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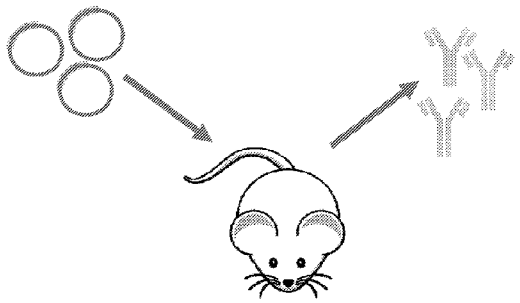

Figure 1

(57) Abstract: Disclosed herein is a composition including a recombinant nucleic acid sequence that encodes an anti-IL-6 and/or anti-CD 126 synthetic antibody. The disclosure also provides a method of preventing and/or treating disease in a subject using said composition and method of generation.

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DNA MONOCLONAL ANTIBODIES TARGETING IL-6 AND CD126

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to and the benefit of U.S. provisional application number 62/332,377, filed May 5, 2016, the content of which is incorporated herein in its entirety.

TECHNICAL FIELD

[0002] The present invention relates to a composition comprising a recombinant nucleic acid sequence for generating one or more synthetic antibodies, including anti-IL-6 and anti-CD126 antibodies, and functional fragments thereof, *in vivo,* and a method of preventing and/or treating disease in a subject by administering said composition.

BACKGROUND

[0003] Pro-inflammatory cytokine IL-6 plays a substantial role in innate inflammation and sepsis. Elevated levels of IL-6 are clinically linked to poor cancer prognoses, as numerous studies demonstrate an association between IL-6 signaling and tumor development. Currently, therapeutic antibodies targeting IL-6 and its receptor, CD126, are approved for treatment of multicentric Castleman disease and rheumatoid arthritis. Unfortunately, manufacture and delivery of purified anti-IL-6 and anti-CD126 antibodies are cost-prohibitive. Furthermore, these antibody therapies must be re-administered weekly-to-monthly – a challenging consideration in treatment of chronic conditions such as cancer and auto-immune disease.

[0004] Thus, there is a need in the art for improved compositions and methods that target IL-6 and CD126 for the treatment of cancer and auto-immune disease.

SUMMARY

[0005] The present invention is directed to a composition comprising one or more nucleic acid molecules encoding one or more synthetic antibodies, wherein the one or more nucleic acid molecules comprise at least one selected from the group consisting of a) a nucleotide

sequence encoding an anti-IL-6 synthetic antibody; b) a nucleotide sequence encoding a fragment of an anti-IL-6 synthetic antibody; c) a nucleotide sequence encoding an anti-CD126 antibody; and d) a nucleotide sequence encoding a fragment of an anti-CD126 antibody.

[0006] In one embodiment, the composition comprises a first nucleotide sequence encoding an anti-IL-6 synthetic antibody; and a second nucleotide sequence encoding an anti-CD126 antibody.

[0007] In one embodiment, the composition comprises a nucleotide sequence encoding a cleavage domain.

[0008] In one embodiment, the composition comprises a nucleotide sequence encoding a variable heavy chain region and a variable light chain region of anti-IL-6.

[0009] In one embodiment, the composition comprises a nucleotide sequence encoding a variable heavy chain region and a variable light chain region of anti-CD126.

[0010] In one embodiment, the composition comprises a nucleotide sequence encoding a constant heavy chain region and a constant light chain region of human IgG1κ.

[0011] In one embodiment, the composition comprises a nucleotide sequence encoding a polypeptide comprising a variable heavy chain region of anti-IL-6; a constant heavy chain region of human IgG1κ; a cleavage domain; a variable light chain region of anti-IL-6; and a constant light chain region of IgG1κ.

[0012] In one embodiment, the composition comprises a nucleotide sequence encoding a polypeptide comprising a variable heavy chain region of anti-CD126; a constant heavy chain region of human IgG1κ; a cleavage domain; a variable light chain region of anti-CD126; and a constant light chain region of IgG1κ.

[0013] In one embodiment, the composition comprises a nucleotide sequence which encodes a leader sequence.

[0014] In one embodiment, the composition comprises an expression vector.

[0015] In various embodiments, the invention provides a composition comprising the nucleic acid molecule. In one embodiment, the composition further comprises a pharmaceutically acceptable excipient.

[0016] In one embodiment, the present invention provides a method of preventing or treating a disease in a subject, comprising administering to the subject a composition described herein. In one embodiment, the disease is cancer. In one embodiment, the disease is an auto-immune disease. In one embodiment, the disease is sepsis. In one embodiment, the disease is a viral infection. In one embodiment, the disease is multicentric Castleman disease.

In one embodiment, the disease is associated with high fever. In one embodiment, the disease is graft-versus-host (GVH) disease. In one embodiment, the disease is cell lysis syndrome.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1 is a schematic representation of a DNA construct encoding anti-IL-6 and anti-CD126.

[0018] Figure 2, comprising Figure 2A through Figure 2C, depicts the results of experiments demonstrating that DMAb constructs are expressed in 293T cells. HEK 293T cells were transfected with plasmid DNA carrying anti-IL-6 (IL-6 1 to 4) or anti-CD126 (CD126 1 to 2) constructs. Empty plasmid served as a negative control. (Figure 2A and Figure 2B) Human IgG1κ expression was determined by quantitative ELISA (N=3 transfection replicates, ±SEM.) (Figure 2C) Representative Western Blot showing supernatant heavy and light-chain peptide cleavage and expression.

[0019] Figure 3, comprising Figure 3A and Figure 3B, depicts the results of experiments demonstrating that DMAb are expressed *in vivo* in mouse serum following intramuscular electroporation. BALB/c mice were injected with 100μg i.m. plasmid DNA followed by electroporation. Seven days later, serum human IgG1κ antibody levels were determined by ELISA. (Figure 3A) Anti-IL-6 DMAb were expressed from 1.5μg/mL to 7.0μg/mL (mean) over baseline Day-0 pre-bleed levels. (Figure 3B) Anti-CD126 DMAb were expressed from 1.6μg/mL to 4.1μg/mL (mean) over baseline Day-0 pre-bleed levels. (N=5, Mean ±SEM.)

[0020] Figure 4 depicts the results of experiments demonstrating that DMAb in serum from muscle-electroporated mice bind their target antigens in vitro. BALB/c mice were injected with 100μg plasmid DNA followed by intramuscular electroporation. One week later, serum human-IgG antibody binding to recombinant human IL-6 (left) and human CD126 (right) was determined by ELISA. (N=5, Mean ±SEM.).

[0021] Figure 5 depicts the results of experiments demonstrating that serum DMAb block IL-6-mediated cell signaling in vitro. HEK-293 cells which were stably transfected with human CD126 and a STAT3-inducible secreted alkaline phosphatase (SEAP) were obtained. Diluted (1:40) serum from untreated mice induced a baseline level of mouse-IL-6-driven SEAP expression, which was normalized to 100% SEAP activity in cell supernatants (gray bar). Day-7 serum from DMAb-electroporated mice was diluted (1:40) and cell supernatants were assayed for SEAP activity as a percentage of untreated control (black bars). Non-

specific cytokine TNFα acted as a control for specific cytokine activation (white bar). (N=4, Mean ±SEM.)

[0022] Figure 6 depicts the results of experiments demonstrating that serum DMAb block IL-6-mediated cell signaling in vitro. HEK-293 cells which were stably transfected with human CD126 and a STAT3-inducible secreted alkaline phosphatase (SEAP) were obtained. Diluted (1:40 – 1:40960) serum from untreated mice induced a baseline level of mouse-IL-6-driven SEAP expression, which was normalized to 100% SEAP activity in cell supernatants (black line). Day-7 serum from DMAb-electroporated mice was diluted (1:40 – 1:40960) and cell supernatants were assayed for SEAP activity as indicated (blue line). Non-specific cytokine TNFα acted as a control for specific cytokine activation (grey line). (N=4, Mean ±SEM.).

DETAILED DESCRIPTION

[0023] The present invention relates to compositions comprising a recombinant nucleic acid sequence encoding an antibody, a fragment thereof, a variant thereof, or a combination thereof. The composition can be administered to a subject in need thereof to facilitate in vivo expression and formation of a synthetic antibody.

[0024] In particular, the heavy chain and light chain polypeptides expressed from the recombinant nucleic acid sequences can assemble into the synthetic antibody. The heavy chain polypeptide and the light chain polypeptide can interact with one another such that assembly results in the synthetic antibody being capable of binding the desired target (e.g., IL-6 and CD126), being more immunogenic as compared to an antibody not assembled as described herein, and being capable of eliciting or inducing an immune response against the desired target.

[0025] Additionally, these synthetic antibodies are generated more rapidly in the subject than antibodies that are produced in response to antigen induced immune response. The synthetic antibodies are able to effectively bind and neutralize a range of targets. The synthetic antibodies are also able to effectively protect against and/or promote survival of disease. Accordingly, with respect to engineered monoclonal antibody (MAb) in the form of synthetic DNA plasmids, the present invention relates to compositions comprising a recombinant nucleic acid sequence encoding an antibody, a fragment thereof, a variant thereof, or a combination thereof. The composition can be administered to a subject in need thereof to facilitate in vivo expression and formation of a synthetic antibody. In one

embodiment, the nucleotide sequence is described herein. For example, in one embodiment, the nucleotide sequence comprises a nucleotide sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, or a variant thereof or a fragment thereof. In another embodiment, the nucleotide sequence comprises a nucleotide sequence encoding the polypeptide sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12, or a variant thereof or a fragment thereof. In one embodiment the nucleotide sequence comprises an RNA sequence transcribed from a DNA sequence described herein. For example, in one embodiment, the nucleotide sequence comprises an RNA sequence transcribed by the DNA sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, or a variant thereof or a fragment thereof. In another embodiment, the nucleotide sequence comprises an RNA sequence transcribed by a DNA sequence encoding the polypeptide sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12, or a variant thereof or a fragment thereof.

[0026] In one embodiment the nucleotide sequence encodes an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, or at least about 95% identity over the entire length of the amino acid sequence to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12. In one embodiment the nucleotide sequence encodes a fragment of an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, or at least about 95% identity over the entire length of the amino acid sequence to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12.

[0027] In one embodiment the nucleotide sequence has at least about 80%, at least about 85%, at least about 90%, or at least about 95% identity over the entire length of the nucleotide sequence to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11. In one embodiment the nucleotide sequence is a fragment of a nucleotide sequence that has at least about 80%, at least about 85%, at least about 90%, or at least about 95% identity over the entire length of the nucleotide sequence to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11.

1. Definitions

[0028] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are

described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0029] The terms "comprise(s)," "include(s)," "having," "has," "can," "contain(s)," and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms "a," "and" and "the" include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments "comprising," "consisting of" and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0030] "Antibody" may mean an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, fragments or derivatives thereof, including Fab, F(ab')2, Fd, and single chain antibodies, and derivatives thereof. The antibody may be an antibody isolated from the serum sample of mammal, a polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom.

[0031] "Antibody fragment" or "fragment of an antibody" as used interchangeably herein refers to a portion of an intact antibody comprising the antigen-binding site or variable region. The portion does not include the constant heavy chain domains (i.e. CH2, CH3, or CH4, depending on the antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include, but are not limited to, Fab fragments, Fab' fragments, Fab'-SH fragments, F(ab')2 fragments, Fd fragments, Fv fragments, diabodies, single-chain Fv (scFv) molecules, single-chain polypeptides containing only one light chain variable domain, single-chain polypeptides containing the three CDRs of the light-chain variable domain, single-chain polypeptides containing only one heavy chain variable region, and single-chain polypeptides containing the three CDRs of the heavy chain variable region.

[0032] "Antigen" refers to proteins that have the ability to generate an immune response in a host. An antigen may be recognized and bound by an antibody. An antigen may originate from within the body or from the external environment.

[0033] "Coding sequence" or "encoding nucleic acid" as used herein means the nucleic acid (RNA or DNA molecule) that comprise a nucleotide sequence which encodes an antibody as set forth herein. The coding sequence may also comprise a DNA sequence which

encodes an RNA sequence. The coding sequence may further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to whom the nucleic acid is administered. The coding sequence may further include sequences that encode signal peptides.

[0034] "Complement" or "complementary" as used herein may mean a nucleic acid may mean Watson-Crick (e.g., A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

[0035] "Constant current" as used herein to define a current that is received or experienced by a tissue, or cells defining said tissue, over the duration of an electrical pulse delivered to same tissue. The electrical pulse is delivered from the electroporation devices described herein. This current remains at a constant amperage in said tissue over the life of an electrical pulse because the electroporation device provided herein has a feedback element, preferably having instantaneous feedback. The feedback element can measure the resistance of the tissue (or cells) throughout the duration of the pulse and cause the electroporation device to alter its electrical energy output (e.g., increase voltage) so current in same tissue remains constant throughout the electrical pulse (on the order of microseconds), and from pulse to pulse. In some embodiments, the feedback element comprises a controller.

[0036] "Current feedback" or "feedback" as used herein may be used interchangeably and may mean the active response of the provided electroporation devices, which comprises measuring the current in tissue between electrodes and altering the energy output delivered by the EP device accordingly in order to maintain the current at a constant level. This constant level is preset by a user prior to initiation of a pulse sequence or electrical treatment. The feedback may be accomplished by the electroporation component, e.g., controller, of the electroporation device, as the electrical circuit therein is able to continuously monitor the current in tissue between electrodes and compare that monitored current (or current within tissue) to a preset current and continuously make energy-output adjustments to maintain the monitored current at preset levels. The feedback loop may be instantaneous as it is an analog closed-loop feedback.

