Treatment of Meningeal Breast Cancer Xenografts in the Rat Using an Anti-P185/HER2 Antibody

Ira Bergman, Mamdouha A. Barmada, Judith A. Griffin, and Dennis J. Slamon

INTRODUCTION

Breast cancer is the most common malignancy in women, with an incidence of 180,000 women/year (1). Metastases to the leptomeninges (LM) occur in 2–5% of patients with metastatic disease, and in 20–25% of these women this is the first or only site of progressive illness (2–5). The prevalence of LM metastases is likely to increase as therapy for systemic metastases becomes more effective and patient survival improves (2). This is attributable to the inability of most systemically delivered therapeutics to cross the blood-brain barrier. This site of metastasis will limit cure, because treatment of LM metastases is usually ineffective or only transiently effective and frequently is accompanied by serious toxicities (2–4). Sixty-five percent of patients with LM metastases respond to therapy with whole brain radiation and IVent methotrexate, but median survival is only 6 months. Median survival in the 35% of patients who do not respond to therapy is 1 month (2–7). Mortality from therapy is 7%, and serious debilitating neurotoxicity occurs in 65% of patients who survive >4 months (6, 7).

Weekly treatment with a humanized antibody to the HER2/neu receptor, recombinant human MAb HER2, was recently found to be effective both as a single agent and in combination with standard chemotherapy in patients with HER2/neu-overexpressing metastatic breast cancer (8–11). Gene amplification of HER2/neu has been noted in 25–30% of primary human breast cancers as well as in 8–11% of ovarian cancers and is an independent predictor of poor prognosis (12–14). Amplification of this gene is thought to be one of the earliest clinical and pathogenic alterations in these tumors (15–17). Expression of HER2/neu appears to be stable over time and generally congruent at different metastatic sites within a given patient (18). MAb 4D5, a murine IgG1, recognizes the extracellular domain of the HER2/neu receptor, produces agonist-like phosphorylation and internalization of the HER2/neu receptor (19), and results in a dose-dependent growth inhibition of HER2/neu-overexpressing cancer cell lines (19–22). It has been used successfully for the treatment of HER2/neu-overexpressing human breast and ovarian cancer xenografts in the s.c. space or subrenal capsule of nude mice (22–25). MAb 4D5 and cisplatin in combination produced a synergistic inhibitory effect in HER2-overexpressing cells both in vitro and in vivo (22, 24). Immunoautoradiography demonstrated that MAb 4D5 administered either i.v. or i.p. rapidly and specifically localized to HER2/neu-overexpressing tumor xenografts in vivo with relatively little antibody accumulation in other body compartments (25).

The current study evaluated the efficacy of MAb 4D5 in the treatment of LM breast cancer xenografts. Continuous IVent administration of 4D5 inhibited the growth of SKBR3 cells that overexpress HER2/neu but not of MCF7 cells, which do not. Inhibition was dose-dependent, with higher doses of 4D5 producing an improved response. I.p. administration of cisplatin in addition to 4D5 did not improve results. Continuous administration of 4D5 into the lumbar, as opposed to the ventricular intrathecal space, was not therapeutically effective. Treatment with 4D5 did not result in outgrowth of cells lacking expression of the HER2/neu receptor. These results suggest that 4D5, administered regionally, may palliate LM metastases from HER2/neu-overexpressing breast carcinoma.

ABSTRACT

The metastatic spread of breast cancer to the leptomeninges (LM) is a painful, debilitating, and usually lethal condition. Current therapies are generally ineffective or extremely toxic. The current study evaluated monoclonal antibody therapy in an animal model of LM human breast cancer. Monoclonal antibody 4D5, which recognizes the extracellular domain of the HER2/neu receptor, was administered into the cerebrospinal fluid of athymic rats implanted with human breast cancer cell lines. Continuous intraventricular administration of 4D5 inhibited growth of SKBR3 cells that overexpress HER2/neu but not of MCF7 cells, which do not. Inhibition was dose-dependent, with higher doses of 4D5 producing an improved response. I.p. administration of cisplatin in addition to 4D5 did not improve results. Continuous administration of 4D5 into the lumbar, as opposed to the ventricular intrathecal space, was not therapeutically effective. Treatment with 4D5 did not result in outgrowth of cells lacking expression of the HER2/neu receptor. These results suggest that 4D5, administered regionally, may palliate LM metastases from HER2/neu-overexpressing breast carcinoma.

