Development of Resistance to Cisplatin Is Associated with Decreased Expression of the gp185<sup>c-erbB-2</sup> Protein and Alterations in Growth Properties and Responses to Therapy in an Ovarian Tumor Cell Line

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Abstract
Overexpression of the c-erbB-2 protein (gp185<sup>c-erbB-2</sup>) is correlated with a tumorigenic phenotype and may contribute to disease progression. We have reported previously an anti-gp185<sup>c-erbB-2</sup> antibody, TAB 250, that inhibits <i>in vitro</i> and <i>in vivo</i> growth of breast and ovarian cell lines that overexpress the protein and enhances the inhibitory activity of cisplatin (CDDP). To assess whether CDDP resistance is related to gp185<sup>c-erbB-2</sup> expression levels, alterations in tumor cell growth characteristics, or efficacy of antibody plus drug combination treatments, an SKOV-3 ovarian tumor cell line was made resistant to escalating doses of CDDP. Parental cells were 12-fold more sensitive to CDDP with 7 times more gp185<sup>c-erbB-2</sup> sites than the most resistant variant (SKOV-3/C12). Additionally, the resistant cells demonstrated a longer lag phase for <i>in vivo</i> growth than the parental cells. While TAB 250 enhanced the <i>in vivo</i> inhibitory effect of CDDP against parental SKOV-3 cells, the antibody did not significantly alter the CDDP responsiveness of the resistant population. Growth inhibition by TAB 250 alone of both the parental and the SKOV-3-resistant variants was similar; however, TAB 250 was able to prolong the lag-phase of tumor growth of the resistant variant by up to 25 days. These results indicate that the development of CDDP resistance is associated with lowered levels of gp185<sup>c-erbB-2</sup> expression, slower tumor cell growth, and enhanced efficacy of antibody treatment of the resistant cells.

Introduction
The c-erbB-2 proto-oncogene encodes a M<sub>c</sub>, 185,000 cell surface growth factor receptor which, when overexpressed, correlates with a poor clinical prognosis in a number of cancers including breast and ovarian carcinomas (1-3). Furthermore, overexpression of the protein in a number of cell lines has been associated with a transformed or tumorigenic phenotype (4, 5). We have described previously a series of monoclonal antibodies reactive with the extracellular domain of the c-erbB-2 protein (6). One of these, TAB 250, inhibited the <i>in vitro</i> and <i>in vivo</i> growth of ovarian and breast tumor cell lines that overexpress this receptor. When coadministered with CDDP<sup>2</sup>, TAB 250 markedly enhanced the <i>in vivo</i> cytotoxicity of this chemotherapeutic agent against xenografts of the ovarian tumor cell line, SKOV-3 (7). Similar reports, involving sensitization by a biological agent to the effects of cisplatin, have been described for tumor necrosis factor (8), EGF (9), antibodies to the EGF receptor (10), and other anti-c-erbB-2 antibodies (11).

The clinical treatment for ovarian cancer often involves platinum-containing drugs such as cisplatin (12). However, dosing protocols frequently result in the emergence of low level drug resistance and a resultant loss of response to therapy. Various mechanisms have been proposed for the development of this resistance including an altered influx or efflux of the drug, improved drug detoxification by elevated metallothionein or glutathione levels, enhanced DNA repair capability, or up-regulation of specific biochemical pathways (13). Investigators have also proposed that changes in oncogene expression may mediate repair of cisplatin-induced DNA damage (14). Clinical data has suggested a relationship between responses to commonly used adjuvant therapies and c-erbB-2 overexpression (15). <i>In vitro</i> studies with breast tumor cell lines have corroborated this clinical data (16). Additionally, transfection of the mammary tumor cell line, MCF-7, with c-erbB-2 results in a decrease in sensitivity to tamoxifen and cisplatin (17). It thus appears that levels of c-erbB-2 expression may be associated with sensitivity to certain chemotherapeutics. It is, however, not known whether the development of drug resistance either in cell lines or in patient tumors that already overexpress the c-erbB-2 protein may affect c-erbB-2 expression levels or associated tumorigenicity.

