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AZD1775 INDUCES TOXICITY THROUGH DOUBLE-STRANDED DNA BREAKS INDEPENDENT OF 5-FU ACTIVITY IN P53 MUTATED COLORECTAL CANCER CELLS

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Introduction: Colorectal cancer is a global health problem with an estimated 700,000 deaths per year. AZD1775 is a small molecule WEE1 inhibitor used in combination with DNA-damaging agents to cause premature mitosis and cell death in p53 mutated cancer cells. Recently, AZD1775 has been shown to have monotherapeutic activity by causing double-stranded DNA (DS-DNA) breaks as a result of nucleotide exhaustion. We sought to determine the dominant mechanism of action of AZD1775 in combination with 5-FU in a p53-mutated colorectal cancer cell line.

Method: HT29 cells (p53 mutated) were treated with 5-FU (1 μM) for 24 hours followed by AZD1775 (300 nM). Proliferation was assessed using a WST-1 assay. Flow cytometry was used to quantify levels of double-stranded DNA breaks and premature mitosis using the specific markers γH2AX and pH3. Caspase-3 dependent apoptosis was quantified using an Incucyte Imaging system.

Result: AZD1775 significantly improved the cytotoxicity of 5-FU decreasing the IC50 from 9.3 μM to 3.5 μM. It caused significantly more mitosis (3.8% vs 56.2%), DS-DNA breaks (5.1% vs. 60.5%) and caspase-3 dependent apoptosis (4% vs. 13%) compared to 5-FU alone. The addition of exogenous nucleosides significantly rescued the increased DS-DNA breaks (60.5% vs 6.9%) and caspase-3 dependent apoptosis (13% vs 4.8%) caused by AZD1775, suggesting this to be the dominant mechanism of action, not premature mitosis.

Conclusion: AZD1775 has independent cytotoxic effects from 5-FU in p53-mutated colorectal cancer cells. This finding is important for designers of future clinical trials when considering the timing and duration of AZD1775 treatment.

Take-home message:
AZD1775 causes DS-DNA breaks and has independent cytotoxic effects from 5-FU in p53-mutated colorectal cancer cells.