

Abstract

Background: ANA773 is an oral prodrug of a small-molecule TLR7-selective agonist discovered at Anadys Pharmaceuticals. Previous preclinical studies with ANA773 have demonstrated pharmacologic activity, notably induction of multiple subtypes of endogenous interferon- α (IFN- α) *in vitro* in human peripheral blood mononuclear cells (PBMCs) and induction of systemic IFN- α in cynomolgus monkeys, which supports the potential utility of ANA773 in HCV. A clinical study was conducted to investigate the pharmacokinetics (PK), pharmacodynamics (PD) and safety of ANA773 in healthy volunteers.

Methods: ANA773 was investigated in a double-blind, placebo-controlled, dose escalation study composed of five dose groups (200, 400, 800, 1200 and 1600 mg). In each group, 6 subjects received oral ANA773 and 2 received placebo. Subjects typically received a single dose of ANA773, followed by a 14-18 day rest period, and were then dosed with ANA773 every other day for 7 days (4 doses).

Results: ANA773 was rapidly converted to the active metabolite with the C_{max} of the latter occurring typically within 1.5 hours after administration. Plasma exposure of the active metabolite was approximately dose proportional and, consistent with its short t_{1/2}, there was no evidence of accumulation. There were dose-related increases in various markers of IFN- α response after administration of ANA773. Notably, significant induction of oligoadenylate synthetase (OAS) activity, neopterin and whole blood OAS1 and ISG15 mRNA levels were observed in most subjects receiving 800 mg or greater. In contrast, substantial levels of circulating IFN- α were seen in only a few subjects confined to the 1200 mg and 1600 mg groups. Importantly, the pharmacologic response to ANA773 continued on off-dosing days and was of comparable magnitude following each dose during the multi-dose phase. ANA773 was generally well tolerated with adverse events (AEs) increasing in frequency and intensity with increasing dose. Mild to moderate AEs were reported, with a few subjects reporting flu-like symptoms in the higher dose groups. No serious AEs were reported.

Conclusions: Oral ANA773 was efficiently converted to the active metabolite, induced a dose-related IFN-dependent response and was typically well tolerated in healthy volunteers. These findings, in conjunction with pharmacologic and toxicologic data from animal studies, justify clinical exploration with ANA773 in patients with chronic HCV infection. Accordingly, a trial with ANA773 in HCV infected patients is currently in progress.

Background

ANA773 is an oral prodrug of a TLR7 agonist in development for the treatment of patients with chronic HCV infection. The active metabolite of ANA773 ("ANA773 active") induced multiple subtypes of IFN- α and functionally activated NK cells *in vitro* in human peripheral blood mononuclear cells (PBMCs). Conditioned media from PBMCs activated by this TLR7 agonist inhibited the replication of HCV *in vitro* (replicon system). Consistent with the *in vitro* findings, oral administration of ANA773 induced systemic IFN- α production in cynomolgus monkeys, with IFN activity being observed in both the periphery and liver. Importantly, ANA773 was well-tolerated in 13-week toxicology studies at doses that produced robust immune induction.

Study Design

- A double-blind, placebo-controlled, single/multiple dose escalation study.
- Healthy subjects were randomized into five sequential cohorts (200, 400, 800, 1200 and 1600 mg). In each group, 6 subjects received oral ANA773 and 2 received placebo.
- Subjects received a single dose of ANA773, followed by a 14-18 day rest period, and were then dosed with ANA773 every other day for 7 days (4 doses).

