# Steroid-free combination of 5-azacytidine and venetoclax for the treatment of multiple myeloma

Lyndsey Flanagan,[1] Aisling Coughlan,[1] Nicola Cosgrove,[1] Andrew Roe,[1] Yu Wang,[1] Stephanie Gilmore,[1] Izabela Drozdz,[2] Claire Comerford,[2,3] Jeremy Ryan,[4] Emma Minihane,[4] Salma Parvin,[4] Michael O'Dwyer,[5] John Quinn,[2,3] Philip Murphy,[2,3] Simon Furney,[1] Siobhan Glavey[2,3#] and Tríona Ní Chonghaile[1#]

[1]Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland; [2]Department of Pathology, Royal College of Surgeons in Ireland, Dublin, Ireland; [3]Department of Haematology, Beaumont Hospital, Dublin, Ireland; [4]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA and [5]Department of Medicine/Haematology, University of Galway, Galway, Ireland

[#]*SGl and TNC contributed equally as senior authors.*

**Correspondence:** T. Ní Chonghaile
tnichonghaile@rcsi.com

**Received:** June 29, 2023.
**Accepted:** March 8, 2024.
**Early view:** March 21, 2024.

**https://doi.org/10.3324/haematol.2023.283771**



## Abstract

Multiple myeloma (MM) is an incurable plasma cell malignancy that, despite an unprecedented increase in overall survival, lacks truly risk-adapted or targeted treatments. A proportion of patients with MM depend on BCL-2 for survival, and, recently, the BCL-2 antagonist venetoclax has shown clinical efficacy and safety in t(11;14) and BCL-2 overexpressing MM. However, only a small proportion of MM patients rely on BCL-2 (approx. 20%), and there is a need to broaden the patient population outside of t(11;14) that can be treated with venetoclax. Therefore, we took an unbiased screening approach and screened epigenetic modifiers to enhance venetoclax sensitivity in 2 non-BCL-2 dependent MM cell lines. The demethylase inhibitor 5-azacytidine was one of the lead hits from the screen, and the enhanced cell killing of the combination was confirmed in additional MM cell lines. Using dynamic BH3 profiling and immunoprecipitations, we identified the potential mechanism of synergy is due to increased NOXA expression, through the integrated stress response. Knockdown of *PMAIP1* or *PKR* partially rescues cell death of the venetoclax and 5-azacytidine combination treatment. The addition of a steroid to the combination treatment did not enhance the cell death, and, interestingly, we found enhanced death of the immune cells with steroid addition, suggesting that a steroid-sparing regimen may be more beneficial in MM. Lastly, we show for the first time in primary MM patient samples that 5-azacytidine enhances the response to venetoclax *ex vivo* across diverse anti-apoptotic dependencies (BCL-2 or MCL-1) and diverse cytogenetic backgrounds. Overall, our data identify 5-azacytidine and venetoclax as an effective treatment combination, which could be a tolerable steroid-sparing regimen, particularly for elderly MM patients.

## Introduction

The median age of onset of multiple myeloma (MM), an aggressive cancer of terminally differentiated plasma cells, is approximately 69 years of age.[1] While triplet and quadruplet combinations of novel therapies, including immunomodulatory agents and cellular therapies, have improved the therapeutic landscape for MM, outcomes remain suboptimal. Survival rates vary by age and cytogenetic status.[2] Patients eligible for autologous stem cell transplant (ASCT) have a median overall survival (OS) of eight years,[3] while for patients >75 years, this falls to five years.[4] Therefore, there is an urgent need to identify tolerable targeted agents for the treatment of elderly MM patients.

Reliance on the anti-apoptotic proteins BCL-2, MCL-1 and BCL-XL has emerged as a vulnerability for MM cells.[5] The anti-apoptotic BCL-2 proteins (BCL-2, MCL-1, BCL-XL, BFL-1) function by binding and inhibiting the activity of pro-apoptotic proteins (BIM, BAX, etc.).[6,7] Due to their function in inhibiting cell death, the anti-apoptotic proteins are attractive therapeutic targets. Previously, it was thought that the pro-survival protein MCL-1 was the main anti-apoptotic protein maintaining the viability of MM cells.[8] However, selective MCL-1 inhibitors (AMG-176, S68345) are associated with potential cardiac toxicity, therefore limiting their clinical utility.[9,10] The selective BCL-2 inhibitor, ABT-199 / venetoclax (ven), is the first BH3 mimetic to be approved by the Food and Drug Administration (FDA) for the treatment of chronic lymphocytic leukemia (CLL)

and acute myeloid leukemia (AML) in combination with a demethylating agent.[11-13]

BCL-2 dependence in MM was initially identified in a subset of patients, characterized by the presence of a translocation in cyclin D1 (CCND1) t(11;14).[14] This was confirmed across a panel of MM cell lines and primary MM samples; sensitivity to Ven was associated with t(11;14) and correlated with BH3 profiling.[15] In the phase III BELLINI trial evaluating the combination of Ven, dexamethasone (Dex) and bortezomib in relapsed/refractory (R/R) MM, the patients with t(11;14) translocation were particularly sensitive.[16] However, a proportion of patients without the t(11;14) translocation were also sensitive to Ven, specifically those with high expression of BCL-2.[17] This highlights the need to identify combination treatments that could induce BCL-2 dependence in MM and broaden the patient population that could be treated with Ven, as only 16-24% of patients have a t(11;14) translocation.[18]

Venetoclax is FDA-approved for the treatment of AML in combination with the demethylating agent 5-azacytidine (5-Aza).[13] 5-Aza is a cytosine analog that is incorporated into both DNA and RNA, where it acts as an epigenetic modifier inhibiting DNA methyltransferases, resulting in hypomethylation.[19] In AML, Ven in combination with 5-Aza is well tolerated in elderly patients who were ineligible for intensive induction therapy.[13,20] Previously, it was shown that methylation regulated BCL-2 expression in mixed lineage leukemia (MLL).[21] Whole exome sequencing analysis of MM revealed mutations in a series of epigenetic modifier genes, including *UTX, MLL, MLL2*, and *HOX9*.[22] This led us to hypothesize that epigenetic modifiers may induce BCL-2 dependence in MM. Our aim was to perform an unbiased screening approach to identify epigenetic modifiers that could induce BCL-2 dependence in MM cells, thereby sensitizing MM cells to Ven, to expand the cohort of patients that could be treated with Ven.

# Methods

For a more detailed description of the materials and methods used in the present study, see the *Online Supplementary Appendix*.

### CD138$^+$ cell isolation from primary multiple myeloma bone marrow samples

Primary MM samples were attained by informed consent from patients from Beaumont Hospital, Dublin, Ireland. Ethical approval was granted from the Beaumont Hospital and the RCSI ethics committee (Study N 19/32). For detailed methods of isolation, see the *Online Supplementary Appendix*.

### Cell culture

Multiple myeloma cell lines (JJN3, KMS18, RPMI-8226, H929, MM1S, KMS27 and U266) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum; KMS-12BM is supplemented with 20% fetal bovine serum (Gibco, Invitrogen, Carlsbad, CA, USA), 1% L-Glutamine (2 mM) (Gibco, Invitrogen, Carlsbad, CA, USA), and 1% v/v penicillin/streptomycin (50 units/mL) (Gibco, Invitrogen, Carlsbad, CA, USA). Primary MM bone marrow stroma cells (MM-BMSC) were cultured in DMEM medium supplemented with 10% fetal bovine serum (Gibco, Invitrogen, Carlsbad, CA, USA), 1% L-Glutamine (2 mM) (Gibco, Invitrogen, Carlsbad, CA, USA), and 1% v/v penicillin/streptomycin (50 units/mL) (Gibco, Invitrogen, Carlsbad, CA, USA).

### Dynamic BH3 profiling

For BH3 profiling, JJN3 cells were seeded at 3x10$^6$ cells in a T25 flask for 24 hours (hr), cells were treated with 3 μM 5-Aza for 20 hr. Cells were resuspended in 100 μM of buffer (1% FBS 0.4% EDTA [2 mM] in PBS, final pH 7.4) and incubated with a panel of BH3 peptides for 60 min at 21°C (Table 1). Details of the methods are provided in the *Online Supplementary Appendix*.

### Statistical analysis

GraphPad Prism 9.0 software was used for all statistical analyses. Dose-response curves and $IC_{50}$ values were calculated using linear regression curve fit (Log inhibitor *vs.* normalized response, variable slope). Unless otherwise

**Table 1.** List of BH3 peptides and interactions with BCL-2 proteins.

| Peptide | Sequence | Interacts |
|---|---|---|
| BIM | MRPEIWIAQELRRIGDEFNA | BAX; BAK; BCL-2; BCL-XL; BCL-w ; MCL-1; A1 |
| BAD | LWAAQRYGRELRRMSDEFEGSFKGL | BCL-2; BCL-XL; BCL-w |
| A12 | RPEIWMGQLRRLGDEINAYYAR | MCL-1 |
| HRK | WSSAAQLTAARLKALGDELHQ | BCL-XL |
| PUMA | EQWAREIGAQLRRMADDLNA | BCL-2; BCL-XL; BCL-w; MCL1; A1 |
| NOXA | AELPPEFAAQLRKIGDKVYC | MCL-1 at lowest doses; BCL-2 at higher doses; BCL-XL, BCL-w |

The BH3 peptides sequence is described along with the pro- and anti-apoptotic proteins that the BH3 peptides bind to.

stated, results are expressed as the mean ± Standard Error of the Mean (SEM) of 3 independent experiments. Webb's fractional product method was used to calculate the synergistic activity of pairs of drugs / inhibitors denoted A and B on cell viability using the following equation:

