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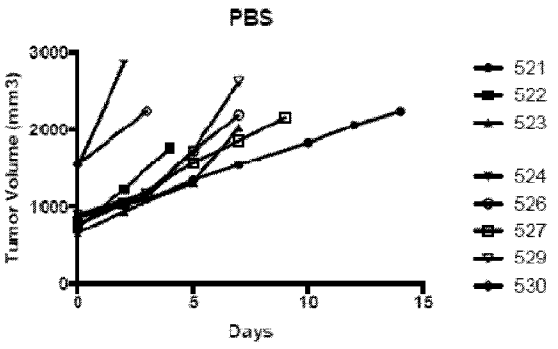


FIG. 1A

(57) Abstract: Described herein are methods, formulations and kits for treating a patient with cancer with anti-VEGF antibodies and albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes. In another aspect, provided herein are methods for enhancing the efficacy of albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes. In another aspect, provided herein are methods for enhancing the therapeutic outcome in a patient suffering from a cancer expressing soluble VEGF which patient is selected to be treated with nanoparticles comprising albumin-bound paclitaxel and anti-VEGF antibodies wherein said antibodies of the nanoparticles are integrated onto and/or into said nanoparticles which method comprises treating said patient with a subtherapeutic amount of said anti-VEGF antibody prior to any subsequent treatment with the nanoparticles.

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METHODS OF TREATING CANCER USING COMPOSITIONS OF ANTIBODIES AND CARRIER PROTEINS WITH ANTIBODY PRETREATMENT

[0001] This application claims benefit of the filing dates of provisional application nos. 62/247,470 filed October 28, 2015 and 62/252,377 filed November 6, 2015, the disclosures of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This disclosure relates to novel methods and kits for treating cancer using vascular endothelial growth factor (VEGF) antibodies and carrier protein/VEGF antibody complexes.

STATE OF THE ART

[0003] Chemotherapy remains a mainstay for systemic therapy for many types of cancer, including melanoma. Most chemotherapeutics are only slightly selective to tumor cells, and toxicity to healthy proliferating cells can be high (Allen TM. (2002) *Cancer* 2:750-763), often requiring dose reduction and even discontinuation of treatment. In theory, one way to overcome chemotherapy toxicity issues as well as improve drug efficacy is to target the chemotherapy drug to the tumor using antibodies that are specific for proteins selectively expressed (or overexpressed) by tumors cells to attract targeted drugs to the tumor, thereby altering the biodistribution of the chemotherapy and resulting in more drug going to the tumor and less affecting healthy tissue. Despite 30 years of research, however, specific targeting rarely succeeds in the therapeutic context.

[0004] Conventional antibody dependent chemotherapy (ADC) is designed with a toxic agent linked to a targeting antibody via a synthetic protease-cleavable linker. The efficacy of such ADC therapy is dependent on the ability of the target cell to bind to the antibody, the linker to be cleaved, and the uptake of the toxic agent into the target cell. Schrama, D. et al. (2006) *Nature reviews. Drug discovery* 5:147-159.

[0005] Antibody-targeted chemotherapy promised advantages over conventional

therapy because it provides combinations of targeting ability, multiple cytotoxic agents, and improved therapeutic capacity with potentially less toxicity. Despite extensive research, clinically effective antibody-targeted chemotherapy remains elusive: major hurdles include the instability of the linkers between the antibody and chemotherapy drug, reduced tumor toxicity of the chemotherapeutic agent when bound to the antibody and the inability of the conjugate to bind and enter tumor cells. In addition, these therapies did not allow for control over the size of the antibody-drug conjugates.

[0006] It was recently discovered that nanoparticle complexes of a carrier protein-bound chemotherapeutic and antibody have superior therapeutic efficacy, including in humans, than either the chemotherapeutic or antibody delivered alone or sequentially, resulting in a significantly reduced tumor size. See, PCT Application No. PCT/US15/54295. It is contemplated that these nanoparticles have improved targeting of the chemotherapeutics to the tumor, and that this targeting may be mediated, at least in part, by the bound antibody. It is further contemplated that the carrier protein-bound chemotherapeutic increases the targeting of the antibody to the tumor or that the existence of the complex shows greater stability *in vivo* than chemotherapeutic alone.

[0007] Even still, there remains a need in the art to improve the efficacy of cancer therapeutics.

SUMMARY

[0008] This invention is directed, in part, on the discovery that treatment of a cancer expressing vascular endothelial growth factor (VEGF) with an anti-VEGF antibody composition enhances the effectiveness of carrier –bound (*e.g.*, albumin) chemotherapeutic/anti-VEGF antibody nanoparticles containing a therapeutically effective amount of the chemotherapeutic. Preferably, such anti-VEGF antibodies are administered prior to treatment with such nanoparticles. Accordingly, in one aspect, provided herein are methods for treating a patient suffering from a cancer which expresses VEGF wherein said patient is treated with a sub-therapeutic amount of an anti-VEGF antibody and carrier –bound (*e.g.*, albumin) chemotherapeutic/anti-VEGF antibody nanoparticle complexes containing a therapeutically effective amount of the chemotherapeutic such that the administration of said sub-therapeutic amount of the anti-VEGF antibody enhances the efficacy of said nanoparticle complexes. It is contemplated

that administration of a sub-therapeutic amount of the anti-VEGF antibody enhances the efficacy of the nanoparticle complexes, at least in part, by clearing the blood stream of at least some undesired, non-tumor VEGF targets. Treatment with a sub- therapeutic amount of anti-VEGF antibody may allow for greater targeting of the nanoparticle complexes to the tumor, decrease the amounts of the chemotherapeutic/antibody complexes administered to a patient, or both.

[0009] In another aspect, provided herein are methods for enhancing the efficacy of albumin- bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes by administering the albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes about 0.5 to 48 hours after pretreatment of a patient with a sub-therapeutic amount of anti-VEGF antibody.

[0010] Preferably, such nanoparticle complexes are administered about 24 hours after the sub- therapeutic amount of anti-VEGF antibody.

[0011] In another aspect, provided herein are methods for enhancing the therapeutic outcome in a patient suffering from a cancer expressing soluble VEGF which patient is selected to be treated with nanoparticles comprising albumin-bound paclitaxel and anti-VEGF antibodies wherein said antibodies of the nanoparticles are integrated onto and/or into said nanoparticles which method comprises treating said patient with a sub-therapeutic amount of said anti-VEGF antibody prior to any subsequent treatment with the nanoparticles.

[0012] In another aspect, provided herein are methods for enhancing the therapeutic outcome in a patient suffering from a cancer overexpressing soluble VEGF, said method comprising treating the patient with a sub-therapeutic amount of said anti-VEGF antibody and co-treating said patients with an effective amount of nanoparticles comprising albumin-bound paclitaxel and anti-VEGF antibodies wherein said antibodies of the nanoparticles are integrated onto and/or into said nanoparticles.

[0013] In one embodiment, the chemotherapeutic is paclitaxel.

[0014] In one embodiment, the anti-VEGF antibody is bevacizumab or a biosimilar version thereof.

[0015] In one embodiment, the sub-therapeutic amount of anti-VEGF antibody is selected from an amount consisting of about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55% or about 60% of the therapeutic dosage of anti-VEGF antibody. It is contemplated that administration of the sub- therapeutic amount of anti-VEGF antibody preferentially blocks circulating VEGF with minimal blocking of VEGF associated with a tumor). In some embodiments, the sub-therapeutic amount of anti-VEGF to be administered to the patient is determined by analyzing the level of circulating VEGF in the blood.

[0016] In one embodiment, the sub-therapeutic amount of anti-VEGF antibody is administered from between about 30 minutes to about 48 hours prior to administration of the albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes.

[0017] In one embodiment, the target of said nanoparticle complexes is a solid cancer.

[0018] In other aspects, provided herein are unit-dose formulations of an anti-VEGF antibody, for example, bevacizumab or a biosimilar version thereof, which formulation comprises from about 1% to about 60% of a therapeutic dose of said antibody wherein said formulation is packaged so as to be administered as a unit dose.

[0019] In some embodiments, the formulation comprises from about 5% to about 20% of a therapeutic dose of bevacizumab or a biosimilar version thereof. The therapeutic dose for bevacizumab for a given approved indication, e.g., treatment for metastatic colorectal cancer, non-squamous non-small cell lung cancer, metastatic breast cancer, glioblastoma, and metastatic renal cell carcinmoma, is recited in the prescribing information. In each case the therapeutic dose is from 5 to 15 mg/kg and preferably a subtherapeutic dose ranges from 5% to 20% of the therapeutic dose. In such a preferred embodiment such a subtherapeutic dose would range from 0.25mg/kg to 3mg/kg, more preferably from 0.5 to 2mg/kg.

[0020] In other aspects, provided herein are kits comprising: (a) an amount of an albumin-bound chemotherapeutic/anti-VEGF antibody complexes, (b) a unit dose of a sub-therapeutic amount of anti-VEGF antibody, and optionally (c) instructions for use.

[0021] In one embodiment, the albumin-bound chemotherapeutic/anti-VEGF antibody complexes of the kits are lyophilized.

[0022] An embodiment of the invention includes a method for increasing the duration of tumor uptake of a chemotherapeutic agent by administering the chemotherapeutic agent in a nanoparticle comprising a carrier protein and the chemotherapeutic agent and having surface complexation with an antibody, e.g., an antibody that specifically binds to an antigen on or shed by the tumor. In some embodiments the subject receives a subtherapeutic amount of the antibody prior to or concurrently with such nanoparticles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The following figures are representative only of the invention and are not intended as a limitation. For the sake of consistency, the nanoparticles of this invention using albumin-bound paclitaxel (e.g., ABRAXANE®) and bevacizumab employ the acronym "AB" and the number after AB such as AB160 is meant to confer the average particle size of these nanoparticles (in nanometers).

[0024] FIGs. 1A-E show in vivo testing of athymic nude mice injected with 1 x 106 A375 human melanoma cells in the right flank and treated with (Fig 1A) PBS, (Fig 1B) 12 mg/kg BEV, (Fig 1C) 30 mg/kg ABX, (Fig 1D) AB160, or (Fig 1E) pretreated with 1.2 mg/kg BEV and, 24hr later, AB160. Data is represented at day 15-post treatment, or longer, as tumor volume in mm^3.

[0025] FIG. 1F summarizes the day 7-post treatment data from FIGs. 1A-E.

[0026] FIG. 1G summarizes the day 10-post treatment data from FIGs. 1A-E.

[0027] FIG. 2 depicts the tumor measurements on day 15-post treatment with either saline (PBS), bevacizumab (BEV), ABRAXANE® (ABX), AB160 or co-treatment with BEV one day (24 hours) prior to administration of AB160 (BEV+AB160).

[0028] FIG. 3 depicts the tumor measurements on day 20-post treatment with either saline (PBS), bevacizumab (BEV), ABRAXANE® (ABX), AB160 or co-treatment with BEV one day (24 hours) prior to administration of AB160 (BEV+AB160).

[0029] FIG. 4 depicts the median survival with either saline (PBS), bevacizumab (BEV), ABRAXANE® (ABX), AB160 or co-treatment with BEV one day (24 hours) prior to administration of AB160 (BEV+AB160).

[0030] FIG. 5 is fluorescent imagery of mice treated with AB160 with or without a bevacizumab (BEV) pre-treatment. The fluorescent imagery was performed at an excitation/emission spectrum of 710/760.

[0031] FIG. 6, depicts the median survival with either saline (PBS), bevacizumab (BEV), ABRAXANE® (ABX), AB160 alone, AB225 and co-treatment with BEV prior to administration of AB160 (BEV+AB160).

[0032] FIG. 7 depicts fluorescence over time of AlexaFluor 750 labeled nanoparticles in the tumors of mice treated with ABRAXANE® or AB160 with or without a pretreatment with bevacizumab. FIG. 7A depicts fluorescence per unit area of tumor ROI's (upper three lines) and background ROIs (lower three lines) for all three treatment groups at 24, 29 and 48 hours post injection. The dotted line represents an extrapolation of fluorescence, indicating an expected complete clearance at about 72 hours. FIG. 7B depicts the clearance from tumor ROIs adjusted for background fluorescence. FIG. 7C is a table of the clearance rates of fluorescent nanoparticles indicating AB160 nanoparticles are cleared nearly twice as slowly as ABRAXANE®.

DETAILED DESCRIPTION

[0033] After reading this description it will become apparent to one skilled in the art how to implement the invention in various alternative embodiments and alternative applications.

[0034] However, all the various embodiments of the present invention will not be described herein. It will be understood that the embodiments presented here are presented by way of an example only, and not limitation. As such, this detailed description of various alternative embodiments should not be construed to limit the scope or breadth of the present invention as set forth below.