The pharmacological half-life of cisplatin in patients at serum concentrations of 1–50 μM is 15–45 min. Treatment regimens include single weekly dosing, followed by a 3–4 week rest interval until dose-limiting toxicity is observed or patients become drug resistant. This suggests that a pharmacologically relevant <i>in vitro</i> drug resistance model could be developed by weekly acute exposures to the drug, followed by rest periods and retreatment, allowing for the selection of populations of cells with increasing drug resistance (12). We report here the establishment of a drug-resistant model of the ovarian tumor cell line, SKOV-3, derived by using acute intermittent <i>in vitro</i> treatments with escalating doses of cisplatin over the course of 2 years. In the present work, we describe and characterize these stable SKOV-3 variants, their <i>in vivo</i> growth properties, and the

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2 The abbreviations used are: CDDP, cis-diaminedichloroplatinum(II); cisplatin; EGF, epidermal growth factor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IC<sub>50</sub>, 50% inhibitory concentration; PBS, phosphate-buffered saline.

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effects of TAb 250 alone and in combination with cisplatin on the growth of tumor xenografts of these drug-resistant variants.

Results

Response to Cisplatin of SKOV-3 and SKOV-3/C12 Cells. The SKOV-3 parental cell line and the CDDP-resistant variants, SKOV-3/C (developed by treatment with repeated intermittent exposure to cisplatin using escalating doses of CDDP from 10–40 μM), were first tested for their in vitro sensitivity to cisplatin. The cells were exposed to varying concentrations of cisplatin for 72 h, and the antiproliferative effects were assayed using the MTT colorimetric assay. The results (Fig. 1) indicate that the SKOV-3 parental cells are 10-fold more sensitive to cisplatin, with an IC50 of 10 μM, than the most CDDP-resistant variant, SKOV-3/C12, with an IC50 of 120 μM. Corresponding IC50s for the intermediate resistant variants fall between those values of the parental and the SKOV-3/C12 cell lines (SKOV-3/C6, 83 μM; SKOV-3/C8, not done; SKOV-3/C10, 101 μM; data not shown).

Expression of the gp185c-erbB-2 in SKOV-3 Cells and SKOV-3/C Variants. The monoclonal antibody, TAb 250, is specific for the extracellular domain of the gp185c-erbB-2 receptor (6). Using competition of radiolabeled TAb 250 binding by unlabeled TAb 250, we were able to determine c-erbB-2 sites per cell by Scatchard analysis. Fig. 2 indicates that the parental and the resistant variants bind TAb 250 with similar dissociation constants (approximately 0.2 nM). The number of TAb 250 binding sites, however, decreased as cells became resistant to CDDP. The SKOV-3 parental cells have 1.8 × 106 gp185c-erbB-2 sites/cell, while the resistant populations have 5- to 7-fold fewer sites, approximately 1.5–2 × 105 sites/cell.

Growth of the SKOV-3 Cells and the SKOV-3/C Variants in Nude Mice. Previous reports have indicated that overexpression of the c-erbB-2 protein in cell lines such as NIH3T3 fibroblasts and the MCF-7 and 184B5 mammary cell lines results in an increase in the tumorigenicity of these cell lines in nude mice (4, 18). In an attempt to determine whether the lowered number of gp185c-erbB-2 sites in the most resistant variants affected tumorigenicity as compared to the parental SKOV-3 line, we implanted the parental and the SKOV-3/C6, SKOV-3/C8, and SKOV-3/C12 variants into nude mice. Time to onset of tumors and doubling times of the tumors that grew were monitored. Tumor growth of CDDP-resistant cells lagged behind parental cells by about 30–40 days. However, once growth was initiated, growth curves for parental and resistant lines were similar, with an approximate doubling time of 5–7 days for each of the lines (Fig. 3). Tumors from several animals in each group of the resistant variants were excised at day 65 and processed; then cells were grown in culture for 2–3 days. Cells were then retreated with the corresponding level of CDDP in order to confirm that tumors retained CDDP resistance after growth in vivo. Less than 20% of these tumor-derived cells were killed after in vitro treatment with CDDP, and the number of TAb 250 binding sites was similar to the number of sites in the cells originally implanted from culture (data not shown).

