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(54) Title: ANTI-NUCLEOLIN ANTIBODIES

(57) Abstract: The present disclosure provides anti-nucleolin antibodies, methods of producing anti-nucleolin antibodies, and cells producing anti-nucleolin antibodies. Also provided are methods of using anti-nucleolin antibodies in treating malignant and non-malignant diseases.

ANTI-NUCLEOLIN ANTIBODIES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/304,742, filed on March 7, 2016, U.S. Provisional Application No. 62/323,159, filed on April 15, 2016, U.S. Provisional Application No. 62/414,316, filed on October 28, 2016, all of which are incorporated herein by reference in their entirety.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] The invention was made with government support under grant number NCI CA109254-04S1 awarded by the National Cancer Institute, National Institutes of Health of the United States, and grants # W81XWH-12-1-0241 and # W81XWH-12-1-0242 awarded by the U.S. Army Medical Research and Materiel Command. The government has certain rights in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 7, 2017, is named 39723-709_601_SL.txt and is 60,062 bytes in size.

INCORPORATION BY REFERENCE

[0004] All publications, patents, and patent applications disclosed herein are incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. In the event of a conflict between a term disclosed herein and a term in an incorporated reference, the term herein controls.

BACKGROUND

[0005] Antibodies are a class of agents known as "biologicals." The source of antibodies can be a polyclonal supply, such as human or horse serum, or derived from a monoclonal source (single cell clone). With the technologic capability to control and select for specific antigen binding, monoclonal antibodies have yielded dramatic therapeutic benefits. However, the difficulty of generating specific antibodies for certain targets has limited the successes, and the potential for therapeutic agents remains largely untapped.

[0006] One impediment to the development of monoclonal antibodies for human therapy is the need to "humanize" such antibodies, which are generally made in mice, rats and rabbits. If human patients are administered such antibodies without humanized constant regions, they can

suffer from "serum sickness," meaning that an endogenous immune response is mounted by the recipient against the non-human antibody sequences. Humanizing monoclonal antibodies produced in research animals can avoid this problem. However, the cost in time and expense for humanization of antibodies can be considerable.

[0007] Nucleolin is expressed on the cell surface of chronic lymphocytic leukemia (CLL) cells, acute myeloid leukemia (AML) cells, some forms of breast carcinoma, as well as other tumors. As such, nucleolin constitutes a promising tumor antigen for targeting of therapeutics, including antibodies.

BRIEF SUMMARY

[0008] In some of many aspects, the present disclosure is directed to specific antibodies that immunologically recognize, bind to, and/or inactivate nucleolin. Also provided herein are light and heavy chain sequences of anti-nucleolin antibodies that may either be used directly to prepare anti-nucleolin antibodies (*e.g.*, human and/or monoclonal), or can be used to provide specific sequence elements (*e.g.*, CDR sequences) that may be incorporated into different, desired antibody backgrounds. Antibodies that comprise the sequences disclosed herein can be used in a wide range of therapies directed to disabling nucleolin.

[0009] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain CDR1 having at least 60% sequence identity to amino acid sequence SEQ ID NO:42; a heavy chain CDR2 having at least 60% sequence identity to amino acid sequence YIS; a heavy chain CDR3 having at least 60% sequence identity to amino acid sequence DM; a light chain CDR1 having at least 60% sequence identity to amino acid sequence SEQ ID NO:65; a light chain CDR2 having at least 60% sequence identity to amino acid sequence SEQ ID NO:54; and a light chain CDR3 having at least 60% sequence identity to amino acid sequence SEQ ID NO:66. In some embodiments, the at least 60% sequence identity is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

[0010] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain CDR1 has an amino acid sequence that comprises SEQ ID NO:42; a heavy chain CDR2 has an amino acid sequence that comprises YIS; a heavy chain CDR3 has an amino acid sequence that comprises DM; a light chain CDR1 has an amino acid sequence that comprises SEQ ID NO:65; a light chain CDR2 has an amino acid sequence that comprises SEQ ID NO:54; and a light chain CDR3 has an amino acid sequence that comprises SEQ ID NO:66.

[0011] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain

CDR1 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 24 to 26; a heavy chain CDR2 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of YIS and SEQ ID NOS. 30 to 32; a heavy chain CDR3 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 37 to 39; a light chain CDR1 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 46 to 48; a light chain CDR2 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 52 to 54; and a light chain CDR3 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 58 to 61. In some embodiments, the at least 60% sequence identity is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

[0012] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: the heavy chain CDR1 has the amino acid sequence of SEQ ID NO:24; the heavy chain CDR2 has the amino acid sequence of SEQ ID NO:30; the heavy chain CDR3 has the amino acid sequence of SEQ ID NO:37; the light chain CDR1 has the amino acid sequence of SEQ ID NO:46; the light chain CDR2 has the amino acid sequence of SEQ ID NO:52; and the light chain CDR3 has the amino acid sequence of SEQ ID NO:58.

[0013] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: the heavy chain CDR1 has the amino acid sequence of SEQ ID NO:25; the heavy chain CDR2 has the amino acid sequence of SEQ ID NO:31; the heavy chain CDR3 has the amino acid sequence of SEQ ID NO:38; the light chain CDR1 has the amino acid sequence of SEQ ID NO:47; the light chain CDR2 has the amino acid sequence of SEQ ID NO:53; and the light chain CDR3 has the amino acid sequence of SEQ ID NO:59.

[0014] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: the heavy chain CDR1 has the amino acid sequence of SEQ ID NO:26; the heavy chain CDR2 has the amino acid sequence of YIS; the heavy chain CDR3 has the amino acid sequence of SEQ ID NO:39; the light chain CDR1 has the amino acid sequence of SEQ ID NO:48; the light chain CDR2 has the amino acid sequence of SEQ ID NO:54; and the light chain CDR3 has the amino acid sequence of SEQ ID NO:60.

[0015] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: the heavy chain CDR1 has the amino acid sequence of SEQ ID NO:24; the heavy chain CDR2 has the amino

acid sequence of SEQ ID NO:32; the heavy chain CDR3 has the amino acid sequence of SEQ ID NO:37;the light chain CDR1 has the amino acid sequence of SEQ ID NO:46; the light chain CDR2 has the amino acid sequence of SEQ ID NO:52; and the light chain CDR3 has the amino acid sequence of SEQ ID NO:61.

[0016] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain variable region (VH) that has at least 60% sequence identity to the amino acid sequence of SEQ ID NO:3; and a light chain variable region (VL) that has at least 60% sequence identity to the amino acid sequence of SEQ ID NO:12. In some embodiments, the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

[0017] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a VH that has an amino acid sequence of SEQ ID NO:3; and a VL has the amino acid sequence of SEQ ID NO:12.

[0018] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain that has at least 60% sequence identity to the amino acid sequence of SEQ ID NO:2; and a light chain that has at least 60% identity to the amino acid sequence of SEQ ID NO:11. In some embodiments, the amino acid sequence of SEQ ID NO:2 is encoded by the nucleotide sequence of SEQ ID NO:1. In some embodiments, the amino acid sequence of SEQ ID NO:11 is encoded by the nucleotide sequence of SEQ ID NO:10. In some embodiments, the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

[0019] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain that has at least 60% sequence identity to the amino acid sequence of SEQ ID NO:14; and a light chain that has at least 60% identity to the amino acid sequence of SEQ ID NO:11. In some embodiments, the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

[0020] In some cases, provided herein is an isolated antibody or fragment thereof that comprises a heavy chain with the amino acid sequence of SEQ ID NO:2; and a light chain with the amino acid sequence of SEQ ID NO:11. In some cases, provided herein is an isolated antibody or fragment thereof that comprises a heavy chain with the amino acid sequence of SEQ ID NO:14; and a light chain with the amino acid sequence of SEQ ID NO:11.

[0021] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain CDR1 having at least 60% sequence identity to an amino acid sequence of DYF; a heavy chain

CDR2 having at least 60% sequence identity to an amino acid sequence of SEQ ID NO:74; a heavy chain CDR3 having at least 60% sequence identity to an amino acid sequence of AR or SEQ ID NO:77; a light chain CDR1 having at least 60% sequence identity to an amino acid sequence of SEQ ID NO:84; a light chain CDR2 having at least 60% sequence identity to an amino acid sequence of NVS; and a light chain CDR3 having at least 60% sequence identity to an amino acid sequence of SEQ ID NO:91. In some embodiments, the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

[0022] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain CDR1 has an amino acid sequence that comprises DYF; a heavy chain CDR2 has an amino acid sequence that comprises SEQ ID NO:74; a heavy chain CDR3 has an amino acid sequence that comprises AR or SEQ ID NO:77; a light chain CDR1 has an amino acid sequence that comprises SEQ ID NO:84; a light chain CDR2 has an amino acid sequence that comprises NVS; and a light chain CDR3 has an amino acid sequence that comprises SEQ ID NO:91.

[0023] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain CDR1 having at least 60% sequence identity to an amino acid sequence of SEQ ID NO:69 or SEQ ID NO:70; a heavy chain CDR2 having at least 60% sequence identity to an amino acid sequence of SEQ ID NO:73 or SEQ ID NO:74; a heavy chain CDR3 having at least 60% sequence identity to an amino acid sequence of AR, SEQ ID NO:77, or SEQ ID NO:78; a light chain CDR1 having at least 60% sequence identity to an amino acid sequence of SEQ ID NO:83 or SEQ ID NO:84; a light chain CDR2 having at least 60% sequence identity to an amino acid sequence of NVS or SEQ ID NO:87; and a light chain CDR3 having at least 60% sequence identity to an amino acid sequence of SEQ ID NO:90 or SEQ ID NO:91. In some embodiments, the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

[0024] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: the heavy chain CDR1 has the amino acid sequence of SEQ ID NO:69; the heavy chain CDR2 has the amino acid sequence of SEQ ID NO:73; the heavy chain CDR3 has the amino acid sequence of SEQ ID NO:77; the light chain CDR1 has the amino acid sequence of SEQ ID NO:83; the light chain CDR2 has the amino acid sequence of SEQ ID NO:87; and the light chain CDR3 has the amino acid sequence of SEQ ID NO:90.

[0025] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: the heavy chain

CDR1 has the amino acid sequence of SEQ ID NO:70; the heavy chain CDR2 has the amino acid sequence of SEQ ID NO:74; the heavy chain CDR3 has the amino acid sequence of SEQ ID NO:78; the light chain CDR1 has the amino acid sequence of SEQ ID NO:84; the light chain CDR2 has the amino acid sequence of NVS; and the light chain CDR3 has the amino acid sequence of SEQ ID NO:90.

[0026] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: the heavy chain CDR1 has the amino acid sequence of SEQ ID NO:70; the heavy chain CDR2 has the amino acid sequence of SEQ ID NO:74; the heavy chain CDR3 has the amino acid sequence of AR; the light chain CDR1 has the amino acid sequence of SEQ ID NO:84; the light chain CDR2 has the amino acid sequence of NVS; and the light chain CDR3 has the amino acid sequence of SEQ ID NO:91.

[0027] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a VH that has at least 60% sequence identity to an amino acid sequence of SEQ ID NO:17; and a VL that has at least 60% sequence identity to an amino acid sequence of SEQ ID NO:19. In some embodiments, the amino acid sequence of SEQ ID NO:17 is encoded by the nucleotide sequence of SEQ ID NO:16. In some embodiments, the amino acid sequence of SEQ ID NO:19 is encoded by the nucleotide sequence of SEQ ID NO:18. In some embodiments, the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

[0028] In some cases, provided herein is an isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a VH has an amino acid sequence of SEQ ID NO:17; and a VL that has an amino acid sequence of SEQ ID NO:19.

[0029] In some cases, provided herein is an isolated antibody or fragment thereof that comprises any combination of a heavy chain CDR, VH, or fragment thereof disclosed herein and a light chain CDR, VL, or fragment thereof disclosed herein.

[0030] In some cases, provided herein is an isolated anti-nucleolin antibody or fragment thereof that binds to amino acid sequence SEQ ID NO:21. In some cases, provided herein is an isolated anti-nucleolin antibody or fragment thereof that binds to an epitope within residues G300 to E466 of amino acid sequence SEQ ID NO:20. In some embodiments, the epitope comprises an amino acid selected from the group consisting of E453, R457, D455, K348, K427, G426, K403, Y402, and any combination thereof. In some embodiments, the isolated antibody or fragment thereof comprises a light chain CDR1 that binds to E453, R457, or a combination thereof. In some embodiments, the isolated antibody or fragment thereof comprises a light chain CDR2 that

binds to D455. In some embodiments, the isolated antibody or fragment thereof comprises a light chain CDR3 that binds to K348. In some embodiments, the isolated antibody or fragment thereof comprises a heavy chain CDR1 that binds to K427. In some embodiments, the isolated antibody or fragment thereof comprises a heavy chain CDR2 that binds to K427, G426, or a combination thereof. In some embodiments, the isolated antibody or fragment thereof comprises a heavy chain CDR3 that binds to K403, Y402, or a combination thereof. In some embodiments, the nucleolin is cell-surface nucleolin. In some embodiments, the isolated antibody or fragment thereof is human or humanized. In some embodiments, the isolated antibody or fragment thereof is an IgG antibody. In some embodiments, the isolated antibody or fragment thereof is an IgG1, IgG2, IgG3, or IgG4 antibody. In some embodiments, the isolated antibody or fragment thereof is a fragment that is a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a Fv fragment, a diabody, a linear antibody, a single-chain antibody, or a multispecific antibody formed from an antibody fragment. In some embodiments, the isolated antibody or fragment thereof is a fragment that comprises an antigen binding region. In some embodiments, the isolated antibody or fragment thereof is nontotoxic to normal cells or tissues. In some embodiments, the isolated antibody or fragment thereof is cytotoxic, *e.g.*, to a tumor or cancer cell. In some embodiments, the isolated antibody or fragment thereof is cytotoxic in presence of human serum. In some embodiments, the isolated antibody or fragment thereof kills at least 10% of a population of tumor or cancer cells, when incubated with the population of tumor or cancer cells for a period of time. In some embodiments, the isolated antibody or fragment thereof kills at least: 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%; or about 100% of the population of tumor or cancer cells. In some embodiments, the incubation is in presence of human serum. In some embodiments, the period of time is about 48-96 hours. In some embodiments, the tumor or cancer cell comprises one, two, or more types of cancer cells selected from the group consisting of human lung cancer, skin cancer, breast cancer, liver cancer, colon cancer, lung cancer, kidney cancer, prostate cancer, leukemia, brain cancer, and pancreas cancer cells. In some embodiments, the tumor or cancer cell comprises one, two, or more types of cancer cells selected from the group consisting of A549, A375, MCF-7, Hep3B, HCT-116, NCI-H358, 786-0, DU-145, MDA-MB-231, MV4-11, U251, CG-EMT, MIA-PaCa2, and PANC-1 cells. In some embodiments, the population of tumor or cancer cells comprises breast cancer cells, *e.g.*, MCF-7 or MDA-MB-231 cells. In some embodiments, the population of tumor or cancer cells comprises acute myeloid leukemia (AML) cells, *e.g.*, MV4-11 cells. In some embodiments, the population of tumor or cancer cells comprises prostate cancer cells, *e.g.*, DU-145 cells, or CG-EMT cells. In some embodiments, the population of tumor or cancer cells comprises lung cancer cells, *e.g.*, A549 or NCI-H358 cells. In some embodiments, the population of tumor or cancer cells comprises skin

malignant melanoma cells, *e.g.*, A375 cells. In some embodiments, the population of tumor or cancer cells comprises hepatocellular carcinoma cells, *e.g.*, Hep3B cells. In some embodiments, the population of tumor or cancer cells comprises colon cancer cells, *e.g.*, HCT-116 cells. In some embodiments, the population of tumor or cancer cells comprises renal cancer cells, *e.g.*, 786-0 cells. In some embodiments, the population of tumor or cancer cells comprises brain tumor cells, *e.g.*, U251 cells. In some embodiments, the population of tumor or cancer cells comprises pancreas carcinoma cells, *e.g.*, MIA-Paca2 or PANC-1 cells.

[0031] In some cases, provided herein is a recombinant cell that produces an isolated antibody or fragment thereof disclosed herein. In some cases, provided herein is an isolated nucleic acid encoding an isolated antibody or fragment thereof disclosed herein. In some cases, provided herein is a vector that comprises a nucleic acid disclosed herein. In some cases, provided herein is a host cell that comprises a nucleic acid disclosed herein or a vector disclosed herein. In some cases, provided herein is a method of producing an antibody or fragment thereof that comprises culturing a host cell disclosed herein so that the antibody or fragment thereof is produced.

[0032] In some cases, provided herein is a pharmaceutical composition that comprises an effective amount of an isolated antibody or fragment thereof disclosed herein and a pharmaceutically acceptable carrier. In some embodiments, the isolated antibody or fragment thereof is a monoclonal antibody. In some embodiments, the isolated antibody or fragment thereof is a polyclonal antibody.

[0033] In some cases, provided herein is a method of treating a cancer, comprising administering to a subject in need thereof a pharmaceutical composition that comprises an isolated antibody or fragment thereof disclosed herein. In some embodiments, the administering is injection. In some embodiments, the administering is intravenous or subcutaneous injection. In some embodiments, the administering occurs 1-3 times per week. In some embodiments, the method reduces a size of tumor in the subject by at least: 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%. In some embodiments, the tumor is a solid tumor. In some embodiments, the pharmaceutical composition is dosed from 0.15 mg to 3 mg per kg of body weight of the subject, *e.g.*, 0.5 mg to 2 mg per kg of body weight of the subject. In some embodiments, the subject is a mammal, *e.g.*, a human. In some embodiments, the cancer comprises one, two, or more types of cancer selected from the group consisting of human lung cancer, skin cancer, breast cancer, liver cancer, colon cancer, lung cancer, kidney cancer, prostate cancer, leukemia, brain cancer, and pancreas cancer.

[0034] In some cases, provided herein is a method of killing cancer cells, comprising contacting with the cancer cells an isolated antibody or fragment thereof disclosed herein. In some cases,

provided herein is a use of an isolated antibody or fragment thereof disclosed herein for treating cancer or killing cancer cells.

[0035] In some cases, provided herein is a use of an isolated antibody or fragment thereof disclosed herein in the manufacture of a medicament. In some embodiments, the medicament is for treatment of a cancer. In some embodiments, the medicament is for killing cancer cells.

[0036] In some cases, provided herein is a recombinant mammalian cell line, wherein the recombinant mammalian cell line comprises one or more cells that comprise a first nucleic acid sequence of SEQ ID NO:1 and a second nucleic acid sequence of SEQ ID NO:10. In some embodiments, the first nucleic acid sequence and the second nucleic acid sequence are in a same construct. In some embodiments, the first nucleic acid sequence and the second nucleic acid sequence are recombinantly or synthetically produced and cloned into an expression vector. In some embodiments, the expression vector is a pTT5 expression vector. In some embodiments, the first nucleic acid sequence and the second nucleic acid sequence are transfected into the one or more cells.

[0037] In some cases, provided herein is a method of activating an immune system in a human subject, comprising administering to the subject an isolated antibody or fragment thereof disclosed herein. In some cases, provided herein is a method of treating cancer by inhibiting Transforming growth factor beta (TGFβ) in a human subject, comprising administering to the subject an isolated antibody or fragment thereof disclosed herein, whereby the TGFβ is inhibited and the cancer is treated. In some embodiments, the TGFβ is TGFβ1, TGFβ2, or TGFβ3.

[0038] In some cases, provided herein is a method of treating a cancer by preventing stabilization of an oncogenic mRNA in a human subject, comprising administering to the subject an isolated antibody or fragment thereof disclosed herein, whereby the oncogenic mRNA is destabilized and the cancer is treated. In some embodiments, the oncogenic mRNA is tumor protein p53 mRNA, B-cell lymphoma-extra large (Bcl-XL) mRNA, (B-cell lymphoma 2) Bcl-2 mRNA, gastrin mRNA, (growth arrest and DNA damage-inducible alpha) Gadd45α mRNA, matrix metallopeptidase 9 (MMP9) mRNA, Arabidopsis thaliana kinesin (Atk1) mRNA, Cyclin 1 mRNA, interleukin-2 (IL-2) mRNA, prostaglandin H synthase-1 (Pghs-1) mRNA, or any combination thereof. In some cases, provided herein is a method of treating a cancer by reducing an expression level of an oncogenic protein in a human subject, comprising administering to the subject an isolated antibody or fragment thereof disclosed herein, whereby the expression level of the oncogenic protein is destabilized and the cancer is treated. In some embodiments, the oncogenic protein is tumor protein p53, Bcl-xL, Bcl-2, gastrin, Gadd45α, MMP9, Atk1, Cyclin 1, IL-2, Pghs-1, or any combination thereof. In some embodiments, the cancer comprises one, two, or more types of cancers selected from the group consisting of human lung cancer, skin

cancer, breast cancer, liver cancer, colon cancer, lung cancer, kidney cancer, prostate cancer, leukemia, brain cancer, and pancreas cancer.

[0039] In some cases, provided herein is an immunoconjugate, wherein the immunoconjugate comprises an antigen binding agent linked to a therapeutic agent, and wherein the antigen binding agent comprises an isolated antibody or fragment thereof disclosed herein. In some embodiments, the immunoconjugate is a fusion protein, and the therapeutic agent is a polypeptide. In some embodiments, the antigen binding agent is a bispecific antibody. In some embodiments, the antigen binding agent is a probody. In some embodiments, the probody comprises an antigen-binding region that is activated by a tumor cell. In some embodiments, the antigen-binding region comprises a peptide linked to the N-terminus of a light chain through a protease cleavable linker. In some embodiments, the antigen binding agent is linked covalently, noncovalently, or recombinantly to the therapeutic agent. In some embodiments, the therapeutic agent is a cytotoxic agent. In some embodiments, the cytotoxic agent is doxorubicin, calicheamicin, auristatin, maytansinoid, brentuximab vedotin, tubulysins, duocarmycins, camptothecin, SN-38, pyrrolobenzodiazepine, methotrexate, α-amanitin, ansamitocin, or any combination thereof. In some embodiments, the therapeutic agent is an immune stimulating agent. In some embodiments, the therapeutic agent is interleukin-2 (IL-2), an immunostimulatory nucleic acid molecule, granulocyte macrophage colony-stimulating factor, resiquimod, Gardiquimod, phycocyanobilin, romiplostim, eltrombopag, or any combination thereof.

[0040] In some cases, provided herein is a pharmaceutical composition that comprises an immunoconjugate disclosed herein and a pharmaceutically acceptable carrier. In some cases, provided herein is a method of treating a cancer, comprising administering to a subject in need thereof a pharmaceutical composition that comprises an immunoconjugate disclosed herein. In some cases, provided herein is a use of an immunoconjugate disclosed herein for treating a cancer. In some cases, provided herein is a use of an immunoconjugate disclosed herein in the manufacture of a medicament for treatment of a cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The present disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0042] FIG. 1 is an image showing SDS PAGE gel of antibody CP1 and IgG and IgM standards. Commassie blue staining of the SDS PAGE gel shows that in the absence of β-mercaptoethanol (lane 10), full length antibody CP1 has a molecular mass of about 150 KDa. Reduction of CP1

with β-mercaptoethanol (lane 11) reveals that the antibody is of class IgG with heavy- and light-chains of about 50 KDa and 26 KDa, respectively.

[0043] FIG. 2 is an image showing SDS PAGE gel of CP1 purified using MabSelect 1.0 ml protein A column.

[0044] FIG. 3 is an image showing western blot analysis of CP1. SDS PAGE was done under reducing conditions with known amounts of antibody CP1 alongwith IgG1 and IgM standards. The transfer membrane was cut into two sections and one section was probed with a HRP-conjugated secondary antibody specific for human IgG1 (lanes 1-7) while the second section was probed with a HRP-conjugated secondary antibody specific for human IgM. Not Shown-Sequencing of the cDNAs of the heavy- and light- chains of CP1 revealed that the antibody is of subclass IgG1kappa.

[0045] FIG. 4 is a bar graph showing binding of CP1 to human recombinant nucleolin. Various amounts of human recombinant nucleolin were bound to a 96 well plate. The plate was washed with blocking buffer and then various amounts of antibody CP1 or human IgG control antibody were added to the wells. The wells were washed with blocking buffer to remove the unbound antibody, and then incubated with a HRP conjugated secondary antibody specific for human IgG1.

[0046] FIG. 5 is a line graph showing cytotoxic effects of CP1 on the viability on tumor and normal cells. Various solid tumor types and AML (MV4-11) and normal breast epithelial cells were incubated in 96 well plates for 24 h in RPMI1640 medium containing 10% human serum. After 24 h the cells were incubated with either human IgG1 antibody (control) or various concentrations of antibody CP1 for 96 h. Cells were then stained with trypan blue and counted in a NEXCELOM Cellometer Auto T4 Plus counter. IC50 results are the means of triplicate determinations plus S.D.

[0047] FIG. 6 is a bar graph showing the effects of CP1 (from B cells or recombinant cells) on various human cell lines. CP1 has potent widespread anticancer activity to both hematological and solid tumor cells *in vitro*, but negligible toxicity to normal cells.

[0048] FIG. 7 is a line graph showing effects of CP1 on the viability MCF7 breast cancer cells and MCF10A normal breast epithelium cells.

[0049] FIG. 8 is a line graph showing ex vivo effects of CP1 on the viability of patient prostate cancer CG-EMT cells. CG-EMT cells are primary cancer cells that were isolated from a patient with hormone-refractory prostate cancer.

[0050] FIG. 9 is a set of images showing cell surface and plasma membrane binding of CP1 to cancer cells. Non-permeabilized MCF-7 cells were incubated for 1 h at room temperature with either a FITC-labeled isotype control antibody or CP1. The incorporation of CP1 into the

plasma membrane was determined by indirect immunofluorescence using CP1 and a FITC-conjugated secondary Ab. Nuclei were counter-stained with propidium iodide. The punctate appearance of nucleolin suggests that it was incorporated within lipid rafts in the plasma membrane.

[0051] FIG. 10 is a set of line graphs showing purified (top lines) and partially purified (bottom lines) CP2 on cell numbers of MCF7 at 96 h.

[0052] FIG. 11 is a set of SDS-PAGE images verifying protein/antibody expression in Example 2.

[0053] FIG. 12 is a line graph showing effect of CP1 recombinant kappa light chain on the viability of MV4-11 cells.

[0054] FIG. 13 is a line graft showing comparing effects of aptamer AS1411 and CP1 on the viability of human MCF-7 breast cancer cells.

[0055] FIG. 14 is a Kaplan-Meier plot showing effects of CP1- 30% long term survivors in an MV411 human leukemia xenograft model with nude mice.

[0056] FIG. 15A is a line chart showing tumor volume changes in mice of Group 1 (10 mice): Control (10 mg/kg, iv, days 1, 4, 7, 10, 13, 16). FIG. 15B is a line chart showing tumor volume changes in mice of Group 2 (10 mice): CP1 (10 mg/kg, iv, days 1, 4, 7, 10, 13, 16).

[0057] FIG. 16 is an image of molecular model of the binding of antibody CP1 to human nucleolin.

DETAILED DESCRIPTION

[0058] The present disclosure provides anti-nucleolin antibodies and methods of use thereof. These antibodies exhibit cytotoxicity towards cells expressing nucleolin in the plasma membrane, such as cells involved in cancer, autoimmune disorders, and viral disorders. Therefore, the antibodies have therapeutic potential for certain forms of cancer, hyperproliferative and neovascular disorders and autoimmune diseases.

[0059] Percent (%) sequence identity with respect to a reference polypeptide sequence is the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes

herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0060] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y, where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0061] The term "about" means the referenced numeric indication plus or minus 15% of that referenced numeric indication.

[0062] In some aspects, the present disclosure provides immortalized B-cells, *e.g.*, human B-cells that express IgG antibodies that bind to and inactivate nucleolin. While these immortalized populations were found to express more than one antibody sequence, through the use of antibody sequencing technologies, the identity and amino acid sequence of the underlying antibodies were determined and can now be provided for use in the construction of immunological therapeutic reagents. Cell culturing was also used to isolate individual antibodies. Subsequently each isolated antibody was tested for potency and binding to cell surface nucleolin.

[0063] Provided herein is an isolated antibody or fragment thereof that can bind to nucleolin, comprising a heavy chain variable region (VH) that can comprise one or more complementarity determining regions (CDRs) each having at least 60% sequence identity to one or more corresponding VH CDRs in Table 7 or Table 8. A VH can comprise one CDR having at least 60% sequence identity to one or more corresponding VH CDR in Table 7 or Table 8. In some cases, a corresponding VH CDR can be CDR H1. In some cases, a corresponding VH CDR can be CDR H2. In some cases, a corresponding VH CDR can be CDR H3. In some aspects, a VH can comprise two CDRs each having at least 60% sequence identity to one or more corresponding VH CDRs in Table 7 or Table 8. In some aspects, the two corresponding VH CDRs can be CDR H1 and CDR H2. In some cases, the two corresponding VH CDRs can be

CDR H1 and CDR H3. In some cases, the two corresponding VH CDRs can be CDR H2 and CDR H3.

[0064] Also provided herein is an antibody or fragment thereof, wherein a VH can comprise three CDRs each having at least 60% sequence identity to one or more corresponding CDR H1, CDR H2, or CDR H3 in Table 7. In some cases, an antibody or fragment thereof wherein the VH can comprise three CDRs having at least 60% sequence identity to corresponding CDR H1, CDR H2, and CDR H3 in Table 8. In some cases, an antibody or fragment thereof can comprise an amino acid sequence identity that can be at least: 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or 100%.

[0065] Also provided herein is an isolated antibody or fragment thereof that binds to nucleolin, comprising a light chain variable region (VL) that comprises one or more complementarity determining regions (CDRs) having at least 60% sequence identity to one or more corresponding VL CDRs in Table 7 or Table 8. In some cases, aVL can comprise one CDR having at least 60% sequence identity to one or more corresponding VL CDR in Table 7 or Table 8. In some cases, a corresponding VL CDR can be CDR L1. In some cases, a corresponding VL CDR can be CDR L2. In some cases, a corresponding VL CDR can be CDR L3.

