Navitoclax Enhances the Efficacy of Taxanes in Non–Small Cell Lung Cancer Models

Nguyen Tan¹, Mehnaz Malek¹, Jiping Zha¹, Peng Yue², Robert Kassees¹, Leanne Berry¹, Wayne J. Fairbrother³, Deepak Sampath¹, and Lisa D. Belmont¹

Abstract

Purpose: To explore the potential of navitoclax in combination with taxane-based chemotherapy in the treatment of non–small cell lung cancer (NSCLC) by defining mechanism of synergy and identifying correlative biomarkers.

Experimental Design: We treated a panel of NSCLC lines with a dose matrix of paclitaxel and navitoclax (formerly ABT-263), an inhibitor of Bcl-2, Bcl-xL, and Bcl-w (1), and evaluated synergy. We next used time-lapse microscopy to explore mechanism of synergy. Finally, we developed an immunohistochemical assay and assessed prevalence of Bcl-xL in NSCLC tumor tissues.

Results: All cell lines exhibit greater than additive response to the combination of navitoclax and a taxane. These results were extended to mouse xenograft tumor models, in which the combination is more efficacious than either single-agent docetaxel or navitoclax. Addition of navitoclax to paclitaxel decreases the time from mitotic entry to cell death and changes cell fate from mitotic slippage to death during mitotic arrest. The relative levels of Bcl-xL and Mcl-1 correlate with the extent of synergy, suggesting that cancers with elevated levels of Bcl-xL will be relatively resistant to taxane-based therapy but could benefit from the addition of navitoclax to taxane treatment. Finally, a significant percentage of NSCLC patient samples exhibit relatively high Bcl-xL levels.

Conclusions: The addition of navitoclax to taxane-based chemotherapy in NSCLC has the potential to increase efficacy, particularly in patients whose tumors express high levels of Bcl-xL.

Introduction

Taxane-based cancer therapy regimens, which are broadly used in the treatment of non–small cell lung cancer (NSCLC), yield overall response rates of 20% to 30% (2). Taxanes, which include paclitaxel and docetaxel (DTX), bind and stabilize microtubules, causing cells to arrest in mitosis, and result in cytostatic or cytotoxic responses. Most studies of mechanisms of resistance to taxanes have focused on modulation of microtubule dynamics or the activity of efflux pumps such as P-gp (3). The first described mechanism of resistance to taxanes was increased expression of efflux pumps. Rare mutations in βIII tubulin that confer decreased sensitivity to taxanes have been isolated from patients undergoing taxane-based treatment (4). Expression of the βIII isotype of tubulin in cancer cells also confers resistance to microtubule-targeting agents in culture and is a marker of poor prognosis in NSCLC patients (5, 6). These mechanisms of resistance negatively impact the primary response of mitotic arrest; however, they are unlikely to account for the majority of NSCLC patients who do not respond to taxane-based therapy. Most cancer cells arrest in mitosis in response to taxanes, suggesting that the subsequent response to mitotic arrest is key to improving efficacy. Indeed, the cellular response to mitotic arrest is highly variable. Time-lapse microscopy of a panel of cancer cell lines showed cell fate after mitotic arrest, ranging from death during mitotic arrest to mitotic slippage and subsequent proliferation (7). A study of 16 murine models of cancer in which animals were treated with paclitaxel found that all models exhibited mitotic arrest in tumors. However, objective response varied considerably and was correlated with the extent of apoptosis rather than mitotic arrest (8). Finally, a study evaluating serial fine needle aspirates from breast cancer patients undergoing paclitaxel treatment similarly showed that mitotic arrest was routinely observed but that the apoptotic index was a better predictor of efficacy (9). These observations suggest that increasing the apoptotic response to mitotic arrest will increase efficacy of taxanes.

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

Note: N. Tan and M. Malek contributed equally to this study.