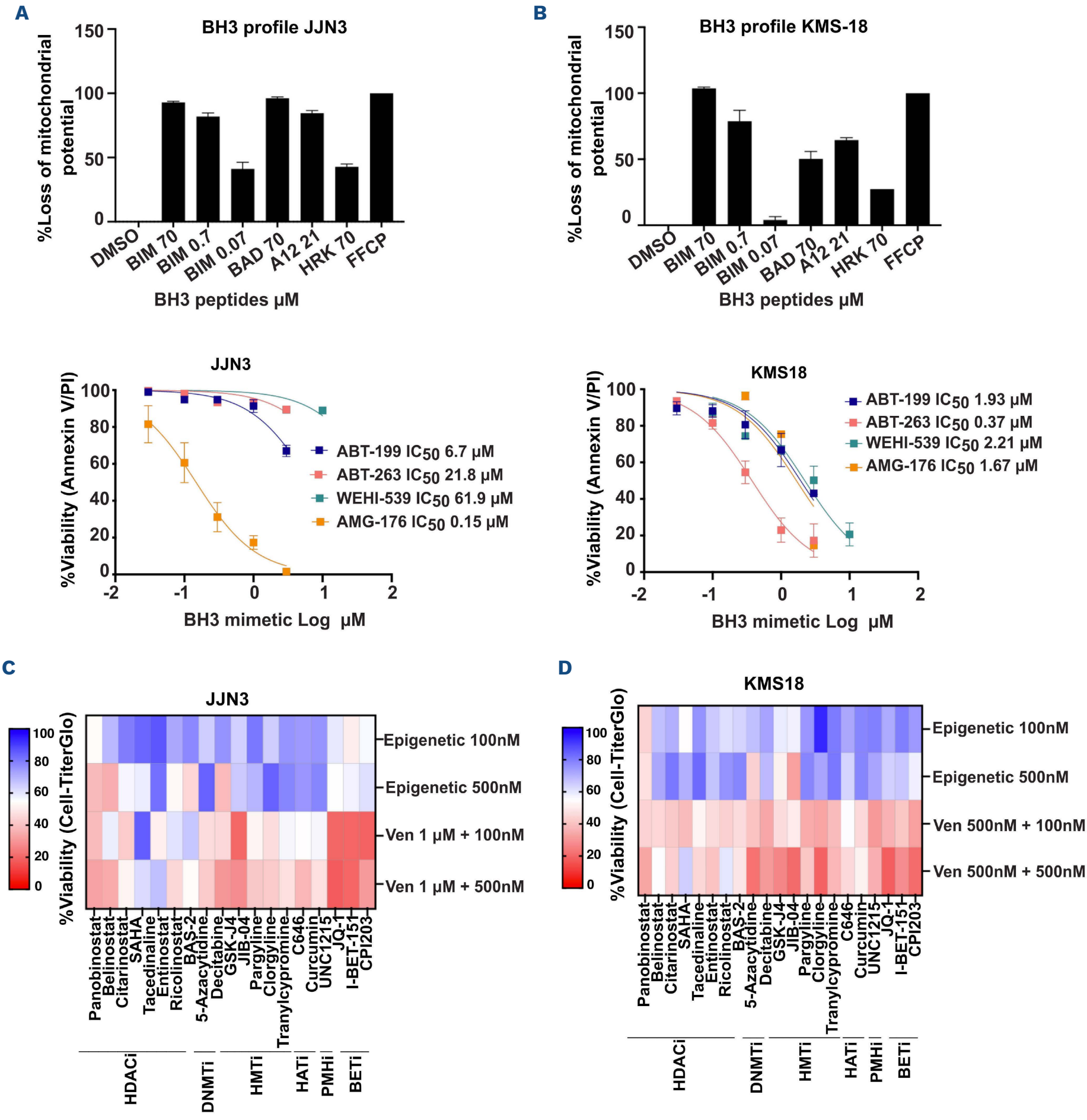
$$CI = AB/((A*B)/100)$$

where CI >1.0 indicates antagonistic activity
CI = 1.0 indicates additive interactions
CI <0.8 indicates strong synergy

# Results

## Epigenetic modifier screen to induce BCL-2 dependence and sensitivity to venetoclax

Taking an unbiased screening approach, we screened 21 epigenetic modifiers in 2 non-BCL-2 dependent MM cell lines to try and induce BCL-2 dependency and enhance sensitivity to Ven. We confirmed that JJN3 mainly relied on MCL-1 for survival, as they were most sensitive to AMG-




Continued on following page.

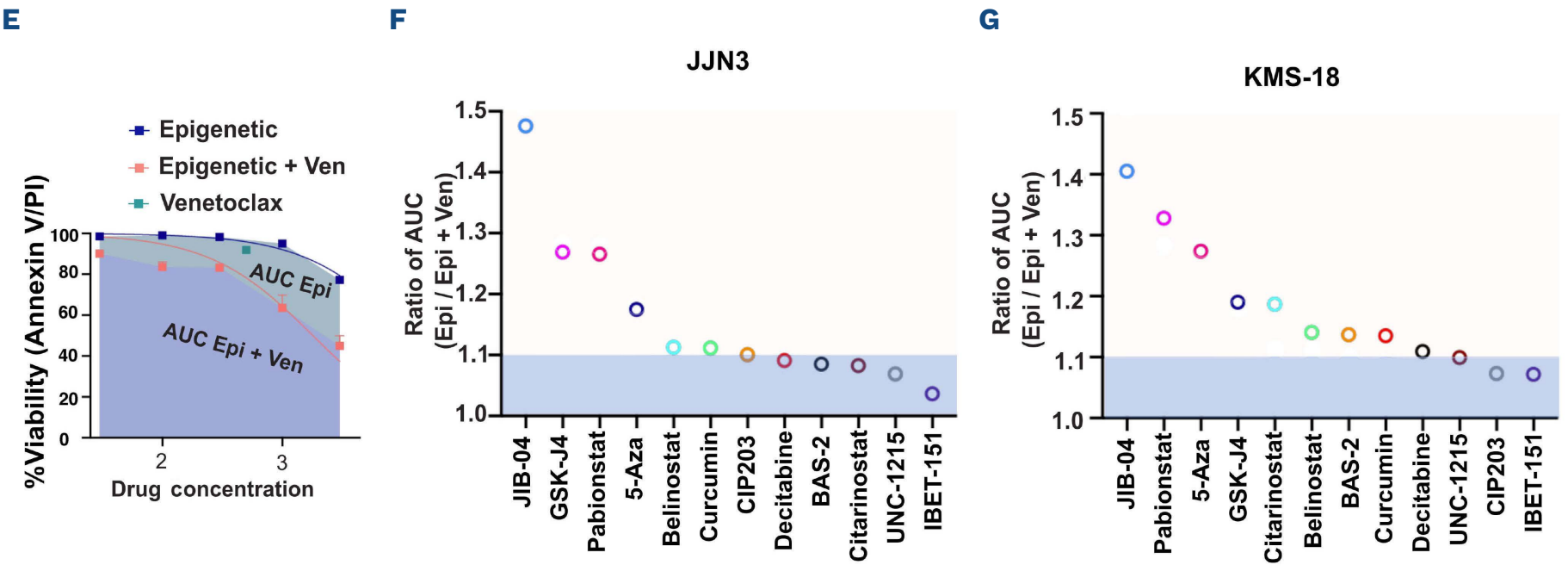


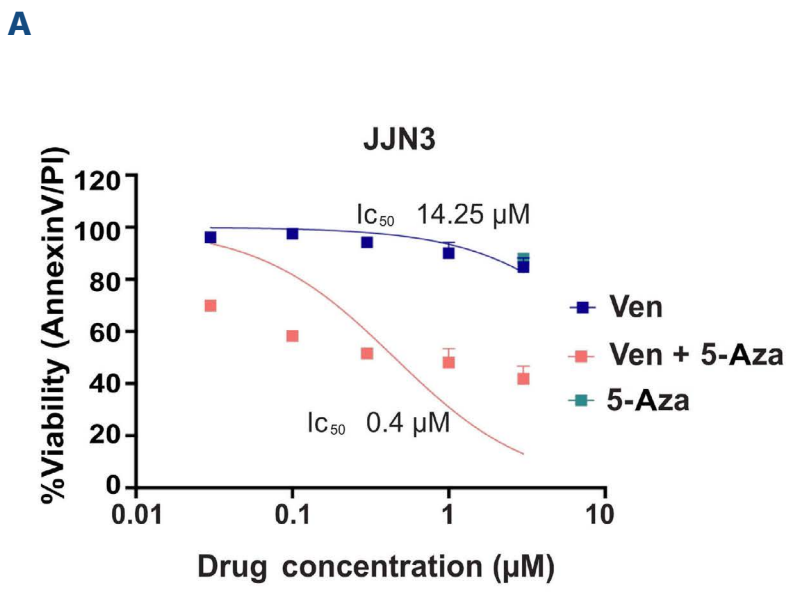

**Figure 1. Screening epigenetic modifiers in combination with venetoclax in multiple myeloma cell lines.** Area under the curve (AUC) of the dose response to BH3 mimetics ABT-199, ABT-263, WEHI-539, and AMG-176 is plotted following 24-hour (hr) treatment as measured by Annexin V / propidium iodide (PI) staining. BH3 profiles of (A) JJN3 and (B) KMS18. Mitochondrial membrane potential was measured using JC-1 following exposure to BH3 peptides and BH3 mimetics over 180 minutes (min). Median ± Standard Error of Mean of 3 independent experiments is shown. (C) JJN3 and (D) KMS18 cells were treated for 24 hr with epigenetic (Epi) modifiers ± venetoclax (Ven). Cells were treated with 2 doses of epigenetic modifier alone, 100 nM and 500 nM, or in combination with 1 μM Ven JJN3 cells or 500 nM Ven in KMS18 cells for 24 hr. Cell viability was assessed following treatment using Cell Titer Glo® and data were graphed using GraphPad prism. The 12 epigenetic modifier hits from (F) JJN3 and (G) KMS18 were assessed for cell death by Annexin V / PI staining. Cells were treated with 30 nM, 100 nM, 300 nM, 1 μM and 3 μM of each epigenetic modifier alone, and 1 μM Ven in JJN3 cells and 500 nM Ven in KMS18 cells for 24 hr. The dose-response curves were graphed in Graphpad. Ratio of AUC values, as shown in (E), were calculated and graphed for each drug in combination with Ven.

