosis, have been reported in association with mexiletine treatment
5	Jaundice	Jaundice (clinically apparent), if caused by drug-induced hepatocellular injury	There is a low incidence of altered liver function or jaundice in patients treated with Marplan®
4	Hyperbilirubinemia	Hyperbilirubinemia without visible jaundice, if not due to other causes like Gilbert syndrome or cholestasis	Ticlopidine therapy has been associated with elevations of alkaline phosphatase, bilirubin, and transaminases, which generally occurred within one to four months of therapy initiation
3	Liver aminotransferases increase	Liver aminotransferases increase (e.g. ALT, AST, transaminase, aminotransferase); abnormal liver/hepatic function test; liver/hepatic injury	Persistent increases (to more than three times the upper limit of normal) in serum transaminases have occurred in ~1% of patients who received simvastatin in clinical studies
2	Cholestasis; steatohepatitis	Steatohepatitis, if probably caused by the drug; cholestasis, cholestatic hepatitis if caused by the drug; liver/hepatic damage/disorder/impairment/toxicity/reaction/hepatitis; hepatopathy	Jaundice of the cholestatic type of hepatitis or liver damage has been reported (in patients receiving trifluoperazine)
1	Steatosis	Steatosis; fatty liver; liver/hepatic steatosis	Lactic acidosis and severe hepatomegaly with steatosis have been reported with the use of nucleoside analogs including zidovudine

Can we compare the different studies?

- **Strictly speaking the answer is NO due to many variables**:
  - Compound classification
  - Fixed concentration versus Cmax and cut offs
  - Endpoints (ATP, GSH, HC, RM, mitochondrial toxicity, …)
  - Presence or absence of serum (protein binding, free concentration)
  - Exposure (1 day versus 5/9 days)
  - Compound purity
  - Source of cells (ECACC vs ATCC)
  - Number of passages, …

→ **Standardization required**: e.g. cpd classification (LTKB Chen et al., 2011),

Cmax recommended (ref cpds), …

4) UCB investigations

4.1) Evaluation of phospholipidosis (Atienzar et al., 2007; Tilmant et al., 2011)

4.2) Characterization of primary Human hepatocytes, HepG2 and HepaRG cells and hepatotoxicity prediction (Gerets et al., 2012)

4.3) ZF investigations with 4 UCB proprietary cpds

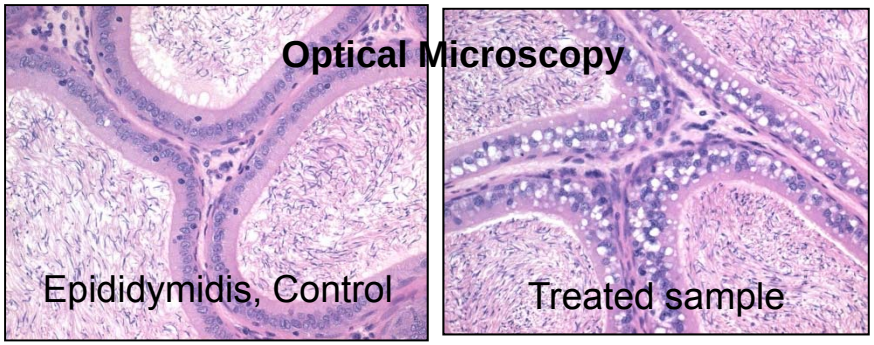
4.4) Hepatotoxicity prediction with different models

→ Up to 58 drugs tested

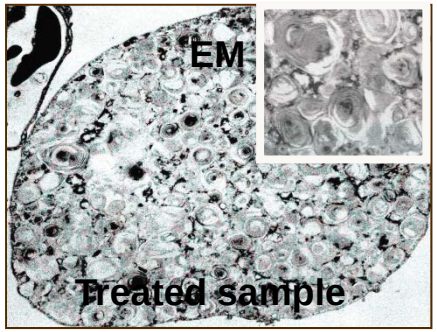
→ Models: HepG2 (glu/gal)
Primary hepatocytes (rat/Human)
HepaRG (Human)
Dog co-culture

4.1) Phospholipidosis: background information

- Phospholipidosis (PLD): accumulation of phospholipids in lysosomes and concurrent development of concentric lamellar bodies
- Generally induced by Cationic Amphiphilic Drugs
- PLD is considered as an adverse effect (Reasor et al., 2006)
- In tox studies: vacuolation (histopathology) suggests PLD
  confirmation obtained by electron microscopy (EM)



ucb-cpd1 – 4-week rat study



ucb-cpd1

- Need for in vitro approach and early biomarkers of PLD:
  - Fluorescent probes: flow cytometry/fluorescence microscopy
  - Toxicogenomic approach (Sawada et al., 2005)

