

BH3 mimetics synergize with the Wee1 inhibitor ZN-c3 by activating caspases which induce DNA damage and degrade key proteins

H. Izadi, N. Ibrahim, T. Hoang, J. Ma, P.R. De Jong, J. Pinchman, K.D. Bunker, A.A. Samatar, F. Doñate

Zentalis Pharmaceuticals, San Diego, CA, USA

INTRODUCTION

- Wee1 is a crucial cell cycle kinase that regulates the G2/M checkpoint in response to DNA damage, and its inhibition can cause mitotic catastrophe and apoptosis in tumor cells.¹
- Wee1 blockade results in inhibition of phosphorylation of cyclin-dependent kinase (CDK) 1/2 and induces the degradation of ribonucleotide reductase M2 (RRM2).²
- RRM2 is involved in synthesis of deoxyribonucleotide triphosphate dNTPs,³ and blockade of RRM2 has been shown to have synergistic anti-tumor activity with Wee1 inhibition.^{2,4} Inhibitors of BCL-2 have been shown to also induce apoptosis.⁵
- ZN-d5 and ZN-c3, currently in clinical development for the treatment of cancer, are highly selective and potent inhibitors of BCL-2 and Wee1, respectively.⁶⁻⁸
- This study provides results regarding the mechanisms of action of ZN-d5 and ZN-c3.

RESULTS

Figure 1. ZN-d5 + ZN-c3 have additive or synergistic anti-proliferative activity in several tumor cell lines

