The selective FXR agonist EYP001 is well tolerated in healthy male subjects and has additive anti-HBV effect with nucleoside analogues in HepaRG cells

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INTRODUCTION

- Nucleos(t)ide analogues (NAs) inhibiting Hepatitis B virus (HBV) polymerase are the main components of approved **HBV therapies.** New approaches to overcome the need for a life-long therapy are however needed.
- HBV interacts with the **human nuclear farnesoid X receptor (FXR)**, an important regulator of bile acid metabolism. FXR agonists have been reported to inhibit HBV replication[1].
- **EYP001** is a selective, synthetic FXR agonist under development for the treatment of chronic HBV infection.

AIM

- To study ***in-vitro*** the **anti-HBV effect of EYP001** alone, and in combination with Tenofovir (TFV) or Entecavir (ETV), commonly used NAs.
- To assess **safety**, tolerance, pharmacokinetics (**PK**) and pharmacodynamics (**PD**) of **oral EYP001 in healthy male subjects.**

METHOD

In-vitro assays

The HepaRG cell line can differentiate and regain phenotypic traits of human hepatocytes after 4 weeks of culture[2]. After differentiation, cells were infected for 24 hr with HBV (100 genome-equivalent per cell), washed and cultured for 48 hr. Cells were then treated with **EYP001 alone, or in combination with TFV or ETV**. Treatment was replaced every 72 hrs. At day 14 post-infection, the **effect on HBV infection and on FXR expression activity** was evaluated: HBV DNA and antigen secretion in cell supernatant, and intracellular cccDNA, pgRNA, *FXR* mRNA, *CYP7A1* mRNA and *BSEP* mRNA expression levels, were quantified as described[1].

Phase I clinical study

EYP001 or placebo were administered to ten cohorts of young males (6 active & 2 placebo subjects per cohort):

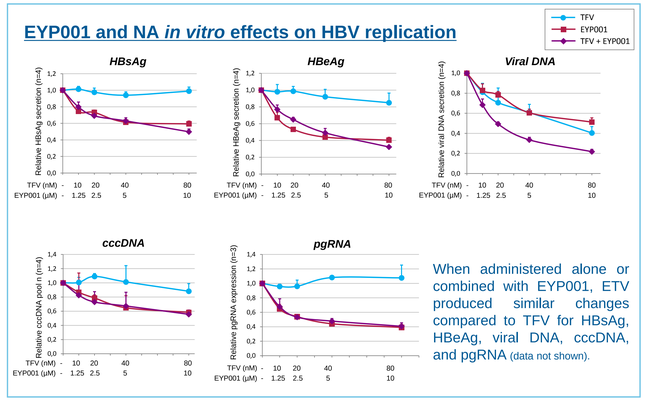
- *Single dose part (SAD):* six cohorts received single EYP001 doses of 30, 60, 120, 250, 500 and 800 mg, respectively.
- *Multiple dose part (MAD):* four cohorts received EYP001 doses of 60, 120, 250 and 500 mg, respectively, once daily during 15 days.

Adverse events (AE), clinical laboratory, ECG parameters and **vital signs** were recorded. Concentrations of **EYP001** and **PD markers** (fibroblast growth factor 19 [**FGF-19**], 7αhydroxy-4-cholesten-3-one [**C4**], Bile Acids) were assessed in plasma.

This paper presents **preliminary results**.

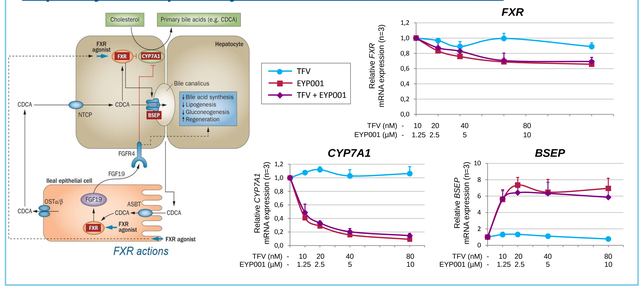
RESULTS

EYP001 and NA *in vitro* effects on HBV replication



When administered alone or combined with EYP001, ETV produced similar changes compared to TFV for HBsAg, HBeAg, viral DNA, cccDNA, and pgRNA (data not shown).

Hepatocyte FXR pathway[3] and *in vitro* effect[1] of EYP001



Safety and clinical tolerance of EYP001 in healthy subjects

EYP001 was well tolerated in all cohorts. No clinically relevant changes in vital signs, ECG, nor laboratory values (in particular liver enzymes, glucose and lipid profiles) occurred.

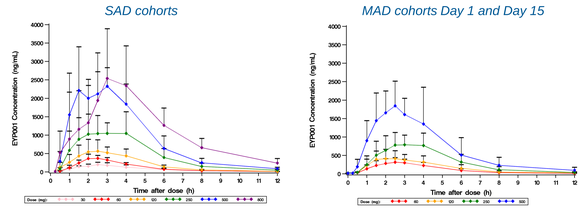
Overall 99 post-dosing **mild and moderate AE** occurred, mostly at the highest doses tested, and of which 28 were categorized as probably drug related. Those were reported up to 2 hours after dosing, **short-lasting** and mostly **mild** gastro-intestinal symptoms.

