



- (51) **International Patent Classification:**
C12N 5/16 (2006.01)
- (21) **International Application Number:**
PCT/US2012/042693
- (22) **International Filing Date:**
15 June 2012 (15.06.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/498,029 17 June 2011 (17.06.2011) US
- (71) **Applicant (for all designated States except US):** CYTO-DYN, INC. [US/US]; 110 Crenshaw Lake Road, Lutz, FL 33548 (US).
- (72) **Inventor; and**
- (75) **Inventor/Applicant (for US only):** TRAUGER, Richard [US/US]; 311 Sanford Street, Leucadia, CA 92024 (US).
- (74) **Agents:** GARCIA, Todd E. et al.; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, MN 55440-1022 (US).
- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

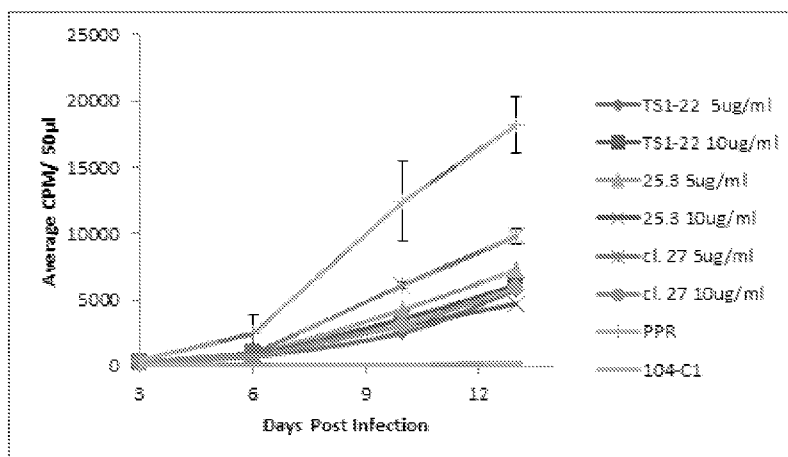
- (84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) **Title:** METHODS OF TREATING RETROVIRAL INFECTIONS IN FELINES

Figure 2



(57) **Abstract:** Provided are methods for treating or reducing the likelihood of developing a retroviral infection in a feline, decreasing retroviral virion entry into a feline cell, decreasing retroviral virion budding from a feline cell, or decreasing syncytium transmission in a feline. These methods require the administration of at least one agent that specifically binds to CD11a and/or CD18, or ICAM-1, and/or decreases or prevents the binding of LFA-1 (CD11a/CD18 heterodimer) to ICAM-1. Also provided are veterinary compositions and methods of identifying candidate agents useful for treating or reducing retroviral infection in a feline, decreasing retroviral virion entry into a feline cell, decreasing retroviral virion budding from a feline cell, or decreasing syncytium transmission in a feline.



METHODS OF TREATING RETROVIRAL INFECTIONS IN FELINES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial
5 No. 61/498,029, filed June 17, 2011, the entire contents of which are herein
incorporated by reference.

TECHNICAL FIELD

This invention relates generally to methods of treating retroviral infections in
felines.

10

BACKGROUND

Felines are susceptible to infection by retroviruses (e.g., feline
immunodeficiency virus (FIV) and feline leukemia virus (FELV)). FIV is the causative
agent of feline immunodeficiency disease syndrome. FELV is caused by a feline
15 retrovirus that is similar to human leukemia virus in humans. Feline leukemia virus
induces the uncontrolled growth of blood cells.

Feline retroviruses affect a significant portion of the feline population. For
example, approximately 2.5% to 4.4% of cats worldwide were estimated to be infected
with FIV in 2005 (Richards, *Biologicals* 33:219, 2005). Approximately 0.5% of
20 domestic felines are persistently infected with FELV. Successful treatment of FIV and
FELV infection in felines (e.g., domestic and feral felines) is desired.

SUMMARY

The present invention is based, at least in part, on the discovery that an antibody
that specifically binds to the cellular antigen LFA-1 (CD11a/CD18 heterodimer)
25 reduces retroviral infection of feline cells in vitro. Provided herein are methods of
treating retroviral infections in felines by administering one or more agents that
specifically bind to CD11a, CD18, an epitope formed by both CD11a and CD18, or
ICAM-1 present on the surface of a feline cell (e.g., a non-infected feline cell) or
present on the surface of a retroviral virion or syncytium. In some embodiments, the
30 one or more agents decrease the binding of LFA-1 to ICAM-1. Also provided are

methods of reducing or preventing retroviral virion entry into a feline cell (e.g., a non-infected feline cell) or reducing or preventing retroviral virion budding from a feline cell by administering at least one agent that specifically binds to CD11a, CD18, an epitope formed by both CD11a and CD18, or ICAM-1, and/or decreases the binding of LFA-1 to ICAM-1. Also provided are methods of reducing or preventing syncytium transmission in a feline by administering at least one agent that specifically binds to CD11a, CD18, an epitope formed by both CD11a and CD18, or ICAM-1, and/or prevents or decreases LFA-1 binding to ICAM-1.

In some embodiments of all of the methods described herein, the at least one agent decreases the interaction (e.g., decreases the K_{on} rate, increases the K_{off} rate, increases the K_D , and/or decreases the K_A) of LFA-1 with ICAM-1. The LFA-1 can be present on the surface of a feline cell (e.g., a non-infected feline cell) with ICAM-1 present on the surface of a retroviral virion or syncytium. In some embodiments, the LFA-1 can be present on the surface of a retroviral virion or syncytium with ICAM-1 present on the surface of a feline cell (e.g., a non-infected feline cell). In some embodiments, the at least one agent decreases the ability of LFA-1 or ICAM-1 to contribute to virion entry into a feline cell (e.g., an non-infected feline cell) or decreases the ability of LFA-1 or ICAM-1 to contribute to virion budding. In some embodiments, the at least one agent decreases the ability of LFA-1 or ICAM-1 to contribute to syncytium transmission in a feline. In any of the embodiments described herein, LFA-1 may be present on the surface of a feline cell (e.g., a non-infected feline cell) or on the surface of a retroviral virion or syncytium. In any of the embodiments described herein, ICAM-1 may be present on the surface of a feline cell (e.g., a non-infected feline cell) or on the surface of a retroviral virion or syncytium.

Compositions containing at least one agent that specifically binds to CD11a, CD18, an epitope formed by both CD11a and CD18, or ICAM-1, and/or decreases the interaction of LFA-1 with ICAM-1 are also provided, as well as methods of identifying such agents.

Provided are methods for treating a retrovirus infection in a feline. These methods include administering to a feline at least one (e.g., one, two, three, or four) small molecule that decreases LFA-1 binding to ICAM-1, where the at least one small molecule is administered in an amount sufficient to treat a retrovirus infection. Also provided are methods for treating a retrovirus infection in a feline that include

administering to a feline at least one (e.g., one, two, three, or four) antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18 (binds specifically to CD11a, CD18, or an epitope that is formed by both CD11a and CD18), wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to treat a retrovirus infection. Also provided are methods for treating a retrovirus infection in a feline that include administering to a feline at least one (e.g., one, two, three, or four) antibody or antigen-binding fragment thereof that specifically binds to ICAM-1, wherein that at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to treat a retrovirus infection.

Also provided are methods for reducing retroviral virion entry into a feline cell (e.g., a non-infected feline cell) or retroviral virion budding from a feline cell in a feline. These methods include administering to a feline at least one (e.g., one, two, three, or four) small molecule that decreases LFA-1 binding to ICAM-1, wherein the at least one (e.g., one, two, three, or four) small molecule is administered in an amount sufficient to reduce retroviral virion entry into a feline cell or retroviral virion budding from a feline cell. Also provided are methods for reducing retroviral virion entry into a feline cell or retroviral virion budding from a feline cell in a feline that include administering at least one (e.g., one, two, three, or four) antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18 (binds specifically to CD11a, CD18, or an epitope formed by both CD11a and CD18), wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to reduce retroviral virion entry into a feline cell or retroviral virion budding from a feline cell. Also provided are methods for reducing retroviral virion entry into a feline cell or retroviral virion budding from a feline cell in a feline that include administering at least one (e.g., one, two, three, or four) antibody or antigen-binding fragment thereof that specifically binds to ICAM-1, wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to reduce retroviral virion entry into a feline cell or retroviral virion budding from a feline cell.

Also provided are methods of reducing syncytium transmission in a feline. These methods include administering to a feline at least one (e.g., one, two, three, or four) small molecule that decreases LFA-1 binding to ICAM-1, wherein the at least one small molecule is administered in an amount sufficient to reduce syncytium transmission in a feline. Also provided are methods of reducing syncytium

transmission in a feline that include administering to a feline at least one (e.g., one, two, three, or four) antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18 (binds specifically to CD11a, CD18, or an epitope formed by both CD11a and CD18), wherein the at least one antibody or antigen-binding fragment
5 thereof is administered in an amount sufficient to reduce syncytium transmission in a feline. Also provided are methods of reducing syncytium transmission in a feline that include administering to a feline at least one (e.g., one, two, three, or four) antibody or antigen-binding fragment thereof that specifically binds to ICAM-1, where the at least one antibody or antigen-binding fragment thereof is administered in an amount
10 sufficient to reduce syncytium transmission in a feline.

In some embodiments, the at least one antibody or antigen-binding fragment thereof binds specifically to CD11a and/or CD18 (binds specifically to CD11a, CD18, or an epitope formed by both CD11a and CD18), or ICAM-1. In some embodiments, the at least one antibody or antigen-binding fragment thereof decreases LFA-1 binding to ICAM-1. In any of the methods described herein, the at least one antibody is a
15 monoclonal antibody.

In some embodiments, the at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a is an antibody or an antigen-binding fragment thereof that contains at least one complementary determining region (CDR) from the
20 light chain or the heavy chain of TS1/22 antibody (produced from ATCC deposit number HB202). In some embodiments, the at least one antibody or antigen-binding fragment thereof is an antibody or an antigen-binding fragment thereof that contains the three CDRs from the light chain of TS1/22 antibody or the three CDRs from the heavy chain of TS1/22 antibody. In some embodiments, the at least one antibody or antigen-
25 binding fragment thereof is an antibody or an antigen-binding fragment thereof that contains the three CDRs from the light chain of TS1/22 antibody and the three CDRs from the heavy chain of TS1/22 antibody.

In some embodiments, the at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a is an antibody or an antigen-binding fragment thereof that contains at least one complementary determining region (CDR) from the
30 light chain or the heavy chain of clone 25.3 antibody (Becker Coulter (PN IM0157 and PN IM1433U)). In some embodiments, the at least one antibody or antigen-binding fragment thereof is an antibody or an antigen-binding fragment thereof that contains the

three CDRs from the light chain of clone 25.3 antibody or the three CDRs from the heavy chain of clone 25.3 antibody. In some embodiments, the at least one antibody or antigen-binding fragment thereof is an antibody or an antigen-binding fragment thereof that contains the three CDRs from the light chain of clone 25.3 antibody and the three
5 CDRs from the heavy chain of clone 25.3 antibody.

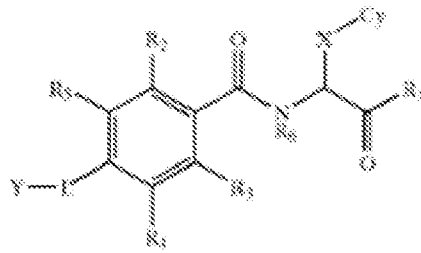
In some embodiments, the at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a is an antibody or an antigen-binding fragment thereof that contains at least one complementary determining region (CDR) from the light chain or the heavy chain of clone 27 antibody (Fisher Scientific No. 610826). In
10 some embodiments, the at least one antibody or antigen-binding fragment thereof is an antibody or an antigen-binding fragment thereof that contains the three CDRs from the light chain of clone 27 antibody or the three CDRs from the heavy chain of clone 27 antibody. In some embodiments, the at least one antibody or antigen-binding fragment thereof is an antibody or an antigen-binding fragment thereof that contains the three
15 CDRs from the light chain of clone 27 antibody and the three CDRs from the heavy chain of clone 27 antibody.

In some embodiments, the at least one antibody or antigen-binding fragment thereof is TS1/22 antibody or an antigen-binding fragment of TS1/22 antibody. In some embodiments, the at least one antibody or antigen-binding fragment is clone 25.3
20 antibody or an antigen-binding fragment of a clone 25.3 antibody. In some embodiments, the at least one antibody or antigen-binding fragment is a clone 27 antibody or an antigen-binding fragment of a clone 27 antibody.

In some embodiments, the at least one antibody is a feline antibody or an antigen-binding fragment thereof, or a felinized antibody or an antigen-binding
25 fragment thereof. In some embodiments of all of the methods described herein, the antibody is a single-chain antibody (e.g., a single-domain antibody). In some embodiments, the at least one antigen-binding fragment thereof is selected from the group consisting of: a Fab fragment, a F(ab')₂ fragment, and a scFv fragment. In some embodiments, the at least one small molecule is lovastatin, simvastatin, a lovastatin
30 derivative (e.g., a 6-exomethylene-modified, an 8-acyl-modified, or an alkylated lovastatin), a simvastatin derivative, LFA703, LFA451, LFA878, or XVA143, or a salt, solvate, or hydrate thereof.

In some embodiments, the at least one small molecule is:

5



, where

Cy is a non-aromatic carbocycle or heterocycle, optionally substituted with hydroxyl, mercapto, thioalkyl, halogen, oxo, thio, amino, aminoalkyl, amidine, guanidine, nitro, alkyl, alkoxy, or acyl; X is a divalent hydrocarbon chain, optionally substituted with hydroxyl, mercapto, halogen, amino, aminoalkyl, nitro, oxo, or thio, and optionally interrupted with N, O, S, SO, or SO₂; Y is a carbocycle or heterocycle, optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, thioalkyl, amino, aminoalkyl, carbocycle, or heterocycle ring, hydrocarbon, a halo-substituted hydrocarbon, amino, amidine, guanidine, cyano, nitro, alkoxy, or acyl; L is a divalent hydrocarbon chain, optionally substituted with hydroxyl, halogen, oxo, or thio; R₁ is H, OH, amino, O-carbocycle, or alkoxy, optionally substituted with amino, a carbocycle, or heterocycle; R₂₋₅ are independently H, hydroxyl, mercapto, halogen, cyano, amino, amidine, guanidine, nitro, or alkoxy; or R₃ or R₄ together form a fused carbocycle or heterocycle, optionally substituted with hydroxyl, halogen, oxo, thio, amino, amidine, guanidine, or alkoxy; and R₆ is H or a hydrocarbon chain, optionally substituted with a carbocycle or a heterocycle. In some embodiments, at least one small molecule can be any of the molecules shown in Figure 1. Any of the small molecules described herein can be in the form of a salt, solvate, or hydrate.

25 In some embodiments, the at least one small molecule and/or the at least one antibody or antigen-binding fragment thereof is administered intravenously, intraarterially, ocularly, orally, subcutaneously, intraperitoneally, or intramuscularly. In some embodiments, the at least one small molecule is administered orally. In some embodiments, the at least one antibody or antigen-binding fragment thereof is administered intravenously.

30

In some embodiments where the feline is administered at least one antibody or antigen-binding fragment thereof (e.g., any of the antibodies or antigen-binding fragments described herein), the feline is further administered at least one (e.g., one,

two, three, or four) small molecule (e.g., any of the small molecules described herein) that prevents LFA-1 from binding to ICAM-1. In some embodiments, the at least one small molecule and the at least one antibody or antigen-binding fragment thereof are administered in the same composition. In some embodiments, the at least one small molecule and the at least one antibody or antigen-binding fragment thereof are administered by different routes of administration.

In some embodiments of any of the methods described herein, the administering results in a decrease (e.g., a significant or observable decrease) in the severity, frequency, or duration of at least one symptom of retroviral infection in the feline. In some embodiments of any of the methods described herein, the administering results in a decrease (e.g., a statistically significant decrease) in retroviral titer in the feline. In some embodiments of any of the methods described herein, the administering results in an increase (e.g., a statistically significant increase) in the ratio of CD4⁺ T-cells to CD8⁺ T-cells in the feline. In some embodiments of any of the methods described herein, the at least one small molecule and/or the at least one antibody and/or antigen-binding fragment thereof is administered to the feline at least once a week (e.g., at least once a day). In some embodiments of any of the methods described herein, the administering does not result in detrimental immunosuppression in the feline.

In any of the methods described herein, the retroviral infection is feline immunodeficiency virus (FIV) or feline leukemia virus (FELV). In some embodiments of any of the methods described herein, the retroviral virion is feline immunodeficiency virus (FIV) or feline leukemia virus (FELV).

Also provided herein are methods of using at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18 (e.g., any of the exemplary antibodies or antibody fragments described herein) in the manufacture of a medicament for treating retrovirus infection in a feline, reducing retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell in a feline, and/or reducing syncytium transmission in a feline.

Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD11a and/or CD18 for use in treating retrovirus infection in a feline, reducing retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell in a feline, and/or reducing syncytium transmission in a feline.

Also provided herein are methods of using at least one small molecule that decreases LFA-1 binding to ICAM-1 (e.g., any of the small molecules that decrease LFA-1 binding to ICAM-1 described herein) in the manufacture of a medicament for treating retrovirus infection in a feline, reducing retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell in a feline, and/or reducing syncytium transmission in a feline.

Also provided herein are small molecules that decrease LFA-1 binding to ICAM-1 for use in treating retrovirus infection in a feline, reducing retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell in a feline, and/or reducing syncytium transmission in a feline.

Also provided herein are methods of using at least one antibody or antigen-binding fragment thereof that specifically binds to ICAM-1 (e.g., any of the exemplary antibodies or antigen-binding fragments thereof that specifically bind to ICAM-1 described herein) in the manufacture of a medicament for treating retrovirus infection in a feline, reducing retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell in a feline, and/or reducing syncytium transmission in a feline.

Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind to ICAM-1 for use in treating retrovirus infection in a feline, reducing retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell in a feline, and/or reducing syncytium transmission in a feline.

By the term “retrovirus infection” or “retrovirus infection in a feline” is meant a disease in a feline where the causative agent is a retrovirus. Non-limiting examples of feline retroviruses are described herein.

By the term “lymphocyte function associated antigen-1” or “LFA-1” is meant a heterodimer of CD11a and CD18. LFA-1 plays a role in lymphocyte adhesion and activation leading to a normal immune response. Intercellular adhesion molecules (ICAMs) -1, -2, and -3 are ligands for LFA-1 expressed in the endothelium, leukocytes, and other cell types. As used herein, CD11a, CD18, and LFA-1 refer to feline CD11a, feline CD18, and feline LFA-1.

By the term “intracellular adhesion molecule-1” or “ICAM-1” is meant a cell surface adhesion receptor that is a member of the immunoglobulin protein super-family. ICAM-1 is expressed on a variety of hematopoietic and non-hematopoietic cells and is up-regulated at sites of inflammation by a variety of inflammatory mediators. ICAM-1

binds to several different cellular receptors, including LFA-1. As used herein, ICAM-1 refers to feline ICAM-1.

By the term “small molecule” is meant any small organic (e.g., peptides, nucleotides, sugars, and/or lipids), small inorganic molecules (e.g., metal complexes), or small organic/inorganic complexes (e.g., metal/protein complexes). In some 5 embodiments, the small molecule can bind directly to CD11a and/or CD18 (bind to CD11a, CD18, or both CD11a and CD18), or ICAM-1. In some embodiments, the small molecule can bind indirectly to CD11a and/or CD18 or ICAM-1. In some embodiments, the small molecule can prevent or decrease LFA-1 binding to ICAM-1.

