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(54) **Title**: COMBINATIONS OF ANTI-HCV-ENTRY FACTOR ANTIBODIES AND DIRECT ACTING ANTIVIRALS FOR THE TREATMENT AND THE PREVENTION OF HCV INFECTION

(57) **Abstract**: The present invention provides combinations for use in the treatment or the prevention of HCV infection. In particular, combinations are provided that comprise at least one anti-HCV-entry factor antibody and at least one direct acting antiviral, wherein the anti-HCV-entry factor antibody and direct acting antiviral act in a highly synergistic manner to inhibit HCV infection of susceptible cells. Also provided are pharmaceutical compositions and kits comprising such combinations and methods of using these compositions and kits for treating or preventing HCV infection.

Combinations of Anti-HCV-Entry Factor Antibodies and Direct Acting Antivirals for the Treatment and the Prevention of HCV Infection

Related Patent Application

The present application claims priority to European Patent Application No. EP 11 306 052.9 filed on August 17, 2011. The European patent application is incorporated herein by reference in its entirety.

Background of the Invention

Hepatitis C virus (HCV) is a major global health problem, with an estimated 150-200 million people infected worldwide, including at least 5 million in Europe (Pawlotsky, Trends Microbiol., 2004, 12: 96-102). According to the World Health Organization, 3 to 4 million new infections occur each year. The infection is often asymptomatic; however, the majority of HCV-infected individuals develop chronic infection (Hoofnagle, Hepatology, 2002, 36: S21-S29; Lauer *et al.*, N. Engl. J. Med., 2001, 345: 41-52; Seeff, Semin. Gastrointest., 1995, 6: 20-27). Chronic infection frequently results in serious liver disease, including fibrosis and steatosis (Chisari, Nature, 2005, 435: 930-932). About 20% of patients with chronic HCV infection develop liver cirrhosis, which progresses to hepatocellular carcinoma in 5% of the cases (Hoofnagle, Hepatology, 2002, 36: S21-S29; Blonski *et al.*, Clin. Liver Dis., 2008, 12: 661-674; Jacobson *et al.*, Clin. Gastroenterol. Hepatol., 2010, 8: 924-933; Castello *et al.*, Clin. Immunol., 2010, 134: 237-250; McGivern *et al.*, Oncogene, 2011, 30: 1969-1983).

Chronic HCV infection is the leading indication for liver transplantations (Seeff *et al.*, Hepatology, 2002, 36: 1-2). Unfortunately, liver transplantation is not a cure for hepatitis C; viral recurrence being an invariable problem and the leading cause of graft loss (Brown, Nature, 2005, 436: 973-978; Watt *et al.*, Am. J. Transplant, 2009, 9: 1707-1713). No vaccine protecting against HCV is yet available. Current therapies include administration of ribavirin and/or interferon-alpha (IFN-α), two non-specific anti-viral agents. Using a combination treatment of pegylated IFN-α and ribavirin, persistent clearance is achieved in about 50% of patients with genotype 1 chronic hepatitis C. However, a large number of patients have contraindications to one of the components of the combination; cannot tolerate the treatment; do not respond to

interferon therapy at all; or experience a relapse when administration is stopped. In addition to limited efficacy and substantial side effects such as neutropenia, haemolytic anemia and severe depression, current antiviral therapies are also characterized by high cost. To improve efficacy of standard of care (SOC), a large number of direct acting antivirals (DAAs) targeting viral polyprotein processing and replication have been developed (Hofmann *et al.*, Nat. Rev; Gastroenterol. Hepatol., 2011, 8: 257-264). These include small molecule compounds targeting HCV non-structural proteins including the HCV protease, polymerase and NS5A protein. Although a marked improvement of antiviral response was observed when protease inhibitors were combined with SOC (Hofmann *et al.*, Nat. Rev; Gastroenterol. Hepatol., 2011, 8: 257-264; Bacon *et al.*, New Engl. J. Med., 2011, 364: 1207-1217; McHutchison *et al.*, New Engl. J. Med., 2010, 362: 1292-1303; Poordad *et al.*, New Engl. J. Med., 2011, 364: 1195-1206; Hezode *et al.*, New Engl. J. Med., 2009, 360: 1839-1850; Kwo *et al.*, Lancet, 2010, 376: 705-716), toxicity of the individual compounds and rapid development of viral resistance in a substantial fraction of patients remain major challenges (Pawlotsky, Hepatology, 2011, 53: 1742-1751; Pereira *et al.*, Nat. Rev. Gastroenterol. Hepatol., 2009, 6: 403-411; Sarrazin *et al.*, Gastroenterol., 2010, 138: 447-462). New therapeutic approaches against HCV are therefore still needed.

HCV entry into target cells is a promising target for antiviral preventive and therapeutic strategies since it is essential for initiation, spread, and maintenance of infection (Timpe *et al.*, Gut, 2008, 57: 1728-1737; Zeisel *et al.*, Hepatology, 2008, 48: 299-307). Indeed, HCV initiates infection by attaching to molecules or receptors on the surface of hepatocytes. Current evidence suggests that HCV entry is a multistep process involving several host factors including heparan sulfate (Barth *et al.*, J. Biol. Chem., 2003, 278: 41003-41012), the tetraspanin CD81 (Pileri *et al.*, Science, 1998, 282: 938-941), the scavenger receptor class B type I (SR-BI) (Zeisel *et al.*, Hepatology, 2007, 46: 1722-1731; Bartosch *et al.*, J. Exp. Med., 2003, 197: 633-642; Grove *et al.*, J. Virol., 2007, 81: 3162-3169; Kapadia *et al.*, J. Virol., 2007, 81: 374-383; Scarselli *et al.*, EMBO J., 2002, 21: 5017-5025), Occludin (Ploss *et al.*, Nature, 2009, 457: 882-886) and Claudin-1 (CLDN1), an integral membrane protein and a component of tight-junction strands (Evans *et al.*, Nature, 2007, 446: 801-805). Furthermore, Niemann-Pick C1-like cholesterol absorption receptor has been

identified as a new hepatitis C virus entry factor (Sainz *et al.*, Nature Medicine, 2012, 18: 281-285).

Identification of these (co)-entry factors or (co)-receptors for HCV has opened up new avenues for the development of therapeutic and prophylactic agents as drug candidates for the prevention and/or treatment of HCV infection. Indeed, proof-of-concept studies in cell culture and animal models have demonstrated that entry inhibitors are a promising novel class of antivirals for prevention and treatment of HCV infection (for review see Zeisel *et al.*, J. Hepatol., 2011, 54: 566-576). Entry inhibitors in preclinical or early clinically development include HCV-receptor- and HCV-envelop-specific antibodies as well as small molecules (Zeisel *et al.*, J. Hepatol., 2011, 54: 566-576; Catanese *et al.*, J. Virol., 2007, 81: 8063-8071; Fafi-Kremer *et al.*, J. Exp. Med., 2010, 207: 2019-2031; Matsumura *et al.*, Gastroenterology, 2009, 137: 673-681; Keck *et al.*, J. Virol., 2005, 79: 13199-13208; Law et al., Nat. Med., 2008, 14: 25-27; Syder *et al.*, J. Hepatol., 2011, 54: 48-55; Fofana *et al.*, Gastroenterology, 2010, 139: 953-964, e1-4).

Cross-neutralizing antibodies inhibiting HCV entry have been shown to be associated with control of HCV infection and prevention of HCV re-infection in cohorts with self-limited acute infection (Osburn *et al.*, Gastroenterology, 2009, 138: 315-324; Pestka *et al.*, Proc. Natl. Acad. Sci. USA, 2007, 104: 6025-630). For example, monoclonal antibodies raised against native human SR-BI have been shown to inhibit HCV E2 binding to SR-BI and to efficiently block HCVcc infection of hepatoma cells in a dose-dependent manner (Catanese *et al.*, J. Virol., 2007, 81: 8063-8071 ; WO 2006/005465). European patent application No. EP 1 256 348 discloses substances, including antibodies, with antiviral effects that inhibit binding of HCV E2 and CD81. International patent application WO 2007/130646 describes *in vitro* and cell-based assays for identifying agents that interfere with HCV interactions with Claudin-1 thereby preventing HCV infection. The present Applicants have generated monoclonal antibodies that efficiently inhibit HCV infection by targeting host entry factor Claudin-1 (EP 08 305 597 and WO 2010/034812).

Recently, the present Applicants have demonstrated that blocking the activity of the newly discovered HCV entry co-factors, epidermal growth factor receptor (EGFR) and ephrin type-A receptor 2 (EphA2), using the approved kinase inhibitors, erlotinib

and dasatinib respectively, broadly impaired infection by all major HCV genotypes and viral escape variants *in vitro* and in the human liver-chimeric Alb-uPA/SCID mouse model (Lupberger *et al.*, Nature Medicine, 2011, 17: 589-595). Furthermore, the Applicants have shown that HCV entry is inhibited by antibodies directed against EGFR and EphA2.

Since the development of novel therapeutic approaches against HCV remains a high-priority goal, these studies are encouraging as they demonstrate that antibodies against receptor or co-receptors that affect HCV entry into susceptible cells may constitute an effective and safe alternative to current HCV therapies.

Summary of the Invention

The present invention relates to systems and improved strategies for the prevention and/or treatment of HCV infection and HCV-related diseases. More specifically, the present Applicants have demonstrated that an anti-claudin-1 antibody, in combination with VX-950, ITMN-191, R7128 or BMS-790052 (which are direct acting antivirals) act in a highly synergistic manner to inhibit HCV infection (see Example 1). Similarly, they have shown that an anti-CD81 antibody or an anti-SRBI antibody in combination with VX-950, ITMN-191, R7128 or BMS-790052, act in a highly synergistic manner to inhibit HCV infection (see Example 1). The Applicants have also demonstrated synergy for combinations of each of the anti-claudin-1 antibody, anti-CD81 antibody and anti-SR-BI antibody with a protease inhibitor (telaprevir, boceprevir, danoprevir or TMC-435), an NS5A inhibitor (daclatasvir) or with a polymerase inhibitor (mericitabine or GS7977). (see Example 5). These results suggest that a combination of a direct acting antiviral and an anti-HCV-entry factor antibody may be an effective antiviral approach to prevent primary HCV infection, such as after liver transplantation, and might also restrain virus spread in chronically infected patients.

Consequently, in one aspect, the present invention provides a combination of at least one direct acting antiviral and at least one anti-HCV-entry factor antibody for use in the treatment or prevention of HCV infection.

In preferred embodiments, the at least one direct acting antiviral is a HCV protease inhibitor or a HCV polymerase inhibitor of an NS5A inhibitor.

In certain embodiments, the at least one direct acting antiviral is selected from the group consisting of telaprevir (also known as VX-950), danoprevir (also known as ITMN-191), mericitabine (also known as RG7128), daclatasvir (also known as BMS-790052), boceprevir, BMS-650032, VX-985, BI 201335, TMC-435, GS-7977, GS 9256, GS 9451, MK-7009, ACH-1625, ABT-450, BMS-791325, VX-985, VX-500, PHX1766, VX-813, AVL-181, AVL-192, ACH-2684, IDX184, PSI-7977, VX-222, PF-868554, ABT-072, ABT-333, ANA598, BI 207127, MK-0608, TMC649128, RG7348, PSI-938, INX-189, VCH-759, IDX375 and A-837093.

In certain preferred embodiments, the at least one direct acting antiviral is telaprevir (also known as VX-950), danoprevir (also known as ITMN-191), mericitabine (also known as RG7128), daclatasvir (also known as BMS-790052), boceprevir, TMC-435, and GS-7977.

The anti-HCV-entry factor antibody of a combination according to the invention may be an antibody against any HCV receptor, co-receptor, entry factor or entry co-factor known in the art or an antibody directed against any cell surface protein involved in the HCV infection process. The anti-HCV-entry factor antibody may be a polyclonal antibody or a monoclonal antibody. Preferably, the anti-HCV-entry factor antibody is a monoclonal antibody. In certain embodiments, the anti-HCV-entry factor antibody is an antibody against a receptor selected from the group consisting of heparan sulfate, the LDL receptor, the tetraspanin CD81, the scavenger receptor class B type I (SR-BI), Occludin, Claudin-1 (CLDN1), or Niemann-Pick C1-like cholesterol absorption receptor. In certain preferred embodiments, the anti-HCV-entry factor antibody is an anti-CLDN1 antibody, in particular a monoclonal anti-CLDN1 antibody such as those developed by the present Applicants and described in EP 08 305 597 and WO 2010/034812. In other preferred embodiments, the anti-HCV-entry factor antibody is an anti-CD81 antibody or an anti-SRBI antibody.

In other embodiments, the anti-HCV-entry factor antibody of a combination according to the invention is an antibody against a HCV entry factor such as an anti-receptor tyrosine kinase antibody.

The anti-HCV-entry factor antibody of a combination according to the present invention may be a full (complete) antibody, or a biologically active fragment of such antibody (*i.e.*, any fragment or portion of such an antibody that retains the ability of

the antibody to interfere with HCV-host cells interactions, and/or to specifically bind to a HCV receptor, and/or to inhibit or block HCV entry into HCV-susceptible cells, and/or to reduce or prevent HCV infection of susceptible cells). Antibodies or fragments thereof that are suitable for use in a combination according to the present invention also include chimeric antibodies, humanized antibodies, de-immunized antibodies and antibody-derived molecules comprising at least one complementary determining region (CDR) from either a heavy chain or light chain variable region of an anti-HCV-entry factor antibody, including molecules such as Fab fragments, $F(ab')_2$ fragments, Fd fragments, Sc antibodies (single chain antibodies), diabodies, individual antibody light single chains, individual antibody heavy chains, chimeric fusions between antibody chains and other molecules, and antibody conjugates, such as antibodies conjugated to a therapeutic agent, so long as these antibody-related molecules retain at least one biologically relevant property of the antibody from which it is "derived". The biologically relevant property may be the ability to interfere with HCV-host cells interactions, to specifically bind to an HCV receptor protein, to inhibit or block HCV entry into HCV-susceptible cells, and/or to reduce or prevent HCV infection of susceptible cells.

In a combination according to the present invention, the direct acting antiviral and the anti-HCV-entry factor antibody act in a highly synergistic manner to inhibit HCV infection. In certain embodiments, the direct acting antiviral decreases the IC_{50} for the inhibition of HCV infection by the anti-HCV-entry factor antibody by a factor of at least 10 fold or at least 15 fold, preferably at least 20 fold or at least 25 fold, more preferably at least 30 fold or 40 fold, and even more preferably 50 fold or more than 50 fold. In other embodiments, the anti-HCV-entry factor antibody decreases the IC_{50} for the inhibition of HCV infection by the direct acting antiviral by a factor of at least 10 fold or at least 15 fold, preferably at least 20 fold or at least 25 fold, more preferably at least 30 fold or 40 fold, and even more preferably 50 fold or more than 50 fold. The combination index (CI) of the at least one direct acting antiviral and at least one anti-HCV-entry factor antibody is lower than 1, preferably lower than 0.50 or lower than 0.25, more preferably lower than 0.15, and even more preferably lower than 0.10.

The combinations of the present invention can find application in a variety of prophylactic and therapeutic treatments. Thus, the combinations are provided for use

in the prevention of HCV infection of a cell (*e.g.*, a susceptible cell or a population of susceptible cells); for preventing or treating HCV infection or a HCV-related disease in a subject; for controlling chronic HCV infection; and for preventing HCV recurrence in a liver transplantation patient. HCV infection may be due to HCV of a genotype selected from the group consisting of genotype 1, genotype 2, genotype 3, genotype 4, genotype 5, genotype 6 and genotype 7, or more specifically of a subtype selected from the group consisting of subtype 1a, subtype 1b, subtype 2a, subtype 2b, subtype 2c, subtype 3a, subtype 4a-f, subtype 5a, and subtype 6a.

In a related aspect, the present invention provides a method of reducing the likelihood of a susceptible cell of becoming infected with HCV as a result of contact with HCV, which comprises contacting the susceptible cell with an effective amount of an inventive combination. Also provided is a method of reducing the likelihood of a subject's susceptible cells of becoming infected with HCV as a result of contact with HCV, which comprises administering to the subject an effective amount of an inventive combination or pharmaceutical composition thereof. The present invention also provides a method of treating or preventing HCV infection or a HCV-associated disease (*e.g.*, a liver disease or pathology) in a subject in need thereof, which comprises administering to the subject an effective amount of an inventive combination or pharmaceutical composition thereof. The invention also provides a method for controlling chronic HCV infection in a subject in need thereof, which comprises administering to the subject an effective amount of an inventive combination or pharmaceutical composition thereof.

Also provided is a method for preventing HCV recurrence in a liver transplantation patient, which comprises administering to the patient an effective amount of an inventive combination or pharmaceutical composition thereof.

In the context of the present invention, the HCV infection or HCV-related disease or HCV re-infection may be caused by a Hepatitis C virus that is resistant to a direct acting antiviral and/or transmitted by cell-cell transmission.

Administration of an inventive combination, or pharmaceutical composition thereof, to a subject may be by any suitable route, including, for example, parenteral, aerosol, oral and topical routes. The inventive combination, or pharmaceutical

composition thereof, may be administered alone or in combination with a therapeutic agent, such as an anti-viral agent.

The inventive combinations may be administered *per se* or as pharmaceutical compositions. Accordingly, in another aspect, the present invention provides for the use of an inventive combination for the manufacture of medicaments, pharmaceutical compositions, or pharmaceutical kits for the treatment and/or prevention of HCV infection and HCV-associated diseases.

In a related aspect, the present invention provides a pharmaceutical composition comprising an effective amount of an inventive combination (*i.e.*, at least one direct acting antiviral and at least one anti-HCV-entry factor antibody, as described herein) and at least one pharmaceutically acceptable carrier or excipient. In certain embodiments, the pharmaceutical composition is adapted for administration in combination with an additional therapeutic agent, such as an antiviral agent. In other embodiments, the pharmaceutical composition further comprises an additional therapeutic agent, such as an antiviral agent. Antiviral agents suitable for use in methods and pharmaceutical compositions of the present invention include, but are not limited to, ribavirin, anti-HCV (monoclonal or polyclonal) antibodies, RNA polymerase inhibitors, protease inhibitors, IRES inhibitors, helicase inhibitors, antisense compounds, ribozymes, entry inhibitors, micro-RNA antagonists, cytokines, therapeutic vaccines, NS5A antagonists, polymerase inhibitors, cyclophilin A antagonists, and any combination thereof.

These and other objects, advantages and features of the present invention will become apparent to those of ordinary skill in the art having read the following detailed description of the preferred embodiments.

Brief Description of the Drawing

Figure 1 is a set of three graphs showing the synergistic effects of an anti-CLDN1 antibody in combination with direct acting antivirals on the inhibition of HCVcc infection. Huh7.5.1 cells were pre-incubated for 1 hour with serial concentrations of anti-CLDN1 monoclonal antibody (OM-7D3-B3) and a fixed concentration (0.001 μM) of VX-950 (**A**), ITMN-191 (**B**) or R7128 (**C**). Huh7.5.1 cells were then incubated with HCVcc in the presence of compounds. HCVcc

infection was analyzed as described in Example 1. Data are expressed relative to HCVcc infection without compound. An unrelated monoclonal isotype control antibody served as negative control.

Figure 2 is a set of three graphs showing the synergistic effects of an anti-CD81 antibody in combination with direct acting antivirals on the inhibition of HCVcc infection. Huh7.5.1 cells were pre-incubated for 1 hour with serial concentrations of an anti-CD81 monoclonal antibody and a fixed concentration (0.001 μM) of VX-950 (**A**), ITMN-191 (**B**) or R7128 (**C**). Huh7.5.1 cells were then incubated with HCVcc in the presence of compounds. HCVcc infection was analyzed as described in Example 1. Data are expressed relative to HCVcc infection without compound. An unrelated monoclonal isotype control antibody served as negative control.

Figure 3 is a set of three graphs showing the synergistic effects of an anti-SRBI antibody in combination with direct acting antivirals on the inhibition of HCVcc infection. Huh7.5.1 cells were pre-incubated for 1 hour with serial concentrations of an anti-SRBI monoclonal antibody and a fixed concentration (0.001 μM) of VX-950 (**A**), ITMN-191 (**B**) or R7128 (**C**). Huh7.5.1 cells were then incubated with HCVcc in the presence of compounds. HCVcc infection was analyzed as described in Example 1. Data are expressed relative to HCVcc infection without compound. An unrelated monoclonal isotype control antibody served as negative control.

Figure 4 is a set of three graphs showing the synergistic effects of an anti-CLDN1-antibody (**A**) or an anti-CD81 antibody (**B**) or an anti-SRBI antibody (**C**) in combination with BMS-790052 on the inhibition of HCVcc infection. Huh7.5.1 cells were pre-incubated for 1 hour with serial concentrations of an anti-HCV-entry factor monoclonal antibody and a fixed concentration of BMS-790052 (0.001 nM). HCVcc infection was analyzed as described in Example 1. Data are expressed relative to HCVcc infection without compound. An unrelated monoclonal isotype control antibody served as negative control.

Figure 5 shows that combination of DAAs and entry inhibitors results in a synergistic activity. Huh7.5.1 cells were pre-incubated for 1 hour with serial concentrations of (**A**) protease inhibitors telaprevir, boceprevir, TMC-435 or danoprevir, (**B**) NS5A inhibitor daclatasvir or (**C**) polymerase inhibitors mericitabine or GS-7977 and 0.01 μg/ml of receptor-specific (anti-CD81, anti-SRBI or anti-

CLDN1) mAbs before incubation with HCVcc Luc-Jc1 in the presence of both compounds. HCVcc infection was analyzed and the CI for an IC_{50} is indicated in Table 2. Dotted lines at combination values of 0.9 and 1.1 indicate the boundaries of an additive interaction. Means±SEM from at least three independent experiments performed in triplicate are shown.

Figure 6 shows antiviral synergy of entry inhibitors and protease inhibitors. Combination of (**A**) boceprevir and anti-CLDN1 mAb or (**B**) TMC-435 and anti-CLDN1 mAb resulted in the highest shift in the IC_{50} of the respective protease inhibitors. Means ± SEM from at least three independent experiments performed in triplicate are shown. (**C-D**) Synergy was confirmed using the method of Prichard and Shipman. One representative experiment is shown.

Figure 7 shows antiviral synergy of entry inhibitors and the NS5A inhibitor, daclatasvir. Combination of daclatasvir and (**A**) the anti-SR-BI mAb, or (**B**) the anti-CLDN1 mAb resulted in the highest shift in the IC_{50} of the respective protease inhibitors. Means ± SEM from at least three independent experiments performed in triplicate are shown. (**C-D**) Synergy was confirmed using the method of Prichard and Shipman. One representative experiment is shown.

Figure 8 shows antiviral synergy of entry inhibitors and the polymerase inhibitor, GS-7977. Combination of GS-7977and (**A**) the anti-SR-BI mAb or (**B**) the anti-CLDN1 mAb decreased the IC_{50} of GS-7977 up to 210 fold. Means ± SEM from at least three independent experiments performed in triplicate are shown. (**C** and **D**) Synergy was confirmed using the method of Prichard and Shipman. One representative experiment is shown.

