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Phase 1 Study of EDP-305, a Novel Once-Daily Oral Farnesoid X Receptor Agonist, in Healthy Subjects and Those with Presumptive Nonalcoholic Fatty Liver Disease

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Abstract: *Introduction:* EDP-305 is a farnesoid X Receptor (FXR) agonist that selectively activates FXR and is currently under investigation for with the treatment of nonalcoholic steatohepatitis (NASH). The primary objective was to assess the safety of EDP-305 in healthy subjects and subjects with presumptive NAFLD. *Methods:* In this placebo-controlled double-blind Phase 1 study, healthy subjects (aged 20-55 years) were randomized to single or multiple oral doses of once daily EDP-305 or placebo and subjects with presumptive NAFLD (aged 25-52 years) were randomized to multiple oral doses of once daily EDP-305 or placebo for 14 days. Six cohorts received EDP-305 1 mg, 5 mg, 10 mg, 20 mg, 40 mg or 80 mg in the SAD phase including a food effect cohort that received EDP-305 10 mg. In the MAD phase, 12 cohorts received EDP-305 0.5 mg, 1 mg, 2.5 mg, 5 mg, 10 mg or 20 mg (six cohorts in healthy subjects and six in presumptive NAFLD). *Results:* In the SAD phase, 38 subjects received EDP-305 and 12 subjects received placebo, and in the MAD phase, 72 subjects received EDP-305 and 24 subjects received placebo. No serious adverse events were reported, and the most common treatment-emergent adverse events (TEAEs) in EDP-305 treated subjects were constipation, headache, and pruritus. The majority of TEAEs were mild to moderate in severity. EDP-305 exposure increased with single and multiple doses in both healthy subjects and those with presumptive NAFLD. EDP-305 exposure was approximately 3-fold higher in fed vs. fasted subjects. Strong FXR target engagement was demonstrated in both healthy and presumptive NAFLD subjects in the MAD phase with FGF19 increases and C4 reductions compared with placebo. *Conclusion:* EDP-305 was well tolerated, with pruritus the most common treatment-emergent adverse event at EDP-305 doses ≥10 mg and with minimal effects on lipids. Dose proportional PK support once daily dosing. Significant elevations of FGF19 and diminutions in C4 demonstrated potent engagement of the FXR receptor at doses that neither elicit adverse effects on lipids nor result in pruritus. These results supported further evaluation of EDP-305 in patients with NASH.

Keywords: Nonalcoholic Fatty Liver Disease, Farnesoid X Receptor, EDP-305, Pharmacokinetics, Safety

1. Introduction

The most common cause of chronic liver disease worldwide is nonalcoholic fatty liver disease (NAFLD) [1-4]. NAFLD is characterized by excessive steatosis [5-6], and while the pathogenesis of NAFLD is a complex, multifactorial process, insulin resistance is a common finding with eventual progression to non-alcoholic steatohepatitis (NASH) [7-9].

A role for FXR in the development of NASH including steatosis, inflammation, and fibrosis, has been demonstrated in animal models [10, 11]. Bile acids play a key role in regulating liver and metabolic homeostasis including regulation of lipid and glucose metabolism mediated through

FXR and G-protein coupled bile acid receptor (TGR5) pathways [12, 13]. FXR regulates bile acid synthesis by suppressing key enzymes in the biosynthetic pathway [14]. A number of therapies are under investigation for the treatment of NAFLD and NASH [5, 15, 16], however, no pharmacological agent is currently approved. FXR agonists are in clinical development for the treatment of NASH as well as other chronic liver diseases.

EDP-305 is an FXR agonist that selectively activates FXR. Results from *in vitro* studies demonstrated that EDP-305 regulates bile acid and lipid metabolism, and decreases the expression of proinflammatory and fibrotic genes [17-21]. In this Phase 1 study, single- and multiple-ascending dose of EDP-305 were administered to healthy subjects and subjects with presumptive NAFLD to evaluate safety, tolerability, pharmacokinetics (PK), pharmacodynamic (PD) and food effects.

2. Methods

The study was conducted in accordance with the principles of the Declaration of Helsinki and The International Council for Harmonisation (ICH) E6 Guideline for Good Clinical Practice. The clinical study protocol and its amendments and the informed consent form and their amendments were reviewed and approved by an Institutional Review Board. All participants provided written informed consent prior to undergoing any study procedures.

2.1. Study Design

This was a Phase 1, randomized, double-blind, placebo-controlled study. The study comprised two phases: a single ascending dose (SAD) phase in healthy subjects, which included a fasted vs. fed two-part cohort to assess food effect, and a multiple ascending dose (MAD) phase in healthy subjects and subjects with presumptive NAFLD (Figure 1). This study was registered at clinicaltrials.gov: NCT02918929.

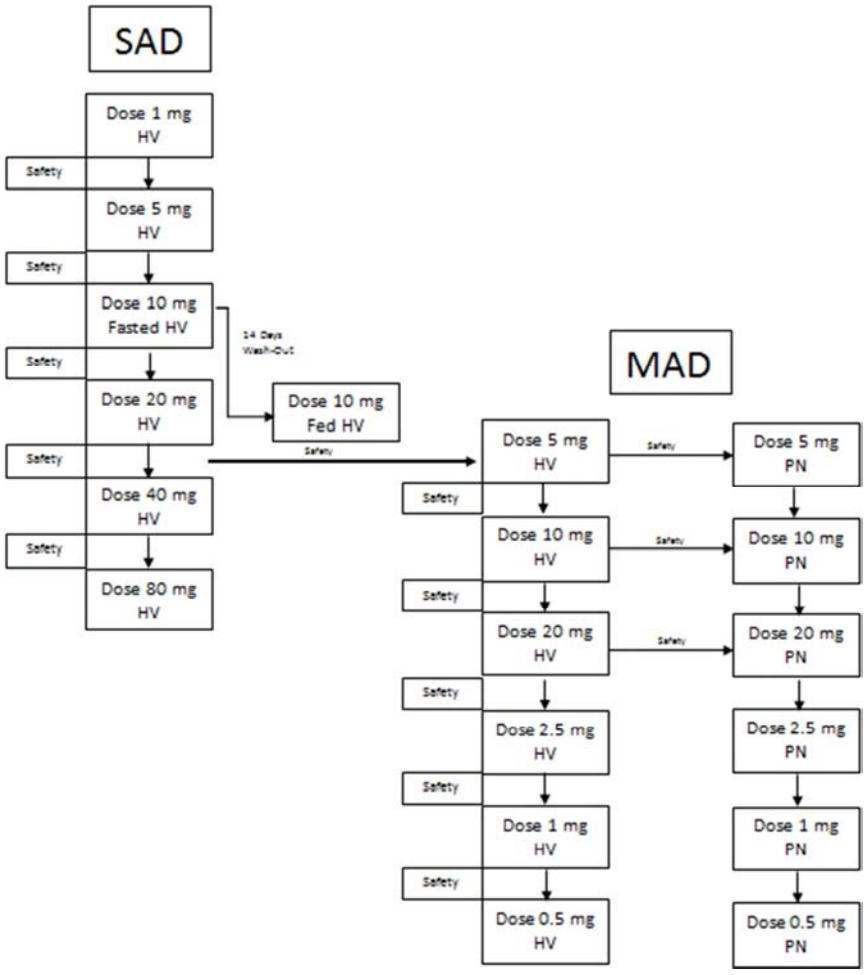


Figure 1. *Study design.*

Subject Selection

Male and female subjects ages 18 to 55 years with a body mass index (BMI) of 18 to 30 kg/m^2 were eligible. Women had to be of non-childbearing potential, and men had to use an effective method of contraception throughout the study period.

