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(54) **Title:** COMBINATION THERAPIES TARGETING TUMOR-ASSOCIATED STROMA OR TUMOR CELLS AND MICROTUBULES

(57) **Abstract:** The present invention provides, inter alia, methods for treating or ameliorating the effects of a disease, such as cancer, in a subject. The methods include: administering to a subject in need thereof (a) a therapeutically effective amount of a microtubule inhibitor; and (b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody contains: (i) a heavy chain variable region (VH), which includes an amino acid sequence selected from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7; and (ii) a light chain variable region (VL), which includes an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8. Compositions, including pharmaceutical compositions, and kits for treating diseases, such as cancer, are also provided herein.

**COMBINATION THERAPIES TARGETING TUMOR-ASSOCIATED STROMA OR
TUMOR CELLS AND MICROTUBULES**

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Patent Application Serial No. 62/009,417, filed on June 9, 2014 which application is incorporated by reference herein in its entirety.

FIELD OF INVENTION

[0002] The present invention provides, *inter alia*, methods for treating or ameliorating the effects of a disease, such as cancer, in a subject. The methods include administering to a subject in need thereof (i) microtubule inhibitors, and (ii) TEM8 antibodies or antigen binding fragments thereof. Compositions, including pharmaceutical compositions, and kits for treating diseases, such as cancer, are also provided herein.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] This application contains references to amino acids and/or nucleic acid sequences that have been filed concurrently herewith as sequence listing text file "0385328.txt", file size of 37 KB, created on June 5, 2015. The aforementioned sequence listing is hereby incorporated by reference in its entirety pursuant to 37 C.F.R. § 1.52(e)(5).

BACKGROUND OF THE INVENTION

[0004] Targeting tumor-associated stroma, especially tumor associated vasculature, is considered a promising approach to cancer therapy. Various classes

of chemotherapeutics directed toward tumor vasculature have been developed, including anti-angiogenic agents and vascular disrupting agents, the former affecting neovascularization and the latter targeting existing blood vessels that supply tumors with nutrients and oxygen. Though these therapies are widely used, particularly in cases of metastatic cancer, they are hampered by their toxicity and off-target effects against healthy vasculature. Thus, there exists, *inter alia*, a need for additional therapeutics that are less toxic and work in combination with current agents to suppress tumor growth by targeting the tumor-associated stroma or the tumor cells themselves. The present invention is directed to meeting these and other needs.

SUMMARY OF THE INVENTION

[0005] One embodiment of the present invention is a method for treating or ameliorating the effects of a disease in a subject. The method comprises:

administering to a subject in need thereof

(a) a therapeutically effective amount of a microtubule inhibitor; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region (VH), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7; and

(ii) a light chain variable region (VL), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

[0006] Another embodiment of the present invention is a method for treating or ameliorating the effects of a disease in a subject. The method comprises:

administering to a subject in need thereof

(a) a therapeutically effective amount of a microtubule inhibitor; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

[0007] A further embodiment of the present invention is a method for treating or ameliorating the effects of a disease in a subject. The method comprises:

administering to a subject in need thereof

(a) a therapeutically effective amount of paclitaxel; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

[0008] An additional embodiment of the present invention is a composition for treating or ameliorating the effects of a disease in a subject. The composition comprises:

(a) a therapeutically effective amount of a microtubule inhibitor; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region (VH), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7; and

(ii) a light chain variable region (VL), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

[0009] Another embodiment of the present invention is a composition for treating or ameliorating the effects of a disease in a subject. The composition comprises:

(a) a therapeutically effective amount of a microtubule inhibitor; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

[0010] A further embodiment of the present invention is a composition for treating or ameliorating the effects of a disease in a subject. The composition comprises:

(a) a therapeutically effective amount of paclitaxel; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

[0011] An additional embodiment of the present invention is a pharmaceutical composition. The pharmaceutical composition comprises any of the compositions disclosed herein and a pharmaceutically acceptable diluent or carrier.

[0012] Another embodiment of the present invention is a kit. The kit comprises any of the compositions or pharmaceutical compositions disclosed herein packaged together with instructions for its use.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIGS. 1A-D show results from an efficacy study of TEM8 antibodies in a HCT116 colorectal carcinoma xenograft model. Treatment was initiated when tumor volumes were approximately 60-80 mm³. TEM 8 antibody (hlgG1) was dosed at 5 and 15 mg/kg, three times per week for three weeks. Bevacizumab was dosed at 5 mg/kg, two times per week for three weeks. FIG. 1A shows a box plot of group median tumor volume distribution on day 21. FIG. 1B shows mean tumor growth over the time course of the study. FIG. 1C shows individual times to the endpoint for each mouse in the study. FIG. 1D shows percent body weight change for each group over the course of the study.

[0014] FIG. 2 shows a line graph of tumor volume in the UACC melanoma model. Treatments were initiated when tumor volumes were approximately 50 mm³.

TEM 8 antibodies (hlgG1) were dosed at 15 mg/kg, three times per week, for 3-4 weeks.

[0015] FIG. 3 shows a line graph of tumor volume over time in the HCT116 colon cancer model. The M825 antibody and drug-conjugated M825-MMAE were given 2 times per week for 3 weeks.

[0016] FIG. 4 shows a line graph of tumor volume over time in an OVCAR3 ovarian cancer model. TEM8 antibody M825 and antibody-drug conjugate (ADC) M825-MMAE were given 2 times per week for 3.5 weeks. The 10 mg/kg ADC group was redosed on Day 56, 2 times per week for the remainder of the study.

[0017] FIG. 5 shows a line graph of tumor volume over time in the MDA-MB231 breast cancer model. M825 and M825-MMAE were given 2 times per week for 3 weeks.

[0018] FIGS. 6A-D show results from a study of treatment with TEM8 M830 antibody, bevacizumab, paclitaxel, and combinations thereof in the H460 non-small cell lung carcinoma xenograft model. FIG. 6A shows a time course of mean tumor growth throughout the study. FIG. 6B shows a Kaplan-Meier survival plot. FIG. 6C shows individual times to endpoint for mice in each group. FIG. 6D shows percent body weight change for each group throughout the study.

[0019] FIGS. 7A-D show results from a study of treatment with M830, doxorubicin, paclitaxel, and combinations thereof in a MDA-MB231 breast cancer model. FIG. 7A shows a time course of mean tumor growth throughout the study. FIG. 7B shows a Kaplan-Meier survival plot. FIG. 7C shows individual times to endpoint for mice in each group. FIG. 7D shows percent body weight change for each group throughout the study.

[0020] FIG. 8 shows a line graph of tumor volume over time from a study of M830, bevacizumab, irinotecan, and combinations thereof in a DLD-1 colorectal adenocarcinoma xenograft model.

[0021] FIG. 9 shows a line graph of tumor volume over time from a study of treatment with combinations of M830, bevacizumab, irinotecan, and the combination of all three treatments in a DLD-1 colorectal adenocarcinoma xenograft model.

[0022] FIG. 10 shows a Kaplan-Meier survival plot demonstrating the effect of M830 antibody in mice in a MC38 colon carcinoma liver metastasis model.

[0023] FIGS. 11A-B show the effect of M825 on 3T3 fibroblasts in the presence of TGF β -1. FIG. 11A shows pictures of the cultured cells over 48 hours and FIG. 11B is a bar graph quantifying the lattice area of the cells.

[0024] FIGS. 12A-B show the effect of M830 Fc mutation on antibody-dependent cell-mediated cytotoxicity (ADCC). FIG. 12A is a schematic showing the antibody binding in the ADCC assay and FIG. 12B is a line graph demonstrating that the Fc mutant form of M830 has significantly enhanced binding to Fc γ RIIIa (CD16a).

DETAILED DESCRIPTION OF THE INVENTION

[0025] One embodiment of the present invention is a method for treating or ameliorating the effects of a disease in a subject. The method comprises:

administering to a subject in need thereof

- (a) a therapeutically effective amount of a microtubule inhibitor; and
- (b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region (VH), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7; and

(ii) a light chain variable region (VL), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

[0026] As used herein, the terms "treat," "treating," "treatment" and grammatical variations thereof mean subjecting an individual subject to a protocol, regimen, process or remedy, in which it is desired to obtain a physiologic response or outcome in that subject, *e.g.*, a patient. In particular, the methods and compositions of the present invention may be used to slow the development of disease symptoms or delay the onset of the disease or condition, or halt the progression of disease development. However, because every treated subject may not respond to a particular treatment protocol, regimen, process or remedy, treating does not require that the desired physiologic response or outcome be achieved in each and every subject or subject population, *e.g.*, patient population. Accordingly, a given subject or subject population, *e.g.*, patient population may fail to respond or respond inadequately to treatment.

[0027] As used herein, the terms "ameliorate", "ameliorating" and grammatical variations thereof mean to decrease the severity of the symptoms of a disease in a subject.

[0028] As used herein, a "subject" is a mammal, preferably, a human. In addition to humans, categories of mammals within the scope of the present invention include, for example, farm animals, domestic animals, laboratory animals, etc. Some

examples of farm animals include cows, pigs, horses, goats, etc. Some examples of domestic animals include dogs, cats, etc. Some examples of laboratory animals include primates, rats, mice, rabbits, guinea pigs, etc.

[0029] As used herein, a “microtubule inhibitor” is a substance that disrupts the functioning of a microtubule, such as the polymerization and the depolymerization of individual microtubule units. In one aspect of the present invention, the microtubule inhibitor may be selected from the group consisting of a microtubule-destabilizing agent, a microtubule-stabilizing agent, and combinations thereof. The microtubule inhibitor of the present invention may also be selected from the group consisting of a taxane, a vinca alkaloid, an epothilone, and combinations thereof. Non-limiting examples of microtubule inhibitors according to the present invention include BT-062 (Biotest), HMN-214 (D. Western Therapeutics), eribulin mesylate (Eisai), vindesine (Eli Lilly), EC-1069 (Endocyte), EC-1456 (Endocyte), EC-531 (Endocyte), vintafolide (Endocyte), 2-methoxyestradiol (EntreMed), GTx-230 (GTx), trastuzumab emtansine (Hoffmann-La Roche), crolibulin (Immune Pharmaceuticals), D1302A-maytansinoid conjugates (ImmunoGen), IMGN-529 (ImmunoGen), lorvotuzumab mertansine (ImmunoGen), SAR-3419 (ImmunoGen), SAR-566658 (ImmunoGen), IMP-03138 (Impact Therapeutics), topotecan/vincristine combinations (LipoCure), BPH-8 (Molecular Discovery Systems), fosbretabulin tromethamine (OXiGENE), estramustine phosphate sodium (Pfizer), vincristine (Pierre Fabre), vinflunine (Pierre Fabre), vinorelbine (Pierre Fabre), RX-21101 (Rexahn), cabazitaxel (Sanofi), STA-9584 (Synta Pharmaceuticals), vinblastine, epothilone A, patupilone (Novartis), ixabepilone (Bristol-Myers Squibb), Epothilone D (Kosan Biosciences), paclitaxel (Bristol-Myers Squibb), docetaxel (Sanofi-Aventis), DJ-927 (Daiichi Sankyo), discodermolide (CAS No: 127943-53-7), eleutherobin (CAS

No.: 174545-76-7), pharmaceutically acceptable salts thereof, and combinations thereof.

[0030] In the present invention, cyclooxygenase-2 (COX-2) inhibitors (COXIBs), non-steroidal anti-inflammatory drugs (NSAIDs), and/or prostaglandin E2 (PGE2) synthase inhibitors may be administered to the subject in addition to or as a replacement for microtubule inhibitor. COXIBs according to the present invention include, but are not limited to, AAT-076 (AskAt), celecoxib (Pfizer), valdecoxib (Pfizer), rofecoxib (Merck), nimesulide, 4-hydroxynimesulide, flosulide, L475L337, bromfenac (InSite Vision), KIT-302 (Kitov), polmacoxib (Pacificpharma), etodolac (Pfizer), parecoxib sodium (Pfizer), etoricoxib (Merck), lumiracoxib (Novartis), VA-964 (Rottapharm Madaus), iguratimod (Toyama), and meloxicam (Boehringer Ingelheim). NSAIDs according to the present invention include, but are not limited to, diclofenac (Novartis), diflunisal (Merck), etodolac (Almirall Limited), fenoprofen (Ranbaxy Pharmaceuticals), flurbiprofen (Pfizer), ibuprofen (Pfizer), indomethacin (Lundbeck, Inc.), ketoprofen (Wyeth), ketorolac (Hospira), mefenamic acid (Shionogi, Inc.), meloxicam (Abbott Laboratories), nabumetone (GlaxoSmithKline), naproxen (Bayer), oxaprozin (Pfizer), piroxicam (Pfizer), sulindac (Mutual Pharmaceutical Company, Inc.), AAT-076 (AskAt), carbasalate calcium (Bristol-Myers Squibb), rimazolium metilsulfate (Chinoin), isonixin (Hermes), lysine clonixinate (Laplex), alminoprofen (Recordati), lornoxicam (Takeda), fosfosal (Uriach), nepafenac (Novartis), diclofenac (Novartis), and tolmetin (Ortho-McNeil-Janssen Pharmaceuticals, Inc.). PGE2 synthase inhibitors according to the present invention include, but are not limited to, NS-398, sulindac sulphide, leukotriene C4, GRC-27864 (Glenmark), OX-MPI (Orexo), PF-04693627 (Pfizer), and those described in, for example, He, et al, 2013.

[0031] As used herein, an “antibody” and “antigen-binding fragments thereof” encompass naturally occurring immunoglobulins (*e.g.*, IgM, IgG, IgD, IgA, IgE, etc.) as well as non-naturally occurring immunoglobulins, including, for example, single chain antibodies, chimeric antibodies (*e.g.*, humanized murine antibodies), heteroconjugate antibodies (*e.g.*, bispecific antibodies), Fab', F(ab')₂, Fab, Fv, and rIgG. See, *e.g.*, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Kuby *et al.*, 1998. As used herein, an “antigen-binding fragment” is a portion of the full length antibody that retains the ability to specifically recognize the antigen, as well as various combinations of such portions.

[0032] Non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly, or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse *et al.*, *Science* 246:1275-1281 (1989), which is incorporated herein by reference. These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies, are well known to those skilled in the art (Winter and Harris, *Immunol. Today* 14:243-246 (1993); Ward *et al.*, *Nature* 341:544-546 (1989); Harlow and Lane, *supra*, 1988; Hilyard *et al.*, *Protein Engineering: A practical approach* (IRL Press 1992); Borrabeck, *Antibody Engineering*, 2d ed. (Oxford University Press 1995); each of which is incorporated herein by reference).

[0033] Full length antibodies can be proteolytically digested down to several discrete, functional antibody fragments, which retain the ability to recognize the antigen. For example, the enzyme papain can be used to cleave a full length immunoglobulin into two Fab fragments and an Fc fragment. Thus, the Fab fragment is typically composed of two variable domains and two constant domains from the

heavy and light chains. The Fv region is usually recognized as a component of the Fab region and typically comprises two variable domains, one from each of the heavy (V_H , "heavy chain variable region", as used herein) and light (V_L "light chain variable region", as used herein) chains. The enzyme pepsin cleaves below the hinge region, so a $F(ab')_2$ fragment and a pFc' fragment is formed. $F(ab')_2$ fragments are intact antibodies that have been digested, removing the constant (Fc) region. Two Fab' fragments can then result from further digestion of $F(ab')_2$ fragments. Examples of antigen-binding fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, $F(ab')_2$, diabodies, tribodies, scFvs, and single-domain antibodies (dAbs).

[0034] Typically, a full length antibody has at least one heavy and at least one light chain. Each heavy chain contains a variable domain (V_H) and typically three or more constant domains (C_{H1} , C_{H2} , C_{H3} , etc.), while each light chain contains a variable domain (V_L) and a constant domain C_L . Light and heavy chain variable regions contain four "framework" regions interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs". The extent of the framework regions and CDRs have been defined. See, e.g., Kabat *et al.*, U.S. Dept. of Health and Human Services, Sequences of Proteins of Immunological Interest (1983) and Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. 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.

[0035] The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically

identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody.

[0036] 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 antigenic epitope. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made, *e.g.*, by the hybridoma method first described by Kohler *et al.*, Nature 256: 495 (1975), and as modified by the somatic hybridization method as set forth above; or may be made by other recombinant DNA methods (*see, e.g.*, U.S. Pat. No. 4,816,567).

[0037] Additional types of antibodies that may be part of the monoclonal antibodies of the present invention include, but are not limited to, chimeric, humanized, and human antibodies. For application in man, it is often desirable to reduce immunogenicity of antibodies originally derived from other species, like mouse. This can be done by construction of chimeric antibodies, or by a process called "humanization". In this context, a "chimeric antibody" is understood to be an antibody comprising a domain (*e.g.* a variable domain) derived from one species (*e.g.* mouse) fused to a domain (*e.g.* the constant domains) derived from a different species (*e.g.* human).

[0038] As used herein, the term "humanized antibody" refers to forms of antibodies that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the framework (FR) regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones *et al.*, Nature 321:522-525 (1986); Riechmann *et al.*, Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol 2:593-596 (1992)). Humanization can be essentially performed, e.g., following the method of Winter and co-workers (Jones *et al.*, Nature 321:522-525 (1986); Riechmann *et al.*, Nature 332:323-3'27 (1988); Verhoeyen *et al.*, Science 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody.

[0039] Furthermore, technologies have been developed for creating antibodies based on sequences derived from the human genome, for example by phage display or using transgenic animals (see, e.g., WO 90/05144; D. Marks, H. R. Hoogenboom, T. P. Bonnert, J. McCafferty, A. D. Griffiths and G. Winter (1991) "By-passing immunisation. Human antibodies from V-gene libraries displayed on phage." J. Mol. Biol., 222, 581-597; Knappik *et al.*, J. Mol. Biol. 296: 57-86, 2000; S. Carmen and L. Jermutus, "Concepts in antibody phage display". Briefings in Functional Genomics and Proteomics 2002 1(2):189-203; Lonberg N, Huszar D. "Human antibodies from transgenic mice". Int Rev Immunol. 1995; 13(1):65-93.; Bruggemann

M, Taussig M J. "Production of human antibody repertoires in transgenic mice". *Curr Opin Biotechnol.* 1997 August; 8(4):455-8.). Such antibodies are "human antibodies" in the context of the present invention.

[0040] As used herein, a "recombinant" antibody is any antibody whose production involves expression of a non-native DNA sequence encoding the desired antibody structure in an organism. In the present invention, recombinant antibodies include tandem scFv (taFv or scFv₂), diabody, dAb₂/VHH₂, knob-into-holes derivatives, SEED-IgG, heteroFc-scFv, Fab-scFv, scFv-Jun/Fos, Fab'-Jun/Fos, tribody, DNL-F(ab)₃, scFv₃-CH1/CL, Fab-scFv₂, IgG-scFab, IgG-scFv, scFv-IgG, scFv₂-Fc, F(ab')₂-scFv₂, scDB-Fc, scDb-CH3, Db-Fc, scFv₂-H/L, DVD-Ig, tandAb, scFv-dhIx-scFv, dAb₂-IgG, dAb-IgG, dAb-Fc-dAb, and combinations thereof.

[0041] Variable regions of antibodies are typically isolated as single-chain Fv (scFv) or Fab fragments. ScFv fragments are composed of V_H and V_L domains linked by a short 10-25 amino acid linker. Once isolated, scFv fragments can be genetically linked with a flexible peptide linker such as, for example, one or more repeats of Ala-Ala-Ala, Gly-Gly-Gly-Gly-Ser, etc. The resultant peptide, a tandem scFv (taFv or scFv₂) can be arranged in various ways, with V_H-V_L or V_L-V_H ordering for each scFv of the taFv. (Kontermann, R.E. In: *Bispecific Antibodies*. Kontermann RE (ed.), Springer Heidelberg Dordrecht London New York, pp. 1-28 (2011)).

[0042] As used herein, the term "epitope" refers to the portion of the antigen which is recognized by the antibody or antigen binding fragment. A single antigen (such as an antigenic polypeptide) may have more than one epitope. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-

linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents.

[0043] Portions of the preferred antibodies and nucleic acids encoding said antibody portions of the present invention are listed below in Table 1.

