

# Venetoclax Is Effective in Small-Cell Lung Cancers with High BCL-2 Expression

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## Abstract

**Purpose:** Small-cell lung cancer (SCLC) is an often-fatal neuroendocrine carcinoma usually presenting as extensive disease, carrying a 3% 5-year survival. Despite notable advances in SCLC genomics, new therapies remain elusive, largely due to a lack of druggable targets.

**Experimental Design:** We used a high-throughput drug screen to identify a venetoclax-sensitive SCLC subpopulation and validated the findings with multiple patient-derived xenografts of SCLC.

**Results:** Our drug screen consisting of a very large collection of cell lines demonstrated that venetoclax, an FDA-approved BCL-2 inhibitor, was found to be active in a substantial fraction of SCLC

cell lines. Venetoclax induced BIM-dependent apoptosis *in vitro* and blocked tumor growth and induced tumor regressions in mice bearing high BCL-2-expressing SCLC tumors *in vivo*. BCL-2 expression was a predictive biomarker for sensitivity in SCLC cell lines and was highly expressed in a subset of SCLC cell lines and tumors, suggesting that a substantial fraction of patients with SCLC could benefit from venetoclax. Mechanistically, we uncover a novel role for gene methylation that helped discriminate high BCL-2-expressing SCLCs.

**Conclusions:** Altogether, our findings identify venetoclax as a promising new therapy for high BCL-2-expressing SCLCs. *Clin Cancer Res*; 24(2); 360–9. ©2017 AACR.

## Introduction

Despite initial sensitivity to platinum-based combination chemotherapies, SCLCs characteristically develop progressive chemotherapy resistance, resulting in a 5-year survival approximating 3%. In contrast to non-small cell lung cancer (NSCLC), which has significant druggable additions to diverse receptor tyrosine kinases (RTKs), such as EGFR and ALK, genomic studies of SCLC tumors have not revealed widespread RTK addiction (1–3).

Instead, these cancers demonstrate a predictable pattern of loss of tumor suppressors *RB* and *p53* in almost all cases, and loss of

*PTEN* or *PIK3CA* through mutations in up to 20% of cases (3, 4). In addition, we have previously demonstrated that BCL-2, the founding prosurvival BCL-2 family member, is highly expressed in 65% of cases (5, 6), as is BIM, a proapoptotic BCL-2 family member, which sensitizes SCLC to targeted therapies (7), in particular BH3 mimetics (8).

As tumor suppressors and transcription factors are currently difficult drug targets, the development of targeted therapies for SCLC has been lacking. However, the recent addition of prosurvival BCL-2 family inhibitors to the repertoire of targeted therapies revealed marked preclinical activity in SCLC (7, 9). Unfortunately, in a phase II trial of the dual BCL-2 and BCL-xL inhibitor, navitoclax (ABT-263) in 39 patients with SCLC, only one patient achieved a partial response, and eight patients had stable disease (10). While the response rate was disappointing, the disease control rate of 26% along with an objective response in a refractory population indicates detectable single-agent activity. Importantly, there was dose-limiting thrombocytopenia in the study (10, 11), which is the result of BCL-xL inhibition (12). This strongly suggests the magnitude of BCL-2 inhibition was limited in this trial by the toxicities induced by BCL-xL inhibition.

The Genomics of Drug Sensitivity in Cancer (GDSC) platform is a comprehensive drug-screening initiative aimed at discovering new drug sensitivities (13). We used this platform to characterize the activity of venetoclax across cancer types and identify potential genomic predictors of response to this drug. We found that a large subset of SCLC cell lines is hypersensitive to the BCL-2-selective inhibitor venetoclax. Moreover, this activity correlated well with BCL-2 expression levels. These data demonstrate strong rationale for venetoclax therapy in high BCL-2-expressing SCLC tumors.

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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### Translational Relevance

Through an unbiased high-throughput drug screen of 791 solid tumor cancer cell lines, we have identified the FDA-approved BCL-2 inhibitor, venetoclax, to be active in high BCL-2-expressing SCLCs. This study should set the stage for clinical evaluation of venetoclax in patients with SCLC with high BCL-2 expression.

## Materials and Methods

### Cell lines

The SCLC cell lines, NCI-H510A, DMS-53, NCI-H187, NCI-H524, SW1271, COR-L303, IST-SL2, NCI-H1105, NCI-H211, NCI-H187, NCI-H146, NCI-H446, NCI-H196, NCI-H1688, NCI-H1048, and NCI-H82 were from the Center for Molecular Therapeutics at Massachusetts General Hospital Cancer Center, and are routinely checked by DNA STR for verification. The NCI-H2171 cell line was from the ATCC. Cell lines were routinely tested for mycoplasma by MycoAlert (Lonza), and if positive, treated with Plasmocure from InvivoGen until MycoAlert assays for the presence of mycoplasma were negative.

### Drugs

Venetoclax for the cell culture and *in vivo* experiments was kindly provided by AbbVie. Cisplatin (catalog No. A8321) and etoposide (catalog No. A1971) were purchased from ApexBio.

### High-throughput drug screen

Venetoclax was screened at the Center for Molecular Therapeutic at the Massachusetts General Hospital across authenticated cell lines from the GDSC collection essentially as described previously (13). Resazurin was used to measure viability for all the cell lines. Drug response estimates [ $IC_{50}$  and AUC (area under the dose-response curve)] were obtained as described previously (13).

