Animal models for novel therapies on fibrosis and NASH: Pharmacological validation and sex differences.

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1 CONTEXT & OBJECTIVES

- **Fibrosis is characterized by excessive deposition of fibrous connective tissue (scarring) resulting in progressive architectural remodeling in almost all tissues and organs (Figure 1).**
- **Fibrotic disorders, such as liver fibrosis, pulmonary fibrosis, renal interstitial fibrosis, myocardial infarction, systemic sclerosis, and graft-versus host-disease (GVHD), result in loss of function of the affected organ and have been estimated to contribute to approximately 45% of all-cause mortality in the United States.**
- **Fibrosis is an extremely complex process that depends on the interactions of many cells, molecules and different pathways. Animal models are valuable means to examine the pathogenesis of these diseases, to identify potential therapeutic targets and develop novel therapies for various fibrotic diseases. At Oncodesign, we offer and develop various models of fibrotic diseases including NASH liver diseases for pharmacology studies.**

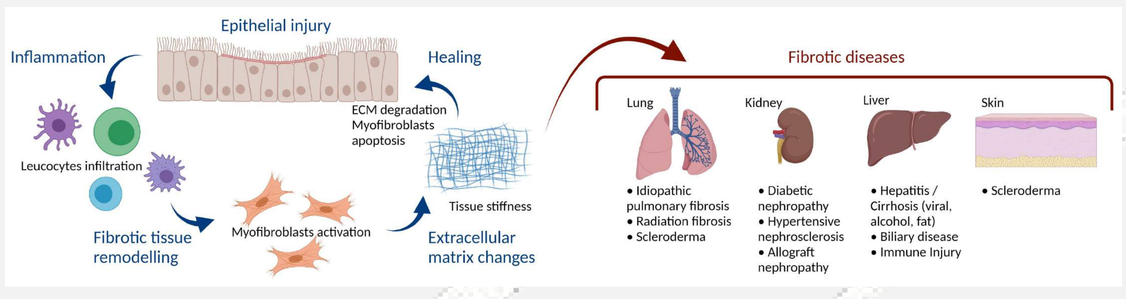


Figure1: Core features shared by pathologic fibrosis among organs (created with Biorender.com)
(Adapted from Friedman et al, 2013; Distler et al, 2019)

- **NAFLDs represent a spectrum of chronic liver disease ranging from simple steatosis to steatohepatitis, and then liver fibrosis or liver cirrhosis. NAFLD are strongly associated with metabolic syndromes including obesity, type 2 diabetes mellitus (T2DM), dyslipidemia and hypertension.**
- **Sex differences exist in the prevalence, risk factors, fibrosis and clinical outcomes of NAFLDs suggesting that a proper consideration of sex, age and hormonal differences are needed to fill current gaps and implement precision medicine for patients with NAFLD (Yang et al, 2014; Leonardo et al, 2019; DiStefano, 2020) (Figure 2).**
- **We have used a model of NASH-HCC with number of cellular and molecular processes that are reflective of human NASH and outcomes (Fujii et al, 2013). Our objectives were to assess:**
  - the effect of Elafibranor (Ela) or/and Obeticholic Acid (OCA) on the development of NASH and fibrosis.
  - the effect of Lenvatinib or/and anti-PD-1 on the development of hepatocellular carcinomas (HCC)
  - to assess the sex differences on the pathogenesis and the response to treatments.

