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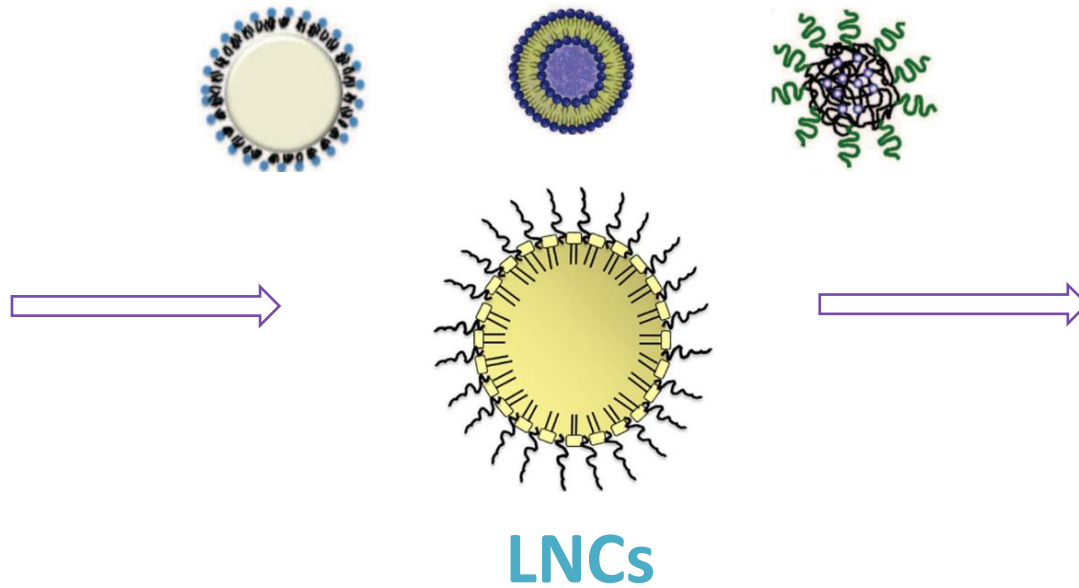
# *In vitro* evaluation of acute and chronic liver toxicity of lipid nanocapsules (LNCs)

Flavien Delaporte

Emilie Roger, Frédéric Lagarce, Camille Savary

MINT (Micro et Nanomédecines Translationelles) UMR 1066 CNRS 6021

# Context



Better kinetics  
Decrease side effects  
Fonctionalization/targeting

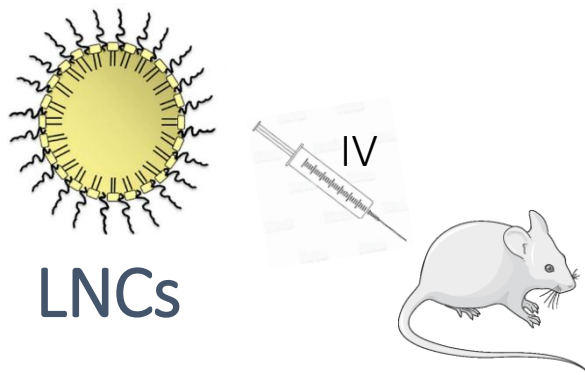
**Cancers**

**Chronic therapy**

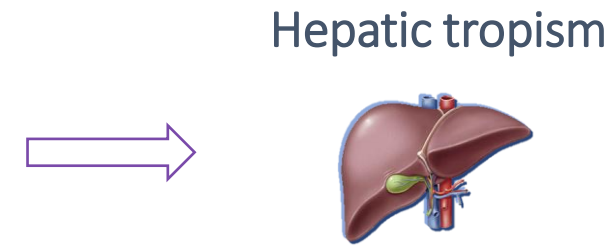
# Context



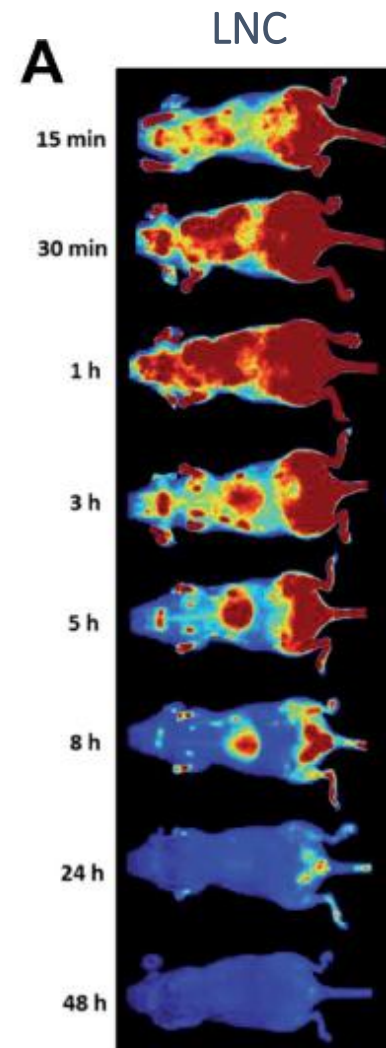
Ovarian  
Breast  
Glioblastoma  
Melanoma



LNCs as treatment  
for hepatic cancer?



