Direct-acting antiviral therapy for chronic hepatitis C

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Randomised clinical trial: anti-viral activity of ANA773, an oral inducer of endogenous interferons acting via TLR7, in chronic hepatitis C patients


*These authors made an equal contribution to this work

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INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major cause of liver cirrhosis and hepatocellular carcinoma. HCV-related end-stage liver disease is now the main indication for liver transplantation in North America and Western Europe. Estimates suggest that there are 170 million HCV-infected patients worldwide and three to four million people are newly infected each year. Approximately 80% of patients who become infected with HCV develop chronic hepatitis C. Combination therapy with pegylated interferon-alfa (peg-IFN-α) and ribavirin (RBV) results in sustained virological response (SVR) in only approximately 40–50% of patients with genotype 1. Thus, there remains an urgent unmet medical need to offer new therapies that may eradicate HCV infection. The most likely agents that will enter clinical practice soon are the protease inhibitors telaprevir and boceprevir. Phase III clinical trials of these drugs combined with peginterferon and ribavirin in genotype-1 patients have shown SVR rates up to 80% in treatment-naïve patients and up to 65% in treatment-experienced patients.

Immune responses against HCV are considered to play an important role in controlling and clearing chronic viral HCV infections. Toll-like receptors (TLRs) are a family of pathogen-recognition receptors that activate the innate immune response. Stimulation of TLRs either directly or indirectly leads to (1) the release of multiple cytokines, including type I and type II IFNs, (2) the induction of pathways and enzymes that destroy intracellular pathogens and (3) the stimulation of antigen-presenting cells which may result in the activation of adaptive immunity. To date, ten functional TLRs have been identified in humans. Among these, TLR7 is of particular interest because small molecule agonists for this receptor have been identified, and like TLR9 agonists, induces type III IFNs. Furthermore, there is precedent for this target in viral diseases. In a clinical proof-of-concept study, a TLR7 agonist, isatoribine, was shown to have an anti-viral effect in the treatment of chronic HCV infection. In addition, topical imiquimod is approved for the treatment of genital papillomavirus infections. Imiquimod is poorly tolerated when administered orally and is both extensively and rapidly metabolised when administered systemically, limiting systemic exposure of the drug.

ANA773 is an oral prodrug of a TLR7 agonist, developed for the treatment of patients with chronic HCV infection. The active metabolite of ANA773 induces multiple subtypes of endogenous IFN-α, induces IFN-dependent anti-HCV activity and functionally activates natural killer (NK) cells in vitro. Oral and intravenous administration of ANA773, induced systemic IFN-α production in Cynomolgus monkeys, with IFN-dependent responses being observed in both the periphery and the liver. The alternate-day dosing schedule in these studies was shown to induce desirable levels of T-cell activation and proliferation, and has been selected as the dosing schedule for clinical evaluation. A single and multiple dose escalation study (200–1600 mg/day every-other-day up to four administrations) of oral ANA773 in 41 healthy volunteers showed a dose-related increase of IFN-α and markers of IFN response. The occurrence of treatment-related adverse events in this study was seen predominantly in the higher dose groups (1200 and 1600 mg/day) and included pronounced IFN-like side effects such as pyrexia, chills, myalgia, headache, nausea and malaise, which confirm the relation with the study drug.
Importantly, ANA773 was well tolerated in 13 week toxicology studies at doses that produced robust immune induction.19

Herein, we report the safety profile, pharmacokinetics, anti-viral activity and the immunological effects of ANA773 administered orally at four dose levels (800, 1200, 1600 and 2000 mg/day every-other-day) to 34 treatment-naïve and treatment-experienced HCV-infected patients.

MATERIALS AND METHODS

Study design and organisation

This randomised, placebo-controlled, double-blind, multiple dose escalation, phase 1b study was conducted at three sites in The Netherlands from October 2008 until August 2009. This study was conducted in accordance with Good Clinical Practice and with the World Medical Association Declaration of Helsinki after approval by the institutional review board at each centre. All patients provided written informed consent before participating in any study-related activity. Initially, HCV patients of any genotype were randomised into three sequential cohorts (800, 1200 and 1600 mg). In the 800, 1200 and 1600 mg groups, six patients received oral ANA773 and two received placebo every-other-day for 28 days (14 doses). As doses in the first three patient dose groups were well tolerated and encouraging anti-viral responses were observed at the highest dose level of 1600 mg, a fourth dose group was added to investigate a higher ANA773 dose of 2000 mg. In this dose group, 10 patients (eight active, two placebo) were administered 2000 mg of ANA773 or placebo every-other-day for 10 days (five doses). The shorter treatment period was considered sufficient since immunological and anti-viral effects of ANA773 were seen shortly after start of treatment of patients in the previous lower dose groups.

