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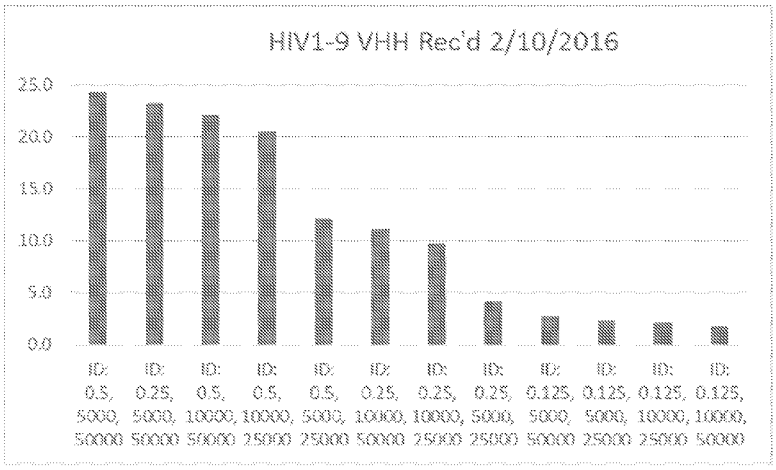

FIG. 1

(57) Abstract: This invention provides compositions and methods to treat a condition or disease without the use of exogenous targeting sequences or chemical compositions. The present invention relates to single-domain antibodies (sdAbs), proteins and polypeptides comprising the sdAbs that are directed against targets that cause a condition or disease. The invention also includes nucleic acids encoding the sdAbs, proteins and polypeptides, and compositions comprising the sdAbs. The invention includes the use of the compositions, sdAbs, and nucleic acids encoding the sdAbs for prophylactic, therapeutic or diagnostic purposes.

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SINGLE DOMAIN ANTIBODIES DIRECTED AGAINST INTRACELLULAR ANTIGENS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This PCT international patent application claims the benefit of United States Provisional Patent Application No. 62/249,898, filed on November 2, 2015, the contents of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in text format in lieu of a paper copy. The Sequence Listing is provided as a file titled "sequence listing.txt," created October 27, 2016, and is 58 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND

[0003] The use of single-domain antibodies (sdAbs) as single antigen-binding proteins or as an antigen-binding domain in larger protein or polypeptide offers a number of significant advantages over the use of conventional antibodies or antibody fragments. The advantages of sdAbs include: only a single domain is required to bind an antigen with high affinity and with high selectivity; sdAbs can be expressed from a single gene and require no post-translational modification; sdAbs are highly stable to denaturing agents or conditions including heat, pH, and proteases; sdAbs are inexpensive to prepare; and sdAbs can access targets and epitopes not accessible to conventional antibodies.

[0004] There are a number of diseases or conditions, such as viral infections or cancer, that are caused by aberrant intracellular or transmembrane components such as nucleotides and proteins. Elimination of the aberrant components can be used to prevent or treat the diseases or conditions. There are a number of pharmacological compounds available for treatment of such diseases, but the compounds can be ineffective, undeliverable, or toxic to unaffected cells.

[0005] Other treatments include the use of therapeutic proteins or agents that contain an exogenous targeting sequence so that the therapeutic agent can be recognized by receptors in the cell membrane, enabling the therapeutic agent to cross the cell membrane and enter the cell. Once the therapeutic agent is inside the cell, the therapeutic agent can interact with the target component in order to treat the disease. However, the use of exogenous targeting sequence can limit the cell type that is targeted by the therapeutic agent, and adds to the cost of manufacturing the therapeutic agent.

[0006] For the foregoing reasons, there is a need for compositions and methods to treat or prevent a disease that do not rely on exogenous targeting sequences or chemical compositions in order to enter the cell, and that are effective in targeting only the affected cells in the body.

[0007] The present invention relates to single-domain antibodies (sdAbs), proteins and polypeptides comprising the sdAbs. The sdAbs are directed against targets that cause a condition or disease. The invention also includes nucleic acids encoding the sdAbs, proteins and polypeptides, and compositions comprising the sdAbs. The invention includes the use of the compositions, sdAbs, proteins or polypeptides for prophylactic, therapeutic or diagnostic purposes. The invention also includes the use of monoclonal antibodies directed towards the sdAbs of the invention.

SUMMARY

[0008] The present invention is directed to sdAbs used to treat or prevent a condition or disease. One embodiment is directed to an anti-Human Immunodeficiency Virus Type 1 (HIV-1) reverse transcriptase single domain antibody (sdAb). In one aspect, the anti-HIV-1 reverse transcriptase sdAb comprises the amino acid sequence as set forth in SEQ ID NO:27. The invention also includes a method of treating a disease, preventing development of a disease, or preventing recurrence of a disease in a subject using an anti-HIV-1 reverse transcriptase sdAb by administration of effective amount of the anti-HIV-1 reverse transcriptase sdAb to a subject in need thereof. The subject can be a mammal, such as a human. The anti-HIV-1 reverse transcriptase sdAb can be administered in combination with one or more compounds such as, for example, a protease inhibitor. Administration of an effective amount of the anti-HIV-1 reverse transcriptase sdAb to a subject in need thereof can

be by intravenous administration, intramuscular administration, oral administration, rectal administration, enteral administration, parenteral administration, intraocular administration, subcutaneous administration, transdermal administration, administered as eye drops, administered as nasal spray, administered by inhalation or nebulization, topical administration, and administered as an implantable drug.

[0009] In another embodiment, the invention is directed to an isolated polypeptide having the amino acid sequence as set forth in SEQ ID NO:27. In another embodiment, the invention includes an antibody directed toward the polypeptide of SEQ ID NO:27.

[0010] It is also contemplated that the invention includes a method of measuring the levels of an anti-HIV-1 reverse transcriptase sdAb in a sample from a subject, the method comprising the steps of: a) generating a mouse monoclonal antibody directed against one or more domains of a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:27; b) obtaining a sample from the subject; c) performing a quantitative immunoassay with the mouse monoclonal antibody and the sample to determine the amount of sdAb in a subject; thus measuring the amount of sdAb in the subject. In one aspect, the quantitative immunoassay comprises an enzyme-linked immunosorbent assay (ELISA), specific analyte labeling and recapture assay (SALRA), liquid chromatography, mass spectrometry, fluorescence-activated cell sorting, or a combination thereof.

[0011] Another embodiment of the invention is directed to an anti-Ebola VP24 sdAb. In one aspect, the anti-Ebola VP 24 sdAb comprises the amino acid sequence as set forth in SEQ ID NO:55. The invention also includes a method of treating a disease, preventing development of a disease, or preventing recurrence of a disease in a subject using an anti-Ebola VP24 sdAb by administration of effective amount of the anti-Ebola VP24 sdAb to a subject in need thereof. The subject can be a mammal, such as a human. The anti-Ebola VP24 sdAb can be administered in combination with one or more compounds such as, for example, a protease inhibitor. Administration of an effective amount of the anti-Ebola VP24 sdAb to a subject in need thereof can be by intravenous administration, intramuscular administration, oral administration, rectal administration, enteral administration, parenteral administration, intraocular administration, subcutaneous administration, transdermal administration,

administered as eye drops, administered as nasal spray, administered by inhalation or nebulization, topical administration, and administered as an implantable drug.

[0012] In another embodiment, the invention is directed to an isolated polypeptide having the amino acid sequence as set forth in SEQ ID NO:55. In another embodiment, the invention includes an antibody directed toward the polypeptide of SEQ ID NO:55.

[0013] It is also contemplated that the invention includes a method of measuring the levels of an anti-Ebola VP24 sdAb in a sample from a subject, the method comprising the steps of: a) generating a mouse monoclonal antibody directed against one or more domains of a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:55; b) obtaining a sample from the subject; c) performing a quantitative immunoassay with the mouse monoclonal antibody and the sample to determine the amount of sdAb in a subject; thus measuring the amount of sdAb in the subject. In one aspect, the quantitative immunoassay comprises an ELISA, SALRA, liquid chromatography, mass spectrometry, fluorescence-activated cell sorting, or a combination thereof.

[0014] Yet another embodiment of the invention is directed to an anti-arachidonate 12-lipoxygenase (ALOX12) sdAb. In one aspect, the anti-ALOX12 sdAb comprises the amino acid sequence as set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, or SEQ ID NO:52. The invention also includes a method of treating a disease, preventing development of a disease, or preventing recurrence of a disease in a subject using an anti-ALOX12 sdAb by administration of effective amount of the anti-ALOX12 sdAb to a subject in need thereof. The subject can be a mammal, such as a human. The anti-ALOX12 sdAb can be administered in combination with one or more compounds. Administration of an effective amount of the anti-ALOX12 sdAb to a subject in need thereof can be by intravenous administration, intramuscular administration, oral administration, rectal administration, enteral administration, parenteral administration, intraocular administration, subcutaneous administration, transdermal administration, administered as eye drops, administered as nasal spray, administered by inhalation or nebulization, topical administration, and administered as an implantable drug.

[0015] In another embodiment, the invention is directed to an isolated polypeptide having the amino acid sequence as set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, or

SEQ ID NO:52. In another embodiment, the invention includes an antibody directed toward the polypeptide of SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, or SEQ ID NO:52.

[0016] It is also contemplated that the invention includes a method of measuring the levels of an anti-HIV-1 reverse transcriptase sdAb in a sample from a subject, the method comprising the steps of: a) generating a mouse monoclonal antibody directed against one or more domains of a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:27; b) obtaining a sample from the subject; c) performing a quantitative immunoassay with the mouse monoclonal antibody and the sample to determine the amount of sdAb in a subject; thus measuring the amount of sdAb in the subject. In one aspect, the quantitative immunoassay comprises an ELISA, SALRA, liquid chromatography, mass spectrometry, fluorescence-activated cell sorting, or a combination thereof.

DRAWINGS

[0017] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying drawings where:

Figures 1 and 2 depict the results of an ELISA using HIV1-9 anti-HIV-1 RT sdAb (SEQ ID NO:27);

Figures 3 and 4 depict the results of an ELISA using a dilution series of HIV1-9 anti-HIV-1 RT sdAb (SEQ ID NO:27);

Figures 5 through 8 depict the results of an ELISA using VP24-5 anti-Ebola VP24 sdAb (SEQ ID NO:55); and

Figures 9 and 10 depict the results of an ELISA using a dilution series of VP24-5 anti-Ebola VP24 sdAb (SEQ ID NO:55).

DESCRIPTION

[0018] As used herein, the following terms and variations thereof have the meanings given below, unless a different meaning is clearly intended by the context in which such term is used.

[0019] The terms "a," "an," and "the" and similar referents used herein are to be construed to cover both the singular and the plural unless their usage in context indicates otherwise.

[0020] The term "antigenic determinant" refers to the epitope on the antigen recognized by the antigen-binding molecule (such as an sdAb or polypeptide of the invention) and more in particular by the antigen-binding site of the antigen-binding molecule. The terms "antigenic determinant" and "epitope" may also be used interchangeably. An amino acid sequence that can bind to, that has affinity for and/or that has specificity for a specific antigenic determinant, epitope, antigen or protein is said to be "against" or "directed against" the antigenic determinant, epitope, antigen or protein.

[0021] As used herein, the term "comprise" and variations of the term, such as "comprising" and "comprises," are not intended to exclude other additives, components, integers or steps.

[0022] It is contemplated that the sdAbs, polypeptides and proteins described herein can contain so-called "conservative" amino acid substitutions, which can generally be described as amino acid substitutions in which an amino acid residue is replaced with another amino acid residue of similar chemical structure and which has little or essentially no influence on the function, activity or other biological properties of the polypeptide. Conservative amino acid substitutions are well known in the art. Conservative substitutions are substitutions in which one amino acid within the following groups (a)-(e) is substituted by another amino acid within the same group: (a) small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro and Gly; (b) polar, negatively charged residues and their (uncharged) amides: Asp, Asn, Glu and Gln; (c) polar, positively charged residues: His, Arg and Lys; (d) large aliphatic, nonpolar residues: Met, Leu, Ile, Val and Cys; and (e) aromatic residues: Phe, Tyr and Trp. Other conservative substitutions include: Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

[0023] A "domain" as used herein generally refers to a globular region of an antibody chain, and in particular to a globular region of a heavy chain antibody, or to a polypeptide that essentially consists of such a globular region.