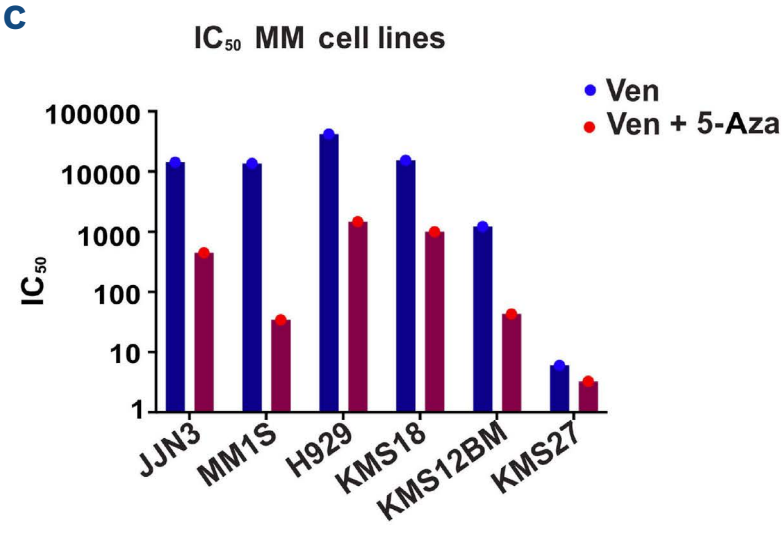
176, a BH3 mimetic that selectively inhibits MCL-1.[23] In the BH3 profile, the A12 BH3 peptide that antagonizes MCL-1[24] caused a large reduction in mitochondrial membrane potential (Figure 1A).[25] The KMS18 cell line was mixed BCL-2- / BCL-XL-dependent, as evidenced by sensitivity to ABT-263 that antagonizes BCL-2, BCL-XL, and BCL-w,[26] and the BH3 peptides BAD and HRK induced loss of mitochondrial membrane potential (Figure 1B).[27] Table 2 contains the list of the epigenetic compounds that were screened in both the KMS18 and JJN3 cell lines using CellTiter-Glo®. The classes of epigenetic modifiers included histone deacetylase inhibitors (HDACi), histone methyltransferase inhibitors (HMTi), DNA methyltransferase inhibitors (DNMTi), histone acetylase inhibitors (HATi), and bromodomain and extra terminal inhibitors (BETi) (Table 2). The 21 epigenetic modifier drugs were screened alone or in combination with Ven in the 2 MM cell lines, at 2 doses: 100 and 500 nM (Figure 1C, D). Based on the initial screen, the top 12 hits were assessed for an ability to induce cell death, as assessed by Annexin V / propium iodide (PI), in combination with Ven in the JJN3 cell line (*Online Supplementary Figure S2*) and the KMS-18 cell line (*Online Supplementary Figure S3*). The hits were ranked based on changes in the area under the curve (AUC) ratio of the epigenetic modifier alone *versus* the epigenetic modifier and Ven (Figure 1E). The lead hits from the cell death analysis in both the JJN3 and KMS18 cell lines were the histone methyltransferase inhibitors (JIB-04 and GSK-J4), the DNA methyltransferase inhibitor (5-aza), and the HDAC inhibitor (panobinostat) (Figure 1F, G). However, as evidenced by the dose-response curves

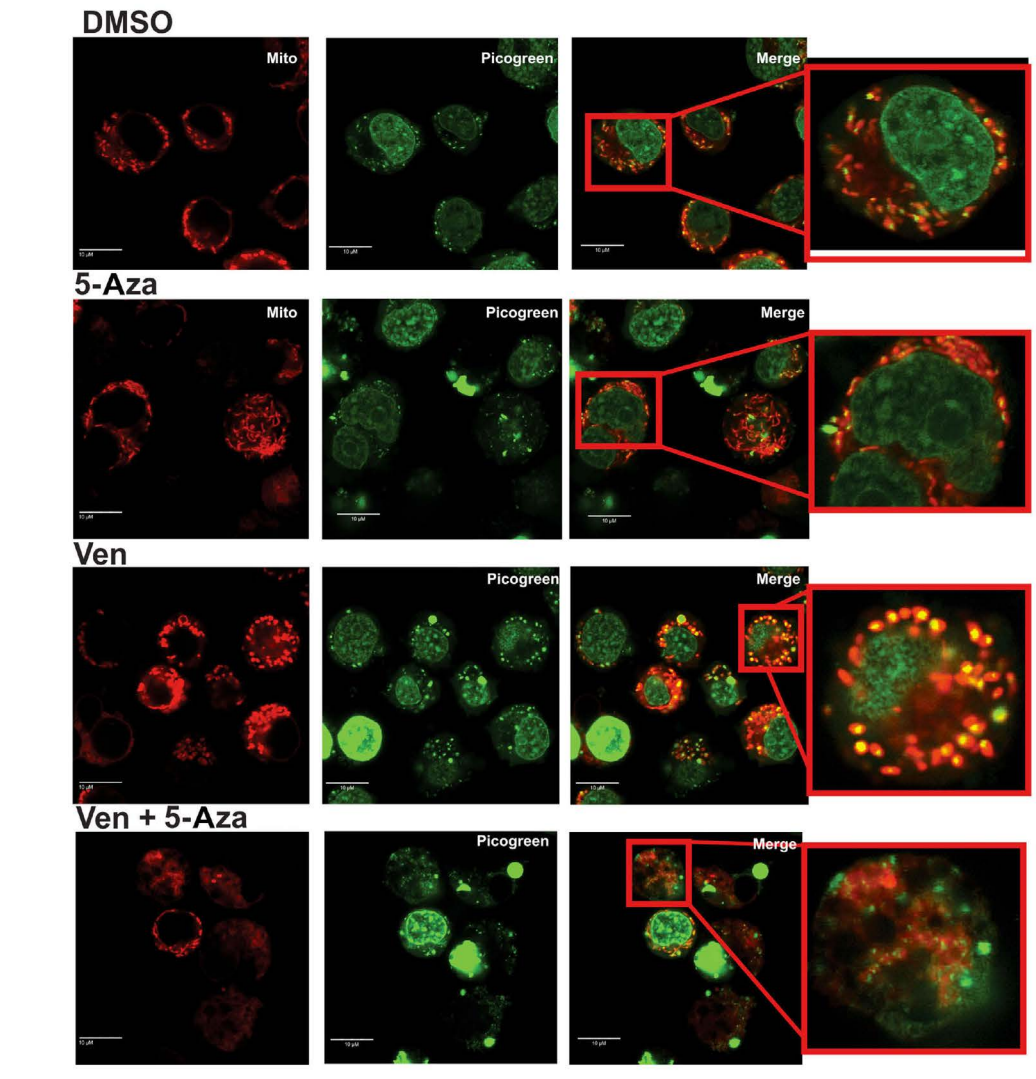
**Table 2.** List of epigenetic modifiers.

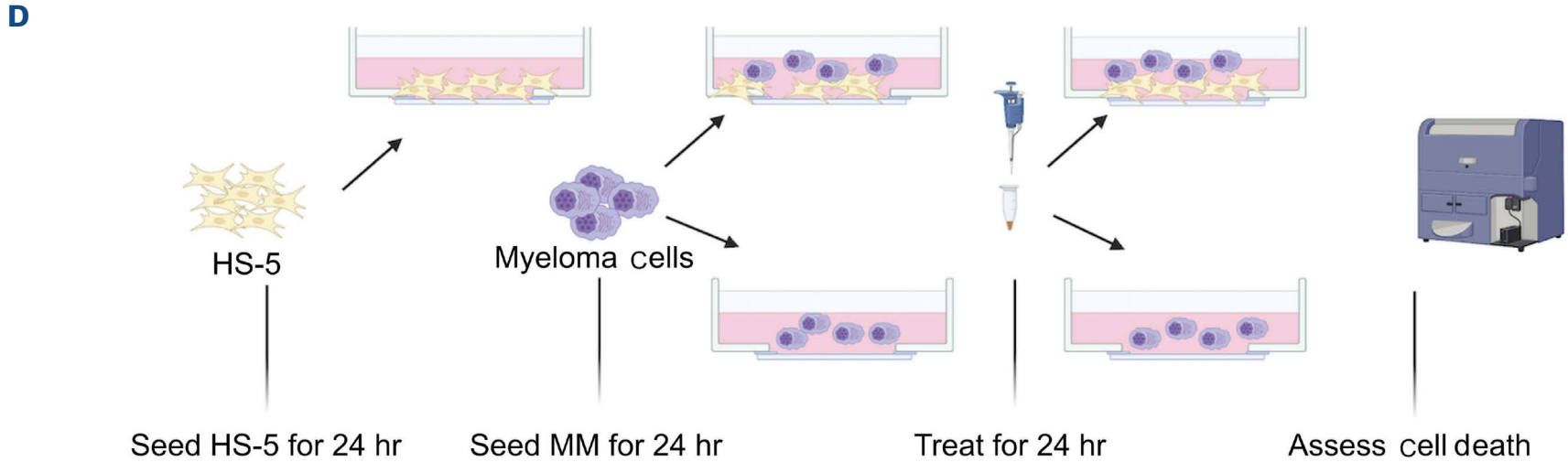
| Class of epigenetic modifier | Drugs |
|---|---|
| HDACi | Panobinostat (pan-HDACi), belinostat (pan-HDACi), ricolinostat (HDAC6), citarinostat (HDAC6), tacedinaline (HDAC1/2/3), BAS-2 (HDAC6), entinostat (HDAC1/2/3), vorinostat (pan-HDACi) |
| HMTi | JIB-04 (Jumonji histone demethylases), GSK-K4 (JMJD3/UTX), pargylcine (MAO-A/B), clorgyline (MAO-A), tranylcypromine (LSD1/MAO-A/B) |
| HATi | Curcumin (p300/CBP), C646 (p300) |
| DNMTi | 5-azacytidine (DNMT1), decitabine (DNMT1) |
| BETi | JQ-1(BRD2/3/4), I-BET-151 (BRD2/3/4), CPI203 (BRD4), UNC-1215 (L3MBTL3) |

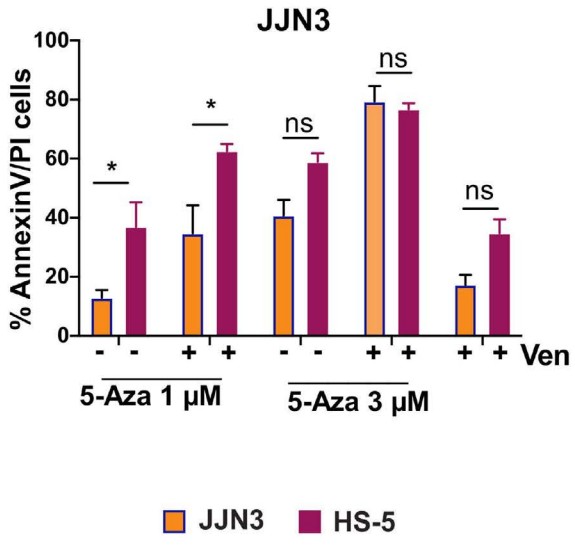
Listed are the types of epigenetic modifiers and drugs in each class used in the screen. They were used at two doses: 100 nM and 500 nM in the initial screen. HDACi: histone deacetylase inhibitors; HMTi: histone methyltransferase inhibitors; HATi: histone acetyltransferase inhibitors; DNMTi: DNA methyltransferase inhibitors; BETi: bromodomain and extra terminal inhibitors.