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1 The abbreviations used are: LM, leptomeninges; leptomeningeal; IVent, intraventricular; MAb, monoclonal antibody; CSF, cerebrospinal fluid; IT, intrathecal; CNS, central nervous system.
chemotherapy and the anti-p185/HER2 MAb Herceptin who developed progressive disease.

MATERIALS AND METHODS

Animals, Cells, and Antibodies. All studies in rats were conducted using female athymic nude (Rnu/nu) rats 6 to 12 weeks of age, weighing 80–200 g, obtained from the National Cancer Institute (Frederick, MD). The human mammary adenocarcinoma cell lines SKBR3 and MCF7 (American Type Culture Collection, Rockville, MD) were grown using standard tissue culture techniques in a humidified incubator at 37°C with 5% CO₂. SKBR3 cells are known HER2/neu-amplified/overexpressing breast cancer cells, whereas MCF7 do not contain HER2/neu amplification and express normal amounts of the receptor. MAb 4D5 and a control IgG1 MAb directed to the herpes simplex virus 1 glycoprotein D were provided by Genentech, Inc. (South San Francisco, CA). Absence of Mycoplasma contamination was confirmed by the Gen-Probe rapid detection system (Gen-Probe, Inc., San Diego, CA). Absence of herpes simplex virus 1 glycoprotein D were provided by Genentech, Inc. (South San Francisco, CA). Absence of Mycoplasma contamination was confirmed by the Gen-Probe rapid detection system (Gen-Probe, Inc., San Diego, CA). All animal studies were approved by the institutional Animal Research and Care Committee.

In Vitro Growth Studies: Cellular Uptake of Tritiated [3H]Thymidine. One hundred µl of cells at a starting concentration of 4 × 10⁴ cells/ml were grown in 96-well, round-bottomed, tissue culture grade plates (Costar, Cambridge, MA) for 4 days with varying concentrations of antibody. On the 4th day, 0.25 uCi of [3H]thymidine (NEN-Lifes Sciences Products, Boston, MA) was added to each well and incubated for 6 h. Cells were then harvested in a cell harvester (model M-12R; Brandel, Gaithersburg, MD), on glass fiber filters (Bellco, Vineland, NJ), placed in 3 ml of scintillation cocktail (Opti-Fluor; Packard, Meriden, CT), in scintillation vials (Fischer, Pittsburgh, PA) and [3H] emissions measured (Wallac 1410 Liquid Scintillation Counter, Pharmacia, Turku, Finland). The percentage of inhibition of cell growth was calculated as follows:

\[
\frac{\text{Number of emissions in control wells} - \text{Number of emissions in experimental wells} \times 100}{\text{Number of emissions in control wells}}
\]

Direct Cell Counts. One milliliter of cells at a starting concentration of 2 × 10⁴ cells/ml were grown in 24-well, flat-bottomed tissue culture grade plates (Falcon; Becton Dickinson Labware, Franklin Lakes, NJ) with 20 µg/ml of anti-HER2/neu or control antibody. On days 2 and 8, cell growth from individual wells was assessed by cell counts using a Coulter counter (Model ZF; Coulter Electronics, Inc., Hialeah, FL).

Flow Cytometry, ELISA, and Immunohistochemistry. Standard techniques that have been described in detail previously were used (26). Briefly, flow cytometry was performed by incubating 1 × 10⁸ cells with 100 µl of antibody and then staining with a FITC-conjugated goat antimouse IgG antibody (F-2653; Sigma Chemical Co., St. Louis, MO). Immunofluorescence was quantitated using a FACScanStarPlus cytometer (Becton Dickinson, Mountainview, CA). ELISA was performed using an antibody capture immunosassay. Wells were coated with recombinant extracellular domain of p185 HER2 at 1 µg/ml. Detection used a rabbit antimouse (H&L) IgG linked to horseradish per-oxidase (Zymed, South San Francisco, CA) and an o-phenylenediamine dihydrochloride substrate. The ELISA could detect concentrations of 4D5 of 9.4 ng/ml using 10-µl samples. Immunohistochemistry to detect the p185 HER2/neu receptor was performed by incubating 10 µm brain sections with 4D5 or a control IgG1 MAb with a subsequent biotin-conjugated rat antimouse F(ab')₂ (Jackson Immunoresearch Laboratory, Westgrove, PA) and then the Vector ABC Elite kit (Burlingame, CA) with 3-amino-9-ethylcarbazole substrate (Scytek, Logan, UT). Detection of residual 4D5 attached to tumor of 4D5-treated animals was performed by direct staining with the biotin-conjugated rat antimouse F(ab')₂. Control tissue was obtained from animals treated with the IgG1 control MAb. The percentage of tumor that was positively stained was estimated visually by a single observer.

