COMBINATION OF HCV NS3 PROTEASE INHIBITOR WITH INTERFERON AND RIBAVIRIN

This application claims the benefit of prior U.S. Provisional Applications: 61/097,753 filed September 17, 2008, 61/109,033 filed October 28, 2008 and 61/171,935 filed April 23, 2009.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to therapeutic combinations comprising Compound (1) as herein described, an interferon alfa and ribavirin. The present invention also relates to methods of using such therapeutic combinations for treating HCV infection or alleviating one or more symptoms thereof in a patient. The present invention also provides kits comprising the therapeutic combinations of the present invention.

BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) infection is a global human health problem with approximately 150,000 new reported cases each year in the United States alone. HCV is a single stranded RNA virus, which is the etiological agent identified in most cases of non-A, non-B post-transfusion and post-transplant hepatitis and is a common cause of acute sporadic hepatitis. It is estimated that more than 50% of patients infected with HCV become chronically infected and 20% of those develop cirrhosis of the liver within 20 years.

Several types of interferons, in particular, alfa-interferons are approved for the treatment of chronic HCV, e.g., interferon-alfa-2a (ROFERON®-A), interferon-alfa-2b (INTRON®-A), consensus interferon (INFERGEN®), as well as pegylated forms of these and other interferons like pegylated interferon alfa-2a (PEGASYS®) and pegylated interferon alfa-2b (PEG-INTRON®). Most patients are unresponsive to interferon-alfa treatment, however, and among the responders, there is a high recurrence rate within 6 months after cessation of treatment (Liang et al., *J. Med. Virol.* 40:69, 1993).

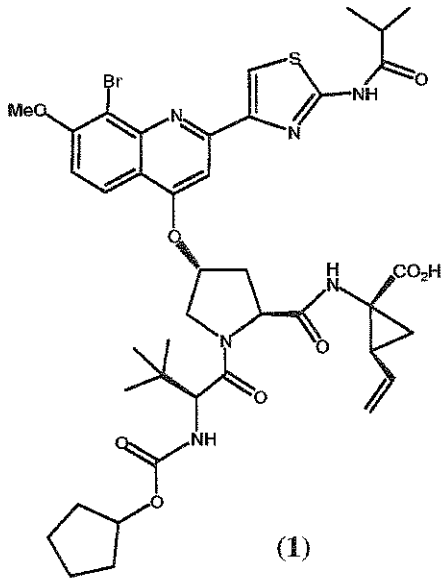
Ribavirin, a guanosine analog with broad spectrum activity against many RNA and DNA viruses, has been shown in clinical trials to be effective against chronic HCV infection

when used in combination with interferon-alfas (see, e.g., Poynard et al., *Lancet* 352:1426-1432, 1998; Reichard et al., *Lancet* 351:83-87, 1998), and this combination therapy has been approved for the treatment of HCV: REBETRON® (interferon alfa-2b plus ribavirin, Schering-Plough); PEGASYS®RBV® (pegylated interferon alfa-2a plus ribavirin combination therapy, Roche); see also Manns et al, *Lancet* 358:958-965 (2001) and Fried et al., 2002, *N. Engl. J. Med.* 347:975-982. However, even with this combination therapy the virologic response rate is still at or below 50%.

Furthermore, there are significant side-effects typically associated with such therapies. Ribavirin suffers from disadvantages that include teratogenic activity, interference with sperm development, haemolysis, fatigue, headache, insomnia, nausea and/or anorexia. Interferon alfa, with or without ribavirin, is associated with many side effects. During treatment, patients must be monitored carefully for flu-like symptoms, depression, rashes and abnormal blood counts. Patients treated with interferon alfa-2b plus ribavirin should not have complications of serious liver dysfunction and such subjects are only considered for treatment of hepatitis C in carefully monitored studies.

Certain interferon-containing combination therapies for treating HCV infection are also disclosed in the following U.S. Patent Application Publications: US 2005/0112093; US 2005/0129659; and US 2008/0138316.

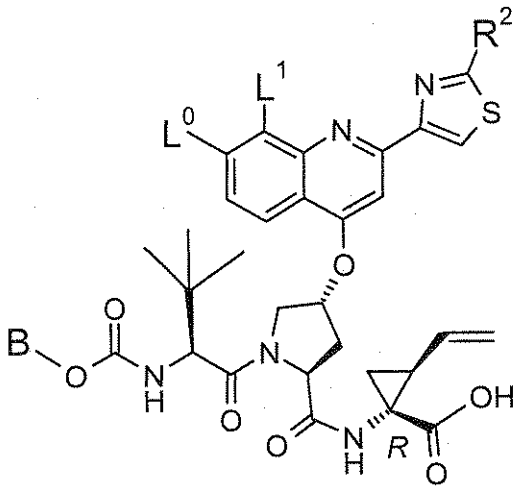
The following Compound (1):

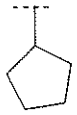
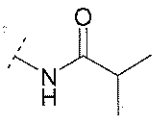


(1)

is known as a selective and potent inhibitor of the HCV NS3 serine protease and useful in the treatment of HCV infection. Compound (1) falls within the scope of the acyclic peptide series of HCV inhibitors disclosed in U.S. Patents 6,323,180, 7,514,557 and 7,585,845. Compound (1) is disclosed specifically as Compound # 1055 in U.S. Patent 7,585,845, and as Compound # 1008 in U.S. Patent 7,514,557. Compound (1), and pharmaceutical formulations thereof, can be prepared according to the general procedures found in the above-cited references, all of which are herein incorporated by reference in their entirety. Preferred forms of Compound (1) include the crystalline forms, in particular the crystalline sodium salt form, which can be prepared as described in the examples section herein.

Compound (1) may also be known by the following alternate depiction of its chemical structure, which is equivalent to the above-described structure:



wherein **B** is  ; $\mathbf{L^0}$ is MeO-; $\mathbf{L^1}$ is Br; and $\mathbf{R^2}$ is  .

Although Compound (1) has been found generally effective in the reduction of viral load and the treatment of HCV infection, a certain amount of viral resistance with resulting viral rebound has been seen. For example, we have observed with Compound (1) given once-daily to treatment-naïve patients as monotherapy for 14 days a strong and very rapid antiviral affect followed by a certain amount of resistance formation after 5-6 days.

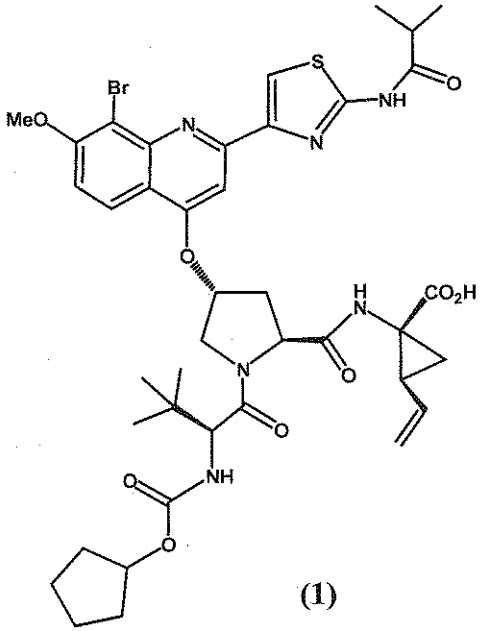
Therefore, there is a continuing need in the field for alternative therapies for the treatment and prevention of HCV infection.

BRIEF SUMMARY

The present disclosure provides a method of treating HCV infection or alleviating one or more symptoms thereof in a patient comprising the step of administering to the patient a therapeutic combination comprising a Compound (1) as defined herein, or a pharmaceutically acceptable salt thereof, together with an interferon alfa and ribavirin, as defined herein. The three actives of the combination can be administered simultaneously or separately, as part of a regimen.

In a first aspect, there is provided the use of the combination of:

(a) a compound of the following formula (1) or a pharmaceutically acceptable salt thereof:



(1)

;

(b) interferon alpha; and

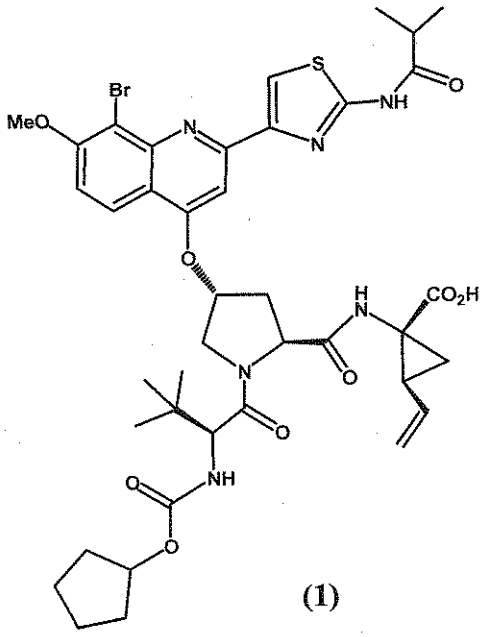
(c) ribavirin

for the manufacture of a medicament for the treatment of hepatitis C viral (HCV) genotype 1 infection or alleviating one or more symptoms thereof.