Results

Table 1. Demographics

DEMOGRAPHIC	200 mg (n=6)	400 mg (n=6)	800 mg (n=6)	1200 mg (n=7)	1600 mg (n=6)	PLACEBO (n=10)
Male, n (%)	6 (100)	5 (83)	5 (83)	3 (42)	1 (16)	6 (60)
Age, mean (range)	33 (19-61)	43 (20-62)	47 (21-61)	51 (37-61)	49 (25-64)	57 (22-65)
White, n (%)	4 (66)	5 (83)	4 (66)	6 (85)	6 (100)	9 (90)
Black/African American, n (%)	2 (33)	0 (0)	1 (16)	0 (0)	0 (0)	1 (10)
BMI, mean (range)	23.9 (20.4 - 29.8)	23.4 (20.4 - 26.5)	25.7 (22.3 - 30.4)	25.9 (21.1 - 32.8)	26.1 (24.0 - 27.3)	25.6 (21.3 - 29.4)

Table 2. Preliminary Listing of Adverse Events Regardless of Causality

ADVERSE EVENT ^a	200 mg (n=6)	400 mg (n=6)	800 mg (n=6)	1200 mg (n=7)	1600 mg (n=6)	PBO (n=10)
Nausea	1	0	3	4	6	1
Headache	0	1	1	2	5	5
Pain ^b	0	1	3	2	4	4
Fatigue	0	0	0	3	4	0
Fever	0	0	1	1	4	0
Myalgia	0	1	1	2	3	0
Chills	0	0	0	1	3	0
Abdominal pain	0	1	1	1	2	2
Dizziness	1	1	1	2	1	0
Lymphocytopenia	0	0	0	1	2	0
Leucopenia	0	0	0	0	2	0
Thrombocytopenia	0	0	0	0	2	0
Vomiting	0	0	0	1	1	0
Drowsiness/sleepiness	0	0	1	1	0	1
Epistaxis	0	0	0	0	3	0
Pruritus	0	0	1	0	1	0
Herpes labialis	0	0	0	0	2	0
Cannula site reaction	1	0	1	1	0	2
Urinary frequency	0	2	0	0	0	1

^a Listed adverse events are for events reported by at least 2 subjects across all dose groups. Subjects reporting multiple episodes of one event are only counted once.

^b back, leg, knee, bone, shoulder, calf, ankle, elbow, mandibular, ear.

- ANA773 was generally well tolerated with adverse events (AEs) increasing in frequency and intensity with increasing dose.
- Mild to moderate AEs were reported, with a few subjects reporting flu-like symptoms in the higher dose groups. No serious AEs were reported.
- Three subjects (one 1200 mg subject and two 1600 mg subjects) discontinued treatment during the multiple-dose period of the study.
- Of these three discontinuations, one subject (with a high baseline APTT) was discontinued due to an increase in APTT, which returned to baseline levels within 48 hours after the last dose. To date, increases in APTT have not been observed in HCV patients treated for 28 days.
- The two other discontinuations were due to lack of tolerability, principally of flu-like symptoms.

Table 3. Mean (SD) Pharmacokinetic Parameters of Plasma ANA773 Active after a Single Oral Dose of ANA773

Dose (mg)	C _{max} (ng/mL)	T _{max} (hr)	AUC ₀₋₁₂ (hr*ng/mL)	AUC _{0-inf} (hr*ng/mL)	T _{1/2} (hr)
200	4158 (506)	0.5 (0.0)	5960 (774)	5977 (774)	2.3 (0.2)
400	8158 (1796)	0.5 (0.0)	14557 (1698)	14579 (1703)	2.5 (0.6)
800	13050 (3034)	0.9 (0.5)	27431 (7538)	27526 (7576)	3.8 (0.2)
1200	13117 (3758)	1.0 (0.6)	40994 (8838)	41096 (8837)	3.3 (0.4)
1600	17683 (3359)	0.9 (0.4)	51574 (9307)	51637 (9302)	2.7 (0.6)

- Systemic exposure to ANA773 active was approximately dose proportional, although the increase in C_{max} with escalating dose was less than dose proportional at doses \geq 800 mg.