Surgical Techniques and Tumor Implantation. The surgical techniques of cisterna magna puncture, insertion of lumbar IT catheters, and insertion of Alzet brain infusion cannulas and osmotic pumps have been described in detail previously (27). Three sizes of pumps were used: (a) Model 2001: 7-day infusion, 1.0 µl/h; (b) Model 2004: 28-day infusion, 0.25 µl/h; and (c) Model 2 ML4: 28-day infusion, 2.5 µl/h (Alza Scientific Products, Palo Alto, CA). LM MCF7 or SKBR3 xenografts were established by implantation of 5 × 10⁷ to 1 × 10⁹ neoplastic cells/kg body weight through lumbar IT catheters. Animals were examined daily and weighed twice a week. Animals were killed if they developed any neurological deficit or their weight decreased to <60% of their starting weight. The numbers of animals included in each experiment are detailed in Tables 1 and 2. Animals in the studies comparing SKBR2 with MCF7 xenografts (Table 1) received a s.c. implant of 1.7 mg 17α estradiol pellets (60-day timed release; Innovative Research of America, Toledo, OH), because MCF7 xenografts are estrogen-dependent. Animals in the studies comparing different doses of 4D5 in the treatment of SKBR3 xenografts (Table 2) did not receive estradiol because SKBR3 LM xenografts were found to grow equally well in animals with or without estradiol supplementation.

Pathological Assessment. These techniques have been described previously in detail (28, 29). CSF for measurement of 4D5 concentration was obtained by cisterna magna puncture.
Table 2  Comparison of therapeutic efficacy of varying doses of MAb 4D5 with and without cisplatin administration in the treatment of Her2/neu-overexpressing LM malignancy

4D5 was administered by continuous intraventricular infusion beginning on the day of tumor implantation. Cisplatin was administered at a dose of 4.5 mg/kg by i.p. injection one day after tumor implantation. There were 11 animals/treatment group of the study administering 0.55 μg/h and 7 animals/treatment group in the others. None of the animals received estrogen.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>MAb dose</th>
<th>Treatment</th>
<th>Duration of study (Days)</th>
<th>Mean pathology grade (0–4)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKBR3</td>
<td>0.55 μg/h</td>
<td>4D5 + cisplatin</td>
<td>22</td>
<td>2.6</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>4D5</td>
<td></td>
<td>2</td>
<td>2.0</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td></td>
<td>2.8</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgG1 control</td>
<td></td>
<td></td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>SKBR3</td>
<td>1 μg/h</td>
<td>4D5 + cisplatin</td>
<td>5</td>
<td>1.6</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>4D5</td>
<td></td>
<td>1.1</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td></td>
<td>2.3</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgG1 control</td>
<td></td>
<td></td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>SKBR3</td>
<td>2.4 μg/h</td>
<td>4D5 + cisplatin</td>
<td>22</td>
<td>1.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>4D5</td>
<td></td>
<td>1</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td></td>
<td>2.1</td>
<td>0.17</td>
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<tr>
<td></td>
<td>IgG1 control</td>
<td></td>
<td></td>
<td>3.4</td>
<td></td>
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</tbody>
</table>

* Treatment group compared with IgG1 control group.

Proof of correct positioning of the IVent cannula was obtained at autopsy by injection of 50 μl of 0.1% methylene blue through the pump catheter and inspection for the presence of dye throughout the ventricular system. In addition, the residual volume in the pump was measured to confirm its proper operation. Brains and spinal cords were harvested from all animals and paraffin embedded blocks prepared as described previously (28). H&E sections, prepared from each case at two standard anatomical locations through the mamillary bodies and at the fourth ventricle/pontine cerebellum level, were reviewed in each case. Sections of the spinal cord at the lumbar and sacral levels were also examined when no tumor was found in the brain sections. Tumor size was graded as follows by a single pathologist blinded to therapy: 0: none; 1: tumor in LM only, scattered tumor cells or small collections ≤5 cell layers in width; 2: tumor in LM only, tumor clumps or confluent collections >5 cell layers in width; 3: tumor in LM and parenchyma, parenchymal invasion ≥10 cell layers in width; and 4: parenchymal invasion >10 cell layers in width.