[0037] "Decentralized current" as used herein may mean the pattern of electrical currents delivered from the various needle electrode arrays of the electroporation devices described herein, wherein the patterns minimize, or preferably eliminate, the occurrence of electroporation related heat stress on any area of tissue being electroporated.

[0038] "Electroporation," "electro-permeabilization," or "electro-kinetic enhancement" ("EP") as used interchangeably herein may refer to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

[0039] "Endogenous antibody" as used herein may refer to an antibody that is generated in a subject that is administered an effective dose of an antigen for induction of a humoral immune response.

[0040] "Feedback mechanism" as used herein may refer to a process performed by either software or hardware (or firmware), which process receives and compares the impedance of the desired tissue (before, during, and/or after the delivery of pulse of energy) with a present value, preferably current, and adjusts the pulse of energy delivered to achieve the preset value. A feedback mechanism may be performed by an analog closed loop circuit.

[0041] "Fragment" may mean a polypeptide fragment of an antibody that is function, i.e., can bind to desired target and have the same intended effect as a full length antibody. A fragment of an antibody may be 100% identical to the full length except missing at least one amino acid from the N and/or C terminal, in each case with or without signal peptides and/or a methionine at position 1. Fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length antibody, excluding any heterologous signal peptide added. The fragment may comprise a fragment of a polypeptide that is 95% or more, 96% or more, 97% or more, 98% or more or 99% or more identical to the antibody and additionally comprise an N terminal methionine or heterologous signal peptide which is not included when calculating percent identity. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a fragment of an antibody.

[0042] A fragment of a nucleic acid sequence that encodes an antibody may be 100% identical to the full length except missing at least one nucleotide from the 5' and/or 3' end, in each case with or without sequences encoding signal peptides and/or a methionine at position 1. Fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more,

75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length coding sequence, excluding any heterologous signal peptide added. The fragment may comprise a fragment that encode a polypeptide that is 95% or more, 96% or more, 97% or more, 98% or more or 99% or more identical to the antibody and additionally optionally comprise sequence encoding an N terminal methionine or heterologous signal peptide which is not included when calculating percent identity. Fragments may further comprise coding sequences for an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The coding sequence encoding the N terminal methionine and/or signal peptide may be linked to a fragment of coding sequence.

[0043] "Genetic construct" as used herein refers to the DNA or RNA molecules that comprise a nucleotide sequence which encodes a protein, such as an antibody. The genetic construct may also refer to a DNA molecule which transcribes an RNA. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered. As used herein, the term "expressible form" refers to gene constructs that contain the necessary regulatory elements operable linked to a coding sequence that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

[0044] "Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

[0045] "Impedance" as used herein may be used when discussing the feedback mechanism and can be converted to a current value according to Ohm's law, thus enabling comparisons with the preset current.

[0046] "Immune response" as used herein may mean the activation of a host's immune system, e.g., that of a mammal, in response to the introduction of one or more nucleic acids and/or peptides. The immune response can be in the form of a cellular or humoral response, or both.

[0047] "Nucleic acid" or "oligonucleotide" or "polynucleotide" as used herein may mean at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

[0048] Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

[0049] "Operably linked" as used herein may mean that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene may be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

[0050] A "peptide," "protein," or "polypeptide" as used herein can mean a linked sequence of amino acids and can be natural, synthetic, or a modification or combination of natural and synthetic.

[0051] "Promoter" as used herein may mean a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter may comprise one or more specific transcriptional regulatory sequences to

further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter may also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter may be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter may regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV 40 late promoter and the CMV IE promoter.

[0052] "Signal peptide" and "leader sequence" are used interchangeably herein and refer to an amino acid sequence that can be linked at the amino terminus of a protein set forth herein. Signal peptides/leader sequences typically direct localization of a protein. Signal peptides/leader sequences used herein preferably facilitate secretion of the protein from the cell in which it is produced. Signal peptides/leader sequences are often cleaved from the remainder of the protein, often referred to as the mature protein, upon secretion from the cell. Signal peptides/leader sequences are linked at the N terminus of the protein.

[0053] "Stringent hybridization conditions" as used herein may mean conditions under which a first nucleic acid sequence (e.g., probe) will hybridize to a second nucleic acid sequence (e.g., target), such as in a complex mixture of nucleic acids. Stringent conditions are sequence dependent and will be different in different circumstances. Stringent conditions may be selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m may be the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions may be those in which the salt concentration is less than about 1.0 M sodium ion, such as about 0.01-1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., about 10-50 nucleotides) and at least about 60°C for long probes (e.g., greater than about 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal may be at least 2 to 10 times background hybridization.

Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

[0054] "Subject" and "patient" as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (e.g., cow, pig, camel, llama, horse, goat, rabbit, sheep, hamsters, guinea pig, cat, dog, rat, and mouse, a non-human primate (for example, a monkey, such as a cynomolgous or rhesus monkey, chimpanzee, etc) and a human). In some embodiments, the subject may be a human or a non-human. The subject or patient may be undergoing other forms of treatment.

[0055] "Substantially complementary" as used herein may mean that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the complement of a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

[0056] "Substantially identical" as used herein may mean that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,or 99% over a region of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

[0057] "Synthetic antibody" as used herein refers to an antibody that is encoded by the recombinant nucleic acid sequence described herein and is generated in a subject.

[0058] "Treatment" or "treating," as used herein can mean protecting of a subject from a disease through means of preventing, suppressing, repressing, or completely eliminating the disease. Preventing the disease involves administering a vaccine of the present invention to a subject prior to onset of the disease. Suppressing the disease involves administering a vaccine of the present invention to a subject after induction of the disease but before its clinical appearance. Repressing the disease involves administering a vaccine of the present invention to a subject after clinical appearance of the disease.

[0059] "Variant" used herein with respect to a nucleic acid may mean (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced

nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

[0060] "Variant" with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant may also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hyrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

[0061] A variant may be a nucleic acid sequence that is substantially identical over the full length of the full gene sequence or a fragment thereof. The nucleic acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the gene sequence or a fragment thereof. A variant may be an amino acid sequence that is substantially identical over

the full length of the amino acid sequence or fragment thereof. The amino acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the amino acid sequence or a fragment thereof.

[0062] "Vector" as used herein may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

[0063] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

2. Composition

[0064] The present invention relates to a composition comprising a recombinant nucleic acid sequence encoding an antibody, a fragment thereof, a variant thereof, or a combination thereof. The invention also includes novel sequences for use for producing antibodies in mammalian cells or for delivery in DNA or RNA vectors including bacterial, yeast, as well as viral vectors. The nucleic acid sequence may be a DNA sequence, an RNA sequence, or a combination and/or derivative thereof. The composition, when administered to a subject in need thereof, can result in the generation of a synthetic antibody in the subject. The synthetic antibody can bind a target molecule (i.e., IL-6 and CD126) present in the subject. Such binding can neutralize the target, block recognition of the target by another molecule, for example, a protein or nucleic acid, and elicit or induce an immune response to the target.

[0065] In one embodiment, the composition comprises a nucleotide sequence encoding a synthetic antibody. In one embodiment, the composition comprises a nucleic acid molecule comprising a first nucleotide sequence encoding a first synthetic antibody and a second nucleotide sequence encoding a second synthetic antibody. In one embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding a cleavage domain.

[0066] In one embodiment, the first nucleotide sequence encoding a first synthetic antibody comprises a first domain encoding the heavy chain region and a second domain encoding the light chain region of the first synthetic antibody. In one embodiment, the second nucleotide sequence encoding a second synthetic antibody comprises a first domain encoding

the heavy chain region and a second domain encoding the light chain region of the second synthetic antibody.

[0067] In one embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding anti-IL-6 antibody. In one embodiment, the nucleotide sequence encoding anti-IL-6 antibody comprises codon optimized nucleic acid sequences encoding the variable VH and VL regions of anti-IL-6. In one embodiment, the nucleotide sequence encoding anti-IL-6 antibody comprises codon optimized nucleic acid sequences encoding CH and CL regions of human IgG1κ.

[0068] In one embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding anti-CD126 antibody. In one embodiment, the nucleotide sequence encoding anti-CD126 antibody comprises codon optimized nucleic acid sequences encoding the variable VH and VL regions of anti-CD126. In one embodiment, the nucleotide sequence encoding anti-CD126 antibody comprises codon optimized nucleic acid sequences encoding CH and CL regions of human IgG1κ.

[0069] In one embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes an anti-IL-6 synthetic antibody comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, fragments thereof, or homologous sequences thereof.

[0070] In one embodiment, the anti-IL-6 synthetic antibody comprises the amino acid sequence of SEQ ID NO: 2, which is encoded by nucleotide sequence of SEQ ID NO: 1. In some embodiments, the anti-IL-6 synthetic antibody can comprise the amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity over an entire length of the amino acid sequence set forth in SEQ ID NO:2.

[0071] Fragments of SEQ ID NO: 2 can be provided. Fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:2. In some embodiments, fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, fragments are free of a leader sequence.

[0072] In one embodiment, the anti-IL-6 synthetic antibody comprises the amino acid sequence of SEQ ID NO: 4, which is encoded by nucleotide sequence of SEQ ID NO: 3. In some embodiments, the anti-IL-6 synthetic antibody can comprise the amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity over an entire length of the amino acid sequence set forth in SEQ ID NO:4.

[0073] Fragments of SEQ ID NO: 4 can be provided. Fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:4. In some embodiments, fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, fragments are free of a leader sequence.

[0074] In one embodiment, the anti-IL-6 synthetic antibody comprises the amino acid sequence of SEQ ID NO: 6, which is encoded by nucleotide sequence of SEQ ID NO: 5. In some embodiments, the anti-IL-6 synthetic antibody can comprise the amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity over an entire length of the amino acid sequence set forth in SEQ ID NO:6.

[0075] Fragments of SEQ ID NO: 6 can be provided. Fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:6. In some embodiments, fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, fragments are free of a leader sequence.

[0076] In one embodiment, the anti-IL-6 synthetic antibody comprises the amino acid sequence of SEQ ID NO: 8, which is encoded by nucleotide sequence of SEQ ID NO: 7. In some embodiments, the anti-IL-6 synthetic antibody can comprise the amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity over an entire length of the amino acid sequence set forth in SEQ ID NO:8.

[0077] Fragments of SEQ ID NO: 8 can be provided. Fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:8. In some embodiments, fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, fragments are free of a leader sequence.

[0078] In certain embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes an anti-IL-6 synthetic antibody, where the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO: 1; SEQ ID NO: 3; SEQ ID NO: 5, SEQ ID NO: 6, fragments thereof, or homologous sequences thereof.

[0079] In one embodiment, the nucleotide sequence encoding the anti-IL-6 synthetic antibody comprises the nucleotide sequence of SEQ ID NO: 1. In certain embodiments, the nucleotide sequence encoding the anti-IL-6 synthetic antibody comprises at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of the nucleic acid sequence set forth in SEQ ID NO:1.

[0080] Some embodiments relate to fragments of SEQ ID NO:1. Fragments can be at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:1.

[0081] In one embodiment, the nucleotide sequence encoding the anti-IL-6 synthetic antibody comprises the nucleotide sequence of SEQ ID NO: 3. In certain embodiments, the nucleotide sequence encoding the anti-IL-6 synthetic antibody comprises at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of the nucleic acid sequence set forth in SEQ ID NO:3.

[0082] Some embodiments relate to fragments of SEQ ID NO:3. Fragments can be at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:3.

[0083] In one embodiment, the nucleotide sequence encoding the anti-IL-6 synthetic antibody comprises the nucleotide sequence of SEQ ID NO: 5. In certain embodiments, the nucleotide sequence encoding the anti-IL-6 synthetic antibody comprises at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of the nucleic acid sequence set forth in SEQ ID NO:5.

[0084] Some embodiments relate to fragments of SEQ ID NO:5. Fragments can be at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:5.

[0085] In one embodiment, the nucleotide sequence encoding the anti-IL-6 synthetic antibody comprises the nucleotide sequence of SEQ ID NO: 7. In certain embodiments, the nucleotide sequence encoding the anti-IL-6 synthetic antibody comprises at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of the nucleic acid sequence set forth in SEQ ID NO:1.

[0086] Some embodiments relate to fragments of SEQ ID NO:7. Fragments can be at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:7.

[0087] In one embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes an anti-CD126 synthetic antibody comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 12, fragments thereof, or homologous sequences thereof.

[0088] In one embodiment, the anti-CD126 synthetic antibody comprises the amino acid sequence of SEQ ID NO: 10, which is encoded by nucleotide sequence of SEQ ID NO: 9. In some embodiments, the anti-CD126 synthetic antibody can comprise the amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity over an entire length of the amino acid sequence set forth in SEQ ID NO:10.

[0089] Fragments of SEQ ID NO: 10 can be provided. Fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:10. In some embodiments, fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, fragments are free of a leader sequence.

[0090] In one embodiment, the anti-CD126 synthetic antibody comprises the amino acid sequence of SEQ ID NO: 12, which is encoded by nucleotide sequence of SEQ ID NO: 11. In some embodiments, the anti-CD126 synthetic antibody can comprise the amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity over an entire length of the amino acid sequence set forth in SEQ ID NO:12.

[0091] Fragments of SEQ ID NO: 12 can be provided. Fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:12. In some embodiments, fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, fragments are free of a leader sequence.

[0092] In certain embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes an anti-CD126 synthetic antibody, where the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO: 9; SEQ ID NO: 11; fragments thereof, or homologous sequences thereof.

