Abstract #7120

Investigating PARP1 selective inhibitor DSB1559 efficacy in BRCA1-associated triple negative breast cancer

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ABSTRACT

Background: Poly (ADP-ribose) polymerase inhibitors (PARPi) have improved outcomes of BRCA-associated breast cancer. However, toxicity can limit patient response as well as the ability to be combined with other therapeutics. Current PARPi broadly inhibit members of the PARP protein family, however only inhibition of PARP1 is needed for PARPi-mediated cell death. As inhibition of PARP2 is associated with hematological toxicity seen in PARPi patients, selectively inhibiting PARP1 may reduce PARPi toxicity and lead to better outcomes for patients. Here we characterize and test the efficacy of the PARP1 selective inhibitor (PARP1i) DSB1559 compared to Olaparib in BRCA1-associated triple negative breast cancer (TNBC).

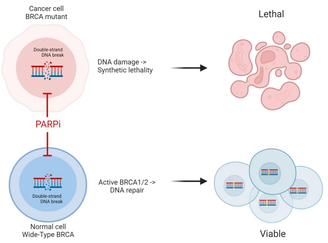
Methods: NanoBRET, PARylation, and pharmacokinetic and pharmacodynamic assays were performed to examine DSB1559 potency, PARP family selectivity, and *in vivo* behavior. CellTiter-Glo and qPCR assays were used to examine DSB1559 effect on cell viability and STING activation, respectively. Immunocompetent FVB/n mice bearing BRCA1-deficient TNBC (*CMV-Cre;Brca1$^{f/f}$;Trp53$^{f/f}$*) tumors were treated daily with PARPi (Olaparib) or PARP1i (DSB1559) for 60 days and followed for survival or treated for 7 days and harvested for flow cytometry.

Results: DSB1559 is a novel and potent PARP1 inhibitor which has superior selectivity for PARP1 compared to Olaparib and PARP1i AZD5305. DSB1559 has highly desirable physio-chemical and in vitro ADME properties, which translate to high oral bioavailability and moderate clearance in rodent PK studies. Additionally, DSB1559 demonstrated superior tumor residence time and tumor/plasma ratio compared to AZD5305. Compared to Olaparib, DSB1559 significantly increased STING activation and reduced cell viability in BRCA1-associated murine TNBC cells. BRCA1-associated TNBC-bearing immune-competent mice treated with DSB1559 had significantly reduced tumor growth and increased overall survival (median survival 74 days) compared to Olaparib (median survival 18 days). Analysis of changes in the tumor microenvironment reveal increased cytotoxic T cells and activated dendritic cells.

Conclusions: Taken together, DSB1559 is a highly selective PARP1 inhibitor that demonstrates superior efficacy compared to Olaparib in a BRCA1-associated immune-competent TNBC model.

BACKGROUND

PARPi induce synthetic lethality in tumors with defects in homologous recombination



Inhibition of PARP2 is associated with adverse effects and is not necessary for PARPi efficacy

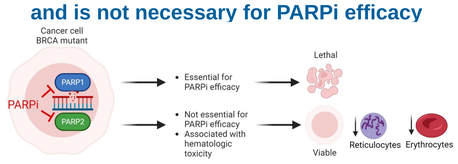


Figure 1. Poly (ADP-Ribose) Polymerase Inhibitors (PARPi) cause cancer cell death by inducing DNA damage and synthetic lethality in cancer cells with defective homologous recombination pathways, such as BRCA1-/- breast cancer (1). Cells with sufficient homologous recombination are able to repair their DNA damage and remain viable. Current PARPi such as Olaparib or Talazoparib inhibit multiple members of the PARP protein family. However, only inhibition of PARP1 is required for PARPi efficacy (2). Furthermore, inhibition of other PARP proteins is associated with adverse effects (3). Therefore, compounds that only inhibit PARP1 (PARP1i) could induce synthetic lethality in homologous recombination deficient cancer cells, while reducing adverse effects for patients.