By the term “antibody” is meant any immunoglobulin or antibody (e.g., human, 10 feline, mouse, cartilagenous fish, or camelid antibodies), and any derivative or conjugate thereof, that specifically binds to an antigen. A wide variety of antibodies are known by those skilled in the art. Non-limiting examples of antibodies include: monoclonal antibodies (e.g., including full-length antibodies), polyclonal antibodies, 15 multi-specific antibodies (e.g., bi-specific antibodies), single-chain antibodies (e.g., single-domain antibodies, camelid antibodies, and cartilagenous fish antibodies), chimeric antibodies, feline antibodies, and feline antibodies. The term antibody also includes antibody derivatives and conjugates (e.g., an antibody conjugated to a stabilizing protein, a detectable moiety, or a therapeutic agent).

By the term “antigen-binding fragment” is meant any portion of a full-length 20 antibody that contains at least one variable domain (e.g., a variable domain of a mammalian (e.g., feline, human, or mouse) heavy or light chain immunoglobulin), a camelid variable antigen-binding domain (VHH), and a cartilagenous fish immunoglobulin new antigen receptor (Ig-NAR) domain) that is capable of specifically 25 binding to an antigen. Non-limiting examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments, diabodies, linear antibodies, and multi-specific antibodies formed from antibody fragments. Additional antibody fragments containing at least one camelid VHH domain or at least one cartilagenous fish Ig-NAR domain include mini-bodies, micro-antibodies, subnano-antibodies, and nano-antibodies, and any of the 30 other forms of antibodies described in U.S. Patent Application Publication No. 2010/0092470.

By the phrase “reduces LFA-1 binding to ICAM-1” is meant a statistically significant decrease (e.g., a detectable decrease, e.g., at least 10%, 15%, 20%, 25%,

30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% decrease) in the binding of LFA-1 to ICAM-1. The reduction in binding of LFA-1 binding to ICAM-1 can be compared to the amount of binding observed in the absence of treatment (e.g., in the absence of any agent as described herein, e.g., an antibody, antigen-binding fragment of an antibody, or small molecule). The binding of LFA-1 to ICAM-1 may be determined, for example, using in vitro cell culture assays or in vitro protein assays using purified proteins (e.g., BioCore technology) or cell lysates. Additional methods for determining binding of LFA-1 to ICAM-1 are known in the art. As used throughout the specification and claims, the phrase “LFA-1 binding to ICAM-1” and “ICAM-1 binding to LFA-1” is used interchangeably.

By the term “retrovirus virion” is meant a single infectious particle of a retrovirus (e.g., a feline retrovirus). A retroviral virion can contain an envelope derived from the plasma membrane of the host feline cell. Retroviral virions contain structural proteins that form a capsid that encloses a nucleic acid encoding the retroviral genome. As is known in the art, the structure of a retroviral virion and its components can vary between different feline retroviruses. In some embodiments, the retroviral virion can contain or present on its surface at least one of CD11a, CD18, or ICAM-1. The CD11a and CD18 present on the surface of a retroviral virion can form LFA-1 (a heterodimer of CD11a and CD18). The CD11a, CD18, or ICAM-1 contained or presented on the surface of the retroviral virion can contribute or play an active role in the entry (e.g., fusion) of the retroviral virion into a feline cell (e.g., a non-infected feline cell) or the budding or release of a retroviral virion into the extracellular space from an infected feline cell.

By the term “syncytium” is meant a large cell-like structure that contains cytoplasm, at least two nuclei, and an infectious retrovirus. A syncytium can express on its surface (plasma membrane) at least one of CD11a, CD18, or ICAM-1. CD11a and CD18 on the surface of a syncytium may form LFA-1 (a heterodimer of CD11a and CD18). The CD11a, CD18, or ICAM-1 present on the surface of the syncytium can contribute or play an active role in the fusion of the syncytium with a feline cell (e.g., a non-infected feline cell). The fusion (transient or permanent fusion) of a syncytium with a non-infected feline cell can promote the transmission of a retrovirus.

By the term “syncytium transmission” is meant the transfer of an infectious retrovirus from a syncytium to a non-infected feline cell that is mediated by fusion

(e.g., transient or permanent fusion) of a syncytium with a non-infected feline cell. In some non-limiting embodiments, syncytium transmission can result in the transfer of a retroviral virion or capsid to a non-infected feline cell. A syncytium can fuse (e.g., transiently or permanently) with the plasma membrane of a non-infected target cell.

5 Syncytial fusion with a non-infected target cell can be measured using any of the methods known in the art, including microscopic studies. A decrease in syncytium transmission in a feline can be detected by observing a reduction or a delay in the onset of retrovirus-induced dementia in a feline.

By the term “budding” is meant the release of a retroviral virion from an
10 infected host cell that includes the step of enveloping a retroviral capsid in the plasma membrane of the infected host cell.

By the term “entry” is meant the natural introduction of at least one retroviral virion into a non-infected feline cell. Entry of a retroviral virion into a non-infected feline cell can include the step of fusion of a retroviral envelope (enclosing the
15 retroviral capsid) with the plasma membrane of a non-infected feline cell. Retroviral virion entry can be measured using any of the methods known in the art, including microscopic studies.

By the term “feline antibody” is meant an antibody that is encoded by a nucleic acid (e.g., rearranged feline immunoglobulin heavy or light chain locus) present in the
20 genome of a feline. In some embodiments, a feline antibody is produced by a feline or in a feline cell culture (e.g., feline hybridoma cells). In some embodiments, a feline antibody is produced in a non-feline cell (e.g., a mouse or human cell line). In some embodiments, a feline antibody is produced in a bacterial or yeast cell.

By the term “felinized antibody” is meant a feline antibody which contains
25 minimal sequence derived from non-feline (e.g., mouse or human) immunoglobulin. In non-limiting examples, felinized antibodies are feline antibodies (recipient antibody) in which hypervariable region residues of the recipient antibody are replaced by hypervariable region residues from a non-feline species antibody (donor antibody), e.g., mouse, rat, rabbit, or human antibody having the desired specificity, affinity, and
30 capacity. In some embodiments, the Fv framework residues of the feline immunoglobulin are replaced by corresponding non-feline residues. In some embodiments, felinized antibodies may contain residues which are not found in the

recipient antibody or in the donor antibody. These modifications can be made to further refine antibody performance.

In some embodiments, the felinized antibody will contain substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops (complementary determining regions) correspond to those of a non-feline immunoglobulin and all or substantially all of the framework regions are those of a feline immunoglobulin sequence. The felinized antibody can also contain at least a portion of an immunoglobulin constant region (Fc), typically, that of a feline immunoglobulin. Felinized antibodies can be produced by molecular biology methods known in the art. Non-limiting examples of methods for generating felinized antibodies are described herein.

By the term "single-chain antibody" is meant a single polypeptide that contains at least one variable binding domain (e.g., a variable domain of a mammalian heavy or light chain immunoglobulin, a camelid variable antigen-binding domain (VHH), or a cartilagenous fish (e.g., shark) immunoglobulin new antigen receptor (Ig-NAR) domain) that is capable of specifically binding to an antigen. Non-limiting examples of single-chain antibodies are described herein, and include single-domain antibodies (described herein).

By the term "single-domain antibody" is meant a polypeptide that contains one camelid variable antigen-binding domain (VHH) or at least one cartilagenous fish (e.g., shark) immunoglobulin new antigen receptor (Ig-NAR) domain that is capable of specifically binding to an antigen. Non-limiting examples of single-domain antibodies are described herein and are known in the art (see, for example, the antibodies described in U.S. Patent Publication No. 2010/0092470).

An antibody or antigen-binding fragment thereof "specifically binds" to a particular antigen, e.g., CD11a and/or CD18 (an epitope on CD11a, CD18, or an epitope that is formed by both CD11a and CD18), or ICAM-1, when it binds to that antigen, but recognizes and binds to a lesser extent (e.g., does not recognize and bind) to other molecules in a sample. In some embodiments, an antibody or an antigen-binding fragment thereof selectively binds to an epitope with an affinity (K_D) equal to or less than 1×10^{-7} M (e.g., less than 1×10^{-8} M or less than 1×10^{-9} M) in phosphate buffered saline. The ability of an agent or molecule to specifically bind a second agent

or molecule may be determined using any of the methods known in the art or those methods described herein.

By the phrase “decrease in the severity, frequency, or duration of at least one symptom” is meant a detectable or observable decrease in the intensity or clinical scoring of at least one symptom of a retroviral infection in a feline, a detectable or observable decrease in the recurrence of at least one symptom of a retroviral infection in a feline, or a detectable or observable decrease in the duration of at least one symptom of a retroviral infection in a feline. Symptoms of a retroviral infection in a feline may be detected, observed, or scored by a veterinary professional or any other individual (e.g., an owner of a domestic feline). A decrease in the severity, frequency, or duration of at least one symptom of a retroviral infection in a feline receiving a treatment (e.g., administered at least one of the agents described herein) may be compared to the severity, frequency, or duration of at least one symptom of a retroviral infection in a control feline (e.g., a feline having the same retroviral infection not receiving treatment or the same feline prior to treatment).

By the term “symptom of retroviral infection” is meant any observable or detectable physiological event that is significantly correlated with a retroviral infection in a feline. A symptom of retroviral infection may be observed by a veterinary professional or a symptom of retroviral infection may be detected by laboratory testing. Non-limiting examples of symptoms of retroviral infection in a feline include: decreased levels of CD4⁺ T-cells (e.g., relative to a control feline or the same feline prior to infection), decreased ratio of CD4⁺ T-cells to CD8⁺ T-cells (e.g., relative to a control feline or the same feline prior to infection), decreased total white cell count (e.g., relative to a control feline or the same feline prior to infection), depression, lack of appetite, discharge from eyes and nose, fever, still and painful joints and muscles, difficulty breathing, sores in the mouth or on the lips, tongue, feet, or nose, weight loss, diarrhea, enlarged lymph nodes, skin infection, bladder infection, upper respiratory infection, seizures, vomiting, bloody diarrhea, weakness, conjunctivitis, stomatitis, odontoclasia, periodontitis, gingivitis, dementia, rhinitis, pneumonitis, enteritis, and dermatitis. Another symptom of a retroviral infection is uncontrolled proliferation of blood cells (feline leukemia). Additional symptoms of retroviral infection in a feline are described herein and are known in the art.

By the term "lovastatin derivative" is meant a modified lovastatin (a modified form of (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2S)-2-methylbutanoate) that has one or more of the following activities: ability to bind (e.g., K_D equal to or less than 1×10^{-6} M) to CD11a, CD18, ICAM-1, or both CD11a and CD18, or the ability to decrease binding of LFA-1 to ICAM-1. Non-limiting examples of lovastatin derivatives are described herein. Additional lovastatin derivatives are known in the art.

By the term "simvastatin derivative" is meant a modified simvastatin (a modified form of (1S,3R,7S,8S,8aR)-8-(2-((2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl)ethyl)-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate) that has one or more of the following activities: ability to bind to CD11a, CD18, ICAM-1, or both CD11a and CD18 (e.g., K_D equal to or less than 1×10^{-6} M), or the ability to decrease binding of LFA-1 to ICAM-1. Non-limiting examples of simvastatin derivatives are described herein. Additional examples of simvastatin derivatives are known in the art.

By the term "complementary determining region" or "CDR" is meant a region within an immunoglobulin (heavy or light chain immunoglobulin) that forms part of an antigen-binding site in an antibody or antigen-binding fragment thereof. As is known in the art, a heavy chain immunoglobulin contains three CDRs: CDR1, CDR2, and CDR3, respectively, and a light chain immunoglobulin contains three CDRs: CDR1, CDR2, and CDR3. In any antibody or antigen-binding fragment thereof, the three CDRs from the heavy chain immunoglobulin and the three CDRs from the light chain immunoglobulin together form an antigen-binding site in the antibody or antigen-binding fragment thereof. The Kabat Database is one system used in the art to number CDR sequences present in a light chain immunoglobulin or a heavy chain immunoglobulin.

By the term "feline" is meant any mammal belonging to the family Felidae. Non-limiting examples of felines include domestic cats, feral cats, jaguars, lions, and tigers.

By the term "non-aromatic" refers to carbocycle or heterocycle rings that do not have the properties of aromaticity. Aromaticity requires a ring to be planar, have π -orbitals that are perpendicular to the plane of the ring at each ring atom and satisfy the Huckel rule, where the number of π electrons in the ring is $(4n + 2)$ and n is an integer

(i.e., the number of π electrons is 2, 6, 10, or 14). Non-aromatic rings do not satisfy one or all of these criteria for aromaticity.

By the term “alkoxy” is meant O-alkyl, O-alkenyl, or an O-alkynyl group. Non-limiting examples of alkoxy groups include methoxy, ethoxy, propoxy, butoxy, i-
5 butoxy, s-butoxy, t-butoxy, pentyloxy, and hexyloxy.

By the term “amino” is meant a primary ($-\text{NH}_2$), secondary ($-\text{NHR}$), tertiary ($-\text{N}(\text{R})_2$), or quaternary ($-\text{N}^+(\text{R})_4$) amine, where R is a hydrocarbon chain, a hydroxy, a carbocycle, a heterocycle, or a hydrocarbon substituted with a carbocycle or a
heterocycle.

10 By the term “carboxyl” is meant a free acid $-\text{COOH}$, as well as esters thereof, such as alkyl, aryl, and aralkyl esters. In some embodiments, the esters are methyl, ethyl, propyl, butyl, i-butyl, s-butyl, and t-butyl esters.

By the term “carbocycle” is meant a mono-, bi-, or tri-cyclic carbon ring or ring system having 4-16 members (including bridged members) which is saturated,
15 unsaturated, or partially unsaturated, including aromatic (aryl) ring systems (unless specified as non-aromatic). Non-limiting examples of non-aromatic carbocyclic rings are cyclopropyl, cyclopropenyl, cyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclohexyl, and cyclohexenyl. Non-limiting examples of aromatic carbocyclic rings are phenyl and naphthyl.

20 By the term “heterocycle” is meant a mono-, bi-, or tri-cyclic ring system having 5-16 members, where at least one ring atom is a heteroatom (i.e., N, O, and S, as well as SO or SO_2). The ring system is saturated, unsaturated, or partially unsaturated and may be aromatic (unless specified as non-aromatic). Non-limiting examples of heterocycles are piperidine, piperazine, pyridine, pyrazine, pyrimidine, pyridazine,
25 morpholine, pyran, pyrrole, furan, thiophene (thienyl), imidazole, pyrazole, thiazole, isothiazole, dithiazole, oxazole, isoxazole, dioxazole, thiadiazole, oxadiazole, tetrazole, triazole, thiatriazole, oxatriazole, thiadiazole, and purine and benzofused derivatives thereof.

By the term “hydrocarbon chain” is meant a saturated, unsaturated, linear, or
30 branched carbon chain (i.e., alkyl, alkenyl, or alkynyl). Non-limiting examples of hydrocarbon chains contain 1-12 carbon atoms, 1-6 carbon atoms, or 1-4 carbon atoms (e.g., methyl, ethyl, propyl, butyl, or allyl).

By the phrase “optionally substituted with” is meant to mean, unless otherwise stated, that one or more of the specified substituents is covalently attached to the substituted moiety. When there is more than one substituent, the substituents can be the same or different groups.

5 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting.
10 All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and
15 advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 shows non-limiting examples of small molecules of Formula I that can be used in any of the methods described herein.

20 Figure 2 is a graph showing the reverse transcriptase activity (counts per minute) in feline 104-C1 T-cells at 3, 6, 9, or 12 days post-infection following a 2-hour spin inoculation with a preparation of FIV-PPR preincubated for 30 minutes with 0 µg/mL (control; PPR), 5 µg/mL, or 10 µg/mL TS1-22 antibody, clone 25.3 antibody, or clone 27 antibody, recovery for 3 hours at 37°C and 5% CO₂, and incubation in fresh media
25 containing the identical concentrations of each antibody for the duration of the study.

DETAILED DESCRIPTION

Provided herein are methods and compositions for treating or reducing retroviral infections in felines.

30

Feline Retroviral Diseases

The feline retroviruses feline immunodeficiency virus (FIV) and feline leukemia virus (FELV) cause disease in felines. FIV is a member of the lentivirus subfamily of retroviruses. It appears to be widely distributed worldwide since the 1960s. There are 5 FIV subtypes (clades A to E) and they all are infectious to a variety of susceptible wild and domestic feline species. In the U.S.A., the prevalence of FIV infection for domestic cats is estimated to be 1-4%, and this number is larger in the feral cat population.

FIV has a primary tropism for lymphocytes and gradually destroys sub-populations of T-lymphocytes. This cytopathic effect causes a progressive loss of CD4⁺ T-cells, a decrease in the CD4⁺ T-cell/CD8⁺ T-cell ratio, and the eventual loss of measurable levels of CD8⁺ T-cells in the late stages of infection. Cell-mediated immunity is impaired to a greater extent than antibody-mediated immunity. In addition, impaired production and dysregulation of various cytokines plays a role in pathogenesis of the disease. Antiretroviral drugs are too toxic for felines, leaving virtually no alternative for treating FIV.

FELV is an RNA retrovirus and was described in 1964 (Jarrett et al., *Nature* 202:566, 1964). FELV and FIV are in the same viral family. Four subgroups of FELV exist: A, B, C, and T. Approximately 0.5% of domestic felines are persistently infected with FELV, but many more domestic felines (>35%) have specific IgG antibodies. Transmission of FELV is mainly through saliva and friendly behaviors, such as sharing feeding bowls and mutual grooming.

FELV causes uncontrolled proliferation of blood cells in felines. Additional symptoms of FELV in felines include infections of the skin, bladder, and respiratory tract, oral disease, seizures, lymphadenopathy, skin lesions, fatigue, fever, weight loss, stomatitis, gingivitis, litter box avoidance, pancytopenia, poor grooming, reoccurring bacterial and viral illnesses, anemia, diarrhea, and jaundice.

Feline retroviral infection can be diagnosed by a skilled veterinary professional (e.g., a veterinarian, a veterinarian assistant, a veterinary technician, or a lab technician). For example, a feline retroviral infection may be determined by the observation of one or more symptoms of a retroviral infection in a feline. Non-limiting examples of symptoms of retroviral infection in a feline include, in addition to those listed above: decreased levels of CD4⁺ T-cells (e.g., relative to a control feline or the

same feline prior to infection), decreased ratio of CD4⁺ T-cells to CD8⁺ T-cells (e.g., relative to a control feline or the same feline prior to infection), decreased total white cell count (e.g., relative to a control feline or the same feline prior to infection), depression, lack of appetite, discharge from eyes and nose, fever, still and painful joints and muscles, difficulty breathing, sores in the mouth or on the lips, tongue, feet, or nose, weight loss, diarrhea, enlarged lymph nodes, skin infection, bladder infection, upper respiratory infection, seizures, dementia, vomiting, bloody diarrhea, weakness, conjunctivitis, stomatitis, odontoclasia, periodontitis, gingivitis, rhinitis, pneumonitis, enteritis, and dermatitis. Another symptom of a feline retroviral infection is dementia.