Results

Figure 1. ANA773 Active After a Single Oral Dose of ANA773 (a) Mean (SEM) Plasma Concentration (b) Mean (SEM) Plasma C_{max} and AUC_{0-inf}

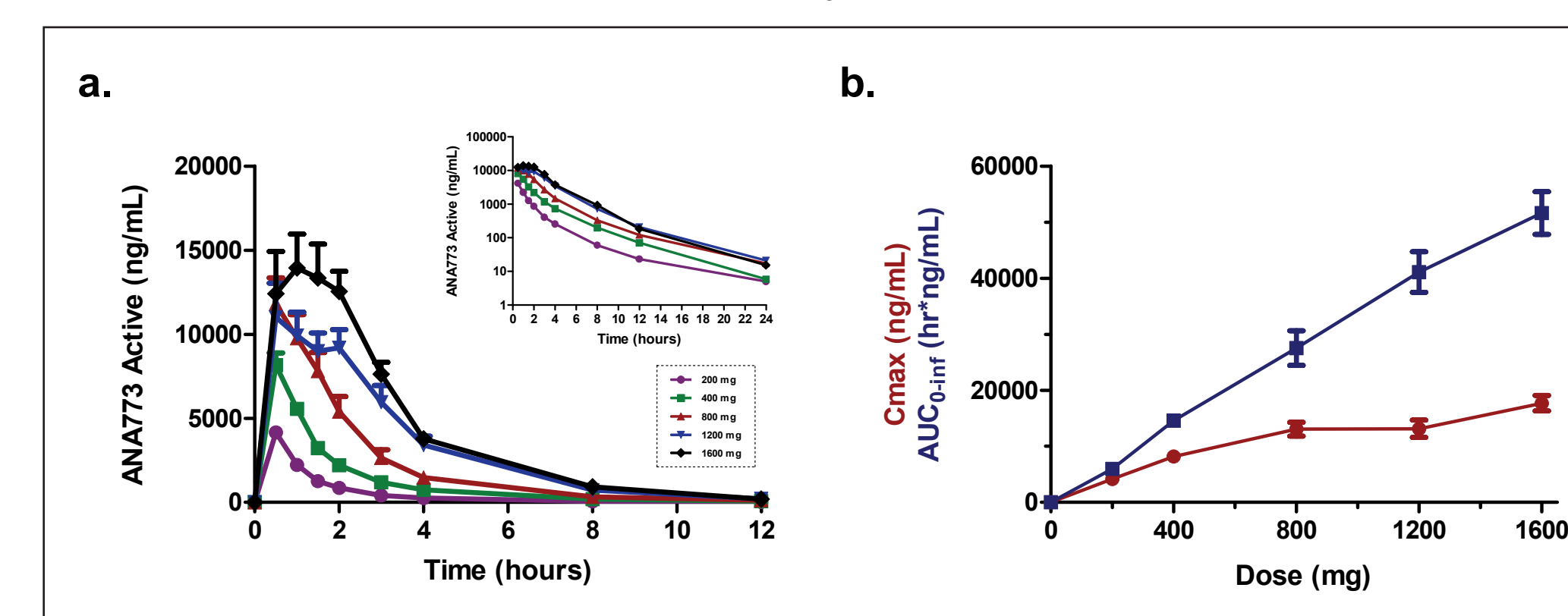
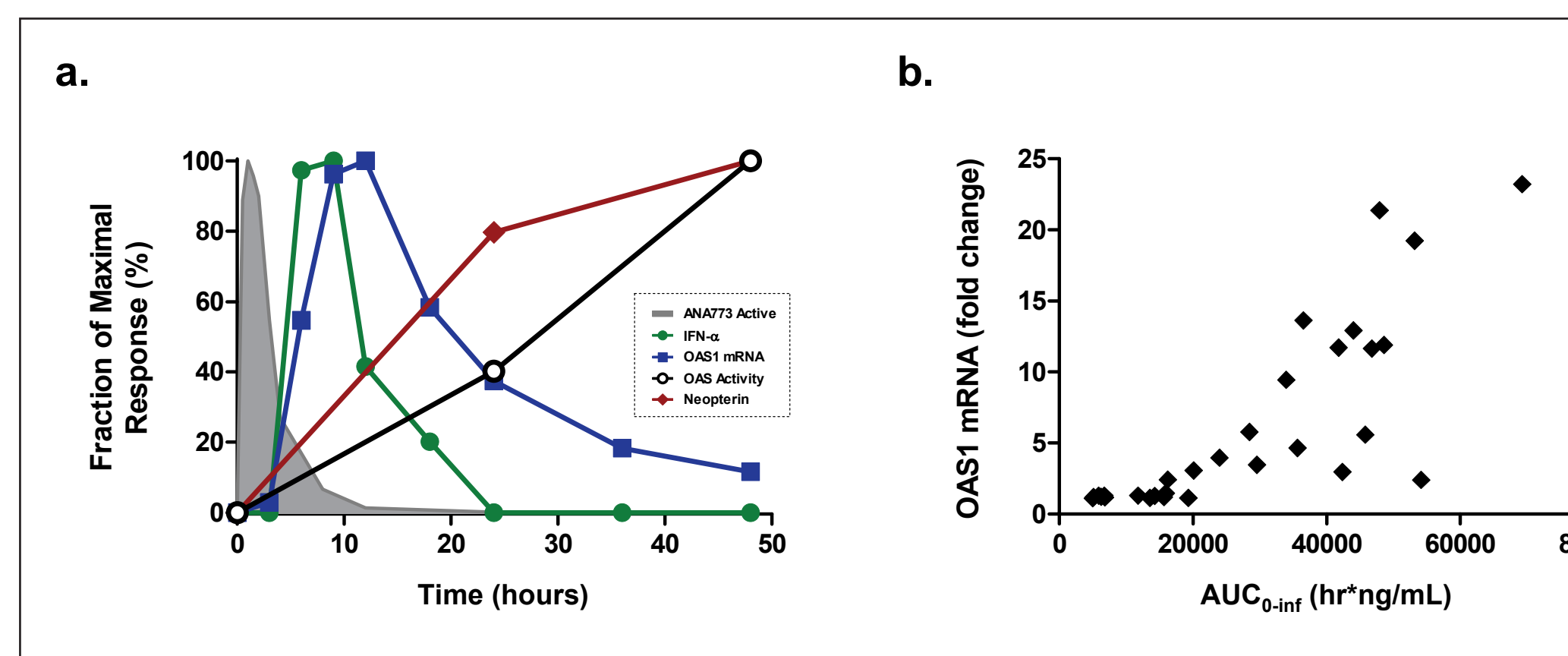


