ABSTRACT

Proteins of the Bcl-2 family are key regulators of caspase activation and apoptosis. Some members of this family, notably Bcl-2 and Bcl-xL, are overexpressed in cancer cells, which have been associated with chemoresistance. We have designed and synthesized a small molecule inhibitor of Bcl-2, named YC137, and studied its role in cancer cells. In vitro studies showed that YC137 inhibits the binding of the Bid BH3 peptide to Bcl-2, thus disrupting an interaction essential for the antiapoptotic activity of Bcl-2. This inhibitor induces apoptosis of hematopoietic progenitors overexpressing Bcl-2 but not Bcl-xL and breast cancer cells that express high levels of Bcl-2. On the contrary, a variety of normal primary cells, including CD34+ progenitors, myoblasts, and peripheral blood mononuclear cells, do not respond to the inhibitor. A breast cancer cell line resistant to YC137 was generated. Analysis of resistant cells revealed a reduced expression of Bcl-2, which correlated with low activation of signal transducer and activator of transcription-3 (Stat3) and reduced expression of the human epidural growth factor receptor-2 (HER2). Of note, YC137-resistant cells were more sensitive to apoptosis induced by chemotherapy. Because HER2 has not been linked previously to the Stat3-Bcl-2 transcriptional pathway, we additionally confirmed that specific blockade of HER2 in breast cancer cells resulted in down-regulation of Stat3 activity and reduced levels of Bcl-2. Consistently, HER2 blockade led to YC137 resistance. These data provide evidence for the selective killing of tumor cells by YC137 and represent the first example of in vitro selection of cancer cells refractory to a Bcl-2 inhibitor.

INTRODUCTION

The expression of genes that regulate apoptotic cell death plays an important role in determining the sensitivity of tumor cells to chemotherapy. High expression of the antiapoptotic protein Bcl-2 is found in a number of human hematologic malignancies and solid tumors (1, 2). The functional blockade of Bcl-2 or other antiapoptotic proteins, such as Bcl-xL, could either induce apoptosis in cancer cells or sensitize these cells for chemotherapy. To this end, it has been reported that down-regulation of Bcl-2 by antisense oligonucleotides induces apoptosis in myeloid leukemia cells, breast and colorectal carcinomas, and lung cancer cells among others, even in the presence of other antiapoptotic genes (3–6). In other cell systems (i.e., MCF7 breast cancer cells, HL-60 myeloid leukemia cell line, and myeloma cells), the use of a Bcl-2 antisense oligonucleotide sensitizes tumor cells to chemotherapeutic drugs (3, 7, 8). Consequently, antiapoptotic members of the Bcl-2 family have attracted intensive interest in drug discovery to develop a new class of anticancer agents (9). BH3-mediated homodimerizations and heterodimerizations play a key role in regulating the apoptotic activity of Bcl-2 family members (10). Structural analysis of the Bcl-xL-Bak BH3 peptide complex (11) and more recently the Bcl-2 protein (12) has made possible the identification of small molecules that inhibit the interaction between the BH3 domain of proapoptotic proteins and the hydrophobic cleft of Bcl-2 or Bcl-xL (13–16). Some of these compounds have been shown to reduce cell viability in cancer cell lines, which suggests that they could lead to the development of new therapeutic agents. However, it has not elucidated the activity of these small molecules on normal cells, such as hematopoietic progenitors and epithelial cells, which are commonly affected after chemotherapy.

The clinical success of small molecule kinase inhibitors has led to the notion that other selective inhibitors may have significant activity against a range of human malignancies (17). However, clinical resistance to these agents has been reported, and it is believed that preexisting mutant cells are selected by the agent to which the mutation confers resistance (18). By analogy, Bcl-2 inhibitors could select for resistant cells. Consequently, research should focus on defining the mechanisms of resistance, so that the emergence of resistant clones might be prevented.

In this study, we show that a novel small molecule inhibitor of Bcl-2, YC137, induces apoptosis in breast cancer cells that express high levels of Bcl-2 but does not have any effect on normal hematopoietic progenitors, peripheral blood mononuclear cells (PBMCs), small intestine epithelial cells, and myoblasts. Furthermore, tumor cells that evade the apoptotic effects of YC137 down-regulate Bcl-2 and become more sensitive to conventional chemotherapy.