Figure 9 is a set of graphs showing that HCV entry inhibitors inhibit cell-free entry of protease inhibitor-resistant variants without cross-resistance. Huh7.5.1 cells were pre-incubated for 1 hour with serial concentrations of (**A**) telaprevir, (**B**) boceprevir, (**C**) CLDN1-specific mAb, (**D**) CD81-specific mAb, or (**E**) SR-BI-specific mAb or respective isotype control reagents before incubation with HCVcc-Jc1-Luc containing the DAA-resistant mutations R155T and A156S, respectively, in the presence of each compound. HCV infection was analyzed 72 hours post-incubation by luciferase reporter gene expression. Means ± SEM from at least three independent experiments performed in triplicate are shown.

Figure 10 is a set of graphs showing cell-cell transmission of protease inhibitor-resistant variants and its inhibition by an HCV entry inhibitor. NS5A+ HCV producer cells (Pi) were transfected with HCV RNA encoding for HCV Luc-Jc1 A156S (**A-C**) or Jc1 L36M R155K (**D-F**). NS5A+ HCV producer cells and target GFP-expressing cells (T) were co-cultivated with an anti-E2 mAb (AP33, 25 μg/mL) to block cell-free transmission. Cell-cell transmission of wild-type or drug resistant strains was determined by quantification of GFP+ NS5A+ target cells (Ti) by flow cytometry. Protease inhibitor-resistant HCV variant Luc-Jc1 A156S (**A-C**) and Jc1 L36M R155K (**D-F**) producer cells (Pi) cultured with uninfected target cells (T) were then incubated with CLDN1-specific mAb (**B** and **E**) or control IgG (**A** and **D**). HCV-infected target cells were quantified by flow cytometry. Cell-cell transmission of DAA-resistant variants, not affected by controls, is inhibited by CLDN1-specific mAb. Percentage of infected target cells is shown as histograms (**C** and **F**) and is represented as means ± SD from three experiments performed in triplicate.

Figure 11 is a graph showing that combination of a HCV-entry inhibitor and a direct acting antiviral (protease inhibitor) prevents DAA-resistance and results in sustained suppression of viral infection in a cell culture model for persistent HCV infection. Huh7.5.1 cells were transfected with RNA encoding wild-type HCV Luc-Jc1. The HCV Luc-Jc1 RNA transfected cells were passaged in the presence of protease inhibitor TMC-435 (250 ng/ml) alone or in combination with CLDN1-specific mAb (10 μg/ml) twice a week. 10^6 cells were harvested at each passage and viral load was assessed by luciferase activity twice a week and normalized by the control. Data are shown as % of HCV Jc-Luc infection in the absence of reagents (=100%). A representative experiment performed in triplicate is shown. The limit of detection (LOD) for positive luciferase reporter protein expression, indicated by a dashed line, was 3×10^3 RLU/assay normalized for the positive control.

Definitions

Throughout the specification, several terms are employed that are defined in the following paragraphs.

As used herein, the term "***subject***" refers to a human or another mammal (*e.g.*, primate, dog, cat, goat, horse, pig, mouse, rat, rabbit, and the like), that can be the host of Hepatitis C virus (HCV), but may or may not be infected with the virus,

and/or may or may not suffer from a HCV-related disease. Non-human subjects may be transgenic or otherwise modified animals. In many embodiments of the present invention, the subject is a human being. In such embodiments, the subject is often referred to as an "***individual***". The term "individual" does not denote a particular age, and thus encompasses newborns, children, teenagers, and adults.

As used herein, the term "***HCV***" refers to any major HCV genotype, subtype, isolate and/or quasispecies. HCV genotypes include, but are not limited to, genotypes 1, 2, 3, 4, 5, 6, and 7; HCV subtypes include, but are not limited to, subtypes 1a, 1b, 2a, 2b, 2c, 3a, 4a-f, 5a and 6a.

The terms "***afflicted with HCV***" or "***infected with HCV***" are used herein interchangeably. When used in reference to a subject, they refer to a subject that has at least one cell which is infected by HCV. The term "***HCV infection***" refers to the introduction of HCV genetic information into a target cell, such as by fusion of the target cell membrane with HCV or an HCV envelope glycoprotein-positive cell.

The terms "***HCV-related disease***" and "***HCV-associated disease***" are herein used interchangeably. They refer to any disease or disorder known or suspected to be associated with and/or caused, directly or indirectly, by HCV. HCV-related (or HCV-associated) diseases include, but are not limited to, a wide variety of liver diseases, such as subclinical carrier state of acute hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The term includes symptoms and side effects of any HCV infection, including latent, persistent and sub-clinical infections, whether or not the infection is clinically apparent.

The term "***treatment***" is used herein to characterize a method or process that is aimed at (1) delaying or preventing the onset of a disease or condition (*e.g.*, HCV infection or HCV-related disease); (2) slowing down or stopping the progression, aggravation, or deterioration of the symptoms of the disease or condition; (3) bringing about amelioration of the symptoms of the disease or condition; or (4) curing the disease or condition. A treatment may be administered prior to the onset of the disease or condition, for a prophylactic or preventive action. Alternatively or additionally, a treatment may be administered after initiation of the disease or condition, for a therapeutic action.

A "***pharmaceutical composition***" is defined herein as comprising an effective amount of a combination of the invention, and at least one pharmaceutically acceptable carrier or excipient.

As used herein, the term "***effective amount***" refers to any amount of a compound, agent, antibody, composition, or combination that is sufficient to fulfil its intended purpose(s), *e.g.*, a desired biological or medicinal response in a cell, tissue, system or subject. For example, in certain embodiments of the present invention, the purpose(s) may be: to prevent HCV infection, to prevent the onset of a HCV-related disease, to slow down, alleviate or stop the progression, aggravation or deterioration of the symptoms of a HCV-related disease (*e.g.*, chronic hepatitis C, cirrhosis, and the like); to bring about amelioration of the symptoms of the disease, or to cure the HCV-related disease.

The term "***pharmaceutically acceptable carrier or excipient***" refers to a carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredient(s) and which is not significantly toxic to the host at the concentration at which it is administered. The term includes solvents, dispersion, media, coatings, antibacterial and antifungal agents, isotonic agents, and adsorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art (see for example "*Remington's Pharmaceutical Sciences*", E.W. Martin, 18th Ed., 1990, Mack Publishing Co.: Easton, PA, which is incorporated herein by reference in its entirety).

The term "***antibody***", as used herein, refers to any immunoglobulin (*i.e.*, an intact immunoglobulin molecule, an active portion of an immunoglobulin molecule, etc.) that binds to a specific epitope. The term encompasses monoclonal antibodies and polyclonal antibodies. All derivatives and fragments thereof, which maintain specific binding ability, are also included in the term. The term also encompasses any protein having a binding domain, which is homologous or largely homologous to an immunoglobulin-binding domain. These proteins may be derived from natural sources, or partly or wholly synthetically produced.

The term "***specific binding***", when used in reference to an antibody, refers to an antibody binding to a predetermined antigen. Typically, the antibody binds with an affinity of at least 1 x 10^7 M^{-1}, and binds to the predetermined antigen with an affinity

that is at least two-fold greater than the affinity for binding to a non-specific antigen (*e.g.*, BSA, casein).

The term "***human Claudin-1*** or ***human CLDN1***" refers to a protein having the sequence shown in NCBI Accession Number NP_066924, or any naturally occurring variants commonly found in HCV permissive human populations. The term "***extracellular domain***" or "***ectodomain***" of Claudin-1 refers to the region of the Claudin-1 sequence that extends into the extracellular space.

The term "***human SR-BI***" refers the scavenger receptor class B member 1, a protein having the sequence shown in NCBI Accession Number NP_005496.4, or any naturally occurring variants commonly found in HCV permissive human populations. The term "***extracellular domain***" or "***ectodomain***" of human SR-BI refers to the region of the SR-BI sequence that extends into the extracellular space (*i.e.*, the space outside a cell).

The term "***human CD81***" refers the Cluster of Differentiation 81), a protein having the sequence shown in NCBI Accession Number NP_004347.1.

The terms "***susceptible cell***" and "***HCV-susceptible cell***" are used interchangeably. They refer to any cell that may be infected with HCV. Susceptible cells include, but are not limited to, liver or hepatic cells, primary cells, hepatoma cells, CaCo2 cells, dendritic cells, placental cells, endometrial cells, lymph node cells, lymphoid cells (B and T cells), peripheral blood mononuclear cells, and monocytes/macrophages.

The term "***preventing, inhibiting or blocking HCV infection***" when used in reference to an inventive combination means reducing the amount of HCV genetic information introduced into a susceptible cell or susceptible cell population as compared to the amount of HCV genetic information that would be introduced in the absence of the combination.

The term "***isolated***", as used herein in reference to a protein or polypeptide, means a protein or polypeptide, which by virtue of its origin or manipulation is separated from at least some of the components with which it is naturally associated or with which it is associated when initially obtained. By "isolated", it is alternatively

or additionally meant that the protein or polypeptide of interest is produced or synthesized by the hand of man.

The terms "***protein***", "***polypeptide***", and "***peptide***" are used herein interchangeably, and refer to amino acid sequences of a variety of lengths, either in their neutral (uncharged) forms or as salts, and either unmodified or modified by glycosylation, side-chain oxidation, or phosphorylation. In certain embodiments, the amino acid sequence is a full-length native protein. In other embodiments, the amino acid sequence is a smaller fragment of the full-length protein. In still other embodiments, the amino acid sequence is modified by additional substituents attached to the amino acid side chains, such as glycosyl units, lipids, or inorganic ions such as phosphates, as well as modifications relating to chemical conversions of the chains such as oxidation of sulfydryl groups. Thus, the term "protein" (or its equivalent terms) is intended to include the amino acid sequence of the full-length native protein, or a fragment thereof, subject to those modifications that do not significantly change its specific properties. In particular, the term "protein" encompasses protein isoforms, *i.e.*, variants that are encoded by the same gene, but that differ in their pI or MW, or both. Such isoforms can differ in their amino acid sequence (*e.g.*, as a result of allelic variation, alternative splicing or limited proteolysis), or in the alternative, may arise from differential post-translational modification (*e.g.*, glycosylation, acylation, phosphorylation).

The term "***analog***", as used herein in reference to a protein, refers to a polypeptide that possesses a similar or identical function as the protein but need not necessarily comprise an amino acid sequence that is similar or identical to the amino acid sequence of the protein or a structure that is similar or identical to that of the protein. Preferably, in the context of the present invention, a protein analog has an amino acid sequence that is at least 30%, more preferably, at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to the amino acid sequence of the protein.

The term "***fragment***" or the term "***portion***", as used herein in reference to a protein, refers to a polypeptide comprising an amino acid sequence of at least 5 consecutive amino acid residues (preferably, at least about: 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 250 or more amino acid residues) of the

amino acid sequence of a protein. The fragment of a protein may or may not possess a functional activity of the protein.

The term "***biologically active***", as used herein to characterize a protein variant, analog or fragment, refers to a molecule that shares sufficient amino acid sequence identity or homology with the protein to exhibit similar or identical properties to the protein. For example, in many embodiments of the present invention, a biologically active fragment of an inventive antibody is a fragment that retains the ability of the antibody to bind to a HCV receptor.

The term "***homologous***" (or "***homology***"), as used herein, is synonymous with the term "identity" and refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both compared sequences is occupied by the same base or same amino acid residue, the respective molecules are then homologous at that position. The percentage of homology between two sequences corresponds to the number of matching or homologous positions shared by the two sequences divided by the number of positions compared and multiplied by 100. Generally, a comparison is made when two sequences are aligned to give maximum homology. Homologous amino acid sequences share identical or similar amino acid sequences. Similar residues are conservative substitutions for, or "allowed point mutations" of, corresponding amino acid residues in a reference sequence. "Conservative substitutions" of a residue in a reference sequence are substitutions that are physically or functionally similar to the corresponding reference residue, *e.g.* that have a similar size, shape, electric charge, chemical properties, including the ability to form covalent or hydrogen bonds, or the like. Particularly preferred conservative substitutions are those fulfilling the criteria defined for an "accepted point mutation" as described by Dayhoff *et al.* ("Atlas of Protein Sequence and Structure", 1978, Nat. Biomed. Res. Foundation, Washington, DC, Suppl. 3, 22: 354-352).

The terms "***approximately***" and "***about***", as used herein in reference to a number, generally include numbers that fall within a range of 10% in either direction of the number (greater than or less than the number) unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

Detailed Description of Certain Preferred Embodiments

As mentioned above, the present invention provides combinations and methods for the treatment and prevention of HCV infection.

I - Combinations

A combination according to the invention comprises at least one direct acting antiviral and at least one anti-HCV-entry factor antibody, and is intended for use in the treatment or the prevention of HCV infection.

A. Anti-HCV-Entry Factor Antibodies

The term "***anti-HCV-entry factor antibody***", as used herein, refers to any antibody raised against a HCV receptor or co-receptor or HCV entry factor or co-factor (or a region of a HCV (co)-receptor or entry (co)-cofactor). The term also refers to any antibody directed against a cell surface protein involved in the HCV infection, in particular in HCV entry into susceptible cells. Examples of such HCV receptors or cell surface proteins include heparan sulfate, the LDL receptor (Agnello *et al.*, Proc. Natl. Acad. Sci. USA, 1999, 96: 12766-12771; Molina *et al.*, J. Hepatol., 2007, 46: 411-419), the tetraspanin CD81, the scavenger receptor class B type I (SR-BI), Occludin, Claudin-1 (CLDN1) or Niemann-Pick C1-like 1 cholesterol absorption receptor. Examples of HCV entry cofactors include the receptor tyrosine kinases.

Thus, anti-HCV-entry factor antibodies that are suitable for use in the practice of the present invention include antibodies against a HCV receptor selected from the group consisting of heparan sulfate, the LDL receptor, CD81, SR-BI, Occludin, CLDN1, and specific regions thereof. In certain preferred embodiments, the anti-HCV-entry factor antibodies are antibodies against CD81, SR-BI or CLDN1 (or specific regions thereof).

Examples of anti-heparan sulfate antibodies that can be used in the practice of the present invention include, but are not limited to, the antibodies described or used in Kurup *et al.*, J. Biol. Chem., 2007, 282: 21032-21042; Briani *et al.*, J. Neurol. Sci., 2005, 229-230; U.S. Pat. Appln. No. 2009/0136964.

Examples of anti-LDL receptor antibodies that can be used in the practice of the present invention include, but are not limited to, the antibodies described or used in Agnello *et al.*, Proc. Natl. Acad. Sci. USA, 1999, 96: 12766-12771; WO 01/68710;

WO 2002/048388; U.S. Pat. Appln. No. US 2008/0213287, and antibodies commercially available, for example, from Amersham International (*e.g.*, Clone C7).

Examples of anti-occludin antibodies that can be used in the practice of the present invention include, but are not limited to, the antibodies described or used in Tokunaga *et al.*, J. Histochem. Cytochem., 2007, 55: 735-744.

Examples of anti-CD81 antibodies that can be used in the practice of the present invention include, but are not limited to, the antibodies described or used in Meuleman *et al.*, Hepatology, 2008, 48: 1761-1769; Dijkstra *et al.*, Exp. Neurol., 2006, 202: 57-66; Azorsa *et al.*, J. Immunol. Methods, 1999, 229: 35-48.

Examples of anti-SR-BI antibodies that can be used in the practice of the present invention include, but are not limited to, the antibodies described or used in Haberstroh *et al.*, Gastroenterology, 2008, 135: 1719-1728; Barth *et al.*, J. Virol., 2008, 82: 3466-3479; Zeisel *et al.*, Hepatology, 2007, 46: 1722-1731; Catanese *et al.*, J. Virol., 2007, 81: 8063-8071; WO 2006/005465.

Examples of anti-CLDN1 antibodies that can be used in the practice of the present invention include, in particular, the polyclonal and monoclonal anti-CLDN1 antibodies that are disclosed in EP 08 305 597 and WO 2010/034812. As described in these documents, eight monoclonal antibodies have been produced by genetic immunization and shown to efficiently inhibit HCV infection by targeting the extracellular domain of CLDN1. Using an infectious HCV model system and primary human hepatocytes, these monoclonal anti-CLDN1 antibodies have been demonstrated to efficiently inhibit HCV infection of all major genotypes as well as highly variable HCV quasispecies in individual patients. Furthermore, these antibodies efficiently blocked entry of highly infectious HCV escape variants that were resistant to neutralizing antibodies in six patients with HCV re-infection during liver transplantation. The monoclonal anti-CLDN1 antibodies are called OM-4A4-D4, OM-7C8-A8, OM-6D9-A6, OM-7D4-C1, OM-6E1-B5, OM-3E5-B6, OM-8A9-A3, and OM-7D3-B3. Other suitable anti-CLDN1 antibodies are monoclonal antibodies secreted by any one of the hybridoma cell lines deposited by the Applicants at the DSMZ (Deutsche Sammlung von Mikro-organismen und Zellkuturen GmbH, Inhoffenstraße 7 B, 38124 Braunschweig, Germany) on July 29, 2008 under Accession Numbers DSM ACC2931, DSM ACC2932, DSM ACC2933, DSM

ACC2934, DSM ACC2935, DSM ACC2936, DSM ACC2937, and DSM ACC2938 (described in EP 08 305 597 and WO 2010/034812).

Other suitable anti-CLDN1 antibodies include those disclosed in European Pat. No. EP 1 167 389 and U.S. Pat. No. 6,627,439.

Other anti-HCV-entry factor antibodies that are suitable for use in the practice of the present invention include antibodies against a HCV entry cofactor selected from the receptor tyrosine kinases (RTKs). The receptor tyrosine kinases may belong to the EGF receptor family; insulin receptor family, PDGF receptor family, VEGF receptor family, HGF receptor family, Trk receptor family, Eph receptor family, AXL receptor family, LTK receptor family, TIE receptor family, ROR receptor family, DDR receptor family, RET receptor family; KLG receptor family, RYK receptor family, or MuSK receptor family.

Examples of anti-RTK monoclonal antibodies suitable for use in the context of the invention include, but are not limited to, anti-EGFR antibodies, such as Cetuximab, Panitumumab, Matuzumab, Zalutumumab, Nimotuzumab, and Necitumumab; anti-EphA2 antibodies such as those developed by MedImmune Inc.; anti-VEGF antibodies such as Bevacizumab and Ranibizumab; anti-Erb2 antibodies such as Trastuzumab; anti-HER2/neu antibodies such as Trastuzumab, Ertimaxomab, and Pertuzumab; anti-VEGFR2 antibodies such as Ramucirumab and Alacizumab pegol; anti-VEGF-A antibodies such as Ranibizumab and Bevacizumab; anti-PDGF-R antibodies such as Olaratumab; and anti-IGF-1 receptor antibodies such as Figitumumab; Robatumumal and Cixutumumab.

The anti-HCV-entry factor antibodies suitable for use in the present invention may be polyclonal antibodies or monoclonal antibodies. In certain preferred embodiments, the anti-HCV-entry factor antibody present in an inventive combination is a monoclonal antibody.

Anti-HCV-entry factor antibodies may be prepared by any suitable method known in the art. For example, an anti-HCV-entry factor monoclonal antibody may be prepared by recombinant DNA methods. These methods generally involve isolation of the genes encoding the desired antibody, transfer of the genes into a suitable vector, and bulk expression in a cell culture system. The genes or DNA encoding the desired monoclonal antibody may be readily isolated and sequenced

using conventional procedures (*e.g.*, using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Hybridoma cell lines may serve as a preferred source of such DNA. Suitable host cells for recombinant production of antibodies include, but are not limited to, appropriate mammalian host cells, such as CHO, HeLa, or CV1. Suitable expression plasmids include, without limitation, pcDNA3.1 Zeo, pIND(SP1), pREP8 (all commercially available from Invitrogen, Carlsbad, CA, USA), and the like. The antibody genes may be expressed *via* viral or retroviral vectors, including MLV-based vectors, vaccinia virus-based vectors, and the like. Cells may be grown using standard methods, in suitable culture media such as, for example, DMEM and RPMI-1640 medium. The anti-HCV-entry factor antibodies may be expressed as single chain antibodies. Isolation and purification of recombinantly produced antibodies may be performed by standard methods. For example, an anti-HCV-entry factor monoclonal antibody may be recovered and purified from cell cultures by protein A purification, ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, such as Protein A column, hydroxylapatite chromatography, lectin chromatography, or any suitable combination of these methods. High performance liquid chromatography (HPLC) can also be employed for purification.

Alternatively, an anti-HCV-entry factor antibody of a combination according to the present invention may be obtained from commercial sources.

In certain embodiments, an anti-HCV-entry factor antibody is used in its native form. In other embodiments, it may be truncated (*e.g.*, *via* enzymatic cleavage or other suitable method) to provide immunoglobulin fragments or portions, in particular, fragments or portions that are biologically active. Biologically active fragments or portions of an anti-HCV-entry factor antibody include fragments or portions that retain the ability of the antibody to interfere with HCV-host cells interactions, and/or to specifically bind to the HCV-receptor, and/or to inhibit or block HCV entry into susceptible cells, and/or to reduce or prevent HCV infection of susceptible cells.

A biologically active fragment or portion of an anti-HCV-entry factor antibody may be an Fab fragment or portion, an $F(ab')_2$ fragment or portion, a variable domain, or one or more CDRs (complementary determining regions) of the antibody. Alternatively, a biologically active fragment or portion of an anti-HCV-entry factor antibody may be derived from the carboxyl portion or terminus of the antibody protein and may comprise an Fc fragment, an Fd fragment or an Fv fragment.

Antibody fragments of the present invention may be produced by any suitable method known in the art including, but not limited to, enzymatic cleavage (*e.g.*, proteolytic digestion of intact antibodies) or by synthetic or recombinant techniques. $F(ab')_2$, Fab, Fv and ScFv (single chain Fv) antibody fragments can, for example, be expressed in and secreted from mammalian host cells or from *E. coli*. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. The various portions of antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques.

Anti-HCV-entry factor antibodies (or fragments thereof) suitable for use in a combination according to the present invention may be produced in a modified form, such as a fusion protein (*i.e.*, an immunoglobulin molecule or portion linked to a polypeptide entity). Preferably, the fusion protein retains the biological property of the antibody. A polypeptide entity to be fused to an anti-HCV-entry factor antibody, or a fragment thereof, may be selected to confer any of a number of advantageous properties to the resulting fusion protein. For example, the polypeptide entity may be selected to provide increased expression of the recombinant fusion protein. Alternatively or additionally, the polypeptide entity may facilitate purification of the fusion protein, for example, by acting as a ligand in affinity purification. A proteolytic cleavage site may be added to the recombinant protein so that the desired sequence can ultimately be separated from the polypeptide entity after purification. The polypeptide entity may also be selected to confer an improved stability to the fusion protein, when stability is a goal. Examples of suitable polypeptide entities include, for example, polyhistidine tags, that allow for the easy purification of the resulting fusion protein on a nickel chelating column. Glutathione-S-transferase

(GST), maltose B binding protein, or protein A are other examples of suitable polypeptide entities.

