AsiDNA™ induces tumor sensitivity to PARP inhibitors in homologous recombination proficient breast cancer

Poster 1110

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Abstract

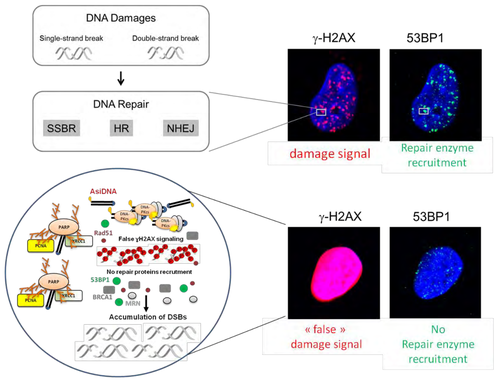
Purpose: PARP inhibitors (PARPi) have shown significant benefits in cancer patients with *BRCA* mutations. However, their major limitations are the necessity of homologous recombination (HR) deficiency and the rapid emergence of resistance. In the current study, we propose a novel therapeutic strategy, based on drug combination to promote sensitivity to PARPi independently of the tumor genetics. **Experimental design:** We used AsiDNA, a DNA repair inhibitor (Dbait concept), consisting in small molecules mimicking double-strand DNA breaks to activate ectopic signaling of DNA damage and prevent recruitment at damage sites of HR repair enzymes. We characterized the inhibition activity of AsiDNA by monitoring repair foci formation and DNA damage and analyzed the cell survival to AsiDNA monotherapy and combination with the PARPi Olaparib of 21 tumor cell lines, and 3 non-tumor cell lines. In vivo efficacy of the combined treatment was analyzed in tumor xenografts derived from the MDA-MB-231 cell line showing reduced sensitivity to AsiDNA and Olaparib in vitro. Frequency of clones resistant to two weeks treatment with AsiDNA or Olaparib was quantified in the 4NQO-mutagenized haploid KBM7 cells.

Results: Molecular analyses of repair foci formed after irradiation demonstrate that AsiDNA inhibits recruitment of RAD51 and 53BP1 whereas Olaparib prevents XRCC1. Combination of both drugs increases the accumulation of unrepaired spontaneous damage resulting in an increase of cell death in all tumor cell lines. The synergy of the association of AsiDNA with PARPi was also confirmed with 6 other PARPi. AsiDNA does not induce any increase in DNA damage or lethality in non-tumor cells when used in monotherapy as well as in association with PARPi. Selection of resistant clones allowed the emergence of resistance to Olaparib (1.45%), Imatinib (0.6%) and 6-Thioguanine (1.6%) but not to AsiDNA (<0.07%), indicating that the drug is able to block several DNA repair pathways. In the MDA-MB-231 xenograft model, Olaparib alone failed to prevent tumor growth while AsiDNA provided 163% increase in tumor growth delay. Interestingly, the combination of Olaparib and AsiDNA increased 216% the mean tumor growth delay. **Conclusion:** Our results highlight the therapeutic interest of combining AsiDNA and PARPi to recapitulate synthetic lethality in all tumors independently of their HR status. Moreover, the low frequency of appearance of resistant clones to AsiDNA suggests a sustained efficacy during treatment unlike most targeted therapies.

Introduction

Multiple DNA Repair pathways inhibition by AsiDNA

Dbait are short and stabilized DNA molecules that mimic DSBs. AsiDNA, a molecule of Dbait family, acts by hijacking and hyper-activating the DNA-dependent protein kinase (DNA-PK), and Poly(ADP-Ribose) Polymerase (PARP), which modify the chromatin and consequently inhibit the recruitment at the damage sites of many proteins involved in the DSB (HR and NHEJ) and SSB (BER) repair pathways. This strategy sensitizes tumors to DNA damaging therapies such as radiotherapy and chemotherapy.



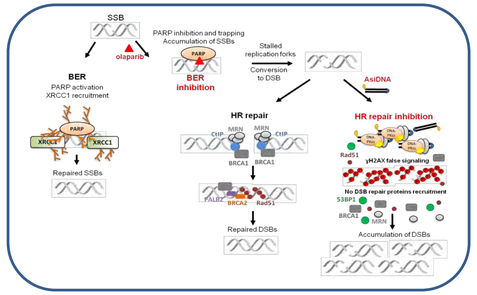
→Inhibition of DNA repair proteins recruitment to damage sites
→no repair → DNA damage accumulation → **Cell death**

Repair inhibition by PARP inhibitors (PARPi)

In cells, damage which arise during normal cellular activity, are repaired by the base excision repair pathway (BER) involving PARP1. PARP inhibition leads to the accumulation of unrepaired SSBs that result in stalled replication forks and DSBs. These DSBs are mainly repaired by Homologous Recombination (HR). Cells with *BRCA1/2* mutations are defective in HR (so-called BRCAness) and are sensitive to PARPi. Cells with functional HR, accurately and efficiently repair DSBs, and are less sensitive to PARPi.

