GENE EXPRESSION ANALYSIS OF THE LIVER-HUMANIZED FRGN KO NASH MOUSE MODEL

Lander Foquet[1], Bin Li[2], Edward Henson[1], Robert Copenhaver[1], Markus Grompe[1,2].
[1]Yecuris, Tualatin, Oregon, United States
[2]Oregon Health and Science University, Portland, Oregon, United States

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INTRODUCTION

NASH progression and fibrosis can vary widely between humans and rodent models, making pre-clinical validation difficult and costly.
In addition, the current models use artificial means to induce only specific components of the disease. These manipulations do not target the biological pathways that contribute to disease progression and are the targets of pharmacological intervention in humans.

AIM

Building on the liver-humanized **FRG** KO model[1], we previously established an in vivo **NAFLD/NASH** model, using only a diet with high levels of fat, sugar, and cholesterol and without additional chemical inducers or nutritional deficiencies.
We transplanted mice with a homozygous **M148I PNPLA3** donor to study the importance of specific genotypes on the development of **NASH**.

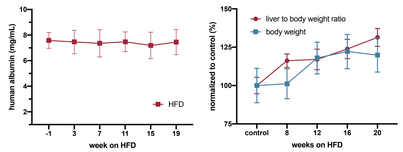
MATERIAL & METHODS

FRG KO/NOD mice were transplanted with human hepatocytes from a female donor homozygous for the G allele on the minus strand of **rs738409** in **PNPLA3**. This **M148I** variant is significantly associated with a high risk of NASH development[2].
After near complete repopulation of the liver with human hepatocytes (13 weeks), three animals per group/time point received either a **control diet** (5LJ5) or a diet with **40 kcal% Fat** (soybean oil and Primex), **20 kcal% fructose**, and **2% cholesterol** (**HFD**; A16091202 from Research Diets).
RNAseq was performed and human and mouse transcripts were separated in silico. Gene Set Enrichment Analysis (GSEA) revealed which functional pathways changed by the HFD.
As a proof-of-concept for treatment, liver-humanized **FRG** KO mice repopulated with hepatocytes carrying the **PNPLA3** mutation, were dosed with **MGL-3196** for eight weeks (6 animals dosed, 4 controls).

MODEL DEVELOPMENT

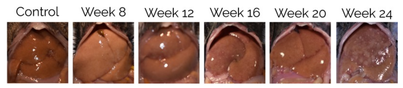
Human hepatocytes with the **PNPLA3** mutation were able to repopulate **FRG** KO/NOD livers up to 95%. Both **control** and **NASH**-induced liver-humanized animals remained highly repopulated over the course of the study.

Bodyweight and liver to bodyweight ratios increased in humanized mice on **HFD** compared to those on the **control diet (controls)**.

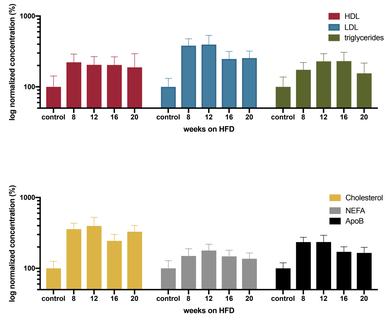


Visually, no adverse effects were observed between mice fed the **control** diet or **HFD**.

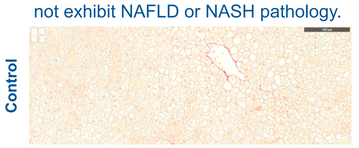
By gross pathology, humanized livers progressed to a **highly fatty state** during the study.



A human-like **LDL/HDL ratio** profile (>1.6) was observed in liver-humanized mice compared to WT mice[3]. The lipid levels of humanized mice on **HFD** were higher than in humanized controls on regular diet.

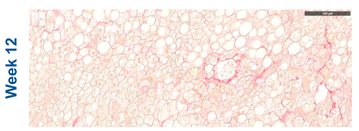


Humanized mice on **control** diet did not exhibit NAFLD or NASH pathology.

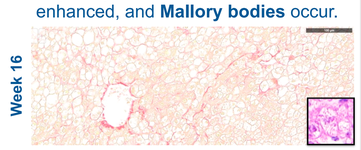


Simple steatosis is pronounced at 8 weeks after starting **HFD**.

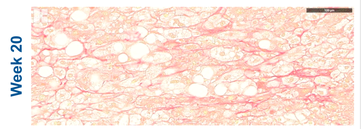
Hepatocyte **ballooning** and perivenular/ pericellular fibrosis become evident.



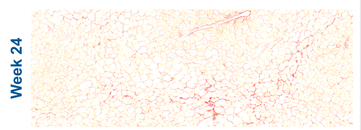
Hepatocyte ballooning and fibrosis are enhanced, and **Mallory bodies** occur.



Collagen deposition within the hepatic sinusoids is apparent.

