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(71) Applicant *(for all designated States except US)*: **MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH** [US/US]; 200 First Street S.W., Rochester, Minnesota 55905 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MARKOVIC, Svetomir N.** [US/US]; 1320 Creek Lane SW, Rochester, Minnesota 55902 (US). **NEVALA, Wendy K.** [US/US]; 71 N. River Court N.E., Rochester, Minnesota 55906 (US).

(74) Agent: **FINN III, J. Patrick**; FISH & RICHARDSON P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

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(54) Title: CANCER TREATMENTS

(57) Abstract:

# CANCER TREATMENTS

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 61/609,689, filed March 12, 2012, and U.S. Provisional Application Serial No. 61/484,151, filed May 9, 2011. The disclosures of the prior applications are considered part of (and are incorporated by reference in) the disclosure of this application.

## BACKGROUND

*1. Technical Field*

This document relates to methods and materials involved in treating cancer (e.g., skin cancers such as melanoma). For example, this document relates to methods and materials involved in using complexes containing albumin-containing nanoparticles (e.g., Abraxane® nanoparticles) and antibodies (e.g., anti-VEGF polypeptide antibodies such as Avastin®) to treat cancer. This document also relates to methods and materials involved in using Abraxane® in combination with an anti-VEGF polypeptide antibody (e.g., Avastin®) to treat skin cancer.

*2. Background Information*

Melanoma is the most serious form of skin cancer. It is a malignant tumor that originates in melanocytes, the cells which produce the pigment melanin that colors skin, hair, and eyes and is heavily concentrated in most moles. While it is not the most common type of skin cancer, melanoma underlies the majority of skin cancer-related deaths. About 48,000 deaths worldwide are registered annually as being due to malignant melanoma. Worldwide, there are about 160,000 new cases of melanoma each year. Melanoma is more frequent in white men and is particularly common in white populations living in sunny climates. Other risk factors for developing melanoma include a history of sunburn, excessive sun exposure, living in a sunny climate or at high altitude, having many moles or large moles, and a family or personal history of skin cancer.

Melanomas fall into four major categories. Superficial spreading melanoma can travel along the top layer of the skin before penetrating more deeply. Lentigo maligna

typically appears as a flat or mildly elevated mottled tan, brown, or dark brown discoloration and is found most often in the elderly. Nodular melanoma can occur anywhere on the body as a dark, protuberant papule or a plaque that varies from pearl to gray to black. Acral-lentiginous melanoma, although uncommon, is the most common form of melanoma in blacks. It can arise on palmar, plantar, or subungual skin. Metastasis of melanoma occurs via lymphatics and blood vessels. Local metastasis results in the formation of nearby satellite papules or nodules that may or may not be pigmented. Direct metastasis to skin or internal organs can occur.

## SUMMARY

This document provides methods and materials involved in treating cancer (e.g., skin cancers such as melanoma). For example, this document provides methods and materials for using complexes containing albumin-containing nanoparticles (e.g., Abraxane® nanoparticles) and antibodies (e.g., anti-VEGF polypeptide antibodies such as Avastin®) to treat cancer. This document also provides methods and materials involved in using Abraxane® in combination with an anti-VEGF polypeptide antibody (e.g., Avastin®) to treat skin cancer (e.g., melanoma). Abraxane® is available from Celgene Corp. and is a nanoparticle formulation that combines paclitaxel with human albumin. Avastin® is also known as bevacizumab and is available from Genentech Corp. and Roche Corp. Avastin® is a humanized monoclonal antibody that binds to vascular endothelial growth factor A. As described herein, *in vitro* mixing of albumin-containing nanoparticles (e.g., Abraxane® nanoparticles) and antibodies (e.g., bevacizumab, bevacizumab, trastuzamab, or rituxan) can result in the formation of macromolecular complexes, the characteristics of which (e.g., size, antibody content, or chemotherapeutic drug content) can be customized depending on need. In some cases, such macromolecular complexes can retain antibody mediated target binding specificity, can retain or exhibit enhanced chemotherapeutic tumor cell cytotoxicity, and can exhibit no additional toxicity beyond that of Abraxane® nanoparticles alone. As also described herein, contacting Abraxane® with an anti-VEGF polypeptide antibody (e.g., Avastin®) prior to administration to a human (e.g., a human melanoma cancer patient) can result in a complex that, when administered as a complex, has an increased ability to treat

melanoma as compared to a treatment regimen that includes administering Abraxane® and the anti-VEGF polypeptide antibody separately in a manner that does not form Abraxane®/anti-VEGF polypeptide antibody complexes.

The methods and materials provided herein can be used to increase the progression-free survival rate in skin cancer patients. Increasing progression-free survival can allow skin cancer patients to live longer.

In general, one aspect of this document features a method for treating a mammal having skin cancer. The method comprises, or consists essentially of, administering to the mammal a composition containing Abraxane®/anti-VEGF polypeptide antibody complexes (or complexes of (a) an anti-VEGF polypeptide antibody and (b) human albumin-containing nanoparticles having an agent other than placitaxel) under conditions wherein the length of progression-free survival is increased. The mammal can be a human. The skin cancer can be melanoma. The skin cancer can be stage IV melanoma. In some cases, a composition comprising Abraxane®/Avastin® complexes can be administered to the mammal. The composition can comprise an alkylating agent. The alkylating agent can be a platinum compound. The platinum compound can be carboplatin. The anti-VEGF polypeptide antibody can be a humanized antibody. The anti-VEGF polypeptide antibody can be bevacizumab. The composition can be administered by injection. The progression-free survival can be increased by 25 percent. The progression-free survival can be increased by 50 percent. The progression-free survival is increased by 75 percent. The progression-free survival can be increased by 100 percent. The composition can be administered under conditions wherein the time to progression is increased.

In another aspect, this document features a method for treating a mammal having cancer. The method comprises, or consists essentially of, administering, to the mammal, a composition comprising albumin-containing nanoparticle/antibody complexes, wherein the average diameter of the complexes is greater than 1 μm (e.g., between 1.1 μm and 5 μm, between 1.5 μm and 5 μm, between 4.5 and 20 μm, or between 5 and 20 μm). The mammal can be a human. The cancer can be skin cancer. The skin cancer can be melanoma. The skin cancer can be stage IV melanoma. The albumin-containing nanoparticle/antibody complexes can be Abraxane®/Avastin® complexes. The

composition or the albumin-containing nanoparticle/antibody complexes can comprise an alkylating agent. The alkylating agent can be a platinum compound. The platinum compound can be carboplatin. The antibodies of the albumin-containing nanoparticle/antibody complexes can be anti-VEGF polypeptide antibodies. The anti-VEGF polypeptide antibodies can be humanized antibodies. The anti-VEGF polypeptide antibodies can be bevacizumab. The composition can be administered by injection. The administration of the composition can be effective to increase progression-free survival by 25 percent. The administration of the composition can be effective to increase progression-free survival by 50 percent. The administration of the composition can be effective to increase progression-free survival by 75 percent. The administration of the composition can be effective to increase progression-free survival by 100 percent. The administration of the composition can be under conditions wherein the median time to progression for a population of mammals with the cancer is at least 150 days. The administration of the composition can be under conditions wherein the median time to progression for a population of mammals with the cancer is at least 165 days. The administration of the composition can be under conditions wherein the median time to progression for a population of mammals with the cancer is at least 170 days. The average diameter of the complexes can be from 1.1 μm to 5 μm. The average diameter of the complexes can be from 2 μm to 5 μm. The average diameter of the complexes can be from 3 μm to 5 μm. The average diameter of the complexes can be from 5 μm to 50 μm. The average diameter of the complexes can be from 10 μm to 50 μm. The average diameter of the complexes can be from 5 μm to 25 μm.

In another aspect, this document features a method for treating a mammal having cancer. The method comprises, or consists essentially of, administering, to the mammal, a composition comprising albumin-containing nanoparticle/antibody complexes, wherein the average diameter of at least 5 percent of the complexes of the composition is greater than 1 μm. The mammal can be a human. The cancer can be skin cancer. The skin cancer can be melanoma. The skin cancer can be stage IV melanoma. The albumin-containing nanoparticle/antibody complexes can be Abraxane®/Avastin® complexes. The composition or the albumin-containing nanoparticle/antibody complexes can comprise an alkylating agent. The alkylating agent can be a platinum compound. The platinum

compound can be carboplatin. The antibodies of the albumin-containing nanoparticle/antibody complexes can be anti-VEGF polypeptide antibodies. The anti-VEGF polypeptide antibodies can be humanized antibodies. The anti-VEGF polypeptide antibodies can be bevacizumab. The composition can be administered by injection. The administration of the composition can be effective to increase progression-free survival by 25 percent. The administration of the composition can be effective to increase progression-free survival by 50 percent. The administration of the composition can be effective to increase progression-free survival by 75 percent. The administration of the composition can be effective to increase progression-free survival by 100 percent. The administration of the composition can be under conditions wherein the median time to progression for a population of mammals with the cancer is at least 150 days. The administration of the composition can be under conditions wherein the median time to progression for a population of mammals with the cancer is at least 165 days. The administration of the composition can be under conditions wherein the median time to progression for a population of mammals with the cancer is at least 170 days. The average diameter of at least 5 percent of said complexes of said composition can be from 1.1 μm to 5 μm. The average diameter of at least 5 percent of said complexes of said composition can be from 2 μm to 5 μm. The average diameter of at least 5 percent of said complexes of said composition can be from 3 μm to 5 μm. The average diameter of at least 5 percent of said complexes of said composition can be from 5 μm to 50 μm. The average diameter of at least 5 percent of said complexes of said composition can be from 10 μm to 50 μm. The average diameter of at least 5 percent of said complexes of said composition can be from 5 μm to 25 μm. The average diameter of at least 10 percent of said complexes of said composition can be greater than 1 μm. The average diameter of at least 50 percent of said complexes of said composition can be greater than 1 μm. The average diameter of at least 75 percent of said complexes of said composition can be greater than 1 μm. The average diameter of at least 90 percent of said complexes of said composition can be greater than 1 μm.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those

described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

## DESCRIPTION OF DRAWINGS

Figure 1 is a diagram of an Abraxane® nanoparticle (labeled A) complexed with an anti-VEGF polypeptide antibody (bevacizumab; labeled B). In two of the three cases, the anti-VEGF polypeptide antibody is shown binding to a VEGF-A polypeptide (labeled V), and a fluorescently-labeled anti-VEGF antibody (labeled aV*) is shown bound to the VEGF-A polypeptide.

Figure 2 contains scatter plots of a flow cytometry analysis plotting the level of yellow fluorescence of A alone, A plus aV*, A plus B plus aV*, A plus V plus aV*, or A plus B plus V plus aV*. The labels are as indicated in Figure 1. These results demonstrate that A and B spontaneously associate and preserve a VEGF polypeptide binding potential.

Figure 3 is graph that contains the flow cytometry data from Figure 2.

Figure 4 is a repeat of the experiment of Figure 3, comparing A alone, A plus aV*, A plus B plus aV*, A plus V plus aV*, or A plus B plus V plus aV*. One difference is in Figure 3, 500 ng of VEGF was used. In Figure 4, 100 ng VEGF was used to visualize the complex.

Figure 5 is a graph plotting flow cytometry data of A plus B incubated in the presence of various concentrations of human plasma (1:1 to 1:16) followed by addition of V and aV*. These results indicate that human plasma diluted in a range of relative volumes (1:1 to 1:16) successfully inhibited the formation of the A+B complex relative to controls.

Figure 6 is a graph plotting flow cytometry data of A plus B incubated in the presence of various concentrations of human serum albumin (500 μg, 50 μg, 5 μg, 0.5 μg, and 0.05 μg/mL) followed by addition of V and aV*. These results indicate that incubation with serum albumin (concentrations ranging from 500 μg/mL to 0.05 μg/mL) did not affect the complexing of A and B.

Figure 7 is a graph plotting flow cytometry data of A plus B incubated in the presence of various concentrations of human polyclonal immunoglobulin (500 μg, 50 μg, 5 μg, 0.5 μg, and 0.05 μg/mL) followed by addition of V and aV*. These results indicate that incubation of A and B with a range of concentrations of human immunoglobulin (IVIG; 500 μg/mL to 0.05 μg/mL) partially inhibited A and B complexing.

Figure 8 contain A plus B complexing results in the presence of plasma (1:1), IVIG (0.5 mg/mL), or albumin (0.5 mg/mL). At the highest concentrations of plasma (1:1), IVIG (0.5 mg/mL), or albumin (0.5mg/mL) tested, the levels of relative inhibition of A plus B complexing differ in diminishing order.

Figure 9 contains photographs of light microscope images of Abraxane® (ABX) or mixtures of Abraxane® (ABX) and bevacizumab (BEV; 0.5, 5, 10, or 25 mg/mL) either 4 or 24 hours after mixing.

Figure 10 is a graph plotting flow cytometry results of Abraxane® alone, ABX:BEV complexes, and 2 μm standard beads.

Figure 11 is graph plotting the proliferation index for A375 cells (a melanoma tumor cell line) exposed to Abraxane® (ABX) only, Abraxane®:Herceptin (non-VEGF targeting) complexes, or Abraxane®:Bevacizumab (VEGF targeting) complexes at the indicated dose.

Figure 12 contains graphs plotting the percent BEV binding for ABX:BEV complexes exposed to 0.9% saline at room temperature or human plasma at 37°C for the indicated times.

Figure 13 contains a line graph plotting the proliferation index for A375 cells exposed to Abraxane® (ABX) only, cisplatin only, or Abraxane®:cisplatin complexes at the indicated dose and contains a bar graph plotting demonstrating that 30% of cisplatin (CDDP) remained unbound after ABX:cisplatin were mixed and incubated for 30 minutes.

Figure 14 contains scatter plots of a flow cytometry analysis of the indicated complexes containing Abraxane®.

Figure 15 contains photographs of Western blot analyses of the indicated materials assessed for bevacizumab or taxol.

Figure 16 contains graphs of the size distributions of the indicated complexes incubated for the indicated time.

Figure 17 contains graphs of the size distributions of the indicated complexes incubated for one hour at room temperature.

Figure 18 is a photograph of a Western blot analysis of ABX:BEV complexes exposed to serum for 15, 30, 45, or 60 minutes. The ABX:BEV complexes were formed by incubating either 6 mg or 8 mg of BEV with ABX for 30 minutes at room temperature. The primary antibody used for the Western blot was an anti-paclitaxel antibody. Lane 1: ABX: BEV (6 mg) exposed to serum for 15 minutes; Lane 2: ABX: BEV (6 mg) exposed to serum for 30 minutes; Lane 3: ABX: BEV (6 mg) exposed to serum for 45 minutes; Lane 4: ABX: BEV (6 mg) exposed to serum for 60 minutes; Lane 5: blank; Lane 6: ABX: BEV (8 mg) exposed to serum for 15 minutes; Lane 7: ABX: BEV (8 mg) exposed to serum for 30 minutes; Lane 8: ABX: BEV (8 mg) exposed to serum for 45 minutes; Lane 9: ABX: BEV (8 mg) exposed to serum for 60 minutes.

Figure 19 is a photograph of a Western blot analysis of mixtures of paclitaxel (0.1, 0.5, 1, or 2 mg) and BEV (4 mg) incubated together for 30 minutes at room temperature. The primary antibody used for the Western blot was an anti-paclitaxel antibody. Lane 1: Bev (4 mg); Lane 2: Taxol (2 mg); Lane 3: Taxol (2 mg) + Bev (4 mg); Lane 4: Taxol (1 mg) + Bev (4 mg); Lane 5: Taxol (0.5 mg) + Bev (4 mg); Lane 6: Taxol (0.1 mg) + Bev (4 mg).

Figure 20 contains graphs plotting the particle size distribution for ABX:BEV complexes as determined using a Mastersizer 2000E (Malvern Instruments Ltd., Worcestershire, England). ABX (20 mg/mL) and BEV (16, 24, or 32 mg/mL) were incubated for 1, 2, or 4 hours at room temperature. After incubation, the mixtures were diluted 1:4 for a final concentration of ABX (5 mg/mL) and BEV (4, 6, or 8 mg/mL), and the diluted samples analyzed using a Mastersizer 2000E.

## DETAILED DESCRIPTION

This document provides methods and materials involved in treating cancer (e.g., skin cancers such as melanoma). For example, this document provides methods and materials for using complexes containing albumin-containing nanoparticles (e.g., Abraxane® nanoparticles) and antibodies (e.g., anti-VEGF polypeptide antibodies such as Avastin®) to treat cancer.

The methods and materials provided herein can be used to treat any type of cancer. For example, the methods and materials provided herein can be used to treat skin cancer (e.g., melanoma) and breast cancer. In some cases, the methods and materials provided herein can be used to treat cancer (e.g., skin cancer) in any type of mammal including, without limitation, mice, rats, dogs, cats, horses, cows, pigs, monkeys, and humans. When treating skin cancer, any type of skin cancer, such as melanoma, can be treated using the methods and materials provided herein. For example, stage I, stage II, stage III, or stage IV melanoma can be treated. In some cases, a lymph node positive, a lymph node negative, or a metastatic melanoma can be treated as described herein.

