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(54) Title: KOMBINATION OF PROTEASOME INHIBITORS AND ANTI-
HEPATITIS MEDICATION FOR TREATING RETROVIRAL
DISEASES

(57) Abstract: The present invention relates to use of proteasome inhibitors in combination therapy with further anti-retroviral
compounds for treating retrovirus infections such as HIV-infections and diseases or conditions induced by a retroviral infection,
e.g. AIDS.
Technical Field of the Invention

The present invention concerns the use of proteasome inhibitors that are suitable to treat and prevent virus infections, particularly retroviral infections such as HIV-infections, and to inhibit release of viruses from infected cells, virus maturation, virus infectivity and replication of said viruses. The proteasome inhibitors according to the present invention may be used in a combination therapy with additional anti-retroviral compounds. The invention relates also to the use of proteasome inhibitors in a combination therapy with additional anti-retroviral compounds for treating therapy-resistant and -refractory retroviral infections. The invention is further concerned with pharmaceutical compositions and kits of pharmaceutical compositions which may be used in methods for treating retroviral diseases, for example for treating therapy-resistant and -refractory retroviral infections such as HIV-infections, particularly HIV-infected individuals that are resistant to conventional therapeutics, such as HAART.
**Background of the Invention**

Proteasome inhibitors are compounds that influence the activity of the cellular ubiquitin/proteasome-pathway, in particular of the 26S and the 20S proteasome complex, which plays an important role in the replication of various viruses, e.g. retroviruses. The use of proteasome inhibitors in the treatment of viral diseases is known, e.g. from EP 1326 632 where it has been shown that these compounds are capable of inhibiting the processing of the Gag-proteins of HIV-1 and HIV-2, inhibit the release of the viruses and reduce their infectivity.


AIDS occurs after an asymptomatic period following infection with HIV-1 or HIV-2. Main characteristics of the syndrome are the progressive degeneration of the immune system and the central nervous system. The infection with HIV itself, does not necessarily cause death. However, infected individuals will eventually suffer from severe immunosuppression, so that various other diseases, such as opportunistic infections, e.g viral infections (e.g. HCV, HSV, CMV, EBV, HHV6), bacterial and fungal infections, or malignancies, especially Kaposi's sarcoma, occur. HIV-infected
individuals eventually succumb to these secondary conditions. In as far as a
treatment is available, such secondary conditions may of course be treated.
However, treatment of secondary infections may adversely affect the already
weakened immune system, and the combination of weak immune response with
often a multitude of secondary adverse conditions can lead to fatal outcomes even for
conditions otherwise easily cured in non-HIV-infected individuals.

AIDS is now a pandemic. In 2007, it was estimated that 33.2 million people lived
with the disease worldwide, and that AIDS killed an estimated 2.1 million people,
including 330,000 children. Over three-quarters of these deaths occurred in sub-
Saharan Africa.

Although treatments for AIDS and HIV can slow the course of the disease, there is
currently no known cure or vaccine leading to the elimination of the virus. Anti-
retroviral treatment reduces both the mortality and the morbidity of HIV infection,
but anti-retroviral compounds or combination products are expensive and routine
access to antiretroviral medication is not available in all countries.

The family of retroviruses which also includes the human immune deficiency viruses
(HIV) belongs to the large group of eukaryotic retrotransposable elements (for a
Said elements are distinguished by the ability to transcribe RNA genomes into DNA
intermediates by using the enzyme reverse transcriptase. Retroviruses are divided
into five subfamilies: (i) spumaviruses; (ii) mammalian type C oncoviruses; (iii) BLV
(bovine leukemia virus)/HTLV (human T-cell leukemia virus) leukemia viruses; (iv)
a heterogeneous group of RSV (Rous sarcoma virus), type A, B and D viruses; and
(v) lentiviruses (for a review, see Doolittle et al, 1990).

Lentiviruses replicate predominantly in lymphocytes and fully differentiated
macrophages and usually cause long-lasting and/or incurable diseases. Retroviruses
contain at least three characteristic genes: gag (groupspecific antigen), pol (polymerase) and env (envelope proteins). Apart from structural and enzymatically active viral proteins, various retroviruses encode additional, usually small proteins with regulatory functions. The lentivirus subfamily includes, in addition to HIV, SIV (simian immunodeficiency virus), EIAV (equine infectious anemia virus), BIV (bovine immunodeficiency virus), FIV (feline immunodeficiency virus) and Visna virus.

HIV Replication Cycle

The HIV replication cycle starts with the virus binding to various cell receptors among which the glycoprotein CD4 acts as the primary receptor and various cellspecific chemokine receptors act as co-receptors, after binding to CD4. After the virus has entered, the viral RNA genome is transcribed by means of reverse transcriptase (RT), RNase H and polymerase into double-stranded DNA which, in association with the preintegration complex, is then transported into the nucleus and incorporated as provirus genome into chromosomal DNA by means of viral integrase. After transcription and translation, Gag/Gag-Pol polyproteins and envelope proteins are transported to the cell membrane where virions are being assembled.

After budding and detachment, virus particles mature due to proteolytic processing of said Gag/Gag-Pol polyproteins (for a review, see Swanstrom, R; Wills, J; In: Coffin, J., et al. (Eds.), Retroviruses, Cold Spring Harbor Press, Plainview, N.Y., 1997, pp. 263-334).

Assembly, Release and Maturation of HIV Particles

The main components of HIV structural proteins are translated in the form-of three polyproteins: Gag and Gag-Pol for the inner core proteins and viral enzymes and Env for proteins of the viral envelope proteins. In the case of HIV-1, complete proteolytic processing of the Gag polyprotein Pr55 results in the formation of the matrix (MA),
capsid (CA) and nucleocapsid (NC) and of the C-terminal p6Gag protein. In general, HIV-1 virions are detached from the plasma membrane as mature noninfectious virus particles, this process being referred to as virus budding. Immediately after or else during budding, proteolytic processing of Gag and Gag-Pol polyproteins commences with the activation of PR (HIV protease). The proteolytic maturation of the virions is accompanied by morphological changes. A characteristic feature is the condensation of the inner core, resulting in the formation of a conical core cylinder typical for the mature virus.

Macrophages as viral reservoirs

The peculiar dynamics of HIV replication in macrophages, their long-term survival after HIV infection, and their ability to spread virus particles to bystander CD4-lymphocytes, make evident their substantial contribution to the pathogenesis of HIV infection. In addition, infected macrophages are able to recruit and activate CD4-lymphocytes through the production of both chemokines and virus proteins (such as nef). In addition, the activation of the oxidative pathway in HIV-infected macrophages may lead to apoptotic death of bystander, not-infected cells. Finally, macrophages are the most important target of HIV in the central nervous system.

The alteration of neuronal metabolism induced by infected macrophages plays a crucial role in the pathogenesis of HIV-related encephalopathy. Taken together, these results strongly support the clinical relevance of therapeutic strategies able to interfere with HIV replication in macrophages. In vitro data show the potent efficacy of all nucleoside analogues inhibitors of HIV-reverse transcriptase in macrophages.

Nevertheless, the limited penetration of some of these compounds in sequestered districts, coupled with the scarce phosphorylation ability of macrophages, suggests that nucleoside analogues carrying preformed phosphate groups may have a potential role against HIV replication in macrophages. This hypothesis is supported by the great anti-HIV activity of tenofovir and other acyclic nucleoside phosphonates in macrophages that may provide a rationale for the remarkable efficacy of tenofovir in
HIV-infected patients. Non-nucleoside reverse transcriptase inhibitors (NNRTI) do not affect HIV-DNA chain termination, and for this reason their antiviral activity in macrophages is similar to that found in CD4-lymphocytes. Interestingly, protease inhibitors, acting at post-integrational stages of virus replication, are the only anti-retroviral compounds able to interfere with virus production and release from macrophages with established and persistent HIV infection (chronically-infected cells). Since this effect is achieved at concentrations and doses higher than those effective in de-novo infected CD4-lymphocytes, it is possible that lack of adherence to therapy, and/or suboptimal dosage leading to insufficient concentrations of protease inhibitors may cause a resumption of virus replication from chronically-infected macrophages, ultimately resulting in therapeutic failure. For all these reasons, therapeutic strategies aimed to achieve the greatest and longest control of HIV replication should inhibit HIV not only in CD4-lymphocytes, but also in macrophages. (Aquaro, S., et al, Antiviral Research 2002, 55:209-225).

Furthermore, macrophages serving as an important reservoir in HIV-infected patients, for example macrophage/microglial cells in the brain of such patients, are an important viral reservoir. Commonly known medicaments affect post-integration stages in macrophages only. It is therefore another objective of the invention to provide compounds capable of interfering with HIV replication in infected macrophages, and preferably of eradicating macrophages with the ability to infect other cells in an organism, potentially after a re-activation of dormant cells.

Ubiquitin/Proteasome Pathway and Retrovirus Replication

Information on the importance of the UPS for particular sections of HIV replication is also known: on the one hand, the system is utilized for proteolysis of de novo synthesized virus receptor CD4. This pathway is mediated by the HIV-1-specific protein Vpu which directs CD4 from the membrane of the endoplasmic reticulum (ER) to the site of proteosomal degradation in the cytoplasm (Schubert et al, 1998, J.
ViroL, 72:2280). Moreover, monoubiquitinated forms of Gag have been described for HIV-1 and Mo-MuLV Gag proteins (Ott et al., 1998, J. Virol, 72:2962).

Although the catalytic activities of the 26S proteasome are completely different from the very specific aspartate-protease activity of the HIV-1/HIV-2 viral proteases, it was observed that a specific inhibitor of the HIV-1 protease, referred to as "ritonavir" (but none of the other previously known HIV protease inhibitors) can inhibit chymotrypsin activity of the 20S proteasome in vitro (Schmidtke et al., 1999, J. Biol. Chem., 274:35734) and proteasome-mediated MHC-I antigen expression in vivo (Andre et al., 1998, Proc. Natl. Acad. Sci. USA, 95:1312; WO 00/33654).

Medical need

As will be clear from the above, there is a need for new anti-retroviral therapies in infected mammals, particularly in humans. New therapeutic approaches intend to prevent the spread of infectious viruses, the development of immunodeficiencies, such as AIDS, HIV-dementia, or other retroviral diseases, etc.

The present invention provides new therapies based on the use of proteasome inhibitors, which may optionally be combined with additional anti-viral therapeutics for use in the treatment of retroviral infections.

**Detailed Description of the Invention**

It is an objective of the invention to provide pharmaceutical compositions, uses thereof and methods which allow treatment and prevention of retroviral infections, such as HIV-infections, particularly infections in therapy-resistant or therapy-refractory patients. The pharmaceutical compositions and methods disclosed herein may be administered or used in the treatment at any stage of retrovirus infection, i.e. including (immediately) after diagnosis of an infection, even in the absence of any
disease symptoms. The compounds and pharmaceutical compositions of the invention may also be administered or used in individuals that are suspected to be infected, even before the infection may be detected using standard assays, e.g. HIV-diagnostic kits that are currently on the market.

These and other objectives as they will become apparent from the ensuing description are attained by the subject matter of the independent claims. The dependent claims refer to some of the preferred embodiments.

Before the above embodiments are described in further detail, further definitions are provided.

Terms as set forth hereinafter are generally to be understood in their common sense unless indicated otherwise.

Where the term "comprising" is used in the present description and claims, it does not exclude other elements. For the purposes of the present invention, the term "consisting of" is considered to be a preferred embodiment of the term "comprising of. If hereinafter a group is defined to comprise at least a certain number of embodiments, this is also to be understood to disclose a group which preferably consists only of these embodiments.

Where an indefinite or definite article is used when referring to a singular noun, e.g. "a", "an" or "the", this includes a plural of that noun unless something else is specifically stated.

In the context of the present invention the terms "about" or "approximately" denote an interval of accuracy that the person skilled in the art will understand to still ensure the technical effect of the feature in question. The term typically indicates deviation from the indicated numerical value of ±10%, and preferably of ±5%.
The terms "retroviral" or "retrovirus" relate to enveloped viruses belonging to the family Retroviridae. This family comprises RNA viruses having a positive-mRNA-stranded RNA genome which is reverse transcribed and replicated in a host cell via the enzyme reverse transcriptase (RT) to produce DNA from its RNA genome. The DNA is integrated as a so-called "provirus" into the host's genome by the viral integrase enzyme. The Retroviridae family comprises such important members as HIV, HTLV, Xenotropic murine leukemia virus-related virus (XMRV), FIV, SIV etc. In the context of the present application an important aspect relates to the treatment of human immunodeficiency virus, i.e. HIV, which encompasses at least two types of HIV, e.g. HIV-1 and HIV-2. While HIV-1 is the dominant type in the Western World, and certain examples provided herein use viruses of this type, the scope of the instant invention shall not be understood as limited to HIV-1, and preferably includes not just types of HI viruses presently known, but also further types which may arise or be characterized and/or typified in the future.

The term retroviral disease refers to diseases or conditions caused by an infection with a retrovirus. The term "HIV-related disease" refers to diseases and conditions including those which are commonly attributed to, or are causally related to, infection with a Human Immunodeficiency Virus, and may include, without limitation, Acquired Immuno-Deficiency Syndrome (AIDS), and all diseases and conditions commonly referred to thereunder, and/or the condition of infection with a Human Immunodeficiency Virus, including in the absence of any overt symptoms of disease or discomfort directly or indirectly linked thereto, and furthermore including before the presence of the virus may be detected with certain diagnostics test as customary for this indication. However, in some embodiments, HIV-related disease will preferably not refer to diseases and conditions ultimately brought about by HIV infection through the associated immunodeficiency, but which, by their nature, cannot be alleviated or cured by removing or eradicating the virus alone, e.g. certain
malignancies which often occur in HIV-positive or AIDS patients, e.g. Karposi Sarcoma.

The expression "pharmaceutically active agent in use against retroviral infections", interchangeably used with the term "anti-retroviral compound", refers to a pharmaceutically active compound for which the art recognizes that it can be used for treatment of retroviral infections, preferably for the treatment of HIV infections. Preferably the term relates to pharmaceutically active agents as they are authorized by regulatory agencies such as the FDA or the European Medicines Agency (herein: EMA) for treating retroviral infections, in particular HIV infections. The term for the purposes of the present invention may not refer to proteasome inhibitors. Recognition in the art of a pharmaceutically active compound as useful for the treatment of retrovirus infection may have occurred at the time the instant invention was made, but nothing herein shall be construed as limiting the instant invention to such compounds. Pharmaceutically active compounds, which are characterized as useful for the therapy of retroviral infections, and/or receive regulatory authorization for these purposes, at a later date, are well within the scope of the instant invention, and are expressly included. Various such compounds are described herein below.

The term "standard therapy for HIV infection" shall mean a therapy of an HIV infection following the recommendations summarized in "Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents" (Panel on Antiretroviral Guidelines for Adults and Adolescents; The U.S. Department of Health and Human Services. Washington, DC, USA, January 10, 2011; pages 1-166), or in a respective future update of this document as indicated by a relevant authority. A "dose recommended for treatment of an HIV-related disease" refers to the doses recommended in the latter document for the respective anti-retroviral compound under applicable circumstances.
"Administering", or "administration of", a compound or agent, e.g. an anti-retroviral compound or a proteasome inhibitor, as used herein, shall be interpreted as equivalent to "administering a pharmaceutical composition comprising" or "administration of a pharmaceutical composition comprising" the respective compound or agent, wherein the pharmaceutical composition will usually further comprise such additional components as described herein below and which are necessary to render the respective compound or agent bioavailable and to minimize adverse effects of administration.

The term "proteasome inhibitor" refers to a compound which is capable of inhibiting, reversibly or irreversibly, an activity of the proteasome, e.g. the proteasome-mediated degradation of ubiquitin-modified peptides and proteins.

The term "semicarbazone proteasome inhibitor" as the term is used herein refers particularly to [1-1-[1-{[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl}-2-(1H-indol-3-yl)-ethylcarbamoyl]-2-(1H-indol)], also referred to as S-2209, pharmaceutically acceptable salts, as well as structural and/or functional analogs thereof.