During the first three doses of ANA773 (800, 1200 and 1600 mg group), and during all five doses in the 2000 mg group, the study was conducted as an in-patient study for careful assessment of safety and tolerability. Study medication (100 mg capsules) and placebo capsules were supplied by Anadys Pharmaceuticals, Inc., San Diego, CA, USA. Patients were allowed to start standard of care (peginterferon + ribavirin) immediately after the study period at the discretion of the study centre. All patients provided written informed consent before participating in any study-related activity. Initially, HCV patients of any genotype were randomised into three sequential cohorts (800, 1200 and 1600 mg). In the 800, 1200 and 1600 mg groups, six patients received oral ANA773 and two received placebo every-other-day for 28 days (14 doses). As doses in the first three patient dose groups were well tolerated and encouraging anti-viral responses were observed at the highest dose level of 1600 mg, a fourth dose group was added to investigate a higher ANA773 dose of 2000 mg. In this dose group, 10 patients (eight active, two placebo) were administered 2000 mg of ANA773 or placebo every-other-day for 10 days (five doses). The shorter treatment period was considered sufficient since immunological and anti-viral effects of ANA773 were seen shortly after start of treatment of patients in the previous lower dose groups.

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Patients

Key inclusion criteria included male and female patients between 18 and 65 years, with body mass index of 18–35 kg/m², HCV RNA level ≥75 x 10^13 copies/mL, and clinical laboratory evaluations consistent with chronic hepatitis C infection as defined by the protocol. Treatment-naïve or relapse patients were allowed. Relapse was defined as undetectable HCV RNA at completion of a previous IFN-based treatment, but positive HCV RNA during follow-up. Key exclusion criteria included decompensated liver disease, findings consistent with Child-Pugh B/C liver cirrhosis, and co-infection with HIV or HBV. Previous nonresponders to IFN-based therapies were excluded due to expected unresponsiveness to immunomodulatory therapy. Patients receiving anti-viral therapy or immunomodulatory therapy within 90 days prior to administration of the first dose of study medication were excluded. Patients with chronic stable haemophilia or on stable methadone substitution treatment were eligible.

Safety assessment

Patients who received at least one dose of ANA773 were considered evaluable for safety. Safety was evaluated by adverse events registration and clinically significant changes from pre-treatment baseline in laboratory values, vital signs, electrocardiogram tracings and findings that were recorded during physical examinations. Dose escalation to the next dose group was based upon the safety and tolerability of ANA773 in all patients in the previous dose group following completion of the 28-day treatment period as determined by the Ethics Committee.

Pharmacokinetic assessment

Plasma pharmacokinetic samples for assay of the active metabolite of ANA773 and two other metabolites were collected at the following times relative to ANA773 dosing: at predose (0 h), 0.5, 1, 1.5, 2, 3, 4, 8, 12 and 24 h after dosing on day 1 in all dose groups. In the 2000 mg group, plasma pharmacokinetic samples were also drawn on day 9 on the same time points. Pharmacokinetic analyses were performed by a central laboratory.

Viral assessment

Siemens INNO-LIPA HCV II assay was used to assess geno(sub)typing of all patients. Serum samples for HCV RNA analysis were obtained on day 1 before the first morning dose, followed by sample collection on day 2, 3, 4, 5, 6, 13, 21, 27, 28, 34 and 41 (800, 1200 and 1600 mg groups) or on day 2, 3, 4, 5, 6, 7, 8, 9, 10 and 16 (2000 mg group). HCV RNA measurements were performed by a central laboratory using the Roche COBAS AmpliPrep/COBAS TaqMan HCV test with a lower limit of quantification (LLQ) of 43 IU/mL. A >1.0 log₁₀ reduction in viral load during treatment was considered a clinically relevant decline.

