Supplemental Information

Eliminating hypoxic tumor cells improves response to PARP inhibitors in homologous recombination deficient cancer models

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A. Vehicle  Olaparib  Talazoparib
Normoxia  Hypoxia

B. Normoxia  Hypoxia
Olaparib  -  +  -  -  +  -
Talazoparib -  -  +  -  -  +

C. SUM149  OVCAR8

D. Normoxia  Hypoxia
Olaparib  -  +  -  -  +  -
Talazoparib -  -  +  -  -  +

E. SUM149  OVCAR8

F. SUM149  OVCAR8

G. Absolute Cell Count (x1000)

H. Survival Fraction

- Normoxia (21% Oxygen)
- Hypoxia (5% Oxygen)
- Hypoxia (2% Oxygen)
**Figure S1. Related to Figure 1:**

(A) Representative images of clonogenic survival assays of SUM149 cells treated with Olaparib (0.1 μM) or Talazoparib (1 nM) for 7 days under normoxia or hypoxia.

(B) Representative Western Blot analysis of PARP1 shows the protein levels are not affected by incubation in hypoxia. SUM149 were treated with Olaparib and Talazoparib for 7 days, β-actin is a loading control.

(C) Anti-PAR ELISA confirms efficiency of inhibition of PAR formation after treatment with PARPi in both normoxia and hypoxia (n=3 per condition), two-way ANOVA, interaction p value (normoxia vs hypoxia for each treatment).

(D) PAR formation, as detected by WB, in OVCAR8 cells treated with PARPi for 7 days in both normoxia and hypoxia. β-actin is a loading control.

(E) Percentage of EDU positive cells after 7 days incubation in normoxia or hypoxia (n=3 per condition).

(F) Cell cycle profiles of OVCAR8 and SUM149, 7 days after incubation in normoxia or hypoxia (n=3 per condition).

(G) Number of OVCAR8, SUM149 and HT1080 cells after 7 days after incubation in normoxia or hypoxia (n=3).

(H) Clonogenic formation of HR deficient OVCAR 8 cells treated with indicated doses of Olaparib or Talazoparib for 7 days under normoxic or hypoxic conditions (2% oxygen and 5% oxygen), followed by 7-10 days culture in the absence of inhibitor Survival relative to vehicle-treated cells is plotted. (n=3 per condition, p values from two-way ANOVA).

Data represented as means ± SEM (represented by error bars).
Figure S2. Related to Figure 2:
(A) Hypoxia is a hallmark of cancer. Hypoxia score (Buffa) of cancers from TCGA cohort.
(B) Dot plot and mean values of hypoxia scores of normal breast tissue and breast cancer
with and without BRCA1/2 deficiency. p Mann Whitney test.
Data represented as means ± SEM (represented by error bars).
Figure S3. Related to Figure 3.

(A) Immunoblots of DDR proteins in OVCAR8 cells treated with vehicle or PARPi for 48 h in normoxia or hypoxia.

(B) Percentage of OVCAR8 and HT1080 cells with more than 10 \(\gamma\)H2AX foci per nucleus after 48 h of PARPi treatment in normoxic or hypoxic culture conditions. \(p\) value calculated by one-way ANOVA, drug treatments vs vehicle (\(n=3\)).

(C) Average number of pDNA-PKcs (T2609) foci per nucleus in OVCAR8 and HT1080 cells. \(p\) value calculated by one-way ANOVA, drug treatments vs vehicle (\(n=3\)).

(D) Number of total 53BP1 foci per nucleus after 48 h of PARPi treatment, average number of foci per cell (red line), percentage of cells with more than 15 foci (blue box).

(E) Hypoxia does not reverse HR status in HR deficient cells. Quantitative real-time PCR (qRT-PCR) measuring BRCA1, BRCA2 and RAD51 mRNA levels in SUM149 cells incubated under different oxygen tensions. Measurements were normalized to 18S mRNA levels and expressed as fold change compared to normoxia (\(n=3\) per condition).

(F) Detection of HR activity in the HR proficient MDA231 cells (\(n=3\)) but not HR deficient SUM149 cells (\(n=5\)) as measured by the TLR system.