Table 1 - Antibody Sequences

SEQ ID NO.	Sequence Name	Nucleic Acid/Polypeptide	Organism
1	M825 mAb VH	Polypeptide	Artificial sequence
2	M825 mAb VL	Polypeptide	Artificial sequence
3	M822 mAb VH	Polypeptide	Artificial sequence
4	M822 mAb VL	Polypeptide	Artificial sequence
5	M830 mAb VH	Polypeptide	Artificial sequence
6	M830 mAb VL	Polypeptide	Artificial sequence
7	M863 mAb VH	Polypeptide	Artificial sequence
8	M863 mAb VL	Polypeptide	Artificial sequence
11	M825 mAb VH cDNA	Nucleic acid	Artificial sequence
12	M825 mAb VL cDNA	Nucleic acid	Artificial sequence
13	M822 mAb VH cDNA	Nucleic acid	Artificial sequence
14	M822 mAb VL cDNA	Nucleic acid	Artificial sequence
15	M830 mAb VH cDNA	Nucleic acid	Artificial sequence
16	M830 mAb VL cDNA	Nucleic acid	Artificial sequence
17	M863 mAb VH cDNA	Nucleic acid	Artificial sequence
18	M863 mAb VL cDNA	Nucleic acid	Artificial sequence

[0044] In one aspect of this embodiment, the disease is characterized by differential expression of a tumor endothelial marker 8 (TEM8) membrane antigen. As used herein, the terms “differential expression”, “differentially expressed”, and grammatical variations thereof mean changes in the production levels of certain

mRNA(s) and/or protein(s) in tumor cells relative to normal cells. Differential expression includes upregulation and downregulation of gene(s). Preferably, the differential expression of TEM8 on, e.g. tumor endothelial cells, is sufficient for the antibodies and/or antigen binding fragments thereof of the present invention to specifically target such cells, leaving normal, non-cancerous tissue substantially untouched.

[0045] Representative human TEM8 nucleic acid and polypeptide sequences are shown in Table 2 below.

Table 2 - TEM8 Sequences

SEQ ID NO.	Sequence Name	Nucleic Acid/Polypeptide	Organism	Other Information
9	TEM8 mRNA	Nucleic acid	Homo sapiens	Transcript variant 1
10	TEM8 protein	Polypeptide	Homo sapiens	Isoform 1 precursor
19	TEM8 mRNA	Nucleic acid	Homo sapiens	Transcript variant 2
20	TEM8 protein	Polypeptide	Homo sapiens	Isoform 2 precursor
21	TEM8 mRNA	Nucleic acid	Homo sapiens	Transcript variant 3
22	TEM8 protein	Polypeptide	Homo sapiens	Isoform 3 precursor

[0046] In another aspect of the invention, the disease is characterized by differential expression of tumor endothelial marker 8 (TEM8) membrane antigen on a tumor cell and/or a tumor stromal cell. As used herein, "tumor cells" comprise cells that have abnormal growth or division. As used herein, "tumors" and "cancers" are used interchangeably. Tumors may be benign or malignant. "Tumor stromal cells" include those cells that are in a tumor cell's microenvironment and support the growth of tumor cells. Tumor vasculature is distinct from normal vasculature in that several genes are differentially expressed in tumor-associated blood vessels (St.

Croix *et al.*, 2000). One of these genes, tumor endothelial marker 8 (TEM8) membrane antigen, is upregulated in the vasculature of malignant solid tumors, with limited expression in healthy tissues.

[0047] In another preferred embodiment, the disease is a cancer that differentially expresses TEM8. More preferably, the cancer is selected from the group consisting of kidney cancer, colon cancer, lung cancer, liposarcomas, brain cancer, breast cancer, melanoma, liver cancer, head and neck cancer, and prostate cancer.

[0048] In another aspect of this embodiment, the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

```
caggtccagctggtgacagtcctggggctgaggtgaagaagcctgggac
ctcagtgaaaggtctcctgcaaggttcttgatacaccttcagcagct
atgctatcagctgggtgacagggccctggacaagggcttgagtgg
atgggagggatcatccctatctttggtacaacaaactacgcacagaa
gttccagggcagagtcacgattaccggggaggaatccacgagcacag
tctacatggagctgagcagcctgagatctgaggacacggccgtgat
tactgtgagagatacggactacatgtttgactactggggccaggg
aacctggtcaccgtgagctca (SEQ ID NO: 11)
```

and

(2) a VL polypeptide encoded by:

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tcttctgagctgactcaggaccctggtgtgtctgtggccttgggaga
gacagtcagtatcacatgccaaaggagacaacctcagagacttttatg
caagctggtaccaacagaagccaggacaggccctctactagtcatg
tatggtaaaaacagggcggcctcagggatcccagaccgattctctgg
ctccacctcaggaaacacactttccttgaccatcactggggctcagg
cggaagatgaggctgactattactgtagctcccgggacaacagtaag
catgtggtgttcggcggggggaccaaggtcaccgtccta (SEQ ID
NO:12).
```

[0049] In an additional aspect of this embodiment, the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

cagggtccagctggtgacagctctggggctgaggtgaagaagcctggggc
ctcagtgaaggctcctgcaaggtttctggatacaccttcagcagct
atgctatcagctgggtgacagggccctggacaagggcttgagtgg
atgggagggatcatccctatctttggtacagcaaactacgcacagaa
gttccagggcagagtcacgattaccgcggacgaatccacgagcacag
cctacatggagctgagcagcctgagatctgaggacacggccgtgtat
tactgtgacgagagatacggactacatgtttgactactggggccaggg
aacctggtcaccgtgagctca (SEQ ID NO:13)

and

(2) a VL polypeptide encoded by:

tcttctgagctgactcaggaccctgttgtgtctgtggccttgggaga
gacagtcagtatcacatgcccaaggagacaacctcagagacttttatg
caagctggtaccaacagaagccaggacagggccctctactagtcatg
tatggtaaaaacagggcggcctcagggatcccagaccgattctctgg
ctccacctcaggaaacacactttccttgaccatcactggggctcagg
cggaagatgaggctgactattactgtagctcccgggacaacagtaag
catgtggtgttcggcggggggaccaaggtcaccgtccta (SEQ ID
NO:14) .

[0050] In another aspect of this embodiment, the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

gaggtgcagctggtggagctctgggggagggcgtggtccagcctgggag
gtccgtgagactctcctgtgcagcctctggattcaccttcagtacct
atactatgcactgggtccgccaggctccaggcaaggggctggagtgg
gtggcaattatctcaaatgatggaagcaataagtactacgcagacc
cgtgagggggccgattcaccatctccagagacaattccaagaacacgc
tgtatctgcaaatgaacagcctgagagctgaggacacggctgtgtat
tactgtgtacgtggcagcagctggatcgcggaaattggttcgacc
ctggggccaggaacctggtcaccgtgagctca (SEQ ID
NO:15)

and

(2) a VL polypeptide encoded by:

gacatccagatgaccagctctccatcctcctgtctgcatctgtagg
agacagagtcaccatcgcttgccgggcaagtcagaccattagtaggt
atthaaattggtatcagcagaaaccagggaaagcccctaagctcctg
atctatgctgcatccagtttgcaagtggggtctcatcaaggttcag

tggcagtgatctgggacagagttcactctcaccatcagcagtcctgc
 agcctgaagatTTTgcaacttatttctgtcaacagacttacagtccc
 ccgatcaccttcggccaagggacacgactggagattaaacga (SEQ
 ID NO:16).

[0051] In a further aspect of this embodiment, the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

gaggtgcagctggtggagaccggggctgaggtgaagaagcctggggc
 ctcagtgaaaggtctcctgcaaggcttctggatacaccttcaccggct
 actatatgcaactgggtgacagggccctggacaagggcttgagtgg
 atgggatggatcaaccctaccagtggtagcacaactatgcacagaa
 gtttcagggcagggtcaccatgaccagggacacgtccatcagcacag
 cctacatggagctgagcgggctgagatctgacgacactgccgtgat
 tactgtgtgagagatccgggttctcctaagtggtggccttcgacc
 ctggggccagggcacctggtcaccgtgagctca (SEQ ID
 NO:17)

(2) a VL polypeptide encoded by:

gacatccagttgaccagtcctccatcctccttgtctgcttctgtagg
 agacagagtcaccatcacttgccgggcaagtcggggccattagtaggt
 atttaaattggatcagcagaaaccagggaaagcccctaagctcctg
 atctatgctgcatccagtttgcaagtggggtctcatcaaggttcag
 tggcagtgatctgggacagagttcactctcaccatcagcagtcctgc
 agcctgaagatTTTgcaacttatttctgtcaacagacttacagtccc
 ccgatcaccttcggccaagggacacgactggagattaaacgt (SEQ
 ID NO:18).

[0052] Another embodiment of the present invention is a method for treating or ameliorating the effects of a disease in a subject. The method comprises:

administering to a subject in need thereof

- (a) a therapeutically effective amount of a microtubule inhibitor; and
- (b) a therapeutically effective amount of a monoclonal antibody or

antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

[0053] Suitable and preferred microtubule inhibitors, subjects, and diseases (including the characteristics of the diseases and various types of cancers) are as disclosed herein.

[0054] A further embodiment of the present invention is a method for treating or ameliorating the effects of a disease in a subject. The method comprises:

administering to a subject in need thereof

(a) a therapeutically effective amount of paclitaxel; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

[0055] Suitable and preferred subjects and diseases (including the characteristics of the diseases and various types of cancers) are as disclosed herein.

[0056] An additional embodiment of the present invention is a composition for treating or ameliorating the effects of a disease in a subject. The composition comprises:

(a) a therapeutically effective amount of a microtubule inhibitor; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region (VH), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7; and

(ii) a light chain variable region (VL), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

[0057] Suitable and preferred microtubule inhibitors, subjects, and diseases (including the characteristics of the diseases and various types of cancers) are as disclosed herein.

[0058] In another aspect of this embodiment, the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

```
caggtccagctggtgacagctctggggctgaggtgaagaagcctgggac
ctcagtgaaggtctcctgcaaggttcctggatacaccttcagcagct
atgctatcagctgggtgacagggcccctggacaagggcttgagtgg
atgggagggatcatccctatctttggtacaacaaactacgcacagaa
gttccagggcagagtcacgattaccggggaggaatccacgagcacag
tctacatggagctgagcagcctgagatctgaggacacggccgtgtat
tactgtgagagagatacggactacatgtttgactactggggccaggg
aaccctggtcaccgtgagctca (SEQ ID NO: 11)
```

and

(2) a VL polypeptide encoded by:

```
tcttctgagctgactcaggaccctgttgtgtctgtggccttgggaga
gacagtcagtatcacatgcccaaggagacaacctcagagacttttatg
caagctggtaccaacagaagccaggacagggcccctctactagtcatg
tatggtaaaaacagggcggccctcagggatcccagaccgattctctgg
```

ctccacctcaggaaacacactttccttgaccatcactggggctcagg
 cggaagatgaggctgactattactgtagctcccgggacaacagtaag
 catgtggtgttcggcggggggaccaaggtcaccgtccta (SEQ ID
 NO:12) .

[0059] In an additional aspect of this embodiment, the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

caggtccagctggtgcagctctggggctgaggtgaagaagcctggggc
 ctcagtgaaaggtctcctgcaaggtttctggatacaccttcagcagct
 atgctatcagctgggtgacagggccctggacaagggcttgagtgg
 atgggagggatcatccctatctttggtacagcaaactacgcacagaa
 gttccagggcagagtcacgattaccgcggaatccacgagcacag
 cctacatggagctgagcagcctgagatctgaggacacggccgtgat
 tactgtgagagatacggactacatgtttgactactggggccaggg
 aaccctggtcaccgtgagctca (SEQ ID NO:13)

and

(2) a VL polypeptide encoded by:

tcttctgagctgactcaggaccctgttgtgtctgtggccttgggaga
 gacagtcagtatcacatgccaaaggagacaacctcagagacttttatg
 caagctggtaccaacagaagccaggacaggccctctactagtcatg
 tatggtaaaaacaggcggccctcagggatcccagaccgattctctgg
 ctccacctcaggaaacacactttccttgaccatcactggggctcagg
 cggaagatgaggctgactattactgtagctcccgggacaacagtaag
 catgtggtgttcggcggggggaccaaggtcaccgtccta (SEQ ID
 NO:14) .

[0060] In another aspect of this embodiment, the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

gaggtgcagctggtggagctctgggggaggcgtggtccagcctgggag
 gtccgtgagactctcctgtgcagcctctggattcaccttcagtacct
 atactatgcactgggtccgccaggctccaggcaaggggctggagtgg
 gtggcaattatctcaaatgatggaagcaataagtactacgcagaccc
 cgtgaggggcccattcaccatctccagagacaattccaagaacacgc
 tgtatctgcaaatgaacagcctgagagctgaggacacggctgtgat
 tactgtgtacgtggcagcagctgggtatcgcggaattggttcgaccc

ctggggccagggaaacctggtcaccgtgagctca (SEQ ID NO:15)

and

(2) a VL polypeptide encoded by:

gacatccagatgaccagttctccatcctcctgtctgcatctgtagg
 agacagagtcaccatcgcttgccgggcaagtcagaccattagtaggt
 atttaaattggtatcagcagaaaccagggaaagcccctaagctcctg
 atctatgctgcatccagtttgcaaagtggggtctcatcaaggttcag
 tggcagtggtatctgggacagagttcactctcaccatcagcagttctgc
 agcctgaagatthttgcaacttatttctgtcaacagacttacagtccc
 ccgatcaccttcggccaaggacacgactggagattaaacga (SEQ ID NO:16) .

[0061] In a further aspect of this embodiment, the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

gaggtgcagctggtggagaccggggctgaggtgaagaagcctggggc
 ctgagtgaaaggtctcctgcaaggcttctggatacaccttcaccggct
 actatatgcaactgggtgacagagccctggacaagggttgagtg
 atgggatggatcaaccctaccagtggttagcacaactatgcacagaa
 gtttcagggcagggtcaccatgaccagggacagtcctcagcag
 cctacatggagctgagcgggctgagatctgacgacactgccgtgat
 tactgtgtgagagatccgggttctcctaagtggctggccttcgacc
 ctggggccagggcaccctggtcaccgtgagctca (SEQ ID NO:17)

(2) a VL polypeptide encoded by:

gacatccagttgaccagttctccatcctccttgtctgcttctgtagg
 agacagagtcaccatcacttgccgggcaagtcgggccattagtaggt
 atttaaattggtatcagcagaaaccagggaaagcccctaagctcctg
 atctatgctgcatccagtttgcaaagtggggtctcatcaaggttcag
 tggcagtggtatctgggacagagttcactctcaccatcagcagttctgc
 agcctgaagatthttgcaacttatttctgtcaacagacttacagtccc
 ccgatcaccttcggccaaggacacgactggagattaaacgt (SEQ ID NO:18) .

[0062] Another embodiment of the present invention is a composition for treating or ameliorating the effects of a disease in a subject. The composition comprises:

- (a) a therapeutically effective amount of a microtubule inhibitor; and
- (b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:
 - (i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and
 - (ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

[0063] Suitable and preferred microtubule inhibitors, subjects, and diseases (including the characteristics of the diseases and various types of cancers) are as disclosed herein.

[0064] A further embodiment of the present invention is a composition for treating or ameliorating the effects of a disease in a subject. The composition comprises:

- (a) a therapeutically effective amount of paclitaxel; and
- (b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:
 - (i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and
 - (ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

[0065] Suitable and preferred subjects and diseases (including the characteristics of the diseases and various types of cancers) are as disclosed herein.

[0066] An additional embodiment of the present invention is a pharmaceutical composition. The pharmaceutical composition comprises any of the compositions disclosed herein and a pharmaceutically acceptable diluent or carrier. For the pharmaceutical compositions of the present invention, suitable and preferred microtubule inhibitors, subjects, monoclonal antibodies or antigen binding fragments thereof (including those comprising specific SEQ ID NOs), and diseases (including the characteristics of the diseases and various types of cancers) are as disclosed herein.

[0067] The pharmaceutical compositions according to the present invention may be in a unit dosage form comprising both agents, namely (a) the microtubule inhibitor, preferably paclitaxel (“(a) agent(s)”), and (b) a monoclonal antibody or antigen binding fragment thereof according to the present invention (“(b) agent”). In another aspect of this embodiment, the agent(s) identified in (a) above is in a first unit dosage form and the agent identified in (b) above is in a second unit dosage form, separate from the first.

[0068] The agents identified in (a) and (b) may be co-administered to the subject, either simultaneously or at different times and in any order, as deemed most appropriate by a physician. If the (a) and (b) agents are administered at different times, for example, by serial administration, the (a) agent(s) may be administered to the subject before the (b) agent. Alternatively, the (b) agent may be administered to the subject before the (a) agent(s).

[0069] Another embodiment of the present invention is a kit. The kit comprises any of the compositions or pharmaceutical compositions of the present invention packaged together with instructions for its use.

[0070] For use in the kits of the invention, compositions and pharmaceutical compositions comprising suitable and preferred microtubule inhibitors, subjects, monoclonal antibodies or antigen binding fragments thereof (including those comprising specific SEQ ID NOs), and diseases (including the characteristics of the diseases and various types of cancers) are as set forth above. The kits may also include suitable storage containers, *e.g.*, ampules, vials, tubes, etc., for each composition or pharmaceutical composition and other reagents, *e.g.*, buffers, balanced salt solutions, etc., for use in administering the compositions or the pharmaceutical compositions to subjects. The compositions or the pharmaceutical compositions and other reagents may be present in the kits in any convenient form, such as, *e.g.*, in a solution or in a powder form. The kits may further include a packaging container, optionally having one or more partitions for housing the composition or pharmaceutical composition and other optional reagents.

[0071] In the present invention, an "effective amount" or a "therapeutically effective amount" of an agent, monoclonal antibody, or fragment thereof or a compound or composition disclosed herein is an amount of such material that is sufficient to effect beneficial or desired results as described herein when administered to a subject. Effective dosage forms, modes of administration, and dosage amounts may be determined empirically, and making such determinations is within the skill of the art. It is understood by those skilled in the art that the dosage amount will vary with the route of administration, the rate of excretion, the duration of the treatment, the identity of any other drugs being administered, the age, size, and

species of mammal, *e.g.*, human patient, and like factors well known in the arts of medicine and veterinary medicine. In general, a suitable dose of any active agent disclosed herein or a composition containing the same will be that amount of the active agent or composition, which is the lowest dose effective to produce the desired effect. The effective dose of an agent, monoclonal antibody, or fragment thereof or a compound or composition of the present invention may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day.

[0072] A suitable, non-limiting example of a dosage of a microtubule inhibitor, a monoclonal antibody, or an antigen binding fragment disclosed herein is from about 1 mg/kg to about 2400 mg/kg per day, such as from about 1 mg/kg to about 1200 mg/kg per day, including from about 50 mg/kg to about 1200 mg/kg per day. Other representative dosages of such agents include about 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 400 mg/kg, 500 mg/kg, 600 mg/kg, 700 mg/kg, 800 mg/kg, 900 mg/kg, 1000 mg/kg, 1100 mg/kg, 1200 mg/kg, 1300 mg/kg, 1400 mg/kg, 1500 mg/kg, 1600 mg/kg, 1700 mg/kg, 1800 mg/kg, 1900 mg/kg, 2000 mg/kg, 2100 mg/kg, 2200 mg/kg, and 2300 mg/kg per day. The effective dose of microtubule inhibitors, monoclonal antibodies, and antibody fragments thereof disclosed herein may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day.

[0073] The compositions and pharmaceutical compositions of the present invention may be administered in any desired and effective manner: for oral ingestion, or as an ointment or drop for local administration to the eyes, or for

parenteral or other administration in any appropriate manner such as intraperitoneal, subcutaneous, topical, intradermal, inhalation, intrapulmonary, rectal, vaginal, sublingual, intramuscular, intravenous, intraarterial, intrathecal, or intralymphatic. Further, the compositions and pharmaceutical compositions of the present invention may be administered in conjunction with other treatments. Each composition and pharmaceutical composition of the present invention may be encapsulated or otherwise protected against gastric or other secretions, if desired.

[0074] The compositions and pharmaceutical compositions of the invention comprise one or more active ingredients in admixture with one or more pharmaceutically-acceptable diluents or carriers and, optionally, one or more other compounds, drugs, ingredients and/or materials. Regardless of the route of administration selected, the agents/compounds of the present invention are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art. See, *e.g.*, Remington, *The Science and Practice of Pharmacy* (21st Edition, Lippincott Williams and Wilkins, Philadelphia, PA.).

[0075] Pharmaceutically acceptable diluents or carriers are well known in the art (see, *e.g.*, Remington, *The Science and Practice of Pharmacy* (21st Edition, Lippincott Williams and Wilkins, Philadelphia, PA.) and *The National Formulary* (American Pharmaceutical Association, Washington, D.C.)) and include sugars (*e.g.*, lactose, sucrose, mannitol, and sorbitol), starches, cellulose preparations, calcium phosphates (*e.g.*, dicalcium phosphate, tricalcium phosphate and calcium hydrogen phosphate), sodium citrate, water, aqueous solutions (*e.g.*, saline, sodium chloride injection, Ringer's injection, dextrose injection, dextrose and sodium chloride injection, lactated Ringer's injection), alcohols (*e.g.*, ethyl alcohol, propyl alcohol, and benzyl alcohol), polyols (*e.g.*, glycerol, propylene glycol, and polyethylene glycol),

organic esters (*e.g.*, ethyl oleate and tryglycerides), biodegradable polymers (*e.g.*, polylactide-polyglycolide, poly(orthoesters), and poly(anhydrides)), elastomeric matrices, liposomes, microspheres, oils (*e.g.*, corn, germ, olive, castor, sesame, cottonseed, and groundnut), cocoa butter, waxes (*e.g.*, suppository waxes), paraffins, silicones, talc, silicylate, etc. Each pharmaceutically acceptable diluent or carrier used in a pharmaceutical composition of the invention must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Diluents or carriers suitable for a selected dosage form and intended route of administration are well known in the art, and acceptable diluents or carriers for a chosen dosage form and method of administration can be determined using ordinary skill in the art.

[0076] The compositions and pharmaceutical compositions of the invention may, optionally, contain additional ingredients and/or materials commonly used in pharmaceutical compositions. These ingredients and materials are well known in the art and include (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (2) binders, such as carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, hydroxypropylmethyl cellulose, sucrose and acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, sodium starch glycolate, cross-linked sodium carboxymethyl cellulose and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, and sodium lauryl sulfate; (10) suspending agents, such as ethoxylated

isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth; (11) buffering agents; (12) excipients, such as lactose, milk sugars, polyethylene glycols, animal and vegetable fats, oils, waxes, paraffins, cocoa butter, starches, tragacanth, cellulose derivatives, polyethylene glycol, silicones, bentonites, silicic acid, talc, salicylate, zinc oxide, aluminum hydroxide, calcium silicates, and polyamide powder; (13) inert diluents, such as water or other solvents; (14) preservatives; (15) surface-active agents; (16) dispersing agents; (17) control-release or absorption-delaying agents, such as hydroxypropylmethyl cellulose, other polymer matrices, biodegradable polymers, liposomes, microspheres, aluminum monostearate, gelatin, and waxes; (18) opacifying agents; (19) adjuvants; (20) wetting agents; (21) emulsifying and suspending agents; (22), solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan; (23) propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane; (24) antioxidants; (25) agents which render the formulation isotonic with the blood of the intended recipient, such as sugars and sodium chloride; (26) thickening agents; (27) coating materials, such as lecithin; and (28) sweetening, flavoring, coloring, perfuming and preservative agents. Each such ingredient or material must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Ingredients and materials suitable for a selected dosage form and intended route of administration are well known in the art, and acceptable ingredients and materials for a chosen

dosage form and method of administration may be determined using ordinary skill in the art.

[0077] Compositions and pharmaceutical compositions of the present invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, powders, granules, a solution or a suspension in an aqueous or non-aqueous liquid, an oil-in-water or water-in-oil liquid emulsion, an elixir or syrup, a pastille, a bolus, an electuary or a paste. These formulations may be prepared by methods known in the art, *e.g.*, by means of conventional pan-coating, mixing, granulation or lyophilization processes.

[0078] Solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules and the like) may be prepared, *e.g.*, by mixing the active ingredient(s) with one or more pharmaceutically-acceptable diluents or carriers and, optionally, one or more fillers, extenders, binders, humectants, disintegrating agents, solution retarding agents, absorption accelerators, wetting agents, absorbents, lubricants, and/or coloring agents. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using a suitable excipient. A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using a suitable binder, lubricant, inert diluent, preservative, disintegrant, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine. The tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein. They may be sterilized by, for example, filtration through a bacteria-retaining

filter. These compositions may also optionally contain opacifying agents and may be of a composition such that they release the active ingredient only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Any active ingredient of the invention can also be in microencapsulated form.

[0079] Liquid dosage forms for oral administration include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. The liquid dosage forms may contain suitable inert diluents commonly used in the art. Besides inert diluents, the oral compositions may also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents. Suspensions may contain suspending agents.

[0080] Compositions and pharmaceutical compositions of the present invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more active ingredient(s) with one or more suitable nonirritating diluents or carriers which are solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound. Compositions and pharmaceutical compositions of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such pharmaceutically-acceptable carriers as are known in the art to be appropriate.

[0081] Dosage forms for topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, drops and inhalants. The active agent(s)/compound(s) may be mixed under sterile conditions with a suitable pharmaceutically-acceptable diluent or carrier. The ointments, pastes, creams and gels may contain excipients. Powders and sprays may contain excipients and propellants.

[0082] Compositions and pharmaceutical compositions of the present invention suitable for parenteral administrations comprise one or more agent(s)/compound(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain suitable antioxidants, buffers, solutes which render the formulation isotonic with the blood of the intended recipient, or suspending or thickening agents. Proper fluidity can be maintained, for example, by the use of coating materials, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. These compositions may also contain suitable adjuvants, such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption.

[0083] In some cases, in order to prolong the effect of a drug (*e.g.*, a composition or a pharmaceutical composition of the present invention), it is desirable to slow its absorption from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility.

[0084] The rate of absorption of the active agent/composition of the invention then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered agent/composition of the invention may be accomplished by dissolving or suspending the active agent/composition in an oil vehicle. Injectable depot forms may be made by forming microencapsule matrices of the active ingredient in

biodegradable polymers. Depending on the ratio of the active ingredient to polymer, and the nature of the particular polymer employed, the rate of active ingredient release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue. The injectable materials can be sterilized for example, by filtration through a bacterial-retaining filter.

[0085] Any formulation of the invention may be presented in unit-dose or multi-dose sealed containers, for example, ampules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid diluent or carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

Additional Definitions

[0086] As used herein, terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers, those containing modified residues, and non-naturally occurring amino acid polymers.

[0087] The term "amino acid" means naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function similarly to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. An "amino

acid analog” means compounds that have the same basic chemical structure as a naturally occurring amino acid, *e.g.*, a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs may have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. An “amino acid mimetic” means a chemical compound that has a structure that is different from the general chemical structure of an amino acid, but that functions similarly to a naturally occurring amino acid.

[0088] “Nucleic acid” or “oligonucleotide” or “polynucleotide” used herein mean at least two nucleotides covalently linked together. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof.

[0089] Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequences. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be synthesized as a single stranded molecule or expressed in a cell (*in vitro* or *in vivo*) using a synthetic gene. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

[0090] The nucleic acid may also be a RNA such as a mRNA, tRNA, short hairpin RNA (shRNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), transcriptional gene silencing RNA (ptgsRNA), Piwi-interacting RNA, pri-

miRNA, pre-miRNA, micro-RNA (miRNA), or anti-miRNA, as described, *e.g.*, in U.S. Patent Application Nos. 11/429,720, 11/384,049, 11/418,870, and 11/429,720 and Published International Application Nos. WO 2005/116250 and WO 2006/126040.

[0091] The nucleic acid may also be an aptamer, an intramer, or a spiegelmer. The term “aptamer” refers to a nucleic acid or oligonucleotide molecule that binds to a specific molecular target. Aptamers are derived from an *in vitro* evolutionary process (*e.g.*, SELEX (Systematic Evolution of Ligands by EXponential Enrichment), disclosed in U.S. Pat. No. 5,270,163), which selects for target-specific aptamer sequences from large combinatorial libraries. Aptamer compositions may be double-stranded or single-stranded, and may include deoxyribonucleotides, ribonucleotides, nucleotide derivatives, or other nucleotide-like molecules. The nucleotide components of an aptamer may have modified sugar groups (*e.g.*, the 2'-OH group of a ribonucleotide may be replaced by 2'-F or 2'-NH₂), which may improve a desired property, *e.g.*, resistance to nucleases or longer lifetime in blood. Aptamers may be conjugated to other molecules, *e.g.*, a high molecular weight carrier to slow clearance of the aptamer from the circulatory system. Aptamers may be specifically cross-linked to their cognate ligands, *e.g.*, by photo-activation of a cross-linker (Brody, E. N. and L. Gold (2000) *J. Biotechnol.* 74:5-13).

[0092] The term “intramer” refers to an aptamer which is expressed *in vivo*. For example, a vaccinia virus-based RNA expression system has been used to express specific RNA aptamers at high levels in the cytoplasm of leukocytes (Blind, M. *et al.* (1999) *Proc. Natl. Acad. Sci. USA* 96:3606-3610).

[0093] The term “spiegelmer” refers to an aptamer which includes L-DNA, L-RNA, or other left-handed nucleotide derivatives or nucleotide-like molecules. Aptamers containing left-handed nucleotides are resistant to degradation by naturally

occurring enzymes, which normally act on substrates containing right-handed nucleotides.

[0094] A nucleic acid will generally contain phosphodiester bonds, although nucleic acid analogs may be included that may have at least one different linkage, e.g., phosphoramidate, phosphorothioate, phosphorodithioate, or O-methylphosphoramidite linkages and peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with positive backbones; non-ionic backbones, and non-ribose backbones, including those disclosed in U.S. Pat. Nos. 5,235,033 and 5,034,506. Nucleic acids containing one or more non-naturally occurring or modified nucleotides are also included within the definition of nucleic acid. The modified nucleotide analog may be located for example at the 5'-end and/or the 3'-end of the nucleic acid molecule. Representative examples of nucleotide analogs may be selected from sugar- or backbone-modified ribonucleotides. It should be noted, however, that also nucleobase-modified ribonucleotides, *i.e.* ribonucleotides, containing a non-naturally occurring nucleobase instead of a naturally occurring nucleobase such as uridines or cytidines modified at the 5-position, e.g. 5-(2-amino)propyl uridine, 5-bromo uridine; adenosines and guanosines modified at the 8-position, e.g. 8-bromo guanosine; deaza nucleotides, e.g. 7-deaza-adenosine; O- and N-alkylated nucleotides, e.g. N6-methyl adenosine are suitable. The 2'-OH-group may be replaced by a group selected from H, OR, R, halo, SH, SR, NH₂, NHR, NR₂ or CN, wherein R is C₁-C₆ alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I. Modified nucleotides also include nucleotides conjugated with cholesterol through, e.g., a hydroxyprolinol linkage as disclosed in Krutzfeldt *et al.*, Nature (Oct. 30, 2005), Soutschek *et al.*, Nature 432:173-178 (2004), and U.S. Patent Application Publication No. 20050107325. Modified nucleotides and nucleic

acids may also include locked nucleic acids (LNA), as disclosed in U.S. Patent Application Publication No. 20020115080. Additional modified nucleotides and nucleic acids are disclosed in U.S. Patent Application Publication No. 20050182005. Modifications of the ribose-phosphate backbone may be done for a variety of reasons, *e.g.*, to increase the stability and half-life of such molecules in physiological environments, to enhance diffusion across cell membranes, or as probes on a biochip. Mixtures of naturally occurring nucleic acids and analogs may be made; alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made.

[0095] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise.

[0096] For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0097] The following examples are provided to further illustrate the methods of the present invention. These examples are illustrative only and are not intended to limit the scope of the invention in any way.

EXAMPLES**Example 1****Materials and Methods****Mice**

[0098] Unless other wise noted, female immunodeficient athymic nude mice (CrI:NU(Ncr)-Foxn/nu, Charles River) were used at nine weeks old with a body weight (BW) range of about 15 to about 30 grams on Day 1 of the study.

In Vivo Implantation and Tumor Growth

[0099] Tumor cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/mL penicillin G sodium, 100 µg/mL streptomycin sulfate, and 25 µg/mL gentamicin. The tumor cells were grown in tissue culture flasks in a humidified incubator at 37°C, in an atmosphere of 5% CO₂ and 95% air.

[0100] On the day of tumor implant, each test mouse was injected with cultured tumor cells, and tumor growth was monitored as the average size approached the target range. Tumors were measured in two dimensions using calipers, and volume was calculated using the formula:

$$\text{Tumor Volume (mm}^3\text{)} = (w^2 \times l) / 2$$

where w = width and l = length, in mm, of the tumor. Tumor weight was estimated with the assumption that 1 mg is equivalent to 1 mm³ of tumor volume. For all therapeutic studies, mice were sorted into groups containing the same average size tumors prior to initiation of therapy.

Controls

[0101] One group in each cohort received 1% vehicle IP three times per week to end, and served as the control group for calculation of % tumor growth delay (TGD).

Endpoint and TGD Analysis

[0102] Tumors were measured using calipers twice per week, and each animal was euthanized when its tumor reached the pre-determined tumor volume endpoint described for each experiment (usually 2000 mm³) or on the final day, whichever came first. Animals that exited the study for tumor volume endpoint were documented as euthanized for tumor progression (TP), with the date of euthanasia. The time to endpoint (TTE) for analysis was calculated for each mouse by the following equation:

$$\text{TTE} = [\log_{10}(\text{endpoint volume}) - b] / m$$

where TTE is expressed in days, endpoint volume is expressed in mm³, b is the intercept, and m is the slope of the line obtained by linear regression of a log-transformed tumor growth data set. The data set consisted of the first observation that exceeded the endpoint volume used in analysis and the three consecutive observations that immediately preceded the attainment of this endpoint volume. The calculated TTE was usually less than the TP date, the day on which the animal was euthanized for determination of tumor size. Animals with tumors that did not reach the endpoint volume were assigned a TTE value equal to the last day of the study. Any animal classified as having died from NTR (non-treatment-related) causes due to accident (NTRa) or due to unknown etiology (NTRu) were excluded from TTE calculations and all further analyses. Animals classified as TR (treatment-related)

deaths or NTRm (non-treatment-related death due to metastasis) were assigned a TTE value equal to the day of death.

[0103] Treatment outcome was evaluated from TGD, defined as the increase in the median TTE in a treatment group compared to the control group:

$$\text{TGD} = T - C,$$

expressed in days, or as a percentage of the median TTE of the control group:

$$\% \text{TGD} = [(T - C) / C] \times 100$$

where:

T = median TTE for a treatment group, and

C = median TTE for the designated control group.

Criteria for Regression Responses

[0104] Treatment efficacy was determined from the incidence and magnitude of regression responses observed during the study. Treatment may cause partial regression (PR) or complete regression (CR) of the tumor in an animal. In a PR response, the tumor volume was 50% or less of its Day 1 volume for three consecutive measurements during the course of the study, and equal to or greater than 13.5 mm³ for one or more of these three measurements. In a CR response, the tumor volume was less than 13.5 mm³ for three consecutive measurements during the course of the study. An animal with a CR response at the termination of the study was additionally classified as a tumor-free survivor (TFS). Animals were monitored for regression responses.

Toxicity

[0105] Animals were weighed daily on Days 1-5, then twice per week until completion of the study. The mice were observed frequently for overt signs of any adverse, treatment related side effects, and clinical signs were recorded when observed. Individual body weight (BW) loss was monitored, and any animal whose weight exceeded the 20% limits for acceptable BW loss was euthanized. Group mean BW loss also was monitored as per protocol. Dosing was suspended in any group that exceeded the 20% limits for acceptable mean BW loss. If mean BW recovered, then dosing was resumed in that group, but at a lower dosage or less frequent dosing schedule. Acceptable toxicity for the maximum tolerated dose (MTD) was defined as a group mean BW loss of less than 20% during the study and not more than 10% TR deaths. A death was classified as TR if attributable to treatment side effects as evidenced by clinical signs and/or necropsy, or was also classified as TR if due to unknown causes during the dosing period or within 14 days of the last dose. A death was classified as NTR if there was no evidence that death is related to treatment side effects.

Statistical and Graphical Analyses

[0106] Prism (GraphPad) for Windows 3.03 was used for statistical analyses. The logrank test, which evaluates overall survival experience, was used to analyze significance of the differences between the TTE values of two groups. Logrank analysis includes data for all animals in a group except those assessed as NTR deaths. Two-tailed statistical analyses were conducted at significance level $P = 0.05$. The statistical tests were not adjusted for multiple comparisons. Test results are identified as not significant "ns" at $P > 0.05$, significant (symbolized by "**") at $0.01 < P < 0.05$, very significant ("**") at $0.001 < P \leq 0.01$, and extremely significant ("****") at $P \leq 0.001$. Groups with regimens above the MTD were not evaluated statistically.

Example 2**Efficacy of TEM8 antibodies in the HCT116 colorectal carcinoma xenograft model**

[0107] 2.5×10^6 to 5×10^6 HCT116 tumor cells were injected subcutaneously into the flank of mice. Tumor weights were calculated following surgical removal. Treatment was initiated when tumor volume reached 60-80 mm³. Treatment groups are shown in Table 3. Dosing volume was 10 mL/kg (0.2 mL / 20 g mouse).

Table 3 - Protocol Design for the HCT116-e401 Study

Group	n	Treatment Regimen				
		Agent	Vehicle	mg/kg	Route	Schedule
1 [#]	15	Vehicle	Histidine Buffer	-	IP	3x / wk x 3
2	15	M825	Histidine Buffer	5	IP	3x / wk x 3
3	15	M825	Histidine Buffer	15	IP	3x / wk x 3
4	15	M822	Histidine Buffer	5	IP	3x / wk x 3
5	15	M822	Histidine Buffer	15	IP	3x / wk x 3
6	15	M863	Histidine Buffer	5	IP	3x / wk x 3
7	15	M863	Histidine Buffer	15	IP	3x / wk x 3
8	15	M830	Histidine Buffer	5	IP	3x / wk x 3
9	15	M830	Histidine Buffer	15	IP	3x / wk x 3
10	10	Bevacizumab	Saline	5	IP	2x / wk x 3

- Control group, IP – intraperitoneal

[0108] FIG. 1A shows the group median tumor volume distribution on day 21 of this study. TEM8 Ab (hlgG1) doses were 5 and 15 mg/kg, three times per week, for 3 weeks. Bevacizumab was dosed at 5 mg/kg, twice per week, for 3 weeks. FIG. 1B and FIG. 1D show mean tumor growth and percent body weight change for each group, respectively, over the time course of the study. FIG. 1C shows the individual times to endpoint for each mouse in the study. The results show that anti-

TEM8 antibodies according to the present invention were effective against HCT116 cancer cells in the HCT116 xenograft model.

Example 3

Comparison of TEM8 antibody efficacy in UACC melanoma model

[0109] UACC melanoma cells were cultured and injected subcutaneously into mice. Treatment was initiated with a tumor volume of 50 mm³, and treatment groups consisted of one of four TEM8 antibodies (M825, M830, M863, and M822), each dosed at 15 mg/kg, 3 times per week for 3-4 weeks. FIG. 2 shows a time course of tumor growth. The results show that TEM 8 antibodies, M830 in particular, were effective at slowing tumor growth in the UACC melanoma model.

Example 4

Efficacy of M825 in xenograft cancer models

HCT116 colon cancer model

[0110] Treatment was initiated when tumor volume reached approximately 100 mm³. M825 antibody was given i.v. at a dose of 10 or 30 mg/kg, and the antibody-drug conjugate (ADC) M825-MMAE was given i.v. at 1, 3, 10, or 30 mg/kg, 2 times per week for 3 weeks. FIG. 3 shows tumor growth over the time course of the study. Results showed that the ADC significantly inhibited tumor growth at 10 and 30 mg/kg in the HCT116 model, even though it showed weak cytotoxicity towards HCT116 cancer cells in vitro (data not shown).

OVCAR3 ovarian cancer model

[0111] Treatment initiated when tumor volume reached approximately 120 mm³. M825 was given i.v. at 10 mg/kg and M825-MMAE was given i.v. at 1, 3, or 10 mg/kg, 2 times per week for 3.5 weeks. The ADC significantly inhibited tumor growth

at 3 mg/kg and regressed tumor growth at 10 mg/kg in this model (FIG. 4). The 10 mg/kg ADC group was redosed on Day 56, 2 times per week. No effect was seen with free MMAE at 0.2 mg/kg, a dose equivalent to the maximum amount of MMAE given in the 10 mg/kg ADC group.

MDA-MB231 triple negative breast cancer model

[0112] Treatment was initiated when tumor volume reached approximately 100 mm³. M825 antibody was given i.v. at a dose of 10 mg/kg, and the antibody-drug conjugate (ADC) M825-MMAE was given i.v. at 3 or 10 mg/kg, 2 times per week for 3 weeks. The anti-TEM8 ADC treatment led to durable tumor stasis (3 mg/kg) or complete regression (10 mg/kg) in this model, even though it showed only very weak cytotoxicity toward MDA-MB231 cancer cells in vitro (data not shown). 50% of animals in the 10 mg/kg ADC group were tumor free on Day 35 (FIG. 5).

Example 5

Efficacy of M830 in the H460 non-small cell lung carcinoma xenograft model

[0113] Cultured H460 cells were harvested during exponential growth and resuspended in phosphate buffered saline at a concentration of 1×10^8 cells/mL. 1.0×10^7 tumor cells were injected subcutaneously into the flank of athymic nude mice. Treatment groups are listed in Table 4. Animals were euthanized when tumors reached the pre-determined volume endpoint of 2000 mm³ or on the final day (Day 88), whichever came first. All regimens were well tolerated, with no treatment related deaths, no noteworthy clinical signs, and acceptable mean body weight losses. Each treatment produced a significant survival difference versus controls ($P < 0.01$). The median TTE for controls was 22.8 days, establishing a maximum possible TGD of 65.2 days (286%) for the 88-day study.

[0114] Both combination therapies were statistically superior to the corresponding monotherapies ($P < 0.05$) but M830 in combination with paclitaxel was the most active treatment regimen tested in the study. Group 7 produced the maximum TGD and 90% regression responses, two PRs and seven CRs, three of which remained TFS at study end. The M830/bevacizumab combination produced TGD of 33.2 days (146%) and 40% regression responses which consisted of 1 PR and 3 CRs, all three of which remained TFS at study end.

Table 4

Group	n	Regimen 1				
		Agent	Vehicle	mg/kg	Route	Schedule
1 [#]	10	Vehicle	PBS	-	IP	3x/wk x 5
2	10	M830	PBS	5	IP	3x/wk x 5
3	10	M830	PBS	15	IP	3x/wk x 5
4	10	Bevacizumab	Saline	5	IP	biwk x 5 /6/ biwk x 4
5	10	M830	PBS	15	IP	3x/wk x 5 /5/ 3x/week x 4
		Bevacizumab	Saline	5	IP	biwk x 5 /6/ biwk x 4
6	10	Paclitaxel	5% EC	30	IV	qod x 5 /29/ qod x 5
7	10	M830	PBS	15	IP	3x/wk x 5 /5/ 3x/week x 4
		Paclitaxel	5% EC	30	IV	qod x 5 /29/ qod x 5

- Control group, EC – 5% ethanol : 5% Cremophor EL in D5W, IP – intraperitoneal, IV – intravenous, qod – every other day

[0115] FIG. 6A shows a time course of mean tumor growth throughout the study. FIG. 6B shows a Kaplan-Meier survival plot and FIG. 6C show individual times to the endpoint for mice in each group. FIG. 6D shows percent body weight change for each group throughout the study. The results show that a combination of M830 and paclitaxel is effective against H460 cancer cells in the xenograft model, and that the combination is more effective than either agent alone.

Example 6**Efficacy of M830 alone and in combination with doxorubicin or paclitaxel in
MDA-MB231 human breast carcinoma orthotopic xenograft model**

[0116] On the day of tumor implant, each test mouse was injected subcutaneously in the mammary fat pad with 5×10^6 cells (0.1 mL cell suspension). Treatment began on Day 1 in seven groups of mice (n=10) with established orthotopic MDA-MB231 tumors (75–126 mm³). The study was designed to evaluate M830 administered i.p. at 5 or 15 mg/kg three times weekly for five weeks alone and in combination with 5 mg/kg dosage of doxorubicin administered i.v. once weekly for four weeks or paclitaxel administered i.v. at 10 mg/kg once weekly for four weeks. The study included the corresponding doxorubicin and paclitaxel monotherapy groups, as well as a vehicle treated tumor growth control group. After the original planned dosing was completed, a second cycle of treatment for all remaining groups (Groups 4, 5, 6 and 7) was initiated on Day 42. The paclitaxel dosage was lowered to 5 mg/kg for the second cycle. Tumors were measured twice per week until the study was ended on Day 79. The endpoint was a tumor volume of 1500 mm³ or the final day, whichever came first. All regimens were well tolerated, with no treatment related deaths, and acceptable mean body weight losses (FIG. 7D). The median TTE for controls was 32.8 days, establishing a maximum possible TGD of 46.2 days (141%) for the 79-day study.

[0117] M830 alone at either 5 or 15 mg/kg did not produce statistically significant survival benefit compared to controls ($P > 0.05$, FIGS. 7B-C). Doxorubicin and paclitaxel monotherapies and both combination therapies resulted in statistically significant survival extensions compared to control. The M830/doxorubicin combination was also statistically superior to the corresponding monotherapies ($P <$

0.01) and was the most active treatment regimen tested in the study with maximum TGD (46.2 days, 141%) and 8 out of 10 79-day survivors. M830 in combination with paclitaxel was statistically superior to M830 alone ($P < 0.001$) but not to the paclitaxel monotherapy ($P > 0.05$) although tumor volume plots indicated improvement over the response to paclitaxel alone (FIG. 7A). The M830/paclitaxel combination produced TGD of 42.4 days (129%).

Example 7

Efficacy of M830 alone and in combination with bevacizumab or irinotecan in the DLD-1 colorectal adenocarcinoma xenograft model

[0118] On the day of tumor implant, each test mouse was injected subcutaneously into the flank with 2.5×10^6 to 5×10^6 cultured DLD-1 tumor cells. Tumor weights were calculated following surgical removal. M830 was administered i.p. at 5 or 15 mg/kg, three times per week, typically on a Monday, Wednesday, and Friday schedule. Irinotecan was given once per week at 60 mg/kg i.v. and bevacizumab was dosed at 5 mg/kg i.p., three times per week.

[0119] FIG. 8 shows a time course of tumor growth throughout the study. The results show that the combinations of M830/bevacizumab and M830/irinotecan were effective against DLD-1 cancer cells in the xenograft model, and that the combinations were more effective than any agent alone.

[0120] In a second study, treatment was initiated with a large tumor volume of approximately 170 mm^3 . M830 alone or in combinations with bevacizumab, irinotecan, or both, was given as a loading dose of 20 mg/kg followed by 5 mg/kg three times per week. The doses of bevacizumab and irinotecan were the same as

in the first study. An additional group received a combination of bevacizumab and irinotecan without the M830 antibody.

[0121] FIG. 9 shows a time course of tumor growth throughout the second study. The results show that all combinations of M830, bevacizumab, and irinotecan were effective against DLD-1 cancer cells in the xenograft model, although the triple combination did not outperform the M830/bevacizumab combination therapy.

[0122] The study is repeated with one group in each cohort receiving irinotecan at 50 mg/kg once per week i.v. One group in each of the two cohorts receives a combination of 5 mg/kg M830 three times a week with Bevacizumab IP at 5 mg/kg twice per week, fluoropyrimidine at 50 mg/kg once per week intravenously, and irinotecan at 50 mg/kg once per week intravenously. Another group in each of the two cohorts receives 15 mg/kg M830 three times a week with Bevacizumab IP at 5 mg/kg twice per week, fluoropyrimidine at 50 mg/kg once per week intravenously, and irinotecan at 50 mg/kg once per week intravenously.

[0123] It is expected that the combination of M830, bevacizumab, and fluoropyrimidine-irinotecan will be effective against cancer cells in the xenograft models and that the combination will be more effective, preferably synergistic compared to any agent alone. The study will be repeated using each of M822, M825, and M863 replacing M830, together with bevacizumab and fluoropyrimidine-irinotecan or other therapeutic agents. It is expected that the combination of M822, M825, or M863 with bevacizumab and fluoropyrimidine-irinotecan or other therapeutic agents will be effective against cancer cells in the xenograft models and that each such combination will be more effective, preferably synergistic, compared to any such agent alone.

Example 8**Comparison of M830 antibody efficacy in xenograft models**

[0124] Percent tumor reduction and percent tumor reduction vs. combination partner for each xenograft model treated with M830 alone or in combination with other chemotherapeutics is shown in Table 5.

Table 5 – M830 TEM8 Antibody Efficacies in Xenograft Models

Antibody	Cancer Model	Combination Partner	% Tumor Reduction vs. Vehicle at Day 21*	% Tumor Reduction vs. combination partner at Day (#)
M830	H460 Lung	Paclitaxel	> 100% (-61% regression)	94% (32)
M830	H460 Lung	Bevacizumab	94.5%	58.5% (32)
M830	H460 Lung	N/A	62%	N/A
M830	DLD1 Colon	Irinotecan	86%	53% (21)
M830	DLD1 Colon	Bevacizumab	86%	48% (21)
M830	DLD1 Colon	N/A	68%	N/A
M830	UACC Melanoma	N/A	65%	N/A
M830	HCT116 Colon	N/A	39.5%	N/A

* - Treatment initiated with tumor volume of 60-80 mm³ for H460 and HCT116 studies or about 50 mm³ for DLD1 and UACC studies; M830 dosed IP, 3 times per week

Example 9**Efficacy of M830 in MC38 colon carcinoma liver metastasis model**

[0125] C57BL/6 mice were challenged with MC38 colon carcinoma liver metastasis by intrasplenic injections of cultured cells. FIG. 10 shows that M830 significantly improved the survival of mice in this model (P = 0.0001), with a median survival rate of 17 days versus 11 days for vehicle treated mice.

Example 10

Efficacy of TEM8 antibodies in combination with molecularly targeted cancer pathway inhibitors

[0126] Athymic nude mice are used. On the day of tumor implant, each test mouse is injected subcutaneously in the right flank with 2.5×10^6 to 5×10^6 DLD-1 cells (cohort 1), or 1×10^7 cells H460 cells (cohort 2), and tumor growth is monitored as the average size approaches the target range of 100 to 150 mm³. The endpoint of the study is a tumor volume of 2000 mm³ or the last day of the study, whichever comes first.

Monotherapy Treatments

[0127] Two groups in each cohort receive M830 at 5 and 15 mg/kg three times a week. Two groups in each cohort receive the COX2 inhibitor celecoxib at 25 and 75 mg/kg daily. (Park et al., 2008). One group in each cohort receives the non-steroidal anti-inflammatory (NSAID) ibuprofen in chow at 1360 mg/kg. (Yao et al., 2005). One group in each cohort receives the prostaglandin E₂ (PGE₂) synthase inhibitor sulindac sulphide orally at 20 mg/kg per day. (Mahmoud et al., 1998).

Combination Treatments

[0128] One group in each of the two cohorts receives a combination of 5 mg/kg M830 three times a week with celecoxib orally at 25 mg/kg daily. Another group in each of the two cohorts receives a combination of 5 mg/kg M830 three times a week with celecoxib orally at 75 mg/kg daily. A further group in each of the two cohorts receives a combination of 15 mg/kg M830 three times a week with celecoxib orally at 25 mg/kg daily. An additional group in each of the two cohorts receives a combination of 15 mg/kg M830 three times a week with celecoxib orally at 75 mg/kg daily.

[0129] One group in each of the two cohorts receives a combination of 5 mg/kg M830 three times a week with ibuprofen in chow at 1360 mg/kg. Another group in each of the two cohorts receives 15 mg/kg M830 three times a week with ibuprofen in chow at 1360 mg/kg.

[0130] One group in each of the two cohorts receives a combination of 5 mg/kg M830 three times a week with sulindac sulphide orally at 20 mg/kg per day. Another group in each of the two cohorts receives 15 mg/kg M830 three times a week with sulindac sulphide orally at 20 mg/kg per day.

Results

[0131] It is expected that the combination of M830 and celecoxib, M830 and ibuprofen, and M830 and sulindac sulphide will be effective against cancer cells and/or tumor associated stroma and/or tumor vasculature in the xenograft models and that the combination will be more effective, preferably synergistic, compared to any agent alone. The study will be repeated using each of M822, M825, and M863 replacing M830, together with celecoxib or other COX-2 inhibitors, ibuprofen or other NSAIDs, and sulindac sulphide or other PGE₂ synthase inhibitors. It is expected that these combinations will also be effective against cancer cells and/or tumor associated stroma and/or tumor vasculature in the xenograft models and that each such combination will be more effective, preferably synergistic, compared to any such agent alone.

Example 11

Affinity and pharmacokinetics of TEM8 antibodies

[0132] Table 6 shows Biocore affinity measurements of anti-TEM8 fragment antigen-binding (Fabs).

Table 6: Binding affinities of TEM8 antibodies

	M830			M825		
	ka (1/Ms)	kd (1/s)	K _D (M)	ka (1/Ms)	kd (1/s)	K _D (M)
mTEM8-Fc	7.749E+5	0.002901	3.744E-9	1.700E+6	8.942E-5	5.261E-11
hTEM8-AP	6.071E+5	9.533E-4	1.570E-9	3.050E+6	1.802E-4	5.909E-11

[0133] Table 7 shows an in silico assessment of TEM8 antibodies. M830 and M863 have more basic V domains, M825 and M822 have more acidic V domains. The inherent polarity between V and C domains may pose downstream purification challenges. Sequence analysis identified no potential glycosylation sites in any CDRs, eliminating one of the biggest risks for development.

Table 7: pI values of TEM8 antibodies

Antibodies	pI calculated based on variable domains only	pI calculated based on IgG1
M822	5.39	7.91
M825	5.47	7.91
M830	10.17	8.75
M863	10.11	8.68

[0134] Tables 8 and 9 show pharmacokinetic properties of TEM8 antibodies according to the present invention in mouse for single dose (Table 8) and multiple dose (Table 9) treatments.

Table 8: Single dose summary

Group	Treatment	C _{MAX} (ng/mL)	T _{MAX} (hr)	AUCI (hr*ng/mL)	AUCT (hr*ng/mL)	HALF (hr)	CLF (mL/hr/kg)	VZF (mL/kg)	KEL (1/hr)
2	M825 5 mg/kg	46800	3	1310519	1310222	6.03	3.82	33.19	0.1149
3	M825 15 mg/kg	144000	3	9239202	8991767	23.77	1.62	55.66	0.0292
4	M822 5 mg/kg	42100	3	1611073	1610845	5.75	3.10	25.73	0.1206
5	M822 15 mg/kg	182000	24	13523442	13522827	10.50	1.11	16.80	0.0660
6	M863 5 mg/kg	51300	3	1330847	1330742	8.59	3.76	46.56	0.0807
7	M863 15 mg/kg	355000	3	10721535	10721535				
8	M830 5 mg/kg	67500	3	2242485	2242286	8.54	2.23	27.48	0.0811
9	M830 15 mg/kg	203000	3	10692742	10692598	14.94	1.40	30.24	0.0464

Table 9: Multiple dose summary

Group	Treatment	C _{MAX} (ng/mL)	T _{MAX} (hr)	AUCI (hr*ng/mL)	AUCT (hr*ng/mL)	HALF (hr)	CLF (mL/hr/kg)	VZF (mL/kg)	KEL (1/hr)
2	M825 5 mg/kg	54300	3	1408345	1408201	9.10	3.55	46.61	0.0762
3	M825 15 mg/kg	314000	3	21649446	21648879	17.05	0.69	17.05	0.0406
4	M822 5 mg/kg	88000	24	4550095	4550095				
5	M822 15 mg/kg	632000	3	68241171	66575247	54.57	0.22	17.31	0.0127
6	M863 5 mg/kg	58600	3	1425735	1425565	14.29	3.51	72.28	0.0485
7	M863 15 mg/kg	147000	3	7272672	7271700	12.64	2.06	37.60	0.0549
8	M830 5 mg/kg	86400	3	4316790	4315918	22.88	1.16	38.24	0.0303
9	M830 15 mg/kg	330000	3	23053875	23052853	18.70	0.65	17.55	0.0371

Example 12

M825-MMAE in vitro and non-GLP toxicology studies

3T3 fibroblasts

[0135] 3T3 cells were cultured and contractility in the presence of TGFb-1 was measured in control or M825 exposed cells. M825 was shown to block the contractility of activated 3T3 fibroblasts in vitro (FIG. 11A-B). Contractile forces are thought to contribute to increased cancer cell invasion.

Toxicology

[0136] In a single rising dose phase study design, female (N=3) and male (N=3) rats were given the ADC at intervals of 3 or more days and were monitored for 2 weeks. Clinical observations, body weight, and necropsy results were looked at, as well as the clinical pathology and histopathology for any unscheduled sacrifices. The first dose was 20 mg/kg. This dose was considered toxic as 1 out of 6 rats were found dead, 2 with erected fur, and 3 with BW loss. There were dark foci in many organs, discoloration, platelet decrease, impaired liver and kidney functions, and skin and testis findings. Part of the findings may have been due to toxicity of MMAE. The next dose was 40 mg/kg, which was considered lethal as all rats were found dead or moribund by Day 5. 15 and 10 mg/kg were chosen for the multiple dose phase study.

[0137] In the multiple dose phase study design, 5 male and 5 female rats for each cohort were given 3 weekly doses at Days 1, 8, and 15, and sacrificed on Day 18. A vehicle control arm was added. In the 15 mg/kg

group, 1 out of 10 animals was found dead (Day 15), 1 moribund (Day 17), 2 had decreased activity (Day 15), 2 had erected fur (Day 15), and 3 had dark foci in the lungs. In the 10 mg/kg group, 1 out of 10 was found dead (Day 16), 2 had decreased activity (Day 15), 2 had erected fur (Day 15), and 1 had dark loci in the lungs. Both groups also had discoloration, skin, thymus, pituitary gland, and lymph node findings, and reduced red blood cell, hemoglobin, and platelet counts.

Example 13

M830 antibody-dependent cell-mediated cytotoxicity (ADCC)

[0138] Fc mutations which increase ADCC are known in the art. For example, substitutions in the IgG constant region such as S239D, A330L, and I332E are known to increase binding to the FC γ receptor. (see, e.g., Lazar *et al.*, *Proc. Natl., Acad. Sci. U.S.A.*, 103:4005-4010, 2006). In the present example, the M830 Fc S239D:I332E double mutant was generated and confirmed by standard molecular biology techniques (830M: S239D/I332E). FIG. 12A is a schematic showing the antibody binding in ADCC and FIG. 12B demonstrates that the Fc mutant form of M830 showed significantly enhanced binding to Fc γ RIIIa (CD16a).

DOCUMENTS

HE, S., *et al.* (2013). Discovery of highly potent microsomal prostaglandin e2 synthase 1 inhibitors using the active conformation structural model and virtual screen. *J Med Chem* 56(8): 3296-309.

ST CROIX, B., *et al.* (2000). Genes expressed in human tumor endothelium. *Science* 289(5482): 1197-1202.

YAMORI, T., *et al.* (1997). Anti-tumor efficacy of paclitaxel against human lung cancer xenografts. *Japan Journal of Cancer Research* 88: 1205-1210.

[0139] All documents cited in this application are hereby incorporated by reference as if recited in full herein.

[0140] Although illustrative embodiments of the present invention have been described herein, it should be understood that the invention is not limited to those described, and that various other changes or modifications may be made by one skilled in the art without departing from the scope or spirit of the invention.

WHAT IS CLAIMED IS:

1. A method for treating or ameliorating the effects of a disease in a subject comprising:

administering to a subject in need thereof

(a) a therapeutically effective amount of a microtubule inhibitor; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region (VH), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7; and

(ii) a light chain variable region (VL), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

2. The method according to claim 1, wherein the subject is a mammal.

3. The method according to claim 2, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

4. The method according to claim 2, wherein the mammal is a human.

5. The method according to claim 1, wherein the disease is characterized by differential expression of tumor endothelial marker 8 (TEM8) membrane antigen.

6. The method according to claim 1, wherein the disease is characterized by differential expression of tumor endothelial marker 8 (TEM8) membrane antigen on a tumor cell and/or a tumor stromal cell.

7. The method according to claim 1, wherein the disease is a cancer that differentially expresses TEM8.

8. The method according to claim 7, wherein the cancer is selected from the group consisting of kidney cancer, colon cancer, lung cancer, liposarcomas, brain cancer, breast cancer, melanoma, liver cancer, head and neck cancer, and prostate cancer.

9. The method according to claim 1, wherein the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