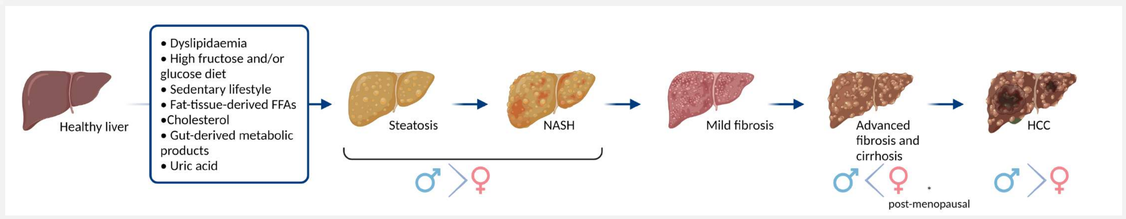
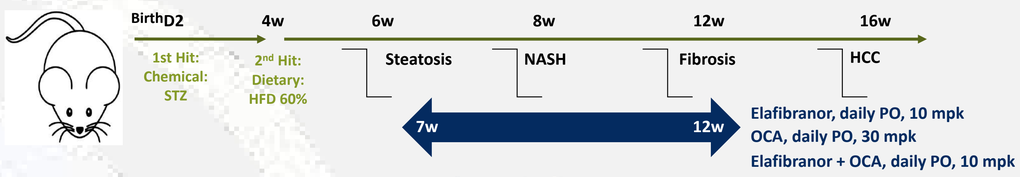


Figure2: NAFLD pathogenesis and sex differences (created with Biorender.com)
(Adapted from Anstee et al, 2019)

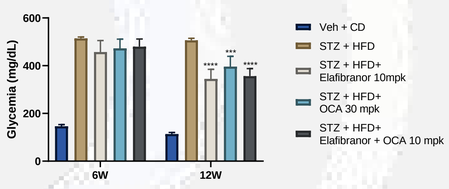
2 RESULTS

1- Pharmacological validation of Elafibranor and OCA treatment in NASH model

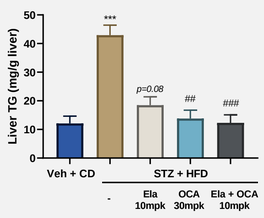


Improvement of metabolic parameters

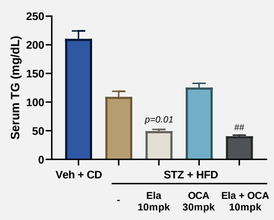
Reduced fasting Hyperglycemia



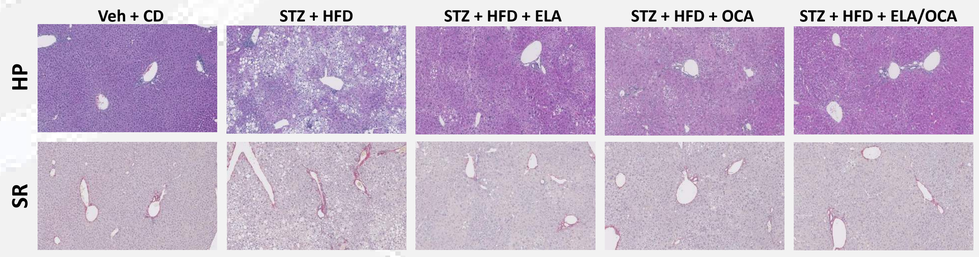
Reduced liver TG



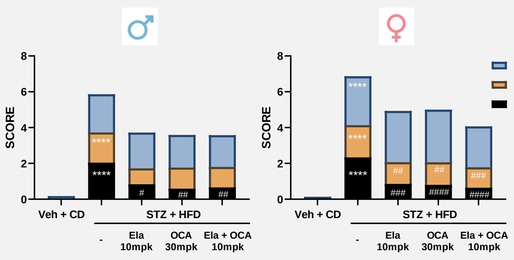
Reduced Serum TG (9W)



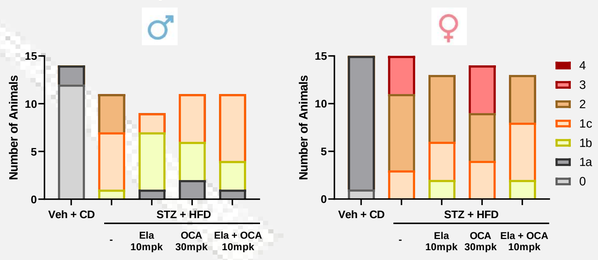
Improvement of histopathological parameters



