



Oral combination therapy with a nucleoside polymerase inhibitor (RG7128) and danoprevir for chronic hepatitis C genotype 1 infection (INFORM-1): a randomised, double-blind, placebo-controlled, dose-escalation trial

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Summary

Background Present interferon-based standard of care treatment for chronic hepatitis C virus (HCV) infection is limited by both efficacy and tolerability. We assessed the safety, tolerability, and antiviral activity of an all-oral combination treatment with two experimental anti-HCV drugs—RG7128, a nucleoside polymerase inhibitor; and danoprevir, an NS3/4A protease inhibitor—in patients with chronic HCV infection.

Methods Patients from six centres in New Zealand and Australia who were chronically infected with HCV genotype 1 received up to 13 days oral combination treatment with RG7128 (500 mg or 1000 mg twice daily) and danoprevir (100 mg or 200 mg every 8 h or 600 mg or 900 mg twice daily) or placebo. Eligible patients were sequentially enrolled into one of seven treatment cohorts and were randomly assigned by interactive voice or web response system to either active treatment or placebo. Patients were separately randomly assigned within each cohort with a block size that reflected the number of patients in the cohort and the ratio of treatment to placebo. The random allocation schedule was computer generated. Dose escalation was started in HCV treatment-naive patients; standard of care treatment-experienced patients, including previous null responders, were enrolled in higher-dose danoprevir cohorts. Investigators, personnel at the study centre, and patients were masked to treatment allocation. However, the pharmacist who prepared the doses, personnel involved in pharmacokinetic sample analyses, statisticians who prepared data summaries, and the clinical pharmacologists who reviewed the data before deciding to initiate dosing in the next cohort were not masked to treatment allocation. The primary outcome was change in HCV RNA concentration from baseline to day 14 in patients who received 13 days of combination treatment. All patients who completed treatment with the study drugs were included in the analyses. This study is registered with ClinicalTrials.gov, NCT00801255.

Findings 88 patients were randomly assigned to a study drug treatment regimen (n=74 over seven treatment groups; 73 received at least one dose of study drug) or to placebo (n=14, all of whom received at least one dose). The median change in HCV RNA concentration from baseline to day 14 ranged from -3.7 to $-5.2 \log_{10}$ IU/mL in the cohorts that received 13 days of combination treatment. At the highest combination doses tested (1000 mg RG7128 and 900 mg danoprevir twice daily), the median change in HCV RNA concentration from baseline to day 14 was $-5.1 \log_{10}$ IU/mL (IQR -5.6 to -4.7) in treatment-naive patients and $-4.9 \log_{10}$ IU/mL in previous standard of care null responders (-5.2 to -4.5) compared with an increase of $0.1 \log_{10}$ IU/mL in the placebo group. The combination of RG7128 and danoprevir was well tolerated with no treatment-related serious or severe adverse events, no grade 3 or 4 changes in laboratory parameters, and no safety-related treatment discontinuations.

Interpretation This oral combination of a nucleoside analogue polymerase inhibitor and protease inhibitor holds promise as an interferon-free treatment for chronic HCV.

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Introduction

Hepatitis C virus (HCV) infection is a rapidly evolving medical problem worldwide;¹⁻³ more than 170 000 000 people are infected, a fifth of whom will develop cirrhosis.^{4,5} HCV-related liver disease is the most common reason for liver transplantation, and HCV-related hepatocellular carcinoma has the fastest growing cancer-related mortality rate in developed countries.⁶ The present standard of care for chronic HCV infection is subcutaneous pegylated interferon alfa plus oral ribavirin.

Many direct-acting antiviral drugs are being developed that are aimed at various HCV targets. Experimental HCV protease inhibitors improved treatment outcomes when added to standard of care in both treatment-naive and treatment-experienced patients.^{7,8} However, the benefits of adding one direct-acting antiviral drug to the present standard of care will be limited by the underlying safety, tolerability, and efficacy drawbacks associated with interferon-based treatment. The successful development of an oral, interferon-free direct-acting antiviral drug

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combination treatment would fill an unmet medical need, and potentially change the existing standard of care for HCV. The rationale for an oral combination treatment for HCV is based on the present HIV treatment regimen, in which multiple direct-acting antiviral drugs that target different steps of viral replication are combined to increase the amount of viral suppression and to prevent or delay the emergence of antiviral resistance.

RG7128 is a 3',5'-di-isobutyric acid ester prodrug of the cytosine nucleoside analogue β -D-2'-deoxy-2'-C-methylcytidine, which, in its triphosphate form, inhibits HCV NS5B RNA polymerase. Danoprevir (RG7227) is a macrocyclic inhibitor of the HCV NS3/4A protease, an enzyme that is needed for viral replication. Both compounds have potent in-vitro and in-vivo activity against HCV,⁹⁻¹² and at the time of this study each were in phase 1 development.

We aimed to assess whether RG7128 and danoprevir could be safely given in combination and whether this combination would provide potent antiviral activity without the emergence of treatment resistance.

