#512 Identification of ZM-0088, a novel Polθ inhibitor that effectively inhibits tumors with homologous recombination deficiency *in vitro* and *in vivo*

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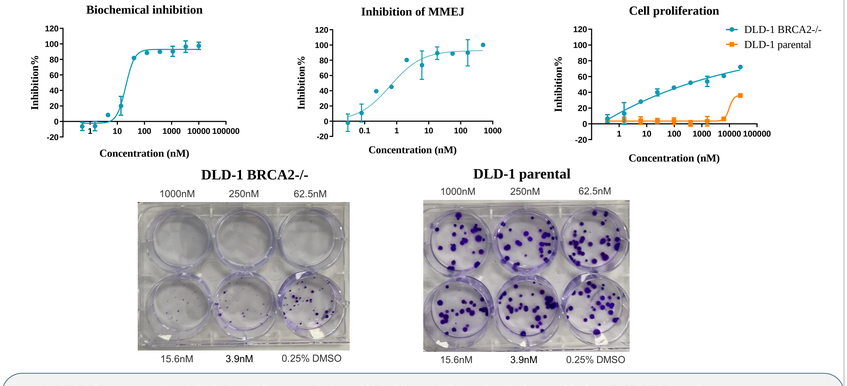
Introduction

- Homologous recombination (HR) is a high-fidelity, error-free DNA double-strand break (DSB) repair pathway, and the dysfunction of HR confers cell genome instability and leads to tumorigenesis.
- In the situation of HR deficiency, microhomology-mediated end joining (MMEJ), in which DNA polymerase theta (Polθ) plays an essential role, is up-regulated to serve as a backup pathway for DSB repair. The inhibition of Polθ may cause synthetic lethality with the deficiency of HR[1-2].
- ZM-0088 is a potent Polθ inhibitor that robustly inhibits MMEJ by blocking the activity of Polθ and elicits a synergetic effect in the combination with PARP inhibitors in HR deficient cells.

Methods

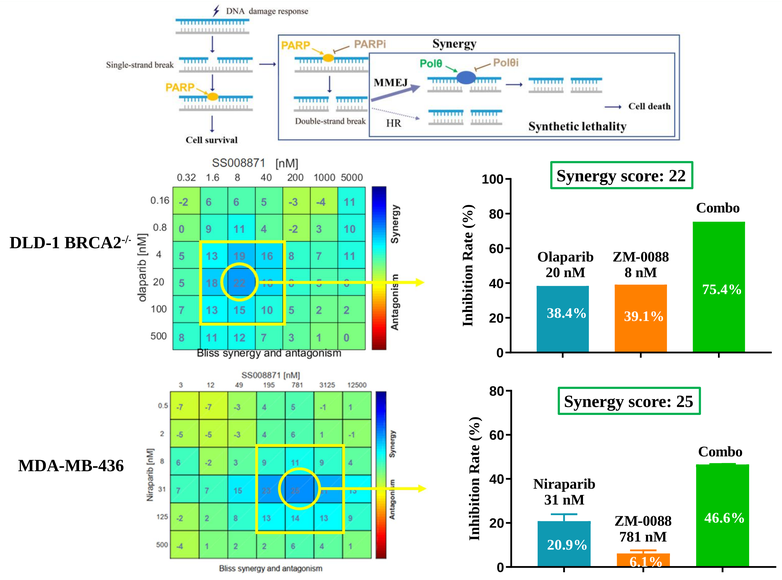
- The enzymatic inhibitory effect was determined by measuring the ATP turnover rate in a NADH oxidation-coupled enzymatic assay.
- The effect of compounds on cellular MMEJ was assessed by a NanoLuciferase reporter assay, in which a NanoLuciferase reporter substrate expresses a functional NanoLuciferase reporter protein only when TMEJ has been correctly performed. The effect on cell proliferation was measured after 7 days of treatment by CellTiter Glo (CTG) or after 14 days of treatment by colony formation assay.
- *In vivo* efficacy was assessed by using a DLD-1 BRCA2-/- xenograft model, in which female nu/nu mice were orally dosed with ZM-0088 twice daily and/or olaparib once daily. γ-H2AX positive cells (nuclei with ≥ 5 foci) were confirmed by immunofluorescence.
- *In vitro* hematotoxicity was evaluated in a CellTiter Glo assay, in which CD34+ mobilized peripheral blood mononuclear cells (PBMCs) were incubated with compounds for 14 days under different lineage-specific cytokine conditions [3]. *In vivo* hematotoxicity was assessed in a DLD-1 BRCA2-/- xenograft model, in which the blood was collected at 2h post last dosing.

