In vitro models for the assessment of antisense oligonucleotide induced hepatotoxicity

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Modeling hepatotoxicity *in vitro*
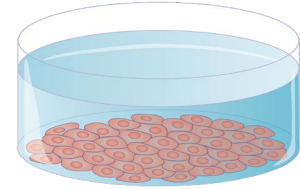
The Problem

- Overcoming hepatotoxicity is a known challenge for the development of therapeutic oligonucleotides[1]

- We aim to develop a scalable system for early & efficient assessment of hepatotoxicity
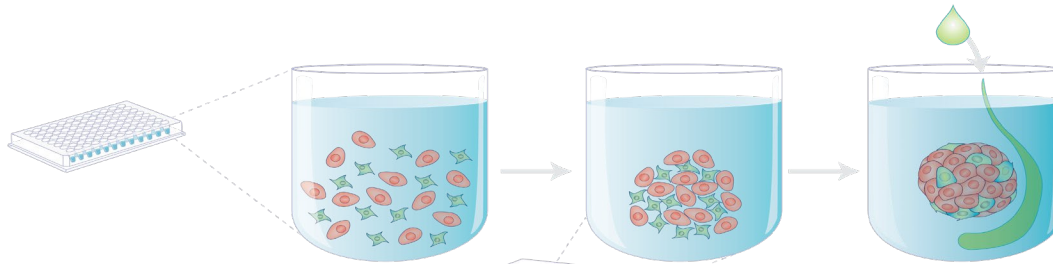
In vivo mouse models

- Sensitive model
- Expensive
- Not scalable

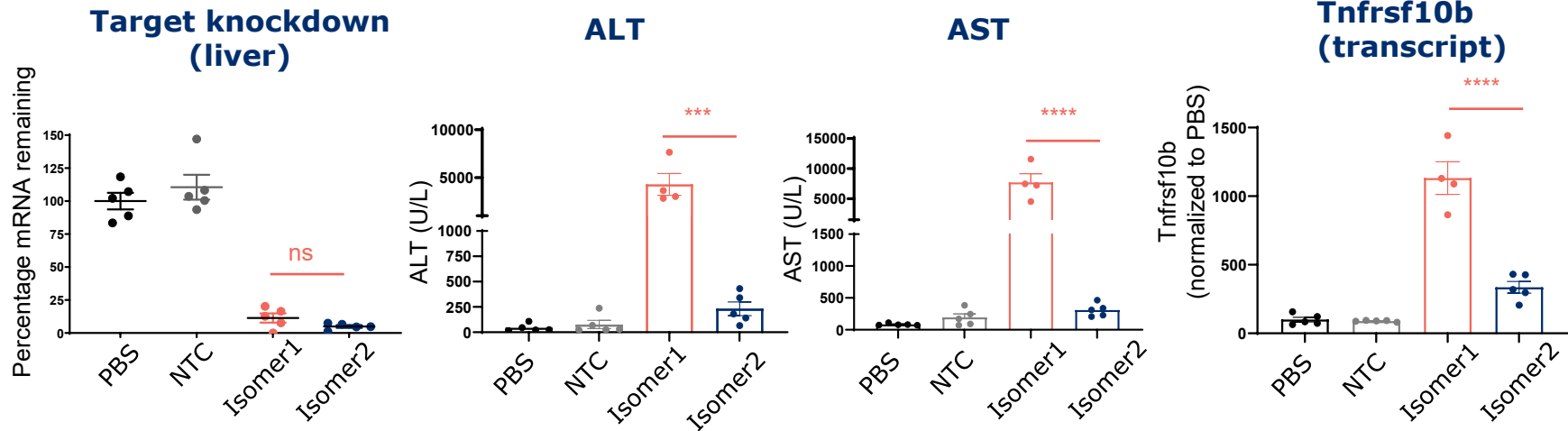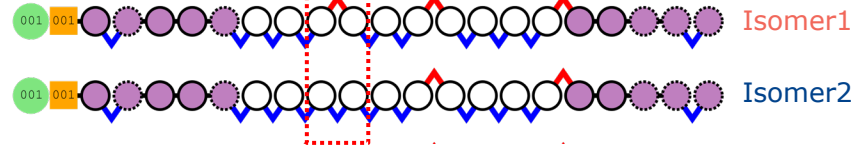## *In vitro* 2D model
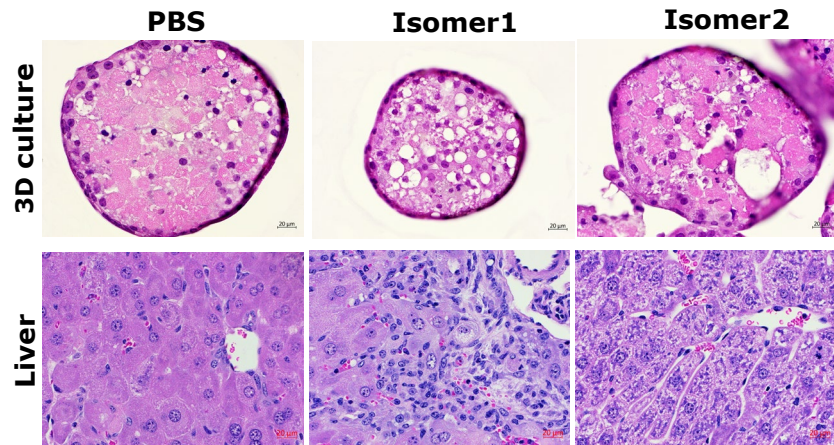
In vitro 3D model