Methods

Patients

INFORM-1 (INterferon-Free regimen FOR the Management of HCV) was a randomised, double-blind, placebo-controlled, dose-escalation trial. Full details of the methods are available in the study protocol. Patients

were enrolled at six centres in New Zealand and Australia; the first patient was screened on Oct 27, 2008, and the last clinical examination was done on Nov 30, 2009. Eligible patients were men and women who were of non-childbearing potential aged 18–65 years, who were chronically infected with HCV genotype 1 but did not have cirrhosis, and who had a minimum HCV RNA of 10⁵ IU/mL. Patients were required to have normal renal and hepatic function and no clinically significant comorbidities. Additional exclusion criteria were co-infection with hepatitis B or HIV, concurrent medical or psychiatric disorder (or history of such), history of any neoplastic disease, history of clinically significant cardiovascular or cerebrovascular disease, use of growth factors, or anticipated use or need for significant concomitant medical treatment. A full list of eligibility criteria is provided in the study protocol.

The study included standard of care treatment-naive and treatment-experienced (null and non-null responder) patients. Patients were classed as non-null responders if they had relapsed (an HCV RNA concentration that was undetectable while receiving standard of care but that became detectable after discontinuation of therapy) or had responded partially (a reduction in HCV RNA concentration by at least 2 log₁₀ IU/mL, after 12 weeks of treatment while on standard of care but not reaching undetectable HCV RNA concentrations). Patients were

For the **INFORM-1** protocol see <http://www.roche-trials.com/trialDetailsGet.action?studyNumber=PP22205>

