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Detailed Terms
Assessment of ABT-263 activity across a cancer cell line collection leads to a potent combination therapy for small-cell lung cancer

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BH3 mimetics such as ABT-263 induce apoptosis in a subset of cancer models. However, these drugs have shown limited clinical efficacy as single agents in small-cell lung cancer (SCLC) and other solid tumor malignancies, and rational combination strategies remain underexplored. To develop a novel therapeutic approach, we examined the efficacy of ABT-263 across >500 cancer cell lines, including 311 for which we had matched expression data for select genes. We found that high expression of the proapoptotic gene Bcl2-interacting mediator of cell death (BIM) predicts sensitivity to ABT-263. In particular, SCLC cell lines possessed greater BIM transcript levels than most other solid tumors and are among the most sensitive to ABT-263. However, a subset of relatively resistant SCLC cell lines has concomitant high expression of the antiapoptotic myeloid cell leukemia 1 (MCL-1). Whereas ABT-263 released BIM from complexes with BCL-2 and BCL-XL, high expression of MCL-1 sequestered BIM released from BCL-2 and BCL-XL, thereby abrogating apoptosis. We found that SCLCs were sensitized to ABT-263 via TORC1/2 inhibition, which led to reduced MCL-1 protein levels, thereby facilitating BIM-mediated apoptosis. AZD8055 and ABT-263 together induced marked apoptosis in vitro, as well as tumor regressions in multiple SCLC xenograft models. In a Tp53; Rb1 deletion genetically engineered mouse model of SCLC, the combination of ABT-263 and AZD8055 significantly repressed tumor growth and induced tumor regressions compared with either drug alone. Furthermore, in a SCLC patient-derived xenograft model that was resistant to ABT-263 alone, the addition of AZD8055 induced potent tumor regression. Therefore, addition of a TORC1/2 inhibitor offers a therapeutic strategy to markedly improve ABT-263 activity in SCLC.

Significance

Small-cell lung cancer (SCLC) is an aggressive carcinoma with few effective treatment options beyond first-line chemotherapy. BH3 mimetics, such as ABT-263, promote apoptosis in SCLC cell lines, but early phase clinical trials demonstrated no significant clinical benefit. Here, we examine the sensitivity of a large panel of cancer cell lines, including SCLC, to ABT-263 and find that high Bcl2-interacting mediator of cell death (BIM) and low myeloid cell leukemia 1 (MCL-1) expression together predict sensitivity. SCLC cells relatively resistant to ABT-263 are sensitized by TORC1/2 inhibition via MCL-1 reduction. Combination of ABT-263 and TORC1/2 inhibition stabilizes or shrinks tumors in xenograft models, in autochthonous SCLC tumors in a genetically engineered mouse model, and in a patient-derived xenograft SCLC model. Collectively, these data support a compelling new therapeutic strategy for treating SCLC.


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Effective cancer-targeted therapies often trigger cell death, commonly via apoptosis, to induce remissions (1–3). For example, we and others previously observed that, among lung cancers with activating EGFR mutations, those with higher expression levels of Bcl2-interacting mediator of cell death (BIM), a key regulator of apoptosis, have a higher response rate and longer progression-free survival on treatment with EGFR inhibitors (2, 4). BH3 mimetics are a class of drugs designed to promote apoptosis. These compounds bind to and inhibit antiapoptotic BCL-2 family members, the molecular sentinels of apoptosis. ABT-263 (5) is a BH3 mimetic that directly binds BCL-2 and BCL-XL, which blocks their binding to BIM and thereby enables BIM-mediated induction of apoptosis (6–8). However, ABT-263 does not bind the prosurvival BCL-2 family member myeloid cell leukemia 1 (MCL-1), and high levels of MCL-1 are associated with resistance to BH3 mimetics such as ABT-263 in both the laboratory and the clinic (9–17).

