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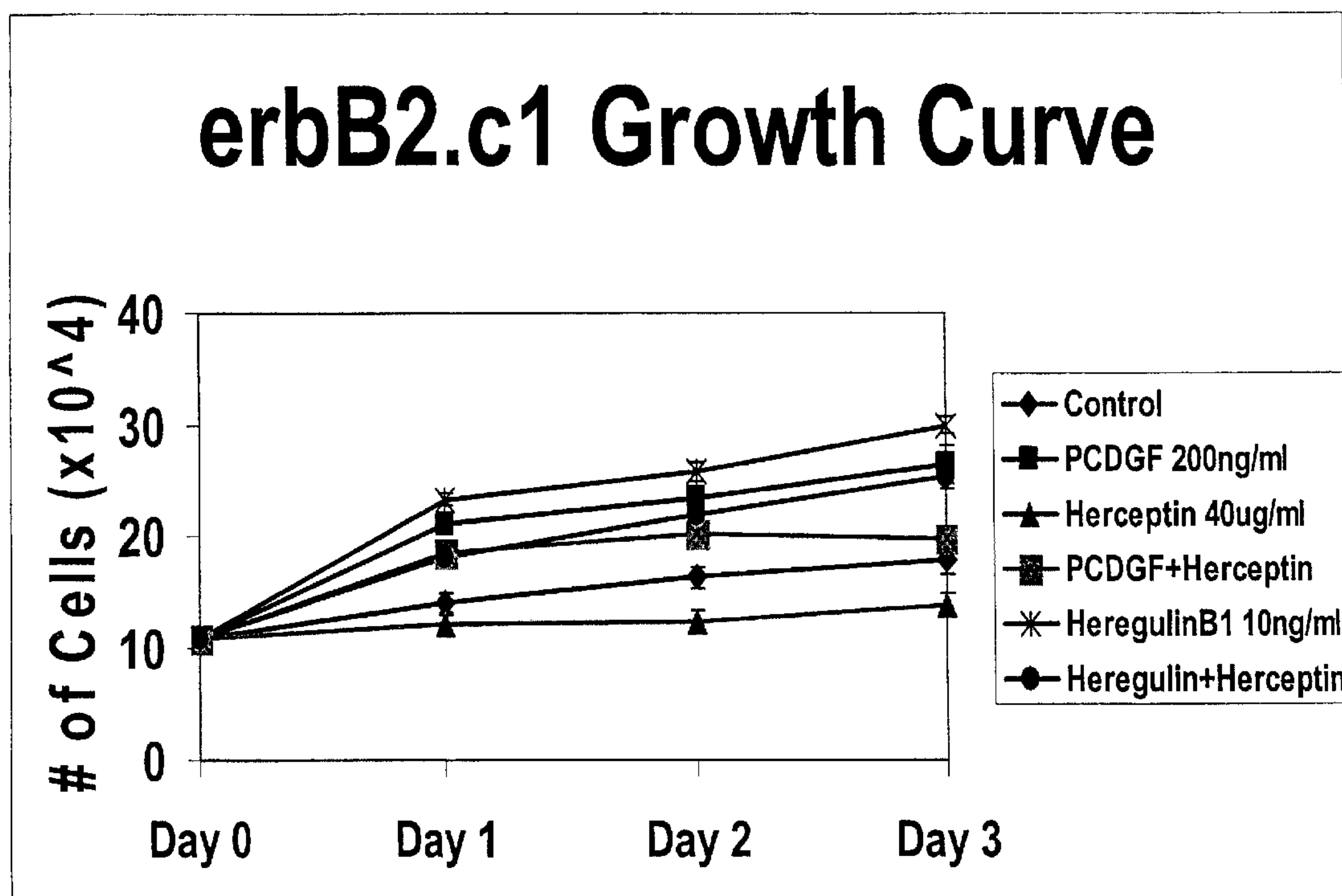
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(54) Titre : COMPOSITIONS ET METHODES POUR RESTAURER LA SENSIBILITE AUX ANTAGONISTES DE HER2
LORS D'UN TRAITEMENT

(54) Title: COMPOSITIONS AND METHODS FOR RESTORING SENSITIVITY TO TREATMENT WITH HER2
ANTAGONISTS



(57) **Abrégé/Abstract:**

Methods and compositions for restoring growth inhibition sensitivity to a tumor cell resistant to growth inhibition by HER2 antagonists. The methods involve administering a PCDGF antagonist to the cell in an amount effective to restore growth inhibition sensitivity to HER2 antagonists. The invention also provides treatment regimens, and therapeutic compositions comprising an HER2 antagonist and a PCDGF antagonist.

ABSTRACT

Methods and compositions for restoring growth inhibition sensitivity to a tumor cell resistant to growth inhibition by HER2 antagonists. The methods involve administering a PCDGF antagonist to the cell in an amount effective to restore growth inhibition sensitivity to HER2 antagonists. The invention also provides treatment regimens, and therapeutic compositions comprising an HER2 antagonist and a PCDGF antagonist.

COMPOSITIONS AND METHODS FOR RESTORING SENSITIVITY TO TREATMENT WITH HER2 ANTAGONISTS

BACKGROUND OF THE INVENTION

[0001] Approximately 25-30% of breast cancer patients overexpress the proto-oncoprotein and cell surface receptor c-erbB2 (human epidermal growth factor receptor 2 protein), also known as HER2/neu. Overexpression of the c-erbB2 oncogene has been linked to poor outcome and decreased survival for patients. The HER-2/neu proto-oncogene is overexpressed in 20-30% of metastatic breast cancers, and is associated with decreased survival and increased recurrence of breast cancer. HER-2/neu is also overexpressed in other cancers types including endometrial cancer, kidney cancer, gastric cancer, and prostate cancer. Presently, the most common form of treatment for these patients is the use of the humanized monoclonal antibody Trastuzumab, also known as Herceptin®.

[0002] Herceptin® is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity ($K_d = 5$ nM) to the extracellular domain of c-erbB2 in a cell-based assay. See Science 1985;230:1132-9 and Cancer Res 1993;53:4960-70 hereby incorporated by reference in its entirety. Herceptin® is an IgG1 kappa antibody that binds to HER2 and contains human framework regions with the complementarity-determining regions of a murine antibody (4D5). Id. However, only 25% of the patients treated with Herceptin® or any other antibody to c-erbB2/HER2 are responsive to this therapy. Several models have been postulated to explain resistance to treatment with c-erbB2/HER2 antibodies.