[0035] Before the present invention is disclosed and described, it is to be understood that the aspects described below are not limited to specific compositions, methods of preparing such compositions, or uses thereof as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

[0036] The detailed description of the invention is divided into various sections only for the reader's convenience and disclosure found in any section may be combined with that in another section. Titles or subtitles may be used in the specification for the convenience of a reader, which are not intended to influence the scope of the present invention.

Definitions

[0037] Unless defined otherwise, 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 invention belongs. In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

[0038] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0039] "Optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

[0040] The term "about" when used before a numerical designation, e.g., temperature, time, amount, concentration, and such other, including a range, indicates approximations which may vary by (+) or (-) 10%, 5%, 1%, or any subrange or subvalue there between. Preferably, the term "about" when used with regard to a dose amount means that the dose may vary by +/- 10%.

[0041] "Comprising" or "comprises" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the stated purpose. Thus, a composition consisting essentially of the elements as defined herein would not exclude other materials or steps that do not materially affect the basic and novel characteristic(s)

of the claimed invention. “Consisting of” shall mean excluding more than trace elements of other ingredients and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention.

[0042] As used herein, the term “sub-therapeutic" is used to describe an amount of antibody that is below the amount of antibody conventionally used to treat a cancer. For example, a sub-therapeutic amount is an amount less than that defined by the manufacturer as being required for therapy.

[0043] The term “nanoparticle” as used herein refers to particles having at least one dimension which is less than 5 microns. In preferred embodiments, such as for intravenous administration, the nanoparticle is less than 1 micron. For direct administration, e.g., into a tumor, the nanoparticle can be larger. Even larger particles are expressly contemplated by the invention.

[0044] In a population of particles, the size of individual particles are distributed about a mean. Particle sizes for the population can therefore be represented by an average, and also by percentiles. D50 is the particle size below which 50% of the particles fall. 10% of particles are smaller than the D10 value and 90% of particles are smaller than D90. Where unclear, the “average” size is equivalent to D50. So, for example, AB160 refers to nanoparticles having an average size of 160 nanometers.

[0045] The term “nanoparticle” may also encompass discrete multimers of smaller unit nanoparticles. For example, a 320 nm particle comprises a dimer of a unit 160 nm nanoparticle. For 160 nm nanoparticles, multimers would therefore be approximately 320 nm, 480 nm, 640 nm, 800 nm, 960 nm, 1120 nm, and so on as determined by a Mastersizer 2000 (available from Malvern Instruments Ltd, Wocestershire, UK) as described in PCT/US15/54295.

[0046] The term “biosimilar” as used herein refers to a biopharmaceutical which is deemed to be comparable in quality, safety, and efficacy to a reference product marketed by an innovator company (Section 351(i) of the Public Health Service Act (42 U.S.C. 262(i)).

[0047] The term “carrier protein” as used herein refers to proteins that function to transport antibodies and/or therapeutic agents. The antibodies of the present disclosure

can reversibly bind to the carrier proteins. Exemplary carrier proteins are discussed in more detail below.

[0048] The term “core” as used herein refers to central or inner portion of the nanoparticle which may be comprised of a carrier protein, a carrier protein and a therapeutic agent, or other agents or combination of agents. In some embodiments, a hydrophobic portion of the antibody may be incorporated into the core.

[0049] As used herein, the term "enhancing the therapeutic outcome" and the like relative to a cancer patient refers to a slowing or diminution of the growth of cancer cells or a solid tumor, or a reduction in the total number of cancer cells or total tumor burden.

[0050] The term “therapeutic agent” as used herein means an agent which is therapeutically useful, e.g., an agent for the treatment, remission or attenuation of a disease state, physiological condition, symptoms, or etiological factors, or for the evaluation or diagnosis thereof. A therapeutic agent may be a chemotherapeutic agent, for example, mitotic inhibitors, topoisomerase inhibitors, steroids, anti-tumor antibiotics, antimetabolites, alkylating agents, enzymes, proteasome inhibitors, or any combination thereof.

[0051] The term “antibody” or “antibodies” as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules (i.e., molecules that contain an antigen binding site that immuno-specifically bind an antigen). The term also refers to antibodies comprised of two immunoglobulin heavy chains and two immunoglobulin light chains as well as a variety of forms including full length antibodies and portions thereof; including, for example, an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR- grafted antibody, a humanized antibody, a Fab, a Fab′, a F(ab′)2, a Fv, a disulfide linked Fv, a scFv, a single domain antibody (dAb), a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, a bispecific antibody, a functionally active epitope-binding fragment thereof, bifunctional hybrid antibodies (e.g., Lanzavecchia et al., Eur. J. Immunol. 17, 105 (1987)) and single chains (e.g., Huston et al., Proc. Natl. Acad. Sci. U.S.A., 85, 5879-5883 (1988) and Bird et al., Science 242, 423-426 (1988), which are incorporated herein by reference). (See, generally, Hood et al., Immunology, Benjamin, N.Y., 2ND ed. (1984); Harlow and Lane, Antibodies. A Laboratory Manual, Cold Spring Harbor

Laboratory (1988); Hunkapiller and Hood, Nature, 323, 15-16 (1986), which are incorporated herein by reference). The antibody may be of any type (e.g., IgG, IgA, IgM, IgE or IgD). Preferably, the antibody is IgG. An antibody may be non-human (e.g., from mouse, goat, or any other animal), fully human, humanized, or chimeric. In an embodiment the antibody is an exogenous antibody. An exogenous antibody is an antibody not naturally produced in a mammal, e.g. in a human, by the mammalian immune system.

[0052] The term "dissociation constant," also referred to as "Kd," refers to a quantity expressing the extent to which a particular substance separates into individual components (e.g., the protein carrier, antibody, and optional therapeutic agent).

[0053] The terms "lyophilized," "lyophilization" and the like as used herein refer to a process by which the material (e.g., nanoparticles) to be dried is first frozen and then the ice or frozen solvent is removed by sublimation in a vacuum environment. An excipient is optionally included in pre-lyophilized formulations to enhance stability of the lyophilized product upon storage. In some embodiments, the nanoparticles can be formed from lyophilized components (carrier protein, antibody and optional therapeutic) prior to use as a therapeutic. In other embodiments, the carrier protein, antibody, and optional therapeutic agent are first combined into nanoparticles and then lyophilized. The lyophilized sample may further contain additional excipients.

[0054] The term "buffer" encompasses those agents which maintain the solution pH in an acceptable range prior to lyophilization and may include succinate (sodium or potassium), histidine, phosphate (sodium or potassium), Tris(tris(hydroxymethyl)aminomethane), diethanolamine, citrate (sodium) and the like. The buffer of this invention has a pH in the range from about 5.5 to about 6.5; and preferably has a pH of about 6.0. Examples of buffers that will control the pH in this range include succinate (such as sodium succinate), gluconate, histidine, citrate and other organic acid buffers.

[0055] The term "pharmaceutical formulation" refers to preparations which are in such form as to permit the active ingredients to be effective, and which contains no additional components which are toxic to the subjects to which the formulation would be administered.

[0056] "Pharmaceutically acceptable" excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

[0057] "Reconstitution time" is the time that is required to rehydrate a lyophilized formulation into a solution.

[0058] A "stable" formulation is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage.

[0059] The term "epitope" as used herein refers to the portion of an antigen which is recognized by an antibody. Epitopes include, but are not limited to, a short amino acid sequence or peptide (optionally glycosylated or otherwise modified) enabling a specific interaction with a protein (e.g., an antibody) or ligand. For example, an epitope may be a part of a molecule to which the antigen-binding site of an antibody attaches.

[0060] The term "treating" or "treatment" covers the treatment of a disease or disorder (e.g., cancer), in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, i.e., arresting its development; (ii) relieving a disease or disorder, i.e., causing regression of the disorder; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. In some embodiments "treating" or "treatment" refers to the killing of cancer cells.

[0061] The term "kill" with respect to a cancer treatment is directed to include any type of manipulation that will lead to the death of that cancer cell or at least a portion of a population of cancer cells.

[0062] The term "dose" refers to an amount of antibody given to a patient in need thereof. The attending clinician will select an appropriate dose from the range based on the patient's weight, age, health, stage of cancer, level of circulating VEGF, and other relevant factors, all of which are well within the skill of the art.

[0063] The term "unit dose" refers to a dose of the antibody that is given to the patient to provide a desired result. In some instances, the unit dose is sold in a sub-therapeutic formulation (e.g., 10% the therapeutic dose). The unit dose may be administered as a single dose or a series of subdoses. The therapeutic dose for an

antibody for a given FDA-approved indication is recited in the prescribing information, for example the therapeutic dose of bevacizumab is 5mg/kg to 15 mg/kg depending on the condition, and preferably a subtherapeutic dose ranges from 5% to 20% of the therapeutic dose. In such a preferred embodiment such a subtherapeutic dose would range from 0.25mg/kg to 3mg/kg, more preferably from 0.5 to 2mg/kg. The therapeutic dose for an antibody for a given indication where the antibody is not yet FDA approved or the antibody is not yet approved for that indication, will be the amount the correlates to the therapeutic that has been approved for other indications, and thus the subtherapeutic dose for the non-FDA approved indications is readily calculated as a percent of the therapeutic dose (e.g., 10% of the therapeutic dose). For example, the therapeutic dose and therefore the subtherapeutic dose of an antibody for the treatment of metastatic melanoma correlates to the therapeutic dose for metastatic cancers in general that has been approved.

[0064] Additionally, some terms used in this specification are more specifically defined below.

Overview

[0065] The current invention is predicated, in part, on the surprising discovery that treatment of a cancer in a patient with an anti-VEGF antibody composition together with albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes provides for unexpectedly improved therapeutic outcomes.

[0066] As will be apparent to the skilled artisan upon reading this disclosure, the present disclosure relates to methods for treating a patient suffering from a cancer by treating the patient with a sub-therapeutic amount of an anti-VEGF antibody and albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes containing a therapeutically effective amount of the chemotherapeutic.

Anti-VEGF antibodies

[0067] In some embodiments, the anti-VEGF antibody is bevacizumab or a biosimilar version thereof.

[0068] Bevacizumab (AVASTIN®, Roche, USA) is a humanized monoclonal

antibody that inhibits angiogenesis by blocking the action of vascular endothelial growth factor (VEGF). As such, bevacizumab can slow the growth of new blood vessels in tumors thereby inhibiting the tumors ability to grow. Bevacizumab has been used to treat various cancers including, non-small cell lung cancer (NSCLC), metastatic colorectal cancer (mCRC), platinum-resistant ovarian cancer (prOC), advanced cervical cancer (CC), metastatic renal cell carcinoma (mRCC), and recurrent glioblastoma (rGBM). Several biosimilar versions of bevacizumab are currently being developed including ABP 215 (Amgen/Allergen, USA), BCD-021 (Biocad, Russia), BI 695502 (Boehringer Ingelheim, Germany), and PF-06439535 (Pfizer, USA), among others.

[0069] In some embodiments, the sub-therapeutic amount of anti-VEGF antibody is selected from an amount consisting of about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55% or about 60% of the therapeutic dosage of anti-VEGF antibody.

[0070] In some embodiments, the sub-therapeutic amount of anti-VEGF antibody is an amount which preferentially blocks circulating VEGF without blocking VEGF associated with tumor.

Complexes

[0071] Carrier protein-bound chemotherapeutic/anti-VEGF antibody complexes and methods of preparing the complexes are described, for example, in U.S. Provisional App. No. 62/060,484, filed October 6, 2014; and U.S. Provisional Patent Application Nos. 62/206,770; 62/206,771; and 62/206,772 filed August 18, 2015, as well as PCT Publication No. PCT/US15/54295, filed October 6, 2015. The contents of each of these applications are specifically incorporated by reference in their entireties. Example 1 below provides one example of a detailed protocol for making such complexes.

[0072] In some embodiments, the anti-VEGF antibody is bevacizumab or a biosimilar version thereof. In some embodiments, the antibodies are a substantially single layer of antibodies on all or part of the surface of the nanoparticle.

[0073] In some embodiments, the at least one chemotherapeutic agent is selected

from the group consisting of abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, cisplatin, chlorambucil, dasatinib, docetaxel, doxorubicin, epirubicin, erlotinib, etoposide, everolimus, gefitinib, idarubicin, imatinib, hydroxyurea, imatinib, lapatinib, leuprorelin, melphalan, methotrexate, mitoxantrone, nedaplatin, nilotinib, oxaliplatin, paclitaxel, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, vinblastine, vinorelbine, vincristine, and cyclophosphamide. In preferred embodiments, the chemotherapeutic is paclitaxel.

