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DESCRIPTION

BACKGROUND

[0001] 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 heat, pH, proteases and other denaturing agents or conditions; sdAbs are inexpensive to prepare; and sdAbs can access targets and epitopes not accessible to conventional antibodies.

[0002] There are a number of diseases or conditions, such as 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, but the compounds can be ineffective, undeliverable, or toxic to unaffected cells.

[0003] 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.

[0004] 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.

[0005] The present invention relates to single-domain antibodies (sdAbs), proteins and polypeptides comprising the sdAbs. The sdAbs are directed against intracellular components 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, WO2010/033913 disclosed single domain antibodies for the targeting of disease-associated biomarkers wherein the biomarker is STAT3. Kodappully et al. (Biochimica et Biophysica Acta 1845(2014) 136-154) disclosed STAT3 as a cytoplasmic target of natural and synthetic inhibitors in cancer treatment.

SUMMARY

[0006] The invention is set out in the appended set of claims. The embodiments and/or examples of the following description which are not covered by the appended claims are considered as not being part of the present invention. The disclosure provides a single-domain antibody (sdAb) directed

against an intracellular component. The intracellular component can be, for example, a protein, nucleic acid, lipid, carbohydrate, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6, TNF-alpha, and KRAS. In one aspect the invention provides an isolated polypeptide, the isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:3.

[0007] In another embodiment, the invention is directed towards an anti-STAT3 sdAb, wherein the anti-STAT3 sdAb comprises an amino acid sequence set forth in SEQ ID NO:3.

[0008] The invention is directed towards an isolated polypeptide comprising an amino acid sequence encoding an anti-STAT sdAb, such as, for example, the polypeptide set forth in SEQ ID NO:3.

[0009] In yet another instance, the disclosure is directed towards a host cell, and the host cell expresses the amino acid sequence of the sdAb such as, for example, the amino acid set forth in SEQ ID NO:3 or SEQ ID NO:4.

[0010] One instance of the disclosure is a pharmaceutical composition comprising a sdAb, or a polypeptide, and a pharmaceutically acceptable carrier. Optionally, the sdAb comprises an anti-STAT3 sdAb comprising an amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4, and the polypeptide comprises an isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4.

[0011] Another instance of the disclosure is a method to diagnose a disorder mediated by STAT3 in a subject, the method comprising the steps of a) contacting a biological sample with the sdAb, or a polypeptide; b) determining the amount of STAT3 in the biological sample; and c) comparing the amount determined in step (b) with a standard, a difference in amount indicating the presence of the disorder.

[0012] Another instance of the disclosure is a method of preventing or treating a disease or disorder, or preventing recurrence of a disease mediated by STAT3, or for use in the treatment of cancer, or diseases caused by abnormal cellular proliferation, comprising administering an anti-STAT3 sdAb, or a polypeptide, to a subject in need thereof. Optionally, the sdAb comprises an anti-STAT3 sdAb comprising an amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4 and the polypeptide comprises an isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4.

[0013] One instance of the disclosure is an anti-TNF-alpha sdAb. Optionally, the anti-TNF-alpha sdAb comprises an amino acid sequence set forth in SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7. The disclosure also comprises an isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7.

[0014] Another embodiment of the disclosure is a host cell expressing the amino acid sequence set forth in SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7.

[0015] In another instance, the disclosure, the invention is also a pharmaceutical composition comprising a sdAb or a polypeptide and a pharmaceutically acceptable carrier. Optionally, the sdAb comprises an anti-TNF-alpha sdAb comprising an amino acid sequence set forth in SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7 and the polypeptide comprises an isolated polypeptide comprising an

amino acid sequence set forth in SEQ ID NO:5, SEQ ID NO: 6 or SEQ ID NO:7.

[0016] Another instance of the disclosure is a method to diagnose a disorder mediated by TNF-alpha in a subject, the method comprising the steps of a) contacting a biological sample with a sdAb or a polypeptide; b) determining the amount of TNF-alpha in the biological sample; and c) comparing the amount determined in step (b) with a standard, a difference in amount indicating the presence of the disorder.

[0017] In one instance, the disclosure describes a method of preventing or treating a disease or disorder or recurrence of a disease or disorder mediated by TNF-alpha, or for use in the treatment of cancer, or diseases caused by abnormal cellular proliferation, comprising administering an anti-TNF-alpha sdAb, or a polypeptide, to a mammal in need thereof.

[0018] Optionally, the anti-TNF-alpha sdAb comprises an amino acid sequence set forth in SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7 and the polypeptide comprises isolated polypeptide, the isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7.

[0019] One instance of the disclosure is an anti-KRAS sdAb. Optionally, the anti-KRAS sdAb comprises an amino acid sequence set forth in SEQ ID NO:2. In one instance, the invention comprises an isolated polypeptide, wherein the isolated polypeptide comprises an amino acid sequence set forth in SEQ ID NO:2. In another instance, the invention comprises a host cell expressing the amino acid sequence set forth in SEQ ID NO:2.

[0020] Another instance of the disclosure is a pharmaceutical composition, comprising a sdAb or a polypeptide, and a pharmaceutically acceptable carrier. Optionally, the sdAb comprises an anti-KRAS sdAb comprising an amino acid sequence set forth in SEQ ID NO:2 and the polypeptide comprises isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO:2.

[0021] An additional instance of the disclosure is method to diagnose a disorder mediated by KRAS in a subject, the method comprising the steps of a) contacting a biological sample with a sdAb or a polypeptide; b) determining the amount of KRAS in said biological sample; and c) comparing the amount determined in step (b) with a standard, a difference in amount indicating the presence of the disorder. Optionally, the sdAb comprises an anti-KRAS sdAb comprising an amino acid sequence set forth in SEQ ID NO: 2 and the polypeptide comprises isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO:2.

[0022] The disclosure also comprises a method of treating a disease using an anti-KRAS sdAb, the method comprising administering an effective amount of an anti-KRAS sdAb to a subject in need thereof.

[0023] In one instance, the disclosure describes a method of preventing or treating a disease or disorder, or the recurrence of a disease or disorder, mediated by KRAS, or for use in the treatment of cancer, or diseases caused by abnormal cellular proliferation, comprising administering an anti-KRAS sdAb or a polypeptide, to a mammal in need thereof. Optionally, the anti-KRAS sdAb comprises an amino acid sequence set forth in SEQ ID NO: 2 and the polypeptide comprises isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 2. In one embodiment the invention provides the sdAb as of the invention, for use as a medicament. In one embodiment the subject is a

mammal; preferably a human.

[0024] In one embodiment, the invention describes a method of administering the sdAb of the invention, the method comprising intravenous administration, intramuscular administration, oral administration, rectal administration, intraocular administration, enteral administration, parenteral 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.

[0025] In another embodiment, the invention describes a method of treating a disease, preventing a disease or preventing the reoccurrence of a disease using the sdAb of the invention in combination with one or more compounds. Optionally, the one or more compounds is a transcriptional inhibitor.

[0026] In another embodiment, the invention describes a method of measuring the levels of a sdAb of the invention, the method comprising the steps of a) generating a mouse monoclonal antibody directed against one or more domains of the sdAb; b) performing a quantitative immunoassay on a sample from the subject, wherein the quantitative immunoassay may optionally comprise 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, with the mouse monoclonal antibody and the sample to determine the amount of sdAb in a subject; and c) quantifying the amount of sdAb in the subject.

DRAWINGS

[0027] 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:

Figure 1 is a schematic map of VHH13 anti-STAT3 sdAb expression vector pTT21-stt VHH13;

Figure 2 is a schematic map of VHH14 anti-STAT3 sdAb expression vector pTT21-stt VHH14;

Figure 3 depicts the results of an immunoprecipitation assay using anti-STAT3 bacterial VHH13 STAT3 (SEQ ID NO:3) and anti-STAT3 bacterial VHH14 STAT3 (SEQ ID NO:4);

Figure 4 depicts the results of an immunoprecipitation assay using anti-STAT3 bacterial VHH13 STAT3 (SEQ ID NO:3);

Figure 5 illustrates the growth inhibition of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the MDA-MB-231 xenograft model, dosed at 0.5mg/kg/day;

Figure 6 illustrates the growth inhibition of anti-STAT3 bacterial VHH13 (SEQ. ID. NO. 3) sdAb in MDA-MB-231 xenograft model at doses ranging from 1 mg/kg twice daily to 2 mg/kg twice daily or 2mg/kg/day;

Figure 7 illustrates the growth inhibition of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the MDA-MB-231 xenograft model, dosed at 5mg/kg/twice daily;

Figure 8 illustrates the growth inhibition of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the

DU145 xenograft model, dosed at 5mg/kg/twice daily;

Figure 9 illustrates the growth inhibition of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the PANC-1 xenograft model, dosed at 5mg/kg/twice daily;

Figure 10 illustrates the growth inhibition of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the MCF-7 xenograft model, dosed at 1mg/kg/three times daily;

Figure 11 illustrates the growth inhibition of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the BT-474 xenograft model, dosed at 1mg/kg/three times daily;

Figure 12 illustrates the cytotoxicity of TNF-alpha in U937 cells;

Figure 13 illustrates the cytotoxicity of Staurosporine in U937 cells; and

Figure 14 illustrates inhibition of TNF-alpha cytotoxicity by anti-TNF-alpha sdAbs.

DESCRIPTION

[0028] 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.

[0029] 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.

[0030] 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.

[0031] 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.

[0032] 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.

[0033] 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.

[0034] 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 to as "Complementarity Determining Region 1" or "CDR1"; as "Complementarity Determining Region 2" or "CDR2"; and as "Complementarity Determining Region 3" or "CDR3," respectively.

[0035] 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.

[0036] 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.

[0037] 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.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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.

[0043] 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 a 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 a sdAb comprises the amino acid residues at positions 1-30, CDR1 of a sdAb comprises the amino acid residues at positions 31-36, FR2 of a sdAb comprises the amino acids at positions 36-49, CDR2 of a sdAb comprises the amino acid residues at positions 50-65, FR3 of a sdAb comprises the amino acid residues at positions 66-94, CDR3 of a sdAb comprises the amino acid residues at positions 95-102, and FR4 of a sdAb comprises the amino acid residues at positions 103-113.

[0044] The term "synthetic" refers to production by *in vitro* chemical or enzymatic synthesis.

[0045] 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.

[0046] 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.

[0047] 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.

[0048] The present invention relates to single-domain antibodies (sdAbs) that are directed against 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.

[0049] 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 Fab fragments or ScFv's fragment in order to bind an antigen.

[0050] 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 cDNAs, (c) constructing a library of cDNA fragments encoding VHH domains, (d) transcribing the VHH domain-encoding cDNAs obtained in step (c) to 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.

[0051] 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.

[0052] 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

[0058] 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 a 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.

[0059] 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.

[0060] The invention further relates to applications and uses of the sdAb, 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. 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.

[0061] In another aspect, a nucleic acid encoding one or more sdAb of the invention can be inserted into the genome of an organism to treat or prevent diseases.

[0062] 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.

[0063] The methods and compositions detailed in the present invention can be used to treat disease 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.

[0064] The sdAbs of the invention, in particular the anti-STAT3 VHH, the anti-KRAS VHH, and the anti-TNF-alpha VHH of the present invention, can be used for treatment and prevention of malignant diseases including, but not limited to: multiple myeloma, leukemias (HTLV-1 dependent, erythroleukemia, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), and large granular lymphocyte leukemia (LGL), lymphomas (EBV-related/Burkitt's, mycosis fungoides, cutaneous T-cell lymphoma, non-Hodgkins lymphoma (NHL), anaplastic large-cell lymphoma (ALCL), breast cancers, triple-negative breast cancers, head and neck cancers, melanoma, ovarian cancers, lung cancers, pancreatic cancers, prostate cancers, sarcomas, osteosarcoma, Kaposi's sarcoma, Ewing's sarcoma, hepatocellular cancers, glioma, neuroblastoma, astrocytoma, colorectal cancers, Wilm's tumors, renal cancers, bladder cancers, endometrial cancers, cervical cancers, esophageal cancers, cutaneous squamous cell cancers, basal cell cancers, and any metastatic cancers. The sdAbs can be used in cancer patients to help prevent or reduce weight loss or cachexia due to cancer.

[0065] The sdAb, in particular the anti-STAT3 and the anti-TNF-alpha sdAbs of the present invention, can also be used for treatment and prevention of diseases such as, but not limited to: autoimmune diseases (e.g., rheumatoid arthritis, ulcerative colitis, Crohn's disease, bacterial induced colitis, asthma, scleroderma, lupus, encephalomyelitis, arteritis, vasculitis, glomerulonephritis, uveitis, uveoretinitis, multiple sclerosis), polycystic kidney disease, dermatologic diseases (e.g., psoriasis, alopecia areata, atopic dermatitis, keloids/hypertrophic scars, lipoma, Padget's disease, and actinic keratosis), Hidradenitis suppurativa, transplantation (e.g., solid organ, bone marrow, hand, face, limbs, any body part), muscular dystrophy and muscle wasting associated with cancers and aging, endometriosis, macular degeneration, retinal degeneration, stroke, epilepsy, traumatic brain and spinal cord injuries, hypertension, cardiac hypertrophy, Alzheimer's disease, pulmonary artery hypertension, type 2 diabetes mellitus, and ankylosing spondylitis. Additionally sdAbs can target orphan diseases. Examples of these rare orphan diseases include, but are not limited to, triple

negative breast cancers, pancreatic cancers, AML (acute myeloid leukemia), head and neck cancers, multiple myeloma, and chemo-resistant cancers.

[0066] Viral infections can be treated by targeting intracellular viral proteins in infected cells. Viral proteins, such as HIV reverse transcriptase, can block viral life-cycle. The sdAb 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. The sdAbs of the invention can be used to target diseases when there is an overexpression of an intracellular molecule. Huntington's disease can be treated with sdAbs.

[0067] 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, & ritonavir), 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 Ruxolitinib Phosphate. The one or more compounds can increase the therapeutic response and augment the effectiveness of the sdAb of the invention. In addition, the effectiveness of the sdAb 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. In addition, anti-STAT3 sdAbs can convert radioresistant cancers to radiosensitive cancers with respect to radiation therapy.

[0068] 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.

[0069] 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.

[0070] After crossing the cell membrane, these sdAbs can target transmembrane or intracellular molecules or antigens. These intracellular or transmembrane 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.

[0071] The sdAb targets can be in humans, animals, plants, fungi, parasites, protists, bacteria,

viruses, prions, prokaryotic cells, and eukaryotic cells. Some examples of inter- 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.

[0072] KRAS is a Kirsten ras oncogene homolog from the mammalian ras gene family. KRAS encodes a protein that is a member of the small GTPase superfamily. The protein is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, and colorectal carcinoma. Under normal conditions, Ras family members influence cell growth and differentiation events in a subcellular membrane compartmentalization-based signaling system. However, oncogenic Ras can deregulate processes that control both cell proliferation and apoptosis.

[0073] Anti-KRAS sdAbs were developed to target wild-type and mutated KRAS (G12D) in order to disrupt its role in malignant cells such as, for example, cells involved in colorectal cancer, pancreatic cancer, biliary tract cancer, lung cancer, leukemias, and other metastatic malignancies. Without being bound by a particular mechanism, it is thought that the anti-KRAS sdAb binds KRAS and blocks the downstream signaling of KRAS in malignant cells. Additionally, the anti-KRAS sdAb may successfully treat malignancies that are resistant to anti-EGFR biologics (e.g., cetuximab and panitumumab).

[0074] Using methods that are well-known in the art, recombinant human mutant KRAS (G12D) protein was used to generate sdAbs that are directed against or can bind to an epitope of KRAS or mutant KRAS (G12D), or other KRAS mutants. Additionally, sdAbs can be generated to other KRAS mutants. To generate the anti-KRAS sdAbs, recombinant full-length human KRAS (Gene ID: 3845) was expressed in *Escherichia coli*.

[0075] Several sdAbs were obtained and screened. The DNA sequence of one anti-KRAS (G12D) sdAb, named KRAS_13 (SEQ ID NO:1), is shown below:

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5'Gagggtgcagctgggtggagtctgggggaggctcgggtgcagactggagggtctctgagactctctgtgcagtttctggaatatcggc
agcagctactgcattggctgggtccgccaggctccagggaagaagcgcgagcggtgcacgtattgtacgtgatgtgccactggct
acgcagactaegtgaaggccgattcaccatctcccagacagcgcgaagaacactctgtatctgcaaatgaacaggetgatactgag
gacactgccatctactgtgggcagacctgccccagggtgttggactcaggcgatttggaaatttgggtatcggggccagggaacct
ggtcaccgtctctca-3'
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[0076] The amino acid sequence of the anti-KRAS (G12D) sdAb (SEQ ID NO. 2), KRAS_13, is shown below, with the CDRs underlined:

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EVOLVESGGGSVOTGGSLRLSCA1VSIGNISSYCMGWFRQAPGKKREAVARIVRDGAT
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GYADYVKGRITISRDSAKNTLYLQMNRLIPEDTAIYYCAADLPPGCLTQAIWNIGYRG
QGTLLTVVSS

[0077] Additionally, the present invention comprises one or more mouse monoclonal antibodies which are directed against one or more domains of the anti-KRAS 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-KRAS sdAb present in a patient's serum. The cytotoxicity of KRAS (G12D) sdAbs on PANC-1 human pancreatic cancer cells was tested, as described below.

[0078] STAT3 is a member of the signal transducers and activators of transcription (STAT) family of proteins that carry both signal transduction and activation of transcription functions. STAT3 is widely expressed and becomes activated through phosphorylation on tyrosine and/or serine as a DNA binding protein in response to a various cytokines and growth factors such as EGF, IL-6, PDGF, IL-2 and G-CSF. The STAT3 phosphoprotein forms homodimers and heterodimers with other members of the STAT family and translocates to the nucleus in order to modulate the transcription of various genes, and as a result plays a key role in many cellular processes such as cell growth, apoptosis, angiogenesis, immune evasion, and survival.

[0079] An anti-STAT3 sdAb can be given to patients and other organisms to treat diseases caused by phosphorylated and non-phosphorylated STAT3, as well as to prevent the development of disease or recurrence of disease. For example, patients who have undergone organ transplant and bone marrow transplant are at higher risk for cutaneous SCCA and BCCA due to the immunosuppressive medications they take. Administration of an anti-STAT3 sdAb can reduce or eliminate this risk. Patients treated for a malignancy who are at risk for recurrence will benefit from treatment with the anti-STAT3 sdAb. Based on family medical history and HLA-type, some individuals will be at increased risk for some types of autoimmune diseases and may benefit from treatment with sdAbs to reduce risk of developing that autoimmune disease. Breast cancer risk can be reduced with administration of anti-STAT3 medication such as GLG-302, as demonstrated in a recent NCI study.

[0080] In addition to inhibiting STAT3, the anti-STAT3 sdAb can also inhibit STAT1, STAT2, STAT4, STAT5a, STAT5b, and STAT6 due to the high degree of homology between these molecules.

[0081] Recombinant human STAT3 protein was used to produce anti-STAT sdAbs that were directed against or can bind to an epitope of STAT3. To generate the anti-STAT3 sdAbs, recombinant full-length human STAT3 (Gene ID: 6774) was expressed by baculovirus in Sf9 insect cells. The anti-STAT sdAbs were cloned into vectors that can be expressed in both bacterial and mammalian cells, as shown in Figures 1 and 2.

[0082] The anti-STAT3 sdAb of the invention can be used to target STAT3 and all other STAT molecules inside the cell in order to inhibit cell growth, such as, for example, suppression of cancer cell growth. In addition, the anti-STAT3 sdAb can inhibit cell growth in other proliferative diseases such as psoriasis and macular degeneration via VEGF.

[0083] Without being limited to a particular mechanism of action, it is thought that anti-STAT3 sdAb

can eliminate cancer induced immune suppression by decreasing STAT3 levels in antigen presenting cells such as, for example, host dendritic cells. STAT3 inhibition promotes anti-cancer response by patient's innate and adaptive immune systems (i.e., dendritic cells, macrophages, neutrophils, T cells, NK cells, and B cells).

[0084] Using methods that are well known in the art, several anti-STAT sdAbs were obtained and screened for the ability to suppress cancer cell growth and induce apoptosis in cancer cell lines, as described below. The cytotoxicity and anti-proliferative activities of the anti-STAT3 sdAbs was tested. In addition, the tolerance of anti-STAT3 sdAbs was tested *in vitro* and *in vivo*. The production of mouse monoclonal antibody directed against one or more domains of the anti-STAT sdAbs is described below.

[0085] The amino acid sequence of one anti-STAT3 sdAb, named VHH13 (SEQ ID NO. 3), is shown below:

HVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREGVSG
ISTGGLITYYADSVKGRFTISQDNTKNTLYIQMNSLKPEDTAMYYCATSR
FDCYRGSWFNRYMYNSWGQGTQVTVSS

[0086] The three CDRs are underlined.

[0087] The amino acid sequence of a second anti-STAT3 sdAb, named VHH14 (SEQ ID NO. 4), is shown below:

QVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
LSSRGFAGHIYTDVKGFRFSISRDYVKNVYLQMNTVKPEDAAMYYCAARE
GWECGETWLDRTAGGIITYWGQGTQVTVSS

[0088] Again, the three CDRs are underlined. The protein sequences of other anti-STAT3 sdAbs that were obtained are as follows:

STAT3_10 (SEQ ID NO. 5):

(1) DVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
 (48) LSSRGFAGHYTDSVKGFRFSISRDYVKNVYLQMNTVKPEDAAMYYCAARE
 (98) GWECGETWLDRTAGGHTYWGQGTQVTVSS

STAT3_34 (SEQ ID NO. 6):

(1) DVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
 (48) LSSRGFAGHYTDSVKGFRFSISRDYVKNVYLQMNTVKPEDAAMYYCAARE
 (98) GWECGETWLDRTAGGIITYWGQGTQVTVSS

STAT3_19 (SEQ ID NO. 7):

(1) HVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
 (48) LSSRGFAGHYTDSVKGFRFSISRDYVKNVYLQMNTVKPEDAAMYYCAARE
 (98) GWECGETWLDRTAGGHTYWGQGTQVTVSS

STAT3_14 (SEQ ID NO. 8):

(1) QVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
 (48) LSSRGFAGHYTDSVKGFRFSISRDYVKNVYLQMNTVKPEDAAMYYCAARE
 (98) GWECGETWLDRTAGGHTYWGQGTQVTVSS

STAT3_35 (SEQ ID NO. 9):

(1) QVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA

(48) LSSRGFAGHYTDSVKGRFSISRDIYVKNVYLQMNTVKPEDAAMYCAARE
 (98) GWECGETWLDRTAGGHTYWGQGLTVTVSS

STAT3_9 (SEQ ID NO. 10):

(1) QVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
 (48) LSSRGFAGHYTDSVKGRFSISRDIYVKNVYLQMNTVKPEDAAMYCAARE
 (98) GWECGETWLDRTAGGHTYWGQGLTVTVSS

STAT3_30 (SEQ ID NO. 11):

(1) QVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
 (48) LSSRGFAGHYTDSVKGRFSISRDIYVKNVYLQMNTVKPEDAAMYCAARE
 (98) GWECGETWLDRTAGGHTYWGQGLTVTVSS

STAT3_23 (SEQ ID NO. 12):

(1) QVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
 (48) LSSRGFAGHYTDSVKGRFSISRDIYVKNVYLQMNTVKPEDAAMYCAARE
 (98) GWECGETWLDRTAGGHTYWGQGLTVTVSS

STAT3_24 (SEQ ID NO. 13):

(1) EVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
 (48) LSSRGFAGHYTDSVKGRFSISRDIYVKNVYLQMNTVKPEDAAMYCAARE
 (98) GWECGETWLDRTAGGHTYWGQGLTVTVSS

STAT3_36 (SEQ ID NO. 14):

(1) DVQLVESGGGSVQAGGSLRLSCVASTYTGCMGWFRQAPGKEREGVAA
 (48) LSSRGFAGHYTDSVKGRFSISRDIYVKNVYLQMNTVKPEDAAMYCAARE
 (98) GWECGETWLDRTAGGHTYWGQGLTVTVSS

STAT3_12 (SEQ ID NO. 15):

(1) QVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREGVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGLTVTVSS

STAT3_16 (SEQ ID NO. 16):

(1) QVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREGVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGLTVTVSS

STAT3_11 (SEQ ID NO. 17):

(1) EVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREGVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGLTVTVSS

STAT3_20 (SEQ ID NO. 18):

(1) DVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREGVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGLTVTVSS

STAT3_2 (SEQ ID NO. 19):

(1) DVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREGVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGLTVTVSS

STAT3_15 (SEQ ID NO. 20):

(1) DVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREGVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGLTVTVSS

STAT3_6 (SEQ ID NO. 21):

(1) HVQLVESEGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREGVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGLTVTVSS

STAT3_33 (SEQ ID NO. 22):

(1) QVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGTQVTVSS

STAT3_17 (SEQ ID NO. 23):

(1) QVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGTQVTVSS

STAT3_25 (SEQ ID NO. 24):

(1) EVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGTQVTVSS

STAT3_32 (SEQ ID NO. 25):

(1) DVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGTQVTVSS

STAT3_13 (SEQ ID NO. 26):

(1) HVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGTQVTVSS

STAT3_39 (SEQ ID NO. 27):

(1) HVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGTQVTVSS

STAT3_4 (SEQ ID NO. 28):

(1) HVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGTQVTVSS

STAT3_29 (SEQ ID NO. 29):

(1) HVQLVESGGGSVQAGGSLRLSCAASGANGGRSCMGWFRQVPGKEREVSG
 (51) ISTGGLITYYADSVKGRFTISQDNTKNTLYLQMNSLKPEDTAMYYCATSR
 (101) FDCYRGSWFNRYMYNSWGQGTQVTVSS

[0089] The corresponding anti-STAT3 DNA sequences are as follows:

Stat3_VHH-10 (SEQ ID NO. 30): 5'-

gatgtgcagctggtgagctctgggggaggctcgggtgcaggctggaggctctctg
 agactctctctgttagcctctacatacaccggctgcatgggctggtccgccaggctcctggaaaggagcgcgaggagtcgcagctctt
 agtagccgtggttttgcgggcaactataccgactccgtgaaggccgattctccatctcccgagactacgtcaagaatcgggtgtatctgc
 aaatgaacactgtgaaacctgaggacgtgccatgtactactgtgcagcacgggaggatgggagtcgggtgagacctggllggaccg
 gaccgccgggggccatacctactctggggccaggggaccacggteaccgtctctctca-3'