The term "structural and/or functional analogs of [1-1-[1-{[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl}-2-(1H-indol-3-yl)-ethylcarbamoyl]-2-(1H-indol)]", as used herein, refers to the group of compounds, but excluding [1-1-[1-{[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl}-2-(1H-indol-3-yl)-ethylcarbamoyl]-2-(1H-indol)], and its pharmaceutically acceptable salts, of the general formula (I), pharmaceutically acceptable salts or a pharmaceutically acceptable salt or a stereoisomer thereof,
wherein

Y is a group of formula (III)

\[ R^a, R^b, R^c, R^d \text{ independently represent } H, -CN, -OH, \text{alkoxy}, -SH, \text{alkyl}, \text{alkenyl- or alkyne}, \text{thio, -C(NR)}_4\text{NR}_4, \text{-N=CR}_4\text{NR}_4, \text{-NR}_4\text{C}(0)\text{R}_4, \text{-NR}_4\text{C}(0)\text{R}_4\text{NR}_4, \text{(CH}_2\text{)}_n\text{aryl, -(CH}_2\text{)}_n\text{N}_R\text{R}_8, \text{-C(0)NR}_4\text{R}_8, \text{-N=CR}_4\text{R}_8, \text{-NR}_4\text{C}(0)\text{R}_8, \text{cycloalkyl, -alkenyl oxy, aryl, arylalkyl, -alkenyl or -alkynyl or a heterocycle; }\]

\[ R^e, R^f, R^g \text{ independently are } H, \text{halogen, alkyl, alkenyl or alkynyl, -C(NR)}_7\text{NR}_7\text{R}_8, \text{-C(NR)}_7\text{NR}_7\text{R}_8, \text{(CH}_2\text{)}_n\text{aryl, -(CH}_2\text{)}_n\text{NR}_7\text{R}_8, \text{-C(0)NR}_7\text{R}_8, \text{-N=CR}_7\text{R}_8, \text{-NR}_7\text{C}(0)\text{R}_8, \text{cycloalkyl, -} \]
alkenyl or -alkynyl, heterocycloalkyl, -alkenyl or -alkynyl, haloalkyl, -alkenyl or-
alkynyl, hydroxyalkyl, -alkenyl or -alkynyl, hydroxyalkyl, -alkenyl or -alkynyl,
aminoalkyl. -alkenyl or -alkynyl, heteroaryl, alkyl-, alkenyl- or alkynylamino, or aryl;

R^7, R^7, R^8 independently are H, halogen, alkyl, -alkenyl or -alkynyl, cycloalkyl, -
alkenyl or -alkynyl, heterocycloalkyl, -alkenyl or -alkynyl, haloalkyl, -alkenyl or -
alkenyl, hydroxyalkyl, -alkenyl or -alkynyl, -alkenyl or -alkynyl amino, alkyl-, alkenyl-
or alkynylamino, heteroaryl, alkylaryl, or aryl;

n is 1; m is 1; r is 1 t is 1; X is O; Z is C=0;

Z1 is (CH\_2\_t)R^2; Z2 is (CH\_2\_t)R^3; Z3 is (CH\_2\_t)R^4; Z4 is H, or methyl;

R^2, R^3, R^4 are independently from each other H, Phenyl, Benzyl, 3-Benzothienyl, 2-
Thienyl, 2-Thiazolyl, 4-Pyridyl, 3-Pyridyl, 2-Pyridyl, 2-Quinolyl, 2-Indolyl, 3-
Indolyl, Ethylbenzene, 2-Naphtyl, 1-Naphtyl, p-Aminobenzyl, p-Azidobenzyl, p-
Bromobenzyl, p-Hydroxyphenyl, p-tButyl-benzyl, p-Carboxybenzyl, p-Chloro-
benzyl, p-Cyanobenzyl, 3,4-Dichlorobenzyl, p-Fluorobenzyl, p-Iodobenzyl, p-
Nitrobenzyl, Pentafluorobenzyl, p-Phenylbenzyl, m-Fluorobenzyl, p-Methyl-benzyl, 
Tryptoline-3-carboxylic acid, 5-Methyl-tryptophan, 4-Methyl-tryptophan, 3-Methyl-
IH-indolyl, 2-Methyl-IH-indolyl, 2-Amino-4-ethylphenol, 2,6-Dibromo-4-ethyl-
phenol, 4-Ethyl-2,6-diiodo-phenol, 1-Ethoxy-4-ethyl-benzene, 1-Ethyl-4-methoxy-
benzene, 4-Ethyl-2-iodo-phenol, (4-Ethyl-phenyl)-phenyl-methane, 1-Thiophen-
2-yl-ethanol, 1,2,3,4-Tetrahydro-isoquinoline-3-carboxylic acid, 7-Hydroxy-1,2,3,4-
tetrahydro-isoquinoline-3-carboxylic acid, Sulfuric acid mono-(4-ethyl-phenyl) ester,
Phosphoric acid mono-(4-ethyl-phenyl) ester, 4-Ethyl-2-nitro-phenol, 1-tert-Butoxy-
4-ethyl-benzene and 4-(4-Ethyl-phenoxo)-phenol;

R^1 is phenyl, 4-pyridyl, 3-pyridyl, 2-pyridyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-
pyrimidinyl, 3-pyridazinyl, 4-pyridazinyl, 2-pyrazinyl, 1-pyrazolyl, 3-pyrazolyl, 4-

pyrazolyl, 2-indolyl, 3-indolyl, 1-imidazolyl, 2-imidazolyl, 4-tetrahydro-thieno[3,4-
J]imidazol-2-one-yl, 4-phenoxy-benz-1-yl, which are optionally substituted by
halogen, alkoxy, haloalkyl, or haloalkoxy; provided the analogous compound is
capable of inhibiting the activity of the proteasome to an extent which corresponds to
at least 25% (50%, 60%, 70%, 80%, 90%, or 95%) of the proteasome inhibitory
activity of S-2209.

In a preferred embodiment, R₂, R³, R⁴ are independently of each other H, benzyl, or
indolyl optionally substituted by halogen.

Specific examples for structural/functional analogs are Compounds 1 to 6 and
and/or Compounds 1 to 10 in WO 2007/017284. S-2209 is a particularly preferred
proteasome inhibitor for use according to the present invention.

"Inhibition of proteasome activity" as the term is used herein, shall mean the
reduction of proteasome activity inside or outside a cell by at least 20%, at least 25%,
at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at
least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, at least
98% or at least 99% compared to the situation where no compound or a compound
which is known to not affect proteasome activity is administered. The proteasome
that is inhibited by a proteasome inhibitor referred to herein thus has a residual
activity of not more than about 80%, more particularly not more than about 75%, yet
more particularly not more than about 70%, yet more particularly not more than
about 65%, yet more particularly not more than about 60%, yet more particularly not
more than about 55%, yet more particularly not more than about 50%, yet more
particularly not more than about 45%, yet more particularly not more than about
40%, yet more particularly not more than about 35%, yet more particularly not more
than about 30%, yet more particularly not more than about 20%, yet more
particularly not more than about 5 to 15%, and particularly not more than about 10% residual activity.

However, the inhibition of proteasome activity may be limited by associated toxicity. Under certain circumstances, a minimum residual proteasome activity may be required for a cell to survive. Hence, the inventive proteasome inhibitors may inhibit the activity of the proteasome in a relevant cell, for example a cell in vitro that is a model for the cell type desired to be impacted by the proteasome inhibitory activity in vivo, by only a limited amount, e.g. by not more than about 20%, not more than about 25%, not more than about 30%, not more than about 35%, not more than about 40%, not more than about 45%, not more than about 50%, not more than about 55%, not more than about 60%, not more than about 70%, or not more than about 75%.

In certain embodiments of the invention, the proteasome inhibitor inhibits one or more of the catalytic activities of the 26-S proteasome, and more specifically one or more of the postglutamyl-peptide-hydrolyzing (caspase-like, B1-subunit), trypsin-like (B2 subunit), and/or chymotrypsin-like (B5 subunit) catalytic activities. Yet more specifically, the proteasome inhibitor inhibits all three, yet more specifically not more than two, and most particularly only one of these 26-S proteasome catalytic activities. In another specific embodiment, the proteasome inhibitor inhibits at least two of these 26-S proteasome catalytic activities. In yet another specific embodiment, the proteasome inhibitor may inhibit at least one activity of the proteasome, but may actually activate another of the 26-S proteasome catalytic activities. In yet another specific embodiment, the proteasome inhibitor does not activate any of the 26-S proteasome catalytic activities.

In certain embodiments of the invention, the inhibitory activity is observed at nanomolar concentrations in cell culture in vitro, e.g. at concentrations ranging between 1 nM and 1 µM, or 10 nM to 1 µM, or 100 nM to 1 µM, or 1 nM to 100 nM,
or 1 nM to 10 nM, or 10 nM to 100 nM, or 1 nM to 10 µM, or 10 nM to 100 nM, or 100 nM to 10 µM, or 100 nM to 10 µM, or 1 µM to 10 µM.

As such caspase-like, trypsin-like, and chymotrypsin-like proteolytic activities can also be found in other cellular proteases, proteasome inhibitors may not only inhibit the proteasome as described above, but also further cellular proteases.

However, in specific embodiments of the invention, a subgroup of proteasome inhibitors, which can be particularly suitable for the pharmaceutical compositions, the kits, the uses and the methods as described herein, comprises so called specific proteasome inhibitors.

"Specific proteasome inhibitor", or "specific inhibition" of an activity of the proteasome by an inhibitor, herein shall mean that the inhibitor reduces the said activity by 50% at a concentration (termed IC50 or IC50) that is lower by at least a factor of 1/2, 1/3, 1/5, 1/10, 1/20, 1/50, 10^2, 5 x 10^3, 2 x 10^3, 10^3, or less, than the IC50 of that same inhibitor for the inhibition of another, or many other, or, specifically, any other relevant activity in question, e.g. a proteolytic activity not associated with the proteasome.

For example, a specific proteasome inhibitor may be at least twice as potent with respect to the inhibition of any one, or more, of the 26S proteasome catalytic activities than with respect to the inhibition of non-proteasomal cellular proteases or microorganism derived proteases, e.g., a lysosomal protease or an HIV protease, or at least three times as potent, or at least five times as potent, etc.. Such specific proteasome inhibitors include, without limitation, the proteasome inhibitors PS-519, PS-341 (Bortezomib) and PS-273. These proteasome inhibitors are potent, specific for the proteasome and substantially do not block other cellular proteases. The proteasome inhibitors PS-341 and PS-519 have moreover been tested pre-clinically in animal models and in humans for clinical studies (cancer patients).
The inhibitory activity on the proteasome of a candidate proteasome inhibitor may be assessed by, for example, the assay described in Adams, J., et al., Cancer Research 1999, 59:2615, or the commercially available Proteasome Glo™ Assay (Promega Corp. Madison WI, USA). These assays may be used to determine an \( IC_{50} \) for the candidate proteasome inhibitor respective the one or more proteasome catalytic activities; any other suitable assay may then be used to determine an \( IC_{50} \) for the candidate proteasome inhibitor respective another relevant activity in question; and these \( IC_{50} \) values may hence be compared to obtain a measure of the specificity of the inhibitory activity of the candidate proteasome inhibitor. Further methods of testing the inhibitory activity of S-2209 and the like are disclosed in Baumann et al., Brit. J. Haematology 144: 875-886, 2009.

Some embodiments of the present invention relate to:

1. A pharmaceutical composition comprising at least one proteasome inhibitor and at least one anti-retroviral compound for use in the treatment of a retroviral disease.

2. The pharmaceutical composition for use according to embodiment 1, wherein the retroviral disease is an HIV-related disease.

3. The pharmaceutical composition for use according to any of embodiment 1 or 2, wherein the anti-retroviral compound is a retrovirus protease inhibitor.

4. The pharmaceutical composition for use according to any of embodiments 1 to 3, wherein the retrovirus protease inhibitor is selected from the group comprising darunavir, atazanavir, indinavir, lopinavir, ritonavir, and saquinavir.
5. The pharmaceutical composition for use according to any of embodiments 1 to 4, wherein the proteasome inhibitor is a semicarbazone proteasome inhibitor, a structural and/or functional analogue or a derivative thereof, a dipeptidyl-boronic acid derivative, or a pharmaceutically acceptable salt of either.

6. The pharmaceutical composition for use according to any of embodiments 1 to 5, wherein the proteasome inhibitor that is a semicarbazone is S-2209 ([1-{1-{[(2,4-Dioxo-imidazolidin-l-ylimino)-methyl]-2-phenyl-ethylcarbamoyl}-2-(IH-indol-3-yl)-ethylcarbamoyl]-2-(IH-indol)}) or the proteasome inhibitor is the dipeptidyl-boronic acid derivative PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), which has the molecular formula C_{19}H_{25}BN_{4}O_{4}.

7. The pharmaceutical composition according to any one of embodiments 1 to 6, wherein the proteasome inhibitor is ([1-{1-{[(2,4-Dioxo-imidazolidin-l-ylimino)-methyl]-2-phenyl-ethylcarbamoyl}]-2-(IH-indol-3-yl)-ethylcarbamoyl]-2-(IH-indol)}) and the retrovirus protease inhibitor is darunavir.

8. The pharmaceutical composition according to any one of embodiments 1 to 6, wherein the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), which has the molecular formula C_{19}H_{25}BN_{4}O_{4} and the retrovirus protease inhibitor is darunavir.

9. A kit comprising (a) at least one anti-retroviral compound or a pharmaceutical composition comprising at least one anti-retroviral compound and (b) at least one proteasome inhibitor or a pharmaceutical composition comprising at least one proteasome inhibitor.
10. The kit according to embodiment 9, wherein the at least one anti-retroviral compound is a retrovirus protease inhibitor, optionally selected from the group comprising darunavir, atazanavir, indinavir, lopinavir, ritonavir, and saquinavir.

11. The kit according to embodiments 9 or 10, wherein the at least one proteasome inhibitor is a semicarbazone proteasome inhibitor, a structural and/or functional analogue or a derivative thereof, a dipeptidyl-boronic acid derivative, or a pharmaceutically acceptable salt of either.

12. The kit according to any of embodiments 9 to 11, wherein the at least one proteasome inhibitor is S-2209 ([l-[1-[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl]-2-([1H-indol-1-3-yl]-ethylcarbamoyl]-2-([1H-indol])) or PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid, which has the molecular formula C19H25BN4O4).

13. The kit according to any one of embodiments 9 to 12, wherein the proteasome inhibitor is ([l-1-[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl]-2-([1H-indol-1-3-yl]-ethylcarbamoyl]-2-([1H-indol])) and the retrovirus protease inhibitor is darunavir.

14. The kit according to any one of embodiments 9 to 12, wherein the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), which has the molecular formula C19H25BN4O4) and the retrovirus protease inhibitor is darunavir.

15. The kit according to any one of embodiments 9 to 14, further comprising instructions for use (e.g. in paper or electronic form), particularly for use in treating retroviral infections (e.g. HIV infections), said instructions optionally advising the user to first administer the anti-retroviral compound, and subsequently, after a specified time period of delay, the proteasome inhibitor.
16. The kit according to embodiment 15, wherein the anti-retroviral compound is darunavir, and the proteasome inhibitor is S-2209.

17. A proteasome inhibitor and at least one anti-retroviral compound for the simultaneous or sequential use in the combined treatment of a retroviral disease.

18. The proteasome inhibitor and at least one anti-retroviral compound for use as defined in embodiment 17, wherein the anti-retroviral compound is a protease inhibitor.

19. The proteasome inhibitor and at least one anti-retroviral compound for use according to embodiment 15 or 16, wherein the proteasome inhibitor is a semicarbazone proteasome inhibitor, a structural and/or functional analogue or a derivative thereof, a dipeptidyl-boronic acid derivative, or a pharmaceutically acceptable salt of either.

20. The proteasome inhibitor and at least one anti-retroviral compound for use according to any one of embodiments 15 to 17, wherein the proteasome inhibitor is S-2209 ([I-1-[1-[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenylethylcarbamoyl]-2-(IH-indol-3-yl)-ethylcarbamoyl]-2-(IH-indol)]) or N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid.

21. The proteasome inhibitor and at least one anti-retroviral compound for use according to any of embodiments 17 to 20, wherein the at least one anti-retroviral compound is a retrovirus protease inhibitor.

22. The proteasome inhibitor and at least one anti-retroviral compound for use according to any of embodiments 17 to 21, wherein the retrovirus protease inhibitor
is selected from the group comprising darunavir, atazanavir, indinavir, lopinavir, ritonavir, and saquinavir.

23. The proteasome inhibitor and at least one anti-retroviral compound for use according to any of embodiments 17 to 22, wherein the proteasome inhibitor is ([1-1-[1-[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl]-2-(1H-indol-3-yl)-ethylcarbamoyl]-2-(1H-indol))] and the retrovirus protease inhibitor is darunavir.

24. The proteasome inhibitor and at least one anti-retroviral compound for use according to any of embodiments 17 to 22, wherein the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), which has the molecular formula C19H25BN4O4) and the retrovirus protease inhibitor is darunavir.

25. The proteasome inhibitor and at least one anti-retroviral compound for use according to any of embodiments 17 to 24, wherein the retroviral disease is an HIV-related disease.

26. The pharmaceutical composition for use according to any of embodiments 1 to 8 or the proteasome inhibitor and at least one anti-retroviral compound as defined in the above embodiments 17 to 25 for the treatment of retrovirus, e.g. HIV-, -positive patients or for the prevention of an infection and/or HIV-related diseases in individuals suspected to be infected with HIV, and/or for the treatment of HIV-infected individuals that are non-responsive (refractory due to the development of resistances) to at least one anti-retroviral compound.

27. The pharmaceutical composition for use according to any of the preceding embodiments 1 to 8 or the proteasome inhibitor and at least one anti-retroviral compound as defined in the above embodiments 17 to 24, wherein the anti-retroviral
compound administered first and the proteasome inhibitor is administered subsequently with a delay.