Depending on the use intended, an anti-HCV-entry factor antibody of a combination of the invention may be re-engineered so as to optimize stability, solubility, *in vivo* half-life, or ability to bind additional targets. Genetic engineering approaches as well as chemical modifications to accomplish any or all of these changes in properties are well known in the art. For example, the addition, removal, and/or modification of the constant regions of an antibody are known to play a particularly important role in the bioavailability, distribution, and half-life of therapeutically administered antibodies. The antibody class and subclass, determined by the Fc or constant region of the antibody (which mediates effector functions), when present, imparts important additional properties.

Additional fusion proteins of the invention may be generated through the techniques of DNA shuffling well known in the art (see, for example, U.S. Pat. Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458).

Anti-HCV-entry factor antibodies suitable for use in a combination according to the present invention may also be "humanized": sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site-directed mutagenesis of individual residues or by grafting of entire regions or by chemical synthesis. Humanized antibodies can also be produced using recombinant methods. In the humanized form of the antibody, some, most or all of the amino acids outside the CDR regions are replaced with amino acids from human immunoglobulin molecules, while some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not significantly modify the biological activity of the resulting antibody. Suitable human "replacement" immunoglobulin molecules include IgG1, IgG2, IgG2a, IgG2b, IgG3, IgG4, IgA, IgM, IgD or IgE molecules, and fragments thereof. Alternatively, the T-cell epitopes present in rodent antibodies can be modified by mutation (de-immunization) to generate non-immunogenic rodent antibodies that can be applied for therapeutic purposes in humans (see www.accurobio.com).

Anti-HCV-entry factor antibodies (or biologically active variants or fragments thereof) suitable for use in a combination according to the invention may be functionally linked (*e.g.*, by chemical coupling, genetic fusion, non-covalent association or otherwise) to one or more other molecular entities. Methods for the preparation of such modified antibodies (or conjugated antibodies) are known in the art (see, for example, "*Affinity Techniques. Enzyme Purification: Part B*", Methods in Enzymol., 1974, Vol. 34, Jakoby and Wilneck (Eds.), Academic Press: New York, NY; and Wilchek and Bayer, Anal. Biochem., 1988, 171: 1-32). Preferably, molecular entities are attached at positions on the antibody molecule that do not interfere with the binding properties of the resulting conjugate, *e.g.*, positions that do not participate in the specific binding of the antibody to its target.

The antibody molecule and molecular entity may be covalently, directly linked to each other. Or, alternatively, the antibody molecule and molecular entity may be covalently linked to each other through a linker group. This can be accomplished by using any of a wide variety of stable bifunctional agents well known in the art, including homofunctional and heterofunctional linkers.

In certain embodiments, an anti-HCV-entry factor antibody (or a biologically active fragment thereof) of a combination of the present invention is conjugated to a therapeutic moiety. Any of a wide variety of therapeutic moieties may be suitable for use in the practice of the present invention including, without limitation, cytotoxins (*e.g.*, cytostatic or cytocidal agents), therapeutic agents, and radioactive metal ions (*e.g.*, alpha-emitters and alpha-emitters attached to macrocyclic chelators such as DOTA). Cytotoxins or cytotoxc agents include any agent that is detrimental to cells. Examples include, but are not limited to, paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, thymidine kinase, endonuclease, RNAse, and puromycin and fragments, variants or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin,

mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin and doxorubicin), antibiotics (*e.g.*, dactinomycin, bleomycin, mithramycin, and anthramycin), and anti-mitotic agents (*e.g.*, vincristine and vinblastine). Combinations comprising the resulting antibody conjugate may find application in the treatment of liver cancer associated with HCV infection (see below).

Other therapeutic moieties include proteins or polypeptides possessing a desired biological activity. Such proteins include, but are not limited to, toxins (*e.g.*, abrin, ricin A, alpha toxin, pseudomonas exotoxin, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin); proteins such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; apoptotic agents (*e.g.*, TNF-α, TNF-β) or, biological response modifiers (*e.g.*, lymphokines, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), or other growth factors).

Thus, an inventive combination of the present invention may comprise anti-HCV-entry factor antibodies under the form of full length antibodies, biologically active variants or fragments thereof, chimeric antibodies, humanized antibodies, and antibody-derived molecules comprising at least one complementary determining region (CDR) from either a heavy chain or light chain variable region of an anti-HCV-entry factor antibody, including molecules such as Fab fragments, $F(ab')_2$ fragments, Fd fragments, Fabc fragments, Sc antibodies (single chain antibodies), diabodies, individual antibody light single chains, individual antibody heavy chains, chimeric fusions between antibody chains and other molecules, and antibody conjugates, such as antibodies conjugated to a therapeutic agent.

B. Direct Acting Antivirals

The combinations of the present invention comprise at least one anti-HCV-entry factor antibody and at least one direct acting antiviral. The terms "***direct acting antiviral***", "***direct acting antiviral agent***", "***DAA***", "***specifically targeted antiviral therapy for hepatitis C***" and "***STAT-C***" are used herein interchangeably. They refer to molecules that interfere with specific steps of the lifecycle of HCV and are thus useful in the prevention or treatment of HCV infection.

A major effort of the pharmaceutical industry is being focused on the development of direct-acting antiviral agents. Direct acting antivirals have been shown to increase the efficacy of the standard of care in randomized clinical trials (for a review, see Hofmann *et al.*, Nat. Rev. Gastroenterol. Hepatol.; 2011, 8: 257-264). However, along with these encouraging results, significant treatment-related adverse events including rash, gastrointestinal side-effects, and anemia as well as the emergence of HCV resistance have been reported (Poordad *et al.*, New Engl. J. Med., 2011, 364: 1195-1206; Hezode *et al.*, New Engl. J. Med., 2009, 360: 1839-1850; Pawlotsky *et al.*, Hepatology, 2011, 53: 1742-1751).

As mentioned above, the present Applicants have shown that an anti-CLDN1 antibody, or an anti-CD81 antibody, or an anti-SRBI antibody, in combination with a direct acting antiviral act in high synergy to inhibit HCV infection. The efficient inhibition observed for these combinations suggest that they may represent a new approach for interferon- or ribavirin-free treatment strategies. Furthermore, such combinations may overcome antiviral resistance as they result in a greater decrease of the viral load in shorter periods of time, thereby limiting the frequency of appearance of resistant variants.

A direct acting antiviral agent suitable for use in a combination of the present invention may exert its effects by any mechanism that interferes with one or more specific steps of the lifecycle of HCV. For example, VX-950 (also known as telaprevir), ITMN-191 (also known as danoprevir), telaprevir, boceprevir, danoprevir and TMC-435 used by the present Applicants are HCV protease inhibitors, mericitabine (also known as RG7128) and GS-7977 are HCV polymerase inhibitors, and daclatasvir (also known as BMS-790052) is an NS5A inhibitor. Thus, in certain preferred embodiments, the at least one direct acting antiviral present in a combination according to the invention is a HCV protease inhibitor or a HCV polymerase inhibitor or an NS5A inhibitor.

Protease inhibitors suitable for use in the context of the present invention include NS3/4A protease inhibitors. Examples of NS3/4A protease inhibitors that can be present in a combination of the present invention include, but are not limited to, VX-950 (also known as telaprevir), ITMN-191 (also known as danoprevir), boceprevir, BMS-650032, VX-985, BI 201335, and TMC435. In certain preferred embodiments,

the at least one direct acting antiviral present in a combination according to the invention is NS3/4A protease inhibitor telaprevir, boceprevir, danoprevir or TMC-435.

Telaprevir (also known as VX-950), marketed under the tradename INCIVEK®, was co-developed by Vertex Pharmaceuticals, Inc. and Johnson & Johnson. In May 2011, the FDA approved telaprevir for the treatment of patients with genotype 1 chronic hepatitis C. ITMN-191 (also known as R7227 or danoprevir) was being co-developed by Roche and InterMune Inc., but is now fully owned by Roche. Boceprevir (initially developed by Schering-Plough, and then by Merck and marketed under the tradename VICTRELIS®) was approved by the FDA for the treatment of hepatitic C genotype 1 in May 2011. BMS-650032 is being developed by Bristol-Myers-Squibb. VX-985 is a NS3/4A protease inhibitor being developed by Vertex Pharmaceuticals, Inc. BI 201335 is being developed by Boehringer Ingelheim and is now in Phase III clinical trials in the United States. TMC435, a NS3/4A protease inhibitor being developed by Medivir/Tibotec/Johnson & Johnson, is also in Phase III clinical trials.

Other examples of NS3/4A protease inhibitors that can be present in a combination according to the invention include, but are not limited to, NS3/4A protease inhibitors that are currently in phase II clinical trials such as GS 9256 and GS 9451 (being developed by Gilead), MK-7009 (also known as vaniprevir, being developed by Merck), ACH-1625 (being developed by Achillion), and ABT-450 (being developed by Abbott/Enanta); NS3/4A protease inhibitors that are currently in phase I clinical trials such as BMS-791325 (being developed by Bristol-Myers Squibb), VX-985 and VX-500 (being developed by Vertex pharmaceuticals), and PHX1766 (being developed by Phenomix); and NS3/4A protease inhibitors that are currently in preclinical trials such as VX-813 (being developed by Vertex), AVL-181 and AVL-192 (being developed by Avila Therapeutics), and ACH-2684 (being developed by Achillion).

Polymerase inhibitors suitable for use in the context of the present invention include NS5B polymerase inhibitors. The NS5B, an RNA-dependent RNA polymerase (RdRp) enzyme, is a highly conserved structure across all hepatitis C genotypes. It is therefore, an ideal target for drug therapy. There are two classes of

polymerase inhibitors: nucleoside/nucleotide analogues and non-nucleoside RdRp inhibitors. Nucleoside inhibitors target the catalytic sites of the enzyme and act as chain terminators. Non-nucleoside inhibitors are allosteric inhibitors. In certain embodiments, the at least one direct acting antiviral present in a combination according to the invention is HCV NS5B polymerase inhibitor, mericitabine or GS-7977.

Mericitabine (also known as RG7128 or RO5024048), is a prodrug of PSI-6130, an oral cytidine nucleoside analogue. It is being developed by Roche and Pharmasset. Mericitabine has shown *in vitro* activity against all of the most common HCV genotypes.

GS-7977 (also known as PSI-7977) is being developed by Gilead Sciences. It is currently in Phase III clinical trials. It is being studied as a treatment to be used in combination with ribavirin. GS-78977 is a prodrug that is metabolized to the active antiviral agent 2'-deoxy-2'-α-fluoro-β-*C*-methyluridine-5'-monophosphate.

Other examples of NS5B polymerase inhibitors that can be present in a combination according to the invention include, but are not limited to, nucleoside/nucleotide polymerase inhibitors that are currently in Phase II clinical trials such as IDX184 (being developed by Idenix) and PSI-7977 (being developed by Pharmasset); non-nucleoside polymerase inhibitors that are currently in Phase II clinical trials such as VX-222 (initially developed by ViroChem, now owned by Vertex); PF-868554 (being developed by Pfizer); ABT-072 and ABT-333 (being developed by Abbott), GS 9190 (being developed by Gilead) and ANA598 (also known as setrobuvir, being developed by Anadys); nucleoside/nucleotide polymerase inhibitors that are currently in Phase I clinical trials such as BI 207127 (being developed by Boehringer Ingelheim), MK-0608 (being developed by Isis/Merck), TMC649128 (being developed by Medivir/Tibotec), RG7348 (being developed by Roche/Ligand (Metabasis)), PSI-938 (being developed by Pharmasset), and INX-189 (being developed by Inhibitex); and non-nucleoside polymerase inhibitors that are currently in Phase I clinical trials such as VCH-759 (initially developed by ViroChem Pharma, now owned by Vertex), IDX375 (being developed by Idenix), and A-837093 (being developed by Abbott).

NS5A inhibitors suitable for use in the context of the present invention include, in particular in particular daclatasvir (also known as BMS-790052), which was developed by Bristol-Myers-Squibb.

C. Properties of the Combinations

A combination according to the present invention is such that (1) it is intended for use in the treatment or the prevention of HCV infection and (2) the at least one anti-HCV-entry factor antibody and at least one direct acting antiviral act in a highly synergistic manner to inhibit HCV infection of susceptible cells.

In certain embodiments, the direct acting antiviral decreases the IC_{50} for the inhibition of HCV infection by the anti-HCV-entry factor antibody by a factor of at least 10 fold or at least 15 fold, preferably at least 20 fold or at least 25 fold, more preferably at least 30 fold or 40 fold, and even more preferably 50 fold or more than 50 fold. In other words, in the presence of the direct acting antiviral, the concentration of anti-HCV-entry factor antibody necessary to obtain a 50% inhibition of HCV entry is at least 10 times or at least 15 times, preferably at least 20 times or at least 25 times, more preferably at least 30 times or at least 40 times, and even more preferably 50 times lower or more than 50 times lower than the concentration of anti-HCV-entry factor antibody that would be necessary to obtain the same HCV entry inhibition in the absence of direct acting antiviral.

In other embodiments, the anti-HCV-entry factor antibody decreases the IC_{50} for the inhibition of HCV infection by the direct acting antiviral by a factor of at least 5 times or 10 fold or at least 15 fold, preferably at least 20 fold or at least 25 fold, more preferably at least 30 fold or 40 fold, and even more preferably 50 fold or more than 50 fold. In other words, in the presence of the anti-HCV-entry factor antibody, the concentration of direct acting antiviral necessary to obtain a 50% inhibition of HCV entry is at least 5 times or at least 10 times or at least 15 times, preferably at least 20 times or at least 25 times, more preferably at least 30 times or at least 40 times, and even more preferably 50 times lower or more than 50 times lower than the concentration of direct acting antiviral that would be necessary to obtain the same HCV entry inhibition in the absence of anti-HCV-entry factor antibody.

In certain embodiments, a combination of the present invention is characterized by a combination index (CI) that is lower than 1 (which is defined as a marked

synergy). A combination of the present invention is preferably characterized by a CI lower than 1, preferably lower than 0.50 or lower than 0.25, more preferably lower than 0.15, and even more preferably lower than 0.10.

II - Treatment or Prevention of HCV infection and HCV-associated Diseases

A. Indications

The combinations according of the present invention may be used in therapeutic and prophylactic methods to treat and/or prevent HCV infection, or to treat and/or prevent a liver disease or a pathological condition affecting HCV-susceptible cells, such as liver cells, lymphoid cells, or monocytes/macrophages.

Methods of treatment of the present invention may be accomplished using an inventive combination or a pharmaceutical composition comprising an inventive combination (see below). These methods generally comprise administration of an effective amount of at least one anti-HCV-entry factor antibody and at least one direct acting antiviral, or a pharmaceutical composition thereof, to a subject in need thereof. The anti-HCV-entry factor antibody and direct acting antiviral may be administered concurrently (*i.e.*, together or separately but at about the same time, *e.g.*, within 5 minutes, 15 minutes or 30 minutes of each other), or alternatively, they may be administered sequentially (*i.e.*, separately and at different times, *e.g.*, different times of the same day or different times of the same week or different times of the same month, etc…).

Administration may be performed using any of the methods known to one skilled in the art. In particular, the combination of an anti-HCV-entry factor antibody and a direct acting antiviral, or a composition thereof, may be administered by various routes including, but not limited to, aerosol, parenteral, oral or topical route.

In general, the combination, or a pharmaceutical composition thereof, will be administered in an effective amount, *i.e.* an amount that is sufficient to fulfill its intended purpose. The exact amount of the combination or pharmaceutical composition to be administered will vary from subject to subject, depending on the age, sex, weight and general health condition of the subject to be treated, the desired biological or medical response (*e.g.*, prevention of HCV infection or treatment of HCV-associated liver disease), and the like. In many embodiments, an effective

amount is one that inhibits or prevents HCV from entering into a subject's susceptible cells and/or infecting a subject's cells, so as to prevent HCV infection, treat or prevent liver disease or another HCV-associated pathology in the subject.

Combinations and compositions of the present invention may be used in a variety of therapeutic or prophylactic methods. In particular, the present invention provides a method for treating or preventing a liver disease or pathology in a subject, which comprises administering to the subject an effective amount of at least one anti-HCV-entry factor antibody and at least one direct acting antiviral (as defined above) (or composition thereof) which inhibits HCV from entering or infecting the subject's cells, so as to treat or prevent the liver disease or pathology in the subject. The liver disease or pathology may be inflammation of the liver, liver fibrosis, cirrhosis, and/or hepatocellular carcinoma (*i.e.*, liver cancer) associated with HCV infection.

The present invention also provides a method for treating or preventing a HCV-associated disease or condition (including a liver disease) in a subject, which comprises administering to the subject an effective amount of at least one anti-HCV-entry factor antibody and at least one direct acting antiviral (as defined above) (or composition thereof) which inhibits HCV from entering or infecting the subject's cells, so as to treat or prevent the HCV-associated disease or condition in the subject. In certain embodiments of the present invention, the combination (or composition thereof) is administered to a subject diagnosed with acute hepatitis C. In other embodiments of the invention, the combination (or composition thereof) is administered to a subject diagnosed with chronic hepatitis C.

Administration of an inventive combination according to such methods may result in amelioration of at least one of the symptoms experienced by the individual including, but not limited to, symptoms of acute hepatitis C such as decreased appetite, fatigue, abdominal pain, jaundice, itching, and flu-like symptoms; symptoms of chronic hepatitis C such as fatigue, marked weight loss, flu-like symptoms, muscle pain, joint pain, intermittent low-grade fevers, itching, sleep disturbances, abdominal pain, appetite changes, nausea, diarrhea, dyspepsia, cognitive changes, depression, headaches, and mood swings; symptoms of cirrhosis such as ascites, bruising and bleeding tendency, bone pain, varices (especially in the stomach and esophagus), steatorrhea, jaundice and hepatic encephalopathy; and symptoms of extrahepatic

manifestations associated with HCV such as thyroiditis, porphyria cutanea tarda, cryoglobulinemia, glomerulonephritis, sicca syndrome, thrombocytopenia, lichen planus, diabetes mellitus and B-cell lymphoproliferative disorders.

Alternatively or additionally, administration of a combination or composition thereof according to such methods may slow down, reduce, stop or alleviate the progression of HCV infection or an HCV-associated disease, or reverse the progression to the point of eliminating the infection or disease. Administration of a combination or composition of the present invention according to such methods may also result in reduction in the number of viral infections, reduction in the number of infectious viral particles, and/or reduction in the number of virally infected cells.

The effects of a treatment according to the invention may be monitored using any of the assays known in the art for the diagnosis of HCV infection and/or liver disease. Such assays include, but are not limited to, serological blood tests, liver function tests to measure one or more of albumin, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), and gamma glutamyl transpeptidase (GGT), and molecular nucleic acid tests using different techniques such as polymerase chain reaction (PCR), transcription mediated amplification (TMA), or branched DNA (bDNA).

Combinations and pharmaceutical compositions of the present invention may also be used in immunization therapies. Accordingly, the present invention provides a method of reducing the likelihood of susceptible cells of becoming infected with HCV as a result of contact with HCV. The method comprises contacting the susceptible cells with an effective amount of at least one anti-HCV-entry factor antibody and at least one direct acting antiviral (as defined above) or a composition thereof which inhibits HCV from entering or infecting the susceptible cells, so as to reduce the likelihood of the cells to become infected with HCV as a result of contact with HCV. The present invention also provides a method of reducing the likelihood of a subject's susceptible cells of becoming infected with HCV as a result of contact with HCV. In this method, contacting the susceptible cells with a combination or a pharmaceutical composition may be performed by administrating the combination or pharmaceutical composition to the subject.

Reducing the likelihood of susceptible cells or of a subject of becoming infected with HCV means decreasing the probability of susceptible cells or a subject to become infected with HCV as a result of contact with HCV. The decrease may be of any significant amount, *e.g.*, at least a 2-fold decrease, more than a 2-fold decrease, at least a 10-fold decrease, more than a 10-fold decrease, at least a 100-fold decrease, or more than a 100-fold decrease.

In certain embodiments, the subject is infected with HCV prior to administration of the inventive composition. In other embodiments, the subject is not infected with HCV prior to administration of the inventive composition. In yet other embodiments, the subject is not infected with, but has been exposed to, HCV. In certain embodiments, the subject may be infected with HIV or HBV.

For example, the methods of the present invention may be used to reduce the likelihood of a subject's susceptible cells of becoming infected with HCV as a result of liver transplant. As already mentioned above, when a diseased liver is removed from a HCV-infected patient, serum viral levels plummet. However, after receiving a healthy liver transplant, virus levels rebound and can surpass pre-transplant levels within a few days (Powers *et al.*, Liver Transpl., 2006, 12: 207-216). Liver transplant patients may benefit from administration of a combination according the invention. Administration may be performed prior to liver transplant, during liver transplant, and/or following liver transplant.

Other subjects that may benefit from administration of a combination of an anti-HCV-entry factor antibody and a direct acting antiviral according to the present invention include, but are not limited to, babies born to HCV-infected mothers, in particular if the mother is also HIV-positive; health-care workers who have been in contact with HCV-contaminated blood or blood contaminated medical instruments; drug users who have been exposed to HCV by sharing equipments for injecting or otherwise administering drugs; and people who have been exposed to HCV through tattooing, ear/body piercing and acupuncture with poor infection control procedures.

Other subjects that may benefit from administration of a combination according to the invention include, but are not limited to, subjects that exhibit one or more factors that are known to increase the rate of HCV disease progression. Such factors include, in particular, age, gender (males generally exhibit more rapid disease

progression than females), alcohol consumption, HIV co-infection (associated with a markedly increased rate of disease progression), and fatty liver.

Still other subjects that may benefit from administration of a combination according to the invention include patients with HCV infections that are resistant to the standard of care or to other combinations of antivirals – antiviral resistance being a major challenge in HCV prevention and treatment (Pawlotsky *et al.*, Hepatology, 2011, 53: 1742-1751).

In certain embodiments, the HCV infection or HCV-related disease to be treated by a combination according to the invention is caused by a Hepatitis C virus that is resistant to a direct acting antiviral. The recent development of direct acting antiviral molecules, together with clinical studies showing that these drugs may lead to the selection of resistant viruses if administered alone or in combination therapy, has raised concerns that resistance may undermine DAA-based therapy (Pawlotsky, Hepatology, 2011, 53: 1742-1751; Schaefer et al., Gastroenterology, 2012, 142: 1340-1350 e1341). However, the present Applicants have shown that an anti-HCV entry factor antibody prevents dissemination of DAA-resistant HCV variants resulting in more rapid and sustained virus elimination. The Applicants have also shown that a combination of an anti-HCV entry factor antibody and of a direct acting antiviral prevents DAA resistance allowing suppression of viral infection below the detection limit in a sustained manner.

