Abstract #2606

ZN-c3, a potent and selective Wee1 inhibitor demonstrates anti-tumor activities in combination with other targeted therapies and overcomes PARP inhibitor resistance

J. Li, J. Ma, P.R. De Jong, H. Izadi, T. Hoang, N. Ibrahim, B. Boren, S.G. Hegde, F. Doñate, A. Samatar

Zentalis Pharmaceuticals, San Diego, CA and New York, NY, USA

INTRODUCTION

- About 78% of all breast cancers (BC) are estrogen receptor positive (ER+) and most of them are dependent on ER signaling.[1,2] About 19% of BC are human epidermal growth factor 2 positive (HER2+) and they tend to be aggressive and fast-growing and are independent of ER signaling.[1,3,4] Triple-negative BC (TNBC) accounts for 12% of all breast cancers it has high rates of recurrence and metastasis rates, and few therapeutic options.[5,6]
- Wee1 plays an essential role in the regulation of G2/M checkpoint and DNA damage response by phosphorylating cyclic-dependent kinase 1/2.[7]
- Abrogating the G2 checkpoint by inhibiting Wee 1 increases genomic instability by inducing replication stress in cancer cells with DNA damage and preferentially sensitizes them to undergo mitotic catastrophe and apoptosis.[7,8]
- We have developed and characterized ZN-c3, a potent and selective small molecule Wee 1 inhibitor with a differentiated kinase selectivity and pharmacokinetics profile.[8]
- This study assessed ZN-c3 activity as a single agent and in combination in multiple experimental efficacy models of BC including cell line- and patient-derived xenografts. We also determined whether ZN-c3 can overcome acquired resistance to poly (ADP-ribose) polymerase (PARP) inhibitors.

MATERIALS AND METHODS

Anti-tumor Activity in ER+ BC Models

ZN-c3 alone or in combination with ZN-c5

- BALB/c nude mice bearing HCC1428 tumors were orally dosed as shown in Fig 1. Animals were subcutaneously (SC) implanted with 17-β-estradiol tablets (0.18 mg, 90-day release) 2 days before cell implantation **(Figure 1)**.
- BALB/c nude mice bearing MCF-7 tumors were dosed orally once daily as shown in **Figures 2A and 3**. All animals also received estradiol benzoate injections SC twice a week.
- BALB/c nude mice bearing T47D tumors were dosed orally as shown in **Figure 2B**. Animals were implanted SC with 17-β-estradiol tablets as shown above.

ZN-c3 and palbociclib

- Growth inhibition in an MCF-7 tumor model treated with ZN-c3 or palbociclib alone and the combination of the two agent simultaneously **(Figure 3A)** or sequentially **(Figure 3B)**. All animals received estradiol benzoate injection SC twice a week.

Anti-tumor Activity in a HER2+ BC Model

- NOD-SCID mice bearing large JIMT-1 (trastuzumab resistant) tumors were dosed with ZN-c3 or trastuzumab alone and the combination of these two, as shown in **Figure 4**.

Anti-tumor Activity in TNBC Models

ZN-c3 in combination with niraparib

- Athymic Nude - Foxn1nu mice with HBCx-10 tumors were orally dosed with ZN-c3 or niraparib alone or the combination of the two agents, as shown in **Figure 5**.

ZN-c3 in the setting of PARP inhibitor resistance

- MDA-MB-436 PARP resistant cells were generated by culturing in the presence of escalating concentrations of niraparib or olaparib. Cell viability was measured by CellTiter-Glo assay.
- NOD-SCID mice bearing parental or olaparib/niraparib resistant MDA-MB-436 tumors were dosed orally as shown in **Figure 6**.