Stat3_VHH-14 (SEQ ID NO. 31): 5'-cagggtgcagctggtggagctctgggggaggctcgggtgcaggctggaggctctc
 tgagactctctgttagcctctacatacaccggctgcatgggctggtccgccaggctcctggaaaggagcgcgaggagtcgcagct
 cttagtagccgtggttttgcgggcaactataccgactccgtgaaggccgattctccatctcccgagactacgtcaagaatcgggtgtatct
 gcaaatgaacactgtgaaacctgaggacgtgccatgtactactgtgcagcacgggaggatgggagtcgggtgagacctggttggac
 cggaccgccgggggccatacctactctggggccaggggaccctggtcaccgtctctctca-3'

Stat3_VIII-12 (SEQ ID NO. 32): 5'-cagggtgcagctggtggagctctgggggaggctcgggtgcaggctggagggtctct
 gagactctctgttagcctctacatacaccggctgcatgggctggtccgccaggctcctggaaaggagcgcgaggagtcgcagct
 ggtttctgtgtattcaaccgggtgtcttattacatactatccgactccgtgaaggccgattcaccatctcccaagacaaccaagaaca
 cgtgtatctgcaaatgaacacctgaaacctgaggacactgccatgtactactgtgcagcacgggaggatgggagtcgggtgagacctggttggac
 cttaaccctatatatataacacttctggggccaggggaccctggtcaccgtctctctca-3'

Stat3_VHH-13 (SEQ ID NO. 33): 5'-catgtgcagctggtggagtctgggggaggctcgggtcaggtcggagggtctctgagactctcctgtgcagcctctggagccaatggtggtcggagctgcatgggtggtccgaggttccagggaaggagcgcgagggggtttctgtatttcaaccgggtggtcttattacatactatgccgactccgtgaaggggccgattcaccatctcccaagacaacaccaagaacacgctgtatctgcaaatgaacagcctgaacctgaggacactgccatgtactactgtgcgacgagtcggtttgactgctatagaggctcttggttcaaccgatatatgtataacagttggggccaggggaccaggtcactgtctctca-3'

Stat3_VHH-20 (SEQ ID NO. 34): 5'-gatgtgcagctggtggagtctgggggaggctcgggtcaggtcggagggtctctgagactctcctgtgcagcctctggagccaatggtggtcggagctgcatgggtggtccgaggttccagggaaggagcgcgagggggtttctgtatttcaaccgggtggtcttattacatactatgccgactccgtgaaggggccgattcaccatctcccaagacaacaccaagaacacgctgtatctgcaaatgaacagcctgaacctgaggacactgccatgtactactgtgcgacgagtcggtttgactgctatagaggctcttggttcaaccgatatatgtataacagttggggccaggggaccctggtcaccgtctctca-3'

Stat3_VHH-23 (SEQ ID NO. 35): 5'-caggtgcagctggtggagtctgggggaggctcgggtcaggtcggagggtctctgagactctcctgtgtagcctctacatacaccggctgcatgggtggttccgaggtcctcggaaaggagcgcgagggagtcgcagctcttagcagccgtggttttggcgggcaactatacagctccgtgaaggggccgattctccatctcccgagactacgtcaagaatgctggtgtatctgcaaatgaacactgtgaacctgaggacgtgccatgtactactgtgcgacggggagggtgggagtgctggtgagacctggttggaccgggaccgcccgggagccatacctactggggccaggggaccctggtcaccgtctctca-3'

Stat3_VHH-24 (SEQ ID NO. 36): 5'-gagggtgcagctggaggagtcgggggaggctcgggtcaggtcggagggtctctgagactctcctgtgtagcctctacatacaccggctgcatgggtggttccgaggtcctcggaaaggagcgcgagggagtcgcagctcttagtagccgtggttttggcgggcaactatacagctccgtgaaggggccgattctccatctcccgagactacgtcaagaatgctggtgtatctgcaaatgaacactgtgaacctgaggacgtgccatgtactactgtgcgacggggagggtgggagtgctggtgagacctggttggaccgaaccgcccggggccatacctactggggccaggggaccctggtcaccgtctctca-3'

Stat3_VHH-25 (SEQ ID NO. 37): 5'-gagggtgcagctggtggagtcgggggaggctcgggtcaggtcggagggtctctgagactctcctgtgcagcctctggagccaatggtggtcggagctgcatgggtggttccgaggttccagggaaggagcgcgagggggtttctgtattcaaccgggtggtcttattacatactatgccgactccgtgaagggtcggattcaccatctcccaagacaacaccaagaacacgctgtatctgcaaatgaacagcctgaacctgaggacactgccatgtactactgtgcgacgagtcggtttgactgctatagaggctcttggttcaaccgatatatgtataacagttggggccaggggaccaggtcaccgtctctca-3'

Stat3_VHH-19 (SEQ ID NO. 38): 5'-catgtgcagctggtggagtctgggggggctcgggtcaggtcggagggtctctgagactctcctgtgtagcctctacatacaccggctgcatgggtggttccgaggtcctcggaaaggagcgcgagggagtcgcagctcttagtagccgtggttttggcgggcaactatacagctccgtgaaggggccgattctccatctcccgagactacgtcaagaatgctggtgtatctgcaaatgaacactgtgaacctgaggacgtgccatgtactactgtgcgacggggagggtgggagtgctggtgagacctggttggaccggaccgcccggggccatacctactggggccaggggaccaggtcaccgtctctca-3'

Stat3_VHH-32 (SEQ ID NO. 39): 5'-gatgtgcagctggtggagtctgggggaggctcgggtcaggtcggagggtctctgagactctcctgtgcagcctctggagccaatggtggtcggagctgcatgggtggttccgaggttccagggaaggagcgcgagggggtttctgtattcaaccgggtggtcttattacatactatgccgactccgtgaaggggccgattcaccatctcccaagacaacaccaagaacacgctgtatctgcaaatgaacagcctgaacctgaggacactgccatgtactactgtgcgacgagtcggtttgactgctatagaggctcttggttcaaccgatatatgtataacagttggggccaggggaccaggtcaccgtctctca-3'

Stat3_VHH-33 (SEQ ID NO. 40): 5'-caggtgcagctggtggagtctgggggaggctcgggtcaggtcggagggtctctgagactctcctgtgcagcctctggagccaatggtggtcggagctgcatgggtggttccgaggttccagggaaggagcgcgagggggtttctgtattcaaccgggtggtcttattacatactatgccgactccgtgaaggggccgattcaccatctcccaagacaacaccaagaacacgctgtatctgcaaatgaacagcctgaacctgaggacactgccatgtactactgtgcgacgagtcggtttgactgctatagaggctcttggttcaaccgatatatgtataacagttggggccaggggaccaggtcaccgtctctca-3'

Stat3_VHH-36 (SEQ ID NO. 41): 5'-gatgtgcagctggtggagtctgggggaggctcgggtcaggtcggagggtctctgagactctcctgtgtagcctctacatacaccggctgcatgggtggttccgaggtcctcggaaaggagcgcgagggagtcgcagctcttagtagccgtggttttggcgggcaactatacagctccgtgaaggggccgattcaccatctcccaagacaacaccaagaacacgctgtatctgcaaatgaacagcctgaacctgaggacactgccatgtactactgtgcgacgagtcggtttgactgctatagaggctcttggttcaaccgatatatgtataacagttggggccaggggaccaggtcaccgtctctca-3'

Stat3_VHH-11 (SEQ ID NO. 42): 5'-gtgcagctggtggagtctgggggaggctcgggtcaggtcggagggtctctgagactctcctgtgcagcctctggagccaatggtggtcggagctgcatgggtggttccgaggttccagggaaggagcgtgagggggtttctgtattcaaccgggtggtcttattacatactatgccgactccgtgaaggggccgattcaccatctcccaagacaacaccaagaacacgctgtatctgcaaatgaacagcctgaacctgaggacactgccatgtactactgtgcgacgagtcggtttgactgctatagaggctcttggttca

ccgatatatgtataacagttggggccaggggaccctgggtcactgtctctca-5

Stat3_VHH-6 (SEQ ID NO. 43): 5'-gtgcagctgggtgagctgagggagctcgggtgcaggtggagggtctctgaga
ctctcctgtgcagccctggagccaatggtggcggagctgcatgggctgggtccgccagggtccagggaaggagcgcgagggggttcc
tggtattcaaccggtgggtcttattacatactatgccgactcctggaaggccgattcaccatctcccaagacaacaccaagaacacgctgt
atctgcaaatgaacagcctgaaacctgaggacactgccatgtactactgtgcgacgagtcgggttgactgctatagaggctcttggttcaac
cgatafatgtataacagttggggccaggggaccctgggtcactgtctctca-3'

Stat3_VIII-1 (SEQ ID NO. 44): 5'-gtgcagctgggtgagctgggggaggtcgggtgcaggtggagggtctctgaga
ctctcctgtgcagccctggagccaatggtggcggagctgcatgggctgggtccgccagggtccagggaaggagcgcgagggggttcc
tggtattcaaccggtgggtcttattacatactatgccgactcctggaaggccgattcaccatctcccaagacaacaccaataacacgctgt
atctgcaaatgaacagcctgaaacctgaggacactgccatgtactactgtgcgacgagtcgggttgactgctatagaggctcttggttcaac
cgatafatgtataacagttggggccaggggaccctgggtcactgtctctca-3'

[0090] Additionally, the present invention comprises one or more mouse monoclonal antibodies which are directed against one or more domains of the anti-STAT3 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 in order to measure the amount of anti-STAT3 sdAb present in a patient's serum. It should be appreciated that the method is not limited to anti-STAT3 sdAbs, and could be used to produce a mouse antibody directed towards any of the sdAbs of the present invention.

[0091] The TNF-alpha gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophages. The cytokine is involved in the regulation of a wide spectrum of biological processes including growth regulation, differentiation, inflammation, viral replication, tumorigenesis, and autoimmune diseases; and in viral, bacterial, fungal, and parasitic infections. Besides inducing hemorrhagic necrosis of tumors, TNF was found to be involved in tumorigenesis, tumor metastasis, viral replication, septic shock, fever, inflammation, cachexia, and autoimmune diseases including Crohn's disease, and rheumatoid arthritis as well as graft-versus-host disease.

[0092] The present invention provides sdAbs, proteins, and polypeptides that are directed against TNF-alpha, in particular against human TNF-alpha inside the cell or cell membrane, so as to prevent the secretion of TNF-alpha by cells.

[0093] It is contemplated that the anti-TNF-alpha 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 TNF-alpha, such as inflammation, rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, multiple sclerosis, Addison's disease, autoimmune hepatitis, autoimmune parotitis, diabetes type 1, epididymitis, glomerulonephritis, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, systemic lupus erythematosus, male infertility, multiple sclerosis, myasthenia gravis, pemphigus, psoriasis, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, spondyloarthropathies, thyroiditis, vasculitis, and weight loss due to cancer and cachexia.

[0094] TNF-alpha exists in different forms; there are monomeric and multimeric forms, including a trimeric form. It is within the scope of the invention that the sdAbs, proteins and polypeptides of the invention bind to TNF-alpha in its different form, i.e., monomeric form or multimeric forms. Thus, when sdAbs, proteins and polypeptides of the invention are directed to TNF-alpha, it should be understood

that this also comprises sdAbs, proteins and polypeptides directed against TNF-alpha in its trimeric form.

[0095] It is known that signal transduction by TNF involves crosslinking by TNF receptors by a trimer of TNF molecules, which contains three receptor binding sites (see, for example, Peppel et al., J. Exp. Med., 174 (1991), 1483-1489).

[0096] Recombinant human TNF-alpha protein was used to generate sdAbs that are directed against or can bind to an epitope of TNF-alpha. To generate the anti-TNF-alpha sdAbs, recombinant full-length human TNF-alpha (Gene ID: 7124) was expressed in *Escherichia coli* and used as the target antigen.

[0097] Thirty-five sdAbs against the TNF-alpha protein were obtained. These anti-TNF-alpha antibodies were divided into three groups based on sequence homology.

[0098] The amino acid sequence of the first anti-TNF-alpha sdAb, named TNF-alpha VHH66 (SEQ ID NO. 45) sdAb, is shown below:

HVQLVESGGGSVEAGGSLRLSCAASGFRYAAAYCMGWFRQADGKERE
 GVATIDIDGLTTHADSVKGRITISRDNAKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRG-QGTLVTVSS

[0099] The three CDRs are underlined.

[0100] In The amino acid sequence of the second anti-TNF-alpha sdAb, named TNF-alpha VHH69 (SEQ ID NO. 46) sdAb, is shown below:

EVQLVESGGGSVLAGGSLRLSCVASGFTSRYNMAWFRQAPGKERE
 GVATIGTASGSADYYGSKDRFTISQDNAKNTVYLQMNSLKPEDTA
 MYYCAARTYGTISLTPSDYRYWGQGITLVTVSS

[0101] The three CDRs are underlined.

[0102] The amino acid sequence of the third anti-TNF-alpha sdAb, named TNF-alpha VHH62 (SEQ ID NO. 47) sdAb, is shown below:

QVQLVESGGGPVQAGETLRSLCTASGFTFAEADMGWYRQAPGHECE
 LVSTITTEGITSEASSYYADSVRGRFTISRDNAKNTLSLQMNSLKPEDTA
 VYYCAPDPYAYSTYSDYCSWAQGTQGITLVTVSS

[0103] The three CDRs are underlined. Other anti-TNF-alpha sdAbs that were found include the sequences below, again with the CDRs underlined:

TNF_2 (SEQ ID NO.48):

QVQLVESGGGSVEAGSLRLSCAASGFRYAAAYCMGWFRQADGKERE
 GVATIDIDGQTTHADSVKGRFTISRDNAKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_46 (SEQ ID NO. 49):

OVQLVESGGGSVEAGGSLRLSCAASGFRYAAAYCMGWFRQADGKERE

GVATIDIDGQTTHADSVKGRFTISRDNVKNLTLQLMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_71 (SEQ ID NO. 50):

QVQLVESGGGSVEAGGSLRLSCAASGFRYAAYCMGWFRQADGKERE
 GVATIDIDGLTTHADSVKGRFTISRDNKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_21 (SEQ ID NO. 51):

QVQLVESGGGSVEAGGSLRLSCAASGFRYAAYCMGWFRQADGKERE
 GVATIDIDGQTTHADSVKGRFTISRDNKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_38 (SEQ ID NO. 52):

EVQLVESGGGSVEAGGSLRLSCAASGFRYAAYCMGWFRQADGKERE
 GVATIDIDGQTTHADSVKGRFTISRDNKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_18 (SEQ ID NO. 53):

EVQLVESGGGSVEAGGSLRLSCAASGFRYAAYCMGWFRQADGKERE
 GVATIDIDGLTTHADSVKGRFTISRDNKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_37 (SEQ ID NO. 54):

DVQLVESGGGSVEAGGSLRLSCAASGFRYAAYCMGWFRQADGKERE
 GVATIDIDGQTTHADSVKGRFTISRDNKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_66 (SEQ ID NO. 55):

HVQLVESGGGSVEAGGSLRLSCAASGFRYAAYCMGWFRQADGKERE
 GVATIDIDGLTTHADSVKGRFTISRDNKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_68 (SEQ ID NO. 56):

HVQLVESGGGSVEAGGSLRLSCAASGFRYAAYCMGWFRQADGKERE
 GVATIDIDGLATHADSVKGRFTISRDNKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_78 (SEQ ID NO. 57):

HVQLVESGGGSVEAGGSLRLSCAASGFRYAAYCMGWFRQADRKERE
 GVATIDIDGQTTHADSVKGRFTISRDNKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_67 (SEQ ID NO. 58):

HVQLVESGGGSVQAGGSLRLSCAASGFRYAAYCMGWFRQADGKVRE
 GVATIDIDGQTTHADSVKGRFTISRDNKNTLSLQMNDLKPEDTA
 MYYCAADRDRCGSIWTYAYKYRGQGTQVTVSS

TNF_6 (SEQ ID NO. 59):

QVQLVESGGGSVQAGGSLRLSCAASGFIDSEGVMAWFRQAPGKERE
 GVAAYVRRAGDTYYADSVKGRFTISRDNKNTVYLQMNSLKPEDSA
 MYYCAARTYGSVSSWTGYKYWGQGTQVTVSS

TNF_7 (SEQ ID NO. 60):

DVQLVESGGGSVQAGGSLRLSCAASGFIDSEGVMAWFRQTPGKERE
 GVAAYVRRAGDTYYADSVKGRFTISRDNKNTVYLQMNSLKPEDSA
 MYYCAARTYGSVSSWTGYKYWGQGTQVTVSS

TNF_13 (SEQ ID NO. 61):

DVQLVESGGGSVQAGGSLRLSCAASGYTDSYGVMAWFRQAPGKERE
 GVASIYRNSGITYYPDSVKGRFTISRDNKNTVLLQMNSLKPEDSA
 TVVCAVDSGGSVCTWAGVVVWGQGTQVTVSS

TTTCAYRSGSVSLWAGTLYLWUQTQVTVSS

TNF_60 (SEQ ID NO. 62):

DVQLVESGGGSVQAGGSLRLSCAASGFDSEGVMAWFRQAPGKERE
GVAAYVRRAGDTYYADSVKGRFTISRDNKNTVYLQMNSLKPEDSA
MYYCAARTYGSVSSWIGYKYWGRGTQVTVSS

TNF_73 (SEQ ID NO. 63):

DVQLVESGGGSVRAGGSLRLSCTASGDTSKSDCMAWFRQAPGKERE
RVGAIYTRNGYTHYADSVNGRFTISQDNAKNALYLQMSGLKPEDTA
MYYCAARFRIYGQCVEDDDIDYWGQGTQVTVSS

TNF_69 (SEQ ID NO. 64):

EVQLVESGGGSVLAGGSLRLSCVASGFTSRYNMAWFRQAPGKERE
GVAITIGTASGSADYYGSVKDRFTISQDNAKNITVYLQMNSLKPEDTA
MYYCAARTYGTISLTPSDYRYWGQGTQVTVSS

TNF_76 (SEQ ID NO. 65):

QVQVVEYGGGSVQAGETVRLSCTASGFTFAEADMGWYRQAPGHEWE
LVSNTITTEGITSEASSYADSVRGRFTIFDNAKNMNVYLQMNSLKHEDTA
VYYCAPDPYAYSTYREYCTWAQGTQGTQVTVSS

TNF_62 (SEQ ID NO. 66):

QVQLVESGGGPVQAGETLRLSCTASGFTFAEADMGWYRQAPGHECE
LVSTITTEGITSEASSYADSVRGRFTISRDNKNMNVYLQMNSLKPEDTA
VYYCAPDPYAYSTYSDYCSWAQGTQGTQVTVSS

TNF_43 (SEQ ID NO. 67):

QVQLVESGGGSVQAGETLRLSCTASGFTFAEADMGWYRQAPGHECE
LVSTITTEGITSEASSYADSVRGRFTISRDNKNMNVYLQMNSLKPEDTA
VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

TNF_15 (SEQ ID NO. 68):

QVQPVESGGGSVQAGETLRLSCTASGFTFAEADMGWYRQAPGHECE
LVSTITTEGITSEASSYADSVRGRFTISRDNKNMNVYLQMNSLKPEDTA
VYYCAPDPYAYSTYSDYCTWAQGAQGTQVTVSS

TNF_11 (SEQ ID NO. 69):

QVQLVESGGGSVQAGETLRLSCTASGFTFAEADMGWYRQAPGHECE
LVSTITTEGITSEASSYADSVRGRFTISRDNKNMNVYLQMNSLKPEDTA
VYYCAPDPYAYSTYSDYCSWAQGTQGTQVTVSS

TNF_17 (SEQ ID NO. 70):

QVQLVESGGGSVQAGETLRLSCTASGFTFAEADMGWYRQAPGHECE
LVSTITTEGITSEASSYADSVRGRFTISRDNKNMNVYLQMNSLKPEDTA
VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

TNF_63 (SEQ ID NO. 71):

QVQLVESGGGSVQAGETLRLSCTASGFTFAEADMGWYRQAPGHECE
LVSTITTEGITSEASSYADSVRGRFTISRDNKNMNVYLQMNSLKPEDTA
VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

TNF_20 (SEQ ID NO. 72):

HVQLVESGGGSVQAGETLRLSCTASGFTFAEADMGWYRQAPGHECE
LVSTITTEGITSEASSYADSVRGRFTISRDNKNMNVYLQMNSLKPEDTA
VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

TNF_58 (SEQ ID NO. 73):

EVQLVESGGGSVQAGETLRLSCTASGFTFAEADMGWYRQAPGHECE
LVSTITTEGITSEASSYADSVRGRFTISRDNKNMNVYLQMNSLKPEDTA
VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

TNF_27 (SEQ ID NO. 74):

EVQLVESGGGSVQAGETLRSLCTASGFTFAEADMGWYRQAPGHECE
 LVSTITTEGITSEASSYYADSVRGRETISRDNNAKNMVYLMNSLKPEDTA
 VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

TNF_28 (SEQ ID NO. 75):

EVQLVESGGGSVQAGETLRSLCTASGFTFAEADMGWYRQAPGHECE
 LVSTITTEGITSEASSYYADSVRGRETISRDNNAKNMVYLMNSLKPEDTA
 VYYCAPDPYAYSTYSDYCSWAQGTQGTQVTVSS

TNF_4 (SEQ ID NO. 76):

EVQLVESGGGSVQAGETLRSLCTASGFTFAEADMGWYRQAPGHECE
 LVSTITTEGITSEASSYYADSVRGRETISRDNNAKNMVYLMNSLKPEDTA
 VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

TNF_14 (SEQ ID NO. 77):

DVQLVESGGGSVQAGETLRSLCTASGFTFAEADMGWYRQAPGHECE
 LVSTITTEGITSEASSYYADSVRGRETISRDNNAKNMVYLMNSLKPEDTA
 VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

TNF_3 (SEQ ID NO. 78):

DVQLVESGGGSVQAGETLRSLCTASGFTFAEADMGWYRQAPGHVCE
 LVSTITTEGITSEASSYYADSVRGRETISRDNNAKNMVYLMNSLKPEDTA
 VYYCAPDPYAYSTYSDYCSWAQGTQGTQVTVSS

TNF_1 (SEQ ID NO. 79):

DVQLVESGGGSVQAGETLRSLCTASGFTFAEADMGWYRQAPGLECE
 LVSTITTEGITSEASSYYADSVRGRETISRDNNAKNMVYLMNSLKPEDTA
 VYYCAPDPYAYSTYSEYCTWAQGTQGTQVTVSS

TNF_45 (SEQ ID NO. 80):

DVQLVESGGGSVQAGETLRSLCTASGFTFAEADMGWYRQAPGHECE
 LVSTITTEGITSEASSYYADSVRGRETISRDNNAKNMVYLMNSLKPEDTA
 VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

TNF_22 (SEQ ID NO. 81):

DVQLVESGGGSVQAGETLRSLCTASGFTFAEADMGWYRQAPGHECE
 LVSTITTEGITSVASSYYADSVRGRETISRDNNAKNMVYLMNSLKPEDTA
 VYYCAPDPYAYSTYSDYCTWAQGTQGTQVTVSS

[0104] The *in vitro* growth inhibition of several TNF-alpha sdAbs was tested, as described below. Additionally, the present invention comprises one or more mouse monoclonal antibodies which are directed against one or more domains of the anti-TNF-alpha sdAb of the invention. The mouse monoclonal antibody can be generated by methods that are known by one of skill in the art, as described above. The mouse monoclonal antibody can be used in diagnostic assays, such as, for example, an immunoassay such as an ELISA in order to measure the amount of anti-TNF-alpha sdAb present in a patient's serum.

[0105] The RAF proteins are a family of serine/threonine-specific kinases that serve as a central intermediate in transmitting extracellular signals to the mitogen-activated protein kinase cascade, which controls cell growth, differentiation and survival. BRAF is a member of the RAF family that is activated by members of the Ras family upon growth factor-induced stimulation. Active Ras can induce heterodimerization of cRaf and BRAF and this may explain the observed cooperativity of cRaf and BRaf in cells responding to growth factor signals. Activating mutations in the BRAF gene are present in

a large percentage of human malignant melanomas and in a proportion of colon cancers. The vast majority of these mutations result in a valine to glutamic acid change at residue 599 within the activation segment of BRAF.

[0106] Anti-BRAF sdAbs were developed to target wild-type and mutated BRAF in order to disrupt its role in malignant cells such as, for example, cells involved in colon cancer and other malignancies.

[0107] Using methods that are well-known in the art, recombinant human BRAF protein was used to generate sdAbs that are directed against or can bind to an epitope of BRAF.

[0108] Additionally, the present invention comprises one or more mouse monoclonal antibodies which are directed against one or more domains of the anti-BRAF sdAb of the invention. The mouse monoclonal antibody can be generated by methods that are known by one of skill in the art. The mouse monoclonal antibody can be used in diagnostic assays, for example, the antibody can be used in an immunoassay such as an ELISA in order to measure the amount of anti-BRAF sdAb present in a patient's serum.

EXAMPLES

EXAMPLE 1: ANTI-STAT3 VHH13 (SEQ ID NO. 3) SDAB BINDS STAT3

[0109] In this example, the affinity of two VHH targets against STAT3 was measured using Octet based label-free binding assay. Anti-STAT3 VHH13 (SEQ ID NO:3) sdAb, anti-KRAS (negative control) and GST-STAT3 (16kDa monovalent antigen, Creative BioMart #STAT3-1476H) were used as antigen probes in this assay. The GST-STAT3 protein was captured at 20µg/ml in PBS using aminopropylsilane (APS) dip and read biosensors, specifically meant for hydrophobic protein. The probes were then dipped into wells with the GST-STAT3 protein, anti-STAT3 VHH13 (SEQ ID NO:3) sdAb or anti-KRAS at a concentration as indicated. The association rate (on rate) of the antigen was measured. The sensors were quenched with 1% BSA in water. The probes were dipped into assay buffer (PBS) and the dissociation rate (off rate) was measured.

[0110] The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding protein (KD) was determined from the obtained affinity constant (KA), and KD using 1:1 global fit analysis Fortebio software as shown below in Table 1. Affinity was determined by averaging KD values for curves with R2 values >0.95. The 250 nM anti-STAT3 VHH13 data point was omitted as it is an outlier. It was determined that the anti-STAT3 VHH13 (SEQ ID NO. 3) sdAb affinity was 1.16×10^{-7} . The affinity of anti-KRAS VHH was not determined.