In certain embodiments, a combination of an anti-HCV-entry factor antibody and a direct acting antiviral or a pharmaceutical composition thereof is administered alone according to a method of treatment of the present invention. In other embodiments, a combination of an anti-HCV-entry factor antibody and a direct acting antiviral or a pharmaceutical composition thereof is administered in combination with at least one additional therapeutic agent. The combination or pharmaceutical composition may be administered prior to administration of the therapeutic agent, concurrently with the therapeutic agent, and/or following administration of the therapeutic agent.

Therapeutic agents that may be administered in combination with an inventive combination or pharmaceutical composition may be selected among a large variety of biologically active compounds that are known to have a beneficial effect in the

treatment or prevention of HCV infection, or a HCV-associated disease or condition. Such agents include, in particular, antiviral agents including, but not limited to, interferons (*e.g.*, interferon-alpha, pegylated interferon-alpha), ribavirin, anti-HCV (monoclonal or polyclonal) antibodies, RNA polymerase inhibitors, protease inhibitors, IRES inhibitors, helicase inhibitors, antisense compounds, ribozymes, micro-RNA antagonists, cytokines, therapeutic vaccines, NS5A antagonists, polymerase inhibitors, cyclophilin A antagonists, and any combination thereof.

B. Administration

An inventive combination (optionally after formulation with one or more appropriate pharmaceutically acceptable carriers or excipients), in a desired dosage can be administered to a subject in need thereof by any suitable route. Various delivery systems are known and can be used to administer combinations of the present invention, including tablets, capsules, injectable solutions, encapsulation in liposomes, microparticles, microcapsules, etc. Methods of administration include, but are not limited to, dermal, intradermal, intramuscular, intraperitoneal, intralesional, intravenous, subcutaneous, intranasal, pulmonary, epidural, ocular, and oral routes. An inventive combination or composition may be administered by any convenient or other appropriate route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral, mucosa, rectal and intestinal mucosa, etc). Administration can be systemic or local. Parenteral administration may be preferentially directed to the patient's liver, such as by catheterization to hepatic arteries or into a bile duct. As will be appreciated by those of ordinary skill in the art, in embodiments where the anti-HCV-entry factor antibody and direct acting antiviral are administered sequentially (*i.e.*, at different times or separately but at substantially the same time), the anti-HCV-entry factor antibody and direct acting antiviral may be administered by the same route (*e.g.*, intravenously) or by different routes (*e.g.*, orally and intravenously). Similarly, in embodiments where an inventive combination is administered along with an additional therapeutic agent, the combination and therapeutic agent may be administered by the same route or different routes.

C. Dosage

Administration of an inventive combination (or a composition thereof) of the present invention will be in a dosage such that the amount delivered is effective for

the intended purpose. The route of administration, formulation and dosage administered will depend upon the therapeutic effect desired, the severity of the HCV-related condition to be treated if already present, the presence of any infection, the age, sex, weight, and general health condition of the patient as well as upon the potency, bioavailability, and *in vivo* half-life of the anti-HCV-entry factor antibody and the direct acting antiviral used, the use (or not) of concomitant therapies, and other clinical factors. These factors are readily determinable by the attending physician in the course of the therapy. Alternatively or additionally, the dosage to be administered can be determined from studies using animal models (*e.g.*, chimpanzee or mice). Adjusting the dose to achieve maximal efficacy based on these or other methods are well known in the art and are within the capabilities of trained physicians. As studies are conducted using the inventive combination of an anti-HCV-entry factor antibody and a direct acting antiviral, further information will emerge regarding the appropriate dosage levels and duration of treatment.

A treatment according to the present invention may consist of a single dose or multiple doses. Thus, administration of an inventive combination, or pharmaceutical composition thereof, may be constant for a certain period of time or periodic and at specific intervals, *e.g.*, hourly, daily, weekly (or at some other multiple day interval), monthly, yearly (*e.g.*, in a time release form). Alternatively, the delivery may occur at multiple times during a given time period, *e.g.*, two or more times per week; two or more times per month, and the like. The delivery may be continuous delivery for a period of time, *e.g.*, intravenous delivery.

In general, the amount of combination administered will preferably be in the range of about 1 ng/kg to about 100 mg/kg body weight of the subject, for example, between about 100 ng/kg and about 50 mg/kg body weight of the subject; or between about 1 µg/kg and about 10 mg/kg body weight of the subject, or between about 100 µg/kg and about 1 mg/kg body weight of the subject.

III - Pharmaceutical Compositions

As mentioned above, a combination of the invention may be administered *per se* or as a pharmaceutical composition. Accordingly, the present invention provides pharmaceutical compositions comprising an effective amount of at least one anti-HCV-entry factor antibody and at least one direct acting antiviral as described herein

and at least one pharmaceutically acceptable carrier or excipient. In some embodiments, the composition further comprises one or more additional biologically active agents.

The combinations and pharmaceutical compositions thereof may be administered in any amount and using any route of administration effective for achieving the desired prophylactic and/or therapeutic effect. The optimal pharmaceutical formulation can be varied depending upon the route of administration and desired dosage. Such formulations may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the administered active ingredient.

The pharmaceutical compositions of the present invention may be formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "unit dosage form", as used herein, refers to a physically discrete unit of an anti-HCV-entry factor antibody or of a direct acting antiviral or of both an anti-HCV-entry factor antibody and a direct acting antiviral for the patient to be treated. It will be understood, however, that the total daily dosage of the compositions will be decided by the attending physician within the scope of sound medical judgement.

A. Formulation

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents, and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 2,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solution or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid may also be used in the preparation of injectable formulations. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration.

Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile

injectable medium prior to use. Liquid pharmaceutical compositions which are sterile solutions or suspensions can be administered by, for example, intravenous, intramuscular, intraperitoneal or subcutaneous injection. Injection may be *via* single push or by gradual infusion. Where necessary or desired, the composition may include a local anesthetic to ease pain at the site of injection.

In order to prolong the effect of an active ingredient (*i.e.*, a combination of an anti-HCV-entry factor antibody and a direct acting antiviral), it is often desirable to slow the absorption of the ingredient from subcutaneous or intramuscular injection. Delaying absorption of a parenterally administered active ingredient may be accomplished by dissolving or suspending the ingredient in an oil vehicle. Injectable depot forms are made by forming micro-encapsulated matrices of the active ingredient in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of active ingredient to polymer and the nature of the particular polymer employed, the rate of ingredient release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations can also be prepared by entrapping the active ingredient in liposomes or microemulsions which are compatible with body tissues.

Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, elixirs, and pressurized compositions. In addition to the active principles, the liquid dosage form may contain inert diluents commonly used in the art such as, for example, water or other solvent, solubilising agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cotton seed, ground nut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols, and fatty acid esters of sorbitan and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, suspending agents, preservatives, sweetening, flavouring, and perfuming agents, thickening agents, colors, viscosity regulators, stabilizes or osmo-regulators. Examples of suitable liquid carriers for oral administration include water (potentially containing additives as above, *e.g.*, cellulose derivatives, such as sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols such as glycols) and their derivatives,

and oils (*e.g.*, fractionated coconut oil and arachis oil). For pressurized compositions, the liquid carrier can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Solid dosage forms for oral administration include, for example, capsules, tablets, pills, powders, and granules. In such solid dosage forms, an inventive combination may be mixed with at least one inert, physiologically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and one or more of: (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannital, and silicic acid; (b) binders such as, for example, carboxymethylcellulose, alginates, gelatine, polyvinylpyrrolidone, sucrose, and acacia; (c) humectants such as glycerol; (d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (e) solution retarding agents such as paraffin; absorption accelerators such as quaternary ammonium compounds; (g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate; (h) absorbents such as kaolin and bentonite clay; and (i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulphate, and mixtures thereof. Other excipients suitable for solid formulations include surface modifying agents such as non-ionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate, and triethanolamine. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatine capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition such that they release the active ingredient(s) only, or preferably, in a certain part of the intestinal tract, optionally, in a delaying manner.

Examples of embedding compositions which can be used include polymeric substances and waxes.

In certain embodiments, it may be desirable to administer an inventive composition locally to an area in need of treatment (*e.g.*, the liver). This may be achieved, for example, and not by way of limitation, by local infusion during surgery (*e.g.*, liver transplant), topical application, by injection, by means of a catheter, by means of suppository, or by means of a skin patch or stent or other implant.

For topical administration, the composition is preferably formulated as a gel, an ointment, a lotion, or a cream which can include carriers such as water, glycerol, alcohol, propylene glycol, fatty alcohols, triglycerides, fatty acid esters, or mineral oil. Other topical carriers include liquid petroleum, isopropyl palmitate, polyethylene glycol, ethanol (95%), polyoxyethylenemonolaurat (5%) in water, or sodium lauryl sulphate (5%) in water. Other materials such as antioxidants, humectants, viscosity stabilizers, and similar agents may be added as necessary.

In addition, in certain instances, it is expected that the inventive compositions may be disposed within transdermal devices placed upon, in, or under the skin. Such devices include patches, implants, and injections which release the active ingredient by either passive or active release mechanisms. Transdermal administrations include all administration across the surface of the body and the inner linings of bodily passage including epithelial and mucosal tissues. Such administrations may be carried out using the present compositions in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

Transdermal administration may be accomplished through the use of a transdermal patch containing an active ingredient (*i.e.,* a combination of an anti-HCV-entry factor antibody and a direct acting antiviral) and a carrier that is non-toxic to the skin, and allows the delivery of the ingredient for systemic absorption into the bloodstream *via* the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may be suitable. A variety of occlusive devices may be used to release the active ingredient into the bloodstream such as a

semi-permeable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient.

Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerine. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

When a pharmaceutical composition of the present invention is used as "vaccine" to prevent HCV-susceptible cells from becoming infected with HCV, the pharmaceutical composition may further comprise vaccine carriers known in the art such as, for example, thyroglobulin, albumin, tetanus toxoid, and polyamino acids such as polymers of D-lysine and D-glutamate. The vaccine may also include any of a variety of well known adjuvants such as, for example, incomplete Freund's adjuvant, alum, aluminium phosphate, aluminium hydroxide, monophosphoryl lipid A (MPL, GlaxoSmithKline), a saponin, CpG oligonucleotides, montanide, vitamin A and various water-in-oil emulsions prepared from biodegradable oils such as squalene and/or tocopherol, Quil A, Ribi Detox, CRL-1005, L-121 and combinations thereof.

Materials and methods for producing various formulations are known in the art and may be adapted for practicing the subject invention. Suitable formulations for the delivery of antibodies can be found, for example, in "*Remington's Pharmaceutical Sciences*", E.W. Martin, 18th Ed., 1990, Mack Publishing Co.: Easton, PA.

B. Additional Biologically Active Agents

In certain embodiments, an inventive combination (*i.e.*, at least one anti-HCV-entry factor antibody and at least one direct acting antibody) is the only active ingredient in a pharmaceutical composition of the present invention. In other embodiments, the pharmaceutical composition further comprises one or more biologically active agents. Examples of suitable biologically active agents include, but are not limited to, vaccine adjuvants and therapeutic agents such as anti-viral agents (as described above), anti-inflammatory agents, immunomodulatory agents, analgesics, antimicrobial agents, antibacterial agents, antibiotics, antioxidants, antiseptic agents, and combinations thereof.

In such pharmaceutical compositions, the anti-HCV-entry factor antibody, a direct acting antiviral and additional therapeutic agent(s) may be combined in one or more preparations for simultaneous, separate or sequential administration of the different components. More specifically, an inventive composition may be formulated in such a way that the anti-HCV-entry factor antibody, direct acting antiviral and therapeutic agent(s) can be administered together or independently from one another. For example, an anti-HCV-entry factor antibody, a direct acting antiviral and a therapeutic agent can be formulated together in a single composition. Alternatively, they may be maintained (*e.g.*, in different compositions and/or containers) and administered separately.

C. Pharmaceutical Packs of Kits

In another aspect, the present invention provides a pharmaceutical pack or kit comprising one or more containers (*e.g.*, vials, ampoules, test tubes, flasks or bottles) containing one or more ingredients of an inventive pharmaceutical composition, allowing administration of a combination of the present invention.

Different ingredients of a pharmaceutical pack or kit may be supplied in a solid (*e.g.*, lyophilized) or liquid form. Each ingredient will generally be suitable as aliquoted in its respective container or provided in a concentrated form. Pharmaceutical packs or kits may include media for the reconstitution of lyophilized ingredients. Individual containers of the kits will preferably be maintained in close confinement for commercial sale.

In certain embodiments, a pharmaceutical pack or kit includes one or more additional therapeutic agent(s) (*e.g.*, one or more anti-viral agents, as described above). Optionally associated with the container(s) can be a notice or package insert in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. The notice of package insert may contain instructions for use of a pharmaceutical composition according to methods of treatment disclosed herein.

An identifier, *e.g.*, a bar code, radio frequency, ID tags, etc., may be present in or on the kit. The identifier can be used, for example, to uniquely identify the kit for

purposes of quality control, inventory control, tracking movement between workstations, etc.

Examples

The following examples describe some of the preferred modes of making and practicing the present invention. However, it should be understood that the examples are for illustrative purposes only and are not meant to limit the scope of the invention. Furthermore, unless the description in an Example is presented in the past tense, the text, like the rest of the specification, is not intended to suggest that experiments were actually performed or data are actually obtained.

Example 1: Inhibition of HCVcc Infection Using an Inventive Combination

Materials and Methods

Cell Lines. Cultures of Huh7.5.1 cells, which have previously been described (Zhong *et al.*, Proc. Natl. Acad. Sci. USA, 2005, 102: 9294-2929), were used in this study.

Production of Anti-CLDN1 mAbs. Anti-CLDN1 mAbs were raised by genetic immunization of Wistar rats using a eukaryotic expression vector encoding the full-length human CLDN1 cDNA as described in EP 08 305 597 and WO 2010/034812. Following completion of immunization, antibodies were selected by flow cytometry for their ability to bind to human CLDN1 expressed on the cell surface of non-permeabilized HEK293T-BOSC23 cells and CHO cells which had been transfected with pCMV-SPORT6/CLDN1. In the present invention, anti-CLDN1 mAb OM-7D3-B3 was used.

Production of Anti-CD81 mAb and Anti-SRBI mAb. The Anti-human SRBI and anti-human CD81 antibodies were produced, as described for anti-CLDN1 antibodies (Fofana *et al.*, Gastroenterology, 2010, 139: 953-964).

Direct Active Antivirals. VX-950, ITMN-191, R7128 and BMS-790052 were obtained from Acme Bioscience.

HCVcc Production and Infection. Cell-culture derived HCVcc (Luc-Jc1) were generated as previously described (Koutsoudakis *et al.*, J. Virol., 2006, 80: 5308-5320; Zeisel *et al.*, Hepatology, 2007, 46: 1722-1731). For infection experiments,

Huh7.5.1 cells were incubated with HCVcc infected as described previously (Fofana *et al.*, Gastroenterology, 2010, 139: 953-964; Lupberger *et al.*, Nature Medicine, 2011, 17: 589-595).

Combination Experiments. The anti-CLDN1 antibody, anti-CD81 antibody, anti-SRBI antibody and direct acting antivirals were tested individually and in combination. Huh7.5.1 cells were pre-incubated with the anti-CLDN1 antibody, anti-CD81 antibody or anti-SRBI antibody and with one of the four direct acting antivirals (VX-950, ITMN-191, R7128, or BMS-790052) for 1 hour. Huh7.5.1 cells were then incubated with HCVcc Luc-Jc1 in the presence of both compounds. HCVcc infection was analyzed two days later by luciferase reporter gene expression as previously described (Krieger *et al.*, Hepatology, 2010, 54: 1144-1157; Fofana *et al.*, Gastroenterology, 2010, 139: 953-964; Koutsoudakis *et al.*, J. Virol., 2006, 80: 5308-5320). The Combination Index (CI) was calculated as described (Zhao *et al.*, Clin. Cancer Res., 2004, 10: 7994-8004). A CI of less than 1 indicates synergy; a CI equal to 1 indicates additivity; and a CI of more than 1 indicates antagonism.

Statistical Analysis. Results are expressed as means ± standard deviation (SD). Statistical analyses were performed using Student's *t* test with a P value of <0.05 being considered statistically significant.

Results and Discussion

The results obtained are presented on Figures 1-4 and in table 1 below.

Synergy was confirmed (CI < 1) for several concentrations of direct acting antivirals and anti-HCV-entry factor antibodies (data not shown).

The combination of an anti-CLDN1 antibody and direct acting antivirals resulted in a high synergistic activity in inhibition of HCVcc infection, with a CI of lower than 0.20. Furthermore, the presence of a direct acting antiviral in the combination decreased the IC_{50} of the anti-CLDN1 antibody from 0.18 µg/mL to 0.015 µg/mL for BMS-790052 (*i.e.*, by a factor 12), to 0.011 µg/mL for R7128 (*i.e.*, by a factor 16), to 0.004 µg/mL for ITMN-191 (*i.e.*, by a factor 45), and to 0.0025 µg/mL for VX-950 (*i.e.*, by a factor 72).

Table 1. IC_{50} and CI for the combinations studied on HCVcc infection. IC for anti-CD81 in combination with NS5A inhibitor BMS-790052 was calculated for an IC_{90}.

Direct Acting Antivirals	anti-HCV-entry factor antibody	IC_{50} (µg/mL)	CI
control	anti-CLDN1	0.18±0.04	
VX-950 (0.001 µM)	anti-CLDN1	0.0025±0.0003	0.02±0.002
ITMN-191 (0.001 µM)	anti-CLDN1	0.004±0.002	0.19±0.01
R7128 (0.1 µM)	anti-CLDN1	0.011±0.001	0.07±0.08
BMS-790052 (0.001 nM)	anti-CLDN1	0.015±0.004	0.16±0.02
control	anti-CD81	0.019±0.006	
VX-950 (0.001 µM)	anti-CD81	0.0055±0.0007	0.29±0.05
ITMN-191 (0.001 µM)	anti-CD81	0.0035±0.004	0.35±0.11
R7128 (0.1 µM)	anti-CD81	0.0013±0.001	0.07±0.03
BMS-790052 (0.001 nM)	anti-CD81	0.05±0.02	0.20±0.1
control	anti-SRBI	1.3±0.4	
VX-950 (0.001 µM)	anti-SRBI	0.013±0.017	0.02±0.002
ITMN-191 (0.001 µM)	anti-SRBI	0.035±0.028	0.19±0.002
R7128 (0.1 µM)	anti-SRBI	0.001±0.003	0.02±0.002
BMS-790052 (0.001 nM)	anti-SRBI	0.01±0.005	0.10±0.01

The combination of anti-CD81 antibody and direct acting antivirals resulted in a high synergistic activity in inhibition of HCVcc infection, with a CI equal to or lower than 0.35. Furthermore, the presence of a direct acting antiviral in the combination decreased the IC_{50} of the anti-CD81 antibody from 0.019 µg/mL to 0.0055 µg/mL for VX-950 (*i.e.*, by a factor 3.4), 0.0035 µg/mL for ITMN-191 (*i.e.*, by a factor 5.4), and 0.0013 µg/mL for R7128 (*i.e.*, by a factor 14.6), and the IC_{90} from 0.3 µg/mL to 0.05 µg/mL for BMS-790052 (*i.e.*, by a factor 6).

The combination of anti-SRBI antibody and direct acting antivirals resulted in a high synergistic activity in inhibition of HCVcc infection, with a CI lower than 0.20. Furthermore, the presence of a direct acting antiviral in the combination decreased the IC_{50} of the anti-SRBI antibody from 1.3 µg/mL to 0.01 µg/mL for BMS-790052 (*i.e.*, by a factor of 130), to 0.035 µg/mL for ITMN-191 (*i.e.*, by a factor 37), to 0.013 µg/mL for VX-950 (*i.e.*, by a factor 100), and to 0.001 µg/mL for R7128 (*i.e.*, by a factor 1300).

These results demonstrate that anti-HCV-entry factor antibodies, in combination with direct acting antivirals could provide a valuable alternative to combination therapy.

Example 2: Further Characterization in *in vitro* Models for HCV Infection

Following screening of combinations including other direct acting antivirals using different HCV genotypes and model systems, combinations according to the invention will be further characterized by comparative analysis of neutralization in state-of-the-art *in vitro* models (Krieger *et al.*, Hepatology, 2010, 51: 1144-1157; Fofana *et al.*, 2010, 139: 953-964).

Example 3: Characterization in an *in vivo* Model for HCV Infection

As a first step to evaluate the combinations according to the present invention, and establish the essential parameters for protection and treatment of HCV infection, the human liver-chimeric SCID/Alb-uPA mouse model will be used in a preclinical study. This model is a well characterized preclinical model for the *in vivo* assessment of antivirals. Pharmacokinetic and toxicity of selected combinations in uPA/SCID mice will be examined as previously described (Law *et al.*, Nat. Med., 2008, 14: 25-27; Vanwolleghem *et al.*, Hepatology, 2008, 47: 1846-1855). Briefly, transplanted SCID/Alb-uPA mice will be infected with HCV-infected human serum intravenously and the effect of combinations of the invention on viral load will be assessed. Treatment outcome will be evaluated clinically (toxicity), virologically (viral load), and morphologically (histopathology of transplanted hepatocytes and other tissues) as described recently (Vanwolleghem *et al.*, Gastroenterology, 2007, 133: 1144-1155). The safety profile will be further assessed in non human primates.

Example 4: Phase I/IIa Clinical Trials

Following completion of the studies in the uPA-SCID mouse model as well as toxicity studies in non human primates, clinical phase I/IIa trials will be initiated in HCV infected humans resistant or not eligible to standard of care using a longstanding collaboration of Inserm U748-University of Strasbourg with the Strasbourg Center for Clinical Investigation (CIC) at Strasbourg. Two study designs are required to assess safety and efficacy for prevention and treatment of HCV infection:

Prevention of HCV infection in subjects undergoing liver transplantation. The combinations will be evaluated for their ability to prevent the universal re-infection of the liver graft following liver transplantation by achieving a reduction in viral load (as measured quantitatively by HCV RT-PCR) post-transplant by ≥ 1 log10 from the baseline value

Treatment of HCV infection in subjects chronically infected patients. The combinations will be evaluated for their ability to achieve reduction in viral load by ≥ 1 log10 from the baseline value.

Example 5: Other Inventive Combinations

Materials and Methods

Cell Lines. Cultures of Huh7.5.1 (Zhong *et al.*, Proc. Natl. Acad. Sci. USA, 2005, 102: 9294-2929) and HEK293T (Pestka *et al.*, Proc. Natl. Acad. Sci. USA, 2007, 104: 6025-6030) cells, which have previously been described, were used in this study.

Production of Monoclonal Antibodies. Anti-CLDN1 mAbs (OM-7D3-B3) was prepared as described in Example 1. Anti-SR-BI and anti-CD81 mAbs were produced by DNA-immunization as described for anti-CLDN1 mAbs (Fofana *et al.*, Gastroenterology, 2010, 139: 953-964, 964.e1-4).

