US 20150202287A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2015/0202287 A1**

(43) **Pub. Date: Jul. 23, 2015**

Zhang et al.

(54) **COMBINATION THERAPIES COMPRISING ANTI-ERBB3 AGENTS**

(71) Applicant: **Merrimack Pharmaceuticals, Inc.**, Cambridge, MA (US)

(72) Inventors: **Bo Zhang**, Lynnfield, MA (US); **Charlotte McDonagh**, Winchester, MA (US); **Alexandra Huhalov**, Cambridge, MA (US)

(73) Assignee: **Merrimack Pharmaceuticals, Inc.**, Cambridge, MA (US)

(21) Appl. No.: **14/424,344**

(22) PCT Filed: **Aug. 30, 2013**

(86) PCT No.: **PCT/US2013/057714**

§ 371 (c)(1),
(2) Date: **Feb. 26, 2015**

Related U.S. Application Data

(60) Provisional application No. 61/695,242, filed on Aug. 30, 2012.

Publication Classification

(51) **Int. Cl.**

A61K 39/395	(2006.01)
A61K 31/567	(2006.01)
A61K 31/138	(2006.01)
A61K 31/4196	(2006.01)
A61K 31/517	(2006.01)
A61K 31/436	(2006.01)
A61K 33/24	(2006.01)
A61K 31/519	(2006.01)
C07K 16/28	(2006.01)
A61K 31/337	(2006.01)
A61K 45/06	(2006.01)
C07K 16/40	(2006.01)
A61K 31/7068	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 39/3955*** (2013.01); ***C07K 16/40*** (2013.01); ***A61K 31/567*** (2013.01); ***A61K 31/138*** (2013.01); ***A61K 31/4196*** (2013.01); ***A61K 31/517*** (2013.01); ***A61K 31/7068*** (2013.01); ***A61K 33/24*** (2013.01); ***A61K 31/519*** (2013.01); ***C07K 16/2863*** (2013.01); ***A61K 31/337*** (2013.01); ***A61K 45/06*** (2013.01); ***A61K 31/436*** (2013.01); *C07K 2317/31* (2013.01); *A61K 2039/505* (2013.01)

(57) **ABSTRACT**

Disclosed are methods and compositions for inhibiting the growth of a tumor (e.g., a malignant 5 tumor) in a subject. In particular, combination therapies for treating a tumor in a subject by coadministering an agent selected from i) an effective amount of an anti-estrogen agent; ii) an effective amount of a receptor tyrosine kinase inhibitor; iii) an effective amount of a MEK/PI3 kinase/AKT inhibitor; iv) an effective amount of MM-151; v) an effective amount of an mTOR inhibitor; and/or vi) an effective amount of trastuzumab or T-DM 1, and/or combinations thereof; and an effective amount of a 10 bispecific anti-ErbB2/anti-ErbB3 antibody.

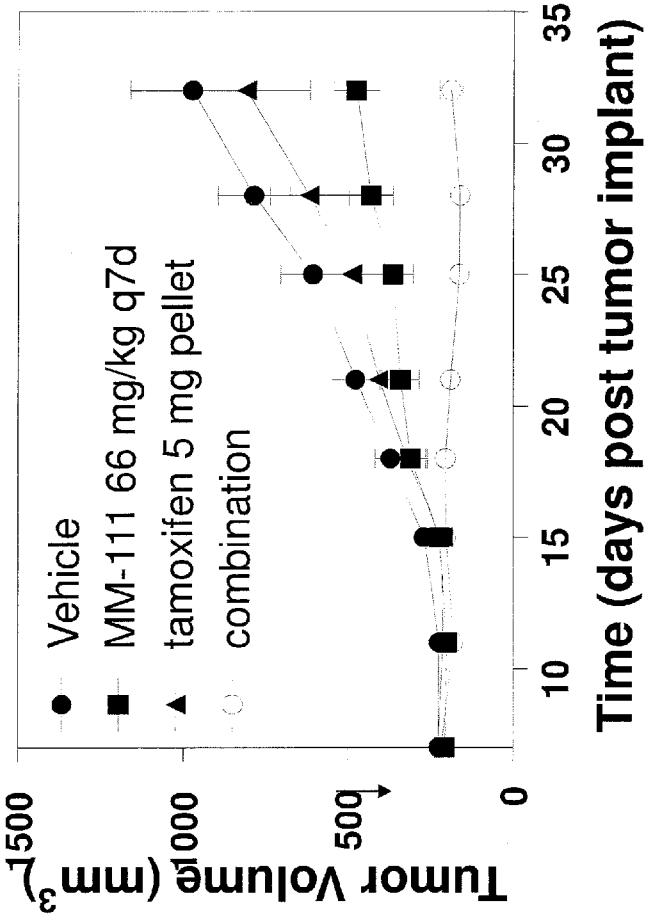
Tumor Volume (mm3)
1500
1000
500
0
10
15
20
25
30
35
Time (days post tumor implant)
Vehicle
MM-111 66 mg/kg q7d
tamoxifen 5 mg pellet
combination

Figure 1

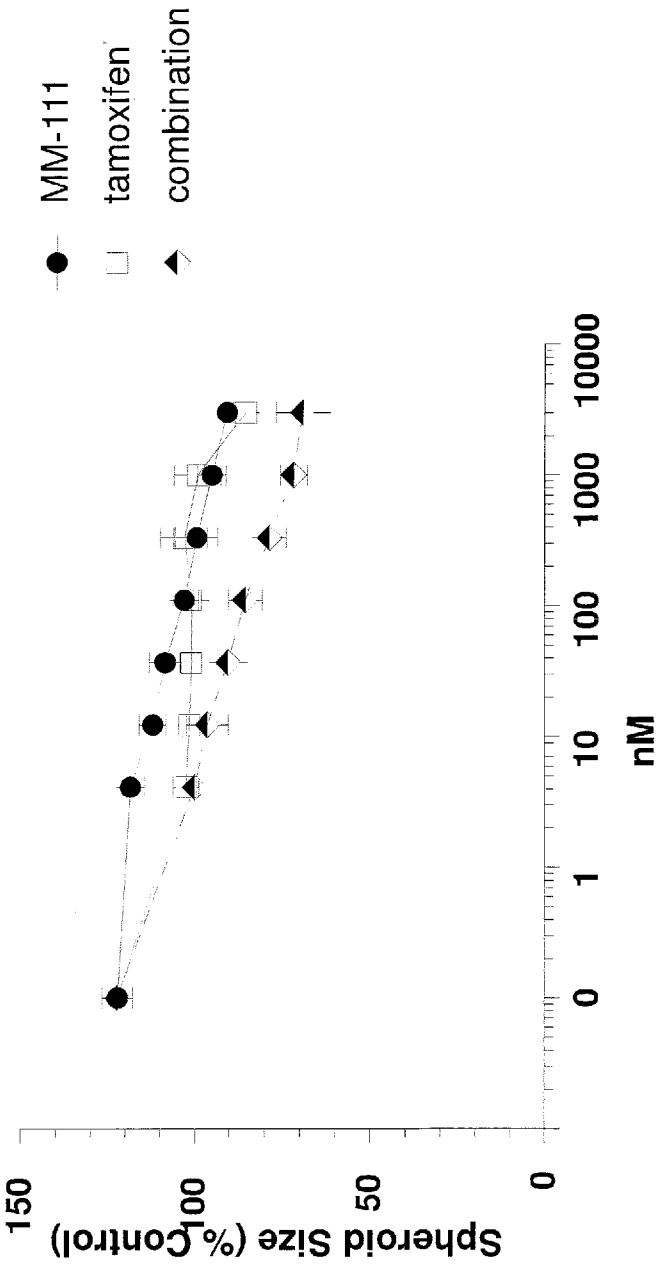
MM-111
tamoxifen
combination
Spheroid Size (%Control)
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2a

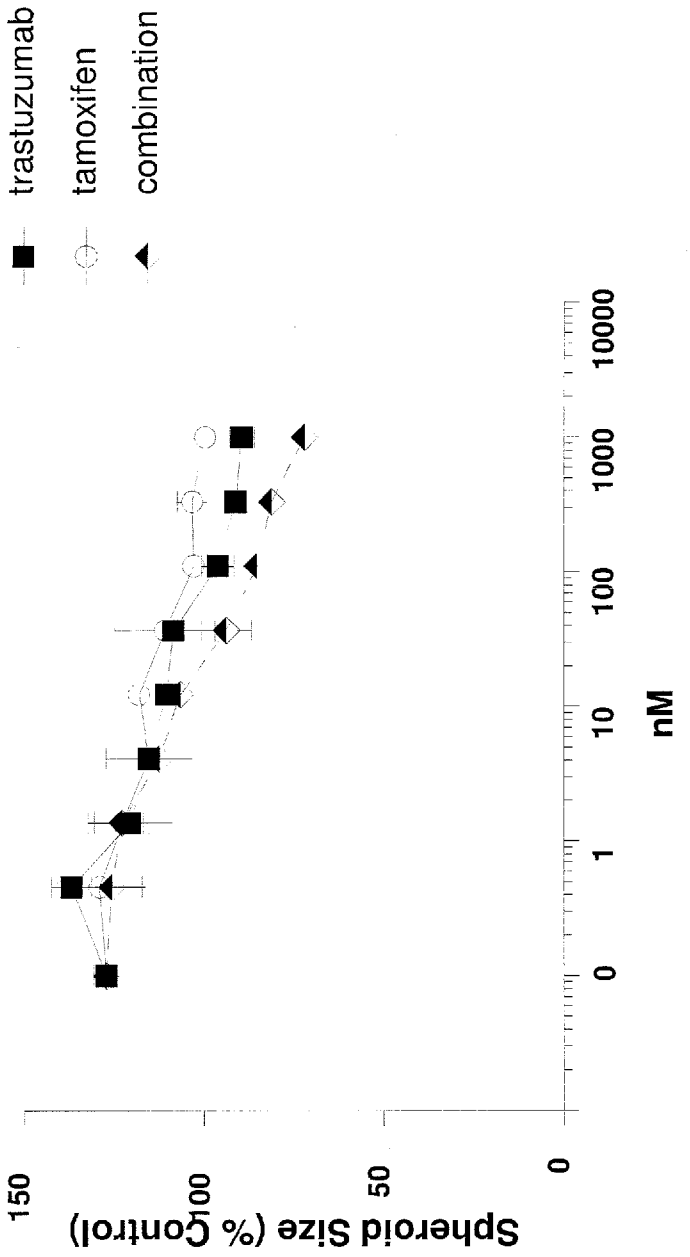
trastuzumab
tamoxifen
combination
Spheroid Size (% Control)
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2b

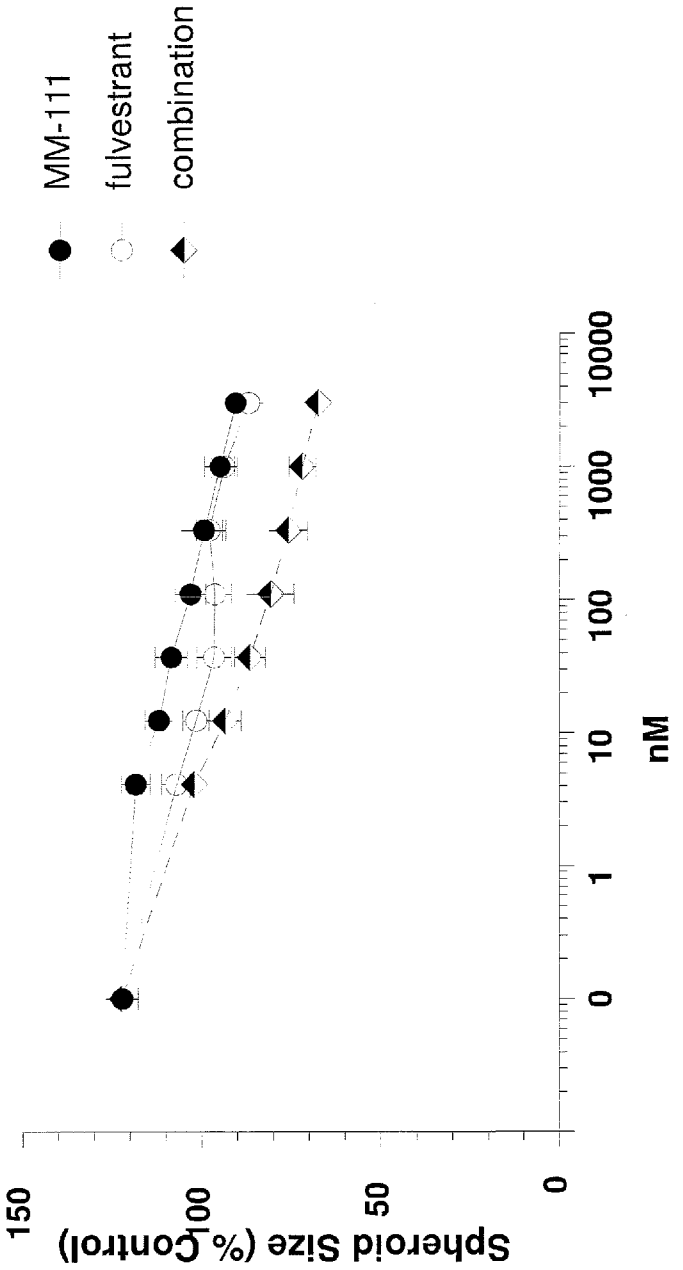
MM-111
fulvestrant
combination
Spheroid Size (% Control)
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2c

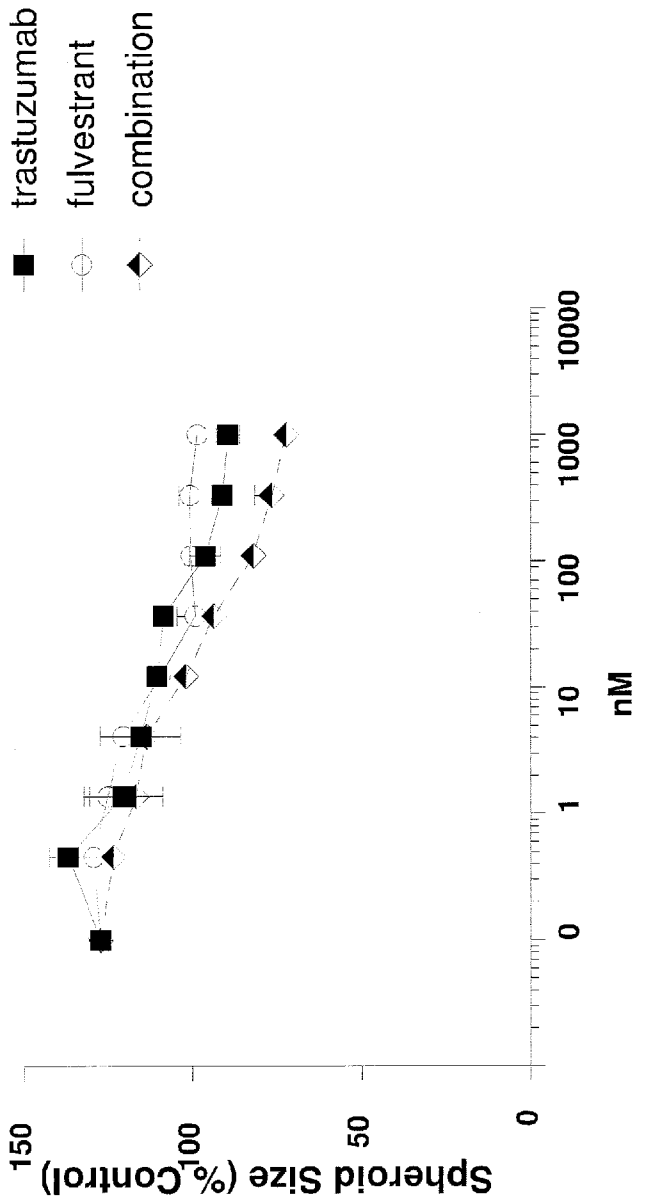
trastuzumab
fulvestrant
combination
Spheroid Size (%Control)
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2d

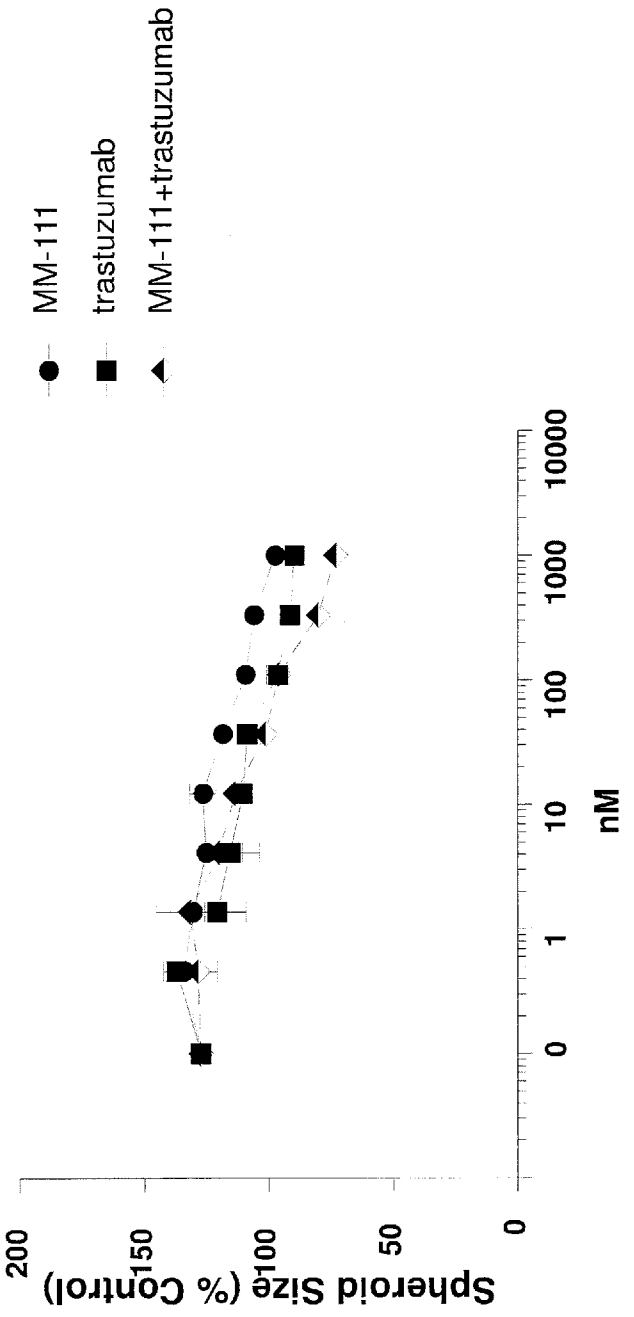
MM-111
trastuzumab
MM-111+trastuzumab
Spheroid Size (% Control)
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2e

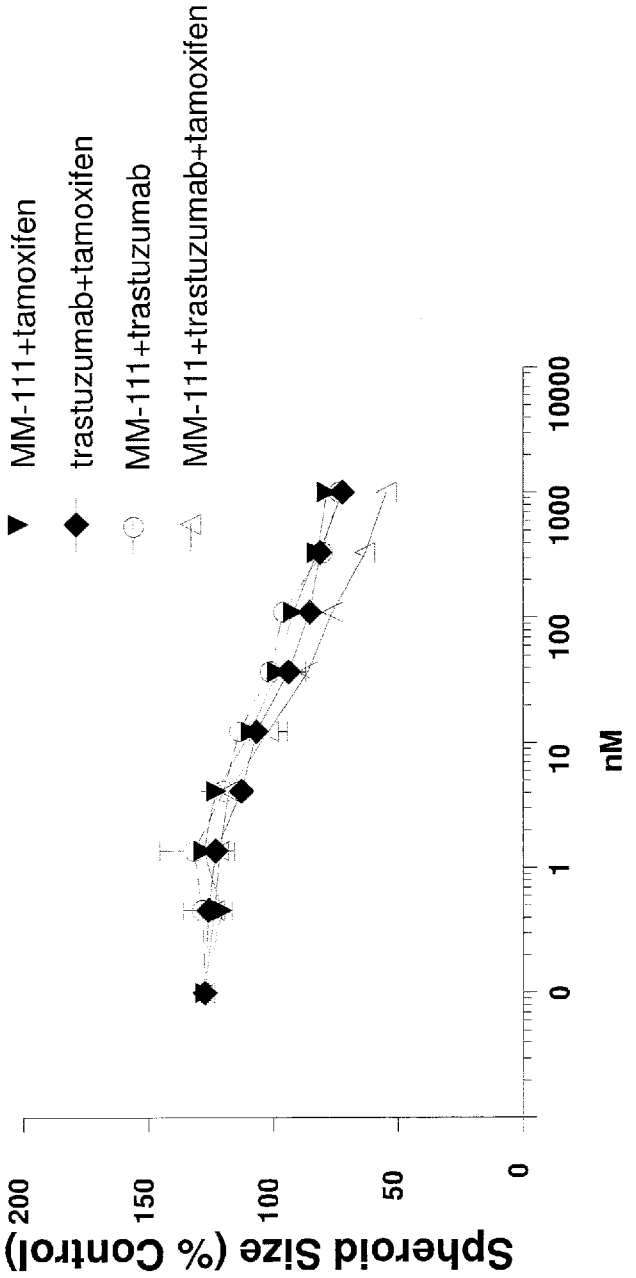
MM-111+tamoxifen
trastuzumab+tamoxifen
MM-111+trastuzumab
MM-111+trastuzumab+tamoxifen
Spheroid Size (% Control)
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2f

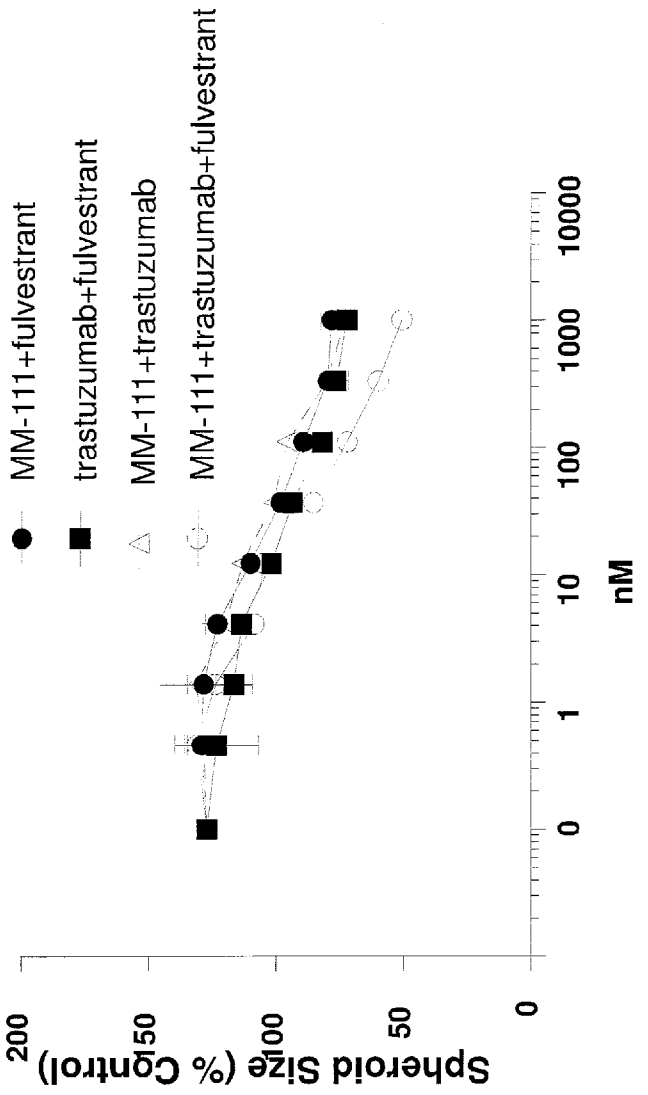
MM-111+fulvestrant
trastuzumab+fulvestrant
MM-111+trastuzumab
MM-111+trastuzumab+fulvestrant
Spheroid Size (% Control)
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 2g

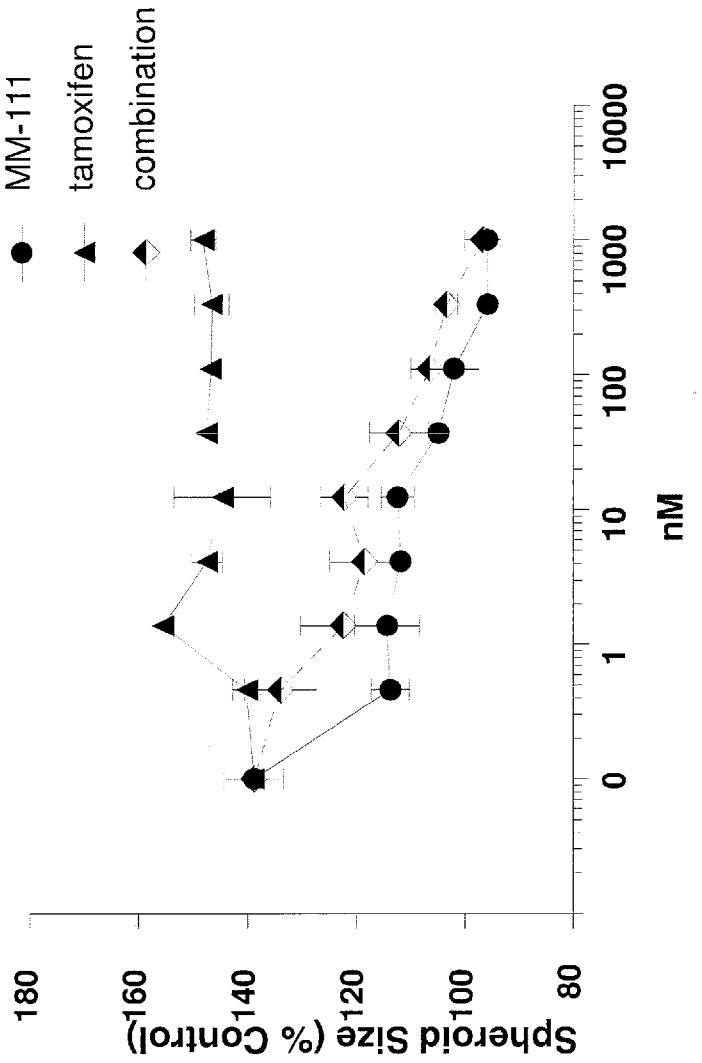
MM-111
tamoxifen
combination
Spheroid Size (% Control)
180
160
140
120
100
80
0
1
10
100
1000
10000
nM

