In Vitro Activity and Efficacy of Novel Dual PARP-HDAC Inhibitors

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Introduction

Poly adenosine diphosphate-ribose polymerase (PARP) inhibition is an FDA-approved treatment for prostate, ovarian, pancreatic and breast cancers harboring homologous recombination (HR) DNA repair deficiencies. Often, these are germline mutation in the BRCA1 or BRCA2 genes. Combining PARP inhibition with inhibitors of other key DNA repair or PARP inhibitor resistance pathways, such as histone deacetylase (HDAC), could sensitize PARP inhibitor-resistant cells to treatment and expand the utility of PARP inhibitors beyond cancers harboring HR deficiencies.

Combination treatment often requires sequential administration due to overlapping toxicities and diverse pharmacokinetics, limiting the clinical utility of combination approaches. Development of a multi-target, single-molecule inhibitor could provide an effective therapy with reduced toxicity.

Here, we describe the *in vitro* activity of kt-3000 series compounds, a novel class of bi-functional PARP-HDAC inhibitors compared to FDA-approved PARP inhibitor olaparib (positive control).

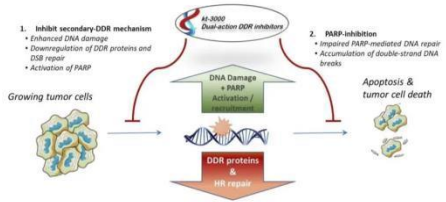
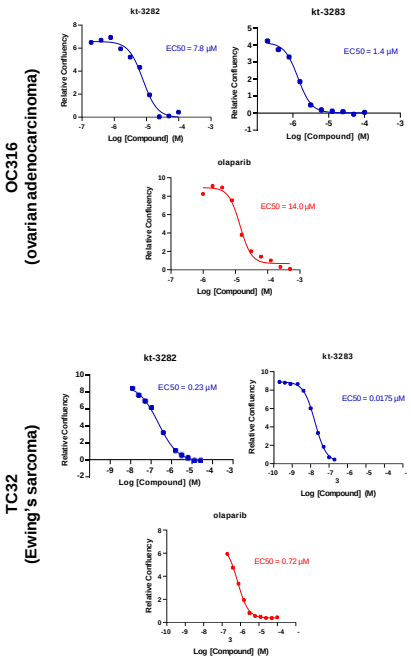


Fig. 1: Proposed mechanism of action of kt-3000 series compounds

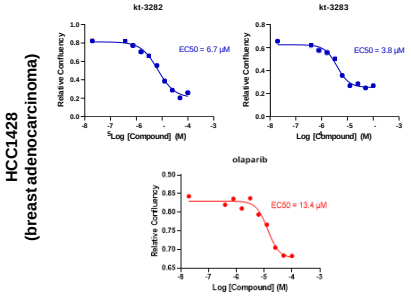
Response to treatment in BRCA wild type cell lines



kt-3000 compounds demonstrate similar or lower EC50 values compared to olaparib control. kt- 3283 is 10-fold lower than olaparib.

Assays are based on cell confluency obtained by Incucyte S3 live cell imaging after 72 hour treatment and normalized to confluency at t=0.

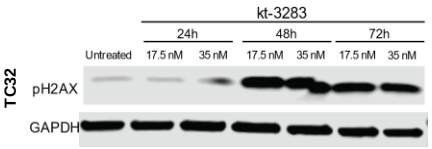
Response to treatment in a BRCA mutant cell line



kt-3000 compounds demonstrate similar or lower EC50 values compared to olaparib control with kt-3282 ~3.5-fold lower than olaparib

Assays are based on cell confluency obtained by Incucyte S3 live cell imaging after 72 hour treatment and normalized to confluency at t=0.

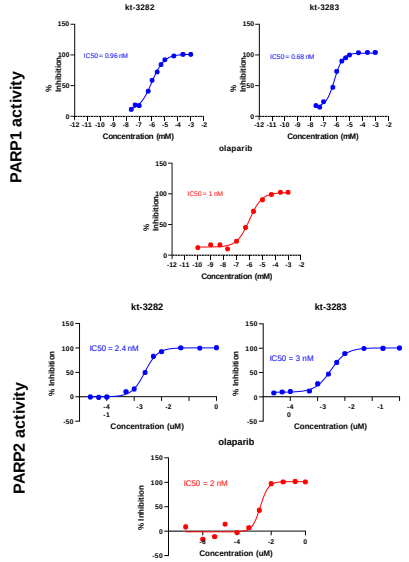
DNA damage induced by kt-3283



Treatment of TC32 cells with kt-3283 at low nM concentrations increased expression of pH2AX, a DNA damage marker, at 48 and 72 hours by western blot

GAPDH is shown as an internal loading control.

Potent inhibition of PARP1/2 activity *in vitro*



IC50 values of kt-3000 series compounds against PARP1 and PARP2 are comparable to olaparib.

PARP1/2 activity was measured using commercially available kits.

Conclusion and next steps

-These data suggest promising activity for kt-3000 series compounds against both BRCA-wt and BRCA mutant cancers.
-Select compounds are being advanced to *in vivo* studies to examine anti-tumor activity, pharmacokinetics and preliminary safety profiles

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