TABLE 1

Local fit analysis, highlighted values used to determine the affinity to be 1.16×10^{-7}							
Sensor Type	Sample ID	Loading Sample ID	VHH Conc. (nM)	KD (M)	k _{on} (1/Ms)	k _{off} (1/s)	Full R ²
APS (Aminopropylsilane)	ANTI-STAT3 VHH13	STAT3 20 µg/ml	1000	1.168E-07	3.16E+05	3.69E-02	0.955
APS (Aminopropylsilane)	ANTI-STAT3 VHH13	STAT3 20 µg/ml	500	1.012E-07	4.84E+05	4.09E-02	0.974
APS (Aminopropylsilane)	ANTI-STAT3 VHH13	STAT3 20 µg/ml	250	<1.0E-12	4.69E+04	5.11E-02	0.980
APS (Aminopropylsilane)	ANTI-STAT3 VHH13	STAT3 20 µg/ml	125	1.474E-07	3.69E+05	4.55E-02	0.991
APS (Aminopropylsilane)	ANTI-STAT3 VHH13	STAT3 20 µg/ml	62.5	9.921E-08	2.71E+05	2.66E-02	0.975
APS (Aminopropylsilane)	ANTI-STAT3 VHH13	STAT3 20 µg/ml	31.3	1.53E-06	8.75E+04	1.03E-01	0.656

Anti-STAT3 Antibody	Cell Line	STAT3 Concentration (µg/ml)	OD ₄₅₀	Relative Activity	Relative Activity	Relative Activity	Relative Activity
APS (Aminopropylsilane)	ANTI-kras	STAT3 20 µg/ml	1000	5.75E-08	1.19E+04	6.01E-04	0.917
APS (Aminopropylsilane)	ANTI-kras	STAT3 20 µg/ml	500	2.916E-08	1.65E+04	4.80E-04	0.890
APS (Aminopropylsilane)	ANTI-kras	STAT3 20 µg/ml	250	4.324E-09	8.93E+03	3.80E-04	0.275
APS (Aminopropylsilane)	ANTI-kras	STAT3 20 µg/ml	125	NA	NA	NA	NA
APS (Aminopropylsilane)	ANTI-kras	STAT3 20 µg/ml	62.5	NA	NA	NA	NA
APS (Aminopropylsilane)	ANTI-kras	STAT3 20 µg/ml	31.3	NA	NA	NA	NA

EXAMPLE 2: IMMUNOPRECIPITATION STUDIES

[0111] The specificity of STAT3 sdAbs was assayed in human breast cancer cells. In this example, MDA-MB-231 human breast cancer cells were grown to 50% to 70% confluence. The cells were then disrupted in freshly prepared ice-cold lysis buffer (20 mM HEPES, pH 7.9, 400 mM NaCl, 0.1% NP-40, 10% glycerol, 1 mM sodium vanadate, 1 mM sodium fluoride, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 µg/mL aprotinin, 10 µg/mL leupeptin) for 45 minutes on ice. Lysates were then centrifuged, the supernatant collected, and protein concentration was determined using a modified Lowry method (Bio Rad, Hercules, CA). Total protein (1 mg) was incubated with 1.5 mg of Dynabeads (Invitrogen) with sdAbs against STAT3, a positive control (STAT3, cat#SC-482, Santa Cruz Biotechnology, Dallas, TX), or negative control (STAT-1, cat# 9172, Cell Signaling, Danvers, MA) for 1 hr at 4 °C. Beads were then washed. Following the final wash, 60 µl of lysis buffer was added, and the resulting supernatant was subject to Western blot analysis. Briefly, samples were separated on 10% polyacrylamide gels and transferred to a nitrocellulose membrane. The membranes were blocked, then incubated with appropriate primary and secondary antibodies. Anti-STAT3 antibody, used as a positive control, was from Cell Signaling (Cat# 4904, Danvers, MA). The chemiluminescence reaction was performed using the ECL system from Santa Cruz Biotechnology (Dallas, TX).

[0112] As illustrated in Figure 3, endogenous STAT3 immunoprecipitated with all sdAbs tested at varying amounts. M is the Marker lane containing the marker, lane 1 contained STAT3 VHH13 (SEQ ID NO:3) produced and isolated from mammalian cells, lane 2 contained STAT3 VHH14 (SEQ ID NO:4) produced and isolated from mammalian cells, lane 3 contained STAT3 VHH13 (SEQ ID NO:3) produced and isolated from bacterial cells, lane 4 contained STAT3 VHH14 (SEQ ID NO:4) produced and isolated from mammalian cells, lane 5 was the positive STAT3 antibody, lane 6, used STAT-1 as a negative control, showed no band.

EXAMPLE 3: ANTI-STAT3 BACTERIAL VHH13 BINDS WITH HIGH AFFINITY TO CELL LINES CONTAINING CONSTITUTIVELY ACTIVATED STAT3

[0113] The specificity of bacterial anti-STAT3 VHH13 (SEQ ID NO:3) using constitutively activated STAT3 in human (PANC-1 and DU145) and murine (4T1) cell lines was assayed. Commercial HeLa cells were also treated with interferon gamma (INFF) in order to induce phosphorylated STAT3. The PC-3 STAT3 null cell line was used as a negative control.

[0114] The cells were grown to 50% to 70% confluence, then disrupted in freshly prepared ice-cold lysis buffer as described above for 45 minutes on ice. Lysates were then centrifuged, the supernatant collected, and protein concentration was determined as described above. Total protein (1 mg) was

incubated with 1.5 mg of Dynabeads (Invitrogen) containing the bacterial anti-STAT3 VHH13 (SEQ ID NO:3) or negative control (KRAS, Creative Biolabs, Shirley, NY) for 1 hour at 4°C. Beads were then washed. Following the final wash, 60 µl of lysis buffer was added, and the resulting supernatant was subject to Western Blot analysis as described in Example 2.

[0115] As illustrated in Figure 4, endogenous STAT3 was immunoprecipitated by bacterial VHH13 STAT3 (SEQ ID NO:3) in the constitutively activated STAT3 cell lines: PANC-1 (lane 1), DU145 (lane 2), and 4T1 (lane 4). Furthermore, bacterial VHH13 STAT3 (SEQ ID NO:3) bound to the Phospho-STAT3 in HeLa lysate (lane 3). No bands were noted for either PANC-1 KRAS, lane 3, and PC-3 (negative control), lane 6.

EXAMPLE 4: CYTOTOXICITY STUDIES OF ANTI-STAT3 SDABS IN MDA-MB-231 CANCER CELL LINES

[0116] In this example, the anti-proliferative effects of anti-STAT3 sdAbs were assayed using the human breast cancer cell line MDA-MB-231. For the experiments, MDA-MB-231 cells were grown until they reached a confluency of 90%. At that time, cells were washed, trypsinized and counted using a Coulter Counter (Beckman, Brea, CA). The proliferation studies were carried out using the 3-[4,5-dimethylthiazolyl]-2,5-diphenyltetrazolium bromide (MTT) assay. For this, cells were seeded in a 96-well plate at a density of 5×10^3 per well as indicated by the manufacturer (Roche Diagnostics Corporation, Indianapolis, IN). Cells were allowed to adhere for 24 hours and then the sdAbs were added at the appropriate concentrations (*i.e.*, 0, 0.5, 1.0, 10.0, or 100 µg/ml). Cells were counted on day 3. For the 5-day treated cells, fresh media containing the sdAbs was refreshed on day 3. At the time of termination, 10 µl of MTT reagent (0.5 mg/mL) was added to each well as indicated by the manufacturer. After a 4 hour incubation period, 100 µl of solubilization solution was added and the plate was placed in the incubator overnight. All the plates were read at 570 nm wavelength using the Biotek plate reader (Winooski, VT).

[0117] All data were analyzed using GraphPad InStat 3 (GraphPad Software, Inc., La Jolla, CA). Treatments groups were compared with vehicle control group using one-way ANOVA. If a significant difference ($p < 0.05$) was observed, the Tukey-Kramer multiple comparison test was conducted.

[0118] Based on the MTT experiment, the bacterial VHH13 anti-STAT3 (SEQ ID NO. 3) sdAb was found to be effective in inhibiting cell growth at days 3 and 5 post-treatment, as shown in Tables 2-5 below.

TABLE 2

Mean Absorbance (570nm) ± S.E. Day 3 Post Treatment with Anti-STAT3 sdAbs in MDA-MB-231 Cells						
Treatment	Control	0.5µg/ml	1.0 µg/ml	10.0 µg/ml	100 µg/ml	p-value*
H.VHH13	0.444 ± 0.030	0.504 ± 0.043	0.545 ± 0.060	0.603 ± 0.025	0.272 ± 0.011	0.001
H.VHH14	0.404 ± 0.011	0.485 ± 0.040	0.402 ± 0.017	0.588 ± 0.020	0.416 ± 0.030	0.002
B.VHH13	0.550 ± 0.036	0.685 ± 0.018	0.716 ± 0.023	0.355 ± 0.033	0.059 ± 0.001	<0.0001

Mean Absorbance (570nM) \pm S.E. Day 3 Post Treatment with Anti-STAT3 sdAbs in MDA-MB-231 Cells

Treatment	Control	0.5 μ g/ml	1.0 μ g/ml	10.0 μ g/ml	100 μ g/ml	p-value*
B.VHH14	0.593 \pm 0.014	0.666 \pm 0.022	0.644 \pm 0.045	0.456 \pm 0.048	0.255 \pm 0.005	<0.0001

*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test

TABLE 3

Effects of Anti-STAT3 sdAb Treatment on MDA-MB-231 Cell Proliferation after 3 Days of Treatment

Treatment	μ g/ml	% Inhibition	p-value*
H.VHH13	0.5		NS
	1.0		NS
	10.0		NS
	100.0	38.7	P< 0.05
H.VHH14	0.5		NS
	1.0	0.5	NS
	10.0		NS
	100.0		NS
B.VHH13	0.5		NS
	1.0		NS
	10.0	35.5	P< 0.001
	100.0	89.3	P< 0.001
B.VHH14	0.5		NS
	1.0		NS
	10.0	23.1	P< 0.05
	100.0	57.0	P< 0.001

*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test

TABLE 4

Mean Absorbance (570nM) \pm S.E. Day 5 Post Treatment with Anti-STAT3 sdAb in MDA-MB-231 Cells

Treatment	Control	0.5 μ g/ml	1.0 μ g/ml	10.0 μ g/ml	100 μ g/ml	p-value*
H.VHH13	1.100 \pm 0.088	0.955 \pm 0.013	0.963 \pm 0.018	0.832 \pm 0.028	0.721 \pm 0.025	0.0012
H.VHH14	0.983 \pm 0.023	0.890 \pm 0.021	0.935 \pm 0.037	0.804 \pm 0.015	0.797 \pm 0.010	0.0007
B.VHH13	0.804 \pm 0.046	0.761 \pm 0.055	0.653 \pm 0.024	0.506 \pm 0.030	0.083 \pm 0.005	<0.0001
B.VHH14	0.677 \pm 0.015	0.733 \pm 0.038	0.794 \pm 0.023	0.640 \pm 0.011	0.549 \pm 0.023	<0.0001

*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test

TABLE 5

Effects of Anti-STAT3 sdAb Treatment on MDA-MB-231 Cell Proliferation After 5 Days of Treatment			
Treatment	µg/ml	% Inhibition	p-value*
H.VHH13	0.5	13.2	NS
	1.0	12.5	NS
	10.0	24.4	P< 0.01
	100.0	34.5	P< 0.001
H.VHH14	0.5	9.5	NS
	1.0	4.9	NS
	10.0	18.2	P< 0.001
	100.0	18.9	P< 0.001
B.VHH13	0.5	5.4	NS
	1.0	18.8	NS
	10.0	37.1	P< 0.001
	100.0	89.7	P< 0.001
B.VHH14	0.5	0	NS
	1.0	0	NS
	10.0	5.5	NS
	100.0	18.9	P< 0.05
*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test			

EXAMPLE 5: CYTOTOXICITY STUDIES OF ANTI-STAT3 SDABS IN HUMAN BREAST (MDA-MB-231) AND PANCREATIC (PANC-1) CANCER CELL LINES

[0119] In this Example, the anti-proliferative effects of anti-STAT3 VHH13 (SEQ ID NO. 3) and the VHH14 (SEQ ID NO. 4) sdAbs were assayed using the human breast cancer cell line MDA-MB-231 and the human pancreatic cancer cell line PANC-1. For the experiments, MDA-MB-231 and PANC-1 cells were grown until they were 90% confluent. At that time, cells were washed, trypsinized and counted using a Coulter Counter (Beckman, Brea, CA). The proliferation studies were carried out using the MTT assay described above. For the 5-day treated cells, fresh media containing the anti-STAT3 sdAbs was refreshed on day 3.

[0120] All data were analyzed using GraphPad InStat 3. Treatments groups were compared with vehicle control group using one-way ANOVA. If a significant difference ($p < 0.05$) was observed, the Tukey-Kramer multiple comparison test was conducted.

[0121] Based on the MTT experiment, both the VHH13 (SEQ ID NO. 3) and the VHH14 (SEQ ID NO.

4) were found to inhibit cell growth in both the MDA-MB-231 and PANC-1 cancer cells, as shown in Tables 6-13 below.

TABLE 6

Mean Absorbance (570nm) \pm S.E. Day 3 Post Treatment With sdAbs in the MDA-MB-231 Cells					
Treatment	Experiment	Control	10.0 μ g/ml	100 μ g/ml	p-value*
B.VHH13	1	0.550 \pm 0.036	0.355 \pm 0.033	0.059 \pm 0.001	<0.0001
	2	0.735 \pm 0.092	0.489 \pm 0.019	0.449 \pm 0.054	0.0355
	3	0.627 \pm 0.033	0.432 \pm 0.060	0.078 \pm 0.001	0.0002
	4	0.648 \pm 0.090	0.576 \pm 0.061	0.063 \pm 0.002	0.0011
Overall Mean		0.640 \pm 0.038	0.463 \pm 0.047	0.163 \pm 0.10	0.0019
B.VHH14	1.	0.593 \pm 0.014	0.456 \pm 0.048	0.255 \pm 0.005	0.0005
	2	0.624 \pm 0.046	0.499 \pm 0.018	0.357 \pm 0.019	0.0025
	3	0.816 \pm 0.088	0.502 \pm 0.048	0.308 \pm 0.021	0.0026
	4	0.729 \pm 0.051	0.559 \pm 0.041	0.287 \pm 0.021	0.0007
Overall Mean		0.691 \pm 0.051	0.504 \pm 0.021	0.302 \pm 0.043	<0.0001
*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test					

TABLE 7

Mean Absorbance (570nm) \pm S.E. Day 5 Post Treatment with Anti-STAT3 sdAbs in MDA-MB-231 Cells					
Treatment	Experiment	Control	10.0 μ g/ml	100 μ g/ml	p-value*
B.VHH13	1	0.804 \pm 0.046	0.506 \pm 0.030	0.083 \pm 0.005	<0.0001
	2	0.561 \pm 0.024	0.417 \pm 0.011	0.266 \pm 0.015	<0.0001
	3	0.970 \pm 0.048	0.814 \pm 0.052	0.105 \pm 0.005	<0.0001
	4	0.757 \pm 0.118	0.665 \pm 0.036	0.087 \pm 0.004	0.011
Overall Mean		0.773 \pm 0.084	0.601 \pm 0.088	0.135 \pm 0.044	0.0005
B.VHH14	1	0.677 \pm	0.640 \pm		0.0047

Mean Absorbance (570nm) \pm S.E. Day 5 Post Treatment with Anti-STAT3 sdAbs in MDA-MB-231 Cells					
Treatment	Experiment	Control	10.0 μ g/ml	100 μ g/ml	p-value*
		0.015	0.011	0.549 \pm 0.023	
	2	0.456 \pm 0.037	0.338 \pm 0.023	0.274 \pm 0.032	0.0166
	3	0.983 \pm 0.019	0.930 \pm 0.044	0.578 \pm 0.039	0.0004
	4	1.092 \pm 0.053	0.842 \pm 0.052	0.499 \pm 0.036	0.0004
Overall Mean		0.802 \pm 0.145	0.688 \pm 0.131	0.475 \pm 0.0690	0.2022
*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test					

TABLE 8

Mean Absorbance (570nm) \pm S.E. Day 3 Post Treatment with Anti-STAT3 sdAbs in the PANC-1 Cells					
Treatment	Experiment	Control	10.0 μ g/ml	100 μ g/ml	p-value*
B.VHH13	1	0.756 \pm 0.045	0.432 \pm 0.015	0.307 \pm 0.012	<0.0001
	2	1.347 \pm 0.189	0.491 \pm 0.087	0.169 \pm 0.094	0.0019
	3	1.025 \pm 0.056	0.493 \pm 0.029	0.166 \pm 0.028	<0.0001
Overall Mean		1.043 \pm 0.171	0.472 \pm 0.020	0.214 \pm 0.047	0.0034
H.VHH13	1	1.541 \pm 0.097	1.066 \pm 0.153	0.732 \pm 0.015	0.0046
	2	1.611 \pm 0.119	1.353 \pm 0.119	0.762 \pm 0.654	0.3527
	3	1.074 \pm 0.040	0.897 \pm 0.154	0.700 \pm 0.082	0.1092
Overall Mean		1.409 \pm 0.169	1.105 \pm 0.133	0.731 \pm 0.181	0.0238
H.VHH14	1	1.195 \pm 0.205	0.920 \pm 0.133	0.808 \pm 0.239	0.4161
	2	1.423 \pm 0.038	1.183 \pm 0.114	0.993 \pm 0.088	0.0338
	3	1.293 \pm 0.169	1.163 \pm 0.044	0.916 \pm 0.088	0.1330
Overall Mean		1.304 \pm 0.066	1.089 \pm 0.085	0.906 \pm 0.054	0.0188
*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test					

TABLE 9

Mean Absorbance (570nm) ± S.E. Day 5 Post Treatment with Anti-STAT3 sdAbs in PANC-1 Cells					
Treatment	Experiment	Control	10.0 µg/ml	100 µg/ml	p-value*
B.VHH13	1	0.687 ± 0.047	0.433 ± 0.036	0.243 ± 0.024	0.0004
	2	1.670 ± 0.196	0.869 ± 0.053	0.211 ± 0.006	0.0004
	3	1.389 ± 0.044	0.627 ± 0.073	0.203 ± 0.013	<0.0001
Overall Mean		1.249 ± 0.292	0.643 ± 0.126	0.219 ± 0.012	0.0208
H.VHH13	1	1.462 ± 0.150	1.128 ± 0.105	0.839 ± 0.117	0.0349
	2	1.792 ± 0.202	1.341 ± 0.095	0.911 ± 0.079	0.0113
	3	1.605 ± 0.289	1.161 ± 0.140	0.820 ± 0.005	0.0638
Overall Mean		1.620 ± 0.096	1.210 ± 0.066	0.857 ± 0.028	0.0007
H.VHH14	1	1.992 ± 0.105	1.859 ± 0.033	0.095 ± 0.003	<0.0001
	2	1.517 ± 0.050	1.165 ± 0.015	1.169 ± 0.050	0.0015
	3	1.579 ± 0.134	1.081 ± 0.103	0.998 ± 0.049	0.0136
Overall Mean		1.696 ± 0.149	1.368 ± 0.247	0.754 ± 0.333	0.0967

*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test

TABLE 10

Mean Growth Inhibition Post 3 Days of Anti-STAT3 sdAbs Treatment on MDA-MB-231 Cell Proliferation						
Treatment	Experiment	P-value ^a	10.0 µg/ml	P-value ^b	100 µg/ml	P-value ^b
B.VHH13	1	P< 0.0001	35.5	P< 0.001	89.3	P< 0.001
	2	P= 0.03	33.5	ns	38.9	P< 0.05
	3	P= 0.0001	31.1	P< 0.05	87.6	P< 0.001
	4	P= 0.0001	11.1	ns	90.3	P< 0.01
Overall Average % Inhibition			27.8		76.5	
B.VHH14	1	P< 0.001	23.1	P< 0.05	57.0	P< 0.001
	2	P= 0.03	20.0	ns	42.8	P< 0.01
	3	P= 0.03	38.5	P< 0.05	62.3	P< 0.01
	4	P= 0.006	23.3	ns	60.6	P< 0.001
Overall Average % Inhibition			26.2		55.7	

a. One-way Analysis of Variance (ANOVA); b. Post test=Tukey-Kramer Multiple Comparisons Test

TABLE 11

Mean Growth Inhibition Post 5 days of Anti-STAT3 sdAbs Treatment on MDA-MB-231 Cell Proliferation						
Treatment	Experiment	P-value ^a	10.0 µg/ml	P-value ^b	100 µg/ml	P-value ^b
B.VHH13	1	P< 0.0001	37.1	P< 0.001	89.7	P< 0.001
	2	P< 0.0001	25.7	P< 0.001	52.6	P< 0.001
	3	P< 0.0001	16.1	ns	89.2	P< 0.001
	4	P= 0.001	12.2	ns	88.5	P< 0.01
Overall Average % Inhibition			22.8		80.0	
B.VHH14	1	P< 0.0001	5.5	ns	18.9	P< 0.05
	2	P= 0.02	25.9	ns	39.9	P< 0.05
	3	P= 0.0004	5.4	ns	41.2	P< 0.001
	4	P= 0.0004	22.9	P< 0.05	54.3	P< 0.001
Overall Average % Inhibition			14.9		38.6	
a. One-way Analysis of Variance (ANOVA); b. Post test=Tukey-Kramer Multiple Comparisons Test						

TABLE 12

Mean Growth Inhibition Post 3 Days of Anti-STAT3 sdAbs Treatment on PANC-1 Cell Proliferation						
Treatment	Experiment	P-value ^a	10.0 µg/ml	P-value ^b	100 µg/ml	P-value ^b
B.VHH13	1	P< 0.0001	42.9	P< 0.001	59.4	P< 0.001
	2	P= 0.03	63.5	P< 0.05	87.5	P< 0.01
	3	P< 0.0001	51.9	P< 0.001	83.8	P< 0.001
Overall Average % Inhibition			52.8		76.9	
H.VHH13	1	P= 0.005	30.8	P< 0.05	52.5	P< 0.01
	2	P= 0.002	16.0	ns	52.7	P< 0.01
	3	P= 0.11	16.5	ns	34.8	ns
Overall Average % Inhibition			21.1		46.7	

Mean Growth Inhibition Post 3 Days of Anti-STAT3 sdAbs Treatment on PANC-1 Cell Proliferation						
Treatment	Experiment	P-value ^a	10.0 µg/ml	P-value ^b	100 µg/ml	P-value ^b
H.VHH14	1	P= 0.42	23.0	ns	32.4	ns
	2	P= 0.03	16.9	ns	30.2	P< 0.05
	3	P= 0.13	10.1	ns	29.2	ns
Overall Average % Inhibition			16.7		30.6	
a. One-way Analysis of Variance (ANOVA); b. Post test = Tukey-Kramer Multiple Comparisons Test						

TABLE 13

Mean Growth Inhibition Post 5 Days of Anti-STAT3 sdAbs Treatment on PANC-1 Cell Proliferation						
Treatment	Experiment	P-value ^a	10.0 µg/ml	P-value ^b	100 µg/ml	P-value ^b
B.VHH13	1	P= 0.0004	37.0	P< 0.01	64.6	P< 0.001
	2	P= 0.0004	48.0	P< 0.01	87.4	P< 0.001
	3	P< 0.0001	54.9	P< 0.001	85.4	P< 0.001
Overall Average % Inhibition			46.6		79.1	
H.VHH13	1	P= 0.03	22.8	ns	42.6	P< 0.05
	2	P= 0.01	25.2	ns	49.2	P< 0.01
	3	P= 0.06	27.7	ns	48.9	ns
Overall Average Inhibition %			25.2		46.9	
H.VHH14	1	P= 0.08	26.8	ns	14.8	ns
	2	P= 0.002	23.2	P< 0.01	22.9	P< 0.01
	3	P= 0.02	31.5	P< 0.05	36.8	P< 0.05
Overall Average Inhibition %			27.2		24.8	
a. One-way Analysis of Variance (ANOVA); b. Post test = Tukey-Kramer Multiple Comparisons Test						

EXAMPLE 6: ANTI-PROLIFERATIVE ACTIONS OF STAT3 SDABS IN THE HUMAN BREAST CANCER AND HUMAN PROSTATE CANCER CELL LINES

[0122] The anti-proliferative effects of the STAT3 VHH13 (SEQ ID NO. 3) sdAb were assayed in the human breast cancer cell line MDA-MB-231 and the human prostate cancer cell lines DU145. For the experiments, cancer cells were grown until they reached 90% confluence. At that time, cells were washed, trypsinized, and counted using a Coulter Counter (Beckman, Brea, CA). The proliferation studies done using the MTT assay as described above.

[0123] The anti-proliferative properties of anti-STAT3 bacterial VHH13 (SEQ ID NO. 3) sdAb on MDA-MB-231 cells were compared to its actions on DU145 cells. As shown in Table 14, MDA-MB-231 cells treated with the anti-STAT3 (SEQ ID NO:3) sdAbs showed an average growth inhibition of 29.6 and 91.2 at 50.0 and 100 µg/ml, respectively. In the DU145 cells, a similar growth inhibition (31.2 and 92.1% for 50.0 and 100 µg/ml, respectively) was seen as set forth in Table 15.

TABLE 14

Anti-proliferative Actions of Anti-STAT3 Bacterial VHH13 sdAbs on MDA-MB-231 Breast Cancer Cells					
	Experiment 1 Absorbance (% Inhibition)	Experiment 2 Absorbance (% Inhibition)	Experiment 3 Absorbance (% Inhibition)	Average Absorbance (% Inhibition)	p- value*
control	0.93	1.25	1.46	1.21	
50 µg	0.82 (12.0)	0.99 (20.5)	0.64 (56.2)	0.82 (32.6)	NS
100 µg	0.07 (93.1)	0.12 (90.1)	0.14 (90.5)	0.11 (91.0)	<0.001
*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test					

TABLE 15

Anti-proliferative Actions of Anti-STAT3 Bacterial VHH13 sdAbs on DU145 Prostate Cancer Cells					
	Experiment 1 Absorbance (% Inhibition)	Experiment 2 Absorbance (% Inhibition)	Experiment 3 Absorbance (% Inhibition)	Average Absorbance (% Inhibition)	p- value*
control	1.05	1.58	1.61	1.41	
50 µg	0.68 (35.7)	1.2 (55.5)	1.03 (35.8)	0.98 (30.5)	NS
100 µg	0.13 (87.4)	0.12 (95.7)	0.06 (96.1)	0.10 (92.7)	<0.001
*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test					

EXAMPLE 7: ANTI-PROLIFERATIVE EFFECTS OF STAT3 VHH13 (SEQ ID NO. 3) SDABS ON HUMAN CANCER CELL LINES

[0124] To test the anti-proliferative effects of the STAT3 VHH13 (SEQ ID NO. 3) sdAbs using the human cancer cell lines: MDA-MB-231, MDA-MB-468, MCF-7, BT474, and DU145 as shown in Table 16.