In some cases, complexes containing albumin-containing nanoparticles (e.g., Abraxane® nanoparticles) and antibodies (e.g., anti-VEGF polypeptide antibodies such as Avastin®) can be designed to have an average diameter that is greater than 1 μm. For example, appropriate concentrations of albumin-containing nanoparticles and antibodies can be used such that complexes having an average diameter that is greater than 1 μm are formed. In some cases, manipulations such as centrifugation can be used to form preparations of albumin-containing nanoparticle/antibody complexes where the average diameter of those complexes is greater than 1 μm. In some cases, the preparations of albumin-containing nanoparticle/antibody complexes provided herein can have an average diameter that is between 1 μm and 5 μm (e.g., between 1.1 μm and 5 μm, between 1.5 μm and 5 μm, between 2 μm and 5 μm, between 2.5 μm and 5 μm, between 3 μm and 5 μm, between 3.5 μm and 5 μm, between 4 μm and 5 μm, between 4.5 μm and 5 μm, between 1.1 μm and 4.5 μm, between 1.1 μm and 4 μm, between 1.1 μm and 3.5 μm, between 1.1 μm and 3 μm, between 1.1 μm and 2.5 μm, between 1.1 μm and 2 μm, or between 1.1 μm and 1.5 μm). Preparations of albumin-containing nanoparticle/antibody complexes provided herein having an average diameter that is

between 1 μm and 5 μm can be administered systemically (e.g., intravenously) to treat cancers located within a mammal's body. In some cases, the preparations of albumin-containing nanoparticle/antibody complexes provided herein can have an average diameter that is between 5 μm and 50 μm (e.g., between 6 μm and 50 μm, between 7 μm and 50 μm, between 10 μm and 50 μm, between 15 μm and 50 μm, between 20 μm and 50 μm, between 25 μm and 50 μm, between 30 μm and 50 μm, between 35 μm and 50 μm, between 5 μm and 45 μm, between 5 μm and 40 μm, between 5 μm and 35 μm, between 5 μm and 30 μm, between 5 μm and 25 μm, between 5 μm and 20 μm, between 5 μm and 15 μm, or between 10 μm and 30 μm). Preparations of albumin-containing nanoparticle/antibody complexes provided herein having an average diameter that is between 5 μm and 50 μm can be administered into a tumor (e.g., intratumorally) or in a region of a tumor located within a mammal's body.

In some cases, a preparation of albumin-containing nanoparticle/antibody complexes provided herein can have greater than 60 percent (e.g., greater than 65, 70, 75, 80, 90, 95, or 99 percent) of the complexes having a diameter that is between 1 μm and 5 μm (e.g., between 1.1 μm and 5 μm, between 1.5 μm and 5 μm, between 2 μm and 5 μm, between 2.5 μm and 5 μm, between 3 μm and 5 μm, between 3.5 μm and 5 μm, between 4 μm and 5 μm, between 4.5 μm and 5 μm, between 1.1 μm and 4.5 μm, between 1.1 μm and 4 μm, between 1.1 μm and 3.5 μm, between 1.1 μm and 3 μm, between 1.1 μm and 2.5 μm, between 1.1 μm and 2 μm, or between 1.1 μm and 1.5 μm). Preparation of albumin-containing nanoparticle/antibody complexes provided herein having greater than 60 percent (e.g., greater than 65, 70, 75, 80, 90, 95, or 99 percent) of the complexes with a diameter that is between 1 μm and 5 μm can be administered systemically (e.g., intravenously) to treat cancers located within a mammal's body. In some cases, a preparation of albumin-containing nanoparticle/antibody complexes provided herein can have greater than 60 percent (e.g., greater than 65, 70, 75, 80, 90, 95, or 99 percent) of the complexes having a diameter that is between 5 μm and 50 μm (e.g., between 6 μm and 50 μm, between 7 μm and 50 μm, between 10 μm and 50 μm, between 15 μm and 50 μm, between 20 μm and 50 μm, between 25 μm and 50 μm, between 30 μm and 50 μm, between 35 μm and 50 μm, between 5 μm and 45 μm, between 5 μm and 40 μm, between 5 μm and 35 μm, between 5 μm and 30 μm, between 5 μm and 25 μm, between 5 μm and

20 μm, between 5 μm and 15 μm, or between 10 μm and 30 μm). Preparation of albumin-containing nanoparticle/antibody complexes provided herein having greater than 60 percent (e.g., greater than 65, 70, 75, 80, 90, 95, or 99 percent) of the complexes with a diameter that is between 5 μm and 50 μm can be administered into a tumor (e.g., intratumorally) or in a region of a tumor located within a mammal's body.

In general, albumin-containing nanoparticles such as Abraxane® can be contacted with an antibody such as an anti-VEGF polypeptide antibody (e.g., Avastin®) prior to administration to a human to form an albumin-containing nanoparticle/antibody complex (e.g., an Abraxane®/anti-VEGF polypeptide antibody complex). Any appropriate albumin-containing nanoparticle preparation and any appropriate antibody can be used as described herein. For example, Abraxane® nanoparticles can be used as described herein. Examples of antibodies that can be used to form albumin-containing nanoparticle/antibody complexes as described herein include, without limitation, bevacizumab (Avastin®), trastuzamab, and rituxan. For example, an appropriate dose of Abraxane® and an appropriate dose of Avastin® can be mixed together in the same container. This mixture can be incubated at an appropriate temperature (e.g., room temperature, between 15 °C and 30 °C, between 15 °C and 25 °C, between 20 °C and 30 °C, or between 20 °C and 25 °C) for a period of time (e.g., about 30 minutes, or between about 5 minutes and about 60 minutes, between about 5 minutes and about 45 minutes, between about 15 minutes and about 60 minutes, between about 15 minutes and about 45 minutes, between about 20 minutes and about 400 minutes, or between about 25 minutes and about 35 minutes) before being administered to a cancer patient (e.g., a melanoma patient). In some cases, Abraxane® can be contacted with an anti-VEGF polypeptide antibody by injecting both Abraxane® and the anti-VEGF polypeptide antibody either individually or as a pre-mixed combination into an IV bag containing an IV bag solution. The contents of the IV bag including Abraxane®/anti-VEGF polypeptide antibody complexes can be introduced into the patient to be treated.

In some cases, albumin-containing nanoparticles such as Abraxane® can be contacted with an antibody such as an anti-VEGF polypeptide antibody (e.g., Avastin®) to form albumin-containing nanoparticle/antibody complexes (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) that are stored prior to being administered to a

cancer patient (e.g., a melanoma patient). For example, a composition containing albumin-containing nanoparticle/antibody complexes can be formed as described herein and stored for a period of time (e.g., days or weeks) prior to being administered to a cancer patient.

Any appropriate method can be used to obtain albumin-containing nanoparticles such as Abraxane® and an antibody such as an anti-VEGF polypeptide antibody. For example, Abraxane® can be obtained from Celgene Corp. or as described elsewhere (U.S. Patent No. 6,537,579). Avastin® can be obtained from Genentech Corp. or Roche Corp. or as described elsewhere (U.S. Patent No. 6,054,297).

In some cases, the combination of an albumin-containing nanoparticle such as Abraxane® and an antibody such as anti-VEGF polypeptide antibody can include one or more other agents such as an alkylating agent (e.g., a platinum compound). Examples of platinum compounds that can be used as an alkylating agent include, without limitation, carboplatin (Paraplatin®), cisplatin (Platinol®), oxaliplatin (Eloxatin®), and BBR3464. Examples of other agents that can be included within an albumin-containing nanoparticle/antibody complex provided herein include, without limitation, bendamustine, bortezomib, cabazitaxel, chlorambucil, dasatinib, docetaxel, doxorubicin, epirubicin, erlotinib, etoposide, everolimus, gefitinib, idarubicin, hydroxyurea, imatinib, lapatinib, melphalan, mitoxantrone, nilotinib, oxaliplatin, pazopanib, pemetrexed, romidepsin, sorafenib, sunitinib, teniposide, vinblastine, and vinorelbine.

Any appropriate method can be used to administer an albumin-containing nanoparticle/antibody complex provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) to a mammal. For example, a composition containing albumin-containing nanoparticle/antibody complexes such as Abraxane®/anti-VEGF polypeptide antibody complexes can be administered via injection (e.g., subcutaneous injection, intramuscular injection, intravenous injection, or intrathecal injection).

Before administering a composition containing an albumin-containing nanoparticle/antibody complex provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) to a mammal, the mammal can be assessed to determine whether or not the mammal has cancer (e.g., skin cancer). Any appropriate method can be used to determine whether or not a mammal has cancer (e.g., skin cancer). For example, a

mammal (e.g., human) can be identified as having skin cancer using standard diagnostic techniques. In some cases, a tissue biopsy can be collected and analyzed to determine whether or not a mammal has skin cancer.

After identifying a mammal as having cancer (e.g., skin cancer), the mammal can be administered a composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes). For example, a composition containing Abraxane®/anti-VEGF polypeptide antibody complexes can be administered prior to or in lieu of surgical resection of a tumor. In some cases, a composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) can be administered following resection of a tumor.

A composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) can be administered to a mammal in any appropriate amount, at any appropriate frequency, and for any appropriate duration effective to achieve a desired outcome (e.g., to increase progression-free survival). In some cases, a composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) can be administered to a mammal having cancer (e.g., skin cancer) to reduce the progression rate of the cancer (e.g., melanoma) by 5, 10, 25, 50, 75, 100, or more percent. For example, the progression rate can be reduced such that no additional cancer progression is detected. Any appropriate method can be used to determine whether or not the progression rate of cancer (e.g., skin cancer) is reduced. For example, the progression rate of skin cancer can be assessed by imaging tissue at different time points and determining the amount of cancer cells present. The amounts of cancer cells determined within tissue at different times can be compared to determine the progression rate. After treatment as described herein, the progression rate can be determined again over another time interval. In some cases, the stage of cancer (e.g., skin cancer) after treatment can be determined and compared to the stage before treatment to determine whether or not the progression rate was reduced.

In some cases, a composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF

polypeptide antibody complexes) can be administered to a mammal having cancer (e.g., skin cancer) under conditions where progression-free survival is increased (e.g., by 5, 10, 25, 50, 75, 100, or more percent) as compared to the median progression-free survival of corresponding mammals having untreated cancer (e.g., untreated skin cancer) or the median progression-free survival of corresponding mammals having cancer (e.g., skin cancer) treated with Abraxane® and an antibody (e.g., an anti-VEGF polypeptide antibody) without forming Abraxane®/antibody complexes (e.g., without forming Abraxane®/anti-VEGF polypeptide antibody complexes). In some cases, a composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) can be administered to a mammal having cancer (e.g., skin cancer) to increase progression-free survival by 5, 10, 25, 50, 75, 100, or more percent as compared to the median progression-free survival of corresponding mammals having cancer (e.g., skin cancer) and having received Abraxane® or an antibody (e.g., an anti-VEGF polypeptide antibody) alone. Progression-free survival can be measured over any length of time (e.g., one month, two months, three months, four months, five months, six months, or longer).

In some cases, a composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) can be administered to a mammal having cancer (e.g., skin cancer) under conditions where the 8-week progression-free survival rate for a population of mammals is 65% or greater (e.g., 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80% or greater) than that observed in a population of comparable mammals not receiving a composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes). In some cases, a composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) can be administered to a mammal having cancer (e.g., skin cancer) under conditions where the median time to progression for a population of mammals is at least 150 days (e.g., at least 155, 160, 163, 165, or 170 days).

An effective amount of a composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF

polypeptide antibody complexes) can be any amount that reduces the progression rate of cancer (e.g., skin cancer), increases the progression-free survival rate, or increases the median time to progression without producing significant toxicity to the mammal. Typically, an effective amount of Abraxane® can be from about 50 mg/m$^2$ to about 150 mg/m$^2$ (e.g., about 80 mg/m$^2$), and an effective amount of an anti-VEGF polypeptide antibody such as bevacizumab can be from about 5 mg/kg to about 20 mg/kg (e.g., about 10 mg/kg). If a particular mammal fails to respond to a particular amount, then the amount of Abraxane® or anti-VEGF polypeptide antibody can be increased by, for example, two fold. After receiving this higher concentration, the mammal can be monitored for both responsiveness to the treatment and toxicity symptoms, and adjustments made accordingly. The effective amount can remain constant or can be adjusted as a sliding scale or variable dose depending on the mammal's response to treatment. Various factors can influence the actual effective amount used for a particular application. For example, the frequency of administration, duration of treatment, use of multiple treatment agents, route of administration, and severity of the cancer (e.g., skin cancer) may require an increase or decrease in the actual effective amount administered.

The frequency of administration can be any frequency that reduces the progression rate of cancer (e.g., skin cancer), increases the progression-free survival rate, or increases the median time to progression without producing significant toxicity to the mammal. For example, the frequency of administration can be from about once a month to about three times a month, or from about twice a month to about six times a month, or from about once every two months to about three times every two months. The frequency of administration can remain constant or can be variable during the duration of treatment. A course of treatment with a composition containing Abraxane®/anti-VEGF polypeptide antibody complexes can include rest periods. For example, a composition containing Abraxane®/anti-VEGF polypeptide antibody complexes can be administered over a two week period followed by a two week rest period, and such a regimen can be repeated multiple times. As with the effective amount, various factors can influence the actual frequency of administration used for a particular application. For example, the effective amount, duration of treatment, use of multiple treatment agents, route of administration, and severity of the skin cancer may require an increase or decrease in

administration frequency.

An effective duration for administering a composition provided herein can be any duration that reduces the progression rate of cancer (e.g., skin cancer), increases the progression-free survival rate, or increases the median time to progression without producing significant toxicity to the mammal. Thus, the effective duration can vary from several days to several weeks, months, or years. In general, the effective duration for the treatment of skin cancer can range in duration from several weeks to several months. In some cases, an effective duration can be for as long as an individual mammal is alive. Multiple factors can influence the actual effective duration used for a particular treatment. For example, an effective duration can vary with the frequency of administration, effective amount, use of multiple treatment agents, route of administration, and severity of the cancer (e.g., skin cancer).

A composition containing albumin-containing nanoparticle/antibody complexes provided herein (e.g., Abraxane®/anti-VEGF polypeptide antibody complexes) can be in any appropriate form. For example, a composition provided herein can be in the form of a solution or powder with or without a diluent to make an injectable suspension. A composition also can contain additional ingredients including, without limitation, pharmaceutically acceptable vehicles. A pharmaceutically acceptable vehicle can be, for example, saline, water, lactic acid, mannitol, or combinations thereof.

After administering a composition provided herein to a mammal, the mammal can be monitored to determine whether or not the cancer (e.g., skin cancer) was treated. For example, a mammal can be assessed after treatment to determine whether or not the progression rate of melanoma was reduced (e.g., stopped). As described herein, any method can be used to assess progression and survival rates.

In some cases, nanoparticles containing albumin (e.g., nanoparticles with an albumin shell) and an agent other than placitaxel can be used as described herein in place of or in combination with Abraxane®. For example, albumin-containing nanoparticles designed to carry a cancer chemotherapeutic agent can be used to form nanoparticle/anti-VEGF polypeptide antibody complexes that can be used as described herein. An example of such a cancer chemotherapeutic agent includes, without limitation, vinblastine.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

## EXAMPLES

### Example 1 – Contacting Abraxane® with Avastin® results in the formation of Abraxane® / Avastin® complexes

Abraxane® (1 mg/mL) and Avastin (25 mg/mL) were stored at 4 °C. 10 μg (10 μL) of Abraxane® nanoparticles and 500 μg (20 μL) of Avastin were mixed in a total volume of 30 μL. The Abraxane® and Avastin were incubated at room temperature for 30 minutes.

After incubation, the Abraxane® nanoparticles were spun and washed three times with 1x PBS to eliminate unbound bevacizumab. The nanoparticles were spun at 5000 rpm for 5 minutes and resuspended in 50 μL of 1x PBS.

100 ng or 500 ng of VEGF was added to each tube for 30 minutes at room temperature, and the washes were repeated to eliminate unbound VEGF. PE anti-human VEGF was added at a 1:50 dilution, and the particles were once again incubated and washed. Visualization was done by flow cytometry, and percentage of PE (VEGF) positive particles was determined (Figures 1-4). Various combinations of agents were tested as indicated in the figures. These results demonstrate that Abraxane® and bevacizumab spontaneously associate in a manner that preserves VEGF binding potential.

Abraxane® nanoparticles were mixed with varying concentrations of bevacizumab (0.5, 5, 10, and 25 mg/mL). The particles were viewed by light microscopy at 4 and 24 hours after mixing. The macromolecular size of the ABX:BEV complexes was dependent on the concentration of the bevacizumab added and the Abraxane® nanoparticles (Figure 9). Once a maximum size was reached, the ABX:BEV complexes began to break down within about 24 hours (Figure 9).