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Translational Significance

Navitoclax is currently the subject of clinical trials, including 2 combination studies with taxane-based regimens (www.clinicaltrials.gov). We show that addition of navitoclax to taxane treatment decreases the time from mitotic arrest to cell death and therefore may increase the window of time when taxanes are efficacious. We also show that non–small cell lung cancer (NSCLC) models with relatively high levels of Bcl-xL are more likely to show a synergistic response to the combination of a taxane and navitoclax and that 30% of NSCLC tumors express high levels of Bcl-xL by immunohistochemistry. Taken together, these data suggest that the combination of navitoclax and paclitaxel (or docetaxel) may be effective in a significant percentage of NSCLC patients who would otherwise have a poor response to taxane-based chemotherapy.

One such strategy is inhibition of antiapoptotic proteins such as Bcl-2 and Bcl-xL (10). The family of Bcl-2-related antiapoptotic proteins includes Bcl-2, Bcl-xL, Bcl-w, Bcl-A1, and Mcl-1. These proteins block cell death by sequestering the proapoptotic proteins Bax and Bak, preventing them from oligomerizing and forming pores in the mitochondrial outer membrane (11). Elevated expression of Bcl-2, the first antiapoptotic protein to be discovered in epithelial cancer cells (14). Thus, inhibition of Bcl-xL might be expected to potentiate the effect of cytotoxic therapy by enhancing the apoptotic response to cellular stress. ABT-737, a small molecule inhibitor of Bcl-2, Bcl-xL, and Bcl-w, was first reported in 2005 (15), followed by navitoclax, an orally bioavailable inhibitor with a similar binding profile, which is being evaluated in clinical trials (1). These molecules inhibit Bcl-2 proteins by mimicking the BH3-domain interactions of the proapoptotic “BH3-only” protein Bad with Bcl-2 family proteins, thereby preventing them from sequestering proapoptotic executioner proteins Bax and Bak and BH3-only proteins (16). Furthermore, navitoclax enhances the efficacy of cytotoxic drugs in hematologic tumors in vivo (17). Bcl-2 is expressed in non–Hodgkins lymphoma (NHL; ref. 12), chronic lymphocytic leukemia (18, 19), and small cell lung cancer (SCLC; ref. 20), whereas Bcl-xL seems to be the major antiapoptotic protein in epithelial tumors (14). A small molecule inhibitor of Bcl-xL potentiates the activity of paclitaxel in vivo and in vivo in NSCLC models (21), similar to what has been observed for the dual inhibitor of Bcl-2 and Bcl-xL (15). In this study, the potential for synergy between navitoclax and a taxane was evaluated across a larger panel of NSCLC models to discover molecular markers that could identify patients more likely to benefit from the combination.

Material and Methods

Chemicals

Navitoclax (formerly ABT-263; Abbott Laboratories), paclitaxel, and DTX (Sigma-Aldrich) were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) at 10 mmol/L, and aliquots were stored at –80°C.

Cell culture, antibodies, and reagents

Cell lines were obtained from the American Type Culture Collection or Deutsche Sammlung von Mikroorganismen und Zellkulturen, expanded, and stored at early passage in a central cell bank. Cell lines were grown in RPMI 1640 medium supplemented with 10% FBS (Invitrogen) and passed no more than 20 times after thawing. A549 green fluorescent protein histone 2B (GFP-H2B) cells were constructed by integrating a GFP-H2B construct (22) into A549 cells by lentiviral delivery (Lentigen, Inc.). Primary antibodies directed against the following proteins were used: Bcl-xL (BD BioSciences), Mcl-1 (BD BioSciences), Bcl-2 (Trevigen), Bak (Sigma-Aldrich), Bax (Cell Signaling Technology), Puma (Cell Signaling Technology), CDC-27 (BD BioSciences), β-actin (Sigma-Aldrich), PARP (Cell Signaling Technology), and horseradish peroxidase (HRP)-conjugated horse anti-mouse and goat anti-rabbit antibodies (Vector Laboratories).

