Translesion Synthesis Limits Temozolomide Response and Modulates PARP Inhibitor Sensitization in MGMT-Methylated GBM

Abstract #4125

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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.

OBJECTIVES: To evaluate TMZ-sensitizing effects of pamperer, and to investigate the molecular mechanisms underlying its sensitizing effect in GBM preclinical models.

METHODS: Cell growth and drug effects were determined by the CyQuant or clonogenic assays. DNA damage and signaling response after TMZ ± pamiparib treatment were assessed *in vitro* by immunofluorescence and immunoblotting. Analyses of *in vivo* efficacy were performed in orthotopic and PD analysis in heterotopic 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. Temozolomide (TMZ) was effective in all three MGMT-hypermethylated PDX models GBM12, GBM22, and GBM84. Combination therapy extended survival in GBM12 and GBM22 but not in GBM84, indicating selective sensitization.
- Mechanistically, TMZ-sensitizing effects of pamiparib were independent of BER. Similarly, silencing SMARCAL1 or inhibiting MRE11 with mirin did not alter pamiparib-mediated sensitization in U251 cells ((data not shown). These results suggest that PARP's roles in BER and fork protection only modestly contribute to TMZ responsiveness.
- We hypothesized that PARP activity 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.

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

RESULTS 1

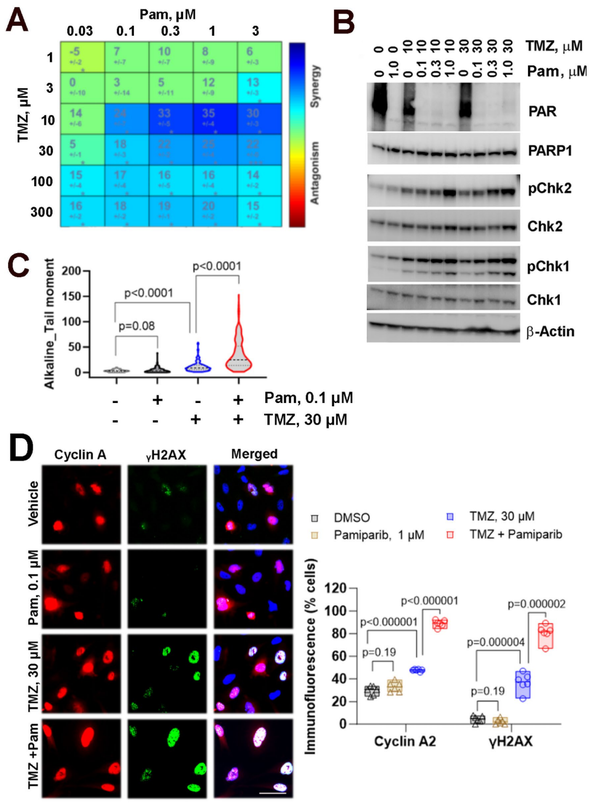

Figure 1. Pamiparib enhances TMZ-induced cytotoxicity in U251 cells. A) Bliss synergy 2D plots across a concentration matrix of TMZ alone or with pamiparib (0.1–3.0 μM). Cell viability was measured after 6 days by CyQuant assay; corresponding surface synergy plots were generated in Combenefit, interactive platform for analysis and visualization of drug combinations, are shown. **B)** DNA damage response signaling was evaluated 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)** Alkaline comet tail moment quantified by CometScore 2 after 24 h treatment. Violin plots show median values (n > 150 nuclei per condition); P values by two-sample t-test. **D)** Representative immunofluorescence images and quantification of Cyclin A2 (red), γH2AX (green), and DAPI (blue) after TMZ (30 μM) ± pamiparib (1 μM) for 48 h. Cells with >20 γH2AX foci/nucleus were scored as DNA damage–positive. Floating bar graphs show mean values (n = 5–6 fields from two independent slides per condition); P values by two-sample t-test. Scale bar, 25 μm.