[0074] In some embodiments, the nanoparticle sizes are between 200 and 800 nm, including 200, 300, 400, 500, 600, 700 or 800nm. In other embodiments, the nanoparticles are larger, e.g. from greater than 800 nm to about 3.5 μm. In some embodiments, the particles are multimers of nanoparticles. In some embodiments the nanoparticles have average particle sizes of about 160nm to about 225 nm either freshly made or after lyophilization and resuspension in an aqueous solution suitable for injection.

Treatment Methods

[0075] In one aspect is provided a method for treating a patient suffering from a cancer which expresses VEGF wherein said patient is treated with a sub-therapeutic amount of an anti-VEGF antibody and albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes containing a therapeutically effective amount of the chemotherapeutic such that the administration of said sub-therapeutic amount of the anti-VEGF antibody enhances the efficacy of said nanoparticle complexes.

[0076] For the sake of clarification, "co-treatment" refers to treatment of the cancer expressing VEGF (a soluble cytokine) with an anti-VEGF antibody prior, concurrent or immediately after administration of the albumin-bound chemotherapeutic/anti-VEGF antibody complex provided that the anti-VEGF antibody is capable of preferentially binding soluble VEGF.

[0077] In one embodiment, the anti-VEGF antibody is administered in a sub-therapeutic dose prior to the nanoparticle complex. In this embodiment, the administration of the anti-VEGF antibody occurs about 0.5 to 48 hours prior to

administration of the nanoparticle complexes.

[0078] In another embodiment, the anti-VEGF antibody composition is administered between 0.5 hours prior to and up to 0.5 hours after administration of the nanoparticle complexes. In this embodiment, it is contemplated that such administration will nevertheless result in binding of some of the circulating VEGF by the antibody composition.

[0079] In yet another embodiment, the antibody composition can be administered up to 2 hours post administration of the nanoparticle complexes.

[0080] In a preferred aspect, there is provided methods for enhancing the efficacy of albumin- bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes by administering the albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes about 0.5 to 48 hours after pretreatment of a patient with a sub-therapeutic amount of anti-VEGF antibody. Preferably, such nanoparticle complexes are administered about 24 hours after the sub- therapeutic amount of anti-VEGF antibody.

[0081] In another aspect, there is provided methods for enhancing the therapeutic outcome in a patient suffering from a cancer expressing soluble VEGF which patient is selected to be treated with nanoparticles comprising albumin-bound paclitaxel and anti-VEGF antibodies wherein said antibodies of the nanoparticles are integrated onto and/or into said nanoparticles which method comprises treating said patient with a sub-therapeutic amount of said anti-VEGF antibody prior to any subsequent treatment with the nanoparticles.

[0082] In another aspect, there is provided methods for enhancing the therapeutic outcome in a patient suffering from a cancer overexpressing soluble VEGF, said method comprising treating the patient with a sub-therapeutic amount of said anti-VEGF antibody and co-treating said patients with an effective amount of nanoparticles comprising albumin-bound paclitaxel and anti-VEGF antibodies wherein said antibodies of the nanoparticles are integrated onto and/or into said nanoparticles.

[0083] In another aspect, there is provided a method for enhancing the therapeutic outcome in a patient suffering from a cancer expressing soluble VEGF which patient is to be treated with nanoparticles comprising albumin-bound paclitaxel and anti-VEGF

antibodies wherein said antibodies of the nanoparticles are integrated onto and/or into said nanoparticles which method comprises treating said patient with a sub-therapeutic amount of said anti-VEGF antibody within +/- 0.5 hours of administration of said nanoparticles.

[0084] In another aspect is provided a method for enhancing the therapeutic outcome in a patient suffering from a cancer overexpressing soluble VEGF which patient has been treated with a sub-therapeutic amount of said anti-VEGF antibody said method comprising treating said patients with an effective amount of nanoparticles comprising albumin-bound paclitaxel and anti-VEGF antibodies wherein said antibodies of the nanoparticles are integrated onto and/or into said nanoparticle antibody within +/- 0.5 hours of administration of said antibodies.

[0085] The patient may be co-treated with a sub-therapeutic amount of an anti-VEGF antibody and albumin-bound chemotherapeutic/anti-VEGF antibody complex.

[0086] In some embodiments the anti-VEGF antibody is administered prior to the albumin- bound chemotherapeutic/anti-VEGF antibody complex, for example, the anti-VEGF antibody can be administered minutes, hours or days prior to administration of the albumin-bound chemotherapeutic/anti-VEGF antibody complex. In some embodiments, the anti-VEGF antibody is administered between about 5 to about 59 minutes, about 10 to about 50 minutes, about 15 to about 45 minutes, about 20 to about 40 minutes, about 25 to about 35 minutes prior to administration of the albumin-bound chemotherapeutic/anti-VEGF antibody complex. In other embodiments, the anti-VEGF antibody can be administered about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 12 hours, about 24 hours, about 48 hours, about 72 hours, or longer prior to administration of the albumin-bound chemotherapeutic/anti-VEGF antibody complex. In other embodiments, the anti-VEGF antibody can be administered about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 10 days, about 12 days, about 15 days, or longer prior to administration of the albumin-bound chemotherapeutic/anti-VEGF antibody complex.

[0087] In some embodiments, the anti-VEGF antibody can be administered concurrently with administration of the albumin-bound chemotherapeutic/anti-VEGF antibody complex, for example, within 10 minutes or less of each other.

[0088] In other embodiments, the anti-VEGF antibody can be administered subsequent to administration of the albumin-bound chemotherapeutic/anti-VEGF antibody complex, for example, within 2 hours after administration of the albumin-bound chemotherapeutic/anti-VEGF antibody complex, provided that the subsequent administration allows the antibody to preferentially bind the soluble VEGF.

[0089] Cancers or tumors that can be treated by the compositions and methods described herein include, but are not limited to: biliary tract cancer; brain cancer, including glioblastomas and medulloblastomas; breast cancer; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer, gastric cancer; hematological neoplasms, including acute lymphocytic and myelogenous leukemia; multiple myeloma; AIDS associated leukemias and adult T-cell leukemia lymphoma; intraepithelial neoplasms, including Bowen's disease and Paget's disease; liver cancer (hepatocarcinoma); lung cancer; lymphomas, including Hodgkin's disease and lymphocytic lymphomas; neuroblastomas; oral cancer, including squamous cell carcinoma; ovarian cancer, including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreas cancer; prostate cancer; rectal cancer; sarcomas, including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma and osteosarcoma; skin cancer, including melanoma, Kaposi's sarcoma, basocellular cancer and squamous cell cancer; testicular cancer, including germinal tumors (seminoma, non-seminoma[teratomas, choriocarcinomas]), stromal tumors and germ cell tumors; thyroid cancer, including thyroid adenocarcinoma and medullar carcinoma; and renal cancer including adenocarcinoma and Wilms tumor. In important embodiments, cancers or tumors include breast cancer, lymphoma, multiple myeloma, and melanoma.

Formulations

[0090] In one aspect, the anti-VEGF is a unit-dose formulation of an anti-VEGF antibody which formulation comprises from about 1% to about 60% of a therapeutic dose of said antibody wherein said formulation is packaged so as to be administered as a unit dose. In an aspect of the invention, the unit-dose formulation of an anti-VEGF antibody comprises about 10% of a therapeutic dose of said antibody. For example 10% of a

therapeutic dose of an anti-VEGF antibody, e.g., bevacizumab, may be 0.5mg/kg to 5mg/kg.

[0091] The unit-dose formulation of an anti-VEGF antibody can be about 1% to about 60%, about 5% to about 50%, about 10% to about 40%, about 15% to about 30%, about 20% to about 25% , of a therapeutic dose of the anti-VEGF antibody. Contemplated values include any value, subrange, or range within any of the recited ranges, including endpoints.

[0092] In some embodiments, the anti-VEGF antibody is bevacizumab or a biosimilar version thereof, which formulation comprises from about 5% to about 20% of a therapeutic dose of bevacizumab or a biosimilar version thereof.

[0093] In another aspect, provided herein is a formulation comprising an anti-VEGF antibody provided herein, and at least one pharmaceutically acceptable excipient.

[0094] In general, the unit-dose formulations provided herein can be formulated for administration to a patient by any of the accepted modes of administration. Various formulations and drug delivery systems are available in the art. See, e.g., Remington's Pharmaceutical Sciences, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990).

[0095] In general, unit-dose formulations provided herein will be administered as pharmaceutical compositions by any one of the following routes: oral, systemic (e.g., transdermal, intranasal or by suppository), or parenteral (e.g., intramuscular, intravenous or subcutaneous) administration.

[0096] The unit-dose formulations may be comprised of, in general, an anti-VEGF antibody, optionally in combination with at least one pharmaceutically acceptable excipient. Acceptable excipients are non-toxic, aid administration, and do not adversely affect the therapeutic benefit of the claimed compounds. Such excipient may be any solid, liquid, semi-solid or, in the case of an aerosol composition, gaseous excipient that is generally available to one of skill in the art.

[0097] Solid pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium

stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc. Preferred liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols. Other suitable pharmaceutical excipients and their formulations are described in Remington's Pharmaceutical Sciences, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990).

[0098] The present formulations may, if desired, be presented in a pack or dispenser device containing a unit-dose of the active ingredient. Such a pack or device may, for example, comprise metal or plastic foil, such as a blister pack, or glass, and rubber stoppers such as in vials. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a unit-dose formulation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Kits

[0099] In some aspects, the current invention relates to kits comprising: (a) an amount of an albumin-bound chemotherapeutic/anti-VEGF antibody complexes, (b) a unit dose of a sub- therapeutic amount of anti-VEGF antibody, and optionally (c) instructions for use.

[0100] In some embodiments, the kits can include lyophilized complexes of the albumin-bound chemotherapeutic/anti-VEGF antibody.

[0101] In some preferred embodiments, the kit components can be configure in such a way that the components are accessed in their order of use. For example, in some aspects the kit can be configured such that upon opening or being accessed by a user, the first component available is the unit dose of a sub-therapeutic amount of anti-VEGF antibody, for example, in a first vial. A second container (e.g., a vial) comprising or containing an amount of the albumin-bound chemotherapeutic/anti-VEGF antibody complexes can then be accessed. As such the kits can be intuitively configured in a way such that the first vial must be opened prior to the second vial being opened. It should be understood that in some embodiments, the order can be different, for example, where it is desired to

administer the complex first, prior to the administration of the antibody. Also, it can be configured such that both are administered at the same time. Finally, it should be understood that additional vials or containers of either or both component(s) can be included, and configured for opening in any desired order. For example, the first vial could be antibody, the second vial could include complex, a third could include either antibody or complex, etc. It is contemplated that a kit configured in such a way would prevent, or at least help to prevent, the components from being administered in an order not intended by the instructions for use.

[0102] In some aspects, the invention is directed to a kit of parts for administration of albumin- bound chemotherapeutic/anti-VEGF antibody complexes and a unit dose of a sub-therapeutic amount of anti-VEGF antibody; and optionally further comprising a dosing treatment schedule in a readable medium. In some embodiments, the dosing schedule includes the sub-therapeutic amount of anti-VEGF antibody required to achieve a desired average serum level is provided. In some embodiments, the kit of parts includes a dosing schedule that provides an attending clinician the ability to select a dosing regimen of the sub-therapeutic amount of anti-VEGF antibody based on the sex of the patient, mass of the patient, and the serum level that the clinician desires to achieve. In some embodiments, the dosing treatment is based on the level of circulating VEGF in the blood of the patient. In some embodiments, the dosing schedule further provides information corresponding to the volume of blood in a patient based upon weight (or mass) and sex of the patient. In an embodiment, the storage medium can include an accompanying pamphlet or similar written information that accompanies the unit dose form in the kit. In an embodiment, the storage medium can include electronic, optical, or other data storage, such as a non-volatile memory, for example, to store a digitally-encoded machine- readable representation of such information.

[0103] The term "readable medium" as used herein refers to a representation of data that can be read, for example, by a human or by a machine. Non-limiting examples of human-readable formats include pamphlets, inserts, or other written forms. Non-limiting examples of machine- readable formats include any mechanism that provides (i.e., stores and/or transmits) information in a form readable by a machine (e.g., a computer, tablet, and/or smartphone). For example, a machine-readable medium includes read-only memory (ROM); random access memory (RAM); magnetic disk storage media; optical

storage media; and flash memory devices. In one embodiment, the machine-readable medium is a CD-ROM. In one embodiment, the machine- readable medium is a USB drive. In one embodiment, the machine-readable medium is a Quick Response Code (QR Code) or other matrix barcode.

EXAMPLES

[0104] The present disclosure is illustrated using a pre-treatment of bevacizumab (i.e., AVASTIN®) followed by nanoparticles composed of albumin-bound paclitaxel (i.e., ABRAXANE®) and bevacizumab (i.e., AVASTIN®).