### Analysis of mRNA expression

Expression of all genes in solid tumors, including venetoclax-sensitive and -resistant SCLC cell lines was obtained through the R2: Genomics Analysis and Visualization Platform (<http://hgserver1.amc.nl>) using the GDSC-based Cellline Cancer Drug (Sanger) dataset (Array Express Accession: E-MTAB-3610). Expression data were also obtained from the R2 platform, using the CCLE expression dataset (GEO ID: GSE36133). For comparison between SCLC, NSCLC, and other solid tumors, blood-type tumors, and all tumors in which histology was not determined were excluded from analyses.

### Analysis of BCL2 promoter methylation

Illumina 450K array data from the GDSC (GEO ID: GSE68379) was used for the analysis of CpG methylation. Methylation status of the BCL2 promoter was performed on SCLCs with either 2- or 3-BCL2 copy-numbers. Raw methylation data was imported into R, and ChAMP (14) was used to normalize the array data. B values were extracted for each cell line, and individual CpG sites for the 1.5-kb region immediately upstream of the BCL2 transcriptional start site were averaged for the top and bottom 50% BCL2 expressers. Gene schema and CpG island notation in Fig. 3B was generated from the UCSC Genome Browser (<http://genome.ucsc.edu>) using the hg19 assembly.

### Lentivirus production

The lentiviral short-hairpin RNA (shRNA)-expressing constructs were purchased from Addgene and Open Biosystems. The constructs were transfected into 293T packaging cells along with the packaging plasmids (Addgene), and the lentivirus-containing supernatants were used to transduce SCLC cells.

### Immunoprecipitations and Western blot analyses

Antibodies were purchased as follows: BCL-2 (clone 100) from Sigma-Aldrich and Cell Signaling Technology [(GAPDH, BIM (C34C5), BCL-X<sub>L</sub> (54H6), and cleaved PARP (D64E10) from Cell Signaling Technology]; Noxa (114C307.1) from Thermo Fisher Scientific; MCL-1 from Enzo Life Sciences; BAX (N-20) from Santa Cruz Biotechnology. Immunoprecipitation and Western blots were performed as described in ref. 19. For Supplementary Fig. S5B12, 500  $\mu$ g of each lysate were incubated with BIM antibody (500 ng; Cell Signaling Technology, catalog No. 2933S), or rabbit IgG (500 ng; Santa Cruz Biotechnology, catalog No. sc-2027). Following the addition of 25  $\mu$ L of 1:1 PBS: prewashed Protein A beads to the antibody/lysate mix, samples were incubated with rotating motion overnight. Equal amounts of extracts (5% of immunoprecipitated protein) were prepared in parallel.

### Cell viability assays

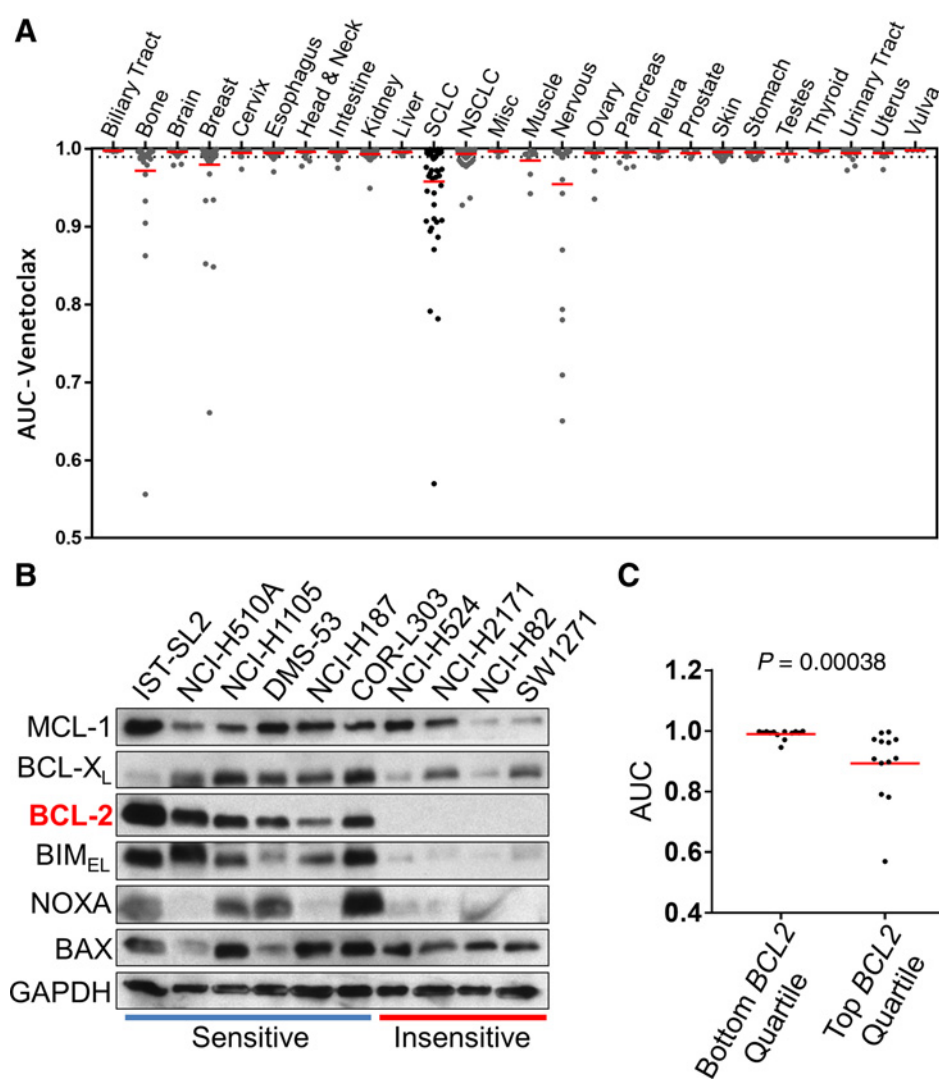
SCLC cells were seeded in triplicate (WST-1 assays) or quadruplicate (CTG-assays) in microtiter plates (96 wells) with a concentration of  $1 \times 10^4$  cells per well in 100- $\mu$ L growth medium (WST-1 assays) or a concentration of  $2 \times 10^3$  cells per well containing 180- $\mu$ L growth medium (CTG assays), and were treated with 0 to 10  $\mu$ M/L venetoclax for 72 hours, 5 days, or 7 days. Cell survival was measured by the WST-1 assay (Fig. 4D) according to the manufacturer's instruction (Roche), and the CellTiter-Glo protocol (Promega; Fig. 2; Supplementary Fig. S2A and S6). For synergy assays in Supplementary Fig. S2C, DMS-53, NCI-H2171, NCI-H82, and SW1271 were seeded at  $1 \times 10^4$  cells/well in 96-well plates. A  $3 \times 4$  dose matrix of venetoclax and the combination of cisplatin and etoposide (ratio cisplatin:etoposide 1/1.5) was performed, and cell viability was measured at the end of 72 hours using WST-1 as above. Drug synergy was determined using the Bliss independence model as described previously (15).

