Title: ANTAGONISTS OF ACID LIPASE FOR PREVENTING VIRUS INFECTION

Abstract: The present invention relates to a pharmaceutical composition for preventing or treating a virus infection or a disease resulting from a virus infection, said composition consisting of one or more active pharmaceutical ingredients and, optionally, at least one pharmaceutically acceptable excipient and/or carrier, wherein at least one active pharmaceutical ingredient is an antagonist of Lysosomal Acid Lipase substantially not inhibiting human pancreatic lipase or bovine milk lipoprotein lipase.
Antagonists of acid lipase for preventing virus infection

The major approach to medical management of virus infection is increasingly the treatment of patients with antiviral drugs, usually targeting viral enzymes essential for virus replication. The various enzymatic processes on which the viral replication cycles rely, present unique approaches for targeted inhibition by pharmacological agents. Due to the high rates of virus production and a relatively high rate of mutation, therapy of virus infection must necessarily also take into account the development of resistance mutations. Thus, treatment regimens often include the administration of multiple therapeutic agents in combination or in succession.

The replication cycle of Hepatitis C Virus (HCV), for example, provides several opportunities for developing and using agents which directly interfere with HCV encoded proteins at various stages of the virus life cycle. These agents have recently been summarized as directly acting antiviral agents (DAAs). In the case of HCV, it appears possible to develop DAAs targeting most of the structural and non-structural proteins, i.e. C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. However, viruses generally lack efficient proofreading functions, allowing them to adapt to selective pressure rather dynamically. With the rapid HCV viral turnover (up to $10^{12}$ virions produced each day), for example, it is estimated that one mutation is contained in every single genome copied. There are thus many variant populations coexisting in a given individual, the patient's quasispecies. Most of these variant populations are susceptible to DAAs because wild-type viruses usually have the advantage of fitness, but some of these preexisting variants are drug-resistant at baseline. With growing selection pressure these preexistent variants are rapidly selected and can become the predominant circulating population, potentially leading to treatment failure. At present, treatment failure is avoided by continuously developing novel DAAs and by administering these in succession and/or in combination to the affected patients.

Increasingly, however, novel treatment strategies focus on targeting components of the cellular machinery on which viruses rely during their replication cycle. Since cellular components, generally, are not affected from the selection pressure induced by antiviral therapy, it is believed that drugs targeting these components induce antiviral barriers which are more difficult to overcome by the virus. The targets of this strategy are rather diverse and
include cellular proteins required for virus attachment, virus uptake, virus assembly, virus intercellular spread and for release of virus particles from the infected cell.

Many different virus families enter host cells either by direct fusion at the plasma membrane or by a process called endocytosis which is further classified into classical clathrin-dependent and clathrin independent pathways. While virus entry via endocytosis pathways often requires, as precondition, binding to specific surface receptors, the lipid composition of the plasma membrane may significantly contribute to efficient virus uptake. Since membranous free cholesterol is enriched in plasma membrane microdomains called lipid rafts and is also present in endosomal membranes it appears to be a key component of the endocytosis pathway. In fact, it is tempting to speculate that it represents a platform that is used by many viruses for entering and infecting the host cell.

A main source of cellular cholesterol is provided by cholesterol containing lipoproteins, which are taken up by LDL-receptor mediated endocytosis. Within lipoproteins most cholesterol is present as cholesterol esters and needs to become hydrolysed first before being incorporated into cellular membranes and further transported intracellularly. Hydrolysis of cholesterol esters and triglycerides of endocytosed lipoproteins is mediated by the endosomal lipase called lysosomal acid lipase/cholesteryl ester hydrolase, beginning early after acidification within early endosomal compartments. The generated free cholesterol is incorporated into the endosomal membrane and then transported out of the endosomal system to the plasma membrane and the endoplasmic reticulum and does not reach the lysosome (Soccio & Breslow, 2004, Arterioscler Thromb Vasc Biol. 24:1150-1160). Once incorporated into the plasma membrane it becomes concentrated within lipid rafts (Lusa et al. 2001, J Cell Sci. 114: 1893-1900). In fact, the lysosomal acid lipase appears to play a key intracellular role in supplying cholesterol for cell growth and membrane function and in the regulation of processes that are mediated by cellular cholesterol flux, including internalization of low density lipoprotein and cholesterol biosynthesis and esterification.

Some serum associated membranous viruses, for example HBV and HCV are associated with substantial amounts of cholesteryl esters, which could participate to an additional shell or surface associated lipoprotein that must be dismantled during virus entry, i.e. before the viral genome can finally be delivered to the cytoplasm of the infected host cell. Additionally, generation of free cholesterol in endosomes might play an important role at an early stage of infection of many virus families. Thus, it was hypothesized by the inventors that, for example, a productive HBV and HCV infection of host cells relies, in several ways (i.e. e.g. entry and
dismantling), on the presence of a functional lipase in the host cell. Hence, it was speculated that the enzymes responsible for hydrolyzing cholesteryl esters represent promising cellular targets for inhibiting virus entry.

Orlistat, a lipase inhibitor interfering with several different human lipases, has recently been used by Yang et al. (Hepatology 2008, 48: 1396-1403) for studying its effect on HCV replication. Rather than reducing HCV replication, however, Orlistat weakly enhanced the viral replication at either the RNA or the protein level. Thus, the authors concluded that Orlistat treatment does not exert any inhibitory effect. Surprisingly, however, the present invention provides proof that the activity of lysosomal acid lipases, in particular of Lyosomal Acid Lipase, is required for efficient HCV infection of host cells and that an inactive lysosomal acid lipase has a strong negative impact on the infectivity of several other viruses. As shown herein below in the examples which illustrate the invention, an antagonist of Lysosomal Acid Lipase has a strong, dose dependent inhibitory effect on HCV infection and on the infection of several other viruses. In fact, additional experimental results shown herein below demonstrate that the therapeutic effect of the antagonist is tightly associated with the mode of entry of the infecting virus.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides in a first aspect a pharmaceutical composition for preventing or treating a virus infection or a disease resulting from a virus infection, said composition comprising one or more active pharmaceutical ingredients and, optionally, at least one pharmaceutically acceptable excipient and/or carrier, wherein at least one active pharmaceutical ingredient is an antagonist of Lysosomal Acid Lipase substantially not inhibiting human pancreatic lipase or bovine milk lipoprotein lipase.

In one embodiment, said antagonist of Lysosomal Acid Lipase is or comprises a thiadiazole carbamate substituted with N-heterocycles, preferably selected from the group consisting of 4-(pyrrolidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate, 4-(pyrrolidin-1-yl)-1,2,5-thiadiazol-3-yl morpholine-4-carboxylate, 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate, 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl morpholine-4-carboxylate, 4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate, 4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl morpholine-4-carboxylate and preferably 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate.
In one embodiment said thia diazole carbamate substituted with N-heterocycles is preferably a 1,2,5-thiadiazole carbamate. Preferably said thia diazole carbamate has the structure

![Chemical Structure 1]

wherein X is a nitrogen containing heterocycle.

In another embodiment said thia diazole carbamate substituted with N-heterocycles is

![Chemical Structure 2]

wherein X is -O- or -CH₂-.

In another embodiment, the thia diazole carbamate substituted with N-heterocycles is 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate.

In another embodiment, the virus infection comprises an infection with a virus selected from the group consisting of Hepatitis C Virus; Hepadnaviridae, preferably Hepatitis B Virus and Hepatitis D Virus; Hepatitis E virus; Flaviviridae preferably Alphaviruses, preferably Chikungunya Virus, Semliki Forest Virus, Sindbis Virus; West-Nile-Virus, Dengue Virus; Yellow-fever; Filoviridae preferably Marburg and Ebola Virus, Orthomyxoviridae, preferably Influenza virus, preferably Influenza Virus A, preferably Influenza Virus of subtype H5N1; Rhabdoviridae, preferably Vesicular Stomatitis Virus and Rabiesvirus; Retroviridae, preferably Human Immunodeficiency Virus, human T-Cell-Leukemia Virus, Avian Leukemia Virus; Paramyxoviridae preferably Measles Virus and Rubella-Virus, New-Castle-Desease Virus and Respiratory-Syncytial-Virus; Arenaviridae, preferably Lymphocytic choriomenigitis virus; Picornaviridae, preferably Hepatitis A Virus, Echovirus, Enterovirus, human Rhinovirus, Coxsackie Virus, Polio Virus; Herpesviridae preferably Herpes Simplex
Virus, Eppstein-Barr-Virus; Cytomegalovirus and Varicella Zoster Virus; Poxviridae preferably Vaccinia Virus; Adenoviridae, preferably human Adenovirus; Papovaviridae preferably human Papillomaviruses (HPV); Polyomaviridae preferably SV40; Burnaviridae preferably Hanta Virus and Rift-Valley-Fever Virus; Reoviridae preferably Rota Virus and Blue-Tongue-Virus; Coronaviridae preferably SARS coronavirus; preferably Hepatitis C Virus.

In another embodiment, said virus is a virus which preferably enters cells by receptor-mediated endocytosis, preferably a virus selected from the group consisting of Hepatitis C Virus; Hepadnaviridae, preferably Hepatitis B Virus and Hepatitis D Virus; Hepatitis E virus; Flaviviridae preferably Alphaviruses, preferably Chikungunya Virus, Semliki Forest Virus, Sindbis Virus; West-Nile-Virus, Dengue Virus; Yellow-fever; Filoviridae preferably Marburg and Ebola Virus, Orthomyxoviridae, preferably Influenza virus, preferably Influenza Virus A, preferably Influenza Virus of subtype H5N1; Rhabdoviridae, preferably Vesicular Stomatitis Virus and Rabiesvirus; Retroviridae, preferably Human Immunodeficiency Virus, human T-Cell-Leukemia Virus, Avian Leukemia Virus; Paramyxoviridae preferably Measles Virus and Rubella-Virus, New-Castle-Desease Virus and Respiratory-Syncytial-Virus; Arenaviridae, preferably Lymphocytic choriomeningitis virus; Picornaviridae, preferably Hepatitis A Virus, Echovirus, Enterovirus, human Rhinovirus, Coxsackie Virus, Polio Virus; Herpesviridae preferably Herpes Simplex Virus, Eppstein-Barr-Virus; Cytomegalovirus and Varicella Zoster Virus; Poxviridae preferably Vaccinia Virus; Adenoviridae, preferably human Adenovirus; Papovaviridae preferably human Papillomaviruses (HPV); Polyomaviridae preferably SV40; Burnaviridae preferably Hanta Virus and Rift-Valley-Fever Virus; Reoviridae preferably Rota Virus and Blue-Tongue-Virus; Coronaviridae preferably SARS coronavirus; preferably Hepatitis C Virus.

In a preferred embodiment, the virus is Hepatitis B Virus, Hepatitis C Virus, Influenza Virus, Dengue Virus, Human Immunodeficiency Virus, Ebola Virus, Chikungunya Virus or Vesicular Stomatitis Virus and the antagonist is 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate.

In another embodiment, the pharmaceutical composition comprises at least one additional active pharmaceutical ingredient or the pharmaceutical composition of the present invention is used in a method of preventing or treating which comprises administering at least one additional pharmaceutical composition comprising at least one additional active pharmaceutical ingredient.
In another embodiment, the additional active pharmaceutical ingredient referred to herein is an inhibitor of activity of a viral protein, wherein the viral protein is a protease or a DNA- or RNA-dependent polymerase, a reverse transcriptase, a viral kinase, a viral integrase, or a viral surface protein.

In another embodiment, the virus is Hepatitis C Virus and the additional active pharmaceutical ingredient is an inhibitor of activity of the virus proteins C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and/or NS5B.