[0024] The amino acid sequence and structure of an sdAb is typically made up of four framework regions or "FRs," which are referred to as "Framework region 1" or "FR1"; as "Framework region 2" or"FR2"; as "Framework region 3" or "FR3"; and as "Framework region 4" or "FR4," respectively. The framework regions are interrupted by three complementarity determining regions or "CDRs," which are referred as "Complementarity Determining Region 1" or "CDR1"; as "Complementarity Determining Region 2" or "CDR2"; and as "Complementarity Determining Region 3" or "CDR3," respectively.

[0025] As used herein, the term "humanized sdAb" means an sdAb that has had one or more amino acid residues in the amino acid sequence of the naturally occurring VHH sequence replaced by one or more of the amino acid residues that occur at the corresponding position in a VH domain from a conventional 4-chain antibody from a human. This can be performed by methods that are well known in the art. For example, the FRs of the sdAbs can be replaced by human variable FRs.

[0026] As used herein, an "isolated" nucleic acid or amino acid has been separated from at least one other component with which it is usually associated, such as its source or medium, another nucleic acid, another protein/polypeptide, another biological component or macromolecule or contaminant, impurity or minor component.

[0027] The term "mammal" is defined as an individual belonging to the class Mammalia and includes, without limitation, humans, domestic and farm animals, and zoo, sports, and pet animals, such as cows, horses, sheep, dogs and cats.

[0028] As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, PBS

(phosphate-buffered saline), and 5% human serum albumin. Liposomes, cationic lipids and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with a therapeutic agent as defined above, use thereof in the composition of the present invention is contemplated.

[0029] A "quantitative immunoassay" refers to any means of measuring an amount of antigen present in a sample by using an antibody. Methods for performing quantitative immunoassays include, but are not limited to, enzyme-linked immunosorbent assay (ELISA), specific analyte labeling and recapture assay (SALRA), liquid chromatography, mass spectrometry, fluorescence-activated cell sorting, and the like.

[0030] The term "solution" refers to a composition comprising a solvent and a solute, and includes true solutions and suspensions. Examples of solutions include a solid, liquid or gas dissolved in a liquid and particulates or micelles suspended in a liquid.

[0031] The term "specificity" refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule or antigen-binding protein molecule can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity. The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding protein (KD), is a measure for the binding strength between an antigenic determinant and an antigen-binding site on the antigen-binding protein: the lesser the value of the KD, the stronger the binding strength between an antigenic determinant and the antigen-binding molecule (alternatively, the affinity can also be expressed as the affinity constant (KA), which is 1/KD). As will be clear to one of skill in the art, affinity can be determined depending on the specific antigen of interest. Avidity is the measure of the strength of binding between an antigen-binding molecule and the antigen. Avidity is related to both the affinity between an antigenic determinant and its antigen binding site on the antigen-binding molecule and the number of pertinent binding sites present on the antigen-binding molecule. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined by any known manner, such as, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays.

[0032] As used herein, the term "recombinant" refers to the use of genetic engineering methods (for example, cloning, and amplification) used to produce the sdAbs of the invention.

[0033] A "single domain antibody," "sdAb" or "VHH" can be generally defined as a polypeptide or protein comprising an amino acid sequence that is comprised of four framework regions interrupted by three complementarity determining regions. This is represented as FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. An sdAb of the invention also includes a polypeptide or protein that comprises the sdAb amino acid sequence. Typically, sdAbs are produced in camelids such as llamas, but can also be synthetically generated using techniques that are well known in the art. As used herein, the variable domains present in naturally occurring heavy chain antibodies will also be referred to as "VHH domains," in order to distinguish them from the heavy chain variable domains that are present in conventional 4-chain antibodies, referred to as "VH domains," and from the light chain variable domains that are present in conventional 4-chain antibodies, referred to as "VL domains." "VHH" and "sdAb" are used interchangeably herein. The numbering of the amino acid residues of an sdAb or polypeptide is according to the general numbering for VH domains given by Kabat et al. ("Sequence of proteins of immunological interest," US Public Health Services, NIH Bethesda, MD, Publication No. 91). According to this numbering, FR1 of an sdAb comprises the amino acid residues at positions 1-30, CDR1 of an sdAb comprises the amino acid residues at positions 31-36, FR2 of an sdAb comprises the amino acids at positions 36-49, CDR2 of an sdAb comprises the amino acid residues at positions 50-65, FR3 of an sdAb comprises the amino acid residues at positions 66-94, CDR3 of an sdAb comprises the amino acid residues at positions 95-102, and FR4 of an sdAb comprises the amino acid residues at positions 103-113.

[0034] The term "synthetic" refers to production by *in vitro* chemical or enzymatic synthesis.

[0035] The term "target" as used herein refers to any component, antigen, or moiety that is recognized by the sdAb. The term "intracellular target" refers to any component, antigen, or moiety present inside a cell. A "transmembrane target" is a component, antigen, or moiety

that is located within the cell membrane. An "extracellular target" refers to a component, antigen, or moiety that is located outside of the cell.

[0036] A "therapeutic composition" as used herein means a substance that is intended to have a therapeutic effect such as pharmaceutical compositions, genetic materials, biologics, and other substances. Genetic materials include substances intended to have a direct or indirect genetic therapeutic effect such as genetic vectors, genetic regulator elements, genetic structural elements, DNA, RNA and the like. Biologics include substances that are living matter or derived from living matter intended to have a therapeutic effect.

[0037] As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of a disease or an overt symptom of the disease. The therapeutically effective amount may treat a disease or condition, a symptom of disease, or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of disease, or the predisposition toward disease. The specific amount that is therapeutically effective can be readily determined by an ordinary medical practitioner, and may vary depending on factors known in the art, such as, *e.g.*, the type of disease, the patient's history and age, the stage of disease, and the administration of other therapeutic agents.

[0038] The present invention relates to single-domain antibodies (sdAbs) that are directed against viral and intracellular components, as well as to proteins and polypeptides comprising the sdAbs and nucleotides encoding the proteins and polypeptides. The invention can also relate to sdAbs that are directed against intercellular, transcellular and extracellular targets or antigens. The invention also includes nucleic acids encoding the sdAbs, proteins and polypeptides, and compositions comprising the sdAbs. The invention includes the use of the compositions, sdAbs, proteins or polypeptides for prophylactic, therapeutic or diagnostic purposes.

[0039] SdAbs have a number of unique structural characteristics and functional properties which make sdAbs highly advantageous for use as functional antigen-binding domains or proteins. SdAbs functionally bind to an antigen in the absence of a light chain variable domain, and can function as a single, relatively small, functional antigen-binding

structural unit, domain or protein. This distinguishes sdAbs from the domains of conventional antibodies, which by themselves do not function as an antigen-binding protein or domain, but need to be combined with conventional antibody fragments such as antigen-binding fragments (Fab) or single chain variable fragments (ScFv) in order to bind an antigen.

[0040] SdAbs can be obtained using methods that are well known in the art. For example, one method for obtaining sdAbs includes (a) immunizing a Camelid with one or more antigens, (b) isolating peripheral lymphocytes from the immunized Camelid, obtaining the total RNA and synthesizing the corresponding complementary DNAs (cDNAs), (c) constructing a library of cDNA fragments encoding VHH domains, (d) transcribing the VHH domain-encoding cDNAs obtained in step (c) to messenger RNA (mRNA) using PCR, converting the mRNA to ribosome display format, and selecting the VHH domain by ribosome display, and (e) expressing the VHH domain in a suitable vector and, optionally purifying the expressed VHH domain.

[0041] Another method of obtaining the sdAbs of the invention is by preparing a nucleic acid encoding an sdAb using techniques for nucleic acid synthesis, followed by expression of the nucleic acid *in vivo* or *in vitro*. Additionally, the sdAb, polypeptides and proteins of the invention can be prepared using synthetic or semi-synthetic techniques for preparing proteins, polypeptides or other amino acid sequences.

[0042] The sdAbs of the invention will generally bind to all naturally occurring or synthetic analogs, variants, mutants, alleles, parts and fragments of the target, or at least to those analogs, variants, mutants, alleles, parts and fragments of the target that contain one or more antigenic determinants or epitopes that are essentially the same as the antigenic determinant or epitope to which the sdAbs of the invention bind in the wild-type target. The sdAbs of the invention may bind to such analogs, variants, mutants, alleles, parts and fragments with an affinity and/or specificity that is the same as, or that is higher than or lower than the affinity and specificity with which the sdAbs of the invention bind to the wild-type target. It is also contemplated within the scope of the invention that the sdAbs of the invention bind to some analogs, variants, mutants, alleles, parts and fragments of the target but not to others. In addition, the sdAb of the invention may be humanized, and may be monovalent or multivalent, and/or multispecific. Additionally, the sdAbs of the invention

can bind to the phosphorylated form of the target protein as well as the unphosphorylated form of the target protein. sdAbs can be linked to other molecules such as albumin or other macromolecules.

[0043] In addition, it is within the scope of the invention that the sdAbs are multivalent, that is, the sdAb can have two or more proteins or polypeptides which are directed against two or more different epitopes of the target. In such a multivalent sdAb, the protein or polypeptide may be directed, for example, against the same epitopes, substantially equivalent epitopes, or different epitopes. The different epitopes may be located on the same target, or it could be on two or more different targets.

[0044] It is also contemplated that the sequence of one or more sdAbs of the invention may be connected or joined with one or more linker sequences. The linker can be, for example, a protein sequence containing a combination of serines, glycines and alanines.

[0045] It is also within the scope of the invention to use parts, fragments, analogs, mutants, variants, alleles and/or derivatives of the sdAbs of the invention, as long as these are suitable for the described uses.

[0046] Since the sdAbs of the invention are mainly intended for therapeutic and/or diagnostic use, they are directed against mammalian, preferably human, targets. However, it is possible that the sdAbs described herein are cross-reactive with targets from other species, for example, with targets from one or more other species of primates or other animals (for example, mouse, rat, rabbit, pig or dog), and in particular in animal models for diseases and disorders associated with the disease associated with the targets.

[0047] In another aspect, the invention relates to a nucleic acid that encodes an sdAb of the invention. Such a nucleic acid may be, for example, in the form of a genetic construct.

[0048] In another aspect, the invention relates to host or host cell that expresses or is capable of expressing an sdAb of the invention, and/or that contains a nucleic acid encoding an sdAb of the invention. Sequences of the sdAbs can be used to insert into the genome of any organism to create a genetically modified organism (GMO). Examples include, but are not limited to, plants, bacteria, viruses, and animals.

[0049] The invention further relates to methods for preparing or generating the sdAbs, nucleic acids encoding the sdAbs, host cells expressing or capable of expressing such sdAbs, products and compositions containing the sdAbs of the invention.

[0050] The invention further relates to applications and uses of the sdAbs, the nucleic acids encoding the sdAbs, host cells, products and compositions described herein. Such a product or composition may, for example, be a pharmaceutical composition for treatment or prevention of a disease, or a product or composition for diagnostic use. The sdAbs can be used in a variety of assays, for example ELISA assays and mass spectrometry assays to measure the serum and tissue levels of the sdAbs.

[0051] In another aspect, a nucleic acid encoding one or more sdAbs of the invention can be inserted into the genome of an organism to treat or prevent diseases.

[0052] The present invention generally relates to sdAbs, as well as to proteins or polypeptides comprising or essentially consisting of one or more of such sdAbs, that can be used for prophylactic, therapeutic and/or diagnostic purposes.

[0053] The methods and compositions detailed in the present invention can be used to treat diseases described herein, and can be used with any dosage and/or formulation described herein or otherwise known, as well as with any route of administration described herein or otherwise known to one of skill in the art.

[0054] The sdAbs of the invention can be used for treatment and prevention of diseases caused by viruses or by aberrant cellular proteins. The sdAbs of the present invention can also be used for treatment and prevention of diseases. The sdAbs of the invention can be used to target diseases when there is an overexpression of an intracellular molecule. They can also be used to treat viral infections by targeting intracellular viral proteins in infected cells. Blocking production of viral proteins, such as, for example, HIV-1 reverse transcriptase, can block the viral life-cycle.

[0055] The sdAbs of the invention can also target intracellular viral proteins such as Ebola VP24 and thus block Ebola's ability to shut down the host's anti-viral immune response.