Statistical Methods. Pathology scores and the percentage of animals with no tumor in the brain were compared between the experimental and control groups using Fisher’s exact test.

RESULTS

In Vitro Studies

Anti-HER2/neu Antibody Produced Dose-dependent Growth Inhibition of SKBR3 but not MCF7 Cells. Four-day in vitro growth assays demonstrated that the anti-HER2/neu antibody 4D5 inhibited growth of SKBR3 cells in a dose-dependent fashion as described previously (19, 24, 30, 31). Growth inhibition averaged 85% at a MAb concentration of 200 μg/ml, 72% at 20 μg/ml, 69% at 2 μg/ml, and 50% at 0.2 μg/ml.

In the same assay, 4D5 produced no measurable inhibition of growth of MCF7 cells. Flow cytometric analyses confirmed high surface expression of HER2/neu receptor on SKBR3 cells and low expression on MCF7 cells (data not shown; Refs. 32, 33). 4D5 antibody was not cytocidal in short-term assays. SKBR3 cells grown for 24 h in media containing 4D5 at a concentration of 20 μg/ml had a viability of 87% by trypan blue exclusion compared with a viability of 85% in cells incubated in media alone (mean of five experiments). Moreover, growth inhibition by 4D5 at a concentration of 20 μg/ml was fully reversible when the antibody was removed (Fig. 1). Growth of SKBR3 for 3 days in media containing 10 μg/ml of 4D5 did not result in cells with a decreased capacity to bind 4D5 MAb on their cell surface (mean fluorescence of 71.1 in cells grown in media with 4D5 compared with 52.6 in cells grown in media alone; mean of three studies), indicating that there was no significant down-modulation of receptor after 4D5 exposure.

In Vivo Studies

Anti-HER2/neu Antibody Inhibited in Vivo LM Growth of HER2/neu-overexpressing Breast Cancer Xenografts. Continuous IVent administration of MAb 4D5 effectively retarded the growth of HER2/neu-overexpressing SKBR3 LM xenografts but not MCF7 human breast cancer xenografts that expressed basal levels of HER2/neu receptor (Table 1). 4D5 doses of 2.4 and 1 μg/h were more effective than 0.55 μg/h (Table 2). A single i.p. dose of cisplatin, 4.5 mg/kg, in addition to 4D5 infusion did not improve outcome data (Table 2). In contrast to the IVent route, treatment of SKBR3 xenografts by continuous lumbar IT infusion was not successful. Seven pairs of animals were treated with lumbar infusion of MAb at a dose of 1 μg/h for 20 days. The mean pathology grade was 3.0 in the 4D5-treated group and 3.4 in the control group (P = 0.18). When all treatment trials involving SKBR3-implanted animals were combined, including animals treated with cisplatin, 12 of 57 animals (21%) treated with continuous IVent 4D5 had no tumor in brain compared with 2 of 60 (3%) animals who did not...
receive 4D5 ($P = 0.001$). The latter two animals were treated with i.p. cisplatin alone. When animals treated with cisplatin are excluded, 8 of 33 animals (24%) treated with 4D5 had no tumor in the brain compared with 0 of 35 animals (0%) treated with control IgG1 MAb ($P = 0.001$). Significantly, 6 of 12 4D5-treated animals that had no tumor in the brain did have tumor cells in the lumbosacral meninges. In all animals, meningeal inflammation was either absent or mild. Histopathological examination showed, at most, only mild meningotheial cell proliferation and a small lymphocytic infiltrate. Survival differences were not evaluated in these studies. Animals were sacrificed at predetermined time points to evaluate pathological differences before end-stage tumors supervened as per animal research subject regulations.