Immunological parameters

Cytokine serum samples (IFN-α) were obtained on day 1, 2, 3, 5, 13, 27, 28, and 34 (800, 1200 and 1600 mg groups). In the 2000 mg group, cytokine serum samples were obtained on day 1, 3, 5, 7 and 9. Samples were analysed by enzyme-linked immunosorbent assay (ELISA) at Alta Analytical Laboratory, San Diego, CA, USA. Serum RNA samples (OAS1 and ISG15) were obtained on day 1, 2, 3, 4, 5, 6, 13, 21, 27, 28, 34 (800, 1200 and 1600 mg groups) and on day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 16 (2000 mg group) by Anadys Pharmaceuticals, San Diego, USA, using branched DNA (bDNA) assay. Neopterin and 2,5-OAS activity samples were collected on day 1, 2, 3, 4, 5, 6, 13, 21, 27, 28, 34, 41 (800, 1200 and 1600 mg groups) and on day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 16 (2000 mg group). Neopterin samples were analysed by ELISA at Alta Analytical Laboratory. 2,5-OAS activity was analysed by radioimmunoassay (RIA) at PLA International, Assen, The Netherlands.
Statistical analysis

The determination of the sample size was based on empirical considerations rather than statistical justification. No formal sample size calculations for this study were performed. The sample size of 24 patients in three dose groups with eight patients per dose group (800, 1200 and 1600 mg groups) and an additional 10 patients in the 2000 mg group were considered appropriate for this type of study. Safety data were tabulated and summarised by descriptive statistics. Pharmacokinetic parameters were summarised by descriptive statistics at each dose level and by study day. Analysis of variance (ANOVA) was used to compare the dose normalised Cmax and AUC values across dose groups. The log transformed HCV RNA values were summarised by descriptive statistics according to dose group and study. ANOVA was used to compare the difference between each time point and baseline, the nadir and baseline, and between the end-of-treatment and baseline across dose groups (including placebo).

Immunological parameters for serum cytokines (IFN-α), RNA (OAS and ISG15), neopterin and 2,5-OAS activity were summarised by descriptive statistics according to dose group and study day. The software used for all summary statistics and statistical analyses was SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA) and comparable desktop parameters. For the calculation of some pharmacokinetic parameters WinNonlin (Pharsight Corp., Mountain View, CA, USA) version 5.0.1 was used. For the calculation of correlation between variables the Spearman nonparametric test was used. Any P-values calculated as a result of the statistical analysis were interpreted in accordance with the exploratory nature of this study.

### Table 1. Demographics.

<table>
<thead>
<tr>
<th></th>
<th>800 mg (n=6)</th>
<th>1200 mg (n=6)</th>
<th>1600 mg (n=6)</th>
<th>2000 mg (n=8)</th>
<th>Placebo (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>4 (67)</td>
<td>7 (88)</td>
<td>7 (88)</td>
</tr>
<tr>
<td>Age, mean (range)</td>
<td>50 (40-55)</td>
<td>44 (35-51)</td>
<td>51 (46-55)</td>
<td>50 (33-65)</td>
<td>52 (38-64)</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>6 (100)</td>
<td>5 (83)</td>
<td>4 (67)</td>
<td>8 (100)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>BMI, mean (range)</td>
<td>27.1 (20.2-33.1)</td>
<td>26.7 (21.4-31.4)</td>
<td>26.0 (20.0-30.0)</td>
<td>26.3 (18.3-34.6)</td>
<td>25.8 (20.2-33.7)</td>
</tr>
<tr>
<td>Baseline log_{10} HCV RNA, mean (SD)</td>
<td>6.42 (0.80)</td>
<td>6.04 (0.46)</td>
<td>6.43 (0.61)</td>
<td>6.06 (0.92)</td>
<td>6.24 (0.80)</td>
</tr>
<tr>
<td>HCV genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, n (%)</td>
<td>5 (83)</td>
<td>5 (83)</td>
<td>3 (50)</td>
<td>0 (0)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>2, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (25)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3, n (%)</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>4 (50)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>4, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (33)</td>
<td>2 (25)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Previous treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naive, n (%)</td>
<td>2 (33)</td>
<td>3 (50)</td>
<td>2 (33)</td>
<td>7 (88)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Relapse, n (%)</td>
<td>4 (67)</td>
<td>3 (50)</td>
<td>4 (67)</td>
<td>1 (12)</td>
<td>4 (50)</td>
</tr>
</tbody>
</table>

BMI, body mass index.

