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(71) Applicant *(for all designated States except US)*: **MERRIMACK PHARMACEUTICALS, INC.** [US/US]; One Kendall Square, Suite B7201, Cambridge, MA 02139 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ZHANG, Bo** [CN/US]; 14 Village Row, Lynnfield, MA 01940 (US). **MCDONAGH, Charlotte** [GB/US]; 11 Sargent Road, Winchester, MA 01890 (US). **HUHALOV, Alexandra** [GB/US]; 47 Sacramento Street #3, Cambridge, MA 02138 (US).

(74) Agent: **ARMSTRONG, Todd**; Clark & Elbing LLP, 101 Federal Street, Boston, MA 02110 (US).

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(54) Title: COMBINATION THERAPIES COMPRISING ANTI-ERBB3 AGENTS

(57) Abstract: Disclosed are methods and compositions for inhibiting the growth of a tumor (e.g., a malignant tumor) in a subject. In particular, combination therapies for treating a tumor in a subject by co-administering either i) an effective amount of an anti-estrogen agent or ii) an effective amount of a receptor tyrosine kinase inhibitor and an effective amount of a bispecific anti-ErbB2/anti-ErbB3 antibody, and optionally an effective amount of trastuzumab. Also disclosed is a bispecific anti-ErbB2/anti-ErbB3 antibody for use in the therapy of a tumor in combination with either i) an anti-estrogen agent or ii) a receptor tyrosine kinase inhibitor, and optionally in use with trastuzumab.

COMBINATION THERAPIES COMPRISING ANTI-ERBB3 AGENTS

FIELD OF THE INVENTION

The various aspects of the invention disclosed herein relate to methods and compositions for the treatment of cancers.

BACKGROUND OF THE INVENTION

Approximately 75% of breast cancers are estrogen receptor (ER) positive. Other cancers are also ER positive (ER+). Estrogen receptors mediate intracellular signaling that can increase the frequency of cell division and drive tumor growth. Although anti-endocrine therapies such as tamoxifen, fulvestrant, and letrozole have demonstrated significant efficacy in treating ER+ breast cancer patients, intrinsic or acquired resistance to such therapies has limited their success.

The prevalence of amplification of the human epidermal growth factor receptor 2 (HER2, or ErbB2) in breast cancer and other cancers has resulted in the research and development of drugs that have ErbB2 as a therapeutic target. Although both the anti-ErbB2 monoclonal antibody trastuzumab and the ErbB1/ErbB2 dual receptor tyrosine kinase inhibitor lapatinib have met with success in the clinic, many patients fail to benefit from these drugs. Additionally, the majority of patients with tumors that initially respond will eventually recrudesce after extended treatment using these therapies.

The ErbB2/ErbB3 heterodimer is the most potent ErbB receptor pairing with respect to strength of interaction, impact on receptor tyrosine phosphorylation, and effects on downstream signaling through mitogen activated protein kinase and phosphoinositide-3 kinase pathways. Heregulin is the primary ligand for ErbB3, and activates signaling by ErbB2/ErbB3 heterodimers. Current ErbB2-targeted therapies do not effectively inhibit heregulin activated signaling. MM-111 is a bispecific anti-ErbB2/anti-ErbB3 antibody that abrogates heregulin binding to ErbB2/ErbB3 and inhibits heregulin activation of ErbB2/ErbB3 without significantly affecting ErbB2 biological activity. In preclinical models of HER-2+ gastric, breast, ovarian and lung cancers, MM-111 inhibits ErbB3 phosphorylation, cell cycle progression, and tumor growth.

Thus, a need exists for therapies and therapeutic strategies providing improved inhibition of ErbB3 activation (e.g., ligand-induced activation) as well as for therapies and therapeutic strategies providing improved inhibition of estrogen receptor signaling activity or of ErB1 and ErbB2 receptor signaling activity.

In the treatment of cancers, the co-administration of pluralities of anti-cancer drugs (combination therapy) often provides better treatment outcomes than monotherapy. Such outcomes can be subadditive, additive, or superadditive. That is to say that the combined effects of two anti-cancer drugs, each of which provides a quantifiable degree of benefit, can be less than, equal to, or greater than the sum of the benefits of each drug. For example, two drug, each of which when used alone to treat a lethal cancer provides an average one year extension of progression free survival, could together provide a <24 month extension (e.g., an 18 month extension), about a 24 month extension, or a >24 month extension (e.g., a 30

month extension) of progression free survival. Typically, combination therapies for cancer treatment provide significantly subadditive outcomes. Outcomes that are near additive, additive, or superadditive are most desirable, but only occur rarely. In addition, many drugs are known to alter the bioavailability, or otherwise affect the safety profile of other drugs when both drugs are co-administered. As new drugs are first used in combination therapies, unforeseen, hazardous drug-drug interactions may be observed that result in drug-drug interaction-mediated toxicity in the patient.

Thus approaches for safely administering combination therapies comprising administration of ErbB2/ErbB3 heterodimer-targeted agents for cancer treatment, and especially combinations that yield near-additive, additive, or superadditive outcomes are needed.

SUMMARY OF THE INVENTION

Provided herein are methods and compositions effective for the inhibition of ErbB3 activation and also effective for the inhibition of estrogen receptor activation. Also provided are methods and compositions effective for the inhibition of ErbB3 activation and also effective for the inhibition of ErB1 and/or ErbB2 activation. These methods and compositions are useful for the treatment of tumors, e.g., malignant tumors, as well as for the treatment of other cancers.

In a first embodiment, a method of treating a subject with a malignant tumor is provided, where the tumor is an ErbB2 expressing or ErbB2 over-expressing tumor (e.g., HER^{++} or HER^{+++} tumors) and the tumor may be a melanoma, clear cell sarcoma, head and neck, endometrial, prostate, breast, ovarian, gastric, colon, colorectal, lung, bladder, pancreatic, salivary gland, liver, skin, brain or renal tumor. The method comprises co-administering to the subject either an effective amount of an anti-estrogen agent or an effective amount of a receptor tyrosine kinase inhibitor, in combination with an effective amount of an anti-ErbB3 agent, e.g., a bispecific anti-ErbB2/anti-ErbB3 antibody (e.g., the antibody comprising the amino acid sequence set forth in SEQ ID NO:1) and optionally an effective amount of trastuzumab.

In one aspect, the combination of the bispecific anti-ErbB2/anti-ErbB3 antibody and either the effective amount of an anti-estrogen agent or the effective amount of the receptor tyrosine kinase inhibitor, and optionally the effective amount of trastuzumab, is characterized as follows: when a first tissue culture medium is prepared comprising the bispecific anti-ErbB2/anti-ErbB3 antibody (e.g., the antibody comprising the amino acid sequence set forth in SEQ ID NO:1) at a first concentration and either the anti-estrogen agent at a second concentration or the receptor tyrosine kinase inhibitor (e.g., lapatinib) at a third concentration (wherein each concentration is the same or different as each other concentration), and the medium is contacted with cancer cells of a cell line in a cell culture, cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or the percentage of cells in the culture that are apoptotic is increased. In certain aspects, cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or the percentage of cells in the culture that are apoptotic is increased to a greater degree than cell growth, or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or percentage of cells in the

culture that are apoptotic is increased, to a lesser degree when cancer cells of the cell line in a cell culture are contacted with each of a second medium that is essentially the same as the first medium except that it does not comprise a bispecific anti-ErbB2/anti-ErbB3 antibody, and a third medium that is essentially the same as the first medium except that it does not comprise any anti-estrogen agent and it does not comprise any receptor tyrosine kinase inhibitor.

In another aspect, all effective amounts are either mouse effective amounts or human effective amounts. In another aspect, all effective amounts are mouse effective amounts and the combination of the bispecific anti-ErbB2/anti-ErbB3 antibody (optionally the antibody comprising the amino acid sequence set forth in SEQ ID NO:1) and either the effective amount of an anti-estrogen agent or the effective amount of the receptor tyrosine kinase inhibitor, is characterized as follows: when co-administered to BT474-M3 xenograft tumor bearing mice with a tumor of a measured volume, the combination is more effective at inhibiting tumor volume increase after 32 days of co-administration than is the mouse effective amount of the bispecific anti-ErbB2/anti-ErbB3 antibody administration without the co-administration of either the effective amount of an anti-estrogen agent or the effective amount of the receptor tyrosine kinase inhibitor. In another aspect, a mouse effective amount of trastuzumab is co-administered with the bispecific anti-ErbB2/anti-ErbB3 antibody.

In a second embodiment, a bispecific anti-ErbB2/anti-ErbB3 antibody (optionally the antibody comprising SEQ ID NO:1) is provided for use in combination therapy of a cancer (optionally a melanoma, clear cell sarcoma, head and neck, endometrial, prostate, breast, ovarian, gastric, colon, colorectal, lung, bladder, pancreatic, salivary gland, liver, skin, brain or renal tumor), where the combination therapy comprises concomitant use of either an anti-estrogen agent or a receptor tyrosine kinase inhibitor and optionally comprises concomitant use of trastuzumab.

In a third embodiment, an aqueous solution is provided comprising a bispecific anti-ErbB2/anti-ErbB3 antibody (optionally the antibody comprising the amino acid sequence set forth in SEQ ID NO:1)at a first concentration and either an anti-estrogen agent at a second concentration or a receptor tyrosine kinase inhibitor at a third concentration. In certain aspects, when a first tissue culture medium is prepared comprising the bispecific anti-ErbB2/anti-ErbB3 antibody at the first concentration and either the anti-estrogen agent at the second concentration or the receptor tyrosine kinase inhibitor at the third concentration, and the medium is contacted with cancer cells of a cell line in a cell culture, cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or percentage of cells in the culture that are apoptotic is increased. In certain aspects, cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or the percentage of cells in the culture that are apoptotic is increased, to a lesser degree when cells of the cell line in a cell culture are contacted with a second tissue culture medium that is essentially the same as the first medium of except that it does not comprise any anti-estrogen agent and it does not comprise any receptor tyrosine kinase inhibitor. In another aspect, cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or the percentage of cells in the culture that are apoptotic is increased, to a

lesser degree when cells of the cell line in a cell culture are contacted with a third tissue culture medium that is essentially the same as the first medium of except that it does not comprise any bispecific anti-ErbB2/anti-ErbB3 antibody.

In another aspect, the aqueous solution is blood plasma in a subject, and the subject does not experience a toxicity that is sufficiently harmful to require a change in a therapy being administered to the subject, which toxicity is mediated by a drug-drug interaction in the subject between the bispecific anti-ErbB2/anti-ErbB3 antibody and the anti-estrogen agent or the receptor tyrosine kinase inhibitor.

In another aspect, the aqueous solution further comprises trastuzumab at a fourth concentration, and the medium also comprises trastuzumab at the fourth concentration.

In another aspect, the method, combination therapy, or aqueous solution does not comprise an aromatase inhibitor or an estrogen receptor antagonist. In one embodiment the method, combination therapy, or aqueous solution comprises nab-paclitaxel.

In each embodiment and aspect thereof above, the anti-estrogen agent may be an estrogen receptor antagonist (e.g., fulvestrant or tamoxifen) or an aromatase inhibitor (e.g., wherein the aromatase inhibitor is letrozole, exemestane, anastrozole, aminoglutethimide, testolactone, vorozole, formestane, or fadrozole. Preferably the aromatase inhibitor is letrozole. Also in each embodiment and aspect thereof above, the receptor tyrosine kinase inhibitor is erlotinib, afatinib, dasatinib, gefitinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib or sorafenib. Preferably the receptor tyrosine kinase inhibitor is lapatinib. Also in each embodiment and aspect thereof above, the bispecific anti ErbB2/anti-ErbB3 antibody is the A5-HSA-ML3.9, ML3.9-HSA-A5, A5-HSA-B1D2, B1D2-HSA-A5, B12-HSA-B1D2, B1D2-HSA-B12, A5-HSA-F5B6H2, F5B6H2-HSA-A5, H3-HSA-F5B6H2, F5B6H2-HSA-H3, F4-HSA-F5B6H2, F5B6H2-HSA-F4, B1D2-HSA-H3, H3-HSA-B1D2, or the antibody comprising the amino acid sequence set forth in SEQ ID NO:1. Each embodiment and aspect thereof above may also further comprise use of capecitabine and/or cisplatin.

In each embodiment and aspect thereof above, one or more of a) - x) that follow may optionally apply: a) the cell line is BT474-M3; b) the culture is a spheroid culture, c) paclitaxel or another taxane or another chemotherapeutic drug is co-administered, optionally in accordance with the manufacturer's directions, d) the anti-estrogen agent is administered in accordance with the manufacturer's directions, e) the receptor tyrosine kinase inhibitor is administered in accordance with the manufacturer's directions, f) the trastuzumab is administered in accordance with the manufacturer's directions, g) the co-administration of the bispecific anti-ErbB2/anti-ErbB3 antibody with an anti-estrogen agent produces an about additive or a superadditive effect, h) the co-administration of the bispecific anti-ErbB2/anti-ErbB3 antibody with a receptor tyrosine kinase inhibitor (e.g., lapatinib) produces about a substantially additive or a superadditive effect. i) the bispecific anti-ErbB2/anti-ErbB3 antibody is the antibody comprising SEQ ID NO:1 and is administered in accordance with any of the regimens (e.g., modes, dosages, dosing intervals, loading and maintenance doses and dosing schemes) described in Examples 12 and 13, below,

j) the lapatinib is administered in accordance with any of the regimens (e.g., modes, dosages, dosing intervals, loading and maintenance doses and dosing schemes) described in Example 16, below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing that the combination of MM-111 and tamoxifen inhibits tumor growth *in vivo* better than either MM-111 or tamoxifen does alone. The x-axis shows time post tumor implant in days and the y-axis shows tumor volume in mm^3. Mice were treated with inhibitors beginning on day 7 post BT474-M3 cell implant.

Figure 2 is seven graphs showing that MM-111 combines positively with anti-estrogen drugs in inhibiting estrogen-stimulated spheroid growth *in vitro*. Figure 2a shows the effect of MM-111, tamoxifen (4-hydroxytamoxifen or 4OHT), or MM-111 and tamoxifen on *in vitro* spheroid growth. Figure 2b shows the effect of trastuzumab, tamoxifen, or trastuzumab and tamoxifen. Figure 2c shows the effect of MM-111, fulvestrant (FVT), or MM-111 and fulvestrant. Figure 2d shows the effect of trastuzumab, fulvestrant, or trastuzumab and fulvestrant. Figure 2e shows the effect of MM-111, trastuzumab, or MM-111 and trastuzumab. Figure 2f shows the effect of MM-111, trastuzumab, and tamoxifen combined compared to that of any of the double combinations. Figure 2g shows the effect of MM-111, trastuzumab, and fulvestrant combined compared to that of any of the double combinations. The x-axes are a log scale of each drug concentration for each experimental condition in nM and the y axis is spheroid size as % of control spheroid size.

Figure 3 is seven graphs showing that MM-111 combines positively with anti-estrogen drugs in inhibiting heregulin (HRG)-stimulated spheroid growth *in vitro*. Figure 3a shows the effect of MM-111, tamoxifen (4-hydroxytamoxifen or 4OHT), or MM-111 and tamoxifen. Figure 3b shows the effect of trastuzumab, tamoxifen, or trastuzumab and tamoxifen. Figure 3c shows the effect of MM-111, fulvestrant (FVT), or MM-111 and fulvestrant. Figure 3d shows the effect of trastuzumab, fulvestrant, or trastuzumab and fulvestrant. Figure 3e shows the effect of MM-111, trastuzumab, or MM-111 and trastuzumab. Figure 3f shows the effect of MM-111, trastuzumab, and tamoxifen combined compared to that of any of the double combinations. Figure 3g shows the effect of MM-111, trastuzumab, and fulvestrant combined compared to that of any of the double combinations. The x-axes are a log scale of each drug concentration for each experimental condition in nM and the y axis is spheroid size as % of control spheroid size.

Figure 4 is seven graphs showing that MM-111 combines positively with anti-estrogen drugs in inhibiting dual ligand (estrogen and heregulin)-stimulated spheroid growth *in vitro*. Figure 4a shows the effect of MM-111, tamoxifen, or MM-111 and tamoxifen. Figure 4b shows the effect of trastuzumab, tamoxifen, or trastuzumab and tamoxifen. Figure 4c shows the effect of MM-111, fulvestrant (FVT), or MM-111 and fulvestrant. Figure 4d shows the effect of trastuzumab, fulvestrant, or trastuzumab and fulvestrant. Figure 4e shows the effect of MM-111, trastuzumab, or MM-111 and trastuzumab. Figure 4f shows the effect of MM-111, trastuzumab, and tamoxifen combined compared to that of any of the

double combinations. Figure 4g shows the effect of MM-111, trastuzumab, and fulvestrant combined compared to that of any of the double combinations. The x-axes are a log scale of each drug concentration for each experimental condition in nM and the y axis is spheroid size as % of control spheroid size.

Figure 5 is a graph summarizing the effect of MM-111, trastuzumab, and tamoxifen combined compared to that of any of the double combinations or MM-111, trastuzumab, and fulvestrant combined compared to that of any of the double combinations at inhibiting single ligand (estrogen or heregulin) or dual-ligand (estrogen and heregulin)-stimulated spheroid growth *in vitro*. The y-axis is % inhibition of spheroid size normalized to stimulated control.

Figure 6 is a graph showing that the combination of MM-111 and lapatinib inhibits tumor growth *in vivo*. The x-axis shows the time post tumor implant in days and the y-axis shows tumor volume in mm^3. Mice were treated with inhibitors on day 7 post tumor implant.

Figure 7 evaluates the ability of lapatinib to inhibit ErbB3 and AKT activation in heregulin-stimulated cells. 7a is a graph comparing computer-generated dose-response curves to experimental results in heregulin-stimulated BT474-M3 cells. 7b is a graph showing lapatinib inhibition (IC50) of ErbB3 and AKT activation in heregulin-stimulated and unstimulated cells following a 1-hour incubation with inhibitor.

Figure 8 is a series of graphs showing MM-111 or lapatinib inhibition of ErbB3 (8a) or AKT (8b) activation in heregulin-stimulated cells incubated with inhibitor for 15 minutes, 1 hour, 4 hours, and 24 hours. Figure 8c shows a comparison of IC50 for MM-111 and lapatinib at 1 hour and 24 hours for both BT474M3 cells and ZR75-30 cells.

Figure 9 is a graph showing the effect of MM-111 and lapatinib combination treatment on AKT activation in heregulin-stimulated BT474-M3 cells.

Figure 10 is a graph showing the effect of lapatinib on cell viability as a measure of proliferation of unstimulated and heregulin-stimulated BT474-M3 cells.

Figure 11 is a graph showing the effect of MM-111, lapatinib, or the combination on BT474-M3 cell apoptosis. The number of dead cells, cells in late apoptosis, early apoptosis, and live cells was quantitated.

Figure 12 is three graphs showing that MM-111 combines positively with anti-estrogen drugs and lapatinib in inhibiting dual ligand (estrogen (E2) and heregulin (HRG))-stimulated spheroid growth *in vitro*. Figure 12a shows the effect of lapatinib alone or the combination of lapatinib and fulvestrant (FVT). Figure 12b shows the effect of lapatinib alone or the combination of lapatinib and MM-111. Figure 12c shows the effect of lapatinib alone, the combination of MM-111 and fulvestrant, or the triple combination of MM-111, FVT, and lapatinib. Lapatinib is given in 3.3, 10, or 30nM doses. The x-axes are a log scale of each of MM-111 and /or FVT concentration in nM and the y axis is spheroid size as % of control (FBS alone) spheroid size.

Figure 13 is four graphs showing the MM-111 combines positively with the aromatase inhibitor letrozole and the tyrosine kinase inhibitor lapatinib in heregulin (HRG) and androstenedione (A4)-

stimulated BT474-M3-Aro cells that stably express human aromatase, which converts androstenedione to estrogen. Figure 13a shows the effect of letrozole, MM-111, or the combination of letrozole and MM-111. Figure 13b shows the effect of lapatinib, MM-111 or the combination of lapatinib and MM-111. Figure 13c shows the effect of lapatinib, letrozole, or the combination of lapatinib and letrozole. Figure 13d shows the effect of the dual combinations of MM-111 and letrozole, MM-111 and lapatinib, lapatinib and letrozole, and the triple combination of MM-111, lapatinib and letrozole. The x-axes are a log scale of MM-111concentration in nM. The drug concentrations are a ratio of 10:20:1 MM-111 to letrozole to lapatinib. The y axis is spheroid size as % of control spheroid size.

DETAILED DESCRIPTION

As herein provided, bispecific anti-ErbB2/anti-ErbB3 antibodies (e.g., MM-111) are co-administered with one or more additional therapeutic agents (e.g. an aromatase inhibitor or tyrosine kinase inhibitor), to provide effective treatment to human patients having a cancer.

The term "anti-ErbB3 agent" refers to any therapeutic agent that binds to ErbB3 or binds to an ErbB3-specific ligand or blocks the expression of ErbB3, and thereby inhibits the activity of cellular signaling mediated by ErbB3. Non-limiting examples of types of anti-ErbB3 agents include antibodies, bispecific antibodies, ligand analogs, soluble forms of ErbB3 or the ErbB3 ectodomain, ErbB3 specific RNAi molecules, and similar biologic agents.

The term "antibody" describes a polypeptide comprising at least one antibody-derived antigen binding site (e.g., V_H/V_L region or Fv, or complementarity determining region - CDR) that specifically binds to a specific antigen, e.g., ErbB3. "Antibodies" include whole antibodies and any antigen binding fragment, e.g., Fab or Fv, or a single chain fragment (e.g., scFv), as well as bispecific antibodies and similar engineered variants, human antibodies, humanized antibodies, chimeric antibodies Fabs, Fab'2s, ScFvs, SMIPs, Affibodies®, nanobodies, or a domain antibodies, and may be of any of the following isotypes: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE. The antibody may be a naturally occurring antibody or may be an antibody that has been altered (e.g., by mutation, deletion, substitution, conjugation to a non-antibody moiety). For example, an antibody may include one or more variant amino acids (compared to a naturally occurring antibody) which change a property (e.g., a functional property) of the antibody. For example, numerous such alterations are known in the art which affect, e.g., half-life, effector function, and/or immune responses to the antibody in a patient. The term "antibody" thus includes whole antibodies and any antigen binding fragment (i.e., "antigen-binding portion," e.g., Fabs) or single chains thereof (e.g., scFvs) as well as bispecific antibodies and similar engineered variants, provided that they retain the binding specificity of an antibody.

An "anti-ErbB3 antibody" is an antibody that immunospecifically binds to the ectodomain of ErbB3 and an "anti-ErbB2 antibody" is an antibody that immunospecifically binds to the ectodomain of ErbB2. The antibody may be an isolated antibody. Such binding to ErbB3 or ErB2 exhibits a Kd with a

value of no greater than 50 nM as measured by a surface plasmon resonance assay or a cell binding assay. Exemplary anti-ErbB3 antibodies inhibit EGF-like ligand mediated phosphorylation of ErbB3, e.g., anti-ErbB2 antibodies that inhibit the binding of heregulin to ErbB2/ErbB3 heterodimers. EGF-like ligands include EGF, TGFα, betacellulin, heparin-binding epidermal growth factor, biregulin, epigen, epiregulin, and amphiregulin, which typically bind to ErbB1 and induce heterodimerization of ErbB1 with ErbB3.

The term "bispecific antibody" as used herein refers to a protein comprising two antigen-binding sites, a first binding site exhibiting immunospecific binding to a first antigen or epitope and a second binding site exhibiting immunospecific binding to a second antigen or epitope distinct from the first. An "anti-ErbB2/anti-ErbB3 bispecific antibody" is an antibody that comprises two binding sites, one that immunospecifically binds to the ectodomain of ErbB3 and another that immunospecifically binds to the ectodomain of ErbB2. Preferably, a bispecific ErbB3, ErbB2 antibody is the antibody comprising SEQ ID NO:1.

An "anti-estrogen agent" as used herein refers to an agent that prevents or reduces production of estrogen or prevents or reduces signaling mediated by estrogen receptors. Anti-estrogen agents include but are not limited to estrogen receptor antagonists and aromatase inhibitors. Estrogen receptor antagonists include but are not limited to raloxifene, fulvestrant, tamoxifen, afimoxifene (4-hydoroxytamoxifen), arzoxifene, toremifene, and lasofoxone. Preferably, the estrogen receptor antagonist is tamoxifen or fulvestrant. Aromatase inhibitors work by blocking the synthesis of estrogen in an animal (e.g., a mouse or a human). This lowers estrogen levels in the animal and thereby inhibits the growth of estrogen-driven cancers. Examples of aromatase inhibitors include but are not limited to exemestane, anastrozole, letrozole, aminoglutethimide, testolactone, vorozole, formestane, and fadrozole. Preferably, the aromatase inhibitor is exemestane or letrozole.

By "cancer" is meant any condition characterized by abnormal, unregulated, malignant cell growth.

By "malignant tumor" is meant any cancer that takes the form of a tumor.

The term "effective amount" refers to an amount of a drug effective to achieve a desired effect, e.g., to ameliorate disease in a subject. Where the disease is a cancer, the effective amount of the drug may inhibit (e.g., slow to some extent, inhibit or stop) one or more of the following characteristics: cancer cell growth, cancer cell proliferation, cancer cell motility, cancer cell infiltration into peripheral organs, tumor metastasis, and tumor growth. Where the disease is a cancer, the effective amount of the drug may alternately do one or more of the following when administered to a subject: slow or stop tumor growth, reduce tumor size (e.g., volume or mass); relieve to some extent one or more of the symptoms associated with the cancer, extend progression free survival, result in an objective response (including a partial response or a complete response), and increase overall survival time. To the extent the drug may prevent growth and/or kill existing cancer cells, it is cytostatic and/or cytotoxic.

A "mouse effective amount" refers to an amount of a drug effective to achieve a desired effect when the subject is a mouse.

A "human effective amount" refers to an amount of a drug effective to achieve a desired effect when the subject is a human patient.

The terms "combination therapy," "concomitant use," "co-administration," co-administering," "co-administered," and the like, refer to the administration of at least two therapeutic agents to a subject either simultaneously or within a time period during which the effects of the earlier-administered therapeutic agent are still operative in the subject when a later-administered therapeutic agent is administered.

A "receptor tyrosine kinase inhibitor" as used herein refers to a member of a class of drugs that specifically inhibit receptor tyrosine kinases and thus reduce or eliminate the activation of various signal transduction pathways. Receptor tyrosine kinase inhibitors useful for the treatment of cancer as disclosed herein include but are not limited to the small molecule inhibitors erlotinib, afatinib, dasatinib, gefitinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib and sorafenib. Receptor tyrosine kinase inhbitors also include antibody-based therapeutics such as cetuximab, panitumumab, zalutumumab, nimotuzumab, and matuzumab). Preferably, the receptor tyrosine kinase inhibitor is lapatinib.

"Dosage" or "dosing regimen" refers to parameters for administering a drug in defined quantities per unit time (e.g., per hour, per day, per week, per month, etc.) to a patient. Such parameters include, e.g., the size of each dose. Such parameters also include the configuration of each dose, which may be administered as one or more units, e.g., taken at a single administration, e.g., orally (e.g., as one, two, three or more pills, capsules, etc.) or injected (e.g., as a bolus). Dosage sizes may also relate to doses that are administered continuously (e.g., as an intravenous infusion over a period of minutes or hours). Such parameters further include frequency of administration of separate doses, which frequency may change over time. A "dosing cycle" or "dosing interval" is the period of time that comprises one cycle of treatment (e.g., 21 days or 28 days) for a dosing regimen.

"Dose" refers to an amount of a drug given in a single administration.

Preferred cancer cells of cell lines are cells of ErbB2 expressing cell lines such as ErbB2 overexpressing cell lines, e.g., BT474-M3 (ATCC® # CRL- HTB-20™, derived from breast ductal carcinoma cells), BT474-M3-Aro (BT474-M3 cells that stably express human aromatase), ZR75-30 (ATCC® # CRL-1504™, derived from breast ductal carcinoma cells), SKOV-3 (ATCC® # HTB-77™, derived from metastatic ovarian adenocarcinoma cells), MCF7 (ATCC® # HTB-22™) clone 18, MDA-MB-453 (ATCC® # HTB-131™, derived from breast carcinoma cells), SK-BR-3 (ATCC® # HTB-30™, derived from breast adenocarcinoma cells), and NCI-N87 (ATCC® # CRL-5822™, derived from gastric carcinoma cells).

Cancers may include, for example, solid tumors such as: sarcomas (e.g., clear cell sarcoma), carcinomas (e.g., renal cell carcinoma), and lymphomas; tumors of the breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, bilecyst, bile duct, small intestine, urinary system (including the kidney, bladder, and epithelium of the urinary tract), female genital system (including the uterine neck, uterus, ovary, chorioma, and gestational trophoblast), male genital system

(including the prostate, seminal vesicle, and testicles), endocrine glands (including the thyroid gland, adrenal gland, and pituitary body), skin (including angioma, melanoma, sarcoma originating from bone or soft tissue, and Kaposi's sarcoma), brain and meninges (including astrocytoma, neuroastrocytoma, spongioblastoma, retinoblastoma, neuroma, neuroblastoma, neurinoma and neuroblastoma), nerves, and eyes.

A cancer may be an estrogen receptor positive (ER+) cancer. Such cancers exemplify candidates for therapy regimens that include anti-estrogen agents. Such cancers may include but are not limited to certain breast, ovarian, uterine, endometrial, lung, bone, brain, bladder, liver and urogenital cancers.

