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(54) Title: ENGINEERED ANTIBODY-INTERFERON FUSION MOLECULES FOR THE TREATMENT OF GLUCOSE-REGULATED PROTEIN 94 (GRP94) EXPRESSING CANCERS

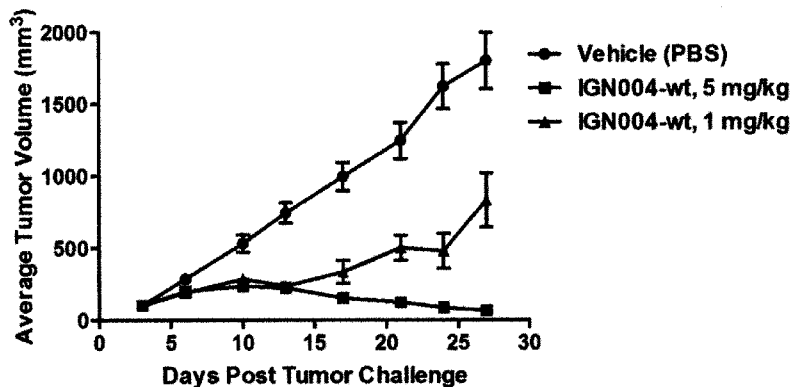


FIG. 8

(57) Abstract: The present invention relates to methods of treating a cancer that expresses or overexpresses GRP94 in an individual, comprising administering to the individual a therapeutically effective amount of an isolated non-naturally occurring fusion molecule comprising an antibody or antigen-binding fragment that binds GRP94 ("GRP94 Ab") attached to an interferon (IFN) molecule (hereinafter "GRP94 Ab-IFN fusion molecule"), as monotherapy at therapeutically effective low doses, or in combination with immunotherapy, wherein the combination therapy provides increased effector cell killing. The methods of the present invention are particularly effective treating recurrent, resistant, or refractory GRP94 expressing cancers.



ENGINEERED ANTIBODY-INTERFERON FUSION MOLECULES
FOR THE TREATMENT OF GLUCOSE-REGULATED PROTEIN 94 (GRP94)
EXPRESSING CANCERS

RELATED PATENT APPLICATIONS

[001] This application claims benefit of U.S. Provisional Application No. 62/175,024, filed on June 12, 2015, incorporated in its entirety by reference herein.

TECHNICAL FIELD

[002] Glucose-regulated protein 94 (GRP94) (also known as endoplasmin) is a member of the HSP90 family that plays an important role in the biology of malignant cells and which has been shown to be expressed or overexpressed on many types of malignant cells including, but not limited to, melanoma, breast cancer, renal cancer, glioma, colorectal cancer, hepatocellular cancer, lung cancer (small cell lung cancer and non-small cell lung cancer), ovarian cancer; pancreatic cancer, prostate cancer, esophageal cancer, gastric cancer, urinary bladder cancer; head and neck cancer, multiple myeloma, acute leukemias (e.g., 11q23-positive acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma (indolent and high grade forms), Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia. It has also been shown that GRP94 has an anti-apoptotic effect on some tumor cells.

[003] Multiple myeloma is a hematological malignancy known to affect approximately 50,000 patients in the United States, with approximately 15,000 new patients diagnosed annually. Multiple myeloma is characterized by the clonal proliferation and accumulation of immunoglobulin-producing plasma B cells in bone marrow, causing the progressive destruction of bone tissue and bone marrow. Other features of multiple myeloma include: low blood counts (e.g., red blood cells, platelets, and white blood cells), bone and calcium problems, infections, kidney problems). If left untreated, the condition ultimately leads to the death of the patient.

[004] Currently available therapies for multiple myeloma include chemotherapy, stem cell transplantation, Thalomid® (thalidomide), Velcade® (bortezomib), Kyprolis® (carfilzomib),

Aredia® (pamidronate), and Zometa® (zoledronic acid). Current treatment protocols, which include a combination of chemotherapeutic agents such as vincristine, BCNU, melphalan, cyclophosphamide, adriamycin, and prednisone or dexamethasone, yield a complete remission rate of only about 5%, and median survival is approximately 36-48 months from the time of diagnosis. Recent advances using high dose chemotherapy followed by autologous bone marrow or peripheral blood mononuclear cell transplantation have increased the complete remission rate and remission duration. Yet overall survival has only been slightly prolonged, and no evidence for a cure has been obtained. Ultimately, all MM patients relapse, even under maintenance therapy with interferon-alpha (IFN- α) alone or in combination with steroids.

[005] Acute myelogenous leukemia (AML), also known as acute myeloid leukemia, acute myeloblastic leukemia, acute granulocytic leukemia and acute nonlymphocytic leukemia is another hematological malignancy. AML affects a group of white blood cells called the myeloid cells, which normally develop into the various types of mature blood cells, such as red blood cells, white blood cells and platelets. AML is characterized by an increase in the number of myeloid cells in the marrow and an arrest in their maturation, frequently resulting in hematopoietic insufficiency (granulocytopenia, thrombocytopenia, or anemia), with or without leukocytosis. The American Cancer Society's estimates for leukemia in the United States for 2015 are about 54,270 new cases of leukemia (all kinds) and 24,450 deaths from leukemia (all kinds); about 20,830 new cases of AML (most will be in adults) and about 10,460 deaths from AML (almost all will be in adults). AML is slightly more common among men than among women, but the average lifetime risk in both sexes is less than $\frac{1}{2}$ of 1%.

[006] Adult AML is not a single disease, but rather a group of related diseases, and patients with different subtypes of AML can have different outlooks and responses to treatment. Depending upon the subtype, several types of treatment may be used for people with AML. Treatment of AML is usually divided into phases: 1) the "remission induction phase" where the goal is to clear the blood of leukemia cells (blasts) and to reduce the number of blasts in the bone marrow to normal; 2) the "consolidation phase" where chemo is given after the patient has recovered from induction and which is intended to kill the small number of leukemia cells that are still present but can't be seen (because there are so few of them); and 3) a "maintenance phase" which involves giving a low dose of chemo for months or years after consolidation is finished. The maintenance phase is most often used for the M3 subtype of AML (also known as acute promyelocytic leukemia, or APL), but is rarely used for other types of AML. Arsenic trioxide (Trisenox) and all-trans retinoic acid (ATRA) are two anti-cancer drugs that can be used

alone or in combination with chemotherapy for remission induction of APL. These drugs cause leukemia cells with a specific gene mutation to mature and die, or to stop dividing.

[007] In spite of all of the recent advances made in the treatment of cancers such as multiple myeloma and AML, the diseases remains largely incurable due, in part, to the low cell proliferation rate and development of tumor cell resistance to conventional therapies. Accordingly, there is currently no known cure for the diseases. As a result, alternative treatment regimens aimed at adoptive immunotherapy targeting surface antigens on tumor cells are being sought.

DISCLOSURE OF THE INVENTION

[008] In one aspect, the present invention relates to a method of treating a cancer that expresses or overexpresses glucose-regulated protein 94 (GRP94) in an individual, said method comprising administering to the individual an isolated non-naturally occurring fusion molecule comprising an antibody or antigen-binding fragment that binds GRP94 ("GRP94 Ab") attached to an interferon (IFN) molecule (hereinafter "GRP94 Ab-IFN fusion molecule"), wherein the GRP94 Ab-IFN fusion molecule is administered to the individual at a dosage (e.g., at a weekly dosage) included in any of the following ranges: about 0.0001 to about 0.0003 mg/kg, about 0.0003 to about 0.001 mg/kg, about 0.001 to about 0.003 mg/kg, about 0.003 to about 0.01 mg/kg, about 0.01 to about 0.03 mg/kg, about 0.03 to about 0.1 mg/kg, about 0.1 to about 0.3 mg/kg, about 0.3 to about 0.4 mg/kg, about 0.4 to about 0.5 mg/kg, about 0.5 to about 0.6 mg/kg, about 0.6 to about 0.7 mg/kg, about 0.7 to about 0.8 mg/kg, and about 0.8 to about 0.9 mg/kg. In various embodiments, the GRP94 Ab-IFN fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of about .0001 mg/kg, about .0003 mg/kg, about .001 mg/kg, about .003 mg/kg, about .01 mg/kg, about .03 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, and about 0.9 mg/kg. In various embodiments, the GRP94 Ab-IFN fusion molecule is administered to the individual at a dosage (e.g., at a weekly dosage) of no greater than about any of: .0001 mg/kg, .0003 mg/kg, .001 mg/kg, .003 mg/kg, .01 mg/kg, .03 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, and 0.9 mg/kg.

[009] In various embodiments, the fusion molecules comprise an antibody or antigen-binding fragment that specifically binds glycosylated GRP94 on the surface of a tumor cell. Thus, the antibody or antigen-binding fragment does not bind non-glycosylated GRP94 or

unrelated antigen. In various embodiments, the antibody or antigen-binding fragment binds to a GRP94 antigen with a dissociation constant (K_D) of at least about 1×10^{-3} M, at least about 1×10^{-4} M, at least about 1×10^{-5} M, at least about 1×10^{-6} M, at least about 1×10^{-7} M, at least about 1×10^{-8} M, at least about 1×10^{-9} M, at least about 1×10^{-10} M, at least about 1×10^{-11} M, or at least about 1×10^{-12} M.

[010] In various embodiments, the GRP94 Ab-IFN fusion molecules comprise an GRP94 Ab selected from group consisting of a fully human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, an antigen-binding antibody fragment, a Fab, a Fab', a Fab₂, a Fab'₂, a IgG, a IgM, a IgA, a IgE, a scFv, a dsFv, a dAb, a nanobody, a unibody, or a diabody. In various embodiments, the GRP94 Ab is a fully human anti-GRP94 Ab. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a monoclonal antibody (mAb), wherein the heavy chain of the antibody comprises one or more of the amino acid sequences set forth as amino acids 26-33 of SEQ ID NO: 3 (CDR1), amino acids 51-58 of SEQ ID NO: 3 (CDR2), and amino acids 97-103 of SEQ ID NO: 3 (CDR3). In various embodiments, the antibody can be a monoclonal antibody, wherein the heavy chain of the antibody comprises the amino acid sequence set forth as amino acids 26-33 of SEQ ID NO: 3 (CDR1), amino acids 51-58 of SEQ ID NO: 3 (CDR2), and amino acids 97-103 of SEQ ID NO: 3 (CDR3) and wherein the light chain of the antibody comprises the amino acid sequence set forth as amino acids 27-32 of SEQ ID NO: 4 (CDR1), amino acids 50-52 of SEQ ID NO: 4 (CDR2), and amino acids 89-97 of SEQ ID NO: 4 (CDR3). In various embodiments, the antibody is a monoclonal antibody comprising the heavy chain variable region of SEQ ID NO: 3 and the light chain variable region of SEQ ID NO: 4.

[011] In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a type 1 interferon molecule. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a type 1 interferon mutant molecule. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises an interferon-alpha (IFN- α) molecule. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a human IFN- α 2b molecule having the amino acid sequence of SEQ ID NO: 5. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a human IFN- α 2b mutant molecule having the amino acid sequence of SEQ ID NO: 6. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a human IFN- α 14 molecule having the amino acid sequence of SEQ ID NO: 7. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises an interferon-beta (IFN- β) molecule. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a human IFN- β -1a molecule having the amino acid sequence of SEQ ID NO: 8. In various embodiments, the GRP94 Ab-IFN fusion molecule

comprises a human IFN- β -1b molecule having the amino acid sequence of SEQ ID NO: 9. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a human IFN- α 2 molecule and a GRP94 Ab that binds a glycosylated GRP94 antigen on the surface of a tumor cell. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a human IFN- α 2 mutant molecule and a GRP94 Ab that binds a glycosylated GRP94 antigen on the surface of a tumor cell. In various embodiments, the GRP94 Ab-IFN fusion molecule comprises a human IFN- α 14 molecule and a GRP94 Ab that binds a glycosylated GRP94 antigen on the surface of a tumor cell.

[012] In various embodiments, the fusion molecules comprise an interferon molecule that is directly attached to the antibody or antigen-binding fragment.

[013] In various embodiments, the fusion molecules comprise an interferon molecule that is attached to the antibody or antigen-binding fragment via a proteolysis resistant peptide linker. In various embodiments, the proteolysis resistant peptide linker is fewer than 20 amino acids in length. In various embodiments, the proteolysis resistant linker is selected from SGGGGS (SEQ ID NO: 10) and AEAAAKEAAKAGS (SEQ ID NO: 11).

[014] In various embodiments, the fusion molecule is a recombinantly expressed fusion molecule.

[015] In various embodiments, the cancer is a cancer that expresses or overexpresses GRP94, selected from the group consisting of: melanoma, breast cancer, renal cancer, glioma, colorectal cancer, hepatocellular cancer, lung cancer (small cell lung cancer and non-small cell lung cancer), ovarian cancer; pancreatic cancer, prostate cancer, esophageal cancer, gastric cancer, urinary bladder cancer; head and neck cancer, multiple myeloma, acute leukemias (e.g., 11q23-positive acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma (indolent and high grade forms), Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

[016] In various embodiments, the present invention relates to a method of treating multiple myeloma in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater

than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[017] In various embodiments, the present invention relates to a method of treating AML in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[018] In various embodiments, the present invention relates to a method of treating non-small cell lung cancer (NSCLC) in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[019] In various embodiments, the present invention relates to a method of treating melanoma in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than

0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[020] In various embodiments, the present invention relates to a method of treating pancreatic cancer in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[021] In another aspect, the present invention provides a method of inhibiting the growth and/or proliferation of a tumor cell that expresses or overexpresses GRP94 in an individual, said method comprising administering to the individual a non-naturally occurring fusion molecule in an amount sufficient to treat said tumor cell, wherein the fusion molecule comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94 ("GRP94 Ab-IFN fusion molecule"), and wherein the GRP94 Ab-IFN fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[022] In another aspect, the present invention provides a combination therapy method of treating a cancer that expresses or overexpresses GRP94 in an individual, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising an anti-GRP94 (endoplasmic) Ab-IFN- α fusion molecule; and b) immunotherapy; wherein the combination therapy provides increased effector cell killing. In various embodiments, the immunotherapy is treatment using agonistic, antagonistic, or blocking antibodies to co-stimulatory or co-inhibitory molecules. In various embodiments, the immunotherapy is treatment using chimeric antigen receptor (CAR)-T cells. In various embodiments, the immunotherapy is treatment using CAR-NK cells. In various embodiments, the immunotherapy is treatment using bispecific T cell engaging antibodies (BiTE®). In various

embodiments, the cancer is selected from the group consisting of NSCLC, acute myeloid leukemia (AML), multiple myeloma, melanoma, and pancreatic cancer. In various embodiments, the immunotherapy will target a tumor associated antigen that is not GRP94.

[023] In another aspect, the present invention provides a pharmaceutical composition which comprises a GRP94 Ab-IFN fusion molecule and a second cancer immunotherapy agent, formulated for administration via a route selected from the group consisting of subcutaneous injection, intraperitoneal injection, intramuscular injection, intrasternal injection, intravenous injection, intraarterial injection, intrathecal injection, intraventricular injection, intraurethral injection, intracranial injection, intrasynovial injection or via infusions.

[024] In other aspects, the present invention provides polynucleotides that encode the fusion molecules of the present invention; vectors comprising polynucleotides encoding fusion molecules of the invention; optionally, operably-linked to control sequences recognized by a host cell transformed with the vector; host cells comprising vectors comprising polynucleotides encoding fusion molecules of the invention; a process for producing a fusion molecule of the invention comprising culturing host cells comprising vectors comprising polynucleotides encoding fusion molecules of the invention such that the polynucleotide is expressed; and, optionally, recovering the fusion molecule from the host cell culture medium.

BRIEF DESCRIPTION OF THE DRAWINGS

[025] Figure 1 is a schematic diagram of an exemplary GRP94 antibody-IFN fusion molecule.

[026] Figure 2 shows the STAT1 phosphorylation and proliferation inhibition activities of IGN004-wt compared to non-fused IFN- α 2b in a non-targeted and a targeted setting using Daudi NHL tumor cells (GRP94-negative) and NCI-H1299 NSCLC tumor cells (GRP94-positive).

[027] Figure 3 depicts the expression of the GRP94 on the surface of three multiple myeloma cell lines, NCI-H929, MM.1s, and U266.

[028] Figure 4 depicts the expression of GRP94 on the surface of four AML cell lines, KG-1, AML-193, Kasumi-1, and Kasumi-6.

[029] Figure 5 shows the anti-proliferative activity of various IGN004 fusion molecules versus non-fused IFN- α 2b-wt in three different multiple myeloma cell lines. The ability of the fusion proteins to inhibit the proliferation of MM.1s, U266, and NCI-H929 multiple myeloma cells

was assessed by MTS assay using human tumor cell lines incubated for 96 hours with titrated concentrations of the various IGN004 fusion molecules or non-fused rIFN- α 2b-wt.

[030] Figure 6 shows the anti-proliferative activity of various IGN004 fusion molecules versus non-fused IFN- α 2b-wt in three different AML cell lines. The ability of the fusion proteins to inhibit the proliferation of AML-193, KG-1, and Kasumi-6 AML cells was assessed by MTS assay using human tumor cell lines incubated for 96 hours with titrated concentrations of the various IGN004 fusion molecules or non-fused rIFN- α 2b-wt.

[031] Figure 7 shows the *in vivo* anti-tumor efficacy of mAb W9 and IGN004-wt fusion molecule in the U266 human multiple myeloma xenograft tumor model. Groups of 8 NOG immunodeficient mice bearing 11-day established subcutaneous U266 human multiple myeloma xenograft tumors were treated with vehicle (PBS), mAb W9, or IGN004-wt fusion molecule at 5, 1, or 0.2 mg/kg intravenously twice per week for 4 weeks. Tumors were measured bidirectionally using calipers and tumor volume calculated as $0.5 \times (L \times W^2)$. Animals were followed for survival and sacrificed when their tumors reached 2000 mm³. Figure 6A shows the average tumor volume \pm SEM of animals treated with PBS vehicle or mAb W9. Figure 6B shows the average tumor volume \pm SEM of animals treated with PBS vehicle or IGN004-wt. Figure 6C shows the survival curve of all groups.

[032] Figure 8 shows the *in vivo* anti-tumor efficacy of IGN004-wt fusion molecule in the KG-1 human AML xenograft tumor model. Groups of 8 NOD-SCID immunodeficient mice bearing 4-day established subcutaneous KG-1 human AML xenograft tumors were treated with vehicle (PBS) or IGN004-wt fusion molecule at 5 or 1 mg/kg intravenously twice per week for 4 weeks. Tumors were measured bidirectionally using calipers and tumor volume calculated as $0.5 \times (L \times W^2)$ and displayed as the average \pm SEM.

[033] Figure 9 shows the *in vivo* anti-tumor efficacy of IGN004-wt fusion molecule in the AML patient-derived xenograft tumor model designated as FLT3-ITD slow. Groups of 5 NSG immunodeficient mice determined to be engrafted with human AML cells in the blood by flow cytometry were treated with vehicle (PBS) or IGN004-wt fusion molecule at 0.2 mg/kg intraperitoneally twice per week for 3 weeks. The tumor burden in the blood was monitored on days -1, 6, 13, and 20 post treatment initiation by flow cytometry of peripheral blood samples. On day 21, animals were sacrificed and blood, spleen, and bone marrow samples were taken and analyzed by flow cytometry for the presence of human AML cells. Figure 9A shows the average \pm SEM of the percentage of human CD33-positive AML cells in the blood. Figure 9B shows the percentage of human CD33-positive AML cells in the spleen for individual mice as well as the average \pm SEM for each group. Figure 9C shows the percentage of human CD33-

positive AML cells in the bone marrow for individual mice as well as the average \pm SEM for each group.

[034] Figure 10 shows the tumor cell killing activity of the human CD8+ NKT cell-like TALL-104 effector cell line (ATCC CRL-11386) assessed in the presence or absence of IGN004 using the A549 human NSCLC tumor cell line (ATCC CCL-185). IGN004 treatment caused a small decrease in the viability of the A549 tumor cells (15.82%). TALL-104 effector cells demonstrated robust killing in the absence of IGN004 (69.2%). However, the combination of IGN004 and TALL-104 cells lead to complete eradication of A549 tumor cells (100% killing). This effect was stronger than the combination of either agent alone (85.02% vs. 100%).

[035] Figure 11 shows the tumor cell killing activity of TALL-104 effector cells assessed in the presence or absence of IGN004 at two different E:T ratios using a different human NSCLC tumor cell line (NCI-H1975; ATCC CRL-5908). IGN004 treatment caused a small decrease in the viability of the A549 tumor cells (5.7% and 10.6%). TALL-104 effector cells demonstrated significant killing in the absence of IGN004 and both 5:1 and 3.3:1 E:T ratios (58.6% and 55.7%, respectively). However, the combination of 50 pM IGN004 and TALL-104 cells lead to much more effective killing of the NCI-H1975 tumor cell targets at both E:T ratios (93.8% and 93.2%, respectively).

[036] Figure 12 shows the potency of the TALL-104 tumor cell killing assessed in the presence of IGN004 using NCI-H1975 NSCLC tumor cells. TALL-104 effector cells killed 17% of the NCI-H1975 tumor cells in the absence of IGN004 co-treatment. Treatment with IGN004 in combination with TALL-104 cells at concentrations from 0.25 to 25 pM caused an increase in tumor cell killing, compared to TALL-104 treatment alone.

[037] Figure 13 shows the tumor cell killing activity of downregulated TALL-104 effector cells assessed on A549 NSCLC tumor cells in the presence or absence of 10 pM IGN004 at different E:T ratios. 10 pM IGN004 alone had no effect on the tumor cells. At the 3:1 E:T ratio TALL-104 cells killed approximately 40% of the A549 tumor cells in the absence of drug but at lower E:T ratios the effector cells were ineffective at tumor cell killing. In the presence of 10 pM IGN004 the TALL-104 cells demonstrated robust tumor cell killing, even at 0.75:1 E:T where TALL-104 had no effect on the tumor cells without drug.

[038] Figure 14 shows the tumor cell killing activity of TALL-104 effector cells assessed in the presence or absence of IGN004 fusion protein or IGN004 non-fused mAb. 10 pM IGN004 non-fused mAb alone had no effect on the tumor cells and 10 pM IGN004 had only a slight effect (<10%). At all E:T ratios TALL-104 cells demonstrated a low level of tumor cell killing in the absence of drug. In the presence of 10 pM IGN004 mAb, the TALL-104 cells killed at an

equivalent rate to TALL-104 cells without drug. However, with 10 pM IGN004 there was a significant increase in the tumor cell killing by TALL-104 cells, compared to no drug (70-80% vs. 10-20% killing).

[039] Figure 15 shows the tumor cell killing activity of TALL-104 effector cells assessed in the presence or absence of IGN004, a control TAA Ab-IFN- α fusion molecule, or the combination of IGN004 non-fused mAb + non-fused IFN- α . 10 pM control TAA Ab-IFN- α fusion molecule alone had no effect on the tumor cells. 10 pM IGN004 or the combination of IGN004 non-fused mAb and non-fused IFN- α 2b had only a slight effect (<10%). At both E:T ratios TALL-104 cells demonstrated a low level of tumor cell killing in the absence of drug (<10%). In the presence of 10 pM control TAA Ab-IFN- α fusion molecule the TALL-104 cells killed at an equivalent rate to TALL-104 cells without drug. With 10 pM of the combination of IGN004 mAb + non-fused IFN- α 2b the TALL-104 effector cells killed more A549 tumor cells (14% and 25% increase in killing at 1:1 and 1.5:1 E:T, respectively). However, with 10 pM IGN004 there was a much higher increase in the tumor cell killing by TALL-104 cells, compared to no drug (34% and 42% increase in killing at 1:1 and 1.5:1, respectively).

[040] Figure 16 shows the tumor cell killing activity of the NK effector cell line NK-92 (ATCC CRL-2407) assessed in the presence or absence of IGN004 or a control TAA Ab-IFN- α fusion molecule at two E:T ratios using the OVCAR-3 ovarian cancer cell line (ATCC HTB-161). 10 pM of either treatment protein had no effect on the tumor cells in the absence of effector cells. NK-92 effector cells demonstrated robust killing of tumor cells in the absence of drug at 1.5:1 E:T ratio (49% killing) and modest killing at 0.5:1 (19% killing). In the presence of 10 pM control TAA Ab-IFN- α fusion molecule the NK-92 cells killed at an equivalent rate to effector cells without drug. With 10 pM IGN004 there was a significant increase in the tumor cell killing by NK-92 cells, compared to no drug (45% and 29% increase in killing at 1.5:1 and 0.5:1, respectively).

[041] Figure 17 shows the tumor cell killing activity of the NK-92 effector cells assessed in the presence or absence of IGN004 or non-fused IFN- α 2b at two E:T ratios using NCI-H1975 NSCLC tumor cells. Treatment with either protein had no effect on the tumor cells in the absence of effector cells. NK-92 effector cells demonstrated little to no killing of tumor cells in the absence of drug. In the presence of 100 pM non-fused IFN- α the NK-92 cells killed more tumor cells than NK-92 cells in the absence of drug. With 10 pM IGN004 there was a significant increase in the tumor cell killing by NK-92 cells, compared to no drug (85% and 62% increase in

killing at 1:1 and 0.3:1, respectively) and non-fused IFN- α 2b (50% and 51% increase in killing at 1:1 and 0.3:1, respectively).

MODE(S) FOR CARRYING OUT THE INVENTION

[042] The present disclosure is based on the inventors' insight that a fusion molecule which combines the specificity of an antibody to the target antigen with the potent cytotoxic effects of the IFN molecule would significantly improve the efficacy and safety profile of current cancer immunotherapies and/or IFN-based therapies; based, in part, on their understanding that use of the GRP94 Ab-IFN fusion molecule will have the following major advantages as compared with non-fused IFN: 1) the potent cytotoxic effects (induced apoptosis and programmed cell death) of IFN is concentrated at the targeted tumor cells by the fusion molecule (as compared with non-fused IFN) and engagement with IFN- α R expressed on the tumor cells will serve to eradicate the tumor cells; 2) the specificity of the GRP94 antibody to the target antigen will spare non-targeted cells, providing for a reduction of the systemic toxicity of IFN; 3) the local actions of IFN- α on dendritic cells (DCs) in the tumor microenvironment will help to negate some of the suppressive actions of tumors on DCs and could potentially lead to more efficient cross presentation of tumor antigens to T cells by DCs; 4) the IFN- α will act directly on T cells including enhancement of the CD8⁺ CTL functions, improving CD8⁺ CTL priming by increased DC cross-presentation which could potentially improve the therapeutic effect of the fusion protein; 5) the fusion molecule will stimulate or activate immune cells in lymphoid organs (e.g., draining lymph nodes, spleen, bone marrow); 6) the fusion molecule will stimulate or activate immune cells (e.g., T cells, natural killer cells, antigen presenting cells, phagocytic cells) that are present in the tumor microenvironment by directly binding to them via antibody-GRP94 and/or IFN-IFN α R interaction; 7) direct activation of the CD8⁺ CTL functions which will allow efficient killing of tumor cells; 8) inducing the up-regulation of the co-inhibitory immune-checkpoint proteins expressed on or associated with tumor cells; 9) inducing the up-regulation of MHC class I expressed on or associated with tumor cells, leading to better antigen presentation to T cells; and 10) directly negating other mechanisms for immune evasion, e.g., the major inhibitory pathways mediated by certain immune-checkpoint proteins on T cells/B cells.

[043] As described herein, the inventors' found that: 1) the GRP94 Ab-IFN fusion molecules and methods described herein can be used to effectively treat cancers, including recurrent, resistant, or refractory cancers, at surprisingly low doses; and 2) the GRP94 Ab-IFN

fusion molecules described herein can be used in combination with immunotherapy to design treatment protocols to provide for increased effector cell killing of tumor cells (i.e., a synergy exists between the GRP94 Ab-IFN fusion molecule and immunotherapy when co-administered). Specifically, the TAA Ab-IFN fusion molecules and methods described herein appear to be optimal for leveraging IFN's multiple properties and demonstrate the following: 1) effective killing of TAA-expressing tumor cells; and 2) the ability to provide for killing of non-TAA expressing tumor cells (also referred to hereinafter as "bystander tumor cells") that are adjacent to or held in close proximity to the tumor cells that express the TAA (i.e., non-TAA expressing tumor cells located in the tumor microenvironment). These observed "bystander effects" on non-TAA expressing tumor cells are surprising, given that the fused IFN has much lower affinity for the IFN receptor than does non-fused IFN, and thus has much lower potency for stimulation of the INF receptor on the non-TAA expressing tumor cells, as compared to non-fused IFN. And, importantly, the apparent bystander effects are only observed on non-TAA expressing tumor cells in the tumor microenvironment, or when immune cells in the tumor microenvironment have been stimulated by any kind of immunotherapy designed to attack the TAA expressing tumor cells. The TAA Ab-IFN fusion molecules and methods of the present invention thus represent promising new effective therapies to treat patients with proliferative diseases, and in particular, patients with recurrent, resistant or refractory proliferative diseases.

[044] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those commonly used and well known in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Green and Sambrook, *Molecular Cloning: A Laboratory Manual*, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012), incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry

described herein are those commonly used and well known in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of individuals.

Definitions

[045] The term “tumor associated antigen” (TAA) refers to, e.g., cell surface antigens that are selectively expressed by cancer cells or over-expressed in cancer cells relative to most normal cells. The terms “TAA variant” and “TAA mutant” as used herein refers to a TAA that comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another TAA sequence. In various embodiments, the number of amino acid residues to be inserted, deleted, or substituted can be, e.g., at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 350, at least 400, at least 450 or at least 500 amino acids in length. In various embodiments, the TAA is GRP94.

[046] As used herein, the term “tumor microenvironment” refers to the cellular environment in which the tumor exists, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules and the extracellular matrix (ECM). Components in the tumor microenvironment can modulate the growth of tumor cells, e.g., their ability to progress and metastasize. The tumor microenvironment can also be influenced by the tumor releasing extracellular signals, promoting tumor angiogenesis and inducing peripheral immune tolerance.

[047] As used herein, a “proliferative disease” includes tumor disease (including benign or cancerous) and/or any metastases. A proliferative disease may include hyperproliferative conditions such as hyperplasias, fibrosis (especially pulmonary, but also other types of fibrosis, such as renal fibrosis), angiogenesis, psoriasis, atherosclerosis and smooth muscle proliferation in the blood vessels, such as stenosis or restenosis following angioplasty. In some embodiments, the proliferative disease is cancer. In some embodiments, the proliferative disease is a non-cancerous disease. In some embodiments, the proliferative disease is a benign or malignant tumor.

[048] As used herein, “proliferation” includes any of a number of growth activities including increase in the number of cells, increase in the rate of cell division, increase in the

number of cell divisions, increase in the size of a cell, change in cellular differentiation, transformation to a malignant state, metastatic transformation, change in cell cycle phase to a more mitotically active cell cycle phase (e.g., S phase), or a combination of two or more of those activities. Cell growth (either in vitro or in vivo) can be a hyper-proliferative condition, such as is characteristic of certain disorders or diseases, for instance neoplasia or tumor formation. Inhibiting proliferation includes any of a number of anti-growth activities that reduce or even eliminate the ability of a cell to proliferate. Inhibiting proliferation includes, for instance, decreasing cell number, decreasing colony forming ability, decreasing the rate of cell division, decreasing the number of cell divisions, stopping cell division, inducing apoptosis, inducing senescence, inducing quiescence, changing cell cycle phase to a less mitotically active cell cycle phase, decreasing cellular de-differentiation, preventing transformation to a malignancy, decreasing malignant potential, decreasing metastatic ability or potential or a combination of two or more of those activities.