### FACS apoptosis

DMS-53, NCI-H510A, NCI-H82, and SW1271 cell lines were seeded at  $1.0$ – $1.5 \times 10^6$  cells in a 6-well tissue culture plate and either untreated or treated for 24, 48, or 72 hours with 1  $\mu$ M/L venetoclax. Following treatment, cells were collected as per BD Biosciences' Annexin V/PI staining protocol. Cells were then assayed on a Millipore Guava flow cytometer. Cells were quantitated as either Annexin V-positive, PI-positive, double positive, or double negative using quadrants. Apoptotic cells were measured as 100% – double negative cell population.

### SCLC cell line xenograft model

The DMS-53 cell line was injected into the right flank of 20 female NOD-SCID-gamma (NSG) mice at a concentration of  $5 \times 10^6$  cells per mouse using a 1:4 ratio of cells to Matrigel (Corning, catalog No. 354248). The NCI-H82 cell line was injected into both flanks of 10 female NSG mice using the same protocol as above. Tumors were monitored until the average size of all tumors increased above the average injection volume of 100

**Figure 1.**

SCLC tumor cell lines are sensitive to venetoclax via increased BCL-2 expression. **A**, Venetoclax drug screen data against 26 different cancer types, with SCLC highlighted. **B**, Expression of the BCL-2 family proteins in SCLC cell lines was determined by Western blot analyses using the indicated antibodies. The SCLC lines are indicated as venetoclax sensitive (AUC < 0.98, blue line) or insensitive (AUC > 0.98, red line). **C**, SCLC expression data from the GDSC database was clustered into the bottom and top 25% BCL-2 RNA-expressing cells, and the AUC data were plotted. The red bar indicates the mean of each group. Statistical significance was determined using the Mann-Whitney *U* test.

mm<sup>3</sup>. Mice were then randomized into untreated and venetoclax-treated cohorts, with 10 mice per cohort. Venetoclax-treated mice were dosed with 100 mg/kg of venetoclax by oral gavage 6 days per week. Every 2 to 3 days, tumors were measured in two dimensions via calipers and tumor volume was calculated as: height × width × width × 0.52, where height was the larger of the two measurements. Mice were euthanized after 29 days of treatment. All animal experiments were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC protocol# AD10001048).

#### Patient-derived xenograft models

The first patient-derived xenograft (PDX) model (Crown Bioscience) is from a 71-year-old female patient with small-cell lung carcinoma (LU5263). The second (LU86) and third (LU95) PDX models have been described previously (16) and were kindly provided by Stemcentrx (currently part of AbbVie). Briefly, a single-cell suspension was mixed with Matrigel at a 1:1 ratio and  $0.5 \times 10^6$  cells were injected per mouse. Enrollment and treatment of this study was performed as above in the xenograft model, with six mice per cohort. Mice were euthanized after 31 days of

treatment. A measure of apoptosis was obtained by treating three mice with venetoclax for 3 days. The mice were euthanized 2 hours after venetoclax treatment on the third day, and the tumors were extracted and immediately snap-frozen in liquid nitrogen for eventual lysate preparation.