A cancer may be an ErbB2 gene-amplified cancer and/or an ErbB2-expressing or overexpressing cancer. ErbB2, also known as HER2 or Neu, is a cell surface transmembrane receptor protein that generates intracellular signals (e.g., upon ligand activation) via its intracellular tyrosine kinase activity. In excess, such signals can promote oncogenesis e.g., by triggering cell division. The ErbB2 gene is amplified and/or overexpressed in many types of human malignancies, including but not limited to breast, ovarian, endometrial, pancreatic, colorectal, prostate, salivary gland, kidney, and lung. ErbB2 overexpressing cancers are designated a $HER2^{+++}$ or $HER2^{++}$ depending on the level of ErbB2 overexpression, with $HER2^{+++}$ indicating the highest levels of HER2 expression. $HER2^{+++}$ and $HER2^{-+}$ status are typically determined by an immunoassay such as immunohistochemistry, e.g., Herceptest®. ErbB2 gene amplification is may be determined by, e.g., FISH (fluorescence in situ hybridization), with HER2-amplified cancer cells being those that have more than two HER2 gene copies being HER2-amplified, and cells and/or tumors comprising HER2-amplified cancer cells being referred to as "FISH positive."

A number of bispecific anti-ErbB2, antiErbB3 antibodies that are scFv HSA conjugates are described in co-pending US patent publication No. 2011-0059076, and PCT publication Nos. WO2009/126920 and WO 2010/059315, each of which is incorporated herein by reference in its entirety and each of which discloses MM-111 (also referred to as B2B3-1) and other bispecific anti-ErbB2/antiErbB3 antibodies that are scFv HSA conjugates and that are suitable for use in the methods and compositions provided herein, including the components of A5-HSA-ML3.9, ML3.9-HSA-A5, A5-HSA-B1D2, B1D2-HSA-A5, B12-HSA-B1D2, B1D2-HSA-B12, A5-HSA-F5B6H2, F5B6H2-HSA-A5, H3-HSA-F5B6H2, F5B6H2-HSA-H3, F4-HSA-F5B6H2, F5B6H2-HSA-F4, B1D2-HSA-H3, and H3-HSA-B1D2, . Other suitable bispecific anti-ErbB2/antiErbB3 antibodies are disclosed and claimed in US Patent Nos. 7,332,580 and 7,332,585, which are incorporated herein by reference. MM-111 is currently undergoing clinical trials, including an open-label Phase 1/2 and pharmacologic study of MM-111 in patients with advanced, refractory HER2 positive cancers, an open-label Phase 1/2 trial of MM-111 in combination with trastuzumab (Herceptin®) in patients with advanced HER2 positive breast cancer, and an open label, Phase 1/2 and pharmacologic study of MM-111with three different combination treatments: MM-111 in combination with cisplatin, capecitabine, and trastuzumab, MM-111 in

combination with lapatinib and trastuzumab, and MM-111 in combination with paclitaxel and trastuzumab.

A bispecific anti-ErbB2/anti-ErbB3 antibody (e.g., MM-111) can be co-administered with other therapeutic agents, (e.g, an anti-estrogen receptor agent or a receptor tyrosine kinase inhibitor) prior to (e.g., neoadjuvant therapy), concurrent with, or following (e.g., adjuvant therapy) radiotherapy of, or surgical intervention to remove, a malignant tumor.

Additional therapeutic agents suitable for combination with anti-ErbB2/anti-ErbB3 antibodies may further include: 1) monoclonal antibody EGFR inhibitors (e.g. cetuximab, panitumumab, zalutumumab, nimotuzumab, and matuzumab), additional small molecule tyrosine kinase inhibitors such as PKI-166, PD-158780, EKB-569, Tyrphostin AG 1478, and pan-HER kinase inhbitors (e.g. CI-1033 (PD 183805), AC480, HM781-36B, AZD8931 and PF299804); 2) microtubule stabilizing agents (e.g. laulimalide, epothilone A, epothilone B, discodermolide, eleutherobin, sarcodictyin A, sarcodictyin B, paclitaxel, nab-paclitaxel or docetaxel); antimetabolites such as 5-fluorouracil (5-FU) and capecitabine; and platinum-based therapeutics such as oxaliplatin, carboplatin and cisplatin. Additional examples of therapeutic agents suitable for combination with anti-ErbB2/anti-ErbB3 antibodies may be found in Table 5 and the Appendix below.

MM-111 is suitable for both large scale production and systemic therapy. MM-111 binds to ErbB2/ErbB3 heterodimers and forms a trimeric complex with ErbB2 and ErbB3, effectively inhibiting ErbB3 signaling. The antitumor activity of MM-111 requires the presence of both ErbB2 and ErbB3, but is particularly dependent on ErbB2 expression. The affinity of its ErbB2 antigen-binding site is about 30 times higher than the affinity of its ErbB3 antigen-binding site, but the ErbB2 antigen-binding site does not by itself inhibit ErbB2 activity when bound to ErbB2. The strong binding of MM-111 to ErbB2 places the ErbB3 antigen-binding site in close proximity to bound ErbB2/ErbB3 heterodimer, resulting in an avidity effect that potentiates the binding of the ErbB3 antigen-binding site to the heterodimer ErbB3, whereby a biological effect is produced. MM-111 is administered to human subjects (patients) at an interval measured in days, as a single loading dose of at least 20 mg/kg of MM-111 followed by at least seven day intervals (e.g., every 2 weeks) by at least one administration of a single maintenance dose of MM-111, where the maintenance dose is generally smaller than the loading dose, e.g., at least 5mg/kg less than the loading dose.

EXAMPLES

The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially the same or similar results.

MM-111 in combination with anti-estrogen therapeutics

Methods:

Spheroid in vitro tumor model assay

BT474-M3 wild type cells (2000 cells/well) are plated in Ultra Low Cluster 96-well plate (Costar). After overnight incubation, indicated treatments are introduced to the plate. Cells are continued to culture for six days. Spheroids are then examined by Nikon microscope and analyzed by MetaMorph Image Analysis Software (Molecular Devices). The spheroid size from cells cultured in medium containing 10% FBS is set as control.

Xenograft model

BT474-M3 cells ($2X10^7$ cells per mice) are inoculated subcutaneously into Nu/Nu immunodeficient mice, which are implanted with an estrogen pellet (0.72mg; 60-day release) one day before the experiment. Tumors are measured after seven days and mice are randomized into four groups: those treated with placebo, MM-111 (60mg/kg, Q7D), 4-hydroxytamoxifen (5mg; 60-day release pellet), and combination of MM-111 and 4-hydroxytamoxifen, respectively. Tumors are measured every three days and the experiment is ended at day 32.

Example 1: MM-111 and tamoxifen combination therapy inhibits tumor growth *in vivo*.

In order to compare the effect of MM-111 and tamoxifen combination therapy on tumor growth *in vivo*, estrogen stimulated mice were prepared in the xenograft model using the methods described above or minor variations thereof. Mice were inoculated with tumor forming BT474-M3 cells and on day 7 given a placebo (vehicle control), MM-111, tamoxifen, or a combination of MM-111 and tamoxifen and tumor growth was measured over time. As shown in Figure 1, this *in vivo* BT474-M3 xenograft model showed resistance to tamoxifen treatment but when mice were given a combination of MM-111 and tamoxifen the combination treatment inhibited tumor growth to a significantly greater extent. Statistical significance ($p<0.05$) was observed for the combination group from day 28 onward when compared to vehicle control, from day 21 onward when compared to MM-111 and from day 25 onward when compared to tamoxifen.

Example 2: MM-111 combines positively with anti-estrogen drugs in inhibiting estrogen-stimulated spheroid growth

Multicellular spheroids are used to simulate the growth and microenvironmental conditions of tumors *in vitro*. To further investigate the ability of MM-111 to inhibit cell growth when in combination with anti-estrogen drugs, spheroids of BT474-M3 cells were prepared using the methods described above or minor variations thereof and treated with an ErbB2-binding therapeutic and/or an anti-estrogen therapeutic. Spheroids of estrogen-stimulated cells were treated with a dose range of MM-111, tamoxifen, or the combination of MM-111 and tamoxifen (Figure 2a); trastuzumab, tamoxifen or the combination of trastuzumab and tamoxifen (Figure 2b); MM-111, fulvestrant, or the combination of MM-111 and fulvestrant (Figure 2c); trastuzumab, fulvestrant, or the combination of trastuzumab and fulvestrant (Figure 2d); or MM-111, trastuzumab, or the combination of MM-111 and trastuzumab (Figure 2e). When used as single agent alone, MM-111, trastuzumab, fulvestrant and tamoxifen showed inhibitory effects on spheroid growth in the estrogen-stimulated BT474-M3 spheroid assay. The combination of tamoxifen or fulvestrant with MM-111 (Figures 2a and 2c, respectively) or trastuzumab (Figures 2b and 2d, respectively) increased the degree of growth inhibition, as did the combination of MM-111 and trastuzumab (Figure 2e). The inhibitory effects were increased still further when estrogen-stimulated spheroids were treated with the triple combination of MM-111, trastuzumab, and tamoxifen (Figure 2f) or MM-111, trastuzumab, and fulvestrant (Figure 2g) as compared to the double combinations of drugs.

Example 3: MM-111 combines positively with anti-estrogen drugs in inhibiting heregulin-stimulated spheroid growth

To further investigate the ability of MM-111 to inhibit cell growth when in combination with anti-estrogen drugs, spheroids of heregulin (HRG)-stimulated BT474-M3 cells were prepared using the methods described above or minor variations thereof and treated with a dose range of MM-111, tamoxifen, or the combination of MM-111 and tamoxifen (Figure 3a); trastuzumab, tamoxifen or the combination of trastuzumab and tamoxifen (Figure 3b); MM-111, fulvestrant, or the combination of MM-111 and fulvestrant (Figure 3c); trastuzumab, fulvestrant, or the combination of trastuzumab and fulvestrant (Figure 3d); or MM-111, trastuzumab, or the combination of MM-111 and trastuzumab (Figure 3e). MM-111 inhibited heregulin-induced spheroid growth but tamoxifen (Figure 3a), trastuzumab (Figure 3b), and fulvestrant (Figure 3c) did not inhibit heregulin stimulated spheroid growth. No significant combinational effect was observed when MM-111 was used with tamoxifen (Figure 3a) or fulvestrant (Figure 3c). The combination of trastuzumab and either tamoxifen (Figure 3b) or fulvestrant (Figure 3d) failed to show inhibitory activity significantly greater than either drug alone. As shown in Figure 3e, MM-111 but not trastuzumab showed inhibitory activity in heregulin-stimulated spheroid growth. Improved inhibitory effects were observed when both drugs were combined. In comparison to the double combination of either MM-111or trastuzumab with tamoxifen or fulvestrant, the triple combination of MM-111, trastuzumab and either tamoxifen (Figure 3f) or fulvestrant (Figure 3g) showed

similar inhibitory effects as those of MM-111 and trastuzumab in combination (Figure 3e) on heregulin-stimulated spheroid growth.

Example 4: MM-111 combines positively with anti-estrogen drugs in inhibiting dual ligand (estrogen and heregulin)-stimulated spheroid growth

Dual ligand (estrogen and heregulin) stimulated spheroids were treated with a dose range of tamoxifen, MM-111 or the combination of MM-111 and tamoxifen (Figure 4a) or trastuzumab, tamoxifen or the combination of trastuzumab and tamoxifen (Figure 4b). While MM-111 and trastuzumab each inhibited spheroid growth (Figure 4a) the combination of MM-111 and tamoxifen showed greater inhibitory effects than either drug alone. In contrast, trastuzumab alone had no significant inhibitory effects and the combination of trastuzumab and tamoxifen showed similar effects to tamoxifen alone.

Dual ligand stimulated spheroids were then treated with a dose range of fulvestrant, MM-111 or the combination of MM-111 and fulvestrant (Figure 4c) or fulvestrant, trastuzumab, or a combination of fulvestrant or trastuzumab (Figure 4d). Again, while MM-111 and fulvestrant each separately inhibited spheroid growth the combination of MM-111 and fulvestrant showed greater inhibitory effects than either drug alone (Figure 4c). Trastuzumab alone had no significant inhibitory effects and the combination of trastuzumab and fulvestrant showed similar effects to tamoxifen alone (Figure 4d).

Dual ligand stimulated spheroids were then treated with MM-111, trastuzumab, or a combination of MM-111 and trastuzumab. MM-111 showed greater inhibitory effects than trastuzumab in dual ligand-stimulated spheroid growth. Enhanced inhibitory effects were observed when both drugs were combined (Figure 4e).

In comparison to the double combination of MM-111or trastuzumab with tamoxifen or fulvestrant, the triple combination of MM-111, trastuzumab and either tamoxifen (Figure 4f) or fulvestrant (Figure 4g) showed similar inhibitory effects to those of MM-111 and trastuzumab in combination (Figure 4e) on estrogen- and heregulin- (dual ligand) stimulated spheroid growth.

The data in the preceding Examples demonstrate that combination therapies comprising MM-111 and an anti-estrogen therapeutic are more effective than each of these therapies alone. The percent of spheroid growth inhibition induced by each treatment under estrogen or heregulin stimulation is summarized in Figure 5 and Table 1. MM-111 was required for inhibition of spheroids stimulated with heregulin. For each stimulated condition tested, the triple combination resulted in the greatest inhibition of spheroid growth, providing a percent inhibition ranging from about 70% to about 90%.

Table 1. Percent inhibitor induced maximal spheroid growth inhibition

Tamoxifen combination				
	MM-111+ Trastuzumab	MM-111 + anti-estrogen	Trastuzumab + anti-estrogen	Triple combination
E2	54%	49%	55%	73%
HRG	65%	43%	0%	71%
E2+HRG	46%	43%	36%	79%
Fulvestrant combination				
E2	54%	49%	55%	77%
HRG	64%	34%	4%	71%
E2+HRG	46%	57%	47%	88%

The percent of spheroid growth inhibition (normalized to untreated, stimulated control) was determined for 1μM doses of inhibitor treatment.

The combination of MM-111 and tamoxifen resulted in potent inhibition of tumor growth *in vivo*. Taken together, these data demonstrate that the combination of MM-111 and anti-estrogen therapies results in potent anti-tumor effects *in vitro* and *in vivo*.

MM-111 in combination with lapatinib

Methods

Computational Modeling

A computational model of HRG-induced phospho-ErbB3 signaling, as well as a model of lapatinib, was used as previously described (Schoeberl, et al 2009).

Cell signaling assay

Serum-starved cells are pre-incubated with serial dilutions of MM-111, lapatinib or combinations at doses and treatment times indicated, followed by stimulation with 5nM heregulin 1-β (R&D Systems, Minneapolis, MN) for 10 minutes. Cell lysates are probed for phospho-ErbB3 (pErbB3), and phospho-AKT (pAKT) by ELISA as described previously (Schoeberl et al, 2009). Inhibitor IC_{50} values are calculated by fitting dose-response data to a 4-parameter sigmoidal curve (GraphPad Prism®, GraphPad Software, Inc., La Jolla, CA).

Cell proliferation assay

Cells (8,000/well) are seeded into 96-well plates and incubated overnight. Inhibitor is added at doses indicated and cells are treated for 24 hours. For experiments with ligand stimulation, cells are serum-starved overnight prior to addition of inhibitor and 2 nM heregulin 1-□(R&D Systems, Minneapolis, MN) is added 1 hour post-inhibitor treatment in media containing 5% FBS. Numbers of viable cells are measured as an indicator of cell proliferation using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI).

Apoptosis assay

BT474-M3 cells (2000 cells/well) are plated in Ultra Low Cluster 96-well plate (Costar®, Corning, NY). After overnight incubation, spheroids are treated with inhibitor at concentrations indicated for 72 hours. Spheroids are then trypsinized and combined with floating cells. Cells are washed twice with cold PBS and suspended in binding buffer (0.01 M HEPES, pH 7.4; 0.14 M NaCl; 2.5 mM $CaCl_2$). Cells are then stained with FITC-conjugated Annexin V and PI. Apoptotic cells are quantified on a FACSCalibur™ FACS machine.

Xenograft efficacy studies

Tumor xenografts are established by subcutaneous injection of BT474-M3 cells into the flank of 5-6 weeks old female athymic nude mice (nu/ nu; Charles River Labs, Wilmington, MA). Mice receive a subcutaneous 60 day, slow-release estrogen implant in the opposite flank (0.72 mg pellet; Innovation Research of America, Sarasota, FL) 24 hours prior to the injection of cells. Once tumors reach a mean volume of 150-500 mm^3, mice are randomized into groups of 8 or 10 and dosed by intraperitoneal injection once every three days with vehicle, MM-111 or lapatinib. For lapatinib combination studies, MM-111 is given once every seven days and lapatinib daily by gavage at doses indicated.

Aromatase-overexpressing BT474-M3 cells and proliferation assay

BT474-M3 cells were transfected with PS100010 vector containing human aromatase (gene accession No: NM_000103.2). Cells with stable expression of aromatase (BT474-M3-Aro) were obtained after selection with 400µg/ml geneticin. For cell proliferation assay, BT474-M3-Aro cells (5000 cells/well) were plated in phenol red-free RPMI-1640 medium containing 5% charcoal-stripped FBS into 96-well plate. After overnight incubation, indicated treatments were introduced in the presence of androstenedione (A-4; 200nM) and heregulin (HRG; 2nM). After three days of treatment, cell viability was determined by WST-1 (Roche; Cat. # 11 644 807 001) according to manufacturer's instruction. Cell viability in the presence of 5% charcoal-stripped FBS was set as control (100%).

Example 5: The combination of MM-111 and lapatinib inhibits tumor growth *in vivo*

The combination of MM-111 with lapatinib was investigated *in vivo* in the BT474-M3 breast cancer xenograft model using the methods described above or minor variations thereof. MM-111 and lapatinib were each dosed at an optimal efficacious dose weekly and daily, respectively. The combination of MM-111 and lapatinib provided more potency compared to either drug alone, reaching statistical significance for MM-111 ($p = 3.9x10-4$) and lapatinib ($p = 5.1x10-3$) on day 13 (Figure 6). The percent change in tumor volume from day 40 to day 7 (inoculation) was calculated for each group (Figure 6b). The combination of MM-111 and lapatinib resulted in a percent change in tumor volume of -69% (about 70%), reflecting tumor regressions, compared to -11% (about 10%) for lapatinib and 14% (about 15%) for MM-111.

Example 6: Simulations predict lapatinib has suboptimal activity in inhibiting heregulin-driven pErbB3 and pAKT

A dose range of lapatinib inhibition of pErbB3 activation was predicted using the computational modeling described above. A dose range of lapatinib was applied to BT474-M3 cells followed by stimulation with 5 nM heregulin for 10 min. The amount of pErbB3 was measured by ELISA using the methods described above or minor variations thereof. Model-generated dose-response curves overlay the experimental data (Figure 7a). A comparison of the inhibitory activity of lapatinib in heregulin-stimulated or unstimulated (basal) cells was performed to demonstrate that heregulin signaling perturbs the activity of lapatinib. Untreated and heregulin-stimulated cells were probed for pErbB3 and pAKT and the IC50 was calculated (Figure 7b). These data show that lapatinib alone is not an effective inhibitor of heregulin-activated signaling.

Example 7: MM-111 is a more potent inhibitor of HRG-driven ErbB3 and AKT phosphorylation than lapatinib

In order to compare the ability of MM-111 and lapatinib to inhibit heregulin-induced ErbB3 activation, BT474-M3, or an additional ErbB2 overexpressing breast tumor cell line, ZR75-30 (ATCC® # CRL-1504™), cells were incubated with serial dilutions of either inhibitor for 15 minutes, 1 hour, 4 hours, and 24 hours followed by stimulation with 5 nM heregulin for 10 min. Amounts of pAKT and pErbB3 were measured by ELISA essentially as described. MM-111 potently reduced pErbB3 levels (inhibited ErbB3 phosphorylation) in BT474-M3 (IC_{50} = 3 nM) cells (Figure 8a) and ZR75-30 cells (IC_{50} = 5 nM) (Figure 8c). Good reduction by MM-111 of pAKT levels (inhibition of AKT phosphorylation) in BT474-M3 (IC_{50} = 10) (Figure 8b) and in ZR75-30 cells (IC_{50} = 4 nM) (Figure 8d) was also observed. The ability of MM-111 to inhibit heregulin-induced ErbB3 activation (phosphorylation) was superior to lapatinib by greater than an order of magnitude and the relative IC_{50} for each inhibitor (Figure 8c) was consistent following up to 24 hours incubation with inhibitors, indicating treatment times had little effect on the potency of the inhibitors.

Example 8: The combination of MM-111 and lapatinib potently inhibits pAKT

The effect of MM-111 combined with lapatinib on pAKT inhibition (reduction of pAKT levels) was assessed in heregulin-stimulated BT474-M3 cells. Cells were incubated for 2 hours with a dose range of MM-111, lapatinib or their combination and pAKT was measured by ELISA. In the presence of heregulin, the combination of MM-111 and lapatinib was extremely effective, inhibiting pAKT well below basal levels at therapeutically relevant concentrations (Figure 9). Treatment with either MM-111 (1μM) or lapatinib (1μM) alone resulted in similar levels of pAKT inhibition (see Figure 8b) while the combination resulted in about 20% more inhibition of pAKT.

Example 9: The ability of lapatinib to inhibit cell proliferation is perturbed under heregulin-stimulated conditions

The effect of lapatinib on cell proliferation was measured in unstimulated and heregulin-stimulated BT474-M3 cells. Cells grown in serum or in serum plus 2 nM heregulin were treated with lapatinib across a dose range for 24 hours. Lapatinib treatment resulted in about a 50% inhibition of unstimulated cells but its effect was reduced to about 23% inhibition in heregulin-stimulated BT474-M3 cells (Figure 10).

Example 10: Treatment with the combination of MM-111 and lapatinib results in increased apoptosis

The effect of the MM-111 combination with lapatinib on apoptosis was assessed in a BT474-M3 spheroid model. Spheroids were prepared using the methods described above or minor variations thereof and treated with MM-111 (100 nM), lapatinib (33 nM), or a combination of 100 nM MM-111 and 33 nM lapatinib. Cells were then stained with Annexin V and propidium iodide (PI) and quantitated using FACS (Figure 11, Table 2). Cell populations staining positive with Annexin V and PI were quantified as late apoptotic, cell populations staining positive with Annexin V but not PI were quantified as early apoptotic, cell populations staining positive for PI but not Annexin V were quantified as dead cells and populations of cells not stained with either Annexin V or PI were considered alive and not apoptotic (Table 2). Spheroids that were treated with both MM-111 and lapatinib had a higher number of total apoptotic cells (about 46%) compared to those treated with only lapatinib (about 31%) or only MM-111 (about 20%; Figure 10).

Table 2. Percent cell population after treatment with MM-111, lapatinib or the combination

	Live cells	Early apoptosis	Late apoptosis	Dead cells
Control	75.2	17.3	7.2	0.42
MM-111	78.9	12.9	7.5	0.74
Lapatinib	67.9	16.8	14.5	0.73
Combination	52.1	30.0	16.2	1.74

Example 11: MM-111 combines positively with anti-estrogen drugs and lapatinib in inhibiting dual ligand (estrogen and heregulin)-stimulated spheroid growth

To further investigate the ability of MM-111 to inhibit cell growth when in combination with both anti-estrogen drugs and tyrosine kinase inhibitors, spheroids of estrogen and heregulin-stimulated BT474-M3 cells were prepared using the methods described above or minor variations thereof and treated with 3.3nM, 10nM, or 30nM lapatinib, either alone or in combination with a dose range of fulvestrant (FVT) (Figure 12a); 3.3nM, 10nM, or 30nM lapatinib, either alone or in combination with a dose range of MM-111 (Figure 12b); or 3.3nM, 10nM, or 30nM lapatinib, either alone or in combination with a dose range of both MM-111 and fulvestrant (Figure 12c). In the presence of dual ligand stimulation the

combination of lapatinib and FVT did not greatly increase inhibition of spheroid growth over lapatinib alone (Figure 12a). In contrast, the addition of MM-111 greatly increased the sensitivity of the spheroids to lapatinib treatment (Figure 12b), and the triple combination of lapatinib, FVT and MM-111 showed an even greater increase of spheroid growth inhibition over lapatinib alone.

Example 12: MM-111 combines positively with anti-estrogen drugs in inhibiting spheroid growth in BT474-M3 cells overexpressing human androstenedione

Androstenedione is a steroid hormone that is converted to estrogen by aromatase. To further investigate the ability of MM-111 to inhibit spheroid growth, aromatase-expressing cells were treated in the presence of androstenedione (A4) and heregulin (HRG) with MM-111, letrozole, or the combination of MM-111 or letrozole (Figure 13a); MM-111, lapatinib, or the combination of MM-111 and lapatinib (Figure 13b); lapatinib, letrozole, or the combination of lapatinib and letrozole (Figure 13c); and each of the dual combination plus the triple combination of MM-111, lapatinib, and letrozole (Figure 13d). In cells treated with A4 and HRG, the letrozole treatment did not result in significant inhibition of spheroid cell growth as compared to control (untreated) cells, whereas cells treated with MM-111 alone or the combination of MM-111 and letrozole inhibited cell proliferation to a similar extent (Figure 13a). Lapatinib treatment of the cells did not result in growth inhibition except at high concentrations, whereas treatment with MM-111 alone or in combination resulted in similar levels of cell growth inhibition except in higher concentrations where the combination showed increased inhibition of cell growth over either of the single treatments (Figure 13b). Treatment with lapatinib alone, letrozole alone, or the combination of lapatinib and letrozole did not result in significant cell growth inhibition except at high concentration (Figure 13c). Similarly, as shown in Figure 13d, the double combination of lapatinib and letrozole resulted in cell growth inhibition only at high drug concentration. In contrast the dual combinations of MM-111 and letrozole or MM-111 and lapatinib both showed an increase in cell growth inhibition as compared to control, and the triple combination of MM-111, lapatinib, and letrozole inhibited cell growth to an even greater degree.

Example 13: amino acid sequence of MM-111(SEQ ID NO:1)

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANINRDGSASYYVD
SVKGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLVTVSSASTGGGG
SGGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKAPKLMIYDVS
DRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYGSSSTHVIFGGGTKVTVLGAASDAHK
SEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHT
LFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNE
ETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAK
QRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRA
DLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAK
DVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL
IKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAED
YLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADICTL
SEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAAS
QAALGLAAALQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYP

GDSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARHDVGYCTDRTCAKWPEWL
GVWGQGTLVTVSSGGGGSSGGGSGGGGSQSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSW
YQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYCASWDYTLSGWV
FGGGTKLTVLG

Dosing and administration of MM-111 in combination one or more additional therapeutics

Example 14: Mode of administration of MM-111

MM-111 is prepared as a formulation containing 25 mg/ml MM-111 in a sterile aqueous solution comprising 20 mM L-histidine hydrochloride, 150 mM sodium chloride, pH 6.5, which is stored at 2-8°C.

MM-111 must be brought to room temperature prior to administration. Containers (e.g., vials) of MM-111 must not be shaken. The appropriate quantity of MM-111 is removed from the container, diluted in 250 mL of 0.9% normal saline and administered as an infusion using a low protein binding in-line filter (e.g., a 0.22 micrometer filter).

MM-111 is initially administered over about 90 minutes (first administration). In the absence of an infusion reaction, subsequent doses are administered over about 60 minutes.

A patient's body weight at the start of a dosing cycle is used to calculate the dose used throughout the cycle. Should a patient's body weight change by more than 10%, a new total dose is calculated to reflect this change.

Example 15: Dosage and Administration of MM-111

Preferred plasma concentrations of MM-111 achieved during treatment are at least 106 mg/L. It has now been discovered that certain combinations of dose frequency and dosage will achieve and maintain this plasma concentration during the course of treatment in at least half, and preferably in more than 60%, 70% or 80% of treated patients.

In certain embodiments a higher initial dose (loading dose - LD) is given, followed as defined intervals by at least one maintenance dose (MD). Intervals of dosing in days are typically indicated as QxD, wherein x represents an integer, so that a QxD of 7 indicates dosing every 7 days. Table 3A, Table 3B, and Table 3C below show doses and dosing intervals of the invention. In Table 3A, Table 3B, and Table 3C the indicated loading doses are optional – initial doses are preferably made at the indicated loading dose (LD), but may (e.g., as directed or at the physician's discretion) be made at the maintenance dose (MD). Table 3A provides a set of exemplary dosing intervals, loading doses and maintenance doses. Table 3B provides a variation of Table 3A allowing for dosage variability (indicated as "about") of up to +/- 3 mg/mL. Table 3C appears below and provides a more extensive set of exemplary dosing intervals, loading doses and maintenance doses. In each cell of Table 3A, Table 3B, and Table 3C, the top figure is the integer x in the interval QxD (e.g., 18 as the top figure in a cell indicates a dosing interval of Q18D or every 18 days), the middle figure represents the (optional) loading dose (LD) in mg/kg, nd the bottom figure represents the maintenance dose (MD) in mg/kg. Thus the top cell in Table 3A indicates a dosing interval (QxD) of once every seven days, a loading dose (optional) of 25 mg per kg of patient body weight, and a maintenance dose of 20 mg per kg of patient body weight; while the cell furthest to the right on the top row of Table 3C

indicates a dosing interval (QxD) of once every seven days, a loading dose (optional) of 30 mg per kg of patient body weight, and a maintenance dose of 15 mg per kg of patient body weight.