[0056] The sdAbs of the invention can be used with one or more compounds. For example, the sdAb of the invention can be used with JAK/STAT inhibitors such as, for example, Curcumin, Resveratrol, Cucurbitacin A, B, E, I, Q, Flavopiridol, Deoxytetrangomycin, Cyclopentenone derivatives, N-Acylhomoserine Lactone, Indirubin derivatives, Meisoindigo, Tyrphostins, Platinum-containing compounds (e.g., IS3-295), Peptidomimetics, antisense oligonucleotides, S3I-201, phosphotyrosin tripeptide derivatives, HIV protease inhibitors (e.g., nelfinavir, indinavir, saquinavir, & ritornavir), JSI-124, XpYL, Ac-pYLPQTV-NH2, ISS 610, CJ-1383, pyrimethamine, Metformin, Atiprimod, S3I-M2001, STX-0119; N-[2-(1,3,4-oxadiazolyl)]-4 quinolinecarboxamide derivative, S3I-1757, LY5; 5,8-dioxo-6(pyridin-3-ylamino)-5,8,-dihydro-naphthalene-1-sulfonamide, withacinstin, Stattic, STA-21, LLL-3, LLL12, XZH-5, SF-1066, SF-1087, 17o, Cryptotanshinone, FLL32, FLL62, C188-9, BP-1108 and BP-1075, Galiellalactone, JQ1, 5, 15 DPP, WP1066, Niclosamide, SD1008, Nifuroxazide, Cryptotanshinone, BBI quinone, and Ruxolitnib Phosphate. The one or more compounds can increase the therapeutic response and augment the effectiveness of the sdAbs of the invention. In addition, the effectiveness of the sdAbs can be increased by combining it with peptides, peptidomimetics, and other drugs, such as, for example, but not limited to, cimetidine, atorvastatin, celecoxib, metformin, and cimetidine.

[0057] It is also contemplated that one or more sdAbs of the invention can be combined, or the sdAbs of the invention can be combined with other sdAbs.

[0058] It is contemplated that certain sdAbs of the invention can cross the cell membrane and enter the cell without the aid of additional targeting protein sequences on the sdAb, and without the aid of exogenous compounds that direct the sdAb to bind to the cell surface receptors and cross the cell membrane.

[0059] After crossing the cell membrane, these sdAbs can target transmembrane or intracellular molecules or antigens. These targets can be, for example, proteins, carbohydrates, lipids, nucleic acids, mutated proteins, viral proteins, and prions. The sdAb targets may function as enzymes, structural proteins of the cell, intracellular portions of cell membrane molecules, molecules within the membranes of organelles, any type of RNA molecule, any regions of DNA or chromosome, methylated or unmethylated nucleic acids, partially assembled molecules within the synthesis mechanism of the cell, second messenger

molecules, and molecules within cell signaling mechanisms. Targets may include all molecules in the cytoplasm, nucleus, organelles, and cell membrane. Molecules destined for secretion or placement in the cell membrane can be targeted within the cytoplasm before leaving the cell.

[0060] The sdAb targets can be in humans, animals, plants, fungi, parasites, protists, bacteria, viruses, prions, prokaryotic cells, and eukaryotic cells. Some examples of intercellular and intracellular signaling molecules and protein groups that can be targeted by the sdAbs of the invention are: oncogene products, hormones, cytokines, growth factors, neurotransmitters, kinases (including tyrosine kinase, serine kinase, and threonine kinase), phosphatases, ubiquitin, cyclic nucleotides, cyclases (adenylyl and guanylyl), G proteins, phosphodiesterases, GTPase superfamily, immunoglobulins (antibodies, Fab fragments, binders, sdAbs), immunoglobulin superfamily, inositol phosphate lipids, steroid receptors, calmodulin, CD group (e.g., CD4, CD8, CD28, etc.), transcription factors, TGF-beta, TNF-alpha and beta, TNF ligand superfamily, notch receptor signaling molecules, hedgehog receptor signaling molecules, Wnt receptor signaling molecules, toll-like receptor signaling molecules, caspases, actin, myosin, myostatin, 12-lipoxygenase, 15-lipoxygenase, lipoxygenase superfamily, reverse transcriptase, viruses and their proteins, amyloid proteins, collagen, G protein coupled receptors, mutated normal proteins, prions, Ras, Raf, Myc, Src, BCR/ABL, MEK, Erk, Mos, Tpl2, MLK3, TAK, DLK, MKK, p38, MAPK, MEKK, ASK, SAPK, JNK, BMK, MAP, JAK, PI3K, cyclooxygenase, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6, Myc, p53, BRAF, NRAS, KRAS, HRAS and chemokines.

[0061] HIV is a retrovirus that causes acquired immunodeficiency syndrome (AIDS) in humans. AIDS results in progressive failure of the infected individual's immune system, which results in the development of life-threatening opportunistic infections and cancers. The average survival time after infection with HIV is estimated to be 9 to 11 years without treatment

[0062] HIV is transmitted as single-stranded, positive-sense, enveloped RNA virus. Upon entry into the target cell, the viral RNA genome is reverse transcribed into double-stranded DNA by a virally encoded reverse transcriptase (RT) that is transported along with the viral genome in the virus particle. RT is an RNA-dependent DNA polymerase and also

has RNaseH activity. The resulting viral DNA is then imported into the host cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors. Once integrated, the virus may become latent for months or years. Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the cell as new virus particles.

[0063] Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is more virulent, more infective, and is the cause of the majority of HIV infections globally. HIV-2 is largely confined to West Africa.

[0064] Anti-HIV RT sdAbs were developed to target HIV-1 reverse transcriptase. The anti-HIV-1 RT sdAb may successfully treat individuals infected with HIV either alone or in combination with other retroviral agents. Using methods that are well-known in the art, recombinant HIV-1 reverse transcriptase protein (Creative Biomart, Shirley, NY) (SEQ ID NO:1) was used to generate sdAbs that are directed against or can bind to an epitope of HIV-1 RT.

[0065] The protein sequence used for immunization of a camel of the recombinant HIV-1 reverse transcriptase protein (SEQ ID NO:1) was
PISPIETVPVKLKPGMDGPKVKQWPLT
EEKIKALVEICAELEEEGKISRIGPENPYNTPVFAIKKKDSTKWRKLVDFRELNKRTQ
DFWEVQLGIPHPAGLKKKKSVTVLDVGDAYFSIPLDEDFRKYTAFTIPSTNNETPGTR
YQYNVLPQGWKGSPAIFQSSMTKILEPFRKQNPDIVIYQYVDDLYVGSDLEIGQHRT
KVEELRQHLWRWGFYTPDKKHQKEPPFLWMGYELHPDKWTVQPIVLPEKDSWTVN
DIQK

[0066] As a result of the immunization, several sdAbs were obtained and screened. The DNA sequences of the anti-HIV-1 RT sdAbs are listed below:

[0067] HIV1-1 (SEQ ID NO:2): 5'-
gatgtgcagctggtggagtctgggggaggctcggtgcaggctggagggtc
tctgagactctcctgtgcagcctctgtttacagctacaacacaaactgcatgggttggttccgccaggctccagggaaggagcgcgag
ggggtcgcagttatttatgctgctggtggattaacatactatgccgactccgtgaagggccgattcaccatctcccaggagaatggcaa

gaatacggtgtacctgacgatgaaccgcctgaaacctgaggacactgccatgtactactgtgcggcaaagcgatggtgtagtagctgg
aatcgcggtgaggagtataactactggggccaggggacccaggtcaccgtctcctca-3'

[0068] HIV1-2 (SEQ ID NO:3): 5'-caggtgcagctggtggagtctgggggaggctcggtgcaggctggaga
ctctctgagactctcctgtgcagcctctggaaacactgccagtaggttctccatgggctggttccgccaggctccagggaaggagcgc
gaggggtcgcggctatttctgctggtggtaggcttacatactatgccgactccgtgaagggccgattcaccatctcccgagacaacg
ccaagaacacgctgtatctggacatgaacaacctgaaacctgaggacactgccatgtactactgtgccgcaattagtgaccggatgac
tggtattcaggctcttgcggctctacccagacttcgcccagaagactacggtaactggggccaggggaccctggtcaccgtctcctca-
3'

[0069] HIV1-7 (SEQ ID NO:4): 5'-gaggtgcagctggtggagtctgggggagactcggtgcaggctgga
gggtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccag
gaaaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctcc
caagacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagaca
gattcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggaccctggtcaccgtctcctc
a-3'

[0070] HIV1-8 (SEQ ID NO:5): 5'-caggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggccaggggacccaggtcaccgtctcctca
-3'

[0071] HIV1-6 (SEQ ID NO:6): 5'-caggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccaatatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggccaggggaccctggtcaccgtctcctca-
3'

[0072] HIV1_28 (SEQ ID NO:7): 5'- aggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca

agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggccaggggaccctggtcaccgtctcctca-
3’

[0073] HIV1-21 (SEQ ID NO:8): 5’-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcaccgtctcctca-
3’

[0074] HIV1-37 (SEQ ID NO:9): 5’-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcaccgtctcctca-
3’

[0075] HIV1-3 (SEQ ID NO:10): 5’-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcactgtctcctca-
3’

[0076] HIV1-5 (SEQ ID NO:11): 5’-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaggcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctaccattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgctaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga

ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcaccgtctcctca-3’

[0077] HIV1-10 (SEQ ID NO:12): 5’-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcagcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcaccgtctcctca-3’

[0078] HIV1_29 (SEQ ID NO:13): 5’-
gaggtgcagctggtggagtctgggggagactcagtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcaccgtctcctca
-3’

[0079] HIV1_32 (SEQ ID NO:14): 5’-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcaccgtctcctca-3’

[0080] HIV1-9 (SEQ ID NO:15): 5’-
gaggtgcagctggtggagtctgggggaggctcggtgcaggctggagg
gtctctgagactctcctgtgcagcctctgtttacagctacaacacaaactgcatgggttggttccgccaggctccagggaaggagcgcg
aggggggtcgcagttatttatgctgctggtggattaacatactatgccgactccgtgaagggccgattcaccatctcccaggagaatggc
aagaacacggtgtacctgacgatgaaccgcctgaaacctgaggacactgccatgtactactgtgcggcaaagcgatggtgtagtagc
tggaatcgcggtgaggagtataactactggggccaggggacccaggtcactgtctcctca-3’

[0081] HIV1-16 (SEQ ID NO:16): 5'-
caggtgcagctggtggagtctgggggaggctcggtgcaggctggagg
gtctctgagactctcctgtgcagcctctggaaacacctacagtagtagctactgcatgggctggttccgccaggctccagggaaggac
cgcgaggggtcgcgcgtattttcactcgaagtggtaccacatactatgccgactccgtgaagggccgattcaccatttcccgtgacaa
cgccaagaacacggtgtatctgcaaatgaacagcctgaaacctgaagacgctgccatgtactactgtgcggcagcccaggggggtg
cctgcatttcgtttacttcgttcgcgaagaatttcgtgtaccggggccaggggaccctggtcactgtctcctca-3'

[0082] HIV1-13 (SEQ ID NO:17): 5'-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggtcctctgactataactactggggtgaggggaccctggtcaccgtctcctca-
3'

[0083] HIV1_35 (SEQ ID NO:18): 5'-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggtcctctgactataactactggggtgaggggaccctggtcaccgtctcctca-
3'

[0084] HIV1-11 (SEQ ID NO:19): 5'-
caggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcactgtctcctca-
3'

[0085] HIV1_22 (SEQ ID NO:20): 5'-
caggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga

aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcaccgtctcctca-
3’

[0086] HIV1-4 (SEQ ID NO:21): 5’- catgtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggaccctggtcaccgtctcctca-
3’

[0087] HIV1_38 (SEQ ID NO:22): 5’-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccaactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga
ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactatgactactggggtgaggggaccctggtcaccgtctcctca-
3’

[0088] HIV1_23 (SEQ ID NO:23): 5’-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaagcctctggatacacctacaatagtagagtcgatatcagatctatgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatggacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacag
attcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcaccgtctcctc
a-3’

[0089] HIV1_25 (SEQ ID NO:24): 5’-
gaggtgcagctggtggagtctgggggagactcggtgcaggctggagg
gtctcttcaactctcctgtaaggcctctggatacacctacaatagtagagtcgatatcagatctgtgggctggttccgccagtatccagga
aaggagcgcgaggggtcgctactattaatattcgtaatagtgtcacatactatgccgactccgtgaagggccgattcaccatctccca
agacaacgccaagaacacggtgtatctgcaaatgaacgccctgaaacctgaggacactgccatgtactactgtgcgttgtcagacaga

ttcgcggcgcaggtacctgccaggtacggaatacggccctctgactataactactggggtgaggggacccaggtcaccgtctcctca-3'

