Antiviral Research 89 (2011) 238–245

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Safety, pharmacokinetics, and antiviral activity of the cyclophilin inhibitor NIM811 alone or in combination with pegylated interferon in HCV-infected patients receiving 14 days of therapy

Eric Lawitz[a], Eliot Godofsky[b], Regine Rouzier[c], Thomas Marbury[d], Tuan Nguyen[e], June Ke[f], MeiMei Huang[g], Jens Praestgaard[f], Denise Serra[f], Thomas G. Evans[g,*]

[a] Alamo Medical Research, San Antonio, TX, USA
[b] University Hepatitis Center at Bach & Godofsky, Bradenton, FL, USA
[c] Centre CAP, Montpellier Cedex, France
[d] Orlando Clinical Research, Orlando, FL, USA
[e] Research and Education Inc., San Diego, USA
[f] Novartis, East Hanover, NJ, USA
[g] Novartis Institutes for Biomedical Research, Cambridge, MA, USA

ARTICLE INFO

Article history:
Received 4 August 2010
Received in revised form 9 December 2010
Accepted 11 January 2011

Keywords:
Cyclophilin
HCV
NIM811
Pegylated interferon
Antiviral

ABSTRACT

Background: Cyclophilin inhibitors have shown activity against a variety of viruses, including HCV. NIM811, a novel, non-immunosuppressive cyclophilin inhibitor was studied in ascending doses in a randomized, double-blind, placebo-controlled 14-day trial in genotype 1 HCV patients. Doses of 10 up to 600 mg were given orally once or twice daily as monotherapy (9:3 randomization of NIM811:placebo). 600 mg or placebo bid for 14 days was then co-administered with pegylated interferon alpha (PEG-IFN-α) administered on days 1 and 8 to genotype 1 relapsers.

Results: NIM811 was well tolerated at all doses. Although lack of antiviral effect was noted in the monotherapy arms, liver transaminase normalization occurred at doses over 75 mg. Mild, clinically non-significant elevations of bilirubin, and significant declines in platelet numbers were observed in the 400 and 600 mg bid groups. In the combination group, the mean HCV RNA decline was 2.85 log, compared to a 0.56 log in the PEG-IFN alone arm. The mean ALT (alanine transaminase) declined significantly by day 14 in the combination, but was unchanged in the PEG-IFN alone group. In the combination therapy group, the mean platelets were 203×10^9/L at baseline and fell to 105×10^9/L by day 14; for patients treated with PEG-IFN the values were 177×10^9/L and 139×10^9/L. There was a significant increase in bilirubin, although this did not reach clinically concerning levels. There were no severe or serious adverse events. The pharmacokinetics in both monotherapy and combination arms were dose linear and not affected by PEG-INF.

Conclusion: NIM811 monotherapy resulted in a normalization of liver transaminases in the absence of significant virological response. The combination of NIM811 and pegylated interferon alpha showed significant antiviral activity compared to interferon alone in genotype 1 HCV relapsers. The use of oral cyclophilin inhibitors as part of a combination regime for treatment of hepatitis C, especially to deter resistance, holds promise.

1. Introduction

Chronic HCV infection leads to progressive fibrogenesis, cirrhosis, liver insufficiency, and hepatocellular cancer, and is the most frequent indication for liver transplantation in the US and EU. Current therapy consists of a combination of pegylated interferon alpha with ribavirin for 6–12 months (Zeuzem, 2008; Vento et al., 2008). Less than half of the patients with genotype 1 successfully sustain an antiviral response, and most patients worldwide remain untreated. Thus, there is a continued unmet medical need for both more efficacious and better tolerated therapeutics in chronic viral hepatitis C, both for patients who are non-responsive to current treatment as well as those who are treatment naive. Such therapies include the newer protease and NS5A inhibitors, although concerns about resistance to these antivirals remain.

* Corresponding author at: 1405 Research Blvd, Rockville, MD 20850, USA. Tel.: +1 301 5472934; fax: +1 301 5472901.
E-mail address: tevans@aeras.org (T.G. Evans).

0166-3542/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2011.01.003

NIM811 (SDZ211-811, MeIle[4]-cyclosporin) is a cyclosporine analog characterized by a lack of immunosuppressive activity and more potent antiviral anti-HCV activity *in vitro* compared to cyclosporine A (CsA) (Rosenwirth et al., 1994; Ma et al., 2006). Unlike CsA, NIM811 does not interfere with the phosphatase activity of calcineurin, which mediates immunosuppression. As a result NIM811 does not interfere with T cells in the mixed lymphocyte reaction assay; it does not interfere with the formation and release of antibodies to a T cell dependent antigen, and it has no effect on the mitogen induced IL-2 expression up to 10 μM concentrations. No significant immunosuppression was observed with NIM811 in three rodent models in preclinical studies. These included a heart allograft rejection model, the formation and release of antibodies in rats, and an effect on graft versus host disease in rats in which cyclosporine was used as a positive control (Novartis internal data).

Similar to CsA, NIM811 displays potent antiviral activities in a number of virus–host systems, including HCV (Nakagawa et al., 2005; Ishii et al., 2006). The antiviral effects are closely correlated with the binding of these compounds to cyclophilins, a class of highly conserved peptidyl–prolyl cis–trans isomerases that facilitate protein folding, and which are thought to be involved in the HCV life cycle. NIM811 has shown a potent, dose-dependent inhibitory effect in the *in vitro* HCV replicon assay, with a 3-log reduction of replicon RNA levels after 9 days at >1 μM (Mathy et al., 2008; Ma et al., 2006). In these studies NIM811 and IFN-α were also additive for *in vitro* inhibition of viral replication, providing a rationale for NIM811–IFN-α combination therapy.

Initial studies by the group of Inoue in Japan had first shown that cyclosporine had clinically relevant activity against HCV in a human study (Inoue et al., 2003). Additional studies on a related non-immunosuppressive compound, Debio-025, were recently reported by Flisiak et al. (2009, 2008). Given the favorable non-immunosuppressive activity of NIM811, its potent *in vitro* activity, and our preclinical data, a proof of concept trial was performed to study whether NIM811 was safe and had antiviral activity in genotype 1 HCV patients.

CNIM811B2102 was a randomized, double-blind, placebo-controlled, dose-escalation study conducted to explore the safety, tolerability, pharmacokinetics and pharmacodynamics of NIM811 in patients with hepatitis C. This was the first multiple dose study, and was amended to include higher doses than originally planned, as well as to add a cohort in which NIM811 was given in combination with pegylated interferon alpha 2a.