Cell viability assay and Western blotting

Cells were seeded in 384-well plates at 2,000 cells/well. After 24 hours, cells were treated with navitoclax (dose range = 0.12–3.3 μmol/L) and paclitaxel or DTX (dose range = 3–100 nmol/L) in a 5 × 5 matrix of concentrations chosen to encompass clinically relevant doses (23, 24). Each treatment was done in quadruplicate. Cells were treated for 72 hours, and cell viability was determined using CellTiter-Glo or Caspase-Glo 3/7 (Promega). A Bliss independence model (25) was used to evaluate combination effects. To examine protein expression, the cells were lysed in ice-cold radioimmunoprecipitation assay (RIPA) buffer (Cell Signaling Technology) containing 1 mmol/L phenylalanine, Pefabloc, Phosphatase Inhibitor Cocktail 1 and 2 (Sigma-Aldrich), and complete EDTA-free protease inhibitor tablet (Roche). Equal amounts of protein were subjected to SDS-PAGE (4%–20% Tris-Glycine; Invitrogen), and protein levels were evaluated by Western blotting.

In vivo efficacy studies

SW1573, A549, and NCI-H1650 NSCLC cells were implanted subcutaneously in the hind flank of female athymic nude mice. After implantation, tumors were monitored until they reached a mean tumor volume of 250 to 350 mm³ and distributed into groups of 10 to 15 animals each before dosing. Navitoclax was dissolved in 60% Phosal 50 PG, 30% PEG (polyethylene glycol) 400, 10% EtOH vehicle and dosed daily or intermittently by oral administration. DTX was diluted in Tween 80 and dosed...
Table 1. Summary of Bliss scores and activity of navitoclax, paclitaxel, or combination treatment in NSCLC cell lines (n = 51)

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<th>SD of % inhibition of navitoclax</th>
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Table 1. Summary of Bliss scores and activity of navitoclax, paclitaxel, or combination treatment in NSCLC cell lines (n = 51) (Cont’d)

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NOTE: Bliss expectation was calculated with the equation \((A + B) - A \times B\), where \(A\) and \(B\) are the fractional growth inhibitions of drug \(A\) and \(B\) at a given dose. The difference between the Bliss expectation and the observed growth inhibition of the combination of drugs \(A\) and \(B\) at the same dose is the "Bliss excess." Bliss excess values were summed across the dose matrix to generate the Bliss sum.

weekly. Tumor volume was measured in 2 dimensions (length and width), using Ultra Cal IV calipers (Model 54 10 111; Fred V. Fowler Company), as follows: tumor volume \((\text{mm}^3) = (\text{length} \times \text{width})^2 \times 0.5\), and analyzed using Excel version 11.2 (Microsoft Corporation). Animal body weights were measured using an Adventurer Pro AV812 scale (Ohaus Corporation). Percent weight change was calculated as follows: body weight change \(\% = \left[\frac{\text{weight}_{\text{day 0}} - \text{weight}_{\text{day 0}}}{\text{weight}_{\text{day 0}}}\right] \times 100\). Tumor sizes and mouse body weights were recorded twice weekly over the course of treatment, and the mice were observed daily. A linear mixed modeling approach was used to analyze the repeated measurement of tumor volumes from the same animals over time (26). This approach addresses both repeated measurements and modest dropouts due to any non–treatment-related death of animals before study end. Cubic regression splines were fit to a nonlinear profile to the time courses of log2 tumor volume at each dose level. These nonlinear profiles were then related to dose within the mixed model. Tumor growth inhibition as a percentage of vehicle control \((\% \text{TGI})\) was calculated as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to the vehicle, using the following formula: \(\% \text{TGI} = 100 \times \left(1 - \frac{\text{AUC}_{\text{drug}}}{\text{AUC}_{\text{veh}}}\right)\). Linear mixed-effects analysis was also used to analyze the repeated measurement of body weight changes from the same animals over time. Data were analyzed using R, version 2.8.1 (R Development Core Team 2008; R Foundation for Statistical Computing), and the mixed models were fitted within R using the nlme package, version 3.1-89. Plotting was carried out in Prism 5 software (GraphPad Software, Inc.).

Time-lapse imaging

GFP-H2B–expressing A549 cells were synchronized with a double thymidine block on glass-bottom 24-well plates (Greiner Bio-one). After the second thymidine block, drug-containing media was added, multiple fields per condition were selected, and fluorescent and phase-contrast images were recorded with a 20× objective every 15 minutes for 72 hours on an AxioObserver inverted microscope (Carl Zeiss), equipped with an environmental chamber (OkoLab) and MS2000 XY stage (Applied Scientific Instruments) and a CoolSnap CCD camera (HP). All devices were controlled using Slidebook (Intelligent Imaging Design). Mitotic events and cell death were scored as described previously (27).