In another embodiment, the inhibitor is selected from the group consisting of ribavirin, telaprevir, boceprevir, simeprevir, asunaprevir, faldaprevir, vaniprevir, danoprevir, ABT-450, GS-9451, GS-9256 (protease inhibitors), ABT-267, daclatasvir, ledipasvir, GS-5816 (NS5A inhibitors), ABT-333, BMS-791325, ABT-072, tegobuvir, deleobuvir, setrobuvir, GS-9669 (non-nucleoside NS5B polymerase inhibitor), sofosbuvir, PSI-6206 (nucleotide analog), mericitabine (nucleoside analog), CPG10101 (synthetic ODN), NOV-205 (formulation of oxidized glutathione and inosine that acts as a hepatoprotective agent with immunomodulating and anti-inflammatory properties), INX-08189 (2’-C-methyl nucleosides).

In another embodiment, the virus is hepatitis B virus (HBV) and the additional active pharmaceutical ingredient is an inhibitor of activity of the virus proteins core, S, M, L, X or the viral polymerase/reverse transcriptase or an inhibitor of HBV entry or an inhibitor of capsid assembly or a TLR-3, TLR-7, TLR-8 or TLR-9 agonist or a ligand of a Rig-I like receptor.

In another embodiment, the virus is Influenza Virus A and the additional active pharmaceutical ingredient is an inhibitor of activity of the neuraminidase (NA) or M2 protein, or an inhibitor of a virus hemagglutinin (HA), or an inhibitor of NP, M1, M2, NS1, NS2, PA, PB1, PB1-F2 and/or PB2.

In another embodiment, the pharmaceutical composition of the present invention comprises at least one additional active ingredient, which is an anti-HCV agent or an anti-HBV agent preferably selected from the group consisting of interferon, preferably interferon-alpha, peginterferon Lambda-1a, peginterferon alfa, IFN gamma, peginterferon alfa-2a, peginterferon alfa-2b; recombinant interleukin 7 (CYT107); lipase inhibitor, preferably Orlistat; Cyclosporin, Cyclophilin inhibitor, preferably alispovir, SCY-635; Silibinin; agonist of the c-mpl (TpoR) receptor, preferably 3’-{(2Z)-2-[1-(3,4-dimethylphenyl)-3-
methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)hydrazino]-2'-hydroxy-3-
biphenylcarboxylic acid; 1-[(4-chlorophenyl)methyl]-2-(pyrrolidin-1-ylmethyl)benzimidazole (clemizole); thiazolide, preferably [2-[(5-nitro-1,3-thiazol-2-yl)carbamoyl]phenyl]ethanoate (nitazoxanide); hydroxychloroquine; antibody against tumor necrosis factor alpha (TNF-α), preferably infliximab, neuraminidase inhibitors, preferably zanamivir (Relenza), Rapivab (peramivir) and/or oseltamivir (Tamiflu); M2 inhibitors, preferably amantadine and rimatadine.

In another aspect, the present invention also relates to a method of preventing or treating in a subject a viral infection or a disease resulting from a viral infection, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of the present invention.

In one embodiment, the disease is selected from the group consisting of an acute or chronic HBV or HCV infection, a virus induced liver abnormality, a liver cirrhosis, a hepatocellular carcinoma, reinfection of liver transplants, HCV induced diabetes, HBV or HCV induced liver fibrosis, non alcoholic fatty liver disease.

In one embodiment, the method of preventing or treating of the present invention comprises administering at least one additional pharmaceutical composition comprising at least one additional active pharmaceutical ingredient.

In another aspect, the present invention also relates to a method of monitoring a virus infection, comprising

(a) obtaining a first sample and a second sample from a subject, wherein the first sample is obtained prior to the second sample and the subject is a subject treated with the pharmaceutical composition of the present invention;

(b) determining in the first and the second sample the amount of a marker, which is indicative for the virus infection; and

(c) comparing the amount of marker determined in the first and the second sample; wherein a reduced amount of said marker in the second sample is indicative for a regression of the virus infection.

In another aspect, the present invention also relates to a method of selecting a patient sensitive to a therapy with the pharmaceutical composition of the present invention, comprising

(a) obtaining a first sample and a second sample from a subject, wherein the first sample is obtained prior to and the second sample is obtained after administering the pharmaceutical composition of the present invention;
(b) determining in the first and the second sample the amount of a marker which is indicative for virus infection; and
(c) comparing the amount of marker determined in the first and the second sample; wherein a reduced amount of said marker in the second sample is indicative for the subject's sensitivity to the pharmaceutical composition.

In one embodiment, said virus is HCV. In another embodiment, said virus is HBV.

Other features and advantages of the instant invention will be apparent from the following detailed description and claims.
BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1:  **Orlistat inhibits HCV infection.**

Huh7.5 cells were preincubated in a medium containing 70μM Orlistat, at which concentration the cells were completely vital (fig 1 A). During infection with HCV, the cell culture supernatant lacked Orlistat ("pre") or also contained a concentration of 70μM Orlistat ("pre+sim"). In a control experiment, 70μM Orlistat was only present during infection ("sim"). Viral titers were determined by TCID50 and significance of inhibitory effects was calculated by student’s t-test (B,D). Orlistat also showed a strong inhibitory effect on protein level (NS3; fig 1C); *p < 0.05, **p < 0.01 and ***p < 0.001.

Figure 2:  **Orlistat blocks early steps of HCV infection, replication and cell-to-cell transmission.**

(A) Huh 7.5 cells were infected with JC1 in the presence or absence of 70μM of Orlistat at 4°C for 2h, washed and incubated at 37°C for 48h. Samples were stained for NS3 as a representative for infection. Here it is clearly shown that there is no effect of Orlistat on the attachment of the virus, which is the only step taking place at 4°C. (B) Huh7.5 cells were infected for 2h with an MOI of 0.1, washed and afterwards covered with an agarose-medium-overlay containing Orlistat or DMSO, which prevents the virus to spread via the supernatant. After 72h agarose was removed and cells were stained with NS3 ab to evaluate the effects on HCV induced cell-to-cell spread, which is strongly inhibited in the presence of Orlistat. (C) HCV (JC1) was incubated with Orlistat, DMSO or untreated at 37°C for 1h and then transferred to Huh7.5 cells. After 48h cells were stained for NS3 and the impact on viral particles was investigated. (D) Expression levels of potential HCV receptors and co- receptors CD81, SRB-I, Claudin-1, Occludin and LDL-R in 70μM Orlistat treated and untreated HUH7.5 cells was measured in relation to β-actin. Hereby no inhibitory effect of Orlistat could be shown on the expression patterns of all potential HCV receptors and coreceptors. (E) LucUbiNeo-ET replicon cells were incubated with 70 μM of Lalistat for 48h. Viral replication was measured by luciferase reporter assay. Orlistat inhibited replication in the replicon system Conl (genotype 1b).
Figure 3: The antiviral effect for Orlistat additionally relies on a clustering of E2 and an accumulation of Core in cellular lipid storages (lipid droplets). (A) Huh7.5 cells were infected with JC1 (MOI 0.5) after a preincubation and simultaneous treatment in the presence or absence of 70μM of Orlistat. Effects were measured by immuno-fluorescent stainings of E2 and Core protein. Representative images are shown. (B) Quantification of Core positive accumulations in donut-like lipid droplet structures in relation to positive events in the control. 10 random Core positive areas were chosen and the positive events for an accumulation of Core in donut structures in relation to negative events was counted.

Figure 4: Orlistat has an antiviral effect on Vesicular Stomatitis Virus (VSV) infection. (A) Huh7.5 cells were infected with single-cycle vector (no reinfection after first entry) VSV-delta-GFP (MOI 0.6) in the presence (A, B) and absence (C) of preincubated and simultaneously (A) or only simultaneously (B) added Orlistat in a concentration of 70μM. Effects were measured by fluorescence and cell viability by bright field microscopy. Representative images are shown. (D) VSV replication was measured by luciferase reporter assay. Error bars represent SD from 7 experiments. Significance was analyzed by student’s t-test *p < 0.05, **p < 0.01 and ***p < 0.001.

Figure 5: Lipase A siRNA transfections show inhibitory effects on HCV expression and infectivity. (A) Huh7.5 cells were transfected with indicated siRNAs two times consecutively, washed and infected for 48h with JC1 (MOI 0.5) followed by RNA extraction and relative qPCR. The percentage of inhibition of Lipase A, HCV and Lipase C (positive control) is given. Error bars represent SD from 2 independent experiments. siRNAs against Lipase A showed a very good knock down efficiency, while the inhibition on HCV was around 25-30%. Lipase C knock down, which was described to be important for the HCV infection cycle, showed an inhibitory effect of 30-40%. Both siRNAs in combination showed additive inhibitory effects. (B) Supernatants from (A) were titrated on Huh7.5 cells to determine the infectivity by TCID50. Error bars represent SD from 4
independent experiments. Strong, significant inhibitory effects of the infectivity of the supernatant from siRNA knock down experiments could be shown. Significance was analyzed by student’s t-test *p < 0.05, **p < 0.01 and ***p < 0.001.

Figure 6: The antiviral effect of Lalistat, a specific Lipase A inhibitor, shows a strong requirement for the lysosomal acid Lipase in the HCV infection cycle.

(A) Huh7.5 cells were infected with HCV (JC1) (MOI 0.5) in the presence and absence of different concentrations of Lalistat. Effects were measured by immuno-fluorescent staining for E2. Representative images are shown. Cell viability was measured by BF microscopy. Lalistat clearly shows a dose-dependent inhibitory effect on HCV infection. (B; C) Lalistat toxicity was tested on Huh7.5 cells, which were cultured in the presence of different concentrations of Lalistat. 48h post incubation Lalistat-containing cell culture medium was removed and viability tested using the neutral red and the CTB viability assay. OD was measured at 540nm and fluorescence measured respectively. Results are means + SD from 3 independent (NR) and one experiment (CTB). Lalistat shows slight toxicity up to 70μM.

Figure 7: Effect of Lalistat on HCV protein levels.

(A) Huh7.5 cells were infected with JC1 (MOI 0.5), preincubated and simultaneously treated with the indicated concentrations of Lalistat. WB lysates were analyzed for expression levels of HCV proteins NS3 and Core. β-actin was used as a loading control. (B) Percentage of Inhibition of the HCV Core and NS3 protein on protein level in relation to the loading control. Lalistat strongly inhibits protein levels of HCV proteins NS3 and Core.

Figure 8: Lalistat treatment shows a reduction of HCV titers in undifferentiated and differentiated Huh7.5 cells.

(A) Huh 7.5 cells were preincubated and simultaneously treated with 70μM of Lalistat and Orlistat for 48h; infection was performed 2h after preincubation with JC1 in the presence or absence of the Lipase inhibitors. Supernatants were collected and the infectivity titers were determined by TCID50. Error bars are SD from 3 independent experiments. (B) Differentiated Huh7.5 cells were
infected and differentiated for 4 weeks before they were treated with 100μM of Orlistat and Lalistat. Supernatants were collected 24h post treatment and the infectivity titers were determined by TCID50. Error bars are SD from 2 independent experiments. (C) Cell Titer blue Assay shows the viability of the differentiated Huh7.5 cells after Lalistat and Orlistat treatment. Fluorescence was measured in the Tecan reader Infinite F200. Up to 100μM of Lalistat does not show any toxic effects on differentiated Huh7.5. In comparison to undifferentiated Huh7.5 cells the differentiated cells are still viable with higher concentrations. Significance was analyzed by student’s t-test *p < 0.05, **p < 0.01 and ***p < 0.001.

Figure 9: Lalistat has no impact on HCV replication in the HCV replicon System.
(A) LucUbiNeo-ET replicon cells were incubated with 10, 70 μM of Lalistat for 48h. Viral replication was measured by luciferase reporter assay. No inhibitory effect was measured on viral replication, which clearly shows that the inhibitory effect of Lalistat plays a role during the early entry steps of infection, which the replicon system are lacking.
(B) Cell viability was measured by NR assay.