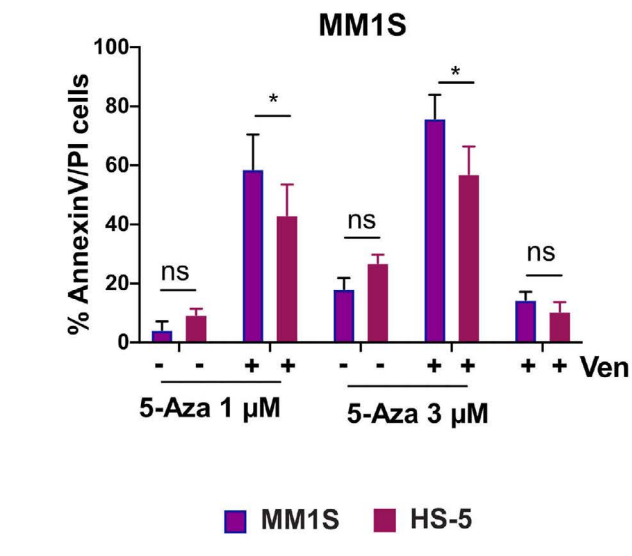


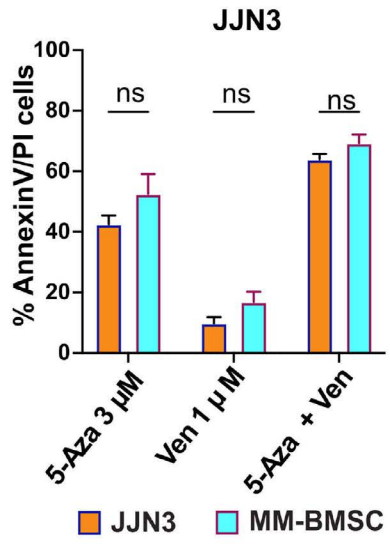




Continued on following page.

**Figure 2. Assessing the effect of bone marrow stroma cells on the combination of 5-azacytidine and venetoclax in multiple myeloma cell lines.** (A) Cell viability was assessed in JJN3 cells using Annexin V / propidium iodide (PI) staining following 24-hour (hr) treatment with dose response of venetoclax (Ven) ± 5-azacytidine (5-Aza) 3 μM. (B) Live cell confocal imaging of JJN3 cells following treatment with DMSO, 3 μM 5-Aza ± 1 μM Ven for 24 hr. Mitochondria were stained with PKMO and DNA stained with Picogreen; 5 images were taken per treatment condition, with images representative of 3 independent experiments. (C) The combination treatment was assessed in a panel of multiple myeloma (MM) cell lines by measuring Annexin V / PI positivity by flow cytometry following 24 hr of treatment. Sensitivity was expressed as $IC_{50}$ μM for Ven alone or Ven + 5-Aza as determined by GraphPad. (D) Workflow of the co-culture experiments. HS-5 cells or MM bone marrow stromal cells (BMSC) from an MM patient sample were seeded for 24 hr. MM cells were co-cultured for 24 hr. and then treated with drugs for 24 hr before assessment by flow cytometry. (E) JJN3 cells were seeded alone (orange) or co-cultured with HS-5 cells for 24 hr (maroon). Cells were then treated with either 5-Aza (1 μM or 3 μM) alone or combined with 1 μM Ven (shown by the plus symbol) for 24 hrs. % cell death of tumor cells was assessed by Annexin V / PI staining on the flow cytometer. (F) The same experiment was carried out for the MM1S cells, seeded alone (purple) or co-cultured with HS-5 (maroon) for 24 hr before treatment. Cells were then treated with either 5-Aza (1 μM or 3 μM) alone or combined with 1 μM Ven (shown by the plus symbol) for 24 hr. (G) The same experiment as described for (E) was carried out with JJN3 cells co-cultured with MM-BMSC cells. Median ± Standard Error of Mean from 3 independent experiments is shown. Two-way ANOVA with Bonferroni multiple comparison test was used to calculate *P* values. ns: not significant; **P*≤0.05, ***P*≤0.01, ****P*≤0.001.

(*Online Supplementary Figures S2, S3*), the top 4 hits all showed similar enhanced cell death and changes in $IC_{50}$ values upon combination treatment.

## 5-azacytidine enhanced venetoclax-induced cell death even in the presence of bone marrow stromal cells

While the lead hits from the screen were the histone demethylase inhibitors, those compounds have only been assessed pre-clinically. Excitingly, the combination of 5-Aza and Ven has not been previously tested in MM; it has been shown to have acceptable tolerability in elderly AML patients and is FDA-approved as a combination treatment.[13] As shown in the JJN3 cells, while Ven caused very little cell death alone ($IC_{50}$ 14.2 μM), when combined with 5-Aza, there is a log fold change in $IC_{50}$ value ($IC_{50}$ 0.4 μM) (Figure 2A). This is also evident from live cell imaging of the JJN3 cells, with enhanced cell killing in the combination treatment and loss of mitochondrial staining with PK Mito Orange (PKMO)[28] (Figure 2B). Interestingly, although Ven did not induce cell death, it did cause a phenotypic change to the mitochondrial structure, as determined by PKMO staining. Next, we assessed the combination of Ven + 5-Aza in a panel of 5 additional MM cell lines (*Online Supplementary Figure S4A-F*). Remarkably, across the panel of cell lines tested, 5-Aza significantly altered the $IC_{50}$ value when combined with Ven in all 5 cell lines (Figure 2C). The KMS27 cell line was most sensitive to Ven treatment alone, probably because this cell line has a t(11;14) translocation, but the addition of 5-Aza reduced the $IC_{50}$ by half. The combination index score for synergy is represented as a heatmap in *Online Supplementary Figure S4G, H*. We also assessed the reverse combination measuring the effect of Ven treatment on a 5-Aza dose-response curve, with 4/5 of the cell lines showing major changes in $IC_{50}$ values upon the addition of Ven (*Online Supplementary Figure S4J-N*), with combination index scores of synergy graphed as a heatmap (*Online Supplementary Figure S4 O*).

The complex interplay between MM cells and the bone marrow microenvironment has been shown to influence cell behavior, particularly MM survival and drug resistance.[29] To assess the impact of bone marrow stroma cells on the 5-Aza and Ven combination, we performed co-culture experiments using HS-5, a bone marrow stroma cell line, and primary MM bone marrow stromal cells (MM-BMSC) (Figure 2D). Surprisingly, co-culture of JJN3 cells with HS-5 cells conferred significant increase in cell death with 5-Aza and Ven, compared to JJN3 cells alone (Figure 2E). Of interest, when MM1S cells were co-cultured with HS-5 cells, they were slightly less sensitive to the 5-Aza and Ven combination (Figure 2F). Next, we co-cultured JJN3 cells with MM-BMSC isolated from a MM patient bone marrow sample (Figure 2G). The JJN3 cells were still sensitive to 5-Aza and Ven combination when co-cultured with the MM-BMSC. In summary, while the MM1S cells benefit from a slight protective effect and the JJN3 cells are slightly sensitized following co-culture with the HS-5 cells, 5-Aza and Ven is still an effective treatment combination in the presence of bone marrow stroma cells across multiple cell lines with diverse anti-apoptotic dependencies.

## 5-azacytidine induces NOXA through the integrated stress response antagonizing MCL-1, sensitizing multiple myeloma cells to venetoclax treatment

To understand the mechanism of action of how 5-Aza may enhance sensitivity to Ven in MM cell lines, a series of approaches were taken. Firstly, we used dynamic BH3 profiling to determine changes in BH3 peptide-induced cytochrome *c* release from mitochondria, following 5-Aza treatment (Figure 3A). As is clear from the BH3 profile, there was a statistically significant increase in the amount of cytochrome *c* released following the addition of the A12 BH3 peptide, that selectively binds MCL-1, following treatment with 5-Aza. In addition, direct Ven treatment, as part of the BH3 profile on permeabilized cells, caused a statistically significant increase in the release of cytochrome *c*, following 5-Aza treatment in the JJN3 cells (Figure 3A). Next, we confirmed the dynamic BH3 profile by assessing the pro-apoptotic proteins that might be interacting with MCL-1 using immunoprecipitation. Following 5-Aza treatment, there was an increase in the amount of both BIM

EL and NOXA bound to MCL-1 (Figure 3B). Previously, it was shown in AML cells, that 5-Aza induced NOXA by the integrated stress response.[30] Indeed, we found a similar induction of NOXA following 5-Aza treatment in the JJN3 cells, which was even greater in the combination of 5-Aza and Ven (Figure 3C). We also assessed if this induction of NOXA was dependent on the integrated stress response. Pre-treatment with ISRIB, which blocks the integrated stress response by targeting eIF1B,[31] also blocked the induction of the NOXA protein following the combination treatment (Figure 3C). Ven alone induces a small amount of NOXA protein, but it enhances the amount of NOXA induced by 5-Aza (Figure 3C). We confirmed that ISRIB did not alter eIF2α phosphorylation but prevented the induction of activating transcription factor 4 (ATF-4) by 5-Aza and 5-Aza + Ven (Figure 3D). Confirming the importance of the integrated stress response in the cell death induced by Ven + 5-Aza, there was a significant reduction in cell death upon pre-treatment with ISRIB (Figure 3E).