[0093] In one embodiment, the nucleotide sequence encoding the anti-CD126 synthetic antibody comprises the nucleotide sequence of SEQ ID NO: 9. In certain embodiments, the

nucleotide sequence encoding the anti-CD126 synthetic antibody comprises at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of the nucleic acid sequence set forth in SEQ ID NO:9.

[0094] Some embodiments relate to fragments of SEQ ID NO:9. Fragments can be at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:9.

[0095] In one embodiment, the nucleotide sequence encoding the anti-CD126 synthetic antibody comprises the nucleotide sequence of SEQ ID NO: 11. In certain embodiments, the nucleotide sequence encoding the anti-CD126 synthetic antibody comprises at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of the nucleic acid sequence set forth in SEQ ID NO:11.

[0096] Some embodiments relate to fragments of SEQ ID NO:11. Fragments can be at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:11.

[0097] The composition of the invention can treat, prevent and/or protect against any disease, disorder, or condition associated with IL-6 and/or CD126 activity. In certain embodiments, the composition can treat, prevent, and or/protect against inflammation. In certain embodiments, the composition can treat, prevent, and or/protect against an auto-immune disease or disorder. In certain embodiments, the composition can treat, prevent, and or/protect against cancer.

[0098] The synthetic antibody can treat, prevent, and/or protect against disease in the subject administered the composition. The synthetic antibody by binding the target can treat, prevent, and/or protect against disease in the subject administered the composition. The synthetic antibody can promote survival of the disease in the subject administered the composition. The synthetic antibody can provide at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% survival of the disease in the subject administered the composition. In other embodiments, the synthetic antibody can provide at least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80% survival of the disease in the subject administered the composition.

[0099] The composition can result in the generation of the synthetic antibody in the subject within at least about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 20 hours, 25

hours, 30 hours, 35 hours, 40 hours, 45 hours, 50 hours, or 60 hours of administration of the composition to the subject. The composition can result in generation of the synthetic antibody in the subject within at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, or 10 days of administration of the composition to the subject. The composition can result in generation of the synthetic antibody in the subject within about 1 hour to about 6 days, about 1 hour to about 5 days, about 1 hour to about 4 days, about 1 hour to about 3 days, about 1 hour to about 2 days, about 1 hour to about 1 day, about 1 hour to about 72 hours, about 1 hour to about 60 hours, about 1 hour to about 48 hours, about 1 hour to about 36 hours, about 1 hour to about 24 hours, about 1 hour to about 12 hours, or about 1 hour to about 6 hours of administration of the composition to the subject.

[00100] The composition, when administered to the subject in need thereof, can result in the generation of the synthetic antibody in the subject more quickly than the generation of an endogenous antibody in a subject who is administered an antigen to induce a humoral immune response. The composition can result in the generation of the synthetic antibody at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, or 10 days before the generation of the endogenous antibody in the subject who was administered an antigen to induce a humoral immune response.

[00101] The composition of the present invention can have features required of effective compositions such as being safe so that the composition does not cause illness or death; being protective against illness; and providing ease of administration, few side effects, biological stability and low cost per dose.

3. Recombinant Nucleic Acid Sequence

[00102] As described above, the composition can comprise a recombinant nucleic acid sequence. The recombinant nucleic acid sequence can encode the antibody, a fragment thereof, a variant thereof, or a combination thereof. The antibody is described in more detail below.

[00103] The recombinant nucleic acid sequence can be a heterologous nucleic acid sequence. The recombinant nucleic acid sequence can include at least one heterologous nucleic acid sequence or one or more heterologous nucleic acid sequences.

[00104] The recombinant nucleic acid sequence can be an optimized nucleic acid sequence. Such optimization can increase or alter the immunogenicity of the antibody. Optimization can also improve transcription and/or translation. Optimization can include one or more of the following: low GC content leader sequence to increase transcription; mRNA stability and

codon optimization; addition of a kozak sequence (e.g., GCC ACC) for increased translation; addition of an immunoglobulin (Ig) leader sequence encoding a signal peptide; and eliminating to the extent possible cis-acting sequence motifs (i.e., internal TATA boxes).

a. Recombinant Nucleic Acid Sequence Construct

[00105] The recombinant nucleic acid sequence can include one or more recombinant nucleic acid sequence constructs. The recombinant nucleic acid sequence construct can include one or more components, which are described in more detail below.

[00106] The recombinant nucleic acid sequence construct can include a heterologous nucleic acid sequence that encodes a heavy chain polypeptide, a fragment thereof, a variant thereof, or a combination thereof. The recombinant nucleic acid sequence construct can include a heterologous nucleic acid sequence that encodes a light chain polypeptide, a fragment thereof, a variant thereof, or a combination thereof. The recombinant nucleic acid sequence construct can also include a heterologous nucleic acid sequence that encodes a protease or peptidase cleavage site. The recombinant nucleic acid sequence construct can include one or more leader sequences, in which each leader sequence encodes a signal peptide. The recombinant nucleic acid sequence construct can include one or more promoters, one or more introns, one or more transcription termination regions, one or more initiation codons, one or more termination or stop codons, and/or one or more polyadenylation signals. The recombinant nucleic acid sequence construct can also include one or more linker or tag sequences. The tag sequence can encode a hemagglutinin (HA) tag.

(1) Heavy Chain Polypeptide

[00107] The recombinant nucleic acid sequence construct can include the heterologous nucleic acid encoding the heavy chain polypeptide, a fragment thereof, a variant thereof, or a combination thereof. The heavy chain polypeptide can include a variable heavy chain (VH) region and/or at least one constant heavy chain (CH) region. The at least one constant heavy chain region can include a constant heavy chain region 1 (CH1), a constant heavy chain region 2 (CH2), and a constant heavy chain region 3 (CH3), and/or a hinge region.

[00108] In some embodiments, the heavy chain polypeptide can include a VH region and a CH1 region. In other embodiments, the heavy chain polypeptide can include a VH region, a CH1 region, a hinge region, a CH2 region, and a CH3 region.

[00109] The heavy chain polypeptide can include a complementarity determining region ("CDR") set. The CDR set can contain three hypervariable regions of the VH region.

Proceeding from N-terminus of the heavy chain polypeptide, these CDRs are denoted "CDR1," "CDR2," and "CDR3," respectively. CDR1, CDR2, and CDR3 of the heavy chain polypeptide can contribute to binding or recognition of the antigen.

(2) Light Chain Polypeptide

[00110] The recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the light chain polypeptide, a fragment thereof, a variant thereof, or a combination thereof. The light chain polypeptide can include a variable light chain (VL) region and/or a constant light chain (CL) region.

[00111] The light chain polypeptide can include a complementarity determining region ("CDR") set. The CDR set can contain three hypervariable regions of the VL region. Proceeding from N-terminus of the light chain polypeptide, these CDRs are denoted "CDR1," "CDR2," and "CDR3," respectively. CDR1, CDR2, and CDR3 of the light chain polypeptide can contribute to binding or recognition of the antigen.

(3) Protease Cleavage Site

[00112] The recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the protease cleavage site. The protease cleavage site can be recognized by a protease or peptidase. The protease can be an endopeptidase or endoprotease, for example, but not limited to, furin, elastase, HtrA, calpain, trypsin, chymotrypsin, trypsin, and pepsin. The protease can be furin. In other embodiments, the protease can be a serine protease, a threonine protease, cysteine protease, aspartate protease, metalloprotease, glutamic acid protease, or any protease that cleaves an internal peptide bond (i.e., does not cleave the N-terminal or C-terminal peptide bond).

[00113] The protease cleavage site can include one or more amino acid sequences that promote or increase the efficiency of cleavage. The one or more amino acid sequences can promote or increase the efficiency of forming or generating discrete polypeptides. The one or more amino acids sequences can include a 2A peptide sequence.

(4) Linker Sequence

[00114] The recombinant nucleic acid sequence construct can include one or more linker sequences. The linker sequence can spatially separate or link the one or more components described herein. In other embodiments, the linker sequence can encode an amino acid sequence that spatially separates or links two or more polypeptides.

(5) Promoter

[00115] The recombinant nucleic acid sequence construct can include one or more promoters. The one or more promoters may be any promoter that is capable of driving gene expression and regulating gene expression. Such a promoter is a cis-acting sequence element required for transcription via a DNA dependent RNA polymerase. Selection of the promoter used to direct gene expression depends on the particular application. The promoter may be positioned about the same distance from the transcription start in the recombinant nucleic acid sequence construct as it is from the transcription start site in its natural setting. However, variation in this distance may be accommodated without loss of promoter function.

[00116] The promoter may be operably linked to the heterologous nucleic acid sequence encoding the heavy chain polypeptide and/or light chain polypeptide. The promoter may be a promoter shown effective for expression in eukaryotic cells. The promoter operably linked to the coding sequence may be a CMV promoter, a promoter from simian virus 40 (SV40), such as SV40 early promoter and SV40 later promoter, a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter may also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, human polyhedrin, or human metalothionein.

[00117] The promoter can be a constitutive promoter or an inducible promoter, which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, the promoter can also be specific to a particular tissue or organ or stage of development. The promoter may also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no. US20040175727, the contents of which are incorporated herein in its entirety.

[00118] The promoter can be associated with an enhancer. The enhancer can be located upstream of the coding sequence. The enhancer may be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, FMDV, RSV or EBV. Polynucleotide function enhances are described in U.S. Patent Nos. 5,593,972, 5,962,428, and W094/016737, the contents of each are fully incorporated by reference.

(6) Intron

[00119] The recombinant nucleic acid sequence construct can include one or more introns. Each intron can include functional splice donor and acceptor sites. The intron can include an enhancer of splicing. The intron can include one or more signals required for efficient splicing.

(7) Transcription Termination Region

[00120] The recombinant nucleic acid sequence construct can include one or more transcription termination regions. The transcription termination region can be downstream of the coding sequence to provide for efficient termination. The transcription termination region can be obtained from the same gene as the promoter described above or can be obtained from one or more different genes.

(8) Initiation Codon

[00121] The recombinant nucleic acid sequence construct can include one or more initiation codons. The initiation codon can be located upstream of the coding sequence. The initiation codon can be in frame with the coding sequence. The initiation codon can be associated with one or more signals required for efficient translation initiation, for example, but not limited to, a ribosome binding site.

(9) Termination Codon

[00122] The recombinant nucleic acid sequence construct can include one or more termination or stop codons. The termination codon can be downstream of the coding sequence. The termination codon can be in frame with the coding sequence. The termination codon can be associated with one or more signals required for efficient translation termination.

(10) Polyadenylation Signal

[00123] The recombinant nucleic acid sequence construct can include one or more polyadenylation signals. The polyadenylation signal can include one or more signals required for efficient polyadenylation of the transcript. The polyadenylation signal can be positioned downstream of the coding sequence. The polyadenylation signal may be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH)

polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β-globin polyadenylation signal. The SV40 polyadenylation signal may be a polyadenylation signal from a pCEP4 plasmid (Invitrogen, San Diego, CA).

(11) Leader Sequence

[00124] The recombinant nucleic acid sequence construct can include one or more leader sequences. The leader sequence can encode a signal peptide. The signal peptide can be an immunoglobulin (Ig) signal peptide, for example, but not limited to, an IgG signal peptide and a IgE signal peptide.

b. Arrangement of the Recombinant Nucleic Acid Sequence Construct

[00125] As described above, the recombinant nucleic acid sequence can include one or more recombinant nucleic acid sequence constructs, in which each recombinant nucleic acid sequence construct can include one or more components. The one or more components are described in detail above. The one or more components, when included in the recombinant nucleic acid sequence construct, can be arranged in any order relative to one another. In some embodiments, the one or more components can be arranged in the recombinant nucleic acid sequence construct as described below.

(1) Arrangement 1

[00126] In one arrangement, a first recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the heavy chain polypeptide and a second recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00127] The first recombinant nucleic acid sequence construct can be placed in a vector. The second recombinant nucleic acid sequence construct can be placed in a second or separate vector. Placement of the recombinant nucleic acid sequence construct into the vector is described in more detail below.

[00128] The first recombinant nucleic acid sequence construct can also include the promoter, intron, transcription termination region, initiation codon, termination codon, and/or polyadenylation signal. The first recombinant nucleic acid sequence construct can further include the leader sequence, in which the leader sequence is located upstream (or 5') of the heterologous nucleic acid sequence encoding the heavy chain polypeptide. Accordingly, the

signal peptide encoded by the leader sequence can be linked by a peptide bond to the heavy chain polypeptide.

[00129] The second recombinant nucleic acid sequence construct can also include the promoter, initiation codon, termination codon, and polyadenylation signal. The second recombinant nucleic acid sequence construct can further include the leader sequence, in which the leader sequence is located upstream (or 5') of the heterologous nucleic acid sequence encoding the light chain polypeptide. Accordingly, the signal peptide encoded by the leader sequence can be linked by a peptide bond to the light chain polypeptide.

[00130] Accordingly, one example of arrangement 1 can include the first vector (and thus first recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH and CH1, and the second vector (and thus second recombinant nucleic acid sequence construct) encoding the light chain polypeptide that includes VL and CL. A second example of arrangement 1 can include the first vector (and thus first recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH, CH1, hinge region, CH2, and CH3, and the second vector (and thus second recombinant nucleic acid sequence construct) encoding the light chain polypeptide that includes VL and CL.

(2) Arrangement 2

[00131] In a second arrangement, the recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide. The heterologous nucleic acid sequence encoding the heavy chain polypeptide can be positioned upstream (or 5') of the heterologous nucleic acid sequence encoding the light chain polypeptide. Alternatively, the heterologous nucleic acid sequence encoding the light chain polypeptide can be positioned upstream (or 5') of the heterologous nucleic acid sequence encoding the heavy chain polypeptide.