Table 3A

7 25 20
7 40 30
14 60 45
14 90 75
21 120 105

Table 3B

7 about 25 about 20
7 about 40 about 30
14 about 60 about 44
14 about 90 about 75
21 about 120 about 105

Table 3C

7 10 5	7 15 5	7 20 5	7 25 5	7 30 5	7 15 10	7 20 10	7 25 10	7 30 10	7 35 10	7 20 15	7 25 15	7 30 15
7 35 15	7 40 15	7 25 20	7 30 20	7 35 20	7 40 20	7 45 20	7 30 25	7 35 25	7 40 25	7 45 25	7 50 25	7 55 25
7 60 25	7 65 25	14 35 30	14 40 30	14 45 30	14 50 30	14 55 30	14 60 30	14 65 30	14 70 30	14 75 30	14 40 35	14 45 35
14 50 35	14 55 35	14 60 35	14 65 35	14 70 35	14 75 35	14 45 40	14 50 40	14 55 40	14 60 40	14 65 40	14 70 40	14 75 40
14 50 45	14 55 45	14 60 45	14 65 45	14 70 45	14 75 45	14 55 50	14 60 50	14 65 50	14 70 50	14 75 50	14 60 55	14 65 55
14 70 55	14 75 55	14 65 60	14 70 60	14 75 60	14 70 65	14 75 65	14 75 70	21 60 55	21 65 55	21 70 55	21 65 60	21 70 60
21 75 60	21 70 65	21 75 70	21 80 75	21 85 80	21 90 85							

Example 16. Dosage and administration of MM-111 with lapatinib and trastuzumab

Treatment for patients with trastuzumab-refractory HER2-overexpressing breast cancer is a critical unmet need in the field of breast oncology, and novel approaches to address this need are required. Although selective tyrosine kinase inhibitors (TKIs) have been highly effective for the treatment of certain tyrosine kinase oncogene-driven cancers, their clinical anti-tumor efficacy in the treatment of HER2-driven breast cancer has been disappointing despite adequate biodistribution and apparent target inhibition. Two completed phase II trials using the most potent HER2 TKI, lapatinib, have reported response rates of only 4%-8% in patients with trastuzumab-refractory HER2-overexpressing breast cancer. It is now known that the effective treatment of HER2+ breast cancer is more complex and resilient than previously thought. Recent evidence has highlighted the role of HER3 and a robust signal buffering capacity inherent in the HER2-HER3 tumor driver that protects it against a two log inhibition of HER2 catalytic activity, placing it beyond the therapeutic index of even the most potent tyrosine kinase inhibitors (TKIs).

Typically, lapatinib is administered at a dosage of 1000 to 1500mg in 250mg tablets taken once daily. Lapatinib is often used in combination with another cancer medication, capecitabine, which is taken for 14 day periods with one week in between.

In order to test whether the full inactivation of the HER2-HER3 driver can be achieved with much higher TKI dosing at an intermittent dosing schedule is more efficacious than continuous dosing, a modified dosing schedule is used wherein an increased dose of lapatinib is administered on days 1-5 of a 14 day cycle, said increased dose being a higher dose than the standard dose of 1000 to 1500mg/day. In some embodiments, the higher lapatinib dose is between 2000 and 9000mg/d. For example, higher lapatinib dose might be 2000, 2250, 3375, 3000, 3250, 3500, 3750, 4000, 4250, 4500, 4750, 5000, 5250, 5500, 5750, 6000, 6250, 6500, 6750, 7000, 7250, 7500, 7750, 8000, 8250, 8500, 8750, or 9000mg/day, and so on.

In certain embodiments a loading dose is given on day 1 of the 14-day cycle that is a higher dose than that given on subsequent days, the maintenance dose. For example, a loading dose given on day 1 of the 14 day cycle might be 7000mg/day, followed by a maintenance dose of 3000mg/day. Non-limiting examples of loading dose and maintenance dose combinations are listed in Table 4 below.

MM-111 is administered as described in Example 15. In some embodiments the treatment further comprises trastuzumab. Trastuzumab is typically given with an initial loading dose followed by a maintenance dose. For example, trastuzumab may be dosed at a loading dose of 8 mg/kg followed by a maintenance dose of 6 mg/kg every three weeks.

Table 4. Exemplary lapatinib dosing schedule: loading dose (top number) and maintenance dose (bottom number) in mg/d

2000 1000	2000 1500	2000 2000	2500 1000	2500 1500	2500 2000	3000 1000	3000 1500	3000 2000	3000 2500	3000 3000	3500 1000	3500 1500
3500 2000	3500 2500	3500 3000	4000 1000	4000 1500	4000 2000	4000 2500	4000 3000	4000 3500	4500 1000	4500 1500	4500 2000	4500 2500
4500 3000	4500 3500	4500 4000	5000 1000	5000 1500	5000 2000	5000 2500	5000 3000	5000 3500	5000 4000	5000 4500	5500 1000	5500 1500
5500 2000	5500 2500	5500 3000	5500 3500	5500 4000	5500 4500	5500 5000	6000 1000	6000 1500	6000 2000	6000 2500	6000 3000	6000 3500
6000 4000	6000 4500	6000 5000	6000 5500	7500 1000	7500 1500	7500 2000	7500 2500	7500 3000	7500 3500	7500 4000	7500 4500	7500 5000
7500 5500	7500 6000	7500 6500	7500 7000	8000 1000	8000 1500	8000 2000	8000 2500	8000 3000	8000 3500	8000 4000	8000 4500	8000 5000
8000 5500	8000 6000	8000 6500	8000 7000	8000 7500	9000 1000	9000 1500	9000 2000	9000 2500	9000 3000	9000 3500	9000 4000	9000 4500
9000 5000	9000 5500	9000 6000	9000 6500	9000 7000	9000 7500	9000 8000	9000 8500					

Example 17: Dosage and Administration of MM-111with Cisplatin, Capecitabine, and Trastuzumab

Administration of MM-111 with cisplatin, capecitabine, and trastuzumab is done, for example, by the following method or minor variations thereof.

Patients are administered therapy on a 21-day treatment cycle. Cisplatin is administered on day 1of each 21-day cycle by intravenous (i.v.) infusion over two hours, at a dose of 80mg/m^2. Capecitabine is administered orally, twice daily, at a dose of 1000 mg/m^2. Up to 21-day cycles of cisplatin and capecitabine are administered. Trastuzumab is administered i.v. at week 1 at an 8 mg/kg loading dose over 90 minutes, followed by a maintenance dose of 6mg/kg every 21 days over 30-90 minutes. MM-111 is administered as described in the above Examples. For example, MM-111 is administered i.v. over 90 minutes for the first dose and then weekly over 60 minutes thereafter.

Example 18: Dosage and Administration of MM-111 with Lapatinib and Trastuzumb

Administration of MM-111 with lapatinib and trastuzumab is done, for example, by the following method or minor variations thereof. Trastuzumab is administered i.v. at a 4 mg/kg loading dose on week 1 over 90 minutes, followed by a 2 mg/kg weekly maintenance dose thereafter. Lapatinib is given by

mouth either at 1000mg daily doses or at the one of the dose regimens described in Example 13. MM-111 is administered as described in the above Examples. For example, MM-111 is administered i.v. over 90 minutes for the first dose and then weekly over 60 minutes thereafter.

Example 19: Dosage and Administration of MM-111with Paclitaxel and Trastuzumab Administration of MM-111 with paclitaxel and trastuzumab is done, for example, by the following method or minor variations thereof. Patients are administered therapy on a 28-day treatment cycle. Paclitaxel dosing begins on day 1 of cycle 1. Paclitaxel is administered at 80 mg/m^2 weekly, as an i.v. infusion over 60 minutes. Trastuzumab is administered at a 4 mg/kg loading dose on week 1, i.v. over 90 minutes, followed by a 2 mg/kg weekly maintenance dose thereafter. MM-111 is administered as described in the above Examples. For example, MM-111 is administered i.v. over 90 minutes for the first dose and then weekly over 60 minutes thereafter.

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Endnotes

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

All patents patent applications and publications mentioned herein are incorporated by reference to the same extent as if each independent patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

APPENDIX

ANTICANCER AGENTS

A bispecific anti-ErbB2/anti-ErbB3 antibody co-administered in combination with an anti-estrogen receptor agent or a receptor tyrosine kinase inhibitor can be further co-administered with at least a third antineoplastic agent selected from any of those disclosed below.

Table 5: Exemplary antineoplastic agents for treatment of breast cancer in combination with a bispecific anti-ErbB2/anti-ErbB3 antibody.

Therapeutic Class	**Exemplary Agent (Generic/ Tradename)**	**Exemplary Dose**
Mitotic Inhibitors	paclitaxel (TAXOL®; ABRAXANE®)	175 mg/m^2
	docetaxel (TAXOTERE®)	60-100 mg/m^2
Topoisomerase Inhibitors	camptothecin	
	topotecan hydrochloride (HYCAMTIN	
	etoposide (EPOSIN®)	
Alkylating Agents	cyclophosphamide (CYTOXAN®)	600 mg/m^2
Platinum-Based Agents	Cisplatin	20-100 mg/m^2
	carboplatin (PARAPLATIN®)	300 mg/m^2
	nedaplatin (AQUPLA®)	
	oxaliplatin (ELOXATIN®)	65-85 mg/m^2
	satraplatin (SPERA®)	
	triplatin tetranitrate	
Selective Estrogen Modulators (SERM)	tamoxifen (NOLVADEX®)	20-40 mg/day
	raloxifene (EVISTA®)	60 mg/day
	toremifene (FARESTON®)	
Antimetabolites	methotrexate	40 mg/m^2
	Fluorouracil (5-FU)	500 mg/m^2
	Raltitrexed	
Antitumor Antibiotics	Doxorubicin (ADRIAMYCIN®)	40-75 mg/m^2
	epirubicin (ELLENCE®)	60-120 mg/m^2
Aromatase Inhibitors	aminoglutethimide (CYTADREN®)	250-2000 mg/day
	anastrozole (ARIMIDEX®)	1 mg/day
	letrozole (FEMARA®)	2.5 mg/day
	Vorozole	
	exemestane (AROMASIN®)	25-50 mg/day

	Testolactone	
	fadrozole (AFEMA®)	
Anti-VEGF Agents	bevacizumab (AVASTIN®)	10 mg/kg
Anti-ErbB2 (HER2/neu) Agents	trastuzumab (HERCEPTIN®)	2-8 mg/kg
	Pertuzumab (OMNITARG®)	
Anti-ErbB3 (HER3) Agents	U3-1287 (AMG 888)	

APPENDIX

ANTICANCER AGENTS

Other anticancer agents for combination with a bispecific anti-ErbB2/anti-ErbB3 antibody	**Brand Name(s)**	**Manufacturer/Proprietor**
Anti-IGF1R Antibodies		
AMG 479 (fully humanized mAb)		Amgen
IMCA12 (fully humanized mAb)		ImClone
NSC-742460		Dyax
19D12 (fully humanized mAb)		
CP751-871 (fully humanized mAb)		Pfizer
H7C10 (humanized mAb)		
alphaIR3 (mouse)		
scFV/FC (mouse/human chimera)		
EM/164 (mouse)		
MK-0646, F50035		Pierre Fabre Medicament, Merck
Small Molecules Targeting IGF1R		
NVP-AEW541		Novartis
BMS-536,924 (1H-benzoimidazol-2-yl)-1H-pyridin-2-one)		Bristol-Myers Squibb
BMS-554,417		Bristol-Myers Squibb
Cycloligan		
TAE226		
PQ401		
Anti-EGFR Monoclonal Antibodies		
INCB7839		Incyte
Bevacizumab	Avastin®	Genentech
Cetuximab	Erbitux®	IMCLONE
mAb 806		
Matuzumab (EMD72000)		
Nimotuzumab (TheraCIM)		
Panitumumab	Vectibix®	Amgen
Anti-ErbB3 Therapeutics		

U3-1287 / AMG888		U3 Pharma/Amgen
MM-121		Merrimack Pharmaceuticals
Anti-ErbB2 Therapeutics		
trastuzumab	Herceptin®	Genentech
HKI-272 - neratinib		Wyeth
KOS-953 - tanespimycin		Kosan Biosciences
Her/ErbB Dimerization Inhibitors		
2C4, R1273 - Pertuzumab	, Omnitarg®	Genentech, Roche
Small Molecules Targeting EGFR		
CI-1033 (PD 183805)		Pfizer, Inc.
EKB-569		
Gefitinib	IRESSA™	AstraZeneca
Lapatinib (GW572016)		GlaxoSmithKline
Lapatinib Ditosylate	Tykerb®	SmithKline Beecham
Erlotinib HCl (OSI-774)	Tarceva®	OSI Pharms
PD158780		
PKI-166		Novartis
Tyrphostin AG 1478 (4-(3-Chloroanillino)-6,7-dimethoxyquinazoline)		
Anti-cmet Antibody Therapies		
AVEO (AV299)		AVEO
AMG102		Amgen
5D5 (OA-5D5)		Genentech
Small Molecules Targeting cmet		
PHA665752		
ARQ-650RP		ArQule
ARQ 197		ArQule
Alkylating Agents		
BCNU→ 1 ,3-bis t2-chloroethyl)-nitrosourea		
Bendamustine		
Busulfan	Myleran	GlaxoSmithKline
Carboplatin	Paraplatin	Bristol-Myers Squibb
Carboquone		
Carmustine		
CCNU→ 1,-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (methyl CCNU)		
Chlorambucil	Leukeran®	Smithkline Beecham
Chlormethine		
Cisplatin (Cisplatinum, CDDP)	Platinol	Bristol-Myers
Cyclophosphamide	Cytoxan	Bristol-Myers Squibb

	Neosar	Teva Parenteral
Dacarbazine (DTIC)		
Fotemustine		
Hexamethylmelamine (Altretamine, HMM)	Hexalen®	MGI Pharma, Inc.
Ifosfamide	Mitoxana®	ASTA Medica
Lomustine		
Mannosulfan		
Melphalan	Alkeran®	GlaxoSmithKline
Nedaplatin		
Nimustine		
Oxaliplatin	Eloxatin®	Sanofi-Aventis US
Prednimustine,		
Procarbazine HCL	Matulane	Sigma-Tau Pharmaceuticals, Inc.
Ribonucleotide Reductase Inhibitor (RNR)		
Ranimustine		
Satraplatin		
Semustine		
Streptozocin		
Temozolomide		
Treosulfan		
Triaziquone		
Tricthylcnc Mclaminc		
ThioTEPA		Bedford, Abraxis, Teva
Triplatin tetranitrate		
Trofosfamide		
Uramustine		
Antimetabolites		
5-azacytidine		
Flourouracil (5-FU)/Capecitabine		
6-mercaptopurine (Mercaptopurine, 6-MP)		
6-Thioguanine (6-TG)	Purinethol®	Teva
Cytosine Arabinoside (Cytarabine, Ara-C)	Thioguanine®	GlaxoSmithKline
Azathioprine	Azasan®	AAIPHARMA LLC
Capecitabine	XELODA®	HLR (Roche)
Cladribine (2-CdA, 2-chlorodeoxyadenosine)	Leustatin®	Ortho Biotech
5-Trifluoromethyl-2'-deoxyuridine		
Fludarabine phosphate	Fludara®	Bayer Health Care
Floxuridine (5-fluoro-2)	FUDR®	Hospira, Inc.
Methotrexate sodium	Trexall	Barr
Pemetrexed	Alimta®	Lilly
Pentostatin	Nipent®	Hospira, Inc.

Raltitrexed Tegafur	Tomudex®	AstraZeneca
Aromatose Inhibitor Ketoconazole		
Glucocorticoids Dexamethasone Prednisolone Prednisone	Decadron® Dexasone, Diodex, Hexadrol, Maxidex Deltasone, Orasone, Liquid Pred, Sterapred®	Wyeth, Inc.
Immunotherapeutics Alpha interferon Angiogenesis Inhibitor IL-12→ Interleukin 12 IL-2→ Interleukin 2 (Aldesleukin)	 Avastin® Proleukin ®	 Genentech Chiron
Receptor Tyrosine Kinase Inhibitors AMG 386 Axitinib ((AG-013736) Bosutinib (SKI-606) Brivanib alalinate (BMS-582664) Cediranib (AZD2171) Dasatinib (BMS-354825) Imatinib mesylate Lestaurtinib (CEP-701) Motesanib diphosphate (AMG-706) Nilotinib hydrochloride monohydrate Pazopanib HCL (GW786034) Semaxanib (SU5416) Sorafenib tosylate Sunitinib malate Vandetanib (AZD647) Vatalanib; PTK-787 XL184, NSC718781	 Recentin Sprycel® Gleevec Tasigna® Armala Nexavar® Sutent® Zactima 	 Amgen Pfizer, Inc Wyeth BMS AstraVeneca Bristol-Myers Squibb Novartis Cephalon Amgen/Takeda Novartis GSK Pharmacia, Bayer Pfizer, Inc. AstraZeneca Novartis; Bayer Schering Pharma Exelixis, GSK
Microtubule-Targeting Agents Colchicine Docetaxel Ixabepilone Larotaxel Ortataxel Nanoparticle paclitaxel (ABI-007) Paclitaxel Tesetaxel	 Taxotere® IXEMPRA™ Abraxane® Taxol®	 Sanofi-Aventis US Bristol-Myers Squibb Sanofi-aventis Spectrum Pharmaceuticals Abraxis BioScience, Inc. Bristol-Myers Squibb Genta

Vinblastine sulfate	Velban®	Lilly
Vincristine	Oncovin®	Lilly
Vindesine sulphate	Eldisine®	Lilly
Vinflunine		Pierre Fabre
Vinorelbine tartrate	Navelbine®	Pierre Fabre
mTOR Inhibitors		
Deforolimus (AP23573, MK 8669)		ARIAD Pharmaceuticals, Inc
Everolimus (RAD001, RAD001C)	Certican®, Afinitor	Novartis
Sirolimus (Rapamycin)	Rapamune®	Wyeth Pharama
Temsirolimus (CCI-779)	Torisel®	Wyeth Pharama
Protein Synthesis Inhibitor		
L-asparaginase	Elspar®	Merck & Co.
Somatostatin Analogue		
Octreotide acetate	Sandostatin®	Novartis
Topoisomerase Inhibitors		
Actinomycin D		
Camptothecin (CPT)		
Belotecan		
Daunorubicin citrate	Daunoxome®	Gilead
Doxorubicin hydrochloride	Doxil®	Alza
	Vepesid®	Bristol-Myers Squibb
Etoposide	Etopophos	Hospira, Bedford, Teva Parenteral, Etc.
Irinotecan HCL (CPT-11)	Camptosar®	Pharmacia & Upjohn
Mitoxantrone HCL	Novantrone	EMD Serono
Rubitecan		
Teniposide (VM-26)	Vumon®	Bristol-Myers Squibb
Topotecan HCL	Hycamtin®	GlaxoSmithKline
Chemotherapeutic Agents		
Adriamycin, 5-Fluorouracil, Cytoxin, Bleomycin, Mitomycin C, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins, Esperamicins Clofarabine, Mercaptopurine, Pentostatin, Thioguanine, Cytarabine, Decitabine, Floxuridine, Gemcitabine (Gemzar), Enocitabine, Sapacitabine		
Hormonal Therapies		
Abarelix	Plenaxis™	Amgen
Abiraterone acetate	CB7630	BTG plc
Afimoxifene	TamoGel	Ascend Therapeutics, Inc.

Anastrazole	Arimidex®	AstraZeneca
Aromatase inhibitor	Atamestane plus toremifene	Intarcia Therapeutics, Inc.
	Arzoxifene	Eli Lilly & Co.
Asentar; DN-101		Novartis; Oregon Health & Science Univ.
Bicalutamide	Casodex®	AstraZeneca
Buserelin	Suprefact®	Sanofi Aventis
Cetrorelix	Cetrotide®	EMD Serono
Exemestane	Aromasin®	Pfizer
Exemestane	Xtane	Natco Pharma, Ltd.
Fadrozole (CGS 16949A)		
Flutamide	Eulexin®	Schering
Flutamide	Prostacur	Laboratorios Almirall, S.A.
Fulvestrant	Faslodex®	AstraZeneca
Goserelin acetate	Zoladex®	AstraZeneca
Letrozole	Femara®	Novartis
Letrozole (CGS20267)	Femara	Chugai Pharmaceutical Co., Ltd.
Letrozole	Estrochek	Jagsonpal Pharmaceuticals, Ltd.
Letrozole	Letrozole	Indchemie Health Specialities
Leuprolide acetate	Eligard®	Sanofi Aventis
Leuprolide acetate	Leopril	VHB Life Sciences, Inc.
Leuprolide acetate	Lupron®/Lupron Depot	TAP Pharma
Leuprolide acetate	Viador	Bayer AG
Megestrol acetate	Megace®	Bristol-Myers Squibb
Magestrol acetate	Estradiol Valerate (Delestrogen)	Jagsonpal Pharmaceuticals, Ltd.
Medroxyprogesterone acetate	Veraplex	Combiphar
MT206		Medisyn Technologies, Inc.
Nafarelin		
Nandrolone decanoate	Zestabolin	Mankind Pharma, Ltd.
Nilutamide	Nilandron®	Aventis Pharmaceuticals
Raloxifene HCL	Evista®	Lilly
Tamoxifen	Taxifen	Yung Shin Pharmaceutical
Tamoxifen	Tomifen	Alkem Laboratories, Ltd.
Tamoxifen citrate	Nolvadex	AstraZeneca
Tamoxifen citrate	Soltamox	EUSA Pharma, Inc.
Tamoxifen citrate	Tamoxifen citrate SOPHARMA	Sopharma JSCo.
Toremifene citrate	Fareston®	GTX, Inc.
Triptorelin pamoate	Trelstar®	Watson Labs
Triptorelin pamoate	Trelstar Depot	Paladin Labs, Inc.
Protein Kinase B (PKB) Inhibitors		
Akt Inhibitor ASTEX		Astex Therapeutics
Akt Inhibitors NERVIANO		Nerviano Medical Sciences

AKT Kinase Inhibitor TELIK		Telik, Inc.
AKT DECIPHERA		Deciphera Pharmaceuticals, LLC
Perifosine (KRX0401, D-21266)		Keryx Biopharmaceuticals, Inc., AEterna Zentaris, Inc.
Perifosine with Docetaxel		Keryx Biopharmaceuticals, Inc., AEterna Zentaris, Inc.
Perifosine with Gemcitabine		AEterna Zentaris, Inc.
Perifosine with Paclitaxel		Keryx Biopharmaceuticals, Inc, AEterna Zentaris, Inc.
Protein Kinase-B inhibitor DEVELOGEN		DeveloGen AG
PX316		Oncothyreon, Inc.
RX0183		Rexahn Pharmaceuticals, Inc.
RX0201		Rexahn Pharmaceuticals, Inc.
VQD002		VioQuest Pharmaceuticals, Inc.
XL418		Exelixis, Inc.
ZEN027		AEterna Zentaris, Inc.
Phosphatidylinositol 3-Kinase (PI3K) Inhibitors		
BEZ235		Novartis AG
BGT226		Novartis AG
CAL101		Calistoga Pharmaceuticals, Inc.
CHR4432		Chroma Therapeutics, Ltd.
Erk/PI3K Inhibitors ETERNA		AEterna Zentaris, Inc.
GDC0941		Genentech Inc./Piramed Limited/Roche Holdings, Ltd.
Enzastaurin HCL (LY317615)	Enzastaurin	Eli Lilly
LY294002/Wortmannin		
PI3K Inhibitors SEMAFORE		Semafore Pharmaceuticals
PX866		Oncothyreon, Inc.
SF1126		Semafore Pharmaceuticals
VMD-8000		VM Discovery, Inc.
XL147		Exelixis, Inc.
XL147 with XL647		Exelixis, Inc.
XL765		Exelixis, Inc.
PI-103		Roche/Piramed
Cyclin-dependent kinase inhibitors		
CYC200, r-roscovitine	Seliciclib	Cyclacel Pharma
NSC-649890, L86-8275, HMR-1275	Alvocidib	NCI
TLr9, CD289		
IMOxine		Merck KGaA
HYB2055		Idera
IMO-2055		Isis Pharma
1018 ISS		Dynavax Technologies/UCSF

PF-3512676		Pfizer
Enzyme Inhibitor		
Lonafarnib (SCH66336)	Sarasar	SuperGen, U Arizona
Anti-TRAIL		
AMG-655		Aeterna Zentaris, Keryx Biopharma
Apo2L/TRAIL, AMG951		Genentech, Amgen
Apomab (fully humanized mAb		Genentech
Other		
Imprime PGG		Biothera
CHR-2797	AminopeptidaseM1	Chroma Therapeutics
E7820, NSC 719239	Integrin-alpha2	Eisai
INCB007839	ADAM 17, TACE	Incyte
CNF2024, BIIB021	Hsp90	Biogen Idec
MP470, HPK-56	Kit/Met/Ret	Shering-Plough
SNDX-275/MS-275	HDAC	Syndax
Zarnestra, Tipifarnib, R115777	Ras	Janssen Pharma
Volociximab; Eos 200-4, M200	alpha581 integrin	Biogen Idec; Eli Lilly/UCSF/PDL BioPharma
Apricoxib (TP2001)	COX-2 Inhibitor	Daiichi Sankyo; Tragara Pharma

CLAIMS

What is claimed is:

1. A method of treating a subject with a malignant tumor, the method comprising co-administering to the subject either i) an effective amount of an anti-estrogen agent or ii) an effective amount of a receptor tyrosine kinase inhibitor and an effective amount of a bispecific anti-ErbB2/anti-ErbB3 antibody, and optionally an effective amount of trastuzumab.

2. The method of claim 1, wherein, the combination of the bispecific anti-ErbB2/anti-ErbB3 antibody and either i or ii, and optionally the effective amount of trastuzumab, is characterized as follows: when a tissue culture medium is prepared comprising the bispecific anti-ErbB2/anti-ErbB3 antibody (at a first concentration) and either the anti-estrogen agent (at a second concentration) or the receptor tyrosine kinase inhibitor (at a third concentration), and the medium is contacted with cancer cells of a cell line in a cell culture, cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or percentage of cells in the culture that are apoptotic is increased.

3. The method of claim 2, wherein cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or percentage of cells in the culture that are apoptotic is increased to a greater degree than cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or percentage of cells in the culture that are apoptotic is increased when cancer cells of the cell line in a cell culture are contacted with each of a) a second medium that is essentially the same as the medium of claim 2 except that it does not comprise a bispecific anti-ErbB2/anti-ErbB3 antibody, and b) a third medium that is essentially the same as the medium of claim 2 except that it does not comprise any anti-estrogen agent and it does not comprise any receptor tyrosine kinase inhibitor.

4. The method of claim 2 or claim 3, wherein the cell line is BT474-M3.

5. The method of any one of claims 2, 3, and 4, wherein the culture is a spheroid culture.

6. The method of claim 1, wherein all effective amounts are either mouse effective amounts or human effective amounts.

7. The method of claim 6, wherein all effective amounts are mouse effective amounts and the combination of either i or ii and the bispecific anti-ErbB2/anti-ErbB3 antibody is characterized as

follows: when co-administered to BT474-M3 xenograft tumor bearing mice with a tumor of a measured volume, the combination is more effective at inhibiting tumor volume increase after 32 days of co-administration treatment than is administration of the mouse effective amount of the bispecific anti-ErbB2/anti-ErbB3 antibody without the co-administration of i) or ii).

8. The method of claim 7, wherein the mouse effective amount of trastuzumab is co-administered with the bispecific anti-ErbB2/anti-ErbB3 antibody.

9. The method of any one of claims 1 to 8, wherein the co-administration to the subject does not create a drug-drug interaction-mediated toxicity in the subject.

10. The method of claim 1, wherein the co-administration to the subject creates a substantially additive or superadditive effect.

11. The method of any one of claims 1 to 10, wherein the anti-estrogen agent is an estrogen receptor antagonist or an aromatase inhibitor.

12. The method of claim 11, wherein the estrogen receptor antagonist is fulvestrant or tamoxifen.

13. The method of claim 11, wherein the aromatase inhibitor is letrozole, exemestane, anastrozole, aminoglutethimide, testolactone, vorozole, formestane, or fadrozole.

14. The method of claim 13, wherein the aromatase inhibitor is letrozole.

15. The method of any one of claims 1 to 14, wherein the bispecific anti-ErbB2/anti-ErbB3 antibody comprises the amino acid sequence set forth in SEQ ID NO:1.

16. The method of any one of claims 1 to 15, wherein the bispecific anti-ErbB2/anti-ErbB3 antibody is chosen from the group consisting of A5-HSA-ML3.9, ML3.9-HSA-A5, A5-HSA-B1D2, B1D2-HSA-A5, B12-HSA-B1D2, B1D2-HSA-B12, A5-HSA-F5B6H2, F5B6H2-HSA-A5, H3-HSA-F5B6H2, F5B6H2-HSA-H3, F4-HSA-F5B6H2, F5B6H2-HSA-F4, B1D2-HSA-H3, and H3-HSA-B1D2.

17. The method of any one of claims 1 to 16, wherein the receptor tyrosine kinase inhibitor is erlotinib, afatinib, dasatinib, gefitinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib or sorafenib.

18. The method of any one of claims 1 to 17, wherein the receptor tyrosine kinase inhibitor is lapatinib.

19. The method of claim 18 wherein the lapatinib is administered via a dosing regimen comprising a 14-day dosing schedule, and wherein the lapatinib is administered intermittently.