Figure 3a

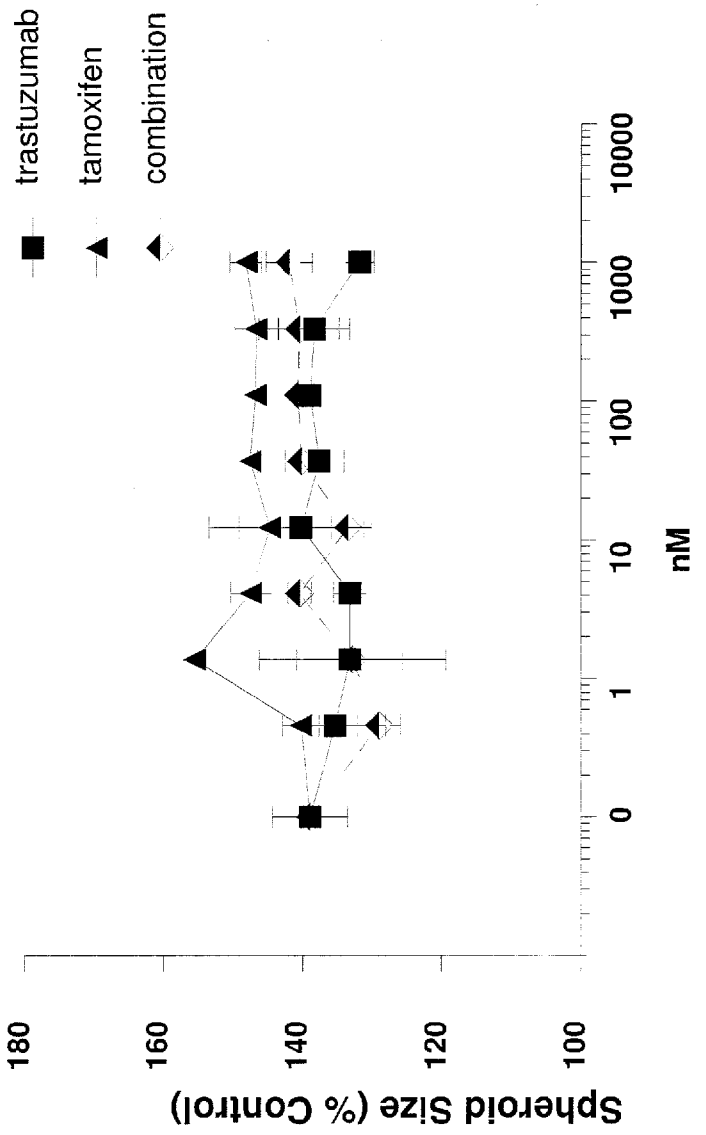
trastuzumab
tamoxifen
combination
Spheroid Size (% Control)
180
160
140
120
100
0
1
10
100
1000
10000
nM

Figure 3b

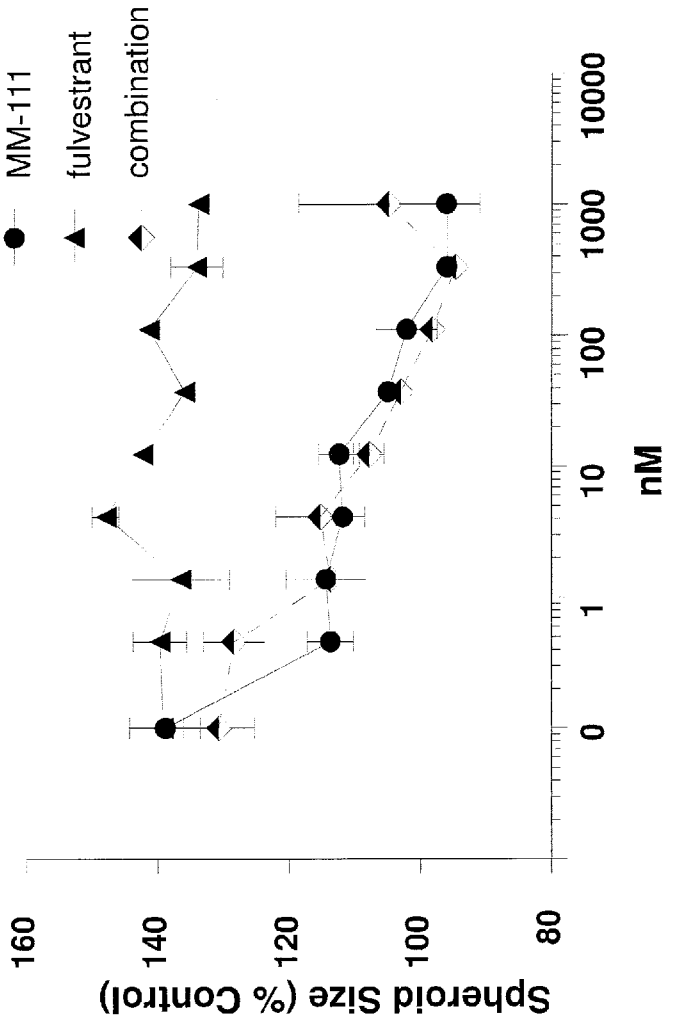
MM-111
fulvestrant
combination
Spheroid Size (% Control)
160
140
120
100
80
0
1
10
100
1000
10000
nM

Figure 3c

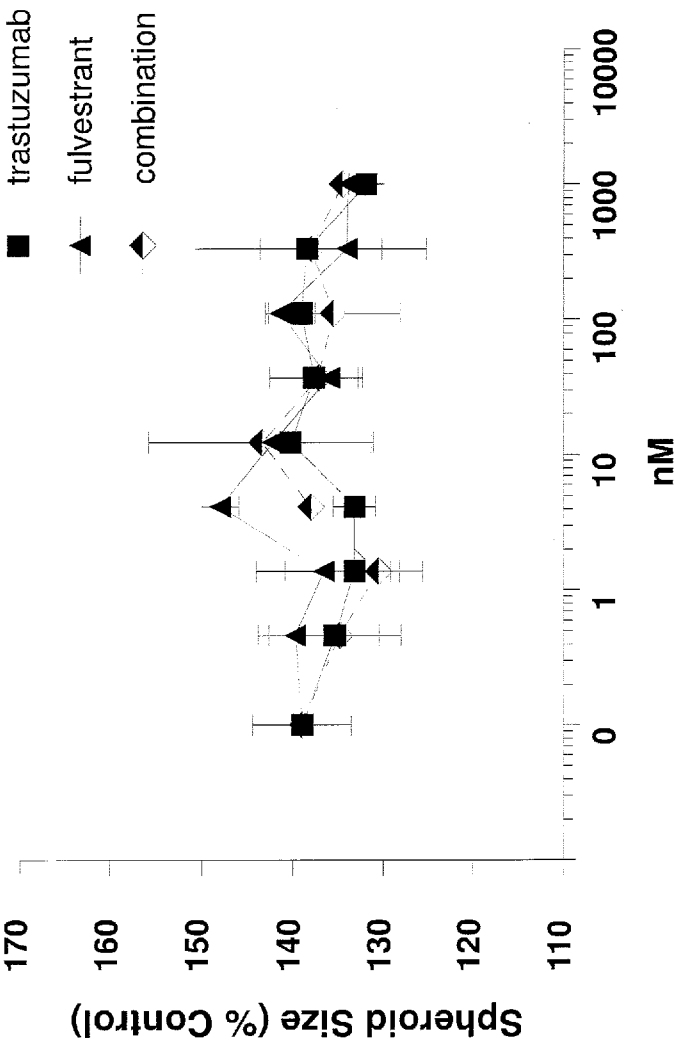
trastuzumab
fulvestrant
combination
Spheroid Size (% Control)
170
160
150
140
130
120
110
0
1
10
100
1000
10000
nM

Figure 3d

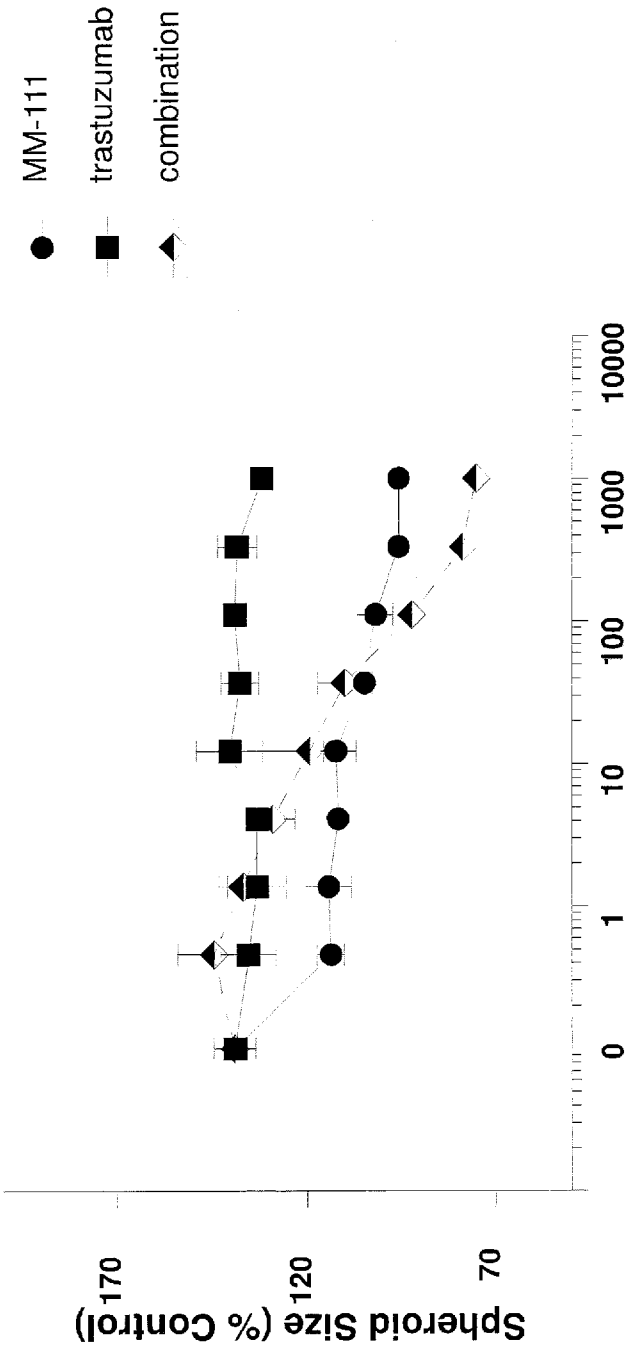

Figure 3e

Figure 3e

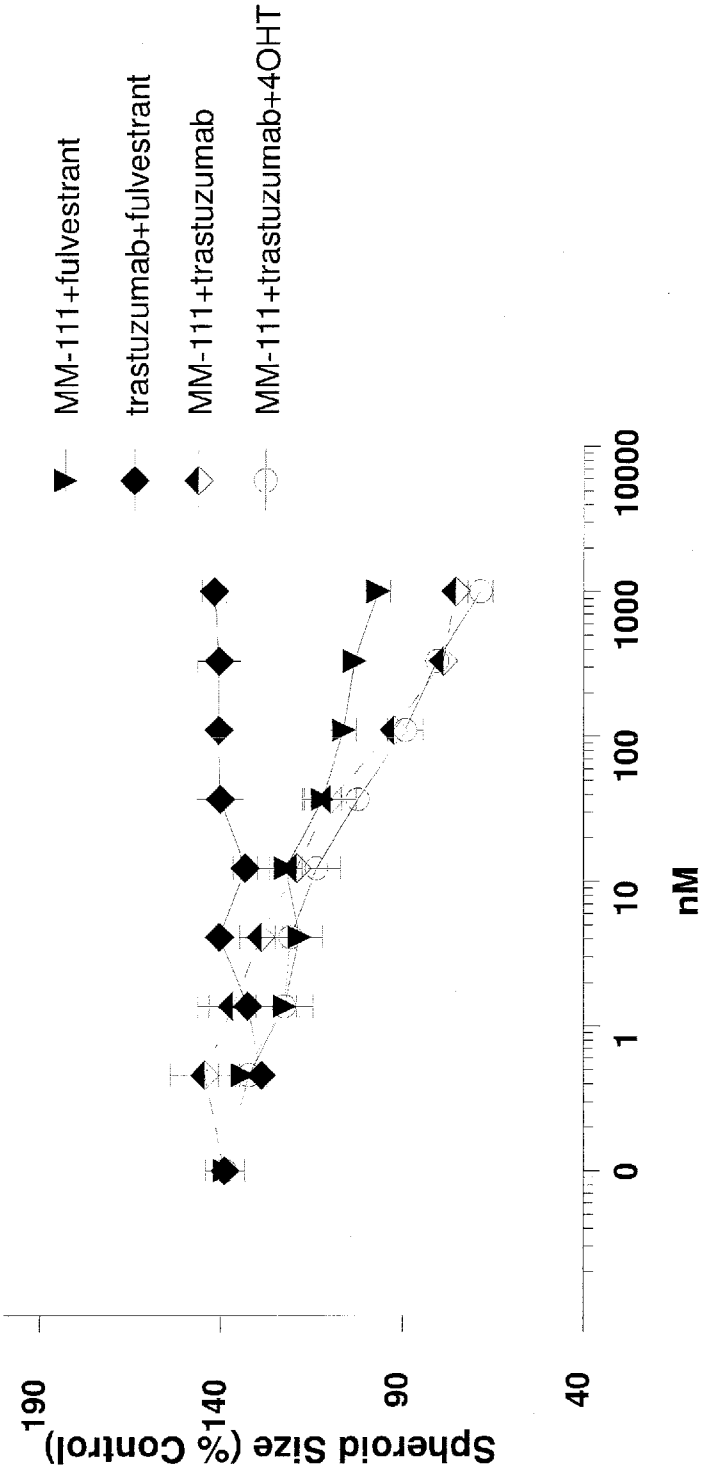
MM-111+fulvestrant
trastuzumab+fulvestrant
MM-111+trastuzumab
MM-111+trastuzumab+4OHT
Spheroid Size (% Control)
190
140
90
40
0
1
10
100
1000
10000
nM

Figure 3f

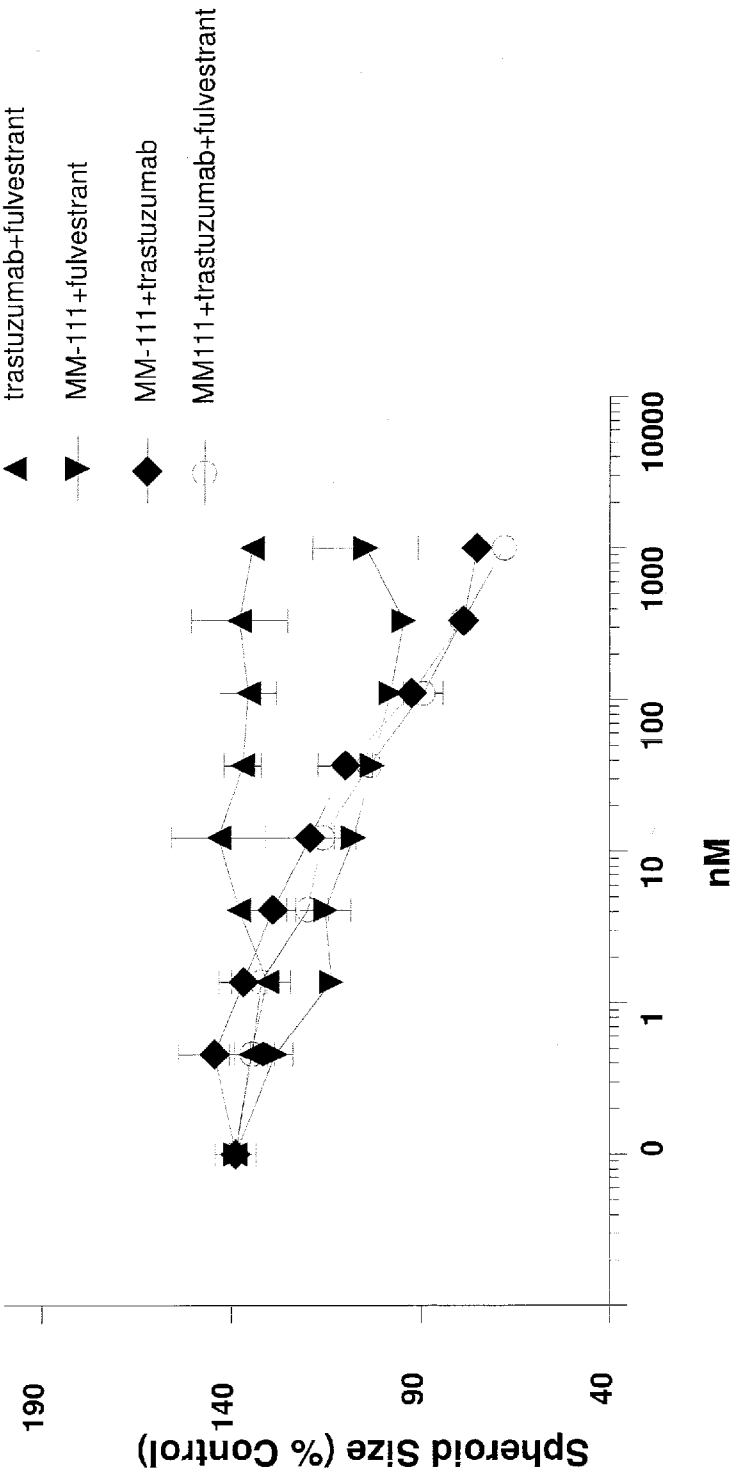
trastuzumab+fulvestrant
MM-111+fulvestrant
MM-111+trastuzumab
MM111+trastuzumab+fulvestrant
Spheroid Size (% Control)
190
140
90
40
0
1
10
100
1000
10000
nM

Figure 3g

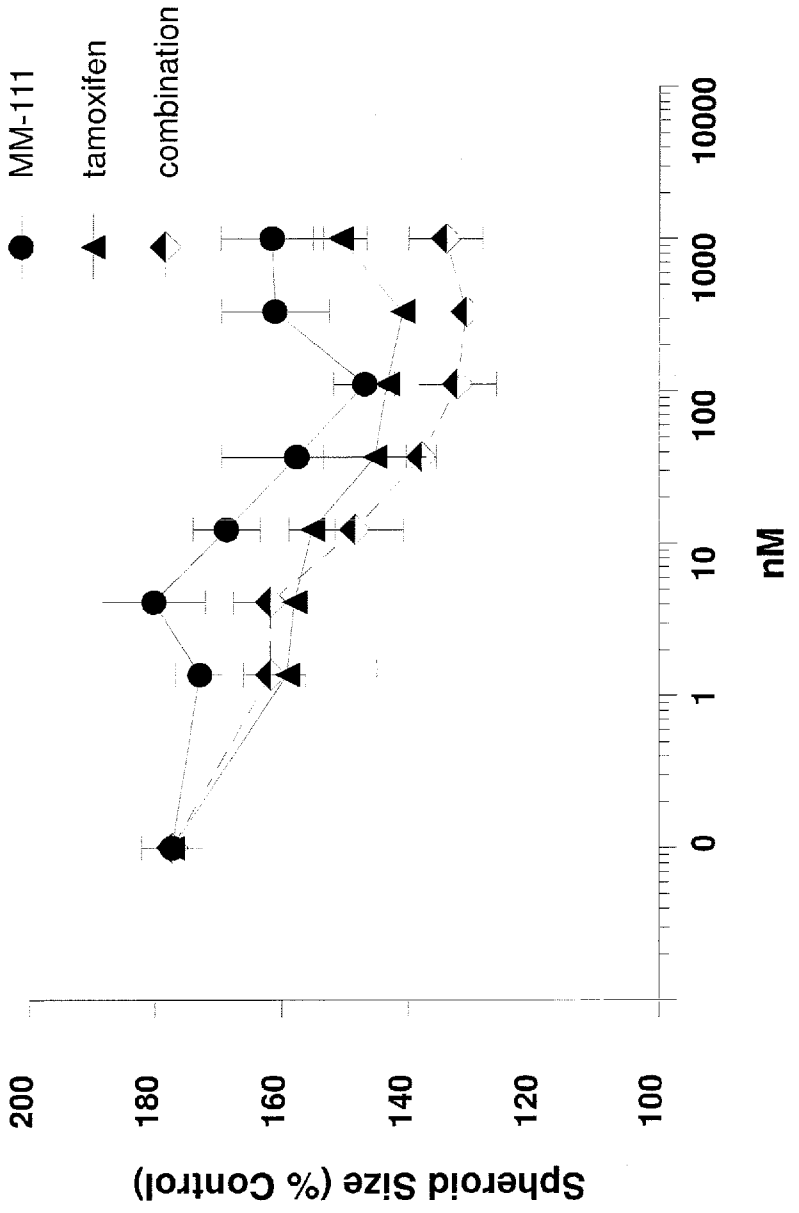
MM-111
tamoxifen
combination
Spheroid Size (% Control)
200
180
160
140
120
100
0
1
10
100
1000
10000
nM

Figure 4a

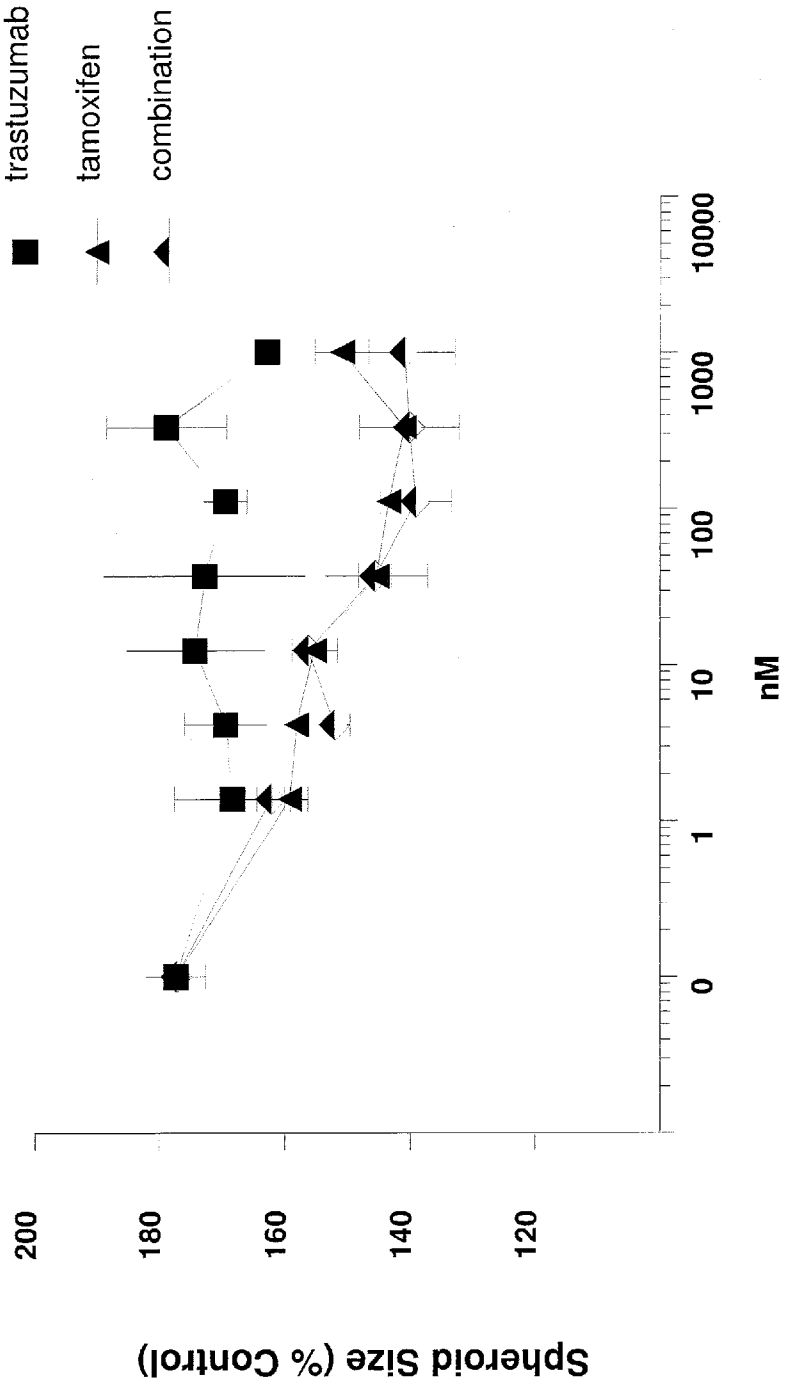
trastuzumab
tamoxifen
combination
Spheroid Size (% Control)
200
180
160
140
120
0
1
10
100
1000
10000
nM

Figure 4b

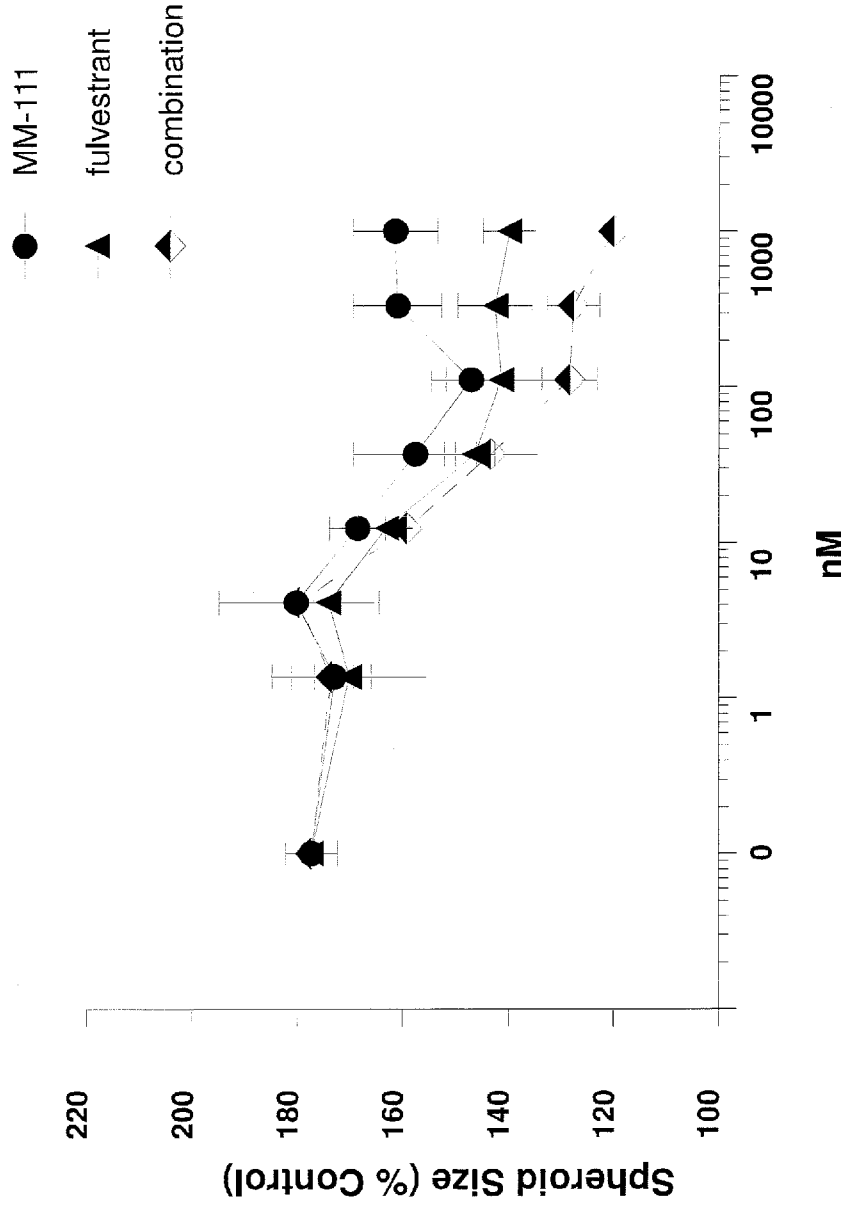
MM-111
fulvestrant
combination
Spheroid Size (% Control)
220
200
180
160
140
120
100
0
1
10
100
1000
10000
nM

