

# Luminescence kits in the development of an *in vitro* system to test fibrosis

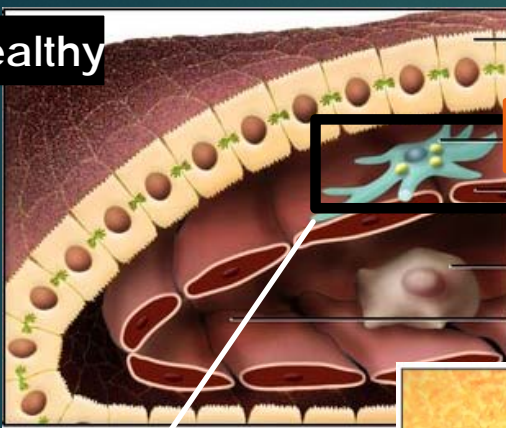
Sofia B. Leite, Inge Mannaerts, Leslie Stradiot,  
Leo A. van Grunsven



# In vivo & in vitro Liver Fibrosis



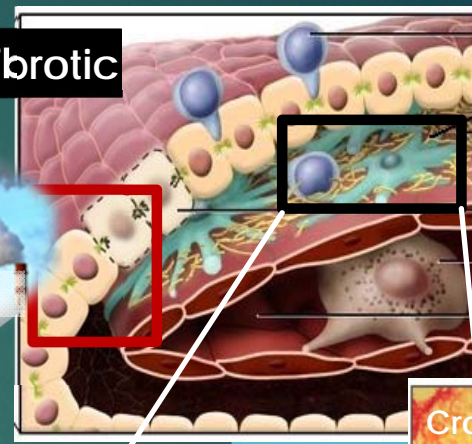
Healthy



Hepatitis A, B, C, D, E  
Hepatic Stellate Cells (HSC)

Drugs      Alcohol      Cosmetics

Fibrotic



Infiltrating lymphocyte  
Hepatic Stellate Cells (HSC)  
Extracellular matrix proteins  
Apoptotic hepatocyte  
Activated Kupffer cell  
Sinusoid lumen with increased resistance to blood flow

injury  
resistance to blood flow

Pollution

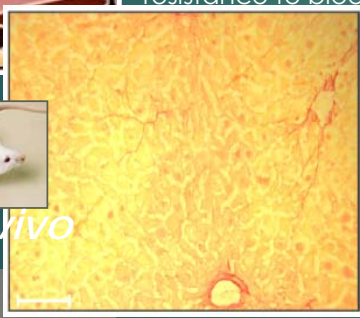
Alcohol

Pesticides

In vitro

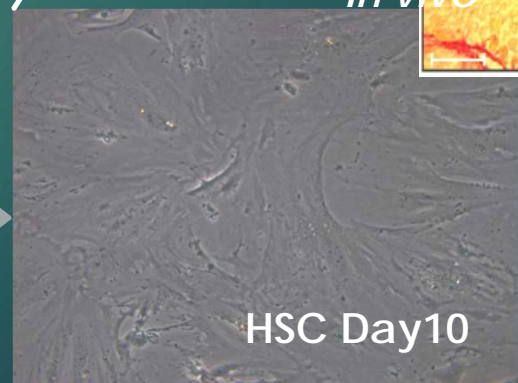


In vivo



✓ 6% of the EU population  
✓ † ~ 70.000/year in l

In vivo

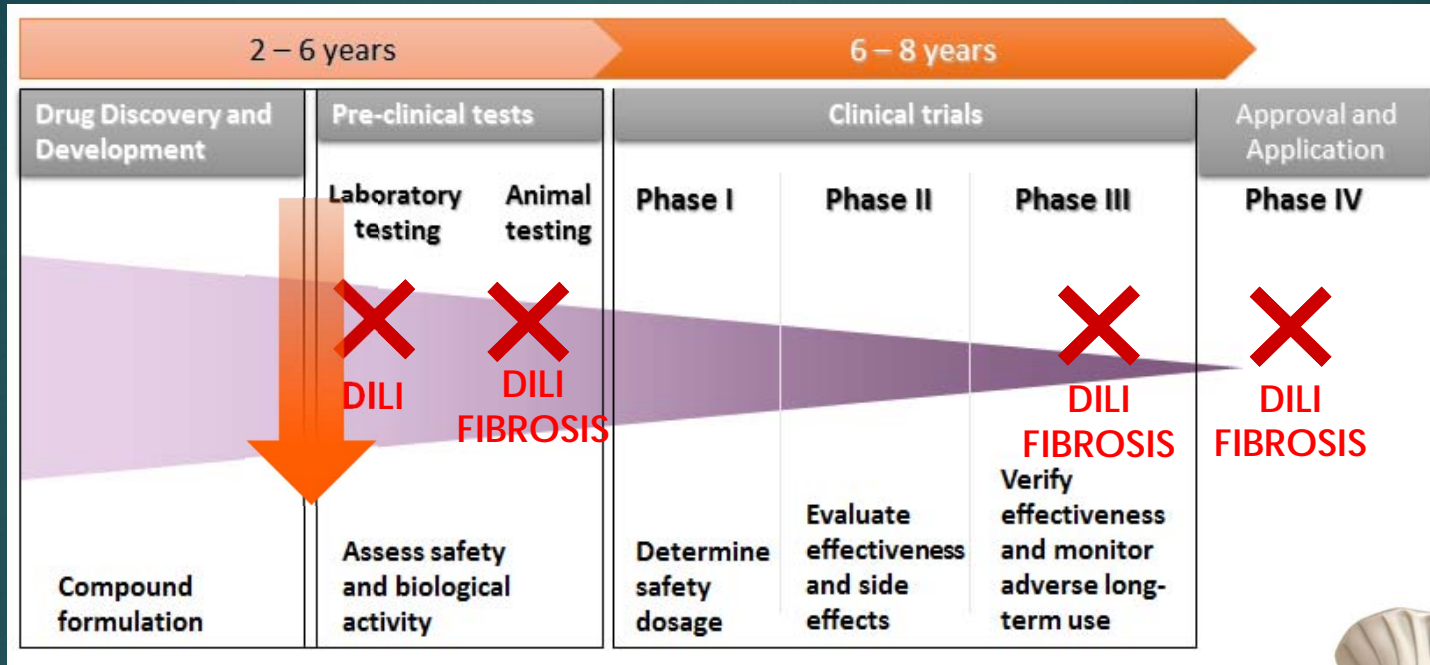


↑ aSMA,  
↑ Col1a1  
↑ Lox  
..

Bata



# Drug toxicity is detected too late...



- ± 90% of compounds entering clinical development fail
  - ± 30% is due to clinical safety and toxicology