There is evidence in the literature that 5-Aza can induce dsRNA, through reactivation of endogenous retrovirus.[32] Therefore, we assessed the amount of dsRNA in JJN3 cells treated with 5-Aza, Ven or the combination (Figure 4A). While all treatments induced some dsRNA, the combination of Ven + 5-Aza induced the most significant amount, as assessed by pixel count (Figure 4B). Next, we assessed if the knockdown of the dsRNA sensor PKR, which can activate the integrated stress response, could protect from the cell death induced by the combination treatment. Knockdown of *PKR* significantly reduced the cell death induced by the combination treatment (Figure 4C). The densitometry on PKR knockdown is shown in *Online Supplementary Figure*

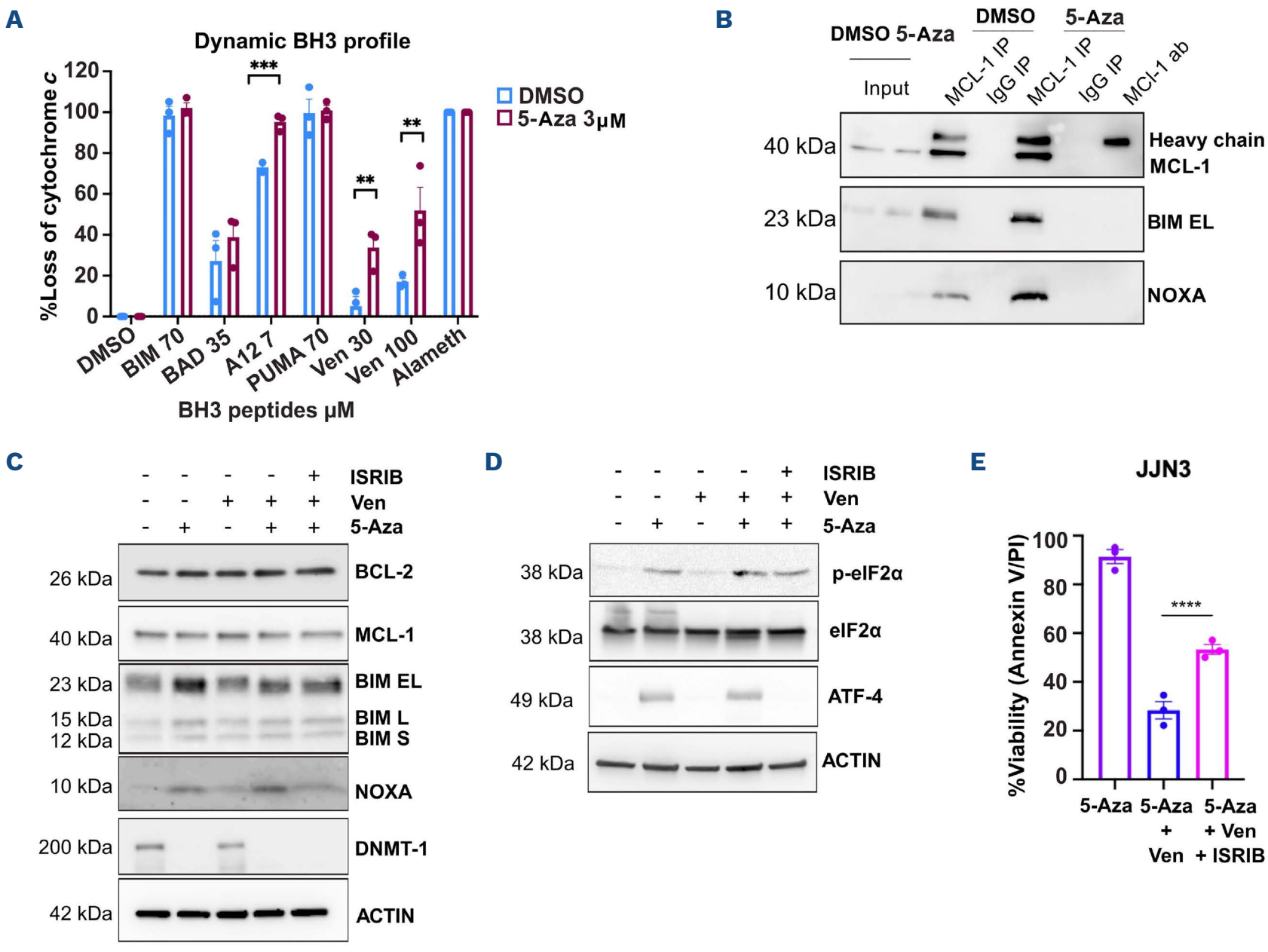



**Figure 3. 5-azacytidine increases NOXA expression through the integrated stress response antagonizing MCL-1.** (A) The effect of 5-azacytidine (5-Aza) 3 μM treatment on JJN3 cells after 24 hours (hr) was assessed by dynamic BH3 profiling (see Methods section). (B) JJN3 cells were treated with 5-Aza 3 μM for 24 hr. The association of NOXA and BIM with MCL-1 was determined by immunoprecipitation and western blot analysis. (C) JJN3 cells were treated with 3 μM 5-Aza; venetoclax (Ven) 1 μM; (3 μM 5-Aza + Ven1 μM); (3 μM 5-Aza + Ven1 μM + 500 nM ISRIB) for 24 hr and the expression of BCL-2, MCL-1, NOXA, DNMT1 and β-actin were determined by western blot analysis. (D) Cells were treated as in (C) for 6 hr and expression of p-eIF2α, total eIF2α, ATF4, and β-actin was measured by western blot. (E) Cell viability was assessed in JJN3 cells by Annexin V / propidium iodide (PI) staining following treatment with 3 μM 5-Aza; (3 μM 5-Aza + Ven1 μM); (3 μM 5-Aza + Ven1 μM + 500 nM ISRIB) for 24 hr. Mean of 3 independent experiments ± Standard Deviation is shown. ***P*≤0.01, ****P*≤0.001, *****P*≤0.0001

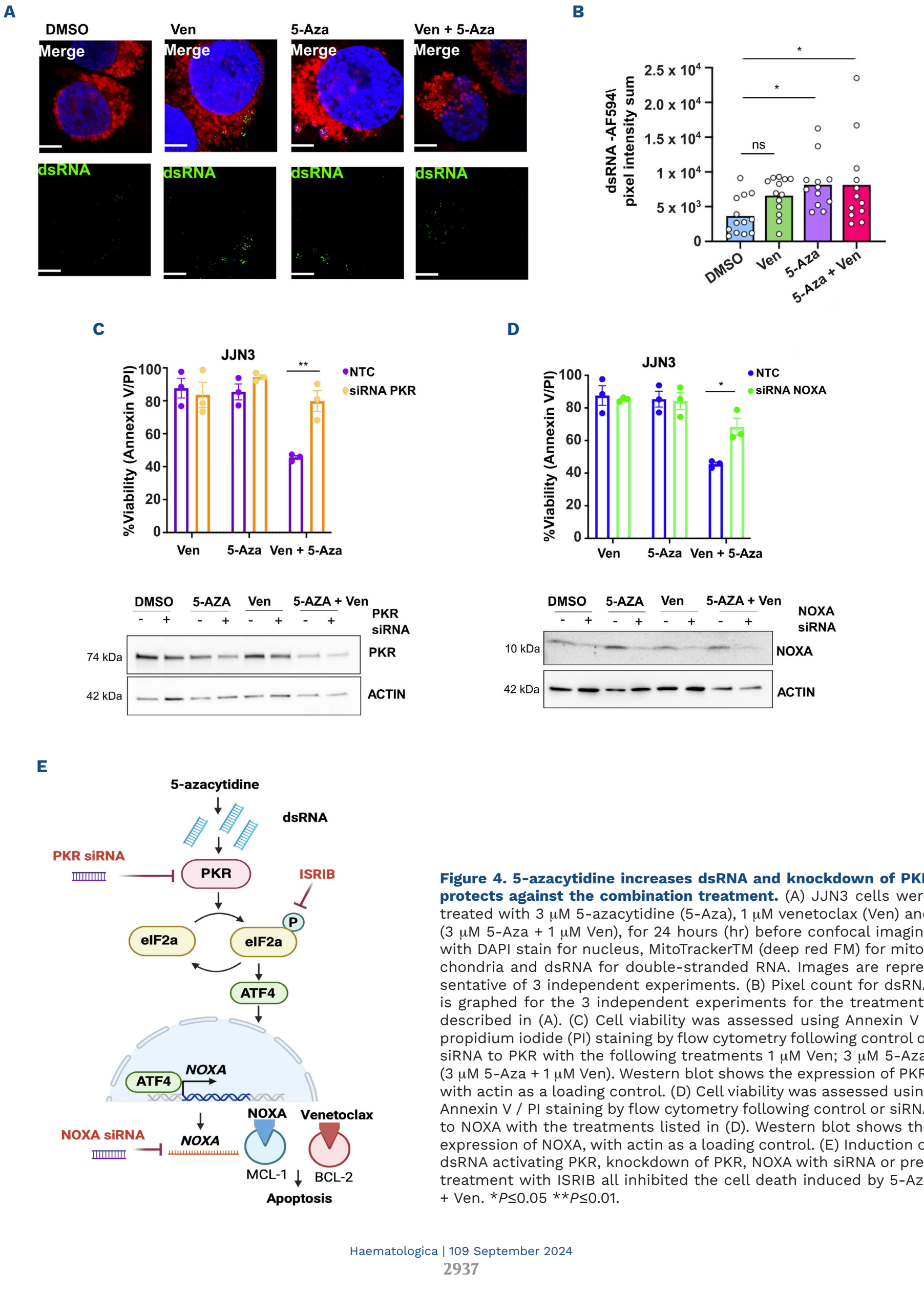



**Figure 4. 5-azacytidine increases dsRNA and knockdown of PKR protects against the combination treatment.** (A) JJN3 cells were treated with 3 μM 5-azacytidine (5-Aza), 1 μM venetoclax (Ven) and (3 μM 5-Aza + 1 μM Ven), for 24 hours (hr) before confocal imaging with DAPI stain for nucleus, MitoTrackerTM (deep red FM) for mitochondria and dsRNA for double-stranded RNA. Images are representative of 3 independent experiments. (B) Pixel count for dsRNA is graphed for the 3 independent experiments for the treatments described in (A). (C) Cell viability was assessed using Annexin V / propidium iodide (PI) staining by flow cytometry following control or siRNA to PKR with the following treatments 1 μM Ven; 3 μM 5-Aza; (3 μM 5-Aza + 1 μM Ven). Western blot shows the expression of PKR, with actin as a loading control. (D) Cell viability was assessed using Annexin V / PI staining by flow cytometry following control or siRNA to NOXA with the treatments listed in (D). Western blot shows the expression of NOXA, with actin as a loading control. (E) Induction of dsRNA activating PKR, knockdown of PKR, NOXA with siRNA or pretreatment with ISRIB all inhibited the cell death induced by 5-Aza + Ven. *$P \leq 0.05$ **$P \leq 0.01$.