Healthy subjects had no clinically relevant abnormality on physical examination, medical history, vital signs or clinical laboratory testing that would interfere with the conduct of the study.

Subjects with NAFLD had to be generally healthy other than conditions associated with NAFLD (obesity, prediabetes, type 2 diabetes). Conditions related to NAFLD were allowed as long as they were stable and not considered to affect the study outcome. Subjects had a BMI of >28 to <35 kg/m^2 with type 2 diabetes OR prediabetes according to American Diabetes Association criteria (plasma glucose >200 mg/dL; fasting glucose >126 mg/dL; 2-h postload glucose >200 mg/dL; or glycated hemoglobin [HbA1c] ≥6.5%) [22]. Fatty liver demonstrated by ultrasound or other imaging technique (magnetic resonance imaging estimated proton density fat fraction, or diagnostic histological findings shown on prior biopsy in the last 2 years) was assessed during screening, if available. If historical assessment was not available, the ultrasound performed before dosing was used for this assessment.

Subjects were excluded for any clinically relevant history of illness or disease that could interfere with the conduct of the study. Also excluded were subjects with a history of febrile illness within 7 days; positive urine drug screen; current tobacco use; any condition effecting drug absorption; history of regular alcohol consumption; clinically significant electrocardiogram (ECG) abnormalities; or clinically significant laboratory abnormalities.

For those with presumptive NAFLD, additional exclusion criteria were subjects taking any antidiabetic medication or fibrates, statins and/or vitamin E within 6 weeks of the first dose of study medication; unstable proliferative retinopathy or macular edema; history of bariatric or gastrointestinal surgery; secondary hepatic steatosis; or abnormal clinical laboratory findings for alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin or international normalized ratio (INR).

2.2. Study Treatments

In the SAD phase, subjects received single 1, 5, 10, 20, 40 and 80 mg doses of EDP-305 or placebo. The fed vs. fasted cohort received a single 10 mg dose of EDP-305 or placebo. In the MAD phase, healthy subjects and presumptive NAFLD subjects received 0.5, 1, 2.5, 5, 10, and 20 mg doses of EDP-305 and placebo once daily for 14 days. For the SAD and MAD phases, subjects in each dose cohort were randomized in a 3:1 ratio to EDP-305 or placebo. For the food effect cohort, 10 subjects were randomized in a 4:1 ratio to EDP-305 10 mg or placebo. In the fasted cohort, subjects fasted from food for at least 8 hours overnight until 4 hours post dose. Subjects in the fed cohort were given a standardized high-fat content meal a maximum of 30 minutes prior to their dose of study drug. Study drug was administered within 10 minutes of completion of the meal.

2.3. Study Assessments

Safety was assessed from adverse events (AEs), clinical laboratory testing (hematology, chemistry, urinalysis), vital signs (heart rate, respiratory rate, blood pressure), and 12-lead ECG. Plasma and urine concentrations were obtained to assess the PK of EDP-305 and its metabolites (EDP-022571, EP-022572, EP-022679). Blood was obtained to measure serum biomarker concentrations including fibroblast growth factor 19 (FGF19), 7α-Hydroxy-4-Cholesten-3-one (C4), and total bile acids. In addition, ALT, AST, cytokeratin-18 (CK-18), alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), and lipid panel (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, lipoprotein A, and apolipoprotein A1 and B) were measured.

2.4. Pharmacokinetic Analysis

Plasma PK parameters for EDP-305 and its metabolites were estimated using noncompartmental methods with Phoenix® WinNonlin® Version 6.3 (Pharsight Corporation, Mountain View, CA). Urine PK parameters were estimated using SAS® for Windows™ Version 9.4 (SAS Institute, Inc., Cary, NC, USA) based on the recorded urine concentrations and volumes.

EDP-305 and its metabolites in human plasma and urine were quantified by high performance liquid chromatography with tandem mass spectrometric detection, with an assay range of 0.030 to 30.0 ng/mL (lower limit of quantification [LLOQ] of 0.030 ng/mL) for EDP-305, EP-022571, EP-022572, and EP-022679. The method was fully validated by assessment of precision, accuracy, sensitivity, and specificity of EDP-305 and its major metabolites by Medpace Bioanalytical Labs. C4 and FGF-19 were measured using validated methods at Medpace Bioanalytical Labs and Covance labs, respectively.

2.5. Statistical Analysis

No formal pre-planned sample size calculations were performed for this Phase 1 study. Final sample size was dependent on post cohort safety review. After evaluating 146 subjects, of which 98 were healthy and 48 had presumptive NAFLD, it was determined that the number of subjects that participated in each EDP-305 dose cohort was sufficient to characterize the safety, tolerability, PK, and biomarker profiles in each of the SAD and MAD phases of the study. One food effect cohort of 10 subjects was considered sufficient to analyze the effect of food on EDP-305 PK parameters.

For both the SAD and MAD phases, plasma PK parameters of C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$ (SAD phase only), and $AUC_{0\text{-}tau}$ (MAD phase only) were compared across each dose level to assess dose proportionality. The MAD phase used Day 14 PK data and was analyzed separately for the healthy subjects and presumptive NAFLD cohorts. For the SAD phase, fasted data from the cohort examining food effect were used for the dose proportionality analyses and fed data were excluded. The power model used the following general form: $\ln(PK) = \alpha + \beta * \ln(dose) + \varepsilon$, where PK was the PK parameter, α was the y-intercept, β was the slope (a value of β=1 indicated linearity), and ε was the error term. The estimates of α and β were reported, along with the 90% confidence interval (CI) for β for each PK parameter. If the

90% CI of the slope of C_{max} and AUC contained 1, then dose proportionality was confirmed.

The effect of food on the PK of EDP-305 was explored using the food effect population from the SAD phase. The results were exploratory, as the study was not powered to formally assess the effect of food. The least squares (LS) geometric mean ratio and associated 90% CI for the PK parameters C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$ were calculated using a linear mixed model with a fixed effect for treatment and a random effect for subject on the natural log-transformed parameters. The LS geometric means for C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$ were provided for each treatment. No adjustments were made for multiplicity.

For both the SAD and MAD phases, summaries of biomarker concentrations, change from baseline (CFB), and percentage CFB by treatment and scheduled visit/time point were provided. Continuous biomarker results were summarized by descriptive statistics (n, mean, standard deviation (SD), minimum, first quartile, median, third quartile, and maximum). Linear plots of mean (SD) biomarker results (raw data) vs. time by treatment were generated. Linear plots of the median biomarker concentrations (raw data) vs. time by treatment were generated for FGF19, C4, and total bile acids. For the MAD phase, a boxplot of the percentage changes from baseline to Day 14 was generated for the lipid-related biomarkers: TC, HDL, TC / HDL ratio, LDL-particles, VLDL, and TG.

3. Results

The study was conducted by PRA Early Development Services, Lenexa, KS between August 2016 and March 2017 (SAD) and between December 2016 and June 2017 (MAD). In the SAD phase, 50 subjects were randomized, 12 to placebo, and 38 subjects to single dose cohorts of EDP-305. One subject discontinued the 20 mg cohort for family emergency, however, the safety population included 50 subjects and the PK population included 38 subjects.

