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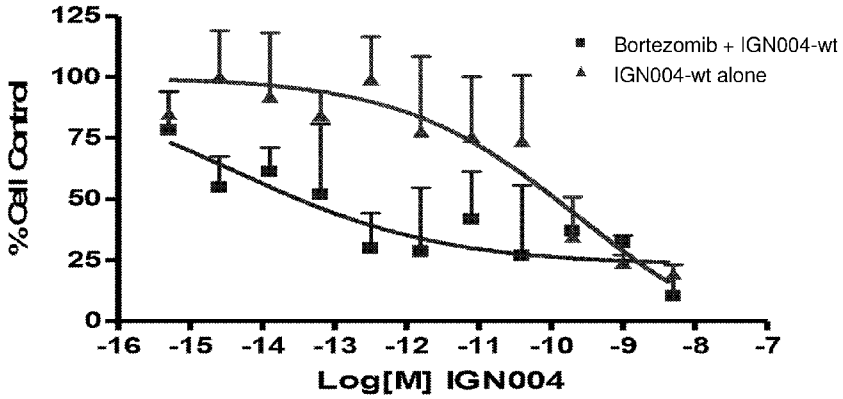
(54) **Title**: COMBINATION THERAPY FOR TREATMENT OF CANCER



	IGN004 5 nM Bort	IGN004 Alone
EC50	4.178e-015	2.425e-010

FIG. 3

(57) **Abstract**: Combinations of agents that have a synergistic effect for the treatment of cancer are disclosed herein. The present disclosure is directed to combination therapy methods designed to prevent, treat or manage a cancer that expresses a selective tumor-associated antigen (TAA) in an individual. Specifically, the disclosure provides combination therapies comprising use of a TAA antibody-IFN fusion molecule and a proteasome inhibitor, so as to prevent, treat or manage the cancer.

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COMBINATION THERAPY FOR TREATMENT OF CANCER

RELATED PATENT APPLICATIONS

[001] This application claims benefit of U.S. Provisional Application No. 62/175,044, filed on June 12, 2015, incorporated in its entirety by reference herein.

TECHNICAL FIELD

[002] Cancer remains a major cause of death worldwide despite the numerous advanced diagnostic and therapeutic methods that have been developed. The major barrier to successful treatment and prevention of cancer lies in the fact that many cancers still fail to respond to the current chemotherapeutic and immunotherapy intervention, and many individuals suffer a recurrence or death, even after aggressive therapy.

[003] Cancer is group of diseases involving abnormal cell growth with the potential to spread or invade other parts of the body. Abnormal growths that form a discrete tumor mass, i.e., do not contain cysts or liquid areas, are defined as solid tumors. Solid tumors may be benign (not cancer), or malignant (cancer). Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors are sarcomas, carcinomas, and lymphomas. Cancers derived from either of the two blood cell linages, myeloid and lymphoid, are defined as hematological malignancies. Such malignancies are also referred to as blood cancers or liquid tumors. Examples of liquid tumors include 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.

[004] The ubiquitin proteasome pathway plays a critical role in regulating many processes in the cell which are important for tumor cell growth and survival. Inhibition of proteasome function has emerged as a powerful strategy for anti-cancer therapy. Bortezomib, a peptide boronate inhibitor, was the first proteasome inhibitor to enter clinical practice and was approved by the FDA in 2003 following two successful single-agent phase II trials in relapsed

MM (Richardson et al., N Engl J Med, 348:2609-17, 2003). Growing evidence from translational research and clinical trials with bortezomib established the proteasome as a novel and legitimate therapeutic target. Unfortunately, there are restrictions to the use of bortezomib including dose limiting toxicity, particularly peripheral neuropathy, resistance and intravenous administration. Moreover, a sizeable proportion of individuals fail to respond to bortezomib therapy, or often relapse. This prompted the development of a new generation of structurally distinct proteasome inhibitors. In addition to bortezomib, there are currently five proteasome inhibitors in clinical development, representing three different structural classes-peptide boronic acids, peptide epoxyketones and β-lactones (Crawford et al, J Cell Commun Signal, 5(2):101-110, 2011).

[005] Interferons (IFNs) are soluble proteins produced naturally by cells in response to viruses. Although first described for their ability to inhibit viral replication, IFN-α's have multiple properties exhibiting anti-proliferative effects, induction of apoptosis (Rodriguez-Villanueva J and TJ McDonnell, Int J Cancer, 61:110, 1995) and induction of the tumor suppressor gene, P53, in tumor cells (Takaoka A et al., Nature, 424:516, 2003). Thus, IFN-α's were the first recombinant proteins used for the treatment of various cancers. However, IFN-α as a single agent is largely ineffective at overcoming the numerous cellular mechanisms that mediate tumor cell resistance to proapoptotic agents. And unfortunately, the use of IFN-α to treat cancer has been limited by its short half-life and associated systemic toxicities (Weiss K, Semin Oncol, 25:9, 1998; Jones GJ and Itri LM, Cancer, 57:1709, 2006). Given these limitations, it is difficult to achieve effective IFN-α concentrations at sites of malignant disease without causing systemic toxicity.

[006] 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.

[007] In spite of the recent advances in cancer treatments, many cancers remain 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 many of these cancers. There remains a significant unmet need for effective therapies to treat individuals with proliferative diseases, and in particular, individuals with refractory and/or recurrent proliferative diseases.

DISCLOSURE OF THE INVENTION

[008] Combinations of agents that have a synergistic effect for the treatment of cancer are disclosed herein. The combination therapy methods described herein are particularly effective against recurrent, resistant and/or refractory cancers.

[009] In one aspect, the present disclosure relates to combination therapy methods of treating a cancer that expresses or overexpresses a tumor associated antigen (TAA), comprising administering to the individual: a) a therapeutically effective amount of a tumor associated antigen antibody-interferon ("TAA Ab-IFN") fusion molecule, and b) a therapeutically effective amount of a proteasome inhibitor; wherein the combination therapy provides increased cell killing of cancer cells, i.e., a synergy exists between the TAA Ab-IFN fusion molecule and the proteasome inhibitor.

[010] In various embodiments, the methods comprise administration of one or more proteasome inhibitors selected from the group consisting of bortezomib, carfilzomib, NPI-0052, MLN9708, CEP-18770, ONX0912, MG132, disulfiram, PR-924, and epoxomicin. In various embodiments, the proteasome inhibitor is bortezomib. In various embodiments, the proteasome inhibitor is carfilzomib.

[011] In various embodiments, the TAA Ab-IFN fusion molecules comprise an TAA Ab selected from 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 Fab_2, 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 antibody is a chimeric antibody. In various embodiments, the antibody is a humanized monoclonal antibody. In various embodiments, the antibody is a fully human monoclonal antibody. In various embodiments, the TAA Ab is a fully human antibody selected from the group consisting of a fully human anti-HER2/neu Ab, a fully human anti-CD20 Ab, a fully human anti-CD138 Ab, a fully human anti-GRP94 (endoplasmin)

Ab, a fully human anti-CD33 Ab, and a fully human anti-CD70 Ab. In various embodiments, the TAA Ab is a fully human anti-GRP94 Ab.

[012] In various embodiments, the TAA Ab-IFN fusion molecule comprises a type 1 interferon molecule. In various embodiments, the fusion molecule comprises a type 1 interferon mutant molecule. In various embodiments, the fusion molecule comprises an interferon-alpha (IFN-α) molecule. In various embodiments, the fusion molecule comprises a human IFN-α2b molecule having the amino acid sequence of SEQ ID NO: 1. In various embodiments, the fusion molecule comprises a human IFN-α2b mutant molecule having the amino acid sequence of SEQ ID NO: 2. In various embodiments, the fusion molecule comprises a human IFN-α14 molecule having the amino acid sequence of SEQ ID NO: 3. In various embodiments, the fusion molecule comprises an interferon-beta (IFN-β) molecule. In various embodiments, the fusion molecule comprises a human IFN-β-1a molecule having the amino acid sequence of SEQ ID NO: 4. In various embodiments, the fusion molecule comprises a human IFN-β-1b molecule having the amino acid sequence of SEQ ID NO: 5.

[013] In various embodiments, the fusion molecules comprise an interferon molecule that is directly attached to the tumor associated antigen antibody.

[014] In various embodiments, the fusion molecules comprise an IFN molecule that is attached to the TAA Ab via a peptide linker. In various embodiments, the peptide linker is fewer than 20 amino acids in length. In various embodiments, the peptide linker is a G/S rich linker. In some embodiments, the peptide linker is an alpha-helical linker. In various embodiments, the peptide linker has the sequence set forth in SEQ ID NO: 18. In various embodiments, the peptide linker has the sequence set forth in SEQ ID NO: 19.

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

[016] In various embodiments, the cancer selected from the group consisting of: B cell lymphoma; a lung cancer (small cell lung cancer and non-small cell lung cancer); a bronchus cancer; a colorectal cancer; a prostate cancer; a breast cancer; a pancreas cancer; a stomach cancer; an ovarian cancer; a urinary bladder cancer; a brain or central nervous system cancer; a peripheral nervous system cancer; an esophageal cancer; a cervical cancer; a melanoma; a uterine or endometrial cancer; a cancer of the oral cavity or pharynx; a liver cancer; a kidney cancer; a biliary tract cancer; a small bowel or appendix cancer; a salivary gland cancer; a thyroid gland cancer; a adrenal gland cancer; an osteosarcoma; a chondrosarcoma; a

liposarcoma; a testes cancer; and a malignant fibrous histiocytoma; a skin cancer; a head and neck cancer; lymphomas; sarcomas; multiple myeloma; and leukemias.

[017] In various embodiments, there is provided a combination therapy method of treating a HER2/neu expressing cancer selected from the group consisting of breast cancer, ovarian cancer and non-small cell lung cancer (NSCLC), comprising administering to the individual a) a therapeutically effective amount of an anti-HER2/neu-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the combination therapy provides increased cell killing of cancer cells.

[018] In various embodiments, there is provided a combination therapy method of treating a CD20 expressing cancer selected from the group consisting of B-cell Non-Hodgkin's lymphoma (NHL) and B-cell chronic lymphocytic leukemia (CLL), comprising administering to the individual a) a therapeutically effective amount of an anti-CD20 Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the combination therapy provides increased cell killing of cancer cells.

[019] In various embodiments, there is provided a combination therapy method of treating CD138 expressing cancer selected from the group consisting of multiple myeloma, breast cancer, and bladder cancer, comprising administering to the individual a) a therapeutically effective amount of an anti-CD138 Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the combination therapy provides increased cell killing of cancer cells.

[020] In various embodiments, there is provided a combination therapy method of treating GRP94 expressing cancer selected from the group consisting of NSCLC, acute myeloid leukemia (AML), multiple myeloma, melanoma, and pancreatic cancer, comprising administering to the individual a) a therapeutically effective amount of an anti-GRP94 (endoplasmin) Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the combination therapy provides increased cell killing of cancer cells.

[021] In various embodiments, there is provided a combination therapy method of treating CD33 expressing cancer selected from the group consisting of AML, chronic myeloid leukemia (CML) and multiple myeloma, comprising administering to the individual a) a therapeutically effective amount of an anti-CD33 Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the combination therapy provides increased cell killing of cancer cells.

[022] In various embodiments, there is provided a combination therapy method of treating CD70 expressing cancer selected from the group consisting of renal cell carcinoma (RCC), Waldenstrom macroglobulinemia, multiple myeloma, and NHL, comprising administering to the individual a) a therapeutically effective amount of an anti-CD70 Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the combination therapy provides increased cell killing of cancer cells.

[023] In various embodiments, the individual previously responded to treatment with an anti-cancer therapy, but, upon cessation of therapy, suffered relapse (hereinafter "a recurrent cancer").

[024] In various embodiments, the individual has resistant or refractory cancer. In various embodiments, the cancer is refractory to targeted treatment with a TAA Ab. In various embodiments, the cancer is refractory to treatment with a proteasome inhibitor. In various embodiments, the cancer is refractory to treatment with a chemotherapeutic agent. In various embodiments, the cancer is refractory to targeted treatment with an immunoconjugate, antibody-drug conjugate (ADC), or fusion molecule comprising a TAA Ab and a cytotoxic agent. In various embodiments, the cancer is refractory to targeted treatment with a small molecule kinase inhibitor. In various embodiments, the cancer is refractory to combination therapy involving, e.g, treatment using surgery, treatment using stem cell transplantation, and treatment using radiation.

[025] In various embodiments, the combination therapy methods comprise administering the proteasome inhibitor and TAA Ab-IFN fusion molecule simultaneously, either in the same pharmaceutical composition or in separate pharmaceutical compositions. In various embodiments, the proteasome inhibitor and the TAA Ab-IFN fusion molecule are administered sequentially, i.e., the proteasome inhibitor is administered either prior to or after the administration of the TAA Ab-IFN fusion molecule. In various embodiments, the administration of the proteasome inhibitor and the TAA Ab-IFN fusion molecule are concurrent, i.e., the administration period of the proteasome inhibitor and that of the TAA Ab-IFN fusion molecule overlap with each other. In various embodiments, the administrations of the proteasome inhibitor and the TAA Ab-IFN fusion molecule are non-concurrent, e.g., in various embodiments the administration of the proteasome inhibitor is terminated before the TAA Ab-IFN fusion molecule is administered, and in various embodiments the administration of the TAA Ab-IFN fusion molecule is terminated before the proteasome inhibitor is administered.

[026] In another aspect, the present disclosure provides a pharmaceutical composition which comprises a TAA Ab-IFN fusion molecule and a proteasome inhibitor as active

ingredients, in a pharmaceutically acceptable excipient or carrier. In various embodiments, the pharmaceutical composition is 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.

[027] In other aspects, the present disclosure provides polynucleotides that encode the fusion molecules of the present disclosure; vectors comprising polynucleotides encoding fusion molecules of the disclosure; 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 disclosure; a process for producing a fusion molecule of the disclosure comprising culturing host cells comprising vectors comprising polynucleotides encoding fusion molecules of the disclosure such that the polynucleotide is expressed; and, optionally, recovering the fusion molecule from the host cell culture medium.

BRIEF DESCRIPTION OF THE DRAWINGS

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

[029] Figure 2 shows the anti-proliferative activity of the proteasome inhibitors bortezomib (Velcade®) and carfilzomib (Kyprolis®) in the MM.1s human multiple myeloma cell line. The ability of bortezomib and carfilzomib to inhibit the proliferation of MM.1s cells was assessed by MTS assay using tumor cells incubated for 96 hours with titrated concentrations of the inhibitors.

[030] Figure 3 shows the anti-proliferative activity of IGN004 and the combination of IGN004 with bortezomib in the MM.1s human multiple myeloma cell line. The ability of IGN004 with or without 5 nM bortezomib was assessed by MTS assay using tumor cells incubated for 96 hours with titrated concentrations of IGN004 in the presence or absence of 5 nM bortezomib.

[031] Figure 4 shows the anti-proliferative activity of IGN004 and the combination of IGN004 with carfilzomib in the MM.1s human multiple myeloma cell line. The ability of IGN004 with or without 5 nM carfilzomib was assessed by MTS assay using tumor cells incubated for 96 hours with titrated concentrations of IGN004 in the presence or absence of 5 nM carfilzomib.

MODE(S) FOR CARRYING OUT THE DISCLOSURE

[032] The present disclosure is directed to combination therapies designed to prevent, treat or manage a cancer that expresses a selective targeted antigen in an individual. Specifically, the disclosure provides combination therapies using a proteasome inhibitor and a tumor-associated antigen (TAA) antibody-IFN fusion molecule which comprises an IFN molecule attached to an antibody or antigen-binding fragment that specifically binds a TAA on a tumor cell, so as to prevent, treat or manage the tumor.

[033] The present disclosure is based on the inventors' insight that combination therapy using a proteasome inhibitor and a TAA Ab-IFN fusion molecule would significantly improve the overall efficacy and safety profiles of current proteasome inhibitor–based therapies, current IFN–based therapies, and current immunotherapies based on utilization of depleting antibodies to specific tumor antigens.

[034] The present disclosure thus provides combination therapy methods of treating a cancer that expresses or overexpresses a tumor associated antigen (TAA), comprising administering to the individual: a) a therapeutically effective amount of a tumor associated antigen antibody-interferon ("TAA Ab-IFN") fusion molecule, and b) a therapeutically effective amount of a proteasome inhibitor; wherein the combination therapy provides increased cell killing of cancer cells, i.e., a synergy exists between the TAA Ab-IFN fusion molecule and the proteasome inhibitor.

[035] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure 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 disclosure 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

[036] 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.

[037] 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.

[038] 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.

[039] 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.

[040] 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.

[041] "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.

[042] 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.

[043] Polypeptides of the disclosure 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)

[044] 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).

[045] 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 $\pm$ 2 is included. In various embodiments, those that are within $\pm$ 1 are included, and in various embodiments, those within $\pm$ 0.5 are included.

[046] 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.

[047] 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 $\pm$ 2 is included, in various embodiments, those that are within $\pm$ 1 are included, and in various embodiments, those within $\pm$ 0.5 are included.

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

Table 1

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
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

[049] 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.

[050] 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.

[051] 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.

[052] 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).

[053] 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 disclosure include fusion proteins.

[054] 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.

[055] 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%.

[056] 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%.

[057] 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.

[058] 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.

[059] 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.

[060] 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.

[061] "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.

[062] “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.

[063] 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.

[064] 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.

[065] 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.

[066] 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.

[067] 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.

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

[069] "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 disclosures of which are incorporated by reference.

[070] 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.

[071] 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.

[072] 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.

[073] In various embodiments, antibodies and antibody fragments used in the constructs of the present disclosure 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 disclosure 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.