BRIEF SUMMARY OF THE INVENTION

[0003] The present invention is based in part on the discovery that an autocrine growth factor, PC-Cell Derived Growth Factor ("PCDGF"), confers resistance to the antineoplastic effects of c-erbB2/HER2 ("HER2") antagonists. Preferred embodiments of this invention are directed to therapeutic compositions and methods for restoring growth inhibition sensitivity to tumor cells resistant to the antineoplastic effects of HER2 antagonists by administering a PCDGF antagonist in an amount effective to restore growth inhibition sensitivity to HER2 antagonists. Another embodiment of the invention provides therapeutic compositions and methods for inhibiting tumor cell growth comprising administering a PCDGF antagonist and a HER2 antagonist in an amount effective to inhibit tumor cell growth.

[0004] The invention also provides preferred compositions comprising a HER2 antagonist and a PCDGF antagonist. In another embodiment, the invention provides a pharmaceutical composition comprising a HER2 antagonist, a PCDGF antagonist, and a pharmaceutically-acceptable carrier (e.g., water, saline, Ringer's solution, dextrose solution, and human serum albumin).

[0005] Further embodiments of the invention provide methods of determining whether a patient is resistant to the antineoplastic effects of HER2 antagonists, comprising obtaining a biological sample containing cells from a patient; detecting PCDGF in the biological sample; and determining the amount of PCDGF in said sample wherein the amount of PCDGF is indicative of resistance to the antineoplastic effects of HER2 antagonists.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] FIG. 1 summarizes pathological studies in paraffin embedded human breast cancer biopsies. PCDGF and erbB2 staining were monitored by

immunohistochemistry. Thus, overexpression of PCDGF in cells overexpressing erbB2 renders the cells HER2 antagonist resistant. It is known that 25% of patients with tumors that overexpress erbB2 will be responsive to HER2 antagonist therapy.

[0007] FIG. 2 shows erbB2 levels in MCF7 cells (breast cancer cells) transfected with erbB2.

[0008] FIG. 3 shows PCDGF expression in MCF7 cells transfected with erbB2.

[0009] FIG. 4 shows PCDGF protein levels in conditioned media of cells transfected with both PCDGF and erbB2 (D.c2) and erbB2 alone (erbB2.c1).

[0010] FIG. 5 shows that PCDGF stimulates erbB2 phosphorylation in erbB2 overexpressing cells.

[0011] FIG. 6 shows that PCDGF stimulates erbB2 phosphorylation in other breast cancer cells that are known to overexpress erbB2 such as BT474 and SKBR3 (see FIG. 12).

[0012] FIG. 7 shows the dose response of PCDGF stimulation of erbB2 in BT474 cells.

[0013] FIG. 8 shows the long term growth of erbB2 cells in response to Herceptin®. Herceptin® inhibits proliferation of erbB2 overexpressing cells as it is a monoclonal antibody that neutralizes erbB2 and inhibits proliferation in erbB2 overexpressing breast cancer cells.

[0014] FIG. 9 shows that when PCDGF is overexpressed in the erbB2 overexpressing cells (e.g., D.c2 cells), Herceptin® no longer inhibits the proliferation of the breast cancer cells.

[0015] FIG. 10 shows the same results as FIG. 9 using thymidine incorporation as a measure of cell growth.

[0016] FIG. 11 shows that PCDGF overexpression prevents Herceptin® inhibition of breast cancer cells in a soft agar (tumorigenesis) assay in erbB2 overexpressing cells. These results demonstrate that overexpression of PCDGF confers Herceptin® resistance in erbB2 overexpressing cells.

[0017] FIG. 12 shows that PCDGF stimulates erbB2 phosphorylation in SKBR3 cells. Overexpression of PCDGF confers Herceptin® resistance in SKBR3 cells overexpressing erbB2.

DETAILED DESCRIPTION OF THE INVENTION

[0018] PCDGF is an 88 kDa autocrine growth factor characterized in our laboratory and shown to be overexpressed in and induce tumorigenesis of a wide variety of human and animal tumor cells (e.g., neuroblastoma, glioblastoma, astrocytoma, sarcomas, and cancers of the prostate, blood, cerebrospinal fluid, liver, kidney, breast, head and neck, pharynx, thyroid, pancreas, stomach, colon, colorectal, uterus, cervix, bone, bone marrow, testes, brain, neural tissue, ovary, skin, and lung). See, e.g., U.S. Patent Number 6,309,826, hereby incorporated by reference in its entirety.

[0019] PCDGF also confers resistance to the antineoplastic effects HER2 antagonists (e.g., Herceptin®, HER2 kinase inhibitors) on tumor cells. As described in U.S. Patent Number 6,309,826, overexpression of PCDGF leads to uncontrolled cell growth and increased tumorigenesis. The degree of PCDGF

overexpression directly correlates with the degree of cellular tumorigenicity. Cells overexpressing PCDGF do not require external signals to maintain uncontrolled cell growth. Loss of regulated cell growth, such as a loss in responsiveness to insulin and/or estrogen, leads to increased malignancy and excessive unregulated cell growth. Development of methods and compositions that interfere with the tumorigenic activity of PCDGF is therefore of great interest for the treatment of cancer.

[0020] HER2 antagonist therapy (e.g., but not limited to, Herceptin® and HER2 kinase inhibitors) is useful for treatment of patients with metastatic breast cancer, including patients whose tumors overexpress the HER2 protein and who have received one or more chemotherapy regimens for their metastatic disease. Herceptin®, in particular, also is approved for combination therapy with paclitaxel for treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have not received chemotherapy for their metastatic disease. However, only 25% of the patients treated with Herceptin® or any other antibody to HER2 are responsive to such therapy.

[0021] PCDGF antagonists, such as anti-PCDGF antibodies, interfere with the biological activity of PCDGF (e.g., tumorigenic activity) by binding PCDGF directly and preventing PCDGF from transmitting cell growth signals to a target cell (e.g., breast cancer cell). An anti-PCDGF antibody may bind the active site of PCDGF (e.g., the PCDGF receptor binding site) and prevent PCDGF from binding to its receptor. Alternatively, anti-PCDGF antibodies may bind to a site on PCDGF other than the active site, alter the conformation of the active site, and thus render PCDGF incapable of binding to its receptor. Anti-PCDGF antibodies include PCDGF neutralizing antibodies. "Neutralizing" antibodies have the ability to inhibit or block the normal biological activity of PCDGF,

including PCDGF's ability to stimulate cell proliferation, increase cell survival, block apoptosis, or induce tumor growth in animals and in humans. PCDGF antagonists also include nucleic acids (antisense, siRNA etc.) and anti-PCDGF receptor antibodies. PCDGF confers resistance to the antineoplastic effects of HER2 antagonists. Immunohistochemistry studies in paraffin embedded human breast cancer biopsies showed that PCDGF was highly expressed in 25% of c-erbB2 positive (+3) invasive ductal carcinomas.