*Kola et al. 2004, Nat Rev Drug Discovery*

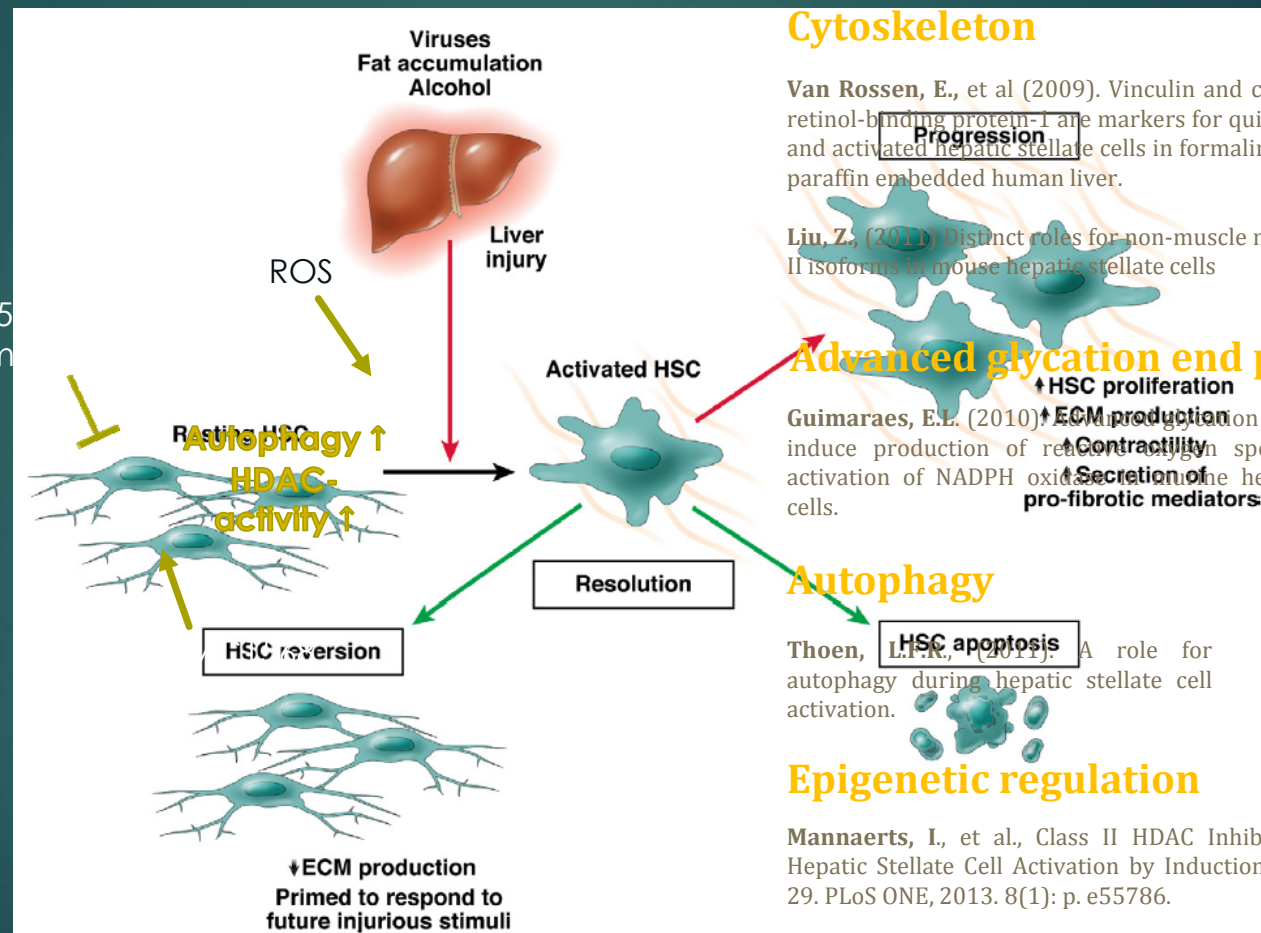
- ± 26.2% of drug withdrawal from the market is due to hepatic toxicity

*Fung et al., 2001, Drug Info J*



# Different fates of HSCs when injury stops....

VPA-MC15  
Bafilomycin



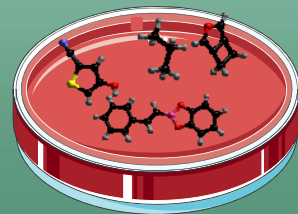
Adapted from Henderson NC, et al. 2012

# *Luminescence assays in fibrosis research*

1. Hepatocyte  
functions in different  
culture conditions



2. Real-time *in vitro* drug testing



3. Enzyme  
activity *in vivo*





# SEURAT-1 Aim

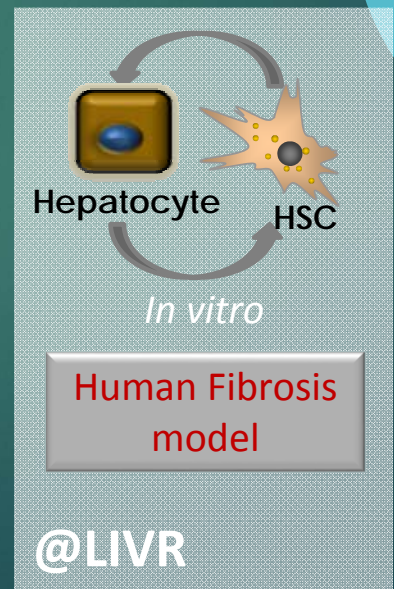
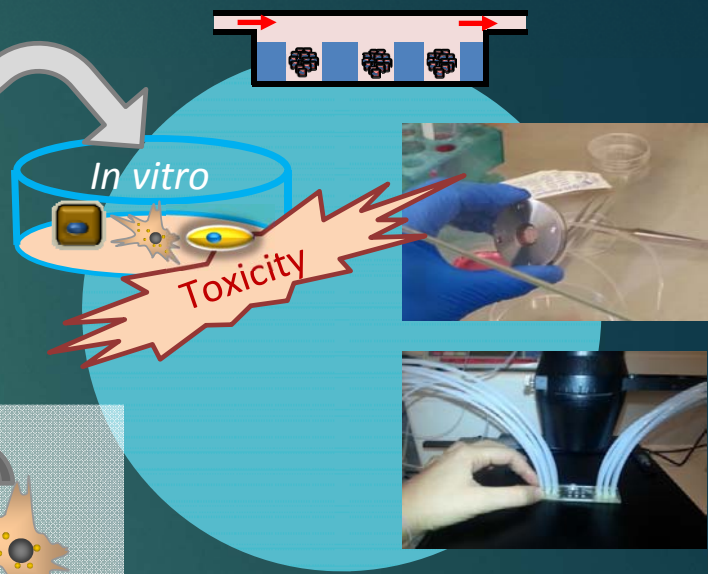
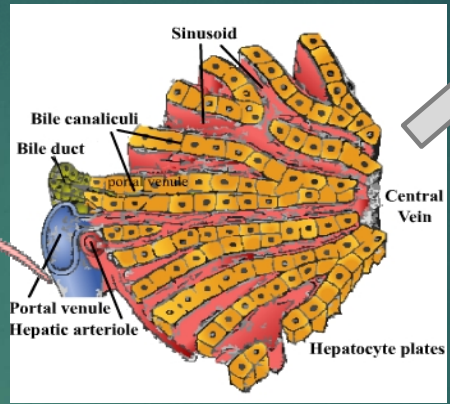


COSMETICS

Paracelsus (1541):  
"The dose makes the poison"



Towards the Replacement of *in vivo* Repeated Dose Systemic Toxicity Testing

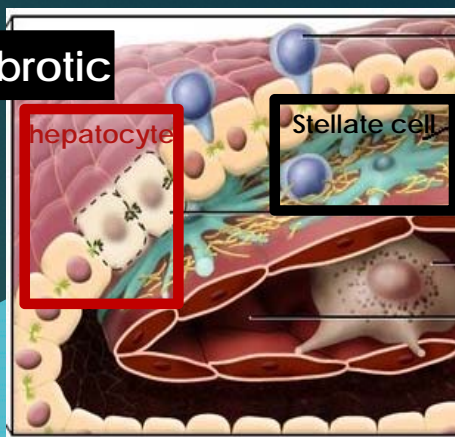


# The ideal in vitro fibrosis model....

Culture Model

Hepatic Stellate Cells quiescent  
+  
Hepatocytes functional  
but  
Still able to activate: *(in)direct drug effect*

Fibrotic

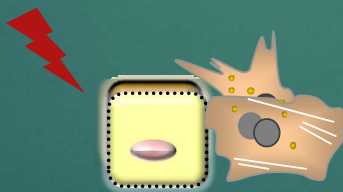


Functional Hepatocytes

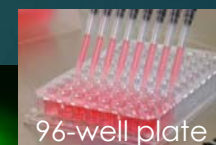
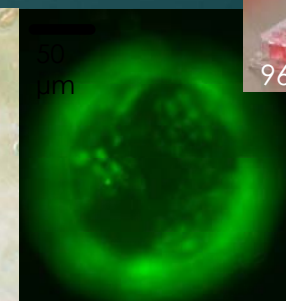
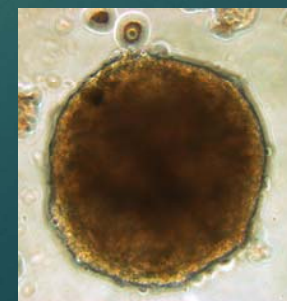


Not activated HSC  
( $\downarrow\alpha$ SMA;  $\downarrow$ Collagen;  $\downarrow$ Lox...)