[0066] In some cases, a VL can comprise two CDRs each having at least 60% sequence identity to one or more corresponding VL CDRs in Table 7 or Table 8. In some cases, two corresponding VL CDRs can be CDR L1 and CDR L2. In some cases, two corresponding VL CDRs can be CDR L1 and CDR L3. In some cases, two corresponding VL CDRs can be CDR L2 and CDR L3. In some aspects, a VL can comprise three CDRs each having at least 60% sequence identity to corresponding CDR L1, CDR L2, or CDR L3 in Table 7. In some cases, a VL can comprise three CDRs each having at least 60% sequence identity to corresponding CDR L1, CDR L2, or CDR L3 in Table 8. In some aspects, an amino acid sequence identity can be at least: 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or 100%.

[0067] Also provided herein is an isolated antibody or fragment thereof that binds to nucleolin, comprising a heavy chain variable region (VH) that comprises one or more complementarity determining regions (CDRs) each having at least 60% sequence identity to one or more corresponding VH CDRs in Table 7 or Table 8; and a light chain variable region (VL) that comprises one or more complementarity determining regions (CDRs) having at least 60% sequence identity to one or more corresponding VL CDRs in Table 7 or Table 8. In some cases, an isolated antibody or fragment thereof that binds to nucleolin, can comprise a VH that has at least 60% sequence identity to VH in Table 7. In some cases, an isolated antibody or fragment thereof that binds to nucleolin, can comprise a VL that has at least 60% sequence identity to VL in Table 7. In some cases, an isolated antibody or fragment thereof that binds to nucleolin, can

comprise a VH that has at least 60% sequence identity to VH in Table 8. In some regards, an isolated antibody or fragment thereof that binds to nucleolin, can comprise a VL that has at least 60% sequence identity to VL in Table 8. In some cases, an antibody or fragment thereof can comprise any combination of one or more CDRs. In some cases, an antibody or fragment thereof can have an amino acid sequence identity that can be or can be at least: 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or 100%.

[0068] In some cases, an antibody or fragment thereof disclosed herein comprises CDR H1 comprising an amino acid sequence in Table 7 or Table 8; CDR H2 comprising the amino acid sequence in Table 7 or Table 8; and/or CDR H3 comprising the amino acid sequence in Table 7 or Table 8. In some cases, an antibody or fragment thereof disclosed herein comprises CDR L1 comprising an amino acid sequence in Table 7 or Table 8; CDR L2 comprising the amino acid sequence in Table 7 or Table 8; and/or CDR L3 comprising the amino acid sequence in Table 7 or Table 8.

[0069] In some cases, a nucleolin disclosed herein can be a cell-surface nucleolin. In some cases, a nucleolin disclosed herein can mean a nucleolin fragment. In some cases, a nucleolin disclosed herein is a human nucleolin.

[0070] Also provided herein is an antibody or fragment thereof that can be human or humanized. An antibody or fragment thereof can be an IgG antibody. In some cases, an antibody or fragment thereof can be an IgG1, IgG2, IgG3, or an IgG4 antibody. In some cases, an antibody or fragment thereof can be a monoclonal antibody. In some cases, an antibody or fragment thereof is nontoxic. In some cases, an antibody or fragment thereof can be cytotoxic. In some cases, an antibody or fragment thereof can be cytotoxic to a tumor or cancer cell. In some cases, an antibody or fragment thereof can be cytotoxic in presence of human serum. In some cases, an antibody or fragment thereof can exhibit or induce complement-dependent cytotoxicity to a tumor or cancer cell. In some cases, an antibody or fragment thereof can exhibit or induce complement-independent cytotoxicity to a tumor or cancer cell.

[0071] In some cases, an antibody or fragment thereof can kill at least 10% of a population of tumor or cancer cells, when incubated with cells for a period of time. In some cases, an antibody or fragment thereof can kill at least: 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the population of tumor or cancer cells. In some cases, an antibody or fragment thereof can kill about 100% of a population of tumor or cancer cells. In some cases, an antibody or fragment thereof can have a period of time is about 48-96 hours. In some cases, a period of time can be about 96 hours. In some cases, cells can be breast cancer cells. In some cases, cells can be MCF-7. In some cases, cells can be acute myeloid leukemia (AML) cells. In some cases, cells can be HCT-116, NCI-H358, DU-145, MDA-MB-231, MV4-11, MIA-PaCa2, or PANC-1 cells. In

some cases, cells can be prostate cancer cells. In some cases, cells can be hormone-refractory prostate cancer cells. In some cases, cells can be CG-EMT cells. In some cases, an isolated monoclonal cytotoxic antibody or fragment thereof can bind to an epitope within residues # 300 to #466 of nucleolin SEQ ID NO:20. In some embodiments, the epitope has/spans about: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, or 80 amino acids. In some cases, an isolated monoclonal cytotoxic antibody or fragment thereof can bind to an epitope comprising one or more amino acids selected from the group consisting of E453, R457, D455, K348, K427, G426, K403, and Y402. In some cases, an antibody or fragment thereof can comprise a VL CDR1 in Table 7 that binds to E453, R457, or a combination thereof. In some cases, an antibody or fragment thereof can comprise a VL CDR2 in Table 7 that can bind to D455. In some cases, an antibody or fragment thereof can comprise a VL CDR3 in Table 7 that can bind to K348. In some cases, an antibody or fragment thereof can comprise a VH CDR1 in Table 7 that can bind to K427. In some cases, an antibody or fragment thereof, can comprise a VH CDR2 in Table 7 that can bind to K427, G426, or a combination thereof. In some cases, an antibody or fragment thereof can comprise a VH CDR3 in Table 7 that binds to K403, Y402, or a combination thereof.

[0072] In some cases, an anti-nucleolin agent disclosed herein has a Kd of about: 10 μM, 1 μM, 0.1 μM, 0.05 μM, 10 nM, 5 nM, 2.5 nM, 1 nM, or less, to nucleolin or a fragment thereof, *e.g.*, amino acid #300 to #466 (SEQ ID NO:21) or an epitope therein. In some embodiments, the Kd is about 2.5 nM or less.

[0073] In some cases, an antibody or fragment thereof can be an IgG antibody. In some cases, a fragment thereof can be a Fab, Fab', $F(ab')_2$, or Fv fragment; diabodie; linear antibody; single-chain antibody; or a multispecific antibody formed from an antibody fragment. In some cases, a fragment thereof can comprise an antigen binding region thereof.

[0074] Also provided herein is a recombinant cell that can produce an antibody or fragment thereof. In some cases, a cell can be a B cell. In some cases, a cell can be a human B cell. In some cases, a cell can be a hybridoma.

[0075] Also provided herein is an isolated nucleic acid encoding an antibody or fragment thereof disclosed herein. In some cases, a vector can comprise a nucleic acid disclosed herein. In some cases, a host cell can comprise a vector disclosed herein.

[0076] Also provided herein is a method of producing an antibody or fragment thereof comprising culturing a host cell so that an antibody or fragment thereof can be produced.

[0077] Also provided herein is a pharmaceutical composition that comprises an antibody or fragment thereof and a pharmaceutically acceptable carrier. In some cases, an antibody or fragment thereof can be used as a medicament.

[0078] Also provided herein is a method of treating cancer with an antibody or fragment thereof. Also provided herein can be a method of killing cancer cells with an antibody or fragment thereof. Also provided herein can be a use of an antibody or fragment thereof treating cancer. In some cases, a use of an antibody or fragment thereof can be used for killing cancer cells. In some cases, a use of an antibody or fragment thereof can be for the manufacture of a medicament. A medicament can be for treatment of cancer. A medicament can be for killing cancer cells.

[0079] Also disclosed herein is a method for making an anti-cancer antibody or a fragment thereof, comprising: culturing a cell in a medium under conditions permitting expression of a polypeptide encoded by a vector and assembling of an antibody or fragment thereof; and purifying an antibody or fragment from cultured cell or medium of a cell.

[0080] In some cases, a recombinant mammalian cell line can be transfected with an antibody construct encoded by a first construct gamma heavy chain disclosed herein and a second construct kappa light chain disclosed herein. In some cases, a recombinant mammalian cell line can be transfected with an antibody construct encoded by a first construct gamma heavy chain disclosed herein and a second construct lambda light chain disclosed herein. In some cases, said heavy chain and said light chain are in the same construct. In some cases, antibody constructs can be synthetically produced and cloned into an expression vector pTT5. In some cases, cells can be maintained at a density of (0.25 to 5) x 10^6 cells/mL.

[0081] In some cases, a cancer treated by an antibody or fragment thereof comprises one, two, three, four, five, or more types selected from the group consisting of human lung cancer, skin cancer, breast cancer, liver cancer, colon cancer, lung cancer, kidney cancer, prostate cancer, leukemia, brain cancer, and pancreas cancer.

[0082] In some cases, a cancer cell killed by an antibody or fragment thereof comprises one, two, three, four, five, or more types selected from the group consisting of human lung cancer, skin cancer, breast cancer, liver cancer, colon cancer, lung cancer, kidney cancer, prostate cancer, leukemia, brain cancer, and pancreas cancer cells. In some embodiments, the cancer cell comprises one, two, three, four, five, or more types selected from the group consisting of A549, A375, MCF-7, Hep3B, HCT-116, NCI-H358, 786-0, DU-145, MDA-MB-231, MV4-11, U251, CG-EMT, MIA-PaCa2, and PANC-1 cells.

1. Nucleolin

1A. General

[0083] Nucleolin is a multi-functional protein that binds to DNA, RNA and the external surface of the plasma membrane. The ability of nucleolin to perform numerous and diverse functions within the cell is related to the multiple structural domains within the protein. Its negatively charged N-terminal domain regulates rDNA transcription by inducing nucleolar chromatin

decondensation (Srivastava *et al.*, 1989), while the central globular domain contains four RNA binding domains (RBDs) (Serin *et al.*, 1997). It has been proposed that nucleolin, via binding of its RBD and its RGG-rich C-terminal domains to pre-ribosomal RNA, functions as an assembly factor by bringing together the correctly folded rRNA and other components necessary for rRNA maturation and ribosome assembly (Ginisty *et al.*, 2001). Nucleolin may also be involved in exporting ribosomes to the cytoplasm while shuttling between the cytoplasm and nucleus (Srivastava and Pollard, 1999). The nucleolin gene coding and protein sequences can be accessed at accession number NM_005381, XM_002342275, NP_005372 and XP_002342316. Nucleolin is also known as C23, FLJ45706, FLJ59041, and NCL.

[0084] Human NCL gene consists of 14 exons with 13 introns and spans approximately 11kb. The nucleolin protein contains several functional domains that mediate its functions. The N-terminal part contains multiple phosphorylation sites and is rich in acidic amino acids. The central part of nucleolin includes four RNA binding domains (RBD) and the C-terminal part contains glycine and arginine rich domain (termed RGG or GAR domain). (Farin *et al.*, 2009)

[0085] A considerable body of evidence supports a role for nucleolin in mRNA stabilization. Nucleolin binds to the 3'-untranslated region (3'-UTR) of amyloid precursor protein mRNA and stabilizes this mRNA (Westmark and Malter, 2001). It is also required for the stabilization of IL-2 mRNA that occurs during T cell activation (Chen *et al.*, 2000).

[0086] Nucleolin is present on the external surface of various types of tumor cells Otake *et al.*, 2007; Soundararajan *et al.*, 2008; Chen *et al.*, 2008; Hovanessian *et al.*, 2000; Sinclair and O'Brien, 2002), despite its lack of a transmembrane domain or signal sequence (Srivastava *et al.*, 1989; Lapeyre *et al.*, 1987). Results show that nucleolin is not secreted from either MV4-11 cells or K-562 cells into the tissue culture medium (Soundararajan *et al.*, 2009). This suggests that the presence of nucleolin on the cell surface is not the result of adsorption of secreted nucleolin by macromolecules on the cell surface of tumor cells. However, nucleolin undergoes extensive posttranslational modification (Srivastava *et al.*, 1989; Lapeyre *et al.*, 1987). It has been isolated as a glyco-phospho-protein from the surface of various types of proliferating cells (Hovanessian *et al.*, 2000; Pfeifle and Anderer, 1983). It is also possible that palmitoylation, prenylation, or myristoylation of nucleolin may allow for insertion or anchoring of these hydrophobic regions of the protein into the plasma membrane. It is thought that nucleolin functions as a shuttling protein between the plasma membrane and nucleus (Hovanessian *et al.*, 2000). In proliferating tumor cells, nucleolin is often associated with endocytotic vesicles that invaginate from the plasma membrane (Hovanessian *et al.*, 2000). Nucleolin also acts as a cell surface receptor for various ligands, since ligands bound to nucleolin within these vesicles become internalized in a temperature-dependent process. For example, plasma membrane nucleolin has been reported to

function as a receptor for intimin-γ of *E.coli* (Sinclair and O'Brien, 2002), the anti-HIV agent midkine (Said *et al.*, 2002), laminin-1 (Kibbey *et al.*, 1995), DNA nanoparticles (Chen *et al.*, 2008), and the anti-angiogenic pseudopeptide HB-19 (Destouches *et al.*, 2008). Nucleolin is an important protein in the nucleolus involved in ribosome biogenesis and maturation in exponentially growing eukaryotic cells. In this regard, one important function of nucleolin is as a shuttling protein between cytoplasm and nucleus involving RNA processing and other cell biological process. While in normal cellular physiology, nucleolin is localized predominantly in the nucleolus and cytoplasm, under certain conditions, especially in various disease states it has also been shown to be present in a phosphorylated form on the cell surface. In this regard, nucleolin in the cell membrane serves as a binding protein for a variety of ligands that drive cell proliferation, differentiation, adhesion, mitogenesis and angiogenesis.

1B. Nucleolin in Cancer

[0087] Several lines of evidence suggest that nucleolin is an excellent tumor antigen for antibody-based immunotherapy. Nucleolin is overexpressed in the plasma membrane and cytoplasm a variety of human tumors including human chronic lymphocytic leukemia (CLL) (Otake *et al.*, 2007), acute myeloid leukemia (AML) (Soundararajan *et al.*, 2008), and breast cancer cells (Soundararajan *et al.*, 2008), but not in normal CD19+ B cells (Otake *et al.*, 2007), CD33+ myeloid cells (Gattoni-Celli *et al.*, 2009), nor in normal mammary epithelial cells (Soundararajan *et al.*, 2008). It is of interest that AML blast cells from patients that engraft in NOD/SCID mice show intense nucleolin staining in the plasma membrane and cytoplasm while the normal mouse bone marrow cells and spleen lymphocytes were negative for nucleolin (Gattoni-Celli *et al.*, 2009). In normal human myeloid cells, nucleolin staining is concentrated in nucleoli, while in patient AML-1 cells extensive nucleolin staining (aberrant expression of nucleolin) was observed in nuclei and in the cytoplasm/cell surface.

[0088] The nucleolin targeting aptamer, AS1411, targets nucleolin. Plasma membrane nucleolin was recently reported to be a receptor for AS1411 in human MV4-11 leukemia cells (Soundararajan *et al.*, 2009).

[0089] AS1411 binds to nucleolin that is overexpressed on the external surface of tumor cells and gains intracellular access when nucleolin is shuttled from the plasma membrane to the cytoplasm and nucleus. AS1411 has been shown to exhibit antiproliferative activity in a broad set of cancer cell lines that over-express nucleolin (Table 1).

Table 1. Cancer Cell Lines That Over-express Nucleolin and/or are Killed Subsequent to Nucleolin Inhibition

Cancer Type:	Cell Line:
Lung cancer	A549, NCI-H322M, NCI-H460, EKVX, HOP-92, NCI-H299, CaLu1,

	NCI-H1385, NCI-H82, CaLu6
Breast cancer	MCF7, T-47D, BT-549, MDA-N, MDA-MB-231, ZR7S-1
Prostate cancer	DU145, PC-3, CA-HPV-10
Colon cancer	HCC 2998, HT-29, KM12, HCT-116, SW620, HCT-15, LS174T
Pancreatic cancer	PANC-1, MIA-PaCa-2
Renal cell carcinoma	786-0, CAKI-1, RXF393, TK10, A498, ACHN, SN12C
Ovarian cancer	IGROV, OVCAR-3, OVCAR-4, OVCAR-5
Cervical cancer	HeLa
Leukemia & Lymphoma	CCRF-CEM, SR, HL60, K-562, RPMI-6226, U937, Meg0, MV4-11
Melanoma	LOX-IMVI, SK-MEL-2, A375, SK-MEL-28, MDA-MB-435
Glioblastoma	SF-268, U87-MG
Neuroblastoma	IMR 32, Lan 5
Sarcoma	HT-1080
Gastric cancer	KATOIII, HGC27

Data from NCI Tumor Cell Line Screen of AS1411 (≥ 50% growth inhibition at 6.3 μM). (Bates *et al.*, 2009).

[0090] Anti-nucleolin antibodies can also exploit the shuttling function of plasma membrane nucleolin and become internalized after binding to cell surface nucleolin. This suggests that anti-nucleolin antibodies can elicit anti-tumor effects through intracellular mechanisms, and/or to antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cellular cytotoxicity (CDCC).

1C. Antibodies or Fragments thereof

[0091] In some embodiments, any of the methods disclosed herein can be practiced with an anti -nucleolin antibody or fragment thereof. In some embodiments an anti -nucleolin antibody or fragment thereof is used to detect a cell expressing nucleolin on its surface. In some embodiments, an anti-nucleolin antibody or fragment thereof is used to inhibit or kill a cell expressing nucleolin on its surface. In some embodiments, an anti -nucleolin antibody or fragment thereof is used to treat or prevent a neoplastic disease (*e.g.,* cancer), an autoimmune disease, an inflammatory disease or condition, a respiratory disease, a viral infection, or macular degeneration.

[0092] In some embodiments, an anti -nucleolin antibody or fragment thereof is conjugated, linked or fused to a toxin, chemotherapeutic, an immunostimulatory nucleic acid sequence (*e.g.*, a CpG sequence), a radionuclide or an immunotherapeutic. In some embodiments, an anti -nucleolin antibody or fragment thereof is conjugated, linked or fused to a radionuclide, a fluorophore, a chemilluminescent compound, a fluorescent compound, or an enzyme. In some embodiments, anti -nucleolin antibody or fragment thereof is used to contact a cell expressing nucleolin on its surface. In some embodiments the cell is pre-cancerous cell, a cancer cell or an immune cell.

[0093] In some embodiments, the anti-nucleolin antibody fragment thereof is a human anti -nucleolin antibody or fragment. In some embodiments the anti -nucleolin antibody fragment thereof is a non-human anti -nucleolin antibody fragment thereof. In some embodiments the anti -nucleolin antibody fragment thereof is a chimeric anti -nucleolin antibody fragment thereof. In some embodiments the anti -nucleolin antibody fragment thereof is a humanized anti -nucleolin antibody fragment thereof.

[0094] In some embodiments, an anti -nucleolin antibody fragment thereof is generated from an anti -nucleolin antibody. In some embodiments the anti-nucleolin antibody fragment has the same binding specificity to nucleolin as the parent antibody. In some embodiments, the anti-nucleolin antibody fragment has improved binding specificity to nucleolin as the parent antibody. In some embodiments the anti-nucleolin antibody fragment has the same binding affinity to nucleolin as the parent antibody. In some embodiments, the anti-nucleolin antibody fragment has improved affinity to nucleolin as the parent antibody. In some embodiments an anti -nucleolin antibody or fragment thereof is an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) fragment.

[0095] "Antibody fragments" comprise a portion of an intact antibody, or the antigen binding region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0096] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0097] "Fv" is a minimum antibody fragment which contains a complete antigen-binding site. In some embodiments, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable region in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable region can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable region interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable region (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0098] The Fab fragment contains the heavy- and light-chain variable regions and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain.

Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab').sub.2 antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0099] "Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, *see, e.g.*, Pluckthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

[0100] The term "diabodies" refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable region (VH) connected to a light-chain variable region (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat. Med. 9:129-134 (2003); and Hollinger et al., PNAS USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat. Med. 9:129-134 (2003).

[0101] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible mutations, *e.g.*, naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of B cell or hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding

sequence can also be a monoclonal antibody. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations can be advantageous in that they are typically uncontaminated by other immunoglobulins.

[0102] The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by a variety of techniques, including, for example, the hybridoma or B cell method (*e.g.*, Kohler and Milstein, Nature, 256:495-97 (1975); Hongo et al., Hybridoma, 14 (3): 253-260 (1995), Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (*see, e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (*see, e.g.*, Clackson et al., Nature, 352: 624-628 (1991); Marks et al., J. Mol. Biol. 222: 581-597 (1992); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, PNAS USA 101(34): 12467-12472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (*see, e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits et al., PNAS USA 90: 2551 (1993); Jakobovits et al., Nature 362: 255-258 (1993); Bruggemann et al., Year in Immunol. 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks et al, Bio/Technology 10: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368: 812-813 (1994); Fishwild et al., Nature Biotechnol. 14: 845-851 (1996); Neuberger, Nature Biotechnol. 14: 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995).

[0103] The modifier "polyclonal" indicates the character of the antibody as being obtained from a source of a nonhomogeneous population of antibodies. A polyclonal antibody comprises more than one antibody, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 antibodies.

[0104] The monoclonal antibodies herein include human, non-human, humanized and "chimeric" antibodies. "Chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived

from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al., PNAS USA 81:6851-6855 (1984)). Chimeric antibodies include PRIMATIZED.RTM. antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with the antigen of interest.

[0105] "Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable regions, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also the following review articles and references cited therein: Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994).

[0106] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147(1):86-95 (1991). See also van Dijk and van de Winkel, Curr. Opin. Pharmacol., 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such

antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (*see, e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE.TM. technology). See also, for example, Li et al., PNAS USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

2. Cloning and Expression of Ig Light and Heavy Chains

[0107] Various methods can be employed for the cloning and expression of immunoglobulin light and heavy chain sequences. Weltschof *et al.* (1995), incorporated herein by reference, describes in detail the methods here. The variable regions, or variable + constant regions, can be cloned.

[0108] In some embodiments, these antibodies can be prepared by a technique described in WO 2011/062997 (incorporated herein by reference in its entirety) that permits one to directly identify, isolate and characterize, for example in terms of amino acid sequence, human anti-nucleolin antibodies from immortalized antibody-producing cells prepared using human immune cells such as tonsil cells.

[0109] Some techniques, such as those described by Takekoshi *et al.* (2001), are also useful. In that reference, total cellular RNA was isolated from pelleted cells using a commercial kit (RNeasy mini kit, Qiagen). Using random 9-mers, nucleotides and reverse transcriptase (Takara, RNA-PCR kit, Ohtsu), cDNAs were synthesized and were amplified by the polymerase chain reaction (PCR), with heavy and light chain primers specific for immunoglobulins (Ig). A "touchdown" PCR protocol was employed, *i.e.*, three cycles each of denaturation at 95°C for 1 min, annealing for 1 min, and elongation at 72°C for 2 min, for a total of 11 cycles. The annealing temperature was varied from 65-55°C in steps of 1°C. The touchdown cycles were followed by 25 cycles using an annealing temperature of 55°C. The resultant PCR product was gel-purified in agarose and extracted using QIAquick spin-columns (Qiagen). The light chain and heavy chain Fc genes were then cloned into the *Nhe*I/*Asc*I and the *Sfi*I/*Not*I sites of the expression vector pFab1-His2. The ligated pFab1-His2 vectors with the light chain (κ and λ) and Fc heavy chain genes (γ and μ) were introduced into competent *E. coli* JM109 cells (Toyobo, Osaka). After transformation, the *E. coli* cells were plated onto Luria-Bertani (LB)/ampicillin (50 μg/ml) plates. Isolated bacterial colonies were incubated at 30°C in 2 ml of Super Broth (SB) with ampicillin (50 μg/ml) and $MgCl_2$ (1.5 mM). Isopropyl-β-D-thiogalactopyranoside (IPTG) was used to induce production of the Fab protein. Cells from the bacterial cultures were pelleted, resuspended in 0.3 ml of B-PER (Pierce) with a protease inhibitor cocktail (Complete, Boehringer Mannheim), and shaken for 5 min at room temperature. Cell lysates were centrifuged

at 15,000G for 10 min, and the resultant supernatant containing the Fab antibody portion was collected.

[0110] In some embodiments, a heavy chain and a light chain can be in the same cloning construct. In some embodiments, a heavy chain and a light chain are found in different cloning constructs. Constructs containing sequences for heavy chain genes, light chain genes, or any combination thereof may be cloned simultaneously. Simultaneous cloning can comprise a vector containing both heavy and light chain genes or two separate vectors introduced simultaneously, each containing either a heavy chain or light chain. In some embodiments, constructs containing sequences for heavy chain genes, light chain genes, or any combination thereof may be cloned sequentially. Sequential cloning may comprise introducing a vector containing a heavy chain gene followed by the introduction of a second vector containing a light chain gene. For example, a cell can be genetically modified with a vector containing gene sequences for both a heavy chain and light chain.

3. Antibody Production

[0111] Once cloned, the nucleic acids for the light and heavy chains can be inserted into appropriate expression vectors and transferred into host cells (*e.g.*, antibody-producing cells) that support production of antibodies. Exemplary cell lines for production are 293 cells, CHO cells, COS cells or various forms of myeloma cells, some lacking IgG. These cells can be exploited for antibodies (*e.g.*, human and/or monoclonal) production in two basic ways. First, myelomas or immortalized cells can be injected (often into the peritoneal cavity) into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion (*e.g.*, a syngeneic mouse), or into an immunodeficient animal for injection of incompatible cells. Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. The injected animal develops tumors secreting the specific monoclonal antibody produced by the transfected myeloma. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide antibodies (*e.g.*, human and/or monoclonal) in high concentration. Second, the individual cell lines could be cultured *in vitro*, where the antibodies (*e.g.*, human and/or monoclonal) are naturally secreted into the culture medium from which they can be readily obtained in high concentrations.

[0112] Antibodies (*e.g.*, human and/or monoclonal) produced by either means can be further purified, if desired, using ultra filtration, centrifugation and various chromatographic methods such as HPLC, affinity chromatography, or ion exchange chromatography. Fragments of the monoclonal antibodies of the present disclosure can be obtained from the monoclonal antibodies so produced by methods which include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction.

[0113] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is produced from an immortalized B cell (*e.g.*, human B cell). In some embodiments an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is produced using a method such as one set forth in PCT/US2008/072124 or US patent application 12/671,936, which are herein incorporated by reference in their entirety.

[0114] In some embodiments, the cDNA of an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) may be produced by cloning cDNA or genomic DNA encoding the immunoglobulin light and heavy chains of the anti-nucleolin antibody from a hybridoma cell (by fusing a specific antibody-producing B cell with a myeloma) that produces an antibody homolog. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is produced by a B cell (*e.g.*, human B cell). In some embodiments, a cell is transfected by one or more polynucleotide sequences isolated from a B cell (*e.g.*, human B cell) where the polynucleotide sequence encodes for anti-nucleolin antibody (*e.g.*, human and/or monoclonal). The cDNA or genomic DNA encoding the polypeptides can be inserted into expression vectors so that both genes are operatively linked to their own transcriptional and translational expression control sequences. The expression vector and expression control sequences can then be chosen to be compatible with the expression host cell used. In some embodiments, separate expression vectors are used for the heavy and light antibody chains.

[0115] Prokaryotic or eukaryotic cells can be used as expression hosts. Expression in eukaryotic host cells may be suitable because such cells are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. However, any antibody produced that is inactive due to improper folding may be renaturable according to well known methods (Kim and Baldwin, 1982). It is possible that the host cells will produce portions of intact antibodies, such as light chain dimers or heavy chain dimers, which also are antibody homologs according to the present disclosure.

[0116] It will be understood that variations on the above procedure are within the scope of the present disclosure. In some embodiments, a host cell is transformed with DNA encoding either the light chain or the heavy chain (but not both) of an antibody homolog. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for nucleolin binding. The molecules expressed from such truncated DNA molecules are antibody homologs. In some embodiments, bifunctional antibodies are produced in which one heavy and one light chain are homologs of an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) and the other heavy and light chain are specific for an antigen other than nucleolin, or another epitope of nucleolin.

[0117] In some embodiments, DNA encoding an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is transferred to a mammalian cell line for expression in "production" or commercial amounts. It has long been recognized that Chinese Hamster Ovary cells (CHO cells) make excellent expression vehicles for recombinant or non-endogenous DNA. See U.S. Patent 4,816,567. There has been developed a series of DHFR deficient CHO cell strains, which permit the amplification of inserted DNA encoding specific proteins or DNA sequences, as set forth in U.S. Patent 5,981,214. Examples of additional mammalian cell lines for expression in "production" or commercial amounts include, but are not limited to 293HEK cells, HeLa cells, COS cells, NIH3T3 cells, Jurkat Cells., NSO cells and HUVEC cells. Other mammalian cell lines suitable for the expression of recombinant proteins have been identified in the literature, and can be equally suitable for use in the present disclosure of this application.

4. Modifications of Antibodies

[0118] In some embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. A variant typically differs from a polypeptide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants can be naturally occurring or can be synthetically generated, for example, by modifying one or more of the above polypeptide sequences and evaluating one or more biological activities of the polypeptide as described herein and/or using any of a number of techniques well known in the art. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding and/or potency.

[0119] In some embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for mutagenesis by substitution include the CDRs and Frameworks (FRs). Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC. Non-limiting examples of amino acid substitutions are shown in **Table 2**.

Table 2. Examples of Amino Acid Substitutions.

Original Residue	Exemplary Conserved Substitutions
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gln; Asn

Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn; Glu
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln; Lys; Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

[0120] Hydrophobic amino acids include: Norleucine, Met, Ala, Val, Leu, and Ile. Neutral hydrophilic amino acids include: Cys, Ser, Thr, Asn, and Gln. Acidic amino acids include: Asp and Glu. Basic amino acids include: His, Lys, and Arg. Amino acids with residues that influence chain orientation include: Gly and Pro. Aromatic amino acids include: Trp, Tyr, and Phe.

