Results of A Phase I Safety, Tolerability and Pharmacokinetic Study of ANA598, A Non-nucleoside NS5B Polymerase Inhibitor, in Healthy Volunteers

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ABSTRACT*

Background: ANA598 is a novel non-nucleoside inhibitor of the hepatitis C virus (HCV) genotype 1 NS5B polymerase in development for the treatment of patients with chronic HCV infection. *In vitro* studies have shown that ANA598 exhibits potent antiviral activity in genotype 1a and 1b replicon systems with EC_{50} values of 50 and 3 nM (28 and 1.7 ng/mL) and EC_{95} values of 500 and 30 nM (280 and 17 ng/mL), respectively. Results from a single-dose escalation trial in healthy subjects are reported here.
Methods: A phase I, randomized, double-blind, ascending, single-dose trial was conducted to evaluate the safety, tolerability and pharmacokinetics of ANA598 formulated as 200 mg capsule. Healthy subjects (18 to 55 years of age) were randomized into treatment cohorts consisting of 6 subjects receiving single ascending oral doses of ANA598 (400, 800, 1400, 2000 [fasted and fed], or 3000 mg) and 2 subjects receiving placebo. An additional cohort received 800 mg twice daily (BID) for 2 doses. All doses were administered in the fasted state with the exception of the 2000 mg-fed group. Safety and pharmacokinetics were evaluated for 7 days after oral administration of ANA598 capsules or matching placebo.
Results: ANA598 was well tolerated at all doses administered. There were no serious adverse events and no withdrawals from the study. All reported adverse events were classified as mild (13 events) or moderate (1 event), with no apparent dose relationship and no pattern of events within any body system. ANA598 systemic exposure generally increased with increasing doses, though the increase was less than dose proportional under fasting conditions (Table 1). Both C_{max} and AUC increased by several fold in subjects who received ANA598 after consuming a high-fat meal. ANA598 plasma elimination $t_{1/2}$ ranged from 22 to 31 hours, and the $t_{1/2}$ was independent of dose.
Conclusion: The favorable pharmacokinetic and tolerability profiles of ANA598 support QD or BID administration. The results support continued development of ANA598 for patients with chronic HCV infection.

Table 1. Mean (SD) ANA598 Concentrations (µg/mL) at 12 and 24 Hours after Administration

	400 mg	800 mg	1400 mg	2000 mg	2000 mg-fed	3000 mg	800 mg BID
C12	8.9 (3.9)	13.4 (4.0)	11.8 (4.2)	23.9 (11.0)	143.5 (26.3)	35.0 (7.6)	17.2 (7.4)
C24	4.8 (2.3)	7.7 (2.4)	6.7 (2.8)	12.0 (5.2)	97.5 (34.3)	20.0 (4.4)	83.4[a] (17.5)

[a] Concentration at 12 hours after the second (PM) dose.

* Updated abstract

Background

ANA598 is a novel non-nucleoside inhibitor of the HCV genotype 1 NS5B polymerase in development for the treatment of patients with chronic HCV infection. In *in vitro* studies, ANA598 has exhibited low nM potency against HCV genotype 1a and 1b replicon systems. Following oral administration of [^{14}C]ANA598 to rats and monkeys, the parent compound in plasma accounted for approximately 99% of the total drug-related radioactivity concentrations, indicating no major circulating metabolites. In rats and monkeys, the majority of radioactivity (81 to 96%) was recovered in feces; this observation in conjunction with high levels of ANA598 radioactivity seen in the liver and/or bile, suggested that biliary excretion is the major route of ANA598 elimination.

Objectives

- To assess the safety and tolerability of ANA598 after oral administration of single ascending doses up to 3000 mg in healthy volunteers.
- To determine the plasma PK of ANA598 after oral administration of single ascending doses in healthy volunteers.
- To evaluate the effect of food on the plasma PK of ANA598 after oral administration of the 2000-mg dose following consumption of a high-fat meal.
- To assess the PK of ANA598 after oral administration of two 800-mg doses given at 12 hours apart.