Hepatocytes respond to injury

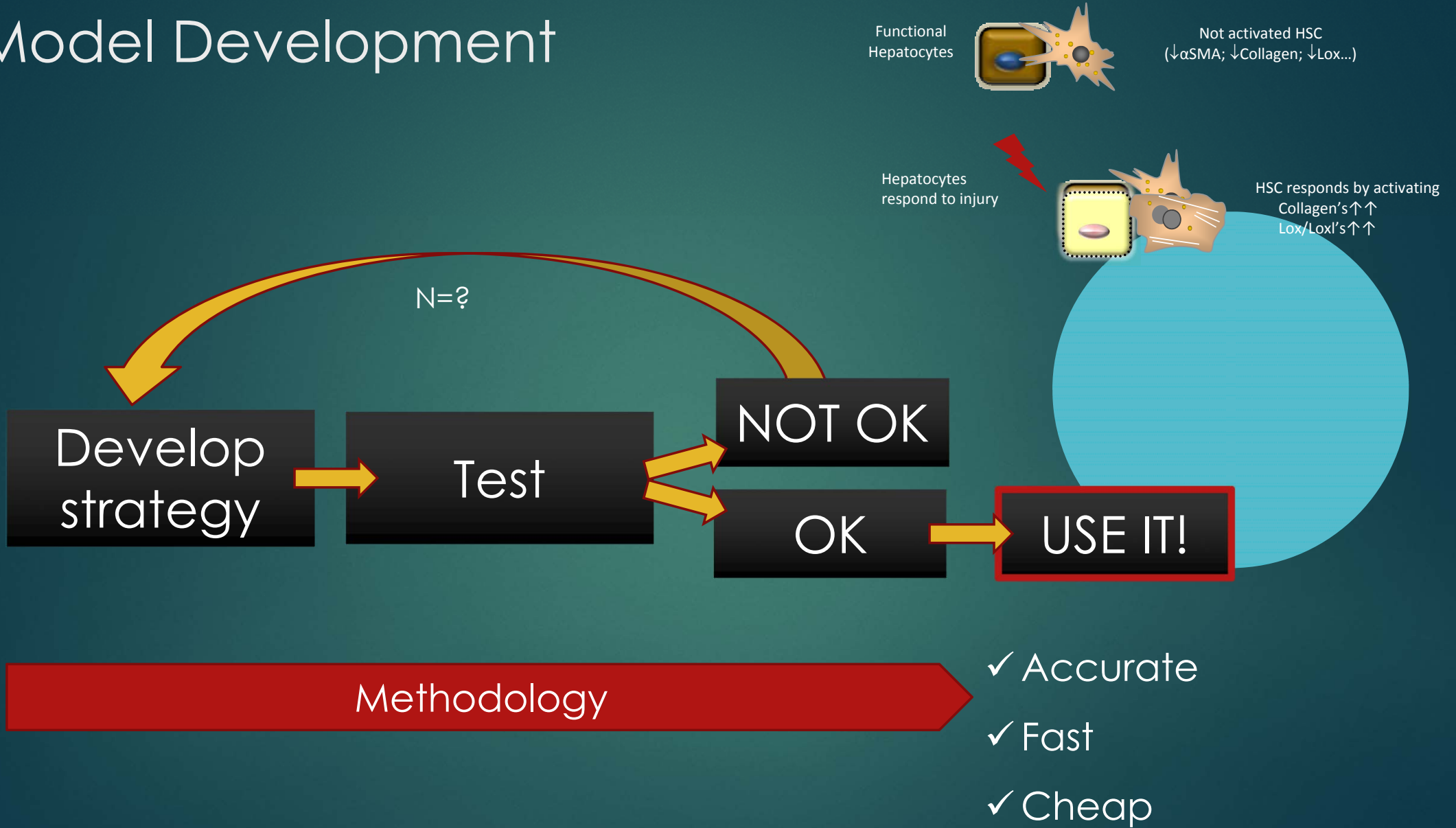


HSC responds by activating  
Collagen's  $\uparrow\uparrow$   
Lox/Loxl's  $\uparrow\uparrow$





# Model Development



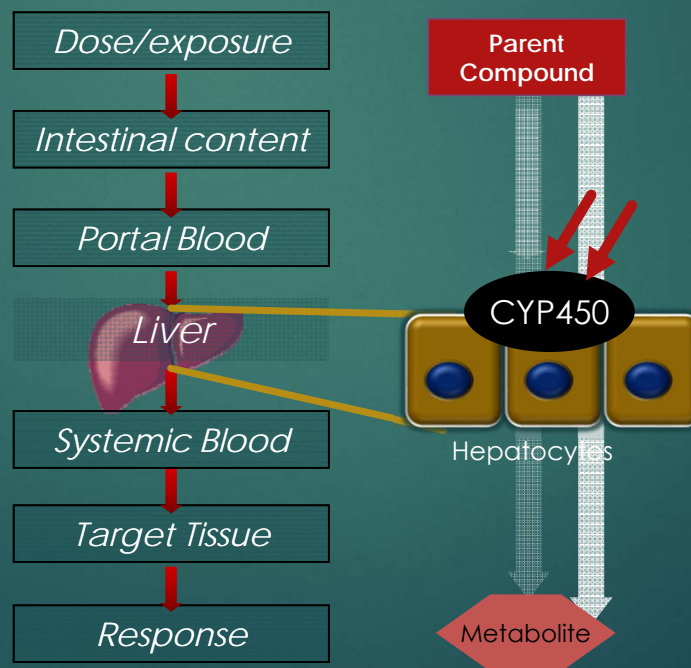
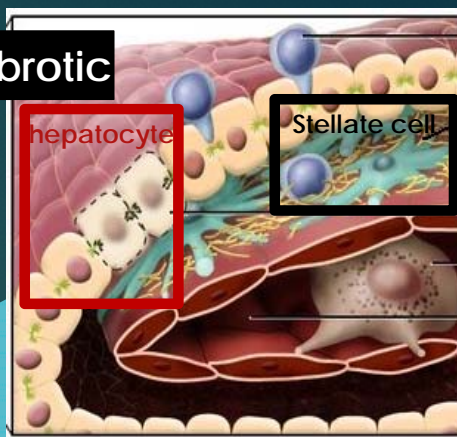


# The ideal in vitro fibrosis model.....

Culture Model

Hepatic Stellate Cells quiescent  
+  
Hepatocytes functional  
but  
Still able to activate: *(in)direct drug effect*

Fibrotic



# Golden standard of P450 activity method

Liquid Chromatography and Mass Spectrometry (LC-MS)



- ✓ Very Accurate
- ✓ Time demanding
- ✓ High qualified skills
- ✓ NOT Cheap

1. Compound incubation

2. Supernatant collection

3. Sample preparation

4. Freeze samples until machine availability

5. Discarding non-pure samples

6. Sample analysis  
(+/-1h/sample)

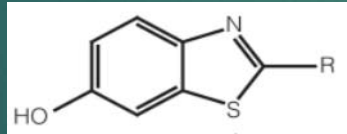
7. Calculate, analyse and compare data

# Promega P450 activity method



P450-Glo™ Substrate  
(proluciferin)