The present disclosure further provides for a kit comprising a first pharmaceutical composition comprising a Compound (1) or a pharmaceutically acceptable salt thereof; a second pharmaceutical composition comprising an interferon alfa; and a third pharmaceutical composition comprising ribavirin.

In a second aspect, there is provided a kit comprising:

(a) a first pharmaceutical composition comprising a compound of the following formula (1) or a pharmaceutically acceptable salt thereof:



(1)

;

(b) a second pharmaceutical composition comprising interferon alpha; and

(c) a third pharmaceutical composition comprising ribavirin,

wherein said kit is for use for the treatment of hepatitis C viral (HCV) genotype 1 infection or for alleviating one or more symptoms thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the average change in HCV viral load in four patient dose groups for treatment-naïve patients with chronic HCV genotype-1 infection treated with Compound (1) sodium salt as monotherapy for 14 days, followed by combination therapy with Compound (1) sodium salt, pegylated interferon alfa-2a and ribavirin for an additional 14 days.

Figure 2 depicts the average change in HCV viral load in three patient dose groups for treatment-experienced patients with chronic HCV genotype-1 infection treated with Compound (1) sodium salt, pegylated interferon alfa-2a and ribavirin as combination therapy for 28 days.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Compound (1)" is as defined above.

"Interferon" means a member of a family of highly homologous species-specific proteins that inhibit viral replication and cellular proliferation and modulate immune response. Human interferons are grouped into three classes based on their cellular origin and antigenicity: α-interferon (leukocytes), β-interferon (fibroblasts) and γ-interferon (B cells). Recombinant forms of each group have been developed and are commercially available. Subtypes in each group are based on antigenic/structural characteristics. At least 24 interferon alfas (grouped into subtypes A through H) having distinct amino acid sequences have been identified by isolating and sequencing DNA encoding these peptides. The terms "α -interferon", "alfa-interferon" and "interferon alfa" are used interchangeably in this

application to describe members of this group. Both naturally occurring and recombinant alfa-interferons, including consensus interferon, may be used in the practice of the invention.

Suitable interferon-alfas for the present invention include, but are not limited to, recombinant interferon alfa-2b such as INTRON®-A interferon and VIRAFERON®; recombinant interferon alfa-2a such as ROFERON® interferon; recombinant interferon alfa-2c such as BEROFOR® alfa 2 interferon; interferon alfa-n1, a purified blend of natural alfa interferons such as SUMIFERON® or WELLFERON® interferon alfa-n1 (INS); or a consensus alfa interferon such as those described in U.S. Pat. Nos. 4,897,471 and 4,695,623; or interferon alfa-n3, a mixture of natural alfa interferons such as ALFERON®. The use of interferon alfa-2a or alfa 2b is preferred. The manufacture of interferon alfa 2b is described in U.S. Pat. No. 4,530,901.

The term "interferon alfa" is further intended to include those "pegylated" analogs meaning polyethylene glycol modified conjugates of interferon alfa, preferably interferon alfa-2a and -2b. The preferred polyethylene-glycol-interferon alfa-2b conjugate is PEG_{12000} - interferon alfa 2b. The term "PEG_{12000}-IFN alfa" as used herein means conjugates such as are prepared according to the methods of International Application No. WO 95/13090 and containing urethane linkages between the interferon alfa-2a or -2b amino groups and polyethylene glycol having an average molecular weight of 12000.

The preferred PEG_{12000}-interferon alfa-2b is prepared by attaching a PEG polymer to the epsilon amino group of a lysine residue in the IFN alfa-2b molecule. A single PEG_{12000} molecule is conjugated to free amino groups on an IFN alfa-2b molecule via a urethane linkage. This conjugate is characterized by the molecular weight of PEG_{12000} attached. The PEG_{12000}-IFN alfa-2b conjugate is formulated as a lyophilized powder for injection. The objective of conjugation of IFN alfa with PEG is to improve the delivery of the protein by significantly prolonging its plasma half-life, and thereby provide protracted activity of IFN alfa.

Especially preferred conjugates of interferon alfa that may be used in the present invention are pegylated alfa-interferons, e.g., pegylated interferon alfa-2a, pegylated interferon alfa-2b, pegylated consensus interferon or pegylated purified interferon alfa product. Pegylated interferon alfa-2a is described, e.g., in European Patent No. EP 0 593 868 and commercially-available, e.g., under the tradename PEGASYS® (Hoffmann-La Roche). Pegylated interferon alfa-2b is described, e.g., in U.S. Patent No. 5,908,621 and WO 98/48840 and commercially-available, e.g., under the tradename PEG-INTRON® A (Schering Plough). Pegylated consensus interferon is described in WO 96/11953. The preferred pegylated alfa interferons are pegylated interferon alfa-2a and pegylated interferon alfa-2b. Also preferred is pegylated consensus interferon.

The term "interferon alfa" further includes other interferon alfa conjugates that can be prepared by coupling an interferon alfa to a water-soluble polymer. A non-limiting list of such polymers includes other polyalkylene oxide homopolymers such as polyethylene glycol (PEG), polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof. As an alternative to polyalkylene oxide-based polymers, effectively non-antigenic materials such as dextran, polyvinylpyrrolidones, polyacrylamides, polyvinyl alcohols, carbohydrate-based polymers and the like can be used. Such interferon alfa-polymer conjugates are described in U.S. Pat. No. 4,766,106, U.S. Pat. No. 4,917,888, European Patent Application No. 0 236 987, European Patent Application Nos. 0510 356, 0 593 868 and 0 809 996 (pegylated interferon alfa-2a) and International Publication No. WO 95/13090.

The term "interferon alfa" further includes fusion proteins of an interferon alfa, for example fusion proteins of interferon-α-2a, interferon- α-2b, consensus interferon or purified interferon-α product, each of which is fused with another protein. Certain preferred fusion proteins comprise an interferon (e.g., interferon-α-2b) and an albumin as described in U.S. Patent 6,972,322 and international publications WO2005/003296 and WO2005/077042. A preferred interferon conjugated to a human albumin is ALBUFERON® which is a longer-acting form of interferon alfa created using albumin

fusion technology. ALBUFERON® results from the genetic fusion of human albumin and interferon alpha. Also included are consensus interferons, such as INFERGEN®.

The term "pharmaceutically acceptable salt" means a salt of a Compound of formula (1) which is, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, generally water or oil-soluble or dispersible, and effective for their intended use.

The term includes pharmaceutically-acceptable acid addition salts and pharmaceutically-acceptable base addition salts. Lists of suitable salts are found in, e.g., S. M. Birge et al., *J. Pharm. Sci.*, 1977, 66, pp. 1-19.

The term "pharmaceutically-acceptable acid addition salt" means those salts which retain the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, sulfamic acid, nitric acid, phosphoric acid, and the like, and organic acids such as acetic acid, trifluoroacetic acid, adipic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, butyric acid, camphoric acid, camphorsulfonic acid, cinnamic acid, citric acid, digluconic acid, ethanesulfonic acid, glutamic acid, glycolic acid, glycerophosphoric acid, hemisulfic acid, hexanoic acid, formic acid, fumaric acid, 2-hydroxyethane-sulfonic acid (isethionic acid), lactic acid, hydroxymaleic acid, malic acid, malonic acid, mandelic acid, mesitylenesulfonic acid, methanesulfonic acid, naphthalenesulfonic acid, nicotinic acid, 2-naphthalenesulfonic acid, oxalic acid, pamoic acid, pectinic acid, phenylacetic acid, 3-phenylpropionic acid, pivalic acid, propionic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, sulfanilic acid, tartaric acid, p-toluenesulfonic acid, undecanoic acid, and the like.

The term "pharmaceutically-acceptable base addition salt" means those salts which retain the biological effectiveness and properties of the free acids and which are not biologically or otherwise undesirable, formed with inorganic bases such as ammonia or hydroxide,

carbonate, or bicarbonate of ammonium or a metal cation such as sodium, potassium, lithium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically-accepta- ble organic nontoxic bases include salts of primary, secondary, and tertiary amines, quaternary amine compounds, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion-exchange resins, such as methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine, triethylamine, isopropylamine, tripropylamine, tributylamine, ethanolamine, diethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, tetramethylammonium compounds, tetraethylammonium compounds, pyridine, N,N-dimethylaniline, N-methylpiperidine, N-methylmorpholine, dicyclohexylamine, dibenzylamine, N,N-dibenzylphenethylamine, 1-ephenamine, N,N'-dibenzylethylenediamine, polyamine resins, and the like. Particularly preferred organic nontoxic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline, and caffeine.