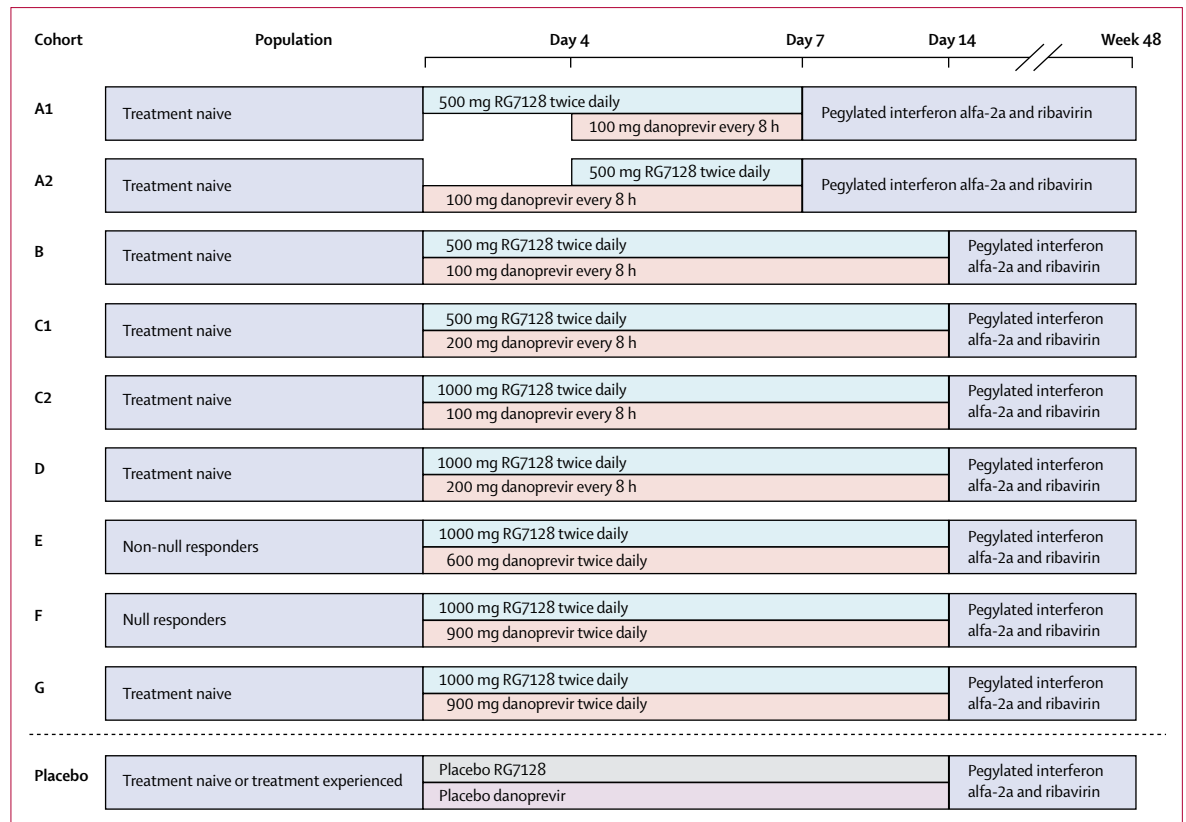


Figure 1: Treatment regimens

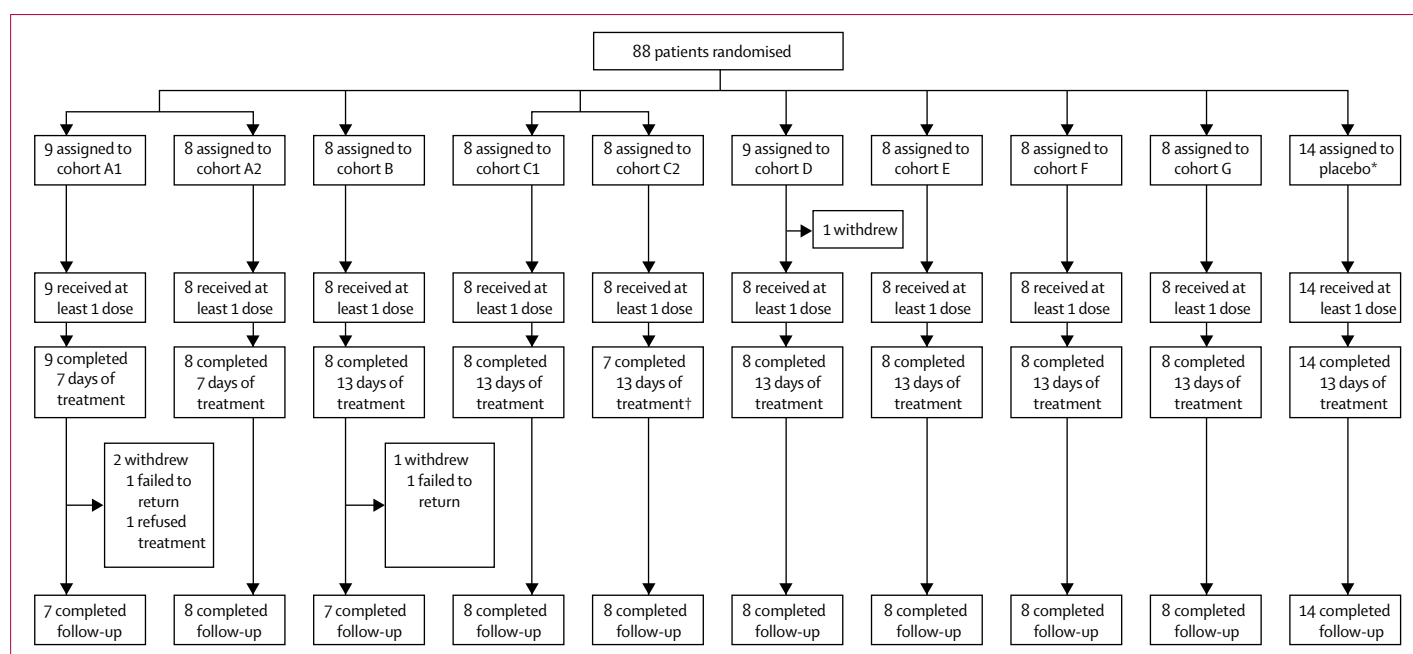


Figure 2: Trial profile

*Placebo patients were randomly assigned from the treatment cohorts: two from B, two from C, four from D, two from E, two from F, and two from G; the main objective of cohort A was to characterise the potential pharmacokinetic interaction, therefore placebo control was not necessary. †One patient did not complete inpatient treatment but completed the day 98 follow-up visit; therefore, this patient was regarded as having completed the study but was not evaluable for the assessment of viral kinetics on day 14.

classified as null responders if they had a less than $1 \log_{10}$ IU/mL reduction in HCV RNA concentration after 1 month or less than $2 \log_{10}$ IU/mL reduction after 12 weeks of previous standard of care.

This trial was approved by local ethics committees and regulatory authorities at each of the six centres and was done in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients before any study-related activities.

Randomisation and masking

Eligible patients were enrolled into one of the seven treatment cohorts and were then randomly assigned by interactive voice or web response system to active treatment or placebo. Random allocation for each cohort was done after completion of treatment in the preceding cohort. Cohorts were treated in the following order: A, B, C, D, E, and F and G (in parallel). Patients were separately randomly assigned within each cohort with a block size that reflected the number of patients in the cohort and the ratio of treatment to placebo. Patients in cohort A were randomly assigned (1:1) to treatment A1 or treatment A2. For cohorts B and E–G, patients were randomly assigned (4:1) to treatment or placebo. Patients in cohort C were randomly assigned (4:4:1) to treatment C1, treatment C2, or placebo. For cohort D, patients were randomly assigned (2:1) to treatment or placebo. The random allocation sequence was computer generated and maintained by the Roche project statistician. Investigators, personnel at the study centre, and patients were masked to treatment

allocation. However, the pharmacist who prepared the doses, personnel involved in pharmacokinetic sample analyses, statisticians who prepared data summaries, and the clinical pharmacologists who reviewed the data before deciding to initiate dosing in the next cohort were not masked to treatment allocation. Patients in cohort C were masked to study drug dose but patients in cohorts A, B, and D–G were not. Study drugs and placebo were identical in colour, size, shape, and taste.

Procedures

Figure 1 shows the treatment regimens for each cohort; each subsequent intervention cohort received a higher dose combination of RG7128 and danoprevir than the previous cohort. Treatment in cohorts A and B was completed sequentially and available safety, antiviral, and pharmacokinetic data were assessed before dosing cohort C. Pending no clinically significant safety issues in cohort C, cohort D was started. After completion of cohort E and the analysis of available safety and pharmacokinetic data, cohorts F and G were started in parallel. Patients remained in a clinical research unit throughout study drug treatment. On completion of study drug dosing and after a washout period of up to 24 h, patients continued on standard of care treatment (180 µg/week pegylated interferon alfa-2a, and ribavirin at 1000 mg/day for patients weighing <75 kg or 1200 mg/day for those weighing ≥75 kg), apart from patients in cohort F, who were unmasked after the last assessment was completed on day 14 to allow the investigator to decide if patients should