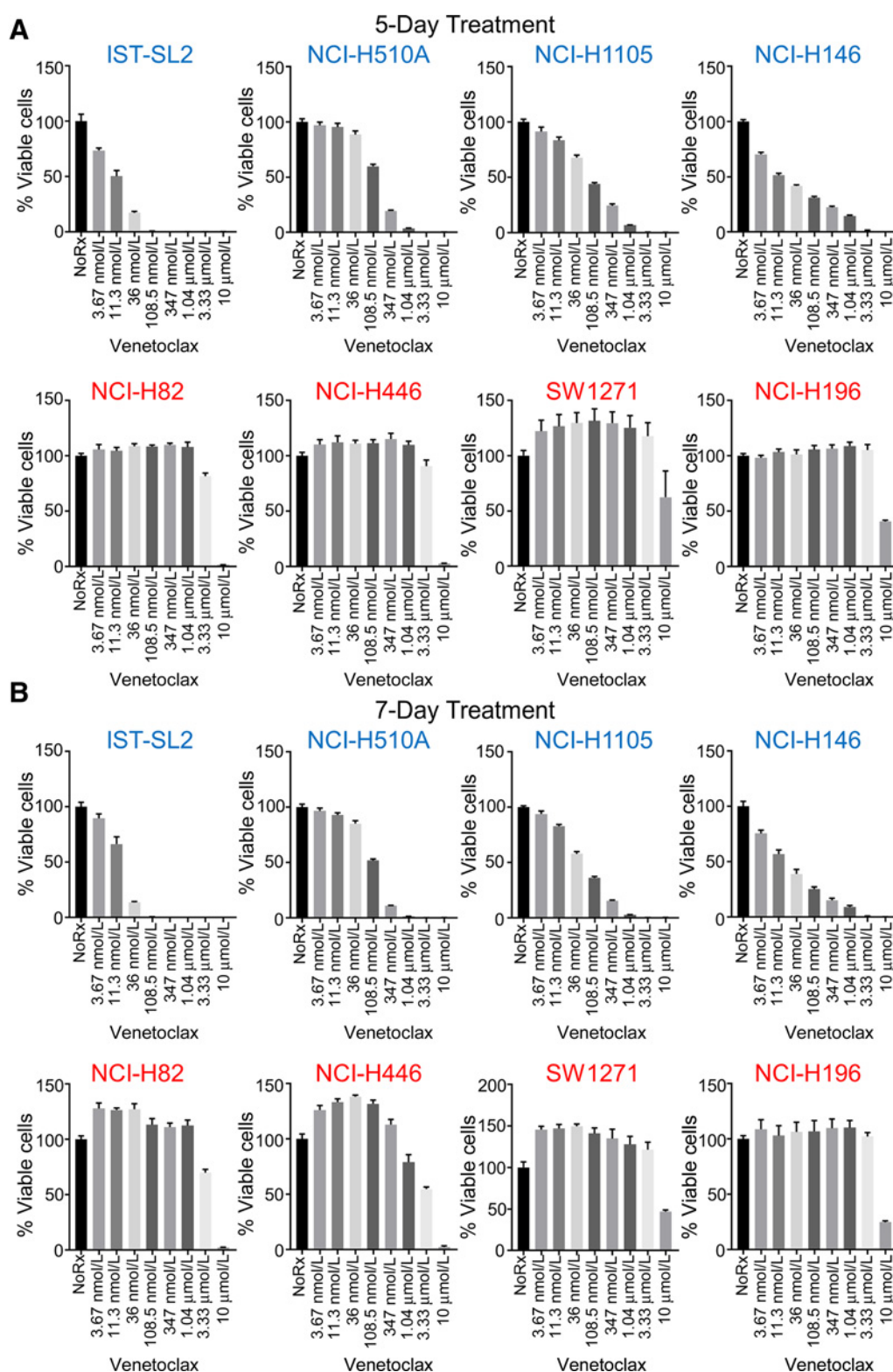
#### Statistical analyses

Mann-Whitney *U* tests were performed to determine significance of relationships in Fig. 1C and Supplementary Fig. S4 and Supplementary Fig. S5. A Spearman correlation test was performed to determine significance and a correlation coefficient in Fig. 3A and Supplementary Figs. S1B and C and S3A. ANOVA analysis was performed to determine the significance in Fig. 3C and D.

## Results

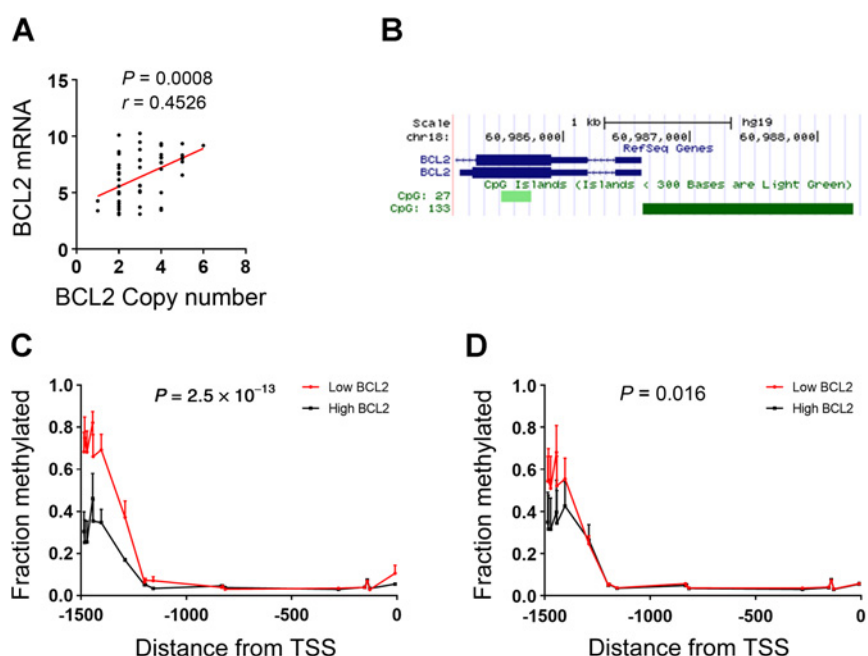
### A subset of SCLC is hypersensitive to venetoclax

Venetoclax is a first in-class BCL-2-selective inhibitor, recently FDA-approved for patients with chronic lymphocytic leukemia (CLL) with 17p deletion who have received at least one prior

**Figure 2.**

Five- and 7-day cell viability assays of venetoclax-sensitive and -resistant SCLC cell lines. **A**, Five-day CellTiter-Glo assays were performed on the indicated venetoclax-sensitive (top) and -resistant (bottom) cell lines. **B**, Seven-day CellTiter-Glo assays were performed on the cell lines in part A. Error bars are plotted as standard error. Venetoclax-sensitive cell lines, blue; venetoclax-resistant cell lines, red.