Data represented as means ± SEM.
Figure S4. Related to Figure 4.
(A) Relative quantity of ROS species (detected by DCF) produced upon treatment of cells with PAPRi for 48h under normoxia or hypoxia. *p* value calculated by one-way ANOVA, *n*=3.
(B) Percentage of HT1080 cells with more than 10 γH2AX foci per nucleus after 2h, 6h, 24h and 48h of PARPi treatment in normoxic or hypoxic culture conditions, *n*=3 for each condition.
(C) Percentage of HT1080 cells with more than 10 γH2AX foci per nucleus after 6h PARPi treatment in normoxic, hypoxic culture conditions and after 48h preincubation in hypoxia showing decreased staining in cell preincubated in hypoxia. *p* value calculated by one-way ANOVA, *n*=3.
(D) Percentage of HT1080 cells with more than 10 γH2AX foci per nucleus after 48h of PARPi treatment in hypoxic culture conditions followed by 2h treatment with 50 μM H₂O₂. *p* values determined by two-way ANOVA, *n*=3.
Data represented as means ± SEM (represented by error bars).
**Figure S5. Related to Figures 6 & 7.**

A) Cell survival of OVCAR8 cells treated with Olaparib (0.001-10 μM) and TPZ (0.1-50 μM) or Talazoparib (0.01-20nM) and TPZ (0.1-50 μM). Cells were treated for 4 days with the indicated drug combinations in normoxia or hypoxia, followed by SRB assay to determine cytotoxicity, p determined by t-tests, combination treatments vs single treatments, n=3 per condition.

(B) HSA synergism analysis of OVCAR8 cells treated with varying doses of TPZ and Olaparib or TPZ and Talazoparib.

(C) Colony formation of OVCAR8 cells. Cells were treated for 96 hours with TPZ (0.1 μM) and Olaparib (1 μM) or Talazoparib (10 nM) in normoxia or hypoxia. Results expressed as survival ratio relative to vehicle treated groups. p value calculated by one-way ANOVA, drug treatments vs vehicle (n=4).

(D) Colony formation of OVCAR8 cells treated for 96 hours with TPZ (1 μM) and Olaparib (1 μM) or Talazoparib (10 nM) in normoxia or hypoxia, p value calculated by t-tests, combination treatments vs single treatments (n=4).

(E) Relative levels of ROS species produced upon treatment of cells with 0.1 μM and 1 μM of TPZ for 96 h, p value calculated by one-way ANOVA, TPZ treatment versus vehicle (n=3).

(F) Schematic diagram of the mechanism of action of Tirapazamine (Adapted from Siim et al., 2004).

Data represented as means ± SEM (represented by error bars).
Figure S6. Related to Figure 8.
(A) **HT1080 xenograft model.** Growth curves of HT1080 xenograft tumors in control mice and mice treated with Olaparib 50mg/kg and/or TPZ 20mg/kg (left panel), (two-way ANOVA: vehicle vs combination interaction $p <0.0001$, Olaparib vs combination interaction $p <0.0001$; TPZ vs combination interaction $p = 0.04$, $n = 5$ mice per group)). Data are presented as mean ± SEM.
(B) Percentage weight loss in mice treated with vehicle, BMN673, TPZ or combination of TPZ and BMN673 ($n = 5$ mice per group). Data are presented as mean ± SEM.
(C) Plot of Leukocytes, white blood cell (WBC), red blood cell (RBC), platelet count, and hematocrit levels in mice with SUM149 allograft treated with vehicle, BMN673 0.1mg/kg, TPZ 20 mg/kg, or combination of BMN673 and TPZ ($n = 5$ mice in each group). *$p < 0.05$, by one-way ANOVA, drug treatment vs vehicle. Data are presented as mean ± SEM across all panels.
(D) Multiple organs were analyzed for histological signs of toxicity. Representative images are shown from each treatment group. Scale bars 50 μm.
Table S1

Primers for QPCR

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<td>BRCA1</td>
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Target sequences for shRNAs and Cas9-CRISPER

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