28. A method of treatment or prevention of a retrovirus infection or diseases relating to said infection with a proteasome inhibitor and at least one anti-retroviral compound as defined in the preceding embodiments, and/or with a pharmaceutical composition comprising a proteasome inhibitor and a pharmaceutical composition comprising at least one anti-retroviral compound as disclosed herein, said method comprising administration of said proteasome inhibitor and at least one anti-retroviral compound and/or with said pharmaceutical composition comprising a proteasome inhibitor and said pharmaceutical composition comprising at least one anti-retroviral compound to a patient selected from one of the following groups of patients:

(i) Those suspected to be infected with a retrovirus, such as HIV;
(ii) Those diagnosed as being infected with a retrovirus, such as HIV, but showing no disease symptoms;
(iii) Those infected with a retrovirus, such as HIV, and suffering from a retroviral disease, e.g. an HIV-related disease;
(iv) Those being retrovirus-positive (e.g. HIV-positive), previously treated with an anti-retroviral compound, but non-responsive to said at least one anti-retroviral compound (also referred to as a refractory or resistant patient).
(v) Those infected with a retrovirus, such as HIV, and not eligible for standard therapy of HIV-related disease;

29. In further embodiments of the kits in embodiments 9 to 16, said kits comprise instructions for use advising an administration of the at least one proteasome inhibitor and at least one anti-retroviral compound to a patient in need of treatment for an HIV-related disease, wherein the dose of at least one of the compounds administered is lower than otherwise recommended doses of that compound for such
patients. Whenever said instructions concern an anti-retroviral compound, the dose of said anti-retroviral compound is lower than that used either in a monotherapy or as part of a standard therapy for HIV-infection (whichever is the lower); alternatively, whenever said instructions concern a proteasome inhibitor, also for administration without anti-retroviral compound, the dose of said proteasome inhibitor is lower than any other recommended dose of said proteasome inhibitor.

In the context of the present invention, a pharmaceutical composition is a composition comprising at least one active ingredient, e.g. at least one proteasome inhibitor and/or at least one anti-retroviral compound. Optionally, the pharmaceutical compositions of the present invention further comprise a pharmaceutically acceptable carrier. Unless specified otherwise, in the embodiments of the present invention the at least one proteasome inhibitor and the at least one anti-retroviral compound may be present together in one pharmaceutical composition. Alternatively, the above compounds may be present in separate compositions.

Separate pharmaceutical compositions may be administered in parallel, i.e. at about the same point in time, or subsequently, wherein either the pharmaceutical compositions(s) comprising the at least one anti-retroviral compound or the pharmaceutical composition(s) comprising the at least one proteasome inhibitor may be administered first, and the respective other pharmaceutical compositions subsequently.

It will be understood by the skilled person that, wherever there is mention herein of a pharmaceutical composition which may be provided according to the invention, such pharmaceutical composition may be provided in a container. Where there is mention of more than one pharmaceutical composition, these compositions may be provided in one container, or they may be provided in separate containers, e.g. one container for each pharmaceutical composition provided. Embodiments wherein different pharmaceutical compositions are provided in separate containers constitute preferred
embodiments of the instant invention. However, nothing herein shall be interpreted as indicating that separate containers may not be packaged together to make up but a single product, including for manufacturing and/or sales purposes.

In one embodiment of the present invention, at least one anti-retroviral compound is first administered and at least one proteasome inhibitor is administered subsequently. In a particular embodiment, the anti-retroviral compound is Darunavir and the proteasome inhibitor is S-2209, a derivative or analog thereof, or a pharmaceutically acceptable salt thereof. In a further particular embodiment, the anti-retroviral compound is Darunavir and the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), a derivative or analog thereof, or a pharmaceutically acceptable salt thereof. In view of the experimental data presented herein, this embodiment is among the presently preferred variations of the invention.

In another embodiment of the present invention, the at least one proteasome inhibitor is first administered and the anti-retroviral compound is administered subsequently. In a particular embodiment, the anti-retroviral compound is Darunavir and the proteasome inhibitor is S-2209, a derivative or analog thereof, or a pharmaceutically acceptable salt thereof. In a further particular embodiment, the anti-retroviral compound is Darunavir and the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), a derivative or analog thereof, or a pharmaceutically acceptable salt thereof.

In further preferred embodiments, the at least one proteasome inhibitor and the at least one anti-retroviral compound may act synergistically, i.e. the effect of a combination of both compounds, e.g. as measured by the IC$_{50}$ value for the combination, is more pronounced than could be expected from the sum of effects of the same compounds used separately. In an alternative embodiment, a synergistic effect is achieved already when at least two proteasome inhibitors are combined, e.g.
S-2209 and PS-341, but it may be even more pronounced in the presence of a further anti-retroviral compound.

The present invention in one aspect relates to a kit comprising the proteasome inhibitors and anti-retroviral compounds herein described or pharmaceutical compositions comprising the same, wherein:

a) at least one pharmaceutical composition comprises at least one proteasome inhibitor;

b) at least one further pharmaceutical composition comprises at least one pharmacetically active agent in use against retroviral infections, in particular against anti-retroviral compounds used in standard therapy for HIV infection.

The at least one proteasome inhibitor(s) may be any of the herein below described proteasome inhibitors. Similarly, the at least one or more pharmacetically active agent(s) in use against retroviral infections may be any of the herein below described anti-retroviral compounds, particularly those anti-retroviral compounds used in standard therapy for HIV infection. Where, indeed, more than one proteasome inhibitor or more than one anti-retroviral compound is present in said kit, the proteasome inhibitors and/or anti-retroviral compound may be different, or, alternatively, they may be identical, but the compositions may be different, e.g. comprise different excipients that allow for varying release rates of the respective ingredients.

In another embodiment the present invention relates to a pharmaceutical composition for use in the treatment or prevention of a retrovirus infection comprising:

a) at least one proteasome inhibitor, and

b) at least one anti-retroviral compound, in particular an anti-retroviral compound used in standard therapy for HIV infection.
In another embodiment, the present invention relates to at least one proteasome inhibitor and at least one anti-retroviral compound described herein below, in particular anti-retroviral compounds used in standard therapy for HIV infection, for use in the treatment of human or animal individuals which do not respond or are refractory to treatment with an anti-retroviral compound, and preferably with (one of) the anti-retroviral compound(s) of said at least one anti-retroviral compound. In a further aspect said at least one proteasome inhibitor and at least one anti-retroviral compound for said use is administered to an individual which does not respond to or is refractory to treatment with an anti-retroviral compound, or a patient that is infected/suspected to be infected with a retrovirus, wherein said at least one anti-retroviral compound is administered before, together with or subsequent to said at least one proteasome inhibitor.

In another embodiment the present invention relates to at least one proteasome inhibitor together with at least one anti-retroviral compound for use in treating patients which do not respond to or are refractory to treatment with an anti-retroviral compound, used in the treatment of retroviral diseases, particularly HIV-related diseases.

In one embodiment of the present invention, the at least one anti-retroviral compound or the pharmaceutical composition comprising the at least one anti-retroviral compound is first administered and the at least one proteasome inhibitor or the pharmaceutical composition comprising the at least one proteasome inhibitor is administered subsequently. In a particular embodiment, the anti-retroviral compound is Darunavir and the proteasome inhibitor is S-2209, a derivative or analog thereof, or a pharmaceutically acceptable salt thereof. In a further particular embodiment, the anti-retroviral compound is Darunavir and the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), a derivative or analog thereof, or a pharmaceutically acceptable salt thereof. In view of the
In another embodiment of the present invention, the at least one proteasome inhibitor or the pharmaceutical composition comprising the at least one proteasome inhibitor is first administered and the at least one anti-retroviral compound or the pharmaceutical composition comprising the at least one anti-retroviral compound is administered subsequently. In a particular embodiment, the anti-retroviral compound is Darunavir and the proteasome inhibitor is S-2209, a derivative or analog thereof, or a pharmaceutically acceptable salt thereof. In a further particular embodiment, the anti-retroviral compound is Darunavir and the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), a derivative or analog thereof, or a pharmaceutically acceptable salt thereof.

In further preferred embodiments the at least one proteasome inhibitor or the pharmaceutical composition comprising the at least one proteasome inhibitor and the at least one anti-retroviral compound or the pharmaceutical composition comprising the at least one anti-retroviral compound upon administration may result in a synergistic effect, i.e. the effect of both compounds present in the respective pharmaceutical compositions, e.g. the IC$_{50}$ value, is more pronounced than the sum of effects of separately used compounds.

Yet another embodiment relates to a method of treating human or animal individuals which do not respond or are refractory to treatment with a pharmaceutically active agent in use against retroviral infections comprising the steps of administering to such individuals first a pharmaceutical composition comprising at least one proteasome inhibitor and subsequent thereto a pharmaceutical composition comprising at least one pharmaceutically active agent in use against retroviral infections. In a currently preferred embodiment of said method of treating human or animal individuals, a pharmaceutical composition comprising the anti-retroviral
compound may be administered first and the proteasome inhibitor containing composition may be administered subsequently.

The methods of the present invention and the uses of the present invention of at least one proteasome inhibitor together with at least one anti-retroviral compound are also intended for treating patients that have previously not been treated with anti-retroviral medicaments. These patients may be patients that have recently been diagnosed as retrovirus-infected, including those patients that do not show any disease symptoms. Thus, the uses, compositions and methods as well as the kits described therein may be used as a first line pharmaceutical defense, e.g. the compositions and methods are used for the prevention of disease and the prevention of development of resistances against anti-retroviral compounds. This applies also to preferred administration orders of the active compounds described herein and pharmaceutical compositions comprising the same.

The methods of treatment in accordance with the invention are practiced on human or animal individuals who are in need of such treatment (sometimes referred to herein as "patients"). These may be individuals suffering from retroviral infections and/or diseases. Preferably such an infection is an HIV-infection, and the disease may be a disorder commonly attributed to AIDS. The animals may be mammals, e.g. cats, monkeys, dogs, sheep etc.

In all of the above-mentioned embodiments, the retroviral infection may be an HIV infection, wherein HIV may stand for, e.g. either or both of HIV-1 and/or HIV-2.

All known proteasome inhibitors may be used in the context of practicing the instant invention. These include, without limitation:

- naturally-occurring proteasome inhibitors e.g. epoxomicine, eponemicin, aclacinomycin A (also known as aclarubicin), lactacystin and its modified variants, e.g. clasto-lactacystin β-lactone;
- synthetically prepared proteasome inhibitors (e.g. modified peptide aldehydes, such as N-carbobenzoxy-L-leucinyl-L-leucinyl-L-leucinal, sometimes referred to as MG132 or zLLL, its boronic acid derivative MG232, N-carbobenzoxy-Leu-Leu-Nva-H, sometimes referred to as MG1 15), N-acetyl-L-leuzinyl-L-leuzinyl-L-norleuzinal, sometimes referred to as LLnL), N-carbobenzoxy-Ile-Glu(Obut)-Ala-Leu-H, sometimes referred to as PSI; synthetic peptides that carry at their C-terminal end an α, β-epoxyketone group vinyl-sulphone group, e.g. carboxbenzoxyl-L-leucinyl-L-leucinyl-L-leucine-vinyl sulfone or 4-hydroxy-5-iodo-3-nitrophenylacetyl-L-leucinyl-L-leucinyl-L-leucin-vinyl-sulfone, also referred to as NLVS), a glyoxal or boronic acid residue, or Benzoyl(Bz)-Phe-boroLeu, Ph-acetyl-Leu-Leu-boroLeu, Cbz-Phe-boroLeu, or a pinacol-ester group, e.g. benzyloxycarbonyl(CbZ)-Leu-Leu-boroLeu-pinacol-ester; and chemically modified derivatives of naturally occurring proteasome inhibitors, such as the β-lacton derivative PS-519 (1R-[1S, 4R, 5S]]-l-(l-hydroxy-2-methylpropyl)-4-propyl-6-oxa-2-azabicyclo[3.2.0]heptanes-3,7-dione, (molecular formula: C12H19NO4), a derivative of lactacystine;
- dipeptidyl-boronic acid derivatives such as PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid, molecular formula: C19H25BN4O4).

Suitable proteasome inhibitors thus include: PS-341; also commonly known as Bortezomib, and the active ingredient in the pharmaceutical preparation sold under the trade name Velcade®, in use for the treatment of multiple myeloma), "PS-273"
and its enantiomer "PS-293", "PS-296" (8-quinolyl-sulfonyl-CONH-(CH-naphthyl)-CONH-(CH-isobutyl)-B(OH)\textsubscript{2}); "PS-303" (NH\textsubscript{2}(CH-Napthyl)-CONH-(CH-isobutyl)-B(OH)\textsubscript{2}); "PS-321" (morpholino-CONH-(CH-napthyl)-CONH-(CH-phenylalanine)-B(OH)\textsubscript{2}); "PS-325" (2-quinol-CONH-(CH-homo-phenylalanin)-CONH-(CH-isobutyl)-B(OH)\textsubscript{2}); "PS-352" (Phenyalanine-CH\textsubscript{2}-CH\textsubscript{2}-CONH-(CH-phenylalanine)-B(OH)\textsubscript{2}); "PS-383" (pyridyl-CONH-(CH/\textsubscript{2}F-phenylalanin)-CONH-(CH-isobutyl)-B(OH)\textsubscript{2}).

Yet further suitable proteasome inhibitors include (Huang, L., Chen, CH, Current Medicinal Chemistry, 2009, 16:931): CEP1612, a dipeptide aldehyde proteasome inhibitor that is highly selective for the chymotrypsin-like proteolytic activity of the proteasome; ZLVS (ZLLL-vs) and YLVS (YLLL-vs), further examples of vinyl sulfones (herein, -vs is used as shorthand for a vinyl sulfone group); MG-262, a boronate analog of MG132, which exhibits a 100-fold increase in anti-proteasome activity compared to its parent compound; Tyropeptin A, a tripeptide aldehyde natural product isolated from Kitasatospora sp. MK993-dF2, preferentially inhibiting the chymotrypsin-like proteasome activity by binding to the β5 subunit of the proteasome (Momose, I.; et al, J. Antibiotics 2001, 54:997; Momose, I., et al, Bioorg. Med. Chem. Lett. 2005, 15:1867); Peptide epoxyketones, isolated from various microbes, are small peptides with a ketone epoxide functional group; for example, epoxomycin was derived from Streptomyces hygroscopicus (Hanada, M., et al, J. Antibiot. (Tokyo) 1992, 45:1746), TMC-86 and TMC-89 were isolated from Streptomyces sp. (Koguchi, Y., et al, J. Antibiot. (Tokyo) 2000, 53:63; Koguchi, Y, et al, J. Antibiot. (Tokyo) 2000, 53:967); peptide epoxyketones inhibit the proteasome by covalently modifying the catalytic sites of the β subunits; Carfilzomib (PR-171), an epoxyketone peptide structurally related to epoxomycin, is in Phase 2 clinical trials for patients with relapsed solid tumors including non-small cell lung cancer, small cell lung cancer, ovarian cancer, and renal cancer (Kuhn, DJ, et al., Blood 2007, 110:3281); it is also in a phase 2 single-agent trial for patients with multiple myeloma and in a phase 1 study for lymphoma patients; some peptide epoxyketone derivatives, such as dihydroepomemycin analogs, were shown to preferentially target the immunoproteasome (Ho, Y.K., et al., Chem. Biol. 2007, 14:419); PR39 is a naturally occurring antibacterial peptide comprising 39 amino acid residues isolated from pig intestine, and was shown to inhibit the proteasome; unlike small tripeptide proteasome inhibitors that bind to the proteolytic active site located at β5 subunit, PR39 binds to the non-proteolytic β7 subunit of the 20S proteasome. PR11 (first 11 residues of PR39 sequence: RRRPRPPYLP) and its analogs exhibit similar activity to that of PR39; Inhibition of the proteasome by
PR1 1 and PR39 results in accumulation of ΙκΒ, a factor that regulates the NF-KB-dependent gene expression pathways; natural products derived from plant sources, such as celastrol, isolated from the traditional herbal medicine "Thunder-god vine", and withaferin A, isolated from Indian winter cherry, which were shown to inhibit the proteasome at low micromolar concentrations (Celastrol is a triterpene and withaferin A is structurally related to steroids); Gliotoxin, a fungal metabolite structurally related to the epipolythiodioxo-piperazines; green tea polyphenolic catechins such as (-)-epigallocatechin-3-gallate {(-)-EGCG} and its analogs have been widely studied for their possible benefits in cancer prevention; EGCG was reported to potently inhibit the chymotrypsin-like activity of the proteasome in vitro and in cultured tumor cells; Disulfiram, a drug for the treatment of alcohol dependence, was shown to inhibit the proteasome; certain acridine derivatives, a class of anti-cancer agents primarily targeting DNA and topoisomerase II, also having proteasome inhibiting activity, e.g. tetra-acridine; certain derivatives of betulinic acid, e.g. 3',3'-dimethylsuccinyl betulinic acid; in contrast to BA a proteasome activator, many BA derivatives inhibit the proteasome; similarly, certain derivatives of glycyrrhetinic acid (GLA) may be potent inhibitors of the proteasome, and such inhibitors are envisaged by the present invention.