```
caggtccagctggtgcagctctggggctgaggtgaagaagcct  
gggacctcagtggaaggtctcctgcaaggttcctggatacacc  
ttcagcagctatgctatcagctgggtgacagggcccctgga  
caagggcttgagtggatgggagggatcatccctatctttggt  
acaacaaactacgcacagaagttccagggcagagtcacgatt
```

accggggaggaatccacgagcacagtctacatggagctgagc
 agcctgagatctgaggacacggccgtgtattactgtgcgaga
 gatacggactacatgtttgactactggggccagggaaaccctg
 gtcaccgtgagctca (SEQ ID NO: 11)

and

(2) a VL polypeptide encoded by:

tcttctgagctgactcaggaccctgttgtgtctgtggccttg
 ggagagacagtcagtatcacatgccaaggagacaacctcaga
 gacttttatgcaagctggtaccaacagaagccaggacaggcc
 cctctactagtcatgtatggtaaaaacaggcggccctcaggg
 atcccagaccgattctctggctccacctcaggaaacacactt
 tccttgaccatcactggggctcaggcgggaagatgaggctgac
 tattactgtagctcccgggacaacagtaagcatgtggtgttc
 ggcggggggaccaaggctcaccgtccta (SEQ ID
 NO:12) .

10. The method according to claim 1, wherein the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

cagggtccagctggtgcagctctggggctgaggtgaagaagcct
 ggggcctcagtgaaggtctcctgcaaggtttctggatacacc
 ttcagcagctatgctatcagctgggtgacagggcccctgga
 caagggttgagtggatgggagggatcatccctatctttggt
 acagcaaactacgcacagaagtccagggcagagtcacgatt
 accgcggacgaatccacgagcacagcctacatggagctgagc
 agcctgagatctgaggacacggccgtgtattactgtgcgaga
 gatacggactacatgtttgactactggggccagggaaaccctg
 gtcaccgtgagctca (SEQ ID NO:13)

and

(2) a VL polypeptide encoded by:

tcttctgagctgactcaggaccctgttgtgtctgtggccttg
 ggagagacagtcagtatcacatgccaaggagacaacctcaga
 gacttttatgcaagctggtaccaacagaagccaggacaggcc
 cctctactagtcatgtatggtaaaaacaggcggccctcaggg
 atcccagaccgattctctggctccacctcaggaaacacactt
 tccttgaccatcactggggctcaggcgggaagatgaggctgac
 tattactgtagctcccgggacaacagtaagcatgtggtgttc

ggcgggggggaccaaggtcaccgtccta (SEQ ID
NO:14) .

11. The method according to claim 1, wherein the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

gaggtgcagctggtggagtctgggggaggcgtggtccagcct
gggagggtccgtgagactctcctgtgcagcctctggattcacc
tcagtacctatactatgcactgggtccgccaggctccaggc
aaggggctggagtgggtggcaattatctcaaagtatggaagc
aataagtaactacgcagacccccgtgaggggcccattcaccatc
tcagagacaattccaagaacacgctgtatctgcaaataaac
agcctgagagctgaggacacggctgtgtattactgtgtacgt
ggcagcagctggtatcgcggaaattggttcgaccctggggc
cagggaaacctggtcaccgtgagctca (SEQ ID NO:15)

and

(2) a VL polypeptide encoded by:

gacatccagatgaccagctctccatcctccctgtctgcatct
gtaggagacagagtcaccatcgcttgccgggcaagtcagacc
attagtaggtatttaaattggtatcagcagaaaccagggaaa
gcccctaagctcctgatctatgctgcatccagtttgcaaagt
ggggtctcatcaaggttcagtgggcagtggtatctgggacagag
ttcactctcaccatcagcagctctgcagcctgaagatthttgca
acttatttctgtcaacagacttacagtcccccgatcaccttc
ggccaagggacacgactggagattaaacga (SEQ ID
NO:16) .

12. The method according to claim 1, wherein the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

gaggtgcagctggtggagaccggggctgaggtgaagaagcct
ggggcctcagtgaaaggtctcctgcaaggcttctggatacacc
ttcaccggctactatatgcactgggtgcgacaggccccctgga
caagggcttgagtggatgggatggatcaaccctaccagtggt
agcacaactatgcacagaagtttcagggcagggtcaccatg
accagggacacgtccatcagcacagcctacatggagctgagc

gggctgagatctgacgacactgccgtgtattactgtgtgaga
 gatccgggttctcctaagtggctggccttcgaccctggggc
 cagggcaccctggtcaccgtgagctca (SEQ ID NO:17)

(2) a VL polypeptide encoded by:

gacatccagttgaccagtcctccatcctccttgtctgcttct
 gtaggagacagagtcaccatcacttgccgggcaagtccggcc
 attagtaggtatttaaattggtatcagcagaaaccagggaaa
 gccctaagctcctgatctatgctgcatccagtttgcaaagt
 ggggtctcatcaagggttcagtggcagtggtctgggacagag
 ttcactctcaccatcagcagtcctgcagcctgaagatcttgca
 acttatttctgtcaacagacttacagtcctcccgatcaccttc
 ggccaagggacacgactggagattaaacgt (SEQ ID
 NO:18) .

13. The method according to claim 1, wherein the microtubule inhibitor is selected from the group consisting of a microtubule-destabilizing agent, a microtubule-stabilizing agent, and combinations thereof.

14. The method according to claim 13, wherein the microtubule inhibitor is selected from the group consisting of a taxane, a vinca alkaloid, an epothilone, and combinations thereof.

15. The method according to claim 13, wherein the microtubule inhibitor is selected from the group consisting of BT-062 (Biotest), HMN-214 (D. Western Therapeutics), eribulin mesylate (Eisai), vindesine (Eli Lilly), EC-1069 (Endocyte), EC-1456 (Endocyte), EC-531 (Endocyte), vintafolide (Endocyte), 2-methoxyestradiol (EntreMed), GTx-230 (GTx), trastuzumab emtansine (Hoffmann-La Roche), crolibulin (Immune Pharmaceuticals), D1302A-maytansinoid conjugates (ImmunoGen), IMGN-529 (ImmunoGen), lorvotuzumab mertansine (ImmunoGen), SAR-3419 (ImmunoGen), SAR-

566658 (ImmunoGen), IMP-03138 (Impact Therapeutics), topotecan/vincristine combinations (LipoCure), BPH-8 (Molecular Discovery Systems), fosbretabulin tromethamine (OXiGENE), estramustine phosphate sodium (Pfizer), vincristine (Pierre Fabre), vinflunine (Pierre Fabre), vinorelbine (Pierre Fabre), RX-21101 (Rexahn), cabazitaxel (Sanofi), STA-9584 (Synta Pharmaceuticals), vinblastine, epothilone A, patupilone (Novartis), ixabepilone (Bristol-Myers Squibb), Epothilone D (Kosan Biosciences), paclitaxel (Bristol-Myers Squibb), docetaxel (Sanofi-Aventis), DJ-927 (Daiichi Sankyo), discodermolide (CAS No: 127943-53-7), eleutherobin (CAS No.: 174545-76-7), pharmaceutically acceptable salts thereof, and combinations thereof.

16. A method for treating or ameliorating the effects of a disease in a subject comprising:

administering to a subject in need thereof

(a) a therapeutically effective amount of a microtubule inhibitor; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

17. A method for treating or ameliorating the effects of a disease in a subject comprising:

administering to a subject in need thereof

(a) a therapeutically effective amount of paclitaxel; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

18. A composition for treating or ameliorating the effects of a disease in a subject comprising:

(a) a therapeutically effective amount of a microtubule inhibitor; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region (VH), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7; and

(ii) a light chain variable region (VL), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

19. The composition according to claim 18, wherein the subject is a mammal.

20. The composition according to claim 19, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

21. The composition according to claim 19, wherein the mammal is a human.

22. The composition according to claim 18, wherein the disease is characterized by differential expression of tumor endothelial marker 8 (TEM8) membrane antigen.

23. The composition according to claim 18, wherein the disease is characterized by differential expression of tumor endothelial marker 8 (TEM8) membrane antigen on a tumor cell and/or a tumor stromal cell.

24. The composition according to claim 18, wherein the disease is a cancer that differentially expresses TEM8.

25. The composition according to claim 24, wherein the cancer is selected from the group consisting of kidney cancer, colon cancer, lung cancer, liposarcomas, brain cancer, breast cancer, melanoma, liver cancer, head and neck cancer, and prostate cancer.

26. The composition according to claim 18, wherein the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