Figure 4c

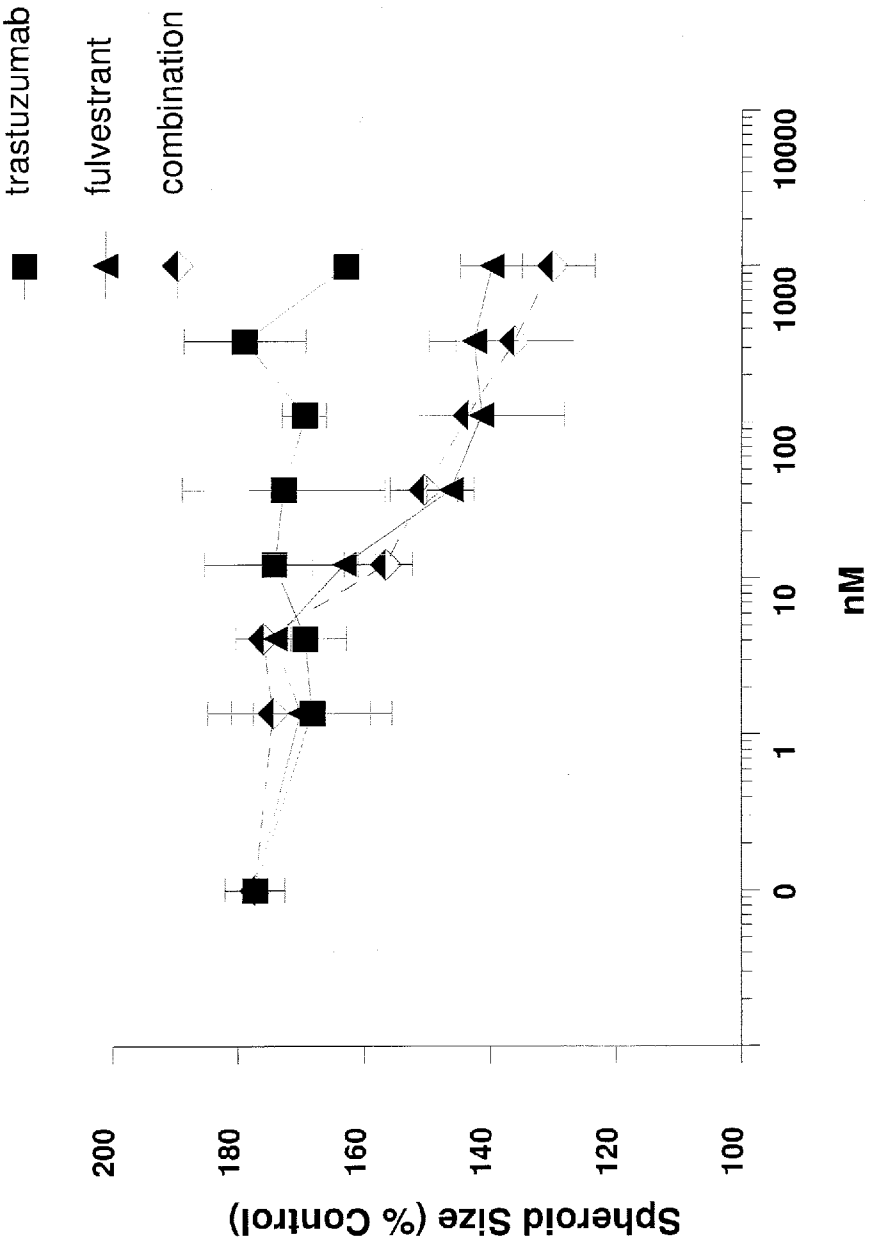
trastuzumab
fulvestrant
combination
Spheroid Size (% Control)
200
180
160
140
120
100
0
1
10
100
1000
10000
nM

Figure 4d

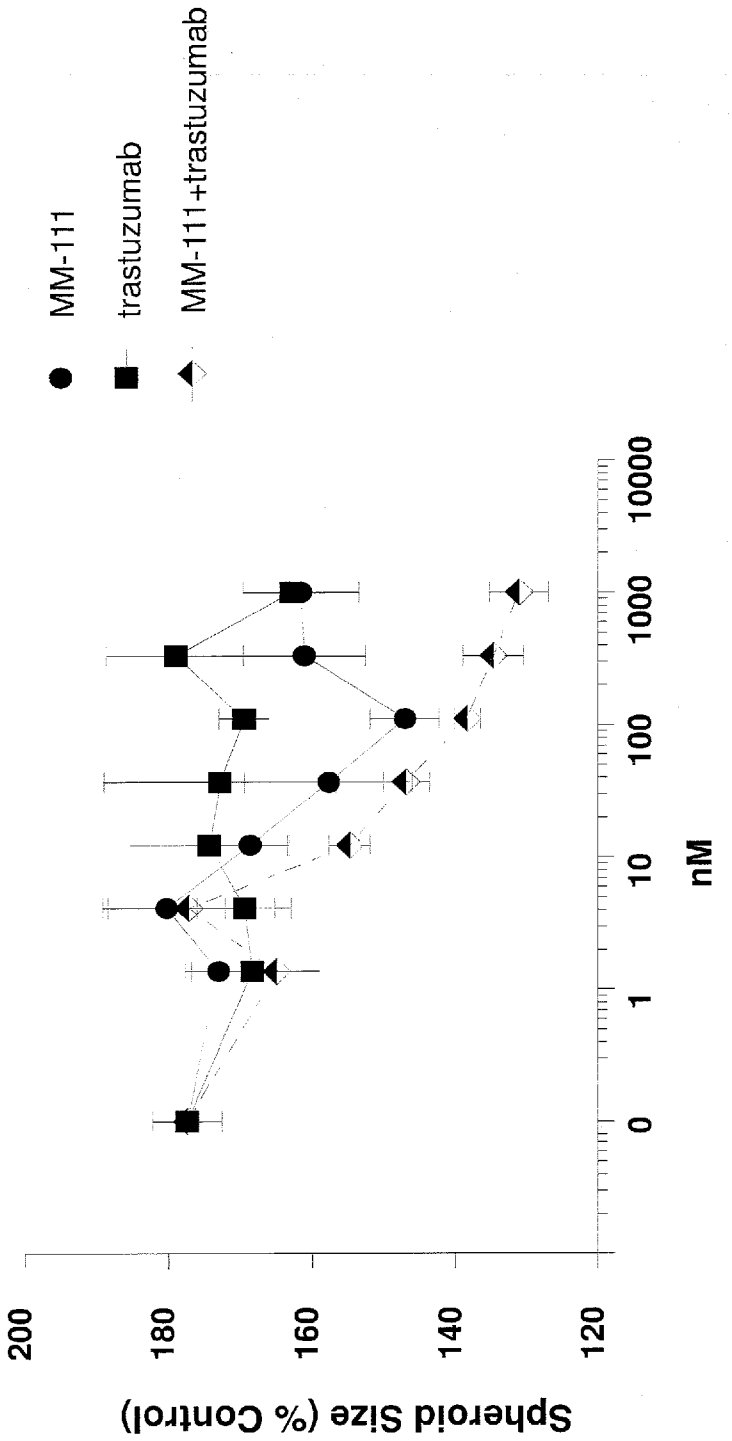
MM-111
trastuzumab
MM-111+trastuzumab
Spheroid Size (% Control)
200
180
160
140
120
0
1
10
100
1000
10000
nM

Figure 4e

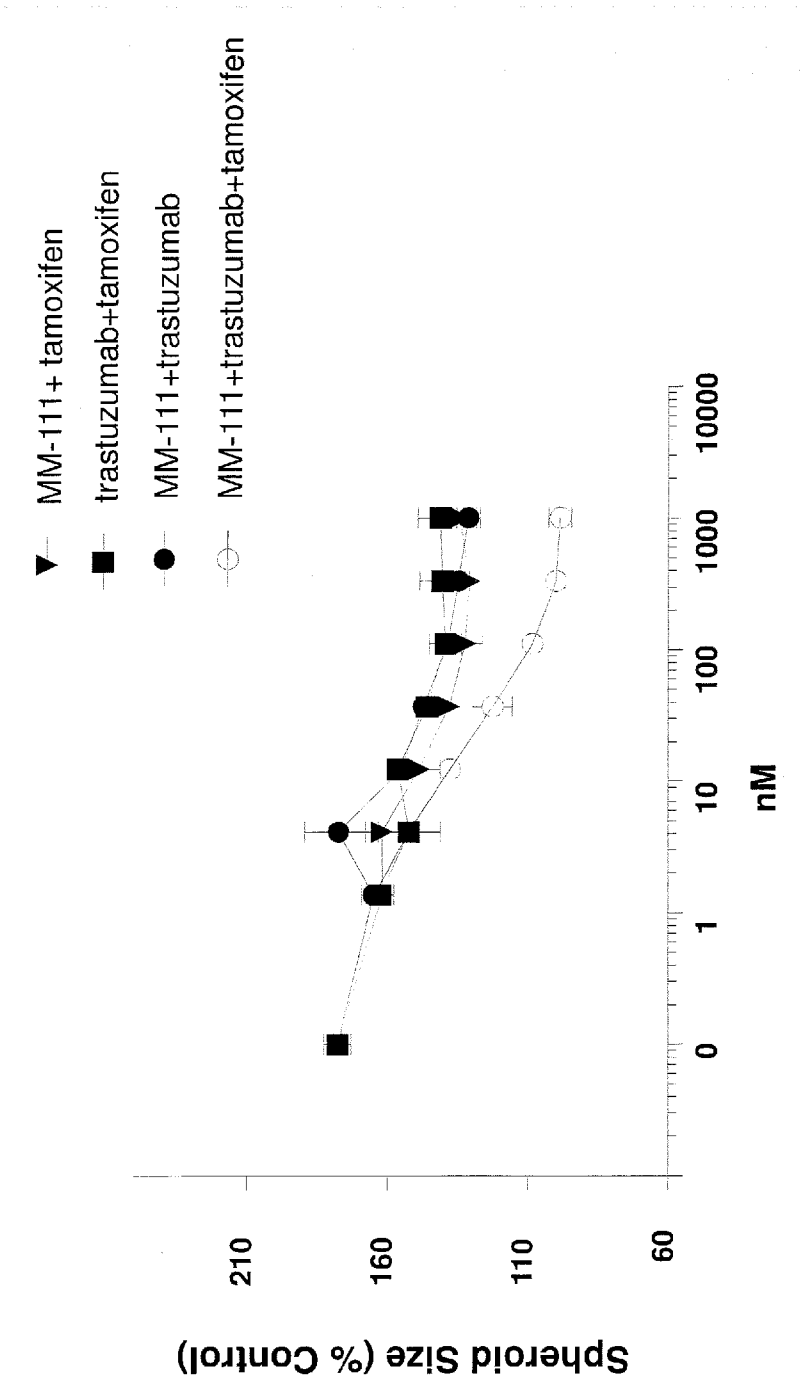
MM-111+ tamoxifen
trastuzumab+tamoxifen
MM-111+trastuzumab
MM-111+trastuzumab+tamoxifen
Spheroid Size (% Control)
210
160
110
60
0
1
10
100
1000
10000
nM

Figure 4f

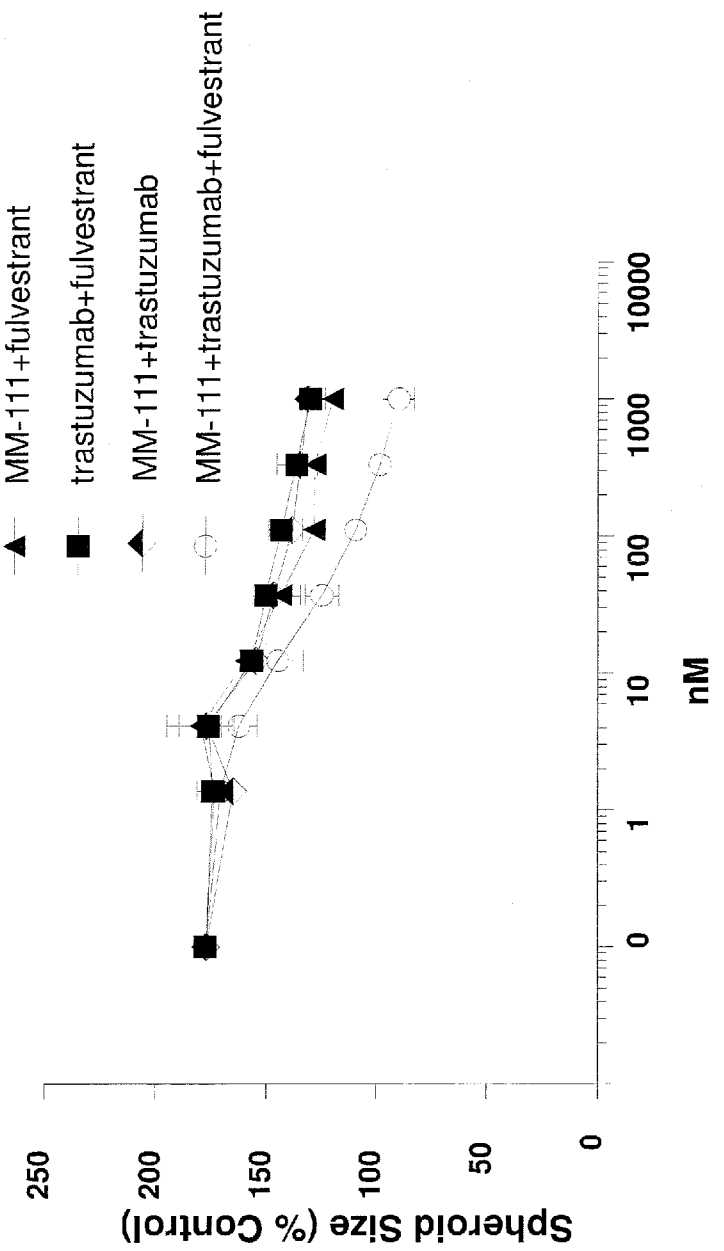
MM-111+fulvestrant
trastuzumab+fulvestrant
MM-111+trastuzumab
MM-111+trastuzumab+fulvestrant
Spheroid Size (% Control)
250
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 4g

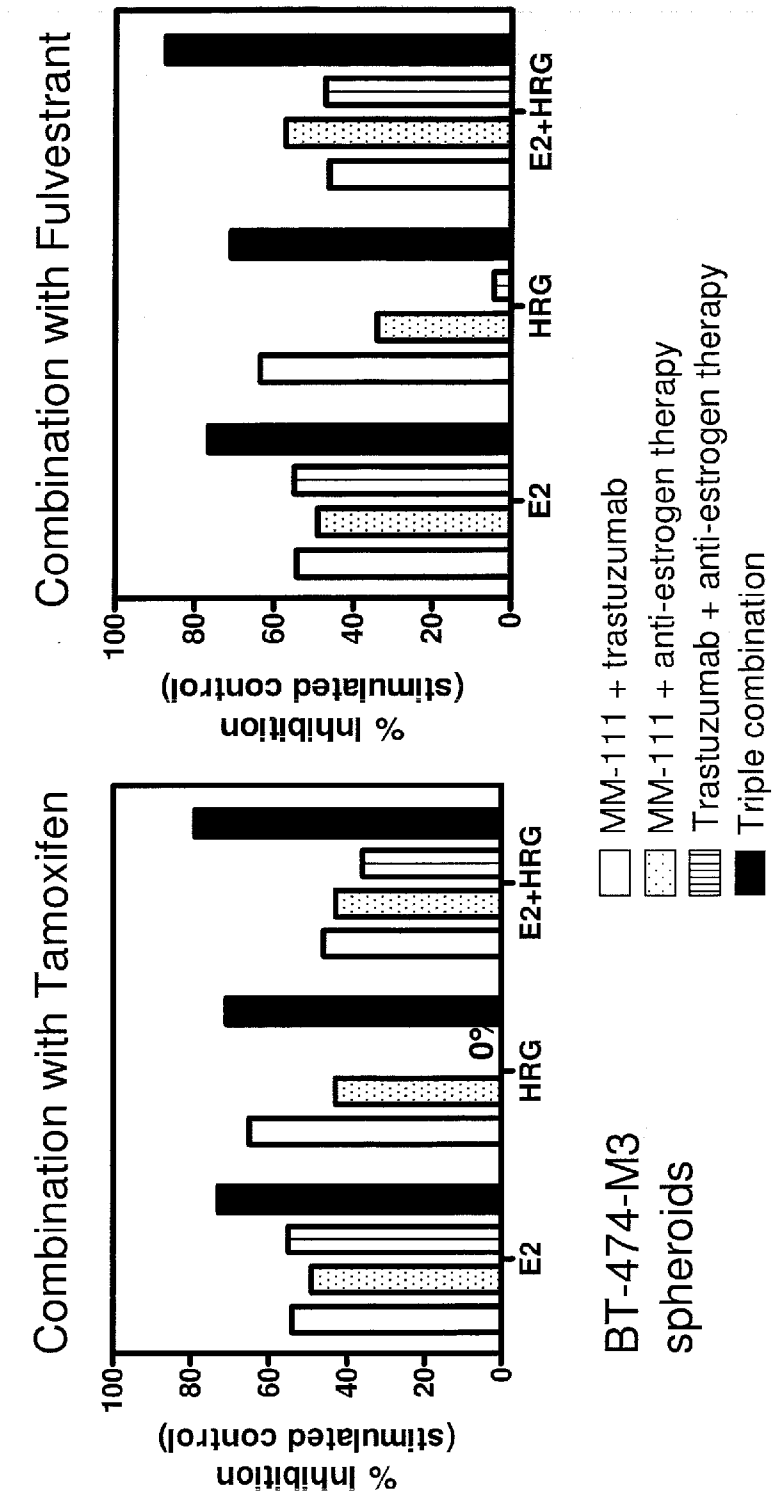
Combination with Tamoxifen
% Inhibition (stimulated control)
100
80
60
40
20
0
E2
HRG
0%
E2+HRG
Combination with Fulvestrant
% Inhibition (stimulated control)
100
80
60
40
20
0
E2
HRG
E2+HRG
BT-474-M3 spheroids
MM-111 + trastuzumab
MM-111 + anti-estrogen therapy
Trastuzumab + anti-estrogen therapy
Triple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

Figure 5

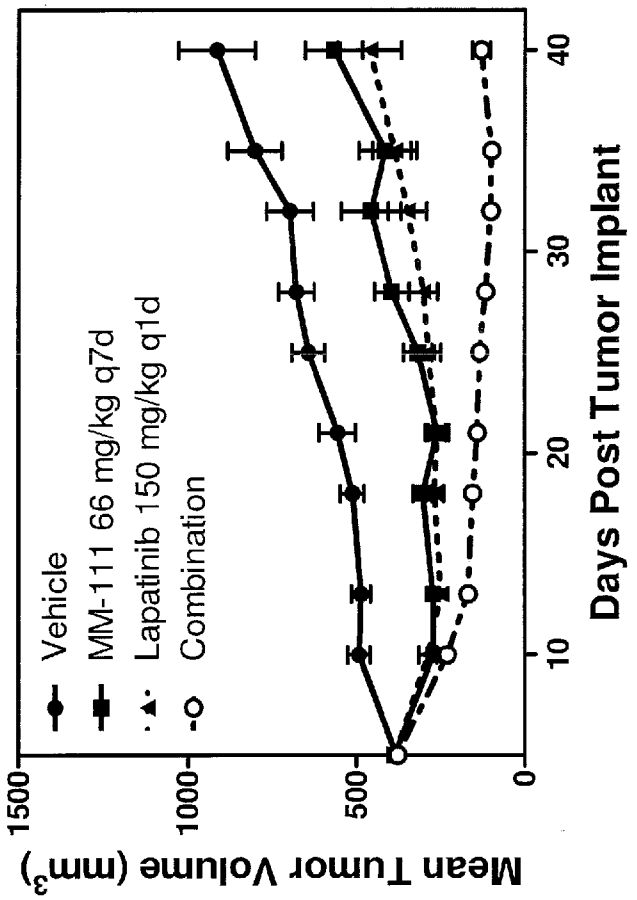
Mean Tumor Volume (mm³)
1500
1000
500
0
10
20
30
40
Days Post Tumor Implant
Vehicle
MM-111 66 mg/kg q7d
Lapatinib 150 mg/kg q1d
Combination

Figure 6

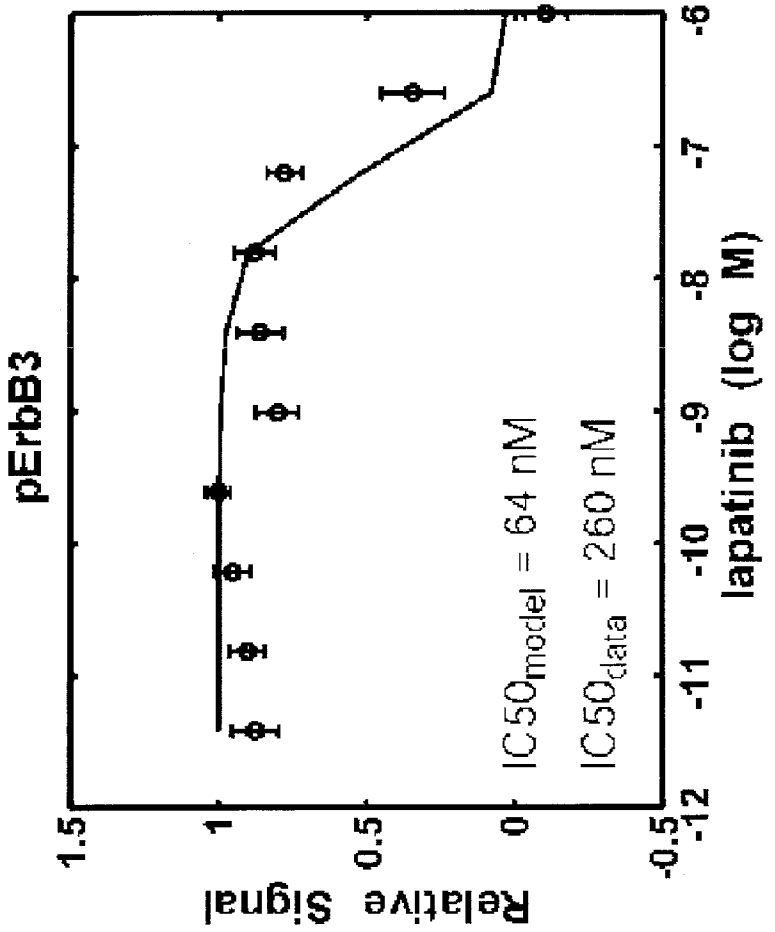
pErbB3
Relative Signal
1.5
1
0.5
0
-0.5
-12
-11
-10
-9
-8
-7
-6
lapatinib (log M)
IC50model = 64 nM
IC50data = 260 nM

Figure 7a

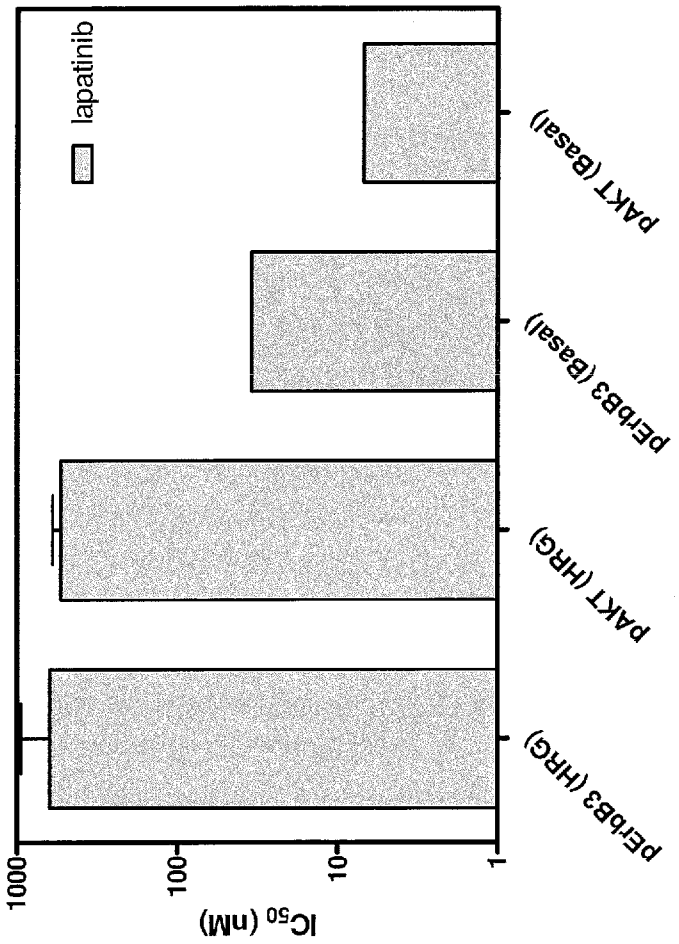
lapatinib
IC50 (nM)
1000
100
10
1
pErbB3 (HRG)
pAKT (HRG)
pErbB3 (Basal)
pAKT (Basal)

Figure 7b

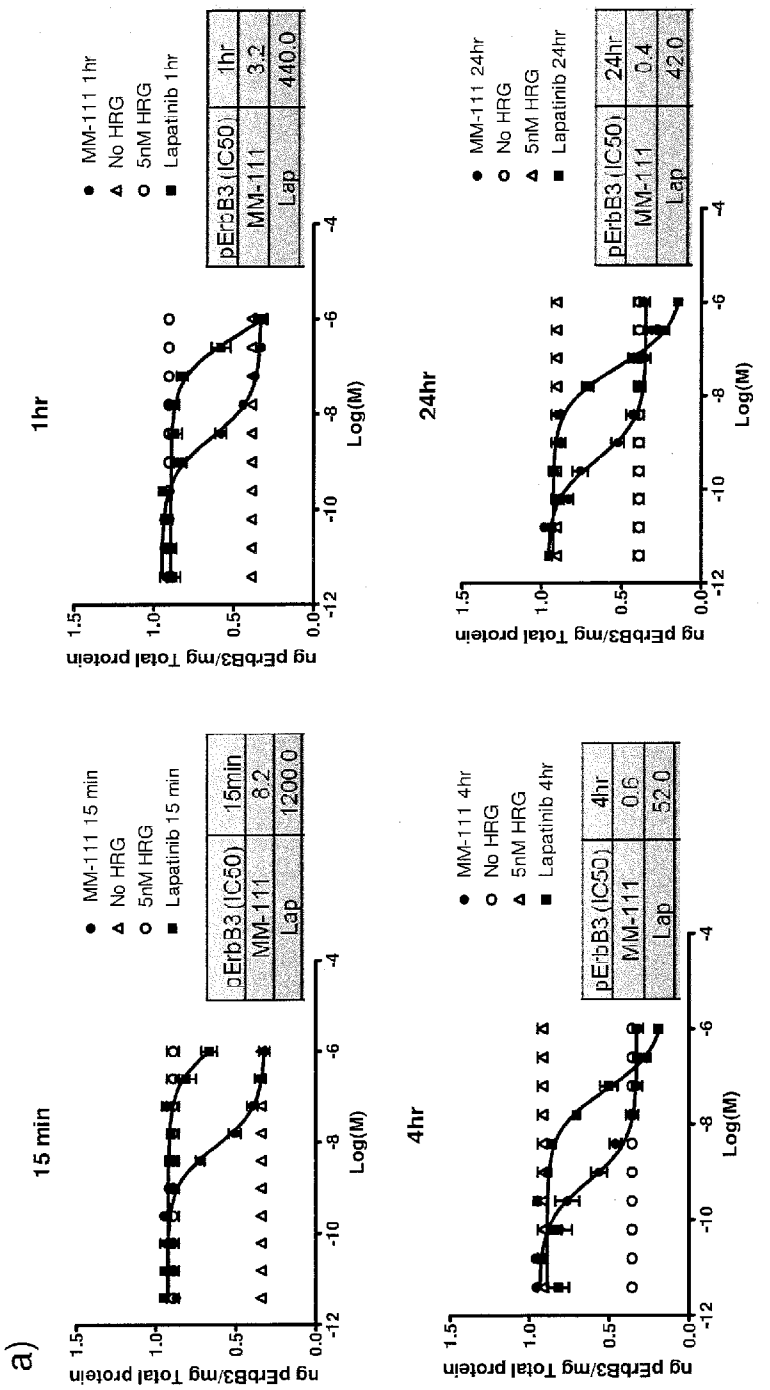
a)
15 min
MM-111 15 min
No HRG
5nM HRG
Lapatinib 15 min
pErbB3 (IC50) 15min
MM-111 8.2
Lap 1200.0
1hr
MM-111 1hr
No HRG
5nM HRG
Lapatinib 1hr
pErbB3 (IC50) 1hr
MM-111 3.2
Lap 440.0
4hr
MM-111 4hr
No HRG
5nM HRG
Lapatinib 4hr
pErbB3 (IC50) 4hr
MM-111 0.6
Lap 52.0
24hr
MM-111 24hr
No HRG
5nM HRG
Lapatinib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