1.1. Methods, trial design and conduct

This multicenter study was conducted in the United States and France in accordance with Good Clinical Practice guidelines and the Helsinki Declaration. Patient informed consent form was approved by local Ethics Committees and Health Authorities.

1.2. Patient demographics

The inclusion criteria required patients age 18–69 years of age, HCV-RNA genotype 1 with $\geq 10^5$ IU/mL copies at screening, and no clinically significant abnormalities in vital signs. Female patients were required to be either postmenopausal or have had tubal ligation or hysterectomy, and male patients were to use double-barrier local contraception during the study and for three months following the last study drug administration. For the monotherapy patients, the HCV treatment status included non-responders, virological relapsers to previously received antiviral treatment, or treatment-naive. Virologic non-responder status was defined as detectable HCV RNA serum levels at the end of treatment; virologic relapser was defined as detectable HCV-RNA serum levels six months after completion of antiviral treatment, with a negative HCV-RNA PCR test result at the end of treatment. For the combination therapy cohort only relapsers who previously received interferon and ribavirin (IFN–RBV) combination treatment for a minimum of 12 weeks were enrolled.

Exclusion criteria included patients who had a weight of less than 50 kg; Child-Pugh B and C liver disease; potential hepatocellular carcinoma, as indicated by measurement of total and the L3 glycoform of alphafetoprotein and liver ultrasound imaging; use of any HCV-specific anti-viral medications within 3 months prior to dosing; participation in any clinical investigation within 4 weeks prior to dosing; donation or loss of 400 mL or more of blood within 8 weeks prior to first dosing; significant illness within two weeks prior to dosing; presence of clinically significant ECG abnormalities such as a prolonged QT-interval syndrome; presence of acute or severe chronic bronchospastic disease; history of clinically significant drug allergy; any surgical or medical condition which might significantly alter the absorption, distribution, metabolism or excretion of drugs; a history suggestive of an immunocompromised status, including a negative HIV test; a positive Hepatitis B surface antigen (HBsAg) test; history or evidence of drug or alcohol abuse within the 6 months prior to dosing; patients with triglycerides > ULN (upper limit normal) as defined by the central laboratory; a positive tuberculin skin test (>5 mm); platelets and or blood polymorphonuclears outside the normal range those are clinically relevant; clinically significant anemia; ALT $\geq 10\times$ ULN (defined as 40 IU/mL). For the combination therapy cohort additional exclusion criteria relevant for interferon use, such as history of severe depression, history of suicide attempt or suicidal ideation, history of a severe seizure disorder, and more stringent hematologic parameters were also used (Hgb <12 g/dL in males or <11 g/dL in females, WBC < 3000/mm^3, ANC < 1500/mm^3, platelet count < 130×10^9/L BUN or serum creatinine > ULN, ALT or AST > $5\times$ ULN, serum amylase or lipase > $2\times$ ULN, PT $\geq$ 3 s prolonged, or total bilirubin > $1\times$ ULN).

Exclusion criteria specific for NIM811 were included due to preclinical toxicology findings. In high doses lenticular changes were noted in dogs, as well as some degree of testicular atrophy. Thus exclusion criteria also included lenticular opacity which according to the investigator would require cataract surgery within 12 months. French centers required exclusion of male patients with clinically significant abnormal semen analysis in the opinion of the investigator.

1.3. Study objectives

The primary objective was to determine the safety, tolerability, and antiviral activity of NIM811 when administered as daily doses for 2 weeks as monotherapy and in combination with pegylated interferon alfa-2a (180 μg as 2 doses over a 14 day interval) in patients with HCV genotype 1. The secondary objectives were designed to determine the pharmacokinetics of NIM811 monotherapy and when coadministered with PEG-IFN after single dose and multiple doses, and to potentially perform exploratory pharmacogenetic and pharmacogenomic assessments to examine whether individual genetic variation conferred differential response to NIM811.

1.4. Experimental study design

NIM811 also known as SDZ211-811, MeIle[4]-cyclosporin, was formulated in a microemulsion preconcentrate and supplied to the site. Pegylated interferon alfa-2a (Pegasys®, Hoffman-La Roche, Inc.) was purchased as 180 μg single use, pre-filled syringes in commercial packaging, and was administered weekly via subcutaneous injection according to the approved product label. Standard dose

reduction modifications to 135 μg and 90 μg for interferon were allowed as per the package insert.

The initial dose escalation phase of the study consisted of six cohorts. In each cohort nine patients were randomly assigned to receive NIM811 and three patients were randomly assigned to receive placebo. Safety data were reviewed prior to starting the next higher dose. The cohorts were dosed with NIM811 or matching placebo at 25 mg per day, 75 mg per day, 100 mg bid, 200 mg bid, 400 mg bid and 600 mg bid. Treatment lasted 14 days. An additional cohort was then included to assess the effect of NIM811 combination therapy. Patients received either 14 daily doses of 600 mg bid of NIM811 or matching placebo plus two 2 doses of 180 μg pegylated interferon administered subcutaneously on days 1 and 8, 15 min prior to the oral dosing of NIM811 or placebo. NIM811 or placebo was given in a fasted condition. The decision to continue pegylated interferon (with ribavirin then added) following the two week therapy for a full treatment course was left to the discretion of the site investigator.

The first few cohorts remained domiciled during the entire two week period as this was the first multiple dose exposure of NIM811, then were followed up weekly on weeks 3 and 4 with a close out visit at day 45. Starting with the 200 mg bid cohort domiciling was no longer required. Safety evaluations included routine chemistry and hematology blood work, as well as FSH and inhibin in males to measure possible testicular dysfunction, ECGs, and 24 h urine collection for GFR at baseline and end of treatment. Due to lenticular changes seen at high doses in canine toxicology study, slit lamp examination was required at screening, end of treatment and end of study. Although NIM811 did not interfere with immunologic assays in preclinical studies, due to its close structural relationship to cyclosporine A, the levels of CD3+, CD4+, CD8+ T cells in peripheral blood at baseline, day 7, day 14, and end of study were measured. IL-2 production in mitogen stimulated PBMCs and reactivation of EBV and CMV DNA were measured at baseline and at end of study in a subset of patients.

1.5. Study outcomes

The primary study outcome of safety was defined in a descriptive manner. The assessment of a virologic effect was measured using a quantitative HCV RNA measurement. HCV RNA quantification was performed centrally using a Roche COBAS® Taqman® assay following the manufacturer's specification. Samples were collected on days 1, 2, 3, 7, 10, 14, 21, 28, and 45.