Direct Active Antivirals. Protease inhibitors (telaprevir, boceprevir, danoprevir and TMC-435), NS5A inhibitor (daclatasvir) and polymerase inhibitors (mericitabine and GS-7977 (formally known as PSI-7977)) were synthesized by Acme Bioscience, Inc.

Analysis of Antiviral Activity of Compounds and Combinations on HCV Infection. The *in vitro* antiviral activity of each compound was tested individually and in combination with a second compound using the HCVcc Huh7.5.1 cell culture described in Example 1. For combination of entry inhibitors (anti-CLDN1, anti-SRBI, and anti-CD81) with DAAs (telaprevir, boceprevir, danoprevir, TMC-435, daclatasvir, mericitabine and GS-7977), Huh7.5.1 cells (cultured in 96-well-plates) were pre-incubated with DAA and the entry inhibitor for 1 hour at 37°C before incubation for 4 hours at 37°C with HCVcc in the presence of both compounds. Viral infection was analyzed by assessing luciferase activity as described in Example 1.

Analysis of Synergy. Synergy was assessed by two independent methods: the combination index as described in Example 1 and the method of Prichard and Shipman (Zhao *et al.*, Clin. Cancer Res., 2004, 10: 7994-8004; Prichard *et al.*, Antiviral Res., 1990, 14: 181-205). A CI of less than 0.9 indicates synergy; a CI equal to 0.9-1.1 indicates additivity; and a CI of more than 1.1 indicates antagonism (Zhao *et al.*, Clin. Cancer Res., 2004, 10: 7994-8004; Zhu *et al.*, J. Infect. Dis., 2012, 205: 656-662). The method of Prichard and Shipman was applied as described (Prichard *et al.*, Antiviral Res., 1990, 14: 181-205). Surface amplitudes > 20% above the zero plane indicate a synergistic effect, while surface amplitudes < 20% below the zero plane indicate antagonism. The validity of the assay and methods were confirmed by comparative analyses of combinations showing a non-synergistic effect.

Toxicity Assays. Huh7.5.1 cells and primary human hepatocytes isolated and cultured as described were incubated with the compounds for 48 hours (Krieger *et al.*, Hepatology, 2010, 51: 1144-1157). Cytotoxic effects were analysed by the ability to metabolize 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as described (Lupberger *et al.*, Nat. Med., 2011, 17: 589-595). An anti-Fas antibody (10 μg/m) was used as a positive control.

Results

The results are presented on Figures 5-8 and Table 2.

Synergy of Entry Inhibitors and Direct Acting Antivirals. A major effort of the pharmaceutical industry and current clinical research is the further improvement of IFN-based therapies using DAAs and the development of IFN-free combinations based on the combination of DAAs with or without ribavirin. Addressing these future concepts, the present Applicants have studied the combined antiviral effect of entry inhibitors with the clinically licensed protease inhibitors, such as telaprevir (McHutchison *et al.*, N. Engl. J. Med., 2010, 362: 1292-1303) and boceprevir (Poordad *et al.*, N. Engl. J. Med., 2011, 364: 1195-1206), as well as second-generation protease inhibitors in late stage development, such as TMC-435 and danoprevir (Gane *et al.*, Hepatology, 2011; 54: 76A – abstract 34) in the HCVcc model system.

Table 2. Synergy of entry inhibitors and direct acting antivirals on inhibition of HCV infection. Huh7.5.1 cells were pre-incubated with serial concentrations of DAAs (protease inhibitors: telaprevir, boceprevir, TMC-435 or danaorevir; NS5A inhibitor: daclatasvir; or polymerase inhibitors: mericitabine or GS-7977) and 0.01 µg/ml receptor specific (anti-CD81, anti-SRBI or anti-CLDN1) or respective isotype control mAbs for 1 hour at 37°C. CI for anti-CD81 in combination with NS5A inhibitor daclatasvir was calculated for an IC_{90} and is indicated by a star (*). Means ± SD of at least three independent experiments performed in triplicate are shown. IC_{50} of entry inhibitors: anti-CD81, 0.015±0.01 µg/ml; anti-SR-BI, 1.3±0.4 µg/ml; anti-CLDN1, 0.18±0.03 µg/ml.

Compound 1	IC_{50} (µM or nM[1])	Compound 2	IC_{50} (µM or nM[1]) for combination	CI
telaprevir	0.15±0.06	anti-CD81	0.004±0.001	0.55±0.007
		anti-SRBI	0.001±0.0001	0.02±0.01
		anti-CLDN1	0.005±0.003	0.09±0.01
boceprevir	0.14±0.02	anti-CD81	0.0004±0.0001	0.53±0.01
		anti-SRBI	0.02±0.002	0.15±0.01
		anti-CLDN1	0.0005±0.001	0.06±0.007
TMC-435	0.013±0.001	anti-CD81	0.0007±0.0001	0.58±0.02
		anti-SRBI	0.006±0.0007	0.49±0.06
		anti-CLDN1	0.0005±0.0002	0.1±0.09
danoprevir	0.006±0.003	anti-CD81	0.0006±0.0004	0.62±0.07
		anti-SRBI	0.0007±0.001	0.15±0.02
		anti-CLDN1	0.0003±0.0004	0.11±0.02
daclatasvir	0.012±0.003	anti-CD81	0.02±0.005 (*)	0.26±0.05 (*)
		anti-SRBI	0.0002±0.0004	0.03±0.004
		anti-CLDN1	0.0006±0.0002	0.11±0.01
mericitabine	0.12±0.03	anti-CD81	0.00015±0.0001	0.52±0.005
		anti-SRBI	0.019±0.007	0.17±0.06
		anti-CLDN1	0.0028±0.00035	0.08±0.02
GS-7977	0.021±0.002	anti-CD81	0.0035±0.0004	0.69±0.02
		anti-SRBI	0.003±0.0007	0.15±0.04
		anti-CLDN1	0.0012±0.0003	0.12±0.02

[1] Telaprevir, boceprevir, danoprevir, TMC-435, mercitabine, PSI-7977: µM ; daclatasvir : nM

Combination of a clinical licensed protease inhibitor, telaprevir or boceprevir and of a sub-IC_{50} concentration of receptor-specific mAbs (which exerts only minimal inhibitory effect on HCV infection) resulted in CIs of 0.02 to 0.55, indicating synergy (see Figure 5A and Table 2). In contrast, combination of two protease inhibitors

(telaprevir and boceprevir) resulted in an additive activity confirming the validity of the assay (Figure 5A).

Second-generation protease inhibitors have beend demonstrated to have a higher genetic barrier for resistance. However, single amino acid substitutions are able to confer drug resistance *in vivo*. Importanly, it has been demonstrated that several telaprevir- and boceprevir-resistance mutations confer cross-resistance to these second-generation protease inhibitors (Sarrazin *et al.*, J. Hepatol., 2012, 56(1): S88-100). Combination of a second-generation protease inhibitor, TMC-435 or danoprevir, and a receptor-specific mAbs resulted in a synergistic activity (CIs of 0.1 to 0.62 – see Figure 5A and Table 2), demonstrating the relevance of adding an entry-inhibitor as a concept to improve antiviral efficacy. Highly effective combinations included the combinations of boceprevir with anti-CLDN1 mAb (Figure 6A), and TMC-435 with anti-CLDN1 mAb (Figure 6B).

A number of novel DDAs have reached early- to late-stage clinical development, including NS5A inhibitors and polymerase inhibitors. The first NS5A inhibitor, daclatasvir (Gao *et al.*, Nature, 2010, 465: 96-100), has been shown to have potent antivial activity against HCV genotype 1 in monotherapy. However, its genetic barrier to resistance is low and resistant variants developed rapidly without improsing a loss of *in vivo* viral fitness (Gao *et al.*, Nature, 2010, 465: 96-100). A marked synergy was observed for the combination of daclatasvir with all the receptor-specific mAbs (CIs of .03 to 0.26, see Figure 5A and Table 2). The most effective combinations included the combination of daclatasvir and the anti-SR-BI mAb, daclatasvir and the anti-CLND1 mAb and daclatasvir and erlotinib, decreasing its IC_{50} up to 60 fold (Figure 7A-B).

Finally, the Applicants investigated the synergy between entry inhibitors and the polymerase inhibitor GS-7977. GS-7977is currenlty in clinical development and has been suggested as having the potential to become the conerstone of an efficacious, all-oral combination regiment for many patients with chronic HCV infection (Zeisel *et al.*, Front Biosci., 2009, 14: 3274-3285; Zeisel *et al.*, J. Hepatol., 2011, 54: 566-576). Thus, the inventors investigated whether entry inhibitors potentiate the antiviral activity of GS-7977. Combination of GS-7977 with each of the receptor-specific mAbs tested resulted in a synergistic activity, with CIs of 0.12 to 0.69 (Figure 5C and

Table 2). Potent combinations included the combinations of GS-7977 and the anti-SR-BI mAb and of GS-7977 and the anti-CLDN1 mAb, decreasing its IC_{50} from 0.021 µM to 0.003 µM and from 0.021 µM to 0.0012 µM, respectively (Figure 8A-B). These data clearly demonstrate the potential of combining GS-7977 with entry inhibitors to improve antiviral activity.

Similar results were obtained for the combinatino of another polymerase inhibitor, mericitabine (Gane *et al.*, Lancet, 2010, 376: 1467-1475) and entry inhibitors (CIs of 0.08 to 0.52 – see Figure 5C and Table 2).

To further confirm the synergistic effect over a broad range of concentrations of both compounds, the inventors performed combinations testing a full checker-board of compounds dose-response curves using the method of Prichard and Shipman (Prichard *et al.*, Antiviral Res., 1990, 14: 181-205). Noteworthy, in particular low doses of both compounds results in an antiviral activity above the expected value (Figure 6C-D, Figure 7C-D and Figure 8C). There results also suggest an opportunity to reduce the doses of both compounds of the combination – a key requirement for improvement of future antiviral treatment.

Noteworthy, none of all the DAA-entry inhibitor combinations tested in the present study resulted in detectable toxicity in primary human hepatocytes (Table 3).

Table 3. Absent toxicity of combinations of compounds in primary human hepatocytes (PHH). Cytotoxic effects on PHH using the highest concentrations of each compound used in combination (DAAs, 10 µM; receptor-specific mAbs, 10 µg/ml) were assessed by analyzing the ability to metabolize MTT). Anti-Fas antibody (10 µg/ml) was used as a positive control of toxicity. Toxicity analyses of the most efficient combinations are shown. Data are presented as relative cell viability compared to PHH cultured in the absence of compounds or solvent (=100%). Means ± SD from one representative experiment performed in triplicate is shown.

Compound 1	Concentration	Compound 2	Concentration	Relative cell viability (%)
boceprevir	10 µM	anti-CLDN1	10 µg/ml	103±3
TMC-435	10 µM	anti-CLDN1	10 µg/ml	97±4
danoprevir	10 µM	anti-CLDN1	10 µg/ml	103±1
daclatasvir	10 µM	anti-SRBI	10 µg/ml	101±2
mericitabine	10 µM	anti-CD81	10 µg/ml	106±5
GS-7977	10 µM	anti-SR-BI	10 µg/ml	110±7
GS-7977	10 µM	anti-CLDN1	10 µg/ml	97±5
anti-Fas	10 µg/ml			16±2

The results obtained suggest an opportunity to reduce the doses of both compounds – a key requirement for improvement of future antiviral treatment. Taken together, the present Applicants have demonstrated that the addition of a sub-IC_{50} concentration of protein kinase inhibitor is sufficient to markedly decrease the IC_{50} concentration of the different DAAs currently evaluated in IFN-free regimens, without displaying any toxic effects *in vitro*. These data demonstrate the proof-of-concept that entry inhibitors and DAAs are highly synergistic and define novel antiviral combinations for further preclinical and clinical development in IFN-free regimens.

Example 6: HCV Entry Inhibitors PreventsAntiviral Resistance by Blocking Cell-Cell Transmission of DAA-Resistant Variants

Materials and Methods

Cell Lines; Monoclonal Antibodies; Direct Acting Antivirals; HCVcc Production and Infection; Combination Experiments; and Statistical Analysis. See Example 1 and Example 5.

Other Antibodies. HCV E2-specific mAb was a gift of Dr. A. Patel (MRC Center for Virology, Glasgow, UK). NS5A-specific mAb was obtained from Virostat.

Production of Recombinant Viruses Containing Resistance Mutations. The drug-resistant mutations were introduced into the Jc1-Luc plasmid (Koutsoudakis *et al.*, J. Virol., 2006, 80: 5308-5320; Pietschmann *et al.*, Proc. Natl. Acad. Sci. USA, 2006, 103: 7408-7413) using *in vitro* site directed mutagenesis (Quickchange XL, Stratagene) as previously described (Zhu *et al.*, J. Infect. Dis., 2012, 205: 656-662). Nucleotide changes were made in the HCV Luc-Jc1 construct to generate the A156S or L36M or R155K amino acid substitutions in the NS3 protein. A one-step polymerase chain reaction (PCR) mutagenesis was performed using mutation primers. The introduction of mutations into Jc1 constructs was confirmed by DNA sequence analysis.

Analysis of HCV Cell-Cell Transmission. Cell-cell transmission of HCV was assessed as previously described (Witteveldt *et al.*, J. Gen. Virol., 20069, 90: 48-58). Briefly, producer Huh7.5.1 cells were electroporated with HCV Jc1 RNA and cultured with naive target Huh7.5-GFP cells in the presence of 1 μg/ml anti-CLDN1 mAb or DMSO solvent/rat IgG control. An HCV anti-E2–neutralizing antibody (Witteveldt *et al.*, J. Gen. Virol., 20069, 90: 48-58) (25 μg/mL) was added to block cell-free

transmission (Witteveldt *et al.*, J. Gen. Virol., 20069, 90: 48-58). After 24 hours of coculture, cells were fixed with paraformaldehyde, stained with an NS5A-specific antibody (0.1 μg/mL) (Virostat) and analyzed by flow cytometer. Cell-cell transmission was defined as percentage HCV infection of Huh7.5-GFP+ target cells in the presence of an HCV E2–specific antibody (Witteveldt *et al.*, J. Gen. Virol., 20069, 90: 48-58).

Long Term HCV Infection of Huh7.5.1 Cells. Huh7.5.1 cells were electroporated with Luc-Jc RNA and cells were passaged in the presence of 10 μg/mL CTRL IgG (Rat IgG), 10 μg/mL anti-CLDN1 mAb, 250 nM TMC-435 or the combination of anti-CLDN1 mAb and TMC-435 or in the absence of any reagents (Mock CTRL) twice a week. 1 x 10^6 cells were harvested at each passage as previously described (Lenz *et al.*, Antimicrob. Agents Chemother., 2010, 54: 1878-1887) and viral load was quantified by measuring luciferase reporter gene expression as described (Krieger *et al.*, Hepatology, 2010, 51: 1144-1157; Fofana *et al.*, Gastroenterology, 2010: 139: 953-964).

Results and Discussion

Infection of DAA-resistant viruses is sensitive to HCV Entry Inhibitors. Clinically approved protease inhibitors telaprevir and boceprevir have a low genetic barrier for resistance in several amino acid substitutions conferring resistance without imposing a large viral fitness cost. First, to assess whether entry inhibitors inhibit protease-inhibitor resistant variants, the present Applicants introduced two well characterized mutations at positions 155 and 156 in the backbone of the HCVcc-Luc genome, known to confer resistance *in vivo* (Sarrazin *et al.*, J. Hepatol., 2012, 56 Suppl. 1, S88-100). As shown on Figure 9A and B, introduction of mutations R155S and A156S into HCVcc-Jc1 increased the IC_{50} of telaprevir and boceprevir up to 10-fold, respectively. In contrast, no differences were observed in the inhibition for both wild-type and protease-resistant viruses by CLDN1-, CD81- and SR-BI-specific mAbs (Fig. 9C-E). These data indicate that erlotinib efficiently inhibits DAAs-resistant variants without exhibiting cross-resistance.

DAA-resistant variants are efficiently transmitted by cell-cell transmission. Cell-cell transmission is considered more rapid and efficient than cell-free spread because it obviates rate-limiting early steps in the virus life cycle, such as virion

attachment (Timpe *et al.*, Hepatology, 2008, 47: 17-24). The cell-cell transmission of DAA-resistant variants may thus accelerate viral spread, leading to viral breakthrough and treatment failure. First, the Applicants investigated whether DAA variants are efficiently transmitted by cell-cell spread. To address this question they used a well-established cell-cell transmission assay (Lupberger *et al.*, Nature Medicine, 2011, 17: 589-595) and recombinant protease inhibitor-resistant virus HCVcc Jc1 containing either the single mutation at position 156 (A156S) or the double mutations at positions 155 and 36 (R155K, L36M). As shown on Figure 10A and D, protease resistant-viruses very efficiently spread through cell-cell transmission indicating that this mode of transmission is relevant for spread of DAA-resistant variants in the infected liver.

Cell-cell transmission of DAA resistant variant is inhibited by HCV entry inhibitor. Since CLDN1 has been suggested as co-factor for cell-cell transmission (Lupberger *et al.*, Nature Medicine, 2011, 17: 589-595), the present Applicants next investigated whether cell-cell transmission of DAA-resistant variants can be inhibited by a CLDN1-specific mAb. The CLDN1-specific mAb efficiently inhibited cell-cell transmission of protease-resistant viruses (Figure 10B and E). These data demonstrate that HCV entry inhibitors efficiently inhibit cell-cell transmission of protease inhibitor-resistant viruses and thus provide a previously undiscovered opportunity to inhibit the dissemination of protease inhibitor-resistant viruses throughout the liver.

Combination of HCV entry inhibitors with DAAs prevents antiviral resistance in a cell-culture model for HCV infection. To investigate whether the inhibition of cell-cell transmission of resistant viruses contributes to resistance during antiviral treatment and whether HCV entry inhibitors prevent viral resistance by preventing dissemination of resistant-viruses, the Applicants performed long-term experiments in passaged HCV-infected Huh7.5.1 cells. Cells replicating wild-type HCV Jc1 were incubated with second-wave protease inhibitor TMC-435 alone or in combination with CLDN1-specific mAb. While TMC-435 monotherapy resulted in viral rebound after two weeks, combination of CLDN1-specific mAb and TMC-435 decreased viral load to levels below the detection limit within one week of treatment and until the end of the six-week-treatment period (Figure 11). These results demonstrate that HCV entry inhibitors prevent antiviral DAA-resistance and the combination of DAAs and HCV entry inhibitors results in rapid and sustained suppression of viral infection.

Other Embodiments

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of the specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope of the invention being indicated by the following claims.

Claims

What is claimed is:

1. A combination of at least one anti-HCV-entry factor antibody and at least one direct acting antiviral for use in the treatment or the prevention of HCV infection, wherein the at least anti-HCV-entry factor antibody and the at least one direct acting antiviral act in synergy to inhibit HCV infection.

2. The combination according to claim 1, wherein the anti-HCV-entry factor antibody is a monoclonal antibody or a biologically active fragment thereof.

3. The combination according to claim 1 or claim 2, wherein the anti-HCV-entry factor antibody is an antibody against a HCV receptor selected from the group consisting of heparan sulfate, LDL receptor, CD81, SR-BI, Occludin and Claudin-1, Niemann-Pick C1-like 1 cholesterol absorption receptor or against a region of such a HCV receptor that is involved in HCV entry into susceptible cells.

4. The combination of claim 3, wherein the anti-HCV-entry factor antibody is an anti-Claudin 1 antibody that binds to the extracellular domain of Claudin 1 and is preferably a monoclonal antibody selected from the group consisting of OM-4A4-D4, OM-7C8-A8, OM-6D9-A6, OM-7D4-C1, OM-6E1-B5, OM-3E5-B6, OM-8A9-A3, OM-7D3-B3 and any biologically active fragment thereof that binds Claudin 1 extracellular domain.

5. The combination according to claim 1 or 2, wherein the anti-HCV-entry factor antibody is an antibody against a receptor tyrosine kinase.

6. The combination of any one of claims 2 to 5, wherein the monoclonal antibody is humanized, de-immunized or chimeric.

7. The combination of any one of claims 1 to 6, wherein the at least one direct acting antiviral is a HCV protease inhibitor, a HCV polymerase inhibitor, or an NS5A inhibitor.

8. The combination according to claim 7, wherein the at least one direct acting antiviral is selected from the group consisting of telaprevir, danoprevir, mericitabine, daclatasvir, boceprevir, BMS-650032, VX-985, BI 201335, TMC-435, GS-7977, GS 9256, GS 9451, MK-7009, ACH-1625, ABT-450, BMS-791325, VX-985, VX-500, PHX1766, VX-813, AVL-181, AVL-192, ACH-2684, IDX184, PSI-7977, VX-222, PF-868554, ABT-072, ABT-333, ANA598, BI 207127, MK-0608, TMC649128, RG7348, PSI-938, INX-189, VCH-759, IDX375 and A-837093.

9. The combination according to claim 8, wherein the at least one direct acting antiviral is telaprevir, danoprevir, mericitabine, daclatasvir, boceprevir, TMC-435, or GS-7977.

10. The combination of any one of claims 1 to 9, wherein the combination index (CI) of the combination is lower than 1, preferably lower than 0.50 or lower than 0.25, more preferably lower than 0.15, and even more preferably lower than 0.10.

11. The combination of any one of claims 1 to 10, wherein the combination is used for the treatment of HCV infection or a HCV-related disease in a subject, or for the control of chronic HCV infection in a subject or for preventing HCV re-infection and recurrence in a liver-transplantation patient.

12. The combination of claim 11, wherein the HCV infection or HCV-related disease or HCV re-infection is caused by a Hepatitis C virus that is resistant to a direct acting antiviral and/or transmitted by cell-cell transmission.

13. A pharmaceutical composition comprising a combination according to any one of claims 1 to 12 and at least one pharmaceutically acceptable carrier or excipient.

14. The pharmaceutical composition according to claim 12 further comprising at least one anti-viral agent.

15. The pharmaceutical composition according to claim 14, wherein the anti-viral agent is selected from the group consisting of interferons, rabivirin, anti-

hepatitis C virus monoclonal antibodies, anti-hepatitis C virus polyclonal antibodies, IRES inhibitors, helicase inhibitors, antisense compounds, ribozymes, micro-RNA antagonists, cytokines, therapeutic vaccines, NS5A antagonists, polymerase inhibitors, cyclophilin A antagonists, and any combination thereof.

16. A kit comprising at least one anti-HCV-entry factor antibody and at least one direct acting antiviral for simultaneous or sequential use in the treatment or the prevention of HCV infection, wherein the at least anti-HCV-entry factor antibody and at least one direct acting antiviral act in synergy to inhibit HCV infection.

17. The kit according to claim 16, wherein the at least one anti-HCV-entry factor antibody is as defined in any one of claims 2 to 6 and the at least one direct acting antiviral is as defined in any one of claims 7 to 9.

18. The kit according to claim 16 or claim 17, wherein the HCV infection is caused by a Hepatitis C virus that is resistant to a direct acting antiviral and/or transmitted by cell-cell transmission.