```
caggtccagctggtgcagtcctggggctgaggtgaagaagcct
gggacctcagtgaaaggtctcctgcaaggttcttgatacacc
ttcagcagctatgctatcagctgggtgacagggccctgga
caagggcttgagtggatgggagggatcatccctatctttggt
acaacaaactacgcacagaagttccagggcagagtcacgatt
accggggaggaatccacgagcacagtcctacatggagctgagc
agcctgagatctgaggacacggccgtgtattactgtgagaga
gatacggactacatgtttgactactggggccagggaaaccctg
gtcaccgtgagctca (SEQ ID NO: 11)
```

and

(2) a VL polypeptide encoded by:

```
tcttctgagctgactcaggaccctggtgtgtctgtggccttg
ggagagacagtcagtatcacatgccaaaggagacaacctcaga
gacttttatgcaagctggtaccaacagaagccaggacaggcc
cctctactagtcatgtatggtaaaaacaggcggccctcaggg
atcccagaccgattctctggctccacctcaggaaacacactt
tccttgaccatcactggggctcaggcgggaagatgaggctgac
tattactgtagctcccgggacaacagtaagcatgtggtgttc
ggcgggggggaccaaggtcaccgtccta (SEQ ID
NO:12) .
```

27. The composition according to claim 18, wherein the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

```
caggtccagctggtgcagctctggggctgaggtgaagaagcct
ggggcctcagtgaaggtctcctgcaaggtttctggatacacc
tcagcagctatgctatcagctgggtgacagggcccctgga
caagggttgagtggatgggagggatcatccctatctttggt
acagcaaactacgcacagaagtccagggcagagtcacgatt
accgcgacgaatccacgagcacagcctacatggagctgagc
agcctgagatctgaggacacggccgtgtattactgtgagaga
gatacggactacatgtttgactactggggccagggaaaccctg
gtcaccgtgagctca (SEQ ID NO:13)
```

and

(2) a VL polypeptide encoded by:

```
tcttctgagctgactcaggaccctggtgtgtctgtggccttg
ggagagacagtcagtatcacatgccaaggagacaacctcaga
gacttttatgcaagctggtaccaacagaagccaggacaggcc
cctctactagtcatgtatggtaaaaacaggcggccctcaggg
atcccagaccgattctctggctccacctcaggaaacacactt
tccttgaccatcactggggctcaggcgggaagatgaggctgac
tattactgtagctcccgggacaacagtaagcatgtggtgttc
ggcgggggggaccaaggtcaccgtccta (SEQ ID
NO:14) .
```

28. The composition according to claim 18, wherein the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