	Placebo (n=14)	B (n=8)	C1 (n=8)	C2 (n=8)	D (n=9)	E (n=8)	F (n=8)	G (n=8)
Age (years)	49.1 (7.9)	48.6 (6.5)	50.8 (3.1)	49.8 (8.9)	43.9 (11.1)	43.3 (12.0)	51.4 (4.4)	43.5 (10.2)
Weight (kg)	70.5 (9.9)	88.7 (16.7)	79.2 (8.3)	79.0 (10.4)	79.6 (14.3)	75.9 (18.3)	76.1 (9.8)	76.0 (13.6)
Men	9	7	8	5	6	6	6	7
Race								
Asian	0	0	0	1	2	0	0	3
White	14	6	8	7	6	8	7	5
Pacific Islander	0	1	0	0	0	0	0	0
Other	0	1	0	0	0	0	1	0
Alanine aminotransferase (IU/mL)	78 (32)	92 (55)	89 (70)	84 (27)	84 (61)	64 (26)	117 (76)	90 (65)
HCV genotype								
1a	13	6	7	5	6	7	7	5
1b	1	2	1	3	2	1	1	3
Baseline log ₁₀ HCV RNA (IU/mL)	6.6 (0.6)	6.3 (0.8)	6.7 (0.5)	6.4 (0.4)	6.2 (0.6)	6.2 (0.6)	6.3 (0.8)	6.5 (0.6)
Log ₁₀ HCV RNA change from baseline to EOT	0.1 (-0.1 to 0.2)	-3.7 (-4.5 to -3.2)	-5.2 (-5.5 to -4.6)	-4.8 (-5.7 to -4.6)*	-4.8 (-5.2 to -4.3)	-4.0 (-4.7 to -3.5)	-4.9 (-5.2 to -4.5)	-5.1 (-5.6 to -4.7)
<LLOQ at EOT	0	1	5	5/7	5/8	4	4	7
<LLOD at EOT	0	1	2	2/7	2/8	1	2	5

Data are mean (SD), number, median (IQR), or n/N. EOT=end of treatment. HCV=hepatitis C virus. LLOD=lower limit of detection. LLOQ=lower limit of quantification. *n=7 for viral kinetics on day 14; one patient did not complete the day 14 assessment for viral kinetics and was excluded from the analysis.

Table 1: Demographics and virological results for patients who received combination treatment or placebo

start standard of care treatment. Patients returned to the clinic for follow-up visits at 1, 4, and 12 weeks after the last dose of study drug. If patients continued on the standard of care, these follow-up visits occurred while they were still receiving standard of care treatment.

The primary outcome was change in HCV RNA concentration from baseline to day 14 in patients who received 13 days of combination treatment.

We collected blood samples from all cohorts for measurement of plasma drug concentrations of both compounds. For cohort A, samples were taken on days 3 (monotherapy) and 7 (combination). For cohorts E, F, and G, samples were taken on days 1, 4, 7, 10, and 14. Samples were assayed by validated liquid chromatography tandem mass spectrometry methods, and standard non-compartmental pharmacokinetic methods were applied. Samples were also taken for cohorts B, C, and D, on days 1, 4, 7, and 14, but we do not present data for these cohorts.

We measured plasma HCV RNA concentrations with the COBAS TaqMan HCV Test (version 2.0; Roche, Burgess Hill, UK), with a lower limit of quantification of 43 IU/mL and a lower limit of detection of 15 IU/mL. HCV RNA was measured at screening, baseline, during and at the end of treatment, and during follow-up.

Serum samples from all timepoints at which HCV RNA was measured were collected and stored for monitoring of drug resistance. Double-stranded DNA population sequencing of NS5B, NS3/4A, and NS3 protease entire coding regions was done for all samples at baseline. For groups A1 and A2, drug susceptibility was assessed at baseline (day 1), at the end of monotherapy (day 4), and at the end of combination treatment (day 7) by direct cloning of patient-derived

sequences into a shuttle replicon. For all other cohorts, drug susceptibility was tested on days 1 and 7. Patients who had virological rebound (defined as a sustained increase of ≥ 0.5 log₁₀ IU/mL HCV RNA above nadir before the end of treatment) and non-responders (defined as an HCV RNA change from baseline ≤ 0.5 log₁₀ IU/mL) also had population and clonal sequencing of samples at baseline and after rebound. We did phenotypic characterisation by cloning the patient's NS5B and NS3 protease region into a replicon shuttle vector to assess drug susceptibility.

Plasma samples collected at baseline and on days 3, 14, and 42 from patients in cohorts B–G were analysed for interferon gamma-inducible protein (IP-10, a marker of reduced interferon responsiveness) concentration by immunoassay (Aushon Biosystems, Billerica, MA, USA).

Biochemical and haematological assessments, urinalysis, electrocardiograms (ECGs), and physical examinations were done at screening, baseline, frequently during study drug treatment, and at the follow-up visit 7 days after the last dose of study medications. Safety events included laboratory abnormalities, adverse events, intercurrent illness, and death.

Statistical analysis

With a sample size of eight participants per group, a two-sided 90% CI for a single mean had an interval that extended no more than 0.7 log₁₀ from the observed mean, with 80% coverage probability (assuming that the true SD is 0.95 log₁₀ and that the CI is based on the *t* statistic). No formal hypothesis testing was planned. Patients in cohorts B–G and the placebo cohort who received at least one dose of drugs were included in the analysis of the primary

outcome. All patients who received at least one dose of study drug were included in the safety, pharmacokinetics, and IP-10 analyses. Adverse events, vital signs, laboratory tests, HCV RNA, and pharmacokinetics were descriptively compared across the various treatment groups.

This study is registered with ClinicalTrials.gov, NCT00801255.