In the MAD phase, 96 subjects were randomized; 48 were healthy subjects and 48 were subjects with presumptive NAFLD. One healthy subject in the EDP-305 20 mg group discontinued for an AE. In the presumptive NAFLD cohort, 1 subject in the placebo group discontinued for physician decision. Two healthy subjects in the EDP-305 20 mg cohort reported AEs and had drug withdrawn for increased ALT and AST levels and for generalized pruritus. These 2 subjects were removed from the PK/PD analysis set.

Baseline characteristics of the study populations were typical for healthy subjects (Table 1). Subjects with presumptive NAFLD had a higher BMI compared with healthy subjects, which was consistent with the eligibility criteria.

Table 1. *Baseline characteristics.*

	Single-Ascending Dose	**Multiple Ascending Dose**	
	Healthy Subjects (n=50)	**Healthy Subjects (n=48)**	**Presumptive NAFLD (n=48)**
Age, years[a]	33.2 ± 8.8	35.2 ± 10.3	39.5 ± 8.1
Male, n (%)	45 (90)	45 (93.8)	40 (83.3)
Race, n (%)			
White	24 (48.0)	26 (54.2)	24 (50.0)
Black or African American	24 (48.0)	19 (39.6)	22 (45.8)
Other	2 (4.0)	3 (6.3)	2 (4.2)
Hispanic or Latino (n (%)	5 (10.0)	6 (12.5)	7 (14.6)
Body mass index, kg/m^2 [a]	24.7 ± 3.2	25.7 ± 2.3	31.6 ± 1.6

[a] Mean ± standard deviation.

3.1. Safety/Tolerability

In the SAD phase, at least one TEAE was reported by 5 (13.2%) subjects with EDP-305 (diarrhea, skin abrasion [2], influenza-like illness, pruritus) compared to 1 (8.3%) subject with placebo (aphthous ulcer). One event of diarrhea (EDP-305 10 mg) and one event of pruritus (EDP-305 20 mg) were considered treatment-related, and all events but one (influenza-like illness of moderate severity) were mild. No serious AEs or discontinuation for AEs occurred, and no deaths were reported. No clinically significant changes in vital signs, ECGs or clinical laboratory assessments were observed.

Among healthy subjects in the MAD phase, 10 (27.8%) subjects in the EDP-305 group and 2 (16.7%) in the placebo group reported at least 1 TEAE (Table 2). Among those with presumptive NAFLD, 10 (27.8%) in the EDP-305 group and 4 (33.3%) in the placebo group reported at least 1 TEAE. The most common TEAEs with EDP-305 were headache and pruritus in healthy subjects and constipation, headache, and pruritus in presumptive NAFLD subjects. Two healthy subjects who received EDP-305 20 mg discontinued for elevated ALT/AST levels (transient, Grade 2 elevation) (1) and pruritus generalized (1). In EDP-305 arms, Pruritus was reported in n=2/12 subjects with 10 mg and n=7/12 in 20 mg, and was of mild severity in n=3, of moderate severity in n=5, and severe n=1 subjects; with n=8 events that were considered drug related. Median time of onset was 10 days (range 6-15 days), and all pruritus events resolved within 9 days (range 3-13 days). No serious TEAEs or deaths occurred. No clinically significant laboratory abnormalities occurred, except for ALT/AST elevations in one subject: Levels of ALT were elevated on Day 7 to >2x ULN and remained elevated to >3x ULN from Day 7 to Day 17 with AST elevations to 2x ULN from Day 7 to Day 16. Elevations were returning to normal thereafter. There were no other changes in other laboratory parameters including bilirubin

and platelets, which remained within the normal range. No clinically relevant changes in the lipid panel including apolipoprotein A1 and B, lipoprotein A, total cholesterol, LDL, HDL, and triglycerides were observed. A numerically higher reduction in HDL was in observed at 20mg in presumptive NAFLD subjects.

Table 2. *Incidence of treatment-emergent adverse events (in ≥2 subjects in EDP-305 arms or leading to drug discontinuation) in the MAD phase (safety population).*

	Number (%) of Subjects/Number of Events						
	EDP-305 Dose						
	0.5 mg (n=6)	**1 mg (n=6)**	**2.5 mg (n=6)**	**5 mg (n=6)**	**10 mg (n=6)**	**20 mg (n=6)**	**Placebo (n=12)**
Healthy Subjects							
Any TEAE	2 (33.3) [5]	0	0	2 (33.3) [2]	1 (16.7) [1]	5 (83.3) [8]	2 (16.7) [4]
Discontinued	0	0	0	0	0	2 (33.3)*	0
Headache	1 (16.7)	0	0	2 (33.3)	0	0	0
Pruritus	0	0	0	0	1 (16.7)	5 (93.3)	1 (8.3)
ALT increased	0	0	0	0	0	1 (16.7)	0
AST increased	0	0	0	0	0	1 (16.7)	0
Presumptive NAFLD Subjects							
Discontinued	0	0	0	0	0	0	0
Any TEAE	0	3 (50.0) [4]	2 (33.3) [3]	1 (16.7 [1]	1 (16.7 [1]	3 (50.0) [7]	4 (33.3) [8]
Constipation	0	2 (33.3)	0	0	0	0	1 (8.3)
Pruritus	0	0	0	0	1 (16.7)	2 (33.3)	0

* Discontinued for elevated ALT/AST levels (1) and pruritus (1).