Study medication related AE	SAD – Placebo	SAD – 30 mg	SAD – 60 mg	SAD – 120 mg	SAD – 250 mg	SAD – 500 mg	SAD – 800 mg
GASTRO-INTESTINAL (N=9)*	-	-	-	-	-	6 (67%)	3 (33%)
	MAD – Placebo		MAD – 60 mg	MAD – 120 mg	MAD – 250 mg	MAD – 500 mg	
GENERAL (N=2)#	-		2 (100%)	-	-	-	
GASTRO-INTESTINAL (N=17) *	1 (6%)		-	2 (12%)	7 (41%)	7 (41%)	

* ABDOMINAL PAIN, DYSPEPSIA, DRY MOUTH, SALIVATION, NAUSEA, one VOMIT, DIARRHOEA

HEADACHE, MUSCULAR CRAMPS LEGS

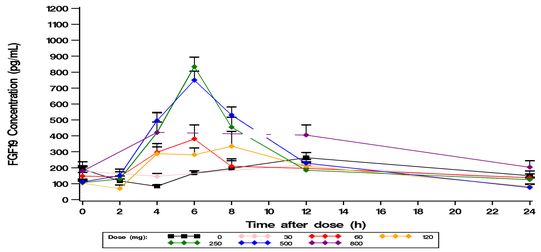
EYP001 plasma-concentration (mean±SD) in healthy subjects



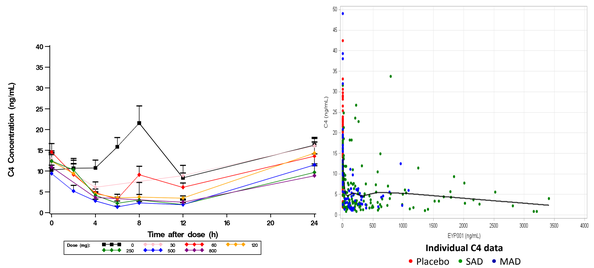
- SAD exposure of EYP001 was linear up to 500 mg and less than linear at 800 mg.
- Tmax was at 2.0 - 2.5 hours ; T 1/2: 1.5 - 2.5 hours and similar in SAD and MAD cohorts.
- No accumulation on Day 15 with AUC ratio of 0.5 to 0.8.

PD markers in healthy subjects

- EYP001-treated subjects showed post-dose time- and dose-level-dependent FGF-19 increase (median ±SEM) in plasma :



- A post-dosing time-dependent C4 decline in plasma (left fig. median ±SEM) was observed at all dose levels and related to EYP001 plasma concentrations (right fig. individual data) :



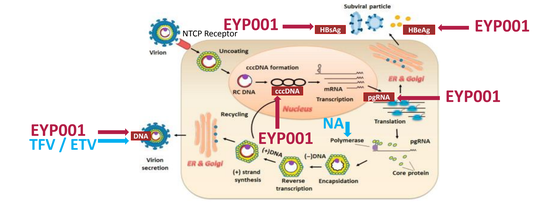
- No relevant changes of total bile acids in plasma in relation to EYP001 dose levels was identified in preliminary analysis.

EYP001 PK profiles MAD Day 15

Cohort	Cmax(ng/mL)	Tmax(h)	AUClast(ng*h/mL)	t1/2z(h)	R-AUC Day15/Day1
Mean MAD 60 Day 15	354	2.75	1106	1.70	0.79
SD	63	0.69	286	0.76	0.20
Mean MAD 120 Day 15	456	2.75	1418	1.66	0.64
SD	157	1.64	331	0.64	0.17
Mean MAD 250 Day 15	963	2.83	2645	1.74	0.54
SD	147	0.68	401	0.61	0.06
Mean MAD 500 Day 15	1902	2.40	5226	2.25	0.48
SD	504	0.55	916	0.67	0.08

CONCLUSIONS

- EYP001 mono-treatment inhibited HBsAg and HBeAg production and reduced cccDNA and pgRNA expression in differentiated hepatic HepaRG cells, while TFV or ETV mono-treatment had negligible effect on these HBV markers.
- EYP001 mono-treatment inhibited HBV DNA similarly to TFV or ETV. The combination of EYP001 with NAs showed an additive effect.



- Single and multiple oral doses of EYP001 were well-tolerated in healthy male subjects. No clinical or biological relevant changes were identified.
- The PK profile of oral EYP001 doses showed dose linearity from 30 to 500 mg and fast kinetics.
- PD marker revealed a dose and time-dependent engagement of bile-acid-regulating FGF-19 in response to FXR stimulation by EYP001 and a time-dependent reduction of bile acids synthesis precursor C4 plasma levels.
- Overall, these results support further clinical testing in an ongoing study in chronically infected HBV subjects and exploration of efficacy in combination with NA.

REFERENCES

[1]Radreau P, Porcherot M, Ramière C, Mouzannar K, Lotteau V, André P. Reciprocal regulation of farnesoid X receptor α activity and hepatitis B virus replication in differentiated HepaRG cells and primary human hepatocytes. *FASEB J,* 30(9):3146-54 (2016).
[2]Hantz O, Parent R, Durantel D, Gripon P, Guguen-Guillouzo C, Zoulim F. Persistence of the hepatitis B virus covalently closed circular DNA in HepaRG human hepatocyte-like cells. *J Gen Virol,* 90(1):127-35 (2009).
[3]Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol,*11(1):55-67 (2014).
[4]Hu Y, Zhu W, Tang G, Mayweg A, Yang G, Wu J, Shen H. Novel therapeutics in discovery and development for treatment of chronic HBV infection. Annu Rep Med Chem. 48:265-281 (2013).

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