Role of the funding source

The sponsor of the study contributed to study design, data collection, data analysis, data interpretation, and writing of the report, with input from the investigators. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

88 patients were enrolled into the trial, of whom 73 received at least one dose of assigned treatment and 14 received placebo (figure 2). Cohort D was over enrolled by one patient; this patient was eligible on screening and was allowed to enrol in case of dropouts. One patient withdrew before receiving the first dose. Of the 73 patients who received at least one dose of study drug, 55 received 13 days of combination treatment (cohorts B–G) and were included in the assessment of viral kinetics on day 14. The baseline characteristics of these patients and those in the placebo cohort were similar (table 1). Most of the 87 patients enrolled were white ($n=78$, 90%), men ($n=70$, 80%), and infected with genotype 1a ($n=69$, 79%). The mean age was 46.8 years (range 27–61) and the mean baseline \log_{10} plasma HCV RNA concentration was 6.4 IU/mL (range 4.6–7.3 IU/mL).

At the end of the study treatment period (day 14), 85 patients started treatment with pegylated interferon alfa-2a and two with pegylated interferon alfa-2b (one patient in each of cohorts A1 and C1), all in combination with oral ribavirin.

Table 1 and figure 3 show the changes in HCV RNA concentrations for cohorts B–G and the placebo group. The median reductions in HCV RNA concentrations were about 5 \log_{10} IU/mL for the cohorts with the highest dose regimens (cohort F, which included previous standard of care null responders, and cohort G, which included treatment-naïve patients; table 1). In a post-hoc analysis, we combined data from cohorts B–G and noted that there was similar antiviral activity in the 42 patients infected with HCV genotypes 1a (median $-4.8 \log_{10}$ IU/mL; IQR -5.1 to -4.1) and the 13 patients infected with genotype 1b ($-5.1 \log_{10}$ IU/mL; -5.4 to -4.5).

In the highest dose cohorts (F and G), five of eight treatment-naïve patients and two of eight null responders had HCV RNA concentrations below the limit of detection (<15 IU/mL) and seven of eight treatment-naïve patients and four of eight null responders had HCV RNA concentrations below the limit of quantification (43 IU/mL, table 1). Combining low-dose danoprevir with

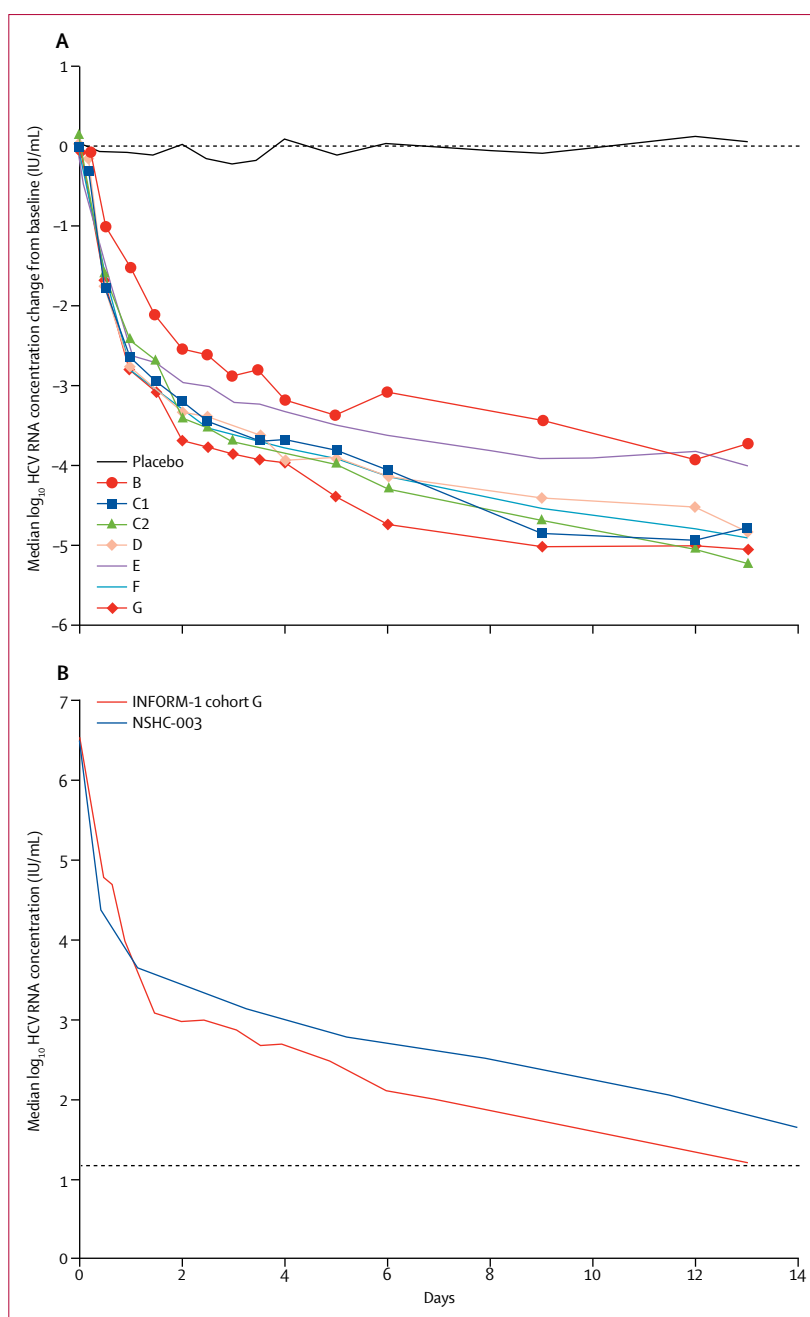


Figure 3: Viral kinetics

(A) Changes in hepatitis C virus (HCV) RNA concentrations over the duration of study drug treatment. (B) Changes in HCV RNA concentrations in treatment-naïve patients given danoprevir 900 mg twice daily co-administered with either RG7128 (cohort G) or with pegylated interferon alfa-2a and ribavirin (NSHC-003 [NS3/4a protease inhibitor of HCV][®]). Dashed line in B shows limit of detection.