Rational for combining AsiDNA and PARPi

AsiDNA induces a transitory «Homologous Recombination Deficient» status that would render all cells sensitive to PARPi.

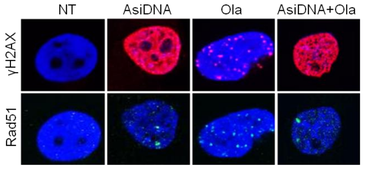


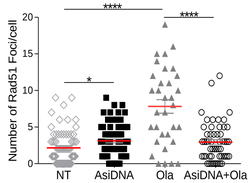
AsiDNA stimulate PARPi efficacy independently of the genetic of the tumors

Molecular mechanisms underlying the combination of AsiDNA and PARPi

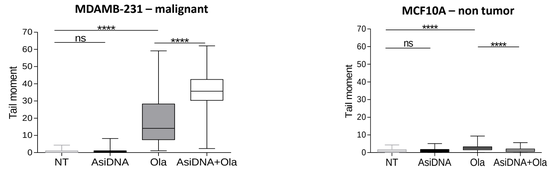
Inhibition of Rad51 recruitment at damage site

Olaparib (Ola) and AsiDNA are both DNA repair inhibitors inhibiting the recruitment of repair enzymes at damage sites.





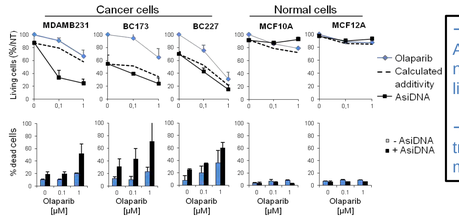
Accumulation of damage in malignant cells but not in normal cells



→ AsiDNA inhibits the repair of Ola-induced DNA damage.
→ AsiDNA acts specifically on tumor cells

AsiDNA and PARPi: Drug-Driven Synthetic Lethality

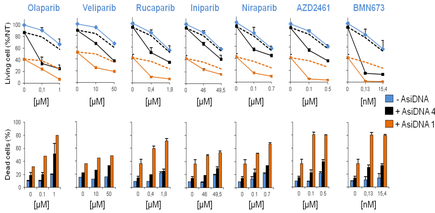
The combined treatment AsiDNA and Olaparib is toxic in HR proficient cells



→ Synergistic effect between AsiDNA and olaparib in Triple negative breast cancer cell lines.

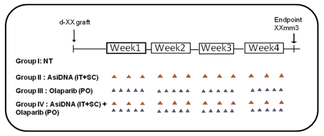
→ No toxicity of the combined treatment in normal mammary cell lines.

AsiDNA is synergic with all PARP inhibitors

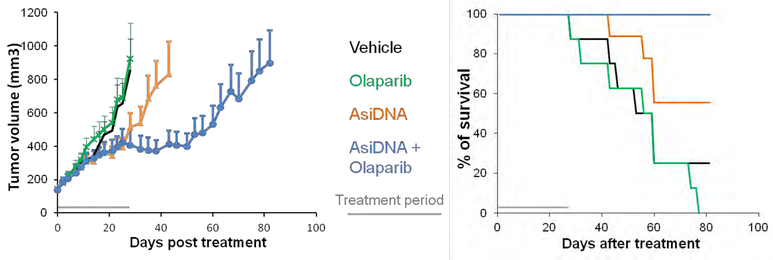


→ Synergistic effects are observed with PARPi targeting catalytic activity or trapping PARP at damage sites.

Synergy of the association of AsiDNA and Olaparib in a HR proficient Breast Cancer model

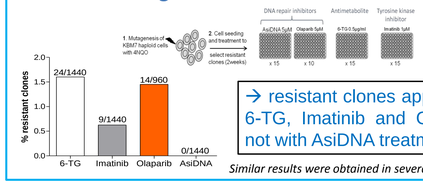


→ AsiDNA+Olaparib combined treatment show better tumor growth delay and longer survival compared to single treatment



Low occurrence of clones resistant to AsiDNA

Chemical mutagenesis and selection of resistant clones



→ resistant clones appear during 6-TG, Imatinib and Olaparib but not with AsiDNA treatment

Similar results were obtained in several malignant cell lines

Related publications:

- C. Le Tourneau, et al. (2016*) Br J Cancer. May 3. doi: 10.1038/bjc.2016.120.* First-in-human phase I study of the DNA repair inhibitor DT01 in combination with radiotherapy in patients with skin metastases from melanoma.
- W. Jdey, et al. (2016) *Clinical. Canc Res.* (2016) Aug 24. pii: clincanres.1193. Drug Driven Synthetic Lethality: bypassing tumor cell genetics with a combination of AsiDNA and PARP inhibitors.
- M. Quanz, et al. (2009) *PloS ONE* 4(7) e6398. Hyperactivation of DNA-PK by Double-Strand Break Mimicking Molecules Disorganizes DNA Damage Response
- M. Quanz, et al.(2009) *Clinical Cancer research* 15(4) 1308-1316. Small molecular drugs mimicking DNA damage (Dbait): a new strategy for sensitizing tumors to radiotherapy

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