Bevacizumab was added to Abraxane® nanoparticles in varying concentrations (0.5, 5, 10, 25 mg/mL) and incubated for 30 minutes at room temperature to allow complex formation. Abraxane® nanoparticles alone, ABX:BEV complexes, and 2 μm standard beads were visualized by flow cytometry. The complex size increased with

increased concentrations of bevacizumab (Figure 10). The larger the particle-size, the further to the right the peak will be. These results demonstrate that complex size can be manipulated by varying the concentration of bevacizumab added.

In another study, Abraxane® nanoparticles and bevacizumab were incubated together for 4 hours and overnight at 1 mg/mL or 10 mg/mL. Abraxane® nanoparticles alone were also incubated for 4 hours and overnight as a control. After the allotted time was reached, the complexes were spun down at 7500 RPM for 5 minutes. The supernatants were collected and mixed 1:1 with Laemmli buffer and boiled at 100 degrees for 3 minutes. 20 μL of sample was loaded onto a 7.5% Tris-HCl Criteron gel. A high range molecular weight marker (BioRad) was added for size determination. The gel was run for 3 hours at 75V.

After the gel ran to completion, the gel was placed in a transfer cassette so the proteins could be moved onto a PVDF membrane. The transfer took place overnight at 4°C running at 20V. The membrane was removed and rocked in TBST containing 5% milk to block for 3 hours at room temperature. The primary antibodies used were Rabbit anti-Taxol (1:500 dilution) and goat anti-mouse IgG-Fab specific-HRP conjugated (1:500 dilution). Antibodies were diluted into 10 mL of TBST with 5% milk. Primary antibodies were allowed to bind overnight at 4°C while rocking.

Primary antibodies were removed, and the membranes were washed three times for 10 minutes with TBST. The taxol blot was incubated in a1:1000 dilution of secondary anti-rabbit IgG-HRP for 1.5 hours rocking at room temperature. The anti-mouse IgG (Bevacizumab) membrane was incubated in ECL detection reagent (GE Amershem) for 5 minutes before it was exposed to film. Membrane was exposed for 10 seconds, 1 minute, and 5 minutes.

After the incubation in secondary antibody, the taxol blot was washed with TBST for 10 minutes three times. The membrane was then placed in ECL detection reagent for 5 minutes and exposed to film. The exposure times were 1 second, 2 seconds, and 10 seconds.

The IgG blot was specific for the mouse portion of the bevacizumab humanized antibody. A clear concentration dependent increase from complexes mixed at 1 mg/mL to 10 mg/mL was observed (Figure 15). Taxol is a small molecule around 20kDa. Free

taxol was observed at the bottom of the blot, but it also was observed running at the bevacizumab molecular weight (149 kDa; Figure 15). These results demonstrate that taxol was bound to the bevacizumab in the supernatant after the large particles were removed by centrifugation.

In another study, Abraxane® nanoparticles and bevacizumab were incubated for various times (1, 4, and 12 hours), and the particle size distribution of the resulting complexes was determined relative to Abraxane® nanoparticles alone using the Malvern Mastersizer 2000E. The size of the complexes generated was a function of antibody concentration and incubation time (Figures 16 and 17). In Figure 16, 1 and 10 mg/mL of bevacizumab was incubated with Abraxane® nanoparticles for 4 hours and overnight. The complexes generated with 10 mg/mL bevacizumab were much larger (8.479 μm) than those with 1 mg/mL bevacizumab (0.165 μm). After an overnight incubation, the larger complexes began to break down.

In Figure 17, complex size increased with concentration of bevacizumab added when incubated for 1 hour at room temperature. In addition, larger complexes were formed when 1 mg/mL bevacizumab was incubated with Abraxane® nanoparticles, spun, and resuspended as compared to the size observed when the same amount (1 mg/mL) of bevacizumab was incubated with Abraxane® nanoparticles without spinning the preparation (Figure 17). These results demonstrate that complex size can be manipulated by altering concentrations, by manual forces (e.g., centrifugation), or by both.

In another study, Abraxane® nanoparticles were dissolved at a concentration of 20 mg/mL, and bevacizumab was added at a final concentration of 16, 24, or 32 mg/mL. The mixtures were incubated at room temperature for various times (1, 2, and 4 hours). After this incubation, the mixture was diluted 1:4 (final concentration of Abraxane = 5 mg/mL; final concentrations of bevacizumab = 4, 6, or 8 mg/mL). The particle size distribution of the resulting complexes was determined relative to Abraxane® nanoparticles alone using the Malvern Mastersizer 2000E. The size of the complexes generated was a function of antibody concentration and incubation time (Figure 20).

Abraxane and bevacizmab were mixed and incubated for 30 minutes at room temperature to allow complex formation. Mice were injected with 100 μL of the

complexes containing 5 mg of Abraxane and 1 mg of bevacizumab in the dorsal tail vein. Injection of the complexes did not harm any mice.

Example 2 – Human plasma inhibits the formation of Abraxane® / Avastin® complexes

10 μL (10 μg) of Abraxane® was added to eppendorf tubes, and 500 μg (25 μL) of avastin was added and resuspended in a final volume of 50 μL. Human plasma was titrated using 1:2 dilutions (1:2, 1:4, 1:8, or 1:16). 50 μL of plasma and 50 μL of each plasma titration were added to the tubes with Abraxane® and avastin. In some cases, human serum albumin (500 μg, 50 μg, 5 μg, 0.5 μg, or 0.05 μg/mL) or human polyclonal immunoglobulin (500 μg, 50 μg, 5 μg, 0.5 μg, and 0.05 μg/mL) was added to the tubes in place of human plasma.

After a 30 minute incubation at room temperature, the Abraxane® nanoparticles were washed in 1x PBS twice. 100 ng of VEGF was added to each tube for 30 minutes at room temperature, and the washes were repeated. PE anti-human VEGF was added at a 1:50 dilution, and particles were once again incubated and washed. Visualization was done by flow cytometry, and percentage of PE (VEGF) positive particles was determined (Figure 5-8).

Example 3 – Abraxane® / Avastin® complexes have a higher level of cell toxicity than Abraxane® alone or Abraxane® / Herceptin complexes

The VEGF producing melanoma tumor cell line, A375, was incubated overnight in the presence of Abraxane® nanoparticles only, Abraxane® / Herceptin (non-VEGF targeting) complexes, and Abraxane® / Avastin® (ABX:BEV; VEGF targeting) complexes. Increasing doses of drug were added to the cells to give 6.25, 12.5, 25, 50, 100, and 200 μg/mL of taxol. After the overnight incubation, cell proliferation was determined by measuring the level of DNA synthesis. A higher level of cell toxicity (less DNA synthesis) of cells incubated with the VEGF targeting complexes (ABX:BEV) relative the ABX alone and non-VEGF targeted complexes (ABX:HER) (Figure 11).

Example 4 – Stability of Abraxane® / Avastin® complexes

Abraxane® / Avastin® complexes were fluorescently labeled such that both the albumin of the Abraxane® and the bevacizumab were directly labeled with a fluorescent marker. The complexes were visualized by flow cytometry after 0, 1, 2, 3, 4, 24, and 48 hours in 0.9% saline at room temperature and after 0, 15, 30, 60, and 120 minutes in human plasma at 37°C. The complexes were stable in saline at room temperature with only about 10% loss at 24 hours (Figure 12). In human plasma at 37°C, the complexes began to break down in about 15 minutes and were completely undetectable by 120 minutes.

Example 5 – Abraxane® / Cisplatin complexes

Abraxane® nanoparticles were incubated with cisplatin (cisplatinum or cis-diamminedichloroplatinum(II) (CDDP)) for 30 minutes at 37°C. The particles were spun, and the supernatant was tested by HPLC to determine how much free cisplatin was in suspension. Cisplatin spontaneously bound to the Abraxane® nanoparticles, and the amount remaining in suspension after the 30 minute incubation with the Abraxane® nanoparticles was only about 30% of the original concentration (Figure 13). These results demonstrate that about 70% of the cisplatin bound to the Abraxane® nanoparticles.

In another experiment, Abraxane® / cisplatin complexes were generated as described above and added to A375 tumor cells. After an overnight incubation, proliferation of the cells was measured by determining the level of DNA synthesis. The toxicity of the Abraxane® / cisplatin complexes was measured relative to the two drugs individually. The Abraxane® / cisplatin complexes were more toxic to cells (lower level of DNA synthesis) than Abraxane® alone but less toxic than cisplatin alone (Figure 13). These results demonstrate that cisplatin can be bound to Abraxane® nanoparticles and delivered to tumors without the highly toxic side effects of cisplatin alone.

Example 6 – Abraxane® / antibody complexes

Three therapeutic monoclonal antibodies (bevacizumab, trastuzamab, and rituxan) were fluorescently labeled and incubated with fluorescently labeled Abraxane® nanoparticles. The particles were spun down, washed, and visualized by flow cytometry.

All three of these recombinant therapeutic antibodies spontaneously formed complexes with Abraxane® nanoparticles (Figure 14). These results demonstrate that albumin-containing nanoparticles can be used to form larger complexes not only with bevacizumab antibodies but also with other antibodies such as trastuzamab and rituxan.

Taken together, the results provided herein demonstrate that *in vitro* mixing of albumin-containing nanoparticles (e.g., Abraxane® nanoparticles) and antibodies (e.g., bevacizumab, trastuzamab, or rituxan) leads to macromolecular complex formation, the characteristics of which (e.g., size, antibody content, or chemotherapeutic drug content) can be customized depending on need. These results also demonstrate that the macromolecular complexes retain antibody mediated target binding specificity, retain or exhibit enhanced chemotherapeutic tumor cell cytotoxicity, and exhibit no additional toxicity beyond that of Abraxane® nanoparticles alone.

Example 7 – Abraxane® / Avastin® complexes disassociate in serum

The following was performed to determine what happens to Abraxane® / Avastin® complexes in serum over time. 6 mg or 8 mg of Avastin® were bound to Abraxane® for 30 minutes at room temperature. The complexes were incubated with serum for 15, 30, 45, or 60 minutes. After this incubation, the serum/complex solution was spun down at 10,000 rpm for 10 minutes at 4 °C. The supernatants were collected, separated using gel electrophoresis, and analyzed via Western blotting with an anti-paclitaxel antibody and an HRP-conjugated secondary antibody.

Incubation in the presence of serum resulted in complex disassociation, not disintegration (Figure 18).

Example 8 – Bevacizumab does not bind free paclitaxel

The following was performed to determine if bevacizumab binds free paclitaxel. 4 mg of bevacizumab was incubated with paclitaxel (0.1, 0.5, 1, or 2 mg) for 30 minutes at room temperature. After this incubation, the mixtures were separated using gel electrophoresis and analyzed via Western blotting with an anti-paclitaxel antibody and an HRP-conjugated secondary antibody.

Bevacizumab did not bind free paclitaxel (Figure 19).

## OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

**WHAT IS CLAIMED IS:**

1. A method for treating a mammal having skin cancer, said method comprising administering to said mammal a composition containing Abraxane®/anti-VEGF polypeptide antibody complexes under conditions wherein the length of progression-free survival is increased.

2. The method of claim 1, wherein said mammal is a human.

3. The method of claim 1, wherein said skin cancer is melanoma.

4. The method of claim 1, wherein said skin cancer is stage IV melanoma.

5. The method of claim 1, wherein a composition comprising Abraxane®/Avastin® complexes is administered to said mammal.

6. The method of claim 1, wherein said composition comprises an alkylating agent.

7. The method of claim 6, wherein said alkylating agent is a platinum compound.

8. The method of claim 7, wherein said platinum compound is carboplatin.

9. The method of claim 1, wherein said anti-VEGF polypeptide antibody is a humanized antibody.

10. The method of claim 1, wherein said anti-VEGF polypeptide antibody is bevacizumab.

11. The method of claim 1, wherein said composition is administered by injection.

12. The method of claim 1, wherein said progression-free survival is increased by 25

percent.

13. The method of claim 1, wherein said progression-free survival is increased by 50 percent.

14. The method of claim 1, wherein said progression-free survival is increased by 75 percent.

15. The method of claim 1, wherein said progression-free survival is increased by 100 percent.

16. The method of claim 1, wherein said composition is administered under conditions wherein the time to progression is increased.

17. A method for treating a mammal having cancer, wherein said method comprises administering, to said mammal, a composition comprising albumin-containing nanoparticle/antibody complexes, wherein the average diameter of said complexes is greater than 1 μm.

18. The method of claim 17, wherein said mammal is a human.

19. The method of claim 17, wherein said cancer is skin cancer.

20. The method of claim 19, wherein said skin cancer is melanoma.

21. The method of claim 19, wherein said skin cancer is stage IV melanoma.

22. The method of claim 17, wherein said albumin-containing nanoparticle/antibody complexes are Abraxane®/Avastin® complexes.

23. The method of claim 17, wherein said composition or said albumin-containing

nanoparticle/antibody complexes comprise an alkylating agent.

24. The method of claim 23, wherein said alkylating agent is a platinum compound.

25. The method of claim 24, wherein said platinum compound is carboplatin.

26. The method of claim 17, wherein the antibodies of said albumin-containing nanoparticle/antibody complexes are anti-VEGF polypeptide antibodies.

27. The method of claim 26, wherein said anti-VEGF polypeptide antibodies are humanized antibodies.

28. The method of claim 26, wherein said anti-VEGF polypeptide antibodies are bevacizumab.

29. The method of claim 17, wherein said composition is administered by injection.

30. The method of claim 17, wherein said administration of said composition is effective to increase progression-free survival by 25 percent.

31. The method of claim 17, wherein said administration of said composition is effective to increase progression-free survival by 50 percent.

32. The method of claim 17, wherein said administration of said composition is effective to increase progression-free survival by 75 percent.

33. The method of claim 17, wherein said administration of said composition is effective to increase progression-free survival by 100 percent.

34. The method of claim 17, wherein said administration of said composition is under conditions wherein the median time to progression for a population of mammals with

said cancer is at least 150 days.

35. The method of claim 17, wherein said administration of said composition is under conditions wherein the median time to progression for a population of mammals with said cancer is at least 165 days.

36. The method of claim 17, wherein said administration of said composition is under conditions wherein the median time to progression for a population of mammals with said cancer is at least 170 days.

37. The method of claim 17, wherein the average diameter of said complexes is from 1.1 μm to 5 μm.

38. The method of claim 17, wherein the average diameter of said complexes is from 2 μm to 5 μm.

39. The method of claim 17, wherein the average diameter of said complexes is from 3 μm to 5 μm.

40. The method of claim 17, wherein the average diameter of said complexes is from 5 μm to 50 μm.

41. The method of claim 17, wherein the average diameter of said complexes is from 10 μm to 50 μm.

42. The method of claim 17, wherein the average diameter of said complexes is from 5 μm to 25 μm.

43. A method for treating a mammal having cancer, wherein said method comprises administering, to said mammal, a composition comprising albumin-containing nanoparticle/antibody complexes, wherein the average diameter of at least 5 percent of

said complexes of said composition is greater than 1 μm.

44. The method of claim 43, wherein said mammal is a human.

45. The method of claim 43, wherein said cancer is skin cancer.

46. The method of claim 45, wherein said skin cancer is melanoma.

47. The method of claim 45, wherein said skin cancer is stage IV melanoma.

48. The method of claim 43, wherein said albumin-containing nanoparticle/antibody complexes are Abraxane®/Avastin® complexes.

49. The method of claim 43, wherein said composition or said albumin-containing nanoparticle/antibody complexes comprise an alkylating agent.

50. The method of claim 49, wherein said alkylating agent is a platinum compound.

51. The method of claim 50, wherein said platinum compound is carboplatin.

52. The method of claim 43, wherein the antibodies of said albumin-containing nanoparticle/antibody complexes are anti-VEGF polypeptide antibodies.

53. The method of claim 52, wherein said anti-VEGF polypeptide antibodies are humanized antibodies.

54. The method of claim 52, wherein said anti-VEGF polypeptide antibodies are bevacizumab.

55. The method of claim 43, wherein said composition is administered by injection.

56. The method of claim 43, wherein said administration of said composition is effective to increase progression-free survival by 25 percent.

57. The method of claim 43, wherein said administration of said composition is effective to increase progression-free survival by 50 percent.

58. The method of claim 43, wherein said administration of said composition is effective to increase progression-free survival by 75 percent.

59. The method of claim 43, wherein said administration of said composition is effective to increase progression-free survival by 100 percent.

60. The method of claim 43, wherein said administration of said composition is under conditions wherein the median time to progression for a population of mammals with said cancer is at least 150 days.

61. The method of claim 43, wherein said administration of said composition is under conditions wherein the median time to progression for a population of mammals with said cancer is at least 165 days.

62. The method of claim 43, wherein said administration of said composition is under conditions wherein the median time to progression for a population of mammals with said cancer is at least 170 days.

63. The method of claim 43, wherein the average diameter of at least 5 percent of said complexes of said composition is from 1.1 μm to 5 μm.

64. The method of claim 43, wherein the average diameter of at least 5 percent of said complexes of said composition is from 2 μm to 5 μm.

