FUNCTIONAL HepaRG CELLS: A breakthrough in 3-R research ?

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eSI meeting –Alicante

17-18 October 2008

INTRODUCTION (1)

- Several hundreds chemicals are potentially hepatotoxic

- More than 50 % new chemical entities (« NCEs ») are stopped during their development due to pharmacokinetics or toxicity problems

- Around one-third hepatotoxic drugs in clinical trials are not found hepatotoxic in animals

- 6 out of 16 drugs withdrawn from the market between 1975 et 1999 in the US were withdrawn for hepatotoxicity

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Critical points:

- Differences in the routes and rates of drug metabolism between humans and animals

- Large interindividual variations iin humans

INTRODUCTION (2)

Obviously the liver is

- A target organ for drugs and other chemicals
- Plays a major role in bioactivation of chemicals

and i*n vitro* human liver models are desirable to mimic the human liver response

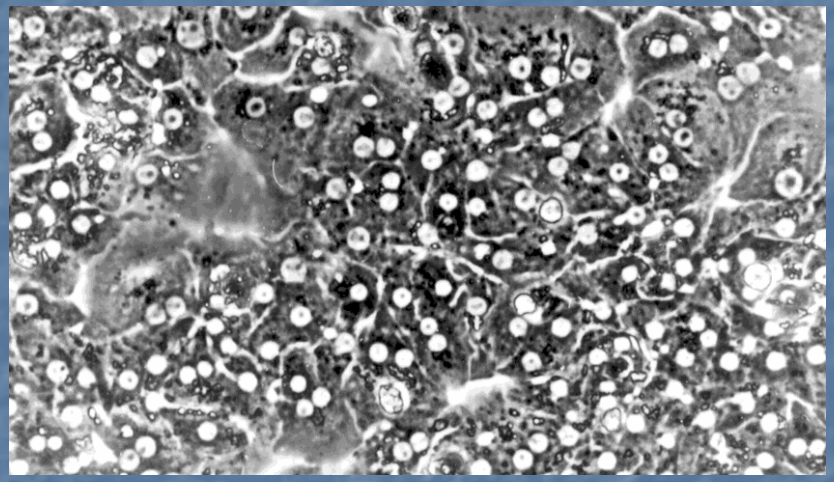
IN VITRO LIVER MODELS FOR **EVALUATION OF CHEMICALS**

- Precision-cut liver tissue slices
- Hepatocyte suspensions
- Primary hepatocyte cultures
- Liver cell lines
- Recombinant cell lines
- (Recombinant) microsomes
- Proteins (enzymes, receptors,…)

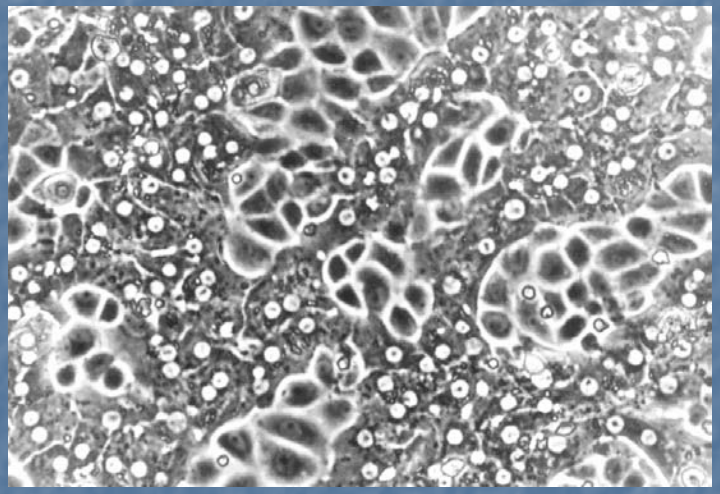
IN VITRO LIVER MODELS FOR **EVALUATION OF CHEMICAL TOXICITY**

- Tissue slices and primary hepatocyte cultures (limited availability, interdonor variability, early phenotypic changes, limited life-span …)
- Cell-based and reporter gene assays (Reporter gene assays for the major nuclear receptors: PXR, CAR,…)
- « Hepatocytes » derived from stem cells (only hepatoblasts can be obtained)
- Liver cell lines (low bioactivation activities, e.g.in HepG2 cells)
- The new human hepatoma cell line HepaRG

PRIMARY HUMAN HEPATOCYTES

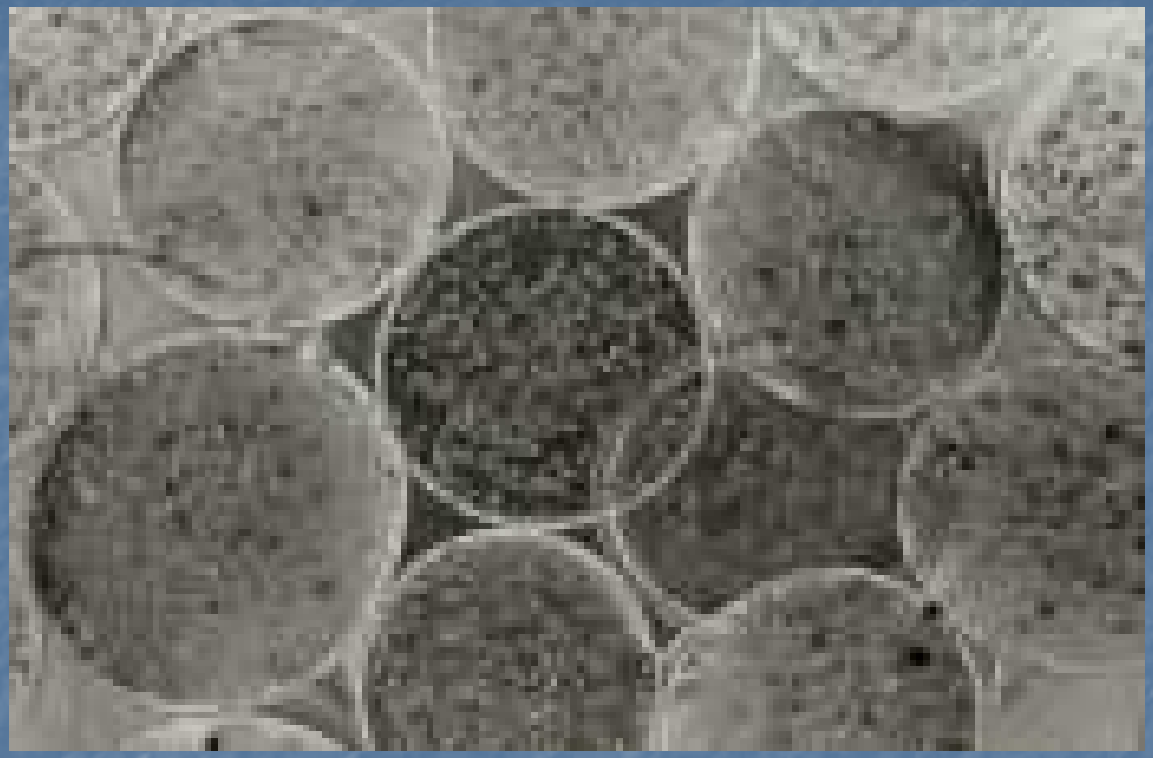


Primary human Hepatocyte culture



Primary human Hepatocyte Coculture with biliary cells (extended survival)

(limited availability, limited life-span, early phenotypic changes,…)



Entrapped Hepatocytes

Cell survival for several days

High viability after cryopreservation

APPLICATIONS OF HUMAN IN VITRO LIVER PREPARATIONS IN DRUG METABOLISM STUDIES

PARAMETER	SLICES	HEPATOCYTES CULTURED	MICROSOMES	RECOMBINANT ENZYMES
Metabolic profile	+	+	-	±
Comparative interspecies metabolism	+	+	-	±
Identification of metabolic pathways	±	±	±	+
Kinetic studies	±	+	+	?
Drug-drug interactions	±	+	+	±
Induction studies	±	+	-	-

Isolated organs not included because of the lack of availability ;

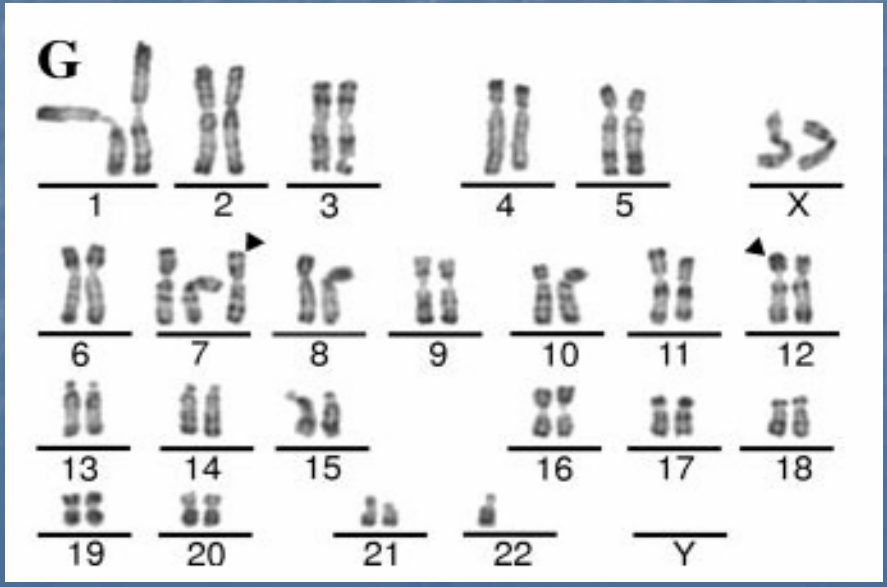
± possible, + currently used; - not suitable.

Acceptance by FDA for drug interactions studies (including cryopreserved hepatocytes

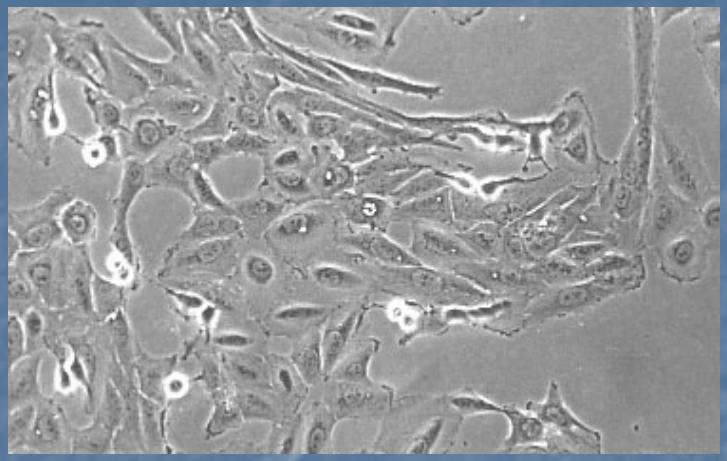
The HepaRG cell line

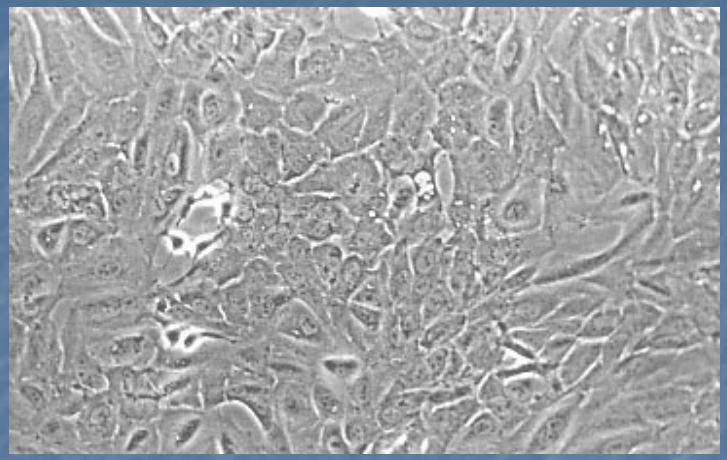
Derived from a differentiated human hepatocarcioma (Gripon,PNAS,2002)