5

10 A feline retroviral disease can also be diagnosed using commercially available diagnostic kits (e.g., an assay that measures antibody titers in a feline that bind to the retrovirus (e.g., bind to a retroviral protein), an assay that measures the presence of proteins present in the retrovirus, or an assay that measures the presence of a nucleic acid present in the retrovirus). For example, diagnostic kits for the diagnosis of FIV or FELV in a feline are commercially available from Anigen Rapid (FIV Ab/FeLV Ag Test Kit) and Idexx (SNAP® Feline Triple® Test). Another diagnostic kit for the diagnosis of FIV is commercially available from Vita-Tech Laboratories (Feline Immunodeficiency Virus (FIV) DNA Confirmatory Test).

15

A skilled veterinary professional can determine the effectiveness of treatment of a retroviral infection in a feline by monitoring the number of symptoms or the severity, frequency, and/or duration of one or more symptoms of a retrovirus infection in a feline. For example, a useful treatment may result in a statistically significant or observable decrease in the severity, duration, or frequency of one or more (e.g., one, two, three, four, or five) symptoms in a feline having a retroviral infection. A useful treatment may also result in a decrease of retroviral titers in the feline (e.g., a decrease in retroviral titers in a specific biological fluid of the feline, e.g., serum, blood, lung fluid, nasal fluid, intestinal fluid, or gastric fluid). A useful treatment of FIV or FELV can also result in one or more (e.g., one, two, three, four, or five) of: an increase in CD4⁺ T-cell level (e.g., relative to a control feline having FIV or FELV, but not receiving treatment or the same feline prior to treatment), an increase in the CD4⁺ T-cell to CD8⁺ T-cell ratio (e.g., relative to a control feline having FIV or FELV, but not receiving treatment or the same feline prior to treatment), a decrease in CD8⁺ T-cell-mediated killing of CD4⁺ T-cells (e.g., relative to a control feline having FIV or FELV,

20

25

30

but not receiving treatment or the same feline prior to treatment), a decrease retroviral virion or syncytium fusion with non-infected feline cells (e.g., relative to a control feline having FIV or FELV, but not receiving treatment or the same feline prior to treatment), a decrease in syncytium transmission in a feline (e.g., relative to the amount of syncytium transmission in a feline without FIV or FELV, or in the same feline prior to treatment), and a decrease in the development or rate of onset of retrovirus-induced dementia (e.g., relative to a control feline having FIV, but not receiving treatment or the same feline prior to treatment). A skilled veterinary professional may adjust the treatment (e.g., dosage of agents and/or frequency or duration of administration of agents) based on the assessment of the effectiveness of the retroviral infection treatment as described herein.

LFA-1 and ICAM-1

Lymphocyte function-associated antigen-1 (LFA-1) is a cell surface heterodimer of CD11a and CD18 proteins that is a variety of cells, including T-cells, B-cells, macrophages, and neutrophils. LFA-1 is involved in the recruitment of cells to the site of infection. LFA-1 also binds to ICAM-1 on antigen-presenting cells, and functions as an adhesion molecule. In any of the embodiments described herein, an antibody or an antigen-binding fragment thereof can bind to CD11a or CD18, or may bind to an epitope formed by both CD11a and CD18. In any of the embodiments described herein, at least one small molecule can bind to CD11a or CD18, or both CD11a and CD18.

Intercellular adhesion molecule 1 (ICAM-1), also known as cluster of differentiation 54 (CD54), is a cell surface glycoprotein that is typically expressed on endothelial cells and cells of the immune system (e.g., antigen-presenting cells). As noted above, ICAM-1 binds to LFA-1.

Provided are methods that treat or reduce (e.g., significantly reduce) the likelihood of developing a retroviral infection in a feline. These methods require administering to a feline at least one (e.g., one, two, three, or four) agent (e.g., a small molecule or antibody or antigen-binding fragment thereof) that specifically binds to CD11a and/or CD18 (specifically binds to CD11a, CD18, or an epitope formed by both CD11a and CD18), or ICAM-1, and/or prevents LFA-1 present in a retroviral virion or syncytium from binding to ICAM-1 expressed on the surface of a feline cell (e.g., a

non-infected cell). In some embodiments, the at least one agent prevents LFA-1 present in a feline cell (e.g., a non-infected feline cell) from binding to ICAM-1 in a virion or syncytium.

5 During a retroviral infection in a feline, virions bud off from or are released from an infected host feline cell. Syncytia fuse (transiently or permanently) with non-infected feline cells. Retroviral virions incorporate cellular proteins (e.g., CD11a, CD18, or ICAM-1) expressed in feline cells during budding from the host cell. A syncytium incorporates cellular proteins (e.g., CD11a, CD18, or ICAM-1) expressed in a feline cell during fusion of the syncytium with a feline cell. CD11a and CD18 present
10 in the virion or syncytium can form LFA-1 (a heterodimer of CD11a and CD18). CD11a, CD18, or ICAM-1 present in the virion or syncytium can aid or facilitate the binding and/or entry of the retroviral virion, or the binding and/or fusion of a syncytium with a non-infected target cell in the feline. In some embodiments, the binding, entry, and/or fusion is facilitated by LFA-1 present on the surface of a virion or syncytium
15 binding to ICAM-1 present on the surface of a non-infected feline cell (e.g., directly or indirectly facilitating retroviral virion binding and/or entry, or syncytium binding and/or fusion with a non-infected feline cell). In some embodiments, the binding, entry, and/or fusion is facilitated by LFA-1 present on the surface of a non-infected feline cell binding to ICAM-1 present on the surface of a virion or syncytium (e.g.,
20 directly or indirectly facilitating retroviral virion binding and/or entry, or syncytium binding and/or fusion with a non-infected feline cell).

In any of the methods described herein and used throughout, LFA-1 or ICAM-1 can be found on the surface of a retroviral virion or syncytium or can be found in a feline cell (e.g., a non-infected feline cell).

25

Agents

Provided herein are methods of treating or reducing the likelihood of developing a retroviral infection that require the administration of at least one agent that prevents or decreases the binding of LFA-1 to ICAM-1.

30 Agents useful in the methods described herein include, without limitation, antibodies and antigen-binding fragments thereof, and small molecules. Non-limiting examples of antibodies and antigen-binding fragments thereof and small molecules that prevent or decrease the binding of LFA-1 to ICAM-1 are described herein. Additional

agents useful for preventing or decreasing the binding of LFA-1 to ICAM-1 are known in the art. Additional agents useful in the methods described herein can be identified using the screening methods described herein. Two or more (e.g., two, three, four, or five) of the agents described herein can be administered to a feline in any combination without limitation.

Antibodies and Antigen-Binding Fragments Thereof

Agents useful in any of the methods described herein include antibodies and antigen-binding fragments thereof. In some embodiments, the antibodies and antigen-binding fragments thereof bind to an epitope on CD11a, CD18, or ICAM-1, or an epitope formed by both CD11a and CD18, that is present in or on the surface of a virion or syncytium or bind to an epitope on CD11a, CD18, or ICAM-1, or an epitope formed by both CD11a and CD18, that is present in or on the surface of a non-infected feline cell. In some embodiments, the antibodies and antigen-binding fragments thereof bind to an epitope on ICAM-1 (e.g., ICAM-1 present in or on the surface of a virion, syncytium, or non-infected feline cell).

In some embodiments, the antibody or antigen-binding fragment thereof specifically binds to (e.g., binds to an epitope present in) feline CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18. In some embodiments, the antibody or antigen-binding fragment thereof prevents or reduces the binding of a LFA-1 present in or on the surface of a virion or syncytium to ICAM-1 present in or on the surface of a feline cell (e.g., a non-infected feline cell). In some embodiments, the antibody or antigen-binding fragment thereof prevents or reduces the binding of a LFA-1 present in or on the surface of a non-infected feline cell to ICAM-1 present in or on the surface of a virion or syncytium. The prevention or reduction of binding of a LFA-1 to ICAM-1 can occur directly (the antibody or antigen-binding fragment thereof binds directly to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18) or indirectly (the antibody or antigen-binding fragment thereof binds to a protein, lipid, and/or carbohydrate that directly binds to CD11a, CD18, ICAM-1, or both CD11a and CD18).

Methods for determining the ability of an antibody or antigen-binding fragment thereof to bind to a target protein (e.g., CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18) can be performed using methods known in the art. Non-limiting examples of such methods include competitive binding assays using antibodies

known to bind the target protein (e.g., CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18), enzyme-linked immunosorbent assays, BioCoRE®, affinity columns, immunoblotting, or protein array technology. In some embodiments, the binding activity of the antibody or antigen-binding fragment thereof is determined by contacting a feline cell (e.g., a CD8⁺ T-cell, a CD4⁺ T-cell, a dendritic cell, a fibroblast, or an epithelial cell) with the antibody or antigen-binding fragment thereof. Additional methods for identifying agents (antibodies or antigen-binding fragments thereof) that bind to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18 are described herein.

In some embodiments of any of the methods described herein, the antibody or antigen-binding fragment thereof binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18 with an K_D equal to or less than 1×10^{-7} M, a K_D equal to or less than 1×10^{-8} M, a K_D equal to or less than 5×10^{-8} M, a K_D equal to or less than 1×10^{-9} M, or a K_D equal to or less than 5×10^{-9} M under physiological conditions (e.g., phosphate buffered saline).

An antibody can be any immunoglobulin or antibody (including, for example, variants, derivatives, and conjugates thereof) that specifically binds an antigen, such as light or heavy chain immunoglobulin molecules and immunologically-active fragments of immunoglobulin molecules. An antibody can also be a single-chain antibody (e.g., a single-domain antibody), such as a single-chain camelid or cartilagenous fish (e.g., shark) antibody, or a single-chain antibody that contains at least one camelid variable antigen-binding domain (VHH) or at least one cartilagenous fish (e.g., shark) immunoglobulin new antigen receptor (Ig-NAR) domain (see, for example, the antibodies described in U.S. Patent Publication No. 2010/0092470). An antibody can be a whole antibody molecule or an antibody multimer.

Antibodies as referred to herein include variants (including derivatives) of antibodies, antibody multimers, and antibody fragments. Examples of antibodies include, but are not limited to: single-chain Fvs (scFvs), single-domain antibodies (e.g., mini-antibodies, micro-antibodies, subnano-antibodies, and nano-antibodies, see for example, the antibodies described in U.S. Patent Application Publication No. 2010/0092470), Fab fragments, Fab' fragments, F(ab')₂, disulfide-linked Fvs (sdFvs), Fvs, and fragments containing, either a VL or a VH domain. The term "single chain

Fv” or “scFv” as used herein refers to a polypeptide comprising at least one VL domain of antibody linked to at least one VH domain of an antibody.

Antibodies useful in the methods described herein include, but are not limited to, polyclonal, monoclonal, multispecific (e.g., bi-specific), feline, human, mouse, 5 rabbit, or rat antibodies, chimeric antibodies (e.g., human-feline chimera, mouse-feline chimera, rat-feline chimera, or rabbit-feline chimera), single chain antibodies (e.g., single-domain antibodies), Fab fragments, F(ab') fragments, intracellularly-made antibodies (i.e., intrabodies), epitope-binding fragments of any of the above, and any of the other antibodies or antigen-binding fragments described herein. The antibodies or 10 antigen-binding fragments thereof can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂) or subclass. In some embodiments, the antibody or antigen-binding fragment thereof is an IgG₁ antibody or fragment thereof. In other embodiments, the antibody or antigen-binding fragment thereof is an IgG₄ antibody or antigen-binding fragment thereof. Immunoglobulins 15 may have both a heavy and light chain.

An isolated feline CD11a, CD18, ICAM-1, or LFA-1 (heterodimer of CD11a and CD18), or fragment thereof can be used as an immunogen to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. For example, feline CD11a can be purified using the methods described in Shimojima et al. 20 (*Microbes Infection* 5:1171-1176, 2003). In this method, feline CD11a is isolated by first cloning feline CD11a from a peripheral blood mononuclear cell (PBMC) cDNA library using the homologue cloning method using the polymerase chain reaction (Nishimura et al., *Immunogenetics* 50:369-370, 1999) and expressing the cDNA in insect cells using a recombinant baculovirus vector containing the cloned cDNA 25 downstream of a polyhedrin promoter (Shimojima et al., *J. Vet. Med. Sci.* 59:467-469, 1997; and Shimojima et al., *Vet. Immunol. Immunopathol.* 61:17-23, 1998).

The full-length polypeptide or protein (e.g., CD11a, CD18, LFA-1, or ICAM-1) can be used or, alternatively, antigenic peptide fragments can be used as immunogens. The antigenic peptide of a protein comprises at least 8 (e.g., at least 10, 15, 20, or 30) 30 amino acid residues of the amino acid sequence of the protein (e.g., CD11a, CD18, or ICAM-1) and encompasses an epitope of the protein such that an antibody raised against the peptide forms a specific immune complex with the protein.

An immunogen typically is used to prepare antibodies by immunizing a suitable subject (e.g., rabbit, goat, mouse, or other mammal). An appropriate immunogenic preparation can contain, for example, a recombinantly expressed or a chemically synthesized polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or a similar immunostimulatory agent.

Polyclonal antibodies can be prepared as described above by immunizing a suitable subject with feline CD11a, CD18, ICAM-1, or LFA-1, or an antigenic peptide thereof, as an immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. If desired, the antibody molecules can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the specific antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler et al. (*Nature* 256:495-497, 1975), the human B cell hybridoma technique (Kozbor et al., *Immunol. Today* 4:72, 1983), the EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96, 1985), or trioma techniques. The technology for producing hybridomas is well known (see, generally, *Current Protocols in Immunology*, 1994, Coligan et al. (Eds.), John Wiley & Sons, Inc., New York, NY). Hybridoma cells producing a monoclonal antibody are detected by screening the hybridoma culture supernatants for antibodies that bind the polypeptide of interest, e.g., using a standard ELISA assay.

As an alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody directed against a polypeptide can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with the polypeptide of interest. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP* Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening an antibody display library can be found in, for example, U.S. Pat. No. 5,223,409; WO

92/18619; WO 91/17271; WO 92/2079; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; WO 90/02809; Fuchs et al., *Bio/Technology* 9:1370-1372, 1991; Hay et al., *Hum. Antibod. Hybridomas* 3:81-85, 1992; Huse et al., *Science* 246:1275-1281, 1989; Griffiths et al., *EMBO J.* 12:725-734, 1993.

5 In some embodiments of any of the methods described herein, the antibodies or antigen-binding fragments are feline antibodies or felinized antibodies. In some embodiments, a felinized antibody is a feline antibody that has been engineered to contain at least one complementary determining region (CDR) present in a non-feline antibody (e.g., a human, rat, mouse, rabbit, or goat antibody). In some embodiments, a
10 felinized antibody or fragment thereof can contain all three CDRs of a light chain of a mouse (e.g., the TS1/22 antibody, the clone 25.3 antibody, or the clone 27 antibody) or human monoclonal antibody that specifically binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18. In some embodiments, the felinized
15 antibody or fragment thereof can contain all three CDRs of a heavy chain of a mouse (e.g., the TS1/22 antibody, the clone 25.3 antibody, or the clone 27 antibody) or human monoclonal antibody that specifically binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18. In some embodiments, the felinized antibody or
20 fragment thereof can contain all three CDRs of a heavy chain and all three CDRs of a light chain of a mouse (e.g., the TS1/22 antibody, the clone 25.3 antibody, or the clone 27 antibody) or human monoclonal antibody that specifically binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18.

 Antibodies of the invention may also include multimeric forms of antibodies. For example, antibodies of the invention may take the form of antibody dimers, trimers, or higher-order multimers of monomeric immunoglobulin molecules. Dimers of whole
25 immunoglobulin molecules or of F(ab')₂ fragments are tetravalent, whereas dimers of Fab fragments or scFv molecules are bivalent. Individual monomers within an antibody multimer may be identical or different, i.e., they may be heteromeric or homomeric antibody multimers. For example, individual antibodies within a multimer may have the same or different binding specificities.

30 Multimerization of antibodies may be accomplished through natural aggregation of antibodies or through chemical or recombinant linking techniques known in the art. For example, some percentage of purified antibody preparations (e.g., purified IgG₁ molecules) spontaneously form protein aggregates containing antibody homodimers

and other higher-order antibody multimers. Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art. For example, heterobifunctional crosslinking agents including, but not limited to, SMCC (succinimidyl 4-(maleimidomethyl)cyclohexane-1-carboxylate) and SATA (N-succinimidyl S-acethylthio-acetate) (available, for example, from Pierce
5 Biotechnology, Inc. (Rockford, IL)) can be used to form antibody multimers. An exemplary protocol for the formation of antibody homodimers is given in Ghetie et al. (*Proc. Natl. Acad. Sci. U.S.A.* 94: 7509-7514, 1997). Antibody homodimers can be converted to Fab₂ homodimers through digestion with pepsin. Another way to form
10 antibody homodimers is through the use of the autophilic T15 peptide described in Zhao et al. (*J. Immunol.* 25:396-404, 2002).

Alternatively, antibodies can be made to multimerize through recombinant DNA techniques. IgM and IgA naturally form antibody multimers through the interaction with the mature J chain polypeptide. Non-IgA or non-IgM molecules, such
15 as IgG molecules, can be engineered to contain the J chain interaction domain of IgA or IgM, thereby conferring the ability to form higher order multimers on the non-IgA or non-IgM molecules (see, for example, Chintalacheruvu et al., *Clin. Immunol.* 101:21-31, 2001, and Frigerio et al., *Plant Physiol.* 123:1483-1494, 2000). IgA dimers are naturally secreted into the lumen of mucosa-lined organs. This secretion is mediated
20 through interaction of the J chain with the polymeric IgA receptor (pIgR) on epithelial cells. If secretion of an IgA form of an antibody (or of an antibody engineered to contain a J chain interaction domain) is not desired, it can be greatly reduced by expressing the antibody molecule in association with a mutant J chain that does not interact well with pIgR (Johansen et al., *J. Immunol.*, 167:5185-192, 2001). ScFv
25 dimers can also be formed through recombinant techniques known in the art; an example of the construction of scFv dimers is given in Goel et al. (*Cancer Res.* 60:6964-71, 2000). Antibody multimers may be purified using any suitable method known in the art, including, but not limited to, size exclusion chromatography.

Any of the antibodies or antigen-binding fragments described herein may be
30 conjugated to a stabilizing molecule (e.g., a molecule that increases the half-life of the antibody or antigen-binding fragment thereof in a feline or in solution). Non-limiting examples of stabilizing molecules include: a polymer (e.g., a polyethylene glycol) or a protein (e.g., serum albumin, such as feline serum albumin). Any of the antibodies or

antigen-binding fragments described herein may be conjugated to a label (e.g., a fluorophore) or a therapeutic agent (e.g., a proteinaceous therapeutic agent).

An exemplary anti-CD11a antibody or antigen-binding fragments thereof that can be used in any of the methods described herein is the antibody produced from clone 25.3 (Becker Coulter (PN IM0157 and PN IM1433U) and antigen-binding fragments of the antibody produced from clone 25.3, the antibody produced from clone 27 (Fisher Scientific No. 610826) and antigen-binding fragments of the antibody produced from clone 27, and TS1/22 and antigen-binding (feline CD11a-binding) fragments of TS1/22. The antibody produced from clone 25.3 has been described as having the ability to bind to an epitope on feline CD11a (Saalmüller et al., *Cell. Immunol.* 236:51-58, 2005). The antibody produced from clone 25.3 and the TS1/22 antibody have been shown to bind to the same epitope (IdeA) within the I domain of CD11a (Champe et al., *J. Biol. Chem.* 270:1388-1394, 1995), thus both the antibody produced by clone 25.3 and the TS1/22 antibody (and antigen-binding fragments of each antibody) can be used to perform the present methods. The data provided herein indicate that the antibody produced by clone 27 binds to CD11a, and prevents FIV infection of feline cells (Figure 2).