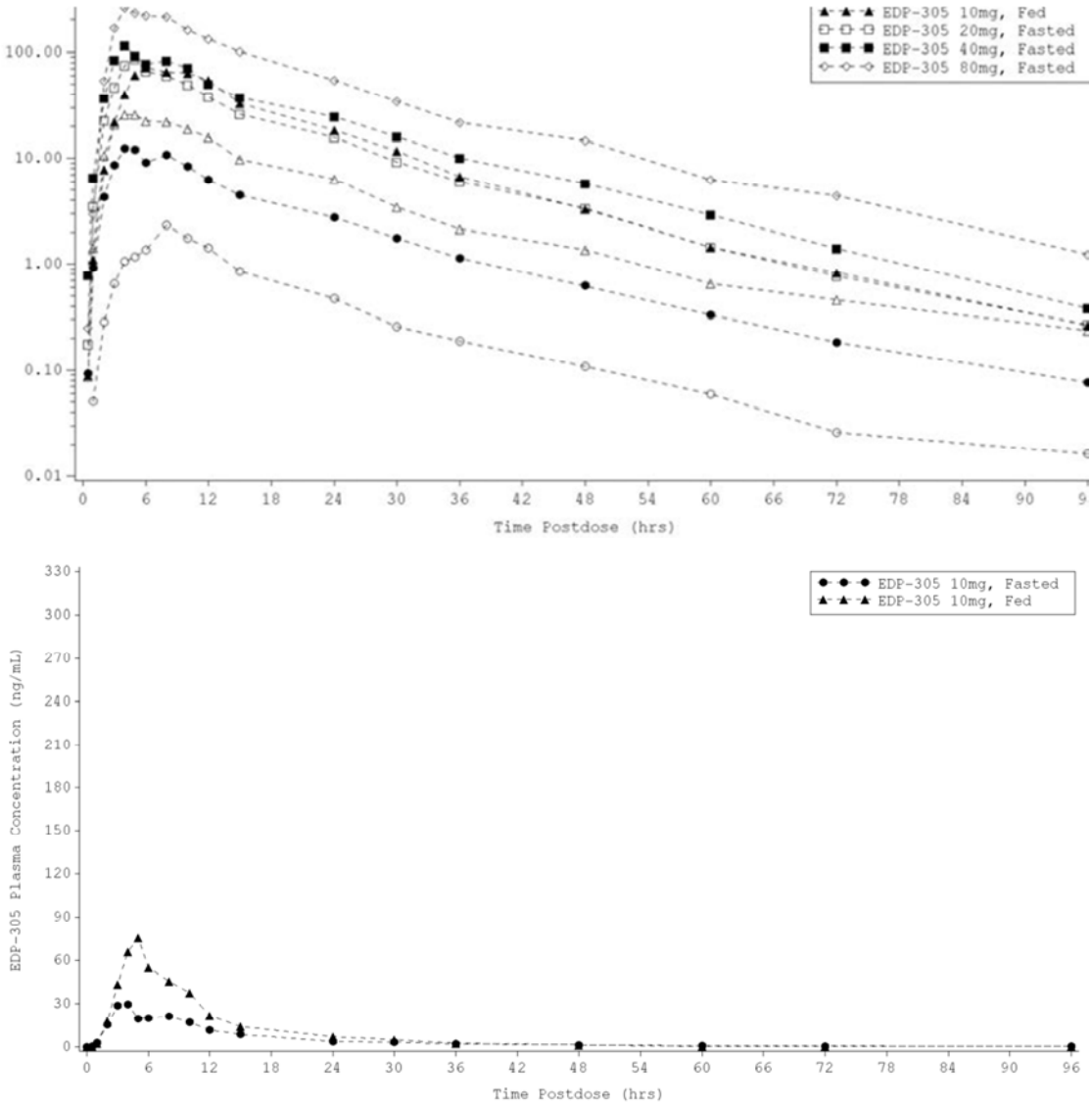


Figure 2. *Mean EDP-305 plasma concentrations over time after single doses (top) and for 10 mg dose in fasted and fed state (bottom) (PK population).*

3.2. Pharmacokinetics

3.2.1. Single Ascending Dose

Following single doses of EDP-305, plasma concentrations increased with dose across a range from 1 mg to 80 mg in fasted subjects, peak concentrations were reached between 3 to 12 hours post dose and decreased gradually thereafter (Figure 2). In the statistical analysis of dose proportionality, estimates for the slope (90% CI) were 1.107 for C_{max} (1.023, 1.192), 1.126 for $AUC_{0\text{-}t}$ (1.042, 1.209), and 1.116 for $AUC_{0\text{-}inf}$ (1.034, 1.197). The 90% CI of the estimates of slope for C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$ vs. dose did not contain 1.0, indicating a lack of dose proportionality.

Following a single 10 mg dose of EDP-305 in the fed state, peak plasma concentrations were typically reached between 4 to 10 hours post dose and decreased gradually thereafter (Figure 2). EDP-305 plasma concentrations and exposure in fed subjects were higher than those in fasted subjects (Table 3). Exposures ($AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$) increased by approximately 3-fold in fed subjects vs. fasted subjects at the same dose. Estimates for the LS mean ratios of fed/fasted (90% CI) were 3.390 for C_{max} (2.451, 4.690), 2.935 for $AUC_{0\text{-}t}$ (2.137, 4.031), and 2.884 for $AUC_{0\text{-}inf}$ (2.108, 3.945).

Table 3. *EDP-305 plasma PK parameters during SAD phase (PK population).*

	EDP-305 Dose						
	1 mg fasted (n=6)	**5 mg fasted (n=6)**	**10 mg fasted (n=8)**	**10 mg fed (n=8)**	**20 mg fasted (n=6)**	**40 mg fasted (n=6)**	**80 mg fasted (n=6)**
C_{max}, ng/mL	2.1 (53.0)	12.7 (43.7)	26.4 (47.3)	89.6 (15.2)	76.6 (75.3)	112.1 (30.7)	266.1 (18.5)
T_{max}, h	8 (8, 10.2)	4.5 (4, 8)	5 (4, 12)	6 (4, 10)	5 (4.1, 8)	4.1 (3, 10)	4 (4, 8)
$AUC_{0\text{-}inf}$, h*ng/mL	28.2 (62.0)	165.7 (55.7)	392.3 (39.6)	1131.3 (20.6)	1006.1 (49.5)	1543.1 (30.7)	3771.0 (22.3)
$t_{1/2}$, h	14.1 (49.8)	13.8 (50.3)	15.0 (38.0)	11.1 (15.5)	11.6 (17.8)	11.6 (16.0)	12.9 (20.9)
CL/F, h	35.4 (50.1)	30.2 (61.9)	25.5 (39.9)	8.8 (26.6)	19.9 (34.6)	25.9 (24.9)	21.2 (21.5)
Vd/F, L	721.2 (92.8)	599.7 (81.0)	551.0 (55.1)	141.8 (20.6)	332.2 (34.2)	434.7 (31.3)	394.1 (23.2)

Values are geometric mean (% coefficient of variation), except for T_{max}, which is median, range.

Table 4. *Plasma PK parameters for EDP-305 on Day 1 and Day 14 for healthy subjects and those with presumptive NAFLD.*