karyotype: subnormal/subdiploide

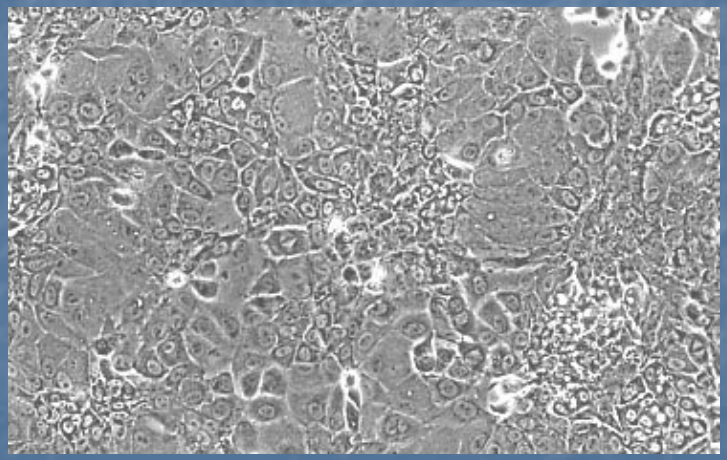


Morphology of HepaRG cells

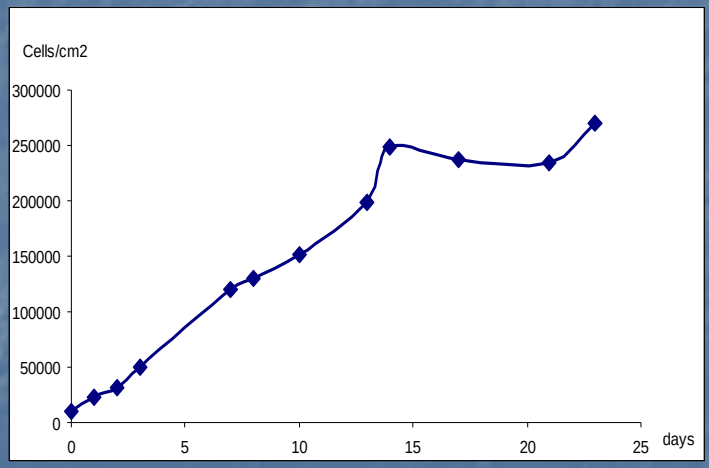




Proliferation



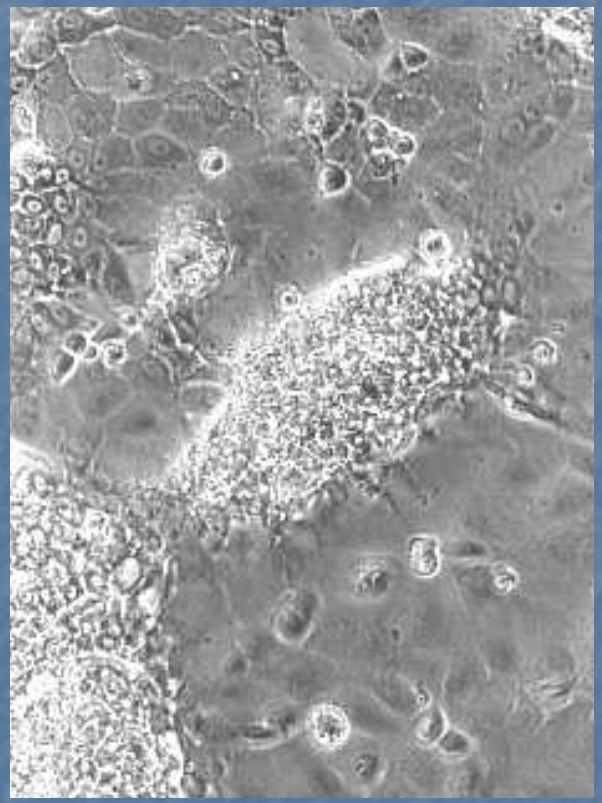
Confluence



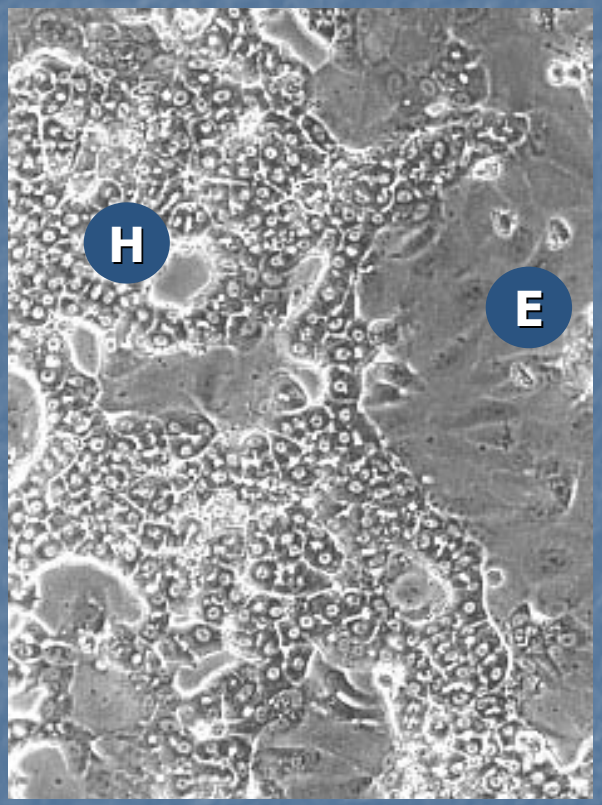
Growth curve

Morphology of HepaRG cells

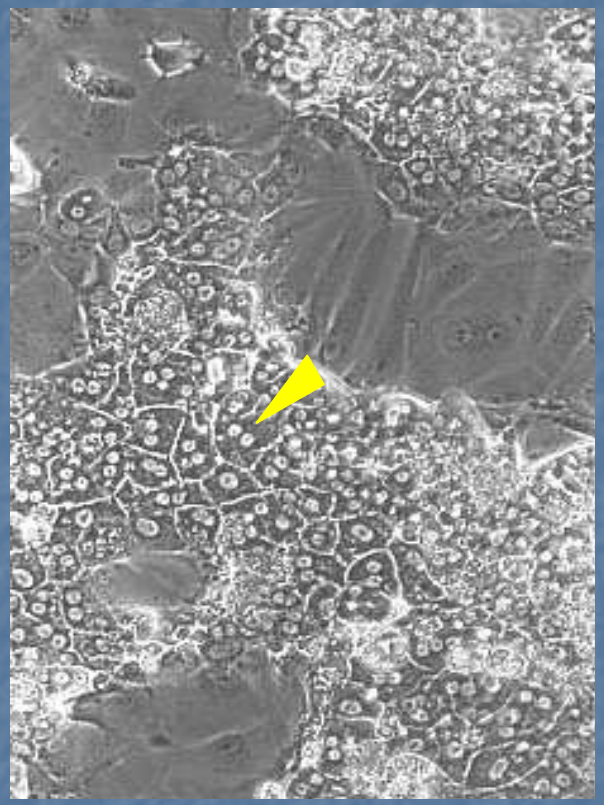
- DMSO



+ DMSO



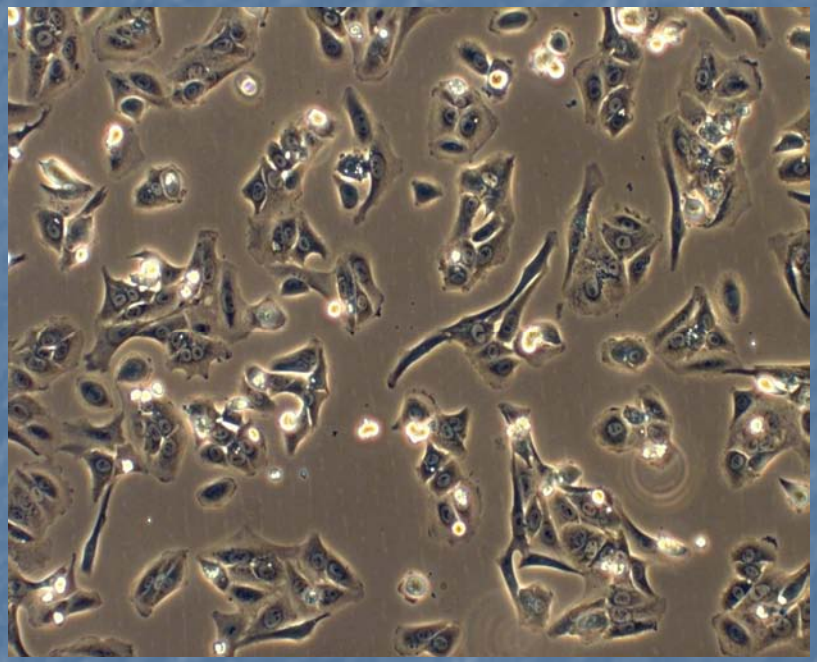
Long term
DMSO-treated culture



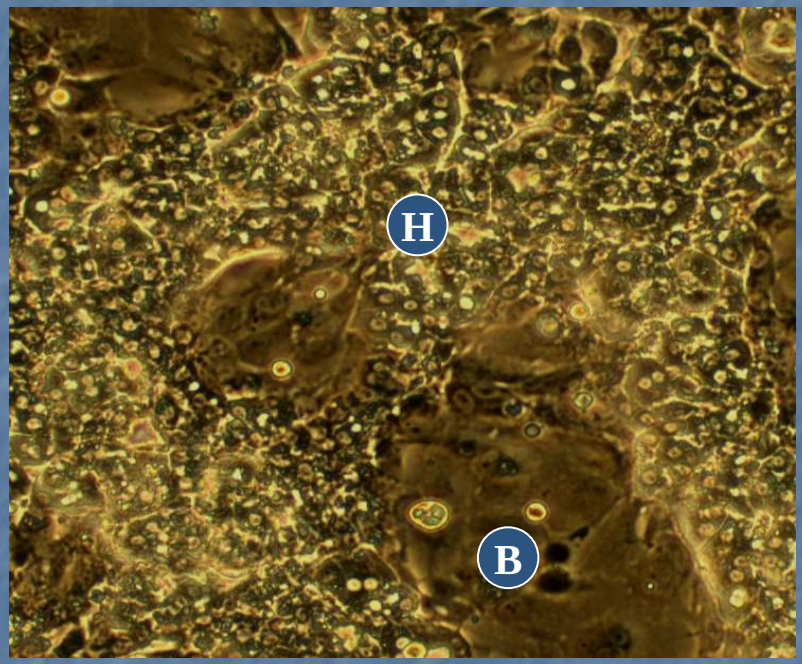
DMSO induces maximum morphological differentiation

Gripon et al. PNAS, 2002 , 99,15655

Differentiation and Transdifferentiation of
hepatocyte-like cells through bipotent progenitors



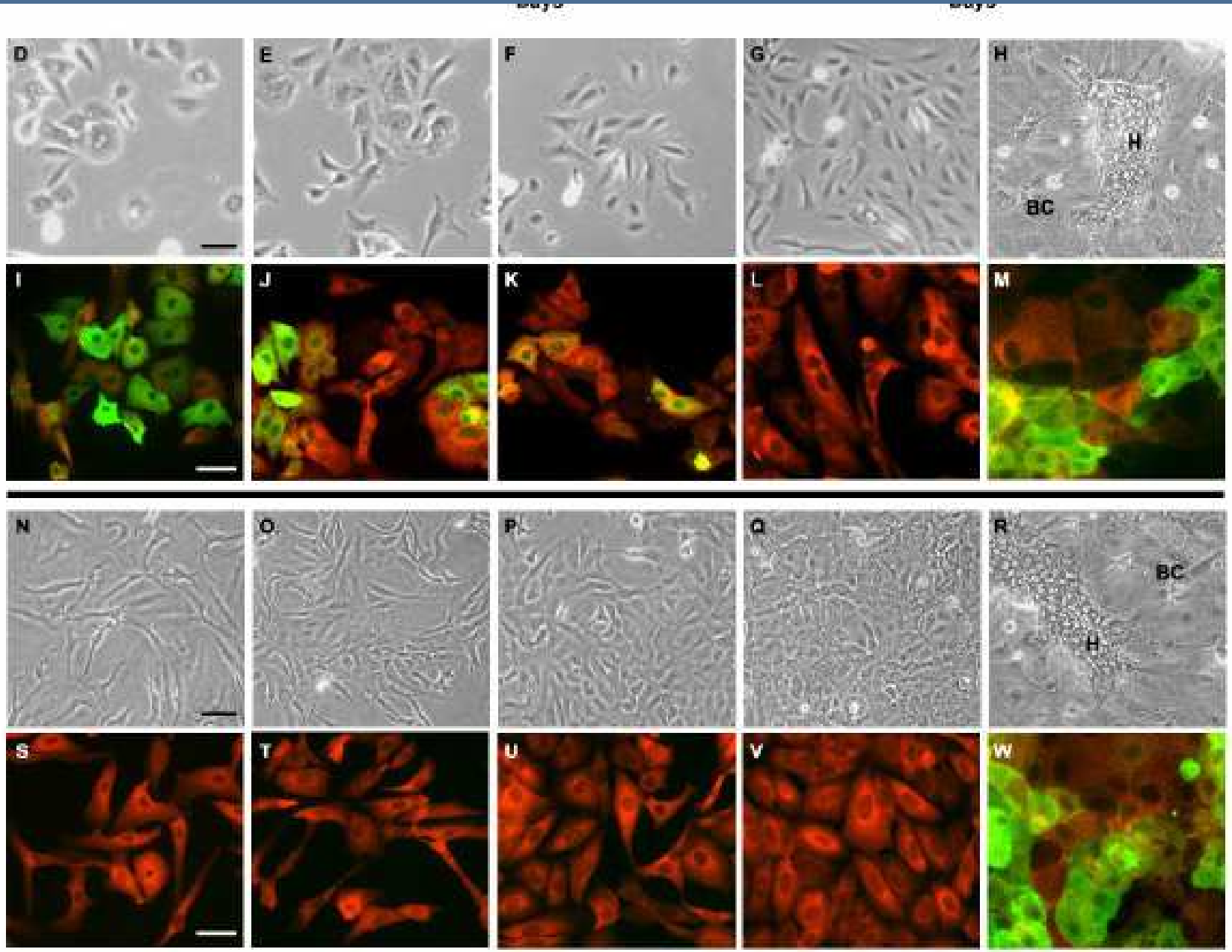
Bipotent Progenitors



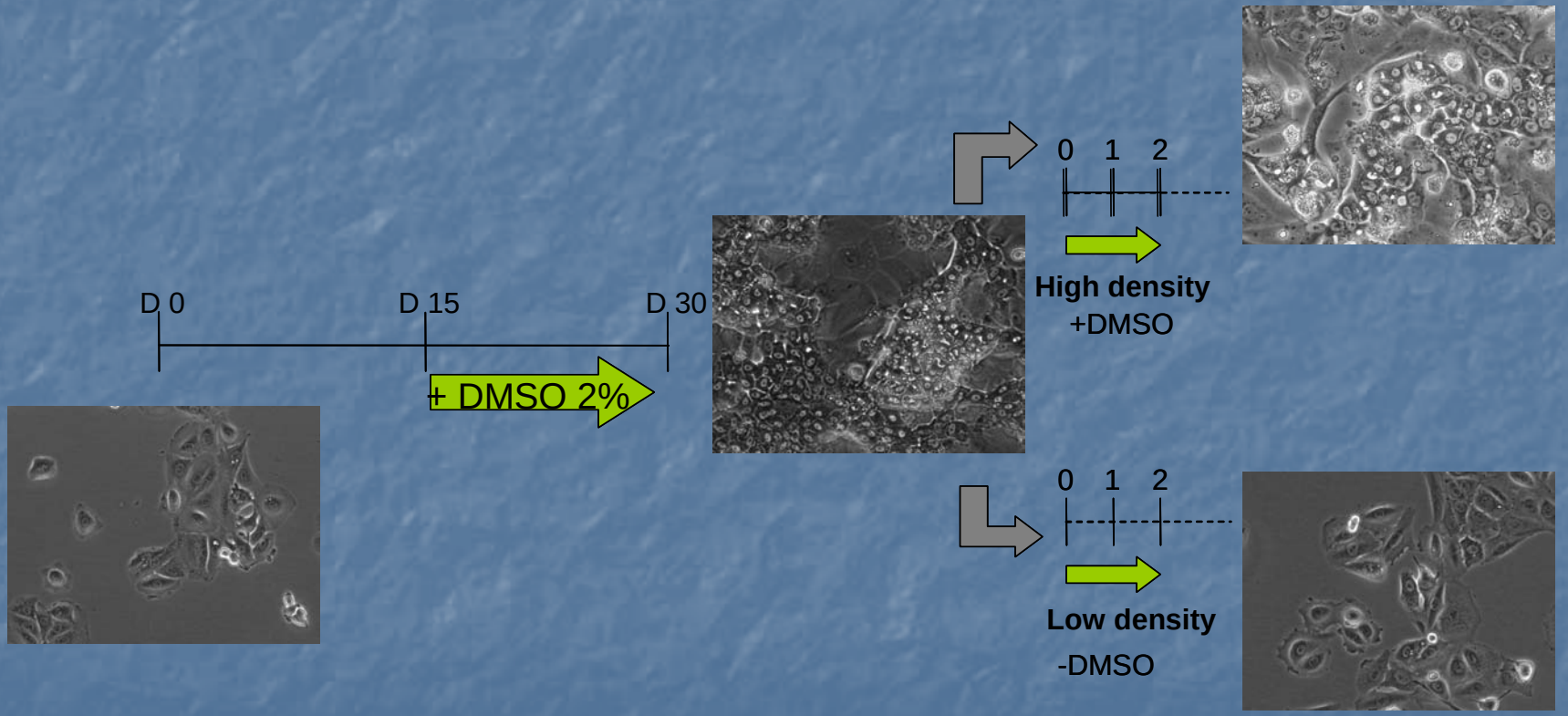
Hepatocyte-like cells (H)
Biliary epithelial-like cells (B)