65. The method of claim 43, wherein the average diameter of at least 5 percent of said

complexes of said composition is from 3 μm to 5 μm.

66. The method of claim 43, wherein the average diameter of at least 5 percent of said complexes of said composition is from 5 μm to 50 μm.

67. The method of claim 43, wherein the average diameter of at least 5 percent of said complexes of said composition is from 10 μm to 50 μm.

68. The method of claim 43, wherein the average diameter of at least 5 percent of said complexes of said composition is from 5 μm to 25 μm.

69. The method of claim 43, wherein the average diameter of at least 10 percent of said complexes of said composition is greater than 1 μm.

70. The method of claim 43, wherein the average diameter of at least 50 percent of said complexes of said composition is greater than 1 μm.

71. The method of claim 43, wherein the average diameter of at least 75 percent of said complexes of said composition is greater than 1 μm.

72. The method of claim 43, wherein the average diameter of at least 90 percent of said complexes of said composition is greater than 1 μm.

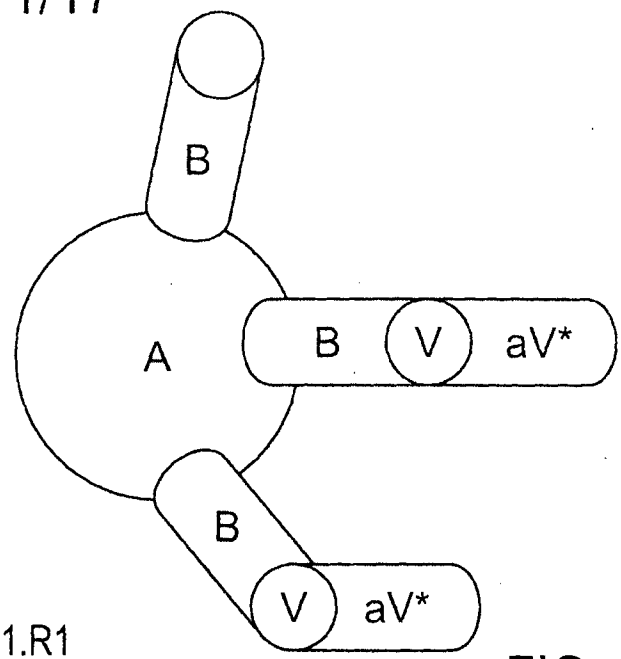
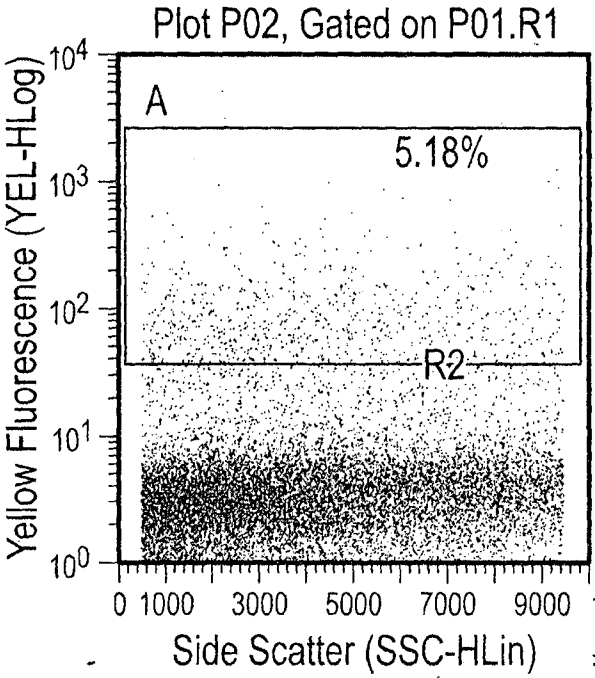

B
A
B
V
aV*
B
V
aV*

FIG. 1


Plot P02, Gated on P01.R1
A
5.18%
R2
Yellow Fluorescence (YEL-HLog)
$10^4$
$10^3$
$10^2$
$10^1$
$10^0$
0 1000 3000 5000 7000 9000
Side Scatter (SSC-HLin)

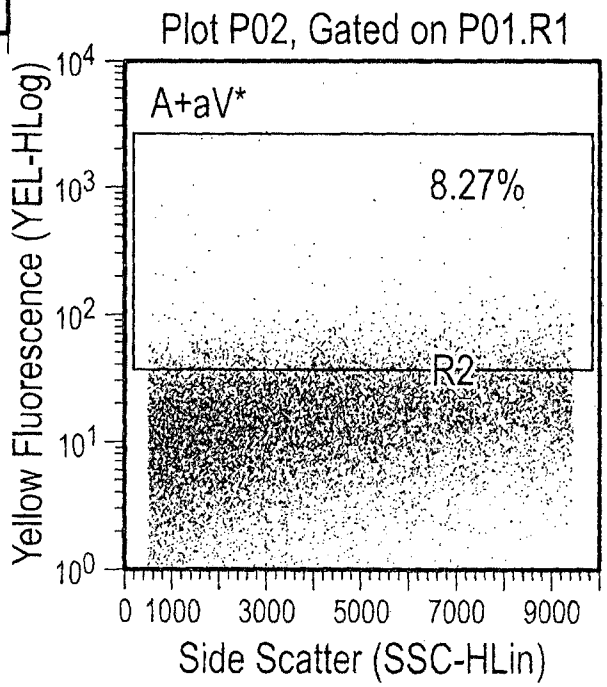

Plot P02, Gated on P01.R1
A+aV*
8.27%
R2
Yellow Fluorescence (YEL-HLog)
$10^4$
$10^3$
$10^2$
$10^1$
$10^0$
0 1000 3000 5000 7000 9000
Side Scatter (SSC-HLin)

FIG. 2

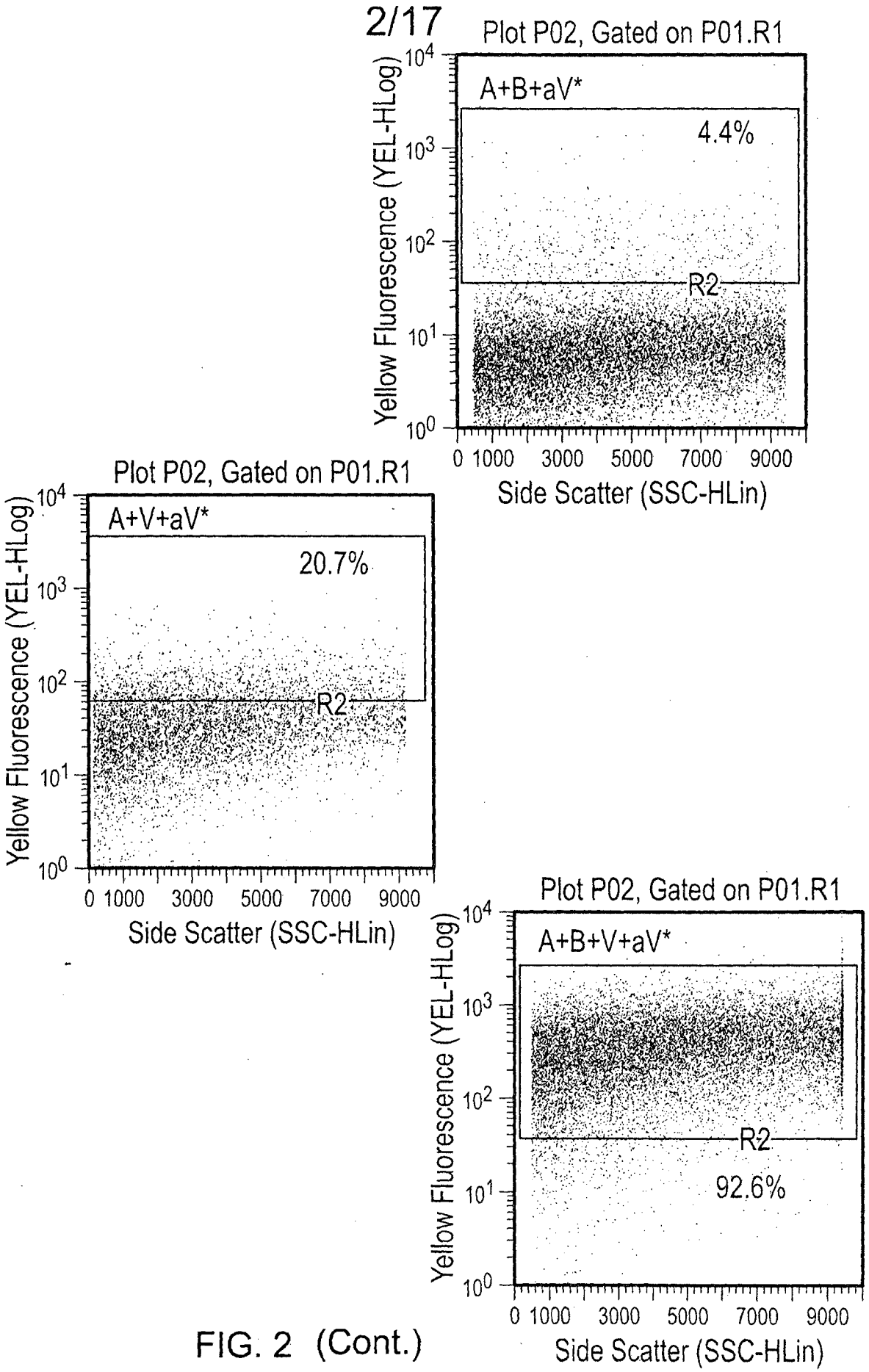

Plot P02, Gated on P01.R1
A+B+aV*
4.4%
R2
Yellow Fluorescence (YEL-HLog)
Side Scatter (SSC-HLin)
Plot P02, Gated on P01.R1
A+V+aV*
20.7%
R2
Yellow Fluorescence (YEL-HLog)
Side Scatter (SSC-HLin)
Plot P02, Gated on P01.R1
A+B+V+aV*
R2
92.6%
Yellow Fluorescence (YEL-HLog)
Side Scatter (SSC-HLin)

FIG. 2 (Cont.)

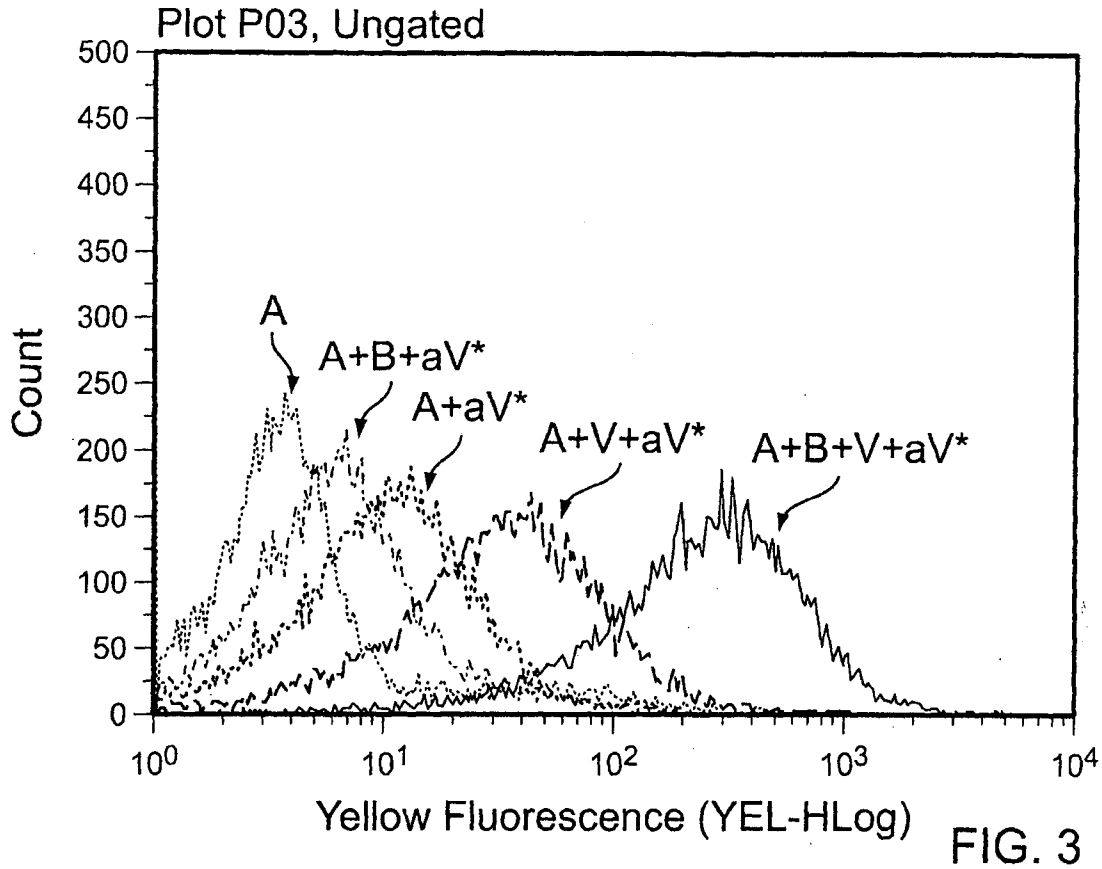

Plot P03, Ungated
Count
Yellow Fluorescence (YEL-HLog)
A
A+B+aV*
A+aV*
A+V+aV*
A+B+V+aV*

FIG. 3

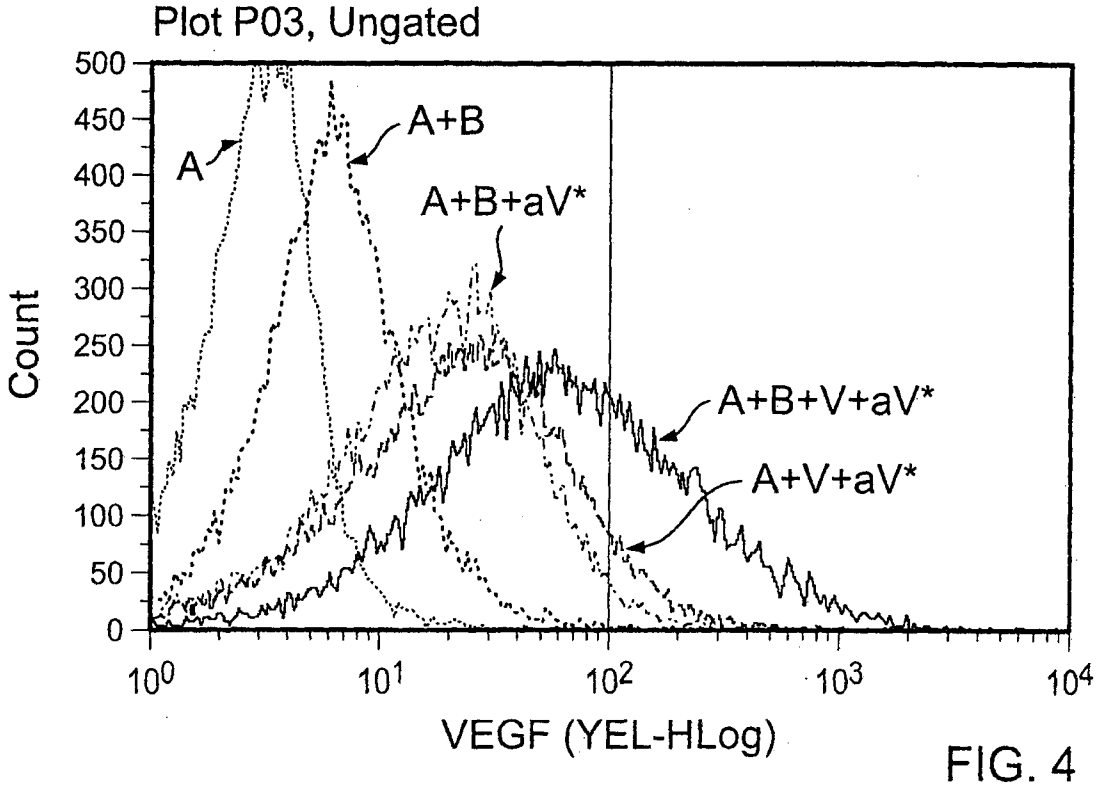

Plot P03, Ungated
Count
VEGF (YEL-HLog)
A
A+B
A+B+aV*
A+B+V+aV*
A+V+aV*

FIG. 4

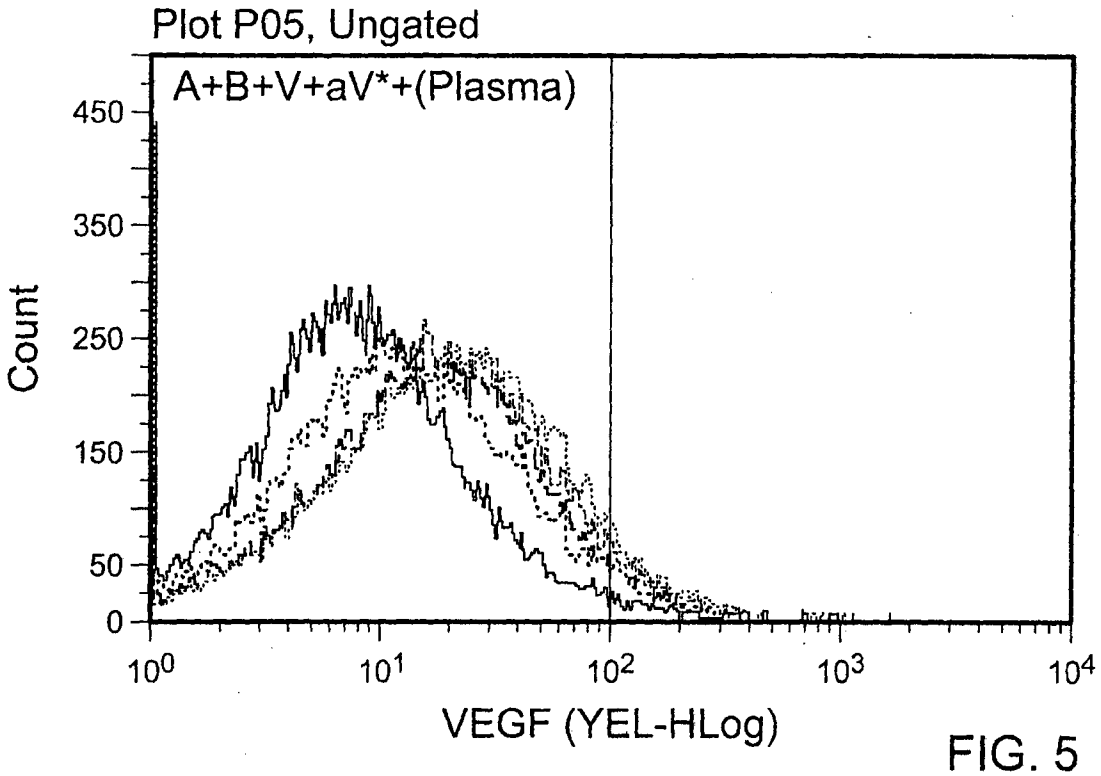

Plot P05, Ungated
A+B+V+aV*+(Plasma)
Count
450
350
250
150
50
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
VEGF (YEL-HLog)

