In vitro and *in vivo* characterization of EYP001, a novel, potent and selective FXR agonist now in a Phase 2 clinical trial in NASH

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a condition defined by excessive fat accumulation (steatosis) in the liver that currently affects 20-40% of the general population. Non-alcoholic steatohepatitis (NASH), the most severe form of NAFLD, is characterized by steatosis, hepatocyte ballooning, inflammation and fibrosis. It may also lead to complications such as cardiovascular disease, cirrhosis, liver failure or liver cancer. 10-20% of NAFLD patients develop NASH, thus making NASH a significant health burden with currently no approved pharmacological treatments. The Farnesoid X receptor (FXR), a nuclear receptor, controls bile acid homeostasis and is a promising target for the treatment of NASH. EYP001, a novel non-bile acid, selective, second generation FXR agonist, is currently in a phase 2 NASH clinical trial.

OBJECTIVES

Characterize EYP001 and its close analog *in vitro* (selectivity towards other nuclear receptors, TGR5 activation and target engagement) and *in vivo* (STAM model) in comparison to OCA.

METHODS

Potency and selectivity: FRET-based FXR functional agonist coactivator recruitment assay was carried out using an SRC1 peptide. TGR5 assay was conducted by Eurofins Discovery Services (assay #3998).

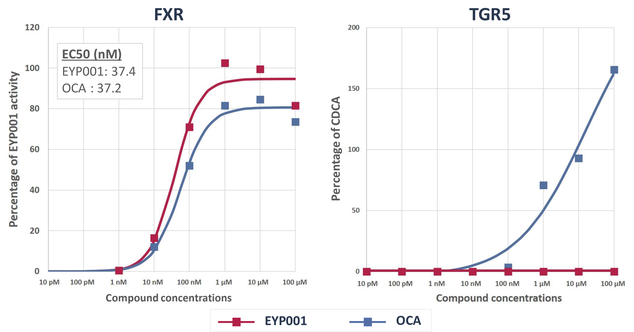
Target engagement: differentiated HepaRG cells were treated with a range of doses of EYP001 for 48 hr. mRNA expression of *Bsep* and *Cyp7a1* were quantified by qRT-PCR.

In vivo STAM™ Study: NASH was induced in C57BL/6 male mice (10 animals per group) by a single subcutaneous injection of streptozotocin (STZ) 2 days after birth and feeding with high fat diet (HFD) from week 4 of age. From week 6 to 10, mice were dosed with a close analog of EYP001 (25, 50 and 100 mg/kg, PO, BID) or OCA (30 mg/kg, PO, QD).

RESULTS

Potency and selectivity

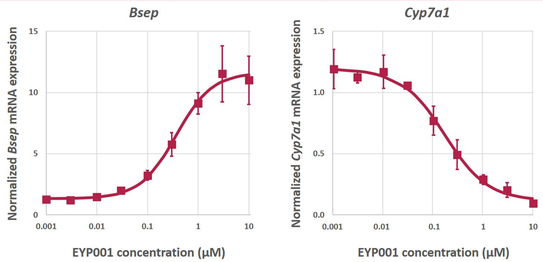
EYP001 is a potent and selective FXR agonist



Potency and Selectivity : Left panel, Efficacy and potency of EYP001 and OCA on the activation of FXR assessed by FRET-based functional agonist coactivator recruitment assay. EYP001 was tested against 20 other human nuclear receptor assays (PPARα/δ/γ, LXRα/β, PXR, CAR, PR, ERα/β, TRα/β, RXRα, GR, ERRα/β/γ, RARα/γ and VDR) and was found strictly selective to FXR (>2000 fold) – Right panel, Unlike OCA, EYP001 does not activate TGR5, a bile acid receptor linked to pruritus.