[0125] All human cancer cell lines were obtained from American Type Culture Collection (Manassas,

VA). Cell lines were maintained and cultured in RPMI 1640 media (MDA-MB-231, MDA-MB-468, MCF-7, BT474) or MEM-E (DU145) containing 10% fetal bovine serum, 2 mM L-glutamine and 1% antibiotic-antimycotic solution (10 units/mL penicillin, 10 µg/mL streptomycin and 25 µg/mL amphotericin B). Cells were kept at 37°C in a humidified atmosphere of 5% CO₂. Cell culture supplies were obtained from Life Technologies, Inc., (Grand Island, NY). The MTT reagent was purchased from Sigma Aldrich (St. Louis, MO).

[0126] For the experiments, cancer cells were grown until they reached 90% confluency. At that time, cells were washed, trypsinized and counted using a Coulter Counter (Beckman, Brea, CA). The proliferation studies were carried out using the MTT assay as described above.

[0127] The anti-proliferative properties of Anti-STAT3 Bacterial VHH13 (SEQ ID NO:3) sdAbs were evaluated on five breast cancer cells of representing various classifications (Table 34). As shown in Table 17, all cell lines at 72 hours post treatment showed significant growth inhibition. The greatest growth inhibition was noted at 100 and 200 µg/ml dose for all cell lines. The half maximal inhibitory concentration (IC₅₀) for growth in the cell lines tested were: 10.1 ± 2.4, 12.36 ± 1.5, 14.8 ± 1.6, and 25.2 ± 14.7 for the MDA-MB-231, MDA-MB-468, MCF-7, and BT474 cell lines, respectively. These data suggest that the triple negative breast cancer cell lines require the lowest concentration of VHH13 (SEQ ID NO:3) sdAbs to achieve the IC₅₀ as compared to estrogen/progesterone positive cell lines (i.e., MCF-7) or HER2 amplified cell lines (i.e., BT474).

TABLE 16

Breast Cancer Cell Line Characteristics			
Cell line	Disease	Immunoprofile	Classification
MDA-MB-231	adenocarcinoma	ER ⁻ , PR ⁻ , HER2 ⁻	Basal; Claudin-low
MDA-MB-468	adenocarcinoma	ER ⁻ , PR ⁻ , Her2 ⁻	Basal
MDA-MB-453	metastatic carcinoma	ER, PR, HER2 ⁻	Unclassified
BT474	ductal carcinoma	Her2 amplified	Luminal B
MCF-7	adenocarcinoma	ER ⁺ , PR ⁺ , HER2 ⁺	Luminal A

TABLE 17

Inhibition of Breast Cancer Cell Lines by Anti-STAT3 VHH13 (SEQ ID NO. 3) sdAbs				
Cell Line	Treatment (µg/ml)	Mean Abs	% Inhibition	p-value
BT474	0	0.634		
	0.39	0.322	49.3	P< 0.001
	0.78	0.462	27.2	P< 0.001
	1.56	0.502	20.8	P< 0.01
	3.13	0.446	29.7	P< 0.001
	6.25	0.469	26.1	P< 0.001
	12.5	0.363	42.7	P< 0.001
	25	0.256	59.6	P< 0.001
	50	0.145	77.2	P< 0.001
	100	0.046	92.8	P< 0.001
	200	0.040	93.8	P< 0.001

Inhibition of Breast Cancer Cell Lines by Anti-STAT3 VHH13 (SEQ ID NO. 3) sdAbs				
Cell Line	Treatment (µg/ml)	Mean Abs	% Inhibition	p-value
MCF-7	0	0.590		
	0.39	0.818	0	
	0.78	0.785	0	
	1.56	0.823	0	
	3.13	0.689	0	
	6.25	0.435	22.1	NS
	12.5	0.327	41.6	P< 0.01
	25	0.212	62.1	P< 0.001
	50	0.057	89.9	P< 0.001
Cell Line	Treatment (µg/ml)	Mean Abs	% Inhibition	p-value
MDA-MB-468	100	0.038	93.2	P< 0.001
	200	0.040	92.9	P< 0.001
	0	0.253		
	0.39	0.311	0	
	0.78	0.289	0	
	1.56	0.201	20.6	
	3.13	0.223	11.9	
	6.25	0.230	9.1	
	12.5	0.130	48.6	P< 0.001
MDA-MB-231	25	0.067	73.5	P< 0.001
	50	0.042	83.4	P< 0.001
	100	0.038	85.0	P< 0.001
	200	0.040	84.4	P< 0.001
	0	0.502		
	0.39	0.603	0	
	0.78	0.576	0	
	1.56	0.570	0	
	3.13	0.445	11.4	P< 0.001
	6.25	0.312	37.8	P< 0.001
	12.5	0.224	55.4	P< 0.001
	25	0.196	60.9	P< 0.001
	50	0.130	74.2	P< 0.001
	100	0.041	91.8	P< 0.001

Cell Line	Treatment (µg/ml)	Mean Abs	% Inhibition	p-value
	200	0.042	91.7	P< 0.001

[0128] The actions of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was also evaluated in the human prostate cancer cell line DU145, as shown in Table 18. The anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb showed dose-dependent growth inhibition in all cancer cells tested.

TABLE 18

Effect of Anti-STAT3 VHH13 sdAbs on Prostate Cancer Cell Lines				
	Treatment (mg/ml)	Mean Abs	% Inhibition	p-value
DU145	0	0.771		
DU145	0.39	0.906	0	
DU145	0.78	1.023	0	
DU145	1.56	0.967	0	
DU145	3.13	0.783	0	
DU145	6.25	0.770	0	
DU145	12.5	0.560	27.4	P< 0.05
DU145	25	0.359	53.5	P< 0.001
DU145	50	0.161	79.1	P< 0.001
DU145	100	0.039	95.0	P< 0.001
DU145	200	0.039	95.0	P< 0.001

EXAMPLE 8: MAXIMUM TOLERATED DOSE OF ANTI-STAT3 BACTERIAL VHH13 (SEQ ID NO:3) IN BALB/C MICE

[0129] In this Example, the tolerance of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was assayed in test animals using the human breast cancer cell line MDA-MB-231. For the experiment, a total of 9 BALB/C nude female mice (6 to 7 weeks old) were divided into three groups according to body weights. (Table 19) Mice (n=3) received either vehicle (PBS) or anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb at 250 or 500 µg/kg body weight/day for five days. During the study, mortality/morbidity was performed twice daily. Body weights were recorded on days 1, 4, and 6 of the study as well as on the day of study termination (Day 13). Toxicity was assessed by body weight measurements and mouse behavior compared to vehicle control mice. Upon completion of treatment phase, animals were followed for an additional week to note any abnormalities in body weights and/or general health post treatment.

TABLE 19

Experimental Design of Maximum Tolerated Dose Study					
Group	# Mice	Treatment	Dose	Route	Frequency
1	3	PBS Vehicle	---	IP	5 days
2	3	Bacterial VHH13	250 µg/kg b.w.	IP	5 days

Experimental Design of Maximum Tolerated Dose Study					
Group	# Mice	Treatment	Dose	Route	Frequency
3	3	Bacterial VHH13	500 µg/kg b.w.	IP	5 days

[0130] As illustrated in Table 20, there was no significant difference in body weights among the groups, and anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was not associated with any drug-related deaths at either dosing level. Additionally, no behavior changes were observed in the animals treated with anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb as compared to the control mice.

TABLE 20

Mean body weights ± S.E					
Group	Randomization	Day 1	Day 4	Day 6	Day 13
Vehicle	17.1 ± 0.06	17.1 ± 0.07	17.8 ± 0.12	18.1 ± 0.09	18.8 ± 0.20
250 µg/kg	17.1 ± 0.06	17.2 ± 0.03	17.2 ± 0.15	17.5 ± 0.15	18.1 ± 0.21
500 µg/kg	17.1 ± 0.17	17.1 ± 0.09	17.8 ± 0.18	18.0 ± 0.20	18.5 ± 0.18
p-value*	> 0.9999	0.52	0.05	0.07	0.11
*One Way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparison Test					

EXAMPLE 9: ACTIVITY OF BACTERIAL ANTI-STAT3 VHH13 (SEQ ID NO:3) IN NUDE BALB/C MICE XENOGRAFT AND HUMAN BREAST CANCER AND HUMAN PANCREATIC CANCER CELLS

[0131] In this example, the activity of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was evaluated in mice using the human breast cancer cell line MDA-MB-231. Briefly, the activity of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was evaluated using: the MDA-MB-231 human breast cancer xenograft model and the PANC-1 human pancreatic cancer xenograft model. Dosing schedules were as follows: Group 1 (n=6; PBS; IP) daily for 14 days [QDx14]; and Group 2 (n=12; 500 µg/kg bw; IP), every day for 14 days [QDx14]. An observation period of 5 days followed the drug administration.

[0132] The human breast cancer cell lines (MDA-MB-231 and PANC-1) were obtained from American Type Culture Collection (ATCC) (Manassas, VA). The MDA-MB-231 cells were grown in MEM (Life Technologies, Grand Island, NY) supplemented with 10% FBS (Atlanta Biologicals, Flowery Branch, GA) and Penicillin-Streptomycin-Glutamine (Life Technologies, Grand Island, NY). The PANC-1 cells were grown in RPMI 1640 media (Life Technologies, Grand Island, NY) supplemented with 10% FBS and Penicillin-Streptomycin-Glutamine. All cells were grown in the presence of 5% CO₂ at 37°C in an incubator.

[0133] Athymic nude-Foxn1^{nu} male mice aged 4 to 5 weeks were purchased from Harlan Laboratories (Indianapolis, IN). Animals were quarantined for one week and housed five mice per cage, with a 12-hr light-dark cycle, and a relative humidity of 50%. Drinking water and diet were supplied to the animals *ad libitum*. All animals were housed under pathogen-free conditions and experiments were performed in accordance with the IIT Research Institute Animal Use and Care Committee. For the MDA-MB-231 xenograft study, cells (4 × 10⁶) in a 100-µL final volume of MEM media were injected

subcutaneously into right flanks of mice. For the PANC-1 xenograft study, cells (5×10^6) in a 100- μ L final volume of RPMI media were injected subcutaneously into right flanks of mice. Tumor measurements for both models were initiated as soon as the tumors were palpable. Thereafter, tumors were measured twice weekly. Animals were randomized when tumors reach a range size of 75 to 175 mm³, control (n = 6) and a treatment (n=12) groups were randomized using the stratified random sampling algorithm. Treatment (anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb) or Vehicle (PBS) was initiated the day following randomization. The treatment was well tolerated and associated with no drug-related deaths. No significant body weight loss was noted.

[0134] For the MDA-MB-231 xenograft study, the randomization Mean (\pm SE) tumor size was: 103.01 \pm 11.89 and 102.61 \pm 9.60 for control and treatment groups respectively. Mean body weights (\pm SE) at randomization were: 32.08 \pm 0.76 and 30.27 \pm 0.75 for Group 1 and Group 2, respectively. Table 21 shows the mean body weights (\pm SE) for the entire study.

TABLE 21

Mean body weights \pm S.E.						
Treatment	Day 1	Day 6	Day 9	Day 12	Day 16	Day 20
Vehicle	31.0 \pm 0.83	32.1 \pm 0.76	31.9 \pm 0.66	32.1 \pm 0.68	32.0 \pm 0.71	32.5 \pm 0.88
Anti-STAT3 VHH13	29.2 \pm 0.71	30.3 \pm 0.75	30.4 \pm 0.79	29.9 \pm 0.72	30.6 \pm 0.74	30.6 \pm 0.77
p-value*	0.16	0.18	0.27	.09	0.28	0.17
* Two-tail T-Test						

[0135] On day 14 of dosing, the mean tumor size (\pm SE) for the control was 179.11 \pm 19.39 versus 118.86 \pm 15.94 for treatment group. Mean body weights (\pm SE) at termination were: 31.98 \pm 0.71 and 30.55 \pm 0.74 for Group 1 and Group 2, respectively. Table 22 summarizes the tumor volumes (\pm SE) for entire study. The % mean tumor growth inhibition in the treatment group was 33.64%. The tumor doubling times were as follows:

Group 1: 44.27 days; and Group 2: 61.06 days. Figure 5 illustrates the growth inhibition of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the MDA-MB-231 xenograft model. Anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb showed significant growth inhibition (p= 0.047). Thus, anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb has chemotherapeutic activity in the MDA-MB-231 human breast cancer model system.

TABLE 22

Individual Tumor Measurements (mm ³) for the MDA-MB-231 Xenograft Model							
Group	Animal #	Day 1	Day 6	Day 9	Day 12	Day 16	Day 20
1	1	117.43	141.72	135.00	139.31	127.93	133.19
	2	130.30	142.83	206.15	256.99	244.06	243.00
	3	78.00	105.97	114.04	144.06	154.50	158.94
	4	118.24	162.41	171.39	225.59	181.32	217.97
	5	71.10	109.03	133.13	168.80	187.73	164.45
Mean		103.01	132.39	151.94	186.95	179.11	183.51
S.E.		11.89	10.82	16.42	23.28	19.39	20.28

Individual Tumor Measurements (mm ³) for the MDA-MB-231 Xenograft Model							
Group	Animal #	Day 1	Day 6	Day 9	Day 12	Day 16	Day 20
2	6	123.94	114.91	129.22	176.04	170.09	162.98
	7	85.93	101.06	112.60	112.24	139.56	96.43
	8	147.34	148.72	169.69	185.08	170.07	256.71
	9	115.91	103.64	108.37	141.21	144.51	119.42
	10	73.23	82.59	110.13	91.22	166.77	285.88
	11	163.73	178.23	183.79	165.52	214.28	129.51
	12	75.54	83.94	103.68	119.88	104.26	99.48
	13	70.04	89.24	102.60	75.25	57.65	95.23
	14	101.62	65.09	82.02	68.01	61.41	61.83
	15	67.83	62.21	59.00	77.04	65.49	82.73
	16	131.93	75.28	76.21	53.55	73.66	51.61
	17	74.28	109.06	111.92	89.94	58.56	100.07
Group	Animal #	Day 1	Day 6	Day 9	Day 12	Day 16	Day 20
Mean		102.61	101.16	112.44	112.92	118.86	128.49
S.E.		9.6	9.8	10.3	12.9	15.9	21.1
P-value		0.98	0.08	0.06	0.01	0.05	0.14

[0136] For the PANC-1 xenograft study, the randomization Mean (+SE) tumor sizes were 107.01 ± 4.54 in the control and 110.58 ± 6.18 in the treatment groups. Mean body weights (± SE) at randomization were: 29.0 ± 0.81 and 28.5 ± 0.70 for Group 1 and Group 2, respectively. Mean body weights (± SE) at termination were: 31.2 ± 0.99 and 30.1 ± 0.75 for Group 1 and Group 2, respectively. Table 23 summarizes the mean body weights (± SE) for entire study. On day 14 of dosing, the mean tumor size (±SE) for control was 287.30 ± 33.94 versus 318.74 ± 29.76 for treatment group. Table 24 summarizes the tumor volumes (± SE) for entire study.

TABLE 23

Mean body weights ±S.E.						
Treatment	2/19	2/24	2/27	3/2	3/6	3/10
Vehicle Control	31.0 ± 0.83	32.1 ± 0.76	31.9 ± 0.66	32.1 ± 0.68	32.0 ± 0.71	32.5 ± 0.88
Anti-STAT3	29.2 ± 0.71	30.3 ± 0.75	30.4 ± 0.79	29.9 ± 0.72	30.6 ± 0.74	30.6 ± 0.77

[0137] The tumor doubling times were as follows: Group 1: 22.44 days; and Group 2: 23.02 days. Anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb showed no significant growth inhibition in the PANC-1 human pancreatic cancer model system.

TABLE 24

Individual Tumor Measurements (mm ³) for the PANC-1 xenograft Model							
Group	Animal #	2/19	2/24	2/27	3/2	3/6	3/10
1	1	99.77	117.96	134.67	161.27	160.79	195.58
	2	117.54	137.14	221.14	241.27	303.70	321.45
	3	120.30	210.99	276.05	322.17	394.96	732.07
	4	111.65	135.91	215.87	340.97	334.08	382.06
	5	90.88	96.35	165.26	156.28	223.17	314.97
	6	107.05	156.56	192.98	324.34	307.13	573.99
Mean		107.87	142.49	201.00	257.72	287.30	420.02
S.E.		11.11	16.01	20.00	34.35	33.94	80.34
2	7	96.31	193.71	275.06	317.53	395.37	540.66
	8	89.24	90.03	112.43	125.51	189.63	235.08
	9	80.62	148.97	196.38	187.24	299.84	530.46
	10	108.03	144.14	234.46	240.39	288.75	421.61
	11	77.66	116.21	313.19	290.38	411.66	197.67
	12	129.68	143.20	290.67	224.92	261.44	343.04
	13	108.99	182.30	239.00	254.64	342.19	464.00
	14	123.27	171.03	223.34	226.88	248.69	324.30
	15	144.53	136.03	198.47	226.04	247.97	273.58
	16	120.96	136.48	226.43	338.06	564.71	883.81
	17	112.69	144.76	167.12	225.70	223.06	326.19
	18	134.95	189.64	193.14	248.01	351.63	364.44
Mean		110.58	149.71	222.47	242.11	318.74	408.74
S.E.		6.18	8.79	15.90	16.30	29.76	53.25
P-value		0.78	0.67	0.43	0.64	0.53	0.91

EXAMPLE 10: MDA-MB-231 XENOGRAFT STUDY

[0138] In this Example, the efficacy of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the MDA-MB-231 human breast xenograft model was further evaluated. The dosing schedules were as follows: Group 1 (n=4; PBS; IP) twice a day for 14 days [BIDx14]; Group 2 (n=4; 1 mg/kg bw; IP), twice a day for 14 days [BIDx14]; Group 3 (n=4; 2 mg/kg bw; IP) twice a day for 14 days [BIDx14]; and Group 4 (n=4; 2 mg/kg bw; IP) once a day for 14 days [QDx14]. An observation period of 7 days followed administration.

[0139] The human breast cancer cell lines MDA-MB-231 and athymic nude-Foxn1^{nu} female mice were described above.

[0140] MDA-MB-231 cells at a density of 5×10^6 were injected subcutaneously into the right flank of the mice at a final volume of 100- μ L in MEM media. Tumor measurements were initiated as soon as the tumors were palpable. Thereafter, tumors were measured twice weekly. Animals are randomized when tumors reach a range size of 55 to 150 mm³ using the stratified random sampling algorithm. Treatment (anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb) or Vehicle (PBS) was initiated the day following randomization.

[0141] The randomization Mean (\pm SE) tumor size was: 92.08 ± 13.24 , 82.38 ± 5.17 , 77.47 ± 7.17 , and 104.71 ± 14.64 for Groups 1, 2, 3, and 4 respectively. As shown in Table 25, mean body weights (\pm SE) at randomization were: 23.65 ± 0.72 , 23.45 ± 0.66 , 23.10 ± 0.20 , and 22.45 ± 1.25 for Groups 1, 2, 3, and 4, respectively.

[0142] As shown in Table 26, at day 14 of dosing, the mean tumor size (\pm SE) for control group was 221.51 ± 57.32 versus 67.12 ± 10.66 , 58.27 ± 22.54 , and 131.44 ± 22.86 , for treatment group 2, 3, and 4, respectively. At the time of termination (day 42) mean tumor size (\pm S.E.) was: 255.42 ± 65.46 , 55.98 ± 6.94 , 41.15 ± 13.21 , and 145.51 ± 52.32 , for groups 1, 2, 3, and 4, respectively. Mean body weights (\pm SE) at termination were: 24.80 ± 0.49 , 23.25 ± 1.20 , 24.00 ± 0.32 , and 23.2 ± 1.46 for Groups 1, 2, 3, and 4, respectively. The max mean % net weight loss (day) was: 0.7 (36), 1.5 (23), 1.8 (36), and 2.2 (29) for Groups 1, 2, 3, and 4, respectively.

[0143] Also as shown in Table 26, the mean growth inhibition in the treatment groups was 78.3, 75.2, and 55.9, for Groups 2, 3, and 4, respectively. The tumor doubling times were: Group 1: 20.56 days; Group 2: 34.54 days; Group 3: 30.07 days; and Group 4: 27.17 days. There was a growth delay of 13.99, 9.52, and 6.61 days for Groups 2, 3 and 4, respectively. The % treatment/control values for treatment groups were: Group 2: -33.75 (tumor stasis); Group 3: -54.4 (tumor regression); and Group 4: 10.28 (tumor inhibition). Figure 6 illustrates the growth inhibition of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the MDA-MB-231 xenograft model.

[0144] Administration of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was associated with a significant growth inhibition in Group 2 ($p=0.02$) [1 mg/kg; BID X 14] and Group 3 ($p= 0.02$) [2 mg/kg; BID x 14]. Furthermore, three out of four tumors showed significant regression. Based on these data, it is concluded that anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb has chemotherapeutic activity in the MDA-MB-231 human breast cancer model system.

TABLE 25

Mean Body Weights \pm S.E.								
		Date/Study Day						
		Dosing					Rec overy	
		6/23	6/26	6/29	7/2	7/6	7/9	7/15
Group	Schedule	20	23	26	29	33	36	42
1	PBS; BID x14	23.65 ± 0.72	23.85 ± 0.60	24.18 ± 0.67	24.05 ± 0.63	24.30 ± 0.67	24.13 ± 0.72	24.80 ± 0.49
2	1 mg/kg; BID x 14	23.45 ± 0.66	23.10 ± 0.68	23.13 ± 0.74	23.13 ± 0.95	23.08 ± 1.01	23.13 ± 1.09	23.25 ± 1.20
3	2 mg/kg; BID x 14	23.10 ± 0.20	23.10 ± 0.14	23.20 ± 0.07	23.85 ± 0.39	23.80 ± 0.24	23.38 ± 0.23	24.00 ± 0.32

Mean Body Weights \pm S.E.								
		Date/Study Day						
		Dosing					Rec overy	
		6/23	6/26	6/29	7/2	7/6	7/9	7/15
Group	Schedule	20	23	26	29	33	36	42
4	2 mg/kg; QD \times 14	22.45 \pm 1.25	22.35 \pm 1.32	22.58 \pm 1.46	22.08 \pm 1.44	22.73 \pm 1.47	22.55 \pm 1.46	23.20 \pm 1.38

TABLE 26

Individual Tumor Measurements (mm ³) for the MDA-MB-231 Xenograft Model								
Group 1	Animal #	6/23/15 (20)	6/26/15 (23)	6/29/15 (26)	7/2/15 (29)	7/6/15 (33)	7/9/15 (36)	7/15/15 (42)
	001	93.38	119.07	159.80	197.91	210.95	243.31	265.61
	002	116.07	241.31	313.16	339.13	362.30	390.48	426.32
	003	55.67	83.45	98.22	135.50	198.19	204.96	218.29
	004	104.82	112.09	118.44	111.07	114.61	115.31	111.45
Mean Absolute		92.49	138.98	172.41	195.90	221.51	238.51	255.42
Mean Relative		100.00%	150.27%	186.41%	211.82%	239.51%	257.89%	276.17%
S.E. Mean		13.12	34.97	48.64	51.12	51.56	57.32	65.46
% Inhibition Mean								
Median Absolute		99.10	115.58	139.12	166.71	204.57	224.13	241.95
Median Relative		100.00%	116.62%	140.38%	168.22%	206.42%	226.16%	244.14%
S.E. Median		13.66	37.49	52.30	53.83	52.48	57.91	65.92
% Inhibition Median								
Group 2	Animal #	6/23/15 (20)	6/26/15 (23)	6/29/15 (26)	7/2/15 (29)	7/6/15 (33)	7/9/15 (36)	7/15/15 (42)
	005	73.15	54.54	59.17	57.21	56.20	37.13	39.17
	006	80.11	76.56	80.34	88.75	99.09	87.42	72.18
	007	97.22	79.99	78.44	59.90	55.90	53.66	60.35
	008	81.21	53.58	54.34	67.43	57.30	29.02	52.23
Mean Absolute		82.92	66.17	68.07	68.32	67.12	51.81	55.98
Mean Relative		100.00%	79.79%	82.09%	82.39%	80.95%	62.48%	67.51%

Group 2	Animal #	6/23/15 (20)	6/26/15 (23)	6/29/15 (26)	7/2/15 (29)	7/6/15 (33)	7/9/15 (36)	7/15/15 (42)
S.E. Mean		5.09	7.03	6.62	7.14	10.66	12.93	6.94
% Inhibition Mean		10.34%	52.39%	60.52%	65.12%	69.70%	78.28%	78.08%
Median Absolute		80.66	65.55	68.80	63.66	56.75	45.40	56.29
Median Relative		100.00%	81.27%	85.30%	78.93%	70.36%	56.28%	69.79%
S.E. Median		5.25	7.04	6.63	7.63	12.23	13.45	6.94
% Inhibition Median		18.61%	43.28%	50.54%	61.81%	72.26%	79.75%	76.74%
Group 3	Animal #	6/23/15 (20)	6/26/15 (23)	6/29/15 (26)	7/2/15 (29)	7/6/15 (33)	7/9/15 (36)	7/15/15 (42)
	009	56.41	43.61	33.13	31.76	34.11	50.33	18.94
	010	84.06	85.18	61.75	80.69	110.72	89.11	73.89
	011	82.87	54.78	34.92	54.38	78.47	78.68	51.30
	012	86.73	44.01	23.09	16.99	9.78	18.71	20.48
Mean Absolute		77.52	56.89	38.22	45.95	58.27	59.21	41.15
Mean Relative		100.00%	73.39%	49.31%	59.28%	75.17%	76.38%	53.09%
S.E. Mean		7.08	9.78	8.26	13.90	22.54	15.79	13.21
% Inhibition Mean		16.19%	59.06%	77.83%	76.54%	73.69%	75.18%	83.89%
Median Absolute		83.46	49.39	34.02	43.07	56.29	64.51	35.89
Median Relative		100.00%	59.18%	40.76%	51.60%	67.44%	77.29%	43.00%
S.E. Median		7.87	10.69	8.61	14.00	22.56	16.08	13.56
% Inhibition Median		15.78%	57.27%	75.54%	74.17%	72.49%	71.22%	85.17%
Group 4	Animal #	6/23/15 (20)	6/26/15 (23)	6/29/15 (26)	7/2/15 (29)	7/6/15 (33)	7/9/15 (36)	7/15/15 (42)
	013	88.56	108.35	105.80	102.94	183.39	159.78	291.06
	014	78.73	51.51	54.20	70.39	84.29	55.83	42.03
	015	113.20	85.29	69.30	103.16	103.20	87.15	130.64

Group 4	Animal #	6/23/15 (20)	6/26/15 (23)	6/29/15 (26)	7/2/15 (29)	7/6/15 (33)	7/9/15 (36)	7/15/15 (42)
	016	141.91	130.82	87.49	145.68	154.89	117.63	118.31
Mean Absolute		105.60	93.99	79.20	105.54	131.44	105.10	145.51
Mean Relative		100.00%	89.01%	75.00%	99.94%	124.47%	99.52%	137.79%
S.E. Mean		14.11	16.94	11.18	15.44	22.86	22.17	52.32
% Inhibition Mean		-14.18%	32.37%	54.06%	46.13%	40.66%	55.94%	43.03%
Median Absolute		100.88	96.82	78.40	103.05	129.05	102.39	124.47
Median Relative		100.00%	95.98%	77.71%	102.15%	127.92%	101.49%	123.38%
S.E. Median		14.37	17.02	11.19	15.50	22.90	22.22	53.72
% Inhibition Median		-1.80%	16.23%	43.65%	38.19%	36.92%	54.32%	48.55%

EXAMPLE 11: EFFICACY OF ANTI-STAT3 BACTERIAL VHH13 (SEQ ID NO:3) SDAB ON THREE HUMAN CANCER XENOGRRAFT MODELS

[0145] In this Example, the efficacy of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was evaluated in the MDA-MB-231 Human Breast, PANC-1 Pancreatic, and DU145 Prostate cancer xenograft models.