```
gaggtgcagctggtggagtctgggggaggcgtggtccagcct
gggaggtccgtgagactctcctgtgcagcctctggattcacc
tcagtacctatactatgcactgggtccgccaggctccaggc
aaggggctggagtgggtggcaattatctcaaagtatggaagc
aataagtactacgcagacccccgtgaggggcccgattcaccatc
tccagagacaattccaagaacacgctgtatctgcaaatgaac
agcctgagagctgaggacacggctgtgtattactgtgtacgt
ggcagcagctggtatcgcgaaattggttcgaccctggggc
cagggaaaccctggtcaccgtgagctca (SEQ ID NO:15)
```

and

(2) a VL polypeptide encoded by:

gacatccagatgaccagctctccatcctcctgtctgcatct
gtaggagacagagtcaccatcgcttgccgggcaagtccagacc
attagtaggtatttaaattggtatcagcagaaaccagggaaa
gcccctaagctcctgatctatgctgcatccagtttgcaaagt
ggggtctcatcaagggttcagtgccagtgatctgggacagag
ttcactctcaccatcagcagctctgcagcctgaagatthtgca
acttatttctgtcaacagacttacagtcccccgatcaccttc
ggccaagggacacgactggagattaaacga (SEQ ID
NO:16) .

29. The composition according to claim 18, wherein the monoclonal antibody or antigen binding fragment thereof comprises:

(1) a VH polypeptide encoded by:

gagggtgcagctgggtggagaccggggctgaggtgaagaagcct
ggggcctcagtgaaaggtctcctgcaaggcttctggatacacc
ttcaccggctactatatgcactgggtgogacaggccccctgga
caagggcttgagtggatgggatggatcaaccctaccagtggt
agcacaactatgcacagaagtttcagggcagggtcaccatg
accagggacacgtccatcagcacagcctacatggagctgagc
gggctgagatctgacgacactgccgtgtattactgtgtgaga
gatccgggttctcctaagtggctggccttcgaccctggggc
cagggcaccctgggtcaccgtgagctca (SEQ ID NO:17)

(2) a VL polypeptide encoded by:

gacatccagttgaccagctctccatcctccttgtctgcttct
gtaggagacagagtcaccatcacttgccgggcaagtccgggcc
attagtaggtatttaaattggtatcagcagaaaccagggaaa
gcccctaagctcctgatctatgctgcatccagtttgcaaagt
ggggtctcatcaagggttcagtgccagtgatctgggacagag
ttcactctcaccatcagcagctctgcagcctgaagatthtgca
acttatttctgtcaacagacttacagtcccccgatcaccttc
ggccaagggacacgactggagattaaacgt (SEQ ID
NO:18) .

30. The composition according to claim 18, wherein the microtubule inhibitor is selected from the group consisting of a microtubule-destabilizing agent, a microtubule-stabilizing agent, and combinations thereof.

