Checkpoint kinase 1 (Chk1) is a key regulator of the cellular response to replication stress, DNA stabilizing damage replication forks, abrogating new fork origin firing, temporarily arresting the cell cycle, and fostering DNA repair including homologous recombination repair (HRR).

The potent, highly selective orally bioavailable small-molecule inhibitor of Chk1, SRA737, is being investigated in clinical trials. PARPi – a distinct class of DDR-inhibitors targeting poly(ADP-ribose) polymerase (PARPi) – are approved for the treatment of ovarian and breast cancers; however, tumors with functional HRR are less sensitive to these agents, thereby limiting their clinical utility.

Several reports have described the synergistic combination of Chk1 and PARPi in vitro and in vivo preclinical settings; however, the mechanistic underpinnings have not been well defined. Here, we explored the efficacy and mechanism of SRA737 in combination with the PARPi, niraparib, in HRR proficient ovarian and breast tumor cell lines.

These findings support the potential therapeutic utility of PARPi in HRR proficient tumors when combined with SRA737.

Figure 1: Network for Potential Synergy Between SRA737 + PARPi.

Figure 2: SRA737 and niraparib demonstrate additive to synergistic cell killing of memory and ovarian cancer cells. (A) BT474 and MCF7 cell viability was measured by cell viability assay (MTT) 8 h after exposure to drug (SRA737, niraparib, or SRA737+niraparib). (B) SRA737 and niraparib were added to BT474 and MCF7 cells at the indicated concentrations for 8 h. Mean ± SD. *p < 0.05 greater than vehicle control; **p < 0.01 greater than vehicle control; ****p < 0.0001 greater than vehicle control.

Figure 3: SRA737 and niraparib synergize to inhibit replication fork protection and enhance DNA repair. BT474 and MCF7 cells were treated with vehicle, different concentrations of SRA737 and/or niraparib for 8 h and then exposed to a high level of replication stress by addition of hydroxyurea (HU) for 1 h. Damage foci were stained with LysosomeTracker Green and DAPI were used as a counterstain. Scale bar, 20 μm. (A) Representative images of control and drug-treated cells. (B) Quantitation of LysosomeTracker Green-positive foci. Data are represented as mean ± SD. *p < 0.05, **p < 0.01, ****p < 0.0001 versus vehicle control.

Figure 4A: SRA737 and niraparib induce multiple cell death pathways.

Figure 4B: SRA737 and niraparib induce multiple cell death pathways.

Figure 5: SRA737 and niraparib combination induce multiple and targeted autophagy. BT474 (25 μM) cells were treated with vehicle, different concentrations of SRA737, or niraparib for 12 h, then exposed to a high level of replication stress by addition of HU for 1 h. Autophagy induction was assessed by immunofluorescence and quantification of autophagosomes. Representative images were acquired with a 60× objective and partially overlap autophagosomes are indicated. Scale bar, 10 μm. (A) Representative images of control and drug-treated cells. (B) Quantitation of autophagosomes. Data are represented as mean ± SD. *p < 0.05, **p < 0.01, ****p < 0.0001 versus vehicle control.

Conclusions

- Checkpoint kinase 1 (CK1) is a key regulator of the cellular response to replication stress, DNA stabilizing damage replication forks, abrogating new fork origin firing, temporarily arresting the cell cycle, and fostering DNA repair, including homologous recombination repair (HRR). The potent, selective oral Chk1 inhibitor, SRA737, is being investigated in clinical trials (NCT02797964, NCT02797977).

- Other DDR inhibitors targeting poly(ADP-ribose) polymerase (PARPi) are approved for the treatment of ovarian and breast cancer; however, tumors with functional HRR are less sensitive to these agents, thereby limiting their clinical potential of these agents.

- In short-term cell viability assays, the combination of SRA737 and niraparib in sensitive cell lines and in advanced cancer cell lines elicits enhanced tumor cell death compared to either agent alone, with evidence of cell death as early as 1.2 h after exposure. Combination index analysis from colorimetric assays indicated highly synergistic activity (CI ≤ 0.5), which was observed across all cell lines and concentrations of each agent.


- Collectively these results argue that autophagic cell death, as well as apoptotic cell death, contributes to the SRA737/niraparib-induced tumor cell killing. The involvement of multiple cell death mechanisms may determine the potential of SRA737/niraparib to overcome resistance in these settings.

- These findings support further clinical investigation of SRA737 in combination with PARPi, such as niraparib, in HRR proficient tumors.

A multicenter Phase 1b/2 study designed to assess the safety, tolerability, pharmacokinetics, and preliminary antitumor activity of SRA737 in combination with niraparib is being planned in subjects with metastatic castration-resistant prostate cancer (NCT02797964).