Inhibition of Polθ activity



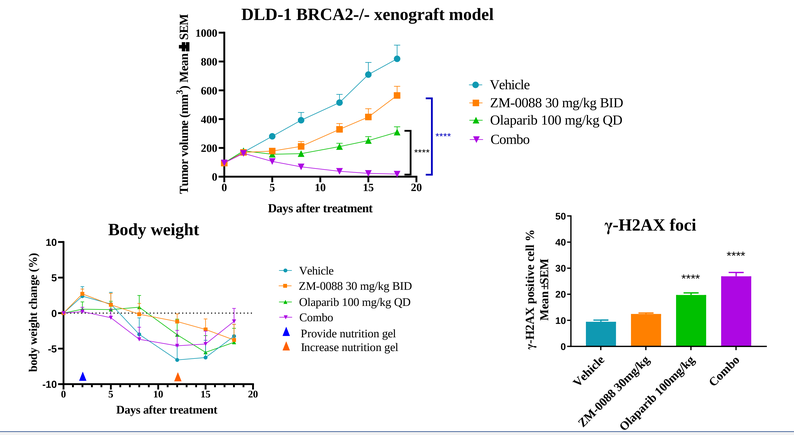
- ZM-0088 potently inhibited the activity of Polθ and blocked the cellular MMEJ pathway.
- ZM-0088 strongly inhibited the proliferation of DLD-1 BRCA2-/- cells and showed a >125× selectivity folds over DLD-1 parent cells in both anti-proliferation CTG assay and colony formation assay, indicating an effect of synthetic lethality between Polθ and HR deficiency.

ZM-0088 in combination with PARP inhibitors



- ZM-0088 elicits synergetic anti-proliferation activities in combination with PARP inhibitors on BRCA2-/- DLD-1 and MDA-MB-436 cells.

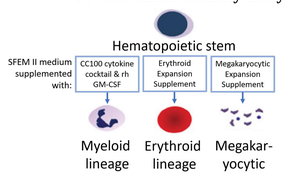
In vivo anti-tumor efficacy of ZM-0088

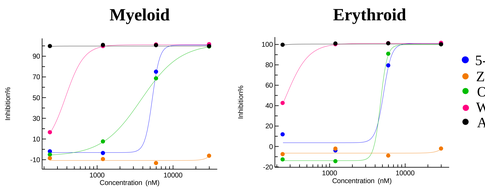


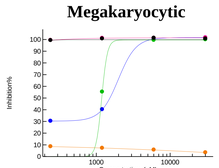
- ZM-0088 reduces the tumor growth as a single agent, and the combination of ZM-0088 and olaparib further results in tumor regression on DLD-1 BRCA2-/- xenografts.

Hematotoxicity of ZM-0088 *in vitro* and *in vivo*

***In vitro* hematotoxicity assay**

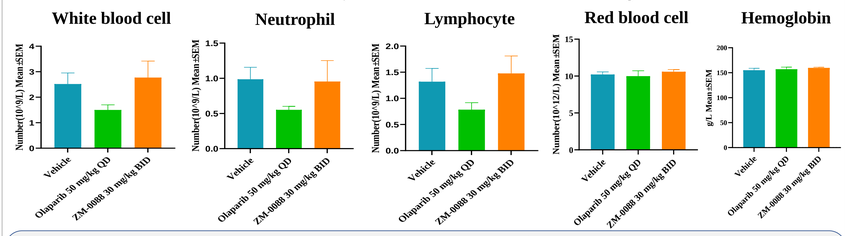






Compound	IC_{50} (nM)		
	Myeloid	Erythroid	Megakaryocytic
5-FU	5325	5091	1515
ZM-0088	>30000	>30000	>30000
Olaparib	3924	4806	1169
WEE1i	379	265	<240
ATRi	<240	<240	<240

Hematotoxicity assessment in DLD-1 BRCA2-/- xenograft model



- In the *in vitro* hematotoxicity assay, ZM-0088 shows no significant inhibition on lineage-specific (myeloid, erythroid and megakaryocytic) cell differentiation and survival.
- In the *in vivo* DLD-1 BRCA2-/- xenograft model, ZM-0088 displays no obvious effect on the numbers of specific blood cells after continuous administration.

Summary

- ZM-0088 is a novel Polθ inhibitor and demonstrated strong inhibition of the cellular MMEJ pathway by blocking the activity of Polθ.
- ZM-0088 strongly inhibits the proliferation of HR deficient BRCA2-/- DLD-1 cells, and shows an excellent selectivity over DLD-1 parent cells, suggesting an effect of synthetic lethality between Polθ and HR deficiency. It elicits synergetic anti-proliferation activities in combination with PARP inhibitors on BRCA2-/- DLD-1 and MDA-MB-436 cells.
- ZM-0088 displays tumor growth inhibition as a single agent and further leads to tumor regression in the combination with olaparib *in vivo*.
- ZM-0088 shows no significant effect on blood cells *in vitro* and *in vivo*, suggesting that targeting Polθ may potentially have a low risk of hematotoxicity.

Reference:

1. Zatreanu, D., Robinson, H.M.R. et al. Nat Commun 12, 3636 (2021).
2. Ceccaldi, R., Liu, J., Amunugama, R. et al. Nature. 2015 February 12; 518(7538): 258–262.
3. Mahalingaiah PK, Palenski T, Van Vleet TR. Curr Protoc Toxicol. 2018 May;76(1):e45.