[0090] The amino acid sequences of the anti-HIV-1 RT sdAbs are shown below:

[0091] HIV1-1 (SEQ ID NO:25):
DVQLVESGGGSVQAGGSLRLSCAASVYSYNTNC
MGWFRQAPGKEREGVAVIYAAGGLTYYADSVKGRFTISQENGKNTVYLTMNRLKP
EDTAMYYCAAKRWCSSWNRGEEYNYWGQGTQVTVSS

[0092] HIV1-2 (SEQ ID NO:26):
QVQLVESGGGSVQAGDSLRLSCAASGNTASRFSM
GWFRQAPGKEREGVAAISAGGRLTYYADSVKGRFTISRDNAKNTLYLDMNNLKPED
TAMYYCAAISDRMTGIQALAALPRLRPEDYGNWGQGTLVTVSS

[0093] HIV1-9 (SEQ ID NO:27):
EVQLVESGGGSVQAGGSLRLSCAASVYSYNTNCM
GWFRQAPGKEREGVAVIYAAGGLTYYADSVKGRFTISQENGKNTVYLTMNRLKPED
TAMYYCAAKRWCSSWNRGEEYNYWGQGTQVTVSS

[0094] HIV1-16 (SEQ ID NO:28):
QVQLVESGGGSVQAGGSLRLSCAASGNTYSSSY
CMGWFRQAPGKDREGVARIFTRSGTTYYADSVKGRFTISRDNAKNTVYLQMNSLKP
EDAAMYYCAAAQGGACISFTSFAKNFVYRGQGTLVTVSS

[0095] HIV1-27 (SEQ ID NO:29):
EVQLGESGGGSVQAGGSLRLSCAASVYSYTTNCM
GWFRQAPGKEREGVAVIYSAGGLTYYADSVKGRFTISQDNGKNTVYLTMNRLKPED
TAMYYCAAKRWCSSWNRGEEYNYWGQGTQVTVSS

[0096] HIV1-30 (SEQ ID NO:30): QVQLVESGGGSVQAGGSLRLSCAASVYSYNTN
CMGWFRQAPGKEREGAAVIYAAGGLTYYADSVKGRFTISQENGKNTVYLTMNRLK
PEDTAMYYCAAKRWCSSWNRGEEYNYWGQGTQVTVSS

[0097] HIV1-21 (SEQ ID NO:31): EVQLVESGGDSVQAGGSLQLSCKASGYTYNSR VDIRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMN ALKPEDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGEGTQVTVSS

[0098] HIV1-4 (SEQ ID NO:32): HVQLVESGGDSVQAGGSLQLSCKASGYTYNSR VDIRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMN ALKPEDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGEGTLVTVSS

[0099] HIV1-6 (SEQ ID NO:33): QVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD IRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNAL KPEDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGQGTLVTVSS

[0100] HIV1-7 (SEQ ID NO:34): EVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD IRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNAL KPEDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGEGTLVTVSS

[0101] HIV1-8 (SEQ ID NO:35): QVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD IRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNAL KPEDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGQGTQVTVSS

[0102] HIV1-11 (SEQ ID NO:36): QVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD IRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNAL KPEDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGEGTQVTVSS

[0103] HIV1-13 (SEQ ID NO:37): EVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD IRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNAL KPEDTAMYYCALSDRFAAQVPARYGIRSSDYNYWGEGTLVTVSS

[0104] HIV1-23 (SEQ ID NO:38): EVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD

IRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMDAL
KPEDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGEGTQVTVSS

[0105] HIV1-24 (SEQ ID NO:39):
HVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD
IRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNAL
KPGDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGQGTLVTVSS

[0106] HIV1-25 (SEQ ID NO:40):
EVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD
IRSVGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNAL
KPEDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGEGTQVTVSS

[0107] HIV1-31 (SEQ ID NO:41):
DVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD
IRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNAL
KPEDTAMYYCALSDRFAAQVPARYGIRPSDYNYWGEGTQVTVSS

[0108] HIV1-38 (SEQ ID NO:42):
EVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD
IRSMGWFRQYPGKEREGVATINIRNSVTYYANSVKGRFTISQDNAKNTVYLQMNAL
KPEDTAMYYCALSDRFAAQVPARYGIRPSDYDYWGEGTLVTVSS

[0109] HIV1-39 (SEQ ID NO:43):
EVQLVESGGDSVQAGGSLQLSCKASGYTYNSRVD
IRSMGWFRQYPGKEREGVATINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNAL
KPEDTAMYYCALSDRFAAQVPTRYGIRPSDYNYWGQGTQVTVSS

[0110] One or more mouse monoclonal antibodies can be generated against one or more domains of the anti-HIV-1 RT sdAbs of the invention. The mouse monoclonal antibody can be generated by methods that are known by one of skill in the art, for example, the mouse monoclonal antibody can be produced by a mouse hybridoma. The mouse monoclonal antibody can be used in diagnostic assays, for example, the antibody can be used in an

immunoassay such as an ELISA or mass spectrometry assay in order to measure the amount of anti-HIV-1 RT sdAb present in a sample from a patient.

[0111] SdAbs were also generated against a recombinant Arachidonate 12-lipoxygenase (ALOX12). ALOX12 is also known as platelet-type 12-lipoxygenase, arachidonate oxygen 12-oxidoreductase, Delta12-lipoxygenase, 12Delta-lipoxygenase, C-12 lipoxygenase, leukotriene A4 synthase, and LTA4 synthase. ALOX12 is a lipoxygenase-type enzyme that participates in arachidonic acid metabolism. ALOX12 has been implicated in the development and complications of dietary-induced and/or genetically-induced diabetes, adipose cell/tissue dysfunction, and obesity. ALOX12 has also been thought to regulate blood vessel contraction, dilation, pressure, remodeling, and angiogenesis. Inhibition of ALOX12 prevents the development of blood vessel formation and thus ALOX12 is a target for reducing neo-vascularization that promotes atherosclerosis, Steatohepatitis, and other arthritic and cancer diseases. Elevated amounts of ALOX12 may contribute to the development of Alzheimer's disease.

[0112] The present invention provides sdAbs, proteins, and polypeptides that are directed against the ALOX12 protein.

[0113] It is contemplated that the anti-ALOX12 sdAbs and polypeptides of the invention can be used for the prevention and/or treatment of diseases and disorders associated with and/or mediated by ALOX12, such as diabetes, adipose cell dysfunction, obesity, atherosclerosis, Steatohepatitis, arthritis and cancer.

[0114] Recombinant human ALOX12 protein was used to generate sdAbs that are directed against or can bind to an epitope of ALOX12. To generate the anti-ALOX12 sdAbs, recombinant human ALOX12 was expressed in *Escherichia coli* and used as the target antigen.

[0115] The recombinant ALOX12 protein sequence (SEQ ID NO:44) used for immunization of camels was:

MGRYRIRVATGAWLFSGSYNRVQLWLVGTRGEAELELQLRPARGEEEEFDHDVAE
DLGLLQFVRLRKHHWLVDDAWFCDRITVQGPGACAEVAFPCYRWVQGEDILSLPEG
TARLPGDNALDMFQKHREKELKDRQQIYCWATWKEGLPLTIAADRKDDLPPNMRF

HEEKRLDFEWTLKAGALEMALKRVYTLLSSWNCLEDFDQIFWGQKSALAEKVRQC
WQDDELFSYQFLNGANPMLLRRSTSLPSRLVLPSGMEELQAQLEKELQNGSLFEADF
ILLDGIPANVIRGEKQYLAAPLVMLKMEPNGKLQPMVIQIQPPNPSSPTPTLFLPSDPP
LAWLLAKSWVRNSDFQLHEIQYHLLNTHLVAEVIAVATMRCLPGLHPIFKFLIPHIRY
TMEINTRARTQLISDGGIFDKAVSTGGGGHVQLLRRAAAQLTYCSLCPPDDLADRGL
LGLPGALYAHDALRLWEIIARYVEGIVHLFYQRDDIVKGDPELQAWCREITEVGLCQ
AQDRGFPVSFQSQSQLCHFLTMCVFTCTAQHAAINQGQLDWYAWVPNAPCTMRMP
PPTTKEDVTMATVMGSLPDVRQACLQMAISWHLSRRQPDMVPLGHHKEKYFSGPK
PKAVLNQFRTDLEKLEKEITARNEQLDWPYEYLKPSCIENSVTI

[0116] As a result of the immunization, several sdAbs were obtained and screened. The DNA sequences of the sdAbs are listed below:

[0117] ALOX_21 (SEQ ID NO:45): 5'-gaggtgcagctggtggagtctgggggaggttcggtgcagg
ctggagggtctctgaggatctcctgtacagcctctggattcacttttgatgacactgacatgggctggtaccgccagactctaggaaatg
ggtgcgagttggtttctcagattagtaatgatggtagtacattctatagagattccgtgaagggccgattcaccatctcctgggaccgcgt
caacaacacggtgtatctgcaaatgagcgccctgagacctgaggacacggccatgtattactgcaatatcaacgggtgtaggagacc
ctcgtacaatcttcacttgaacgcatggggccaggggacacaggtcaccgtctcctca-3'

[0118] ALOX_41 (SEQ ID NO:46): 5'-caggtgcagctggtggagtctgggggaggctcggtgcagg
ctggagggtctctgacactgtcctgtgtagcctctggatacggctacagtgccacgtgcatgggctggttccgccaggctccagggaa
ggagcgcgaggggtcgcgtctatttcaccttatggtgttagaaccttctatgccgactccgcgaaaggccgattcaccgtctcccgag
acaacgccaagaacacgctgtatctgcaaatgaacagcctgaaacctgaggacacgtccgtgtactactgtgcggccggttcgggcg
ttggtgtttgttcactttcgtatccatacacctactggggccaggggacccaggtcaccgtctcctca-3'

[0119] ALOX_43 (SEQ ID NO:47): 5'-caggtgcagctggtggagtctgggggaggctcggtgcgg
gctggagagtctctgagactctcctgtgtagcctctagatccatctatgtttggtactgcatgggctggttccgccaggctgcagggaag
gagcgcgaggggtcggaagtatgttcgttggtggcggtaggacatattatgacgactccgtcaagggccgattcaccatctcccaag
acaaggccaagaacacgctgtatctgcaaatggacaacctggcacctgaagacactgccatgtattactgtgcggctgggcgctgcg
gtggcaactggctgagaagcaatgctttcgacaaatggggccaggggacactggtcaccgtctcctca-3'

[0120] ALOX_46 (SEQ ID NO:48): 5'-gatgtgcagctggtggagtctgggggaggctcggtgcagg
ctggagggtctctgagactctcctgtgcagccactggaaacacctacattagccgctgcatgggctggttccgccagcctccagggaa
ggagcgcgaggtggtcgcacgtatttataccgactctggtaatacatactatcccgacgccgtggagggccgattcaccatctcccaa

gacaacgccaagaacacgatatatctgcaaatgaacagcctgaaacctgacgacaccgccgtgtactactgtgtgctctcagaggcc gtctgtacaaaagaacctggggactttcgttactggggccaggggacccaggtcactgtctcctca-3'

[0121] The protein sequences of the anti-ALOX sdAbs generated are as follows:

[0122] ALOX_21 (SEQ ID NO:49): EVQLVESGGGSVQAGGSLRISCTAS GFTFDDTDMGWYRQTLGNGCELVSQISNDGSTFYRDSVKGRFTISWDRVNNTVYLQ MSALRPEDTAMYYCNINGCRRPSYNLHLNAWGQGTQVTVSS

[0123] ALOX_41 (SEQ ID NO:50): QVQLVESGGGSVQAGGSLTLSCVAS GYGYSATCMGWFRQAPGKEREGVASISPYGVRTFYADSAKGRFTVSRDNAKNTLYL QMNSLKPEDTSVYYCAAGSGVGVCSLSYPYTYWGQGTQVTVSS

[0124] ALOX_43 (SEQ ID NO:51): QVQLVESGGGSVRAGESLRLSCVAS RSIYVWYCMGWFRQAAGKEREGVGSMFVGGGRTYYDDSVKGRFTISQDKAKNTLY LQMDNLAPEDTAMYYCAAGRCGGNWLRSNAFDKWGQGTLVTVSS

[0125] ALOX_46 (SEQ ID NO:52): DVQLVESGGGSVQAGGSLRLSCAAT GNTYISRCMGWFRQPPGKEREVVARIYTDSGNTYYPDAVEGRFTISQDNAKNTIYLQ MNSLKPDDTAVYYCVLSEAVCTKEPGDFRYWGQGTQVTVSS

[0126] One or more mouse monoclonal antibodies can be generated against one or more domains of the anti-ALOX12 sdAbs of the invention. The mouse monoclonal antibody can be generated by methods that are known by one of skill in the art, for example, the mouse monoclonal antibody can be produced by a mouse hybridoma. The mouse monoclonal antibody can be used in diagnostic assays, for example, the antibody can be used in an immunoassay such as an ELISA or mass spectrometry assay in order to measure the amount of anti-ALOX12 sdAb present in a sample from a patient.

