Novel Combination Therapy of DNMT inhibitor SGI-110 and PARP inhibitor BMN-673 (Talzaporib) for *BRCA*-proficient Ovarian Cancer

Nicholas Pulliam[1], Jay Pilrose[2], Pietro Taverna[3], John Lyons[4], Kenneth P. Nephew[1,2,5]

[1]Indiana University, Molecular and Cellular Biochemistry, Bloomington, IN; [2] Indiana University, Medical Sciences; [3]Astex Pharmaceuticals, Inc., Pleasanton, CA; [4]Astex Pharmaceuticals, Inc., Cambridge, United Kingdom; [5]Indiana University, Department of Cellular and Integrative Physiology, Bloomington, IN

ABSTRACT

•Ovarian cancer (OC) is initially chemoresponsive but the majority of patients relapse after first line platinum-, taxane-based chemotherapy.

•Recurrence has been shown to be associated with increased DNA damage response (DDR) mediated by poly-(ADP)-ribose polymerase 1/2 (PARP1/2), which can be therapeutically targeted by PARP inhibitors (PARPi). Although PARPi are indicated for platinum-responsive, *BRCA*-mutated OC, most OC patients have *BRCA*-proficient disease.

•Based on our previous studies supporting a role for DNA methylation in chemoresistant OC, mediated by the enzyme DNA methyltransferase 1 (DNMT1), and reports on a functional role for DNMT1 in DNA double strand break repair mediated by BRCA1/2, we hypothesize that combining the DNMTi SGI-110 and the PARPi talazoparib (BMN673) will impair *BRCA*-mediated DDR, resulting in cytotoxicity.

CONCLUSION: Combination SGI-110 + talazoparib treatment significantly reduced cancer cell colony formation. Regardless of *BRCA* and platinum sensitivity status, co-administration of SGI-110 and talazoparib reduced cell survival, albeit %survival was dependent on drug dose and cancer cell line.

MATERIALS AND METHODS

Fig. 1 Schema utilized for combination treatments

Co-Administration Treatment

- Day 0: Plate Cells (40-50% confluent)
- Day 1-3: Daily SGI-110 Treatment (5, 20, 100nM)
- Day 1: Talazoparib Treatment (1 or 10nM)
- Day 4: Examine For Colony Growth, Proliferation, WB, Luciferase

Sequential ("Priming") Treatment

- Day 0: Plate Cells (40-50% confluent)
- Day 1-3: Daily SGI-110 Treatment (5, 20, 100nM)
- Day 4: Wash Cells, Recover 24hrs
- Day 5: Talazoparib Treatment (1 or 10nM)
- Day 8: Examine For Colony Growth, Proliferation, WB, Luciferase

Cell Line (ovarian cancer)	*BRCA* Status
Kuramochi	*BRCA2* Mutant
Ovcar8	Hypermethylated *BRCA1*
A2780P	*BRCA* wt
HeyC2	*BRCA* wt
PEO1&4	*BRCA* mut/wt

Cell Line (breast cancer)	*BRCA* Status
MCF7	*BRCA1* wt
SKBR3	Hypermethylated *BRCA1*
MDA-MB-231	*BRCA* wt

RESULTS

Fig. 2 Combination of the DNMT inhibitor SGI-110 and PARP inhibitor Talazoparib demonstrate increased efficacy in ovarian cancer cell lines

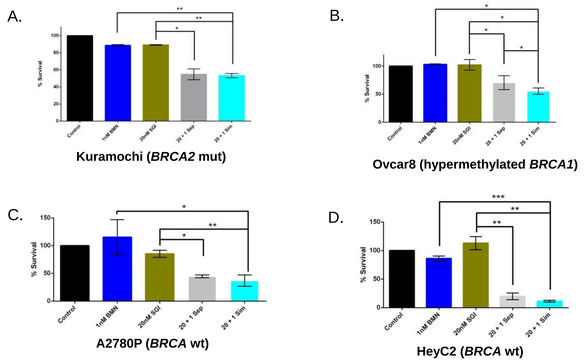

Fig 2. Clonogenic survival assay of ovarian cancer cells Kuramochi (*BRCA2* mutant), Ovcar8 (hypermethylated *BRCA1*), A2780P and HeyC2 (*BRCA* wt), was performed following the treatment schema described in the Materials and Methods. Results are representative of three separate experiments, performed in duplicate.

Fig. 3 Combination DNMT inhibitor SGI-110 and PARP inhibitor talazoparib demonstrate increased efficacy in breast cancer cell lines MCF7, MDA-MB-231 and SKBR3

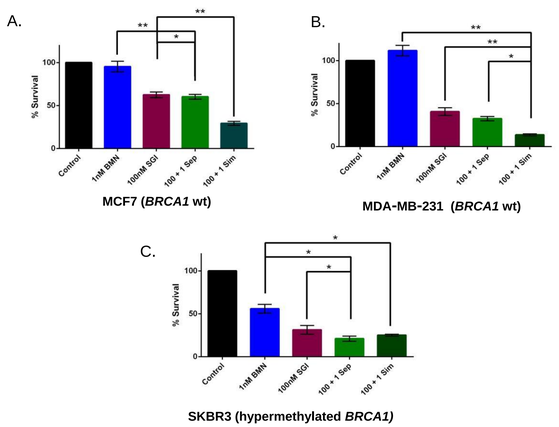

Fig. 3. Clonogenic survival assay of breast cancer cell lines MCF7 and MDA-MB-231 (both *BRCA1* wt) and SKBR3 (hypermethylated *BRCA1*), was performed following the treatment schema described in the Materials and Methods. Results are representative of three separate experiments, performed in duplicate.