RESULTS 2

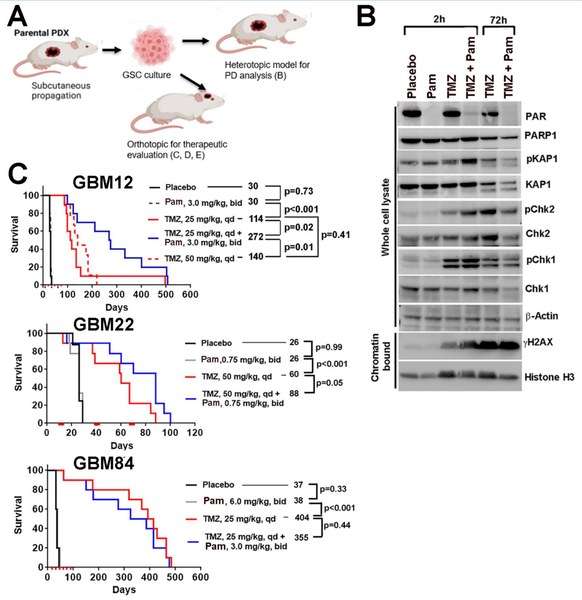

Figure 2. In vivo efficacy of TMZ ± pamiparib in GBM PDX models. A) Schematic overview of xenograft models. **B)** Western blot analysis of DNA damage response signaling in pooled tumor lysates (n = 3/group) from GBM12 flank xenografts (≥300 mm³) treated for 5 days with placebo, pamiparib (3 mg/kg BID), TMZ (25 mg/kg QD), or TMZ + pamiparib. Tumors were collected 2 or 72 h after TMZ. **C)** Kaplan–Meier survival of athymic nude mice bearing orthotopic tumors treated with placebo, pamiparib, and/or TMZ (days 1–5, three 28-day cycles). Survival was analyzed by log-rank test.

RESULTS 3

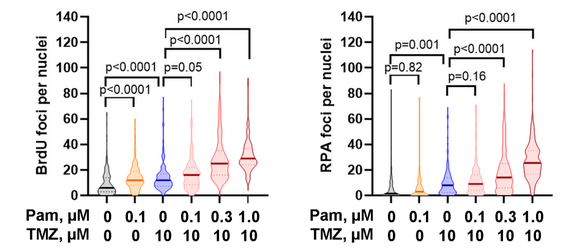

Figure 3. TMZ ± Pam treatment increases single-stranded DNA gaps. Violin plots show quantification of native BrdU foci (left) and RPA foci (right) in U251 cells 24 h after the indicated treatments. Immunofluorescence images were analyzed using ImageJ (n > 200 nuclei per condition). P values were calculated by two-sample t-test.

RESULTS 4

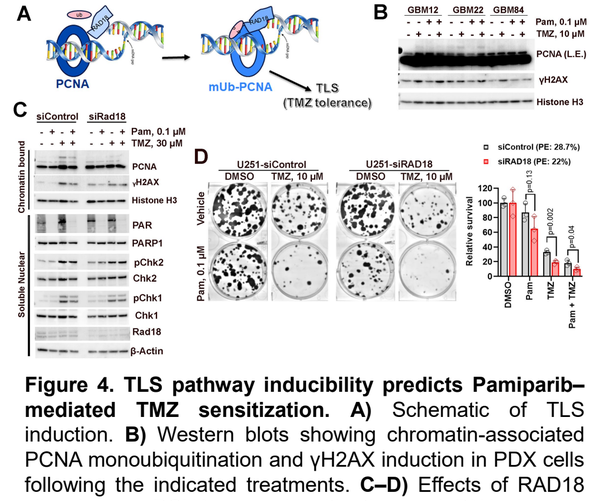

Figure 4. TLS pathway inducibility predicts Pamiparib–mediated TMZ sensitization. A) Schematic of TLS induction. **B)** Western blots showing chromatin-associated PCNA monoubiquitination and γH2AX induction in PDX cells following the indicated treatments. **C–D)** Effects of RAD18 silencing on PCNA monoubiquitination and DNA damage signaling (C) and clonogenic survival (D) in U251 cells.

CONCLUSIONS

- Pamiparib is a brain-penetrant PARP inhibitor, which can sensitize a subset of MGMT-hypermethylated tumors.
- The sensitizing effect of Pamiparib is potentially linked to accumulation of single strand DNA gaps, 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 fork-protective role of PARP 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 contribute to PARPi-mediated TMZ sensitization, potentially reflecting a role for PARP in promoting TLS 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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