TARGETING THE DNA DAMAGE RESPONSE PATHWAY BY COMBINATION OF ATR AND PARP INHIBITION IMPARTS SYNTHETIC LETHALITY TO CERVICAL CANCER CELLS

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Introduction: Cervical cancer (CC) is the fourth most common cancer among women worldwide. Most CC cases are due to high-risk human papillomavirus (HPV) infection, which expresses oncoproteins that inhibit tumor suppressors causing loss of G1-S cell cycle checkpoint control and defective DNA repair. Targeting the DNA damage response (DDR) pathway is being studied as an effective treatment strategy in metastatic CC, with specific focus on increasing dependence upon the Poly (ADP-ribose) Polymerase (PARP) DNA repair pathway. We hypothesized that increasing dependence upon the PARP pathway through abrogation of the G2 cell cycle checkpoint by ATR inhibition would sensitize CC cells to subsequent PARP inhibition.

Methods: The impact of drug sequencing on cytotoxic cell death was determined in two HPV+ cervical cancer cells lines (Caski and SiHa) by treatment with the ATR inhibitor (ATRi) AZD6738, PARP inhibitor (PARPi) olaparib, or both, given either simultaneously or by pre-treatment with ATRi. The degree of drug interaction was determined by isobologram analysis to determine the combination index for CC cells exposed to simultaneous ATRi/PARPi treatment and ATRi pre-treatment followed by sequential PARPi. Changes in cell viability in the drug combination versus PARPi alone groups was measured using MTS cell viability assays. Expression of proteins involved in the DDR pathway including PARP and ATR, as well as downstream cell cycle checkpoint proteins, were evaluated by western blot.

Results: A synergistic effect was observed when CC cells were simultaneously treated and when pre-treated with ATRi followed by PARPi, likely due to the observed upregulation of the PARP pathway after ATR inhibition. Cell viability in the drug combination groups, for both 48- and 72-hour incubation periods, showed a drop in cell survival below 50% compared to the PARPi alone groups. Lower IC50 values were identified when comparing PARPi alone versus combination treatment, indicating the potential for PARPi dose de-escalation in treatment with ATRi. Western blotting of Caski cells, with ATRi pre-treatment followed by sequential PARPi, demonstrated both a decrease in total PARP levels and an increase in cleaved PARP indicating increased

cell death, while also demonstrating a decrease in Chk-1, P-Chk1 and P-Chk2 indicating G2 checkpoint blockade.
Conclusion: HPV oncoproteins reduce HPV+ CC cells' ability to repair DNA damage via homologous recombination. Treatment with ATRi may increase reliance upon the PARP pathway for DNA repair through the more error-prone nonhomologous end joining, leaving this pathway more vulnerable to PARP inhibition. Combination of ATRi and PARPi is more effective at decreasing CC cell viability compared to PARPi or ATRi treatment alone.