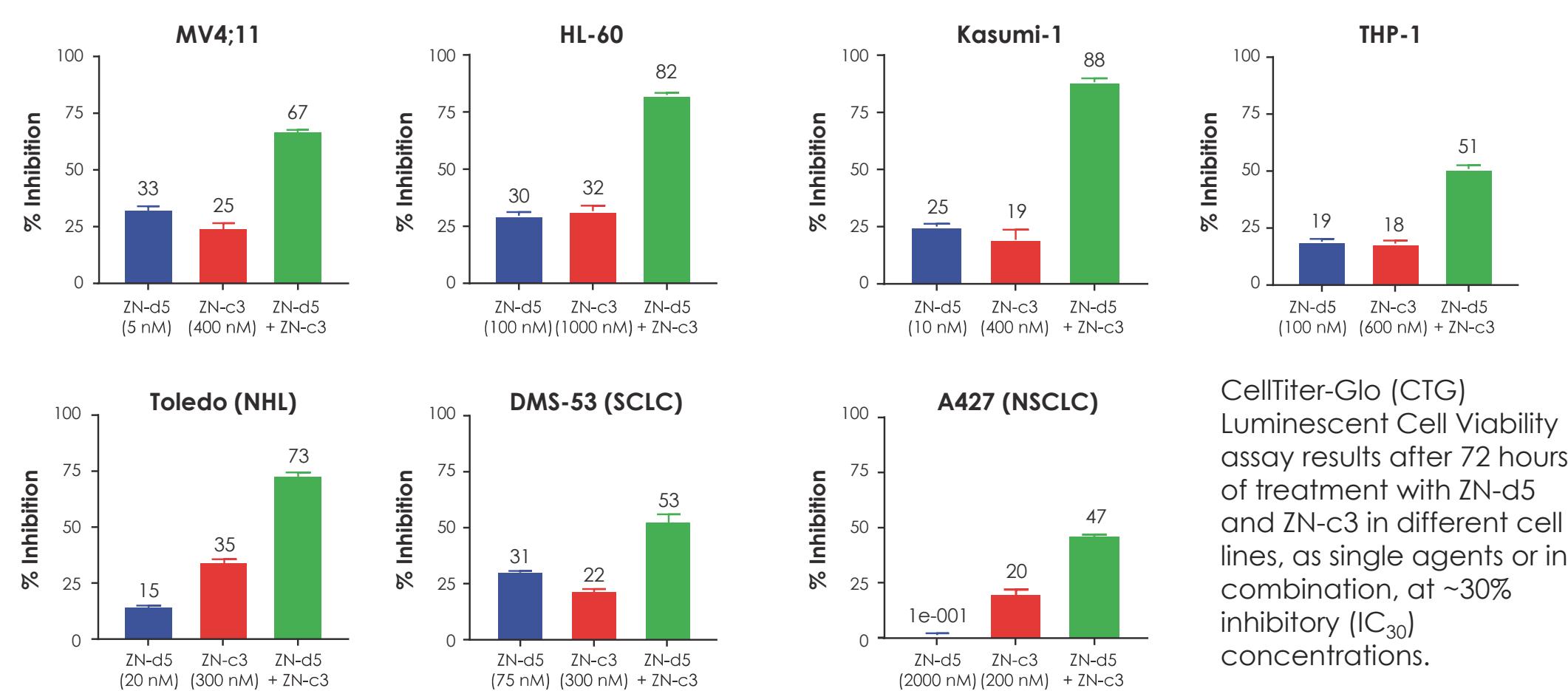
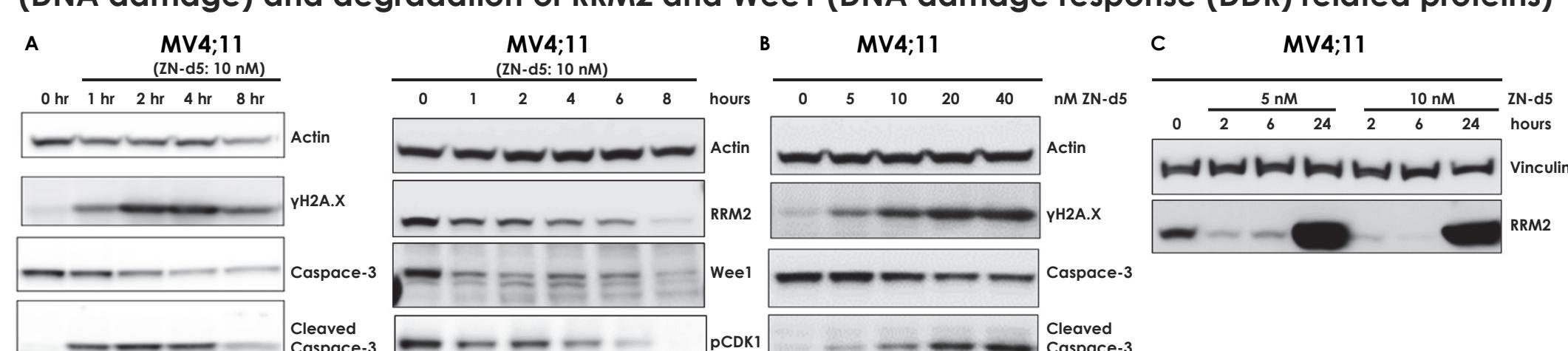
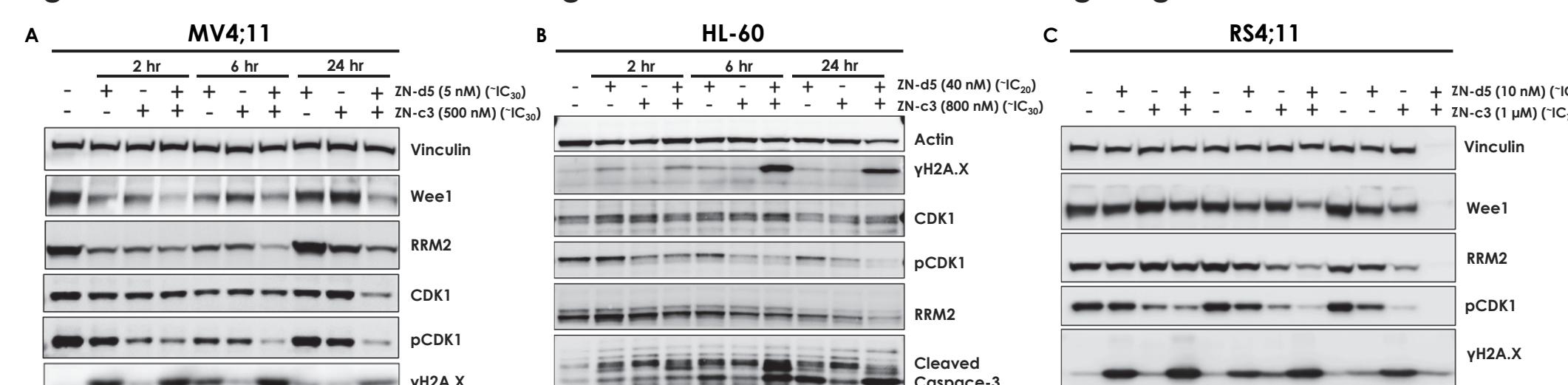