[0105] One skilled in the art would understand that making and using the nanoparticles, as well as administration of a co-treatment of bevacizumab, of the Examples are for the sole purpose of illustration, and that the present disclosure is not limited by this illustration.

[0106] Any abbreviation used herein, has normal scientific meaning. All temperatures are °C unless otherwise stated. Herein, the following terms have the following meanings unless otherwise defined:

ABX	=	ABRAXANE®/(albumin-bound
ADC	=	antibody dependent chemotherapy
BEV	=	bevacizumab
BSA	=	bovine serum albumin
dH_2O	=	distilled water
kg	=	kilogram
nM	=	nano molar
mg	=	milligram
ml or mL	=	milliliter
m^2	=	square meters
mm^3	=	cubic millimeter
µg	=	microgram

µl	=	microliter
µm	=	micrometer/micron
PBS	=	Phosphate buffered saline

Example 1: Nanoparticle Preparation

[0107] ABRAXANE® (ABX) (10 mg) was suspended in bevacizumab (BEV) (4 mg [160 µl] unless otherwise indicated), and 840 µl of 0.9% saline was added to give a final concentration of 10 mg/ml and 2 mg/ml of ABX and BEV, respectively. The mixture was incubated for 30 minutes at room temperature (or at the temperature indicated) to allow particle formation. For Mastersizer experiments to measure particle size of ABX:BEV complexes, 10 mg of ABX was suspended in BEV at concentrations of 0 to 25 mg/ml. Complexes of ABX with rituximab (0-10 mg/ml) or trastuzumab (0-22 mg/ml) were formed under similar conditions.

[0108] For use in humans, the ABX:BEV complexes may be prepared by obtaining the dose appropriate number of 4 mL vials of 25 mg/mL BEV and diluting each vial per the following directions to 4 mg/mL. The dose appropriate number of 100 mg vials of ABX can be prepared by reconstituting to a final concentration containing 10 mg/mL ABX nanoparticles. Using a sterile 3 mL syringe, 1.6 mL (40 mg) of bevacizumab (25 mg/mL) can be withdrawn and slowly injected, over a minimum of 1 minute, onto the inside wall of each of the vials containing 100 mg of ABX. The bevacizumab solution should not be injected directly onto the lyophilized cake as this will result in foaming. Then, using a sterile 12 mL sterile syringe, 8.4 mL 0.9% Sodium Chloride Injection, USP, can be withdrawn and slowly injected, over a minimum of 1 minute, 8.4 mL onto the inside wall of each vial containing ABX 100 mg and BEV 40 mg. Once the addition of BEV 1.6 mL and 0.9% Sodium Chloride Injection, USP 8.4 mL is completed, each vial can be gently swirled and/or inverted slowly for at least 2 minutes until complete dissolution of any cake/powder occurs. Generation of foam should be avoided. At this point, the concentration of each vial should be 100 mg/10 mL ABX and 40 mg/10 mL BEV. The vials containing the ABX and BEV should sit for 60 minutes. The vial(s) should be gently swirled and/or inverted every 10 minutes to continue to mix the

complex. After 60 minutes has elapsed, the calculated dosing volume of ABX and BEV should be withdrawn from each vial and slowly added to an empty viaflex bag. An equal volume of 0.9% Sodium Chloride Injection, USP is then added to make the final concentration of ABX 5 mg/mL and BEV 2 mg/mL. The bag should then be gently swirled and/or inverted slowly for 1 minute to mix. The ABX:BEV nanoparticles can be stored for up to 4 hours at room temperature following final dilution.

Example 2: Co-treatment with BEV improves targeting of ABX/BEV complexes

[0109] Athymic nude mice were injected with 1 x 106 A375 human melanoma cells in the right flank and then treated with PBS, 12 mg/kg BEV, 30 mg/kg ABX, AB160, or pretreated with 1.2 mg/kg BEV and, 24hr later, AB160. AB160 was prepared as described in PCT Application No. PCT/US15/54295 and Example 1 above. FIG. 1A-E track tumor size over 40 days (all mice in the PBS, BEV, and ABX died by day 20) . Data is represented as tumor volume in mm^3. Only mice treated with AB160 (with or without pretreatment with BEV) showed a reduction in average tumor volume. See also FIG. 1F and FIG. 1G.

[0110] The day 7-post treatment data, as summarized in FIG. 1F, show that pretreatment with BEV was associated with a statistically significant reduction in tumor volume over control or BEV alone ($p \leq 0.0001$), or ABX alone ($p \leq 0.0001$).

[0111] The day 10-post treatment data, as summarized in FIG. 1G, again show that pretreatment with BEV was associated with a statistically significant reduction in tumor volume over control or BEV alone ($p \leq 0.0001$), or ABX alone ($p \leq 0.0001$). Pretreatment with BEV before AB160 was also associated with a reduction in tumor volume over AB160 alone ($p=0.02$), with complete response in two mice.

[0112] In this experiment, a 12 mg/kg dose of BEV was not therapeutic. The amount of BEV added in the pretreatment group was only 1.2 mg/kg, which is 1/10 the usual dose in mice. Yet pretreatment with a subtherapeutic dose improved the efficacy of the AB160 nanoparticles. This data support the idea that pretreatment with a subtherapeutic amount of BEV can clear systemic levels of VEGF, leaving a greater relative concentration at the tumor such that tumor-associated VEGF targeting by the AB160 nanoparticles is more

effective.

[0113] Tumors were measured on day 15 following treatment with either saline (PBS), AVASTIN® (BEV), ABRAXANE® (ABX), AB160 or a pretreatment of BEV one day before AB160 (BEV+AB160). A 10% sub-therapeutic dose of BEV, as compared to the dose give to the BEV alone or AB160 cohort, was given to the BEV+AB160 cohort 24 hours prior to administration of the AB160. The BEV+AB160 cohort presented with delayed tumor growth, even when compared to AB160, with two mice presenting with complete cures. FIG. 2. As summarized in Table 1, these experiments also show that pre-treatment with BEV + AB160, increases survival. At day 15, 100% of the mice in the BEV + AB160 cohort were alive, whereas 86% of these mice in the AB160 cohort were still alive. Both treatment groups had substantially greater survival than the PBS, BEV-only, or ABX-only treatment groups which exhibited a 0%, 0% and 11% survival rate, respectively. At day 20, 92% of the mice in the BEV + AB160 cohort were alive, whereas 67% of the mice in the AB160 cohort were still alive. FIG. 3.

Table 1: Percentage of mice alive on day 15

Treatment Group	PBS	BEV	ABX	AB160	BEV + AB160
Percent Alive on day 15	0 %	0 %	11 %	86 %	100 %

[0114] Survival was again assessed at day 40. FIG. 4. As shown median survival of both the mice treated with PBS or BEV was 7 days. Mice treated with ABX alone shown median survival of 14 days, while those treated with AB160 exhibited a median survival of 25 days.

[0115] Median survival of mice co-treated with BEV and AB160 remained undefined, with approximately 50% of the mice co-treated with BEV and AB160 still alive, at day 40.

Example 3 Fluorescence over time of AlexaFluor 750 labeled nanoparticles

[0116] Mice received IV injections of equal amounts of either labeled ABRAXANE®,

or nanoparticles of ABRAXANE® having surface complexation with bevacizumab (BEV) as per Example 1 above (AB160); one AB160 group of mice received a pre-treatment 1.2mg/kg of bevacizumab. Fluorescent imagery was done at an excitation/emission spectrum of 710/760 (FIG. 5). Regions of interest (ROI) in the mice of FIG. 5 were assigned by software to track tumor accumulation based on a fluorescence threshold. Fluorescence per unit area of background ROIs and tumor ROIs for all three treatment groups were determined at 24, 29, and 48 hours post injection

[0117] The amount of fluorescence (and thus paclitaxel) in the tumor and background ROIs at 24, 29 and 48 hour presented in FIG. 7A and 7B. The data demonstrates that pretreatment with BEV results in higher levels of tumor fluorescence as compared AB160 alone or ABRAXANE alone. Furthermore, the clearance rate for AB160-treated mice with or without a BEV pre-treatment is nearly twice as slow as the ABRAXANE treated mice (FIG. 7C). These results evidence that pretreatment with BEV and use of ABRAXANE® nanoparticles having surface complexation with BEV provides for a method for increasing the duration of tumor uptake of albumin containing a chemotherapeutic agent both at 24 hours and 48 hours. Likewise, use of ABRAXANE® nanoparticles having surface complexation with BEV also provides for increasing the duration of tumor uptake of these albumin containing nanoparticles with or without pretreatment with BEV at 48 hours. Both of these results evidence continued uptake for 48 hours and possibly out to 72 hours. On the other hand, background fluorescence of the corresponding nanoparticles is projected to go to about 0 at 72 hours evidencing that systemic circulation of the drugs have disappeared from all parts of the animal but for the tumor. All told, the incorporation of the BEV into ABRAXANE® has a stabilizing impact, which is most evident by the concentration of the drug in the tumor at 48 hours and projected for 72 hours. Without being limited to any theory, the antibody coating of the albumin nanoparticles imparts stability possibly by reducing liver or kidney clearance and/or by reducing protease degradation of the albumin carrier. This approach allows targeting antibodies to complex with a protein carrier such as albumin and any chemotherapeutic drug, such as, e.g., abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, cisplatin, chlorambucil, dasatinib, docetaxel, doxorubicin, epirubicin, erlotinib, etoposide, everolimus, 5-fluoruracil, gefitinib, idarubicin, imatinib, hydroxyurea, imatinib, lapatinib, leuprorelin, melphalan, methotrexate, mitoxantrone, nedaplatin, nilotinib, oxaliplatin, paclitaxel, pazopanib, pemetrexed, picoplatin,

romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, vinblastine, vinorelbine, vincristine, and cyclophosphamide thereby providing prolonged delivery of such drugs to the tumor.

[0118] FIG. 7A indicates that at 24 hours the total amount of fluorescence accumulating in the tumor of AB160-treated mice without a BEV pretreatment was less than that achieved in ABRAXANE®-treated mice. However, the rate of clearance of ABRAXANE® in treated mice was higher than AB160 in treated mice. Therefore if one were to extrapolate the data in FIG. 7A, as shown by the dotted line, the level of fluorescence (and thus paclitaxel) would be maintained at a higher level in the tumors of AB160-treated mice with or without BEV pre-treatment than the level maintained by ABRAXANE treatment.

[0119] FIG. 7 also demonstrates that the background fluorescence in AB160-treated mice with or without pretreatment is higher than that in ABRAXANE-treated mice. While not wishing to be bound by theory, this suggests that the antibodies confer additional stability to the nanoparticles of paclitaxel and albumin such that they are cleared more slowly from the circulation than ABRAXANE alone. This would be advantageous as it would provide higher levels of the nanoparticles for longer periods of time for subsequent infiltration into the tumor.

Example 16: Nanoparticles having a size of 225nm

[0120] To make a nanoparticle having a size of 225nm, the particles were prepared in accordance with Example 1 but the ratio of BEV to ABRAXANE® was 4:5, i.e., 4 parts BEV and 5 parts ABRAXANE. This ratio produced nanoparticles having a size of 225nm (AB225). The effect of AB225 was assayed in animals as set forth above. FIG. 6 demonstrates that the survival of the mice treated with a single dose of saline, BEV24 (24 mg/kg), ABX30(30 mg/kg), AB160 (12mg/kg BEV and 30mg/kg ABX) and AB225 (24 mg/kg BEV and 30 mg/kg ABX) and with AB160 with a BEV (1.2 mg/kg) pretreatment. At 30 days the survival of mice treated with AB225, and with AB160 with or without pretreatment with BEV far exceeded the survival of mice treated with BEV alone of ABRAXANE® alone.

WHAT IS CLAIMED IS:

1. A method for treating a patient suffering from a cancer which expresses VEGF wherein said patient is treated with a sub-therapeutic amount of an anti-VEGF antibody and albumin- bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes containing a therapeutically effective amount of the chemotherapeutic such that the administration of said sub-therapeutic amount of the anti-VEGF antibody enhances the efficacy of said nanoparticle complexes.

2. The method of claim 1, wherein the chemotherapeutic is paclitaxel.

3. The method of claim 1, wherein the anti-VEGF antibody is bevacizumab or a biosimilar version thereof.

4. The method of claim 1, wherein the sub-therapeutic amount of anti-VEGF antibody is selected from an amount consisting of about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55% or about 60% of the therapeutic dosage of anti-VEGF antibody.

5. The method of claim 1, wherein the sub-therapeutic amount of anti-VEGF antibody is an amount which blocks circulating VEGF without blocking VEGF on a target of said nanoparticle complexes.

6. The method of claim 5, wherein the target of said nanoparticle complexes is a solid cancer.

7. The method of claim 1, wherein the sub-therapeutic amount of anti-VEGF to be administered to the patient is determined by analyzing the level of circulating VEGF in the blood.