20. The method of claim 19, wherein the lapatinib is administered on days 1 to 3, 1 to 4, 1 to 5, 1 to 6, or 1 to 7 of the 14-day dosing schedule.

21. The method of claim 20, wherein the lapatinib is administered on days 1 to 5 of the 14-day dosing schedule.

22. The method of any one of claims 19 to 21, wherein the lapatinib is administered at a dose that is between 2000 and 9000 mg/d.

23. The method of claim 22, wherein the dose is 3000 mg/day.

24. The method of claim any of claims 19 to 23, wherein the dose of lapatinib administered on day 1 of the 14-day dosing cycle comprises a loading dose.

25. The method of any one of claims 1 to 24, further comprising an effective amount of capecitabine and/or cisplatin.

26. A bispecific anti-ErbB2/anti-ErbB3 antibody for use in combination therapy of a malignant tumor, wherein the combination therapy comprises concomitant use of either i) an anti-estrogen agent or ii) a receptor tyrosine kinase inhibitor and optionally comprises use of trastuzumab.

27. The combination therapy of claim 26, wherein the anti-estrogen agent is an estrogen receptor antagonist or an aromatase inhibitor.

28. The combination therapy of claim 26 or 27, wherein the estrogen receptor antagonist is fulvestrant or tamoxifen.

29. The combination therapy of claim 26 or 27, wherein the anti-estrogen agent is an aromatase inhibitor chosen from the group consisting of letrozole, exemestane, anastrozole, aminoglutethimide, testolactone, vorozole, formestane, and fadrozole.

30. The combination therapy of any one of claims 26, 27, and 29, wherein the aromatase inhibitor is letrozole.

31. The combination therapy of any one of claims 26 to 30, wherein the bispecific anti-ErbB2/anti-ErbB3 antibody comprises the amino acid sequence set forth in SEQ ID NO:1.

32. The combination therapy of any one of claims 26 to 31, wherein the anti-ErbB2/anti-ErbB3 antibody is chosen from the group consisting of A5-HSA-ML3.9, ML3.9-HSA-A5, A5-HSA-B1D2, B1D2-HSA-A5, B12-HSA-B1D2, B1D2-HSA-B12, A5-HSA-F5B6H2, F5B6H2-HSA-A5, H3-HSA-F5B6H2, F5B6H2-HSA-H3, F4-HSA-F5B6H2, F5B6H2-HSA-F4, B1D2-HSA-H3, and H3-HSA-B1D2.

33. The combination therapy of any one of claims 26 to 32, wherein the receptor tyrosine kinase inhibitor is chosen from the group consisting of erlotinib, afatinib, dasatinib, gefitinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib and sorafenib.

34. The combination therapy any one of claims 26 to 33, wherein the receptor tyrosine kinase inhibitor is lapatinib.

35. The combination therapy of any one of claims 26 to 34, said combination therapy further comprising concomitant use of capecitabine and/or cisplatin.

36. An aqueous solution comprising a bispecific anti-ErbB2/anti-ErbB3 antibody at a first concentration and either i) an anti-estrogen agent at a second concentration or ii) a receptor tyrosine kinase inhibitor at a third concentration, wherein, when a tissue culture medium is prepared comprising the bispecific anti-ErbB2/anti-ErbB3 antibody at the first concentration and either the anti-estrogen agent at the second concentration or the receptor tyrosine kinase inhibitor at the third concentration and the medium is contacted with cancer cells of a cell line in a cell culture, cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or percentage of cells in the culture that are apoptotic is increased.

37. The aqueous solution of claim 36, wherein cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or percentage of cells in the culture that are apoptotic is increased, to a lesser degree than when cells of the cell line in a cell culture are contacted with a second tissue culture medium that is essentially the same as the medium of claim 15 except that it does not comprise any anti-estrogen agent and it does not comprise any

receptor tyrosine kinase inhibitor.

38. The aqueous solution of claim 36, wherein cell growth or cell proliferation or production of pErbB3 or production of pAKT in the cells is inhibited, or percentage of cells in the culture that are apoptotic is increased, to a lesser degree than when cells of the cell line in a cell culture are contacted with a third tissue culture medium that is essentially the same as the medium of claim 15 except that it does not comprise any bispecific anti-ErbB2/anti-ErbB3 antibody.

39. The aqueous solution of any one of claims 36-38, further comprising trastuzumab at a fourth concentration, wherein the medium further comprises trastuzumab at the fourth concentration.

40. The aqueous solution of any one of claims 36 to 39, wherein the cell line is BT474-M3.

41. The aqueous solution of any one of claims 36 to 40, wherein the culture is a spheroid culture.

42. An aqueous solution comprising a bispecific anti-ErbB2/anti-ErbB3 antibody at a first concentration and either i) an anti-estrogen agent at a second concentration or ii) a receptor tyrosine kinase inhibitor at a third concentration, wherein each concentration is an effective concentration and when the aqueous solution is blood plasma in a subject (optionally a human patient), the subject does not experience a toxicity that is sufficiently harmful to require a change in a therapy being administered to the subject, which toxicity is mediated by a drug-drug interaction in the subject between the bispecific anti-ErbB2/anti-ErbB3 antibody and the anti-estrogen agent or the receptor tyrosine kinase inhibitor.

43. The aqueous solution of any one of claims 36 to 42, wherein the anti-estrogen agent is fulvestrant or tamoxifen.

44. The aqueous solution ofany one of claims 36, 37, and 39 to 43, wherein the bispecific anti-ErbB2/anti-ErbB3 antibody comprises the amino acid sequence set forth in SEQ ID NO:1.

45. The aqueous solution of any one of claims 37, 37, and 39 to 44, wherein the bispecific anti ErbB3, anti-ErbB2 antibody is chosen from the group consisting of A5-HSA-ML3.9, ML3.9-HSA-A5, A5-HSA-B1D2, B1D2-HSA-A5, B12-HSA-B1D2, B1D2-HSA-B12, A5-HSA-F5B6H2, F5B6H2-HSA-A5, H3-HSA-F5B6H2, F5B6H2-HSA-H3, F4-HSA-F5B6H2, F5B6H2-HSA-F4, B1D2-HSA-H3, and H3-HSA-B1D2.

46. The aqueous solution of any one of claims 36 and 38 to 45, wherein the receptor tyrosine kinase inhibitor is chosen from the group consisting essentially of erlotinib, afatinib, dasatinib, gefitinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib and sorafenib.

47. The aqueous solution of any one of claims 36 and 38 to 46, wherein the receptor tyrosine kinase inhibitor is lapatinib.

48. A method of inhibiting the growth of a malignant tumor comprising tumor cells, said method comprising contacting the tumor cells with the aqueous solution of any one of claims 36 to 47.

49. The method of claim 1, wherein the estrogen receptor antagonist is fulvestrant or tamoxifen.

50. The method of claim 1, wherein the aromatase inhibitor is letrozole.

51. The method of claim 1, wherein the bispecific anti-ErbB2/anti-ErbB3 antibody comprises the amino acid sequence set forth in SEQ ID NO:1.

52. The method of claim 1, wherein the receptor tyrosine kinase inhibitor is lapatinib.

53. The method of claim 1, further comprising an effective amount of capecitabine and/or cisplatin.

54. The combination therapy of claim 26, wherein the anti-estrogen agent is fulvestrant or tamoxifen.

55. The combination therapy of claim 26, wherein the bispecific anti-ErbB2/anti-ErbB3 antibody comprises the amino acid sequence set forth in SEQ ID NO:1.

56. The combination therapy of claim 26, wherein the aromatase inhibitor is letrozole.

57. The combination therapy of claim 26, wherein the receptor tyrosine kinase inhibitor is lapatinib.

58. The combination therapy of claim 26, further comprising concomitant use of capecitabine and/or cisplatin.

59. The aqueous solution of claim 36, wherein the anti-estrogen agent is fulvestrant or tamoxifen.

60. The aqueous solution of claim 36, wherein the bispecific anti-ErbB2/anti-ErbB3 antibody comprises the amino acid sequence set forth in SEQ ID NO:1.

61. The aqueous solution of claim 36, wherein the aromatase inhibitor is letrozole.

62. The aqueous solution of claim 36, wherein the receptor tyrosine kinase inhibitor is lapatinib.

63. The aqueous solution of claim 36, further comprising concomitant use of capecitabine and/or cisplatin.

64. A method of treating a subject with a malignant tumor, the method comprising co-administering to the subject, 1) an effective amount of a bispecific anti-ErbB2/anti-ErbB3 antibody, 2) an effective amount of trastuzumab, 3) an effective amount of cisplatin, and 4) an effective amount of capecitabine.

65. A method of treating a subject with a malignant tumor, the method comprising co-administering to the subject, 1) an effective amount of a bispecific anti-ErbB2/anti-ErbB3 antibody, 2) an effective amount of trastuzumab, and 3) an effective amount of nab-paclitaxel.

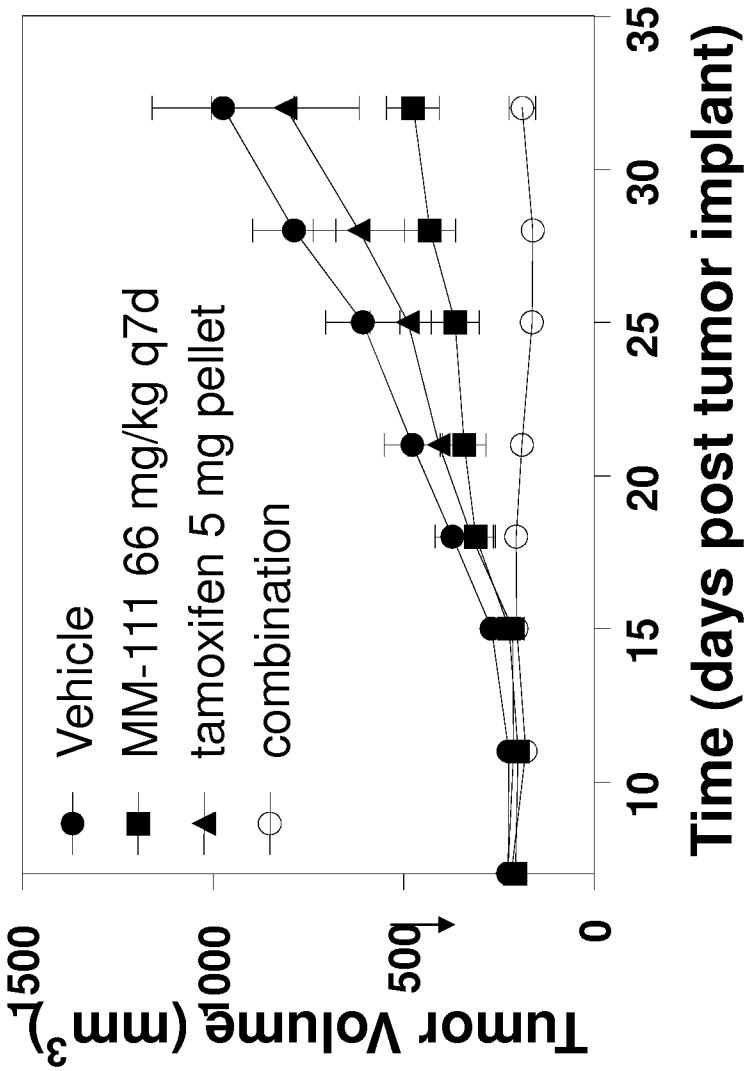
Tumor Volume (mm3)
1500
1000
500
0
10
15
20
25
30
35
Time (days post tumor implant)
Vehicle
MM-111 66 mg/kg q7d
tamoxifen 5 mg pellet
combination

Figure 1

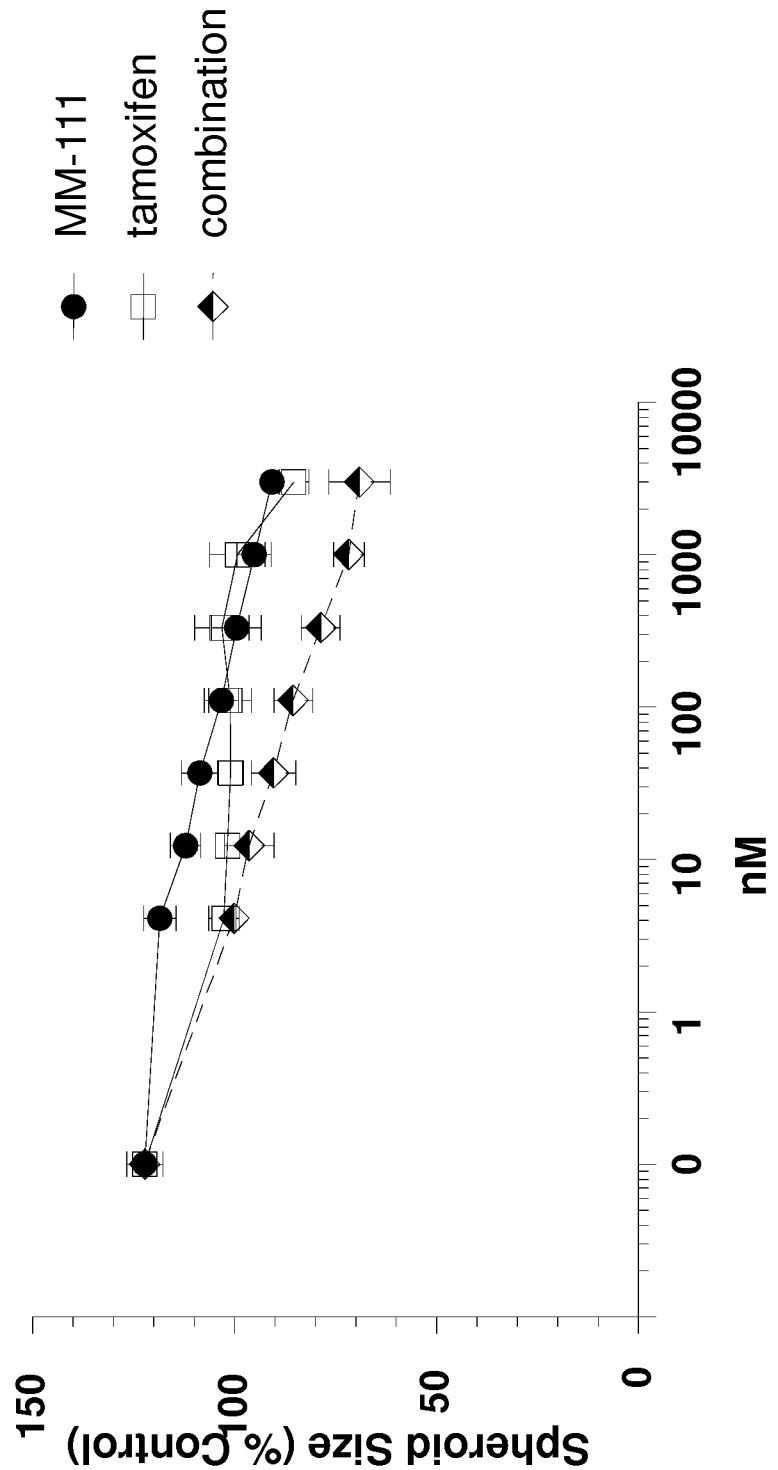
MM-111
tamoxifen
combination
Spheroid Size (%Control)
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2a

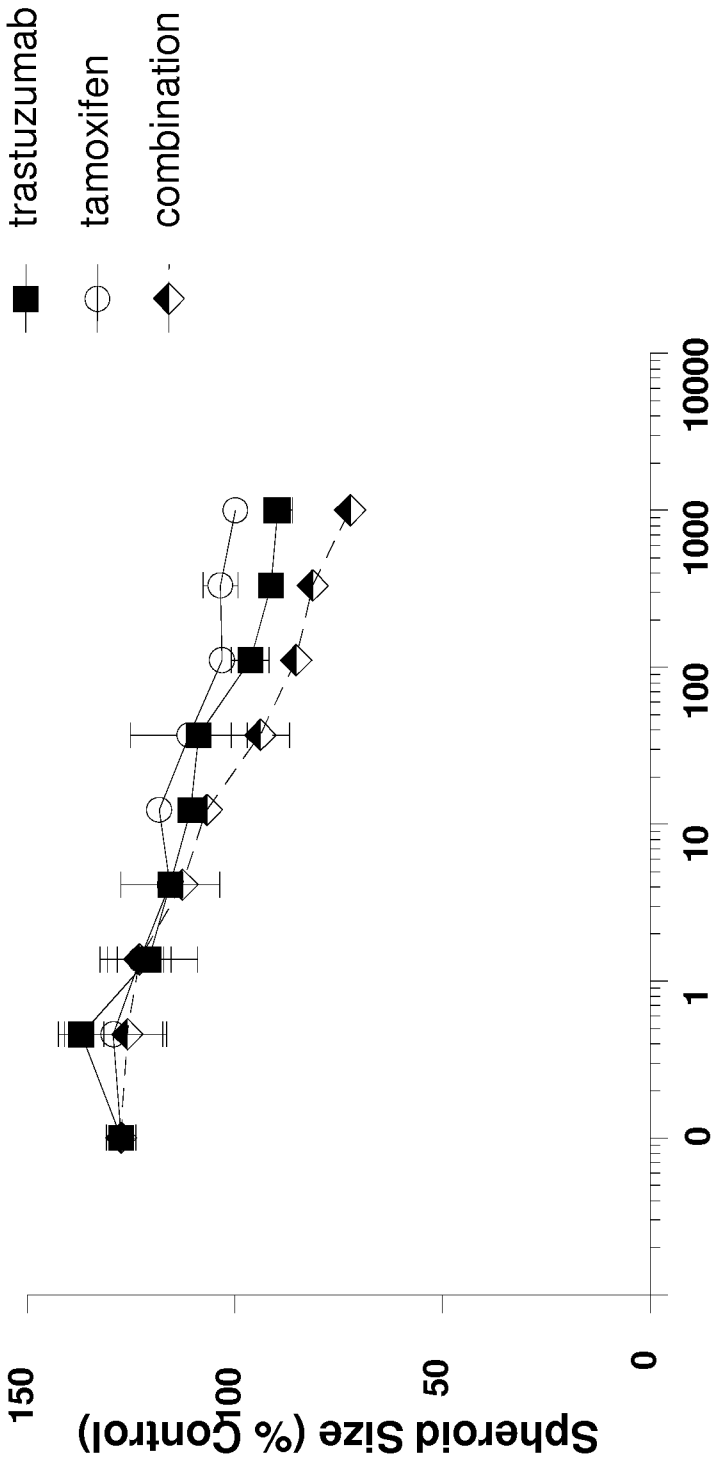
trastuzumab
tamoxifen
combination
Spheroid Size (% Control)
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2b

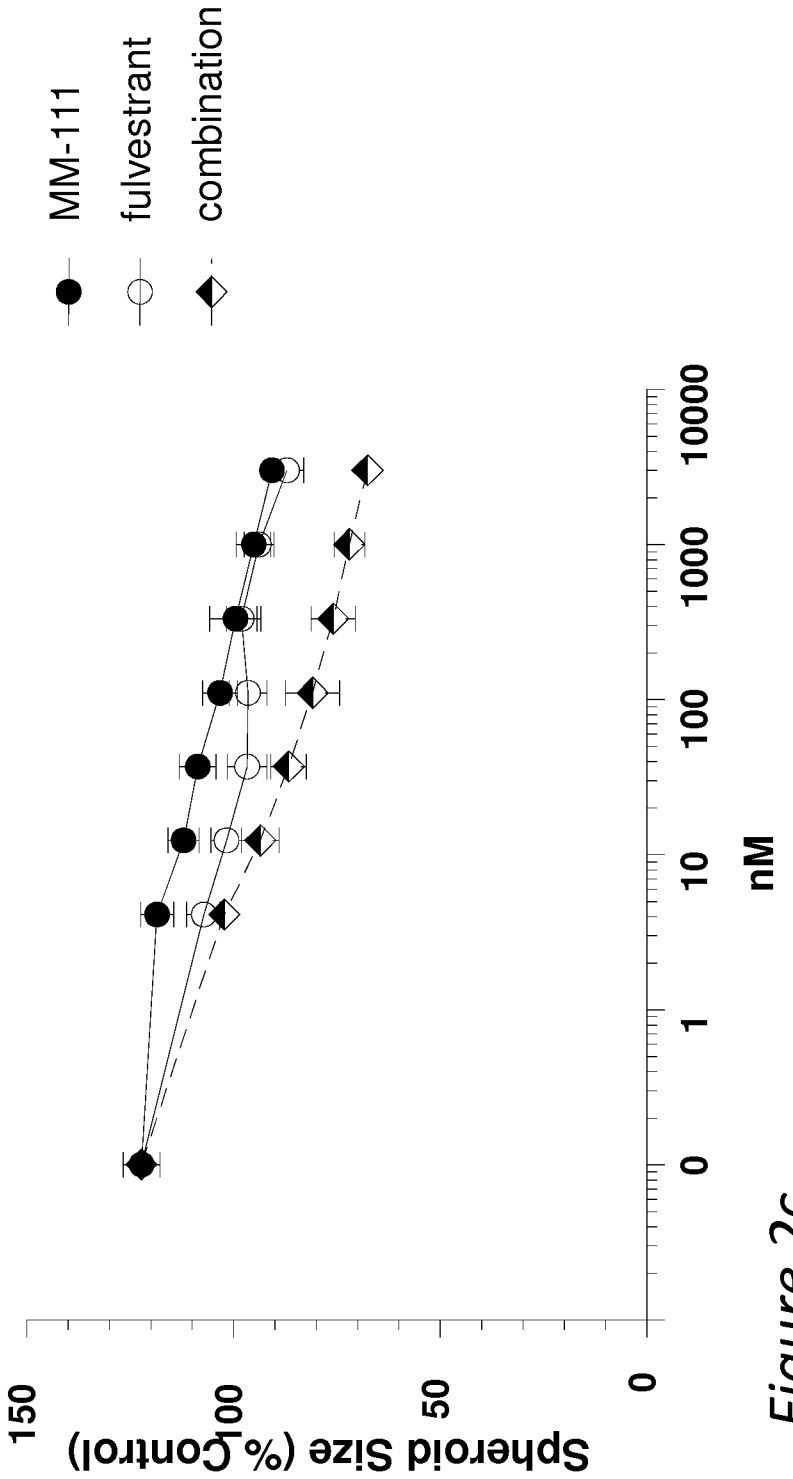
MM-111
fulvestrant
combination
Spheroid Size (% Control)
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2c

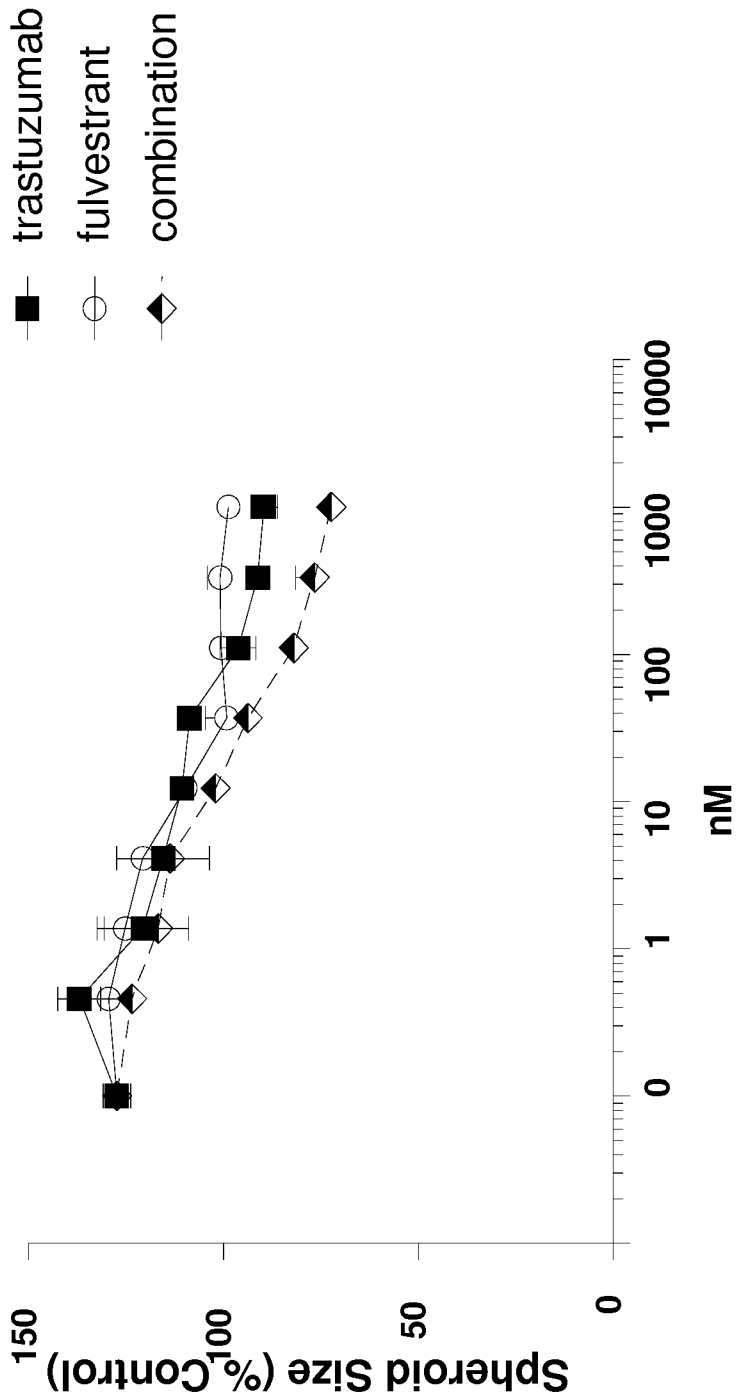
trastuzumab
fulvestrant
combination
Spheroid Size (%Control)
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2d

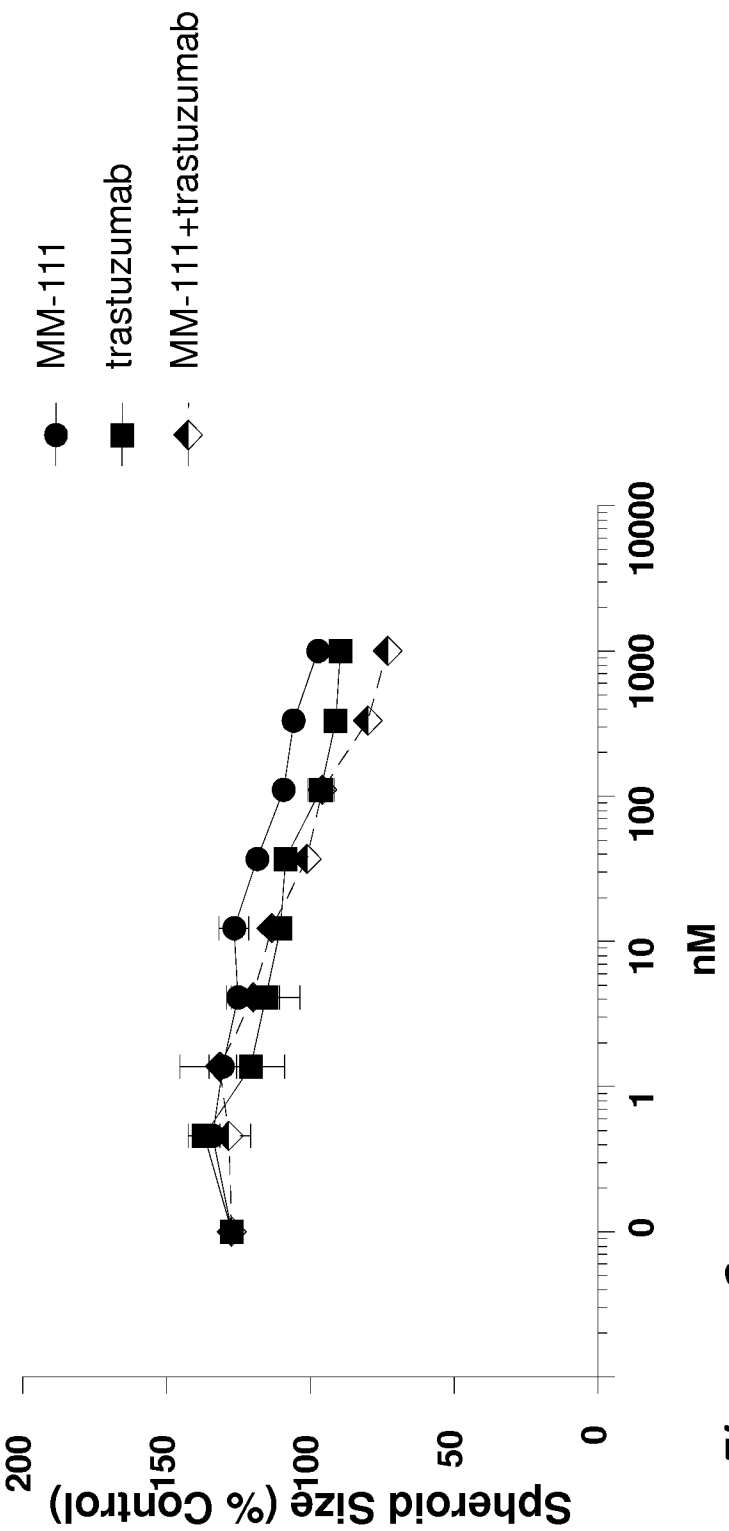
MM-111
trastuzumab
MM-111+trastuzumab
Spheroid Size (% Control)
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2e