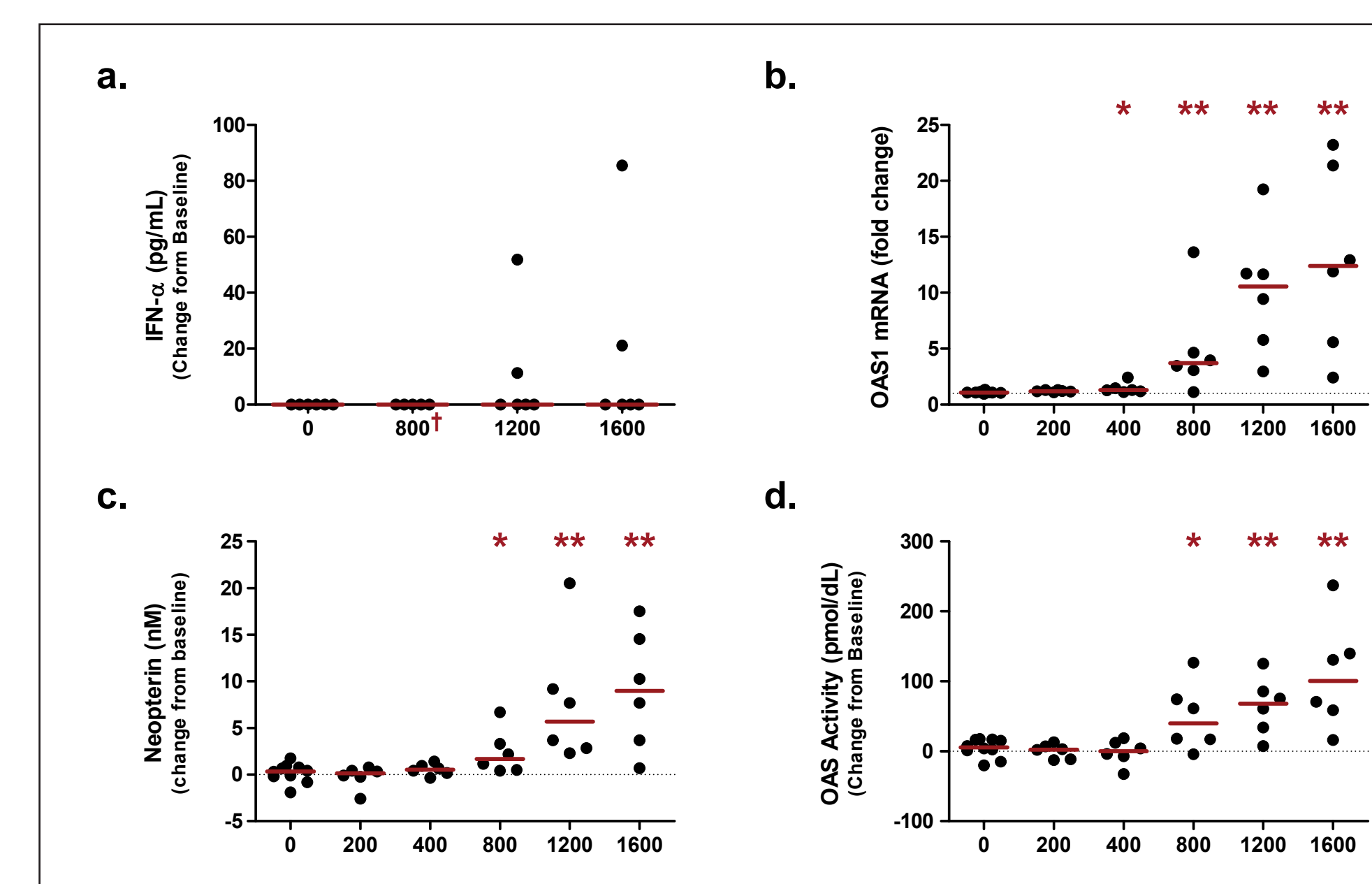
Figure 2. PK-PD Assessment after a Single Oral Dose of ANA773. (a) Comparative Kinetics of ANA773 Active and Associated PD Responses in the 1600 mg Cohort (b) Correlation between Exposure to ANA773 Active and OAS1 Expression in all Dose Groups