Cerec,... **Guguen-Guillouzo**, **Corlu**, Hepatology, 2007,45, 957

HepaRG cells: Transdifferentiation of hepatocytes and biliary cells

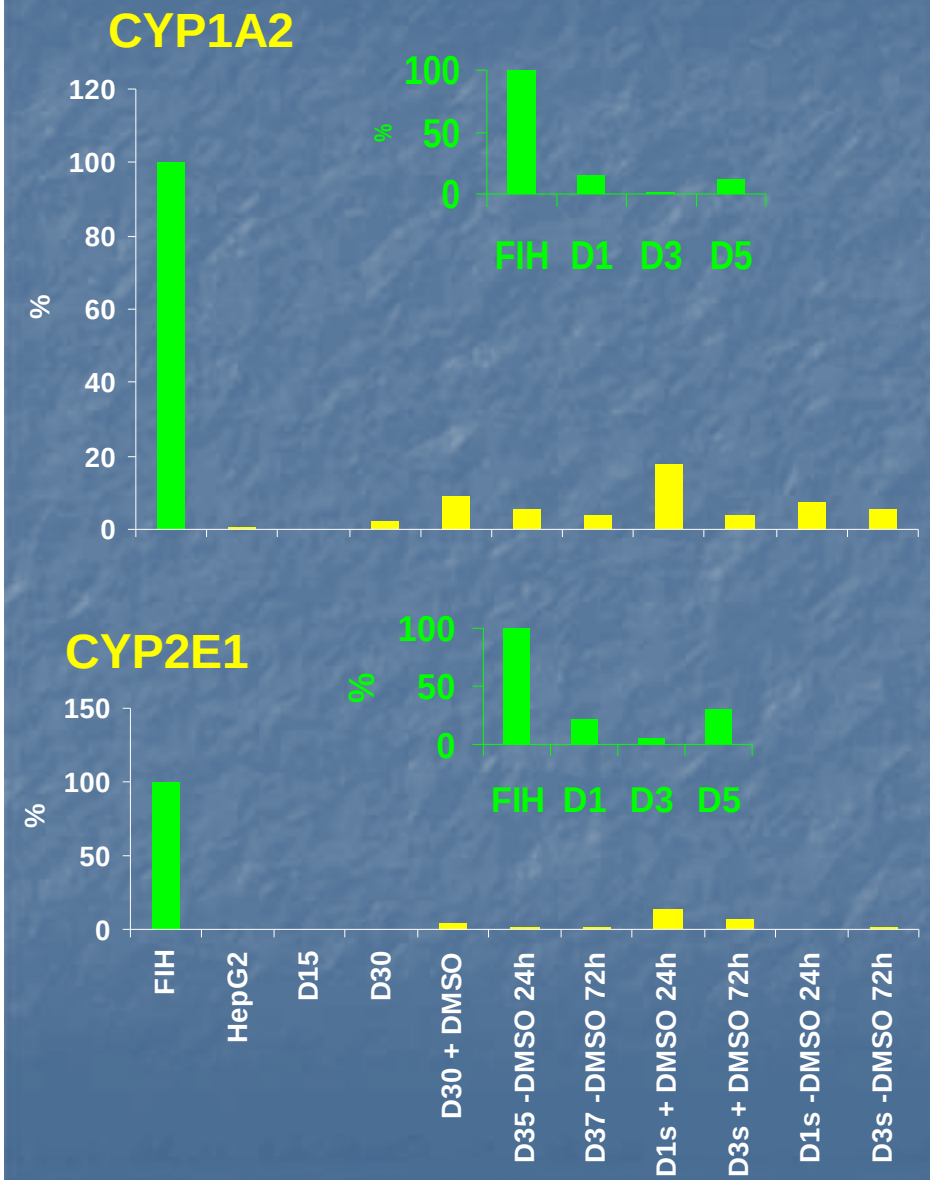


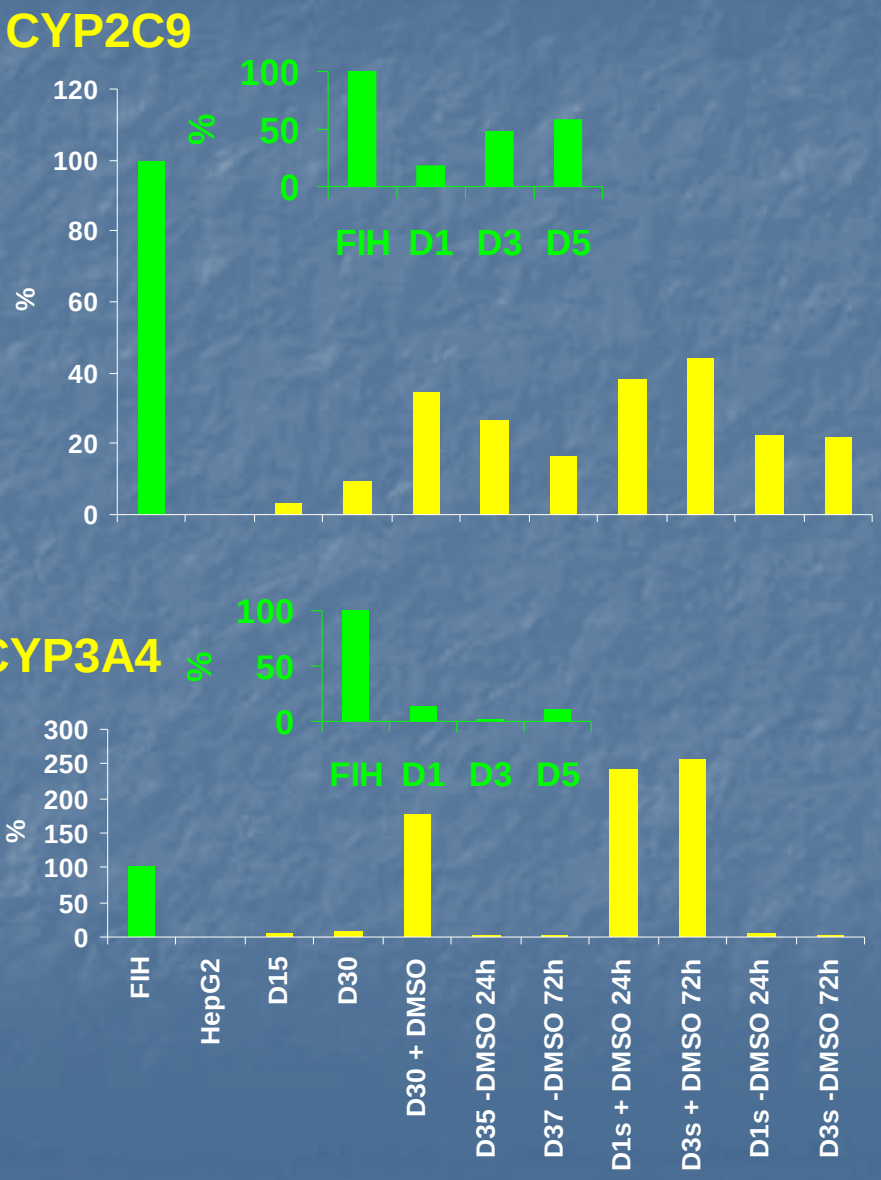
HepaRG cells after low and high density seeding