[074] 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.

[075] 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.

[076] 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 disclosure 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 CDR_3. 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.

[077] 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.

[078] 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.

[079] 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 disclosure. 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.

[080] 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 Kb,

as defined below) value of at least 1 x 10^{-6} M, or at least 1 x 10^{-7} M, or at least 1 x 10^{-8} M, or at least 1 x 10^{-9} M, or at least 1 x 10^{-10} M, or at least 1 x 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.

[081] The phrase "inhibition of growth and/or proliferation" of a tumor cell refers to decrease in the growth rate and/or proliferation rate of a tumor cell. In various embodiments this includes death of a tumor 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.

[082] "Resistant or refractory cancer" refers to tumor cells or cancer that do not respond to previous anti-cancer therapy including, e.g., chemotherapy, surgery, radiation therapy, stem cell transplantation, and immunotherapy. Tumor cells can be resistant or refractory at the beginning of treatment, or they may become resistant or refractory during treatment. Refractory tumor cells include tumors that do not respond at the onset of treatment or respond initially for a short period but fail to respond to treatment. Refractory tumor cells also include tumors that respond to treatment with anticancer therapy but fail to respond to subsequent rounds of therapies. For purposes of this disclosure, refractory tumor cells also encompass tumors that appear to be inhibited by treatment with anticancer therapy but recur up to five years, sometimes up to ten years or longer after treatment is discontinued. The anticancer therapy can employ chemotherapeutic agents alone, radiation alone, targeted therapy alone, surgery alone, or combinations thereof. For ease of description and not limitation, it will be understood that the refractory tumor cells are interchangeable with resistant tumor.

[083] "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.

[084] The terms "subject" and "individual" 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.

[085] As used herein, the terms "co-administration", "co-administered" and "in combination with", referring to the TAA Ab-IFN fusion molecules of the disclosure and one or more other therapeutic agents, is intended to mean, and does refer to and include the following: simultaneous administration of such combination of TAA Ab-IFN fusion molecules of the disclosure and one or more proteasome inhibitors to an individual in need of treatment, when such components are formulated together into a single dosage form which releases said components at substantially the same time to said individual; substantially simultaneous administration of such combination of TAA Ab-IFN fusion molecules of the disclosure and one or more proteasome inhibitors to an individual in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at substantially the same time by said individual, whereupon said components are released at substantially the same time to said individual; sequential administration of such combination of TAA Ab-IFN fusion molecules of the disclosure and one or more proteasome inhibitors to an individual in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at consecutive times by said individual with a significant time interval between each administration, whereupon said components are released at substantially different times to said individual; and sequential administration of such combination of TAA Ab-IFN fusion molecules of the disclosure and one or more proteasome inhibitors to an individual in need of treatment, when such components are formulated together into a single dosage form which releases said components in a controlled manner whereupon they are concurrently, consecutively, and/or overlappingly released at the same and/or different times to said individual, where each part may be administered by either the same or a different route.

[086] The term “therapeutic protein" refers to proteins, polypeptides, antibodies, peptides or fragments or variants thereof, having one or more therapeutic and/or biological

activities. Therapeutic proteins encompassed by the disclosure include but are not limited to, proteins, polypeptides, peptides, antibodies, and biologics (the terms peptides, proteins, and polypeptides are used interchangeably herein). It is specifically contemplated that the term "therapeutic protein" encompasses the fusion molecules of the present disclosure.

[087] 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.

[088] 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 a t 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 inteferons (*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.

[089] Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The term "comprises" means "includes." All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present

specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Interferon and interferon mutants

[090] In the fusion molecules of the present disclosure, either the N- or C- terminus of a TAA 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), including any biosimilar, biogeneric, follow-on biologic, or follow-on protein version of an interferon taught in the art.. 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. Various such interferons have been extensively described in the literature and are well known to one of ordinary skill in the art (see, e.g., Pestka, Immunological Reviews, 202(1):8-32, 2004). FDA-approved interferons include, e.g., ROFERON®-A (Roche), INTRON® A (Schering), INFERGEN® (InterMune, Inc), AVONEX® (Biogen, Inc.), BETASERON® (Chiron Corporation) and REBIF® (EMD Serono and Pfizer).

[091] In various embodiments, the TAA 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.

[092] In various embodiments, the TAA 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.

[093] In various embodiments, the TAA 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.

[094] 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.

[095] In various embodiments, the TAA 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 TAA to which the antibody binds over cells that do not express the TAA, when compared to interferon having the same amino acid sequence not attached to an antibody.

[096] In various embodiments of the present disclosure, 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 know 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).

[097] In various embodiments of the present disclosure, the interferon mutant comprises one or more amino acid substitutions, insertions, and/or deletions. Means of

identifying such modified 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 know 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).

[098] The use of chemically modified interferons is also contemplated. For example, in certain embodiments, the interferon is chemically modified to increase serum half-life. Thus, for example, (2-sulfo-9-fluorenylmethoxycarbony1)$_7$-interferon-α2 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).

[099] 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-α5 (NP_002160.1), IFN-α6 (NP_066282.1), IFN-α7 (NP_066401.1), IFN-α8 (NP_002161.2), IFN-α10 (NP_002162.1), IFN-α16 (NP_002164.1), IFN-α17 (NP_067091.1), and IFN-α21 (NP_002166.2).

[0100] 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-α2b sequence provided below as SEQ ID NO: 1 (referred to hereinafter as "IFN-α2b-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-α2b-wt provided below as SEQ ID NO: 1. In some embodiments, the interferon has less than any of about 70%, 75%, 80%, 85%, 90%, or 95%, activity of IFN-α2b-wt provided below as SEQ ID NO: 1.

CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKA
ETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVI
QGVGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRS
FSLSTNLQESLRSKE (SEQ ID NO: 1)

[0101] 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-αR1 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.); PCT WO 2013/059885 (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.

[0102] In various embodiments, the interferon is an IFN-α2b mutant molecule having the amino acid sequence set forth in SEQ ID NO: 1, 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.

[0103] In various embodiments, the interferon is an IFN-α2b mutant molecule wherein the arginine at amino acid residue 149 of SEQ ID NO: 1 is replaced with an alanine (R149A) and the arginine at amino acid residue 162 of SEQ ID NO: 1 is replaced with an alanine (R162A). This IFN-α2b mutant molecule is referred to hereinafter as "IFN-α2b-M8". The amino acid sequence of IFN-α2b-M8 is provided below as SEQ ID NO: 2.

CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKA
ETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVI
QGVGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMAS
FSLSTNLQESLASKE (SEQ ID NO: 2)

[0104] 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-α14 sequence provided below as SEQ ID NO: 3 (referred to hereinafter as "IFN-α14-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-α14-wt provided below as SEQ ID NO: 3. In some embodiments, the interferon has less than any of about 70%, 75%, 80%, 85%, 90%, or 95%, activity of IFN-α14-wt provided below as SEQ ID NO: 3:

CNLSQTHSLNNRRTLMLMAQMRRISPFSCLKDRHDFEFPQEEFDGNQFQKAQAISVL
HEMMQQTFNLFSTKNSSAAWDETLLEKFYIELFQQMNDLEACVIQEVGVEETPLMNED
SILAVKKYFQRITLYLMEKKYSPCAWEVVRAEIMRSLSFSTNLQKRLRRKD
(SEQ ID NO: 3)

[0105] 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: 4 (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: 4. 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: 4:

MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKE
DAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLE
KEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRL
TGYLRN (SEQ ID NO: 4)

[0106] 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: 5 (referred to hereinafter as "IFN-β-1b-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-1b-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-β-1b-wt provided below as SEQ ID NO: 5:

MSYNLLGFLQRSSNFQSQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKE
DAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKL
EKEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINR
LTGYLRN (SEQ ID NO: 5)

[0107] 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-Δ1, IFN-Δ2, IFN-Δ3, IFN-Δ4, IFN-Δ5, IFN-Δ6, IFN-Δ7, IFN-Δ8, IFN-Δ9, IFN-Δ10 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.

Tumor Associated Antigen Antibodies

[0108] The methods of the present disclosure utilize isolated non-occurring genetically engineered TAA Ab-IFN fusion molecules comprising at least one tumor associated antigen antibody, or antigen-binding fragment thereof, attached to at least one interferon, or interferon

mutant molecule. The TAA can be a protein that comprises more than one polypeptide subunit. Thus, the TAA can be any peptide, polypeptide, protein, nucleic acid, lipid, carbohydrate, or small organic molecule, or any combination thereof. For example, the protein can be a dimer, trimer, or higher order multimer. In various embodiments, the TAA is a peptide that comprises about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 150, about 200, about 250, about 300, about 400, about 500, about 600, about 700, about 800, about 900 or about 1000 amino acids.

[0109] Tumor antigens expressed on the cell membrane are potential targets in immunotherapy, with the ideal tumor antigen absent on normal cells and overexpressed on the tumor cell surface. The TAA Ab-IFN fusion molecules used in the methods of the present disclosure may comprise an antibody, or antigen binding antibody fragment, specific to any of the tumor associated antigens described in the art, including any biosimilar, biogeneric, follow-on biologic, or follow-on protein version of any TAA described in the art. The TAA can be any peptide, polypeptide, protein, nucleic acid, lipid, carbohydrate, or small organic molecule, or any combination thereof, against which the skilled artisan wishes to induce an immune response.

[0110] In various embodiments, the TAA, TAA variant, or TAA mutant contemplated for use in the combination methods of the present disclosure is selected from, or derived from, the list provided in Table 2.

Table 2

Tumor Associated Antigen	RefSeq (protein)
Her2/neu	NP_001005862
Her3	NP_001005915
Her4	NP_001036064
EGF	NP_001171601
EGFR	NP_005219
CD2	NP_001758
CD3	NM_000732
CD5	NP_055022
CD7	NP_006128
CD13	NP_001141
CD19	NP_001171569
CD20	NP_068769
CD21	NP_001006659

CD23	NP_001193948
CD30	NP_001234
CD33	NP_001234.3
CD34	NP_001020280
CD38	NP_001766
CD46	NP_002380
CD55	NP_000565
CD59	NP_000602
CD69	NP_001772
CD70	NM_001252
CD71	NP_001121620
CD97	NP_001020331
CD117	NP_000213
CD127	NP_002176
CD134	NP_003318
CD137	NP_001552
CD138	NP_001006947
CD146	NP_006491
CD147	NP_001719
CD152	NP_001032720
CD154	NP_000065
CD195	NP_000570
CD200	NP_001004196
CD212	NP_001276952
CD223	NP_002277
CD253	NP_001177871
CD272	NP_001078826
CD274	NP_001254635
CD276	NP_001019907
CD278	NP_036224
CD279	NP_005009
CD309 (VEGFR2)	NP_002244
DR6	NP_055267
Kv1.3	NP_002223
5E10	NP_006279
MUC1	NP_001018016
uPA	NM_002658
SLAMF7 (CD319)	NP_001269517
MAGE 3	NP_005353
MUC 16 (CA-125)	NP_078966
KLK3	NP_001025218
K-ras	NP_004976
Mesothelin	NP_001170826

p53	NP_000537
Survivin	NP_001012270
G250 (Renal Cell Carcinoma Antigen)	GenBank CAB82444.1
PSMA	NP_001014986
Endoplasmin (GRP94)	NM_003299

[0111] In various embodiments, the TAA 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 any one of the sequences disclosed in Table 2.

[0112] Methods of generating antibodies that bind to the TAAs described herein are known to those skilled in the art. For example, a method for generating a monoclonal antibody that binds specifically to a targeted antigen polypeptide may comprise administering to a mouse an amount of an immunogenic composition comprising the targeted antigen polypeptide effective to stimulate a detectable immune response, obtaining antibody-producing cells (e.g., cells from the spleen) from the mouse and fusing the antibody-producing cells with myeloma cells to obtain antibody-producing hybridomas, and testing the antibody-producing hybridomas to identify a hybridoma that produces a monocolonal antibody that binds specifically to the targeted antigen polypeptide. Once obtained, a hybridoma can be propagated in a cell culture, optionally in culture conditions where the hybridoma-derived cells produce the monoclonal antibody that binds specifically to targeted antigen polypeptide. The monoclonal antibody may be purified from the cell culture. A variety of different techniques are then available for testing an antigen/antibody interaction to identify particularly desirable antibodies.

[0113] Other suitable methods of producing or isolating antibodies of the requisite specificity can used, including, for example, methods which select recombinant antibody from a library, or which rely upon immunization of transgenic animals (e.g., mice) capable of producing a full repertoire of human antibodies. See e.g., Jakobovits et al., Proc. Natl. Acad. Sci. (U.S.A.), 90: 2551-2555, 1993; Jakobovits et al., Nature, 362: 255-258, 1993; Lonberg et al., U.S. Pat. No. 5,545,806; and Surani et al., U.S. Pat. No. 5,545,807.

[0114] Antibodies can be engineered in numerous ways. They can be made as single-chain antibodies (including small modular immunopharmaceuticals or SMIPs™), Fab and $F(ab')_2$ fragments, etc. Antibodies can be humanized, chimerized, deimmunized, or fully human. Numerous publications set forth the many types of antibodies and the methods of engineering

such antibodies. For example, see U.S. Pat. Nos. 6,355,245; 6,180,370; 5,693,762; 6,407,213; 6,548,640; 5,565,332; 5,225,539; 6,103,889; and 5,260,203.

[0115] Chimeric antibodies can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted (see Robinson et al., International Patent Publication PCT/US86/02269; Akira, et al., European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Application WO 86/01533; Cabilly et al. U.S. Pat. No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al., Science, 240:1041-1043, 1988; Liu et al., Proc. Natl. Acad. Sci. (U.S.A.), 84:3439-3443, 1987; Liu et al., J. Immunol., 139:3521-3526, 1987; Sun et al., Proc. Natl. Acad. Sci. (U.S.A.), 84:214-218, 1987; Nishimura et al., Canc. Res., 47:999-1005, 1987; Wood et al., Nature, 314:446-449, 1985; and Shaw et al., J. Natl Cancer Inst., 80:1553-1559, 1988).

[0116] Methods for humanizing antibodies have been described in the art. In some embodiments, a humanized antibody has one or more amino acid residues introduced from a source that is nonhuman, in addition to the nonhuman CDRs. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525, 1986; Riechmann et al., Nature, 332:323-327, 1988; Verhoeyen et al., Science, 239:1534-1536, 1988), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable region has been substituted by the corresponding sequence from a nonhuman species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some framework region residues are substituted by residues from analogous sites in rodent antibodies.

[0117] U.S. Patent No. 5,693,761 to Queen et al, discloses a refinement on Winter et al. for humanizing antibodies, and is based on the premise that ascribes avidity loss to problems in the structural motifs in the humanized framework which, because of steric or other chemical incompatibility, interfere with the folding of the CDRs into the binding-capable conformation found in the mouse antibody. To address this problem, Queen teaches using human framework sequences closely homologous in linear peptide sequence to framework sequences of the mouse antibody to be humanized. Accordingly, the methods of Queen focus on comparing

framework sequences between species. Typically, all available human variable region sequences are compared to a particular mouse sequence and the percentage identity between correspondent framework residues is calculated. The human variable region with the highest percentage is selected to provide the framework sequences for the humanizing project. Queen also teaches that it is important to retain in the humanized framework, certain amino acid residues from the mouse framework critical for supporting the CDRs in a binding-capable conformation. Potential criticality is assessed from molecular models. Candidate residues for retention are typically those adjacent in linear sequence to a CDR or physically within 6Å of any CDR residue.

[0118] In other approaches, the importance of particular framework amino acid residues is determined experimentally once a low-avidity humanized construct is obtained, by reversion of single residues to the mouse sequence and assaying antigen binding as described by Riechmann et al, 1988. Another example approach for identifying important amino acids in framework sequences is disclosed by U.S. Patent No. 5,821,337 to Carter et al, and by U.S. Patent No. 5,859,205 to Adair et al. These references disclose specific Kabat residue positions in the framework, which, in a humanized antibody may require substitution with the correspondent mouse amino acid to preserve avidity.

[0119] Another method of humanizing antibodies, referred to as "framework shuffling", relies on generating a combinatorial library with nonhuman CDR variable regions fused in frame into a pool of individual human germline frameworks (Dall'Acqua et al., Methods, 36:43, 2005). The libraries are then screened to identify clones that encode humanized antibodies which retain good binding.

[0120] The choice of human variable regions, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable region of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the rodent is then accepted as the human framework region (framework region) for the humanized antibody (Sims et al., J. Immunol., 151:2296, 1993; Chothia et al., J. Mol. Biol., 196:901, 1987). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chain variable regions. The same framework may be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. (U.S.A.), 89:4285, 1992; Presta et al., J. Immunol., 151:2623, 1993).

[0121] The choice of nonhuman residues to substitute into the human variable region can be influenced by a variety of factors. These factors include, for example, the rarity of the amino acid in a particular position, the probability of interaction with either the CDRs or the antigen, and the probability of participating in the interface between the light and heavy chain variable domain interface. (See, for example, U.S. Patent Nos. 5,693,761, 6,632,927, and 6,639,055). One method to analyze these factors is through the use of three-dimensional models of the non-human and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available that illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, e.g., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, nonhuman residues can be selected and substituted for human variable region residues in order to achieve the desired antibody characteristic, such as increased affinity for the target antigen(s).

