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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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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 antimtumor activity of BH3 mimetics in laboratory models of SCLC (5, 19–21). These findings spurred clinical trials of the BH3 mimetic ABT-263

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 survivals upon 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 mimic 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).

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– small-cell lung cancer (SCLC) cell line model, and in a patient-derived xenograft model, 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.

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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ABT-263 was well tolerated in the clinic with a dose-limiting toxicity of thrombocytopenia, an on-target toxicity of BCL-2 inhibition. Unfortunately, phase II trials of ABT-263 monotherapy revealed unimpressive activity. Sixteen of 26 evaluable patients had progression of disease, 9 had stable disease, and one had a partial response. Consequently, there has been no further clinical development of ABT-263 as a monotherapy in SCLC.

Despite these findings, several questions remain: which solid tumor malignancies are most sensitive to single-agent ABT-263, and why is SCLC among the most sensitive? In addition, why does SCLC fail to respond to single-agent ABT-263 in the clinic, and how could efficacy be enhanced? Here, we assess the sensitivity of a large panel of cell lines spanning multiple cancer types to ABT-263. We observe that cell lines with high expression of BIM are highly sensitive to ABT-263 and that SCLC expresses higher levels of BIM than other solid tumor malignancies. Although SCLC is among the most sensitive to single-agent ABT-263, efficacy is substantially limited by MCL-1, which is also elevated in SCLC. We find that SCLC can be sensitized to ABT-263 via TORC1/2 inhibition, which leads to reduction of MCL-1 protein levels, thereby permitting BIM-mediated apoptosis.

In two SCLC mouse xenograft models, either drug alone has little activity. However, the combination of ABT-263 and AZD8055 induces tumor stabilization or regression. Furthermore, we examined the efficacy of this combination in autochthonous lung tumors arising in a genetically engineered mouse model (GEMM) of SCLC, where the combination of ABT-263 and AZD8055 also induces tumor stabilization or regression. By contrast, most tumors progress when treated with either drug alone. Finally, in a patient-derived xenograft model of SCLC in which ABT-263 alone is ineffective, the combination of ABT-263 and AZD8055 potently suppresses tumor progression across a variety of preclinical SCLC experimental models.

Results
BIM/MCL-1 Ratio Predicts Sensitivity to ABT-263. Our initial studies stemmed from the observation that 5 of 11 SCLC human xenografts tested by Shoemaker and colleagues (19) did not respond to ABT-263 and that the majority of patients treated in a phase II study had progression of disease (24). To more broadly identify mediators of response to ABT-263, we examined data collected from a high-throughput drug screen (13) assessing over 500 human cancer cell lines for sensitivity to ABT-263. Because the mechanism underlying ABT-263 activity putatively relies on releasing BIM to promote apoptosis, 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. S1A). 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. S1 B 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. 2 A and B and...
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 therapy in a subset of SCLC cell lines (Fig. 2F and 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 re-activation 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 mTORC 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 not be the most 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 SCUs 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. 4A). 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...
AZD8055 versus ABT-263/AZD8055: 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 nude NSG mice. There was no in vitro intermediate. Once tumors were established, nude mice were treated with vehicle for both drugs, (K–N) AZD8055 (16 mg/kg qd), (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 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).
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AZD8055 (16 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. 3B 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).

These data indicate substantial activity of this combination in a third type of SCLC preclinical model and demonstrate that, in a SCLC PDX model, AZD8055 down-regulates MCL-1 to sensitize SCLC to ABT-263.

Efficacy of Low-Dose ABT-263 Plus AZD8055 in Vitro and in Vivo.

Therapeutic dosing in humans is sometimes limited by drug toxicity. We therefore anticipated that lower doses of either AZD8055 or ABT-263 might be more effective than the relatively high doses used in the xenograft model. AZD8055 down-regulates MCL-1 to sensitize SCLC to ABT-263. The ABT-263/TORC1/2 inhibitor combination further sensitized cells to ABT-263 (Fig. 6E). This suggests that 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.