Need for hepatic  
toxicity studies



# Objective



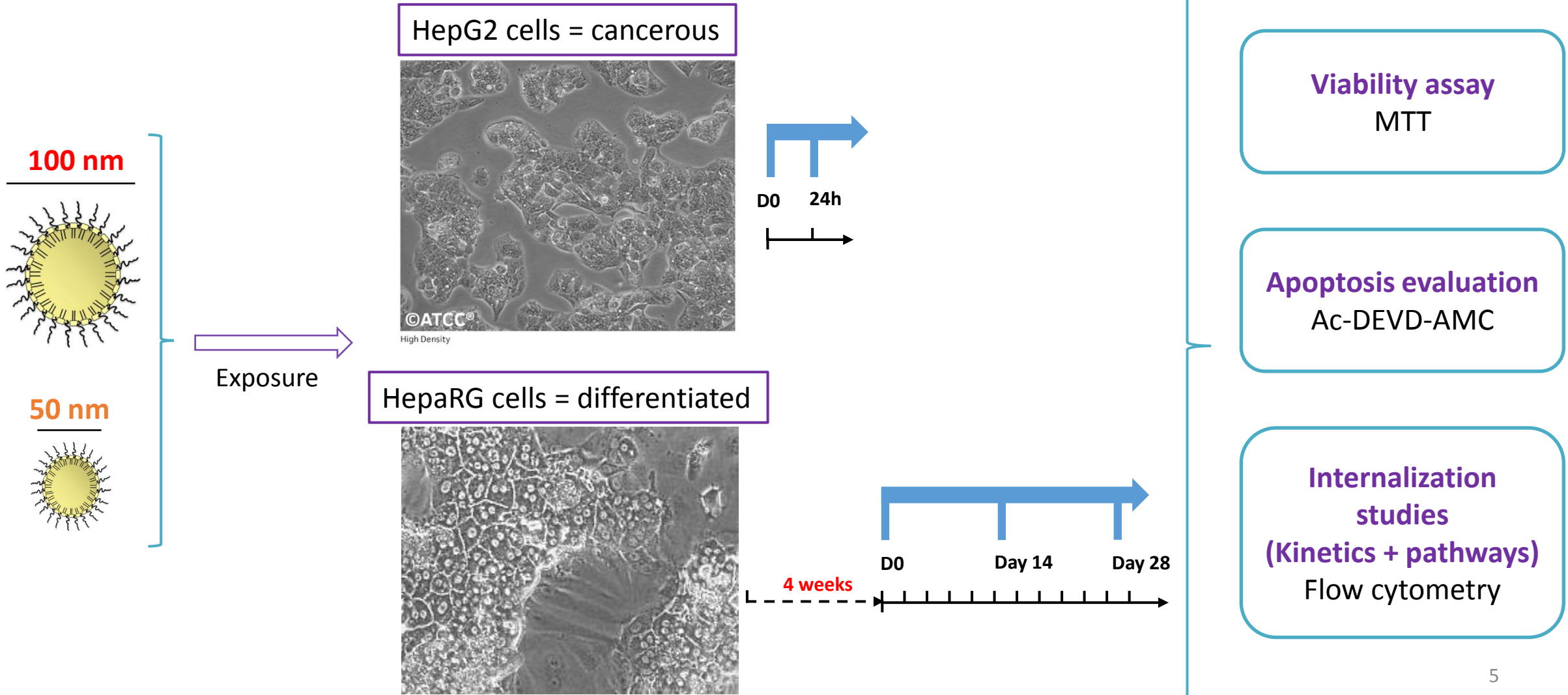
Few studies on  
hepatotoxicity



*In vitro* evaluation of acute and chronic liver toxicity of  
**LNCs**

1. Is there a **size effect** on LNCs's toxicity?
2. Are there **chronic effects** after repeated exposure?

# Methods



# Objective



Few studies on  
hepatotoxicity



*In vitro* evaluation of acute and chronic liver toxicity of  
**LNCs**

1. Is there a **size effect** on LNCs's toxicity?
2. Are there chronic effects after repeated exposure?

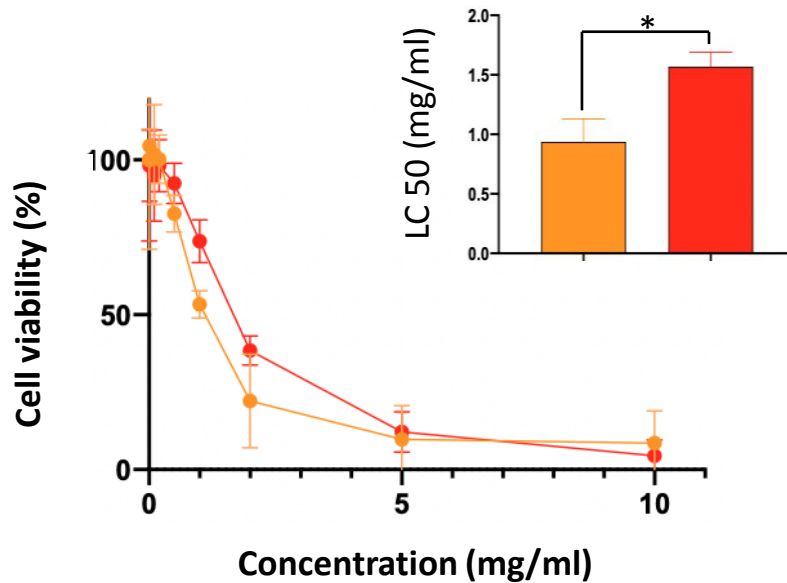
# Results: is there a size effect?



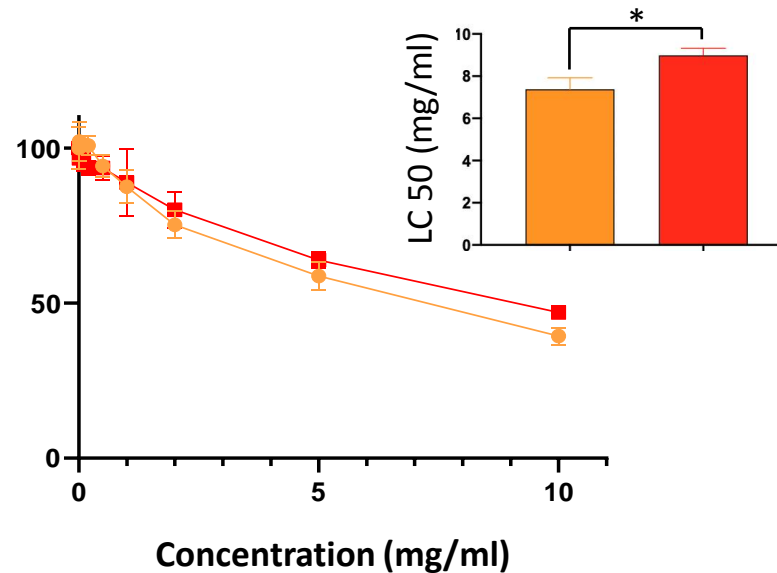
Viability on **HepG2 cells** (n = 4)

Viability on **HepaRG cells** (n = 3)