[0022] Preferred embodiments of the invention are directed to methods of restoring growth inhibition sensitivity to tumor cells resistant to the antineoplastic effects of HER2 antagonists by administering a PCDGF antagonist to a human patient in an amount effective to restore the patient's growth inhibition sensitivity to HER2 antagonists.

[0023] The term "HER2 antagonist" refers to any molecule (e.g., protein, peptide, small molecule, nucleic acid, antisense, or siRNA) that is capable of binding, interfering with, or inhibiting the activity of HER2 or any analogs or derivatives of HER2 that retain the neoplastic properties of HER2 (e.g., but not limited to Herceptin® and HER2 kinase inhibitors).

[0024] In one embodiment, a HER2 antagonist includes a molecule that can target or selectively bind to HER2 and, for example, deliver a toxin or other compound or molecule to kill a cell or inhibit cell growth. For example, a HER2 antibody can be coupled to a toxin or chemotherapeutic agent that is delivered to a tumor cell after the antibody binds to HER2. HER2 antagonists also include molecules (e.g., peptides, small molecules, antisense molecules, and siRNA) that modulate the biological activity of molecules that regulate the activity of HER2. A HER2 antagonist can be an antibody that recruits an immune response, e.g., through ADCC (antibody dependent cell cytotoxicity).

[0025] The term "PCDGF antagonist" refers to any molecule (e.g., protein, peptide, small molecule, nucleic acid, antisense, or siRNA) that is capable of selectively binding, interfering with, or inhibiting the biological activity of PCDGF including, but not limited to, the HER2 antagonist resistance-conferring activity of PCDGF or any analogs or derivatives of PCDGF that retain the properties of PCDGF. In one embodiment, a PCDGF antagonist includes PCDGF receptor antibodies that can target or selectively bind to the PCDGF receptor and, for example, deliver a toxin or other compound or molecule to kill a cell or inhibit cell growth.

[0026] The invention also provides pharmaceutical compositions containing one or more HER2 antagonists and one or more PCDGF antagonists. Patients receiving both HER2 and PCDGF antagonists will benefit from the antineoplastic effects of each antagonist in that the PCDGF antagonist can restore the HER2 antagonist sensitivity of patients resistant to HER therapy. Pharmaceutical compositions comprising a HER2 antagonist, a PCDGF antagonist, and an acceptable pharmaceutical carrier (such as water, saline, Ringer's solution, dextrose solution, and human serum albumin) are also provided.

[0027] Preferred embodiments of the invention also include methods of determining whether a patient is resistant to the antineoplastic effects of HER2 antagonist therapy by obtaining a biological sample containing cells from the patient; detecting PCDGF in the biological sample; and determining the amount of PCDGF in the sample wherein the amount of PCDGF is indicative of resistance to the antineoplastic effects of HER2 antagonist therapy.

[0028] The term antibody herein includes but is not limited to human and non-human polyclonal antibodies, human and non-human monoclonal

antibodies (mAbs), chimeric antibodies, anti-idiotypic antibodies (anti-IdAb), neutralizing antibodies, non-neutralizing antibodies, and humanized antibodies. Polyclonal antibodies are heterogeneous populations of antibody molecules derived either from sera of animals immunized with an antigen or from chicken eggs. Monoclonal antibodies ("mAbs") are substantially homogeneous populations of antibodies to specific antigens. mAbs may be obtained by any suitable method. Such antibodies may be of any immunological class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The term antibody is also meant to include both intact molecules as well as fragments thereof such as, for example, Fab and F(ab')₂, which are capable of binding to the antigen. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to generate Fab fragments) and pepsin (to generate F(ab')₂ fragments).

[0029] In yet another embodiment, a subject's cells (e.g., tumor cells) are removed from the body, transfected with a polynucleotide encoding a HER2 antagonist and a PCDGF antagonist, and injected at the site of the tumor. Expression of the polynucleotides encoding the HER2 antagonist and PCDGF antagonist localizes the HER2 and PCDGF antagonists at the tumor site. A subject's cells (e.g., tumor cells) can also be directly transfected in the body (e.g., *in situ*) with a construct containing a nucleic acid encoding a HER2 antagonist and/or a PCDGF antagonist. Expression of the nucleic acid results in production of the HER2 antagonist and/or PCDGF antagonist inside the transfected cell.

[0030] For *in vivo* applications, PCDGF antagonists, and HER2 antagonists can be provided to a subject by a variety of administration routes and

dosage forms. A subject, preferably a human subject, suffering from a neoplastic condition, including but not limited to breast cancer, or other disease condition associated with increased HER2 and/or PCDGF expression, is treated consecutively or simultaneously with an HER2 antagonist and a PCDGF antagonist. In one embodiment, the HER2 antagonist and PCDGF antagonist are co-administered. In another embodiment, the HER2 antagonist and PCDGF antagonist are sequentially administered, preferably the level of PCDGF ascertained and, if elevated, the PCDGF antagonist being administered first.

[0031] Treatment with a PCDGF antagonist can precede, follow, or be conducted concurrently with treatment with a HER2 antagonist. Treatment with a PCDGF antagonist may precede or follow treatment with a HER2 antagonist by intervals ranging from minutes to weeks. In another embodiment, a PCDGF antagonist and a HER2 antagonist are administered in a way to ensure that a prolonged period of time does not elapse between the time of administration of each agent. For example, each antagonist can be administered to a patient within seconds, minutes, or hours of the other antagonist.

[0032] In a preferred embodiment, treatment with a PCDGF antagonist increases c-erbB2 phosphorylation by at least 10%, more preferably by at least 50%. In a further preferred embodiment, treatment with a PCDGF antagonist increases HER2 sensitivity by at least two-fold, more preferably by at least three-fold.

[0033] The antagonists of the present invention may be administered by any means that achieves their intended purpose. For example, antibody administration may be by various routes including but not limited to subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, and oral. Parenteral administration can be by bolus injection or by gradual perfusion over

time. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions, which may contain auxiliary agents or excipients. Pharmaceutical compositions such as tablets and capsules can also be prepared. It is understood that the dosage will be dependent upon the age, sex and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired.