Yet further suitable proteasome inhibitors may include: NEOSH-101, also known as OSH-101, a tetrapeptide aldehyde in clinical trials for androgenetic alopecia; CEP-18770, a P2 threonine boronic acid derivative under development for, e.g., multiple myeloma; IPSI001, IPSI007, as well as MLN2238 and its prodrug MLN9708, under development for indications in oncology by Millennium Pharmaceuticals/Takeda; ONX 0912 (formerly PR-047 (1) by Proteolix, Inc.; Peese, K., Drug Discovery
Today 2009, 14:905), a proteasome inhibitor based on the same novel chemistry as carfilzomib, and ONX 0914 (formerly PR-957; Muchamuel, T., et al., Nature Medicine 2009, 15:7) an inhibitor of the immunoproteasome, both being developed by Onyx Pharmaceuticals; AA-102, an anti-cancer agent being developed by Bionovo, Inc., 26 SPI, a proteasome inhibitor being developed by Ergon Pharmaceuticals for oncology and other indications; AVR-147, a development candidate by Advanced Viral Research, Corp., in oncology; BU-32 (pyrazy 1,2,5-bis-CONH(CHPhe)CONH(CHisobutyl)-B(OH)_2, NSC D750499-S; Aygin, JK, et al., Breast Cancer Res. 2009, 11:R74), 4E12, a non-peptidyl small molecule proteasome inhibitor identified by Telik, Inc., and intended for development in oncology; and Compound 13 and Compound 20 (Purandare, AS, et al, Am. Assoc. Cancer Res. Annual Meeting 2007, 98th: April 15, Abs. 717), two lactam boronic acid proteasome inhibitors having high activity (low nM IC_{50} values) as well as high specificity (>100 fold selective against chymotrypsin, trypsin, elastase and Factors Xa, Xia and Vila).

Yet further suitable proteasome inhibitors may include: ALLnL, ALLnM; LLnV; DFLB (dansyl-Phe-Leu-boronate); Ada-(Ahx)3-(Leu) 3-vs; YU101 (Ac-hFLFL-ex), MLN519, and the semicarbazone S-2209 (Baumann et al., Brit. J. Haematology 144:875-886, 2009) as well as its structural analogues Compound 1-6 and Compound 8 (Leban, J., et al., Bioorg. Med. Chem. 2008, 16:4579). Moreover, a combination of S-2209 or a structurally related compound as proteasome inhibitor with a further
anti-(retro)viral agent in the compositions, uses, kits, and/or methods of the invention is particularly suitable embodiment in the context of the present invention. In a very preferred embodiment of the present invention, S-2209 or an analogue is used in combination with Darunavir or an analog thereof. This combination is intended with respect to methods, compositions, methods, uses and/or kits disclosed within the context of the present invention.

Proteasome inhibitors may further include di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-peptide aldehydes or peptide aldehydes having ten or more amino acids such as 15, 20, 30, 40 or more amino acids.

Such di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-peptide aldehydes or peptide aldehydes having ten or more amino acids may carry at their C-terminus and a,B-epoxyketone functionality, a vinyl-sulphone functionality, a glyoxal functionality, a boronic acid functionality, a pinacol ester functionality or other functionalities.

Such di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-peptide aldehydes or peptide aldehydes having ten or more amino acids may comprise natural or non-natural amino acids.

Such di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-peptide aldehydes or peptide aldehydes having ten or more amino acids may also be chemically modified by hydrogenation, dehydrogenation, hydroxylation, dehydroxylation, acylation, deacylation, alkylation, dealkylation, pegylation, hesylation, glycosylation and the like.

Such di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-peptide aldehydes or peptide aldehydes having ten or more amino acids and which optionally may carry a,B-epoxyketone functionality, a vinyl-sulphone functionality, a glyoxal functionality, a
boronic acid functionality, a pinacol ester functionality or other functionalities at their C-terminus may also may comprise natural or non-natural amino acids.

Such di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-peptide aldehydes or peptide aldehydes having ten or more amino acids and which optionally may carry a,B-epoxyketone functionality, a vinyl-sulphone functionality, a glyoxal functionality, a boronic acid functionality, pinacol ester functionality or other functionalities at their C-terminus may also be further chemically modified by hydrogenation, dehydrogenation, hydroxylation, dehydroxylation, acylation, deacylation, alkylation, dealkylation, pegylation, hesylation, glycosylation and the like; and may comprise natural or non-natural amino acids.

Anti-retroviral compounds to be combined with (a) proteasome inhibitor(s) according to the invention include, but are not limited to, HIV protease inhibitors, HIV reverse transcriptase inhibitors, HIV integrase inhibitors, inhibitors of HIV TAT, budding/maturation inhibitors, entry inhibitors, including fusion inhibitors, inhibitors of the CD4 receptor, inhibitors of the CCR5 co-receptor and inhibitors of the CXCR4 coreceptor.

Reverse transcriptase inhibitors include, but are not limited to BCH-189, AzdU, carbovir, ddA, d4C, d4T (stavudine), 3TC (lamivudine), DP-AZT, FLT (fluorothymidine), BCH-189, 5-halo-3'-thia-dideoxycytidine, PMEA, bis-POMPMEA, Retrovir (AZT), zidovudine (AZT), MSA-300, troviridine, R82193, L-697,661, R82150, U-87201E, amdoxovir, alovudine, cidoivir, ribavarin, viramidine, vidarabine, cytarabine, idoxuridine, trifluridine, valopitacabine, BIT225, R1728, BE-868554, ITMN-5489, SP754, capavirine, emivirine, calanolide A, GW5634, BMS-56190 (DPC-083), DPC-961, MTV-150, MTV-160, MIV-210, MIV-310, MIV-410, alovudine (FLT), zalcitabine (ddC), didanosine (ddl), abacavir, ABC, emtricitabine (FTC), racivir (racemic FTC), adefovir (ADV), entecavir (BMS 30 200475), alovudine (FLT), tenofovir (TNF), amdoxavir (DAPD), D-d4FC (DPC-8 17), -dOTC
(SPD754), elvucitabine (ACH-126443), BCH 10681, SPD-756, D-FDOC, GS7340, INK-20 (thioether phospholipid AZT), 2'3'-dideoxy-3'-fluoroguanosine (FLG) & its prodrugs such as MIV-210 and reverse (RVT, D-D4FC, DPC-817), delavirdine, efavirenz (DMP-266), (+)calanolide A and B, capravirine (AG1549f S-1 153), GW-695634 (GW-8248), MV026048 (R-1495), NV-05 2 2, R-278474, RS-1588, UC-781, YM215389, RDEA900, CMX157, Festinavir, APD ([]-beta-d-2-Aminopurine Dioxolane), DXG (2'-Deoxyguanosine analog), Fosalvudine Tidoxil / HDP 99.0003, KP-1461, KP-1212, 3-fluoro-2,3-Dideoxyguanosine (FLG), Amdoxovir (DAPD), Apricitabine, Hydroxyurea, VS41 1, Stavudine, Aciclovir / Acyclovir, Lamivir, Lamivir-S, Truvada, Zidovudine Transdrug, Dexelvucitabine, RDEA640, IDX-989, RDEA806, IDX899, UK-453.061, Dapivirine (TMC-120), Etravirine (TMC125, R-165335), Rilpivirine (TMC-278), Nevirapine (BI-RG-587), Cipla-Nevirapine, RDEA427, Non-Nucleoside Reverse Transcriptase Inhibitor NUMERATE, oxetanocin, oxetanocin-G, BV ara-U, penciclovir, L-697,639 and the like.


HIV integrase inhibitors include, but are not limited to, S-l 360, zintevir (AR-177), L-870812, L-870810, L-c-2507 and S(RSC)-1838 , TG-10, INH001, S-247303, S-265744, S-349572, Raltegravir, Elvitegravir, HIV Integrase Inhibitors by AMBRILIA (e.g. AMBRILIA Compound 1, AMBRILIA Compound 2, AMBRILIA Compound 3), HIV Integrase Inhibitors by AVEXA, HIV Integrase Inhibitors by
BIOALLIANCE (e.g. derivatives of styrylquinolines), HIV Integrase Inhibitors by CRITICAL OUTCOME, HIV Integrase Inhibitors by VIROCHEM, and the like

TAT inhibitors include, for example, RO-24-7429 and the like.

HIV budding/maturation inhibitors include, but are not limited to, PA-457 and the like.

HIV entry/fusion inhibitors include, but are not limited to, AMD-070 (AMD 11070), BlockAide/CR, BMS 806 (BMS-378806), Enfurvirtide (T-20, R698, Fuzeon), KRH1636, ONO-4128 (GW-873140, AK-602, E-913), PRO-140, PRO-542, Schering C (SCH-C), SCH-D (SCH-417690), T-1249 (R724), TAK-220, TNX-355 and UK 427,857, PRO 2000, AMD-3100, Peptide T, FP21399 and the like.

CCR5 antagonists include, but are not limited to: TAK-779, Vicriviroc (SCH-417690), Aplaviroc (GW871340), Maraviroc, INCB9471, PF-232798, and Compounds 1 and 3-96 as disclosed in Yang, H., Expert opinion on therapeutic patents 2010, 20:325

Other anti-retroviral compounds to be administered in combination with at least one proteasome inhibitor according to the present invention may include AL-721, polymannoacetate, HPA-23, trisodium phosphonoformate, foscarinet, eflophidine, Reticulose, UA001, cylobut-G, cyclobut-A, ara-M, BW882C87, BW256U87, L-693,989, FIAC, HOE-602, ganciclovir, rCD4/CD4-IgG, CD4-PE40, butyl-DNJ, oxamyristic acid, and dextran sulfate.

It is also possible to administer one or more compounds of the combination of compounds according to the invention, e.g. one or more anti-retroviral compound, and/or one or more proteasome inhibitor, in combination with ritonavir. Such a
combination is especially useful for inhibiting or treating an HIV-related disease in a human.

When administered in combination with a compound, ritonavir may cause an improvement in the pharmacokinetics (i.e., increases half-life, increases the time to peak plasma concentration, increases blood levels) of such compound, e.g. of one or more anti-retroviral compound, and/or of one or more proteasome inhibitor.

It will be understood that anti-retroviral compounds which can be combined with (a) proteasome inhibitor(s) according to the instant invention for the inhibition, treatment or prophylaxis of HIV-related diseases are not limited to those listed above, but include in principle any agents useful for the treatment or prophylaxis of an HIV-related disease.

Retrovirus treatment

The main focus of retroviral therapy currently relates to the treatment of HIV-infected individuals.

Other currently known retrovirus infections in humans are, e.g. infections with HTLV-I causing blood cancer. The infection with HTLV-I is believed to occur from mother to child. HTLV-I infected individuals are frequently treated with anti-cancer drugs, but the treatment with the nucleoside analogue reverse transcriptase inhibitor azidothymide and cyclophosphamide, hydroxydaunorubicin (doxorubicin), Oncovin (vincristine), and prednisone/prednisolone has also been attempted (Taylor GP, Matsuoka M (September 2005), *Oncogene* 24 (39): 6047-57).

In HIV-infections, current treatment frequently consists of "Highly-Active-Anti-Retroviral-Activity" or HAART. This has been highly beneficial to many HIV-infected individuals since its introduction in 1996 when the protease inhibitor-based
HAART initially became available (Palella FJ Jr, et al, (1998), N. Engl. J. Med 338 (13): 853-860). Current optimal HAART options consist of combinations consisting of at least three anti-retroviral compounds belonging to at least two types, or classes, of anti-retroviral compounds. Typical regimens consist of two "Nucleoside analogue reverse transcriptase inhibitor" (NARTIs or NRTIs) plus either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI). Standard goals of HAART include improvement in the patient's quality of life, reduction in complications, and reduction of HIV viremia below the limit of detection, but it does not cure the patient of HIV nor does it prevent the return, once treatment is stopped, of high blood levels of HIV, often then HAART resistant.

For some patients, which can be more than fifty percent of patients, HAART achieves far less than optimal results, due to medication intolerance/side effects, prior ineffective antiretroviral therapy and infection with a anti-retroviral compound-resistant strain of HIV. Non-adherence and non-persistence with therapy are the major reasons why some people do not benefit from HAART. The reasons for non-adherence and non-persistence are varied and include poor access to medical care, inadequate social support, psychiatric disease and drug abuse. HAART regimens can also be complex and thus hard to follow, with large numbers of pills taken frequently.

A huge number of anti-retroviral compounds has been authorized in various countries or is clinically investigated. These compounds may be broadly classified by the phase of the retrovirus life-cycle that is inhibited:

- Reverse transcriptase inhibitors that inhibit reverse transcription by being incorporated into the newly synthesized viral DNA and preventing its further elongation.
Reverse transcriptase inhibitors that inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function.

Protease inhibitors target viral assembly by inhibiting the activity of an enzyme used by HIV to cleave nascent proteins for final assembly of new virions.

Integrase inhibitors that inhibit the enzyme integrase, which is responsible for integration of viral DNA into the DNA of the infected cell. There are several integrase inhibitors currently under clinical trial, and raltegravir became the first to receive FDA approval in October 2007.

Entry inhibitors (or fusion inhibitors) interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets. Maraviroc and enfuvirtide are the two currently available agents in this class.

Maturation inhibitors inhibit the last step in gag processing in which the viral capsid polyprotein is cleaved, thereby blocking the conversion of the polyprotein into the mature capsid protein (p24). Because these viral particles have a defective core, the virions released consist mainly of non-infectious particles. There are no drugs in this class currently available, though two are under investigation, bevirimat and Vivecon.

AV-HALTs (Antiviral HyperActivation Limiting Therapeutics or 'virostatics') combine immunomodulating and antiviral properties to inhibit a specific antiviral target while also limiting the hyper-elevated state of immune system activation driving disease progression.
Some natural antivirals, such as extracts from certain species of mushrooms like Shiitake and Oyster mushrooms, may contain multiple pharmacologically active compounds, which inhibit the virus at various different stages in its life cycle. Researchers have also isolated a protease inhibitor from the Shiitake mushroom.

A patient which does not respond to anti-retroviral treatment, and in particular to HIV treatment, may be designated as a "ηοη-responder" or "therapy resistant" patient. The terms "therapy" and "treatment" respectively, may be used interchangeably.

Resistances may not only be determined in clinical trials but also in cell culture tests in vitro. For example, the antiviral activity of a drug in cell culture systems may be determined using the inhibition of replication as a read-out parameter. The concentration of a compound under investigation to inhibit virus replication by 50 percent (EC$_{50}$ for cell-based assays; IC$_{50}$ for biochemical or subcellular assays) should be determined. A large number of tools to determine this value are known to the person skilled in the art. A well-characterized wild-type (WT) HIV laboratory strain should serve as a reference standard. Other parameters that may be used to determine the efficiency of a compound on HIV replication as a measure for the development of resistances are for example cytotoxicity and therapeutic indices, protein binding assays to human serum proteins, genotypic and phenotypic assays using, for example, nucleic acid sequencing methodology to determine mutations that have evolved in viruses under investigation, and standard virus assays, such as p24, viral RNA, RT assay, MTT cytotoxic assay and reporter gene expression assays.

Anti-retroviral compounds or combination products comprising the same that are currently used in the treatment of HIV are e.g. the multi-class combination product comprising efavirenz, emtricitabine and tenovir disoproxil fumarate; Nucleoside Reverse Transcriptase Inhibitors (NRTIs) such as lamivudine, zidovudine,
emtricitabine, lamivudine, abacavir, zalcitabine, dideoxycytidine, azidothymidine, enteric coated didanosine, stavudine, abacavir sulfate; Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs) such as etravirine, delavirdine, efavirenz, nevirapine; Protease Inhibitors such as amprenavir, tipranavir, indinavir, saquinavir, saquinavir mesylate, lopinavir, ritonavir, fosamprenavir calcium, darunavir, atazanavir sulfate, nelfinavir mesylate; Fusion Inhibitors such as enfuvirtide; Entry inhibitors/CCR5 co-receptor antagonists such as maraviroc; HIV integrase strand transfer inhibitors such as raltegravir. Other anti-retroviral compounds that are currently tested include monoclonal antibodies and fragments thereof, etc. Any of these pharmaceutical compounds or combination products may be used in combination with the proteasome inhibitors described above. A preferred combination comprises methods, kits, compositions, uses etc., as described above which involve S-2209 or analogues thereof and/or PS-341 or related molecules as proteasome inhibitor(s) and darunavir or related active agents as a further anti-retroviral compound.

HIV resistances are mainly due to its high rate of replication (often of a magnitude of $10^9$ to $10^{10}$ virions per person per day) and error-prone polymerase. Therefore, HIV can easily develop mutations that alter susceptibility to anti-retroviral compounds and/or combination products. As a result, the emergence of resistance to one or more anti-retroviral compound and/or combination product is one reason for therapeutic failure in the treatment of HIV. In addition, the emergence of resistance to one anti-retroviral compound sometimes confers a reduction in or a loss of susceptibility to other or all pharmaceutical agents of the same class (U.S. Dept. of Health and Human Services, FDA, CDER, 2007, in "Guidance for Industry - Role of HIV resistance testing in antiretroviral drug development). The present invention provides a new strategy to fight against retroviral, e.g. HIV-infections. In one embodiment the combined therapy presented herein is particularly well-suited to fight against the development of resistances, or may be used in patients that have
already developed resistances to one or more of the above described anti-retroviral therapies.