Figure 8a

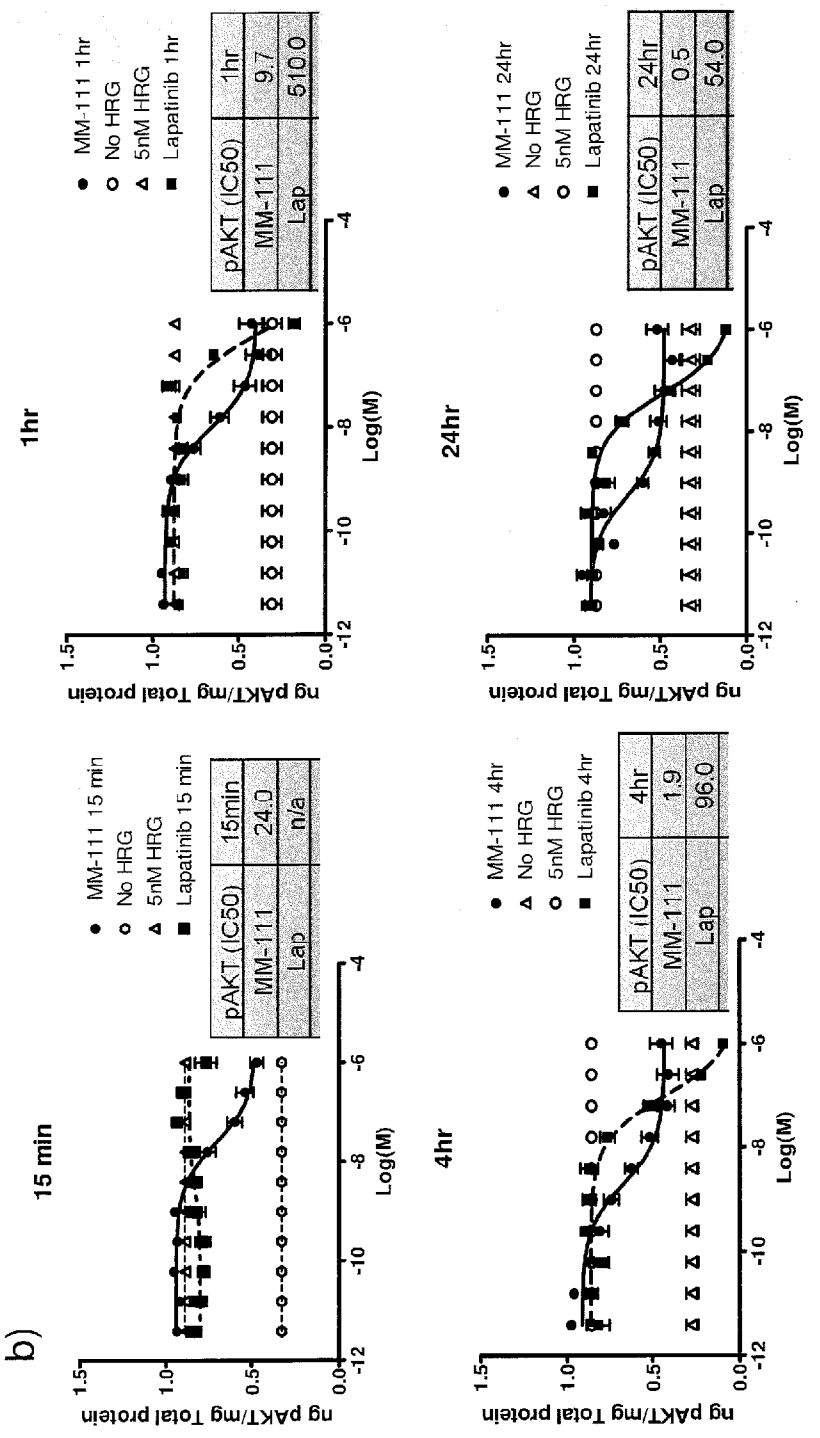
b)
15 min
MM-111 15 min
No HRG
5nM HRG
Lapatinib 15 min
pAKT (IC50) 15min
MM-111 24.0
Lap n/a
1hr
MM-111 1hr
No HRG
5nM HRG
Lapatinib 1hr
pAKT (IC50) 1hr
MM-111 9.7
Lap 510.0
4hr
MM-111 4hr
No HRG
5nM HRG
Lapatinib 4hr
pAKT (IC50) 4hr
MM-111 1.9
Lap 96.0
24hr
MM-111 24hr
No HRG
5nM HRG
Lapatinib 24hr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

Figure 8b

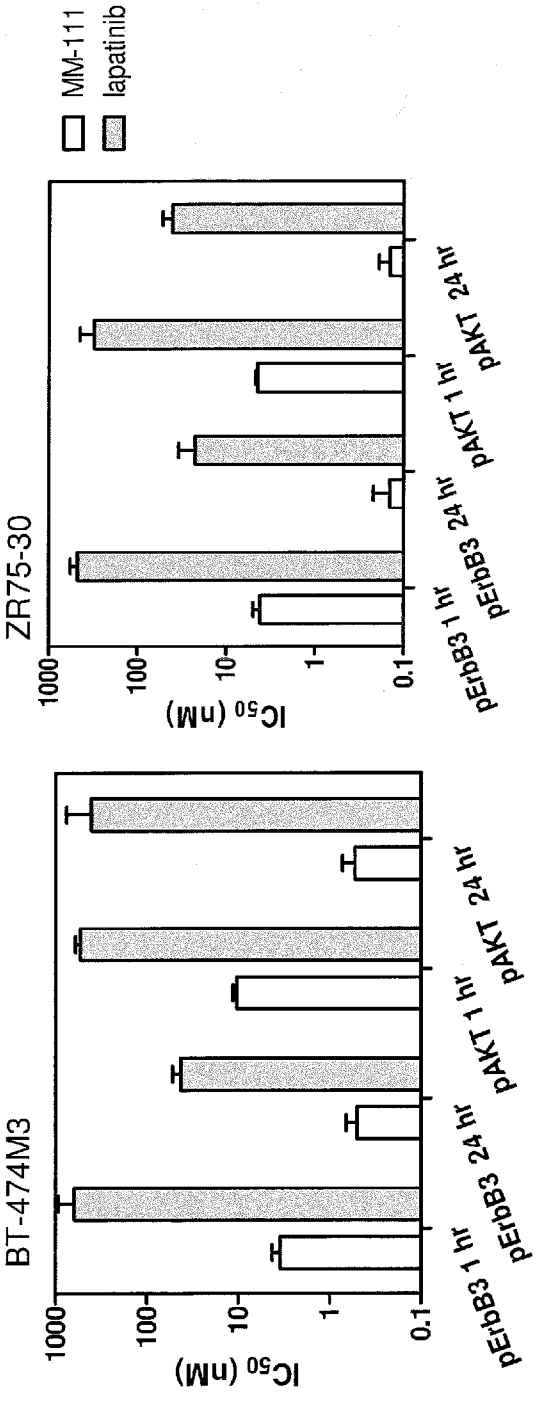
MM-111
lapatinib
ZR75-30
BT-474M3
IC50 (nM)
1000
100
10
1
0.1
pErbB3 1 hr
pErbB3 24 hr
pAKT 1 hr
pAKT 24 hr

Figure 8c

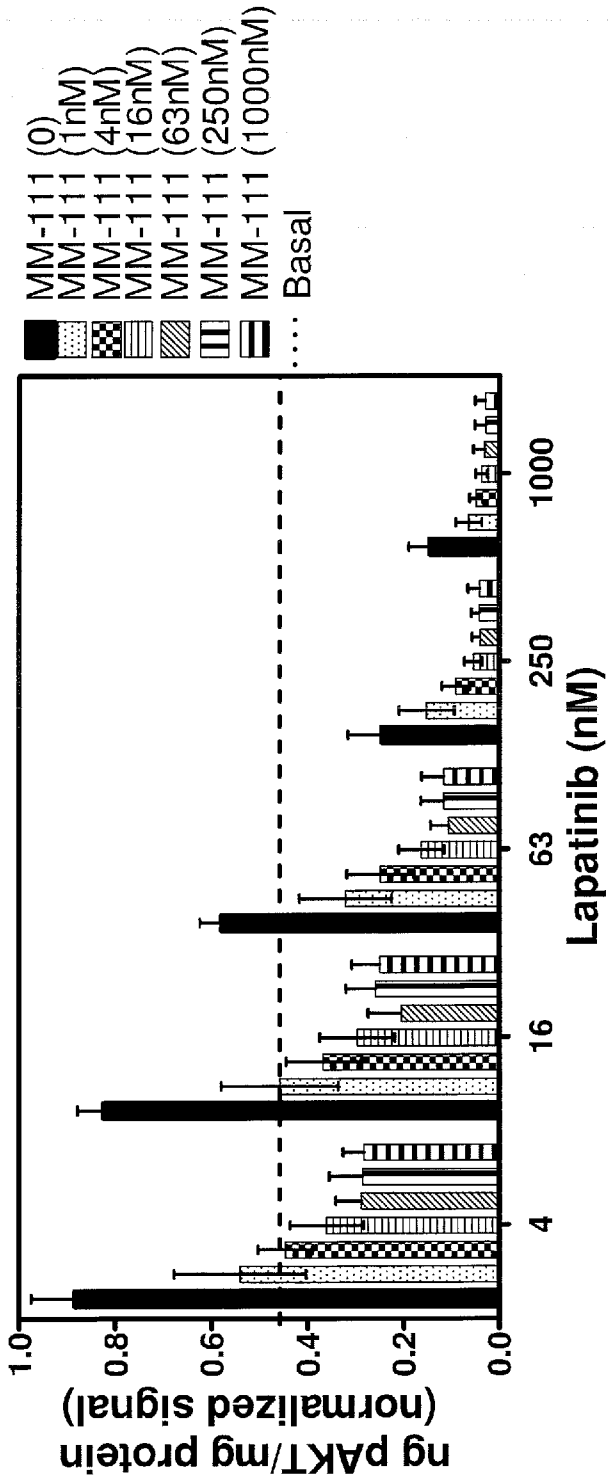
MM-111 (0)
MM-111 (1nM)
MM-111 (4nM)
MM-111 (16nM)
MM-111 (63nM)
MM-111 (250nM)
MM-111 (1000nM)
Basal
ng pAKT/mg protein
(normalized signal)
1.0
0.8
0.6
0.4
0.2
0.0
4
16
63
250
1000
Lapatinib (nM)

Figure 9

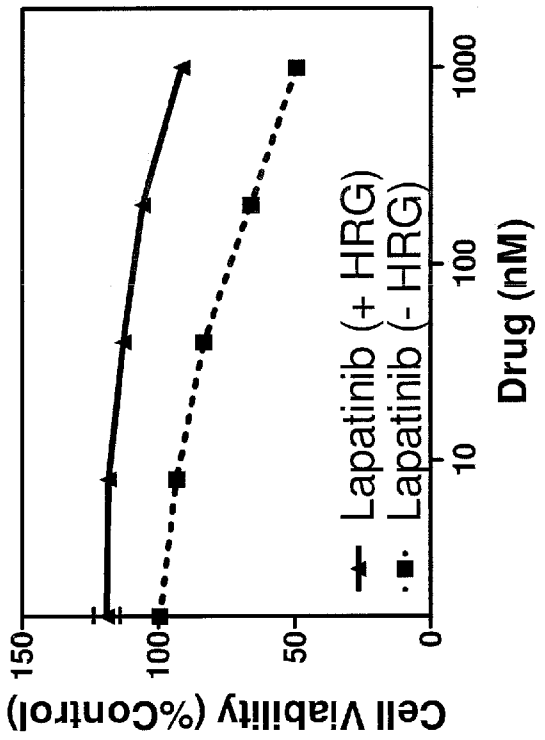
Cell Viability (%Control)
150
100
50
0
Drug (nM)
10
100
1000
Lapatinib (+ HRG)
Lapatinib (- HRG)

Figure 10

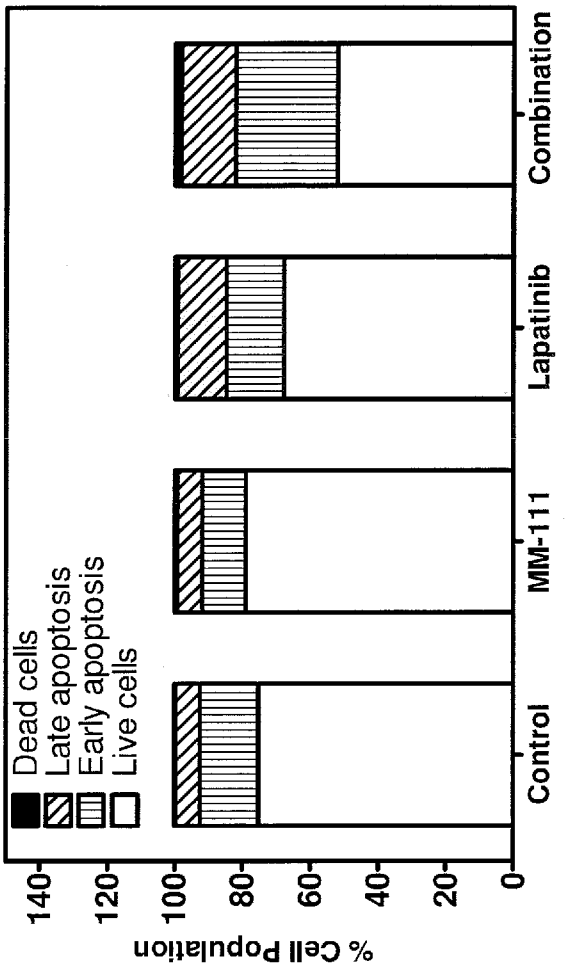
Dead cells
Late apoptosis
Early apoptosis
Live cells
% Cell Population
0
20
40
60
80
100
120
140
Control
MM-111
Lapatinib
Combination

Figure 11

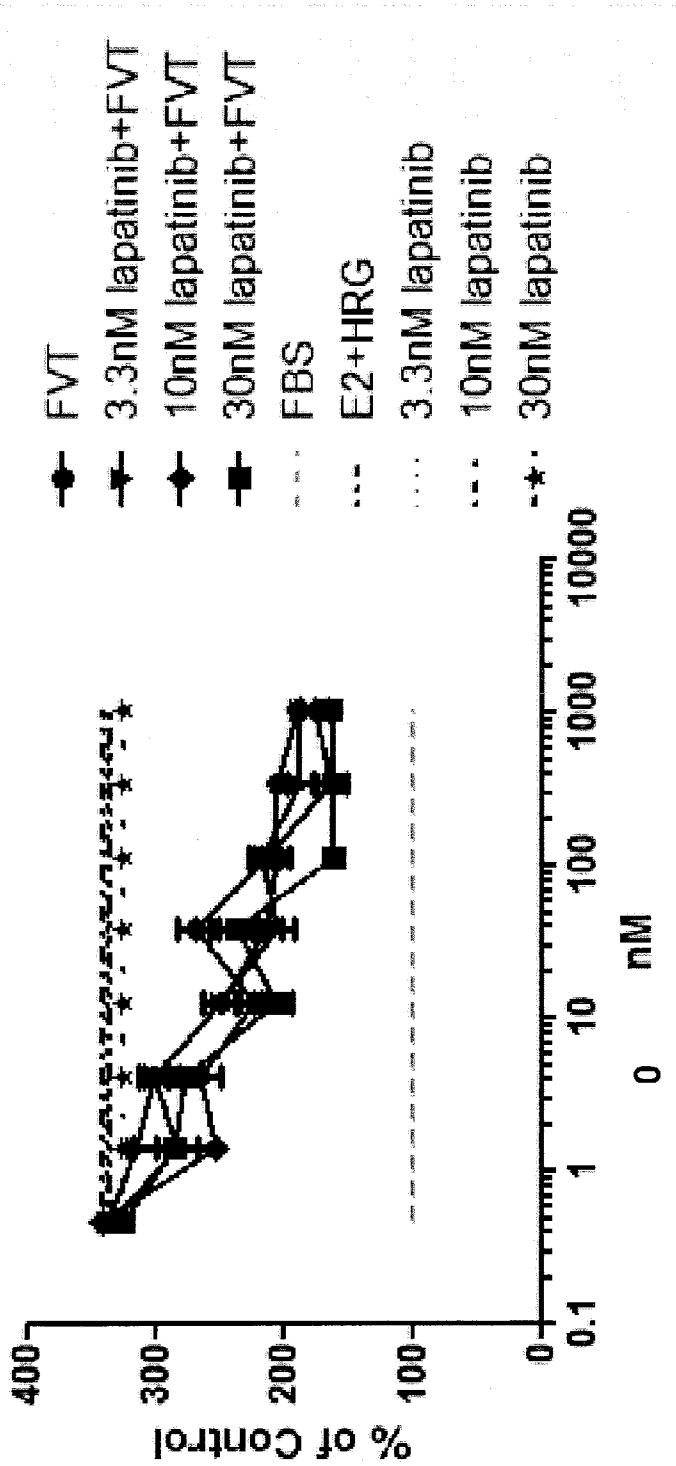
FVT
3.3nM lapatinib+FVT
10nM lapatinib+FVT
30nM lapatinib+FVT
FBS
E2+HRG
3.3nM lapatinib
10nM lapatinib
30nM lapatinib
% of Control
400
300
200
100
0
0.1
1
10
100
1000
10000
nM

Figure 12a

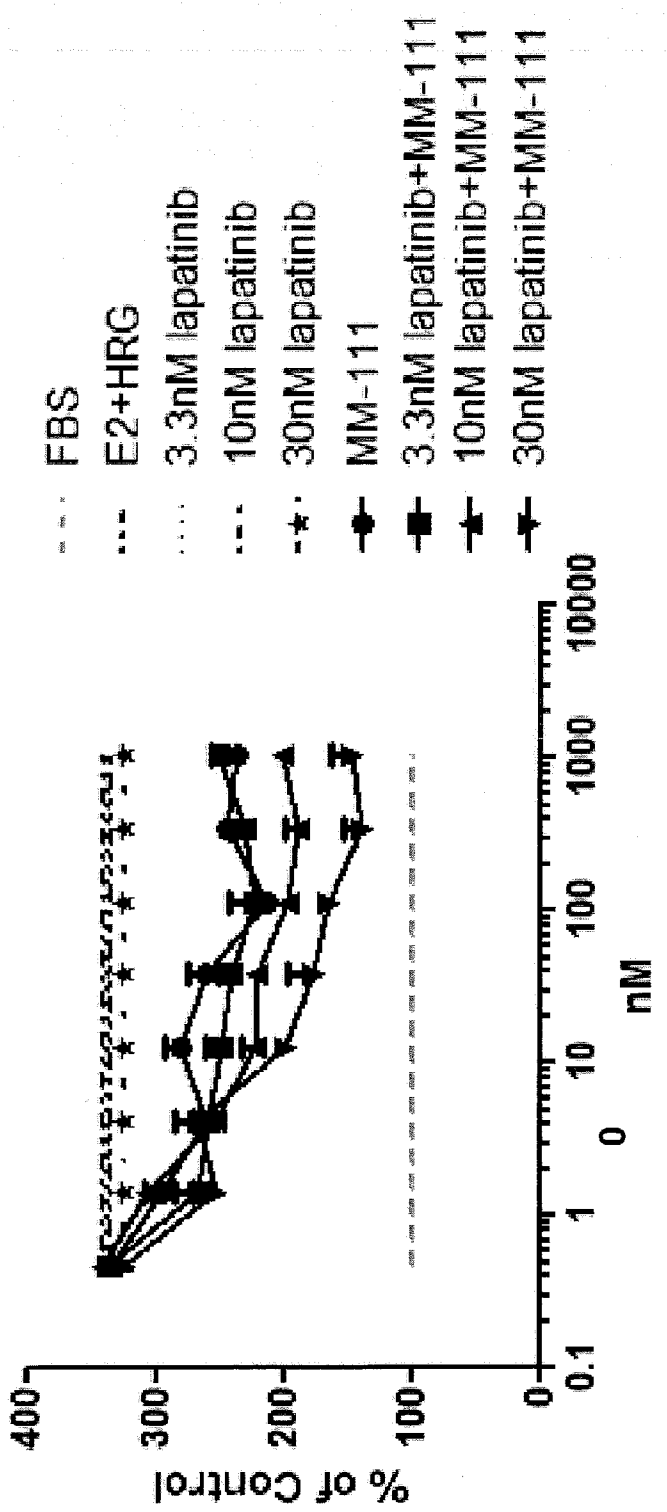
FBS
E2+HRG
3.3nM lapatinib
10nM lapatinib
30nM lapatinib
MM-111
3.3nM lapatinib+MM-111
10nM lapatinib+MM-111
30nM lapatinib+MM-111
% of Control
400
300
200
100
0
0.1
1
0
10
100
1000
10000
nM

Figure 12b

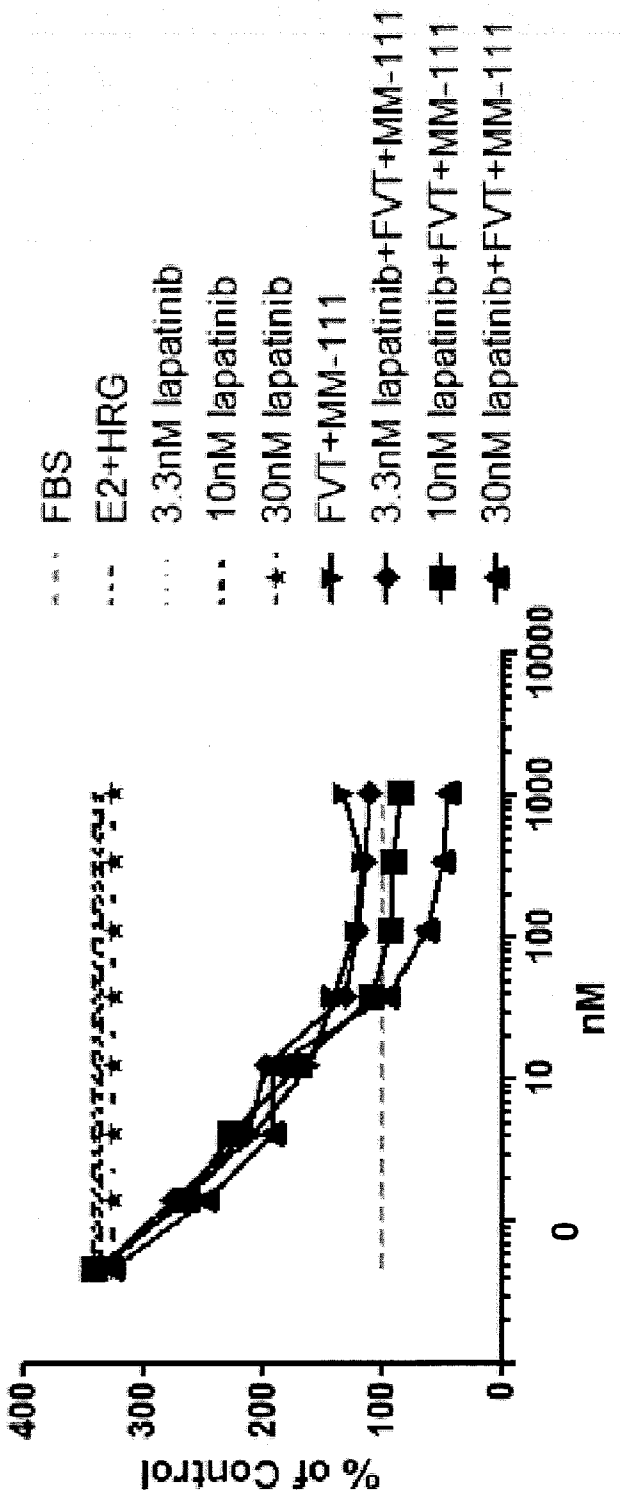
FBS
E2+HRG
3.3nM lapatinib
10nM lapatinib
30nM lapatinib
FVT+MM-111
3.3nM lapatinib+FVT+MM-111
10nM lapatinib+FVT+MM-111
30nM lapatinib+FVT+MM-111
% of Control
400
300
200
100
0
0
10
100
1000
10000
nM

Figure 12c

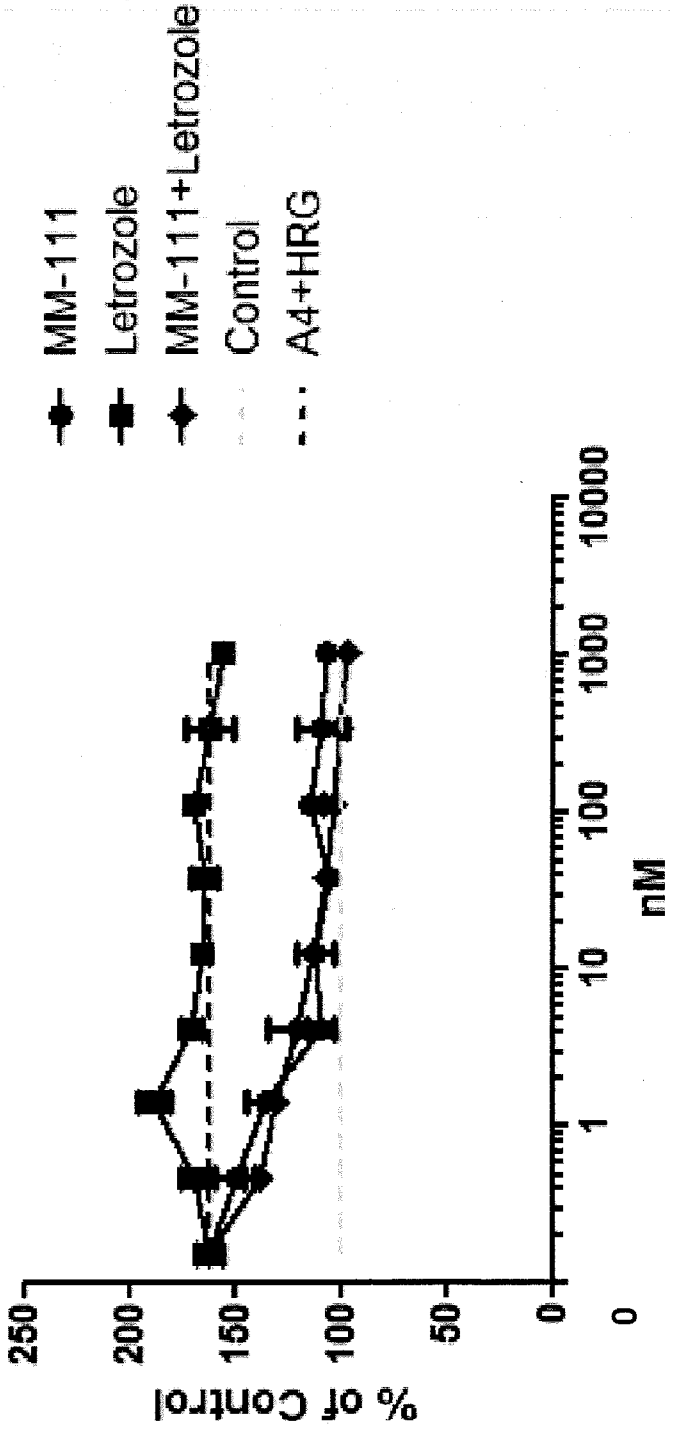
MM-111
Letrozole
MM-111+Letrozole
Control
A4+HRG
% of Control
250
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 13a