[0121] In some embodiments, substitutions, insertions, or deletions, *e.g.*, by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, may occur within constant regions or one or more CDRs, wherein the substitutions, insertions, or deletions do not substantially reduce antibody binding to antigen. For example, conservative substitutions that do not substantially reduce binding affinity may be made in CDRs. Such alterations may be outside of CDR "hotspots" or SDRs. In some embodiments of the variant V_H and V_L sequences, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0122] Alterations (*e.g.*, substitutions) may be made in CDRs, *e.g.*, by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, to improve antibody affinity. Such alterations may be made in CDR encoding codons with a high mutation rate during somatic maturation (*See, e.g.,* Chowdhury, Methods Mol. Biol. 207:179-196 (2008)), and the resulting variant can be tested for binding affinity. Affinity maturation (*e.g.*, using error-prone PCR, chain shuffling, randomization of CDRs, or oligonucleotide-directed mutagenesis) can be used to improve antibody affinity (*See, e.g.,* Hoogenboom et al. in Methods in Molecular Biology 178:1-37 (2001)). CDR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling (*See, e.g.,* Cunningham and Wells Science, 244:1081-1085 (1989)). CDR-H3 and CDR-L3 are often targeted. In some embodiments, a crystal structure of an antigen-antibody

complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0123] Amino acid sequence insertions and deletions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions and deletions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.,* for ADEPT) or a polypeptide which increases the serum half-life of the antibody. Examples of intrasequence insertion variants of the antibody molecules include an insertion of 3 amino acids in the light chain. Examples of terminal deletions include an antibody with a deletion of 7 or less amino acids at an end of the light chain.

[0124] In some embodiments, an anti-nucleolin antibody may be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.* (1985); Hwang *et al.* (1980); and U.S. Patents 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent 5,013,556.

[0125] Useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present disclosure can be conjugated to the liposomes as described in Martin *et al.* (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.* (1989).

[0126] In some embodiments, an anti-nucleolin antibody is used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (*e.g.*, a peptidyl chemotherapeutic agent, see WO 81/01145) to an active drug. *See,* for example, WO 88/07378 and U.S. Patent 4,975,278. The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active form exhibiting the desired biological properties.

[0127] Enzymes that can be useful include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases,

useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. In some embodiments, antibodies with enzymatic activity, also known in the art as "abzymes," can be used to convert the prodrugs of the present disclosure into free active drugs (*see, e.g.*, Massey, 1987). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a desired cell population.

[0128] The enzymes can be covalently bound to the anti-nucleolin antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. In some embodiments, fusion proteins comprising at least the antigen binding region of an antibody of the present disclosure linked to at least a functionally active portion of an enzyme of the present disclosure can be constructed using recombinant DNA techniques well known in the art (*see, e.g.*, Neuberger *et al.,* 1984).

[0129] In some embodiments, an anti-nucleolin antibody comprises an antibody fragment, rather than an intact antibody. In this case, the antibody fragment may be modified in order to increase its serum half-life. This may be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment (*e.g.*, by mutation of the appropriate region in the antibody fragment or by incorporating the epitope into a peptide tag that is then fused to the antibody fragment at either end or in the middle, *e.g.*, by DNA or peptide synthesis). See WO 96/32478 published Oct. 17, 1996.

[0130] The salvage receptor binding epitope generally constitutes a region wherein any one or more amino acid residues from one or two loops of an Fc domain are transferred to an analogous position of the antibody fragment. For example, three or more residues from one or two loops of the Fc domain are transferred. For example, the epitope is taken from the CH2 domain of the Fc region (*e.g.*, of an IgG) and transferred to the CH1, CH3, or V_H region, or more than one such region, of the antibody. For example, the epitope is taken from the CH2 domain of the Fc region and transferred to the C_L region or V_L region, or both, of the antibody fragment.

[0131] In some embodiments, an anti-nucleolin antibody is modified by covalent linkages. Covalent linkages may include but are not limited to by chemical synthesis or by enzymatic or chemical cleavage of the antibody. Other types of covalent modifications of the antibody are introduced into the molecule by reacting targeted amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues. Exemplary covalent modifications of polypeptides are described in U.S. Patent

5,534,615, specifically incorporated herein by reference. One type of covalent modification of the antibody comprises linking the antibody to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patents 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

[0132] In some embodiments, an anti-nucleolin antibody (such as a human antibody) is modified by conjugating it to another, heterologous polypeptide or amino acid sequence. In some embodiments, an anti-nucleolin antibody (such as a human antibody) is modified to comprise targeted immunoconjugate moieties which enable the effective generation of innate and adaptive immune responses against tumors or pathogens. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is modified to comprise targeted immunoconjugate moieties which enable the effective generation of innate and adaptive immune responses against tumors or pathogens. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) produced by a B cell (*e.g.*, human B cell) is modified to comprise targeted immunoconjugate moieties which enable the effective generation of innate and adaptive immune responses against tumors or pathogens. The immunoconjugates can be capable of simultaneously satisfying multiple key requirements for mounting effective antibody- and/or cell-mediated immune responses against the targeted tumor or pathogen, which include but are not limited to : (i) Inducing or augmenting uptake and cross-presentation of tumor- or pathogen antigen(s) or antigenic determinant(s) by antigen presenting cells (APC)/dendritic cells (DC); (ii) promoting the maturation of dendritic cells (DCs) in the target cell milieu; (iii) providing CD4+ T cell help to generate CD8+ T cell memory and antibodies against the tumor or pathogen; (iv) sensitizing the targeted tumor cell to antibody dependent cell cytotoxicity (ADCC) and T-cell mediated death. Such immunoconjugated antibodies can be used for targeted immunotherapy or immunoprophylaxis of neoplastic diseases, infectious diseases, and other disorders. For example, pattern recognition receptors (PRRs), such as Toll like Receptors, recognize pathogen-associated molecular patterns (PAMPs) expressed by diverse infectious microorganisms (bacteria, fungi, protozoa, viruses) and molecules released by damaged host tissues (damage associated molecular patterns/alarmins). The addition of a PAMP conjugated to an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) provides a moiety comprising a nucleic acid or protein that is recognized by a PRR, ultimately leading to an immune response which eliminates the target cell with the anti-nucleolin antibody bound to it. Examples of PAMPS that can be conjugated to an anti-nucleolin antibody include but are limited to known viral and pathogenic epitopes, such as polyinosine-polycytidylic acid, lipopolysaccharide (LPS), lipid A, flagellin, GU-rich short single-stranded RNA, unmethylated CpG-oligodeoxynucleotides.

[0133] In some embodiments, an anti-nucleolin antibody (such as a human antibody) is modified by fusing, or conjugating it to another, heterologous polypeptide or amino acid sequence. In some embodiments an anti-nucleolin antibody (such as a human antibody) is fused or conjugated with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag can be placed at the amino- or carboxyl-terminus of the anti-nucleolin antibody. The presence of such epitope-tagged forms of an anti-nucleolin antibody can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the anti-nucleolin antibody to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag and its antibody 12CA5 (Field *et al.*, 1988); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan *et al.*, 1985); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky *et al.*, 1990). Other tag polypeptides include the Flag-peptide (Hopp *et al.*, 1988); the KT3 epitope peptide (Martin *et al.*, 1992); an α-tubulin epitope peptide (Skinner *et al.*, 1991); and the T7 gene 10 protein peptide tag (Lutz-Freyermuth *et al.*, 1990).

[0134] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) or fragment is linked to a nanoparticle. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is linked to a nanoparticle. In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) produced by a B cell (*e.g.*, human B cell) is linked to a nanoparticle. Cell surface nucleolin has been reported to serve as receptor for DNA nanoparticles composed of PEGylated polylysine and DNA (Chen *et al.*, 2008). In some embodiments, the antibody-nanoparticle conjugate can penetrate a cell expressing nucleolin on its surface more rapidly and extensively than the unconjugated antibody. In some embodiments, the cell is a cancer cell, tumor cell, virally infected cell, lymphocyte, or activated lymphocyte.

5. Therapeutic Use

5.1. Anti-nucleolin antibodies

[0135] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is provided that can be used to inhibit or kill a cancer cell. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is provided that can be used to inhibit or kill a cancer cell. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) expressed by a B cell (*e.g.*, human B cell) is provided that can be used to inhibit or kill a cancer cell. In some embodiments the cancer cell expresses nucleolin on its surface or in it cytoplasm. Examples of cancer cells that can be inhibited or killed by an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) are described hereinbelow.

[0136] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to reduce cell viability of a cancer cell in a subject sample by 30 to 80% as compared to cells not exposed to an anti-nucleolin antibody (*e.g.*, human and/or monoclonal). In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to reduce cell viability of a cancer cell in a subject sample by 30 to 80% as compared cells not exposed to an anti-nucleolin antibody (*e.g.*, human and/or monoclonal). In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) produced from a B cell (*e.g.*, human B cell) is provided and used to reduce cell viability of a cancer cell in a subject sample by 30 to 80% as compared cells not exposed to an anti-nucleolin antibody (*e.g.*, human and/or monoclonal).

[0137] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to reduce cell viability of a cancer cell in a subject by 30 to 80% as compared cells not exposed to an anti-nucleolin antibody (*e.g.*, human and/or monoclonal). In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to reduce cell viability of a cancer cell in a subject by 30 to 80% as compared cells not exposed to an anti-nucleolin antibody (*e.g.*, human and/or monoclonal). In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) produced from a B cell (*e.g.*, human B cell) is provided and used to reduce cell viability of a cancer cell in a subject by 30 to 80% as compared cells not exposed to an anti-nucleolin antibody (*e.g.*, human and/or monoclonal).

[0138] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is administered to a human subject with one or more forms of cancer. In some embodiments an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is administered to a human subject with one or more forms of cancer. In some embodiments at least one of the forms of cancer is inhibited or killed by an anti-nucleolin antibody (*e.g.*, human and/or monoclonal). In some embodiments an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is administered to a human subject where the cancer is resistant to other cancer treatments. In some embodiments an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) produced from a B cell (*e.g.*, human B cell) is provided is administered to a human subject where the cancer is resistant to other cancer treatments. For example, cancers can be resistant to radiation therapy, chemotherapy, or biological therapy. In some embodiments the immune system of the human subject is more tolerant to the isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) than to an isolated non anti-nucleolin antibody (*e.g.*, human and/or monoclonal). In some embodiments, the immune system of the human subject is more tolerant to the isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) than to an isolated humanized anti-nucleolin antibody. In some embodiments, the immune system of the human

subject is more tolerant to the isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) than to an isolated chimeric anti-nucleolin antibody.

[0139] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill a cell as part of an adjuvant therapy. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill a cell as part of an adjuvant therapy. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) expressed by a B cell (*e.g.*, human B cell) is provided and used as part of an adjuvant therapy. Adjuvant therapy can include chemotherapy, radiation therapy, hormone therapy, targeted therapy, or biological therapy. Adjuvant therapy as used herein refers to treatment given after the primary treatment to lower the risk that the cancer will come back.

[0140] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill a cell used in combination with an adjuvant therapy. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill a cell used in combination with an adjuvant therapy. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) expressed by a B cell (*e.g.*, human B cell) is provided as part of an adjuvant therapy. Adjuvant therapy may include chemotherapy, radiation therapy, hormone therapy, targeted therapy, or biological therapy.

[0141] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill a cell of a non-malignant cell proliferative disorder wherein nucleolin is expressed on the cell surface or in the cytoplasm. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill a cell of a non-malignant cell proliferative disorder wherein nucleolin is expressed on the cell surface or in the cytoplasm. In some embodiments, the isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is expressed by a B cell (*e.g.*, human B cell). For example, specific non-limiting examples of non-malignant cell proliferative disorders that can treat or inhibited with an anti-nucleolin antibody include but are not limited to warts, benign prostatic hyperplasia, skin tags, and non-malignant tumors. For example, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) can be used to determine such cell proliferative disorders as benign prostatic hyperplasia or unwanted genital warts by targeting the undesirable cells that characterize such conditions for removal. Expression of nucleolin on the cell surface of endothelial cells in tumors has been shown to be a unique marker of tumor angiogenesis (Christian *et al.*, 2003). In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill in a subject a cell comprising an angiogenic tumor. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill in a subject a cell comprising an angiogenic tumor. In some embodiments, an isolated anti-nucleolin antibody

(*e.g.*, human and/or monoclonal) expressed by a B cell (*e.g.*, human B cell) is provided that can inhibit or kill in a subject a cell comprising an angiogenic tumor. An angiogenic tumor as used herein a tumor cell with a proliferation of a network of blood vessels that penetrate into cancerous growths, supplying nutrients and oxygen and removing waste products.

[0142] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill in a subject a tumor cell under conditions of tumor hypoxia. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill in a subject a tumor cell under conditions of tumor hypoxia. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) expressed by a B cell (*e.g.*, human B cell) is provided that can inhibit or kill in a subject a tumor cell under conditions of tumor hypoxia. Tumor hypoxia occurs in the situation where tumor cells have been deprived of oxygen. Tumor hypoxia can be a result of the high degree of cell proliferation undergone in tumor tissue, causing a higher cell density, and thus taxing the local oxygen supply.

[0143] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill in subject a lymphocyte cell expressing nucleolin on its surface. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill in subject a lymphocyte cell expressing nucleolin on its surface. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) expressed by a B cell (*e.g.*, human B cell) is provided that is used to inhibit or kill in subject a lymphocyte cell expressing nucleolin on its surface. In some embodiments, the lymphocyte cell comprises a B cell, T cell, or natural killer cell. In some embodiments, the lymphocyte cell comprises a CD4-positive or CD8-positive cells.

[0144] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill in a subject an activated lymphocyte or memory cell expressing nucleolin on its surface. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill in a subject an activated lymphocyte or memory cell expressing nucleolin on its surface. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) expressed by a B cell (*e.g.*, human B cell) is provided that is used to inhibit or kill in subject an activated lymphocyte cell or memory cell expressing nucleolin on its surface. In a further embodiment, the activated lymphocyte comprises an activated B cell, T cell, or natural killer cell. In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill a cell in a subject having an autoimmune disorder. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is used to inhibit or kill a cell in a subject having an autoimmune disorder. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal)

expressed by a B cell (*e.g.*, human B cell) is provided that is used to inhibit or kill a cell in a subject having an autoimmune disorder, including but not limited to alopecia greata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, asthma, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, diabetes, type 1 diabetes mellitus, diabetic retinopathy, eosinophilic fascites, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barre, Hashimoto's thyroiditis, Henoch-Schonlein purpura, idiopathic pulmonary fibrosis, idiopathic/autoimmune thrombocytopenia purpura (ITP), IgA neuropathy, juvenile arthritis, lichen planus, lupus erthematosus, Meniere's disease, mixed connective tissue disease, multiple sclerosis, type 1 or immune-mediated diabetes mellitus, myasthenia gravis, pemphigus-related disorders (*e.g.*, pemphigus vulgaris), pernicious anemia, polyarteritis nodosa, polychrondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomenon, Reiter's syndrome, Rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, stiff-man syndrome, systemic lupus erythematosus (SLE), Sweet's syndrome, Still's disease, lupus erythematosus, takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vasculitis such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis. Examples of inflammatory disorders include, but are not limited to, asthma, encephalitis, inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), allergic disorders, septic shock, pulmonary fibrosis, undifferentiated spondyloarthropathy, undifferentiated arthropathy, arthritis, inflammatory osteolysis, graft versus host disease, urticaria, Vogt-Koyanagi-Hareda syndrome, chronic inflammatory pneumonitis, and chronic inflammation resulting from chronic viral or bacteria infections.

[0145] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) or fragment is used to inhibit or kill a cell in a subject infected by a virus, including but not limited to cells infected with Retroviridae (*e.g.,* human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III); and other isolates, such as HIV-LP); Picornaviridae (*e.g.,* polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); Calciviridae (*e.g.,* strains that cause gastroenteritis); Togaviridae (*e.g.,* equine encephalitis viruses, rubella viruses); Flaviridae (*e.g.,* dengue viruses, encephalitis viruses, yellow fever viruses); Coronoviridae (*e.g.,* coronaviruses); Rhabdoviradae

(*e.g.,* vesicular stomatitis viruses, rabies viruses); Filoviridae (*e.g.,* ebola viruses); Paramyxoviridae (*e.g.,* parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (*e.g.,* influenza viruses); Bungaviridae (*e.g.,* Hantaan viruses, bunga viruses, phleboviruses and Nairo viruses); Arenaviridae (hemorrhagic fever viruses); Reoviridae (*e.g.,* reoviruses, orbiviurses and rotaviruses); Bimaviridae; Hepadnaviridae (Hepatitis B virus); Parvovirida (parvoviruses); Papovaviridae (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus); Rous sarcoma virus (RSV), avian leukemia virus (ALV), and avian myeloblastosis virus (AMV)) and C-type group B (including feline leukemia virus (FeLV), gibbon ape leukemia virus (GALV), spleen necrosis virus (SNV), reticuloendotheliosis virus (RV) and simian sarcoma virus (SSV)), D-type retroviruses include Mason-Pfizer monkey virus (MPMV) and simian retrovirus type 1 (SRV-1), the complex retroviruses including the subgroups of lentiviruses, T-cell leukemia viruses and the foamy viruses, lentiviruses including HIV-1, HIV-2, SIV, Visna virus, feline immunodeficiency virus (FIV), and equine infectious anemia virus (EIAV), simian T-cell leukemia virus (STLV), and bovine leukemia virus (BLV), the foamy viruses including human foamy virus (HFV), simian foamy virus (SFV) and bovine foamy virus (BFV), Poxyiridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (*e.g.,* African swine fever virus); and unclassified viruses (*e.g.,* the etiological agents of Spongiform encephalopathies, the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1 = internally transmitted; class 2 = parenterally transmitted (*i.e.*, Hepatitis C); Norwalk and related viruses, and astroviruses), Mycobacterium (Mycobacterium tuberculosis, *M. bovis*, *M. avium-intracellulare*, *M. leprae*), *Pneumococcus*, *Streptococcus*, *Staphylcococcus*, *Diphtheria*, *Listeria*, *Erysipelothrix*, Anthrax, Tetanus, *Clostridium*, Mixed Anaerobes, *Neisseria*, *Salmonella*, *Shigella*, *Hemophilus*, *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Serratia*, *Pseudomonas*, *Bordatella*, *Francisella tularensis*, *Yersinia*, *Vibrio cholerae*, *Bartonella*, *Legionella*, *Spirochaetes* (*Treponema*, *Leptospira*, *Borrelia*), Fungi, *Actinomyces*, *Rickettsia*, *Mycoplasma*, *Chlamydia*, *Protozoa* (including *Entamoeba*, *Plasmodium*, *Leishmania*, *Trypanosoma*, *Toxoplasma*, *Pneumocystis*, *Babasia*, *Giardia*, *Cryptosporidium*, *Trichomonas*), Helminths (*Trichinella*, *Wucheraria*, *Onchocerca*, *Schistosoma*, Nematodes, Cestodes, Trematodes), and viral pneumonias. Additional examples of antigens which can be targets for compositions of the present disclosure are known, such as those disclosed in U.S. Patent Publication No. 2007/0066554. In a further aspect, a conjugate can comprise an antigen or cellular component as described herein, but in addition to a targeting moiety and an immunostimulatory nucleic acid molecule.

5.2. Antibody Conjugates

[0146] In some embodiments, the present disclosure provides for an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) linked to at least one therapeutic agent to form an antibody conjugate. In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal), is linked, or covalently bound, or complexed to at least one therapeutic agent, such as a molecule or moiety. Therapeutic agents comprise molecules having a desired activity, *e.g.*, cytotoxic activity. In some embodiments, a therapeutic agent which can be attached to an antibody includes but is not limited to a toxin (such as a peptide immunotoxin that catalytically inhibit the elongation step of protein synthesis) an anti-tumor agent, a therapeutic enzyme, a radionuclide, an antiviral agent, a chelating agent as described herein, a cytokine, a growth factor, or a oligo- or polynucleotide.

[0147] In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is conjugated to an enzymatically active toxin or fragment thereof. Examples of enzymatically active toxins and fragments thereof include, but are not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), pokeweed antiviral protein, momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, calicheamicins orthe tricothecenes.

[0148] In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is conjugated to a radionuclide. Examples of suitable radionuclides include, but are not limited to, ^{124}antimony, ^{125}antimony, ^{74}arsenic, ^{211}astatine, ^{103}barium, ^{140}barium, ^{7}beryllium, ^{206}bismuth, ^{207}bismuth, ^{212}Bi, ^{109}cadmium, ^{115}cadmium, ^{45}calcium, ^{14}carbon, ^{139}cerium, ^{141}cerium, ^{144}cerium, ^{137}cesium, ^{51}chromium, ^{36}chlorine, ^{56}cobalt, ^{57}cobalt, ^{58}cobalt, ^{60}cobalt,^{67}copper, ^{169}erbium, ^{152}eurpium, ^{67}gallium, ^{153}gadolinium, ^{195}gold, ^{199}gold, ^{175}hafnium, $^{175+181}$hafnium, ^{181}hafnium, ^{3}hydrogen, ^{123}iodine, ^{125}iodine, ^{131}iodine, ^{111}indium, ^{131}In, ^{192}iridium, ^{55}iron, ^{59}iron, ^{85}krypton, ^{210}lead, ^{177}lutecium, ^{54}manganese, ^{197}mercury, ^{203}mercury, ^{99}molybdenum, ^{147}neodynium, ^{237}neptunium, ^{63}nickel, ^{95}niobium, $^{185+191}$osmium, ^{103}palladium, ^{32}phosphorus, ^{184}platinum,^{143}praseodymium, ^{147}promethium, ^{233}protactinium, ^{226}radium, rhenium^{186}, ^{188}rhenium, ^{86}rubidium, ^{130}ruthenium, ^{106}ruthenium, ^{44}scandium, ^{46}scandium, ^{45}selenium, ^{75}selenium, ^{110m}silver, ^{111}silver, ^{22}sodium, ^{85}strontium, ^{89}strontium, ^{90}strontium, ^{35}sulphur, ^{182}tantalum, ^{99m}technicium, ^{125m}tellurium, ^{132}tellurium, ^{160}terbium, ^{204}thallium, ^{228}thorium, ^{232}thorium, ^{170}thullium, ^{113}tin, ^{44}titanium, ^{185}tungsten, ^{48}vanadlum, ^{49}vanadium, ^{88}yttrium, ^{90}yttrium, ^{91}yttrium, ^{169}ytterbium, ^{65}zinc, and/or ^{95}zirconium.

[0149] Conjugates of the antibody and cytotoxic agent can be made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.* (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 94/11026.

[0150] In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is conjugated to a cytokine. The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF- β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1a, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

[0151] In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is conjugated to a chemotherapeutic agent. A variety of chemical compounds, also described as "chemotherapeutic agents," function to induce DNA damage. Categories of chemotherapeutic agents suitable for conjugation with a an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) include, but are not limited to, alkylating agents, anthracyclines,

cytoskeletal disruptors, epothilones, inhibitors of topoisomerase I, inhibitors of topoisomerase II, nucleoside and nucleotide analogs and precursor analogs, peptide antibiotics, platinum-based agents, retinoids, or vinca alkaloids and derivatives. Specific chemotherapeutic agents within these groups include, but are not limited to, actinomycin-D, all-trans retinoic acid azacitidine, adriamycin azathioprine, bleomycin, camptothecin, carboplatin, capecitabine, cisplatin, chlorambucil, cyclophosphamide, cytarabine, daunorubicin, docetaxel, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, fluorouracil, 5-fluorouracil (5FU), gemcitabine, hydroxyurea, hydrogen peroxide, idarubicin, imatinib, mechlorethamine, mercaptopurine, methotrexate, mitomycin C, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, teniposide, tioguanine, valrubicin, vinblastine , vincristine, vindesine, vinorelbine. The present disclosure also encompasses the use of a combination of one or more DNA damaging agents, whether radiation-based or actual compounds, such as the use of X-rays with cisplatin or the use of cisplatin with etoposide.

[0152] In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is conjugated to an anti-viral agent. Example of anti-viral agents that can be used with an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) include, but are not limited to, substrates and substrate analogs, inhibitors and other agents that severely impair, debilitate or otherwise destroy virus-infected cells. Substrate analogs include amino acid and nucleoside analogs. Substrates can be conjugated with toxins or other virucidal substances. Inhibitors include integrase inhibitors, protease inhibitors, polymerase inhibitors and transcriptase inhibitors such as reverse transcriptase inhibitors.

5.3. Pharmaceutical Compositions and Administration

[0153] It is envisioned that, for administration to a subject in need thereof, an antibody will be suspended in a composition suitable for administration to a host. In some embodiments the antibody is a monoclonal antibody. In some embodiments the monoclonal antibody is an anti-nucleolin antibody. In some embodiments the monoclonal anti-nucleolin antibody is a human monoclonal anti-nucleolin antibody. Aqueous compositions of the present disclosure comprise an effective amount of an antibody dispersed in a pharmaceutically acceptable composition and/or aqueous medium. The phrases "pharmaceutically and/or pharmacologically acceptable" refer to compositions that do not produce an adverse, allergic and/or other untoward reaction when administered to an animal, and specifically to humans, as appropriate.

[0154] As used herein, "pharmaceutically acceptable carrier" includes any solvents, dispersion media, coatings, antibacterial and/or antifungal agents, isotonic and/or absorption delaying agents and the like. The use of such media or agents for pharmaceutical active substances is well known in the art. Supplementary active ingredients can also be incorporated into the

compositions. For administration to humans, preparations should meet sterility, pyrogenicity, general safety and/or purity standards as required by FDA Office of Biologics standards.

[0155] In some embodiments, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) of the present disclosure can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. Any of these molecules can be administered to a patient alone, or in combination with other agents, drugs or hormones, in pharmaceutical compositions where it is mixed with suitable excipient(s), adjuvants, and/or pharmaceutically acceptable carriers. In some embodiments of the present disclosure, the pharmaceutically acceptable carrier is pharmaceutically inert.

[0156] Administration of pharmaceutical compositions is accomplished orally or parenterally. Methods of parenteral delivery include topical, intra-arterial (*e.g.*, directly to a tumor), intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration. In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically acceptable carriers comprising excipients and other compounds that facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of "Remington's Pharmaceutical Sciences" (Maack Publishing Co, Easton Pa.). Antibody compositions can be lyophilized. Antibody compositions can be aqueous antibody. The compositions to be used for *in vivo* administration are generally sterile (*e.g.*, by filtration through sterile filtration membranes).

Some compositions can be suitable for targeted delivery to the brain or the spinal fluid of a subject. The composition can be substantially free of preservatives. Some compositions are stable for at least about 12 months, at least about 18 months, at least about 24 months, or at least about 30 months. Some compositions are stable at about −80° C. to about 40° C., at about 0° C. to about 25° C., at about 0° C. to about 10° C., such as at about −80° C. to about −50° C. or at about 2° C. to about 8° C.

[0157] Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, *etc.*, suitable for ingestion by the patient. See PCT publication WO 93/23572.

[0158] Pharmaceutical preparations for oral use may be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable additional compounds, if desired, to obtain tablets or

dragee cores. Suitable excipients are carbohydrate or protein fillers include, but are not limited to sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; as well as proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

[0159] Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound (*i.e.*, dosage).

[0160] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

[0161] Pharmaceutical compositions for parenteral administration include aqueous solutions of active compounds. For injection, the pharmaceutical compositions of the present disclosure may be formulated in aqueous solutions, such as in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0162] For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the composition. Such penetrants are generally known in the art.

[0163] The pharmaceutical compositions of the present disclosure may be manufactured in a manner similar to that known in the art (*e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes).

[0164] The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.* Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In some embodiments, the preparation may be a lyophilized powder in 1 mM-50 mM histidine, 0.1%-2% sucrose, 2%-7% mannitol, at a pH range of 4.5 to 5.5, which is combined with buffer prior to use.

[0165] The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial ad antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it may be suitable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0166] Pharmaceutical compositions suitable for use in the present disclosure include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. "Therapeutically effective amount" or "pharmacologically effective amount" are well recognized phrases and refer to that amount of an agent effective to produce the intended pharmacological result. Thus, a therapeutically effective amount is an amount sufficient to ameliorate the symptoms of the disease being treated. One useful assay in ascertaining an effective amount for a given application (*e.g.*, a therapeutically effective amount) is measuring the effect on cell survival. The amount actually administered will be dependent upon the individual to which treatment is to be applied, and will probably be an optimized amount such that the desired effect is achieved without significant side-effects.

[0167] For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in any appropriate animal model. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

[0168] In an animal, a "therapeutically effective amount" is the quantity of compound which results in an improved clinical outcome as a result of the treatment compared with a typical

clinical outcome in the absence of the treatment. An "improved clinical outcome" refers, for, example, to a longer life expectancy, fewer complications, fewer symptoms, less physical discomfort and/or fewer hospitalizations as a result of the treatment. Improved clinical outcome can be quantified as a certain percent of subjects receiving administration and improving in their disease state over certain period of time. The certain percent of subjects receiving administration and improving in their disease state may be about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%. The certain percent of subjects receiving administration and improving in their disease state may be about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 85%. The certain percent of subjects receiving administration and improving in their disease state may be about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. The certain period of time to measure improved clinical outcome may be 1, 2, 3, 4, 5, 6, or 7 days. The certain period of time to measure improved clinical outcome may be 1, 2, 3, or 4 weeks. The certain period of time to measure improved clinical outcome may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more years.

[0169] With respect to cancer, an "improved clinical outcome" includes a longer life expectancy. It can also include slowing or arresting the rate of growth of a tumor, causing shrinkage in the size of the tumor, a decreased rate of metastasis or an improved quality of life (*e.g.*, a decrease in physical discomfort or an increase in mobility).

[0170] With respect to modulation of the immune system, "an improved clinical outcome" refers to an increase in the magnitude of the immune response in the individual, if the individual has a disease involving immune suppression. "An improved clinical outcome" for individuals with suppressed immune systems can also refer to a lesser susceptibility to infectious diseases. For diseases involving an overactive immune system, "an improved clinical outcome" can refer to a decrease in the magnitude of the immune response. In both cases, an improved clinical outcome can also involve an improvement in the quality of life, as described above.

[0171] The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state (*e.g.*, tumor size and location; age, weight and gender of the patient; diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy). Administration may be every day, every other day, every week, every other week, every month, every other month, or any variation thereof. Administration of a dosage form comprising an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) may be for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days. Administration of a dosage form comprising an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) may be for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or

10 weeks. Administration of a dosage form comprising an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) may be for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. Administration of a dosage form comprising an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) may be for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 years. Administration of one or more agents (*e.g.*, an anti-nucleolin antibody (*e.g.*, human and/or monoclonal) and another agent) can be intermittent; for example, administration can be once every two days, every three days, every five days, once a week, once or twice a month, and the like. Long acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular composition. Guidance as to particular dosages and methods of delivery is provided in the literature (*see,* U.S. Patents 4,657,760; 5,206,344; and 5,225,212, herein incorporated by reference). In some embodiments, the dosage of a composition comprising anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is administered to a patient is about 0.1 mg/kg to 500 mg/kg of the patient's body weight. The amount, forms, and/or amounts of the different forms can be varied at different times of administration.