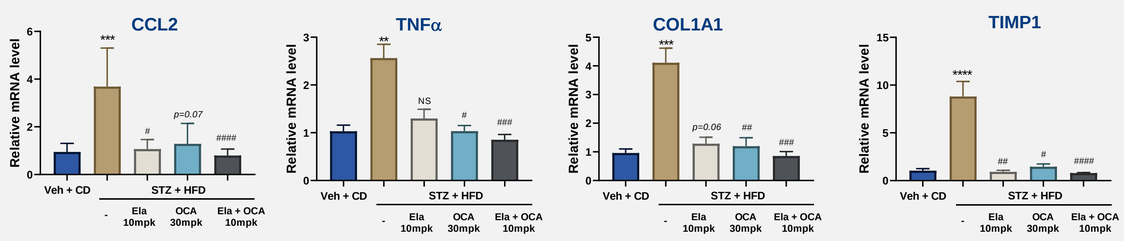
Improvement of NASH score



Improvement of Fibrosis score

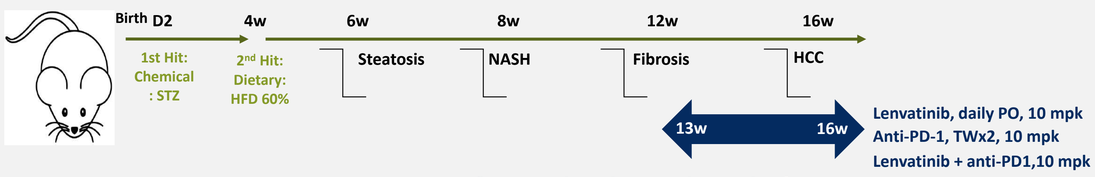


Decreased induction of inflammation and fibrosis-related genes

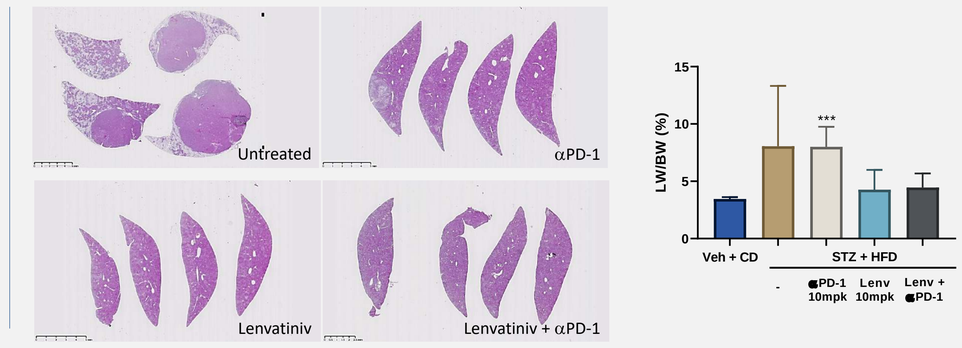


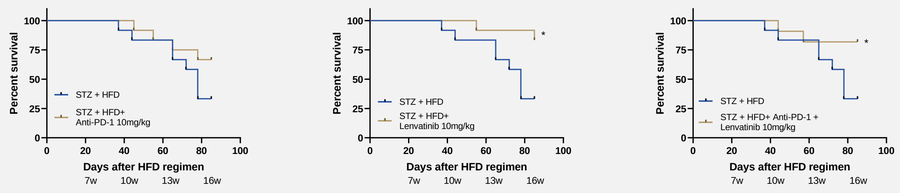
1) Reduction of pathologic parameters after treatment with Elafibranor or/and OCA: Neonatal male mice were injected with (streptozotocin) STZ on day 2 and fed a high fat diet (HFD) from week 4. Mice were treated from wee k7 to week 12 for the fibrosis study. Analyses were done on male mice unless otherwise indicated. Asterisks and hashtags denote significant changes compared to Vehicle or same group at week 6 for glycemia parameters and to STZ+HFD respectively (*or# p <0.05, **or## p <0.01, ***or ###p <0.001 ; one way ANOVA followed by Dunn's test). Males: Veh+CD, n=14; STZ+HFD, n=10; Ela, n = 9; OCA, n = 11; Ela + OCA, n=11. Females: Veh+CD, n=15; STZ+HFD, n=14; Ela, n = 13; OCA, n = 14; Ela + OCA, n=13.