[0034] In another embodiment of the invention, a HER2 antagonist in combination with a PCDGF antagonist and/or chemotherapy (e.g., paclitaxel, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil, vincristin, vinblastin and methotrexate, or any analog or derivative or variant of the foregoing) can be used to treat metastatic breast cancer or cancers of a variety of tissues (e.g., neuroblastoma, glioblastoma, astrocytoma, sarcomas, and cancers of the prostate, blood, liver, kidney, breast, head and neck, pharynx, thyroid, pancreas, stomach, colon, colorectal, uterus, cervix, bone, bone marrow, testes, brain, neural tissue, ovary, skin, and lung). Combination therapy can be administered independently of diagnostic evaluation.

[0035] In a preferred modality of the invention, a breast cancer patient (e.g., having metastatic breast cancer and overexpressing HER2) is given HER2 antagonist treatment with, for example, Herceptin. Although, the patient exhibits some degree of tumor growth inhibition, her progress declines, and she becomes resistant to HER2 antagonist therapy. A biopsy or serum sample is

conducted and reveals that the patient has elevated levels of PCDGF. In another embodiment, the patient's PCDGF levels are determined prior to beginning HER2 antagonist therapy. The patient is then treated with a PCDGF antagonist alone, or co-administration of PCDGF antagonist and HER2 antagonist, to restore sensitivity to HER2 antagonist therapy. Following treatment with PCDGF antagonist, the patient is again responsive to HER2 antagonist therapy. The PCDGF level of the patient is periodically monitored, for example, weekly or monthly throughout the HER2 therapy and a PCDGF antagonist provided to again restore HER2 sensitivity if needed. Alternatively, the patient can continue to receive co-administration of PCDGF antagonist and HER2 antagonist.

[0036] The ranges of effective doses provided below are not intended to limit the invention and merely represent illustrative dose ranges. However the most preferred dosage will be tailored to the individual subject as is understood and determinable by one of ordinary skill in the art given the teachings herein. The total dose required for each treatment may be administered by multiple doses or in a single dose. In one embodiment, effective amounts of each of a HER2 antibody and a PCDGF antibody are from about 0.01 ng to about 500 µg/ml and preferably from about 10 ng to about 100 µg/ml. An HER2 antagonist and a PCDGF antagonist may be administered alone, administered together, or in conjunction with other therapeutics. In another embodiment, the amount of each antagonist administered will typically be in the range of about 0.1 to about 10 mg/kg of patient weight, so long as the HER2 and PCDGF antagonists are administered to the patient in therapeutically effective amounts (i.e., amounts that eliminate and/or reduce the patient's tumor burden or restore sensitivity to the antineoplastic effects of any HER2 antagonists).

[0037] A preferred treatment regimen comprises co-administration of an effective amount of a HER2 antagonist and a PCDGF antagonist over a period of one or several weeks and including between about one week and six months. In another embodiment, a HER2 antagonist can be provided in an initial dose of 4 mg/ kg followed by 2 mg/kg intravenous (i.v.) weekly; or dose of 8 mg/kg initial dose followed by 4 mg/kg i.v. weekly. Treatment with a PCDGF antagonist can be provided, for example, in an initial dose of 4 mg/ kg followed by 2 mg/kg intravenous (i.v.) weekly; or a dose of 8 mg/kg initial dose followed by 4 mg/kg i.v. weekly. Additional examples of regimens for cancer treatment that can be used for treatment with a HER2 antagonist, a PCDGF antagonist, and/or chemotherapeutic agents are disclosed in the following articles, hereby incorporated by reference in their entirety: Hamid, O., *J Am Pharm Assoc*, 2004 Jan-Feb;44(1):52-8; Kubo et al., *Anticancer Res*. 2003 Nov-Dec; 23(6a):4443-9; Slamon et al., *N Engl J Med*. 2001;344:783-792.; Baselga et al., *Cancer Res* 1998;58:2825-2831; and Jones, et al., *Ann Oncol*. 2003 Dec;14(12):1697-704.

EXAMPLE 1

PCDGF CONFERS RESISTANCE TO THE ANTINEOPLASTIC EFFECTS OF HER2 ANTAGONIST THERAPY

[0038] To investigate the role of PCDGF in HER2 antagonist resistance of c-erbB2 overexpressing cells, MCF-7 breast cancer cells were stably transfected with c-erbB2 cDNA. A c-erbB2 overexpressing clone (erbB2.c1) was selected, and used for the subsequent stable transfection with PCDGF cDNA. Clones overexpressing both c-erbB2 and PCDGF were obtained and further examined in comparison to single erbB2 overexpressing cells. We show in the appended Figures that dual-overexpressing clones (D.c2) (i.e., clones overexpressing both PCDGF and erbB2) were HER2 antagonist resistant with respect to their ability to proliferate in vitro, whereas the erbB2.c1 cells were sensitive to Herceptin®

treatment. These Herceptin®-sensitive erbB2.c1 cells also had a growth advantage when treated with PCDGF. Moreover PCDGF stimulates c-erbB2 phosphorylation in these cells. We also show that the dual expressing D.c2 cells were more resistant than the erbB2.c1 cells to the antiestrogen ICI 182,780. Our findings demonstrate that PCDGF confers Herceptin® resistance in c-erbB2 overexpressing tumors. See Figures. Blocking PCDGF action would be beneficial for patients undergoing HER2 antagonist treatment by reversing the initial resistance.