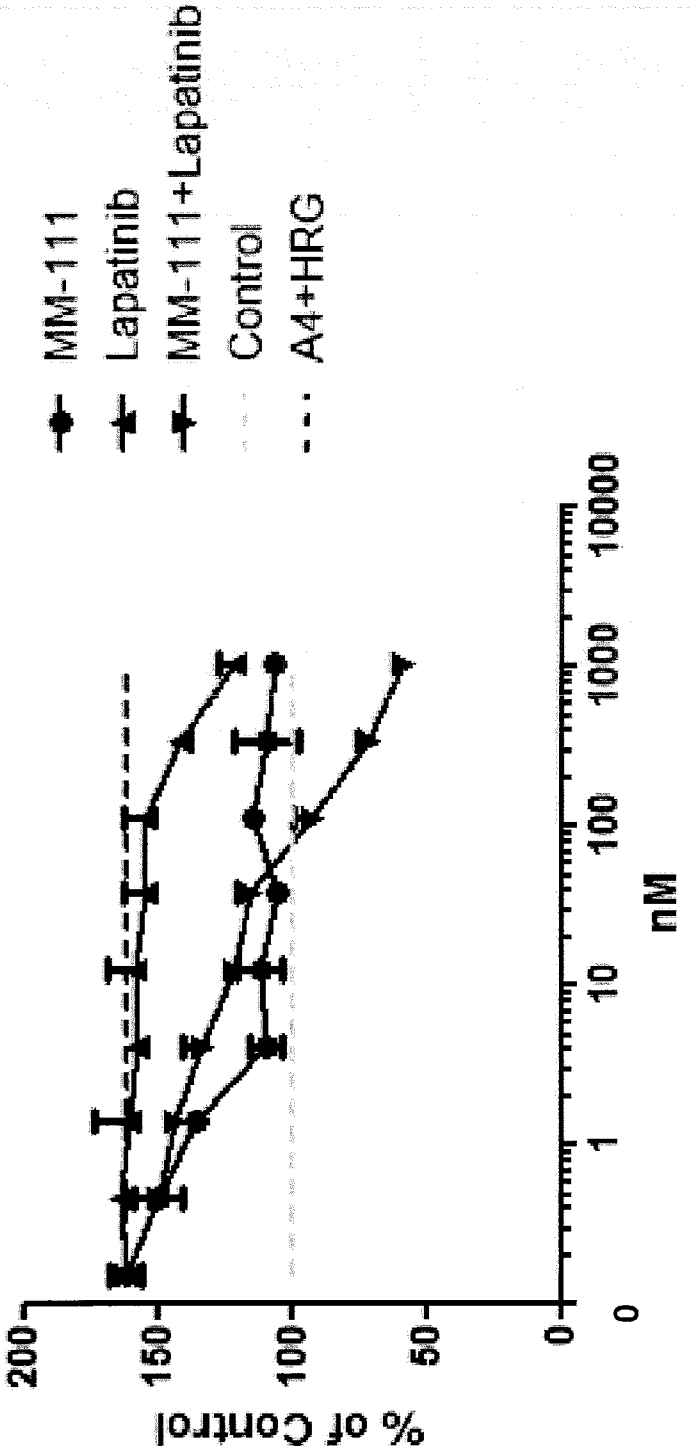
MM-111
Lapatinib
MM-111+Lapatinib
Control
A4+HRG
% of Control
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 13b

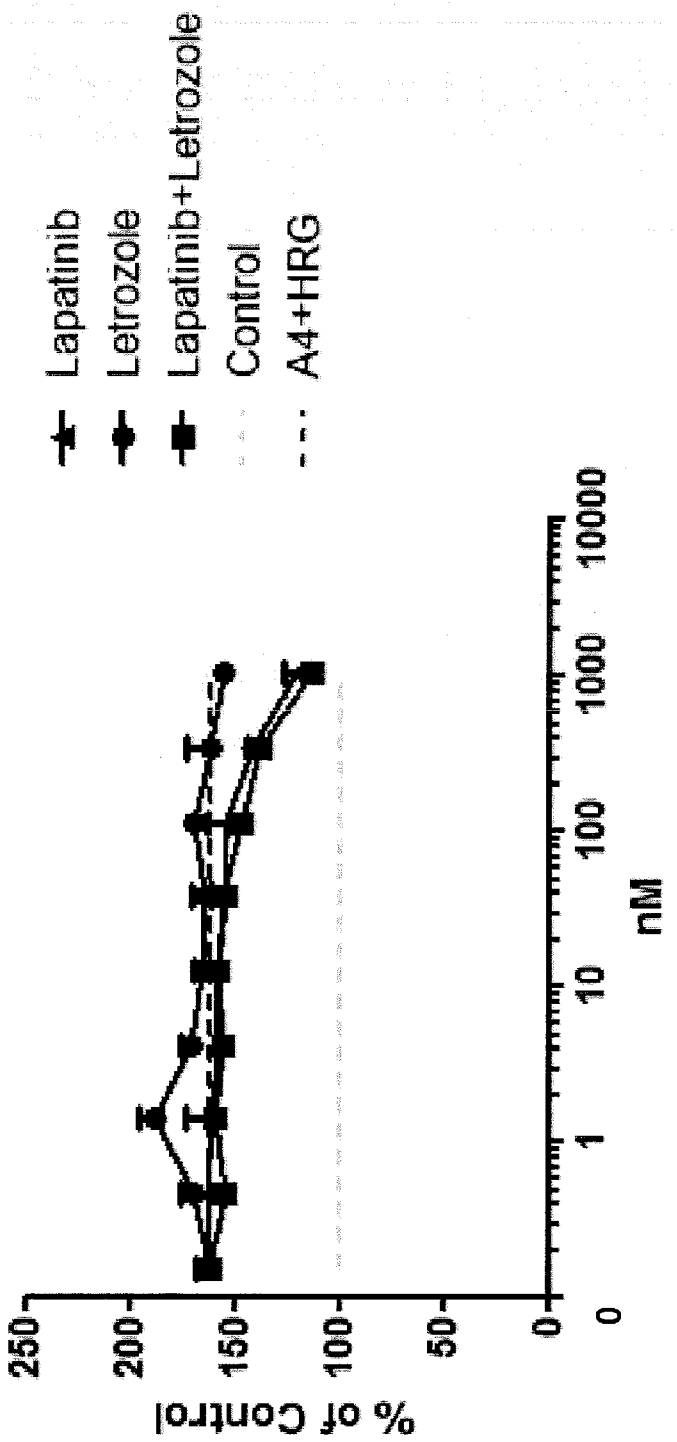
Lapatinib
Letrozole
Lapatinib+Letrozole
Control
A4+HRG
% of Control
250
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 13c

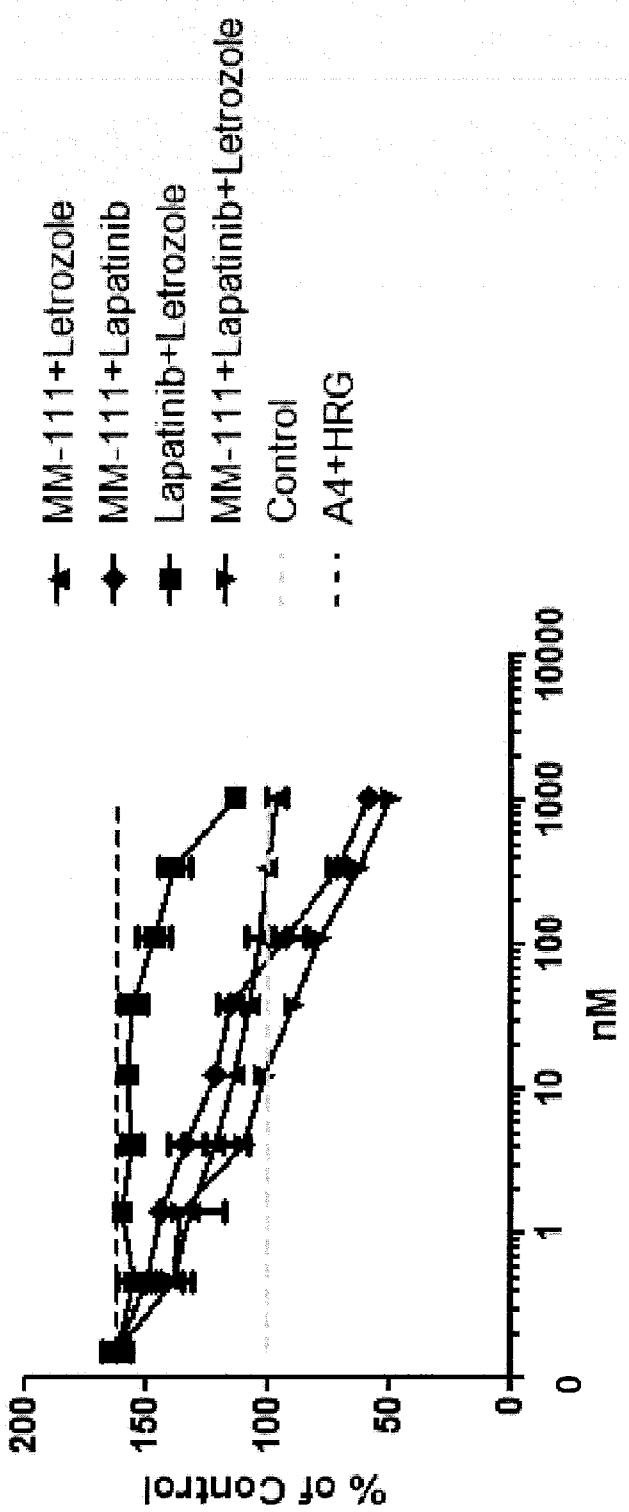
MM-111+Letrozole
MM-111+Lapatinib
Lapatinib+Letrozole
MM-111+Lapatinib+Letrozole
Control
A4+HRG
% of Control
200
150
100
50
0
0
1
10
100
1000
10000
nM

Figure 13d

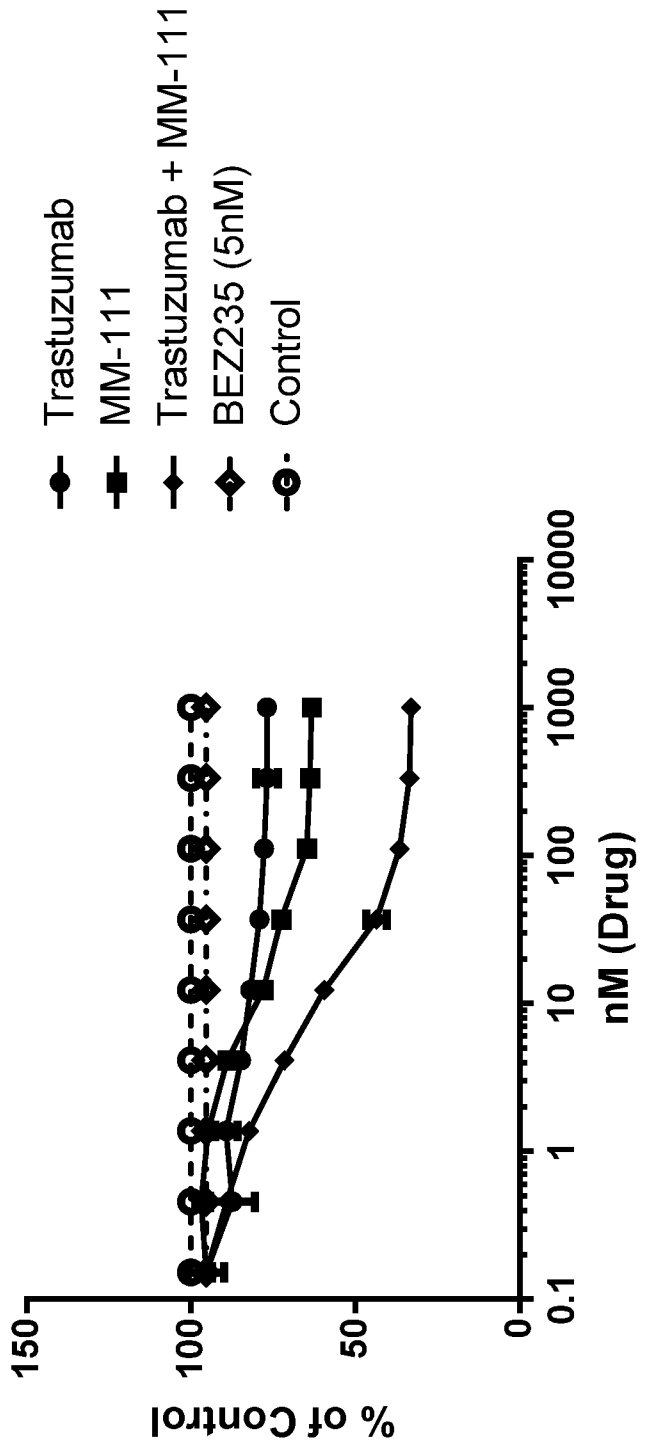
NCI-N87 2D
Trastuzumab
MM-111
Trastuzumab + MM-111
BEZ235 (5nM)
Control
% of Control
150
100
50
0
0.1
1
10
100
1000
10000
nM (Drug)

Figure 14a

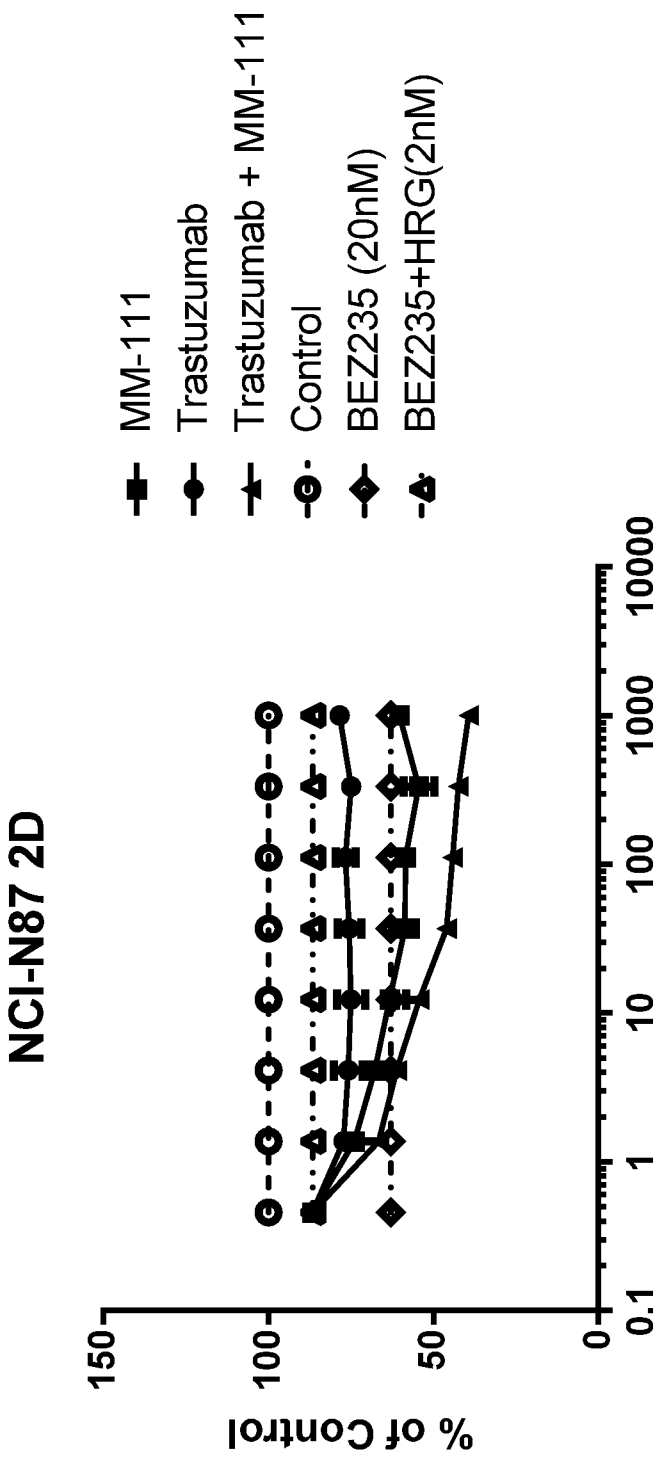
NCI-N87 2D
MM-111
Trastuzumab
Trastuzumab + MM-111
Control
BEZ235 (20nM)
BEZ235+HRG(2nM)
% of Control
150
100
50
0
0.1
1
10
100
1000
10000
nM (Drug)

Figure 14b

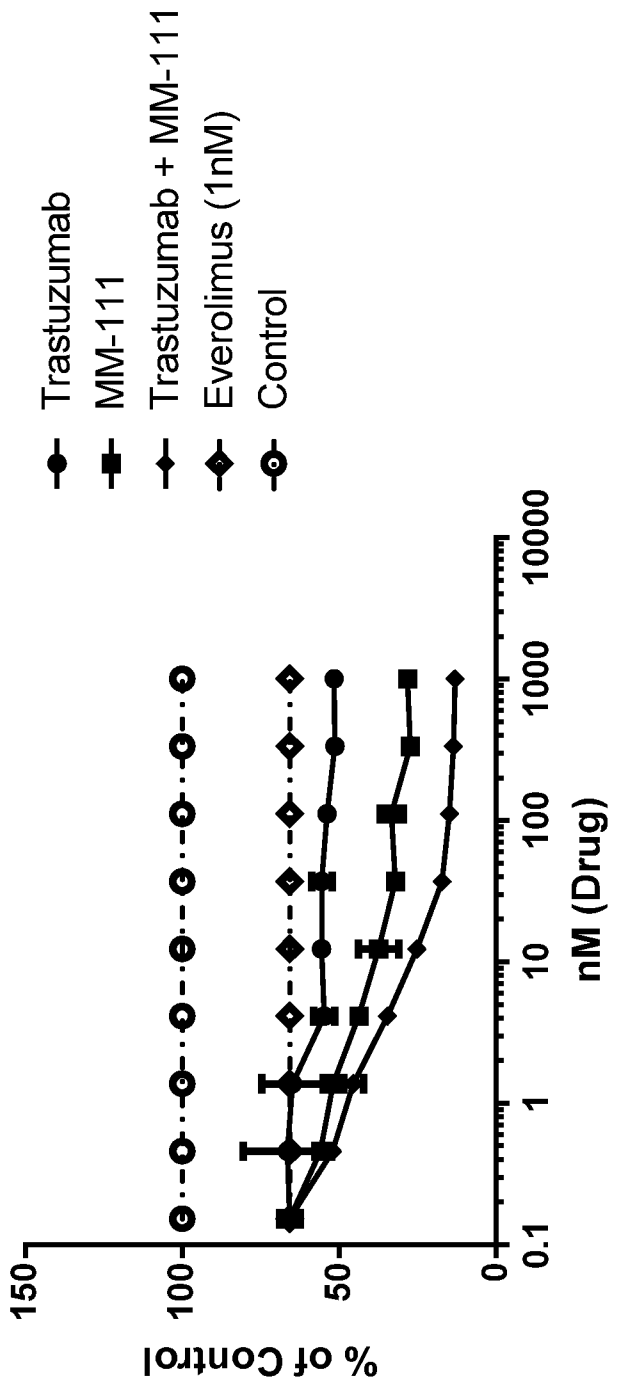
BT474M3 2D
Trastuzumab
MM-111
Trastuzumab + MM-111
Everolimus (1nM)
Control
% of Control
150
100
50
0
0.1
1
10
100
1000
10000
nM (Drug)

Figure 15a

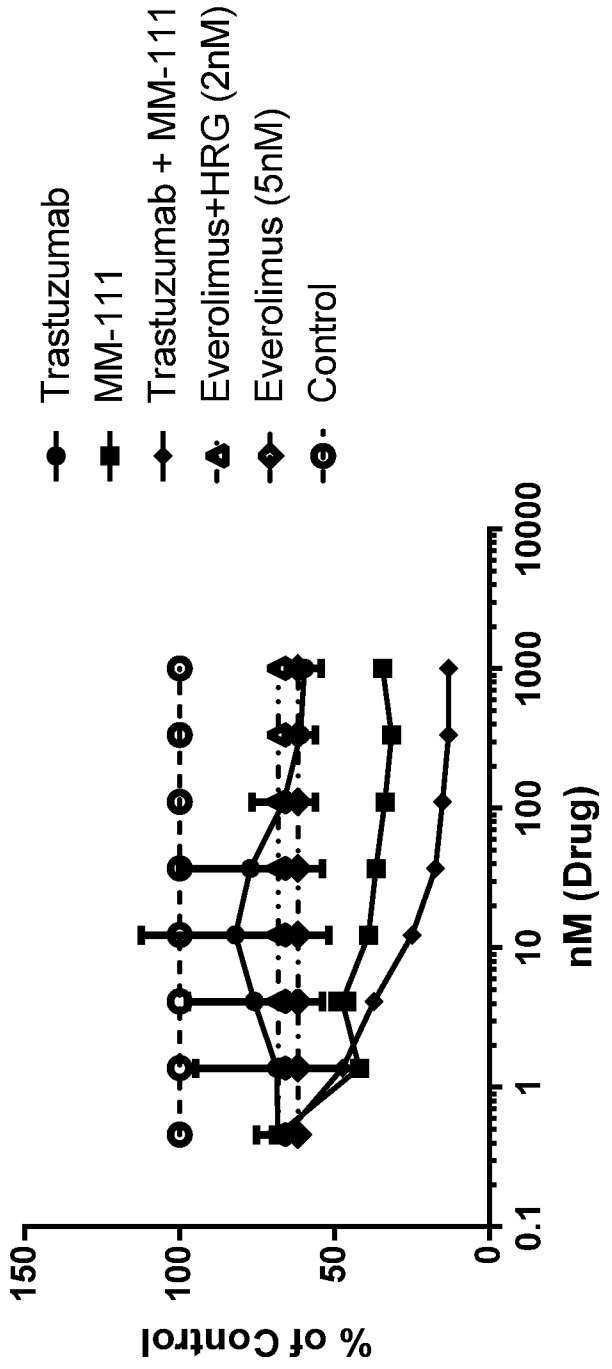
BT474M3 2D
Trastuzumab
MM-111
Trastuzumab + MM-111
Everolimus+HRG (2nM)
Everolimus (5nM)
Control
% of Control
150
100
50
0
0.1
1
10
100
1000
10000
nM (Drug)

Figure 15b

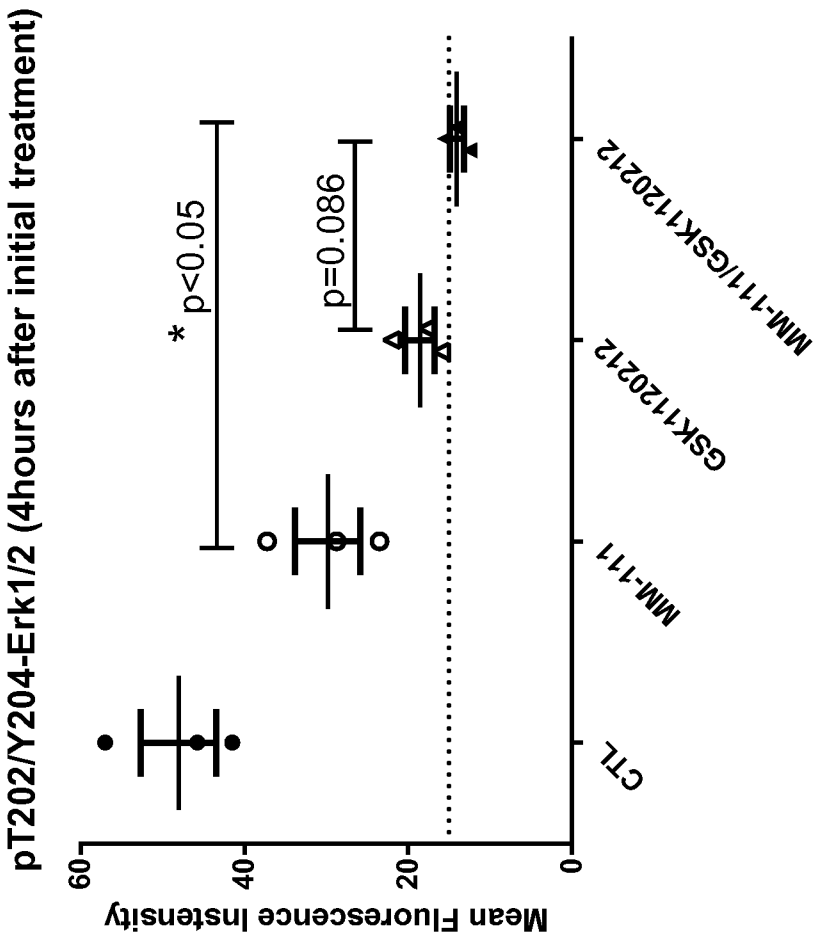
pT202/Y204-Erk1/2 (4hours after initial treatment)
Mean Fluorescence Instensity
60
40
20
0
* p<0.05
p=0.086
CTL
MM-111
GSK1120212
MM-111/GSK1120212

Figure 16a

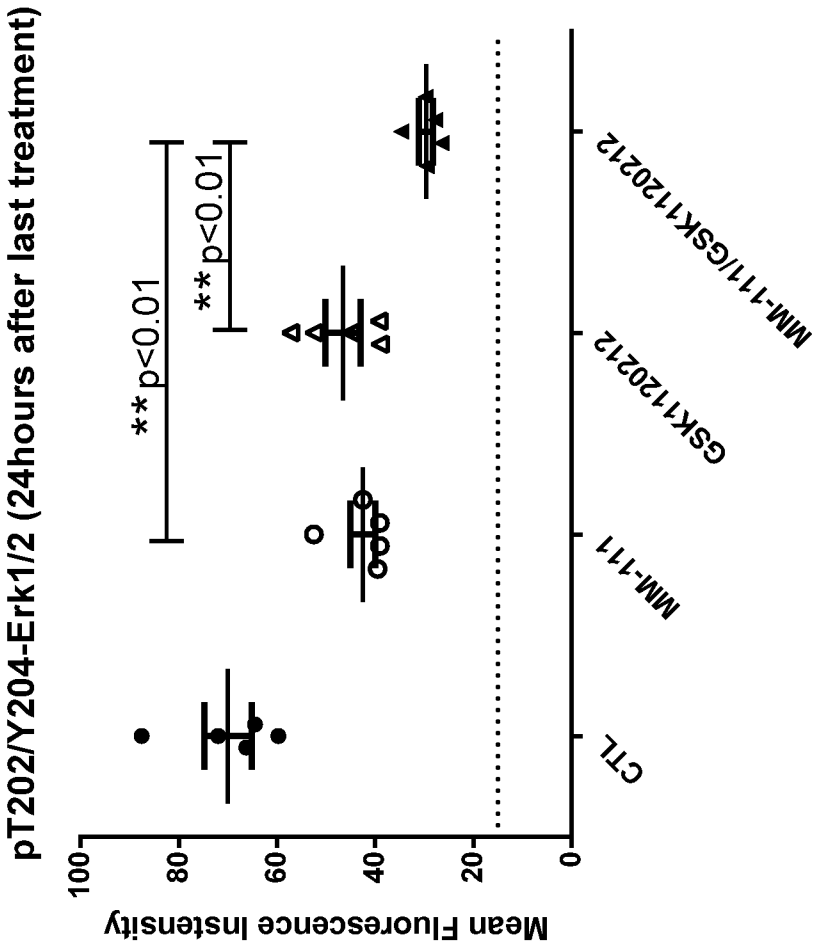
pT202/Y204-Erk1/2 (24hours after last treatment)
Mean Fluorescence Instensity
100
80
60
40
20
0
** p<0.01
** p<0.01
CTL
MM-111
GSK1120212
MM-111/GSK1120212