[0172] An antibody or composition disclosed herein (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route. For example, dosing can be by injections (*e.g.*, intravenous or subcutaneous injections). Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0173] Antibody disclosed herein can be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the composition, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0174] For the prevention or treatment of disease, the appropriate dosage of an antibody disclosed herein (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. About 1 μg/kg to 15 mg/kg (*e.g.,* 0.1 mg/kg-10 mg/kg) of antibody can be an initial candidate dosage for administration to the patient (*e.g.*, by one or more separate administrations, or by continuous infusion). A daily dosage might range from about 1 μg/kg to 100 mg/kg or more. For repeated administrations over several days or longer the treatment would generally be sustained until a desired suppression of infection or disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently (*e.g.*, every week or every three weeks). An initial higher loading dose, followed by one or more lower doses may be administered.

[0175] Pharmaceutical compositions of an antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*See*, *e.g.*, Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized compositions or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed. Exemplary pharmaceutical acceptable carriers include buffers (*e.g.*, phosphate, citrate, and other organic acids); antioxidants (*e.g.*, ascorbic acid and methionine); preservatives (*e.g.*, octadecyldimethylbenzyl ammonium chloride); hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens (*e.g.*, methyl or propyl paraben); catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol; low molecular weight (less than about 10 residues) polypeptides; proteins, (*e.g.*, serum albumin, gelatin, or immunoglobulins); hydrophilic polymers (*e.g.*, polyvinylpyrrolidone); amino acids (*e.g.*, glycine, glutamine, asparagine, histidine, arginine, or lysine); monosaccharides, disaccharides, and other carbohydrates (*e.g.*, glucose, mannose, or dextrins); chelating agents (*e.g.*, EDTA); sugars (*e.g.*, sucrose, mannitol, trehalose or sorbitol); salt-forming counter-ions (*e.g.*, sodium); metal complexes (*e.g.,* Zn-protein complexes); and/or non-ionic surfactants (*e.g.*, polyethylene glycol (PEG)). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents (*e.g.*, soluble neutral-active hyaluronidase

glycoproteins (sHASEGP)). In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases (*e.g.*, chondroitinases).

[0176] Active ingredients may be entrapped in microcapsules (*e.g.*, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) Active ingredients may be entrapped in microcapsules in colloidal drug delivery systems (*e.g.*, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles (*e.g.*, films or microcapsules).

[0177] In some liquid compositions, the concentration of the antibody is about 0.1 mg/ml to about 60 mg/ml, about 40 mg/ml to about 60 mg/ml, about 17 mg/ml to about 23 mg/ml, about 50 mg/ml, about 30 mg/ml, about 17 mg/ml to about 23 mg/ml, about 20 mg/ml, about 17 mg/ml, about 10 mg/ml, about 5 mg/ml, about 2 mg/ml, or about 1 mg/ml. In some compositions, at least one tonicity agent (*e.g.*, D-mannitol) and is present at a concentration of about 1% w/v to about 10% w/v, about 2% w/v to about 6% w/v, or about 4% w/v. In some compositions, at least one buffering agent (*e.g.*, histidine, succinate) is present at a concentration of about 0.1 mM to about 25 mM, about 5 mM to about 15 mM, about 5 mM or about 10 mM. In some compositions, an antioxidant (*e.g.*, methionine) is present at a concentration of about 0.1 mM to about 25 mM, about 5 mM to about 15 mM, or about 10 mM. In some compositions, a stabilizer (*e.g.*, polysorbate 80) is present at a concentration of about 0.001% w/v to about 0.01% w/v, about 0.005% w/v to about 0.01% w/v, or about 0.005% w/v. A composition disclosed herein can have a pH of about 4 to about 8, about 4.5 to about 7.5, about 5 to about 7, about 5.5 to about 6.5, about 6.0 to about 6.5, about 6.2, about 6.0, or about 5.5.

[0178] In some embodiments, an antibody disclosed herein is present in a composition from about 0.1 mg/ml to about 100 mg/ml, from about 0.1 mg/ml to about 75 mg/ml, from about 0.1 mg/ml to about 50 mg/ml, from about 0.1 mg/ml to about 40 mg/ml, from about 0.1 mg/ml to about 30 mg/ml, from about 10 mg/ml to about 20 mg/ml, about 12 mg/ml to about 17 mg/ml, about 17 mg/ml to about 23 mg/ml, from about 20 mg/ml to 30 mg/ml, or higher, for example, up to about 100 mg/ml, about 200 mg/ml, about 500 mg/ml, or about 1000 mg/ml or more. In various embodiments, the antibody is present at about 1, 2, 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or 30 mg/ml. Ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

[0179] In some embodiments, compositions disclosed herein are stable to freezing, lyophilization and/or reconstitution. Moreover, exemplary embodiments are stable over extended periods of time. For example, the compositions are stable for at least about 6, 7, 8, 9, 10, 11, 12,

13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 months. In some embodiments, the compositions are stable for at least about 12 months, for at least about 18 months, for at least about 24 months, or for at least about 30 months. In some embodiments, the composition may be stored at temperatures from about −80° C. to about 40° C., from about 0° C. to about 25° C., from about 0° C. to about 15° C., or from about 0° C. to about 10° C., for example from about 2° C. to about 8° C. In various embodiments, the composition may be stored at about 0° C., 1° C., 2° C., 3° C., 4° C., 5° C., 6° C., 7° C., 8° C., 9° C. or 10° C. In some embodiments, the composition is stored at about 5° C. Generally, the composition is stable and retains biological activity at these ranges. Ranges intermediate to the above recited temperatures, for example, from about 2° C. to about 17° C., are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

[0180] Dosage

[0181] Effective doses of the compositions vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals including transgenic mammals can also be treated. Treatment dosages need to be titrated to optimize safety and efficacy.

[0182] For passive immunization with an antibody, exemplary dosages are from about 0.0001 mg/kg to about 100 mg/kg, about 0.01 mg/kg to about 5 mg/kg, about 0.15 mg/kg to about 3 mg/kg, 0.5 mg/kg to about 2 mg/kg, for example about 1 mg/kg to about 2 mg/kg of the host body weight. In some exemplary embodiments, dosages can be about 0.5, 0.6, 0.7, 0.75, 0.8, 0.9, 1.0, 1.2, 1.25, 1.3, 1.4, 1.5, 1.6, 1.7, 1.75, 1.8, 1.9, or 2.0 mg/kg. Other exemplary dosages for passive immunization are from about 1 mg/kg to about 20 mg/kg. In some exemplary embodiments, dosages can be about 5, 10, 15 or 20 mg/kg. Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months. Exemplary dosage schedules include 1-10 mg/kg or 15 mg/kg on consecutive days, 30 mg/kg on alternate days or 60 mg/kg weekly. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated.

[0183] Antibody is usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of 1-1000 μg/ml and in some methods 25-300 μg/ml. In some embodiments, antibody can be administered as a sustained release composition, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half-life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies.

[0184] The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in the disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactic effective dose." In this use, the precise amounts again depend upon the patient's state of health and general immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per dose. A relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives.

[0185] In some therapeutic applications, a relatively high dosage (for example, from about 0.5 or 1 to about 200 mg/kg of antibody per dose (for example 0.5, 1, 1.5, 2, 5, 10, 20, 25, 50, or 100 mg/kg), with dosages of from 5 to 25 mg/kg being more commonly used) at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and for example until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patent can be administered a prophylactic regime.

[0186] It is especially advantageous to provide compositions in unit dosage form for ease of administration and uniformity of dosage. Compositions may be presented in capsules, ampules, lyophilized form, or in multi-dose containers. The term "container" refers to something, for example, a holder, receptacle, or vessel, into which an object or liquid can be placed or contained, for example, for storage. The unit dosage form may comprise any composition described herein including suspensions, solutions or emulsions of the active ingredient together with formulating agents such as suspending, stabilizing and/or dispersing agents. In an exemplary embodiment, the pharmaceutical dosage unit form may be added to an intravenous drip bag (for example a 50 ml, 100 ml, or 250 ml, or 500 ml drip bag) with a suitable diluent, for example, sterile pyrogen-free water or saline solution, before administration to the patient, for example, by intravenous infusion. Some pharmaceutical unit dosage forms may require reconstitution with a suitable diluent prior to addition to an intravenous drip bag, for example lyophilized forms. In exemplary embodiments, the pharmaceutical unit dosage form is a

container containing a composition described herein. For example, the container may be a 10 mL glass, type I, tubing vial. Generally, the container should maintain the sterility and stability of the composition. For example, the vial may be closed with a serum stopper. Furthermore, in various embodiments, the container should be designed so as to allow for withdrawal of about 100 mg of composition or active ingredient (for example, for single use). In some embodiments, the container may be suitable for larger amounts, of composition or active ingredient, for example, from about 10 mg to about 5000 mg, from about 100 mg to about 1000 mg, and from about 100 mg to about 500 mg, about 40 mg to about 250 mg, about 60 mg to about 80 mg, about 80 mg to about 120 mg, about 120 mg to about 160 mg, or ranges or intervals thereof, for example, about 100 mg to about 200 mg. Ranges intermediate to the above recited amounts, for example, from about 25 mg to about 195 mg, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. In some embodiments, the composition often is supplied as a liquid in unit dosage form.

[0187] In some aspects, the present disclosure provides a kit including a pharmaceutical dosage unit form (for example, a container with a composition disclosed herein), and instructions for use. Accordingly, the container and the kit may be designed to provide enough composition for multiple uses. In various embodiments, the kit may further include diluent. The diluent may include excipients, separate or combined. For example, the diluent may include a tonicity modifier such as mannitol, a buffering agent such as histidine, a stabilizer such as polysorbate 80, an anti-oxidant such as methionine, and/or combinations thereof. The diluent may contain other excipients, for example, lyoprotectant, as deemed necessary by one skilled in the art.

[0188] Excipients

[0189] In various embodiments, the present disclosure provides a composition that may include various excipients, including, but not limited to, buffer, anti-oxidant, a tonicity agent, and a stabilizer. In addition, the compositions may contain an additional agent for pH adjustment (for example, HCl) and a diluent (for example, water). In some embodiments, different forms of histidine can be used for pH adjustment. In part, the excipients serve to maintain the stability and the biological activity of the antibody (for example, by maintaining the proper conformation of the protein), and/or to maintain pH.

[0190] Buffering Agent

[0191] In various aspects, the composition includes a buffering agent (buffer). The buffer serves to maintain a physiologically suitable pH. In addition, the buffer can serve to enhance isotonicity and chemical stability of the composition. Generally, the composition should have a physiologically suitable pH. In various embodiments, the composition has a pH of about 5 to

about 7, about 5.5 to about 6.5, for example about 6.0 to about 6.5. In some embodiments, the composition has a pH of about 6. Ranges intermediate to the above recited pH levels, for example, about pH 5.2 to about pH 6.3, for example pH 6.0 or pH 6.2, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. The pH may be adjusted as necessary by techniques known in the art. For example, HCl may be added as necessary to adjust the pH to desired levels or different forms of histidine may be used to adjust the pH to desired levels.

[0192] The buffer may include, but is not limited to, succinate (sodium or phosphate), histidine, phosphate (sodium or potassium), Tris (tris (hydroxymethyl) aminomethane), diethanolamine, citrate, other organic acids and mixtures thereof. In some embodiments, the buffer is histidine (for example, L-histidine). In some embodiments, the buffer is succinate. In some embodiments, the composition includes an amino acid such as histidine that is present in an amount sufficient to maintain the composition at a physiologically suitable pH. Histidine is an exemplary amino acid having buffering capabilities in the physiological pH range. Histidine derives its buffering capabilities spanning from its imidazole group. In one exemplary embodiment, the buffer is L-histidine (base) (for example $C_6H_9N_3O_2$, FW: 155.15). In some embodiments, the buffer is L-histidine monochloride monohydrate (for example $C_6H_9N_3O_2.HCl.H_2O$, FW: 209.63). In some embodiments, the buffer is a mixture of L-histidine (base) and L-histidine monochloride monohydrate.

[0193] In some embodiments, the buffer (for example, L-histidine or succinate) concentration is present from about 0.1 mM to about 50 mM, from about 0.1 mM to about 40 mM, from about 0.1 mM to about 30 mM, about 0.1 mM to about 25 mM, from about 0.1 mM to about 20 mM, or from about 5 mM to about 15 mM, for example 5 mM or 10 mM. In various embodiments, the buffer may be present at about 6 mM, 7 mM, 8 mM, 9 mM, 11 mM, 12 mM, 13 mM, 14 mM, or 15 mM. In some embodiments, the buffer is present at about 10 mM. Ranges intermediate to the above recited concentrations, for example, about 12 mM to about 17 mM, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. In certain embodiments, the buffer is present in an amount sufficient to maintain a physiologically suitable pH.

[0194] Tonicity Agent

[0195] In various aspects, the composition includes a tonicity agent. In part, the tonicity agent contributes to maintaining the isotonicity of the composition, and to maintaining protein levels. In part, the tonicity agent contributes to preserving the level, ratio, or proportion of the therapeutically active polypeptide present in the composition. As used herein, the term "tonicity"

refers to the behavior of biologic components in a fluid environment or solution. Isotonic solutions possess the same osmotic pressure as blood plasma, and so can be intravenously infused into a subject without changing the osmotic pressure of the subject's blood plasma. In some embodiments, tonicity agent is present in an amount sufficient to render the composition suitable for intravenous infusion. Often, the tonicity agent serves as a bulking agent as well. As such, the agent may allow the protein to overcome various stresses such as freezing and shear.

[0196] The tonicity agent may include, but is not limited to, $CaCl_2$, NaCl, $MgCl_2$, lactose, sorbitol, sucrose, mannitol, trehalose, raffinose, polyethylene glycol, hydroxyethyl starch, glycine and mixtures thereof. In some embodiments, the tonicity agent is mannitol (for example, D-mannitol, for example, $C_6H_{14}O_6$, FW: 182.17).

[0197] In some embodiments, the tonicity agent is present at about 2% to about 6% w/v, or about 3% to about 5% w/v. In some embodiments, the tonicity agent is present at about 3.5% to about 4.5% w/v. In some embodiments, the tonicity agent is percent at about 20 mg/ml to about 60 mg/ml, at about 30 mg/ml to about 50 mg/ml, or at about 35 mg/ml to about 45 mg/ml. For example, the tonicity agent is present at about 4% w/v or at about 40 mg/ml. In some embodiments, the tonicity agent is present at about 6% w/v. In some embodiments, the tonicity agent is present at about 10% w/v.

[0198] Ranges intermediate to the above recited concentrations, for example, about 3.2% to about 4.3% w/v or about 32 to about 43 mg/ml, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. The tonicity agent should be present in a sufficient amount so as to maintain tonicity of the composition.

[0199] Anti-Oxidant

[0200] In various aspects, the composition includes an anti-oxidant so as to, in part, preserve the composition (for example, by preventing oxidation). The anti-oxidant may include, but is not limited to, GLA (gamma-linolenic acid)-lipoic acid, DHA (docosahexaenoic acid)-lipoic acid, GLA-tocopherol, di-GLA-3,3′-thiodipropionic acid and in general any of, for example, GLA, DGLA (dihomo-gamma-linolenic acid), AA (arachidonic acid), SA (salicylic acid), EPA (eicosapentaenoic acid) or DHA (docosahexaenoic acid) with any natural or synthetic anti-oxidant with which they can be chemically linked. These include phenolic anti-oxidants (for example, eugenol, carnosic acid, caffeic acid, BHT (butylated hydroxyanisol), gallic acid, tocopherols, tocotrienols and flavenoid anti-oxidants (such as myricetin and fisetin)), polyenes (for example, retinoic acid), unsaturated sterols (for example, Δ^5-avenosterol), organosulfur compounds (for example, allicin), terpenes (for example, geraniol, abietic acid) and amino acid antioxidants (for example, methionine, cysteine, carnosine). In some embodiments, the anti-

oxidant is ascorbic acid. The anti-oxidant is methionine, or an analog thereof, for example, selenomethionine, hydroxy methyl butanoic acid, ethionine, or trifluoromethionine.

[0201] In some embodiments, the anti-oxidant (for example, a methionine such as L-methionine, for example $CH_3SCH_2CH_2CH(NH_2)CO_2H$, FW=149.21) is present from about 0.1 mM to about 50 mM, from about 0.1-mM to about 40 mM, from about 0.1 mM to about 30 mM, from about 0.1 mM to about 20 mM, or from about 5 mM to about 15 mM. In various embodiments, the anti-oxidant may be present at about 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, or 15 mM. For example, the anti-oxidant is present at about 10 mM. In some embodiments, the anti-oxidant is present at about 15 mM. Ranges intermediate to the above recited concentrations, for example, about 12 mM to about 17 mM, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. In certain embodiments, the anti-oxidant should be present in a sufficient amount so as to preserve the composition, in part, by preventing oxidation.

[0202] Stabilizer

[0203] In various aspects, the composition includes a stabilizer, also known as a surfactant. Stabilizers are specific chemical compounds that interact and stabilize biological molecules and/or general pharmaceutical excipients in a composition. In certain embodiments, stabilizers may be used in conjunction with lower temperature storage. Stabilizers generally protect the protein from air/solution interface induced stresses and solution/surface induced stresses, which may otherwise result in protein aggregation. The stabilizer may include, but is not limited to, glycerin, polysorbates such as polysorbate 80, dicarboxylic acids, oxalic acid, succinic acid, adipic acid, fumaric acid, phthalic acids, and combinations thereof. In some embodiments, the stabilizer is polysorbate 80.

[0204] In some embodiments, the stabilizer (for example, polysorbate 80) concentration is about 0.001% w/v to about 0.01% w/v, about 0.001% w/v to about 0.009% w/v, or about 0.003% w/v to about 0.007% w/v. For example, the stabilizer concentration is about 0.005% w/v. In some embodiments, the stabilizer is present at about 0.01% w/v. Ranges intermediate to the above recited concentrations, for example, about 0.002% w/v to about 0.006% w/v, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

[0205] In some embodiments, the composition is substantially free of preservatives. In some embodiments, preservatives may be added as necessary. For example, cryoprotectants or lyoprotectants may be included, for example, should the composition be lyophilized.

5.4. Nucleolin-Expressing Cancers and Non-Malignant Cells

[0206] In some embodiments, an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) produced in accordance with the present disclosure is used in treating a variety of cells, including both cancerous and non-cancerous cells. In some embodiments the isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is a monoclonal antibody. In some embodiments, the isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) is a polyclonal antibody. The term "cancer" is described previously herein. Examples of types cancer that can be inhibited or treated with an isolated anti-nucleolin antibody (*e.g.*, human and/or monoclonal) include, but are not limited to: Acute Lymphoblastic Leukemia; Myeloid Leukemia; Acute Myeloid Leukemia; Chronic Myeloid Leukemia; Adrenocortical Carcinoma Adrenocortical Carcinoma; AIDS-Related Cancers; AIDS-Related Lymphoma; Anal Cancer; Astrocytoma, Childhood Cerebellar; Astrocytoma, Childhood Cerebral; Basal Cell Carcinoma; Bile Duct Cancer, Extrahepatic; Bladder Cancer; Bladder Cancer; Bone Cancer, osteosarcoma/Malignant Fibrous Histiocytoma; Brain Stem Glioma; Brain Tumor; Brain Tumor, Brain Stem Glioma; Brain Tumor, Cerebellar Astrocytoma; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma; Brain Tumor, Ependymoma; Brain Tumor, Medulloblastoma; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors; Brain Tumor, Visual Pathway and Hypothalamic Glioma; Breast Cancer, Female; Breast Cancer, Male; Bronchial Adenomas/Carcinoids; Burkitt's Lymphoma; Carcinoid Tumor; Central Nervous System Lymphoma; Cerebellar Astrocytoma; Cerebral Astrocytoma/Malignant Glioma; Cervical Cancer; Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic Myeloproliferative Disorders; Colon Cancer; Colorectal Cancer; Cutaneous T-Cell Lymphoma; B-Cell Lymphoma Endometrial Cancer; Ependymoma; Esophageal Cancer; Esophageal Cancer; Ewing's Family of Tumors; Extracranial Germ Cell Tumor; Extragonadal Germ Cell Tumor; Extrahepatic Bile Duct Cancer; Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer; Gastric (Stomach) Cancer; Gastrointestinal Carcinoid Tumor; Germ Cell Tumor, Extracranial; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma; Glioma, Childhood Brain Stem; Glioma, Childhood Cerebral Astrocytoma; Glioma, Childhood Visual Pathway and Hypothalamic; Hairy Cell Leukemia; Head and Neck Cancer; Hepatocellular (Liver) Cancer, Adult (Primary); Hepatocellular (Liver) Cancer, Childhood (Primary); Hodgkin's Lymphoma; Hodgkin's Lymphoma During Pregnancy; Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma; Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma; Kidney (Renal Cell) Cancer; Kidney Cancer; Laryngeal Cancer; Leukemia, Acute Lymphoblastic; Leukemia, Acute Lymphoblastic; Leukemia, Acute Myeloid; Leukemia, Acute Myeloid; Leukemia, Chronic Lymphocytic; Leukemia; Chronic Myelogenous; Lip and Oral Cavity Cancer; Liver Cancer,

Adult (Primary); Liver Cancer, Childhood (Primary); Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoma, AIDS-Related; Lymphoma, Burkitt's; Lymphoma, Cutaneous T-Cell, see Mycosis Fungoides and Sezary Syndrome; Lymphoma, Hodgkin's; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's; Lymphoma, Non-Hodgkin's During Pregnancy; Lymphoma, Primary Central Nervous System; Macroglobulinemia, Waldenstrom's; Malignant Fibrous Histiocytoma of Bone/Osteosarcoma; Medulloblastoma; Melanoma; Melanoma, Intraocular (Eye); Merkel Cell Carcinoma; Mesothelioma, Adult Malignant; Mesothelioma; Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia Syndrome; Multiple Myeloma/Plasma Cell Neoplasm' Mycosis Fungoides; Myelodysplastic Syndromes; Myelodysplastic/Myeloproliferative Diseases; Myelogenous Leukemia, Chronic; Myeloid Leukemia, Adult Acute; Myeloid Leukemia, Childhood Acute; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Neuroblastoma; Non-Hodgkin's Lymphoma; Non-Hodgkin's Lymphoma During Pregnancy; Oral Cancer; Oral Cavity Cancer, Lip and; Oropharyngeal Cancer; Osteosarcoma/Malignant Fibrous Histiocytoma of Bone; Ovarian Cancer; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor; Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Pancreatic Cancer; Pancreatic Cancer, Islet Cell; Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors; Pituitary Tumor; Plasma Cell Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer; Pregnancy and Hodgkin's Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma; Primary Central Nervous System Lymphoma; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Cell (Kidney) Cancer; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma; Salivary Gland Cancer; Salivary Gland Cancer; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; Sarcoma, Soft Tissue; Sarcoma, Soft Tissue; Sarcoma, Uterine; Sezary Syndrome; Skin Cancer (non-Melanoma); Skin Cancer; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma; Soft Tissue Sarcoma; Squamous Cell Carcinoma, see Skin Cancer (non-Melanoma); Squamous Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Stomach (Gastric) Cancer; Supratentorial Primitive Neuroectodermal Tumors; T-Cell Lymphoma, Cutaneous, see Mycosis Fungoides and Sezary Syndrome; Testicular Cancer; Thymoma; Thymoma and Thymic Carcinoma; Thyroid Cancer; Thyroid Cancer; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, Gestational; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Cancer, Endometrial; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma; Vulvar Cancer; Waldenstrom's Macroglobulinemia; and Wilms' Tumor.

[0207] Cancer cells known to express nucleolin include lung cancers (*e.g.*, non-small cell lung cancers), breast cancers, prostate cancers, colon cancers, pancreatic cancers, renal cell carcinomas, ovarian cancers, leukemias (*e.g.*, AML, CLL), melanomas, glioblastomas, neuroblastomas, sarcomas and gastric cancers. In addition, non-cancer cells that express nucleolin include immune cells such as dendritic cells, peripheral blood monocytes, macrophages, and glial cells, as well as vascular smooth muscle cells and endothelial cells. In some embodiments, an antibody of the present disclosure is used in a treatment for subjects with hyper-immune and hyper-angiogenic diseases, the latter being described in U.S. Patent Publication No. 2009/0191244, incorporated herein by reference.

6. Certain Antibody Properties

6.1. Mutation Frequency

[0208] An antibody disclosed herein can comprise a heavy chain sequence with a mutation frequency of at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13 %, 14%, 15%, 16%, 17%, 18%, 19%, or 20%, or higher from a germline sequence. The antibodies can comprise a CDR3 region that is a light chain sequence with a mutation frequency of at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13 %, 14%, 15%, 16%, 17%, 18%, 19%, or 20%, or higher from a germline sequence. The antibodies can comprise a heavy chain and a light chain sequence with a mutation frequency of at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13 %, 14%, 15%, 16%, 17%, 18%, 19%, or 20%, or higher from a germline sequence. The antibodies can comprise a V_H region from a V_H family selected from the group consisting of any one of V_H family 4-59.

6.2. Heavy and Light Chain Lengths

[0209] An antibody disclosed herein can comprise a CDR3 region that is a length of at least about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids in length. The antibodies can comprise a CDR3 region that is at least about 18 amino acids in length.

[0210] An antibody disclosed herein can comprise a deletion at an end of a light chain. The antibodies can comprise a deletion of 3 or more amino acids at an end of the light chain. The antibodies can comprise a deletion of 7 or less amino acids at an end of the light chain. The antibodies can comprise a deletion of 3, 4, 5, 6, or 7 amino acids at an end of the light chain.

[0211] An antibody disclosed herein can comprise an insertion in a light chain. The antibodies of can comprise an insertion of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more amino acids in the light chain. The antibodies can comprise an insertion of 3 amino acids in the light chain.

6.3. Affinity

[0212] Affinity is the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.,* an antibody) and its binding partner (*e.g.,* an antigen). Unless

indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.,* antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (k_d). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0213] In some embodiments, an antibody disclosed herein has a dissociation constant (K_D) of about 1 μM, 100 nM, 10 nM, 5 nM, 2 nM, 1 nM, 0.5 nM, 0.1 nM, 0.05 nM, 0.01 nM, or 0.001 nM or less (*e.g.,* 10^{-8}M or less, *e.g.,* from 10^{-8}M to 10^{-13}M, *e.g.*, from 10^{-9}M to 10^{-13} M) for human nucleolin. An affinity matured antibody is an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.These antibodies can bind to human nucleolin with a K_D of about 5×10^{-9}M, 2×10^{-9}M, 1×10^{-9}M, $5\times10^{-1\circ}$ M, 2×10^{-9}M, 1×10^{-10} M, 5×10^{-11}M, 1×10^{-11}M, 5×10^{-12} M, 1×10^{-12} M, or less.

[0214] K_D can be measured by any suitable assay. For example, K_D can be measured by a radiolabeled antigen binding assay (RIA) (*See, e.g.*, Chen et al., J. Mol. Biol. 293:865-881 (1999); Presta et al., Cancer Res. 57:4593-4599 (1997)). For example, K_D can be measured using surface plasmon resonance assays (*e.g.*, using a BIACORE®-2000 or a BIACORE®-3000).

6.4. Cysteine Engineered Antibody Variants

[0215] In some embodiments, it may be desirable to create cysteine engineered antibodies, *e.g.*, "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In some embodiments, the substituted residues occur at accessible sites of the antibody. Reactive thiol groups can be positioned at sites for conjugation to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate. In some embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described (*See, e.g.*, U.S. Pat. No. 7,521,541).

6.5. Antibody Derivatives

[0216] In some embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol,

carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water.

[0217] The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if two or more polymers are attached, they can be the same or different molecules.

[0218] In some embodiments, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (*See, e.g.*, Kam et al., Proc. Natl. Acad. Sci. USA 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

7. Recombinant Methods and Compositions

[0219] Antibodies may be produced using recombinant methods and compositions (*See, e.g.*, U.S. Pat. No. 4,816,567). In some embodiments, an isolated nucleic acid encoding a nucleolin antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the V_L and/or an amino acid sequence comprising the V_H of the antibody. In a further embodiment, one or more vectors comprising such nucleic acid are provided. A vector is a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked.

[0220] In some embodiments, a host cell comprising such nucleic acid is provided. Host cells are cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. In one such embodiment, a host cell comprises (*e.g.*, has been transformed with) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_L of the antibody and an amino acid sequence

comprising the V_H of the antibody or a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_L of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_H of the antibody. In some embodiments, the host cell is eukaryotic, *e.g.,* a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell). In some embodiments, a method of making a nucleolin antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell or host cell culture medium.

[0221] For recombinant production of a nucleolin antibody, an isolated nucleic acid encoding an antibody, *e.g.*, as described above, is inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures.

[0222] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, *e.g.*, when glycosylation and Fc effector function are not needed (*See, e.g.*, U.S. Pat. Nos. 5,648,237, 5,789,199, and 5,840,523; Charlton, Methods in Molecular Biology, Vol. 248, pp. 245-254 (2003)). After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0223] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors (*See, e.g.*, Gerngross, Nat. Biotech. 22:1409-1414 (2004), and Li et al., Nat. Biotech. 24:210-215 (2006)). Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms, including invertebrates and vertebrates. Examples of invertebrates include plant and insect cells (*See, e.g.*, U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429). Examples of vertebrate cells include mammalian cell lines, monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.*, in Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TR1 cells; MRC 5 cells; FS4 cells; Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells; and myeloma cell lines such as Y0, NS0 and Sp2/0. (*See, e.g.*, Yazaki and Wu, Methods in Molecular Biology, Vol. 248, pp. 255-268 (2003).

8. EXAMPLES

Example 1. Subcloning and Purification of Antibody CP1 from Human B Cells (CP1)

[0224] Cells from each parental B-cell lines were thawed and expanded. The cells were cultured in regular growth media (RPMI) supplemented with 10% FBS. The culture was subcloned by seeding 96-well plates at three different densities (2 cells, 1 cell, and 0.5 cells/well). The clonal outgrowth was monitored over at least a 2 week timeframe. An antigen-specific ELISA ("nucleolin ELISA") was used to detect anti-nucleolin antibodies and screen single-cell clones. Positive wells were counter-screened using an anti-human IgG ELISA to estimate the antibody titers in the positive wells. Up to ten positive subclones per parental B cell line were expanded and cryopreserved in banks of at least twenty vials each. Cells were frozen in RPMI containing 5% DMSO. A TRIZOL extract was prepared from 1 x 10^7 cells from each subclone frozen above at the time of banking. RNA and oligo-dT primed cDNA were prepared from each sample. The cDNA from each TRIZOL extract was polyadenylated and used as template in a PCR reaction using 3' specific heavy chain and light chain constant region primers and oligo-dT as the 5' primer so as to amplify the variable regions of the expressed antibodies. The 500 bp inserts were gel-isolated and ligated into pBluescript. For each of the insert sets, six V_H inserts and six V_L inserts were sequenced and aligned.