The present invention in one embodiment relates to a kit of compounds or pharmaceutical compositions comprising:

a) at least one first pharmaceutical composition comprising at least one proteasome inhibitor;

b) at least one second pharmaceutical composition comprising at least one pharmaceutically active agent in use against retroviral infections, particularly further pharmaceutical compositions comprising more than one anti-retroviral compound, e.g. those used in standard therapy for HIV infection.

In another embodiment the present invention relates to a pharmaceutical composition comprising:

a) at least one proteasome inhibitor;

b) at least one pharmaceutically active agent in use against retroviral infections, particularly more than one anti-retroviral compound such as those used in standard therapy for HIV infection.

In these embodiments specific proteasome inhibitors such as S-2209, PS-519, PS-341 (Bortezomib) and PS-273, and any of the above-mentioned anti-retroviral/anti-HIV compounds and/or combination products comprising the same may be used.

The pharmaceutically active agent that is not a proteasome inhibitor in use against retroviral infections may be one that, for example, may stimulate or assist the body's own functions, and particularly the body's natural defenses, more specifically the natural defenses against viruses, and/or prevent or limit the capacities of retroviruses to efficiently fuse with a target cell, to reverse transcribe, to integrate as a provirus...
into the host genome, to replicate in the infected cell, to maturate to virions in the infected cells and/or to be released as infectious virus.

The rapid development of resistances against single therapeutic compounds in retroviruses, particularly in HIV, necessitates a multi-pronged approach if control, reduction of number, eradication, elimination etc. of the pathologic agent is to be achieved. Therefore, the inventive pharmaceutical composition preferably comprises at least two pharmaceutically active agents in use against retroviral infections, e.g. HIV-infections, more preferably at least 3, and most preferably at least 4 pharmaceutically active agents in use against retroviral infections.

Hence, the inventive pharmaceutical compositions and kits preferably comprise a total of at least 3, more preferably at least 4, yet more preferably at least 5, yet more preferably at least 6, and most preferably at least 7 pharmaceutically active ingredients.

The compounds described herein and the pharmaceutical compositions and kits may be used for treating patients suffering from retroviral infections, in particular from HIV infections, or they may be used to prevent infections or the onset of retrovirus-infection related diseases.

It may be particularly preferred to use the herein described compounds, methods, uses, pharmaceutical compositions and kits for treating patients suffering from retrovirus infections and related diseases, and in particular from HIV infections/AIDS which are considered as "non-responding" or "refractory" patients.

One advantage of the kits in accordance with the present invention is that they comprise the pharmaceutical compositions in separate form, e.g. as different solutions, tablets etc. This may allow for a timely ordered, i.e. subsequent administration of the separate pharmaceutical compositions which can be important
when treating "non-responding" or "refractory" patients suffering e.g. from retroviral infections, e.g. from HIV infections/AIDS.

The kits in accordance with the present invention may thus comprise instructions in paper or electronic form advising the user to first administer a pharmaceutical composition comprising a proteasome inhibitor and to administer at least one further pharmaceutical composition, e.g. a pharmaceutical comprising an anti-retroviral compound, subsequently.

Alternatively, kits in accordance with the present invention may thus comprise instructions in paper or electronic form advising the user to first administer a pharmaceutical composition comprising an anti-retroviral compound, in particular darunavir, and to administer at least one further pharmaceutical composition, in particular a pharmaceutical composition comprising a herein described proteasome inhibitor(s), subsequently.

The instructions may further instruct the user to wait a specified time period, e.g. a delay between administering the first pharmaceutical composition and further pharmaceutical composition(s).

The instructions may thus advise to wait for about 12 h to 24 h, about 24 h to 48 h, about 2 to 4 days, about 4 to 6 days, about 1 week, about 1 to 2 weeks, about 2 to 3 weeks, about 3 to 4 weeks, or more than 4 weeks after treatment with the first to be administered compound or pharmaceutical composition has ended and before commencing treatment with the second and/or third compound or pharmaceutical composition. It is currently preferred to administer the further compounds or compositions after a delay of about 12h to 24 h or after a delay of about 24 h to 48 h.
The instructions may additionally advise that treatment with the proteasome inhibitor may on a three to four daily basis such as e.g. on day 1, 4, 8 and 11, or on day 1, 4, 7, 10 or on day 1, 5, 9 and 13 and the like.

The pharmaceutical compositions may be formulated for oral, subcutaneous, transdermal, rectal, peritoneal or intravenous administration and may contain suitable pharmaceutically acceptable excipients.

In a further embodiment, the kit may comprise instructions to administer at least one proteasome inhibitor and at least one anti-retroviral compound or the pharmaceutical compositions comprising the same, to a human or animal in need of treatment for an HIV-related disease, wherein the dose of at least one of the compounds administered is lower than otherwise recommended doses of that compound for such human or animal when said compound is used either in a monotherapy or in standard therapy for HIV infections for anti-retroviral compounds, whichever is the lower, or when it is used without anti-retroviral compound(s) for proteasome inhibitors. Optionally, and for the case of proteasome inhibitors, said dose is lower by at least a factor of 1/4, by at least a factor of 1/3, or by at least a factor 1/2 than the otherwise recommended doses suggested in the treatment of proliferative diseases as defined in WO 2007/017284 (page 30, lines 1 to 24). Furthermore, for those anti-retroviral compounds having been authorized for marketing in the US, the said dose is optionally lower by at least a factor of 1/4, by at least a factor of 1/3, or by at least a factor 1/2 than the otherwise recommended doses suggested in, for example "Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents" (Panel on Antiretroviral Guidelines for Adults and Adolescents; The U.S. Department of Health and Human Services. Washington, DC, USA, January 10, 2011; pages 1-166).

With the administration of a proteasome inhibitor, it may be possible to advantageously influence even the efficacy of the standard of care treatment, or at
any rate the treatment with said anti-retroviral compound. For example, said anti-
retroviral compound may be administered for a reduced duration compared to the
duration otherwise recommended for the respective patient under the respective
circumstances for the treatment of the retroviral infection with the respective agents,
e.g. a duration of not more than about 95%, 90% 85%, 80%, 75% 70%, 66%, 50%,
40%, 33%, 25%, 15%, or 10%> of the recommended duration. Such shortened
treatment would greatly ease the discomfort felt by patients on therapies of such
duration which are associated with side effects as potentially severe as those
associated with long term standard treatment for HIV-related disease.

In a particularly preferred embodiment, the treatment with said anti-retroviral
compound is continued for only about 50% of the duration of the treatment
recommended without the proteasome inhibitor. In another preferred embodiment,
the treatment with said anti-retroviral compound is continued for only about 25% of
the duration of the treatment recommended without the proteasome inhibitor.

Instead of, or at the same time as, reducing the time of treatment necessary to achieve
the desired effect, the proteasome inhibitor treatment may enable the physician to
reduce the dose of said anti-retroviral compound. For example, the treatment with
said anti-retroviral compound may involve administration of a reduced dose
compared to the dose otherwise recommended for the respective agent in the
treatment of a retroviral infection with the respective anti-retroviral compound for
the respective patient under the respective circumstances, e.g. not more than about
95%, 90% 85%, 80%, 75% 70%, 66%, 50%, 40%, 33%, 25%, 15% or 10% of the
recommended dose. In a particularly preferred embodiment, not more than about
66% of the recommended dose of at least one of the anti-retroviral compound is
administered. In a particularly preferred embodiment, the dose is 66% of the
recommended dose. Such treatment with reduced doses could equally ease the
discomfort felt by patients on such therapy.
While 50% of the recommended duration and 66% of the recommended dose are preferred embodiments, these are primarily directed at the goal of enabling a patient to undergo treatment he or she is otherwise ineligible for, or which is otherwise too unpleasant to undergo. This may be achieved already at lesser, or may need a higher, degree of reduction in the duration and or dose. It is therefore preferred in the context of the instant invention, that a level of dose/duration reduction is established, which leads to a significant improvement in the side effect profile compared to standard therapy for HIV-infection, whereby certain patients previously ineligible for, or unwilling to undergo, standard therapy for HIV-infection, are enabled to undergo an inventive treatment as provided herein.

These limitations may optionally be spelled out in instructions in paper or electronic form which accompany the packaged form of the inventive compositions, kits etc. provided herein, as also further provided for the inventive kits herein.

In another embodiment, the present invention relates to the use of at least one proteasome inhibitor and at least one anti-retroviral compound, preferably more anti-retroviral compounds, e.g. those used in standard therapy for HIV infection, for treating a human or animal individual which does not respond or is refractory to treatment with one or more pharmaceutical agent in use against retroviral infections, wherein:

a) said at least one proteasome inhibitor is first administered to said individual

b) said at least one anti-retroviral compound is administered subsequent to treatment with said at least one proteasome inhibitor.

In another embodiment, the present invention relates to the use of at least one proteasome inhibitor and at least one anti-retroviral compound, preferably more anti-retroviral compounds, e.g. those used in standard therapy for HIV infection, for treating a human or animal individual which does not respond or are refractory to
treatment with one or more pharmaceutical agent in use against retroviral infections, wherein:

i) said at least one anti-retroviral compound is first administered to said individual;

ii) said at least one proteasome inhibitor is administered subsequent to treatment with said at least one anti-retroviral compound.

In these embodiments it can be preferred to use proteasome inhibitors such as S-2209, PS-519, PS-341 (Bortezomib) and PS-273.

The treatments of step a) or (i) may be initiated in patients which received therapy for retroviral infections and in particular for HIV infections such as standard therapy for HIV infection and which have been classified as non responders or refractory, or in patients which have recently received the diagnosis of a retrovirus infection, i.e. before the treatment with any conventionally anti-retroviral compounds or combination products. In the latter case the treatment may prevent or delay the onset of disease/symptoms associated with the retroviral infection, e.g. HIV-infection.

In certain embodiments, the patient can be any patient infected with, or at risk for infection with a retrovirus, e.g. HIV. Infection or risk for infection can be determined according to any technique deemed suitable by the practitioner of skill in the art.

In certain embodiments, the patient has never received therapy or prophylaxis for a retrovirus infection, more particularly an HIV infection. In further embodiments, the patient has previously received therapy or prophylaxis for a retroviral infection, or more particularly an HIV infection. For instance, in certain embodiments, the patient has not responded to treatment for a retroviral disease, or more particularly an HIV infection. In certain embodiments, the patient can be a patient that received therapy but continued to suffer from viral infection or one or more symptoms thereof. In certain embodiments, the patient can be a patient that received therapy but failed to
achieve a sustained virologic response. In certain embodiments, the patient has received therapy for a retroviral disease, or more particularly an HIV-related disease, but has failed to show, for example, a 2 log₁₀ decline in viral RNA levels after 12 weeks of therapy.

In certain embodiments, the patient is a patient that discontinued therapy for a retroviral disease, or more particularly HIV-related disease, because of one or more adverse events associated with the therapy. In certain embodiments, the patient is a patient where current therapy is not indicated.

The kits, methods and compositions provided herein may reduce or eliminate the need for exposing patients to the agents of current therapy, either by reducing the dose needed or reducing the required time of exposure to these agents, or by facilitating the replacement of certain agents of current therapy.

Accordingly, provided are methods of treating or preventing a retroviral disease, or more particularly an HIV-related disease. In one embodiment, provided are methods of treating or preventing a retroviral disease, more particularly an HIV-related disease. Further provided are methods of treating a retroviral disease, or an HIV-related disease, in patients where a neuropsychiatric event, such as depression, or risk of such indicates a different treatment of the current HIV therapy.

In further embodiments, the patient has received treatment for a retroviral disease, or more particularly an HIV-related disease, and discontinued that therapy prior to administration of a method provided herein. In further embodiments, the patient has received therapy and continues to receive that therapy along with administration of a method provided herein.

Treatment with at least one proteasome inhibitor may include concentrations of the proteasome inhibitors used within the range of about 1 nM to about 50 µM,
preferably about 10 nM to about 10 μM in the pharmaceutical composition. The
proteasome inhibitors may be used at doses of about 0.25 to about 5, of about 0.4 to
about 2.5, or of about 0.7 to about 1.5 mg/m², or at doses of about 2.5 to about 50, of
about 4 to about 25, or of about 7 to about 15 mg/m², or at doses of about 25 to about
500, of about 40 to about 250, or of about 70 to about 150 mg/m² body surface.

Treatment with proteasome inhibitors may be performed over several days and
weeks up to months. Usually a proteasome inhibitor may be administered every day,
or every second day, or every third day, or twice a week, or once a week, or once
every two weeks, or once every month

In one embodiment, the proteasome inhibitors described herein may be used in the
treatment or prevention of HIV infections, particularly in patients that have
developed resistances to conventional therapy such as standard therapy for HIV
infection. In the treatment forms disclosed herein, the protease inhibitor may be used
before or after or simultaneously with at least one, or alternatively before, following
or simultaneously with more than one conventionally used compounds that are
administered to patients infected with or suspected of being infected, with
retroviruses, e.g. HIV.

In another embodiment, the present invention thus relates to the use of at least one
proteasome inhibitor together with at least one pharmaceutically active agent in use
against retroviral infections in the manufacture of a medicament for treating patients
which do not respond or are refractory to treatment with a pharmaceutically active
agent in use against retroviral infections (e.g. HIV infections).

Yet another embodiment of the present invention relates to the use of at least one
proteasome inhibitor together with at least one first and at least one second
pharmaceutically active agent in use against retroviral infections in the manufacture
of a medicament for treating patients which do not respond or are refractory to
treatment with a pharmaceutically active agent in use against retroviral infections.

In these embodiments it can be preferred to use specific proteasome inhibitors such
as S-2209, PS-5 19, PS-341 (Bortezomib) and PS-273. If only one additional
different pharmaceutical agent is present, this may be selected from the above
mentioned anti-retroviral compounds used in the treatment of retroviral, e.g. HIV-
infection.

With the above mentioned pharmaceutical compositions, kits, uses and methods, in
particular where they relate to a combination of a proteasome inhibitor, and another
anti-retroviral or anti-HIV compound it is possible to reduce the virus load or to even
completely remove the virus.

In any of the embodiments of the present invention (kits, uses, pharmaceutical
compositions, methods of treatment), the proteasome inhibitor may be selected from
the list of peptides carrying at their C-terminal α, β-epoxyketone, vinyl- sulphones,
glyoxal or boronic acid-residues pinacol-esters, chemically modified derivatives of
naturally occurring proteasome inhibitors, epoxomycin, carfilzomib, eponemycin,
aclacinomycine A (also known as aclarubicine), celestrol, withaferin A, Gliotoxin,
epipolythiodioxo- piperazines, green tea polyphenolic catechins such as (-)-
epigallocatechin- 3-gallate, Disulfiram, acridine derivatives including tetra-acridine
derivatives with betulinic acid such as 3’,3’-dimethylsucciinyl betulinic acid,
dihydroeponemycin analogs, PR39, PR1 1, argyrin A, Tyropeptin A, TMC-86, TMC-
89 calpain inhibitor 1, Mal-P-Ala-Val-Arg-al, fellutamide B, syringolin A,
glidobactin A, syrbactins, TMC-95 family of cyclic tripeptides such as TMC-95 A, its
endocyclic oxindole-phenyl clamp (BIA-la) and endocyclic biphenyl- ether clamp
(BIA-2a) derivatives, lactacystine, Omuralide, Homobelactosin C, Salinosporamide
A, NEOSH-101, CEP-18770, IPSI001, IPSI007, MLN2238, MLN9708, ONX 0912,
ONX 0914, AA-102, 26 S PI, AVR-147, 4E12, N-carbobenzoxy-L-leucinyl-L-

In further specific embodiments of the present invention, the proteasome inhibiting compounds disclosed herein in a particular embodiment S-2209 and or PS-341 (Bortezomib) and the anti-retroviral compounds referred to herein (in particular Darunavir), the treatment schemes, the pharmaceutical compositions comprising the above-mentioned proteasome inhibiting compounds and or the anti-retroviral compounds surprisingly are particularly suitable in inhibiting the replication of retroviruses, particularly of HIV, in cells of the monocyte-/macrophage lineage. These cells are target cells of HIV and represent an important reservoir for HIV.
Furthermore, the proteasome inhibitors can be formulated into pharmaceutical compositions using methods available in the art and those disclosed herein.

The methods provided herein encompass administering pharmaceutical compositions comprising at least one proteasome inhibitor as described herein, and further comprising one or more compatible and pharmaceutically acceptable carriers, such as, e.g., excipients, diluents or adjuvants, and/or further at least one pharmaceutically active agent in use against retroviral infections.

In certain embodiments, the at least one pharmaceutically active agent in use against retroviral infections can be formulated or packaged with the proteasome inhibitor. Of course, the at least one pharmaceutically active agent in use against retroviral infections will only be formulated with the proteasome inhibitor when, according to the judgment of those of skill in the art, such co-formulation should not interfere with the activity of either agent or the method of administration. In certain embodiments, the proteasome inhibitor and the at least one pharmaceutically active agent in use against retroviral infections are formulated separately. They can be packaged together, or packaged separately, for the convenience of the practitioner of skill in the art.