### RESULTS

Demographic and baseline characteristics

A total of 54 patients were screened of whom 33 patients were considered eligible. One patient participated twice in the study under two different subject numbers. This was allowed per protocol as the patient received placebo during his first treatment period. The different subject numbers were treated as if they were two individuals and therefore the total number of approved and included subjects was 34. There were no dropouts or withdrawals; all patients completed the study as per protocol. Eighteen patients (53%) were treatment-naïve; the remaining 16 patients (47%) had relapsed from a prior full course of IFN-α based therapy. Patients were infected with genotype 1 (n = 17), 2 (n = 2), 3 (n = 10) or 4 (n = 5). Of the 34 patients, 30 (88%) were male patients and 4 (12%) were female patients. Thirty-one (91%) patients were White/Caucasian. The mean age was 49.5 years (range, 32–64 years) and mean BMI was 26.35 kg/m² (range, 18.3–34.6 kg/m²). Demographic and other baseline characteristics of the randomised patients are shown in Table 1.

Safety

Safety data including serious adverse events, premature discontinuations and haematological effects are shown in Table 2. Overall, multiple oral doses of ANA773 were safe and generally well tolerated at all dose levels tested up to 2000 mg. There were no deaths or other SAEs during the study. The majority of AEs were of mild or moderate intensity. The total number of AEs was highest in the 1600 mg (66 AEs) and 2000 mg (58 AEs) dose group. The number of AEs reported by the placebo-treated patients was only slightly less (49 AEs). A total of 167 AEs were considered possibly or probably related to the study medication (71% of the total number of AEs). The most frequently reported related AEs were headache, myalgia, fatigue, abdominal pain and dizziness. The occurrence of headache appeared to increase with dose, whereas for the other frequently reported related AEs a relation with dose was not observed. In addition, nausea (total of six events) considered related to the study medication occurred in the highest dose groups only. At the 1600 mg dose level 2 patients experienced flu-like symptoms (e.g. headache, myalgia, chills, malaise) at the beginning of their dosing period. However, these symptoms rapidly disappeared during continued dosing, even when they were initially of moderate to severe intensity. At the 2000 mg dose level three patients treated with ANA773 experienced flu-like symptoms, mainly at the first two dosing occasions. One of these patients started to use paracetamol as prophylactic treatment and the remaining doses were well tolerated. Individual dose reductions, as were allowed per protocol, were not necessary. Trends for decreases in leucocytes (nadir -4.06 x 10^3/L on day 6), thrombocytes (nadir -38.4 x 10^3/L on day 6) and lymphocytes (nadir -1.23 x 10^3/L on day 2) were observed for the highest dose level (2000 mg ANA773), but were not considered clinically significant. No other clinically significant abnormalities in vital signs, ECG, clinical laboratory values and physical examination were observed.
Table 3. Pharmacokinetic parameters of plasma ANA773 active metabolite.

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Day</th>
<th>Cmax (µg/mL)</th>
<th>tmax (h)</th>
<th>AUC∞ (µg.h/mL)</th>
<th>t1/2 (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>1</td>
<td>10.69 (7.57-15.3)</td>
<td>0.509 (0.50-1.00)</td>
<td>24.96 (21.9-29.1)</td>
<td>3.254 (2.09-3.96)</td>
</tr>
<tr>
<td>1200</td>
<td>1</td>
<td>11.99 (8.10-16.9)</td>
<td>1.000 (0.50-1.50)</td>
<td>31.53 (26.2-34.7)</td>
<td>2.901 (1.87-3.75)</td>
</tr>
<tr>
<td>1600</td>
<td>1</td>
<td>13.94 (9.17-19.0)</td>
<td>1.000 (0.50-2.00)</td>
<td>40.76 (33.3-49.4)</td>
<td>3.375 (2.66-3.85)</td>
</tr>
<tr>
<td>2000</td>
<td>1</td>
<td>15.87 (10.9-23.9)</td>
<td>1.000 (0.50-1.50)</td>
<td>51.06 (36.7-69.4)</td>
<td>2.995 (1.55-4.29)</td>
</tr>
<tr>
<td>2000</td>
<td>9</td>
<td>15.50 (13.1-19.3)</td>
<td>1.000 (0.50-1.50)</td>
<td>46.42 (35.0-60.4)</td>
<td>3.237 (2.67-3.65)</td>
</tr>
</tbody>
</table>

For all parameters except tmax the geometric mean (range) is presented; for tmax the median (range) is presented.