[0127] Ebola, also known as Ebola virus disease (EVD) and Ebola hemorrhagic fever (EHF), is a viral hemorrhagic fever of humans and other primates caused by Ebolavirus. The disease has a high risk of death, killing between 25 and 90 percent of those infected, typically six to sixteen days after symptoms appear.

[0128] Ebola interferes with proper functioning of the infected individual's innate immune system. Ebola proteins weaken the immune system's response to viral infections by interfering with the cells ability to produce and respond to interferon proteins such as interferon-alpha, interferon-beta, and interferon gamma. Ebola's structural proteins, VP24 and VP35, play a key role in this interference. The V24 protein blocks the production of the host cell's antiviral proteins. By inhibiting the host's immune responses, Ebola quickly spreads throughout the body.

[0129] As described herein, anti-VP24 sdAbs were developed to target Ebola's VP24 protein. The anti-VP24 sdAb may successfully treat individuals infected with Ebola either alone or in combination with other retroviral agents. Using methods that are well-known in the art, recombinant VP24 protein (SEQ ID NO:53) was used to generate sdAbs that are directed against or can bind to an epitope of VP24.

[0130] The protein sequence recombinant VP24 protein (SEQ ID NO:53) used for immunization of a camel was:
AKATGRYNLISPKKDLEKGVVLSDLCNFLVSQTIQGWKVYWAGIEFDVTHKGMALL
HRLKTNDFAPAWSMTRNLFPHLFQNPNSTIESPLWALRVILAAGIQDQLIDQSLIEPLA
GALGLISDWLLTTNTNHFNMRTQRVKEQLSLKMLSLIRSNILKFINKLDALHVVNYN
GLLSSIEI ILEFNSSLAI

[0131] As a result of the immunization, one anti-VP24 sdAb, VP24_5 was obtained and screened for binding to VP24. The DNA sequence of VP24_5 (SEQ ID. NO:54) is:
5'- ATGGGTGAT GTGCAGCTGGTGGAGTCT GGGGGAGAC TCGGTGCGG
GCTGGAGGG TCTCTTCAAATGGGTGAT GTGCAGCTG GTGGAGTCT
GGGGGAGAC TCGGTGCGGGCTGGAGGGTCTCTTCAA CTCTCCTGT AAAGCCTCT
GGATACACC TACAATAGTAGAGTCGATATCAGATCT ATGGGCTGG
TTCCGCCAG TATCCAGGA AAGGAGCGCGAGGGGGTCGCTACTATT
AATATTCGT AATAGTGTC ACATACTAT GCCGACTCCGTGAAGGGCCGATTCACC
ATCTCCCAA GACAACGCC AAGAACACG
GTGTATCTGCAAATGAACGCCCTGAAA CCTGAGGAC ACTGCCATG TACTACTGT
GCGTTGTCAGACAGATTCGCGGCGCAG GTACCTGCC AGGTACGGA

ATACGGCCC TCTGACTAT AACTACTGG GGTGAGGGG ACCCTGGTC ACCGTCTCC TCAAGCTCT GGTCTCGAG-3'

[0132] The amino acid sequence of the VP24_5 sdAb (SEQ ID NO:55) is shown below, with the CDRs underlined:

MGDVQLVESGGDSVRAGGSLQLSCKASGYTYNSRVDIRSMGWFRQYPGKEREGVA TINIRNSVTYYADSVKGRFTISQDNAKNTVYLQMNALKPEDTAMYYCALSDRFAAQ VPARYGIRPSDYNYWGEGTLVTVSSSSGLE

[0133] One or more mouse monoclonal antibodies can be generated against one or more domains of the anti-VP24 sdAb of the invention. The mouse monoclonal antibody can be generated by methods that are known by one of skill in the art, for example, the mouse monoclonal antibody can be produced by a mouse hybridoma. The mouse monoclonal antibody can be used in diagnostic assays, for example, the antibody can be used in an immunoassay such as an ELISA or mass spectrometry assay in order to measure the amount of anti-VP24 sdAb present in a sample from a patient.

EXAMPLES

EXAMPLE 1: GENERATION OF SDABS

[0134] SdAbs were produced from a camel that was immunized with several proteins including ALOX12 (SEQ ID NO:44), VP24 (SEQ ID NO:53), and HIV-1 reverse transcriptase (SEQ ID NO:1).

[0135] Using standard techniques, a phage display library was constructed using the pCDisplay-3M vector (Creative Biogene, Shirley, NY) and M13K07 helper phage (New England Biolabs, Ipswich, MA). Single clones of sdAbs were confirmed by ELISA, and the DNA and protein sequences determined using standard methods.

EXAMPLE 2: HIV1-9 (SEQ ID NO:27) SDAB BINDS HIV-1 REVERSE TRANSCRIPTASE AND EBOLA VP-24

[0136] Protein binding experiments were performed on a Biacore 3000 (General Electric Company, Fairfield, CT) at 25°C. The assay buffer contained 10 mM HEPES buffer (pH

7.4), 150 mM NaCl, 3mM EDTA, 0.05% P20. The regeneration buffer contained 10mM glycine HCl pH 1.75, and the immobilization buffer contained 10 mM sodium acetate, pH 5.0. The flow rate used for capturing the ligand was 5ul/min. The flow rate used for kinetics analysis was 30 ul/min.

[0137] The ligands used for the protein binding experiment were HIV1-9 (SEQ ID NO:27) and STAT3-VHH 14 (SEQ ID NO:56). The ligands were directly immobilized by amine coupling (EDC/NHS) at a response unit (RU) of 1200 and 550 on flow cell 2 and 4, respectively, of a CM5 sensor chip. Flow cell 1 was kept blank and used for background subtraction. The un-occupied sites on the CM5 chip were blocked with 1M ethanol amine. For binding analysis, the analyte, rHIV-1 (SEQ ID NO:1) was flowed over the sensor chip. Binding of analyte to the ligand was monitored in real time. The affinity constant ($K_{D=}$ kd/ka) was calculated from the observed on rate (ka) of off rate (kd), as shown in Table 1.

[0138] The negative control for the protein binding experiments was an anti-STAT3 sdAb, VHH14 (SEQ ID NO:56):
QVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQ
APGKEREGVAALSSRGFAGHYTDSVKGRFSISRDYVKNAVYLQMNTVKPEDAAMY
YCAAREGWECGETWLDRTAGGHTYWGQGTLVTVSS

[0139] Chi square (χ^2) analysis was carried out between the actual sensorgram and the sensorgram generated from the BIAnalysis software to determine the accuracy of the analysis. A χ^2 value within 1- 2 is considered accurate and below 1 is highly accurate.

TABLE 1

Ligand	Analyte	ka (1/Ms)	kd(1/s)	Rmax	KD (M)	Conc. (nM)	Chi square
HIV1-9 VHH	rHIV-1	$8.91x10^{4}$	$3.79x10^{-4}$	71.3	$4.25x10^{-9}$	100	0.0321
STAT3 VHH14	rHIV-1	N/A	N/A	N/A	N/A	100	N/A

[0140] Full kinetic analysis was performed at analyte concentrations as indicated in Table 2 with 2 fold serial dilution of the highest analyte concentration. The HIV1-9 anti-RT sdAb bound both HIV-1 and Ebola VP24 analytes.

TABLE 2

Ligand	Analyte	ka (1/Ms)	kd(1/s)	Rmax	KD (M)	Conc. (nM)	Chi square
HIV1-9 VHH (1200 RU)	rHIV-1	$1.90x10^{5}$	$7.31x10^{-4}$	126	$3.85x10^{-9}$	0-200	0.226
STAT3 VHH14 (550 RU)	rHIV-1	NA	NA	NA	NA	0-200	NA
HIV1-9 VHH (1200 RU)	VP-24	$4.38x10^{2}$	$1.66x10^{-4}$	1190	$3.79x10^{-7}$	0-200	0.199

EXAMPLE 3: HIV1-9 (SEQ ID NO:27) SDAB BINDS HIV-1 REVERSE TRANSCRIPTASE IN ELISA

[0141] Two different samples of the HIV1-9 anti-HIV-1 RT sdAb (SEQ ID NO:27) was assessed at 1 µg/mL against a checkerboard of coating antigen, 2° antibody and HRP concentrations in an ELISA. The coating antigen was recombinant HIV-1 RT (Creative BioMart) (SEQ ID NO:1) at 0.5, 0.025 and 0.125 µg/mL per well. The secondary antibody was a rabbit anti-llama biotinylated diluted at 1:5,000, and 1:10,000, HRP at 1:25,000 and 1:50,000. Signal-to-noise ratios >20 were seen with several of the concentrations. The results of the ELISA are shown in Figures 1 and 2.

[0142] Three combinations were chosen to assess a dilution series of the HIV1-9 anti-HIV-1 RT sdAb (SEQ ID NO:27) (1µg/mL to 0.0001 µg/mL).

Coating Antigen	2° antibody	HRP
0.5 µg/mL	1:10,000	1:25,000
0.5 µg/mL	1:5,000	1:50,000
0.5 µg/mL	1:10,000	1:50,000

[0143] The results are shown in Figures 3 and 4. The two HIV1-9 anti-HIV-1 RT sdAb (SEQ ID NO:27) preparations used have very similar results. Results with 0.5 µg/mL coating, 1:5,000 dilution of 2° antibody and 1:50,000 dilution of HRP showed binding of HIV1-9 anti-HIV-1 RT sdAb (SEQ ID NO:27) to HIV1 RT (SEQ ID NO:1) with the highest signal-to-noise ratio and a slightly lower blank value.

EXAMPLE 4: VP24-5 (SEQ ID NO:55) SDAB BINDS VP24

[0144] Protein binding experiments were performed as described in Example 2. The ligands used for protein binding were VP24-5 (SEQ ID NO:55) and STAT3-VHH 14 (SEQ ID NO:56). The ligands were directly immobilized by amine coupling (EDC/NHS) at a response unit (RU) of 427 and 550 on flow cell 2 and 4, respectively, of a CM5 sensor chip. Flow cell 1 was kept blank and used for background subtraction. The un-occupied sites on the CM5 chip were blocked with 1M ethanol amine. For binding analysis, the analytes, VP24 (SEQ ID NO:53) was flowed over the sensor chip and monitored in real time. The affinity constant ($K_{D=}$ kd/ka) was calculated from the observed on rate (ka) of off rate (kd), as shown in Table 3.

TABLE 3

Ligand	Analyte	ka (1/Ms)	kd(1/s)	Rmax	KD (M)	Conc. (nM)	Chi2
VP24-5-VHH	VP-24	$1.39x10^{5}$	$8.77x10^{-4}$	6.84	$6.31x10^{-9}$	100	0.0481
STAT3 VHH14	VP-24	NA	NA	NA	NA	100	NA

[0145] Full kinetic analysis was performed at different analyte concentrations with 2 fold serial dilution of the highest analyte concentration, as shown in Table 4.

TABLE 4

Ligand	Analyte	ka (1/Ms)	kd(1/s)	Rmax	KD (M)	Conc. (nM)	Chi2
VP24-5-	VP-24	$1.61x10^{3}$	$4.73x10^{-5}$	222	$2.94x10^{-8}$	0-200	0.187

VHH							
STAT3 VHH14 (550 RU)	VP-24	NA	NA	NA	NA	0-200	NA

EXAMPLE 5: VP24-5 (SEQ ID NO:55) SDAB BINDS EBOLA VP24 TARGET IN ELISA

[0146] Two different samples of the VP24-5 anti-Ebola VP24 sdAb (SEQ ID NO:55) was assessed at 1 µg/mL against a checkerboard of coating antigen, 2° antibody and HRP concentrations in an ELISA. The coating antigen was recombinant Ebola VP24 (Creative BioMart) (SEQ ID NO:53) at 0.5, 0.025 and 0.125 µg/mL per well. The secondary antibody was a rabbit anti-llama biotinylated diluted at 1:5,000, and 1:10,000. HRP was used at a dilution of 1:10,000 and 1:25,000. The results of the ELISA are shown in Figures 5 and 6. The signal-to-noise ratios were low and the analysis was repeated with higher concentrations.