Figure 10: Lalistat shows a strong effect on HCV cell-to-cell-spread.
Huh7.5 cells were infected with JC1 (MOI 0.1) for 2h, washed and covered with an Agarose-Medium-Overlay containing 70μM Lalistat, Orlistat or DMSO in given concentrations. 72h post infection cells were fixed with 8% of PFA and agarose overlay was removed. Effects are shown by immuno-fluorescent staining for E2. Representative images are shown. Cell viability was measured by bright field microscopy. The experimental results demonstrate that the cell-to-cell spread of HCV is strongly inhibited by Lalistat resulting in a size-reduction of the HCV-induced foci.

Figure 11: Lalistat displays significant effects on HCVpp and VSVpp entry, but not on entry of MLVpp.
Huh7.5 cells were preincubated for 2h with 70μM Lalistat or DMSO and transduced with HCVpp, VSVpp or MLVpp. 5h post transduction cells were washed and supplied with media containing Lalistat. 72h after transduction
relative luciferase units were measured by luciferase reporter assay in the Tecan reader Infinite F200. Significance was analyzed by student’s t-test *p < 0.05, **p < 0.01 and ***p < 0.001.

Figure 12: Inhibitory effects of Orlistat and Lalistat in early HBV infection.
(Fig 12 A) 2h before and during HBV infection cells were incubated with Orlistat at indicated concentrations and cccDNA was measured as a marker for early infection. (Figure 12B) Addition of Orlistat 2h before and during or only before HBV infection and the % of HBeAg expression in the supernatant was measured. (Figure 12C) 2h before and during HBV infection cells were incubated with Lalistat at indicated concentrations. Results are expressed in percentage of non-treated cells (mock) and show the impact on the HBV markers HBeAg and cccDNA. Significance was analyzed by student’s t-test . **p < 0.01 and ***p < 0.001.

Figure 13: Lalistat in Adenovirus infection
HEK 293 cells (A) or HepG2 cells (B) were infected with Adeno-GFP-Virus (MOI 10) in the presence and absence of preincubated and simultaneously administered Orlistat or Lalistat in given concentrations. Effects were measured by fluorescence intensity measurement of GFP in the Tecan Infinite F200 reader. Error bars represent SD from 5 independent experiments. Cell viability of the used concentrations was determined by CTB-Assay in 293 cells (C) and HepG2 cells (D). Error bars represent SD from 3 independent experiments. These observations support the conclusion that Orlistat and Lalistat do not affect Adeno-GFP-Virus (AdGOva) infection.

Figure 14: Lalistat in SARS-Coronavirus Virus infection
Huh7 cells were infected with an MOI of 0.1 (FFU/ml) in the presence and absence of preincubated and simultaneously administered Lalistat in given concentrations for 48h. Effects on the viral titers were determined by plaque assay (A). Cell viability of the used concentrations was determined by XTT-Assay (B). Error bars represent SD from 3 independent experiments. These observations support the conclusion that Lalistat does not have an antiviral effect on HCoV- 229E in Huh7 cells.
Figure 15: Lalistat in Measles Virus infection
Vero cells were infected with an MOI of 0.1 (PFU/ml) in the presence and absence of preincubated and simultaneously administered Lalistat in given concentrations for 48h. Effects on the viral titers were determined by plaque assay (A) and observations documented with a fluorescence microscope (C). Cell Viability of the used concentrations was determined by XTT-Assay (B). Error bars represent SD from 3 independent experiments. The experimental observations support the conclusion that Lalistat does not affect measles virus infection.

Figure 16: Lalistat in Chikungunya Virus infection
BHk-21 cells were infected with Gaussia CHIKV of LaReunion (strain: LR 2006-OPY1) (MOI 0.01) in the presence and absence of 70µΜ Lalistat preincubated and/or simultaneously added. CHIKV replication was measured by luciferase reporter assay (A). Error bars represent SD from 3 experiments. MTT vitality assay was performed in BHk-21 cells with different concentrations of Lalistat (B). These experimental observations support the conclusion of an inhibitory effect of Lalistat on Chikungunya Virus infection.

Figure 17: Lalistat in VSV infection
Huh7.5 cells were infected with VSVpseudo particles (VSVpp) (A) or VSV-delta-GFP (MOI 0.6) (B) in the presence and absence of 70µΜ Lalistat 2h preincubated and simultaneously added. VSV replication was measured by luciferase reporter assay. Error bars represent SD from 3 experiments. Error bars represent SD from 3 independent experiments. These experimental observations support the conclusion of an inhibitory effect of Lalistat on VSV infection.

Figure 18: Lalistat in Ebola Zaire Virus infection
Huh7 cells were infected with ZEBOV (MOI 0.1) in the presence and absence of 70µΜ Lalistat preincubated and simultaneously added. Impact on ZEBOV infection was observed as the cytopathic effect on cell level. Cells incubated with Lalistat showed less cytotoxic effect induced by the virus than in the untreated samples. Representative pictures are shown. These experimental
observations support the conclusion of an inhibitory effect of Lalistat on Ebola Virus infection.

**Figure 19: Lalistat in Ebola Zaire Virus infection**

Huh7 cells were infected with EBOV (MOI 0.1) in the presence and absence of 70μM Lalistat preincubated and simultaneously added. ZEBOV titers were analyzed by performing TCID$_{50}$. A clear reduction of ZEBOV infection could be detected after Lalistat treatment. These experimental observations support the conclusion of an inhibitory effect of Lalistat on Ebola Virus infection.

**Figure 20: Lalistat in HIV infection**

LC5-RIC receptor cells were infected with HIV-1 and treated with different concentrations of Lalistat. After 48h the fluorescence intensity was measured, which is proportional to the infection strength. Cell viability was measured by performing a cell titer blue assay. These experimental observations support the conclusion of an inhibitory effect of Lalistat on early steps of HIV infection.

**Figure 21: Lalistat in Dengue virus replication**

Huh7 cells were electroporated with a DENV reporter subgenomic replicon expressing Renilla luciferase (R-Luc), preincubated and simultaneously added in the presence and absence of 70μM LalistatR-Luc activity was determined 48h later from cell lysates. An aliquot of electroporated cells were seeded in a 96-well plates and treated with Lalistat 70μM or DMSO (B). Viability was determined 48h later by measuring cellular ATP content using a bioluminescent assay kit (A). These experimental observations support the conclusion of an inhibitory effect of Lalistat on Dengue virus infection.

**Figure 22: Lalistat in Influenza Virus infection**

MDCK cells were infected with highly pathogenic avian Influenza Virus R65 of serotype H5N1 (HPAIV A/Swan/Germany/R65/06 (H5N1) (R65)). 48h p.I., the supernatant was titrated. The figure shows titers (TCID50/ml) 72h after infection. When compared to the titer observed from the infection in the absence of Lalistat ("R65"), the presence of Lalistat ("R65 Lalistat"), starting from a titer of $10^4.97$ (n=1), resulted in a significant reduction of the titer. These
experimental observations support the conclusion of an inhibitory effect of Lalistat on Influenza Virus infection.

**Figure 23:  Lalistat toxicity assay in mice**

In order to determine the compatibility and tolerance of mammalian subjects to Lalistat, BL6 mice were repeatedly treated with 400μM Lalistat by intravenous injection. No toxicity could be observed after Lalistat treatment.
DETAILED DESCRIPTION OF THE INVENTION

Although the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments described throughout the specification should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all elements described herein should be considered disclosed by the description of the present application unless the context indicates otherwise.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", H.G.W. Leuenberger, B. Nagel, and H. Kölbl, Eds., (1995) Helvetica Chimica Acta, CH-4010 Basel, Switzerland. However, definitions and explanations used herein prevail.

The practice of the present invention will employ, unless otherwise indicated, conventional methods of biochemistry, cell biology, immunology, and recombinant DNA techniques which are explained in the literature in the field (cf., e.g., Molecular Cloning: A Laboratory Manual, 2nd Edition, J. Sambrook et al. eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989).

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated member, integer or step or group of members, integers or steps but not the exclusion of any other member, integer or step or group of
members, integers or steps although in some embodiments such other member, integer or step or group of members, integers or steps may be excluded, i.e. the subject-matter consists in the inclusion of a stated member, integer or step or group of members, integers or steps. The terms "a" and "an" and "the" and similar reference used in the context of describing the invention (especially in the context of the claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein.

All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as"), provided herein is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The terms "subject", "individual", "organism" or "patient" are used interchangeably and relate to vertebrates, preferably mammals including human and non-human mammals. For example, mammals in the context of the present invention are humans, non-human primates, domesticated animals such as dogs, cats, sheep, cattle, goats, pigs, horses etc., laboratory animals such as mice, rats, rabbits, guinea pigs, etc. The term "animal" as used herein also includes humans. The term "subject" may also include a patient, i.e., an animal, preferably a human having a disease or condition, preferably a disease or condition as described herein or suspected of having a disease or condition. Alternatively, the subject may be free of a virus infection.
The term "pharmaceutical composition" as used herein, relates to any preparation of the active pharmaceutical ingredient described herein suitable for preserving the biological activity of the active pharmaceutical ingredient. The term active pharmaceutical ingredient (API) refers to any pharmaceutically acceptable salt or form of the API.

As used herein, "pharmaceutically acceptable excipient and/or carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The term "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see e.g., Berge, S. M., et al. (1977) J. Pharm. Sci. 66: 1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfonic, hydrobromic, hydriodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylendiamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for the preparation of such formulations are generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.
To administer a compound of the invention by certain routes of administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan et al. (1984) J. Neuroimmunol. 7: 27).

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration.

Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying.
(lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alphatocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

For the therapeutic compositions, formulations of the present invention include those suitable for intrahepatic administration but also for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate. Dosage forms for the topical or transdermal
administration of compositions of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the presence of microorganisms may be ensured both by sterilization procedures, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

According to the invention, the term "disease" refers to any pathological state, including any asymptomatic, acute or chronic virus infection and any state caused by or associated with such virus infection. Treatment of a disease or treating a disease includes curing, shortening the duration, ameliorating, preventing, slowing down or inhibiting progression or worsening, or preventing or delaying the onset of a disease or the symptoms thereof.
As used herein, the terms "prevent," "preventing" and "prevention" in the context of the administration of a therapy(ies) to a subject to prevent a viral infection refer, inter alia, to one or more of the following effects resulting from the administration of a therapy or a combination of therapies: (i) the inhibition of the development or onset of a viral infection and/or a symptom associated therewith; and (ii) the inhibition of the recurrence of a viral infection and/or a symptom associated therewith.

As used herein, the prevention relates to the prophylactic treatment of a subject, wherein an active pharmaceutical ingredient or the pharmaceutical composition of the present invention is used as a prophylactic agent. Terms such as "prophylactic agent" and "prophylactic agents" refer to any agent(s) which can be used in the prevention of a viral infection or a symptom associated therewith. Preferably, a prophylactic agent is an agent which is known to be useful to or has been or is currently being used to prevent or impede the onset, development, progression and/or severity of a viral infection or a symptom associated therewith. The prophylactic agent is generally applied in a prophylactically effective amount. As used herein, the term "prophylactically effective amount" refers to the amount of a therapy (e.g., prophylactic agent) which is sufficient to prevent a viral infection or a symptom thereof in a subject.

As used herein, the term "small molecules" and analogous terms include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, other organic and inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, organic or inorganic compounds having a molecular weight less than about 100 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. Salts, esters, and other pharmaceutically acceptable forms of such compounds are also encompassed.

As used herein, the terms "subject" or "patient" are used interchangeably. As used herein, the terms "subject" and "subjects" refer to an animal (e.g., birds, reptiles, and mammals), preferably a mammal including a non-primate (e.g., a camel, donkey, zebra, cow, pig, horse, goat, sheep, cat, dog, rat, and mouse) and a primate (e.g., a monkey, chimpanzee, and a human), and most preferably a human.
As used herein, the terms "therapies" and "therapy" can refer to any protocol(s), method(s), compositions, formulations, and/or agent(s) that can be used in the prevention, treatment, management, or amelioration of a viral infection or a symptom associated therewith. In certain embodiments, the terms "therapies" and "therapy" refer to biological therapy, supportive therapy, and/or other therapies useful in treatment, management, prevention, or amelioration of a viral infection or a symptom associated therewith known to one of skill in the art.