In clinical practice the active agents provided herein may be administered by any conventional route. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, intramuscular, subcutaneous, oral, buccal, sublingual, inhalation (e.g. in the form of aerosols), intranasal, transdermal, topical, transmucosal, intra-tumoral, intra-synovial and rectal administration. In certain embodiments, the proteasome inhibitor and/or the at least one pharmaceutically active agent in use against retroviral infections is or are administered orally.
A pharmaceutical composition is formulated to be compatible with its intended route of administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal or topical administration to human beings. In an embodiment, a pharmaceutical composition is formulated in accordance with routine procedures for subcutaneous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

Use may be made, as solid compositions for oral administration, of tablets, pills, hard gelatin capsules, powders or granules. In these compositions, the active product is mixed with one or more inert excipients, diluents or adjuvants, such as sucrose, lactose or starch.

These compositions can comprise substances other than diluents, for example a lubricant, such as magnesium stearate, or a coating intended for controlled release.

Use may be made, as liquid compositions for oral administration, of solutions which are pharmaceutically acceptable, suspensions, emulsions, syrups and elixirs containing inert diluents, such as water or liquid paraffin. These compositions can also comprise substances other than diluents, for example wetting, sweetening or flavoring products.

The compositions for parenteral administration can be emulsions or sterile solutions. Use may be made, as solvent or vehicle, of propylene glycol, a polyethylene glycol, vegetable oils, in particular olive oil, or injectable organic esters, for example ethyl oleate. These compositions can also contain adjuvants, in particular wetting, isotonizing, emulsifying, dispersing and stabilizing agents. Sterilization can be
carried out in several ways, for example using a bacteriological filter, by radiation or by heating. They can also be prepared in the form of sterile solid compositions which can be dissolved at the time of use in sterile water or any other injectable sterile medium.

The compositions for rectal administration are suppositories or rectal capsules which contain, in addition to the active principle, excipients such as cocoa butter, semi-synthetic glycerides or polyethylene glycols.

The compositions can also be aerosols. For use in the form of liquid aerosols, the compositions can be stable sterile solutions or solid compositions dissolved at the time of use in apyrogenic sterile water, in saline or any other pharmaceutically acceptable vehicle. For use in the form of dry aerosols intended to be directly inhaled, the active principle is finely divided and combined with a water-soluble solid diluent or vehicle, for example dextran, mannitol or lactose.

In one embodiment, a composition provided herein is a pharmaceutical composition or a single unit dosage form. Pharmaceutical compositions and single unit dosage forms provided herein comprise a prophylactically or therapeutically effective amount of one or more prophylactic or therapeutic agents (e.g., a proteasome inhibitor, or other prophylactic or therapeutic agent), and a typically one or more pharmaceutically acceptable carriers. In a specific embodiment and in this context, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" includes a diluent, adjuvant (e.g., Freund's adjuvant (complete and incomplete)), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water can be used as a
carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well-known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient and the specific active ingredients in the dosage form. The composition or single unit dosage form, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

Lactose-free compositions provided herein can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmacopeia (USP) SP(XXI)/NF (XVI). In general, lactose free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Exemplary lactose free dosage forms comprise an active ingredient, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

Further encompassed herein are anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long term storage in order to determine characteristics such as shelf life or the stability of formulations over time.
See, e.g., Jens T. Carstensen, Drug Stability: Principles & Practice, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379–80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms provided herein can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine can be anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions can be packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

Further provided are pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

The pharmaceutical compositions and single unit dosage forms can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such compositions and dosage
forms will contain a prophylactically or therapeutically effective amount of a prophylactic or therapeutic agent, in certain embodiments, in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. In a certain embodiment, the pharmaceutical compositions or single unit dosage forms are sterile and in suitable form for administration to a patient, for example, an animal patient, such as a mammalian patient, for example, a human patient.

A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, intramuscular, subcutaneous, oral, buccal, sublingual, inhalation, intranasal, transdermal, topical, transmucosal, intra-synovial and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal or topical administration to human beings. In an embodiment, a pharmaceutical composition is formulated in accordance with routine procedures for subcutaneous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non aqueous liquid suspensions, oil in water emulsions, or a water in oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or
amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms provided herein will typically vary depending on their use. For example, a dosage form used in the initial treatment of viral infection may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the maintenance treatment of the same infection. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed herein will vary from one another will be readily apparent to those skilled in the art. See, e.g. Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing, Easton Pa. (2000).

Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachets indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

Typical dosage forms comprise a proteasome inhibitor, or a pharmaceutically acceptable salt, solvate or hydrate thereof lie within the range of from about 0.1 mg to about 1000 mg per day, given as a single once-a-day dose in the morning or as divided doses throughout the day taken with food. Particular dosage forms can have about 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0, 50.0, 100, 200, 250, 400, 500, 600, 750, 800, 1000, 1250 or 1500 mg of the active compound.
Pharmaceutical compositions that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing, Easton Pa. (2000).

In certain embodiments, the oral dosage forms are solid and prepared under anhydrous conditions with anhydrous ingredients, as described in detail in the sections above. However, the scope of the compositions provided herein extends beyond anhydrous, solid oral dosage forms. As such, further forms are described herein.

Typical oral dosage forms are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general,
intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.
Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL PH 101, AVICEL PH 103, AVICEL RC 581, AVICEL PH 105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, PA), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC 581. Suitable anhydrous or low moisture excipients or additives include AVICEL PH 103, TM, and Starch 1500 LM.

Disintegrants are used in the compositions to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms include, but are not limited to, agar agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, pregelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc
stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Piano, Tex.), CAB O SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

Active ingredients such as the compounds provided herein can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; 5,639,480; 5,733,566; 5,739,108; 5,891,474; 5,922,356; 5,972,891; 5,980,945; 5,993,855; 6,045,830; 6,087,324; 6,113,943; 6,197,350; 6,248,363; 6,264,970; 6,267,981; 6,376,461; 6,419,961; 6,589,548; 6,613,358; 6,699,500 each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions.

Suitable controlled release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients provided herein. Thus encompassed herein are single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled release.

All controlled release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non controlled counterparts. Ideally, the use of an optimally designed controlled release preparation in medical treatment is
characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

In certain embodiments, the drug may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see, Sefton, CRC Crit. Ref. Biomed. Eng. 1987, 14:201; Buchwald et al, Surgery 1980, 88:507; Saudek et al, N. Engl. J. Med. 1989, 321:574). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in a patient at an appropriate site determined by a practitioner of skill, i.e., thus requiring only a fraction of the systemic dose (see, e.g., Goodson, Medical Applications of Controlled Release, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (Science 1990, 249:1527). The active ingredient can be dispersed in a solid inner matrix, e.g., polymethylmethacrylate, polybutylmethacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethylene-terephthalate, natural
rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinylacetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinylalcohol and cross-linked partially hydrolyzed polyvinyl acetate, that is surrounded by an outer polymeric membrane, e.g., polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinylacetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinylchloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinylkoxyethanol copolymer, that is insoluble in body fluids. The active ingredient then diffuses through the outer polymeric membrane in a release rate controlling step. The percentage of active ingredient in such parenteral compositions is highly dependent on the specific nature thereof, as well as the needs of the patient.

In one embodiment, provided are parenteral dosage forms. Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are typically, sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride
Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms.

Also provided are transdermal, topical, and mucosal dosage forms. Transdermal, topical, and mucosal dosage forms include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences, 16.sup.th, 18th and 20.sup.th eds., Mack Publishing, Easton Pa. (1980, 1990 & 2000); and Introduction to Pharmaceutical Dosage Forms, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed herein are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane 1,3 diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical

Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients provided. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery enhancing or penetration enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

In human therapeutics, the physician will determine the posology which he considers most appropriate according to a preventive or curative treatment and according to the age, weight, stage of the infection and other factors specific to the patient to be treated. In certain embodiments, doses are from about 1 to about 5000 mg per day
for an adult, or from about 1 to about 2500 mg per day, or from about 1 to about
1000 mg per day, or from about 500 to about 5000 mg per day, or from 250 to about
2500 mg per day, or from about 5 to about 250 mg per day or from about 10 to 50
mg per day for an adult. In certain embodiments, doses are from about 5 to about
400 mg per day or 25 to 200 mg per day per adult. In certain embodiments, dose
rates of from about 50 to about 500 mg per day are also contemplated.

In further aspects, provided are methods of treating or preventing a retroviral
infection in a patient by administering, to a patient in need thereof, inter alia an
effective amount of a proteasome inhibitor, or a pharmaceutically acceptable salt
thereof. The amount of the compound or composition which will be effective in the
prevention or treatment of a disorder or one or more symptoms thereof will vary with
the nature and severity of the disease or condition, and the route by which the active
ingredient is administered. The frequency and dosage will also vary according to
factors specific for each patient depending on the specific therapy (e.g., therapeutic
or prophylactic agents) administered, the severity of the disorder, disease, or
condition, the route of administration, as well as age, body, weight, response, and the
past medical history of the patient. Effective doses may be extrapolated from dose-
response curves derived from in vitro or animal model test systems.

In certain embodiments, exemplary doses of a composition include milligram or
microgram amounts of the active compound per kilogram of patient or sample
weight (e.g., about 10 micrograms per kilogram to about 50 milligrams per kilogram,
about 100 micrograms per kilogram to about 25 milligrams per kilogram, or about
100 microgram per kilogram to about 10 milligrams per kilogram). For
compositions provided herein, in certain embodiments, the dosage administered to a
patient is 0.140 mg/kg to 35 mg/kg of the patient's body weight, based on weight of
the active compound. In certain embodiments, the dosage administered to a patient
is between 0.20 mg/kg and 20.0 mg/kg, or between 0.30 mg/kg and 15.0 mg/kg of
the patient's body weight. Alternatively, an estimate of the surface area of the
patient's body may be used to scale the dose, as the surface area is sometimes a more accurate predictor of certain properties related to drug distribution and clearance (see, for example, Pinkel, D., Cancer Res. 1958, 18:853). The proteasome inhibitors may be used at doses of about 0.25 to about 5, of about 0.4 to about 2.5, or of about 0.7 to about 1.5 mg/m², or at doses of about 2.5 to about 50, of about 4 to about 25, or of about 7 to about 15 mg/m², or at doses of about 25 to about 500, of about 40 to about 250, or of about 70 to about 150 mg/m² body surface.

In certain embodiments, the recommended daily dose range of a composition provided herein for the conditions described herein lie within the range of from about 0.1 mg to about 5000 mg per day, given as a single once-a-day dose or as divided doses throughout a day. In one embodiment, the daily dose is administered twice daily in equally divided doses. In certain embodiments, a daily dose range should be from about 1 to about 2500 mg per day, or from about 1 to about 1000 mg per day, or from about 500 to about 5000 mg per day, or from 250 to about 2500 mg per day, or from about 5 to about 250 mg per day or from about 10 to 50 mg per day. It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with patient response.

Different therapeutically effective amounts may be applicable for different conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to prevent, manage, treat or ameliorate such conditions, but insufficient to cause, or sufficient to reduce, adverse effects associated with the composition provided herein are also encompassed by the above described dosage amounts and dose frequency schedules. Further, when a patient is administered multiple dosages of a composition provided herein, not all of the dosages need be the same. For example, the dosage administered to the patient may be increased to
improve the prophylactic or therapeutic effect of the composition or it may be
decreased to reduce one or more side effects that a particular patient is experiencing.

In certain embodiment, the dosage of the composition provided herein, based on
weight of the active compound, administered to prevent, treat, manage, or ameliorate
a disorder, or one or more symptoms thereof in a patient is 0.1 mg/kg, 1 mg/kg, 2
mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25
mg/kg, 35 mg/kg or 50 mg/kg or more of a patient's body weight. In another
embodiment, the dosage of the composition or a composition provided herein
administered to prevent, treat, manage, or ameliorate a disorder, or one or more
symptoms thereof in a patient is a unit dose of 0.1 mg to 1500 mg, 0.1 mg to 1000
mg, 0.1 mg to 500 mg, 0.1 mg to 200 mg, 0.1 mg to 100 mg, 0.1 mg to 50 mg, 0.1
mg to 25 mg, 0.1 mg to 20 mg, 0.1 mg to 15 mg, 0.1 mg to 10 mg, 0.1 mg to 7.5 mg,
0.1 mg to 5 mg, 0.1 to 2.5 mg, 0.25 mg to 20 mg, 0.25 to 15 mg, 0.25 to 12 mg, 0.25
to 10 mg, 0.25 mg to 7.5 mg, 0.25 mg to 5 mg, 0.5 mg to 2.5 mg, 1 mg to 20 mg, 1
mg to 15 mg, 1 mg to 12 mg, 1 mg to 10 mg, 1 mg to 7.5 mg, 1 mg to 5 mg, or 1 mg
to 2.5 mg.

In certain embodiments, treatment or prevention can be initiated with one or more
loading doses of a compound or composition provided herein followed by one or
more maintenance doses. In such embodiments, the loading dose can be, for
instance, about 40 to 4000 mg per day, 60 to about 2000 mg per day, or about 100 to
about 1000 mg per day for one day to five weeks. The loading dose can be followed
by one or more maintenance doses. In certain embodiments, each maintenance dose
is, independently, about from about 10 mg to about 2000 mg per day, between about
25 mg and about 1500 mg per day, or between about 25 and about 800 mg per day.
Maintenance doses can be administered daily and can be administered as single
doses, or as divided doses.
In certain embodiments, a dose of a compound or composition provided herein can be administered to achieve a steady-state concentration of the active ingredient in blood or serum of the patient. The steady-state concentration can be determined by measurement according to techniques available to those of skill or can be based on the physical characteristics of the patient such as height, weight and age. In certain embodiments, a sufficient amount of a compound or composition provided herein is administered to achieve a steady-state concentration in blood or serum of the patient of from about 300 to about 4000 ng/mL or higher, from about 400 to about 1600 ng/mL, or from about 600 to about 1200 ng/mL. In some embodiments, loading doses can be administered to achieve steady-state blood or serum concentrations of about 1200 to about 8000 ng/mL or higher, or about 2000 to about 4000 ng/mL for one to five days. In certain embodiments, maintenance doses can be administered to achieve a steady-state concentration in blood or serum of the patient of from about 300 to about 4000 ng/mL, from about 400 to about 1600 ng/mL, or from about 600 to about 1200 ng/mL.

In certain embodiments, administration of the same composition may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months. In other embodiments, administration of the same prophylactic or therapeutic agent may be repeated and the administration may be separated by at least at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months.

In certain aspects, provided herein are unit dosages comprising a compound, or a pharmaceutically acceptable salt thereof, in a form suitable for administration. Such forms are described in detail above. In certain embodiments, the unit dosage comprises 1 to 5000 mg, 5 to 2500 mg or 10 to 1000 mg active ingredient. In particular embodiments, the unit dosages comprise about 1, 5, 10, 25, 50, 100, 125, 250, 400, 500, 600, 800, 1000, 1500, 2000, 2500, 3000, 4000, or 5000 mg active
ingredient. Such unit dosages can be prepared according to techniques familiar to those of skill in the art.

The dosages of the at least one pharmaceutically active agent in use against retroviral infections are to be used in the combination therapies provided herein. In certain embodiments, dosages lower than those which have been or are currently being used to prevent or treat retrovirus infections are used in the combination therapies provided herein. The recommended dosages of at least one pharmaceutically active agent in use against retrovirus infections can be obtained from the knowledge of those of skill. For those at least one pharmaceutically active agent in use against retrovirus infections that are approved for clinical use, recommended dosages are described in, for example, Hardman et al, eds., 1996, Goodman & Gilman's The Pharmacological Basis Of Basis Of Therapeutics, Ed, Mc-Graw-Hill, New York; Physician's Desk Reference (PDR) 57.sup.th Ed., 2003, Medical Economics Co., Inc., Montvale, N.J., which are incorporated herein by reference in its entirety.

In various embodiments, the therapies (e.g., the at least one proteasome inhibitor and the at least one pharmaceutically active agent in use against retroviral infections) are administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, at about 12 hours to about 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours part. In various embodiments, the therapies are administered no more than 24 hours apart or no more than 48 hours apart. In certain embodiments, two or more therapies are administered
within the same patient visit. In other embodiments, the at least one proteasome inhibitor and the at least one pharmaceutically active agent in use against retrovirus infections are administered concurrently.

In other embodiments, the at least one proteasome inhibitor and the at least one pharmaceutically active agent in use against retrovirus infections are administered at about 12 h to 24 h apart, 24 h to 48 h apart, 2 to 4 days apart, at about 4 to 6 days apart, at about 1 week part, at about 1 to 2 weeks apart, at about 2 to 3 weeks apart, at about 3 to 4 weeks apart, or more than 4 weeks apart.

In certain embodiments, administration of the same agent may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months.

In certain embodiments, the at least one proteasome inhibitor and at least one and, optionally, further anti-retroviral compounds are administered to a patient, for example, a mammal, such as a human, in a sequence and within a time interval such that the proteasome inhibitor can act together with the other agent(s) to provide an increased benefit than if they were administered otherwise. For example, where more than one anti-retroviral compound is administered, they can be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. In one embodiment, the proteasome inhibitor and the anti-retroviral compound exert their effect at times which overlap. Each different pharmaceutically active agent can be administered separately, in any appropriate form and by any suitable route. In other embodiments, the proteasome inhibitor is administered before administration of the different anti-retroviral compounds. In yet other embodiments, the reverse order is observed.
In certain embodiments, the proteasome inhibitor and at least one, and, optionally, further pharmaceutically active agents in use against retroviral infections are cyclically administered to a patient. Cycling therapy involves the administration of a first agent (e.g. a first prophylactic or therapeutic agent) for a period of time, followed by the administration of at least one further agent for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improve the efficacy of the treatment.

