

***TP53* Loss Creates a Replication Stress Dependency Targetable by ATR and PARP Inhibition in Waldenström Macroglobulinemia**

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Background

Loss of *TP53* disrupts the G1 checkpoint and promotes cell cycle progression creating barriers to faithful DNA replication. In high replicative conditions, to preserve genome stability, cells activate replication stress (RS) response (RSR) and DNA damage response (DDR), including ATR-mediated signaling pathways that coordinate cell-cycle arrest and facilitate DNA repair. Effective RSR supports cell survival while unresolved RS leads to apoptosis.

TP53 alterations occur in 10–20% of Waldenström Macroglobulinemia (WM) cases and identify a high-risk subgroup with worse outcomes. The biological mechanisms underlying this inferior prognosis—including the DDR and RSR dependencies that sustain *TP53*-altered WM—remain poorly defined and are the focus of this study.

Methods

WM patient-derived cell lines MWCL (*TP53*^{del/mut}) and BCWM.1 (*TP53*^{WT}-WM) were used. *TP53* knockout BCWM.1 clones (*TP53*^{KO}-WM) were generated using CRISPR–Cas9. DNA damage was assessed by alkaline comet assay. RSR function was evaluated by treating cells with hydroxyurea (HU) for 24h, releasing them into fresh media, and analyzing cell-cycle progression by flow cytometry. DDR and RSR activation were evaluated by Western blot (WB) analysis of pathway related phosphorylated proteins (pRPA, pCHK1, pKAP1). ATR inhibitor ART0380 (ATRi, Artios Pharma), and PARP inhibitors olaparib, niraparib, talazoparib and saruparib (PARPi) were used to assess drug sensitivity via CellTiter-Glo® assay in *TP53*^{WT}-WM and *TP53*^{KO}-WM cells. *In vivo* safety and efficacy were explored in a *TP53*^{KO}-WM NRG xenograft mouse model.

Results

Alkaline comet assays showed higher tail moments in MWCL (*TP53*^{del/mut}) compared with BCWM.1 (*TP53*^{WT}-WM), indicating increased DNA damage associated with *TP53* loss (Fig.1A). Compared to *TP53*^{WT}-WM cells, isogenic *TP53*^{KO}-WM cells

accumulate in S-phase 16h following HU release, suggesting *TP53* loss induces increased RS and DNA damage which needs to be resolved before progressing to G2-phase (Fig 1B). WB showed increased levels of pRPA and pCHK1 in *TP53*^{KO}-WM cells at 16h, corroborating RS-recovery delay (Fig.1C).

Since increased pCHK1 levels observed in *TP53*^{KO}-WM, we tested whether *TP53*^{KO}-WM cells depend on RSR for survival by challenging them with ATRi. ATRi treatment increased levels of pRPA, pKAP1 in *TP53*^{KO}-WM (Fig.1D) and caused significant antitumor effect compared to *TP53*^{WT}-WM cells (Fig. 1D, E). When ATRi is combined with agents that exacerbate RS and DNA damage, such as PARPi, significant synergistic antitumor activity was seen, especially in *TP53*^{KO}-WM cells (Bliss Score >10, Fig 1F). This synergy was validated using four different PARPi, including a PARP1-specific inhibitor (saruparib) with ATRi. Using the comet assay, we assessed unrepaired DNA damage following ATRi-PARPi combination. Indeed, increased DNA damage in *TP53*^{KO}-WM cells was seen with ATRi or ATRi-PARP combination treatment compared to *TP53*^{WT}-WM cells (Fig.1G). *In vivo* assessment of antitumor activity confirmed that the ARTi/PARPi combination was safe and effective in killing *TP53*^{KO}-WM cells.

Conclusions

TP53-loss increases RS and creates RSR pathway dependence in WM. ATR inhibition exploits this vulnerability, leading to enhanced DNA damage and decreased viability, particularly when combined with PARP inhibition in *TP53*^{KO}-WM models. These findings identify RSR-targeted therapy as a rational strategy for *TP53*-altered WM and support further clinical evaluation of ATR–PARP inhibitor combinations for this high-risk subgroup.