[0122] Methods for making fully human antibodies have been described in the art. By way of example, a method for producing a TAA antibody or antigen-binding fragment thereof comprises the steps of synthesizing a library of human antibodies on phage, screening the library with TAA or an antibody-binding portion thereof, isolating phage that bind TAA, and obtaining the antibody from the phage. By way of another example, one method for preparing the library of antibodies for use in phage display techniques comprises the steps of immunizing a non-human animal comprising human immunoglobulin loci with TAA or an antigenic portion thereof to create an immune response, extracting antibody-producing cells from the immunized animal; isolating RNA encoding heavy and light chains of antibodies of the disclosure from the extracted cells, reverse transcribing the RNA to produce cDNA, amplifying the cDNA using primers, and inserting the cDNA into a phage display vector such that antibodies are expressed on the phage. Recombinant anti-TAA antibodies of the disclosure may be obtained in this way.

[0123] Again, by way of example, recombinant human anti-TAA antibodies of the disclosure can also be isolated by screening a recombinant combinatorial antibody library. Preferably the library is a scFv phage display library, generated using human V_L and V_H cDNAs prepared from mRNA isolated from B cells. Methods for preparing and screening such libraries are known in the art. Kits for generating phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, catalog no. 27-9400-01; and the Stratagene SurfZAP™ phage display kit, catalog no. 240612). There also are other methods

and reagents that can be used in generating and screening antibody display libraries (see, e.g., U.S. Pat. No. 5,223,409; PCT Publication Nos. WO 92/18619, WO 91/17271, WO 92/20791, WO 92/15679, WO 93/01288, WO 92/01047, WO 92/09690; Fuchs et al., Bio/Technology, 9:1370-1372 (1991); Hay et al., Hum. Antibod. Hybridomas, 3:81-85, 1992; Huse et al., Science, 246:1275-1281, 1989; McCafferty et al., Nature, 348:552-554, 1990; Griffiths et al., EMBO J., 12:725-734, 1993; Hawkins et al., J. Mol. Biol., 226:889-896, 1992; Clackson et al., Nature, 352:624-628, 1991; Gram et al., Proc. Natl. Acad. Sci. (U.S.A.), 89:3576-3580, 1992; Garrad et al., Bio/Technology, 9:1373-1377, 1991; Hoogenboom et al., Nuc. Acid Res., 19:4133-4137, 1991; and Barbas et al., Proc. Natl. Acad. Sci. (U.S.A.), 88:7978-7982, 1991), all incorporated herein by reference.

[0124] Human antibodies are also produced by immunizing a non-human, transgenic animal comprising within its genome some or all of human immunoglobulin heavy chain and light chain loci with a human IgE antigen, e.g., a XenoMouse™ animal (Abgenix, Inc./Amgen, Inc.- Fremont, Calif.). XenoMouse™ mice are engineered mouse strains that comprise large fragments of human immunoglobulin heavy chain and light chain loci and are deficient in mouse antibody production. See, e.g., Green et al., Nature Genetics, 7:13-21, 1994 and U.S. Pat. Nos. 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598, 6,130,364, 6,162,963 and 6,150,584. XenoMouse™ mice produce an adult-like human repertoire of fully human antibodies and generate antigen-specific human antibodies. In some embodiments, the XenoMouse™ mice contain approximately 80% of the human antibody V gene repertoire through introduction of megabase sized, germline configuration fragments of the human heavy chain loci and kappa light chain loci in yeast artificial chromosome (YAC). In other embodiments, XenoMouse™ mice further contain approximately all of the human lambda light chain locus. See Mendez et al., Nature Genetics, 15:146-156, 1997; Green and Jakobovits, J. Exp. Med., 188:483-495, 1998; and WO 98/24893.

[0125] In various embodiments, the fusion molecules of the present disclosure utilize an antibody or antigen-binding fragment thereof is a polyclonal antibody, a monoclonal antibody or antigen-binding fragment thereof, a recombinant antibody, a diabody, a chimerized or chimeric antibody or antigen-binding fragment thereof, a humanized antibody or antigen-binding fragment thereof, a fully human antibody or antigen-binding fragment thereof, a CDR-grafted antibody or antigen-binding fragment thereof, a single chain antibody, an Fv, an Fd, an Fab, an Fab', or an $F(ab')_2$, and synthetic or semi-synthetic antibodies.

[0126] In various embodiments, the fusion molecules of the present disclosure utilize an antibody or antigen-binding fragment that binds to a TAA with a dissociation constant (K_D) of,

e.g., at least about $1x10^{-3}$ M, at least about $1x10^{-4}$ M, at least about $1x10^{-5}$ M, at least about $1x10^{-6}$ M, at least about $1x10^{-7}$ M, at least about $1x10^{-8}$ M, at least about $1x10^{-9}$ M, at least about $1x10^{-10}$ M, at least about $1x10^{-11}$ M, or at least about $1x10^{-12}$ M. In various embodiments, the fusion molecules of the present disclosure utilize an antibody or antigen-binding fragment that binds to a TAA with a dissociation constant (K_D) in the range of, e.g., at least about $1x10^{-3}$ M to at least about $1x10^{-4}$ M, at least about $1x10^{-4}$ M to at least about $1x10^{-5}$ M, at least about $1x10^{-5}$ M to at least about $1x10^{-6}$ M, at least about $1x10^{-6}$ M to at least about $1x10^{-7}$ M, at least about $1x10^{-7}$ M to at least about $1x10^{-8}$ M, at least about $1x10^{-8}$ M to at least about $1x10^{-9}$ M, at least about $1x10^{-9}$ M to at least about $1x10^{-10}$ M, at least about $1x10^{-10}$ M to at least about $1x10^{-11}$ M, or at least about $1x10^{-11}$ M to at least about $1x10^{-12}$ M.

[0127] In various embodiments, the fusion molecules of the present disclosure utilize an antibody or antigen-binding fragment that cross-competes for binding to the same epitope on the TAA as a reference antibody which comprises the heavy chain variable region and light chain variable region set forth in the references and sequence listings provided herein.

[0128] Anti-HER2 Antibodies. The ergB 2 gene, more commonly known as (HER2/neu), is an oncogene encoding a transmembrane receptor. Several antibodies have been developed against HER2/neu, including trastuzumab (e.g., HERCEPTIN®); Fornier et al., Oncology (Huntingt) 13: 647-58 (1999)), TAB-250 (Rosenblum et al., Clin. Cancer Res. 5: 865-74 (1999)), BACH-250 (Id.), TA1 (Maier et al., Cancer Res. 51: 5361-9 (1991)), and the mAbs described in U.S. Pat. Nos. 5,772,997; 5,770,195 (mAb 4D5; ATCC CRL 10463); and U.S. Pat. No. 5,677,171, 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 antibody is an anti-HER2/neu antibody which comprises a heavy chain having an amino acid sequence as set forth in SEQ ID NO: 6:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYT
RYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK (SEQ ID NO: 6)

[0129] In various embodiments, the heavy chain of the anti-HER2/neu 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: 6. In various embodiments, the heavy chain of the anti-HER2/neu antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 6.

[0130] In various embodiments the antibody is an anti-HER2/neu antibody which comprises a light chain having an amino acid sequence as set forth in SEQ ID NO: 7:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVP
SRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFE
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 7)

[0131] In various embodiments, the light chain of the anti-HER2/neu 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: 7. In various embodiments, the light chain of the anti-HER2/neu antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 7.

[0132] In various embodiments, the anti-HER2/neu antibody specifically binds to the same epitope as the antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 6 and a light chain having the amino acid sequence of SEQ ID NO: 7. In various embodiments, the anti-HER2/neu antibody competes for binding to the HER2/neu antigen with the antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 6 and a light chain having the amino acid sequence of SEQ ID NO: 7.

[0133] Anti-CD20 Antibodies. The FDA approved anti-CD20 antibody, Rituximab (IDEC C2B8; RITUXAN; ATCC No. HB 11388) has also been used to treat humans. Ibritumomab, is the murine counterpart to Rituximab (Wiseman et al., Clin. Cancer Res. 5: 3281s-6s (1999)). Other reported anti-CD20 antibodies include the anti-human CD20 mAb 1F5 (Shan et al., J. Immunol 162: 6589-95 (1999)), the single chain Fv anti-CD20 mouse mAb 1H4 (Haisma et al., Blood 92: 184-90 (1998)) and anti-B1 antibody (Liu et al., J. Clin. Oncol. 16: 3270-8 (1998)) 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 antibody is an chimeric anti-CD20 antibody which comprises a heavy chain having an amino acid sequence as set forth in SEQ ID NO: 8:

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNG
DTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGA

GTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK (SEQ ID NO: 8)

[0134] In various embodiments, the heavy chain of the anti-CD20 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: 8. In various embodiments, the heavy chain of the anti-CD20 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 8.

[0135] In various embodiments the antibody is an anti-CD20 antibody which comprises a light chain having an amino acid sequence as set forth in SEQ ID NO: 9:

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPV
RFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 9)

[0136] In various embodiments, the light chain of the anti-CD20 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: 9. In various embodiments, the light chain of the anti-CD20 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 9.

[0137] In various embodiments, the anti-CD20 antibody specifically binds to the same epitope as the antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 8 and a light chain having the amino acid sequence of SEQ ID NO: 9. In various embodiments, the anti-CD20 antibody competes for binding to the CD20 antigen with the antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 8 and a light chain having the amino acid sequence of SEQ ID NO: 9.

[0138] Anti-CD138 Antibodies. Murine and chimeric anti-CD138 antibodies are described in, e.g., US Patent Application Publication No. 20070183971 (Goldmakher) and 20090232810 (Kraus 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 antibody is an anti-CD138 antibody which comprises a heavy chain having an amino acid sequence as set forth in SEQ ID NO: 10:

QVQLQQSGSELMMPGASVKISCKATGYTFSNYWIEWVKQRPGHGLEWIGEILPGTGR
TIYNEKFKGKATFTADISSNTVQMQLSSLTSEDSAVYYCARRDYYGNFYYAMDYWGQG
TSVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 10)

[0139] In various embodiments, the heavy chain of the anti-CD138 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: 10. In various embodiments, the heavy chain of the anti-CD138 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 10.

[0140] In various embodiments the antibody is an anti-CD138 antibody which comprises a light chain having an amino acid sequence as set forth in SEQ ID NO: 11:

DIQMTQSTSSLSASLGDRVTISCSASQGINNYLNWYQQKPDGTVELLIYYTSTLQSGVP
SRFSGSGSGTDYSLTISNLEPEDIGTYYCQQYSKLPRTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 11)

[0141] In various embodiments, the light chain of the anti-CD138 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: 11. In various embodiments, the light chain of the anti-CD138 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 11.

[0142] In various embodiments, the anti-CD138 antibody specifically binds to the same epitope as an antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 10 and a light chain having the amino acid sequence of SEQ ID NO: 11. In various embodiments, the anti-CD138 antibody competes for binding to the CD138 antigen with an antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 10 and a light chain having the amino acid sequence of SEQ ID NO: 11.

[0143] Anti-GRP94 (endoplasmin) Antibodies. Isolated monoclonal antibodies, including fully human antibodies that specifically bind endoplasmin (GRP94) and use in detecting tumors that express endoplasmin, methods of treatment using the antibodies, and immunoconjugates comprising the antibodies are described in US Patent No. 8,497,354 (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 antibody is an anti-GRP94 antibody which comprises a heavy chain having an amino acid sequence as set forth in SEQ ID NO: 12:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMHWVRQAPGQRLEWMGWINAGN
GNTKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCARAHFDYWGQGTLVTVS
AASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 12)

[0144] In various embodiments, the heavy chain of the anti-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: 12. In various embodiments, the heavy chain of the anti-GRP94 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 12.

[0145] In various embodiments the antibody is an anti-GRP94 antibody which comprises a light chain having an amino acid sequence as set forth in SEQ ID NO: 13:

EIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 13)

[0146] In various embodiments, the light chain of the anti-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: 13. In various embodiments, the light chain of the anti-GRP94 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 13.

[0147] In various embodiments, the anti-GRP94 antibody specifically binds to the same epitope as an antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 12 and a light chain having the amino acid sequence of SEQ ID NO: 13. In various embodiments, the anti-GRP94 antibody competes for binding to the GRP94 antigen with an antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 12 and a light chain having the amino acid sequence of SEQ ID NO: 13.

[0148] Anti-CD33 Antibodies. CD33 is a glycoprotein expressed on early myeloid progenitor and myeloid leukemic (e.g., acute myelogenous leukemia, AML) cells, but not on stem cells. An IgG_1 monoclonal antibody was prepared in mice (M195) and also in a humanized form (HuM195) that reportedly has antibody-dependent cellular cytotoxicity (Kossman et al., Clin. Cancer Res. 5: 2748-55 (1999)). An anti-CD33 immunoconjugate (CMA-676) consisting of a humanized anti-CD33 antibody linked to the antitumor antibiotic calicheamicin reportedly demonstrated selective ablation of malignant hematopoiesis in some AML patients (Sievers et al., Blood 93: 3678-84 (1999) 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 antibody is an anti-CD33 antibody which comprises a heavy chain having an amino acid sequence as set forth in SEQ ID NO: 14:

QVQLVQSGAEVKKPGSSVKVSCKASGYTITDSNIHWVRQAPGQSLEWIGYIYPYNGGTD
YNQKFKNRATLTVDNPTNTAYMELSSLRSEDTAFYYCVNGNPWLAYWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 14)

[0149] In various embodiments, the heavy chain of the anti-CD33 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: 14. In various embodiments, the heavy chain of the anti-CD33 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 14.

[0150] In various embodiments the antibody is an anti-CD33 antibody which comprises a light chain having an amino acid sequence as set forth in SEQ ID NO: 15:

DIQLTQSPSTLSASVGDRVTITCRASESLDNYGIRFLTWFQQKPGKAPKLLMYAASNQG
SGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQTKEVPWSFGQGTKVEVKRTVAAP

SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 15)

[0151] In various embodiments, the light chain of the anti-CD33 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: 15. In various embodiments, the light chain of the anti-CD33 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 15.

[0152] In various embodiments, the anti-CD33 antibody specifically binds to the same epitope as an antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 14 and a light chain having the amino acid sequence of SEQ ID NO: 15. In various embodiments, the anti-CD33 antibody competes for binding to the CD33 antigen with an antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 14 and a light chain having the amino acid sequence of SEQ ID NO: 15.

[0153] Anti-CD70 (CD27L) Antibodies. Antibodies that bind CD70 are described in, e.g., US Patent No. 7,491,390 (Law et al) and US Patent No. 8,124,738 (Terret 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 antibody is an anti-CD70 antibody which comprises a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 16:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYIMHWVRQAPGKGLEWVAVI
SYDGRNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDTDGY
DFDYWGQGTLVTVSS (SEQ ID NO: 16)

[0154] In various embodiments, the heavy chain variable region of the anti-CD70 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: 16. In various embodiments, the heavy chain of the anti-CD70 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 16.

[0155] In various embodiments the antibody is an anti-CD70 antibody which comprises a light chain variable region having an amino acid sequence as set forth in SEQ ID NO: 17:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASN

RATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRTNWPLTFGGGTKVEIK
(SEQ ID NO: 17)

[0156] In various embodiments, the light chain variable region of the anti-CD70 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: 17. In various embodiments, the light chain of the anti-CD70 antibody comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 17.

[0157] In various embodiments, the anti-CD70 antibody specifically binds to the same epitope as an antibody having a heavy chain variable region having the amino acid sequence of SEQ ID NO: 16 and a light chain variable region having the amino acid sequence of SEQ ID NO: 17. In various embodiments, the anti-CD70 antibody competes for binding to the CD70 antigen with an antibody having a heavy chain having the amino acid sequence of SEQ ID NO: 16 and a light chain having the amino acid sequence of SEQ ID NO: 17.

Fusion Molecules

[0158] Generally speaking, the TAA antibody molecule and interferon molecule of the TAA Ab-IFN fusion 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. 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.

[0159] 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. 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).

[0160] 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.

[0161] The term "linker" is used herein to denote polypeptides comprising one or more amino acid residues joined by peptide bonds and are used to link the TAA antibody and interferon molecules of the present disclosure. 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 linker is capable of forming covalent bonds to both the antibody and to the interferon. 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 certain 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 certain preferred 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. Such linker polypeptides are well known in the art (see e.g., Holliger, P., et al., Proc. Natl. Acad. Sci. (U.S.A.), 90:6444, 1993; Poljak, R. J., et al.,

Structure, 2:1121, 1994). Linker length contemplated for use can vary from about 5 to 200 amino acids.

[0162] 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 alpha-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 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.), hereby incorporated by reference in its entirety for the proteolysis-resistant linkers and sequences provided therein). In various embodiments, the linker is a proteolysis-resistant linker set forth in Table 3 below:

Table 3

Examples of Proteolysis-Resistant Linkers

Linker Sequence	SEQ ID NO
SGGGGS	18
AEAAAKEAAAKAGS	19
GGGGS	20
SGGGGSGGGGS	21
GGGGG	22
GAGAGAGAGA	23
AEAAAKAGS	24
GGGGGGGG	25
AEAAAKEAAAKA	26
AEAAAKA	27
GGAGG	28

[0163] In various embodiments, the linker comprises SGGGGS (SEQ ID NO: 18). In various embodiments, the linker comprises AEAAAKEAAAKAGS (SEQ ID NO: 19).

[0164] In various embodiments, the fusion molecule is a recombinantly expressed fusion molecule and will comprise interferon molecules attached to the antibody via a peptide linker as

described herein and as depicted in Figure 1. In various embodiments, the preparation of the TAA Ab-IFN fusion molecules of the present disclosure can be generally described as follows: the heavy chain of the TAA Ab is recombinantly engineered with an interferon, or mutant thereof, at the carboxy-terminus using a peptide linker. After verifying that the fusion protein containing vector has the correct nucleotide sequence, it is transfected, along with the vector containing the light chain into, e.g., CHO cells. 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 is used to confirm the expected size of the molecule.