As used herein, the term "synergistic," in the context of the effect of therapies, refers to a combination of therapies which is more effective than the additive effects of any two or more single therapies. In a specific embodiment, a synergistic effect of a combination of therapies permits the use of lower dosages of one or more of therapies and/or less frequent administration of said therapies to a subject with a viral infection. In certain embodiments, the ability to utilize lower dosages of therapies (e.g., prophylactic or therapeutic agents) and/or to administer said therapies less frequently reduces the toxicity associated with the administration of said therapies to a subject without reducing the efficacy of said therapies in the prevention or treatment of a viral infection. In some embodiments, a synergistic effect results in improved efficacy of therapies (e.g., prophylactic or therapeutic agents) in the prevention, management and/or treatment of a viral infection. In some embodiments, a synergistic effect of a combination of therapies (e.g., prophylactic or therapeutic agents) avoids or reduces adverse or unwanted side effects associated with the use of any single therapy.

As used herein, the term "therapeutically effective amount" refers to the amount of a therapy, which is sufficient to treat and/or manage a viral infection. As used herein, the terms "therapeutic agent" and "therapeutic agents" refer to any agent(s) which can be used in the prevention, treatment and/or management of a viral infection or a symptom associated therewith. Preferably, a therapeutic agent is an agent which is known to be useful for, or has been or is currently being used for the prevention, treatment, and/or management of a viral infection or a symptom associated therewith.

As used herein, the terms "treat," "treatment," and "treating" refer in the context of administration of a therapy(ies) to a subject to treat a viral infection refer to one, two, three, four, five or more of the following effects resulting from the administration of a therapy or a combination of therapies: (i) the reduction or amelioration of the severity of a viral infection and/or a symptom associated therewith; (ii) the reduction in the duration of a viral infection
and/or a symptom associated therewith; (iii) the regression of a viral infection and/or a symptom associated therewith; (iv) the reduction of the titer of a virus; (v) the reduction in organ failure associated with a viral infection; (vi) the reduction in hospitalization of a subject; (vii) the reduction in hospitalization length; (viii) the increase in the survival of a subject; (ix) the elimination of a virus infection; (x) the inhibition of the progression of a viral infection and/or a symptom associated therewith; (xi) the prevention of the spread of a virus from a cell, tissue or subject to another cell, tissue or subject; and/or (xii) the enhancement or improvement the therapeutic effect of another therapy.

The term “Lysosomal Acid Lipase” refers to an enzyme which is found, inter alia, in endosomes/lysosomes and which hydrolyses cholesteryl esters and triglycerides. The term includes Lysosomal Acid Lipase (LAL), Acid Cholesteryl ester hydrolase, cholesteryl ester hydrolase; cholesterol esterase, Lipase A (LIPA) preferably Homo sapiens lipase A, lysosomal acid, cholesterol ester hydrolase. Lysosomal acid Lipase is preferably encoded by a nucleic acid consisting of or comprising SEQ ID NO: 1 (transcript variant 1, NM_001127605.2), SEQ ID NO: 2 (transcript variant 2, NM_000235.3) or SEQ ID NO: 3 (transcript variant 3, NM_001288979.1), encoding the corresponding polypeptides of SEQ ID NO: 4 (polypeptide encoded by transcript variant 1, NP_001121077.1), SEQ ID NO: 5 (polypeptide encoded by transcript variant 2, NP_000226.2) and SEQ ID NO: 6 (polypeptide encoded by transcript variant 3, NP_001275908.1) and any variant or mutant form thereof.

A variant as used herein refers to any nucleic acid molecule or polypeptide with at least at least about 60%, at least about 70%, at least about 80%, at least about 90% or about 100% sequence identity. As used herein, the degree of identity is given preferably for a sequence which is at least about 60%, at least about 70%, at least about 80%, at least about 90% or about 100% of the entire length of the reference sequence. In preferred embodiments, the degree of identity is given for the entire length of the conserved region. The alignment for determining sequence identity can be done with tools known in the art, preferably using the best sequence alignment, for example, using Align, using standard settings, preferably EMBOSS: needle, Matrix: Blosum62, Gap Open 10.0, Gap Extend 0.5.

"Sequence identity" between two sequences indicates the percentage of nucleotides or polypeptides that are identical between the sequences. The term "percentage identity" is intended to denote a percentage of nucleotides or amino acids which are identical between the two sequences to be compared, obtained after the best alignment, this percentage being purely statistical and the differences between the two sequences being distributed randomly and over
their entire length. Sequence comparisons between two sequences are conventionally carried out by comparing these sequences after having aligned them optimally, said comparison being carried out by segment or by "window of comparison" in order to identify and compare local regions of sequence similarity. The optimal alignment of the sequences for comparison may be produced, besides manually, by means of the local homology algorithm of Smith and Waterman, 1981, Ads App. Math. 2, 482, by means of the local homology algorithm of Needleman and Wunsch, 1970, J. Mol. Biol. 48, 443, by means of the similarity search method of Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85, 2444, or by means of computer programs which use these algorithms (GAP, BESTFIT, FASTA, BLAST N and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.). The percentage identity is calculated by determining the number of identical positions between the two sequences being compared, dividing this number by the number of positions compared and multiplying the result obtained by 100 so as to obtain the percentage identity between these two sequences.

The term "antagonist of Lysosomal Acid Lipase" refers to a compound which is capable of reducing the amount and/or biological activity of Lysosomal Acid Lipase, preferably its hydrolase activity, i.e. its capability to catalyze the hydrolysis of cholesteryl esters and triglycerides. Reducing the amount includes reducing the local amount of Lysosomal Acid Lipase in endosomes/lysosomes or in a particular endosomal compartment. Reducing the amount and/or biological activity preferably means a reduction by up to or by at least 10%, by up to or by at least 20%, by up to or by at least 30%, by up to or by at least 40%, by up to or by at least 50%, by up to or by at least 60%, by up to or by at least 70%, by up to or by at least 80%, by up to or by at least 90% or by up to 100%. The term includes any kind of inhibitor such as a reversible or irreversible inhibitor. For determining the biological activity, the following assay conditions as published in Rosenbaum et al. 2010 (J. Med. Chem. 2010, 53: 5281-5289) are to be used.

A first example of an antagonist of Lysosomal Acid Lipase is siRNA or antisense nucleic acid, where said siRNA and antisense nucleic acid preferably comprises the nucleic acid sequence encoding Lysosomal Acid Lipase or a fragment thereof. A second example of an antagonist is a reversible or irreversible inhibitor, preferably an inhibitor binding to the catalytic center of the enzyme.

Preferably the antagonist does not or not substantially inhibit human pancreatic lipase or bovine milk lipoprotein lipase. An antagonist which does not substantially inhibit human
pancreatic lipase or bovine milk lipoprotein lipase preferably does not reduce the catalytic activity of human pancreatic lipase or of bovine milk lipoprotein lipase by more than 1%, 2%, 5%, 10% or 20%, using assay conditions as described in Rosenbaum et al., 2010. Preferably, the antagonist is not Orlistat or (S)-(S)-1-((2S,3S)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl) 2-formamido-4-methylpentanoate.

In one embodiment, said antagonist of Lysosomal Acid Lipase is or comprises a thia diazole carbamate substituted with N-heterocycles, preferably selected from the group consisting of 4-(pyrrolidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate, 4-(pyrrolidin-1-yl)-1,2,5-thiadiazol-3-yl morpholine-4-carboxylate, 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate (compound 12 of Rosenbaum et al., J. Med. Chem. 2010, 53: 5281-5289 also referred to as “Lalistat 2”), 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl morpholine-4-carboxylate (compound 13 of Rosenbaum et al., J. Med. Chem. 2010, 53: 5281-5289 also referred to as “Lalistat 1”), 4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate, 4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl morpholine-4-carboxylate and preferably 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate (compound 12 of Rosenbaum et al., 2010, i.e. Lalistat 2).

The term Lalistat includes Lalistat 1 and Lalistat 2.

In one embodiment said thia diazole carbamate substituted with N-heterocycles is preferably a 1,2,5-thiadiazole carbamate. Preferably said thia diazole carbamate has the structure

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\text{S} & \quad \text{N}
\end{align*}
\]

wherein X is a nitrogen containing heterocycle.

In another embodiment said thia diazole carbamate substituted with N-heterocycles is

\[
\begin{align*}
\text{X} & \quad \text{N} \\
\text{O} & \quad \text{N}
\end{align*}
\]
wherein X is -O- or -CH$_2$-.

In another embodiment, the thiadiazole carbamate substituted with N-heterocycles is 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate.

In another embodiment, said virus is a virus which enters cells by receptor-mediated endocytosis, preferably a virus selected from the group consisting of Hepatitis C Virus; Hepadnaviridae, preferably Hepatitis B Virus and Hepatitis D Virus; Hepatitis E virus; Flaviviridae preferably Alphaviruses, preferably Chikungunya Virus, Semliki Forest Virus, Sindbis Virus; West-Nile-Virus, Dengue Virus; Yellow-fever; Filoviridae preferably Marburg and Ebola Virus, Orthomyxoviridae, preferably Influenza virus; Rhabdoviridae, preferably Vesicular Stomatitis Virus and Rabiesvirus; Retroviridae, preferably Human Immunodeficiency Virus, human T-Cell-Leukemia Virus, Avian Leukemia Virus; Paramyxoviridae preferably Measles Virus and Rubella-Virus, New-Castle-Desease Virus and Respiratory-Syncytial-Virus; Arenaviridae, preferably Lymphocytic choriomeningitis virus; Picornaviridae, preferably Hepatitis A Virus, Echovirus, Enterovirus, human Rhinovirus, Coxsackie Virus, Polio Virus; Herpesviridae preferably Herpes Simplex Virus, Eppstein-Barr-Virus; Cytomegalovirus and Varicella Zoster Virus; Poxviridae preferably Vaccinia Virus; Adenoviridae, preferably human Adenovirus; Papovaviridae preferably human Papillomaviruses (HPV); Polyomaviridae preferably SV40; Burnaviridae preferably Hanta Virus and Rift-Valley-Fever Virus; Reoviridae preferably Rota Virus and Blue-Tongue-Virus; Coronaviridae preferably SARS coronavirus; preferably Hepatitis C Virus.

In another embodiment, the virus is selected from the group consisting of Orthomyxoviridae, preferably Influenza Virus preferably Influenza Virus A, preferably an Influenza Virus of subtype H5N1; Filoviridae preferably Ebola and Marburg Virus, Flaviviridae preferably Dengue Virus; preferably Alphaviruses, preferably Chikungunya Virus, Semliki Forest Virus, Sindbis Virus; West-Nile-Virus; Yellow-fever.

In a preferred embodiment, the virus is a virus which requires lipid rafts (cholesterol microdomains) for virus entry. In another embodiment, the virus is a virus which requires endosomal free cholesterol for virus endocytosis and/or viral membrane fusion with the endosomal membrane. In another preferred embodiment, the virus is a virus, which requires lipid rafts for assembly, budding and/or release and intracellular virus spread.
In a preferred embodiment, the virus is Hepatitis B Virus, Hepatitis C Virus, Influenza Virus, Dengue Virus, Human Immunodeficiency Virus, Ebola Virus, Chikungunya Virus or Vesicular Stomatitis Virus and the antagonist is 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate.

In another embodiment, the pharmaceutical composition comprises at least one additional active pharmaceutical ingredient or the pharmaceutical composition of the present invention is used in a method of preventing or treating which comprises administering at least one additional pharmaceutical composition comprising at least one additional active pharmaceutical ingredient.

The “additional active pharmaceutical ingredient” may be an inhibitor of activity of a viral protein, wherein the viral protein may be a protease or a DNA- or RNA-dependent polymerase, a reverse transcriptase, a viral kinase, a viral integrase, or a viral surface protein. The additional active pharmaceutical ingredient may induce an additive or synergistic therapeutic effect.