31. The composition according to claim 18, wherein the microtubule inhibitor is selected from the group consisting of a taxane, a vinca alkaloid, an epothilone, and combinations thereof.

32. The composition according to claim 18, wherein the microtubule inhibitor is selected from the group consisting of BT-062 (Biotest), HMN-214 (D. Western Therapeutics), eribulin mesylate (Eisai), vindesine (Eli Lilly), EC-1069 (Endocyte), EC-1456 (Endocyte), EC-531 (Endocyte), vintafolide (Endocyte), 2-methoxyestradiol (EntreMed), GTx-230 (GTx), trastuzumab emtansine (Hoffmann-La Roche), crolibulin (Immune Pharmaceuticals), D1302A-maytansinoid conjugates (ImmunoGen), IMGN-529 (ImmunoGen), lorvotuzumab mertansine (ImmunoGen), SAR-3419 (ImmunoGen), SAR-566658 (ImmunoGen), IMP-03138 (Impact Therapeutics), topotecan/vincristine combinations (LipoCure), BPH-8 (Molecular Discovery Systems), fosbretabulin tromethamine (OXIGENE), estramustine phosphate sodium (Pfizer), vincristine (Pierre Fabre), vinflunine (Pierre Fabre), vinorelbine (Pierre Fabre), RX-21101 (Rexahn), cabazitaxel (Sanofi), STA-9584 (Synta Pharmaceuticals), vinblastine, epothilone A, patupilone (Novartis), ixabepilone (Bristol-Myers Squibb), Epothilone D (Kosan Biosciences), paclitaxel (Bristol-Myers Squibb), docetaxel (Sanofi-Aventis),

DJ-927 (Daiichi Sankyo), discodermolide (CAS No: 127943-53-7), eleutherobin (CAS No.: 174545-76-7), pharmaceutically acceptable salts thereof, and combinations thereof.

33. A composition for treating or ameliorating the effects of a disease in a subject comprising:

(a) a therapeutically effective amount of a microtubule inhibitor; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

34. A composition for treating or ameliorating the effects of a disease in a subject comprising:

(a) a therapeutically effective amount of paclitaxel; and