CYP Enzyme



Luciferin  
Detection  
Reagent



1. Compound incubation

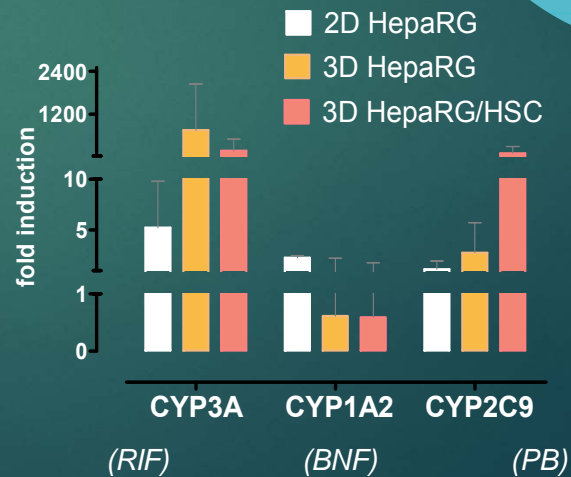
2. Add Luciferin Detection  
Reagent

3. Read Luminescence

4. Check values

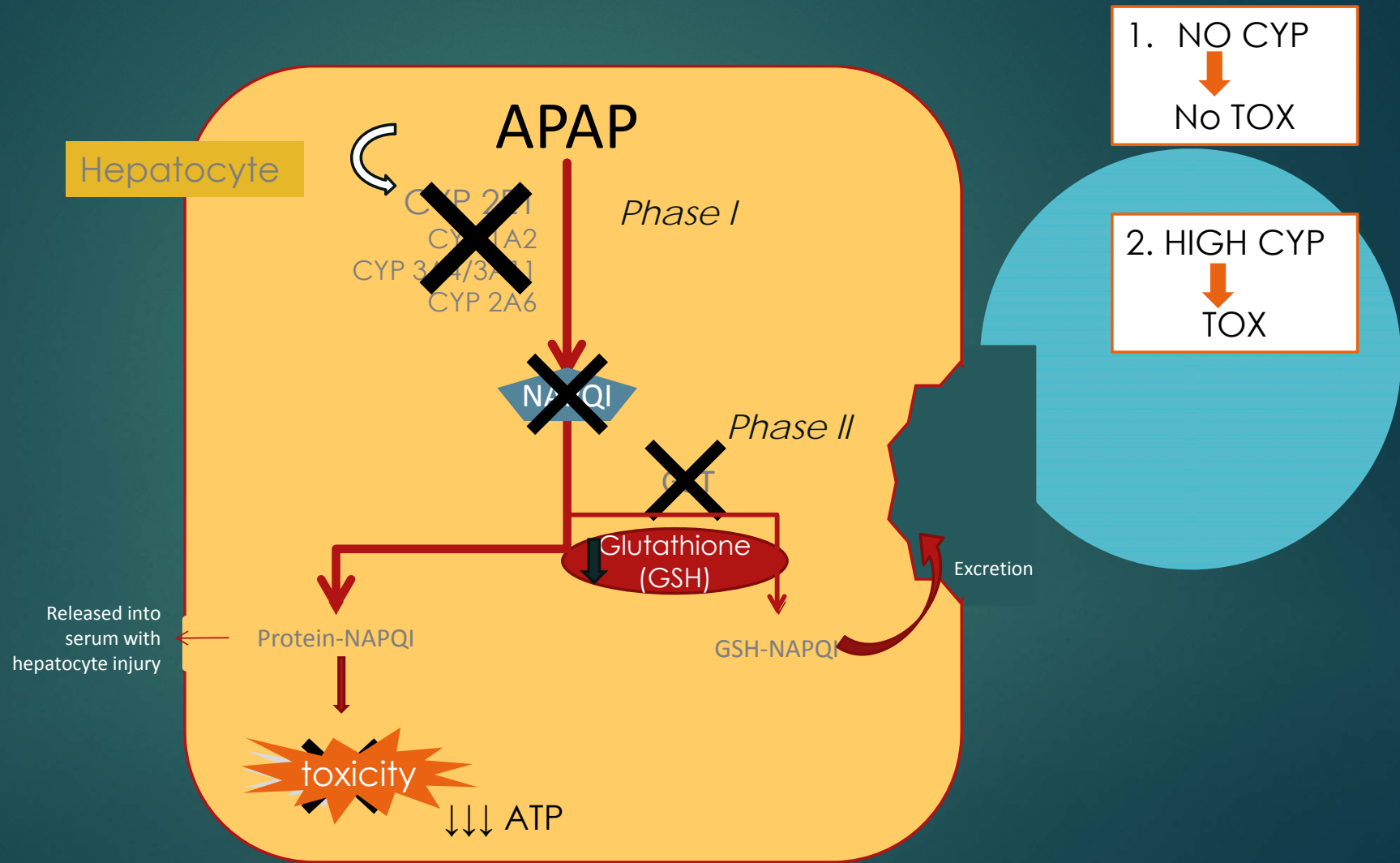


CYP induction - Day 21





# APAP tox mechanism



GST = glutathione-S-transferase

# Drug toxicity

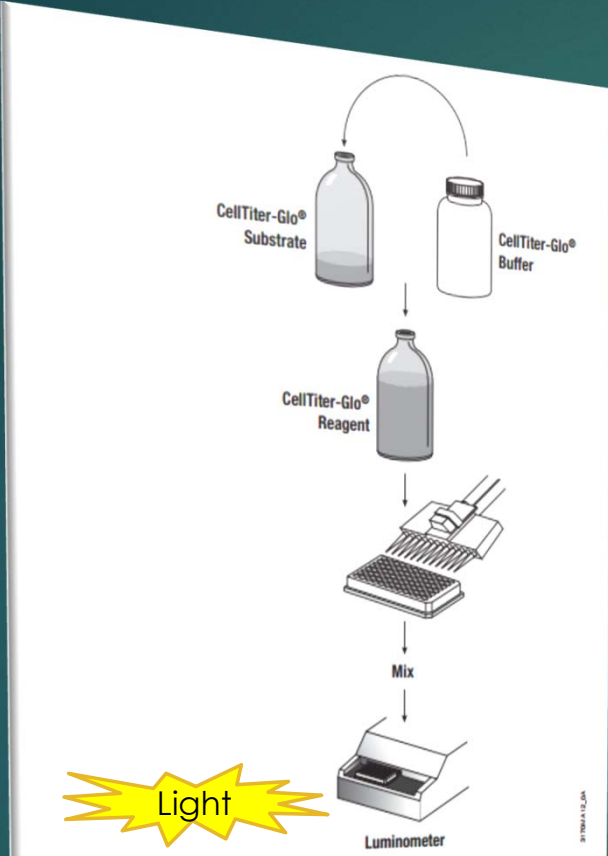
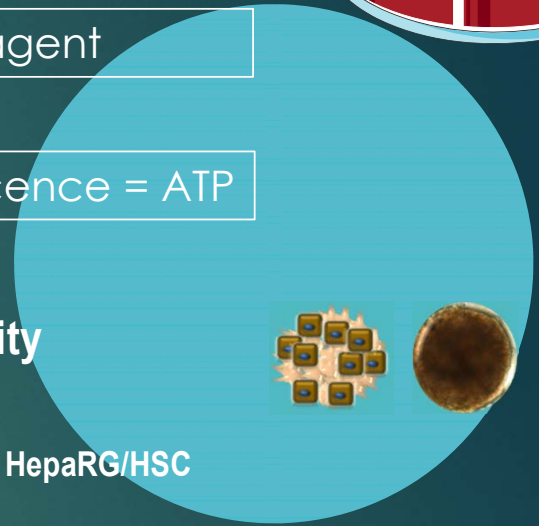
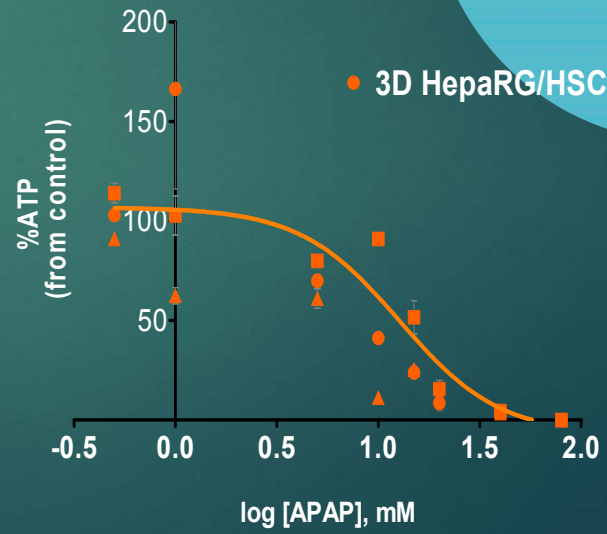


Figure 1. Flow diagram showing preparation and use of CellTiter-Glo® Reagent.