Figure 16b

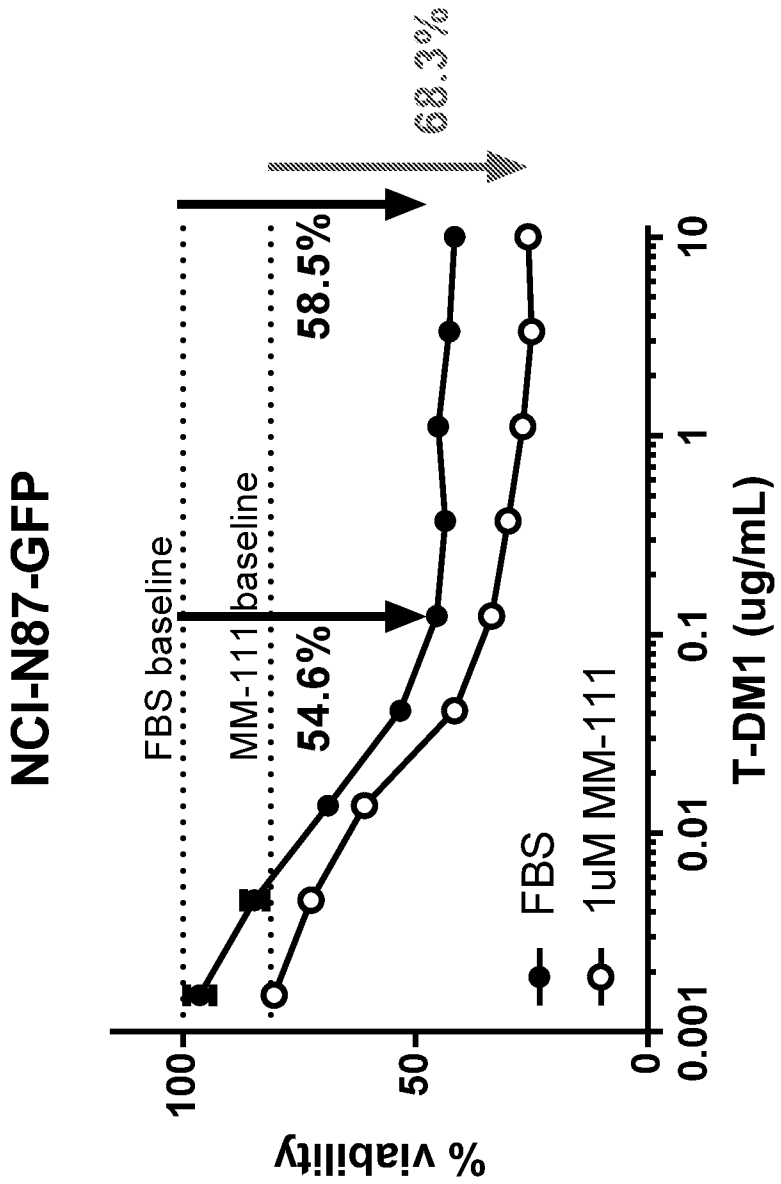
NCI-N87-GFP
% viability
100
50
0
0.001
0.01
0.1
1
10
T-DM1 (ug/mL)
FBS
1uM MM-111
FBS baseline
MM-111 baseline
54.6%
58.5%
68.3%

Figure 17a

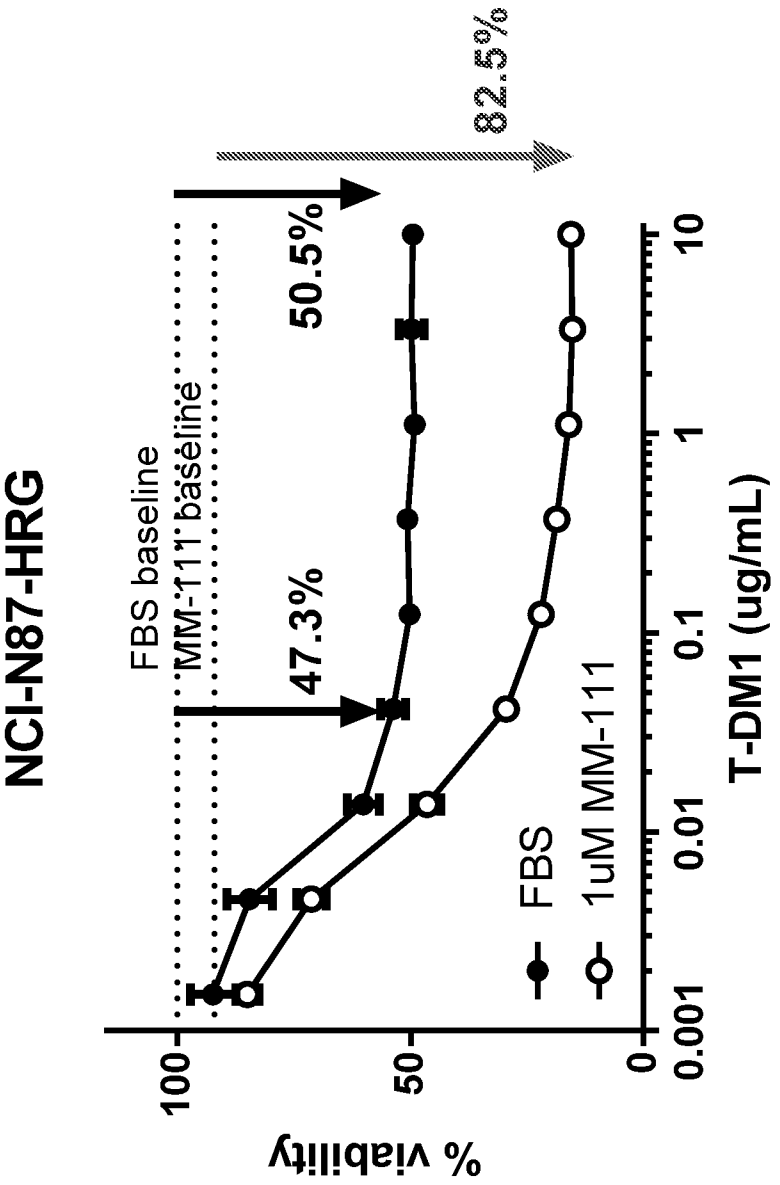
NCI-N87-HRG
FBS baseline
MM-111 baseline
47.3%
50.5%
82.5%
FBS
1uM MM-111
% viability
T-DM1 (ug/mL)
100
50
0
0.001
0.01
0.1
1
10

Figure 17b

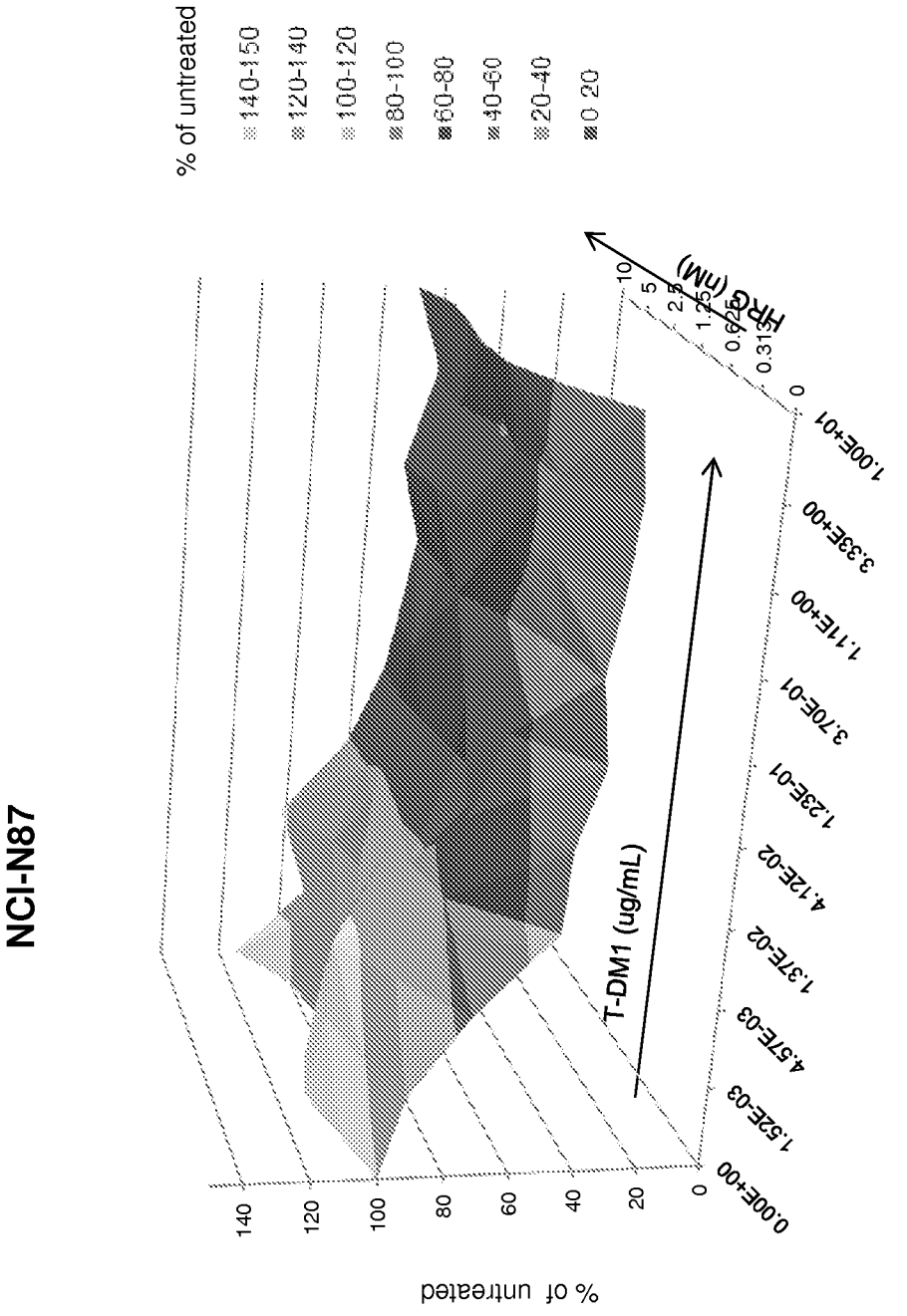
NCI-N87
% of untreated
140-150
120-140
100-120
80-100
60-80
40-60
20-40
0-20
% of untreated
140
120
100
80
60
40
20
0
T-DM1 (ug/mL)
0.00E+00
1.52E-03
4.57E-03
1.37E-02
4.12E-02
1.23E-01
3.70E-01
1.11E+00
3.33E+00
1.00E+01
HRG (nM)
0
0.313
0.625
1.25
2.5
5
10

Figure 18a

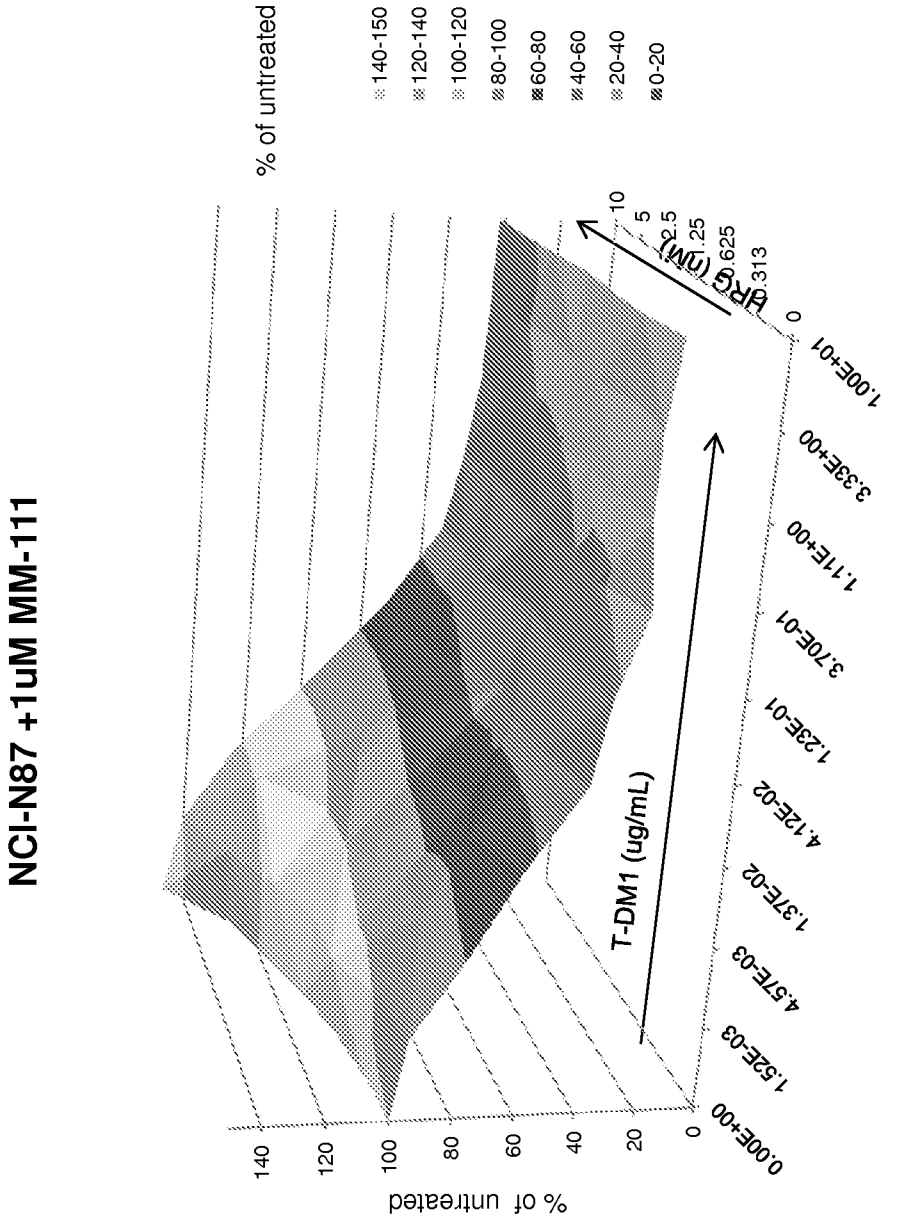
NCI-N87 +1uM MM-111
% of untreated
140-150
120-140
100-120
80-100
60-80
40-60
20-40
0-20
HRG (nM)
10
5
2.5
1.25
0.625
0.313
0
T-DM1 (ug/mL)
0.00E+00
1.52E-03
4.57E-03
1.37E-02
4.12E-02
1.23E-01
3.70E-01
1.11E+00
3.33E+00
1.00E+01
% of untreated
0
20
40
60
80
100
120
140

Figure 18b

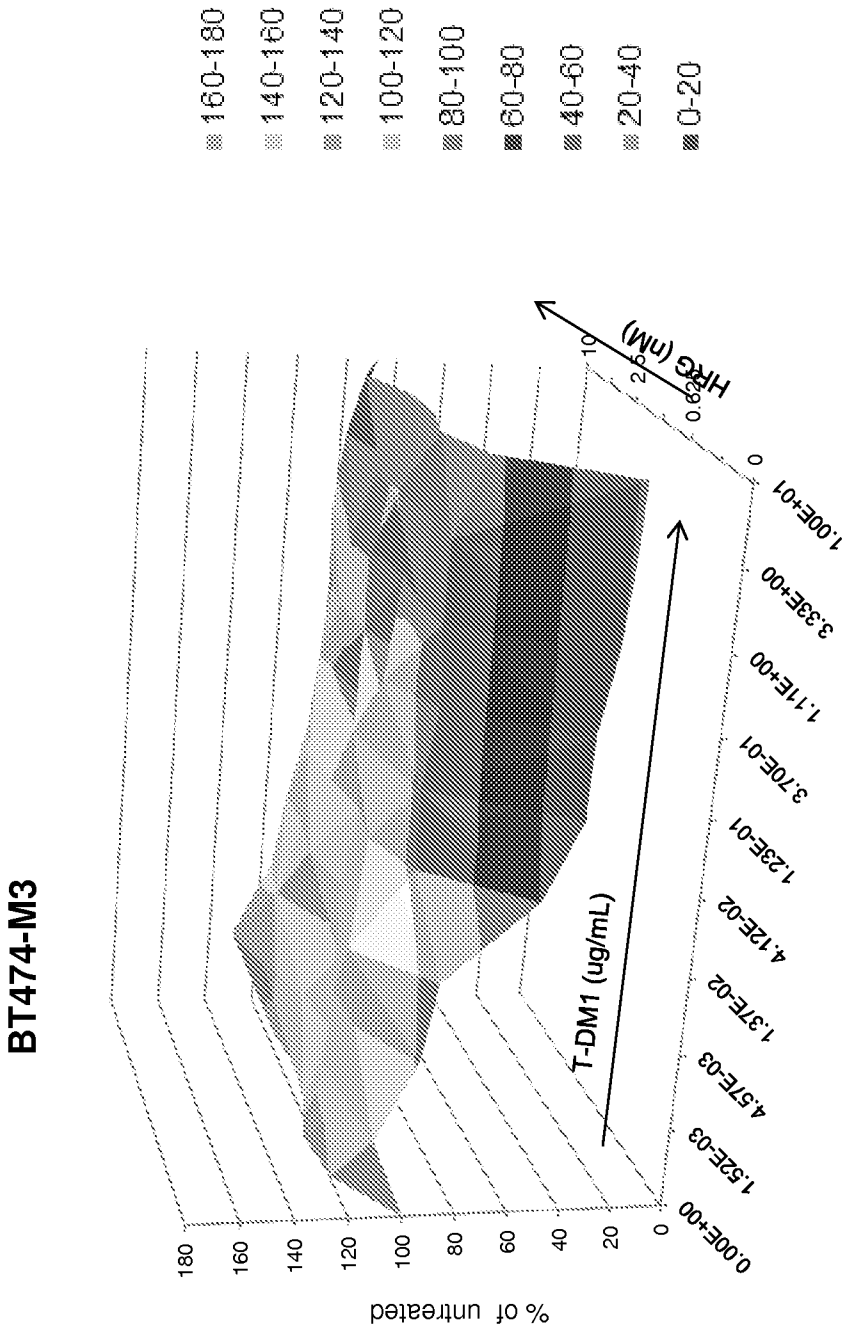
BT474-M3
160-180
140-160
120-140
100-120
80-100
60-80
40-60
20-40
0-20
% of untreated
180
160
140
120
100
80
60
40
20
0
T-DM1 (ug/mL)
0.00E+00
1.52E-03
4.57E-03
1.37E-02
4.12E-02
1.23E-01
3.70E-01
1.11E+00
3.33E+00
1.00E+01
HRG (nM)
0
0.6
2
10

Figure 18c

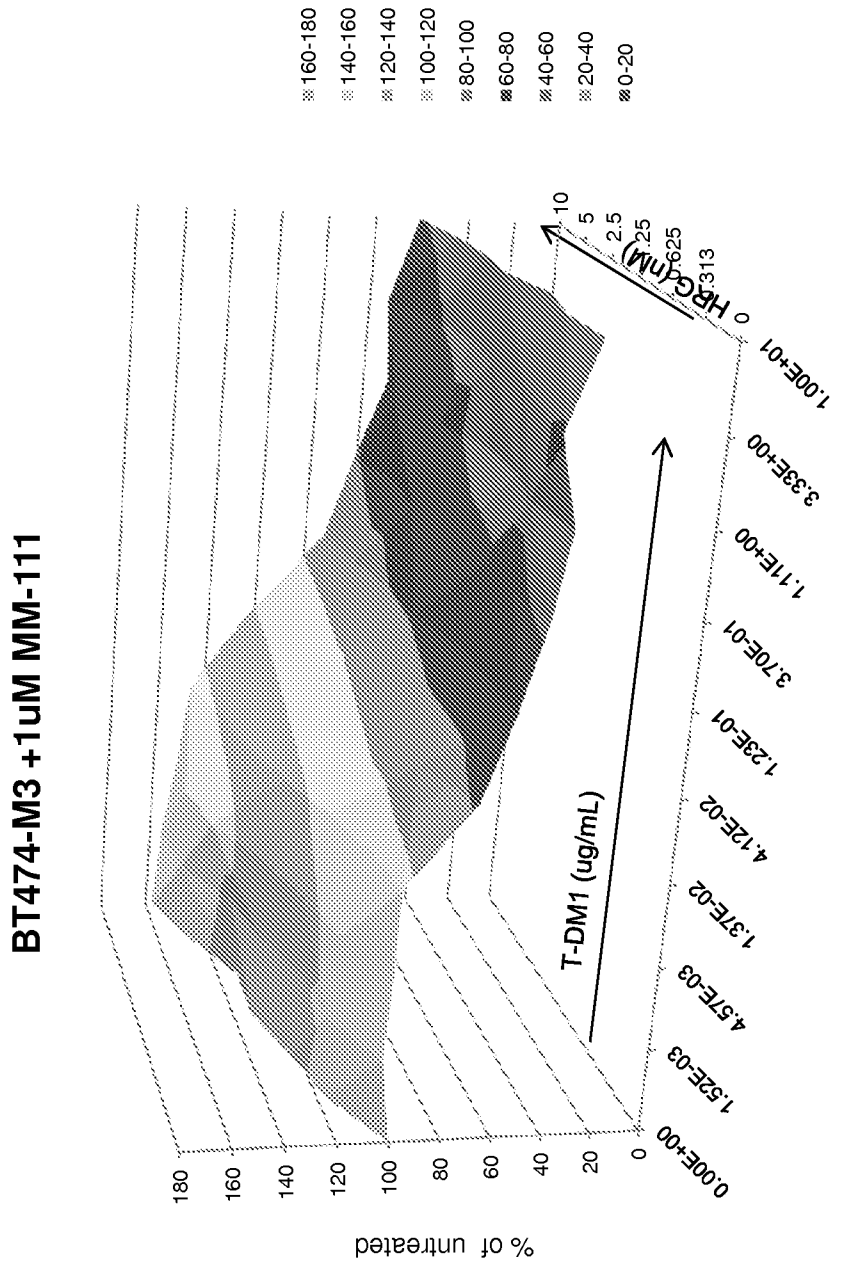
BT474-M3 +1uM MM-111
160-180
140-160
120-140
100-120
80-100
60-80
40-60
20-40
0-20
% of untreated
180
160
140
120
100
80
60
40
20
0
T-DM1 (ug/mL)
0.00E+00
1.52E-03
4.57E-03
1.37E-02
4.12E-02
1.23E-01
3.70E-01
1.11E+00
3.33E+00
1.00E+01
HRG (nM)
0
.313
.625
1.25
2.5
5
10

Figure 18d

COMBINATION THERAPIES COMPRISING ANTI-ERBB3 AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 61/695,242, filed Aug. 30, 2012, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The various aspects of the invention disclosed herein relate to methods and compositions for the treatment of cancers.

BACKGROUND OF THE INVENTION

[0003] 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.

[0004] 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, the anti-ErbB2 monoclonal antibody drug conjugate T-DM1 (ado-trastuzumab emtansine) 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.

[0005] ErbB2 can function as a homodimer or as a heterodimer with related epidermal growth factor-type receptors such as ErbB3. 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 intracellular 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. Such signaling is believed to drive proliferation of cancer cells. 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.

[0006] 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, as well as inhibition of the downstream intracellular signaling pathways such as the mitogen activated protein kinase and phosphoinositide-3 kinase pathways.

[0007] 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.

[0008] 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

[0009] 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.

[0010] 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., $HER2^{+++}$ or $HER2^{++}$ tumors) and the tumor may be a melanoma, clear cell sarcoma, head and neck, endometrial, prostate, breast, ovarian, esophageal, gastric, gastro-esophageal, colon, colorectal, lung, bladder, pancreatic, salivary gland, liver, skin, brain or renal tumor. The method comprises co-administering to the subject an effective amount an agent selected from i) an anti-estrogen agent, ii) a receptor tyrosine kinase inhibitor, iii) a MEK inhibitor, (e.g., selumetinib, trametinib, PD0325901, UO126), iv) a PI3 kinase inhibitor (e.g., BEZ235, BKM120, GDC0941), v) an AKT inhibitor (e.g., MK-2206, triciribine), vi) an mTOR inhibitor (e.g., BEZ235, AZD8055, everolimus, temsirolimus, sirolimus, ridaforolimus), vii) trastuzumab, viii) T-DM1, ix) capecitabine, x) cisplatin, and xi) MM-151; and combinations thereof, in combination with an effective amount of an anti-ErbB3 agent, e.g., a bispecific anti-ErbB2/anti-ErbB3 antibody (e.g., an antibody comprising the amino acid sequence set forth in SEQ ID NO:1). Additional agents for use in combination with the anti-ErbB3 agent, e.g., a bispecific anti-ErbB2/anti-ErbB3 antibody, are described in the Appendix.

[0011] 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 or T-DM1, 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.

[0012] 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 or T-DM1 is co-administered with the bispecific anti-ErbB2/anti-ErbB3 antibody.

[0013] 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, esophageal, gastro-esophageal junction, 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 an effective amount an agent selected from i) an anti-estrogen agent, ii) a receptor tyrosine kinase inhibitor, iii) a MEK inhibitor, iv) a PI3 kinase inhibitor, v) an AKT inhibitor, vi) an mTOR inhibitor, vii) trastuzumab, viii) T-DM1, ix) capecitabine, x) cisplatin, and xi) MM-151; and combinations thereof.

[0014] In a third embodiment, an aqueous solution is provided comprising a bispecific anti-ErbB2/anti-ErbB3 antibody (optionally an antibody comprising the amino acid sequence set forth in SEQ ID NO:1) at a first concentration and an agent selected from i) an anti-estrogen agent, ii) a receptor tyrosine kinase inhibitor, iii) an effective amount of a MEK inhibitor, iv) a PI3 kinase inhibitor, v) an AKT inhibitor, vi) an mTOR inhibitor, vii) trastuzumab, viii) T-DM1, ix) capecitabine, x) cisplatin, and xi) MM-151; and combinations thereof, at a second 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 the agent at the second 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 the agent(s). 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.

[0015] 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.

[0016] In another aspect, the aqueous solution further comprises trastuzumab or T-DM1 at a third concentration.

[0017] 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.

[0018] 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.

[0019] In each embodiment and aspect thereof above, one or more of a)-i) 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 agent i)-xi) is administered in accordance with the manufacturer's directions, e) the trastuzumab or T-DM1 is administered in accordance with the manufacturer's directions, f) the co-administration of the bispecific anti-ErbB2/anti-ErbB3 antibody with the agent g)-vi) produces an about additive or a superadditive effect, h) 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, i) 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

[0020] FIG. **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.

[0021] FIG. **2** is seven graphs showing that MM-111 combines positively with anti-estrogen drugs in inhibiting estrogen-stimulated spheroid growth in vitro. FIG. **2***a* shows the effect of MM-111, tamoxifen (4-hydroxytamoxifen or 4OHT), or MM-111 and tamoxifen on in vitro spheroid growth. FIG. **2***b* shows the effect of trastuzumab, tamoxifen, or trastuzumab and tamoxifen. FIG. **2***c* shows the effect of MM-111, fulvestrant (FVT), or MM-111 and fulvestrant. FIG. **2***d* shows the effect of trastuzumab, fulvestrant, or trastuzumab and fulvestrant. FIG. **2***e* shows the effect of MM-111, trastuzumab, or MM-111 and trastuzumab. FIG. **2***f* shows the effect of MM-111, trastuzumab, and tamoxifen combined compared to that of any of the double combinations. FIG. **2***g* 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.