FIG. 5

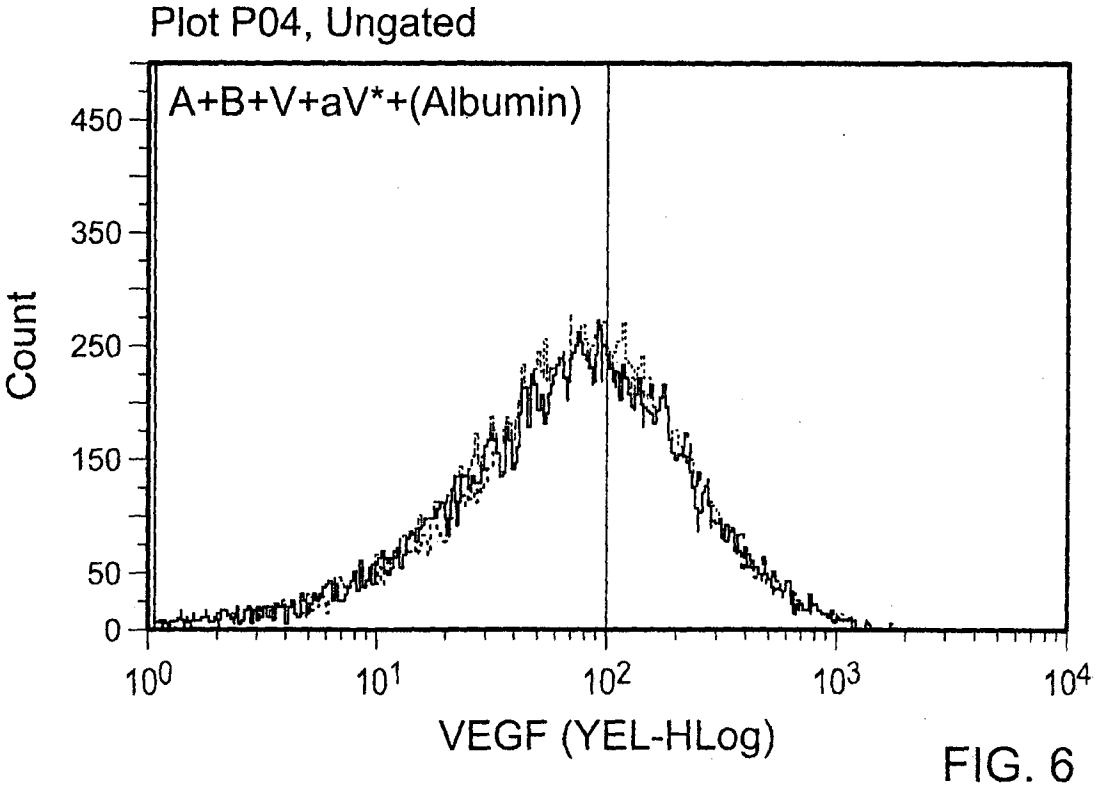

Plot P04, Ungated
A+B+V+aV*+(Albumin)
Count
450
350
250
150
50
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
VEGF (YEL-HLog)

FIG. 6

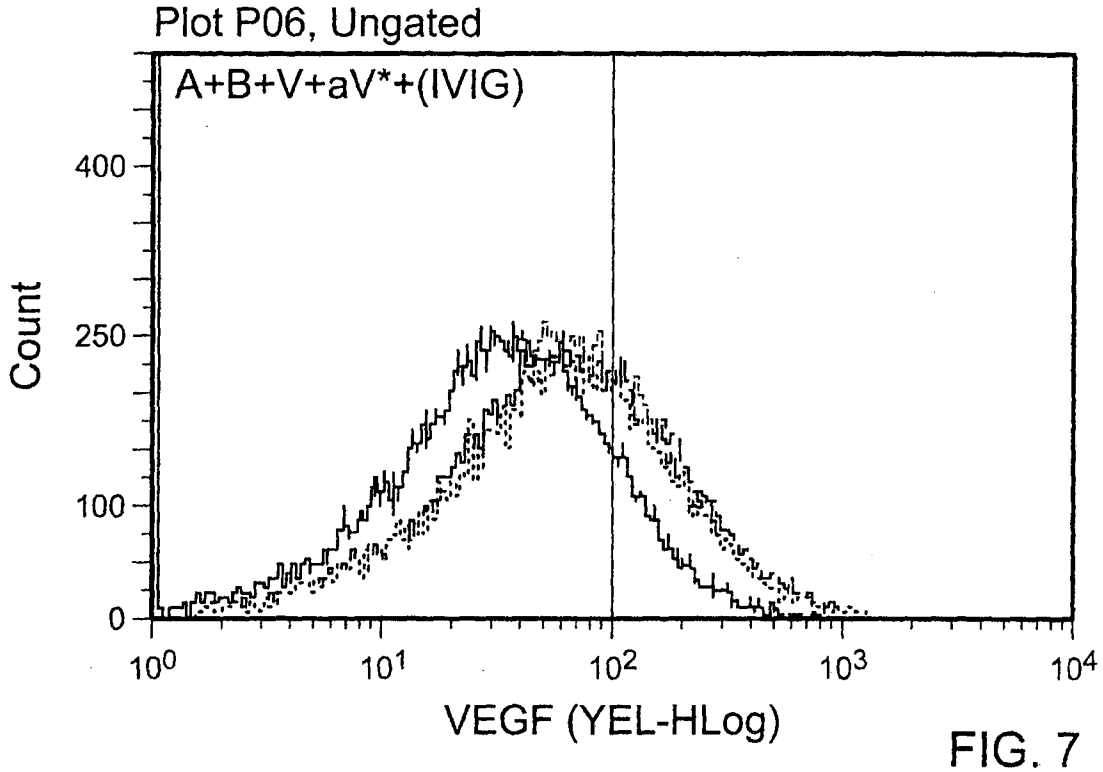

Plot P06, Ungated
A+B+V+aV*+(IVIG)
Count
400
250
100
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
VEGF (YEL-HLog)

FIG. 7

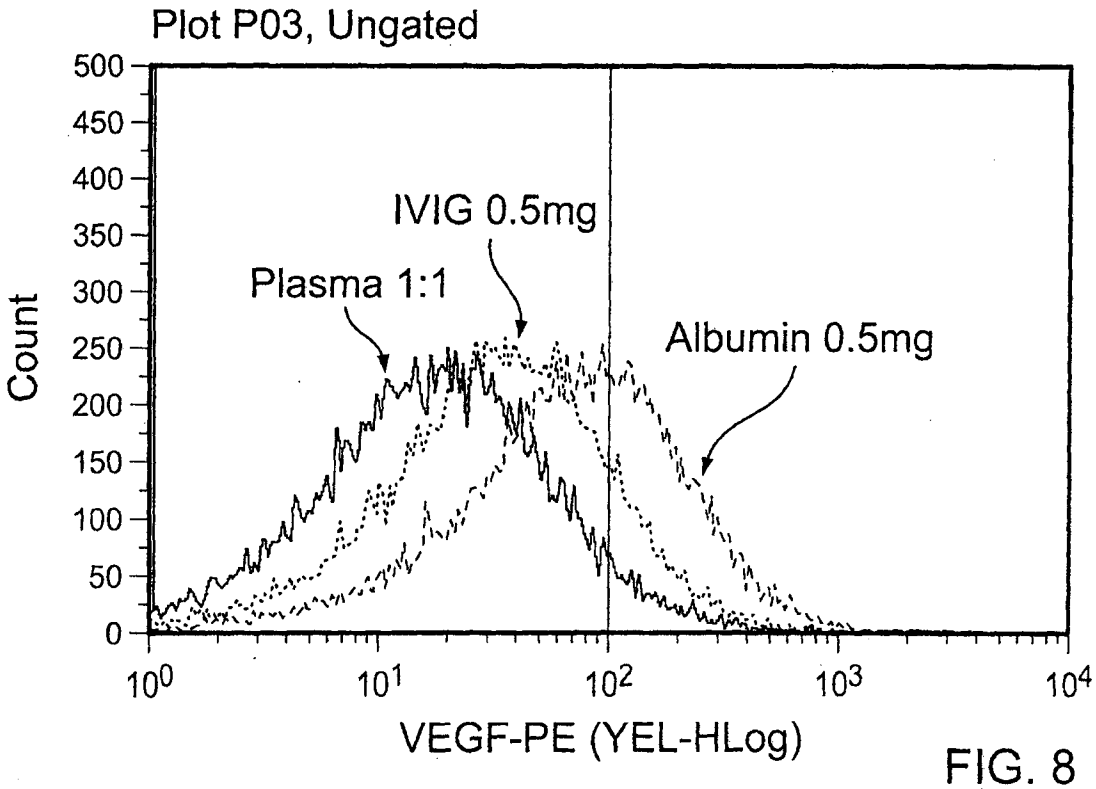

Plot P03, Ungated
Count
500
450
400
350
300
250
200
150
100
50
0
IVIG 0.5mg
Plasma 1:1
Albumin 0.5mg
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
VEGF-PE (YEL-HLog)

FIG. 8

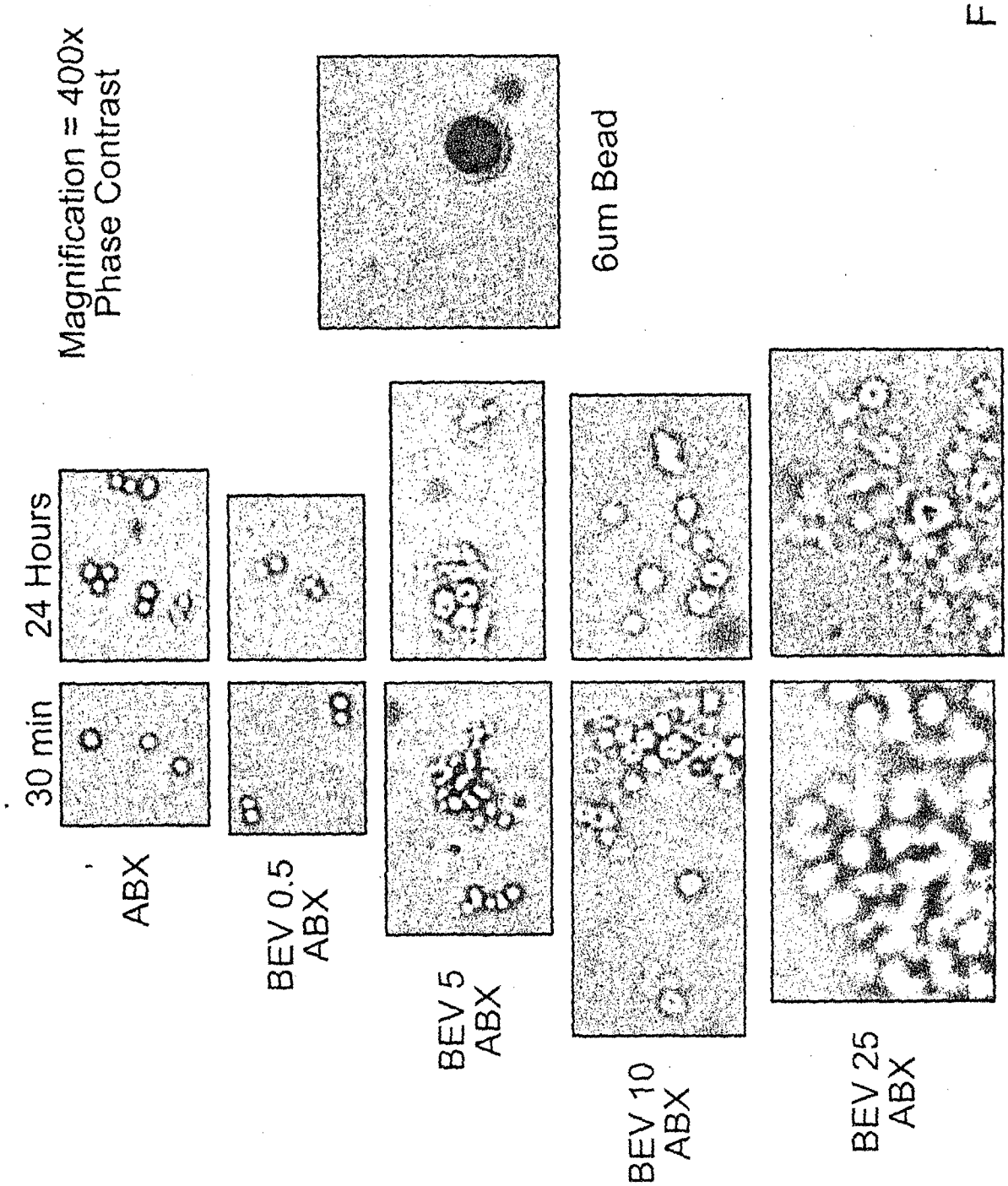

Magnification = 400x
Phase Contrast
30 min
24 Hours
ABX
BEV 0.5 ABX
BEV 5 ABX
BEV 10 ABX
BEV 25 ABX
6um Bead