Figure 2. Treatment of MV4;11 acute myeloid leukemia cells with ZN-d5 resulted in induction of γH2AX (DNA damage) and degradation of RRM2 and Wee1 (DNA damage response (DDR) related proteins)



Western blot analysis of MV4;11 cells treated with ZN-d5. (A) shows that ZN-d5 treatment at 10 nM ($\sim IC_{40}$) induced an increase in DNA damage ($\gamma H2AX$), increase in cleaved caspase 3, and degradation of Wee1 and RRM2. Treatment also decreased levels of pCDK1 in agreement with the degradation of Wee1. (B) shows dose dependent increase in $\gamma H2AX$ and caspase activity at 4 hours. (C) shows that treatment with ZN-d5 at 5 nM or 10 nM ($\sim IC_{30}$ and $\sim IC_{40}$) decreased the levels of RRM2 at 2 and 6 h, which recovered at 24 h suggesting reversibility. The results suggest that treatment with ZN-d5 at subtherapeutic doses causes increases in DNA damage and degradation of DDR related proteins in a reversible manner. These effects may be mediated by caspase activation and may explain the synergistic effects when combined with ZN-c3 shown in Figure 1.

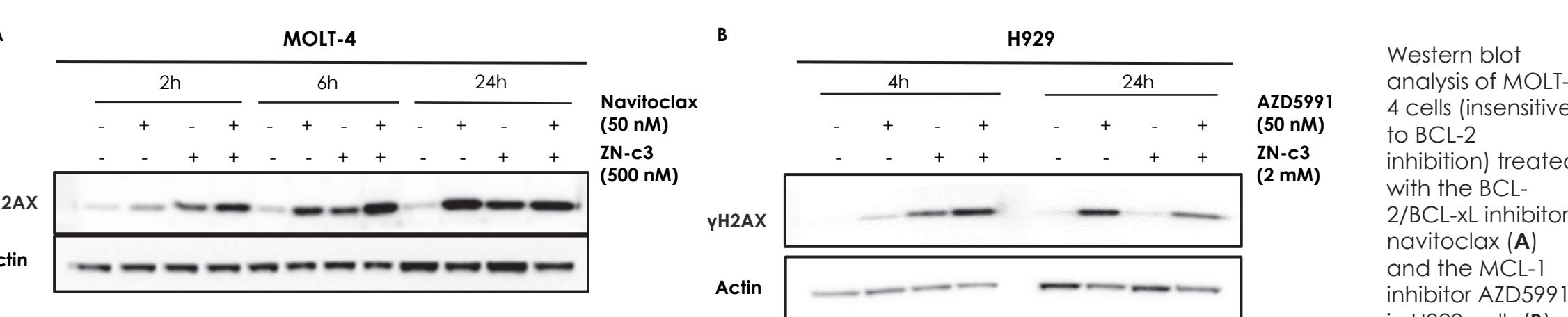
Figure 3. ZN-d5 + ZN-c3 results in larger effects on tumor cells than single agent treatment



Western blot analysis of (A) MV4;11, (B) HL-60, and (C) RS4;11 cells treated with ZN-d5, ZN-c3 and the combination. The ZN-d5 concentrations used are subtherapeutic (IC_{30} , IC_{20} and IC_{60} , respectively). ZN-c3 treatment leads to modest increases in DNA damage, reductions in pCDK1, Wee1 and RRM2 protein and increases in apoptotic markers similar to what is seen for ZN-d5 alone (Figures 2 and 3). The effect for the combination is larger than single agent alone for all markers. The effects for ZN-d5 alone seem to decrease at the 24-hour time point suggesting reversibility.