[0022] FIG. **3** is seven graphs showing that MM-111 combines positively with anti-estrogen drugs in inhibiting heregulin (HRG)-stimulated spheroid growth in vitro. FIG. **3***a* shows the effect of MM-111, tamoxifen (4-hydroxytamoxifen or 4OHT), or MM-111 and tamoxifen. FIG. **3***b* shows the effect of trastuzumab, tamoxifen, or trastuzumab and tamoxifen. FIG. **3***c* shows the effect of MM-111, fulvestrant (FVT), or MM-111 and fulvestrant. FIG. **3***d* shows the effect of trastuzumab, fulvestrant, or trastuzumab and fulvestrant. FIG. **3***e* shows the effect of MM-111, trastuzumab, or MM-111 and trastuzumab. FIG. **3***f* shows the effect of MM-111, trastuzumab, and tamoxifen combined compared to that of any of the double combinations. FIG. **3***g* 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.

[0023] FIG. **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. FIG. **4***a* shows the effect of MM-111, tamoxifen, or MM-111 and tamoxifen. FIG. **4***b* shows the effect of trastuzumab, tamoxifen, or trastuzumab and tamoxifen. FIG. **4***c* shows the effect of MM-111, fulvestrant (FVT), or MM-111 and fulvestrant. FIG. **4***d* shows the effect of trastuzumab, fulvestrant, or trastuzumab and fulvestrant. FIG. **4***e* shows the effect of MM-111, trastuzumab, or MM-111 and trastuzumab. FIG. **4***f* shows the effect of MM-111, trastuzumab, and tamoxifen combined compared to that of any of the double combinations. FIG. **4***g* 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.

[0024] FIG. **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.

[0025] FIG. **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.

[0026] FIG. **7** evaluates the ability of lapatinib to inhibit ErbB3 and AKT activation in heregulin-stimulated cells. **7***a* is a graph comparing computer-generated dose-response curves to experimental results in heregulin-stimulated BT474-M3 cells. **7***b* 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.

[0027] FIG. **8** is a series of graphs showing MM-111 or lapatinib inhibition of ErbB3 (**8***a*) or AKT (**8***b*) activation in heregulin-stimulated cells incubated with inhibitor for 15 minutes, 1 hour, 4 hours, and 24 hours. FIG. **8***c* shows a comparison of IC50 for MM-111 and lapatinib at 1 hour and 24 hours for both BT474M3 cells and ZR75-30 cells.

[0028] FIG. **9** is a graph showing the effect of MM-111 and lapatinib combination treatment on AKT activation in heregulin-stimulated BT474-M3 cells.

[0029] FIG. **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.

[0030] FIG. **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.

[0031] FIG. **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. FIG. **12***a* shows the effect of lapatinib alone or the combination of lapatinib and fulvestrant (FVT). FIG. **12***b* shows the effect of lapatinib alone or the combination of lapatinib and MM-111. FIG. **12***c* 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 30 nM 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.

[0032] FIG. **13** is four graphs showing that 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. FIG. **13***a* shows the effect of letrozole, MM-111, or the combination of letrozole and MM-111. FIG. **13***b* shows the effect of lapatinib, MM-111 or the combination of lapatinib and MM-111. FIG. **13***c* shows the effect of lapatinib, letrozole, or the combination of lapatinib and letrozole. FIG. **13***d* 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-111 concentration 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.

[0033] FIG. **14** is two graphs showing that MM-111 combines positively with the PI3K/mTOR inhibitor BEZ235 and trastuzumab in unstimulated (FIG. **14***a*) and heregulin-stimulated (FIG. **14***b*) NCI-N87 cells in vitro. Cells were plated and either untreated (Control, open circle), treated with BEZ235 alone (open diamond), or treated with BEZ235 (5 nM FIG. **14***a,* 20 nM FIG. **14***b*) and either trastuzumab (closed circle), MM-111 (closed square), or the combination of MM-111 and trastuzumab (closed diamond). Closed circles (trastuzumab) may appear as squares or rectangles on trastuzumab curves when they comprise error bars. The x-axes are a log scale of the concentrations of each of MM-111 and/or trastuzumab.

[0034] FIG. **15** is two graphs showing that MM-111 combines positively with the mTOR inhibitor everolimus and trastuzumab in unstimulated (FIG. **15***a*) and heregulin-stimulated (FIG. **15***b*) NCI-N87 cells in vitro. Cells were plated and either untreated (Control, open circle), treated with everolimus alone (open diamond), or treated with everolimus (1 nM FIG. **15***a,* 5 nM FIG. **15***b*) and either trastuzumab (closed circle), MM-111 (closed square), or the combination of MM-111 and trastuzumab (closed diamond). Closed circles may appear as squares or rectangles on trastuzumab curves when they comprise error bars. The x-axes are a log scale of the concentrations of each of MM-111 and/or trastuzumab.

[0035] FIG. **16** is two graphs showing that MM-111 combines positively with the MEK inhibitor GSK1120212 (trametinib) in a mouse xenograft study. Phospho-ERK was measured as a function of ERK activity via a Luminex® immunosandwich assay at 4 hours after the initial treatment (FIG. **16**A) and 24 hours after the initial treatment (FIG. **16**B) as a function of ERK activity via a Luminex immunosandwich assay. The x-axes show type of treatment and the y-axes show mean fluorescent intensity.

[0036] FIG. **17** is two graphs showing that MM-111 combines positively with T-DM1 (ado-trastuzumab emtansine) in both the absence (FIG. **17**A) and presence (FIG. **17**B) of endogenous heregulin. The x-axes show a log scale of T-DM1 concentration and the y-axes show viability as % of control.

[0037] FIG. **18** is a series of 3D graphs showing that MM-111 combines positively with TDM-1 (ado-trastuzumab emtansine) in the presence of exogenous heregulin. NCI-N87 (FIGS. **18**A and **18**B) or BT-474-M3 (FIGS. **18**C and **18**D) cells were incubated with a dose range of both T-DM1 and heregulin, either without MM-111 (FIGS. **18**A and **18**C) or with MM-111 (FIGS. **18**B and **18**D). The x-axes are the concentration of T-DM1, the y-axes are cell viability as % of untreated cells, and the z-axes are heregulin concentration. The data presented in these graphs is also set forth below in Tables 1-4.

DETAILED DESCRIPTION

[0038] 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. Such co-administrations beneficially have an additive or superadditive effect on suppressing tumor cell growth, which effect on suppressing tumor cell growth is measured, e.g., in a mouse xenograft model e.g., using BT-474 or NCI-N87 cells.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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. An exemplary bispecific ErbB3, ErbB2 antibody is an antibody comprising SEQ ID NO:1.

[0043] 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.

[0044] By "cancer" is meant any condition characterized by abnormal, unregulated, malignant cell growth.

[0045] By "malignant tumor" is meant any cancer that takes the form of a tumor.

[0046] 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.

[0047] A "mouse effective amount" refers to an amount of a drug effective to achieve a desired effect when the subject is a mouse.

[0048] A "human effective amount" refers to an amount of a drug effective to achieve a desired effect when the subject is a human patient.

[0049] 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.

[0050] 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 inhibitors also include antibody-based therapeutics such as cetuximab, panitumumab, zalutumumab, nimotuzumab, and matuzumab). Preferably, the receptor tyrosine kinase inhibitor is lapatinib.

[0051] "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.

[0052] "Dose" refers to an amount of a drug given in a single administration.

[0053] In combination, the components of the combination of the anti-ErbB2/ErbB3 antibody have an additive or super-additive effect on suppressing tumor cell growth, as compared to monotherapy with the anti-ErbB2/ErbB3 antibody or treatment with the other agents in the absence of antibody therapy. By "additive" is meant a result that is greater in extent (e.g., in the degree of reduction of tumor mitotic index or of tumor growth or in the degree of tumor shrinkage or the frequency and/or duration of symptom-free or symptom-reduced periods) than the best separate result achieved by monotherapy with each individual component, while "super-additive" is used to indicate a result that exceeds in extent the sum of such separate results. In one embodiment, the additive effect is measured as slowing or stopping of tumor growth or tumor cell proliferation. The additive effect can also be measured as, e.g., reduction in size of a tumor, reduction of tumor mitotic index, reduction in number of metastatic lesions over time, increase in overall response rate, or increase in median or overall survival.

[0054] One non-limiting example of a measure by which effectiveness of a therapeutic treatment can be quantified is by calculating the log 10 cell kill, which is determined according to the following equation:

$$\log 10 \text{ cell kill} = TC(\text{days})/3.32 \times Td$$

[0055] in which T C represents the delay in growth of the cells, which is the average time, in days, for the tumors of the treated group (T) and the tumors of the control group (C) to have reached a predetermined value (1 g, or 10 mL, for example), and Td represents the time, in days necessary for the volume of the tumor to double in the control animals. When applying this measure, a product is considered to be active if log 10 cell kill is greater than or equal to 0.7 and a product is considered to be very active if log 10 cell kill is greater than 2.8. Using this measure, a combination, used at its own maximum tolerated dose, in which each of the constituents is present at a dose generally less than or equal to its

maximum tolerated dose, exhibits therapeutic synergy when the log 10 cell kill is greater than the value of the log 10 cell kill of the best constituent when it is administered alone. In an exemplary case, the log 10 cell kill of the combination exceeds the value of the log 10 cell kill of the best constituent of the combination by at least 0.1 log cell kill, at least 0.5 log cell kill, or at least 1.0 log cell kill.

[0056] 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® # CRL1504™, 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).

[0057] 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, gastric esophageal junction, stomach, pancreas, liver, bile-cyst, 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.

[0058] 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.

[0059] 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 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."

[0060] 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, 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 U.S. Pat. Nos. 7,332,580 and 7,332,585. 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, an open label, Phase 1/2 and pharmacologic study of MM-111 with five 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, MM-111 in combination with lapatinib, paclitaxel and trastuzumab, and MM-111 in combination with docetaxel and trastuzumab, and an open label, Phase 2 study of MM-111 and paclitaxel with or without trastuzumab in patients with HER-2 expressing carcinomas of the distal esophagus, gastroesophageal junction and stomach.

[0061] 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.

[0062] Additional therapeutic agents suitable for combination with anti-ErbB2/anti-ErbB3 antibodies may further include: 1) antibody EGFR inhibitors (e.g. MM-151, Sym004, cetuximab, panitumumab, zalutumumab, nimotuzumab, and matuzumab), additional small molecule tyrosine kinase inhibitors such as PKI-166, PD-158780, EKB-569 (pelitinib), tyrphostin AG-1478, and pan-HER kinase inhibitors (e.g. CI-1033 (canertinib, PD 183805), AC480/BMS-599626, HM781-36B, AZD8931 (sapitinib) and PF299804 (dacomitinib)); 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; 3) platinum-based therapeutics such as oxaliplatin, carboplatin and cisplatin; 4) mTOR inhibitors such as BEZ235 (a dual PI3K/mTOR inhibitor), AZD8055, everolimus, temsirolimus, siroplimus/rapamycin, and ridaforolimus; and 5) additional anti-ErbB2 therapeutic agents, such as trastuzumab, pertuzumab, and T-DM1 (ado-trastuzumab emtansine). Additional examples of therapeutic agents suitable for combination with anti-ErbB2/anti-ErbB3 antibodies may be found in Table 5 and the Appendix below.

[0063] 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 5 mg/kg less than the loading dose.

EXAMPLES

[0064] 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

[0065] 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

[0066] BT474-M3 cells (2×10^7 cells per mice) are inoculated subcutaneously into Nu/Nu immunodeficient mice, which are implanted with an estrogen pellet (0.72 mg; 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 (60 mg/kg, Q7D), 4-hydroxytamoxifen (5 mg; 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

[0067] 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 FIG. **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

[0068] 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 (FIG. **2***a*); trastuzumab, tamoxifen or the combination of trastuzumab and tamoxifen (FIG. **2***b*); MM-111, fulvestrant, or the combination of MM-111 and fulvestrant (FIG. **2***c*); trastuzumab, fulvestrant, or the combination of trastuzumab and fulvestrant (FIG. **2***d*); or MM-111, trastuzumab, or the combination of MM-111 and trastuzumab (FIG. **2***e*). 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 (FIGS. **2***a* and **2***c*, respectively) or trastuzumab (FIGS. **2***b* and **2***d*, respectively) increased the degree of growth inhibition, as did the combination of MM-111 and trastuzumab (FIG. **2***e*). The inhibitory effects were increased still further when estrogen-stimulated spheroids were treated with the triple combination of MM-111, trastuzumab, and tamoxifen (FIG. **2***f*) or MM-111, trastuzumab, and fulvestrant (FIG. **2***g*) as compared to the double combinations of drugs.

Example 3

MM-111 Combines Positively with Anti-Estrogen Drugs in Inhibiting Heregulin-Stimulated Spheroid Growth

[0069] 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 (FIG. **3***a*); trastuzumab, tamoxifen or the combination of trastuzumab and tamoxifen (FIG. **3***b*); MM-111, fulvestrant, or the combination of MM-111 and fulvestrant (FIG. **3***c*); trastuzumab, fulvestrant, or the combination of trastuzumab and fulvestrant (FIG. **3***d*); or MM-111, trastuzumab, or the combination of MM-111 and trastuzumab (FIG. **3***e*). MM-111 inhibited heregulin-induced spheroid growth but tamoxifen (FIG. **3***a*), trastuzumab (FIG. **3***b*), and fulvestrant (FIG. **3***c*) did not inhibit heregulin stimulated

spheroid growth. No significant combinational effect was observed when MM-111 was used with tamoxifen (FIG. **3***a*) or fulvestrant (FIG. **3***c*). The combination of trastuzumab and either tamoxifen (FIG. **3***b*) or fulvestrant (FIG. **3***d*) failed to show inhibitory activity significantly greater than either drug alone. As shown in FIG. **3***e*, 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-111 or trastuzumab with tamoxifen or fulvestrant, the triple combination of MM-111, trastuzumab and either tamoxifen (FIG. **3***f*) or fulvestrant (FIG. **3***g*) showed similar inhibitory effects as those of MM-111 and trastuzumab in combination (FIG. **3***e*) 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

[0070] 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 (FIG. **4***a*) or trastuzumab, tamoxifen or the combination of trastuzumab and tamoxifen (FIG. **4***b*). While MM-111 and trastuzumab each inhibited spheroid growth (FIG. **4***a*) 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.

[0071] Dual ligand stimulated spheroids were then treated with a dose range of fulvestrant, MM-111 or the combination of MM-111 and fulvestrant (FIG. **4***c*) or fulvestrant, trastuzumab, or a combination of fulvestrant or trastuzumab (FIG. **4***d*). 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 (FIG. **4***c*). Trastuzumab alone had no significant inhibitory effects and the combination of trastuzumab and fulvestrant showed similar effects to tamoxifen alone (FIG. **4***d*).

[0072] 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 (FIG. **4***e*).

[0073] In comparison to the double combination of MM-111 or trastuzumab with tamoxifen or fulvestrant, the triple combination of MM-111, trastuzumab and either tamoxifen (FIG. **4***f*) or fulvestrant (FIG. **4***g*) showed similar inhibitory effects to those of MM-111 and trastuzumab in combination (FIG. **4***e*) on estrogen- and heregulin-(dual ligand) stimulated spheroid growth.

[0074] 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 FIG. **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

	MM-111 + Trastuzumab	MM-111 + anti-estrogen	Trastuzumab + anti-estrogen	Triple combination
Tamoxifen 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 104 doses of inhibitor treatment.

[0075] 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

[0076] 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

[0077] 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 5 nM heregulin 143 (R&D Systems, Minneapolis, Minn.) 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, Calif.).

Cell Proliferation Assay

[0078] 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, Minn.) 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, Wis.).

Apoptosis Assay

[0079] BT474-M3 cells (2000 cells/well) are plated in Ultra Low Cluster 96-well plate (Costar®, Corning, N.Y.). 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

[0080] 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, Mass.). Mice receive a subcutaneous 60 day, slow-release estrogen implant in the opposite flank (0.72 mg pellet; Innovation Research of America, Sarasota, Fla.) 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

[0081] 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 400 μg/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; 200 nM) and heregulin (HRG; 2 nM). 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

[0082] 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=3.9×10-4) and lapatinib (p=5.1×10-3) on day 13 (FIG. **6**). The percent change in tumor volume from day 40 to day 7 (inoculation) was calculated for each group (FIG. **6***b*). 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

[0083] 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 (FIG. **7***a*). 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 (FIG. **7***b*). 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

[0084] 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® #

[0085] 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 (FIG. **8***a*) and ZR75-30 cells (IC_{50}=5 nM) (FIG. **8***c*). Good reduction by MM-111 of pAKT levels (inhibition of AKT phosphorylation) in BT474-M3 (IC_{50}=10) (FIG. **8***b*) and in ZR75-30 cells (IC_{50}=4 nM) (FIG. **8***d*) 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 (FIG. **8***c*) 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

[0086] 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 (FIG. **9**). Treatment with either MM-111 (1 μM) or lapatinib (1 μM) alone resulted in similar levels of pAKT inhibition (see FIG. **8***b*) 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

[0087] 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 (FIG. **10**).

Example 10

Treatment with the Combination of MM-111 and Lapatinib Results in Increased Apoptosis

[0088] 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 (FIG. **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%; FIG. **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

[0089] 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 3.3 nM, 10 nM, or 30 nM lapatinib, either alone or in combination with a dose range of fulvestrant (FVT) (FIG. **12***a*); 3.3 nM, 10 nM, or 30 nM lapatinib, either alone or in combination with a dose range of MM-111 (FIG. **12***b*); or 3.3 nM, 10 nM, or 30 nM lapatinib, either alone or in combination with a dose range of both MM-111 and fulvestrant (FIG. **12***c*). In the presence of dual ligand stimulation the combination of lapatinib and FVT did not greatly increase inhibition of spheroid growth over lapatinib alone (FIG. **12***a*). In contrast, the addition of MM-111 greatly increased the sensitivity of the spheroids to lapatinib treatment (FIG. **12***b*), 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

[0090] 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 (FIG. **13***a*); MM-111, lapatinib, or the combination of MM-111 and lapatinib (FIG. **13***b*); lapatinib, letrozole, or the combination of lapatinib and letrozole (FIG. **13***c*); and each of the dual combination plus the triple combination of MM-111, lapatinib, and letrozole (FIG. **13***d*). 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 (FIG. **13***a*). 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 (FIG. **13***b*). 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 (FIG. **13***c*) Similarly, as shown in FIG. **13***d*, 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)

[0091]

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANINRDGSASYYVDS

VKGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLVTVSSASTGGGGS

-continued

GGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKAPKLMIYDVS

DRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYGSSSTHVIFGGGTKVTVLGAASDAHK

SEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHT

LFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNE

ETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAK

QRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRA

DLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAK

DVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL

IKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAED

YLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADICTL

SEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAAS

QAALGLAAALQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYP

GDSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARHDVGYCTDRTCAKWPEWL

GVWGQGTLVTVSSGGGGSSGGGSGGGGSQSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSW

YQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYCASWDYTLSGWVF

GGGTKLTVLG

Dosing and Administration of MM-111 in Combination with One or More Additional Therapeutics

Example 14

Mode of Administration of MM-111

[0092] 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.

[0093] 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).

[0094] 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.

[0095] 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

[0096] 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.

[0097] 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, and 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 3B

7 about 25 about 20

TABLE 3B-continued

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

TABLE 3A

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

TABLE 3C

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

Example 16

Dosage and Administration of MM-111 with Lapatinib and Trastuzumab

[0098] 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).

[0099] Typically, lapatinib is administered at a dosage of 1000 to 1500 mg in 250 mg 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.

[0100] 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 1500 mg/day. In some embodiments, the higher lapatinib dose is between 2000 and 9000 mg/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 9000 mg/day, and so on.

[0101] 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 7000 mg/day, followed by a maintenance dose of 3000 mg/day. Non-limiting examples of loading dose and maintenance dose combinations are listed in Table 4 below.

[0102] 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	2000	2000	2500	2500	2500	3000	3000	3000	3000	3000	3500	3500
1000	1500	2000	1000	1500	2000	1000	1500	2000	2500	3000	1000	1500
3500	3500	3500	4000	4000	4000	4000	4000	4000	4500	4500	4500	4500
2000	2500	3000	1000	1500	2000	2500	3000	3500	1000	1500	2000	2500
4500	4500	4500	5000	5000	5000	5000	5000	5000	5000	5000	5500	5500

TABLE 4-continued

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

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

Example 17

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

[0103] Administration of MM-111 with cisplatin, capecitabine, and trastuzumab is done, for example, by the following method or minor variations thereof.

[0104] Patients are administered therapy on a 21-day treatment cycle. Cisplatin is administered on day 1 of each 21-day cycle by intravenous (i.v.) infusion over two hours, at a dose of 80 mg/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 6 mg/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

[0105] 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 1000 mg 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-111 with Paclitaxel and Trastuzumab

[0106] 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.

Example 20

Coadministration of MM-111 and Other Therapeutic Agents

[0107] MM-111 (at dosages described herein; see, e.g., Example 15) can be administered in combination with one or more additional agents to a patient in need thereof for the treatment of a cancer. In particular, MM-111 can be administered in combination with MM-151 (oligoclonal anti-EGFR mixture), TDM-1 (ado-trastuzumab emtansine, an antibody-drug conjugate of the antibody trastuzumab linked to maytansine derivative (DM1)), an mTOR inhibitor (e.g., AZD8055, sirolimus, everolimus, temsirolimus, ridaforolimus, or the dual PI3K/mTOR inhibitor BEZ235), and combinations thereof.

[0108] MM-151 is an oligoclonal therapeutic that is a mixture of three fully human monoclonal antibodies designed to bind to non-overlapping epitopes of the epidermal growth factor receptor, or EGFR (also known as ErbB1). An oligoclonal therapeutic is a mixture of two or more distinct monoclonal antibodies. MM-151 is disclosed, e.g., in copending PCT Application No. PCT/US12/45235.

[0109] MM-111 can be administered in the same dosage form as MM-151, TDM-1, and/or the mTOR inhibitor(s), or the agents can be administered in separate dosage forms.

[0110] In an embodiment, MM-111 and one or more of MM-151, TDM-1, and/or the mTOR inhibitor(s) is administered to a patient for the treatment of a malignant tumor, e.g., an ErbB2-expressing or ErbB2 over-expressing tumor (e.g., HER or HER tumors). 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.

[0111] In another embodiment, MM-111 and MM-151 are co-administered to treat a solid tumor (e.g., an advanced refractory solid tumor) in a patient in need thereof.

[0112] The effect of MM-111 combined with trastuzumab and the dual PI3K/mTOR inhibitor BEZ235 on cell proliferation was assessed by the methods described above or with minor variations thereof in unstimulated and heregulin-stimulated NCI-N87 cells in vitro. Cells were incubated for 2 hours with a dose range of MM-111, trastuzumab, or their combination, in the presence of BEZ235 as shown in FIG. **14**. Cells were plated and either untreated (Control, open circle), treated with BEZ235 alone (open diamond), or treated with

BEZ235 (5 nM FIG. **14***a*, 20 nM FIG. **14***b*) and either trastuzumab (closed circle), MM-111 (closed square), or the combination of MM-111 and trastuzumab (closed diamond). Treatment with the triple combination of MM-111, trastuzumab, and BEZ235 resulted in a significant reduction in cell number compared to control cells in both the presence and absence of heregulin stimulation.

[0113] The effect of MM-111 combined with the mTOR inhibitor everolimus and trastuzumab was assessed in unstimulated (FIG. **15***a*) and heregulin-stimulated (FIG. **15***b*) BT474M3 cells in vitro. Cells were plated and either untreated (Control, open circle), treated with everolimus alone (open diamond), or treated with everolimus (1 nM FIG. **15***a*, 5 nM FIG. **15***b*) and either trastuzumab (closed circle), MM-111 (closed square), or the combination of MM-111 and trastuzumab (closed diamond). Treatment with the triple combination of MM-111, trastuzumab, and everolimus resulted in a significant reduction in cell number compared to control cells in both the presence and absence of heregulin stimulation.

Example 21

Combination Treatment with MM-111 and MEK/PI3k/AKT Pathway Inhibitors

[0114] MM-111, at dosages described herein (see, e.g., Example 15), can be administered in combination with one or more MAP/ERK kinase (MEK)/phosphatidylinositol 3-kinase (PI3k)/AKT pathway inhibitors to a patient in need thereof for the treatment of a cancer. MM-111 can be administered in the same dosage form as the MEK/PI3k/AKT pathway inhibitor(s) or these agents can be administered in separate dosage forms.

[0115] In one embodiment, MM-111 and a MEK/PI3k/AKT pathway inhibitor is administered to a patient for the treatment of a malignant tumor, e.g., an ErbB2-expressing or ErbB2 over-expressing tumor (e.g., HER^{+++} or HER^{++} tumors). 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.

[0116] To determine whether the combination of MM-111 with a MEK inhibitor results in decreased Erk1/2 activity in a tumor model, MM-111 and the MEK inhibitor trametinib, and were tested in a mouse xenograft model as described below:

Cell Culture and Xenograft Study

[0117] BT474-M3 cells are transduced with full length HRG1-β1 (Origene)-constructs (encoding PAC-PA-turboGFP for selection) by lentiviral infection. A HRG overexpressing polyclonal cell line (BT474-M3-GFP-HRG) is established after puromycin selection and sorting for high GFP expressing cell population. A control cell line (BT474-M3-GFP) is engineered to express EGFP in the same manner. Cells are maintained in culture in RPMI supplemented with 10% FBS, penicillin, streptomycin and puromycin. MM-111 is produced in-house at Merrimack Pharmaceuticals and MK2206, trametinib, and UO126 (UO126-EtOH) are purchased from Selleckchem. 7-9 week old female NCRNU-mice (Taconic) are implanted subcutaneously with 0.72 mg 60-day release 17 β-estradiol pellets (Innovative Research of America).

MM-111 Combination Treatment with Trametinib

[0118] 48 hours after implantation of the β-estradiol pellets, 15×106 BT474-M3-GFP-HRG cells were implanted in the second axillary mammary fat pad. When tumors reached an average volume of 450-500 mm3 (day 0), mice were randomized into groups to receive control (phosphate buffered saline, intraperitoneally), MM-111 (48 mg/kg, intraperitoneally using phosphate buffered saline as vehicle), trametinib (3 mg/kg, by oral gavage in 0.5% methylcellulose/0.1% Tween®-80) or a combination of MM-111 and trametinib. A set (3 per group) of mice are euthanized 4 h after the initial treatment. Tumors were excised and snap-frozen in liquid nitrogen for subsequent protein analysis. Another set of animals continued on their assigned treatment regimens for another 7 days receiving phosphate buffered saline control (intraperitoneally) on days 3 and 6, MM-111 (48 mg/kg, intraperitoneally) on days 3 and 6, trametinib (3 mg/kg, by oral gavage) on days 1, 2, 5 and 6. 24 hours after the last treatment, tumors were excised and snap-frozen in liquid nitrogen for subsequent protein analysis. Frozen tumor samples were pulverized and protein lysates were prepared in Tissue Extraction Reagent (Invitrogen) supplemented with Protease Inhibitor Cocktail Set III (Calbiochem, EMD) and Halt™ Phosphatase Inhibitor Cocktail (Thermo Scientific). Lysates were diluted in assay buffer (1% Bovine Serum Albumin in Tris buffered saline, pH 7.4, containing 0.1% Tween-20) to 1 mg/ml concentration and analyzed for phopsho- and total Erk1/2 using Luminex® immunosandwich assay on a FLEXMAP 3D instrument (Luminex). Antibodies from Cell Signaling Technologies were used to detect total Erk1/2 (3374) and pT202/Y204-sites on Erk1/2 (4370BF). 4 h after the start of treatment, addition of MM-111 to trametinib showed a trend to decreasing Erk1/2 activity (decreasing pT202/Y204-Erk1/2, $p=0.086$, Student's t-test) compared to treatment withtrametinib alone (FIG. **16**A). 24 h after the last treatment, addition of MM-111 to trametinib significantly decreased Erk1/2 activity (decreasing pT202/Y204-Erk1/2, $p<0.001$, Student's t-test) compared to treatment with trametinib alone (FIG. **16**B).