"Ribavirin" refers to 1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide, available from ICN Pharmaceuticals, Inc., Costa Mesa, Calif. and is described in the Merck Index, compound No. 8199, Eleventh Edition. Its manufacture and formulation is described in U.S. Pat. No. 4,211,771. Preferred marketed ribavirin products include REBETOL® and COPEGUS®. The term further includes derivatives or analogs thereof, such as those described in U.S. Pat. Nos. 6,063,772, 6,403,564 and 6,277,830. For example, derivatives or analogs include modified ribavirins such as 5'-amino esters, ICN Pharmaceutical's L-enantiomer of ribavirin (ICN 17261), 2'-deoxy derivatives of ribavirin and 3-carboxamidine derivatives of ribavirin, viramidine (previously known as ribamidine) and the like.

The term "therapeutic combination" as used herein means a combination of one or more active drug substances, i.e., compounds having a therapeutic utility. Typically, each such

compound in the therapeutic combinations of the present invention will be present in a pharmaceutical composition comprising that compound and a pharmaceutically acceptable carrier. The compounds in a therapeutic combination of the present invention may be administered simultaneously or separately, as part of a regimen.

Embodiments of the Invention

According to a general embodiment, the present invention provides for a method of treating HCV infection or alleviating one or more symptoms thereof in a patient comprising the step of administering to the patient a therapeutic combination comprising a Compound (1) as defined herein, or a pharmaceutically acceptable salt thereof, together with an interferon alfa and ribavirin. In another embodiment, the present invention teaches the use of a Compound (1) as defined herein, or a pharmaceutically acceptable salt thereof, an interferon alfa, and ribavirin for the preparation of a pharmaceutical kit to treat a hepatitis C viral (HCV) infection or alleviating one or more symptoms thereof in a patient. Although this combination therapy is expected to be effective against all HCV genotypes, it has been demonstrated to be particularly effective in treating HCV genotype 1 infection, including subgenotypes 1a and 1b.

The patient population to be treated with the combination therapy of the present invention can be further classified into "treatment-naïve" patients, i.e., those patient who have not received any prior treatment for HCV infection and "treatment experienced" patients, i.e, those patients who have undergone prior treatment for HCV. Either of these classes of patients may be treated with the combination therapy of the present invention. A particular class of patients that are preferably treated are those treatment experienced patients that have undergone prior interferon plus ribavirin therapy but are non-responsive to said therapy (herein "non-responders"). Such non-responders include three distinct groups of patients: (1) those who experienced $< 1x \log_{10}$ maximum reduction in HCV RNA levels during treatment with interferon plus ribavirin ("null responders"), (2) those who experienced $\geq 1x \log_{10}$ maximum reduction in HCV RNA levels during treatment with

interferon plus ribavirin but never achieve HCV RNA levels below level of detection ("partial responders"), and (3) those who achieved a virologic response with and during interferon plus ribavirin therapy but had a viral load rebound either during treatment (other than due to patient non-compliance) or after treatment has completed ("relapser"). As will be detailed below, particularly surprising results have been obtained with the treatment of certain non-responder patients using the combination therapy regimen of the present invention.

According to an alternative embodiment, the present invention provides a method of reducing HCV-RNA levels in a patient in need thereof, comprising the step of administering to said patient a therapeutic combination according to the present invention. Preferably, the method of the present invention reduces the HCV-RNA levels in a patient to a less than detectable level. The terms "less than detectable level" and "below level of detection" are used interchangeably herein and have the same meaning as being less than a detectable level of HCV RNA. A detectable level of HCV RNA as used in the present invention means at least 50 International Units (IU) per ml of serum of a patient as measured by quantitative, multi-cycle reverse transcriptase PCR methodology according to the WHO international standard (Saladanha J, Lelie N and Heath A, Establishment of the first international standard for nucleic acid amplification technology (NAT) assays for HCV RNA. WHO Collaborative Study Group. Vox Sang 76:149-158, 1999). Such methods are well known in the art. In a preferred embodiment, the method of the present invention reduces the HCV-RNA levels in a patient to less than 25 IU per ml of serum, even more preferably to less than 10 IU per ml of serum.

The usual duration of the treatment for standard interferon plus ribavirin therapy is at least 48 weeks for HCV genotype 1 infection, and at least 24 weeks for HCV genotypes 2 and 3. However, with the addition of Compound (1), or a pharmaceutically acceptable salt thereof, in the triple combination therapy of the present invention, it may be possible to have a much shorter duration of treatment. With the triple combination therapy of the present invention the contemplated durations of treatment include at least 4 weeks, preferably at least 12 weeks, e.g., from about 12 weeks to about 24 weeks, although

treatment up to and even beyond 48 weeks is possible as well. Thus, further embodiments include treatment for at least 24 weeks and for at least 48 weeks. The time period for different HCV genotypes, e.g. HCV genotypes 2,3 or 4 is expected to be similar. Also contemplated is an initial treatment regimen with the triple combination therapy of the present invention, followed by a continuation of only the interferon plus ribavirin double combination therapy. Thus, possible scenarios for the initial triple and then double combination therapy include, for example: (1) 4 weeks of the triple combination therapy, followed by 20 to 44 weeks of the interferon plus ribavirin only therapy; (2) 12 weeks of the triple combination therapy, followed by 12 to 36 weeks of the interferon plus ribavirin only therapy; and (3) 24 weeks of the triple combination therapy, followed by 12 to 24 weeks of the interferon plus ribavirin only therapy.

The first component of the therapeutic combination, namely, Compound (1) or a pharmaceutically acceptable salt thereof is comprised in a composition. Such a composition comprises Compound (1), or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable adjuvant or carrier. Typical pharmaceutical compositions that may be used for Compound (1), or a pharmaceutically acceptable salt thereof, are as described in U.S. Patent 7,585,845.

In general, the Compound (1) or a pharmaceutically acceptable salt thereof may be administered at a dosage of at least 40 mg/day (in single or divided doses). Additional embodiments for dosage amounts and ranges may include (in single or divided doses):

(a) at least 48 mg/day
(b) at least 100 mg/day
(c) at least 120 mg/day
(d) at least 200 mg/day
(e) at least 240 mg/day
(f) at least 360 mg/day
(g) at least 480 mg/day
(h) from about 40 mg/day to about 480 mg/day
(i) from about 48 mg/day to about 240 mg/day

(j) from about 100 mg/day to about 300 mg/day

(k) from about 120 mg/day to about 300 mg/day

(l) from about 120 mg/day to about 240 mg/day

(m) from about 240 mg/day to about 480 mg/day

(n) about 48 mg/day

(o) about 120 mg/day

(p) about 240 mg/day

(q) about 360 mg/day

(r) about 480 mg/day

Although Compound (1) or a pharmaceutically acceptable salt thereof may be administered in single or divided daily doses, once a day administration of the daily dose is preferred. As the skilled artisan will appreciate, however, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the infection, the patient's disposition to the infection and the judgment of the treating physician. In general, the compound is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious side effects.

The second component of the therapeutic combination, namely interferon-alfa, is comprised in a pharmaceutical composition. Typically, such compositions are injectible formulations comprising interferon-alfa and a pharmaceutically acceptable adjuvant or carrier and are well known in the art, including in a number of marketed interferon-alfa formulations. See, e.g., the various marketed interferon-alfa products and various patent and other literature related to interferon-alfa cited hereinabove.

The types of interferon-alfas that may be used in the combination are as outlined hereinabove in the definitions section. In one preferred embodiment, the interferon alfa is a pegylated interferon alfa. In a further embodiment, the interferon alfa is a pegylated

interferon alfa-2a or pegylated interferon alfa-2b. In a particularly preferred embodiment, the interferon alfa is PEGASYS® or PEG-INTRON®.

When using known, marketed interferon alfa products, such products may be administered at their labeled dosage levels indicated for interferon plus ribavirin combination therapy for the treatment of HCV infection. Of course, with the triple combination therapy of the present invention it may be possible to use a lower dosage of interferon alfa, e.g., significantly lower than is used the current standard interferon plus ribavirin therapy, while delivering the same or better efficacy than the current standard therapy with less side-effects usually associated with such therapy.

In one embodiment, the interferon alfa may be administered parenterally one to three times per week, preferably once or twice a week. With respect to pegylated interferon alfas, these are typically administered once per week and the total weekly dose ranges, e.g., from about 0.5 μg /kg/week to about 2 μg /kg/week in case of pegylated interferon alfa-2b, and with respect to pegylated interferon alfa-2a the dosage is independent from the body weight of the host and is typically about 90 to 200 μg/week, more preferably about 160 to about 200 μg/week. In combination with ribavirin, a standard dosage of pegylated interferon alfa-2b is about 1.5 μg/kg/week and a standard dosage of pegylated interferon alfa-2a is about 180 μg/week, together with about 600-1200 mg/day, in particular, 800-1200 mg/day of oral ribavirin.

