6215

Peposertib, a DNA-PK inhibitor, enhances the antitumor efficacy of anthracyclines in triple-negative breast cancer models *in vitro* and *in vivo*

Steffie Revia[1], Christian Sirrenberg[1], Antonia Schach[1#], Astrid Zimmermann[1], Frank T. Zenke[2], and **Joachim Albers[1]***

[1]the healthcare business of Merck KGaA, Darmstadt, Germany; [2]EMD Serono, Billerica, MA, USA
*Presenting author (**joachim.albers@emdgroup.com**)
#At the time of the study

CONCLUSIONS

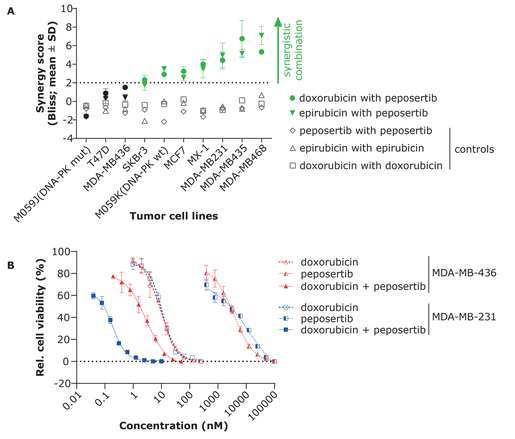
- **Co-treatment with the DNA-dependent protein kinase (DNA-PK) inhibitor peposertib has the potential to enhance the antitumor efficacy of anthracycline/topoisomerase II (TOPO II) inhibitor-based chemotherapy regimens in patients with triple-negative breast cancer (TNBC)**
- **Based on *in vitro* studies, the combined treatment of peposertib with TOPO II inhibitors may result in an immuno-stimulatory tumor microenvironment**
- **Future research is needed to validate this finding *in vivo* and to test rational combination approaches with immune-activating anti-cancer therapies**

INTRODUCTION

- TNBC is the most lethal breast cancer subtype, exhibiting poor response rates toward current standard of care chemotherapy regimens[1,2]
- DNA-PK is a member of the phosphoinositide 3-kinase–related kinase protein family and promotes non-homologous end joining (NHEJ) as part of the DNA damage response
- Peposertib is a potent, selective, and orally bioavailable DNA-PK inhibitor, which in pre-clinical models, strongly potentiates the antitumor effects of ionizing radiation and DNA double-strand break (DSB)–inducing agents, including anthracyclines[3]
- Here, we report the synergistic antitumor effects of peposertib combined with TOPO II inhibitors, particularly anthracyclines, in human TNBC xenograft models, both *in vitro* and *in vivo*

RESULTS

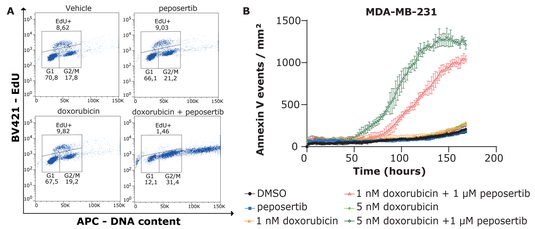
Figure 1. Peposertib exhibits synergistic antiproliferative activity with TOPO II inhibitors



(A) Bliss synergy scores for peposertib with doxorubicin or epirubicin were assessed in a panel of breast cancer cell lines using combination dose matrices. A synergy score of >2 is indicative of synergism and black data points indicate absence of synergy. M059J and M059K glioblastoma cell lines served as controls. **(B)** MDA-MB-231 and MDA-MB-436 TNBC cells were exposed to increasing concentrations of doxorubicin, peposertib, or combined treatment for 7 days, and dose-response was evaluated on day 7.

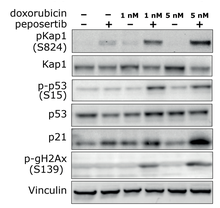
TNBC, triple-negative breast cancer; **TOPO II,** topoisomerase-II

Figure 2. Peposertib, in combination with doxorubicin, induces cell cycle arrest and apoptosis in MDA-MB-231 cells



MDA-MB-231 cells were exposed to doxorubicin, peposertib, or their combination for a period of 7 days, and subjected to **(A)** cell cycle analyses and **(B)** live-cell apoptosis analysis using Annexin V.