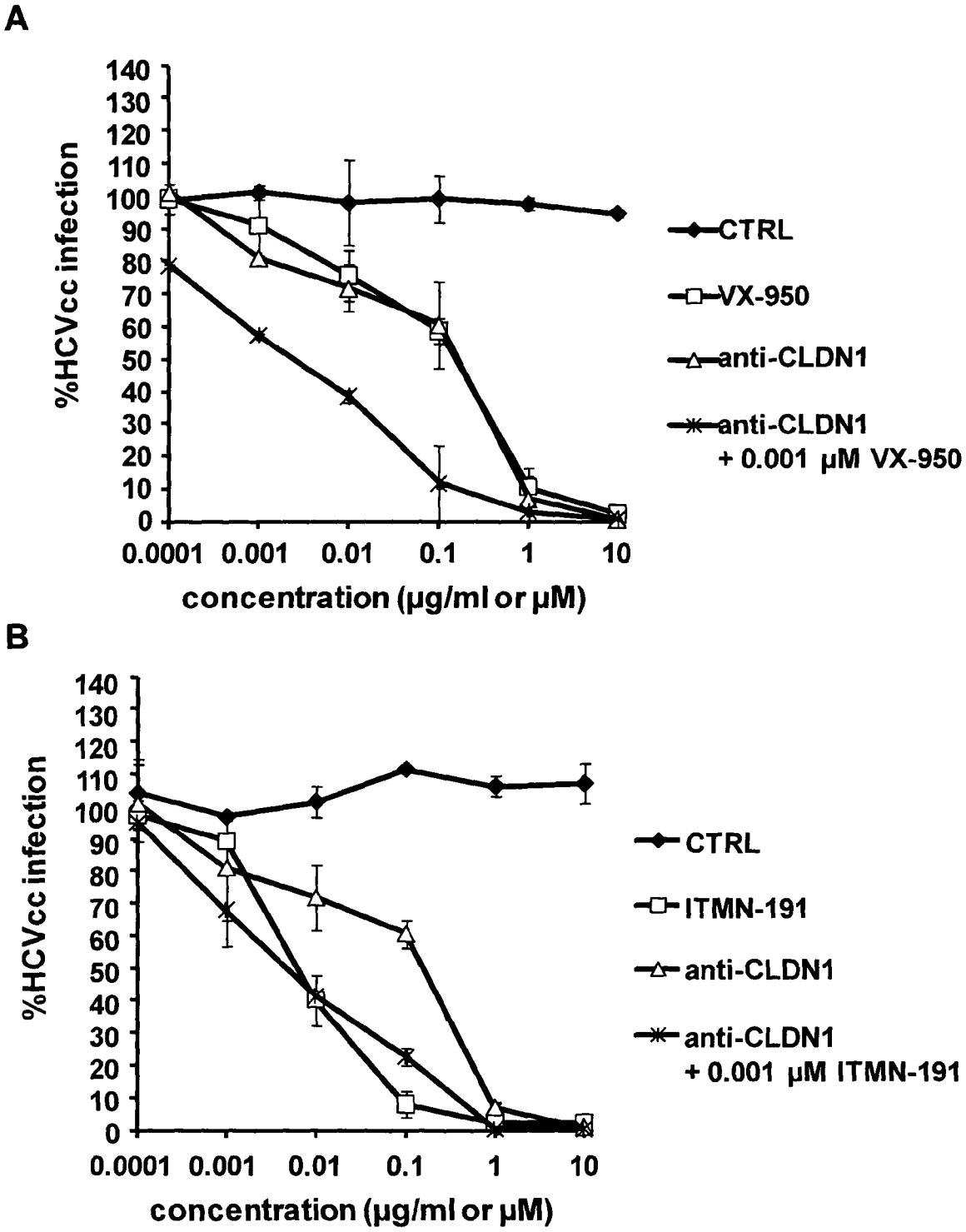

A
%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001 0.001 0.01 0.1 1 10
concentration (µg/ml or µM)
CTRL
VX-950
anti-CLDN1
anti-CLDN1
+ 0.001 µM VX-950
B
%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001 0.001 0.01 0.1 1 10
concentration (µg/ml or µM)
CTRL
ITMN-191
anti-CLDN1
anti-CLDN1
+ 0.001 µM ITMN-191

Figure 1 (A)-(B)

C

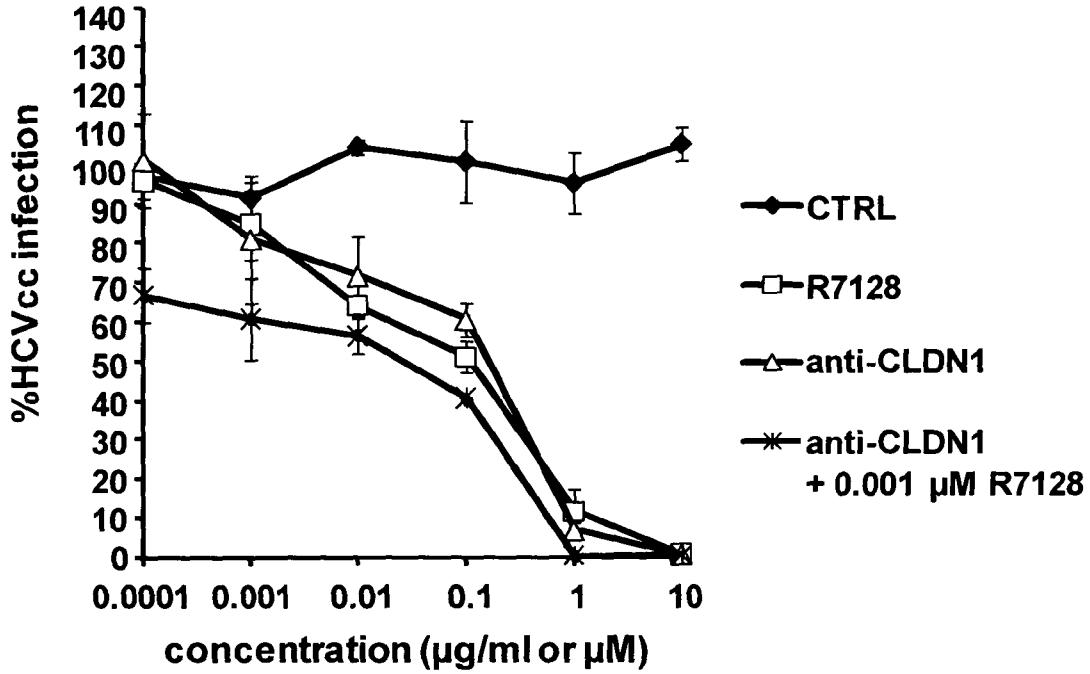

%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001
0.001
0.01
0.1
1
10
concentration (µg/ml or µM)
CTRL
R7128
anti-CLDN1
anti-CLDN1
+ 0.001 µM R7128

Figure 1 (C)

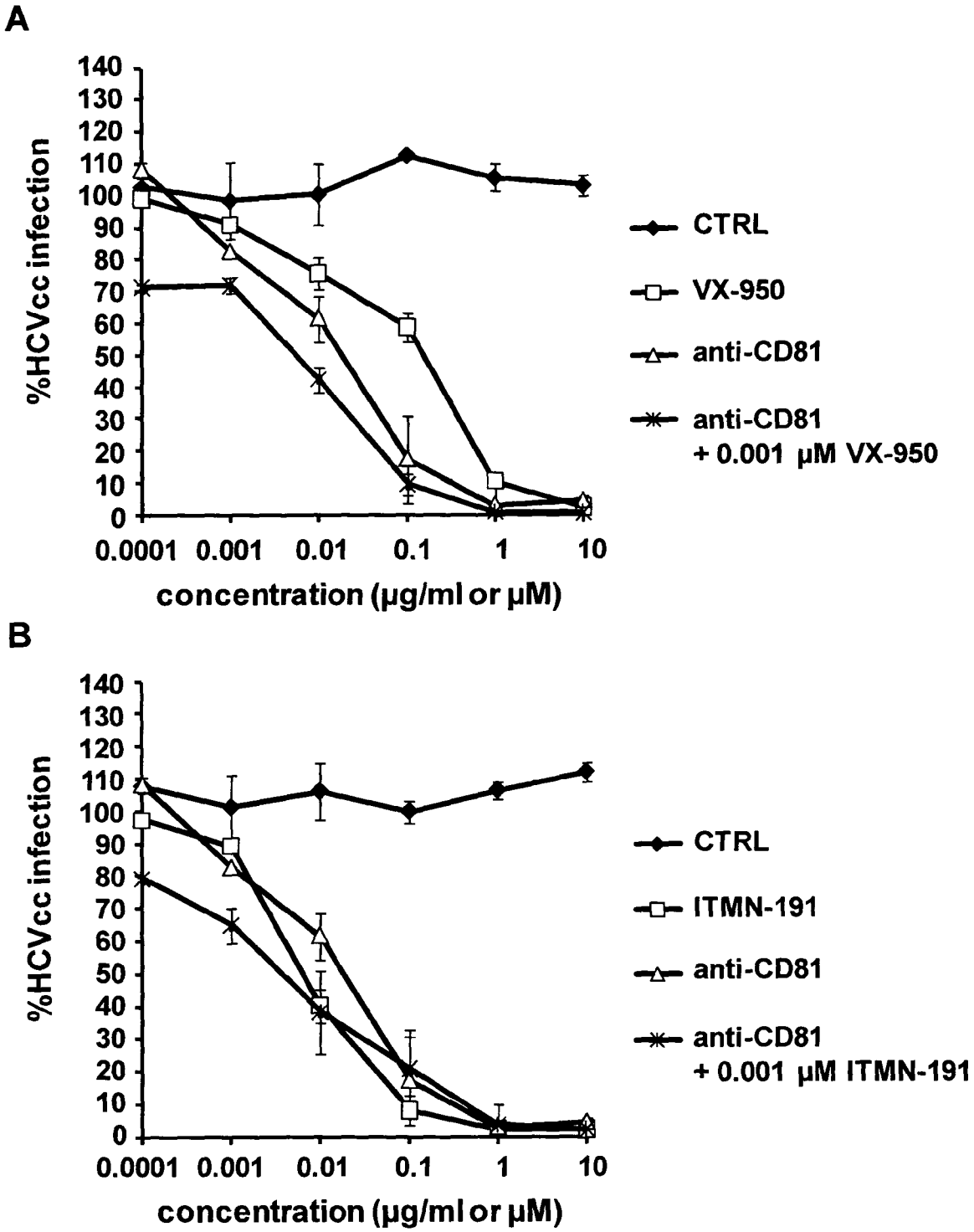

A
%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001 0.001 0.01 0.1 1 10
concentration (µg/ml or µM)
CTRL
VX-950
anti-CD81
anti-CD81
+ 0.001 µM VX-950
B
%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001 0.001 0.01 0.1 1 10
concentration (µg/ml or µM)
CTRL
ITMN-191
anti-CD81
anti-CD81
+ 0.001 µM ITMN-191

Figure 2 (A)-(B)

C

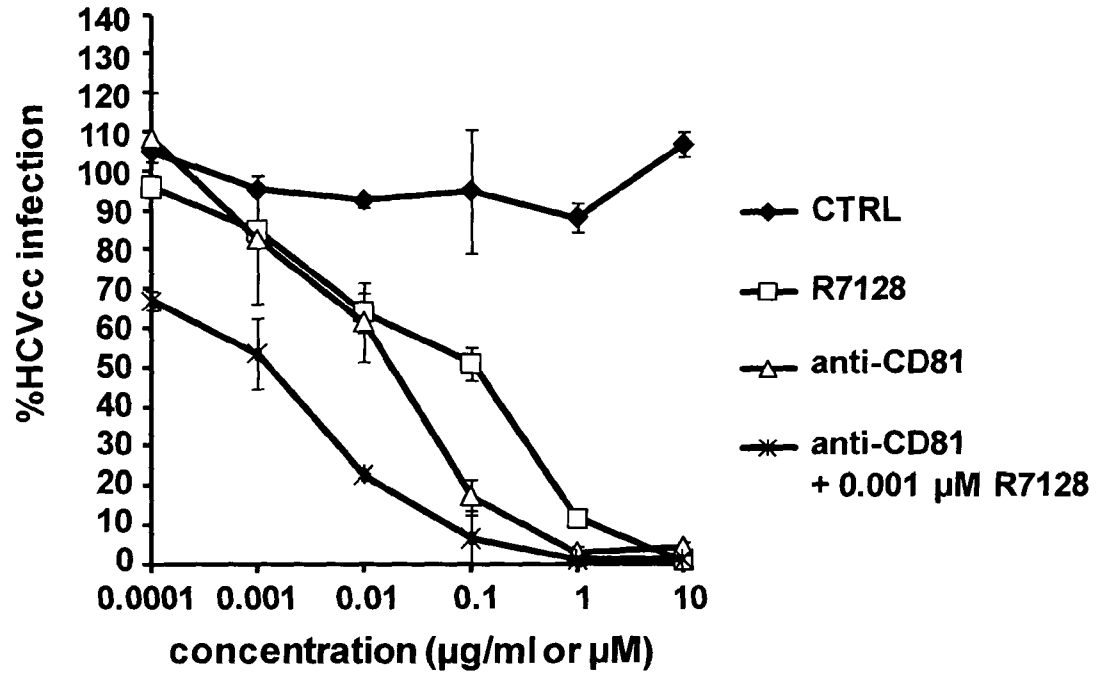

%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001
0.001
0.01
0.1
1
10
concentration (µg/ml or µM)
CTRL
R7128
anti-CD81
anti-CD81
+ 0.001 µM R7128

Figure 2 (C)

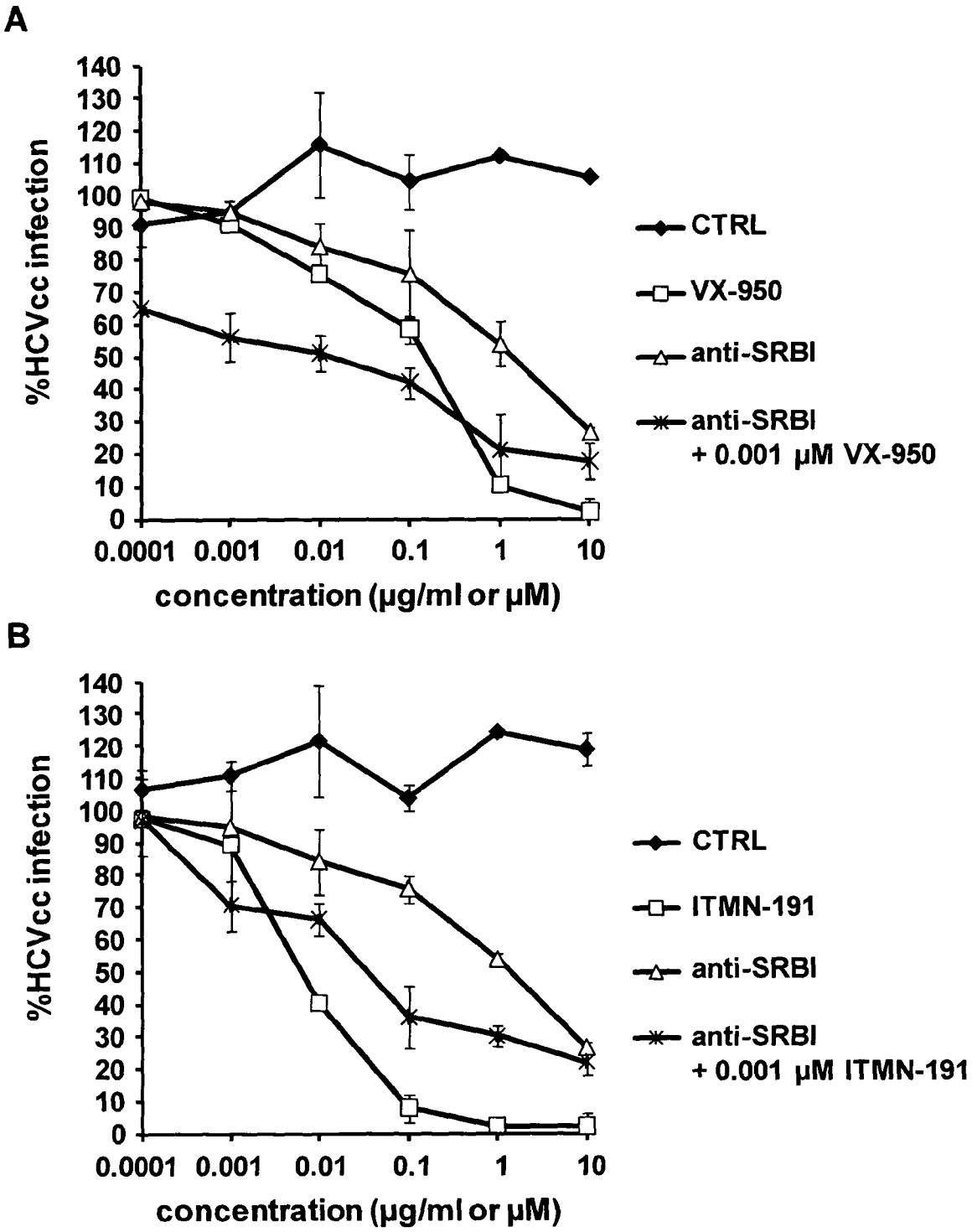

A
%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001 0.001 0.01 0.1 1 10
concentration (µg/ml or µM)
CTRL
VX-950
anti-SRBI
anti-SRBI
+ 0.001 µM VX-950
B
%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001 0.001 0.01 0.1 1 10
concentration (µg/ml or µM)
CTRL
ITMN-191
anti-SRBI
anti-SRBI
+ 0.001 µM ITMN-191

Figure 3 (A)-(B)

C

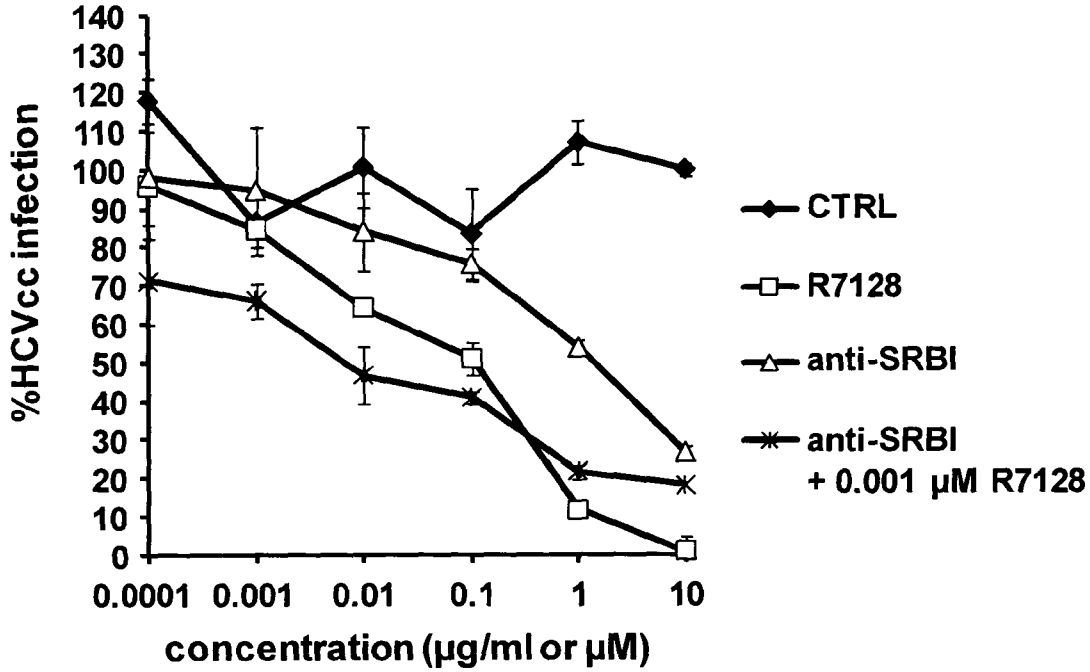

%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001
0.001
0.01
0.1
1
10
concentration (µg/ml or µM)
CTRL
R7128
anti-SRBI
anti-SRBI
+ 0.001 µM R7128

Figure 3 (C)

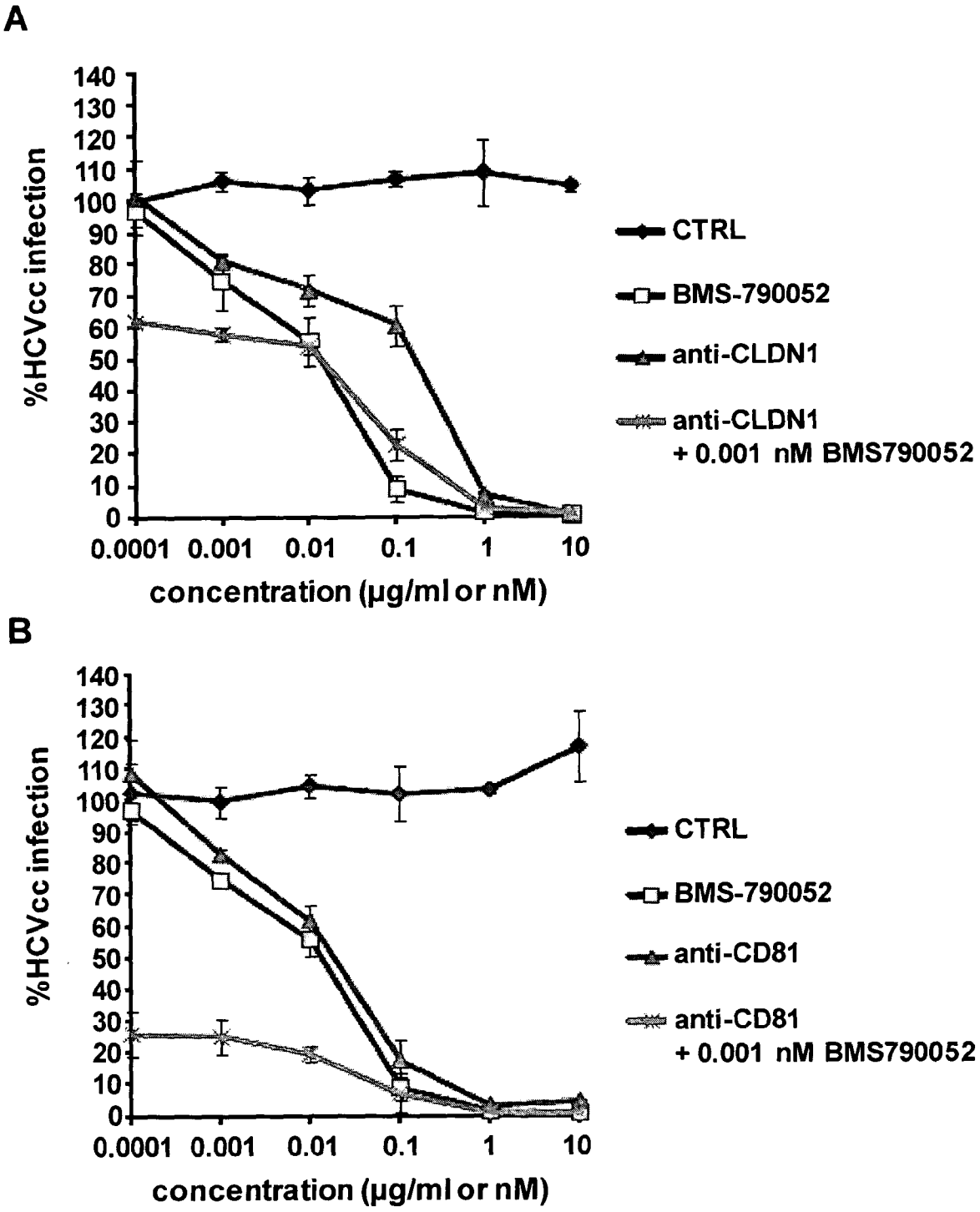

A
%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001 0.001 0.01 0.1 1 10
concentration (µg/ml or nM)
CTRL
BMS-790052
anti-CLDN1
anti-CLDN1
+ 0.001 nM BMS790052
B
%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001 0.001 0.01 0.1 1 10
concentration (µg/ml or nM)
CTRL
BMS-790052
anti-CD81
anti-CD81
+ 0.001 nM BMS790052