Synchronized cell-cycle study

A549 cells were synchronized with a double thymidine block and released into drug-containing media. At indicated time points, adherent cells were washed twice with 10 mL of cold PBS and lysed in 750 μL of RIPA lysis buffer (Teknova) containing phosphatase (Pierce) and protease (Roche) inhibitors. Mitotic cells were collected by mitotic shake, washed twice with cold PBS, and lysed in cold RIPA buffer. All lysates were centrifuged for 10 minutes at 12,000 rpm at 4°C and frozen in liquid nitrogen.

Clonogenic viability assay

SW1573 cells were treated with drug for 24 hours, then trypsinized, diluted, and replated with biological and technical replicates. Plates were incubated for 10 days, stained with crystal violet (0.2%), and visible colonies (>50 cells) were counted.

Immunochemistry

Formalin-fixed and paraffin-embedded specimens were sectioned at 4 μm onto slides. After deparaffinization and rehydration, sections were processed for Bcl-xl, immuno-histochemical (IHC) analysis. Antigen retrieval was done using CC1 standard buffer (Ventana Medical Systems).
Sections were incubated with 1:1,000 dilution of rabbit anti-Bcl-xL (clone 54H6) monoclonal antibody (Cell Signaling Technology) for 60 minutes at 37°C, followed by incubation with HRP-conjugated secondary anti-rabbit antibody and detection by OmniMap DAB system (Ventana Medical Systems). A minimum of 50% of the cancer cells on a specimen were required to have the staining intensity of the assigned score.

Results

Effects of navitoclax and paclitaxel in cultured cells

Fifty-one human NSCLC cell lines were treated with navitoclax and paclitaxel in a 5 x 5 dose matrix, and the combination effect was evaluated using the Bliss independence model. This model was chosen over combination index analyses because it allows for evaluation of drug interactions.
interaction when single-agent IC\textsubscript{50} values cannot be determined, as is the case with navitoclax. The data are expressed as percentage difference in cell growth compared with the expectation if the agents act independently. Bliss = 0 indicates that the combination treatment is additive (as expected for independent pathway effects); Bliss > 0 indicates activity greater than additive (synergy); and Bliss < 0 indicates the combination is less than additive (antagonism). The Bliss scores for all dose combinations were added to generate a Bliss sum. The combination of navitoclax and paclitaxel was synergistic in all cell lines tested, with combined growth inhibition greater than 90% in 36 cell lines (Table 1). Three-dimensional (3D) graphs of percent inhibition and Bliss sums across the dose matrix of representative cell lines highlight the differences between lines with relatively high or low Bliss sums. In SW1573 cells with relatively high Bliss sum, the inhibition curves are shifted upward with the addition of increasing doses of navitoclax, resulting in positive Bliss scores for most dose combinations (Fig. 1A). In contrast, the inhibition curves for H1703 cells are relatively unchanged by the addition of navitoclax, resulting in Bliss scores close to zero (Fig. 1B). Interestingly, cell lines with relatively low Bliss sums were more sensitive to single-agent paclitaxel (Spearman \( r = -0.66; P < 0.0001 \)), indicating that the addition of navitoclax was only slightly advantageous over the strong single-agent activity of paclitaxel (Fig. 1C).

Bliss sums were calculated using an assay that measures ATP. Because this assay does not unambiguously distinguish cytostatic from cytotoxic effects, we employed a caspase-3/7 activation assay on a subset of cell lines that were chosen to cover the range of Bliss sums obtained from the cell viability assay. A similar pattern in Bliss sum was observed using a caspase-3/7 assay as compared with a cell viability assay (\( r^2 = 0.64; P < 0.006 \); Fig. 1D). Because DTX is increasingly used in the treatment of NSCLC patients, we evaluated a set of 15 human NSCLC cell lines with navitoclax and DTX, using the same dose matrix as that used for the paclitaxel studies. A significant correlation of Bliss sum between the 2 combinations was observed (\( r^2 = 0.78; P < 0.0001 \); Supplementary Fig. 1).