Small-cell lung cancer (SCLC) is a high-grade neuroendocrine carcinoma that accounts for 10–15% of all lung cancers and is commonly associated with a significant tobacco history. Patient outcomes have not improved substantially over the past 30 y, underscoring the need for more effective treatment strategies (18). Recent studies have demonstrated the antitumor activity of BH3 mimetics in laboratory models of SCLC (5, 19–21). These findings spurred clinical trials of the BH3 mimetic ABT-263

small-cell lung cancer targeted therapies BH3 mimetics apoptosis BIM
expression and sensitivity to ABT-263 were measured in SCLC cell lines consistently suppressed at predicting response (0.0014, P = 0.00043). Among cell lines, those with the highest ratio of either biomarker alone (Fig. 1C) predicted sensitivity to ABT-263. By matching cell line sensitivity to ABT-263 activity putatively relies on releasing BIM to promote apoptosis (6), we hypothesized that BIM levels may predict responsiveness to ABT-263. By matching cell line sensitivity to two independent gene expression data sets, we found a modest, but significant, correlation between BIM expression and sensitivity to ABT-263 (Fig. L4 and SI Appendix, Fig. S1A4). As we and others have reported (10, 13–17), high MCL-1 expression correlated with resistance to ABT-263 and the related ABT-737, and we also observed MCL-1–based resistance to ABT-263 across both data sets (SI Appendix, Fig. S1B and C). However, the ratio of BIM to MCL-1 predicted sensitivity to ABT-263 more effectively than the expression of either biomarker alone (Fig. 1B and SI Appendix, Fig. S1D). Moreover, among cell lines, those with the highest BIM levels that also expressed high levels of MCL-1 were not sensitive to ABT-263 nor were those cancers with low expression of both BIM and MCL-1, underscoring the added value of measuring the ratio of BIM to MCL-1 in predicting sensitivity (Fig. 1C and SI Appendix, Fig. S1E). It is notable that the ratio of either BCL-2 or BCL-XL to MCL-1 was inferior to the ratio of BIM to MCL-1 at predicting response to ABT-263 across solid tumor cancers (SI Appendix, Fig. S2). These findings underlie the potential capacity for MCL-1 to mitigate the therapeutic benefit of ABT-263 in cancers with high BIM levels.

We found that SCLC lines have increased BIM expression compared with other solid tumor types (Fig. 1D and SI Appendix, Fig. S3) along with enhanced sensitivity to ABT-263 compared with other solid tumor types across a large panel of cancer cell lines (Fig. 1E and SI Appendix, Fig. S4). To determine whether ABT-263 sensitivity in SCLC was mediated by high BIM expression, we knocked down BIM using siRNA. Knockdown with two different siRNAs designed against BIM in SCLC cell lines consistently suppressed ABT-263–induced apoptosis by >50% (Fig. 2A and B and...
BIM and MCL-1 mediate ABT-263-induced apoptosis in SCLC, and the ratio of BIM to MCL-1 expression predicts the magnitude of apoptosis in SCLC cell lines. (A) SCLC SW1271 and H1048 cells were treated with either 10 nM scrambled (sc) or BIM siRNA (2), and, the next day, cells were treated with or without ABT-263. Then, cells were (A) prepared and stained with propidium iodide and Annexin-V. Apoptosis was measured by FACS analysis of the percentage of cells positive for Annexin-V 72 h after treatment (the amount of apoptotic cells caused by ABT-263 treatment minus no drug treatment) or (B) lysed and separated by SDS/PAGE, subjected to Western blot, and probed with the indicated antibodies. In A, error bars are SD (n = 3); in B, the asterisk indicates a nonspecific band. (C) H1048 cells transduced with lentiviral particles containing plasmids that express GFP alone (control) or GFP-IRES-MCL-1. MCL-1-overexpressing cells were sorted to isolate cells expressing low and high amounts of MCL-1 based on GFP fluorescence intensity. Protein lysates were prepared and probed with the indicated antibodies. (D) The cells were treated with either no drug (control) or ABT-263 for 48 h. Cells were prepared and stained with propidium iodide and Annexin-V. Apoptosis was measured by FACS analysis of the percentage of cells with Annexin-V positivity. Bars represent mean percentage of apoptotic cells, ABT-263 treatment minus control. Error bars are SD (n = 3). (E) BIM antibody (or IgG control) was added to lysates following treatment with or without ABT-263 for 6 h derived from H1048 cells expressing GFP, low MCL-1, or high MCL-1 (as in C), and BIM-containing complexes were immunoprecipitated and separated by SDS/PAGE, subjected to Western blot, and probed with the indicated antibodies. (F) BIM RNA levels or (G) MCL-1 RNA levels in a panel of 10 human SCLC cell lines were determined by quantitative PCR, and the average of three replicates was plotted versus the amount of apoptosis (n = 3) induced by 1 μM of ABT-263 over 72 h. Each dot represents a unique human SCLC line labeled in red font, with RNA levels relative to GAPDH abundance. A linear regression analysis was used to assign, for F, a coefficient of determination (R²) of 0.142 and a P value of 0.284 (P = not significant), and for G, a coefficient of determination (R²) of 0.135 and a P value of 0.296 (P = not significant). (H) BIM/MCL-1 RNA levels were plotted versus apoptosis in the same lines. A linear regression analysis was used to assign a coefficient of determination (R²) of 0.456 and a P value of 0.032. (I) SCLC H1048 cells were treated with either 50 nM scrambled (sc) or MCL-1 siRNA for 24 h. Cells were reseeded and treated the following day with no drug (control) or ABT-263 and then prepared for FACS analysis of percentage of cells with Annexin-V positivity 24 h after treatment. Error bars are SD (n = 3). (Right) Cell lysates were prepared from the transfected cells and separated by SDS/PAGE, subjected to Western blot, and probed with the indicated antibodies.