[0165] In various embodiments, the fusion molecules of the present disclosure will comprise the antibody, peptide linker, and interferon molecule combinations recited in Table 4.

Table 4

Examples of TAA Ab-IFN Fusion Molecules

TAA Antibody	Peptide Linker	Interferons
Anti-HER2neu	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-CD20	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-CD138	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-endoplasmin	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-CD33	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-CD70	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-CD38	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-BCMA	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-CD40	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-CS1	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-WT1	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2)

		wtIFN-β (SEQ ID NO: 4 or 5)
Anti-B7H3	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-PD-L1	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-PD-1	SEQ ID NO: 18 or SEQ ID NO: 19	wtIFN-α (SEQ ID NO: 1 or 2) wtIFN-β (SEQ ID NO: 4 or 5)
Anti-HER2neu	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-CD20	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-CD138	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-endoplasmin	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-CD33	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-CD70	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-CD38	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-BCMA	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-CD40	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-CS1	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-WT1	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-B7H3	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-PD-L1	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-PD-1	SEQ ID NO: 18 or SEQ ID NO: 19	IFN-α Mutant (SEQ ID NO: 3)
Anti-HER2neu	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-CD20	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-CD138	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-endoplasmin	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-CD33	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-CD70	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-CD38	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-BCMA	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-CD40	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-CS1	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-WT1	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-B7H3	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-PD-L1	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106
Anti-PD-L1	SEQ ID NO: 18 or SEQ ID NO: 19	Truncated IFN-β's IFN-Δ1-Δ10 US 2009/0025106

Proteasome Inhibitors

[0166] The ubiquitin proteasome pathway plays a critical role in regulating many processes in the cell which are important for tumor cell growth and survival. The proteasome is a multicatalytic proteinase complex responsible for the degradation of most intracellular proteins, including proteins crucial to cell cycle regulation and programmed cell death, or apoptosis. Catalytic activities of the proteasome are classified into three major categories, based upon preference to cleave a peptide bond after a particular amino acid residue. These activities are referred to as chymotrypsin-like (CT-L), trypsinlike (T-L) and caspase-like (C-L) and are associated with β5, β2 and β1 subunits respectively. The CT-L activity cleaves after hydrophobic residues, the T-L activity cleaves after basic residues and the C-L activity cleaves after acidic residues. Proteasome inhibitors were initially synthesized as in vitro probes to investigate the function of the proteasome's catalytic activity. As the essential role of the proteasome in cell function was unravelled, the possibility that proteasome inhibitors may have potential as therapeutic agents was considered.

[0167] Multiple studies have been performed over the years to design proteasome inhibitors and various classes have been identified and reported, including, e.g., reversible synthetic proteasome inhibitors (e.g., peptide aldehydes, peptide boron acids, α-keto carbonyl, α-keto amide, α-keto aldehyde); irreversible synthetic proteasome inhibitors (e.g., 3,4-dichloroisocoumarin, peptide chloromethyl/ diazomethyl ketones, α, β-epoxyketones, peptide vinyl sulfones); synthetic bivalent inhibitors of the proteasome; natural product small molecule inhibitors (e.g., eponemycin, epoxomicin, lactacystin, TMC-95 A-D); and protein inhibitors of the proteasome. (Bogyo and Wang, The Proteasome-Ubiquitin Protein Degradation Pathway, Springer Press, Vol 268, pgs. 185-204, 2002). All such proteasome inhibitors are contemplated for use in various embodiments of the present disclosure.

[0168] In preclinical cancer models, proteasome inhibitors (PIs) induce apoptosis, have *in vivo* antitumor efficacy, and sensitize malignant cells and tumors to the proapoptotic effects of conventional chemotherapeutics and radiation therapy. Interestingly, transformed cells display greater susceptibility to proteasome inhibition than nonmalignant cells. Proteasome functions as a gatekeeper, which controls the execution of protein degradation and plays a critical role in the ubiquitin-proteasome pathway. Inhibition of proteasome function causes cell cycle arrest and cell death. Pre-clinical studies have demonstrated that malignant cells are more susceptible to the cytotoxic effects of proteasome inhibition than normal cells. The mechanisms behind the

higher sensitivity of malignant cells are unclear, however, it is likely that they exploit the proteasome to regulate proliferation and anti-apoptotic pathways. Most tumor cells are highly proliferative and have an increased requirement for protein synthesis which would make them more vulnerable to proteasome inhibition. As a result, inhibition of proteasome function has emerged as a powerful strategy for anti-cancer therapy.

[0169] Clinical validation of the proteasome as a therapeutic target was achieved with bortezomib and has prompted the development of a second generation of proteasome inhibitors with improved pharmacological properties (Crawford et al, J Cell Commun Signal, 5(2):101-110, 2011). Bortezomib (Velcade®) was the first proteasome inhibitor to enter clinical practice and was approved by the FDA in 2003. However, dose limiting toxicity, particularly peripheral neuropathy, resistance and intravenous administration, and the fact that a sizeable proportion of individuals fail to respond to bortezomib therapy, or often relapse, prompted the development of a new generation of structurally distinct proteasome inhibitors.

[0170] Carfilzomib (Kyprolis®) is an intravenous, irreversible tetrapeptide epoxyketone second-generation proteasome inhibitor. The irreversible binding and higher affinity for proteasome translates into superior biological activity and cytotoxicity in bortezomib resistant cell lines *in vitro* and *in vivo*. It was approved by the FDA on July 20, 2012, for treatment of patients with multiple myeloma who have received at least two prior therapies, including bortezomib and a IMiD, and have demonstrated disease progression within 60 days of completion of last therapy (Siegel DS, Martin T, Wang M, et al., Blood, 120:2817-2825, 2012).

[0171] Ixazomib citrate (MLN9708) is a reversible, oral/intravenous boronate peptide PI currently being tested in clinical trials (Kupperman E, Lee EC, Cao Y, et al., Cancer Res., 70:1970-1980, 2010).

[0172] Oprozomib (ONX 0912) is an oral, irreversible, tripeptide epoxyketone that exerts its activity via inhibition of chymotrypsin- like activity of the proteasome. Biochemically, it is the oral analogue of carfilzomib, and demonstrates similar antiangiogenic and proapoptotic activity *in vitro* and *in vivo* (C Potts B, X Albitar M, C Anderson K, et al., Curr Cancer Drug Targets, 11:254-284, 2011).

[0173] Marizomib (NPI-0052) is an oral, irreversible β-lactone derivative that binds selectively to the active proteasomal sites. In vivo studies with marizomib demonstrate reduced tumor growth without significant toxicity in myeloma xenograft models (Singh AV et al., Br J Haematol., 149:550-559, 2010).

[0174] Delanzomib (CEP-18770) is an oral/intravenous, reversible boronate peptide agent which demonstrated equivalent antiproliferative activity of delanzomib and bortezomib in MM cell lines and xenograft models (Piva R et al., Blood, 111:2765-2775, 2008).

[0175] Other promising agents in preclinical development include immunoproteasome inhibitors such as PR-924 (Kuhn DJ, et al., Blood, 113:4667- 4676, 2009) and ISPI-101 (Hurchla MA, et al., Leukemia, 27:430-440, 2013).

[0176] Proteasome inhibitors contemplated for use herein include, but are not limited to, those set forth in Table 5.

Table 5

Examples of Proteasome Inhibitors

Proteasome Inhibitor	NCBI PubChem
Bortezomib	93860 387447
Bortezomib Intermediates II	86346386
Carfilzomib	11556711
NPI-0052 Marizomib	11347535
MLN9708 ixazomib	49867936
CEP-18770 Delanzomib	24800541
ONX0912 Oprozomib	25067547
MG132	462382
epoxomicin	16760412
disulfiram	3117
PR-924	92135659

Pharmaceutical Compositions

[0177] In another aspect, the present disclosure provides pharmaceutical compositions comprising a TAA Ab-IFN fusion molecule and a proteasome inhibitor as described above. The pharmaceutical compositions, methods and uses of the disclosure thus also encompass

embodiments of combinations (co-administration) with other active agents.

[0178] Generally, the TAA Ab-IFN fusion molecules and/or proteasome inhibitors of the disclosure 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 disclosure. 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 disclosure 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.

[0179] The protease inhibitors, e.g., bortezomib or carfilzomib, used in the present disclosure may be administered in any suitable chemical form, including as prodrugs, such as a pharmaceutically acceptable salt form and/or pharmaceutically acceptable ester form of the parent compound. In various embodiments, the pharmaceutically acceptable salt or ester derivative of the parent compound converts to the parent compound upon administration. As used herein, "pharmaceutically acceptable salt" refers to a derivative of the parent compound in which the compound is modified by making an acid or base salt thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids or boronic acids; and the like. As used herein, "pharmaceutically acceptable ester" refers to a derivative of the parent compound in which an acid residue is modified by making an ester thereof. Examples of pharmaceutically acceptable esters include, for example, boronic esters,

i.e., an ester derivative of a boronic acid compound, and cyclic boronic esters. Examples of cyclic boronic esters include, but are not limited to, pinanediol boronic ester, pinacol boronic ester, 1,2-ethanediol boronic ester, 1,3-propanediol boronic ester, 1,2-propanediol boronic ester, 2,3-butanediol boronic ester, 1,1,2,2-tetramethylethanediol boronic ester, 1,2-diisopropylethanediol boronic ester, 5,6-decanediol boronic ester, 1,2-dicyclohexylethanediol boronic ester, bicyclohexyl-1,1'-diol, and 1,2-diphenyl-1,2-ethanediol boronic ester.

[0180] A pharmaceutical composition of the disclosure 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.

[0181] The pharmaceutical compositions of the disclosure 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.

[0182] 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.

[0183] For example, in one aspect, sterile injectable solutions can be prepared by incorporating the fusion molecule and/or proteasome inhibitor 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.

[0184] The fusion molecules and/or proteasome inhibitors of the disclosure 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.

[0185] The pressurized container, pump, spray, atomizer, or nebulizer generally contains a solution or suspension of a fusion molecule and/or proteasome inhibitor of the disclosure comprising, for example, a suitable agent for dispersing, solubilizing, or extending release of the active, a propellant(s) as solvent.

[0186] 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.

[0187] 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 disclosure, a suitable powder base and a performance modifier.

[0188] Suitable flavours, such as menthol and levomenthol, or sweeteners, such as saccharin or saccharin sodium, may be added to those formulations of the disclosure intended for inhaled/intranasal administration.

[0189] 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.

[0190] 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 disclosure are typically arranged to administer a metered dose or "puff" of an antibody of the disclosure. The overall daily dose will typically be administered in a single dose or, more usually, as divided doses throughout the day.

[0191] The fusion molecules and/or proteasome inhibitor of the disclosure 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.

[0192] 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 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.

[0193] In various embodiments, the pharmaceutical compositions are formulated as orally deliverable tablets containing 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.

[0194] In various embodiments, the pharmaceutical compositions are formulated as hard gelatin capsules wherein the fusion molecule is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate, or kaolin or as soft gelatin capsules wherein the fusion molecule is mixed with an aqueous or an oil medium, for example, arachis oil, peanut oil, liquid paraffin or olive oil.

[0195] 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.

[0196] Any method for formulating and administering peptides, proteins, antibodies, and/or immunoconjugates accepted in the art may suitably be employed for administering the fusion molecules and/or proteasome inhibitors of the present disclosure.

Combination Therapy Methods of Use

[0197] As used herein, the terms "co-administration", "co-administered" and "in combination with", referring to the TAA Ab-IFN fusion molecules of the disclosure and one or more other therapeutic agents, is intended to mean, and does refer to and include the following: simultaneous administration of such combination of TAA Ab-IFN fusion molecules of the disclosure and one or more proteasome inhibitors to an individual in need of treatment, when such components are formulated together into a single dosage form which releases said components at substantially the same time to said individual; substantially simultaneous administration of such combination of TAA Ab-IFN fusion molecules of the disclosure and one or more proteasome inhibitors to an individual in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at substantially the same time by said individual, whereupon said components are released at substantially the same time to said individual; sequential administration of such combination of TAA Ab-IFN fusion molecules of the disclosure and one or more proteasome inhibitors to an individual in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at consecutive times by said individual with a significant time interval between each administration, whereupon said components are released at substantially different times to said individual; and sequential administration of such combination of TAA Ab-IFN fusion molecules of the disclosure and one or more proteasome inhibitors to an individual in need of treatment, when such components are formulated together into a single dosage form which releases said components in a controlled manner whereupon they are concurrently, consecutively, and/or overlappingly released at the same and/or different times to said individual, where each part may be administered by either the same or a different route.

[0198] The present disclosure relates to combination therapy methods of treating a cancer that expresses or overexpresses a tumor associated antigen (TAA), comprising administering to the individual: a) a therapeutically effective amount of a tumor associated antigen antibody-interferon (“TAA Ab-IFN”) fusion molecule, and b) a therapeutically effective

amount of a proteasome inhibitor; wherein the combination therapy provides increased cell killing of cancer cells, i.e., a synergy exists between the TAA Ab-IFN fusion molecule and the proteasome inhibitor.

[0199] The methods of the present disclosure are useful in treating certain cellular proliferative diseases, e.g., cancers. Such diseases include, but are not limited to, the following: a) proliferative diseases of the breast, which include, but are not limited to, invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma, lobular carcinoma in situ and metastatic breast cancer; b) proliferative diseases of lymphocytic cells, which include, but are not limited to, various T cell and B cell lymphomas, non-Hodgkins lymphoma, cutaneous T cell lymphoma, Hodgkins disease, and lymphoma of the central nervous system; (c) multiple myeloma, chronic neutrophilic leukemia, chronic eosinophilic leukemia/hypereosinophilic syndrome, chronic idiopathic myelofibrosis, polycythemia vera, essential thrombocythemia, chronic myelomonocytic leukemia, atypical chronic myelogenous leukemia, juvenile myelomonocytic leukemia, refractory anemia with ringed sideroblasts and without ringed sideroblasts, refractory cytopenia (myelodysplastic syndrome) with multilineage dysplasia, refractory anemia (myelodysplastic syndrome) with excess blasts, 5q-syndrome, myelodysplastic syndrome with t(9;12)(q22;p12), and myelogenous leukemia (e.g., Philadelphia chromosome positive (t(9;22)(qq34;q11)); d) proliferative diseases of the skin, which include, but are not limited to, basal cell carcinoma, squamous cell carcinoma, malignant melanoma and Kaposi's sarcoma; e) leukemias, which include, but are not limited to, acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia, f) proliferative diseases of the digestive tract, which include, but are not limited to, anal, colon, colorectal, esophageal, gallbladder, stomach (gastric), pancreatic cancer, pancreatic cancer-Islet cell, rectal, small-intestine and salivary gland cancers; g) proliferative diseases of the liver, which include, but are not limited to, hepatocellular carcinoma, cholangiocarcinoma, mixed hepatocellular cholangiocarcinoma, primary liver cancer and metastatic liver cancer; h) proliferative diseases of the male reproductive organs, which include, but are not limited to, prostate cancer, testicular cancer and penile cancer; i) proliferative diseases of the female reproductive organs, which include, but are not limited to, uterine cancer (endometrial), cervical, ovarian, vaginal, vulval cancers, uterine sarcoma and ovarian germ cell tumor; j) proliferative diseases of the respiratory tract, which include, but are not limited to, small cell and non-small cell lung carcinoma, bronchial adema, pleuropulmonary blastoma and malignant mesothelioma; k) proliferative diseases of the brain, which include, but are not limited to, brain stem and hyptothalamic glioma, cerebellar and cerebral astrocytoma,

medullablastoma, ependymal tumors, oligodendroglial, meningiomas and neuroectodermal and pineal tumors; l) proliferative diseases of the eye, which include, but are not limited to, intraocular melanoma, retinoblastoma, and rhabdomyosarcoma; m) proliferative diseases of the head and neck, which include, but are not limited to, laryngeal, hypopharyngeal, nasopharyngeal, oropharyngeal cancers, and lip and oral cancer, squamous neck cancer, metastatic paranasal sinus cancer; n) proliferative diseases of the thyroid, which include, but are not limited to, thyroid cancer, thymoma, malignant thymoma, medullary thyroid carcinomas, papillary thyroid carcinomas, multiple endocrine neoplasia type 2A (MEN2A), pheochromocytoma, parathyroid adenomas, multiple endocrine neoplasia type 2B (MEN2B), familial medullary thyroid carcinoma (FMTC) and carcinoids; o) proliferative diseases of the urinary tract, which include, but are not limited to, bladder cancer; p) sarcomas, which include, but are not limited to, sarcoma of the soft tissue, osteosarcoma, malignant fibrous histiocytoma, lymphosarcoma, and rhabdomyosarcoma; q) proliferative diseases of the kidneys, which include, but are not limited to, renal cell carcinoma, clear cell carcinoma of the kidney; and renal cell adenocarcinoma; r) precursor B-lymphoblastic leukemia/lymphoma (precursor B-cell acute lymphoblastic leukemia), B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone B-cell lymphoma, hairy cell leukemia, plasma cell myeloma/plasmacytoma, extranodal marginal zone B-cell lymphoma of MALT type, nodal marginal zone B-cell lymphoma, follicular lymphoma, mantle-cell lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell lymphoma, primary effusion lymphoma and Burkitt's lymphoma/Burkitt cell leukemia; (s) precursor T-lymphoblastic lymphoma/leukemia (precursor T-cell acute lymphoblastic leukemia), T-cell prolymphocytic leukemia, T-cell granular lymphocytic leukemia, aggressive NK-cell leukemia, adult T-cell lymphoma/leukemia (HTLV-1), extranodal NK/T-cell lymphoma, nasal type, enteropathy-type T-cell lymphoma, hepatosplenic gamma-delta T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, mycosis fungoides/Sezary syndrome, anaplastic large-cell lymphoma, T/null cell, primary cutaneous type, peripheral T-cell lymphoma, not otherwise characterized, angioimmunoblastic T-cell lymphoma, anaplastic large-cell lymphoma, T/null cell, and primary systemic type; (t) nodular lymphocyte-predominant Hodgkin's lymphoma, nodular sclerosis Hodgkin's lymphoma (grades 1 and 2), lymphocyte-rich classical Hodgkin's lymphoma, mixed cellularity Hodgkin's lymphoma, and lymphocyte depletion Hodgkin's lymphoma; and (u) AML with t(8;21)(q22;q22), AML1(CBF-alpha)/ETO, acute promyelocytic leukemia (AML with t(15;17)(q22;q11-12) and variants, PML/RAR-alpha), AML with abnormal bone marrow eosinophils (inv(16)(p13q22) or t(16;16)(p13;q11), CBFb/MYH11.times.), and AML with 11q23

(MLL) abnormalities, AML minimally differentiated, AML without maturation, AML with maturation, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroid leukemia, acute megakaryocytic leukemia, acute basophilic leukemia, and acute panmyelosis with myelofibrosis.