MATERIALS AND METHODS

Cell Lines and Primary Cells. Breast cancer cell lines MDA-MB435L (lung metastatic variant of MDA-MB435), MDA-MB435B (brain metastatic variant of MDA-MB435), MDA-MB231, MCF7, and SKBR3 were cultured as described previously (19). SUM159, SUM159 and SUM229 were incubated in DMEM/Ham’s F-12 (Biochrom KG, Berlin, Germany), supplemented with 5% fetal calf serum, 5 μg/mL insulin, and 1 μg/mL hydrocortisone (both from Sigma, St. Louis, MO). Normal small intestine FHs cells were grown in DMEM/Ham’s F-12 with 10% fetal calf serum, 10 ng/mL cholera toxin, 5 μg/mL transferrin, 5 μg/mL insulin, and 100 ng/mL hydrocortisone (Sigma). HCD-57 and FL5.12 hematopoietic cells and their derivative cell lines transfected with Bcl-2 or Bcl-xL were cultured as described previously (20).

PBMCs were obtained from normal donors. CD34+ cells were selected from the PBMC population of donors undergoing mobilization for allogeneic progenitor cell transplantation as described previously (21). All of the donors signed informed consent according to Guidelines from the Committee for the Protection of Human Subjects at the University Hospital Marques de Valdecilla. Myoblasts were obtained from the Cell Therapy Unit, at the University Clinic of Navarra following a protocol described previously (22). In some experiments, MB435B cells were treated with the recombinant humanized anti-human epidermal growth factor receptor (HER2) monoclonal antibody trastuzumab (F. Hoffmann-La Roche, Basel, Switzerland) and then additionally analyzed for the expression of Bcl-2.

Chemical Synthesis of YC137. Briefly, 1.35 g (5.95 mmol) of 2,3-dichloro-1,4-naphthoquinone were dissolved in 20 mL of 1,4-dioxane, and
then 1 g (5.95 mmol) of 4-hydroxyphenylthiourea was added. The mixture was refluxed for 20 minutes. When the color of the reaction mixture turned to deep purple, 20 mL of EtOH and 1.344 g (12 mmol) of 1,4-diaze-
bicyclo(2,2,2)octane (Dabco) were added and refluxed for 5 hours. The reaction mixture was then cooled down to room temperature, and the solvent was removed under vacuum. Then, the intermediate (deep violet solid) was purified by flash column chromatography. To a solution of 10 mL of dichloromethane and 110 mg (0.53 mmol) of N-methyl-formate-L-methio-
nine, 110 mg (0.53 mmol) of dicyclohexylcarbodiimide were added in ice-cold bath, followed by the addition of 142 mg (0.44 mmol) of the intermediate. Then, the reaction mixture was allowed to warm up to room temperature and stirred for 16 hours. The solid of the reaction mixture was filtered out and washed with brine, dried over sodium sulfate, and purified via flash chroma-
tography.

YC137 was solubilized at 10 mmol/L in dimethylsulfoxide and kept at −20°C in the dark until use. This stock solution was diluted >104-fold for both binding assays and cell culture experiments.

Competitive Fluorescence Polarization Binding Assay. A sensitive and quantitative in vitro binding assay using a fluorescence polarization-based method was developed and used to determine the binding affinity of YC137 to Bcl-2 protein. For this assay, 6-carboxy fluorescein succinimidyl ester was coupled to a 21-residue Bid peptide (Flu-QUDEIRNAIRHLAQVGDMSDR), which binds with high affinity to Bcl-2 protein ($K_i = 47$ nmol/L).

Fluorescence polarization experiments were performed in Dynex 96-well, black, and round-bottomed plates (Fisher Scientific, Hampton, NH) using the Ultra Plate Reader (Tecan U.S., Inc., Research Triangle Park, NC). YC137, diluted in assay buffer, was incubated with recombinant His-fused soluble Bcl-2 protein (120 mmol/L) and Flu-Bid peptide (5 mmol/L). It is worthy of note that the assay buffer conditions have a great influence on the binding affinity of YC137. Thus, we tried a number of assay conditions until consistent results were obtained in three independent experiments with a buffer containing

50 mmol/L Bis-Tris (pH 7.4) and 0.01% bovine y globulin. The plates were mixed and incubated at room temperature for 4 hours to reach equilibrium. Curve fitting was performed using GraphPad Prism software. A nonlabeled Bid BH3 peptide was used as the positive control. The $K_i$ values were calculated using a recently developed equation for fluorescence polarization-based binding assays (23). For determination of the binding affinity to Bcl-xL protein, we used human Bcl-xL recombinant His-tagged protein without the COOH-terminal hydrophobic tail (120 nmol/L) and Flu-Bid peptide (5 nmol/ L), and the competitive binding assay was performed in the same way as for the Bcl-2 protein.