RG7128 (cohort B) resulted in a reduction in HCV RNA concentration from baseline after 3 days of combination treatment that was 0.6 \log_{10} IU/mL greater than the sum of the reductions achieved with each drug as monotherapy for 3 days (cohort A, data not shown).

In a post-hoc analysis, the viral kinetics reported for cohort G were qualitatively similar to those recorded with

	RG7128		Danoprevir	
	C _{max} (µg/mL [%])	AUC _{0-24h} (µg/h/mL [%])	C _{max} (ng/mL [%])	AUC _{0-24h} (ng/h/mL [%])
A1 (day 3)	5.7 (17%)	27.7 (12%)
A1 (day 7)	5.1 (14%)	27.8 (13%)
A2 (day 3)	12.3 (96%)	20.9 (73%)
A2 (day 7)	15.4 (86%)	23.0 (64%)
E	10.2 (26%)	60.4 (24%)	331 (83%)	546 (69%)
F	9.3 (19%)	60.0 (19%)	2340 (69%)	3490 (77%)
G	10.2 (16%)	60.2 (17%)	774 (139%)	1050 (78%)

Data are mean (coefficient of variation). C_{max}=maximum observed plasma concentration. AUC_{0-24h}=area under the concentration-time curve from time zero to end of dosing interval.

Table 2: Selected pharmacokinetic results

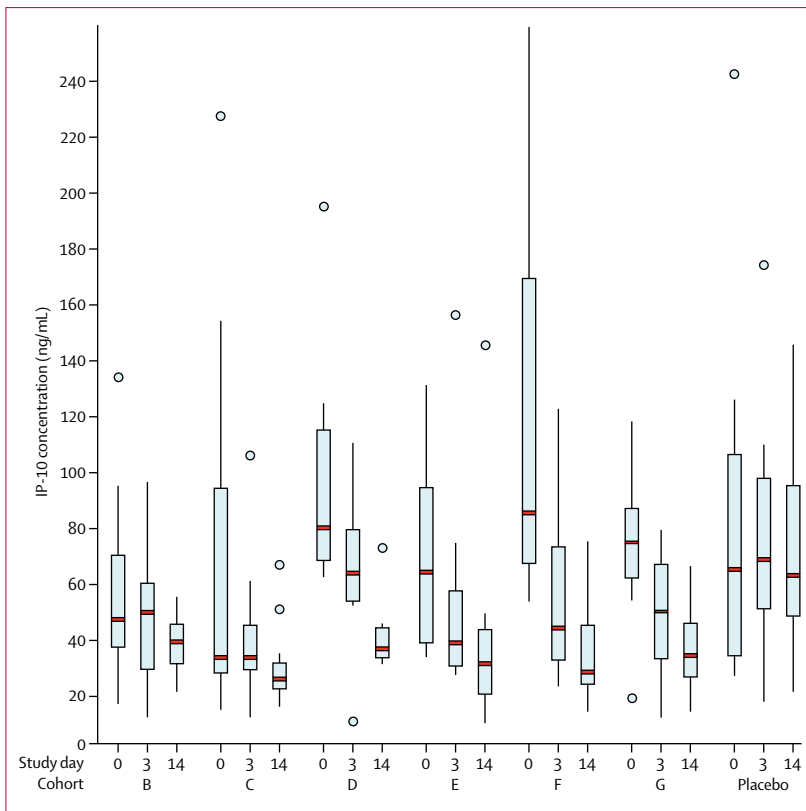


Figure 4: IP-10 concentrations
Boxed areas=IQR. Lines=lower (0-25%) and upper (75-100%) quartile ranges. Red lines=median. Circles=outliers.

previous data⁹ from a similar patient population who were given the same danoprevir regimen with standard of care (figure 3B).

No evidence of treatment-emergent resistance to either compound was identified during the study, and 72 of 73 patients in the treatment groups had a continuous decline in viral load, which was maintained throughout dosing. For cohort A, we identified the population and clonal sequence for NS3/4A (or NS3 protease domain) and NS5B for samples with a viral load of at least 1000 IU/mL (limit of amplification). In the ten patients

with a viral load of at least 1000 IU/mL, there was no evidence of drug resistance by population, clonal sequencing, or phenotypic analysis (data not shown).

One patient in group C1 had a 1.4 log₁₀ IU/mL increase in HCV RNA above nadir on day 13 of treatment and thus met the protocol definition of viral rebound. Sequence and phenotypic analyses were done on the first PCR-amplifiable sample for this patient after the HCV RNA rebound (day 15, 24 h after the last dose of study drugs). No resistance mutations to either drug were identified by population sequence, whereas clonal analysis identified one clone (1/88) harbouring a danoprevir-associated resistance mutation (F43S). Phenotypic characterisation studies showed full susceptibility to both drugs.

Two patients had pre-existing mutations associated with protease inhibitor resistance; one patient in the placebo group had a R155K mutation in the protease NS3 region and one patient in cohort D had a pre-existing D168E mutation. The baseline danoprevir EC₅₀ was 37 times higher in this patient than in patients with the wild-type virus. Despite this reduced susceptibility to danoprevir, HCV RNA concentrations decreased by 2.7 log₁₀ IU/mL from baseline to the end of treatment in this patient.