[0200] In various embodiments, the cancer selected from the group consisting of: B cell lymphoma; a lung cancer (small cell lung cancer and non-small cell lung cancer); a bronchus cancer; a colorectal cancer; a prostate cancer; a breast cancer; a pancreas cancer; a stomach cancer; an ovarian cancer; a urinary bladder cancer; a brain or central nervous system cancer; a peripheral nervous system cancer; an esophageal cancer; a cervical cancer; a melanoma; a uterine or endometrial cancer; a cancer of the oral cavity or pharynx; a liver cancer; a kidney cancer; a biliary tract cancer; a small bowel or appendix cancer; a salivary gland cancer; a thyroid gland cancer; a adrenal gland cancer; an osteosarcoma; a chondrosarcoma; a liposarcoma; a testes cancer; and a malignant fibrous histiocytoma; a skin cancer; a head and neck cancer; lymphomas; sarcomas; multiple myeloma; and leukemias.

[0201] Multiple myeloma is a hematological malignancy that comprises approximately 10% of all hematological malignancies, Multiple myeloma is known to affect approximately 50,000 individuals in the United States, with approximately 15,000 new individuals 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, white blood cells), bone and calcium problems, infections, kidney problems). If left untreated, the condition ultimately leads to the death of the individual. 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.

[0202] 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

[0203] 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.

[0204] In various embodiments, there is provided a combination therapy method of treating multiple myeloma, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising an anti-GRP94 Ab-IFN fusion molecule, and b) an effective amount of a pharmaceutical composition comprising a proteasome inhibitor. In various

embodiments, the anti-GRP94 Ab is a fully human antibody comprising the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13, and the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

[0205] In various embodiments, the present disclosure relates to a combination therapy method of treating multiple myeloma in an individual who has been diagnosed as having Stage I multiple myeloma, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising an anti-GRP94 Ab-IFN fusion molecule, and b) an effective amount of a pharmaceutical composition comprising a proteasome inhibitor.

[0206] In various embodiments, the present disclosure relates to a combination therapy method of treating multiple myeloma in an individual who has been diagnosed as having Stage II multiple myeloma, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising an anti-GRP94 Ab-IFN fusion molecule, and b) an effective amount of a pharmaceutical composition comprising a proteasome inhibitor.

[0207] In various embodiments, the present disclosure relates to a combination therapy method of treating multiple myeloma in an individual who has been diagnosed as having Stage III multiple myeloma, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising an anti-GRP94 Ab-IFN fusion molecule, and b) an effective amount of a pharmaceutical composition comprising a proteasome inhibitor.

[0208] In various embodiments, the present disclosure relates to a combination therapy method of treating recurrent multiple myeloma in an individual, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising a proteasome inhibitor, and b) an effective amount of a pharmaceutical composition comprising an anti-GRP94 Ab-IFN fusion molecule.

[0209] In various embodiments, the present disclosure relates to a combination therapy method of treating refractory multiple myeloma in an individual, comprising administering to the individual a) an effective amount of a pharmaceutical composition comprising an anti-GRP94 Ab-IFN fusion molecule, and b) an effective amount of a pharmaceutical composition comprising a proteasome inhibitor.

[0210] Treating multiple myeloma with the combination therapy of the presently disclosed subject matter can further include one or more treatment courses with a radiotherapeutic agent to induce DNA damage. Radiotherapeutic agents include, for example, gamma irradiation, X-rays, UV-irradiation, microwaves, electronic emissions, radioisotopes and the like. Therapy can be achieved by irradiating the localized tumor site with the above-described forms of radiation.

[0211] In various embodiments, the present disclosure relates to a method of treating a HER2/neu expressing cancer in an individual, comprising administering to the individual a) a therapeutically effective amount of an anti-HER2/neu-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the cancer is selected from the group consisting of breast cancer, ovarian cancer and non-small cell lung cancer (NSCLC), and wherein the combination therapy provides increased cell killing of cancer cells. In various embodiments, the anti-HER2/neu Ab is a fully human antibody comprising the heavy chain of SEQ ID NO: 6 and the light chain of SEQ ID NO: 7, and the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

[0212] In various embodiments, the present disclosure relates to a method of treating a CD20 expressing cancer in an individual, comprising administering to the individual a) a therapeutically effective amount of an anti-CD20 Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the cancer is selected from the group consisting of B-cell Non-Hodgkin's lymphoma (NHL) and B-cell chronic lymphocytic leukemia (CLL), and wherein the combination therapy provides increased cell killing of cancer cells. In various embodiments, the anti-CD20 Ab is a fully human antibody comprising the heavy chain of SEQ ID NO: 8 and the light chain of SEQ ID NO: 9, and the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

[0213] In various embodiments, the present disclosure relates to a method of treating a CD138 expressing cancer in an individual, comprising administering to the individual a) a therapeutically effective amount of an anti-CD138 Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the cancer is selected from the group consisting of multiple myeloma, breast cancer, and bladder cancer, and wherein the combination therapy provides increased cell killing of cancer cells. In various embodiments, the anti-CD138 Ab is a fully human antibody comprising the heavy chain of SEQ ID NO: 10 and the light chain of SEQ ID NO: 11, and the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

[0214] In various embodiments, the present disclosure relates to a method of treating a GRP94 expressing cancer in an individual, comprising administering to the individual a) a therapeutically effective amount of an anti-GRP94 Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the cancer is selected from the group consisting of NSCLC, acute myeloid leukemia (AML), melanoma, and pancreatic cancer, and wherein the combination therapy provides increased cell killing of cancer cells. In various embodiments, the anti-GRP94 Ab is a fully human antibody comprising the heavy chain

of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13, and the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

[0215] In various embodiments, the present disclosure relates to a method of treating a CD33 expressing cancer in an individual, comprising administering to the individual a) a therapeutically effective amount of an anti-CD33 Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the cancer is selected from the group consisting of AML, chronic myeloid leukemia (CML) and multiple myeloma, and wherein the combination therapy provides increased cell killing of cancer cells. In various embodiments, the anti-CD33 Ab is a fully human antibody comprising the heavy chain of SEQ ID NO: 14 and the light chain of SEQ ID NO: 15, and the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

[0216] In various embodiments, the present disclosure relates to a method of treating a CD70 expressing cancer in an individual, comprising administering to the individual a) a therapeutically effective amount of an anti-CD70 Ab-IFN-α fusion molecule; and b) a therapeutically effective amount of a proteasome inhibitor; wherein the cancer is selected from the group consisting of renal cell carcinoma (RCC), Waldenstrom macroglobulinemia, multiple myeloma, and NHL, and wherein the combination therapy provides increased cell killing of cancer cells. In various embodiments, the anti-CD70 Ab is a fully human antibody comprising the heavy chain variable region of SEQ ID NO: 16 and the light chain variable region of SEQ ID NO: 17, and the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

[0217] In various embodiments, the individual has a recurrent cancer. In various embodiments, the individual has resistant or refractory cancer. In various embodiments, the cancer is refractory to an anti-cancer treatment selected form the group consisting of targeted treatment with a TAA Ab, treatment with a proteasome inhibitor, treatment with a chemotherapeutic agent, targeted treatment with an immunoconjugate, targeted treatment with a small molecule kinase inhibitor, treatment using surgery, treatment using stem cell transplantation, and treatment using radiation.

[0218] In various embodiments, the combination methods of the present disclosure treat the individual by inhibiting the growth or proliferation of tumor cells which express or overexpress a specific TAA. These combination methods may inhibit the growth or proliferation of the tumor cells by, e.g., 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 cancer 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%.

[0219] The inhibition of tumor cell growth in an individual may be assessed by monitoring the cancer growth in an individual, for example in an animal model or in human individuals. 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.

[0220] 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 [^{3}H]-thymidine incorporation is A (under the treatment of modulators) and C (under negative control condition), the percentage of inhibition would be (C-A)/Cx100%.

[0221] The antiproliferative effect of a combination therapy of the disclosure may be assessed by administering the active ingredients of the combination therapy to a cultured tumor cell line. In the context of an *in vitro* assay, 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 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 Green and Sambrook, Molecular Cloning--A Laboratory Manual, 4th ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2012). Alternatively, the antiproliferative effect of a combination therapy of the disclosure may be assessed by administering the active ingredients of the combination therapy to an animal in an approved *in vivo* model for cell proliferation.

[0222] Examples of cancer 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, RPM1-8226, SR, P388 and P388/ADR); non-small cell lung cancer cell lines (e.g., A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522 and LXFL 529); small cell lung cancer cell lines (e.g., DMS 114 and SHP-77); colon cancer cell lines (e.g., COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620, DLD-1 and KM20L2); central nervous system (CNS) cancer cell lines (e.g., SF-268, SF-295, SF-539, SNB-19, SNB-75, U251, SNB-78 and XF 498); melanoma cell lines (e.g., LOX I MVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62, RPMI-7951 and M19-MEL); ovarian cancer cell lines (e.g., IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8 and SK-OV-3); renal cancer cell lines (e.g., 786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31, RXF-631 and SN12K1); prostate cancer cell lines (e.g., PC-3 and DU-145); breast cancer cell lines (e.g., MCF7, NCI/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, BT-549, T-47D and MDA-MB-468); thyroid cancer cell lines (e.g., SK-N-SH); 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, and OCI-My 5).

[0223] In various embodiments, there is provided a combination therapy method of treating a solid tumor selected from, e.g., colon cancer, pancreatic cancer, breast cancer (including basal breast carcinoma, ductal carcinoma and lobular breast carcinoma), lung cancers (including NSCLC), ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, renal cell carcinoma, cervical cancer, bladder cancer, and brain tumors.

[0224] In various embodiments, there is provided a combination therapy method of treating a liquid tumor selected from the group consisting of multiple myeloma, 11q23-positive acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia, myeloblastic leukemia, promyelocytic leukemia, myelomonocytic leukemia, monocytic leukemia, erythroleukemia, chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, 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.

Dosing

[0225] Dosage amounts and dosing regimens for the pharmaceutical compositions of the present disclosure can be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus can be administered, or several divided

doses (multiple or repeat or maintenance) can be administered over time and the dose can 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. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian individuals to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the present disclosure will be dictated primarily by the unique characteristics of the fusion molecule and proteasome inhibitor and the particular therapeutic effect to be achieved.

[0226] The precise dose of TAA Ab-IFN fusion molecule and/or proteasome inhibitor to be employed in the methods of the present disclosure 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 disclosure 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 disclosure 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.

[0227] Thus, the skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen is adjusted in accordance with methods well-known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a individual may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the individual. Accordingly, while various dose and

administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to an individual in practicing the present disclosure.

[0228] It is to be further understood that for any particular individual, 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 disclosure may be based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the individual, 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 disclosure encompasses intra-individual 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.

[0229] An exemplary, non-limiting weekly dosing range for a therapeutically effective amount of the fusion molecules of the disclsoure 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 0.1 to about 0.1 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.

[0230] For administration to human individuals, the total monthly dose of the fusion molecules of the disclosure can be in the range of 0.02-1200 mg per individual, 0.02-1100 mg per individual, 0.02-1000 mg per individual, 0.02-900 mg per individual, 0.02-800 mg per individual, 0.02-700 mg per individual, 0.02-600 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, 0.05-1200 mg per individual, 0.05-1100 mg per individual, 0.05-1000 mg per individual, 0.05-900 mg per individual, 0.05-800 mg per individual, 0.05-700 mg per individual, 0.05-600 mg per individual, 0.05-500 mg per individual, 0.05-400 mg per individual, 0.05-300 mg per individual, 0.05-200 mg per individual, 0.05-100 mg per individual, 0.05-50 mg per individual, 0.1-1200 mg per individual, 0.1-1100 mg per individual, 0.1-1000 mg per individual, 0.1-900 mg per individual, 0.1-800 mg per individual, 0.1-700 mg per individual, 0.1-600 mg per individual, 0.1-500 mg per individual, 0.1-400 mg per individual, 0.1-300 mg per individual, 0.1-200 mg per individual, 0.1-100 mg per individual, 0.1-50 mg per individual, 0.25-1200 mg per individual, 0.25-1100 mg per individual, 0.25-1000 mg per individual, 0.25-900 mg per individual, 0.25-800 mg per individual, 0.25-700 mg per individual, 0.25-600 mg per individual, 0.25-500 mg per individual, 0.25-400 mg per individual, 0.25-300 mg per individual, 0.25-200 mg per individual, 0.25-100 mg per individual, 0.25-50 mg per individual, 0.5-1200 mg per individual, 0.5-1100 mg per individual, 0.5-1000 mg per individual, 0.5-900 mg per individual, 0.5-800 mg per individual, 0.5-700 mg per individual, 0.5-600 mg per individual, 0.5-500 mg per individual, 0.5-400 mg per individual, 0.5-300 mg per individual, 0.5-200 mg per individual, 0.5-100 mg per individual, 0.5-50 mg per individual, 1-1200 mg per individual, 1-1100 mg per individual, 1-1000 mg per individual, 1-900 mg per individual, 1-800 mg per individual, 1-700 mg per individual, 1-600 mg per individual, 1-500 mg per individual, 1-400 mg per individual, 1-300 mg per individual, 1-200 mg per individual, 1-100 mg per

individual, or 1-50 mg per individual depending, of course, on the mode of administration. For example, an intravenous monthly dose can require about 1-1000 mg/individual. In various embodiments, the fusion molecules of the disclosure can be administered at about 0.02-200 mg per individual, 0.02-150 mg per individual or 0.02-100 mg/individual. 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.

[0231] An exemplary, non-limiting daily dosing range for a therapeutically effective amount of a fusion molecule of the disclosure can be 0.0001 to 100 mg/kg, 0.0001 to 90 mg/kg, 0.0001 to 80 mg/kg, 0.0001 to 70 mg/kg, 0.0001 to 60 mg/kg, 0.0001 to 50 mg/kg, 0.0001 to 40 mg/kg, 0.0001 to 30 mg/kg, 0.0001 to 20 mg/kg, 0.0001 to 10 mg/kg, 0.0001 to 5 mg/kg, 0.0001 to 4 mg/kg, 0.0001 to 3 mg/kg, 0.0001 to 2 mg/kg, 0.0001 to 1 mg/kg, 0.001 to 100 mg/kg, 0.001 to 90 mg/kg, 0.001 to 80 mg/kg, 0.001 to 70 mg/kg, 0.001 to 60 mg/kg, 0.001 to 50 mg/kg, 0.001 to 40 mg/kg, 0.001 to 30 mg/kg, 0.001 to 20 mg/kg, 0.001 to 10 mg/kg, 0.001 to 5 mg/kg, 0.001 to 4 mg/kg, 0.001 to 3 mg/kg, 0.001 to 2 mg/kg, 0.001 to 1 mg/kg, 0.010 to 50 mg/kg, 0.010 to 40 mg/kg, 0.010 to 30 mg/kg, 0.010 to 20 mg/kg, 0.010 to 10 mg/kg, 0.010 to 5 mg/kg, 0.010 to 4 mg/kg, 0.010 to 3 mg/kg, 0.010 to 2 mg/kg, 0.010 to 1 mg/kg, 0.1 to 50 mg/kg, 0.1 to 40 mg/kg, 0.1 to 30 mg/kg, 0.1 to 20 mg/kg, 0.1 to 10 mg/kg, 0.1 to 5 mg/kg, 0.1 to 4 mg/kg, 0.1 to 3 mg/kg, 0.1 to 2 mg/kg, 0.1 to 1 mg/kg, 1 to 50 mg/kg, 1 to 40 mg/kg, 1 to 30 mg/kg, 1 to 20 mg/kg, 1 to 10 mg/kg, 1 to 5 mg/kg, 1 to 4 mg/kg, 1 to 3 mg/kg, 1 to 2 mg/kg, or 1 to 1 mg/kg body weight. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood 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.

