

PIM1 Kinase Inhibition Induces Apoptosis and Synergizes with Venetoclax in Waldenström Macroglobulinemia via Suppression of NF- κ B Signaling

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Background: Despite advances in targeted therapy, Waldenström Macroglobulinemia (WM) cells frequently exhibit intrinsic or acquired resistance, underscoring the need for novel therapeutic strategies. Our previous scRNA-seq data from 79 unique WM tumor samples (Dang et al. Blood. 2025;146[Suppl 1]:460) revealed upregulated expression levels of PIM1 & BCL2 in both neoplastic B cells and plasma cells. PIM1 is a serine/threonine kinase known to be involved in the regulation of cell survival, proliferation, and apoptosis of several hematologic malignancies, but its role in WM is not well defined. Nuvisertib (TP-3654), a selective PIM1 inhibitor, has demonstrated encouraging clinical activity and a favorable safety profile in myeloproliferative neoplasms such as myelofibrosis. We hypothesized that nuvisertib could overcome the apoptosis-resistant phenotype of WM cells by regulating the NF- κ B axis and enhancing sensitivity to venetoclax, a BCL-2 inhibitor.

Methods: WM cell lines were first treated with single-agent nuvisertib to determine median inhibitory (IC₅₀) values using a WST-1 assay. Combination studies with venetoclax were then performed across a matrix of doses, and synergy was evaluated using the Bliss independence model by Synergy Finder. A clonogenic assay (colony formation) was performed to understand the therapeutic impact of nuvisertib on WM cell lines, and apoptosis induction was evaluated by western blot for BCL-2, BAD, cleaved caspase-3, c-MYC and MCL-1. Finally, NF- κ B pathway activity was assessed by detection of phospho-p65/RelA presence through western blot to determine downstream signaling effects of nuvisertib treatment.

Results: Nuvisertib demonstrated potent single-agent cytotoxicity, with IC₅₀ values of 0.44 μ M (BCWM.1), 0.16 μ M (RPCI-WM1), and 1.8 μ M (MWCL-1) (Figure 1A). Combination treatment with venetoclax (single agent IC₅₀ values of 0.18 μ M (BCWM.1), 1.94 μ M (RPCI-WM1), and 0.94 μ M (MWCL-1); Figure 1B) also demonstrated significant synergy on Bliss scores [Bliss score +10.93 (BCWM.1), Bliss score -2.30 (RPCI-WM1)] across tested dose ranges (Table. 1, Figure 1D). The early apoptotic changes were evaluated through Annexin v/PI (Figure 1C). The growth-inhibitory efficacy of nuvisertib was validated using a clonogenic assay (Figure

2A). Nuvisertib treatment downregulated anti-apoptotic BCL-2 and phospho-BAD, while increasing cleavage of caspase-3 and PARP, confirming robust activation of the intrinsic apoptotic pathway. Furthermore, nuvisertib downregulated c-MYC and MCL-1, key drivers of the anti-apoptotic network that maintains WM cell survival. Additionally, nuvisertib inhibited NF- κ B signaling, as demonstrated by reduced p65 phosphorylation, suggesting that suppression of this pro-survival pathway contributes to nuvisertib-induced apoptosis.

Conclusion: Collectively, these findings establish nuvisertib as a potent and mechanistically distinct PIM1 kinase inhibitor, capable of overcoming the apoptosis-resistant phenotype characteristic of WM. Nuvisertib monotherapy induced robust intrinsic apoptotic signaling, evidenced by BCL-2/BAD rebalancing and caspase-3/PARP cleavage, and concurrently attenuated NF- κ B-mediated pro-survival signaling, a pathway central to WM pathogenesis and therapeutic resistance. The pronounced synergy observed with venetoclax further indicates that dual PIM1 and BCL-2 inhibition has potential as a therapeutic strategy against resistant WM cells. Taken together, this preclinical data provides mechanistic rationale for PIM1 inhibition as a novel therapeutic vulnerability in WM and supports the clinical development of nuvisertib, alone or in combination with venetoclax.

Table 1. Single agent and combination pharmacology

Cell line	Nuvisertib IC50 (μ M)	Venetoclax IC50 (μ M)	Bliss Synergy Score
BCWM.1	0.44	0.18	+ 10.93
RPCI-WM1	0.16	1.94	- 2.30
MWCL-1	1.80	0.94	ns

Figure 1.