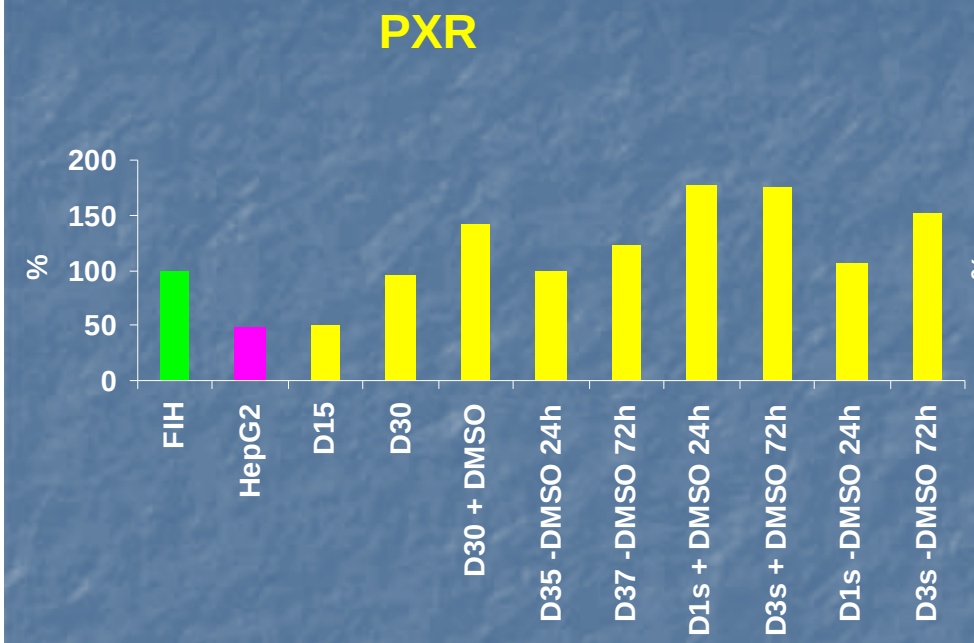
Aninat et al. DMD, 2006, 34, 75

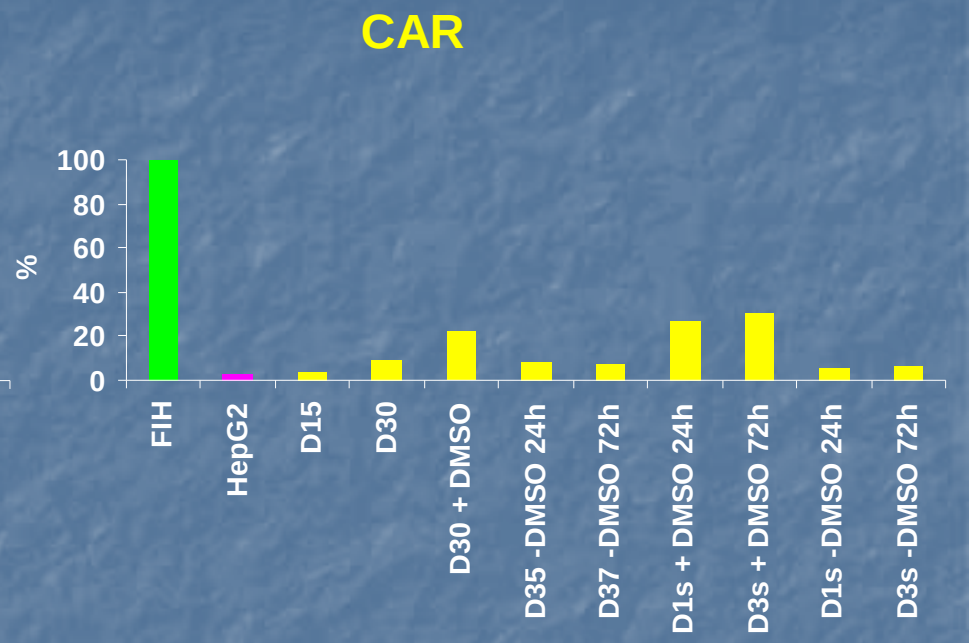
Phase I enzyme mRNAs

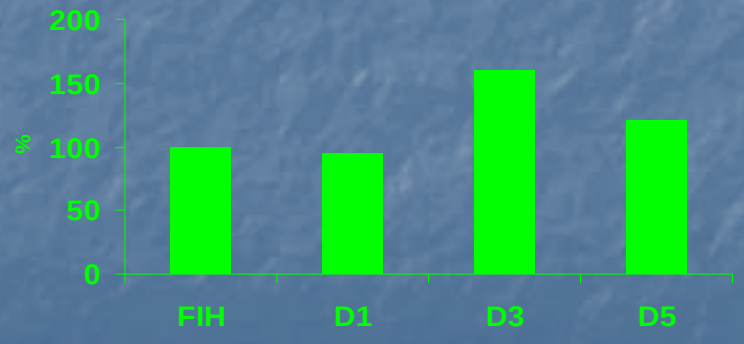




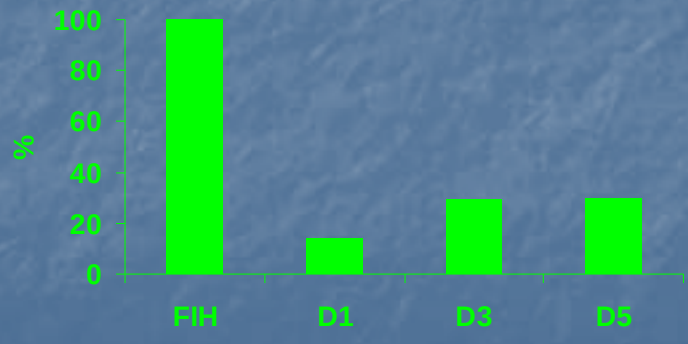
Nuclear receptor mRNAs







Human hepatocytes



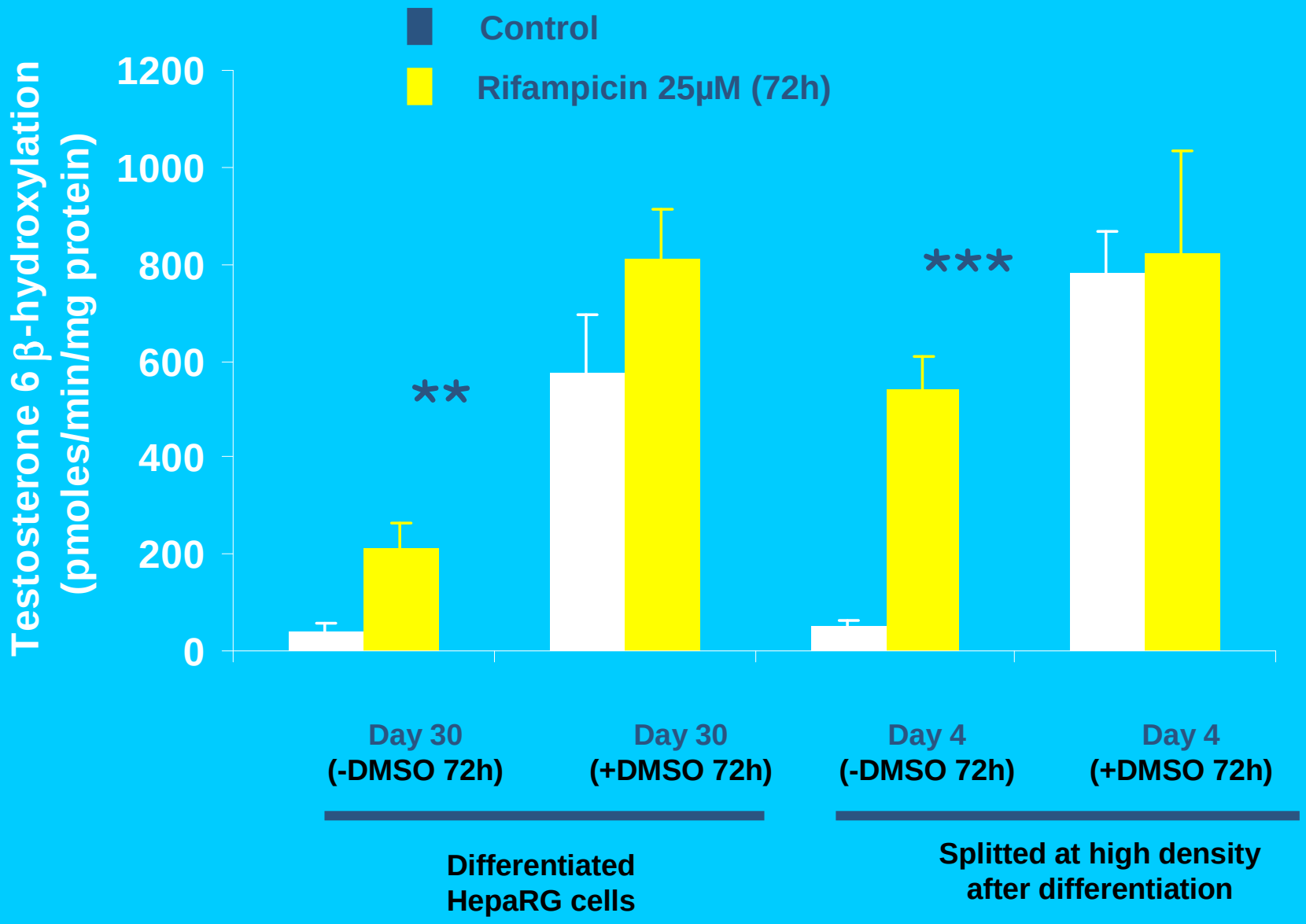
Human hepatocytes

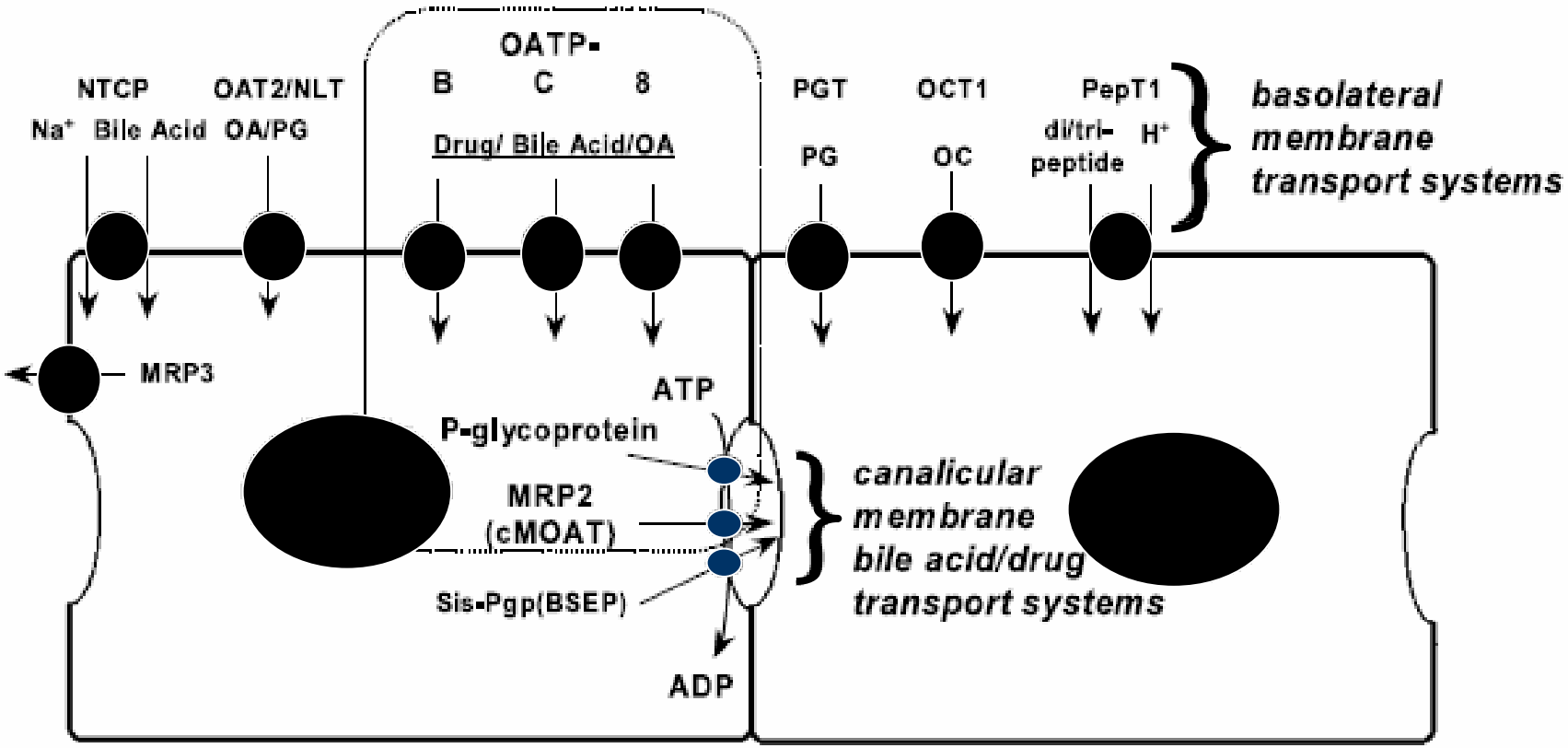
Aninat et al. DMD 2006,34, 75

mRNA levels (% of FIH values set at 100%)

	CYP2B6	CYP2C9	CYP3A4	CAR
Differentiated hepatocytes (high density seeding)				
+ DMSO 24 h	72.6	38.1	24.3	26.4
- DMSO 24 h	4.9	22.1	6.8	5.3
- DMSO 72 h	11.9	22	2.8	6.4

Testosterone 6β-hydroxylation (CYP3A4)



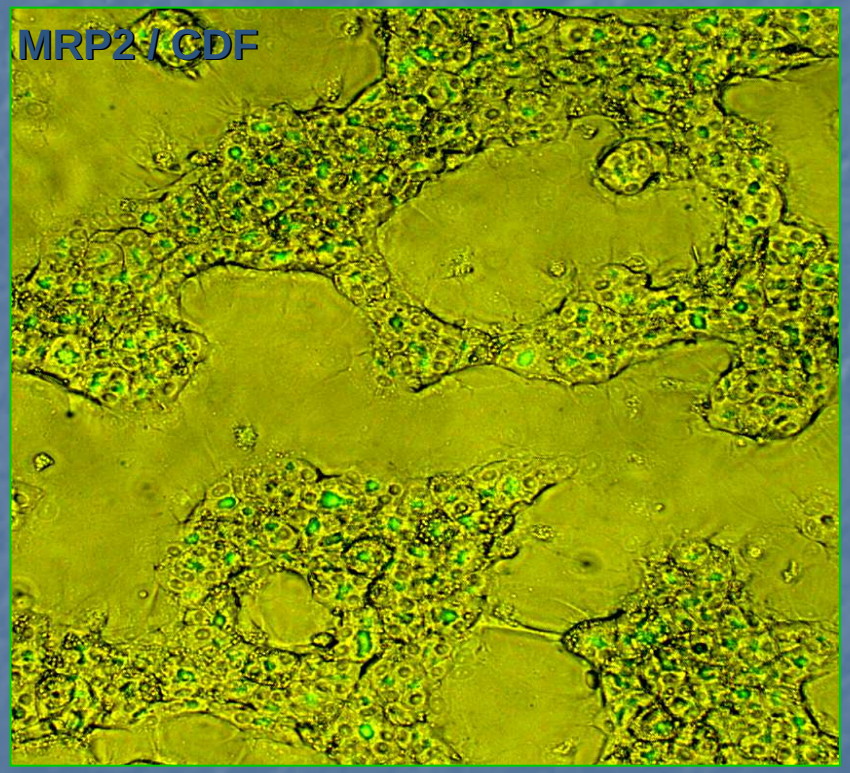


Plasma membrane transporters in HepaRG cells

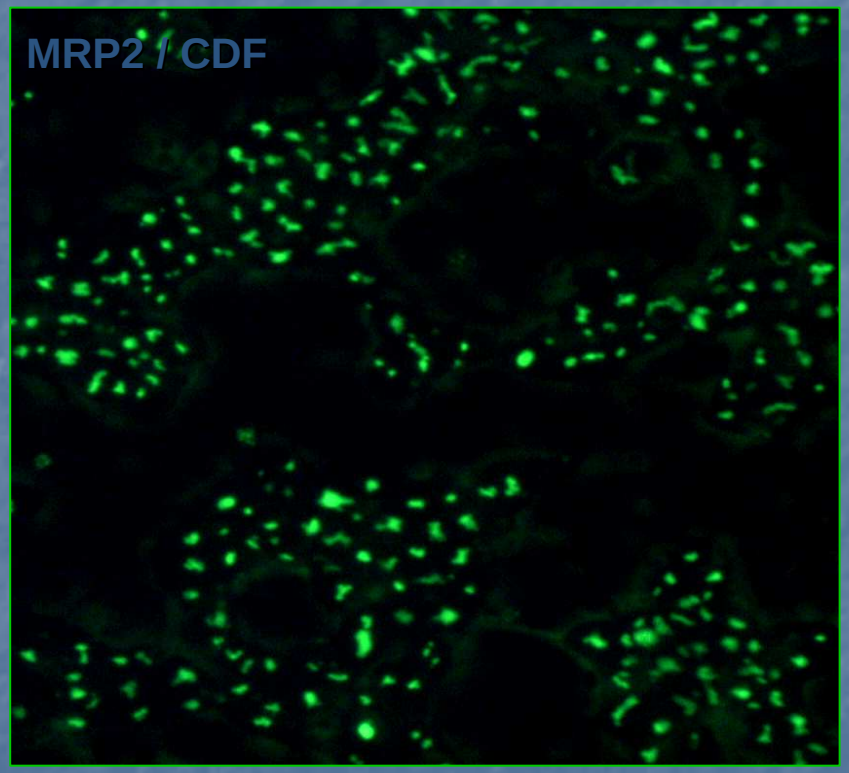
Organic anion (OA) and cation (OC) transporters of bile acids and xenobiotics on the basolateral membrane of hepatocytes and efflux canalicular transporters are expressed

Functional activity of efflux transporters in differentiated HepaRG cells

Phase-contrast/Fluorescent microscopy



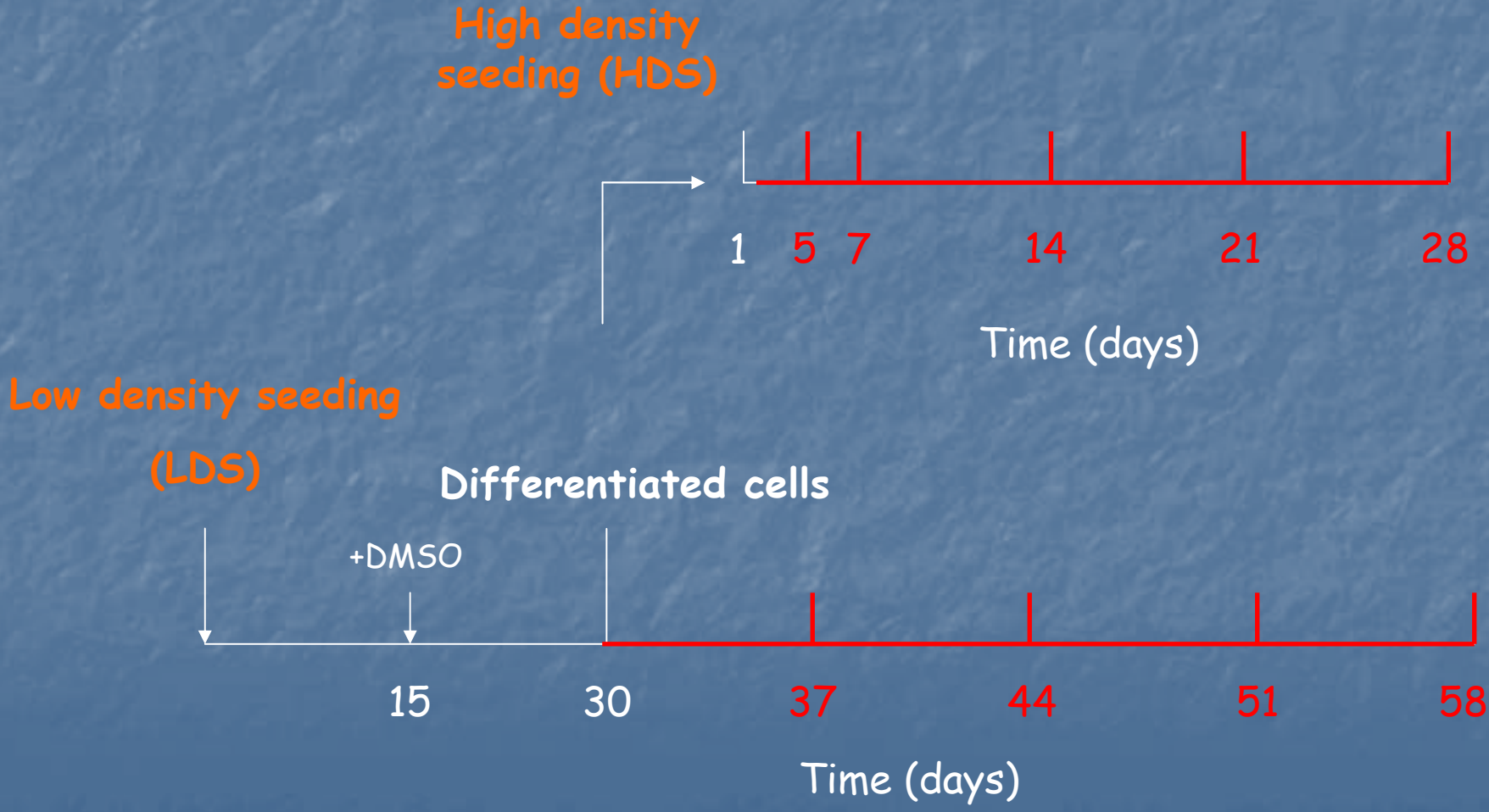
Fluorescence image



Fluorescent Substrate of MRP2:
Carboxy-dichloro-fluorescein diacetate *(CDF)*

Evaluation of long-term stability of human HepaRG hepatocytes

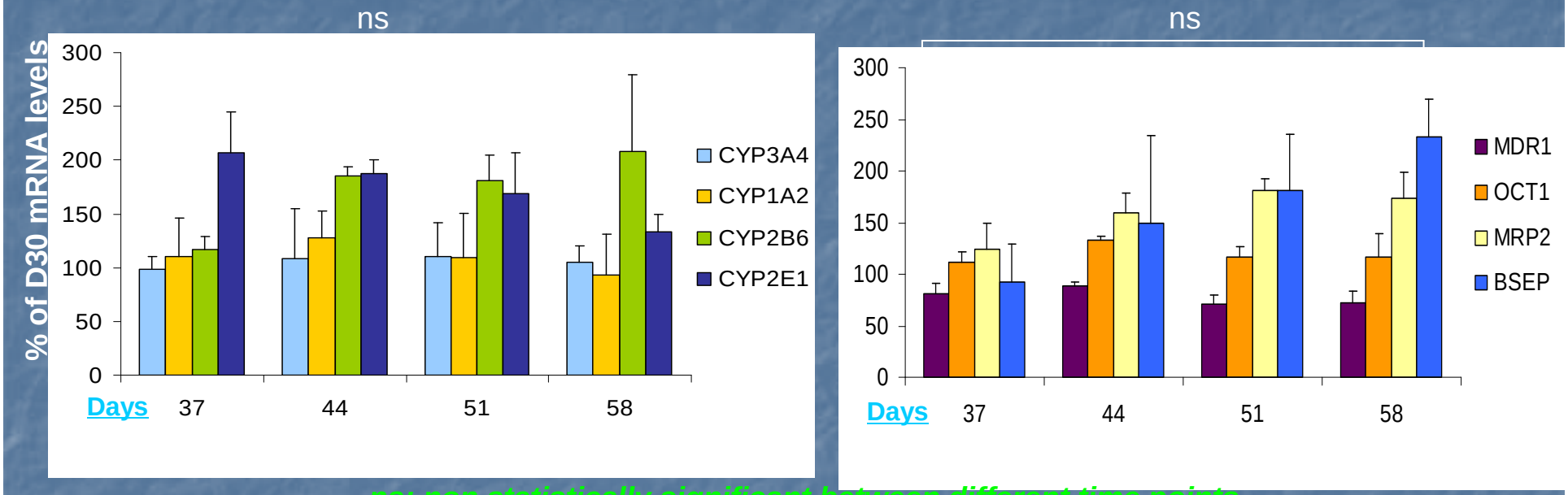
Experimental Design



Gene expression after seeding at **low density**

CYPs

Membrane transporters



ns: non statistically significant between different time points

Stable long-term expression of several liver transcripts:

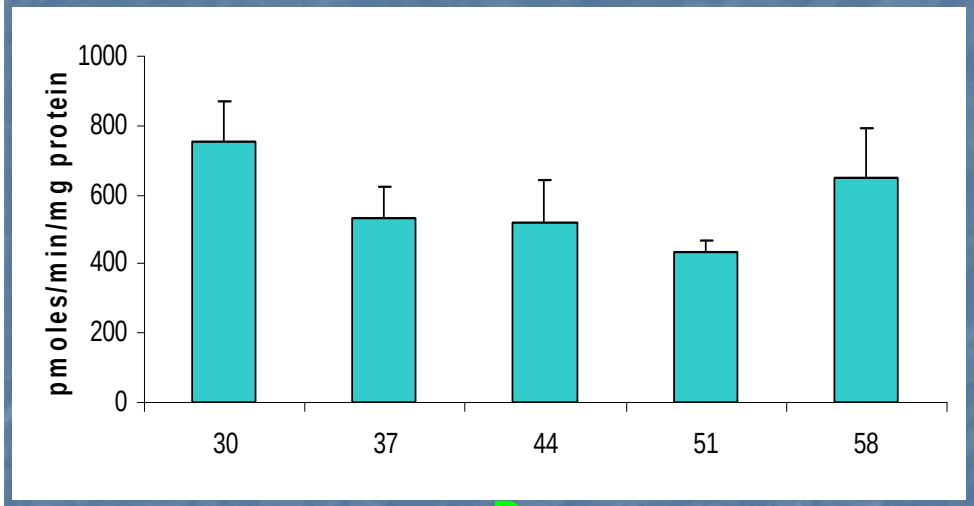
- **CYPs and membrane transporters**
- **phase II (GSTA1, UGT1A1, mEH)**
- **antioxidant enzymes (catalase, MnSOD)**
- **nuclear receptors (CAR, PXR)**

Stability also maintained when cells were seeded at high density.

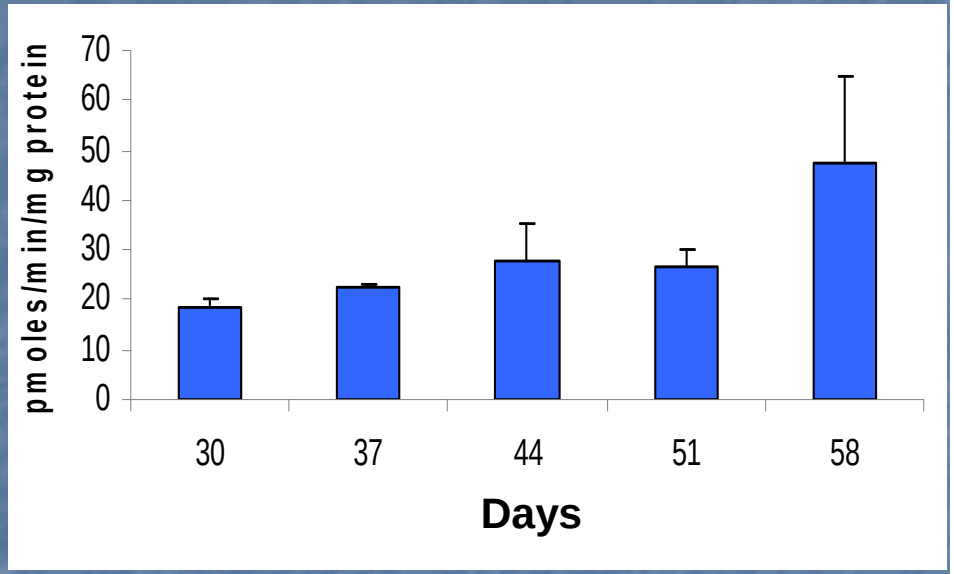
Jossé et al DMD 2008, 36, 1111

Cytochrome P450 enzyme activities of CYP3A4 and 1A2 (LD)

CYP3A4
6- β hydroxylation of testosterone



CYP1A2
phenacetin deethylation



- **Stable activities of CYP3A4 and 1A2 for at least 4 weeks.**
- **Stability also maintained when cells were seeded at high density.**
- **Comparable activity levels after either high and low density-seeded cultures.**
- **Responsiveness to inducers well maintained.**

Kanebratt and Andersson, DMD 2008, 36, 1444

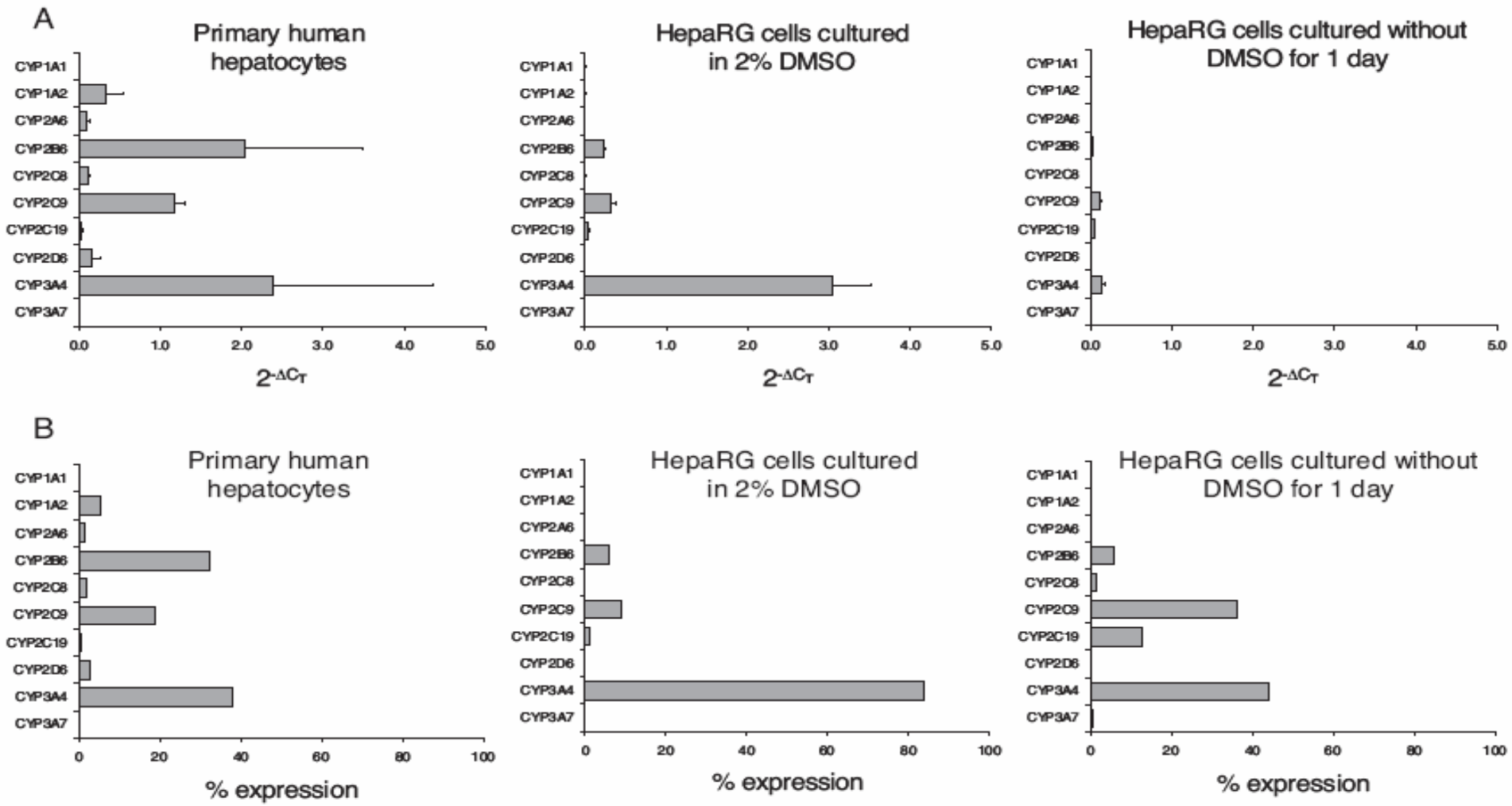


FIG. 3. mRNA expression levels of drug-metabolizing P450 calculated as $2^{-\Delta C_T}$ (A) and relative expression calculated as percentage of total expression of drug-metabolizing P450s (B) in primary human hepatocytes (batches 1, 4, and 5), differentiated HepaRG cells cultured with 2% DMSO, and differentiated HepaRG cells cultured without DMSO for 1 day. Results in A are mean + S.D., $n = 3$.

1A2 2B6 2C9 3A4

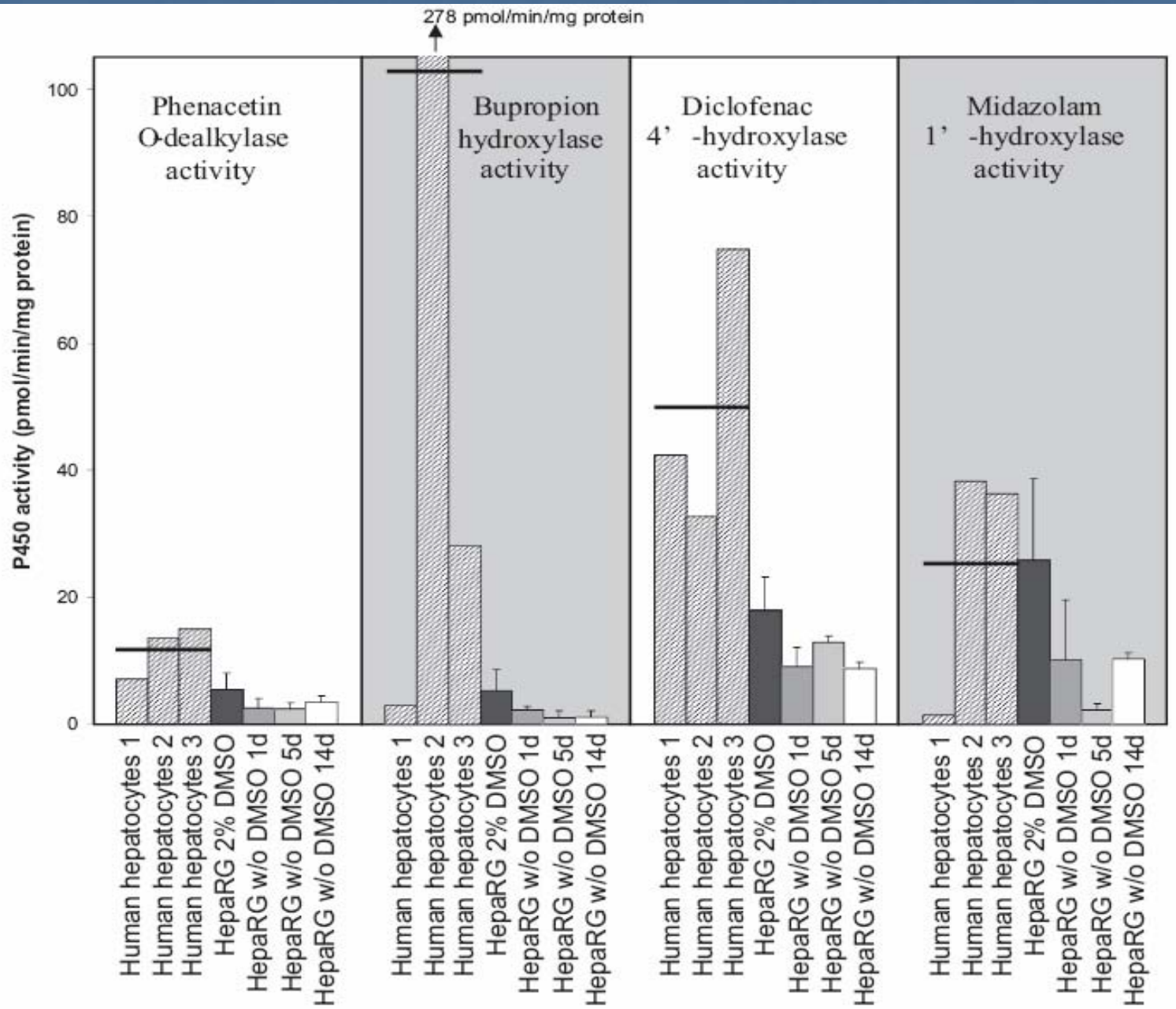


FIG. 5. P450-selective activities using phenacetin, bupropion, diclofenac, and midazolam in primary human hepatocytes (individual values of batches 1–3, average is marked by a line), differentiated HepaRG cells cultured with DMSO and without DMSO for 1, 5, or 14 days. Results for HepaRG cells are mean + S.D., $n = 6$.