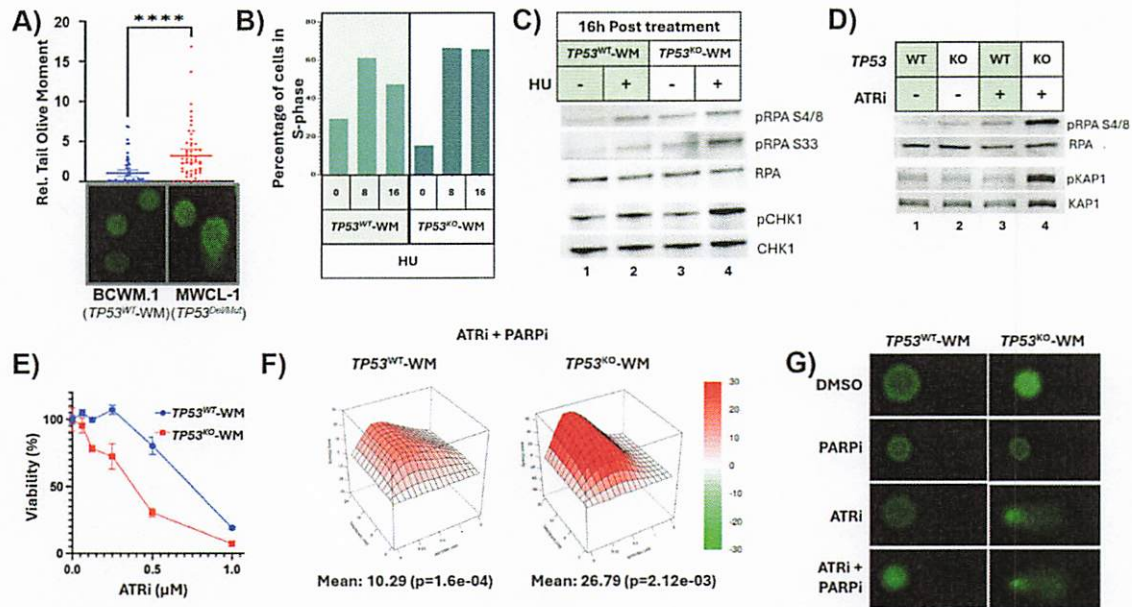


Figure 1. $TP53$ loss in WM increases RS and creates a dependency on RSR pathways for survival.

A) Alkaline comet assay showed increased tail moment, indicative of DNA damage, in WM-derived cell line with $TP53$ -altered genotype. **B)** BCWM.1 ($TP53^{WT}$ -WM) and its isogenic $TP53$ knockout clone ($TP53^{KO}$ -WM) cells were treated with HU for 24h and subsequently released in fresh media. Cell-cycle progression was evaluated at three timepoints, immediately after the treatment (0h), and 8h and 16h after release. S-phase population remained elevated in $TP53^{KO}$ -WM cells 16h after release. In contrast, $TP53^{WT}$ -WM cells exhibited an increase in the S-phase population at 8h after release, followed by a reduction at 16h, suggesting recovery from RS. **C)** Consistent with persistent RS, increased levels of RSR-associated phosphorylated proteins (pRPA pCHK1) were detected in $TP53^{KO}$ -WM cells at 16h post-release by WB. **D)** ATRi (ART0380) treatment increased the phosphorylation of proteins associated with DDR levels pRPA and pKAP1 in $TP53^{KO}$ -WM cells. **E)** Additionally, CellTiter-Glo® viability assays demonstrated that $TP53^{KO}$ cells were more sensitive to ATRi (ART0380). **F)** Bliss Score shows synergy antitumor activity in the ATR-PARPi (ART0380-Talazoparib) combination treatment especially in $TP53^{KO}$ -WM cells. **G)** This treatment also increased DNA damage compared with either agent alone (representative alkaline comet assay images showing DNA damage caused by ART0380 and Talazoparib treatment). **** $p < 0.0001$.

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