[00132] The recombinant nucleic acid sequence construct can be placed in the vector as described in more detail below.

[00133] The recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the protease cleavage site and/or the linker sequence. If included in the recombinant nucleic acid sequence construct, the heterologous nucleic acid sequence encoding the protease cleavage site can be positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide. Accordingly, the protease cleavage site

allows for separation of the heavy chain polypeptide and the light chain polypeptide into distinct polypeptides upon expression. In other embodiments, if the linker sequence is included in the recombinant nucleic acid sequence construct, then the linker sequence can be positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00134] The recombinant nucleic acid sequence construct can also include the promoter, intron, transcription termination region, initiation codon, termination codon, and/or polyadenylation signal. The recombinant nucleic acid sequence construct can include one or more promoters. The recombinant nucleic acid sequence construct can include two promoters such that one promoter can be associated with the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the second promoter can be associated with the heterologous nucleic acid sequence encoding the light chain polypeptide. In still other embodiments, the recombinant nucleic acid sequence construct can include one promoter that is associated with the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00135] The recombinant nucleic acid sequence construct can further include two leader sequences, in which a first leader sequence is located upstream (or 5') of the heterologous nucleic acid sequence encoding the heavy chain polypeptide and a second leader sequence is located upstream (or 5') of the heterologous nucleic acid sequence encoding the light chain polypeptide. Accordingly, a first signal peptide encoded by the first leader sequence can be linked by a peptide bond to the heavy chain polypeptide and a second signal peptide encoded by the second leader sequence can be linked by a peptide bond to the light chain polypeptide.

[00136] Accordingly, one example of arrangement 2 can include the vector (and thus recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH and CH1, and the light chain polypeptide that includes VL and CL, in which the linker sequence is positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00137] A second example of arrangement of 2 can include the vector (and thus recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH and CH1, and the light chain polypeptide that includes VL and CL, in which the heterologous nucleic acid sequence encoding the protease cleavage site is positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00138] A third example of arrangement 2 can include the vector (and thus recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH, CH1, hinge region, CH2, and CH3, and the light chain polypeptide that includes VL and CL, in which the linker sequence is positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00139] A forth example of arrangement of 2 can include the vector (and thus recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH, CH1, hinge region, CH2, and CH3, and the light chain polypeptide that includes VL and CL, in which the heterologous nucleic acid sequence encoding the protease cleavage site is positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

c. Expression from the Recombinant Nucleic Acid Sequence Construct

[00140] As described above, the recombinant nucleic acid sequence construct can include, amongst the one or more components, the heterologous nucleic acid sequence encoding the heavy chain polypeptide and/or the heterologous nucleic acid sequence encoding the light chain polypeptide. Accordingly, the recombinant nucleic acid sequence construct can facilitate expression of the heavy chain polypeptide and/or the light chain polypeptide.

[00141] When arrangement 1 as described above is utilized, the first recombinant nucleic acid sequence construct can facilitate the expression of the heavy chain polypeptide and the second recombinant nucleic acid sequence construct can facilitate expression of the light chain polypeptide. When arrangement 2 as described above is utilized, the recombinant nucleic acid sequence construct can facilitate the expression of the heavy chain polypeptide and the light chain polypeptide.

[00142] Upon expression, for example, but not limited to, in a cell, organism, or mammal, the heavy chain polypeptide and the light chain polypeptide can assemble into the synthetic antibody. In particular, the heavy chain polypeptide and the light chain polypeptide can interact with one another such that assembly results in the synthetic antibody being capable of binding the antigen. In other embodiments, the heavy chain polypeptide and the light chain polypeptide can interact with one another such that assembly results in the synthetic antibody being more immunogenic as compared to an antibody not assembled as described herein. In still other embodiments, the heavy chain polypeptide and the light chain polypeptide can

interact with one another such that assembly results in the synthetic antibody being capable of eliciting or inducing an immune response against the antigen.

d. Vectors

[00143] Vectors include, but are not limited to, plasmids, expression vectors, recombinant viruses, any form of recombinant "naked DNA" vector, and the like. A "vector" comprises a nucleic acid which can infect, transfect, transiently or permanently transduce a cell. It will be recognized that a vector can be a naked nucleic acid, or a nucleic acid complexed with protein or lipid. The vector optionally comprises viral or bacterial nucleic acids and/or proteins, and/or membranes (e.g., a cell membrane, a viral lipid envelope, etc.). Vectors include, but are not limited to replicons (e.g., RNA replicons, bacteriophages) to which fragments of DNA may be attached and become replicated. Vectors thus include, but are not limited to RNA, autonomous self-replicating circular or linear DNA or RNA (e.g., plasmids, viruses, and the like, see, e.g., U.S. Pat. No. 5,217,879), and include both the expression and non-expression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector" this includes both extra-chromosomal circular and linear DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome. The recombinant nucleic acid sequence construct described above can be placed in one or more vectors. The one or more vectors can contain an origin of replication. The one or more vectors can be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. The one or more vectors can be either a self-replication extra chromosomal vector, or a vector which integrates into a host genome.

[00144] The one or more vectors can be a heterologous expression construct, which is generally a plasmid that is used to introduce a specific gene into a target cell. Once the expression vector is inside the cell, the heavy chain polypeptide and/or light chain polypeptide that are encoded by the recombinant nucleic acid sequence construct is produced by the cellular-transcription and translation machinery ribosomal complexes. The one or more vectors can express large amounts of stable messenger RNA, and therefore proteins.

(1) Expression vector

[00145] The one or more vectors can be a circular plasmid or a linear nucleic acid. The circular plasmid and linear nucleic acid are capable of directing expression of a particular

nucleotide sequence in an appropriate subject cell. The one or more vectors comprising the recombinant nucleic acid sequence construct may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components.

(2) Plasmid

[00146] The one or more vectors can be a plasmid. The plasmid may be useful for transfecting cells with the recombinant nucleic acid sequence construct. The plasmid may be useful for introducing the recombinant nucleic acid sequence construct into the subject. The plasmid may also comprise a regulatory sequence, which may be well suited for gene expression in a cell into which the plasmid is administered.

[00147] The plasmid may also comprise a mammalian origin of replication in order to maintain the plasmid extrachromosomally and produce multiple copies of the plasmid in a cell. The plasmid may be pVAX, pCEP4 or pREP4 from Invitrogen (San Diego, CA), which may comprise the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which may produce high copy episomal replication without integration. The backbone of the plasmid may be pAV0242. The plasmid may be a replication defective adenovirus type 5 (Ad5) plasmid.

[00148] The plasmid may be pSE420 (Invitrogen, San Diego, Calif.), which may be used for protein production in *Escherichia coli* (E.coli). The plasmid may also be p YES2 (Invitrogen, San Diego, Calif.), which may be used for protein production in Saccharomyces cerevisiae strains of yeast. The plasmid may also be of the MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.), which may be used for protein production in insect cells. The plasmid may also be pcDNAI or pcDNA3 (Invitrogen, San Diego, Calif.), which may be used for protein production in mammalian cells such as Chinese hamster ovary (CHO) cells.

(3) RNA Vectors

[00149] In one embodiment, the nucleic acid is an RNA molecule. In one embodiment, the RNA molecule is transcribed from a DNA sequence described herein. For example, in some embodiments, the RNA molecule is encoded by one of SEQ ID NOs:1, 3, 5, 7, 9, 11, or a variant thereof or a fragment thereof. In another embodiment, the nucleotide sequence comprises an RNA sequence transcribed by a DNA sequence encoding the polypeptide sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, or a variant thereof or a fragment thereof. Accordingly, in one embodiment, the invention provides an RNA molecule encoding one or

more of the antibodies or other molecules disclosed herein. The RNA may be plus-stranded. Accordingly, in some embodiments, the RNA molecule can be translated by cells without needing any intervening replication steps such as reverse transcription. A RNA molecule useful with the invention may have a 5′ cap (e.g. a 7-methylguanosine). This cap can enhance in vivo translation of the RNA. The 5′ nucleotide of a RNA molecule useful with the invention may have a 5′ triphosphate group. In a capped RNA this may be linked to a 7-methylguanosine via a 5′-to-5′ bridge. A RNA molecule may have a 3′ poly-A tail. It may also include a poly-A polymerase recognition sequence (e.g. AAUAAA) near its 3′ end. A RNA molecule useful with the invention may be single-stranded.

(4) Circular and Linear Vector

[00150] The one or more vectors may be one or more circular plasmids, which may transform a target cell by integration into the cellular genome or exist extrachromosomally (e.g., autonomous replicating plasmid with an origin of replication). The vector can be pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleic acid sequence construct.

[00151] Also provided herein is a linear nucleic acid, or linear expression cassette ("LEC"), that is capable of being efficiently delivered to a subject via electroporation and expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleic acid sequence construct. The LEC may be any linear DNA devoid of any phosphate backbone. The DNA may encode one or more antibodies. The LEC may comprise a promoter, an intron, a stop codon, a polyadenylation signal. The LEC may not contain any antibiotic resistance genes and/or a phosphate backbone. The LEC may not contain other nucleic acid sequences unrelated to the desired antibody expression. The LEC is capable of being efficiently delivered to a subject via electroporation and expressing one or more desired antibodies.

[00152] The LEC may be derived from any plasmid capable of being linearized. These can also be made synthetically without bacterial growth and not from linearized sequences. The plasmid may be capable of expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleic acid sequence construct. The plasmid can be pNP (Puerto Rico/34) or pM2 (New Caledonia/99). The plasmid may be WLV009, pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing the heavy chain

polypeptide and/or light chain polypeptide encoded by the recombinant nucleic acid sequence construct.

[00153] The LEC can be pcrM2. The LEC can be pcrNP. pcrNP and pcrMR can be derived from pNP (Puerto Rico/34) and pM2 (New Caledonia/99), respectively.

(5) Viral Vectors

[00154] In one embodiment, viral vectors are provided herein which are capable of delivering a nucleic acid of the invention to a cell. The expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001), and in Ausubel et al. (1997), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector comprises an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers. (See, e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

(6) Method of Preparing the Vector

[00155] Provided herein is a method for preparing the one or more vectors in which the recombinant nucleic acid sequence construct has been placed. After the final subcloning step, the vector can be used to inoculate a cell culture in a large scale fermentation tank, using known methods in the art.

[00156] In other embodiments, after the final subcloning step, the vector can be used with one or more electroporation (EP) devices. The EP devices are described below in more detail.

[00157] The one or more vectors can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using a plasmid manufacturing technique that is described in a licensed, co-pending U.S. provisional application U.S. Serial No. 60/939,792, which was filed on May 23, 2007. In some examples, the DNA plasmids described herein can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those

described in U.S. Serial No. 60/939792, including those described in a licensed patent, US Patent No. 7,238,522, which issued on July 3, 2007. The above-referenced application and patent, US Serial No. 60/939,792 and US Patent No. 7,238,522, respectively, are hereby incorporated in their entirety.

4. Antibody

[00158] As described above, the recombinant nucleic acid sequence can encode the antibody, a fragment thereof, a variant thereof, or a combination thereof. The antibody can bind or react with the antigen, which is described in more detail below.

[00159] The antibody can treat, prevent, and/or protect against disease in the subject administered a composition of the invention. The antibody by binding the antigen can treat, prevent, and/or protect against disease in the subject administered the composition. The antibody can promote survival of the disease in the subject administered the composition. In one embodiment, the antibody can provide increased survival of the disease in the subject over the expected survival of a subject having the disease who has not been administered the antibody. In various embodiments, the antibody can provide at least about a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or a 100% increase in survival of the disease in subjects administered the composition over the expected survival in the absence of the composition. In one embodiment, the antibody can provide increased protection against the disease in the subject over the expected protection of a subject who has not been administered the antibody. In various embodiments, the antibody can protect against disease in at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of subjects administered the composition over the expected protection in the absence of the composition.

[00160] The antibody may comprise a heavy chain and a light chain complementarity determining region ("CDR") set, respectively interposed between a heavy chain and a light chain framework ("FR") set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. The CDR set may contain three hypervariable regions of a heavy or light chain V region. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted as "CDR1," "CDR2," and "CDR3," respectively. An antigen-binding site, therefore, may include six CDRs, comprising the CDR set from each of a heavy and a light chain V region.

[00161] The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the F(ab) fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the $F(ab')_2$ fragment, which comprises both antigen-binding sites. Accordingly, the antibody can be the Fab or $F(ab')_2$. The Fab can include the heavy chain polypeptide and the light chain polypeptide. The heavy chain polypeptide of the Fab can include the VH region and the CH1 region. The light chain of the Fab can include the VL region and CL region.

[00162] The antibody can be an immunoglobulin (Ig). The Ig can be, for example, IgA, IgM, IgD, IgE, and IgG. The immunoglobulin can include the heavy chain polypeptide and the light chain polypeptide. The heavy chain polypeptide of the immunoglobulin can include a VH region, a CH1 region, a hinge region, a CH2 region, and a CH3 region. The light chain polypeptide of the immunoglobulin can include a VL region and CL region.

[00163] The antibody can be a polyclonal or monoclonal antibody. The antibody can be a chimeric antibody, a single chain antibody, an affinity matured antibody, a human antibody, a humanized antibody, or a fully human antibody. The humanized antibody can be an antibody from a non-human species that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule.