According to further embodiments, the pegylated interferon alfa-2b may be administered at dosages of:

(a) about 0.5 μg/kg/week to about 2 μg/kg/week;

(b) about 1 μg/kg/week to about 2 μg/kg/week;

(c) about 1.5 μg/kg/week to about 2 μg/kg/week;

(d) about 1.5 μg/kg/week

According to further embodiments, the pegylated interferon alfa-2a may be administered at dosages of:

(a) about 90 to about 200 µg/week;

(b) about 160 to about 200 µg/week;

(b) about 180 µg/week

The third component of the therapeutic combination, namely ribavirin, is comprised in a pharmaceutical composition. Typically, such compositions comprise ribavirin and a pharmaceutically acceptable adjuvant or carrier and are well known in the art, including in a number of marketed ribavirin formulations. Formulations comprising ribavirin are also disclosed, e.g., in US Patent 4,211,771.

The types of ribavirin that may be used in the combination are as outlined hereinabove in the definitions section. In one preferred embodiment, the ribavirin is either REBETOL® or COPEGUS® and they may be administered at their labeled dosage levels indicated for interferon plus ribavirin combination therapy for the treatment of HCV infection. Of course, with the triple combination therapy of the present invention it may be possible to use a lower dosage of ribavirin, e.g., lower than is used the current standard interferon plus ribavirin therapy, while delivering the same or better efficacy than the current standard therapy with less side-effects usually associated with such therapy.

According to various embodiments, the ribavirin may be administered at dosages of (in single or divided doses):

(a) between 400 mg/day to about 1200 mg/day;

(b) between about 800 mg/day to about 1200 mg/day;

(c) between about 1000 mg/day to about 1200 mg/day;

(d) about 1000 mg/day

(e) about 1200 mg/day

(f) between about 300 mg/day to about 800 mg/day

(g) between about 300 mg/day to about 700 mg/day

(h) between 500 mg/day to about 700 mg/day

(i) between 400 mg/day to about 600 mg/day

(j) about 400 mg/day

(k) about 600 mg/day

(l) about 800 mg/day

According to one embodiment, the ribavirin composition comprises ribavirin in a formulation suitable for dosing once a day, twice daily, thrice daily, four times a day, five times a day, or six times a day. For example, if a therapeutic combination comprises about 1000 mg/day dosage of ribavirin, and a dosing of five times a day is desired, then the therapeutic combination will comprise ribavirin in a formulation, e.g., a tablet, containing, e.g., about 200 mg of ribavirin.

With respect to the Compound (1) or a pharmaceutically acceptable salt thereof plus interferon alfa plus ribavirin triple combination therapy of the present invention, the present invention contemplates and includes all combinations of the various preferred embodiments and sub-embodiments as set forth hereinabove.

For example, in one embodiment the present invention contemplates a method of treating hepatitis C viral (HCV) infection or alleviating one or more symptoms thereof in a patient comprising the step of administering to the patient a therapeutic combination comprising:

(a) Compound (1) or a pharmaceutically acceptable salt thereof at a dosage between about 48 mg per day and about 480 mg per day;

(b) pegylated interferon alfa -2a at a dosage of about 160 to about 200 µg/week or pegylated interferon alfa -2b at a dosage of about 0.5 µg/kg/week to about 2 µg/kg/week; and

(c) ribavirin at a dosage of between about 400 mg/day to about 1200 mg/day.

In another embodiment the present invention contemplates a method of treating hepatitis C viral (HCV) infection or alleviating one or more symptoms thereof in a patient comprising the step of administering to the patient a therapeutic combination comprising:

(a) Compound (1) or a pharmaceutically acceptable salt thereof at a dosage between about 48 mg per day and about 480 mg per day;
(b) pegylated interferon alfa -2a at a dosage of about 180 µg/week; and
(c) ribavirin at a dosage of between about 1000 mg/day to about 1200 mg/day.

In another embodiment the present invention contemplates a method of treating hepatitis C viral (HCV) infection or alleviating one or more symptoms thereof in a patient comprising the step of administering to the patient a therapeutic combination comprising:

(a) Compound (1) or a pharmaceutically acceptable salt thereof at a dosage between about 48 mg per day and about 480 mg per day;
(b) pegylated interferon alfa -2b at a dosage of about 1.5 µg/kg/week; and
(c) ribavirin at a dosage of about 800 mg/day.

Further embodiments include any of the above-mentioned embodiments, and where:

(a) the HCV infection is genotype 1 and the patient is a treatment-naïve patient; or
(b) the HCV infection is genotype 1 and the patient is a treatment-experienced patient who is non-responsive to a combination therapy of interferon plus ribavirin.

Further embodiments include any of the above-mentioned embodiments, and where the Compound (1) or a pharmaceutically acceptable salt thereof is administered once a day, the interferon alpha is administered once a week and the ribavirin is administered twice a day.

According to a another embodiment, the therapeutic regimen of the present invention comprises administering to a patient for at least about 4 weeks, more preferably either at

least about 12 weeks or at least about 24 weeks:

(i) a therapeutically effective amount of Compound (1) or a pharmaceutically acceptable salt thereof once a day;
(ii) a therapeutically effective amount of interferon alpha once a week; and
(iii) a therapeutically effective amount of ribavirin twice a day.

According to another embodiment, the present invention provides kits for use in treating HCV infection in a patient. The kits of the present invention may comprise any of the therapeutic combinations of the present invention. The kits further comprise instructions for utilizing the therapeutic combinations. The kits may be tailored to the needs of classes or types of patients or other clinically relevant factors such as age, body weight, concomitant diseases/conditions, severity and stage of HCV infection, responsiveness or non-responsiveness to prior treatments, propensity for side effects, etc.

According to another embodiment, the present invention provides a kit comprising:
(a) a first pharmaceutical composition comprising a Compound (1) or a pharmaceutically acceptable salt thereof:
(b) a second pharmaceutical composition comprising interferon alfa;
(c) a third pharmaceutical composition comprising ribavirin; and.
(d) instructions for utilizing the above compositions.

Additionally, surprising results have been seen in the suppression of HCV viral resistance during the combination therapy treatment contemplated by the present invention. Dosing of Compound (1) (sodium salt) as a monotherapy resulted in a rapid viral load rebound in the first 14 days during treatment in a majority of the patients from all dose groups of treatment-naïve patients in whom viral load reductions were observed. In contrast, among the 19 treatment experienced patients who received Compound (1) (sodium salt) once daily (qd) doses of 48 mg (n=6), 120 mg (n=7), or 240 mg (n=6) in a combination with pegylated interferon alfa 2a and ribavirin (PegIFN/RBV) for 28 days, virologic rebound during the 28 day treatment was only observed in 2/6 patients in the 48 mg dose group,

and in 1/7 patients in the 120 mg dose group. Notably, no rebound was seen during the first 28 days in the treatment-experienced dose group that received Compound (1) (sodium salt) 240 mg qd treatment in combination with PegIFN/RBV.

Accordingly, in an additional embodiment, there is limited or no emergence of viral resistance during the combination therapy of the present invention. In a more specific embodiment, there is limited or no emergence of HCV variants that encode HCV NS3 protease amino acid substitutions at one or more of R155 and/or D168 and/or A156 during the combination therapy of the present invention.

Further embodiments include any of the above-mentioned embodiments, and where either:

(a) the HCV infection is genotype 1a and the patient is a treatment-naïve patient; or
(b) the HCV infection is genotype 1a and the patient is a treatment-experienced patient who is non-responsive to a combination therapy of interferon plus ribavirin;

and wherein there is limited or no emergence of variants that encode substitutions at NS3 protease amino acid R155 during the combination therapy of the present invention.

Further embodiments include any of the above-mentioned embodiments, and where either:

(a) the HCV infection is genotype 1b and the patient is a treatment-naïve patient; or
(b) the HCV infection is genotype 1b and the patient is a treatment-experienced patient who is non-responsive to a combination therapy of interferon plus ribavirin;

and wherein there is limited or no emergence of the variants that encode substitutions at NS3 protease amino acid D168 during the combination therapy of the present invention.

Examples

I. Methods for Preparing Compound (1)

Methods for preparing amorphous Compound (1) can be found in US Patents 6,323,180, 7,514,557 and 7,585,845, which are herein incorporated by reference. The following Examples 1 to 5 provide methods for preparing additional forms of Compound (1) that may be used in the present invention.

Example 1 – Preparation of Type A crystalline form of Compound (1)

Amorphous Compound (**1**) (Batch 7, 13.80g) was added to a 1000 ml three neck flask. Absolute ethanol (248.9g) was added to the flask. While stirring, the contents of the flask were heated at 60 degrees C/hr to ~ 74 degrees C. (Solids do not dissolve at 74 degrees C). Water (257.4g) was then added linearly over 4 hr to the resulting slurry while stirring and maintaining the temperature at 74 degrees C. After the water addition was complete, the temperature was reduced linearly to ambient temperature at 8 degrees C/hr and then held at ambient temperature for 6 hrs while stirring. The resulting solids were collected by filtration and washed with 50 ml of 1/1 (w/w) EtOH/Water. The wet solids were dried on the funnel for 30 minutes by sucking N_2 through the cake. (XRPD analysis on this sample indicates that the pattern is similar to the EtOH solvate). The solids were then dried at 65-70 degrees C under vacuum (P = 25 in Hg) and a nitrogen bleed for 1.5 hr. The resulting solids (12.6g, 95.5 % corrected yield) were confirmed by XRPD as being Type A Compound (**1**).