	0.5 mg (n=6)	**1 mg (n=6)**	**2.5 mg fasted (n=6)**	**5 mg (n=6)**	**10 mg (n=6)**	**20 mg (n=6)**
Healthy Day 1						
T_{max}, h	4 (4, 8)	4.5 (4, 8)	8 (4, 10)	6 (4, 23.8)	9 (3, 12)	4.5 (4, 8)
C_{max}, ng/mL	2.0 (92.7)	3.0 (26.7)	8.7 (21.0)	15.2 (57.0)	31.2 (46.5)	64.3 (57.5)
C_{12}, ng/mL	1.1 (88.0)	1.8 (34.4)	5.1 (35.7)	7.2 (72.6)	24.8 (49.7)	36.0 (28.1)
C_{24}, ng/mL	0.32 (91.8)	0.48 (20.1)	1.55 (16.6)	3.94 (101.0)	7.67 (61.4)	13.77 (32.8)
$AUC_{0\text{-}tau}$, h*ng/mL	20.9 (98.9)	32.5 (16.9)	93.4 (27.1)	149.5 (60.6)	366.0 (51.6)	705.1 (43.9)
Healthy Day 14						
T_{max}, h	4 (4, 10)	4 (4, 8)	4.5 (4, 10)	8 (4, 12)	11 (4, 12)	4.5 (3, 12)
C_{max}, ng/mL	2.6 (47.8)	3.3 (39.2)	12.3 (22.2	19.6 (46.4)	53.2 (89.5)	145.7 (81.5)
$AUC_{0\text{-}tau}$, h*ng/mL	28.2 (50.2)	37.0 (41.4)	128.9 (36.2)	245.5 (52.2)	810.8 (92.5)	2400 (82.7)
$AUC_{0\text{-}t}$, h*ng/mL	33.4 (52.9)	44.2 (40.3)	153.7 (38.8)	320.4 (52.3)	1234.3 (107.9)	4884.8 (112.5)
$t_{1/2}$, h	10.6 (23.6)	10.6 (32.7)	12.5 (23.5)	13.5 (25.8)	14.6 (34.4)	21.2 (45.7)
CL/F, h	17.7 (85.8)	27.0 (63.9)	19.3 (23.2)	20.4 (86.8)	12.3 (69.9)	8.3 (90.8)
Vd/F, L	270.1 (54.3)	413.9 (82.3)	342.7 (36.0)	395.3 (74.3)	259.0 (43.8)	363.3 (18.4)
Accumulation Index	1.35	1.14	1.38	1.64	2.21	4.10
NAFLD Day 1						
T_{max}, h	8 (4, 12)	4 (4, 4)	8 (4, 10)	9 (4, 12.1)	4.5 (3, 10)	4 (4, 12)
C_{max}, ng/mL	1.3 (65.0)	3.7 (56.7)	6.9 (64.9)	13.6 (87.8)	22.0 (30.5)	58.3 (60.6)
C_{12}, ng/mL	0.88 (49.0)	2.16 (68.6)	4.50 (90.0)	11.02 (68.0)	14.02 (39.4)	33.43 (46.0)
C_{24}, ng/mL	0.29 (57.7)	0.62 (87.3)	1.20 (107.8)	3.46 (94.0)	4.51 (40.8)	9.45 (72.8)
$AUC_{0\text{-}tau}$, h*ng/mL	15.9 (53.2)	39.5 (63.6)	76.0 (77.5)	170.6 (85.5)	259.5 (30.9)	636.2 (53.5)
NAFLD Day 14						
T_{max}, h	5 (2, 10)	8 (4, 12)	6 (4, 8.1)	4.5 (4, 10)	8.5 (4, 12.1)	5 (4, 12)
C_{max}, ng/mL	1.8 (116.6)	3.9 (51.9)	10.8 (85.1)	23.7 (75.7)	44.5 (35.7)	227.7 (87.8)
$AUC_{0\text{-}tau}$, h*ng/mL	24.1 (117.8)	48.1 (62.5)	121.8 (98.9)	297.2 (76.2)	635.0 (37.1)	3504.2 (95.8)
$AUC_{0\text{-}t}$ h*ng/mL	29.8 (125.2)	60.9 (69.5)	142.9 (111.4)	442.8 (74.3)	1005.6 (48.6)	6867.6 (117.4)
$t_{1/2}$, h	11.7 (26.3)	12.0 (16.2)	9.9 (26.0)	13.6 (19.1)	16.9 (26.5)	17.2 (22.2)
CL/F, h	20.8 (45.3)	20.8 (61.7)	20.5 (40.9)	16.8 (103.1)	15.8 (64.7)	5.7 (82.6)
Vd/F, L	350.9 (44.2)	360.5 (65.3)	290.9 (45.5)	312.6 (94.5)	384.4 (34.7)	260.4 (36.3)
Accumulation Index	1.51	1.22	1.60	1.74	2.44	5.50

Values are geometric mean (% coefficient of variation), except for T_{max}, which is median, range.

For each of the three metabolites of EDP-305, EP-022571, EP-022572, EP-022679, exposures increased with dose and were 2-fold to 3-fold higher in the fed state. Half-life and T_{max} of the metabolites were generally comparable to the parent compound. Minimal urinary excretion occurred with EDP-305, and metabolites were not quantifiable in urine.

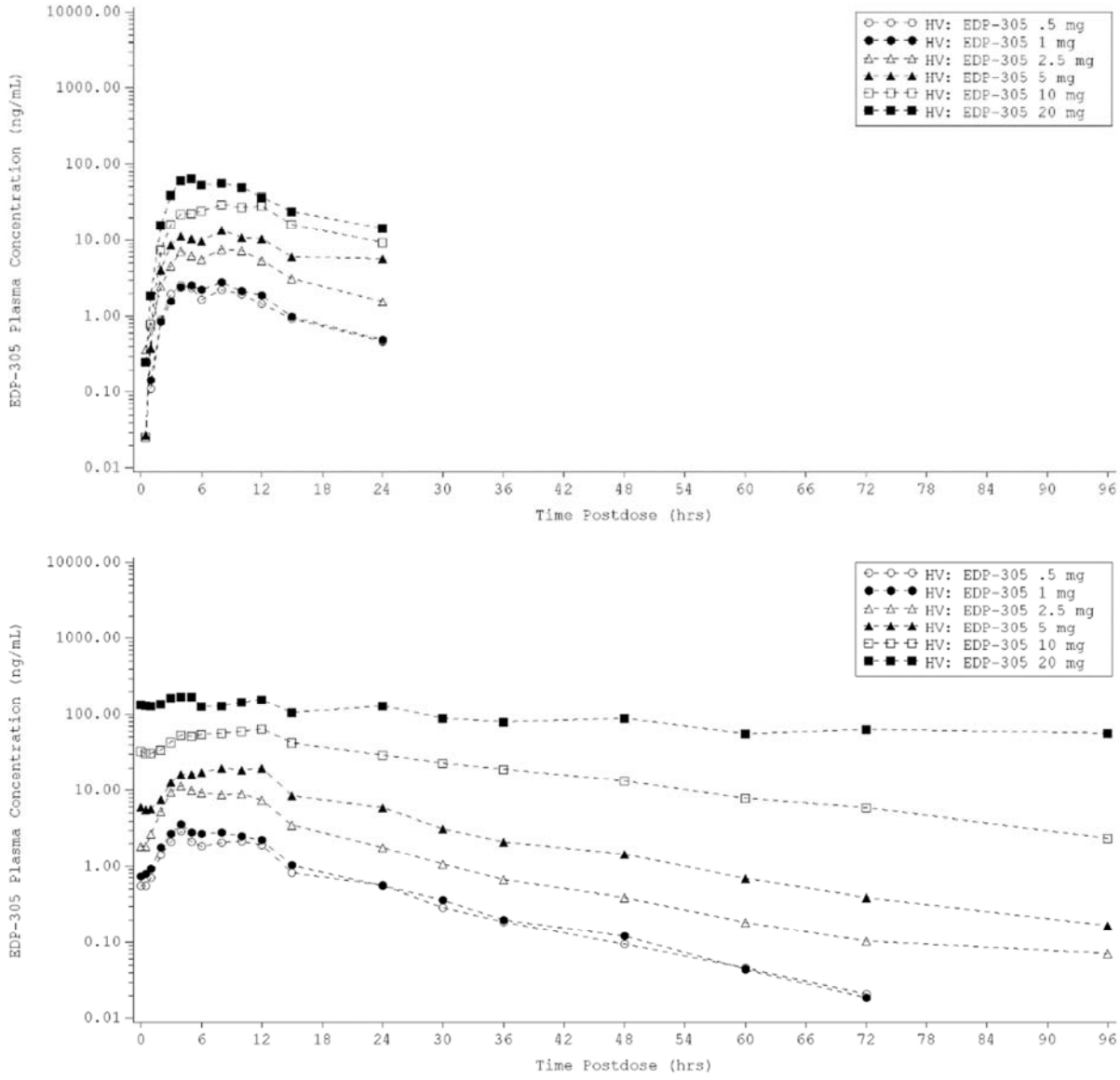
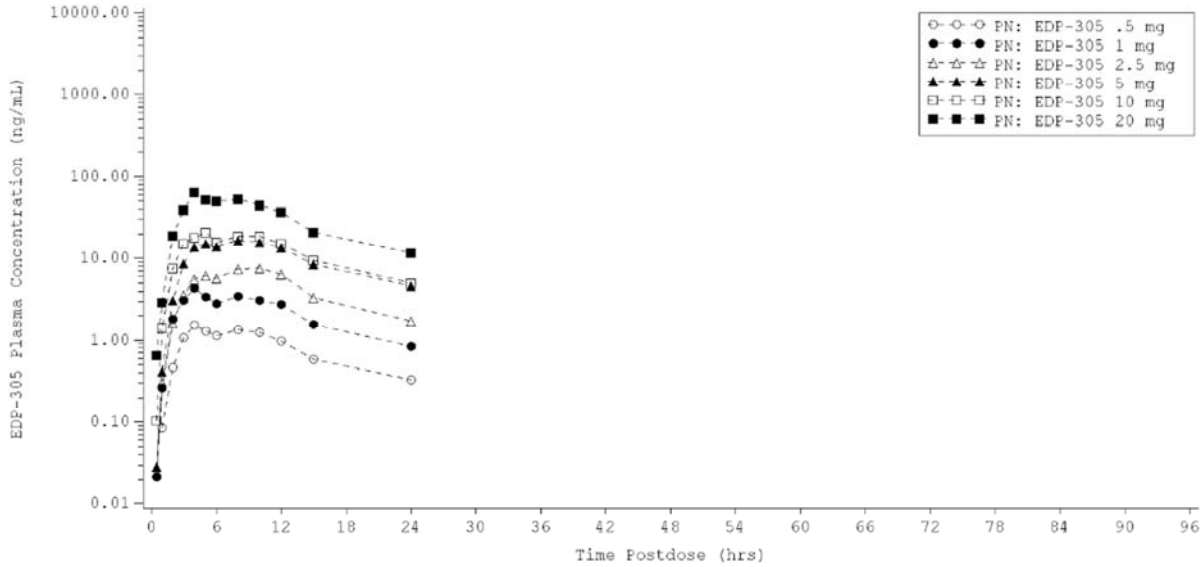