Transfection and Flow Cytometry Analysis. CD34+ progenitors ($1 \times 10^6$ cells) were cotransfected with 1 μg of a vector encoding enhanced green fluorescence protein (pEGFP-C1, BD Biosciences, Palo Alto, CA) and 4 μg of pSFFV-Bcl-2 (24) by using the human CD34 cell nucleofector kit (Axama, Cologne, Germany). After nucleofection, cells were cultured for 16 h with or without stem cell factor and interleukin (IL)-3 at a final concentration of 100 ng/mL. When 0.25 μmol/L YC137 was added to the culture, and after 24 hours of incubation, apoptotic cells were detected, within the EGFP + population, with annexin V labeled with phycoerythrin (Becton Dickinson, San Jose, CA) by flow cytometry using a FACSscan analyzer (Becton Dickinson).

MB435B cells were cotransfected with 1 μg of pEGFP-C1 and 4 μg of either a constitutively active form of Stat3 (RCCMV-Stat3-C; ref. 25) or a constitutively active form of Stat3 (Santa Cruz Biotechnology, Santa Cruz, CA). The binding affinity of YC137 to Stat3 was analyzed using fluorescence polarization-based methods (Fig. 1B). We determined that YC137 has a $K_i$ value of 1.3 μmol/L when assayed in Bis-Tris buffer. As a positive control, the 21-residue Bid-BH3 peptide was subjected to the same binding assay conditions (Fig. 1B), and we determined a $K_i$ of 54 mmol/L. Using a similar fluorescence polarization-based method for Bcl-xL, we determined that YC137 has a $K_i$ value >100 μmol/L (data not shown), suggesting that this small molecule preferentially binds to Bcl-2 protein.

A model of Bcl-2 in complex with YC137 was generated by docking YC137 into the BH3 domain-binding pocket of Bcl-2 (Fig. 2). The three-dimensional structure of the Bcl-2 protein (isoform-2) was obtained from the Protein Data Bank (entry 1GJH), and molecular docking was performed with the GOLD program (version 2.1, dis-

tributed by Cambridge Crystallographic Data Center). According to this predicted binding model, the tri-cyclic moiety of YC137 occupies two hydrophobic sites in the Bcl-2-binding pocket and forms hydrogen bonds with residues Arg107, Tyr108, Arg146, and Tyr202 of the Bcl-2 protein.

YC137 Induces Apoptosis of Bcl-2-Dependent Cells. To confirm the specificity of YC137 for the Bcl-2 protein in cellular models, we analyzed its activity in cells dependent on Bcl-2 or Bcl-xL for survival, e.g., H9262 and H9263 cells

RESULTS

Characterization of a Small Molecule Inhibitor of Bcl-2. We have used a structure-based computer screening strategy to discover novel small molecule inhibitors that bind to the crucial BH3-binding groove of Bcl-2 protein (16). On the basis of the structure of initial lead compounds, we have designed and synthesized new analogues to improve their binding affinity and cellular activity. One of these compounds, YC137 (Fig. 1A), was selected for binding assays and additional biological studies to determine its specificity and activity.

The binding affinity of YC137 to Bcl-2 protein was analyzed using fluorescence polarization-based methods (Fig. 1B). We determined that YC137 has a $K_i$ value of 1.3 μmol/L when assayed in Bis-Tris buffer. As a positive control, the 21-residue Bid-BH3 peptide was subjected to the same binding assay conditions (Fig. 1B), and we determined a $K_i$ of 54 mmol/L. Using a similar fluorescence polarization-based method for Bcl-xL, we determined that YC137 has a $K_i$ value >100 μmol/L (data not shown), suggesting that this small molecule preferentially binds to Bcl-2 protein.

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Affinity for Bcl-2 was measured by a competitive fluorescence polarization assay. Each presence of increasing concentrations of YC137 or unlabeled Bid peptide, and the binding soluble Bcl-2 protein was incubated with a fluorescein-labeled Bid-BH3 peptide in the point.