RESULTS

ER+ BC Models

Figure 1. ZN-c3 exhibits significant anti-tumor activity in ER+ BC xenografts (HCC1428 tumor model)

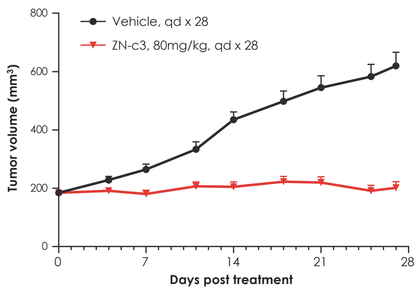
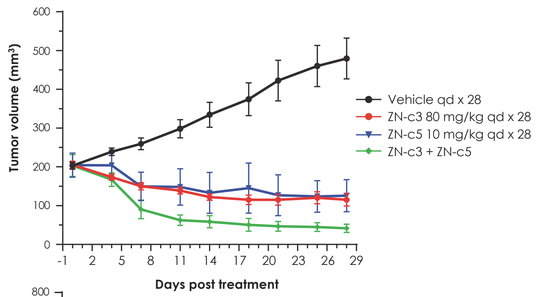
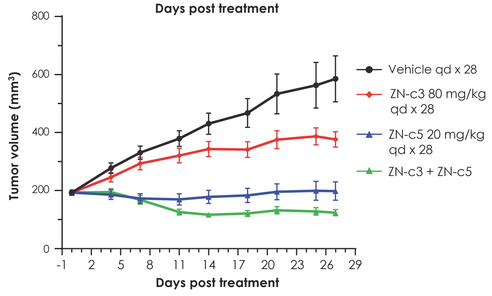


Figure 2. ZN-c3 is active as a single agent and in combination with ZN-c5 in ER+ BC xenografts: MCF-7 (2A) and T47D tumor models (2B)

2A

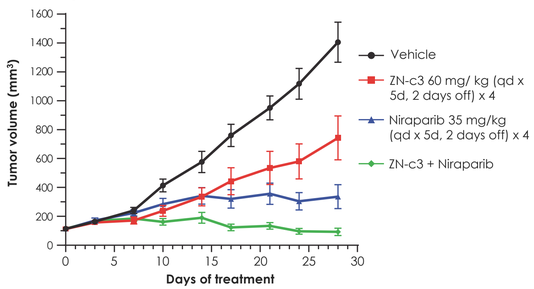


2B



TNBC Models

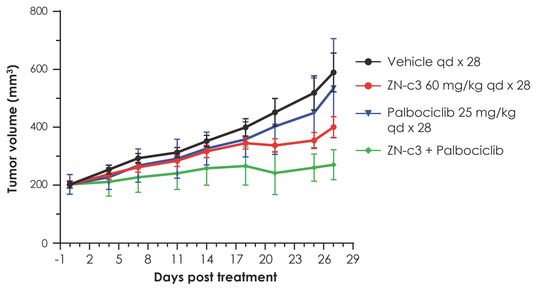
Figure 5. ZN-c3 in combination with niraparib induces tumor regression in a BRCA mutant, CCNE1$_{amp}$ TNBC patient-derived xenograft model (HBCx-10 model)



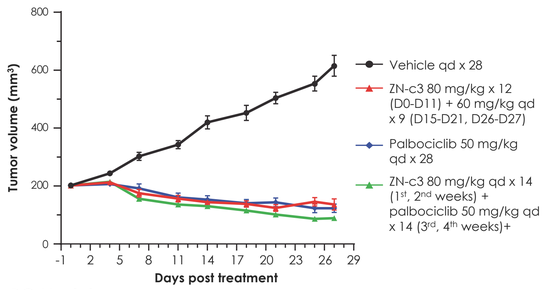
ER+ BC Models

Figure 3. ZN-c3 in combination with palbociclib shows enhanced activity against ER + BC xenografts (MCF-7 tumor model)