**Figure 3.**

Methylation of the *BCL2* promoter correlates with RNA expression. **A**, *BCL2* expression correlates with DNA copy-number. Correlation analysis was performed using Spearman correlation. **B**, A CpG island exists within a 1.5-kb region upstream of the *BCL2* transcriptional start site. **C**, Methylation within the CpG island of *BCL2*. Top 50% of *BCL2*-expressing SCLC cell lines (black line;  $n = 8$ ) with two copies of *BCL2* are compared with the bottom 50% *BCL2* expressors (red line;  $n = 8$ ). **D**, Methylation of within the CpG island of *BCL2*. Top 50% of *BCL2* expressing SCLC cell lines (black line;  $n = 6$ ) with three copies of *BCL2* are compared to the bottom 50% *BCL2* expressors (red line;  $n = 7$ ). Statistical significance between methylation status was determined using ordinary ANOVA analysis.

treatment. On the basis of its safety profile and potent anticancer activity, we performed a high-throughput screen of venetoclax across multiple cancer types with the goal of identifying other cancers that may respond to venetoclax. We screened over 950 cell lines including 791 lines derived from solid tumors. Among solid tumor cell lines, a subset of neuroblastomas (*MYCN*-amplified; ref. 17) and SCLCs were particularly sensitive to venetoclax (Fig. 1A; Supplementary Fig. S1A; B1A; Supplementary Table S1). Longer treatment times of 5 and 7 days did not change sensitivity profiles of the different SCLC cell lines to venetoclax (Fig. 2). Furthermore, a clear dose response to venetoclax of the high *BCL2* NCI-H187 (AUC = 0.965) cell line was observed (Supplementary Fig. S2A). In contrast, the low *BCL2* NCI-H524 (AUC = 0.987) was unaffected until the maximum dosage given (Supplementary Fig. S2C). As SCLCs are sensitive to initial platinum-based chemotherapy (18), we tested venetoclax with cisplatin-etoposide at a 1:1.5 ratio (16). Here, we found some weak synergy at higher concentrations of chemotherapy in four of the six lines (Supplementary Fig. S2C) across different venetoclax concentrations. These data suggest potential new indications for this clinical compound.

#### The sensitive SCLCs are distinguished by high *BCL2* expression

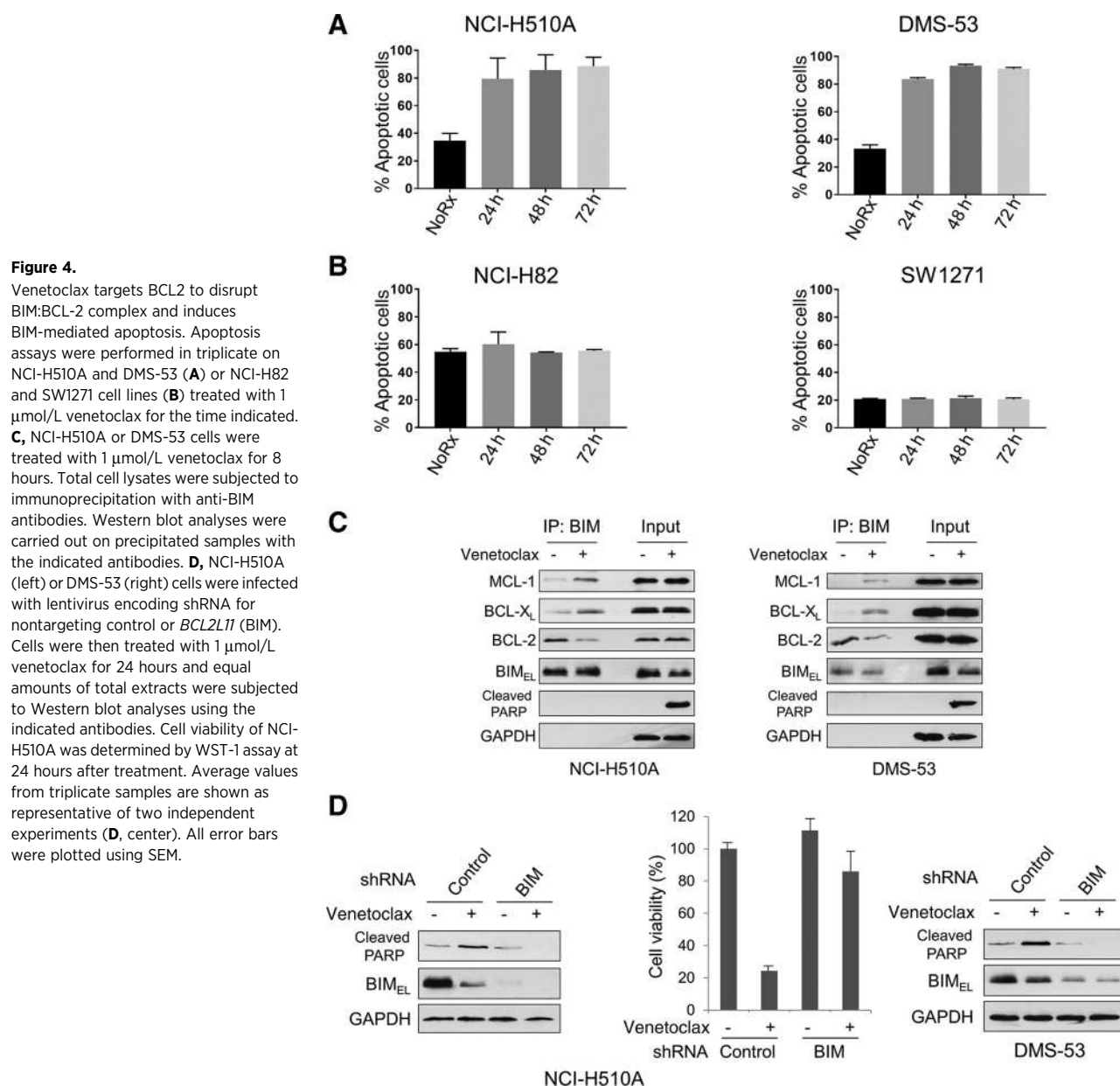
We next sought to investigate whether the expression level of *BCL2*, the direct target of venetoclax, might predict response within SCLC. Through the GDSC database, RNA expression levels of *BCL2* were available in 52 of the 56 SCLC cell lines screened. We found sensitivity to venetoclax positively correlated with *BCL2* expression (Fig. 1B and C; Supplementary Fig. S1B). In contrast, the mRNA and protein expression profiles of other prominent *BCL2* members, including *BCL2L11* (BIM), did not further predict sensitivity (Fig. 1B; Supplementary Fig. S3 and S4). While large drug screen studies have shown that target expression is not consistently predictive of drug response (19), these data