8. The method of claim 1, wherein the sub-therapeutic amount of anti-VEGF antibody is administered from between about 30 minutes to about 48 hours prior to administration of the albumin-bound chemotherapeutic/anti-VEGF antibody

nanoparticle complexes.

9. A method for enhancing the efficacy of albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes by administering the albumin-bound chemotherapeutic/anti- VEGF antibody nanoparticle complexes about 24 hours after administrating a sub-therapeutic amount of anti-VEGF antibody to a patient in need thereof.

10. The method of claim 9, wherein the anti-VEGF antibody is bevacizumab or a biosimilar version thereof.

11. The method of claim 9, wherein the sub-therapeutic amount of anti-VEGF antibody is selected from an amount consisting of about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55% or about 60% of the therapeutic dosage of anti-VEGF antibody.

12. The method of claim 9, herein the sub-therapeutic amount of anti-VEGF antibody is an amount which blocks circulating VEGF without blocking VEGF on a target of said nanoparticle complexes.

13. The method of claim 12, wherein the target of said nanoparticle complexes is a solid cancer.

14. The method of claim 9, wherein the sub-therapeutic dose of anti-VEGF to be administered to the patient is determined by analyzing the level of circulating VEGF in the blood.

15. The method of claim 9, wherein the sub-therapeutic dose of the anti-VEGF antibody is administered from between about 30 minutes to about 48 hours prior to administration of the albumin-bound chemotherapeutic/anti-VEGF antibody nanoparticle complexes.

16. A method for enhancing the therapeutic outcome in a patient suffering from a cancer expressing soluble VEGF which patient is to be treated with nanoparticles comprising albumin- bound paclitaxel and anti-VEGF antibodies wherein said

antibodies of the nanoparticles are integrated onto and/or into said nanoparticles which method comprises treating said patient with a sub-therapeutic amount of said anti-VEGF antibody.

17. A method for enhancing the therapeutic outcome in a patient suffering from a cancer overexpressing soluble VEGF which patient has been treated with a sub-therapeutic amount of said anti-VEGF antibody said method comprising treating said patients with an effective amount of nanoparticles comprising albumin-bound paclitaxel and anti-VEGF antibodies wherein said antibodies of the nanoparticles are integrated onto and/or into said nanoparticles.

18. A unit-dose formulation of an anti-VEGF antibody which formulation comprises from about 1% to about 60% of a therapeutic dose of said antibody wherein said formulation is packaged so as to be administered as a unit dose.

19. The formulation of claim 18, wherein the anti-VEGF antibody is bevacizumab or a biosimilar version thereof.

20. The unit-dose formulation of claim 19, which formulation comprises from about 5% to about 20% of a therapeutic dose of bevacizumab or a biosimilar version thereof.

21. A kit comprising: (a) an amount of an albumin-bound chemotherapeutic/anti-VEGF antibody complexes, (b) a unit dose of a sub-therapeutic amount of anti-VEGF antibody, and optionally (c) instructions for use.

22. The kit of claim 21, wherein the albumin-bound chemotherapeutic/anti-VEGF antibody complexes are lyophilized.

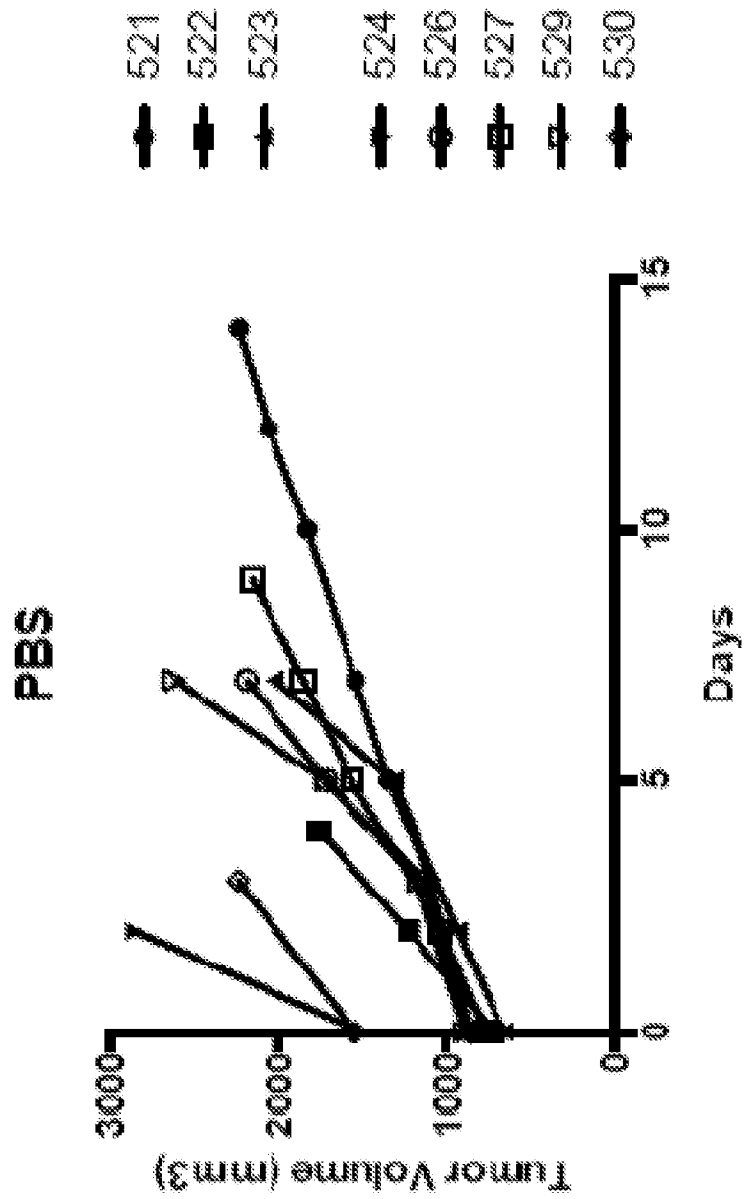

521
522
523
524
526
527
529
530
PBS
Tumor Volume (mm3)
3000
2000
1000
0
0
5
10
15
Days

FIG. 1A

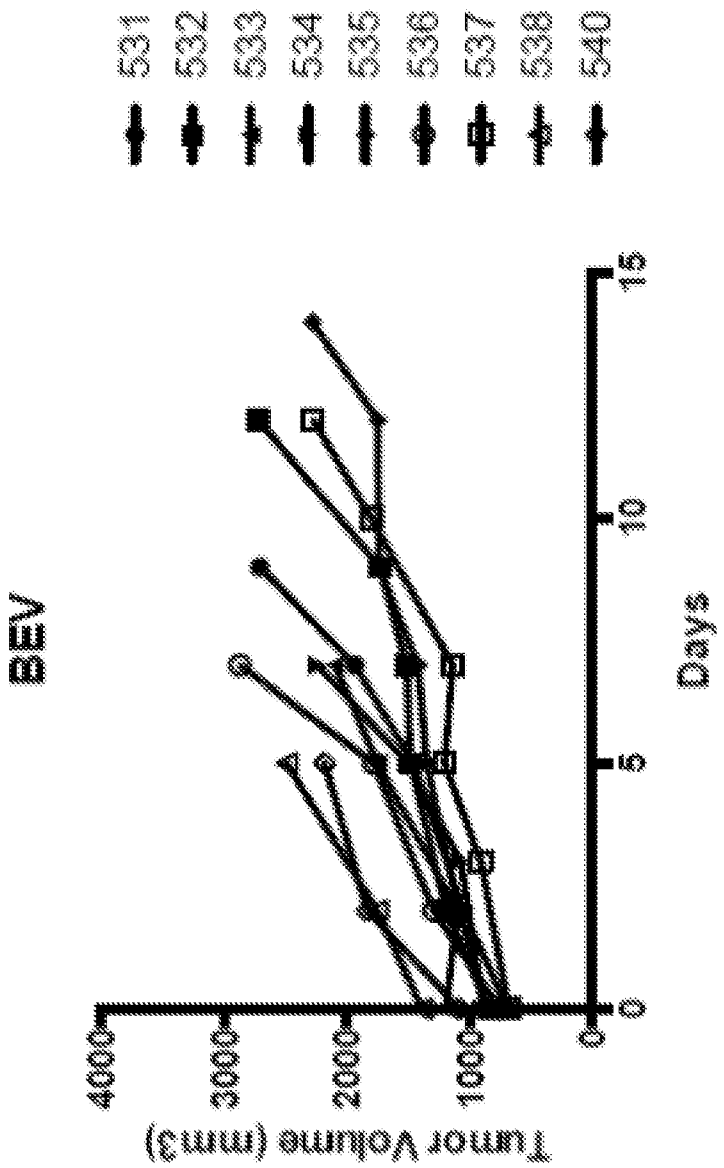

531
532
533
534
535
536
537
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540
BEV
Tumor Volume (mm3)
4000
3000
2000
1000
0
0
5
10
15
Days

FIG. 1B

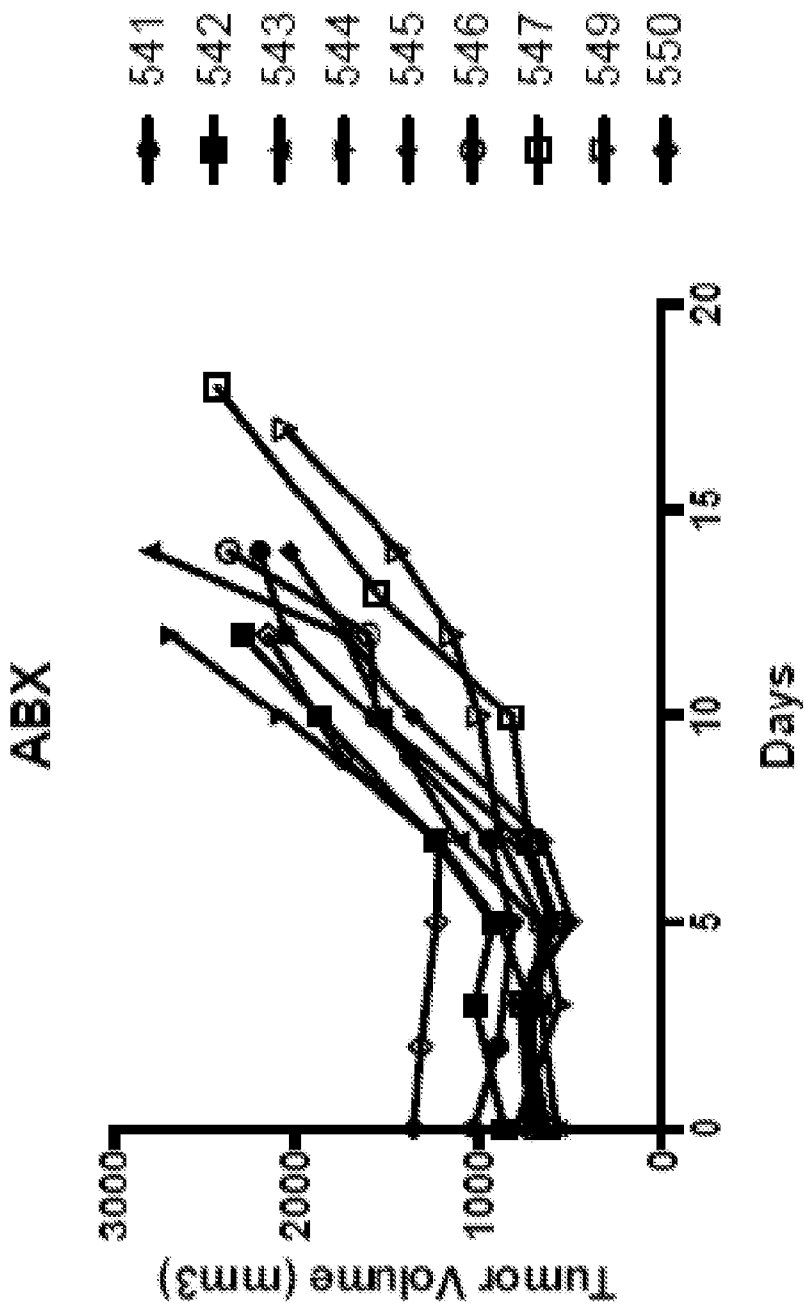

541
542
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549
550
ABX
Tumor Volume (mm3)
3000
2000
1000
0
5
10
15
20
Days