Additional anti-CD11a antibodies are known in the art and include, for example antibodies produced from clone 2D7, HI111, M17/4, TS2/4, WT.1, or H155-78 (BioLegend, San Diego, CA), efalizumab, the antibody produced from clone MEM-25 (Antibodies Online, Aachen, Germany), and the anti-feline CD11a antibody described in Shimojima et al. (*Microbes and Infection* 5:1171-1176, 2003), or antigen-binding fragments thereof. Additional anti-CD11a antibodies can contain at least one CDR (e.g., at least three light chain CDRs and/or at least three heavy chain CDRs) of the antibody produced from clone 25.3 (Becker Coulter (PN IM0157 and PN IM1433U), the antibody produced from clone 27 (Fisher Scientific No. 610826), or the anti-feline CD11a antibody described in Shimojima et al. (*Microbes and Infection* 5:1171-1176, 2003).

Additional antibodies or antigen-binding fragments useful in any of the methods described herein bind competitively with the antibody TS1/22 to feline CD11a (e.g., binds competitively with the antibody TS1/22 to a cell that expresses feline CD11a). Additional antibodies or antigen-binding fragments useful in any of the methods described herein bind competitively with the clone 25.3 antibody to feline CD11a (e.g.,

binds competitively with the clone 25.3 antibody to a cell that expresses feline CD11a) or bind competitively with the clone 27 antibody to feline CD11a (e.g., binds competitively with the clone 27 antibody to a cell that expresses feline CD11a). Additional anti-CD11a antibodies are known in the art.

5 Non-limiting examples of anti-CD18 antibodies or antigen-binding fragments thereof that can be used in any of the methods described herein include antibodies produced from clone M18/2, TS1/18, or WT.3 (BioLegend, San Diego, CA), the antibodies described in U.S. Patent Application Publication No. 2004/0101527, and the antibodies described in U.S. Patent Nos. 6,689,869 and 5,914,112, or antigen-binding
10 fragments thereof. Additional anti-CD18 antibodies are known in the art.

 Non-limiting examples of anti-ICAM-1 antibodies or antigen-binding fragments thereof that can be used in any of the methods described herein include antibody #4915 (Cell Signaling Technology), sc-107 (Santa Cruz Biotechnology), RabMab® (Epitomics), anti-CD54 (Cell Applications, Inc.), the antibodies described in U.S.
15 Patent Application Publication No. 2011/0052601, and the antibodies described in U.S. Patent No. 5,324,510, 5,695,760, or antigen-binding fragments thereof. Additional anti-ICAM-1 antibodies are known in the art.

Small Molecules

20 Agents useful in the methods described herein also include small molecules. Small molecules useful in the methods described herein can be small organic (e.g., peptides, nucleotides, sugars, and/or lipids) or small inorganic molecules (e.g., metal complexes). Small molecules useful in the described methods may be identified using any of the screening methods described herein. Small molecules can block or decrease
25 binding of LFA-1 to ICAM-1 by binding directly to CD11a, CD18, ICAM-1, or both CD11a and CD18. In some embodiments, the small molecule can bind LFA-1 at or around the same site that ICAM-1 binds to LFA-1. In some embodiments, the small molecule can bind LFA-1 at a site that is remote from the site that ICAM-1 binds to LFA-1 (e.g., the binding of the small molecule induces a change in the tertiary structure
30 of LFA-1 that prevents or decreases binding of LFA-1 to ICAM-1, or the binding of the small molecule prevents the oligomerization or heterodimerization of LFA-1 that prevents or decreases the the binding of LFA-1 to ICAM-1).

In some embodiments, the small molecule can bind ICAM-1 at or around the same site that LFA-1 binds to ICAM-1. In some embodiments, the small molecule can bind ICAM-1 at a site that is remote from the site that LFA-1 binds to ICAM-1 (e.g., the binding of the small molecule induces a change in the tertiary structure of ICAM-1 that prevents or decreases binding of ICAM-1 to a LFA-1, or the binding of the small molecule prevents the oligomerization of ICAM-1 that prevents or decreases the the binding of ICAM-1 to LFA-1).

In some embodiments, the small molecules can block or decrease binding of LFA-1 to ICAM-1 by binding to another molecule (e.g., protein, lipid, and/or carbohydrate) that binds to CD11a, CD18, ICAM-1, or both CD11a and CD18.

Non-limiting examples of small molecules that can be used in any of the methods described herein include statin or statin derivatives. Lovastatin and simvastatin have been demonstrated to inhibit LFA-1 activation (Wang et al., *Biol. Blood Marrow Transplant.* 15:1513-1522, 2009; Almog, *Chest* 124:740-743, 2003). Some statin derivatives (e.g., LFA878, LFA703, LFA451, and XVA143) have been identified as preventing the ability of CD11a to bind to ICAM-1 (Weitz-Schmidt et al., *J. Biol. Chem.* 279:46764-46771, 2004; Welzenbach et al., *J. Biol. Chem.* 277:10590-10598, 2002).

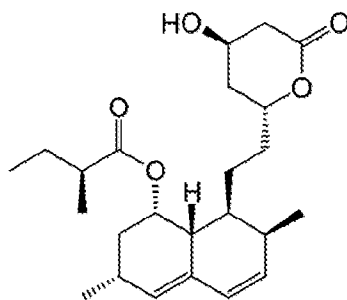
Thus, lovastatin, simvastatin, lovastatin derivatives, simvastatin derivatives, LFA878, LFA703, LFA451, and XVA143, as well as other statins may prevent or decrease the ability of LFA-1 (comprising CD11a and CD18) from binding to ICAM-1.

Non-limiting examples of small molecules that can be used in any of the methods described herein include: lovastatin, simvastatin, pravastatin, atorvastatin, fluvastatin, rosuvastatin, amlodipine, cerivastatin, mevastatin, pitavastatin, lovastatin derivatives, simvastatin derivatives, LFA878, LFA703, LFA451, and XVA143. In some embodiments of any of the methods described herein the small molecule is lovastatin, simvastatin, a lovastatin derivative, a simvastatin derivative, LFA878, LFA703, LFA451, and XVA143, or a salt, solvate, or hydrate thereof.

30

Lovastatin has the following chemical structure:

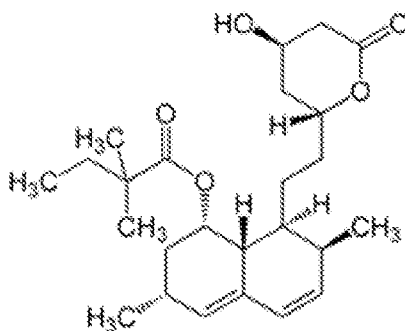
5



Derivatives of lovastatin can be generated using methods of chemical
10 modification known in the art. For example, methods of generating derivatives of
lovastatin, as well as lovastatin derivatives (e.g., exomethylene-modified, an 8-acyl-
modified, or an alkylated lovastatin derivatives) are described in U.S. Patent Nos.
4,866,186 and 6,472,542).

Simvastatin has the following chemical structure:

15



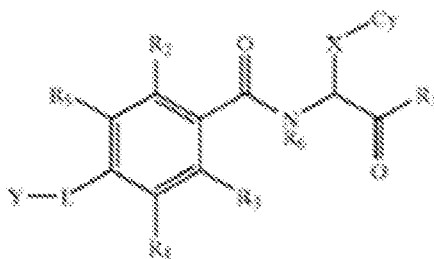
20

Derivatives of simvastatin can be generated using methods of chemical
modification known in the art. For example, methods of generating derivatives of
simvastatin, as well as simvastatin derivatives (e.g., 3-keto, 5-hydroxy-derivatives, di-
25 and hydroxy derivatives) are described in U.S. Patent Nos. 6,252,091; 6,541,511;
6,384,238; 6,252,091; 6,100,407; 5,393,893; 5,159,104; and 4,965,200; and in U.S.
Patent Application Publication Nos. 2003/0176501 and 2002/0035274.

Additional methods for the modification of statins (e.g., lovastatin and
simvastatin) are described in U.S. Patent Nos. 5,134,124; 7,855,302; 5,462,716;
30 7,563,909; 7,420,078; 7,304,091; 7,297,808; and 7,166,638; and U.S. Patent
Application Publication Nos. 2011/0054193; 2009/0118317; 2008/0289056;
2008/0096908; 2008/0090857; 2007/0072942; 2005/0228042; 2005/0165084;
2005/0148654; 2004/0235935; and 2004/0186313.

Statin derivatives (e.g., lovastatin and simvastatin derivatives) can be used in any of the methods described herein. The ability of a statin derivative, a simvastatin derivative, or a lovastatin derivative to decrease LFA-1 binding to ICAM-1 can be determined using affinity assays known in the art (e.g., BioCore technology) and assays described herein.

Additional small molecules that are useful in any of the methods described have the structure of Formula I:



In Formula I, Cy is a non-aromatic carbocycle or heterocycle, optionally substituted with hydroxyl (-OH), mercapto (-SH), thioalkyl, a halogen (e.g., F, Cl, Br, or I), oxo (=O), thio (=S), amino, aminoalkyl, amidine (-C(NH)-NH₂), guanidine (-NH₂-C(NH)-NH₂), nitro, alkyl, or alkoxy. In some embodiments, Cy is a 3-5 member ring. In some embodiments, Cy is a 5- or 6-member non-aromatic heterocycle, optionally substituted with hydroxyl, mercapto, halogen (e.g., F or Cl), oxo (=O), thio (=S), amino, amidine, guanidine, nitro, alkyl, or alkoxy. In some embodiments, Cy is a 5-member non-aromatic heterocycle, optionally substituted with hydroxyl, oxo, thio, Cl, C₁₋₄ alkyl (e.g., methyl), or C₁₋₄ alkanoyl (e.g., acetyl, propanoyl, or butanoyl). In some embodiments, the non-aromatic heterocycle contains one or heteroatoms (N, O, or S) and is optionally substituted with hydroxyl, oxo, mercapto, thio, methyl, acetyl, propanoyl, or butyl. In some embodiments, the non-aromatic heterocycle contains at least one nitrogen atom that is optionally substituted with methyl or acetyl. In some embodiments, the non-aromatic heterocycle is selected from: piperidine, piperazine, morpholine, tetrahydrofuran, tetrahydrothiophene, oxazolidine, and thiazolidine, optionally substituted with hydroxy, oxo, mercapto, thio, alkyl, or alkanoyl. In some embodiments, Cy is a non-aromatic heterocycle selected from: tetrahydrofuran-2-yl, thiazolidin-5-yl, thiazolidin-2-one-5-yl, thiazolidin-2-thione-5-yl, and cyclopropapyrrolidine.

In some embodiments Cy is a 3-6 member carbocycle, optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, amino, amidine, guanidine, alkyl, alkoxy,

or acyl. In some embodiments, the carbocycle is saturated or partially unsaturated. In some embodiments Cy is a carbocycle selected from the group consisting of cyclopropyl, cyclopropenyl, cyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclohexyl, and cyclohexenyl.

5 In Formula I, X is a C₁₋₅ divalent hydrocarbon linker, optionally having one or more carbon atoms replaced with N, O, S, SO, or SO₂, and is optionally substituted with hydroxyl, mercapto, halogen, amino, aminoalkyl, nitro, oxo, or thio. In some embodiments X will have at least one carbon atom. Replacements and substitutions can form an amide moiety (-NRC(O)- or -C(O)NR-) within the hydrocarbon chain or at
10 either or both ends. Other moieties can include sulfonamide (-NRSO₂- or -SO₂NR), acyl, ether, thioether, and amine. In some embodiments, X is the group -CH₂-NR₆-C(O)-, where the carbonyl -C(O)- portion thereof is adjacent (i.e., covalently bonded) to Cy and R₆ is alkyl (e.g., methyl) or H.

In Formula I, Y is a carbocycle or heterocycle, optionally substituted with
15 hydroxyl, mercapto, halogen, oxo, thio, a hydrocarbon, a halo-substituted hydrocarbon, amino, amidine, guanidine, cyano, nitro, alkoxy, or acyl. In some embodiments, Y is aryl or heteroaryl, optionally substituted with halogen or hydroxyl. In some embodiments, Y is phenyl, furan-2-yl, thiophene-2-yl, phenyl substituted with a halogen (e.g., Cl) or hydroxyl (e.g., at the meta position).

20 In Formula I, L is a divalent hydrocarbon, optionally having one or more carbon atoms replaced with N, O, S, SO, or SO₂, and optionally being substituted with hydroxyl, halogen, oxo, or thio; or three carbon atoms of the hydrocarbon are replaced with an amino acid residue. In some embodiments, L is less than 10 atoms or less than 5 atoms in length. In some embodiments, L is 5 or 3 atoms in length. In some
25 embodiments, L is selected from: -CH=CH-C(O)-NR₆-CH₂-, -CH₂-NR₆-C(O)-, -C(O)-NR₆-CH₂-, -CH(OH)-(CH₂)₂-, -(CH₂)₂-CH(OH)-, -(CH₂)₃-, -C(O)-NR₆-CH(R₇)-C(O)-NR₆-, -NR₆-C(O)-CH(R₇)-NR₆-C(O)-, -CH(OH)-CH₂-O-, and -CH(OH)-CF₂-CH₂-, where each R₆ is independently H or alkyl, and R₇ is an amino acid side chain. Amino acid side chains can include non-naturally occurring side chains, such as phenyl, or
30 naturally-occurring side chains. In some embodiments the side chains are Phe, Tyr, Ala, Gln, and Asn. In some embodiments, L is -CH=CH--C(O)-NR₆-CH₂-, where the -CH=CH- moiety is adjacent (i.e., covalently bounded) to Y. In some embodiments, L is -CH₂-NR₆-C(O)-, where the methylene moiety (-CH₂-) is adjacent to Y.

In Formula I, R₁ is H, OH, amino, O-carbocycle, or alkoxy, optionally substituted with amino, a carbocycle, or a heterocycle. In some embodiments, R₁ is H, phenyl, or C₁₋₄ alkoxy, optionally substituted with a carbocycle, such as phenyl. In some embodiments, R₁ is H. In some embodiments, R₁ is methoxy, ethoxy, propyloxy, butyloxy, isobutyloxy, s-butyloxy, t-butyloxy, phenoxy, or benzyloxy. In some
5
embodiments R₁ is NH₂. In some embodiments, R₁ is ethoxy. In some embodiments, R₁ is isobutyloxy. In some embodiments, R₁ is an alkoxy substituted with amino, for example, 2-aminoethoxy, N-morpholinoethoxy, N,N-dialkylaminoethoxy, or quaternary ammonium hydroxy alkoxy (e.g., trimethylammoniumhydroxyethoxy).

10
In Formula I, R₂₋₅ are independently H, hydroxyl, mercapto, halogen, cyano, amino, amidine, guanidine, nitro, or alkoxy; or R₃ and R₄ together form a fused carbocycle or heterocycle, optionally substituted with hydroxyl, halogen, oxo, thio, amino, amidine, guanidine, or alkoxy. In some embodiments, R₂ and R₃ are independently H, F, Cl, Br, or I. In some embodiments, R₄ and R₅ are both H. In some
15
embodiments, one of R₂ and R₃ is a halogen, while the other is hydrogen or a halogen. In some embodiments, R₃ is Cl, while R₂, R₄, and R₅ are each H. In some embodiments, R₂ and R₃ are both Cl, while R₄ and R₅ are both H.

R₆ is H or a hydrocarbon chain, optionally substituted with a carbocycle or a heterocycle. In some embodiments, R₆ is H or alkyl (e.g., methyl, ethyl, propyl, butyl, i-butyl, s-butyl, or t-butyl). In some embodiments R₆ is H. Non-limiting specific
20
examples of compounds of Formula I are shown in Figure 1. Additional examples of small molecules of Formula I are described in U.S. Patent No. 6,872,735 (herein incorporated by reference in its entirety).

Small molecules of Formula I can be prepared according to established organic
25
synthesis techniques from starting materials and reagents that are commercially available. Many standard chemical techniques and procedures are described in March, J., "Advanced Organic Chemistry," McGraw-Hill, New York, 1977; and Collman, J., "Principles and Applications of Organotransition Metal Chemistry," University Science, Mill Valley, 1987; and Larock, R., "Comprehensive Organic Transformations,"
30
Verlag, New York, 1989. Depending on the particular substituents present on the compounds, suitable protection and deprotection procedures may be required. Numerous protecting groups are described in Greene and Wuts, "Protective Groups in Organic Chemistry," 2nd Edition, John Wiley and Sons, 1991, as well as detailed

protection and deprotection procedures. For example, suitable amino protecting groups include t-butyloxycarbonyl (Boc), fluorenylmethyloxycarbonyl (Fmoc), 2-trimethylsilylethoxy carbonyl (Teoc), 1-methyl-1-(4-biphenyl)ethoxycarbonyl (Bpoc), allyloxycarbonyl (Alloc), and benzyloxycarbonyl (Cbz). Carboxyl groups can be protected as fluorenylmethyl groups or alkyl esters (e.g., methyl, ethyl, or alkenyl esters, such as allyl). Hydroxyl groups can be protected with trityl, monomethoxytrityl, dimethoxytrityl, and trimethoxytrityl groups. Additional specific methods for generating small molecules of Formula I are described in U.S. Patent No. 6,872,735 (herein incorporated by reference in its entirety).

10

Veterinary Compositions

Also provided herein are veterinary compositions that contain at least one (e.g., one, two, three, four, or five) of the agents described herein. In some embodiments, the at least one agent decreases (e.g., significantly decreases) or inhibits binding of a LFA-1 to ICAM-1 in a feline.

15

Two or more (e.g., two, three, four, or five) agents can be present in a veterinary composition in any combination, e.g., two or more proteins (e.g., antibodies and/or antigen-binding antibody fragments), two or more small molecules (e.g., lovastatin, simvastatin, lovastatin derivatives, simvastatin derivatives, LFA703, LFA451, LFA878, XVA143, or any compound of Formula I, or any combination thereof), or combinations of at least one protein (e.g., antibodies and/or antigen-binding antibody fragments) and at least one small molecule (e.g., lovastatin, simvastatin, lovastatin derivatives, simvastatin derivatives, LFA703, LFA451, LFA878, XVA143, or any compound of Formula I, or any combination thereof).

20

The veterinary compositions may be formulated in any manner known in the art. Veterinary compositions are formulated to be compatible with their intended route of administration, whether oral or parenteral (e.g., intravenous, intradermal, subcutaneous, intraperitoneal, transmucosal, or transdermal (e.g., topical ointments, salves, gels, patches or creams as generally known in the art)). The compositions can include a sterile diluent (e.g., sterile water or saline), a fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvents; antibacterial or antifungal agents such as benzyl alcohol or methyl parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like; antioxidants such as ascorbic acid or sodium bisulfite;

30

chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates; and isotonic agents such as sugars (e.g., dextrose), polyalcohols (e.g., mannitol or sorbitol), or salts (e.g., sodium chloride). Liposomal suspensions (including liposomes targeted to affected cells with monoclonal antibodies specific for the target feline cell) can also be used as pharmaceutically acceptable carriers (see, e.g., 5 U.S. Patent No. 4,522,811). Preparations of the compositions can be formulated and enclosed in ampules, disposable syringes, or multiple dose vials. Where required (as in, for example, injectable formulations), proper fluidity can be maintained by, for example, the use of a coating such as lecithin, or a surfactant. Absorption of the active 10 ingredient can be prolonged by including an agent that delays absorption (e.g., aluminum monostearate and gelatin). Alternatively, controlled release can be achieved by implants and microencapsulated delivery systems, which can include biodegradable, biocompatible polymers (e.g., ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid; Alza Corporation and Nova 15 Pharmaceutical, Inc.).