Figure 3. *Mean EDP-305 plasma concentrations over time in healthy subjects for the MAD phase on Day 1 (top) and Day 14 (bottom) (PK population).*



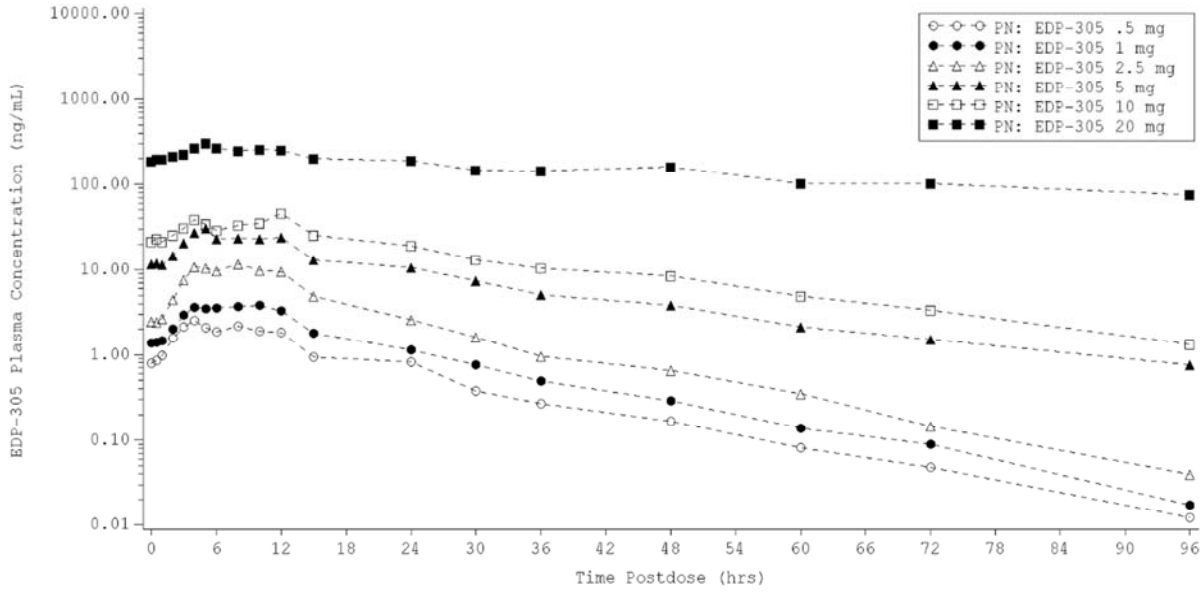


Figure 4. *Mean EDP-305 plasma concentrations over time in presumptive NAFLD subjects for the MAD phase on Day 1 (top) and Day 14 (bottom) (PK population).*

3.2.2. Multiple Ascending Dose

Following oral administration of EDP-305 in healthy subjects on Day 1, mean peak plasma concentrations were reached between 3 to 12 hours post dose for all doses, and decreased slowly thereafter until 24 hours (Figure 3). Median T_{max} ranged from 4.0 to 9.0 hours across dose groups, and geometric mean C_{max} and $AUC_{0\text{-}tau}$ generally increased with dose from 0.5 mg to 20 mg (Table 4). Mean concentrations at 12 hours (C_{12}) and 24 hours (C_{24}) generally increased with increasing dose. On Day 14 in healthy subjects, mean peak EDP-305 plasma concentrations were reached between 3 to 12 hours post dose, which was similar to Day 1, and decreased gradually thereafter (Figure 3). Steady state appeared to be reached by Day 3 for the 0.5 mg, 1 mg, and 2.5 mg doses, Day 5 for the 5 mg dose, and Day 9 for the 10 mg and 20 mg doses. Median T_{max} ranged from 4.0 to 11.0 hours across dose groups (Table 4), which was generally similar to Day 1. Geometric mean $t_{1/2}$ was for doses of 0.5 mg to 10 mg ranged from 10.6 to 14.6 hours, which was similar to $t_{1/2}$ in the SAD phase. Geometric mean C_{max} and $AUC_{0\text{-}tau}$ and $AUC_{0\text{-}t}$ increased with dose at steady state. Variability of exposure parameters (%CV 81.5% to 112.5%) was observed at 10 mg and 20 mg doses. Compared with Day 1, exposures appeared to increase with multiple dosing, which was more evident at higher doses of 10 mg and 20 mg. The accumulation index ranged from 1.14 to 1.64 for doses less than 10 mg, and from 2.21 to 4.10 for the higher doses of 10 mg and 20 mg.

Following oral administration of EDP-305 in subjects with presumptive NAFLD on Day 1, mean peak plasma concentrations were reached between 3 and 12 hours post dose, and decreased slowly thereafter until 24 hours (Figure 4). EDP-305 plasma concentrations increased with increasing dose across the range from 0.5 mg to 20 mg. Median T_{max} ranged from 4.0 to 9.0 hours across dose groups, and geometric mean C_{max} and systemic exposure ($AUC_{0\text{-}tau}$) increased with dose (Table 4). On Day 14 in presumptive NAFLD subjects, mean peak plasma concentrations were reached between 2 to 12 hours post dose similar to Day 1 (Figure 3). Steady state appeared to be reached by Day 3 for the 0.5 mg and 1 mg doses, Day 5 for the 2.5 mg dose, Day 7 for the 5 mg and 10 mg doses, and Day 9 for the 20 mg dose (Table 4). EDP-305 steady-state plasma concentrations in subjects with presumptive NAFLD were similar to those observed in healthy subjects. Median T_{max} ranged from 4.5 to 8.5 hours across doses, which was similar to Day 1. Geometric mean $t_{1/2}$ was similar from 0.5 mg to 20 mg and ranged from 9.9 to 17.2 hours. Geometric mean C_{max} and systemic exposures ($AUC_{0\text{-}tau}$ and $AUC_{0\text{-}t}$) increased with dose at steady state. Geometric mean exposures appeared to be dose proportional up to 2.5 mg and greater than dose proportional at higher doses. Compared with Day 1, exposures appeared to increase with multiple dosing, which was more evident at the higher doses of 10 mg and 20 mg. Geometric mean accumulation index ranged from 1.22 to 1.74 for doses less than 10 mg, and from 2.44 to 5.50 for the higher doses of 10 mg and 20 mg indicating low accumulation at doses less than 10 mg, and moderate to high accumulation at the 10 mg and 20 mg doses.