Treatment of SKOV-3/C12 Tumors with TAb 250 or CDDP. In order to determine whether in vitro drug resistance correlated with in vivo resistance or whether the altered c-erbB-2 expression levels or the altered growth patterns of the resistant variants would affect responses to anti-gp185 antibodies or CDDP, tumor xenografts of the SKOV-3 parental and SKOV-3/C12-resistant variant were treated with antibody or CDDP. Using a treatment regimen of once a week for 3 weeks, parental SKOV-3 tumors established for 1 week respond to CDDP at 50 μg/dose/animal, with an inhibition of tumor growth of 35–55% 40–50 days after the onset of tumor growth (Table 1). Animals treated with TAb 250 at 500 μg/dose/animal show similar levels of inhibition (Table 1). Using an identical dosing regimen, parental SKOV-3 tumors are inhibited 60–80% when treated with CDDP at 100 μg/dose/animal and 65–85% when treated with TAb 250 at 1000 μg/dose/animal (Table 1). In Fig. 4, the response of tumors of the SKOV-3/C12 variant to TAb 250, CDDP, and an isotype control antibody are shown (summarized in Table 1).
CDDP was able to inhibit the growth of this CDDP variant, but only at the higher concentration (41% inhibition at 100 μg/dose/animal, 40 days after the onset of tumor growth), and had no effect at the lower concentration. TAB 250, on the other hand, effectively inhibited tumor growth at both the lower 500-μg dose (63%) and at the higher 1000-μg dose (77%) 40 days after onset of tumor growth. Additionally, the higher dose of TAB 250 prolonged the lag phase to onset of tumor growth of the resistant variant by approximately 25 days. The growth of the resistant tumors in the presence of the nonspecific control antibody was unaffected relative to untreated tumors (data not shown).

**Treatment of SKOV-3 or SKOV-3/C12 Tumor Xenografts with TAB 250 Coadministered with CDDP.** Table 1 summarizes the effect of the combination treatment of TAB 250 plus CDDP on the parental tumors or the SKOV-3/C12-resistant variant. The data in Table 1 corroborate in vitro data and indicate that the CDDP-resistant variant is more resistant to CDDP in vivo than is the parental SKOV-3 tumor. The parental SKOV-3 tumors are inhibited 40% at the 50-μg dose as compared to the resistant SKOV-3/C12 tumors, which are inhibited 40%, but only at the higher 100-μg dose. An enhanced inhibition of tumor growth of the parental SKOV-3 tumors was observed when CDDP was combined with TAB 250 (74–85%) as compared to treatment with either drug or antibody alone. In contrast, while TAB 250 still effectively inhibited tumor growth of the SKOV-3/C12 variant (up to 77% at the 1000-μg dose CDDP), no additional inhibition was observed when the antibody was coadministered with CDDP. These results indicate that the inhibition of these CDDP-resistant tumors is probably due to the effects of the antibody when the combination therapy is administered.

**Discussion**
In the clinical setting, a patient with an ovarian cancer will likely be followed with a course of chemotherapy including CDDP. It is also possible that the patient’s tumor may be diagnosed as overexpressing the c-erbB-2 protein at the time of presentation. The patient is treated until dose-limiting toxicity or drug resistance develops. In the current study, we attempted to model this clinical profile in vitro by taking an ovarian cell line that already overexpresses c-erbB-2 and making it resistant to a chemotherapeutic, such as cisplatin, using intermittent treatment cycles with the drug. This treatment was designed to mimic the clinical regimens in which effective drug concentrations are maintained for relatively short periods of 12 hours to 2–3 days. The results we present indicate that stable drug-resistant variants of the SKOV-3 ovarian tumor cell line can be derived using this regimen. These variants demonstrate both in vitro and in vivo resistance to CDDP. When these lines were characterized for their ability to bind the anti-c-erbB-2 antibody, TAB 250, we were surprised to discover that the development of drug resistance was associated with a marked reduction in the expression levels of the c-erbB-2 protein. Previous studies attempting to correlate c-erbB-2 expression with drug resistance suggested that gp185°-erbB-2 overexpression is either causative or an associated variable of poor drug response. These studies included both the analysis of clinical samples using retrospective samples as

![Figure 3](image-url) In vivo growth curves of SKOV-3 parental and SKOV-3/C12 CDDP-resistant ovarian cells. SKOV-3 parental (□) or CDDP-resistant SKOV-3/C6 (■), SKOV-3/C8 (●), or SKOV-3/C12 (▲) were grown to 80–90% confluency and harvested. Cells were resuspended in PBS at ~1 x 10^6 cells/ml, and 100 μl of the cell suspension was implanted s.c. into 6–7-week-old athymic BALB/c mice on day 0 (8 mice/group). Tumors were measured twice per week using Vernier calipers, and volumes were calculated: V = length x width x height. Mean tumor volume/group was plotted as a function of day postimplantation. Bars, SEM.