[00164] The antibody can be a bispecific antibody as described below in more detail. The antibody can be a bifunctional antibody as also described below in more detail.

[00165] As described above, the antibody can be generated in the subject upon administration of the composition to the subject. The antibody may have a half-life within the subject. In some embodiments, the antibody may be modified to extend or shorten its half-life within the subject. Such modifications are described below in more detail.

[00166] The antibody can be defucosylated as described in more detail below.

[00167] The antibody may be modified to reduce or prevent antibody-dependent enhancement (ADE) of disease associated with the antigen as described in more detail below.

a. Bispecific Antibody

[00168] The recombinant nucleic acid sequence can encode a bispecific antibody, a fragment thereof, a variant thereof, or a combination thereof. The bispecific antibody can bind or react with two antigens, for example, two of the antigens described below in more detail. The bispecific antibody can be comprised of fragments of two of the antibodies

described herein, thereby allowing the bispecific antibody to bind or react with two desired target molecules, which may include the antigen, which is described below in more detail, a ligand, including a ligand for a receptor, a receptor, including a ligand-binding site on the receptor, a ligand-receptor complex, and a marker, including a cancer marker.

b. Bifunctional Antibody

[00169] The recombinant nucleic acid sequence can encode a bifunctional antibody, a fragment thereof, a variant thereof, or a combination thereof. The bifunctional antibody can bind or react with the antigen described below. The bifunctional antibody can also be modified to impart an additional functionality to the antibody beyond recognition of and binding to the antigen. Such a modification can include, but is not limited to, coupling to factor H or a fragment thereof. Factor H is a soluble regulator of complement activation and thus, may contribute to an immune response via complement-mediated lysis (CML).

c. Extension of Antibody Half-Life

[00170] As described above, the antibody may be modified to extend or shorten the half-life of the antibody in the subject. The modification may extend or shorten the half-life of the antibody in the serum of the subject.

[00171] The modification may be present in a constant region of the antibody. The modification may be one or more amino acid substitutions in a constant region of the antibody that extend the half-life of the antibody as compared to a half-life of an antibody not containing the one or more amino acid substitutions. The modification may be one or more amino acid substitutions in the CH2 domain of the antibody that extend the half-life of the antibody as compared to a half-life of an antibody not containing the one or more amino acid substitutions.

[00172] In some embodiments, the one or more amino acid substitutions in the constant region may include replacing a methionine residue in the constant region with a tyrosine residue, a serine residue in the constant region with a threonine residue, a threonine residue in the constant region with a glutamate residue, or any combination thereof, thereby extending the half-life of the antibody.

[00173] In other embodiments, the one or more amino acid substitutions in the constant region may include replacing a methionine residue in the CH2 domain with a tyrosine residue, a serine residue in the CH2 domain with a threonine residue, a threonine residue in

the CH2 domain with a glutamate residue, or any combination thereof, thereby extending the half-life of the antibody.

d. Defucosylation

[00174] The recombinant nucleic acid sequence can encode an antibody that is not fucosylated (i.e., a defucosylated antibody or a non-fucosylated antibody), a fragment thereof, a variant thereof, or a combination thereof. Fucosylation includes the addition of the sugar fucose to a molecule, for example, the attachment of fucose to N-glycans, O-glycans and glycolipids. Accordingly, in a defucosylated antibody, fucose is not attached to the carbohydrate chains of the constant region. In turn, this lack of fucosylation may improve FcγRIIIa binding and antibody directed cellular cytotoxic (ADCC) activity by the antibody as compared to the fucosylated antibody. Therefore, in some embodiments, the non-fucosylated antibody may exhibit increased ADCC activity as compared to the fucosylated antibody.

[00175] The antibody may be modified so as to prevent or inhibit fucosylation of the antibody. In some embodiments, such a modified antibody may exhibit increased ADCC activity as compared to the unmodified antibody. The modification may be in the heavy chain, light chain, or a combination thereof. The modification may be one or more amino acid substitutions in the heavy chain, one or more amino acid substitutions in the light chain, or a combination thereof.

e. Reduced ADE Response

[00176] The antibody may be modified to reduce or prevent antibody-dependent enhancement (ADE) of disease associated with the antigen, but still neutralize the antigen.

[00177] In some embodiments, the antibody may be modified to include one or more amino acid substitutions that reduce or prevent binding of the antibody to FcyR1a. The one or more amino acid substitutions may be in the constant region of the antibody. The one or more amino acid substitutions may include replacing a leucine residue with an alanine residue in the constant region of the antibody, i.e., also known herein as LA, LA mutation or LA substitution. The one or more amino acid substitutions may include replacing two leucine residues, each with an alanine residue, in the constant region of the antibody and also known herein as LALA, LALA mutation, or LALA substitution. The presence of the LALA substitutions may prevent or block the antibody from binding to FcyR1a, and thus, the modified antibody does not enhance or cause ADE of disease associated with the antigen, but still neutralizes the antigen.

5. Target

[00178] The synthetic antibody is directed to a target or fragment or variant thereof. The target can be a nucleic acid sequence, an amino acid sequence, or a combination thereof. The nucleic acid sequence can be DNA, RNA, cDNA, a variant thereof, a fragment thereof, or a combination thereof. The amino acid sequence can be a protein, a peptide, a variant thereof, a fragment thereof, or a combination thereof.

[00179] In one embodiment, the target is IL-6. In one embodiment, the target is CD126. IL-6 and its receptor, CD126, stimulate the inflammatory and auto-immune processes in many diseases including, but not limited to, diabetes, atherosclerosis, depression, Alzheimer's Disease, systemic lupus erythematosus, multiple myeloma, cancer, Behçet's disease, and rheumatoid arthritis.

6. Excipients and Other Components of the Composition

[00180] The composition may further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient can be functional molecules such as vehicles, carriers, or diluents. The pharmaceutically acceptable excipient can be a transfection facilitating agent, which can include surface active agents, such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

[00181] The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and the poly-L-glutamate may be present in the composition at a concentration less than 6 mg/ml. The transfection facilitating agent may also include surface active agents such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid may also be used administered in conjunction with the composition. The composition may also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS),

or lipid. Concentration of the transfection agent in the vaccine is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than 0.050 mg/ml, or less than 0.010 mg/ml.

[00182] The composition may further comprise a genetic facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

[00183] The composition may comprise DNA at quantities of from about 1 nanogram to 100 milligrams; about 1 microgram to about 10 milligrams; or preferably about 0.1 microgram to about 10 milligrams; or more preferably about 1 milligram to about 2 milligram. In some preferred embodiments, composition according to the present invention comprises about 5 nanogram to about 1000 micrograms of DNA. In some preferred embodiments, composition can contain about 10 nanograms to about 800 micrograms of DNA. In some preferred embodiments, the composition can contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the composition can contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the composition can contain about 25 to about 250 micrograms, from about 100 to about 200 microgram, from about 1 nanogram to 100 milligrams; from about 1 microgram to about 10 milligrams; from about 0.1 microgram to about 10 milligrams; from about 1 milligram to about 2 milligram, from about 5 nanogram to about 1000 micrograms, from about 10 nanograms to about 800 micrograms, from about 0.1 to about 500 micrograms, from about 1 to about 350 micrograms, from about 25 to about 250 micrograms, from about 100 to about 200 microgram of DNA.

[00184] The composition can be formulated according to the mode of administration to be used. An injectable pharmaceutical composition can be sterile, pyrogen free and particulate free. An isotonic formulation or solution can be used. Additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol, and lactose. The composition can comprise a vasoconstriction agent. The isotonic solutions can include phosphate buffered saline. The composition can further comprise stabilizers including gelatin and albumin. The stabilizers can allow the formulation to be stable at room or ambient temperature for extended periods of time, including LGS or polycations or polyanions.

7. Method of Generating the Synthetic Antibody

[00185] The present invention also relates a method of generating the synthetic antibody. The method can include administering the composition to the subject in need thereof by using the method of delivery described in more detail below. Accordingly, the synthetic antibody is generated in the subject or in vivo upon administration of the composition to the subject.

[00186] The method can also include introducing the composition into one or more cells, and therefore, the synthetic antibody can be generated or produced in the one or more cells. The method can further include introducing the composition into one or more tissues, for example, but not limited to, skin and muscle, and therefore, the synthetic antibody can be generated or produced in the one or more tissues.

8. Method of Identifying or Screening for the Antibody

[00187] The present invention further relates to a method of identifying or screening for the antibody described above, which is reactive to or binds the antigen described above. The method of identifying or screening for the antibody can use the antigen in methodologies known in those skilled in art to identify or screen for the antibody. Such methodologies can include, but are not limited to, selection of the antibody from a library (e.g., phage display) and immunization of an animal followed by isolation and/or purification of the antibody.

9. Method of Delivery of the Composition

[00188] The present invention also relates to a method of delivering the composition to the subject in need thereof. The method of delivery can include, administering the composition to the subject. Administration can include, but is not limited to, nucleic acid (i.e., DNA and/or RNA, or modified versions thereof) injection with and without in vivo electroporation, liposome mediated delivery, and nanoparticle facilitated delivery.

[00189] The mammal receiving delivery of the composition may be human, primate, non-human primate, cow, cattle, sheep, goat, antelope, bison, water buffalo, bison, bovids, deer, hedgehogs, elephants, llama, alpaca, mice, rats, and chicken.

[00190] The composition may be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal. The composition may be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gone guns", or other physical methods such as electroporation (“EP”), “hydrodynamic method”, or ultrasound.

a. Electroporation

[00191] Administration of the composition via electroporation may be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal, a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation device may comprise an electroporation component and an electrode assembly or handle assembly. The electroporation component may include and incorporate one or more of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The electroporation may be accomplished using an in vivo electroporation device, for example CELLECTRA EP system (Inovio Pharmaceuticals, Plymouth Meeting, PA) or Elgen electroporator (Inovio Pharmaceuticals, Plymouth Meeting, PA) to facilitate transfection of cells by the plasmid.

[00192] The electroporation component may function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component may function as more than one element of the electroporation devices, which may be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device may not limited as the elements can function as one device or as separate elements in communication with one another. The electroporation component may be capable of delivering the pulse of energy that produces the constant current in the desired tissue, and includes a feedback mechanism. The electrode assembly may include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism may receive the measured impedance and can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

[00193] A plurality of electrodes may deliver the pulse of energy in a decentralized pattern. The plurality of electrodes may deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed

sequence is input by a user to the electroporation component. The programmed sequence may comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

[00194] The feedback mechanism may be performed by either hardware or software. The feedback mechanism may be performed by an analog closed-loop circuit. The feedback occurs every 50 μs, 20 μs, 10 μs or 1 μs, but is preferably a real-time feedback or instantaneous (i.e., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode may measure the impedance in the desired tissue and communicates the impedance to the feedback mechanism, and the feedback mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism may maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

[00195] Examples of electroporation devices and electroporation methods that may facilitate delivery of the composition of the present invention, include those described in U.S. Patent No. 7,245,963 by Draghia-Akli, et al., U.S. Patent Pub. 2005/0052630 submitted by Smith, et al., the contents of which are hereby incorporated by reference in their entirety. Other electroporation devices and electroporation methods that may be used for facilitating delivery of the composition include those provided in co-pending and co-owned U.S. Patent Application, Serial No. 11/874072, filed October 17, 2007, which claims the benefit under 35 USC 119(e) to U.S. Provisional Applications Ser. Nos. 60/852,149, filed October 17, 2006, and 60/978,982, filed October 10, 2007, all of which are hereby incorporated in their entirety.

[00196] U.S. Patent No. 7,245,963 by Draghia-Akli, et al. describes modular electrode systems and their use for facilitating the introduction of a biomolecule into cells of a selected tissue in a body or plant. The modular electrode systems may comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The biomolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical

pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the biomolecule into the cell between the plurality of electrodes. The entire content of U.S. Patent No. 7,245,963 is hereby incorporated by reference.

[00197] U.S. Patent Pub. 2005/0052630 submitted by Smith, et al. describes an electroporation device which may be used to effectively facilitate the introduction of a biomolecule into cells of a selected tissue in a body or plant. The electroporation device comprises an electro-kinetic device ("EKD device") whose operation is specified by software or firmware. The EKD device produces a series of programmable constant-current pulse patterns between electrodes in an array based on user control and input of the pulse parameters, and allows the storage and acquisition of current waveform data. The electroporation device also comprises a replaceable electrode disk having an array of needle electrodes, a central injection channel for an injection needle, and a removable guide disk. The entire content of U.S. Patent Pub. 2005/0052630 is hereby incorporated by reference.

[00198] The electrode arrays and methods described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/0052630 may be adapted for deep penetration into not only tissues such as muscle, but also other tissues or organs. Because of the configuration of the electrode array, the injection needle (to deliver the biomolecule of choice) is also inserted completely into the target organ, and the injection is administered perpendicular to the target issue, in the area that is pre-delineated by the electrodes The electrodes described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/005263 are preferably 20 mm long and 21 gauge.

[00199] Additionally, contemplated in some embodiments that incorporate electroporation devices and uses thereof, there are electroporation devices that are those described in the following patents: US Patent 5,273,525 issued December 28, 1993, US Patents 6,110,161 issued August 29, 2000, 6,261,281 issued July 17, 2001, and 6,958,060 issued October 25, 2005, and US patent 6,939,862 issued September 6, 2005. Furthermore, patents covering subject matter provided in US patent 6,697,669 issued February 24, 2004, which concerns delivery of DNA using any of a variety of devices, and US patent 7,328,064 issued February 5, 2008, drawn to method of injecting DNA are contemplated herein. The above-patents are incorporated by reference in their entirety.