[1]Frazier, Toxicol Pathol 2015

Stereoisomers have similar pharmacodynamic effects but different hepatotoxicity profiles in mice
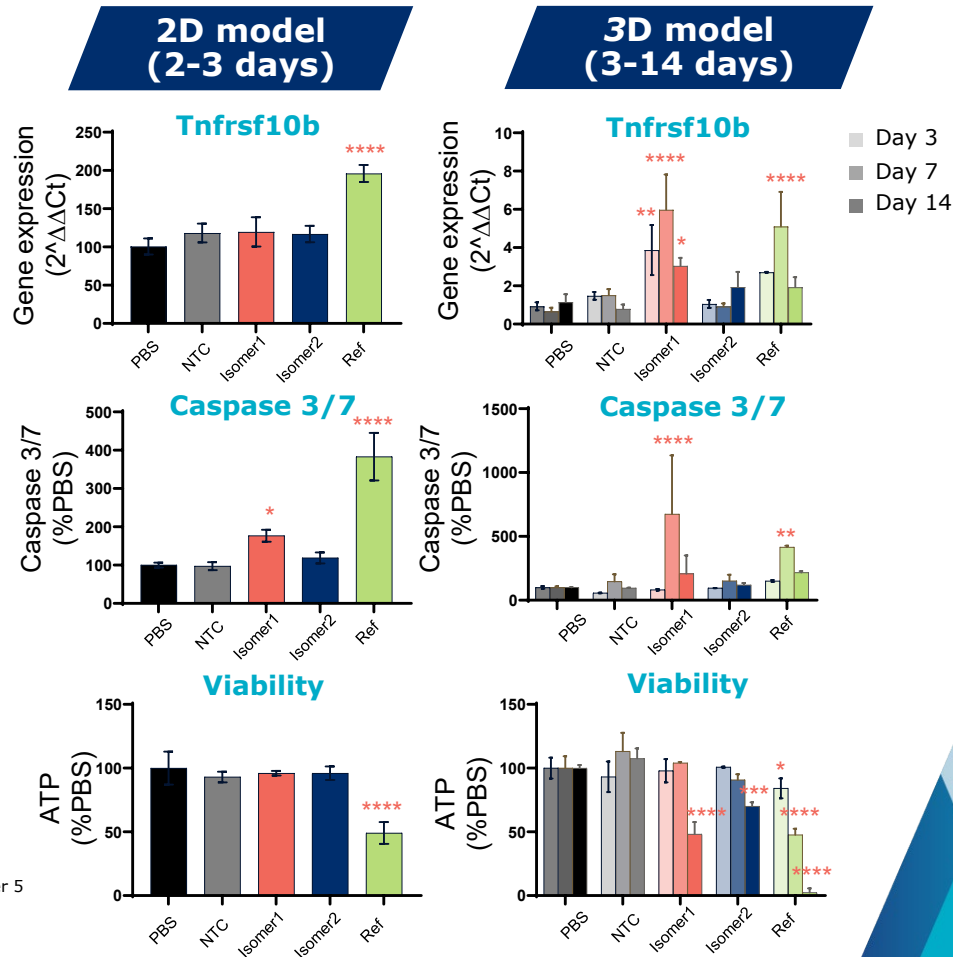
GalNAc-conjugated stereoisomers

Isomer1

Isomer2

Target knockdown (liver)

ALT

AST

Tnfrsf10b (transcript)

3D model identifies acute & long-term cytotoxicity

3D culture — PBS, Isomer1, Isomer2 (H&E stain of 3D model)

Liver — PBS, Isomer1, Isomer2 (mouse liver tissue)