[0147] The ELISA was repeated with 1 and 0.5 µg/mL VP24-5 anti-Ebola VP24 sdAb (SEQ ID NO:55). Recombinant VP24 (SEQ ID NO:53) was used at either 0.5 or 1 µg/mL per well. The secondary antibody was a rabbit anti-llama biotinylated diluted at 1:1,000, 1:4,000, 1:10,000, and 1:10,000. HRP was used at a dilution of 1:25,000 and 1:50,000. The results of the ELISA are shown in Figures 7 and 8.

[0148] Three combinations were chosen to assess a dilution series of the VP24-5 anti-Ebola VP24 sdAb (SEQ ID NO:55) (1µg/mL to 0.0001 µg/mL).

Coating Antigen	2° antibody	HRP
0.5 µg/mL	1:1,000	1:1,000
0.5 µg/mL	1:10,000	1:25,000
1 µg/mL	1:4,000	1:25,000

[0149] The results are shown in Figures 9 and 10. The two VP24-5 anti-Ebola VP24 sdAb (SEQ ID NO:55) preparations used have very similar results, and show binding of VP24-5 anti-Ebola VP24 sdAb (SEQ ID NO:55) to recombinant VP24 (SEQ ID NO:53).

[0150] Although the present invention has been described in considerable detail with reference to certain preferred embodiments, other embodiments are possible. The steps disclosed for the present methods, for example, are not intended to be limiting nor are they intended to indicate that each step is necessarily essential to the method, but instead are exemplary steps only. Therefore, the scope of the appended claims should not be limited to the description of preferred embodiments contained in this disclosure. All references cited herein are incorporated by reference in their entirety.

What is claimed is:

1. An anti-Human Immunodeficiency Virus Type 1 (HIV-1) reverse transcriptase single domain antibody (sdAb).

2. An anti-HIV-1 reverse transcriptase sdAb, wherein the anti-HIV-1 reverse transcriptase sdAb comprises the amino acid sequence as set forth in SEQ ID NO:27.

3. A method of treating a disease, preventing development of a disease, or preventing recurrence of a disease in a subject using the anti-HIV-1 reverse transcriptase sdAb according to claims 1 or 2, the method comprising administering an effective amount of the anti-HIV-1 reverse transcriptase sdAb to a subject in need thereof.

4. The method of claim 3, wherein the subject is a mammal.

5. The method of claim 4, wherein the mammal is a human.

6. The method of claim 3, wherein the anti-HIV-1 reverse transcriptase sdAb is administered in combination with one or more compounds.

7. The method of claim 6, wherein the one or more compounds is a protease inhibitor.

8. The method of claim 3, wherein administering an effective amount of the anti-HIV-1 reverse transcriptase sdAb to a subject in need thereof comprises intravenous administration, intramuscular administration, oral administration, rectal administration, enteral administration, parenteral administration, intraocular administration, subcutaneous administration, transdermal administration, administered as eye drops, administered as nasal spray, administered by inhalation or nebulization, topical administration, and administered as an implantable drug.

9. An isolated polypeptide, the isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:27.

10. An antibody directed toward the polypeptide of claim 9.

11. A method of measuring the levels of an anti-HIV-1 reverse transcriptase sdAb in a sample from a subject, the method comprising the steps of:

a) generating a mouse monoclonal antibody directed against one or more domains of a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:27;

b) obtaining a sample from the subject;

c) performing a quantitative immunoassay with the mouse monoclonal antibody and the sample to determine the amount of sdAb in a subject; and

d) quantifying the amount of sdAb in the subject.

12. The method of claim 11 wherein the quantitative immunoassay comprises an enzyme-linked immunosorbent assay (ELISA), specific analyte labeling and recapture assay (SALRA), liquid chromatography, mass spectrometry, fluorescence-activated cell sorting, or a combination thereof.

13. An anti-Ebola VP24 single domain antibody (sdAb).

14. An anti-Ebola VP24 sdAb, wherein the anti-Ebola VP24 sdAb comprises the amino acid sequence as set forth in SEQ ID NO:55.

15. A method of treating a disease, preventing development of a disease, or preventing recurrence of a disease in a subject using the anti-Ebola VP24 sdAb according to claims 13 or 14, the method comprising administering an effective amount of the anti-Ebola VP24 sdAb to a subject in need thereof.

16. The method of claim 15, wherein the subject is a mammal.

17. The method of claim 16, wherein the mammal is a human.

18. The method of claim 15, wherein the anti-Ebola VP24 sdAb is administered in combination with one or more compounds.

19. The method of claim 18, wherein the one or more compounds is an anti-viral compound.

20. The method of claim 14, wherein administering an effective amount of the anti-Ebola VP24 sdAb to a subject in need thereof comprises intravenous administration, intramuscular administration, oral administration, rectal administration, enteral administration, parenteral administration, intraocular administration, subcutaneous administration, transdermal administration, administered as eye drops, administered as nasal spray, administered by inhalation or nebulization, topical administration, and administered as an implantable drug.

21. An isolated polypeptide, the isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:55.

22. An antibody directed toward the polypeptide of claim 21.

23. A method of measuring the levels of an anti-Ebola VP24 sdAb in a sample from a subject, the method comprising the steps of:

a) generating a mouse monoclonal antibody directed against one or more domains of a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:55;

b) obtaining a sample from the subject;

c) performing a quantitative immunoassay with the mouse monoclonal antibody and the sample to determine the amount of sdAb in a subject; and

d) quantifying the amount of sdAb in the subject.

24. The method of claim 23 wherein the quantitative immunoassay comprises an enzyme-linked immunosorbent assay (ELISA), specific analyte labeling and recapture assay (SALRA), liquid chromatography, mass spectrometry, fluorescence-activated cell sorting, or a combination thereof.

25. An anti-arachidonate 12-lipoxygenase (ALOX12) single domain antibody (sdAb).

26. An anti-ALOX12 sdAb, wherein the anti-ALOX12 sdAb comprises the amino acid sequence as set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, or SEQ ID NO:52.

27. A method of treating a disease, preventing development of a disease, or preventing recurrence of a disease in a subject using the anti-ALOX12 sdAb according to claims 25 or 26, the method comprising administering an effective amount of the anti-ALOX12 sdAb to a subject in need thereof.

28. The method of claim 27, wherein the subject is a mammal.

29. The method of claim 28, wherein the mammal is a human.

30. The method of claim 27, wherein the anti-ALOX12 sdAb is administered in combination with one or more compounds.

31. The method of claim 27, wherein administering an effective amount of the anti-ALOX12 sdAb to a subject in need thereof comprises intravenous administration, intramuscular administration, oral administration, rectal administration, enteral administration, parenteral administration, intraocular administration, subcutaneous administration, transdermal administration, administered as eye drops, administered as nasal spray, administered by inhalation or nebulization, topical administration, and administered as an implantable drug.

32. An isolated polypeptide, the isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, or SEQ ID NO:52.

33. An antibody directed toward the polypeptide of claim 32.

34. A method of measuring the levels of an anti-ALOX12 sdAb in a sample from a subject, the method comprising the steps of:

a) generating a mouse monoclonal antibody directed against one or more domains of a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, or SEQ ID NO:52;

b) obtaining a sample from the subject;

c) performing a quantitative immunoassay with the mouse monoclonal antibody and the sample to determine the amount of sdAb in a subject; and

d) quantifying the amount of sdAb in the subject.

35. The method of claim 34 wherein the quantitative immunoassay comprises an enzyme-linked immunosorbent assay (ELISA), specific analyte labeling and recapture assay (SALRA), liquid chromatography, mass spectrometry, fluorescence-activated cell sorting, or a combination thereof.

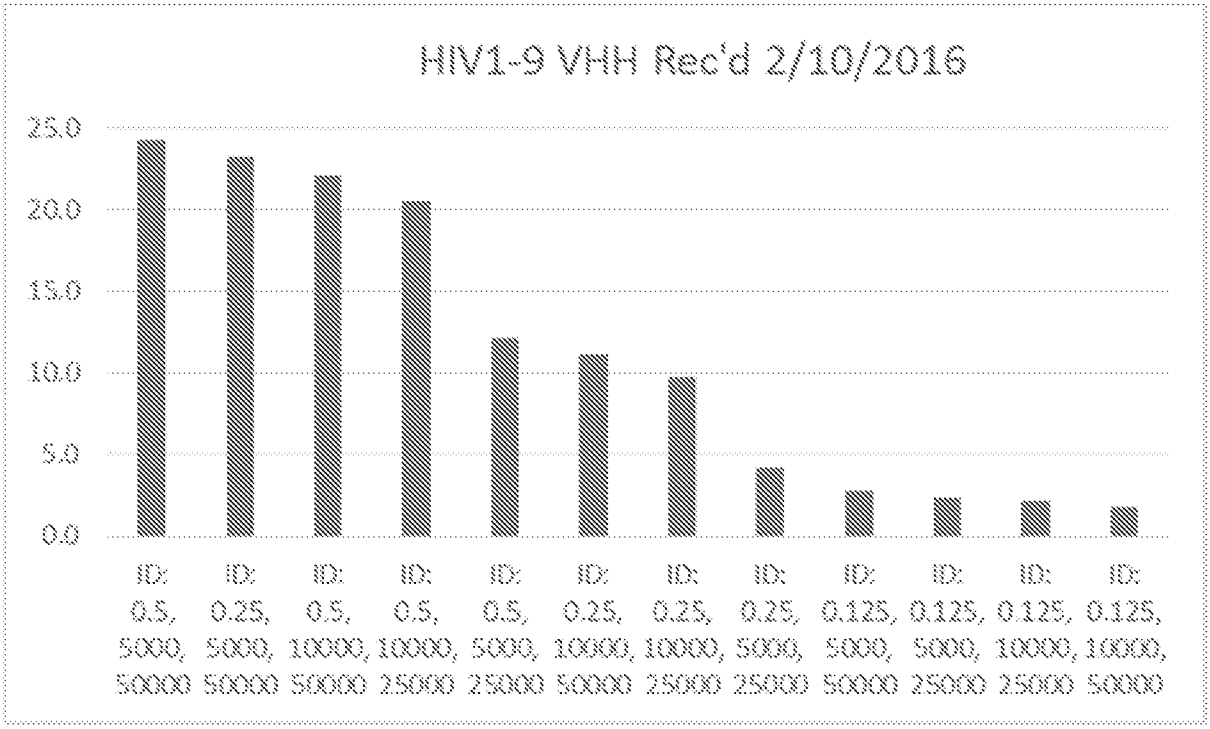
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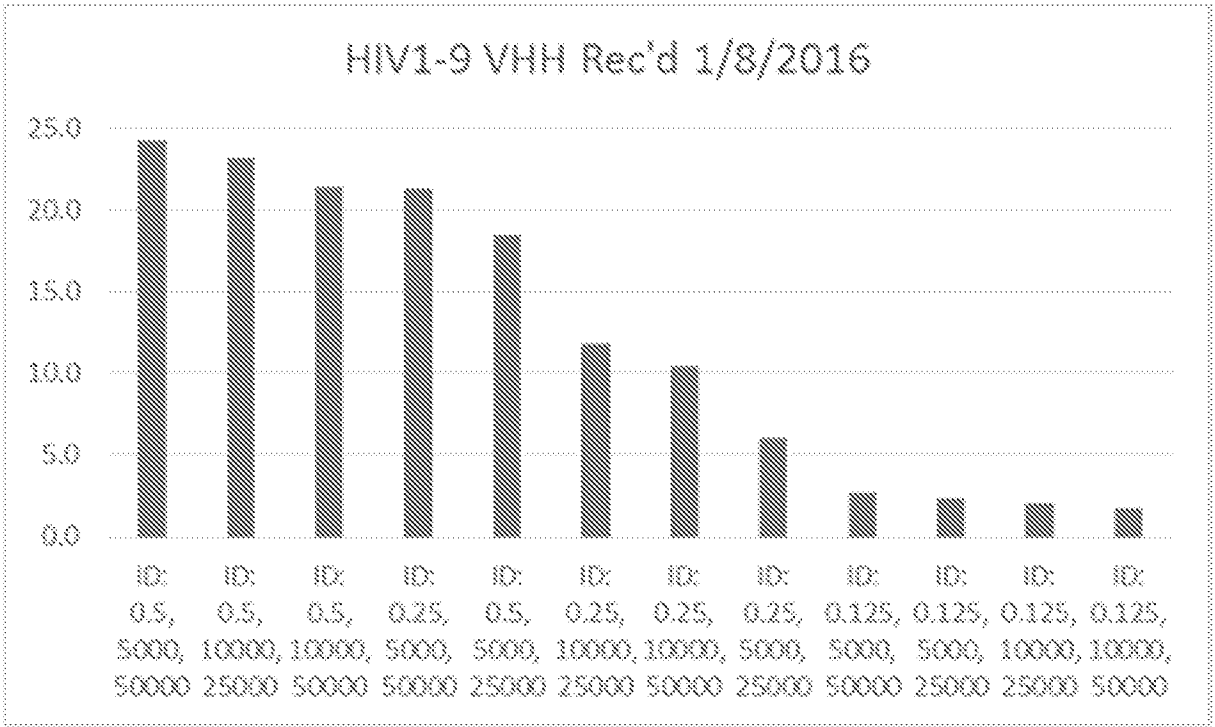
FIG. 1