1.6. Pharmacokinetics assessment

Pharmacokinetics of blood NIM811were evaluated on days 1 and 13, and trough (pre-morning dose) levels on days 2, 3, 7, 10, 12 and 14. Blood levels of NIM811 were measured with a validated high performance liquid chromatographic assay with mass spectrometric detection (LC/MS/MS). The assay lower limit of quantitation was 1 ng/mL, and intra- and inter-day precision and accuracy (percent deviation) were below 10%. Pharmacokinetic parameters were determined with WinNonlin Pro (Version 5.01) using non-compartmental methods.

1.7. Sample size calculation

The initial sample size was calculated to determine safety and to examine for a meaningful virologic effect. It was assumed that the 14 day mean drop from baseline is 1 log unit higher in the combination therapy group than in the PEG-IFN monotherapy group. Assuming a standard deviation of 1 log unit in both treatment groups, a one side *t*-test at nominal level 5% would have power 86% to detect a significant difference. The analysis used, conducted after 10 patients in each group completed day 14, was Bayesian. The posterior probability, calculated when 10:10 patients would have completed day 14 under a non-informative prior, of a positive treatment effect would, with probability 86%, exceed 95%.

1.8. Randomization, allocation, blinding and masking

Only the pharmacist preparing the NIM811 or placebo was unblinded to the patient allocation. Standard emergency unblinding procedures were in place in event of serious or severe AEs requiring patient or protocol modifications.

1.9. Statistical methods

The main efficacy parameter was the change from baseline to day 14 in serum HCV RNA level. This measure was computed in all patients and compared between treated groups and placebo. For the final analysis, the posterior distribution of the drop from baseline in HCV RNA was computed in each cohort.

A planned interim analysis took place for the combination therapy cohort. After 10 patients in each treatment group completed day 14, the log 10 viral load at day 14 was analyzed by Bayesian analysis of covariance including treatment group as a categorical covariate and baseline viral load as a continuous covariates. A non-informative prior was used for all model parameters including the treatment effect, the residual variance, and the regression coefficient for the baseline covariate. The posterior probability for a positive treatment difference was calculated. If this probability exceeded 95%, the study was to be stopped for success. If the posterior probability was below 50%, corresponding to a negative, observed treatment effect, then the study was to be ended for futility. If the posterior probability was between 50% and 95%, a simulation was to be conducted to assess whether a treatment difference exists which can be detected by enrollment of an additional group not to exceed 30 patients.

2. Results

72 patients were enrolled in the monotherapy arm (approximately 12 per cohort) and 21 patients in the combination therapy arm. In each monotherapy cohort there were 9 patients scheduled to receive NIM811 and 3 to receive placebo. One patient withdrew after randomization but before dosing. This patient was not replaced. One additional patient received therapy in the 600 mg bid monotherapy arm ($N = 10$). The final combination therapy cohort enrolled 21 patients, all of whom received pegylated interferon and 11 of whom received placebo and 10 NIM811. One patient did not receive the final dose of pegylated interferon due to neutropenia.

2.1. Major protocol deviations

There were no major protocol deviations. One patient in the combination cohort was naive to therapy rather than a relapser. This was due to the fact that the decision to recruit only relapsers in the combination therapy cohort was made after review with the health authorities only after the first patient had been enrolled. An unplanned interim analysis took place after the first 6 cohorts, but followed the standard procedure established by Novartis.

2.2. Demographics

The demographics were similar between the placebo and NIM811 groups (Table 1). There were no significant differences in any of the baseline characteristics. Based on *in vitro* data using resistance isolates, there was no reason to assume that NIM811 monotherapy would be affected by any prior treatment status other

Table 1
Patient demographics.

		Placebo	25 mg	75 mg	100 mg bid	200 mg bid	400 mg bid	600 mg bid	600 mg bid + PEG	Placebo + PEG
N		19	9	9	9	9	9	10	10	11
Age	Mean (SD)	51.2 (5.6)	50.8 (5.8)	50.4 (10.7)	53.2 (5.2)	48.6 (6.3)	52.0 (6.3)	54.9 (7.9)	51.5 (9.6)	52.5 (3.0)
Gender	Male	13	6	6	5	6	6	6	8	9
	Female	6	3	3	4	3	3	4	2	2
Race	Caucasian	17	8	7	8	9	8	7	7	7
	Black	2	1	2	1			3	1	
	Other						1		2	4

than another cyclophilin inhibitor. Due to restrictions on women of child bearing potential, almost all participants were male.

2.3. Pharmacokinetics

Single-dose and steady-state NIM811 pharmacokinetics were analyzed using a non-compartmental approach. Single dose PK from a dose escalation trial in healthy volunteers showed that there was no difference between HCV patients and those patients (data not shown). The pharmacokinetics of NIM811 in this trial are shown in Table 2. NIM811 was rapidly absorbed after oral administration with a mean T_{max} ranging from 1 to 1.5 h post-dosing across cohorts. After reaching C_{max}, NIM811 blood concentrations decreased in a bi-exponential manner with a terminal elimination half-life ranging from 8 to 10 h. Following daily multiple bid dosing, NIM811 accumulated slightly upon reaching steady state with mean accumulation factor of approximately 1.2 based on AUC. The mean C_{max} values were similar for both day 1 and day 13 across doses. Dose-related but less than dose proportional increases in AUC, C_{max} and C_{trough} were found in the tested dose range. The NIM811 pharmacokinetic parameters, such as T_{max}, C_{max} and C_{trough} were similar between NIM811 600 mg bid monotherapy and in combination with pegylated interferon, suggesting that the concomitant use of pegylated interferon with that same dose had no effect on NIM811 pharmacokinetics.

2.4. Efficacy

There was no effect of NIM811 monotherapy on the HCV viral load, at doses from 25 mg qd to 600 mg po bid (Fig. 1A). In contrast there was a significant reduction in viral load in the combination therapy arm ($p = 0.0001$) compared to the use of pegylated interferon alone (Fig. 1B). The difference was noted by 7 days, and continued through the 14 day period. At day 14 the viral load reduction from baseline was a $2.85 \pm 1.02 \log_{10}$ reduction for the combination therapy arm, as opposed to a drop of $0.56 \pm 0.77 \log_{10}$ for the pegylated interferon therapy alone arm. Of note, the effect was universal in that almost all patients in the combination therapy arm had a greater reduction than those in the monotherapy arm; that is, the overall effect was in almost all patients rather than due to one or two outliers in one of the arms. All patients in the combination arms were offered combination therapy of PEG-IFN plus ribavirin following the 21 day time point. As this resulted in variable viral load responses at that time point and later, a rebound in the NIM811 plus PEG-IFN following cessation of NIM811 therapy was difficult to document.