[049] As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this disclosure, beneficial or desired clinical results include, but are not limited to, any one or more of: alleviation of one or more symptoms, diminishment of extent of disease, preventing or delaying spread (e.g., metastasis, for example metastasis to the lung or to the lymph node) of disease, preventing or delaying recurrence of disease, delay or slowing of disease progression, amelioration of the disease state, and remission (whether partial or total). Also encompassed by "treatment" is a reduction of pathological consequence of a proliferative disease. As used herein, to "alleviate" a disease, disorder or condition means reducing the severity and/or occurrence frequency of the symptoms of the disease, disorder, or condition.

[050] The term "effective amount" or "therapeutically effective amount" as used herein refers to an amount of a compound or composition sufficient to treat a specified disorder, condition or disease such as ameliorate, palliate, lessen, and/or delay one or more of its symptoms. In reference to NHL and other cancers or other unwanted cell proliferation, an effective amount comprises an amount sufficient to: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer. An effective amount can be administered in one or more administrations.

[051] "Adjuvant setting" refers to a clinical setting in which an individual has had a history of a proliferative disease, particularly cancer, and generally (but not necessarily) been responsive to therapy, which includes, but is not limited to, surgery (such as surgical resection), radiotherapy, and chemotherapy. However, because of their history of the proliferative disease (such as cancer), these individuals are considered at risk of development of the disease. Treatment or administration in the "adjuvant setting" refers to a subsequent mode of treatment. The degree of risk (i.e., when an individual in the adjuvant setting is considered as "high risk" or "low risk") depends upon several factors, most usually the extent of disease when first treated.

[052] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. In various embodiments, "peptides", "polypeptides", and "proteins" are chains of amino acids whose alpha carbons are linked through peptide bonds. The terminal amino acid at one end of the chain (amino terminal) therefore has a free amino group, while the terminal amino acid at the other end of the chain (carboxy terminal) has a free carboxyl group. As used herein, the term "amino terminus" (abbreviated N-terminus) refers to the free α -amino group on an amino acid at the amino terminal of a peptide or to the α -amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" refers to the free carboxyl group on the carboxy terminus of a peptide or the carboxyl group of an amino acid at any other location within the peptide. Peptides also include essentially any polyamino acid including, but not limited to, peptide mimetics such as amino acids joined by an ether as opposed to an amide bond.

[053] Polypeptides of the invention include polypeptides that have been modified in any way and for any reason, for example, to: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (5) confer or modify other physicochemical or functional properties. For example, single or multiple amino acid substitutions (e.g., conservative amino acid substitutions) may be made in the naturally occurring sequence (e.g., in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). A "conservative amino acid substitution" refers to the substitution in a polypeptide of an amino acid with a functionally similar amino acid. The following six groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Serine (S), and Threonine (T)
- 2) Aspartic acid (D) and Glutamic acid (E)

- 3) Asparagine (N) and Glutamine (Q)
- 4) Arginine (R) and Lysine (K)
- 5) Isoleucine (I), Leucine (L), Methionine (M), and Valine (V)
- 6) Phenylalanine (F), Tyrosine (Y), and Tryptophan (W)

[054] A “non-conservative amino acid substitution” refers to the substitution of a member of one of these classes for a member from another class. In making such changes, according to various embodiments, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[055] The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art (see, for example, Kyte et al., 1982, J. Mol. Biol. 157:105-131). It is known that various amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, in various embodiments, the substitution of amino acids whose hydropathic indices are within ± 2 is included. In various embodiments, those that are within ± 1 are included, and in various embodiments, those within ± 0.5 are included.

[056] It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functional protein or peptide thereby created is intended for use in immunological embodiments, as disclosed herein. In various embodiments, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

[057] The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0+-1); glutamate (+3.0+-1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5+-1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, in various embodiments, the substitution of amino

acids whose hydrophilicity values are within ± 2 is included, in various embodiments, those that are within ± 1 are included, and in various embodiments, those within ± 0.5 are included.

[058] Exemplary amino acid substitutions are set forth in Table 1.

Table 1

Amino Acid Substitutions

<u>Original Residues</u>	<u>Exemplary Substitutions</u>	<u>Preferred Substitutions</u>
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	
Asp	Glu	
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[059] A skilled artisan will be able to determine suitable variants of polypeptides as set forth herein using well-known techniques. In various embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In other embodiments, the skilled artisan can identify residues and portions of the molecules that are conserved among similar polypeptides. In further embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

[060] Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, the skilled artisan can predict the importance of amino acid residues in a polypeptide that correspond to amino acid residues important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[061] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of a polypeptide with respect to its three-dimensional structure. In various embodiments, one skilled in the art may choose to not make radical changes to amino acid residues predicted to be on the surface of the polypeptide, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change can be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

[062] The term "polypeptide fragment" and "truncated polypeptide" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion as compared to a corresponding full-length protein. In various embodiments, fragments can be, *e.g.*, at least 5, at least 10, at least 25, at least 50, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 600, at least

700, at least 800, at least 900 or at least 1000 amino acids in length. In various embodiments, fragments can also be, *e.g.*, at most 1000, at most 900, at most 800, at most 700, at most 600, at most 500, at most 450, at most 400, at most 350, at most 300, at most 250, at most 200, at most 150, at most 100, at most 50, at most 25, at most 10, or at most 5 amino acids in length. A fragment can further comprise, at either or both of its ends, one or more additional amino acids, for example, a sequence of amino acids from a different naturally-occurring protein (*e.g.*, an Fc or leucine zipper domain) or an artificial amino acid sequence (*e.g.*, an artificial linker sequence).

[063] The terms "polypeptide variant" and "polypeptide mutant" as used herein refers to a polypeptide that comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. In various embodiments, the number of amino acid residues to be inserted, deleted, or substituted can be, *e.g.*, at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 350, at least 400, at least 450 or at least 500 amino acids in length. Variants of the present invention include fusion proteins.

[064] A "derivative" of a polypeptide is a polypeptide that has been chemically modified, *e.g.*, conjugation to another chemical moiety such as, for example, polyethylene glycol, albumin (*e.g.*, human serum albumin), phosphorylation, and glycosylation.

[065] The term "% sequence identity" is used interchangeably herein with the term "% identity" and refers to the level of amino acid sequence identity between two or more peptide sequences or the level of nucleotide sequence identity between two or more nucleotide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% identity means the same thing as 80% sequence identity determined by a defined algorithm, and means that a given sequence is at least 80% identical to another length of another sequence. In various embodiments, the % identity is selected from, *e.g.*, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% or more sequence identity to a given sequence. In various embodiments, the % identity is in the range of, *e.g.*, about 60% to about 70%, about 70% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 99%.

[066] The term "% sequence homology" is used interchangeably herein with the term "% homology" and refers to the level of amino acid sequence homology between two or more peptide sequences or the level of nucleotide sequence homology between two or more

nucleotide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% homology means the same thing as 80% sequence homology determined by a defined algorithm, and accordingly a homologue of a given sequence has greater than 80% sequence homology over a length of the given sequence. In various embodiments, the % homology is selected from, *e.g.*, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% or more sequence homology to a given sequence. In various embodiments, the % homology is in the range of, *e.g.*, about 60% to about 70%, about 70% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 99%.

[067] Exemplary computer programs which can be used to determine identity between two sequences include, but are not limited to, the suite of BLAST programs, *e.g.*, BLASTN, BLASTX, and TBLASTX, BLASTP and TBLASTN, publicly available on the Internet at the NCBI website. See also Altschul et al., 1990, *J. Mol. Biol.* 215:403-10 (with special reference to the published default setting, *i.e.*, parameters $w=4$, $t=17$) and Altschul et al., 1997, *Nucleic Acids Res.*, 25:3389-3402. Sequence searches are typically carried out using the BLASTP program when evaluating a given amino acid sequence relative to amino acid sequences in the GenBank Protein Sequences and other public databases. The BLASTX program is preferred for searching nucleic acid sequences that have been translated in all reading frames against amino acid sequences in the GenBank Protein Sequences and other public databases. Both BLASTP and BLASTX are run using default parameters of an open gap penalty of 11.0, and an extended gap penalty of 1.0, and utilize the BLOSUM-62 matrix. See *id.*

[068] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, *e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA*, 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability ($P(N)$), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is, *e.g.*, less than about 0.1, less than about 0.01, or less than about 0.001.

[069] The term "isolated molecule" (where the molecule is, for example, a polypeptide or a polynucleotide) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically

synthesized, or expressed in a cellular system different from the cell from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For various purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[070] A protein or polypeptide is "substantially pure," "substantially homogeneous," or "substantially purified" when at least about 60% to 75% of a sample exhibits a single species of polypeptide. The polypeptide or protein may be monomeric or multimeric. A substantially pure polypeptide or protein will typically comprise about 50%, 60%, 70%, 80% or 90% W/W of a protein sample, more usually about 95%, and e.g., will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For various purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[071] "Linker" refers to a molecule that joins two other molecules, either covalently, or through ionic, van der Waals or hydrogen bonds, e.g., a nucleic acid molecule that hybridizes to one complementary sequence at the 5' end and to another complementary sequence at the 3' end, thus joining two non-complementary sequences. A "cleavable linker" refers to a linker that can be degraded or otherwise severed to separate the two components connected by the cleavable linker. Cleavable linkers are generally cleaved by enzymes, typically peptidases, proteases, nucleases, lipases, and the like. Cleavable linkers may also be cleaved by environmental cues, such as, for example, specific enzymatic activities, changes in temperature, pH, salt concentration, etc. when there is such a change in environment following transcytosis of the fusion molecules across a polarized epithelial membrane.

[072] "Antibody" refers to a protein comprising one or more polypeptides substantially or partially encoded by immunoglobulin genes or fragments of immunoglobulin genes and having specificity to a tumor antigen or specificity to a molecule overexpressed in a pathological state. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as subtypes of these genes and myriad of immunoglobulin variable region genes. Light chains (LC) are classified as either kappa or

lambda. Heavy chains (HC) are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. A typical immunoglobulin (e.g., antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition.

[073] In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_{H1} , C_{H2} and C_{H3} (and in some instances, C_{H4}). Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L . The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR_1 , CDR_1 , FR_2 , CDR_2 , FR_3 , CDR_3 , FR_4 . The extent of the framework region and CDRs has been defined. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species, such as humans. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

[074] Antibodies exist as intact immunoglobulins or as a number of well characterized fragments. Such fragments include Fab fragments, Fab' fragments, Fab_2 , $F(ab)'_2$ fragments, single chain Fv proteins ("scFv") and disulfide stabilized Fv proteins ("dsFv"), that bind to the target antigen. A scFv protein is a fusion protein in which a light chain variable region of an immunoglobulin and a heavy chain variable region of an immunoglobulin are bound by a linker, while in dsFvs, the chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, as used herein, the term antibody encompasses e.g., monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, human antibodies, humanized antibodies,

camelised antibodies, chimeric antibodies, single-chain Fvs (scFv), single-chain antibodies, single domain antibodies, domain antibodies, Fab fragments, F(ab')₂ fragments, antibody fragments that exhibit the desired biological activity, disulfide-linked Fvs (sdFv), intrabodies, and epitope-binding fragments or antigen binding fragments of any of the above.

[075] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site. A "Fab fragment" comprises one light chain and the C_{H1} and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule.

[076] A "Fab' fragment" comprises one light chain and a portion of one heavy chain that contains the V_H domain and the C_{H1} domain and also the region between the C_{H1} and C_{H2} domains, such that an interchain disulfide bond can be formed between the two heavy chains of two Fab' fragments to form an F(ab')₂ molecule.

[077] Pepsin treatment of an antibody yields an F(ab')₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen. A "F(ab')₂ fragment" contains two light chains and two heavy chains containing a portion of the constant region between the C_{H1} and C_{H2} domains, such that an interchain disulfide bond is formed between the two heavy chains. A F(ab')₂ fragment thus is composed of two Fab' fragments that are held together by a disulfide bond between the two heavy chains.

[078] The "Fv region" comprises the variable regions from both the heavy and light chains, but lacks the constant regions.

[079] "Single-chain antibodies" are Fv molecules in which the heavy and light chain variable regions have been connected by a flexible linker to form a single polypeptide chain, which forms an antigen-binding region. Single chain antibodies are discussed in detail in International Patent Application Publication No. WO 88/01649, U.S. Patent No. 4,946,778 and 5,260,203, the inventions of which are incorporated by reference.

[080] The terms "an antigen-binding fragment" and "antigen-binding protein" as used herein means any protein that binds a specified target antigen. In the instant application, the specified target antigen is human Axl protein or fragment thereof. "Antigen-binding fragment" includes but is not limited to antibodies and binding parts thereof, such as immunologically functional fragments. An exemplary antigen-binding fragment of an antibody is the heavy chain and/or light chain CDR(s), or the heavy and/or light chain variable region.

[081] The term "immunologically functional fragment" (or simply "fragment") of an antibody or immunoglobulin chain (heavy or light chain) antigen-binding protein, as used herein, is a species of antigen-binding protein comprising a portion (regardless of how that portion is

obtained or synthesized) of an antibody that lacks at least some of the amino acids present in a full-length chain but which is still capable of specifically binding to an antigen. Such fragments are biologically active in that they bind to the target antigen and can compete with other antigen-binding proteins, including intact antibodies, for binding to a given epitope. In some embodiments, the fragments are neutralizing fragments. In some embodiments, the fragments can block or reduce the likelihood of the interaction between Axl and Gas6. In one aspect, such a fragment will retain at least one CDR present in the full-length light or heavy chain, and in some embodiments will comprise a single heavy chain and/or light chain or portion thereof. These biologically active fragments can be produced by recombinant DNA techniques, or can be produced by enzymatic or chemical cleavage of antigen-binding proteins, including intact antibodies. Immunologically functional immunoglobulin fragments include, but are not limited to, Fab, a diabody, Fab', F(ab')₂, Fv, domain antibodies and single-chain antibodies, and can be derived from any mammalian source, including but not limited to human, mouse, rat, camelid or rabbit. It is further contemplated that a functional portion of the antigen-binding proteins disclosed herein, for example, one or more CDRs, could be covalently bound to a second protein or to a small molecule to create a therapeutic agent directed to a particular target in the body, possessing bifunctional therapeutic properties, or having a prolonged serum half-life.

[082] Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V_H and V_L regions joined by a linker that is too short to allow for pairing between two regions on the same chain, thus allowing each region to pair with a complementary region on another polypeptide chain (see, e.g., Holliger et al., 1993, Proc. Natl. Acad. Sci. USA 90:6444-48 (1993), and Poljak et al., Structure 2:1121-23 (1994)). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen-binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen-binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen-binding sites, respectively, which can be the same or different.

[083] In various embodiments, antibodies and antibody fragments used in the constructs of the present invention can be bispecific. Bispecific antibodies or fragments can be of several configurations. For example, bispecific antibodies may resemble single antibodies (or antibody fragments) but have two different antigen-binding sites (variable regions). In various embodiments bispecific antibodies can be produced by chemical techniques (Kranz et al., Proc. Natl. Acad. Sci., USA, 78:5807, 1981), by "polydoma" techniques (see, e.g., U.S. Patent No. 4,474,893), or by recombinant DNA techniques. In various embodiments bispecific antibodies

of the present invention can have binding specificities for at least two different epitopes at least one of which is a tumor associate antigen. In various embodiments the antibodies and fragments can also be heteroantibodies. Heteroantibodies are two or more antibodies, or antibody binding fragments (e.g., Fab) linked together, each antibody or fragment having a different specificity.

[084] 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 naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method.

[085] The term "chimeric antibody" as used herein refers to an antibody which has framework residues from one species, such as human, and CDRs (which generally confer antigen-binding) from another species, such as a murine antibody that specifically binds targeted antigen.

[086] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR₃. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[087] The term "humanized antibody" as used herein refers to an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. A humanized antibody binds to the same antigen as the donor antibody that provides the CDRs. The acceptor framework of a humanized immunoglobulin or antibody may have a limited number of substitutions by amino acids taken from the donor framework. Humanized or other monoclonal antibodies can have additional conservative amino acid substitutions which have substantially no effect on antigen-binding or other immunoglobulin functions.

[088] The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell; antibodies isolated from a recombinant, combinatorial human antibody library; antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes; or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In various embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. All such recombinant means are well known to those of ordinary skill in the art.

[089] The term "epitope" as used herein includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. Epitopic determinants generally consist of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and generally have specific three dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., using the techniques described in the present invention. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct cross-competition studies to find antibodies that competitively bind with one another, e.g., the antibodies compete for binding to the antigen.

[090] The term "specifically binds" or "is specific for", as used herein, refers to measurable and reproducible interactions, such as binding between a target and an antibody (or antibody moiety) that is determinative of the presence of the target in the presence of a

heterogeneous population of molecules, including biological molecules. For example, an antibody or antibody moiety that specifically binds to a target (which can be an epitope) is an antibody or antibody moiety that binds this target with greater affinity, avidity, more readily, and/or with greater duration than its bindings to other targets. In various embodiments, an antibody or antigen-binding fragment "specifically binds" to an antigen if it binds to the antigen with a high binding affinity as determined by a dissociation constant (K_D , or corresponding K_b , as defined below) value of at least 1×10^{-6} M, or at least 1×10^{-7} M, or at least 1×10^{-8} M, or at least 1×10^{-9} M, or at least 1×10^{-10} M, or at least 1×10^{-11} M. An antigen-binding protein that specifically binds to the human antigen of interest may be able to bind to the same antigen of interest from other species as well, with the same or different affinities. The term " K_D " as used herein refers to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[091] The term "interferon" refers to a full-length interferon or to an interferon fragment (truncated interferon) or interferon mutant, that substantially retains the biological activity of the full length wild-type interferon (*e.g.*, retains at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 95%, or at least 98%, or at least 99% of the full-length interferon in its free form (*e.g.*, when not a component of a chimeric construct). Interferons include type I interferons (*e.g.*, interferon-alpha and interferon-beta) as well as type II interferons (*e.g.*, interferon-gamma). The interferon (*e.g.*, IFN- α) can be from essentially any mammalian species. In various embodiments, the interferon is from a species selected from the group consisting of human, equine, bovine, rodent, porcine, lagomorph, feline, canine, murine, caprine, ovine, a non-human primate, and the like. In various embodiments the mutated interferon comprises one or more amino acid substitutions, insertions, and/or deletions.

[092] The phrase "inhibiting the growth and/or proliferation" of a tumor or cancer cell refers to decrease in the growth rate and/or proliferation rate of a cancer cell. In various embodiments this includes death of a cancer cell (*e.g.* via apoptosis). In various embodiments this term also refers to inhibiting the growth and/or proliferation of a solid tumor and/or inducing tumor size reduction or elimination of the tumor.

[093] The terms "subject," "individual," and "patient" may be used interchangeably and refer to a mammal, preferably a human or a non-human primate, but also domesticated mammals (*e.g.*, canine or feline), laboratory mammals (*e.g.*, mouse, rat, rabbit, hamster, guinea pig), and agricultural mammals (*e.g.*, equine, bovine, porcine, ovine). In various embodiments, the subject can be a human (*e.g.*, adult male, adult female, adolescent male, adolescent female, male child, female child) under the care of a physician or other health worker in a hospital,

psychiatric care facility, as an outpatient, or other clinical context. In various embodiments, the subject may not be under the care or prescription of a physician or other health worker.

[094] "Pharmaceutical composition" refers to a composition suitable for pharmaceutical use in an animal. A pharmaceutical composition comprises a pharmacologically effective amount of an active agent and a pharmaceutically acceptable carrier. "Pharmacologically effective amount" refers to that amount of an agent effective to produce the intended pharmacological result. "Pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, vehicles, buffers, and excipients, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents and/or adjuvants. Suitable pharmaceutical carriers and formulations are described in Remington's Pharmaceutical Sciences, 21st Ed. 2005, Mack Publishing Co, Easton. A "pharmaceutically acceptable salt" is a salt that can be formulated into a compound for pharmaceutical use including, e.g., metal salts (sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines.

[095] The phrase "cause to be administered" refers to the actions taken by a medical professional (e.g., a physician), or a person controlling medical care of a subject, that control and/or permit the administration of the agent(s)/compound(s) at issue to the subject. Causing to be administered can involve diagnosis and/or determination of an appropriate therapeutic regimen, and/or prescribing particular agent(s)/compounds for a subject. Such prescribing can include, for example, drafting a prescription form, annotating a medical record, and the like. Where administration is described herein, "causing to be administered" is also contemplated.

GRP94 (Endoplasmin) and Anti-GRP94 Antibodies

[096] Glucose-regulated protein 94 (GRP94) is the HSP90-like protein in the lumen of the endoplasmic reticulum and therefore it chaperones secreted and membrane proteins. It has essential functions in development and physiology of multicellular organisms, at least in part because of this unique clientele. GRP94 shares many biochemical features with other HSP90 proteins, in particular its domain structure and ATPase activity, but also displays distinct activities, such as calcium binding, necessitated by the conditions in the endoplasmic reticulum. GRP94's mode of action varies from the general HSP90 theme in the conformational changes induced by nucleotide binding, and in its interactions with co-chaperones, which are very different from known cytosolic co-chaperones.

[097] It has been shown that GRP94 is expressed or overexpressed in tumor cells, including, e.g., melanoma, breast cancer, renal cancer, glioma, lung cancer (small cell lung cancer and non-small cell lung cancer), colorectal cancer, hepatocellular cancer, ovarian cancer; pancreatic cancer, prostate cancer, esophageal cancer, gastric cancer, urinary bladder cancer; head and neck cancer, multiple myeloma, acute leukemias (e.g., 11q23-positive acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma (indolent and high grade forms), Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia, and myelodysplasia, and that GRP94 has an anti-apoptotic effect on some tumor cells.

[098] An exemplary human cell surface GRP94 as used herein comprises the amino acid sequence set forth in SEQ ID NO: 1:

MRALWVLGLCCVLLTFGSVRADDEVVDVGTVEEDLGKSREGSRTDDEVVQREEEAIQLDGLN
 ASQIRELREKSEKFAFQAEVNRMMKLIINSLYKNKEIFLRELISNASDALDKIRLISLTDENALSGN
 EELTVKIKCDKEKNLLHVTDTGVGMTREELVKNLGTIAKSGTSEFLNKMTEAQEDGQSTSELIG
 QFGVGFYSAFLVADKVIVTSKHNNDTQHIWESDSNEFSVIADPRGNTLGRGTTITLVLKEEASD
 YLELDTIKNLVKKYSQFINFPIYVWSSKTETVEEPMEEEEAAKEEKEESDDEAAVEEEEEEEKPK
 TKKVEKTVDWELMNDIKPIWQRPSKEVEEDEYKAFYKSFESKESDDPMAYIHFTAEGEVTFKSI
 LfvPTSAPRGLFDEYgSKKSDYIKLYVRRVFITDDFHDMMPKYLNfVKGVDSDDLPLNVSRET
 LQQHKLLKVirKKLVrKtLDMIKKIADDKYNDTFWKEFGTNIKLGVIEDHSNRTRLAKLLRFQSS
 HHPTDITSLDQYVERMKEKQDKIYFMAGSSRKEAESSPFVERLLKKGYEVIYLTEPVDEYCIQAL
 PEFDGKRFQNVAKEGVKFDESEKTKEsREAVEKEFEPLLNWMKDKALKDKIEKAVVSQRLTES
 PCALVASQYGWsgNMERIMKAQAYQTGKDISTNYYASQKKTfEINPRHPLIRDMLRRIKEDED
 DKTvLDLAVVLFETAtLRSGYLLPDTKAYGDRIERMLRLSLNIDPDAKVEEEPEEEPEETAEDTT
 EDTEQDEDEEMDVGTDDEEEETAKESTAekDEL(SEQ ID NO: 1)

[099] In various embodiments, the GRP94 has an amino acid sequence that shares an observed homology of, e.g., at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 1.

[0100] An exemplary nucleic acid encoding human cell surface GP94 as used herein is set forth in SEQ ID NO: 2:

gattggtgggttcattgtttcccgccccgccccgcccgggaagtgggggtgaaaagcggcccgacctgcttgcgggtgtagtgg
 gcggaccgcgcggtctggagggtgaggatccgaaccagggtgggggggtggaggcggctcctgcgatcgaagggg
 acttgagactcaccggccgcacgcatgagggccctgtgggtgctgggcctctgctgcgtcctgctgcacctcgggtcggctc

agagctgacgatgaagttgatgtggatggtacagtagaagaggatctgggtaaaagtagagaaggatcaaggacggat
gatgaagtagtacagagagaggaagaagctattcagttggatggattaaatgcatcacaataagagaacttagagaga
agtcggaaaagtttcctccaagccgaagtaacagaatgatgaaacttatcatcaattcattgtataaaaaataagagatt
ttcctgagagaactgattcaaatgctctgatgctttagataagataaggctaatactactgactgatgaaatgctcttctgg
aaatgaggaactaacagtcaaaattaagtgatgataaggagaagaacctgctgcatgtcacagacaccgggttaggaatg
accagagaagagttggtaaaaaccttggtaccatagccaaatctgggacaagcgagttttaacaaaatgactgaagc
acaggaagatggccagtcaacttctgaattgattggccagtttgggtcggtttctattccgcctcctttagcagataaggta
ttgctacttcaaaacacaacaacgataaccagcacatctgggagtctgactccaatgaatttctgtaattgctgaccaaga
ggaaacactctaggacggggaacgacaattaccttctttaaagaagaagcatctgattacctgaattggatacaatta
aaaaactcgtcaaaaaatattcacagttcataaactttcctattatgtatggagcagcaagactgaaactgttagggagccc
atggaggaagaagaagcagccaaagaagagaaagaagaatctgatgatgaagctgcagtagaggaagaagaaga
agaaaagaaccaaagactaaaaaagttgaaaaactgtctgggactgggaacttatgatgatacaaaccaatatgg
cagagaccatcaaaagaagtagaagaagatgaatacaaaagctttctacaaatcatttcaaaggaaagtgatgacccat
ggcttatattcactttactgctgaaggggaagttaccttcaaatcaattttattgtaccacatctgctccacgtggctgtttgac
gaatatggatctaaaaagagcgattacattaagctctatgtgcgccgtgtattcatcacagacgacttccatgatgatgcct
aaatacctcaattttgcaaggggtgtgggactcagatgatctcccctgaatgttcccgcgagactctcagcaacataaa
ctgcttaaggtgattaggaagaagctgttcgtaaaacgctggacatgatcaagaagattgctgatgataatacaatgata
cttttggaaagaatttggtaccaacatcaagcttgggtgattgaagaccactcgaatcgaacacgcttctgtaaaacttcttag
gttccagcttctcatcatccaactgacattactagccttagaccagtatgtggaagaatgaaggaaaaacaagacaaaat
ctactcatggctgggtccagcagaaaagaggctgaatcttctccattgttagcgcacttctgaaaaagggtatgaattat
ttacctcacagaacctgtggatgaatactgtattcaggccctcccgaattgtatgggaagaggttccagaatgttccaagg
aaggagtgaaagtgatgaaagtgagaaaactaaggagagtcgtgaagcagttgagaagaattgagcctctgctga
attggatgaaagataaagcccttaaggacaagattgaaaaggctgtgggtctcagcgcctgacagaatctccgtgtgcttt
ggtggccagccagtagcggatggctggaacatggagagaatcatgaaagcacaagcgtaccaaacgggcaaggac
atcttacaataactatgtagcagtagaagaaaacattgaaattaatcccagacacccgctgatcagagacatgcttcgac
gaattaaggaagatgaagatgataaaacagtttggatcttctgtgtgttttggaaacagcaacgcttcgggtcagggatct
ttaccagacactaaagcatatggagatagaatagaagaatgcttgcctcagttgacattgacctgatgcaagggtg
gaagaagagcccgaagaagaacctgaagagacagcagaagacacaagaagacacagagcaagacgaagatg
aagaaatggatgtgggaacagatgaagaagaagaacagcaaggaaatctacagctgaaaaagatgaattgtaatt
atacttcaccatttggatcctgtgtggagagggaaatgtgaaattacatcatttcttttgggagagactgttttggatgccccct
aatccccttctcccctgactgtaaaatgtgggattatgggtcacaggaaaaagtgggtttttagttgaatttttaacattcct
catgaatgtaaattgtactatttaactgactattctgtatgtaaaatctgtcatgtatataaaaaataaaaaagatcccaaat
(SEQ ID NO: 2)

[0101] In various embodiments, the GRP94 contains an nucleic acid sequence that shares an observed homology of, *e.g.*, at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 2.

[0102] In various embodiments, GRP94 (endoplasmic) may be encoded by the following genes, but not limited to, GENBANK® Accession Nos. NM_003299, BC066656 (Homo sapiens); NM_011631 (Mus musculus); NM_001045763: (Xenopus (Silurana) tropicalis); NM_214103 (Sus scrofa) NM_98210 (Danio rerio); NM_001012197 (Rattus norvegicus); NM_001134101: Pongo abelii; NM_001003327 (Canis lupus familiaris) heat shock protein 90 kDa beta (GRP94); or NM_204289 (Gallus gallus).

[0103] Once of skill in the art can readily use a nucleic acid sequence to produce a polypeptide, such as GRP94 using standard method in molecular biology (see, for example, . See, e.g., Green and Sambrook, *Molecular Cloning: A Laboratory Manual*, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)).

[0104] Antibodies (Abs) to endoplasmin are known to those of skill in the art and can be obtained commercially or readily produced. For example, antibodies can be produced by immunizing an animal with a target antigen or an immunogenic fragment thereof and raising the antibodies in that animal, and single chain antibodies can be produced using phage-display technology according to methods well known to those of skill in the art. Isolated monoclonal antibodies (mAbs), including fully human antibodies that specifically bind GRP94 and are contemplated for use in the fusion molecules and methods of treatment described herein include, but are not limited to, those described in, e.g., US Patent No. 8.771,687 (Ferrone et al) and US 20040001789 (Young et al), each of which is hereby incorporated by reference in its entirety for purposes of providing such antibodies and antigen-binding fragments). In various embodiments, the GRP94 antibody is an antibody or antigen-binding fragment that specifically binds glycosylated GRP94 on the surface on a cell, e.g., a tumor cell. Thus, in various embodiments, the antibody or antigen-binding fragment does not bind non-glycosylated GRP94 or unrelated antigen.

[0105] In various embodiments the antibody is a GRP94 antibody which comprises a heavy chain having an amino acid sequence as set forth in SEQ ID NO: 3:

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QVQLVQSGAEVKKPGASVKVSCASGYTFTSYAMHWVRQAPGQRLEWMGWINAGN
GNTKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCARAHFDYWGQGLVTVS
AASKGPSVFLPAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSQVHTFPVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 3)

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[0106] In various embodiments, the heavy chain of the GRP94 antibody has an amino acid sequence that shares an observed homology of, e.g., at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 3.

[0107] In various embodiments the antibody is a GRP94 antibody which comprises a light chain having an amino acid sequence as set forth in SEQ ID NO: 4:

EIELTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS
RFGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYSLSS
TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 4)

[0108] In various embodiments, the light chain of the GRP94 antibody has an amino acid sequence that shares an observed homology of, *e.g.*, at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 4.

[0109] In various embodiments, the antibody can be a monoclonal antibody (mAb), wherein the heavy chain of the antibody comprises one or more of the amino acid sequences set forth as amino acids 26-33 of SEQ ID NO: 3 (CDR1), amino acids 51-58 of SEQ ID NO: 3 (CDR2), and amino acids 97-103 of SEQ ID NO: 3 (CDR3). In various embodiments, the antibody can be a monoclonal antibody, wherein the heavy chain of the antibody comprises the amino acid sequence set forth as amino acids 26-33 of SEQ ID NO: 3 (CDR1), amino acids 51-58 of SEQ ID NO: 3 (CDR2), and amino acids 97-103 of SEQ ID NO: 3 (CDR3) and wherein the light chain of the antibody comprises the amino acid sequence set forth as amino acids 27-32 of SEQ ID NO: 4 (CDR1), amino acids 50-52 of SEQ ID NO: 4 (CDR2), and amino acids 89-97 of SEQ ID NO: 4 (CDR3). In various embodiments, the antibody can be a monoclonal antibody that comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 3 and a light chain having the amino acid sequence set forth in SEQ ID NO: 4; referred to hereinafter as "mAb W9".

