# *In vitro* investigation of the anti-NASH properties of elafibranor and lanifibranor using a human stem cell-derived disease model

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## Introduction

Metabolic syndrome is the key driver of non-alcoholic steatohepatitis (NASH) [1]. No anti-NASH drug has been approved so far to treat this disease. The discovery and development of anti-NASH drugs are hampered by a lack of adequate models that recapitulate the human pathophysiology [2]. Peroxisome proliferator-activated receptor (PPAR) agonists are a major drug class that is administered to metabolic syndrome patients. However, none of the on-the-market PPAR-agonists has been shown to resolute NASH. Two first-in-class PPAR-agonists, lanifibranor (a pan-PPAR agonist [3]) and elafibranor (a dual PPAR-α/δ agonist [4]), are currently being tested as potential anti-NASH drugs in clinical test phases II and III, respectively. In this study we use hepatic cells derived from human skin precursors (hSKP-HPC), previously shown to predict drug-induced fatty liver disease [5], to investigate the anti-NASH properties of elafibranor and lanifibranor.

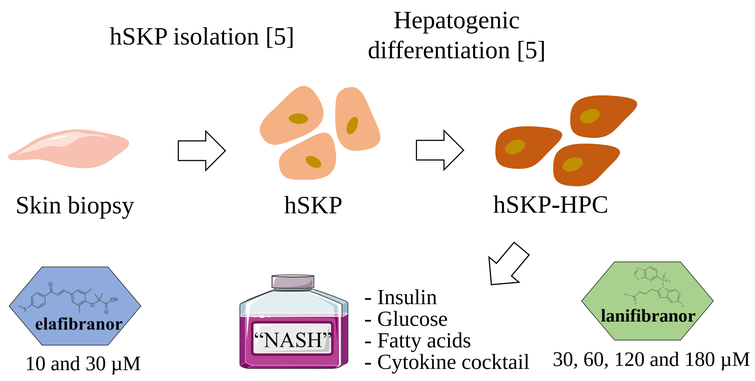
## Aim

The aim of the present study is:

1. To establish a fit-for-purpose and human-relevant *in vitro* NASH model using hSKP-HPC.

2. To compare anti-NASH drugs under development using this *in vitro* disease model.

## Materials & methods




- Lipid accumulation was assessed by fluorescence microscopy and flow-cytometry using a neutral lipids dye (BODIPY™).
- Whole genome transcriptomics data were generated using Affymetrix Human Genome U133 plus 2.0 microarrays.
- Inflammatory responses were investigated by anti-body arrays (Abcam) for inflammatory cytokines, microarrays and RT-qPCR (Applied Biosystems).
- NFκB protein levels were evaluated using immunoblot and immunocytochemistry (Abcam).
- Transcriptomics data analyses were conducted using Robust Multiarray Analysis Express, Transcriptomics Analysis Console (Thermo Fisher Scientific) and Ingenuity Pathway Analysis (Qiagen).

## Results

### Human relevance

***In vitro*** **Patients**

hSKP-HPC NASH (3086) ∩ Frades *et al.* (33) = 87

hSKP-HPC NASH (2537) ∩ Moylan *et al.* (advanced NASH) (2740) = 636

hSKP-HPC NASH (2622) ∩ Moylan *et al.* (mild NASH) (2446) = 551

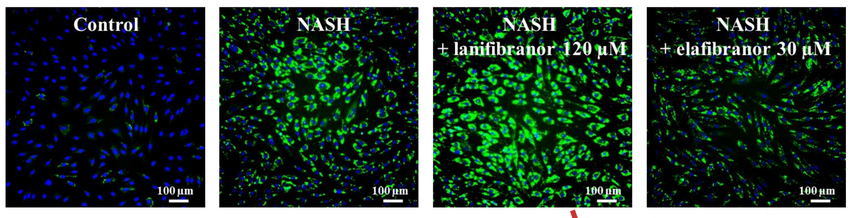
\+

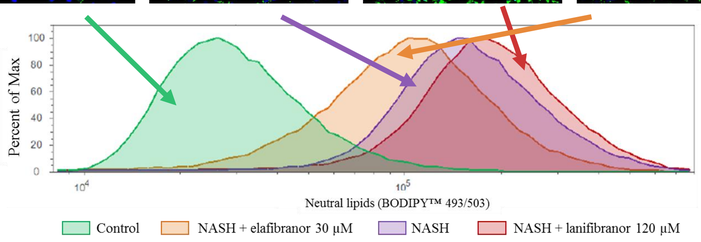
(overlapping genes) - duplicates = **651 genes**