Where oral administration is intended, the agent can be included in pills, capsules, troches, and the like, and can contain any of the following ingredients, or compounds of a similar nature: a binder, such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient, such as starch or lactose, a disintegrating agent, such 20 as alginate, Primogel, or corn starch; a lubricant, such as magnesium stearate; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; or a flavoring agent, such as methyl salicylate or fish flavoring.

Compositions containing one or more of any of the agents described herein can be formulated for oral or parenteral administration in dosage unit form (i.e., physically 25 discrete units containing a predetermined quantity of active compound for ease of administration and uniformity of dosage). In some embodiments, one or more agents can be administered to the feline as a component of a food composition (e.g., a pellet, powder, or semi-solid slurry) or liquid (e.g., a syrup) for oral ingestion.

Toxicity and therapeutic efficacy of compositions can be determined by 30 standard pharmaceutical procedures in cell cultures or experimental animals (e.g., felines). One can, for example, determine the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population), the therapeutic index being the ratio of LD50:ED50. Agents that exhibit high

therapeutic indices are preferred. Where an agent exhibits an undesirable side effect, care should be taken to target that agent to the site of the affected tissue (the aim being to minimize potential damage to unaffected cells and, thereby, reduce side effects). Toxicity and therapeutic efficacy can be determined by other standard pharmaceutical procedures.

5 Data obtained from cell culture assays and animal studies can be used in formulating an appropriate dosage of any given agent for use in felines. A therapeutically effective amount of the one or more agent will be an amount that treats or decreases the risk of developing a retroviral infection in a feline, decreases the severity, frequency, and/or duration of one or more symptoms of a retroviral infection in a feline, increases the ratio of CD4⁺ T-cells to CD8⁺ T-cells in a feline with a retroviral infection, increases the number of CD4⁺ T cells in a feline with a retroviral infection, and/or increases the total white blood cell count in a feline with a retroviral infection (e.g., as compared to a control feline having a retroviral infection or the same feline prior to treatment). The effectiveness and dosing of any of the agents described herein can be determined by a veterinary professional using methods known in the art, as well as by the observation of one or more symptoms of retroviral infection in a feline. Certain factors may influence the dosage and timing required to effectively treat a feline (e.g., the severity of the infection or disease, previous treatments, the general health and/or age of the feline, and the presence of other diseases).

As noted herein, agents administered according to the methods described herein can be small molecules (e.g., peptides, peptidomimetics (e.g., peptoids), amino acid residues (or analogs thereof), polynucleotides (or analogs thereof), nucleotides (or analogs thereof), or organic or inorganic compounds (e.g., heteroorganic or organometallic compounds)). Typically, such molecules will have a molecular weight less than about 10,000 grams per mole (e.g., less than about 7,500, 5,000, 2,500, 1,000, or 500 grams per mole). Salts, esters, and other pharmaceutically acceptable forms of any of these compounds can be assayed and, if a desirable activity is detected, administered according to the therapeutic methods described herein. The agents administered according to the methods described herein can be proteins (e.g., antibodies or antigen-binding fragments thereof).

Exemplary doses include milligram or microgram amounts of any of the agents described herein per kilogram of the feline's weight (e.g., about 1 µg – 500 mg/kg;

about 100 µg -- 500 mg/kg; about 100 µg -- 50 mg/kg; 10 µg -- 5 mg/kg; 10 µg -- 0.5 mg/kg; or 1 µg -- 50 µg/kg). While these doses cover a broad range, one of ordinary skill in the art will understand that therapeutic agents, including small molecules, vary in their potency, and effective amounts can be determined by methods known in the art.

5 Typically, relatively low doses are administered at first, and the attending veterinary professional (in the case of therapeutic application) or a researcher (when still working at the development stage) can subsequently and gradually increase the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular feline will depend upon a variety of factors including the

10 activity of the specific compound employed, the age, body weight, general health, gender, and diet of the feline, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of activity to be modulated.

The veterinary compositions can be included in a container, pack, or dispenser together with instructions for administration.

15

Methods of Treating Retroviral Infection in a Feline

Provided herein are methods for treating or reducing the likelihood of developing a retroviral infection in a feline. Also provided are methods for reducing retroviral virion entry (e.g., fusion) into a feline target cell (e.g., a non-infected cell), retroviral

20 virion budding from a feline cell, and syncytium transmission in a feline. All of the methods described herein require administering to the feline at least one agent that prevents or reduces the binding of LFA-1 (e.g., present in or on the surface of a non-infected feline cell, a virion, or a syncytium) from binding to ICAM-1 (e.g., present in a non-infected feline cell, a virion, or a syncytium). In some embodiments, the at least

25 one agent specifically binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18 (e.g., present in or on the surface of a non-infected target cell and/or present in or on the surface of a virion and/or syncytium). In some embodiments, the at least one agent binds directly to CD11a, CD18, ICAM-1, or an epitope formed by both

30 CD11a and CD18. In some embodiments, the at least one agent indirectly binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18. In any of the methods described herein, the at least one agent can be formulated or administered as one or more of the veterinary compositions described herein.

In some embodiments, the feline is a domestic feline. In some embodiments, the feline that is treated can be previously diagnosed as having a retroviral infection (e.g., FIV or FELV). A feline can be diagnosed as having a retroviral infection by a veterinary professional using any of the methods described herein (e.g., by the
5 observation of at least one symptom of a retroviral infection in a feline) or any methods known in the art. In some embodiments, the feline that is treated can be identified as having an increased risk of developing a retroviral infection (e.g., FIV and FELV). For example, a feline can be determined to have an increased risk of retroviral infection in view of a local pandemic of retroviral infection or by its proximity to one or more other
10 felines having or suspected of having a retroviral infection. A determination or prediction of the incidence of retroviral infection in a population of felines can be assessed or determined by public health officials. In any of the methods described herein, the feline may be treated by its owner (e.g., a domestic feline), a veterinary professional, or a public health worker.

In some embodiments, the agent is an antibody or an antigen-binding fragment thereof that binds specifically to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18. In some embodiments, the antibody or an antigen-binding fragment thereof prevents or reduces LFA-1 from binding to ICAM-1. Any of the antibodies or antibodies fragments described herein can be used alone or in any combination in any
20 of the methods described herein. In a non-limiting example, the at least one agent used in any of the methods described herein can be a monoclonal antibody, a chimeric antibody, a single-chain antibody (e.g., a single-domain antibody), a feline antibody, or a felinized antibody.

In some embodiments of any of the methods described herein, the at least one
25 agent can be any of the small molecules described herein. In a non-limiting example, the small molecule can be a statin or a statin derivative (e.g., any of the statins or statin derivatives described herein, such as lovastatin, simvastatin, a lovastatin derivative (6-exomethylene-modified, an 8-acyl-modified, or an alkylated lovastatin), and/or a simvastatin derivative). Additional small molecules that can be used in any of the
30 methods described herein include LFA703, LFA451, LFA878, and XVA143. Additional small molecules that can be used in any of the methods described herein include molecules of Formula I (as described herein). Non-limiting examples of small molecules of Formula I that can be used in any of the methods described herein are

shown in Figure 1. Additional examples of small molecules of Formula I that can be used in any of the methods described herein are listed in U.S. Patent No. 6,872,735 (herein incorporated by reference in its entirety).

In some embodiments of any of the methods described herein, the methods
5 include administering to a feline at least one small molecule that prevents or decreases LFA-1 binding to ICAM-1 and/or at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18, where the at least one small molecule or the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to treat a
10 retrovirus infection, reduce the entry (e.g., fusion) of a retroviral virion into a feline cell, reduce the budding of retroviral virions from a feline cell, and/or reduce syncytium transmission in a feline. The effect of a small molecule and/or an antibody or antigen-binding fragment thereof (as described herein) on virion fusion and/or budding from a feline cell, as well as syncytium transmission can be studied in vitro (in tissue culture)
15 using, for example, microscopic techniques. The data from these in vitro studies can be used to predict the effect of the small molecule and/or antibody or antigen-binding fragment thereof (as described herein) on syncytium transmission in a feline. A decrease in syncytium transmission can also result in a decrease in or a delay in the onset of dementia in a feline having a retroviral (FIV) infection. Feline dementia can
20 be diagnosed by a veterinary care professional or by a feline's owner (for a domestic feline) by the observation of specific behaviors by the feline (e.g., the feline seems to get lost or confused in familiar surroundings, inability or difficulty in finding a litter box (for a domestic feline), or a decrease in the response to an owner's vocal greeting or command).

In some embodiments, the feline is administered at least one antibody or
25 antigen-binding fragment thereof that specifically binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18 (e.g., present in or on the surface of a non-infected feline cell or present in or on the surface of a virion or syncytium). In some embodiments of any of the methods described herein, the feline is administered at least
30 one antibody or antigen-binding fragment thereof that prevents LFA-1 from binding to ICAM-1.

In some embodiments, the feline can be administered at least one antibody or antigen-binding fragment thereof that contains at least one complementary determining

region (CDR) from the light chain or the heavy chain of TS1/22 antibody, the clone 25.3 antibody, or the clone 27 antibody. In some embodiments, the feline can be administered at least one antibody or antigen-binding fragment thereof that contains the three CDRs from the light chain of TS1/22 antibody, the clone 25.3 antibody, or the clone 27 antibody or the three CDRs from the heavy chain of TS1/22 antibody, the clone 25.3 antibody, or the clone 27 antibody. In some embodiments, the feline can be administered an antibody or an antigen-binding fragment thereof that contains the three CDRs from the light chain of TS1/22 antibody and the three CDRs from the heavy chain of TS1/22 antibody. In some embodiments, the feline can be administered an antibody or an antigen-binding fragment thereof that contains the three CDRs from the light chain of the clone 25.3 antibody and the three CDRs from the heavy chain of the clone 25.3 antibody. In some embodiments, the feline can be administered an antibody or an antigen-binding fragment thereof that contains the three CDRs from the light chain of the clone 27 antibody and the three CDRs from the heavy chain of the clone 27 antibody.

In some embodiments of any of the methods described herein, the at least one antibody can be a feline antibody or an antigen-binding fragment thereof, or a felinized antibody or an antigen-binding fragment thereof. In some embodiments of any of the methods described herein, the antibody can be a single-chain antibody (e.g., a single-domain antibody). In some embodiments of any of the methods described herein, the at least one antigen-binding fragment thereof can be a Fab fragment, a F(ab')₂ fragment, or a scFv fragment.

In some embodiments of any of the methods described herein, the feline is administered at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30) dose of a composition containing at least one of any of the agents described herein (e.g., administered at least one dose of any of the veterinary compositions described herein). In any of the methods described herein, at least one agent (e.g., any of the agents described herein) or at least one veterinary composition (e.g., any of the veterinary compositions described herein) can be administered intracenosusly, intaarterially, ocularly, orally, subcutaneously, intraperitoneally, or intramuscularly to the feline. In some embodiments, the at least one agent (e.g., small molecule) or at least one veterinary composition containing a small molecule is administered orally. In some embodiments, at least one antibody or antigen-binding fragment thereof or at least one

veterinary composition containing an antibody or antigen-binding fragment thereof is administered intravenously. In some embodiments of any of the methods described herein, a feline is administered at least one agent that is a small molecule (e.g., a small molecule that prevents or reduces LFA-1 from binding to ICAM-1, e.g., any of the small molecules described herein) or at least one veterinary composition that contains a
5 small molecule, and at least one antibody or antigen-binding fragment thereof (e.g., an antibody or antigen-binding fragment thereof that specifically binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18) or at least one veterinary composition that contains an antibody or antigen-binding fragment thereof (e.g., an
10 antibody or antigen-binding fragment thereof that specifically binds to CD11a, CD18, ICAM-1, or an epitope formed by both CD11a and CD18). In some embodiments, at least one small molecule agent and at least one antibody or antigen-binding fragment thereof are administered in the same composition (e.g., the same veterinary composition). In some embodiments, at least one small molecule and at least one
15 antibody or antigen-binding fragment thereof are administered to the feline using different routes of administration.

In some embodiments, the administering of at least one of any of the agents or at least one of any of the veterinary compositions described herein results in a decrease in the severity, frequency, or duration of at least one symptom of a retroviral
20 infection in the feline. In some embodiments, the administering of at least one of any of the agents or at least one of any of the veterinary compositions described herein results in a decrease in retroviral titer in the feline. In some embodiments, the administering of at least one agent or at least one veterinary composition as described herein results in an increase (e.g., a significant increase in the ratio of CD4⁺ T-cells to
25 CD8⁺ T-cells in the feline (e.g., a feline having FIV or SELV).

In any of the methods described herein, the at least one agent or the at least one veterinary composition can be administered to the feline at least once a week (e.g., twice a week, three times a week, four times a week, once a day, twice a day, or three
30 times a day). In some embodiments, at least one small molecule and/or at least one antibody and/or antigen binding fragment thereof is administered to the feline at least once a week (e.g., twice a week, three times a week, four times a week, once a day, twice a day, or three times a day). In some embodiments, at least two different agents are administered in the same composition (e.g., a solid composition or liquid

composition). In some embodiments, at least two different agents are administered in two different compositions (e.g., a solid composition and a liquid composition). In some embodiments, the at least one agent is administered as a component of a feed composition (e.g., pellets, a liquid, or a semi-solid slurry).

5 In some embodiments, the feline can be administered the at least one agent or at least one veterinary composition over an extended period of time (e.g., over a period of at least 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months). A skilled veterinary profession may determine the length of treatment period using any of
10 the methods described herein for diagnosing or following the effectiveness of retroviral treatment (e.g., the observation of at least one symptom of a retroviral infection in a feline or retroviral titers in the feline). As described herein, a skilled veterinary professional can also change the identity and number (e.g., increase or decrease) of agents administered to the feline and can also adjust (e.g., increase or decrease) the
15 dosage or frequency of administration of at least one agent to the feline based on assessment of the effectiveness of retroviral treatment (e.g., using any of the methods described herein and known in the art).

 In some embodiments, the feline can be administered the at least one agent or the at least one veterinary composition during a time of feline retroviral outbreak (e.g.,
20 starting at any time before or during the retroviral outbreak and ending at a time when the incidence of infected felines in the population has significantly decreased).

 In some embodiments, the administration of at least one agent or at least one veterinary composition described herein does not cause detrimental immunosuppression in the feline. Detrimental immunosuppression, for example, can
25 be indicated by a nearly complete suppression of CD8⁺ T-cell activation (cytotoxic T-lymphocyte activation) in a feline following therapeutic treatment.

 In some embodiments, the retroviral infection treated is FIV or FELV. In some embodiments, the virion or syncytium is FIV or FELV.

 In some embodiments of the methods described herein, the feline may be
30 further administered one or more (e.g., one, two, three, or four) additional therapeutic agents (e.g., a sedative, an analgesic, or an anti-inflammatory agent). The one or more additional therapeutic agents and the at least one agent (e.g., any of the agents described herein) can be administered in the same dose. In some embodiments, the one

or more additional therapeutic agents and the at least one agent (e.g., any of the agents described herein) can be administered in separate dosage forms. In some embodiments, the one or more additional therapeutics can be administered to the feline prior to administering at least one agent (e.g., any of the agents described herein). In some
5 embodiments, the one or more additional therapeutics can be administered to the feline after administering at least one agent (e.g., any of the agents described herein). In some embodiments, the one or more additional therapeutics and the at least one agent are administered to the feline such that there is overlap in the bioactive period of the one or more additional therapeutics and the at least one agent in the feline.

10

Methods of Screening for Anti-Retroviral Agents

Also provided herein are methods of identifying candidate agents that can be useful for treating or decreasing the risk of retroviral infection in a feline, for decreasing virion entry (e.g., fusion) into a feline cell, for decreasing virion budding
15 from a feline cell, or for decreasing syncytium transmission in a feline. These methods include providing LFA-1 and ICAM-1, and contacting the LFA-1 to ICAM-1 in the presence of the candidate agent, and determining the binding of LFA-1 to ICAM-1 in the presence of the candidate agent, where a decrease in the amount of binding between LFA-1 to ICAM-1 compared to the binding observed in the absence of the candidate
20 agent indicates that the candidate agent can be useful for treating or decreasing the risk of retroviral infection in a feline, for decreasing virion entry into a feline cell, for decreasing virion budding from a feline cell, or for decreasing syncytium transmission in a feline.

20

In some embodiments of these methods, the LFA-1 may be expressed on the
25 surface of a cell (e.g., a feline cell) and ICAM-1 is a recombinant soluble form of ICAM-1 protein. In some embodiments of these methods, the LFA-1 may be a recombinant form of soluble LFA-1 protein and ICAM-1 is expressed on the surface of a cell (e.g., a feline cell). In some embodiments, both LFA-1 and ICAM-1 are recombinant soluble proteins (e.g., and interactions identified by co-
30 immunoprecipitation reactions).

30

In some embodiments of these methods, LFA-1 can be attached to a solid surface (e.g., a magnetic bead) and ICAM-1 is a recombinant soluble ICAM-1 protein.

In some embodiments, ICAM-1 is attached to a solid surface (e.g., a magnetic bead) and LFA-1 is a recombinant soluble LFA-1 protein.

In some embodiments of any of the methods described herein, LFA-1 and/or ICAM-1 (including recombinant soluble forms of LFA-1 and/or ICAM-1) may be
5 labelled (e.g., a fluorescent label, a radioisotope, or peptide-tag) for detection (e.g., detection by fluorescence, luminescence, or binding by a secondary antibody).

Candidate agents identified using any of the assays described herein can further tested in a feline model of retroviral infection. The efficacy of the candidate agent to treat or reduce the number, severity, duration, or frequency of one or more symptoms of
10 a retroviral infection in a feline can be determined using any of the methods described herein.

EXAMPLE

The invention is further described in the following examples, which do not limit
15 the scope of the invention described in the claims.

Example 1. Antibodies that Specifically Bind to LFA-1 Decrease Retroviral Infection

An experiment was performed to determine whether an antibody that
20 specifically binds to LFA-1 would decrease FIV infection in a FIV-susceptible, feline T-cell line (104-C1). In these experiments, FIV strain PPR ("PPR") was produced in 104-C1 cells. The resulting viral preparation was incubated with 0 µg/mL antibody (control), or 5 µg/mL or 10 µg/mL of anti-LFA1 antibody TS1-22 (produced from ATCC deposit number HB202), clone 25.3 (Becker Coulter (PN IM0157 and PN
25 IM1433U), or clone 27 (Fisher Scientific No. 610826) for 30 minutes (at 37 °C and 5% CO₂) before being added to 150,000 104-C1 cells, and spin inoculated for 2 hours at 3,000 rpm. After spinning, the cells were recovered for 3 hours at 37°C and 5% CO₂, before the viral media was replaced with fresh media containing the same concentrations of diluted antibody. After 6, 12, and 14 days of incubation in fresh
30 medium, the cells were lysed and the reverse transcriptase activity in the cells was determined (counts per minute) as generally described in Thompson et al., *PLoS One* 2011; 6(8): e24020, 2011. The resulting data show that three different antibodies that specifically bind to LFA-1 significantly decrease FIV infection in feline T-cells (Figure

2). These data indicate that antibodies or antigen-binding fragments thereof that specifically bind to LFA-1, or its binding partner ICAM-1, can decrease retrovirus infection (e.g., FIV infection) in a feline and can reduce retrovirus virion entry (e.g., FIV virion entry) into a feline cell. These data further indicate that small molecules that decrease binding of LFA-1 to its binding partner ICAM-1 can decrease retrovirus infection (e.g., FIV infection) in a feline and can reduce retrovirus virion entry (e.g., FIV virion entry) into a feline cell.