2 - Pharmacological validation of Lenvatinib on HCC development and Survival

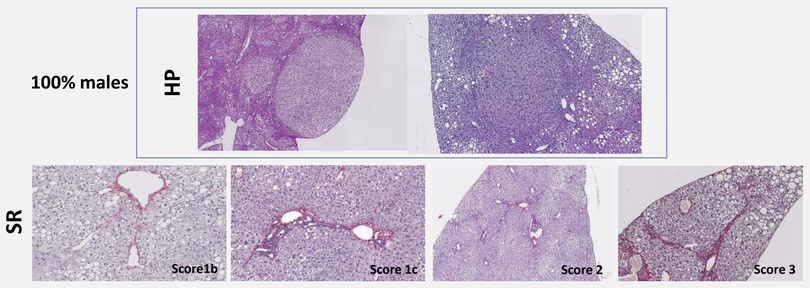


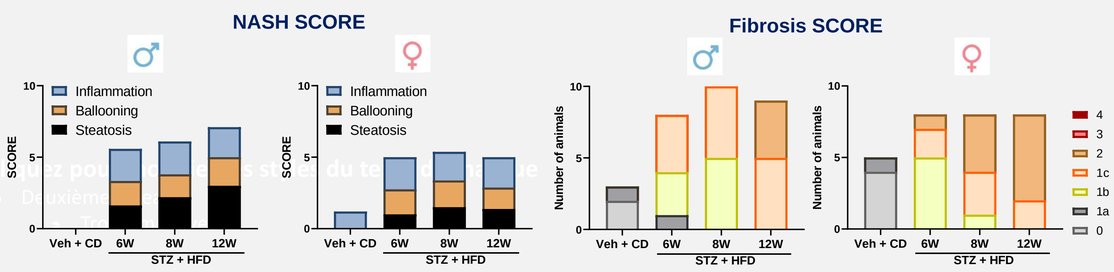
Improvement of tumor burden and survival





3- Sex differences in the course of the NASH-HCC model





2) Increased survival after treatment with Lenvatinib or/and anti PD-1: neonatal male mice were injected with STZ on day 2 and fed a high fat diet from week4. Mice were treated from week 13 to week 16 for the HCC study. LW/BW ratio; Asterisks denote significant changes compared to Vehicle; ** p < 0.01, one way ANOVA; Veh+CD, n=15; STZ+HFD,n=3; Anti PD-1, n = 7; Lenv, n = 8; combo, n=9. Survival curve; LogRank Mantel-Cox test; ** p < 0.01 compared to STZ+HFD (n=12/group)

3) Sex differences in NASH-HCC model: 100% of males develop HCC; Females develop higher grade of fibrosis. Representative images of HCC stained by HE; and of scoring methods using Sirius Red staining. Males: Veh + CD, n=3; STZ+HFD, n=8-10; Females: Veh + CD, n=5; STZ+HFD, n=10

3 CONCLUSION

- **Treatment with PPAR α/δ agonist (Elafibranor) or/and FXR agonist (OCA) led to a significant decrease of the metabolic parameters and NASH score in both male and female mice. However, while a lower grade of fibrosis was observed in all Elafibranor-treated mice, only male mice were protected from advanced fibrosis after OCA treatment.**
- **Treatment with Lenvatinib increased survival and reduced tumor burden as shown with reduced LW/BW ratio at 16 weeks.**
- **Further analyses are needed to assess how sex differences (hormones, genetics, immune system …) contribute to the differential response to treatments in NASH.**
- **Altogether, these results show that this model is very useful for the development of novel treatments, from therapies targeting the cause of the disease, i.e. dysregulated metabolism, to therapies targeting inflammation and fibrosis directly but also to assess sex differences and treatment responses in preclinical studies, in order to achieve precision medicine.**

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