**IVent Administration Resulted in Excellent Delivery of MAb to CSF and Tumor.** Continuous IVent infusion of MAb 4D5, at a rate of 1 $\mu$g/h yielded mean concentrations in the CSF at 5 days of 3.8 $\mu$g/ml (range, 0.1–13.4; SD 2.8; $n = 22$). Immunohistochemical staining of SKBR3 xenografts demonstrated attachment of 4D5 to 100% of the surface area of persistent tumor deposits in the brain after 5 days of continuous IVent infusion of MAb at 1 $\mu$g/h and to 92% of persistent brain tumor after 20 days of infusion (mean of six animals at each time point; Fig. 2). All tumor deposits stained uniformly and highly with the 4D5 antibody, indicating that in vivo treatment did not result in outgrowth of tumor cells that stopped overexpressing the HER2/neu receptor (Fig. 2).

**DISCUSSION**

These studies in a rat model of LM human breast cancer demonstrated that regional IT therapy with MAb 4D5, directed against the HER2/neu receptor, inhibits growth of HER2/neu overexpressing xenografts. Metastatic breast cancer lesions outside of the CNS respond to therapy with i.v. anti-HER2/neu antibody (9, 10), but MAbs administered i.v. do not cross the blood-brain barrier and cannot be detected in the CSF or in LM tumor tissue (26, 27). A recently completed clinical trial using chemotherapy plus anti-HER2/neu antibody (9, 10), but MAbs administered i.v. do not cross the blood-brain barrier and cannot be detected in the CSF or in LM tumor tissue (26, 27). A recently completed clinical trial using chemotherapy plus anti-HER2/neu antibody (9, 10), but MAbs administered i.v. do not cross the blood-brain barrier and cannot be detected in the CSF or in LM tumor tissue (26, 27). A recently completed clinical trial using chemotherapy plus anti-HER2/neu antibody (9, 10), but MAbs administered i.v. do not cross the blood-brain barrier and cannot be detected in the CSF or in LM tumor tissue (26, 27). A recently completed clinical trial using chemotherapy plus anti-HER2/neu antibody (9, 10), but MAbs administered i.v. do not cross the blood-brain barrier and cannot be detected in the CSF or in LM tumor tissue (26, 27).

Fig. 2 Immunohistochemistry of 10 $\mu$m rat brain sections from animals implanted with SKBR3 and treated by continuous IVent infusion of either MAb 4D5 or control IgG1 MAb for 21 days. Sections were incubated with either MAb 4D5 or control IgG1 MAb with a subsequent biotin-conjugated rat antirat MAb. A and B are contiguous sections from a 4D5-treated animal; C and D are from an animal treated with control MAb. A and C were stained with 4D5 as the primary antibody, and B and D were stained with control IgG1 MAb as the primary antibody.
continuous IVent infusion produced therapeutic concentrations of the antibody in the CSF and resulted in antibody attachment to LM human breast cancer deposits. There was no observed reversion of the tumor cells to a HER2/neu receptor-negative phenotype, in vitro or in vivo. LM metastases may be particularly susceptible to MAb therapy because tumors grow in thin layers or as small nodules that are accessible to circulating antibody in the CSF.

The mechanism of the antitumor effect was not defined in this study. 4D5 was not directly cytocidal in vitro. An indirect immunological effect such as antibody-mediated cellular or complement-dependent cytotoxicity is unlikely because pathological examination of the tissue did not reveal active inflammation in the meninges or brain of 4D5-treated animals. In addition, no beneficial effect was seen against xenografts of MCF7 cells that were transfected with the HER2 gene despite high surface expression of the HER2/neu receptor and excellent attachment of the 4D5 MAb to these cells (data not shown). It should be noted, however, that the levels of HER2/neu overexpression in these MCF7 cells is considerably lower than that seen in SKBR3 cells. Consistent with previous reports, we found that 4D5 inhibited SKBR3 growth in vitro (19, 24, 30, 31). It is possible that continuous growth inhibition in vivo may have produced tumor cell apoptosis or terminal differentiation (34, 35). An alternative mechanism may be in part related to the fact that MAb 4D5 produces a dose-dependent reduction of vascular endothelial cell growth factor and vascular permeability factor protein expression in SKBR3 cells (36). Finally, overexpression of the HER2/neu receptor may be necessary to make the cancer cells independent of a growth factor that is not present in the CSF. Many molecules present in the systemic circulation, including peptide growth factors, are precluded from entering the brain and CSF by the blood-brain barrier (37). Overexpression of HER2/neu has been demonstrated to change the phenotype of tumor cells from estrogen-dependent to estrogen-independent (38, 39), and similar effects may be true for other growth factors. Blockade of the HER2/neu receptor may prevent LM tumor establishment by restoring cell dependence on growth factors not present in the CSF.