[0110] In various embodiments, the anti-GRP94 antibody specifically binds to the same epitope as the antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 3 and a light chain having the amino acid sequence of SEQ ID NO: 4. In various embodiments, the anti-GRP94 antibody competes for binding to the GRP94 antigen with the antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 3 and a light chain having the amino acid sequence of SEQ ID NO: 4.

[0111] The monoclonal antibodies contemplated for use herein can be of any isotype. The monoclonal antibody can be, for example, an IgA, IgM or an IgG antibody, such as IgG₁, IgG₂ or an IgG₄. The class of an antibody that specifically binds GRP94 can be switched with another. For example, a nucleic acid molecule encoding V_L or V_H is isolated using methods well-known in the art, such that it does not include any nucleic acid sequences encoding the constant region of the light or heavy chain, respectively. The nucleic acid molecule encoding V_L

or V_H is then operatively linked to a nucleic acid sequence encoding a C_L or C_H from a different class of immunoglobulin molecule. This can be achieved using a vector or nucleic acid molecule that comprises a C_L or C_H chain, as known in the art. For example, an antibody that specifically binds GRP94 that was originally IgM may be class switched to an IgG. Class switching can be used to convert one IgG subclass to another, such as from IgG₁ to IgG₂.

[0112] In various embodiments, the antibody or antigen-binding fragment binds to a GRP94 antigen with a dissociation constant (K_D) of at least about 1×10^{-3} M, at least about 1×10^{-4} M, at least about 1×10^{-5} M, at least about 1×10^{-6} M, at least about 1×10^{-7} M, at least about 1×10^{-8} M, at least about 1×10^{-9} M, at least about 1×10^{-10} M, at least about 1×10^{-11} M, or at least about 1×10^{-12} M.

Interferon and interferon mutants

[0113] In various embodiments of the present invention, either the N- or C- terminus of an antibody, or antigen-binding fragment heavy or light chain will be genetically constructed with one of the several contemplated interferons or interferon mutants. Interferons include type I interferons (e.g., IFN- α , IFN- β) as well as type II interferons (e.g., IFN- γ). The term "interferon" as used herein refers to a full-length interferon or to an interferon fragment (truncated interferon) or to an interferon mutant (truncated interferon and interferon mutant collectively referred to herein as 'modified interferon'), that substantially retains the biological activity of the full length wild-type interferon (e.g., retains at least 50%, for example at least about any of 60%, 70%, 80%, 90%, or more biological activity of the full length wild-type interferon). The interferon can be from essentially any mammalian species. In various embodiments, the interferon is from a species selected from the group consisting of human, equine, bovine, rodent, porcine, lagomorph, feline, canine, murine, caprine, ovine, a non-human primate, and the like.

[0114] In various embodiments, the GRP94 antibody-IFN fusion molecules comprise an interferon or a modified interferon that possesses, e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 100%, of the endogenous activity of the wild-type interferon having the same amino acid sequence but not attached to an antibody.

[0115] In various embodiments, the GRP94 antibody-IFN fusion molecules will comprise an interferon or a modified interferon that possesses, e.g., less than 10%, less than 20%, less than 30%, less than 40%, less than 50%, less than 55%, less than 60%, less than 65%, less

than 70%, less than 75%, less than 80%, less than 85%, less than 90%, less than 95%, less than 96%, less than 97%, less than 98%, less than 99%, less than 100%, of the endogenous activity of the wild-type interferon having the same amino acid sequence but not attached to an antibody.

[0116] In various embodiments, the GRP94 antibody-IFN fusion molecules will comprise an interferon or a modified interferon that possesses, e.g., more than 5 times, more than 10 times, more than 15 times, more than 20 times, more than 25 times, more than 30 times, more than 35 times, more than 40 times, more than 50 times, more than 60 times, more than 70 times, more than 80 times, more than 90 times, more than 100 times, more than 125 times, more than 150 times, more than 175 times, more than 200 times, more than 250 times, more than 300 times, more than 400 times, more than 500 times, more than 750 times, and more than 1000 times, the endogenous activity of the wild-type interferon having the same amino acid sequence but not attached to an antibody.

[0117] Interferon activity can be assessed, for example, using the various anti-viral and anti-proliferative assays described in art (see, e.g., U.S. Patent No. 8,563,692, U.S. Pat. Public. No. 20130230517, U.S. Pat. Public. No. 20110158905, PCT WO/2014/028502, and PCT WO/2013/059885) as well as the assays described in the Examples section below.

[0118] In various embodiments, the GRP94 antibody-IFN fusion molecules will show at least 10, at least 100, at least 1000, at least 10,000, or at least 100,000 fold selectivity toward cells that express the GRP94 antigen to which the antibody binds over cells that do not express the GRP94 antigen, when compared to interferon having the same amino acid sequence not attached to an antibody.

[0119] In various embodiments of the present invention, the interferon is an interferon mutant which comprises one or more amino acid substitutions, insertions, and/or deletions. Means of identifying such mutant interferon molecules are routine to those of skill in the art. In one illustrative approach, a library of truncated and/or mutated IFN- α is produced and screened for IFN- α activity. Methods of producing libraries of polypeptide variants are well known to those of skill in the art. Thus, for example, error-prone PCR can be used to create a library of mutant and/or truncated IFN- α (see, e.g., U.S. Patent No. 6,365,408). The resultant library members can then be screened according to standard methods known to those of skill in the art. Thus, for example, IFN- α activity can be assayed by measuring antiviral activity against a particular test virus. Kits for assaying for IFN- α activity are commercially available (see, e.g., ILITE™ alphabeta kit by Neutekbio, Ireland).

[0120] The use of chemically modified interferons is also contemplated. For example, in various embodiments, the interferon is chemically modified to increase serum half-life. Thus, for example, (2-sulfo-9-fluorenylmethoxycarbonyl)₇-interferon- α 2 undergoes time-dependent spontaneous hydrolysis, generating active interferon (Shechter et al., Proc. Natl. Acad. Sci., USA, 98(3): 1212-1217, 2001). Other modifications, include for example, N-terminal modifications in including, but not limited to the addition of PEG, protecting groups, and the like (see, e.g., U.S. Patent No. 5,824,784).

[0121] In various embodiments, the interferon contains an amino acid sequence that shares an observed homology of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% with the wildtype IFN- α sequence selected from the group consisting of IFN- α 5 (NP_002160.1), IFN- α 6 (NP_066282.1), IFN- α 7 (NP_066401.1), IFN- α 8 (NP_002161.2), IFN- α 10 (NP_002162.1), IFN- α 16 (NP_002164.1), IFN- α 17 (NP_067091.1), and IFN- α 21 (NP_002166.2).

[0122] In various embodiments, the interferon contains an amino acid sequence that shares an observed homology of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% with the wildtype IFN- α 2b sequence provided below as SEQ ID NO: 5 (referred to hereinafter as "IFN- α 2b-wt"). In some embodiments, the interferon has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 1x, at least 1.5x, at least 2x, at least 2.5x, or at least 3x activity of IFN- α 2b-wt provided below as SEQ ID NO: 5. In some embodiments, the interferon has less than any of about 70%, 75%, 80%, 85%, 90%, or 95%, activity of IFN- α 2b-wt provided below as SEQ ID NO: 5.

CDLPQTHSLGSRRTLMLLAQMRRISLFSCCLKDRHDFGFPQEEFGNQFQKA
 ETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNLEACVI
 QGVGVTTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRS
 FSLSTNLQESLRSKE (SEQ ID NO: 5)

[0123] In various embodiments, the use of a mutated IFN- α is contemplated. Single point mutations contemplated for use herein include, but are not limited to, a series of mostly single point mutants that are considered important to the binding affinity of IFN- α to IFN- α R1 based on published information on NMR structure with the assumption that a single point mutation may change the binding affinity but will not completely knock off the activity of IFN- α , therefore still retaining the anti-proliferative properties albeit at much higher concentrations.

This will potentially improve the therapeutic index of the fusion molecules comprising an antibody fused to the interferon-alpha mutants. A single mutation will be identified by the particular amino acid substitution at a specific amino acid position within the full length wild type interferon sequence. For example, a mutation comprising a tyrosine substituted for the full length wild type histidine at amino acid 57 is identified as H57Y. IFN- α mutants contemplated for use herein include, but are not limited to those described in US Patent No. 8,980,267 (Grewal et al.) which is hereby incorporated by reference in its entirety for the interferon mutants and sequences provided therein. Additional interferon mutants contemplated for use include those described in, e.g., PCT WO 2013/059885 (Wilson et al.), U.S. 20140248238 (Wilson et al) and U.S. Pat. No. 8,258,263 (Morrison et al), each of which is hereby incorporated by reference in its entirety for the interferon mutants and sequences provided therein.

[0124] In various embodiments, the interferon is an IFN- α 2b mutant molecule having the amino acid sequence set forth in SEQ ID NO: 5, and comprising one or more single point mutations selected from L15A, A19W, R22A, R23A, S25A, L26A, F27A, L30A, L30V, K31A, D32A, R33K, R33A, R33Q, H34A, D35E, Q40A, H57A, H57S, H57Y, E58A, E58N, E58S, Q61A, Q61S, D114R, L117A, R120A, R125A, R125E, K131A, E132A, K133A, K134A, R144A, R144D, R144E, R144G, R144H, R144I, R144K, R144L, R144N, R144Q, R144S, R144T, R144V, R144Y, A145D, A145E, A145G, A145H, A145I, A145K, A145L, A145M, A145N, A145Q, A145R, A145S, A145T, A145V, A145Y, M148A, R149A, S152A, L153A, N156A, R162A, or E165D.

[0125] In various embodiments, the interferon is an IFN- α 2b mutant molecule wherein the arginine at amino acid residue 149 of SEQ ID NO: 5 is replaced with an alanine (R149A) and the arginine at amino acid residue 162 of SEQ ID NO: 5 is replaced with an alanine (R162A). This IFN- α 2b mutant molecule is referred to hereinafter as "IFN- α 2b-M8". The amino acid sequence of IFN- α 2b-M8 is provided below as SEQ ID NO: 6.

CDLPQTHSLGSRRTLMLLAQMRRISLFSLKDRHDFGFPQEEFGNQFQKA
 ETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQQLNDLEACVI
 QGVGVTTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMAS
 FSLSTNLQESLASKE (SEQ ID NO: 6)

[0126] In various embodiments, the interferon contains an amino acid sequence that shares an observed homology of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% with the

wildtype IFN- α 14 sequence provided below as SEQ ID NO: 7 (referred to hereinafter as “IFN- α 14-wt”). In some embodiments, the interferon has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 1x, at least 1.5x, at least 2x, at least 2.5x, or at least 3x activity of IFN- α 14-wt provided below as SEQ ID NO: 7. In some embodiments, the interferon has less than any of about 70%, 75%, 80%, 85%, 90%, or 95%, activity of IFN- α 14-wt provided below as SEQ ID NO: 7.

CNLSQTHSLNNRRTLMLMAQMRRISPFSCCLKDRHDFEFPQEEFDGNQFQKAQAISVL
HEMMQQTFNLFSTKNSSAAWDETLLEKFYIELFQQMNDLEACVIQEVGVEETPLMNED
SILAVKKYFQRITLYLMEKKYSPCAWEVVRAEIMRSLSFSTNLQKRLRRKD
(SEQ ID NO: 7)

[0127] In various embodiments, the interferon contains an amino acid sequence that shares an observed homology of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% with the wildtype IFN- β -1a sequence provided below as SEQ ID NO: 8 (referred to hereinafter as “IFN- β -1a-wt”). In some embodiments, the interferon has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 1x, at least 1.5x, at least 2x, at least 2.5x, or at least 3x activity of IFN- β -1a-wt provided below as SEQ ID NO: 8. In some embodiments, the interferon has less than any of about 70%, 75%, 80%, 85%, 90%, or 95%, activity of IFN- β -1a-wt provided below as SEQ ID NO: 8:

MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKE
DAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLE
KEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNIFYFINRL
TGYLRN (SEQ ID NO: 8)

[0128] In various embodiments, the interferon contains an amino acid sequence that shares an observed homology of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% with the wildtype IFN- β -1b sequence provided below as SEQ ID NO: 9 (referred to hereinafter as “IFN- β -1a-wt”). In some embodiments, the interferon has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 1x, at least 1.5x, at least 2x, at least 2.5x, or at least 3x activity of IFN- β -1a-wt provided

below as SEQ ID NO: 9. In some embodiments, the interferon has less than any of about 70%, 75%, 80%, 85%, 90%, or 95%, activity of IFN- β -1a-wt provided below as SEQ ID NO: 9:

MSYNLLGFLQRSSNFQSQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKE
DAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKL
EKEDFTRGKLMSSLHLKRYYGRIHLHYLKAKEYSHCAWTIVRVEILRNIFYFINR
LTGYLRN (SEQ ID NO: 9)

[0129] In various embodiments use of a mutated IFN- β is contemplated. A mutated IFN- β comprising a serine substituted for the naturally occurring cysteine at amino acid 17 of IFN- β -1a has also been demonstrated to show efficacy (Hawkins et al., Cancer Res., 45:5914-5920, 1985). Various C-terminally truncated IFN- β -1a's have been shown to have increased activity (see, e.g., U.S. Patent Publication 2009/0025106 A1). Accordingly, in various embodiments the interferons used in the fusion molecules described herein include the C-terminally truncated IFN- β described as IFN- Δ 1, IFN- Δ 2, IFN- Δ 3, IFN- Δ 4, IFN- Δ 5, IFN- Δ 6, IFN- Δ 7, IFN- Δ 8, IFN- Δ 9, IFN- Δ 10 in US 2009/0025106 A1. In various embodiments, the interferon is an IFN- β -1a mutant molecule having the amino acid sequence set forth in SEQ ID NO: 9, and comprising one or more single point mutations selected from R27A, R35T, E42K, D54N, M62I, G78S, K123A, C141Y, A142T, E149K, and R152H. This reference is hereby incorporated by reference in its entirety herein for purposes of the interferon mutants and sequences provided therein.

Fusion Molecules

[0130] In one aspect, the present invention relates to genetically engineered fusion molecules comprising at least one antibody, or antigen-binding fragment thereof, attached to at least one interferon, or interferon mutant molecule. Generally speaking, the antibody portion and interferon molecule can be joined together in any order. Thus, for example, the interferon molecule(s) can be joined to either the amino or carboxy terminal of the antibody. Alternatively, the antibody can be joined to either the amino or carboxy terminal of the interferon molecule. Figure 1 is a schematic diagram of an exemplary GRP94 antibody-IFN fusion molecule.

[0131] In various embodiments, the antibody and interferon molecule are linked directly to each other without an intervening peptide linker sequence and synthesized using recombinant DNA methodology. By "linked" we mean that the first and second sequences are

associated such that the second sequence is able to be transported by the first sequence to a target cell, i.e., fusion molecules in which the antibody is linked to a IFN- α molecule via their polypeptide backbones through genetic expression of a DNA molecule encoding these proteins, directly synthesized proteins, and coupled proteins in which pre-formed sequences are associated by a cross-linking agent.

[0132] In various embodiments, the antibody portion is chemically conjugated to the interferon molecule. Means of chemically conjugating molecules are well known to those of skill.

[0133] The procedure for conjugating two molecules varies according to the chemical structure of the agent. Polypeptides typically contain variety of functional groups; e.g., carboxylic acid (COOH) or free amine ($--NH_2$) groups, that are available for reaction with a suitable functional group on the other peptide, or on a linker to join the molecules thereto. Alternatively, the antibody and/or the interferon can be derivatized to expose or attach additional reactive functional groups. The derivatization can involve attachment of any of a number of linker molecules such as those available from Pierce Chemical Company, Rockford Ill. A bifunctional linker having one functional group reactive with a group on the antibody and another group reactive on the interferon, can be used to form the desired conjugate. Alternatively, derivatization can involve chemical treatment of the antibody portion. Procedures for generation of, for example, free sulfhydryl groups on polypeptides, such as antibodies or antibody fragments, are known (See U.S. Pat. No. 4,659,839).

[0134] Many procedures and linker molecules for attachment of various compounds including radionuclide metal chelates, toxins and drugs to proteins such as antibodies are known. See, for example, European Patent Application No. 188,256; U.S. Pat. Nos. 4,671,958, 4,659,839, 4,414,148, 4,699,784; 4,680,338; 4,569,789; and 4,589,071; and Borlinghaus et al. (1987) Cancer Res. 47: 4071-4075. In particular, production of various immunotoxins is well-known within the art and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982); Waldmann (1991) Science, 252: 1657; U.S. Pat. Nos. 4,545,985 and 4,894,443, and the like.

[0135] In various embodiments, the two molecules can be separated by a peptide linker consisting of one or more amino acids. Generally the linker will have no specific biological activity other than to join the proteins or to preserve some minimum distance or other spatial relationship between them. In various embodiments, however, the constituent amino acids of the linker can be selected to influence some property of the molecule such as the folding, net

charge, or hydrophobicity. In various embodiments, the fusion molecule is a recombinantly expressed fusion molecule and will comprise an interferon molecule attached to the antibody via a peptide linker as described herein and as depicted in Figure 1. In various embodiments, the antibody portion and the interferon portion of the fusion molecule are linked (e.g., fused) without a linker.

[0136] Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. In various embodiments, the linker(s) can be joined to the constituent amino acids of the antibody and/or the interferon through their side groups (e.g., through a disulfide linkage to cysteine). In various embodiments, the linkers are joined to the alpha carbon amino and/or carboxyl groups of the terminal amino acids of the antibody and/or the interferon. Linker length contemplated for use can vary from about 5 to 200 amino acids. In various embodiments, the linker may be a proteolysis-resistant linker of 1 to 20 amino acids in length (see, e.g., U.S. Pat. No. 8,258,263 (Morrison et al.); PCT/WO2014/089354 (Morrison); U.S. Pat. No. 8,378,076 (Shen et al), each hereby incorporated by reference in its entirety for the proteolysis-resistant linkers and sequences provided therein).

[0137] In various embodiments, the linker is an α -helical linker. In various embodiments, the linker is rich in G/S content (e.g., at least about 60%, 70%, 80%, 90%, or more of the amino acids in the linker are G or S. In various embodiments, the linker is rich in G/C content and is less than about any of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 amino acid long. In various embodiments, the linker is an α -helical linker and is less than about any of 7, 8, 9, 10, 15, 20, 25, or 30 amino acid long. In various embodiments, the linker comprises SGGGGS (SEQ ID NO: 10). In various embodiments, the linker comprises AEAAAKEAAKAGS (SEQ ID NO: 11). In various embodiments, there is no linker. Linkers contemplated for use include, but are not limited to, those set forth in Table 2.

Table 2

Examples of Proteolysis-Resistant Linkers

Linker Sequence	SEQ ID NO
SGGGGS	10
AEAAAKEAAKAGS	11

GGGGS	12
SGGGGSGGGGS	13
GGGGG	14
GAGAGAGAGA	15
AEAAKAGS	16
GGGGGGGG	17
AEAAKEAAKA	18
AEAAKA	19
GGAGG	20

Pharmaceutical Compositions

[0138] In another aspect, the present invention provides a pharmaceutical composition comprising a GRP94 Ab-IFN fusion molecule as described herein, with one or more pharmaceutically acceptable excipient(s).

[0139] Generally, the GRP94 Ab-IFN fusion molecules of the invention are suitable to be administered as a formulation in association with one or more pharmaceutically acceptable excipient(s). The term 'excipient' is used herein to describe any ingredient other than the compound(s) of the invention. The choice of excipient(s) will to a large extent depend on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form. As used herein, "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Some examples of pharmaceutically acceptable excipients are water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable substances are wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody. Pharmaceutical compositions of the present invention and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company, 1995). The

pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all GMP regulations of the U.S. Food and Drug Administration.

[0140] A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[0141] The pharmaceutical compositions of the invention are typically suitable for parenteral administration. As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue, thus generally resulting in the direct administration into the blood stream, into muscle, or into an internal organ. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal, intravenous, intraarterial, intrathecal, intraventricular, intraurethral, intracranial, intrasynovial injection or infusions; and kidney dialytic infusion techniques. Various embodiments include the intravenous and the subcutaneous routes.

[0142] Formulations of a pharmaceutical composition suitable for parenteral administration typically generally comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampoules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and the like. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition. Parenteral formulations also include aqueous solutions which may

contain excipients such as salts, carbohydrates and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. Exemplary parenteral administration forms include solutions or suspensions in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, or in a liposomal preparation. Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

[0143] For example, in one aspect, sterile injectable solutions can be prepared by incorporating the GRP94 Ab-IFN fusion molecule in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution 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. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0144] The GRP94 Ab-IFN fusion molecules of the invention can also be administered intranasally or by inhalation, typically in the form of a dry powder (either alone, as a mixture, or as a mixed component particle, for example, mixed with a suitable pharmaceutically acceptable excipient) from a dry powder inhaler, as an aerosol spray from a pressurized container, pump, spray, atomiser (preferably an atomiser using electrohydrodynamics to produce a fine mist), or nebulizer, with or without the use of a suitable propellant, or as nasal drops.

[0145] The pressurized container, pump, spray, atomizer, or nebulizer generally contains a solution or suspension of a GRP94 Ab-IFN fusion molecule of the invention comprising, for example, a suitable agent for dispersing, solubilizing, or extending release of the active, a propellant(s) as solvent.

[0146] Prior to use in a dry powder or suspension formulation, the drug product is generally micronized to a size suitable for delivery by inhalation (typically less than 5 microns). This may be achieved by any appropriate comminuting method, such as spiral jet milling, fluid bed jet milling, supercritical fluid processing to form nanoparticles, high pressure homogenization, or spray drying.

[0147] Capsules, blisters and cartridges for use in an inhaler or insufflator may be formulated to contain a powder mix of the fusion molecule of the invention, a suitable powder base and a performance modifier.

[0148] Suitable flavours, such as menthol and levomenthol, or sweeteners, such as saccharin or saccharin sodium, may be added to those formulations of the invention intended for inhaled/intranasal administration.

[0149] Formulations for inhaled/intranasal administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

[0150] In the case of dry powder inhalers and aerosols, the dosage unit is determined by means of a valve which delivers a metered amount. Units in accordance with the invention are typically arranged to administer a metered dose or "puff" of an antibody of the invention. The overall daily dose will typically be administered in a single dose or, more usually, as divided doses throughout the day.

[0151] The GRP94 Ab-IFN fusion molecules of the invention may also be formulated for an oral administration. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, and/or buccal, lingual, or sublingual administration by which the compound enters the blood stream directly from the mouth. Formulations suitable for oral administration include solid, semi-solid and liquid systems such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids, or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

[0152] Pharmaceutical compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents in order to provide a pharmaceutically elegant and palatable preparation. For example, to prepare orally deliverable tablets, the GRP94 Ab-IFN fusion molecule is mixed with at least one pharmaceutical excipient, and the solid formulation is compressed to form a tablet according to known methods, for delivery to the gastrointestinal tract. The tablet composition is

typically formulated with additives, e.g. a saccharide or cellulose carrier, a binder such as starch paste or methyl cellulose, a filler, a disintegrator, or other additives typically usually used in the manufacture of medical preparations. To prepare orally deliverable capsules, DHEA is mixed with at least one pharmaceutical excipient, and the solid formulation is placed in a capsular container suitable for delivery to the gastrointestinal tract. Compositions comprising fusion molecules may be prepared as described generally in Remington's Pharmaceutical Sciences, 18th Ed. 1990 (Mack Publishing Co. Easton Pa. 18042) at Chapter 89, which is herein incorporated by reference.

[0153] In various embodiments, the pharmaceutical compositions are formulated as orally deliverable tablets containing GRP94 Ab-IFN fusion molecules in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for manufacture of tablets. These excipients may be inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, maize starch, gelatin or acacia, and lubricating agents, for example, magnesium stearate, stearic acid, or talc. The tablets may be uncoated or they may be coated with known techniques to delay disintegration and absorption in the gastrointestinal track and thereby provide a sustained action over a longer period of time. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

[0154] In various embodiments, the pharmaceutical compositions are formulated as hard gelatin capsules wherein the GRP94 Ab-IFN fusion molecule is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate, or kaolin or as soft gelatin capsules wherein the GRP94 Ab-IFN fusion molecule is mixed with an aqueous or an oil medium, for example, arachis oil, peanut oil, liquid paraffin or olive oil.

[0155] Liquid formulations include suspensions, solutions, syrups and elixirs. Such formulations may be employed as fillers in soft or hard capsules (made, for example, from gelatin or hydroxypropylmethylcellulose) and typically comprise a carrier, for example, water, ethanol, polyethylene glycol, propylene glycol, methylcellulose, or a suitable oil, and one or more emulsifying agents and/or suspending agents. Liquid formulations may also be prepared by the reconstitution of a solid, for example, from a sachet.

[0156] Any method for formulating and administering peptides, proteins, antibodies, and/or immunoconjugates accepted in the art may suitably be employed for administering the GRP94 Ab-IFN molecules of the present invention.

Dosing

[0157] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. The term "dosage unit form," as used herein, refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0158] The precise dose of GRP94 Ab-IFN fusion molecule to be employed in the methods of the present invention will depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. It is to be noted that dosage values may include single or multiple doses, and that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Further, the dosage regimen with the compositions of this invention may be based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the subject, the severity of the condition, the route of administration, and the particular antibody employed. Thus, the dosage regimen can vary widely, but can be determined routinely using standard methods. For example, doses may be adjusted based on pharmacokinetic or pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. Thus, the present invention encompasses intra-subject dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

[0159] For administration to human subjects, the total monthly dose of the GRP94 Ab-IFN fusion molecules of the invention can be in the range of 0.002-500 mg per individual, 0.002-400 mg per individual, 0.002-300 mg per individual, 0.002-200 mg per individual, 0.002-100 mg per individual, 0.002-50 mg per individual, 0.006-500 mg per individual, 0.006-400 mg per individual, 0.006-300 mg per individual, 0.006-200 mg per individual, 0.006-100 mg per individual, 0.006-50 mg per individual, 0.02-500 mg per individual, 0.02-400 mg per individual,

0.02-300 mg per individual, 0.02-200 mg per individual, 0.02-100 mg per individual, 0.02-50 mg per individual, 0.06-500 mg per individual, 0.06-400 mg per individual, 0.06-300 mg per individual, 0.06-200 mg per individual, 0.06-100 mg per individual, 0.06-50 mg per individual, 0.2-500 mg per individual, 0.2-400 mg per individual, 0.2-300 mg per individual, 0.2-200 mg per individual, 0.2-100 mg per individual, 0.2-50 mg per individual, 0.6-500 mg per individual, 0.6-400 mg per individual, 0.6-300 mg per individual, 0.6-200 mg per individual, 0.6-100 mg per individual, or 0.6-50 mg per individual, 2-500 mg per individual, 2-400 mg per individual, 2-300 mg per individual, 2-200 mg per individual, 2-100 mg per individual, 2-50 mg per individual, 6-500 mg per individual, 6-400 mg per individual, 6-300 mg per individual, 6-200 mg per individual, 6-100 mg per individual, or 6-50 mg per individual, depending, of course, on the mode of administration. The total monthly dose can be administered in single or divided doses and can, at the physician's discretion, fall outside of the typical ranges given herein.

[0160] An exemplary, non-limiting weekly dosing range for a therapeutically effective amount of the GRP94 Ab-IFN fusion molecules of the invention can be about 0.0001 to about 0.9 mg/kg, about 0.0001 to about 0.8 mg/kg, about 0.0001 to about 0.7 mg/kg, about 0.0001 to about 0.6 mg/kg, about 0.0001 to about 0.5 mg/kg, about 0.0001 to about 0.4 mg/kg, about 0.0001 to about 0.3 mg/kg, about 0.0001 to about 0.2 mg/kg, about 0.0001 to about 0.1 mg/kg, about 0.0003 to about 0.9 mg/kg, about 0.0003 to about 0.8 mg/kg, about 0.0003 to about 0.7 mg/kg, about 0.0003 to about 0.6 mg/kg, about 0.0003 to about 0.5 mg/kg, about 0.0003 to about 0.4 mg/kg, about 0.0003 to about 0.3 mg/kg, about 0.0003 to about 0.2 mg/kg, about 0.0003 to about 0.1 mg/kg, about 0.001 to about 0.9 mg/kg, about 0.001 to about 0.8 mg/kg, about 0.001 to about 0.7 mg/kg, about 0.001 to about 0.6 mg/kg, about 0.001 to about 0.5 mg/kg, about 0.001 to about 0.4 mg/kg, about 0.001 to about 0.3 mg/kg, about 0.001 to about 0.2 mg/kg, about 0.0001 to about 0.1 mg/kg, about 0.003 to about 0.9 mg/kg, about 0.003 to about 0.8 mg/kg, about 0.003 to about 0.7 mg/kg, about 0.003 to about 0.6 mg/kg, about 0.003 to about 0.5 mg/kg, about 0.003 to about 0.4 mg/kg, about 0.003 to about 0.3 mg/kg, about 0.003 to about 0.2 mg/kg, about 0.003 to about 0.1 mg/kg, about 0.01 to about 0.9 mg/kg, about 0.01 to about 0.8 mg/kg, about 0.01 to about 0.7 mg/kg, about 0.01 to about 0.6 mg/kg, about 0.01 to about 0.5 mg/kg, about 0.01 to about 0.4 mg/kg, about 0.01 to about 0.3 mg/kg, about 0.01 to about 0.2 mg/kg, about 0.01 to about 0.1 mg/kg, about 0.03 to about 0.9 mg/kg, about 0.03 to about 0.8 mg/kg, about 0.03 to about 0.7 mg/kg, about 0.03 to about 0.6 mg/kg, about 0.03 to about 0.5 mg/kg, about 0.03 to about 0.4 mg/kg, about 0.03 to about 0.3 mg/kg, about 0.03 to about 0.2 mg/kg, about 0.03 to about 0.1 mg/kg, about 0.1 to about 0.9 mg/kg, about 0.1 to about 0.8 mg/kg, about 0.1 to about 0.7 mg/kg, about 0.1 to about 0.6 mg/kg, about 0.1 to

about 0.5 mg/kg, about 0.1 to about 0.4 mg/kg, about 0.1 to about 0.3 mg/kg, about 0.1 to about 0.2 mg/kg, about 0.3 to about 0.9 mg/kg, about 0.3 to about 0.8 mg/kg, about 0.3 to about 0.7 mg/kg, about 0.3 to about 0.6 mg/kg, about 0.3 to about 0.5 mg/kg, about 0.3 to about 0.4 mg/kg, about 0.3 to about 0.3 mg/kg, about 0.3 to about 0.2 mg/kg, about 0.3 to about 0.1 mg/kg.

[0161] In various embodiments, the GRP94 Ab-IFN fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of about 0.0001 to about 0.0003 mg/kg, about 0.0003 to about 0.001 mg/kg, about 0.001 to about 0.003 mg/kg, about 0.003 to about 0.01 mg/kg, about 0.01 to about 0.03 mg/kg, about 0.03 to about 0.1 mg/kg, about 0.1 to about 0.3. In various embodiments, the GRP94 Ab-IFN fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[0162] In various embodiments, the weekly dose for a therapeutically effective amount of a GRP94 Ab-IFN fusion molecule of the invention will be no greater than 0.0001 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a fusion molecule of the invention will be no greater than 0.0003 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a GRP94 Ab-IFN fusion molecule of the invention will be no greater than 0.001 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a GRP94 Ab-IFN fusion molecule of the invention will be no greater than 0.003 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a GRP94 Ab-IFN fusion molecule of the invention will be no greater than 0.01 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a GRP94 Ab-IFN fusion molecule of the invention will be no greater than 0.03 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a GRP94 Ab-IFN fusion molecule of the invention will be no greater than 0.1 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a GRP94 Ab-IFN fusion molecule of the invention will be no greater than 0.3 mg/kg body weight. In various embodiments the fusion molecules will be administered via intravenous (IV) infusion for up to three cycles of eight once weekly doses.