1. Compound incubation
2. Add Lysing Reagent
3. Read Luminescence = ATP



## APAP toxicity

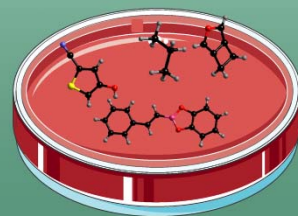


# *Luminescence assays in fibrosis stories*

1. Hepatocyte  
functions at  
different culture  
conditions



2. *In vitro* drug  
testing  
on-line

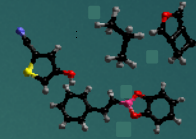


3. Enzyme  
activity *in vivo*





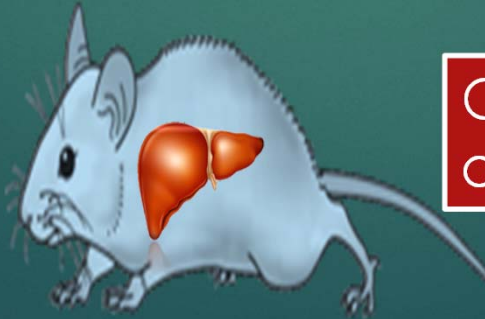
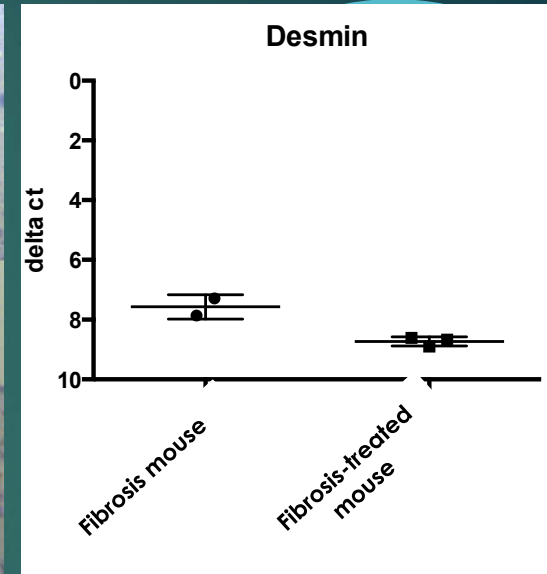
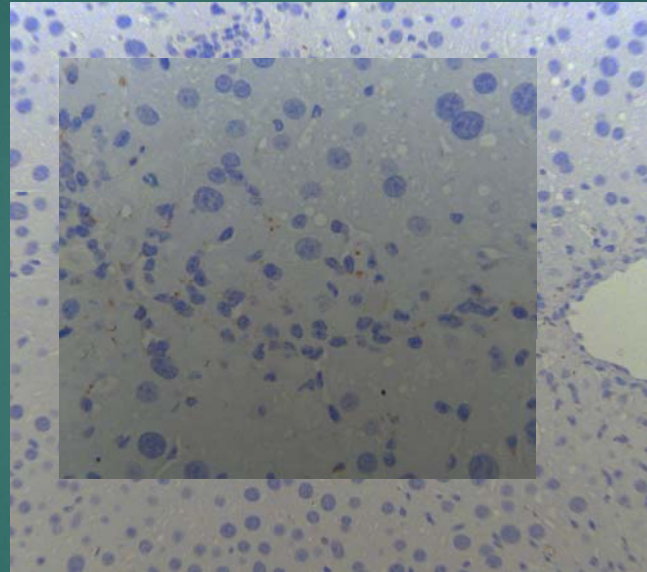
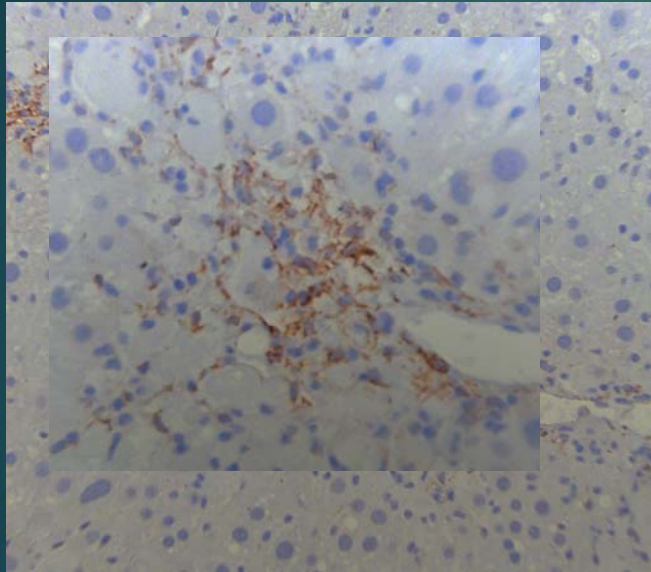
# Anti-fibrotic compound



Fibrotic mouse

Fibrotic mouse + comp X

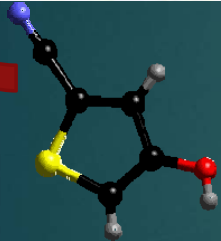
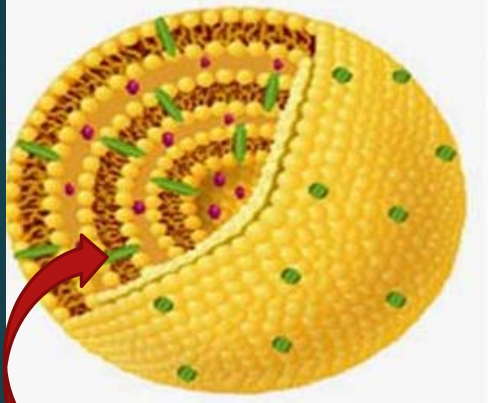
Desmin -HSC