4.1) Validation study: transcriptomics screen

- HepG2 exposed to cpds known to induce PLD
- Measurement of 17 genes biomarkers of PLD (Sawada et al., 2005)
- Selection of the best 11 biomarkers (Atienzar et al., 2007):
- Test of 15 positive and 6 negative cpds

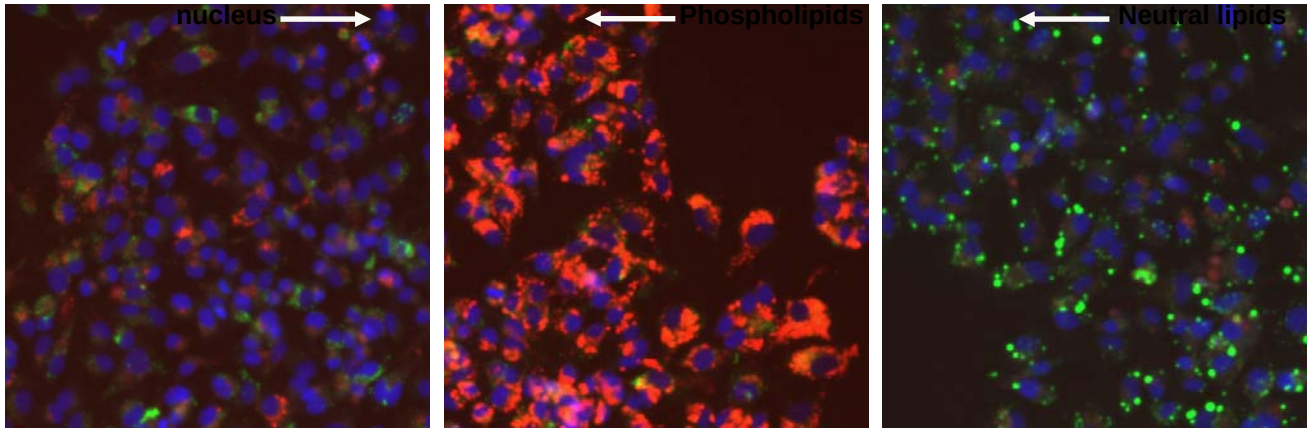
  Transcriptomic screen: sensitivity: 93.3% (14/15)

  specificity: 100% (6/6)

- Gene quantification: simplex → multiplex
- Main drawbacks: Time consuming, expensive

4.1) Replacement of the transcriptomic screen

- LipidTox assay: Quicker, more sensitive (Nioi et al., 2007)
  - Multiplexing: determination of PLD, lipidosis and mortality
  - 3 dyes bind to the targets: fluorescence detection (Cellomics)



Solvent Control | Amitriptyline (5 µM) | Cyclosporine A (40 µM)

Validation with reference cpds:

sensitivity: 100% (15/15)
specificity: 100% (6/6)

4.2) Human hepatocytes, HepG2 and HepaRG cells: characterization and hepatotoxicity predictivity

- **Gerets et al., 2012:** metabolism and cytotoxicity comparison in primary Human hepatocytes, HepG2 and HepaRG cells

Three models exposed to 16 human hepatotoxic compounds and 5 non human hepatotoxic drugs for 3 days using the xCELLigence platform.

→ HepG2 and HepaRG: experiments repeated 3 times
→ 3 donors used for primary Human hepatocytes

Specificity: 100 % for the 3 cellular models (Top concentration = 100 µM)

Sensitivity (if LC_{50}<10 µM, cpd classified as hepatotoxic)
30-50%: Human primary hep
12.5 %: HepaRG cells
6.3%: HepG2 cells

Conclusion: Primary Human Hepatocytes were the best models

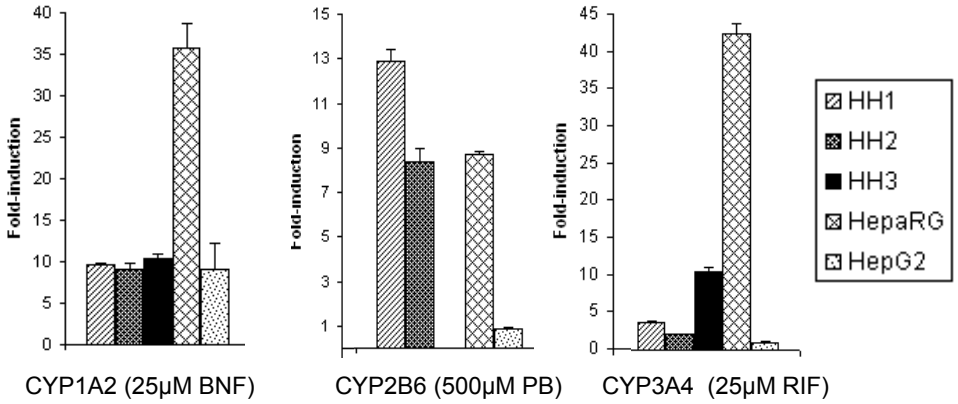
NB: sensitivity (Human Hep = 44-75% if LC_{50}<50 µM)