RESULTS

DSB1559 has superior selectivity compared to Olaparib and AZD5305

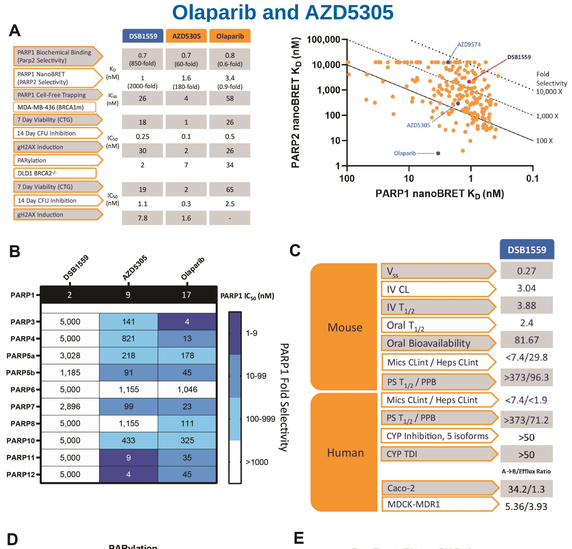


Figure 2. (A) Comparison of Olaparib, DSB1559, and AZD5035 ability to inhibit PARP1 and PARP2 using an NanoBRET assay and induce cell death in homologous recombinant impaired cancer cells. **(B)** Comparison of DSB1559, AZD5305, AZD9574, and Olaparib to inhibit different members of the PARP protein family. Inhibition was determined using Promega NanoBRET kit. **(C)** *In vitro* ADME properties of DSB1559 in mice, and humans showing that DSB1559 has desirable physio-chemical and *in vitro* ADME properties. **(D-E)** Mice bearing MDA-MB-436 BRCA1-/- tumors were treated for 5 days with DSB1559 (1-10mg/kg daily PO) and AZD5305 (10mg/kg daily PO). Tumors were isolated at 1, 4 or 24 hours post final dose and PARylation was determined by ELISA. DSB1559 demonstrates higher tumor:plasma ratio versus AZD5305.

DSB1559 reduces viability and increases STING signaling of TNBC BRCA1-/- cells

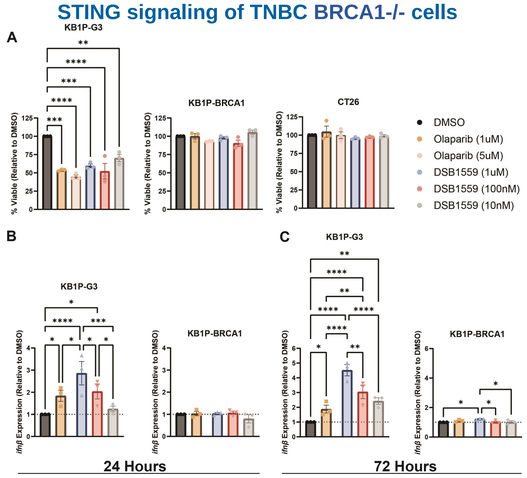


Figure 3. (A) KB1P-G3 (BRCA1-/-), KB1P-BRCA1, and CT26 cells were treated with Olaparib or DSB1559 at different concentrations for 72 hours. CellTitre-Glo assay was performed following the manufacturer's protocol to determine cell viability. **(B)** KB1P-G3 (BRCA1-/-) and KB1P-BRCA1 cells were treated with Olaparib or DSB1559 at different concentrations for 24 or 72 hours. RNA from cells were harvested using a Qiagen Rneasy kit. IFNβ expression was determined by qPCR. n=3 biological replicates per group. Error bars represent ±S.E.M. Statistical analyses were performed comparing each group using one-way ANOVA with multiple comparisons.

DSB1559 improves overall survival in BRCA1-associated TNBC compared to Olaparib

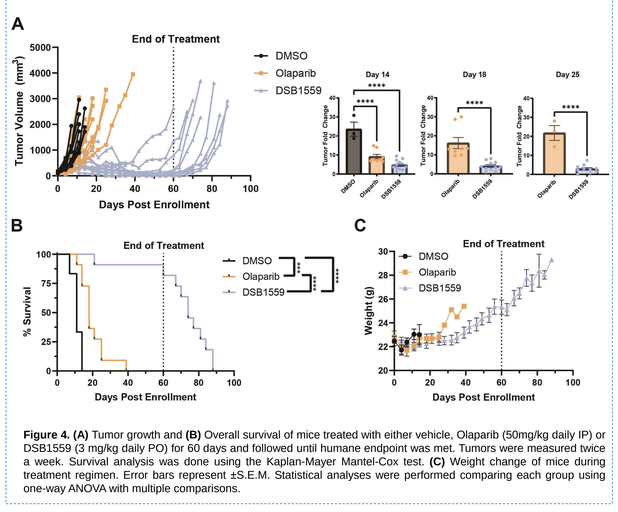


Figure 4. (A) Tumor growth and **(B)** Overall survival of mice treated with either vehicle, Olaparib (50mg/kg daily IP) or DSB1559 (3 mg/kg daily PO) for 60 days and followed until humane endpoint was met. Tumors were measured twice a week. Survival analysis was done using the Kaplan-Mayer Mantel-Cox test. **(C)** Weight change of mice during treatment regimen. Error bars represent ±S.E.M. Statistical analyses were performed comparing each group using one-way ANOVA with multiple comparisons.

Olaparib and DSB1559 increase CD8+ T cell cytotoxicity

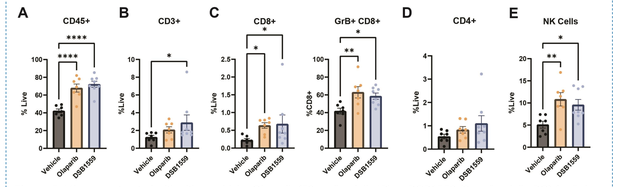


Figure 5. Mice were treated with either vehicle, Olaparib (50 mg/kg daily IP) or DSB1559 (3 mg/kg daily PO) for 7 days to capture changes in the myeloid and T cell compartment related to the treatments. Tumors were harvested for immunophenotyping by flow cytometry for the indicated markers: **(A)** CD45 (leukocyte), **(B)** CD3 (T cells), **(C)** CD8 (CD8 T cells) and Granzyme B (Cytotoxic CD8 T cells), **(D)** CD4 (CD4 T cells), **(E)** NKp46 (NK cells). Error bars represent ±S.E.M with n=7-8 mice per group. Statistical analyses were performed using one-way ANOVA with multiple comparisons.