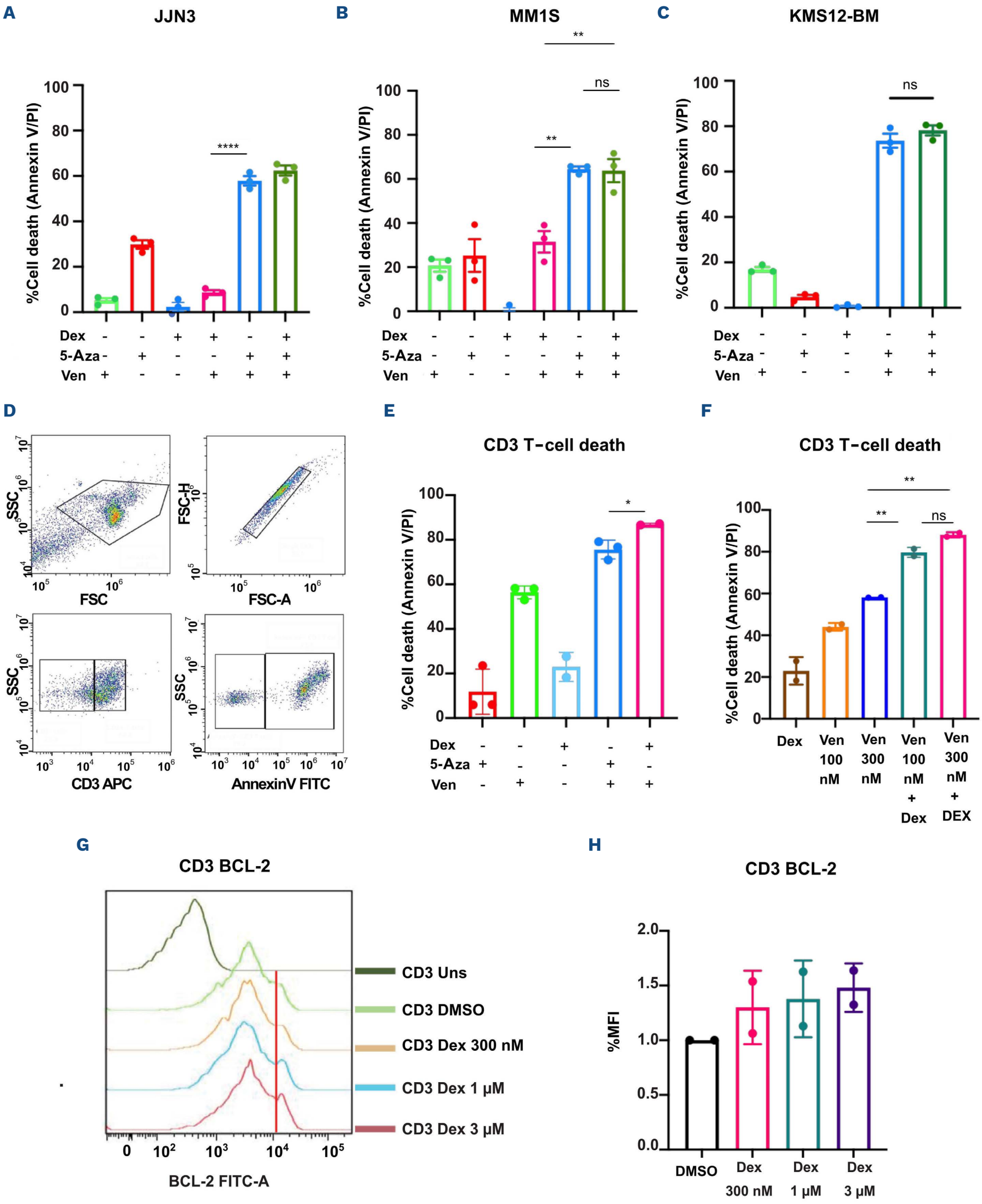



**Figure 5. Dexamethasone does not enhance venetoclax + 5-azacytidine cell death and induces T-cell death.** (A) JJN3 cells were treated with 1 µM venetoclax (Ven); 3 µM 5-azacytidine (5-Aza); 1 µM dexamethasone (Dex); (1 µM Ven + 1 µM Dex); (1 µM Ven + 3 µM

Continued on following page.

5-Aza); (1 μM Ven + 3 μM 5-Aza + 1 μM Dex) for 24 hours (hr). Cell viability was measured using Annexin V/ propidium iodide (PI) staining. Mean of 3 independent repeats + Standard Deviation is shown. (B) MMIS cells were treated with 1 μM Ven; 3 μM 5-Aza; 1 μM Dex; (1 μM Ven + 1 μM Dex); (1 μM Ven + 3 μM 5-Aza); (1 μM Ven + 3 μM 5-Aza + 1 μM Dex) for 24 hours (hr). Cell viability was assessed as in (A). (C) KMS12BM were treated with 0.5 μM Ven; 3 μM 5-Aza; 1 μM Dex; (0.5 μM Ven + 3 μM 5-Aza); (0.5 μM Ven + 3 μM 5-Aza + 1 μM Dex) for 24 hr. Cell viability was assessed and graphed as in (A). (D) Gating strategy used for identifying CD3 T cells. (E) Cell viability was assessed in $CD3^+$ T cells isolated from peripheral blood mononuclear cells using Annexin V / PI staining following 16-hr treatment with 3 μM 5-Aza; 300 nM Ven; 1 μM Dex; (300 nM Ven + 3 μM 5-Aza); (1 μM Dex + 300 nM Ven). Mean of 3 independent experiments is shown. (F) Cell viability was assessed in $CD3^+$ T cells following treatment with Ven at 100 nM and 300 nM with and without 1 μM Dex. Graphed are the mean of 2 independent experiments ± SD. (G, H) Intracellular BCL-2 staining was measured in T cells following 16 hr treatment with 300 nM, 1 μM and 3 μM Dex. One-way ANOVA was performed to determine *P* values. ns: not significant; **P*≤0.05, ***P*≤0.01, ****P*≤0.001, *****P*≤0.0001.

*S5C*. Validating the importance of NOXA in the cell death mechanism, siRNA knockdown of *PMAIP1* (NOXA gene), also significantly protected against the combination induced cell death (Figure 4D). Similarly, densitometry for NOXA knockdown is shown in *Online Supplementary Figure S5D*. A proposed model is shown in Figure 4E. Combined, the data suggest the mechanism of synergy following 5-Aza + Ven is due, at least in part, to an increase in dsRNA, activating PKR, activating the integrated stress response, with an increase in eIF2α phosphorylation, inducing NOXA protein that antagonizes MCL-1. By antagonizing MCL-1, the cells are more sensitive to Ven (Figure 4E).

## Assessing if a steroid enhances the cell death induced by 5-azacytidine and venetoclax in multiple myeloma and immune cells

It is standard practice for a steroid, such as Dex, to be added to a combination treatment regimen in MM.[33] Therefore, we assessed the combination of Ven, 5-Aza and Dex in a panel of MM cells. Interestingly, we did not find any significant increase in cell death upon the addition of 1 μM Dex to the combination of Ven and 5-Aza, across 3 different MM cell lines (Figure 5A-C). Indeed, the combination of Ven and 5-Aza caused significantly more cell death than Dex (1 μM) and Ven in both the JJN3 and MM1S cell lines (Figure 5A, B). Next, we measured the effect of the combination treatment on immune cells, Ven has been shown to induce cell death in B[34] and T cells.[35] Using peripheral blood mononuclear cells from healthy donors, we treated the cells *ex vivo* for 16 hr and measured cell death of $CD3^+$ T cells by annexin V/PI staining (Figure 5D). In the $CD3^+$ T cells, 5-Aza caused a minimal amount of cell death alone. Ven induced some cell death in $CD3^+$ T cells, as was found previously in mouse $CD8^+$ T cells,[35] and additive cell death was seen with the combination treatment of 5-Aza and Ven (Figure 5E). The combination of Dex and Ven caused slightly more cell death, which was significant, than Ven and 5-Aza (Figure 5E). Dex combined with Ven caused a dose-dependent increase in T-cell death (Figure 5F). Potentially, the reason for this enhanced cell death is due to Dex inducing a BCL-2 expression in T cells. There was a dose-dependent increase in BCL-2 expression following Dex treatment, as measured by intercellular BCL-2 staining by flow cytometry (Figure 5G, H). Our data potentially suggest that the addition of a steroid may induce more cell death in the immune cells while having little effect on the tumor cell killing, at least *ex vivo*. Based on this result, there is a potential to use a steroid-sparing regimen to test the combination of Ven/5-Aza in the clinic. This steroid effect on T cells may also have implications for the efficacy of many other therapies in MM.

## Venetoclax and 5-azacytidine show enhanced killing of primary multiple myeloma samples irrespective of stage or cytogenetics

As the combination treatment had only been assessed in MM cell lines, we next wanted to confirm our findings that the combination of 5-Aza and Ven was effective in heterogeneous MM patient samples. Following isolation of $CD138^+$ patients' cells, the cells were seeded and treated with either increasing doses of BH3 mimetics for 16 hr or the combination of 5-Aza and Ven and cell death was measured using Annexin V/PI staining on the flow cytometer (Figure 6A, *Online Supplementary Figures S6A-K, S7A-K*). Of the 11 primary MM bone marrow samples analyzed *ex vivo* (Figure 6A), 8 were non-t(11;14) patients (*Online Supplementary Table S1*). The combination of 5-Aza and Ven showed enhanced cell killing in 6 out of the 8 non-t(11;14) (Figure 6B) and 2 out of 3 t(11;14) samples (Figure 6C), with 6 samples showing a CI score of less than 1 (*Online Supplementary Figure S6A-K*). Patient cytogenetics were not known at the time of Ven treatment. The dose of Ven used induced substantial cell death alone in the t(11;14) patient samples, with a statistical difference in the *ex vivo* sensitivity of non-t(11;14) samples *versus* the t(11;14) samples (Figure 6D). These data highlight the use of BH3 mimetic profiling as a biomarker to identify patients who may respond to Ven. There was a diverse anti-apoptotic dependence across the MM patient sample (*Online Supplementary Figure S6*). Four of the primary samples $CD138^+$ patient cells were more BCL-2 dependent, 4 were more MCL-1 dependent, and 3 samples were a mix, with the BCL-XL dependent samples showing the lowest cell death following combination treatment (Figure 6E). Lastly, there was no statistical difference in the combination treatment of Ven and 5-Aza in upfront *versus* relapsed patient samples (Figure 6F) or in patients with normal or altered cytogenetics (Figure 6G). Importantly, here we have shown for the first time that 5-Aza and Ven are effective at killing primary MM samples *ex vivo*, over-

coming high-risk cytogenetic features and multiple lines of prior therapies.