FIG. 9

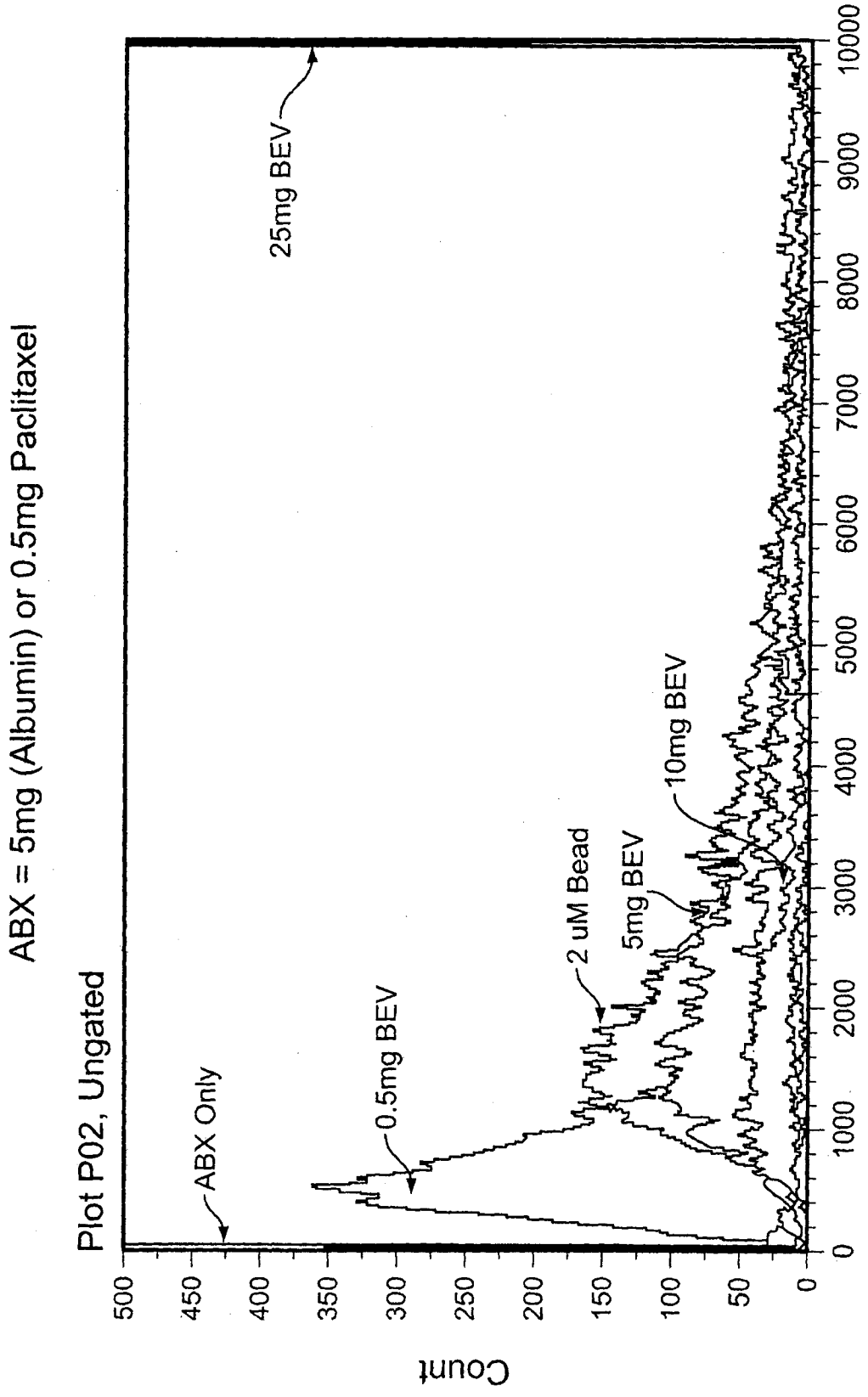

ABX = 5mg (Albumin) or 0.5mg Paclitaxel
Plot P02, Ungated
ABX Only
0.5mg BEV
2 uM Bead
5mg BEV
10mg BEV
25mg BEV
Count
Side Scatter (SSC-HLin)
0
50
100
150
200
250
300
350
400
450
500
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000

FIG. 10

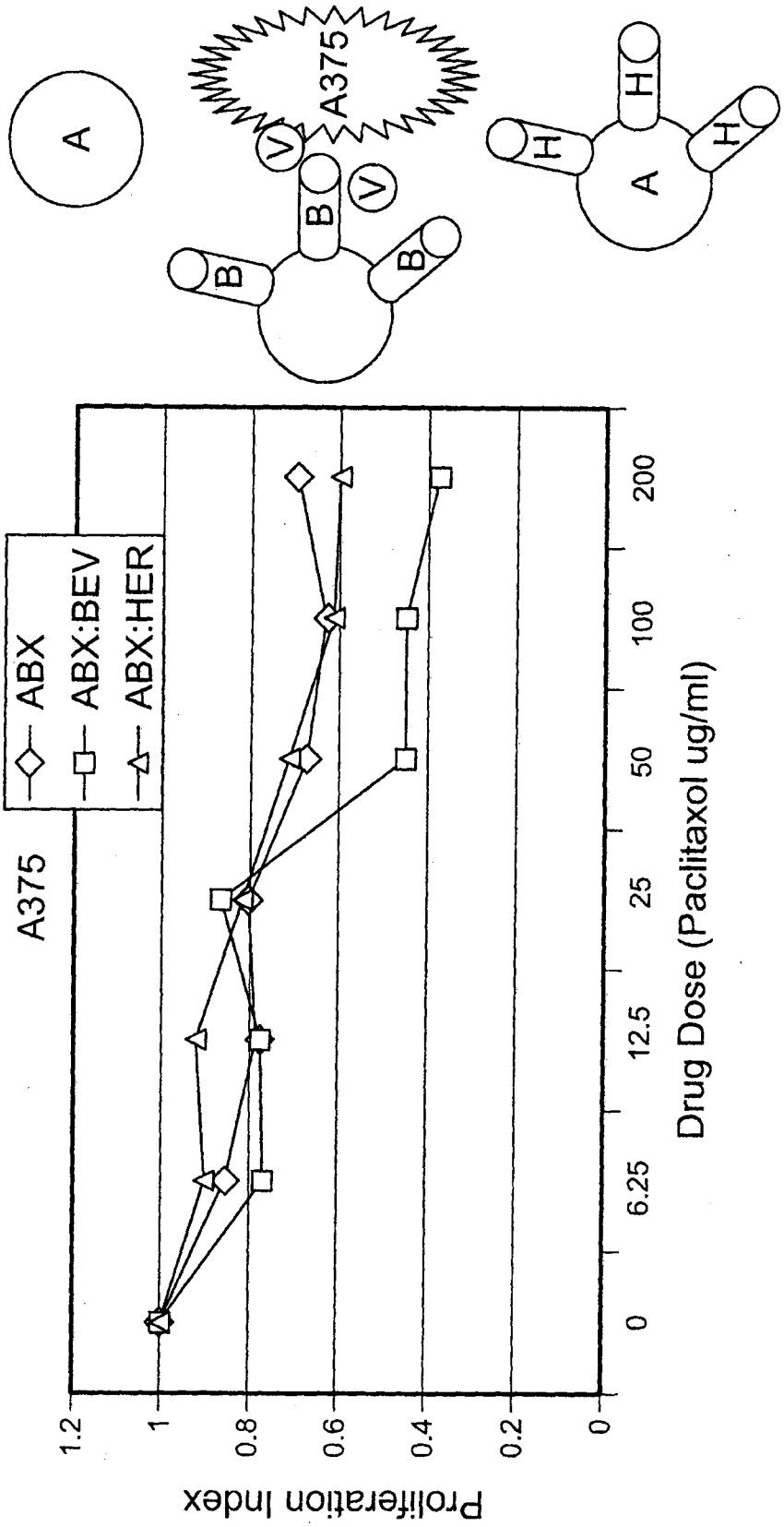

A
A375
V
V
B
B
B
H
H
A
A375
ABX
ABX:BEV
ABX:HER
Proliferation Index
1.2
1
0.8
0.6
0.4
0.2
0
0
6.25
12.5
25
50
100
200
Drug Dose (Paclitaxol ug/ml)

FIG. 11

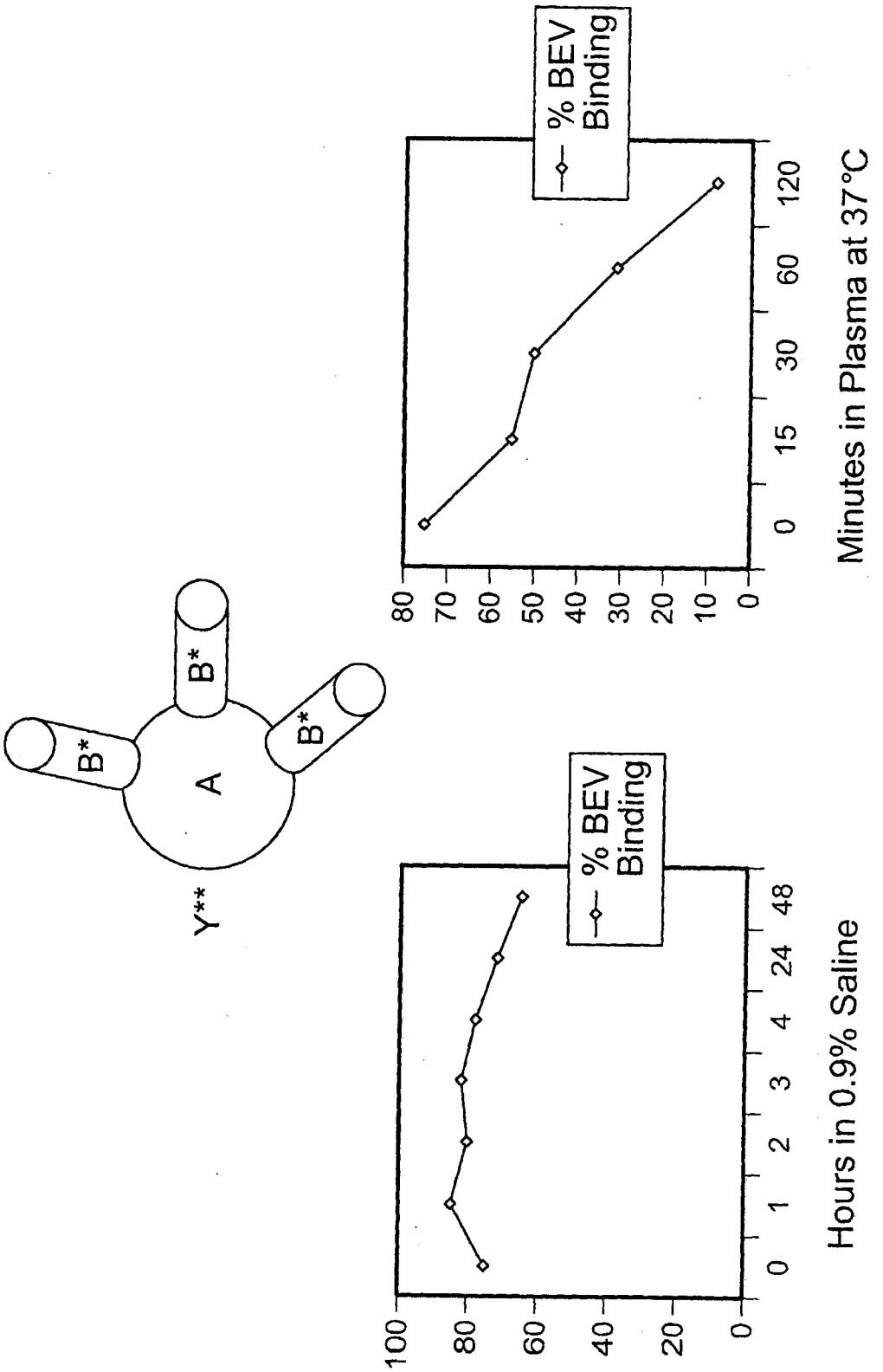
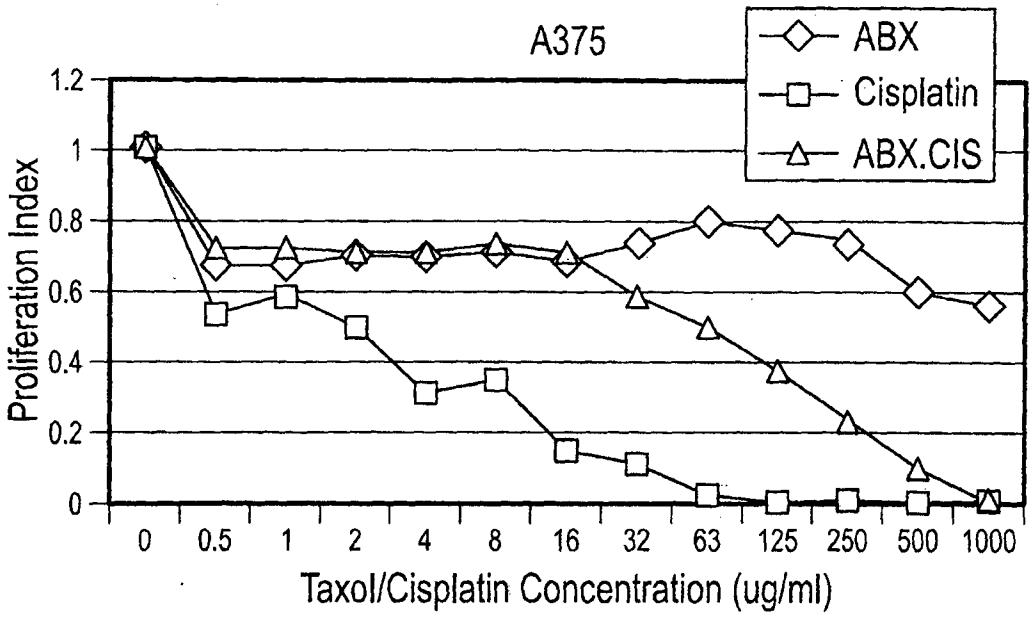

% BEV Binding
Minutes in Plasma at 37°C
0
15
30
60
120
0
10
20
30
40
50
60
70
80
% BEV Binding
Hours in 0.9% Saline
0
1
2
3
4
24
48
0
20
40
60
80
100
B*
B*
B*
A
Y**

FIG. 12


A375
ABX
Cisplatin
ABX.CIS
Proliferation Index
1.2
1
0.8
0.6
0.4
0.2
0
0 0.5 1 2 4 8 16 32 63 125 250 500 1000
Taxol/Cisplatin Concentration (ug/ml)

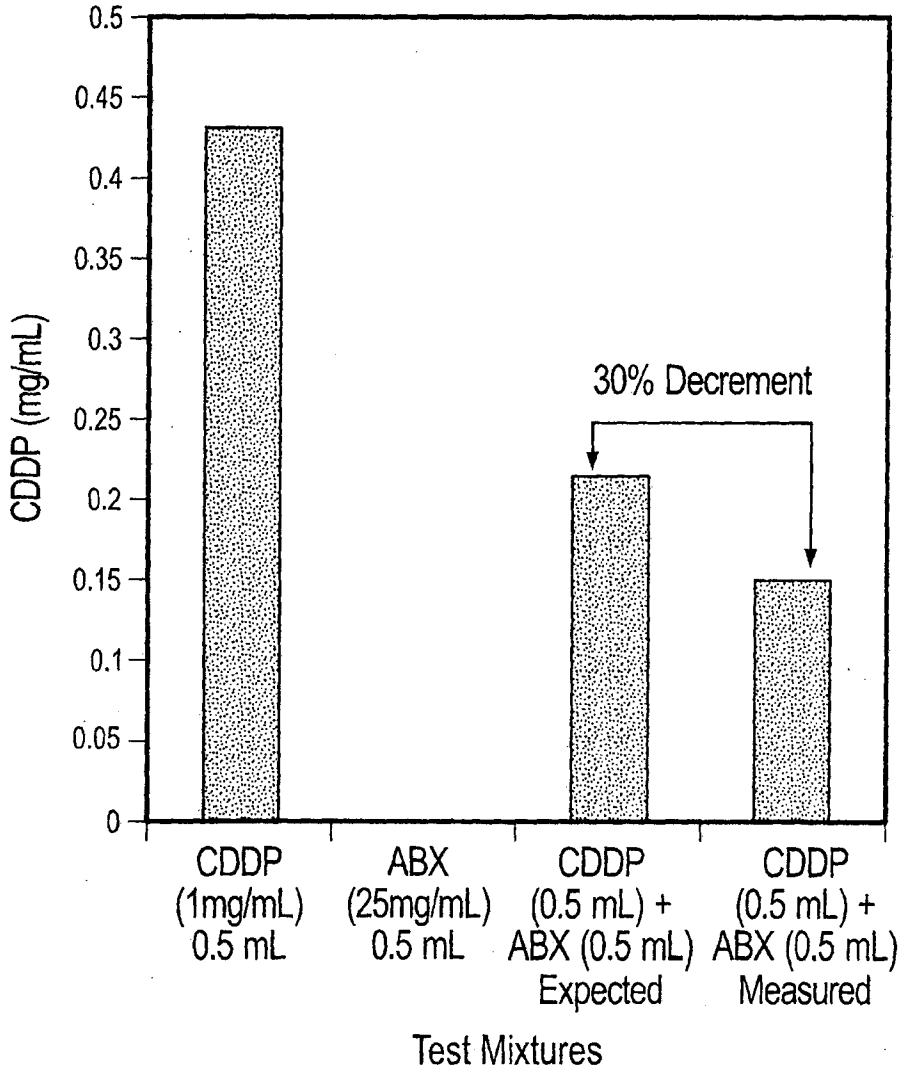

CDDP (mg/mL)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
30% Decrement
CDDP (1mg/mL) 0.5 mL
ABX (25mg/mL) 0.5 mL
CDDP (0.5 mL) + ABX (0.5 mL) Expected
CDDP (0.5 mL) + ABX (0.5 mL) Measured
Test Mixtures