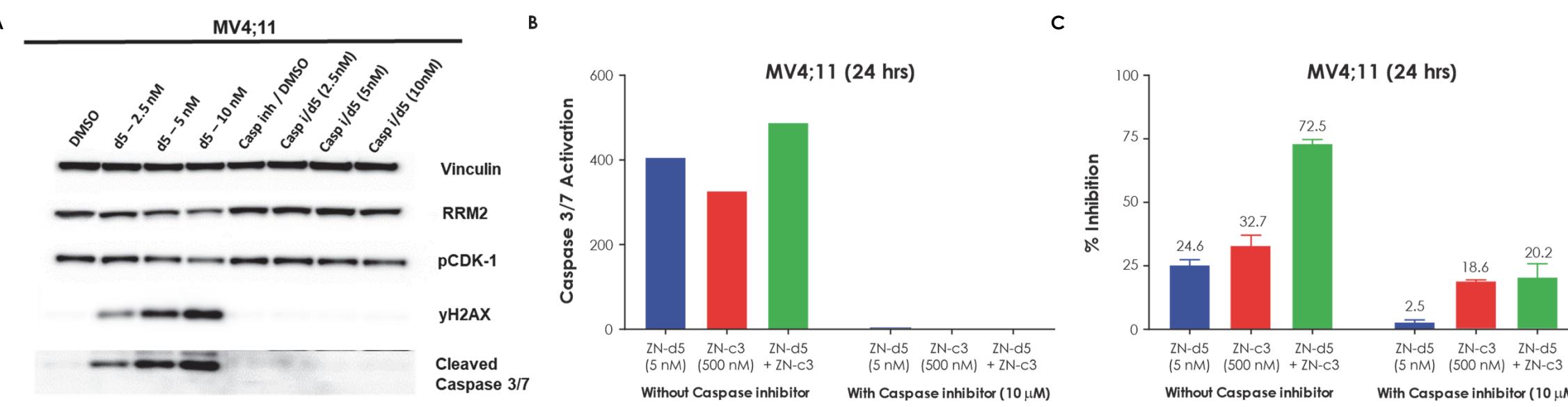
RESULTS

Figure 4. Other BH3 mimetics also induce γH2AX in tumor cells similar to ZN-d5



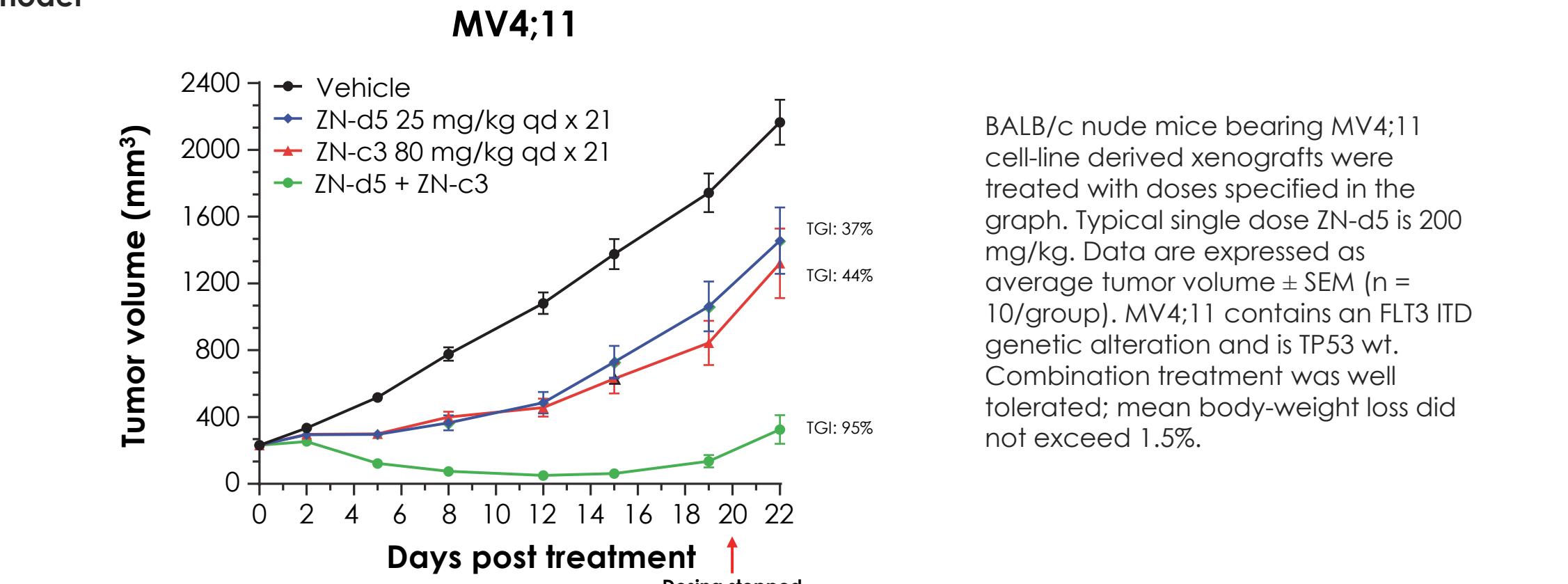
Western blot analysis of MOLT-4 cells (insensitive to BCL-2 inhibition) treated with the BCL-2/BCL-XL inhibitor navitoclax (A) and the MCL-1 inhibitor AZD5991 in H929 cells (B).