FIG. 1C

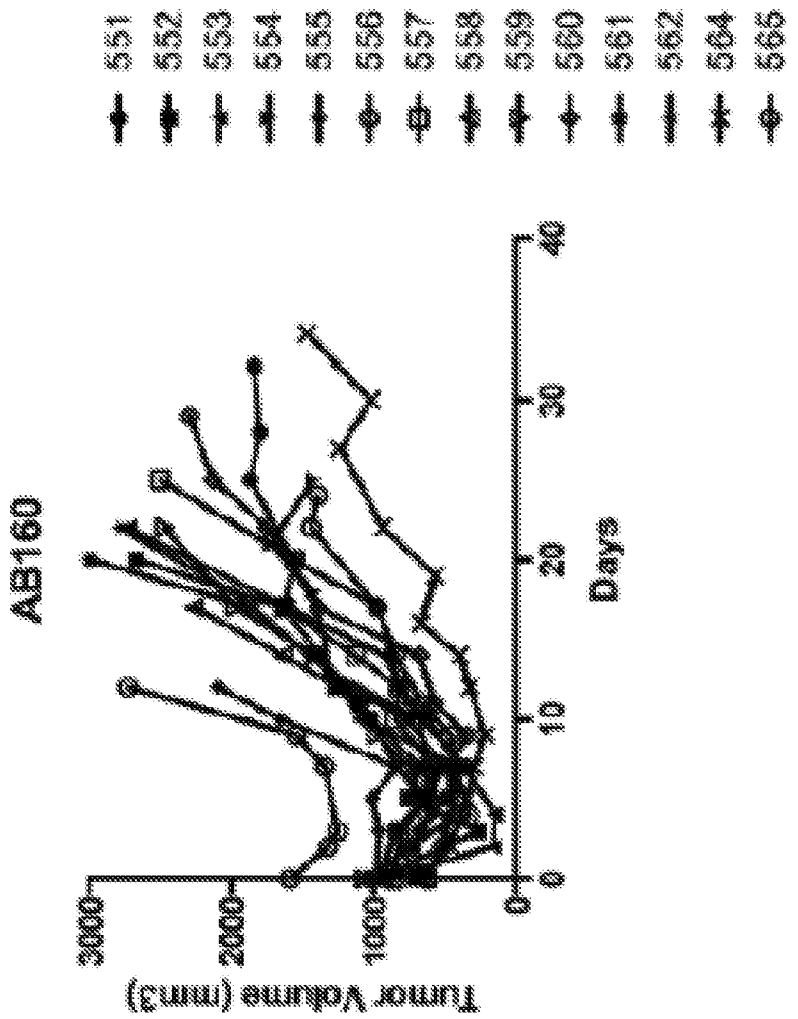

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564
565
AB160
Tumor Volume (mm3)
3000
2000
1000
0
10
20
30
40
Days

FIG. 1D

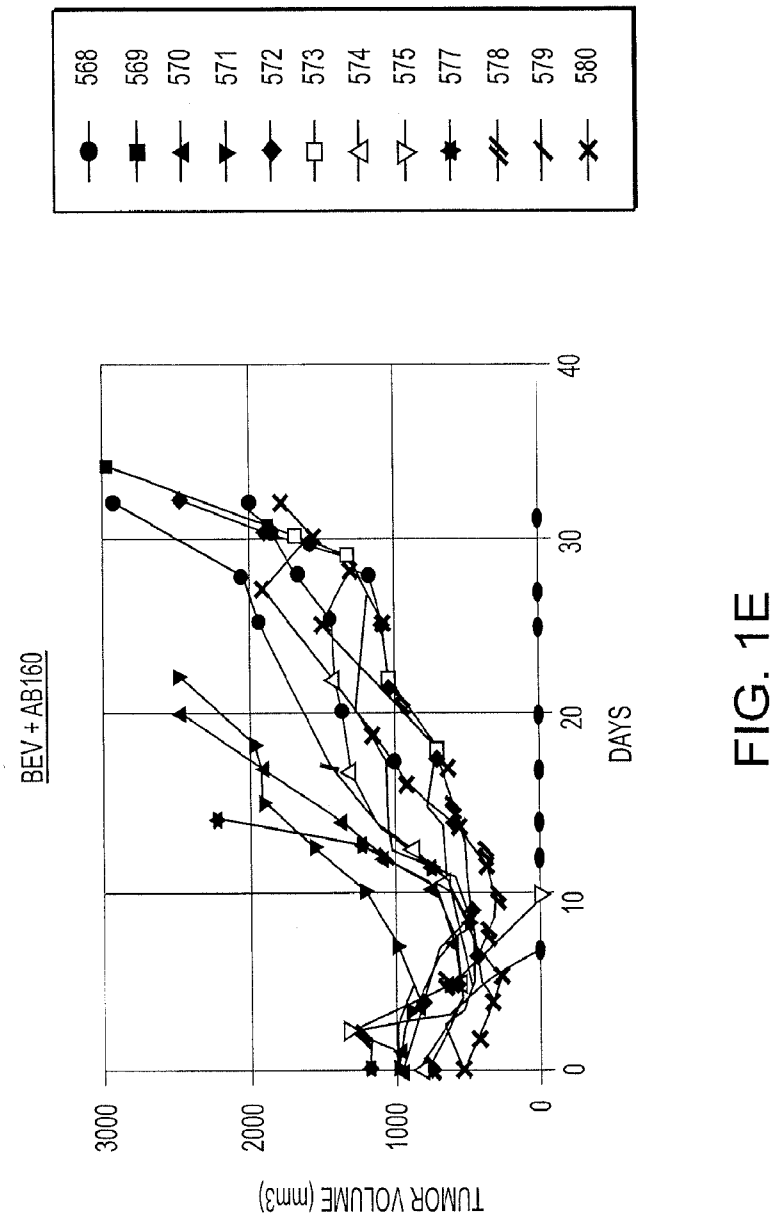

568
569
570
571
572
573
574
575
577
578
579
580
BEV + AB160
TUMOR VOLUME (mm3)
3000
2000
1000
0
0
10
20
30
40
DAYS

FIG. 1E

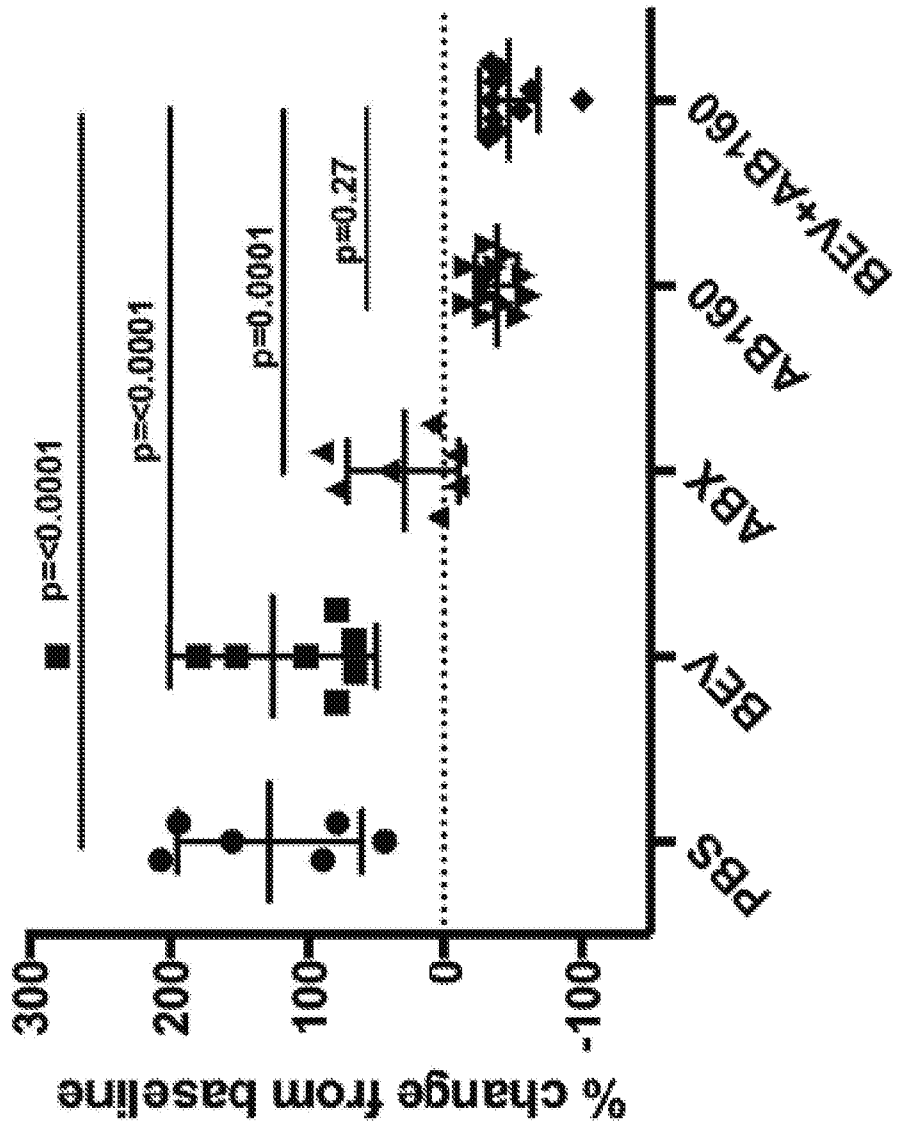

Day 7 % Change
p=<0.0001
p=<0.0001
p=0.0001
p=0.27
300
200
100
0
-100
% change from baseline
PBS
BEV
ABX
AB160
BEV+AB160
Treatment Group

FIG. 1F

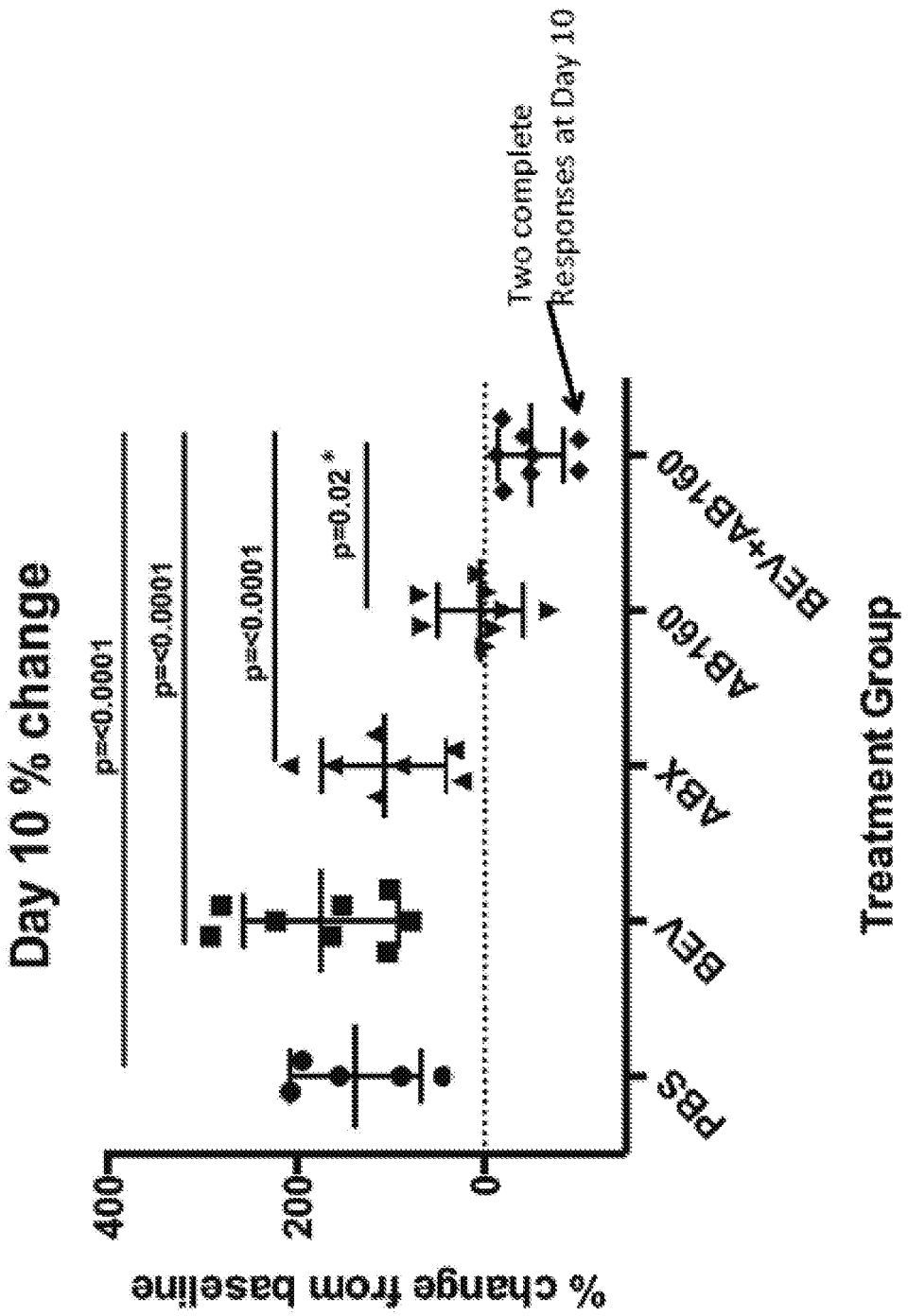

Day 10 % change
p=<0.0001
p=<0.0001
p=<0.0001
p=0.02*
Two complete
Responses at Day 10
400
200
0
% change from baseline
PBS
BEV
ABX
AB160
BEV+AB160
Treatment Group