Figure 1. Mean plasma concentration of the active metabolite of ANA773 on day 1 (all groups) and day 9 (2000 mg only).

A response to ANA773 treatment was seen in both treatment-naïve and relapse patients. The two patients with a clinically relevant virological response in the 1600 mg dose group had relapsed from previous treatment, whereas the five patients with a clinically relevant virological response in the 2000 mg dose group were all treatment-naïve.

The PK profile on day 9 after multiple doses of ANA773 was similar to the PK profile on day 1 after a single dose of ANA773 (Figure 1). This indicates that no accumulation during multiple dosing was observed. Two other metabolites of ANA773 (metabolite A and metabolite B) also appeared in plasma within 0.5 h after dosing. Metabolite A is an intermediate in the conversion of ANA773 to the active metabolite and metabolite B is a minor degradation product. The maximum mean concentrations were observed for metabolite A at 0.5 to 1 h postdose and for metabolite B at 1.5 to 2.5 h postdose. The plasma concentrations of metabolite A were...
on average approximately 2–3-fold lower than that of the active metabolite, whereas for metabolite B the plasma concentrations were more than 100-fold lower. Evaluation of the dose-normalised individual values and geometric mean PK parameters and evaluation of dose proportionality with an exploratory ANOVA analysis showed that $C_{\text{max}}$ of the active metabolite of ANA773 increased with dose in a less than dose proportional manner [estimate of slope of 0.44 (95% CI 0.12–0.75)]. The AUC for the active metabolite of ANA773 increased at slightly lesser than dose proportionality [estimate of slope 0.79 (95% CI 0.60–0.97)].

Figure 2. Mean maximum HCV RNA response of all patients during treatment with ANA773. Mean (+S.E.M.) viral load nadir values are plotted for each dose group. The maximum viral load decline at 2000 mg is statistically significant relative to the placebo group. The number of patients in each group with a maximum decline in viral load of >1.0 log$_{10}$ IU/mL is displayed above the plot. Patients in the 800, 1200 and 1600 mg group were dosed for 28 days. Patients in the 2000 mg group were dosed for 10 days.

HCV RNA levels
At the end of treatment one patient in the 1600 mg dose group and three patients in the 2000 mg dose group, achieved a viral load decline >1.0 log$_{10}$ IU/mL, which was considered a clinically relevant decline. Another three patients (one patient in the 1600 mg dose group and two patients in the 2000 mg dose group) showed transient declines of >1.0 log$_{10}$ IU/mL reduction in viral load at any time point. The mean maximum decline of serum HCV RNA per dose group is shown in Figure 2. Individual viral load data of the seven patients with >1.0 log$_{10}$ IU/mL decline during treatment are shown in Table 4 and in Figure 3. The time of nadir for these patients varied between 3 and 9 days after start of treatment and was always 24 h after ANA773 dosing. After reaching nadir, HCV viral load started to increase in several of these patients (Figure 3). Serum viral load levels generally reverted to baseline after cessation of treatment. Viral load reductions of >1.0 log$_{10}$ IU/mL during treatment were observed in all genotypes. The pre-treatment viral load ranged from 5.11 to 7.04 log$_{10}$ IU/mL in the seven patients who showed a maximum virological response of >1 log$_{10}$ IU/mL.

Table 4. Individual HCV RNA data for patients with a maximal viral decline of >1.0 log$_{10}$ IU/mL.