(a) Plasma (ANA773 active), serum (IFN- α and neopterin) and whole blood (OAS1 mRNA) samples were collected from subjects (n=6) for 48 hours after a single oral dose of 1600 mg ANA773. (b) OAS1 mRNA maximum fold change (R_{max}) is plotted. Each point represents an individual subject. Fold change in mRNA expression is calculated relative to the average of two pre-treatment samples. Oligoadenylate synthetase; OAS.

- Systemic exposure to ANA773 active induces IFN- α , which in turn triggers IFN-dependent responses (Figure 2a); induction of interferon-stimulated gene (ISG) expression (e.g. OAS1), OAS activity and neopterin.
- OAS1 mRNA expression and exposure to ANA773 active are significantly correlated ($r = 0.84$, $p < 0.0001$), although there was considerable subject-to-subject variation in response at a given exposure (Figure 2b).

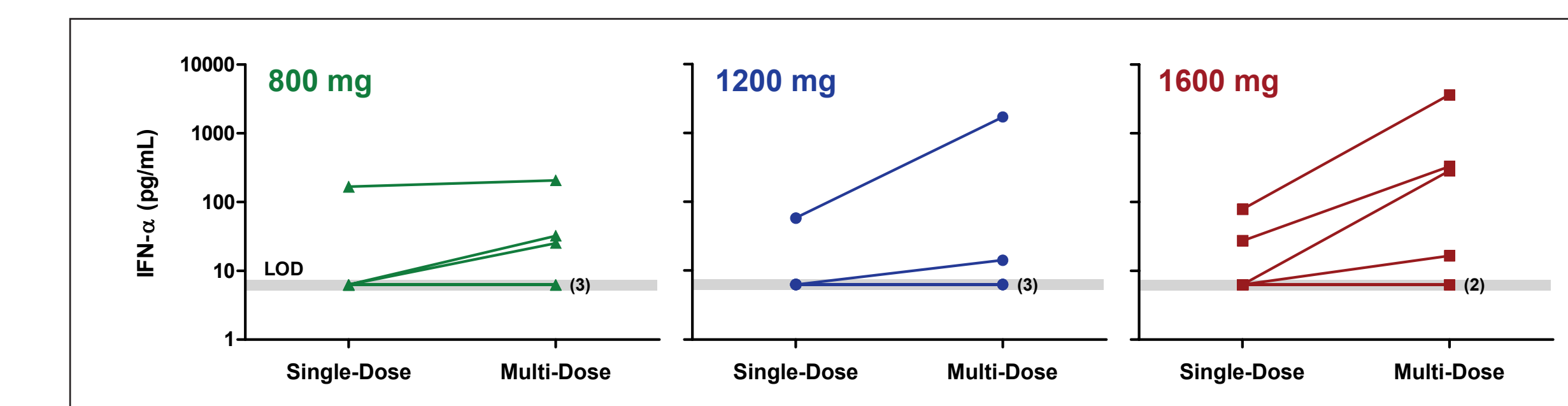
Figure 3. Pharmacodynamic Response to a Single Oral Dose of ANA773: Significant IFN-Dependent Response in the Absence of Detectable Circulating IFN- α



Serum (a, c & d) and whole blood (b) samples were collected for 48 hours after a single oral dose of ANA773 or placebo. Maximum response (R_{max}) is plotted relative to dose of ANA773 (mg). Each point represents an individual subject. Red lines indicates median. Fold change in mRNA expression is calculated relative to the average of two pre-treatment samples. * $p < 0.05$, ** $p < 0.01$ relative to placebo. †A single subject with a high and somewhat variable baseline IFN- α level (mean: 148 pg/mL), which did not increase appreciably after dosing, was removed from the analysis to better visualize the general trend.