![Figure 4](image-url) In vivo efficacy of TAB 250 or CDDP against SKOV-3/C12 CDDP-resistant tumors. SKOV-3/C12 ovarian tumor cells were implanted (8 mice/group) and tumors were measured; data (mean; bars, SEM) were plotted as described in Fig. 3. Mice were treated i.p. on days 7, 14, and 21 and again on days 76, 82, and 90 (arrows) with either a nonspecific control antibody, IgG1 (□), or TAB 250 at 500 μg (●) or 1000 μg (○), or CDDP at 50 μg (▲) or 100 μg (■).

**Table 1** In vivo efficacy of TAB 250 ± CDDP against SKOV-3 parental and SKOV-3/C12 CDDP-resistant tumors

<table>
<thead>
<tr>
<th>Treatment (dose/mouse)</th>
<th>% Maximal inhibition</th>
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<tbody>
<tr>
<td></td>
<td>SKOV-3 Parental</td>
</tr>
<tr>
<td>IgG1 (1000 μg)</td>
<td>0 ± 5</td>
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<tr>
<td>CDDP (50 μg)</td>
<td>45 ± 10</td>
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<tr>
<td>CDDP (100 μg)</td>
<td>67 ± 9</td>
</tr>
<tr>
<td>TAB 250 (500 μg)</td>
<td>50 ± 12</td>
</tr>
<tr>
<td>TAB 250 (1000 μg)</td>
<td>63 ± 11</td>
</tr>
<tr>
<td>TAB 250 (500 μg) + CDDP (50 μg)</td>
<td>85 ± 15</td>
</tr>
<tr>
<td>TAB 250 (1000 μg) + CDDP (50 μg)</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>TAB 250 (500 μg) + CDDP (100 μg)</td>
<td>ND*</td>
</tr>
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<td>TAB 250 (1000 μg) + CDDP (100 μg)</td>
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*ND, not done.*
well as in vitro studies with cell lines overexpressing gp185\textsuperscript{c-erbB-2} or cells transfected with gp185\textsuperscript{c-erbB-2}. The findings from the clinical studies indicated that overexpression of the protein was correlated with both a poor prognosis as well as a lack of responsiveness to a variety of adjuvant therapies including cytoxan/methotrexate/5-fluorouracil (15, 16) and tamoxifen (19). Due to their retrospective nature, it is not clear from these clinical studies whether the levels of the c-erbB-2 protein could have changed as a result of the drug treatments or whether the therapeutic responsiveness to the drug was lowered as a result of consistently overexpressed levels of gp185\textsuperscript{c-erbB-2}.

In an attempt to design in vitro correlates for clinical responsiveness to chemotherapy, Paik (16) described studies with a variety of breast cancer cell lines in which c-erbB-2 overexpression was also correlated to resistance to 5-fluorouracil and mitomycin C but not to the anthracyclines. In studies in which the low-expressing mammary cell line, MCF-7, was transfected with c-erbB-2 to overexpress the protein, a concomitant decrease in sensitivity to both taxol and cisplatin was reported (17). Similarly, we have described that transfection/infection of the immortalized human mammary cell line, MCF-10A, with c-erbB-2 alone or in combination with either the ras or transforming growth factor \(\alpha\) genes, resulted in increased resistance to cisplatin (20). From this previous work, it is clear that c-erbB-2 is definitely associated or even correlated with decreased sensitivity to chemotherapeutic drugs. Both the clinical data and the in vitro data make the assumption that gp185\textsuperscript{c-erbB-2} overexpression is causative of drug resistance. Our results, on the other hand, indicate that the development of CDDP resistance is correlated with a lowered c-erbB-2 expression. At best, the levels of c-erbB-2 expression may be an associated variable of poor drug response.

When we examined the responsiveness of the most drug-resistant variant to therapy with the anti-erbB-2 antibody or a combination of cisplatin with the antibody, we discovered both the parental cell line and the drug-resistant variant were effectively inhibited by the antibody alone. In contrast, while TAb 250 enhanced the inhibitory effects of CDDP against the parental SKOV-3 tumors, no such enhancement was observed when the combination treatment was used against the resistant xenografts.