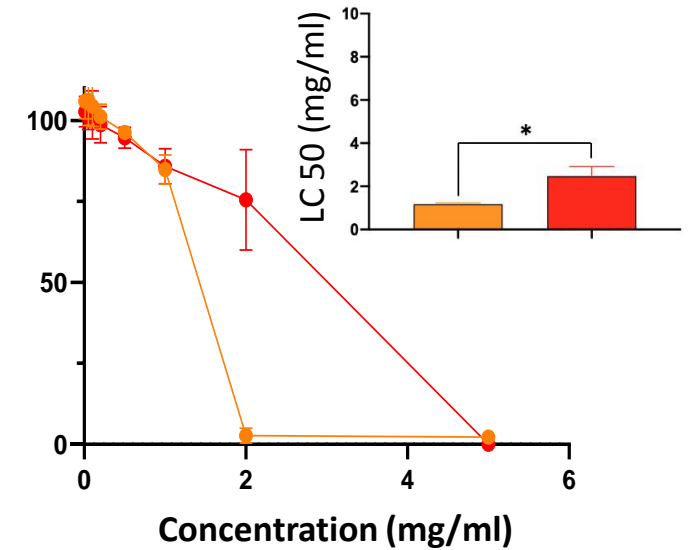
24h treatment



24h treatment



28D treatment



- Captex® 50 nm
- Captex® 100 nm

Higher toxicity induced by **LNCs Captex® 50 nm** in both cell lines

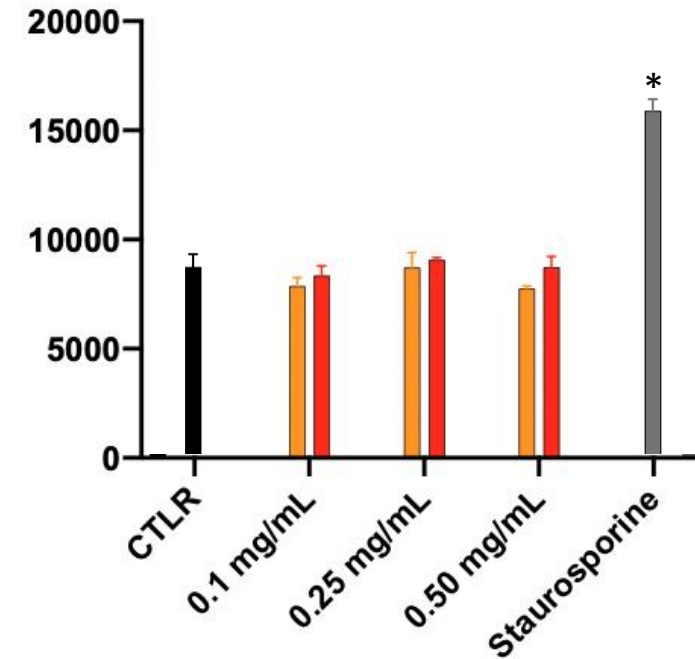
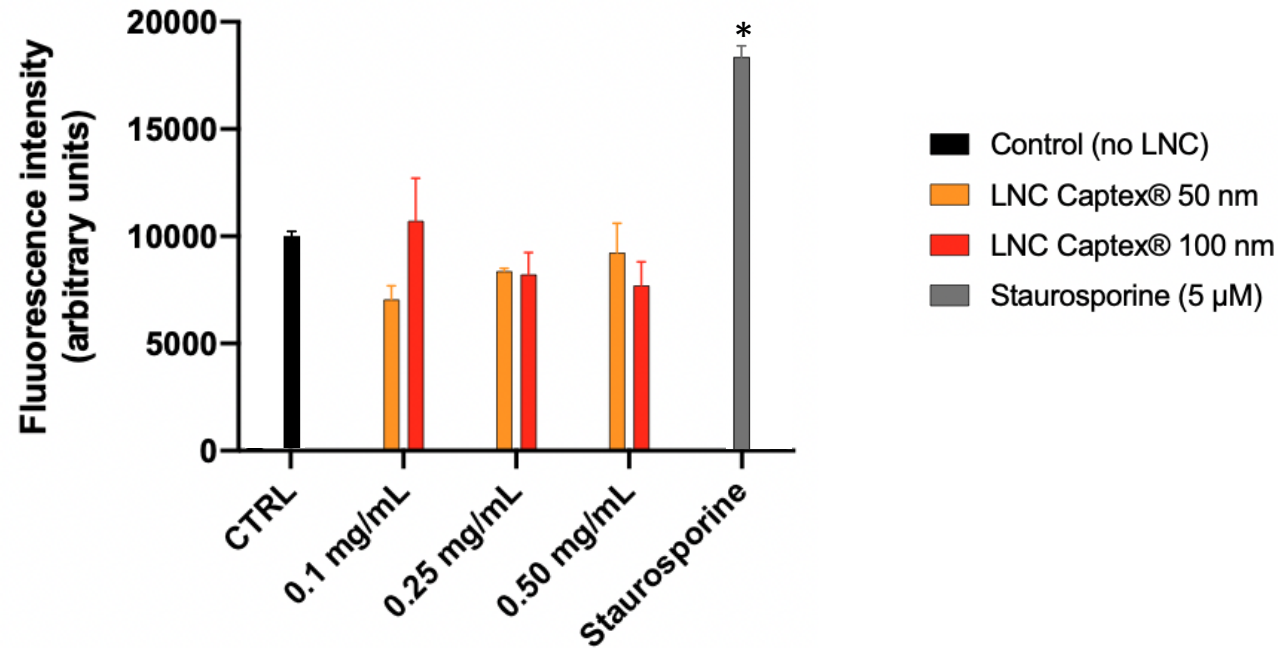
# Results: is there a size effect?



Assessment of caspase 3 activity (Ac-DEVD-AMC assay) after 24h of exposure

HepG2 cells (n = 4)

HepaRG cells (n = 3)



No apoptosis was triggered by LNCs exposure at these concentrations

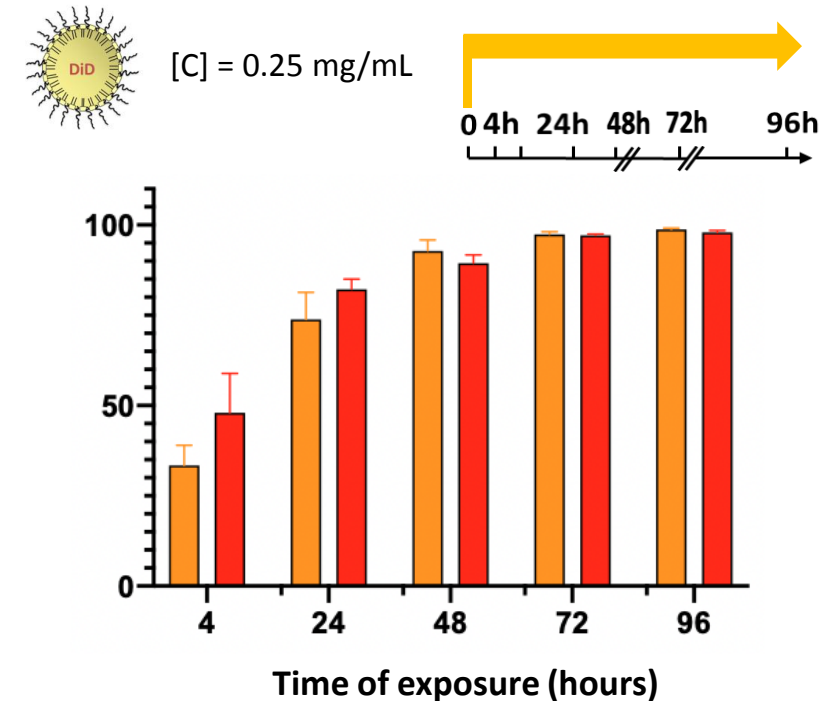
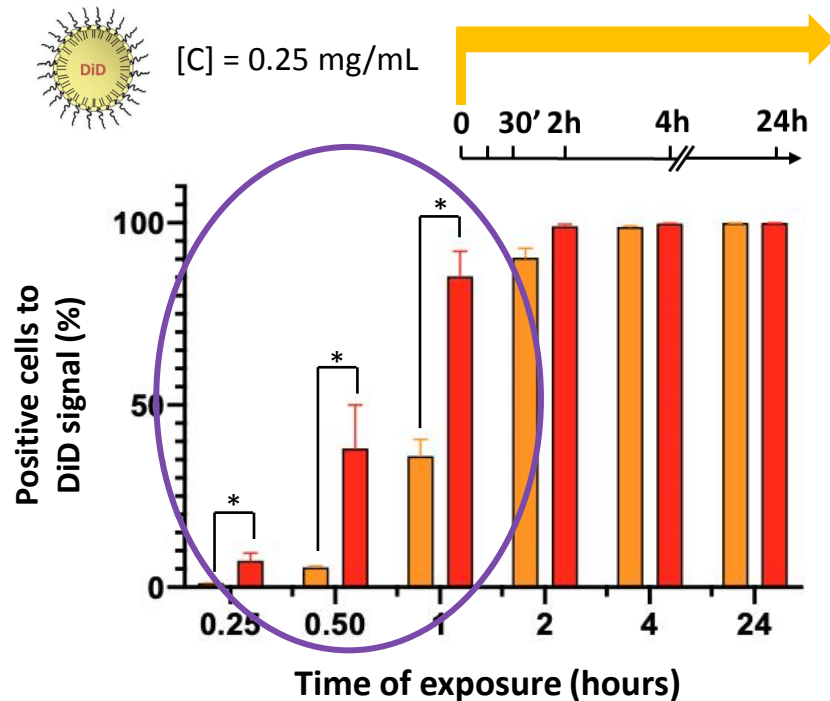
# Results: is there a size effect?