Generally, PK parameters with metabolites were similar to those of the parent compound, and exposure to metabolites increased with increasing dose. EDP-305 and metabolite urine concentrations were BLQ suggesting limited renal clearance.

3.3. Biomarkers

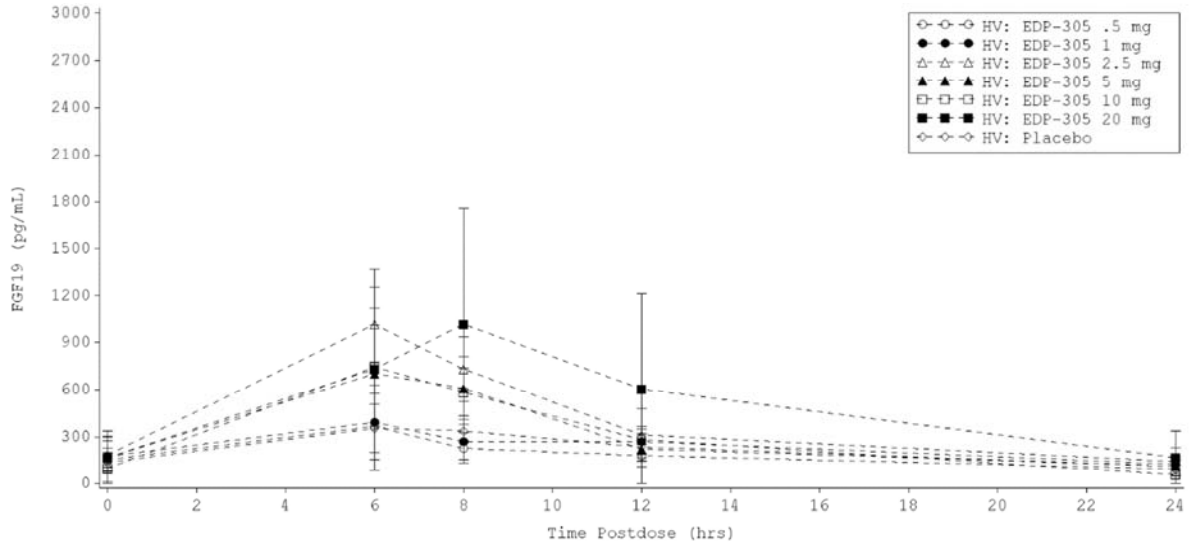
In the SAD phase at single doses of 1 to 80 mg, EDP-305 demonstrated target engagement consistent with FXR agonism with increases in FGF19 (C_{max} range: 485 – 1519 pg/mL for EDP-305 doses of 1 to 80 mg vs. 320 pg/mL for

placebo) and reductions in C4 (C_{min} range: 9.5 – 0.9 ng/mL for EDP-305 1 to 80 mg vs. 9.4 ng/mL for placebo) that were greater with increasing dose. Total bile acid levels were below the assay quantification limit of 5 µmol/L.

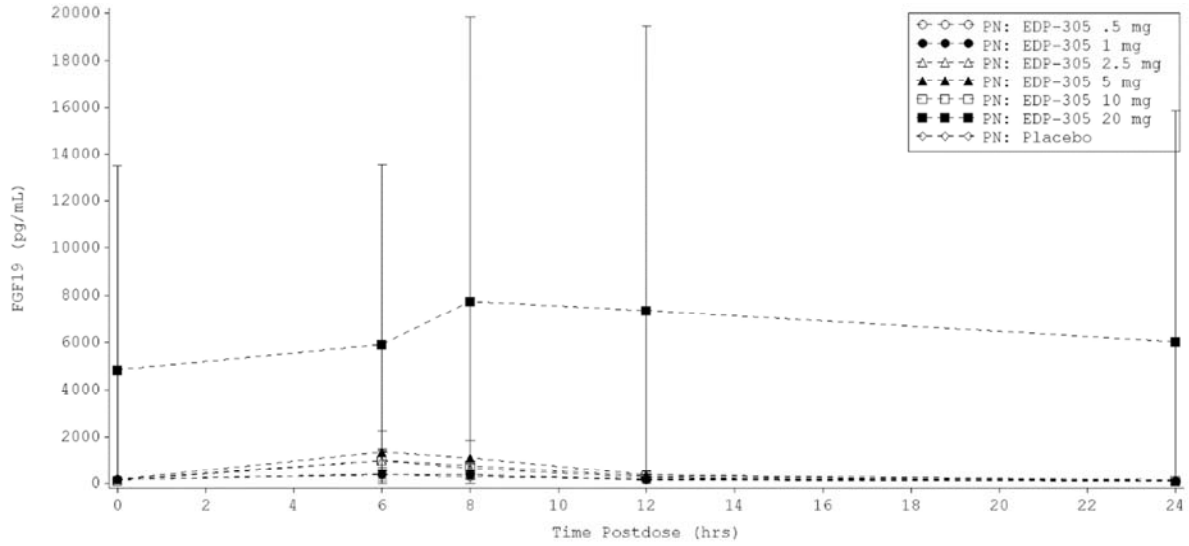
In the MAD phase, FGF19 levels increased and C4 levels decreased with all doses supporting robust target engagement of FXR by EDP-305 (Figure 5). Following multiple doses of EDP-305 ranging from 0.5 to 20 mg, increases in FGF-19 were observed post dose in healthy subjects and subjects with presumptive NAFLD. Mean percent increases in peak FGF19 concentrations (6-hour post dose on Day 14) ranged from approximately 304% to 1739% (compared to 481% with placebo) and from approximately 431% to 4095% (compared to 215% with placebo) in healthy subjects and subjects with presumptive NAFLD, respectively. FGF19 increases measured by C_{max} and AUC were higher than placebo at EDP-305 doses ≥1 mg in healthy subjects, while in subjects with presumptive NAFLD, they were higher than placebo at doses ≥2.5 mg (Day 14).

Over the same multiple dose range of 0.5 to 20 mg, reductions in C4 were observed post dose in both healthy subjects and subjects with presumptive NAFLD (Figure 6). Mean percent decreases in C4 (12-hour post dose on Day 14) ranged from approximately -1 to -96% (compared to -63% with placebo) and from approximately -40% to -97% (compared to -8% with placebo) in healthy subjects and subjects with presumptive NAFLD, respectively. C4 reductions (C_{min} [minimum concentration] and AUC) were more pronounced than with placebo at doses >1 mg in healthy subjects, while in subjects with presumptive NAFLD, they were more pronounced than placebo at all EDP-305 doses.

No notable changes occurred in total bile acids levels (data on file).