FIG. 1G

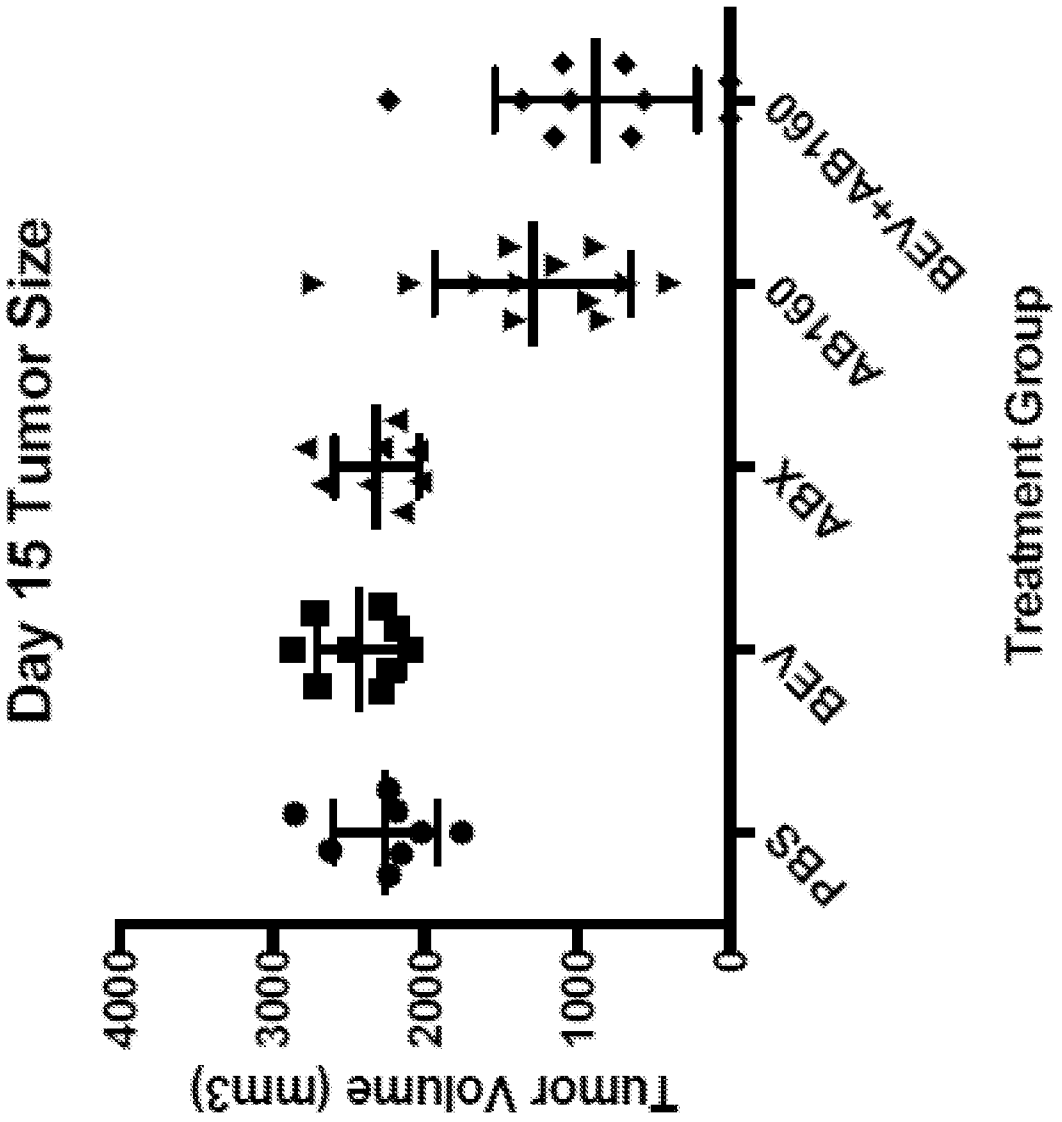

Day 15 Tumor Size
Tumor Volume (mm3)
4000
3000
2000
1000
0
PBS
BEV
ABX
AB160
BEV+AB160
Treatment Group

FIG. 2

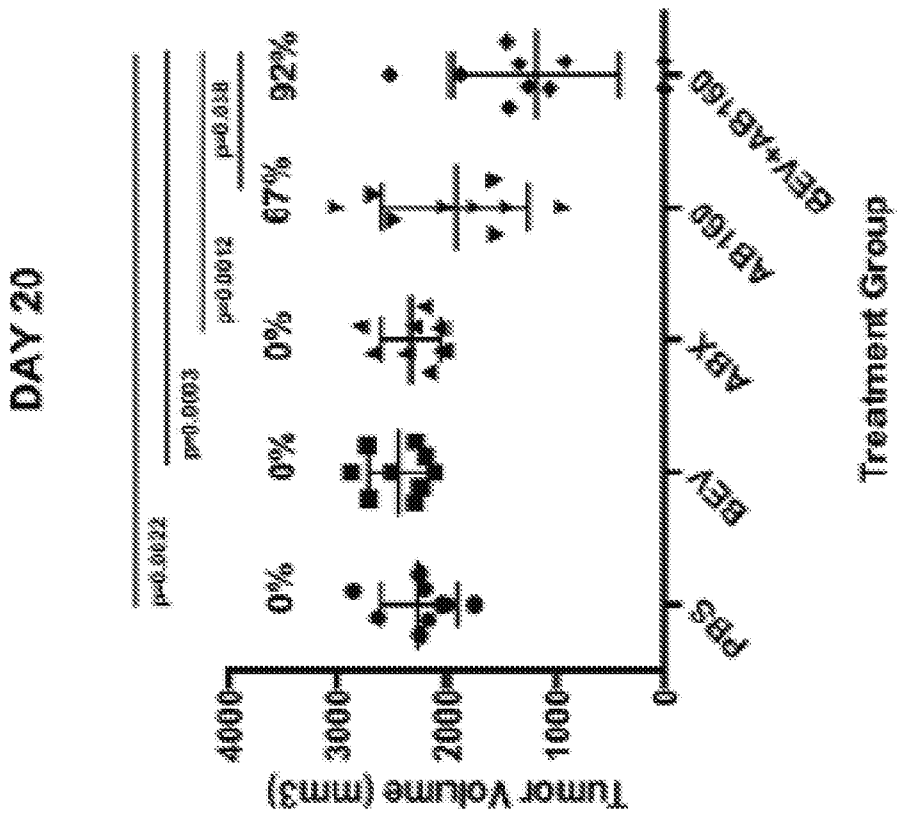
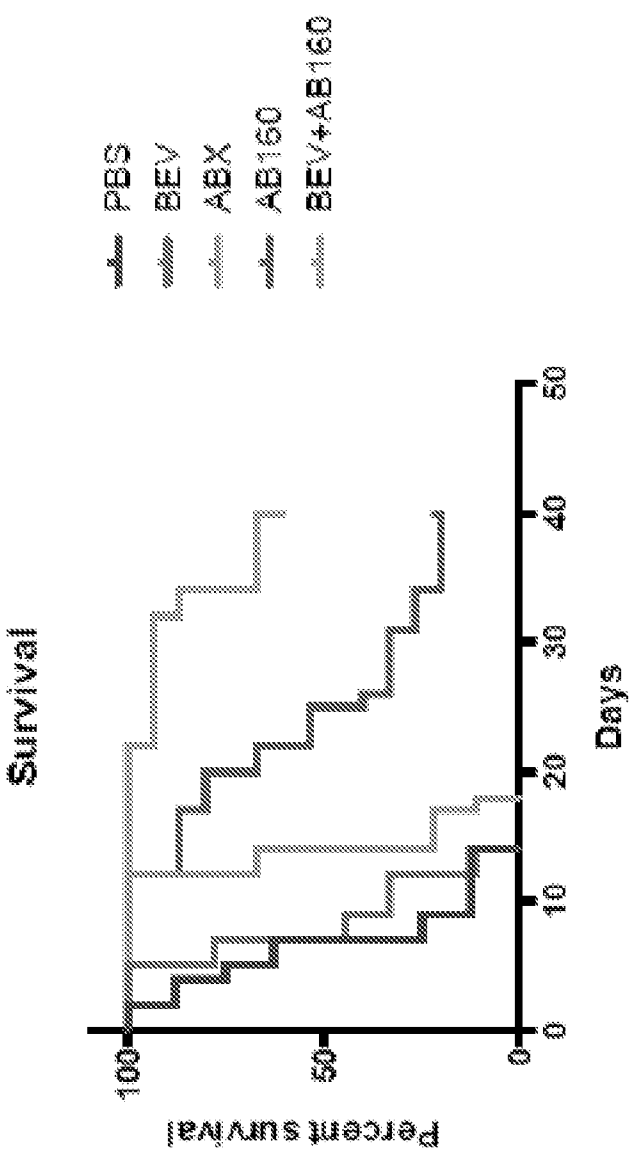

DAY 20
Tumor Volume (mm3)
4000
3000
2000
1000
0
0%
0%
0%
67%
92%
p=0.0022
p=0.0003
p=0.0012
p=0.0138
PBS
BEV
ABX
AB160
BEV+AB160
Treatment Group

FIG. 3



	PBS	BEV	ABX	AB160	BEV+AB160
Median survival	7	7	14	25	Undefined

FIG. 4

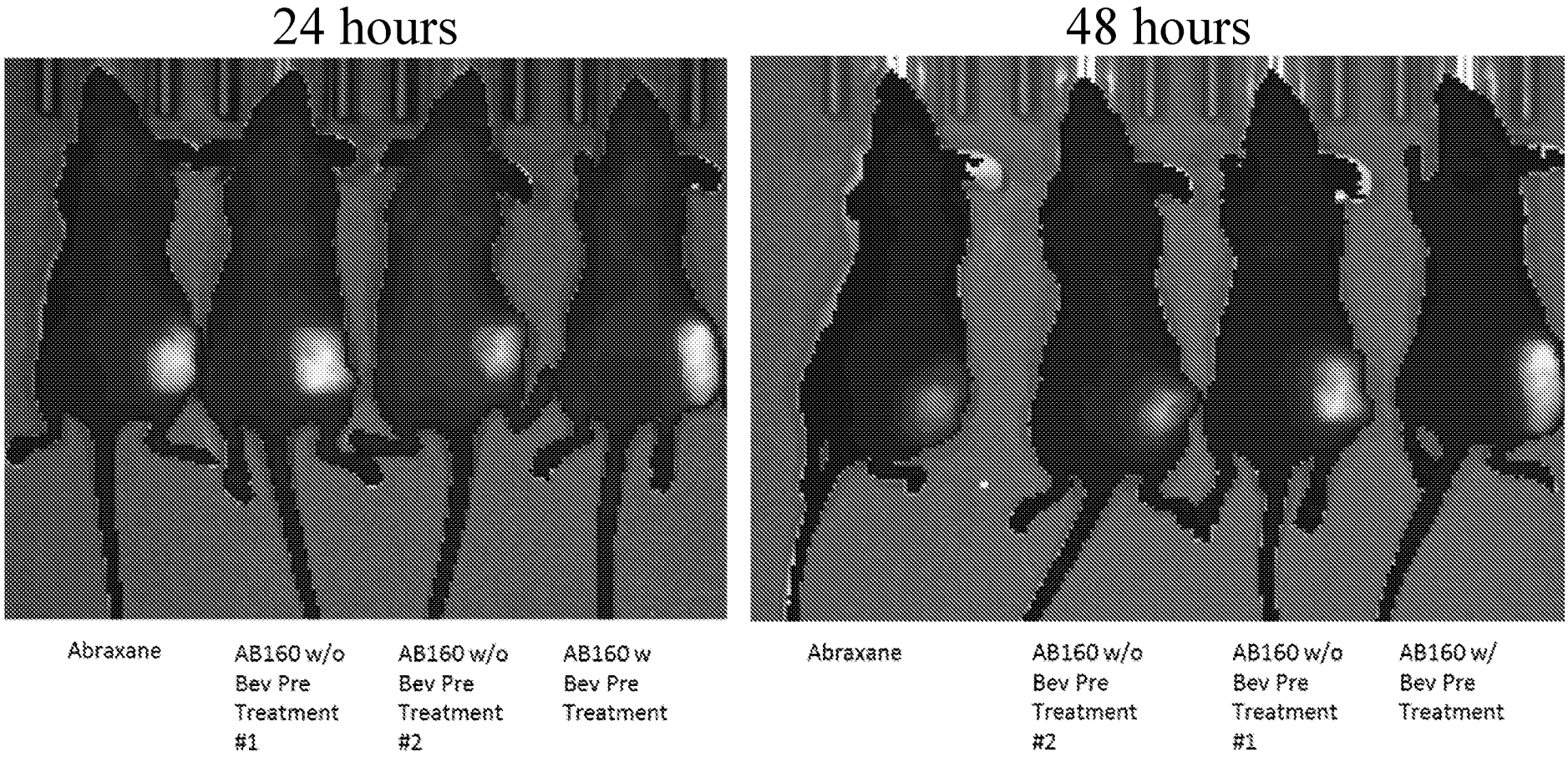
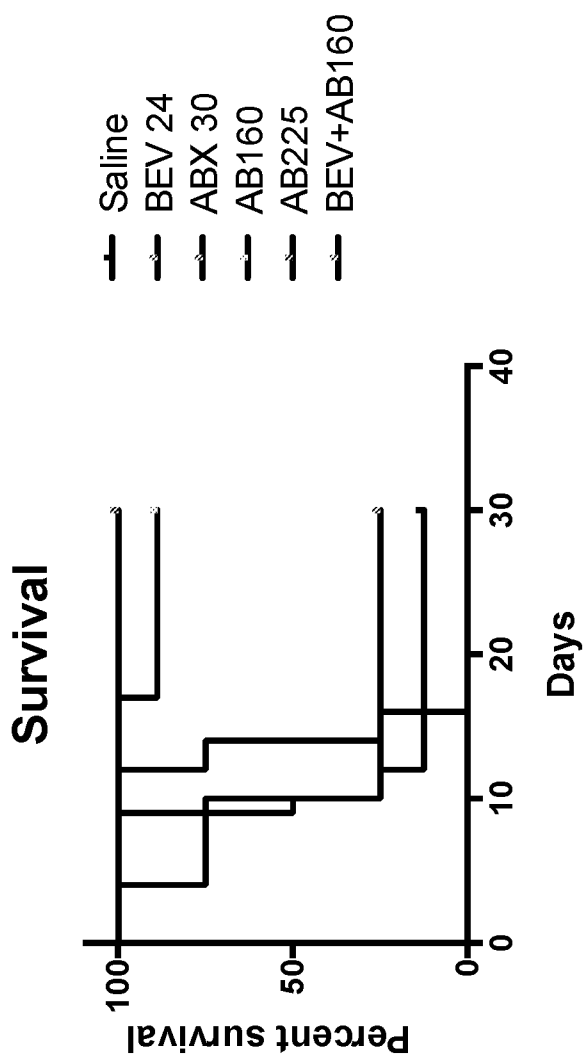