Example 2 - Preparation of the Sodium Salt of Compound (1) - Method 1

2.1 g of amorphous sodium salt of Compound (1) and 8.90g of acetone was added to a vial and stirred at ambient temperature for 3 hr. The slurry was filtered off mother liquors and

the resulting solids were dried for 20 minutes under nitrogen flow for 20 minutes. 1.51 g of crystalline sodium salt of Compound (1) as solids was collected.

Example 3 - Preparation of the Sodium Salt of Compound (1) - Method 2

15.6 g of Type A of Compound (1), 175 ml of acetone and 3.6 ml of water was added to a 250 ml reactor and heated to 53 degrees C to dissolve the solids. 900 ul of 10.0 N NaOH was added to reactor and the solution was seeded with Type A. The seeded solution was stirred at 53 degrees C for 10 minutes. A second 900 ul portion of 10.0 N NaOH was added and the system was stirred at 53 degrees C for 30 minutes over which a slurry developed. The slurry was cooled to 19 degrees C at a cooling rate of 15 degrees C per hour and held overnight at 19 degrees C. The final resulting slurry was filtered and the wet solids were washed with 15 ml of acetone. Dried solids for 1 hr at 52 degrees C under vacuum with a nitrogen flow and then exposed the solids to lab air for one hour. Collected 12.1 g of Compound (1) crystalline sodium salt solids.

Example 4 - Preparation of the Sodium Salt of Compound (1) - Method 3

25.4 Kg of amorphous Compound (1), 228 L of THF and 11.1 Kg of 10 wt % NaOH (aq) was added to a reactor. The components were mixed at 25 degrees C to dissolve all solids. The resulting solution was filtered and the reactor and filter was washed with 23 L of THF. 180 L of solvent was removed using atmospheric distillation at 65 degrees C. 195 L of MIBK was added and 166 L of solvent was removed by vacuum distillation at ~ 44 degrees C. 161 L of MIBK and 0.41 Kg of water was added back to the reactor and the contents were heated to 70 degrees C. 255 g of Compound (1) sodium salt seeds were added at 70 degrees C and 1.42 L of water was added over 1.5 hours. After the water addition the slurry was held at 70 degrees C for 45 minutes and then cooled to 45 degrees C over 1 hr. The resulting slurried was filtered and washed with 64 L of MIBK containing ~0.8 weight % water. The wet cake was dried at 55 degrees C to give ~ 25 Kg of crystalline sodium salt of Compound (1).

Example 5 - Preparation of the Sodium Salt of Compound (1) - Method 4

2.00 g of amorphous Compound (1), 9.96g of THF and 0.11 g of water was added to a reactor and stirred at ambient temperature to dissolve solids. 0.820ml of 21 weight% NaOET in ethanol was added drop-wise while stirring the solution to get solution A. 15.9 g of n-BuAc and 160 ul of water was added to a second reactor and heated to 65 degrees C (solution B). 2.56 g of Solution A was added to Solution B at 65 degrees C and the resulting mixture was seeded with 40 mg of Compound (1) sodium salt seeds. The seeded mixture was aged at 65 degrees C for 45 minutes. 2.56 g of Solution B was added to Solution A and aged for 45 minutes in four separate intervals. After the final addition and aging, the slurry was cooled to 50 degrees C over 1 hour and filtered. The wet cake was washed with 6 ml of n-BuAc containing 0.5 weight % water. The final solids were dried at 50 degrees C under vacuum using a nitrogen purge. Compound (1) crystalline sodium salt solids were collected.

II. Clinical Results

For the clinical trials described below, the drug product administered was an oral solution of Compound (1) sodium salt. The Compound (1) sodium salt was provided to the clinical site(s) as a powder for preparing an oral solution using a co-supplied solvent. The solvent was also used as the placebo.

Example 6 - Clinical Study with Treatment-Naïve Patients

Safety and antiviral activity of Compound (1) sodium salt, a new HCV NS3 protease inhibitor, in treatment-naive patients with chronic hepatitis C genotype-1 infection given as monotherapy and in combination with Peginterferon alfa 2a (P) and Ribavirin (R)

Background: Compound (1) sodium salt is a HCV NS3 protease inhibitor (EC50 of 3-6 nM). A multiple rising dose study evaluated safety and antiviral activity in treatment-naive patients (pts) with chronic HCV genotype-1 infection as monotherapy for 14 days followed by triple combination therapy with P+R for an additional 14 days.

Methods: 34 patients (France, Germany, Spain, USA) with a Metavir fibrosis score of 0-3 and no prior therapy with any interferon or R were randomized (2 placebo:6 or 7active) to 4 dose groups of once-daily (qd) Compound (1) sodium salt: 20 mg (n=8), 48 mg (n=9), 120 mg (n=9), or 240 mg (n=8). Compound (1) sodium salt was given as monotherapy for 14 days. Pts with <1 log10 decrease in Day 10 viral load (VL) had Compound (1) sodium salt discontinued after Day 14. Pts with a ≥1 log10 decrease in Day 10 VL continued Compound (1) sodium salt on Day 15 and added P(180μg/week) + R(weight based) for triple combination therapy through Day 28. The primary endpoint was ≥2 log10 VL reduction at any time to Day 14. Plasma HCV-RNA levels were measured with Roche COBAS® TaqMan® assay (LLOQ 25 IU/m L). After day 28, at the discretion of the investigator, patients could continue to receive standard of care treatment, i.e., P + R.

Results: 33 pts were white, 1 was Asian, 27 were male, mean age = 48.9±11.1 years, mean body weight 79.1±17.5 kg, and median (range) baseline VL was 6.8 (4.7-7.7) log10. There were no significant demographic differences between dose groups. Compound (1) sodium salt was well tolerated. No pts discontinued treatment during monotherapy due to adverse events (AEs). AEs observed were typical for P+R. One serious AE, asthenia, occurred in the 20 mg dose cohort 6 days after initiating P+R. Rapid decline of VL was observed in all pts with maximal decline typically 2-4 days after starting Compound (1) sodium salt. With the exception of 1 pt in the 20 mg cohort, all pts on Compound (1) sodium salt achieved > 2 log10 VL decline during the monotherapy period. Median (range) maximal reductions in VL during 14 day monotherapy for the 20 mg, 48 mg, 120 mg, and 240 mg groups were 3.0 (1.5-3.9), 3.6 (3.1-3.8), 3.7 (3.3-4.1), and 4.2 (3.6-4.8) log10 IU/ml, respectively. No significant change in VL was observed with placebo. VL rebound during treatment was seen in the first 14 days of monotherapy in a majority of patients from all dose groups.

Conclusion: Compound (1) sodium salt as monotherapy for 14 days followed by combination with P+R for additional 14 days was well tolerated, and induced a strong and rapid antiviral response among treatment naïve patients.

Figure 1 depicts the average change in HCV viral load in the four patient dose groups of this study, i.e., treatment-naïve patients with chronic HCV genotype-1 infection treated with Compound (1) sodium salt as monotherapy for 14 days, followed by combination therapy with Compound (1) sodium salt, pegylated interferon alfa-2a and ribavirin for an additional 14 days. In the figure, the change in viral load is in IU/ml log10 units and the four patient dose groups are labeled as follows:

"20 naiv av" = average viral load change for 20 mg dose group

"48 naiv av" = average viral load change for 48 mg dose group

"120 naiv av" = average viral load change for 120 mg dose group

"240 naiv av" = average viral load change for 240 mg dose group

Emergence of Variants During Treatment-Naïve Patient Therapy

Population sequencing of the NS3/4A protease at baseline and rebound during treatment revealed selection of variants that confer in vitro resistance to Compound (1) sodium salt. There were seen notable changes at key residues within the NS3 protease domain relative to subtype reference (sub-genotype 1a: AF009606 or sub-genotype1b: AJ238799) during therapy in various dose groups. See Tables 1 to 4 below for details. In all tables, Gt represents the HCV subgenotype and Day 1 represents baseline sequence and the indicated amino acid substitutions are naturally occurring NS3 polymorphisms at key residues. Additional changes, that may encode or be associated with drug-resistance, are noted at different days during treatment.