At the dose of 100 mg po bid NIM811, rapid normalization of ALT and AST was observed in almost all patients (Fig. 2). This was seen in most of the NIM811 monotherapy arms (Fig. 2A) and in the dual therapy arm (Fig. 2B), but not in the pegylated interferon/placebo arm. The effect was noted early (by day 3), and the effect was observed in most patients.

2.5. Adverse event profile

In general NIM811 was well tolerated. There were no severe or serious adverse reactions in the study, and a single patient discontinuation was due to neutropenia. This patient in the combination therapy arm had a decline to an ANC of 500/mm^3 by day 7. The most common adverse events in the monotherapy and dual therapy arms are listed in Table 3. Of note, in the combination therapy arm, the usual symptoms in response to interferon, such as malaise, fever, and headache were observed. In the combination therapy arm containing NIM811 more patients had mild nausea than in

Table 2
Single and multiple oral dose pharmacokinetics of NIM811 in HCV-1 patients ($N = 9$).

Cohort	NIM dose (mg/d)	Day	T_{max} (h)	C_{max} (ng/mL)	[a]C_t (C_{trough}) (ng/mL)	[b]AUC0-t (ng h/mL)	T1/2 (h)
1	25	1	1 (0.5–1.5)	176 ± 62.3	3.07 ± 0.92	558 ± 199	7.69 ± 2.24
	(QD)	13	1 (0.5–3)	174 ± 99.1	6.82 ± 4.27	657 ± 273	9.60 ± 3.80
2	75	1	1.5 (1–2)	628 ± 230	11.9 ± 5.86	2102 ± 722	8.38 ± 1.29
	(QD)	13	1 (1–1.5)	599 ± 216	21.2 ± 16.0	2139 ± 636	9.15 ± 4.14
3	200	1	1.5 (0.5–2)	1150 ± 486	59.2 ± 32.1	3676 ± 1629	–
	(100 b.i.d.)	13	1 (0.5–2)	1114 ± 514	136 ± 76.7	4517 ± 2098	–
4	400	1	1 (1–1.5)	1663 ± 236	120 ± 44.2	5598 ± 1222	–
	(200 b.i.d.)	13	1 (1–1.5)	1638 ± 265	261 ± 113	6969 ± 1491	–
[c]5	800	1	1.5 (1–4)	1885 ± 740	276 ± 91.3	8087 ± 2589	–
	(400 b.i.d.)	13	1.5 (1–1.5)	1763 ± 502	467 ± 168	9241 ± 1698	–
6	1200	1	1 (0.5–2)	2252 ± 356	[d]NS	[e]ND	–
	(600 b.i.d.)	13	1.5 (1–2)	2403 ± 432	542 ± 115	ND	–
[f]7	1200	1	1 (1–2)	2573 ± 562	364 ± 150	10,779 ± 2694	–
	(600 b.i.d.)	13	1.5 (1–1.5)	2413 ± 519	561 ± 186	12,011 ± 2548	–

Values are mean ± SD except for T_{max} which is median (range). Terminal elimination half-life (T1/2) was calculated only for QD dose regimen.

[a] C_t: blood concentration at last sampling time points: $t = 12$ h for bid and $t = 24$ h for QD.

[b] AUC0-t: AUC from time zero to last sampling time points.

[c] $N = 7$.

[d] No sample collected at 12 h after dosing.

[e] Not determined since PK sampling up to 4 h.

[f] Combination cohort: $N = 10$ and $N = 9$ for day 1 and day 13, respectively.