[0163] In various embodiments, single or multiple administrations of the pharmaceutical compositions are administered depending on the dosage and frequency as required and tolerated by the individual. In any event, the composition should provide a sufficient quantity of at least one of the fusion molecules disclosed herein to effectively treat the individual. The dosage can be administered once but may be applied periodically until either a therapeutic result is achieved or until side effects warrant discontinuation of therapy.

[0164] The dosing frequency of the administration of the GRP94 Ab-IFN fusion molecule pharmaceutical composition depends on the nature of the therapy and the particular disease being treated. The individual can be treated at regular intervals, such as weekly or monthly, until a desired therapeutic result is achieved. Exemplary dosing frequencies include, but are not limited to: once weekly without break; once weekly, every other week; once every 2 weeks; once every 3 weeks; weakly without break for 2 weeks, twice weekly without break for 2 weeks, twice weekly without break for 3 weeks, twice weekly without break for 4 weeks, twice weekly without break for 5 weeks, twice weekly without break for 6 weeks, twice weekly without break for 7 weeks, twice weekly without break for 8 weeks, monthly; once every other month; once every three months; once every four months; once every five months; or once every six months, or yearly. In various embodiments the fusion molecules will be administered via intravenous (IV) infusion for up to three cycles of eight once weekly doses of about 0.0001 to about 0.0003 mg/kg. In various embodiments the fusion molecules will be administered via intravenous (IV) infusion for up to three cycles of eight once weekly doses of about 0.0003 to about 0.001 mg/kg. In various embodiments the fusion molecules will be administered via intravenous (IV) infusion for up to three cycles of eight once weekly doses of about 0.001 to about 0.003 mg/kg. In various embodiments the fusion molecules will be administered via intravenous (IV) infusion for up to three cycles of eight once weekly doses of about 0.003 to about 0.01 mg/kg. In various embodiments the fusion molecules will be administered via intravenous (IV) infusion for up to three cycles of eight once weekly doses of about 0.01 to about 0.03 mg/kg. In various embodiments the fusion molecules will be administered via intravenous (IV) infusion for up to three cycles of eight once weekly doses of about 0.03 to about 0.1 mg/kg. In various embodiments the fusion molecules will be administered via intravenous (IV) infusion for up to three cycles of eight once weekly doses of about 0.1 to about 0.3 mg/kg.

Therapeutic Methods of Use

[0165] In one aspect, the present invention relates to a method of inhibiting the growth and/or proliferation of a tumor cell that expresses or overexpresses GRP94, said method comprising contacting said cell with a non-naturally occurring fusion molecule in an amount sufficient to treat said cell, wherein the fusion molecule comprises an IFN molecule attached to an antibody or antigen-binding fragment that binds GRP94.

[0166] In various embodiments, there is provided a method of preferentially treating a cancer selected from the group consisting of: melanoma, breast cancer, renal cancer, glioma, lung cancer (small cell lung cancer and non-small cell lung cancer), colorectal cancer, hepatocellular cancer, ovarian cancer; pancreatic cancer, prostate cancer, esophageal cancer, gastric cancer, urinary bladder cancer; head and neck cancer, multiple myeloma, acute leukemias (e.g., 11q23-positive acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma (indolent and high grade forms), Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia, and myelodysplasia, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of at least about .0001 mg/kg, at least about .0003 mg/kg, at least about .001 mg/kg, at least about .003 mg/kg, at least about .01 mg/kg, at least about .03 mg/kg, at least about 0.1 mg/kg, at least about 0.2 mg/kg, at least about 0.3 mg/kg, at least about 0.4 mg/kg, at least about 0.5 mg/kg, at least about 0.6 mg/kg, at least about 0.7 mg/kg, at least about 0.8 mg/kg, and at least about 0.9 mg/kg. In various embodiments, the anti-GRP94-IFN- α fusion molecule is administered to the individual at a dosage (e.g., at a weekly dosage) included in any of the following ranges: about 0.0001 to about 0.0003 mg/kg, about 0.0003 to about 0.001 mg/kg, about 0.001 to about 0.003 mg/kg, about 0.003 to about 0.01 mg/kg, about 0.01 to about 0.03 mg/kg, about 0.03 to about 0.1 mg/kg, about 0.1 to about 0.3 mg/kg, about 0.3 to about 0.4 mg/kg, about 0.4 to about 0.5 mg/kg, about 0.5 to about 0.6 mg/kg, about 0.6 to about 0.7 mg/kg, about 0.7 to about 0.8 mg/kg, and about 0.8 to about 0.9 mg/kg. In various embodiments, the anti-GRP94-IFN- α fusion molecule is administered to the individual at a dosage (e.g., at a weekly dosage) of no greater than about any of: .0001 mg/kg, .0003 mg/kg, .001 mg/kg, .003 mg/kg, .01 mg/kg, .03 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, and 0.9 mg/kg.

[0167] Multiple myeloma is a hematological malignancy characterized by the clonal proliferation and accumulation of immunoglobulin-producing plasma B cells in bone marrow, causing the progressive destruction of bone tissue and bone marrow. Other features of multiple myeloma include: low blood counts (e.g., red blood cells, platelets, white blood cells), bone and calcium problems, infections, kidney problems). If left untreated, the condition ultimately leads to the death of the individual. The stage of multiple myeloma is one of the most important factors in evaluating treatment options. There are currently two ways of staging multiple myeloma, both of which divide myeloma into three stages indicated by Roman numerals I-III. These two multiple myeloma staging systems differ in the factors that are evaluated: 1) the Durie-Salmon System which considers the levels of monoclonal immunoglobulin, calcium and hemoglobin in the blood as well as the number of bone lesions (indicating the severity of bone damage); and 2) the International Staging System which relies on two main factors to stage multiple myeloma: the levels of albumin and beta-2-microglobulin in the blood.

[0168] Multiple myeloma can be staged as follows:

Smoldering myeloma (also called asymptomatic myeloma), a slow-growing type of multiple myeloma, is characterized by increased plasma cells in the bone marrow and the presence of monoclonal proteins, without the presence of symptoms.

Stage I - In stage I, tests indicate there are a relatively small number of myeloma cells. The levels of beta-2 microglobulin may be slightly higher than normal and the levels of albumin (a water soluble protein) and hemoglobin may have decreased.

Stage II - an intermediate stage of multiple myeloma that is determined if the levels tested fall between the standards set for stage I and stage III.

Stage III - the number of myeloma cells is considered high. The most advanced stage of multiple myeloma is characterized by high levels of beta-2 microglobulin and low levels of albumin and hemoglobin. Calcium levels are high, indicating large amount of bone destruction and X-rays show more than three areas of bone destruction

[0169] As used herein, "refractory and/or relapsed multiple myeloma" is refractory to one or more of chemotherapy, and/or resistant to one or more of chemotherapy or other therapy, and/or relapsed after treatment with one or more of chemotherapy or other therapy, where the chemotherapy includes, without limitation, monotherapy and combination therapy involving cyclophosphamide, dexamethasone, doxorubicin, etoposide, interferon-alpha,

melphalan, pegylated interferon-alpha, vincristine, and the like, corticosteroids, such as prednisone, dexamethasone (e.g., decadron), and the like, and immune modulating agents such as thalidomide, lenalidomide (Revlimid®, Celgene), and bortezomib (Velcade®, Millennium Pharmaceuticals), and the like. Various combinations of such agents intended for treating multiple myeloma is well known to the skilled artisan. For example, and without limitation, such combination chemotherapy include revlimid/melphalan/prednisone, revlimid/decadron, velcade/decadron, velcade/revlimid/decadron, and EPOCH (etoposide, prednisone, vincristine (oncovin), doxorubicin, and cyclophosphamide). As used herein, "other therapy" includes, without limitation, radiation therapy and autologous stem cell transplant therapy. For example, and without limitation, such radiation therapy is preferably administered to the humerus, thoracic/lumbar spine and/or the sacrum.

[0170] In various embodiments, the present invention relates to a method of treating multiple myeloma in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[0171] In various embodiments, the present invention relates to a method of treating multiple myeloma in an individual who has been diagnosed as having smoldering myeloma, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0172] In various embodiments, the present invention relates to a method of treating multiple myeloma in an individual who has been diagnosed as having Stage I multiple myeloma, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0173] In various embodiments, the present invention relates to a method of treating multiple myeloma in an individual who has been diagnosed as having Stage II multiple myeloma, comprising administering to the individual a therapeutically effective amount of a

fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0174] In various embodiments, the present invention relates to a method of treating multiple myeloma in an individual who has been diagnosed as having Stage III multiple myeloma, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0175] In various embodiments, the present invention relates to a method of treating relapsed multiple myeloma in an individual, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0176] In various embodiments, the present invention relates to a method of treating refractory multiple myeloma in an individual, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0177] AML affects a group of white blood cells called the myeloid cells, which normally develop into the various types of mature blood cells, such as red blood cells, white blood cells and platelets. AML is characterized by an increase in the number of myeloid cells in the marrow and an arrest in their maturation, frequently resulting in hematopoietic insufficiency (granulocytopenia, thrombocytopenia, or anemia), with or without leukocytosis. The stage of AML is one of the most important factors in evaluating treatment options. Most cancers are staged based on the size and spread of tumors. However, because leukemia already occurs in the developing blood cells within the bone marrow, leukemia staging is a little bit different. The stages of leukemia are often characterized by blood cell counts and the accumulation of leukemia cells in other organs, like the liver or spleen. Two of the main systems that have been used to classify AML into subtypes are the French-American-British (FAB) classification and the newer World Health Organization (WHO) classification.

[0178] Using the FAB classification, AML can be subtyped and staged in eight subtypes, M0 through M7, based on: the number of healthy blood cells; the size and number of leukemia cells; the changes that appear in the chromosomes of the leukemia cells; and any other genetic abnormalities that have occurred. The eight AML stages are classified as follows:

Undifferentiated AML - M0: In this stage of acute myelogenous leukemia, the bone marrow cells show no significant signs of differentiation.

Myeloblastic leukemia - M1: Bone marrow cells show some signs of granulocytic differentiation with or without minimal cell maturation.

Myeloblastic leukemia - M2: Maturation of the bone marrow cells is beyond the promyelocyte (early granulocyte) stage. Varying amounts of granulocyte maturation may be observed.

Promyelocytic leukemia - M3: Most of the abnormal cells are early granulocytes, between myeloblasts and myelocytes in their stage of development. The cells contain many small particles and have nucleuses of varying size and shape.

Myelomonocytic leukemia - M4: In this stage of acute myelogenous leukemia, the bone marrow and circulating blood have variable amounts of monocytes and differentiated granulocytes in them. The percentage of monocytes and promonocytes in the bone marrow is greater than 20 percent. There may also be an increased number of granular leukocytes called eosinophils, a type of granulocyte that often has a two-lobed nucleus.

Monocytic leukemia - M5: This subset is further divided into two different categories. The first is characterized by poorly differentiated monoblasts with lacy-appearing genetic material. The second subset is characterized by a large number of monoblasts, promonocytes and monocytes. The proportion of monocytes in the bloodstream may be higher than that in the bone marrow.

Erythroleukemia - M6: This form of leukemia is characterized by abnormal red blood cell-forming cells, which make up over half of the nucleated cells in the bone marrow.

Megakaryoblastic leukemia - M7: The blast cells in this form of leukemia look like immature megakaryocytes (giant cells of the bone marrow) or lymphoblasts (lymphocyte-forming cells). M7 leukemia may be distinguished by extensive fibrous tissue deposits (fibrosis) in the bone marrow.

[0179] Although the FAB classification is still commonly used to group AML into subtypes, the World Health Organization (WHO) has proposed a new acute myelogenous leukemia staging system in an attempt to more clearly communicate the individual's prognosis (outlook). This system divides AML into broad groups:

Acute myelogenous leukemia with certain genetic abnormalities: Cellular testing is capable of identifying specific genetic abnormalities: 1) AML with a translocation between chromosomes 8 and 21; 2) AML with a translocation or inversion in

chromosome 16; 3) AML with changes in chromosome 11; and 4) APL (M3), which usually has translocation between chromosomes 15 and 17.

AML with multi-lineage dysplasia: More than one type of abnormal myeloid cell is involved.

AML from previous chemotherapy/radiation

AML not otherwise specified: This includes AML cases that don't fall into any of the above groups, such as:

Undifferentiated AML - M0

AML with Minimal Maturation - M1

AML with Maturation - M2

Acute Myelomonocytic Leukemia - M4

Acute Monocytic Leukemia - M5

Acute Erythroid Leukemia - M6

Acute Megakaryoblastic Leukemia - M7

Acute Basophilic Leukemia

Acute Panmyelosis with Fibrosi

Myeloid Sarcoma - Also called Granulocytic Sarcoma or Chloroma

Undifferentiated/biphenotypic acute leukemias: This type of leukemia may exhibit features of both lymphocytic and myelogenous leukemia. This may also be referred to as ALL with myeloid markers, AML with lymphoid markers or mixed lineage leukemia.

[0180] As used herein, "refractory and/or relapsed AML" is refractory to one or more of chemotherapy, and/or resistant to one or more of chemotherapy or other therapy, and/or relapsed after treatment with one or more of chemotherapy or other therapy, where the chemotherapy includes, without limitation, monotherapy and combination therapy, involving ,e.g, chemotherapy, stem cell transplantation, and agents under investigation which include arsenic trioxide (Trisenox®), all-trans retinoic acid (ATRA), cytotoxic drugs such as clofarabine, as well as targeted therapies, such as farnesyl transferase inhibitors, decitabine, and inhibitors of MDR1 (multidrug-resistance protein).

[0181] In various embodiments, the present invention relates to a method of treating AML in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than

.0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[0182] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML M0, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0183] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML M1, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0184] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML M2, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0185] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML M3, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0186] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML M4, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0187] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML M5, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN

molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0188] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML M6, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0189] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML M7, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0190] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML with a translocation between chromosomes 8 and 21, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0191] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML with a translocation or inversion in chromosome 16, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0192] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML with changes in chromosome 11, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0193] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having APL (M3), comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0194] In various embodiments, the present invention relates to a method of treating an individual who has been diagnosed as having AML with lymphoid markers or mixed lineage

leukemia, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0195] In various embodiments, the present invention relates to a method of treating relapsed AML in an individual, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0196] In various embodiments, the present invention relates to a method of treating refractory AML in an individual, comprising administering to the individual a therapeutically effective amount of a fusion molecule that comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94.

[0197] In various embodiments, the present invention relates to a method of treating non-small cell lung cancer (NSCLC) in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[0198] In various embodiments, the present invention relates to a method of treating melanoma in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[0199] In various embodiments, the present invention relates to a method of treating pancreatic cancer in an individual, comprising administering to the individual an therapeutically effective amount of an anti-GRP94-IFN- α fusion molecule that specifically binds glycosylated

GRP94, wherein the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

[0200] In various embodiments, the present invention provides for methods of inhibiting the growth or proliferation of tumor cells which express the GRP94 antigen in an individual comprising administering an effective amount of the GRP94 Ab-IFN fusion molecule of the present invention to the individual. These methods may inhibit or prevent the growth of the tumor cells of said individual, such as for example, by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%. As a result, where the tumor is a solid tumor, the modulation may reduce the size of the solid tumor by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%.

[0201] The inhibition of the tumor cell proliferation can be measured by cell-based assays, such as bromodeoxyuridine (BRDU) incorporation (Hoshino et al., *Int. J. Cancer* 38, 369, 1986; Campana et al., *J. Immunol. Meth.* 107:79, 1988; [3 H]-thymidine incorporation (Chen, J., *Oncogene* 13:1395-403, 1996; Jeoung, J., *J. Biol. Chem.* 270:18367-73, 1995; the dye Alamar Blue (available from Biosource International) (Voytik-Harbin et al., *In Vitro Cell Dev Biol Anim* 34:239-46, 1998); the MTT and MTS tetrazolium salts (available from Promega) (Mosmann, T., *J. Immunol. Meth.* 65:55-63, 1983; Khabar, K. et al., *J. Interferon and Cytokine Res.* 16:31-33, 1996). The anchorage independent growth of tumor cells is assessed by colony formation assay in soft agar, such as by counting the number of tumor cell colonies formed on top of the soft agar (see Examples and Sambrook et al., *Molecular Cloning*, Cold Spring Harbor, 1989).

[0202] The inhibition of tumor cell growth in a subject may be assessed by monitoring the tumor growth in a subject, for example in an animal model or in human subjects. One exemplary monitoring method is tumorigenicity assays. In one example, a xenograft comprises human cells from a pre-existing tumor or from a tumor cell line. Tumor xenograft assays are known in the art and described herein (see, e.g., Ogawa et al., *Oncogene* 19:6043-6052, 2000).

In another embodiment, tumorigenicity is monitored using the hollow fiber assay, which is described in U.S. Patent No. 5,698,413, which is incorporated herein by reference in its entirety.

[0203] The percentage of the inhibition is calculated by comparing the tumor cell proliferation, anchorage independent growth, or tumor cell growth under modulator treatment with that under negative control condition (typically without modulator treatment). For example, where the number of tumor cells or tumor cell colonies (colony formation assay), or PRDU or [³H]-thymidine incorporation is A (under the treatment of modulators) and C (under negative control condition), the percentage of inhibition would be $(C-A)/Cx100\%$.

[0204] Examples of tumor cell lines derived from human tumors and available for use in the *in vitro* and *in vivo* studies include, but are not limited to, leukemia cell lines (e.g., CCRF-CEM, HL-60(TB), K-562, MOLT-4, SR, P388 and P388/ADR), AML cell lines (e.g., AML-193, KG-1, Kasumi-1, and Kasumi-6 AML cells); and multiple myeloma cell lines (e.g., XG-1, XG-2, OPM-1, OPM-2, S6B45, Delta 47, 8266/Dox40, 8266/S, NCI-H929, ANBL-6, MM144, MM.1s, U266, RPMI 8226, and OCI-My 5), NSCLC tumor cells NCI-H1299 (ATCC CRL-5803).

Immunotherapy

[0205] Cancer immunotherapy is enjoying a renaissance, and in the past few years the rapidly advancing field has produced several new methods of treating cancer. Numerous cancer immunotherapy strategies have been the focus of extensive research and clinical evaluation including, but not limited to, treatment using depleting antibodies to specific tumor antigens (see, e.g., reviews by Blattman and Greenberg, *Science*, 305:200, 2004; Adams and Weiner, *Nat Biotech*, 23:1147, 2005; Vogal et al. *J Clin Oncology*, 20:719, 2002; Colombat et al., *Blood*, 97:101, 2001); treatment using antibody-drug conjugates (see, e.g., Ducry, Laurent (Ed.) *Antibody Drug Conjugates*. In: *Methods in Molecular Biology*. Book 1045. New York (NY), Humana Press, 2013; *Nature Reviews Drug Discovery* 12, 259-260, April 2013); treatment using agonistic, antagonistic, or blocking antibodies to co-stimulatory or co-inhibitory molecules (immune checkpoints) such as CTLA-4 (ipilimumab), PD-1 (nivolumab; pembrolizumab; pidilizumab) and PD-L1 (BMS-936559; MPLD3280A; MEDI4736; MSB0010718C)(see, e.g., Philips and Atkins, *International Immunology*, 27(1); 39-46, Oct 2014), OX-40, CD137, GITR, LAG3, TIM-3, and VISTA (see, e.g., Sharon et al., *Chin J Cancer*., 33(9): 434–444, Sep 2014; Hodi et al., *N Engl J Med*, 2010; Topalian et al., *N Engl J Med*, 366:2443-54, 2012); treatment using bispecific T cell engaging antibodies (BiTE®) such as blinatumomab (see, e.g., US Pat. No. 9,260,522; US Patent Application No. 20140302037); treatment involving administration of

biological response modifiers such as IL-2, IL-12, IL-15, IL-21, GM-CSF, IFN- α , IFN- β , and IFN- γ (see, e.g., Sutlu T et al., *Journ of Internal Medicine*, 266(2):154-181, 2009; Joshi S *PNAS USA*, 106(29):12097-12102, 2009; Li Y et al., *Journal of Translational Medicine*, 7:11, 2009); treatment using therapeutic vaccines such as sipuleucel-T (see, e.g., Kantoff PW *New England Journal of Medicine*, 363(5):411-422, 2010; Schlom J., *Journal of the National Cancer Institutes*, 104(8):599-613, 2012); treatment using dendritic cell vaccines, or tumor antigen peptide vaccines; treatment using chimeric antigen receptor (CAR)-T cells (see, e.g., Rosenberg SA *Nature Reviews Cancer*, 8(4):299-308, 2008; Porter DL et al, *New England Journal of Medicine*, 365(8):725-733, 2011; Grupp SA et al., *New England Journal of Medicine*, 368(16):1509-151, 2013); treatment using CAR-NK cells (see, e.g., Glienke et al., *Front Pharmacol*, 6(21):1-7, Feb 2015); treatment using tumor infiltrating lymphocytes (TILs)(see e.g., Wu et al, *Cancer J.*, 18(2): 160-175, 2012); treatment using adoptively transferred anti-tumor T cells (ex vivo expanded and/or TCR transgenic)(see e.g., Wrzesinski et al., *J Immunother*, 33(1): 1-7, 2010); treatment using TALL-104 cells; and treatment using immunostimulatory agents such as Toll-like receptor (TLR) agonists CpG and imiquimod (see, e.g., Krieg, *Oncogene*, 27:161-167, 2008; Lu, *Front Immunol*, 5(83):1-4, March 2014).

[0206] Immunotherapy focused on utilization of depleting antibodies to specific tumor antigens have been explored with much success (see, e.g., reviews by Blattman and Greenberg, *Science*, 305:200, 2004; Adams and Weiner, *Nat Biotech*, 23:1147, 2005). A few examples of such tumor antigen-specific, depleting antibodies are HERCEPTIN® (anti-Her2/neu mAb)(Baselga et al., *J Clin Oncology*, Vol 14:737, 1996; Baselga et al., *Cancer Research*, 58:2825, 1998; Shak, *Semin. Oncology*, 26 (Suppl12):71, 1999; Vogal et al. *J Clin Oncology*, 20:719, 2002); and RITUXAN® (anti-CD20 mAb)(Colombat et al., *Blood*, 97:101, 2001). Unfortunately, while clearly having made a mark in oncology treatment, as monotherapy they generally work in only about 30% of the individuals and with a partial response. Moreover, many individuals eventually become refractory or relapse after treatment with these antibody-containing regimens.

[0207] Treatment using agonistic, antagonistic, or blocking antibodies to co-stimulatory or co-inhibitory molecules (immune checkpoints) has been an area of extensive research and clinical evaluation. Under normal physiological conditions, immune checkpoints are crucial for the maintenance of self-tolerance (that is, the prevention of autoimmunity) and protect tissues from damage when the immune system is responding to pathogenic infection. It is now also clear that tumors co-opt certain immune-checkpoint pathways as a major mechanism of immune resistance, particularly against T cells that are specific for tumor antigens (Pardoll DM., *Nat Rev*

Cancer, 12:252-64, 2012). Accordingly, treatment utilizing antibodies to immune checkpoint molecules including, e.g., CTLA-4 (ipilimumab), PD-1 (nivolumab; pembrolizumab; pidilizumab) and PD-L1 (BMS-936559; MPLD3280A; MEDI4736; MSB0010718C)(see, e.g., Philips and Atkins, *International Immunology*, 27(1); 39-46, Oct 2014), and OX-40, CD137, GITR, LAG3, TIM-3, and VISTA (see, e.g., Sharon et al., *Chin J Cancer.*, 33(9): 434–444, Sep 2014; Hodi et al., *N Engl J Med*, 2010; Topalian et al., *N Engl J Med*, 366:2443-54) are being evaluated as new, alternative immunotherapies to treat patients with proliferative diseases such as cancer, and in particular, patients with refractory and/or recurrent cancers.

[0208] Treatment using chimeric antigen receptor (CAR) T cell therapy is an immunotherapy in which the patient's own T cells are isolated in the laboratory, redirected with a synthetic receptor to recognize a particular antigen or protein, and reinfused into the patient. CARs are synthetic molecules that minimally contain: (1) an antigen-binding region, typically derived from an antibody, (2) a transmembrane domain to anchor the CAR into the T cells, and (3) 1 or more intracellular T cell signaling domains. A CAR redirects T cell specificity to an antigen in a human leukocyte antigen (HLA)-independent fashion, and overcomes issues related to T cell tolerance (Kalos M and June CH, *Immunity*, 39(1):49-60, 2013). Over the last 5 years, at least 15 clinical trials of CAR-T cell therapy have been published. A new wave of excitement surrounding CAR-T cell therapy began in August 2011, when investigators from the University of Pennsylvania (Penn) published a report on 3 patients with refractory chronic lymphocytic leukemia (CLL) who had long-lasting remissions after a single dose of CAR T cells directed to CD 19 (Porter DL, et al., *N Engl J Med.*, 365(8):725-733, 2011).

[0209] In contrast to donor T cells, natural killer (NK) cells are known to mediate anti-cancer effects without the risk of inducing graft-versus-host disease (GvHD). Accordingly, alloreactive NK cells are now also the focus of considerable interest as suitable and powerful effector cells for cellular therapy of cancer. Several human NK cell lines have been established, e.g., NK-92, HANK-1, KHYG-1, NK-YS, NKG, YT, YTS, NKL and NK3.3 (Kornbluth, J., et al., *J. Immunol.* 134, 728–735, 1985; Cheng, M. et al., *Front. Med.* 6:56, 2012) and various CAR expressing NK cells (CAR-NK) have been generated. Immunotherapy using CAR expressing NK cells (CAR-NK) is an active area of research and clinical evaluation (see, e.g., Glienke et al., *Front Pharmacol*, 6(21):1-7, Feb 2015).

[0210] Bispecific T-cell engager molecules (BiTE®s) constitute a class of bispecific single-chain antibodies for the polyclonal activation and redirection of cytotoxic T cells against pathogenic target cells. BiTE®s are bispecific for a surface target antigen on cancer cells, and for CD3 on T cells. BiTE®s are capable of connecting any kind of cytotoxic T cell to a cancer

cell, independently of T-cell receptor specificity, costimulation, or peptide antigen presentation. a unique set of properties that have not yet been reported for any other kind of bispecific antibody construct, namely extraordinary potency and efficacy against target cells at low T-cell numbers without the need for T-cell co-stimulation (Baeuerle et al., *Cancer Res*, 69(12):4941–4, 2009). BiTE antibodies have so far been constructed to more than 10 different target antigens, including CD19, EpCAM, Her2/neu, EGFR, CD66e (or CEA, CEACAM5), CD33, EphA2, and MCSP (or HMW-MAA)(Id.) Treatment using BiTE® antibodies such as blinatumomab (Nagorsen, D. et al., *Leukemia & Lymphoma* 50(6): 886–891, 2009) and solitomab (Amann et al., *Journal of Immunotherapy* 32(5): 452–464, 2009) are being clinically evaluated.

Methods of Combination Therapy

[0211] In another aspect, the present invention relates to combination therapies designed to treat a cancer that expresses or overexpresses GRP94 in an individual, comprising administering to the individual: a) a therapeutically effective amount of a GRP94 Ab-IFN fusion molecule, and b) a therapeutically effective amount of immunotherapy, wherein the combination therapy provides increased effector cell killing of tumor cells, i.e., a synergy exists between the GRP94 Ab-IFN fusion molecule and the immunotherapy when co-administered.

[0212] In various embodiments, there is provided a combination therapy method of treating cancer selected from the group consisting of NSCLC, acute myeloid leukemia, multiple myeloma, melanoma, and pancreatic cancer, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising an anti-GRP94 Ab-IFN- α fusion molecule; and b) immunotherapy; wherein the combination therapy provides increased effector cell killing. In various embodiments, the immunotherapy is treatment using agonistic, antagonistic, or blocking antibodies to co-stimulatory or co-inhibitory molecules. In various embodiments, the immunotherapy is treatment using chimeric antigen receptor (CAR)-T cells. In various embodiments, the immunotherapy is treatment using CAR-NK cells. In various embodiments, the immunotherapy is treatment using bispecific T cell engaging antibodies (BiTE®). In various embodiments, the anti-GRP94-IFN- α fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no

greater than 0.9 mg/kg. In various embodiments, the cancer expresses GRP94. In various embodiments, the cancer is a non-GRP94 expressing cancer in the tumor microenvironment of a GRP94 expressing cancer. In various embodiments, the immunotherapy will target a TAA that is different than GRP94.

[0213] In various embodiments, the combination therapy methods comprise administering the GRP94 Ab-IFN fusion molecule and immunotherapy simultaneously, either in the same pharmaceutical composition or in separate pharmaceutical compositions. Alternatively, the GRP94 Ab-IFN fusion molecule and immunotherapy are administered sequentially, i.e., the GRP94 Ab-IFN fusion molecule is administered either prior to or after the immunotherapy.

[0214] In various embodiments, the administration of the GRP94 Ab-IFN fusion molecule and immunotherapy are concurrent, i.e., the administration period of the GRP94 Ab-IFN fusion molecule and immunotherapy overlap with each other.

[0215] In various embodiments, the administrations of the GRP94 Ab-IFN fusion molecule and immunotherapy are non-concurrent. For example, in some embodiments, the administration of the GRP94 Ab-IFN fusion molecule is terminated before the immunotherapy is administered. In some embodiments, the administration of immunotherapy is terminated before the GRP94 Ab-IFN fusion molecule is administered.

[0216] In various embodiments, the methods described herein may be used in combination with other conventional anti-cancer therapeutic approaches directed to treatment or prevention of proliferative disorders, such approaches including, but not limited to chemotherapy, small molecule kinase inhibitor targeted therapy, surgery, radiation therapy, and stem cell transplantation. For example, such methods can be used in prophylactic cancer prevention, prevention of cancer recurrence and metastases after surgery, and as an adjuvant of other conventional cancer therapy. The present disclosure recognizes that the effectiveness of conventional cancer therapies (e.g., chemotherapy, radiation therapy, phototherapy, immunotherapy, and surgery) can be enhanced through the use of the fusion molecules described herein.

[0217] A wide array of conventional compounds has been shown to have anti-neoplastic activities. These compounds have been used as pharmaceutical agents in chemotherapy to shrink solid tumors, prevent metastases and further growth, or decrease the number of malignant T-cells in leukemic or bone marrow malignancies. Although chemotherapy has been effective in treating various types of malignancies, many anti-neoplastic compounds induce undesirable side effects. It has been shown that when two or more different treatments are

combined, the treatments may work synergistically and allow reduction of dosage of each of the treatments, thereby reducing the detrimental side effects exerted by each compound at higher dosages. In other instances, malignancies that are refractory to a treatment may respond to a combination therapy of two or more different treatments.

[0218] When the GRP94 Ab-IFN fusion molecule disclosed herein is administered in combination with another conventional anti-neoplastic agent, either concomitantly or sequentially, such fusion molecule may enhance the therapeutic effect of the anti-neoplastic agent or overcome cellular resistance to such anti-neoplastic agent. This allows decrease of dosage of an anti-neoplastic agent, thereby reducing the undesirable side effects, or restores the effectiveness of an anti-neoplastic agent in resistant T-cells. In various embodiments, a second anti-cancer agent, such as a chemotherapeutic agent, will be administered to the patient. The list of exemplary chemotherapeutic agent includes, but is not limited to, daunorubicin, dactinomycin, doxorubicin, bleomycin, mitomycin, nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, bendamustine, cytarabine (CA), 5-fluorouracil (5-FU), floxuridine (5-FUdR), methotrexate (MTX), colchicine, vincristine, vinblastine, etoposide, teniposide, cisplatin, carboplatin, oxaliplatin, pentostatin, cladribine, cytarabine, gemcitabine, pralatrexate, mitoxantrone, diethylstilbestrol (DES), fluradabine, ifosfamide, hydroxyureataxanes (such as paclitaxel and doxorubicin) and/or anthracycline antibiotics, as well as combinations of agents such as, but not limited to, DA-EPOCH, CHOP, CVP or FOLFOX. In various embodiments, the dosages of such chemotherapeutic agents include, but is not limited to, about any of 10 mg/m², 20 mg/m², 30 mg/m², 40 mg/m², 50 mg/m², 60 mg/m², 75 mg/m², 80 mg/m², 90 mg/m², 100 mg/m², 120 mg/m², 150 mg/m², 175 mg/m², 200 mg/m², 210 mg/m², 220 mg/m², 230 mg/m², 240 mg/m², 250 mg/m², 260 mg/m², and 300 mg/m².