FIG. 13

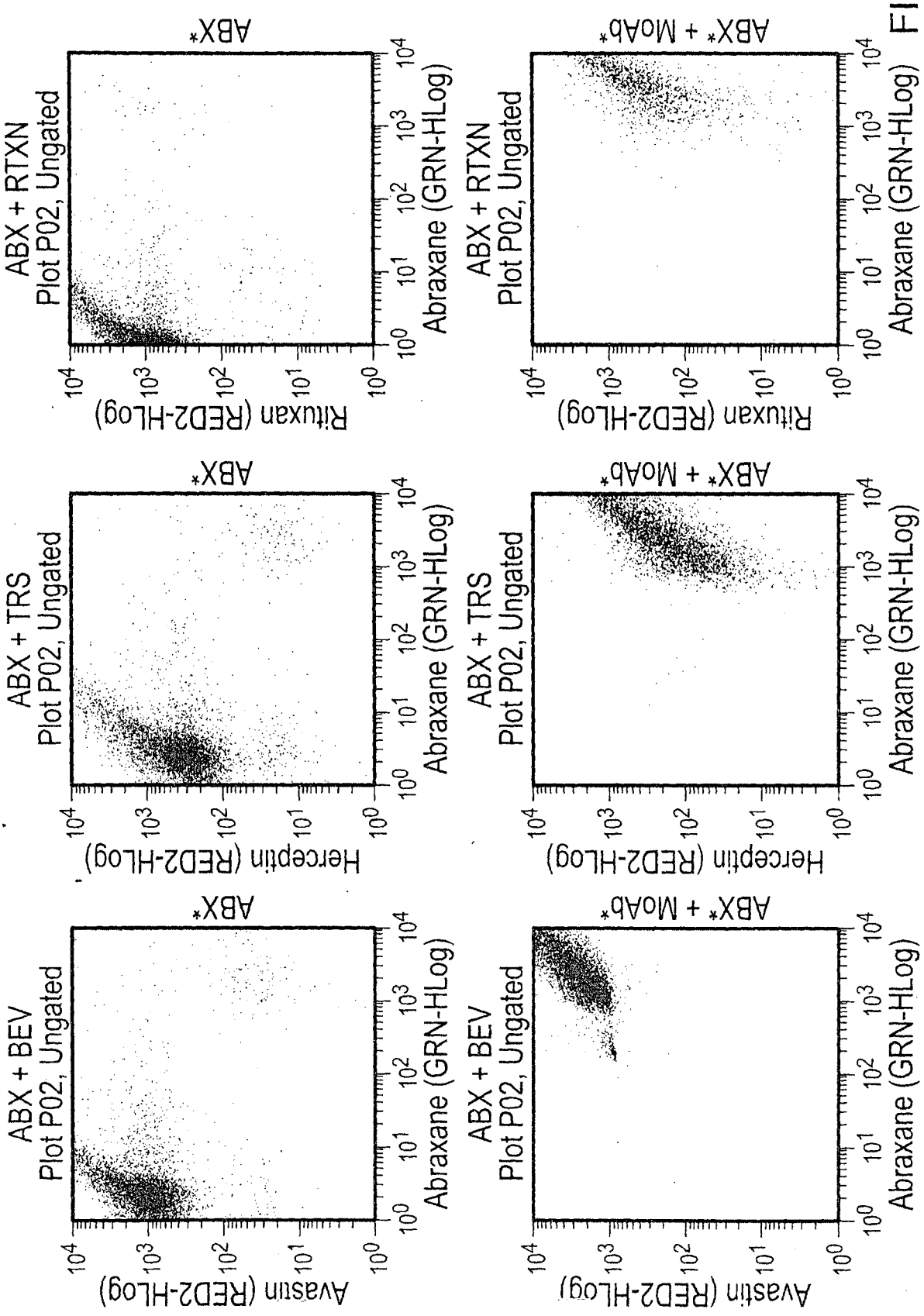
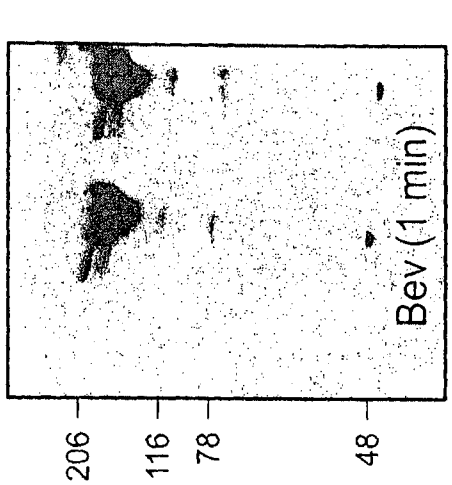

ABX*
ABX* + MoAb*
ABX + RTXN
ABX + TRS
ABX + BEV
Plot P02, Ungated
Rituxan (RED2-HLog)
Herceptin (RED2-HLog)
Avastin (RED2-HLog)
Abraxane (GRN-HLog)

FIG. 14



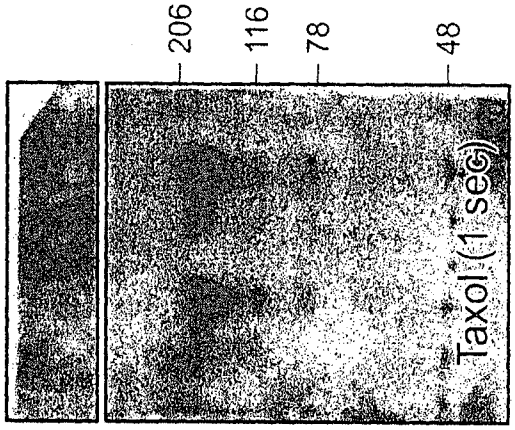




FIG. 15

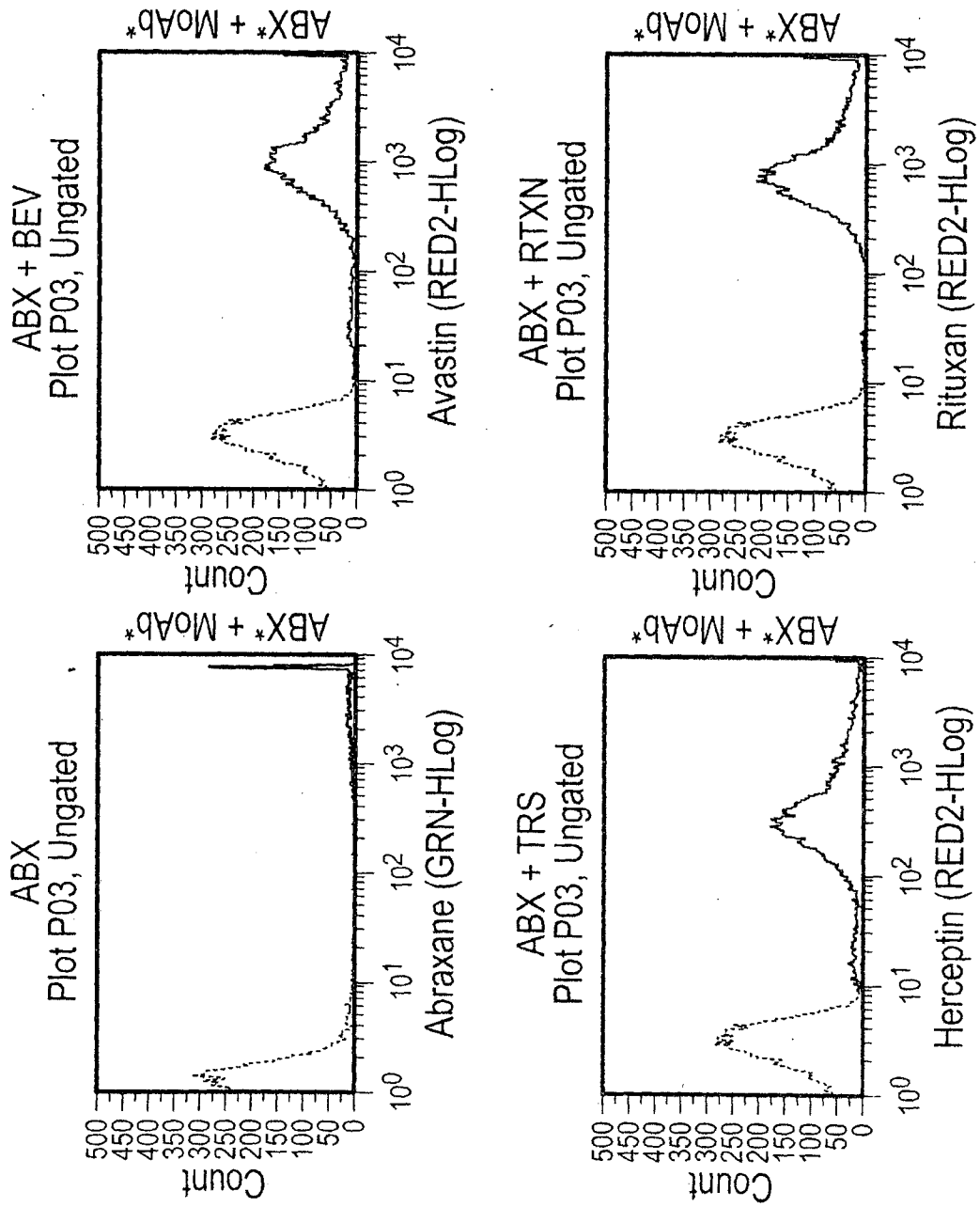



FIG. 14(Cont.)

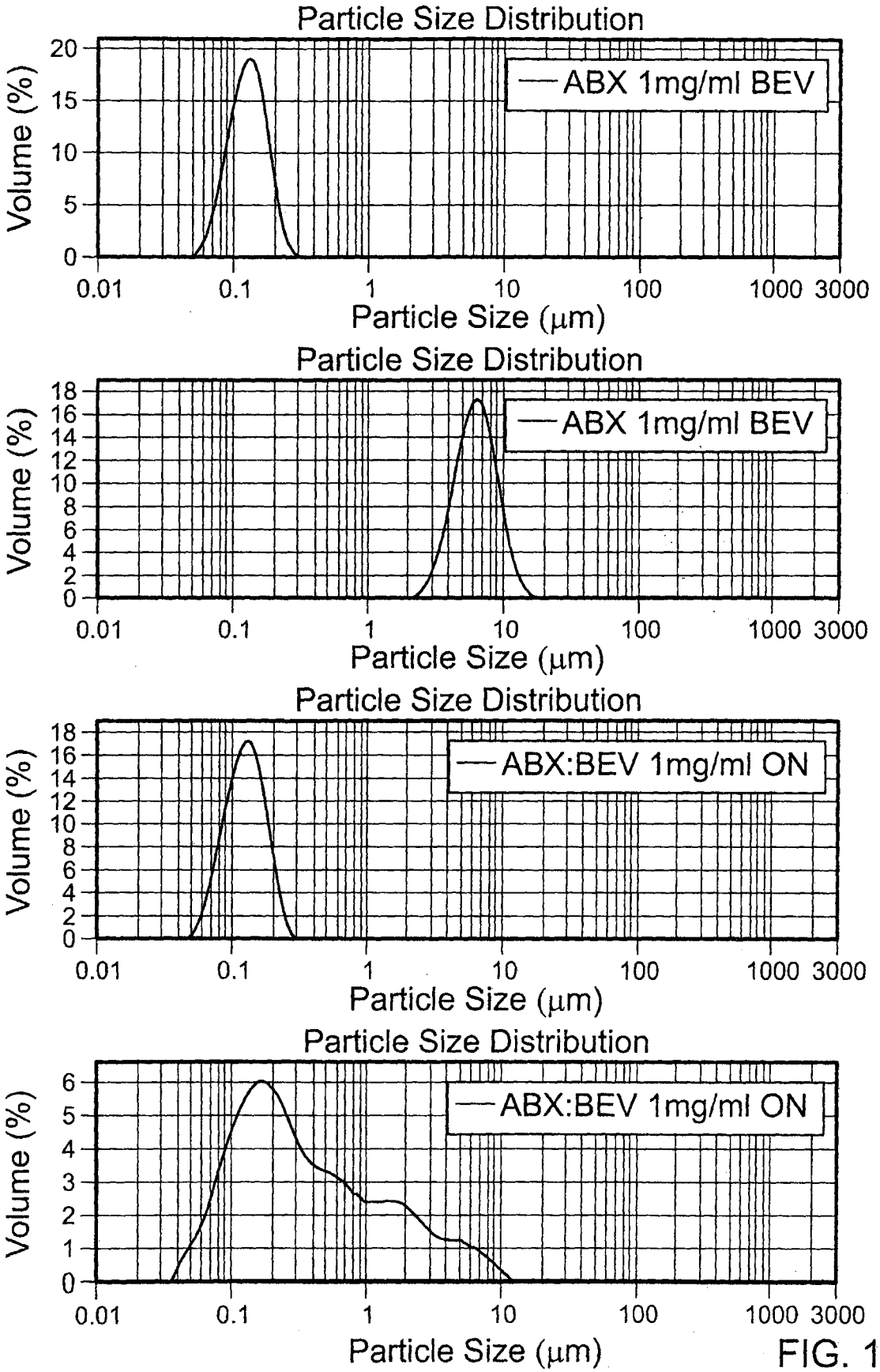
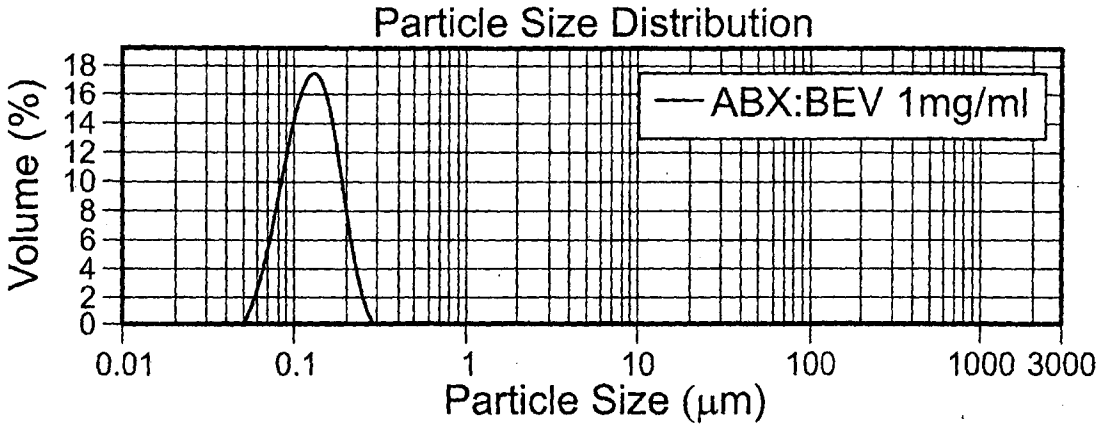
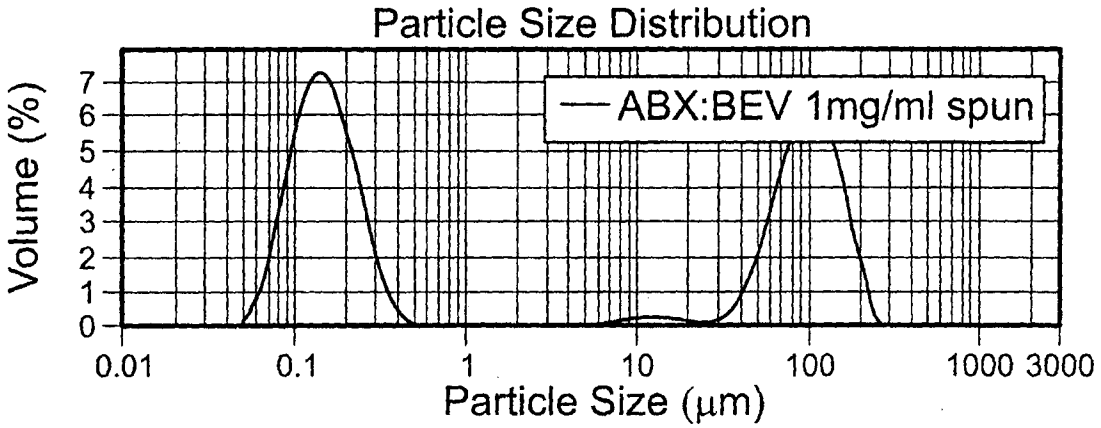

Particle Size Distribution
Volume (%)
20
15
10
5
0
ABX 1mg/ml BEV
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)
Particle Size Distribution
Volume (%)
18
16
14
12
10
8
6
4
2
0
ABX 1mg/ml BEV
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)
Particle Size Distribution
Volume (%)
18
16
14
12
10
8
6
4
2
0
ABX:BEV 1mg/ml ON
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)
Particle Size Distribution
Volume (%)
6
5
4
3
2
1
0
ABX:BEV 1mg/ml ON
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)

FIG. 16


Particle Size Distribution
Volume (%)
18
16
14
12
10
8
6
4
2
0
ABX:BEV 1mg/ml
0.01
0.1
1
10
100
1000 3000
Particle Size (μm)


Particle Size Distribution
Volume (%)
7
6
5
4
3
2
1
0
ABX:BEV 1mg/ml spun
0.01
0.1
1
10
100
1000 3000
Particle Size (μm)