SI Appendix, Fig. S5 A and B). Next, we engineered H1048 SCLC cells to have either modest (“low”) or marked (“high”) overexpression of MCL-1 (Fig. 2C and SI Appendix, Fig. S5C). Increasing expression of MCL-1 protected cells from ABT-263–induced apoptosis (Fig. 2D). These results support the model that high BIM and low MCL-1 promote sensitivity to ABT-263, whereas high MCL-1 mediates ABT-263 resistance.

To further assess the mechanism of ABT-263 response, we performed immunoprecipitation of BIM complexes from whole-cell lysates. Please note that the BIM immunoprecipitations successfully depleted >90% of the cellular BIM (SI Appendix, Fig. S5D). In control cells, ABT-263 treatment led to loss of BIM binding to BCL-XL and BCL-2, whereas it induced BIM binding to MCL-1 (Fig. 2E). This indicates that MCL-1 binds the BIM that is...
released from complexes with BCL-2 and BCL-XL upon treatment with ABT-263. This likely contributes to the mechanism whereby MCL-1 expression mitigates the apoptotic response induced by ABT-263. Incremental overexpression of MCL-1 resulted in more BIM/MCL-1 complexes (Fig. 2E) and fewer BIM/BCL-2 complexes, consistent with the abrogation of apoptosis induced by MCL-1 overexpression. Consistent with these results and similar to the analyses of the large cell line panel described above, BIM or MCL-1 expression alone was not significantly predictive of apoptosis following ABT-263 treatment in a subset of SCLC cell lines (Fig. S5, panels C–G). However, the ratio of BIM/MCL-1 correlated significantly with apoptotic response in SCLC cell lines (Fig. 2H). Thus, BIM and MCL-1 levels substantially impact sensitivity to ABT-263-mediated apoptosis in SCLC cell lines, with high BIM and low MCL-1 expression associated with enhanced sensitivity to ABT-263.