Table 2 summarises pharmacokinetic results for selected cohorts. For cohort A, on the basis of differing routes of elimination (RG7128 is excreted renally and danoprevir is metabolised in the liver), no substantial changes in pharmacokinetics were reported when either drug was given alone or in combination. The pharmacokinetics of RG7128 were similar to historical controls¹³ in both treatment-naïve (cohort G) and treatment-experienced populations (cohorts E and F). The pharmacokinetics of RG7128 did not seem to differ between treatment-naïve patients and null responders (cohorts F and G). For danoprevir, the pharmacokinetics in treatment-naïve patients (cohort G) were similar to previous data;¹⁴ however, the mean danoprevir exposure in the null-responder cohort were substantially higher than previously reported in treatment-naïve HCV-infected patients.¹⁴

Baseline plasma concentrations of IP-10 seemed to be higher in standard of care null responders (cohort F; figure 4). IP-10 concentrations decreased in all cohorts while they were receiving active direct-acting antiviral drug combination therapy and reduced in parallel with viral load.

The combination of RG7128 and danoprevir was well tolerated in all cohorts over the study treatment period (table 3). 80 of 87 patients had at least one adverse event during treatment up to day 14. The most common adverse event was headache, which occurred with similar frequency in the treatment groups (median 50%, range 13-88%) to the placebo group (57%). Other commonly reported adverse events included lethargy, rash, gastrointestinal disorders, and nausea. With the exception of one case of severe back pain in cohort E and one case of severe influenza-like illness in cohort G, all other

adverse events up to day 14 were of mild or moderate intensity. There were no treatment-related study withdrawals or dose reductions. One patient in group C2 withdrew from the in-house portion of study for personal reasons on day 10; however, this patient did complete long-term follow-up visits.

Two serious adverse events were reported in the follow-up standard of care period (one multiple-drug overdose and one ankle fracture); neither was judged by the investigator to be related to the study drugs. There were no deaths reported in the study, and there were no grade 3 or 4 changes in laboratory parameters.¹⁵ No clinically significant changes in vital signs or ECGs were reported in any of the dose groups (data not shown).

Discussion

The INFORM-1 study provides proof of concept for an oral approach to the treatment of HCV, in which a combination of direct-acting antiviral drugs is safely co-administered without pegylated interferon. INFORM-1 combines drugs that, at the time of the study, were still in phase 1 development, which differ from traditional development pathways, in which studies of treatment combinations are delayed until each drug is in the late stages of development or has been approved. Even though INFORM-1 is a short-term phase 1 study, the findings show that an interferon-free regimen can suppress viral replication. However, we did not show that interferon-free regimens can eradicate HCV (ie, produce a sustained virological response). Second, the study did not enrol patients with cirrhosis, which is a large and important group of patients at great need of treatment advances (especially safety).

Co-administration of RG7128 and danoprevir was generally safe and well tolerated. Although patients were masked to treatment allocation, because of the cohort dose-escalation design, patients were not fully masked to the doses of study treatments they might receive. The knowledge of treatment dose could have influenced tolerability in the higher dose cohorts. However, there was no evidence of poorer tolerability among patients who were randomly assigned to the higher dose cohorts. The combination treatment resulted in potent antiviral activity in standard of care treatment-naïve, treatment-experienced, and null-responder patients. The potent activity in null responders, some of whom had undetectable HCV RNA at 14 days, is particularly impressive because this population did not achieve a clinically significant reduction in viral load during previous treatment with standard of care.

The pronounced antiviral activity in patients who received the combination treatment was maintained throughout the duration of dosing, and there was no evidence of development of resistance, albeit only after 13 days of treatment in a small cohort of patients. Although one patient in group C1 met the definition of viral rebound, phenotypic characterisation showed full

	Placebo (n=14)	A1 (n=9)	A2 (n=8)	B (n=8)	C1 (n=8)	C2 (n=8)	D (n=8)	E (n=8)	F (n=8)	G (n=8)
Headache	8	5	3	2	4	7	4	4	6	1
Lethargy	2	0	0	1	1	1	0	1	0	2
Nausea	2	0	1	1	1	0	0	1	4	1
Constipation	1	0	0	1	1	2	0	1	1	1
Diarrhoea	1	0	1	1	1	0	0	2	2	0
Abdominal discomfort	1	1	0	0	0	0	0	3	0	2
Dry mouth	0	0	0	2	0	0	1	0	0	0
Abdominal distension	0	0	0	0	0	2	0	0	0	0
Rash	1	2	1	3	0	0	1	1	0	1
Skin irritation	2	0	0	1	0	0	0	0	2	0
Pain in extremity	1	0	0	0	0	0	1	0	2	0
Dry eye	1	0	0	1	0	2	0	0	0	0
Anxiety	0	0	0	0	0	0	0	2	0	1

Table 3: Adverse events during treatment occurring in at least two patients in any treatment group

susceptibility to both study drugs, suggesting that viral rebound was not caused by the development of resistance, and that the detection of one resistance mutation as a minority species after treatment could be a consequence of random mutagenesis during ongoing viral replication. These results lend support to further testing of this interferon-free treatment regimen in studies of longer duration to assess the possibility of achieving a sustained virological response.

