Pamiparib-induced Replication Stress Augments the Efficacy of Temozolomide in GBM PDX Models

ABSTRACT #2910

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SUMMARY

BACKGROUND: Temozolomide (TMZ) is the standard chemotherapy for glioblastoma (GBM), but resistance develops in nearly all patients, highlighting the need for sensitizing strategies. Poly (ADP)-ribose polymerase inhibitors (PARPi) disrupt DNA repair and are under investigation as TMZ-sensitizing agents. This study evaluates pamiparib, alone and in combination with TMZ, and investigates the molecular mechanisms underlying its sensitizing effect in GBM preclinical models.

METHODS: Cell growth and drug effects were determined by the CyQuant or neurosphere assays. DNA damage and signaling response after TMZ ± pamiparib treatment were assessed *in vitro* by comet assey, immunofluorescence and immunoblotting. Analyses of *in vivo* efficacy were performed in orthotopic PDX models.

RESULTS:

- Pamiparib (3 μM or higher) exhibited direct cytotoxicity, while lower concentrations (0.1-1.0 μM) synergized with TMZ, particularly during the S- and G2/M phases of the cell cycle, suggesting replication-associated stress.
- In vivo studies showed that pamiparib alone (0.75 or 3 mg/kg, twice daily) had no antitumor effect. In contrast, temozolomide (TMZ) was effective in three patient-derived xenograft (PDX) models GBM12, GBM22, and GBM84. Combination therapy significantly extended survival in GBM12 and GBM22 but not in GBM84, indicating selective sensitization in TMZ-sensitive, MGMT-hypermethylated models.
- Mechanistically, A) PARP activity contributes to the repair of TMZ-induced DNA lesions via base excision repair (BER). B) PARP facilitates the recruitment of translocases such as SMARCAL1 to stalled replication forks. C) PARP regulates endonucleases like MRE11 and EXO1 to protect stressed replication forks and nascent DNA.
- TMZ-sensitizing effects of pamiparib were independent of PARP mediated BER inhibition (data not shown). Similarly, silencing SMARCAL1 or inhibiting MRE11 with mirin did not alter pamiparib-mediated sensitization in U251 cells. While EXO1 silencing attenuated DNA damage signaling, it had minimal impact on TMZ sensitivity or pamiparib-enhanced cytotoxicity. These results suggest that PARP's roles in BER and fork protection contribute only modestly to TMZ responsiveness in this context.
- Alternatively, PARP may mitigate replication stress by promoting lesion bypass and/or replication gap repair through translesion synthesis (TLS), where replicative polymerases are replaced with low-fidelity polymerases.
- Consistent with a role for TLS, RAD18 silencing enhanced sensitivity to both TMZ and pamiparib, with the combination showing superior efficacy. Similarly, pretreatment with the TLS inhibitor JH-RE06 sensitized a subset of TMZ-resistant GBM12 sublines, which were hypersensitive to the pamiparib/TMZ combination.

CONCLUSIONS: Pamiparib enhances TMZ efficacy in a subset of primary GBM. While further *in vivo* validation is needed, TLS deficiency may be a key determinant of pamiparib-mediated sensitization.

RESULTS

FIGURE 1: *In vitro* analysis of the biological effects of Pamiparib in GBM cells