TORC1/2 Inhibition Suppresses MCL-1 and Sensitizes SCLC Cells to ABT-263. Despite some of the preclinical promise of ABT-263 against SCLC (Fig. 1E) (5, 19), ABT-263 has demonstrated minimal clinical activity in SCLC as monotherapy (22, 24). BIM levels are higher in SCLC relative to other solid tumor cell lines (Fig. 1D and SI Appendix, Fig. S3), but MCL-1 levels are also relatively high in SCLC (SI Appendix, Fig. S5E), suggesting that high MCL-1 levels may be mitigating the efficacy of ABT-263 in SCLC. Consistent with this notion, knockdown of MCL-1 using two different siRNAs sensitized the H1048 SCLC cell line to ABT-263 (Fig. 2F and SI Appendix, Fig. S6D). We therefore sought to identify pharmacological strategies that could suppress MCL-1 levels and increase sensitivity to ABT-263. We did not pursue obatoclax, which does target MCL-1 in addition to other BCL-2 family members, because early clinical trials suggest that obatoclax causes toxicity independent of its effects on BCL-2 family proteins, potentially limiting its clinical utility (25–27). Rather, we considered TORC1/2 inhibitors because they suppress MCL-1 protein levels in some cancers (7, 28–31). We observed that the TORC1/2 catalytic inhibitor AZD8055 (32) potently suppressed protein levels of MCL-1 in SCLC cells (Fig. 3A) and markedly enhanced ABT-263-induced apoptosis in four different SCLC cell lines examined (Fig. 3B). BIM immunoprecipitations confirmed that AZD8055 abrogated the formation of MCL-1/BIM complexes normally induced by ABT-263 treatment (Fig. 3C and SI Appendix, Fig. S6B; compare the combination to ABT-263). Consistent with the proposed mechanism of apoptosis induced by this combination, overexpression of MCL-1 blocked apoptosis induced by the combination (Fig. 3D and SI Appendix, Fig. S6 C and D). Importantly, AZD8055 and ABT-263 interfered with cell cycle progression in all SCLC cell lines (SI Appendix, Fig. S7). Thus, the combination of ABT-263 and AZD8055 both increased apoptosis and induced growth arrest of SCLCs, suggesting that this combination could be of superior efficacy compared with treatment uniquely targeting BCL-2 family members. Interestingly, we found that treatment of SCLC cells with rapamycin, an allosteric mTOR inhibitor, diminished expression of pS6, a downstream target of TORC1, but, unlike AZD8055 therapy, failed to suppress p4E-BP1 signaling, lower MCL-1 levels, or sensitize to ABT-263 as well as AZD8055 (SI Appendix, Fig. S8 A and B). These data are consistent with other studies demonstrating that TORC1/2 catalytic inhibitors more effectively suppress 4E-BP1 phosphorylation and cap-dependent translation than rapamycin or rapalogs (31, 33). In fact, resistance to single-agent AZD8055 has been reported to emerge through reactivation of cap-dependent translation, including that of MCL-1, in the presence of the drug (34). Of note, although others found that BCL2-associated X protein (BAX) plays a role in rapamycin sensitization of ABT-737 (35), we did not observe an increase in BAX expression in the ABT-263/AZD8055 combination treatment compared with ABT-263 treatment (SI Appendix, Fig. S8C) despite the induction of substantial apoptosis (∼20%) at the same time point (SI Appendix, Fig. S8D).

Previous studies have suggested that mTOR is necessary for efficient cap-dependent translation of MCL-1, and thus mTOR inhibitors may decrease MCL-1 expression by decreasing translation. To determine if AZD8055 affected the half-life of MCL-1 protein, we treated H1048 cells with the cytoplasmic protein synthesis inhibitor, cycloheximide (CHX), alone or in combination with AZD8055. We found the rate of MCL-1 protein degradation was comparable over time in the CHX-treated cells versus the CHX+AZD8055 combination-treated cells, suggesting a similar mode of MCL-1 inhibition between CHX and AZD8055 (SI Appendix, Fig. S9A). Treatment at 6 h with AZD8055 alone left a nearly equivalent amount of cellular MCL-1 as CHX alone or the combination of CHX and AZD8055 (SI Appendix, Fig. S9 A and B). These data are consistent with translational inhibition being the major mechanism underlying suppression of MCL-1 protein levels following mTORC1 inhibition.

Combination of ABT-263 and AZD8055 Causes Tumor Regression in Xenograft Models. We next assessed the efficacy of AZD8055 and ABT-263 in vivo using H1048 and H82 SCLC cell line xenograft tumor models. ABT-263 has been shown to be only modestly effective in the H1048 model and minimally effective in the H82 model (19) and therefore may be more representative of SCLC clinical outcomes with single-agent BH3 mimetics such as ABT-263 (22, 24). Consistent with previous data (19), ABT-263 (80 mg/kg/qd) was partially effective in established H1048 xenografts, whereas single-agent AZD8055 (16 mg/kg/qd) modestly slowed tumor growth (Fig. 3E). However, the combination induced marked tumor regressions to nearly undetectable sizes (Fig. 3E). In the faster-growing H82 xenografts, ABT-263 was ineffective as previously reported (19). Single-agent AZD8055 had a modest effect on tumor growth (Fig. 3E). However, the combination almost completely blocked growth of these tumors (Fig. 3E). Pharmacodynamic studies confirmed that AZD8055 suppressed phosphorylation of TORC1 targets S6 and 4E-BP1 and decreased MCL-1 protein levels in vivo (Fig. 3F). Moreover, cleaved caspase 3, a marker of apoptosis, was significantly increased following combination treatment (Fig. 3G and SI Appendix, Fig. S10A), consistent with the in vitro studies. No overt toxicities were observed in the tumor bearing mice treated with the combination (SI Appendix, Fig. S10B).