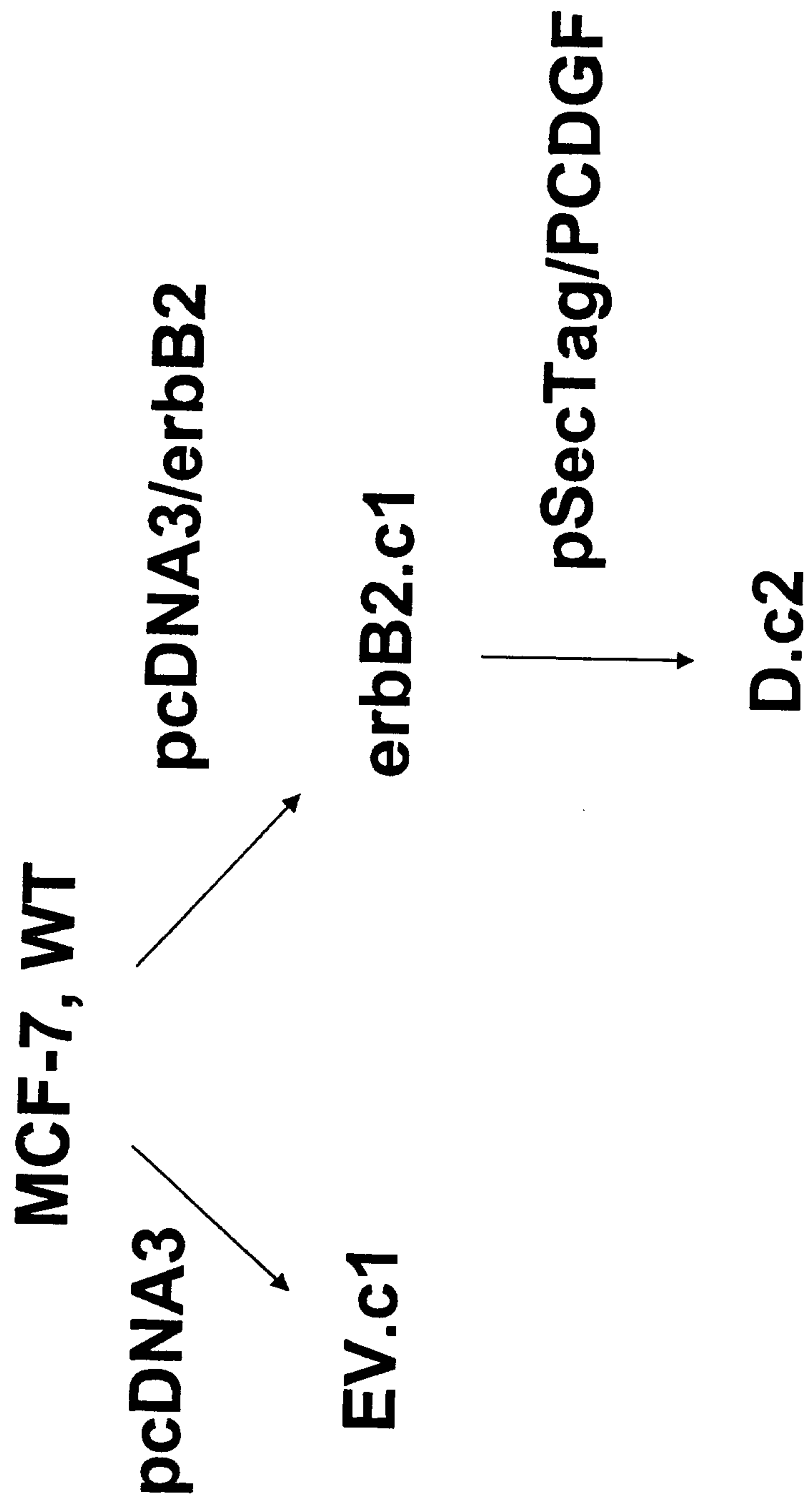
The increase of the endogenous levels of PCDGF in erbB2-overexpressing human breast cancer cells provides Herceptin resistance to these cells.

Methods

- **Stable transfections were carried out by the Calcium phosphate method. Clones were established in the presence of Zeocin and/or G418.**
- **Cell proliferation assays: Cells were plated in DMEM/F12 (+5% FBS). 24 hrs. later, cells were washed, and fresh PFMEM was added along with the various treatments. Cells were counted for the next three days using a haemocytometer.**

- **Thymidine incorporation assays:** Cells were plated in DMEM/F12 (+5% FBS). 48 hrs. later, cells were treated with various drugs. 48 hrs. later, 1 μ Ci of ³H-Thymidine was added to each well. 24 hrs. later, cell lysates were collected, and levels of ³H-Thymidine were measured by liquid scintillation counter.
- **Activation of erbB2:** Cells were plated in DMEM/F12 (+5% FBS). 48 hrs. later, PFMEM was added. 24 hrs. later, various treatments were added. Samples were lysed with RIPA buffer, and separated on a SDS-PAGE gel, and transferred onto a PVDF membrane. Membranes were blocked, and incubated with anti-phospho-erbB2, followed by incubation with secondary antibody. Proteins were detected with ECL.

Stable Transfectants



CLAIMS

1. A method of restoring growth inhibition sensitivity to a tumor patient resistant to growth inhibition by an HER2 antagonist, comprising administering a PCDGF antagonist to the patient in an amount effective to restore growth inhibition sensitivity to an HER2 antagonist.
2. A method of inhibiting tumor cell growth comprising administering a PCDGF antagonist and an HER2 antagonist to the patient in an amount effective to inhibit tumor growth.
3. The method of claim 2, wherein the antagonists are co-administered.
4. A pharmaceutical composition comprising an HER2 antagonist, and a PCDGF antagonist.
5. The method/composition of claims 1-4, wherein the HER2 antagonist is Herceptin.
6. The method/composition of claims 1-4, wherein the PCDGF antagonist is an antibody.
7. The method/composition of claims 1-4 is an antisense oligonucleotide.

8. The method/composition of claims 1-4 is a small molecule.
9. The method/composition of claims 1-4 is siRNA.
10. The method of claim 2, further comprising monitoring the patient's level of PCDGF.

Application number / numéro de demande: 2459622

Figures: 2,3,5,6,7

Pages: _____

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au
10^{ème} étage)

**PCDGF and erbB2 expression
in human breast cancer biopsies:**

ErbB2	Cases #	PCDGF staining		
		0	1	2
				3
0	n = 9	1	1	4
				3
2	n = 6	2	3	1
				0
3	n = 7	0	4	2
				1