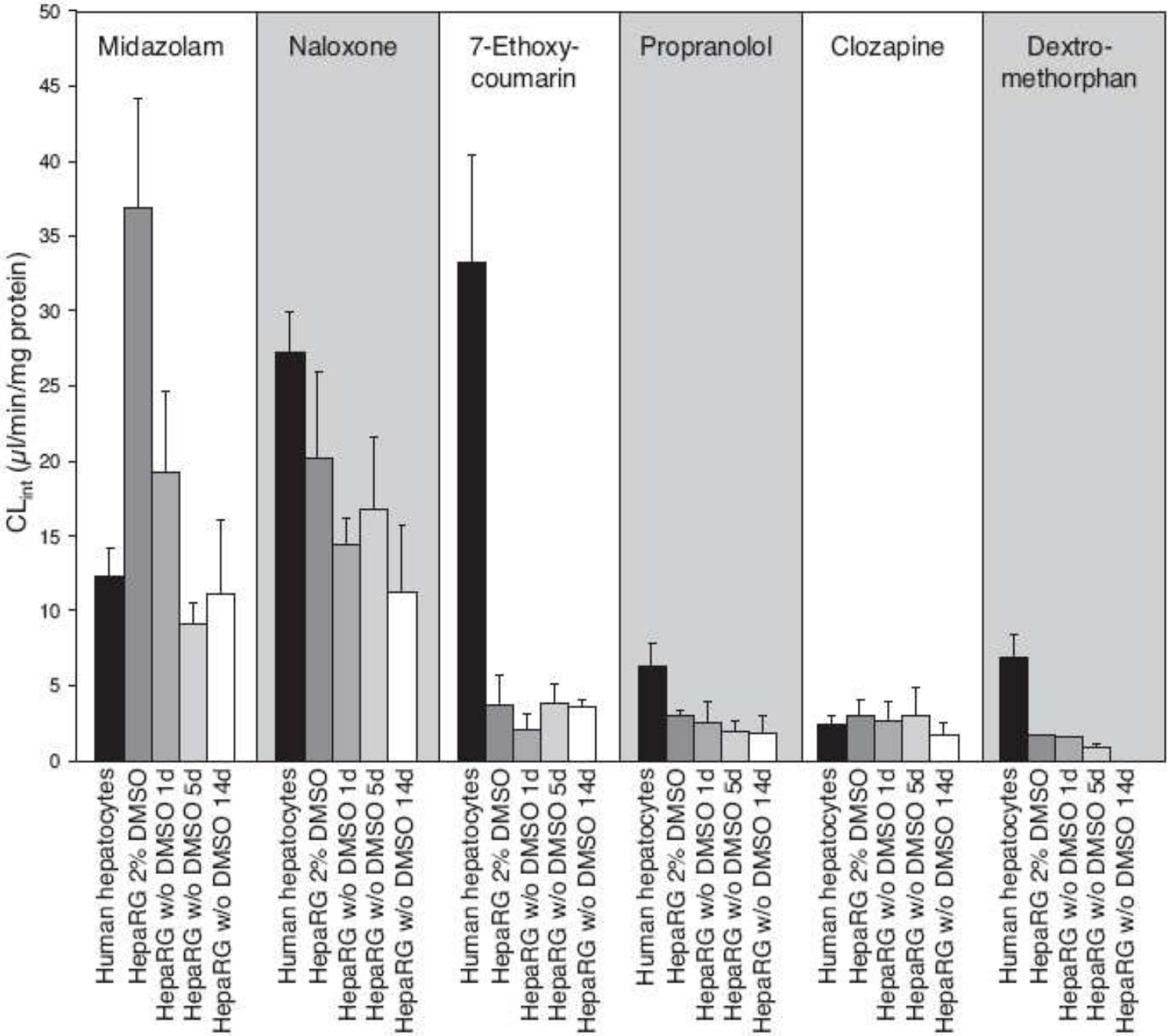


FIG. 4. Measurement of CL_{int} in primary human hepatocytes (batches 6–8) and differentiated HepaRG cells cultured with DMSO and without DMSO for 1, 5, or 14 days. Results are mean + S.D., human hepatocyte results are mean for three individuals, $n = 5$ for HepaRG results.

Correlation between in vitro and in vivo induction data

$$y = \frac{\%\ \text{decrease of } \textit{in vivo}\ \text{AUCmax} \cdot (\text{AUC}/F2)}{\%\ \text{decrease of } \textit{in vivo}\ \text{AUC50} + \text{AUC}/F2}$$

AUC : Area under the plasma concentration versus time curve

F2 Values: twofold increase of the baseline CYP levels

Kanebratt and Andersson, DMD 2008, 36, 137

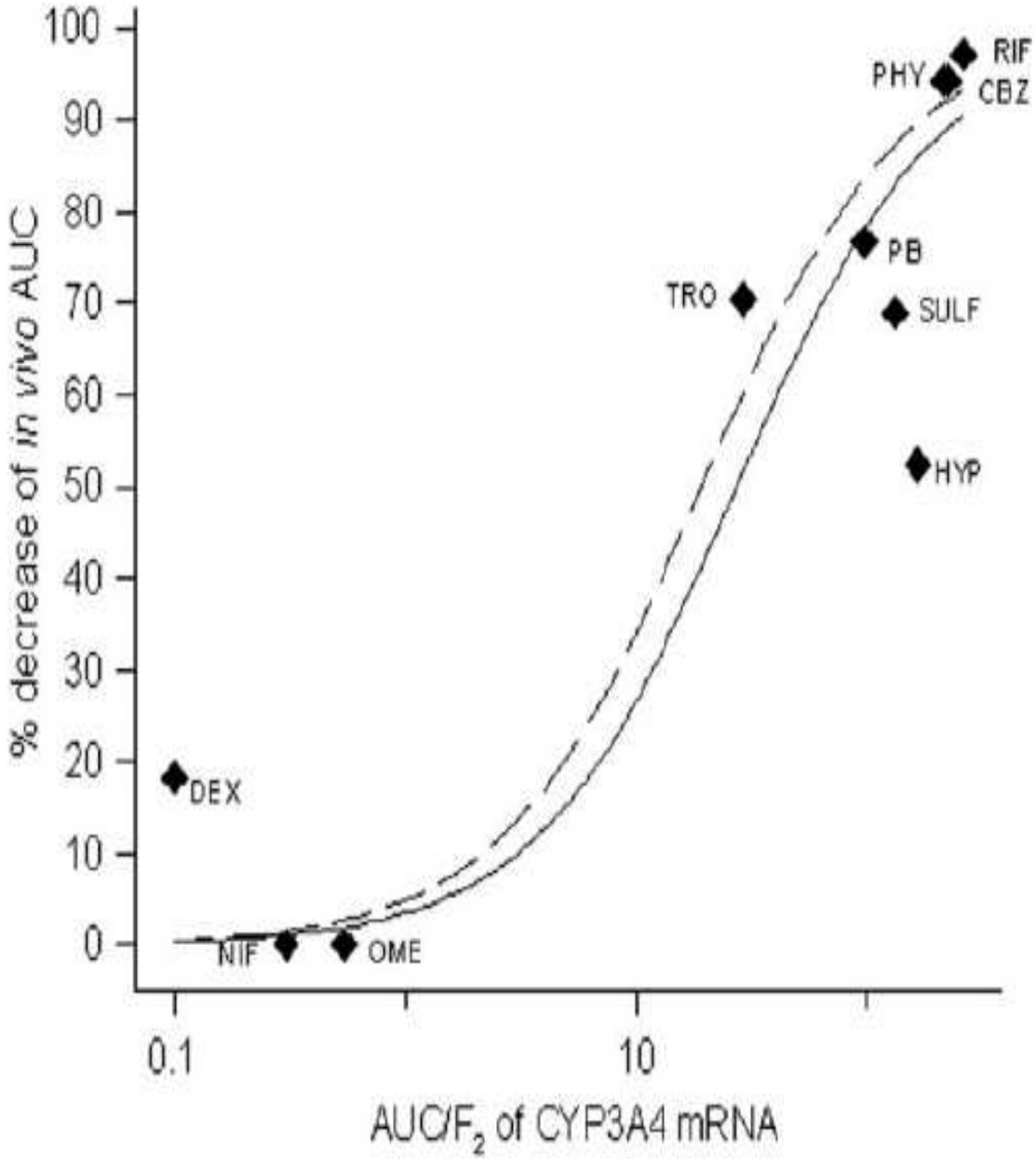


FIG. 4. Correlation of AUC/F_2 of CYP3A4 mRNA in HepaRG cells with percentage decrease of in vivo AUC for CYP3A probe drugs (whole line), R^2 = 0.863. The equation used is described under *Materials and Methods*. Correlation when hyperforin is excluded (dashed line), R^2 = 0.943. Compound abbreviations: CBZ, carbamazepine; DEX, dexamethasone; HYP, hyperforin; NIF, nifedipine; OME, omeprazole; PB, phenobarbital; PHY, phenytoin; RIF, rifampicin; SULF, sulfinpyrazone; TRO, troglitazone.

…..« Thus the HepaRG cell line could be used as an important in vitro model for investigation of enzyme induction in drug discovery. »

HepaRG CELLS for **EVALUATION OF CHEMICALS**

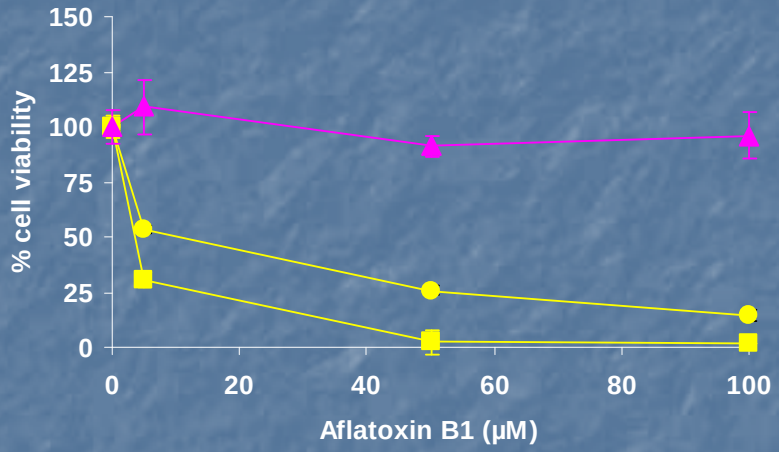
- Acute toxicity
- Chronic toxicity
- Genotoxicity

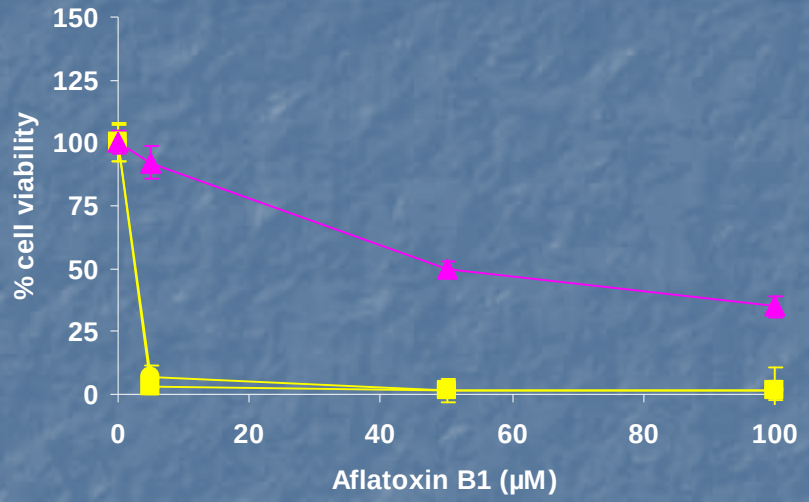
  -single or reiterated exposures?

  -evaluation of mixtures of compounds?

  - High throughput sceening
  - « Omics » approaches?

Aflatoxin B_1

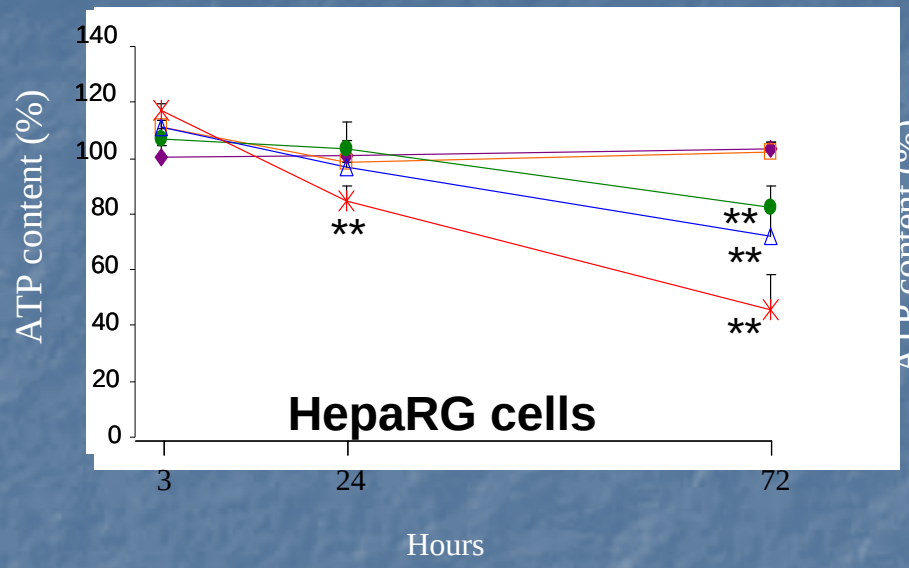


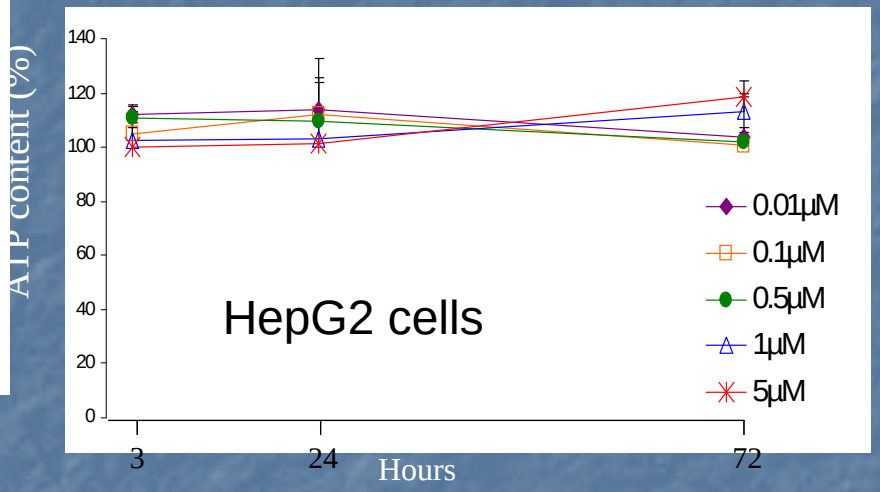


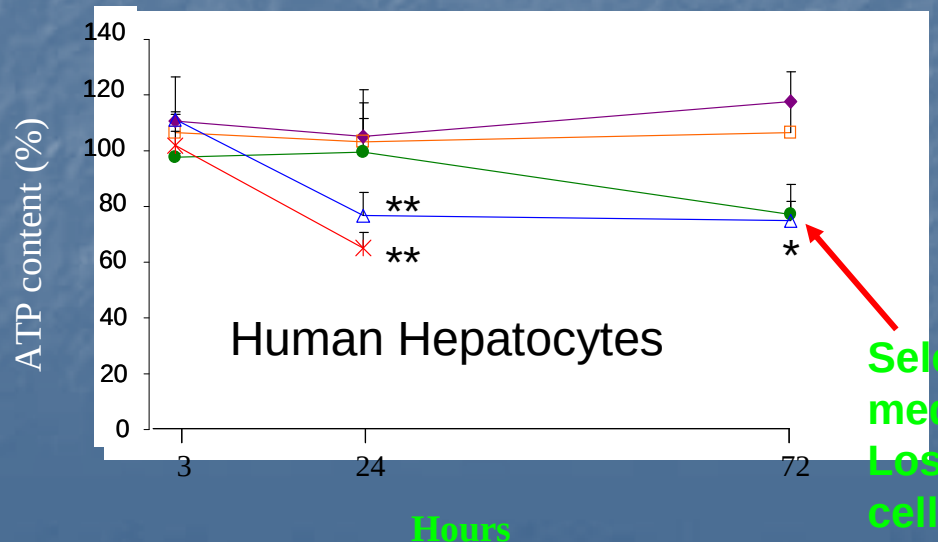
24h **72h**

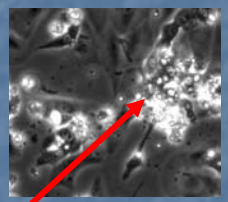
- Differentiated HepaRG cells
- HepaRG seeding at high density after differentiation
- HepG2 cells