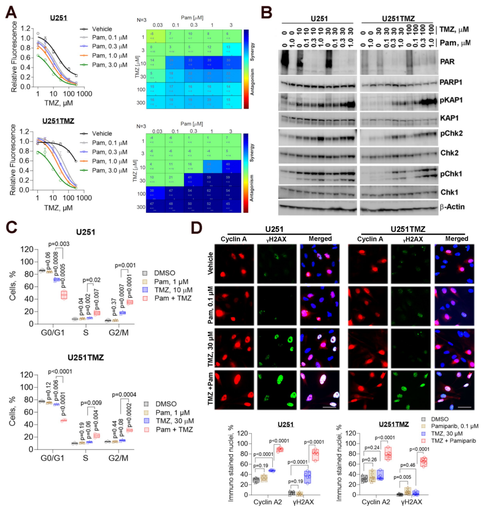

Figure 1. Pamiparib enhances TMZ-induced cytotoxic effects. A) U251 and U251TMZ cells were treated with increasing concentrations of temozolomide (TMZ), either alone or in combination with Pamiparib (0.1–3.0 μM). Cell viability was assessed one week later using the CyQuant assay. Corresponding surface Bliss synergy plots are shown (right). **B)** DNA damage response signaling was evaluated in U251 and U251TMZ cells following a 48-hour pre-treatment with TMZ ± Pamiparib (0.1–1.0 μM). Expression levels of key DNA damage response proteins were analyzed by western blot; β-Actin served as a loading control. **C)** Cell cycle distribution was analyzed by ModFit, following a 48-hour treatment with the indicated conditions. Results are presented as floating bar graphs with the mean indicated (n = 3). Statistical analysis was performed using a two-sample t-test. **D)** Representative immunofluorescence images (top) and quantification (bottom) of cell nuclei stained for Cyclin A2 (red), γH2AX foci (green), and DAPI (blue) after treatment with TMZ (30 μM) ± Pamiparib (0.1 μM) for 48 hours. Cells with >20 γH2AX foci per nucleus were scored as positive for DNA damage. Quantification is shown as floating bar graphs with the mean indicated (n = 5–6 view fields from two independent slides, ~200 nuclei per condition), analyzed using a two-sample t-test. Scale bar = 50 μm.

FIGURE 2: Pamiparib-mediated sensitization of GBM12 primary cell cultures

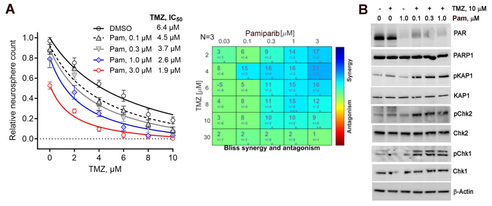

Figure 2: *In vitro* evaluation of pamiparib-mediated sensitization in GBM12. A) GBM12 cells were treated with TMZ (0-10 μM), alone or with pamiparib (0.1–1.0 μM), and analyzed via neurosphere assay; corresponding Bliss surface plots are shown (right). **B)** Western blots showing the effects of a 48-hour pre-treatment with TMZ ± pamiparib on DNA damage signaling, β-Actin was a loading control.

FIGURE 3: *In vivo* evaluation of Pamiparib-mediated TMZ sensitization in GBM PDX models

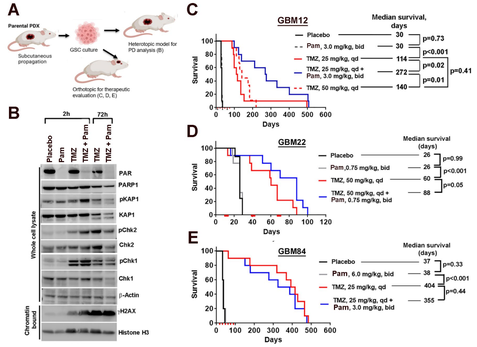

Figure 3. In vivo efficacy of TMZ ± Pamiparib in GBM PDX models.. A) Schematic overview of xenograft models used. **B)** Western blot analysis of DNA damage response signaling from pooled tumor lysates (n = 3 per group) in GBM12 flank xenografts (≥300 mm³) treated for 5 days with: (i) placebo, (ii) Pamiparib (3.0 mg/kg BID), (iii, v) TMZ (25 mg/kg QD), or (iv, vi) TMZ + Pamiparib. Tumors were collected either 2 or 72 hours post-final TMZ dose. **C–E)** Kaplan–Meier survival curves of mice bearing orthotopic PDX tumors: GBM12 (C), GBM22 (D), and GBM84 (E). Mice received a placebo, Pamiparib, and/or TMZ (days 1–5, three cycles, every 28 days). Survival was monitored and analyzed by the log-rank test.