Design & Methods

- Randomized, double-blind, placebo-controlled, single ascending dose
- Healthy subjects were randomized into sequential cohorts consisting of 6 subjects administered ANA598 capsules and 2 subjects administered matching placebo
- Safety assessments included physical examination, vital signs, hematology, blood chemistry, urinalysis, ECG and adverse event reporting throughout the study
- For pharmacokinetic evaluations, serial plasma samples were collected for up to 144 hours (6 days) post dose and analyzed using a validated high performance liquid chromatography-tandem mass spectrometery (LC-MS/MS) method

Results

Note: The clinic portion of the study was recently completed but the clinical database has not been locked. Final analysis of the safety data is pending database lock.

Table 2. Demographics

Dose (mg)	400 (n=6)	800 (n=6)	1400 (n=6)	2000 (n=6)	3000 (n=6)	800(BID) (n=6)	Placebo (n=12)
Gender							
M	3	4	5	6	5	3	9
F	3	2	1	0	1	3	3
Age (yr)							
18-30	3	1	4	2	2	4	8
31-40	1	3	1	3	2	0	1
41-50	0	1	1	1	2	0	2
51-55	2	1	0	0	0	2	1
Race							
Caucasian	3	5	1	0	0	0	3
Hispanic	3	1	3	6	6	5	9
Black	0	0	2	0	0	0	0
A. Indian	0	0	0	0	0	1	0
Weight (kg) Range	55.7 – 97.6	51.3 – 90.1	62.4 – 97.2	60.1 – 82.3	56.2 – 72.9	59.8 – 76.6	50.4 – 87.6

Table 3. Summary of Reported Adverse Events

Dose (mg)	Subj	Event	Severity
Placebo	004	Swelling, left antecubital area secondary to blood draw	Mild
	046	Bilateral foot pain	Mild
400	005	Constipation	Mild
	005	Intoxicated feeling	Mild
	008	Headache	Moderate
	008	Nausea	Mild
	008	Vomited (x2)	Mild
	008	Headache	Mild
	008	Nausea	Mild
800	013	Late menses	Mild
800 BID	040	Sinus pressure	Mild
1400	023	Pain right and left antecubital fossa at venipuncture site	Mild
	023	Vaginal irritation	Mild
2000 (fasted)[1]	No AE		
2000 (fed)[1]	030	Dyspepsia	Mild
3000	No AE		

[1] Same cohort

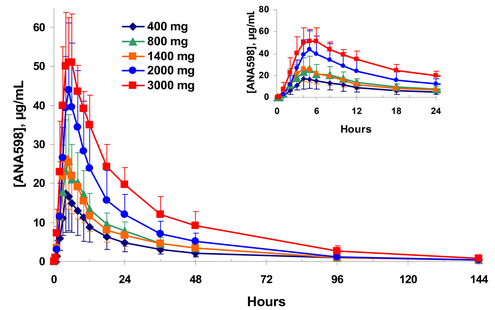
- All reported AEs were classified as mild (13 events) or moderate (1 event), with no apparent dose relationship and no pattern of events within any body system.
- There were no clinically important changes in physical examinations, vital signs, ECGs, or in clinical laboratory results. There was one instance of mild elevation in total bilirubin in the 3000 mg cohort.

Table 4. Mean (SD) Pharmacokinetic Parameters of ANA598 after Single Dose Administration in Fasted and Fed States

Dose (mg)	C_{max} µg/mL	T_{max} h	AUC_{0-t} µg*h/mL	AUC_{0-inf} µg*h/mL	$T_{1/2}$ h
400	18 (8.6)	4.0 (1.1)	387 (169)	401 (178)	31 (5.2)
800	28 (13)	4.8 (1.9)	576 (184)	598 (188)	30 (16)
1400	27 (12)	4.2 (0.8)	556 (245)	569 (260)	25 (4.6)
2000	44 (17)	4.7 (0.5)	909 (349)	921 (355)	24 (3.2)
2000-fed	182 (43)	8.0 (1.3)	5603 (2103)	5672 (2163)	22 (2.4)
3000	57 (9.8)	6.2 (3.0)	1429 (297)	1478 (312)	27 (2.1)

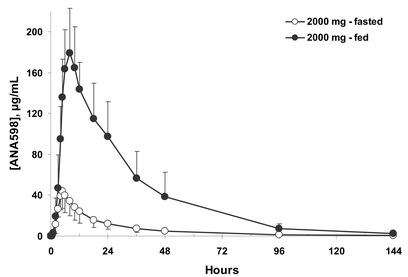
Non-compartmental PK analysis was performed on individual ANA598 plasma concentrations versus time data using WinNonlin (Professional Edition software, v.5.1).