Short-term toxicity of Aflatoxin B1 (AFB1) to human hepatocytes, HepaRG and HepG2 cells: Comparable sensitivity of human hepatocytes and HepaRG cells





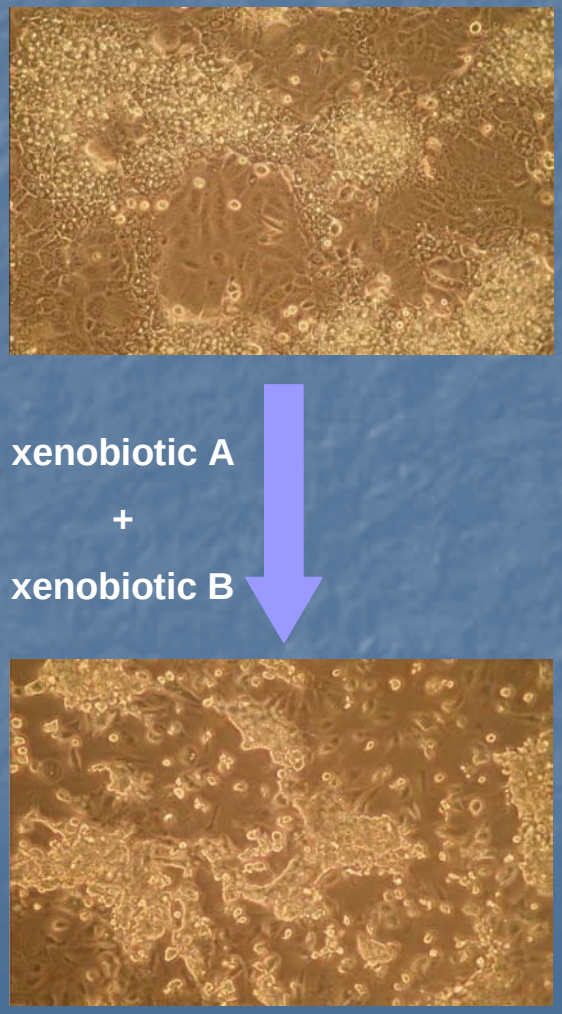


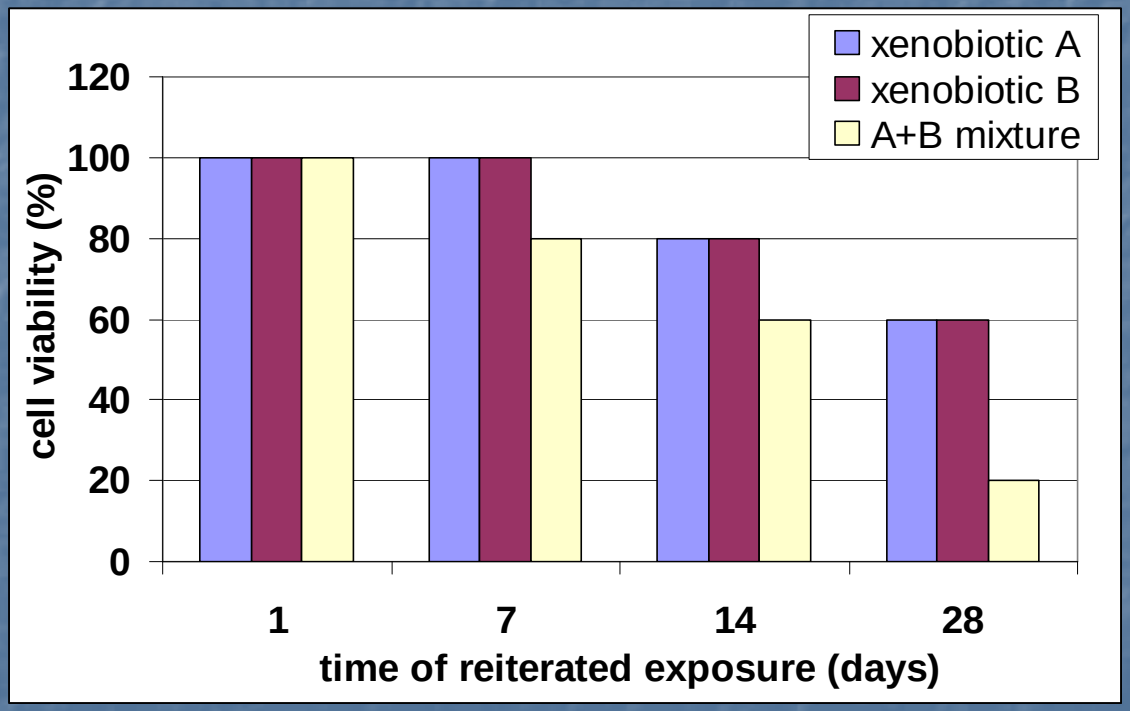


Selective metabolism-mediated cytotoxic effect: Loss of hepatocyte-like cells; epithelial-like cells survive

Jossé et al. DMD. 2008, 36, 1111-8

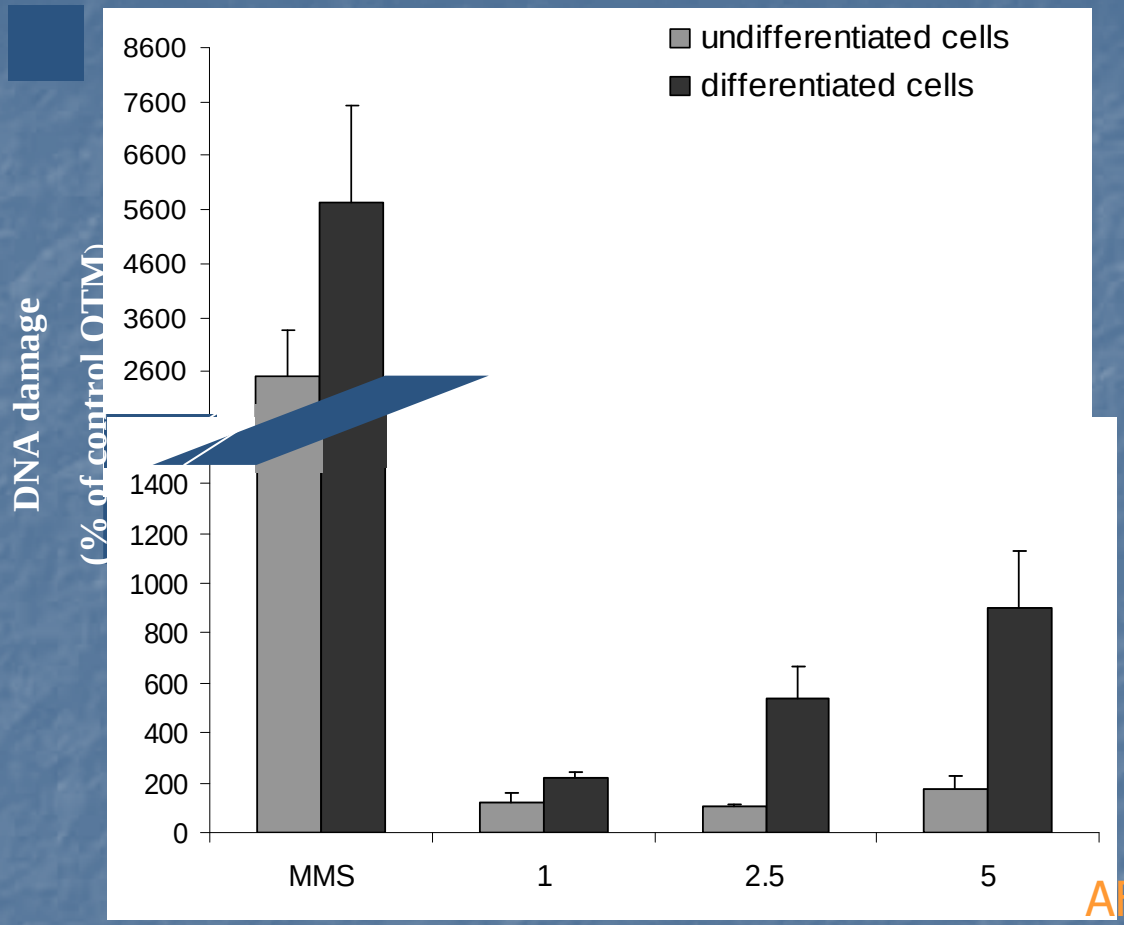
Cumulative cytotoxic effects of xenobiotics as a function of time of exposure





See the presentation of Julie Dumont

Effects of AFB1 on DNA damage in HepaRG cells: Comet assay

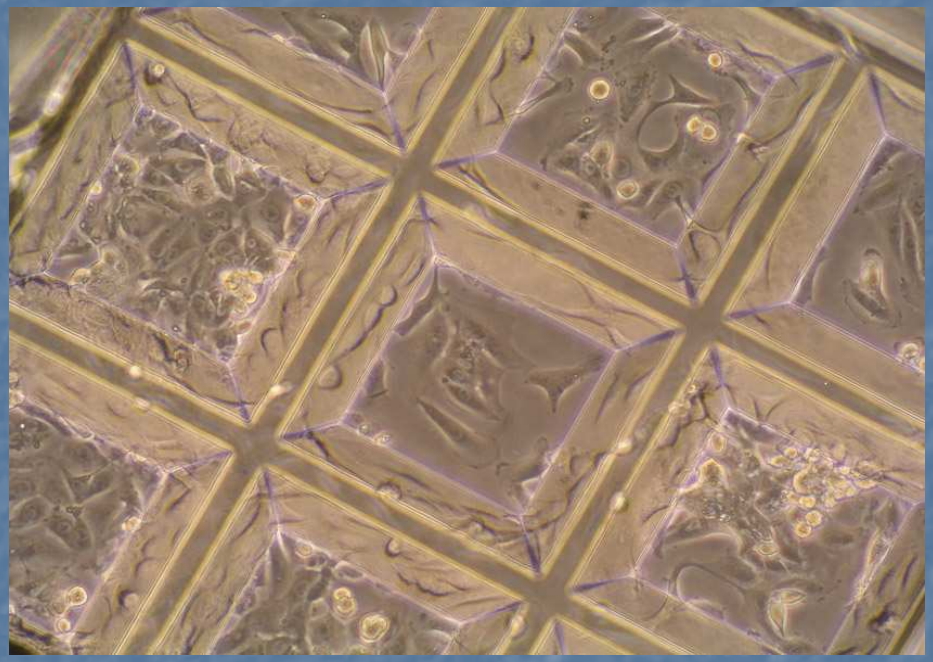


Undifferentiated and differentiated HepaRG cells were exposed to varying AFB1 concentrations for 3 h. Results are the mean ± SEM of 3 independent experiments. DNA damage was expressed as percentage of corresponding negative control: Olive tail moment (OTM). MMS (5µg/ml) was used as a positive control. *p<0.05, statistically significant difference between undifferentiated and differentiated cells.

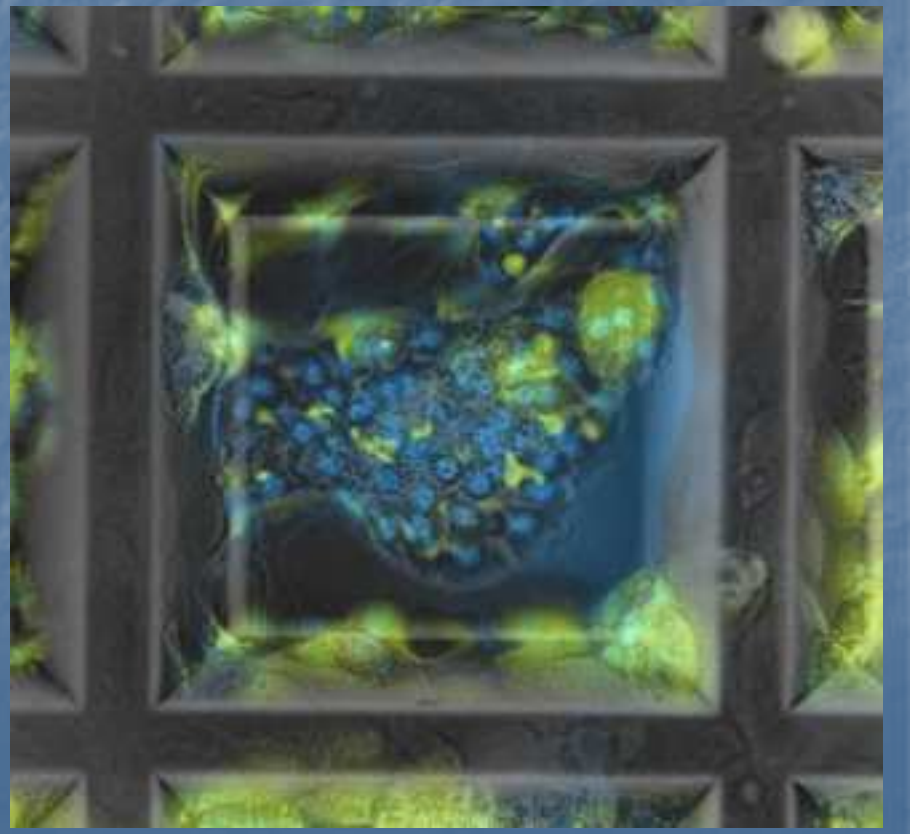
CELL CHIP COUPLED WITH HIGH CONTENT IMAGING:

("Cell chip and imaging" platform, OGP, Rennes :CH. Guillouzo Inserm U522)

CELL PROLIFERATION

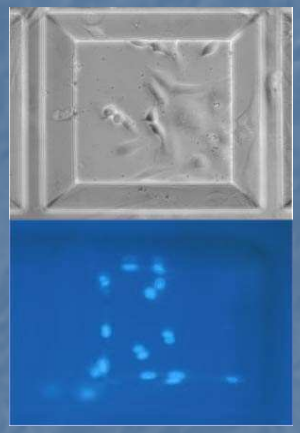


HepaRG cells



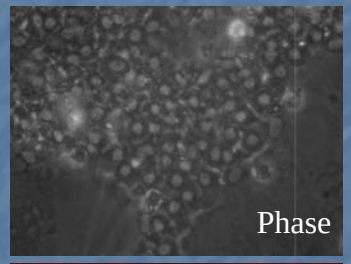
CELL DIFFERENTIATION →

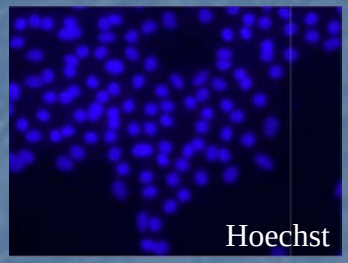
I- Automated cell seeding

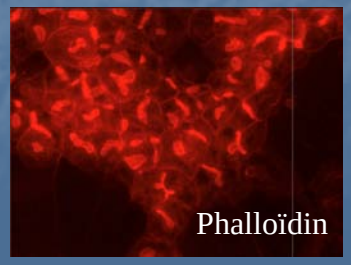


- **Transfer of viable cells**
- **One drop each 2-5 milli-sec**
- **Minimum volume <200pl (maximum 1µl)**