[0219] These various combination therapies may provide a "synergistic effect", i.e., the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately.

Nucleic acid Molecules and Fusion Molecule Expression

[0220] The present application further provides nucleic acid molecules comprising nucleotide sequences encoding the recombinant, genetically engineered fusion molecules described herein. Because of the degeneracy of the genetic code, a variety of nucleic acid sequences encode each fusion molecule amino acid sequence. The application further

provides nucleic acid molecules that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined herein, to nucleic acid molecules that encode a fusion molecule. Stringent hybridization conditions include, but are not limited to, hybridization to filter-bound DNA in 6xSSC at about 45°C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65°C, highly stringent conditions such as hybridization to filter-bound DNA in 6xSSC at about 45°C followed by one or more washes in 0.1xSSC/0.2% SDS at about 60°C, or any other stringent hybridization conditions known to those skilled in the art (see, for example, Ausubel, F. M. et al., eds. 1989 Current Protocols in Molecular Biology, vol. 1, Green Publishing Associates, Inc. and John Wiley and Sons, Inc., NY at pages 6.3.1 to 6.3.6 and 2.10.3).

[0221] The nucleic acid molecules may be obtained, and the nucleotide sequence of the nucleic acid molecules determined by, any method known in the art. For example, if the nucleotide sequence of the fusion molecule is known, a nucleic acid molecule encoding the fusion molecule may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242, 1994), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR. In one embodiment, the codons that are used comprise those that are typical for human or mouse (see, e.g., Nakamura, Y., *Nucleic Acids Res.* 28: 292, 2000).

[0222] A nucleic acid molecule encoding a fusion molecule may also be generated from nucleic acid from a suitable source. For example, if a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably polyA+RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0223] In one embodiment of the present invention, nucleic acid sequences encoding the appropriate antibody framework are optionally cloned and ligated into appropriate vectors (e.g., expression vectors for, e.g., prokaryotic or eukaryotic organisms). Additionally, nucleic acid sequences encoding the appropriate interferon molecule are optionally cloned into the same vector in the appropriate orientation and location so that expression from the vector

produces an antibody-interferon molecule fusion molecule. Some optional embodiments also require post-expression modification, e.g., assembly of antibody subunits, etc. The techniques and art for the above (and similar) manipulations are well known to those skilled in the art. Pertinent instructions are found in, e.g., Sambrook et al., *Molecular Cloning--A Laboratory Manual* (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989 and *Current Protocols in Molecular Biology*, F. M. Ausubel et al., eds., *Current Protocols*, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (supplemented through 1999).

[0224] The present invention is also directed to host cells that express the fusion molecules of the invention. Host cells suitable for replicating and for supporting recombinant expression of fusion protein are well known in the art. Such cells may be transfected or transduced as appropriate with the particular expression vector and large quantities of vector containing cells can be grown for seeding large scale fermenters to obtain sufficient quantities of the protein for clinical applications. Such cells may include prokaryotic microorganisms, such as *E. coli*; various eukaryotic cells, such as Chinese hamster ovary cells (CHO), NSO, 293; HEK Yeast; insect cells; hybridomas; human cell lines; and transgenic animals and transgenic plants, and the like. Standard technologies are known in the art to express foreign genes in these systems. The recombinant protein gene is typically operably linked to appropriate expression control sequences for each host. For *E. coli* this includes a promoter such as the T7, trp, or lambda promoters, a ribosome binding site and preferably a transcription termination signal. For eukaryotic cells, the control sequences will include a promoter and preferably an enhancer derived from immunoglobulin genes, SV40, cytomegalovirus, etc., and a polyadenylation sequence, and may include splice donor and acceptor sequences.

[0225] To express an antibody-IFN fusion molecule recombinantly, a host cell is transformed, transduced, infected or the like with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and/or heavy chains of the antibody and attached interferon such that the light and/or heavy chains are expressed in the host cell. The heavy chain and the light chain may be expressed independently from different promoters to which they are operably-linked in one vector or, alternatively, the heavy chain and the light chain may be expressed independently from different promoters to which they are operably-linked in two vectors one expressing the heavy chain and one expressing the light chain. Optionally, the heavy chain and light chain may be expressed in different host cells.

[0226] Additionally, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody light and/or heavy chain from a host cell. The antibody light

and/or heavy chain gene can be cloned into the vector such that the signal peptide is operably-linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide. Preferably, the recombinant antibodies are secreted into the medium in which the host cells are cultured, from which the antibodies can be recovered or purified.

[0227] An isolated DNA encoding a HCVR can be converted to a full-length heavy chain gene by operably-linking the HCVR-encoding DNA to another DNA molecule encoding heavy chain constant regions. The sequences of human, as well as other mammalian, heavy chain constant region genes are known in the art. DNA fragments encompassing these regions can be obtained e.g., by standard PCR amplification. The heavy chain constant region can be of any type, (e.g., IgG, IgA, IgE, IgM or IgD), class (e.g., IgG₁, IgG₂, IgG₃ and IgG₄) or subclass constant region and any allotypic variant thereof as described in Kabat (supra).

[0228] An isolated DNA encoding a LCVR region may be converted to a full-length light chain gene (as well as to a Fab light chain gene) by operably linking the LCVR-encoding DNA to another DNA molecule encoding a light chain constant region. The sequences of human, as well as other mammalian, light chain constant region genes are known in the art. DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region.

[0229] Additionally, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and one or more selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced. For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin, or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (dhfr) gene (for use in dhfr-minus host cells with methotrexate selection/amplification), the neo gene (for G418 selection), and glutamine synthetase (GS) in a GS-negative cell line (such as NSO) for selection/amplification.

[0230] For expression of the light and/or heavy chains with attached interferon, the expression vector(s) encoding the heavy and/or light chains is introduced into a host cell by standard techniques e.g. electroporation, calcium phosphate precipitation, DEAE-dextran transfection, transduction, infection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, eukaryotic cells and most specifically mammalian host cells, are more typical because such cells are more likely to assemble and secrete a properly folded and immunologically active antibody. Mammalian host

cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) [including dhfr minus CHO cells, as described in Urlaub and Chasin, Proc. Natl. Acad. Sci. USA 77:4216-20, 1980, used with a DHFR selectable marker, e.g. as described in Kaufman and Sharp, J. Mol. Biol. 159:601-21, 1982], NSO myeloma cells, COS cells, and SP2/0 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown under appropriate conditions known in the art. Antibodies can be recovered from the host cell and/or the culture medium using standard purification methods.

[0231] Once expressed, the intact antibodies, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, ion exchange, affinity (e.g., Protein A), reverse phase, hydrophobic interaction column chromatography, hydroxyapatite chromatography, gel electrophoresis, and the like. Standard procedures for purification of therapeutic antibodies are described, for example, by Feng L1, Joe X. Zhou, Xiaoming Yang, Tim Tressel, and Brian Lee in an article entitled "Current Therapeutic Antibody Production and Process Optimization" (BioProcessing Journal, September/October 2005), for example. Additionally, standard techniques for removing viruses from recombinantly expressed antibody preparations are also known in the art (see, for example, Gerd Kern and Mani Krishnan, "Viral Removal by Filtration: Points to Consider" (Biopharm International, October 2006)). The effectiveness of filtration to remove viruses from preparations of therapeutic antibodies is known to be at least in part dependent on the concentration of protein and/or the antibody in the solution to be filtered. The purification process for antibodies of the present invention may include a step of filtering to remove viruses from the mainstream of one or more chromatography operations. Preferably, prior to filtering through a pharmaceutical grade nanofilter to remove viruses, a chromatography mainstream containing an antibody of the present invention is diluted or concentrated to give total protein and/or total antibody concentration of about 1 g/L to about 3 g/L. Even more preferably, the nanofilter is a DV20 nanofilter (e.g., Pall Corporation; East Hills, N.Y.). Substantially pure immunoglobulins of at least about 90%, about 92%, about 94% or about 96% homogeneity are preferred, and about 98 to about 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the sterile antibodies may then be used therapeutically, as directed herein.

[0232] In view of the aforementioned discussion, the present invention is further directed to a fusion molecule obtainable by a process comprising the steps of culturing a host cell including, but not limited to a mammalian, plant, bacterial, transgenic animal, or transgenic plant cell which has been transformed by a nucleic acid molecule or a vector comprising nucleic acid molecules encoding antibodies of the invention so that the nucleic acid is expressed and, optionally, recovering the antibody from the host cell culture medium.

Kits

[0233] In various embodiments, this invention provides for kits for the treatment of cancer and/or in an adjunct therapy. Kits typically comprise a container comprising a pharmaceutical composition containing a GRP94 Ab-IFN fusion molecule of the present invention. In various embodiments, the kits will comprise a container comprising a second cancer immunotherapy agent.

[0234] In addition the kits can optionally include instructional materials disclosing means of use of the GRP94 Ab-IFN fusion molecule to treat a cancer. The instructional materials may also, optionally, teach preferred dosages, counter-indications, and the like.

[0235] The kits can also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, and additionally comprise means for disinfecting a wound, for reducing pain, for attachment of a dressing, and the like.

[0236] While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

[0237] The following examples are provided to describe the invention in further detail.

Example 1

[0238] This example generally describes the preparation of genetically engineered fusion molecules comprising an interferon attached to an anti-GRP94 antibody, wherein the interferon is attached to the antibody via proteolysis resistant peptide linker. The fusion

molecules were initially constructed as depicted in Figure 1, with the interferon molecule attached via a linker to the C-terminus of heavy chain of the antibody. All of the fusion molecules described in these examples are prepared using recombinant DNA methods and techniques that are well known and understood by one of ordinary skill in the art.

[0239] The preparation of the GRP94 mAb-IFN fusion molecules for evaluation and testing as described in the Examples below can be generally described as follows: a vector encoding the heavy chain of the selected antibody is recombinantly engineered with an interferon, or interferon mutant, at the carboxy-terminus using a proteolysis resistant peptide linker. After verifying that the vector has the correct fusion molecule nucleotide sequence, it is transfected, along with a vector encoding the light chain of the selected antibody into mammalian cells, e.g., NSO, CHO, or HEK293. Transfectants are screened by ELISA for the production of the complete fusion molecule. The clone giving the highest signal is expanded and following sub-cloning is grown in roller bottles. Conditioned medium is collected, concentrated, and the protein of interest purified using a single Protein A affinity chromatography step or appropriate alternative chromatography methods. The final product is formulated in a desired buffer and at a desired concentration (the protein concentration is confirmed by UV absorption). The purity of the final product is determined by SDS-PAGE both under reducing and non-reducing conditions. Western blot analysis was used to confirm the expected size of the molecule.

[0240] Using the methods described above and using monoclonal Ab W9 (mAb that comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 3 and a light chain having the amino acid sequence set forth in SEQ ID NO: 4), the following GRP94 Ab-IFN fusion molecules were prepared: 1) an interferon molecule having the amino acid sequence set forth in SEQ ID NO: 5 was attached to the C-terminus of the mAb W9 heavy chain using a linker having the amino acid sequence of SEQ ID NO: 10 (this fusion hereinafter referred to as "IGN004-wt" or "IGN004"); 2) an interferon mutant molecule having the amino acid sequence set forth in SEQ ID NO: 6 was attached to the C-terminus of the mAb W9 heavy chain using a linker having the amino acid sequence of SEQ ID NO: 10 (this fusion hereinafter referred to as "IGN004-M8"); 3) an interferon molecule having the amino acid sequence set forth in SEQ ID NO: 7 was attached to the C-terminus of the mAb W9 heavy chain using a linker having the amino acid sequence of SEQ ID NO: 10 (this fusion hereinafter referred to as "IGN004- α 14"); and 4) an interferon molecule having the amino acid sequence set forth in SEQ ID NO: 7 was attached to the C-terminus of the mAb W9 heavy chain using a linker having the amino acid sequence of SEQ ID NO: 11 (this fusion hereinafter referred to as "IGN004- α 14-alpha").

[0241] The fusion molecules prepared as described above are evaluated and tested using the various *in vitro* functional assays and *in vivo* assays described in the Examples below.

Example 2

[0242] In this example, the expression of GRP94 on various solid tumor and hematological cancer cell lines was assessed by flow cytometry using IGN004-wt fusion molecule from Example 1. Cells were incubated with IGN004-wt at 2 μg per 10^6 cells for 1 hour on ice. After incubation, cells were washed twice then bound mAb was detected with anti-human IgG(Fc)-FITC Ab. Samples were analyzed by flow cytometry using a Beckman Coulter Cytomics FC500 or Galios flow cytometer instrument and data analyzed using WinMDI software. Controls included IGN004-wt followed by an IgG2a, κ -FITC isotype control and anti-human IgG (Fc-specific)-FITC alone. The flow cytometry results are depicted in Table 3. IGN004-wt bound to the majority of tumor cell lines investigated, including melanoma, NSCLC, AML, and MM.

Table 3

Tumor Type	# Positive Cell Types
AML	10/10
LUNG	6/10
MELANOMA	5/5
BREAST	3/5
MULTIPLE MYELOMA	3/4
OVARIAN	2/3
COLORECTAL	2/2

[0243] The expression of GRP94 on various primary human tumors and patient-derived human xenograft tumors (grown in immunodeficient mice) was also evaluated using Immunohistochemistry (IHC) and using IGN004-wt. The IHC results are depicted in Table 4.

Table 4

Primary Tumor	# Positive Tumors
LUNG	10/10
LUNG PDX	50/54
MELANOMA	7/7
PANCREATIC	4/7
PANCREATIC PDX	40/43
BREAST	2/5
CANCER	2/5
COLORECTAL	1/5

[0244] Again, IGN004-wt bound to nearly 100% of the primary solid tumor samples tested by IHC.

Example 3

[0245] In this example, the STAT1 phosphorylation and proliferation inhibition activities of IGN004-wt were compared to non-fused IFN- α 2b in a non-targeted and a targeted setting, respectively.

[0246] For the non-targeted STAT1 phosphorylation experiment, Daudi NHL tumor cells (GRP94-negative) were incubated with the indicated concentration of IGN004-wt or IFN- α 2b for 15 minutes, then cells were fixed, permeabilized and intracellularly stained with PE-labeled anti-STAT1 (pY701) or PE-labeled isotype control. After washing, samples were analyzed by flow cytometry. Dose response curves were generated by non-linear regression analysis using Prism software. For the targeted proliferation inhibition experiment, GRP94-positive NCI-H1299 NSCLC tumor cells (ATCC CRL-5803) were treated with the indicated concentration of IGN004-wt or IFN- α 2b for 96 hours at 37 °C in a 5% CO₂ atmosphere. After incubation, standard MTS assay (Promega Cell Titer96; Promega, Madison, WI) was performed to assess cellular proliferation. Dose response curves were generated by non-linear regression analysis using Prism software.

[0247] As depicted in Figure 2, IGN004-wt relative IFN activity was reduced on antigen-negative cells (Daudi) and enhanced on antigen-positive cells (NCI-H1299). The STAT1-phosphorylation activity was attenuated by 54-fold, compared to non-fused IFN- α 2b (EC_{50} (IFN- α 2b) = 0.154 nM and EC_{50} (IGN004-wt) = 8.39 nM), on Daudi cells that do not express the

antibody target antigen. The proliferation inhibition activity was enhanced by 275-fold, compared to non-fused IFN- α 2b (EC_{50} (IFN- α 2b) = 30.3 pM and EC_{50} (IGN004-wt) = 0.11 pM), on NCI-H1299 cells that express the antibody target antigen.

Example 4

[0248] In this example, the expression of GRP94 on various multiple myeloma cells was assessed by flow cytometry using the human multiple myeloma cell lines NCI-H929, MM.1s, and U266.

[0249] Cells were incubated with mAb W9 at 2 μ g per 10^6 cells for 1 hour on ice. After washing unbound mAb, bound mAb W9 was detected using anti-human IgG (Fc-specific)-FITC diluted 1:5. Samples were analyzed by flow cytometry using a Beckman Coulter Cytomics FC500 or Galios flow cytometer instrument and data analyzed using WinMDI software. Controls included mAb W9 followed by an IgG2a, κ -FITC isotype control and anti-human IgG (Fc-specific)-FITC alone.

[0250] As depicted in Figure 3, all three multiple myeloma cell lines demonstrated surface expression of GRP94 with NCI-H929 and MM.1s having the highest intensity of staining. This demonstrates that human multiple myeloma tumors can express GRP94 on their surface.

Example 5

[0251] In this example, the expression of GRP94 on various AML cells was assessed by flow cytometry using the human AML cell lines KG-1, AML-193, Kasumi-1, and Kasumi-6.

[0252] Cells were incubated with mAb W9 at 2 μ g per 10^6 cells for 1 hour on ice. After washing unbound mAb, bound mAb W9 was detected using anti-human IgG (Fc-specific)-FITC diluted 1:5. Samples were analyzed by flow cytometry using a Beckman Coulter Cytomics FC500 or Galios flow cytometer instrument and data analyzed using WinMDI software. Controls included mAb W9 followed by an IgG2a, κ -FITC isotype control and anti-human IgG (Fc-specific)-FITC alone.

[0253] As depicted in Figure 4, all four AML cell lines demonstrated surface expression of GRP94 with a similar high intensity of staining. This demonstrates that human AML tumors can express GRP94 on their surface.

Example 6

[0254] In this example, fusion molecules IGN004-wt, IGN004-M8, IGN004- α 14 and IGN004- α 14-alpha were assessed for the ability to inhibit the proliferation of various multiple myeloma cell lines.

[0255] The anti-proliferative activity of various IGN004 fusion molecules versus non-fused IFN- α 2b-wt was investigated in three different multiple myeloma cell lines. MM.1s (A,B), U266 (C,D), and NCI-H929 (E,F) cells were seeded in 96-well tissue culture plates at a density of $1-2 \times 10^4$ cells per well and serial dilutions of different fusion proteins added. After 96 hours incubation at 37°C in a 5% CO₂ atmosphere, proliferation was assessed by MTS assay. 20 μ L of MTS solution (Promega, Madison, WI) was added to each well and absorbance at 490 nm was read on an ELISA plate reader and data analyzed in GraphPad Prism with nonlinear regression analysis using variable slope sigmoidal dose response curve fit, and the EC₅₀ values were determined.

[0256] As depicted in Figure 5 and Table 5, against all three multiple myeloma cell lines IGN004-wt fusion molecule or IGN004- α 14 fusion molecule inhibited proliferation to a greater degree than non-fused rIFN- α 2b-wt (13-47-fold lower EC₅₀). The IGN004-M8 fusion molecule demonstrated 2.2 – 2.9-fold reduced anti-proliferative potency compared to non-fused rIFN- α 2b-wt. The linker used for the fusion molecule constructs containing IFN- α 14 did not affect the anti-proliferative potency, as both IGN004- α 14 and IGN004- α 14-alpha had equivalent potency.

Table 5

Cell Line	IGN004- α 14-alpha	IGN004-wt	IGN004-M8	IGN004- α 14	rIFN- α 2b-wt
MM.1s	0.78 pM	2.9 pM	107 pM	0.89 pM	37 pM
U266	0.15 pM	0.095 pM	ND	0.16 pM	2.7 pM
NCI-H929	1.7 pM	1.1 pM	65 nM	1.3 pM	30 pM

Anti-proliferative potency (EC₅₀) of IGN004 fusion molecules tested in Figure 4 was determined by nonlinear regression analysis using GraphPad Prism software. ND = not determined due to poor curve fit.

Example 7

[0257] In this example, fusion molecules IGN004-wt, IGN004-M8, IGN004- α 14 and IGN004- α 14-alpha were assessed for the ability to inhibit the proliferation of various AML cell lines.

[0258] The anti-proliferative activity of various IGN004 fusion molecules versus non-fused rIFN- α 2b-wt was investigated in three different AML cell lines. AML-193 (A,B), KG-1 (C,D), and Kasumi-6 (E,F) cells were seeded in 96-well tissue culture plates at a density of $1-2 \times 10^4$ cells per well and serial dilutions of different fusion proteins added. After 96 hours incubation at 37°C in a 5% CO₂ atmosphere, proliferation was assessed by MTS assay. 20 μ L of MTS solution was added to each well and absorbance at 490 nm was read on an ELISA plate reader and data analyzed in GraphPad Prism with nonlinear regression analysis using variable slope sigmoidal dose response curve fit, and the EC₅₀ values were determined.

[0259] As depicted in Figure 6 and Table 6, against all three AML cell lines IGN004- α 14-alpha fusion molecule, IGN004-wt fusion molecule, and IGN004- α 14 fusion molecule inhibited proliferation to a greater degree than non-fused rIFN- α 2b-wt (52- to 7917-fold lower EC₅₀). The IGN004-M8 fusion molecule demonstrated 16-fold increased anti-proliferative potency compared to non-fused rIFN- α 2b-wt against the AML-193 cell line, but reduced potency compared to non-fused rIFN- α 2b-wt against KG-1 and Kasumi-6 AML cells.

Table 6

Cell Line	IGN004- α 14-alpha	IGN004-wt	IGN004-M8	IGN004- α 14	rIFN- α 2b-wt
AML-193	0.044 pM	0.012 pM	5.9 pM	0.017 pM	95 pM
KG-1	2 pM	6 pM	0.79 nM	0.71 pM	0.31 nM
Kasumi-6	0.01 pM	0.01 pM	>10 nM	0.061 pM	14 pM

Anti-proliferative potency (EC₅₀) of IGN004 fusion molecules tested in Figure 5 was determined by nonlinear regression analysis using GraphPad Prism software.

Example 8

[0260] In this example, the *in vivo* anti-tumor activity of mAb W9 and IGN004-wt was investigated in a xenograft model of human multiple myeloma where U266 tumors were grown in NOG (NOD/Shi-*scid*/IL-2R γ^{null}) immunodeficient mice (Ito et al, Blood, 100(9): 3175-82, 2002).

[0261] In this study, groups of 8 11-day established subcutaneous tumor-bearing animals (average tumor volume = 138 mm³) were treated intravenously, twice per week, for 4 weeks with 5, 1 or 0.2 mg/kg mAb W9 or IGN004-wt fusion protein. Vehicle (PBS) treatment served as a negative control. Animals treated with PBS had a median survival time of 39.5 days, and treatment with 0.2 mg/kg of mAb W9 and IGN004-wt did not significantly extend

survival (median survival = 38 days and 41 days, respectively) (Figure 7). However, treatment with 1 mg/kg mAb W9 and IGN004-wt significantly improved survival, with median survival of 47 days ($p = 0.02$ vs. PBS) and 73 days ($p = 0.0002$ vs. PBS), respectively. Treatment of animals with 5 mg/kg mAb W9 resulted in prolonged survival (median survival = 84.5 days; $p = 0.0002$ vs. PBS) and complete regression of established tumors in 6 of 8 mice, though for a short duration before tumor relapse. Treatment of animals with 5 mg/kg IGN004-wt resulted in complete regression of established tumors in 100% of the mice with a much longer duration of response than mAb W9, with only 3 of 8 tumors relapsing after initial regression ($p = 0.0002$ vs. PBS). Comparing the anti-tumor efficacy of the two treatment proteins, there was no difference in efficacy at the 0.2 mg/kg dose level ($p = 0.2$ for mAb W9 vs. IGN004-wt). However, at the 1 mg/kg and the 5 mg/kg dose levels, IGN004-wt had superior anti-tumor efficacy, compared to mAb W9 ($p = 0.02$ and 0.0002 , respectively). These data demonstrate that IGN004-wt fusion protein can effectively treat human multiple myeloma xenograft tumors and that there is a significant enhancement in *in vivo* anti-tumor activity of mAb W9 by fusing wild-type IFN- α 2b to the C-terminus via a short peptide linker.

Example 9

[0262] In this example, the *in vivo* anti-tumor activity of IGN004-wt fusion molecule was investigated in a xenograft model of human AML where KG-1 tumors were grown in NOD-SCID (NOD.CB17-Prkdc^{scid}) immunodeficient mice (Schultz et al, J Immunol, 154(1): 180-91, 1995).

[0263] In this study, groups of 8 4-day established subcutaneous tumor-bearing mice (average tumor volume = 103 mm³) were treated intravenously, twice per week, for 4 weeks with 5 or 1 mg/kg mAb IGN004-wt fusion protein. Vehicle (PBS) treatment served as a negative control. Treatment with 5 mg/kg IGN004-wt caused regression of all tumors, including two complete regressions by day 27 ($p < 0.0001$ vs. vehicle control for tumor volume at day 27) (Figure 8). Treatment with 1 mg/kg IGN004-wt caused a significant delay in tumor progression ($p = 0.003$ vs. vehicle control for tumor volume at day 27). These data demonstrate that IGN004-wt fusion protein can effectively treat human AML xenograft tumors.

Example 10

[0264] In this example, the *in vivo* anti-tumor activity of IGN004-wt fusion molecule was investigated in a patient-derived xenograft model of human AML where primary human AML

tumor cells were engrafted in NSG (NOD/LtSz-Prkdc^{scid}/IL-2R γ ^{null}) immunodeficient mice (Schultz et al, J Immunol, 174(10): 6477-89, 2005).

[0265] In this study, groups of 5 mice confirmed to be engrafted by FLT3-ITD slow primary human AML cells (The Jackson Laboratory) by flow cytometry were treated intraperitoneally, twice per week, for 3 weeks with 0.2 mg/kg mAb IGN004-wt fusion protein. Vehicle (PBS) treatment served as a negative control. The percentage of human CD33-positive AML cells in the blood was determined weekly by flow cytometry of peripheral blood samples from all mice. On day 21 post treatment initiation, animals were sacrificed and blood, spleen, and bone marrow samples were obtained and analyzed by flow cytometry for the presence of human CD33-positive AML tumor cells. While the level of human AML tumor cells in the blood of control mice increased over time, treatment with IGN004-wt caused a reduction in the percentage of human CD33-positive AML cells (Figure 9A). While there was a reduction in the percentage of human CD33-positive AML cells in the spleen at day 21 in animals treated with IGN004-wt compared to vehicle (43.6% vs. 60.8%, respectively) this result did not reach statistical significance ($p = 0.2$) (Figure 9B). However, IGN004-wt treatment caused a highly significant reduction in the percentage of human CD33-positive AML cells in the bone marrow, compared to control mice (0.8% vs. 80.3%, respectively; $p = 0.001$) (Figure 9C). These data demonstrate that IGN004-wt fusion protein can effectively treat patient-derived human AML xenograft tumors.

Example 11

[0266] In this example, the in vivo anti-tumor activity of IGN004-wt was assessed against a panel of 15 different human NSCLC patient-derived xenograft (PDX) tumors grown in immunodeficient mice.

[0267] Groups of 5 BALB/c nude immunodeficient mice bearing established NSCLC PDX tumors with an average tumor volume of 150 mm³ were treated with either PBS or 2 mg/kg IGN004-wt intravenously twice per week for the duration of the experiment. Tumor size was measured bidirectionally using calipers twice weekly, and tumor volume calculated using the formula: $V = 0.5 a \times b^2$ where a and b are the long and short diameters of the tumor, respectively. Average tumor volume at each time point for each tumor model was plotted using Excel software (Microsoft) and efficacy of IGN004-wt was sorted into 4 categories: +++ = tumor regression, ++ = stable disease, + = slowing of tumor growth, and - = no response.

[0268] As depicted in Table 7, IGN004-wt demonstrated in vivo efficacy on 10/15 PDX

tumors (66.7%), including tumor regression in 4 tumor models. There did not appear to be a correlation with known gene mutations nor NSCLC tumor type and response to treatment. These results show that GRP94 Ab-IFN fusion molecules like IGN004-wt can be highly effective against clinically-relevant NSCLC PDX tumors, even in the absence of immune cells which may potentially play a role in the mechanism of action of GRP94 Ab-IFN fusion molecules in human cancer patients.

Table 7

Model #	Type	Mutations	Efficacy
1	Adenosquamous	p53	+++
2	Adenocarcinoma	ND	+++
3	Large-cell carcinoma	ND	+++
4	Squamous cell carcinoma	p53, ALK	+++
5	Squamous cell carcinoma	AKT, p53	++
6	Squamous cell carcinoma	EGFR	++
7	Adenocarcinoma	EGFR, p53	+
8	Adenocarcinoma	EGFR	+
9	Adenosquamous	c-Met	+
10	Adenocarcinoma	p53	+
11	Squamous cell carcinoma	ND	-
12	Adenocarcinoma	ND	-
13	Squamous cell carcinoma	p53	-
14	Squamous cell carcinoma	p53, PTEN	-
15	Squamous cell carcinoma	p53	-

Example 12

[0269] In this example, the tumor cell killing activity of the human CD8+ NKT cell-like TALL-104 effector cell line (ATCC CRL-11386) was assessed in the presence or absence of IGN004 using the A549 human NSCLC tumor cell line (ATCC CCL-185).

[0270] TALL-104 cells growing in 300 U/mL IL-2 were washed twice to remove IL-2 and placed back into culture overnight. A549 tumor cells were plated in 24-well plates and incubated overnight at 37°C in a 5% CO₂ atmosphere. The next day, cells were incubated with 3 nM IGN004 for 4 hours then wells were washed to remove unbound protein. After overnight incubation in the absence of IL-2, TALL-104 effector cells were then added to the wells containing A549 tumor cells to achieve an effector:target ratio (E:T) of 5:1. Co-cultures were incubated for 24 hours at 37°C in a 5% CO₂ atmosphere then viability of the tumor cells was assessed by standard MTS assay after washing away the non-adherent effector cells. Wells were washed twice and then 0.5 mL of 4:1 mix RPMI + 10% FBS and Promega Cell Titer96 was added and incubated for 1 hour at 37°C. Media was transferred to a 96 well plate and the plate was read at 490 nm using a spectrophotometer. Data was plotted in GraphPad Prism taking untreated tumor cells as 100% cell control and the mix of media and Cell Titer incubated for 1 hour at 37°C as 0% cell control. Controls included A549 tumor cells alone, A549 tumor cells + IGN004 (no effectors), and A549 tumor cells + TALL-104 effector cells (no IGN004). Plates were set up with quadruplicate samples.

[0271] As depicted in Figure 10, IGN004 treatment caused a small decrease in the viability of the A549 tumor cells (15.82%). TALL-104 effector cells demonstrated robust killing in the absence of IGN004 (69.2%). However, the combination of IGN004 and TALL-104 cells lead to complete eradication of A549 tumor cells (100% killing). This effect was stronger than the combination of either agent alone (85.02% vs. 100%), leading to the conclusion that IGN004 and TALL-104 can have a synergistic effect upon A549 tumor cells leading to much more robust tumor cell killing.

Example 13

[0272] In this example, the tumor cell killing activity of TALL-104 effector cells was assessed in the presence or absence of IGN004 at two different E:T ratios using a different human NSCLC tumor cell line (NCI-H1975; ATCC CRL-5908).

[0273] TALL-104 cells growing in 300 U/mL IL-2 were washed twice to remove IL-2 and placed back into culture overnight. NCI-H1975 tumor cells were plated in 24-well plates and

incubated overnight at 37 °C in a 5% CO₂ atmosphere. The next day, cells were incubated with 50 pM IGN004 for 4 hours then wells were washed to remove unbound protein. After overnight incubation in the absence of IL-2, TALL-104 effector cells were then added to the wells containing tumor cells to achieve an E:T ratio of 5:1 or 3.3:1. Co-cultures were incubated for 48 hours at 37 °C in a 5% CO₂ atmosphere then viability of the tumor cells was assessed as described previously in Example 1. Controls included NCI-H1975 tumor cells alone, NCI-H1975 tumor cells + IGN004 (no effectors), and NCI-H1975 tumor cells + TALL-104 effector cells (no IGN004). Plates were set up with duplicate samples.