3A

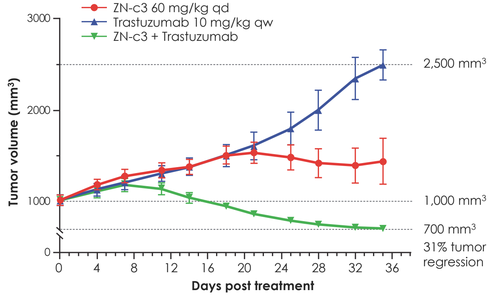


3B



HER2+ BC Model

Figure 4. ZN-c3 + trastuzumab produces regression in HER2+ trastuzumab-resistant tumors (JIMT-1 model)



Activity against PARP inhibitor-resistant MDA-MB-436 cells

Table 1. ZN-c3 is active against niraparib- and olaparib-resistant MDA-MB-436 cells

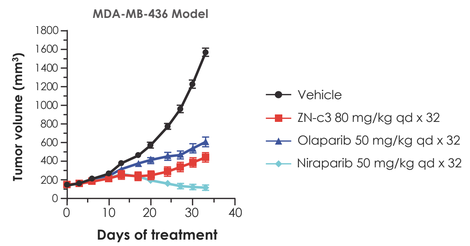
Compound	IC_{50}nM		
	MDA-MB-436 NR	MDA-MB-436 OR	MDA-MB-436
ZN-c3	184.6	174.5	282.2
Niraparib	8648.1	7731.2	102.7
Olaparib	>10000	>10000	94.9

MDA-MB-436 NR: niraparib-resistant cells; MDA-MB-436 OR: Olaparib-resistant cells.
IC_{50}: 50% inhibitory concentration

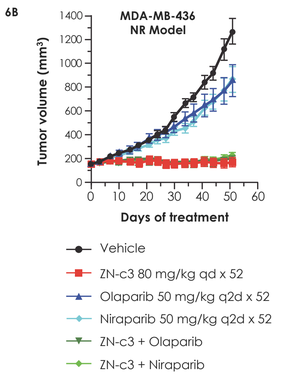
Activity of ZN-c3 against PARP inhibitor-resistant MDA-MB-436 tumors

Figure 6. ZN-c3 induces tumor regression in PARP inhibitor-resistant breast cancer efficacy model

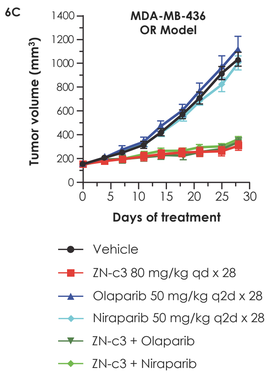
6A



6B



6C



CONCLUSIONS

- The data shown indicate that ZN-c3 is active in BC as single agent and its anti-tumor activity is enhanced in combination settings
- No significant body weight-loss was found in any combination treatment
- Moreover, ZN-c3 can overcome the acquired resistance to PARP inhibitors and trastuzumab
- Taken together, these findings support the clinical development of ZN-c3 in breast cancer
- ZN-c3 is currently undergoing Phase 1/2 clinical trials as a single agent and in combinations for several indications and has demonstrated promising clinical activity and good tolerability[9]

REFERENCES

1. NCI. 2022. https://seer.cancer.gov/statfacts/html/breast-subtypes.html; **2.** Chi D, et al. *Proc Natl Acad Sci* U S A. 2019;116(23):11437-11443; **3.** Lee SH, et al. *Medicine* (Baltimore). 2016;95(37): e4874; **4.** Ellis MJ, et al, *J Clin Oncol.* 2006;19:3019-3025; **5.** Howard FM, Olopade OI. *Cancer J.* 2021;27(1):8-16; **6.** Caparica R, et al. *ESMO Open.* 2019;4(Suppl 2):e000504; **7.** Osman AA, et al. *Mol Cancer Ther.* 2015;14(2):608-619; **8.** Li J, et al. *Cancer Res.* 2021;61(13 Suppl):1965; **9.** Tolcher A, et al. *Cancer Res.* 2021;61(13 Suppl):CT016.