Combination of ABT-263 and AZD8055 Is Superior to Either Drug Alone in a SCLC Genetically Engineered Mouse Model. Although cell line xenograft models of SCLC are valuable tools for assessing drug activity in vivo, they may not fully model the tissue environment and heterogeneity of autochthonous tumors (those growing in their native tissue context). To rigorously assess the activity of the combination of ABT-263 and AZD8055 in vivo, we used a GEMM of SCLC (36). Tumors are initiated in this model via conditional inactivation of both alleles of Tp53 and Rb1 in pulmonary neuroendocrine cells, resulting in the development of tumors that model the histology, metastatic spread, and acquired genetic alterations observed in human SCLC (37–39). Tumor-bearing GEMM GEMMs were randomized to receive no treatment, AZD8055 alone (10mg/kg/qd), ABT-263 alone (80 mg/kg/qd), or both ABT-263 and AZD8055 and were treated for 21 d (Fig. 4). Magnetic resonance imaging (MRI) of the thorax was performed 1 day before starting treatment and on day 21 of treatment, and lung tumor volumes pre- and posttreatment were quantified. Most animals had exactly one measurable lung tumor. If more than one tumor was observed in separate lobes of the lungs, the larger tumor was measured. Only tumors that were histologically confirmed to be high-grade neuroendocrine tumors or had clear evidence of distant metastatic spread on MRI were included in the analysis. Tumors progressed in all untreated animals (n = 7), although we observed significant variability in the rate of progression over the 21-d period (Fig. 4 B–D).
and SI Appendix, Table S1). All tumors treated with AZD8055 alone progressed (n = 5) (Fig. 4 B, E, and F and SI Appendix, Table S1). Of six tumors treated with ABT-263, five progressed and one regressed (Fig. 4 B, G, and H and SI Appendix, Table S1). By contrast, in six animals treated with the combination of ABT-263 and AZD8055, three tumors regressed and three others showed relatively limited tumor progression (Fig. 4 B, I, and J and SI Appendix, Table S1). The responses of tumors to the combination of ABT-263 and AZD8055 were significantly superior to responses either to the drug alone or to no treatment. Consistent with these findings and those in the human xenograft models (Fig. 3 E–G), CC3 staining in allografted SCLC GEMM tumors was markedly apparent in the combination treatment at the 3-d time point (Fig. 4 K–N), again indicating a strong apoptotic response following administration of this regimen. Ex vivo cell lines derived from SCLC GEMMs treated with AZD8055 had reduction of MCL-1 protein levels and were sensitized...
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Fig. 4. Combination ABT-263 and AZD8055 induce tumor regressions in a GEMM of SCLC. (A) Schematic of GEMM experiment. Gray space in mouse drawing indicates lung; red circle indicates lung tumor. (B) SCLC GEMMs harboring radiographically measurable lung tumors were randomized to receive treatment for 21 d with 80 mg/kg/day ABT-263, 16 mg/kg/day AZD8055, combination ABT-263/AZD8055, or no treatment. Chest and upper abdomen were imaged by MRI on the day before starting treatment and on day 21 of treatment. For each animal, the largest single lung tumor that was histologically confirmed to be a high-grade neuroendocrine tumor was measured radiographically, and volume was computed. The percentage of tumor volume change for each animal is plotted as a single circle, with black bars indicating mean and SEM. Also included for comparison is the percentage of tumor volume change by MRI when equivalent GEMMs were treated with cisplatin (7 mg/kg IP on days 1 and 8) and etoposide (10 mg/kg on days 2 and 9). Notably, the follow-up imaging time point for the chemotherapy-treated animals was 28 d. Unpaired two-tailed t tests for statistical significance comparing tumor volume percentage change between pairs of datasets were performed. Untreated versus ABT-263/AZD8055: **P = 0.0222. AZD8055 versus ABT-263/AZD8055: P = 0.00350. ABT-263 versus ABT-263/AZD8055: P = 0.0332. Cisplatin/etoposide versus AZD8055/AZD8055: 0.1589 (not significant). (C–J) Representative examples of MRI images (with tumors outlined in green) on day 1 and day 21 of a 21-d treatment period, with the treatment indicated. The asterisk in G and H indicates additional tumor in a separate lobe, which is not included in measurements. (Scale bar: 5.0 mm.) (K–N) A GEMM SCLC tumor was dissected from the lung and implanted s.c. into a NSG mouse. An established tumor in the NSG mouse was then divided and s.c. implanted directly into four nu/nu NSG mice. There was no in vitro intermediate. Once tumors were established, nu/nu mice were treated with 80 mg/kg/day ABT-263, 16 mg/kg/day AZD8055, combination ABT-263/AZD8055, or vehicle for both drugs, (K) vehicle for both drugs, (L) AZD8055 (16 mg/kg/day), (M) ABT-263 (80 mg/kg/qd), (N) ABT-263 (80 mg/kg PO daily), and AZD8055 (16 mg/kg PO daily). Tumors were collected and fixed 3 h after the final treatment. Sections from tumor were stained to detect CC3, shown as brown stain. (Scale bar: 100 μm.)