Figure 1

PCDGF Levels in Conditioned Media of D.c2 vs. erbB2.c1

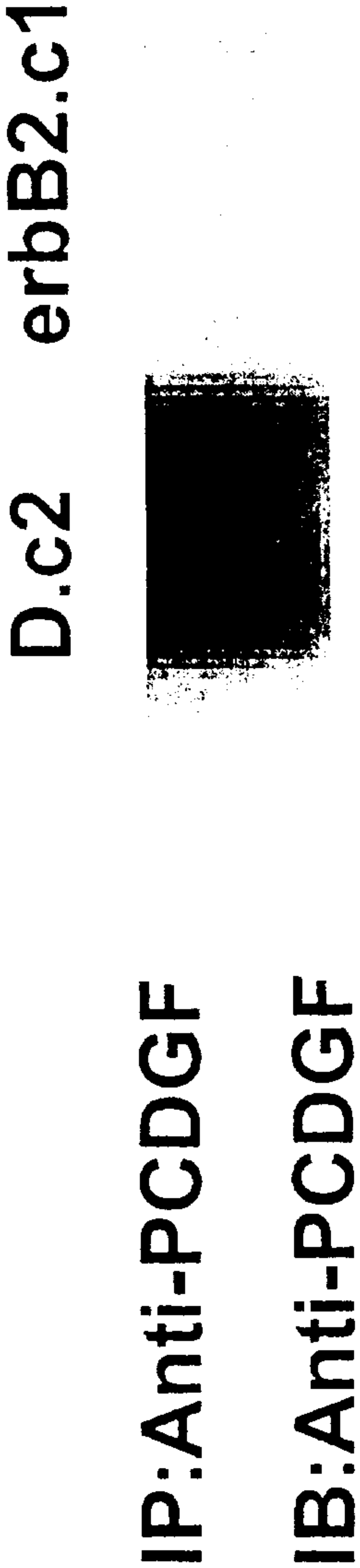


Figure 4

erbB2.c1 Growth Curve

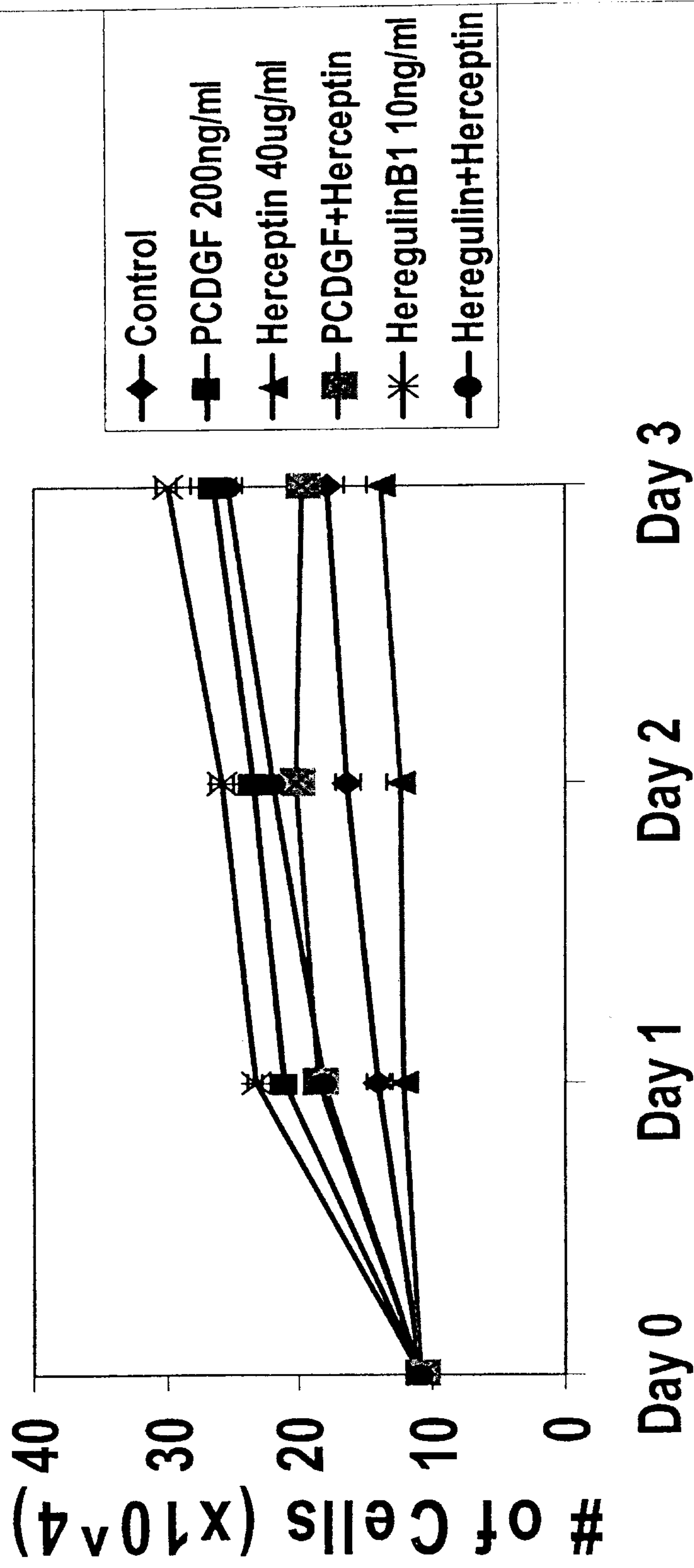


Figure 8