## Kinetics of internalization (% of positive cells)

### HepG2 cells (n = 3)

### HepaRG cells (n = 3)



LNCs Captex® 100 nm were internalized faster in HepG2 cells

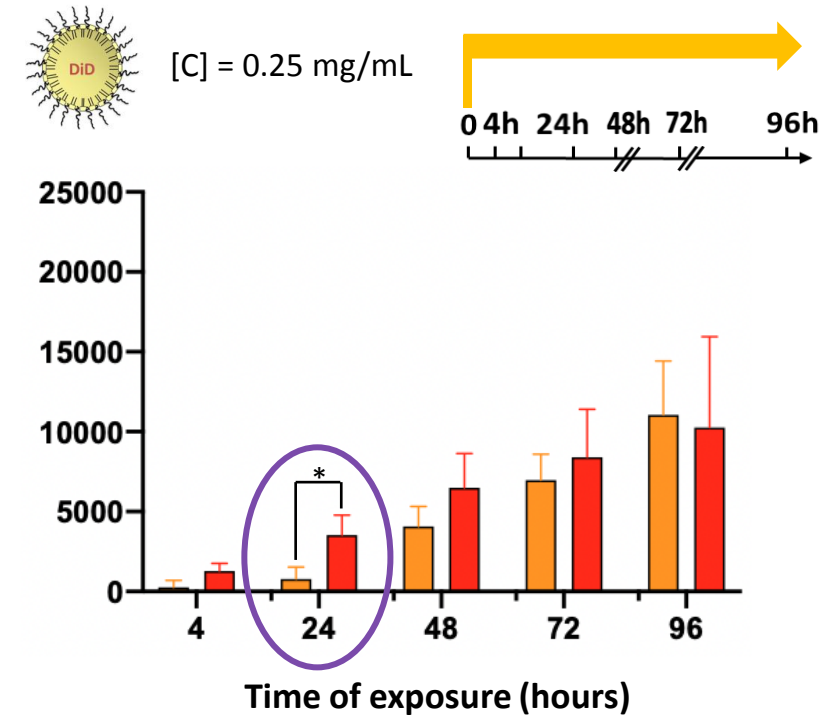
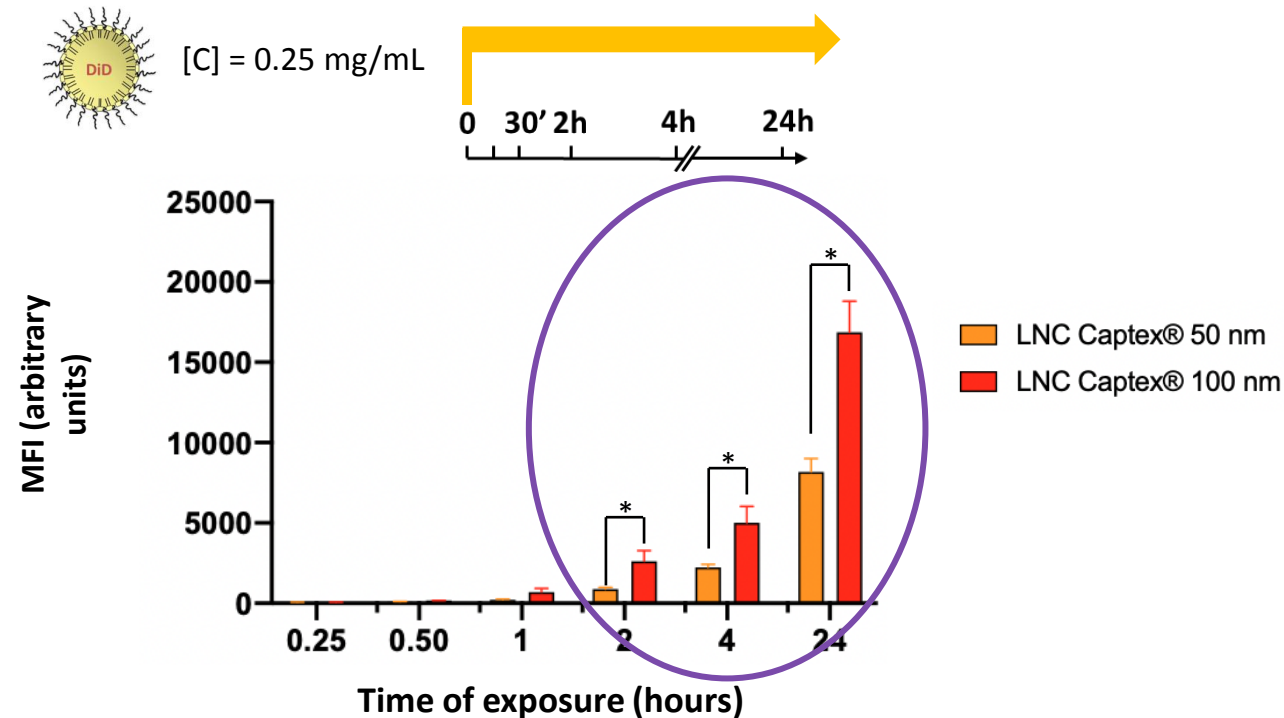
# Results: is there a size effect?



Kinetics of internalization (MFI = mean fluorescence intensity)

HepG2 cells (n = 3)

HepaRG cells (n = 3)