Zol-2-ylamino)-phenyl ester, a small organic compound that binds Bcl-2. In carbonylamino-4-methylsulfanyl-butyric acid 4-(4,9-dioxo-4,9-dihydro-naphtho[2,3-]thiazol-2-ylamino)-phenyl ester, a small organic compound that binds Bcl-2. In B, His-fused soluble Bcl-2 protein was incubated with a fluorescein-labeled Bid-BH3 peptide in the presence of increasing concentrations of YC137 or unlabeled Bid peptide, and the binding affinity for Bcl-2 was measured by a competitive fluorescence polarization assay. Each data point represents the mean ± SD of at least three independent experiments.

Are dependent on IL-3 and erythropoietin, respectively. In the absence of the growth factor, these cells stop proliferation and undergo apoptosis. However, apoptosis induced by IL-3 or erythropoietin depri-vation can be inhibited or delayed in cells overexpressing Bcl-2 or Bcl-xL (20). On the basis of this, FL5.12 and HCD-57 transfected with Bcl-2 or Bcl-xL were treated with different concentrations of YC137, and then apoptosis was determined by an immunoenzymatic assay. Fig. 3, A and B, show Western blot analyses of the overexpressed proteins in transfected cells. After 20 hours of culture in the absence of IL-3 or erythropoietin, YC137 induced apoptosis of Bcl-2-transfected cells in a dose-dependent manner (Fig. 3C). At 0.2 μmol/L of YC137, most of the cells were apoptotic. Conversely, the viability of nontransfected control cells and cells transfected with Bcl-xL remained unaffected (Fig. 3C), even at concentrations ≥5 μmol/L (data not shown). Of note, all of the other described Bcl-2/Bcl-xL inhibitors tested, whereas MB435B, used here as a positive control, reached the highest level of apoptosis at 0.3 μmol/L YC137.

Normal primary cell survival is not modified by treatment with YC137. A key issue to validate a new chemotherapeutic drug is to determine its tumor specificity. For this purpose, we treated normal small intestine epithelial cells (FHS), primary myoblasts, and PBMCs with YC137. Cells were incubated with increasing concentrations of YC137 (0.1 to 0.5 μmol/L) for 20 hours and then analyzed for the presence of histone-associated DNA fragments. As shown in Fig. 5A, apoptosis was not detected in these cells at any of the concentrations tested, whereas MB435B, used here as a positive control, reached the highest level of apoptosis at 0.3 μmol/L YC137.

Ex vivo expanded peripheral blood progenitor cells have been proposed as a source of hematopoietic support to decrease or eliminate the period of neutropenia after high-dose chemotherapy (28). However, this procedure will only be advantageous if contaminating tumor cells are not expanded concomitantly. This model served us to additionally analyze the specificity of the Bcl-2 inhibitor. We cocultured defined numbers of MB435B cells with CD34 + cells selected from normal donors in the presence of 0.4 μmol/L YC137. By 20 hours of treatment, 27% of MB435B cells (CD34 + cell population) were annexin V positive as determined by flow cytometry analysis, the effect of YC137 on these cells and other breast cancer cell lines (MB435L, MB231, SKBR3, MCF7, SUM159, SUM185, and SUM229) with different levels of Bcl-2 expression. Interestingly, the expression level of Bcl-2 correlated with the apoptotic response to the inhibitor (Fig. 4A and B). Thus, MB435B and SUM159 cells, which express the highest levels of Bcl-2 protein, showed also the strongest apoptotic response after 20 hours of treatment with YC137. To determine whether YC137 induces apoptosis via caspase activation, we treated MB435B cells with the inhibitor in the presence of a cell-permeable fluorescent peptide that irreversibly binds to activated caspase 9, a caspase strongly associated with chemotherapy-induced apoptosis. As shown in Fig. 4C, after 2 hours of culture with the inhibitor, activated caspase 9 was present in the great majority (>85%) of tumor cells. Furthermore, we studied whether YC137 induces the release of cytochrome c from mitochondria, which is needed for caspase 9 activation. Analysis of soluble and heavy membrane fractions by Western blot revealed that YC137 induced cytochrome c release (present in soluble fraction) in MB435B cells treated with the inhibitor for 24 hours (Fig. 4D).