[0274] As depicted in Figure 11, IGN004 treatment caused a small decrease in the viability of the A549 tumor cells (5.7% and 10.6%). TALL-104 effector cells demonstrated significant killing in the absence of IGN004 and both 5:1 and 3.3:1 E:T ratios (58.6% and 55.7%, respectively). However, the combination of 50 pM IGN004 and TALL-104 cells lead to much more effective killing of the NCI-H1975 tumor cell targets at both E:T ratios (93.8% and 93.2%, respectively). This effect was stronger than the combination of either agent alone, leading to the conclusion that IGN004 and TALL-104 can have a synergistic effect upon NCI-H1975 tumor cells leading to much more robust tumor cell killing.

Example 14

[0275] In this example, the potency of the TALL-104 tumor cell killing enhancement by IGN004 was assessed using NCI-H1975 NSCLC tumor cells.

[0276] Co-cultures were set up in 24-well plates as described in Examples 6 and 7 using NCI-H1975 tumor cells as targets and TALL-104 cells as effectors, after incubating the tumor cells with the indicated concentration of IGN004 for 3 hours. Unbound IGN004 was washed away prior to adding effector cells to achieve an E:T ratio of 3.3:1. Incubation time for the co-cultures was 48 hours at 37 °C.

[0277] As depicted in Figure 12, TALL-104 effector cells killed 17% of the NCI-H1975 tumor cells in the absence of IGN004 co-treatment. Treatment with IGN004 in combination with TALL-104 cells at concentrations from 0.25 to 25 pM caused an increase in tumor cell killing, compared to TALL-104 treatment alone. This result demonstrates that the enhancement in immune cell killing is a very potent effect and can occur at very low concentrations of drug.

Example 15

[0278] In this example, the tumor cell killing activity of downregulated TALL-104 effector cells was assessed on A549 NSCLC tumor cells in the presence or absence of 10 pM IGN004 at different E:T ratios.

[0279] Co-cultures were set up in 24-well plates as described in Examples 6 and 7 using A549 tumor cells as targets and TALL-104 cells as effectors, after incubating the tumor cells with 10 pM IGN004 for 3 hours. Unbound IGN004 was washed away prior to adding effector cells to achieve an E:T ratio of 3:1, 1.5:1, or 0.75:1. The TALL-104 cells were washed and IL-2 removed from the media 2 days prior to the assay setup in an effort to reduce their activation status and killing activity. Incubation time for the co-cultures was 5 days at 37°C.

[0280] As depicted in Figure 13, 10 pM IGN004 alone had no effect on the tumor cells. At the 3:1 E:T ratio TALL-104 cells killed approximately 40% of the A549 tumor cells in the absence of drug but at lower E:T ratios the effector cells were ineffective at tumor cell killing. In the presence of 10 pM IGN004 the TALL-104 cells demonstrated robust tumor cell killing, even at 0.75:1 E:T where TALL-104 had no effect on the tumor cells without drug. These results demonstrate that IGN004 is able to reverse the downregulation in killing activity of TALL-104 effector cells achieved by IL-2 starvation.

Example 16

[0281] In this example, the tumor cell killing activity of TALL-104 effector cells was assessed in the presence or absence of IGN004 or IGN004 non-fused mAb.

[0282] Co-cultures were set up in 24-well plates as described in Examples 6 and 7 using A549 tumor cells as targets and TALL-104 cells as effectors, after incubating the tumor cells with 10 pM of either IGN004 or IGN004 non-fused mAb for 3 hours. Unbound protein was washed away prior to adding effector cells to achieve an E:T ratio of 1.5:1, 0.75:1, or 0.375:1. The TALL-104 cells were washed and IL-2 removed from the media 2 days prior to the assay setup. Incubation time for the co-cultures was 4 days at 37°C.

[0283] As depicted in Figure 14, 10 pM IGN004 non-fused mAb alone had no effect on the tumor cells and 10 pM IGN004 had only a slight effect (<10%). At all E:T ratios TALL-104 cells demonstrated a low level of tumor cell killing in the absence of drug. In the presence of 10 pM IGN004 mAb, the TALL-104 cells killed at an equivalent rate to TALL-104 cells without drug. However, with 10 pM IGN004 there was a significant increase in the tumor cell killing by TALL-104 cells, compared to no drug (70-80% vs. 10-20% killing). These results demonstrate that the

antibody portion of the IGN004 is not solely responsible for the synergistic effects on TALL-104-mediated tumor cell killing.

Example 17

[0284] In this example, the tumor cell killing activity of TALL-104 effector cells was assessed in the presence or absence of IGN004, a control TAA Ab-IFN- α fusion protein, or the combination of IGN004 non-fused mAb + non-fused IFN- α .

[0285] Co-cultures were set up in 24-well plates as described in Examples 6 and 7 using A549 tumor cells as targets and TALL-104 cells as effectors, after incubating the tumor cells with 10 pM of either IGN004, control TAA Ab-IFN- α fusion protein, or the combination of IGN004 non-fused mAb + non-fused IFN- α 2b for 3 hours. Effector cells were then added without washing away treatment protein to achieve an E:T ratio of 1:1 or 1.5:1. The TALL-104 cells were washed and IL-2 removed from the media 2 days prior to the assay setup. Incubation time for the co-cultures was 5 days at 37 °C.

[0286] As depicted in Figure 15, 10 pM control antibody-IFN- α 2b alone had no effect on the tumor cells. 10 pM IGN004 or the combination of IGN004 non-fused mAb and non-fused IFN- α 2b had only a slight effect (<10%). At both E:T ratios TALL-104 cells demonstrated a low level of tumor cell killing in the absence of drug (<10%). In the presence of 10 pM control TAA Ab-IFN- α fusion the TALL-104 cells killed at an equivalent rate to TALL-104 cells without drug. With 10 pM of the combination of IGN004 mAb + non-fused IFN- α 2b the TALL-104 effector cells killed more A549 tumor cells (14% and 25% increase in killing at 1:1 and 1.5:1 E:T, respectively). However, with 10 pM IGN004 there was a much higher increase in the tumor cell killing by TALL-104 cells, compared to no drug (34% and 42% increase in killing at 1:1 and 1.5:1, respectively). These results demonstrate that the TAA Ab-IFN- α fusion protein must bind to the tumor cell to exert its function of enhancing immune cell killing of tumor cells, and that the TAA Ab and IFN must be fused together to have the complete effect. Therefore, the enhancement in immune cell function should only occur at sites where the antibody target antigen is expressed.

Example 18

[0287] In this example, the tumor cell killing activity of the NK effector cell line NK-92 (ATCC CRL-2407) was assessed in the presence or absence of IGN004 or a control TAA-Ab-

IFN- α fusion protein at two E:T ratios using the OVCAR-3 ovarian cancer cell line (ATCC HTB-161).

[0288] The NK-92 tumor cell killing assay was performed similarly to the TALL-104 killing assays described in Examples 6 and 7. Co-cultures were set up in 24-well plates using OVCAR-3 tumor cells as targets and NK-92 cells as effectors, after incubating the tumor cells with 10 pM of either IGN004 or control TAA Ab-IFN- α fusion protein for 3 hours. Effector cells were then added without washing away treatment protein to achieve an E:T ratio of 1.5:1 or 0.5:1. The NK-92 cells were washed and IL-2 removed from the media 1 day prior to the assay setup. Incubation time for the co-cultures was 2 days at 37°C.

[0289] As depicted in Figure 16, 10 pM of either treatment protein had no effect on the tumor cells in the absence of effector cells. NK-92 effector cells demonstrated robust killing of tumor cells in the absence of drug at 1.5:1 E:T ratio (49% killing) and modest killing at 0.5:1 (19% killing). In the presence of 10 pM control TAA Ab-IFN- α fusion the NK-92 cells killed at an equivalent rate to effector cells without drug. With 10 pM IGN004 there was a significant increase in the tumor cell killing by NK-92 cells, compared to no drug (45% and 29% increase in killing at 1.5:1 and 0.5:1, respectively). These results demonstrate that IGN004 is able to enhance the NK cell mediated killing of tumor cells, and that the TAA Ab-IFN fusion protein must bind to the tumor cell to exert this function. Therefore, the enhancement in immune cell function should only occur at sites where the antibody target antigen is expressed.

Example 19

[0290] In this example, the tumor cell killing activity of the NK-92 effector cells was assessed in the presence or absence of IGN004 or non-fused IFN- α 2b at two E:T ratios using NCI-H1975 NSCLC tumor cells.

[0291] The NK-92 tumor cell killing assay was performed as described in Example 12. Co-cultures were set up in 24-well plates using NCI-H1975 cells as targets and NK-92 cells as effectors, after incubating the tumor cells with 10 pM IGN004 or 100 pM non-fused IFN- α 2b for 3 hours. Effector cells were then added without washing away treatment protein to achieve an E:T ratio of 1:1 or 0.3:1. The NK-92 cells were washed and IL-2 removed from the media 1 day prior to the assay setup. Incubation time for the co-cultures was 4 days at 37°C.

[0292] As depicted in Figure 17, treatment with either protein had no effect on the tumor cells in the absence of effector cells. NK-92 effector cells demonstrated little to no killing of tumor cells in the absence of drug. In the presence of 100 pM non-fused IFN- α 2b the NK-92

cells killed more tumor cells than NK-92 cells in the absence of drug. With 10 pM IGN004 there was a significant increase in the tumor cell killing by NK-92 cells, compared to no drug (85% and 62% increase in killing at 1:1 and 0.3:1, respectively) and non-fused IFN- α 2b (50% and 51% increase in killing at 1:1 and 0.3:1, respectively). These results demonstrate that IGN004 is able to enhance the NK cell mediated killing of tumor cells, and that the TAA Ab-IFN fusion protein mediates this effect much better than non-fused IFN- α 2b demonstrating the importance of targeting of the IFN to the tumor cell surface via TAA antibody.

Example 20

[0293] This Example describes the use of the methods described herein to treat an individual who has been diagnosed with multiple myeloma. To test the safety and clinical efficacy of the methods described herein in human individuals, individuals diagnosed with multiple myeloma, individuals with relapsed multiple myeloma, or individuals with refractory multiple myeloma are identified and randomized to a treatment group.

[0294] Treatment groups are treated with a fusion molecule of the present invention. Individuals receive weekly doses of, e.g., between 0.0001 mg/kg and 1.0 mg/kg body weight fusion molecule. In one exemplary study, treatment will commence with an dose-escalation stage where individuals are treated with 0.0001 mg/kg, or 0.0003 mg/kg, or 0.001 mg/kg, or 0.003 mg/kg, or 0.01 mg/kg, or 0.03 mg/kg, or 0.1 mg/kg body weight (2 doses weekly) administered via intravenous (IV) infusion for weeks 1-2, followed by a 6 week treatment-free interval, until the maximum tolerated dose (MTD) is identified. The dose-escalation stage is followed by an expansion stage comprising up to 24 weekly doses at the MTD (administered in three 8-week cycles) with no treatment-free interval.

[0295] Tumor response will be assessed at the end of each 8-week cycle in both stages using the Lugano Classification based on CT imaging (and BM biopsy, as applicable). Subject disposition at the end of Cycles 1 and 2 guided by tumor response as follows:

- a) Progressive disease (PD): withdrawn from the study
- b) Stable disease (SD) or partial response (PR): continue to the next cycle
- c) Complete response (CR) at the end of Cycle 1 or 2: treated for 4 weeks in the next cycle and undergo repeat tumor assessment at Cycle Week 4
 - if a subject remains with CR during Cycle Week 4, study drug will be discontinued (i.e., after the 4th dose of Cycle 2 or 3)

- if a subject has SD or PR during Cycle Week 4, study drug will be continued for the duration of the 8-week cycle, and tumor response will be reassessed during Cycle Week 8.

Example 21

[0296] This Example describes the use of the methods described herein to treat an individual who has been diagnosed with AML. To test the safety and clinical efficacy of the methods described herein in human individuals, individuals diagnosed with AML, individuals with relapsed AML, or individuals with refractory AML are identified and randomized to a treatment group.

[0297] Treatment groups are treated with a fusion molecule of the present invention. Individuals receive weekly doses of, e.g., between 0.0001 mg/kg and 1.0 mg/kg body weight fusion molecule. In one exemplary study, treatment will commence with an dose-escalation stage where individuals are treated with 0.0001 mg/kg, or 0.0003 mg/kg, or 0.001 mg/kg, or 0.003 mg/kg, or 0.01 mg/kg, or 0.03 mg/kg, or 0.1 mg/kg body weight (2 doses weekly) administered via intravenous (IV) infusion for weeks 1-2, followed by a 6 week treatment-free interval, until the maximum tolerated dose (MTD) is identified. The dose-escalation stage is followed by an expansion stage comprising up to 24 weekly doses at the MTD (administered in three 8-week cycles) with no treatment-free interval.

[0298] Tumor response will be assessed at the end of each 8-week cycle in both stages using the Lugano Classification based on CT imaging (and BM biopsy, as applicable). Subject disposition at the end of Cycles 1 and 2 guided by tumor response as follows:

- d) Progressive disease (PD): withdrawn from the study
- e) Stable disease (SD) or partial response (PR): continue to the next cycle
- f) Complete response (CR) at the end of Cycle 1 or 2: treated for 4 weeks in the next cycle and undergo repeat tumor assessment at Cycle Week 4
 - if a subject remains with CR during Cycle Week 4, study drug will be discontinued (i.e., after the 4th dose of Cycle 2 or 3)
 - if a subject has SD or PR during Cycle Week 4, study drug will be continued for the duration of the 8-week cycle, and tumor response will be reassessed during Cycle Week 8.

[0299] All of the articles and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present invention. While the articles and methods of this invention have been described in terms of various embodiments, it will be apparent to those of skill in the art that variations may be applied to the articles and methods without departing from the spirit and scope of the invention. All such variations and equivalents apparent to those skilled in the art, whether now existing or later developed, are deemed to be within the spirit and scope of the invention as defined by the appended claims. All patents, patent applications, and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All patents, patent applications, and publications are herein incorporated by reference in their entirety for all purposes and to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in its entirety for any and all purposes. The invention illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein. Thus, for example, in each instance herein any of the terms “comprising”, “consisting essentially of”, and “consisting of” may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by various embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

Sequence Listings

The amino acid sequences listed in the accompanying sequence listing are shown using standard three letter code for amino acids, as defined in 37 C.F.R. 1.822.

SEQ ID NO: 1 is the amino acid sequence of endoplasmin (GRP94).

SEQ ID NO: 2 is an exemplary nucleic acid sequence encoding GRP94.

SEQ ID NO: 3 is the amino acid sequence of the heavy chain of an anti-GRP94 antibody.

SEQ ID NO: 4 is the amino acid sequence encoding the light chain of an anti-GRP94 antibody.

SEQ ID NO: 5 is the amino acid sequence of a human wildtype IFN- α 2b molecule.

SEQ ID NO: 6 is the amino acid sequence of a IFN- α 2b mutant molecule.

SEQ ID NO: 7 is the amino acid sequence of a wildtype IFN- α 14 molecule.

SEQ ID NO: 8 is the amino acid sequence of a wildtype IFN- β -1a molecule.

SEQ ID NO: 9 is the amino acid sequence of a wildtype IFN- β -1b molecule.

SEQ ID NOs: 10-20 are the amino acid sequences of various peptide linkers.

SEQUENCE LISTINGS

SEQ ID NO: 1 - Amino acid sequence of an exemplary human endoplasmin (GRP94)

MRALWVLGLCCVLLTFGSVRADDEVDVDGTVEEDLGKSREGSRTDDEVVQREEEAIQLDGLN
ASQIRELREKSEKFAFQAEVNRMMKLIINSLYKNKEIFLRELISNASDALDKIRLISLTDENALSGN
EELTVKIKCDKEKNLLHVTDTGVGMTREELVKNLGTIAKSGTSEFLNKMTEAQEDGQSTSELIG
QFGVGFYSAFLVADKIVITSKHNNDTQHIWESDSNEFSVIADPRGNTLGRGTTITLVLKEEASD
YLELDTIKNLVKKYSQFINFPIYWSSKTETVEEPMEEEEAAKEEKEESDDEAAVEEEEEKPK
TKKVEKTVWDWELMNDIKPIWQRPSKEVEEDEYKAFYKFSKESDDPMAYIHFTAEGEVTFKSI
LFVPTSAPRGLFDEYGSKKSDYIKLYVRRVFITDDFHMMPKYLNFVKGVVDSDDLPLNVSRET
LQQHKLLKVIKKLVKRLTDMIKKIADDKYNDTFWKEFGTNIKLGVIEDHSNRTRLAKLLRFQSS
HHPTDITSLDQYVERMKEKQDKIYFMAGSSRKEAESSPFVERLLKKGVEVIYLTEPVDEYCIQAL
PEFDGKRFQNVAKEGVKFDESEKTESREAVEKEFEPLLNWMKDKALKDKIEKAVVSQRLTES
PCALVASQYGWSGNMERIMKAQAYQTGKDISTNYYASQKKTFEINPRHPLIRDMLRRIKEDED
DKTVLDLAVVLFETATLRSGYLLPDTKAYGDRIERMLRSLNIDPDAKVEEPEEEPEETAEDTT
EDTEQDEDEEMDVGTDDEEETAKESTA EKDEL

SEQ ID NO: 2 - An exemplary nucleic acid sequence encoding GRP94

gattggtgggttcatttcccgccccgccccgcccgcggaagtggggtgaaaagcggccgacctgctgctgtagtggcgccgac
cgcgcggtcgagggtgtaggatccgaaccaggggtgggggggtggaggcggctcctgcatcgaaggggacttgagactcacc
ggccgcacgcatgagggccctgtgggtgctgggcctctgctgctgctgctgacctcgggtcgggtcagagctgacgatgaagtgat
gtggatggtacagtagaagaggatctgggtaaaagtagagaaggatcaaggacggatgatgaagtagtacagagagaggaga
agctattcagttgatgattaaatgcatcacaataagagaacttagagagaagtcggaaaagttgcctccaagccgaagtaac
agaatgatgaaactatcatcaattcattgtataaaaataaagagatttctgagagaactgattcaaatgcttctgatgctttagataag
ataaggctaatactgactgatgaaaatgctcttctggaatgaggaactaacagtcaaaaattaagtgtgataaggagaagaacc
tgctgcatgtcacagacaccggtgtaggaatgaccagagaagagttggttaaaaacctgtaccatagccaaatctgggacaagc
gagttttaaacaaaatgactgaagcacaggaagatggccagtcacttctgaattgattggccagtttggtgctggttctattccgctt
cctgtagcagataaggtattgtcacttcaaacacacaacgataccagcacatctgggagctgactccaatgaatttctgtaatt
gctgaccaagaggaacactctaggacggggaacgacaattacctgtctaaagaagaagcatctgattacctgaaattggat
acaattaaaaatctgtaaaaaatattcacagttcataaacttctctattatgtatggagcagcaagactgaaactgttgaggagccc
atggaggaagaagaagcagccaagaagagaagaagaatctgatgatgaagctgcagtagaggaagaagaagaagaaaa
gaaaccaagactaaaaaagttgaaaaactgtctgggactgggaactatgaatgatatcaaaccaatattggcagagaccatca
aaagaagtagaagaagatgaatacaaaagctttctacaaatcatttcaaaaggaagtgatgacccatggcttatattcactttactgct
gaaggggaagttaccttcaaatcaattttattgtaccacacatctgctccacgtggtctgtttgacgaatatggatctaaaaagagcgatta
cattaagctctatgtgcgcccgtattcatcacagacgacttccatgatgatgcctaaatacctcaattttgcaaggggtgtggtgactc
agatgatctcccctgaatgttcccgcgagactctcagcaacataaactgcttaagggtgattaggaagaagctgttcgtaaaacgctg

gacatgatcaagaagattgctgatgataaatacaatgatacttttggaaagaatttggtagcaaacatcaagcttggtgattgaagac
 cactcgaatcgaacacgtcttgctaaacttcttaggtccagctcttcatcatccaactgacattactagccttagaccagatgaggaaag
 aatgaaggaaaaacaagacaaaatctacttcatggctgggtccagcagaaaagaggctgaatcttccattgtgagcgacttctg
 aaaaagggtatgaagttattacctcacagaacctgtggatgaatactgtattcaggccctcccgaattgatgggaagagggtccag
 aatgttgccaaggaaggagtgaagttcgatgaaagtgagaaaactaaggagagtcgtgaagcagttgagaaagaatttgagcctct
 gctgaattggatgaaagataaagcccttaaggacaagattgaaaaggctgtgggtctcagcgcctgacagaatctccgtgtgctttg
 tggccagccagtagcggatggtctggcaacatggagagaatcatgaaagcacaagcgtaccaaacgggcaaggacatctctaaa
 attactatgtagcagaagaaaacatttgaattaatcccagacacccgctgacagagacatgctcagcgaattaaggaagatg
 aagatgataaaacagtttggatcttctgtgtgtttgttgaacagcaacgctcgggtcagggtatctttaccagacactaaagcatatg
 gagatagaatagaagaatgcttcgctcagtttgaacattgacctgatgcaaaggtggaagaagagcccgaagaagaacctga
 agagacagcagaagacacaacagaagacacagagcaagacgaagatgaagaaatggatgtgggaacagatgaagaagaag
 aaacagcaaaggaatctacagctgaaaaagatgaattgtaattatactctcaccattggatcctgtgtggagaggaatgtgaaatt
 tacatcattcttttgggagagactgttttggatgcccctaataccccttctcccctgactgtaaatgtgggattatgggtcacaggaaa
 aagtgggttttttagttgaatttttaacattcctcatgaatgtaaatgttactatctaactgactattctgatgtaaatcttgcattgtataa
 aaataaaaaagatcccaaat

SEQ ID NO: 3 - Amino acid sequence encoding the heavy chain of an anti-GRP94 antibody.

QVQLVQSGAEVKKPGASVKVSCASGYTFTSYAMHWVRQAPGQRLEWMGWINAGNGNTKY
 SQKFQGRVTITRDTASASTAYMELSSLRSED TAVYYCARAHFDYWGQGLVTVSAASTKGPSVF
 PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
 SSSLGTQTYICNVNHKPSNTKVDKKEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
 SRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
 GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
 WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQKSL
 SLSPGK

SEQ ID NO: 4 - Amino acid sequence encoding the light chain of an anti-GRP94 antibody.

EIELTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPKAPKLLIYAASSLQSGVPSRFGSGS
 GSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTA
 SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYLSSTLTLSKADYEKHKVYA
 CEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 5 - Amino acid sequence of a human wildtype IFN- α 2b molecule.

CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIF
 NLFSTKDSSAAWDETLLEKDYTELYQQLNDLEACVIQGVGTETPLMKEDSILAVRKYFQRITLY
 LKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSKE

SEQ ID NO: 6 - Amino acid sequence of an IFN- α 2b mutant molecule.

CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIF
 NLFSTKDSSAAWDETLLEKDYTELYQQLNDLEACVIQGVGTETPLMKEDSILAVRKYFQRITLY
 LKEKKYSPCAWEVVRAEIMASFSLSTNLQESLASKE

SEQ ID NO: 7 - Amino acid sequence of a wildtype IFN- α 14 molecule.

CNLSQTHSLNRRRTLMLMAQMRRISPFSCCLKDRHDFEFPQEEFDGNQFQKAQAISVLHEMMQ
 QTFNLFSTKNSSAAWDETLLEKDYIELFQQMNDLEACVIQEVGVEETPLMNEDSILAVKKYFQRI
 TLYLMEKKYSPCAWEVVRAEIMRSLSFSTNLQKRLRRKD

SEQ ID NO: 8 - Amino acid sequence of a wildtype IFN- β -1a molecule.

MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEML
 QNIFAIRQDSSSTGWNENIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSSLHLKRYYYGR
 ILHYLKAKEYSHCAWTIVRVEILRNIFYFINRLTGYLRLN

SEQ ID NO: 9 - Amino acid sequence of a wildtype IFN- β -1b molecule.

MSYNLLGFLQRSSNFQSQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEML
QNIFAIFRQDSSSTGWNENIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYGR
ILHYLKAKEYSHCAWTIVRVEILRNIFYFINRLTGYLRLN

SEQ ID NO: 10 - Amino acid sequence of a peptide linker.
SGGGGS

SEQ ID NO: 11 - Amino acid sequence of a peptide linker.
AEAAAKEAAKAGS

SEQ ID NO: 12 - Amino acid sequence of a peptide linker.
GGGGS

SEQ ID NO: 13 - Amino acid sequence of a peptide linker.
SGGGGSGGGGS

SEQ ID NO: 14 - Amino acid sequence of a peptide linker.
GGGGG

SEQ ID NO: 15 - Amino acid sequence of a peptide linker.
GAGAGAGAGA

SEQ ID NO: 16 - Amino acid sequence of a peptide linker.
AEAAAKAGS

SEQ ID NO: 17 - Amino acid sequence of a peptide linker.
GGGGGGGG

SEQ ID NO: 18 - Amino acid sequence of a peptide linker.
AEAAAKEAAKA

SEQ ID NO: 19 - Amino acid sequence of a peptide linker.
AEAAKA

SEQ ID NO: 20 - Amino acid sequence of a peptide linker.
GGAGG

What is claimed is:

1. A method of treating a cancer that expresses or overexpresses glucose-regulated protein 94 (GRP94) in an individual, said method comprising administering to the individual a non-naturally occurring fusion molecule in an amount sufficient to treat said cancer, wherein the fusion molecule comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94 ("GRP94 Ab-IFN fusion molecule"), and wherein the GRP94 Ab-IFN fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.
2. The method according to claim 1, wherein the GRP94 Ab-IFN fusion molecule comprises an GRP94 Ab selected from the group consisting of a fully human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, an antigen-binding antibody fragment, a Fab, a Fab', a Fab₂, a Fab'₂, a IgG, a IgM, a IgA, a IgE, a scFv, a dsFv, a dAb, a nanobody, a unibody, or a diabody.
3. The method according to claim 2, wherein the GRP94 Ab is an isolated human monoclonal antibody, and wherein the heavy chain variable domain comprises the amino acid sequences set forth as amino acids 26-33 of SEQ ID NO: 3 (CDR1), amino acids 51-58 of SEQ ID NO: 3 (CDR2), and amino acids 97-103 of SEQ ID NO: 3 (CDR3).
4. The method according to claim 2, wherein the GRP94 Ab is an isolated human monoclonal antibody, and wherein the heavy chain variable domain comprises the amino acid sequences set forth as amino acids 26-33 of SEQ ID NO: 3 (CDR1), amino acids 51-58 of SEQ ID NO: 3 (CDR2), and amino acids 97-103 of SEQ ID NO: 3 (CDR3) and wherein the light chain variable domain comprises the amino acid sequence set forth as amino acids 27-32 of SEQ ID NO: 4 (CDR1), amino acids 50-52 of SEQ ID NO: 4 (CDR2), and amino acids 89-97 of SEQ ID NO: 4 (CDR3).

5. The method according to claim 2, wherein the GRP94 Ab is a fully human antibody comprising the heavy chain variable region of SEQ ID NO: 3 and the light chain variable region of SEQ ID NO: 4.
6. A method according to any one of claims 1-5, wherein the GRP94 Ab-IFN fusion molecule comprises a type 1 interferon molecule selected from the group consisting of an interferon (IFN)- α molecule, an IFN- β -1a molecule, an IFN- β -1b molecule, an IFN- α mutant molecule, and an IFN- β mutant molecule.
7. The method according to claim 6, wherein the type 1 interferon molecule is the IFN- α 2b molecule which comprises the amino acid sequence set forth is SEQ ID NO: 5.
8. The method according to claim 6, wherein the type 1 interferon molecule is the IFN- α 2b mutant molecule which comprises the amino acid sequence set forth is SEQ ID NO: 6.
9. The method according to claim 6, wherein the type 1 interferon molecule is the IFN- α 14 molecule which comprises the amino acid sequence set forth is SEQ ID NO: 7.
10. The method according to claim 1, wherein the cancer is selected from the group consisting of: a melanoma, breast cancer, renal cancer, glioma, colorectal cancer, hepatocellular cancer, lung cancer (small cell lung cancer and non-small cell lung cancer), ovarian cancer; pancreatic cancer, prostate cancer, esophageal cancer, gastric cancer, urinary bladder cancer; head and neck cancer, multiple myeloma, acute leukemias (e.g., 11q23-positive acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma (indolent and high grade forms), Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia tumor cell.
11. The method according to claim 10, wherein the cancer is multiple myeloma.

12. The method according to claim 11, wherein the multiple myeloma is relapsed multiple myeloma.
13. The method according to claim 11, wherein the multiple myeloma is refractory multiple myeloma.
14. The method according to claim 10, wherein the cancer is acute myelogenous leukemia (AML).
15. The method according to claim 14, wherein the AML is relapsed AML.
16. The method according to claim 14, wherein the AML is refractory AML.
17. The method according to claim 10, wherein the cancer is non-small cell lung cancer (NSCLC).
18. The method according to claim 17, wherein the NSCLC is relapsed NSCLC.
19. The method according to claim 17, wherein the NSCLC is refractory NSCLC.
20. The method according to claim 10, wherein the cancer is pancreatic cancer.
21. The method according to claim 20, wherein the pancreatic cancer is relapsed pancreatic cancer.
22. The method according to claim 20, wherein the pancreatic cancer is refractory pancreatic cancer.
23. A method of inhibiting the growth and/or proliferation of a tumor cell that expresses or overexpresses GRP94 in an individual, said method comprising administering to the individual an isolated non-naturally occurring GRP94 Ab-IFN fusion molecule that specifically binds glycosylated GRP94 in an amount sufficient to treat said tumor cell, wherein the GRP94 Ab-IFN fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001

mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg.

24. A combination therapy method of treating a cancer that expresses or overexpresses GRP94 in an individual, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising an anti-GRP94-IFN- α fusion molecule; and b) immunotherapy.

25. The method of claim 24, wherein the immunotherapy comprises treatment using agonistic, antagonistic, or blocking antibodies to co-stimulatory or co-inhibitory molecules (immune checkpoints).

26. The method of claim 24, wherein the immunotherapy comprises treatment using chimeric antigen receptor (CAR)-T cells.

27. The method of claim 24, wherein the immunotherapy comprises treatment using CAR-NK cells.

28. The method of claim 24, wherein the immunotherapy comprises treatment using bispecific T cell engaging antibodies (BiTE®).

29. A method according to any one of claims 1-28, wherein the GRP94 Ab-IFN fusion molecule comprises an interferon molecule that is directly joined to the antibody or antigen-binding fragment.

30. A method according to any one of claims 1-28, wherein the GRP94 Ab-IFN fusion molecule comprises an interferon molecule that is directly joined to the antibody or antigen-binding fragment with a proteolysis resistant peptide linker.

31. The method according to claim 30, wherein the linker is selected from the group consisting of: SGGGGS (SEQ ID NO: 10) and AEAAAKEAAKAGS (SEQ ID NO: 11).

32. A method according to any one of claims 1-31, wherein the GRP94 Ab-IFN fusion molecule is a recombinantly expressed fusion molecule.

33. A kit comprising: a) a pharmaceutical composition containing a GRP94 Ab-IFN fusion molecule of the present invention, and b) instructional materials disclosing means of use of the GRP94 Ab-IFN fusion molecule to treat a cancer.

34. A kit comprising: a) a pharmaceutical composition containing a GRP94 Ab-IFN fusion molecule of the present invention, and b) a second cancer immunotherapy agent.