[0232] In various embodiments, the weekly dose for a therapeutically effective amount of a TAA Ab-IFN fusion molecule of the disclosure will be at least about 0.0001 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a fusion molecule of the disclosure will be at least about 0.0003 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a fusion molecule of the disclosure will be at least about 0.001 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a fusion molecule of the disclosure will be at least about 0.003 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a fusion molecule of the disclosure will be at least about 0.01 mg/kg body

weight. In various embodiments, the weekly dose for a therapeutically effective amount of a fusion molecule of the disclosure will be at least about 0.03 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a fusion molecule of the disclosure will be at least about 0.1 mg/kg body weight. In various embodiments, the weekly dose for a therapeutically effective amount of a fusion molecule of the disclosure will be at least about 0.3 mg/kg body weight. In various embodiments the fusion molecules will be administered via intravenous (IV) infusion twice weekly.

[0233] In various embodiments, the total dose administered will achieve a plasma antibody concentration in the range of, e.g., about .000001 to 1000 μg/ml, about .000001 to 750 μg/ml, about .000001 to 500 μg/ml, about .000001 to 250 μg/ml, about .00001 to 1000 μg/ml, about .00001 to 750 μg/ml, about .00001 to 500 μg/ml, about .00001 to 250 μg/ml, about .0001 to 1000 μg/ml, about .0001 to 750 μg/ml, about .0001 to 500 μg/ml, about .0001 to 250 μg/ml, about .001 to 1000 μg/ml, about .001 to 750 μg/ml, about .001 to 500 μg/ml, about .001 to 250 μg/ml, about .01 to 1000 μg/ml, about .01 to 750 μg/ml, about .01 to 500 μg/ml, about .01 to 250 μg/ml, about .1 to 1000 μg/ml, about .1 to 750 μg/ml, about .1 to 500 μg/ml, about .1 to 250 μg/ml, about 1 to 1000 μg/ml, about 1 to 750 μg/ml, about 1 to 500 μg/ml, about 1 to 250 μg/ml, about 10 to 1000 μg/ml, about 10 to 750 μg/ml, about 10 to 500 μg/ml, about 10 to 250 μg/ml, about 20 to 1000 μg/ml, about 20 to 750 μg/ml, about 20 to 500 μg/ml, about 20 to 250 μg/ml, about 30 to 1000 μg/ml, about 30 to 750 μg/ml, about 30 to 500 μg/ml, about 30 to 250 μg/ml.

[0234] Toxicity and therapeutic index of the pharmaceutical compositions of the disclosure can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effective dose is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compositions that exhibit large therapeutic indices are generally preferred.

[0235] 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.

[0236] The dosing frequency of the administration of the 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, then monthly; weakly without break for 3 weeks, then monthly; monthly; once every other month; once every three months; once every four months; once every five months; or once every six months, or yearly.

[0237] In the combination therapy methods of the present disclosure, the dosing regimen may be similar to or different from the approved multiple myeloma regimens, including those presented above. For example, the bortezomib or carfilzomib may be administered more or less frequently than in the approved regimens, and may optionally be administered at higher or lower doses.

[0238] The combination therapy methods of the present disclosure may be used as part of a treatment course further involving attempts to surgically remove part or all of a cancerous growth. For instance, the combination therapy may be administered after surgical treatment of a subject to treat any remaining neoplastic or metastasized cells. Treatment can also precede surgery, in an effort to shrink the size of the tumor to reduce the amount of tissue to be excised, thereby making the surgery less invasive and traumatic.

[0239] An exemplary, non-limiting weekly dosing range for a therapeutically effective amount of the proteasome inhibitors of the disclosure can be in the range of about 0.2 mg/m^2 to about 70 mg/m^2, about 0.2 mg/m^2 to about 60 mg/m^2, about 0.2 mg/m^2 to about 50 mg/m^2, about 0.2 mg/m^2 to about 40 mg/m^2, about 0.2 mg/m^2 to about 30 mg/m^2, about 0.2 mg/m^2 to about 20 mg/m^2, and about 0.2 mg/m^2 to about 10 mg/m^2. The preceding doses are suitable for any method of proteasome inhibitor administration, and are especially suitable for subcutaneous or intravenous dosing, with intravenous dosing preferred at the lower end of the preceding ranges, while oral doses of proteasome inhibitor will typically be at the high end of the preceding ranges.

[0240] Using the proteasome inhibitor bortezomib as one example, bortezomib may be administered in conjunction with a TAA Ab-IFN fusion molecule at any suitable dose. In various embodiments, a suitable bortezomib dose can be in the range of about 0.5 mg/m^2 to about 7 mg/m^2, about 0.5 mg/m^2 to about 6 mg/m^2, about 0.5 mg/m^2 to about 4 mg/m^2, about 0.5 mg/m^2 to about 3 mg/m^2, about 0.5 mg/m^2 to about 2 mg/m^2, and about 0.5 mg/m^2 to about 1 mg/m^2. In various embodiments, the bortezomib dose is in the range of about 0.6 mg/m^2 to about 1.5 mg/m^2. In various embodiments, the bortezomib dose is in the range of about 0.7 mg/m^2 to about 1.3 mg/m^2. In various embodiments, bortezomib doses include, but are not limited to, 0.7 mg/m^2, 1 mg/m^2, or 1.3 mg/m^2. The preceding doses are suitable for any method of bortezomib

administration, and are especially suitable for subcutaneous or intravenous dosing, with intravenous dosing preferred. Oral doses of bortezomib will typically be at the high end of the preceding ranges, such as about 1 mg/m^2 to about 5 mg/m^2, about 1.5 mg/m^2 to about 4 mg/m^2, or about 2 mg/m^2 to about 3 mg/m^2.

[0241] The bortezomib may be administered at the above-described doses with a TAA Ab-IFN fusion molecule according to any suitable schedule. The bortezomib dose amounts may be constant or varied within the dosing schedule. In various embodiments, the bortezomib dose is maintained at a constant level during the schedule unless significant drug-related toxicity is observed, in which case subsequent doses can be reduced, for example, by about 5-100%, by about 10-20%, by about 20-30%, and by about 30-40%. The bortezomib may be administered on the same or different days as the TAA Ab-IFN fusion molecule. In one embodiment, the bortezomib and TAA Ab-IFN fusion molecule are administered on the same days during the schedule. A suitable bortezomib schedule will typically range from once-daily dosing to once-weekly dosing or even once-monthly dosing. In various embodiments, the bortezomib is administered less frequently than once-daily, such as one dose every 2-14 days. In various embodiments, the bortezomib is administered every 3 to 7 days, such as every 3 to 4 days. In various embodiments, the schedule includes, after treatment with bortezomib for one or more weeks, such as 2, 3, or 4 weeks, a period of at least 5 days during which bortezomib is not administered, such as a period of about 7 to 21 days. In various embodiments, the rest period is about 10 to 17 days, such as about 10 days or about 17 days. For example, the bortezomib can be administered on days 1, 4, 8 and 11 of a 21 day cycle, wherein days 12-21 are a rest period. As another example, the bortezomib can be administered on days 1, 4, 8, and 11 of a 28 day cycle, wherein days 12-28 are a rest period. As another example, the bortezomib can be administered once weekly for 4 weeks (e.g., days 1, 8, 15 and 22 of a 35 day cycle) followed by a 13-day rest period (e.g., days 23 to 35 of the 35 day cycle). The scheduled dosing cycles can be repeated one or more times. For example, the scheduled cycle may be repeated until maximum response is observed, plus one or two additional cycles. As another example, the scheduled cycle may be repeated for 6 to 12 cycles. Optionally, after the initial cycles are completed, a "maintenance schedule" may be used in which the bortezomib is administered less frequency than in the initial schedule, such as once per week or once every two weeks. The maintenance schedule may be continued either for a fixed period of time, generally 1-2 years, or indefinitely as long as the individual is continuing to show no signs of progressive disease and is tolerating the treatment without significant toxicity.

[0242] Using the proteasome inhibitor carfilzomib as another example, carfilzomib may be administered in conjunction with a TAA Ab-IFN fusion molecule at any suitable dose. In various embodiments, a suitable carfilzomib dose can be in the range of about 5 mg/m^2 to about 70 mg/m^2, about 10 mg/m^2 to about 60 mg/m^2, about 15 mg/m^2 to about 50 mg/m^2, about 20 mg/m^2 to about 40 mg/m^2, about 25 mg/m^2 to about 35 mg/m^2, and about 20 mg/m^2 to about 30 mg/m^2. In various embodiments, the carfilzomib dose is in the range of about 10 mg/m^2 to about 60 mg/m^2. In various embodiments, the carfilzomib dose is in the range of about 20 mg/m^2 to about 30 mg/m^2. In various embodiments, carfilzomib doses include, but are not limited to, 20 mg/m^2, 27 mg/m^2, or 56 mg/m^2. The preceding doses are suitable for any method of carfilzomib administration, and are especially suitable for subcutaneous or intravenous dosing, with intravenous dosing preferred.

[0243] The carfilzomib may be administered at the above-described doses with a TAA Ab-IFN fusion molecule according to any suitable schedule. The carfilzomib dose amounts may be constant or varied within the dosing schedule. In various embodiments, the carfilzomib dose is maintained at a constant level during the schedule unless significant drug-related toxicity is observed, in which case subsequent doses can be reduced, for example, by about 5-100%, by about 10-20%, by about 20-30%, and by about 30-40%. The carfilzomib may be administered on the same or different days as the TAA Ab-IFN fusion molecule. In one embodiment, the carfilzomib and TAA Ab-IFN fusion molecule are administered on the same days during the schedule. A suitable carfilzomib schedule will typically range from once-weekly dosing, or twice weekly dosing, or even once-monthly dosing. In various embodiments, the carfilzomib is administered less frequently than once-daily, such as one dose every 2-14 days. In various embodiments, the schedule includes, after treatment with carfilzomib for one or more weeks, such as 2, 3, or 4 weeks, a period of at least 5 days during which carfilzomib is not administered, such as a period of about 7 to 21 days. In various embodiments, the rest period is about 10 to 17 days, such as about 12 days. For example, the carfilzomib can be administered on days 1, 2, 8, 9 and 15, 16 of a 28 day cycle, wherein days 17-28 are a rest period. In various embodiments, 15 mg/m^2 carfilzomib will be administered intravenously on two consecutive days weekly (e.g., for 3 weeks (days 1, 2, 8, 9, 15, 16), followed by a 12 day rest period (days 17 to 28), and, if tolerated, the carfilzomib dose for the second and succeeding cycles is 27 mg/m^2/day. In various embodiments, 20 mg/m^2 carfilzomib will be administered intravenously on two consecutive days weekly (e.g., for 3 weeks (days 1, 2, 8, 9, 15, 16), followed by a 12 day rest period (days 17 to 28), and, if tolerated, the carfilzomib dose for the second and succeeding cycles is 27 mg/m^2/day. In various embodiments, 20 mg/m^2 carfilzomib will be administered

intravenously on days 1-2, and 27 mg/m^2 carfilzomib will be administered intravenously on days 8, 9, and 15, and 16), followed by a 12 day rest period (days 17 to 28), and, if tolerated, the carfilzomib dose for the second and succeeding cycles is 27 mg/m^2/day. In various embodiments, 20 mg/m^2 carfilzomib will be administered intravenously on days 1-2, and 56 mg/m^2 carfilzomib will be administered intravenously on days 8, 9, and 15, and 16), followed by a 12 day rest period (days 17 to 28), and, if tolerated, the carfilzomib dose for the second and succeeding cycles is about 45 mg/m^2/day to 36 mg/m^2/day. The scheduled dosing cycles can be repeated one or more times. For example, the scheduled cycle may be repeated until maximum response is observed, plus one or two additional cycles. As another example, the scheduled cycle may be repeated for 6 to 12 cycles. Optionally, after the initial cycles are completed, a "maintenance schedule" may be used in which the carfilzomib is administered less frequency than in the initial schedule, such as once per week or once every two weeks. The maintenance schedule may be continued either for a fixed period of time, generally 1-2 years, or indefinitely as long as the individual is continuing to show no signs of progressive disease and is tolerating the treatment without significant toxicity.

[0244] In various embodiments, the combination therapy will comprise an initial 28-day cycle comprising bi-weekly intravenous doses of about 0.0001 mg/kg to 0.0003 mg/kg body weight TAA Ab-IFN fusion molecule during the first two weeks and intravenous doses of about 0.7 mg/m^2 to about 1.3 mg/m^2 bortezomib on days 1, 2, 8, 11.

[0245] In various embodiments, the combination therapy will comprise an initial 28-day cycle comprising bi-weekly intravenous doses of about 0.001 mg/kg to 0.003 mg/kg body weight TAA Ab-IFN fusion molecule during the first two weeks and intravenous doses of about 0.7 mg/m^2 to about 1.3 mg/m^2 bortezomib on days 1, 2, 8, 11.

[0246] In various embodiments, the combination therapy will comprise an initial 28-day cycle comprising bi-weekly intravenous doses of about 0.01 mg/kg to 0.03 mg/kg body weight TAA Ab-IFN fusion molecule during the first two weeks and intravenous doses of about 0.7 mg/m^2 to about 1.3 mg/m^2 bortezomib on days 1, 2, 8, 11.

[0247] In various embodiments, the combination therapy will comprise an initial 28-day cycle comprising bi-weekly intravenous doses of about 0.1 mg/kg to 0.3 mg/kg body weight TAA Ab-IFN fusion molecule during the first two weeks and intravenous doses of about 0.7 mg/m^2 to about 1.3 mg/m^2 bortezomib on days 1, 2, 8, 11.

[0248] In various embodiments, the combination therapy will comprise an initial 28-day cycle comprising bi-weekly intravenous doses of about 0.0001 mg/kg to 0.0003 mg/kg body

weight TAA Ab-IFN fusion molecule during the first two weeks and intravenous doses of about 15 mg/m^2 to about 30 mg/m^2 carfilzomib on days 1, 2, 8, 9, 15, 16.

[0249] In various embodiments, the combination therapy will comprise an initial 28-day cycle comprising bi-weekly intravenous doses of about 0.001 mg/kg to 0.003 mg/kg body weight TAA Ab-IFN fusion molecule during the first two weeks and intravenous doses of about 15 mg/m^2 to about 30 mg/m^2 carfilzomib on days 1, 2, 8, 9, 15, 16.

[0250] In various embodiments, the combination therapy will comprise an initial 28-day cycle comprising bi-weekly intravenous doses of about 0.01 mg/kg to 0.03 mg/kg body weight TAA Ab-IFN fusion molecule during the first two weeks and intravenous doses of about 15 mg/m^2 to about 30 mg/m^2 carfilzomib on days 1, 2, 8, 9, 15, 16.

[0251] In various embodiments, the combination therapy will comprise an initial 28-day cycle comprising bi-weekly intravenous doses of about 0.1 mg/kg to 0.3 mg/kg body weight TAA Ab-IFN fusion molecule during the first two weeks and intravenous doses of about 15 mg/m^2 to about 30 mg/m^2 carfilzomib on days 1, 2, 8, 9, 15, 16.

Nucleic acid Molecules and Fusion Molecule Expression

[0252] 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).

[0253] 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).

[0254] 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.

[0255] In one embodiment of the present disclosure, 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., Green and Sambrook, Molecular Cloning--A Laboratory Manual, 4th ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2012.

[0256] The present disclosure is also directed to host cells that express the fusion molecules of the disclosure. 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.

[0257] 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.

[0258] 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.

[0259] 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., IgG_1, IgG_2, IgG_3 and IgG_4) or subclass constant region and any allotypic variant thereof as described in Kabat (supra).

[0260] 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.

[0261] Additionally, the recombinant expression vectors of the disclosure 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.

[0262] 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 disclosure 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 disclosure 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.

[0263] Once expressed, the intact antibodies, individual light and heavy chains, or other immunoglobulin forms of the present disclosure 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 disclosure 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 disclosure 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.

[0264] In view of the aforementioned discussion, the present disclosure 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 disclosure so that the nucleic acid is expressed and, optionally, recovering the antibody from the host cell culture medium.

Kits

[0265] In various embodiments, this disclosure provides for kits for the treatment of cancer and/or in an adjunct therapy. Kits typically comprise a container containing a TAA Ab-IFN fusion molecule of the present disclosure and a container containing a proteasome inhibitor. The TAA Ab-IFN fusion molecule and proteasome inhibitor can be present in a pharmacologically acceptable excipient.

[0266] In addition the kits can optionally include instructional materials disclosing means of use of the TAA Ab-IFN fusion molecule and proteasome inhibitor to treat a cancer. The

instructional materials may also, optionally, teach preferred dosages, counter-indications, and the like.

[0267] 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.

[0268] 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 disclosure. 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.

[0269] The disclosure having been described, the following examples are offered by way of illustration, and not limitation.

Example 1

[0270] The preparation of an anti-GRP94 Ab-IFN fusion molecule 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.

[0271] Using the method described above, the following anti-GRP94 Ab-IFN fusion molecule was prepared: an interferon molecule having the amino acid sequence set forth in SEQ ID NO: 1 was attached to the C-terminus with an anti-GRP94 Ab that comprises a heavy

chain having the amino acid sequence set forth in SEQ ID NO: 12 and a light chain having the amino acid sequence set forth in SEQ ID NO: 13 (this fusion hereinafter referred to as "IGN004-wt").

Example 2

[0272] In this example, the proteasome inhibitors bortezomib (Velcade®) and carfilzomib (Kyprolis®) (Selleck Chemicals, Houston, TX) were assessed for their ability to inhibit the proliferation of MM.1s human multiple myeloma cells.

[0273] Cells were seeded in 96-well tissue culture plates at a density of $2x10^4$ cells per well and serial dilutions of the inhibitors added. After 96 hours incubation at 37°C in a 5% CO_2 atmosphere, proliferation was assessed by MTS assay. 20 µl 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 EC_{50} values were determined. % Cell Control was calculated as A490 (experimental) / A490 (control) x 100 where control refers to untreated cells.

