PNKP Inhibition Demonstrates Selective Activity in PTEN-Deficient Osteosarcoma and Is Enhanced by NRF2 Blockade

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Significance and Hypothesis:
Osteosarcoma is a radioresistant malignancy with a high propensity for pulmonary metastasis, for which outcomes remain poor and therapeutic progress has stagnated. PTEN pathway disruption is common and associated with aggressive disease and inferior survival. Synthetic lethality offers a strategy to selectively target tumors with defined genetic vulnerabilities. PNKP has been identified as a synthetic lethal partner of PTEN in other cancer models, reflecting increased reliance on DNA repair pathways following PTEN loss–associated genomic instability. However, this interaction has not been evaluated in osteosarcoma.

We evaluated whether inhibition of the DNA repair enzyme PNKP using A12B4C3 (A12) selectively targets PTEN-deficient osteosarcoma, and whether this effect is enhanced by inhibition of NRF2-mediated antioxidant defenses. We hypothesized that NRF2 inhibition would increase oxidative stress and DNA damage, thereby augmenting the cytotoxic effects of PNKP inhibition.

Innovation:
This study translates a validated synthetic lethal interaction between PTEN loss and PNKP inhibition into osteosarcoma, where it has not previously been explored. We further investigate whether this vulnerability can be enhanced through disruption of NRF2-mediated antioxidant defenses.

Approach:
Three PTEN-deficient human osteosarcoma cell lines (143b, SaOS-2, U2OS) and a PTEN-wildtype murine cell line (LM8) were treated with A12 (5–20 μM) alone and in combination with the NRF2 inhibitor ML385 (1–5 μM). PTEN expression was confirmed by Western blotting. Cell viability and clonogenic survival were assessed using Alamar Blue and clonogenic assays. Drug interactions were evaluated using the Bliss independence model. Experiments were performed in technical and independent biological replicates. Statistical significance was determined using appropriate ANOVA models with post hoc testing ($p < 0.05$).

Results:
A12 treatment resulted in a dose-dependent reduction in viability and clonogenic survival across all PTEN-deficient cell lines (143b, SaOS-2, U2OS), consistent with a synthetic lethal interaction (Figure 1). Co-treatment with ML385 further reduced clonogenic survival, with near-complete loss of colony formation at higher combination doses. In contrast, the PTEN wild-type LM8 cell line demonstrated minimal sensitivity to A12 alone, while ML385 reduced viability at higher doses. Combination treatment produced modest additive effects without evidence of enhanced sensitivity to A12 (Figure 2). Bliss independence analysis did not demonstrate consistent synergy between A12 and ML385, despite enhanced cytotoxicity with combination treatment.

Discussion & Impact:
PNKP inhibition demonstrates a vulnerability in PTEN-deficient osteosarcoma that is enhanced by NRF2 blockade. These findings support further investigation of a dual-targeting strategy combining DNA repair inhibition and oxidative stress modulation within a synthetic lethality framework. This approach may have translational relevance

for overcoming therapeutic resistance in osteosarcoma, including potential applications in radiosensitization. Ongoing studies include development of PTEN-matched isogenic models and *in vivo* studies to further validate this relationship.