[0225] Frozen culture was thawed and expanded. B cell supernatant preparation: Cells were seeded at ~ [0.8 x 10^6 cells/ml] into 28 x T225 flasks (A) (75 ml each, 2100 ml total) and 10 x T75 flasks (B) (40 ml each, 400 ml total) in 75:25 AIM media: RPMI (1% antibiotics), total 2500 ml. When the cells reached a total volume of ~ 4,400 ml (36x T225 flasks), supernatant CP1 was harvested with Centricon centrifugation. Cell #: mean cell number = 8.8 x 10^5 cells/ml Mean cell viability = 70.5% Centrifuge Filtration: Centricon Plus-70 Centrifugal filter device, 100K. Melon Gel Purification of IgG: Melon Gel IgG Purification 200 ml resin (or 1L total volume), Thermo Scientific (cat# PI-45214). Total 1L gel solution (containing 20% beads slurry, 200 ml). Combined 400 ml used gel solution + 600 ml unused gel solution ~ 1 L total.

Example 2. Production of Antibody CP1 from Recombinant Cells (CP1(RC))

[0226] CHO3E7 (Chinese hamster ovary) cells with an initial density of 2.1 x 10^6 cells/mL, in a culture medium of F17 supplemented with 0.1% Pluronic F-68, 4 mM GlutaMAX, was transfected with either CP1 gamma heavy chain/kappa light chain or CP1 gamma heavy chain/lambda light chain. cDNA encoding was synthetically produced with codon optimization for mammalian cell expression and cloned into expression vector pTT5 at the indicated restriction sites by standard methods. Two, 30 mL cultures of CHO3E7 cells in 125 mL shake flasks were transfected with 1 mg of plasmid DNA/L culture using PolyPlus linear Q-PEI at a 1:4 (w/v) DNA:PEI ratio. Cultures were supplemented with 4.5 mL CD Efficient Feed B (Life Technologies) 24 hours post transfection. Culture parameters were monitored using a ViCell XR for density and viability.

[0227] Culture supernatants were harvested 10 days post transfection (6 days for the second transfection) via centrifugation for 5 minutes at 1000 xg. The conditioned culture supernatants (CCS) were clarified by centrifugation for 30 minutes at 9100 xg, filter sterilized with a 0.2 μm PES filter system, and stored at 4 °C.

[0228] Protein expression was analyzed via reducing and non-reducing SDS-PAGE. *See* FIG. 11. Expression verification SDS-PAGE. Forty microliter samples of conditioned culture supernatant (CCS) were evaluated via reducing (left panels) and non-reducing (right panels) SDS-PAGE on 4-20% Tris-glycine TGX gels (Bio-Rad) and stained with Instant Blue gel stain.

[0229] CP1(RC) bound tightly to human recombinant nucleolin (Kd =2.6 ± 0.7 nM, SEM.) and to plasma membrane nucleolin of human tumor cells. Confocal microscopy of Panc-1 and DU-145 tumor cells incubated at 37 °C with CP1(RC) revealed punctate localization of the antibody in the plasma membranes of these cells and internalization of the antibody into the cytoplasm. The localization of the antibody within foci in the plasma membrane suggested that the antibody was bound to nucleolin that was incorporated into lipid rafts within the plasma membrane.

[0230] Plasmid isolation and transient expression in CHO3E7 cells at the 6 L culture scale. The goal was to isolate endotoxin-free expression plasmid DNA encoding CP1 gamma heavy chain. 6 L of CHO3E7 cells was transiently transfect with gamma heavy chain and kappa light chain plasmid DNA using linear PEI. The culture parameters were monitored, and the conditioned culture supernatant (CCS) was harvested when the viability has dropped to ~85-90% (targeting 90%). It was expected to hit viability target 3-4 days post transfection. Protein expression in the CCS was analyzed by reducing and non-reducing SDS-PAGE. Clarified CCS was sterile filtered and stored at 4 °C.

Example 3. Binding to Recombinant Nucleolin by ELISA Assay

[0231] Reagents: Goat anti-human IgG Ab-HRP Antibody produced by Santa Cruz as nonspecific IgG Control, recombinant nucleolin quantitation by nanodrop (Yoko Otake), and *femto*-ELISA-HRP kit.

[0232] Preparation: 100 μl binding buffer (control) or recombinant nucleolin (containing 200, 400 or 800 ng) was added to designated wells in an Immuno 96 well plate. The plate was incubated for 1 h on orbital shaker @ 100 rpm at room temp. After 1 h incubation, the plate was carefully inverted to empty and gently tap out residual liquid. 200 μl diluted 1x NAP-blocker was added to each well and incubate the plate for 15 min on orbital shaker @ 100 rpm. After incubation, carefully invert the plate to empty and gently tap out residual liquid.

[0233] Primary Antibody incubation: 100 μl of serial diluted supernatant solution in blocking buffer was incubated for 1h at room temp on an orbital shaker @ 100 rpm. After incubation, the plate was carefully inverted to empty and gently tap out residual liquid. To wash out the

antibody, each well was filled with 1x *femto*-TBST (200 µl) and waited for 30 sec then the plate was inverted and tapped to empty the residual liquid from each well.

[0234] Secondary Antibody Incubation: 100 µl of *1→500 diluted 2° anti-human IgG Ab-HRP stock was incubated at RT for 1 h on an orbital shaker @ 100 rpm. To wash out the antibody, each well was filled with 1x *femto*-TBST (200 µl) and waited for 30 sec then the plate was inverted and tapped to empty the residual liquid from each well.

[0235] Preparation for Reading: 100 µl of *femto*-ELISA-HRP substrate was added into each well. A soluble blue color developed, which is read at 620 nm using substrate as a blank. Monitoring was done every 10 min for 1 h.

[0236] Results: ELISA Analysis of the binding of antibody CP1 to truncated human recombinant nucleolin with an N-terminal deletion (Δ1-283) and 6x-His (**SEQ ID NO:94**) yields an equilibrium dissociation constant of 2.6 ± 0.7 nM S.E.M, N=4, *see* FIG. 4.

Example 4. Activity/Potency Tests

[0237] Melon Gel Purified Antibodies on MCF-7 and MCF-10A cells by Cell Counting/TBE at 96 h. Antibody was spin column purified by Melon gel (CP expt 407); IgG conc. [533.26 µg/ml] (CP expt 409); ~ 2.0 ml; 1x PBS buffer with 100 mM arginine. MCF7 cells and MCF10A cells were seeded at [1 x 10^4 cells/ml] (or 2x10^3 cells/200 µl per well). MCF-7 and MCF-10A cells were set up at [1x10^4 cells/ml]$_{1x}$ (or 2x10^3 cells/200 µl per well) in a 96 well plate (MCF7 cells A-D, MCF10A cells E-H). For 25 ml at [1 x10^4 cells/ml]$_{1x}$, collect 2.5 x 10^5 cells. 200 µl of cell solution were seeded per well of a 96 well each cell line as indicated below. Incubated overnight. Next day, confluency checked: ~30% confluency for MCF7 cells, ~ 10% confluency for MCF10A cells.

[0238] A 96 h drug treatment was conducted using the following protocol. For 1/2 plate of MCF7 cells, make up 5 ml media with 20% human serum (type AB) using appropriate media (RPMI with 1% antibiotics) or 1 ml of human serum and 4 ml RPMI media for MCF7 cells, or HuMEC complete medium for MCF10A cells. Test various concentrations of CP1M4.2 at 0, 0.1, 0.25, 0.5, 1, 2, and 4 µg/ml IgG in triplicate. First make up the stock solution of CP1 at [4 µg/ml]$_{1x}$ (or [8 µg/ml]$_{2x}$) (0.7 ml) in RPMI media-1% antibiotics for MCF7 cells or HuMEC complete media for MCF10A cells. For IgG control, first make up a stock solution of the same conc. as that of CP1M4 sample [53.26µg/ml] in the same solution, 1x PBS buffer with 100 mM arginine. Then, prepare the serial dilution exactly the same as for CP1M4.2 for each cell line (see step 4). Start the drug treatment. Discard all solution (200 µl) by pipetting out. Add 100 µl of media with 20% human serum each well (x3). Add 100 µl of regular media without human serum (*e.g.,* RPMI/1% antibiotics), IgG control or supernatant as indicated below in the sample

set up. Put back in the incubator. Count cell numbers and perform TBE assay at 96 h (Tuesday) to determine the effects of antibodies CP1 and the control IgG antibody.

[0239] Cytotoxicity: at Time = 96 h. The following protocol was used to assay cytotoxicity. At 96 h, check cell morphology under microscope briefly. Working with a group of sample at a time (*e.g.,* IgG controls), remove the supernatant. Add *30 µl trypsin to each well, and incubate for 15 min. Add 170 µl PBS (total volume 200 µl. Transfer cell solution to 0.5 ml tube and pipet well to break up the cells. Into another 0.5 ml, add 10 µl 0.2% Trypan blue in PBS (filtered). Working with 2 samples, add in 20 µl of cell solution to 10 µl 0.2%Trypan blue. Mix well, load onto a chamber, then incubate for 1 min before counting viability and live cell numbers with Cellometer (*e.g.,* 20 µl per sample).

[0240] Results are shown in **FIG. 7**. Compared to IgG control, CP1 has potent killing activity to MCF7 breast cancer cells, but negligible toxicity to normal breast cells.

Comparison with Aptamer AS1411

[0241] MCF-7 cells were incubated for 96 hours with either the aptamer AS1411 (A) or with an anti-nucleolin antibody consistent with current claims (B) at a concentration recited below. Cell viability was measured using a chromogenic assay involves the biological reduction by viable cells of the tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (or MTS assay) (A) or by the trypan blue exclusion assay (B). Results are the means ± S.E.M. (Standard Error of Mean) of six experiments (A) or 3 experiments (B).

[0242] As a result, the concentration of AS1411 required to reduce MCF-7 cell viability to 50% (IC_{50}) was about 7 µM, while the IC_{50} value of the claimed antibody to MCF-7 cells was as low as about 0.8 nM or 0.0008 µM, shown in **FIG. 13**. Thus, the claimed antibody was about 9000 times more potent than AS1411 in killing MCF-7 cells.

Example 5. Effects of Antibodies on MV411 Human Leukemia Xenograft Model in Nude Mice

[0243] Female NCr nu/nu mice (8-12 week of age; n=40) were injected with $1x10^7$ MV411 tumor cells in 50% (v/v) Matrigel subcutaneously in their flanks. When tumors reach an average size of 100 - 150 mm^3, a pair match was performed to sort mice into two groups of ten each. Mice were then treated with IgG isotype control antibody (Group 1) or an antibody as claimed such as CP1 (Group 2). Each group had 10 female CRL *nnu/nu* mice and was administered intravenously with a dosage of 10 mg/kg in a 0.1 ml injection volume on days 1, 4, 7, 10, 13, 16. Animals were monitored individually. Body Weight measurements were taken daily for the first week and then biweekly. Caliper measurements of tumor sizes were taken biweekly. The

endpoint of the study was a tumor volume of 2000 mm^3 or 76 days, whichever came first. When the endpoint was reached, the animals were euthanized.

[0244] CP1 was well tolerated with the only adverse event being a 16% transient loss in mean body weight. CBC was normal. Necropsy results of all major organs were normal.

[0245] In this MV4-11 human xenograft mouse model, CP1 treatment resulted in 30% long-term survivors (Hazard ratios of 0.22-0.29) without inducing any serious toxicity to the mice. This was a statistically significant increase in survival of nude mice treated with CP1 compared to isotype control antibody with no significant organ or hematologic toxicity. No other biologic is known to show survival benefit in this model as a single agent. **FIG. 14** shows that in Group 2, 30% of the mice survived for 80 days. Even more surprisingly, 20% of the mice showed complete tumor regression by about Day 40 (data not shown). In marked contrast, all of the mice in the control group (Group 1) showed rapid tumor growth to an endpoint volume 2000 mm^3 and had to be euthanized on about Day 40.

[0246] **FIG. 15A** and **FIG. 15B** are another way of comparison of Group 1 and Group 2 in view of tumor volume changes. Surprisingly 3 out of the 10 treated mice (Group 2) survived for at least 76 days and more surprisingly, two of the survived mice showed complete tumor regression by about Day 40. In marked contrast, all of the 10 mice in the control group (Group 1) showed rapid tumor growth and had to be euthanized on about Day 40. FIG. 15A shows that all the mice in Group 1 showed rapid tumor growth after treatment with an IgG control antibody. In contrast, FIG. 15B shows that the tumor volume of mice # 1 and # 3 in Group 2 dropped to non-detectable levels by about Day 40 after treatment with a claimed antibody. Mice #10 also survived and its tumor volume became generally static from about Day 60.

Example 6. Methods of Predicting CDRs

[0247] Amino acid numbering based on framework and complementary determining regions (CDR) are defined by one of the following:

[0248] **Rosie Rosetta.** Reference: Lyskov S, et al., "Serverification of Molecular Modeling Applications: The Rosetta Online Server That Includes Everyone (ROSIE)". PLoS One. 2013 May 22;8(5):e63906. doi: 10.1371/journal.pone.0063906. Print 2013. The ROSIE app interface to the RosettaAntibody3 program was used to model the 3-D structure of the hypervariable region of antibody CP1 and identify the six CDRs. The first stage utilizes canonical template selection and assembly based on the Chothia definition described below and the lowest energy structures. They are assembled using a Rosetta protocol, resulting in a crude structure. In the second stage, CDR-H3 is remodeled de novo. Paratope side chains and loop backbones were refined simultaneously based on the Paratome method described below. CDR-H3 contained less

than 10 amino acids, which indicates that the root mean square deviation is approximately 1.5 Angstroms and the model is highly accurate.

[0249] Paratome. Reference: Kunik V, et al. (2012). Paratome: An online tool for systematic identification of antigen binding regions in antibodies based on sequence or structure. Nucleic Acids Res. 2012 Jul;40(Web Server issue):W521-4. doi: 10.1093/nar/gks480. Epub 2012 Jun 6. The Paratome web server (http://www.ofranlab.org/paratome/) was used to identify the six Antibody Binding Regions (ABRs) within the hypervariable region of antibody CP1. The ABRs are similar to CDRs but also contain amino acid side-chains near the CDRs that also contribute to binding of the antibody to the antigen. From the primary structure of antibody CP1, the Paratome web server was able to identify the ABRs of the antibody by comparison to the structural consensus regions within a multiple structure alignment of a non-redundant set of all antibody–antigen complexes.

[0250] P.I.G.S. (Prediction of Immunoglobulin Structure) numbering system. Reference: Marcatili P. et al. PIGS: automatic prediction of antibody structures. Bioinformatics 2008 24: 1953.

[0251] Chothia Definition. Reference: Chothia C et al. Conformations of immunoglobulin hypervariable regions. Nature 1989 342: 887). The Chothia definition is a scheme for numbering the amino acid residues of the hypervariable regions of antibodies and the beginning and ending of each of the six complementary regions (CDRs) within the hypervariable regions. The scheme is based on the analysis of the canonical structures of numerous antibodies. Using the Chothia definition, we were able to identify the positions of the amino acids at the beginning and ending of the six CDRs and the remaining amino acids within the CDRs by comparison to the primary structure of antibody CP1.

[0252] Kabat. Reference: Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991).

[0253] A set of rules were followed to identify the CDRs, as published Dr. Andrew C.R. Martin's Bioinformatics Group at University College London in UK, or below.

[0254] CDR-L1 (L24-L34)

Start	Approx residue 24
Residue before	always a Cys
Residue after	always a Trp. Typically Trp-Tyr-Gln, but also, Trp-Leu-Gln, Trp-Phe-Gln, Trp-Tyr-Leu
Length	10 to 17 residues

[0255] CDR-L2 (L50-L56)

Start	always 16 residues after the end of L1

Residues before	generally Ile-Tyr, but also, Val-Tyr, Ile-Lys, Ile-Phe
Length	always 7 residues (except NEW (7FAB) which has a deletion in this region)

[0256] CDR-L3 (L89-L97)

Start	always 33 residues after end of L2 (except NEW (7FAB) which has the deletion at the end of CDR-L2)
Residue before	always Cys
Residues after	always Phe-Gly-XXX-Gly
Length	7 to 11 residues

[0257] CDR-H1 (H31-H35B)

Start	Approx residue 31
Residues before	always Cys-XXX-XXX-XXX
Residues after	always a Trp. Typically Trp-Val, but also, Trp-Ile, Trp-Ala
Length	10 to 12 residues [AbM definition]; Chothia definition excludes the last 4 residues

[0258] CDR-H2 (H50-H65)

Start	always 15 residues after the end of CDR-H1
Residues before	typically Leu-Glu-Trp-Ile-Gly (**SEQ ID NO:95**), but a number of variations
Residues after	Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala
Length	Kabat definition 16 to 19 residues; AbM (and recent Chothia) definition ends 7 residues earlier

[0259] CDR-H3 (H95-H102)

Start	always 33 residues after end of CDR-H2 (always 2 after a Cys)
Residues before	always Cys-XXX-XXX (typically Cys-Ala-Arg)
Residues after	always Trp-Gly-XXX-Gly
Length	3 to 25 residues

[0260] IMGT(ImMunoGeneTics). The following rules of IMGT are followed to identify the CDRs. Reference: Lefranc, M.-P., The Immunologist, 7, 132-136 (1999)).

Table 3. Definition of the FR and CDR according to IMGT

	FR1-IMGT	CDR1-IMGT	FR2-IMGT	CDR2-IMGT	FR3-IMGT	Specific to V-REGION of Germline V-GENEs	Specific to V-DOMAIN; for rearranged V-J-GENES and V-D-J-GENES	
						Germline CDR3-IMGT (1)	Rearranged CDR3-IMGT (2)	FR4-IMGT (2)
Amino acid numbering	1-> 26 (C 23)	27->38	39->55 (W 41)	56->65	66->104 (C 104)	105->116	105->117	118->129
Number of amino acids	25-26	5-12	16-17	0-10	36-39	2-12	2-13	10-12

(C 23) 1st-CYS, (W 41) CONSERVED-TRP, (C 104) 2nd-CYS, position 118 corresponds to J-PHE or J-TRP as described in the text. IMGT notes: (1) The germline CDR3-IMGT is specific of the V-REGION of germline V-GENEs. It comprises 0, 1 or 2 nucleotide(s) before the V-HEPTAMER. (2) The rearranged CDR3-IMGT and the FR4-IMGT are specific of the V-DOMAIN (V-J-REGION or V-D-J-REGION). They are characteristic of rearranged V-J-GENEs and V-D-J-GENEs, and corresponding cDNAs and proteins.

Example 7. Methods of computer modeling the binding of anti-nucleolin antibody to nucleolin

[0261] ClusPro 2.0 protein-protein docking software (available at http://cluspro.bu.edu) was utilized to predict the interaction between the Rosetta ROSIE-predicted structure of antibody CP1(RC) and the solution structure of RNA-binding domains (RBD) 1 and 2 of human nucleolin. The solution structure of human nucleolin RBDs 1 and 2, also known as "2KRR", is publically available through the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB).

[0262] To begin docking in ClusPro 2.0, the .pdb file containing the Rosetta ROSIE-predicted structure of the antibody was assigned as the "Receptor" and the .pdb file containing the 2KRR structure was assigned as the "Ligand". Under "Advanced Options", "Use Antibody Mode" was selected. In Antibody Mode, ClusPro 2.0 uses an asymmetric potential for docking antibody and antigen pairs. This asymmetric potential was arrived at following the discovery that antibody-antigen interactions do not exhibit high degrees of surface complementarity, as in enzyme-substrate interactions. Rather, antibody-antigen interactions exhibit mostly flat, less hydrophobic interfaces. Finally, since antibodies interact with their antigens via their complementarity-determining regions (CDRs), the option to "Automatically Mask non-CDR Regions" was selected.

[0263] To execute docking, ClusPro 2.0 utilizes the protein-protein docking program PIPER, an FFT (fast Fourier transform)-based docking program that uses a structure-based pairwise potential as one component of its energy function; in Antibody Mode, the asymmetric potential is used. While the antibody is held in place on a three-dimensional grid, the ligand is rotated in increments of 1.0 Å every 5°, resulting in 70,000 rotations total. For each rotation, the ligand is translated in x,y,z relative to the receptor on a grid. The lowest scoring 1000 structures/translation combinations from PIPER are then exported to ClusPro 2.0, where they are clustered within a 9 Angstrom C-alpha rmsd radius. This means that ClusPro 2.0 finds the ligand position with the most "neighbors" within 9 Angstroms, and it becomes a cluster center, and its neighbors the members of the cluster. These are then removed from the set and ClusPro 2.0 then looks for a second cluster center, and so on. The docking predictions can be ranked by cluster size. Those with the highest number of cluster members scored better than those with fewer. The model having the highest number of cluster members was chosen as the working model.

[0264] CP1 and CP1(RC) bind to cell surface nucleolin and the complex appears to utilize lipid raft mediated endocytosis for cellular entry. In the cytoplasm CP1 and CP1(RC) bind to RNA binding domains 1 and 2 of human nucleolin.

[0265] FIG. 16 is an image of molecular model of the binding of antibody CP1(RC) to human nucleolin, where the CDRs in contact with nucleolin are labeled. ClusPro 2.0 protein-protein docking software was utilized in antibody mode to predict the interaction between the hypervariable region of CP1(RC) and the structures of various nucleolin fragments in the Protein Data Bank (PDB). The model as shown was obtained with the binding of CP1(RC) (its CDR predictions are done with Rosie Rosetta) and the RNA-binding domains 1 and 2 (RBD 1 & 2) of human nucleolin (PDB 2krr, amino acid sequence of residues 300-466 of SEQ ID NO: 20). Some of the closest distances between residues in antibody CDRs and residues of nucleolin (NCL) are listed in **Table 4**.

Table 4. Distances of select amino acids in the nucleolin –antibody CP1(RC) binding model.

	Distance	Antibody Residue	Side Chain Property	2KRR Residue	Side Chain Property	NCL Residue #
Light Chain CDR1	0.266 nm	[ARG]31:L.HH12 #284	Basic, electrically charged	[GLU]154:A.OE2 #1485	Polar	E453
	0.351 nm	[TRP]32:L.HE1 #300	Nonpolar	[ARG]158:A.HH12 #1522	Basic, electrically charged	R457
	0.333 nm	[TRP]32:L.NE1 #299	Nonpolar	[ARG]158:A.HH12 #1522	Basic, electrically charged	R457
	0.198 nm	[ARG]31:L.HH22 #287	Basic, electrically charged	[GLU]154:A.OE1 #1484	Polar	E453
Light Chain CDR2	0.172 nm	[LYS]50:L.HZ3 #490	Basic, electrically charged	[ASP]156:A.O #1505	Acidic, electrically charged	D455
	0.188 nm	[LYS]50:L.HZ2 #489	Basic, electrically charged	[ASP]156:A.OD1 #1502	Acidic, electrically charged	D455
	0.185 nm	[LYS]50:L.HZ2 #489	Basic, electrically charged	[ASP]156:A.OD2 #1503	Acidic, electrically charged	D455
Light Chain CDR3	0.164 nm	[TYR]94:L.OH #897	Polar	[LYS]49:A.HZ3 #456	Basic, electrically charged	K348
	0.245 nm	[TYR]94:L.HH #898	Polar	[LYS]49:A.HZ3 #456	Basic, electrically charged	K348
Heavy Chain CDR1	0.172 nm	[TYR]33:H.OH #1353	Polar	[LYS]128:A.HZ3 #1233	Basic, electrically charged	K427
Heavy Chain CDR2	0.164 nm	[TYR]53:H.OH #1567	Polar	[LYS]128:A.HZ1 #1231	Basic, electrically charged	K427
	0.338 nm	[TYR]53:H.HH #1568	Polar	[GLY]127:A.O #1222	Nonpolar	G426
	0.31 nm	[TYR]53:H.HH #1568	Polar	[LYS]128:A.HZ2 #1232	Basic, electrically charged	K427
Heavy Chain CDR3	0.175 nm	[ASP]98:H.OD2 #2033	Acidic, electrically charged	[LYS]104:A.HZ1 #999	Basic, electrically charged	K403
	0.304 nm	[ASP]98:H.OD2 #2033	Acidic, electrically charged	[LYS]104:A.HZ2 #1000	Basic, electrically charged	K403
	0.321 nm	[ASN]97:H.OD1 #2021	Polar	[TYR]103:A.CD2 #984	Polar	Y402

Example 8. Effects of CP1 on the Viability of Tumor and Normal Cells

[0266] CP1 is a potent inhibitor of tumor cell viability *in vitro*. **FIG. 5 and FIG. 6** show that IC_{50} values of less than 1 μg/ml were obtained for CP1 versus a broad range of tumor cells. For example, IC_{50} of CP1 against human MV4-11 AML cells is 0.4 μg/ml. In contrast, the IC_{50} concentrations of CP1 versus normal human B and myeloid cells, breast epithelial cells and lung fibroblasts were greater than 10 μg/ml. Unlike the tumor cells, these normal cells did not express detectable levels of nucleolin in either the plasma membrane or cytoplasm. The widespread and aberrant expression of the multi-functional protein nucleolin in human tumor cells, in contrast to the corresponding normal cells, explains both the broad-spectrum anticancer activity and tumor selectivity of antibody CP1.

[0267] Patient-derived CG-EMT prostate cancer cells were obtained from Michael B. Lilly, M.D. of the Hollings Cancer Center at the Medical University of South Carolina. The cells were cultured in RPMI media with 10% heat-inactivated fetal bovine serum and 1% antibiotics for 32 passages until cell growth became consistent. To test the potency of purified CP1 in killing CG-EMT cells, the cells were seeded onto 96 well plate at a density of 6000 cells per well. On the following day, the cells were incubated in triplicate with either 0 to 8 μg/ml of CP1 or with 0 to 8 μg/ml of isotype control human IgG_1 in the presence of 10% human A/B serum. After 96 hours, cell viability was assayed using trypan blue exclusion and cell counting with a NEXCELOM Cellometer. Results are shown in **FIG. 8**.

Examle 9. Effects of CP1(RC) on the Viability of Tumor Cells

Illustrative IC_{50} Value Determination

[0268] The antiproliferative activity of CP1(RC) against the human tumor cell lines was investigated with Promega's Cell Titer-Glo® Luminescent Cell Viability assay.

Table 5. Cells and antibodies used in the IC_{50} study.

Tumor Type	Cell Line	Dilution	Test Agent; Top Conc.
Human Kidney Renal	786-0	1:2	CP1(RC): 8 μg/mL IgG Control 1: 8 μg/mL
Human Colon	HCT-116		
Human Glioma	U251		
Human Lung	A549		
Human Liver	Hep3B		
Human Melanoma	A375		

[0269] The human tumor cells were seeded in a clear polystyrene 96-well microculture plate (Corning® Costar® 96-well flat bottom plate, Cat.# 3997) in a total volume of 90 μL/well. After 24 hours of incubation in a humidified incubator at 37 °C with 5% CO2 and 95% air, 10 μL of

10X, serially diluted test agents in growth medium were added to each well in duplicate (10 pt dose response, highest concentration 8 µg/mL). After 72 hours of culture in a humidified incubator at 37°C, in an atmosphere of 5% CO_2 and 95% air, the plated cells and Cell Titer-Glo® (Promega G7571) reagents were brought to room temperature to equilibrate for 30 minutes. A picture was taken of the control wells to depict confluency at endpoint. 100 µL of Cell Titer-Glo® reagent was added to each well. The plate was shaken for two minutes and then left to equilibrate for ten minutes. The medium/Cell Titer-Glo® reagent was transferred to a white polystyrene 96-well microculture plate (Corning® Costar® 96-well flat bottom plate, Cat.# 3917) before reading luminescence on the BioTek Synergy II microplate reader. The IC_{50} value for the test agents were estimated using Graph Pad Prism 7.0 by plotting compound concentration (Log µM) versus % C and fitting the four parameter logistic equation to the normalized data by nonlinear regression.

Table 6. Results of IC_{50} measurements.