to ABT-263 (SI Appendix, Fig. S11 A and B), consistent with the mechanism delineated in human cell lines and the activity observed in vivo.

SCLC tumors often respond to first-line chemotherapy in patients, although these responses are almost invariably transient. To provide context for the responsiveness to the AZD8055/ABT-263 combination in the SCLC GEMM model, we treated mice with a combination of cisplatin (7 mg/kg IP on days 1 and 8) and etoposide (10 mg/kg on days 2 and 9), the standard chemotherapies used to treat SCLC in patients. When we compared responses to the combination chemotherapy at day 28 to responses to the targeted therapy combination at day 21, the regimen of ABT-263 and AZD8055 improved responses, although the differences did not reach statistical significance (Fig. 4B). These data show an encouraging efficacy of the ABT-263/AZD8055 combination therapy in reference to standard of care chemotherapy in this model.

AZD8055 Sensitizes a SCLC Patient-Derived Xenograft Model to ABT-263.

To further gain insight into the translational potential of our findings, we next determined the efficacy of the combination in a third type of in vivo model, a patient-derived xenograft (PDX). This type of in vivo model may more faithfully recapitulate the high degree of genomic complexity of the human disease. Patient-derived xenografts derived from a histologically confirmed SCLC case were implanted into NOD scid gamma (NSG) mice, and mice were monitored for tumor growth (Fig. 5A and SI Appendix, Fig. S12). Growing tumors were subsequently treated with either single-agent ABT-263 (80 mg/kg/qd) (n = 4) or the combination of ABT-263 (80 mg/kg/qd) and AZD8055 (16 mg/kg/qd) (n = 4) (there were not enough tumor-bearing mice to treat with single-agent AZD8055). Consistent with a recent report highlighting overall modest activity of ABT-737 against PDXs of SCLC (35), the tumors in all mice treated with single-agent ABT-263 grew fairly rapidly despite drug treatment (Fig. 5A). In contrast, the addition of AZD8055 to ABT-263 led to consistent tumor regressions and showed no sign of regrowth when the experiment was ended ~50 d after drug treatments began (Fig. 5A). Additionally, pharmacodynamic analyses of the tumors demonstrated marked decrease in MCL-1 expression in the combination cohort (Fig. 5B).
Efficacy of Low-Dose ABT-263 Plus AZD8055 in Vitro and in Vivo.

Therapeutic dosing in humans is sometimes limited by drug toxicities that often preclude sufficient target inhibition in patients. The synergistic effect of the ABT-263/AZD8055 combination prompted us to test whether lower doses of either AZD8055 or ABT-263 could still efficiently suppress cell viability and demonstrate in vivo efficacy. In human SCLC cell lines, we found that even low-nanomolar doses of AZD8055 (5 or 16.66 nM) were sufficient to sensitize SCLCs to ABT-263 (100 nM) (Fig. 6D). This sensitization to low-dose AZD8055 was also observed in the ex vivo cell lines derived from SCLC GEMMs (SI Appendix, Fig. S11B). Similarly, a low dose of ABT-263 (3.3 nM) was sufficient to strongly suppress cell viability in combination with 50 nM AZD8055 (SI Appendix, Fig. S13). In the H211 cell line, which is sensitive to single-agent ABT-263 in vitro and in vivo (19), addition of AZD8055 further sensitized cells to ABT-263 (Fig. 6F). Furthermore, in the presence of ABT-263, p4E-BP1 was almost fully suppressed and MCL-1 was significantly down-regulated by low-nanomolar doses of AZD8055 (Fig. 6B). In the dose–response analyses, the amount of apoptosis induced by the combination was inversely related with the amount of MCL-1 protein remaining in response to increasing doses of AZD8055 (Fig. 6C). These in vitro findings prompted us to assess lower doses of AZD8055 in vivo. Strikingly, a low dose of 2 mg/kg of AZD8055 (Fig. 6D, compared with 16 mg/kg, Fig. 3E) in combination with ABT-263 (80 mg/kg) was sufficient to induce regressions in the H1048 SCLC xenograft model.