Example 2. Anti-CD11a Antibody and FIV Infection

10 Additional experiments may be performed to determine whether an anti-CD11a antibody would decrease or prevent FIV infection in feline cells. For example, in such experiments, TS1/22 antibody (anti-CD11a antibody) may be incubated with either feline peripheral blood mononuclear cells or the feline T-cell line 104-C1 and binding assayed by fluorescence-assisted cell sorting (FACS).

15 In additional experiments, feline 104-C1 T-cells may be left untreated or pre-incubated for 15 minutes with TS1-22 (10 µg/mL or 50 µg/mL). The cells may then be washed and infected with FIV-PPR. The presence of virus may be determined by measuring reverse transcriptase activity at days 4, 7, 10, and 14. In one set of controls, the cells may be left untreated with TS1/22 and uninfected with FIV.

20

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

25

What is claimed is:

1. A method for treating a retrovirus infection in a feline, the method comprising administering to a feline at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18, wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to treat a retrovirus infection.

2. A method for treating a retrovirus infection in a feline, the method comprising administering to a feline at least one small molecule that decreases LFA-1 binding to ICAM-1, wherein the at least one small molecule is administered in an amount sufficient to treat a retrovirus infection.

3. A method for reducing retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell in a feline, the method comprising administering to a feline at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18, wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to reduce retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell.

4. A method for reducing retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell in a feline, the method comprising administering to a feline at least one small molecule that decreases LFA-1 from binding to ICAM-1, wherein the at least one small molecule is administered in an amount sufficient to reduce retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell.

5. A method for reducing syncytium transmission in a feline, the method comprising administering to a feline at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18, wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to reduce syncytium transmission in a feline.

6. A method for reducing syncytium transmission in a feline, the method comprising administering to a feline at least one small molecule that decreases LFA-1 binding to ICAM-1, wherein the at least one small molecule is administered in an amount sufficient to reduce syncytium transmission in a feline.

7. The method of claim 1, 3, or 5, wherein the at least one antibody or antigen-binding fragment thereof binds specifically to CD11a.

8. The method of claim 1, 3, or 5, wherein the at least one antibody or antigen-binding fragment thereof binds specifically to CD18.

9. The method of claim 1, 3, or 5, wherein the at least one antibody or antigen-binding fragment thereof binds specifically to a surface formed by both CD11a and CD18.

10. The method of claim 1, 3, or 5, wherein the at least one antibody or antigen-binding fragment thereof decreases LFA-1 binding to ICAM-1.

11. The method of claim 1, 3, or 5, wherein the at least one antibody is a monoclonal antibody.

12. The method of claim 1, 3, or 5, wherein the at least one antibody or antigen-binding fragment thereof is an antibody or an antigen-binding fragment thereof that contains at least one complementary determining region (CDR) from the light chain or the heavy chain of TS1/22 antibody.

13. The method of claim 12, wherein the at least one antibody or antigen-binding fragment thereof is an antibody or an antigen-binding fragment thereof that contains the three CDRs from the light chain of TS1/22 antibody or the three CDRs from the heavy chain of TS1/22 antibody.

14. The method of claim 12, wherein the at least one antibody or antigen-binding fragment thereof is an antibody or an antigen-binding fragment thereof that

contains the three CDRs from the light chain of TS1/22 antibody and the three CDRs from the heavy chain of TS1/22 antibody.

15. The method of claim 14, wherein the at least one antibody or antigen-binding fragment thereof is TS1/22 antibody or an antigen-binding fragment of TS1/22 antibody.

16. The method of claim 1, 3, or 5, wherein the at least one antibody is a feline antibody or an antigen-binding fragment thereof, or a felinized antibody or an antigen-binding fragment thereof.

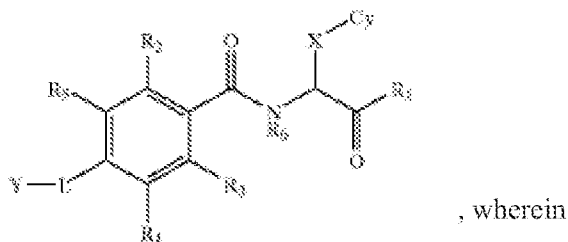
17. The method of claim 1, 3, or 5, wherein the antibody is a single-chain antibody.

18. The method of claim 17, wherein the single-chain antibody is a single-domain antibody.

19. The method of claim 1, 3, or 5, wherein the at least one antigen-binding fragment thereof is selected from the group consisting of: a Fab fragment, a F(ab')₂ fragment, and a scFv fragment.

20. The method of claim 2, 4, or 6, wherein the at least one small molecule is lovastatin, simvastatin, a lovastatin derivative, a simvastatin derivative, LFA703, LFA451, LFA878, or XVA143, or a salt, solvate, or hydrate thereof.

21. The method of claim 2, 4, or 6, wherein the at least one small molecule is:



Cy is a non-aromatic carbocycle or heterocycle, optionally substituted with hydroxyl, mercapto, thioalkyl, halogen, oxo, thio, amino, aminoalkyl, amidine, guanidine, nitro, alkyl, alkoxy, or acyl;

X is a divalent hydrocarbon chain, optionally substituted with hydroxyl, mercapto, halogen, amino, aminoalkyl, nitro, oxo, or thio, and optionally interrupted with N, O, S, SO, or SO₂,

Y is a carbocycle or heterocycle, optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, thioalkyl, amino, aminoalkyl, carbocycle, or heterocycle ring, hydrocarbon, a halo-substituted hydrocarbon, amino, amidine, guanidine, cyano, nitro, alkoxy, or acyl;

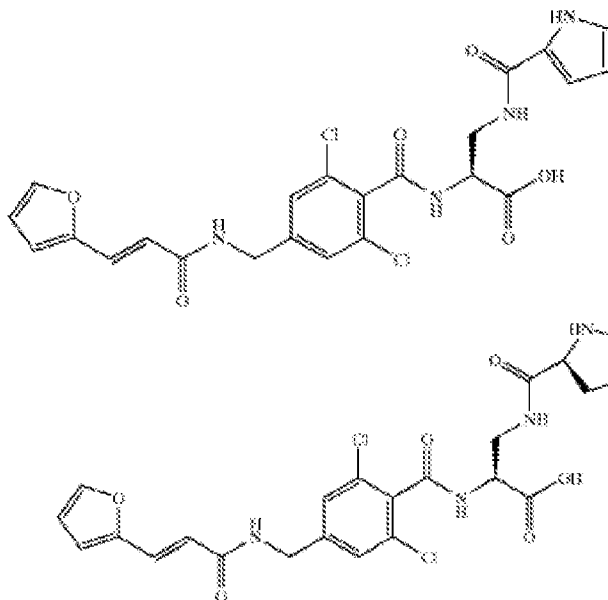
L is a divalent hydrocarbon chain, optionally substituted with hydroxyl, halogen, oxo, or thio;

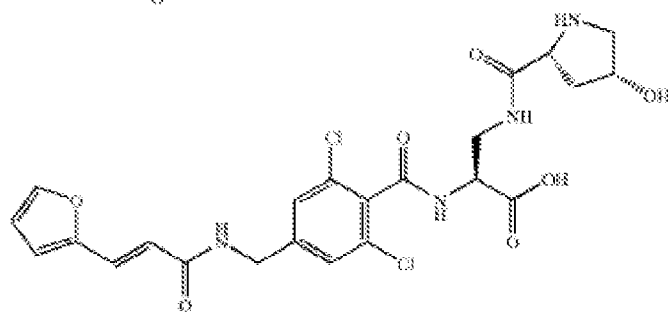
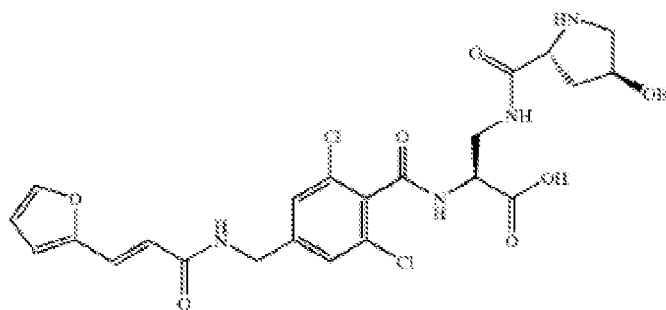
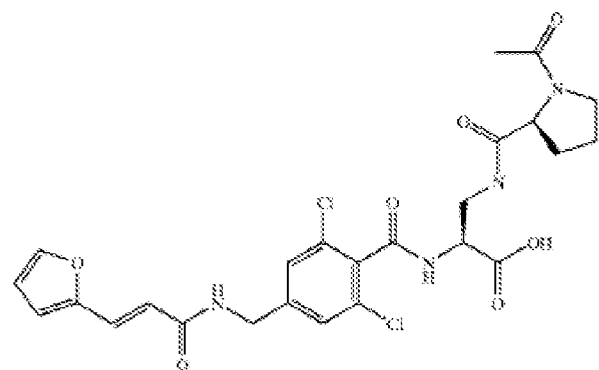
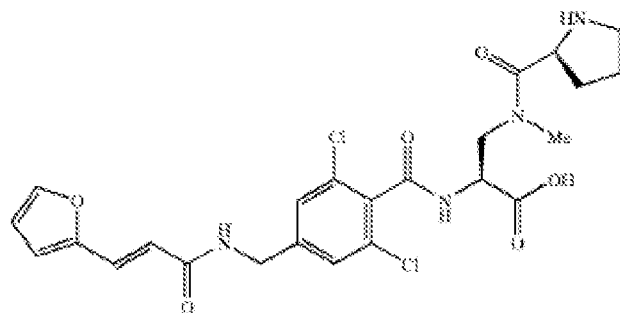
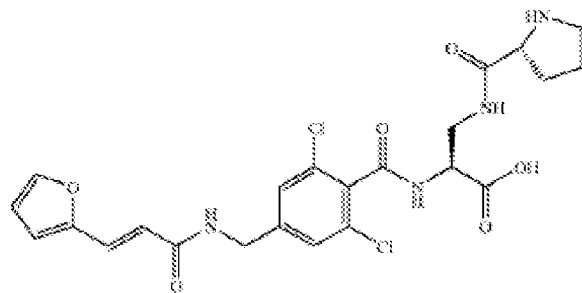
R₁ is H, OH, amino, O-carbocycle or alkoxy, optionally substituted with amino, a carbocycle, or heterocycle;

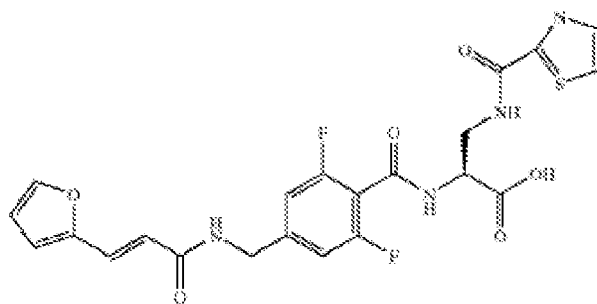
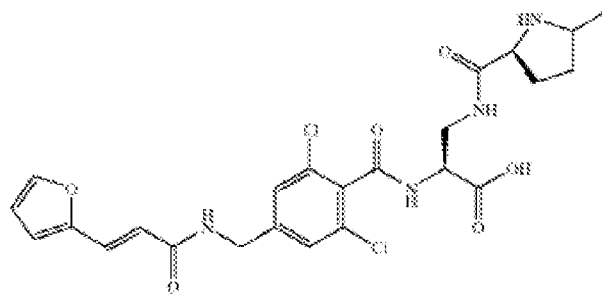
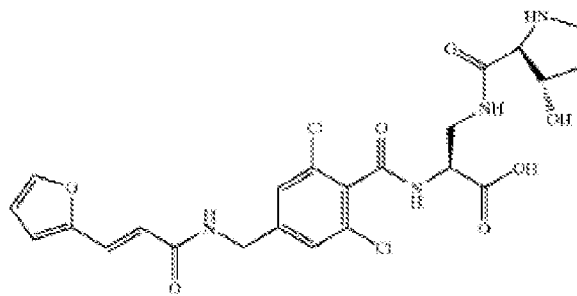
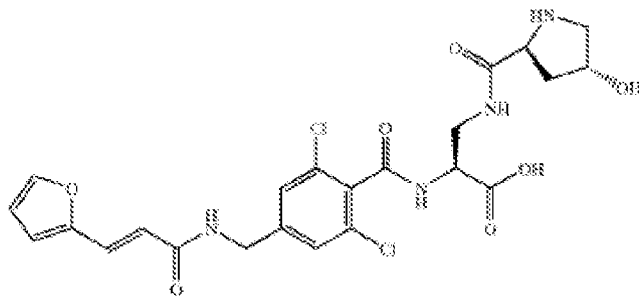
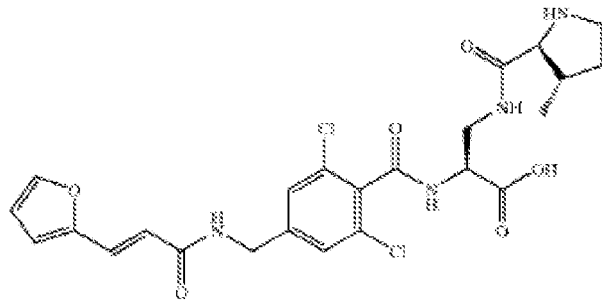
R₂₋₅ are independently H, hydroxyl, mercapto, halogen, cyano, amino, amidine, guanidine, nitro, or alkoxy; or R₃ or R₄ together form a fused carbocycle or heterocycle optionally substituted with hydroxyl, halogen, oxo, thio, amino, amidine, guanidine, or alkoxy; and

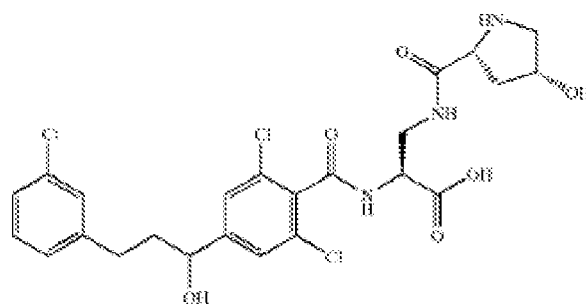
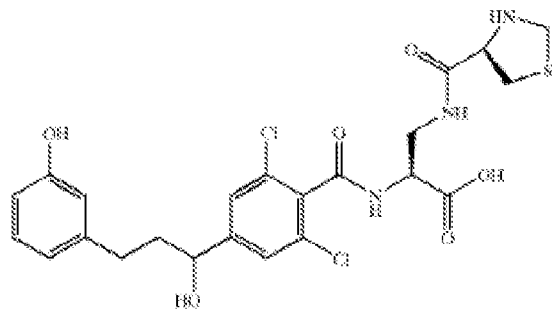
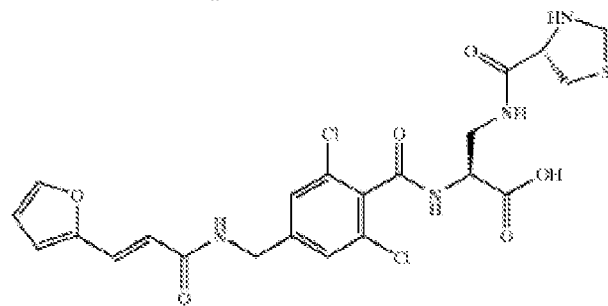
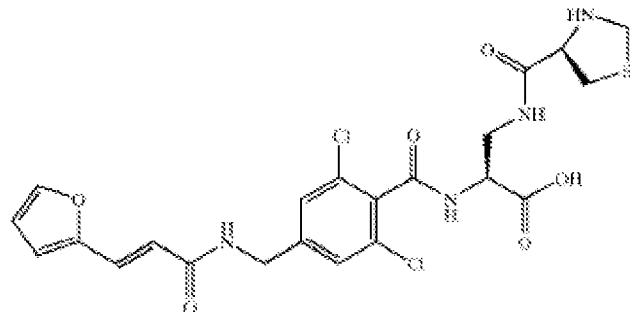
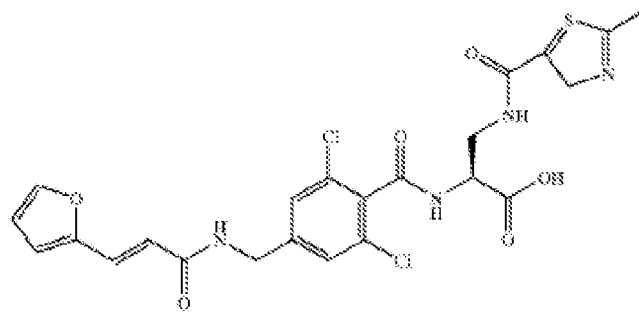
R₆ is H or a hydrocarbon chain, optionally substituted with a carbocycle or a heterocycle; or a salt, solvate, or hydrate thereof.