[0146] Athymic Nude-Foxn1^{nu} mice, MDA-MB-231 breast cancer cells, PANC-1 pancreatic cancer and the DU145 prostate cancer cell lines were described above. The body weight of the mice ranged from 17 to 19 g (34 females) and 21 to 23 g (16 males) on Day 1 of the study.

[0147] Cells in early passages (4 to 10) were used for implantation into the mice and were harvested during log phase growth. MDA-MB-231 (5×10^6), DU145 (5×10^6), and PANC-1 (1.5×10^6) were injected subcutaneously into the right flank of the mice at a final volume of 100- μ L of media. Tumor measurements were initiated as soon as the tumors were palpable. Thereafter, tumors were measured twice weekly.

[0148] Animals were randomized using the stratified random sampling algorithm when tumors reach a range size of: 74-120 mm³ (MDA-MB-231), 89-146 mm³ (DU145), or 60-160 mm³ (PANC-1). Treatment (containing anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb and referred to herein as SBT-100) or Vehicle (PBS) was initiated the day following randomization, referred to as day 1.

[0149] Anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was supplied as a pre-formulated solution at a concentration of 0.651 mg/ml and was stored at -20°C until ready to use. The stock solution was diluted in sterile PBS pH 7.6 to provide a 5 mg/kg in a dosing volume of 10 mL/kg. The working solution was prepared every 7 days, aliquoted onto seven vials and stored at 4°C. On each day of treatment, only the needed vial was brought to room temperature. All leftover sdAb material was retained at 4°C as need for the next dose. At day 8, any remaining sdAb material was discarded and a fresh batch prepared.

[0150] Two groups (control and SBT-100) of mice per tumor model were dosed according to the protocol shown in Table 27. Dosing schedules were as follows: Group 1 (n=4; PBS) twice a day for 14 days [BIDx14]; Group 2 (n=4; SBT-100, 5 mg/kg bw), twice a day for 14 days [BIDx14]. Both the vehicle (PBS pH 7.6) and SBT100 were administered intraperitoneally (i.p.) twice a day, six hours apart for fourteen days. Dosing was conducted according to individual animal weights. A recovery period of 7 days followed administration.

TABLE 27

Experimental Design of Xenograft Study

Model	# of cells inoculated/mouse	Group	# Mice	Agent	Dose (mg/Kg)	Route	Schedule
MDA-MB-231	5×10^5	1	4	Control (PBS)	0	IP	BIDx14
		2	4	SBT-100	5	IP	BIDx14
PANC-1	1.5×10^6	1	4	Control (PBS)	0	IP	BIDx14
		2	4	SBT-100	5	IP	BIDx14
DU145	5×10^5	1	4	Control (PBS)	0	IP	BIDx14
		2	4	SBT-100	5	IP	BIDx14

[0151] Study Log Study Director Animal Study Management Software (San Francisco, CA) was used to randomize animals, collect data (e.g., dosing, body weights, tumors measurements, clinical observations), and conduct data analyses.

[0152] In the MDA-MB-231 tumor xenograft model, animals were randomized on day 23 post-inoculation with a mean (\pm SE) tumor size of: 77.98 ± 21.58 and 84.71 ± 5.56 for Groups 1 and 2, respectively. Mean body weights (\pm SE) at randomization were: 20.04 ± 0.62 and 23.7 ± 1.84 for Groups 1 and 2, respectively. Table 28 summarizes the mean body weights (\pm SE) for entire study. At last day of dosing (Day 14), the mean tumor size (\pm SE) for control group was 168.28 ± 51.57 versus 83.81 ± 22.65 for SBT-100 treated mice. Table 29 summarizes the tumor volumes (\pm SE) for entire study. At the time of termination (day 28) mean tumor size (\pm S.E.) was: 270.49 ± 112.35 and 91.72 ± 33.17 , for Groups 1 and 2, respectively. Mean body weights (\pm SE) at termination were: 25.36 ± 1.07 and 24.25 ± 1.68 for Groups 1 and 2, respectively. At the end of the study, the mean tumor growth inhibition in the SBT-100 treated group was 85.8% ($p = 0.006$). Figure 7 illustrates the mean tumor volume. The tumor doubling times were 25.78 days versus 111.6 days for Group 1 and Group 2, respectively. The % treatment/control for Group 2 was 13.35 (tumor inhibition).

TABLE 28

Mean Body Weights for Mice in MDA-MB-231

Group	Animal ID	Phase						
		Dosing					Recovery	
		8/28	9/1	9/4	9/8	9/11	9/15	9/19
Control	001	23.40	24.80	24.60	25.00	24.70	23.30	25.10
Control	002	22.40	22.50	22.60	22.70	22.80	20.60	23.10
Control	003	23.70	24.80	25.20	24.60	24.30	23.30	25.20

Control	004	23.70	24.70	25.10	25.30	24.90	22.90	25.40
Mean		23.30	24.20	24.38	24.45	24.18	22.53	24.70
Median		23.55	24.75	24.85	24.90	24.50	23.10	25.15
SD		0.62	1.13	1.21	1.18	0.95	1.30	1.07
% Change		0.00	3.82	4.56	4.89	3.73	-3.38	5.97
SBT-100	005	21.70	21.70	21.70	22.40	22.50	21.40	22.20
SBT-100	006	25.00	24.30	24.30	24.70	25.30	24.40	25.00
SBT-100	007	22.50	23.00	23.10	23.10	23.80	22.80	23.70
SBT-100	008	25.50	25.30	25.50	26.10	25.90	25.50	26.10
Mean		23.7	23.575	23.65	24.075	24.375	23.55	24.25
Median		23.8	23.65	23.7	23.9	24.55	23.6	24.35
SD		1.84	1.56	1.63	1.65	1.46	1.84	1.68
% Change		0.00	-0.45	-0.15	1.58	2.96	-0.63	2.38

TABLE 29

Tumor Volumes for MDA-MB-231

Group	Animal ID	Pre-Dosing		Phase						Recovery	
		8/21	8/24	8/27	8/28	9/1	9/4	9/8	9/11	9/15	9/18
Control	001	51.00	55.80	80.54	78.35	83.66	94.11	110.78	123.98	162.81	184.15
Control	002	75.19	77.22	121.13	120.73	145.12	179.21	209.15	234.66	309.70	428.44
Control	003	57.04	57.81	75.32	81.06	93.35	114.27	181.87	242.88	255.93	408.67
Control	004	40.82	51.07	108.54	92.23	116.90	142.00	181.58	213.48	288.91	388.19
Mean		56.54	58.63	95.88	92.59	109.75	132.55	171.84	205.10	263.59	331.11
Median		54.02	56.80	93.74	88.64	105.10	128.44	186.72	223.76	291.42	355.80
SD		13.71	11.38	21.58	18.81	27.42	36.92	41.83	51.57	57.78	112.36
SBT-100	005	72.25	64.45	90.03	74.07	56.81	48.44	68.75	73.54	93.33	116.07
SBT-100	006	61.50	63.00	90.67	76.00	71.02	67.03	87.54	115.80	119.57	120.44
SBT-100	007	37.41	35.15	91.83	81.55	50.02	50.32	46.10	63.85	68.57	80.57
SBT-100	008	43.80	56.95	95.22	79.94	55.29	60.10	54.10	82.57	79.47	49.78
Mean		53.74	54.91	94.71	81.37	59.49	56.76	64.11	83.81	88.08	91.72
Median		52.55	53.02	83.45	79.77	53.82	55.25	61.40	77.81	85.49	88.32
SD		16.00	13.57	5.56	7.49	9.16	8.43	18.21	22.56	21.96	33.17
%TC		0.0	32.3	84.9	81.6	13.8	5.8	9.2	29.7	17.8	13.4
p-values		0.909	0.542	0.351	0.332	0.913	0.907	0.903	0.905	0.993	0.996

[0153] In the DU145 tumor xenograft model, animals were randomized on day 17 post-inoculation with a mean (\pm SE) tumor size of: 111.87 ± 20.53 and 111.23 ± 25.16 for Groups 1 and 2, respectively. Mean body weights (\pm SE) at randomization were: 29.10 ± 1.94 and 30.68 ± 1.56 for Groups 1 and 2, respectively. Table 30 summarizes the mean body weights (\pm SE) for entire study. At last day of dosing (Day 14), the mean tumor size (\pm SE) for control group was 621.81 ± 276.25 versus 364.14 ± 51.64 for SBT-100 treated mice. Table 31 summarizes the tumor volumes (\pm SE) for entire study. At the time of termination (day 28) mean tumor size (\pm S.E.) was: 819.42 ± 351.88 and 601.83 ± 131.51 , for Groups 1 and 2, respectively. Mean body weights (\pm SE) at termination were: 29.20 ± 2.33 and 29.60 ± 1.04 for Groups 1 and 2, respectively. At the end of the study, the mean tumor growth inhibition in the SBT-100 treated group was 26.6% ($p=0.29$). Figure 8 illustrates the mean tumor volume. The tumor doubling times were 14.57 days versus 18.19 days for Group 1 and Group 2, respectively. The % treatment/control for Group 2 was 74.8.

TABLE 30

Mean Body Weights for Mice in DU145

Group	Animal ID	Phase						
		Dosing					Recovery	
		8/4	8/8	9/11	9/15	9/18	9/22	9/25
Control	001	29.60	28.10	29.30	28.40	28.30	29.00	29.90
Control	002	29.30	30.10	31.20	30.10	30.40	29.80	30.00
Control	003	30.80	30.10	31.00	31.70	31.20	31.10	31.30
Control	004	25.30	25.70	25.60	25.20	25.10	25.20	25.80
Mean		29.10	28.58	29.63	28.85	28.90	29.05	28.80
Median		29.55	28.10	29.15	29.35	29.35	29.45	29.95
SD		1.94	2.09	2.13	2.73	2.29	2.59	2.33
% Change		0.00	-2.07	1.46	-0.89	-0.37	-0.19	0.27
SBT-100	005	30.00	30.00	32.80	30.80	28.50	30.50	30.50

SBT-100	006	28.40	28.20	27.30	28.90	27.50	29.10	28.50
SBT-100	007	31.20	31.20	31.50	30.40	30.70	31.20	30.80
SBT-100	008	31.30	29.70	30.20	28.20	28.10	28.80	29.00
Mean		30.63	29.33	29.23	28.83	28.65	29.30	29.50
Median		31.30	29.95	29.95	29.00	29.00	29.80	29.55
SD		1.55	2.17	1.97	1.53	1.50	1.14	1.04
% Change		0.00	-4.47	-4.74	-6.90	-5.23	-2.39	-2.40

TABLE 31
Tumor Volumes for DU145

Group	Animal ID	Phase										
		Pre-Dosing				Dosing				Recovery		
		9/24	9/27	9/31	9/3	9/4	9/8	9/11	9/15	9/18	9/22	9/25
Control	001	36.18	41.27	58.41	92.80	93.22	121.16	203.79	310.41	409.15	430.01	450.89
Control	002	45.05	35.99	64.98	85.50	101.89	138.42	228.62	327.76	473.14	524.45	595.87
Control	003	40.55	22.37	58.76	127.88	141.49	212.34	304.15	500.74	1,023.13	1,004.09	1,198.53
Control	004	17.56	36.41	65.73	151.20	159.33	227.23	288.73	338.70	576.83	526.30	1,027.50
Mean		36.98	34.02	64.47	111.87	119.22	174.25	275.94	475.93	821.91	743.95	819.42
Median		42.11	36.22	65.38	111.74	121.09	174.30	267.79	323.27	527.49	730.69	815.93
SD		13.67	8.12	20.55	20.53	24.32	53.70	80.81	302.77	278.25	311.67	351.88
SBT-100	005	33.80	23.32	35.67	93.37	89.21	101.30	145.67	336.92	325.85	474.31	498.63
SBT-100	006	59.44	41.00	54.21	96.56	121.39	148.44	206.10	357.62	391.62	518.25	586.67
SBT-100	007	42.30	30.11	77.00	144.00	145.78	116.06	106.70	248.10	318.24	454.70	526.83
SBT-100	008	69.37	50.19	71.23	116.28	118.70	134.16	147.52	330.22	423.46	604.72	790.96
Mean		51.23	37.40	59.75	111.23	118.77	137.39	151.50	328.22	354.14	513.91	591.63
Median		50.87	38.06	62.72	107.42	120.05	141.30	146.50	338.92	350.43	498.20	568.75
SD		16.13	11.25	19.90	25.16	23.17	16.75	40.38	59.97	51.64	68.65	131.51
% TIC		0.0	-26.9	29.1	78.8	81.7	55.4	42.1	79.6	56.9	69.9	74.8
p-value		0.225	0.643	0.747	0.979	0.889	0.239	0.034	0.372	0.116	0.197	0.291

[0154] In the PANC-1 tumor xenograft model, animals were randomized on day 22 post-inoculation with a mean (\pm SE) tumor size of: 78.74 ± 40.21 and 93.84 ± 36.31 for Groups 1 and 2, respectively. Mean body weights (\pm SE) at randomization were: 22.50 ± 1.47 and 24.23 ± 1.63 for Groups 1 and 2, respectively. Table 32 summarizes the mean body weights (\pm SE) for entire study. At last day of dosing (Day 14), the mean tumor size (\pm SE) for control group was 204.95 ± 178.90 versus 159.03 ± 28.01 for SBT-100 treated mice. Table 33 summarizes the tumor volumes (\pm SE) for entire study. At the time of termination (day 28) mean tumor size (\pm S.E.) was: 284.77 ± 288.88 and 203.02 ± 30.34 , for groups 1 and 2, respectively. Mean body weights (\pm SE) at termination were: 27.38 ± 1.07 and 26.23 ± 1.19 for Groups 1 and 2, respectively. At the end of the study, the mean tumor growth inhibition in the SBT-100 treated group was 41.78% ($p = 0.35$). Figure 9 illustrates the mean tumor volume. The tumor doubling times were 18.51 days versus 35.70 days for Group 1 and Group 2, respectively. The % treatment/control for Group 2 was 52.79.

TABLE 32
Mean Body Weights for Mice in PANC-1

Group	Animal ID	Phase						
		Dosing				Recovery		
		9/9	9/11	9/15	9/19	9/22	9/25	9/29
Control	001	26.50	26.60	25.90	27.10	25.70	26.10	27.20
Control	002	24.30	24.60	23.90	25.10	24.40	25.00	25.00
Control	003	27.60	26.50	26.90	26.20	26.10	27.50	28.20
Control	004	25.10	25.30	24.20	25.10	24.70	25.80	26.90
Mean		23.50	23.80	23.94	24.30	24.50	25.80	27.30
Median		25.80	25.90	25.90	25.65	25.20	26.30	27.05
SD		1.47	0.97	1.18	0.97	0.91	1.03	1.07
% Change		0.00	-0.39	-3.15	0.12	-2.41	1.85	4.33
SBT-100	005	22.60	22.80	21.40	22.50	22.00	22.50	24.00
SBT-100	006	26.00	25.10	24.90	25.70	25.10	25.40	27.10
SBT-100	007	23.10	22.30	22.40	22.70	23.10	23.50	25.70
SBT-100	008	25.20	25.00	25.00	25.40	26.20	25.40	27.00
Mean		24.23	23.80	23.48	24.08	24.25	24.30	26.23
Median		24.15	23.90	23.65	24.05	24.10	24.45	26.40
SD		1.63	1.48	1.07	1.71	1.89	1.28	1.19
% Change		0.00	-1.71	-3.14	-0.63	0.13	0.39	8.39

TABLE 33
Tumor Volumes for PANC-1

Group	Animal ID	Phase									
		Pre-Dosing			Dosing					Recovery	
		9/21	9/21	9/28	9/28	9/11	9/18	9/18	9/22	9/25	9/29
Control	001	54.91	58.79	94.22	94.37	94.69	122.00	135.77	206.74	220.21	223.91
Control	002	46.39	75.43	51.36	31.62	65.44	130.01	131.95	140.52	145.62	202.22
Control	003	0.00	27.56	57.30	55.60	55.77	107.02	143.23	140.55	168.69	187.27
Control	004	0.00	0.00	152.17	158.58	227.02	360.94	500.08	514.93	574.44	731.45
Mean		20.26	32.54	78.74	80.91	104.18	151.29	189.83	204.95	224.81	284.77
Median		23.19	42.15	88.11	87.99	97.23	126.86	145.64	173.65	194.50	213.96
SD		29.45	33.13	40.21	43.18	65.52	130.48	173.63	178.90	209.56	288.89
SBT-100	005	39.60	64.75	78.44	78.07	87.54	93.17	112.98	140.09	173.32	245.94
SBT-100	006	40.31	97.27	68.67	73.13	76.46	113.90	190.49	192.56	205.55	198.42
SBT-100	007	85.71	91.27	147.61	149.02	123.95	116.01	187.50	171.29	175.63	200.97
SBT-100	008	48.72	65.19	62.73	63.19	86.90	102.49	106.05	132.19	158.93	175.94
Mean		53.58	82.12	93.94	86.85	88.21	106.34	126.95	159.03	173.02	203.02
Median		44.51	59.97	78.69	80.62	81.68	107.09	121.73	155.93	174.80	195.19
SD		21.82	22.62	36.31	35.69	27.84	19.49	23.06	29.91	24.10	38.34
% T/C		0.0	0.0	42.6	44.2	27.4	34.4	39.0	49.7	51.0	52.6
p-value		0.174	0.110	0.927	0.917	0.296	0.272	0.285	0.360	0.343	0.355

EXAMPLE 12: EFFICACY OF ANTI-STAT3 BACTERIAL VHH13 (SEQ ID NO:3) SDAB IN THE ER+/PR+ (MCF-7) HUMAN BREAST TUMOR XENOGRAFT MODEL

[0155] This Example demonstrates the efficacy of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb in the MCF-7 human breast tumor xenograft model in nude mice.

[0156] Female athymic nude mice (CrI:NU(Ncr)-Foxn1^{nu}, Charles River) were twelve weeks old with a body weight (BW) range of 23.0 to 30.1 g on Day 1 of the study. The animals were fed and housed as described above.

[0157] MCF-7 human breast carcinoma cells were obtained and cultured as described above, and used for the mouse xenograph. Three days prior to tumor cell implantation, estrogen pellets (0.36 mg estradiol, 60-day release, Innovative Research of America, Sarasota, FL) were implanted subcutaneously between the scapulae of each test animal using a sterilized trocar.

[0158] The tumor cells used for implantation were harvested during log phase growth and resuspended in phosphate buffered saline (PBS) at a concentration of 1×10^8 cells/mL. On the day of implantation, each test mouse received 1×10^7 MCF-7 cells (0.1 mL cell suspension) implanted subcutaneously in the right flank and tumor growth was monitored as the average size approached the target range of 100-150 mm³. Twenty-one days later, designated as Day 1 of the study, the animals were sorted into two groups each consisting of four mice with individual tumor volumes ranging from 108 to 144 mm³ and group mean tumor volumes from 117 to 123 mm³.

[0159] Anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was provided as a pre-formulated ready to dose solution at a concentration 0.41867 mg/mL in 1 mL aliquots and were stored at -20 °C until needed. The 0.41867 mg/mL solution provided 1 mg/kg dosage in a dosing volume of 23.88 mL/kg. On each day of treatment, only needed vials of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb were thawed to room temperature. All leftover dosing suspensions were retained at 4 °C as needed for the

next dose.

[0160] Two groups of athymic nude mice were dosed according to the protocol shown in Table 34. All vehicle (control) and anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb doses were administered intraperitoneally (i.p.) three times daily, six hours apart for fourteen days, with two doses delivered on Day 1 and one dose delivered on the morning of Day 15 (tid x 14, first day 2 doses). The dosing volume for vehicle and anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was 0.478 mL per 20 grams of body weight (23.88 mL/kg) and was scaled to the body weight of each individual animal. Group 1 received the vehicle and served as the benchmark group for tumor engraftment and progression, as well as the control. Group 2 was given anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb at 1 mg/kg.

TABLE 34

Protocol Design for the Study					
Treatment Regimen					
Group	n	Agent	mg/kg	Route	Schedule
1	4	vehicle	-	ip	tid x 14 first Day 2 doses
2	4	VHH13	1	ip	tid x 14 first Day 2 doses

[0161] Tumors were measured twice weekly, and each animal was euthanized when its neoplasm reached the predetermined endpoint volume (1000 mm³) or at the end of the study, day 39, whichever came first. When a tumor reached the endpoint volume, the animal was documented as euthanized for tumor progression (TP), with the date of euthanasia. The time to endpoint (TTE) for each mouse was calculated by the following equation:

$$TTE = \frac{\log_{10}(\text{endpoint volume}) - b}{m}$$

where TTE is expressed in days, endpoint volume is expressed in mm³, b is the intercept, and m is the slope of the line obtained by linear regression of a log-transformed tumor growth data set. The data set consists of the first observation that exceeded the endpoint volume used in analysis and the three consecutive observations that immediately preceded the attainment of this endpoint volume. The calculated TTE is usually less than the TP date, the day on which the animal was euthanized for tumor size. Animals that did not reach the endpoint volume were assigned a TTE value equal to the last day of the study (D39). Any animal classified as having died from treatment-related (TR) causes was to be assigned a TTE value equal to the day of death. Any animal classified as having died from non-treatment-related (NTR) causes was to be excluded from TTE calculations.

[0162] Treatment efficacy was determined from tumor growth delay (TGD), which is defined as the increase in the median TTE, in days, for a treatment group compared to the control group:

$$TGD = T - C$$

The percent increase in the median TTE, relative to the control group, is

where:

$$\%TGD = \frac{T - C}{C} \times 100$$

T = median TTE for a treatment group, and

C = median TTE for the designated control group.

[0163] Treatment efficacy in each group may be indicated by the median tumor volume, MTV(n), which was defined as the median tumor volume on the last day of the study (D39) in the number of animals remaining (n) whose tumors had not attained the endpoint volume.

[0164] Treatment efficacy may also be determined from the incidence and magnitude of regression responses observed during the study. Treatment may cause partial regression (PR) or complete regression (CR) of the tumor in an animal. In a PR response, the tumor volume was 50% or less of its D1 volume for three consecutive measurements during the course of the study, and equal to or greater than 13.5 mm³ for one or more of these three measurements. In a CR response, the tumor volume was less than 13.5 mm³ for three consecutive measurements during the course of the study. Any animal with a CR response at the end of the study was additionally classified as a tumor-free survivor (TFS).

[0165] Animals were weighed daily for the first five days, then twice weekly for the remainder of the study. The mice were observed frequently for health and overt signs of any adverse treatment related TR side effects, and noteworthy clinical observations were recorded. Individual body weight loss was monitored per protocol, and any animal with weight loss exceeding 30% for one measurement, or exceeding 25% for three measurements, was to be euthanized for health as a TR death. If group mean body weight recovered, dosing may resume in that group, but at a lower dose or less frequent dosing schedule. Acceptable toxicity was defined as a group mean BW loss of less than 20% during the study and not more than one TR death among ten treated animals, or 10%. Any dosing regimen resulting in greater toxicity is considered above the maximum tolerated dose (MTD). A death was to be classified as TR if it was attributable to treatment side effects as evidenced by clinical signs and/or necropsy, or may also be classified as TR if due to unknown causes during the dosing period or within 14 days of the last dose. A death was classified as NTR if there was evidence that the death was related to the tumor model, rather than treatment-related. NTR deaths are further categorized as NTRa (due to accident or human error), NTRm (due to necropsy-confirmed tumor dissemination by invasion or metastasis), and NTRu (due to unknown causes).

[0166] Prism 6.07 (GraphPad) for Windows was employed for graphical analyses. Statistics were not employed due to small sample size.

[0167] A scatter plot was constructed to show TTE values for individual mice, by group; this plot shows NTR deaths, which were excluded from all other figures. Individual animal, group median and mean tumor volumes were plotted as functions of time. When an animal exited the study because of tumor size or TR death, its final recorded tumor volume was included with the data used to calculate the median volume at subsequent time points. A Kaplan-Meier plot was constructed to show the percentage of animals in each group remaining on study versus time. Tumor growth curves were truncated after two TR deaths occurred in the same group. Group mean BW changes over the course of the study were graphed as percent change, \pm SEM, from Day 1. Tumor growth and BW change curves were truncated after more than half the assessable mice in a group exited the study. Figure 10 illustrates the mean tumor volume in the study.