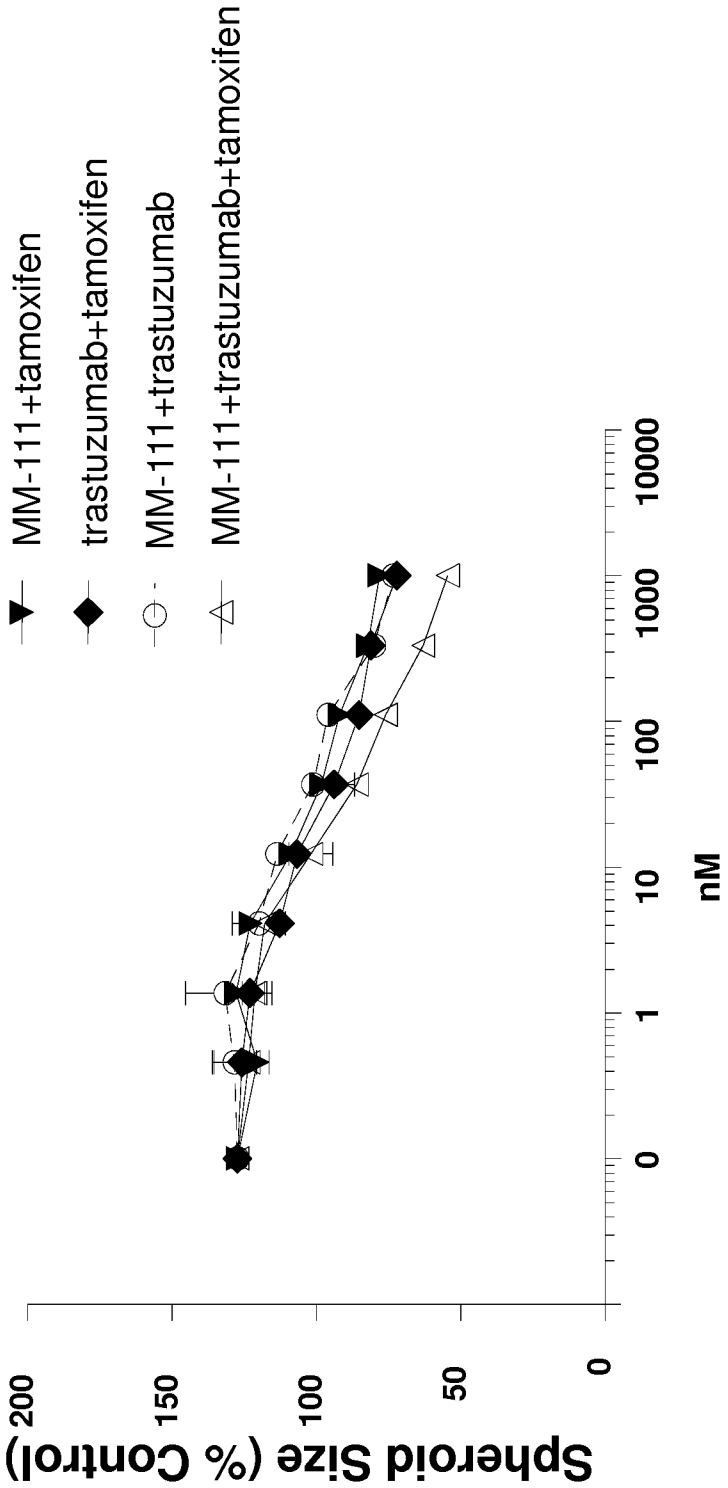
MM-111+tamoxifen
trastuzumab+tamoxifen
MM-111+trastuzumab
MM-111+trastuzumab+tamoxifen
Spheroid Size (% Control)
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2f

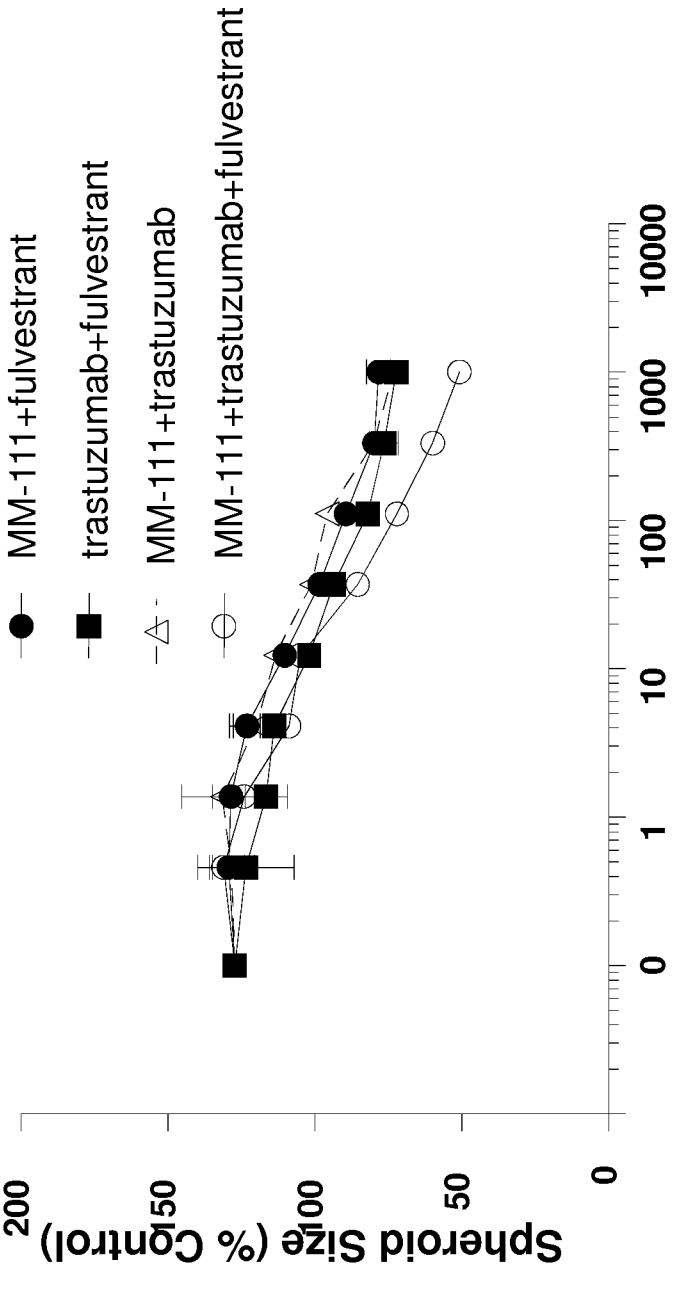
MM-111+fulvestrant
trastuzumab+fulvestrant
MM-111+trastuzumab
MM-111+trastuzumab+fulvestrant
Spheroid Size (% Control)
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2g

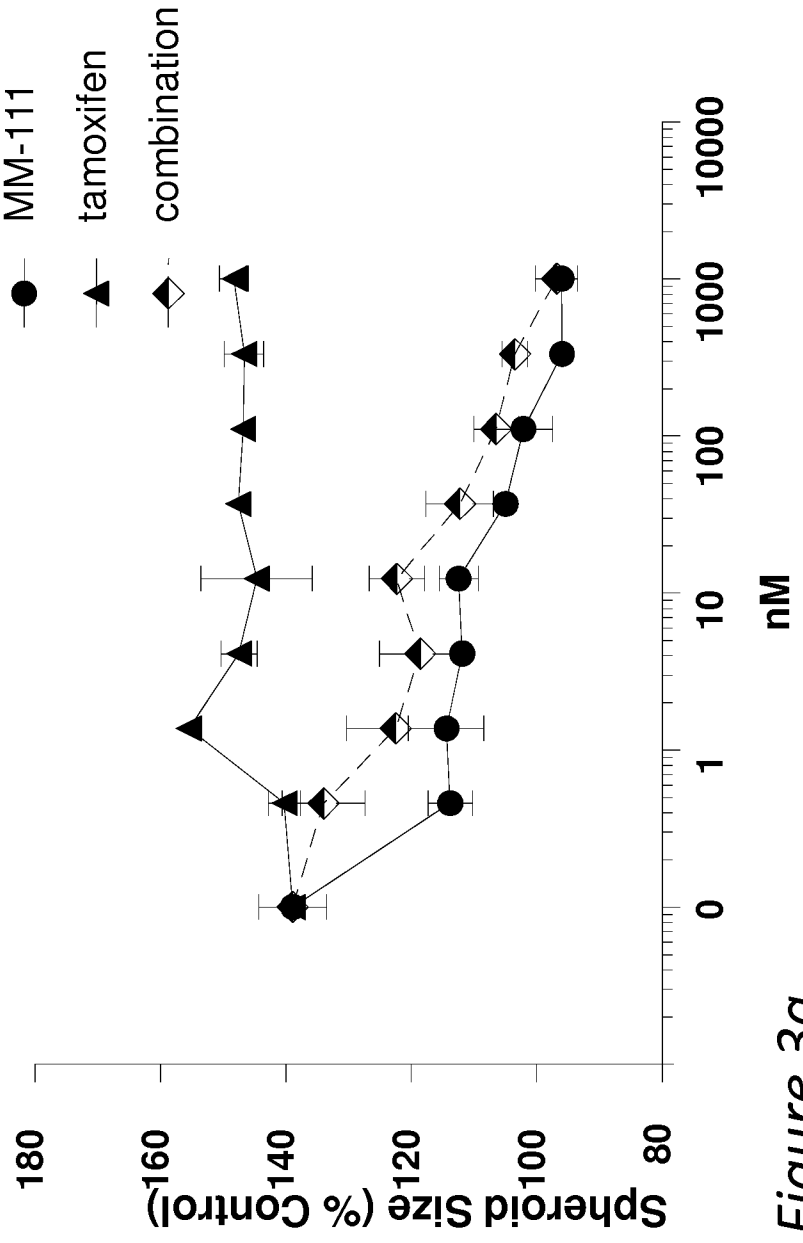
MM-111
tamoxifen
combination
Spheroid Size (% Control)
180
160
140
120
100
80
0
1
10
100
1000
10000
nM

Figure 3a

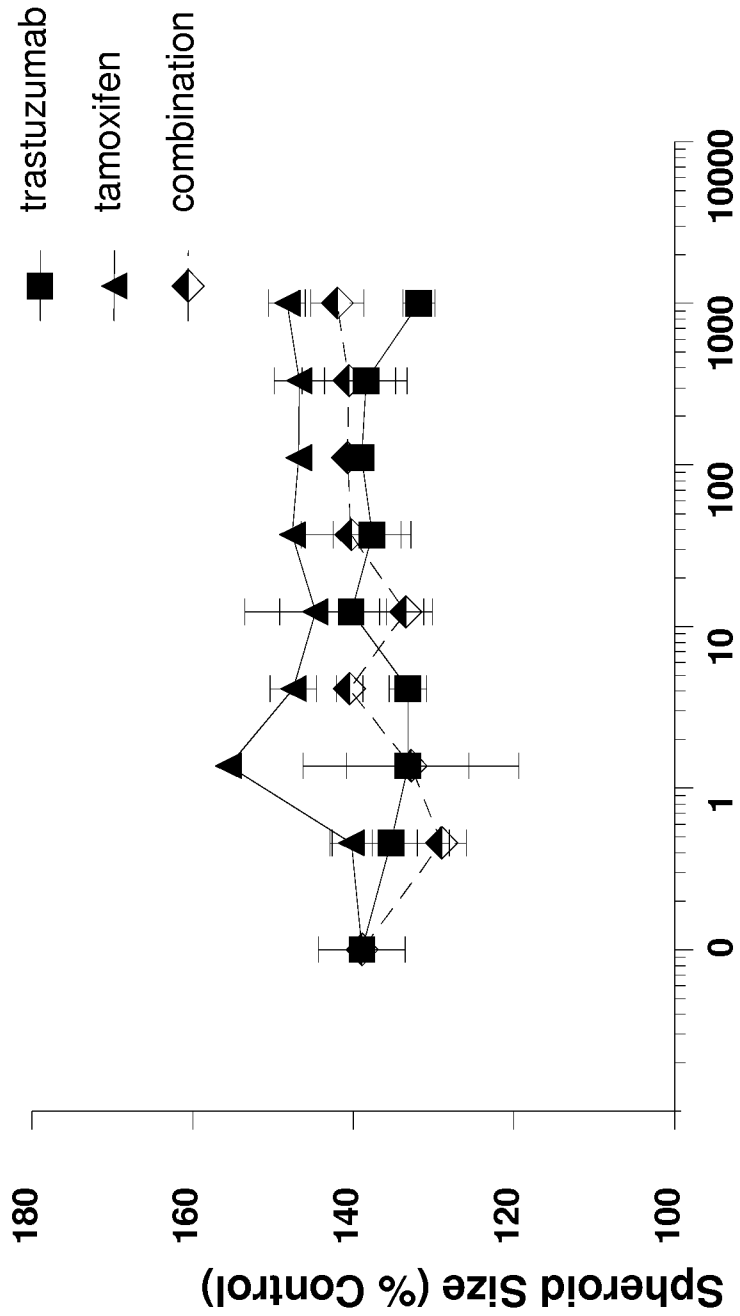
trastuzumab
tamoxifen
combination
Spheroid Size (% Control)
180
160
140
120
100
0
1
10
100
1000
10000
nM

Figure 3b

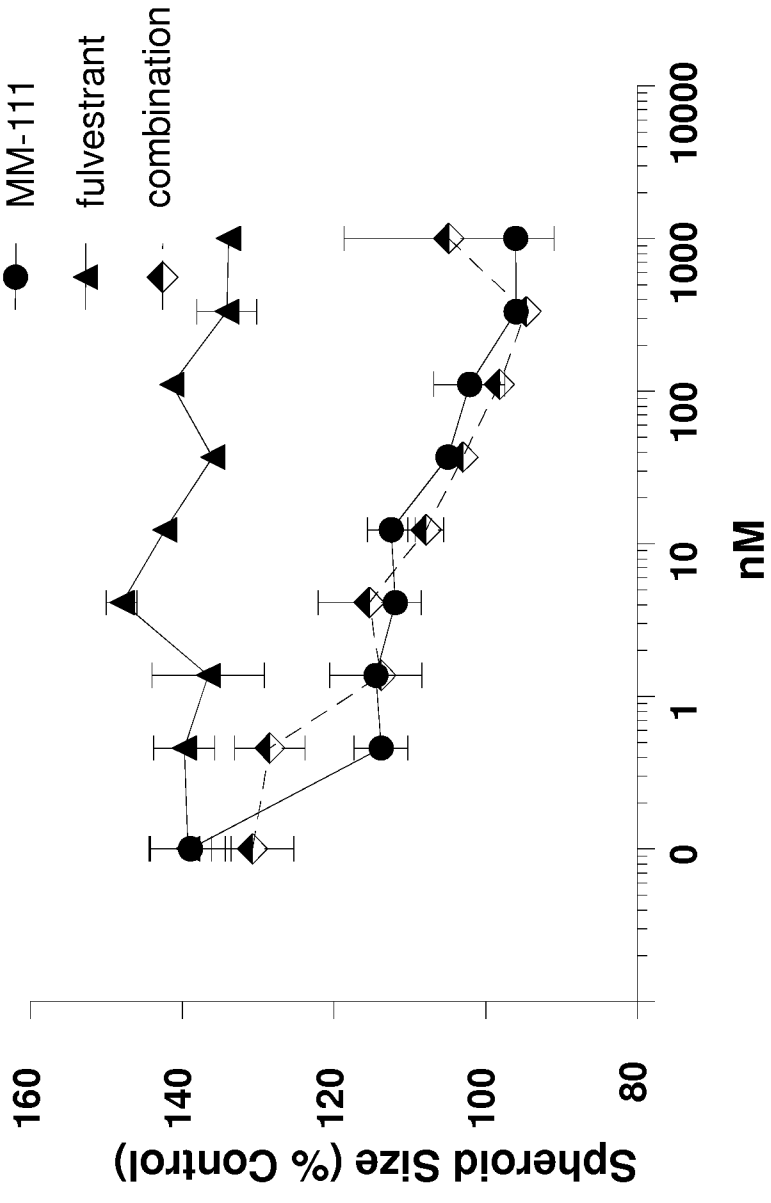
MM-111
fulvestrant
combination
Spheroid Size (% Control)
160
140
120
100
80
0
1
10
100
1000
10000
nM

Figure 3c

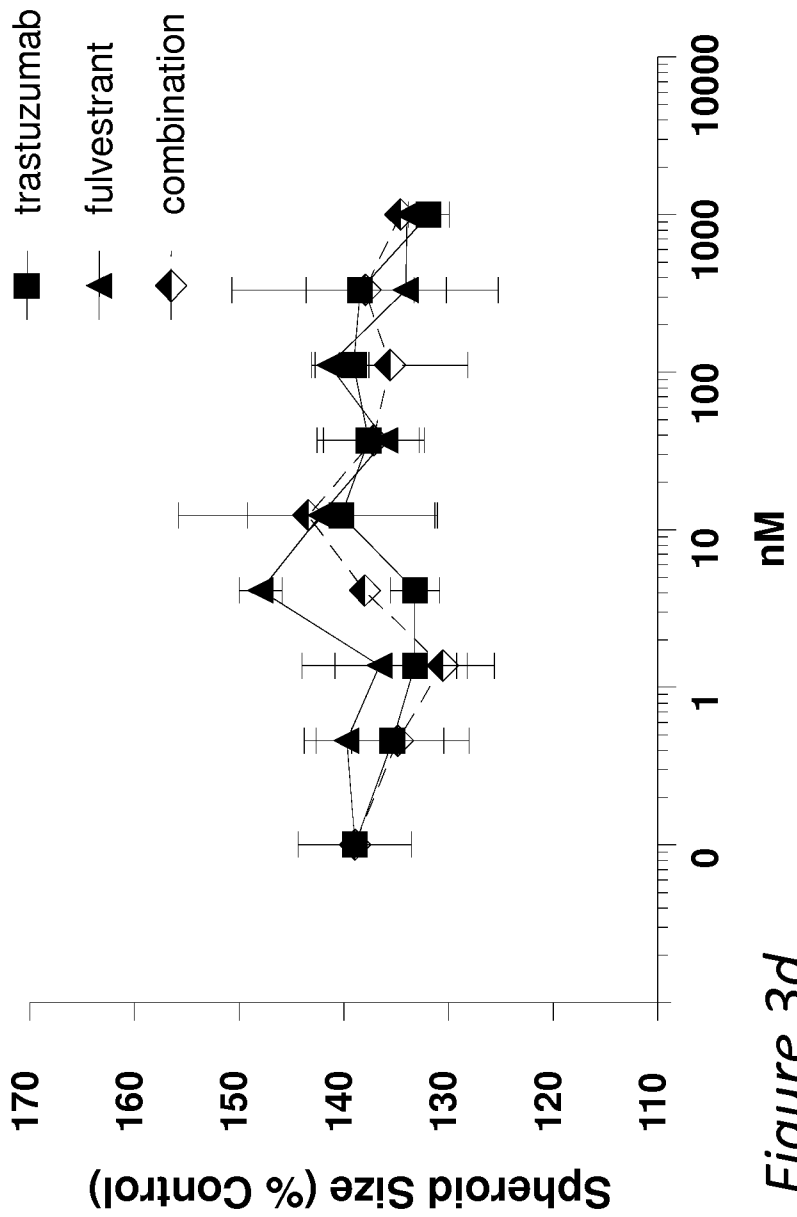
trastuzumab
fulvestrant
combination
Spheroid Size (% Control)
170
160
150
140
130
120
110
0
1
10
100
1000
10000
nM

Figure 3d

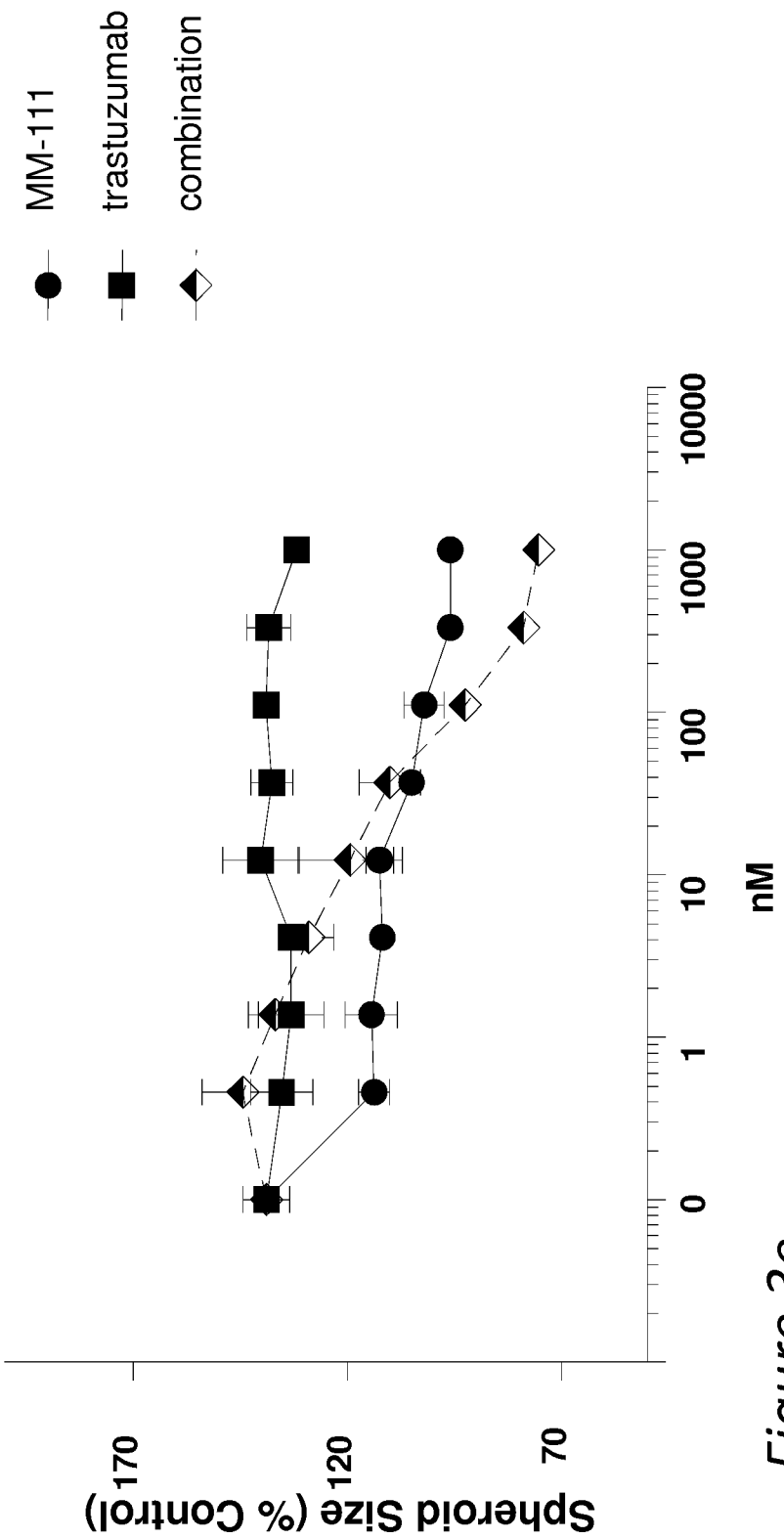
MM-111
trastuzumab
combination
Spheroid Size (% Control)
170
120
70
0
1
10
100
1000
10000
nM

Figure 3e

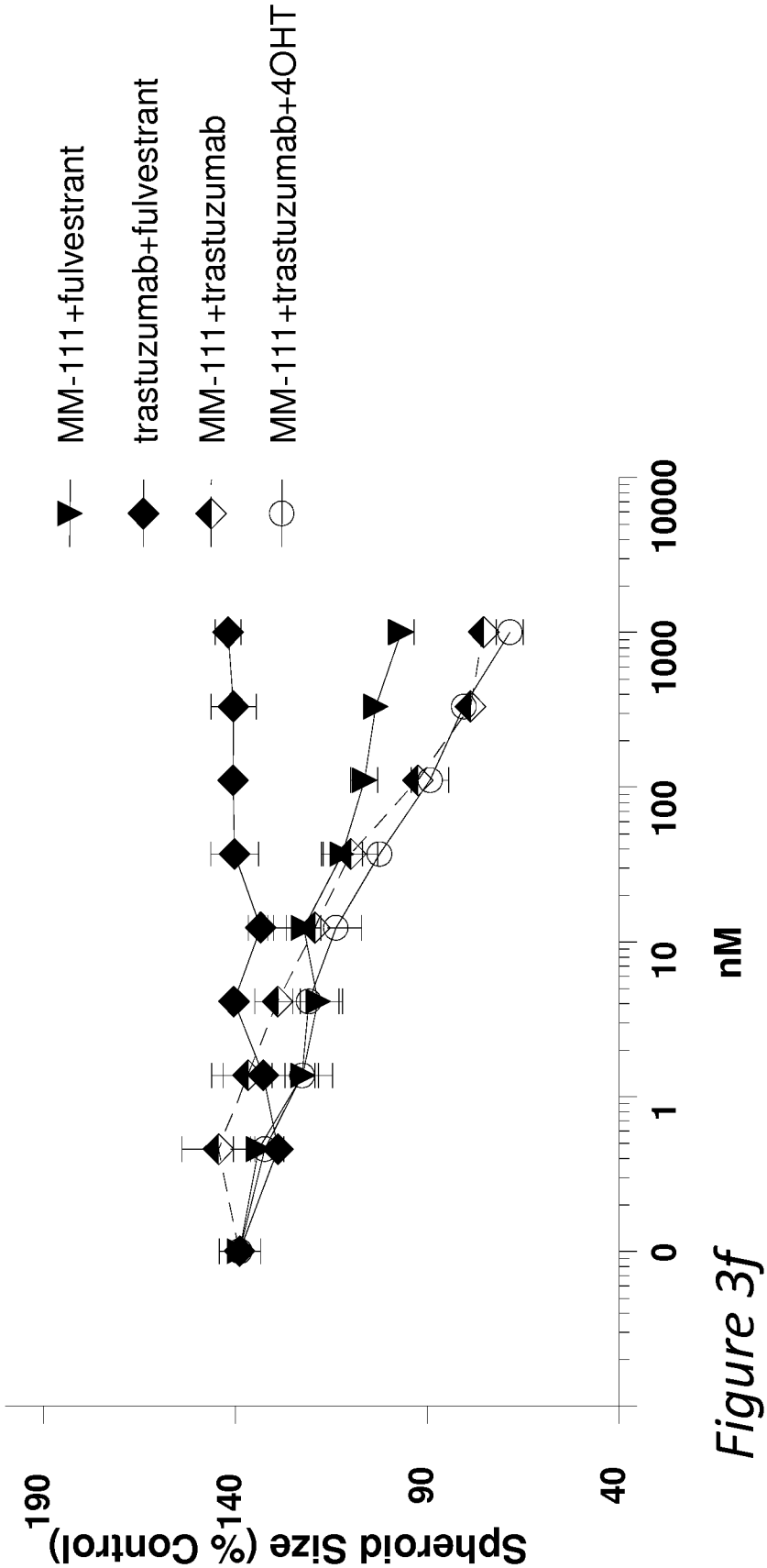
MM-111+fulvestrant
trastuzumab+fulvestrant
MM-111+trastuzumab
MM-111+trastuzumab+4OHT
Spheroid Size (% Control)
190
140
90
40
0
1
10
100
1000
10000
nM

Figure 3f

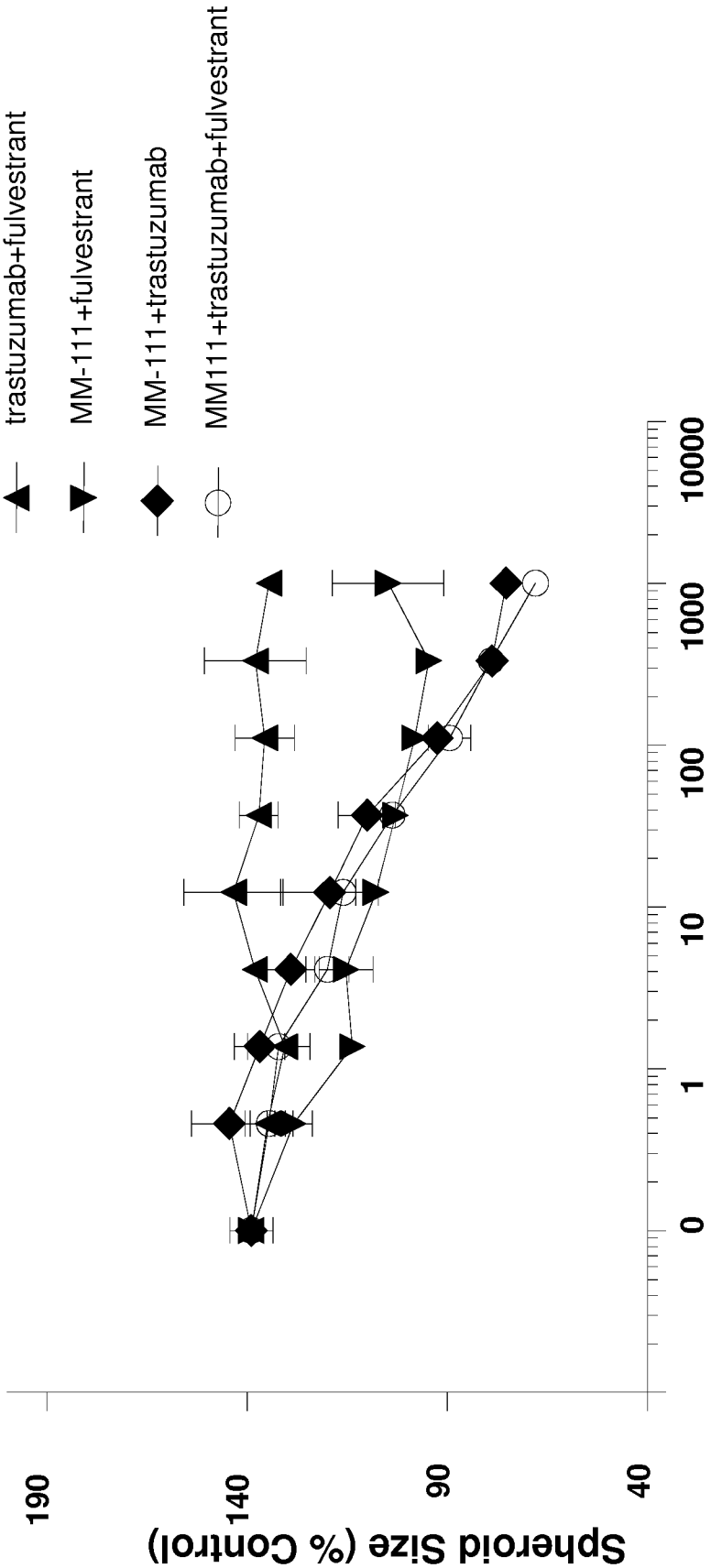
trastuzumab+fulvestrant
MM-111+fulvestrant
MM-111+trastuzumab
MM111+trastuzumab+fulvestrant
Spheroid Size (% Control)
190
140
90
40
0
1
10
100
1000
10000
nM