Figure 1. Mean (SD) Plasma Concentrations of ANA598 after Single Dose Administration in Fasted State



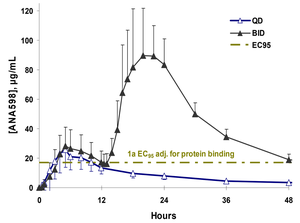
- Systemic exposure of ANA598 increased with increasing doses, though the increase was less than dose proportional.
- The elimination $t_{1/2}$ was relatively consistent across doses.

Figure 2. Mean (SD) Plasma Concentrations of ANA598 after Single Dose Administration in Fasted and Fed States



- The rate and extent of ANA598 absorption (C_{max} and AUC) were increased significantly, while intersubject variability (%CV) was reduced when ANA598 was administered following consumption of a high-fat meal

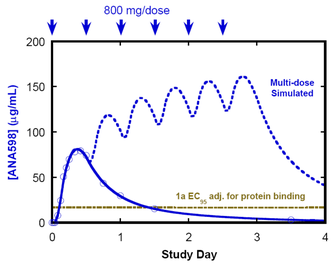
Figure 3. Mean (SD) Plasma Concentrations of ANA598 after 800 mg QD or BID Administration



The dashed line depicts EC_{95} for genotype 1a replicon after adjustment for the relative amount of free ANA598 in human plasma and replicon media. The EC_{95} for genotype 1b is ~17-fold lower than the EC_{95} for genotype 1a.

- Exposure after the AM dose (800 mg) was consistent with the 800 mg single-dose cohort (overnight fast for 10h before and 4h after dosing).
- Exposure after the PM dose (800 mg) increased several fold, most likely because of food effect (subjects ate 2h before and 2h after dosing), and partly due to expected accumulation.

Figure 4. Multiple-Dose Simulation of ANA598 Based on the Observed Plasma Concentrations in Healthy Subjects (800 mg PM Dose of the BID Cohort)



ANA598 plasma profile after administration of the PM dose (800 mg) from the BID cohort is shown as the solid blue line. The predicted level of ANA598 when administered at 800 mg BID for 3 days (the duration of treatment being investigated in ongoing Phase 1b trial in HCV patients) is depicted by the dotted blue line.

- The predicted plasma concentrations of ANA598 exceed the genotype 1a replicon EC95 when ANA598 800 mg is administered twice daily with food.

Conclusions

- ANA598 was very well tolerated after administration of single oral doses up to 3000 mg.
- There were no serious AEs or discontinuations from the study.
- ANA598 systemic exposure (C_{max} and AUC) generally increased with increasing doses, though the increase was less than dose proportional under fasting conditions.
- ANA598 plasma elimination $t_{1/2}$ ranged from 22 to 31 hours and was relatively consistent across doses.
- Following consumption of a high fat-meal, both C_{max} and AUC increased significantly relative to the fasted state.
- Exposure was higher after the PM dose (800 mg) in the BID cohort, most likely because of food effect and partly due to expected accumulation.
- The favorable PK and tolerability profiles of ANA598 support QD or BID administration.
- The results support continued development of ANA598 for patients with chronic HCV infection.

Disclosures

Mohamad Rahimy - Employee of Anadys Pharmaceuticals, Inc., Stock Holder of Anadys Pharmaceuticals, Inc., Discussion will include off-label / investigative use of medicine(s), medical devices or procedure(s); Constance Crowley - Employee of Anadys Pharmaceuticals, Inc., Stock Holder of: Anadys Pharmaceuticals, Inc.; James Freddo - Employee of Anadys Pharmaceuticals, Inc., Stock Holder of: Anadys Pharmaceuticals, Inc.; Maria Sergeeva - Employee of Anadys Pharmaceuticals, Inc., Stock Holder of: Anadys Pharmaceuticals, Inc.; Brent Golec - Employee of Anadys Pharmaceuticals, Inc., Stock Holder of: Anadys Pharmaceuticals, Inc.