**The ratio of Bcl-x\textsubscript{L} to Mcl-1 correlates with Bliss sum for navitoclax and paclitaxel**

Because there is variability in the synergy values across different NSCLC models, we wanted to identify molecular markers that predict high synergy. There was no correlation between Bliss sums and common genetic lesions in epidermal growth factor receptor, KRAS, TP53, or phosphatidylinositol 3\textsuperscript{\textprime}kinase (Supplementary Table 1). It has been reported that high levels of Bcl-2 and low levels of Mcl-1 correlate with single-agent response to ABT-737 or navitoclax in NHL and SCLC cell lines (28–30). Because we see little single-agent activity of navitoclax in NSCLC cell lines, we wanted to identify predictive markers of synergy between navitoclax and paclitaxel. We conducted Western blots of 25 cell lines (Fig. 2A) and found that Bcl-x\textsubscript{L} protein levels are positively correlated with Bliss sum (Spearman \( r = 0.53; P < 0.007 \); Fig. 2B) whereas Mcl-1 level is inversely correlated with Bliss sum (Spearman \( r = -0.50; P < 0.01 \); Fig. 2C). The findings were extended by considering the ratio of Bcl-x\textsubscript{L} and Mcl-1 expression and comparing it with the Bliss sum; indeed, the Bcl-x\textsubscript{L}/Mcl-1 ratio is better correlated with Bliss sum than Bcl-x\textsubscript{L} expression alone (Spearman \( r = 0.67; P < 0.0002 \); Fig. 2D). To test this model, we evaluated 19 additional NSCLC lines for Bcl-x\textsubscript{L} and Mcl-1 protein levels (Supplementary Fig. 2). Similar to the initial study, Bcl-x\textsubscript{L} levels correlated with Bliss sum.

![Figure 2](https://www.aacrjournals.org/clinres/17/6/2011/mmc1.png)
Mcl-1 levels did not correlate with Bliss sum in this test set. However, the ratio of Bcl-x\(_L\) and Mcl-1 levels exhibited a better correlation with Bliss sum than Bcl-x\(_L\) expression alone (Spearman \(\rho = 0.57; P < 0.01\); Supplementary Fig. 2D). Bcl-2, Bim, Bak, Bax, and Puma expression did not correlate with Bliss sum (Supplementary Fig. 3). Together, these data suggest that high levels of Bcl-x\(_L\) predict combination efficacy of navitoclax and paclitaxel in NSCLC and that evaluating a ratio of Bcl-x\(_L\) to Mcl-1 may provide better predictive power.

**Combination of navitoclax and DTX leads to enhanced antitumor efficacy in NSCLC xenograft models**

To extend these studies to an *in vivo* setting, we selected SW1573, a model with relatively high Bliss sum and high Bcl-x\(_L\) to Mcl-1 ratio. Mice bearing SW1573 xenograft tumors were dosed weekly (QW \(\times\) 3) with DTX at 7.5 mg/kg in combination with navitoclax, dosed daily (QD \(\times\) 21) or on an intermittent schedule (days 1–3 of a weekly cycle times for 3 cycles) at 100 mg/kg. The intermittent schedule was designed to mimic the combination schedule in phase 1B clinical trials (www.clinicaltrials.gov). Because DTX was very efficacious in this model, we monitored tumor regrowth to evaluate combination effects. The addition of navitoclax to DTX resulted in sustained TGI and tumor regressions that were evident up to 29 days post-dosing of navitoclax (Fig. 3A and B). More important, equivalent antitumor responses were observed when navitoclax was administered on the intermittent schedule versus daily dosing (Fig. 3A and B). The latter suggests that maintaining navitoclax drug exposure when DTX is at peak bioavailable levels is sufficient for a combinatorial effect. Intermittent and daily dosing schedules of navitoclax either alone or in combination with DTX were well tolerated (Fig. 3C and D). To expand on these studies, we evaluated 2 additional NSCLC models for combination efficacy, A549 and NCI-H1650 cells. We observed a statistically significant decrease in tumor volume during dosing in the combination versus DTX single agent in both models with continuous or intermittent navitoclax dosing (Supplementary Fig. 4). Thus, the combination of navitoclax and DTX...
resulted in tumor regressions and enhanced antitumor responses in 3 NSCLC xenograft tumor models in which a synergistic effect was observed in vitro.