In another embodiment, the virus is Hepatitis C Virus and the additional active pharmaceutical ingredient referred to herein is an inhibitor of activity of a virus protein selected from the group consisting of C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B.

The additional active pharmaceutical ingredient may induce an additive or synergistic therapeutic effect. The pharmaceutical composition of the present invention may comprise, for example, 2, 3, 4, 5, 6, 7 or 8 active pharmaceutical ingredients. According to the present teaching, the additional active pharmaceutical ingredient, for example the inhibitor of activity of a viral protein, may be formulated together with the antagonist referred to herein in a single pharmaceutical composition. Alternatively, the pharmaceutical composition may be structured as kit of parts, wherein the antagonist is provided in a first formulation and the additional active pharmaceutical ingredient, for example the inhibitor of activity of the viral protein, is provided in a second formulation, i.e. a second pharmaceutical composition. The first and the second pharmaceutical composition may be combined prior to use. In other words, before administering the pharmaceutical composition, a formulation comprising the additional active pharmaceutical ingredient may be added to the first pharmaceutical composition comprising the antagonist. Alternatively, the present teaching envisages administering the antagonist formulated in a first pharmaceutical composition and administering the additional active pharmaceutical ingredient formulated in a second pharmaceutical composition. The
pharmaceutical compositions may be administered concomitantly or in succession. For example, the first pharmaceutical composition may be administered at a first point in time and the second pharmaceutical composition may be administered at a second point in time, wherein the points in time may be separated by, for example, 0, or up to 1, 2, 3, 4, 5 or 10 min, ours, days or years.

The term "inhibitor" as used herein above refers to any compound capable of reducing the biological activity. The biological activity as used herein includes the enzymatic or catalytic activity or the capability of a structural protein to form infectious virus particles. In general terms, the inhibitor may be for example a small molecule or a peptide, polypeptide or peptidomimetic. The term polypeptide includes antibodies.

For example, an inhibitor of the core protein, "C", may be a compound, preferably a small molecule or a peptide, peptide analog or peptidomimetic, interfering with or preventing assembly of the viral core or interfering with or preventing budding of the virus particle.

Likewise, an inhibitor of "E1" and/or "E2" may be a compound, preferably a small molecule or a peptide, peptide analog or peptidomimetic, preventing the virus particle from incorporating sufficient E1 and/or E2 or, for example, a compound interfering with the formation of surface proteins capable of mediating receptor interaction or membrane fusion or a compound capable of interfering with maturation of E1 and/or E2. Likewise, an inhibitor of NS2, may be a compound, preferably a small molecule or a peptide, peptide analog or peptidomimetic, interfering with or preventing assembly of virus particles or inhibiting the protease activity of the NS2 domain.

Likewise, an inhibitor of NS3, may be a compound, preferably a small molecule or a peptide, peptide analog or peptidomimetic, interfering with the protease activity or helicase activity or NTPase activity of the NS3 domain. For example, the protease inhibitor may be Incivek (telaprevir, VX-950), Victrelis (boceprevir, SCH503034), Simeprevir (TMC435), Faldaprevir (BI201335), Danoprevir (RG7227), Vaniprevir (MK-7009), MK-5172, Asunaprevir (BMS-650032), ACH-1625, GS-9256, ACH-2684, GS-9451, Narlaprevir (optionally boosted by ritonavir), IDX320. For example, the helicase inhibitor may be selected from DRBT or TBBT (Borowski et al., European Journal of Biochemistry, 270: 1645–1653, 2003), Soluble blue HT (Chen et al., Journal of Medicinal Chemistry, 52: 2716–2723, 2009), Ring-expanded (fat) nucleoside analogues (Zhang et al., Journal of Medicinal Chemistry, 46: 4149–4164, 2003), AICAR analogue (compound 4) (Ujjinamatada et al., Bioorganic and Medicinal Chemistry Letters, 17: 2285–2288, 2007), QU663 (Maga et al., Biochemistry, 44: 9637–9644, 2005),

In another embodiment, the inhibitor is selected from the group consisting of ribavirin, telaprevir, boceprevir, simeprevir, asunaprevir, faldaprevir, vaniprevir, danoprevir, ABT-450, GS-9451, GS-9256 (protease inhibitors), ABT-267, daclatasvir, ledipasvir, GS-5816 (NS5A inhibitors), ABT-333, BMS-791325, ABT-072, tegobuvir, deleobuvir, sotrobuvin, GS-9669 (non-nucleoside NS5B polymerase inhibitor), sofosbuvir, PSI-6206 (nucleotide analog), mericitabine (nucleoside analog), CPG10101 (synthetic ODN), NOV-205 (formulation of oxidized glutathione and inosine that acts as a hepatoprotective agent with immunomodulating and anti-inflammatory properties), INX-08189 (2'-C-methyl nucleosides).

In another embodiment, the virus is hepatitis B virus (HBV) and the additional active pharmaceutical ingredient is an inhibitor of activity of the virus proteins core, S, M, L, X or the viral polymerase / reverse transcriptase or an inhibitor of HBV entry or an inhibitor of capsid assembly or a TLR-3, TLR-7, TLR-8 or TLR-9 agonist or a ligand of a Rig-I like receptor.

In another embodiment, the virus is Influenza Virus A and the additional active pharmaceutical ingredient is an inhibitor of activity of the neuraminidase (NA) or M2 protein, or an inhibitor of a virus hemagglutinin (HA), or an inhibitor of NP, M1, M2, NS1, NS2, PA, PB1, PB1-F2 and/or PB2.
In another embodiment, the pharmaceutical composition of the present invention comprises at least one additional active ingredient, which is an anti-HBV agent, an anti-Influenza agent or an anti-HCV agent preferably selected from the group consisting of interferon, preferably interferon-alpha, peginterferon Lambda-1a, peginterferon alfa, IFN gamma, peginterferon alfa-2a, peginterferon alfa-2b; recombinant interleukin 7 (CYT107); lipase inhibitor, preferably Orlistat or (S)-(S)-1-((2S,3S)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl 2-formamido-4-methylpentanoate; Cyclosporin, Cyclophilin inhibitor, preferably alisporivir, SCY-635; Silibinin; agonist of the c-mpl (TpoR) receptor, preferably 3'-{(2Z)-2-[1-((3,4-dimethylphenyl)-3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)hydrazino]-2'-hydroxy-3-biphenylcarboxylic acid; 1-[(4-chlorophenyl)methyl]-2-(pyrrolidin-1-ylmethyl)benzimidazole (clemizole); thiazolide, preferably [2-[(5-nitro-1,3-thiazol-2-yl)carbamoyl]phenyl]ethanoate (nitazoxanide); hydroxychloroquine; antibody against tumor necrosis factor alpha (TNF-α), preferably infliximab, neuraminidase inhibitors, preferably zanamivir (Relenza) and oseltamivir (Tamiflu); M2 inhibitors, preferably amantadine and rimantadine.

In another aspect, the present invention also relates to method of preventing or treating in a subject a viral infection or a disease resulting from a viral infection, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of the present invention.

In one embodiment, the disease is selected from the group consisting of an acute or chronic HBV or HCV infection, a virus induced liver abnormality, a liver cirrhosis, a hepatocellular carcinoma, reinfection of liver transplants, HCV induced diabetes, HBV or HCV induced liver fibrosis, non alcoholic fatty liver disease.

In another embodiment, the method of preventing or treating of the present invention comprises administering at least one additional pharmaceutical composition comprising at least one additional active pharmaceutical ingredient.

In another aspect, the present invention also relates to a method of monitoring a virus infection, comprising
(a) obtaining a first sample and a second sample from a subject, wherein the first sample is obtained prior to the second sample and the subject is a subject treated with the pharmaceutical composition of the present invention;
(b) determining in the first and the second sample the presence or amount of a marker, which is indicative for the virus infection; and
(c) comparing the amount of marker determined in the first and the second sample; wherein a reduced amount of said marker in the second sample is indicative for a regression of the virus infection.

In one embodiment, the virus is HBV. In another embodiment, the virus is HCV, VSV, Chikungunya virus, Ebola virus, HIV, Influenza Virus or Dengue virus.

In more general terms, the method of monitoring according to the invention generally concern the use of means for the detection and/or the determination or the monitoring of the quantity of (i) a nucleic acid, which may be a genomic DNA or RNA or which may code for a viral protein, or a part thereof and/or (ii) a viral polyprotein and/or (iii) an antibody against a viral protein or polyprotein or a part thereof and/or (iv) cytotoxic or T helper lymphocytes, which are specific for a viral protein or polyprotein or a part thereof, in a biologic sample isolated from a patient. A part of a protein or polyprotein encoded by the virus is, for example, a protein or polypeptide a subdomain of the protein or polyprotein or an antigenic fragment of the protein or polyprotein. An antigenic fragment may comprise at least or up to 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 50, 100 amino acids or more.

Regarding HCV, the method of monitoring according to the invention generally concern the use of means for the detection and/or the determination or the monitoring of the quantity of (i) a nucleic acid, which may be a genomic RNA or which may code for an HCV protein, or a part thereof and/or (ii) the polyprotein encoded by HCV or a part thereof and/or (iii) an antibody against the HCV polyprotein or a part thereof and/or (iv) cytotoxic or T helper lymphocytes, which are specific for an HCV protein or a part thereof, in a biologic sample isolated from a patient. A part of the polyprotein encoded by HCV is, for example, a protein or polypeptide selected from the group consisting of C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B.

Accordingly, the marker which is indicative for HCV infection may be viral RNA or a viral polypeptide or protein such as the genomic RNA or a subgenomic RNA of HCV or the polyprotein encoded by the HCV or any of C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B.

According to the invention, detection of a nucleic acid or of a part thereof or determining or monitoring the amount of a nucleic acid or of a part thereof may be carried out using a polynucleotide probe which hybridizes specifically to said nucleic acid or said part thereof or
may be carried out by selective amplification of said nucleic acid or said part thereof. In one embodiment, the polynucleotide probe comprises a sequence of 6-50, in particular 10-30, 15-30 or 20-30, contiguous nucleotides of said nucleic acid. In one embodiment, detection comprises a step of amplifying the nucleic acid or the part thereof by PCR or RT-PCR.

According to the invention, detection of the HCV polyprotein or of a part thereof such as C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B or of a part thereof or determining or monitoring their amounts may be carried out using an antibody binding specifically to any of said HCV antigens. Preferably, a part of the HCV polyprotein or of an HCV polypeptide comprises at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 consecutive amino acids of the HCV polyprotein. In a preferred embodiment, said part is selected from the group consisting of C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B.

In certain embodiments, HCV infection is monitored by detecting a complex of the HCV polyprotein or a said part thereof with an MHC molecule, in particular an HLA molecule.

According to the invention, detection of an antibody or determining or monitoring the amount of antibodies may be carried out using a protein or peptide binding specifically to said antibody. For example, the HCV polyprotein or a part thereof such as C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B or of a part thereof may be used accordingly.

According to the invention, detection of cytolytic T cells or of T helper cells or determining or monitoring the amount of cytolytic T cells or of T helper cells which are specific for complexes between an antigen or a part thereof and MHC molecules may be carried out using a cell presenting the complex between said antigen or said part thereof and an MHC molecule.

The polynucleotide probe, the antibody, the protein or peptide or the cell, which is used for detection or determining or monitoring, is preferably labeled in a detectable manner. In particular embodiments, the detectable marker is a radioactive marker or an enzymic marker, T lymphocytes may additionally be detected by detecting their proliferation, their cytokine production, and their cytotoxic activity triggered by specific stimulation with the complex of MHC and the HCV polypeptide or the part thereof. T lymphocytes may also be detected via a recombinant MHC molecule or else a complex of two or more MHC molecules which are loaded with an immunogenic fragment.