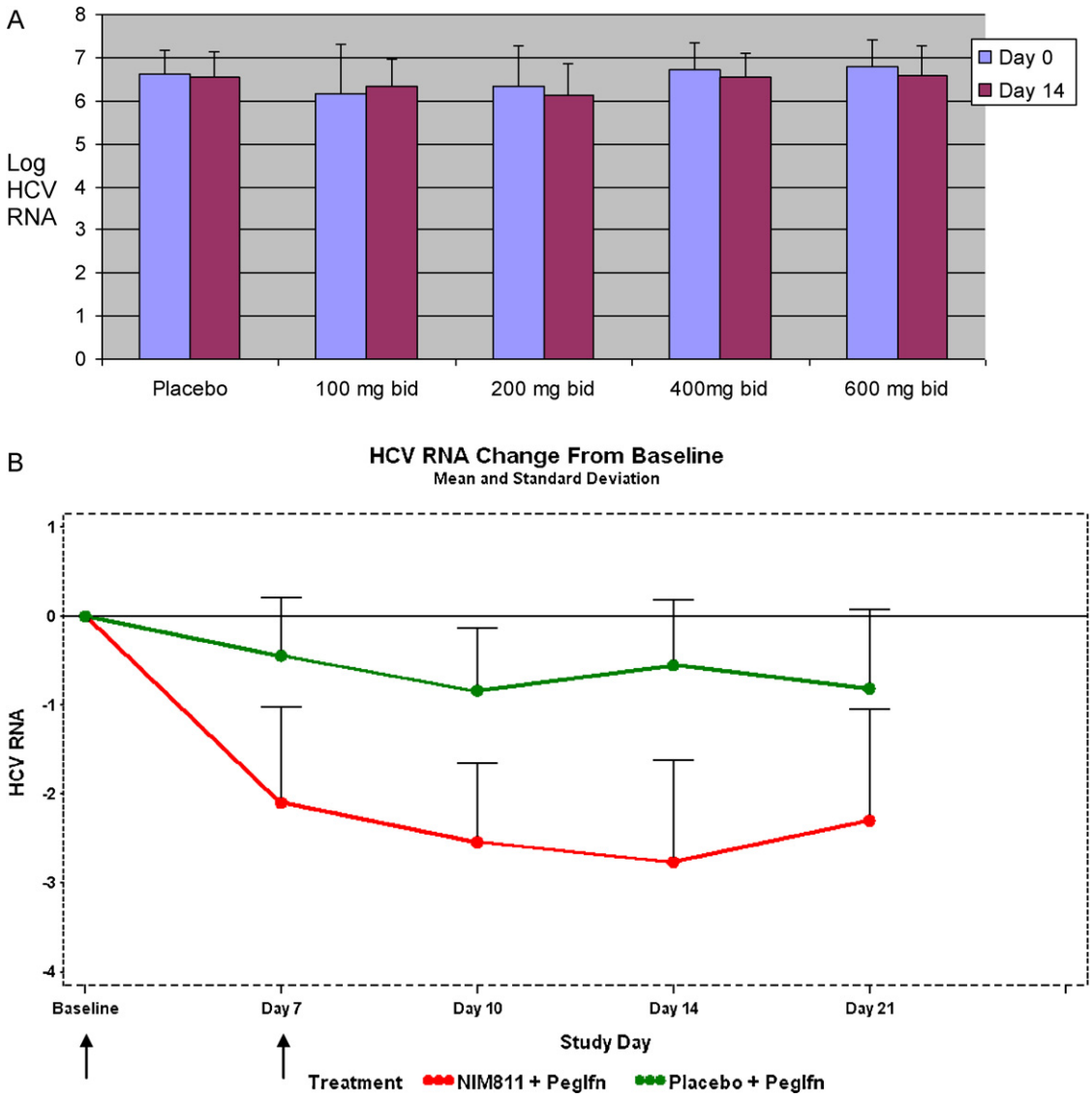

Fig. 1. Effect of NIM 811 on HCV viral load. (A) Baseline and day 14 values for the 100–600 mg bid groups as compared to the pooled placebo arms (log_{10} IU/mL). (B) Time course of viral load change from baseline (in log_{10} IU/mL) for the combination therapy arm for NIM811 600 mg po bid plus pegylated interferon versus pegylated interferon alone. Arrows show time of pegylated interferon injections.

the pegylated interferon alone arm. 3 patients complained of an episode of vomiting. None of these symptoms required discontinuation of therapy.

The use of NIM811 was associated with a rise in bilirubin, first noted as statistically significant at the 400 mg bid dose (Fig. 3A). This effect was, however, to levels that were not considered clinically significant, and no patient had a rise greater than 1.5× ULN. A similar effect was observed in the combination therapy arms (Fig. 3B). Also noted were minor increases in triglycerides at day 14 ranging from a 0.63 mmol increase (1.70–2.33 mmol) at 600 mg po monotherapy, 0.61 mmol increase (1.26–1.87 mmol) at 600 mg combination therapy, 0.40 mmol increase in Peg-INF alone (1.54–1.94 mmol) and no change in the placebo monotherapy combined patients.

Thrombocytopenia that was statistically significant was first noted at the 200 mg po bid dose (Fig. 3C). This effect was more

Table 3
Most common adverse events.

	Placebo $N=19$	100 mg bid $N=9$	200 mg bid $N=9$	400 mg bid $N=9$	600 mg bid $N=10$	600 mg bid + Peg-IFN $N=10$	Placebo + Peg-IFN $N=11$
Neutropenia	0	0	0	0	0	1	1
Diarrhea	0	1	0	0	1	2	2
Nausea	1	0	0	0	1	5	1
Vomiting	0	0	0	1	0	3	0
Flu like	3	2	1	1	0	6	4
Myalgia	0	0	0	0	0	1	2
Headache	1	2	3	2	0	3	3

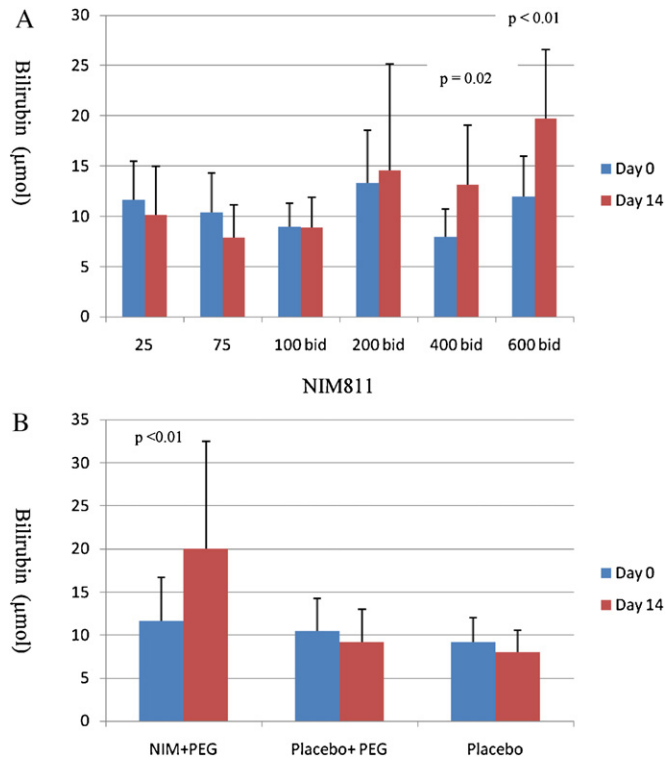

Fig. 2. Effect of NIM811 on SGPT (ALT, IU/mL) levels at 14 days compared to baseline (mean plus standard deviation). (A) Monotherapy arms and (B) combination therapy for NIM811 600 mg po bid plus pegylated interferon versus pegylated interferon alone, as well as pooled placebo. *p* values shown for significance of <0.05.

pronounced in the combination therapy arm, and the thrombocytopenia was more significant, although no dose adjustment of the pegylated interferon was needed as the study was only of two weeks duration (Fig. 3D). The difference in fall from baseline for the NIM811 plus pegylated interferon arm was significantly greater than the pegylated interferon alone group ($p = 0.025$). There was no effect on ANC or white blood counts in the monotherapy arms, but in the combination therapy group the effect on WBC was greater in the combination therapy arm ($6.36 \pm 1.38/mm^3$ at baseline to $2.89 \pm 0.78/mm^3$ at 14 days) versus the pegylated interferon alone arm ($5.48 \pm 1.23/mm^3$ to $3.35 \pm 1.18/mm^3$) ($p = 0.05$). The declines in ANC for the combination arm were $3.62 \pm 1.03/mm^3$ at baseline to $1.05 \pm 0.51/mm^3$ at day 14, as compared to $3.31 \pm 0.93/mm^3$ to 1.47 ± 0.53 in the pegylated interferon arm ($p = 0.12$).

There was no trend toward an effect of NIM811 on thyroid function, inhibin or FSH levels as compared to placebo. Of note, many abnormalities at baseline were detected in the inhibin values in this HCV positive patient population (data not shown). Likewise no effect on lenticular structure was observed in the slit lamp eye examinations. Over the course of the study there were no significant changes in CD3, CD4, or C8 cell counts, nor in IL-2 secretion in a small subset of patients (data not shown).