**Addition of navitoclax to paclitaxel changes cell fate from mitotic slippage to death during mitotic arrest**

To observe and quantify the effects of navitoclax and paclitaxel on cell-cycle timing and fate, we carried out live cell time-lapse microscopy on GFP-H2B–expressing cells. Mitotic entry (nuclear condensation), metaphase alignment, and anaphase segregation of chromosomes were scored by fluorescence imaging of GFP-histone, and mitotic exit, cytokinesis, and membrane blebbing in cell death were scored by phase contrast (Fig. 4A and B). Control cells completed mitosis within an average of 48 minutes, and this timing was not significantly impacted by navitoclax. Paclitaxel caused prolonged mitotic arrest (~12 hours on average), and the addition of navitoclax to paclitaxel reduced the duration of mitotic arrest by nearly 4-fold (Fig. 4C). The reduction in the duration of mitotic arrest reflects a change in cell fate from mitotic slippage to death during mitotic arrest (Fig. 4D). Thus, when combined with paclitaxel, navitoclax accelerates cell death and causes cells to die prior to mitotic exit, eliminating the opportunity for cellular escape.

**Addition of navitoclax to paclitaxel causes an increase in cell death**

For biochemical evaluation of mechanism, we treated synchronized A549 cells with navitoclax, paclitaxel, or a combination of both. Sustained phosphorylation of CDC27 in cells exposed to paclitaxel confirmed mitotic arrest. Mcl-1 is slowly degraded in cells treated with paclitaxel, whereas Bcl-xL levels remain unaffected regardless of the treatment. Paclitaxel-treated cells exhibit increasing levels of PARP cleavage, starting with the mitotic time point. Moreover, cells treated with a combination of both agents show greater levels of cleaved PARP, confirming that the kinetics and extent of cell death in response to paclitaxel increase with the addition of navitoclax (Fig. 5A).
confirm that the combination effects of navitoclax and paclitaxel are durable, we measured clonogenic survival (Fig. 5B and Supplementary Fig. 5). Cells were treated with navitoclax combined with various concentrations of paclitaxel. Navitoclax alone has a very modest effect on cell survival but enhances the cytotoxicity of paclitaxel at all doses. The effects are particularly dramatic at the low paclitaxel concentration of 2 nmol/L, where the percent clonogenic survival is reduced from 73% to 31% ($P = 0.008$) with the addition of navitoclax (Fig. 5B).

**Bcl-xL is highly expressed in a significant percentage of NSCLC tumors**

We evaluated the prognostic significance of Bcl-xL expression on a large NSCLC data set, using patient outcome and correlative expression microarrays (31). There was a trend toward poor prognosis with high Bcl-xL expression (Supplementary Fig. 6), but it did not reach statistical significance ($P = 0.058$). It may be necessary to evaluate protein level rather than mRNA to get an accurate assessment of Bcl-xL. We therefore assessed the dynamic range of Bcl-xL protein in NSCLC patient tissues. We validated IHC methods using cells with known Bcl-xL expression, developed an IHC scoring system, and evaluated tissues from 66 NSCLC patients (Fig. 5C). On pathologist review, 30% of samples were found to have IHC scores of 2 or 3, which were considered to be "Bcl-xL high." Among the NSCLC subtypes, the "Bcl-xL high" samples are preferentially found in non-squamous histology, constituting 40% of those cases (Supplementary Table 2). These data suggest that a significant percentage of nonsquamous NSCLC patients may benefit from the addition of navitoclax to a taxane-based therapy.