Herceptin Resistance of D.c2

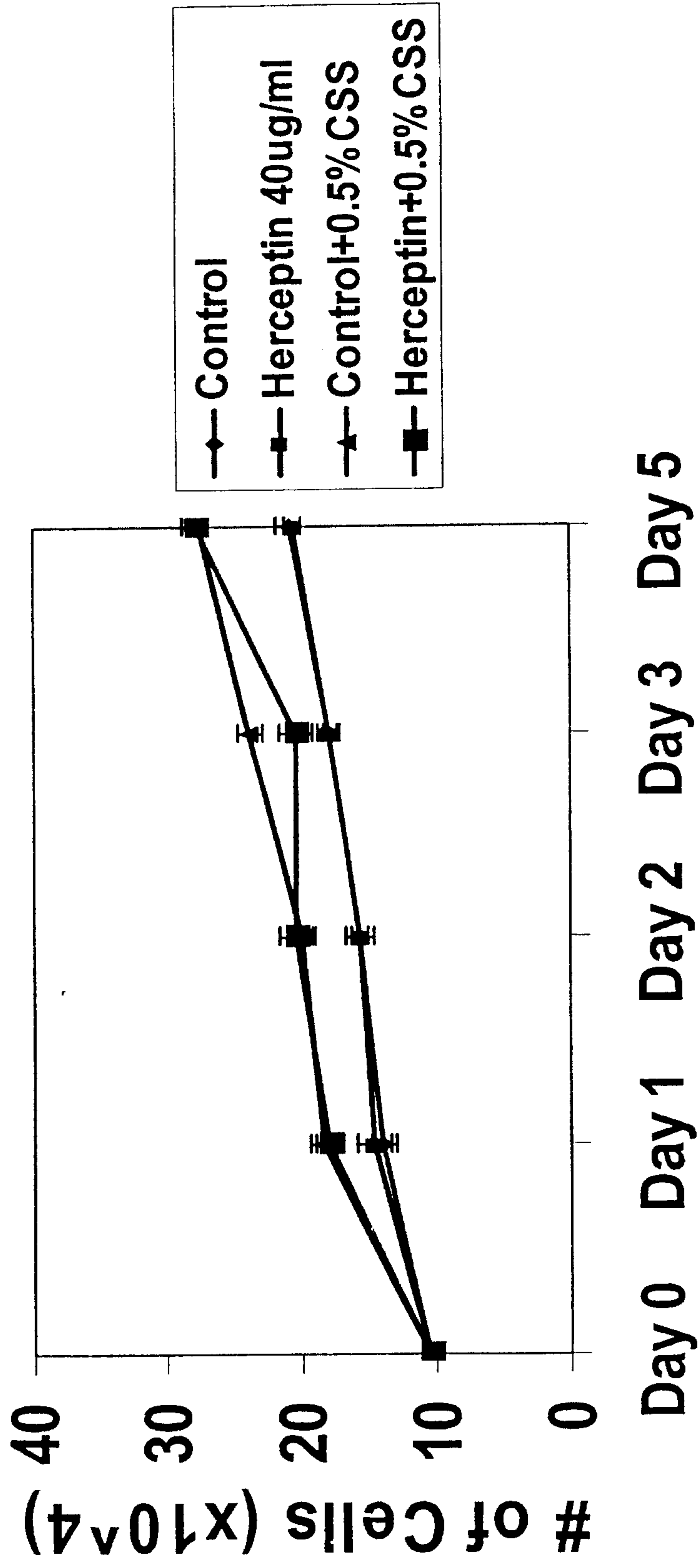


Figure 9

Thymidine Incorporation: D.c2 Herceptin Response

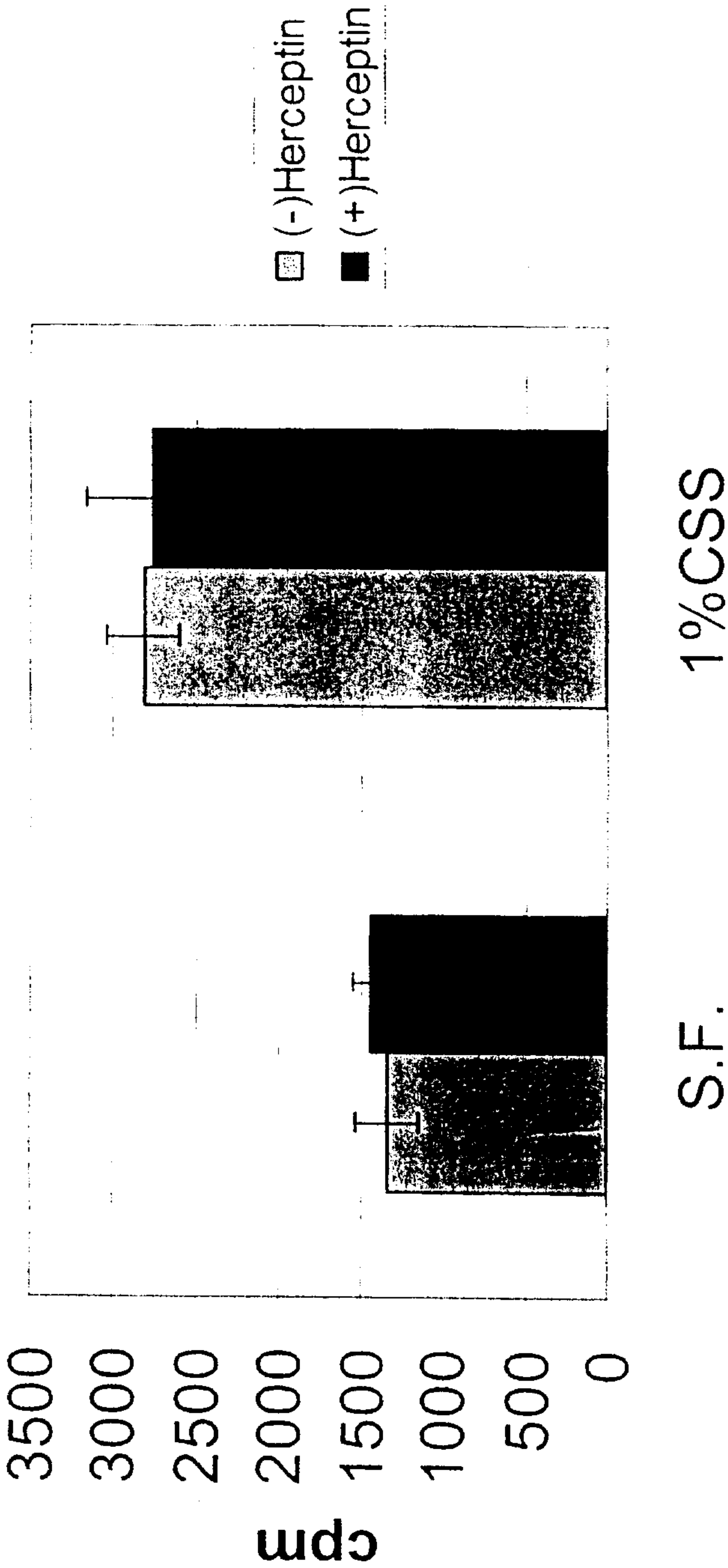


Figure 10