[0168] Table 35 provides the mean BW losses, TR and NTR deaths for the mice. Clinical signs were recorded when observed, as shown in Tables 36-38. No TR deaths occurred during the study. Bodyweight losses were variable, severe for one animal in each group, and resulted from estrogen

effects. Clinical observations including weight loss, enlarged uterine horns, and bladder crystals were present in both groups and were also attributable to estrogen effects. Estrogen toxicity resulted in two non-treatment related deaths in each group. The treatment evaluated in the study was acceptably tolerated.

TABLE 35
Response Summary

Group	n	Treatment Regimen					Median		MTV ₅₀		Regression			Mean BW		Deaths	
		Agent	mg/kg	qd	Route	Schedule	ITE	TC	OTCD	D ₅₀	PR	CR	TP ₅₀	Male		TR	NTR
1	10	0.001% E2	0	q	ip	BD x 14 First Day 2 dose	15.1	1	1	1	0	0	0	33.08 Day 22	0	0	2
2	10	0.001% E2	1	q	ip	BD x 14 First Day 2 dose	12.6	17	40	1	0	0	0	31.26 Day 22	0	0	2

TABLE 36
Body Weight

		Body Weight																			
		Group 1 Vehicle (0.001% E2 First Day 2 dose)																			
Date of Study	N	07/27/15	07/28/15	07/29/15	07/30/15	07/31/15	08/01/15	08/02/15	08/03/15	08/04/15	08/05/15	08/06/15	08/07/15	08/08/15	08/09/15	08/10/15	08/11/15	08/12/15	08/13/15	08/14/15	09/03/15
		Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV
1	10	27.50	2.80	10	28.00	2.50	10	28.50	2.20	10	29.00	2.00	10	29.50	1.80	10	30.00	1.60	10	30.50	1.40
2	10	26.00	2.50	10	26.50	2.20	10	27.00	2.00	10	27.50	1.80	10	28.00	1.60	10	28.50	1.40	10	29.00	1.20
3	10	30.00	3.00	10	30.50	2.80	10	31.00	2.60	10	31.50	2.40	10	32.00	2.20	10	32.50	2.00	10	33.00	1.80
4	10	23.00	2.00	10	23.50	1.80	10	24.00	1.60	10	24.50	1.40	10	25.00	1.20	10	25.50	1.00	10	26.00	0.80
Mean		26.7	2.78		27.6	2.55		28.2	2.22		28.8	2.00		29.4	1.78		30.0	1.56		30.6	1.34
STDEV		2.9	2.8		2.2	2.0		1.9	1.7		1.6	1.4		1.4	1.2		1.2	1.0		0.9	0.7
n		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

		Group 2 Vehicle (0.001% E2 First Day 2 dose)																			
Date of Study	N	07/27/15	07/28/15	07/29/15	07/30/15	07/31/15	08/01/15	08/02/15	08/03/15	08/04/15	08/05/15	08/06/15	08/07/15	08/08/15	08/09/15	08/10/15	08/11/15	08/12/15	08/13/15	08/14/15	09/03/15
		Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV	n	Mean	STDEV
1	10	28.90	2.90	10	29.00	2.80	10	29.10	2.70	10	29.20	2.60	10	29.30	2.50	10	29.40	2.40	10	29.50	2.30
2	10	25.90	2.50	10	26.00	2.40	10	26.10	2.30	10	26.20	2.20	10	26.30	2.10	10	26.40	2.00	10	26.50	1.90
3	10	27.10	2.70	10	27.20	2.60	10	27.30	2.50	10	27.40	2.40	10	27.50	2.30	10	27.60	2.20	10	27.70	2.10
4	10	27.60	2.70	10	27.70	2.60	10	27.80	2.50	10	27.90	2.40	10	28.00	2.30	10	28.10	2.20	10	28.20	2.10
Mean		27.3	2.73		27.4	2.66		27.5	2.59		27.6	2.52		27.7	2.45		27.8	2.38		27.9	2.31
STDEV		1.5	1.6		1.7	1.8		1.8	1.9		1.9	2.0		2.0	2.1		2.1	2.2		2.2	2.3
n		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE 37
Tumor Measurements

LIQUOR MEASUREMENT

[illegible]

TABLE 38
Tumor Volume

5.2 doses)					
7.5	08.20.15	08.24.15	08.27.15	08.31.15	09.03.15
2.5	25	29	32	36	39
	TV (num ³)	TV (num ³)	TV (num ³)	TV (num ³)	TV (num ³)
Ra on 08.01.15					
0.00	TP on 08.17.15				
8.7	100.8	TP on 08.20.15			
Ra on 08.03.15					
1.3	100.8				
6.8					
1. Day 2 doses)					
7.5	08.20.15	08.24.15	08.27.15	08.31.15	09.03.15
2.5	25	29	32	36	39
	TV (num ³)	TV (num ³)	TV (num ³)	TV (num ³)	TV (num ³)
30	864	1099	TP on 08.24.15		
405	500	550	864	1099	
on 07.30.15					
NRa on 08.08.15					
7.5	634.5	799.3	550	864	1098.5
2.5	229.5	299.3	2.5		
37222111					

Tumor Volume										
Group 1: vehicle (p.tid x 14 first Day										
Date Day of Study A#	07/27/15 TV (mm ³)	14 07/30/15 TV (mm ³)	08/03/15 8 TV (mm ³)	08/06/15 11 TV (mm ³)	08/10/15 13 TV (mm ³)	08/13/15 15 TV (mm ³)	08/17/15 18 TV (mm ³)	08/20/15 21 TV (mm ³)	08/23/15 24 TV (mm ³)	08/27/15 28 TV (mm ³)
1	108	196	384	527	650	650	650	650	650	650
2	108	221	405	600	726	726	726	726	726	726
3	113	245	405	600	726	726	726	726	726	726
4	126	270	405	600	726	726	726	726	726	726
Mean	122.6	232.8	394.5	563.3	688	688	688	688	688	688
SEM	8.1	15.8	10.5	36.8	38	38	38	38	38	38
n	4	4	4	4	4	4	4	4	4	4
Group 2: VHH13 (1 mg/kg p.tid x 14 first Day										
Date Day of Study A#	07/27/15 TV (mm ³)	14 07/30/15 TV (mm ³)	08/03/15 8 TV (mm ³)	08/06/15 11 TV (mm ³)	08/10/15 13 TV (mm ³)	08/13/15 15 TV (mm ³)	08/17/15 18 TV (mm ³)	08/20/15 21 TV (mm ³)	08/23/15 24 TV (mm ³)	08/27/15 28 TV (mm ³)
1	108	196	320	405	405	405	405	405	405	405
2	108	126	196	196	283	283	283	283	283	283
3	126	144	245	320	405	405	405	405	405	405
4	126	144	245	320	405	405	405	405	405	405
Mean	117	155.3	253.7	307	346.5	346.5	346.5	346.5	346.5	346.5
SEM	5.2	21	36.1	60.7	58.5	58.5	58.5	58.5	58.5	58.5
n	4	3	3	3	3	3	3	3	3	3

[0169] Because two out of the four mice in the control group and also in the treatment group died of estrogen toxicity, no statistical conclusion could be determined. With the data available, the median tumor growth and mean tumor volume were reduced in the treatment group when compared to the control group. This difference was present during the 14 days of treatment but also to day 25 of the study. It took the control group 25 days to reach a tumor volume of 1000 mm³, whereas the treatment group took 36 days to reach a tumor volume of 1000 mm³. This suggests that anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb slows the growth of MCF-7 tumor *in vivo*. Throughout the study both the control group and the treatment group maintained similar weights. This suggests that the anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb did not cause toxicity with respect to weight loss.

EXAMPLE 13: TREATMENT OF HUMAN HER2+ (BT474) BREAST CANCER WITH ANTI-STAT3 BACTERIAL VHH13 (SEQ ID NO:3) SDAB IN XENOGRAFT MICE

[0170] In this Example, the efficacy of anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was determined in the BT474 human breast tumor xenograft in CB.17 SCID mice.

[0171] Two groups of 8-12 week old CB.17 SCID mice containing xenografts of 1 mm³ BT474 tumor fragments in their flank were treated according to the protocol shown in Table 39 when the tumors reached an average size of 100-150 mm³. All vehicle (PBS control) and anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb (shown in Table 39 as SB-01) doses were administered intraperitoneally (i.p.) three times daily, six hours apart for fourteen days, with two doses delivered on Day 1 (tid x 14, first day 2 doses). The dosing volume for vehicle and anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was 0.478 mL per 20 grams of body weight (23.88 mL/kg) and was scaled to the body weight of each individual animal. Group 1 received the vehicle and served as the benchmark group for tumor engraftment and progression, as well as the control. Group 2 was given anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb at 1 mg/kg.

TABLE 39
Study protocol

Gr.	N	Regimen 1				
		Agent	Vehicle	mg/kg	Route	Schedule
1 [#]	4	vehicle		-	ip	tid x 14 first day 2 doses
2	4	SB-01		1	ip	tid x 14 first day 2 doses

[0172] During the first 14 days of the study, the treatment group received anti-STAT3 B VHH13 and the control group only received the vehicle. As shown in Table 40, during this time, the treatment group maintained and gained weight throughout the study while the control group had lower weights throughout the study. This suggests that the treatment group did not experience toxicity from anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb with respect to weight loss. Both groups mean tumor volume and median tumor volume were similar, and exactly the same on day 15 of the study. On day 59 of the study, both groups reached a tumor volume of 700 cubic mm³. This suggests that the anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb did not reduce the growth of BT474 tumors in vivo when compared to the control group. Figure 11 illustrates the group mean tumor volume.

TABLE 40

BT474 Response Summary

Group	n	Treatment Regimen 1					Median TTE	T-C	%TGD	Stat Sign	MTV (n), Day 60	PR	CR	TFS	BW Nadir	TR	NTRn	NTR
		Agent	Vehicle	mg/kg	Route	Schedule												
1 [#]	4	vehicle		-	ip	tid x 14 first day 2 doses	40.2	--	-		266 (2)	0	0	0	-9.1% (3)	0	0	0
2	4	SB-01		1	ip	tid x 14 first day 2 doses	60.0	10.8	22		550 (3)	0	0	0	--	0	0	0

- Control Group

EXAMPLE 14: PRODUCTION OF MOUSE MONOCLONAL ANTIBODY DIRECTED AGAINST ANTI-STAT3 BACTERIAL VHH13 (SEQ ID NO:3) SDAB

[0173] In this Example, mouse monoclonal antibodies were generated towards the sdAb of the invention. The animals used were BALB/c female mice, 8-10 week. A water-soluble adjuvant was used (CBL). The HAT and the HT used were from Sigma-Aldrich.

[0174] Anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb was used to immunize three mice and make hybridoma cell lines. The mice were immunized three times each with water-soluble adjuvant. In one mouse, the serum titer reached 1/51200. The mouse was sacrificed and hybridoma cell lines were made by fusing spleen cells with myeloma cell line Sp2/0.

[0175] The fused cells were seeded into 96 well plates by limited dilution. The fused cells were cultured in the presence of HAT, and 651 single clones were tested. Of the 651 single clones, 27 positive clones were identified that specifically bound to anti-STAT3 bacterial VHH13 (SEQ ID NO:3) sdAb antigen.

EXAMPLE 15: CYTOTOXICITY OF KRAS (G12D) SINGLE DOMAIN ANTIBODIES ON PANC-1 HUMAN PANCREATIC CANCER CELLS

[0176] This Example demonstrates the anti-proliferative effects of the anti-KRAS (G12D) (SEQ ID NO:2) sdAb using the human pancreatic cancer cell line PANC-1. For the experiments, the PANC-1 cells were grown until they reached a confluency of 90%. At that time, proliferation studies were carried out using the MTT assay as described above.

[0177] The anti-proliferative properties of anti-KRAS (G12D) (SEQ ID NO:2) sdAB on PANC-1 cells three days post treatment are shown in Table 41. PANC-1 cells treated with the anti-KRAS (G12D) (SEQ ID NO:2) sdAb showed an average growth inhibition of 19.9 and 37.7 at 50.0 and 100 µg/ml, respectively.

TABLE 41

Anti-proliferative Actions of Anti-KRAS (G12D) (SEQ ID NO:2) sdAb on PANC-1 Cancer Cells		
	Mean Abs ± SE	% Inhibition
control	0.281± 0.017	
50 µg/ml	0.225± 0.006	19.9
100 µg/ml	0.175± 0.016	37.7

[0178] Thus, the anti-KRAS (G12D) (SEQ ID NO:2) sdAb showed dose-dependent growth inhibition in the PANC-1 human pancreatic cancer cells.

EXAMPLE 16: IN VITRO GROWTH INHIBITION BY TNF-ALPHA SDAB

[0179] This Example demonstrates the method development to determine TNF-alpha concentration and evaluation of the inhibition of TNF-alpha function. The concentration of TNF-alpha required to show measurable modulation of activity in the U937 human lung lymphoblast cell line was evaluated by quantitation of the ATP present, which signals the presence of metabolically active cells using Promega's Cell Titer-Glo® Luminescent Cell Viability assay.

[0180] The U937 cells were seeded in a clear polystyrene 96-well microculture plate (Corning® Costar® 96-well flat bottom plate, Cat. #3997) in a total volume of 90 µL/well. After 24 hours of incubation in a humidified incubator at 37°C with 5% CO₂ and 95% air, 5 µL of 20X, serially diluted TNF-alpha in growth medium was added to each well in duplicate (10 pt dose response, highest concentration 20 ng/mL). Additionally, 5 µL of 20X, diluted staurosporine in growth medium was added to each well in duplicate (concentration 1 nM).

[0181] After 24 hours of culture in the presence of test agents, the concentration of compound required to show measurable modulation of TNF-alpha activity in the U937 cell line as evaluated by quantitation of the ATP present. Percent cell growth was calculated relative to untreated control wells. All tests were performed in duplicate at each concentration level.

[0182] The EC₅₀ value for the test agents was estimated using Prism 6.05 by curve-fitting the data using the following four parameter-logistic equation:

$$Y = \frac{Top - Bottom}{1 + e^{-\frac{(X - EC_{50})}{HillSlope}}} + Bottom$$

$$1 + \left(\frac{Y - \text{Bottom}}{\text{Top} - \text{Bottom}} \right)^{1/n}$$

where Top is the maximal % of control absorbance, Bottom is the minimal % of control absorbance at the highest agent concentration, Y is the % of control absorbance, X is the agent concentration, IC₅₀ is the concentration of agent that inhibits cell growth by 50% compared to the control cells, and n is the slope of the curve.

[0183] Figures 12 and 13 demonstrate that TNF-alpha is cytotoxic to the U937 cells. The IC₅₀ for TNF-alpha against U937 is 95.10pg/ml. The TNF-alpha curve shows a dose titration killing effect.

[0184] Figure 14 demonstrates that TNF-alpha cytotoxicity against U937 is inhibited by the three different anti-TNF-alpha VHHs. When anti-TNF-alpha VHH62 (SEQ ID NO:47) sdAb, anti-TNF-alpha VHH 66 (SEQ ID NO:45) sdAb, and anti-TNF-alpha VHH69 (SEQ ID NO:46) sdAb were incubated with a constant dose of TNF-alpha, at EC₅₀, all three anti-TNF-alpha VHHs inhibit killing of U937 by TNF-alpha. The IC₅₀ of anti-TNF-alpha VHH62 (SEQ ID NO:47) sdAb was approximately 713.6ug/ml. The IC₅₀ of anti-TNF-alpha VHH69 (SEQ ID NO:46) sdAb was greater than 208.055ug/ml. The IC₅₀ of anti-TNF-alpha VHH66 (SEQ ID NO:45) sdAb could not be determined because it completely inhibited the cytotoxicity of TNF-alpha from concentrations of about 1X10² ug/ml to 1 X 10² ug/ml of anti-TNF-alpha VHH66 (SEQ ID NO:45) sdAb. In this concentration range of anti-TNF-alpha VHH66 (SEQ ID NO:45) sdAb, there is an increase in U937 cell growth, and thus complete inhibition of TNF-alpha activity.

SEQUENCE LISTING

[0185]

<110> Singh, Sunanda

<120> Single Domain Antibodies Against Intracellular Antigens

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Cys Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Lys Arg Glu Ala Val
          35          40          45

Ala Arg Ile Val Arg Asp Gly Ala Thr Gly Tyr Ala Asp Tyr Val Lys
          50          55          60

Gly Arg Phe Thr Ile Ser Arg Asp Ser Ala Lys Asn Thr Leu Tyr Leu
65          70          75          80

Gln Met Asn Arg Leu Ile Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
          85          90          95

Ala Asp Leu Pro Pro Gly Cys Leu Thr Gln Ala Ile Trp Asn Phe Gly
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Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
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Phe Ser Ile Ser Arg Asp Tyr Val Lys Asn Ala Val Tyr Leu Gln Met
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Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val Ala Ala Leu
35 40 45

Ser Ser Arg Gly Phe Ala Gly His Tyr Thr Asp Ser Val Lys Gly Arg
50 55 60

Phe Ser Ile Ser Arg Asp Tyr Val Lys Asn Ala Val Tyr Leu Gln Met
65 70 75 80

Asn Thr Val Lys Pro Glu Asp Ala Ala Met Tyr Tyr Cys Ala Ala Arg
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20

25

30

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35 40 45

Ser Ser Arg Gly Phe Ala Gly His Tyr Thr Asp Ser Val Lys Gly Arg
50 55 60

Phe Ser Ile Ser Arg Asp Tyr Val Lys Asn Ala Val Tyr Leu Gln Met
65 70 75 80

Asn Thr Val Lys Pro Glu Asp Ala Ala Met Tyr Tyr Cys Ala Ala Arg
85 90 95

Glu Gly Trp Glu Cys Gly Glu Thr Trp Leu Asp Arg Thr Ala Gly Gly
100 105 110

His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 9

<211> 126

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 9

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Thr Tyr Thr Gly Cys Met Gly
20 25 30

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val Ala Ala Leu
35 40 45

Ser Ser Arg Gly Phe Ala Gly His Tyr Thr Asp Ser Val Lys Gly Arg
50 55 60

Phe Ser Ile Ser Arg Asp Tyr Val Lys Asn Ala Val Tyr Leu Gln Met
65 70 75 80

Asn Thr Val Lys Pro Glu Asp Ala Ala Met Tyr Tyr Cys Ala Ala Arg
85 90 95

Glu Gly Trp Glu Cys Gly Glu Thr Trp Leu Asp Arg Thr Ala Gly Gly
100 105 110

His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 10

<211> 126

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 10

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Thr Tyr Thr Gly Cys Met Gly
 20 25 30

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val Ala Ala Leu
 35 40 45

Ser Ser Arg Gly Phe Ala Gly His Tyr Thr Asp Ser Val Lys Gly Arg
 50 55 60

Phe Ser Ile Ser Arg Asp Tyr Val Lys Asn Ala Val Tyr Leu Gln Met
 65 70 75 80

Asn Thr Val Lys Pro Glu Asp Ala Ala Met Tyr Tyr Cys Ala Ala Arg
 85 90 95

Glu Gly Trp Glu Cys Gly Glu Thr Trp Leu Asp Arg Thr Ala Gly Gly
 100 105 110

His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

<210> 11

<211> 126

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 11

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Thr Tyr Thr Gly Cys Met Gly
 20 25 30

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val Ala Ala Leu
 35 40 45

Ser Ser Arg Gly Phe Ala Gly His Tyr Thr Asp Ser Val Lys Gly Arg
 50 55 60

Phe Ser Ile Ser Arg Asp Tyr Val Lys Asn Ala Val Tyr Leu Gln Met
 65 70 75 80

Asn Thr Val Lys Pro Glu Asp Ala Ala Met Tyr Tyr Cys Ala Ala Arg
 85 90 95

Glu Gly Trp Glu Cys Gly Glu Thr Trp Leu Asp Arg Thr Ala Gly Gly
 100 105 110

His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 12

<211> 126

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 12

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Thr Tyr Thr Gly Cys Met Gly
20 25 30

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val Ala Ala Leu
35 40 45

Ser Ser Arg Gly Phe Ala Gly His Tyr Thr Asp Ser Val Lys Gly Arg
50 55 60

Phe Ser Ile Ser Arg Asp Tyr Val Lys Asn Ala Val Tyr Leu Gln Met
65 70 75 80

Asn Thr Val Lys Pro Glu Asp Ala Ala Met Tyr Tyr Cys Ala Ala Arg
85 90 95

Glu Gly Trp Glu Cys Gly Glu Thr Trp Leu Asp Arg Thr Ala Gly Ser
100 105 110

His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 13

<211> 126

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 13

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Thr Tyr Thr Gly Cys Met Gly
20 25 30

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val Ala Ala Leu
35 40 45

Ser Ser Arg Gly Phe Ala Gly His Tyr Thr Asp Ser Val Lys Gly Arg
50 55 60

Phe Ser Ile Ser Arg Asp Tyr Val Lys Asn Ala Val Tyr Leu Gln Met
65 70 75 80

Asn Thr Val Lys Pro Glu Asp Ala Ala Met Tyr Tyr Cys Ala Ala Arg
85 90 95

Glu Gly Trp Glu Cys Gly Glu Thr Trp Leu Asp Arg Thr Ala Gly Gly
100 105 110

His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 14

<211> 126

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 14

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Thr Tyr Thr Gly Cys Met Gly
20 25 30

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val Ala Ala Leu
35 40 45

Ser Ser Arg Gly Phe Ala Gly His Tyr Thr Asp Ser Val Lys Gly Arg
50 55 60

Phe Ser Ile Ser Arg Asp Tyr Val Lys Asn Ala Val Tyr Leu Gln Met
65 70 75 80

Asn Thr Val Lys Pro Glu Asp Ala Ala Met Tyr Tyr Cys Ala Ala Arg
85 90 95

Glu Gly Trp Glu Cys Gly Glu Thr Trp Leu Asp Arg Thr Ala Gly Gly
100 105 110

His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 15

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 15

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 16

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 16

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Asn Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 17

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 17

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
 20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
 35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
 100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

<210> 18

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 18

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
 20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
 35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
 100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

115 120 125

<210> 19
 <211> 127
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Camelid

<400> 19
 Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
 20 25 30
 Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
 35 40 45
 Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95
 Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
 100 105 110
 Met Tyr Asn Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

<210> 20
 <211> 127
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Camelid

<400> 20
 Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
 20 25 30
 Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
 35 40 45
 Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr

Asp Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 21

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 21

His Val Gln Leu Val Glu Ser Glu Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 22

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 22

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 23

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 23

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 24

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 24

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 25

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 25

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser

- 115 - - 120 125
 <210> 26
 <211> 127
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Camelid

 <400> 26
 His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15

 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
 20 25 30

 Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
 35 40 45

 Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

 Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
 65 70 75 80

 Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

 Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
 100 105 110

 Met Tyr Asn Ser Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
 115 120 125

<210> 27
 <211> 127
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Camelid

<400> 27
 His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15

 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
 20 25 30

 Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
 35 40 45

 Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

 Lys Gly Arg Phe Thr Ile Ser Gln Asn Asn Thr Lys Asn Thr Leu Tyr

Tyr Val Arg Phe Thr Ser Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 28

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 28

His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser
20 25 30

Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr
100 105 110

Met Tyr Asn Ser Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 29

<211> 127

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 29

His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ala Asn Gly Gly Arg Ser

20	25	30
Cys Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg Glu Gly Val		
35	40	45
Ser Gly Ile Ser Thr Gly Gly Leu Ile Thr Tyr Tyr Ala Asp Ser Val		
50	55	60
Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Thr Lys Asn Thr Leu Tyr		
65	70	80
Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys		
85	90	95
Ala Thr Ser Arg Phe Asp Cys Tyr Arg Gly Ser Trp Phe Asn Arg Tyr		
100	105	110
Met Tyr Asn Ser Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser		
115	120	125

<210> 30

<211> 378

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 30

gatgtgcagc tgggtggagtc tggggggaggc tcggtgcagg ctggaggctc tctgagactc	60
tcctgtgtag cctctacata caccggctgc atgggctggt tccgccaggc tcctggaaag	120
gagcgcgagg gagtgcgagc tcttagtagc cgtgggttttg ccgggcacta taccgactcc	180
gtgaagggcc gattctccat ctcccagac tacgtcaaga atgcggtgta tctgcaaata	240
aacactgtga aacctgagga cgctgccatg tactactgtg cagcacggga gggatgggag	300
tgcggtgaga cctggttga cgggaccgcc gggggccata cctactgggg ccaggggacc	360
caggtcaccg tctcctca	378

<210> 31

<211> 378

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 31

caggtgcagc tgggtggagtc tggggggaggc tcggtgcagg ctggaggctc tctgagactc	60
tcctgtgtag cctctacata caccggctgc atgggctggt tccgccaggc tcctggaaag	120
gagcgcgagg gagtgcgagc tcttagtagc cgtgggttttg ccgggcacta taccgactcc	180
gtgaagggcc gattctccat ctcccagac tacgtcaaga atgcggtgta tctgcaaata	240
aacactgtga aacctgagga cgctgccatg tactactgtg cagcacggga gggatgggag	300
tgcggtgaga cctggttga cgggaccgcc gggggccata cctactgggg ccaggggacc	360

tcgggtgcagc tgggtggagtc tgggggaggc tcgggtgcagg ctggaggggc tctgagactc 360
ctgggtcaccg tctcctca 378

<210> 32

<211> 381

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 32

cagggtgcagc tgggtggagtc tgggggaggc tcgggtgcagg ctggaggggc tctgagactc 60
tcctgtgcag cctctggagc caatgggtgt cggagctgca tgggctggtt ccgccaggtt 120
ccagggaagg agcgcgaggg ggtttctggt atttcaaccg gtggtcttat tacatactat 180
gccgactccg tgaaggggccg attcaccatc tccaagaca acaccaagaa cacgctgtat 240
ctgcaaatga acagcctgaa acctgaggac actgccatgt actactgtgc gacgagtcgg 300
tttgactgct atagaggctc ttggttcaac cgatatatgt ataacagttg gggccagggg 360
accctggtca ccgtctctc a 381

<210> 33

<211> 381

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 33

catgtgcagc tgggtggagtc tgggggaggc tcgggtgcagg ctggaggggc tctgagactc 60
tcctgtgcag cctctggagc caacgggtgt cggagctgca tgggctggtt ccgccaggtt 120
ccagggaagg agcgcgaggg ggtttctggt atttcaaccg gtggtcttat tacatactat 180
gccgactccg tgaaggggccg attcaccatc tccaagaca acaccaagaa cacgctgtat 240
ctgcaaatga acagcctgaa acctgaggac actgccatgt actactgtgc gacgagtcgg 300
tttgactgct atagaggctc ttggttcaac cgatatatgt ataacagttg gggccagggg 360
accaggtca ctgtctctc a 381