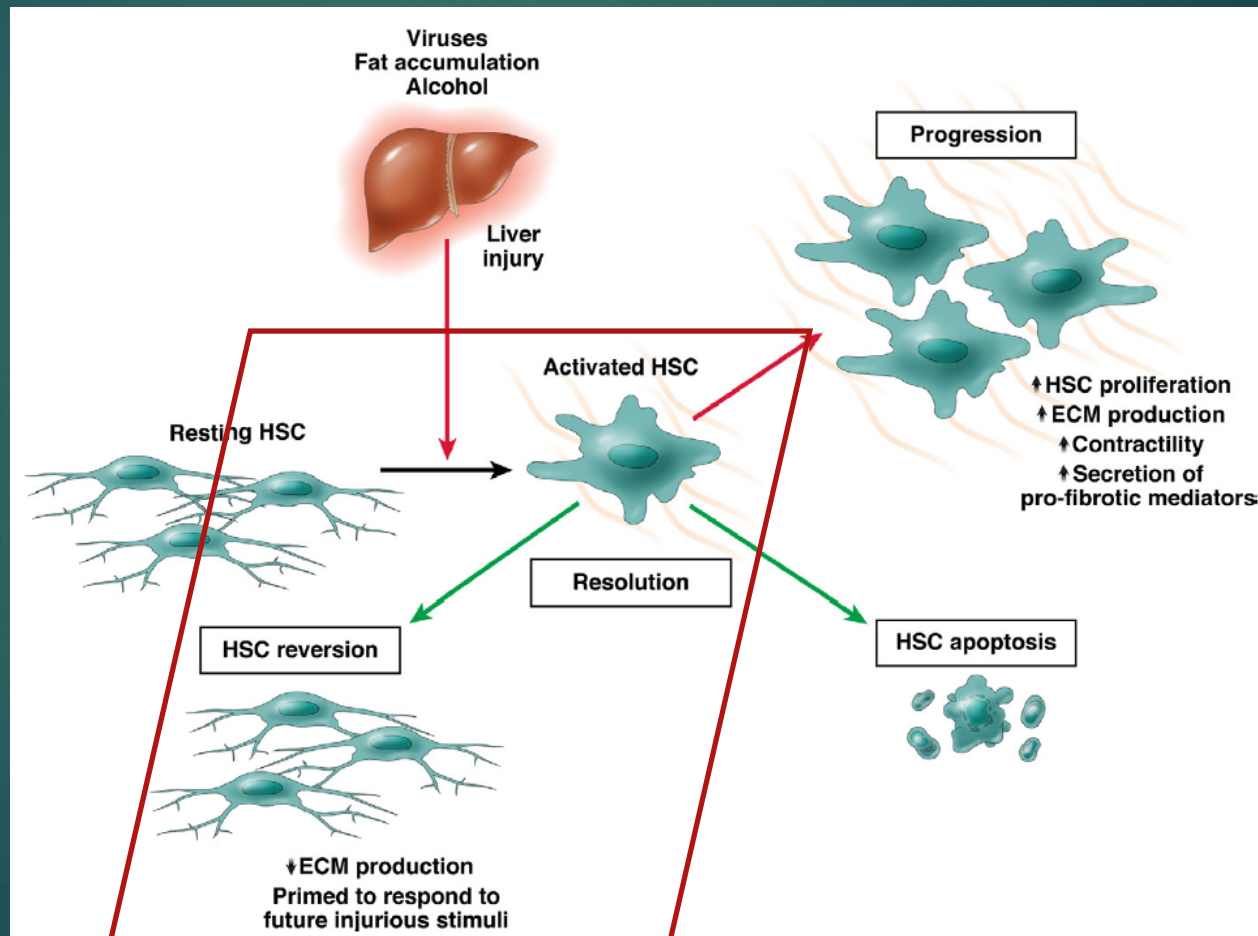
Compound effects  
other organs

# Specific delivery of the Anti-fibrotic to the HSC

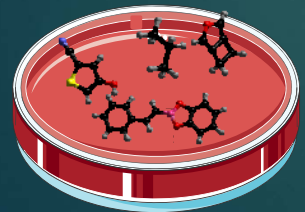
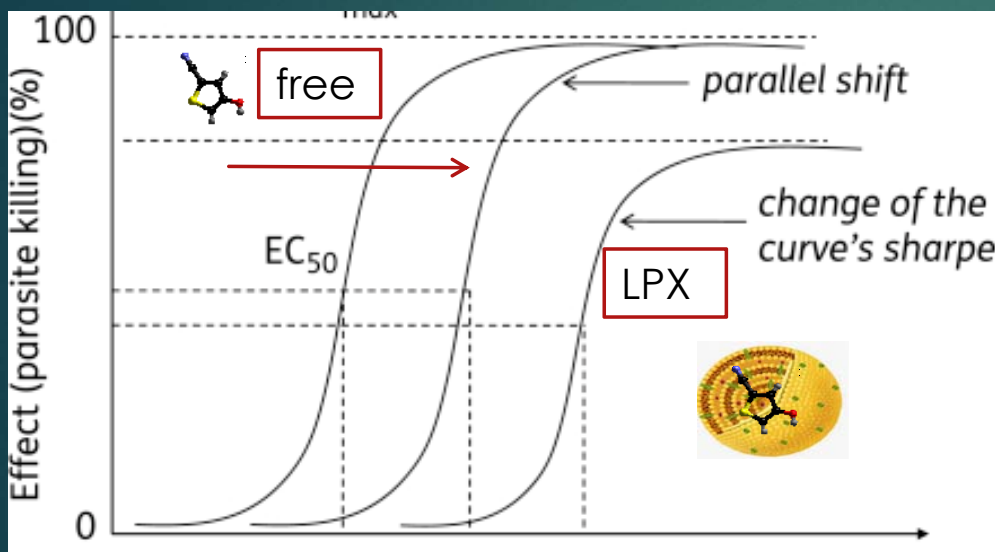
## LIPOSOMES



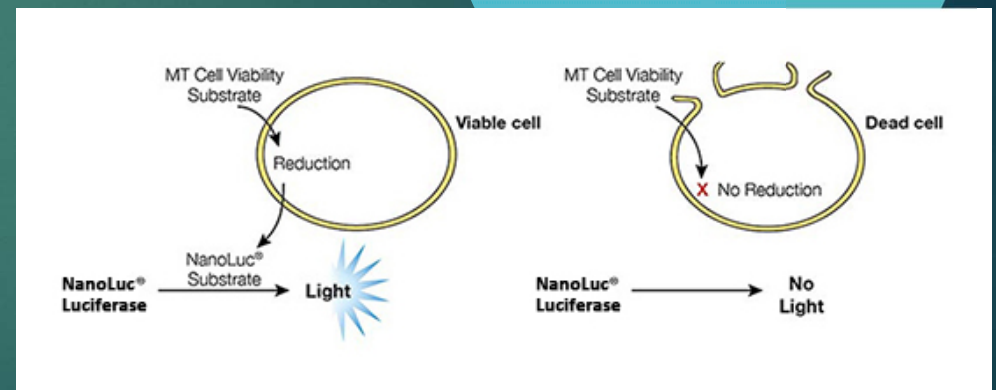
Concentration???



# Which liposomal concentration correlates with the effective HSC free concentration?



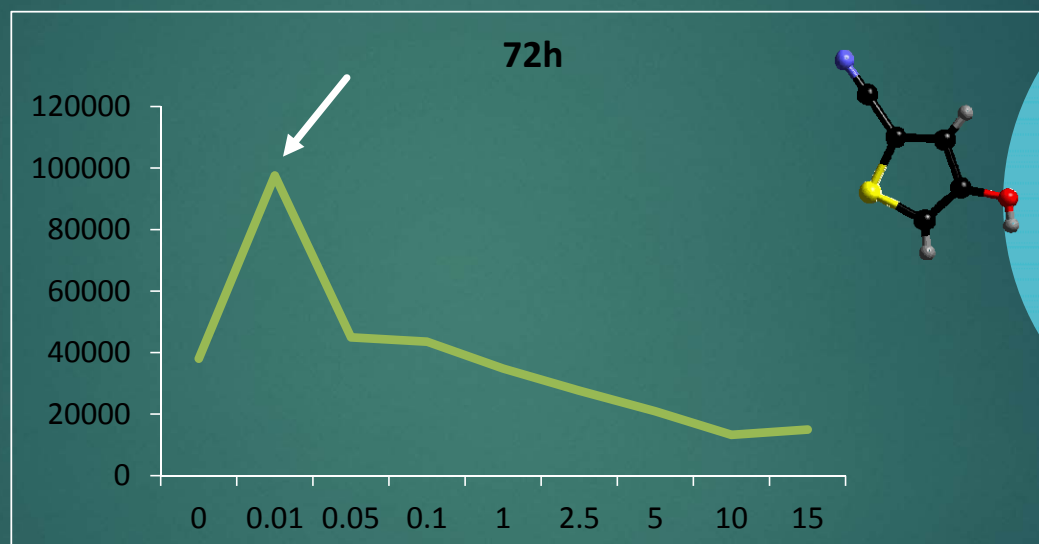
RealTime-Glo™ Metabolism  
cell Viability assay