HIV1-9 VHH Rec'd 1/8/2016
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15.0
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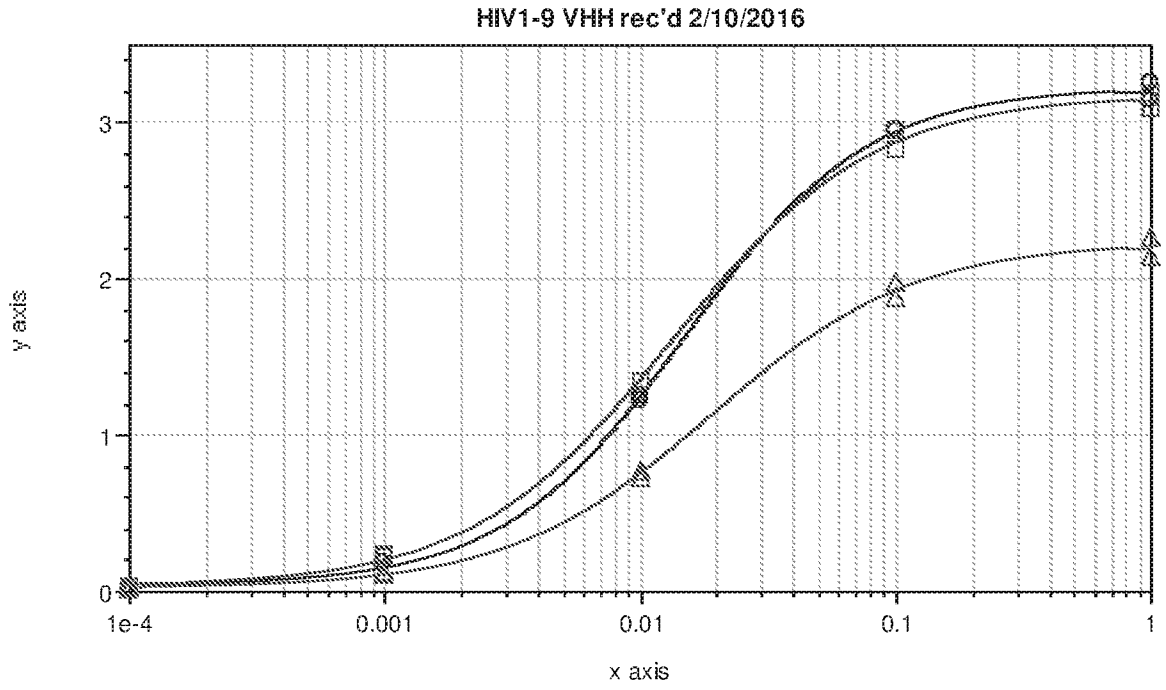
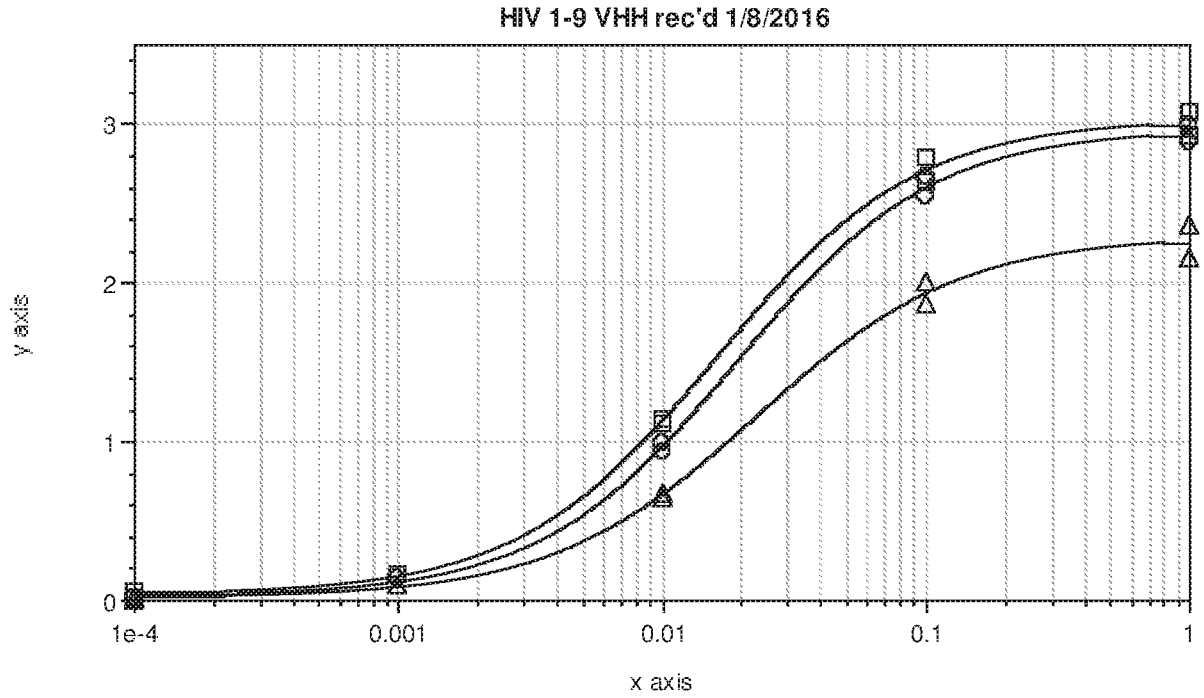
FIG. 2



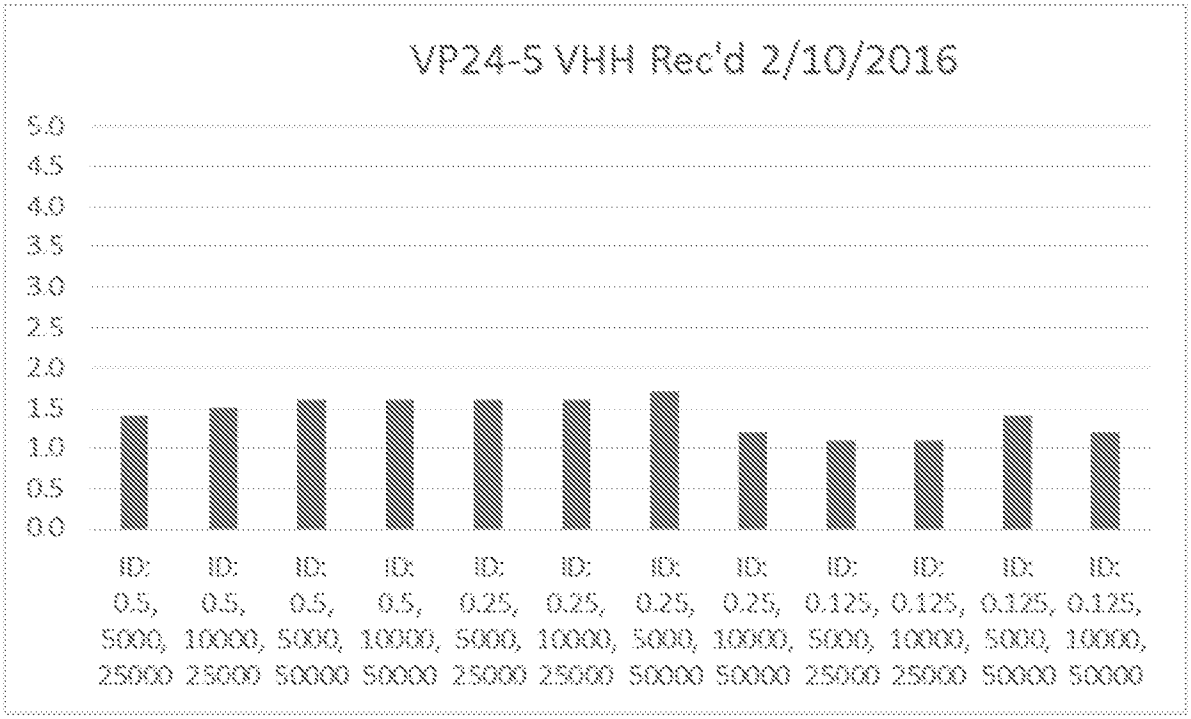
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
STD (0.5/10/25 HIV1-9 2/10/16: Concentration vs V...	0.0353	1.22	0.015	3.23	0.999
STD (0.5/5/50 HIV1-9 2/10/16: Concentration vs V...	0.0301	1.11	0.0133	3.17	0.999
STD (0.5/10/50 HIV1-9 2/19/16: Concentration vs V...	0.0195	1.09	0.019	2.23	0.998

FIG. 3



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
○ STD (0.5/10/25 HIV1-9 1/8/16: Concentration vs V...	0.0231	1.17	0.0191	2.97	0.999
□ STD (0.5/5/50 HIV1-9 1/8/16: Concentration vs Val...	0.0344	1.16	0.0161	3.03	0.998
△ STD (0.5/10/50 HIV1-9 1/8/16: Concentration vs Va...	0.0153	1.13	0.0227	2.29	0.997

