Abstract #2591

Combination of the BCL-2 inhibitor ZN-d5 with the WEE1 inhibitor ZN-c3 shows additive or synergistic anti-tumor activity in acute myeloid leukemia (AML) models

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INTRODUCTION

- The BCL-2 inhibitor venetoclax is approved in combination with hypomethylating agents, such as azacitidine, for the treatment of newly diagnosed elderly patients with acute myeloid leukemia (AML).[1]
- However, relapse eventually occurs in most patients, especially those with *TP53* mutations who have poor prognosis.[2,3]
- Wee1 is a crucial cell cycle checkpoint kinase that regulates the G2/M checkpoint in response to DNA damage, and its inhibition can cause mitotic catastrophe and apoptosis.[4,5]
- ZN-d5 and ZN-c3 are highly selective and potent inhibitors of Bcl-2 and Wee1, respectively, in clinical development by Zentalis.[4-6]
- This study evaluated the activity of ZN-d5 + ZN-c3 in preclinical models of AML.

MATERIALS AND METHODS

ZN-d5 and ZN-c3 in AML cell lines (Figure 1)

- In vitro CellTiter Glo (CTG assays) were carried out to evaluate ZN-d5 and ZN-c3 as single agents and in combination at 30% inhibitory concentrations (IC_{30}) concentrations in different AML cell lines.

ZN-d5 or venetoclax in combination with ZN-c3 (Figure 2)

- BALB/c nude mice bearing HL-60 cell-line derived xenografts were treated orally as described.

ZN-d5 in combination with ZN-c3 in an MV4;11 AML xenograft model (Figure 3)

- BALB/c nude mice bearing MV4;11 cell line-derived xenografts were treated orally as described.

ZN-d5 with ZN-c3 and azacitidine at low doses in the HL-60 in vivo model (Figure 4)

- BALB/c nude mice bearing HL-60 cell line-derived xenografts were treated orally as described. Lower doses were used in this study to observe optimal triple-combination efficacy.

ZN-d5 or ZN-c3 in patient-derived AML samples (Tables 1-5)

- AML blasts from 29 subjects were treated with ZN-d5, ZN-c3, or the combination for 6 days, and cell viability was assessed with the CTG assay. Single-agent dose titration (3-fold) was done for each model, and 4 doses for each compound were selected for a matrix combination assay. Synergistic activity was defined as % inhibition with the combination that exceed the sum of the % inhibition when each drug was delivered alone. Additive activity was defined as equivalence to the % inhibition of the combination vs the sum of the % inhibition for each drug delivered alone. Additive(†) activity was defined as % inhibition of the combination that was >10 points higher than the highest % inhibition of any single agent. Inconclusive activity was defined as a single-agent activity too high to determine any combination effect.

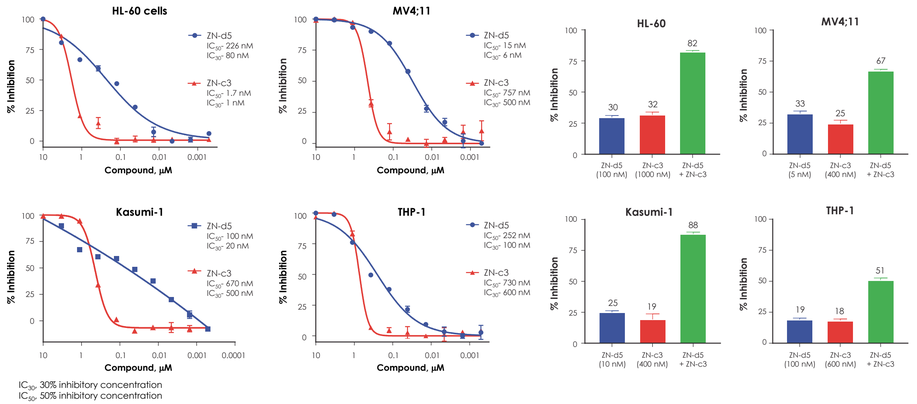
In vivo patient-derived xenograft (PDX) model (Figure 5)

- This PDX model contained 11 relevant mutations including *TP53, IDH1, RUNX1, STAG2, TET2,* and *ASXL1*. The patient received 9 lines of therapy but was not treated with venetoclax-based combinations. Sub-lethally irradiated NOG-EXL mice were inoculated with 1 x 10^6 AML blasts. Treatment started when satellite animals showed ≥ 20% bone marrow engraftment. The PDX model was treated orally with vehicle, ZN-d5 (200 mg/kg/day), ZN-c3 (80 mg/kg/day), or the combination of ZN-d5 with ZN-c3 at the same doses for 17 days.