<table>
<thead>
<tr>
<th>Subject</th>
<th>HCV genotype</th>
<th>Gender</th>
<th>Baseline viral load</th>
<th>Nadir</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 28 (N)</th>
<th>Day 10 (N)</th>
<th>EOT / EOT follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>3A</td>
<td>M</td>
<td>6.23</td>
<td>-1.35</td>
<td>6</td>
<td>-1.01</td>
<td>-1.35</td>
<td>-0.55</td>
<td>6.10</td>
</tr>
<tr>
<td>Patient 2</td>
<td>4D</td>
<td>M</td>
<td>5.11</td>
<td>-1.53</td>
<td>4</td>
<td>-0.65</td>
<td>-1.12</td>
<td>-0.28</td>
<td>4.72</td>
</tr>
<tr>
<td>Patient 3</td>
<td>3A</td>
<td>M</td>
<td>6.99</td>
<td>-3.10</td>
<td>4</td>
<td>-2.16</td>
<td>-3.07</td>
<td>-1.68</td>
<td>5.25</td>
</tr>
<tr>
<td>Patient 4</td>
<td>2A</td>
<td>M</td>
<td>7.04</td>
<td>-1.15</td>
<td>4</td>
<td>-0.74</td>
<td>-0.70</td>
<td>-0.49</td>
<td>6.52</td>
</tr>
<tr>
<td>Patient 5</td>
<td>4A</td>
<td>M</td>
<td>6.59</td>
<td>-2.52</td>
<td>6</td>
<td>-1.64</td>
<td>-2.52</td>
<td>-2.24</td>
<td>4.41</td>
</tr>
<tr>
<td>Patient 6</td>
<td>1A</td>
<td>F</td>
<td>6.52</td>
<td>-1.63</td>
<td>10</td>
<td>-1.62</td>
<td>-2.52</td>
<td>-2.24</td>
<td>4.41</td>
</tr>
<tr>
<td>Patient 7</td>
<td>3A</td>
<td>M</td>
<td>5.35</td>
<td>-1.63</td>
<td>4</td>
<td>-1.30</td>
<td>-1.63</td>
<td>-1.35</td>
<td>6.10</td>
</tr>
</tbody>
</table>

1Mean of day -1 and pre-dose day 1.  
2Maximum negative change from baseline value at day 28 or at day 10.  
3Average of the day 27 and day 28 or day 9 and day 10 (2000 mg only) HCV RNA concentrations.

Figure 3. Viral load time course in patients with >1.0 log$_{10}$ decline during treatment. Five 2000 mg patients (closed circles) and two 1600 mg patients (open circles) had a maximum decline in viral load of >1 log$_{10}$ during treatment. Each line represents the viral load change for one of these patients during the treatment period (10 days for the 2000 mg group, 28 days for the 1600 mg group). Patients in the 2000 mg group had samples taken pre-treatment (PT) and on days 1 through 10 (24 h after the last dose). Patients in the 1600 mg group had samples taken pretreatment, on days 1–6, and then on days 13, 21, 27 and 28 (24 h after the last dose). For these patients, the period between days 6–28 is represented by a dashed line. Patient genotypes (GT): green; GT3, orange; GT1A, light blue; GT2, black; GT3, dark blue; GT4, red; GT4, purple; GT3. See page 285 for full colour figure.
Immunological parameters

In total, 10 patients had a measurable IFN-α response, defined as having one or more IFN-α concentrations above LLQ that increased after ANA773 dosing. For the patients treated with placebo no IFN-α concentrations above LLQ were observed. The incidence of the IFN-α responses appeared to increase with increasing dose of ANA773. The response was rapid: IFN-α concentrations >LLQ were observed at 3 h postdose in several patients. In most cases, the peak IFN-α concentrations were observed at 6–9 h postdose. The IFN-α concentrations rapidly decreased thereafter and were below LLQ prior to the administration of the next ANA773 dose. Due to the small number of patients that showed a quantifiable response, it was not clear whether the magnitude of the response increased with dose. Of the seven subjects who showed a decline in viral load of >1.0 log_{10} IU/mL, four patients also had a measurable IFN-α response (one patient in the 1600 mg group and three patients in the 2000 mg group). However, for three of the subjects with a virological response of >1.0 log_{10} IU/mL no IFN-α concentrations above LLQ were observed (one patient) or at only one time point (two patients). In one patient (800 mg group) measurable IFN-α concentrations were present throughout the study period. Since these substantial levels of circulating IFN-α in this subject were present even at baseline, this is considered to be unrelated to the study treatment. Nonetheless, the IFN-α concentrations increased following ANA773 dosing on 4 of the 5 days with postdose IFN-α assessments; this subject is therefore considered as having a measurable IFN-α response. The expression of two different IFN-response genes, OAS1 and ISG15, was evaluated as markers of IFN response. The extent of the increase (mean fold change in mRNA levels) was higher for ISG15 than for OAS1, but other than that the profiles were very similar with regard to timing and dose-response relationship. As previously reported in healthy volunteers dosed with ANA773, the maximum increases in mRNA levels were observed approximately 6–12 h postdose and mean mRNA levels were near baseline by 48 h postdose. The extent of the induction of these genes appeared to be approximately constant for the first 3–5 dosing occasions. All seven patients who showed a decline in viral load of >1.0 log_{10} IU/mL also had clearly increased expression of OAS1 and ISG15.