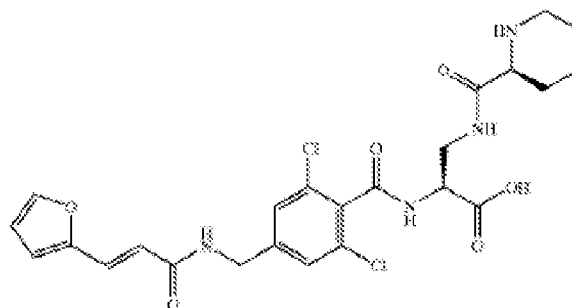
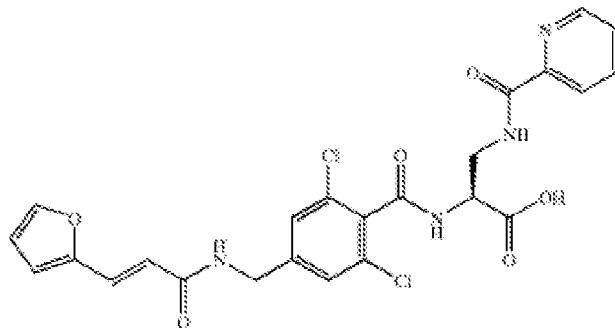
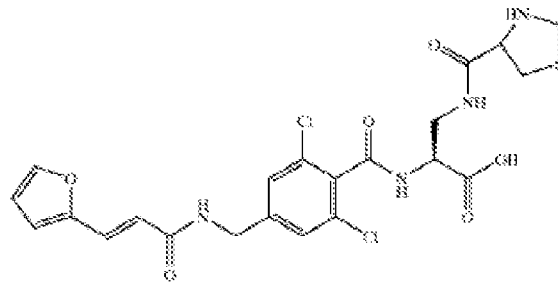
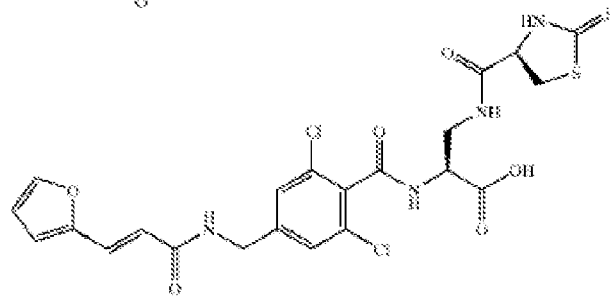
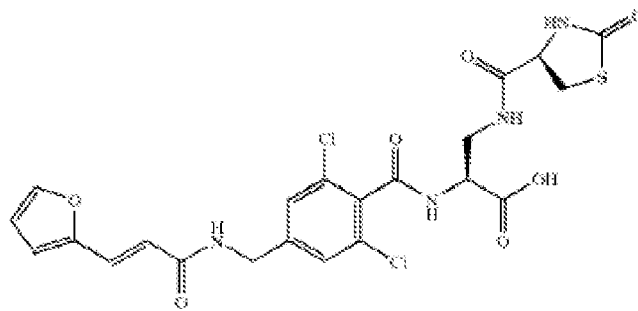
22. The method of claim 21, wherein the small molecule is selected from the group consisting of:

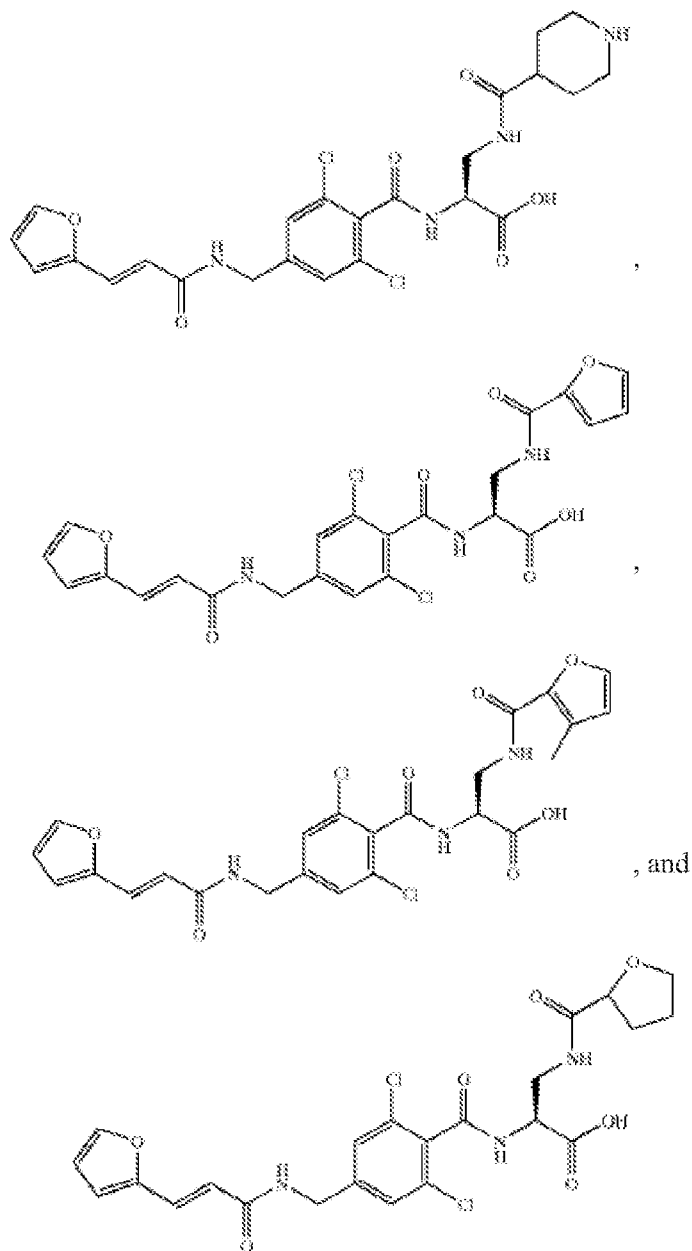












23. The method of claim 1, 3, or 5, wherein the at least one antibody or antigen-binding fragment thereof is administered intravenously, intraarterially, ocularly, orally, subcutaneously, intraperitoneally, or intramuscularly.

24. The method of claim 23, wherein the at least one antibody or antigen-binding fragment thereof is administered intravenously.

25. The method of claim 2, 4, or 6, wherein the at least one small molecule is administered intravenously, intaarterially, ocularly, orally, subcutaneously, intraperitoneally, or intramuscularly.

26. The method of claim 25, wherein the at least one small molecule is administered orally.

27. The method of claim 1, 3, or 5, wherein the feline is further administered at least one small molecule that decreases LFA-1 binding to ICAM-1.

28. The method of claim 27, wherein the at least one small molecule and the at least one antibody or antigen-binding fragment thereof are administered in the same composition.

29. The method of claim 27, wherein the at least one small molecule and the at least one antibody or antigen-binding fragment thereof are administered by different routes of administration.

30. The method of any one of claims 1-6, wherein the administering results in a decrease in the severity, frequency, or duration of at least one symptom of a retroviral infection in the feline.

31. The method of any one of claims 1-6, wherein the administering results in a decrease in retroviral titer in the feline.

32. The method of any one of claims 1-6, wherein the administering results in an increase in the ratio of CD4⁺ T-cells to CD8⁺ T-cells in the feline.

33. The method of claim 1, 3, or 5, wherein the at least one antibody and/or antigen-binding fragment thereof is administered to the feline at least once a week.

34. The method of claim 33, wherein the at least one antibody or antigen-binding fragment thereof is administered to the feline at least once a day.

35. The method of claim 2, 4, or 6, wherein the at least one small molecule is administered to the feline at least once a week.

36. The method of claim 35, wherein the at least one small molecule is administered to the feline at least once a day.

37. The method of any one of claims 1-6, wherein the administering does not result in detrimental immunosuppression in the feline.

38. The method of claim 1 or 2, wherein the retroviral infection is feline immunodeficiency virus (FIV).

39. The method of claim 1 or 2, wherein the retroviral infection is feline leukemia virus (FELV).

40. The method of claim 3 or 4, wherein the retroviral virion is feline immunodeficiency virus (FIV).

41. The method of claim 3 or 4, wherein the retroviral virion is feline leukemia virus (FELV).

42. A method for treating a retrovirus infection in a feline, the method comprising administering to a feline at least one antibody or antigen-binding fragment thereof that specifically binds to ICAM-1, wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to treat a retrovirus infection.

43. A method for reducing retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell in a feline, the method comprising administering to a feline at least one antibody or antigen-binding fragment thereof that specifically binds to ICAM-1, wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to reduce retrovirus virion entry into a feline cell or retrovirus virion budding from a feline cell.

44. A method for reducing syncytium transmission in a feline, the method comprising administering to a feline at least one antibody or antigen-binding fragment thereof that specifically binds to ICAM-1, wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to reduce syncytium transmission in a feline.

45. The method of any one of claims 42-44, wherein the at least one antibody or antigen-binding fragment thereof decreases LFA-1 binding to ICAM-1.

46. The method of any one of claims 42-44, wherein the at least one antibody is a monoclonal antibody.

47. The method of any one of claims 42-44, wherein the at least one antibody is a feline antibody or an antigen-binding fragment thereof, or a felinized antibody or an antigen-binding fragment thereof.

48. The method of any one of claims 42-44, wherein the antibody is a single-chain antibody.

49. The method of claim 48, wherein the single-chain antibody is a single-domain antibody.

50. The method of any one of claims 42-44, wherein the at least one antigen-binding fragment thereof is selected from the group consisting of: a Fab fragment, a F(ab')₂ fragment, and a scFv fragment.

51. The method of any one of claims 42-44, wherein the at least one antibody or antigen-binding fragment thereof is administered intravenously, intraarterially, ocularly, orally, subcutaneously, intraperitoneally, or intramuscularly.

52. The method of claim 51, wherein the at least one antibody or antigen-binding fragment thereof is administered intravenously.

53. The method of any one of claims 42-44, wherein the feline is further administered at least one small molecule that decreases LFA-1 binding to ICAM-1.

54. The method of claim 53, wherein the at least one small molecule and the at least one antibody or antigen-binding fragment thereof are administered in the same composition.

55. The method of claim 53, wherein the at least one small molecule and the at least one antibody or antigen-binding fragment thereof are administered by different routes of administration.

56. The method of any one of claims 42-44, wherein the administering results in a decrease in the severity, frequency, or duration of at least one symptom of retroviral infection in the feline.

57. The method of any one of claims 42-44, wherein the administering results in a decrease in retroviral titer in the feline.

58. The method of any one of claims 42-44, wherein the administering results in an increase in the ratio of CD4⁺ T-cells to CD8⁺ T-cells in the feline.

59. The method of any one of claims 42-44, wherein the at least one antibody and/or antigen-binding fragment thereof is administered to the feline at least once a week.

60. The method of claim 59, wherein the at least one antibody or antigen-binding fragment thereof is administered to the feline at least once a day.

61. The method of claim 53, wherein the at least one small molecule is administered to the feline at least once a week.

62. The method of claim 61, wherein the at least one small molecule is administered to the feline at least once a day.

63. The method of any one of claims 42-44, wherein the administering does not result in detrimental immunosuppression in the feline.

64. The method of claim 42, wherein the retroviral infection is feline immunodeficiency virus (FIV).

65. The method of claim 42, wherein the retroviral infection is feline leukemia virus (FELV).

66. The method of claim 43, wherein the retroviral virion is feline immunodeficiency virus (FIV).

67. The method of claim 43, wherein the retroviral virion is feline leukemia virus (FELV).

Figure 1 (Page 1)

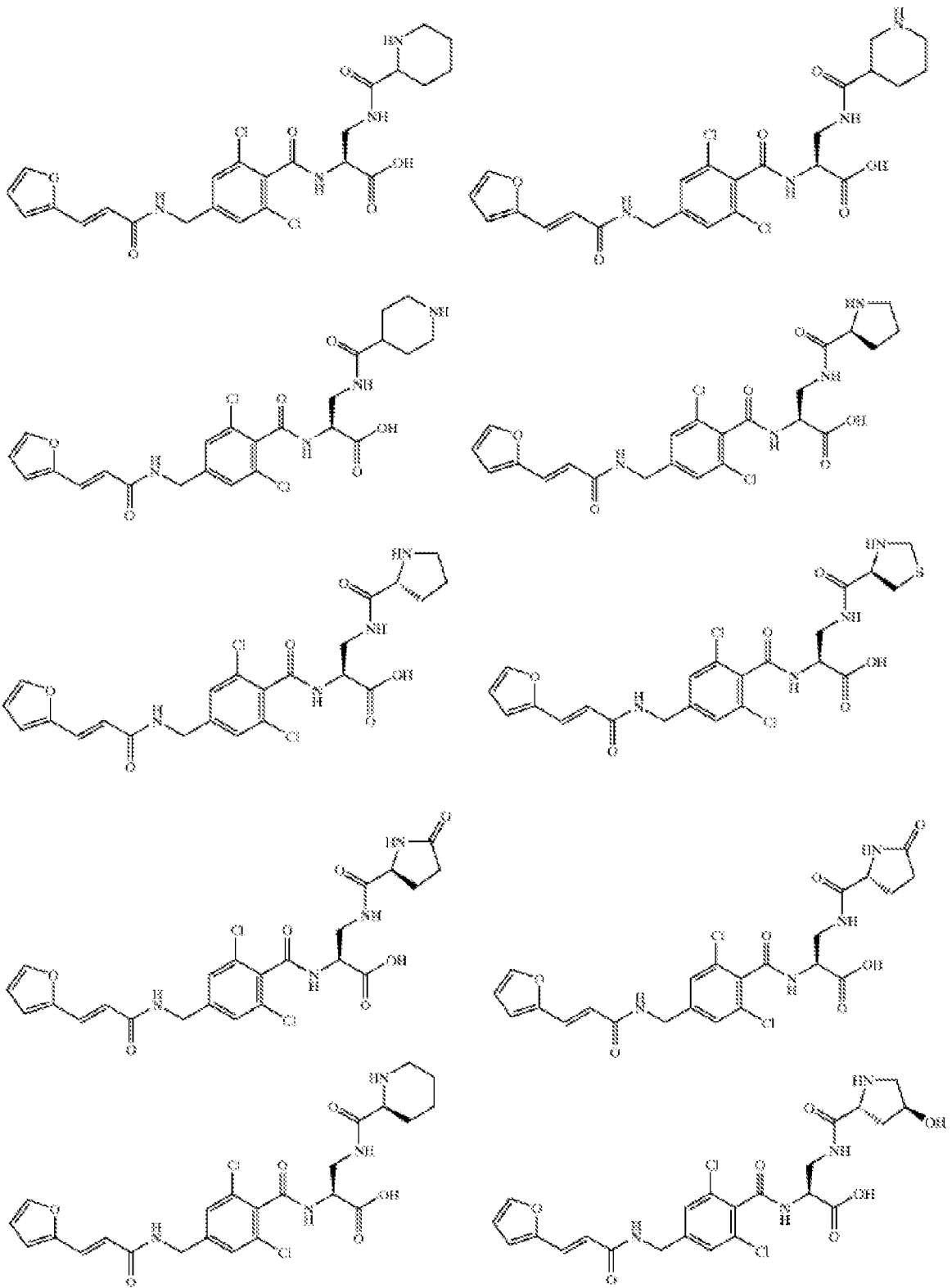


Figure 1 (Page 2)

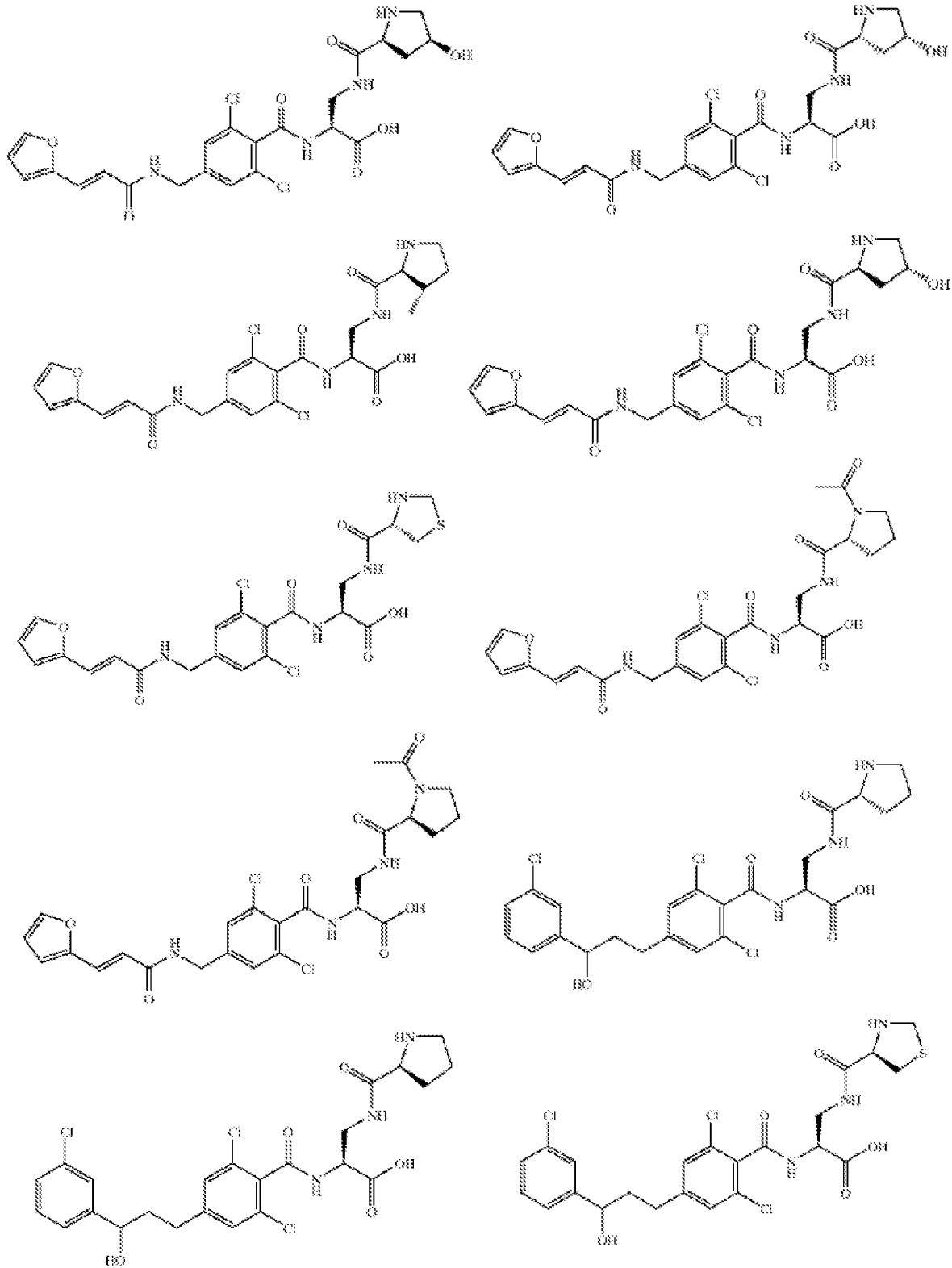


Figure 1 (Page 3)

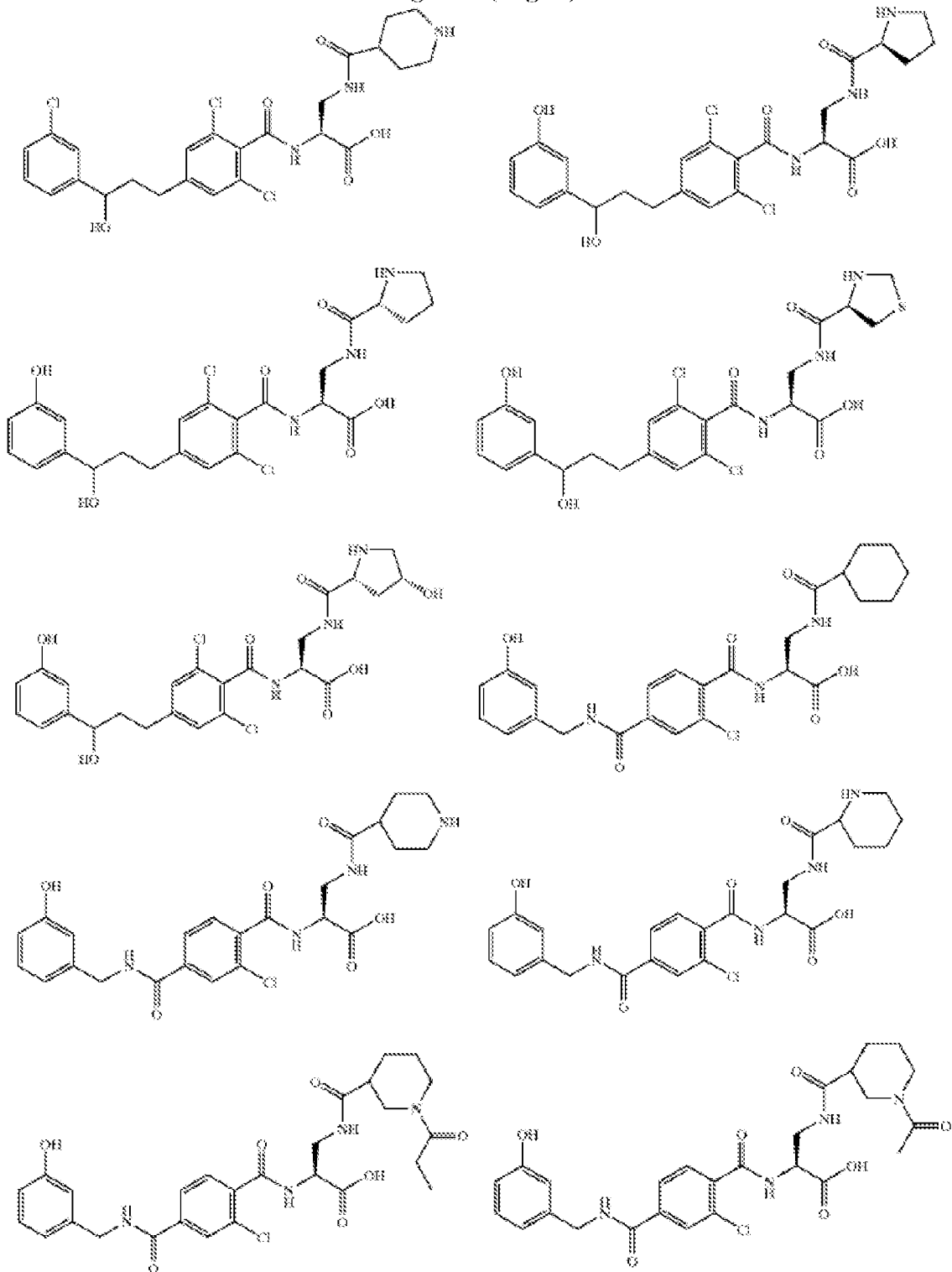


Figure 1 (Page 4)