LNC Captex® 100 nm accumulate more than  
LNC Captex® 50 nm in HepG2 cells

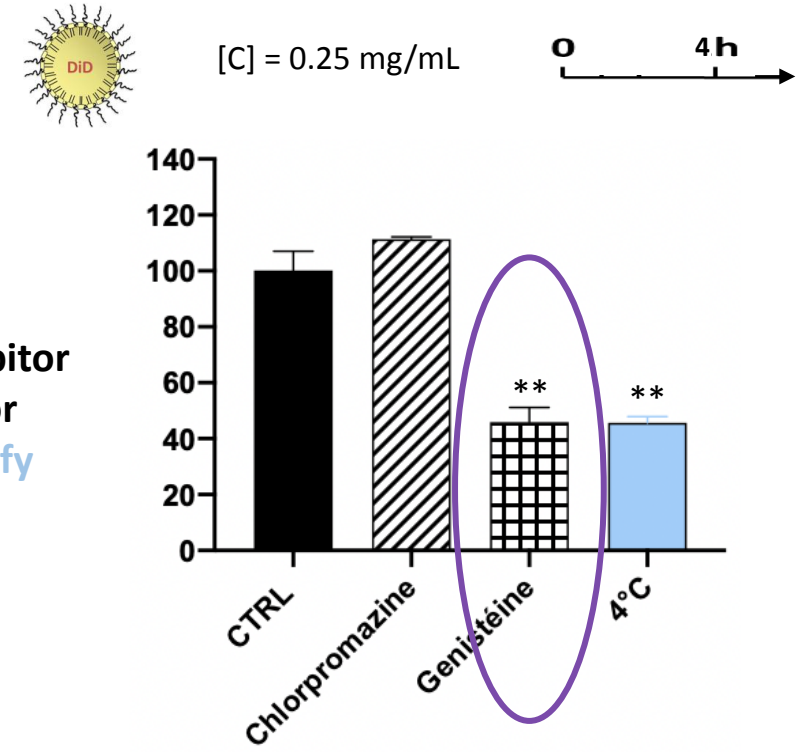
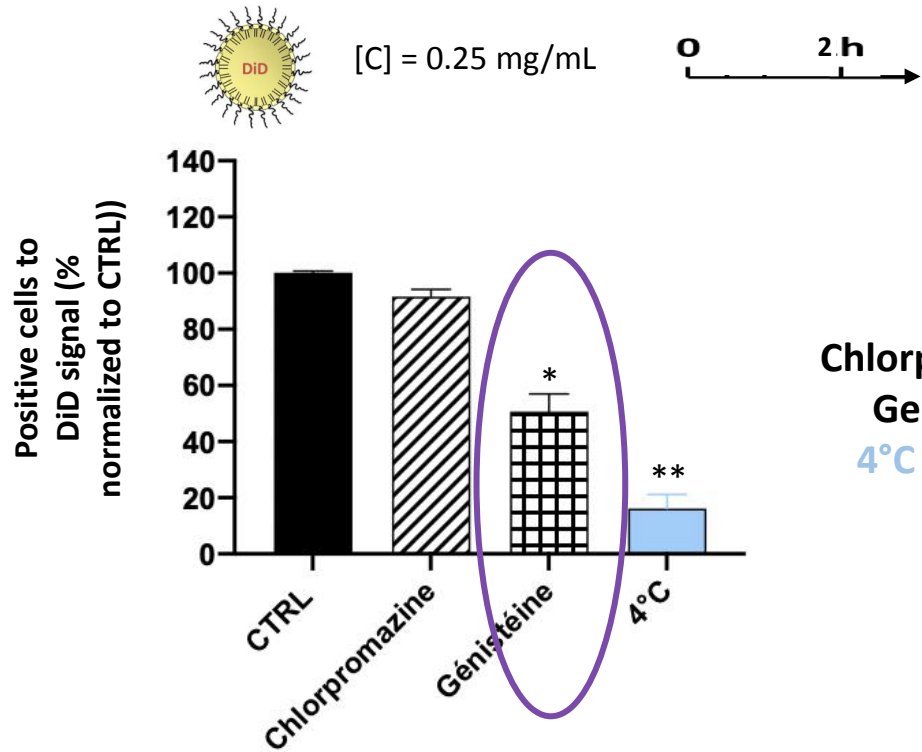
# Results: is there a size effect?



## Pathways of internalization

HepG2 cells (n = 3)

HepaRG cells (n = 3)



LNCs 50 nm

Chlorpromazine = clathrin-endocytosis inhibitor  
 Genistein = caveolin-endocytosis inhibitor  
 4°C condition = low metabolism and rigidify membranes

LNCs enter mainly via caveolin-mediated endocytosis in both models



Few studies on  
hepatotoxicity



*In vitro* evaluation of acute and chronic liver toxicity of  
**LNCs**

1. Is there a size effect on LNCs's toxicity?
2. Are there **chronic effects** after repeated exposure?

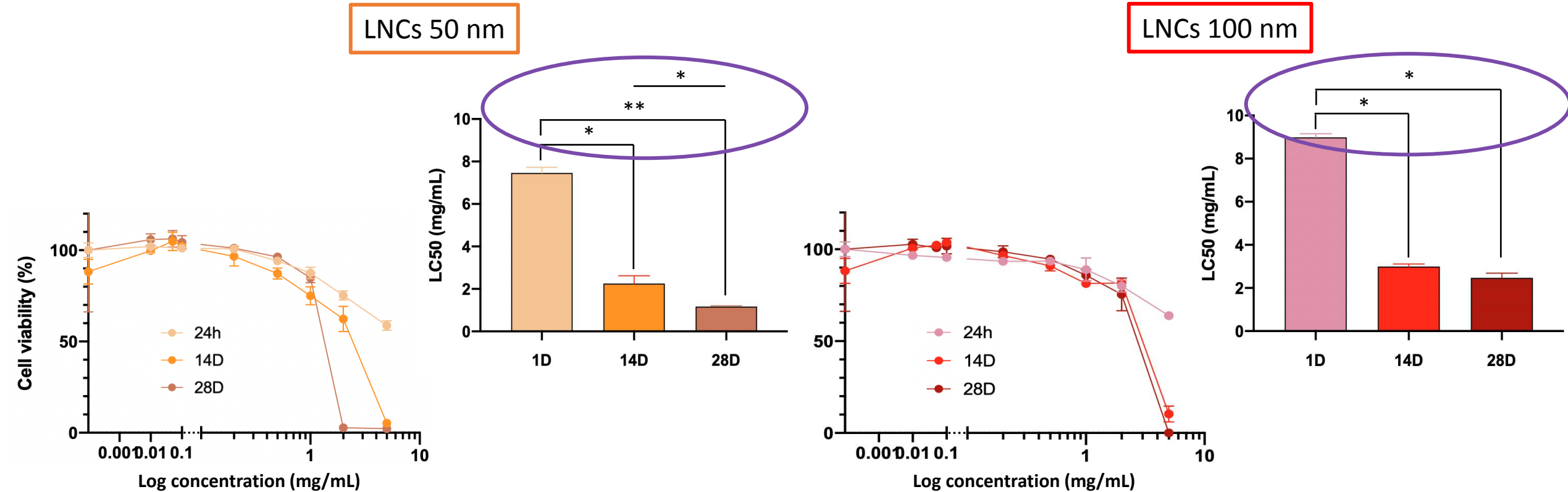
# Results: are there chronic effects?