Mean neopterin concentrations increased for all ANA773 dose levels tested (Figure 4a). There was a dose-response relationship up to the 1600 mg ANA773 dose. Following the 2000 mg ANA773 dose, the maximum increase in mean neopterin concentrations was slightly lower than that in following the 1600 mg dose. The highest increases in neopterin concentrations were observed at the beginning of the treatment period. After the first 3–4 doses, the neopterin concentrations waned over time such that in most patients neopterin concentrations had returned to values near baseline by the end of the treatment period. In the patients with a viral decline >1.0 log_{10} IU/mL a clear increase in neopterin concentrations was observed. At the 2000 mg ANA773 dose level, the five patients with a virological response >1.0 log_{10} IU/mL had a neopterin mean maximum change (R_{max, FC}) ranging from 2.02 to 6.48-fold. These patients also had the highest maximum absolute neopterin concentrations. The three patients in this dose group with no virological response had a neopterin R_{max, FC} similar to values measured for placebo, indicating no clear increase in neopterin.

Figure 4. (a) Mean neopterin concentration throughout the study. Patients in the 800, 1200 and 1600 mg groups were dosed for 28 days. Patients in the 2000 mg group were dosed for 10 days.
(b) Mean 2,5-OAS activity throughout the study.

An increased 2,5-OAS activity was observed for the 1200 and 1600 mg dose level (Figure 4b). For the 2000 mg dose group no 2,5-OAS activity was determined. Although the mean plots do not indicate a clear dose-response relationship, the mean values for R_{max, FC} did seem to indicate the presence of a dose-response relationship. Since the samples of the 2000 mg dose group were not analysed, only a limited evaluation of a possible relation between increased 2,5-OAS activity and virological response could be done. The two patients who had a decrease in viral load of >1.0 log_{10} IU/mL, did not appear to have the highest increases or absolute values of 2,5-OAS activity in their dose groups. The increase in mean 2,5-OAS activity was observed starting from 24 h after the first dose. A further increase was observed after the second dose; thereafter the 2,5-OAS activity appeared to remain constant throughout the treatment period.
DISCUSSION

The present study is the first clinical trial to evaluate ANA773, an oral inducer of endogenous interferons that acts via TLR7, in chronic hepatitis C patients. This multiple dose escalation study was designed to explore the safety, tolerability, pharmacokinetics, pharmacodynamics and anti-viral activity of ANA773.

Our primary objective was to investigate safety and tolerability of ANA773. There were no serious AEs observed and there were no premature discontinuations. The most frequently reported AEs, which were considered to be related to the study medication, were flu-like symptoms, which were only observed early following the start of treatment. Since these symptoms are known IFN-like side effects, the observed AE profile is consistent with the expected mechanism of action of the compound. With increasing dose of ANA773, a trend was seen towards a higher frequency and stronger intensity of flu-like symptoms. Overall, treatment with multiple doses of ANA773 at dose levels up to 2000 mg were well tolerated by the 26 patients chronically infected with HCV. The same mild profile of side effects was seen with an intravenous TLR7 agonist (isatoribine), although only few flu-like symptoms were seen in this study. In contrast, oral administration of resiquimod (a TLR7 and TLR8 agonist) was associated with typical interferon related adverse events which limited dose escalation.