4.2) HepaRG (Biopredic/Life Technologies)

- Cells obtained from a liver tumor of a female patient suffering from hepatocarcinoma (mixture of hepatocytes and epithelial cells)
- Gerets et al., 2012: metabolism studies

  - *Toxicogenomics evaluation*: according to principal component analysis HepaRG are closer to 3 donors of Human hepatocytes compared to HepG2 cells

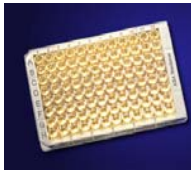
  - *Cyp450 activities*: HepaRG cells responded well to different inducers



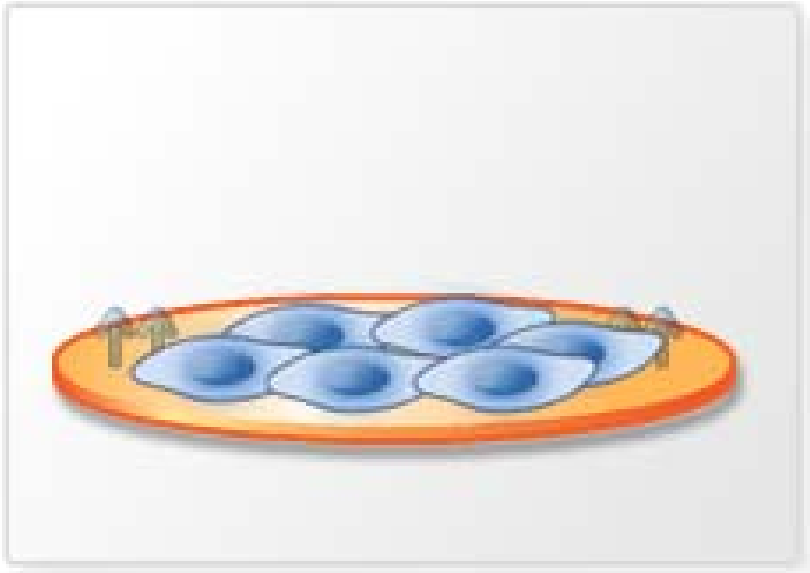
NB: HepaRG hepatotox pred. unknown with 'large' sets of cpds

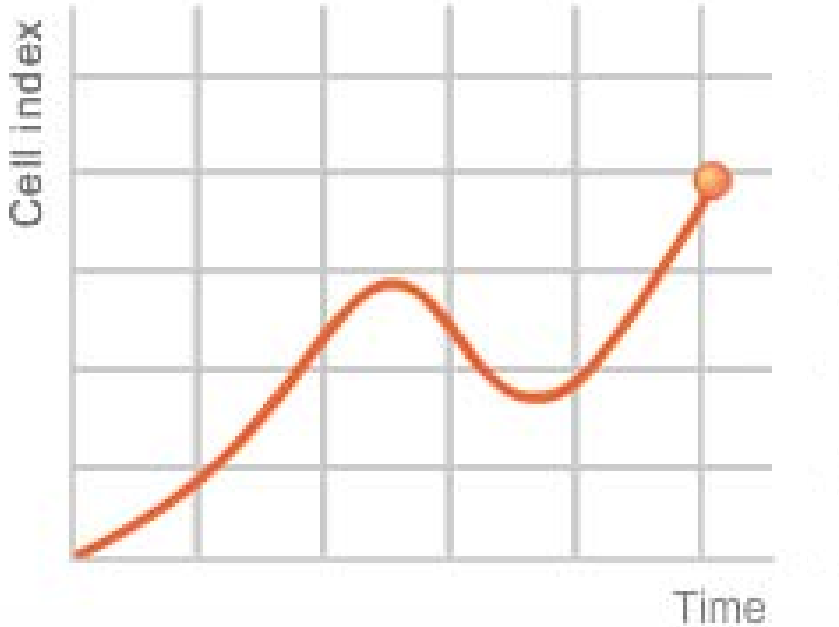
4.2) Endpoint: Real Time Cell Analyser (RTCA)

- Gerets et al., 2011: multiplexing for cytotox investigations (cell viability, caspase, LDH, ATP): main drawback: single point measurement
- Electronic impedance: - 6 x 96 well plate device, kinetic measurement
  - Monitor cellular events (e.g. cytotox)
  - Signatures: specific profiles obtained for antimitotic, DNA damaging, nuclear receptor modulator, protein synthesis inhibitor, calcium modulator, GPCR, …