- 'hSKP-HPC NASH' shares 651 co-modulated genes with liver samples of patients suffering from NASH (obtained from publicly available datasets [6,7]).
- Pathway analysis using these co-modulated genes shows a predicted activation of 'Chemotaxis', 'Recruitment of cells' and 'Accumulation of lipids', indicating the human relevance of the *in vitro* model.

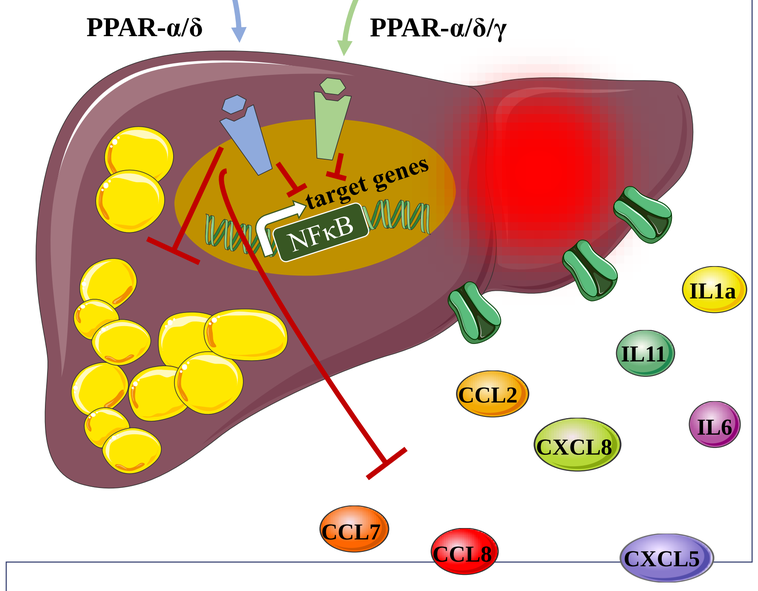
### Steatosis

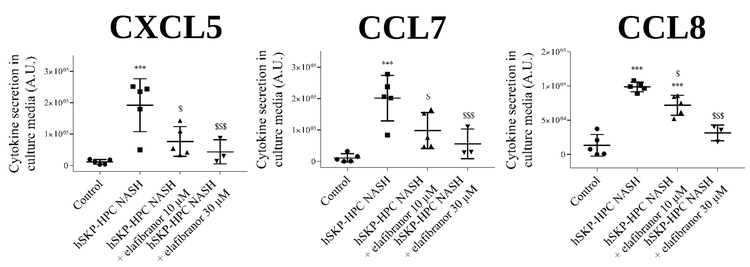
**Lanifibranor *vs* elafibranor**




- Elafibranor significantly restricts the increase in lipid load in 'hSKP-HPC NASH'.
- On the contrary, lanifibranor increases the lipid load in 'hSKP-HPC NASH', pointing to a possible PPAR-γ-mediated side effect.

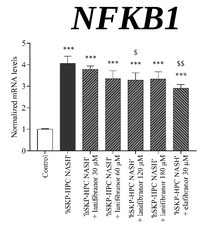



- Elafibranor weakens in a dose-dependent way the inflammatory response by reducing the expression of chemokine ligands and inflammatory interleukins (top right).
- All secreted inflammatory cytokines, with the exception of IL6, are reduced by elafibranor treatment (top left).
- Pathway analysis reveals a general inhibition of chemotaxis-related gene classes induced by elafibranor (right-middle).
- The reduced inflammatory response is (at least partly) NFκB-mediated (down).