indicate that *BCL2* alone may constitute an efficient predictive biomarker for venetoclax in SCLC, with high *BCL2*-expressing SCLCs yielding better responses.

#### Methylation of *BCL2* contributes to its expression

We next evaluated *BCL2* expression in SCLCs compared with other cancers, to confirm prior findings (20) that *BCL2* expression was generally higher in SCLC, suggestive of an underlying reliance on *BCL2*. We used *BCL2* mRNA expression data from both the GDSC and the Cancer Cell Line Encyclopedia (CCLE; refs. 21, 22) resource and indeed found SCLC cell lines have higher *BCL2* expression on average than other solid tumor cell lines including NSCLC (Supplementary Fig. S5A). We next asked whether this relationship held true in patient tumors. Biomarker assessment in SCLC tumors is challenging due to the limitation of available tumor material. We identified two datasets (23, 24) with gene expression from SCLC tumors and found significantly ( $P < 0.001$ ) higher *BCL2* expression in SCLC tumors than in NSCLC tumors (Supplementary Fig. S5B and S5C). These data confirm *BCL2* expression is high in a substantial subset of SCLC cell lines and tumors.

As *BCL2* is amplified in SCLC (9), we explored whether DNA amplification alone was responsible for increased levels of *BCL2* expression. We found that *BCL2* amplification did indeed correlate with *BCL2* expression (Fig. 3A). However, we noticed that *BCL2* RNA levels varied greatly within each copy-number category. The *BCL2* gene has a CpG island immediately upstream of the transcriptional start site (TSS; Fig. 3B). Given that CpG islands are often involved in the regulation of gene expression, we therefore asked whether DNA methylation may play a role in the expression of *BCL2*. Using methylation data available from the GDSC, we found that both the 2 and 3 *BCL2* copy-number cell lines had differential methylation within this CpG island, with lower *BCL2*-expressing cells having higher methylation (Fig. 3C

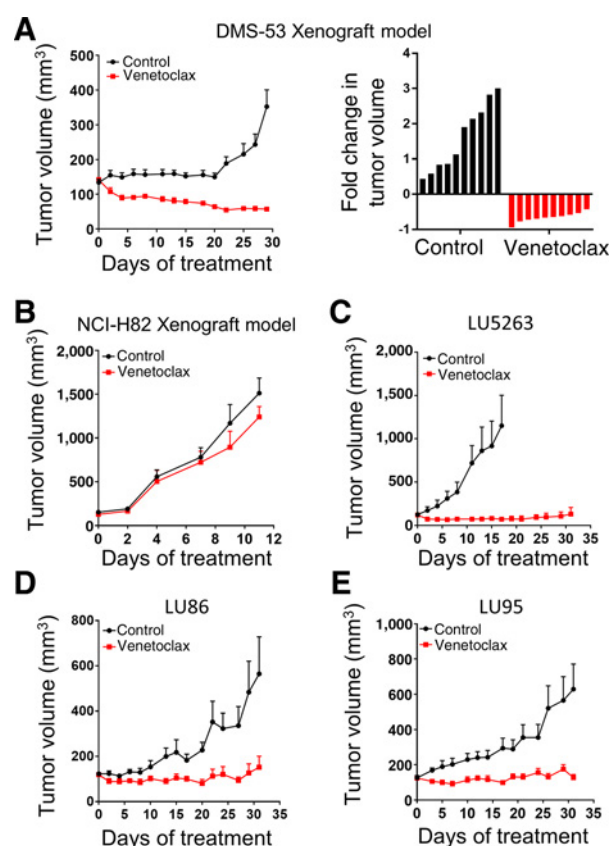


and D). These results suggest that epigenetic regulation of BCL-2 expression plays an important role in SCLC, independent of *BCL2* copy-number.

#### Venetoclax kills SCLC cell lines by disrupting BIM:BCL-2 complexes

We next investigated the mode of venetoclax killing in SCLC cell lines. FACS analysis of PI/Annexin V-Cy5-stained SCLC cells demonstrated that 1  $\mu$ mol/L of venetoclax induced marked apoptosis over the treatment period of 72 hours in high BCL-2-expressing NCI-H510A and DMS-53 cells (Fig. 4A), corresponding to a sharp reduction in viable cells over the same treatment period (Supplementary Fig. S6A and S6B). In contrast, venetoclax-resistant cell lines demonstrated no significant increase of apoptosis within the same 72-hour treatment

(Fig. 4B). Importantly, the concentration used in these assays is clinically relevant based on steady-state pharmacokinetics observed with venetoclax (25–27). Immunoprecipitation of BIM in NCI-H510A and DMS-53 whole-cell lysates treated with 1  $\mu$ mol/L venetoclax revealed on-target activity of venetoclax as judged by the disassociation of BIM:BCL-2 complexes (Fig. 4C, left and right, respectively). Consistent with past reports, venetoclax led to an increase in BIM:MCL-1 complexes (17, 28) and we also noted increases in BIM:BCL-x<sub>L</sub> complexes (Fig. 4C). BIM knockdown mitigated venetoclax efficacy in NCI-H510A and DMS-53 cells (Fig. 4D), further demonstrating venetoclax kills in a BIM-mediated manner (17, 29). Altogether, these data demonstrate on-target killing by venetoclax in SCLC through disruption of BIM:BCL-2 complexes, leading to BIM-dependent apoptosis.