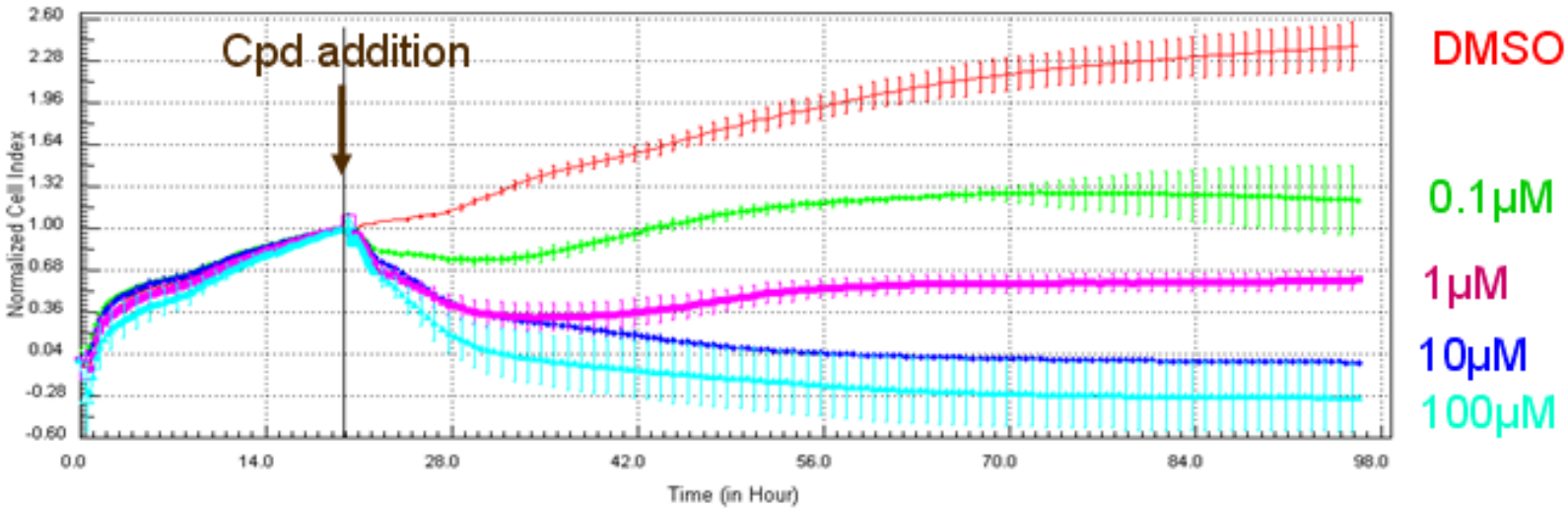
Atienzar et al., 2011; 2013: The use of RTCA in drug discovery





4.2) Example of cytotoxicity curves and correlation

Endpoints (RTCA): HepG2 cells exposed to Cerivastatin (0.1, 1, 10 and 100 µM)