FIG. 4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0.5
0.0
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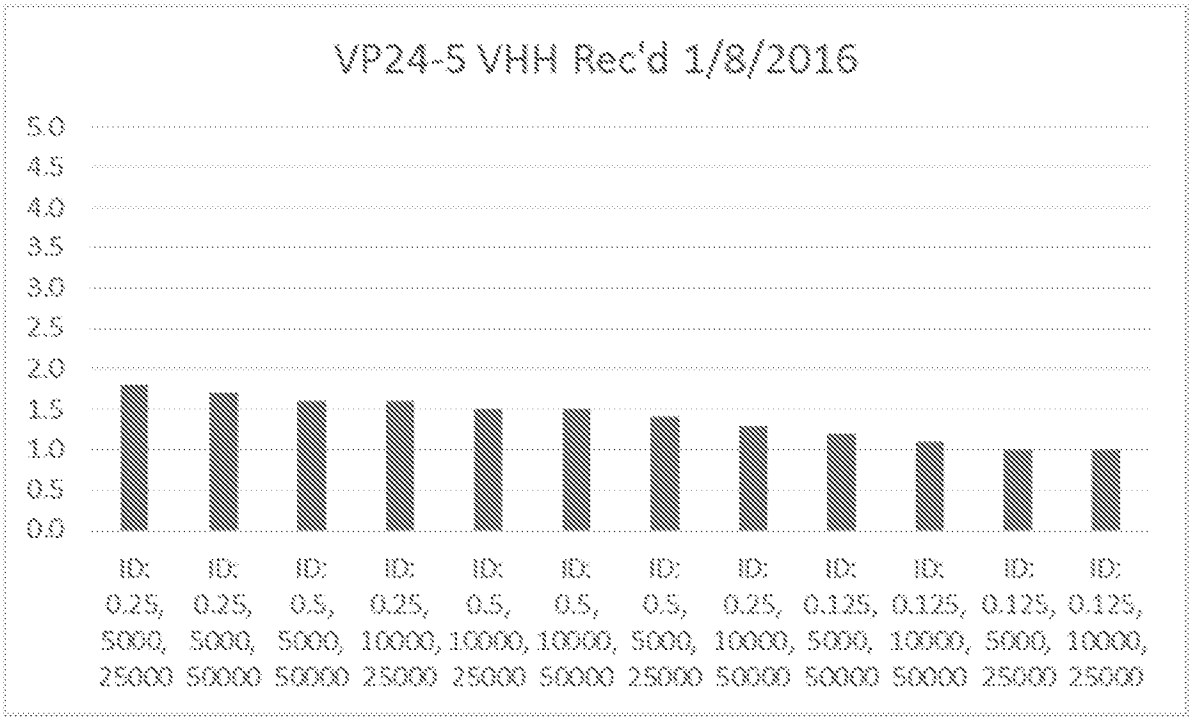
FIG. 5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3.0
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2.0
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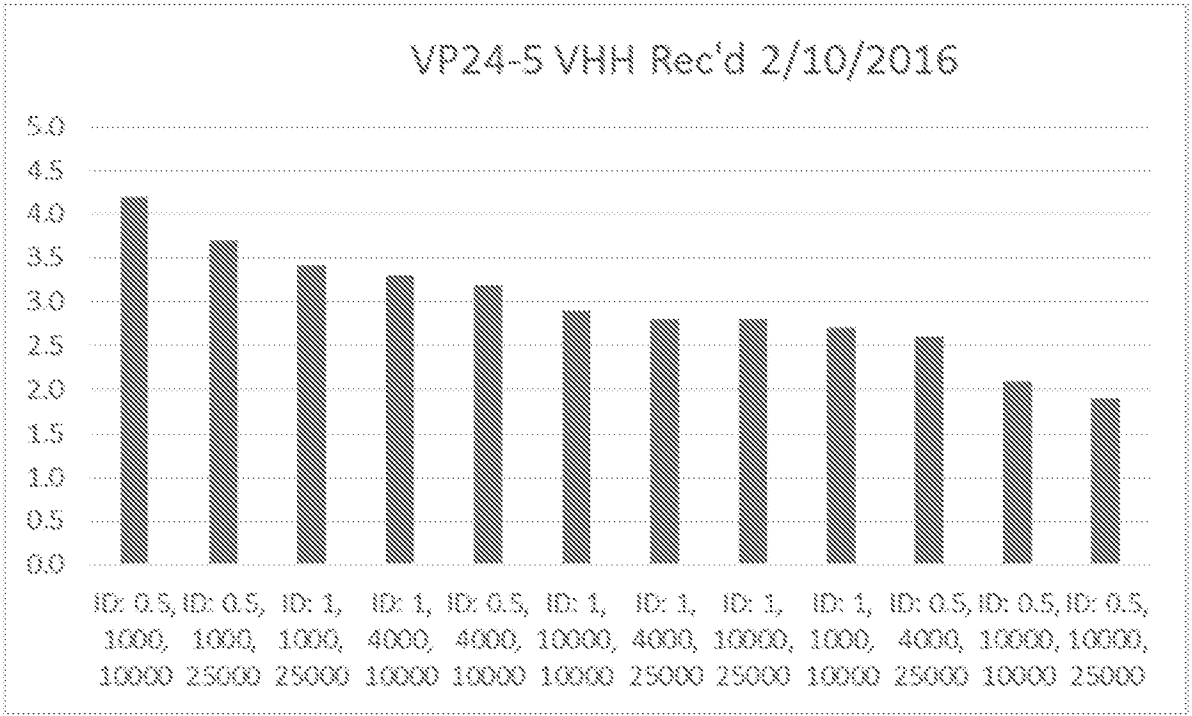
FIG. 6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3.0
2.5
2.0
1.5
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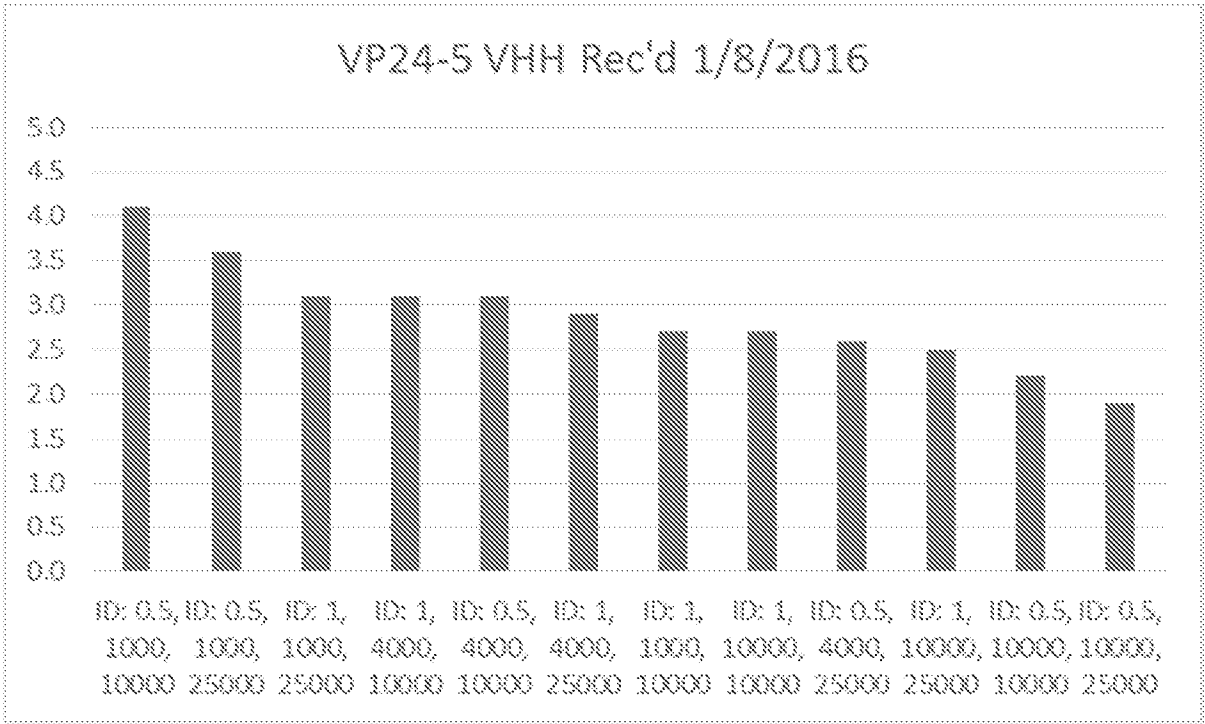
FIG. 7


VP24-5 VHH Rec'd 1/8/2016
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4.0
3.5
3.0
2.5
2.0
1.5
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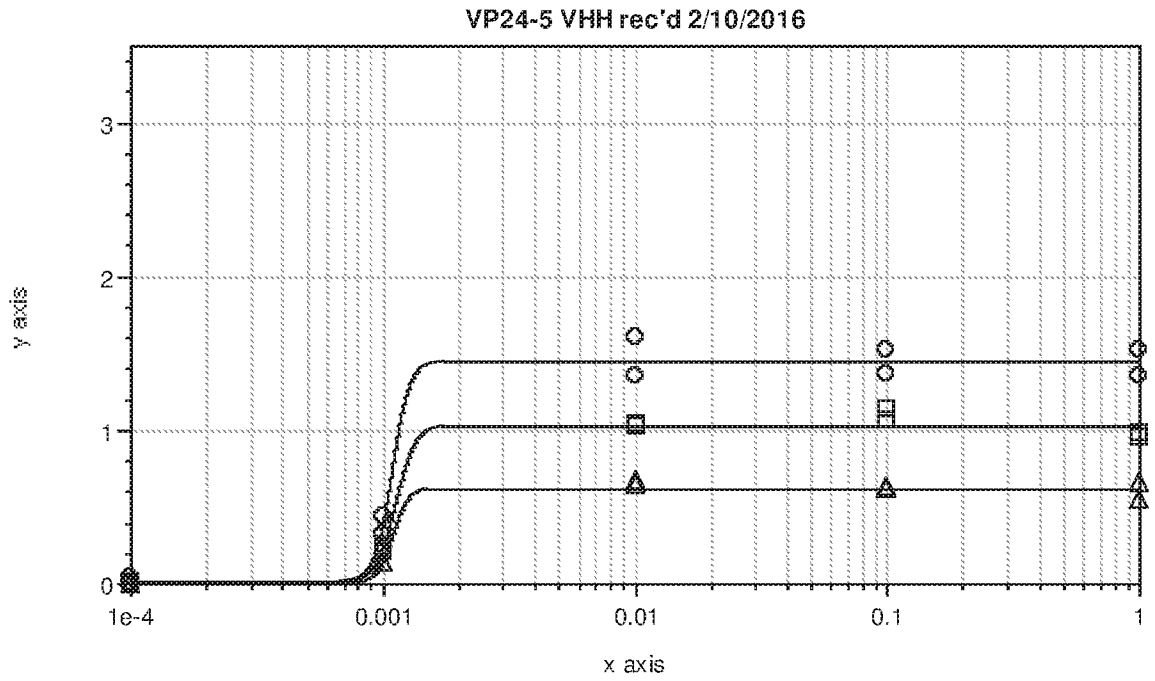
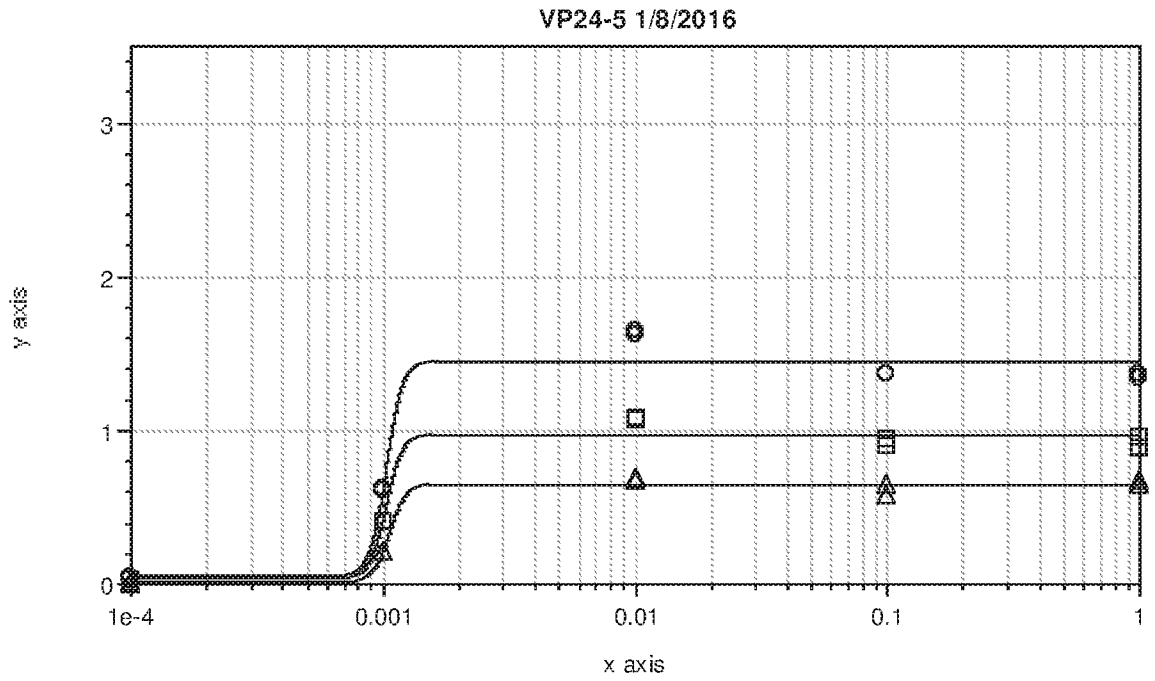
FIG. 8



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
○ STD (0.5/1/10 VP24-5 New : Concentration vs Val...	0.0114	12.9	0.00109	1.45	0.983
□ STD (0.5/1/25 VP24-5 New : Concentration vs Val...	0.0049	11.2	0.00112	1.04	0.99
△ STD (1/4/25 VP24-5 New : Concentration vs Value...	-0.0035	13.3	0.0011	0.632	0.988

FIG. 9



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
○ STD (0.5/1/10 VP24-5: Concentration vs Values)	0.0455	13.5	0.00103	1.45	0.971
□ STD (0.5/1/25 VP24-5: Concentration vs Values)	0.0306	13.5	0.00103	0.974	0.977
△ STD (1/4/25 VP24-5: Concentration vs Values)	0.0025	13.1	0.00106	0.653	0.99

FIG. 10