II- High content imaging analysis

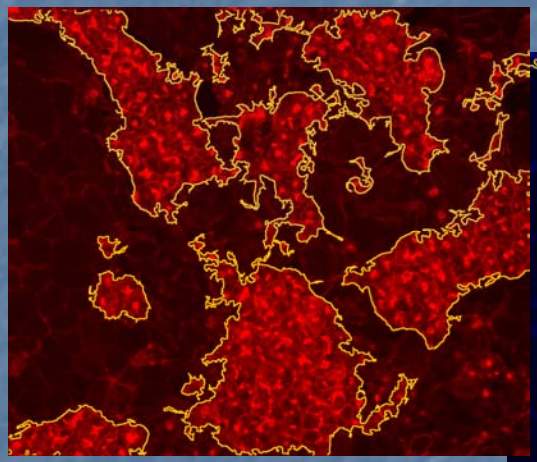




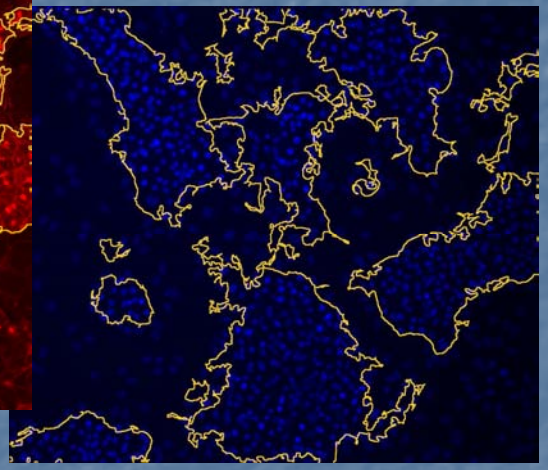


Quantification of biological activities

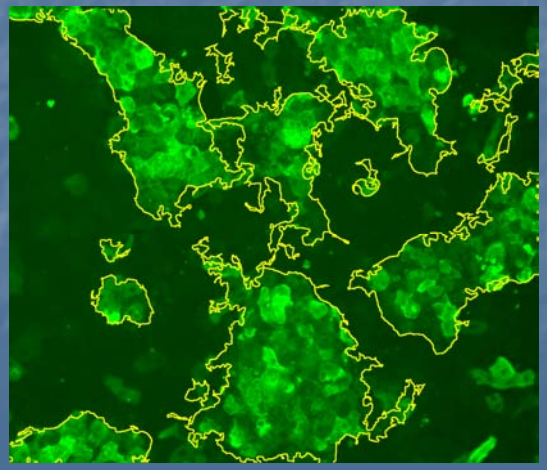
Imaging and fluorescence quantification



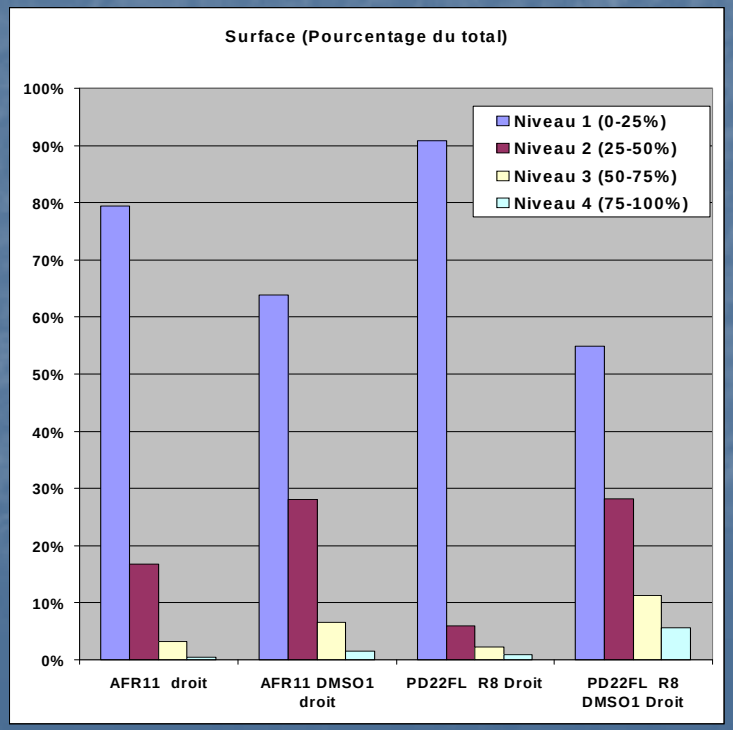
F-actin



Nuclei

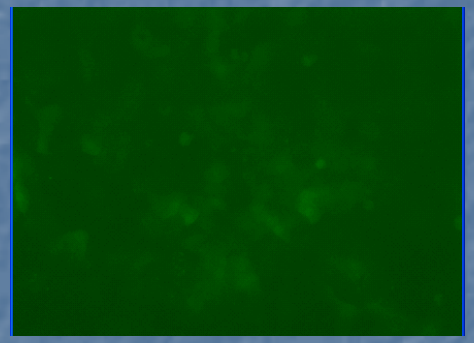


CYP 3A4

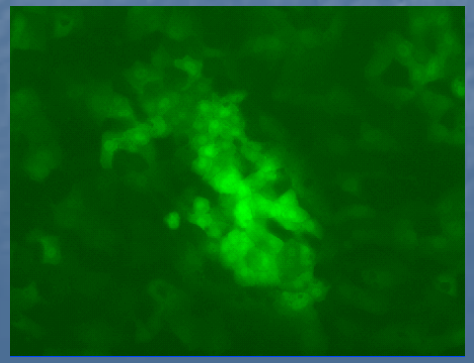


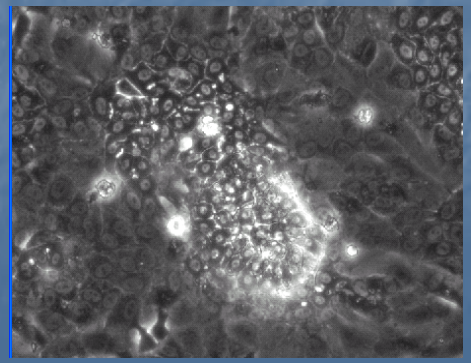
Recombinant HepaRG cel lines

Negative Control



Induction of the transgene





Expression of GFP only in HepaRG hepatocyte-like cells

(Cell chip and imaging"platform, OGP, Rennes :Ch. Guillouzo Inserm U522)

Use of HepaRG cells for gene profiling expression analysis

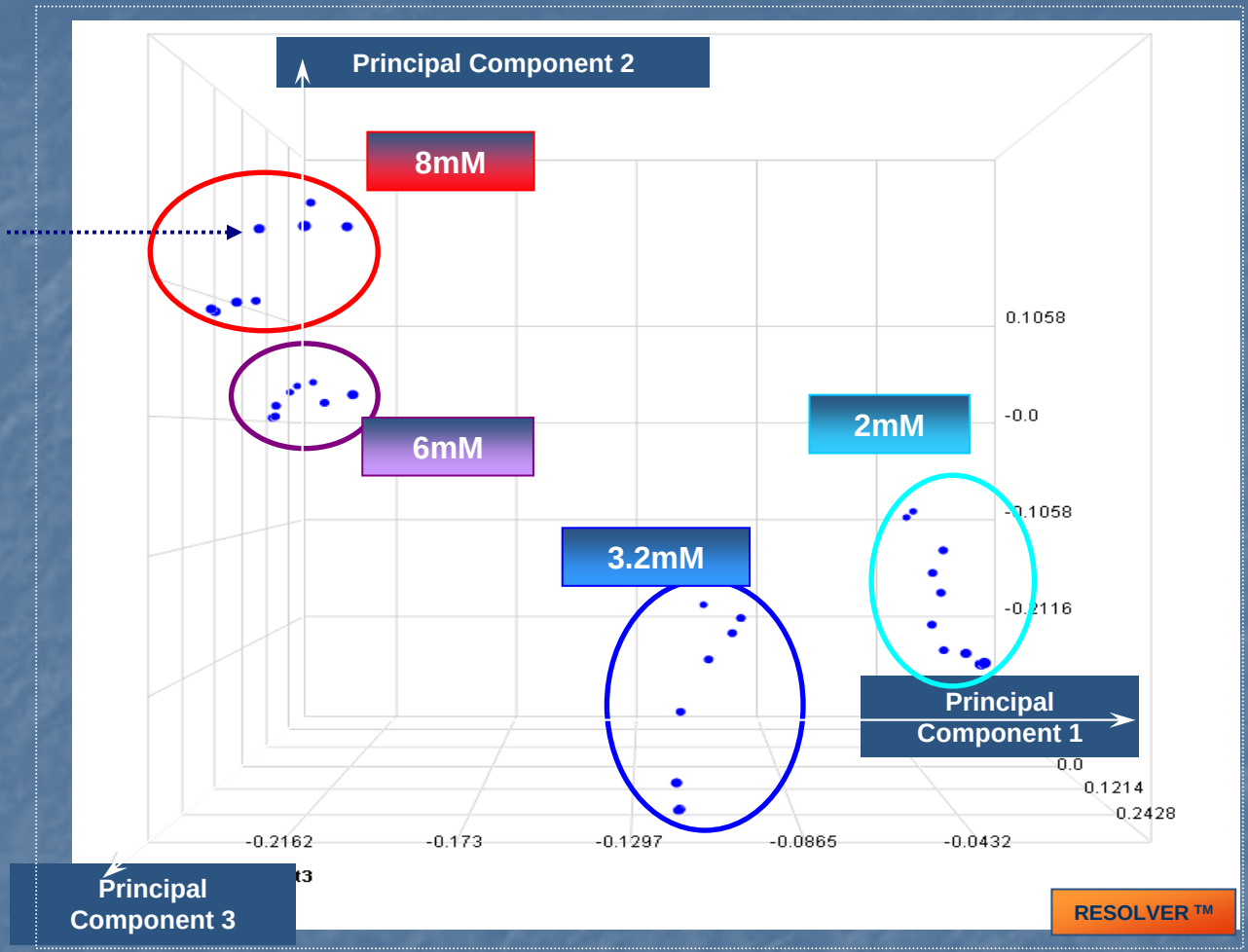
Trancriptomic analysis:

⇨ Concentration- and time-dependent response:

Phenobarbital

Lambert et al. submitted

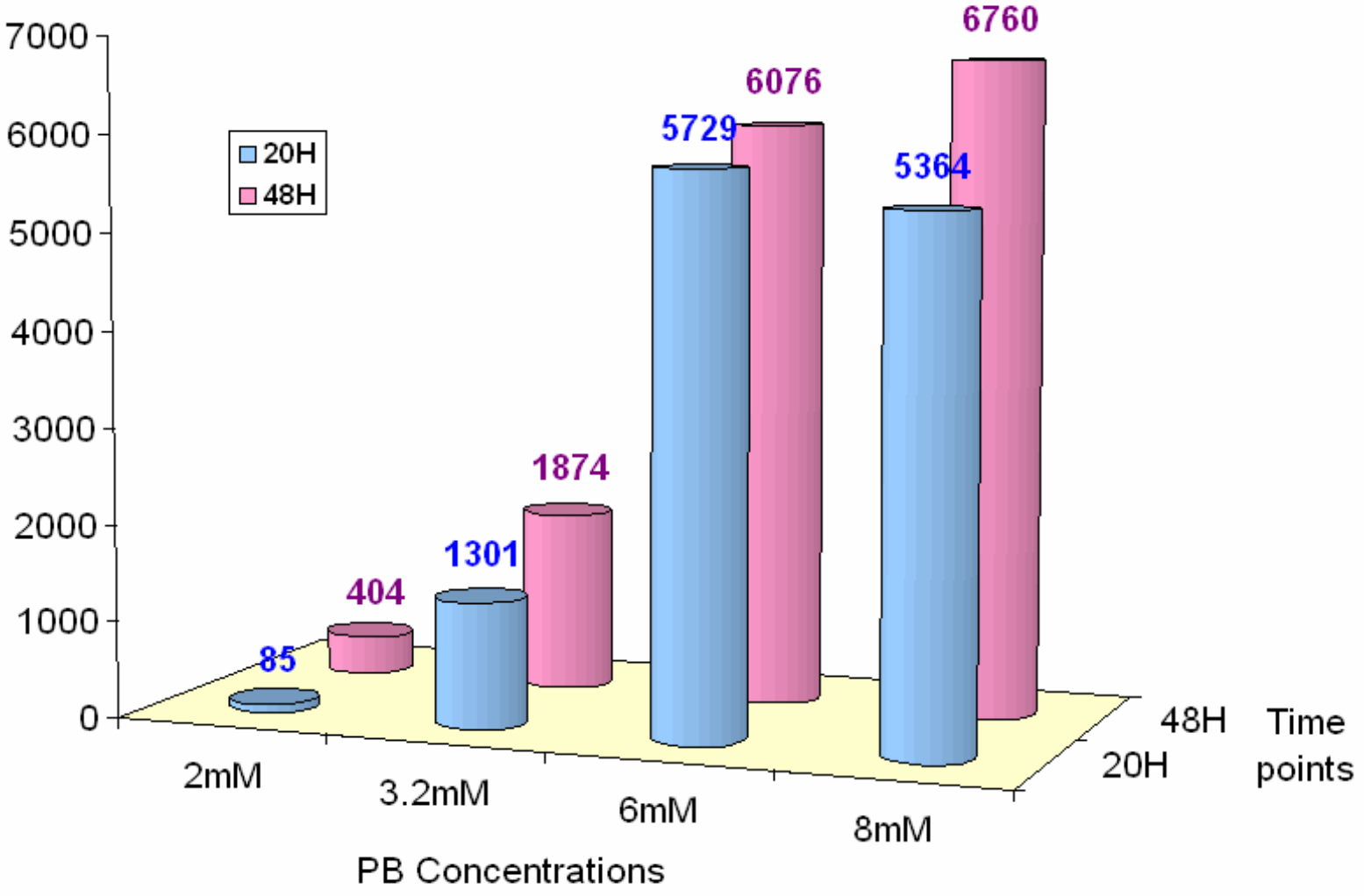
Principal Componant analysis (20h treatment with phenobarbital)



Number of modulated genes (3-fold change and p ≤ 0.01)

Dose- and time-dependent effects

Total number of significantly modulated genes



Use of HepaRG cells for gene profiling expression analysis

Trancriptomic analysis:

⇨ Comparative analysis of various conpounds:

Lambert et al. in preparation

Comparative gene profiling in HepaRG cells

Trancriptomic analysis:

- ⇨ Chemical-specific modulated genes
- ⇨ Specific biomarkers
- ⇨ Mechanistic study

CONCLUSIONS

- The human HepaRG cell line expresses a unique set of liver functions including the major CYPs involved in drug metabolism and the key nuclear receptors (CAR,PXR, AhR, PPARs)
- This cell line possesses both the functional capacities of adult hepatocytes in primary culture and the indefinite growth capacity of hepatoma cells
- This cell line can be used for various applications including hepatitis B infection (PNAS, 2002)
- However it must be kept in mind that these cells are transformed and derived from a single individual (female)

Guillouzo and Guguen-Guillouzo Expert Opin. Drug Metab. Toxicol. 2008, 4, 1279

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