Fig. 5. Addition of AZD8055 to ABT-263 sensitizes a PDX model of SCLC. (A) Pieces of a patient-derived SCLC tumor were implanted into NSG mice and monitored for subsequent growth. Upon growing to ∼150–300 mm³, tumors were treated with ABT-263 (80 mg/kg/d) or ABT-263 (80 mg/kg/d) plus AZD8055 (16 mg/kg/d). AZD8055 was given ∼1.5 h before ABT-263 for combination treatments. Tumor measurements were performed approximately three times per week by calipers, and the average tumor volume + SEM for each cohort is displayed. Treatment start is indicated by an arrow. (B) Approximately 3 h after final drug administration, tumors were surgically excised and tumor lysates were prepared and subjected to Western blot analyses and probed with the indicated antibodies. ABT-263-treated and ABT-263 and AZD8055 combination–treated (ABT+AZD) tumors were compared to two untreated tumors (No Rx). Each number represents a tumor from a unique mouse.

Fig. 6. AZD8055 plus ABT-263 is synergistic in vitro, in mouse xenografts, and in human xenografts. (A) Relative activity of rapamycin, AZD8055, and ABT-263 evaluated by inducing apoptosis in NCI-H82 mouse xenografts. These data were analyzed as described (16), showing that AZD8055 and ABT-263 individually induced apoptosis, whereas the combination was more effective. (B) Boxplot analysis of apoptosis in NCI-H82 cells treated with rapamycin, AZD8055, ABT-263, or a combination of all three. The middle line in each box represents the median, and the edges of the box represent the first and third quartiles. The whiskers represent the 10th and 90th percentiles.

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

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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 decrease 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. 3B 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
We extended our study beyond the more traditional preclinical strategies by using both a SCLC GEMM model and a PDX from this combination therapy may specifically be effective for cancers in which TORC1/2 inhibition suppresses MCL-1 expression. The possibility for this difference is that Gardner et al. (35) assessed for changes in BAX expression after more prolonged treatment (1 d to 1 w). Although we did not detect BAX changes after >14 d of treatments in the H1048 xenograft model (Fig. 3F) or in the PDX model of SCLC (∼50 d of treatment), we assayed for BAX expression only at shorter time points in vitro (16 h). Thus, BAX regulation through TORC1 inhibition may be a later event in some models of SCLC. Importantly, Gardner et al. (35) observed that the mTOR pathway was active in all of the PDX models that they studied, further supporting the concept that combining mTORC catalytic site inhibitors with BH3 mimetics in SCLC could be beneficial.

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.

Fig. 6. Low doses of AZD8055 sensitize SCLC to ABT-263. (A) SCLC cell lines were treated with no drug (Media), 100 nM ABT-263 (ABT-263 only), or 100 nM ABT-263 in combination with the indicated dose of AZD8055 (ranging from 5 to 5,000 nM AZD8055) for 7 d. The number of viable cells was determined by CellTitre-Glo and presented as fold-change of cells compared with day 0 (i.e., negative values represent loss of cells from day 0). Error bars are SD of cells treated in triplicate. (B) H1048 cells were treated with the indicated dose of AZD8055 with 100 nM ABT-263 or 100 nM ABT-263 alone (−) for 16 h, and equal amounts of lysates were subjected to Western blot analyses and probed with the indicated antibodies. (C) The amount of MCL-1 protein relative to GAPDH as determined by quantification on a SynGene GBox was plotted against the amount of apoptosis induced by the corresponding 72-h drug treatments (as determined by FACS analysis of the percentage of Annexin-V-positive cells, treatment minus no-treatment control). Note: AZD8055 treatments were in the presence of 100 nM ABT-263, as in (B). For the linear regression analysis, the R² value was 0.939 and the P value was 0.007. (D) H1048 cells were grown as xenograft tumors in Nu/Nu mice. When tumors reached ∼100–200 mm³, mice were randomized into treatment cohorts: control (no drug), 2 mg/kg AZD8055, or the combination of AZD8055 (2 mg/kg) and ABT-263 (80 mg/kg). AZD8055 was given −1.5 h before ABT-263 for combination treatments. Tumor measurements were performed approximately three times per week by calipers, and the average tumor volume ± SEM for each cohort is displayed.

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 platinum and etoposide (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
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The authors declare no conflict of interest. All studies in GEMM and allo-MFM were performed under an Institutional Animal Care and Use Committee of the Massachusetts Institute of Technology Committee on Animal Care-approved animal protocol.

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 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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