FXR target engagement

FXR target engagement was confirmed through the regulation of *Bsep* and *Cyp7a1*, two genes under the regulation of FXR



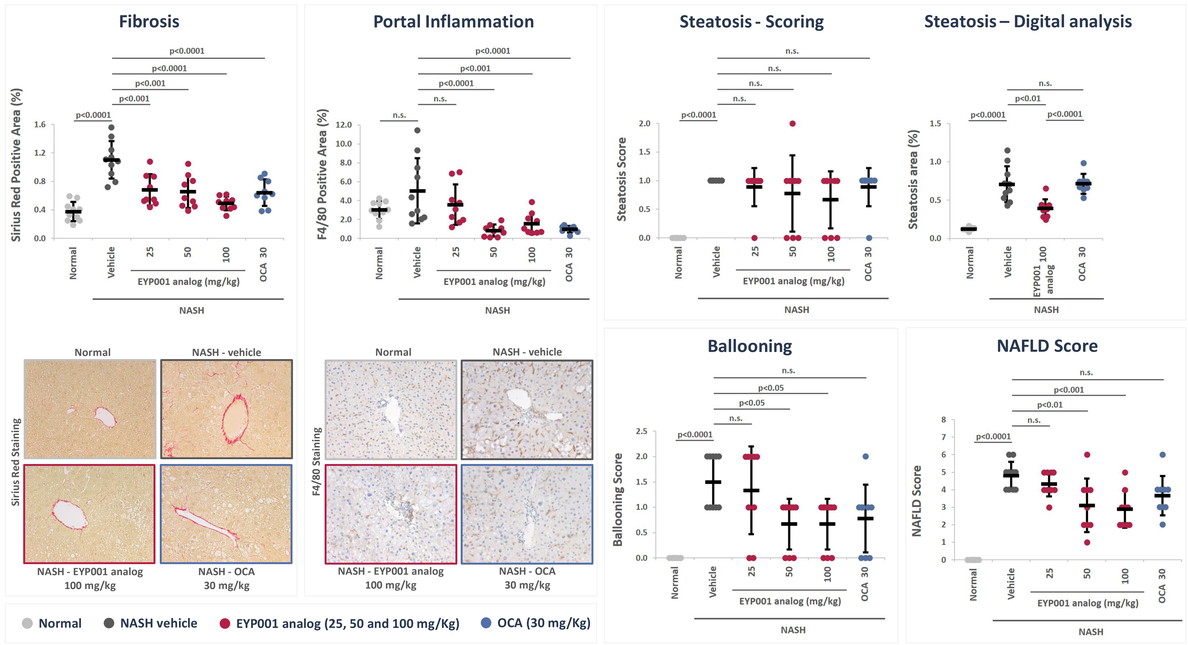
FXR target engagement: Differentiated HepaRG cells were treated for 48 hr with a range of doses of EYP001 (0.001 – 10 µM). mRNA expression of Bsep (left) and Cyp7a1 (right) was quantified by qRT-PCR.

In vivo efficacy in STAM™ NASH model

A close analog of EYP001 demonstrated dose dependent reduction in

- **Fibrosis (Sirius red positive area decreased by 55%)**
- **Portal inflammation (F4/80 positive area decreased by 69%)**
- **Ballooning (score decreased by 56%)**
- **Steatosis (positive area decreased by 45%)**
- **NAFLD Activity Score (NAS decreased by 40%)**

with statistically significant lowering at 50 and 100 mg/kg, while OCA showed significant effects only on fibrosis and inflammation



***In vivo* efficacy in STAM™ NASH model**: NASH phenotype was induced (except for the "normal" group) with streptozotocin and HFD, as described in Methods. Animals were dosed PO with an EYP001 close analog (25, 50 and 100 mg/kg, *BID*) or OCA (30 mg/kg, *QD*) for 4 weeks. NASH parameters such as fibrosis, steatosis, ballooning and portal inflammation were investigated by histology and immunological staining. In addition, steatosis positive area was measured by automated digital analysis (carried out by Biocellvia).

CONCLUSIONS

- **The non-bile acid second generation FXR agonist EYP001 was shown to be potent, selective, and to induce FXR target gene mRNA expression *in vitro*.**
- **A close analog of EYP001 significantly improved NASH parameters such as fibrosis, steatosis, ballooning, inflammation and NAS in a murine model of NASH.**
- **Based on these preclinical efficacy findings, and safety, tolerability, pharmacokinetics and pharmacodynamics data obtained in Phase 1 clinical trials, EYP001 has now entered a Phase 2 clinical trial examining benefits in NASH patients.**

DISCLOSURES

JV : Stock Shareholder at Obseva SA and Hoffman La Roche AG ; Board member at Obseva SA, Inatherys SAS, Step Pharma SAS and Inotrem SA.
PS : Consultant at ENYO Pharma

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