**Figure 5.**

Venetoclax is effective in high BCL2-expressing xenograft and PDX mouse models. **A**, Growth curves of the DMS-53 xenograft model. Mice were either untreated (black lines) or administered 100 mg/kg of venetoclax (red lines) by oral gavage 6 days per week. Right, the total tumor change over the total course of treatment. **B**, Growth curves of the venetoclax-resistant cell line, NCI-H82. Mice were treated identical as above, and are plotted as untreated (black lines) or venetoclax treated (red lines). **C–E**, Growth curves of the PDX models LU5263 (**C**), LU86 (**D**), and LU95 (**E**), either untreated (black lines) or venetoclax treated (red lines). Dosing was identical to the xenograft model above. All error bars were plotted as SEM.

### Venetoclax is effective in high BCL-2-expressing SCLC mouse models

We next assessed the ability of venetoclax to impede tumor growth in the high BCL-2-expressing DMS-53 mouse model. Treatment with 100 mg/kg of venetoclax delivered once daily (30) led to marked tumor shrinkage (Fig. 5A). Reflecting the established safety profile of venetoclax in humans (31), these mice exhibited little to no weight loss, nor other overt signs of toxicity (Supplementary Fig. S7A). Consistent with our hypothesis, the low BCL-2 xenograft model, NCI-H82, was unresponsive to venetoclax treatment (Fig. 5B).

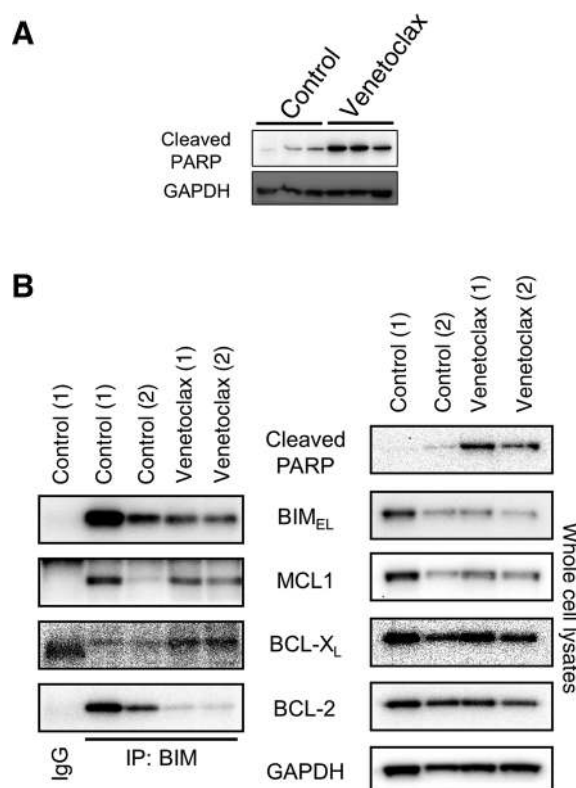
We expanded our analysis to three patient-derived xenografts (PDX) that had high BCL-2 expression as measured by Western blot analysis (Supplementary Fig. S8), including the chemorefractory LU86 model (16). Using the same treatment schedule, we found these tumors were also uniformly sensitive to venetoclax, with marked regressions in the LU5263 PDX model, and near total growth inhibition in the LU86 and LU95 models (Fig. 5C–E).

Analysis of LU5263 tumors treated for 3 days with venetoclax demonstrated marked apoptosis (Fig. 6A), consistent with the mode of activity of venetoclax (Fig. 4A; Supplementary Fig. S6D; ref. 30). Furthermore, the *in vitro* mechanism of BIM:BCL-2 complex disruption was observed in the LU5263 tumors *in vivo*, including a noticeable increase in BIM:BCL-xL complexes (Fig. 6B).

## Discussion

Preclinical studies have demonstrated that venetoclax is an effective BCL-2 inhibitor that lacks the platelet-killing capacity of dual BCL-2/BCL-xL inhibitors (30). This translates to more potent BCL-2 inhibition, and venetoclax has proven effective against BCL-2-dependent CLL without inducing marked thrombocytopenia (27, 31).

Despite promising preclinical activity, the BCL-2/BCL-xL inhibitor navitoclax did not translate to successful clinical activity in SCLC (10, 11). A leading hypothesis to explain this failure is that BCL-2 itself was not sufficiently inhibited due to the dose-limiting thrombocytopenia caused by BCL-xL inhibition. In this study, we have demonstrated that a significant population of SCLC cell lines, those bearing high BCL-2 levels, are susceptible to

**Figure 6.**

Venetoclax disrupts BIM:BCL-2 complexes *in vivo* in the LU5263 PDX model. **A**, Induction of the apoptosis marker cleaved PARP in three control and three, 3-day venetoclax-treated LU5263 tumors. GAPDH was used as a loading control. **B**, Tumor lysates from 3-day venetoclax treated LU5263 tumors were immunoprecipitated using an anti-BIM antibody. Western blot analysis was carried out on precipitated and input samples using the antibodies indicated.