On line measurements for 72h with same cells



RealTime-Glo™  
Metabolism cell  
Viability assay



Induction effect at  
very low  
concentrations

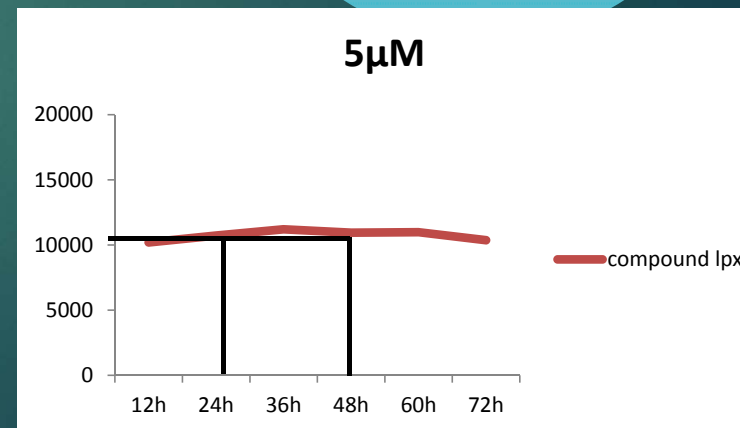
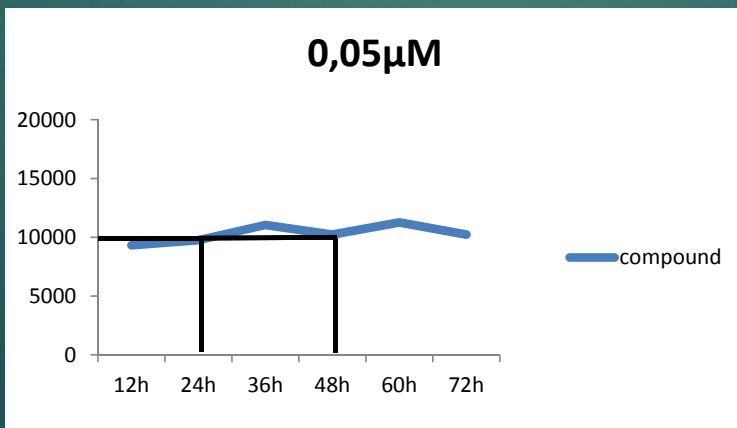
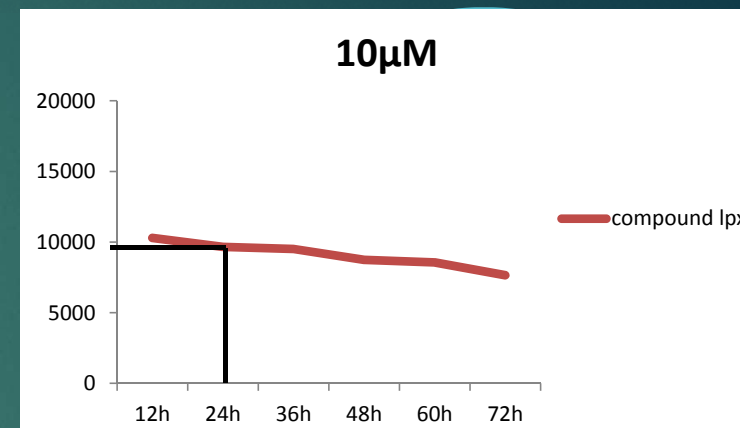
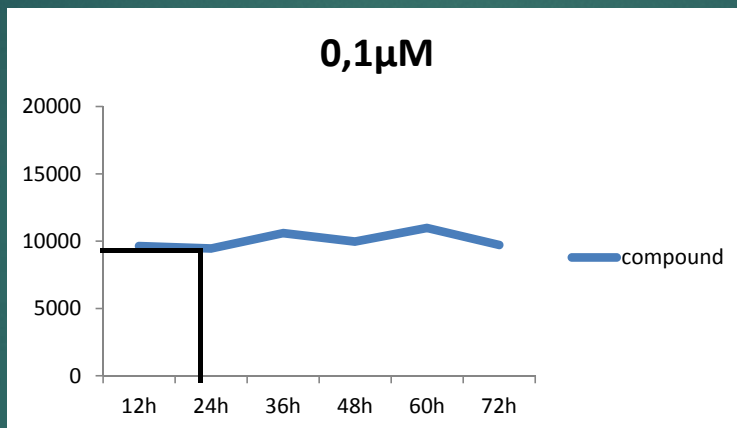
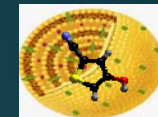
*Confirming previous  
results*

RealTime-Glo™  
Metabolism cell  
Viability assay



Free

LPX

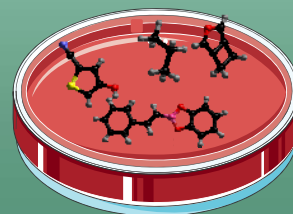


# *Luminescence assays in fibrosis stories*

1. Hepatocyte  
functions at  
different culture  
conditions



2. *In vitro* drug  
testing  
on-line



3. Enzyme  
activity *in vivo*





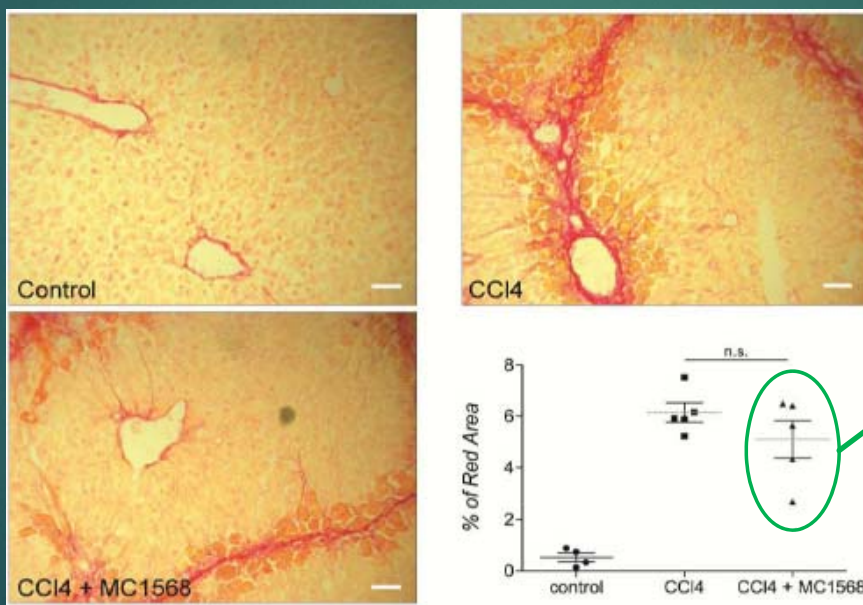
# Class II HDAC Inhibition Hampers Hepatic Stellate Cell Activation by Induction of MicroRNA-29

Inge Mannaerts<sup>1</sup>, Nathalie Eysackers<sup>1</sup>, Oscar O. Onyema<sup>1</sup>, Katrien Van Beneden<sup>2</sup>, Sergio Valente<sup>3</sup>, Antonello Mai<sup>3</sup>, Margarete Odenthal<sup>4</sup>, Leo A. van Grunsven<sup>1\*</sup>

**1** Department of Cell Biology, Liver Cell Biology Lab, Vrije Universiteit Brussel, Brussels, Belgium, **2** Department of Human Anatomy, Liver Cell Biology Lab, Vrije Universiteit Brussel, Brussels, Belgium, **3** Istituto Pasteur - Fondazione Cenci Bolognetti, Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Universita' di Roma, Roma, Italy, **4** Institute of Pathology, University Hospital of Cologne, Cologne, Germany