Healthy – FGF19



Presumptive NAFLD – FGF19

Figure 5. *Mean (SD) FGF19 concentrations (pg/mL) in healthy subjects (top) and presumptive NAFLD subjects (bottom) over time (Day 14) in the MAD phase (PD Population).*

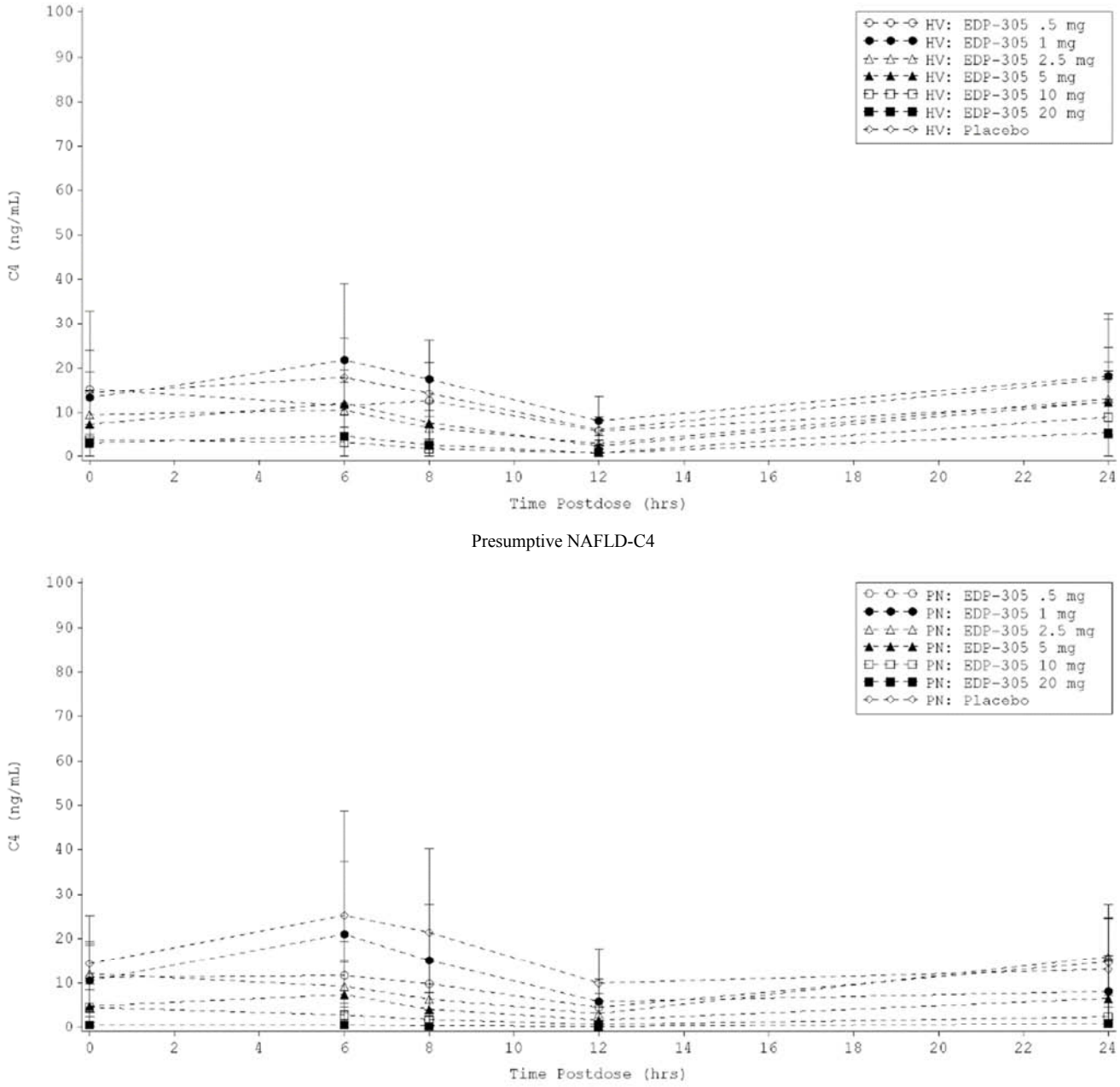


Figure 6. *C4 concentrations (ng/mL) in healthy subjects (top) and presumptive NAFLD subjects (bottom) over time (Day 14) in the MAD phase Healthy-C4 (PD Population).*

4. Discussion

EDP-305 is a potent FXR agonist that is being developed for the treatment of patients with NASH. Here we report the results of a Phase 1 in healthy subjects and in subjects with presumptive NAFLD (e.g., obese with or without pre-diabetes or diabetes mellitus). In this single and multiple ascending dose Phase 1 study, EDP-305 was generally safe and well tolerated at multiple oral once daily doses. No serious AEs were reported. Regarding safety, a higher proportion of treatment-emergent-adverse events were observed in subjects receiving EDP-305 compared with placebo. The difference in the proportion of TEAEs was largely driven by pruritus. The majority of pruritus events were of mild or moderate severity and occurred with highest multiple doses of EDP-305 at 10 and 20 mg; no pruritus was reported at doses below 10mg. Pruritus is common with FXR agonists [23, 24]. The mechanism for pruritus may involve activation of TGR5 by the FXR agonist or active metabolites [12, 13], or alternatively could reflect increases in the concentration of total bile acids [25]. EDP-305 had minimal effect on total bile acid levels over the short study duration.

The results demonstrated an increase in C_{max} and AUC with increasing doses of EDP-305 among healthy subjects as well as those with presumptive NAFLD. Increased exposure (AUC) was observed during the fed state due to lower apparent clearance that could be attributed to increased oral

bioavailability when EDP-305 was administered with food. In addition, EDP-305 metabolites also displayed increased exposure (AUC) with increasing dose (data not shown). Urine concentrations of EDP-305 and its metabolites were minimal, indicating that urinary clearance plays a limited role in EDP-305 excretion. In this first-in-human study of EDP-305, PK results from both the SAD and MAD phases supported once daily dosing.

Strong FXR target engagement was observed with EDP-305 with increased levels of FGF19 and reduced levels of C4 at doses of EDP-305 >1 mg. Overall PK/PD profiles were similar between healthy volunteers and presumptive NAFLD with a more pronounced PD effect in presumptive NAFLD than healthy volunteers at all doses when compared to placebo. Increases in FGF19 and decreases in C4 are consistent with previous reports with other FXR agonists [26-30]. In the MAD phase, no significant changes in the lipid profile were observed in healthy subjects at any dose, and no dose-related changes in lipids were observed in presumptive NAFLD subjects except for reductions of total cholesterol and HDL cholesterol at the 20 mg dose; no increase in LDL cholesterol was observed. This is in contrast to the FXR agonist, obeticholic acid, which caused significant increases in total and LDL cholesterol in healthy subjects [31] and those with NASH [23, 32].

Overall, these data were consistent with a predictable pharmacokinetic profile amenable to once-daily dosing, minimal accumulation of the compound in plasma after repeat dose except for the highest dose 20mg, and steady state achievement within 3 days for doses≤5mg.

EDP-305 was safe and generally well tolerated except for pruritus at EDP-305 doses ≥10 mg and with minimal effects on lipids. Significant elevations of FGF19 and diminutions in C4 demonstrated potent engagement of the FXR receptor at doses that neither elicit adverse effects on lipids nor result in pruritus.

5. Conclusion

These results supported further clinical evaluation of EDP-305 in patients with NASH. A 12-week study (ARGON-1; NCT03421431) is completed, and a 72-week study (ARGON-2; NCT04378010) currently is ongoing. Combinations of an FXR agonist such as EDP-305 with agents of complementary mechanisms of action may also provide value for patients. Further studies will be required to determine if such combination therapies can facilitate both reduction of fibrosis along with improvement in steatosis and other metabolic parameters, and allow the use of an optimal dose that doesn't elicit too much pruritus.

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