Olaparib and DSB1559 increase the infiltration of activated tumor associated macrophages

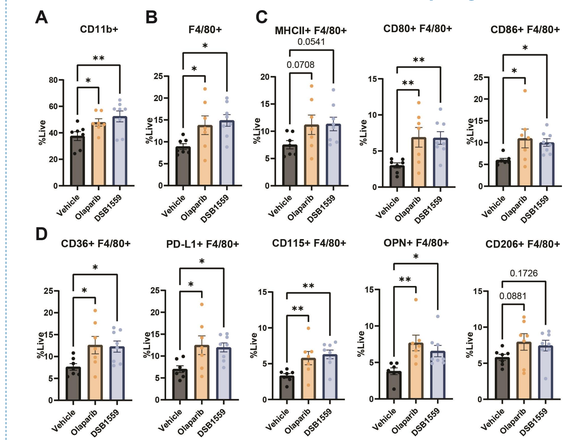


Figure 6. Mice were treated with either vehicle, Olaparib (50 mg/kg daily IP) or DSB1559 (3 mg/kg daily PO) for 7 days to capture changes in the myeloid and T cell compartment related to the treatments. Tumors were harvested for immunophenotyping by flow cytometry for the indicated markers: **(A)** CD11b (Myeloid), **(B)** F4/80 (Macrophages), **(C)** their expression of antigen presentation machinery (MHCII, CD80,CD86), **(D)** and suppressive markers (CD36, PD-L1, CD115, OPN, and CD206). Error bars represent ±S.E.M with n=7-8 mice per group. Statistical analyses were performed using one-way ANOVA with multiple comparisons.

DSB1559, but not Olaparib, increases the infiltration and activation of dendritic cells

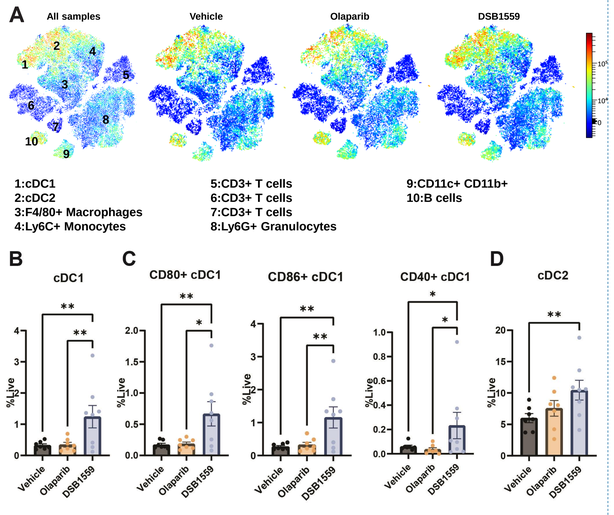


Figure 7. Mice were treated with either vehicle, Olaparib (50 mg/kg daily IP) or DSB1559 (3 mg/kg daily PO) for 7 days to capture changes in the myeloid and T cell compartment related to the treatments. Tumors were harvested for immunophenotyping by flow cytometry for the indicated markers: **(A)** Dot plot representation of t-distributed stochastic neighbor embedding (tSNE) analysis showing CD11c expression **(B)** CD11c+ CD11b- MHCII+ (cDC1), **(C)** their expression of co-stimulatory receptors (CD80, CD86, and CD40), and **(D)** CD11c+ CD11b+ MHCII+ (cDC2). Error bars represent ±S.E.M with n=7-8 mice per group. Statistical analyses were performed using one-way ANOVA with multiple comparisons.

CONCLUSIONS

- DSB1559 demonstrates superior PARP1 selectivity compared to Olaparib and AZD5305.
- DSB1559 has highly desirable *in vitro* ADME properties and *in vivo* oral bioavailability.
- DSB1559 has superior tumor residence time and tumor/plasma ratio compared to AZD5305.
- DSB1559 reduces BRCA1-/- p53-/- TNBC cell viability in a dose-dependent manner.
- DSB1559 significantly increased IFN-beta expression compared to Olaparib.
- DSB1559 demonstrates superior efficacy in BRCA1-/- p53-/- TNBC compared to Olaparib.

Immune changes with DSB1559
- Immune cell infiltration
- CD8 T cell infiltration and cytotoxicity
- NK cell infiltration
- TAM infiltration and expression of suppressive markers
- DC infiltration and activation

- Strategies to target suppressive TAMs and/or activate DCs may further enhance the anti-tumor responses seen with the PARP1-selective inhibitor.

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