Figure 3g

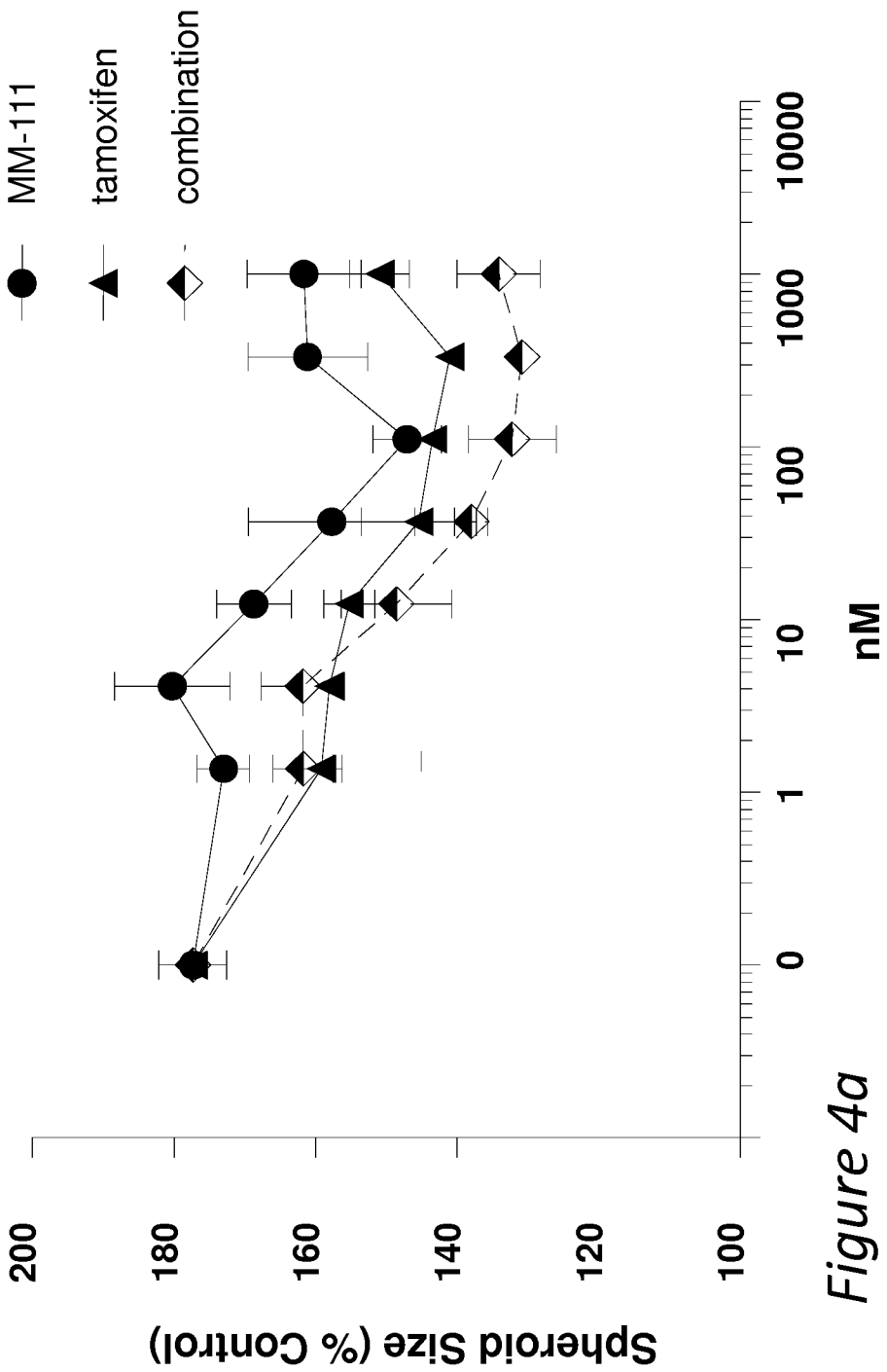
MM-111
tamoxifen
combination
Spheroid Size (% Control)
200
180
160
140
120
100
0
1
10
100
1000
10000
nM

Figure 4a

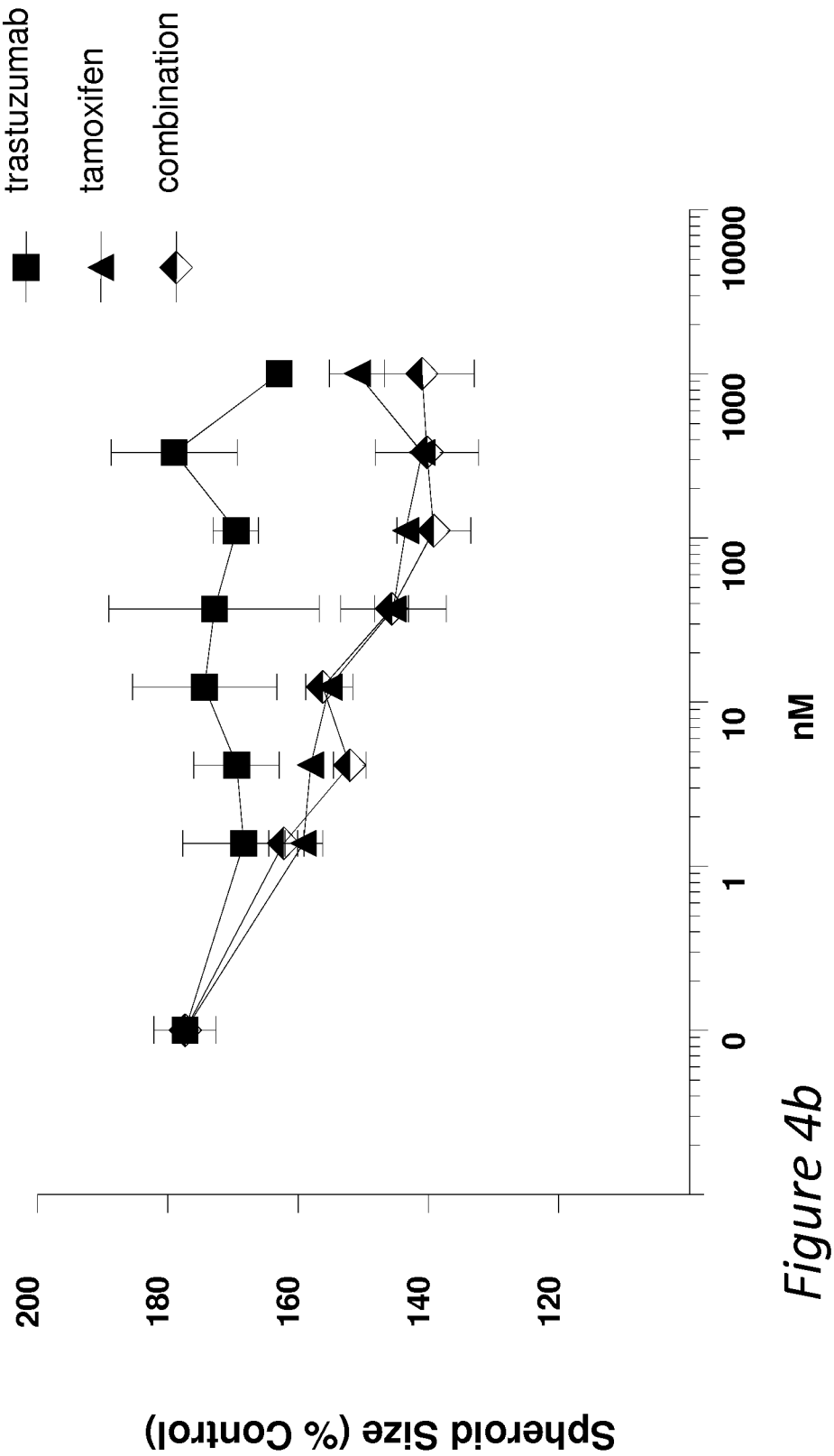
trastuzumab
tamoxifen
combination
Spheroid Size (% Control)
200
180
160
140
120
0
1
10
100
1000
10000
nM

Figure 4b

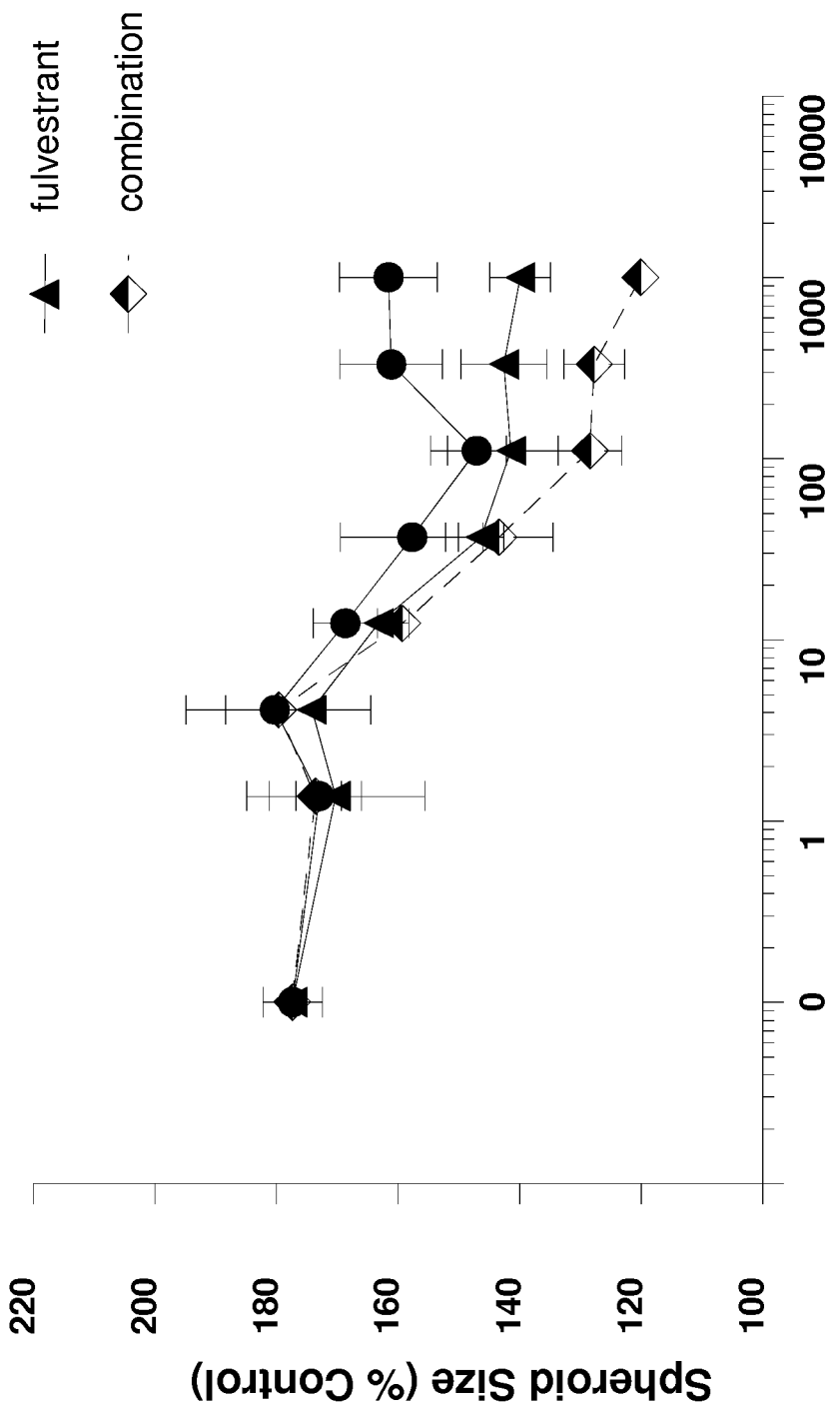
MM-111
fulvestrant
combination
Spheroid Size (% Control)
220
200
180
160
140
120
100
0
1
10
100
1000
10000
nM

Figure 4c

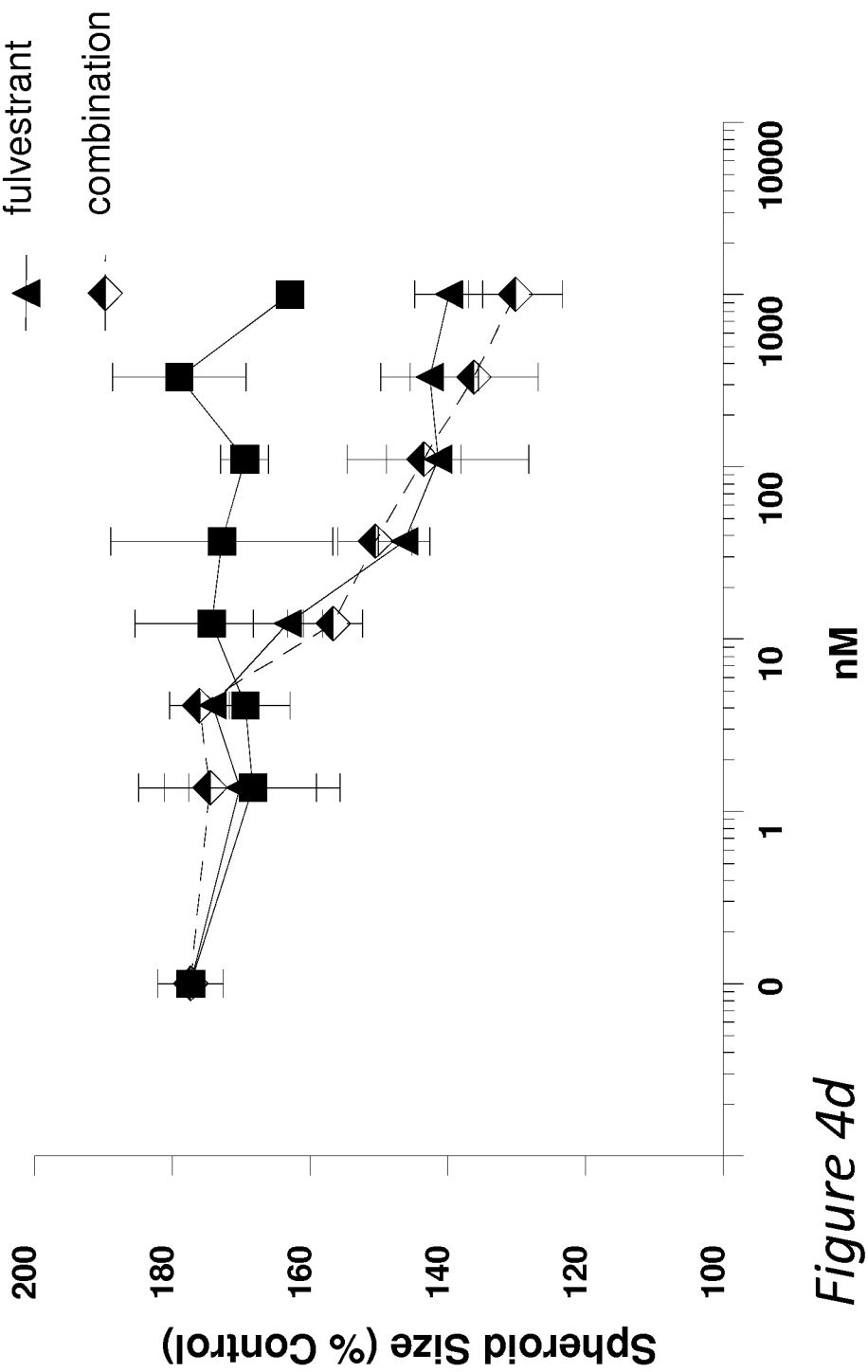
trastuzumab
fulvestrant
combination
Spheroid Size (% Control)
200
180
160
140
120
100
0
1
10
100
1000
10000
nM

Figure 4d

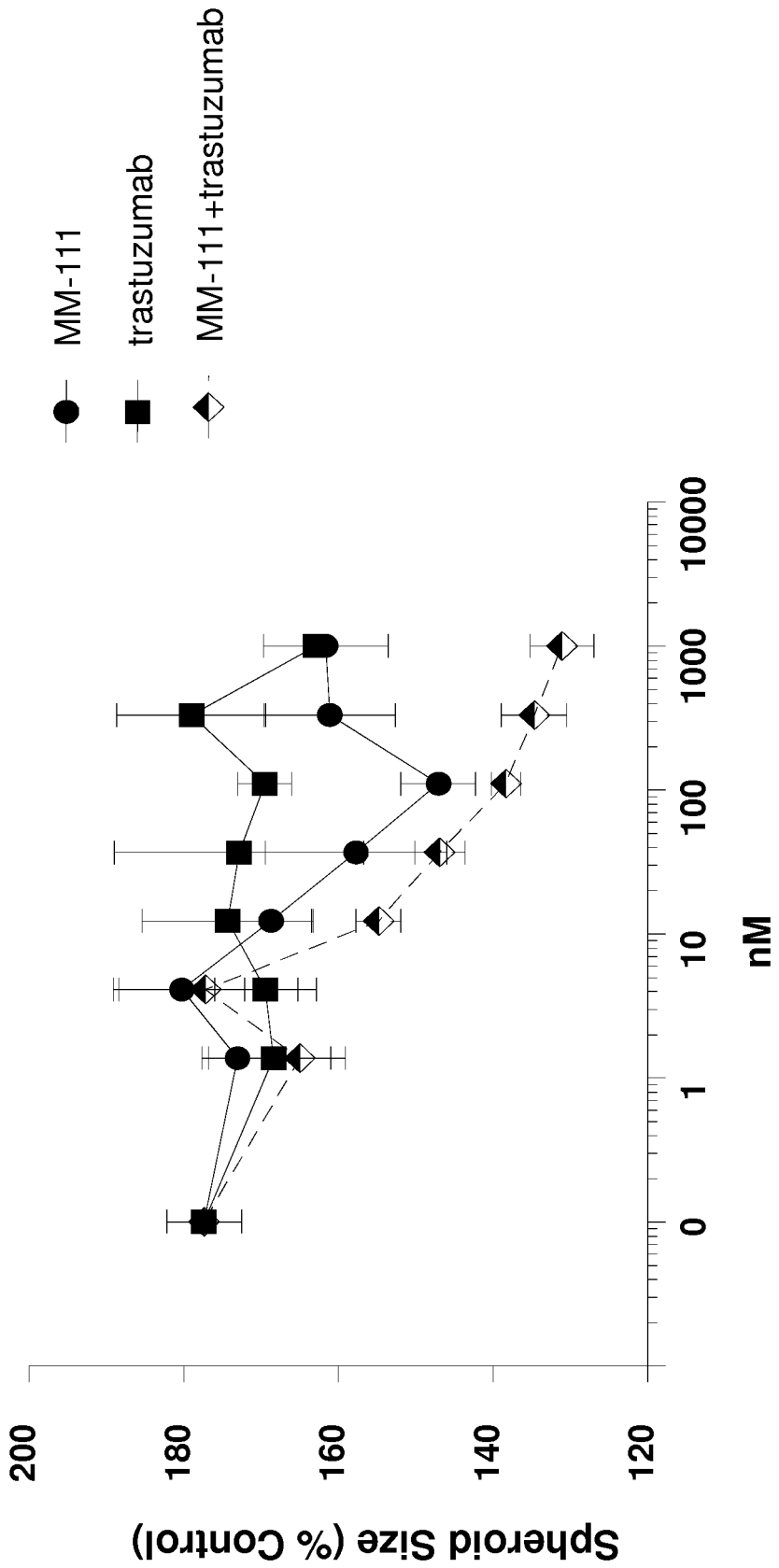
MM-111
trastuzumab
MM-111+trastuzumab
Spheroid Size (% Control)
200
180
160
140
120
0
1
10
100
1000
10000
nM

Figure 4e

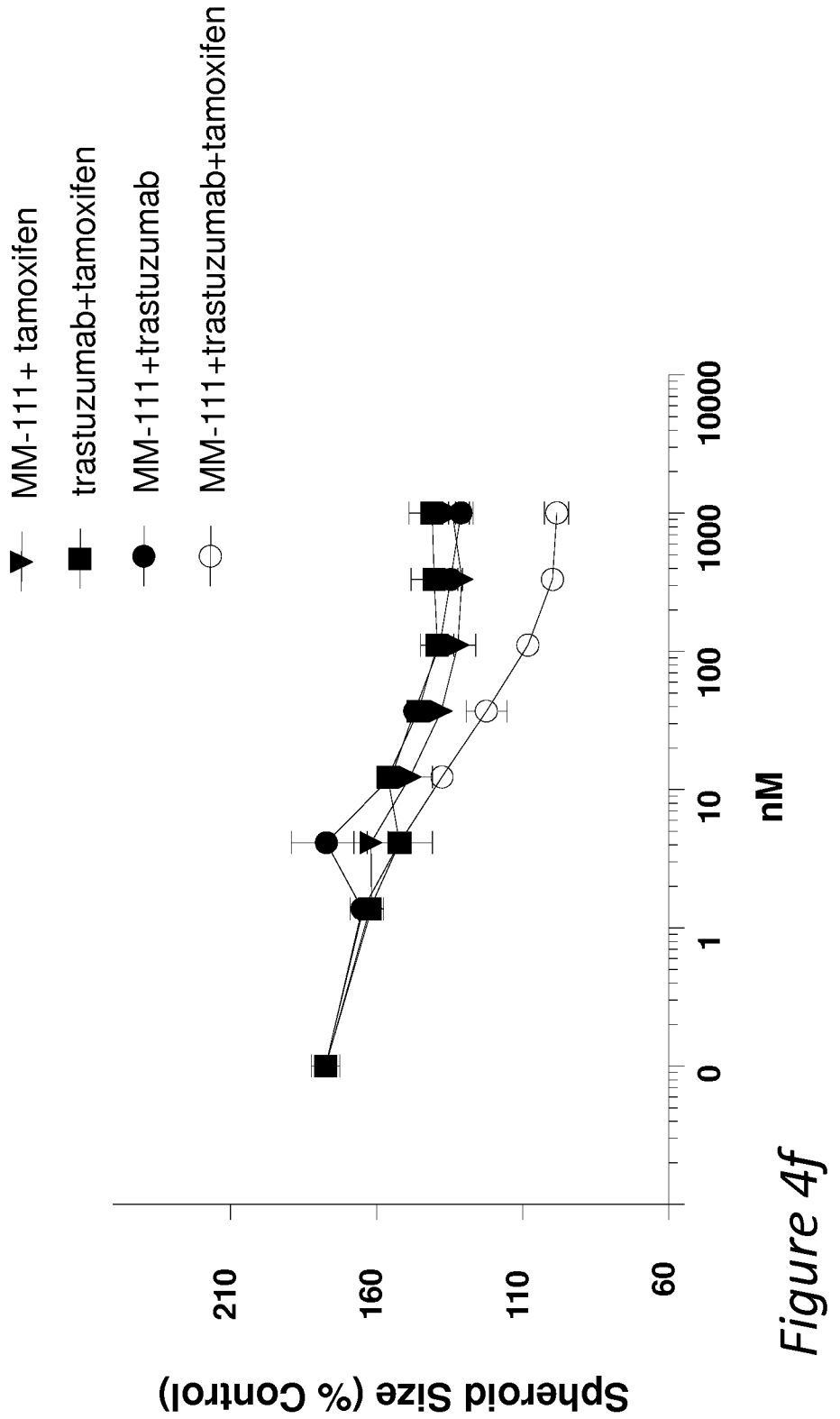
MM-111+ tamoxifen
trastuzumab+tamoxifen
MM-111+trastuzumab
MM-111+trastuzumab+tamoxifen
Spheroid Size (% Control)
210
160
110
60
0
1
10
100
1000
10000
nM

Figure 4f

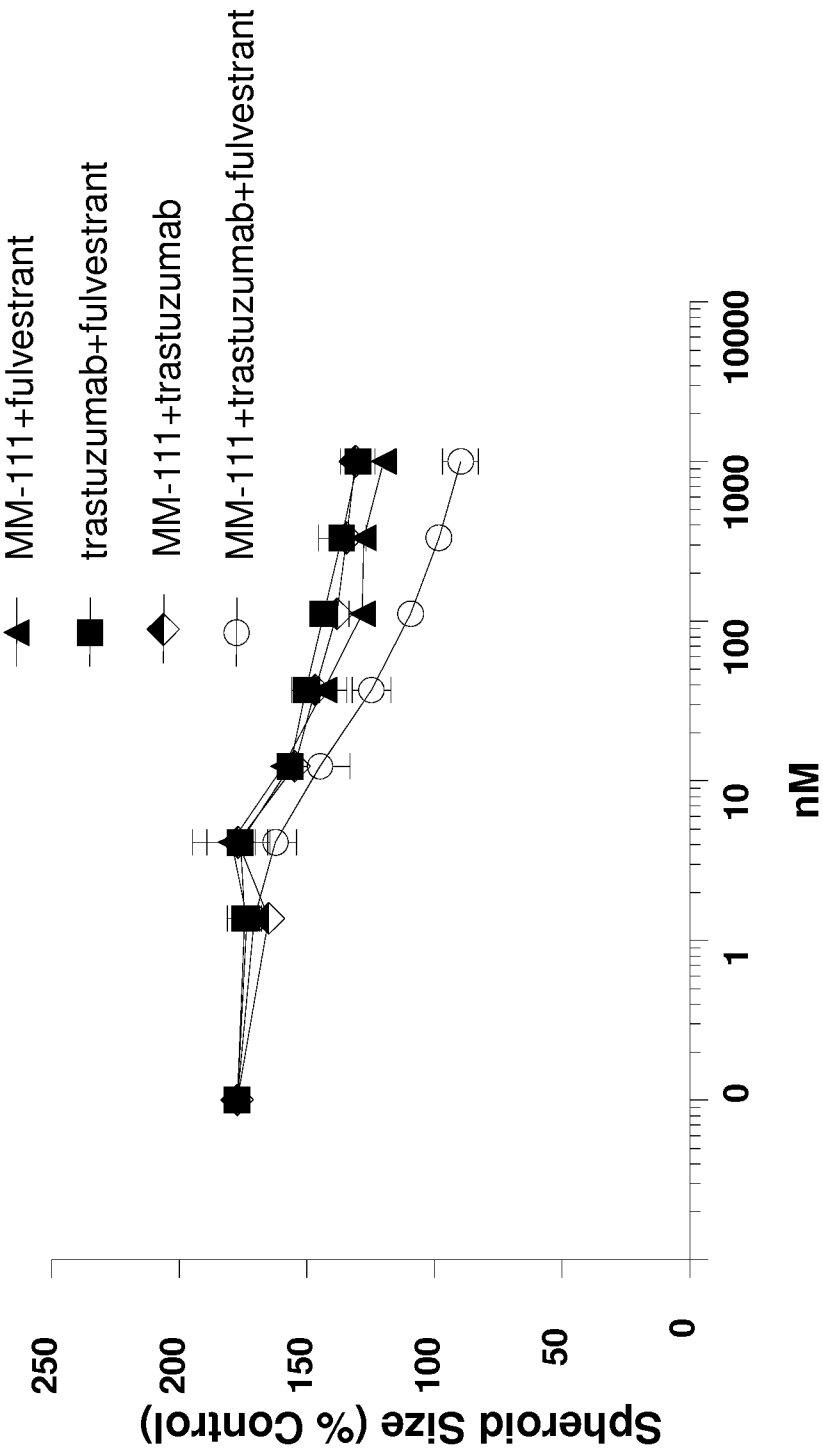
MM-111+fulvestrant
trastuzumab+fulvestrant
MM-111+trastuzumab
MM-111+trastuzumab+fulvestrant
Spheroid Size (% Control)
250
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 4g