# MC1568 does not seem to inhibit fibrogenesis in a CCl<sub>4</sub> mouse model

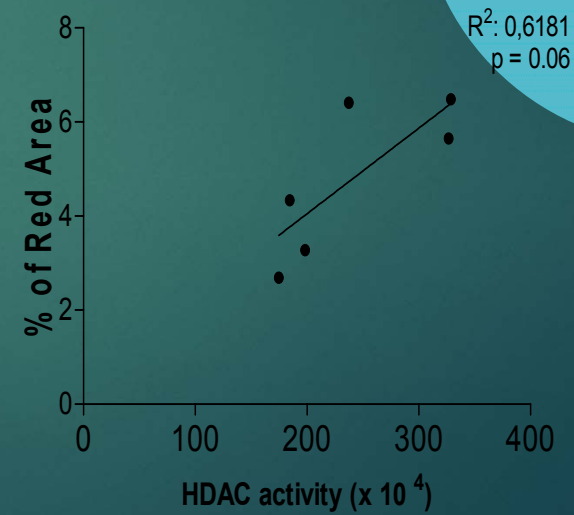
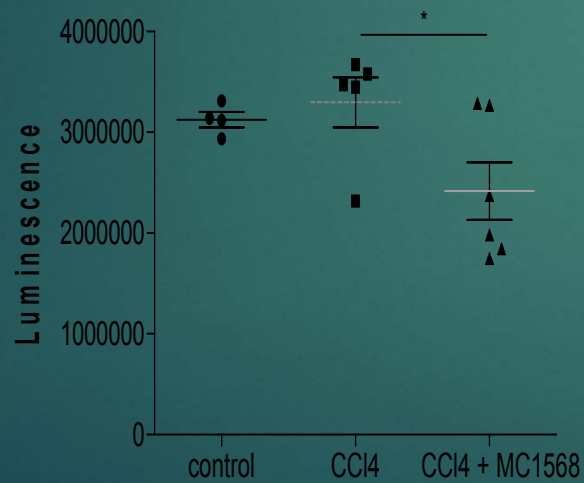
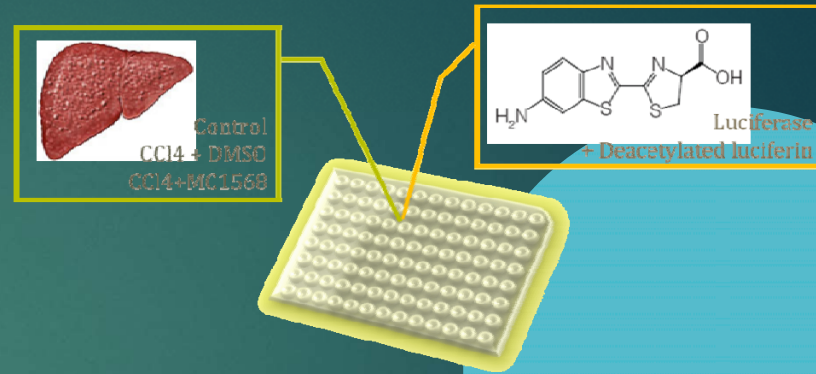


Variations due to difference in inhibition level of HDAC-activity? => Activity assay



# HDAC inhibition reduced fibrogenesis

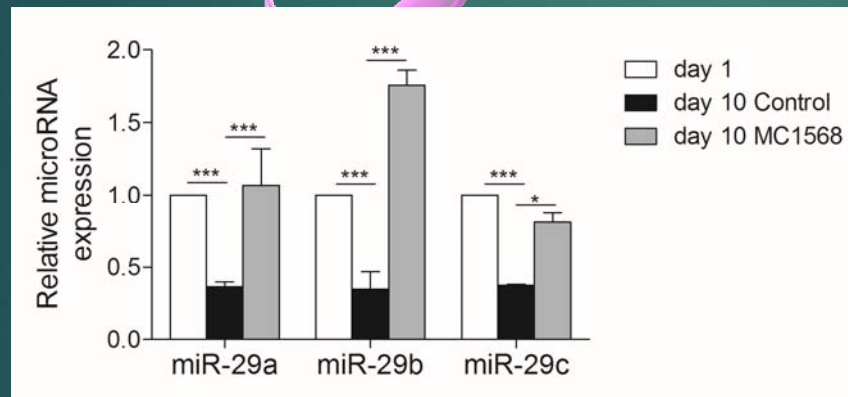
- Role for HDACs
  - Class II inhibitor MC1568





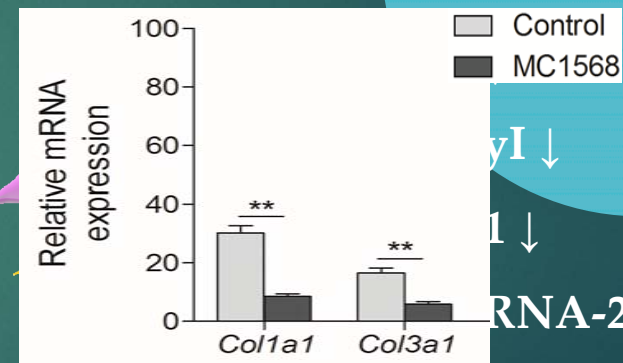
# Class II HDAC inhibition induces miR-29 expression

- Role for HDACs
- Role for microRNAs
  - Regulation of microRNAs by epigenetic modifications



Quiescent  
HSCs

HDAC-I

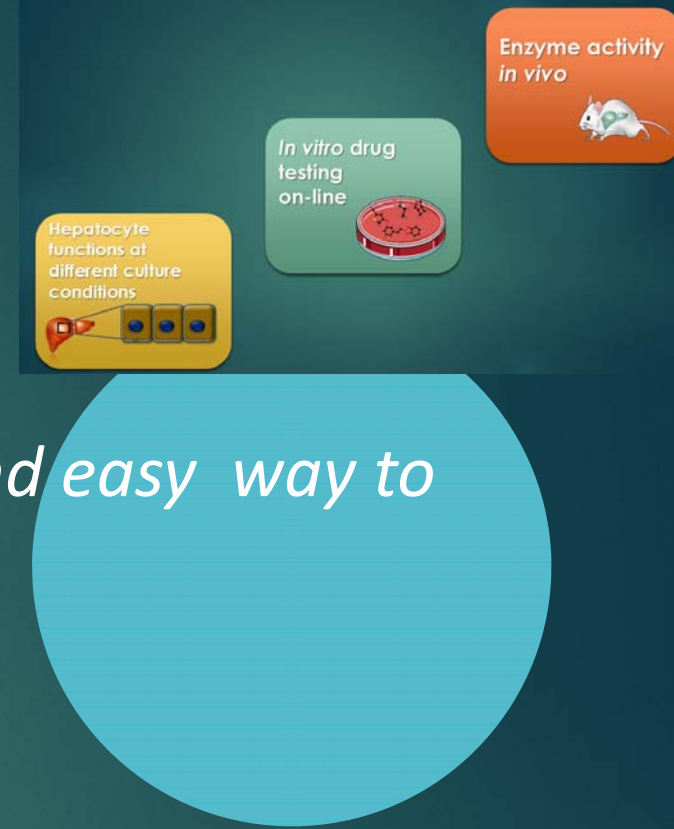


Activated  
HSCs

miR-29 ↑

# Conclusion

*Luminescence assays are a reliable, fast and easy way to*  
*-test and optimize in vitro cultures*  
*-evaluate compound toxicity in vitro*  
*-HDAC activity at the organ level*



# Acknowledgments

## Head of the Group

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## Post-Docs

Laurent Dolle  
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Sofia B. Leite

## PhD Students

Leslie Stradiot  
Adil El Taghdouini  
Tiffany Roosens  
Lien Thoen  
Joeri Lambrecht  
Stefaan Verhulst

## Master student

Maryam Marefati

## Administrative and Technical Staff

Jean-Marc Lazou  
Chris Derom  
Danielle Blyweert  
Nathalie Eysackers  
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(LIVR)

Leslie Stradiot

Boss

Inge Mannaerts





Thank you



QUESTIONS?