Example 22

Combination Treatment with MM-111 and ErbB2-Targeted Therapeutic Agents

[0119] MM-111, at dosages described herein (see, e.g., Example 15), can be administered in combination with one or more additional ErbB2-targeted therapeutic agents, such as trastuzumab, T-DM1, pertuzumab, etc. In order to assess the effect of MM-111 in combination with an additional ErbB2-targeted therapeutic, cells with (FIG. **17**B) and without (FIG. **17**A) heregulin (HRG) were treated with a dose escalation of T-DM1 and then treated with MM-111 as follows.

Materials

[0120] NCI-N87 cells are transduced with full length HRG1-β1 (Origene)-constructs (encoding PAC-PA-turboGFP for selection) by lentiviral infection. A HRG overexpressing polyclonal cell line (NCI-N87-GFP-HRG) is established after puromycin selection and sorting for high GFP expressing cell population. A control cell line (NCI-N87-GFP) is engineered to express EGFP in the same manner.

Cells are maintained in culture in RPMI supplemented with 10% fetal bovine serum, penicillin, streptomycin and puromycin (1 μg/ml).

[0121] MM-111 is manufactured at Merrimack Pharmaceuticals. Recombinant human NRG-1-β1 EGF domain is from R&D Systems. T-DM1 (KADCYLA, Genentech) is obtained from pharmacy. CellTiter-Glo® Luminescent Cell Viability Assay is from Promega.

[0122] 700 NCI-N87 or BT-474-M3 cells were seeded on 384-well flat clear bottom polystyrene microplates in 20 μl of culture media (RPMI supplemented with 10% fetal bovine serum and penicillin and streptomycin). Cells were allowed to adhere 16-20 hours at +37° C., 5% CO_2. Cells were stimulated for 4 hours by adding 5 μl of culture media containing recombinant human NRG-1-β1 EGF domain (HRG1) bringing the final HRG1 stimulation concentration to 0, 0.313, 0.625, 1.25, 2.5, 5 or 10 nM. After 4 hours, T-DM1 was added in 5 μl of culture media with or without MM-111 bringing the T-DM1 concentration to 0, 0.001524, 0.004572, 0.013717, 0.041152, 0.123457, 0.37037, 1.11111, 3.333333 or 10 μg/ml and the MM-111 concentration to 0 or 1 μM. All conditions were assayed in biological quadruplicates. Cells were incubated for 72 h at +37° C., 5% CO_2. Cell viability was determined by CellTiter-Glo® Luminescent Cell Viability Assay by adding 20 μl of reconstituted CellTiter-Glo reagent per well, incubating plated for 10 minutes at RT in an orbital shaker and reading the luminescence signal using Biotek™ Synergy H1 Hybrid Reader.

[0123] 700 NCI-N87-GFP or NCI-N87-GFP-HRG1 cells were seeded on 384-well flat clear bottom polystyrene microplates in 20 μl of culture media (RPMI supplemented with 10% fetal bovine serum and penicillin and streptomycin). Cells were allowed to adhere 16-20 hours at +37° C., 5% CO2. T-DM1 was added in 5 μl of culture media with or without MM-111 bringing the T-DM1 concentration to 0, 0.001524, 0.004572, 0.013717, 0.041152, 0.123457, 0.37037, 1.11111, 3.333333 or 10 μg/ml and the MM-111 concentration to 0 or 1 μM. All conditions were assayed in biological quadruplicates. Cells were incubated for 72 h at +37° C., 5% CO_2. Cell viability was determined by CellTiter-Glo® Luminescent Cell Viability Assay by adding 20 ul of reconstituted CellTiter-Glo reagent per well, incubating plated for 10 minutes at RT in an orbital shaker and reading the luminescence signal using Biotek Synergy H1 Hybrid Reader.

[0124] As shown in FIG. **17**, In the absence of endogenous heregulin, treatment of cells with T-DM1 reduces cell viability up to approximately 54.6% with 0.1 μg/mL T-DM1, at which point the remaining cells become resistant. The addition of MM-111 restores sensitivity of the cells to T-DM1. This effect is significantly increased in the presence of endogenous heregulin where treatment of cells with T-DM1 reduces cell viability up to approximately 47.3% with 0.04111152 μg/mL T-DM1, at which point the remaining cells become resistant. The addition of MM-111 restores sensitivity of the cells to T-DM1 further reducing cell viability by approximately 30% to approximately 80% (FIG. **17**B).

[0125] To test the combination therapy with endogenous heregulin (HRG), NCI-N87 cells and BT-474-M3 cells were prepared as described above and treated with a dose range of both T-DM1 and heregulin in the presence and absence of MM-111 (3D graphs, FIG. **18**). Cell viability was tested and numbers are given as % of control and summarized in Tables 1-4 below. As can be seen in FIG. **18**, while exogenous HRG decreases the activity of T-DM1, co-treatment of the cells with a combination of MM-111 and T-DM1 greatly reduced cell viability in the presence of endogenous heregulin in both NCI-N87 (FIG. **18**B) and BT-474-M3 cells (FIG. **18**D).

TABLE 1

NCI-N87 cells without MM-111

HRG (nM)	0	0.001524	0.004572	0.013717	0.041152	0.123457	0.37037	1.111111	3.333333	10	T-DM1 (μg/ml)
0	100.0001	93.36976	75.62197	51.2155	50.04061	42.64956	46.5761	42.8517	40.80667	43.84619	
0.313	105.2683	100.3208	92.59442	79.62461	74.71907	64.7733	50.76463	55.8238	57.24998	57.69454	
0.625	110.9354	106.4739	106.0159	84.03995	61.45342	71.29415	58.90545	65.075	62.77755	67.86139	
1.25	105.9291	102.2426	94.15791	81.15374	64.90047	66.01914	69.29675	69.40256	58.90233	68.76759	
2.5	95.5206	90.80036	84.09815	83.76757	69.34041	74.67369	67.23904	72.16217	66.05516	65.68811	
5	99.9019	94.98233	89.09868	80.41984	71.01775	73.54437	68.99217	75.72861	64.94796	63.21516	
10	114.0075	88.14249	101.4929	80.41022	69.82144	64.32714	61.13489	62.15488	59.45985	68.79469	

TABLE 2

NCI-N87 cells with MM-111

HRG (nM)	0	0.001524	0.004572	0.013717	0.041152	0.123457	0.37037	1.111111	3.333333	10	T-DM1 (μg/ml)
0	99.99994	95.67672	78.87085	64.23961	46.34934	41.8017	32.86071	35.79531	35.56626	32.20002	
0.313	101.4158	98.3686	87.83576	74.46012	52.5808	44.82781	41.52759	37.6864	34.50943	36.02112	
0.625	105.024	108.5211	86.78032	66.85997	55.32827	45.12097	40.0349	38.27817	38.6461	36.36282	
1.25	110.2781	107.8277	99.94741	73.38117	51.00783	43.11336	43.031	40.2014	37.52794	35.5527	
2.5	118.0867	117.2117	99.28302	90.13422	63.70836	52.75964	43.73032	41.26843	36.41708	38.52877	
5	126.5145	128.6171	118.6616	95.04545	70.74315	51.52235	43.87818	43.0434	42.54104	40.95163	
10	146.1417	140.3695	122.2993	95.17032	70.81566	52.81674	47.2081	43.46258	42.60197	42.12973	

TABLE 3

BT-474-M3 cells without MM-111

HRG (nM)	0	0.001524	0.004572	0.013717	0.041152	0.123457	0.37037	1.111111	3.333333	10	T-DM1 (μg/ml)
0	100	113.2862	98.62592	94.0068	59.46435	46.28945	46.03028	40.94169	38.39982	37.44111	
0.313	119.8347	116.4401	115.5001	120.231	97.75329	98.67097	94.19504	95.98138	95.80208	84.02226	
0.625	123.4093	117.8323	116.7982	112.1619	108.0231	104.9562	107.4638	97.33409	94.15624	94.01721	
1.25	116.9189	108.1483	107.0419	108.9035	104.0392	109.1902	91.67416	94.82696	100.5096	88.67155	
2.5	114.6797	113.1709	104.357	114.8767	108.3647	96.44198	100.0466	99.7191	99.2052	89.80503	
5	117.0592	123.3681	119.6607	111.0487	111.2081	98.30082	102.3885	99.45587	90.43802	99.39723	
10	116.0498	130.2768	115.6098	111.159	104.5468	101.7859	99.51807	101.389	98.00674	87.40396	

TABLE 4

BT-474-M3 cells with MM-111

HRG (nM)	0	0.001524	0.004572	0.013717	0.041152	0.123457	0.37037	1.111111	3.333333	10	T-DM1 (μg/ml)
0	100.0001	105.1766	103.9555	103.6039	77.39243	66.65368	57.99249	52.81845	61.04489	49.52486	
0.313	116.4349	118.4495	115.8389	106.7296	91.78999	68.56924	61.57601	59.03206	58.89703	52.39394	
0.625	123.4726	120.7381	123.5142	115.1022	91.45484	68.63193	61.65257	53.11713	54.6567	47.24296	
1.25	132.3855	131.1941	131.9924	125.6464	100.4235	78.41731	64.38723	54.49712	56.83025	54.51656	
2.5	131.572	136.6084	146.7699	134.055	109.0098	80.11692	73.45718	64.84097	55.26672	53.33503	
5	146.0121	152.0284	151.8639	138.9854	116.4752	86.2649	70.99611	74.74774	61.73628	55.30379	
10	157.2453	154.9672	149.674	145.9666	121.6431	91.79307	81.23282	69.08484	71.97691	61.17057	

Endnotes

[0126] 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.

[0127] 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. In particular, WO 2012/116317 is incorporated herein by reference in its entirety.

APPENDIX

Anticancer Agents

[0128] The Table and Appendix below describe effective an anti-estrogen agents, receptor tyrosine kinase inhibitors; MEK/PI3 kinase/AKT inhibitors, and mTOR inhibitors that can be used in the methods and compositions of the invention.

[0129] The bispecific anti-ErbB2/anti-ErbB3 antibody co-administered in combination with an agent selected from i) an effective amount of an anti-estrogen agent; ii) an effective amount of a receptor tyrosine kinase inhibitor; iii) a MEK/PI3 kinase/AKT inhibitor; iv) MM-151; v) an mTOR inhibitor; and/or vi) trastuzumab or T-DM1, and combinations thereof, can be further co-administered with at least a third antineoplastic agent selected from any of those disclosed in the Table and Appendix 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	

TABLE 5-continued

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
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

[0130]

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 Antibodies		
INCB7839		Incyte
Bevacizumab	Avastin ®	Genentech
Cetuximab	Erbitux ®	EMCLONE
mAb 806		
Matuzumab (EMD72000)		
Nimotuzumab (TheraCIM)		
Panitumumab	Vectibix ®	Amgen
MM-151		Merrimack
Sym004		Symphogen
Zalutumumab		Humax
Anti-ErbB3 Therapeutics		
U3-1287/AMG888		U3 Pharma/Amgen
MM-121		Merrimack Pharmaceuticals

-continued

Other anticancer agents for combination with a bispecific anti-ErbB2/anti-ErbB3 antibody	Brand Name(s)	Manufacturer/Proprietor
Anti-ErbB2 Therapeutics		
trastuzumab	Herceptin ®	Genentech
HKI-272—neratinib		Wyeth
KOS-953—tanespimycin		Kosan Biosciences
T-DM1—ado-trastuzumab emtansine	Kadcyla ®	Genentech
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)		
Afatinib (BIBW 2992)		Boehringer Ingelheim
Small Molecules Targeting MEK		
CI-1040 (PD184352)		
AZD6244 (selumetinib)		
RDEA119 (BAY 869766)		
GSK1120212 (trametinib)		Glaxo Smith Kline
PD-0325901		
GDC-0973		Genentech
UO126-EtOH		Cell Signaling Technology
Anti-cMet Antibody Therapies		
AVEO (AV299)		AVEO
AMG102		Amgen
5D5 (OA-5D5)		Genentech
Small Molecules Targeting cMet		
PHA665752		
ARQ-650RP		ArQule
ARQ 197		ArQule
Alkyaling 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
	Cytoxan	Bristol-Myers Squibb
Cyclophosphamide	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		

-continued

Other anticancer agents for combination with a bispecific anti-ErbB2/anti-ErbB3 antibody	Brand Name(s)	Manufacturer/Proprietor
Semustine		
Streptozocin		
Temozolomide		
Treosulfan		
Triaziquone		
Triethylene Melamine		
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	Tomudex ®	AstraZeneca
Tegafur		
Aromatose Inhibitor		
Ketoconazole		
Glucocorticoids		
Dexamethasone	Decadron ® Dexasone, Diodex, Hexadrol, Maxidex	Wyeth, Inc.
Prednisolone		
Prednisone	Deltasone, Orasone, Liquid Pred, Sterapred ®	
Immunotherapeutics		
Alpha interferon		
Angiogenesis Inhibitor	Avastin ®	Genentech
IL-12→ Interleukin 12		
IL-2→ Interleukin 2 (Aldesleukin)	Proleukin ®	Chiron
Receptor Tyrosine Kinase Inhibitors		
AMG 386		Amgen
Axitinib ((AG-013736)		Pfizer, Inc
Bosutinib (SKI-606)		Wyeth
Brivanib alalinate (BMS-582664)		BMS
Cediranib (AZD2171)	Recentin	AstraVeneca
Dasatinib (BMS-354825)	Sprycel ®	Bristol-Myers Squibb
Imatinib mesylate	Gleevec	Novartis
Lestaurtinib (CEP-701)		Cephalon
Motesanib diphosphate (AMG-706)		Amgen/Takeda
Nilotinib hydrochloride monohydrate	Tasigna ®	Novartis
Pazopanib HCL (GW786034)	Armala	GSK
Semaxanib (SU5416)		Pharmacia,
Sorafenib tosylate	Nexavar ®	Bayer
Sunitinib malate	Sutent ®	Pfizer, Inc.
Vandetanib (AZD647)	Zactima	AstraZeneca
Vatalanib; PTK-787		Novartis; Bayer Schering Pharma
XL184, NSC718781		Exelixis, GSK
Microtubule-Targeting Agents		
Colchicine		
Docetaxel	Taxotere ®	Sanofi-Aventis US
Ixabepilone	IXEMPRA ™	Bristol-Myers Squibb
Larotaxel		Sanofi-aventis

-continued

Other anticancer agents for combination with a bispecific anti-ErbB2/anti-ErbB3 antibody	Brand Name(s)	Manufacturer/Proprietor
Ortataxel		Spectrum Pharmaceuticals
Nanoparticle paclitaxel (ABI-007)	Abraxane ®	Abraxis BioScience, Inc.
Paclitaxel	Taxol ®	Bristol-Myers Squibb
Tesetaxel		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, Ridaforolimus)		ARIAD Pharmaceuticals, Inc
Everolimus (RAD001, RAD001C)	Certican ®, Afinitor	Novartis
Sirolimus (Rapamycin)	Rapamune ®	Wyeth Pharma
Temsirolimus (CCI-779)		
BEZ235	Torisel ®	Wyeth Pharma
AZD8055		
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

-continued

Other anticancer agents for combination with a bispecific anti-ErbB2/anti-ErbB3 antibody	Brand Name(s)	Manufacturer/Proprietor
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 Inhibitor Triciribine		
AKT DECIPHERA		Deciphera Pharmaceuticals, LLC
Perifosine (KRX0401, D-21266)		Keryx Biopharmaceuticals, Inc., AEtenta Zentaris, Inc.
Perifosine with Docetaxel		Keryx Biopharmaceuticals, Inc., AEtenta Zentaris, Inc.
Perifosine with Gemcitabine		AEtenta Zentaris, Inc.
Perifosine with Paclitaxel		Keryx Biopharmaceuticals, Inc, AEtenta 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		AEtenta 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		Eli Lilly
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
BKM120		
Cvelin-dependent kinase inhibitors		
CYC200, r-roscovitine	Seliciclib	Cyclacel Pharma
NSC-649890, L86-8275, HMR-1275	Alvocidib	NCI
TLR9, CD289		
IMOxine		Merck KGaA
HYB2055		Idera

-continued

Other anticancer agents for combination with a bispecific anti-ErbB2/anti-ErbB3 antibody	Brand Name(s)	Manufacturer/Proprietor
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)	ICOX-2 Inhibitor	Daiichi Sankyo; Tragara Pharma

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 1

<210> SEQ ID NO 1
<211> LENGTH: 1095
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 1

```
Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Asn Ile Asn Arg Asp Gly Ser Ala Ser Tyr Tyr Val Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Arg Gly Val Gly Tyr Phe Asp Leu Trp Gly Arg Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser Ala Ser Thr Gly Gly Gly Gly Ser Gly Gly
        115                 120                 125

Gly Gly Ser Gly Gly Gly Gly Ser Gln Ser Ala Leu Thr Gln Pro Ala
    130                 135                 140

Ser Val Ser Gly Ser Pro Gly Gln Ser Ile Thr Ile Ser Cys Thr Gly
145                 150                 155                 160
```

-continued

```
Thr Ser Ser Asp Val Gly Gly Tyr Asn Phe Val Ser Trp Tyr Gln Gln
                165                 170                 175

His Pro Gly Lys Ala Pro Lys Leu Met Ile Tyr Asp Val Ser Asp Arg
            180                 185                 190

Pro Ser Gly Val Ser Asp Arg Phe Ser Gly Ser Lys Ser Gly Asn Thr
        195                 200                 205

Ala Ser Leu Ile Ile Ser Gly Leu Gln Ala Asp Asp Glu Ala Asp Tyr
    210                 215                 220

Tyr Cys Ser Ser Tyr Gly Ser Ser Ser Thr His Val Ile Phe Gly Gly
225                 230                 235                 240

Gly Thr Lys Val Thr Val Leu Gly Ala Ala Ser Asp Ala His Lys Ser
                245                 250                 255

Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala
            260                 265                 270

Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Ser Pro Phe Glu
        275                 280                 285

Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys
    290                 295                 300

Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu
305                 310                 315                 320

Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly
                325                 330                 335

Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys
            340                 345                 350

Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg
        355                 360                 365

Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr
    370                 375                 380

Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe
385                 390                 395                 400

Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe
                405                 410                 415

Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys
            420                 425                 430

Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg
        435                 440                 445

Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala
    450                 455                 460

Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala
465                 470                 475                 480

Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys
                485                 490                 495

Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala
            500                 505                 510

Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu
        515                 520                 525

Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val
    530                 535                 540

Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe
545                 550                 555                 560

Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val
```

-continued

```
               565                 570                 575

Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr
            580                 585                 590

Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu
        595                 600                 605

Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val
    610                 615                 620

Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys
625                 630                 635                 640

Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn
                645                 650                 655

Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro
            660                 665                 670

Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys
        675                 680                 685

Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu
    690                 695                 700

Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val
705                 710                 715                 720

Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg
                725                 730                 735

Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu
            740                 745                 750

Phe Gln Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser
        755                 760                 765

Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val
    770                 775                 780

Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp
785                 790                 795                 800

Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu
                805                 810                 815

Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala
            820                 825                 830

Ala Leu Gly Leu Ala Ala Ala Leu Gln Val Gln Leu Val Gln Ser Gly
        835                 840                 845

Ala Glu Val Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly
    850                 855                 860

Ser Gly Tyr Ser Phe Thr Ser Tyr Trp Ile Ala Trp Val Arg Gln Met
865                 870                 875                 880

Pro Gly Lys Gly Leu Glu Tyr Met Gly Leu Ile Tyr Pro Gly Asp Ser
                885                 890                 895

Asp Thr Lys Tyr Ser Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Val
            900                 905                 910

Asp Lys Ser Val Ser Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Pro
        915                 920                 925

Ser Asp Ser Ala Val Tyr Phe Cys Ala Arg His Asp Val Gly Tyr Cys
    930                 935                 940

Thr Asp Arg Thr Cys Ala Lys Trp Pro Glu Trp Leu Gly Val Trp Gly
945                 950                 955                 960

Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Ser Gly
                965                 970                 975
```

-continued

```
Gly Gly Ser Gly Gly Gly Gly Ser Gln Ser Val Leu Thr Gln Pro Pro
            980                 985                 990

Ser Val Ser Ala Ala Pro Gly Gln  Lys Val Thr Ile Ser  Cys Ser Gly
        995                 1000                1005

Ser Ser  Ser Asn Ile Gly Asn  Asn Tyr Val Ser Trp  Tyr Gln Gln
    1010                1015                1020

Leu Pro  Gly Thr Ala Pro Lys  Leu Leu Ile Tyr Asp  His Thr Asn
    1025                1030                1035

Arg Pro  Ala Gly Val Pro Asp  Arg Phe Ser Gly Ser  Lys Ser Gly
    1040                1045                1050

Thr Ser  Ala Ser Leu Ala Ile  Ser Gly Phe Arg Ser  Glu Asp Glu
    1055                1060                1065

Ala Asp  Tyr Tyr Cys Ala Ser  Trp Asp Tyr Thr Leu  Ser Gly Trp
    1070                1075                1080

Val Phe  Gly Gly Gly Thr Lys  Leu Thr Val Leu Gly
    1085                1090                1095
```

What is claimed is:

1. A method of treating a subject having a malignant tumor, the method comprising administering to the subject an effective amount a combination therapy comprising and effective amount of each of one or more agents selected from i) an anti-estrogen agent, ii) a receptor tyrosine kinase inhibitor, iii) a MEK inhibitor, iv) a PI3 kinase inhibitor, v) an AKT inhibitor, vi) an mTOR inhibitor, vii) trastuzumab, viii) ado-trastuzumab emtansine, ix) capecitabine, x) cisplatin, and xi) nab-paclitaxel; and an effective amount of an agent that is a bispecific anti-ErbB2/anti-ErbB3 antibody that comprises the amino acid sequence set forth in SEQ ID NO:1.

2. The method of claim **1**, wherein the anti-estrogen agent is an estrogen receptor antagonist or an aromatase inhibitor.

3. The method of claim **2**, wherein the estrogen receptor antagonist is fulvestrant or tamoxifen.

4. The method of claim **2**, wherein the aromatase inhibitor is letrozole, exemestane, anastrozole, aminoglutethimide, testolactone, vorozole, formestane, or fadrozole.

5. The method of claim **4**, wherein the aromatase inhibitor is letrozole.

6. The method of claim **1**, wherein the administration to the subject of the combination therapy does not create a drug-drug interaction-mediated toxicity in the subject.

7. The method of claim **1**, wherein the administration to the subject of the combination therapy creates a substantially additive or superadditive effect as compared to the separate administration of each of the combination therapy agents alone.

8. The method of any one of claims **1** to **7**, wherein the receptor tyrosine kinase inhibitor is erlotinib, afatinib, dasatinib, gefitinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib, or sorafenib.

9. The method of claim **8**, wherein the receptor tyrosine kinase inhibitor is lapatinib.

10. The method of any one of claims **1** to **9**, further comprising co-administration to the patient of an effective amount of either or both of capecitabine and cisplatin.

11. Use of a bispecific anti-ErbB2/anti-ErbB3 antibody for combination therapy together with one or more agents selected from i) an anti-estrogen agent, ii) a receptor tyrosine kinase inhibitor, iii) a MEK inhibitor, iv) a PI3 kinase inhibitor, v) an AKT inhibitor, vi) an mTOR inhibitor, vii) trastuzumab, viii) ado-trastuzumab emtansine, ix) capecitabine, and x) cisplatini) an anti-estrogen agent, ii) a receptor tyrosine kinase inhibitor, iii) a MEK inhibitor, iv) a PI3 kinase inhibitor, v) an AKT inhibitor, vi) an mTOR inhibitor, vii) trastuzumab, viii) ado-trastuzumab emtansine, ix) capecitabine, and x) cisplatin; for treatment of a malignant tumor.

12. The use of claim **11**, wherein the anti-estrogen agent is an estrogen receptor antagonist or an aromatase inhibitor.

13. The use of claim **12**, wherein the estrogen receptor antagonist is fulvestrant or tamoxifen.

14. The use of claim **12**, wherein the aromatase inhibitor chosen from the group consisting of letrozole, exemestane, anastrozole, aminoglutethimide, testolactone, vorozole, formestane, and fadrozole.

15. The use of claim **14**, wherein the aromatase inhibitor is letrozole.

16. The use of any one of claims **11** to **15**, wherein the receptor tyrosine kinase inhibitor is chosen from the group consisting of erlotinib, afatinib, dasatinib, gefitinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib and sorafenib.

17. The use of claim **16**, wherein the receptor tyrosine kinase inhibitor is lapatinib.

18. An aqueous solution comprising a bispecific anti-ErbB2/anti-ErbB3 antibody and one or more of i) an anti-estrogen agent, ii) a receptor tyrosine kinase inhibitor, iii) a MEK inhibitor, iv) a PI3 kinase inhibitor, v) an AKT inhibitor, vi) an mTOR inhibitor, vii) trastuzumab, viii) ado-trastuzumab emtansine, ix) capecitabine, x) cisplatin, and xi) nab-paclitaxel.

19. 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 claim **18**.

20. The method of claim **1**, wherein said MEK inhibitor is selumetinib, trametinib, UO126, or PD0325901, said PI3K inhibitor is BKM-120 or GDC-0941, and said AKT inhibitor is triciribine or MK-2206.

21. The use of claim **11**, wherein said MEK inhibitor is selumetinib, trametinib, UO126, or PD0325901, said PI3K inhibitor is BKM-120 or GDC-0941, and said AKT inhibitor is triciribine or MK-2206.

22. The aqueous solution of claim **18**, wherein said MEK inhibitor is selumetinib, trametinib, UO126, or PD0325901, said PI3K inhibitor is BKM-120 or GDC-0941, and said AKT inhibitor is triciribine or MK-2206.

23. The method of claim **1**, comprising co-administering the MEK inhibitor and the bispecific anti-ErbB2/anti-ErbB3 antibody.

24. The method of claim **1**, comprising co-administering the PI3K inhibitor and the bispecific anti-ErbB2/anti-ErbB3 antibody.

25. The method of claim **1**, comprising co-administering the mTOR inhibitor and the bispecific anti-ErbB2/anti-ErbB3 antibody.

26. The method of claim **1**, comprising co-administering trastuzumab or ado-trastuzumab emtansine and the bispecific anti-ErbB2/anti-ErbB3 antibody.

27. The method of claim **1**, wherein said mTOR inhibitor is BEZ235, AZD8055, everolimus, temsirolimus, sirolimus, or ridaforolimus.

28. The use of claim **11**, wherein said mTOR inhibitor is BEZ235, AZD8055, everolimus, temsirolimus, sirolimus, or ridaforolimus.

29. The aqueous solution of claim **18**, wherein said mTOR inhibitor is BEZ235, AZD8055, everolimus, temsirolimus, sirolimus, or ridaforolimus.

* * * * *