Figure 4 (A)-(B)

C

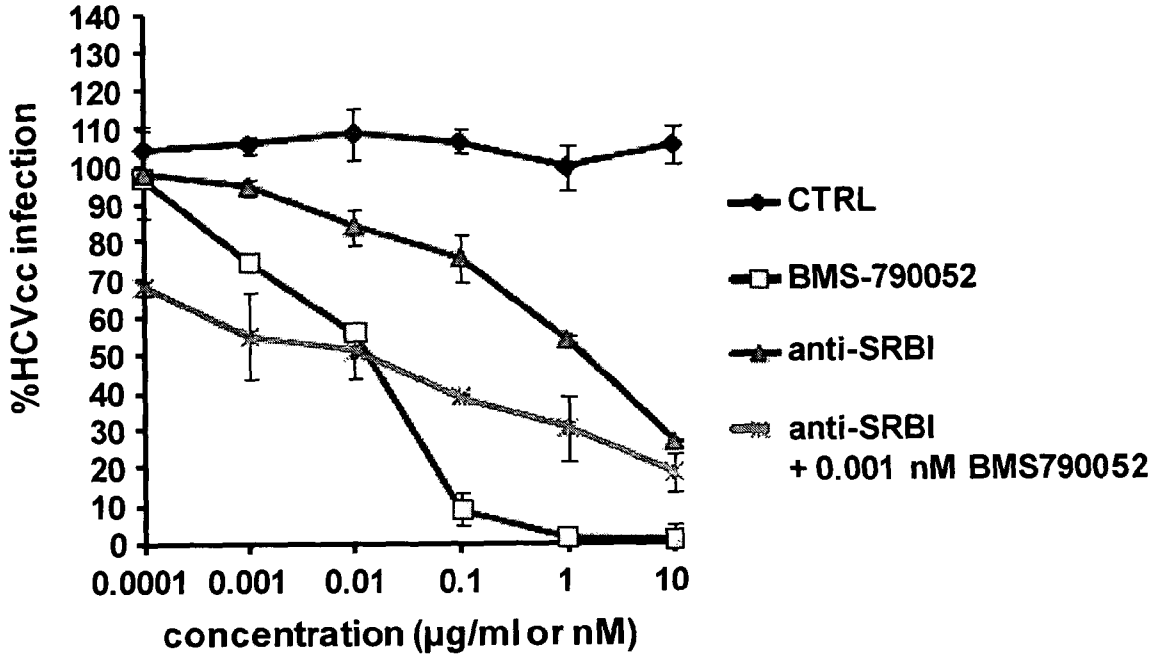

%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001
0.001
0.01
0.1
1
10
concentration (µg/ml or nM)
CTRL
BMS-790052
anti-SRBI
anti-SRBI
+ 0.001 nM BMS790052

Figure 4 (C)

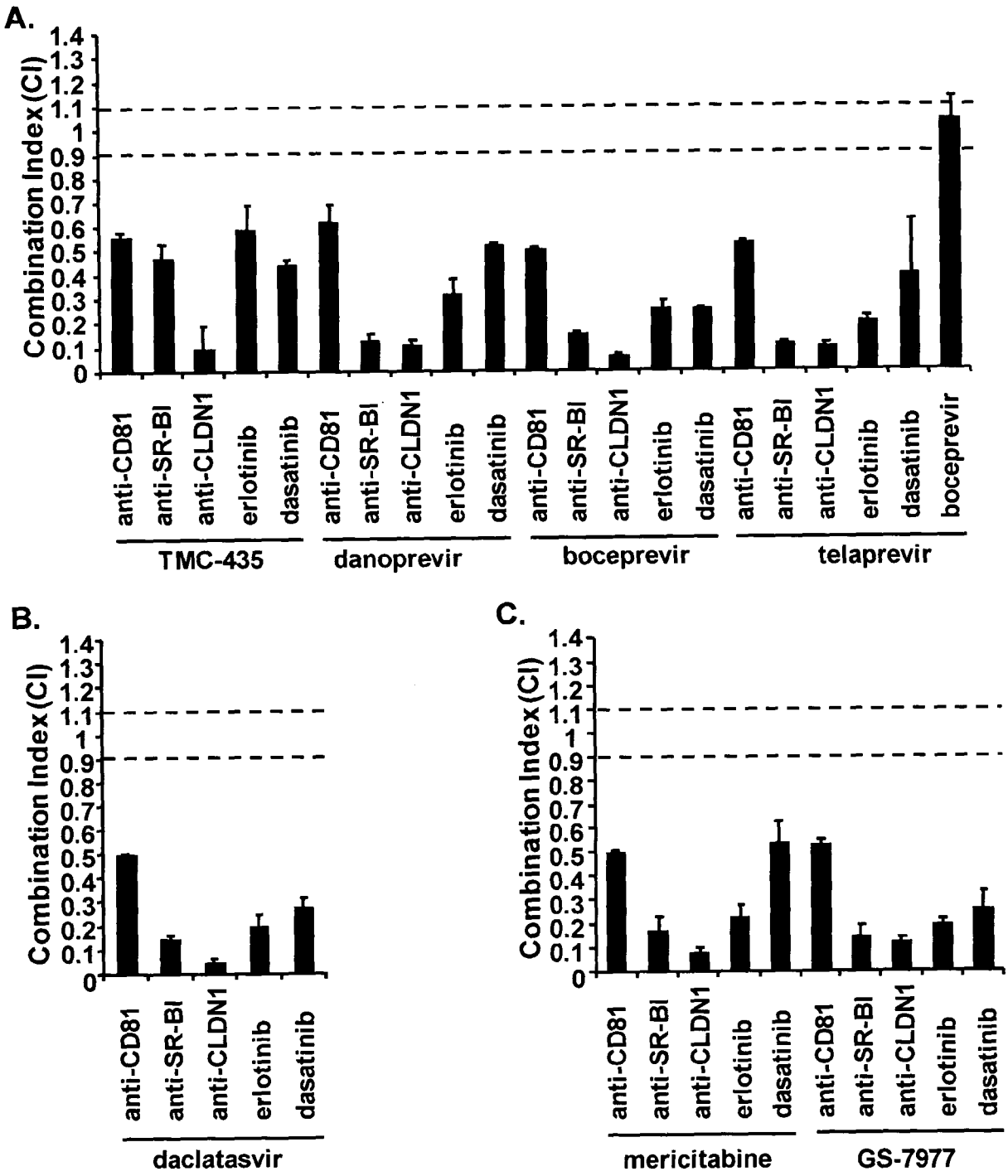

A.
Combination Index (CI)
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
anti-CD81
anti-SR-BI
anti-CLDN1
erlotinib
dasatinib
anti-CD81
anti-SR-BI
anti-CLDN1
erlotinib
dasatinib
anti-CD81
anti-SR-BI
anti-CLDN1
erlotinib
dasatinib
anti-CD81
anti-SR-BI
anti-CLDN1
erlotinib
dasatinib
boceprevir
TMC-435
danoprevir
boceprevir
telaprevir
B.
Combination Index (CI)
anti-CD81
anti-SR-BI
anti-CLDN1
erlotinib
dasatinib
daclatasvir
C.
Combination Index (CI)
anti-CD81
anti-SR-BI
anti-CLDN1
erlotinib
dasatinib
anti-CD81
anti-SR-BI
anti-CLDN1
erlotinib
dasatinib
mericitabine
GS-7977

Figure 5

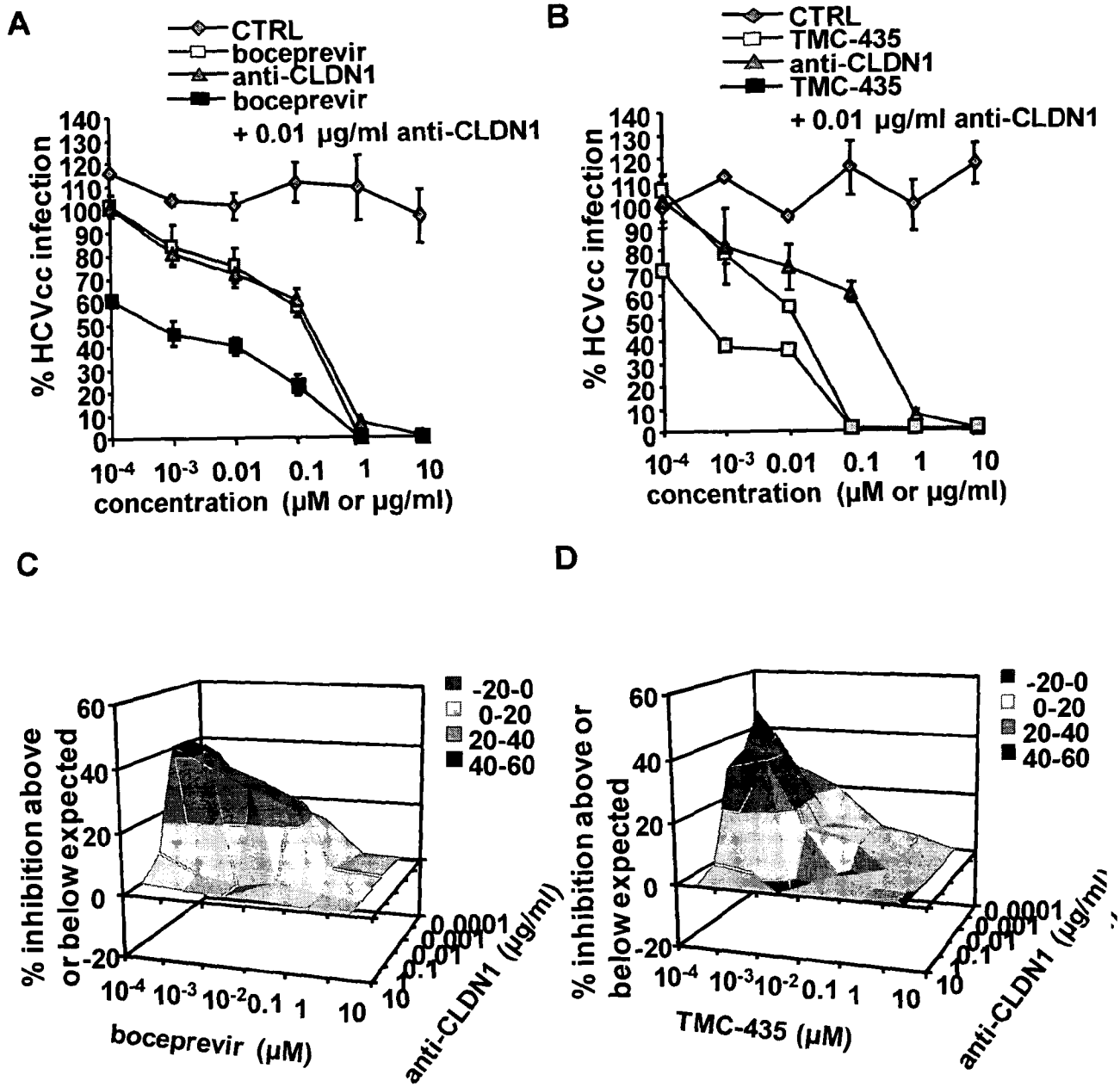

A
CTRL
boceprevir
anti-CLDN1
boceprevir
+ 0.01 µg/ml anti-CLDN1
% HCVcc infection
concentration (µM or µg/ml)
B
CTRL
TMC-435
anti-CLDN1
TMC-435
+ 0.01 µg/ml anti-CLDN1
% HCVcc infection
concentration (µM or µg/ml)
C
-20-0
0-20
20-40
40-60
% inhibition above or below expected
boceprevir (µM)
anti-CLDN1 (µg/ml)
D
-20-0
0-20
20-40
40-60
% inhibition above or below expected
TMC-435 (µM)
anti-CLDN1 (µg/ml)

Figure 6

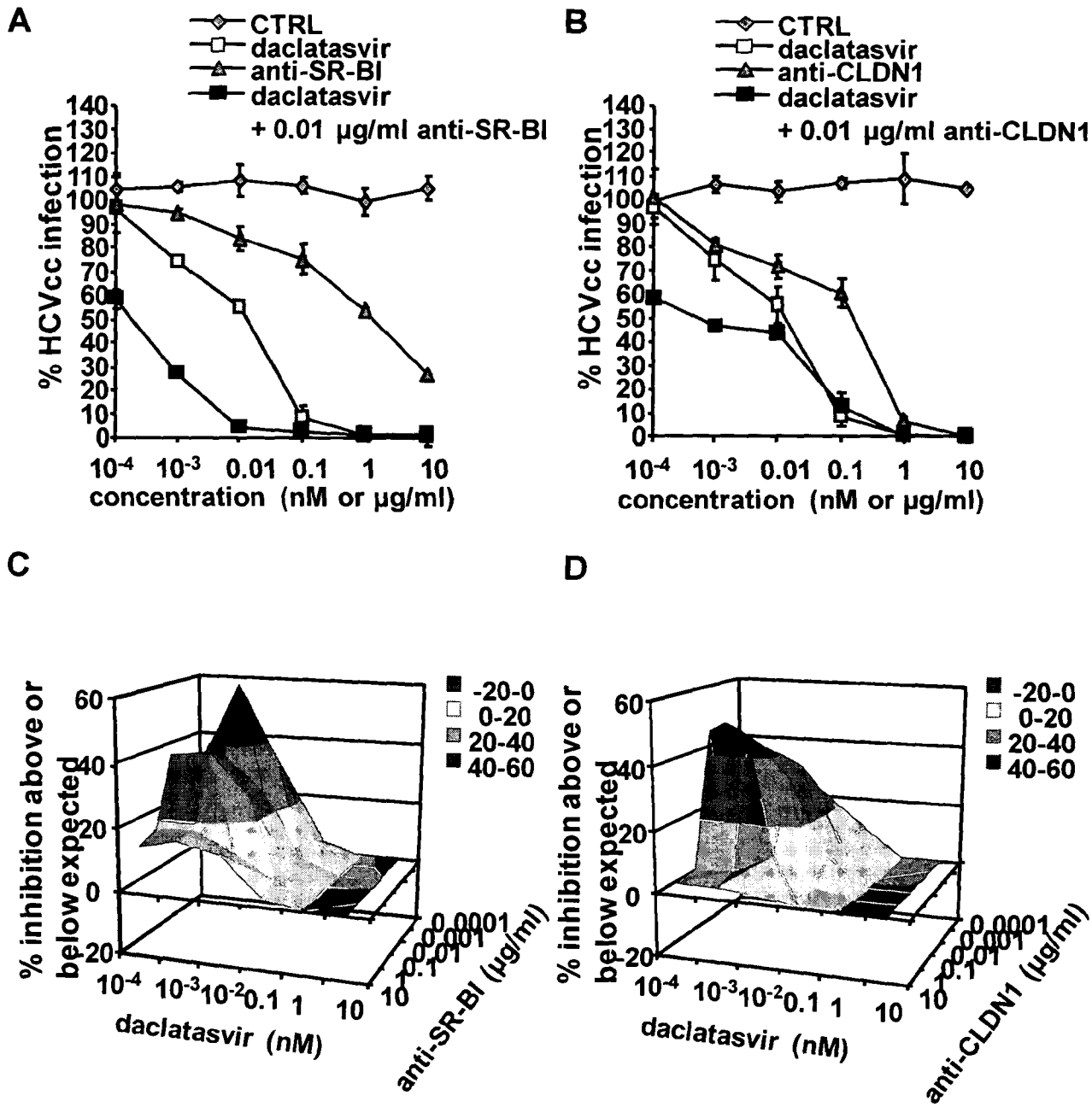

A
CTRL
daclatasvir
anti-SR-BI
daclatasvir
+ 0.01 µg/ml anti-SR-BI
% HCVcc infection
concentration (nM or µg/ml)
B
CTRL
daclatasvir
anti-CLDN1
daclatasvir
+ 0.01 µg/ml anti-CLDN1
% HCVcc infection
concentration (nM or µg/ml)
C
% inhibition above or below expected
-20-0
0-20
20-40
40-60
daclatasvir (nM)
anti-SR-BI (µg/ml)
D
% inhibition above or below expected
-20-0
0-20
20-40
40-60
daclatasvir (nM)
anti-CLDN1 (µg/ml)

Figure 7

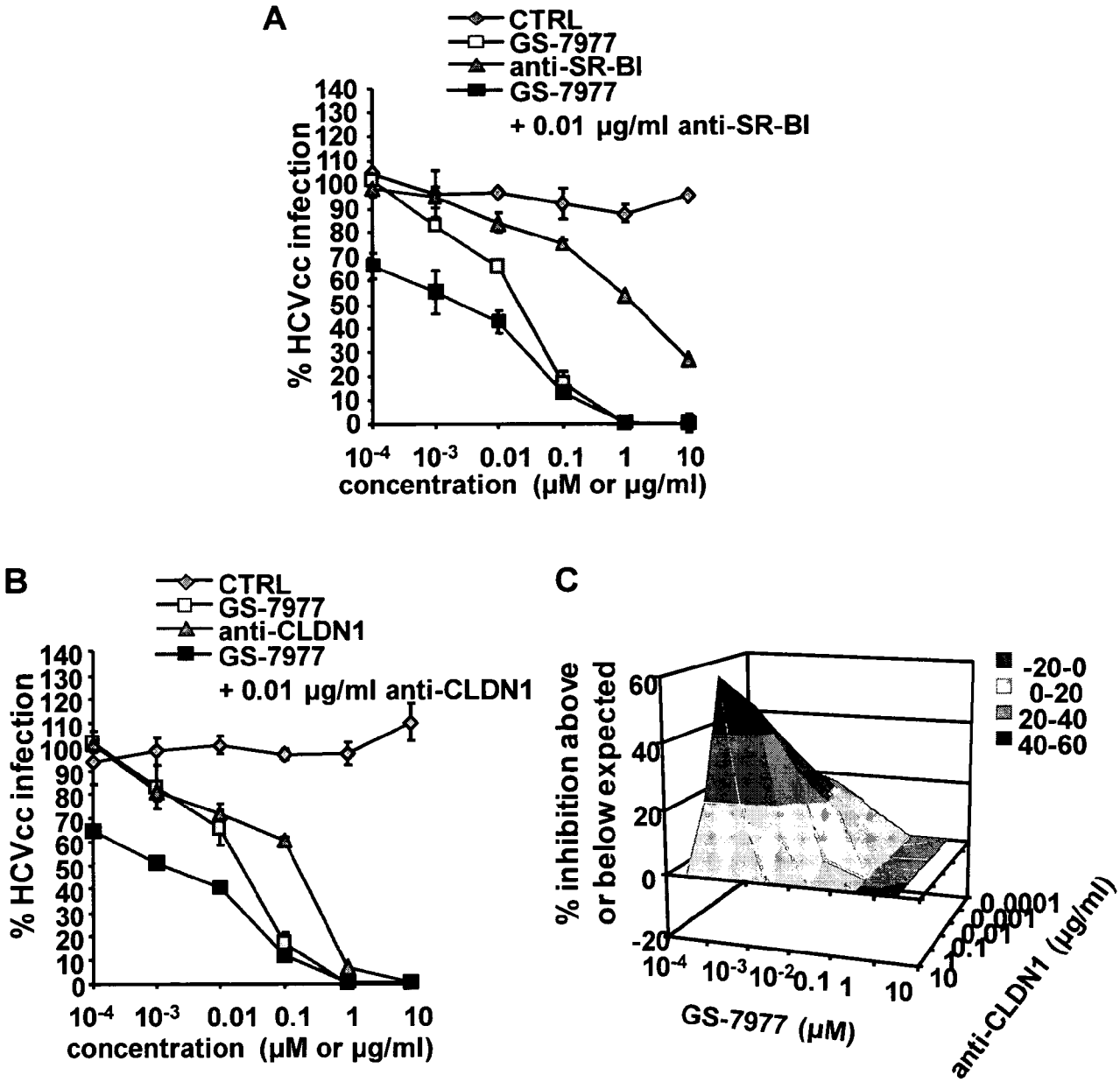
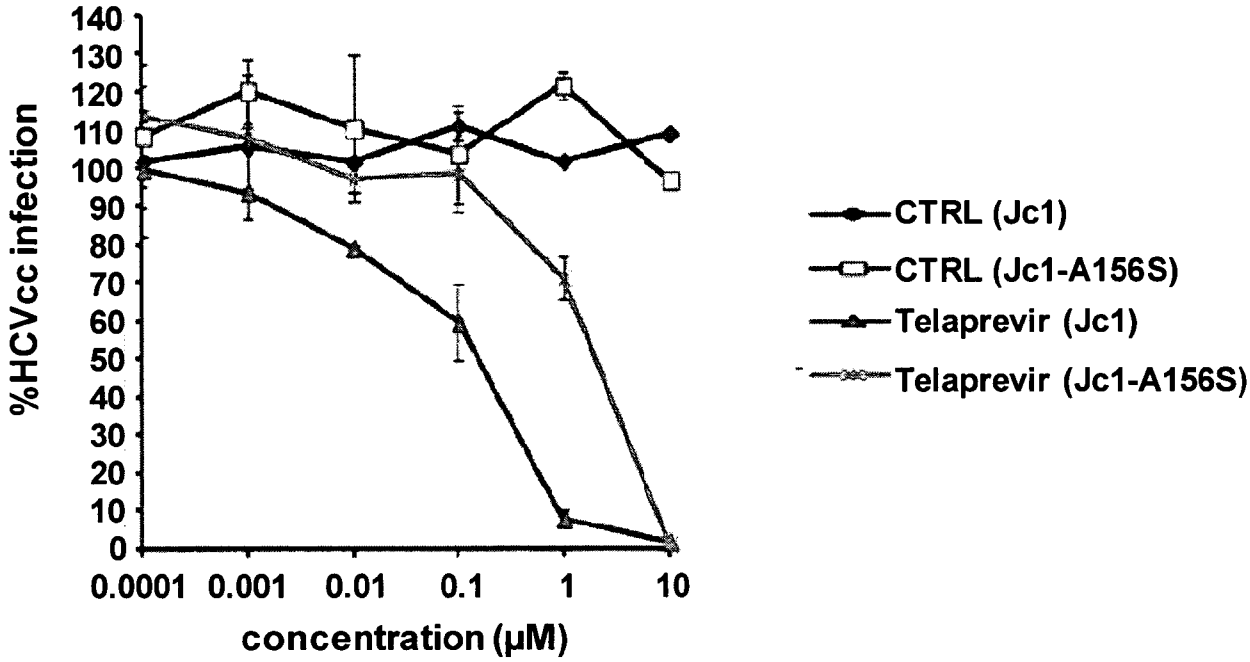