In HCV, the rapid replication rate and low fidelity of the RNA polymerase results in pre-existing drug-resistant mutations and the rapid development of treatment resistance for some classes of direct-acting antiviral drugs.¹⁶ When given as monotherapy, most direct-acting antiviral drugs rapidly select HCV variants with reduced drug susceptibility, resulting in virological rebound and treatment failure.¹⁷ In patients receiving the HCV protease inhibitor telaprevir as monotherapy, resistant mutations can be detected as early as day 4.¹⁷ The selection of mutations that cause an increase in the EC₅₀ against HCV nucleoside polymerase inhibitors, such as RG7128, in vitro is more difficult than with other classes of direct-acting antiviral drugs.

Nucleoside resistance in vivo has not been reported so far, probably because of decreased viability of the in-vitro-selected resistance mutation.¹⁸ Development of a treatment regimen of direct-acting antiviral drugs with one or more drugs with a high resistance barrier will be crucial to the success of this interferon-free treatment approach. The combination of RG7128 with direct-acting antiviral drugs that have a lower barrier to resistance, such as protease inhibitors, reduces drug resistance in vitro.¹⁹ The INFORM-1 study confirms this benefit in vivo, because the combination of RG7128 and danoprevir prevented resistance-associated virological breakthrough that has been reported with monotherapy with a protease inhibitor, including danoprevir.²⁰

HCV viral kinetics are biphasic, with the first rapid phase representing clearance of free virions, and the more shallow second phase slope representing the loss of HCV-infected hepatocytes.^{21,22} The viral kinetic profile during combination treatment with danoprevir and RG7128 is similar to that recorded previously during treatment with danoprevir plus standard of care.⁹ This similarity between the kinetic profiles suggests that treatment of HCV without the use of interferon might be possible. On the basis of the phase 2 viral decline reported in this study and the estimated total body viral burden of 10¹¹, between 8 and 12 weeks of combination direct-acting antiviral drug therapy might be sufficient to cure patients with HCV, provided there is no emergence of antiviral resistance. However, the number and type of direct-acting antiviral drugs needed for success, along with the optimal duration of treatment, will need to be tested in longer-term clinical studies.

IP-10 is a chemokine involved in lymphocyte chemotaxis.²³ Higher concentrations of IP-10 are presumed to be indicative of greater endogenous activation of host interferon pathways.^{24,25} Previous studies have shown that concentrations of serum and intrahepatic IP-10 before treatment are inversely associated with the likelihood of response to standard of care; null-responder cohorts consistently had higher IP-10 concentrations than did patients who achieved a sustained virological response.^{26,27} The reduction of IP-10 in these patients suggests that rapid viral suppression with interferon-free direct-acting antiviral drug therapy effectively reduces activation of endogenous interferon.

The pharmacokinetics of antiretrovirals and anti-HCV drugs (including danoprevir), which usually rely on cytochrome P450 3A4 metabolism, differ between healthy control individuals and patients infected with HCV who are treatment naive.^{28–33} However, the higher danoprevir exposure between null responders and patients who are treatment naive when given the same dose was unexpected. This difference might represent a continuum of differences in cytochrome P450 3A4 metabolic activity between healthy volunteers, patients who are treatment naive, and interferon null responders. Despite the higher exposure to danoprevir in null responders, the viral kinetic profiles and the number of patients reaching undetectable concentrations of HCV RNA might be lower in these patients than in those who are treatment naive. Therefore, the possibility that interferon non-responders might also have a reduced antiviral response to direct-acting antiviral drugs cannot be ruled out.

The combination of RG7128 and danoprevir should be further developed and might be a viable interferon-free, all-oral regimen for patients with chronic HCV infection. Promising results have been published for use of direct-acting antivirals as monotherapy. However, treatment of patients with an all-oral, interferon-free dual direct-acting antiviral drug combination, as assessed in our study,

marks a major shift in the future management of HCV infection and the biggest development in treatment of the disease for the past two decades.

Contributors

EJG designed the study, was lead recruiter, interpreted and presented the data, and wrote the report. SKR collected, interpreted, and analysed the data and wrote the report. CAMS designed the study, recruited patients, reviewed and interpreted the data, and wrote the report. PWA recruited patients, interpreted the data, and wrote the report. BR, DI, PNM, IN, TC, and UL designed the study, collected and interpreted data, and wrote the report. RE designed the study, analysed data, and reviewed the report. LB collected data and critically reviewed the report. MMB and PFS designed the study, analysed and interpreted the data, and wrote the report. WB designed the study, analysed and interpreted the data, and wrote the report. ML designed the study and analysed the data. NSS designed the study, analysed data, and was medical lead and monitor for the study.

Conflicts of interest

EJG was the recipient of the Roche grant for this clinical trial, and has received consultancy fees and support for travel to meetings from Roche, consultancy fees for acting as an advisory board member for Novartis and Merck, and payment for lectures including service on speaker bureaus for Novartis and GlaxoSmithKline. SKR is a member of a Roche advisory board (not related to this study) and receives consultancy fees from Roche. PWA received travel expenses to attend an Australian Roche advisory board meeting from Roche. BR received a fee for monitoring patients as required during a clinical trial and received financial support for travel to meetings for this study from Roche. RE, DI, PNM, LB, IN, TC, UL, MMB, ML, NSS, and PFS are Roche employees. UL, ML, NSS, and PFS receive stock options from Roche. WB is an InterMune employee and receives stock options from InterMune. CAMS declares that she has no conflicts of interest.

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