(b) a therapeutically effective amount of a monoclonal antibody or antigen binding fragment thereof, wherein the monoclonal antibody comprises:

(i) a heavy chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:5; and

(ii) a light chain variable region, which comprises an amino acid sequence selected from SEQ ID NO:6.

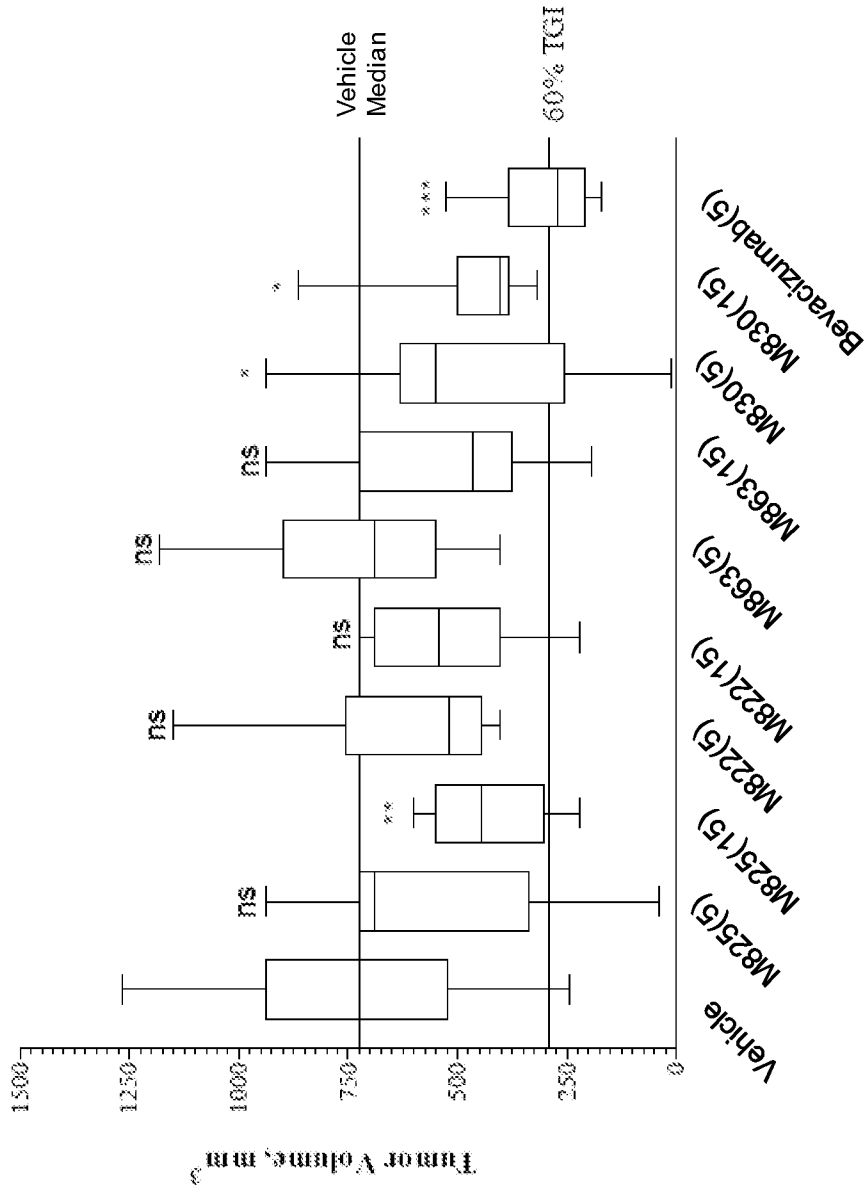
35. A pharmaceutical composition comprising the composition of any one of claims 18, 33, and 34 and a pharmaceutically acceptable diluent or carrier.

36. A kit comprising the composition of any one of claims 18, 33, and 34 packaged together with instructions for its use.

37. A kit comprising the pharmaceutical composition of claim 35 packaged together with instructions for its use.

FIG. 1

1A



ns = not significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

FIG. 1 continued

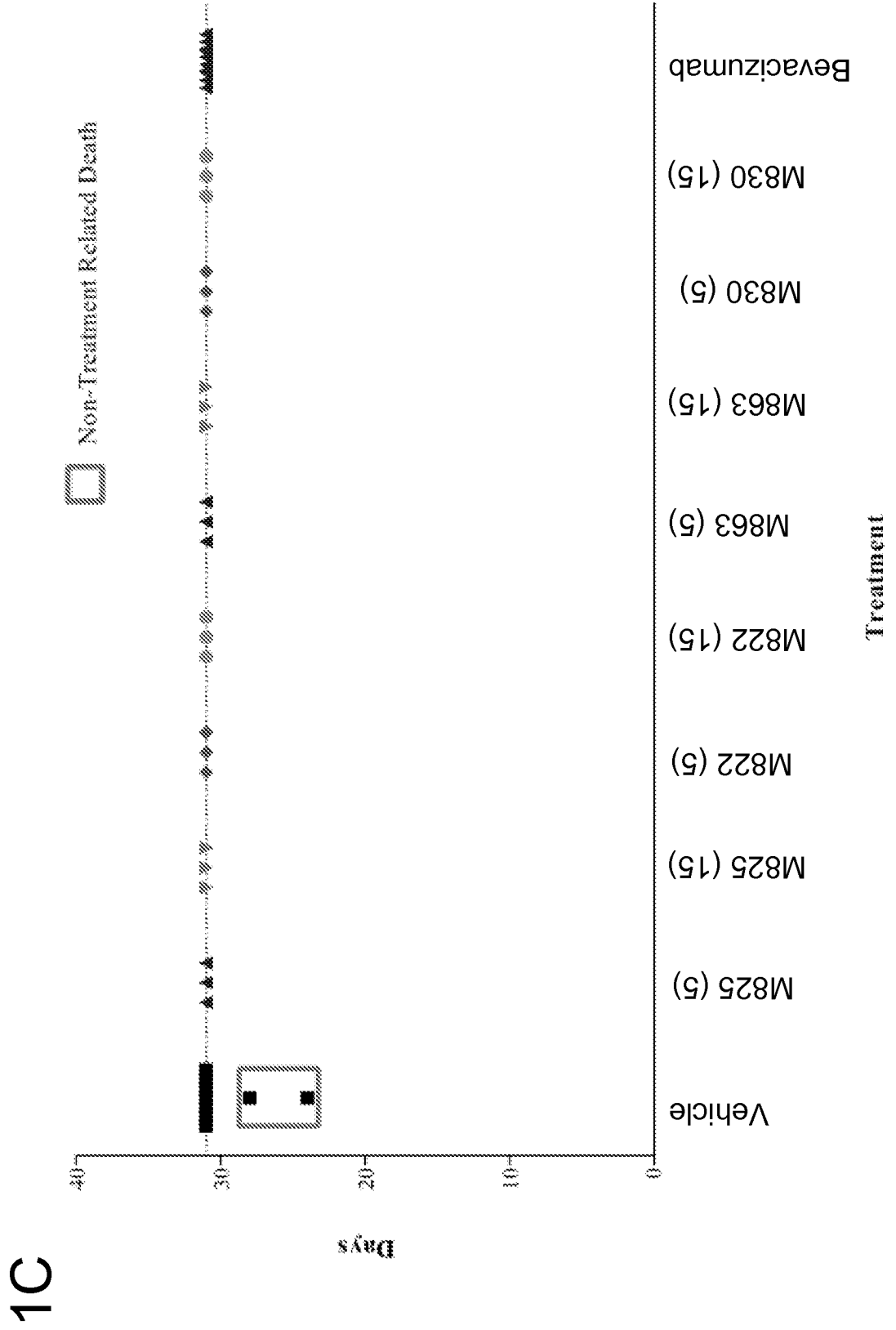


FIG. 1 continued

1D

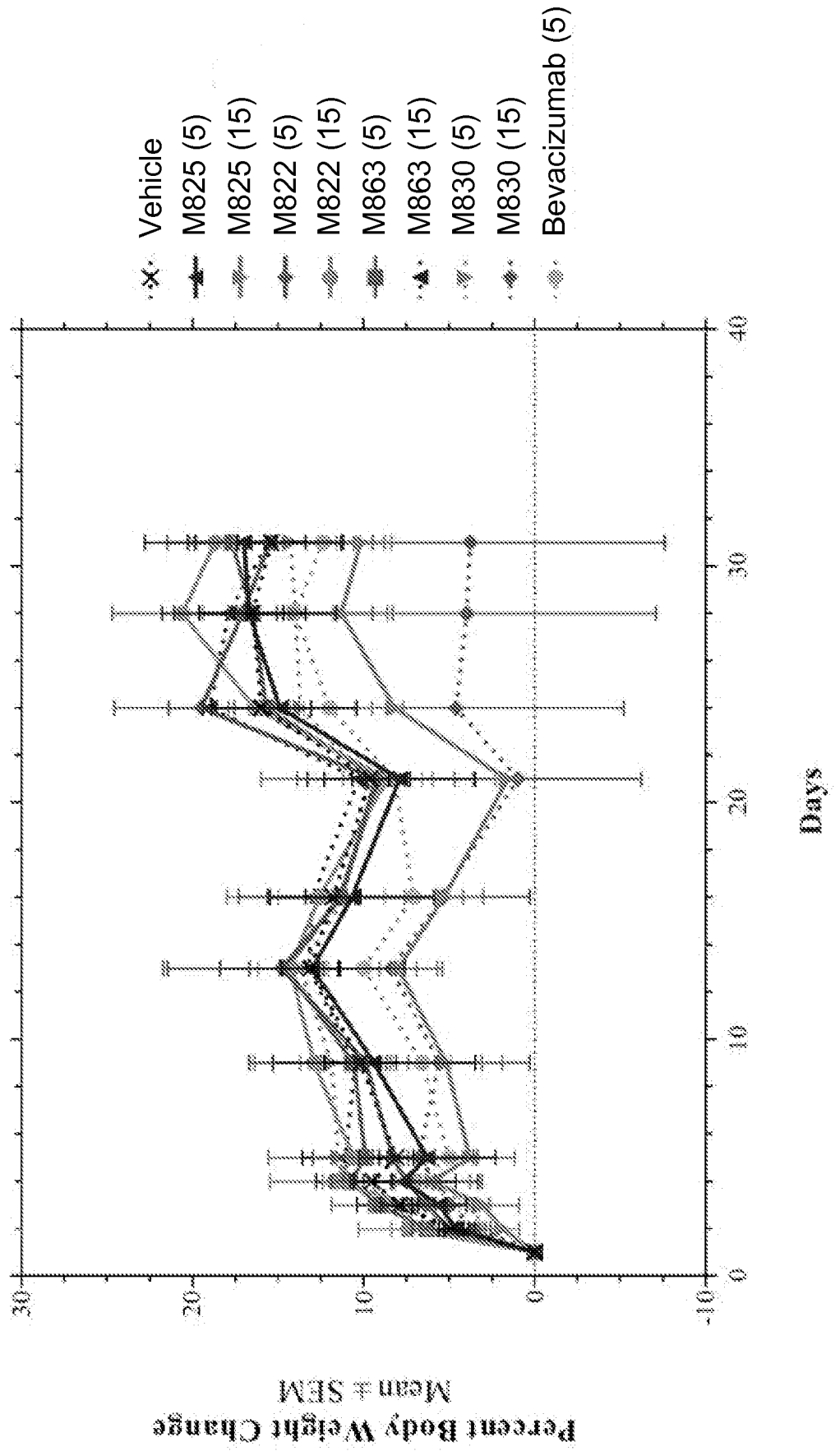


FIG. 2

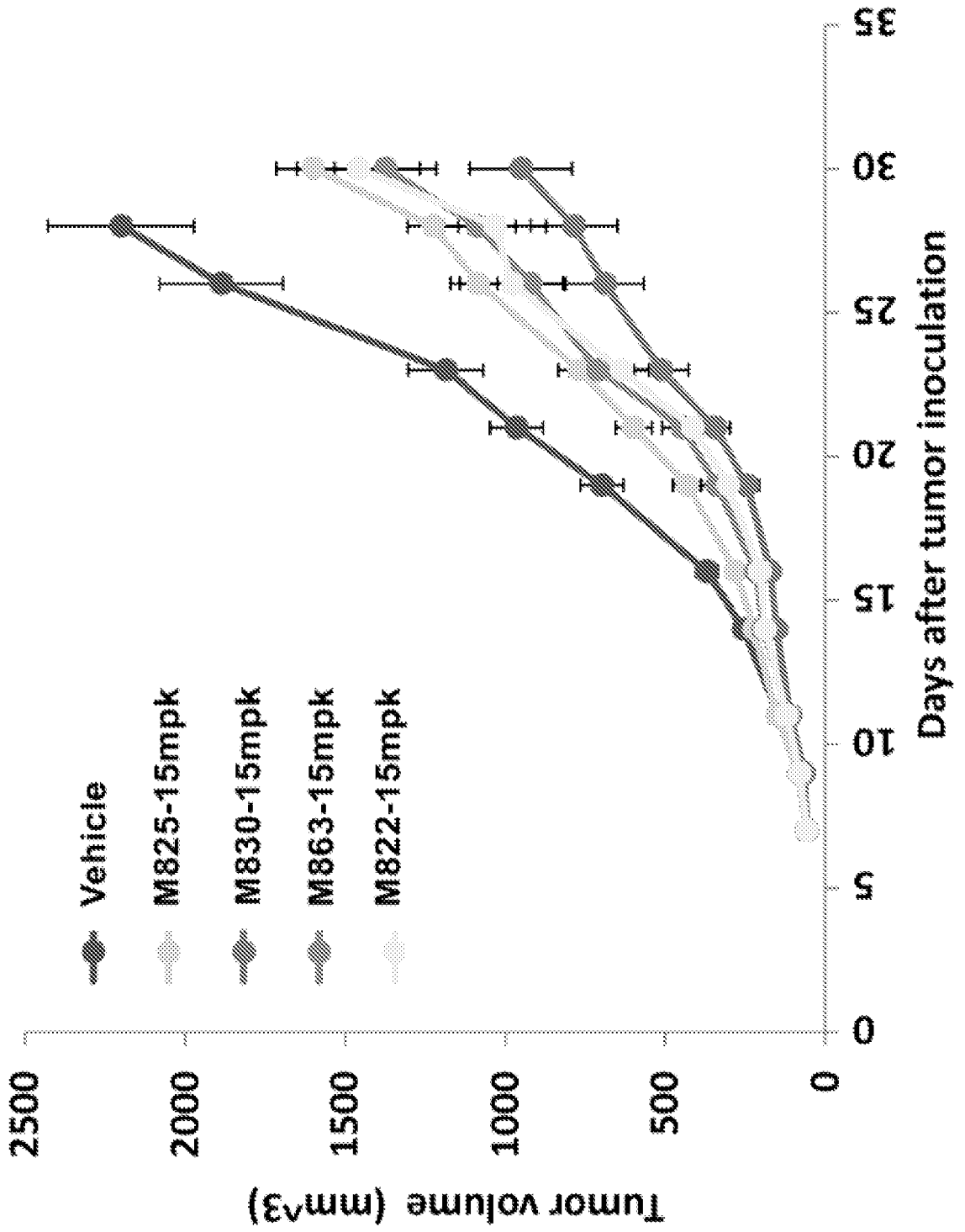


FIG. 4

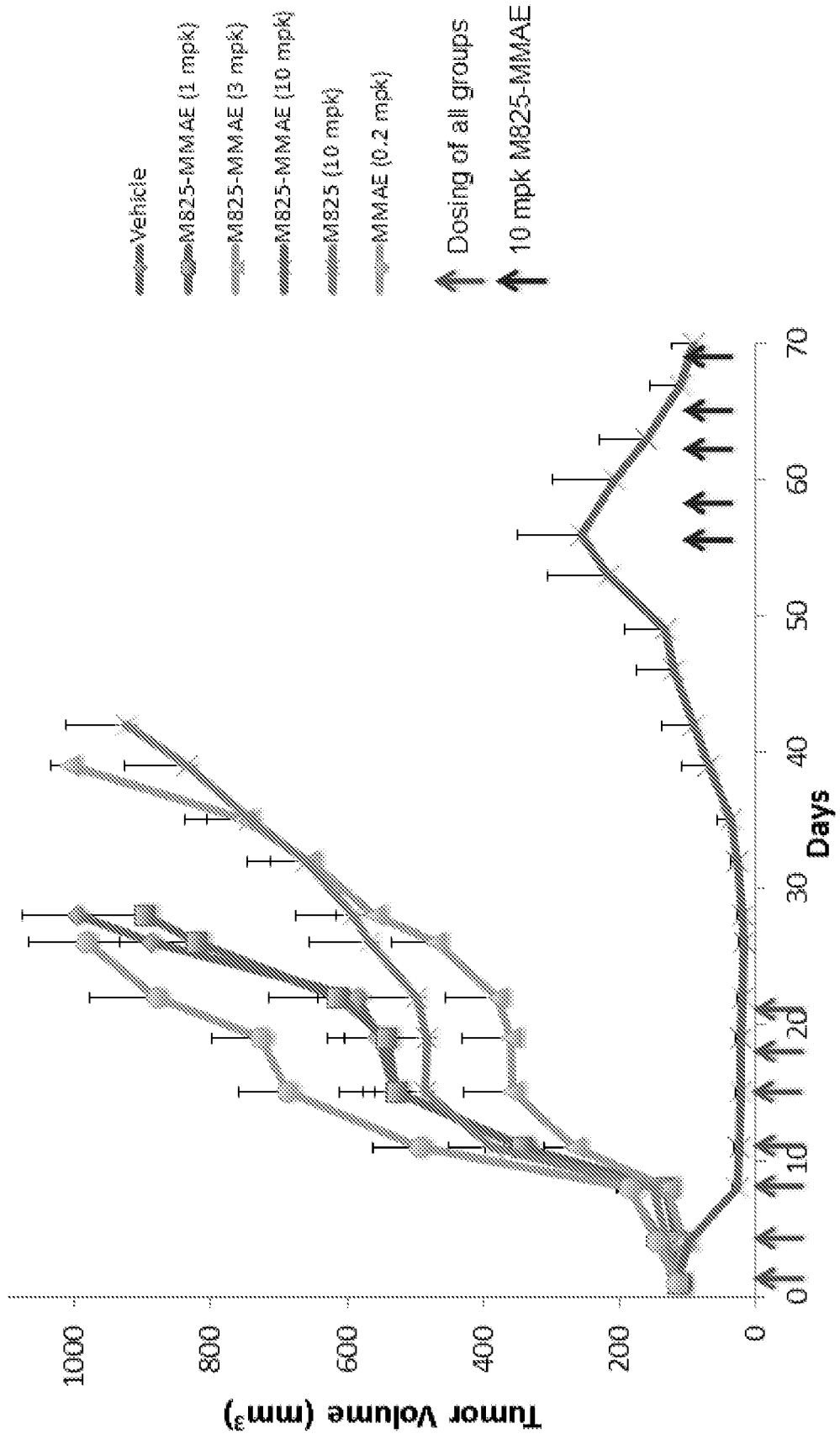


FIG. 5

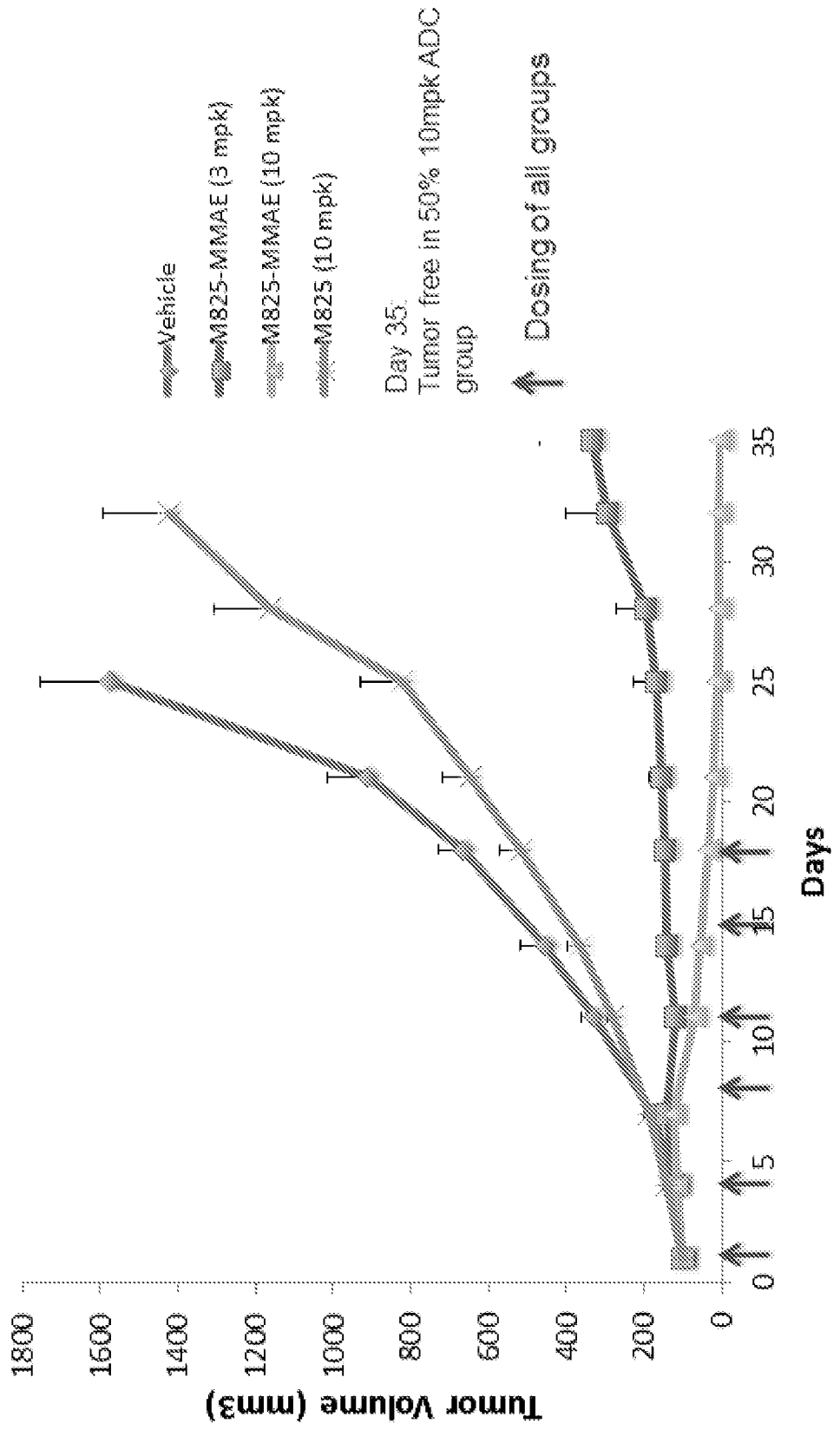


FIG. 6

6A

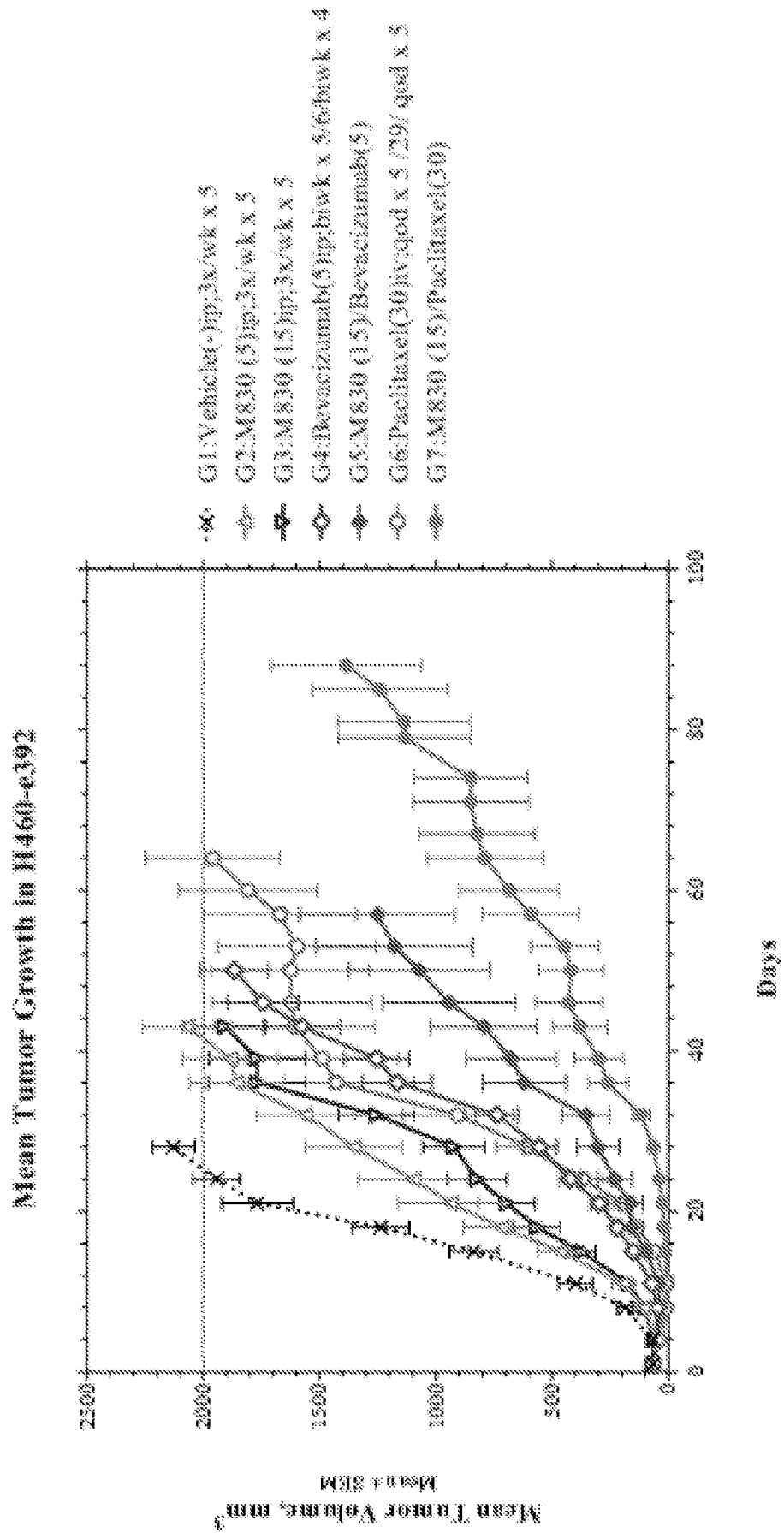


FIG. 6 continued

6B

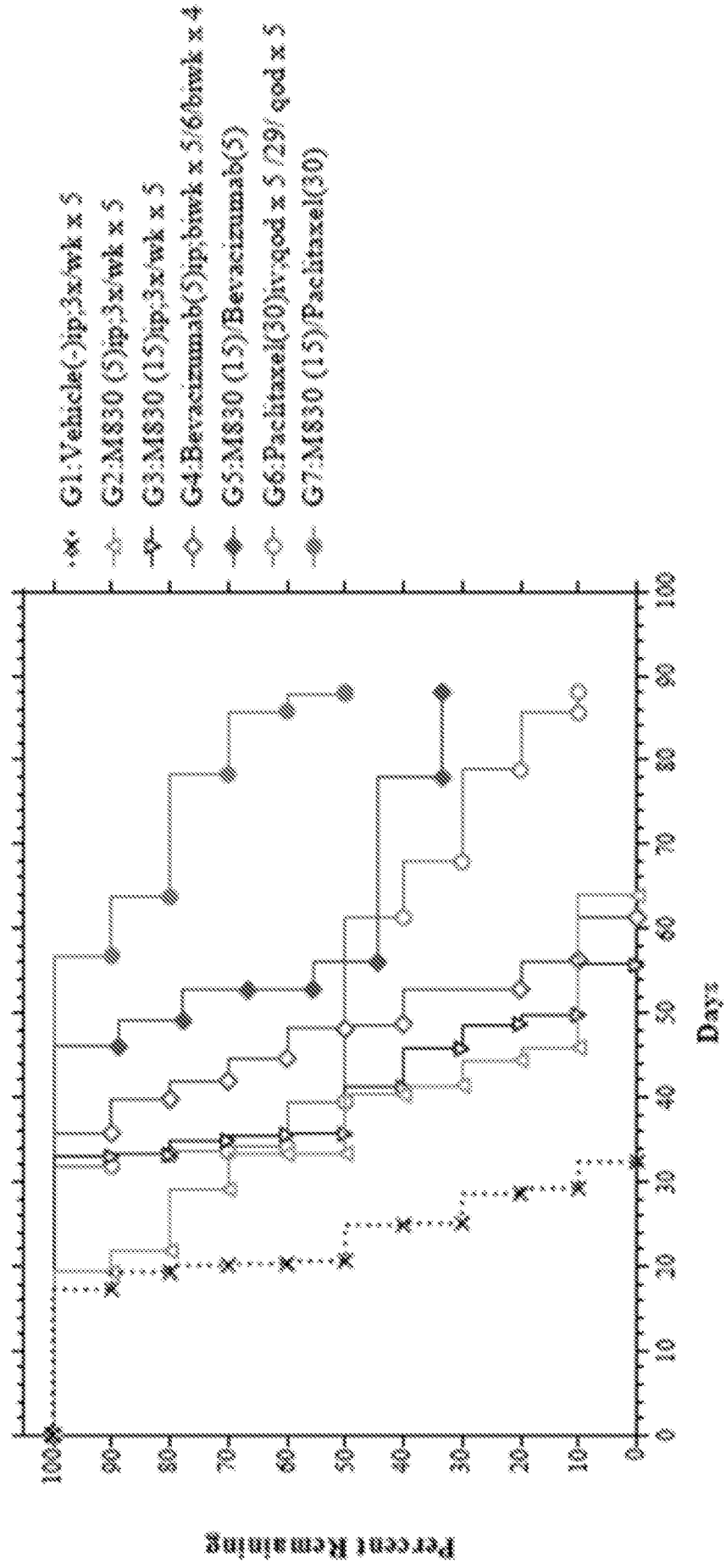


FIG. 6 continued

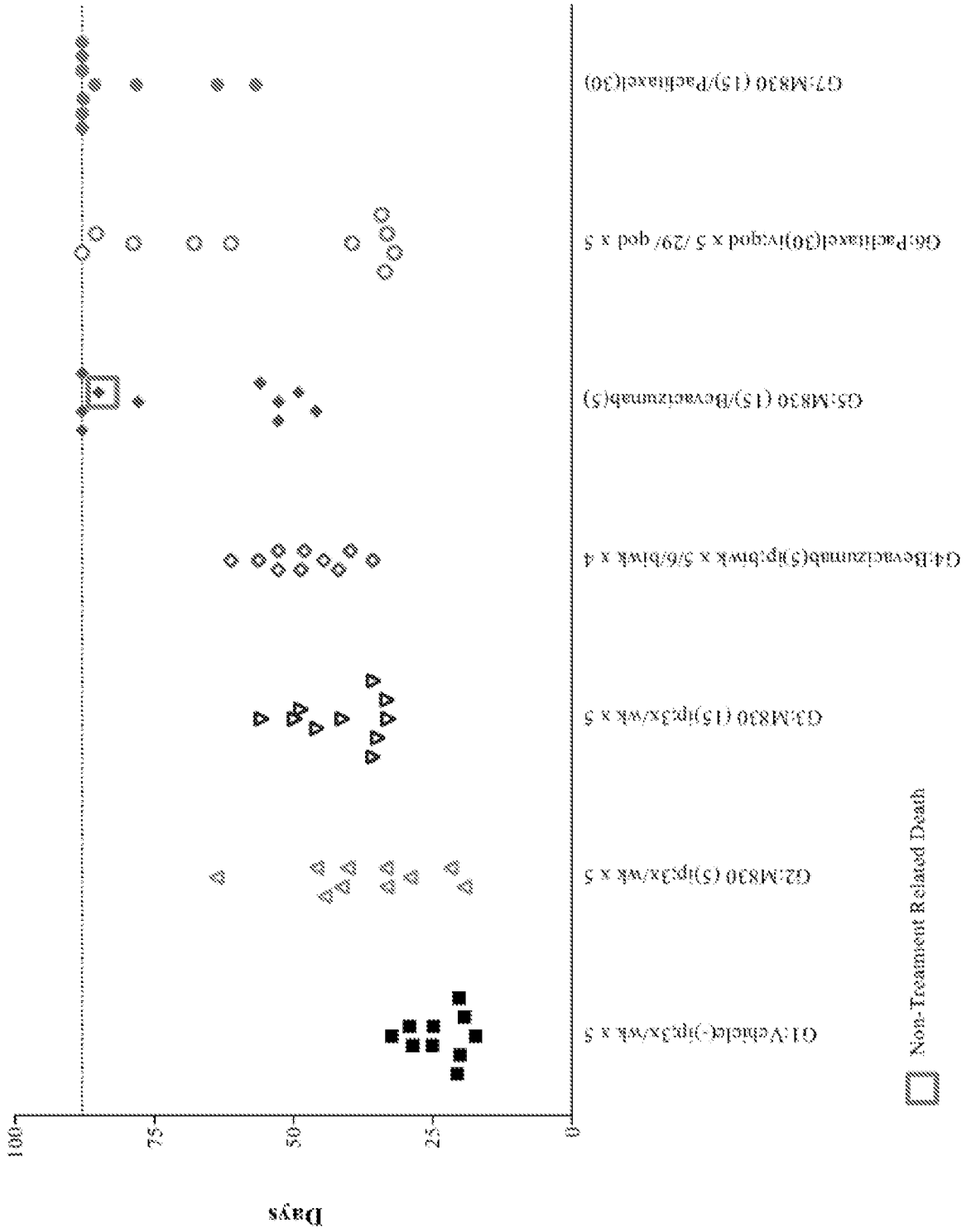


FIG. 6 continued

6D

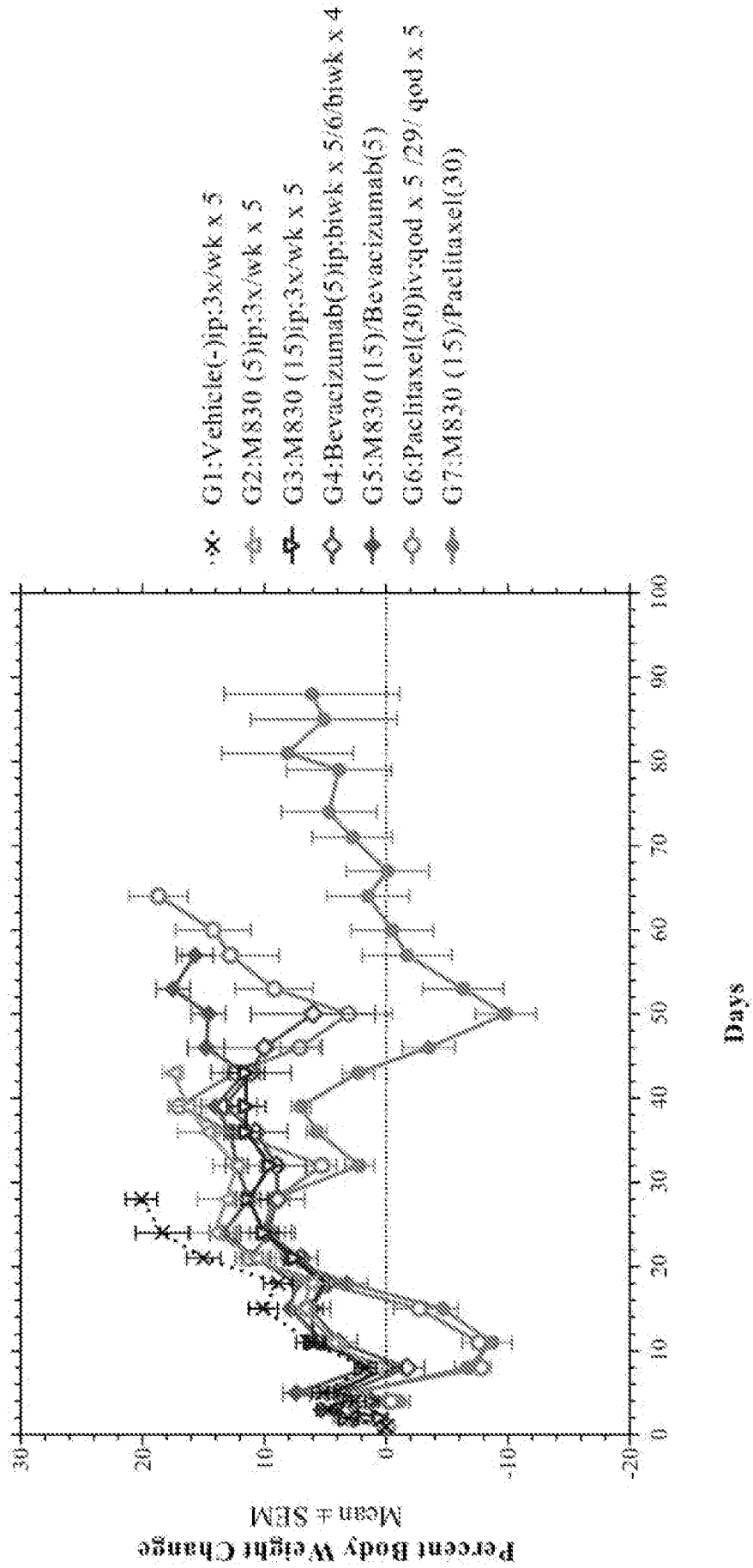


FIG. 7

7A

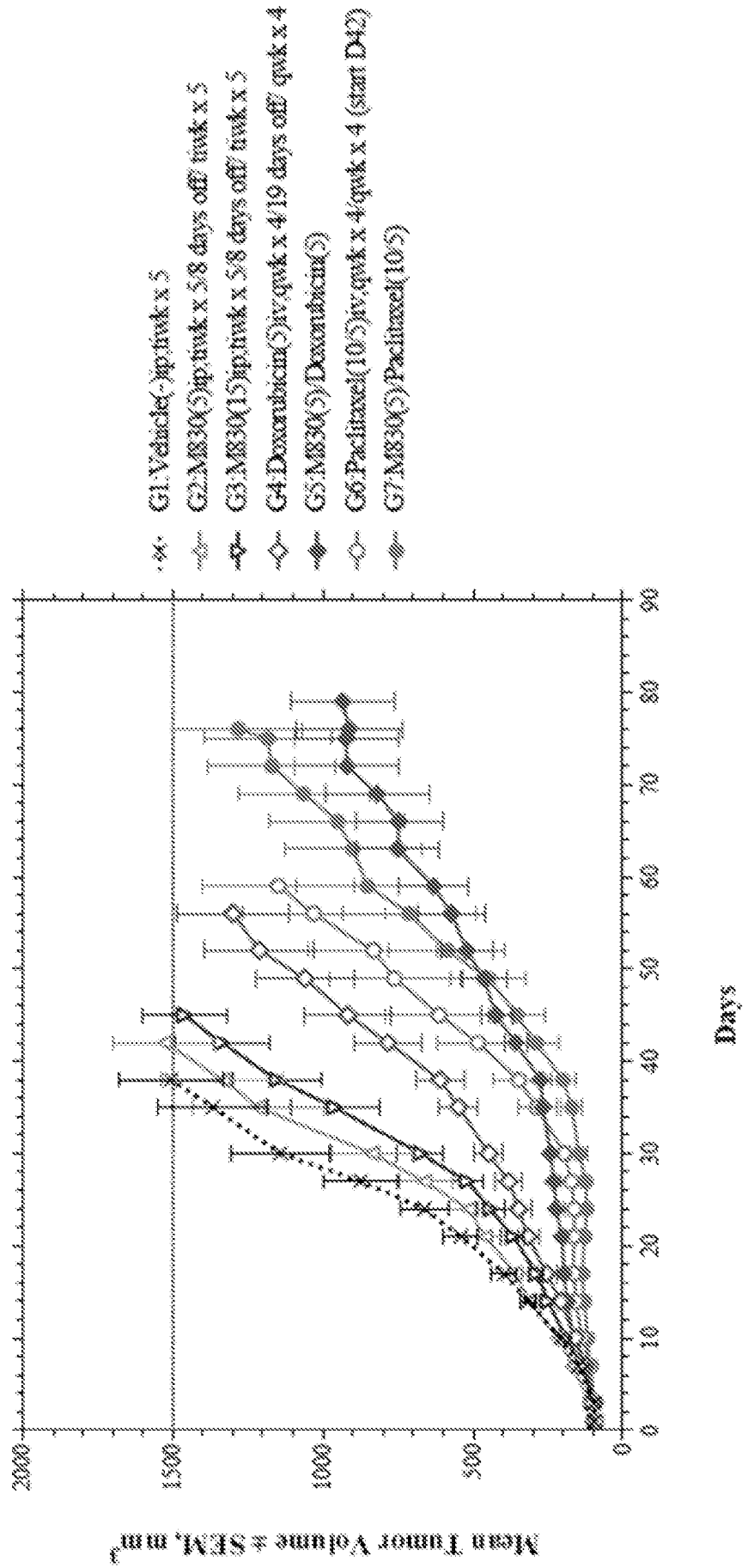


FIG. 7 continued

7B

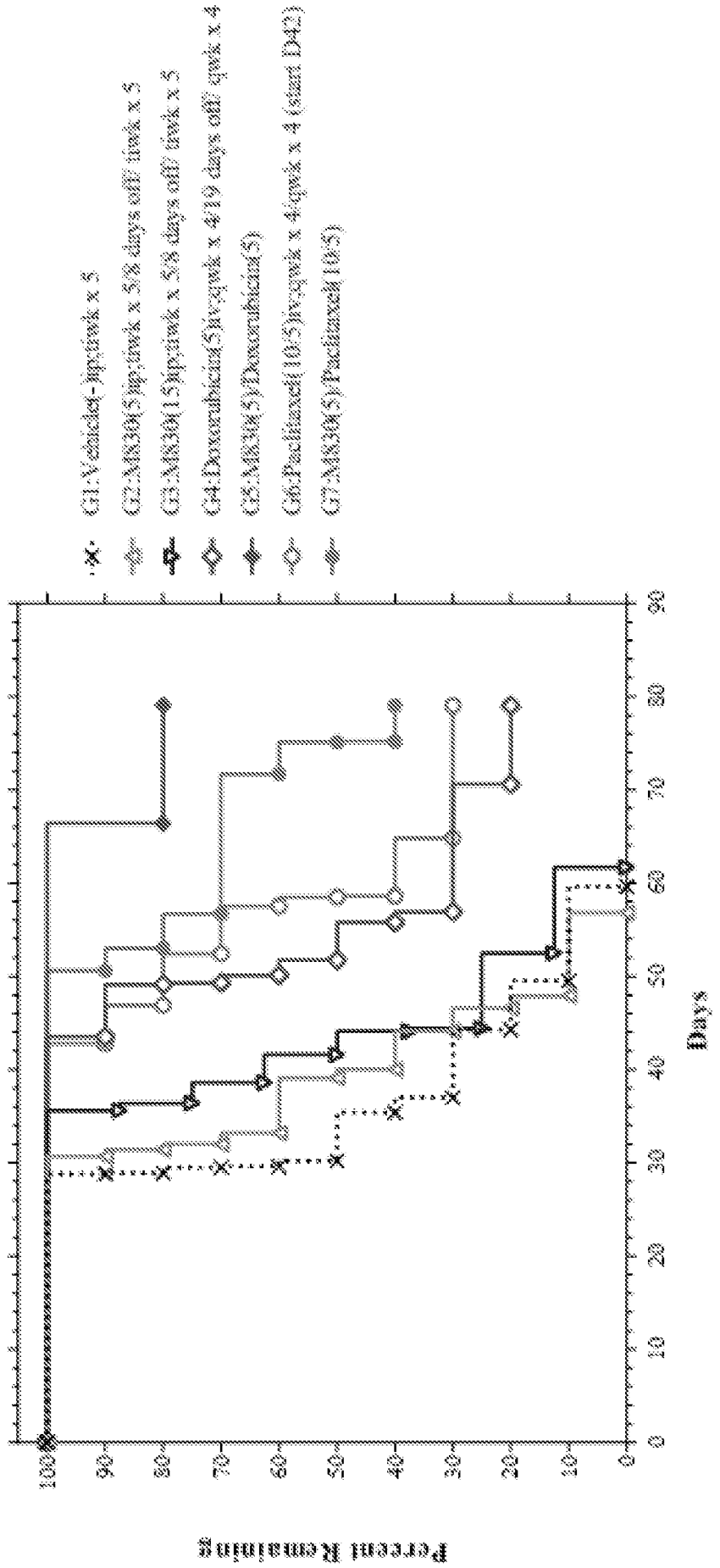
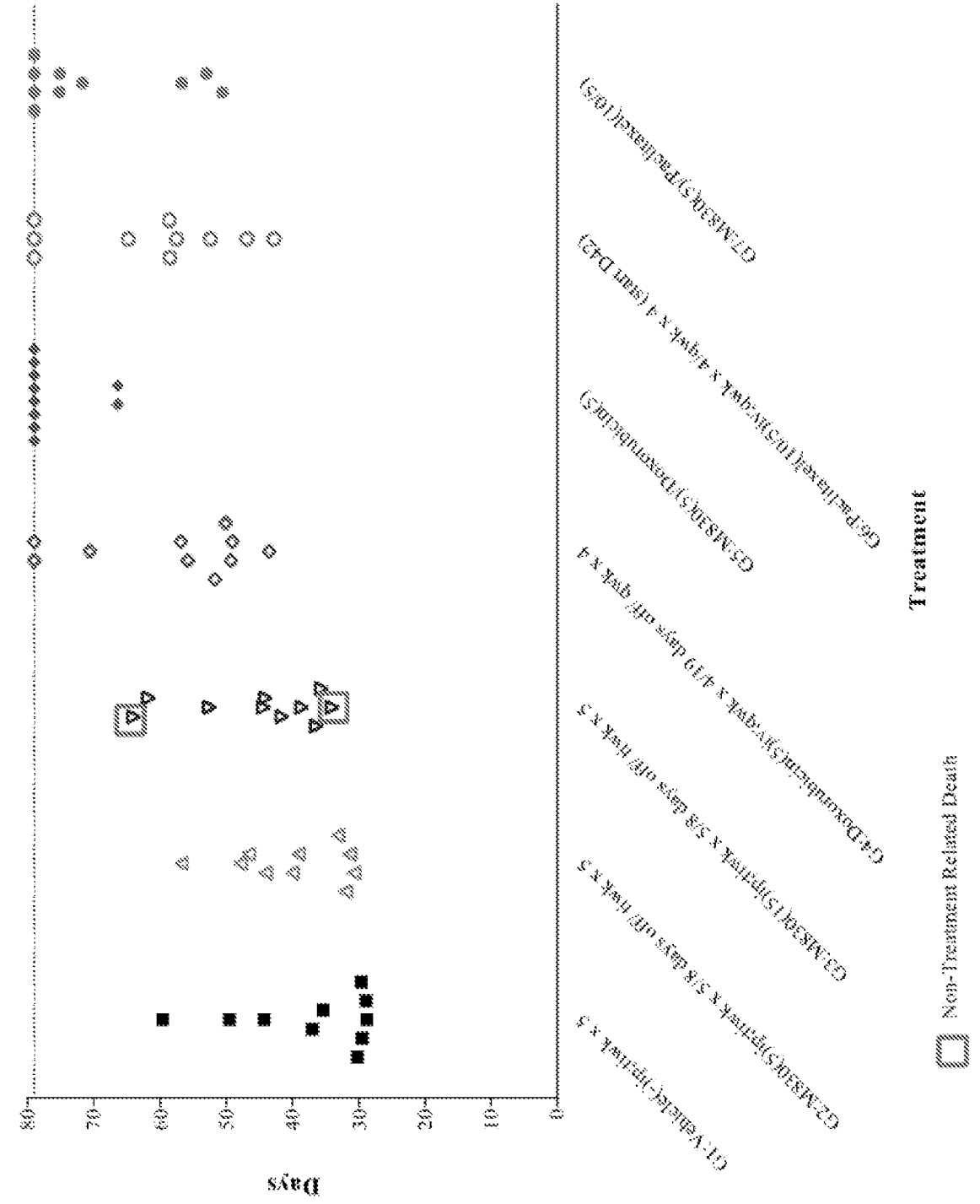


FIG. 7 continued



7C

FIG. 7 continued

7D

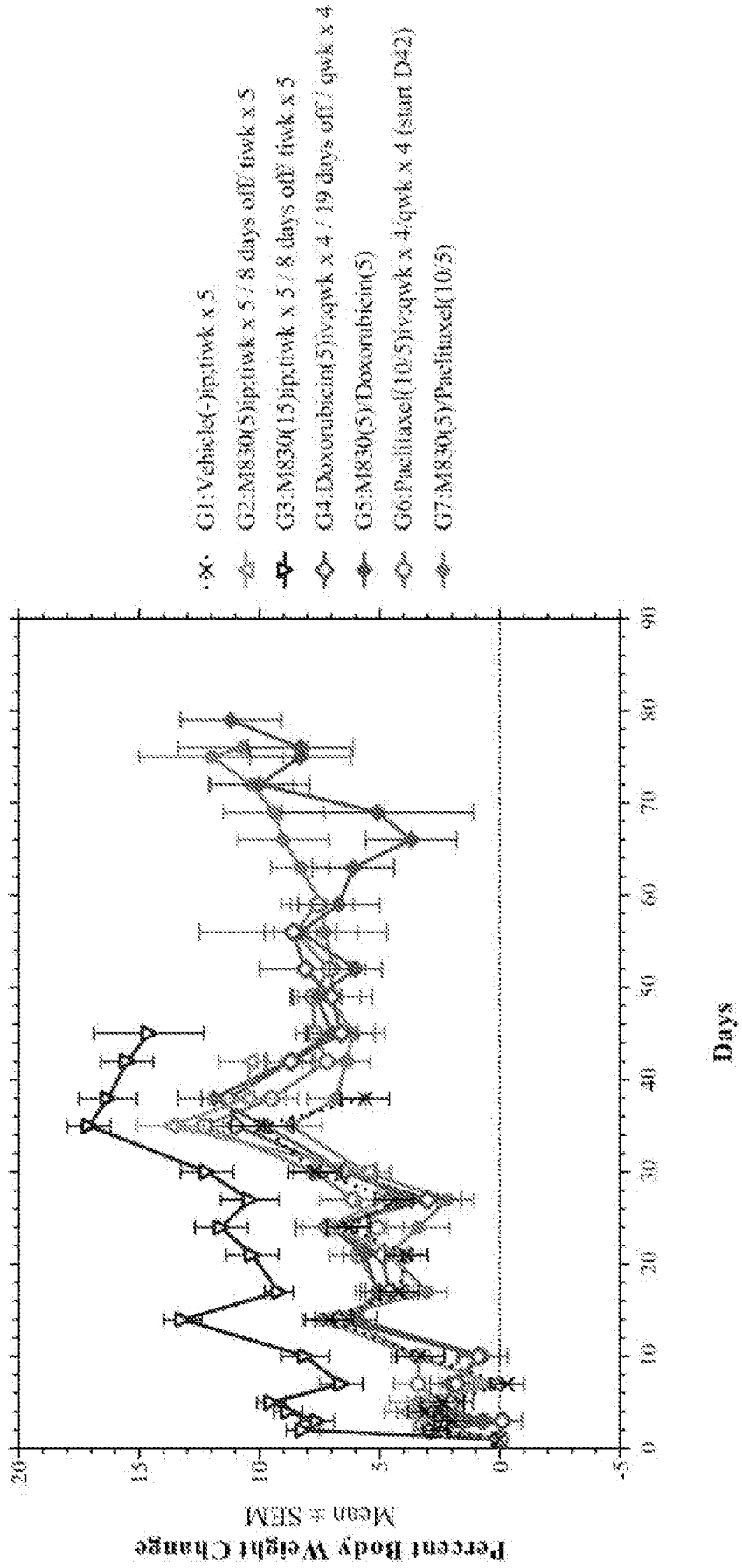


FIG. 8

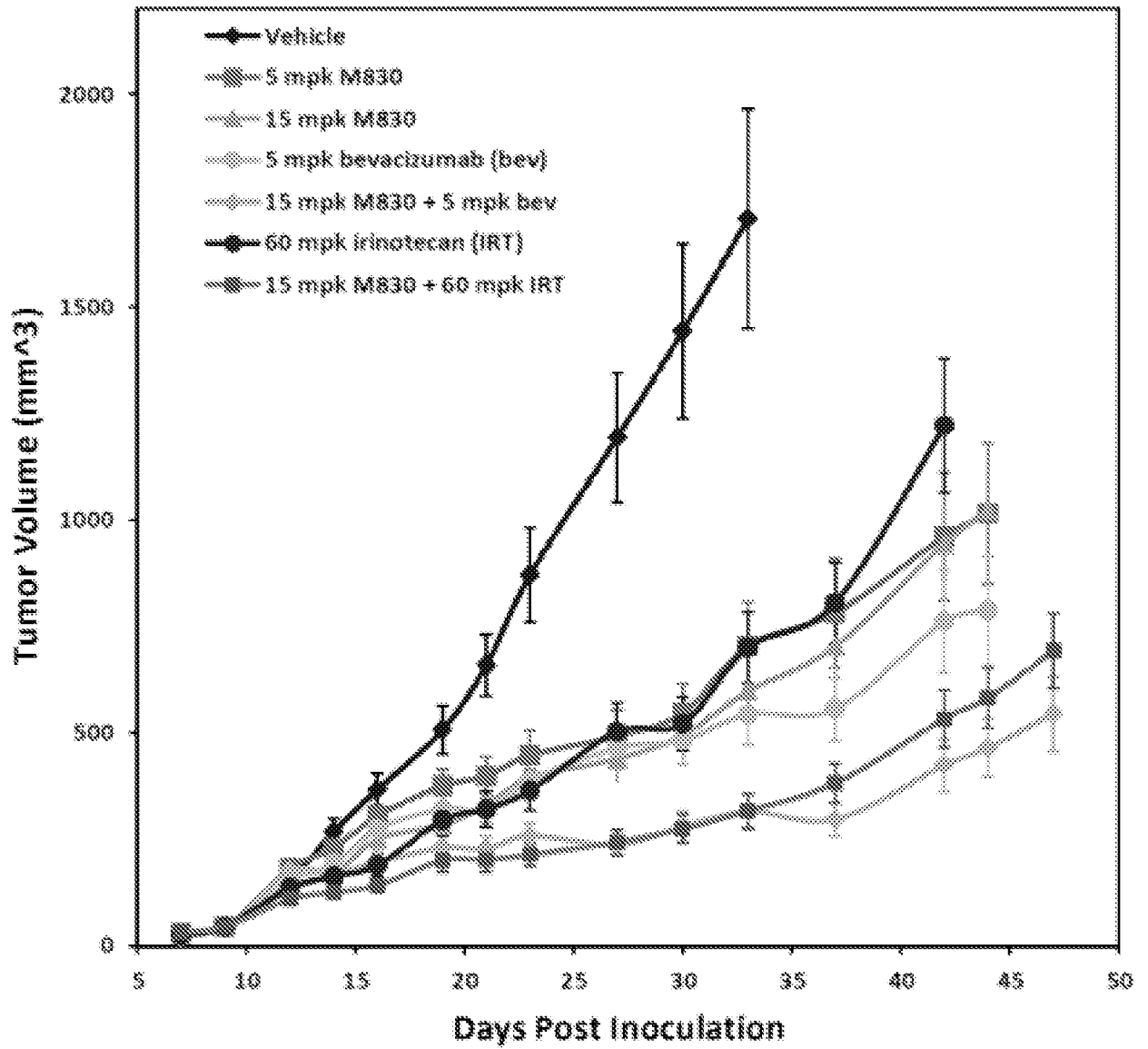


FIG. 9

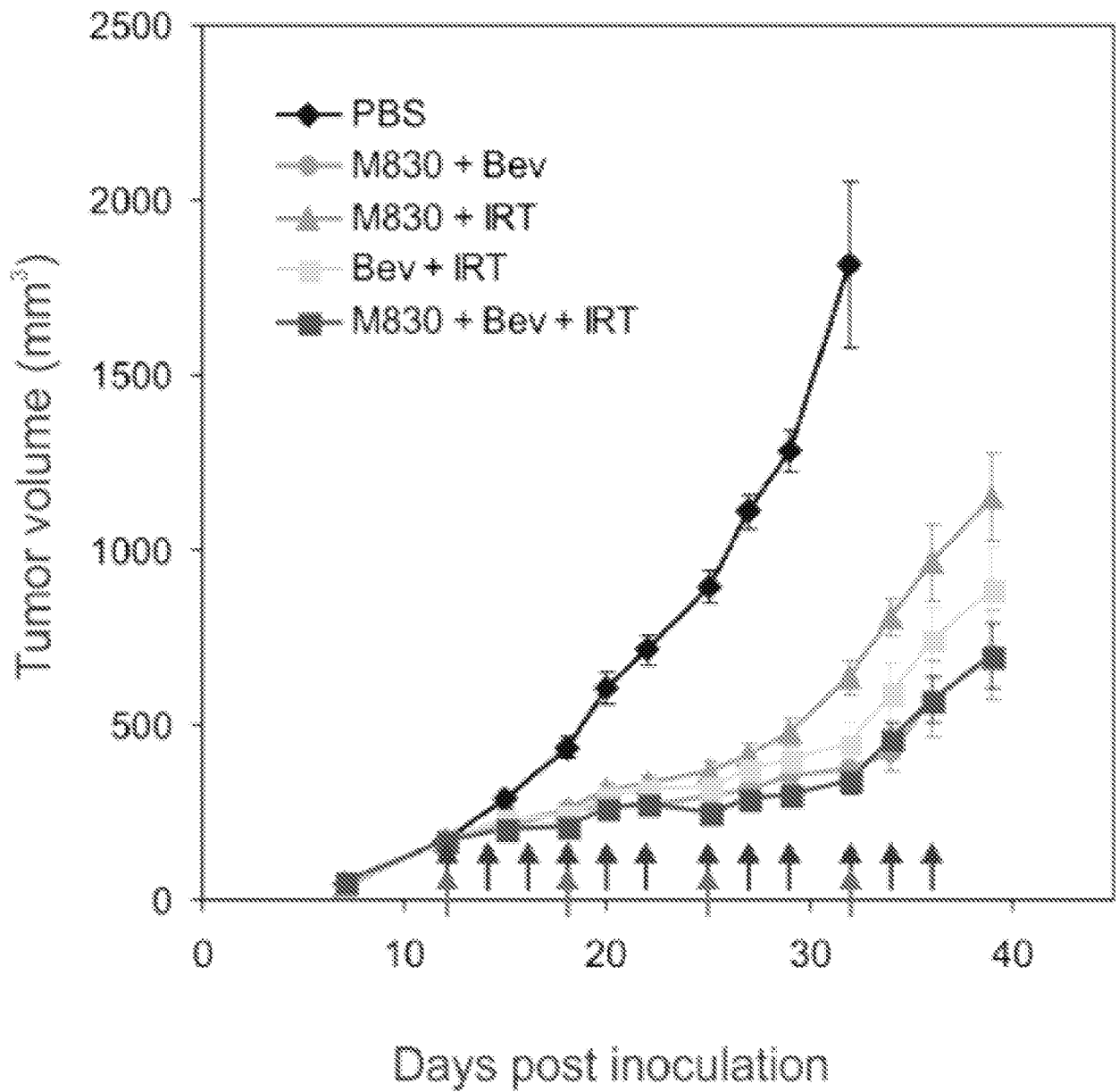


FIG. 10

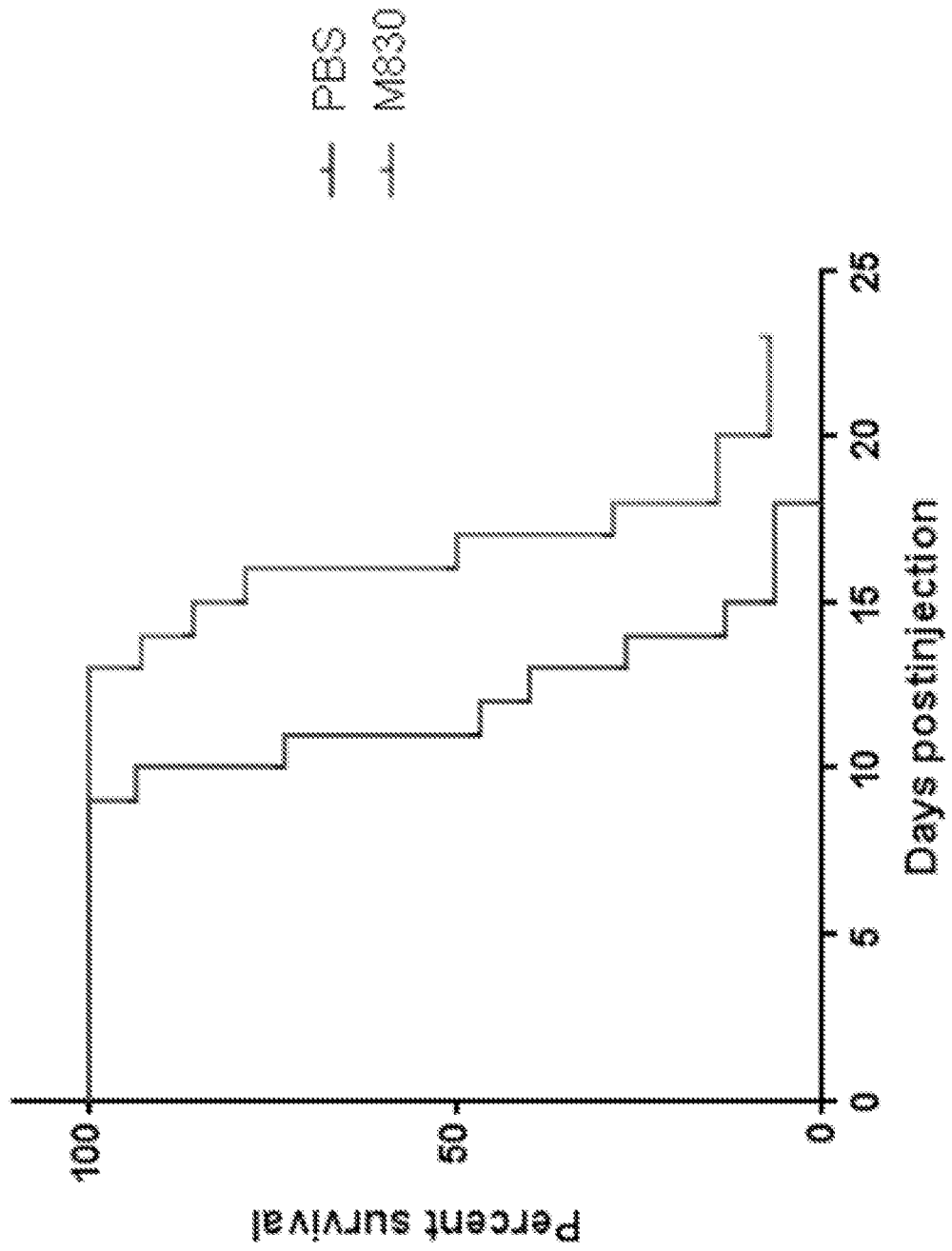


FIG. 11

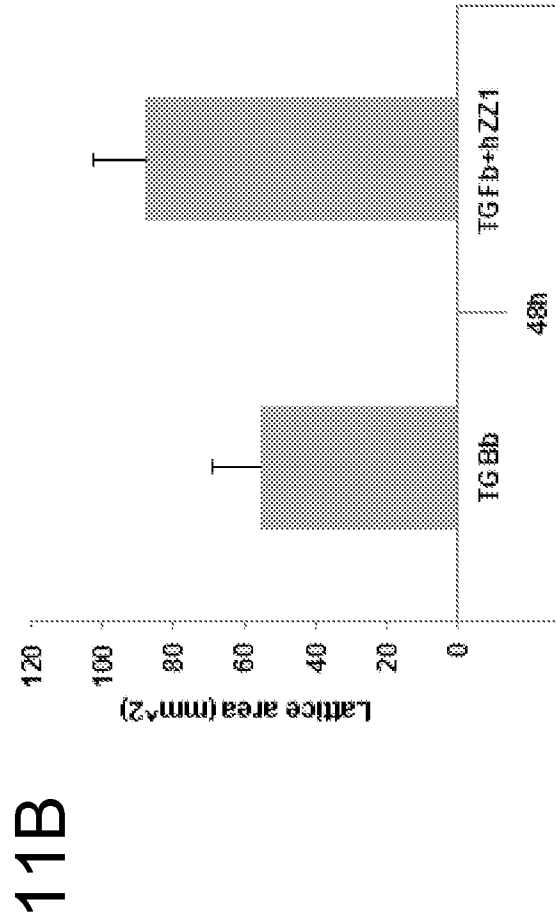
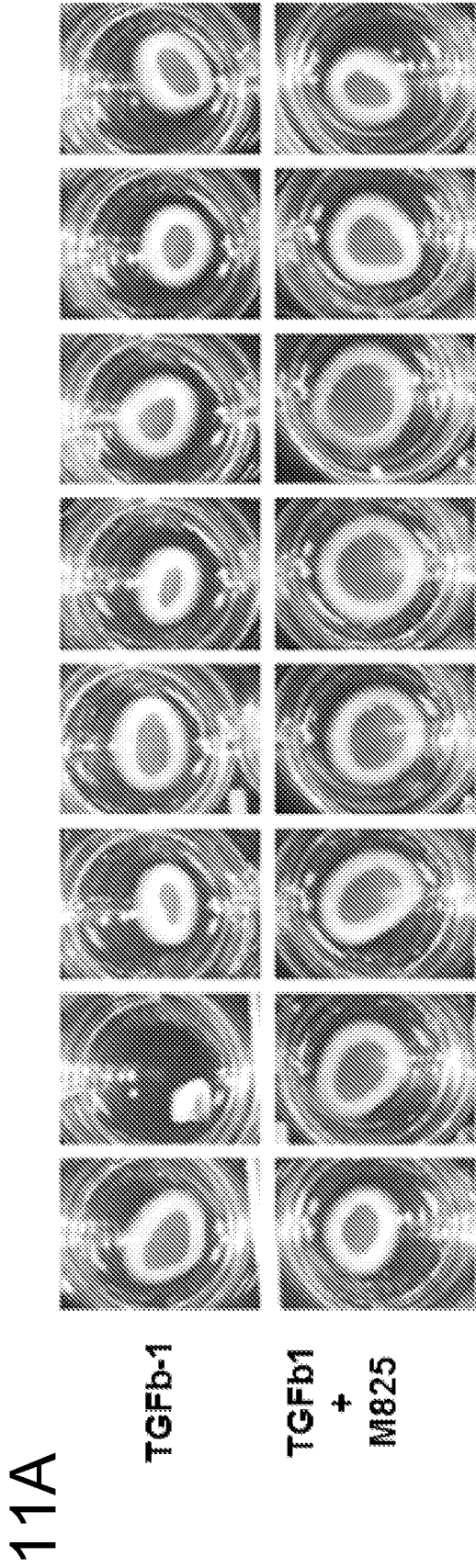
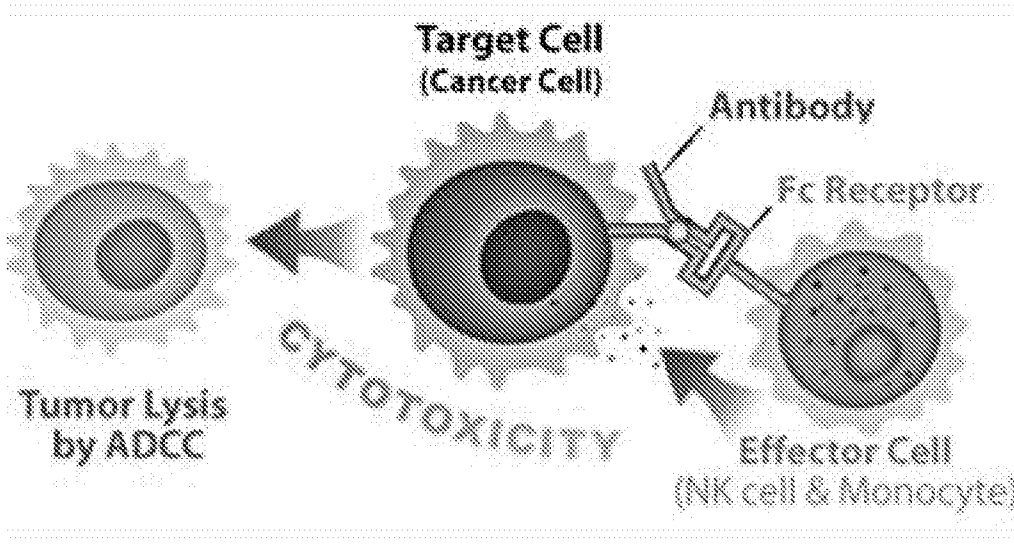


FIG. 12

12A



12B

ELISA binding to Fc γ R11a (CD16a)