Table 1 Predominant variants (relative to subtype reference) at key residues in the population-based amino acid sequences from the treatment-naïve patients in the 20 mg dose group

Patient	Gt	Day of treatment					
		1	6	14	28	56	168
20N1	1b	I71V, Q86P	I71V, Q86P	I71V, Q86P	ND	ND	ND
20N2	1b	none	BLD	D168V	R155[K,Q,R], D168[D,V]	R155[K,Q,R], D168[D,V]	BLD
20N3*	1b	Q86A, V/I170T	Q86A, V/I170T	Q86A, V/I170T	BLD	BLD	BLD

20N4	1a	none	V/I170[I,T]	R155K	R155K	R155K	BLD
20N5	1b	Q80L,	Q80L, D168[G,A]	Q80L, D168[E,D]	BLD	BLD	BLD
20N6	1a	none	none	none	ND	ND	ND
20N7	1a	none	R155K	R155K	R155K	R155K	R155K
20N8	1a	none	R155[R,K]	R155K	R155K	R155K	BLD

None: no substitutions at the selected positions were observed

BLD: Below limit of detection for PCR amplification

ND: Not determined

* Encodes V/I170T baseline polymorphism.

Table 2 Predominant variants (relative to subtype reference) at key residues in the population-based amino acid sequences from the treatment-naïve patients in the 48 mg dose group.

Patient	Gt	Day of treatment		
		1	14	28
48N1	1a	T54[T,S]	T54[T,S]	ND
48N2	1b	I71V, Q86P	I71V, Q86P V170[E,V]	ND
48N3	1b	P89S	P89S, D168V	P89S, D168V
48N4	1b	I71V	I71V, D168V	T54[T, A], I71V, D168V
48N5	1a	none	R155K	R155K
48N6	1b	none	D168[T,I,A,V]	D168[T,I,A,V]
48N7	1b	none	BLD	BLD
48N8	1a	Q80K	Q80K, R155K	BLD
48N9	1b	I71V, Q80[Q,R]	I71V, Q80[Q,R] R155[R,W], D168[D,V]	ND

None: no substitutions at the selected positions were observed

BLD: Below limit of detection for sequencing

ND: Not determined

Table 3 Predominant variants (relative to subtype reference) at key residues in the population-based amino acid sequences from the treatment-naïve patients in the 120 mg dose group

Patient	Gt	Day of treatment			
		1	14	28	84
120N1	1a	Q80K	Q80K	ND	ND
120N2	1	D168D/E	D168D/E	ND	ND
120N3	1a	none	R155K	BLD	BLD
120N4	1a	Q80K	BLD	ND	ND
120N5	1b	none	D168V	BLD	BLD
120N6	1b	P89T	P89T, D168V	P89T, D168V	P89T, D168V
120N7	1a	none	R155K	BLD	BLD
120N8	1b	none	D168V	D168V	BLD
120N9	1b	Q86P	Q86P, A156[T, A], D168[D, V]	Q86P, D168[D,I, A, V]	Q86P, R155K

None: no substitutions at the selected positions were observed

BLD: Below limit of detection for sequencing

ND: Not determined

Table 4 Predominant variants (relative to subtype reference) at key residues in the population-based amino acid sequences from the treatment-naïve patients in the 240 mg dose group

Patient	Gt	Day of Treatment			
		1	14	28	84
240N1	1b	Q8OL, Q86P	Q80L, Q86P	ND	ND
240N2	1b	none	none	ND	ND
240N3	1a	V36[L, V], Q80K	V36L, Q80K, R155K	ND	V36L, Q80K, R155K
240N4	1b	none	BLD	BLD	BLD
240N5	1b	P89A	P89A, D168V	P89A, D168V	BLD
240N6	1	none	D168V	D168V	BLD
240N7	1b	none	D168V	BLD	BLD
240N8	1a	Q89P	BLD	BLD	BLD

None: no substitutions at the selected positions were observed

BLD: Below limit of detection for PCR amplification and sequencing

ND: Not determined

Example 7 - Clinical Study with Treatment-Experienced Patients

Safety and antiviral activity of Compound (1) sodium salt, a new HCV NS3 protease inhibitor, in combination therapy with Peginterferon alfa 2a (P) and Ribavirin (R) for 28 days in P+R treatment-experienced patients with chronic hepatitis C genotype-1 infection.

Background: Compound (1) sodium salt is a HCV NS3 protease inhibitor (EC50 of 3-6 nM). A multiple rising dose study evaluated the safety and antiviral activity in P+R treatment-experienced patients (pts) with chronic hepatitis C genotype-1 infection for 28 days as combination therapy with P + R.

Methods: 19 pts (France, Germany, Spain, USA) with a Metavir fibrosis score of 0-3, who experienced previous virologic failure with P+R combination therapy, were assigned to receive Compound (1) sodium salt once-daily (qd) doses of 48 mg (n=6), 120 mg (n=7), or 240 mg (n=6) in combination with P (180μg/wk)+R (weight based) for 28 days. All patients were monitored for safety and tolerability of study drugs. The primary endpoint was a ≥2 log10 reduction in HCV viral load (VL) from baseline at any time up to Day 28. Plasma HCV-RNA levels were measured using the Roche COBAS® TaqMan® assay (LLOQ 25 IU/mL). The treatment experienced patients in this study included P+R null responders and partial respoders. After day 28, at the discretion of the investigator, patients could continue to receive standard of care treatment, i.e., P + R.

Results: 19 pts were white, 11 were male, mean age was 48±9 years, mean body weight was 81±15 kg, and median (range) baseline VL was 6.9 (5.9-7.4) log10. There were no significant demographic differences between dose groups. Compound (1) sodium salt was well tolerated and no serious or severe adverse events (AEs) were observed among pts in this study. AEs were typical for P + R. One subject discontinued treatment due to an AE (anxiety). A rapid, dose-related decline of VL was observed in all pts. All pts treated with Compound (1) sodium salt + P +R achieved > 2 log10 VL decline with triple combination therapy. Median (range) maximal decline in VL during 28 day combination therapy for 48 mg, 120 mg, and 240 mg dose cohorts was 4.8 (3.4-5.9), 5.2 (3.9-6.0), and 5.3 (4.8-6.1) log10 IU/ml, respectively. Virologic rebound during treatment was observed during the first 28 days of Compound (1) sodium salt + P + R dosing in 2/6 pts in the 48 mg and in 1/7 pts in the 120 mg dose groups. In these patients, population sequencing of the NS3/4A protease at baseline and at viral rebound during treatment revealed selection of variants in the NS3 protease domain shown to confer in vitro resistance to Compound (1) sodium salt. One patient in the 120 mg dose group did not display virologic rebound, but plateaued with ~500 copies IU/ml VL by day 28 and encoded a R155K mutant; this viral load sample was below the lower limit of detection in our phenotypic resistance assays.

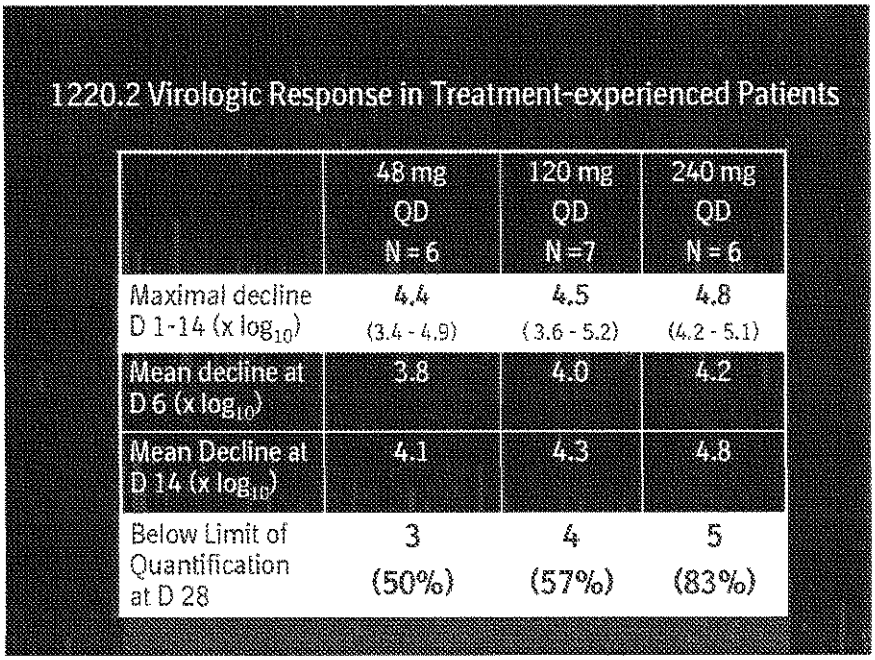
No rebound during the 28-day BI 201335 treatment was seen in the 240 mg qd dose cohort: 5/6 pts had VL < 25 IU/mL at Day 28. The sixth pt had a 4.7 log10 decline in VL from baseline on Day 28 and VL was < 25 IU/ml at next visit, Day 42.

Conclusion: Compound (1) sodium salt given once-daily in combination therapy with P + R for 28 days was well tolerated, and induced a strong and rapid antiviral response among treatment experienced patients.