Figure 5. Effects of ZN-d5 and anti-tumor combination effect of ZN-d5 + ZN-c3 in MV4;11 cells are mainly mediated by caspase activation



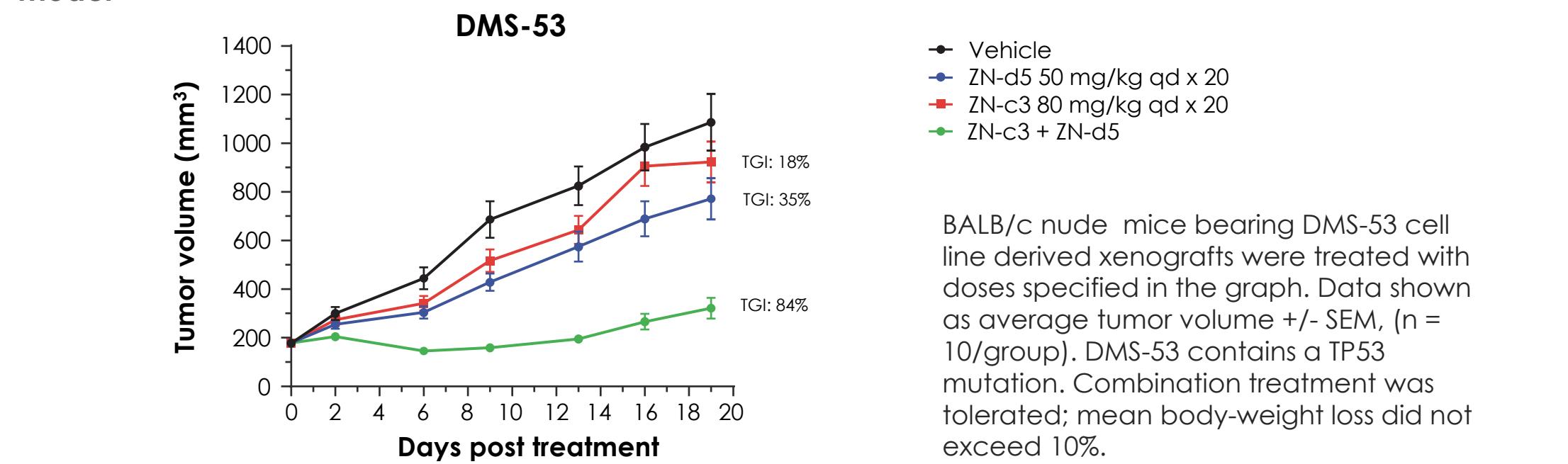
(A) Western blot analysis of MV4;11 cells after 6 hours of treatment with sublethal doses of ZN-d5 (IC_{15} to IC_{40}) with and without the pan-caspase-inhibitor (Q-VD-OPh hydrate). ZN-d5 modestly decreased levels of RRM2 and of pCDK1, increased DNA damage, and cleaved caspase 3 levels. These effects were all abrogated by pre-treatment with a caspase inhibitor. (B) and (C) treatment of MV4;11 cells. CTG and caspase activity were assessed in the same well. Caspase inhibition was maximal (B) and led to abrogation of the anti-tumor activity of ZN-d5, partial inhibition of the ZN-c3 effect, and total abrogation of the anti-tumor combination effect of ZN-d5 and/or ZN-c3 (% inhibition of ZN-d5 + ZN-c3 = % inhibition of ZN-d5 plus % inhibition of ZN-c3) (C). These results suggest that ZN-d5 at subtherapeutic doses is activating caspases and that synergistic anti-tumor activity is primarily mediated by caspase activation.