Macro (Excel): automatic LC_{50} calculation at different time points

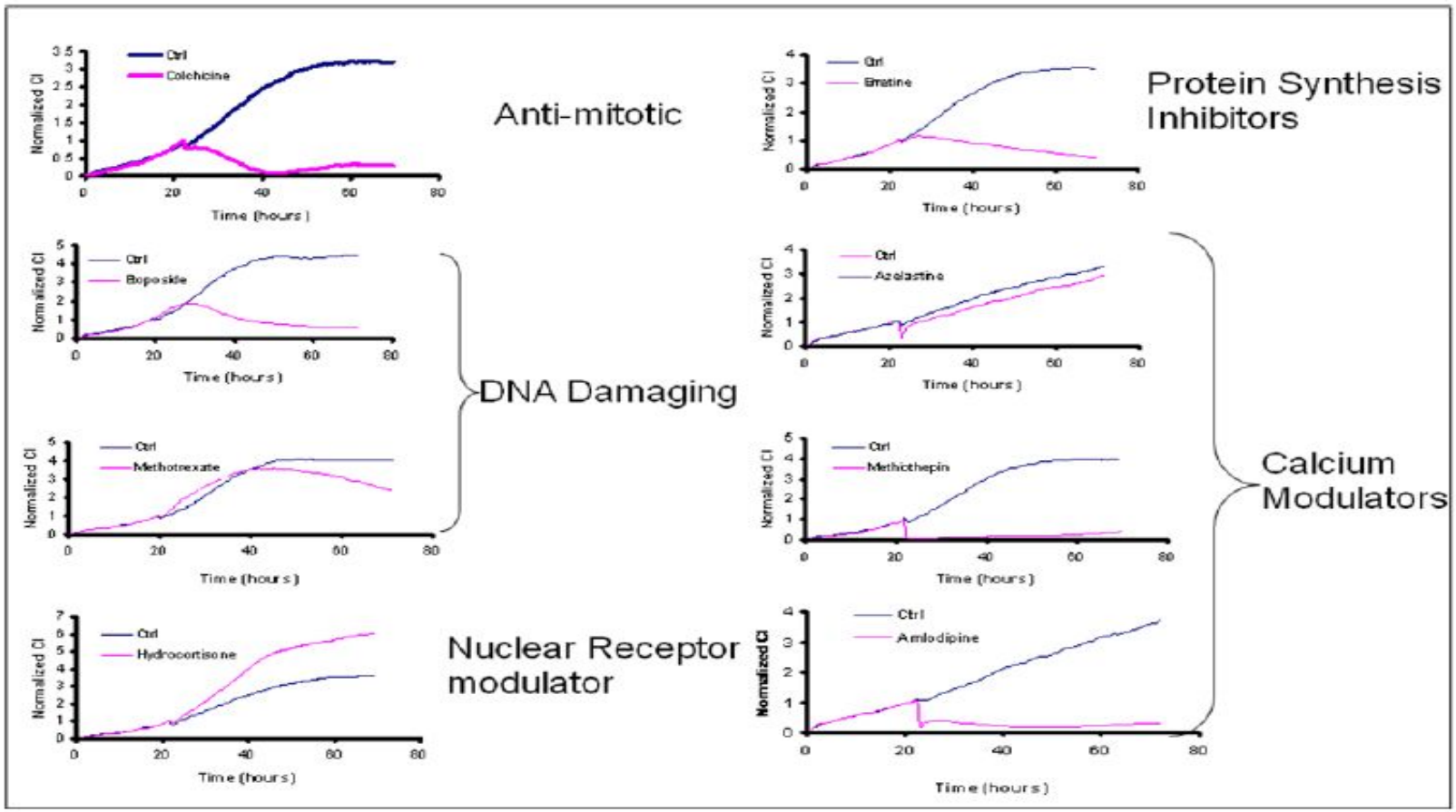
Correlation cell impedance versus classical toxicity endpoints?

- Limited number of studies in the literature (Atienzar et al., 2011)
- Coefficient of correlation (RTCA vs cell number measured by cell imaging) of 76 and 88% when HepG2 and HepaRG cells were exposed to a set of 21 cpds
- Coefficient of correlation between ATP and RTCA of 88.5% in HepG2 cells exposed to 50 compounds

4.2) RTCA signatures

→ Signatures are directly linked to mechanisms of actions

(Abassi et al., 2009; Atienzar et al., 2013)

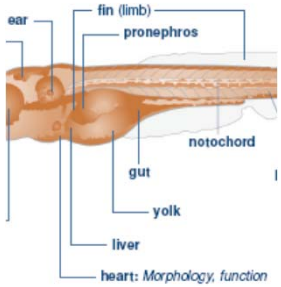


(Roche/ACEA data)

4.3) Comparison HepG2 / Rat / Dog / ZF

UCB study on 4 proprietary compounds: 16 larvae/concentration (2 replicates) exposed to 3, 10, 30, 100, 300 and 1000 µM for 48 h, endpoints of toxicity: liver degradation, changes in liver size, yolk retention and lethality, bioanalysis (LCMS-MS): measurements on well solution & in the larvae

Example of data with UCB4



Compounds	HepG2	Rat	Dog	ZF
UCB1		4 day study Hypertrophy (70 mg/kg) Coagulative necrosis (200 mg/kg)	No data	Liver abnormality Hepatomegaly Yolk retention 214 ng/larvae
UCB2		4 week study No AEL	4 week study porphyrin-like pigment deposits at 200 mg/kg	(low exposure: max 32 ng)
UCB3		2 week study centrilobular hepatocyte hypertrophy Minimal at 200 and 500 mg/kg	2 week dog study from 120mg/kg - Accumulation of lipids - Hepatocyte vacuolation - Hepatocyte degeneration	Yolk retention (low incidence) 81,6 ng
UCB4		4 day study Bile duct hyperplasia at 1000 mg/kg	4 day study Bile duct hyperplasia at 30 mg/kg	Liver abnormality Yolk retention 34,4 ng/larvae

Rat/Dog/ZF:

hepatotoxic

non hepatotoxic

HepG2:

LC50>100 µM

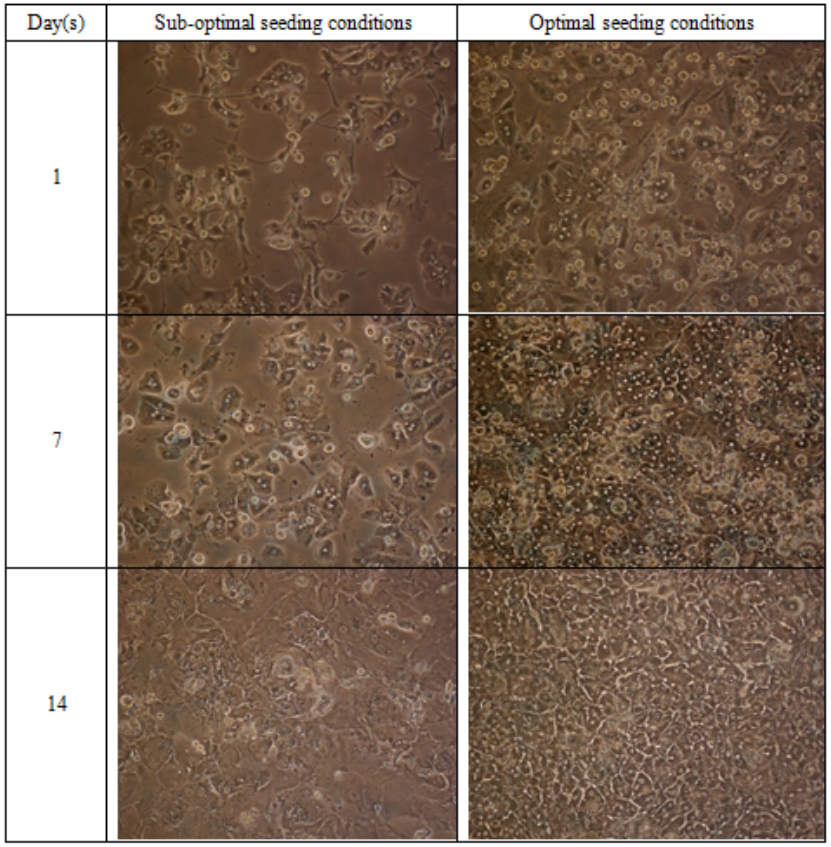
→ *Good correlation between ZF and rat data*

4.4) Hepatotoxicity prediction with different models

- Different cellular models exposed to Human-hepatotoxic and non-hepatotoxic drugs (most of the drugs are part of the LTKB, Chen et al., 2011)
  - Up to 58 drugs tested (depending on models)
  - Multiple of Cmax used (12.5, 25, 50 and 100 Cmax)
  - Cellular models: HepG2 (glu/gal), primary hepatoxytes (rat/Human), HepaRG as well as the dog co-culture model
  - 5 day exposure: drug added twice on day 0 and 2
  - Endpoints (cell models): impedance and more classical toxicity endpoints
  - Cut offs:100 Cmax

UCB/Hurel collaboration: Development of a dog co-culture model

- Hardly any publications available on dog hepatocytes
- Optimisation: cell morphology, co-culture ratio, medium composition, extracellular matrix for coating as well as phase I and II activities

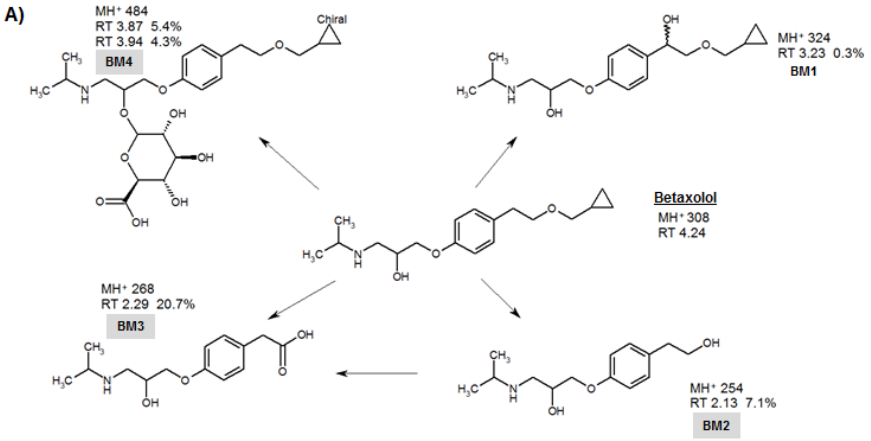


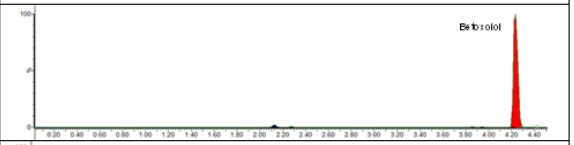
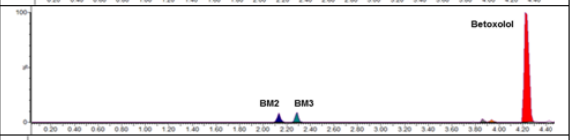
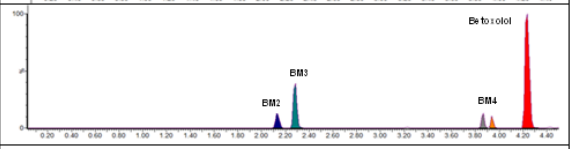
NB: Cell-cell connectivity and cell-surface interactions are maintained up to 21 days (unpublished data)