Figure 2 depicts the average change in HCV viral load in three patient dose groups for this study, i.e., treatment-experienced patients with chronic HCV genotype-1 infection treated with Compound (1) sodium salt, pegylated interferon alfa-2a and ribavirin as combination therapy for 28 days. In the figure, the change in viral load is in IU/ml log10 units and the four patient dose groups are labeled as follows:

"48 NR av" = average viral load change for 48 mg dose group
"120 NR av" = average viral load change for 120 mg dose group
"240 NR av" = average viral load change for 240 mg dose group

Certain parameters relating to the virologic response (reduction in viral load) in the patients in this study are shown in the following table (where "D" = day, and "QD" = once per day; "N" = # of patients; "Below Limit of Quantification" means less than 25 IU per ml of serum).



Emergence of Variants During Treatment-Experienced Patient Therapy

There are notable changes at key residues within the NS3 protease domain relative to subtype reference (sub-genotype 1a: AF009606 or sub-genotype1b: AJ238799) during therapy in various dose groups. See Tables 1 to 4 below for details. In all tables, Gt represents the HCV subgenotype and Day 1 represents baseline sequence and the indicated amino acid substitutions are naturally occurring NS3 polymorphisms at key residues. Additional changes, that may encode or be associated with drug-resistance, are noted at different days during treatment.

Table 5 Predominant variants (relative to subtype reference) at key residues in the population-based amino acid sequences from the 48 mg treatment-experienced dose group.

Patient	Gt	Day of treatment			
		1	14	28	168
48TE1	1a	Q80K	Q80K	Q80K, R155K	Q80K, R155K
48TE2	1a	Q80L	BLD	BLD	BLD
48TE4	1a	Q80R	BLD	BLD	BLD

48TE3	1b	none	D168V	D168V	R155R/K/Q
48TE5	1b	Q86P	BLD	BLD	BLD
48TE6	1b	Q86P	BLD	BLD	Q86P, R155K

None: no substitutions at the selected positions were observed

BLD: Below limit of detection for PCR amplification and sequencing

Table 6 Predominant variants (relative to subtype reference) at key residues in the population-based amino acid sequences from the 120 mg treatment-experienced dose group.

Patient	Gt	Day of Treatment		
		1	14	28
120TE1	1a	Q80K	Q80K, R155K	Q80K, R155K
120TE2	1a	Q89Y	BLD	BLD
120TE3	1b	Q86P, P89S	BLD	BLD
120TE4	1b	Q86A	BLD	BLD
120TE5	1b	P89[P, S]	BLD	BLD
120TE6	1a	none	BLD	BLD
120TE7	1a	none	R155K*	R155K*

None: no substitutions at the selected positions were observed

BLD: Below limit of detection for PCR amplification and sequencing

*VL plateau at < 1000 IU/ml at these time points.

Table 7 Predominant variants (relative to subtype reference) at key residues in the population-based amino acid sequences from the 240 mg treatment-experienced dose group.

Patient	Gt	Day of Treatment	
		1	28
240TE1	1a	none	BLD
240TE2	1a	none	BLD
240TE3	1a	none	BLD
240TE4	1b	none	BLD
240TE5	1b	Q86P	BLD
240TE6	1a	none	BLD

None: no substitutions at the selected positions were observed

BLD: Below limit of detection for PCR amplification and sequencing

Overall Assessment

These results demonstrate a strong and very rapid antiviral affect in treatment-naïve patients treated with Compound (1) sodium salt given once-daily as monotherapy for 14 days, which is followed by anti-viral resistance formation and increase in viral load after 5-6 days of monotherapy. Upon initiation of the triple combination therapy of the present invention at day 14 (Compound (1) sodium salt, with pegylated interferon alfa 2a and ribavirin), however, viral load progressively decreased demonstrating the antiviral effectiveness of the triple combination therapy of the present invention in treatment-naïve patients. See Figure 1.

When Compound (1) sodium salt was given once-daily to treatment-experienced patients (interferon plus ribavirin null responders and partial responders) in combination with pegylated interferon alfa 2a and ribavirin for 28 days, the results demonstrate the same strong and very rapid antiviral response but with reduced resistance formation. See Figure 2. One would have expected in such treatment-experienced, non-responder patients that a similar viral resistance would have occurred after day 5 as these patients were selected for being non-responsive to pegylated interferon plus ribavirin therapy. However, a continuous viral suppression occurred.

In essence, it has been shown that the triple combination therapy of the present invention can effectively reduce the viral load of treatment-naïve and treatment-experienced patients with chronic genotype-1 hepatitis C viral infection and, at least in some patients, keep it at less than detectable level, that is defined as below 50 International Units per ml serum of a patient as measured by quantitative, multi-cycle reverse transcriptase PCR methodology according to the WHO international standard. In preferred embodiments, the triple combination therapy of the present invention can effectively reduce the viral load in HCV genotype 1-infected patients to below 10 International Units per ml serum.

Definitely surprising results include:

(1) no early resistance formation (e.g., variants encoding NS3 amino acid substitutions at R155 and/or D168) in treatment-experienced patients who are treated with Compound (1) sodium salt in combination with pegylated interferon alfa 2a and ribavirin at standard doses (Figure 2) as compared to the effect of the same dose of Compound (1) sodium salt alone in treatment-naïve patients who experienced viral resistance (see Figure 1, first 14 days). One would have expected in treatment experienced patients that a similar viral resistance would have occurred after day 5 even with the addition of pegylated interferon alfa 2a and ribavirin as these patients were selected for being non-responsive to previous pegylated interferon plus ribavirin combination therapy; and

(2) that a dose of only 120 mg QD (once daily) in combination with pegylated interferon and ribavirin induced a viral depletion to below the level of quantification (defined as less than 25 IU per ml of serum) in more than 50% of the treatment-experienced patients who have been previously non-responsive to pegylated interferon plus ribavirin therapy (see table).

Example 8 - Methods for Identifying HCV NS3 Variants

VIRAL RNA EXTRACTION AND PCR AMPLIFICATION

Viral RNA was isolated from plasma of HCV-infected subjects and a DNA fragment of 2.4 kbp containing the complete NS3-NS4A region was first synthesized using SUPERSCRIPT™ III one step RT-PCR System (Invitrogen) and two gene-specific primers spanning positions 3276 in NS2 and 5650 in NS4B. After purification of the first PCR product, two different second-round, semi-nested PCR products of either 2.3 or 0.7 kbp (spanning the entire NS3/NS4A or only the NS3 protease domain, respectively) were then generated using KOD Hot Start DNA polymerase (Novagen). The limit of detection of the RT-PCR amplification method restricted the analysis to patient samples with VL above 1000 IU/ml.

SEQUENCE ANALYSIS

The 2.3 kbp DNA product was then used for direct population-based sequencing of the entire NS3-NS4A 2055 nt region using BIG DYE® Terminator V3.1 (Applied Biosystems) and an ABI PRISM® 3130XL Genetic Analyzer (Applied Biosystems). Sequences were obtained from 10 primers to achieve at least 90% double strand coverage for the NS3-NS4A region. The resulting nucleotide sequences were analyzed with SEQSCAPE® v2.5 (Applied Biosystems).

The 0.7 kbp DNA fragment was used to generate the clonal-based (ZEROBLUNT® TOPO® Cloning Kit, Invitrogen) sequences of the 543 nt NS3 protease region covering the first 181 aa of NS3; for each sample 96 clones were picked and sequenced using universal primers with ABI PRISM® BIG DYE™ Terminator Cycle PCR Sequencing. Two single pass sequences were performed for each clone resulting in 90-100% double strand coverage of the 543 nt region analyzed with MUTATION SURVEYOR™ v3.0 (Softgenetics LLC). For each patient sample, the clones with low quality sequence or containing deletions, insertions or stop codons were not included for further analysis such

that the number of clones analyzed varied from 74 to 89 (with a median and average of 80 clones).