In a preferred embodiment, the method of monitoring is performed by Immunoblotting of the aforementioned viral proteins or by an ELISA, preferably by detecting the HCV Core-Ag "C".
In another aspect, the present invention also relates to a method of selecting a patient sensitive to a therapy with the pharmaceutical composition of the present invention, comprising
(a) obtaining a first sample and a second sample from a subject, wherein the first sample is obtained prior to and the second sample is obtained after administering the pharmaceutical composition of the present invention;
(b) determining in the first and the second sample the amount of a marker which is indicative for the virus infection; and
(c) comparing the amount of marker determined in the first and the second sample;
wherein a reduced amount of said marker in the second sample is indicative for the subject’s sensitivity to the pharmaceutical composition.

In one embodiment, the virus is HCV. In another embodiment, the virus is HCV.

Other features and advantages of the instant invention will be apparent from the following detailed description and claims.
The following examples illustrate the invention:

**Example 1: Experimental summary**

During the last years there is evidence for a role of host cell lipid metabolism in the HCV replication cycle whereas HCV seems to hijack different pathways and properties of the cellular lipid machinery. Thus it was shown that Lipoprotein lipase (LPL) mediates hepatitis C virus (HCV) cell entry, the expression of hepatic triglyceride lipase (HTGL) increases HCV infectivity and an efficient assembly requires the triglyceride-synthesizing enzyme diacylglycerol acyltransferase 1 (DGAT1). Because HCV exploits many steps of the host cell, in particular its lipid metabolism, we first investigated the impact of lipase inhibitor Orlistat, an approved drug inhibiting triglyceride and cholesterol ester lipases. The experimental data provided herein below demonstrates that a 2 hours pre-incubation had a strong impact on HCV infection, resulting in a reduction of the infection efficiency as well as an effect on viral replication and the distribution of E2 and Core protein. As this relatively broad lipase inhibitor seemed to act on very early steps of the infection cycle, we hypothesized that Lysosomal acid lipase, which is present in the late endosome, might play an essential role, especially during the entry and uncoating process, during infection of HCV and other virus families entering cells by receptor-mediated endocytosis.

Lalistat (Lalistat 2), a specific inhibitor of Lysosomal acid lipase, was used to test this hypothesis. Surprisingly, we observed a strong impact of Lalistat on the efficiency of early infection steps of replication competent virus particles (HCVcc) as well as subviral particles (HCV pseudoparticles, (HCVpp)), indicating that Lalistat has a therapeutic effect on HCV infection. Knock-down of acid lipase expression by siRNA alone and in combination with Lipase C siRNAs resulted in a significant additive reduction on viral RNA expression confirming the essential role of acid lipase.

Our data underscore the importance of host cell lipases for the viral infection cycle of HCV and other virus families entering host cells by receptor-mediated endocytosis. We identified the Lysosomal acid lipase, to be essential for productive HCV infection. Acid lipase might therefore represent a novel target for HCV therapy.

**Example 2: Orlistat inhibits early steps of HCV infection**

To test toxicity of Orlistat on Huh7.5 cells, the cells were cultured in the presence of different concentrations of Orlistat (Figure 1A). 48h post incubation Orlistat-containing cell culture medium was removed and viability tested using a neutral red based viability assay. OD was
measured at 540nm. Results are means ± SD from 3 independent experiments. The results indicate that a concentration of Orlistat of 70μM is non-toxic for Huh7.5 cells. Subsequently, Huh 7.5 cells were infected with HCV (JC1) in the presence or absence of 70μM of Orlistat for 48h using different treatment conditions (Figure 1B). Supernatants were collected and the infectivity titers were determined by TCID50. Error bars show standard deviation (SD) from 3 independent experiments. The results shown in Figure 1B demonstrate that the treatment of Huh7.5 cells with Orlistat reduces infectivity in the cell culture supernatant by more than 90%. Figure 1C show Western Blot lysates of infected Huh7.5 cells, which were treated with 30μM and 70μM of Orlistat and stained for NS3 protein levels. As a loading control β-actin was used. The experimental results demonstrate that Orlistat reduces the amount of NS3 in the lysate of cells, which were infected with HCV and treated with 70μM of Orlistat. Figure 1D shows Huh7.5 cells infected with JC1 (MOI 0.5) in the presence or absence of 70μM of Orlistat at different time points. Effects were measured by immunofluorescent staining for NS3. Representative images are shown. Cell viability was measured by bright field microscopy. Preincubation of Huh7.5 with Orlistat and its presence during infection (pre+sim) showed the most prominent reduction of the number of cells infected with HCV (JC1). Removing the step of preincubation with Orlistat (70μM sim) HCV infection is still clearly inhibited but the effect is not as strong as with additional preincubation. Adding 70μM Orlistat 2h post infection (70μm p.i.) still showed clear inhibitory effects, which are not as strong as the effects induced by Orlistat preincubation alone, which reduced the number of cells positive for NS3.

Example 3: Orlistat blocks HCV replication and cell-to cell transmission
To further explore the effect of Orlistat on HCV infection, Huh7.5 cells were infected with HCV (JC1) in the presence or absence of 70μM of Orlistat at 4°C for 2h, washed and incubated at 37°C for 48h. Samples were stained for NS3 as a representative for infection. Here it is clearly shown that there is no effect of Orlistat on the attachment of the virus, which is the only step taking place at 4°C. Figure 2B shows immuno-fluorescence stainings of Huh7.5 cells, which were infected for 2h with an MOI of 0.1 and afterwards covered with Orlistat or DMSO containing agarose, which prevents the virus to spread via the supernatant. After 72h cells were fixed, agarose was removed and cells were stained with NS3 antibody to evaluate the effects on HCV induced cell-to-cell spread. The experimental results demonstrate by the reduction of the size of the foci that HCV cell-to-cell spread was clearly reduced by the treatment with Orlistat. Figure 2C shows Huh7.5 cells after infection with HCV (JC1),
wherein prior to infection the virus preparation was incubated at 37°C for 1h with Orlistat, DMSO or remained untreated. After 48h cells were stained for NS3 and no impact on viral particles could be observed as both samples showed the same efficiency of infection. Figure 2D shows expression levels of potential HCV receptors and co-receptors CD81, SRB-I, Claudin-1, Occludin and LDL-R in 70μM Orlistat treated and untreated HUH7.5 cells was measured in relation to β-actin. Hereby no inhibitory effect of Orlistat could be shown on the expression patterns of all potential HCV receptors and coreceptors. (Fig 2E) LucUbiNeo-ET replicon cells were incubated with 70 μM of Lalistat for 48h. Viral replication was measured by luciferase reporter assay. Orlistat inhibited replication in the replicon system Con1 (genotype 1b).

Summarizing the data from figure 2 it could be clearly shown that Orlistat treatment inhibits cell-to-cell transmission as well as viral replication, while the viral attachment, the viral particle itself nor the expression pattern of viral receptors and coreceptors was not influenced.

The antiviral effect of Orlistat was further explored by studying the intracellular localization of E2 and HCV core proteins 3 days post infection with HCV (JC1). More specifically, Huh7.5 cells were infected with JC1 (MOI 0.5) after a preincubation and simultaneous treatment in the presence or absence of 70μM of Orlistat (Figure 3A). Orlistat appears to induce a clustering of E2 and an accumulation of Core in donut-like lipid droplet structures, as shown in Figure 3A. Effects were measured by immuno-fluorescent staining of E2 and Core protein. Representative images are shown. This accumulation could possibly be interpreted as an interruption of the HCV assembly, which under normal circumstances takes place at Lipid-droplet structures associated with the Endoplasmatic Reticulum (ER). In a second inhibitory step Orlistat seems to disturb virus assembly, which as a consequence leads to an accumulation of viral proteins like E2 or Core. Core positive localization in donut structures was quantified in relation to positive events in the control (Figure 3B). 10 random Core positive areas were chosen and the positive events for a localization of core in donut structures in relation to negative events was counted. Although Core proteins are stored in donut-like lipid droplet structures until their processing for the formation of viral particles also in untreated cells, positive events of Core accumulations in Orlistat treated cells is more often observable, strongly suggesting that will ultimately lead to less production of virions and therefore to reduction of viral titers.
Example 4: Orlistat impairs infection of VSV

VSV was used to study the effect of Orlistat on entry of other viruses making use of receptor-mediated endocytosis. To this end, Huh7.5 cells were infected with the single-cycle vector VSV-delta-GFP at an MOI of 0.6 in the presence (Figure 4A, 4B) or absence (Figure 4C) of preincubated and/or simultaneously added Orlistat (Figure 4A/B). Effects were measured by immunofluorescence and cell viability by bright field microscopy. Representative images are shown. (Figure 4D) VSV replication was measured by luciferase reporter assay. Error bars represent SD from 3 experiments. The results demonstrate that Orlistat impaired virus entry of VSV by at least 50%.

Example 5: HCV expression after siRNA knockdown of different Lipases

Orlistat broadly interferes with various components of the lipid metabolism, thus preventing a conclusion on the requirement of specific Lipases for promoting virus infection. To elucidate the role of specific Lipases for replication of HCV, gene expression of Lipase A and/or Lipase C were knocked-out by siRNA. To this end, Huh7.5 cells were transfected with indicated siRNAs two times consecutively, washed and infected for 48h with HCV (JC1) at an MOI of 0.5 followed by RNA extraction and relative qPCR. The percentage of inhibition of Lipase A, HCV and Lipase C (positive control) is shown in Figure 5A. Error bars represent SD from 2 independent experiments. (Fig 5B) Supernatants from Figure 5A were titrated on Huh7.5 cells to determine the infectivity by TCID50. Error bars represent SD from 4 independent experiments. The results demonstrate that the knock-down of Lipase A is essentially inhibiting HCV infection up to 20-25%. In comparison, Lipase C, which is shown to be essential in HCV infection, showed an inhibitory effect of up to 30%. Both siRNAs in combination showed an additive inhibitory effect, which demonstrates the importance of both lipases in the HCV infection cycle. Supernatants from those siRNA transfected cells showed strong inhibitory effects on the infectious dose of the released virus particles respectively, which further underlines the importance of Lipase A in HCV infections.

Example 6: Lipase A is required for efficient HCV infection

A specific inhibitor of Lipase A activity, Lalistat, was used for studying the putative requirement for Lipase A activity during HCV replication. More specifically, Huh7.5 cells were infected with JC1 (MOI 0.5) in the presence and absence of different concentrations of
Lalistat (Figure 6A). Effects were measured by immuno-fluorescent staining for E2. Representative images are shown. Cell viability was measured by BF microscopy. (Figure 6B and Figure 6C) Lalistat toxicity was tested on Huh7.5 cells, which were cultured in the presence of different concentrations of Lalistat. 48h post incubation Lalistat-containing cell culture medium was removed and viability tested using the neutral red based and the CTB viability assay. OD was measured at 540nm and fluorescence measured respectively. Results are means + SD from 3 independent (NR) and one experiments (CTB). The results of Figure 6 demonstrate that the specific Lipase A inhibitor Lalistat showed strong dose-dependent inhibitory effects on HCV infection, while cell viability up to 70μM was reasonable.

The impairment of HCV infection was also reflected on the protein level (Figure 7). Huh7.5 cells infected with HCV (JC1) at an MOI of 0.5 and preincubated and simultaneously treated with the indicated concentrations of Lalistat (Figure 7A) were analyzed for expression levels of HCV proteins NS3 and Core. β-actin was used as a loading control. In this experimental setting, the treatment with Lalistat reduced expression of HCV structural proteins (core) by approximately 50%, suggesting that Lalistat may be useful for preventing or treating HCV infection.

**Example 7: Impact of Lipase A inhibition on HCV titres**

To further explore the effect of Lalistat on HCV replication, the infectivity of cell culture supernatants of HCV infected cells was determined. In the experimental setting of Figure 8A, undifferentiated Huh 7.5 cells were preincubated and simultaneously treated with 70μM of Lalistat and Orlistat for 48h; infection was performed 2h after preincubation with HCV (JC1) in the presence or absence of the Lipase inhibitors. Supernatants were collected and the infectivity titers were determined by TCID50. Error bars are SD from 3 independent experiments.