UCB/Hurel collaboration: Development of a dog co-culture model (Cont'd)

- Metabolic activities:
  - Gene expression phase I and II maintained after 2 weeks and even longer
  - Metabolic activities were also maintained after 2 weeks of culture
  - Example of metabolites identification: Betoxolol

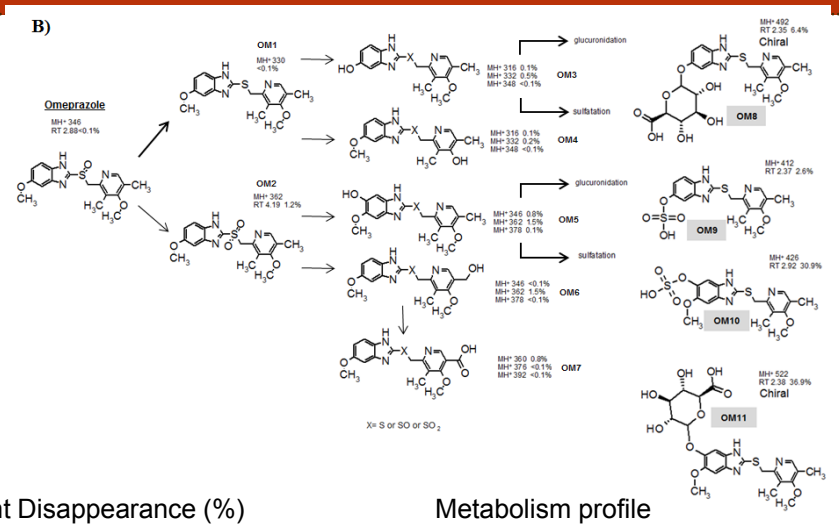
A)

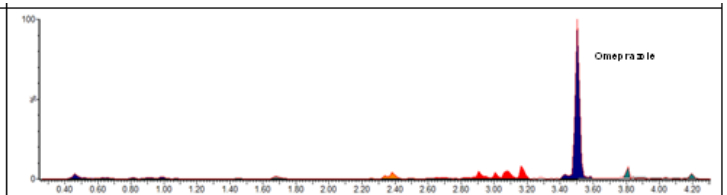
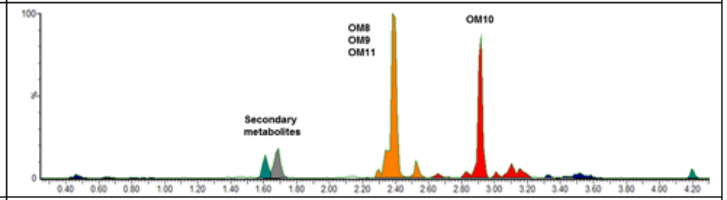
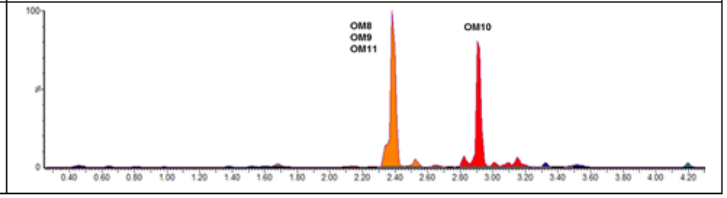


Drugs	Time points	Disappearance (%)	Metabolism profiles
Betoxolol	4h	3	
	2 d	14	
	7d	38	

UCB/Hurel collaboration: Development of a dog co-culture model (Cont'd)