REFERENCES

1. Venetoclax PI. 2021. https://www.rxabbvie.com/pdf/venclexta.pdf; **2.** DiNardo CD, et al. *N Engl J Med*. 2020;383(7):617-629; **3.** Saliba AN, et al. *Cancer Drug Resist*. 2021;4:125-142; 4. Osman AA, et al. *Mol Cancer Ther*. 2015;14(2):608-619; **4.** Li J, et al. *Cancer Res*. 2021;61 (13 Suppl):1965; **5.** Tolcher A, et al. *Cancer Res.* 2021;61(13 Suppl):CT016; **6.** Zaucha JM, et al. *Ann Oncol.* 2021;32(suppl_5): S583-S620.

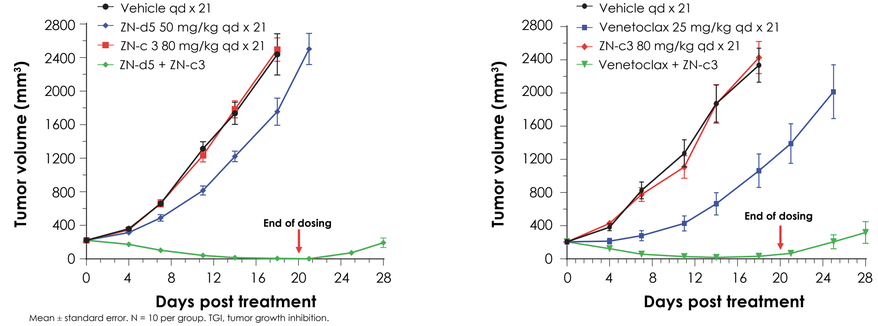
Figure 1. In vitro combination of ZN-d5 and ZN-c3 in various AML cell lines resulted in synergistic activity



IC_{30}: 30% inhibitory concentration
IC_{50}: 50% inhibitory concentration

- The combination of ZN-d5 and ZN-c5 exhibited synergistic activity in AML cell lines (**Figure 1**).

Figure 2. ZN-d5 + venetoclax (A) or ZN-c3 + venetoclax (B) in an HL-60 AML Xenograft model



Mean ± standard error, N = 10 per group, TGI, tumor growth inhibition.

- ZN-d5 or venetoclax in combination with ZN-c3 had highly synergistic anti-tumor activity in an HL-60 AML xenograft model (**Figure 2**).

Figure 3. ZN-d5 + ZN-c3 in an MV4;11 AML model

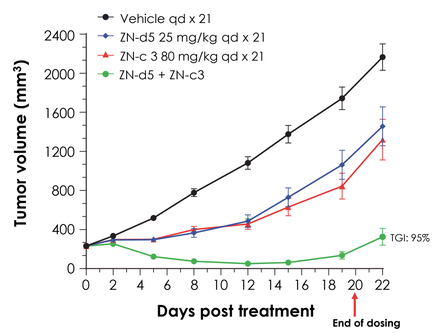
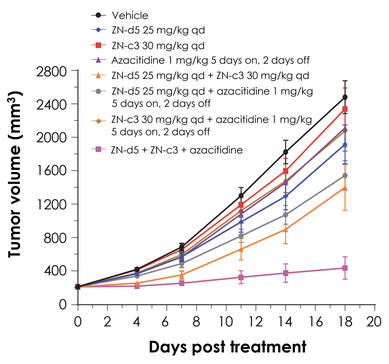


Figure 4. ZN-d5 + ZN-c3 + azacitidine in an HL-60 AML model



Mean ± standard error, N = 10 per group, TGI, tumor growth inhibition.

- ZN-d5 + ZN-c3 is highly synergistic in an MV4;11 AML xenograft model (**Figure 3**).
- The triple combination of ZN-d5, ZN-c3, and azacitidine at low doses in the HL-60 in vivo model resulted in synergistic anti-tumor efficacy compared with single- and double-agent treatments (**Figure 4**).
- Only minor reductions in body weight (<10%) were observed in the mice treated in the in vivo studies.

RESULTS

Table 1. ZN-d5 or ZN-c3 in 29 patient-derived AML samples

ZN-d5, IC_{50} (nM)	Number of Samples
0-50	13
51-200	4
201-1000	12
ZN-c3, IC_{50} (nM)	**Number of Samples**
0-150	15
151-450	8
451-1000	6

IC_{50}, 50% inhibitory concentration.

- Treatment with ZN-d5 or ZN-c3 showed significant activity in patient-derived AML samples. In 17 of 29 samples, the IC_{50} for ZN-d5 was < 200 nM and in 23 of 29 samples the IC_{50} for ZN-c3 was < 450 nM (**Table 1**).