[0274] As depicted in Figure 2, bortezomib and carfilzomib treatment caused a dose-dependent decrease in cellular proliferation of MM.1s cells, with EC_{50} values of 0.87 nM and 1.3 nM, respectively. Thus, MM.1s multiple myeloma cells are sensitive to the growth inhibitory effects of proteasome inhibitors.

Example 3

[0275] In this example, the combination of the TAA Ab-IFN fusion molecule designated IGN004-wt and bortezomib was assessed in an anti-proliferation assay using the MM.1s multiple myeloma cell line.

[0276] Cells were seeded in 96-well tissue culture plates at a density of $2x10^4$ cells per well and serial dilutions of IGN004-wt ± 5 nM bortezomib added. After 96 hours incubation at 37°C in a 5% CO_2 atmosphere, proliferation was assessed by MTS assay. 20 µl 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 EC_{50} values were determined. % Cell Control was calculated as A490(experimental) / A490(control) x 100 where control refers

to untreated cells for the IGN004-wt alone samples and cells treated with 5 nM bortezomib alone for the IGN004-wt + bortezomib samples.

[0277] As depicted in Figure 3, IGN004-wt treatment alone caused a dose-dependent decrease in cellular proliferation of MM.1s cells with an EC_{50} value of 0.24 nM. The addition of 5 nM bortezomib to IGN004-wt caused a leftward shift in the dose-response curve of IGN004-wt, resulting in >58000-fold increased potency (EC_{50} = 4.2 x 10^{-3} pM). The combinations of these agents thus demonstrate a synergistic effect for the treatment of cancers such as MM.

EXAMPLE 4

[0278] In this example, the combination of IGN004-wt and carfilzomib was assessed in an anti-proliferation assay using the MM.1s multiple myeloma cell line.

[0279] Cells were seeded in 96-well tissue culture plates at a density of $5x10^4$ cells per well and serial dilutions of IGN004-wt ± 5 nM carfilzomib added. After 96 hours incubation at 37°C in a 5% CO_2 atmosphere, proliferation was assessed by MTS assay. 20 µl 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 EC_{50} values were determined. % Cell Control was calculated as A490(experimental) / A490(control) x 100 where control refers to untreated cells for the IGN004-wt alone samples and cells treated with 5 nM carfilzomib alone for the IGN004-wt + carfilzomib samples.

[0280] As depicted in Figure 4, IGN004-wt treatment alone caused a dose-dependent decrease in cellular proliferation of MM.1s cells with an EC_{50} value of 24.2 pM. The addition of 5 nM carfilzomib to IGN004-wt caused a leftward shift in the dose-response curve of IGN004, resulting in 22-fold increased potency (EC_{50} = 1.1 pM). The combinations of these agents thus demonstrate a synergistic effect for the treatment of cancers such as MM.

Example 5

[0281] 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.

[0282] In one exemplary study, treatment groups are treated with an anti-GRP94 Ab-IFN fusion molecule of the present disclosure in combination with a proteasome inhibitor, bortezomib. A series of 21-day cycles will comprise groups of individuals receiving two weekly intravenous doses of between about 0.0001 mg/kg to about 0.3 mg/kg body weight anti-GRP94 Ab-IFN fusion molecule during each of the first two weeks followed by 7 days of rest, in combination with intravenous doses of between about 0.7 mg/m^2 to about 1.3 mg/m^2 bortezomib on days 1, 2, 8, 11, followed by 10 days of rest. This study will evaluate different dose levels of anti-GRP94 Ab-IFN fusion molecule and bortezomib to see which dose level seems to be the best for most people. There will be at least 8 dose levels with three to six individuals treated at each dose level and observed for one full cycle. Depending upon the side effects, the dose level will increase, stay the same or be decreased by one level for the next group. After each cycle, a physical exam (including vital signs), ECG, neurological examination, blood tests, urine tests, bone marrow aspiration, x-rays and MRI or CT scan is performed to assess how the individual's disease is responding to the treatment. Additional tests such as bone marrow biopsy, x-rays or scans may be performed. If the disease is stable or getting better, individuals will continue to receive repeated cycles of treatment. Once 8 cycles of treatment have been completed, the disease will be fully assessed again by blood tests, bone marrow biopsy, x-rays or scans. Again, if it is determined that the disease is stable of getting better, additional treatment cycles can be performed. If the disease is getting worse, treatment will be stopped.

[0283] In another exemplary study, treatment groups are treated with an anti-GRP94 Ab-IFN fusion molecule of the present disclosure in combination with a proteasome inhibitor, carfilzomib. A series of 28-day cycles will comprise groups of individuals receiving two weekly intravenous doses of between about 0.0001 mg/kg to about 0.3 mg/kg body weight anti-GRP94 Ab-IFN fusion molecule during each of the first two weeks followed by 14 days of rest, in combination with intravenous doses of about 15 mg/m^2 to about 30 mg/m^2 carfilzomib on days 1, 2, 8, 9, 15, 16, followed by 12 days of rest. This study will evaluate different dose levels of anti-GRP94 Ab-IFN fusion molecule and carfilzomib to see which dose level seems to be the best for most people. There will be at least 8 dose levels with three to six individuals treated at each dose level and observed for one full cycle. Depending upon the side effects, the dose level will increase, stay the same or be decreased by one level for the next group. After each cycle, a physical exam (including vital signs), ECG, neurological examination, blood tests, urine tests, bone marrow aspiration, x-rays and MRI or CT scan is performed to assess how the individual's disease is responding to the treatment. Additional tests such as bone marrow biopsy, x-rays or scans may be performed. If the disease is stable or getting better, individuals will continue to

receive repeated cycles of treatment. Once 8 cycles of treatment have been completed, the disease will be fully assessed again by blood tests, bone marrow biopsy, x-rays or scans. Again, if it is determined that the disease is stable of getting better, additional treatment cycles can be performed. If the disease is getting worse, treatment will be stopped.

[0284] All of the articles and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the articles and methods of this disclosure have been described in terms of preferred 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 disclosure. 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 disclosure 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 disclosure 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 disclosure 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 disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred 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 disclosure as defined by the appended claims.

Sequence Listings

[0285] 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. This

disclosure includes a Sequence Listing in computer readable form (ST25 format text file) prepared through the use of software program PatentIn and is identical to the accompanying sequence listings.

[0286] SEQ ID NO: 1 is the amino acid sequence of a human wildtype IFN-α2b molecule.

[0287] SEQ ID NO: 2 is the amino acid sequence of an IFN-α2b mutant molecule.

[0288] SEQ ID NO: 3 is the amino acid sequence of a wildtype IFN-α14 molecule.

[0289] SEQ ID NO: 4 is the amino acid sequence of a wildtype IFN-β-1a molecule.

[0290] SEQ ID NO: 5 is the amino acid sequence of a wildtype IFN-β-1b molecule.

[0291] SEQ ID NO: 6 is the amino acid sequence encoding the heavy chain of an anti-HER2/neu antibody. SEQ ID NO: 7 is the amino acid sequence encoding the light chain of an anti-HER2/neu antibody.

[0292] SEQ ID NO: 8 is the amino acid sequence encoding the heavy chain of an anti-CD20 antibody. SEQ ID NO: 9 is the amino acid sequence encoding the light chain of an anti-CD20 antibody.

[0293] SEQ ID NO: 10 is the amino acid sequence encoding the heavy chain of an anti-CD138 antibody. SEQ ID NO: 11 is the amino acid sequence encoding the light chain of an anti-CD138 antibody.

[0294] SEQ ID NO: 12 is the amino acid sequence encoding the heavy chain of an anti-GRP94 antibody. SEQ ID NO: 13 is the amino acid sequence encoding the light chain of an anti-GRP94 antibody.

[0295] SEQ ID NO: 14 is the amino acid sequence encoding the heavy chain of an anti-CD33 antibody. SEQ ID NO: 15 is the amino acid sequence encoding the light chain of an anti-CD33 antibody.

[0296] SEQ ID NO: 16 is the amino acid sequence encoding the heavy chain of an anti-CD70 antibody. SEQ ID NO: 17 is the amino acid sequence encoding the light chain of an anti-CD70 antibody.

[0297] SEQ ID NOs: 18-28 are the amino acid sequences of various peptide linkers.

SEQUENCE LISTINGS

SEQ ID NO: 1 - Amino acid sequence of a human wildtype IFN-α2b molecule.

CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIF
NLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTETPLMKEDSILAVRKYFQRITLY
LKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE

SEQ ID NO: 2 - Amino acid sequence of an IFN-α2b mutant molecule.
CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIF
NLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTETPLMKEDSILAVRKYFQRITLY
LKEKKYSPCAWEVVRAEIMASFSLSTNLQESLASKE

SEQ ID NO: 3 - Amino acid sequence of a wildtype IFN-α14 molecule.
CNLSQTHSLNNRRTLMLMAQMRRISPFSCLKDRHDFEFPQEEFDGNQFQKAQAISVLHEMMQ
QTFNLFSTKNSSAAWDETLLEKFYIELFQQMNDLEACVIQEVGVEETPLMNEDSILAVKKYFQRI
TLYLMEKKYSPCAWEVVRAEIMRSLSFSTNLQKRLRRKD

SEQ ID NO: 4 - Amino acid sequence of a wildtype IFN-β-1a molecule.
MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEML
QNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYGR
ILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN

SEQ ID NO: 5 - Amino acid sequence of a wildtype IFN-β-1b molecule.
MSYNLLGFLQRSSNFQSQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEML
QNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYGR
ILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN

SEQ ID NO: 6 - Amino acid sequence encoding the heavy chain of an anti-HER2/neu antibody.
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADS
VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

SEQ ID NO: 7 - Amino acid sequence encoding the light chain of an anti-HER2/neu antibody.
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS
GSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFEPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 8 - Amino acid sequence encoding the heavy chain of an anti-CD20 antibody.
QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYN
QKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK

SEQ ID NO: 9 - Amino acid sequence encoding the light chain of an anti-CD20 antibody.

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGS
GSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 10 - Amino acid sequence encoding the heavy chain of an anti-CD138 antibody.
QVQLQQSGSELMMPGASVKISCKATGYTFSNYWIEWVKQRPGHGLEWIGEILPGTGRTIYNEK
FKGKATFTADISSNTVQMQLSSLTSEDSAVYYCARRDYYGNFYYAMDYWGQGTSVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

SEQ ID NO: 11 - Amino acid sequence encoding the light chain of an anti-CD138 antibody.
DIQMTQSTSSLSASLGDRVTISCSASQGINNYLNWYQQKPDGTVELLIYYTSTLQSGVPSRFSG
SGSGTDYSLTISNLEPEDIGTYYCQQYSKLPRTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 12 - Amino acid sequence encoding the heavy chain of an anti-GRP94 antibody.
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMHWVRQAPGQRLEWMGWINAGNGNTKY
SQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCARAHFDYWGQGTLVTVSAASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

SEQ ID NO: 13 - Amino acid sequence encoding the light chain of an anti-GRP94 antibody.
EIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 14 - Amino acid sequence encoding the heavy chain of an anti-CD33 antibody.
QVQLVQSGAEVKKPGSSVKVSCKASGYTITDSNIHWVRQAPGQSLEWIGYIYPYNGGTDYNQKF
KNRATLTVDNPTNTAYMELSSLRSEDTAFYYCVNGNPWLAYWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

SEQ ID NO: 15 - Amino acid sequence encoding the light chain of an anti-CD33 antibody.
DIQLTQSPSTLSASVGDRVTITCRASESLDNYGIRFLTWFQQKPGKAPKLLMYAASNQGSGVPS
RFSGSGSGTEFTLTISSLQPDDFATYYCQQTKEVPWSFGQGTKVEVKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK

HKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 16 - Amino acid sequence encoding the heavy chain of an anti-CD70 antibody.
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYIMHWVRQAPGKGLEWVAVISYDGRNKYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDTDGYDFDYWGQGTLVTVSS

SEQ ID NO: 17 - Amino acid sequence encoding the light chain of an anti-CD70 antibody.
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSG
SGSGTDFTLTISSLEPEDFAVYYCQQRTNWPLTFGGGTKVEIK

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

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

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

SEQ ID NO: 21 - Amino acid sequence of a peptide linker.
SGGGGSGGGGS

SEQ ID NO: 22 - Amino acid sequence of a peptide linker.
GGGGG

SEQ ID NO: 23 - Amino acid sequence of a peptide linker.
GAGAGAGAGA

SEQ ID NO: 24 - Amino acid sequence of a peptide linker.
AEAAAKAGS

SEQ ID NO: 25 - Amino acid sequence of a peptide linker.
GGGGGGGG

SEQ ID NO: 26 - Amino acid sequence of a peptide linker.
AEAAAKEAAAKA

SEQ ID NO: 27 - Amino acid sequence of a peptide linker.
AEAAAKA

SEQ ID NO: 28 - Amino acid sequence of a peptide linker.
GGAGG

What is claimed is:

1. A method of treating a cancer that expresses or overexpresses a tumor associated antigen (TAA) in an individual, comprising administering to the individual: a) a therapeutically effective amount of a tumor associated antigen antibody-interferon ("TAA Ab-IFN") fusion molecule, and b) a therapeutically effective amount of a proteasome inhibitor.

2. The method of claim 1, wherein the proteasome inhibitor is selected from the group consisting of bortezomib, carfilzomib, NPI-0052, MLN9708, CEP-18770, ONX0912, MG132, disulfiram, PR-924, and epoxomicin.

3. A method according to any one of claims 1-2, wherein the TAA Ab-IFN fusion molecule comprises an TAA 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 Fab_2, a Fab'_2, a IgG, a IgM, a IgA, a IgE, a scFv, a dsFv, a dAb, a nanobody, a unibody, or a diabody.

4. The method of claim 3, wherein the TAA Ab is a fully human antibody selected from the group consisting of a fully human anti-HER2/neu Ab, a fully human anti-CD20 Ab, a fully human anti-CD138 Ab, a fully human anti-GRP94 (endoplasmin) Ab, a fully human anti-CD33 Ab, and a fully human anti-CD70 Ab.

5. A method according to any one of claims 1-4, wherein the TAA 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.

6. The method according to claim 5, wherein the IFN-α molecule is a human IFN-α2b molecule having the amino acid sequence of SEQ ID NO: 1.

7. A method according to any one of claims 1-6, wherein the cancer selected from the group consisting of: B cell lymphoma; a lung cancer (small cell lung cancer and non-small cell lung cancer); a bronchus cancer; a colorectal cancer; a prostate cancer; a breast cancer; a pancreas cancer; a stomach cancer; an ovarian cancer; a urinary bladder cancer; a brain or

central nervous system cancer; a peripheral nervous system cancer; an esophageal cancer; a cervical cancer; a melanoma; a uterine or endometrial cancer; a cancer of the oral cavity or pharynx; a liver cancer; a kidney cancer; a biliary tract cancer; a small bowel or appendix cancer; a salivary gland cancer; a thyroid gland cancer; a adrenal gland cancer; an osteosarcoma; a chondrosarcoma; a liposarcoma; a testes cancer; and a malignant fibrous histiocytoma; a skin cancer; a head and neck cancer; lymphomas; sarcomas; multiple myeloma; and leukemias.

8. The method of claim 7, wherein the cancer is a HER2/neu expressing cancer selected from the group consisting of breast cancer, ovarian cancer and non-small cell lung cancer (NSCLC), wherein the combination therapy provides increased cell killing of cancer cells, and wherein the TAA Ab-IFN fusion molecule is an anti-HER2/neu-IFN-α fusion molecule, and wherein the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

9. The method of claim 7, wherein the cancer is a CD20 expressing cancer selected from the group consisting of B-cell Non-Hodgkin's lymphoma (NHL) and B-cell chronic lymphocytic leukemia (CLL), wherein the TAA Ab-IFN fusion molecule is an anti-CD20-IFN-α fusion molecule, and wherein the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

10. The method of claim 7, wherein the cancer is a CD138 expressing cancer is selected from the group consisting of multiple myeloma, breast cancer, and bladder cancer, wherein the TAA Ab-IFN fusion molecule is an anti-CD138-IFN-α fusion molecule, and wherein the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

11. The method of claim 7, wherein the cancer is a GRP94 expressing cancer selected from the group consisting of NSCLC, acute myeloid leukemia (AML), multiple myeloma, melanoma, and pancreatic cancer, wherein the TAA Ab-IFN fusion molecule is an anti-GRP94 Ab-IFN-α fusion molecule, and wherein the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

12. The method of claim 7, wherein the cancer is a CD33 expressing cancer selected from the group consisting of AML, chronic myeloid leukemia (CML) and multiple myeloma, wherein

the TAA Ab-IFN fusion molecule is an anti-CD33-IFN-α fusion molecule, and wherein the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

13. The method of claim 7, wherein the cancer is a CD70 expressing cancer selected from the group consisting of renal cell carcinoma (RCC), Waldenstrom macroglobulinemia, multiple myeloma, and NHL, wherein the TAA Ab-IFN fusion molecule is an anti-CD70-IFN-α fusion molecule, and wherein the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

14. A method according to any one of claim 1-13, wherein the individual has a recurrent cancer.

15. A method according to any one of claim 1-13, wherein the individual has resistant or refractory cancer.

16. The method of claim 15, wherein the cancer is refractory to an anti-cancer treatment selected form the group consisting of targeted treatment with a TAA Ab, treatment with a proteasome inhibitor, treatment with a chemotherapeutic agent, targeted treatment with an immunoconjugate, targeted treatment with a small molecule kinase inhibitor, treatment using surgery, treatment using stem cell transplantation, and treatment using radiation.