In the experimental setting of Figure 8B, differentiated Huh7.5 cells were infected for 4 weeks before they were treated with 100μM of Orlistat and Lalistat. Supernatants were collected after 24h and the infectivity titers were determined by TCID50. Error bars are SD from 2 independent experiments. Cell Titer blue Assay shows the viability of the differentiated Huh7.5 cells after Lalistat and Orlistat treatment (Figure 8C). Fluorescence was measured in the Tecan reader Infinite F200. Up to 100μM of Lalistat does not show any toxic effects on differentiated Huh7.5. In comparison to undifferentiated Huh7.5 cells the
differentiated cells are still viable with higher concentrations. Significance was analyzed by student’s t-test *p < 0.05, **p < 0.01 and ***p < 0.001.

The experimental results demonstrate a reduction of HCV titers in undifferentiated and differentiated Huh7.5 cells after Lalistat treatment, which clearly shows the importance of Lipase A in HCV infection. While the effect by Orlistat is induced because of targeting more than one lipase, Lalistat inhibits HCV infection because of the specific inhibition of Lipase A very efficiently in undifferentiated Huh7.5 cells. Differentiated Huh7.5 cells, which show all typical markers of real human liver cells, also show a clear reduction of HCV titers, which further supports the use of Lalistat for medical treatment.

Example 8: Lalistat has no effect on a subviral replication system of HCV

A subviral expression system of HCV (Lohmann et al., 1999) was used for elucidating if Lalistat was specifically showing an effect on lipase A at the first entry steps of HCV infection. LucUbiNeo-ET replicon cells (Genotype 1b replicon cells), which completely lack the entry step and constantly replicate, were incubated with 10, 70 μM of Lalistat for 48h. (Figure 9A) Viral replication was measured by luciferase reporter assay. Cell viability was measured by NR assay (Figure 9B). The experimental results demonstrate that Lalistat has no impact on HCV replication in the subviral expression system. Since this subviral system does not involve the entry steps of the viral life cycle, we could clearly show that Lalistat is exclusively inhibiting Lipase A, which is essential for the early entry steps of HCV infection.

Example 9: Lipase A inhibition leads to an impaired cell-to-cell spread of HCV

To study the effect of Lalistat on cell-to-cell spread, virus infected cells were covered with Agarose to prevent spread of virus particles through the cell culture supernatant. More specifically, Huh7.5 cells were infected with HCV (JC1) (MOI 0.1) for 2h, washed and covered with an Agarose-Medium-Overlay containing Lalistat, Orlistat or DMSO in given concentrations (see Figure 10). 72h post infection cells were fixed with 8% of PFA and the agarose overlay was removed. Effects are shown by immuno-fluorescent staining for E2. Representative images are shown. Cell viability was measured by bright field microscopy. Here we clearly show that the cell-to-cell spread of HCV is strongly inhibited by Lalistat and Orlistat resulting in a size-reduction of the HCV-induced foci. In the DMSO controls HCV induced foci resemble the size of the untreated controls.
The results of Figure 10 demonstrate that Lipase A inhibition leads to an impaired cell-to-cell spread of HCV, which is itself kind of a reinfection with another entry step.

Example 10: Lalistat displays significant effects on HCVpp and VSVpp entry, but not on entry of MLVpp.

Huh7.5 cells were preincubated for 2h with 70μM Lalistat or DMSO and transduced with HCVpp, VSVpp or MLVpp. Pseudoparticles were generated by transfecting 293T cells with plasmids encoding a HIV provirus expressing luciferase (pNL4-3.luc.R-E-) and vesicular stomatitis virus G (VSV-G), murine leukemia virus (MLV) or HCV envelope glycoprotein H77 (genotype 1) (Dowd et al., 2009) or a no-envelope control, as previously reported (Hsu et al., 2003). Virus-containing medium was harvested after 48h and added to pre-treated Huh7.5 cells. 5h post transduction cells were washed and supplied with media containing Lalistat. At 72h post infection relative luciferase units were measured. Specific infectivity was calculated by expressing the HCV, MLV, or VSV-G luciferase signal (relative light units, RLU) normalised to the no envelope control RLU value. Statistical comparisons were made using student's t-test *p < 0.05, **p < 0.01 and ***p < 0.001.

The experimental results obtained with HCVpp and VSVpp strongly suggest that viruses, which enter host cells by receptor-mediated endocytosis, are sensitive to Lalistat. It was unclear, however, as to whether or not the experimental observations with HCV and VSV can be extended to other virus families, in particular those, which enter the host cells by direct fusion at the plasma membrane. Therefore, Huh7.5 cells were preincubated for 2h with Lalistat or DMSO and transduced with pseudoparticles of HCV, VSV or MLV (HCVpp, VSVpp or MLVpp, resp.). MLV (Murine Leukemia Virus) is a prototypic C-type retroviruses which enter host cells by direct fusion at the plasma membrane.

The results of 3 independent experiments demonstrate that Lalistat has no significant impact on MLVpp entry into Huh7.5 cells, while a strong impact was observed for entry of HCVpp and VSVpp (Figure 11).

The effect of Lalistat on VSV was further studied in Huh7.5 cells infected with VSVpseudo particles (VSVpp) (Figure 17A) or VSV-delta-GFP (MOI 0.6) (Figure 17B) in the presence and absence of 70μM Lalistat 2h preincubated and simultaneously added. VSV replication was measured by luciferase reporter assay. The experimental observation shown in Figure 17 further support the conclusion of a strong inhibitory effect of Lalistat on VSV infection.
Example 11: Inhibitory effects of Orlistat and Lalistat in early HBV infection

In order to establish as to whether or not the requirement for lipase activity is limited to the particular virus families tested, i.e. to rhabdoviridae (VSV) and flaviviridae (HCV), the present study was extended to Hepatitis B Virus (HBV), a virus of the family of Hepadnaviridae.

Figure 12 shows treatment of HepaRG cells. (Figure 12 A) 2h before and during HBV infection cells were incubated with Orlistat at indicated concentrations and cccDNA was measured as a marker for early infection. (Figure 12B) Addition of Orlistat 2h before and during or only 2h before HBV infection and the % of HBeAg expression in the supernatant was measured. (Figure 12C) 2h before to and during HBV infection cells were incubated with Lalistat at indicated concentrations. Results are expressed in percentage of non-treated cells (mock) and show the impact on the HBV markers HBeAg and cccDNA. Significance was analyzed by student’s t-test. **p < 0.01 and ***p < 0.001.

Treatment of HepaRG cells with Orlistat clearly interfered in a dose dependent fashion with the establishment of HBV infection if added either prior to or prior to and during with the latter having an essentially stronger effect (Figure 12A and Figure 12B). Similar to Orlistat, the more specific acid lipase inhibitor Lalistat (Lalistat 2) inhibited productive HBV infection in a dose dependent fashion (Figure 12C).

Thus, Hepadnaviridae like HBV also seem to have a strong requirement for acid lipase activity and, thus, infection is sensitive to treatment with specific inhibitors of acid lipase.

Example 12: Lalistat and Orlistat display no significant effect on Adenovirus infection

In order to explore the effect of Lipase A inhibition on Adenovirus infection, HEK 293 cells (Figure 13A) or HepG2 cells (Figure 13B) were infected with Adeno-GFP-Virus at an MOI of 10 in the presence and absence of preincubated and simultaneously administered Orlistat or Lalistat in given concentrations. Effects were measured by fluorescence intensity measurement of GFP in the Tecan Infinite F200 reader. Cell viability of the used concentrations was determined by CTB-Assay in 293 cells (C) and HepG2 cells (D). Based on the experimental observations it is concluded that Lipase A inhibition does not affect Adenovirus infection in 293 cells or HepG2 cells.
Example 13: Lalistat displays no significant effect on SARS-Coronavirus Virus infection

In order to explore the effect of Lipase A inhibition on human SARS coronavirus (SARS-coronavirus) infection, permissive hepatocyte carcinoma cells (Huh7 cells) were infected with HCoV-229E at an MOI of 0.1 (FFU/ml) in the presence and absence of preincubated and simultaneously administered Lalistat in given concentrations for 48h. Effects on the viral titers were determined by plaque assay (Figure 14A). Cell viability of the used concentrations was determined by a conventional proliferation assay XTT cell proliferation assay (XTT-assay) (Figure 14B). The experimental observations support the conclusion that Lalistat does not have an antiviral effect on HCoV-229E in Huh7 cells.

Example 14: Lalistat does not affect measles virus infection

In order to explore the effect of Lalistat on measles virus infection, Vero cells were infected at an MOI of 0.1 (PFU/ml) with the recombinant measles virus MV-eGFP (rMV-Edtag-eGFP, Radecke et al., 1995) expressing enhanced Green Fluorescent Protein (eGFP). Cells were infected in the presence and absence of preincubated and simultaneously administered Lalistat in given concentrations for 48h. Effects on the viral titers were determined by plaque assay (Figure 15A) and observations documented with a fluorescence microscope (Figure 15C). Cell Viability of the used concentrations was determined by XTT-Assay (Figure 15B). The experimental observations support the conclusion that Lalistat does not affect measles virus infection.

Example 15: Lalistat significantly impairs Chikungunya Virus infection

In order to explore the effect of Lalistat on Chikungunya Virus (CHIKV) infection, BHK-21 cells were infected with CHIKV (MOI 0.01) in the presence and absence of 70μM Lalistat preincubated and/or simultaneously added. CHIKV replication was measured by luciferase reporter assay (A). Error bars represent SD from 3 experiments. MTT vitality assay was performed in BHK-21 cells with different concentrations of Lalistat (B). These experimental observations support the conclusion of a significant inhibitory effect of Lalistat on Chikungunya Virus infection.
Example 16: Lalistat significantly impairs Ebola Virus infection

In order to explore the effect of Lalistat on Ebola Virus infection, permissive Huh7 cells were infected with Zaire EBOV (ZEBOV) (MOI 0.1) in the presence and absence of 70μM Lalistat preincubated and simultaneously added. The impact of Lalistat on ZEBOV infection was monitored in Figure 18 by observing the cytopathic effect induced on cells exposed to ZEBOV. Infected cells incubated with Lalistat showed significantly less cytopathic effect than infected cells not treated with Lalistat.

In further experiments, Huh7 cells were infected with ZEBOV (MOI 0.1) in the presence and absence of 70μM Lalistat preincubated and simultaneously added. ZEBOV titers were analyzed by performing TCID$_{50}$ (Figure 19). A significant reduction of ZEBOV infection could be detected after Lalistat treatment. These experimental observations strongly support the conclusion of a significant inhibitory effect of Lalistat on Ebola Virus infection.

Example 17: Lalistat impairs HIV infection

In order to explore the effect of Lalistat on HIV infection, LC5-RIC receptor cells were incubated with infectious HIV-1 virus and treated with different concentrations of Lalistat. After 48h the fluorescence intensity was measured, which is proportional to the infection strength. Cell viability was measured by performing a cell titer blue assay. The experimental observations shown in Figure 20 support the conclusion of a significant inhibitory effect of Lalistat on early steps of HIV infection.

Example 18: Lalistat impairs Dengue virus replication

In order to explore the effect of Lalistat on Dengue virus replication, Huh7 cells were electroporated with a subgenomic reporter replicon of Dengue virus of serotype 2 (DENV-2, strain 16681) expressing Renilla luciferase (R-Luc). (Fischl W, Bartenschlager R. Methods Mol Biol. 2013;1030:205-19), preincubated and simultaneously added in the presence and absence of 70μM Lalistat. R-Luc activity was determined 48h later from cell lysates. An aliquot of electroporated cells was seeded in a 96-well plates and treated with Lalistat 70μM or DMSO (Figure 21B). Viability was determined 48h later by measuring cellular ATP content using a bioluminescent assay kit (Figure 21A). The experimental observations shown in Figure 21 support the conclusion of an inhibitory effect of Lalistat on Dengue virus infection.
Example 19: Lalistat impairs Influenza virus replication
Permissive MDCK cells were infected with highly pathogenic avian Influenza Virus R65 of serotype H5N1 (HPAIV A/Swan/Germany/R65/06 (H5N1) (R65)). 48h p.i., the supernatant was titrated. Figure 22 shows titers (TCID50/ml) 72h after infection. When compared to the titer observed from the infection in the absence of Lalistat ("R65"), the presence of Lalistat ("R65 Lalistat"), starting from a titer of \(10^4.97\) (n=1), resulted in a significant reduction of the titer. These experimental observations support the conclusion of an inhibitory effect of Lalistat on Influenza Virus infection.