Apoptosis of breast cancer cells induced by treatment with YC137 correlates with the levels of Bcl-2. We have shown previously that a metastatic variant of an estrogen receptor-negative breast cancer cell line, MB435B, expresses high levels of Bcl-2 as compared with the parental cell line MB435 (19). Thus, we studied the structure of YC137 [2-methoxy-carbonylamino-4-methylsulfanyl-butyric acid 4-(4,9-dioxo-4,9-dihydro-naphtho[2,3-])thiazol-2-ylamino)-phenyl ester], a small organic compound that binds Bcl-2. In B, His-fused soluble Bcl-2 protein was incubated with a fluorescein-labeled Bid-BH3 peptide in the presence of increasing concentrations of YC137 or unlabeled Bid peptide, and the binding affinity for Bcl-2 was measured by a competitive fluorescence polarization assay. Each data point represents the mean ± SD of at least three independent experiments.
whereas $>95\%$ of hematopoietic progenitors (CD34$^+$ cell population) were viable annexin V-negative cells (Fig. 5B).

As shown in Fig. 4C and Fig. 5B, there is a difference in the percentage of apoptotic cells between annexin V and activated caspase 9 assays in MB435B cells treated with YC137. This may reflect the fact that activation of caspase 9 measures early apoptosis, whereas annexin V measures late apoptosis, and that both methods assess different cellular responses to apoptosis.

**YC137-Resistant Breast Cancer Cells Down-Regulate the Expression of Bcl-2.** Resistance to small molecules that inhibit defined tyrosine kinases has been reported in both cell lines and primary cells (17). As a first step to know whether tumor cells may develop resistance to Bcl-2 inhibitors, we cultured MB435B cells with increasing concentrations of YC137. After 3 months of culture, a MB435B subline growing at 0.225 $\mu$mol/L YC137 (a lethal concentration for parental cells) was isolated. Comparative genomic hybridization analysis revealed no chromosome abnormalities (deletions or amplifications) in the resistant variant (data not shown). Interestingly, these cells had low expression of Bcl-2 at the protein and mRNA level (Fig. 6A and B); however, the expression of Bcl-xL was the same as that of the parental cell line. We have described previously that Bcl-2 is transcriptionally regulated by Stat3 in MB435B cells (19). Therefore, we analyzed the DNA binding activity of this transcription factor and found a reduced constitutive activation as measured by electrophoretic...
with trastuzumab for 6 hours significantly reduced the activation of Stat3, as determined by electrophoretic mobility shift assay, which continued to decrease by 12 hours of treatment. This pattern of Stat3-DNA binding complex formation correlated with the protein and mRNA levels of Bcl-2, which were clearly down-regulated after 24 hours of treatment with trastuzumab (Fig. 7D). Furthermore, consistent with our previous data, trastuzumab-treated cells became more resistant to YC137. Pretreatment with trastuzumab for 24 hours reduced ~2-fold the number of apoptotic MB435B cells cultured in the presence of YC137, as determined by flow cytometry with FITC-labeled annexin V (Fig. 7E).

To additionally confirm this transcriptional pathway in a more direct way, we analyzed the effect of a constitutively activated form of Stat3 (Stat3-C) on the Bcl-2 expression in MB435B treated with trastuzumab. Cells were cotransfected with a vector expressing EGFP and vector encoding Stat3-C. After 36 hours of transfection in the presence of trastuzumab, the control cells had significantly reduced the expression of Bcl-2 (~35%), whereas those expressing Stat3-C had no variation in the levels of Bcl-2 as determined by flow cytometry (Fig. 7F).