10. Method of Treatment

[00200] Also provided herein is a method of treating, protecting against, and/or preventing disease in a subject in need thereof by generating the synthetic antibody in the subject. The

method can include administering the composition to the subject. Administration of the composition to the subject can be done using the method of delivery described above.

[00201] In certain embodiments, the invention provides a method of treating protecting against, and/or preventing a disease associated with IL-6 and/or CD126. For example, in one embodiment, the method treats, protects against, and/or prevents an auto-immune disorder. In one embodiment, the method treats, protects against, and/or prevents cancer. Exemplary diseases or disorders treated or prevented by way of the administration of the composition of the invention, includes, but is not limited to diabetes, atherosclerosis, depression, Alzheimer's Disease, systemic lupus erythematosus, multiple myeloma, cancer, Behçet's disease, rheumatoid arthritis, sepsis, bacterial infection, viral infection, fungal infection, multicentric Castleman disease, any disease associated with high fever, graft-versus-host (GVH) disease, cell lysis syndrome, and the like.

[00202] Upon generation of the synthetic antibody in the subject, the synthetic antibody can bind to or react with the antigen. Such binding can neutralize the antigen, block recognition of the antigen by another molecule, for example, a protein or nucleic acid, and elicit or induce an immune response to the antigen, thereby treating, protecting against, and/or preventing the disease associated with the antigen in the subject.

[00203] The composition dose can be between 1 μg to 100 mg active component/kg body weight/time, and can be 20 μg to 100 mg component/kg body weight/time. The composition can be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days. The number of composition doses for effective treatment can be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00204] The present invention has multiple aspects, illustrated by the following non-limiting examples.

11. Examples

[00205] The present invention is further illustrated in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in

the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Example 1

[00206] The studies presented herein demonstrate the generation of functional anti-IL-6 and anti-CD126 "DNA monoclonal antibodies" (DMAb) via intramuscular electroporation of plasmid DNA.

[00207] These studies demonstrate functional DNA monoclonal antibodies (DMAb) targeting IL-6 and CD126 are expressed in vivo. Codon-optimized variable region DNA sequences from four anti-IL-6 and two anti-CD126 monoclonal antibodies on the human IgG1 constant domain were constructed. Plasmid DNA encoding each antibody was delivered intramuscularly with electroporation to nude and immune-competent mice. multiple aspects of DMAb delivery were optimized- including antibody sequence, plasmid heavy and light chain arrangement, and formulation – to enhance in vivo expression.

[00208] Anti-IL-6 and anti-CD126 DMAb were expressed in serum with levels ranging from 1.5 μg/mL to 7.1 μg/mL in BALB/c mice. Also, long-term DMAb expression in nude mice was observed. Serum DMAb retained functional binding to purified IL-6 and CD126. Serum DMAb also blocked downstream IL-6 cell signaling in vitro. Studies are conducted to investigate anti-IL-6 and anti-CD126 DMAb for their role in controlling sepsis, limiting inflammation during acute viral infection, and slowing tumor progression. These studies not only provide a novel method to further define the role of in vivo IL-6 signaling in immune pathologies, but also define DMAb as an alternative to protein antibody therapies.

[00209] This study supports DMAb as an alternative to existing biologic therapies, and provides a novel method to further define the role of in vivo IL-6 signaling in immune pathologies.

[00210] The methods and materials are now described

Antibody DNA Sequences & Cloning:

[00211] Variable VH and VL amino acid sequences of anti-IL6 antibodies (Clazakizumab [Alder Biopharmaceuticals], Olokizumab [R-Pharm], Siltuximab [Sylvant®, Janssen Biotech], Sirukumab [Centocor/GSK]) and anti-CD126 antibodies (Sarilumab [Regeneron Pharmacauticals], Tocilizumab [Actemra®, Genentech]) were codon-optimized. DNA sequences were synthesized with codon-optimized constant human IgG1κ and cloned into a

modified pVax-1 (Invitrogen) mammalian expression plasmid. A furin/2A peptide cleavage site was included for separation of heavy and light-chain peptides (Figure 1).

Transfections:

[00212] $1x10^6$ 293T cells were transfected with 0.5μg plasmid DNA using GeneJammer (Agilent Technologies). Cell supernatants and whole lysates were collected 48 hours post-transfection.

DMAb Electroporation:

[00213] BALB/c mice received 100μg of formulated plasmid DNA delivered intramuscularly to the quadriceps followed by electroporation with a CELLECTRA® 3P device (Inovio Pharmaceuticals, Plymouth Meeting, PA) as previously described (Flingai et al., 2015, Sci Rep, 5:12616; Muthumani et al., 2013, Hum Vaccin Immunother, 9(10): 2253-63.

ELISA & Western Blots:

[00214] Human IgG1κ were captured using anti-human-Fc fragments and detected with secondary anti-kappa-light-chain HRP conjugated antibody, with quantification against a human IgG1κ control (Bethyl). Binding to recombinant human IL-6 and CD126 (Sino Biological) was detected with HRP-conjugated anti-human-IgG secondary antibody (Sigma). Western blots were developed with conjugated anti-human IgG 800nm antibody (Licor).

STAT3 Signaling Assay:

[00215] HEK-BlueTM 293 cells stably transfected with human CD126 and STAT3-induced secreted alkaline phosphatase were purchased from InVivoGen. Mouse serum was diluted 1:40 in culture media and added to cells treated with 1ng/mL recombinant human IL-6. Supernatant SEAP was assayed 24 hours later by calorimetric QuantiBlueTM assay (InVivoGen). Absorbance values were normalized to SEAP expression in cells receiving sera from un-treatead (No DMAb) mice. 10μg/mL TNFalpha served as control.

[00216] The results of the experiments are now described

Intramuscular electroporation of plasmid DNA containing anti-IL-6 and anti-CD126 antibody sequences generates monoclonal antibodies from muscle tissue in vivo

[00217] Codon-optimized variable region DNA sequences from anti-IL-6 and anti-CD126 monoclonal antibodies were synthesized onto a human IgG1 constant domain. Plasmid DNA encoding antibody was delivered to BALB/c mice (Figure 1). Monoclonal antibody variable VH and VL amino acid sequences were DNA codon optimized. The codon optimized DNA was synthesized with human IgG1κ antibody constant CH and CL region DNA sequences.

The engineered DNA sequence was cloned into a modified pVax-1 expression vector. The plasmid construct was injected intramuscularly followed by electroporation with CELLECTRA® device (Inovio Pharmaceuticals). Expression and function of human IgG1κ produced in vivo was measured.

DMAb constructs are expressed and secreted from transfected 293T cells

[00218] Experiments were conducted to evaluate the expression and secretion of anti-IL-6 and anti-CD126 encoded by the DMAb construct. HEK 293T cells were transfected with plasmid DNA carrying anti-IL-6 or anti-CD126 constructs. Empty plasmid served as a negative control. Human IgG1κ expression was determined by quantitative ELISA and Western blots were performed to detect supernatant heavy and light-chain peptide cleavage and expression (Figure 2A – Figure 2C). As shown in Figure 2A and Figure 2B, anti-IL-6 and anti-CD126 is observed in HEK 293T supernatant and HEK 293T lysate demonstrating the ability for the DMAb construct to induce the expression and secretion of anti-IL-6 and anti-CD126.

Robust serum levels of anti-IL-6 and anti-CD126 DNA monoclonal antibodies following DNA electroporation in mice

[00219] Experiments were conducted to evaluate whether the DMAb induced the expression of anti-IL-6 and anti-CD126 in vivo. BALB/c mice were injected with 100μg i.m. plasmid DNA followed by electroporation. Seven days later, serum human IgG1κ antibody levels were determined by ELISA. As shown in Figure 3A and Figure 3B, high levels of anti-IL-6 and anti-CD126 antibody are produced in mouse serum following DNA electroporation of muscle.

Serum DNA monoclonal antibodies bind target antigens IL-6 and CD126

[00220] Experiments were conducted to investigate the functionality of expressed anti-IL-6 and anti-CD126. BALB/c mice were injected with 100μg plasmid DNA followed by intramuscular electroporation. One week later, serum human-IgG antibody binding to recombinant human IL-6 and human CD126 was determined by ELISA. As shown in Figure 4, the expressed antibodies bind to target IL-6 and CD126 antigens.

Serum DNA monoclonal antibodies block IL-6-mediated cell signaling in vitro

[00221] Experiments were conducted to investigate whether the expressed antibodies can inhibit IL-6 mediated signaling. HEK-293 cells which were stably transfected with human CD126 and a STAT3-inducible secreted alkaline phosphatase (SEAP) were obtained. Diluted (1:40) serum from untreated mice induced a baseline level of mouse-IL-6-driven SEAP

expression, which was normalized to 100% SEAP activity in cell supernatants. Day-7 serum from DMAb-electroporated mice was diluted (1:40) and cell supernatants were assayed for SEAP activity as a percentage of untreated control. The HEK-293 cells secrete SEAP in response to IL-6 signaling. As shown in Figure 5, serum from mice treated with the DMAb construct encoding anti-IL-6 blocked SEAP activity, demonstrating that the encoded antibodies can block IL-6 mediated signaling.

[00222] The experiments presented herein demonstrate that anti-IL-6 and anti-CD126 DNA Monoclonal Antibodies (DMAb) are expressed in vivo at high levels in mouse serum following intramuscular electroporation of plasmid DNA constructs expressing codon-optimized antibody variable sequences. Antibodies produced from muscle cells in vivo are functional, binding and signaling in vitro. DMAb provide a safe, economical, practical alternative to purified protein monoclonal antibody therapies targeting IL-6 and CD126. The role of IL-6 in controlling sepsis, limiting inflammation during acute viral infection, and slowing tumor progression is conducted.

[00223] DMAb have several advantages over purified protein mAb and viral-vectors. With respect to protein mAb, DMAb is relatively inexpensive to manufacture; thermally stable; easy to distribute; modifiable; and induces persistent expression without need for frequent re-administration. With respect to viral vectors, DMAb is safe and non-integrating; non-immunogenic; can be delivered repeatedly; no pre-existing serology; and induces acute expression for rapid administration. Potent & persistent expression of DMAb provides a substantial benefit in treatment of chronic conditions with potential need for re-dosing, such as cancer and auto-immune disease. Inexpensive DNA vector production & distribution provides enhanced affordability, especially in the developing world and where there is chronic need.

[00224] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents.

[00225] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, compositions, formulations, or methods of use of the invention, may be made without departing from the spirit and scope thereof.

CLAIMS

What is claimed is:

1. A composition comprising one or more nucleic acid molecules encoding one or more synthetic antibodies, wherein the one or more nucleic acid molecules comprise at least one selected from the group consisting of

a) a nucleotide sequence encoding an anti-IL-6 synthetic antibody;

b) a nucleotide sequence encoding a fragment of an anti-IL-6 synthetic antibody;

c) a nucleotide sequence encoding an anti-CD126 antibody; and

d) a nucleotide sequence encoding a fragment of an anti-CD126 antibody.

2. The composition of claim 1, comprising a nucleotide sequence encoding an anti-IL-6 synthetic antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, a fragment of SEQ ID NO: 2, an amino acid sequence having greater than 90% sequence identity to SEQ ID NO: 2, SEQ ID NO: 4, a fragment of SEQ ID NO: 4, an amino acid sequence having greater than 90% sequence identity to SEQ ID NO: 4, SEQ ID NO: 6, a fragment of SEQ ID NO: 6, an amino acid sequence having greater than 90% sequence identity to SEQ ID NO: 6, SEQ ID NO: 8, a fragment of SEQ ID NO: 8, or an amino acid sequence having greater than 90% sequence identity to SEQ ID NO: 8.

3. The composition of claim 1, wherein the nucleotide sequence encoding an anti-IL-6 synthetic antibody comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, a fragment of SEQ ID NO: 1, a nucleotide sequence having greater than 90% sequence identity to SEQ ID NO: 1, SEQ ID NO: 3, a fragment of SEQ ID NO: 3, a nucleotide sequence having greater than 90% sequence identity to SEQ ID NO: 3, SEQ ID NO: 5, a fragment of SEQ ID NO: 5, a nucleotide sequence having greater than 90% sequence identity to SEQ ID NO: 5, SEQ ID NO: 7, a fragment of SEQ ID NO: 7, or a nucleotide sequence having greater than 90% sequence identity to SEQ ID NO: 7.

4. The composition of claim 1, comprising a nucleotide sequence encoding an anti-CD126 synthetic antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10, a fragment of SEQ ID NO: 10, an amino acid sequence having greater than 90% sequence identity to SEQ ID NO: 10, SEQ ID NO: 12, a fragment of SEQ ID NO: 12, and an amino acid sequence having greater than 90% sequence identity to SEQ ID NO: 12.

5. The composition of claim 1, wherein the nucleotide sequence encoding an anti-CD126 synthetic antibody comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 9, a fragment of SEQ ID NO: 9, a nucleotide sequence having greater than 90% sequence identity to SEQ ID NO: 9, SEQ ID NO: 11, a fragment of SEQ ID NO: 11, and a nucleotide sequence having greater than 90% sequence identity to SEQ ID NO: 11.

6. The composition of claim 1, comprising a first nucleotide sequence encoding an anti-IL-6 synthetic antibody; and a second nucleotide sequence encoding an anti-CD126 antibody.

7. The composition of claim 1, further comprising a nucleotide sequence encoding a cleavage domain.

8. The composition of claim 1, comprising a nucleotide sequence encoding a variable heavy chain region and a variable light chain region of anti-IL-6.