In trying to understand how TAb 250 alters the efficacy to CDDP in the parental cells but not in the drug-resistant population, we offer two potential hypotheses. It is possible that a threshold level of TAb 250 binding sites is required in order for the effects of the CDDP to be enhanced by TAb 250. The levels of c-erbB-2 expression were reduced from greater than \(1.8 \times 10^4\) sites/cell in the parental line to approximately \(1.5 \times 10^4\) sites/cell in the resistant variants. Using a variety of mammary and ovarian cell lines, we have previously observed that a level of \(> 300,000\) binding sites/cell is necessary for TAb 250 to enhance the inhibitory effects of CDDP.\(^3\) Consistent with this observation, the enhancement of CDDP effects by the antibody were no longer observed when receptor levels fell below this threshold. Nevertheless, it is important to remember that the drug-resistant cells continue to express sufficient levels of receptor to permit inhibition of tumor cell growth by the antibody alone.

Another possibility to account for the lack of enhancement of CDDP effects by the antibody against the resistant tumors may involve intracellular events subsequent to drug uptake or antibody binding. We have observed previously that treatment of cells that overexpress gp185\textsuperscript{c-erbB-2} with TAb 250 results in phosphorylation of the c-erbB-2 receptor and subsequent receptor down-modulation. This treatment also results in activation of certain signal transduction intermediates, including tyrosine phosphorylation of phospholipase C-\(\gamma\)I and activation of protein kinase C (21). These events are similar to what can be expected when cells are treated with the natural ligand. By analogy with other growth factor receptors, phosphorylation of the receptor and activation of such signaling intermediates would be expected to result in growth stimulation. The antibody, however, effectively inhibits cell proliferation. Similar observations of growth inhibition by anti-gp185\textsuperscript{c-erbB-2} antibodies with potential agonistic activities have been made by other investigators (22). The activation of signal transduction intermediates may play a role in the interaction with cisplatin. It was recently shown that stimulation of protein kinase A by forskolin increased the sensitivity to CDDP (23). Similarly, stimulation of protein kinase C by the phorbol ester, 12-O-tetradecanoyl-phorbol 13-acetate, also lead to enhanced cisplatin cytotoxicity (24). The activation of a growth factor signal transduction pathway leading to the modulation of cisplatin sensitivity has been proposed previously by Christen et al. (9). In their studies, EGF enhanced the sensitivity of CDDP against the ovarian carcinoma 2008 line. Similar to our observations for the c-erbB-2 receptor, these investigators found that the CDDP-resistant variants of the 2008 line had approximately 3-fold fewer EGF receptors than the parent cells. EGF also failed to induce sensitivity to CDDP in these resistant variants, consistent with the results we present in this paper. The authors suggest that, because EGF resulted in similar receptor autophosphorylation in both parental and resistant variants, downstream signaling events may be implicated in the sensitization to CDDP. We have observed that autophosphorylation of the c-erbB-2 receptor by TAb 250 in the parental cells is slightly higher than in the resistant variants, after correcting for the lower level of receptor expression (data not shown). Studies are under way to assess whether signaling pathways involving protein kinase A or C or perhaps subsequent effects on DNA repair enzymes may modulate the sensitivity to CDDP and whether activation of these pathways or the particular pathways themselves differ in the parental and resistant cells.

At this time, we can only speculate that an agent directed at a growth factor receptor such as c-erbB-2 could be used to modulate the sensitivity to chemotherapeutic agents. In the present study, we observed that ovarian tumor cells overexpressing c-erbB-2 could be made resistant to cisplatin, and this resistance is associated with a decrease in the levels of the receptor protein. By inference, one could suggest that, at least in certain tumor cell populations, the original overexpressing population may be maximally sensitive to the drug. Since only the cells that overexpress the receptor (comparable to levels observed in the mammary tumor cell lines or in the transfected cells tested to date) are growth inhibited by agents directed against the receptor (such as the monoclonal antibody), one could envision using this approach in combination with the chemotherapies which would normally be used against these tumors. The results we present further suggest that, for cancers that

\(^3\) Manuscript in preparation.
overexpress gp185c-erbB-2, potential therapeutic approaches involving conventional chemotherapies in combination with an anti-c-erbB-2 agent may still be effective, even when the tumor cells start to develop drug resistance.