**DISCUSSION**

Most of the currently available small molecule inhibitors are designed to block the enzymatic activity of key proteins in cancer cells (17), and there are very few examples of inhibitors that disrupt protein–protein interactions. Bcl-2 family members play key roles in the regulation of apoptosis and may contribute to cancer development and resistance to chemotherapy, which have attracted intensive inter-
est in the discovery of small molecules that inhibit the activity of antiapoptotic proteins of this family, mainly Bcl-2 and Bcl-xL (13–16). Here, we have designed and synthesized an inhibitor of Bcl-2, YC137, which shows high Bcl-2-binding activity. However, it is much less effective in binding to Bcl-xL. This is consistent with our data, which demonstrate that YC137 selectively induces apoptosis in breast cancer cells with high levels of Bcl-2. Interestingly, the concentration of YC137 needed for the selective killing of tumor cells is >20 times lower than that reported for the other Bcl-2/Bcl-xL inhibitors. Of note, a complete apoptotic response was achieved at concentrations about four to six times lower than the Ki value of YC137. There are two main explanations for this discrepancy: (1) the concentration of Bcl-2 protein used in our fluorescence polarization-based binding assays is 120 nmol/L, which might be considerably higher than the cellular concentration of Bcl-2, and (2) YC137 has a poor solubility in our binding assay conditions, and consequently, it is possible that the binding affinity of YC137 was underestimated. The effectiveness of this inhibitor in hematopoietic precursors that become dependent on Bcl-2 for survival suggests that the sensitivity to Bcl-2 inhibitors will be given not only by the levels of Bcl-2 but also the contribution of this antiapoptotic protein to the overall survival of particular tumor cells. Consistently, apoptosis can be induced in a number of tumor cell lines by decreasing the amount of Bcl-2 protein (4, 29). Anticancer drugs used in chemotherapy for tumors and leukemias cause severe toxicity in normal tissues, leading to side effects such as myelosuppression (30). Thus, it is of particular interest our finding that normal cells, including a small intestine epithelial cell line (FHs), myoblasts, PBMCs, and CD34+ hematopoietic progenitors, are not sensitive to YC137. A likely explanation is that Bcl-2 is absent in the crypts of the small intestine (31). Additionally, it has been described that Bcl-2 expression is limited to a small proportion (1% to 4%) of neonatal and adult primary muscle cells in culture (32) and that other Bcl-2 family members, such as Mcl-1 and Bcl-xL, instead of Bcl-2, are critical in the regulation of apoptosis in peripheral blood lymphocytes (33, 34). Furthermore, several authors have shown that in quiescent hematopoietic progenitors, Bcl-2 is not expressed or does not contribute significantly to the survival of this cell population (35, 36). This is the first study that shows the specificity of a Bcl-2 inhibitor for tumor cells as compared with a variety of normal primary cells.

Chronic myelogenous leukemia represents the first human malignancy to be successfully treated with a small molecule inhibitor specific for a tyrosine kinase (17). However, clinical resistance to this inhibitor has been reported in a number of patients, which led to the notion that the effectiveness of other selective inhibitors may be hampered by the presence of resistant tumor cells. We have described a variant of the MB435B cell line that is resistant to YC137-induced apoptosis. This resistant subline has reduced levels of Bcl-2 attributable to down-regulation of the HER2-Stat3 transcriptional pathway, which contributes to chemosensitivity. In support of this, reduced expression of Bcl-2 by antisense oligonucleotides has been shown to sensitize tumor cells to chemotherapeutic drugs (3, 37). We demonstrated previously that Bcl-2 is transcriptionally regulated by Stat3 in MB435B cells (19). However, this pathway seems to be triggered by EGFR, and we show here that resistant cells down-regulate the expression of HER2 but not EGFR. This result may be explained by the formation of receptor complexes between these two members of the EGFR family, which allow the participation of HER2 in apoptosis regulation, because no direct ligand appears to exist for HER2 (38). Trastuzumab, used for the treatment of some forms of breast cancer, is another example of a gene-based cancer drug. This humanized
monoclonal antibody binds HER2 and induces receptor internalization and degradation, inhibits cell cycle progression, and sensitization to chemotherapy-induced apoptosis (39, 40). We show that trastuzumab down-regulates the Stat3-dependent expression of Bcl-2 in MB435B cells, and consistent with our previous data, these cells become more resistant to YC137. This novel result supports the relevance of Bcl-2 levels in the efficacy of Bcl-2 inhibitors and sheds light on the mechanisms by which trastuzumab may modulate the apoptotic response to chemotherapy in tumor cells. It also identifies other molecular targets, such as Stat3 and Bcl-2, that can lead to a rational design of therapies that increase drug sensitivity.

In conclusion, we have described a small molecule inhibitor of Bcl-2, YC137, and showed for the first time that a Bcl-2–specific inhibitor is able to kill selectively tumor cells that overexpress or depend on this antiapoptotic protein for survival but has no effect on a variety of primary cells. We also describe that cancer cells may develop resistance to YC137. Resistant cells reduce the levels of Bcl-2 and become more sensitive to chemotherapy. These data support the rationale for preclinical and clinical studies of small molecule inhibitors specific for Bcl-2 and represent the first example of in vitro selection of cancer cells refractory to a Bcl-2 inhibitor.

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Breast Cancer Cells Can Evade Apoptosis-Mediated Selective Killing by a Novel Small Molecule Inhibitor of Bcl-2

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