Delivery of MAb to tumor by IVent infusion was excellent, with 92% of persistent tumor deposits staining with 4D5 after 20 days of continuous infusion. Lumbar IT therapy was ineffective most likely because the natural flow of CSF is from the ventricles to the spinal subarachnoid space and then over the convexities of the brain. As a result, a greater concentration of drug will be delivered to wider areas of the subarachnoid space after IVent than after intralumbar administration. However, even IVent administration does not result in uniform distribution of therapy throughout the CSF space (26). There is a higher concentration of drug in the brain than in the sacral thecal sac, perhaps accounting for those animals that had absence of tumor in the brain but persistent tumor in the lumbosacral meninges.

The therapeutic response was directly related to the dose of 4D5, as reported previously for treatment of s.c. xenografts (23). In this study, an infusion rate of 1 μg/h produced therapeutic benefit that was present at 5 days and sustained for 20 days (Tables 1 and 2). In separate treatment trials lasting 20 to 22 days, the mean pathology grade in animals treated with MAb 4D5 was 1 at a dose of 2.4 μg/h, 1.44 at a dose of 1 μg/h, and 2 at a dose of 0.55 μg/h, indicating a dose response (Tables 1 and 2). An initial infusion rate of 1 μg/h was chosen because previous studies using this model and rate achieved a mean CSF MAb concentration of 2 μg/ml (29). In this study, an infusion of 1 μg/h achieved a mean CSF MAb concentration of 3.8 μg/ml. Our in vitro growth assays demonstrated excellent and near-maximal inhibition of SKBR3 growth at this 4D5 concentration. There is a steep decline in growth inhibition at lower MAb concentrations that probably accounts for the less effective therapeutic response at the lower infusion rate. Although limitation of CSF sampling precluded correlation of CSF MAb concentration with therapeutic effects, it is noteworthy that infusion rates of 1 μg/h yielded a wide range of CSF MAb concentrations that varied from 0.1 to 13.4 μg/ml. Some therapeutic failures may have resulted from inadequate delivery of MAb to the small ventricles of the rat rather than a failure of antibody efficacy. Similar difficulties are less likely in humans, whose CSF volume is 350 times that of the rat and in whom IVent infusion is easy and consistent (40).

Contrary to previous reports (8, 10, 22, 23, 41, 42), we did not find that cisplatin augmented the therapeutic effects of 4D5 in the LM model. We used a therapeutic cisplatin dose of 4.5 mg/kg because it was well tolerated and previously shown to produce therapeutic CSF concentrations (43). Administration of a higher dose, 6 mg/kg, resulted in high mortality in the rats (data not shown). A proposed mechanism explaining the synergistic effects of combined cisplatin and 4D5 therapy in other models is that cisplatin-induced DNA adducts are repaired poorly in HER2/neu receptor-amplified breast cancer cells treated with anti-HER2/neu receptor MAb (22, 23, 42). We did not measure CSF cisplatin concentrations and thus could not correlate CSF cisplatin concentrations with therapeutic efficacy. It is possible that concentrations of either cisplatin or 4D5 in the CSF were insufficient to demonstrate the synergistic effect seen in other models.

Toxicity from 4D5 infusion was not specifically tested in this study. However, no overt clinical toxicity was noted in the treated animals. In addition, the HER2/neu receptor is not detectable in normal adult human brain, spinal cord, or meninges by immunohistochemistry or Northern blotting (44). On the other hand, i.v. therapy with 4D5 has produced significant cardiotoxicity despite failure to detect receptors in human heart by the same methods (10, 44), and HER2/neu is required in the mouse embryo for normal myocardial and neural crest development (45). Importantly, HER2/neu mRNA has been detected in adult rat myocardium by RNase protection assays (46). The clinical safety of IVent infusion of anti-HER2/neu antibody in humans can be determined only by careful clinical trials. The efficacy data shown in the current study would indicate that this approach should be evaluated in the clinic.

ACKNOWLEDGMENTS

We acknowledge Genentech, Inc. for supplying 4D5 and Cheryl Schofield, Research Associate, Bioanalytical Technology, Genentech, Inc., who determined the concentration of 4D5 in the CSF samples. We also acknowledge the expert secretarial assistance of Holly Schaupp.
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