Materials and Methods

Cell Culture. The human tumor cell line, SKOV-3 (parental), was obtained from the American Type Culture Collection (HTB 77, Rockville, MD) and was grown and passaged in Iscove's Modified Dulbecco's Medium containing 10% fetal bovine serum and 2 mM L-glutamine. The SKOV-3/C cisplatin-resistant variants were developed by intermittent repeated exposure to escalating doses (10–40 μM) of CDDP. Cell populations were treated for 24–48 h with 10 μM CDDP (Platino; Bristol-Meyers Squibb, Syracuse, NY), solubilized in sterile water to 1 mg/ml, and further diluted with PBS; surviving cell populations were grown to confluence (1–4 weeks) and retreated until no cell death was observed at this drug concentration (approximately 3–4 months). The resultant population was then treated with an escalating dose of CDDP (20 μM), and the above selection procedure was repeated. The treatment was repeated with 27, 33, and 40 μM CDDP over a total of approximately 24 months to obtain lines that were resistant to treatment with the different concentrations of CDDP: SKOV-3/C6 (20 μM); SKOV-3/C8 (27 μM); SKOV-3/C10 (33 μM); and SKOV-3/C12 (40 μM). Stable resistant populations were maintained in the absence of CDDP and were treated every 3–5 weeks with the appropriate dose of CDDP (less than 10% cell death upon retreatment was established as a criteria for stable populations). All lines were maintained in a humidified incubator at 5% CO₂ and 37°C.

MTT Assay. Assays were carried out as described previously (7). Briefly, on day 1, 70–90% confluent cells (parental or SKOV-3/C variants) were harvested, plated in triplicate at 1 × 10⁴ cells/well in a 96-well plate, and incubated at 37°C in 5% CO₂. On day 2, CDDP diluted in PBS was added at the appropriate concentrations, and control wells received only PBS. On day 5, MTT (Sigma Chemical Co., St. Louis, MO) was added to each well, and the plates were incubated an additional 4 h at 37°C, after which the contents of the wells were solubilized with isopropl alcohol/0.04 n HCL/0.3% sodium dodecyl sulfate. Absorbance of the wells was measured at 570 nm using the wells containing media alone plus dye as background.

% of control = \frac{\text{Average } A_{570 \text{ of test well}}}{\text{Average } A_{570 \text{ of control well}}} \times 100

Competition Assay. TAB 250 (6) was radiolabeled with ¹²⁵I (Amersham, Arlington, IL) to a specific activity of 2–5 μCi/μg as described previously (6, 25) and stored at 4°C for up to 8 weeks with no apparent loss of binding activity. Before each competition assay, an appropriate amount of ¹²⁵I-labeled TAB 250 was desalted using a NAP-5 column (Pharmacia, Piscataway, NJ) equilibrated in PBS containing 0.1% bovine serum albumin. Parental SKOV-3 or SKOV-3/C cells (70–95% confluent) were harvested from tissue culture flasks with calcium- and magnesium-free PBS containing 2 mM EDTA and 1% glucose. Cells were washed once in PBS, centrifuged (200 x g for 10 min), and resuspended to 1–2 × 10⁵/ml in binding buffer (minimal essential medium (GIBCO, Gaithersburg, MD) plus 0.1% bovine serum albumin buffered with 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4; Whitaker Bioproducts, Walkersville, MD). Cells (3.6 × 10⁵/tube) were incubated with 10 μl of labeled TAB 250 (approximately 8000 cpm/tube) alone (total binding) or in combination with 10 μl of varying concentrations of cold TAB 250 on ice for 4 h on a rotary shaker. Cold binding buffer (800 μl) was then added to each tube; the tubes were centrifuged (3800 × g for 10 min), and the supernatants were aspirated and cell-associated radioactivity was determined using a gamma counter (Isodata Series 500; Iso Data, Rolling Meadows, IL). Percent binding was defined as:

% binding = \frac{\text{Experimental cpm}}{\text{Total cpm}} \times 100

The program LIGAND (26) was used to determine dissociation constants and c-erbB-2 binding sites/cell, assuming bivalent binding of the antibody.

In Vivo s.c. Xenografts. SKOV-3 parental or SKOV-3/C variants were implanted s.c. (1 × 10⁷ cells/mouse) into 4–6-week-old athymic (nu/nu) BALB/c mice (Simonsen, Gilroy, CA) on day 0 (8 mice/group). Tumors were measured twice per week with Vernier calipers as described previously (7) and volumes (length × width × height) were calculated. Mean tumor volume was plotted as a function of day postimplant. In the experiments in which animals were treated, SKOV-3 parental or SKOV-3/C12 tumors were grown and measured as described above and treated i.p. on days 7, 14, and 21 and again on days 76, 82, and 90 postimplant with either 100 μg of a nonspecific control IgG1 antibody or 500 or 1000 μg TAB 250 alone or in combination with 50 or 100 μg CDDP.

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