<210> 34

<211> 381

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 34

gatgtgcagc tgggtggagtc tgggggaggc tcgggtgcagg ctggaggggc tctgagactc 60
tcctgtgcag cctctggagc caatgggtgt cggagctgca tgggctggtt ccgccaggtt 120
ccagggaagg agcgcgaggg ggtttctggt atttcaaccg gtggtcttat tacatactat 180

gcccactccg tgaagggccg attcaccatc tcccaagaca acaccaagaa cacgctgtat 240
ctgcaaatga acagcctgaa acctgaggac actgccatgt actactgtgc gacgagtcgg 300
tttgactgct atagaggctc ttggttcaac cgatatatgt ataacagttg gggccagggg 360
accctggtca ccgtctcttc a 381

<210> 35

<211> 378

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 35

caggtgcagc tggtaggagtc tgggggaggc tcggtgcagg ctggaggctc tctgagactc 60
tcctgtgtag cctctacata caccggctgc atgggctggt tccgccaggc tcctggaaag 120
gagcgcgagg gagtgcgagc tcttagcagc cgtggttttg ccgggcacta taccgactcc 180
gtgaagggcc gattctccat ctcccagac tacgtcaaga atgcggtgta tctgcaaatg 240
aacactgtga aacctgagga cgctgccatg tactactgtg cagcacggga ggatgggag 300
tgcggtgaga cctggttga ccggaccgcc gggagccata cctactgggg ccaggggacc 360
ctggtcaccg tctctca 378

<210> 36

<211> 378

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 36

gaggtgcagc tggtaggagtc tgggggaggc tcggtgcagg ctggaggctc tctgagactc 60
tcctgtgtag cctctacata caccggctgc atgggctggt tccgccaggc tcctggaaag 120
gagcgcgagg gagtgcgagc tcttagtagc cgtggttttg ccgggcacta taccgactcc 180
gtgaagggcc gattctccat ctcccagac tacgtcaaga atgcggtgta tctgcaaatg 240
aacactgtga aacctgagga cgctgccatg tactactgtg cagcacggga ggatgggag 300
tgcggtgaga cctggttga ccgaaccgcc gggggccata cctactgggg ccaggggacc 360
ctggtcaccg tctctca 378

<210> 37

<211> 381

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 37

gaggtgcagc tgggtggagtc tgggggaggc tcggtgcagg ctggagggtc tctgagactc	60
tcctgtgcag cctctggagc caatggtggt cggagctgca tgggctggtt ccgccaggtt	120
ccagggaagg agcgcgaggg gggttctggt atttcaaccg gtggtcttat tacatactat	180
gccgactccg tgaagggtcg attcaccatc tccaagaca acaccaagaa cacgctgtat	240
ctgcaaatga gcagcctgaa acctgaggac actgccatgt actactgtgc gacgagtcgg	300
tttgactgct atagaggctc ttggttcaac cgatatatgt ataacagttg gggccagggg	360
acccaggtca ccgtctcctc a	381

<210> 38

<211> 378

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 38

catgtgcagc tgggtggagtc tggggggggc tcggtgcagg ctggagggtc tctgagactc	60
tcctgtgtag cctctacata caccggctgc atgggctggt tccgccaggc tcctggaaag	120
gagcgcgagg gagtgcagc tcttagtagc cgtgggtttg ccgggcacta taccgactcc	180
gtgaagggcc gatttcccat ctcccagac tacgtcaaga atgcggtgta tctgcaaatg	240
aacactgtga aacctgagga cgctgccatg tactactgtg cagcacggga gggatgggag	300
tgcggtgaga cctggttga cgggaccgcc gggggccata cctactgggg ccaggggacc	360
caggtcaccg tctcctca	378

<210> 39

<211> 381

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 39

gatgtgcagc tgggtggagtc tgggggaggc tcggtgcagg ctggagggtc tctgagactc	60
tcctgtgcag cctctggagc caatggtggt cggagctgca tgggctggtt ccgccaggtt	120
ccagggaagg agcgcgaggg gggttctggt atttcaaccg gtggtcttat tacatactat	180
gccgactccg tgaagggccg attcaccatc tccaagaca acaccaagaa cacgctgtat	240
ctgcaaatga acagcctgaa acctgaggac actgccatgt actactgtgc gacgagtcgg	300
tttgactgct atagaggctc ttggttcaac cgatatatgt ataacagttg gggccagggg	360
acccaggtca ccgtctcctc a	381

<210> 40

<211> 381

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 40

caggtgcagc tggaggagtc tgggggaggc tcggtgcagg ctggagggtc tctgagactc	60
tcctgtgcag cctctggagc caatggtggt cggagctgca tgggctggtt ccgccagggt	120
ccagggaagg agcgcgaggg ggtttctggt atttcaaccg gtggtcttat tacatactat	180
gccgactccg tgaagggccg attcaccatc tccaagaca acaccaagaa cacgctgtat	240
ctgcaaatga acagcctgaa acctgaggac actgccatgt actactgtgc gacgagtcgg	300
tttgactgct atagaggctc ttggttcaac cgatatatgt ataacagttg gggccagggg	360
accaggtca ccgtctcctc a	381

<210> 41

<211> 378

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 41

gatgtgcagc tggaggagtc tgggggaggc tcggtgcagg ctggagactc tctgagactc	60
tcctgtgtag cctctacata caccggctgc atgggctggt tccgccaggc tcctggaaag	120
gagcgcgagg gagtgcgagc tcttagtagc cgtggttttg ccgggcacta taccgactcc	180
gtgaagggcc gattctccat ctcccagac tacgtcaaga atgcggtgta tctgcaaatg	240
aacactgtga aacctgagga cgctgccatg tactactgtg cagcacggga gggatgggag	300
tgcggtgaga cctggttga ccggaccgcc gggggccata cctactgggg ccaggggacc	360
ctggtcactg tctcctca	378

<210> 42

<211> 378

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 42

gtgcagctgg tggagtctgg gggaggctcg gtgcaggctg gagggctctt gagactctcc	60
tgtgcagcct ctggagccaa tgggtggtcgg agctgcatgg gctggttccg ccaggttcca	120
gggaaggagc gtgagggggg ttctggtatt tcaaccggtg gtcttattac atactatgcc	180
gactccgtga agggccgatt caccatctcc caagacaaca ccaagaacac gctgtatctg	240
caaatgaaca gcctgaaacc tgaggacact gccatgtact actgtgcgac ggtcggttt	300
gactgctata gaggtctctt gttcaaccga tatatgtata acagttgggg ccaggggacc	360
ctggtcactg tctcctca	378

<210> 43

<211> 378

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 43

gtgcagctgg tggagtctga gggaggctcg gtgcaggctg gagggctctt gagactctcc	60
tgtgcagcct ctggagccaa tggtaggtcgg agctgcatgg gctgggtccg ccagggtcca	120
gggaaggagc gcgaggggggt ttctggtatt tcaaccgggtg gtcttattac atactatgcc	180
gactccgtga agggccgatt caccatctcc caagacaaca ccaagaacac gctgtatctg	240
caaatgaaca gcctgaaacc tgaggacact gccatgtact actgtgcgac gagtcgggtt	300
gactgctata gaggtctctg gttcaaccga tatatgtata acagttgggg ccaggggacc	360
ctggtcaccg tctcctca	378

<210> 44

<211> 378

<212> DNA

<213> Artificial Sequence

<220>

<223> Camelid

<400> 44

gtgcagctgg tggagtctgg gggaggctcg gtgcaggctg gagggctctt gagactctcc	60
tgtgcagcct ctggagccaa tggtaggtcgg agctgcatgg gctgggtccg ccagggtcca	120
gggaaggagc gcgaggggggt ttctggtatt tcaaccgggtg gtcttattac atactatgcc	180
gactccgtga agggccgatt caccatctcc caagacaaca ccaataacac gctgtatctg	240
caaatgaaca gcctgaaacc tgaggacact gccatgtact actgtgcgac gagtcgggtt	300
gactgctata gaggtctctg gttcaaccga tatatgtata acagttgggg ccaggggacc	360
ctggtcactg tctcctca	378

<210> 45

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 45

His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Gly	
1 5 10 15	
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr	
20 25 30	
Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val	
35 40 45	

Ala Thr Ile Asp Ile Asp Gly Leu Thr Thr His Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
100 105 110

Arg Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 46

<211> 124

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 46

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Leu Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Thr Ser Arg Tyr Asn
20 25 30

Tyr Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ala Thr Ile Gly Thr Ala Ser Gly Ser Ala Asp Tyr Tyr Gly Ser Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Gln Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Ala Arg Thr Tyr Gly Thr Ile Ser Leu Thr Pro Ser Asp Tyr Arg
100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 47

<211> 129

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 47

Ala Thr Ile Asp Ile Asp Gly Leu Thr Thr His Ala Asp Ser Val Lys

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Pro Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Ser Trp Ala Gln Gly Thr Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 48

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 48

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
20 25 30

Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val
35 40 45

Ala Thr Ile Asp Ile Asp Gly Gln Thr Thr His Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
100 105 110

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 49
 <211> 123
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Camelid

<400> 49
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
 20 25 30
 Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val
 35 40 45
 Ala Thr Ile Asp Ile Asp Gly Gln Thr Thr His Ala Asp Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Val Lys Asn Thr Leu Ser Leu
 65 70 75 80
 Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
 85 90 95
 Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
 100 105 110
 Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
 115 120

<210> 50
 <211> 123
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Camelid

<400> 50
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
 20 25 30
 Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val
 35 40 45
 Ala Thr Ile Asp Ile Asp Gly Leu Thr Thr His Ala Asp Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
 65 70 75 80
 Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
 85 90 95

85

90

95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
 100 105 110

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
 115 120

<210> 51

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 51

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
 20 25 30

Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val
 35 40 45

Ala Thr Ile Asp Ile Asp Gly Gln Thr Thr His Ala Asp Ser Val Lys
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
 65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
 85 90 95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
 100 105 110

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
 115 120

<210> 52

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 52

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
 20 25 30

Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val
 35 40 45

Ala Thr Ile Asp Ile Asp Gly Gln Thr Thr His Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
100 105 110

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 53

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 53

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
20 25 30

Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val
35 40 45

Ala Thr Ile Asp Ile Asp Gly Leu Thr Thr His Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
100 105 110

Arg Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 54

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 54

Asp Val Gln Leu Val Glu Ser Glv Glv Glv Ser Val Glu Ala Glv Glv

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1      5      10      15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
    20          25          30

Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val
    35          40          45

Ala Thr Ile Asp Ile Asp Gly Gln Thr Thr His Ala Asp Ser Val Lys
    50          55          60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
    65          70          75          80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
    85          90          95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
    100          105          110

Arg Gly Gln Gly Thr Leu Val Thr Val Ser Ser
    115          120

<210> 55
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> Camelid

<400> 55
His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Gly
1      5      10      15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
    20          25          30

Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val
    35          40          45

Ala Thr Ile Asp Ile Asp Gly Leu Thr Thr His Ala Asp Ser Val Lys
    50          55          60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
    65          70          75          80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
    85          90          95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
    100          105          110

Arg Gly Gln Gly Thr Leu Val Thr Val Ser Ser
    115          120

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<210> 56

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 56

His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
20 25 30

Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Glu Arg Glu Gly Val
35 40 45

Ala Thr Ile Asp Ile Asp Gly Leu Ala Thr His Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
100 105 110

Arg Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 57

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 57

His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Glu Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
20 25 30

Cys Met Gly Trp Phe Arg Gln Ala Asp Arg Lys Glu Arg Glu Gly Val
35 40 45

Ala Thr Ile Asp Ile Asp Gly Gln Thr Thr His Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
 100 105 110

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
 115 120

<210> 58

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 58

His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Arg Tyr Ala Ala Tyr
 20 25 30

Cys Met Gly Trp Phe Arg Gln Ala Asp Gly Lys Val Arg Glu Gly Val
 35 40 45

Ala Thr Ile Asp Ile Asp Gly Gln Thr Thr His Ala Asp Ser Val Lys
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Ser Leu
 65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
 85 90 95

Ala Asp Arg Asp Arg Cys Gly Ser Ile Trp Thr Tyr Ala Tyr Lys Tyr
 100 105 110

Arg Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 59

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 59

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Asp Ser Phe Gly
 20 25 30

Val Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
 35 40 45

Ala Ala Val Tyr Arg Arg Ala Gly Asp Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Ser Ala Met Tyr Tyr Cys
85 90 95

Ala Ala Arg Thr Tyr Gly Ser Val Ser Ser Trp Thr Gly Tyr Lys Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 60

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 60

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Asp Ser Phe Gly
20 25 30

Val Met Ala Trp Phe Arg Gln Thr Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ala Ala Val Tyr Arg Arg Ala Gly Asp Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Ser Ala Met Tyr Tyr Cys
85 90 95

Ala Ala Arg Thr Tyr Gly Ser Val Ser Ser Trp Thr Gly Tyr Lys Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 61

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 61

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Val Gly Gly
1 5 10 15

Ser Leu Thr Leu Ser Cys Ala Val Ser Gly Tyr Thr Asp Ser Tyr Gly
20 25 30

Val Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ala Ser Ile Tyr Arg Asn Ser Gly Ile Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Leu
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Ser Ala Thr Tyr Tyr Cys
85 90 95

Ala Val Arg Ser Phe Gly Ser Val Ser Thr Trp Ala Gly Tyr Val Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 62

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 62

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Asp Ser Phe Gly
20 25 30

Val Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ala Ala Val Tyr Arg Arg Ala Gly Asp Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Ser Ala Met Tyr Tyr Cys
85 90 95

Ala Ala Arg Thr Tyr Gly Ser Val Ser Ser Trp Thr Gly Tyr Lys Tyr
100 105 110

Trp Gly Arg Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 63

<211> 125

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 63

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Arg Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Asp Thr Ser Lys Ser Asp
20 25 30

Cys Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Arg Val
35 40 45

Gly Ala Ile Tyr Thr Arg Asn Gly Tyr Thr His Tyr Ala Asp Ser Val
50 55 60

Asn Gly Arg Phe Thr Ile Ser Gln Asp Asn Ala Lys Asn Ala Leu Tyr
65 70 75 80

Leu Gln Met Ser Gly Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Ala Arg Phe Arg Ile Tyr Gly Gln Cys Val Glu Asp Asp Asp Ile
100 105 110

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 64

<211> 124

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 64

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Leu Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Thr Ser Arg Tyr Asn
20 25 30

Tyr Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ala Thr Ile Gly Thr Ala Ser Gly Ser Ala Asp Tyr Tyr Gly Ser Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Gln Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Ala Arg Thr Tyr Gly Thr Ile Ser Leu Thr Pro Ser Asp Tyr Arg
100 105 110

Met Met Gly Gly Gly Thr Val Thr Val Ser Ser

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 65

<211> 128

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 65

Gln Val Gln Val Val Glu Tyr Gly Gly Gly Ser Val Gln Ala Gly Glu
1 5 10 15

Thr Val Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Trp Glu Leu Val
35 40 45

Ser Asn Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Ser Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Phe Asp Asn Ala Lys Asn
65 70 75 80

Met Val Tyr Leu Gln Met Asn Ser Leu Lys His Glu Asp Thr Ala Val
85 90 95

Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Arg Glu Tyr
100 105 110

Cys Thr Trp Ala Gln Gly Thr Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 66

<211> 129

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 66

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Pro Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Ser Trp Ala Gln Gly Thr Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 67

<211> 129

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 67

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Thr Trp Ala Gln Gly Thr Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 68

<211> 129

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 68

Gln Val Gln Pro Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Thr Trp Ala Gln Gly Ala Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 69

<211> 129

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 69

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Ser Trp Ala Gln Gly Thr Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 70
 <211> 129
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Camelid

<400> 70
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
 1 5 10 15
 Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
 20 25 30
 Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
 35 40 45
 Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
 50 55 60
 Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
 65 70 75 80
 Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
 85 90 95
 Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
 100 105 110
 Tyr Cys Thr Trp Ala Gln Gly Thr Gln Gly Thr Gln Val Thr Val Ser
 115 120 125

Ser

<210> 71
 <211> 129
 <212> PRT
 <213> Artificial Sequence

<220>
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 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
 1 5 10 15
 Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
 20 25 30
 Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
 35 40 45
 Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
 50 55 60
 Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
 65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Thr Trp Ala Gln Gly Thr Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 72

<211> 129

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 72

His Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Thr Trp Ala Gln Gly Thr Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 73

<211> 129

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 73

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Thr Trp Ala Gln Gly Thr Gln Gly Ala Leu Val Thr Val Ser
115 120 125

Ser

<210> 74

<211> 129

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 74

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Thr Trp Ala Gln Gly Thr Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 75
 <211> 129
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Camelid

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 Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
 20 25 30
 Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
 35 40 45
 Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
 50 55 60
 Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
 65 70 75 80
 Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
 85 90 95
 Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
 100 105 110
 Tyr Cys Ser Trp Ala Gln Gly Thr Gln Gly Thr Gln Val Thr Val Ser
 115 120 125

Ser

<210> 76
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 1 5 10 15
 Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
 20 25 30
 Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
 35 40 45
 Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
 50 55 60
 Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys

65	70					75					80				
Asn	Met	Val	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Pro	Glu	Asp	Thr	Ala
				85			90				95				
Val	Tyr	Tyr	Cys	Ala	Pro	Asp	Pro	Tyr	Ala	Tyr	Ser	Thr	Tyr	Ser	Asp
			100				105						110		
Tyr	Cys	Thr	Trp	Ala	Gln	Gly	Thr	Gln	Gly	Thr	Gln	Val	Thr	Val	Ser
			115				120						125		

Ser

<210> 77

<211> 129

<212> PRT

<213> Artificial Sequence

$\langle 220 \rangle$

<223> Camelid

<400> 77

Asp	Val	Gln	Leu	Val	Glu	Ser	Arg	Gly	Gly	Ser	Val	Gln	Ala	Gly	Glu
1			5						10					15	
Thr	Leu	Arg	Leu	Ser	Cys	Thr	Ala	Ser	Gly	Phe	Thr	Phe	Ala	Glu	Ala
			20					25					30		
Asp	Met	Gly	Trp	Tyr	Arg	Gln	Ala	Pro	Gly	His	Glu	Cys	Glu	Leu	Val
		35					40					45			
Ser	Thr	Ile	Thr	Thr	Glu	Gly	Ile	Thr	Ser	Glu	Ala	Ser	Ser	Tyr	Tyr
	50					55					60				
Ala	Asp	Ser	Val	Arg	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys
65					70					75					80
Asn	Met	Val	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Pro	Glu	Asp	Thr	Ala
				85					90					95	
Val	Tyr	Tyr	Cys	Ala	Pro	Asp	Pro	Tyr	Ala	Tyr	Ser	Thr	Tyr	Ser	Asp
			100					105					110		
Tyr	Cys	Thr	Trp	Ala	Gln	Gly	Thr	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser
		115				120						125			

Ser

<210> 78

<211> 129

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 78

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Val Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Ser Trp Ala Gln Gly Thr Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 79

<211> 128

<212> PRT

<213> Artificial Sequence

<220>

<223> Camelid

<400> 79

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly Leu Glu Cys Glu Leu Val
35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Ala
50 55 60

Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
65 70 75 80

Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val
85 90 95

Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Glu Tyr
100 105 110

Asp Thr Trp Ser Ala Gln Gly Thr Gln Gly Thr Gln Val Thr Val Ser

Cys Thr Trp Ala Gln Gly Thr Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

<210> 80

<211> 129

<212> PRT

<213> Artificial Sequence

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<400> 80

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
 1 5 10 15

Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
 20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
 35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Glu Ala Ser Ser Tyr Tyr
 50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
 65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
 85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
 100 105 110

Tyr Cys Thr Trp Ala Gln Gly Thr Gln Gly Thr Leu Val Thr Val Ser
 115 120 125

Ser

<210> 81

<211> 129

<212> PRT

<213> Artificial Sequence

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<223> Camelid

<400> 81

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Glu
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Thr Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ala Glu Ala
 20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly His Glu Cys Glu Leu Val
 35 40 45

Ser Thr Ile Thr Thr Glu Gly Ile Thr Ser Val Ala Ser Ser Tyr Tyr
50 55 60

Ala Asp Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Asn Met Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Pro Asp Pro Tyr Ala Tyr Ser Thr Tyr Ser Asp
100 105 110

Tyr Cys Thr Trp Ala Gln Gly Thr Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

REFERENCES CITED IN THE DESCRIPTION

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- US6206790B [0185]
- US6214865B [0185]
- US6218835B [0185]
- US6221079B [0185]

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- **KODAPPULLY et al.** Biochimica et Biophysica Acta, 2014, vol. 1845, 136-154 [0005]
- **PEPPEL et al.** J. Exp. Med., 1991, vol. 174, 1483-1489 [0095]

PATENTKRAV

1. Isoleret polypeptid, hvilket isoleret polypeptid omfatter et anti-STAT3 enkeltdomænantistof (sdAb), der omfatter aminosyresekvensen ifølge SEQ ID NO: 3.
2. Isoleret polypeptid ifølge krav 1, hvor polypeptidet er et anti-STAT3 enkeltdomæne-
5 antistof (sdAb), hvor anti-STAT3 sdAb omfatter aminosyresekvensen ifølge SEQ ID NO: 3.
3. SdAb ifølge krav 2 til anvendelse som et lægemiddel.
4. SdAb til anvendelse ifølge krav 3, hvor individet er et pattedyr; fortrinsvis et menneske.
5. SdAb til anvendelse ifølge krav 3 eller krav 4, hvor sdAb administreres i kombination med én eller flere forbindelser, fortrinsvis hvor den ene eller flere forbindelser er en
10 transskriptionshæmmer.
6. SdAb til anvendelse ifølge et hvilket som helst af krav 3 til 5, hvor lægemidlet er til intravenøs administration, intramuskulær administration, oral administration, rektal administration, enteral administration, parenteral administration, intraokulær administration, subkutan administration, transdermal administration, administration som øjendråber,
15 administration som næsespray, administration ved inhalation eller forstøvning, topisk administration og administration som et implanterbart lægemiddel.
7. Fremgangsmåde til måling af niveauerne af et sdAb ifølge krav 2 i en prøve fra et individ, hvilken fremgangsmåde omfatter følgende trin:
 - a) generering af et monoklonalt museantistof rettet mod ét eller flere domæner af
20 et polypeptid, der omfatter aminosyresekvensen ifølge SEQ ID NO: 3;
 - b) udførelse af et kvantitativt immunassay på en prøve fra individet, hvor det kvantitative immunassay eventuelt kan omfatte et enzym-bundet immunosorbent assay (ELISA), specifik analysandmarkerings- og genvindingsassay (SALRA), væskechromatografi, massespektrometri, fluorescens-aktiveret cellosortering, eller en kombination deraf, med det
25 monoklonale museantistof og prøven for at bestemme mængden af sdAb hos et individ; og
 - d) kvantificering af mængden af sdAb hos individet.