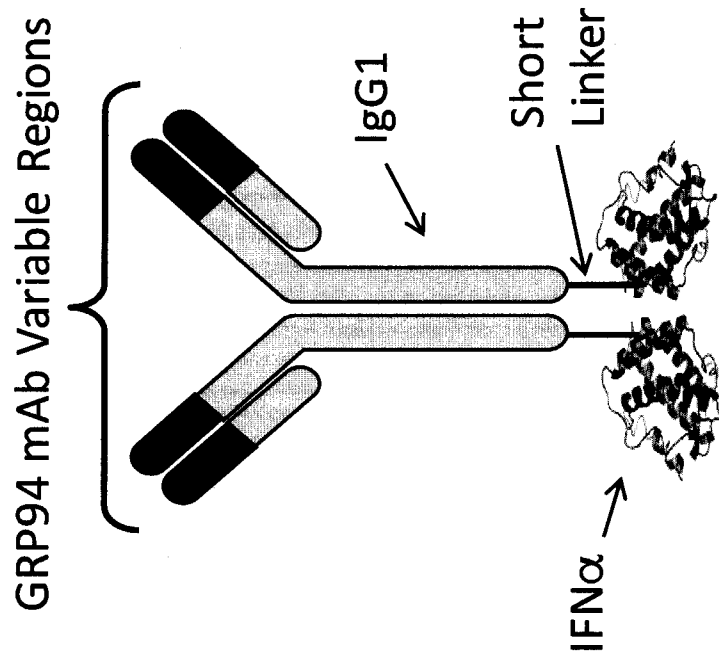


FIG.1

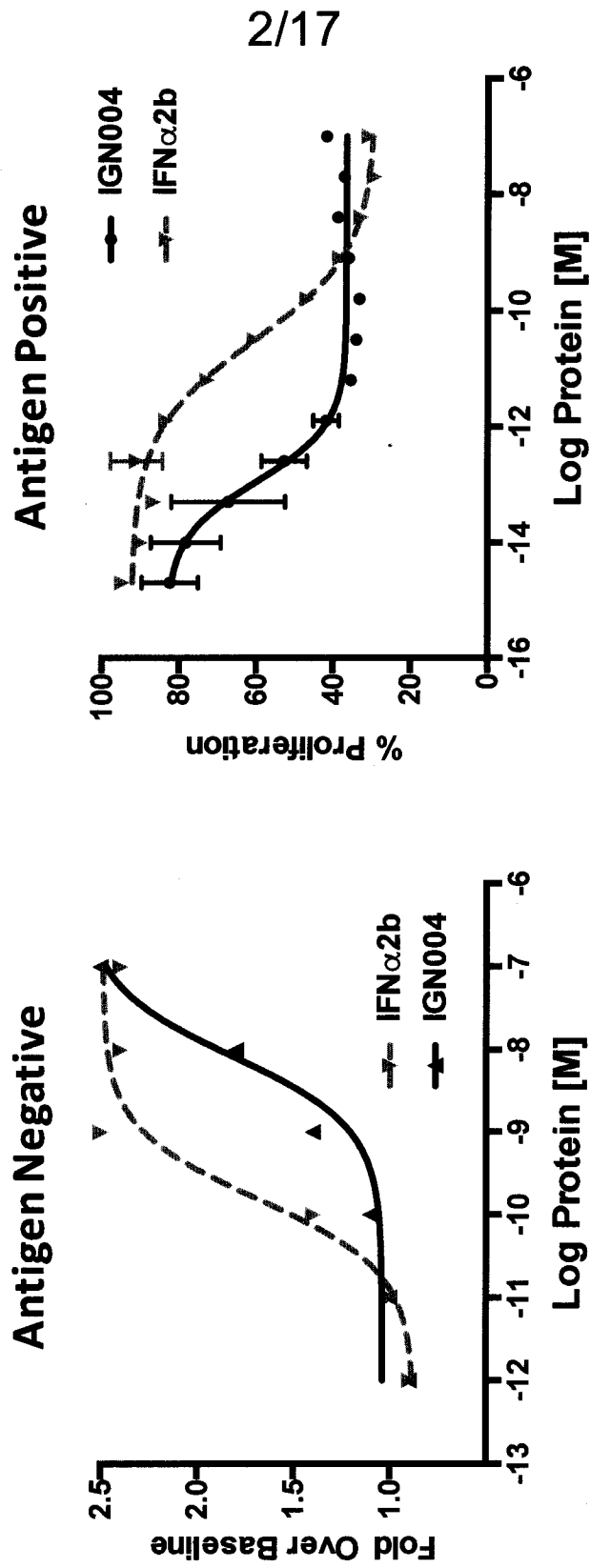


FIG.2

3/17

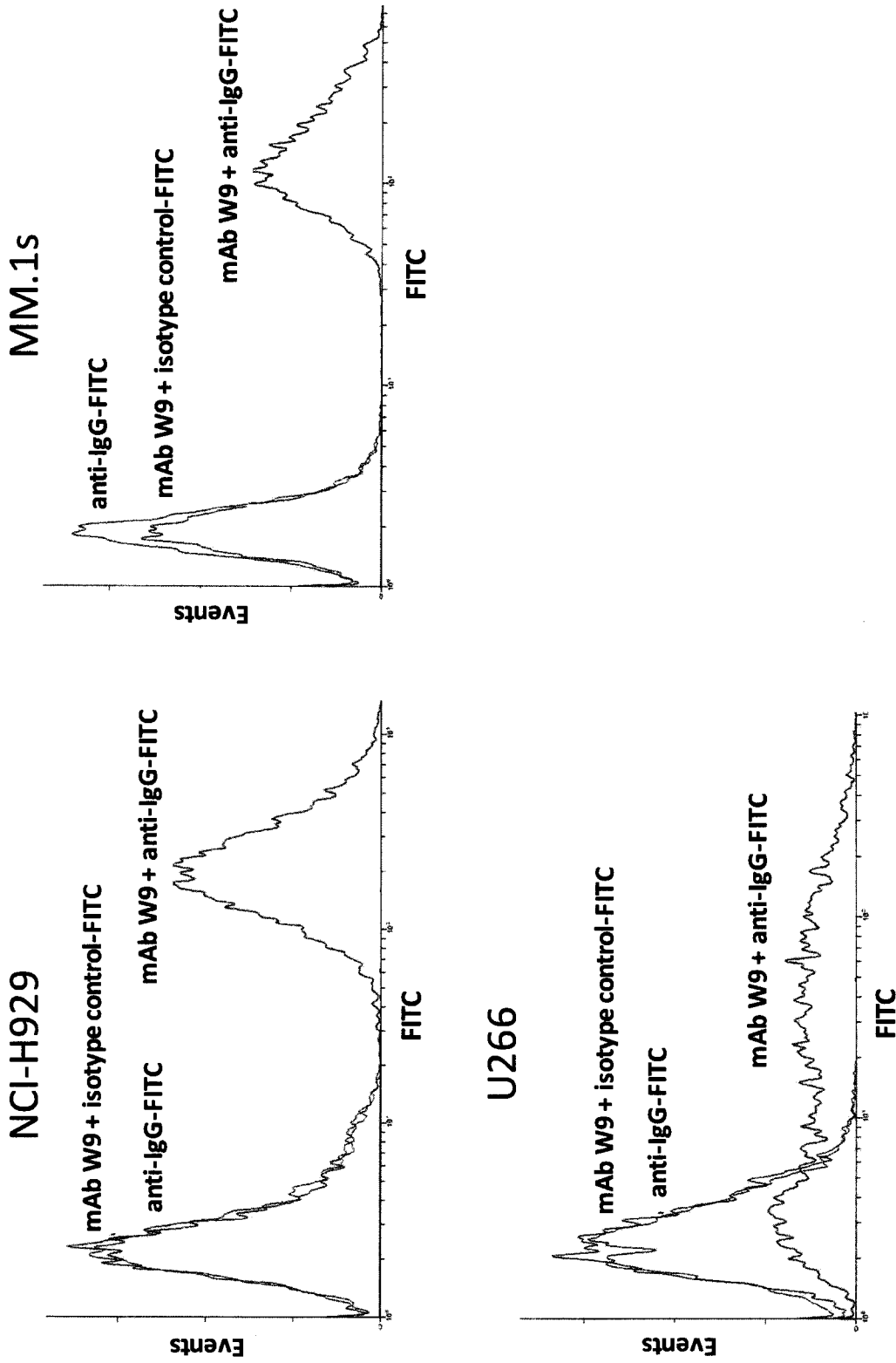


FIG.3

4/17

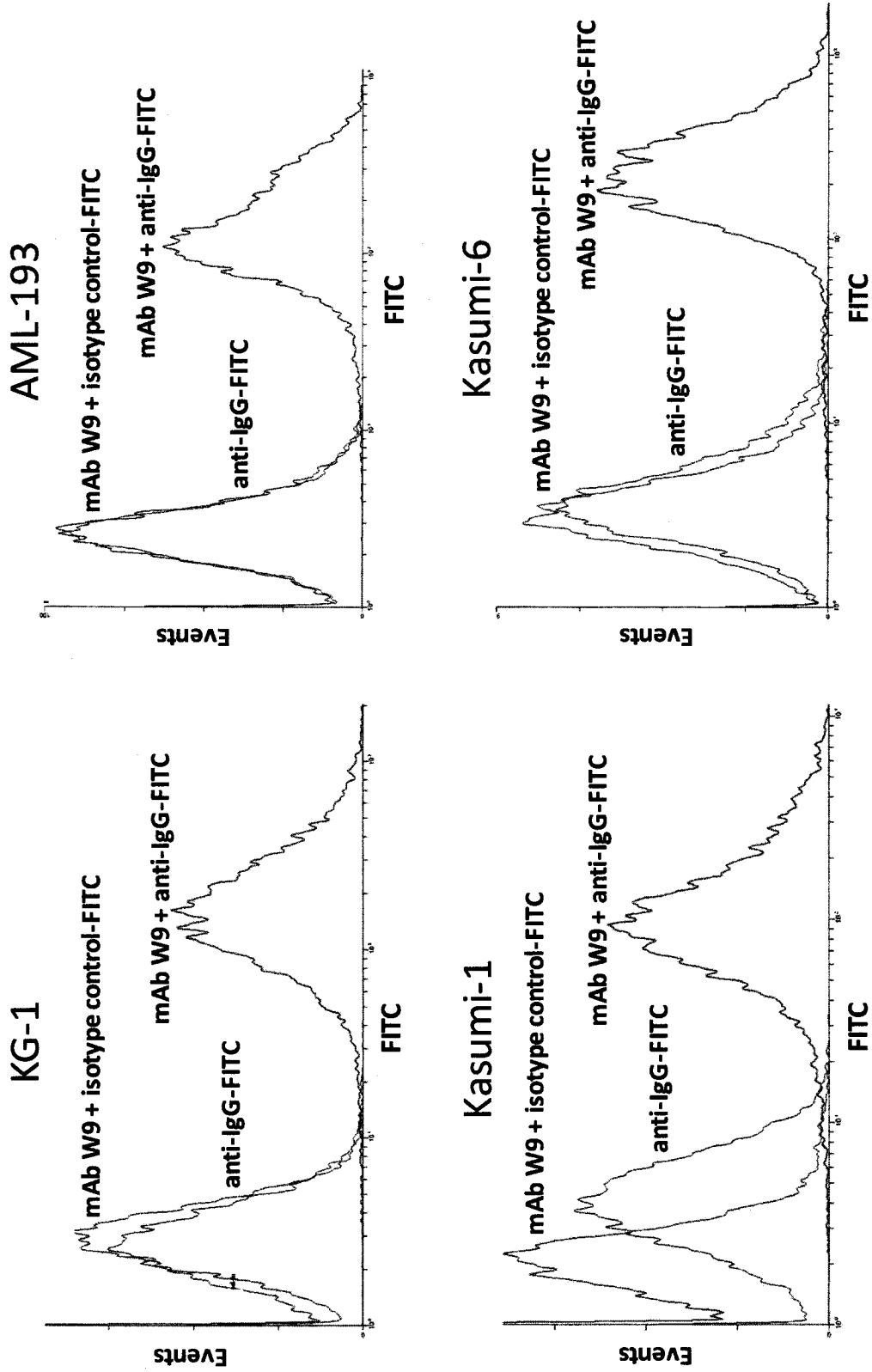


FIG.4

5/17

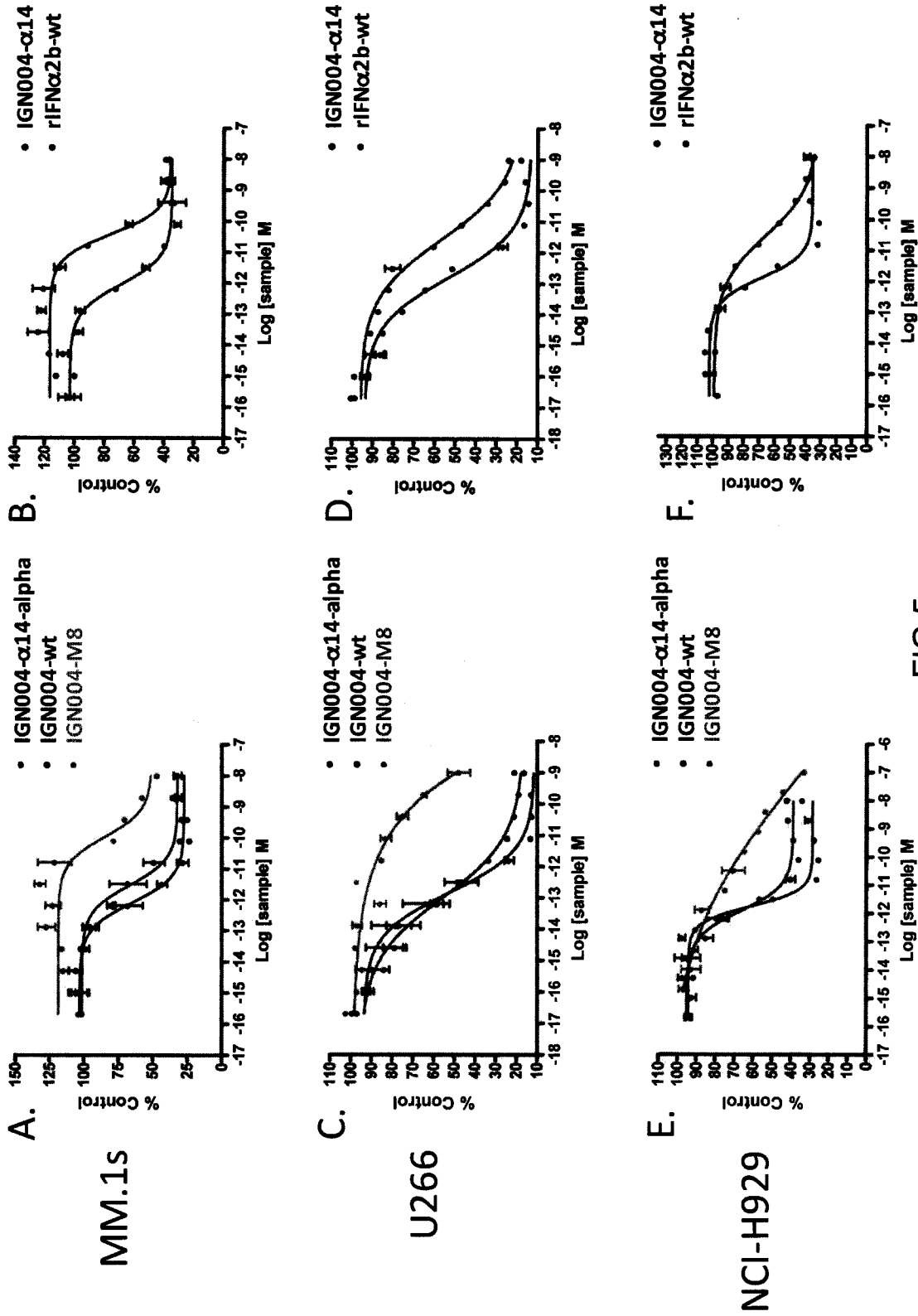


FIG.5

6/17

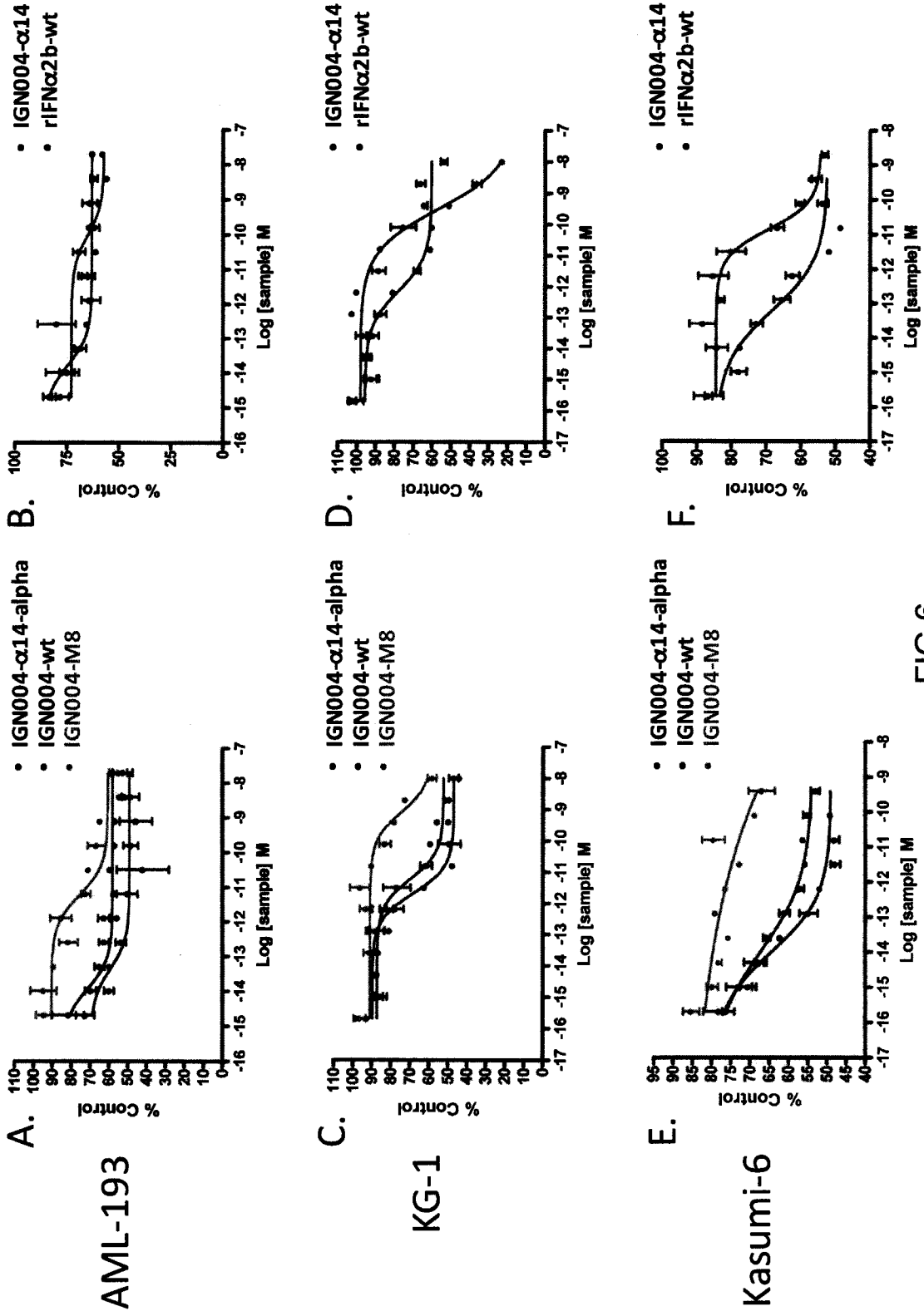


FIG.6

7/17

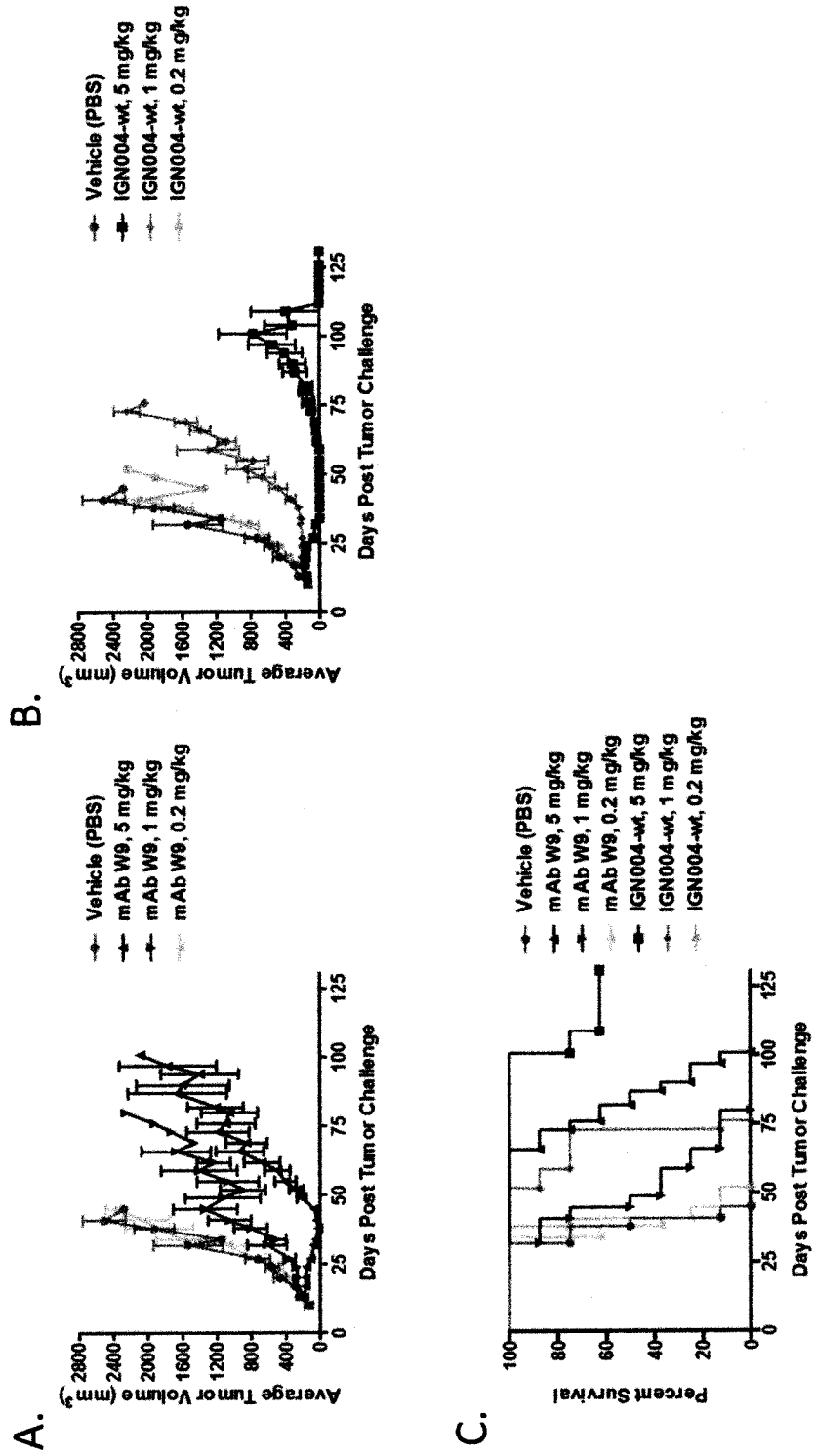


FIG.7

8/17

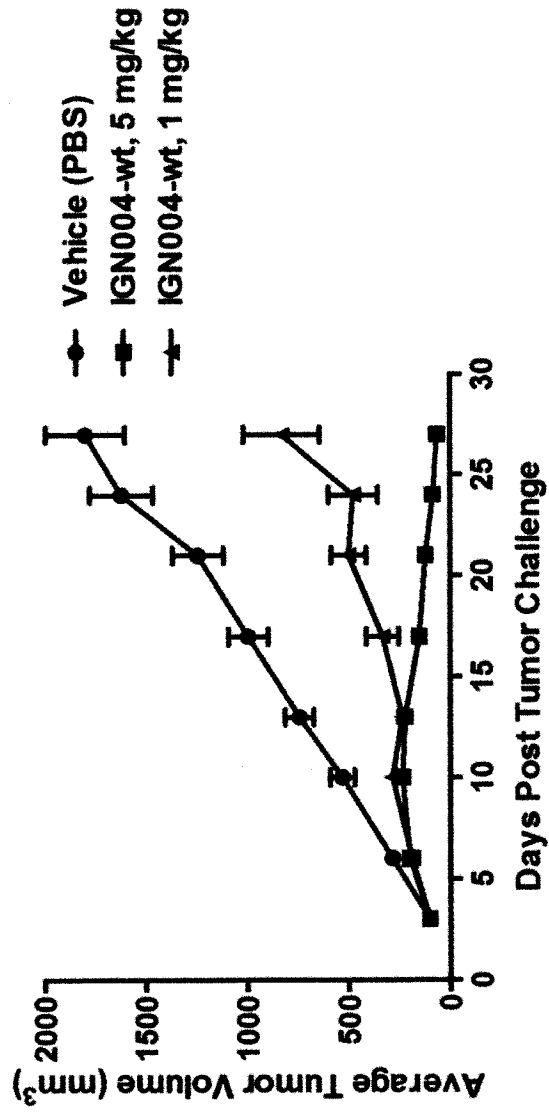


FIG.8

9/17

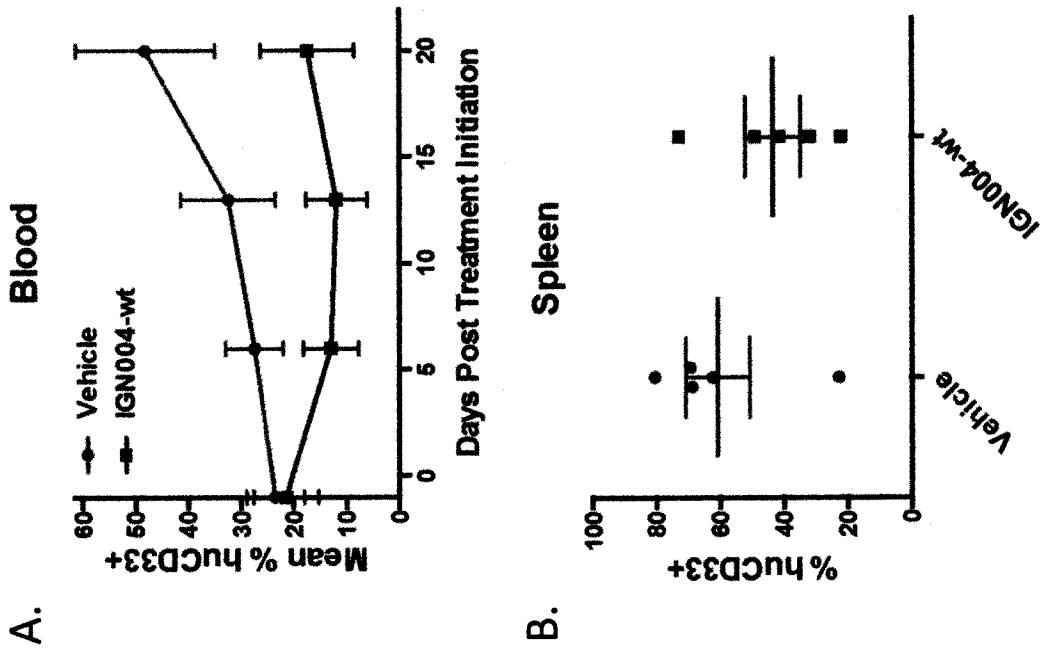


FIG.9

10/17

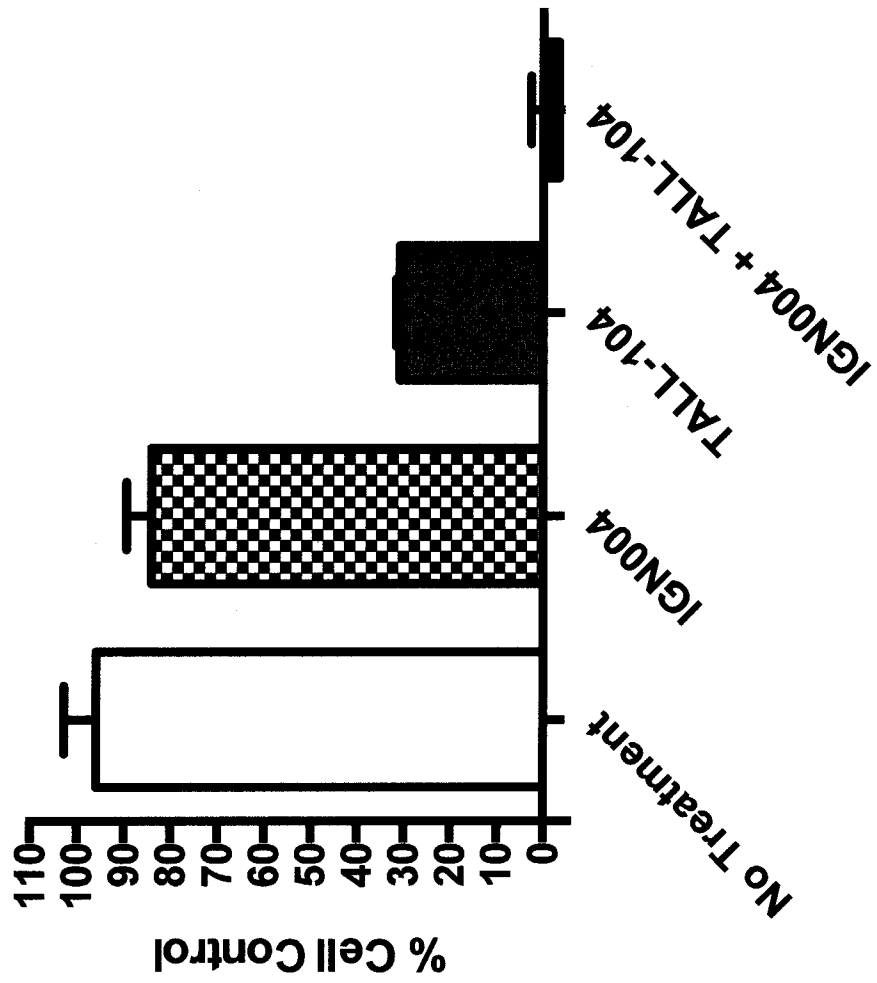


FIG.10

11/17

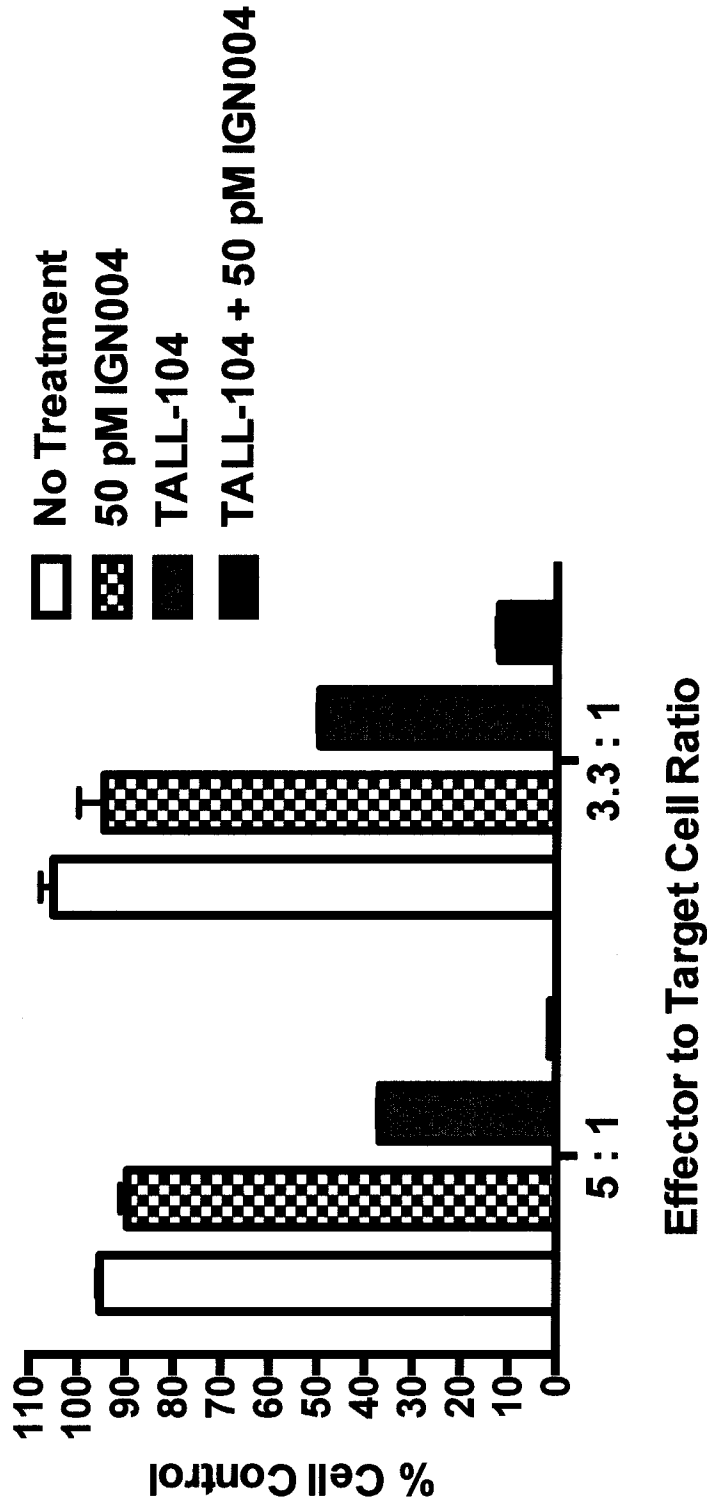


FIG.11

12/17

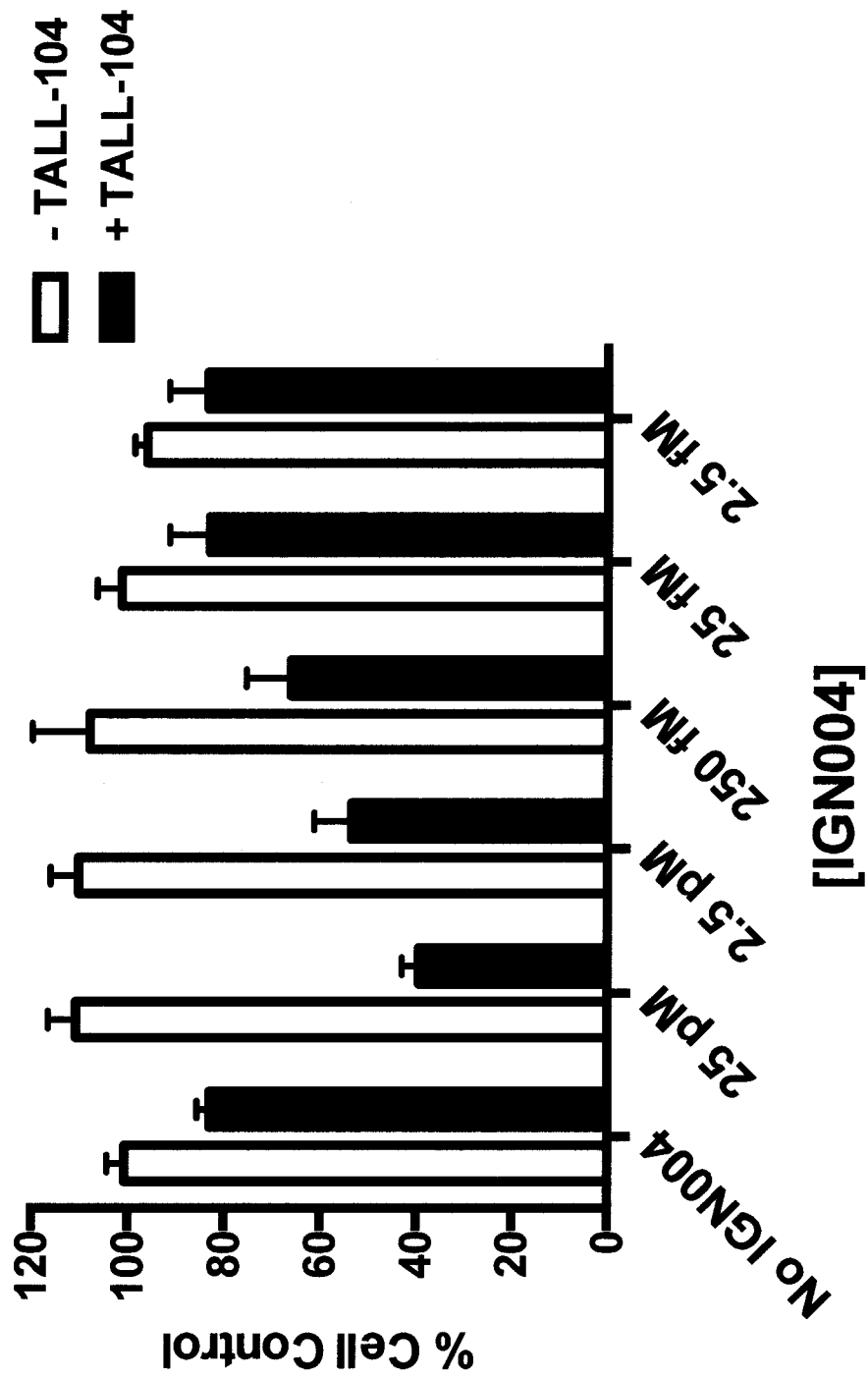


FIG.12

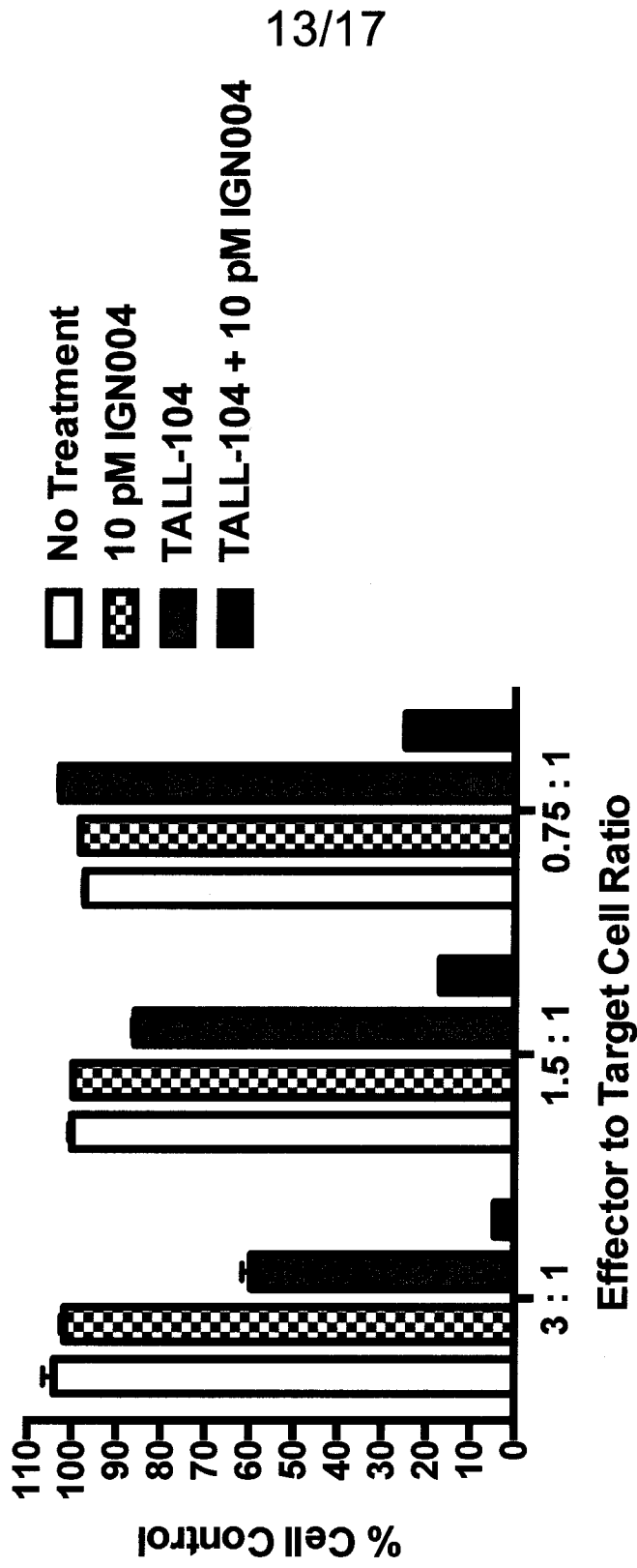


FIG.13

14/17

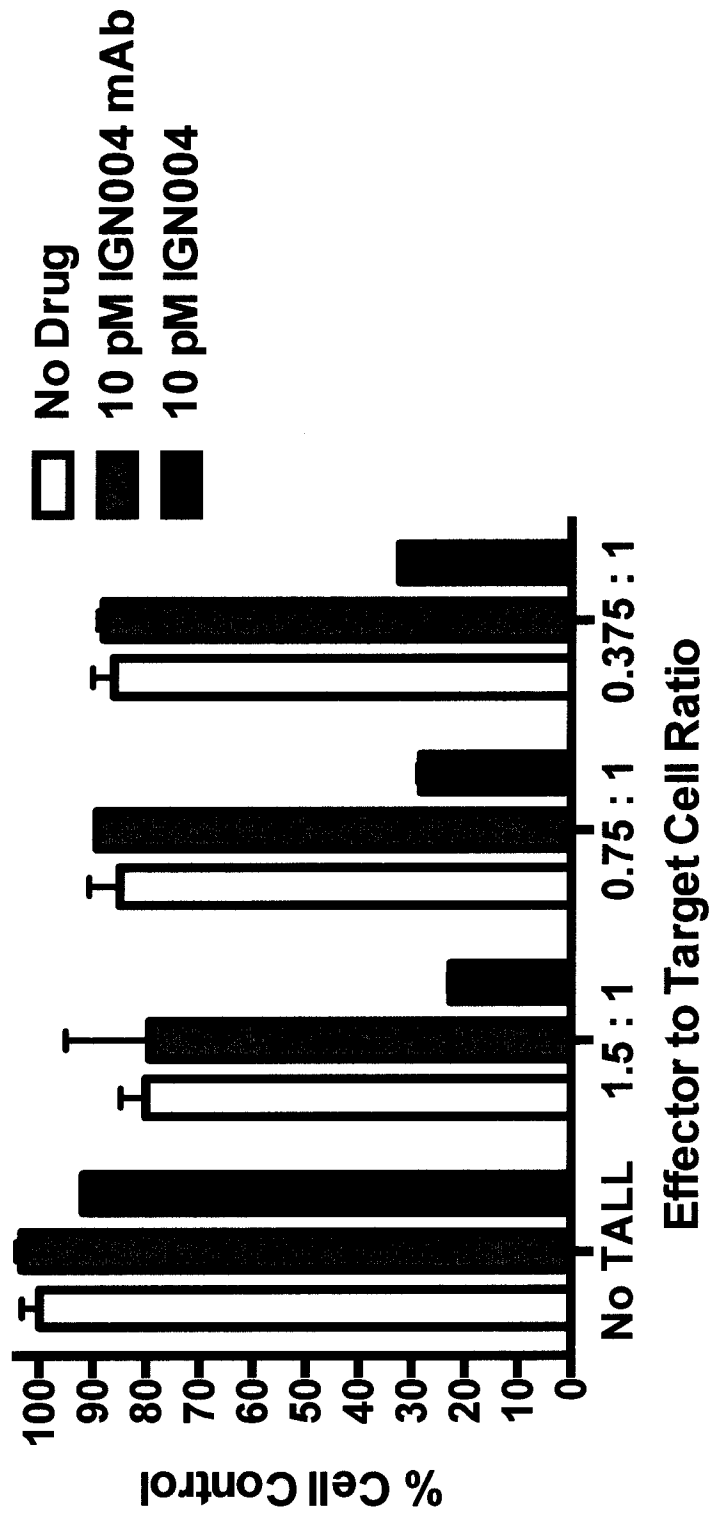


FIG.14

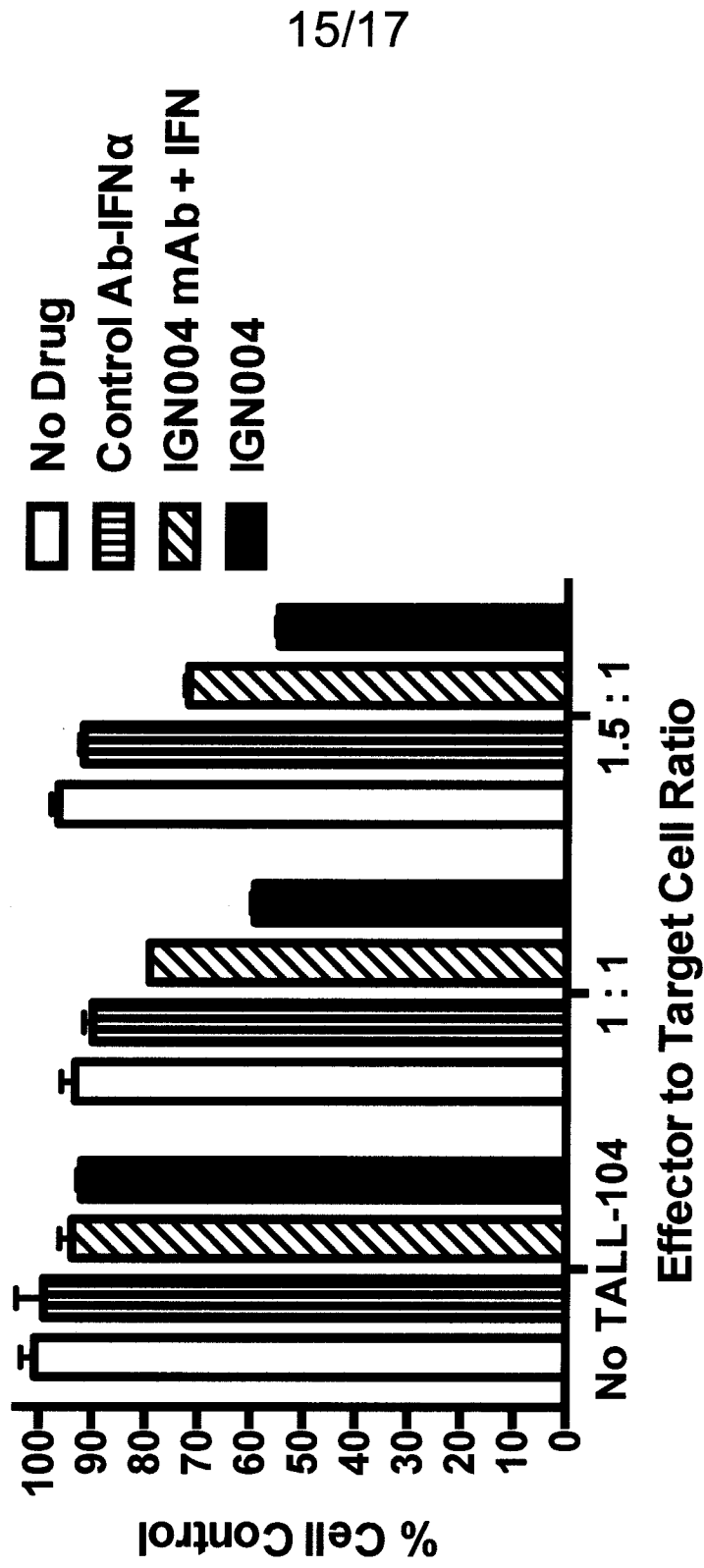


FIG.15

16/17

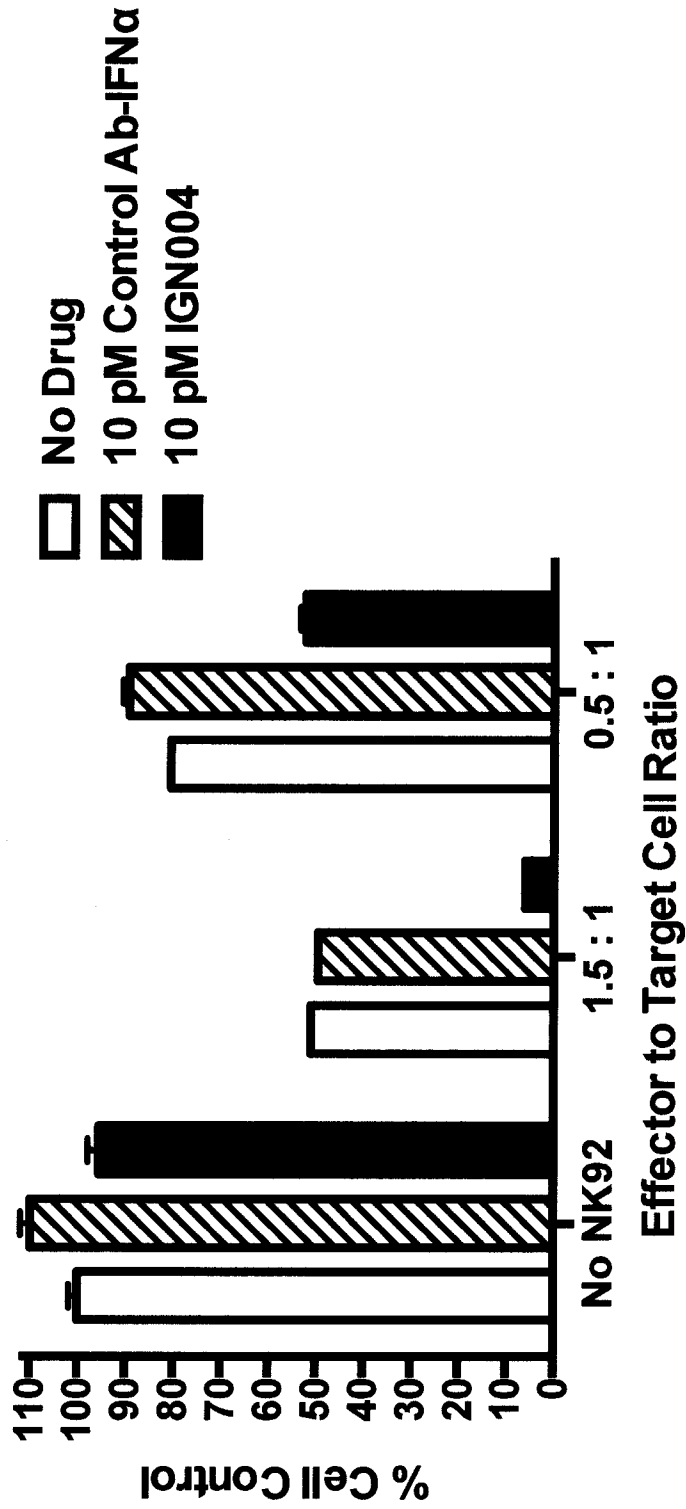


FIG.16

17/17

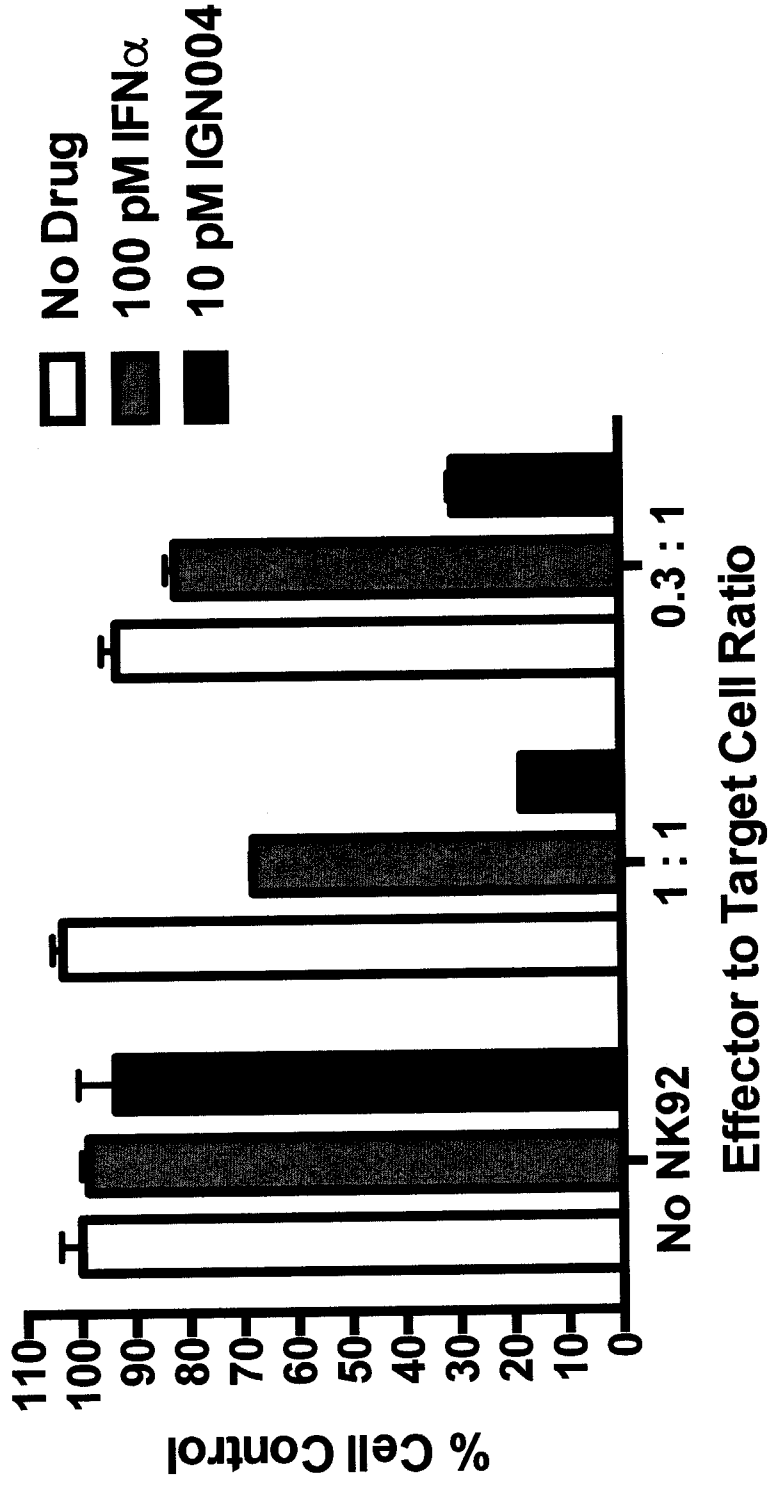


FIG.17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/37038

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C07K 14/56, 14/565 (2016.01) CPC - C07K 14/56, 14/565 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) IPC(8): A61K 39/00; C07K 14/00, 14/56, 14/565, 16/30 (2016.01) CPC: A61K 39/00, 39/39558, 2039/505, 2039/5152, 2039/5158; C07K 14/00, 14/56, 14/565, 16/30 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) PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); EBSCO Discovery; PubMed; Google; Google Scholar; Google Patents; KEYWORDS: cancer, fusion, antibody, bind, Grp94, interferon		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2014/0010811 A1 (FERRONE, S et al.) January 9, 2014; abstract; paragraphs [0006], [0023], [0102], [0137], [0206], [0225], [0235], [0237], [0262], [0263], [0274], [0281], [0282], [0289], [0305], [0409]; claim 16	1-2, 6/1-2, 10, 23, 33 --- 3-5, 6/3-5, 11-22, 24-28, 34
Y	US 2013/0230517 A1 (IMMUNGENE Inc.) September 5, 2013; paragraphs [0035], [0051], [0059], [0061], [0153]	3-5, 6/3-5
Y	US 2015/0118251 A1 (SANOFI) April 30, 2015; abstract; paragraph [0017]	11-13
Y	US 2014/0112920 A1 (CELGENE CORPORATION) April 24, 2014; paragraph [0073]	14-16
Y	US 2011/0319355 A1 (NGUYEN, A et al.) December 29, 2011; abstract; paragraphs [0041], [0151]	17-19
Y	US 2013/0190285 A1 (SHESHBARADARAN, H et al.) July 25, 2013; abstract; paragraph [0017]	20-22
Y	WO 2015/069922 A2 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) May 14, 2015; page 8, lines 35-37; page 25, lines 25-38; page 47, line 39 to page 48, line 4; page 58, line 2-3	24-27, 34
Y	US 2015/0132217 A1 (IBC PHARMACEUTICALS, INC.) May 14, 2015; paragraphs [0024], [0131]	25, 28
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 24 October 2016 (24.10.2016)		Date of mailing of the international search report 27 NOV 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/37038

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

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

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

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

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

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

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

-Please See Supplemental Page-

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 6/1-5, 10-28, 33, 34

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

-Continued from Previous Supplemental Box--

Ferrone does not disclose a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg; and immunotherapy; and a kit comprising: a) a pharmaceutical composition containing a GRP94 Ab-IFN fusion molecule of the present invention, and b) instructional materials disclosing means of use of the GRP94 Ab-IFN fusion molecule to treat a cancer; and a kit comprising: a) a pharmaceutical composition containing a GRP94 Ab-IFN fusion molecule of the present invention, and b) a second cancer immunotherapy agent.

Honjo discloses compositions for immunopotentialiation for the treatment of cancer (compositions for immunopotentialiation for the treatment of cancer; abstract), wherein said compositions comprise alpha interferon (wherein said compositions comprise alpha interferon; paragraph [0084]) and an immunotherapeutic agent (and an immunosuppressive signal inhibitor of PD-1 (and an immunotherapeutic agent); paragraph [0014]).

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Ferrone to have included the use of maintenance dosages for treatment of a patient, wherein the weekly dosages were in the range indicated for the initial dose, as disclosed by Ferrone, in order to enable effective treatment of a subject with cancer, while minimizing potential side-effects due to excessive dosing of the active compounds. Additionally, it would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have provided a kit comprising: a) a pharmaceutical composition containing a GRP94 Ab-IFN fusion molecule of the present invention, and b) instructional materials disclosing means of use of the GRP94 Ab-IFN fusion molecule to treat a cancer; and optionally, a second cancer immunotherapy agent, based on the disclosure of Ferrone, in order to enable a medical practitioner to appropriately prepare, select a dosage, and administer the treatment disclosed by Ferrone, optionally including a second agent for combination with the antibody disclosed by Ferrone, to a patient with cancer to effectively treat said cancer. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Ferrone to have included the use of additional effective treatments in combination with those disclosed by Ferrone, including the immunopotentiative compositions disclosed by Honjo, in order to better reduce immune suppression in a patient with cancer in order to provide more effective treatment.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Ferrone and Honjo references, unity of invention is lacking.