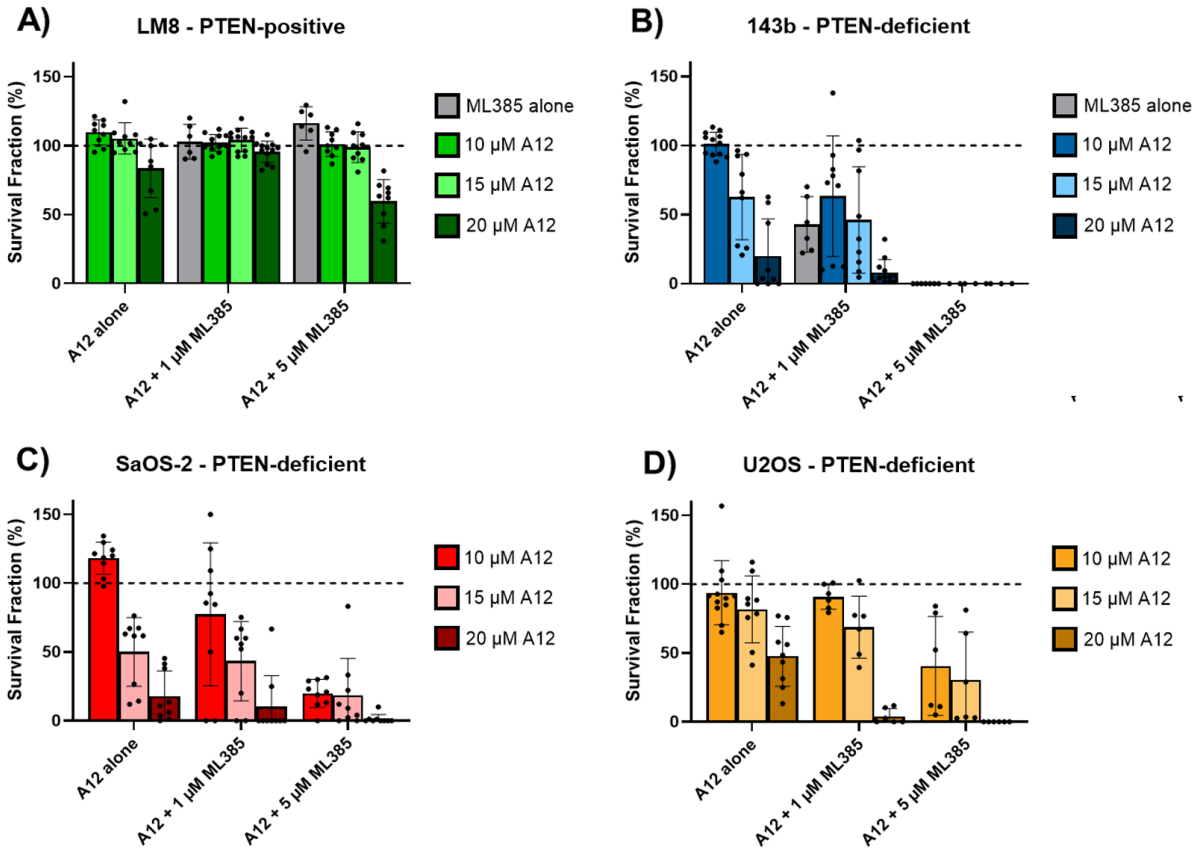

Figure 1. Clonogenic survival of osteosarcoma cell lines following treatment with A12B4C3 (A12) and ML385. Survival fraction (%) of (A) LM8, (B) 143b, (C) SaOS-2, and (D) U2OS cells treated with A12 (10, 15, or 20 µM) and ML385 (1 or 5 µM) as single agents or in combination. Panel titles indicate the phosphatase and tensin homolog (PTEN) status of each cell line. Bars represent mean survival fraction normalized to vehicle control (dimethyl sulfoxide; dashed line at 100%), with individual technical replicates shown ($n \geq 3$). Representative independent biological replicates are shown.

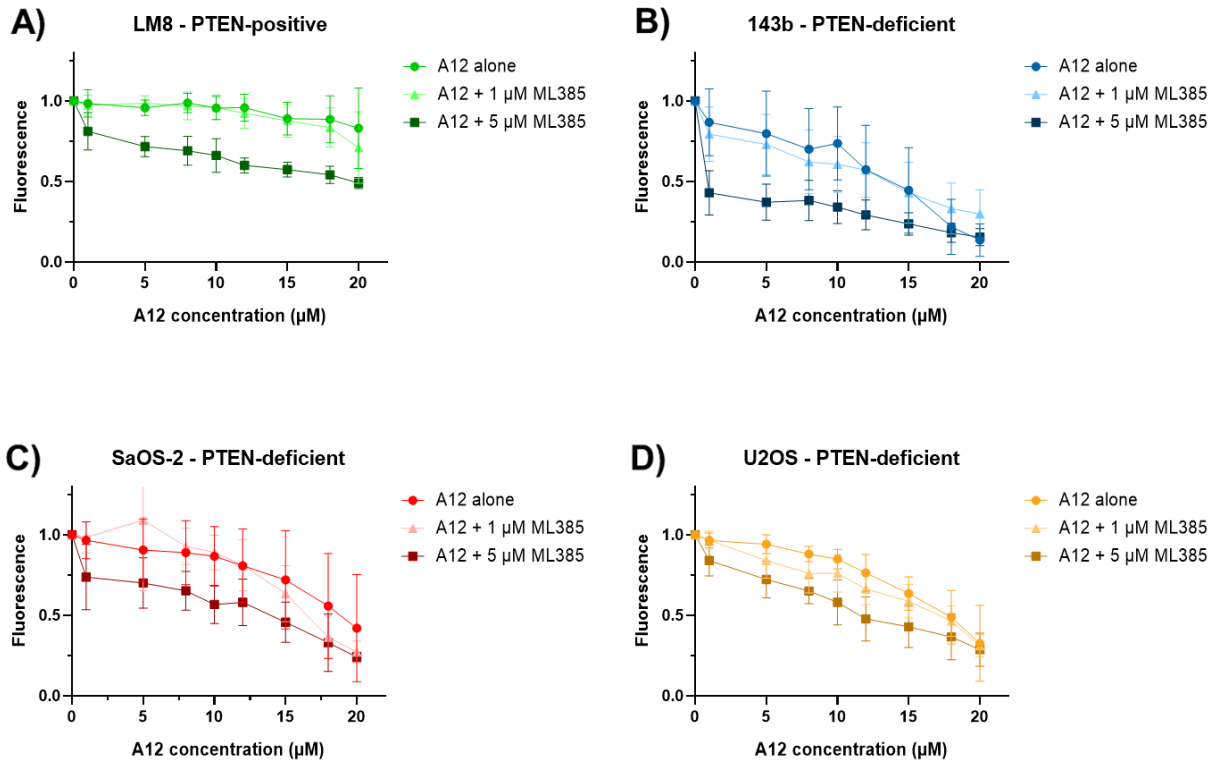

Figure 2. A12 selectively reduces viability in PTEN-deficient osteosarcoma cell lines and is enhanced by NRF2 inhibition.

Alamar Blue fluorescence of (A) LM8 (PTEN wild-type), (B) 143b, (C) SaOS-2, and (D) U2OS (PTEN-deficient) following treatment with A12 (0–20 µM) alone or in combination with ML385 (1 or 5 µM).

A12 induces a consistent, dose-dependent reduction in viability across PTEN-deficient cell lines, with further reduction observed in combination with ML385. In contrast, PTEN wild-type LM8 cells demonstrate minimal response to A12. Data are normalized to vehicle control (100%) and presented as mean ± SD of technical replicates (n ≥ 3), with representative independent biological replicates shown.