Figure 6. ZN-d5 + ZN-c3 had synergistic anti-tumor activity in the acute myeloid leukemia MV4;11 xenograft model



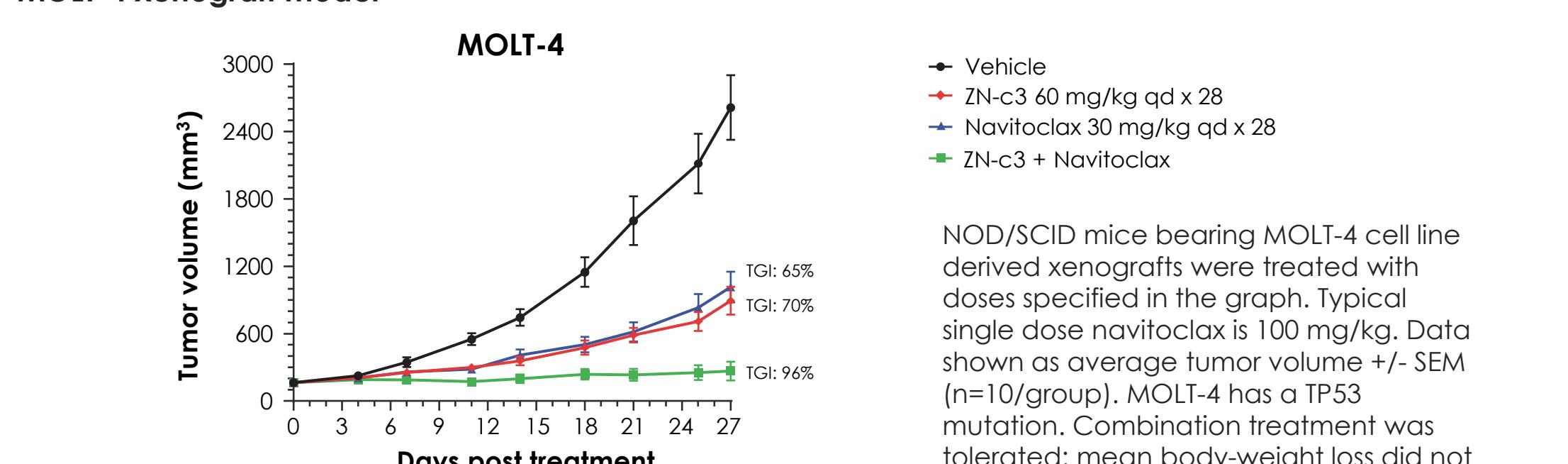
BALB/c nude mice bearing MV4;11 cell-line derived xenografts were treated with doses specified in the graph. Typical single dose ZN-d5 is 200 mg/kg. Data are expressed as average tumor volume ± SEM (n = 10/group). MV4;11 contains an FLT3 ITD genetic alteration and is TP53 wt. Combination treatment was well tolerated; mean body-weight loss did not exceed 1.5%.

Figure 8. ZN-d5 + ZN-c3 had synergistic anti-tumor activity in the small cell lung cancer DMS-53 xenograft model