### Inflammation

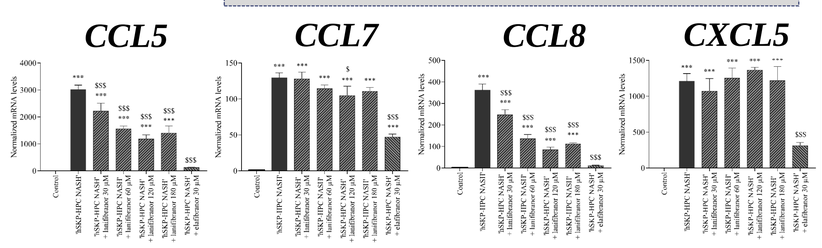
***NFKB1***



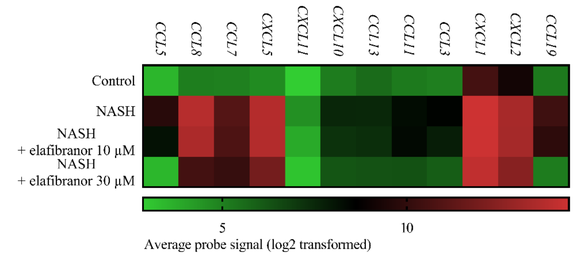

**Lanifibranor *vs* elafibranor**

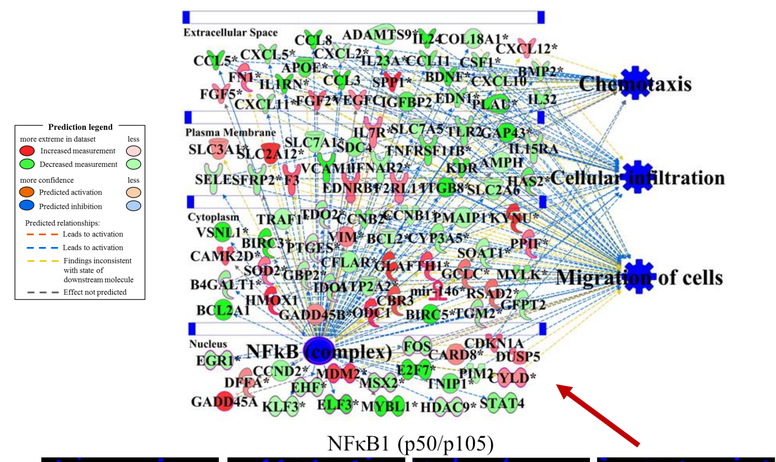
- Lanifibranor and elafibranor attenuate the expression of *CCL5*, *CCL7*, *CCL8* and *NFKB1*.
- Unlike elafibranor, lanifibranor does not reduce *CXCL5* expression.

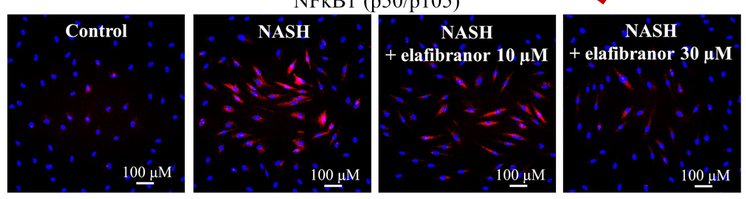
***CCL5*** ***CCL7*** ***CCL8*** ***CXCL5***




**Elafibranor**




## CONCLUSION

- **'hSKP-HPC' represent a powerful tool to investigate NASH *in vitro* and assess the efficacy and underlying mechanisms of potential anti-NASH compounds.**
- **Elafibranor exhibits stronger anti-NASH properties than lanifibranor in the 'hSKP-HPC NASH' model.**

**Statistics:** One-way ANOVA (*, **and ***) *vs* control samples with post-hoc Sidak's multiple comparisons test *vs* 'hSKP-HPC NASH' ($, $$ and $$$) $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$
Pathway analysis (IPA): analysis cut-off: fold change [-2 ; +2], $p \leq 0.05$ (Fischer's exact test)

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**References:** [1] J. Boeckmans *et al.*, Pharmacol. Res. 134, 257–267 (2018).
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