venetoclax therapy. Interestingly, while high BCL-2 (7, 32–35), high NOXA (36), high BIM (7), and low MCL-1 (7, 9) are key factors governing BH3 mimetic sensitivity in SCLC, high *BCL2* mRNA alone predicted venetoclax sensitivity in SCLC (Fig. 1; Supplementary Figs. S1, S3, and S4A), and neither *BCL2L1*, *BCL2L11*, nor *MCL1* mRNA expression improved the ability of BCL-2 to predict response (Supplementary Fig. S4B). In addition, all of the sensitive cell lines we assayed for protein expression displayed varying levels of BCL-X<sub>L</sub>, MCL-1, and NOXA (Fig. 1B; Supplementary Fig. S8), while displaying high levels of BCL-2 and BIM. However, not all high *BCL2* mRNA expressers were sensitive to venetoclax (Fig. 1C; Supplementary Fig. S1B). Furthermore, another report using therapeutics that decrease MCL-1 expression found a more robust response with both navitoclax and venetoclax in a single SCLC xenograft and PDX model (34). Therefore, while our data indicate high BCL-2 expression may locate potential responders to venetoclax, more research is required to determine other potential cofactors in SCLC susceptibility to venetoclax.

Our *in vivo* data demonstrated that venetoclax effectively shrank or controlled tumors in high BCL-2–expressing xenografts and PDX models, while a low BCL-2–expressing xenograft was unresponsive (Fig. 5). Other studies using ABT-737 across a longer-scale treatment have found that tumors develop resistance (35, 37). Given the better tolerance for venetoclax due to a lack of thrombocytopenia development (27, 30, 31), it is also possible that this agent would be more efficacious in the treatment of SCLC. However, it remains unclear whether extended treatment times would lead to similar development of resistance using venetoclax.

Downregulation of BIM inhibited apoptosis following venetoclax treatment (Fig. 4D), despite the variable levels of BIM even in sensitive cell lines (Fig. 1B; Supplementary Figs. S3 and S4). In addition, the disruption of BIM:BCL-2 complexes is triggered by venetoclax (Fig. 4C), both suggesting BIM is necessary for venetoclax-induced apoptosis in SCLCs. Consistently, SW1271, in which the expression of BCL-2 and BIM are markedly low, was highly resistant to venetoclax (Fig. 1B).

It should be noted that our previously published large cell line screen of navitoclax revealed that cell lines from many different solid tumors were susceptible to this agent (7). This was not the case for venetoclax; however, where the only solid tumors with noticeable sensitivity were SCLCs and MYCN-amplified neuroblastomas (Fig. 1A; Supplementary Fig. S1A; Supplementary Table S1). We and others previously reported enhanced sensitivity of MYCN-amplified neuroblastoma to venetoclax (17, 38). Although MYCN is the predominant MYC family member amplified in neuroblastoma (39), in SCLC all three members of the MYC family are amplified independently of each other, with L-Myc (MYCL) being the most frequently amplified at 9% (3). Thus, future evaluation of how MYC family status could affect BCL-2 levels to predict venetoclax responses would be valuable.

We also report that while BCL-2 amplification plays a role in high BCL-2 expression in SCLC (9), methylation in the *BCL2* promoter helps distinguish low from high BCL-2 expressers. Methylation of *BCL2* has been linked to increased sensitivity to antimetabolic agents in patients with breast cancer (40), where no methylation was found in normal breast tissue. This is also consistent with studies demonstrating a reliance on another antiapoptotic BCL-2 family member, MCL-1, for antimetabolic

efficacy (41, 42). However, in the case of BCL-2 inhibitors, lack of *BCL2* methylation in SCLCs seem to have the opposite effect, sensitizing these cancers to venetoclax (Figs. 1C and 3), likely through the priming of BIM:BCL-2 complexes (Fig. 4).

Our data are consistent with a recent report identifying *in vitro* activity of venetoclax in SCLC cell lines, and a correlation between BCL-2 expression and venetoclax activity (32). In addition, Lam and colleagues very recently reported that venetoclax sensitizes SCLC cell lines to BET inhibition (33). These previous reports support the potential of venetoclax in SCLC. Here, we expand significantly on these findings by demonstrating that a subset of SCLC is uniquely sensitive to venetoclax among solid tumor cell types, with the exception of MYCN-amplified neuroblastoma (Fig. 1A; Supplementary Table S1), and that single-agent venetoclax demonstrates antitumor efficacy in high BCL-2–expressing patient-derived xenografts (Fig. 5C–E), and a cell-line derived xenograft (Fig. 5A). These results provide a rationale for assessing the clinical efficacy of venetoclax in a biomarker selected SCLC population. Importantly, we also demonstrate that the expression of other BCL-2 family genes does not improve predictive power over BCL-2 expression alone (Supplementary Fig. S3B). Finally, we identify that methylation of the *BCL2* promoter is increased in cell lines that express low levels of *BCL2* mRNA despite evidence of gene amplification (Fig. 3), identifying a potential epigenetic component in the regulation of *BCL2* in SCLC.

In conclusion, screening venetoclax against approximately 800 solid tumor cancer cell lines uncovered activity across a large subset of SCLC lines. These sensitive cancers have high BCL-2 expression at the protein level and a high level of *BCL2* mRNA expression. Together, this work suggests rationale for biomarker-directed clinical trials of the FDA-approved drug venetoclax in high BCL-2–expressing SCLCs.

## Disclosure of Potential Conflicts of Interest

S.J. Dylla and J.D. Levenson have ownership interests (including patents) in AbbVie Stemcentrx, LLC. No potential conflicts of interest were disclosed by the other authors.

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