## Discussion

Multiple myeloma is a disease with a diverse genetic background and, to date, the development of molecularly targeted therapies has proven elusive. Our aim was to take an unbiased screening approach to identify epigenetic modifiers that could induce BCL-2 dependence in MM cells to broaden the patient population that could benefit from treatment with Ven.[15] The DNA methyltransferase inhibitor 5-Aza emerged as one of the top hits from our screen. Ven in combination with 5-Aza has never been tested in MM, even though it is an approved combination regimen in AML.[36]

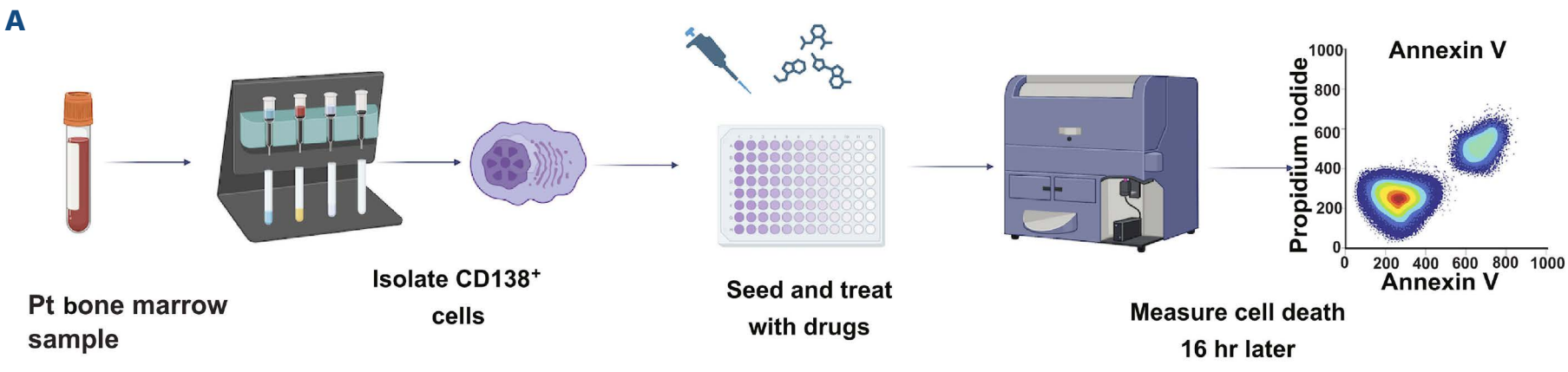



**Figure 6. Venetoclax and 5-azacytidine in combination enhance cell death in primary multiple myeloma samples *ex vivo*.** Cell viability was assessed using Annexin V / propidium iodide (PI) staining following 16-hour (hr) treatment. (A) Workflow of isolating CD138+ cells from patient (Pt) bone marrow samples and treating them *ex vivo*. (B) Summary of the response across the 8 non-t(11;14) patients to 3 μM 5-azacytidine (5-Aza), 300 nM venetoclax (Ven), or combination. (C) Summary of the response across 3 t(11;14) patients to 5-Aza, Ven or in combination. (D) Response of non-t(11;14) *versus* t(11;14) patients to 300 nM Ven following 16-hr treatment. Summary of response to 5-Aza and Ven in 11 patients across (E) anti-apoptotic dependencies, (F) diagnosis, and (G) cytogenetics; ns: not significant; ***P*≤0.01, ****P*≤0.001.

We identified a mechanism of synergism by which 5-Aza in combination with Ven induces NOXA protein inhibiting MCL-1, sensitizing MM cells to Ven. The induction of NOXA is reliant on the integrated stress response, with evidence of phosphorylation of eIF2-α. A similar mechanism was identified for 5-Aza in AML, ATF4 was responsible for inducing NOXA expression.[30] Importantly, we demonstrated that *PMAIP1* (NOXA) knockdown partially rescued the cell death induced by the combination treatment, as did inhibition of the integrated stress response with ISRIB. We also showed for the first time, the translational potential of 5-Aza and Ven in combination, which demonstrated strong cell killing effect in primary CD138$^+$ MM cells *ex vivo*, irrespective of cytogenetic background.

In the case of AML, either 5-Aza or decitabine were both effective at enhancing the response to Ven treatment.[13,20] In contrast, in our study, while 5-Aza enhanced the cell death induced by Ven, decitabine did not cause a similar enhanced cell death. Decitabine is a deoxyribonucleotide and is incorporated into DNA, whereas 5-Aza is a ribonucleoside and is incorporated into both DNA and RNA.[19,37] Potentially, this difference could explain why 5-Aza enhances cell death in MM and may suggest the integration into mRNA may lead to activation of the integrated stress response. A series of studies have also shown evidence that 5-Aza acts through activation of endogenous retroviral elements (ERV), inducing viral mimicry and activating the dsRNA sensing pathways (e.g., MDA5, TLR3).[32,38] We showed that the combination treatment induced dsRNA in the cells, and that knockdown of the dsRNA sensor *PKR* partially rescued the cell death induced by the combination. We did not investigate if the dsRNA also activates RIG-1 to signal through the mitochondrial antiviral-signaling protein (MAVS), to induce type 1 interferons and proinflammatory cytokines.[32,39] The combination of Ven + 5-Aza works very effectively in the clinic in AML.[13,36] Activation of the dsRNA pathway by the combination treatment, as shown in our data, could also activate an immune response; this could potentially play an important role in the mechanism of action *in vivo*. However, this was not assessed in this study.

Steroid utilization in MM is standard practice.[40] Therefore, we assessed if the addition of the steroid Dex enhanced the cell death induced by the combination treatment (Ven + 5-Aza). We did not detect any enhanced killing in 3 different MM cell lines, with diverse anti-apoptotic dependence, following the addition of Dex to the combination. The combination of 5-Aza + Ven was more effective at killing than Dex + Ven, which was previously identified as an effective combination in MM.[41,42] Potentially, our data may help to explain the latest phase III CANOVA trial data with Ven and Dex in t(11;14) patients, which did not reach a significant endpoint in comparison to pomalidomide + Dex, despite evidence of longer progression-free survival.[43] Next, we assessed the combination on immune cell killing *ex vivo* on T cells, as it was previously identified that mature T cells express high BCL-2[27,44] and are sensitive to Ven treatment alone.[35,45] We found a similar induction of cell death in human CD3 T cells with Ven treatment alone, and the addition of 5-Aza did enhance cell killing. However, Dex + Ven caused significantly more death of the T cells than 5-Aza + Ven, and Dex induced BCL-2 expression in the CD3 cells. These data would suggest that a steroid has little therapeutic benefit, as it did not enhance the MM cell killing; but it may enhance the killing of immune cells, potentially limiting the capacity of immune cells to kill tumor cells.[17] In a randomized trial comparing lenalidomide with high-dose *versus* low-dose Dex, the low-dose Dex regimen was associated with better short-term overall survival.[46] Indeed, a high-dose Dex regimen was associated with toxicities, particularly in frail, elderly patients. In a recent abstract, the results of a phase III trial comparing daratumumab and lenalidomide without long-term Dex to lenalidomide and Dex were described.[47] This trial showed that the overall response rates were higher in the Dex-sparing regimen. This study highlighted that there is a case to not add Dex to MM regimens, particularly in frail, elderly patients with MM, in the situation where the patient is receiving highly effective therapy.

To assess the translational relevance of 5-Aza and Ven, we screened the combination in a panel of primary MM CD138$^+$ cells from MM patients, with different cytogenetic backgrounds. In total, 11 patient samples were assessed: 4 were MCL-1 dependent, 3 were mixed anti-apoptotic dependent, and 4 samples were BCL-2 dependent. The 2 samples that did not respond to the combination treatment had a higher sensitivity to the BCL-XL antagonist. Given the mechanism of the synergy described, induction of the NOXA protein binding to MCL-1 could potentially explain the lack of response in these 2 patient samples. Of the 4 samples that were BCL-2 dependent, it was later revealed by cytogenetics that 3 had t(11;14) translocation. Gomez-Bougie *et al*. looked at the anti-apoptotic dependence in MM samples.[48] They saw that BCL-2 dependence was mostly found in the t(11;14) subgroup and in 35% of the samples there was a co-dependency on either BCL-2/MCL-1 or BCL-XL/MCL-1. While we only assessed a small cohort of samples, the results corroborated previous studies, confirming that there are diverse anti-apoptotic dependencies in MM primary samples.[25]

In our study, we used patient-specific sensitivity measurements by carrying out *ex vivo* functional cell death measurements of primary MM samples treated with BH3 mimetics to determine the anti-apoptotic dependence. Our group has previously identified BCL-2 dependence in a patient with secondary plasma cell leukemia.[49] BH3 profiling can also be used to identify resistance mechanisms to Ven, including switching of anti-apoptotic dependence.[50-53] An important study by Gupta *et al*. used *ex vivo* assessment of MM patients' samples to Ven as an indicator of PFS.[54] As we have also shown here, assays such as precision testing and BH3 profiling are dynamic and give a functional measurement of cellular response to treatment; as such, they can be useful for identifying sensitive patients *a priori*.

Collectively, our data identify 5-Aza and Ven as an effective therapeutic combination for the treatment of MM, including in patients with non-t(11;14) disease with high-risk cytogenetics, such as 17p and 1q amplification. This combination has the potential to be effective across a broad patient population and should be considered in a steroid-free regimen for assessment in a clinical trial setting.