3. Discussion

In this randomized, double-blind, placebo-controlled study the combination of NIM811, a host factor targeting inhibitor that binds to a number of human cyclophilins, showed a statistically significant antiviral effect within one week when combined with pegylated interferon alpha-2a in the treatment of HCV genotype 1 relapsers. This study followed a single Phase 1 study of single dose NIM811 in which the drug was administered to 63 healthy volunteers at doses ranging from 1 to 1600 mg. Essentially no laboratory or other adverse events were seen in that study and the pharmacokinetics were dose proportional.

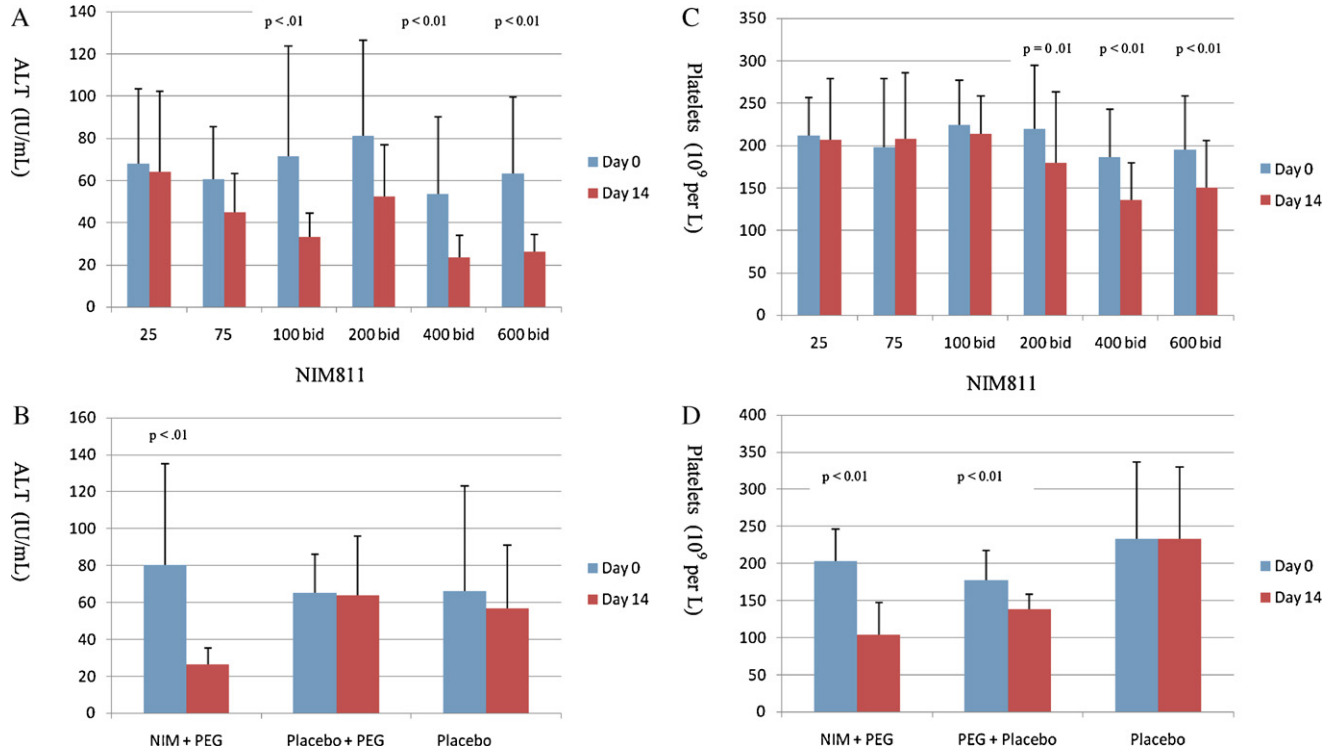

Fig. 3. Laboratory effects of NIM811 on bilirubin and platelets (mean plus standard deviation). (A) Bilirubin change in monotherapy arms (μmol) from baseline to day 14. (B) Bilirubin change in combination therapy arms, and pooled placebo from baseline to day 14. (C) Platelet change in monotherapy arms from baseline to day 14. (D) Platelet change in combination therapy arm, and pooled placebo from baseline to day 14. *p* values shown for significance of <0.05.

Using the pharmacokinetic data from that trial, *in vitro* antiviral potency of the compound, and the reported activities of CsA (Inoue et al., 2003), we originally projected that NIM811 would be efficacious at approximately 400 mg bid when given orally. However, even at 600 mg of monotherapy no antiviral effect was observed. Given the steep *in vitro* dose–response curve in our preclinical studies, further escalation to 800 mg po bid monotherapy had been planned.

However, given the additive to synergistic nature of the compound with interferon (Ma et al., 2006; Mathy et al., 2008), the reported low effective dose of cyclosporine A in a human trial conducted in Japan when used in combination (Inoue et al., 2003), we predicted that NIM811 would more likely show antiviral activity when administered with interferon alpha. There was also concern that dose absorption may be limited at higher doses.

This prediction of an effect in combination was substantiated by our highly statistically significant findings ($p = 0.0001$). The study was limited by the small number of patients studied and the short length of therapy. Of note, NIM811 was only effective when added to an interferon arm. This is in contrast to the results obtained by Flisiak et al. (2009) who were able to demonstrate activity of Debio-025 as monotherapy. Of note, in *in vitro* assays Debio-025 is 2–4-fold more potent than NIM811 (data not shown), and NIM811 exhibits a steep dose response curve in such assays (Ma et al., 2006; Mathy et al., 2008). As relative oral exposure of the two compounds is somewhat comparable, and no major differences have been observed in mechanism of action studies, we may not have administered a large enough dose of NIM811 to achieve antiviral activity as a monotherapy.

NIM811 has a number of features which make it a very attractive candidate for HCV therapy. There is no *in vitro* cross resistance with interferon, nor with a number of the polymerase or protease inhibitors in development (Ma et al., 2006; Mathy et al., 2008). In those studies the compound had additive to synergistic effects when used in combination with interferon or HCV NS3 protease and NS5 polymerase inhibitors. More importantly, as would be predicted for host targeting agent, the barrier of generating *in vitro* resistance is high with difficulty in our hands of generating resistance replicon mutants to NIM811, in contrast to the known low barrier for non-nucleoside polymerase or most protease inhibitors.