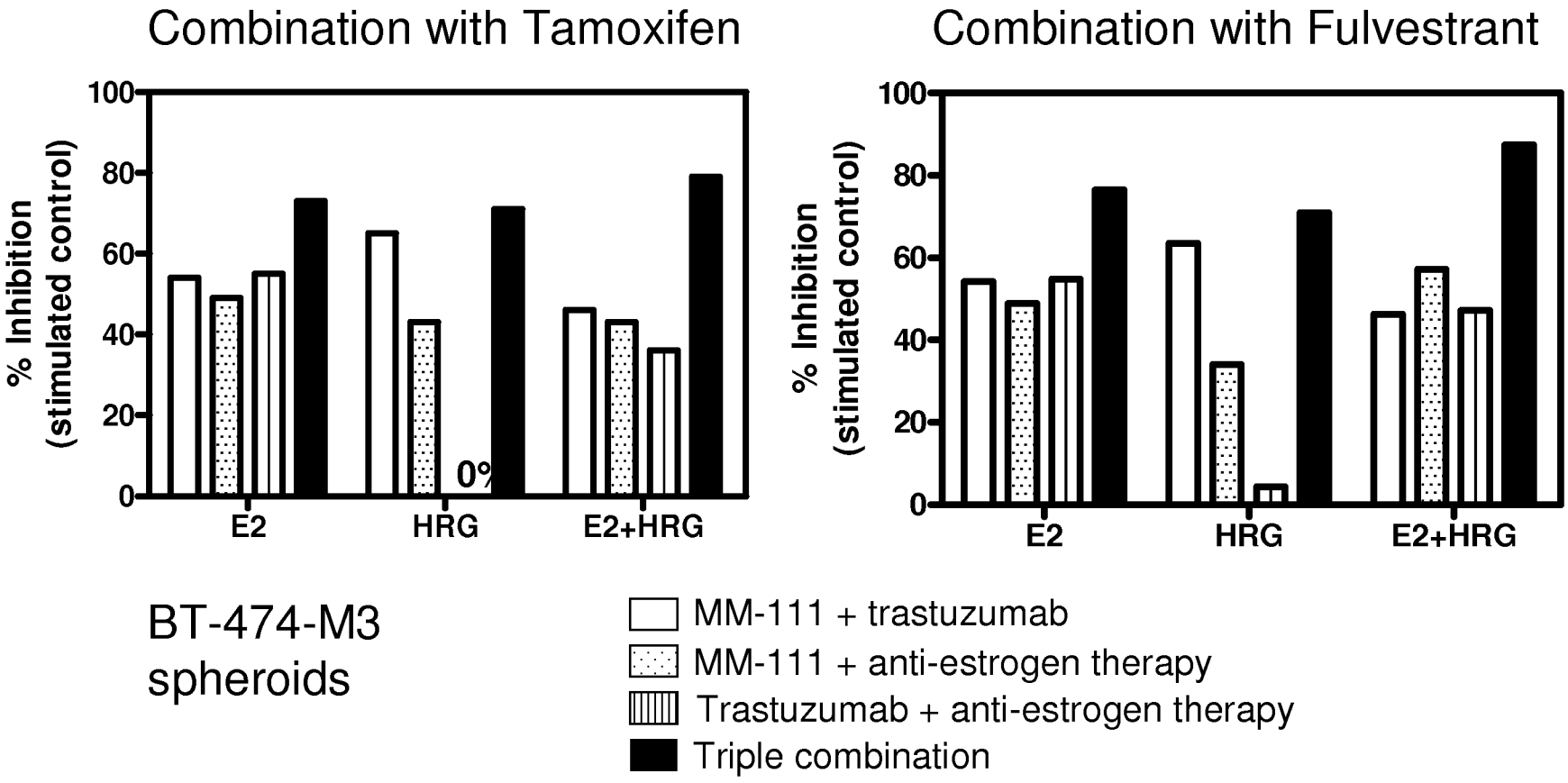
Combination with Tamoxifen
Combination with Fulvestrant
% Inhibition (stimulated control)
100
80
60
40
20
0
0%
E2
HRG
E2+HRG
BT-474-M3 spheroids
MM-111 + trastuzumab
MM-111 + anti-estrogen therapy
Trastuzumab + anti-estrogen therapy
Triple combination

Figure 5

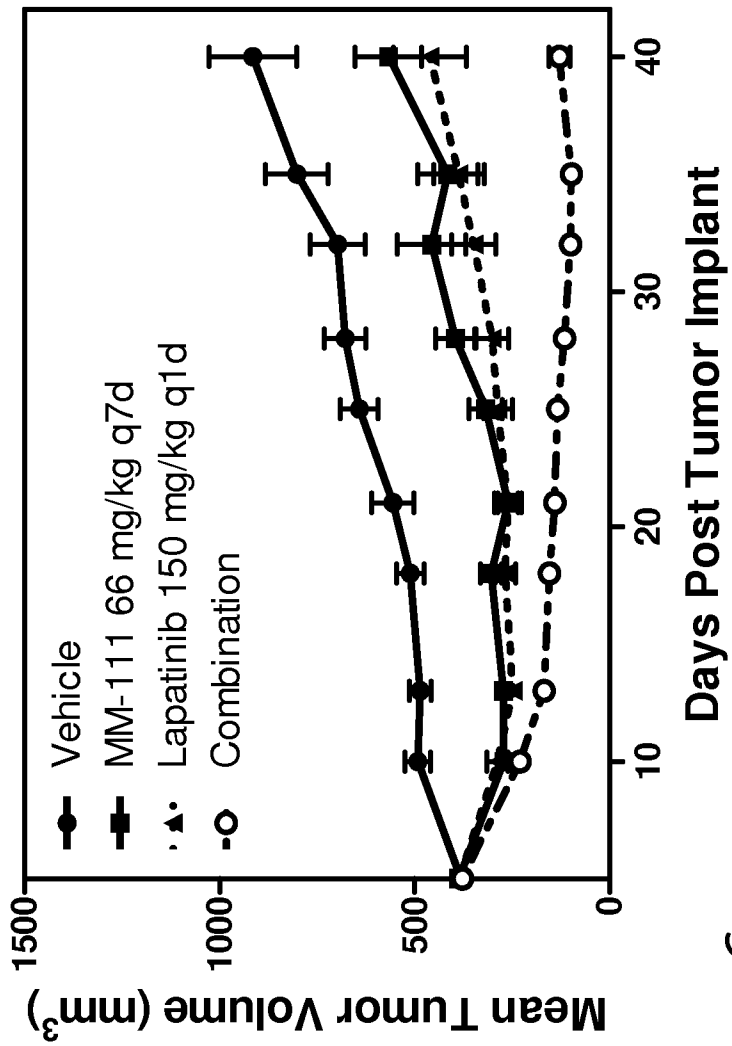
Mean Tumor Volume (mm3)
1500
1000
500
0
10
20
30
40
Days Post Tumor Implant
Vehicle
MM-111 66 mg/kg q7d
Lapatinib 150 mg/kg q1d
Combination

Figure 6

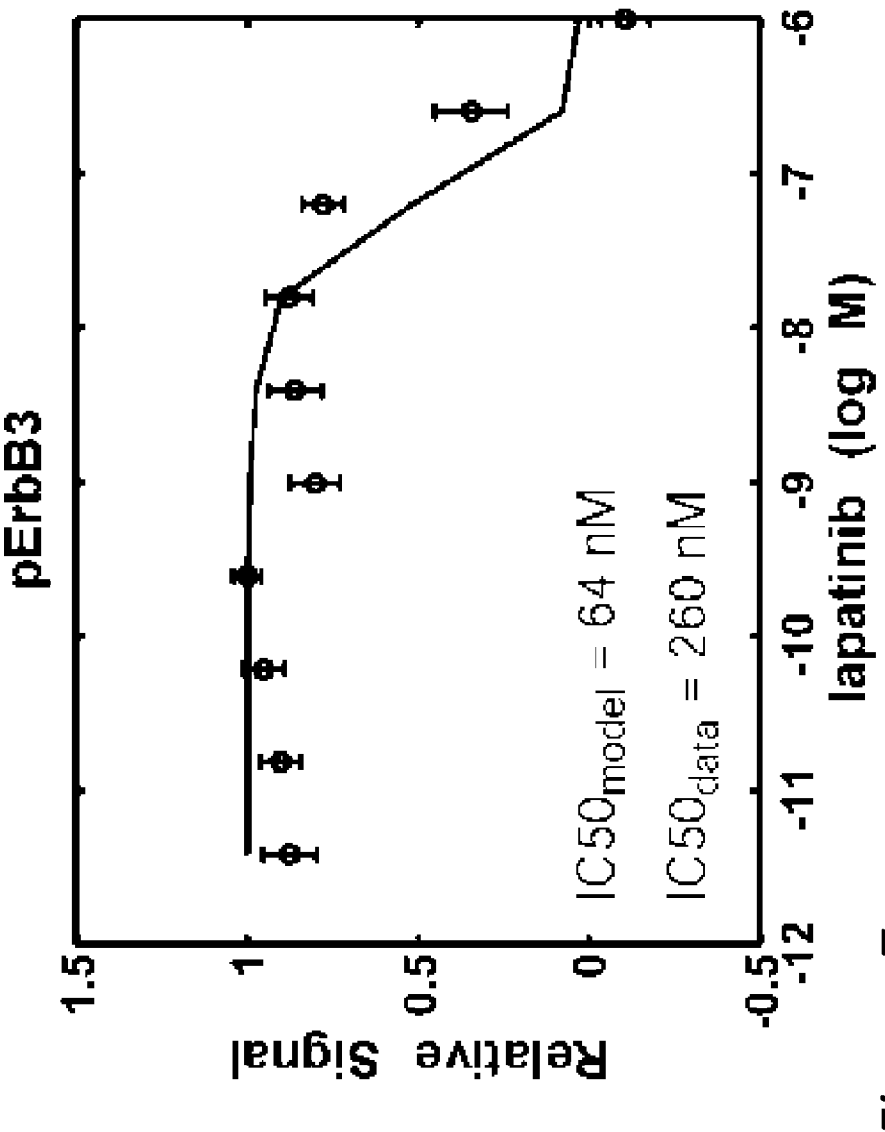
pErbB3
Relative Signal
1.5
1
0.5
0
-0.5
-12
-11
-10
-9
-8
-7
-6
lapatinib (log M)
IC50model = 64 nM
IC50data = 260 nM

Figure 7a

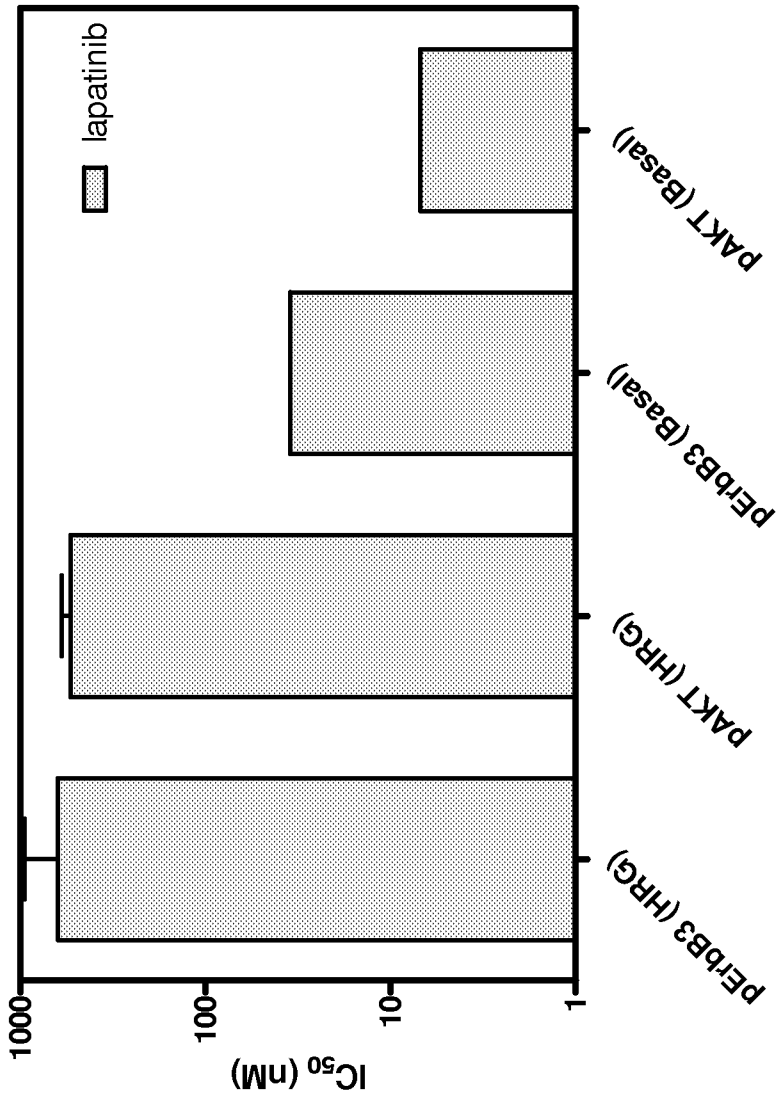
lapatinib
IC50 (nM)
1000
100
10
1
ErbB3 (HRG)
pAKT (HRG)
ErbB3 (Basal)
pAKT (Basal)

Figure 7b

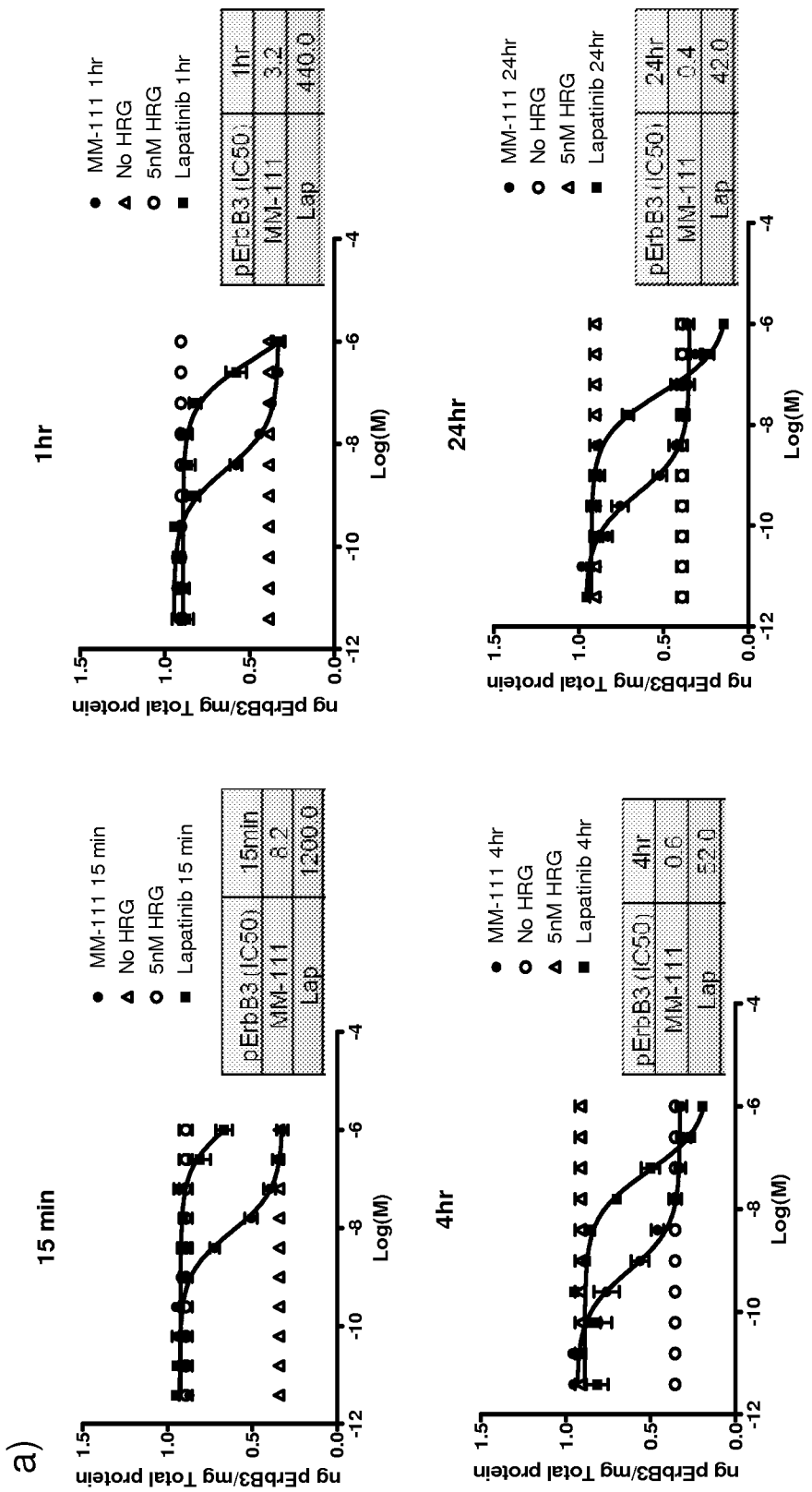
a)
15 min
MM-111 15 min
No HRG
5nM HRG
Lapatinib 15 min
pErbB3 (IC50) 15min
MM-111 8.2
Lap 1200.0
ng pErbB3/mg Total protein
Log(M)
1hr
MM-111 1hr
No HRG
5nM HRG
Lapatinib 1hr
pErbB3 (IC50) 1hr
MM-111 3.2
Lap 440.0
4hr
MM-111 4hr
No HRG
5nM HRG
Lapatinib 4hr
pErbB3 (IC50) 4hr
MM-111 0.6
Lap 52.0
24hr
MM-111 24hr
No HRG
5nM HRG
Lapatinib 24hr
pErbB3 (IC50) 24hr
MM-111 0.4
Lap 42.0

Figure 8a

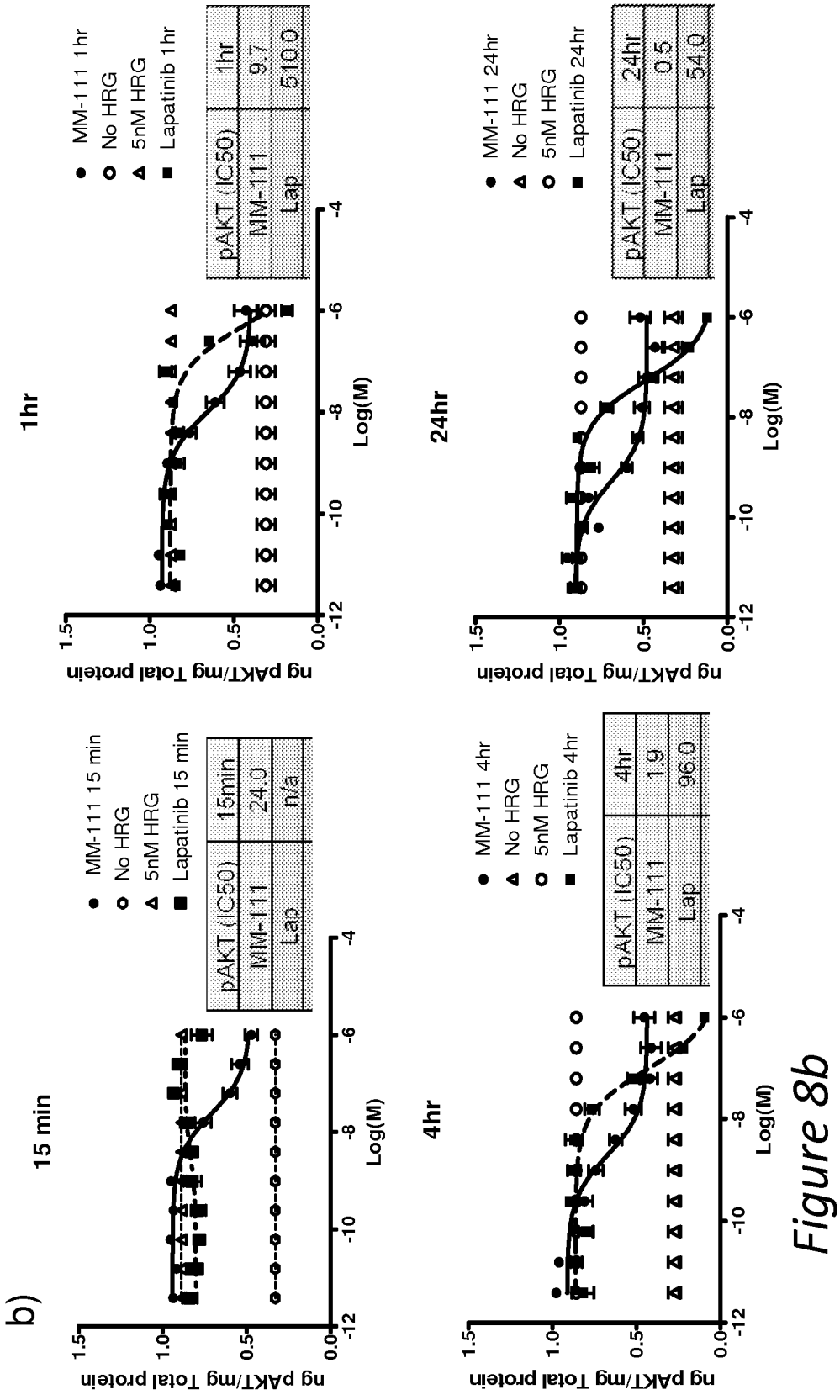
b)
15 min
MM-111 15 min
No HRG
5nM HRG
Lapatinib 15 min
pAKT (IC50) 15min
MM-111 24.0
Lap n/a
1hr
MM-111 1hr
No HRG
5nM HRG
Lapatinib 1hr
pAKT (IC50) 1hr
MM-111 9.7
Lap 510.0
4hr
MM-111 4hr
No HRG
5nM HRG
Lapatinib 4hr
pAKT (IC50) 4hr
MM-111 1.9
Lap 96.0
24hr
MM-111 24hr
No HRG
5nM HRG
Lapatinib 24hr
pAKT (IC50) 24hr
MM-111 0.5
Lap 54.0
ng pAKT/mg Total protein
Log(M)

Figure 8b

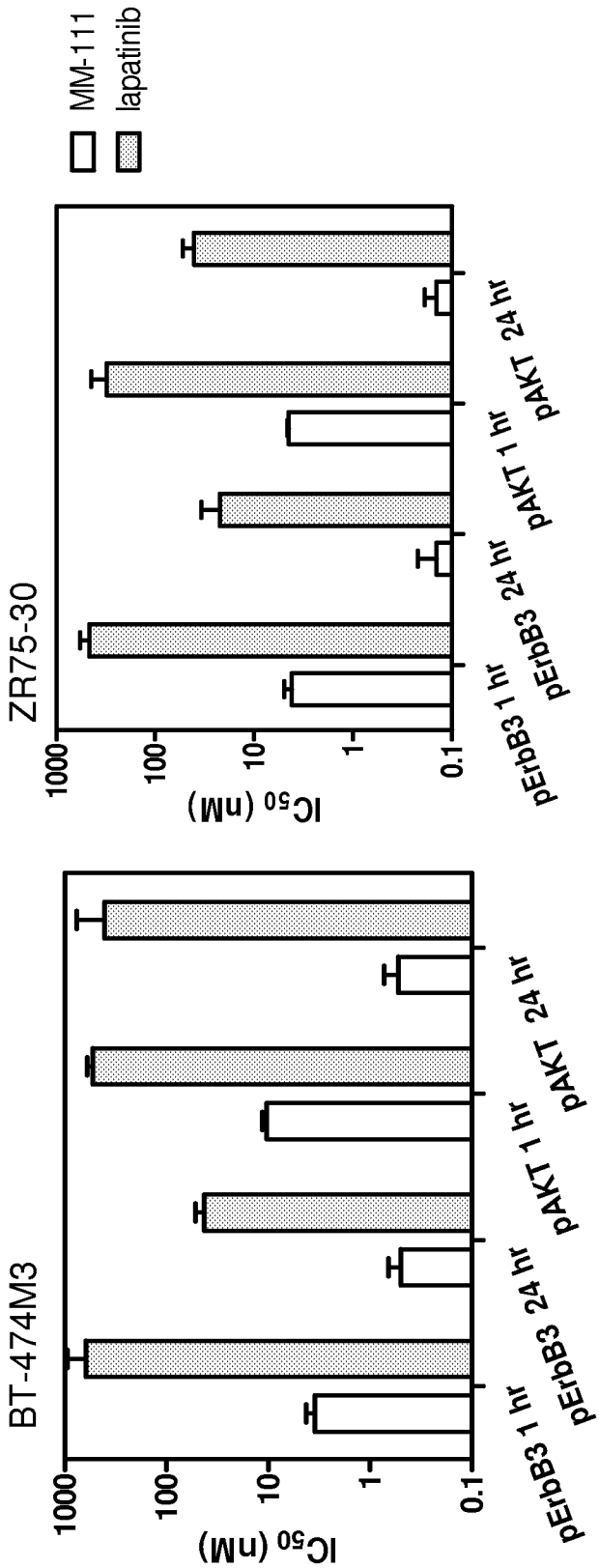
MM-111
lapatinib
ZR75-30
BT-474M3
IC50 (nM)
1000
100
10
1
0.1
pErbB3 1 hr
pErbB3 24 hr
pAKT 1 hr
pAKT 24 hr

Figure 8c

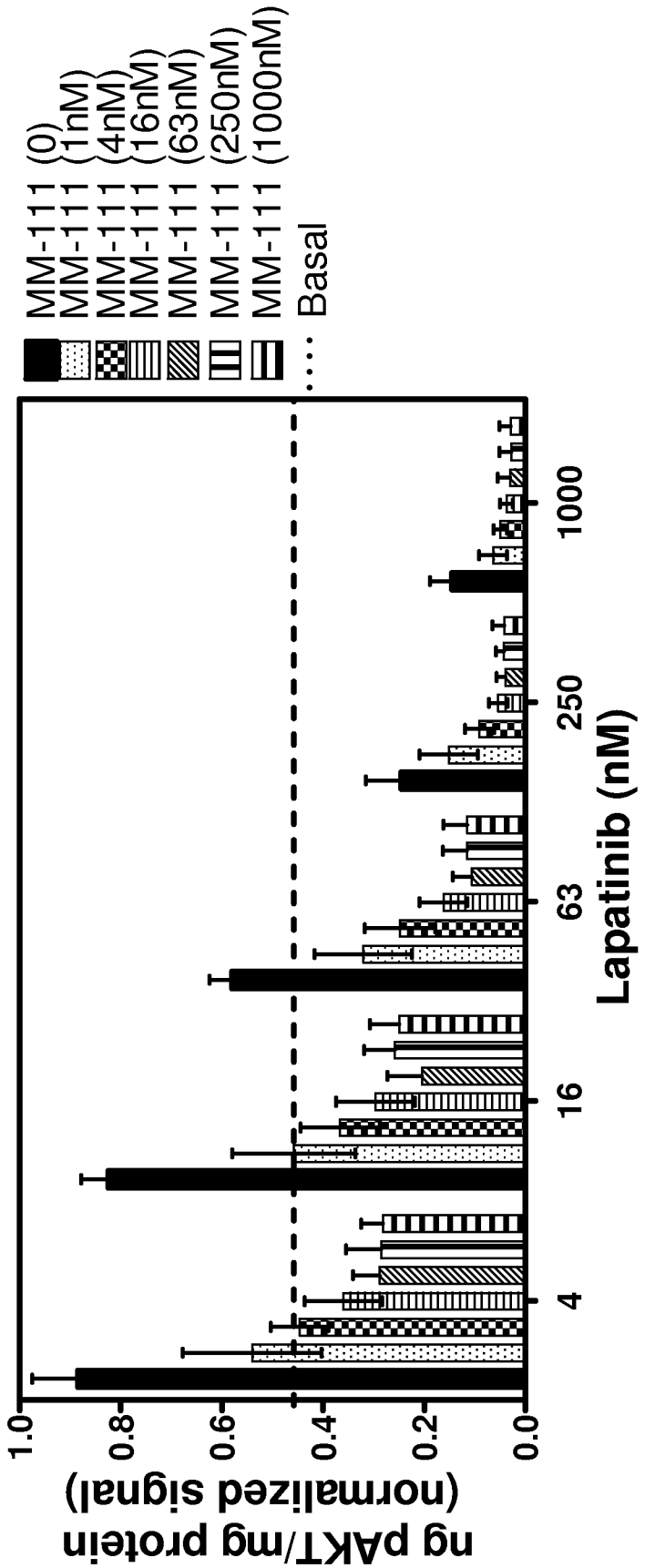
MM-111 (0)
MM-111 (1nM)
MM-111 (4nM)
MM-111 (16nM)
MM-111 (63nM)
MM-111 (250nM)
MM-111 (1000nM)
Basal
ng pAKT/mg protein
(normalized signal)
1.0
0.8
0.6
0.4
0.2
0.0
4
16
63
250
1000
Lapatinib (nM)