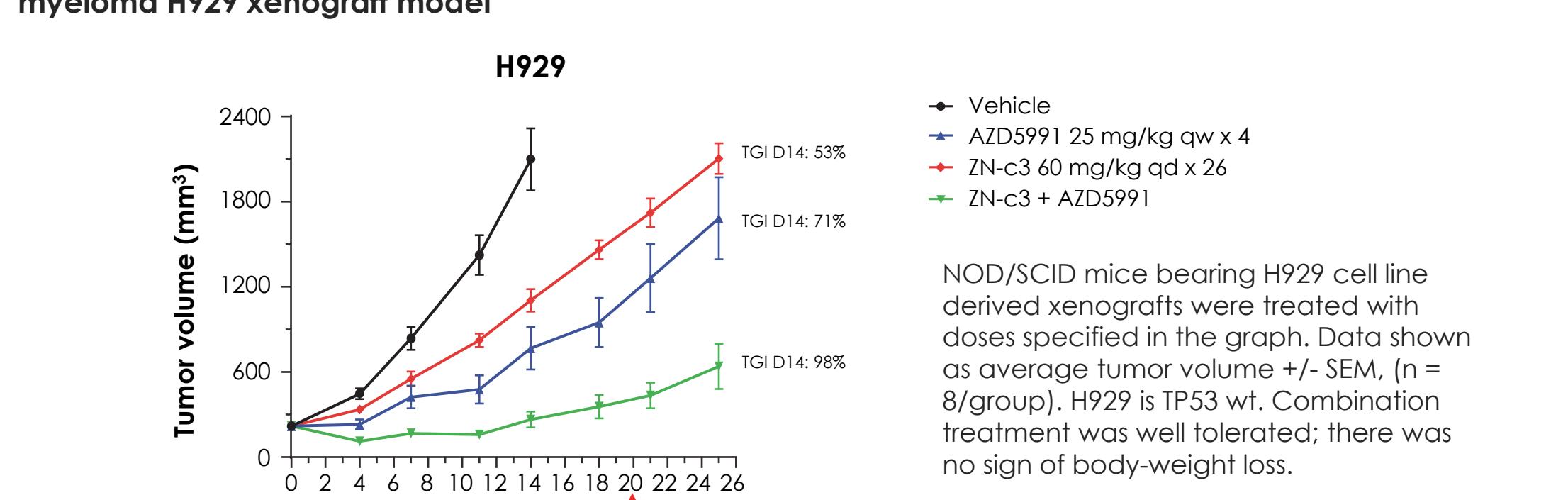
BALB/c nude mice bearing DMS-53 cell line derived xenografts were treated with doses specified in the graph. Data shown as average tumor volume +/- SEM, (n = 10/group). DMS-53 contains a TP53 mutation. Combination treatment was tolerated; mean body-weight loss did not exceed 10%.

Figure 9. Navitoclax + ZN-c3 resulted in enhanced anti-tumor activity in the acute lymphoblastic leukemia MOLT-4 xenograft model



NOD/SCID mice bearing MOLT-4 cell line derived xenografts were treated with doses specified in the graph. Typical single dose navitoclax is 100 mg/kg. Data shown as average tumor volume +/- SEM (n = 10/group). MOLT-4 has a TP53 mutation. Combination treatment was tolerated; mean body-weight loss did not exceed 7%.

Figure 10. The MCL-1 inhibitor AZD5991 + ZN-c3 resulted in enhanced anti-tumor activity in the multiple myeloma H929 xenograft model



NOD/SCID mice bearing H929 cell line derived xenografts were treated with doses specified in the graph. Data shown as average tumor volume +/- SEM (n = 8/group). H929 is TP53 wt. Combination treatment was well tolerated; there was no sign of body-weight loss.

TGI, tumor growth inhibition; Regn, regression. Mean ± standard error n=10 per group

- ZN-d5 at subtherapeutic doses ($\sim IC_{30}$) activates caspases in a reversible manner leading to DNA damage and degradation of both Wee1 and RRM2 proteins in tumor cells
- ZN-d5 + ZN-c3 increases levels of DNA damage, degradation of RRM2 and Wee1, and apoptosis than single agent alone
- Inhibition of other anti-apoptotic proteins such as BCL-XL or MCL-1 also synergizes with ZN-c3 in several tumor models
- The enhanced anti-tumor activity of BH3 mimetics (anti-apoptotic protein inhibitors) plus ZN-c3 is seen in models of AML, NHL, SCLC, NSCLC, ALL and MM supporting potential clinical trials in multiple indications

Figure 7. ZN-d5 + ZN-c3 had enhanced anti-tumor activity vs. single agents in the non-Hodgkin's lymphoma Toledo xenograft model