FIGURE 4: Effect of Fork remodelers on Pamiparib-mediated sensitization

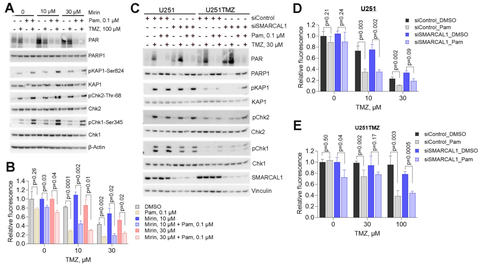

Figure 4. Fork remodeling factors SMARCAL1 and MRE11 do not affect sensitivity to TMZ ± Pamiparib. A) DNA damage signaling in U251 cells following indicated treatments. **B)** U251 cells pretreated with Mirin (10 or 30 μM) or vehicle were treated with TMZ ± Pamiparib; cell growth was measured by CyQuant assay. **C)** DNA damage signaling in U251 cells transfected with control or SMARCAL1 siRNA and treated as indicated. **D-E)** U251 cells with control or SMARCAL1 siRNA were treated with TMZ ± Pamiparib; cell growth was assessed by CyQuant assay and analyzed using a two-sample t-test.

FIGURE 5: Effect of EXO1 loss on TMZ ± Pamiparib induced DNA damage signaling

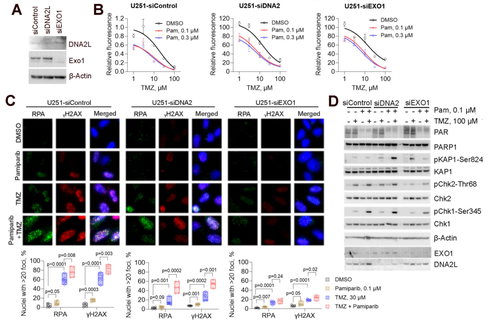

Figure 5: EXO1, but not DNA2, modulates the response to TMZ ± Pamiparib. A-B) Immunoblot validation of DNA2 and EXO1 knockdown (A) in U251 cells transfected with control or target-specific siRNA, followed by treatment with TMZ ± Pamiparib and cell growth assessment (B). **C)** Representative images (top) and quantification (bottom) of RPA (green), γH2AX (red), and DAPI (blue) staining after 24-hour treatment with 30 μM TMZ ± 0.1 μM Pamiparib. **D)** DNA damage signaling in U251 cells post-siRNA-transfection and indicated treatments.

FIGURE 6: TLS activity predicts response to Pamiparib-mediated TMZ sensitization

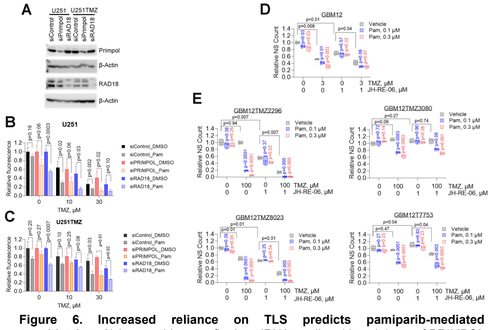

Figure 6. Increased reliance on TLS predicts pamiparib-mediated sensitization. A) Immunoblots confirming siRNA-mediated knockdown of PRIMPOL and RAD18, β-Actin was a loading control. **B–C)** Bar graphs illustrating the effect of PRIMPOL or RAD18 knockdown on sensitivity to TMZ ± pamiparib in U251 (B) and U251TMZ (C) cells. **D–E)** Floating bar plots depicting neurosphere (NS) formation as a surrogate of growth showing the impact of TLS inhibitor JH-RE06 on TMZ ± pamiparib response in GBM12 (D) and selected TMZ-resistant GBM12 sublines (E).

DISCUSSION

- Pamiparib is a brain-penetrant PARP inhibitor, which can sensitize a subset of TMZ-sensitive tumors.
- The sensitizing effect of Pamiparib is potentially linked to impaired DNA damage response during S and G2 phases, suggesting replication-associated vulnerability as an underlying mechanism.
- Modulators of stalled replication forks, such as SMARCAL1 or MRE11, do not appear to mediate sensitization, suggesting that the PARP-trapping effect of Pamiparib may be dispensable in this context.
- Although EXO1 activity amplifies DNA damage signaling, it does not significantly alter sensitivity to TMZ or TMZ/PARPi, suggesting that EXO1 or DNA2 have a limited role in pamiparib-mediated sensitization.
- TLS-associated tolerance to TMZ appears to contribute to PARPi-mediated sensitization, potentially reflecting a role for PARP in promoting TLS activity or generating substrates repaired via TLS.
- Further analysis will identify deficiencies in replicative or post-replication repair pathways, rendering a subset of GBM susceptible to Pamiparib-mediated sensitization.

ACKNOWLEDGEMENTS

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