The resulting sequences were compared to reference sequences according to their respective subtypes which were previously determined during the trial screening phase with the TRUEGENE™ HCV 5'NC genotyping assay. AF009606 served as the reference for subtype 1a and AJ238799 for subtype 1b. Particular attention was focused on mutations that resulted in amino acid substitutions at 15 positions in the NS3 protease domain. These positions were all previously reported as potentially conferring resistance to this class of compounds. These positions are: 36, 41, 54, 71, 80, 86, 89, 109, 111, 155, 156, 168, 170, 176 and 178 (References: [1.] Tong X, Bogen S, Chase R, Girijavallabhan V, Guo Z, Njoroge FG, Prongay A, Saksena A, Skelton A, Xia E, Ralston R. Characterization of resistance mutations against HCV ketoamide protease inhibitors. Antiviral Res. 2008 March 77(3):177-185. [2.] Lagacé L, Marquis M, Bousquet C, Do F, Gingras R, Lamarre D, Lamarre L, Maurice R, Pause A, Pellerin C, Spickler C, Thieault D, and Kukolj G. BILN 2061 and beyond: pre-clinical evaluation of HCV subgenomic replicon resistance to a NS3 protease inhibitor. In Framing the Knowledge of Therapeutics for Viral Hepatitis. RF Schinazi and ER Schiff (eds).P263-278 IHL press, 2006 [3.]Koev G, Kati W. The emerging field of drug resistance. Expert Opinion Invest Drugs 17(3), 303-319, 2008 (P08-03895)

DRUG SENSITIVITY ASSAYS

A bicistronic HCV replicon shuttle vector (pIT2) comprising a luciferase reporter gene and an adapted Con-1 NS3 /NS5B region was modified to create two unique restriction sites (*Mlu* I and *Spe* I) at NS3 codons 11 and 225 which enabled the insertion of compatible NS3 amplicons isolated from HCV-infected patient plasma samples. The first-round PCR product synthesized from the patient plasma-purified RNA (also used for generating fragments for population- and clonal-based sequencing) was used to amplify a 0.65 kbp fragment with primer pairs that respectively contain the unique *Mlu*I and *Spe*I restriction sites for insertion into the shuttle vector. Amplicons were ligated in the pIT2 shuttle vector

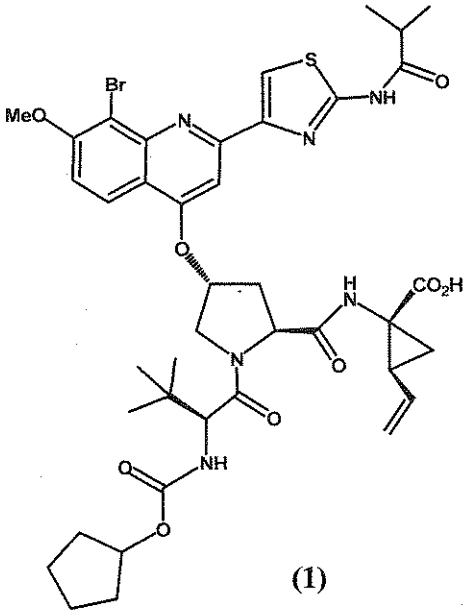
and reconstituted plasmid DNA was used to generate HCV subgenomic replicon RNA transcripts (T7 RIBOMAX™ kit, Promega). The *in vitro* transcribed RNA was transiently transfected by electroporation in Huh-7.5 cells which were then seeded in 96 well plates for 24 hours and treated with a range of Compound (1) sodium salt (or IFN-α) concentrations for a period of 72 hours. At the end of the incubation, luciferase activity was measured with the BRIGHT-GLO™ substrate as the luminescence quantified (CPS) in each well of the culture plate reflected the amount of HCV RNA replication. The level of inhibition (% inhibition) in each well containing inhibitor was calculated with the following equation: % inhibition = 100 - [100 x CPS (inhibitor) / CPS (control)]. The concentration giving 50 % inhibition of HCV RNA replication (EC_{50}) was determined by the non-linear regression routine NLIN procedure of SAS. The EC_{50} of mutant NS3 were compared to the baseline EC_{50} and used to generate fold-change values.

The predominant genotype 1a resistance mutations in on-treatment viral rebound samples encoded an R155K substitution, and other minor variants were detected by clonal sequence analysis at this position. R155K variants conferred reductions in sensitivity to BI 201335 with the range of EC_{50} values of 1.8 - 6.5 μM. The genotype 1b viruses mainly encoded changes at D168, with valine as a predominant substitution, and other minor variants detected by sensitive clonal sequencing. EC_{50} values for D168 variants ranged 3.6 - 15 μM. This profile may be attributable, in part, to a different mutational barrier to resistance at the R155 codon in genotype 1a (a single nucleotide transition changes the codon to a lysine) versus genotype 1b (that requires two nucleotide substitutions to encode a change to lysine.

CLAIMS

1. The use of the combination of:

(a) a compound of the following formula (1) or a pharmaceutically acceptable salt thereof:



(1)

;

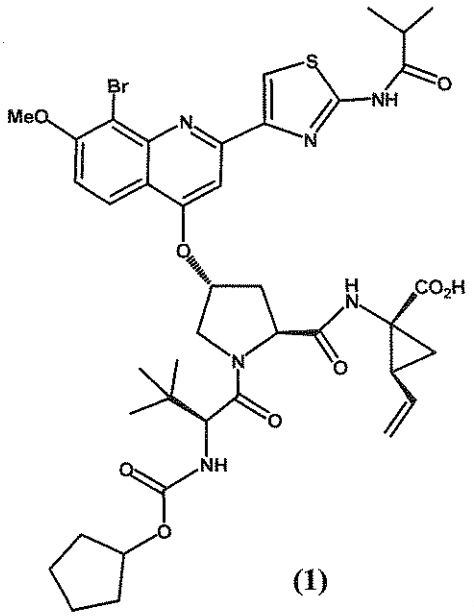
(b) interferon alpha; and

(c) ribavirin

for the manufacture of a medicament for the treatment of hepatitis C viral (HCV) genotype 1 infection or alleviating one or more symptoms thereof.

2. The use according to claim 1, whereby the medicament comprises

(a) a first pharmaceutical composition comprising a compound of the following formula (1) or a pharmaceutically acceptable salt thereof:



(I)

;

(b) a second pharmaceutical composition comprising interferon alpha; and

(c) a third pharmaceutical composition comprising ribavirin.

3. The use according to claims 1 or 2, whereby the components (a), (b) and (c) are present in the medicament in a therapeutically effective amount.

4. The use according to claim 1 or 2, wherein the medicament is for the treatment of hepatitis C viral (HCV) genotype 1 infection or alleviating one or more symptoms thereof in a treatment-naive patient.

5. The use according to claim 1 or 2, wherein the medicament is for the treatment of hepatitis C viral (HCV) genotype 1 infection or alleviating one or more symptoms thereof in a patient that is non-responsive to a combination therapy using ribavirin and an interferon alpha.

6. The use according to claim 1 or 2, wherein the medicament is for the treatment of hepatitis C viral (HCV) genotype 1 infection or alleviating one or more symptoms thereof in a patient

whereby the HCV-RNA levels in the patient are reduced to a less than detectable level as a result of the treatment.

7. The use according to claim 1 or 2, wherein said medicament is administered for at least 4 weeks.

8. The use according to claim 1 or 2, wherein the administered amount of compound (1) or a pharmaceutically acceptable salt thereof is at least 40 mg per day.

9. The use according to claim 1 or 2, wherein the administered amount of ribavirin is selected from 400 mg/day, 600 mg/day, 800 mg/day, 1000 mg/day, or 1200 mg/day.

10. The use according to claim 1 or 2, wherein interferon alpha is administered once a week.

11. The use according to claim 1 or 2, wherein said interferon alpha is a pegylated interferon alfa.

12. The use according to claim 11, wherein the pegylated interferon alfa is pegylated interferon alfa-2a administered at a dosage of about 90 to about 200 μg/week or is pegylated interferon alfa-2b administered at a dosage of about 0.5 μg/kg/week to about 2 μg/kg/week.

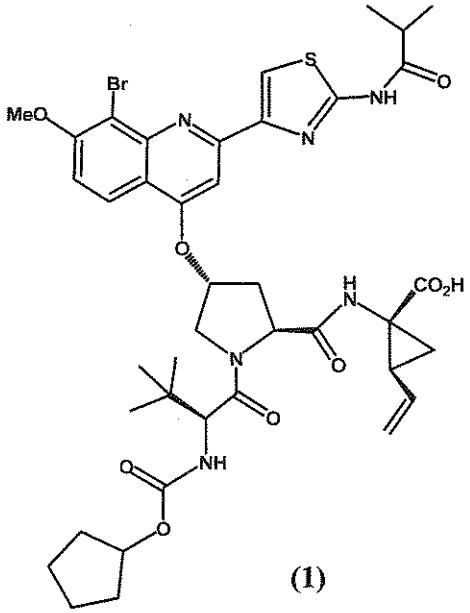
13. The use according to claim 1 or 2, wherein the treatment is for HCV infection of genotype 1 in a patient that is non-responsive to a combination therapy using ribavirin and an interferon, the compound (1) or a pharmaceutically acceptable salt thereof is administered in an amount of between about 48 mg per day and about 240 mg per day and wherein the interferon alfa is pegylated interferon alfa-2a or pegylated interferon alfa-2b.

14. The use according to claim 1 or 2, wherein the compound (1) or a pharmaceutically acceptable salt thereof is administered once a day; the ribavirin is administered twice a day; and the interferon alfa is administered once a week.

15. The use according to claim 1 or 2, wherein there is limited or no emergence of HCV variants that encode HCV NS3 protease amino acid substitutions at one or more of: R155, D168, or A156.

16. A kit comprising:

(a) a first pharmaceutical composition comprising a compound of the following formula (1) or a pharmaceutically acceptable salt thereof:



(1)

;

(b) a second pharmaceutical composition comprising interferon alpha; and

(c) a third pharmaceutical composition comprising ribavirin,

wherein said kit is for use for the treatment of hepatitis C viral (HCV) genotype 1 infection or for alleviating one or more symptoms thereof.