**Disclosures**

*TNC received research funding from AbbVie. JQ has received Honoraria from Janssen and Takeda, and travel funding from Roche. MOD has no competing interest but has employment and equity in ONK Therapeutics. All the other authors have no conflicts of interest to disclose.*

**Contributions**

*TNC and SGl designed the study and co-wrote the paper. LF performed experiments, analyzed data and co-wrote the manuscript. AC, NC, AR, YW, SGi, ID, CC, JR, EM, SGl and SP performed experiments and analyzed data. MOD, JQ, PM and SF designed experiments, and all authors read and edited the manuscript.*

**Funding**

*The authors would like to thank the following funding agencies for their support: the Leukemia Research Foundation (LRF-RCSI-2020) and Science Foundation Ireland (19/FFP/6461) (to TNC) and Irish Research Council (IRCLA/2022/2822) Breakthrough Cancer Research (BCR-RCSI-2020) (to LF).*

**Data-sharing statement**

*All data will be made freely available to any researcher wishing to use it for non-commercial purposes.*

# References

1. Cancer Factsheet Multiple Myeloma (ICD-10 C90) NCRI. https://www.ncri.ie/factsheets Accessed June 28, 2023.
2. Rajkumar SV. Multiple myeloma: 2020 update on diagnosis, risk-stratification and management. Am J Hematol. 2020;95(5):548-567.
3. Landgren O, Weiss BM. Patterns of monoclonal gammopathy of undetermined significance and multiple myeloma in various ethnic / racial groups: support for genetic factors in pathogenesis. Leukemia. 2009;23(10):1691-1697.
4. Moreau P, Attal M, Facon T. Frontline therapy of multiple myeloma. Blood. 2015;125(20):3076-3084.
5. Maji S, Panda S, Samal SK, et al. Bcl-2 antiapoptotic family proteins and chemoresistance in cancer. Adv Cancer Res. 2018;137:37-75.
6. Ni Chonghaile T, Letai A. Mimicking the BH3 domain to kill cancer cells. Oncogene. 2008;27(Suppl 1):S149-S157.
7. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer. 2002;2(9):647-656.
8. Wuilleme-Toumi S, Robillard N, Gomez P, et al. Mcl-1 is overexpressed in multiple myeloma and associated with relapse and shorter survival. Leukemia. 2005;19(7):1248-1252.
9. Thomas RL, Roberts DJ, Kubli DA, et al. Loss of MCL-1 leads to impaired autophagy and rapid development of heart failure. Genes Dev. 2013;27(12):1365-1377.
10. Wang X, Bathina M, Lynch J, et al. Deletion of MCL-1 causes lethal cardiac failure and mitochondrial dysfunction. Genes Dev. 2013;27(12):1351-1364.
11. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. N Engl J Med. 2016;374(4):311-322.
12. DiNardo CD, Stein EM, de Botton S, et al. Durable remissions with ivosidenib in IDH1-mutated relapsed or refractory AML. N Engl J Med. 2018;378(25):2386-2398.
13. DiNardo CD, Pratz K, Pullarkat V, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. Blood. 2019;133(1):7-17.
14. Bodet L, Gomez-Bougie P, Touzeau C, et al. ABT-737 is highly effective against molecular subgroups of multiple myeloma. Blood. 2011;118(14):3901-3910.
15. Touzeau C, Dousset C, Le Gouill S, et al. The Bcl-2 specific BH3 mimetic ABT-199: a promising targeted therapy for t(11;14) multiple myeloma. Leukemia. 2014;28(1):210-212.
16. Kumar S, Kaufman JL, Gasparetto C, et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t(11;14) multiple myeloma. Blood. 2017;130(22):2401-2409.
17. Kumar S, Harrison SJ, Cavo M, et al. Final overall survival results from BELLINI, a phase 3 study of venetoclax or placebo in combination with bortezomib and dexamethasone in relapsed/refractory multiple myeloma. Blood. 2021;138(Suppl 1):84.
18. Bal S, Kumar SK, Fonseca R, et al. Multiple myeloma with t(11;14): unique biology and evolving landscape. Am J Cancer Res. 2022;12(7):2950-2965.
19. Stresemann C, Lyko F. Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. Int J Cancer. 2008;123(1):8-13.
20. DiNardo CD, Pratz KW, Letai A, et al. Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. Lancet Oncol. 2018;19(2):216-228.
21. Benito JM, Godfrey L, Kojima K, et al. MLL-rearranged acute lymphoblastic leukemias activate BCL-2 through H3K79 methylation and are sensitive to the BCL-2-specific antagonist ABT-199. Cell Rep. 2015;13(12):2715-2727.
22. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med. 2011;364(11):1046-1060.
23. Caenepeel S, Brown SP, Belmontes B, et al. AMG 176, a selective MCL1 inhibitor, is effective in hematologic cancer models alone and in combination with established therapies. Cancer Discov. 2018;8(12):1582-1597.
24. Foight GW, Ryan JA, Gulla SV, Letai A, Keating AE. Designed BH3 peptides with high affinity and specificity for targeting Mcl-1 in cells. ACS Chem Biol. 2014;9(9):1962-1968.
25. Touzeau C, Ryan J, Guerriero J, et al. BH3 profiling identifies heterogeneous dependency on Bcl-2 family members in multiple myeloma and predicts sensitivity to BH3 mimetics. Leukemia. 2016;30(3):761-764.
26. Tse C, Shoemaker AR, Adickes J, et al. ABT-263: a potent and

orally bioavailable Bcl-2 family inhibitor. Cancer Res. 2008;68(9):3421-3428.
27. Chonghaile TN, Roderick JE, Glenfield C, et al. Maturation stage of T-cell acute lymphoblastic leukemia determines BCL-2 versus BCL-XL dependence and sensitivity to ABT-199. Cancer Discov. 2014;4(9):1074-1087.
28. Liu T, Stephan T, Chen P, et al. Multi-color live-cell STED nanoscopy of mitochondria with a gentle inner membrane stain. Proc Natl Acad Sci U S A. 2022;119(52):e2215799119.
29. Vincent T, Mechti N. Extracellular matrix in bone marrow can mediate drug resistance in myeloma. Leuk Lymphoma. 2005;46(6):803-811.
30. Jin S, Cojocari D, Purkal JJ, et al. 5-azacitidine induces NOXA to prime AML cells for venetoclax-mediated apoptosis. Clin Cancer Res. 2020;26(13):3371-3383.
31. Sidrauski C, Acosta-Alvear D, Khoutorsky A, et al. Pharmacological brake-release of mRNA translation enhances cognitive memory. Elife. 2013;2:e00498.
32. Chiappinelli KB, Strissel PL, Desrichard A, et al. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. Cell. 2015;162(5):974-986.
33. Shah UA, Mailankody S. Emerging immunotherapies in multiple myeloma. BMJ. 2020;370:m3176.
34. Khaw SL, Merino D, Anderson MA, et al. Both leukaemic and normal peripheral B lymphoid cells are highly sensitive to the selective pharmacological inhibition of prosurvival Bcl-2 with ABT-199. Leukemia. 2014;28(6):1207-1215.
35. Ludwig LM, Hawley KM, Banks DB, et al. Venetoclax imparts distinct cell death sensitivity and adaptivity patterns in T cells. Cell Death Dis. 2021;12(11):1005.
36. DiNardo CD, Rausch CR, Benton C, et al. Clinical experience with the BCL2-inhibitor venetoclax in combination therapy for relapsed and refractory acute myeloid leukemia and related myeloid malignancies. Am J Hematol. 2018;93(3):401-407.
37. Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. Nat Rev Drug Discov. 2006;5(1):37-50.
38. Roulois D, Loo Yau H, Singhania R, et al. DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. Cell. 2015;162(5):961-973.
39. Patel JR, Jain A, Chou YY, et al. ATPase-driven oligomerization of RIG-I on RNA allows optimal activation of type-I interferon. EMBO Rep. 2013;14(9):780-787.
40. Rajkumar SV, Kumar S. Multiple myeloma current treatment algorithms. Blood Cancer J. 2020;10(9):94.
41. Matulis SM, Gupta VA, Nooka AK, et al. Dexamethasone treatment promotes Bcl-2 dependence in multiple myeloma resulting in sensitivity to venetoclax. Leukemia. 2016;30(5):1086-1093.
42. Matulis SM, Gupta VA, Neri P, et al. Functional profiling of venetoclax sensitivity can predict clinical response in multiple myeloma. Leukemia. 2019;33(5):1291-1296.
43. AbbVie presents results from phase 3 CANOVA study of venetoclax in patients with relapsed or refractory multiple myeloma. News release. AbbVie. September 29. https://news.abbvie.com/2023-09-29-AbbVie-Presents-Results-from-Phase-3-CANOVA-Study-of-Venetoclax-in-Patients-with-Relapsed-or-Refractory-Multiple-Myeloma Accessed December 3, 2023.
44. Veis DJ, Sentman CL, Bach EA, Korsmeyer SJ. Expression of the Bcl-2 protein in murine and human thymocytes and in peripheral T lymphocytes. J Immunol. 1993;151(5):2546-2554.
45. Rohner L, Reinhart R, Iype J, et al. Impact of BH3-mimetics on human and mouse blood leukocytes: a comparative study. Sci Rep. 2020;10(1):222.
46. Rajkumar SV, Jacobus S, Callander NS, et al. Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial. Lancet Oncol. 2010;11(1):29-37.
47. Manier S, Corre J, Hulin C, et al. A dexamethasone-sparing regimen with daratumumab and lenalidomide in frail patients with newly-diagnosed multiple myeloma: efficacy and safety analysis of the phase 3 IFM2017-03 trial. Blood. 2022;140(Suppl 1):1369-1370.
48. Gomez-Bougie P, Maiga S, Tessoulin B, et al. BH3-mimetic toolkit guides the respective use of BCL2 and MCL1 BH3-mimetics in myeloma treatment. Blood. 2018;132(25):2656-2669.
49. Glavey SV, Flanagan L, Bleach R, et al. Secondary plasma cell leukaemia treated with single agent venetoclax. Br J Haematol. 2020;190(4):e242-e245.
50. Di Grande A, Peirs S, Donovan PD, et al. The spleen as a sanctuary site for residual leukemic cells following ABT-199 monotherapy in ETP-ALL. Blood Adv. 2021;5(7):1963-1976.
51. Sullivan GP, Flanagan L, Rodrigues DA, Ni Chonghaile T. The path to venetoclax resistance is paved with mutations, metabolism, and more. Sci Transl Med. 2022;14(674):eabo6891.
52. Ni Chonghaile T, Sarosiek KA, Vo TT, et al. Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. Science. 2011;334(6059):1129-1133.
53. Bhatt S, Pioso MS, Olesinski EA, et al. Reduced mitochondrial apoptotic priming drives resistance to BH3 mimetics in acute myeloid leukemia. Cancer Cell. 2020;38(6):872-890.e6.
54. Gupta VA, Matulis SM, Barwick BG, et al. Venetoclax ex vivo functional profiling predicts improved progression-free survival. Blood Cancer J. 2022;12(8):115.