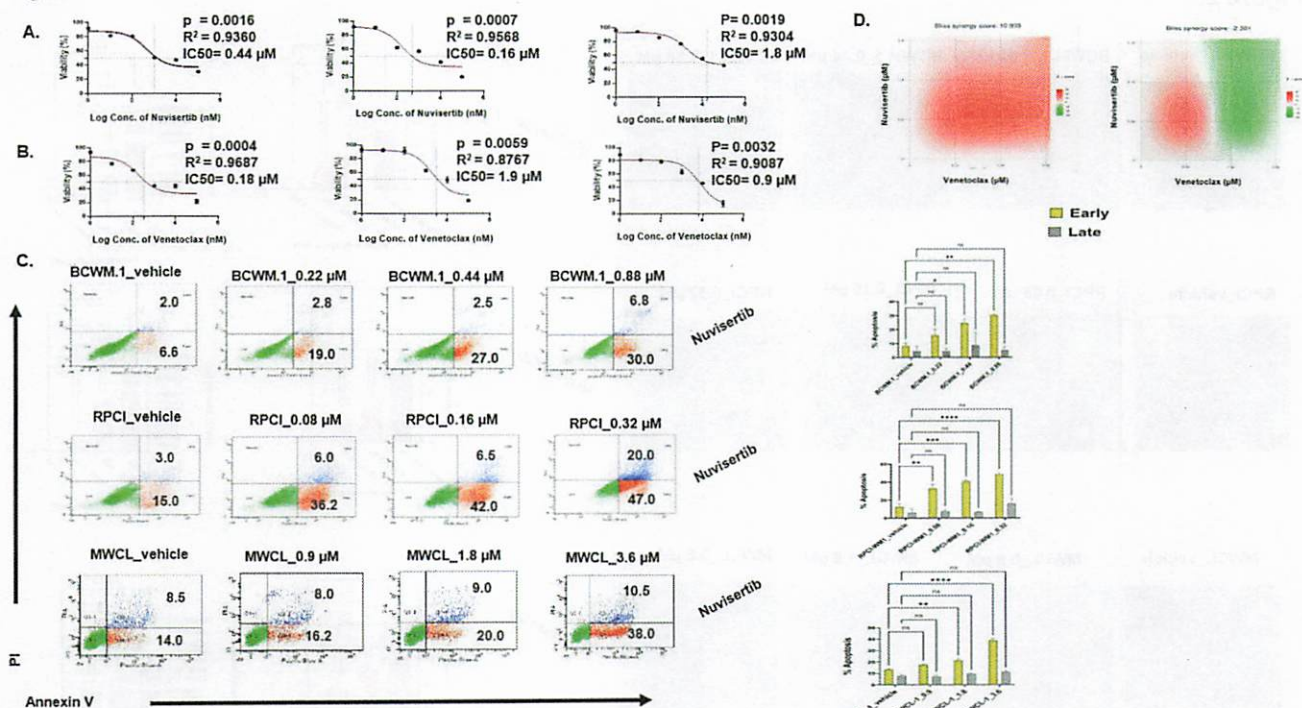


Figure 1: Nuvisertib exhibits dose-dependent cytotoxicity and induces apoptosis in WM cell lines, with synergistic activity in combination with venetoclax. A. Dose-response curves showing viability (%) of BCWM.1 (left), RPCI-WM1 (middle), and MWCL-1 (right) cells following 24 & 48-hour treatment with increasing concentrations of Nuvisertib, assessed by WST-1 assay. **B.** Dose-response curves showing viability (%) of BCWM.1, RPCI-WM1, and MWCL-1 cells following treatment with increasing concentrations of venetoclax, assessed by WST-1 assay. **C.** Annexin V/PI flow cytometry analysis of apoptosis in BCWM.1, RPCI-WM1, and MWCL-1 cells treated with vehicle or increasing concentrations of Nuvisertib. Representative dot plots show early apoptotic (lower right, Annexin V⁺/PI⁻), and late apoptotic/necrotic (upper right, Annexin V⁺/PI⁺) populations, with percentages indicated in each quadrant. Corresponding bar graphs (right) quantify the percentage of cells in early (olive) versus late (gray) apoptosis across treatment conditions. Data are shown as mean $\pm$ SEM; statistical comparisons to vehicle were performed by [Two-way ANOVA], with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, not significant. **D.** Bliss synergy landscape plots for the combination of Nuvisertib and venetoclax, showing synergy (δ -score) across the tested dose matrix (Nuvisertib, μ M, y-axis; venetoclax, μ M, x-axis) in BCWM.1 (left) and RPCI-WM1 (right). Red regions indicate positive Bliss synergy; green regions indicate antagonism.