-***-Continued from Box III Observations where unity of invention is lacking-***-

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

Groups I+, Claims 1-28, SEQ ID NO: 5 are directed toward a method of treating a cancer, or inhibiting the growth or proliferation of a tumor cell, that expresses or overexpresses glucose-regulated protein 94 (GRP94); a related combination therapy method; and kits for said methods.

The methods and kits will be searched to the extent that they comprise an interferon encompassing SEQ ID NO: 5 (first exemplary interferon sequence). Applicant is invited to elect additional interferon(s), with specified SEQ ID NO: for each, to be searched. Additional interferon sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-6, 10-28, 33 and 34 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 5 (interferon sequence). Applicants must specify the claims that encompass any additionally elected interferon sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be an interferon encompassing SEQ ID NO: 6 (first exemplary elected interferon sequence).

No technical features are shared between the interferon sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a method of treating a cancer, and inhibiting the growth and/or proliferation of a tumor cell that expresses or overexpresses glucose-regulated protein 94 (GRP94) in an individual, said method comprising administering to the individual a non-naturally occurring fusion molecule in an amount sufficient to treat said cancer, wherein the fusion molecule comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds glycosylated GRP94 ("GRP94 Ab-IFN fusion molecule"), and wherein the GRP94 Ab-IFN fusion molecule is administered to the individual at a weekly dosage selected from the group consisting of no greater than .0001 mg/kg, no greater than .0003 mg/kg, no greater than .001 mg/kg, no greater than .003 mg/kg, no greater than .01 mg/kg, no greater than .03 mg/kg, no greater than 0.1 mg/kg, no greater than 0.2 mg/kg, no greater than 0.3 mg/kg, no greater than 0.4 mg/kg, no greater than 0.5 mg/kg, no greater than 0.6 mg/kg, no greater than 0.7 mg/kg, no greater than 0.8 mg/kg, and no greater than 0.9 mg/kg; a combination therapy method of treating a cancer that expresses or overexpress GRP94 in an individual, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising an anti-GRP94-IFN-a fusion molecule; and b) immunotherapy; a kit comprising: a) a pharmaceutical composition containing a GRP94 Ab-IFN fusion molecule of the present invention, and b) instructional materials disclosing means of use of the GRP94 Ab-IFN fusion molecule to treat a cancer; and a kit comprising: a) a pharmaceutical composition containing a GRP94 Ab-IFN fusion molecule of the present invention, and b) a second cancer immunotherapy agent.

However, these shared technical features are previously disclosed by US 2014/0010811 A1 to Ferrone et al. (hereinafter 'Ferrone') in view of US 2014/0314714 A1 to Honjo et al. (hereinafter 'Honjo').

Ferrone discloses a method of treating a cancer (treating cancer (a method of treating cancer; abstract, paragraph [0006], Claim 16), and inhibiting the growth and/or proliferation of a tumor cell (inhibiting the growth and/or proliferation of a tumor cell; paragraph [0023]) that expresses or overexpresses glucose-regulated protein 94 (GRP94) (that express endoplasmic (that expresses or overexpresses glucose-regulated protein 94 (GRP94); abstract, paragraphs [0006], [0023]) in an individual (in an individual; paragraph [0102], Claim 16), said method comprising administering to the individual (said method comprising administering to the individual; paragraph [0102], Claim 16) a non-naturally occurring fusion molecule (an immunoconjugate including an antibody linked to a cytokine or chemokine, including an interferon; paragraphs [0262], [0263]) in an amount sufficient to treat said cancer (a therapeutically effective amount (in an amount sufficient to treat said cancer); Claim 16), wherein the fusion molecule comprises an IFN molecule attached to an antibody (wherein the fusion molecule comprises an IFN molecule attached to an antibody; paragraphs [0262], [0263]) that specifically binds glycosylated GRP94 (that specifically binds glycosylated endoplasmic (GRP94); paragraph [0225]), and wherein the GRP94 Ab-IFN fusion molecule is administered to the individual at a dosage of 0.5 mg/kg (wherein the GRP94 Ab-IFN fusion molecule is administered to the individual at a dosage of 0.5 mg/kg; paragraph [0305]); a combination therapy (a combination therapy; paragraph [0102], Claim 16) method of treating a cancer (method of treating a cancer; paragraph [0006], Claim 16) that expresses GRP94 (that express endoplasmic (that expresses or overexpresses glucose-regulated protein 94 (GRP94); abstract, paragraphs [0006], [0023]) in an individual (in an individual; paragraph [0102], Claim 16), comprising administering to the individual (said method comprising administering to the individual; paragraph [0102], Claim 16) a) an effective amount (a therapeutically effective amount; Claim 16) of a pharmaceutical composition (a pharmaceutical composition; paragraph [0187]) comprising an anti-GRP94-IFN-a fusion molecule (comprising an anti-GRP94-IFN-a conjugate (fusion molecule); paragraphs [0006], [0262], [0263]); a kit (a kit; paragraph [0281]) comprising: a) a GRP94 Ab-IFN fusion molecule (a GRP94 Ab-IFN fusion molecule; paragraphs [0006], [0262], [0263], [0281]), and b) instructional materials (and instructional materials; paragraph [0282]).

-***-Continued Within the Next Supplemental Box-***-