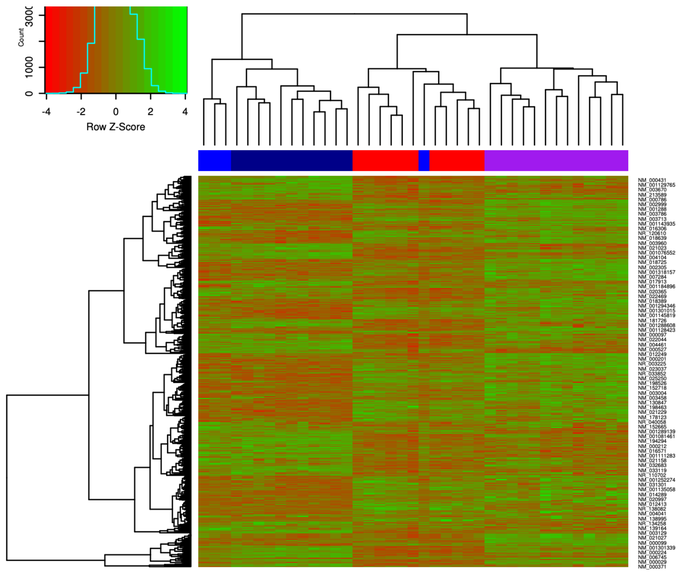


Bridging fibrosis is clear.



RNAseq ANALYSIS

GSEA showed transcriptomic changes in control liver-humanized mice and animals receiving HFD for 12 weeks (purple) or 16-24 weeks (red). The derangement is intermediate by week 12 and severe at the later timepoints. These results were comparable to human RNAseq data from controls vs NAFLD or NASH[4]. in contrast, murinized control mice fed the HFD do not overlap with human or liver-humanized mouse data (not shown).

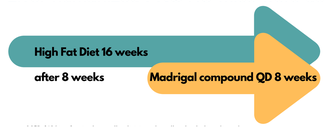


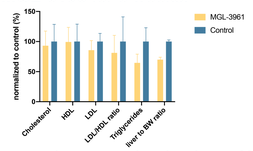
INTERVENTION STUDY RESULTS

MGL-3196 is a **thyroid hormone receptor (THR) ß-selective agonist**.

Pre-clinical, toxicology, Phase 1 and Phase 2 clinical data suggest **MGL-3196** has a good profile as a treatment for **NASH** and dyslipidemias. Patients treated with MGL-3196 achieved a two-point reduction in **NAS** (NAFLD activity score) on biopsy and had sustained, significant reductions in liver fat.

Liver-humanized **FRG** KO mice were dosed with 10mg/kg **MGL-3196** for 8 weeks.



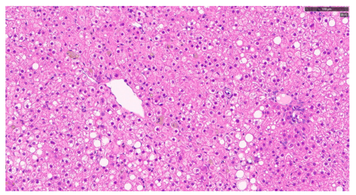


Animals dosed with **MGL-3196** showed a smaller liver compared to control animals on **HFD**: liver to body weight ratio of **10.3% vs 15.7%** for control (p=0.0003).

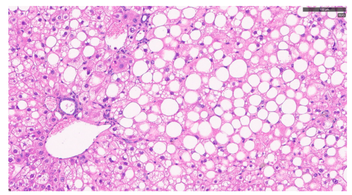
Blood **LDL** was reduced by **14.3%** vs control. **Triglycerides** were reduced by **35.4%.**

NAS (NAFLD activity score) reduced from 4.25 to 2.17 in treated vs control animals. This was mainly due to a reduction in liver fat (**steatosis**).

Fibrosis was not affected by the treatment and ranged from mild to perivenular/ pericellular (score 1 to 2).



H&E MGL-3196



H&E Control

CONCLUSION

FRG KO mice repopulated with **human M148I PNPLA3** hepatocytes and fed an **HFD** developed key aspects of **NASH**. Unlike current rodent models, the blood **lipid profiles** closely resembled those in humans with **NAFLD**. Histological analysis showed time-dependent **progressive accumulation** of typical **NASH features**. Similarly, **GSEA** showed a similar overlap between liver-humanized mice on HFD and NASH patients.
These results suggest that liver-humanized **FRG** KO mice fed an **HFD** may be a **superior model** for **NASH**.
As a proof-of-concept, **FRG** KO mice were dosed with **MGL-3196**. Steatosis was reduced as shown by a significant reduction in the **NAS score**.

FRG MODEL BACKGROUND

FRG KO mice have a knockout of the **F**ah, **R**ag2, and Il2r**g** genes. Fah deficiency kills mouse hepatocytes, while the immune deficiency (Rag2 and IL2rg) allows the mice to be rescued with hepatocytes from any species including human. Hepatocytes from nearly **any donor of interest** can be engrafted in **FRG** mice, including those with specific genotypes. Liver-humanized animal models are useful for in vivo studies in many application areas including infectious diseases, NAFLD/NASH, gene editing/therapy, metabolism, and toxicology.

REFERENCES

[1]Azuma, et al. "Robust expansion of human hepatocytes in Fah−/−/Rag2−/−/Il2rg−/− mice." *Nature biotech* (2007).
[2]Valenti, et al. "Homozygosity for I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease." *Hepatology* (2010).
[3]Ellis, et al. "Mice with chimeric livers are an improved model for human lipoprotein metabolism." *PLoS One* (2013).
[4]Teufel, et al. "Comparison of Gene Expression Patterns Between Mouse Models of Nonalcoholic Fatty Liver Disease and Liver Tissues from Patients." *Gastroenterology* (2016).

Contact information

info@yecuris.com
P.O. Box 4645
Tualatin, OR 97062, USA
Tel: +1 503.352.4663

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