**Discussion**

This study evaluates the mechanism of synergy between navitoclax and paclitaxel in NSCLC models. Previous studies in breast cancer cell lines have shown that increased levels of Bcl-2 or decreased levels of Bim can lead to taxane resistance that is reversible with ABT-737 (10). Similarly, ABT-737 enhances the activity of paclitaxel in prostate cancer models that have been engineered to express high...
levels of Bcl-2 (32). In this study, we explored the innate sensitivity of NSCLC models to the combination of paclitaxel and navitoclax, with the goal of elaborating mechanism of synergy and identifying predictive biomarkers. Fifty-one NSCLC cell lines were tested for combination effects of navitoclax and paclitaxel. Although navitoclax had little single-agent activity, it enhanced the cytotoxic effects of paclitaxel in all cell lines. The primary synergy effect observed is an increase in maximal inhibition rather than a shift in the IC_{50}. Thus, we propose that navitoclax increases the cell killing after paclitaxel has caused its primary effect of inducing mitotic arrest. To test this, we used live cell time-lapse imaging of A549 cells expressing GFP-H2B. Navitoclax did not affect cells as a single agent, whereas paclitaxel caused A549 cells to arrest in mitosis for prolonged periods (average of 12 hours), usually followed by mitotic slippage and multinucleation. However, the addition of navitoclax to paclitaxel decreased the duration of mitotic arrest by 4-fold and changed the cell fate from mitotic slippage to death during mitotic arrest. Thus, cells die more quickly when navitoclax is added to paclitaxel, thereby reducing significantly the opportunity for escape via mitotic exit. This mechanism of action could have significant impact in increasing the efficacy for taxane-based therapies by reducing the time that the taxane must be at or above an efficacious dose to kill cancer cells.

Some steps associated with the mitotic induction of apoptosis have been elucidated. Although the initiating events are unclear, the proapoptotic Bim is activated during mitosis (33–35) but cells do not routinely die in mitosis because this proapoptotic event is antagonized by Mcl-1, Bcl-2, and Bcl-x_L. However, Bcl-2 and Bcl-x_L are inhibited by phosphorylation during mitotic arrest (36–38). Inhibitory phosphorylations of Bcl-x_L and Bcl-2 increase during mitotic arrest over the course of several hours (39), suggesting that there may be a threshold level of phosphorylation necessary to fully inhibit their activity. Furthermore, Mcl-1 is slowly degraded over the course of several hours during mitotic arrest (40). The slow inhibition of these protective proteins could act as a “clock” that allows a cell to distinguish a short mitotic arrest, which can give a cell time to correct misaligned chromosomes, from a long mitotic arrest (as in the case of microtubule-disrupting drugs) from which it is unlikely to recover. Thus, the longer a cell persists in mitotic arrest, the more likely it is to undergo apoptosis. The length of time a given cell can persist in mitotic arrest is highly variable (7), and Gascoigne and Taylor propose a dependence on cyclin B degradation rate for the duration of mitotic arrest. We further propose that the probability of undergoing apoptosis before mitotic exit is determined by the relative dependence on Bcl-2 family proteins for survival (Fig. 5D). A549 cells have relatively high levels of Bcl-x_L and, in our experiments, acute inhibition of Bcl-x_L dramatically increases the rate at which cells die after entry into the mitotic arrest state. It has been previously shown that agents synergistic with ABT-737 inhibit the activity of Mcl-1, for example, by increasing levels of Bim (41–43); however, our observations of synergy with antimitotic agents are unique in showing that the kinetics of Mcl-1 destabilization have a role in determining cell fate.

Because the extent of synergy observed is variable, there is a need to identify markers that predict synergy. In the context of the model described earlier, we hypothesize that greater dependence on Mcl-1 will render cancer cells sensitive to single-agent paclitaxel whereas greater dependence on Bcl-x_L requires the combination of paclitaxel and a Bcl-x_L inhibitor. Indeed, a correlation was identified between the Bliss sum and the ratio of Bcl-x_L to Mcl-1 protein levels in the NSCLC panel. Furthermore, we found by immunohistochemical analysis that 40% of nonsquamous NSCLC patient tissues had relatively high levels of Bcl-x_L (2 or 3+). Thus, we propose that a significant percentage of NSCLC patients may benefit from the combination of navitoclax and a taxane.

**Disclosure of Potential Conflict of Interest**

All authors are employees or former employees of Genentech, Inc., which is co-developing Navitoclax in collaboration with Abbott Laboratories.

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Navitoclax Enhances the Efficacy of Taxanes in Non–Small Cell Lung Cancer Models

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