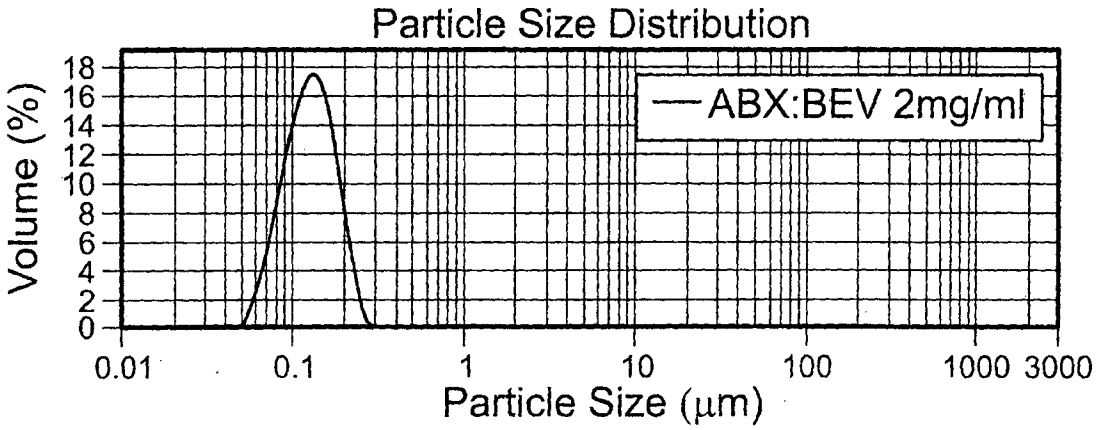
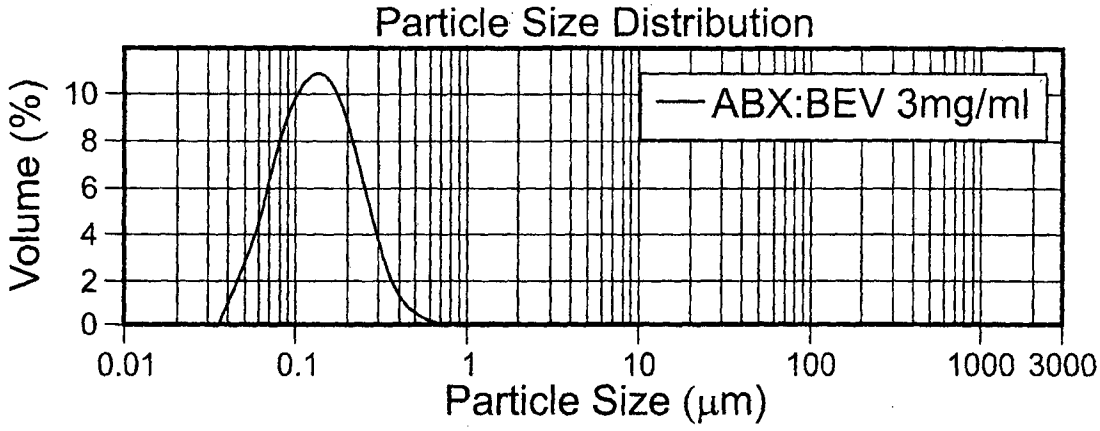
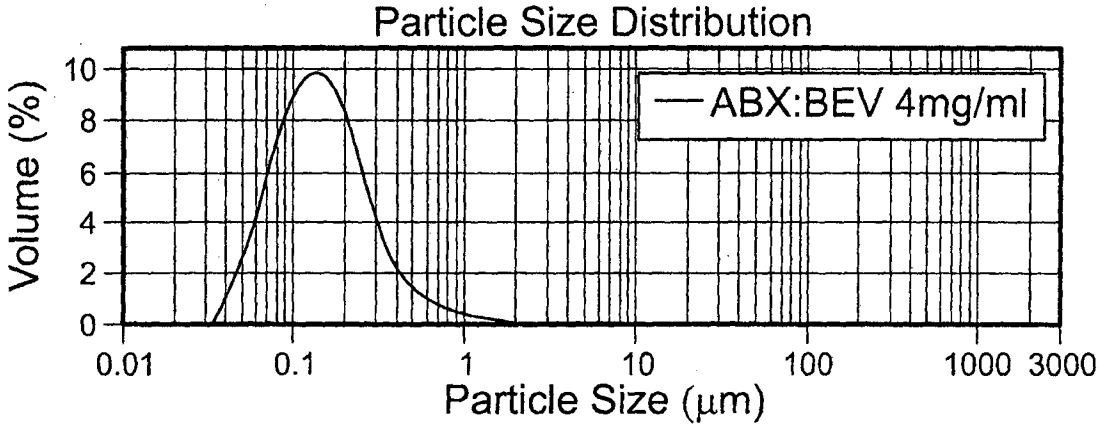

Particle Size Distribution
Volume (%)
18
16
14
12
10
8
6
4
2
0
ABX:BEV 2mg/ml
0.01
0.1
1
10
100
1000 3000
Particle Size (μm)

FIG. 17


Particle Size Distribution
ABX:BEV 3mg/ml
Volume (%)
0
2
4
6
8
10
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)


Particle Size Distribution
ABX:BEV 4mg/ml
Volume (%)
0
2
4
6
8
10
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)

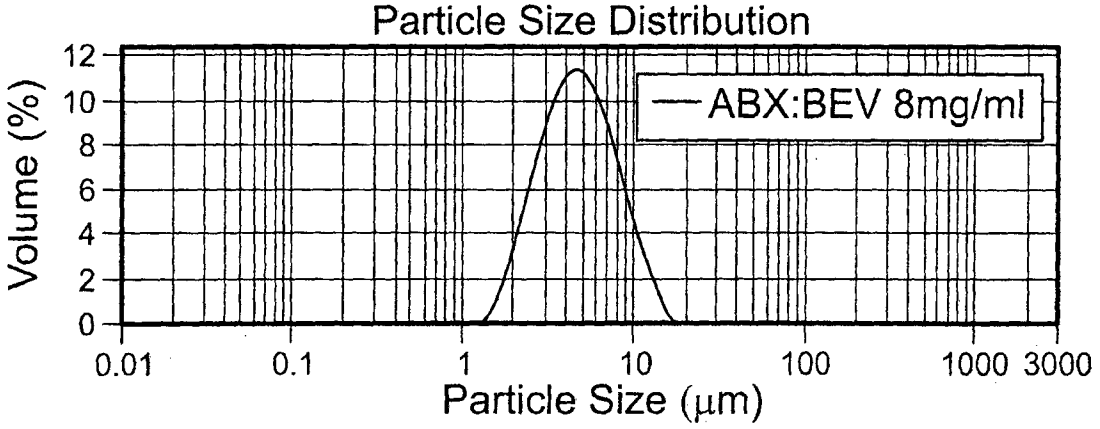

Particle Size Distribution
ABX:BEV 8mg/ml
Volume (%)
0
2
4
6
8
10
12
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)

FIG. 17 (Cont.)

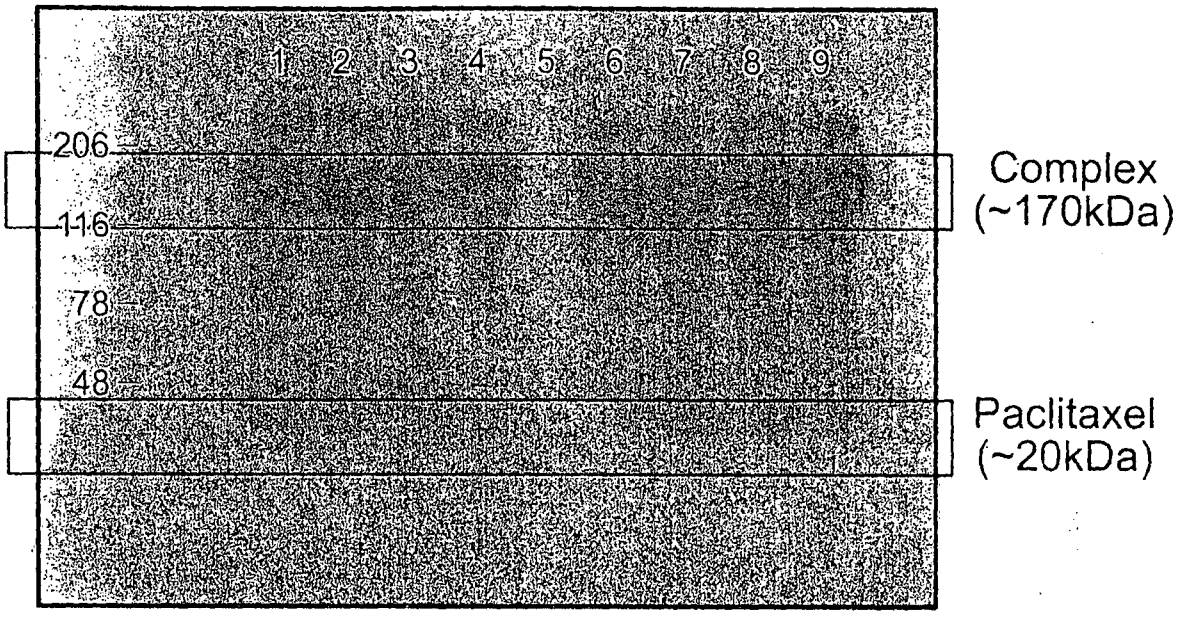

1
2
3
4
5
6
7
8
9
206
116
78
48
Complex
(~170kDa)
Paclitaxel
(~20kDa)

FIG. 18

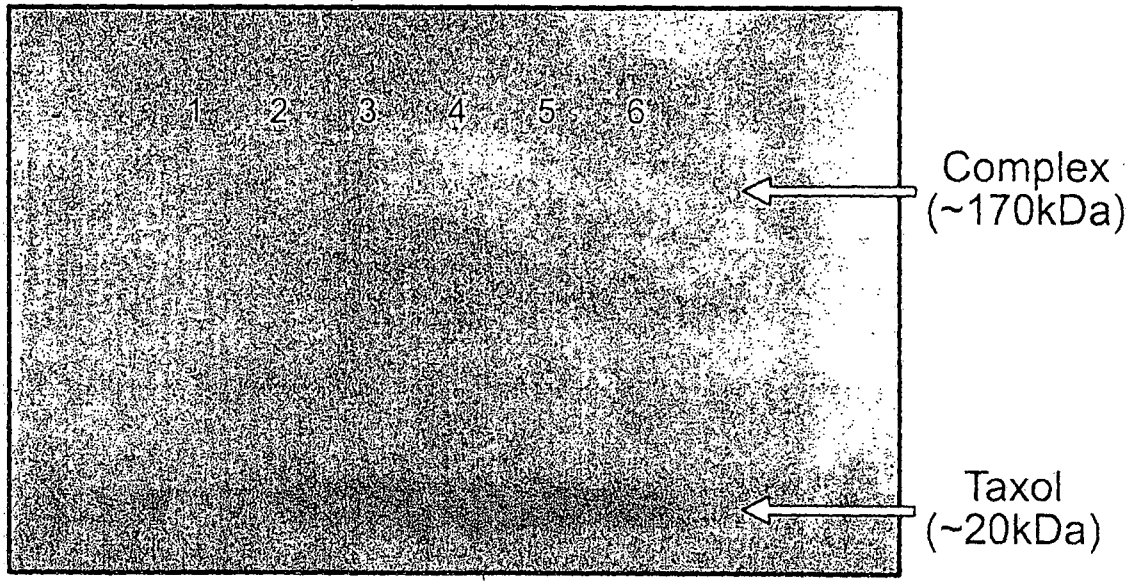

1
2
3
4
5
6
Complex
(~170kDa)
Taxol
(~20kDa)

FIG. 19

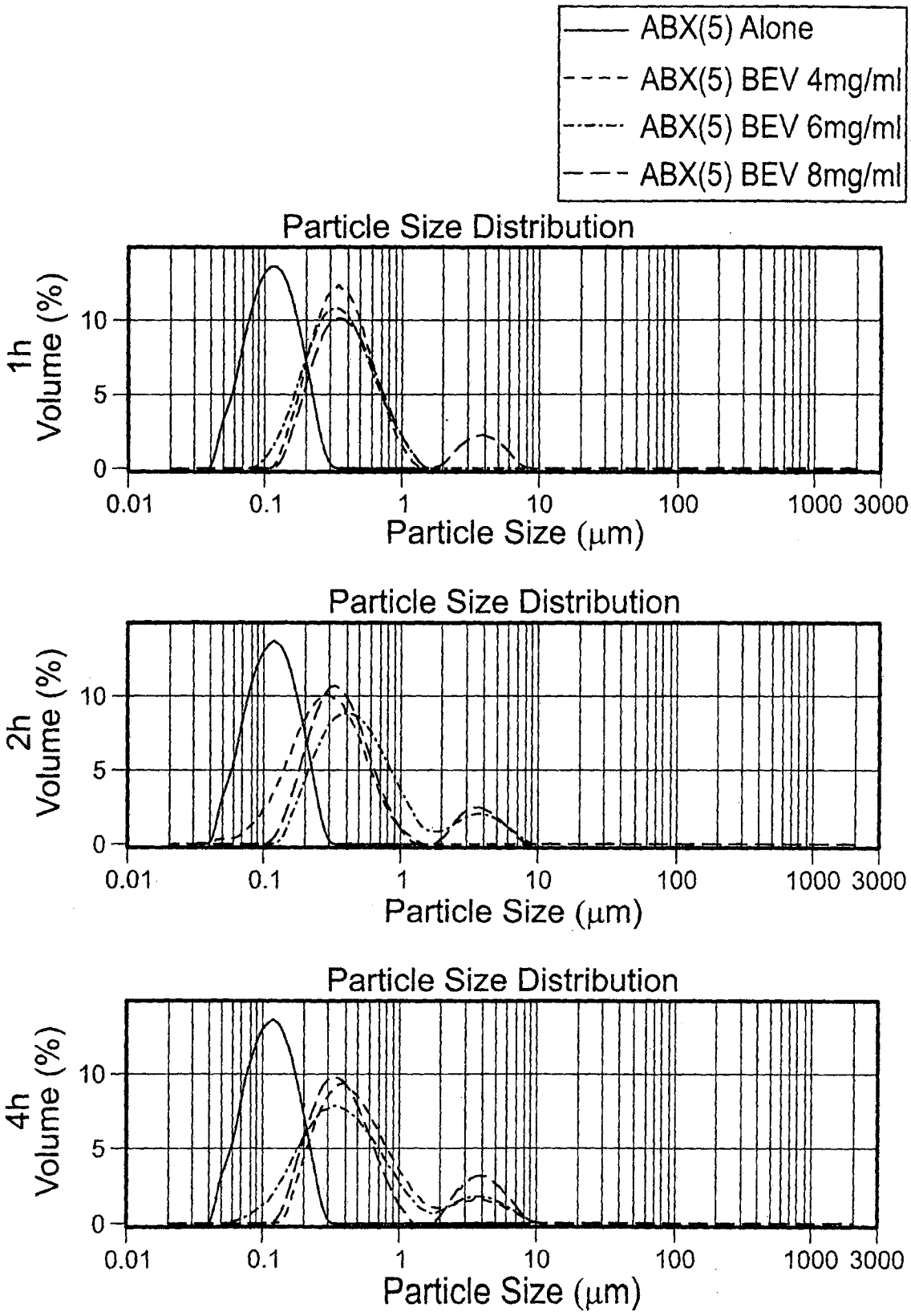

ABX(5) Alone
ABX(5) BEV 4mg/ml
ABX(5) BEV 6mg/ml
ABX(5) BEV 8mg/ml
Particle Size Distribution
1h Volume (%)
10
5
0
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)
Particle Size Distribution
2h Volume (%)
10
5
0
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)
Particle Size Distribution
4h Volume (%)
10
5
0
0.01
0.1
1
10
100
1000
3000
Particle Size (μm)

FIG. 20

**DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT**
**(PCT Article 17(2)(a), Rules 13*ter*.1(c) and (d) and 39)**

| Applicant's or agent's file reference | **IMPORTANT DECLARATION** | Date of mailing (*day/month/year*) |
|---|---|---|
| 070391070WO1 | | 28 SEPTEMBER 2012 (28.09.2012) |
| International application No. | International filing date (*day/month/year*) | (Earlist) Priority date *(day/month/year)* |
| **PCT/US2012/037137** | **09 MAY 2012 (09.05.2012)** | 09 MAY 2011 (09.05.2011) |

International Patent Classification (IPC) or both national classification and IPC

***A61K 39/395(2006.01)i, A61K 31/337(2006.01)i, A61P 35/00(2006.01)i***

Applicant

**MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH et al**

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☒ The subject matter of the international application relates to:
   - a. ☐ scientific theories.
   - b. ☐ mathematical theories.
   - c. ☐ plant varieties.
   - d. ☐ animal varieties.
   - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
   - f. ☐ schemes, rules or methods of doing business.
   - g. ☐ schemes, rules or methods of performing purely mental acts.
   - h. ☐ schemes, rules or methods of playing games.
   - i. ☒ methods for treatment of the human body by surgery or therapy.
   - j. ☐ methods for treatment of the animal body by surgery or therapy.
   - k. ☐ diagnostic methods practised on the human or animal body.
   - l. ☐ mere presentation of information.
   - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.
2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

   ☐ the description ☐ the claims ☐ the drawings
3. ☐ A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:
   - ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
   - ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
   - ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b)
4. Further coments:


Name and mailing address of ISA/KR

Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

Choi Sung Hee

Telephone No. 82-42-481-8740