17. A method of treating multiple myeloma in an individual, comprising administering to the individual a) a therapeutically effective amount of an anti-GRP94 Ab-IFN-α fusion molecule, and b) a therapeutically effective amount of a proteasome inhibitor; wherein the anti-GRP94 Ab is a fully human antibody comprising the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13; wherein the proteasome inhibitor is selected from the group consisting of bortezomib and carfilzomib.

18. The method according to claim 17, wherein the individual has relapsed multiple myeloma.

19. The method according to claim 17, wherein the individual has refractory multiple myeloma.

20. A method according to any one of claim 1-19, wherein the combination therapy methods comprise administering the TAA Ab-IFN fusion molecule and proteasome inhibitor simultaneously.

21. A method according to any one of claim 1-19, wherein the administration of the TAA Ab-IFN fusion molecule and proteasome inhibitor are concurrent.

22. A method according to any one of claim 1-19, wherein the administration of the TAA Ab-IFN fusion molecule and proteasome inhibitor are non-concurrent.

23. A method according to any one of claims 1-22, wherein the TAA Ab-IFN fusion molecules comprise an interferon molecule that is directly attached to the tumor associated antigen antibody.

24. A method according to any one of claims 1-22, wherein the TAA Ab-IFN fusion molecules comprise an IFN molecule that is attached to the TAA Ab via a peptide linker, wherein the peptide linker is fewer than 20 amino acids in length.

25. The method according to claim 24, wherein the peptide linker has the sequence selected from the group consisting of SEQ ID NO: 18 and SEQ ID NO: 19.

26. A pharmaceutical composition which comprises a TAA Ab-IFN fusion molecule and a proteasome inhibitor as active ingredients, in a pharmaceutically acceptable excipient or carrier.

27. The pharmaceutical composition according to claim 26, wherein the pharmaceutical composition is 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 and via infusions.

28. A kit comprising: 1) a pharmaceutical composition comprising a therapeutically effective amount of a proteasome inhibitor, and 2) a pharmaceutical composition comprising a therapeutically effective amount of a TAA Ab-IFN fusion molecule.

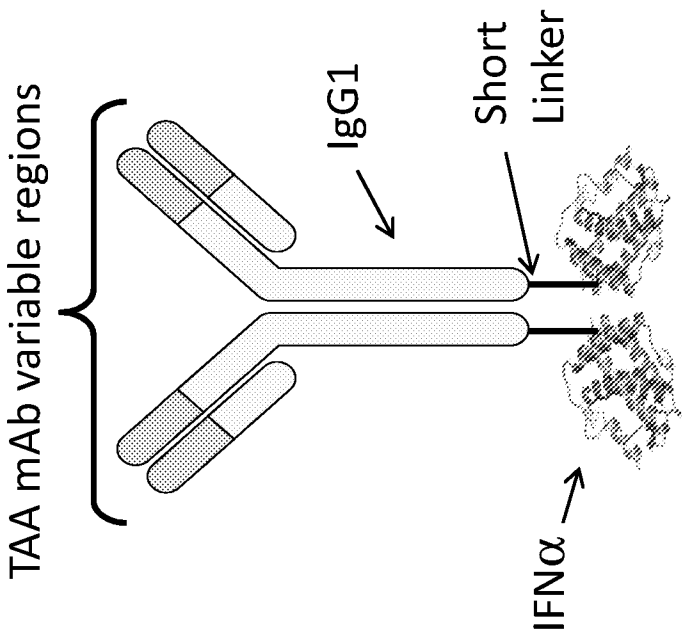

FIG. 1

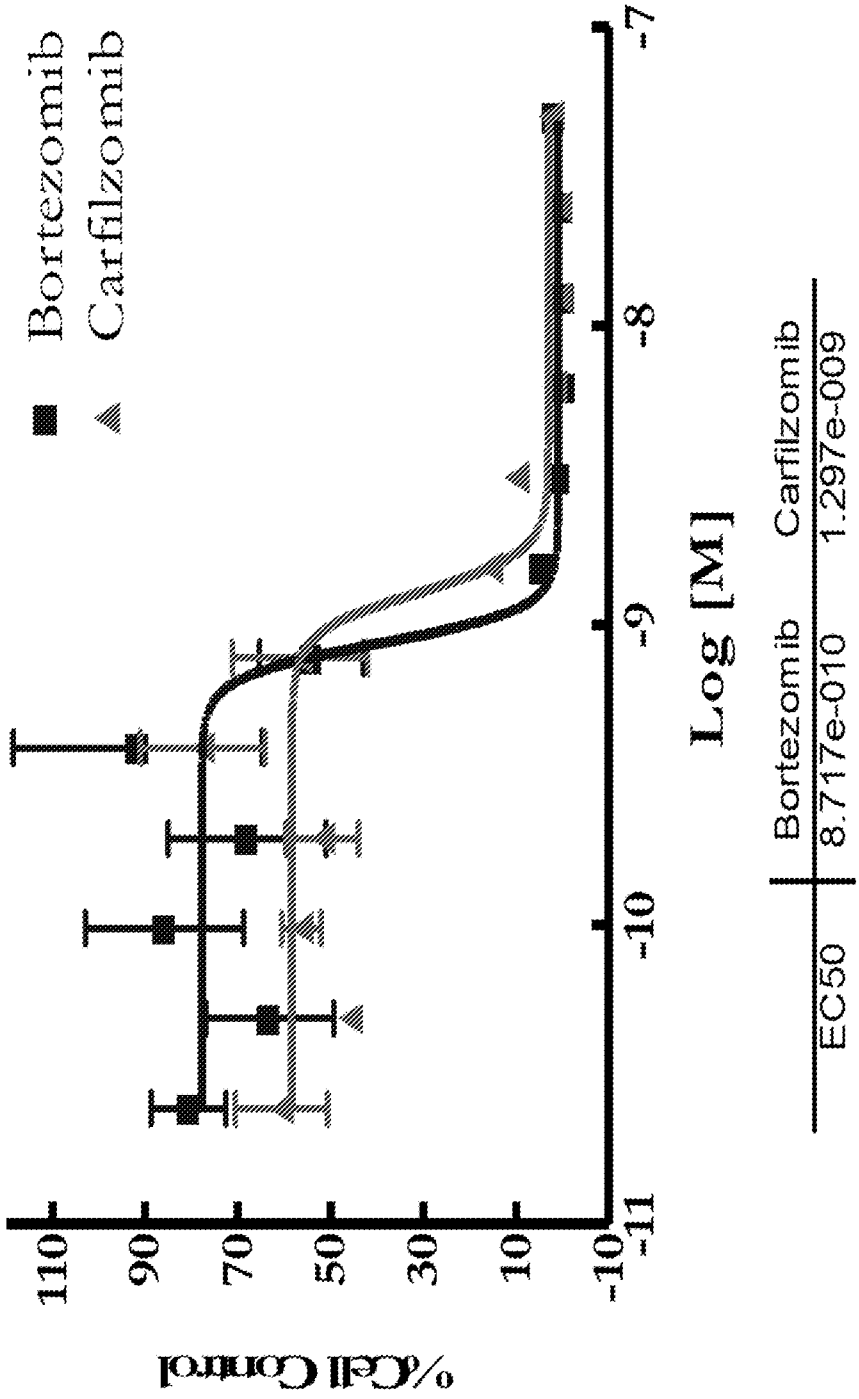
Bortezomib
Carfilzomib
% Cell Control
110
90
70
50
30
10
-10
-11
-10
-9
-8
-7
Log [M]
Bortezomib Carfilzomib
EC50 8.717e-010 1.297e-009

FIG. 2

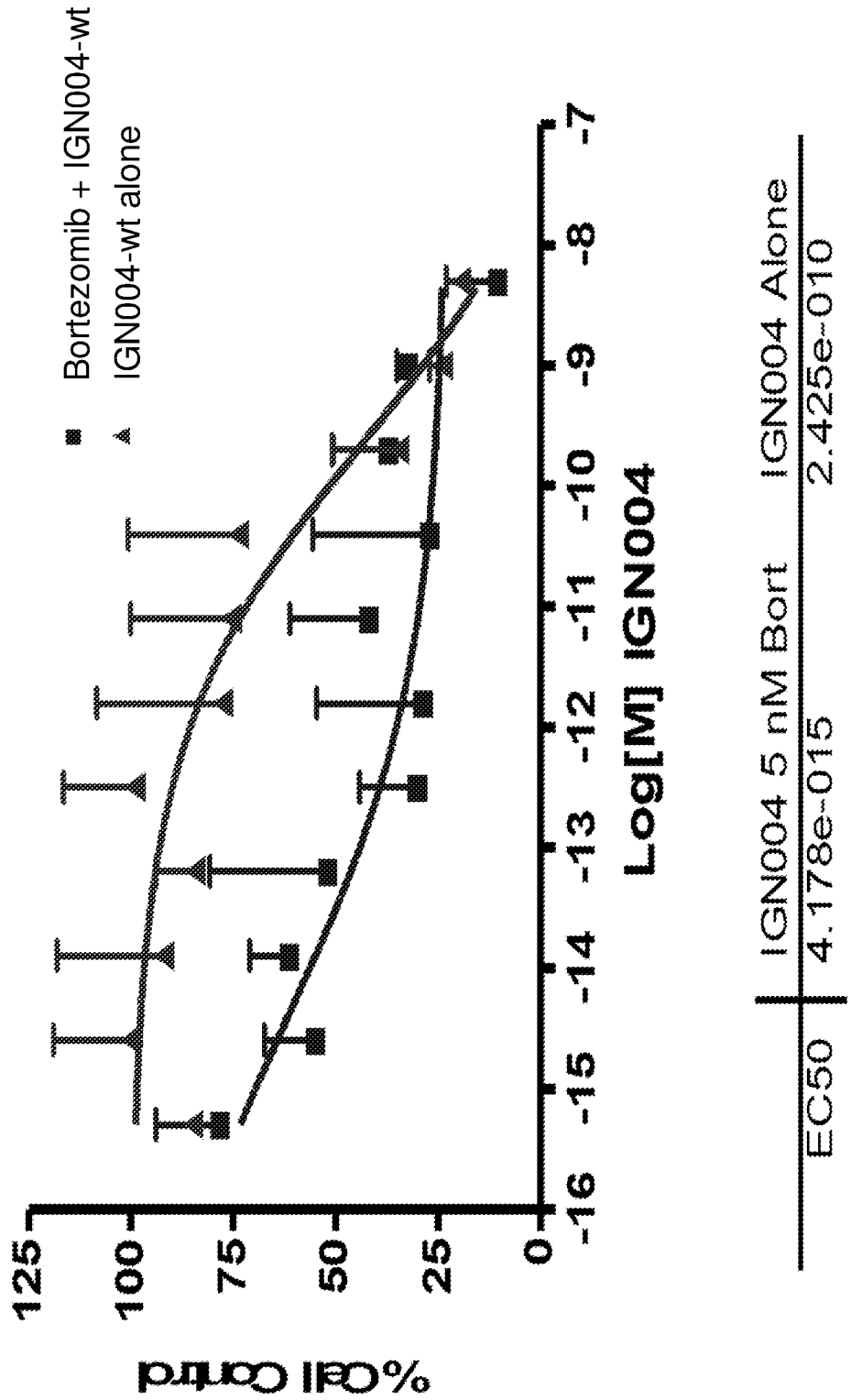
Bortezomib + IGN004-wt
IGN004-wt alone
% Cell Cntrl
125
100
75
50
25
0
-16
-15
-14
-13
-12
-11
-10
-9
-8
-7
Log[M] IGN004
IGN004 5 nM Bort
IGN004 Alone
EC50
4.178e-015
2.425e-010

FIG. 3

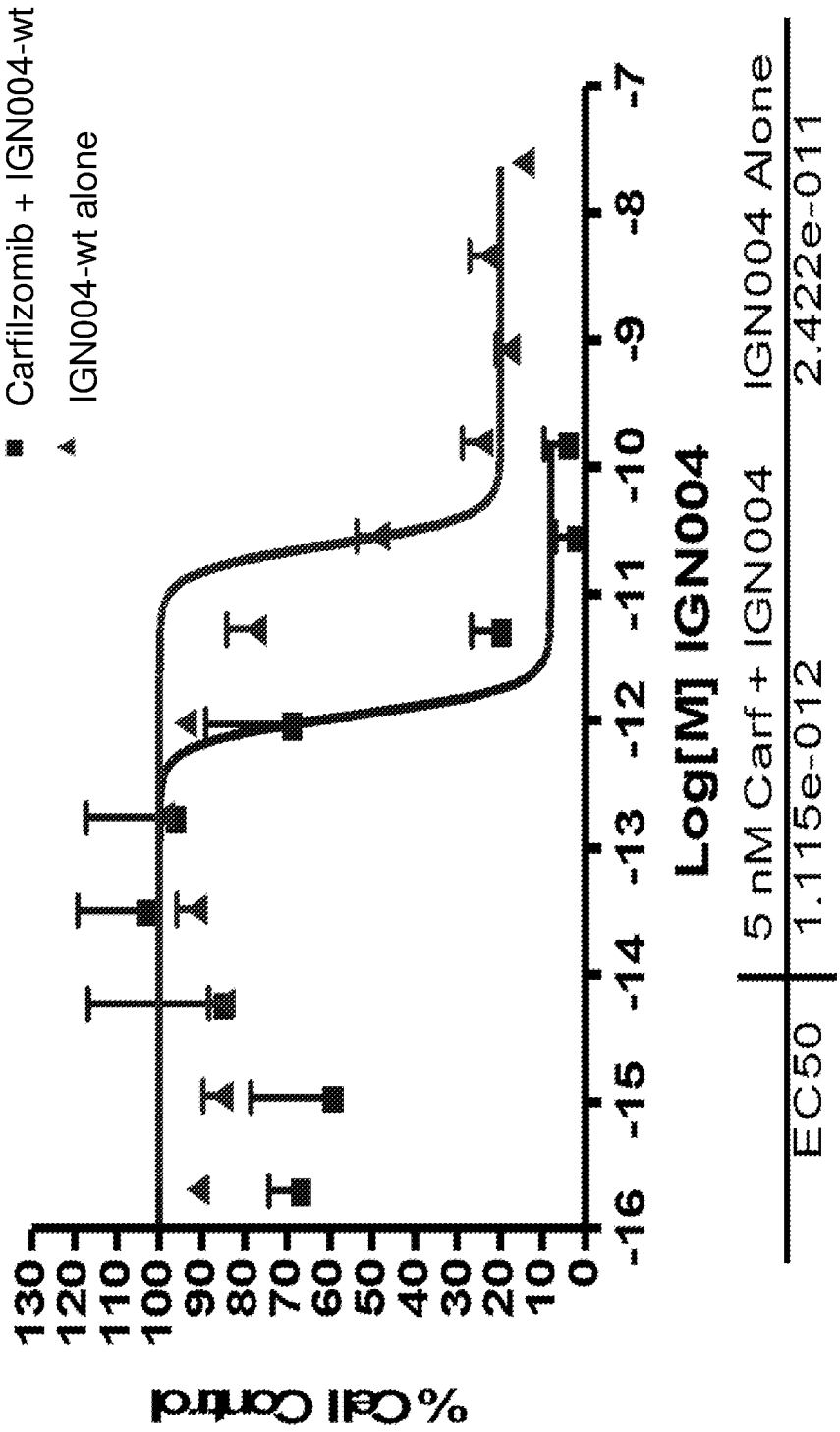
Carfilzomib + IGN004-wt
IGN004-wt alone
% Cell Cntrl
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-16
-15
-14
-13
-12
-11
-10
-9
-8
-7
Log[M] IGN004
5 nM Carf + IGN004
IGN004 Alone
EC50
1.115e-012
2.422e-011

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/37054

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 16/24, 16/46, 14/555, 14/715; A61K 38/21; C12N 15/19, 15/20, 15/21 (2016.01)
CPC - C07K 16/249, 16/462, 14/555, 14/7156; A61K 38/212, 38/215
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): C07K 16/24, 16/28, 16/46, 14/555, 14/715; A61K 38/21, 31/506, 31/519; C12N 15/19, 15/20, 15/21; C07D 487/04 (2016.01)
CPC: C07K 16/249, 16/462, 14/555, 14/7156; A61K 38/212, 38/215; C07D 409/12, 493/04, 405/12, 413/12

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); Google Scholar; Pubmed; EBSCO
Keywords: cancer, tumor, carcinoma, antigen, myeloma, interferon, antibody, administer, kit, proteasome, inhibit

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2014/089354 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 12 June, 2014; paragraphs [0005]-[0006], [0082], [0103], [0130], [0205], [0208], [0233], [0249], [0260]-[0261], [0310]; claims 1-2, 67	1-2, 3/1-2, 26-28 ---------- 4/3/1-2, 17-19
Y	US 2014/0112865 A1 (IMMUNOMEDICS, INC.,) 24 April, 2014; paragraphs [0034], [0079]	4/3/1-2
Y	US 2013/0230517 A1 (IMMUNGENE, INC.,) 05 September, 2013; paragraphs [0020], [0035]; SEQ ID NO.s 7-8	17-19
Y	(DIMOPOULOS, MA et al.) Emerging Therapies For The Treatment of Relapsed or Refractory Multiple Myeloma. European Journal of Haematology. 25 November 2010; Vol. 86, No. 1; pages 1-15; page 1, column 2, paragraph 2; page 3, column 1, paragraph 2; page 3, column 2, paragraph 3; DOI: 10.1111/j.1600-0609.2010.01542.x.	18-19

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 2016 (26.08.2016)	23 SEP 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

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/37054

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.: 5-16, 20-25
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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.:

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.