Discussion

Direct targeting of apoptotic regulators has emerged as an effective therapeutic approach in cancer. One such class of compounds includes BH3 mimetics, such as ABT-263, which block the binding of BCL-2 and BCL-XL to BIM and other proapoptotic proteins. In this study, we examined the efficacy of ABT-263 across a panel of cancer cell lines and observed that the ratio of BIM to MCL-1 predicted sensitivity (Fig. 1 and SI Appendix, Fig. S1). These data agree with a recent report by Roberts et al. showing that a high BIM to MCL-1 ratio indicated a favorable response to ABT-263 in nine patients with chronic lymphocytic leukemia (11).

There is significant interest in further developing BH3 mimetics to treat SCLC, and thus we specifically sought to improve the efficacy of ABT-263 in these cancers. We found that, although levels of BIM were relatively high in SCLC lines (Fig. 1D), MCL-1 levels were also relatively high, contributing to ABT-263 resistance (SI Appendix, Fig. S5E). Thus, high BIM levels may “prime” SCLC for apoptosis and in this way contribute to the sensitivity of SCLC to ABT-263. However, the lack of sensitivity of SCLC to ABT-263 in the clinic may be a result of high MCL-1 levels in these cancers. Therefore, we used these data as a rationale to develop a novel targeted therapy for SCLC based on the intrinsic high levels of BIM and the need to suppress MCL-1 (via TORC inhibition) to sensitize to ABT-263. The ABT-263/TORC1/2 inhibitor combination strategy was more effective than either agent alone, not only in increasing the induction of apoptosis, but also in suppressing proliferation. Inducing both apoptosis and growth arrest recapitulates the effects of other successful targeted therapy paradigms (2), including EGFR inhibitors for EGFR mutant lung cancers and ALK inhibitors for ALK-positive lung cancers.

Notably, the enhanced induction of apoptosis and tumor regression by the combination of ABT-263 and AZD8055 was achieved even with low concentrations of AZD8055 in both cell lines and mouse xenografts and was directly correlated with the relative reduction in MCL-1 protein level (Fig. 6). This suggests that AZD8055 may be effectively combined with ABT-263 even at doses that would otherwise be subtherapeutic as a single agent, widening the potential therapeutic window of this combination. As other BH3 mimetics are being currently developed in the clinic, it will be interesting to determine the differential activity of each to build on the promising concept of combining TORC inhibitors with BH3 mimetics.

A recent report by Gardner et al. demonstrated that rapamycin sensitized several SCLC human cell lines and PDxS to the structurally related BH3-mimetic ABT-737 (35). However, our study reveals significant differences between the two approaches. Unlike the TORC catalytic site inhibitor, rapamycin fails to significantly down-regulate MCL-1 (SI Appendix, Fig. S8A). This finding is consistent with results demonstrating that TORC1/2 catalytic inhibitors more effectively suppress 4E-BP1 phosphorylation and cap-dependent translation than rapamycin or rapalogs (31, 33). Thus, the TORC catalytic inhibitor is uniquely capable of down-regulating MCL-1 protein expression, which is key for the combination to induce apoptosis. Indeed, we found that addition of rapamycin did not consistently increase the amount of apoptosis induced by ABT-263 (SI Appendix, Fig. S8B). Another major difference between these therapeutic approaches is that the AZD8055/ABT-263 combination appears effective in low-BCL-2-expressing SCLCs (NCI-H82 and NCI-H446, Figs. 3 B and E and 6D), which Gardner et al. (35) found were resistant to the combination of rapamycin with ABT-737. In contrast to the findings reported by Gardner et al. (35), we did not observe that ABT-263, rapamycin, or AZD8055 consistently affected BAX levels (Figs. 3F and 5B and SI Appendix, Fig. S8C). One
and BRAF mutant colorectal cancers (7). I wild-type colorectal cancers, where Rb1 value was 0.939 and the Low doses of AZD8055 sensitize SCLC to ABT-263. (Published online March 3, 2015 E1295 PNAS) SEM for each cohort is displayed.