The mechanism of action of NIM811 and other cyclophilin inhibitors in the treatment of HCV is the focus of intensive investigation. Knock-down of cyclophilins in the replicon cells with specific siRNAs led to an inhibition of HCV replication, suggesting that cyclophilins are essential for viral replication (Gaither et al., 2010; Nakagawa et al., 2005; Watashi et al., 2005). However, there have been some discrepancies on specifically which cyclophilins are required and how they are involved in HCV replication. Watashi et al. (2005) reported that CypB but not CypA was required for HCV replication through its interaction with NS5B. This finding was confirmed by Heck et al. (2009) who further demonstrated that CypB stimulated RNA synthesis by NS5B *in vitro* and the PPIase activity of CypB was not required for this function. In contrast, Yang et al. (2008) suggested that CypA was the main mediator of HCV replication. Moreover, the PPIase activity of CypA was essential for its function in HCV replication (Liu et al., 2009). NS5A domain 2 directly interacted with CypA and CypB and was a substrate of CypA and CypB PPIase *in vitro* (Hanoulle et al., 2009). In addition, there have also been data suggesting cyclophilin inhibitors may interfere with the self cleavage of NS2–NS3 (Ciesek et al., 2009) and the cleavage of NS5A–NS5B by NS3 (Kaul et al., 2009); however, whether such an effect is genotype-specific (2a, JFH-1) remains to be determined. Thus, increasing evidences suggest that cyclophilins are involved in HCV replication by (1) interacting directly with viral proteins as part of the replication complex and/or (2) mediating the correct folding and trafficking of viral proteins to the site of replication (cytosolic side of ER membrane) through their PPIase activity.

The effect on transaminases at relatively low doses of NIM811, despite the lack of antiviral activity was quite remarkable. NIM811 and related cyclosporine-like compounds are able to effectively bind cyclophilin D, and thereby block the apoptosis induced by the mitochondrial pore transition complex (Argaud et al., 2005; Hansson et al., 2004; Waldmeier et al., 2002). This action has led to the preclinical study of these compounds in fibrosis (Rehman et al., 2008), reperfusion injury (Zhong et al., 2008; Theruvath and Lemasters, 2008), stroke (Korde et al., 2007), neural injury (Mbye et al., 2008; McEwen et al., 2007), and a variety of other conditions (Raisky et al., 2004). Whether the transaminase normalization was due to an anti-apoptotic effect of the compound, or has another mechanism involving cyclophilin inhibition deserves further study. Nonetheless, the present cyclophilin inhibitors, all of which have effects on multiple human cyclophilins, are likely to have differential effects on a number of host pathways. NIM811 only varies from cyclosporine A through a single methyl group, and yet has profoundly different properties; thus generalization of potential effects of this class of compounds may be risky.

An unexpected result from the trial was the concerning thrombocytopenia that appeared when added to pegylated interferon. The combination therapy for two weeks produced a degree of thrombocytopenia that was greater than that seen with either pegylated interferon or NIM811 used at the same dose. The mechanism for this effect is not yet clear, and further studies will be required to ascertain whether longer courses of therapy will result in better or worsened results, but it is possible that this may be a class effect, as a similar finding was seen with Debio-025 (Flisiak et al., 2009). Of note, no platelet effect was seen at the lowest dose required for ALT normalization, again pointing to a different mechanism or threshold effect.

Besides the thrombocytopenia, the drug was relatively well tolerated. The only other laboratory abnormalities were mild increases in bilirubin and triglycerides, which did not attain clinical significance. The bilirubin elevations in this trial did not appear as severe as though seen with very high doses of a related compound (Flisiak et al., 2008). A possible increase in nausea may have been observed in the combination therapy arm, and warrants further monitoring.

It is now clear that cyclophilin inhibitors are active *in vivo* against hepatitis C virus (Flisiak et al., 2009). NIM811 did not show the effect in monotherapy reported in trial of Debio-025, and a direct comparison of the trial of combination therapy with interferon is difficult, as the patient populations (naive versus relapsers) were not the same (Flisiak et al., 2008, 2009). The role of these compounds in treatment is likely to be as part of combination regimens, and the high *in vitro* barrier to resistance is one of the more promising aspects of this class of compounds. Further studies to determine the long term safety, efficacy, and appropriate dosing of these compounds as part of combination therapy approach to HCV are needed.

Acknowledgements

We would like to thank Michael P. Cooreman, Roger Fujimoto, Kai Lin, Brigitte Wiedmann, Jennifer Philips, Kiran Dole, Christine Garrett, Margaret Koziel, and countless others for their support in the NIM811 project and clinical trial.

Funding: Novartis Institutes of Biomedical Research.

References

Argaud, L., Gateau-Roesch, O., Muntean, D., Chalabreysse, L., Loufouat, J., Robert, D., Ovize, M., 2005. Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury. J. Mol. Cell Cardiol. 38, 367–374.

Ciesek, S., Steinmann, E., Wedemeyer, H., Manns, M.P., Neyts, J., Tautz, N., Madan, V., Bartenschlager, R., von, H.T., Pietschmann, T., 2009. Cyclosporine A inhibits hepatitis C virus nonstructural protein 2 through cyclophilin A. Hepatology 50, 1638–1645.

Flisiak, R., Feinman, S.V., Jablkowski, M., Horban, A., Kryczka, W., Pawlowska, M., Heathcote, J.E., Mazzella, G., Vandelli, C., Nicolas-Metral, V., Grosgurin, P., Liz, J.S., Scalfaro, P., Porchet, H., Crabbe, R., 2009. The cyclophilin inhibitor Debio 025 combined with PEG IFNalpha2a significantly reduces viral load in treatment-naive hepatitis C patients. Hepatology 49, 1460–1468.

Flisiak, R., Horban, A., Gallay, P., Bobardt, M., Selvarajah, S., Wiercinska-Drapalo, A., Siwak, E., Cielniak, I., Higersberger, J., Kierkus, J., Aeschlimann, C., Grosgurin, P., Nicolas-Metral, V., Dumont, J.M., Porchet, H., Crabbe, R., Scalfaro, P., 2008. The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus. Hepatology 47, 817–826.

Gaither, L.A., Borawski, J., Anderson, L.J., Balabanis, K.A., Devay, P., Joberty, G., Rau, C., Schirle, M., Bouwmeester, T., Mickanin, C., Zhao, S., Vickers, C., Lee, L., Deng, G., Baryza, J., Fujimoto, R.A., Lin, K., Compton, T., Wiedmann, B., 2010. Multiple cyclophilins involved in different cellular pathways mediate HCV replication. Virology 397, 43–55.

Hanoulle, X., Badillo, A., Wieruszeski, J.M., Verdegem, D., Landrieu, I., Bartenschlager, R., Penin, F., Lippens, G., 2009. Hepatitis C virus NS5A protein is a substrate for the peptidyl–prolyl cis/trans isomerase activity of cyclophilins A and B. J. Biol. Chem. 284, 13589–13601.