Figure 3. Peposertib in combination with doxorubicin activates ATM/p53 signaling and suppresses DNA DSB repair

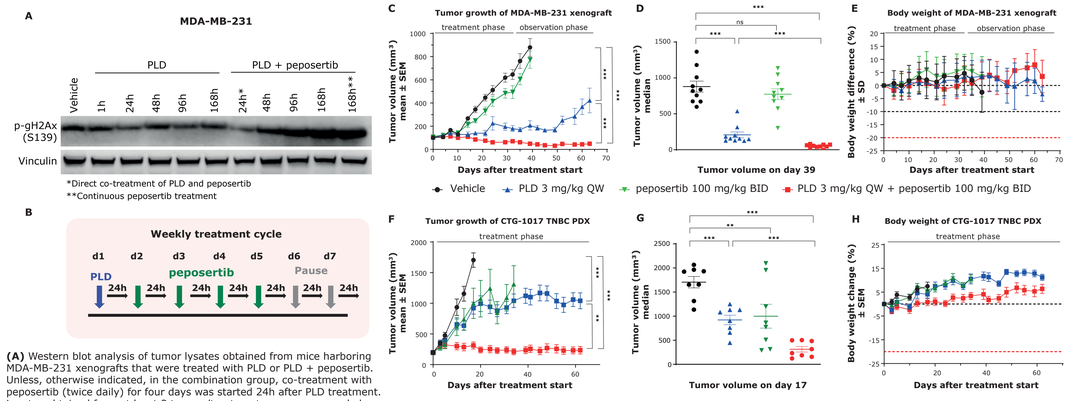


MDA-MB-231 cells were exposed to doxorubicin, 1 µM peposertib or their combination for a period of 7 days. Protein lysates were analyzed by Western blotting using antibodies against phospho-Kap1(S824), Kap1, phospho-p53(S15), p53, p21, phospho-gH2Ax(S139), and Vinculin (loading control). Enhanced ATM activity was evaluated as upregulation of its direct phosphorylation targets p-Kap1 (Ser824) and p-p53 (Ser15). Accumulation of DNA-damage is indicated by an increase in p-gH2AX in the combination groups.

ATM, ataxia-telangiectasia mutated; **DSB,** double-strand break

RESULTS

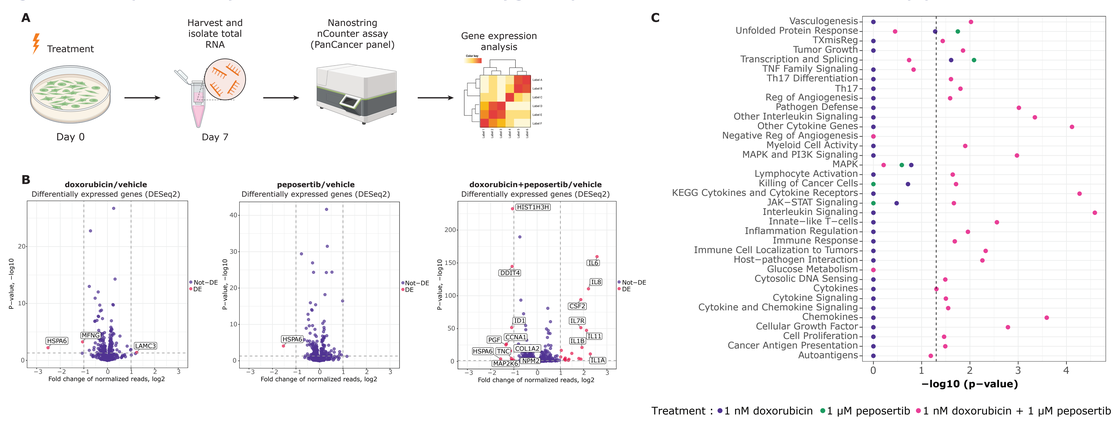
Figure 4. Combined treatment of peposertib with pegylated liposomal doxorubicin (PLD) in human TNBC xenograft models *in vivo* results in DNA damage accumulation, is well tolerated, and suppresses tumor growth