Cell Line	Agent	IC_{50} (µg/mL)
786-0	CP1(RC)	0.31
786-0	IgG	>8
HCT-116	CP1(RC)	0.69
HCT-116	IgG	3.87
U251	CP1(RC)	0.81
U251	IgG	6.77
A549	CP1(RC)	0.08
A549	IgG	>8
Hep3B	CP1(RC)	0.11
Hep3B	IgG	>8
A375	CP1(RC)	0.08
A375	IgG	7.37

9. Sequences of CP1(RC), CP1, CP2, Nucleolin

9.1. CP1(RC)

CP1(RC) Gamma heavy chain, nucleotide sequence SEQ ID NO:1

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                         ATGAAACAC CTGTGGTTCT TTCTCCTGCT GGTGGCTGCT
CCCAGGTGGG TGCTGAGCCA GGTGCAGCTG CAGGAAAGCG GACCTGGCCT GGTCAAACCC
TCCCAGACAC TGAGCCTGAC CTGCACCGTC AGCGGCGGAT CCATCAACTC CGGCGGCTTC
TACTGGAGCT GGATCAGACA GCATCCTGGC AAGGGCCTCG AGTGGATCGG CTACATTAGC
TATACCGGCA GCACCTACTA CAATCCCTCC CTGAAGAGCA GGGTGAACAT TAGCGCCGAC
ACCTCCAAGA ACAGGTTCAG CCTGAAGCTC AGCAGCGTCA CCGCCGCCGA TACCGCCGTG
TACTACTGCG CCAGGGACAT GAACGACGGC CTGCAGATCT GGGGACAGGG CACACTGGTC
ACCGTGTCCG CTGCCAGCAC CAAGGGACCC AGCGTGTTCC CCCTGGCTCC CTCCTCCAAG
AGCACCTCCG GAGGCACCGC CGCCCTGGGC TGCCTGGTGA AGGATTACTT CCCCGAGCCC
GTGACCGTGA GCTGGAACAG CGGAGCCCTG ACAAGCGGAG TGCACACATT CCCTGCCGTG
CTGCAGAGCA GCGGCCTGTA CTCCCTGAGC TCCGTGGTCA CAGTGCCTAG CTCCTCCCTC
GGCACCCAGA CCTACATCTG CAACGTGAAC CATAAGCCCT CCAATACCAA GGTGGACAAG
AGGGTCGAGC CCAAATCCTG CGACAAGACA CACACCTGTC CTCCTTGCCC CGCCCCCGAA
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CTGCTGGGCG GACCCTCCGT CTTCCTCTTC CCTCCTAAGC CCAAGGATAC CCTGATGATC
AGCAGGACAC CTGAGGTGAC CTGCGTGGTG GTGGACGTCT CCCACGAGGA CCCCGAGGTG
AAGTTCAACT GGTACGTGGA TGGCGTGGAG GTCCACAACG CCAAGACCAA GCCCAGAGAG
GAGCAGTACA ACAGCACATA CAGGGTGGTC TCCGTCCTGA CAGTGCTCCA CCAGGACTGG
CTGAATGGCA AGGAGTACAA GTGCAAGGTC AGCAACAAAG CCCTGCCCGC CCCTATCGAG
AAGACCATCA GCAAGGCTAA GGGCCAGCCC AGGGAGCCCC AGGTCTATAC CCTGCCCCCC
AGCAGGGAAG AGATGACCAA GAATCAGGTC TCCCTGACCT GTCTGGTGAA GGGCTTCTAC
CCTAGCGACA TCGCCGTGGA GTGGGAGAGC AACGGCCAGC CTGAAAACAA CTACAAGACC
ACCCCTCCTG TGCTGGACTC CGACGGATCC TTCTTCCTGT ACTCCAAGCT GACCGTGGAT
AAAAGCAGGT GGCAACAGGG CAACGTGTTC TCCTGCTCCG TCATGCACGA AGCTCTGCAC
AACCACTACA CCCAGAAGAG CCTGTCCCTG AGCCCTGGCA AG

CP1(RC) Gamma heavy chain, amino acid sequence SEQ ID NO:2

1 MKHLWFFLLL VAAPRWVLSQ VQLQESGPGL VKPSQTLSLT CTVSGGSINS GGFYWSWIRQ
61 HPGKGLEWIG YISYTGSTYY NPSLKSRVNI SADTSKNRFS LKLSSVTAAD TAVYYCARDM
121 NDGLQIWGQG TLVTVSAAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS
181 GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTQTYIC NVNHKPSNTK VDKRVEPKSC
241 DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
301 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
361 GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
421 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK

R234, E376, M378 are allotype residues identified by comparing the sequencing of the CP1 antibody to known allotype sequences in other human antibodies.

Residues Q20 to A137 of CP1(RC) Gamma heavy chain: variable region, amino acid sequence SEQ ID NO:3

QVQLQESGPGLVKPSQTLSLTCTVSGGSINSGGFYWSWIRQHPGKGLEWIGYISYTGSTYYNPSLKSRVN
ISADTSKNRFSLKLSSVTAADTAVYYCARDMNDGLQIWGQGTLVTVSA

Residues A138 to K467 of CP1(RC) Gamma heavy chain: constant region, amino acid sequence SEQ ID NO:4

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Residues M1 to S19 of CP1(RC) Gamma heavy chain: native signal peptide (leader), amino acid sequence SEQ ID NO:5

MKHLWFFLLLVAAPRWVLS

Residues A138 to V235 of CP1(RC) Gamma heavy chain: human CH1, amino acid sequence SEQ ID NO:6

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKRV

Residues E236 to P250 of CP1(RC) Gamma heavy chain: human G1 Hinge, amino acid sequence SEQ ID NO:7

EPKSCDKTHTCPPCP

Residues A251 to G361 of CP1(RC) Gamma heavy chain: human CH2, amino acid sequence SEQ ID NO:8

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG

Residues Q362 to K467 of CP1(RC) Gamma heavy chain: human CH3, amino acid sequence SEQ ID NO:9

QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

CP1(RC) Kappa light chain, nucleotide sequence SEQ ID NO:10

ATGGACATG AGGGTGCCTG CCCAGCTGCT CGGACTGCTG
CTGCTGTGGC TGCCCGGAGC TAAGTGCGAC ATCCAGATGA CCCAGAGCCC TTCCACACTC
TCCGCCAGCG TGGGCGATAG GGTGACCATC ACCTGCAGGG CCAGCCAGTC CATCAGCAGG
TGGCTGGCCT GGTACCAGCA GAAGCCCGGC AAGGCCCCCA AGCTGCTGAT CTACAAGGCC
AGCACACTCG AGTCCGGCGT GCCCAGCAGA TTCAGCGGAA GCGGCAGCGG CACCGAGTTT
ACCCTGACCA TCAGCAGCCT GCAGCCCGAC GACTTCGCCA CCTACTACTG CCAGCAGTAC
AACTCCTATA GCAGGGCCTT CGGCCAGGGC ACCAAAGTGG AGATCAAGAG GACCGTGGCC
GCCCCTAGCG TCTTCATCTT CCCCCCCTCC GACGAGCAGC TGAAGAGCGG CACAGCCTCC
GTGGTGTGCC TGCTGAACAA CTTCTACCCC AGGGAGGCCA AGGTGCAGTG GAAGGTGGAC
AACGCCCTGC AGAGCGGCAA CTCCCAGGAG AGCGTGACCG AGCAGGACTC CAAGGACAGC
ACCTACAGCC TGAGCAGCAC CCTCACCCTG AGCAAGGCCG ACTACGAGAA GCACAAGGTG
TACGCCTGCG AGGTGACACA CCAGGGCCTG AGCAGCCCTG TGACCAAGTC TTTTAACAGG
GGCGAATGC

CP1(RC) Kappa light chain, amino acid sequence SEQ ID NO:11

1 MDMRVPAQLL GLLLLWLPGA KCDIQMTQSP STLSASVGDR VTITCRASQS ISRWLAWYQQ
61 KPGKAPKLLI YKASTLESGV PSRFSGSGSG TEFTLTISSL QPDDFATYYC QQYNSYSRAF
121 GQGTKVEIKR TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN
181 SQESVTEQDS KDSTYSLSST LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC

A175 and V213 are allotype residues identified by comparing the sequencing of the CP1 antibody to known allotype sequences in other human antibodies.
Residues M1 to C22 are a native signal peptide (leader).

Residues D23 to K129 of CP1(RC) Kappa light chain: variable region, amino acid sequence SEQ ID NO:12

DIQMTQSPSTLSASVGDRVTITCRASQSISRWLAWYQQKPGKAPKLLIYKASTLESGVPSRFSGSGSGTE
FTLTISSLQPDDFATYYCQQYNSYSRAFGQGTKVEIK

Residues R130 to C236 of CP1(RC) Kappa light chain: constant region, amino acid sequence SEQ ID NO:13

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS
TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

9.2. CP1

CP1 heavy chain, amino acid sequence SEQ ID NO:14

1 MKHLWFFLLL VAAPRWVLSQ VQLQESGPGL VKPSQTLSLT CTVSGGSINS GGFYWSWIRQ
61 HPGKGLEWIG YISYTGSTYY NPSLKSRVNI SADTSKNRFS LKLSSVTAAD TAVYYCARDM
121 NDGLQIWGQG TLVTVSAAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS
181 GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC
241 DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
301 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
361 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
421 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK

Residues 20-137 of CP1 heavy chain: variable region, amino acid sequence SEQ ID NO:3

QVQLQESGPGLVKPSQTLSLTCTVSGGSINSGGFYWSWIRQHPGKGLEWIGYISYTGSTYYNPSLKSRVN
ISADTSKNRFSLKLSSVTAADTAVYYCARDMNDGLQIWGQGTLVTVSA

Residues 138-467 of CP1 heavy chain: constant region, amino acid sequence SEQ ID NO:15

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

CP1 light chain, amino acid sequence SEQ ID NO:11

1 MDMRVPAQLL GLLLLWLPGA KCDIQMTQSP STLSASVGDR VTITCRASQS ISRWLAWYQQ
61 KPGKAPKLLI YKASTLESGV PSRFSGSGSG TEFTLTISSL QPDDFATYYC QQYNSYSRAF
121 GQGTKVEIKR TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN
181 SQESVTEQDS KDSTYSLSST LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC

Residues D23 to K129 of CP1 light chain: variable region, amino acid sequence SEQ ID NO:12

DIQMTQSPSTLSASVGDRVTITCRASQSISRWLAWYQQKPGKAPKLLIYKASTLESGVPSRFSGSGSGTE
FTLTISSLQPDDFATYYCQQYNSYSRAFGQGTKVEIK

Residues R130 to C236 of CP1 light chain: constant region, amino acid sequence SEQ ID NO:13

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS
TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

9.3. Antibody CP2

CP2 Heavy chain variable region, nucleotide sequence SEQ ID NO:16

GGAAGGTGTGCACGCCGCTGGTCAGGGCGCCTGAGTTCCACGACACCGTCACCGGTTCGGGGAAGTAGTC

CTTGACCAGGCAGCCCAGGGCCGCTGTGCCCCCAGAGGTGCTCTTGGAGGAGGGTGCCAGGGGGAAGACC

GATGGGCCCTTGGTGGAGGCTGAGGAGACGGTGACCAGGGTTCCCAGGCCCCAGTAAACTCCTATAATAA

TCACTTCAGAAATCCTTCCGGAAGTGGTGCAATAATACACGGCTGTGTCCTCGGTTTGCAGGCTGTTCAT

TTGCAGATACATCGTATTTTTTGAATCATCTCTTGAGATGGTGAACCTGTCATTCACGGCTGCGGCGTAG

TCTGTTGTCCCACCATCAGATCTGCCCTTAATACGGCCAACCCACTCCAGCCCCTTCCCTGGAACCCGGC

GGACCCAATTCATCCAACTGTTCGCGAGACTGAGTCCAGAGACTGTACAGGAGAGTCTAAGGGACCCCCC

CGGCTGTACCAAGTCTCCCCCCGACTCCTCCAACTGCACCTCACACTGGACACCTT

CP2 Heavy chain variable region, amino acid sequence SEQ ID NO:17

1 EVKLQESGPE LVKPGASVKI SCKASGYTFT DYFMIWVKQS HGKSLEWIGD INPSNGGSSY
61 NLKFKDKATL TVDKSSNTAY MDLRSLTSED SAVYYCARGQ FRLPAWFAYW GQGALVTVSA

CP2 Light chain variable region, nucleotide sequence SEQ ID NO:18

ATGAGGCTCCCTGCTCAGCTGCTGGGGCTGCTAATGCTCAGCGTCCCAGGGTCCAGTGGGGATGTTGTGC

TGACTCAGTCTCCACTCTCCCTGCCCGTAACCCCTGGACAGCCGGCCTCCATATCCTGCACGTCTACTCA

AAGCCTCGCACACAGCAATGGAGACACCTACTTGAATTGGTTTCTGCAGAGGCCAGGCCAAGCTCCAAGG

CGCCTATTTTATAACGTTTCTGACCGCGACTTTGGGGTCCCGGACAGATTCAGCGGCAGTGGGTCAGGCA

CTGATTTCACACTGAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGCATTTATTACTGCATGCAGGGTAC

ACTCTGGCCTCCGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGAACTGTGGCTGCACCATCTGTC

TTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACT

TCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAG

TGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCC

CP2 Light chain variable region, amino acid sequence SEQ ID NO:19

1 DVVLTQSPLS LPVTPGQPAS ISCTSTQSLA HSNGDTYLNW FLQRPGQAPR RLFYNVSDRD
61 FGVPDRFSGS GSGTDFTLKI SRVEAEDVGI YYCMQGTLWP PTFGQGTKVE IK

9.4. Nucleolin

Nucleolin, amino acid sequence SEQ ID NO:20

1 MVKLAKAGKN QGDPKKMAPP PKEVEEDSED EEMSEDEEDD SSGEEVVIPQ KKGKKAAATS
61 AKKVVVSPTK KVAVATPAKK AAVTPGKKAA ATPAKKTVTP AKAVTTPGKK GATPGKALVA
121 TPGKKGAAIP AKGAKNGKNA KKEDSDEEED DDSEEDEEDD EDEDEDEDEI EPAAMKAAAA
181 APASEDEDDE DDEDDEDDDD DEEDDSEEEA METTPAKGKK AAKVVPVKAK NVAEDEDEEE
241 DDEDEDDDDD EDDEDDDDED DEEEEEEEEE EPVKEAPGKR KKEMAKQKAA PEAKKQKVEG
301 TEPTTAFNLF VGNLNFNKSA PELKTGISDV FAKNDLAVVD VRIGMTRKFG YVDFESAEDL
361 EKALELTGLK VFGNEIKLEK PKGKDSKKER DARTLLAKNL PYKVTQDELK EVFEDAAEIR
421 LVSKDGKSKG IAYIEFKTEA DAEKTFEEKQ GTEIDGRSIS LYYTGEKGQN QDYRGGKNST
481 WSGESKTLVL SNLSYSATEE TLQEVFEKAT FIKVPQNQNG KSKGYAFIEF ASFEDAKEAL
541 NSCNKREIEG RAIRLELQGP RGSPNARSQP SKTLFVKGLS EDTTEETLKE SFDGSVRARI
601 VTDRETGSSK GFGFVDFNSE EDAKAAKEAM EDGEIDGNKV TLDWAKPKGE GGFGGRGGGR
661 GGFGGRGGGR GGRGGFGGRG RGGFGGRGGF RGGRGGGGDH KPQGKKTKFE

Residues G300 to E466 of nucleolin, amino acid sequence SEQ ID NO:21

GTEPTTAFNLFVGNLNFNKSAPELKTGISDVFAKNDLAVVDVRIGMTRKFGYVDFESAEDLEKALELTGL
KVFGNEIKLEKPKGKDSKKERDARTLLAKNLPYKVTQDELKEVFEDAAEIRLVSKDGKSKGIAYIEFKTE
ADAEKTFEEKQGTEIDGRSISLYYTGE

Table 7 Part 1. Complementarity Determining Region (CDR) of Heavy Chain Variable Region (VH) of Antibody CP1/ Antibody CP1(RC), SEQ ID NOS. 22 to 42

VH	Framework H1	CDR H1	Framework H2	CDR H2	Framework H3	CDR H3	Framework H4
Rosie Rosetta	QVQLQESGPGLVKPSQTLSLTCTVS (SEQ ID NO:22)	G**GSINSGG**FYWS (SEQ ID NO:24)	WIRQHPGKGLEWIG (SEQ ID NO:27)	**YIS**YTGSTYYNPSLKS (SEQ ID NO:30)	RVNISADTSKNRFSLKLSSVTAADTAVYYCAR (SEQ ID NO:33)	**DM**NDGLQI (SEQ ID NO:37)	WGQGTLVTVSA (SEQ ID NO:40)
Paratome	QVQLQESGPGLVKPSQTLSLTCTVSG (SEQ ID NO:23)	**GSINSGG**FYWS (SEQ ID NO:25)	WIRQHPGKGLE (SEQ ID NO:28)	WIG**YIS**YTGSTYY (SEQ ID NO:31)	NPSLKSRVNISADTSKNRFSLKLSSVTAADTAVYYCA (SEQ ID NO:34)	R**DM**NDGLQI (SEQ ID NO:38)	WGQGTLVTVSA (SEQ ID NO:40)
P.I.G.S.	QVQLQESGPGLVKPSQTLSLTCTVS (SEQ ID NO:22)	G**GSINSGG** (SEQ ID NO:26)	FYWSWIRQHPGKGLEWIG (SEQ ID NO:29)	**YIS**	YTGSTYYNPSLKSRVNISADTSKNRFSLKLSSVTAADTAVY (SEQ ID NO:35)	YCAR**DM** (SEQ ID NO:39)	NDGLQIWGQGTLVTVSA (SEQ ID NO:41)
Chothia Definition	QVQLQESGPGLVKPSQTLSLTCTVS (SEQ ID NO:22)	G**GSINSGG**FYWS (SEQ ID NO:24)	WIRQHPGKGLEWIG (SEQ ID NO:27)	**YIS**YTGSTYYNPSL (SEQ ID NO:32)	KSRVNISADTSKNRFSLKLSSVTAADTAVYYCAR (SEQ ID NO:36)	**DM**NDGLQI (SEQ ID NO:37)	WGQGTLVTVSA (SEQ ID NO:40)

GSINSGG (SEQ ID NO:42)

Table 7 Part 2. CDR of Light Chain Variable Region (VL) of Antibody CP1/ Antibody CP1(RC), SEQ ID NOS. 43 to 66

VL	Framework L1	CDR L1	Framework L2	CDR L2	Framework L3	CDR L3	Framework L4
Rosie Rosetta	DIQMTQSPSTLSASVGDRVTITC (SEQ ID NO:43)	RAS**QSISRWL**A (SEQ ID NO:46)	WYQQKPGKAPKLLIY (SEQ ID NO:49)	**KAST**LES (SEQ ID NO:52)	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC (SEQ ID NO:55)	QQ**YNSY** (SEQ ID NO:58)	SRAFGQGTKVEIK (SEQ ID NO:62)
Paratome	DIQMTQSPSTLSASVGDRVTITCRAS (SEQ ID NO:44)	**QSISRWL**A (SEQ ID NO:47)	WYQQKPGKAPK (SEQ ID NO:50)	LLIY**KAST**LES (SEQ ID NO:53)	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC (SEQ ID NO:55)	QQ**YNSY**SRA (SEQ ID NO:59)	FGQGTKVEIK (SEQ ID NO:63)
P.I.G.S.	DIQMTQSPSTLSASVGDRVTITCRA (SEQ ID NO:45)	S**QSISRWL** (SEQ ID NO:48)	AWYQQKPGKAPKLLIY (SEQ ID NO:51)	**KAST** (SEQ ID NO:54)	LESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQ (SEQ ID NO:56)	**YNSY**SR (SEQ ID NO:60)	AFGQGTKVEIK (SEQ ID NO:64)
Chothia Definition	DIQMTQSPSTLSASVGDRVTITC (SEQ ID NO:43)	RAS**QSISRWL**A (SEQ ID NO:46)	WYQQKPGKAPKLLIY (SEQ ID NO:49)	**KAST**LES (SEQ ID NO:52)	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQ (SEQ ID NO:57)	**YNSY**SRA (SEQ ID NO:61)	FGQGTKVEIK (SEQ ID NO:63)

QSISRWL (SEQ ID NO:65)

YNSY (SEQ ID NO:66)

Table 8 Part 1. CDR of VH of antibody CP2 (SEQ ID NOS. 67 to 80)

VH	Framework H1	CDR H1	Framework H2	CDR H2	Framework H3	CDR H3	Framework H4
KABAT	EVKLQESGPEL VKPGASVKISC KASGYTFT (SEQ ID NO:67)	**DYF**MI (SEQ ID NO:69)	WVKQSHGKSL EWIG (SEQ ID NO:71)	DI**NPSNGGS**SY NLKFKD (SEQ ID NO:73)	KATLTVDKSS NTAYMDLRSL TSEDSAVYYC AR (SEQ ID NO:75)	GQFRLPAWFA Y (SEQ ID NO:77)	WGQGALVTVS A (SEQ ID NO:79)
IMGT	EVKLQESGPEL VKPGASVKISC KAS (SEQ ID NO:68)	GYTFT**DYF** (SEQ ID NO:70)	MIWVKQSHGK SLEWIGD (SEQ ID NO:72)	**INPSNGGS** (SEQ ID NO:74)	SYNLKFKDKA TLTVDKSSNT AYMDLRSLTS EDSAVYYC (SEQ ID NO:76)	**AR**GQFRLPAW FAY (SEQ ID NO:78)	WGQGALVTVS A (SEQ ID NO:79)
Molecular Cloning Laboratories (MCLAB)	EVKLQESGPEL VKPGASVKISC KAS (SEQ ID NO:68)	GYTFT**DYF** (SEQ ID NO:70)	MIWVKQSHGK SLEWIGD (SEQ ID NO:72)	**INPSNGGS** (SEQ ID NO:74)	SYNLKFKDKA TLTVDKSSNT AYMDLRSLTS EDSAVYYC (SEQ ID NO:76)	**AR**	GQFRLPAWFA YWGQGALVTV SA (SEQ ID NO:80)

Table 8 Part 2. CDR of VL of antibody CP2 (SEQ ID NOS. 81 to 93)

VL	Framework L1	CDR L1	Framework L2	CDR L2	Framework L3	CDR L3	Framework L4
KABAT	DVVLTQSPLSLP VTPGQPASISC (SEQ ID NO:81)	TST**QSLAHSNG DTY**LN (SEQ ID NO:83)	WFLQRPGQAP RRLFY (SEQ ID NO:85)	**NVS**DRDF (SEQ ID NO:87)	GVPDRFSGSGS GTDFTLKISRVE AEDVGIYYC (SEQ ID NO:88)	**MQGTLWPP**T (SEQ ID NO:90)	FGQGTKVEIK (SEQ ID NO:92)
IMGT	DVVLTQSPLSLP VTPGQPASISCT ST (SEQ ID NO:82)	**QSLAHSNGDTY** (SEQ ID NO:84)	LNWFLQRPGQA PRRLFY (SEQ ID NO:86)	**NVS**	DRDFGVPDRFS GSGSGTDFTLKI SRVEAEDVGIYY C (SEQ ID NO:89)	**MQGTLWPP**T (SEQ ID NO:90)	FGQGTKVEIK (SEQ ID NO:92)
Molecular Cloning Laboratories (MCLAB)	DVVLTQSPLSLP VTPGQPASISCT ST (SEQ ID NO:82)	**QSLAHSNGDTY** (SEQ ID NO:84)	LNWFLQRPGQA PRRLFY (SEQ ID NO:86)	**NVS**	DRDFGVPDRFS GSGSGTDFTLKI SRVEAEDVGIYY C (SEQ ID NO:89)	**MQGTLWPP** (SEQ ID NO:91)	TFGQGTKVEIK (SEQ ID NO:93)

[0270] All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. It will be apparent to those of skill in the art that variations can be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related can be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:
   a heavy chain CDR1 having at least 60% sequence identity to amino acid sequence SEQ ID NO:42;
   a heavy chain CDR2 having at least 60% sequence identity to amino acid sequence YIS;
   a heavy chain CDR3 having at least 60% sequence identity to amino acid sequence DM;
   a light chain CDR1 having at least 60% sequence identity to amino acid sequence SEQ ID NO:65;
   a light chain CDR2 having at least 60% sequence identity to amino acid sequence SEQ ID NO:54; and
   a light chain CDR3 having at least 60% sequence identity to amino acid sequence SEQ ID NO:66.
2. The isolated antibody or fragment thereof of claim 1, wherein the at least 60% sequence identity is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.
3. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:
   a heavy chain CDR1 has an amino acid sequence that comprises SEQ ID NO:42;
   a heavy chain CDR2 has an amino acid sequence that comprises YIS;
   a heavy chain CDR3 has an amino acid sequence that comprises DM;
   a light chain CDR1 has an amino acid sequence that comprises SEQ ID NO:65;
   a light chain CDR2 has an amino acid sequence that comprises SEQ ID NO:54; and
   a light chain CDR3 has an amino acid sequence that comprises SEQ ID NO:66.
4. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:
   a heavy chain CDR1 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 24 to 26;
   a heavy chain CDR2 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of YIS and SEQ ID NOS. 30 to 32;
   a heavy chain CDR3 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 37 to 39;

a light chain CDR1 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 46 to 48;

a light chain CDR2 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 52 to 54; and

a light chain CDR3 having at least 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS. 58 to 61.

5. The isolated antibody or fragment thereof of claim 4, wherein the at least 60% sequence identity is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

6. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 has an amino acid sequence of SEQ ID NO:24;

a heavy chain CDR2 has an amino acid sequence of SEQ ID NO:30;

a heavy chain CDR3 has an amino acid sequence of SEQ ID NO:37;

a light chain CDR1 has an amino acid sequence of SEQ ID NO:46;

a light chain CDR2 has an amino acid sequence of SEQ ID NO:52; and

a light chain CDR3 has an amino acid sequence of SEQ ID NO:58.

7. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 has an amino acid sequence of SEQ ID NO:25;

a heavy chain CDR2 has an amino acid sequence of SEQ ID NO:31;

a heavy chain CDR3 has an amino acid sequence of SEQ ID NO:38;

a light chain CDR1 has an amino acid sequence of SEQ ID NO:47;

a light chain CDR2 has an amino acid sequence of SEQ ID NO:53; and

a light chain CDR3 has an amino acid sequence of SEQ ID NO:59.

8. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 has an amino acid sequence of SEQ ID NO:26;

a heavy chain CDR2 has an amino acid sequence of YIS;

a heavy chain CDR3 has an amino acid sequence of SEQ ID NO:39;

a light chain CDR1 has an amino acid sequence of SEQ ID NO:48;

a light chain CDR2 has an amino acid sequence of SEQ ID NO:54; and

a light chain CDR3 has an amino acid sequence of SEQ ID NO:60.

9. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 has an amino acid sequence of SEQ ID NO:24;

a heavy chain CDR2 has an amino acid sequence of SEQ ID NO:32;

a heavy chain CDR3 has an amino acid sequence of SEQ ID NO:37;

a light chain CDR1 has an amino acid sequence of SEQ ID NO:46;

a light chain CDR2 has an amino acid sequence of SEQ ID NO:52; and

a light chain CDR3 has an amino acid sequence of SEQ ID NO:61.

10. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain variable region (VH) that has at least 60% sequence identity to amino acid sequence SEQ ID NO:3; and a light chain variable region (VL) that has at least 60% sequence identity to amino acid sequence SEQ ID NO:12.
11. The isolated antibody or fragment thereof of claim 10, wherein the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.
12. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a VH that has an amino acid sequence of SEQ ID NO:3; and a VL has an amino acid sequence of SEQ ID NO:12.
13. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain that has at least 60% sequence identity to amino acid sequence SEQ ID NO:2; and a light chain that has at least 60% identity to amino acid sequence SEQ ID NO:11.
14. The isolated antibody or fragment thereof of claim 13, wherein the amino acid sequence SEQ ID NO:2 is encoded by nucleotide sequence SEQ ID NO:1.
15. The isolated antibody or fragment thereof of claim 13 or 14, wherein the amino acid sequence SEQ ID NO:11 is encoded by nucleotide sequence SEQ ID NO:10.
16. The isolated antibody or fragment thereof of any one of claims 13-15, wherein the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.
17. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a heavy chain that has at least 60% sequence identity to amino acid sequence SEQ ID NO:14; and a light chain that has at least 60% identity to amino acid sequence SEQ ID NO:11.
18. The isolated antibody or fragment thereof of claim 17, wherein the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

19. An isolated antibody or fragment thereof that comprises a heavy chain having an amino acid sequence of SEQ ID NO:2; and a light chain having an amino acid sequence of SEQ ID NO:11.

20. An isolated antibody or fragment thereof that comprises a heavy chain having an amino acid sequence of SEQ ID NO:14; and a light chain having an amino acid sequence of SEQ ID NO:11.

21. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 having at least 60% sequence identity to amino acid sequence DYF;

a heavy chain CDR2 having at least 60% sequence identity to amino acid sequence SEQ ID NO:74;

a heavy chain CDR3 having at least 60% sequence identity to amino acid sequence AR or SEQ ID NO:77;

a light chain CDR1 having at least 60% sequence identity to amino acid sequence SEQ ID NO:84;

a light chain CDR2 having at least 60% sequence identity to amino acid sequence NVS; and

a light chain CDR3 having at least 60% sequence identity to amino acid sequence SEQ ID NO:91.

22. The isolated antibody or fragment thereof of claim 21, wherein the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

23. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 has an amino acid sequence that comprises DYF;

a heavy chain CDR2 has an amino acid sequence that comprises SEQ ID NO:74;

a heavy chain CDR3 has an amino acid sequence that comprises AR or SEQ ID NO:77;

a light chain CDR1 has an amino acid sequence that comprises SEQ ID NO:84;

a light chain CDR2 has an amino acid sequence that comprises NVS; and

a light chain CDR3 has an amino acid sequence that comprises SEQ ID NO:91.

24. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 having at least 60% sequence identity to amino acid sequence SEQ ID NO:69 or SEQ ID NO:70;

a heavy chain CDR2 having at least 60% sequence identity to amino acid sequence SEQ ID NO:73 or SEQ ID NO:74;

a heavy chain CDR3 having at least 60% sequence identity to amino acid sequence AR, SEQ ID NO:77, or SEQ ID NO:78;

a light chain CDR1 having at least 60% sequence identity to amino acid sequence SEQ ID NO:83 or SEQ ID NO:84;

a light chain CDR2 having at least 60% sequence identity to amino acid sequence NVS or SEQ ID NO:87; and

a light chain CDR3 having at least 60% sequence identity to amino acid sequence SEQ ID NO:90 or SEQ ID NO:91.

25. The isolated antibody or fragment thereof of claim 24, wherein the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.

26. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 has an amino acid sequence of SEQ ID NO:69;

a heavy chain CDR2 has an amino acid sequence of SEQ ID NO:73;

a heavy chain CDR3 has an amino acid sequence of SEQ ID NO:77;

a light chain CDR1 has an amino acid sequence of SEQ ID NO:83;

a light chain CDR2 has an amino acid sequence of SEQ ID NO:87; and

a light chain CDR3 has an amino acid sequence of SEQ ID NO:90.

27. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 has an amino acid sequence of SEQ ID NO:70;

a heavy chain CDR2 has an amino acid sequence of SEQ ID NO:74;

a heavy chain CDR3 has an amino acid sequence of SEQ ID NO:78;

a light chain CDR1 has an amino acid sequence of SEQ ID NO:84;

a light chain CDR2 has an amino acid sequence of NVS; and

a light chain CDR3 has an amino acid sequence of SEQ ID NO:90.

28. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises:

a heavy chain CDR1 has an amino acid sequence of SEQ ID NO:70;

a heavy chain CDR2 has an amino acid sequence of SEQ ID NO:74;

a heavy chain CDR3 has an amino acid sequence of AR;

a light chain CDR1 has an amino acid sequence of SEQ ID NO:84;

a light chain CDR2 has an amino acid sequence of NVS; and

a light chain CDR3 has an amino acid sequence of SEQ ID NO:91.

29. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a VH that has at least 60% sequence identity to amino acid sequence SEQ ID NO:17; and a VL that has at least 60% sequence identity to amino acid sequence SEQ ID NO:19.
30. The isolated antibody or fragment thereof of claim 29, wherein the amino acid sequence of SEQ ID NO:17 is encoded by nucleotide sequence SEQ ID NO:16.
31. The isolated antibody or fragment thereof of claim 29 or 30, wherein the amino acid sequence of SEQ ID NO:19 is encoded by nucleotide sequence SEQ ID NO:18.
32. The isolated antibody or fragment thereof of any one of claims 29-31, wherein the at least 60% sequence identity is at least: 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%, or 100% sequence identity.
33. An isolated antibody or fragment thereof, wherein the isolated antibody or fragment thereof binds to human nucleolin and comprises: a VH has an amino acid sequence of SEQ ID NO:17; and a VL that has an amino acid sequence of SEQ ID NO:19.
34. An isolated antibody or fragment thereof that comprises any combination of a heavy chain CDR, VH, or fragment thereof recited in any preceding claim and a light chain CDR, VL, or fragment thereof recited in any preceding claim.
35. An isolated anti-nucleolin antibody or fragment thereof that binds to amino acid sequence SEQ ID NO:21.
36. An isolated anti-nucleolin antibody or fragment thereof that binds to an epitope within residues G300 to E466 of amino acid sequence SEQ ID NO:20.
37. The isolated antibody or fragment thereof of claim 36, wherein the epitope comprises an amino acid selected from the group consisting of E453, R457, D455, K348, K427, G426, K403, Y402, and any combination thereof.
38. The isolated antibody or fragment thereof of claim 36 or 37, wherein the isolated antibody or fragment thereof comprises a light chain CDR1 that binds to E453, R457, or a combination thereof.
39. The isolated antibody or fragment thereof of any one of claims 36 to 38, wherein the isolated antibody or fragment thereof comprises a light chain CDR2 that binds to D455.
40. The isolated antibody or fragment thereof of any one of claims 36 to 39, wherein the isolated antibody or fragment thereof comprises a light chain CDR3 that binds to K348.
41. The isolated antibody or fragment thereof of any one of claims 36 to 40, wherein the isolated antibody or fragment thereof comprises a heavy chain CDR1 that binds to K427.

42. The isolated antibody or fragment thereof of any one of claims 36 to 41, wherein the isolated antibody or fragment thereof comprises a heavy chain CDR2 that binds to K427, G426, or a combination thereof.
43. The isolated antibody or fragment thereof of any one of claims 36 to 42, wherein the isolated antibody or fragment thereof comprises a heavy chain CDR3 that binds to K403, Y402, or a combination thereof.
44. The isolated antibody or fragment thereof of any preceding claim, wherein the nucleolin is cell-surface nucleolin.
45. The isolated antibody or fragment thereof of any preceding claim, wherein the isolated antibody or fragment thereof is human or humanized.
46. The isolated antibody or fragment thereof of any preceding claim, wherein the isolated antibody or fragment thereof is an IgG antibody.
47. The isolated antibody or fragment thereof of any preceding claim, wherein the isolated antibody or fragment thereof is an IgG1, IgG2, IgG3, or IgG4 antibody.
48. The isolated antibody or fragment thereof of any preceding claim, wherein the isolated antibody or fragment thereof is a fragment that is a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a Fv fragment, a diabody, a linear antibody, a single-chain antibody, or a multispecific antibody formed from an antibody fragment.
49. The isolated antibody or fragment thereof of any preceding claim, wherein the isolated antibody or fragment thereof is a fragment that comprises an antigen binding region.
50. The isolated antibody or fragment thereof of any preceding claim, wherein the isolated antibody or fragment thereof is nontotoxic to normal cells or tissues.
51. The isolated antibody or fragment thereof of any preceding claim, wherein the isolated antibody or fragment thereof is cytotoxic to a tumor or cancer cell.
52. The isolated antibody or fragment thereof of any preceding claim, wherein the isolated antibody or fragment thereof kills at least 10% of a population of tumor or cancer cells, when incubated with the population of tumor or cancer cells for a period of time.
53. The isolated antibody or fragment thereof of claim 52, wherein the isolated antibody or fragment thereof kills at least: 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%; or about 100% of the population of tumor or cancer cells.
54. The isolated antibody or fragment thereof of claim 52 or 53, wherein the incubation is in presence of human serum.
55. The isolated antibody or fragment thereof of any one of claims 52-54, wherein the period of time is about 48-96 hours.

56. The isolated antibody or fragment thereof of any one of claims 51-55, wherein the tumor or cancer cell comprises one, two, or more types of cancer cells selected from the group consisting of human lung cancer, skin cancer, breast cancer, liver cancer, colon cancer, lung cancer, kidney cancer, prostate cancer, leukemia, brain cancer, and pancreas cancer cells.
57. The isolated antibody or fragment thereof of any one of claims 51-56, wherein the tumor or cancer cell comprises one, two, or more types of cancer cells selected from the group consisting of A549, A375, MCF-7, Hep3B, HCT-116, NCI-H358, 786-0, DU-145, MDA-MB-231, MV4-11, U251, CG-EMT, MIA-PaCa2, and PANC-1 cells.
58. The isolated antibody or fragment thereof of any one of claims 52-57, wherein the population of tumor or cancer cells comprises breast cancer cells.
59. The isolated antibody or fragment thereof of claim 58, wherein the breast cancer cells are MCF-7 or MDA-MB-231 cells.
60. The isolated antibody or fragment thereof of any one of claims 52-59, wherein the population of tumor or cancer cells comprises acute myeloid leukemia (AML) cells.
61. The isolated antibody or fragment thereof of claim 60, wherein the AML cells are MV4-11 cells.
62. The isolated antibody or fragment thereof of any one of claims 52-61, wherein the population of tumor or cancer cells comprises prostate cancer cells.
63. The isolated antibody or fragment thereof of claim 62, wherein the prostate cancer cells are DU-145 cells.
64. The isolated antibody or fragment thereof of claim 62, wherein the prostate cancer cells are CG-EMT cells.
65. The isolated antibody or fragment thereof of any one of claims 52-64, wherein the population of tumor or cancer cells comprises lung cancer cells.
66. The isolated antibody or fragment thereof of claim 65, wherein the lung cancer cells are A549 or NCI-H358 cells.
67. The isolated antibody or fragment thereof of any one of claims 52-66, wherein the population of tumor or cancer cells comprises skin malignant melanoma cells.
68. The isolated antibody or fragment thereof of claim 67, wherein the skin malignant melanoma cells are A375 cells.
69. The isolated antibody or fragment thereof of any one of claims 52-68, wherein the population of tumor or cancer cells comprises hepatocellular carcinoma cells.
70. The isolated antibody or fragment thereof of claim 69, wherein the hepatocellular carcinoma cells are Hep3B cells.

71. The isolated antibody or fragment thereof of any one of claims 52-70, wherein the population of tumor or cancer cells comprises colon cancer cells.
72. The isolated antibody or fragment thereof of claim 71, wherein the colon cancer cells are HCT-116 cells.
73. The isolated antibody or fragment thereof of any one of claims 52-72, wherein the population of tumor or cancer cells comprises renal cancer cells.
74. The isolated antibody or fragment thereof of claim 73, wherein the renal cancer cells are 786-0 cells.
75. The isolated antibody or fragment thereof of any one of claims 52-74, wherein the population of tumor or cancer cells comprises brain tumor cells.
76. The isolated antibody or fragment thereof of claim 75, wherein the brain tumor cells are U251 cells.
77. The isolated antibody or fragment thereof of any one of claims 52-76, wherein the population of tumor or cancer cells comprises pancreas cancer cells.
78. The isolated antibody or fragment thereof of claim 77, wherein the pancreas cancer cells are MIA-Paca2 or PANC-1 cells.
79. A recombinant cell that produces the isolated antibody or fragment thereof of any one of claims 1-78.
80. An isolated nucleic acid encoding the isolated antibody or fragment thereof of any one of claims 1-78.
81. A vector that comprises the nucleic acid of claim 80.
82. A host cell that comprises the nucleic acid of claim 80 or the vector of claim 81.
83. A method of producing an antibody or fragment thereof that comprises culturing the host cell of claim 82 whereby the antibody or fragment thereof is produced.
84. A pharmaceutical composition that comprises an effective amount of the isolated antibody or fragment thereof of any one of claims 1-78 and a pharmaceutically acceptable carrier.
85. The pharmaceutical composition of claim 84, wherein the isolated antibody or fragment thereof is a monoclonal antibody.
86. The pharmaceutical composition of claim 84, wherein the isolated antibody or fragment thereof is a polyclonal antibody.
87. A method of treating a cancer, comprising administering to a subject in need thereof a pharmaceutical composition that comprises the isolated antibody or fragment thereof of any one of claims 1-78.
88. The method of claim 87, wherein the administering is injection.

89. The method of claim 88, wherein the administering is intravenous or subcutaneous injection.
90. The method of any one of claims 87-89, wherein the administering occurs 1-3 times per week.
91. The method of any one of claims 87-90, wherein the method reduces a size of tumor in the subject by at least: 25%, 50%, 75%, or 95%.
92. The method of claim 91, wherein the tumor is a solid tumor.
93. The method of any one of claims 87-92, wherein the pharmaceutical composition is dosed from 0.15 mg to 3 mg per kg of body weight of the subject.
94. The method of claim 93, wherein the dose is from 0.5 mg to 2 mg per kg of body weight of the subject.
95. The method of any one of claims 87-94, wherein the subject is a mammal.
96. The method of claim 95, wherein the subject is a human.
97. The method of any one of claims 87-96, wherein the cancer comprises one, two, or more types of cancer selected from the group consisting of human lung cancer, skin cancer, breast cancer, liver cancer, colon cancer, lung cancer, kidney cancer, prostate cancer, leukemia, brain cancer, and pancreas cancer.
98. A method of killing cancer cells, comprising contacting with the cancer cells the isolated antibody or fragment thereof of any one of claims 1-78.
99. Use of the isolated antibody or fragment thereof of any one of claims 1-78 for treating cancer or killing cancer cells.
100. Use of the isolated antibody or fragment thereof of any one of claims 1-78 in the manufacture of a medicament.
101. The use of claim 100, wherein the medicament is for treatment of a cancer.
102. The use of claim 100, wherein the medicament is for killing cancer cells.
103. A recombinant mammalian cell line, wherein the recombinant mammalian cell line comprises one or more cells that comprise a first nucleic acid sequence of SEQ ID NO:1 and a second nucleic acid sequence of SEQ ID NO:10.
104. The recombinant mammalian cell line of claims 103, wherein the first nucleic acid sequence and the second nucleic acid sequence are in a same construct.
105. The recombinant mammalian cell line of claim 103 or 104, wherein the first nucleic acid sequence and the second nucleic acid sequence are recombinantly or synthetically produced and cloned into an expression vector.
106. The recombinant mammalian cell line of claim 105, wherein the expression vector is a pTT5 expression vector.

107. The recombinant mammalian cell line of any one of claims 103-106, wherein the first nucleic acid sequence and the second nucleic acid sequence are transfected into the one or more cells.
108. A method of activating an immune system in a human subject, comprising administering to the subject the isolated antibody or fragment thereof of any one of claims 1-78.
109. A method of treating cancer by inhibiting Transforming growth factor beta (TGFβ) in a human subject, comprising administering to the subject the isolated antibody or fragment thereof of any one of claims 1-78, whereby the TGFβ is inhibited and the cancer is treated.
110. The method of claim 109, wherein the TGFβ is TGFβ1, TGFβ2, or TGFβ3.
111. A method of treating a cancer by preventing stabilization of an oncogenic mRNA in a human subject, comprising administering to the subject the isolated antibody or fragment thereof of any one of claims 1-78, whereby the oncogenic mRNA is destabilized and the cancer is treated.
112. The method of claim 111, wherein the oncogenic mRNA is tumor protein p53 mRNA, B-cell lymphoma-extra large (Bcl-XL) mRNA, (B-cell lymphoma 2) Bcl-2 mRNA, gastrin mRNA, (growth arrest and DNA damage-inducible alpha) Gadd45α mRNA, matrix metallopeptidase 9 (MMP9) mRNA, Arabidopsis thaliana kinesin (Atk1) mRNA, Cyclin 1 mRNA, interleukin-2 (IL-2) mRNA, prostaglandin H synthase-1 (Pghs-1) mRNA, or any combination thereof.
113. A method of treating a cancer by reducing an expression level of an oncogenic protein in a human subject, comprising administering to the subject the isolated antibody or fragment thereof of any one of claims 1-78, whereby the expression level of the oncogenic protein is destabilized and the cancer is treated.
114. The method of claim 113, wherein the oncogenic protein is tumor protein p53, Bcl-xL, Bcl-2, gastrin, Gadd45α, MMP9, Atk1, Cyclin 1, IL-2, Pghs-1, or any combination thereof.
115. The method of any one of claims 109-114, wherein the cancer comprises one, two, or more types of cancers selected from the group consisting of human lung cancer, skin cancer, breast cancer, liver cancer, colon cancer, lung cancer, kidney cancer, prostate cancer, leukemia, brain cancer, and pancreas cancer.
116. An immunoconjugate, wherein the immunoconjugate comprises an antigen binding agent linked to a therapeutic agent, and wherein the antigen binding agent comprises the isolated antibody or fragment thereof of any one of claims 1-78.
117. The immunoconjugate of claim 116, wherein the immunoconjugate is a fusion protein, and wherein the therapeutic agent is a polypeptide.

118. The immunoconjugate of claim 116, wherein the antigen binding agent is a bispecific antibody.
119. The immunoconjugate of claim 116, wherein the antigen binding agent is a probody.
120. The immunoconjugate of claim 119, wherein the probody comprises an antigen-binding region that is activated by a tumor cell.
121. The immunoconjugate of claim 120, wherein the antigen-binding region comprises a peptide linked to the N-terminus of a light chain through a protease cleavable linker.
122. The immunoconjugate of any one of claims 116-121, wherein the antigen binding agent is linked covalently, noncovalently, or recombinantly to the therapeutic agent.
123. The immunoconjugate of any one of claims 116-122, wherein the therapeutic agent is a cytotoxic agent.
124. The immunoconjugate of claim 123, wherein the cytotoxic agent is doxorubicin, calicheamicin, auristatin, maytansinoid, brentuximab vedotin, tubulysins, duocarmycins, camptothecin, SN-38, pyrrolobenzodiazepine, methotrexate, α-amanitin, ansamitocin, or any combination thereof.
125. The immunoconjugate of any one of claims 116-122, wherein the therapeutic agent is an immune stimulating agent.
126. The immunoconjugate of claim 125, wherein the therapeutic agent is interleukin-2 (IL-2), an immunostimulatory nucleic acid molecule, granulocyte macrophage colony-stimulating factor, resiquimod, Gardiquimod, phycocyanobilin, romiplostim, eltrombopag, or any combination thereof.
127. A pharmaceutical composition that comprises the immunoconjugate of any one of claims 116-126 and a pharmaceutically acceptable carrier.
128. A method of treating a cancer, comprising administering to a subject in need thereof a pharmaceutical composition that comprises the immunoconjugate of any one of claims 116-126.
129. Use of the immunoconjugate of any one of claims 116-126 for treating a cancer.
130. Use of the immunoconjugate of any one of claims 116-126 in the manufacture of a medicament for treatment of a cancer.

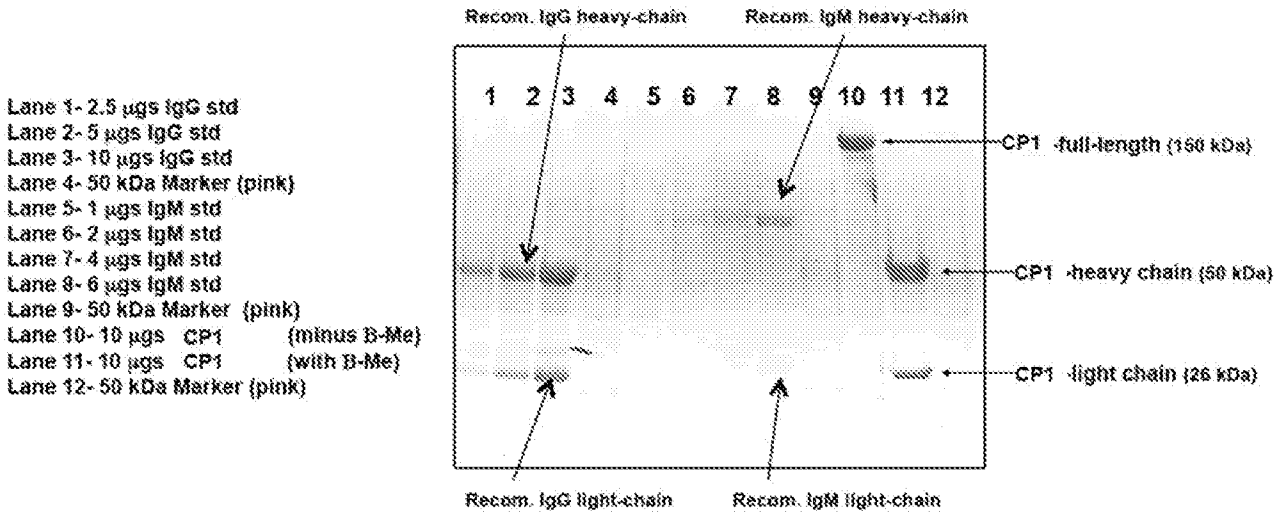

Lane 1- 2.5 µgs IgG std
Lane 2- 5 µgs IgG std
Lane 3- 10 µgs IgG std
Lane 4- 50 kDa Marker (pink)
Lane 5- 1 µgs IgM std
Lane 6- 2 µgs IgM std
Lane 7- 4 µgs IgM std
Lane 8- 6 µgs IgM std
Lane 9- 50 kDa Marker (pink)
Lane 10- 10 µgs CP1 (minus B-Me)
Lane 11- 10 µgs CP1 (with B-Me)
Lane 12- 50 kDa Marker (pink)
Recom. IgG heavy-chain
Recom. IgM heavy-chain
1 2 3 4 5 6 7 8 9 10 11 12
CP1 -full-length (150 kDa)
CP1 -heavy chain (50 kDa)
CP1 -light chain (26 kDa)
Recom. IgG light-chain
Recom. IgM light-chain

FIG. 1

Lane-1- Marker (pre-stained)
Lane-2-IgG-5.0 ugs IgG (-)ME
Lane-3-IgG-2.5 ugs + ME
Lane-4-IgG-5.0 ugs + ME
Lane-5-IgG-10 ugs+ME
Lane-6- Marker
Lane -7-IgM-3.5 ugs (-)ME
Lane-8-IgM-1.75ugs+ME
Lane-9-IgM-3.50 ugs +ME
Lane-10-IgM-7.0 ugs+ME
Lane-11-marker
Lane-12-CP1- 1.170 ml after dialysis and 200 ul concentrated to 100 ul and 25 ul loaded (-) ME
Lane-13-CP1-25 ul of concentrated loaded (+) ME
Lane-14-Marker

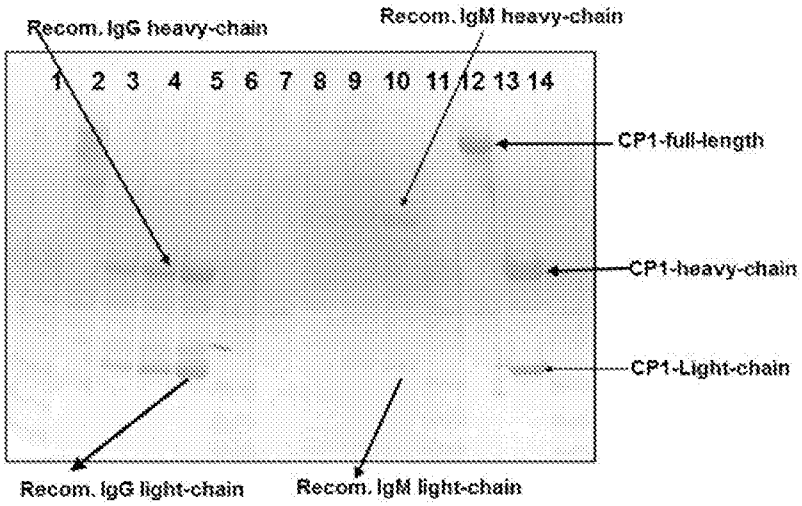


FIG. 2

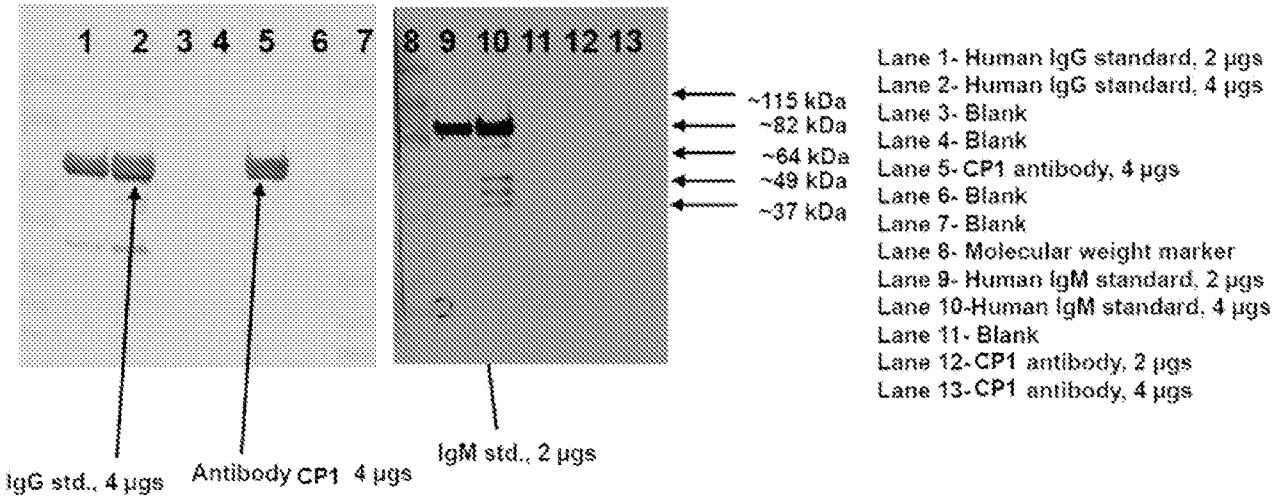

1 2 3 4 5 6 7
8 9 10 11 12 13
~115 kDa
~82 kDa
~64 kDa
~49 kDa
~37 kDa
Lane 1- Human IgG standard, 2 µgs
Lane 2- Human IgG standard, 4 µgs
Lane 3- Blank
Lane 4- Blank
Lane 5- CP1 antibody, 4 µgs
Lane 6- Blank
Lane 7- Blank
Lane 8- Molecular weight marker
Lane 9- Human IgM standard, 2 µgs
Lane 10-Human IgM standard, 4 µgs
Lane 11- Blank
Lane 12-CP1 antibody, 2 µgs
Lane 13-CP1 antibody, 4 µgs
IgG std., 4 µgs
Antibody CP1 4 µgs
IgM std., 2 µgs

FIG. 3

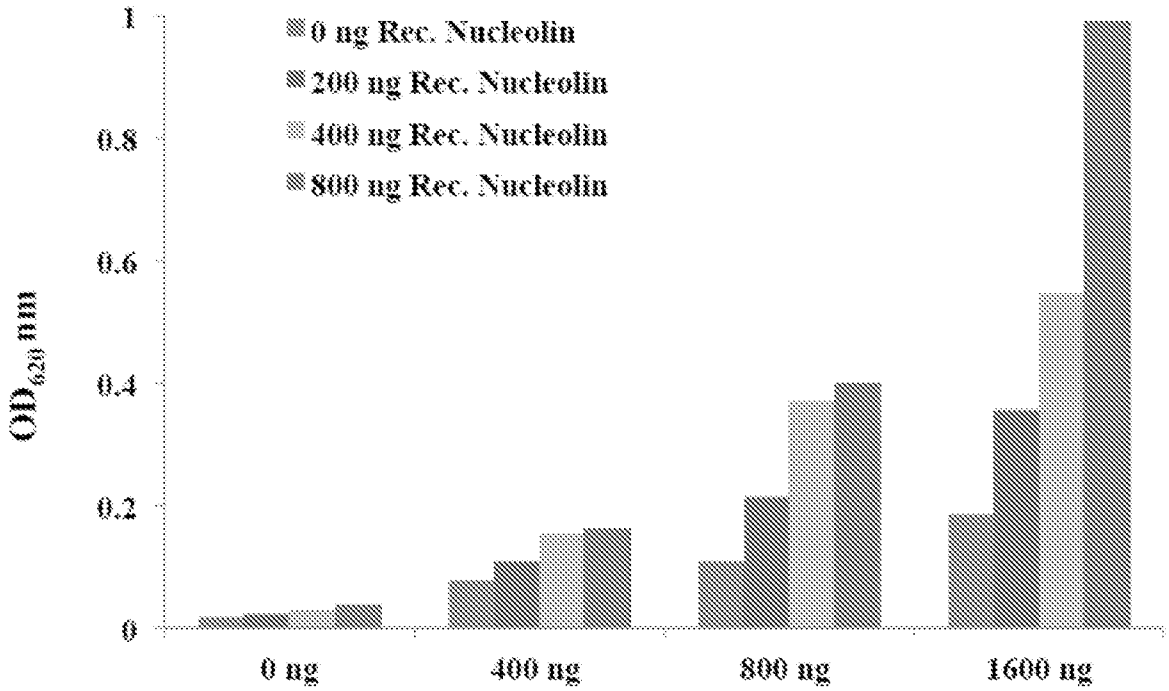

0 ng Rec. Nucleolin
200 ng Rec. Nucleolin
400 ng Rec. Nucleolin
800 ng Rec. Nucleolin
OD620 nm
1
0.8
0.6
0.4
0.2
0
0 ng
400 ng
800 ng
1600 ng

FIG. 4

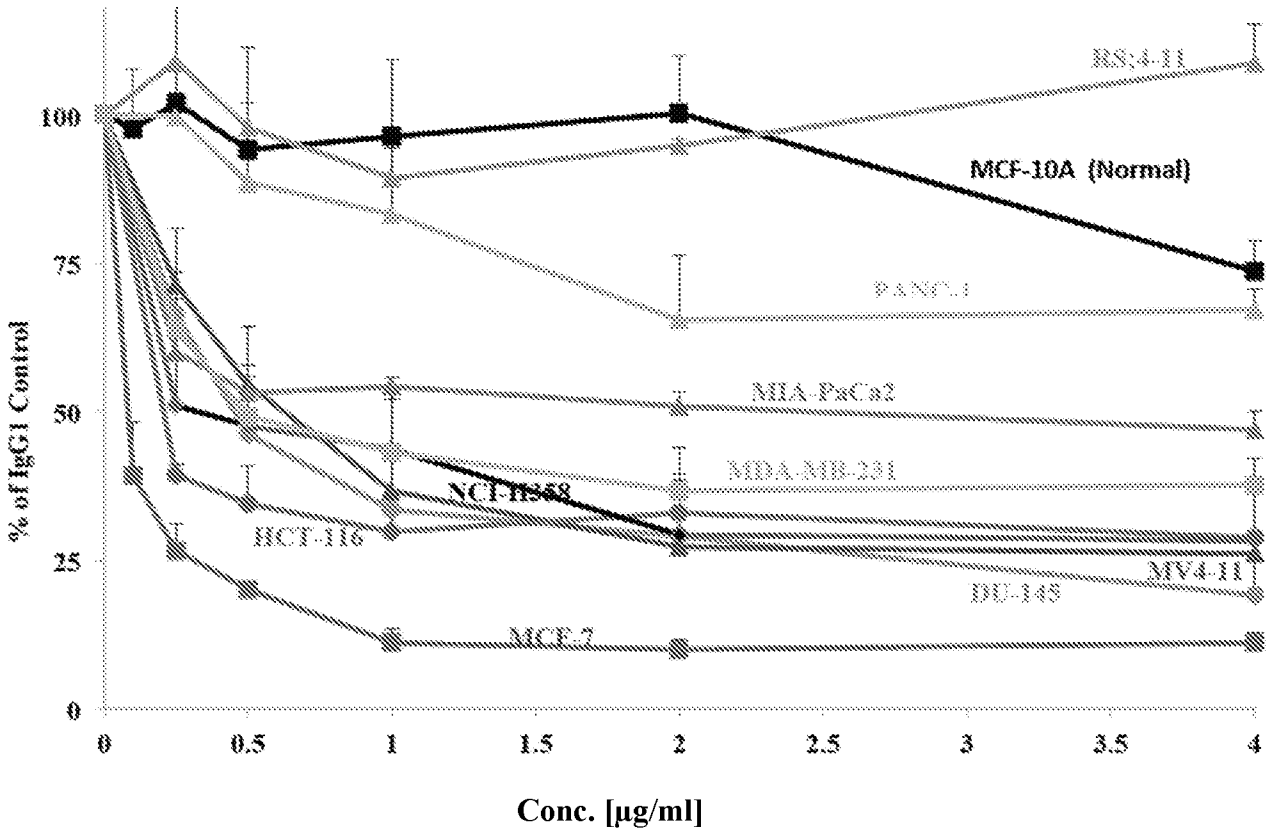

RS;4-11
MCF-10A (Normal)
PANC-1
MIA-PaCa2
MDA-MB-231
NCI-H358
HCT-116
MV4-11
DU-145
MCF-7
100
75
50
25
0
% of IgG1 Control
0
0.5
1
1.5
2
2.5
3
3.5
4
Conc. [µg/ml]

FIG. 5

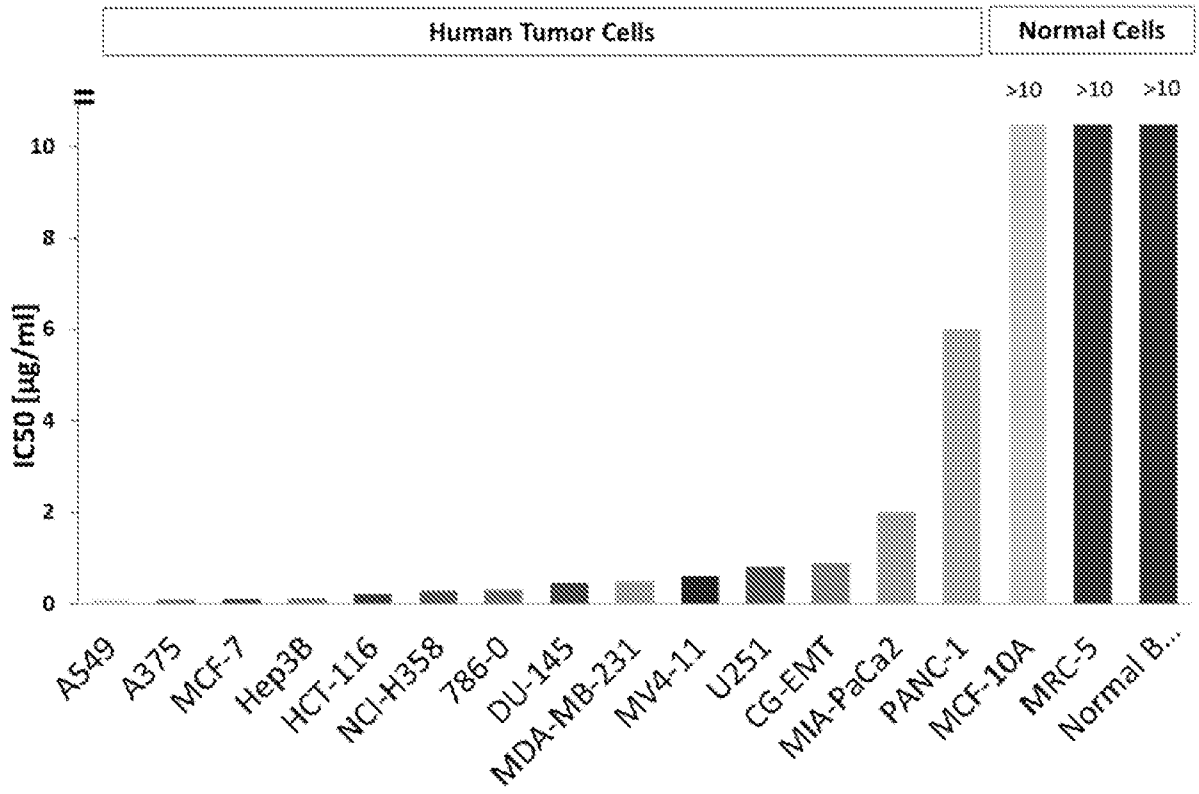

Human Tumor Cells
Normal Cells
IC50 [µg/ml]
10
8
6
4
2
0
>10
>10
>10
A549
A375
MCF-7
Hep3B
HCT-116
NCI-H358
786-0
DU-145
MDA-MB-231
MV4-11
U251
CG-EMT
MIA-PaCa2
PANC-1
MCF-10A
MRC-5
Normal B...