A
CTRL
GS-7977
anti-SR-BI
GS-7977
+ 0.01 µg/ml anti-SR-BI
% HCVcc infection
concentration (µM or µg/ml)
B
CTRL
GS-7977
anti-CLDN1
GS-7977
+ 0.01 µg/ml anti-CLDN1
% HCVcc infection
concentration (µM or µg/ml)
C
% inhibition above or below expected
-20-0
0-20
20-40
40-60
GS-7977 (µM)
anti-CLDN1 (µg/ml)

Figure 8

A



B

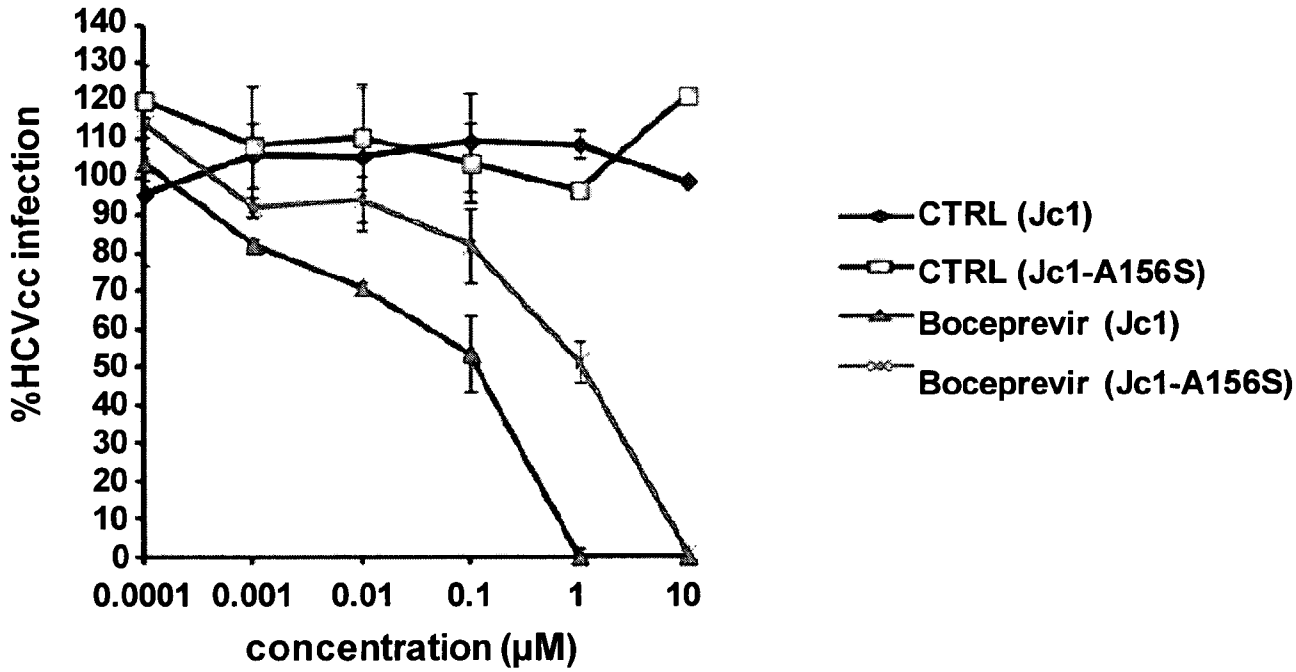
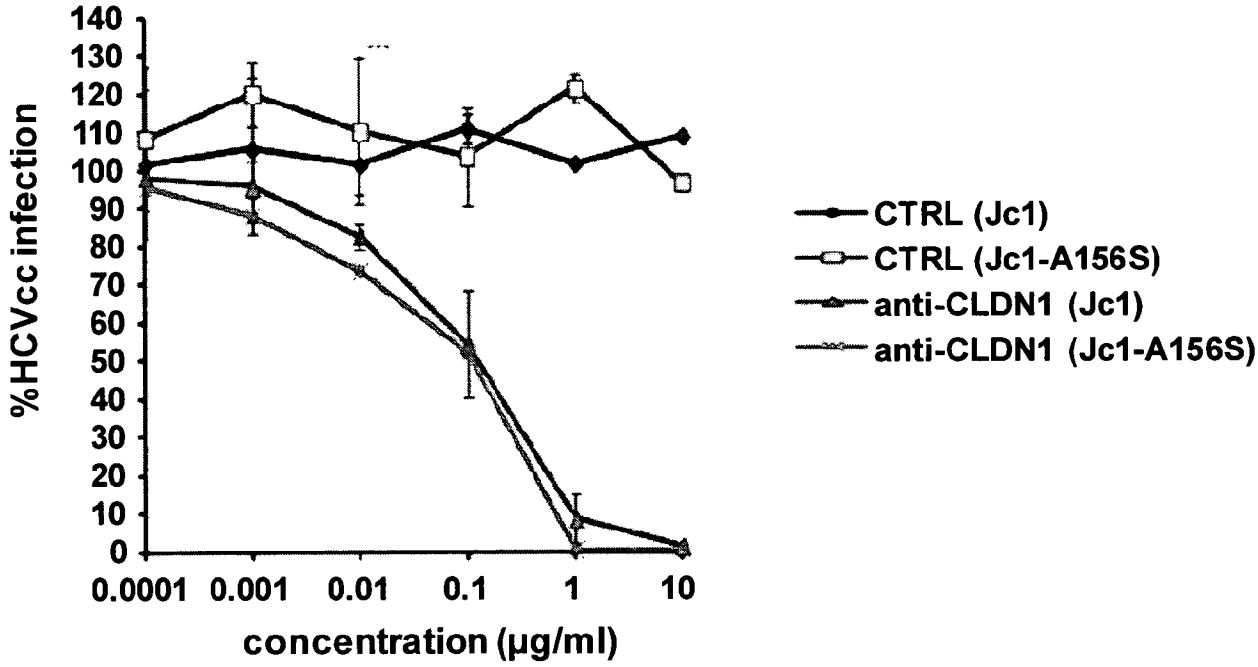


Figure 9(A)-(B)

C



D

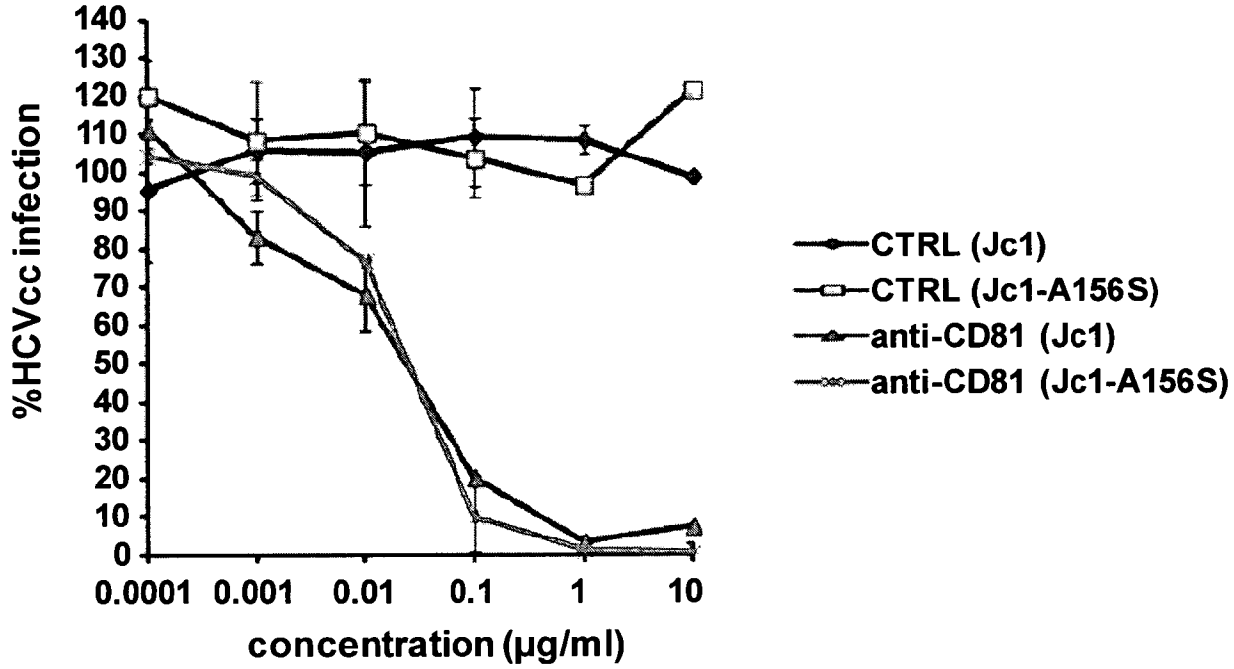


Figure 9(C)-(D)

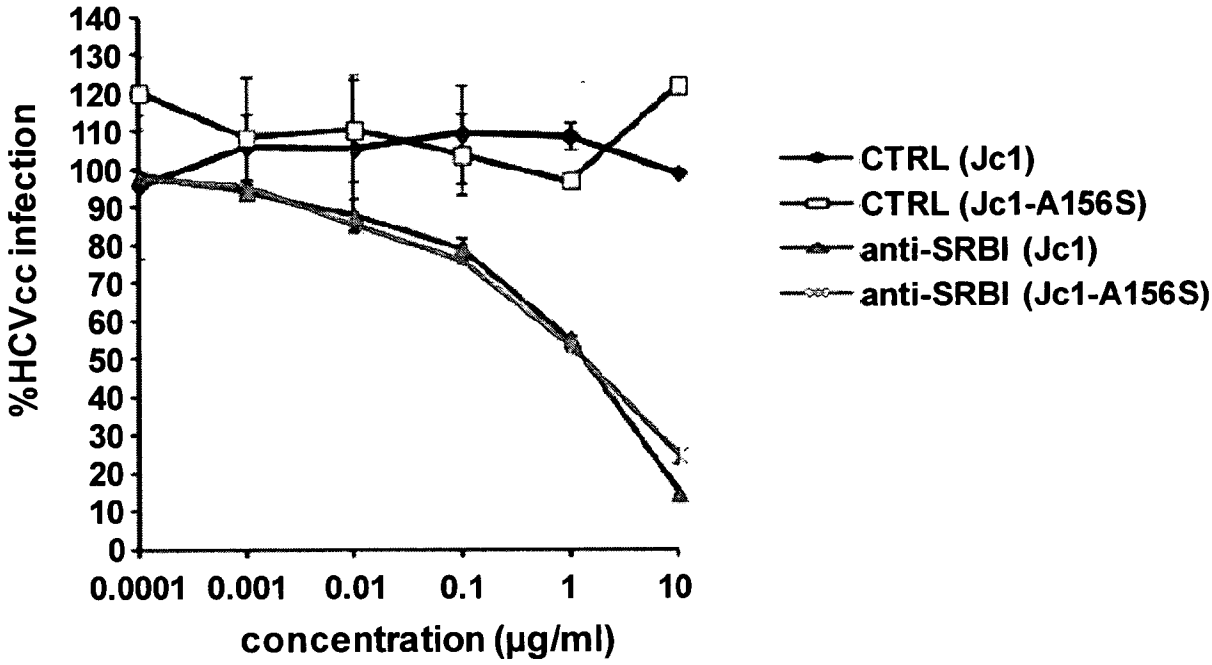

%HCVcc infection
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0001
0.001
0.01
0.1
1
10
concentration (µg/ml)
CTRL (Jc1)
CTRL (Jc1-A156S)
anti-SRBI (Jc1)
anti-SRBI (Jc1-A156S)

Figure 9 (E)

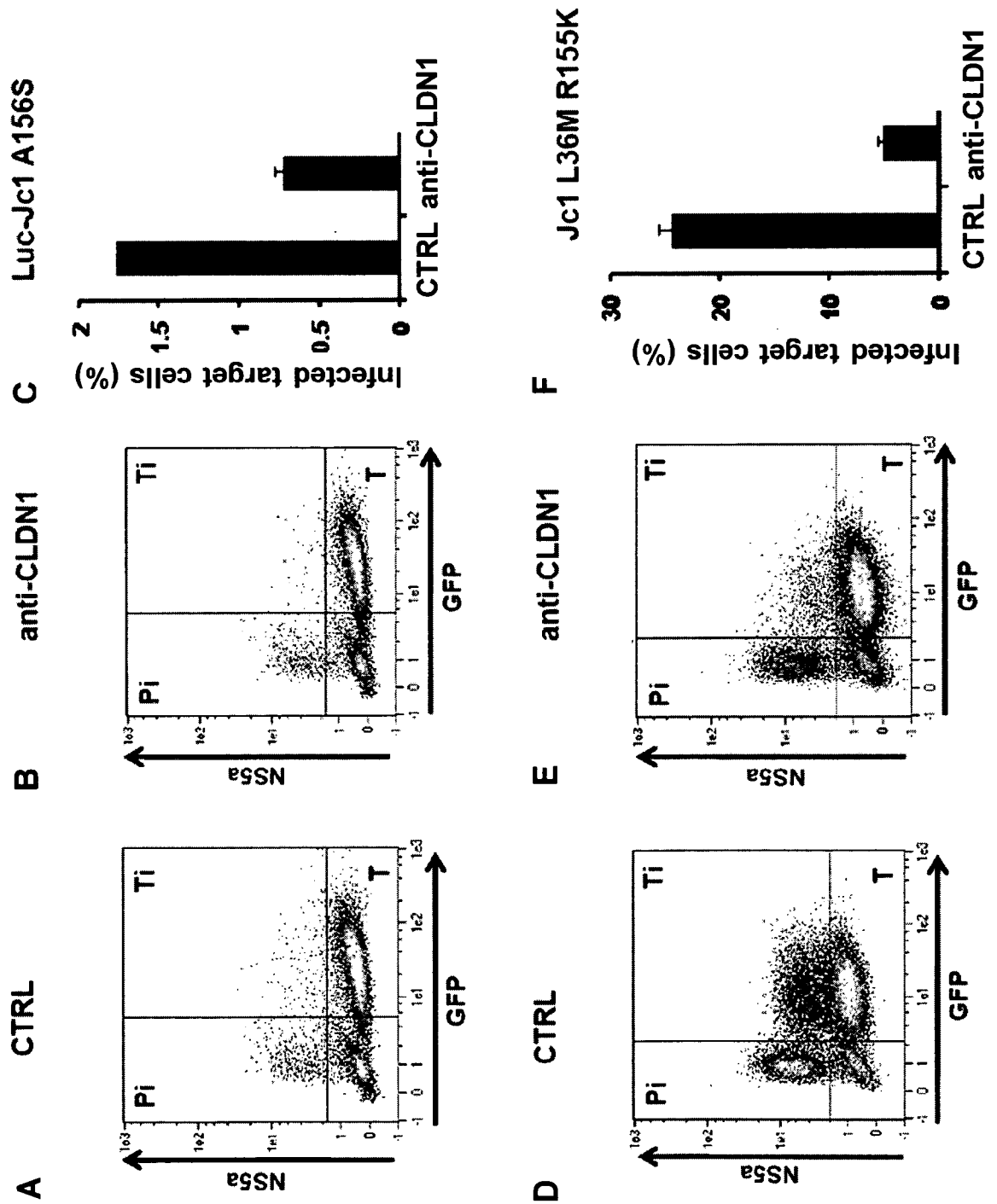

A
CTRL
B
anti-CLDN1
C
Luc-Jc1 A156S
D
CTRL
E
anti-CLDN1
F
Jc1 L36M R155K
Ti
Pi
T
NS5a
GFP
Infected target cells (%)
CTRL anti-CLDN1

Figure 10

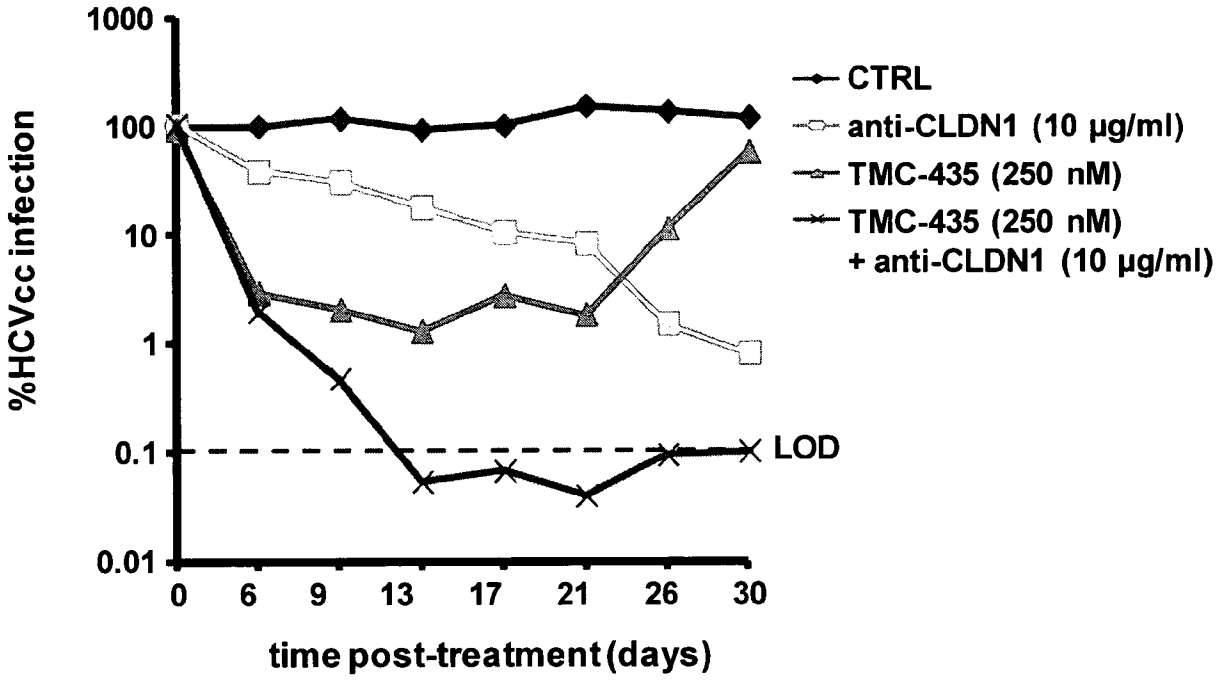

1000
100
10
1
0.1
0.01
%HCVcc infection
0
6
9
13
17
21
26
30
time post-treatment (days)
CTRL
anti-CLDN1 (10 µg/ml)
TMC-435 (250 nM)
TMC-435 (250 nM)
+ anti-CLDN1 (10 µg/ml)
LOD

Figure 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/066098

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61P31/12 C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/034812 A1 (INST NAT SANTE RECH MED [FR]; GENOVAC [DE]; UNIV STRASBOURG [FR]; BAUM) 1 April 2010 (2010-04-01) cited in the application	1,2,6-18
Y	claims 2, 3, 7, 12,18, 20, 21; table 1	3

Y	BARTH HEIDI ET AL: "Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulfate", JOURNAL OF BIOLOGICAL CHEMISTRY, THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC, US, vol. 278, no. 42, October 2003 (2003-10), pages 41003-41012, XP002666885, ISSN: 0021-9258 the whole document	3

	-/--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 2012	11. 12. 2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Weikl, Martina

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/066098

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZEISEL MIRJAM B ET AL: "Hepatitis C virus entry into hepatocytes: Molecular mechanisms and targets for antiviral therapies", JOURNAL OF HEPATOLOGY, MUNKSGAARD INTERNATIONAL PUBLISHERS, COPENHAGEN, DK, vol. 54, no. 3, 1 March 2011 (2011-03-01), pages 566-576, XP002666426, ISSN: 0168-8278 cited in the application the whole document -----	1-3,6-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2012/066098

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-3, 6-18(all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3, 6-18(all partially)

   Claims relating to a combination of at least one anti-HCV-entry factor antibody and at least one 'direct acting' antiviral for use in the treatment or prevention of HCV infection wherein the at least one anti-HCV-entry factor antibody is an antibody against heparan sulfate

   ---

2. claims: 1-3, 6-18(all partially)

   Claims relating to a combination of at least one anti-HCV-entry factor antibody and at least one 'direct acting' antiviral for use in the treatment or prevention of HCV infection wherein the at least one anti-HCV-entry factor antibody is an antibody against LDL receptor

   ---

3. claims: 1-3, 6-18(all partially)

   Claims relating to a combination of at least one anti-HCV-entry factor antibody and at least one 'direct acting' antiviral for use in the treatment or prevention of HCV infection wherein the at least one anti-HCV-entry factor antibody is an antibody against CD81

   ---

4. claims: 1-3, 6-18(all partially)

   Claims relating to a combination of at least one anti-HCV-entry factor antibody and at least one 'direct acting' antiviral for use in the treatment or prevention of HCV infection wherein the at least one anti-HCV-entry factor antibody is an antibody against SR-BI

   ---

5. claims: 1-3, 6-18(all partially)

   Claims relating to a combination of at least one anti-HCV-entry factor antibody and at least one 'direct acting' antiviral for use in the treatment or prevention of HCV infection wherein the at least one anti-HCV-entry factor antibody is an antibody against Occludin

   ---

6. claims: 4(completely); 1-3, 6-18(partially)

   Claims relating to a combination of at least one anti-HCV-entry factor antibody and at least one 'direct acting' antiviral for use in the treatment or prevention of HCV infection wherein the at least one anti-HCV-entry factor antibody is an antibody against claudin-1

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

---

7. claims: 1-3, 6-18(all partially)

Claims relating to a combination of at least one anti-HCV-entry factor antibody and at least one 'direct acting' antiviral for use in the treatment or prevention of HCV infection wherein the at least one anti-HCV-entry factor antibody is an antibody against Niemann-Pick C1-like 1 cholesterol absorption receptor

---

8. claims: 5(completely); 1, 2, 6-18(partially)

Claims relating to a combination of at least one anti-HCV-entry factor antibody and at least one 'direct acting' antiviral for use in the treatment or prevention of HCV infection wherein the at least one anti-HCV-entry factor antibody is an antibody against a receptor tyrosine kinase

---

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/066098

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010034812 A1	01-04-2010	CA 2738027 A1	01-04-2010
		CN 102224171 A	19-10-2011
		EP 2328933 A1	08-06-2011
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