Figure 9

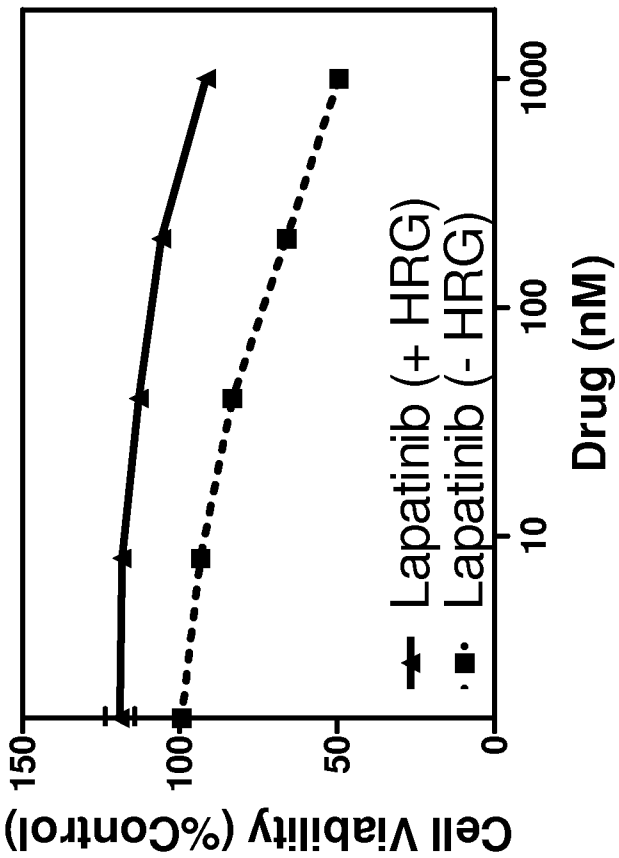
Cell Viability (%Control)
150
100
50
0
10
100
1000
Drug (nM)
Lapatinib (+ HRG)
Lapatinib (- HRG)

Figure 10

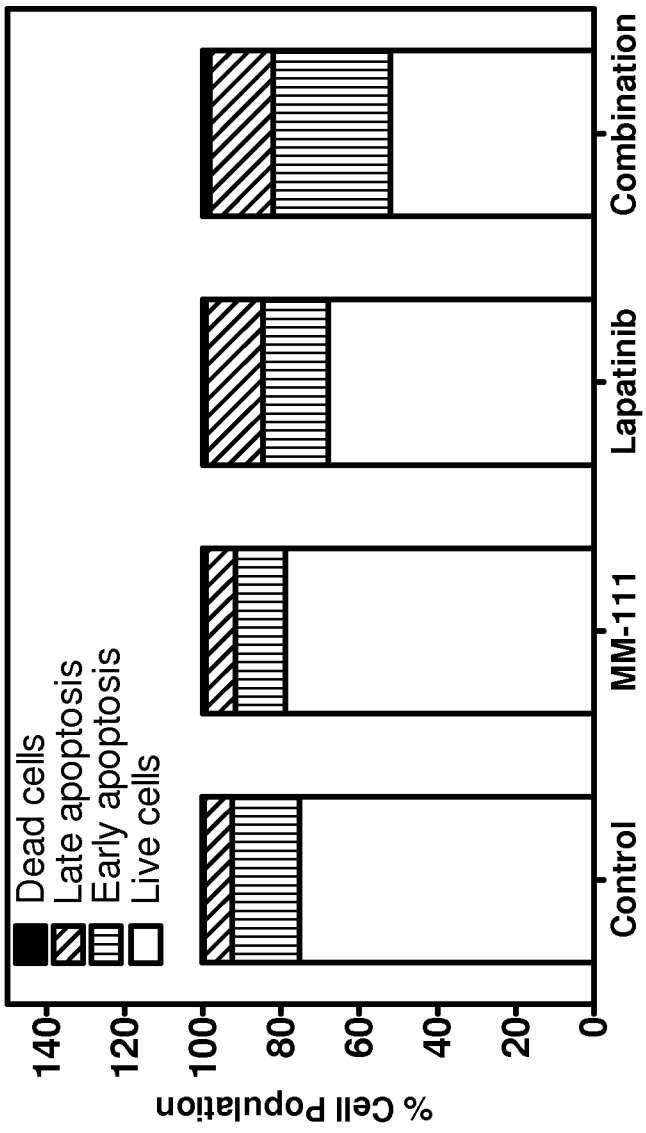
Dead cells
Late apoptosis
Early apoptosis
Live cells
% Cell Population
0
20
40
60
80
100
120
140
Control
MM-111
Lapatinib
Combination

Figure 11

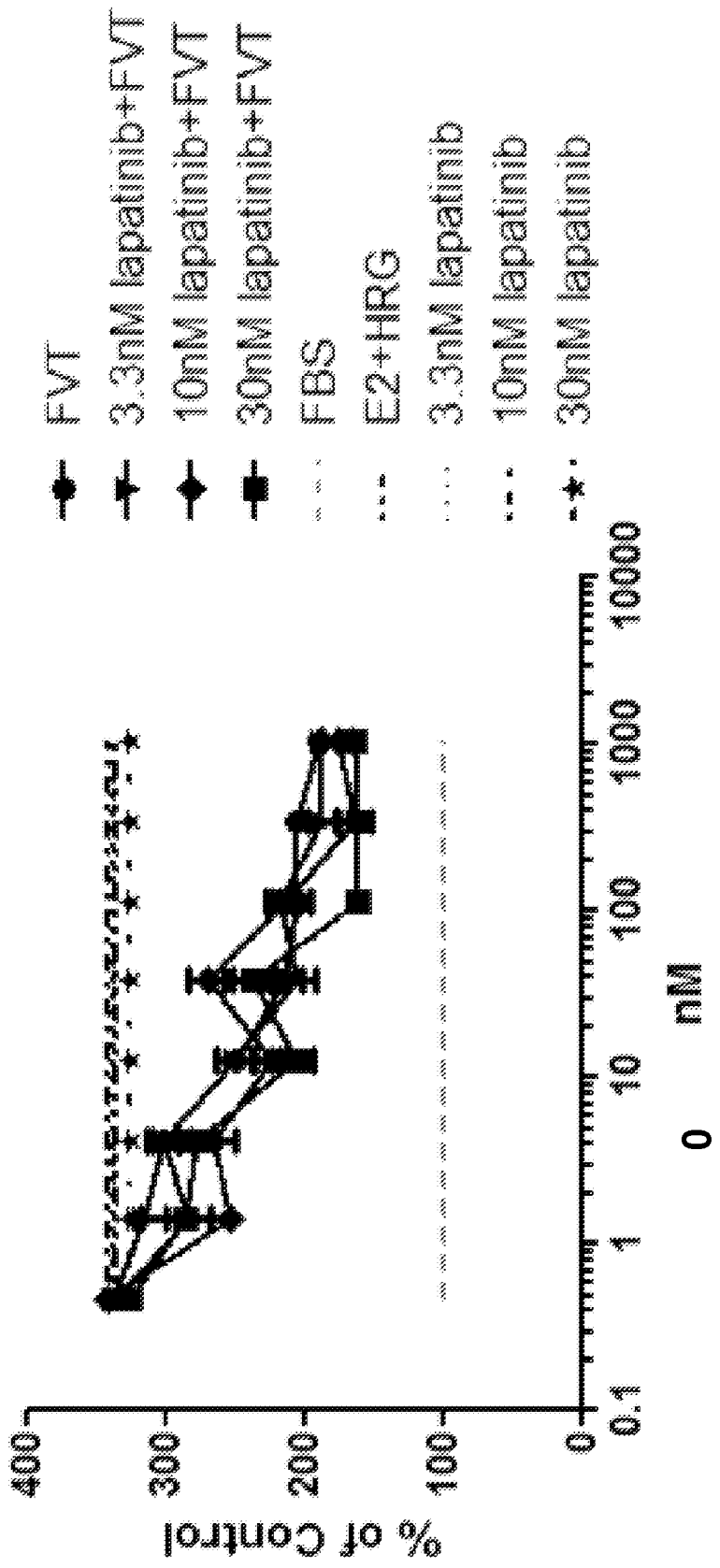
FVT
3.3nM lapatinib+FVT
10nM lapatinib+FVT
30nM lapatinib+FVT
FBS
E2+HRG
3.3nM lapatinib
10nM lapatinib
30nM lapatinib
% of Control
400
300
200
100
0
0.1
1
10
100
1000
10000
nM

Figure 12a

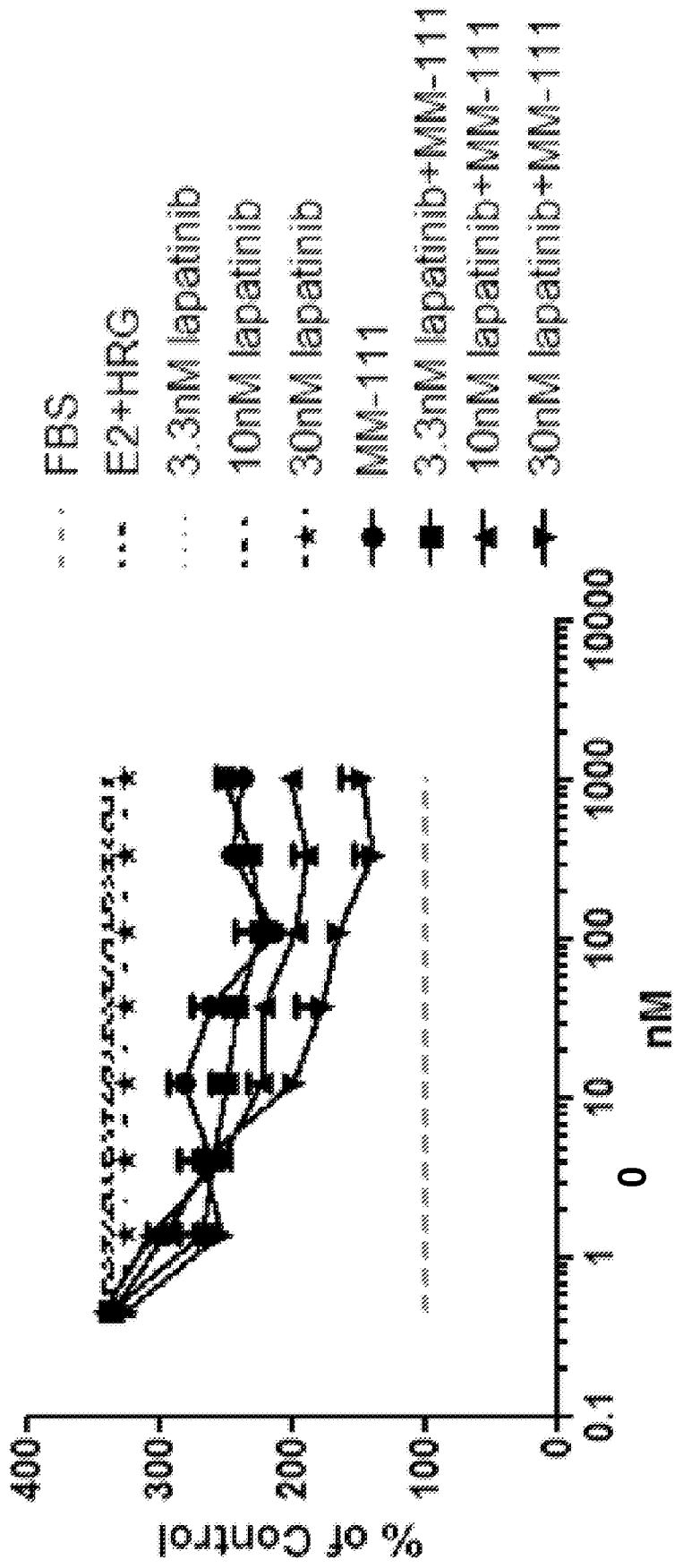
FBS
E2+HRG
3.3nM lapatinib
10nM lapatinib
30nM lapatinib
MM-111
3.3nM lapatinib+MM-111
10nM lapatinib+MM-111
30nM lapatinib+MM-111
% of Control
400
300
200
100
0
0.1
1
10
100
1000
10000
nM

Figure 12b

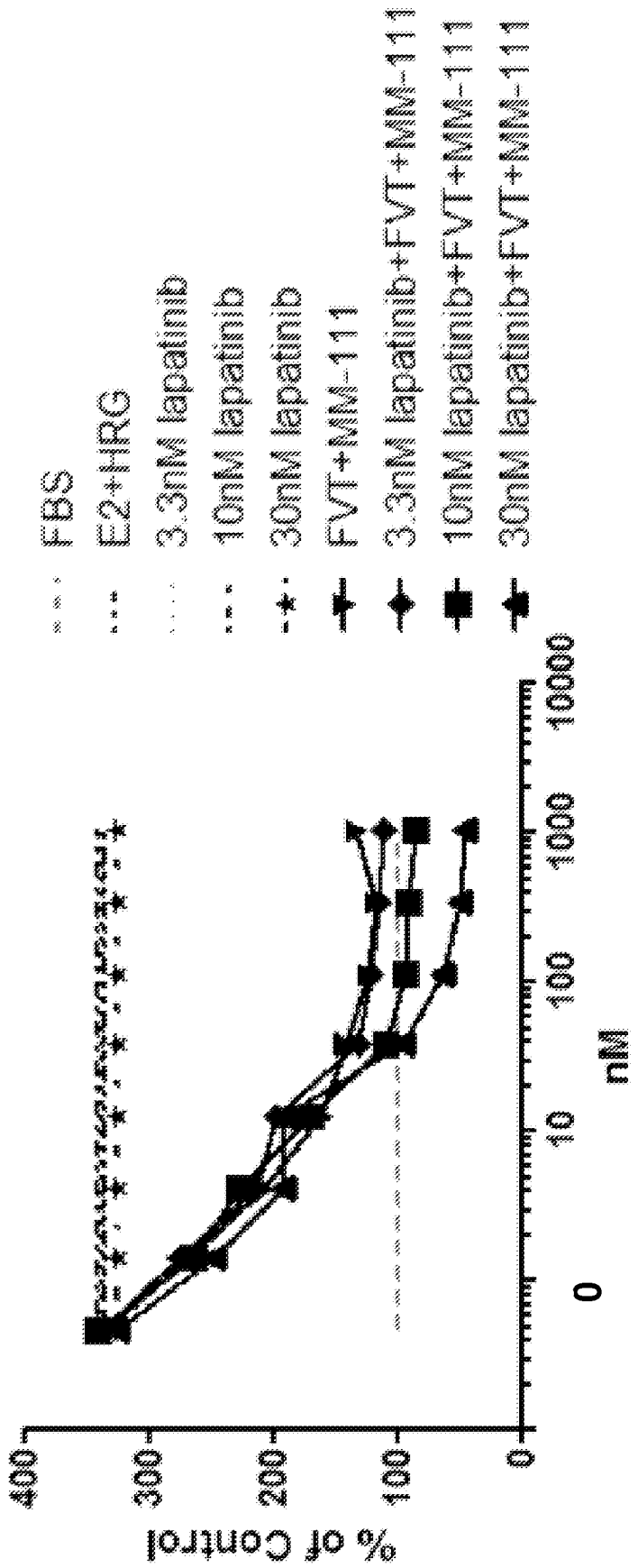
FBS
E2+HRG
3.3nM lapatinib
10nM lapatinib
30nM lapatinib
FVT+MM-111
3.3nM lapatinib+FVT+MM-111
10nM lapatinib+FVT+MM-111
30nM lapatinib+FVT+MM-111
% of Control
400
300
200
100
0
0
10
100
1000
10000
nM

Figure 12c

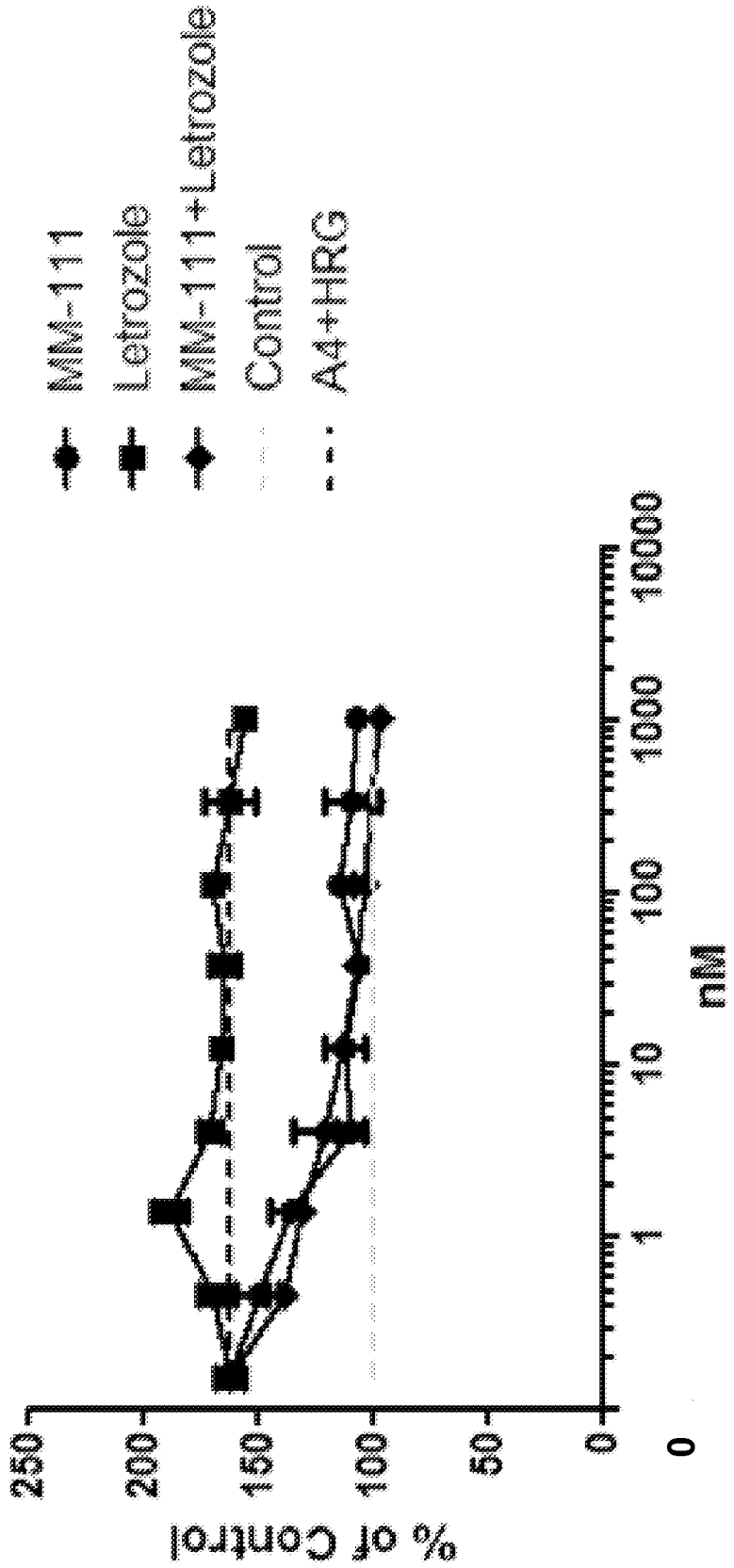
MM-111
Letrozole
MM-111+Letrozole
Control
A4+HRG
% of Control
250
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 13a

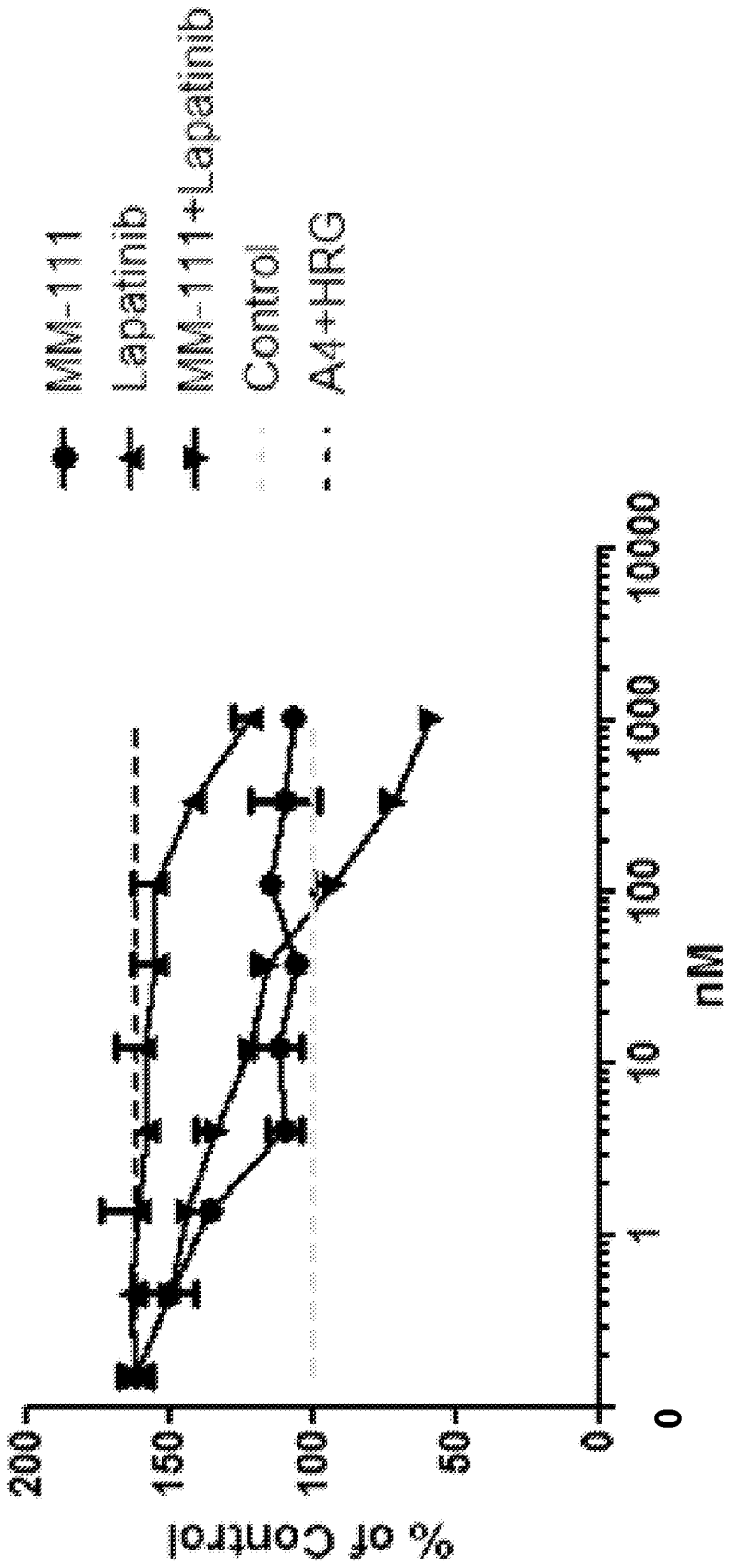
MM-111
Lapatinib
MM-111+Lapatinib
Control
A4+HRG
% of Control
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 13b

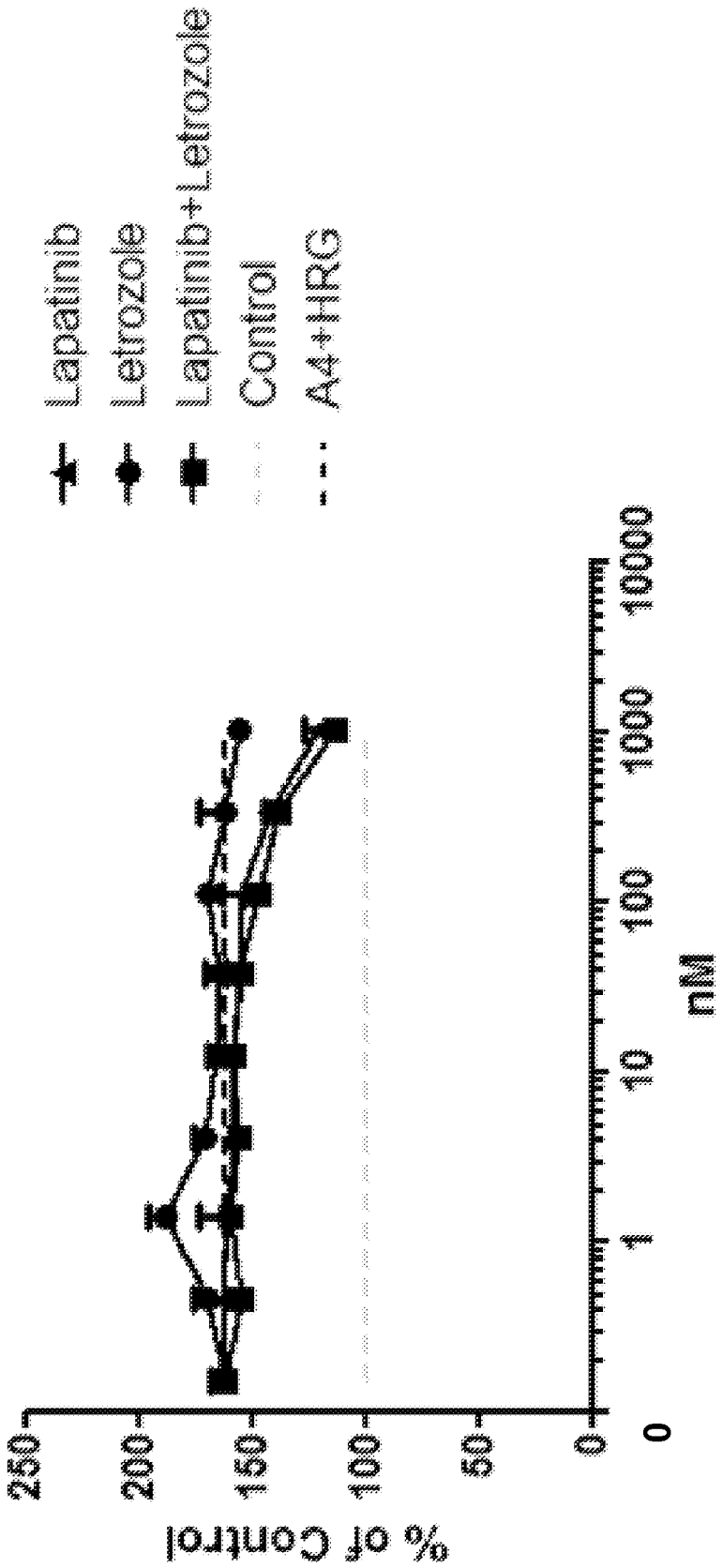
Lapatinib
Letrozole
Lapatinib+Letrozole
Control
A4+HRG
% of Control
250
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 13c

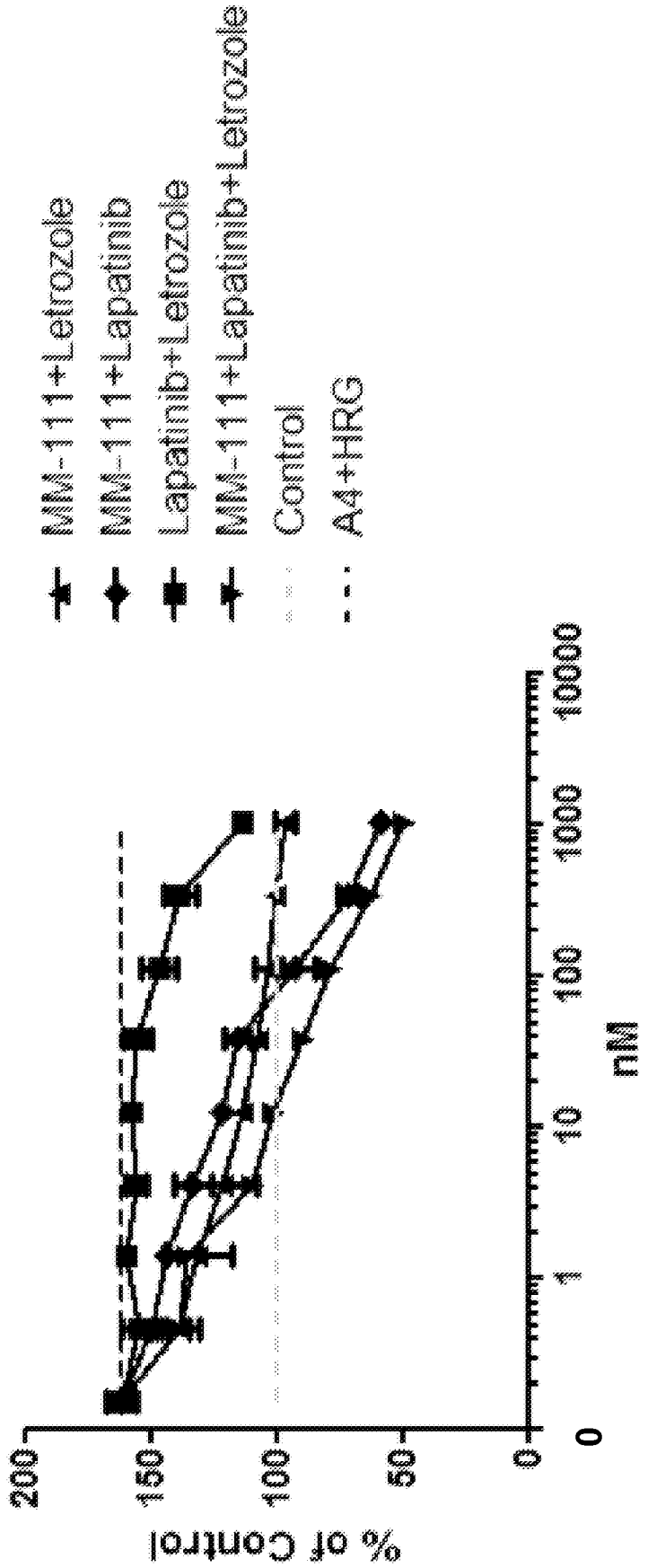
MM-111+Letrozole
MM-111+Lapatinib
Lapatinib+Letrozole
MM-111+Lapatinib+Letrozole
Control
A4+HRG
% of Control
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 13d