Example 20: Lalistat toxicity assay in mice
In order to determine the compatibility and tolerance of mammalian subjects to Lalistat, BL6 mice were repeatedly treated with 400μM Lalistat by intravenous injection (Figure 23). No toxicity as well as no liver injury could be observed after Lalistat treatment. The indicated concentration of 400μM Lalistat is the calculated final blood concentration in mice.
Claims

1. Pharmaceutical composition for use in a method of preventing or treating a virus infection or a disease resulting from a virus infection, said composition comprising one or more active pharmaceutical ingredients and, optionally, at least one pharmaceutically acceptable excipient and/or carrier, wherein at least one active pharmaceutical ingredient is an antagonist of Lysosomal Acid Lipase substantially not inhibiting human pancreatic lipase or bovine milk lipoprotein lipase.

2. The pharmaceutical composition of claim 1, wherein said antagonist of Lysosomal Acid Lipase is or comprises a thiazole carbamate substituted with N-heterocycles, preferably selected from the group consisting of 4-(pyrrolidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate, 4-(pyrrolidin-1-yl)-1,2,5-thiadiazol-3-yl morpholine-4-carboxylate, 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate, 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl morpholine-4-carboxylate, 4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate, 4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl morpholine-4-carboxylate and preferably 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate.

3. The pharmaceutical composition of claim 2, wherein said thiazole carbamate substituted with N-heterocycles is a 1,2,5-thiadiazole carbamate, which preferably has the structure

\[
\text{\begin{tikzpicture}
\draw (0,0) circle (0.5cm);
\draw (0,0) -- (1,0);
\draw (0,0) -- (0,1);
\draw (0,0) -- (-0.5,0.5);
\draw (0,0) -- (-0.5,-0.5);
\draw (1,0) -- (1.5,0);
\draw (1,0) -- (1.5,1);
\draw (1,0) -- (0.5,0.5);
\draw (1,0) -- (0.5,-0.5);
\text{X}
\end{tikzpicture}}
\]

wherein X is a nitrogen containing heterocycle.

4. The pharmaceutical composition of claim 3, wherein said thiazole carbamate substituted with N-heterocycles is
wherein X is -O- or -CH2-.

5. The pharmaceutical composition of claim 4, wherein the thiadiazole carbamate substituted with N-heterocycles is 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate.

6. The pharmaceutical composition of any one of claims 1 to 5, wherein said virus is a virus which enters cells by receptor-mediated endocytosis or a virus selected from the group consisting of Hepatitis C Virus; Hepadnaviridae, preferably Hepatitis B Virus and Hepatitis D Virus; Hepatitis E virus; Flaviviridae preferably Alphaviruses, preferably Chikungunya Virus, Semliki Forest Virus, Sindbis Virus; West-Nile-Virus, Dengue Virus; Yellow-fever; Filoviridae preferably Marburg and Ebola Virus, Orthomyxoviridae, preferably Influenza virus; Rhabdoviridae, preferably Vesicular Stomatitis Virus and Rabiesvirus; Retroviridae, preferably Human Immunodeficiency Virus, human T-Cell-Leukemia Virus, Avian Leukemia Virus; New-Castle-Disease Virus and Respiratory-Syncytial-Virus; Arenaviridae, preferably Lymphocytic choriomeningitis virus; Picornaviridae, preferably Hepatitis A Virus, Echovirus, Enterovirus, human Rhinovirus, Coxsackie Virus, Polio Virus; Herpesviridae preferably Herpes Simplex Virus, Eppstein-Barr-Virus; Cytomegalovirus and Varicella Zoster Virus; Poxviridae preferably Vaccinia Virus; Papovaviridae preferably human Papillomaviruses (HPV); Polyomaviridae preferably SV40; Burnaviridae preferably Hanta Virus and Rift-Valley-Fever Virus; Reoviridae preferably Rota Virus and Blue-Tongue-Virus; preferably Hepatitis C Virus.

7. The pharmaceutical composition of any one of claims 1 to 6, wherein the virus is Hepatitis B Virus, Hepatitis C Virus, Influenza Virus, Dengue Virus, Human Immunodeficiency Virus, Ebola Virus, Chikungunya Virus or Vesicular Stomatitis Virus and wherein the antagonist is 4-(piperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate.
8. The pharmaceutical composition of any one of claims 1 to 7, wherein the pharmaceutical composition comprises at least one additional active pharmaceutical ingredient or wherein the method of preventing or treating comprises administering at least one additional pharmaceutical composition comprising at least one additional active pharmaceutical ingredient.

9. The pharmaceutical composition of claim 8, wherein the additional active pharmaceutical ingredient is an inhibitor of activity of a viral protein, wherein the viral protein is a protease or a DNA- or RNA-dependent polymerase, a reverse transcriptase, a viral kinase, a viral integrase, or a viral surface protein.

10. The pharmaceutical composition of any one of claims 7 to 9, wherein the virus is Hepatitis C Virus and wherein the additional active pharmaceutical ingredient is an inhibitor of activity of the virus proteins C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and/or NS5B.

11. The pharmaceutical composition claim 10, wherein said inhibitor is selected from the group consisting of ribavirin, telaprevir, boceprevir, simeprevir, asunaprevir, faldaprevir, vaniprevir, danoprevir, ABT-450, GS-9451, GS-9256, ABT-267, daclatasvir, ledipasvir, GS-5816, ABT-333, BMS-791325, ABT-072, tegobuvir, deleobuvir, setrobuvir, GS-9669, sofosbuvir, PSI-6206, meritabite, CPG10101, NOV-205, INX-08189.

12. The pharmaceutical composition of claim 10 or 11, comprising at least one additional active pharmaceutical ingredient, which is an anti-HCV agent preferably selected from the group consisting of interferon, preferably interferon-alpha, peginterferon Lambda-1a, peginterferon alfa, IFN gamma, peginterferon alfa-2a, peginterferon alfa-2b; recombinant interleukin 7; lipase inhibitor, preferably Orlistat; Cyclosporin, Cyclophilin inhibitor, preferably alisporivir, SCY-635; Silibinin; agonist of the c-mpl (TpoR) receptor, preferably 3'-{(2Z)-2-[1-(3,4-dimethylphenyl)-3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)hydrazino}-2'-hydroxy-3-biphenylcarboxylic acid; 1-[(4-chlorophenyl)methyl]-2-(pyrrolidin-1-ylmethyl)benzimidazole; thiazolide, preferably [2-[(5-nitro-1,3-thiazol-2-yl)carbamoyl]phenyl]ethanoate (nitazoxanide); hydroxychloroquine; antibody against tumor necrosis factor alpha, preferably infliximab.
13. A method of preventing or treating in a subject a viral infection or a disease resulting from a viral infection, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition according to any one of claims 1 to 12.

14. The pharmaceutical composition of any one of claims 1 to 12 or the method of claim 11, wherein the disease is selected from the group consisting of: an acute or chronic HBV or HCV infection, a virus induced liver abnormality, a liver cirrhosis, a hepatocellular carcinoma, reinfection of liver transplants, HCV induced diabetes, HBV or HCV induced liver fibrosis, non alcoholic fatty liver disease.

15. The method of claim 13 or 14, comprising administering at least one additional pharmaceutical composition comprising at least one additional active pharmaceutical ingredient.

16. A method of monitoring a virus infection infection, comprising
(a) obtaining a first sample and a second sample from a subject, wherein the first sample is obtained prior to the second sample and the subject is a subject treated with the pharmaceutical composition of any one of claims 1 to 12 or 14;
(b) determining in the first and the second sample the amount of a marker, which is indicative for the virus infection;
(c) comparing the amount of marker determined in the first and the second sample; wherein a reduced amount of said marker in the second sample is indicative for a regression of the virus infection.

17. A method of selecting a patient sensitive to a therapy with the pharmaceutical composition according to any one of claims 1 to 12 or 14, comprising
(a) obtaining a first sample and a second sample from a subject, wherein the first sample is obtained prior to and the second sample is obtained after administering the pharmaceutical composition of any one of claims 1 to 12 or 14;
(b) determining in the first and the second sample the amount of a marker which is indicative for the virus infection;
(c) comparing the amount of marker determined in the first and the second sample; wherein a reduced amount of said marker in the second sample is indicative for the subject’s sensitivity to the pharmaceutical composition.
18. The method of claim 16 or claim 17, wherein the virus is HCV.
N=3 independent experiments, standard deviations are given; student's two-tailed t test, unpaired, * p ≤ 0.05; ** p ≤ 0.01.
Figure 4

D

VSV-Luc

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<td>70μM pres.</td>
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<td>70μM sim</td>
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N=7 independent experiments, standard deviations are given; student's two-tailed t test, unpaired, *p ≤ 0.05.
Figure 5

A

% of Inhibition

Lipase A
Lipase C
HCV

siRNA Lipase A
siRNA Lipase C
siRNA Lipase C+A

transfection I
Wash
transfection II
Wash
infection (48 h p.t.)
48 h p.i. RNA extraction
N=4 independent experiments, standard deviations are given; student's two-tailed t test, unpaired, *** p ≤ 0.0001.
Figure 7

A

NS3 (27 kDa)
Core (22 kDa)
β-actin (45 kDa)

B

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Percentage Inhibition
Figure 8

A Undifferentiated Huh7.5

N=3 independent experiments, standard deviations are given; student's two-tailed t test, unpaired, *** p ≤ 0.0001.
Figure 8

Differentiated Huh7.5
24h post treatment

B

TCD50/ml

100E+00 100E+01 100E+02 100E+03 100E+04

ctrl 50 Orlistat 50 Lalistat 100 Orlistat 100 Lalistat
Figure 11

MLV/VSV/pp Results

控制
Lalistat (70uM)

BASA

HCVpp Results
Figure 14

A

Lalistat

cell line: Huh7
time point p.i.: 48h

virus titer [pfu/ml]

1.0x10^3
1.0x10^2
1.0x10^1
1.0x10^0
1.0x10^-1
1.0x10^-2
1.0x10^-3

100 50 25 12.5 6.25 3.13 1.56 0

concentration [μM]

B

cell viability [%]

140
120
100
80
60
40
20
0

CC_{50} = 65μM

concentration [μM]
Figure 20

EC_{50} = 37.07 ± 5.2 μM

% Infection/ Viability
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K45/06 A61K31/433 A61K31/435 A61K31/5375 A61P31/12
A61P31/14 A61P31/16 A61P31/18 G01N33/50

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 A61P G01N

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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>EP 0 785 193 A1 (RATIONAL DRUG DESIGN LAB [JP]) 23 July 1997 (1997-07-23)</td>
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See patent family annex.

Further documents are listed in the continuation of Box C.

Date of the actual completion of the international search 27 April 2015

Date of mailing of the international search report 11/05/2015

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

Authorized officer
Albayrak, Timur

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<td>FUJIWARA M ET AL: &quot;THIADIAZOLE DERIVATIVES: HIGHLY POTENT AND SELECTIVE INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) REPLICATIONS IN VITRO&quot;, MICROBIOLOGY AND IMMUNOLOGY, CENTER FOR ACADEMIC PUBLICATIONS JAPAN , JP, vol. 41, no. 4, 1 January 1997 (1997-01-01), pages 301-308, XP000983184, ISSN: 0385-5600 the whole document</td>
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