Table 3. Combination activity in AML samples according to mutation status

	Synergistic*	Additive	Additive (†)	Inconclusive
Number of models	12	3	11	3
***TP53* Mutation**	6	1	6	3
FLT3-ITD	4	2	4	1
NPM	1	0	7	0

*†See materials and methods for definition.
Some models had multiple mutations.

- ZN-d5 + ZN-c3 showed anti-tumor activity independent of mutation status (**Table 3**).

Table 2. ZN-d5 + ZN-c3 resulted in additive or synergistic activity in AML samples

ZN-d5+ZN-c3 (% inhibition)	Synergistic*	Additive	Additive (†)	Inconclusive	Total
0-50	2	0	0	0	2
50-80	6	0	0	0	6
80-100	4	3	11	3	21

*, † See materials and methods for definition.

- The combination of ZN-d5 and ZN-c3 in vitro resulted in higher anti-tumor activity than single-agent treatment in all the AML samples, with anti-tumor activity of 80% or higher in 21 of the models (**Table 2**).

Table 4. Synergism was observed in 10 models insensitive to ZN-d5 as single agent (IC_{50} > 590 nM)

Model #	ZN-d5 (IC_{50}) nM	ZN-c3 (IC_{50}) nM	ZN-d5 (% inhibition)	ZN-c3 (% inhibition)	Combination (% inhibition)
CTG-2228	10000	427	3	60	66
CTG-2702	10000	10000	2	14	50
CTG-3440	10000	90	0	93	98.5
CTG-3660	10000	4000	10	27	47
CTG-2233	592	109	13	76	91
CTG-2457	10000	105	1	78	88.2
CTG-2704	2409	1730	22	28	56
CTG-2775	10000	542	7	55	66.2
CTG-3439	2389	450	0	51	70
CTG-3661	2347	88	30	44	78

- ZN-d5 + ZN-c3 had synergistic activity in patient samples not sensitive to ZN-d5 alone (**Table 4**).

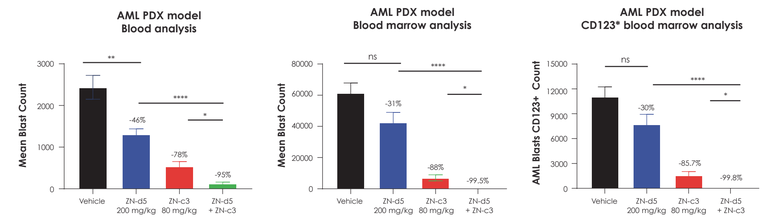
Table 5. ZN-d5 + ZN-c3 in samples from patients who progressed on venetoclax

	Clinical			In Vitro (ZN-d5+ZN-c3)	
Sample #	Blasts % (before treatment)	Post-Collection Treatment	Blasts % (after treatment)	ZN-d5/ZN-c3 Treatment (nM)	Blasts % (after treatment)
3930	93.4	Azacitidine/Venetoclax	Residual AML (33% blast; ~2 months post treatment)	120/500	**4.6**
3977	62.1	Azacitidine/Venetoclax	Residual AML (68% blast; ~2 months post treatment)	65/100	**0**
3978	41.1	Gilteritinib/Venetoclax	Residual AML (32% blast; ~1 month post treatment)	65/500	**3.6**

Samples taken prior to treatment.

- ZN-d5 + ZN-c3 was active in vitro in all 3 samples from patients who progressed on venetoclax (**Table 5**).
- In an in vivo PDX model, the ZN-d5/ ZN-c3 combination was significantly more effective than the single agents for inhibiting tumor growth; it also resulted in complete abrogation of AML blasts in bone marrow (**Figure 5**).

Figure 5. ZN-d5 + ZN-c3 in a TP53-mutated AML PDX model



Data shown as an average blast count from bone marrow analysis by flow cytometry (n = 10 per group; combination group n=6). Based on CD123+ cell expression, which is present on more than 98% of leukemic stem cells (LSC), the combination of ZN-d5 + ZN-c3 may abrogate the majority of LSC, *p = 0.0216, **p = 0.0021, ****p < 0.0001, ns, non-significant

CONCLUSIONS

- The combination of ZN-d5 and ZN-c3 was synergistic in AML xenograft models and highly active in an AML patient-derived, PDX model . The triple combination of ZN-d5, ZN-c3, and azacitidine resulted in significantly higher anti-tumor activity than single agents or doublets
- The in vitro combination of ZN-d5 and ZN-c3 was highly active in 29 samples derived from AML patients
  - Independently of *TP53* mutation status
  - In samples with insensitivity to BCL-2 inhibition
- The combination of ZN-d5 and ZN-c3 was highly active in vitro in 3 samples from patients with AML who later progressed on venetoclax-based therapies
- These results support testing the ZN-d5 and ZN-c3 combination or possibly the triple combination including azacitidine in AML patients independently of *TP53* status