MTT on HepaRG cells (n = 3)

LNCs 50 nm

LNCs 100 nm

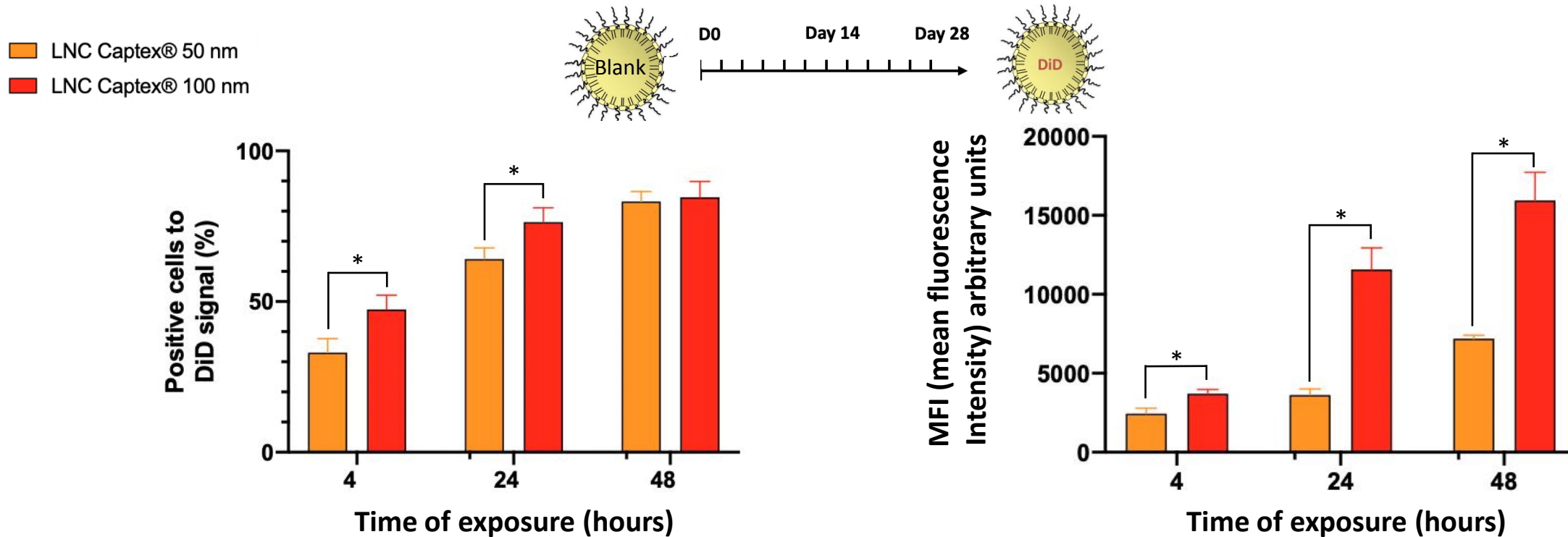


Time effect for **50 nm LNCs** – Stability after 2 weeks for **100 nm LNCs**

# Results: are there chronic effects?



Kinetics of internalization after **28 days of exposure** on HepaRG cells (n = 3)

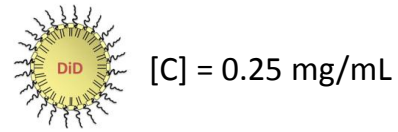


**100 nm LNCs** are still internalized faster  
&  
accumulate much more than **50 nm ones**

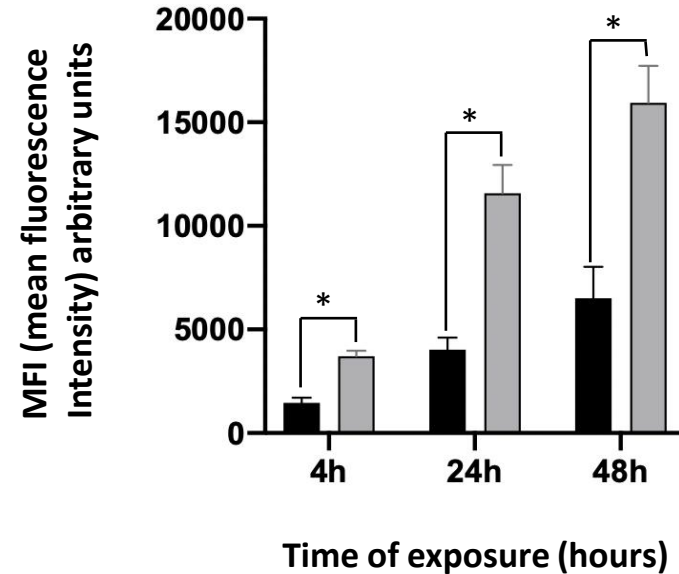
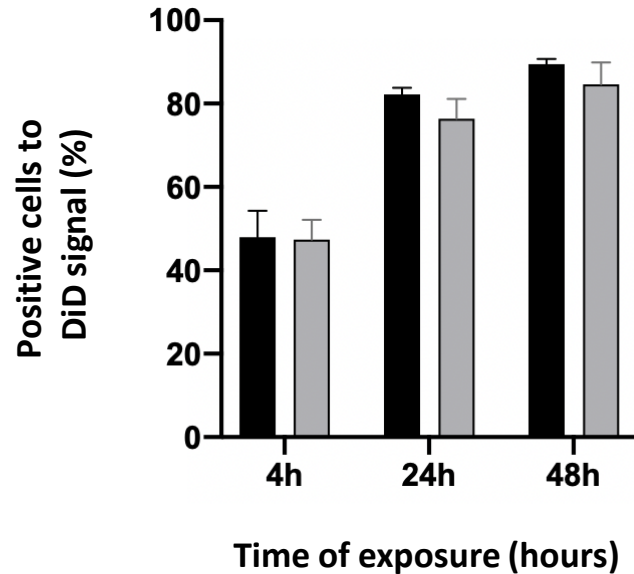
# Results: are there chronic effects?



Comparison of LNCs' internalization **acute vs chronic** on HepaRG cells (n =3)



LNCs 100 nm



No difference in % of positive cells but much more accumulation after a 28 days chronic exposure

# Results: cancerous VS differentiated?

(HepG2)