Interestingly, we observed that the BIM to MCL-1 ratio predicted sensitivity to ABT-263 not only in SCLC, but also across a large panel of cancer cells encompassing a wide range of malignancies (Fig. 1B and SI Appendix, Fig. S1D). We indeed recently reported that combination ABT-263 and AZD8055 also showed efficacy in KRAS and BRAF mutant colorectal cancers (7). In that study, we demonstrated that AZD8055 led to loss of MCL-1 in those cancers, thereby sensitizing to ABT-263. However, this combination therapy was not effective in KRAS and BRAF wild-type colorectal cancers, where TORC1/2 inhibition failed to suppress MCL-1. Thus, it is likely that this combination therapy may specifically be effective for cancers in which TORC1/2 inhibition suppresses MCL-1 expression.

We extended our study beyond the more traditional preclinical strategies by using both a SCLC GEMM model and a PDX from a patient with SCLC. Importantly, each autochthonous tumor in the SCLC GEMM model, although initiated by inactivation of Tp53 and Rb1, develops a unique set of additional genetic alterations (39). Furthermore, tumors themselves are heterogeneous, harboring multiple distinct subclones (39). Autochthonous GEMM tumors may therefore more closely recapitulate the genetic heterogeneity found in human SCLC tumors (24, 40, 41) and may be a more rigorous assay for therapeutic efficacy than in vitro studies or mouse xenograft models. They also model the tumor microenvironmental features and immune interactions of human disease more fully than s.c. xenografts in immune-compromised hosts. The fact that we consistently observed stabilization or regression of tumors in response to combination of ABT-263 and AZD8055 in the GEMM, compared with either drug alone, further supports the notion that this combination may be more broadly active in patients than ABT-263 monotherapy. Notably, of six autochthonous tumors treated with ABT-263 alone, one showed significant regression whereas the other five significantly progressed. This range of sensitivities also mirrors the range of sensitivities to ABT-263 in human cell lines and in patients (19, 24).

There was a trend toward GEMM tumors responding more to ABT-263 and AZD8055 than to the standard of care chemotherapies used in patients, cisplatin and etoposide. The relative lack of response of the GEMM tumors to cisplatin and etoposide is interesting because, by contrast, human SCLC tumors have a response rate of ~50% to combination carboplatin and irinotecan (42). Consistent with our findings, Singh and colleagues (43) showed that Tp53/ Rb1-deleted SCLC GEMMs were only modestly sensitive to combination carboplatin and irinotecan, which have similar clinical efficacy as cisplatin and etoposide in patients (42, 44). The SCLC GEMM may therefore more closely resemble a chemotherapy-resistant subset of human SCLC. If this is true, then our
study supports the use of the combination of ABT-263 and TORC1/2 inhibition even in second line setting in patients. Alternatively, it is possible that we did not achieve sufficient levels of cisplatin and etoposide in the mice to induce the extent of tumor regression often seen in patients.

Our PDX study demonstrated lack of efficacy of single-agent ABT-263, consistent with the lack of efficacy observed with single-agent ABT-737 by Gardner et al. (35). The combination, however, induced regressions of all of the combination-treated tumors (Fig. 5A). Taken together with the potent efficacy demonstrated in the human xenograft mouse models of SCLC (Fig. 3) and the GEMM model (Fig. 4), the effect of the combination of AZD8055 and ABT-263 was consistent and profound across several mouse models, even at low concentrations of AZD8055 (Fig. 6D).

Overall, we found that high BIM levels helped promote sensitivity to BCL-2/BCL-XL inhibitors in SCLC, but efficacy was mitigated by MCL-1. The addition of TORC1/2 inhibitors to BCL-2/ BCL-XL inhibitors in SCLCs can lead to marked tumor responses in an array of complementary mouse models and therefore may improve the efficacy of BH3 mimetics for the treatment of SCLC.

Materials and Methods

All materials and methods are described in SI Appendix. These include information on cell lines, western blotting, immunoprecipitation, plasmid preparation, siRNA transfections and shRNA transductions, viability assays, FACS death and cell cycle assays, mouse experiments, and statistical methods. All studies in GEMM and allograft models were performed under an Institutional Animal Care and Use Committee approval of the Massachusetts Institute of Technology Committee on Animal Care-approved animal protocol. The mouse xenografts and patient-derived xenografts were performed in accordance with the Massachusetts General Hospital Subcommittee on Research Animal Care.

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