(A) Western blot analysis of tumor lysates obtained from mice harboring MDA-MB-231 xenografts that were treated with PLD or PLD + peposertib. Unless, otherwise indicated, in the combination group, co-treatment with peposertib (twice daily) for four days was started 24h after PLD treatment. Lysates obtained from at least 3 tumors/treatment groups were pooled for this analysis. **(B)** Scheme of the treatment schedule used in the *in vivo* efficacy studies for PLD + peposertib. **(C)** Tumor growth of MDA-MB-231 xenografts treated with vehicle control, peposertib, PLD or combinations thereof (n=10 for all groups, mean ± SEM). **(D)** Median tumor volumes on day 39 and, **(E)** corresponding mouse bodyweight change over time. **(F)** Tumor growth of CTG-1017 TNBC PDX xenografts treated with vehicle control, peposertib, PLD or combinations thereof (n=8 for all groups, mean ± SEM). **(G)** Median tumor volume on day 17, and **(H)** corresponding mouse bodyweight change over time.

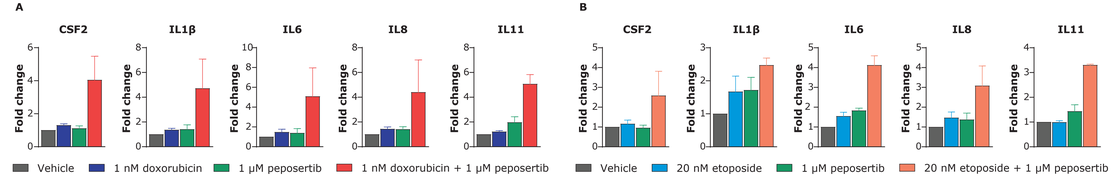
PDX, patient-derived xenograft; **TNBC,** triple-negative breast cancer

Figure 5. Gene expression analysis revealed induction of inflammatory genes upon combined treatment with doxorubicin and peposertib



(A) Schematic of the experimental setup for gene expression analysis in MDA-MB-231 cells using the Nanostring nCounter PanCancer panel. **(B)** Volcano plot of differentially expressed (DE) genes in MDA-MB-231 cells after exposure to doxorubicin, peposertib or their combination for a period of 7 days. **(C)** Pathway enrichment analysis of nCounter PanCancer expression data.

Figure 6. Combined treatment of peposertib + TOPO II inhibitors (doxorubicin or etoposide) induces upregulation of inflammatory chemokines and cytokines



qPCR analysis of selected inflammatory genes in MDA-MB-231 cells exposed for a period of 7 days to **(A)** doxorubicin, peposertib or their combination, and **(B)** etoposide, peposertib or their combination. Data represent mean ± SD.

CSF, colony stimulating factor; **IL,** interleukin

References: 1. Davison C, et al. *NPJ Breast Cancer*. 2021 6;7(1):38; 2. Bianchini G, et al. *Nat Rev Clin Oncol.* 2016;13(11):674-690; 3. Zenke FT, et al. *Mol Cancer Ther.* 2020;19(5):1091-1101

Disclosures: Steffie Revia, Christian Sirrenberg, Antonia Schach, Astrid Zimmermann, and Joachim Albers are employees of the healthcare business of Merck KGaA, Darmstadt, Germany; Frank T Zenke is an employee of EMD Serono, Billerica, MA, USA. The study was funded by the healthcare business of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945)

Acknowledgements: Editorial assistance was provided by Vasuprada Iyengar, of Merck Specialties Pvt. Ltd., Bengaluru, India, an affiliate of Merck KGaA, Darmstadt, Germany, and funded by the healthcare business of Merck KGaA, Darmstadt, Germany.

Presented at the American Association for Cancer Research Annual Meeting 2023 | April 14–19 | Orlando, FL, USA