- Example of metabolites identification: Omeprazole

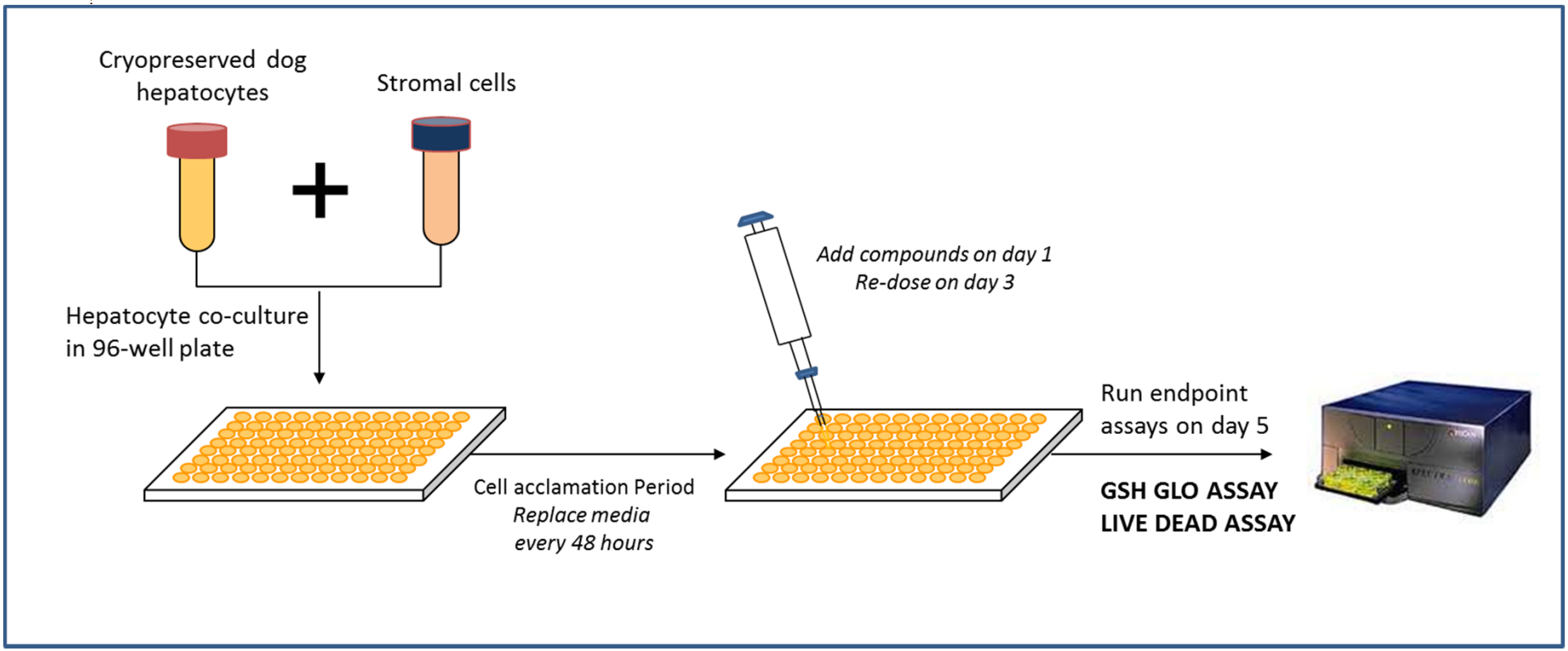


Drug	Time point	Disappearance (%)	Metabolism profile
Omeprazole	4h	19	
	2 d	99	
	7d	100	

→ Metabolites generated in vitro with the dog co culture model were also observed in dogs

UCB/Hurel collaboration: Development of a dog co-culture model (Cont'd)

- HμRELstatic™ dog hepatocyte co-culture model



Predictivity of the different models (100x C_{max})

		Sensitivity (n)	Specificity (n)
Primary cells:	Rat	**72 % (47)**	36 % (11)
	Human	**83 % (47)**	46 % (11)
Human Cell lines:	HepG2 (glu)	**78 % (46)**	36 % (11)
	HepG2 (gal)	**89 % (47)**	**73 % (11)**
	HepaRG	60 % (47)	**91 % (11)**
Hurel:	Dog	**78 % (40)**	**73 % (11)**

→ *Highest sensitivity:* *> 70 % all models (except HepaRG)*
→ *Highest specificity:* *HepaRG + HepG2 (Gal) + co-culture model (dog)*
→ *Best models:* *HepG2 (Gal) + dog co-culture model (Hurel)*

NB: Surprising gain in specificity with HepG2 gal (compared to HepG2 glu)
Results need to be confirmed

Take Home message

- Many recent and relevant investigations in the hepatotoxicity field but comparison is not always possible
- UCB studies:
  - Important to have access to different cellular models from different species (rat, dog, monkey, human) but more validations are required
  - Dog co-culture model is a promising model for chronic studies in metabolism and hepatotoxicity evaluations
  - Most models allowed to detect human hepatotoxic drugs with a reasonable sensitivity
  - An issue remains the low specificity particularly at 100 Cmax (except for the dog co-culture model, HepaRG and HepG2 (Gal)): data need to be confirmed with a higher number of non-hepatotoxic drugs (n=11)
  - Need to integrate different models to better evaluate hepatotoxicity (and other organ toxicities) as well as results from different disciplines (i.e. DMPK and pharmacology) for a better risk evaluation

→ Standardization required to better compare the different studies

Acknowledgements

In Vitro Tox Team (left to right): Stéphane Dhalluin, Sébastien Speeckaert, Helga Gerets, Gaëlle Toussaint, Karen Tilmant, Franck Atienzar

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Hurel (dog co-culture): Eric Novik (CTO)

Robert Freedman (CEO)