24 hours
48 hours
Abraxane
AB160 w/o Bev Pre Treatment #1
AB160 w/o Bev Pre Treatment #2
AB160 w Bev Pre Treatment
Abraxane
AB160 w/o Bev Pre Treatment #2
AB160 w/o Bev Pre Treatment #1
AB160 w/ Bev Pre Treatment

FIG. 5



	Saline	BEV 24	ABX 30	AB160	AB225	BEV+AB160
Median survival	9.5	14	10	Undefined	Undefined	Undefined

FIG. 6

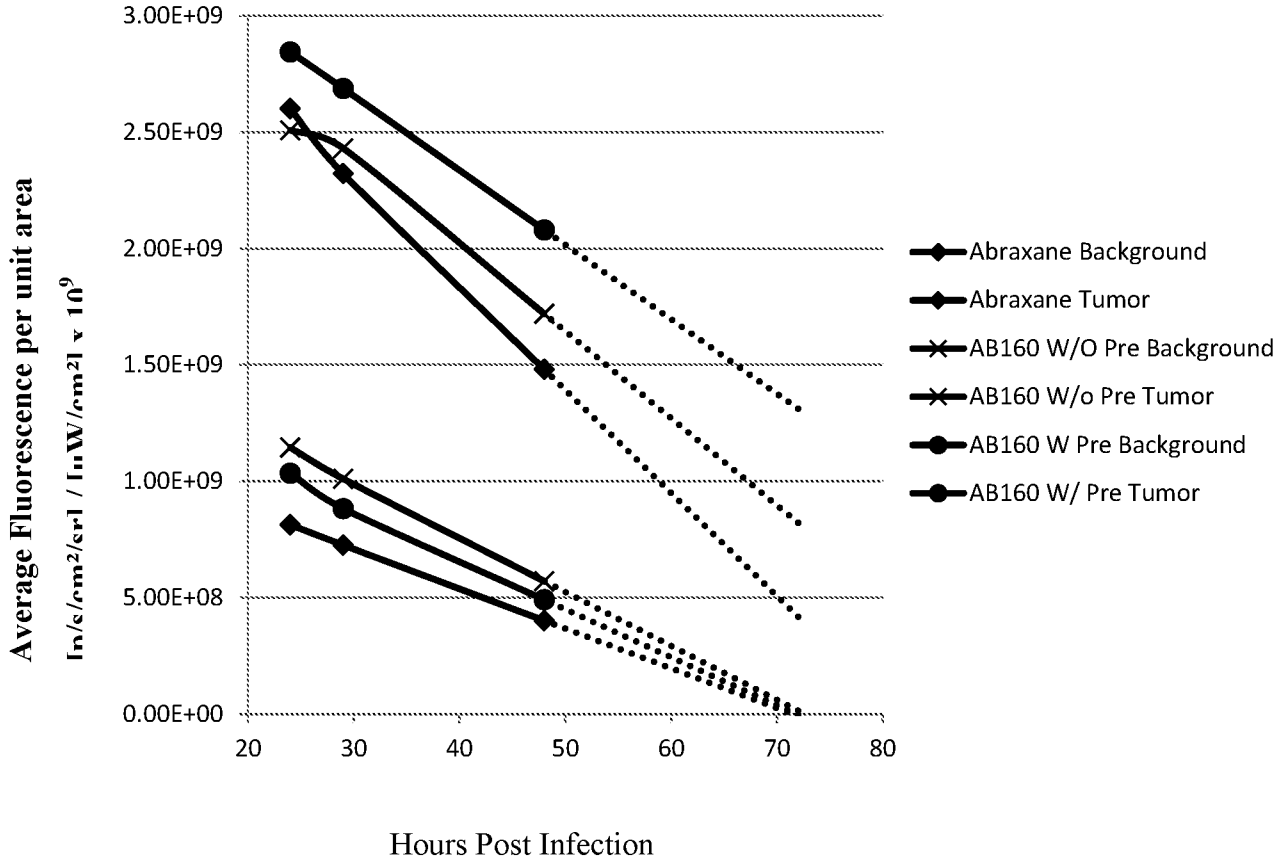

Average Fluorescence per unit area
3.00E+09
2.50E+09
2.00E+09
1.50E+09
1.00E+09
5.00E+08
0.00E+00
20
30
40
50
60
70
80
Hours Post Infection
Abraxane Background
Abraxane Tumor
AB160 W/O Pre Background
AB160 W/o Pre Tumor
AB160 W Pre Background
AB160 W/ Pre Tumor

FIG. 7A

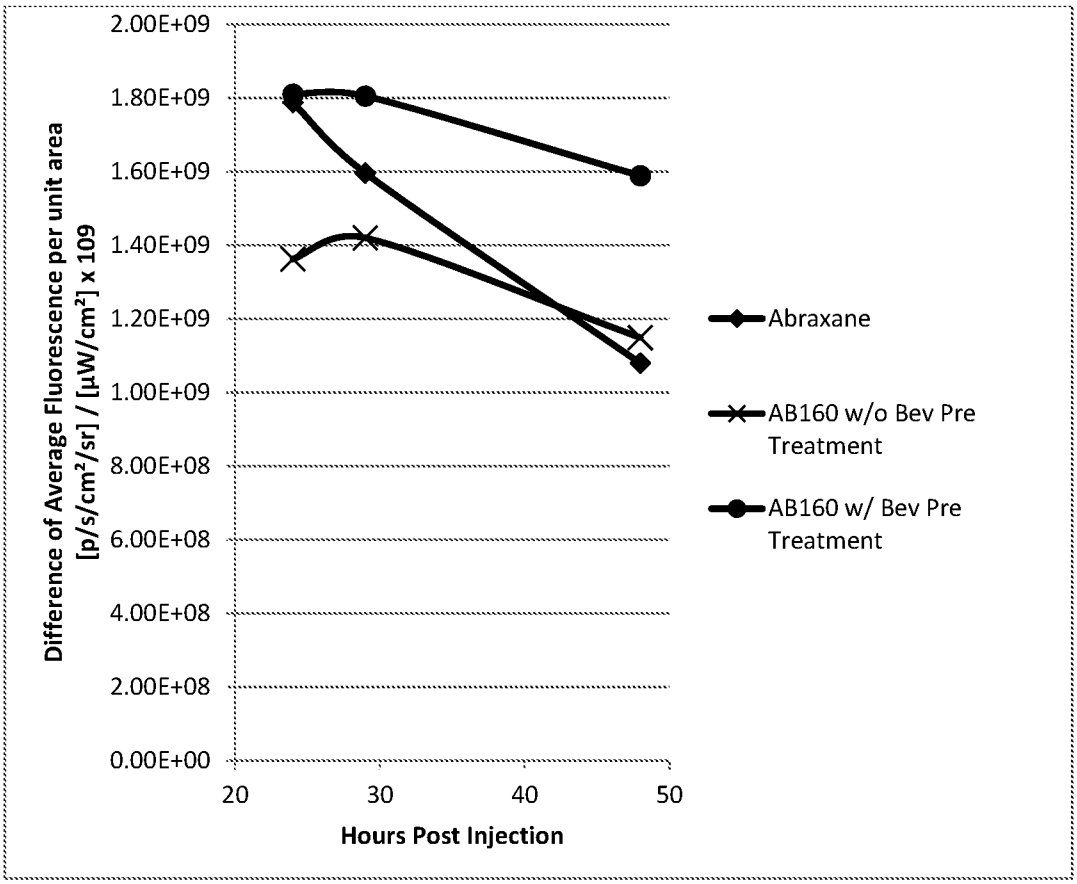


FIG. 7B

	Particle Clearance (Δ Fluorescene/Hour)
	Rate of Tumor Clearance Compared to Background (Tumor/Background)
Abraxane	2.717127608
AB160 w/o Pre Treating	1.371746888
AB160 w/ Pre Treatment	1.407543698
	Tumor Clearance Rate Relative to Abraxane
Abraxane	1
AB160 w/o Pre Treating	0.504851846
AB160 w/ Pre Treatment	0.51802635

FIG. 7C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/026267

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/64; A61K 39/395; A61P 35/00 (2016.01)
CPC - A61K 9/64; A61K 39/395; A61P 35/00 (2016.05)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 9/64; A61K 39/395; A61P 35/00 (2016.01)
CPC - A61K 9/64; A61K 39/395; A61P 35/00 (2016.05)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/400; 424/489; 424/499; 514/252.01 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, Google Scholar.
Search terms used: "circulating vegf" unit dose lyophilized paclitaxel Bevacizumab cancer albumin nanoparticle complexes Synergistic combination chemotherapy sequential administration kit abraxane

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2010/0112077 A1 (DESAI et al) 06 May 2010 (06.05.2010) entire document	1-4, 8-11, 15, 18-22 --- 5-7, 12-14, 17
Y	MEADOWS et al. "Anti-VEGF Therapies in the Clinic," Cold Spring Harbor Perspectives in Medicine, 01 October 2012 (01.10.2012), Vol. 2, Pgs. 1-27. entire document	5, 6, 12, 13
Y	US 2010/0260679 A1 (SHACHAR et al) 14 October 2010 (14.10.2010) entire document	7, 14
Y	US 2013/0149238 A1 (AFFITECH RESEARCH AS et al) 13 June 2013 (13.06.2013) entire document	17
A	HEGDE et al. "Predictive Impact of Circulating Vascular Endothelial Growth Factor in Four Phase III Trials Evaluating Bevacizumab," Clinical Cancer Research, 15 February 2013 (15.02.2013), Vol. 19, Pgs. 929-937. entire document	1-15, 17-22
A	PETRELLI et al. "Targeted Delivery for Breast Cancer Therapy: the History of Nanoparticle-Albumin-Bound Paclitaxel," Expert Opinion on Pharmacotherapy, 01 June 2010 (01.06.2010), Vol. 11, Pgs. 1413-32. entire document	1-15, 17-22

☐ 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
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"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
07 June 2016	12 JUL 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774