Fig. 4 SGI-110 plus talazoparib is effective in *BRCA2*-deficient (Peo1) and *BRCA2*–proficient (Peo4)OC cell lines

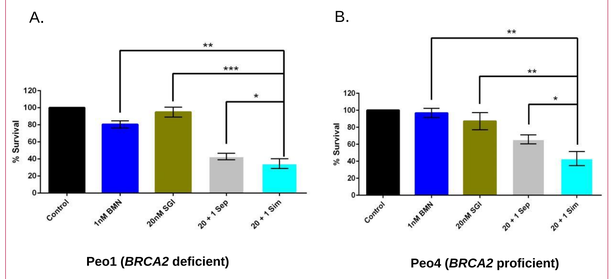

Fig. 4. Cell lines derived from the same patient before therapy (Peo1, *BRCA2* mut; platinum sensitive) and following therapy (Peo4, *BRCA2* proficient, platinum resistant), differing notably in their BRCA2 status were treated as described in the Materials and Methods and clonogenic survival assay performed. Results are representative of three separate experiments, performed in duplicate.

Fig. 5. Low-dose SGI-110 increases PARP expression, while low-dose talazoparib increases DNMT1 expression

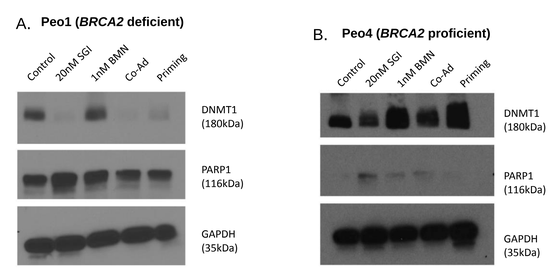

Fig. 5. Western blot analysis of DNMT1, PARP1, and GAPDH (loading control), respectively, for Peo1 and Peo4 cell lines. Cells were treated as described in the Materials and Methods.

Fig. 6 Low-dose talazoparib reduces PARP enzymatic activity, while SGI-110 induces PARylation

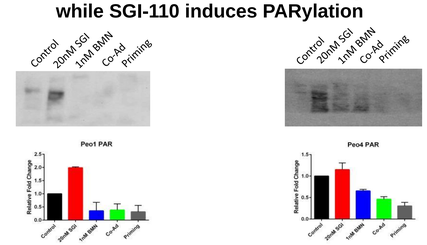

Fig. 6. Western blot analysis of whole cell lysate for PAR. Cells were treated as described in the Materials and Methods.

Fig. 7 Combination therapy reduces proliferation and promotes caspase 3-mediated cell death

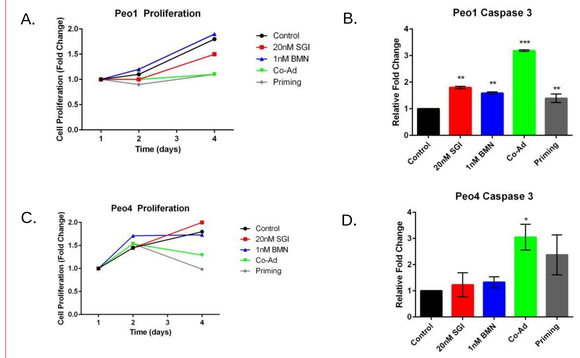

Fig. 7. Peo1 (A,B) and Peo4 (C,D) cells were treated as described in Materials and Methods. MTT assay was performed following treatment to determine proliferation rate. Caspase 3 activity was measured by luminescence.

Fig. 8. A proposed model

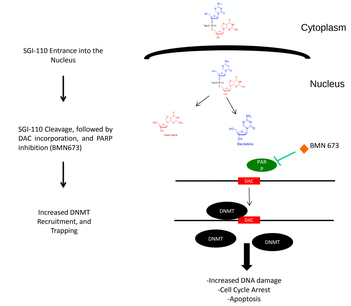

Fig.8. A proposed model for the efficacy of combination DNMT inhibitor, SGI-110 and PARP inhibitor, talazoparib

CONCLUSIONS

1. Combination therapy resulted in decreased clonogenic survival in *BRCA* deficient or proficient ovarian cancer and breast cancer cell lines, suggesting that combinations of SGI-110 and talazoparib are effective irrespective of BRCA status.
2. Combination therapy resulted in decreased proliferative capacity and increased apoptosis in paired *BRCA2*-deficient and –proficient ovarian cancer cell lines.
3. SGI-110 increased PARP expression, as well as PARP enzymatic activity (PARylation).
4. Talazoparib reduced PARylation through PARP stabilization

ACKNOWLEDGMENTS

- National Cancer Institute Award U54 CA13001, Integrated Cancer Biology Prog.
- Dr. Len Post (Biomarin Pharmaceutical Inc.) for helpful discussions.
- Ali Ozes for technical help and discussions.