ANA773 was rapidly absorbed following oral administration, and all three metabolites were detected in plasma at the first sampling time point 30 min after start of treatment. Systemic exposure to the active metabolite of ANA773 was dose proportional, although the C_{max} increased with dose in a less than dose proportional manner. It is possible that the rate of conversion of ANA773 into the active metabolite may become more limiting at the higher doses. An additional factor contributing to the less than dose proportional enhancement of the C_{max} of the active metabolite of ANA773 could be the high number of capsules administered which may limit the efficiency of the dissolution and absorption process. For the C_{max} of metabolite A no deviations from dose proportionality were observed, which would also be expected if dissolution and absorption was a major factor. The pharmacokinetic profile of ANA773 in chronic HCV-infected patients was similar to healthy volunteers (data not shown). For the 2000 mg group it was shown that all three metabolites were below LLQ at predose on day 9, indicating that no accumulation during multiple dosing occurred.

Repeated administration of 1600 mg or 2000 mg of ANA773 resulted in a mean maximum viral load decrease of -0.97 and -1.26 log_{10} IU/mL, respectively, compared to -0.34 log_{10} IU/mL in placebo-treated HCV patients (P = 0.037 at 2000 mg relative to the placebo group). At the 1600 mg dose level 2 of 6 HCV patients and at the 2000 mg dose level 5 of 8 HCV patients had a decline in viral load of >1.0 log_{10} IU/mL. The decline of serum HCV RNA levels was generally observed within the first several days, and HCV RNA levels remained reduced until the end of therapy in most patients. Interestingly, HCV viral load decline did not resemble the typical biphasic pattern as seen with interferon-based regimens, which may be due to a different anti-viral mechanism, suboptimal drug dosing, or due to the specific dosing regimen.

Stimulation with TLR7 agonists induces the production of type I interferons, pro-inflammatory cytokines and the expression of co-stimulatory molecules on various cell populations. In all patients with a decline of viral load of more than 1.0 log_{10}, these factors were induced or activated, demonstrating the immunomodulatory activity of ANA773 leading to anti-viral effects. Our findings demonstrated that the decline in serum HCV RNA levels correlated with exposure to the active metabolite of ANA773 as well as neopterin levels, respectively (Figure 5).

Further studies are needed to examine why some patients respond to ANA773 as demonstrated by activation of innate immune responses, whereas no decline of viral load is observed in these patients. We observed that HCV genotype and the baseline HCV RNA levels were not predictive of the responsiveness to administration of the TLR7 agonist.

Figure 5. Correlation of anti-viral effect of ANA773 with (a) exposure to ANA773 and (b) induction of IFN-dependent response. Each point represents an individual patient. The plots display the correlation between maximum change in viral load with (a) exposure to ANA773 after the first dose; r = 0.699, P < 0.0001, and (b) maximum change in neopterin levels during treatment; r = 0.457, P = 0.0066. Placebo values are not included in (a).

Whereas current peginterferon administration consists of a single IFN subtype (2a or 2b), TLR activation induces several IFN subtypes which may potentially improve antiviral efficacy, but may also lead to more cytokine-mediated adverse events; however, improved anti-viral efficacy and deterioration of the AE profile were not seen (with respect to peginterferon), suggesting that the optimal dosage of ANA773 remains to be determined.

In this proof of concept study conclusions are limited by the number of subjects and the heterogeneity of the study population. But due to its heterogeneous population it is possible to conclude that immune activation and viral decline can be achieved by ANA773 in HCV infected patients, irrespective of genotype, viral load, gender or previous response to IFN-based therapies.
In conclusion, oral administration of ANA773 was safe and modest anti-HCV activity was seen in combination with marked therapy induced activation of the immune system. Potential advantages of ANA773 relative to weekly subcutaneous injections with peginterferon include the route of administration (oral vs. injection) and a good safety profile. For the near future it is likely that peginterferon will remain the cornerstone of antiviral combination therapies. However, with the advent of direct anti-viral compounds targeting the HCV protease and polymerase proteins and leading to strong viral suppression, the clinical development of immune activators, such as TLR-agonists, may be advantageous. In this study, we show that forced viral decline combined with activation of the immune system by the TLR7 agonist ANA773 is safe and exhibits modest anti-HCV activity. In addition, our findings pave the way for future studies to explore whether or not higher doses of ANA773 administered via the oral route will further enhance the anti-viral efficacy of this TLR7 agonist in lowering the serum HCV RNA levels in chronic HCV patients. It will be of particular interest to investigate this drug in combination with direct anti-virals or with ribavirin, as currently is being planned.

REFERENCES