Hansson, M.J., Mattiasson, G., Mansson, R., Karlsson, J., Keep, M.F., Waldmeier, P., Ruegg, U.T., Dumont, J.M., Besseghir, K., Elmer, E., 2004. The nonimmunosuppressive cyclosporin analogs NIM811 and UNIL025 display nanomolar potencies on permeability transition in brain-derived mitochondria. J. Bioenerg. Biomembr. 36, 407–413.

Heck, J.A., Meng, X., Frick, D.N., 2009. Cyclophilin B stimulates RNA synthesis by the HCV RNA dependent RNA polymerase. Biochem. Pharmacol. 77, 1173–1180.

Inoue, K., Sekiyama, K., Yamada, M., Watanabe, T., Yasuda, H., Yoshiba, M., 2003. Combined interferon alpha2b and cyclosporin A in the treatment of chronic hepatitis C: controlled trial. J. Gastroenterol. 38, 567–572.

Ishii, N., Watashi, K., Hishiki, T., Goto, K., Inoue, D., Hijikata, M., Wakita, T., Kato, N., Shimotohno, K., 2006. Diverse effects of cyclosporine on hepatitis C virus strain replication. J. Virol. 80, 4510–4520.

Kaul, A., Stauffer, S., Berger, C., Pertel, T., Schmitt, J., Kallis, S., Zayas, M., Lohmann, V., Luban, J., Bartenschlager, R., 2009. Correction: essential role of cyclophilin a for hepatitis C virus replication and virus production and possible link to polyprotein cleavage kinetics. PLoS Pathog. 5.

Korde, A.S., Pettigrew, L.C., Craddock, S.D., Pocernich, C.B., Waldmeier, P.C., Maragos, W.F., 2007. Protective effects of NIM811 in transient focal cerebral ischemia suggest involvement of the mitochondrial permeability transition. J. Neurotrauma 24, 895–908.

Liu, Z., Yang, F., Robotham, J.M., Tang, H., 2009. Critical role of cyclophilin A and its prolyl-peptidyl isomerase activity in the structure and function of the hepatitis C virus replication complex. J. Virol. 83, 6554–6565.

Ma, S., Boerner, J.E., TiongYip, C., Weidmann, B., Ryder, N.S., Cooreman, M.P., Lin, K., 2006. NIM811, a cyclophilin inhibitor, exhibits potent *in vitro* activity against hepatitis C virus alone or in combination with alpha interferon. Antimicrob. Agents Chemother. 50, 2976–2982.

Mathy, J.E., Ma, S., Compton, T., Lin, K., 2008. Combinations of cyclophilin inhibitor NIM811 with hepatitis C Virus NS3-4A Protease or NS5B polymerase inhibitors enhance antiviral activity and suppress the emergence of resistance. Antimicrob. Agents Chemother. 52, 3267–3275.

Mbye, L.H., Singh, I.N., Sullivan, P.G., Springer, J.E., Hall, E.D., 2008. Attenuation of acute mitochondrial dysfunction after traumatic brain injury in mice by NIM811, a non-immunosuppressive cyclosporin A analog. Exp. Neurol. 209, 243–253.

McEwen, M.L., Sullivan, P.G., Springer, J.E., 2007. Pretreatment with the cyclosporin derivative NIM811, improves the function of synaptic mitochondria following spinal cord contusion in rats. J. Neurotrauma 24, 613–624.

Nakagawa, M., Sakamoto, N., Tanabe, Y., Koyama, T., Itsui, Y., Takeda, Y., Chen, C.H., Kakinuma, S., Oooka, S., Maekawa, S., Enomoto, N., Watanabe, M., 2005. Suppression of hepatitis C virus replication by cyclosporin a is mediated by blockade of cyclophilins. Gastroenterology 129, 1031–1041.

Raisky, O., Gomez, L., Chalabreysse, L., Gateau-Roesch, O., Loufouat, J., Thivolet-Bejui, F., Ninet, J., Ovize, M., 2004. Mitochondrial permeability transition in cardiomyocyte apoptosis during acute graft rejection. Am. J. Transplant. 4, 1071–1078.

Rehman, H., Ramshesh, V.K., Theruvath, T.P., Kim, I., Currin, R.T., Giri, S., Lemasters, J.J., Zhong, Z., 2008. NIM811 (N-methyl-4-isoleucine cyclosporine), a mitochondrial permeability transition inhibitor, attenuates cholestatic liver injury but not fibrosis in mice. J. Pharmacol. Exp. Ther. 327, 699–706.

Rosenwirth, B., Billich, A., Datema, R., Donatsch, P., Hammerschmid, F., Harrison, R., Hiestand, P., Jaksche, H., Mayer, P., Peichl, P., 1994. Inhibition of human immunodeficiency virus type 1 replication by SDZ NIM 811, a nonimmunosuppressive cyclosporine analog. Antimicrob. Agents Chemother. 38, 1763–1772.

Theruvath, T.P., Lemasters, J.J., 2008. Cyclosporine in acute myocardial infarction. N. Engl. J. Med. 359, 2286–2289.

Vento, S., Cainelli, F., Temesgen, Z., 2008. Perspectives in therapy for hepatitis C. Expert. Opin. Investig. Drugs 17, 1635–1639.

Waldmeier, P.C., Feldtrauer, J.J., Qian, T., Lemasters, J.J., 2002. Inhibition of the mitochondrial permeability transition by the nonimmunosuppressive cyclosporin derivative NIM811. Mol. Pharmacol. 62, 22–29.

Watashi, K., Ishii, N., Hijikata, M., Inoue, D., Murata, T., Miyanari, Y., Shimotohno, K., 2005. Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. Mol. Cell 19, 111–122.

Yang, F., Robotham, J.M., Nelson, H.B., Irsigler, A., Kenworthy, R., Tang, H., 2008. Cyclophilin A is an essential cofactor for hepatitis C virus infection and the principal mediator of cyclosporine resistance *in vitro*. J. Virol. 82, 5269–5278.

Zeuzem, S., 2008. Interferon-based therapy for chronic hepatitis C: current and future perspectives. Nat. Clin. Pract. Gastroenterol. Hepatol. 5, 610–622.

Zhong, Z., Ramshesh, V.K., Rehman, H., Currin, R.T., Sridharan, V., Theruvath, T.P., Kim, I., Wright, G.L., Lemasters, J.J., 2008. Activation of the oxygen-sensing signal cascade prevents mitochondrial injury after mouse liver ischemia-reperfusion. Am. J. Physiol. Gastrointest. Liver Physiol. 295, 823–832.