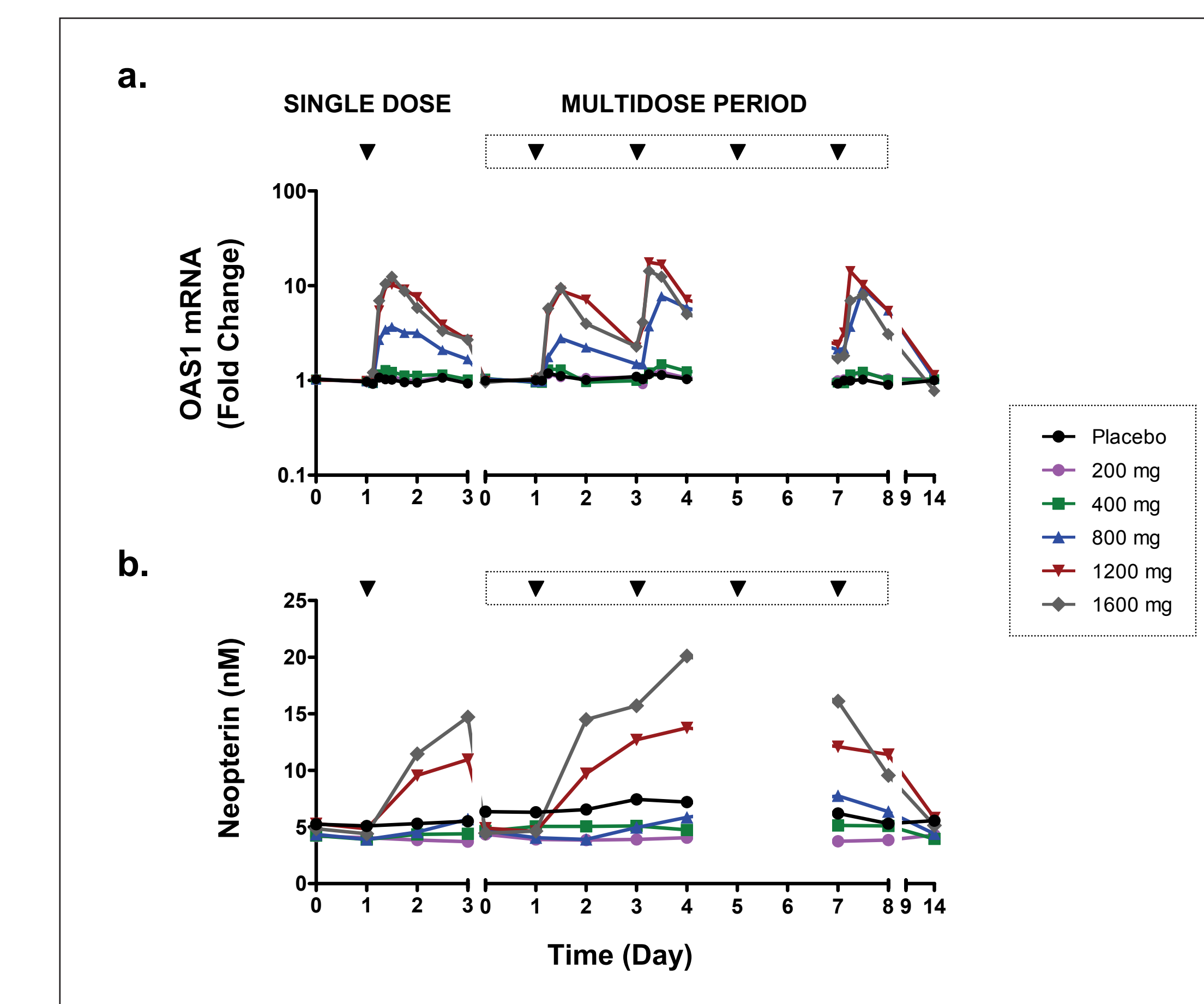
- A single dose of ANA773 induced substantial levels of circulating IFN- α in only a few subjects in the highest dose groups (Figure 3a). In contrast, significant, dose-related increases in various markers of IFN- α response were observed at lower doses of ANA773 (Figure 3b-d).
- There was substantial inter-subject variability in the pharmacologic response to a single dose (Figure 3) and multiple doses (data on file) of ANA773.

Figure 4. Multiple Doses of ANA773 Induce Higher IFN- α Levels than a Single Dose



The maximum IFN- α level (R_{max}) is plotted. Each point represents an individual subject. The limit of detection (LOD) of the assay was 6.3 pg/mL (PBL IFN- α Serum Sample ELISA kit). IFN- α levels in the placebo group were all below the limit of detection.

Figure 5. Pharmacodynamic Response to Single and Multiple Doses of ANA773



Group median values are plotted. Fold change is calculated relative to the average of two pre-treatment samples; fold change values for the single dose and multi-dose periods were calculated independently. The blank area denotes a time period during which no samples were collected.

- ANA773 elicited dose-related, repeated induction of ISG expression (Figure 5a), together with robust induction of neopterin, the levels of which remained elevated throughout the dosing period (Figure 5b).
- The higher doses of ANA773 induced neopterin to comparable levels as clinical treatment with rIFN- α 2.
- The pharmacologic response to ANA773 continued on off-dosing days and there was no evidence for the development of TLR tolerance.

Conclusions

- ANA773 was generally well tolerated in healthy subjects, with AEs increasing in frequency and intensity with increasing dose.
- There were no serious AEs reported.
- Three subjects discontinued treatment; two due to tolerability and one due to a change in clinical labs that readily reversed upon cessation of treatment.
- Oral ANA773 was efficiently converted to the active metabolite (ANA773 active), with exposure being approximately dose-proportional.
- ANA773 induced dose-related, IFN-dependent-responses, even in the absence of detectable levels of circulating IFN- α .
- The pharmacologic response to ANA773 continued on off-dosing days and there was no evidence for tachyphylaxis.
- These results support clinical exploration with ANA773 in patients with chronic HCV infection.