FIG. 6

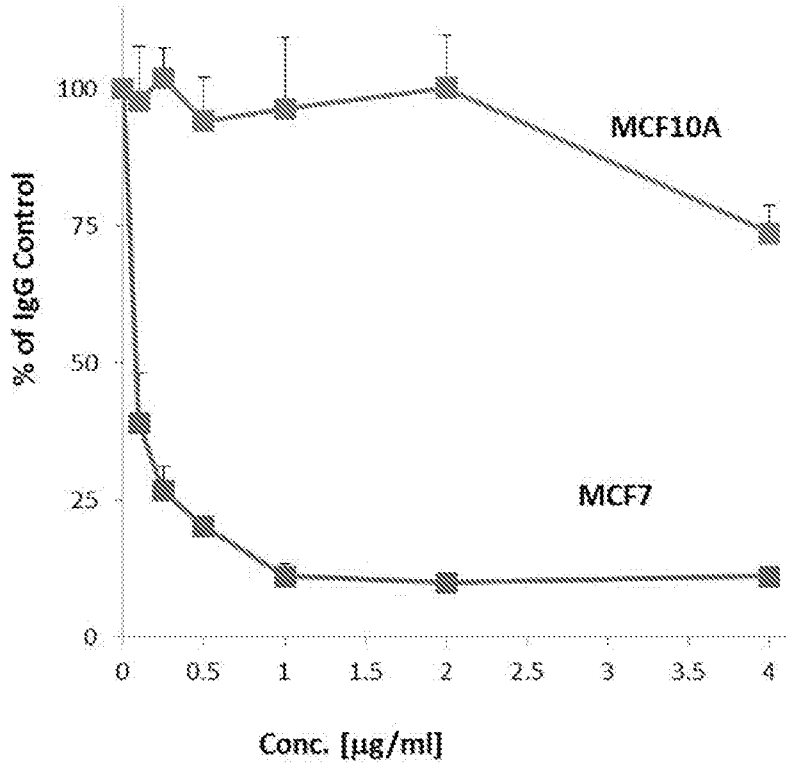

100
75
50
25
0
% of IgG Control
MCF10A
MCF7
0
0.5
1
1.5
2
2.5
3
3.5
4
Conc. [µg/ml]

FIG. 7

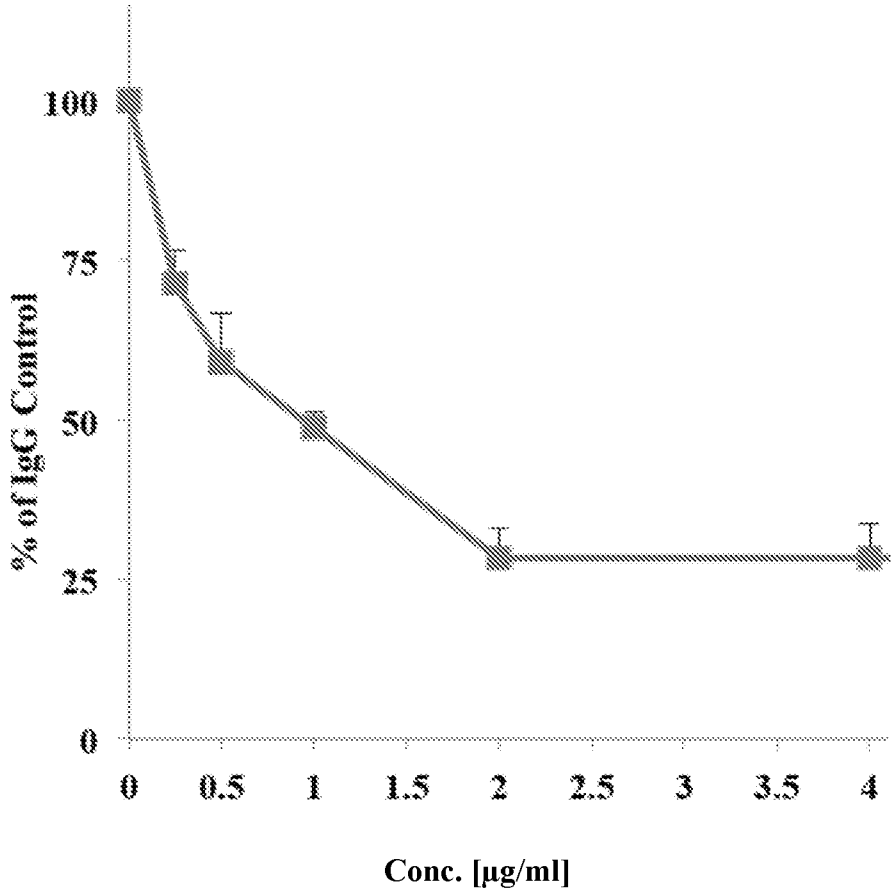


FIG. 8

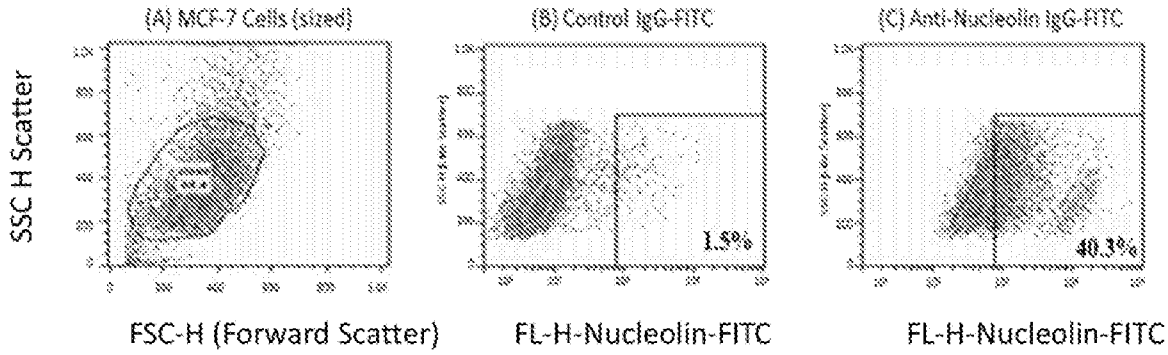

(A) MCF-7 Cells (sized)
(B) Control IgG-FITC
(C) Anti-Nucleolin IgG-FITC
SSC H Scatter
1.5%
40.3%
FSC-H (Forward Scatter)
FL-H-Nucleolin-FITC
FL-H-Nucleolin-FITC

FIG. 9

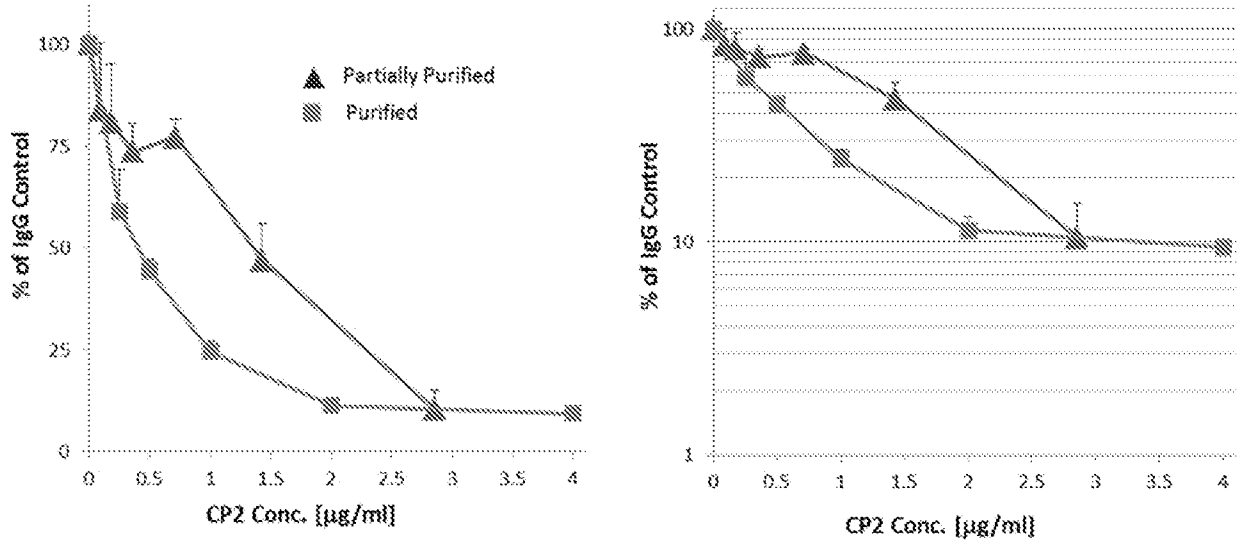
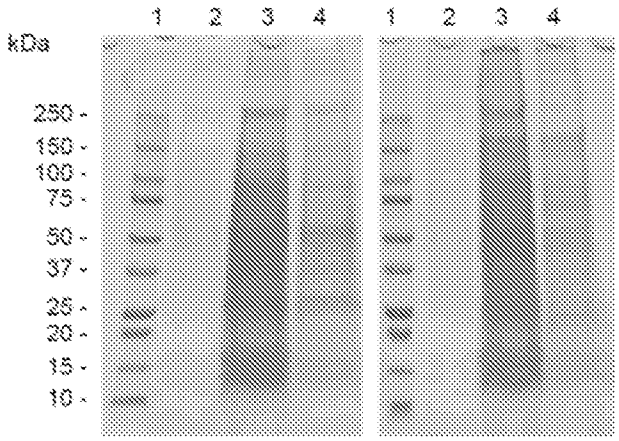
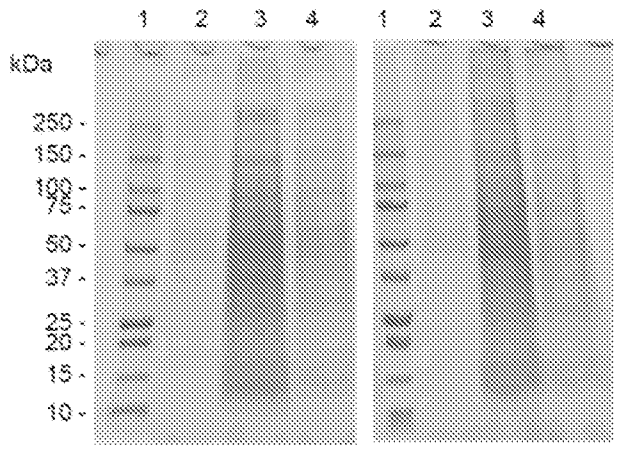

100
75
50
25
0
% of IgG Control
Partially Purified
Purified
0 0.5 1 1.5 2 2.5 3 3.5 4
CP2 Conc. [µg/ml]
100
10
1
% of IgG Control
0 0.5 1 1.5 2 2.5 3 3.5 4
CP2 Conc. [µg/ml]

FIG. 10



1. MW markers
2. CHO3E7 negative control CCS
3. CP1 gamma heavy chain / kappa light chain CHO3E7 CCS
4. CP1 gamma heavy chain / kappa light chain (second transfection) CHO3E7 CCS



1. MW markers
2. CHO3E7 negative control CCS
3. CP1 gamma heavy chain / lambda light chain CHO3E7 CCS
4. CP1 gamma heavy chain / lambda light chain (second transfection) CHO3E7 CCS

FIG. 11

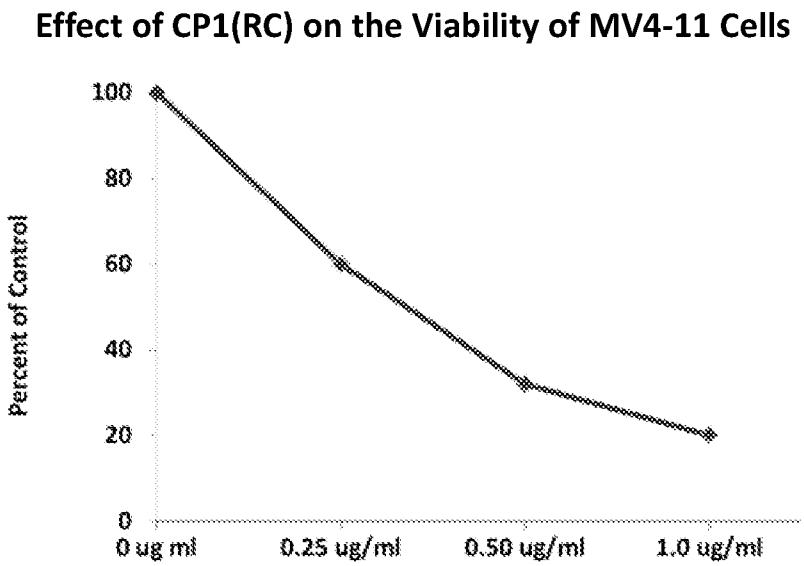

Effect of CP1(RC) on the Viability of MV4-11 Cells
Percent of Control
100
80
60
40
20
0
0 ug ml
0.25 ug/ml
0.50 ug/ml
1.0 ug/ml

FIG. 12

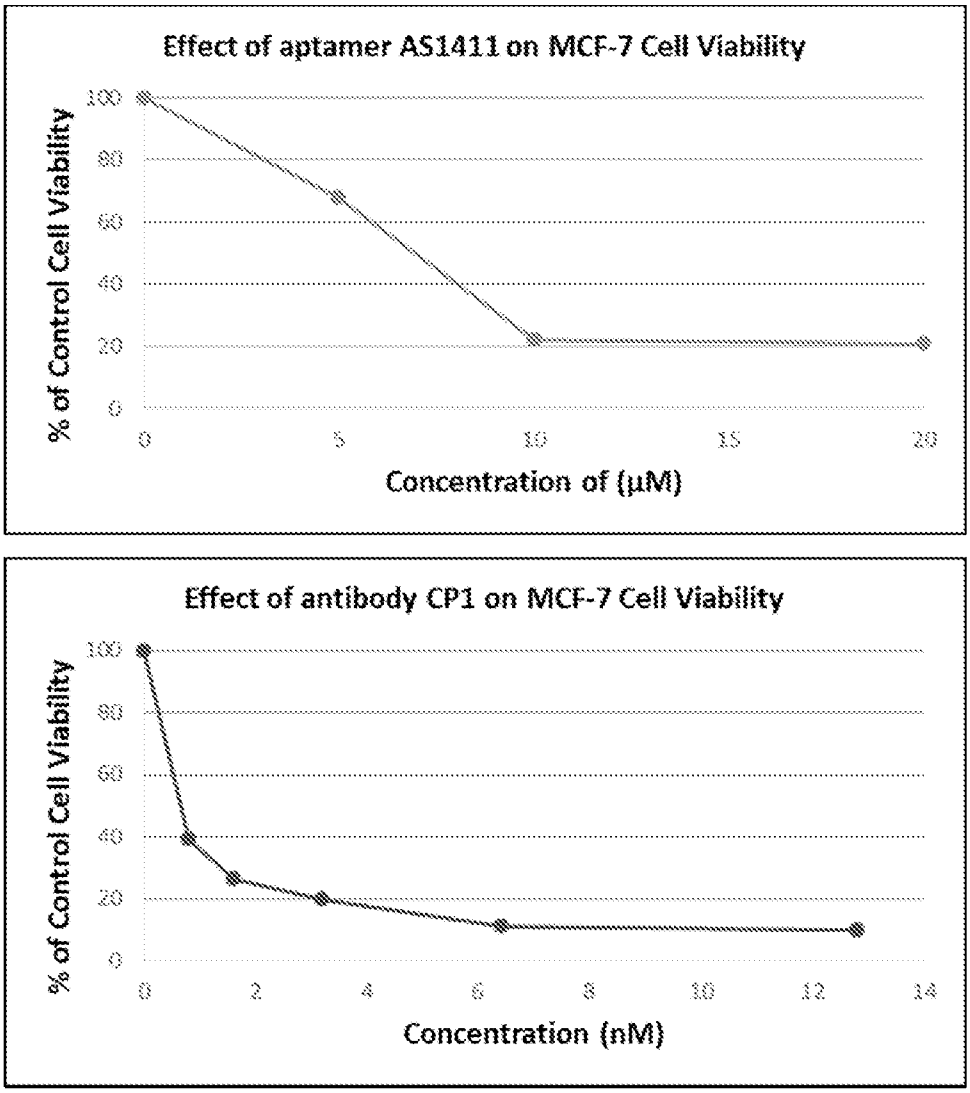

Effect of aptamer AS1411 on MCF-7 Cell Viability
% of Control Cell Viability
100
80
60
40
20
0
0
5
10
15
20
Concentration of (μM)
Effect of antibody CP1 on MCF-7 Cell Viability
% of Control Cell Viability
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Concentration (nM)

FIG. 13

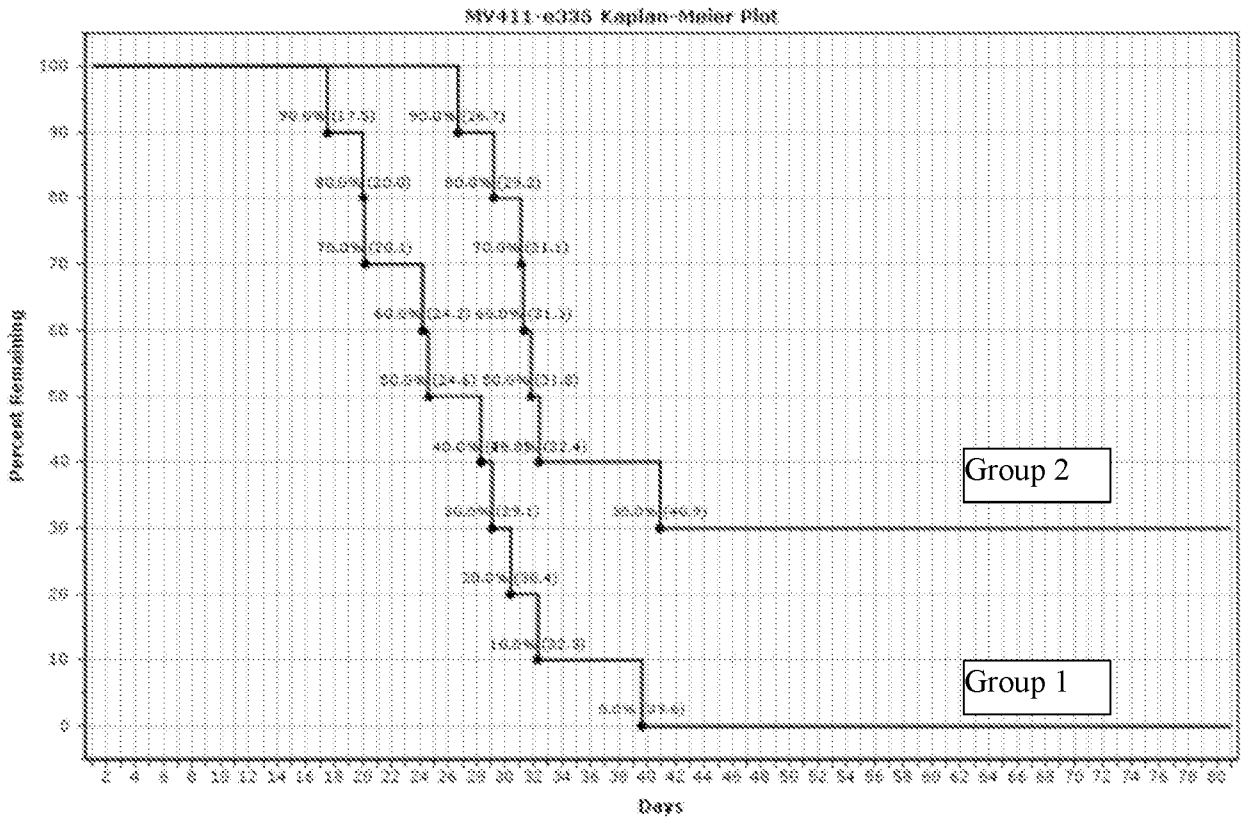
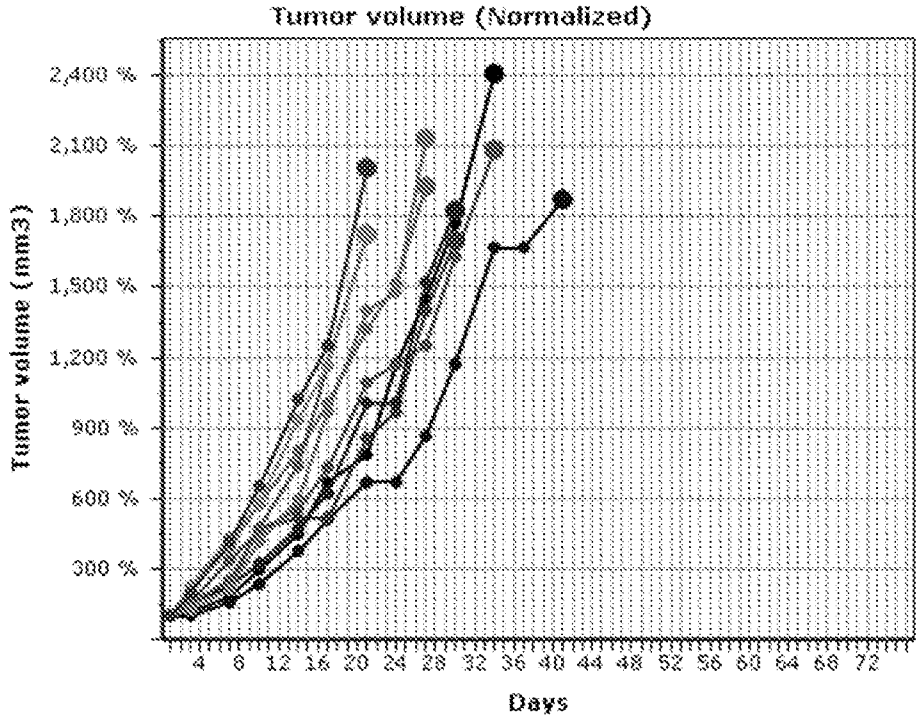
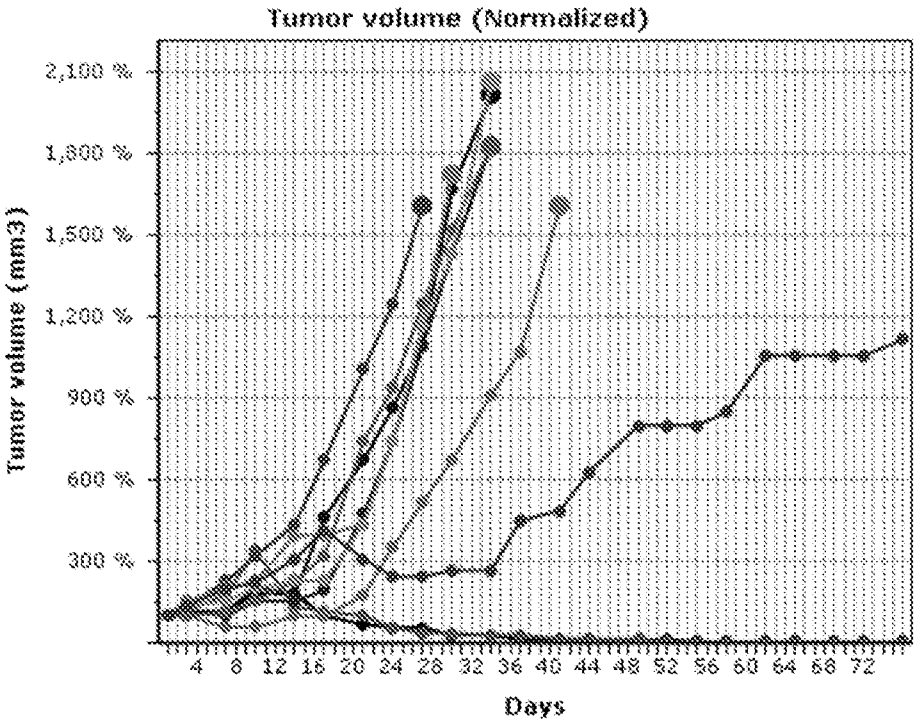

MV411-e335 Kaplan-Meier Plot
Percent Remaining
Days
Group 2
Group 1

FIG. 14



Group 1

FIG. 15A.



Mouse # 10

Mice # 1, # 3

Group 2

FIG. 15B.

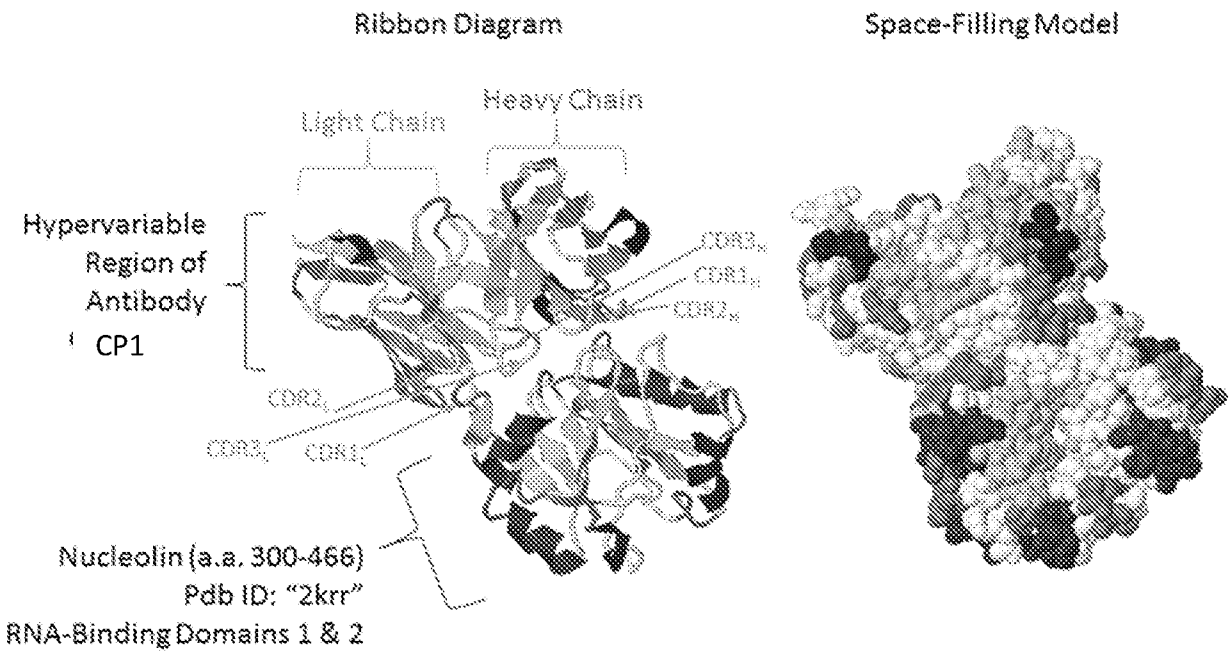

Ribbon Diagram
Space-Filling Model
Light Chain
Heavy Chain
Hypervariable Region of Antibody
CP1
Nucleolin (a.a. 300-466)
Pdb ID: "2krr"
RNA-Binding Domains 1 & 2

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/021203

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/30 (2006.01) A61K 39/395 (2006.01)

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)

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

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

EPODOC, WPIAP, MEDLINE, BIOSIS, CAPLUS, EMBASE (nucleolin, c23 protein, NCL, antibody, cancer, treat and similar terms)

GENOMEQUEST (SEQ ID NOs: 2, 3, 11, 12, 14, 17 AND 19 and CDR motif search at 100% sequence identity)

PATENTSCOPE, AUSPAT, GOOGLE, GOOGLE ADVANCED PATENT SEARCH, GOOGLE SCHOLAR, ESPACENET, PUBMED, IPONZ (CHARLESTONPHARMA, LLC; MUSC FOUNDATION FOR RESEARCH DEVELOPMENT; FERNANDES, D; SCHWARTZ, L; SUTKOWSKI, N; HOEL, B; RUBINCHIK, S.)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] 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

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Date of the actual completion of the international search	Date of mailing of the international search report
15 May 2017	15 May 2017

Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Anita Premkumar AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 0262832013

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