Soft Agar Assay: Herceptin Response

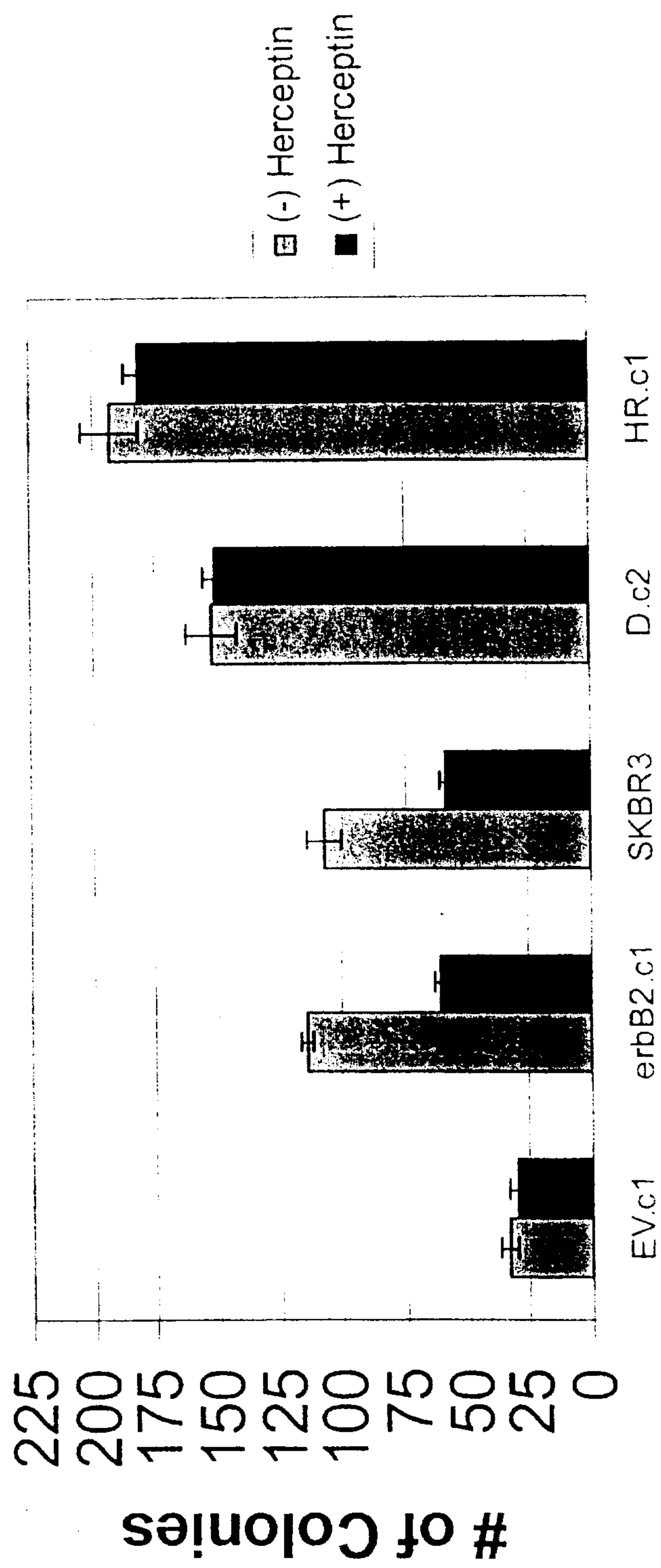


Figure 11

SKBR3 Herceptin Response

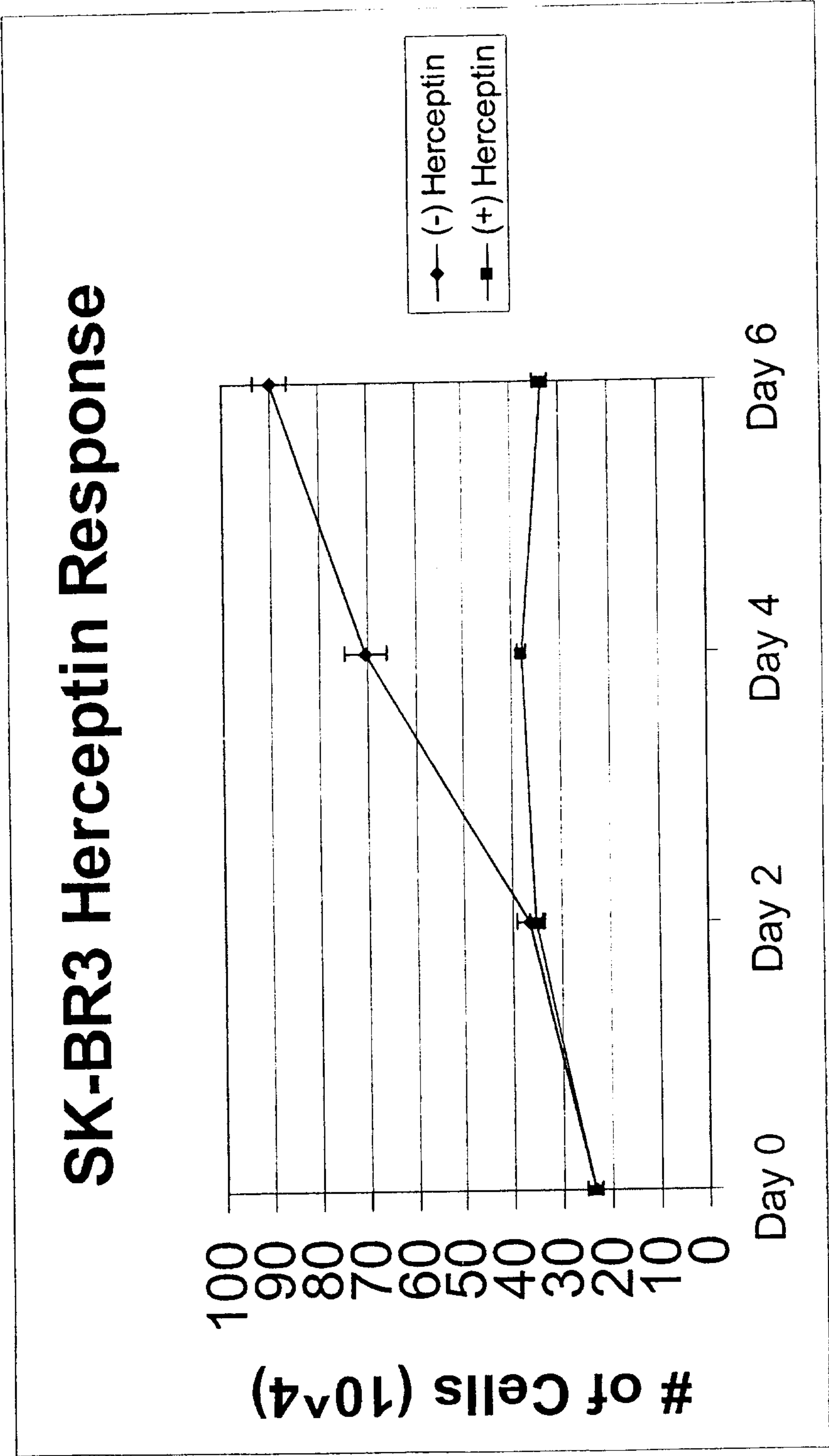


Figure 12

erbB2.c1 Growth Curve