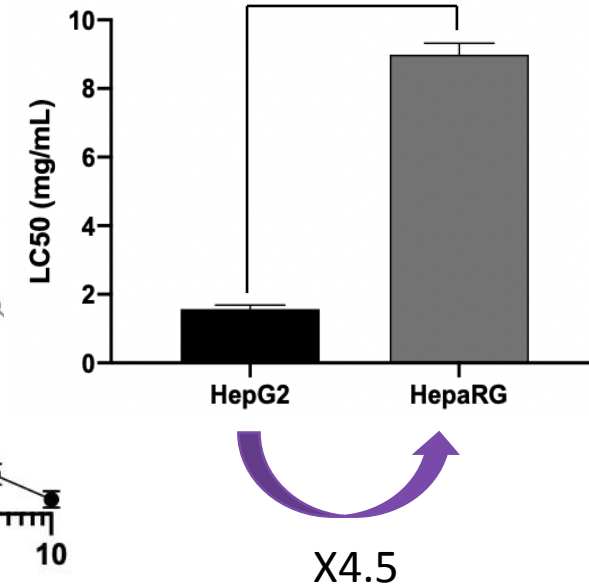
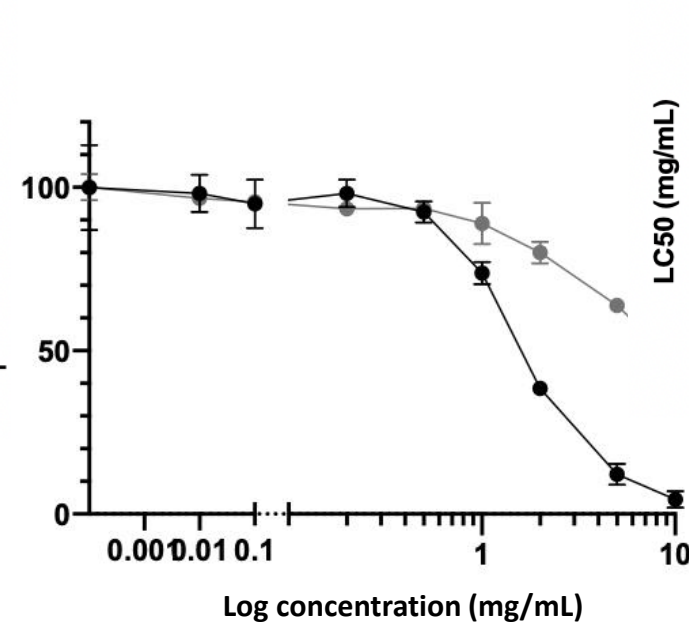
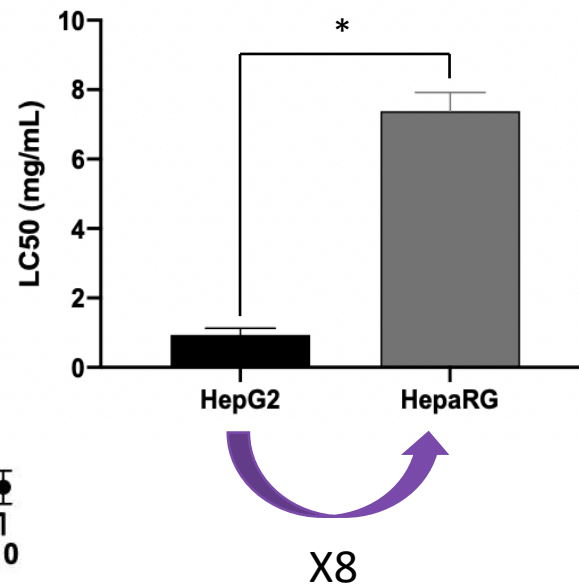
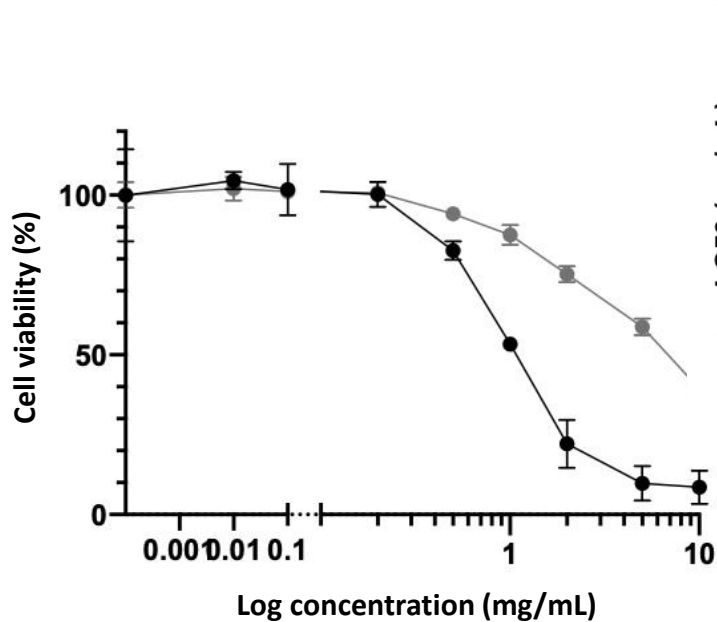
(HepaRG)



MTT after 24h of exposure (n = 3)

LNCs 50 nm

LNCs 100 nm



Higher toxicity of LNCs on cancerous model, especially 50 nm LNCs

# Results: cancerous VS differentiated?

(HepG2)

(HepaRG)



Kinetics of internalization (n = 3)

% of positive cells

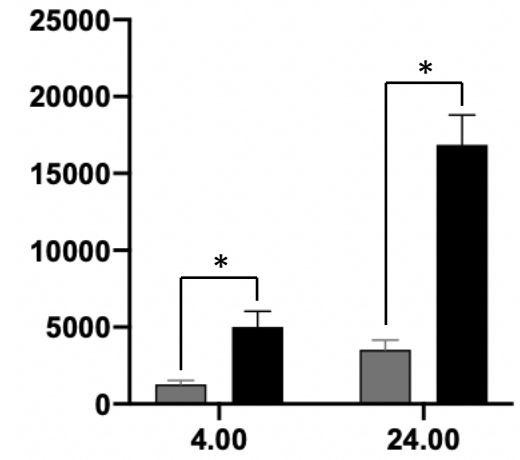
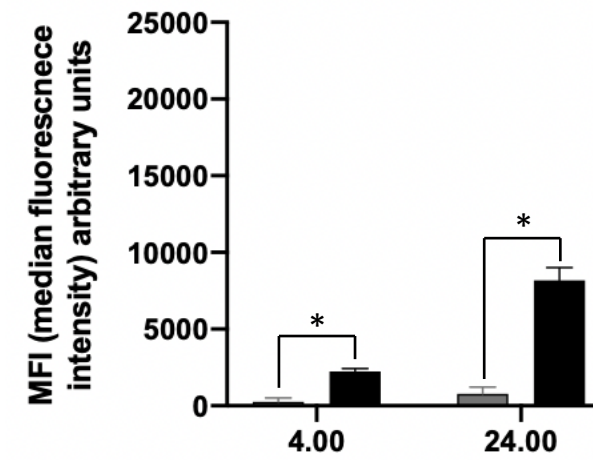
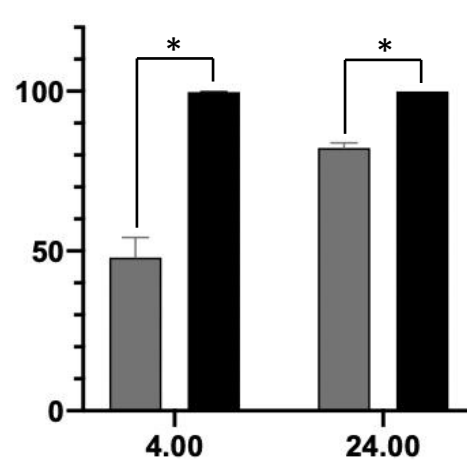
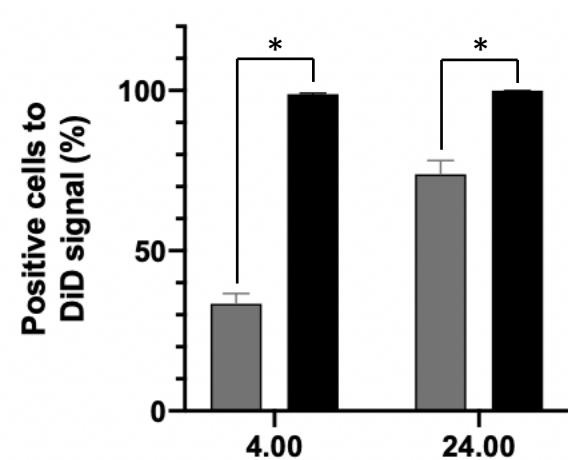
MFI

LNCs 50 nm

LNCs 100 nm

LNCs 50 nm

LNCs 100 nm



Time of exposure (hours)

Time of exposure (hours)

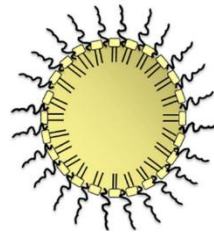
■ HepaRG  
■ HepG2

Both LNCs' sizes were internalized faster in HepG2 cells and also accumulated much more

# Conclusion / perspectives



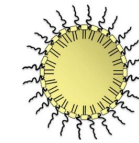
Is there a **size effect**?