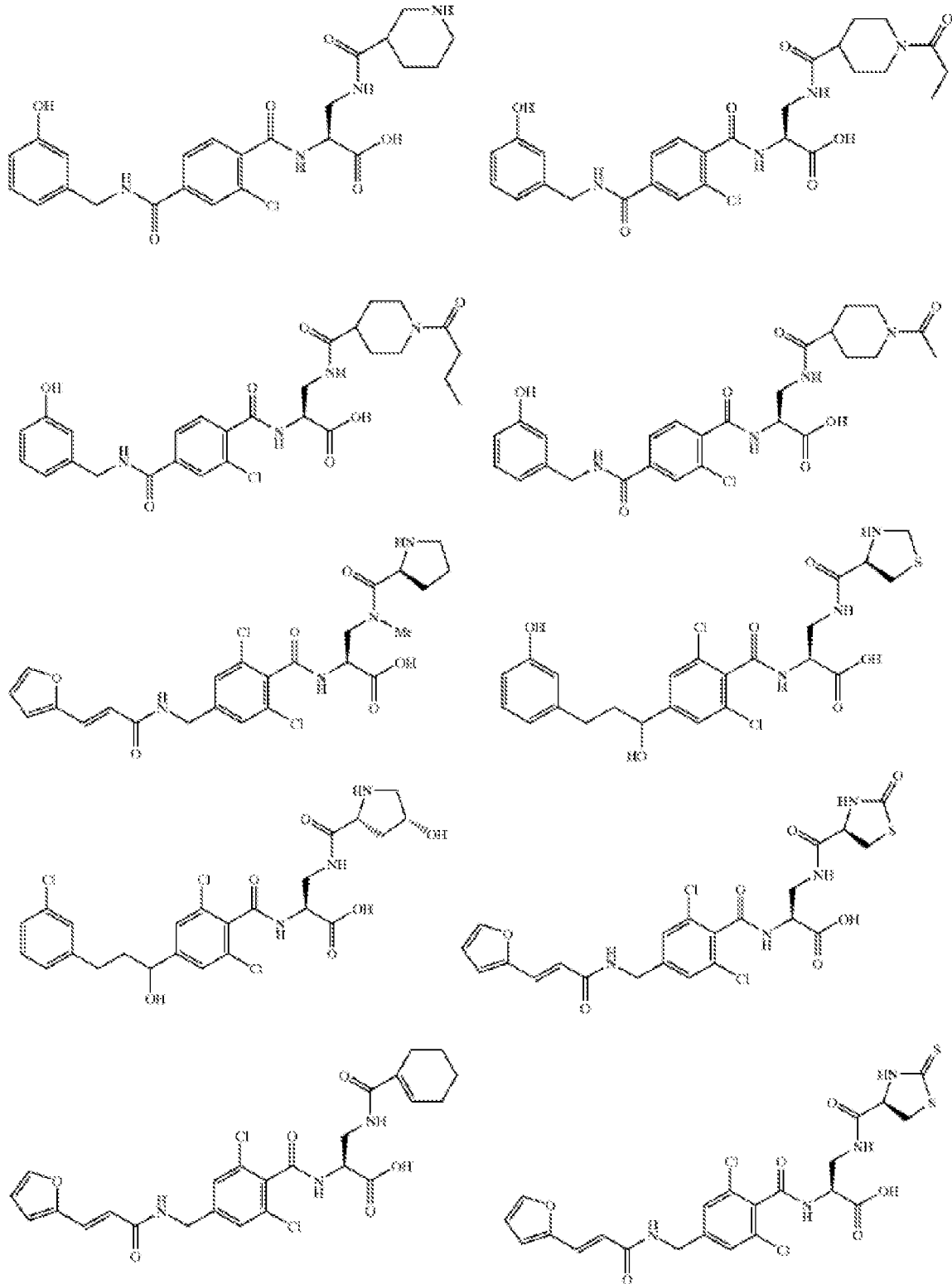


Figure 1 (Page 5)

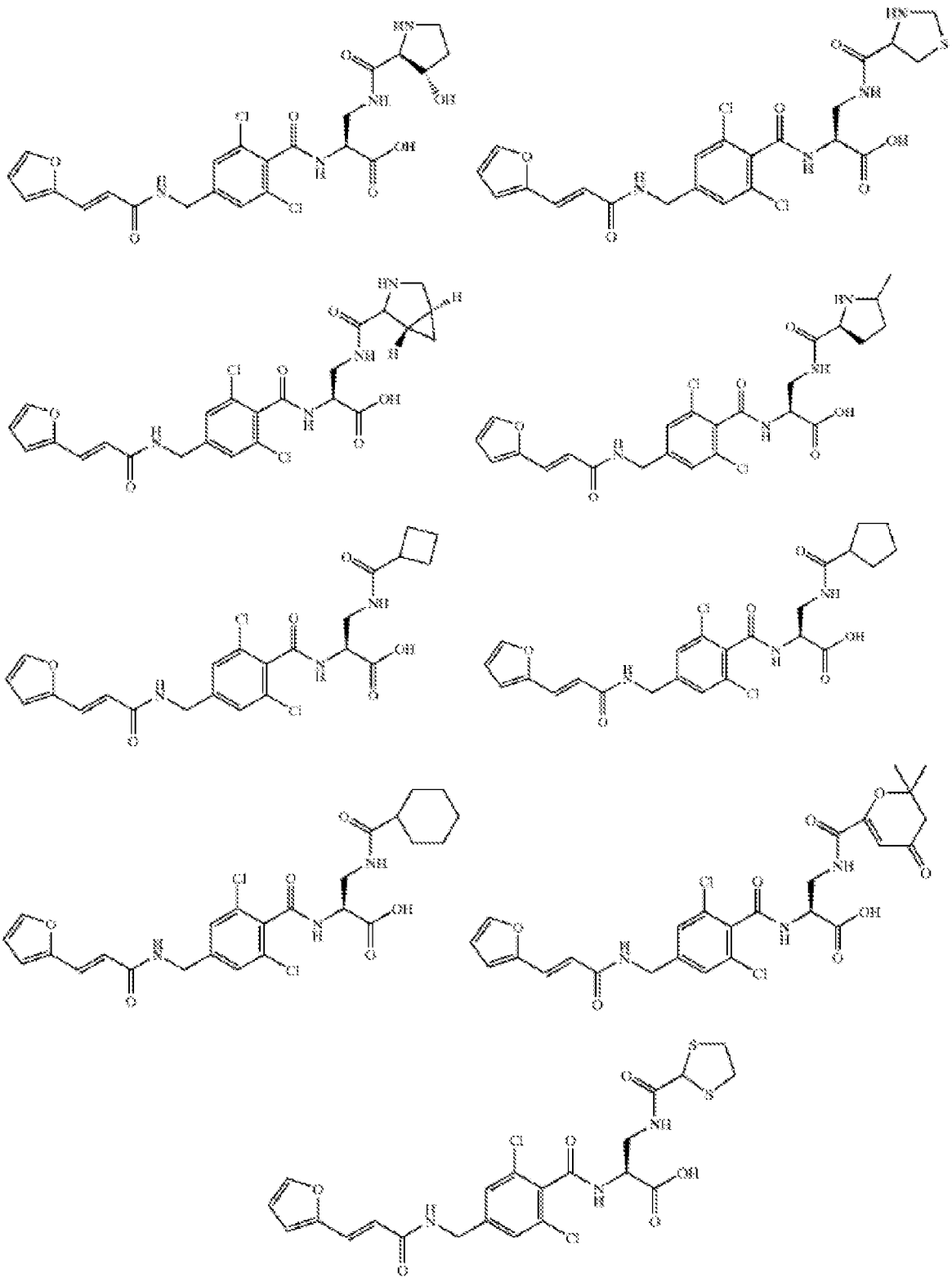


Figure 1 (Page 6)

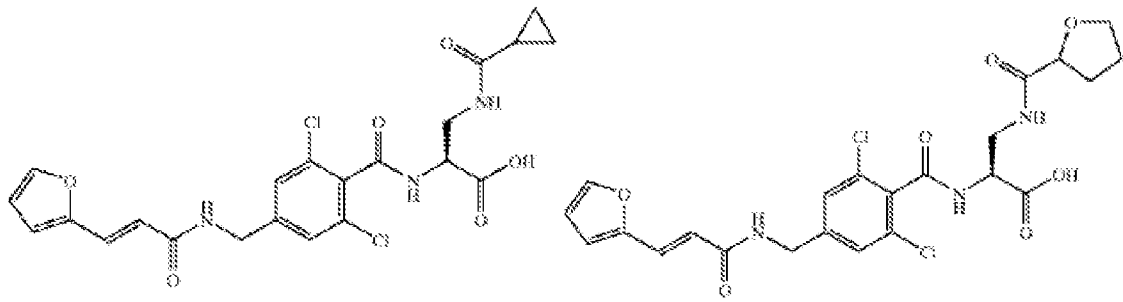
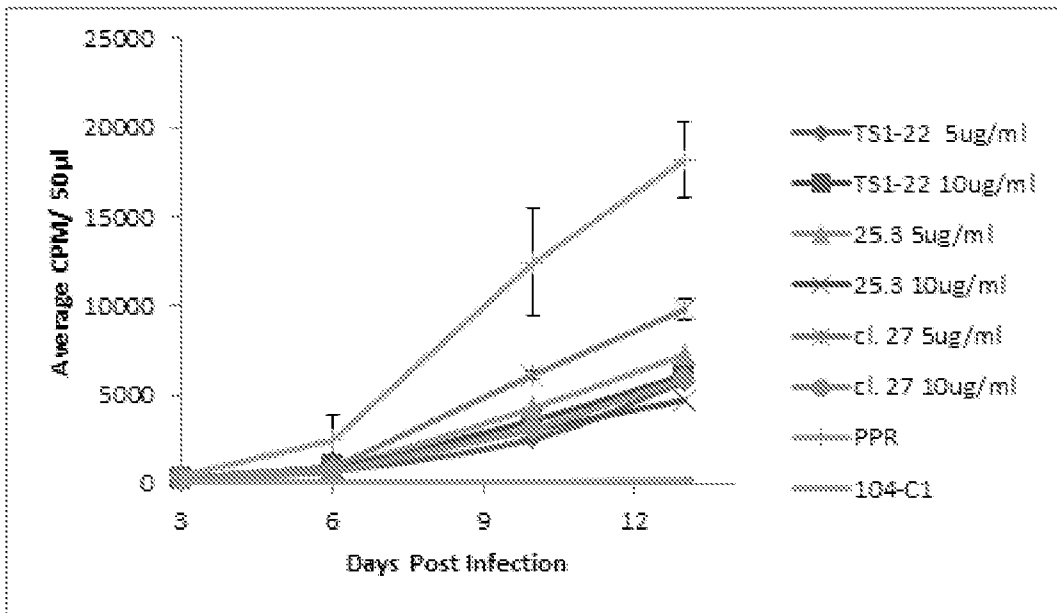


Figure 2



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/42693

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C12N 5/16 (2012.01) USPC - 435/339.1 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) USPC - 435/339.1 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC - 424/130.1, 184.1; 435/235.1; 514/408, 449, 453, 461, 563, 613 (see search terms below) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatBase, Google Scholar, Dialog retrovirus, virion, FELV, FIV, titer, budding, feline, antibody, CD11a, CD18, syncytium, LFA-1, ICAM-1, TS 1/22, light chain, heavy chain, complementary determining region (CDR), single-chain antibody, single domain antibody, Fab fragment, scFv fragment, intravenously		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2002/0039577 A1 (Townsend et al.) 4 April 2002 (04.04.2002) para[0052], para[0058], para[0061], para[0064], para[0065], para[0066], para[0067], para[0070], para[0071], para[0074]	1, 3, 5, 7-19, 23-24, 27-34, and 37-41
Y	Shimojima et al. Phenotypic changes in CD8+ peripheral blood lymphocytes in cats infected with feline immunodeficiency virus. <i>Microbes and Infection</i> 2003, Vol.5, pp 1171-1176. pg 1174	1, 3, 5, 7-19, 23-24, 27-34, and 37-41
Y	Rizzuto et al. Contribution of Virion ICAM-1 to Human Immunodeficiency Virus Infectivity and Sensitivity to Neutralization. <i>JOURNAL OF VIROLOGY</i> , June 1997, Vol.71(6), pp. 4847-4851. pg 4848, col 2; Fig 3	1, 3, 5, 7-19, 23-24, 27-34, and 37-41
Y	Li et al. Efalizumab binding to the LFA-1 alphaL I domain blocks ICAM-1 binding via steric hindrance. <i>PNAS</i> March 17, 2009, Vol. 106(11), pp 4349-4354. pg 4349, col 1, pg 4352	12-15, 19 and 27-29
Y	Dobson et al. Diagnosis and management of leukaemia in dogs and cats. <i>In Practice</i> 2006, Vol.28, pp 22-31 doi:10.1136/inpract.28.1.22 pg 24/Table	39 and 41
A	Petruzzelli et al. Activation of Lymphocyte Function-Associated Molecule (CD11a/CD18) and Mac-1 (CD11 b/CD18) Mimicked by an Antibody Directed Against CD18. <i>Journal of Immunology</i> . 1995, 155: 854-866. pg 860 col 2 - pg 861 col 1	1, 3, 5, 7-19, 23-24, 27-34, and 37-41
A	Rice et al. INDUCIBLE CELL ADHESION MOLECULE 110 (INCAM-110) IS AN ENDOTHELIAL RECEPTOR FOR LYMPHOCYTES. <i>J.Exp.Med.</i> April 1990, Vol.171, pp 1369-1374. Abstract	1, 3, 5, 7-19, 23-24, 27-34, and 37-41
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 21 October 2012 (21.10.2012)		Date of mailing of the international search report 05 NOV 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/42693

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

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

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

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

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

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

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

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1, 3, 5, 7-19, 23-24, 27-34, and 37-41

- Remark on Protest**
- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
 - The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
 - No protest accompanied the payment of additional search fees.

-----ATTACHMENT TO BOX. No. III-----

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1, 3, 5, 7-19, 23-24, 27-29, 30-32 (in part), 33-34, and 37-41 (in part), drawn to a method for treating a retrovirus infection in a feline, the method comprising administering to a feline at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18, wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to treat a retrovirus infection.

Group II, claims 2, 4, 6, 20-22, 25-26, 30-32 (in part), 35-36, and 37-41(in part), drawn to a method for treating a retrovirus infection in a feline, the method comprising administering to a feline at least one small molecule that decreases LF A-I binding to ICAM -I, wherein the at least one small molecule is administered in an amount sufficient to treat a retrovirus infection.

Group III, claims 42-67 drawn to a method for treating a retrovirus infection in a feline, the method comprising administering to a feline at least one antibody or antigen-binding fragment thereof that specifically binds to ICAM-I, wherein the at least one antibody or antigen binding fragment thereof is administered in an amount sufficient to treat a retrovirus infection.

The inventions listed as Groups I, II and III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The invention of Group II does not include the inventive concept of treating a feline with an antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18, as required by Group I.

The invention of Group I does not represent a contribution over prior art as being made obvious by the journal article entitled 'Phenotypic changes in CD8+ peripheral blood lymphocytes in cats infected with feline immunodeficiency virus.' by Shimojima et al. (hereinafter 'Shimojima') in view of the journal article entitled 'Contribution of Virion ICAM-1 to Human Immunodeficiency Virus Infectivity and Sensitivity to Neutralization.' by Rizzuto et al. (hereinafter 'Rizzuto').

Shimojima teaches that there is an expectation that an antibody to CD11a in a feline will treat FIV (page 1174 'These results indicate that CD8+ PBLs rather than CD4+ PBLs profoundly respond to FIV infection in cats. CD11a (an alphaL chain of leukocyte function-associated antigen-1, a member of the beta2 integrin family) is expressed in all leukocytes and functions as an adhesion molecule between the leukocytes and their target cells. CD11a expression in CD8+ memory/activated lymphocytes is upregulated when compared with that in naive cells; therefore, the upregulation can be used as a reliable marker for memory/activated lymphocytes in various mammals, including humans [25], [26] and [27], monkeys [28], mice [29] and [30] and rats [31]. It is reasonable that felineCD8alpha+ betalow or CD8alpha+beta- PBLs, which increase with FIV infection and have anti-FIV activities ([9] and [10], Shimojima et al., unpublished), show the upregulation of the CD11a molecule (Fig. 2c). Besides CD11a, the expression levels of CD44, CD49d and CD62L were also reported to change within CD8alpha+ PBLs of FIV-infected cats[32]; however, the use of these molecules is shown to be difficult for division into different immune stages [2], [33] and [34]. In the future, anti-CD11a antibody TMM11a will be a useful reagent with which to analyze immunological states in FIV infection.').

Rizzuto teaches that the CD11a antibody blocks HIV viral infectivity (page 4848, col 2: 'We sought to determine if an antibody against CD11a, a component of the LFA-1 receptor, could block the effects of ICAM-1 on viral infectivity.' and 'In a representative experiment (Fig. 3), the anti-CD11a monoclonal antibody reduced the ICAM-1-mediated enhancement of virus infection in a concentration-dependent manner.'). It would have been obvious to one of ordinary skill in the art to combine the references since they are both directed to treating retroviruses. As combined, one of ordinary skill in the art would have found the suggestion to use CD11a antibody in feline studies and the results that CD11a antibodies did reduced retroviral infectivity to create an expectation of success of actually using the CD11a antibodies for treating FIV for example. As combined, the claim to a method for treating a retrovirus infection in a feline, the method comprising administering to a feline at least one antibody or antigen-binding fragment thereof that specifically binds to CD11a and/or CD18, wherein the at least one antibody or antigen-binding fragment thereof is administered in an amount sufficient to treat a retrovirus infection would have been obvious to one of ordinary skill in the art.

Additionally, Groups I and II are directed to different agents. Group I is directed to an antibody and Group II is directed to a small molecule.

Finally, Groups II and III are directed to different agents since Group II is directed to small molecule and Group III is directed to an antibody.

Groups I, II and III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.