9. The composition of claim 1, comprising a nucleotide sequence encoding a variable heavy chain region and a variable light chain region of anti-CD126.

10. The composition of claim 1, comprising a nucleotide sequence encoding a constant heavy chain region and a constant light chain region of human IgG1κ.

11. The composition of claim 1, comprising a nucleotide sequence encoding a polypeptide comprising a variable heavy chain region of anti-IL-6; a constant heavy chain region of human IgG1κ; a cleavage domain; a variable light chain region of anti-IL-6; and a constant light chain region of IgG1κ.

12. The composition of claim 1, comprising a nucleotide sequence encoding a polypeptide comprising a variable heavy chain region of anti-CD126; a constant heavy chain region of human IgG1κ; a cleavage domain; a variable light chain region of anti-CD126; and a constant light chain region of IgG1κ.

13. The composition of claim 1, wherein the nucleotide sequence encodes a leader sequence.

14. The composition of any one of claims 1-13, wherein the nucleic acid molecule comprises an expression vector.

15. A composition comprising the nucleic acid molecule of any one of claims 1-14.

16. The composition of claim 15, further comprising a pharmaceutically acceptable excipient.

17. A method of treating a disease in a subject, the method comprising administering to the subject the composition of any of claims 1-14 or a composition of any of claims 15-16.

18. The method of claim 17, wherein the disease is cancer.

19. The method of claim 17, wherein the disease is an auto-immune disease.

20. The method of claim 17, wherein the disease is sepsis.

21. The method of claim 17, wherein the disease is a viral infection.

22. The method of claim 17, wherein the disease is multicentric Castleman disease.

23. The method of claim 17, wherein the disease is associated with high fever.

24. The method of claim 17, wherein the disease is graft-versus-host (GVH) disease.

25. The method of claim 17, wherein the disease is cell lysis syndrome.

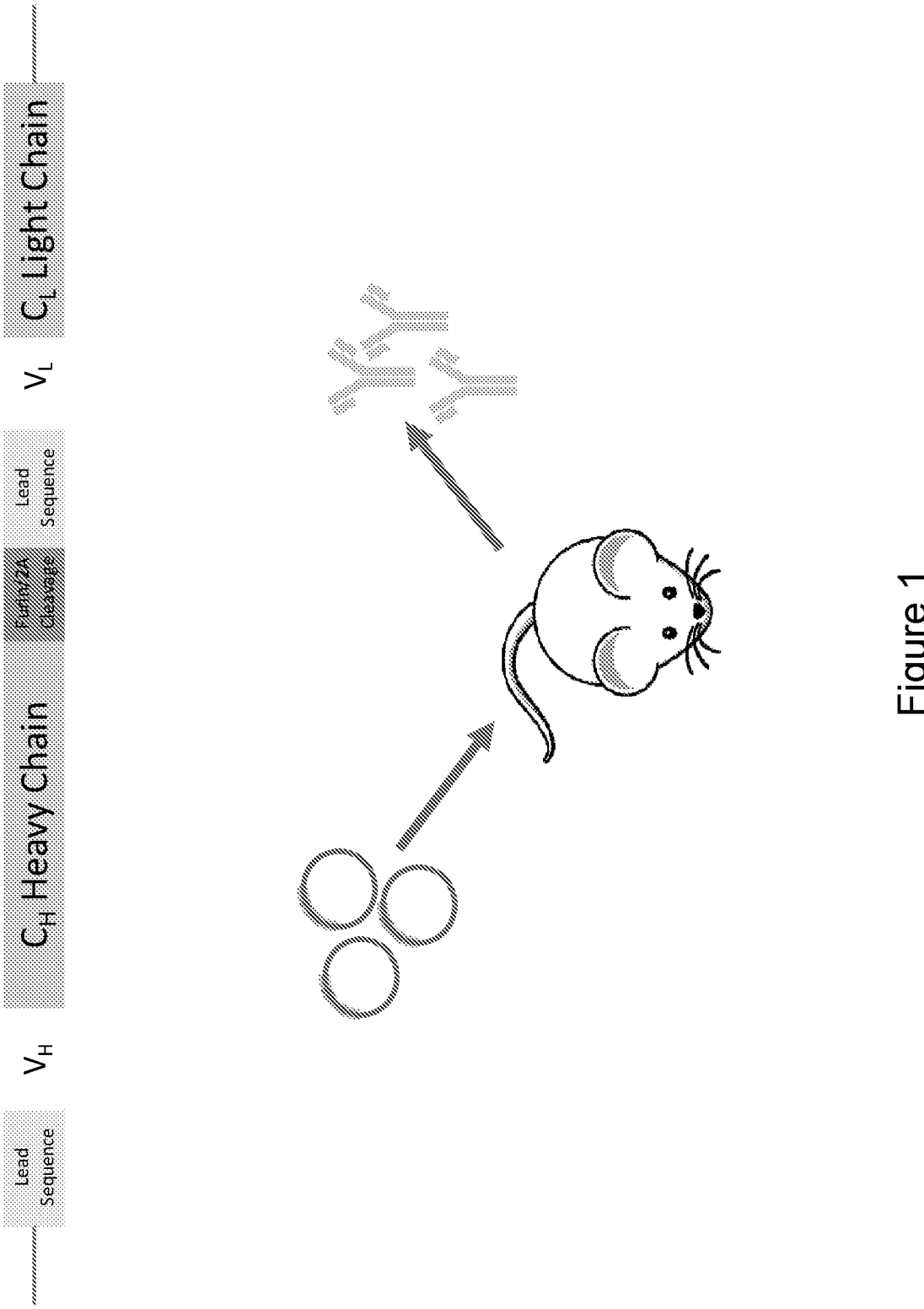

Lead Sequence
V_H
C_H Heavy Chain
Furin/2A Cleavage
Lead Sequence
V_L
C_L Light Chain

Figure 1

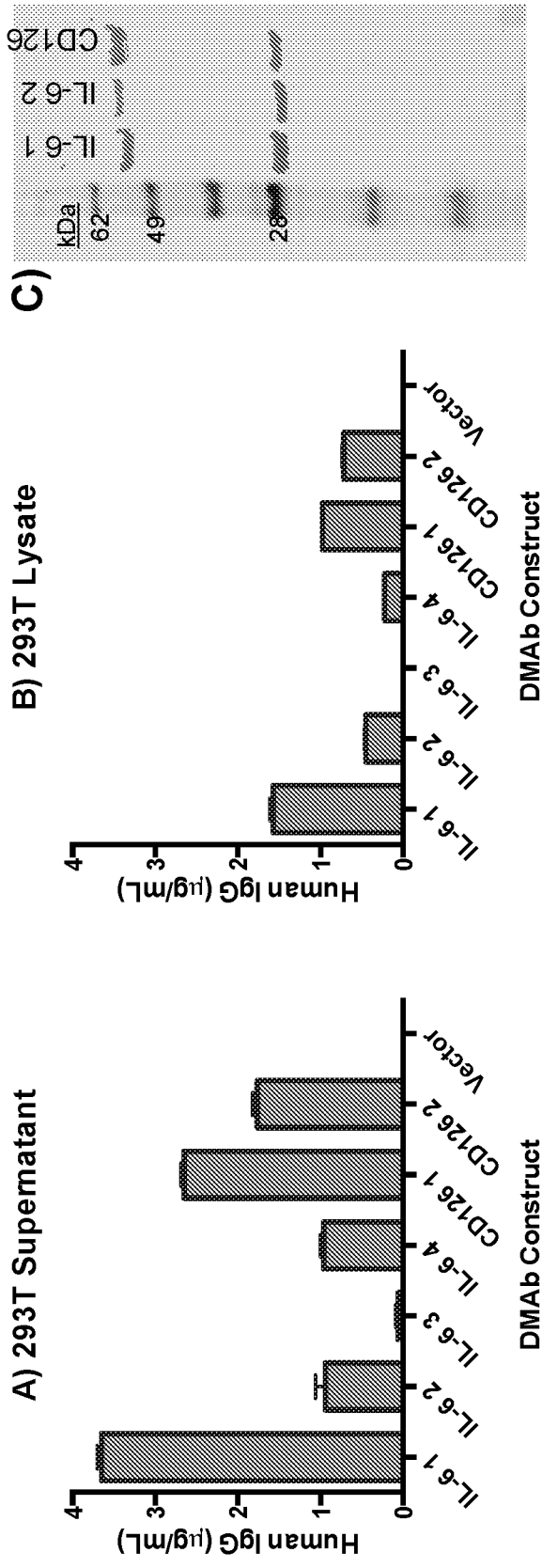

A) 293T Supernatant
Human IgG (µg/mL)
0
1
2
3
4
IL-6 1
IL-6 2
IL-6 3
IL-6 4
CD126 1
CD126 2
Vector
DMAb Construct
B) 293T Lysate
Human IgG (µg/mL)
0
1
2
3
4
IL-6 1
IL-6 2
IL-6 3
IL-6 4
CD126 1
CD126 2
Vector
DMAb Construct
C)
kDa
62
49
28
IL-6 1
IL-6 2
CD126 1

Figure 2

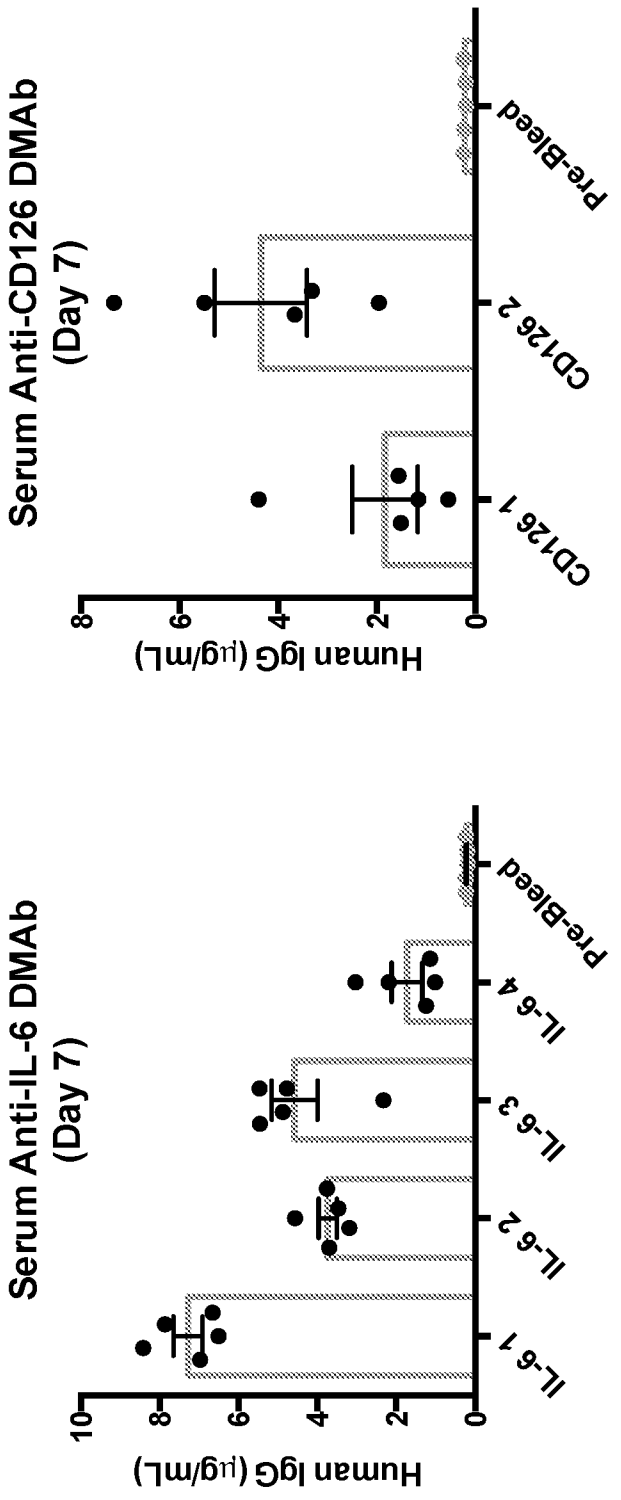

Serum Anti-IL-6 DMAb
(Day 7)
Human IgG (μg/mL)
0
2
4
6
8
10
IL-6 1
IL-6 2
IL-6 3
IL-6 4
Pre-Bleed
Serum Anti-CD126 DMAb
(Day 7)
Human IgG (μg/mL)
0
2
4
6
8
CD126 1
CD126 2
Pre-Bleed