DRAWINGS

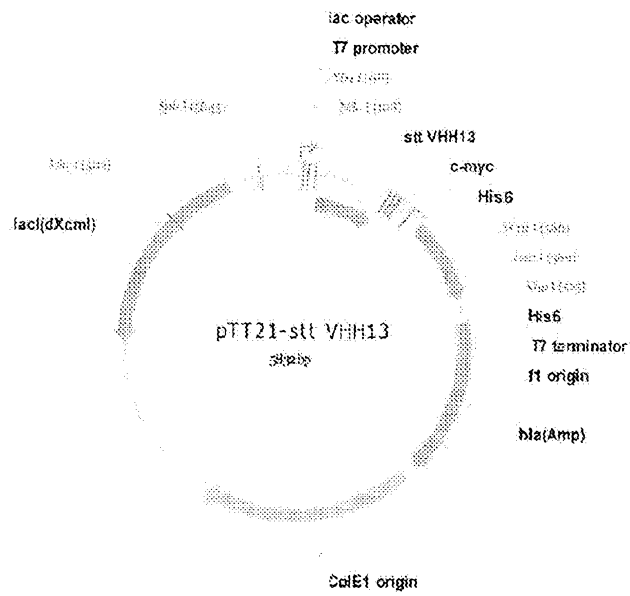


FIG. 1

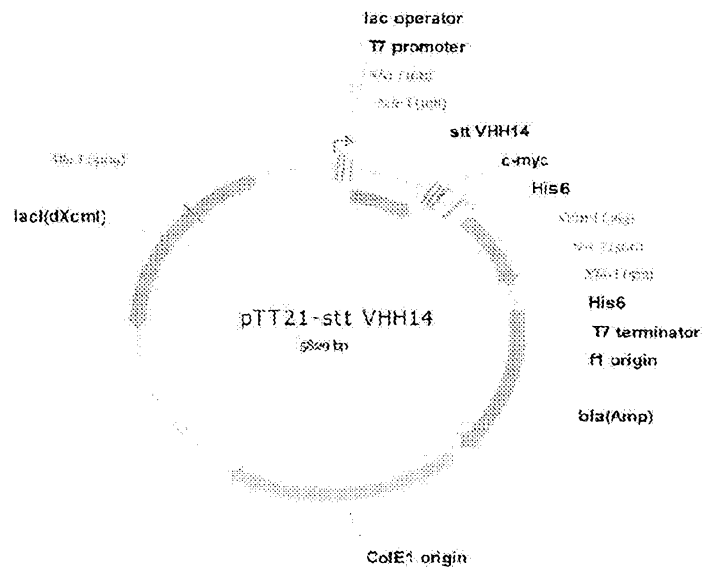
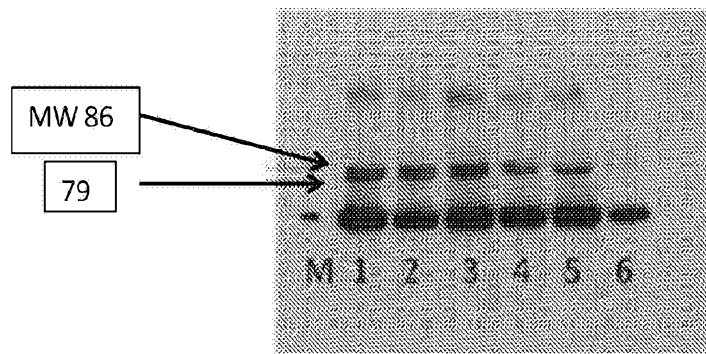


FIG. 2



Lanes:

M- Marker

1- STAT3 VHH13 (mammalian)

2- STAT3 VHH14 (mammalian)

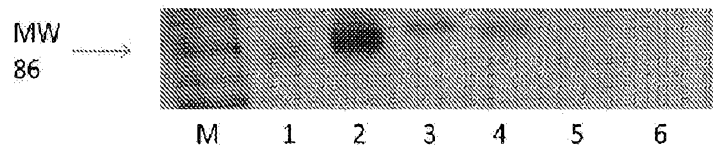
3- STAT3 VHH13 (bacterial)

4- STAT3- VHH14 (bacterial)

5- Positive control (STAT-3)

6- Negative control (STAT-1)

FIG. 3



Lanes:

M= Marker

1= PANC-1 with anti-STAT3 B VHH13

2= DU145 with anti-STAT3 B VHH13

3= HeLa + IFN-gamma with anti-STAT3 B VHH13

4= 4T1 with anti-STAT3 B VHH13

5= PANC-1 with anti-KRAS (G12D) VHH

6= PC-3 (negative control) with anti-STAT3 B VHH13

FIG. 4

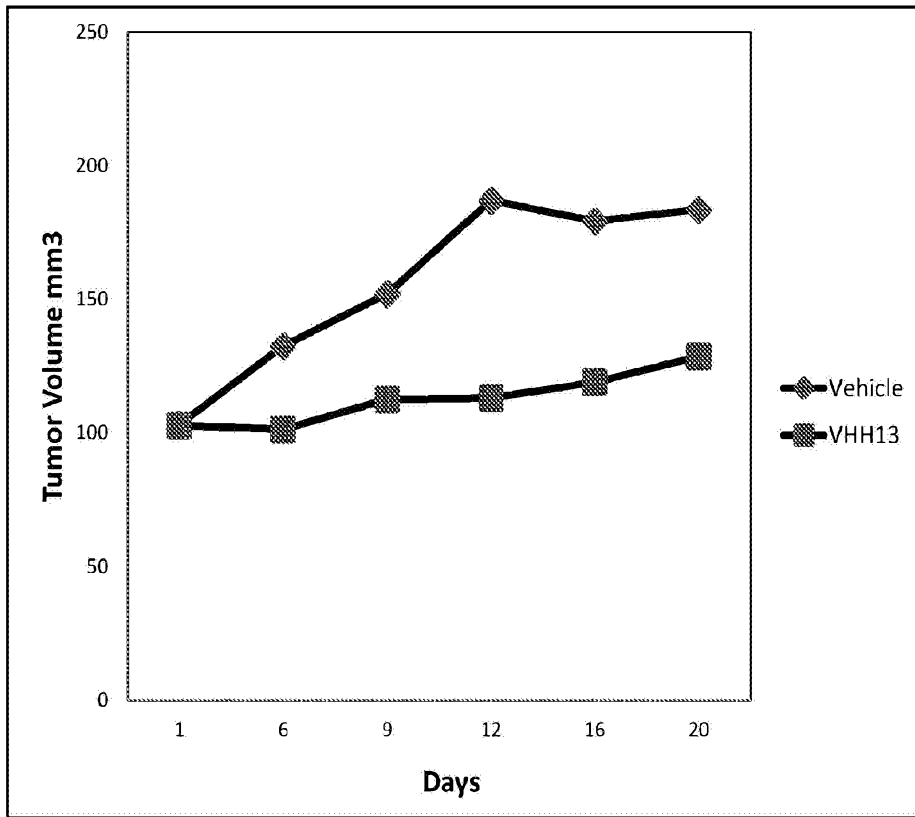
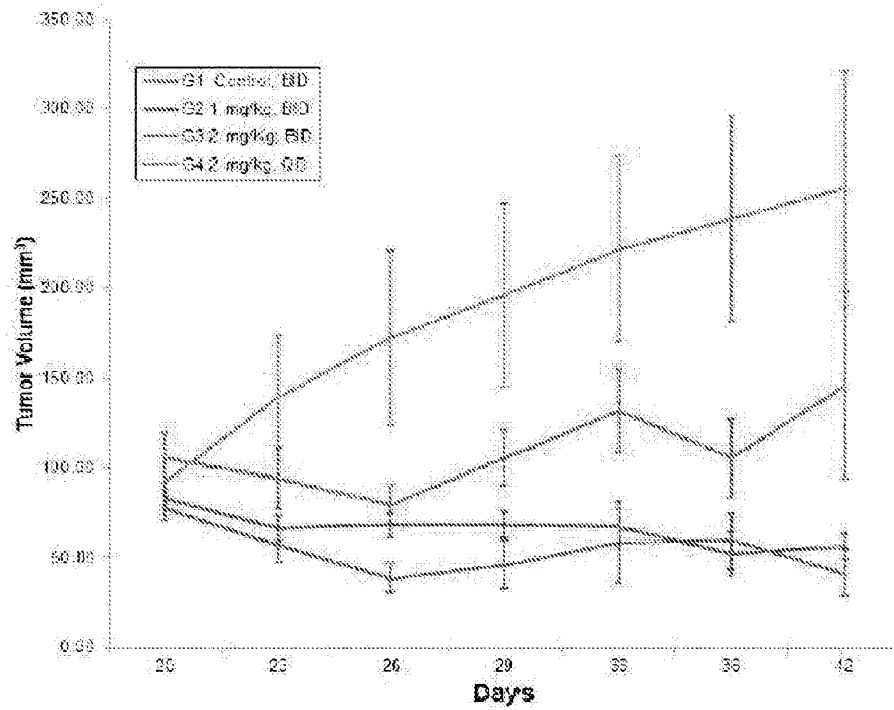


FIG. 5



Top curve: G1 is control group with vehicle only IP; given twice daily
 Second curve: G2 is given anti-STAT3 B VHH13 IP; 1mg/kg/twice daily
 Third curve at the beginning: G3 is given anti-STAT3 B VHH13 IP; 2mg/kg/twice daily
 Bottom curve at the beginning: G4 is given anti-STAT3 B VHH13 2mg/kg/once daily

FIG. 6

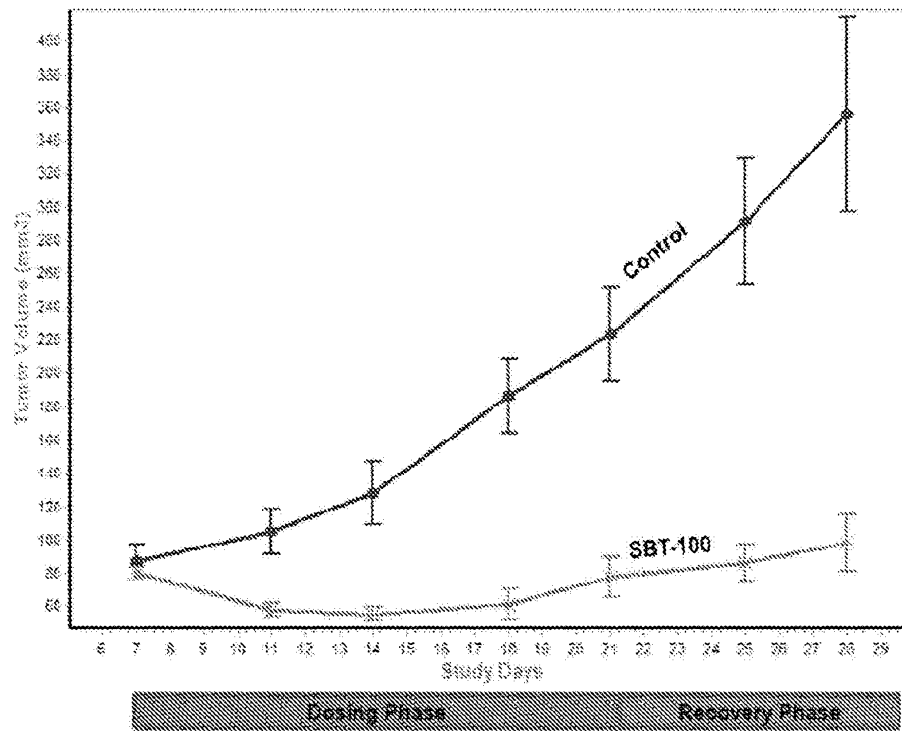


FIG. 7

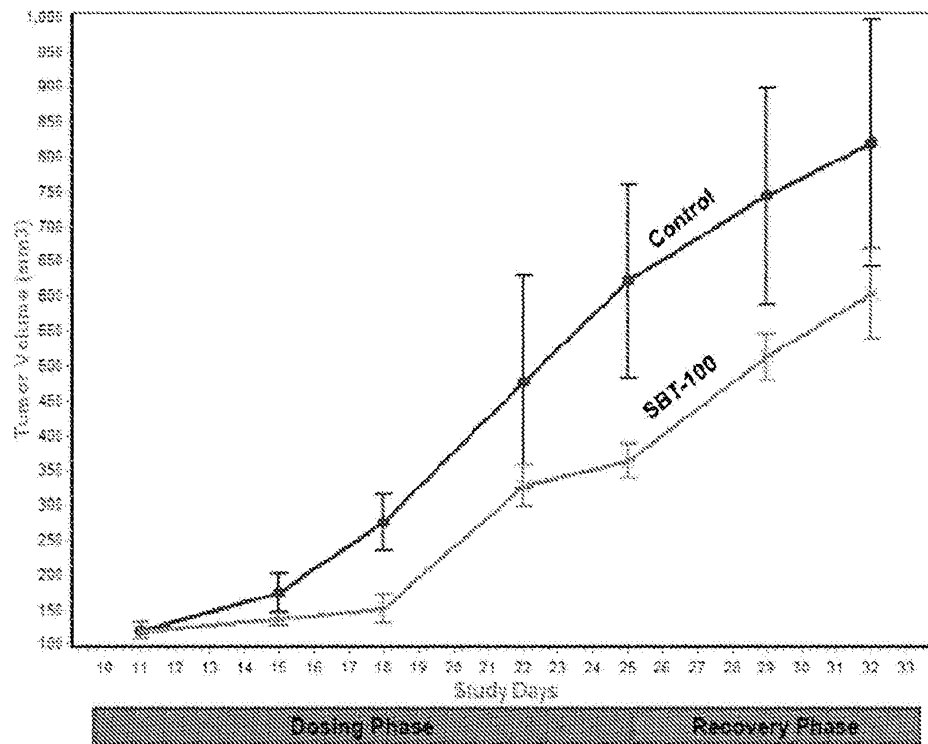


FIG. 8

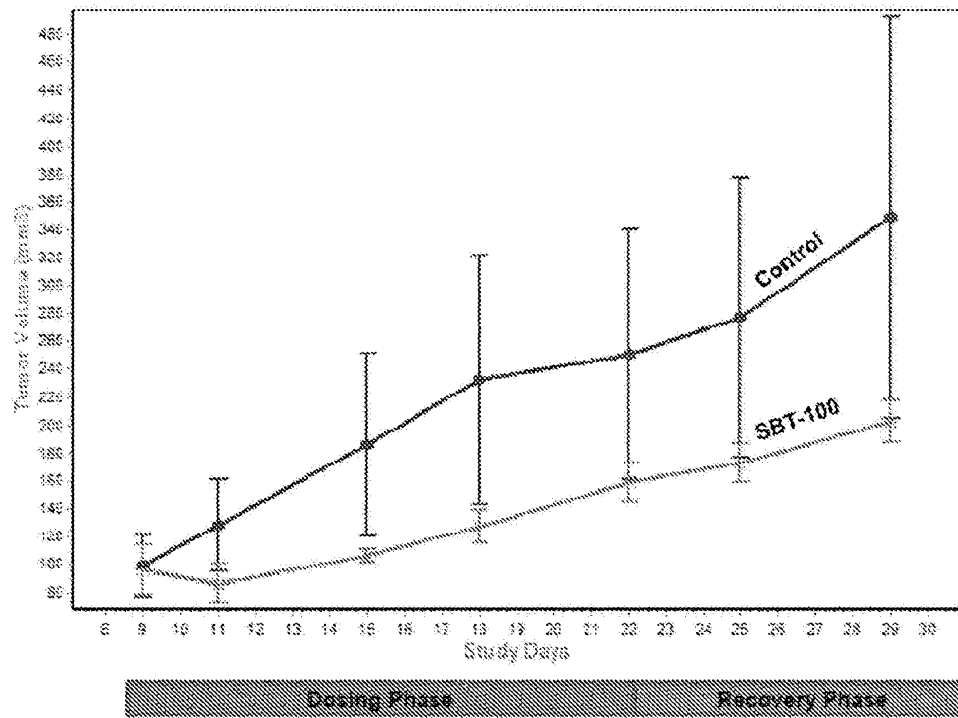


FIG. 9

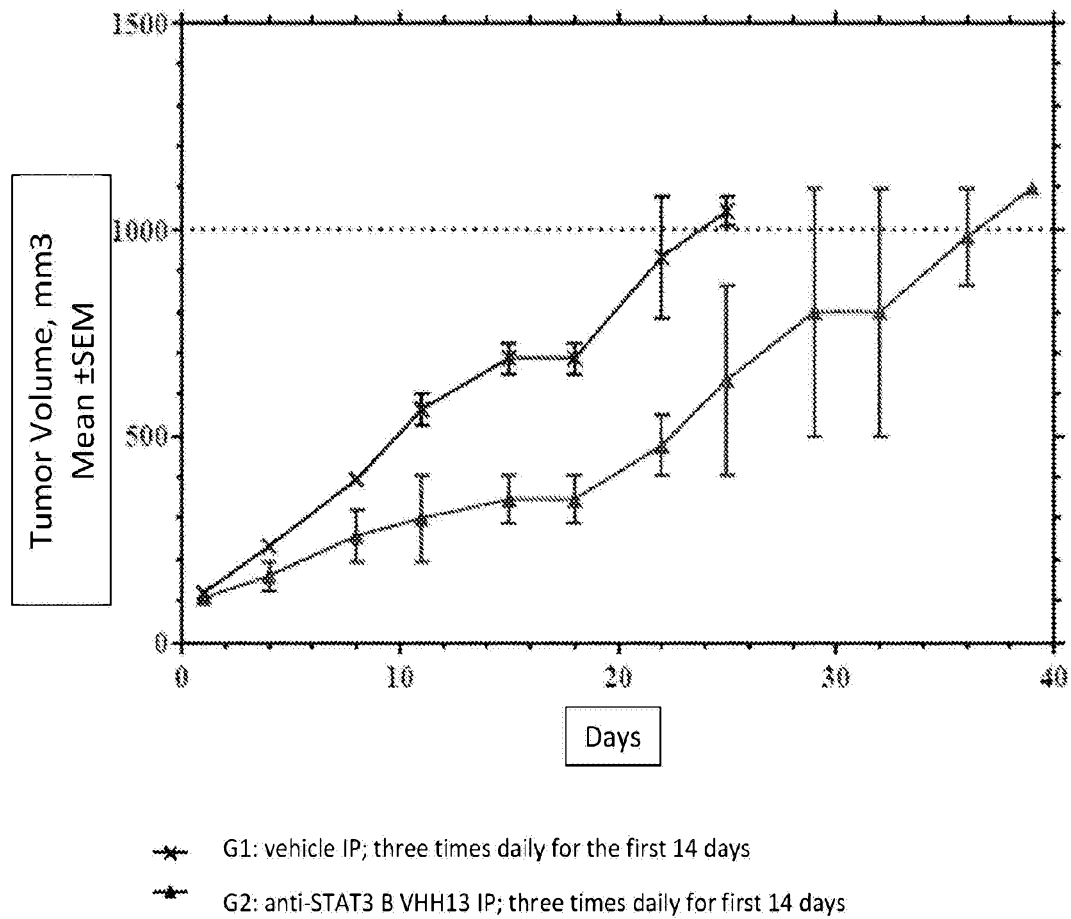


FIG. 10

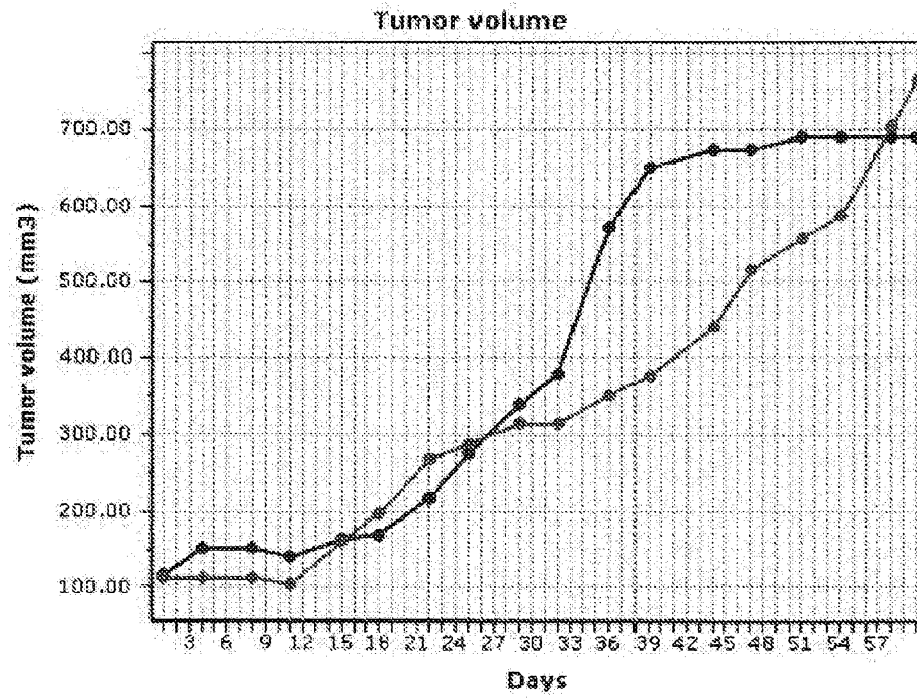
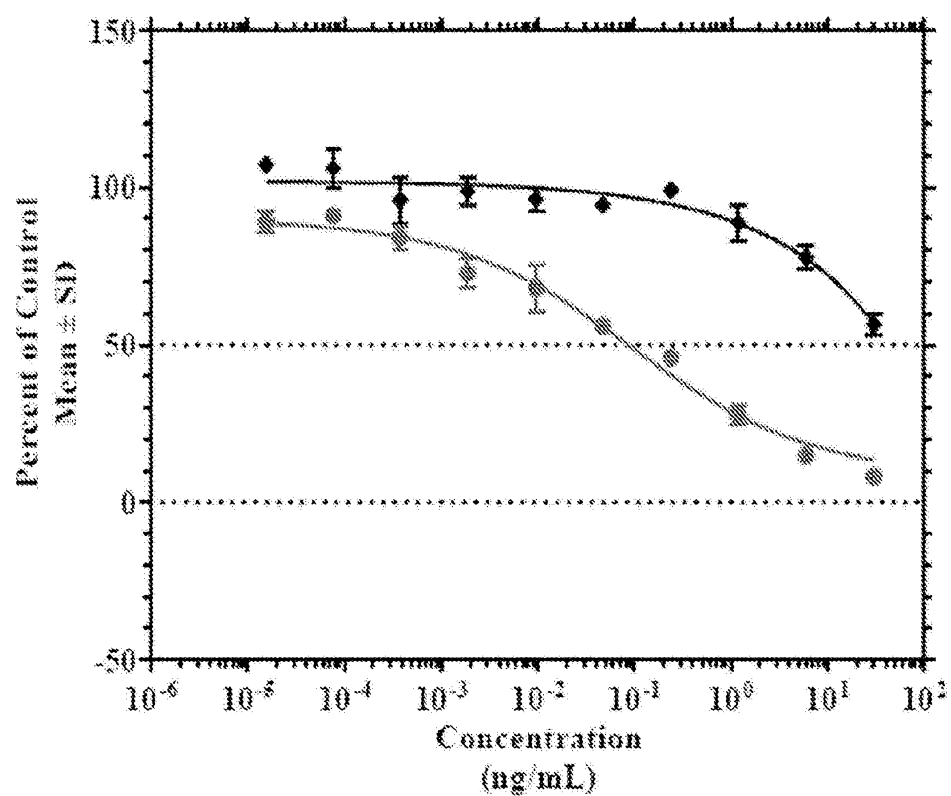


FIG. 11

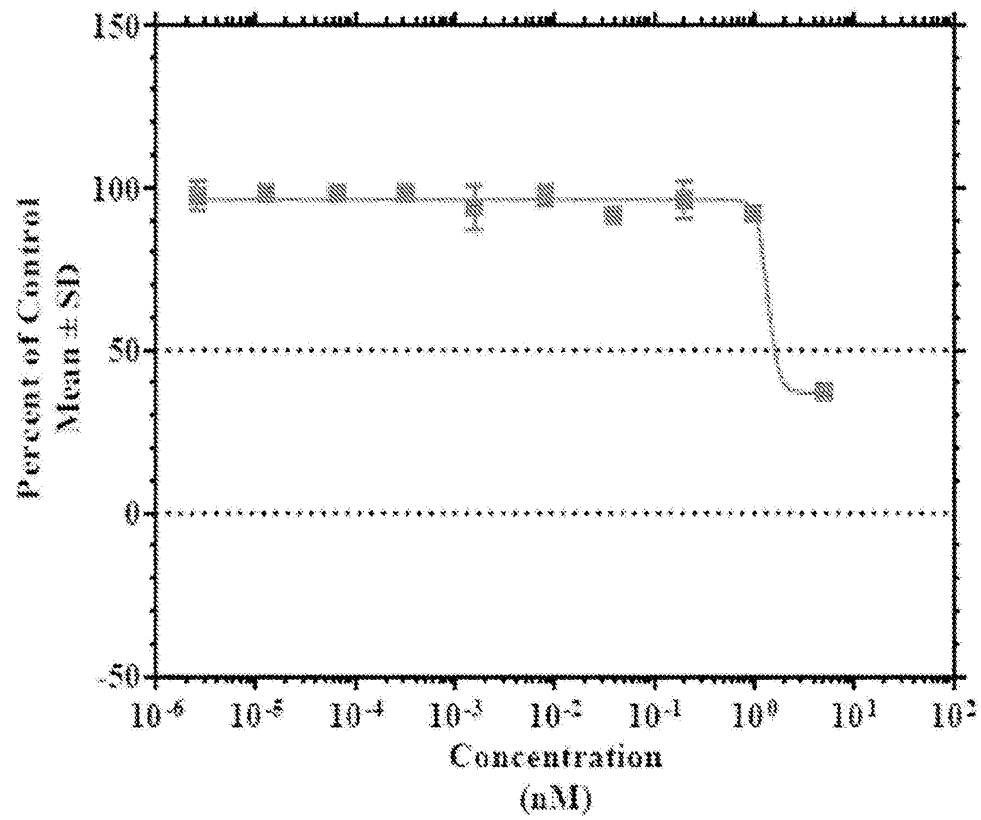


Mean IC_{50}

—•— TNF α (IC_{50} – 95.10 pg/mL)

- - - - - TNF α + constant Staurosporine (1 nM) (IC_{50} > 30 ng/mL)

FIG. 12



Mean IC₅₀

■ Staurosporine (IC₅₀ Not determined)

FIG. 13

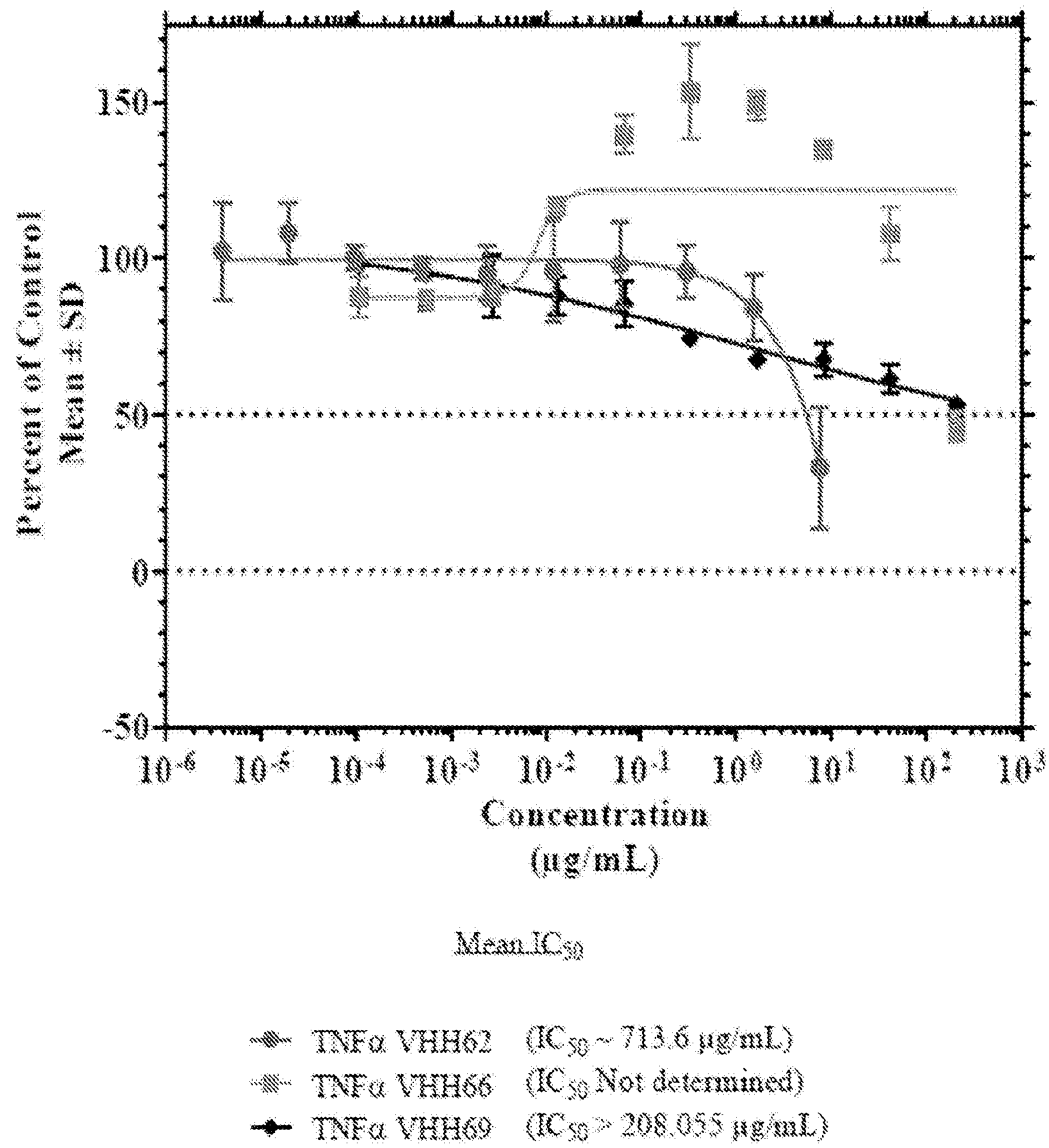


FIG. 14