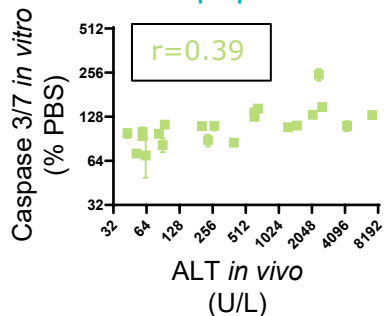|	PBS	NTC	Isomer1	Isomer2	Ref
Serum ALT (U/L)	43	59	4280	262	2368

- 2D model detects acute (3 days, Ref) cytotoxicity
- 3D model detects acute (Ref) & long-term (7-14 days, Isomer1) cytotoxicity

2D model (2-3 days)

Tnfrsf10b — Gene expression ($2^{\wedge}\Delta\Delta Ct$): PBS, NTC, Isomer1, Isomer2, Ref ****

Caspase 3/7 (%PBS): PBS, NTC, Isomer1 *, Isomer2, Ref ****

Viability — ATP (%PBS): PBS, NTC, Isomer1, Isomer2, Ref ****

3D model (3-14 days)

Day 3, Day 7, Day 14

Tnfrsf10b — Gene expression ($2^{\wedge}\Delta\Delta Ct$): Isomer1 ** **** *; Ref ****

Caspase 3/7 (%PBS): Isomer1 ****; Ref **

Viability — ATP (%PBS): Isomer1 ****; Isomer2 *** ; Ref * **** ****

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(Left) H&E stain of 3D model (top) and mouse liver tissue (bottom) 3 days after 5 mpk dosage at Day 1, 3, 5, 8. NTC: Non-targeting control; Ref: Reference hepatotoxic oligonucleotide (Sewing et al., 2016 PLoS One); Stats: One-way ANOVA for 2D, Two-way ANOVA for 3D; PBS phosphate buffered saline

3D hepatic models demonstrate superior *in vitro-in vivo* correlation than 2D model

2D model

Apoptosis

r=0.39

Caspase 3/7 *in vitro* (% PBS) vs ALT *in vivo* (U/L)

Viability

r=-0.27

ATP *in vitro* (% PBS) vs ALT *in vivo* (U/L)

3D models

Apoptosis

■ Hep+NPC
■ Hep+MEF

r=0.71
r=0.57

Caspase 3/7 *in vitro* (% PBS) vs ALT *in vivo* (U/L)
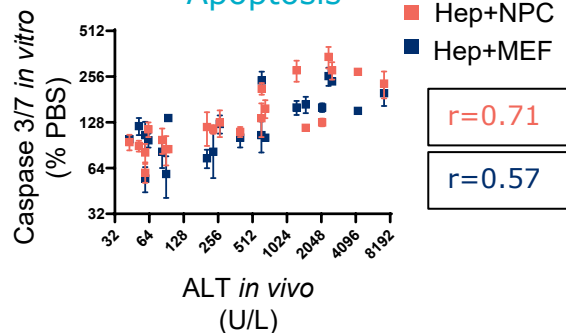
Viability

r=-0.66
r=-0.37

ATP *in vitro* (% PBS) vs ALT *in vivo* (U/L)
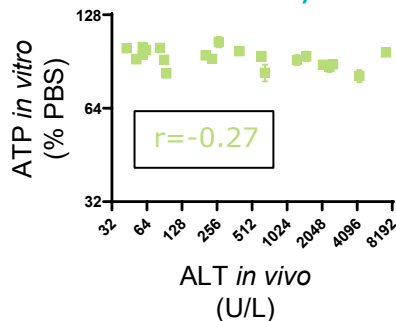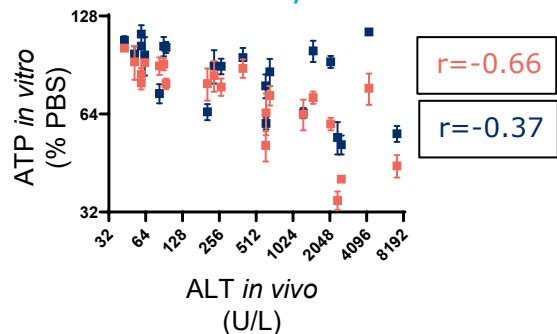
- 3D models are better correlated to *in vivo* hepatotoxicity than 2D models

- Inclusion of NPCs improves *in vivo* correlation compared with MEFs

- 3D models may be a sensitive, affordable and scalable model for predicting hepatotoxicity

In vitro toxicity data from 2D (left) or 3D (center) models are plotted with respect to in vivo ALT measurements for the same series of oligonucleotides.
r, Pearson's r; Hep, hepatocyte; NPC, non-parenchymal cell; MEF mouse embryonic fibroblast

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