Figure 3

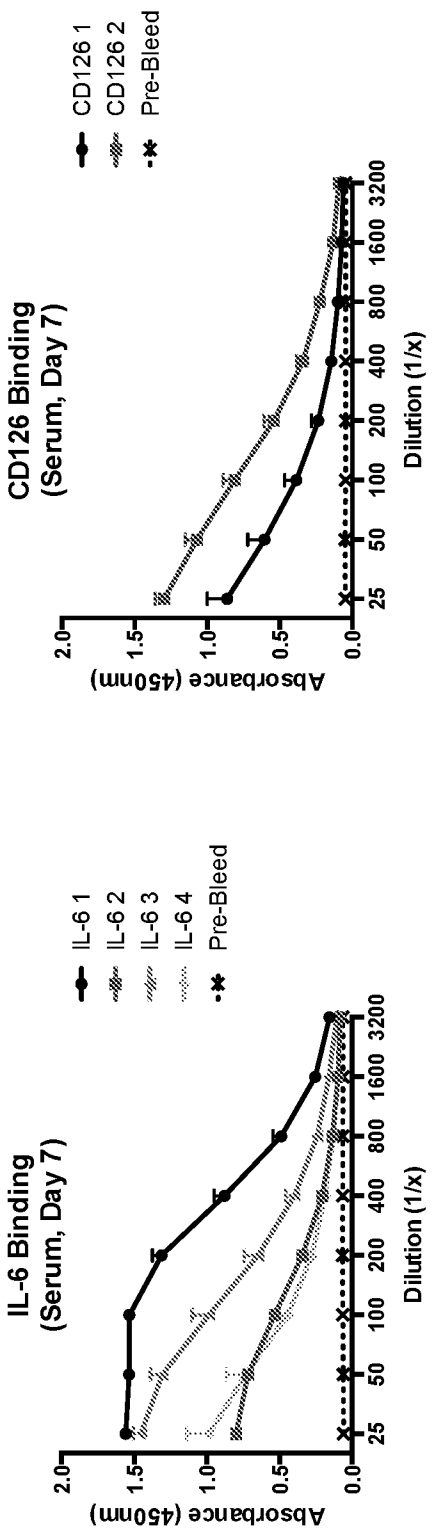

CD126 Binding
(Serum, Day 7)
CD126 1
CD126 2
Pre-Bleed
Absorbance (450nm)
Dilution (1/x)
IL-6 Binding
(Serum, Day 7)
IL-6 1
IL-6 2
IL-6 3
IL-6 4
Pre-Bleed
Absorbance (450nm)
Dilution (1/x)

Figure 4

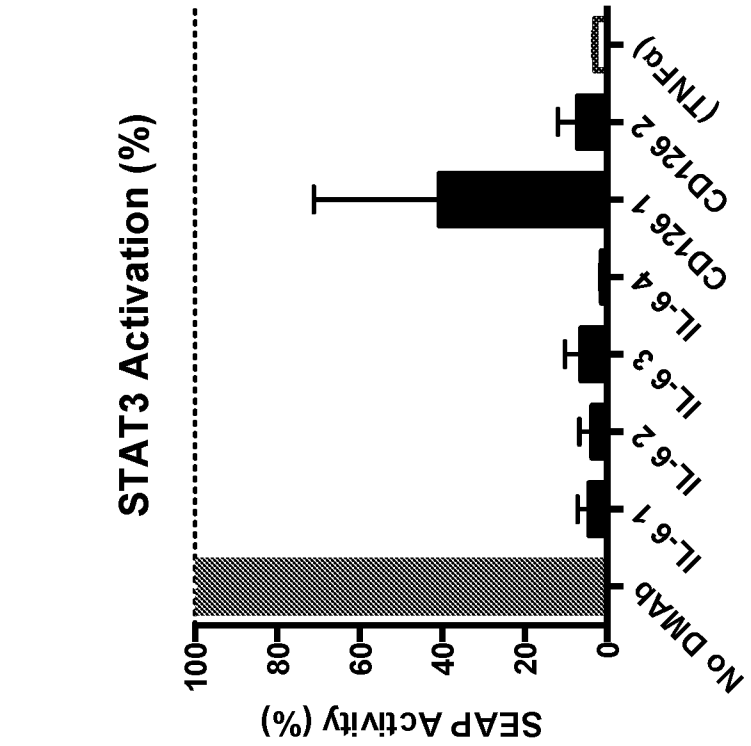
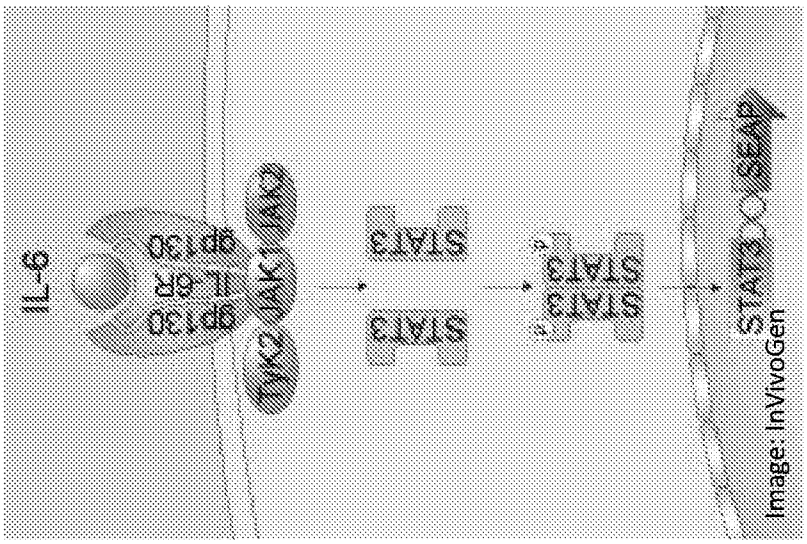

STAT3 Activation (%)
SEAP Activity (%)
100
80
60
40
20
0
No DMAb
IL-6 1
IL-6 2
IL-6 3
IL-6 4
CD126 1
CD126 2
(TNFα)

Figure 5


IL-6
gp130
IL-6R
gp130
JAK1
JAK2
TyK2
STAT3
STAT3
P
P
STAT3
STAT3
SEAP
Image: InVivoGen

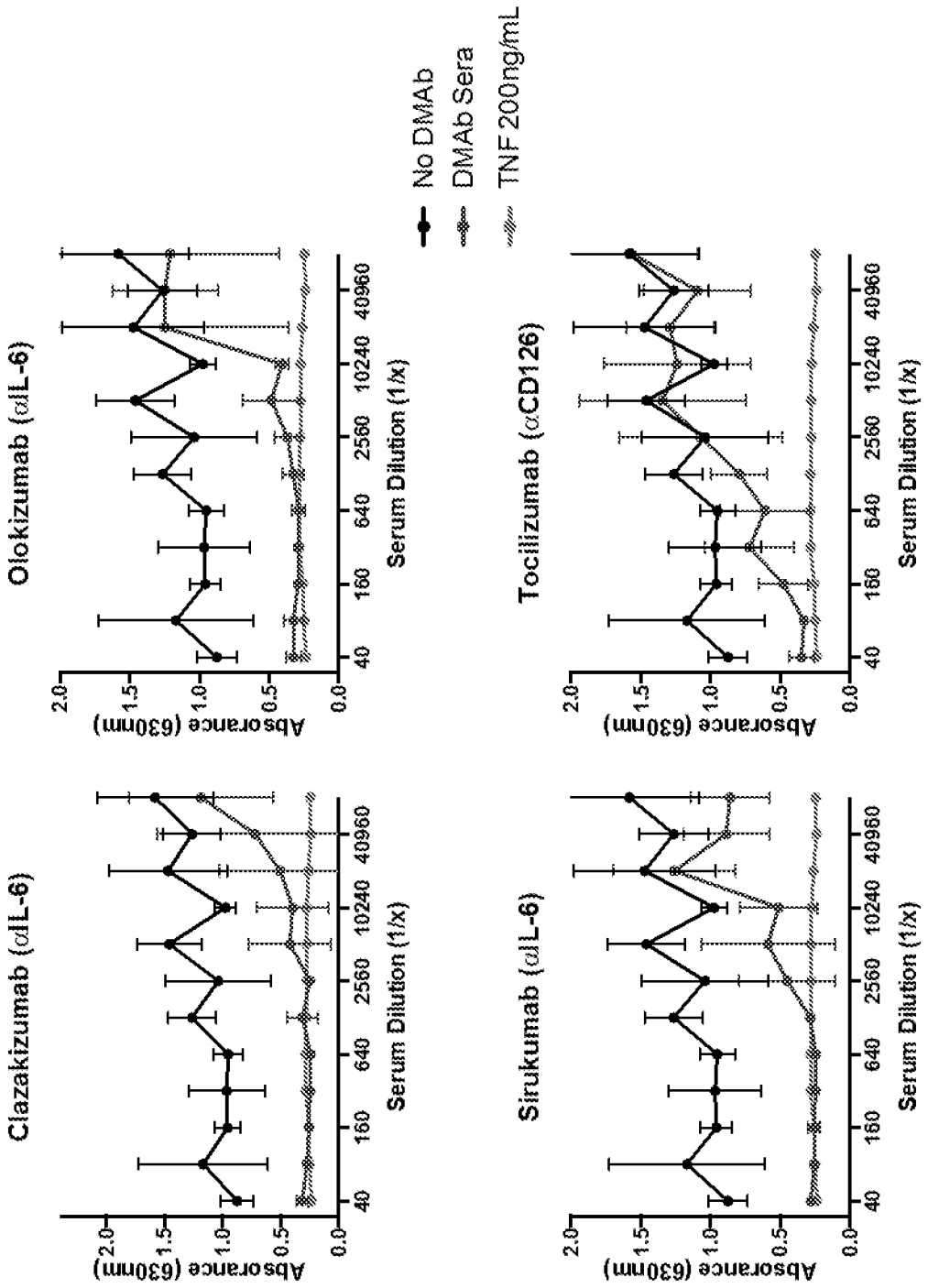

Olokizumab (αIL-6)
Clazakizumab (αIL-6)
Tocilizumab (αCD126)
Sirukumab (αIL-6)
Absorance (630nm)
Serum Dilution (1/x)
No DMAb
DMAb Sera
TNF 200ng/mL

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/31193

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 48/00; A61K 39/395; C07K 16/24 (2017.01)
CPC - C07K 16/248, 2317/21, 2317/51, 2317/515, 2317/52, 2317/76, 2319/50; A61K 48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- A	US 2015/0284448 A1 (The Trustees of the University of Pennsylvania) 8 October 2015 (08.10.2015). Especially para [0009], [0025], [0213], [0214], [0290], [0378], [0379], [0382], SEQ ID NOs: 1, 62	1, 7, 8, 10, 11, 13, 14/(1,7,8,10,11,13) ---------- 2, 3, 14/(2, 3)
A	US 2008/0075726 A1 (Smith et al.) 9 October 2008 (09.10.2008). Especially para [0012], [0016]	1, 7, 8, 10, 11, 13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 July 2017	29 SEP 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/31193

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☒ on paper or in the form of an image file.

   b. ☒ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
GenCore ver 6.4.1 SEQ ID NOs: 1, 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/31193

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

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

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

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

3. ☒ Claims Nos.: 15-25
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
-----go to Extra Sheet for continuation-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 7, 8, 10, 11, 13, 14 limited to SEQ ID NOs: 1-2

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/31193

----continuation of Box III (Lack of Unity of Invention)-----

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

Group I+: Claims 1-14, drawn to nucleic acid molecule composition encoding a synthetic antibody.
The synthetic antibody will be searched to the extent that it comprises an anti-IL-6 antibody (claim 1), with the amino acid sequence SEQ ID NO: 2 (claim 2) and nucleotide sequence SEQ ID NO: 1 (claim 3). It is believed that claims 1-3, 7, 8, 10, 11, 13, 14 (in part) read on this first named invention and thus these claims will be searched without fee to the extent that they encompass anti-IL-6 synthetic antibody, SEQ ID NOs: 1, 2. Additional synthetic antibodies and their amino acid and nucleotide sequences will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected synthetic antibodies. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election anti-CD126 synthetic antibody, SEQ ID NOs: 10, 9 (claims 1, 4-6, 9, 10, 12, 13, 14 (in part)).

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

Special Technical Features:

Among the inventions listed as Groups I+ are the specific synthetic antibodies, recited therein. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained among the nucleic acid sequences and the amino acid sequences of each synthetic antibody.

Common Technical Features:

Group I+ inventions share the common technical features of:

1. a nucleotide sequence encoding a synthetic antibody.

2. an anti-IL6 antibody

3. an anti-CD126 antibody [CD126 is also known as IL-6 Receptor alpha subunit (IL-6Ra)]

However, said common technical features do not represent a contribution over the prior art, and are obvious over US 2015/0284448 A1 to The Trustees of the University of Pennsylvania (hereinafter "Penn"), in view of US 2008/0075726 A1 to Smith et al. (hereinafter "Smith") and US 2010/0098709 A1 to Bowers et al. (hereinafter "Bowers").

As to common technical feature #1, Penn teaches a nucleotide sequence encoding a synthetic antibody (para [0009]; "The present invention is directed to a method of generating a synthetic antibody in a subject. The method can comprise administering to the subject a composition comprising a recombinant nucleic acid sequence encoding an antibody or fragment thereof. The recombinant nucleic acid sequence can be expressed in the subject to generate the synthetic antibody").

As to common technical feature #2, Smith teaches polynucleotides and amino acids encoding an anti-IL6 antibody (para [0012]; "The present invention provides novel monoclonal antibodies that bind specifically to IL-6. The antibodies of the invention comprise a variable heavy chain (VH) region selected from any of the VH regions disclosed herein as well as amino acid variants thereof, and/or a variable light chain (VL) region selected from any of the VL regions disclosed herein as well as amino acid variants thereof"; para [0016]; "The invention also includes nucleic acid molecules that encode any of the VH and/or VL regions disclosed herein, and vectors and host cells comprising the nucleic acid molecules").

As to common technical feature #3, Bowers teaches an anti-CD126 [IL-6Ra] antibody (para [0021]; a humanised anti-IL-6Ra antibody Tocilizumab (also known as hPM-1, MRA and Actemra). This is a humanised version of the murine anti-IL6Ra antibody PM-1"; para [0064]; IL-6 receptor a, IL-6Ra, is the receptor for interleukin 6. IL-6Ra is also known as IL-6Ralpha, IL-6Ra, IL-6R and CD126")

As the common technical features were known in the art at the time of the invention, they cannot be considered a common special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Group I+ inventions lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Continuation of item 4: Claims 15-25 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).