**100 nm LNCs**



Quicker internalization with a lower toxicity



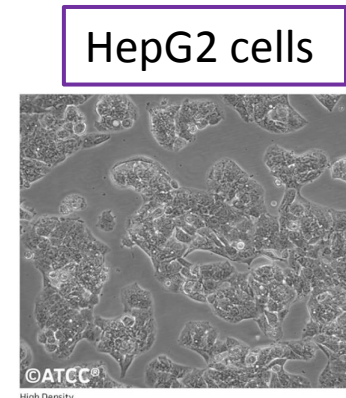
**50 nm LNCs**

Higher accumulation with a lower toxicity

Are there **chronic effects**?

## ❖ Common points

- 1) No size neither chronic effects for internalization pathways
- 2) No apoptosis & no size effect



# Acknowledgments



## Mint laboratory

**Camille Savary  
Emilie Roger  
Samuel Legeay  
Frédéric Lagarce**



## Funding

**La ligue contre le cancer 49**

## Platforms

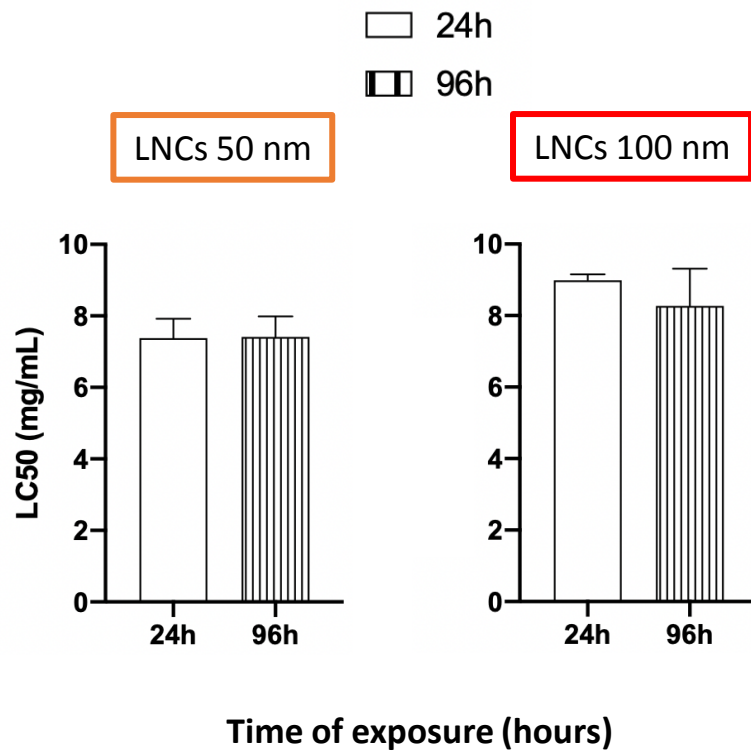
**Catherine Guillet (PACEM)**

# Supplementary datas

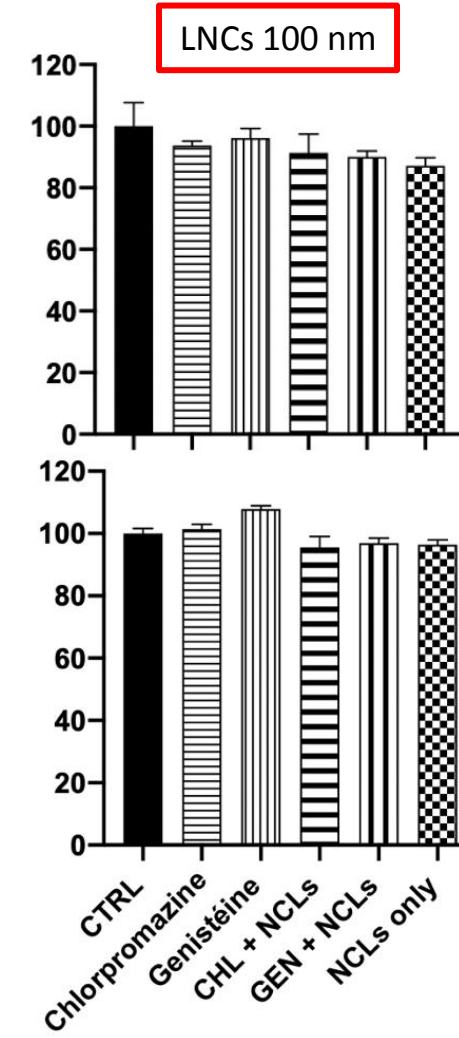
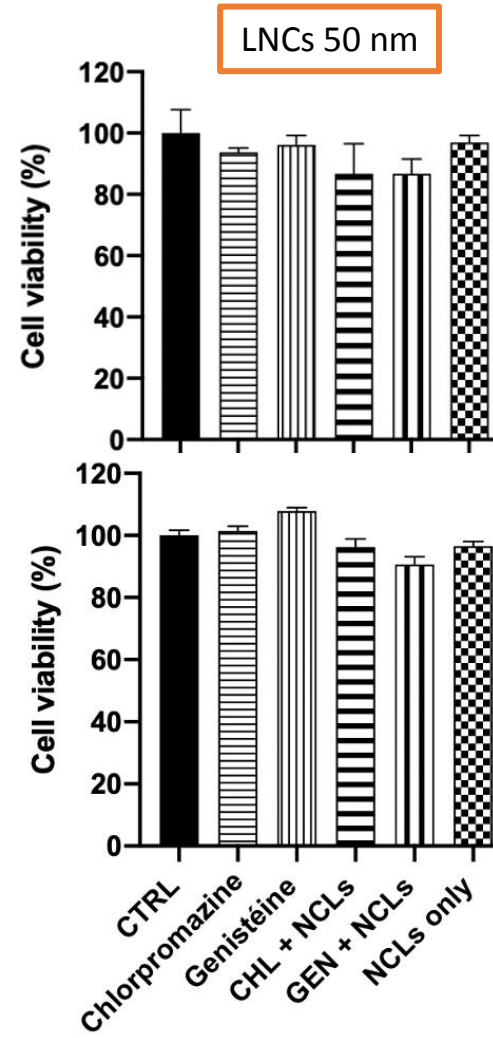


MTT on HepaRG cells (n = 3)

Pathways of internalization → toxicity of inhibitors by MTT (n = 3)



No difference observed



HepG2 cells

- CTRL
- ▨ Chlorpromazine (CHL)
- ▩ Genistéine (GEN)
- ▧ CHL + LNCs
- ▦ GEN + NCLs
- ▤ LNCs only

HepaRG cells