In certain embodiments, the proteasome inhibitor and at least one, and, optionally, further different pharmaceutically active agent are administered in a cycle of less than about 6 weeks, about once every four weeks, about once every three weeks, about once every two weeks, about once every 10 days or about once every week. One cycle can comprise the administration of a proteasome inhibitor and the at least one first and/or second pharmaceutically active agent in use against retrovirus infections by infusion over about 480 minutes every cycle, about 360 minutes every cycle, about 240 minutes every cycle, about 180 minutes every cycle, about 120 minutes every cycle, about 90 minutes every cycle, about 1 hour every cycle, about 45 minutes every cycle. Each cycle can comprise at least 1 week of rest, at least 2 weeks of rest, at least 3 weeks of rest, or at least 4 weeks of rest. The number of cycles administered is from about 1 to about 12 cycles, more typically from about 2 to about 10 cycles, and more typically from about 2 to about 8 cycles.

In other embodiments, courses of treatment are administered concurrently to a patient, i.e., individual doses of the at least one pharmaceutically active agent in use against retrovirus infections are administered separately yet within a time interval such that the proteasome inhibitor can have an additive and/or synergistic effect with the at least one first and/or second different pharmaceutically active agent. For example, one component can be administered once per week in combination with the other components that can be administered once every two weeks or once every three
weeks. In other words, the dosing regimens are carried out concurrently even if the therapeutics are not administered simultaneously or during the same day.

The at least one first pharmaceutically active agent in use against retroviral infections can act additively or synergistically with the proteasome inhibitor. In one embodiment, the proteasome inhibitor is administered concurrently with one or more at least one pharmaceutically active agent in use against retroviral infections in the same pharmaceutical composition. In another embodiment, a proteasome inhibitor is administered concurrently with one or more first and/or second pharmaceutically active agents in use against retrovirus infections in separate pharmaceutical compositions. In still another embodiment, a proteasome inhibitor is administered prior to administration of one or more pharmaceutically active agents in use against retroviral infections. Also contemplated are administration of a proteasome inhibitor and one or more pharmaceutically active agents in use against retrovirus infections by the same or different routes of administration, e.g., oral and parenteral. In certain embodiments, when the proteasome inhibitor is administered concurrently with a at least one pharmaceutically active agent in use against retrovirus infections that potentially produces adverse side effects including, but not limited to, toxicity, the one or more first and/or second pharmaceutically active agents in use against retrovirus infections can Advantageously be administered at a dose that falls below the threshold that the adverse side effect is elicited.

Kits

Also provided are kits for use in methods of treatment of a retroviral disease, e.g. an HIV-related disease. The kits can include at least one proteasome inhibitor, at least one pharmaceutically active agent in use against retrovirus infections, and instructions providing information to a health care provider regarding usage for treating the disorder. Instructions may be provided in printed form or in the form of an electronic medium such as a floppy disc, CD, or DVD, or in the form of a website.
address where such instructions may be obtained. A unit dose of at least one proteasome inhibitor, and/or of at least one pharmaceutically active agent in use against retrovirus infections, can include a dosage such that when administered to a patient, a therapeutically or prophylactically effective plasma level of the active ingredients(s) can be maintained in the patient for at least 1 day. In some embodiments, a composition can be included as a sterile aqueous pharmaceutical composition or dry powder (e.g., lyophilized) composition.

In a further embodiment, the kit may comprise instructions to administer at least one proteasome inhibitor and at least one anti-retroviral compound or the pharmaceutical compositions comprising the same, to a human or animal in need of treatment for an HIV-related disease, wherein the dose of at least one of the compounds administered is lower than otherwise recommended doses of that compound for such human or animal when said compound is used either in a monotherapy or in standard therapy for HIV infections for anti-retroviral compounds, whichever is the lower, or when it is used without anti-retroviral compound(s) for proteasome inhibitors. Optionally, and for the case of proteasome inhibitors, said dose is lower by at least a factor of 1/4, by at least a factor of 1/3, or by at least a factor 1/2 than the otherwise recommended doses suggested in the treatment of proliferative diseases as defined in WO 2007/017284 (page 30, lines 1 to 24). Furthermore, for those anti-retroviral compounds having been authorized for marketing in the US, the said dose is optionally lower by at least a factor of 1/4, by at least a factor of 1/3, or by at least a factor 1/2 than the otherwise recommended doses suggested in, for example "Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents" (Panel on Antiretroviral Guidelines for Adults and Adolescents; The U.S. Department of Health and Human Services. Washington, DC, USA, January 10, 2011; pages 1-166).

In some embodiments, suitable packaging is provided. As used herein, "packaging" includes a solid matrix or material customarily used in a system and capable of
holding within fixed limits at least one proteasome inhibitor and/or at least one pharmaceutically active agent in use against retrovirus infections suitable for administration to a patient. Such materials include glass and plastic (e.g., polyethylene, polypropylene, and polycarbonate) bottles, vials, paper, plastic, and plastic-foil laminated envelopes and the like. If e-beam sterilization techniques are employed, the packaging should have sufficiently low density to permit sterilization of the contents.

The invention is illustrated in the following with respect to an example which however is not be construed as being limiting. It will be clear that the scope of claimed subject matter may be practiced otherwise than as particularly described herein. Numerous modifications and variations of the subject matter are possible in view of the teachings herein and, therefore, are within the scope of the claimed subject matter.

**Examples**

**Example 1: General methods**

*Preparation of peripheral blood mononuclear cell (PBMC) cultures*

Pathogen-free human buffy coat preparations were obtained from a blood bank (lymphocyte concentrate from 500 ml whole blood, Institut fur Transfusionsmedizin Suhl gemeinnützige GmbH, Suhl, Germany), were transferred into sterile 50 ml tubes and diluted 1:1 with PBS. The diluted preparation was overlaid in another sterile 50 ml tube on 15 ml Ficoll-Histopaque (Sigma-Aldrich, Cat. H8889) and centrifuged at 2.200 rpm (Heraeus Multifuge 3SR) for 20 min without brakes. The white interphase layer formed by peripheral blood mononuclear cells (PBMC) was transferred in a fresh tube and washed twice with ice cold PBS. Subsequently, the cell count was determined by Trypan Blue staining and the cells were adjusted to 5 x 10⁶ per ml and cultured in supplemented RPMI 1640.
For viral infection, cells were pre-stimulated with 10 µg/ml PHA-P (Sigma-Aldrich, Cat. No. L1668-5mg) and 100 U/ml IL-2 (Proleukin, Roche) and the culture was allowed to grow for at least 72 h.

**Preparation of human lymphoid aggregate cultures from fresh tonsils**

Tonsil tissue was removed during routine tonsillectomy from HIV, HBV, HCV-negative patients. Tonsils were washed carefully with PBS and capsula, brown necrotic and bloody regions were removed. To prepare a Human Lymphoid Aggregate Culture (HLAC), tonsil tissue was mechanically dispersed by cutting tissue in 2- to 3-mm blocks and passing them through 70-µm cell strainers with the plunger of a 2-ml syringe. The cell strainer was washed with PBS and cells were spun down for 5 min at 500 rcf at room temperature. PBS was removed and cells were suspended in HLAC medium (RPMI 1640 containing 15% fetal bovine serum, 2 mM L-glutamine, 100 U/ml PenStrep, 2.5 µg/ml fungizone, 1 mM sodium pyruvate, 1% non-essential amino acids, 50 µg/ml gentamycin). Cells were counted and diluted with fresh HLAC medium to a final concentration of 1 x 10⁷ cells/ml. Cells were seeded in 96-well plates (200 µl/well; 2 x 10⁶ cells/well) and incubated overnight at 37°C. Cultures were used within 24 h of preparation.

**HIV virus stock preparation by transfection of 293T cells**

Virus stocks were generated by transient transfection of 293T cells using Lipofectamine 2000™ according to manufacturer’s protocols. One day before transfection, 0.5 x 10⁵ 293T cells were seeded in 15 ml DMEM in T75 flasks. At a confluence of 50-75% the cells were used for transfection. 48 h post transfection, supernatants were collected and centrifuged at 1200 rpm for 5 min (Heraeus Multifuge 3SR). After centrifugation, the supernatant was passed through a 45 µm sterile filter. 1 ml supernatant was overlaid on 200 µl 2% sucrose in a eppendorf tube and centrifuged at 14000 rpm (Eppendorf Centrifuge, model 5417R) for 90 min at 4°C. Each virus pellet was re-suspended in 50 µl RPMI 1640 and pellets were collected to
a final volume of 750 µι virus stock. The HIV-1 p24 antigen concentration of virus
stocks was determined by p24 antigen enzyme-linked immunosorbent assay (ELISA).

Staining of apoptotic/necrotic cells

Apoptosis is a tightly regulated process, characterized by DNA fragmentation, shrinkage of cytoplasm, and reassembly of membranes. In viable cells, phosphatidylserine (PS) is located on the cytoplasmic surface of the cell membrane. In apoptotic cells, PS is translocated from the inner to the outer leaflet of the membrane and is therefore exposed to the extracellular environment. Annexin V, a human anticoagulant conjugated to a fluorophore, binds to PS and can therefore identify apoptotic cells. Propidium iodide (PI) is impermeant to live and apoptotic cells, but stains dead cells with red fluorescence by binding tightly to nucleic acids made accessible to membrane impermeant agents only by cell death. Staining a population of cells with Annexin V and PI leads to different fluorescent cell populations, of which apoptotic cells show a green fluorescence, dead cells show a green and red fluorescence, whereas live cells appear unstained. Cells were stained following the protocols described in Immunol Cell Biol 1998, 76:1.

P24 ELISA:

The p24 antigen ELISA is a standard method for detection of released HIV viral particles in the supernatant of tissue cell culture or blood samples of patients. The assay was performed using a commercially available assay kit following manufacturer's instructions (Aalto Bio Reagents, Dublin, Ireland; see also: Moore et al, Science 1990, 250:1139-1142; Moore et al, J.Virol. 1991, 65:852-860).

Briefly, 15 µι of HIV-1 containing supernatants are inactivated with 135 µι 2% Empigen. After one hour, 100µl are transferred onto a 96-well plate coated with a polyclonal sheep-anti-HIV-l-p24 gag antibody. After washing, the samples are incubated with horseradish peroxidase (HRP-) conjugated p24 antibody, a HRP
substrate is added after repeat washing, and luminescence is quantitated in a standard ELISA reader.

**RT-Assay**

The RT-Assay is a standard method for detecting the amount of mature retroviruses present in cell culture supernatants (Willey RL, et al, J Virol 1988, 62:139-147). It is used to assess potential anti-viral effects of test substances.

Briefly, supernatants from cultures of cells infected with the HI virus are used for assessing HIV replication in such cells, by measuring the incorporation of 32P-dTT into DNA generated by viral reverse transcriptase liberated by the lysis of infected cells. 32P-incorporation is measured by the scintillation counting of 32P-radioactivity in supernatant samples of defined volume. An average reading obtained from uninfected cells is subtracted as background. Counts per minute (cpm) in these supernatant samples quantitatively reflects the amount of active viral reverse transcriptase as an indicator of mature viruses present in the supernatant.

To gauge an inhibitory effect of inhibitor candidates on HIV-1 replication, the area under the curve is calculated for a plot of 32P-radioactivity in supernatant samples vs. time as an indicator for total viral replication. The average area under the curve obtained from untreated infected samples is defined as 100%, and the results for samples having been treated with test substances are expressed relative to this value.

**Example 2**: Inhibition of HIV replication in human lymphoid aggregate cultures

Within 24 h of preparation, HLAC were infected with HIV-1NL4/3 (1 ng p24 per well).

**Example 2.1: Inhibitory potency and cytotoxicity of a proteasome inhibitor (S-2209)**

To first compare and contrast the potency of S-2209 in inhibiting HIV replication with its toxicity to HLAC, IC50 (concentration yielding 50% inhibition) and CC50...
(concentration yielding 50% cytotoxicity) for the treatment of HLAC with S-2209 under the experimental conditions to be used further on were first determined. To this effect, HLAC medium (RPMI 1640 containing 15% fetal bovine serum, 2 mM L-glutamine, 100 U/ml PenStrep, 2.5 µg/ml fungizone, 1 mM sodium pyruvate, 1% non-essential amino acids, 50 µg/ml gentamycin) containing S-2209 at concentrations ranging from 0 nM (mock treated) to 100 µM was added to HLAC infected with HIV-1NΔ4I immediately post-infection (for S-2209 IC50) or uninfected HLAC (for cytotoxicity assessment), cultures were incubated for 24 h, the medium exchanged for fresh medium again containing S-2209 at the same concentration as before, and the cultures incubated for another 48 h. Subsequently, the medium containing S-2209 was exchanged for fresh HLAC medium, and the cultures incubated for another 12 days (up to day 15 post infection) under HLAC medium, exchanging the HLAC medium for fresh HLAC medium every 72 h. Culture supernatants were removed on days 1, 3, 6, 9, 12 and 15, and HIV-1 replication in infected cultures assessed by RT assay. The amounts of radioactivity measured in these samples were summed up to yield a number roughly corresponding to an aggregate viral production. The co-treated uninfected cultures were subjected to Annexin/PI staining for assessment of cytotoxicity on the day of peak HIV-1 replication in the infected culture treated with the same inhibitor concentration. The average value obtained for mock treated cells was set to 100% replication and 100% viable cells, respectively. All other results were expressed relative to these values. Polynomial regression (4-parameter, GraphPad Prism, GraphPad Software, La Jolla, CA, USA) on the resulting data points yielded an IC50 for inhibiting HIV-1 replication by S-2209 of approximately 375 nM (see Figure 1), and a CC50 of approximately 5500 nM under the conditions of the experiment as described (see Figure 2).

It was therefore established, that the inhibition of HIV-1 replication was not due to S-2209 rendering the cells non-viable.
Example 2.2: Comparison of the time course of inhibition of a proteasome inhibitor (S-2209) and an agent inhibiting a viral target (protease inhibitor darunavir)

In a further set of experiments, the time course of the inhibitory efficacy of a concentration of S-2209 giving near 100% inhibition of peak replication in Example 2.1 (750 nM S-2209) was compared to the comparable activity of an agent inhibiting a viral target, which is used routinely in the therapy of HIV infection (darunavir, DRV). To this effect, HLAC infected with HIV-1\textsubscript{NL4/3} were treated with HLAC medium containing 750 nM S-2209 or 15 nM DRV (IC\textsubscript{50} for DRV: 8 nM under the conditions of the experiment, data not shown) for 24 h, the HLAC medium containing the respective agent exchanged for fresh HLAC medium containing the respective agent, and the incubation continued for another 48 h. Subsequently, the medium containing S-2209 or DRV was exchanged for fresh HLAC medium, and the cultures incubated for another 18 days (up to day 21 post infection) under HLAC medium, exchanging the HLAC medium for fresh HLAC medium every 72 h.

Culture supernatants were removed on days 1, 3, 6, 9, 12, 15, 18, and 21, and HIV-1 replication in the culture assessed by RT assay. Mock infected cells served as negative controls, cells treated with HLAC medium not containing any agent as positive controls. In positive controls, HIV-1 replication sharply increased on day 12, and peak replication was observed on day 15 post infection, gradually decreasing thereafter, most likely due to the depletion of uninfected cells available for further infection. In cells treated with DRV for the first 72h post infection, replication was observed slightly shifted, with peak replication observed on day 18, and gradually decreasing thereafter. In cultures treated with S-2209, virtually no increase of radioactivity readings over those obtained for mock infected cells were observed.

It was thus established, that S-2209 is capable of suppressing HIV-1 replication over the full time course of the experiment, while treatment with the protease inhibitor DRV merely delays HIV-1 replication until removal of the protease inhibitor.
Example 2.3: Synergistic action of treatment with a combination of proteasome inhibitor (S-2209) with an agent inhibiting a viral target (protease inhibitor darunavir)

In further sets of experiments, HLAC were treated with the following regimens (see also Figure 3):

(i) Addition of HLAC medium containing DRV at 15 nM or 7.5 nM concentrations, as indicated below, directly following infection, exchanging the HLAC medium for fresh HLAC medium containing DRV at the same concentration 24 h post infection, and again replacing the HLAC medium with plain HLAC medium (i.e. not containing DRV), 72 h post infection; the plain HLAC medium was subsequently exchanged for fresh plain HLAC medium every 72 h until day 21 post infection;

(ii) Addition of HLAC medium containing DRV at 15 nM or 7.5 nM, as indicated below, directly following infection, exchanging the HLAC medium for fresh HLAC medium containing DRV at the same concentration 24 h post infection, and again replacing the HLAC medium with HLAC medium comprising S-2209 at 500 nM or 100 nM concentrations, as indicated below, but not DRV, 72 h post infection; the HLAC medium comprising S-2209 was subsequently replaced with fresh HLAC medium comprising S-2209 every 72 h until day 12 post infection; it was then replaced by plain HLAC medium (i.e. not containing S-2209 or DRV), and the plain HLAC medium refreshed every 72 h until day 21 post infection;

(iii) Addition of plain HLAC medium directly following infection, exchanging the HLAC medium for fresh plain HLAC medium 24 h post infection, and again replacing the HLAC medium with HLAC medium comprising S-2209 at 500 nM or 100 nM concentrations, as indicated below, but not DRV, 72 h post infection; the HLAC medium comprising S-2209 was subsequently replaced.
with fresh HLAC medium comprising S-2209 every 72 h until day 12 post infection; it was then replaced by plain HLAC medium (i.e. not containing S-2209 or DRV), and the plain HLAC medium refreshed every 72 h until day 21 post infection;

(iv) Addition of plain HLAC medium directly following infection, exchanging the HLAC medium for fresh plain HLAC medium 24 h post infection, and again replacing the HLAC medium with fresh plain HLAC medium every 72 h until day 21 post infection (i.e. no treatment with either DRV or S-2209).