Figure 2.

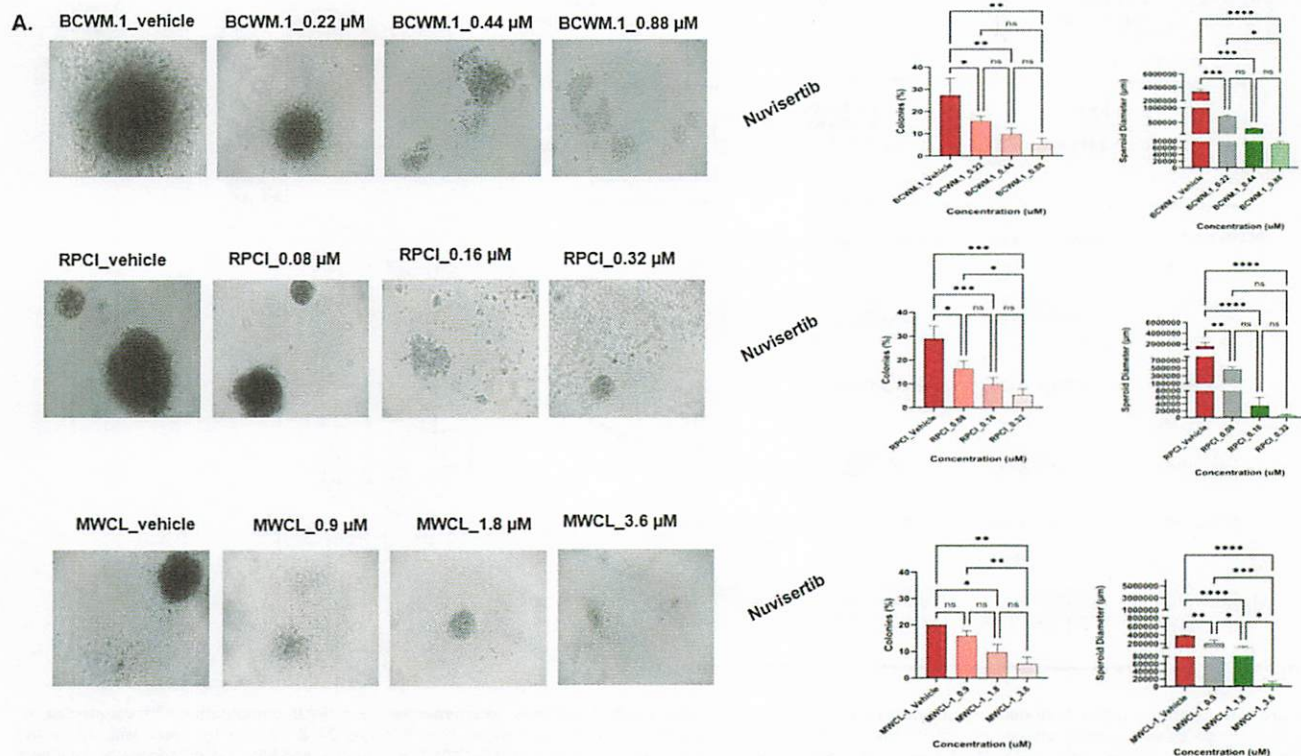


Figure 2. Nuvisertib dose-dependently inhibits clonogenic growth and spheroid formation in WM cell lines. A. Representative bright-field images (left) of colonies/spheroids formed by BCWM.1 (top), RPCI-WM1 (middle), and MWCL-1 (bottom) cells treated with vehicle or increasing concentrations of Nuvisertib, assessed by clonogenic (colony formation) assay. Nuvisertib treatment produced a dose-dependent reduction in both colony number and spheroid size across all three WM cell lines. Data are shown as mean $\pm$ SEM; statistical comparisons were performed by [One-way ANOVA], with * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001; ns, not significant.

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