Mock infected cells served as negative (background) controls.

Two sets of experiments were performed. In the first, concentrations of 15 nM DRV and 500 nM S-2209 were used for the cells treated with the either DRV or S-2209, and 15 nM DRV and 100 nM S-2209 were used on cells sequentially treated with both substances; supernatant samples were harvested and stored at -80°C for subsequent analysis by RT-Assay or p24-ELISA with every medium exchange. The results of this set of experiments are shown in Figure 4. In the supernatants from cells infected with HIV -1NL43 and not treated with either substance, viral loads increased rapidly from day 6 to day 12, returning to almost baseline by day 21 due to the lytic destruction of essentially all cells in the culture (Figure 4, filled circles). In cells treated with DRV but not S-2209, supernatant viral loads showed a similar profile of increase followed by a decrease, but shifted to later time points by approximately 6 days (Figure 4, filled squares). Likely, viral replication was suppressed in the presence of 15 nM DRV, but could resume almost unhampered once DRV was removed. In cells treated with 500 nM S-2209 only under the above conditions (NB: onset of S-2209 treatment 72 h post infection, hence the IC_{50} determined above is not applicable here), the viral loads increased between day 6 and day 12, as in untreated cells, but did not reach the levels seen in untreated controls (Figure 4, unfilled triangles). When using S-2209 at the lower concentration of 100 nM under these conditions, no effect on viral loads was observed (data not shown).
However, when cells were treated sequentially with both 15 nM DRV and 100 nM S-2209, viral loads in supernatants stayed essentially at baseline over the whole course of the experiment (Figure 4, filled triangles).

In the second set of experiments, a concentration of DRV was used that had previously been found to inhibit viral replication under the conditions of the experiment by about 50% (IC$_{50}$) compared to untreated controls, up to about the point in time when viral loads start to decrease in untreated cells due to a lack of uninfected cells available for new productive infection 7.5 nM DRV. Post infection, cells were hence treated with 7.5 nM DRV on days 1 to 3, 100 nM S-2209 on days 4 to 12, or a combination of both treatments, and supernatant samples were collected for $^{32}$P analysis on days 1, 3, 6, 9, 12 and 15. The amounts of radioactivity measured in these samples were summed up to yield a number roughly corresponding to an aggregate viral production. Here, neither the treatment with 7.5 nM DRV nor with 100 nM S-2209 had any effect on the sums over supernatant viral loads, but the combination treatment with 7.5 nM DRV followed subsequently by 100 nM S-2209 reduced supernatant viral loads to baseline (see Figure 5).

**Example 3: Eradication of HIV from human macrophages**

Cells of macrophage lineage represent a key target of human immunodeficiency virus (HIV) in addition to CD4-lymphocytes. The peculiar dynamics of HIV replication in macrophages, their long-term survival after HIV infection, and their ability to spread virus particles to bystander CD4-lymphocytes, make evident their substantial contribution to the pathogenesis of HIV infection.

The following experiment set out to assess the capability of proteasome inhibitors, and particularly in combination with anti-retroviral compounds, of interfering with HIV replication in infected macrophages, and preferably of eradicating macrophages with the ability to infect other cells in an organism.
Macrophage differentiation

For differentiation to monocyte derived macrophages (MDM), monocytes were seeded in 24-well Nunc UpCell™ Surface plates (Nunc GmbH & Co KG, Langenselbold, Germany) at a density of $10^6$ cells per well. Macrophage medium (DMEM containing 10% human serum, 5 mM glutamine, 5 mM PenStrep, 1 mM sodium pyruvate) was replaced 24h post seeding and every 3-4 days thereafter. After 10-14 days, completion of differentiation was determined visually by assessment of macrophage morphology.

For isolation, cells were detached by reducing the temperature below the threshold temperature of 32°C for the Nunc UpCell™ Surface. These plates are coated with a polymer surface having either hydrophobic (cell adhesion) or hydrophilic (cell detachment) character depending on the temperature, with a threshold temperature of 32°C. Cells were pelleted by centrifugation, $5 \times 10^4$ cells seeded into 24 well plates and allowed to adhere for at least 48h.

Determination of HIV replication in monocyte derived macrophages

Before infection with 1 ng macrophage-tropic HIV - NL4/3, the medium was replaced to get a final volume after infection of 250 μl. On day one post infection, 250 μl macrophage medium was added, 200 μl supernatant samples were transferred to 96 well plates, and the samples stored at -80°C until further analysis. In one set of experiments, the remaining supernatant was replaced by 500 μl fresh macrophage medium, optionally containing an inhibitor of HIV replication; on day 4, a 200 μl supernatant sample was taken and stored in a 96 well plates at -80°C until analysis, the remaining supernatant discarded and replaced by 500 μl fresh macrophage medium, again optionally containing an inhibitor of HIV replication. On day 8 post infection, another 200 μl supernatant sample was taken, the reminder of the supernatant discarded, and replaced by 500 μl of plain macrophage medium. Further supernatant samples were harvested every 3-4 days, terminating after 29 days (see
Figure 6), by transferring 200 µl supernatant to 96 well plates for storage at -80°C until analysis. Each time, the remaining supernatant was discarded, and 500 µl fresh macrophage medium added to each well. The results of this experiment are graphically represented in Figure 7.

In a second set of experiments, the cells were incubated with plain medium between days 1 and 3, and 1 µM S-2209 was added to the medium for some cells only with medium changes on day 4 and on day 7, or only on day 7, the medium replaced by fresh plain macrophage medium on day 11 for all cells, and the experiment terminated on day 18. HIV replication was quantitated by performing RT-assays on the stored supernatant samples.

HIV infection may be eradicated from human macrophages by treatment with proteasome inhibitors

The luminescence activities found in supernatant samples of MDM cultures treated with 500 nM, 750 nM and 1 µM solutions of S-2209 between days 1 and 8 post infection are depicted in Fig. 7. Evidently, S-2209 severely represses HIV replication even at the lowest concentration employed, and fully disallows replication of HIV at the highest concentration of 1 µM under experimental conditions. Furthermore, as is evident from Figure 8, viral replication can be severely reduced, if not abolished, in macrophages by the addition of S-2209 to their culture medium even after the infection has established itself firmly, e.g. 4 and/or 7 days post in vitro infection. Corroborating evidence is found in the fact that when monocyte derived macrophage cultures were infected with the green fluorescent protein expressing HIV-1-NL4/3-IRES-GFP, the green fluorescence was easily detectable in untreated cells on day 3 post infection and thereafter up to the end of the experiment (day 10), but no green fluorescence was observed in MDM infected with HIV-1-NL4/3-IRES-GFP and treated with 1 µM solutions of S-2209 for up to 10 days following infection.
It was thus established, that HIV infection can effectively be suppressed in monocyte derived macrophages by treatment with a proteasome inhibitor, and preferably by treatment with S-2209, thereby reducing or eliminating the potential of infected macrophages to act as a viral reservoir.

Eradication of HIV infection from human macrophages can be achieved with lower concentrations of the active agent if a combination treatment is used

A similar experiment is subsequently performed using the following treatment regimens:

(i) Addition of macrophage medium containing DRV at 15 nM or 7.5 nM concentrations, immediately post infection, exchanging the macrophage medium for fresh macrophage medium containing DRV at the same concentration after 24 h of incubation, and again replacing the macrophage medium with plain macrophage medium (i.e. not containing DRV), 72 h post infection; the plain medium is subsequently exchanged for fresh plain medium every 72 h until day 29 post infection;

(ii) Addition of macrophage medium containing DRV at 15 nM or 7.5 nM, directly following infection, exchanging the macrophage medium for fresh macrophage medium containing DRV at the same concentration 24 h post infection, and again replacing the macrophage medium with macrophage medium comprising S-2209 at 750 nM, 500 nM, 250 nM, or 100 nM concentrations, but not DRV, 72 h post infection; the macrophage medium comprising S-2209 is subsequently replaced with fresh macrophage medium comprising S-2209 every 72 h until day 9, 12 or 15 post infection; it is then replaced by plain macrophage medium (i.e. not containing S-2209 or DRV), and the plain macrophage medium refreshed every 72 h until day 29 post infection;
(iii) Addition of plain macrophage medium directly following infection, exchanging the macrophage medium for fresh plain macrophage medium 24 h post infection, and again replacing the macrophage medium with macrophage medium comprising S-2209 at 750 nM, 500 nM, 250 nM, or 100 nM concentrations, but not DRV, 72 h post infection; the macrophage medium comprising S-2209 is subsequently replaced with fresh macrophage medium comprising S-2209 every 72 h until day 9, 12 or 15 post infection; it is then replaced by plain macrophage medium (i.e. not containing S-2209 or DRV), and the plain macrophage medium refreshed every 72 h until day 29 post infection;

(iv) Addition of plain macrophage medium directly following infection, exchanging the macrophage medium for fresh plain macrophage medium 24 h post infection, and again replacing the macrophage medium with fresh plain macrophage medium every 72 h until day 29 post infection (i.e. no treatment with either DRV or S-2209).

This experiment shows, that HIV infection of macrophages is amenable to eradication when a protease inhibitor, e.g. DRV, is added to the treatment regimen. In particular, lower doses of the combination components suffice to achieve an effect as compared to treatment with a single agent.
CLAIMS

1. A pharmaceutical composition comprising at least one proteasome inhibitor and at least one anti-retroviral compound for use in the treatment of a retroviral disease, wherein said retroviral disease preferably is an HIV-related disease.

2. The pharmaceutical composition for use according to claims 1, wherein the anti-retroviral compound is a retrovirus protease inhibitor, optionally selected from the group comprising darunavir, atazanavir, indinavir, lopinavir, ritonavir, and saquinavir.

3. The pharmaceutical composition for use according to any of claims 1 or 2, wherein the proteasome inhibitor is a semicarbazone proteasome inhibitor, a structural and/or functional analogue or a derivative thereof, a dipeptidyl-boronic acid derivative, or a pharmaceutically acceptable salt of either, optionally selected from the group comprising the semicarbazone S-2209 ([l-{l-{l-[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl}] -2-(1H-indol 1-3-yl)-ethylcarbamoyl]-2-(1H-indol)) and the dipeptidyl-boronic acid derivative PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), which has the molecular formula C19H25BN4O4).

4. The pharmaceutical composition according to any one of claims 1 to 3, wherein the proteasome inhibitor is ([l-[l-{l-{2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl}] -2-(1H-indol 1-3-yl)-ethylcarbamoyl]-2-(1H-indol)) and the retrovirus protease inhibitor is darunavir.

5. The pharmaceutical composition according to any one of claims 1 to 3, wherein the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), which has the molecular formula C19H25BN4O4) and the retrovirus protease inhibitor is darunavir.
6. A kit comprising (i) at least one anti-retroviral compound or a pharmaceutical composition comprising said anti-retroviral compound and (ii) at least one proteasome inhibitor or a pharmaceutical composition comprising said anti-retroviral compound, wherein said anti-retroviral compound optionally is a retrovirus protease inhibitor that is selected from the group comprising darunavir, atazanavir, indinavir, lopinavir, ritonavir, and saquinavir.

7. The kit according to claim 6, wherein the at least one proteasome inhibitor is a semicarbazone proteasome inhibitor, a structural and/or functional analogue or a derivative thereof, a dipeptidyl-boronic acid derivative, or a pharmaceutically acceptable salt of either, optionally selected from the group comprising S-2209 ([I-1-[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl] -2-(1H-indol-3-yl)-ethylcarbamoyl] -2-(1H-indol1)) and PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid, which has the molecular formula C19H25BN4O4).

8. The kit according to any one of claims 6 to 7, wherein the proteasome inhibitor is ([I-1-[(2,4-Dioxo-imidazolidin-1-ylimino)-methyl]-2-phenyl-ethylcarbamoyl] -2-(1H-indol-3-yl)-ethylcarbamoyl] -2-(1H-indol1)) and the retrovirus protease inhibitor is darunavir.

9. The kit according to any one of claims 6 to 7, wherein the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), which has the molecular formula C19H25BN4O4) and the retrovirus protease inhibitor is darunavir.

10. The kit according to any one of claims 6 to 9, further comprising instructions for the user to
a) administer at least one proteasome inhibitor first to a patient in need of treatment for an HIV-related disease, and to administer at least one anti-retroviral compound subsequently with delay; or

b) administer at least one anti-retroviral compound first to a patient in need of treatment for an HIV-related disease, and to administer at least one proteasome inhibitor subsequently with delay; or

c) administer at least one proteasome inhibitor and at least one anti-retroviral compound to a patient in need of treatment for an HIV-related disease, wherein the dose of at least one of the compounds administered is lower than otherwise recommended doses of that compound for such patient when said compound is used either in a monotherapy or standard therapy for HIV infection, whichever is lower, or without anti-retroviral compound, and optionally said dose is lower by at least a factor of \( \frac{1}{4} \), by at least a factor of \( \frac{1}{3} \), or by at least a factor \( \frac{1}{2} \) than said otherwise recommended doses; or

d) administer at least one proteasome inhibitor and at least one anti-retroviral compound to a patient in need of treatment for an HIV-related disease, wherein the HIV-infection of said patient is resistant or refractory to treatment with at least one anti-retroviral compound.

11. A proteasome inhibitor and at least one anti-retroviral compound for the simultaneous or sequential use in the combined treatment of a retroviral disease, wherein the anti-retroviral compound optionally is a retrovirus protease inhibitor selected from the group comprising darunavir, atazanavir, indinavir, lopinavir, ritonavir, and saquinavir.

12. The proteasome inhibitor and at least one anti-retroviral compound for use according to claim 11, wherein the proteasome inhibitor is a semicarbazone proteasome inhibitor, a structural and/or functional analogue or a derivative thereof, a dipeptidyl-boronic acid derivative, or a pharmaceutically acceptable salt of either,
and wherein said proteasome inhibitor is optionally selected from the group comprising S-2209 (1-[1-1-{2,4-Dioxo-imidazolidin-1-ylmino}-methyl]-2-phenyl-ethylcarbamoyl]-2-(IH-indol-3-yl)-ethylcarbamoyl]-2-(IH-indol)) and N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid.

13. The proteasome inhibitor and at least one anti-retroviral compound for use according to any of claims 11 to 12, wherein (a) the proteasome inhibitor is (1-[1-{2,4-Dioxo-imidazolidin-1-ylmino}-methyl]-2-phenyl-ethylcarbamoyl]-2-(IH-indol-3-yl)-ethylcarbamoyl]-2-(IH-indol)) and the retrovirus protease inhibitor is darunavir, or wherein (b) the proteasome inhibitor is PS-341 (N-(2,3-pyrazine)carbonyl-L-Phenylalanine-L-leucine-boronic acid), which has the molecular formula C_{19}H_{25}BN_{4}O_{4}) and the retrovirus protease inhibitor is darunavir.

14. The proteasome inhibitor and at least one anti-retroviral compound for use according to any of claims 11 to 13, wherein said retroviral disease is an HIV-related disease.

15. The pharmaceutical composition for use according to any of claims 1 to 5 or the at least one proteasome inhibitor and at least one anti-retroviral compound as defined in the above embodiments 11 to 14 for the treatment of an HIV-positive patient or for the prevention of an infection and/or HIV-related disease in an individual suspected to be infected with HIV, optionally for the treatment of HIV-infected individual selected from the following patients groups:

(i) patients suspected to be infected with HIV,
(ii) patients infected with a HIV, but not showing disease symptoms,
(iii) patients infected with a retrovirus and suffering from an HIV-related disease,
(iv) patients being HIV-positive and refractory to at least one anti-HIV compound.
16. The novel kits, compositions, methods and uses essentially as provided herein before.
Figure 1

**S-2209**
mean of 8 tonsils

\[ \text{IC}_{50} = 374 \text{nM} \]
\[ R^2 = 0.9998 \]

Figure 2

**S-2209**
Annexin/PI

\[ \text{CC}_{50} = 5366 \text{nM} \]
\[ R^2 = 0.9762 \]
Figure 8

- untreated
- 1μM (day 4/7)
- 1μM (day 7)

virus replication [fu/ml]

0 5 10 15 20
dpi

0 500000 1000000 1500000 2000000
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

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 practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search

16 May 2011

Date of mailing of the international search report

20/05/2011

Authorized officer

Al bayrak, Timur

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