The Chk1 Inhibitor, SRA737, Synergizes with the PARP Inhibitor, Niraparib, to Kill Carcinoma Cells via Multiple Cell Death Pathways

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Rationale

- Targeting the DNA damage response (DDR) network is a promising strategy for the development of new cancer therapies.
- Checkpoint kinase 1 (Chk1) is a key regulator of the cellular response to replication stress (RS), stabilizing stalled replication forks, abrogating new 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 (PARP) 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 Chk1i and PARPi, even in HRR proficient contexts; however, the mechanism of anti-tumor activity has not been well defined.
- We explored the efficacy and mechanism of cytotoxicity 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. Rationale for Potential Synergy Between SRA737 + PARPi

- Chk1's role regulating replication stress and HRR facilitates several potential SRA737 + PARPi therapeutic scenarios via 'chemical synthetic lethality', the synergistic, contemporaneous inhibition of both Chk1 and PARP.
- Consequently, SRA737 combination therapy with PARPi may (i) deepen responses in HRR deficient tumors; (ii) overcome resistance following PARPitherapy or (iii) expand therapeutic utility into HRR proficient tumor indications.

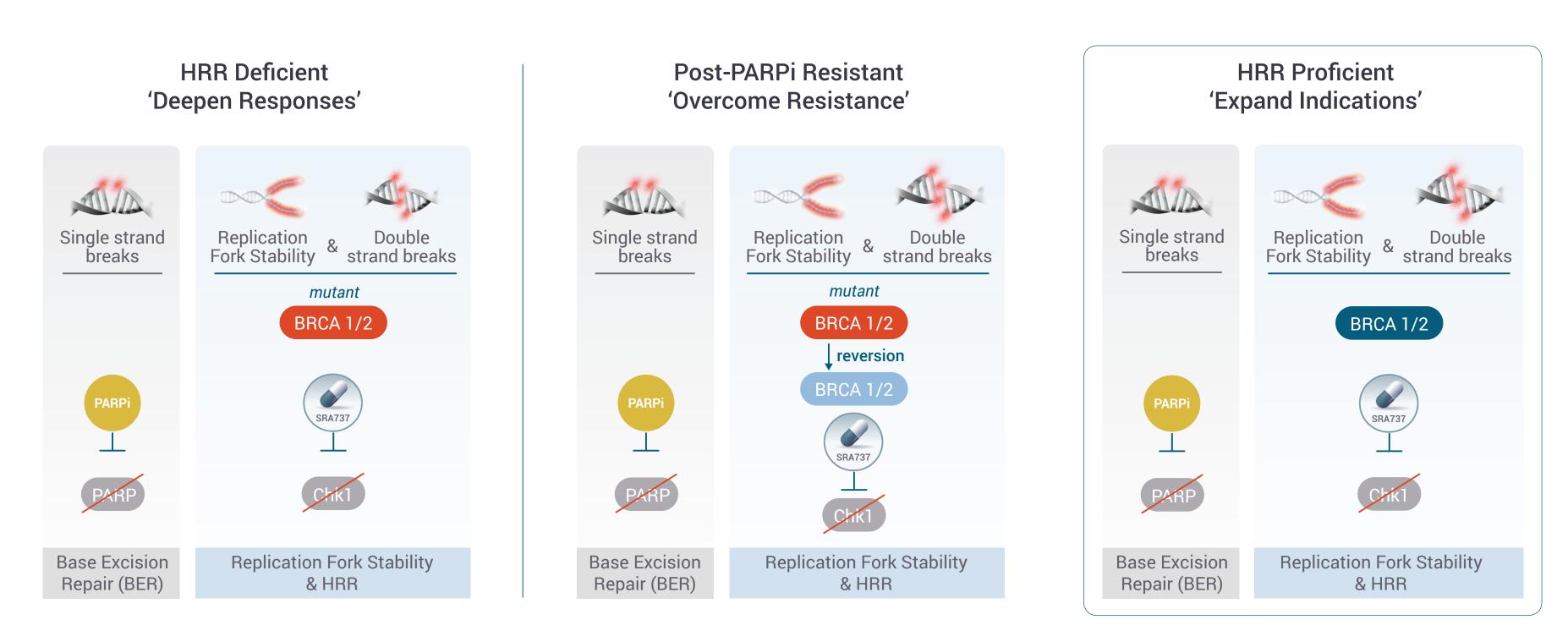
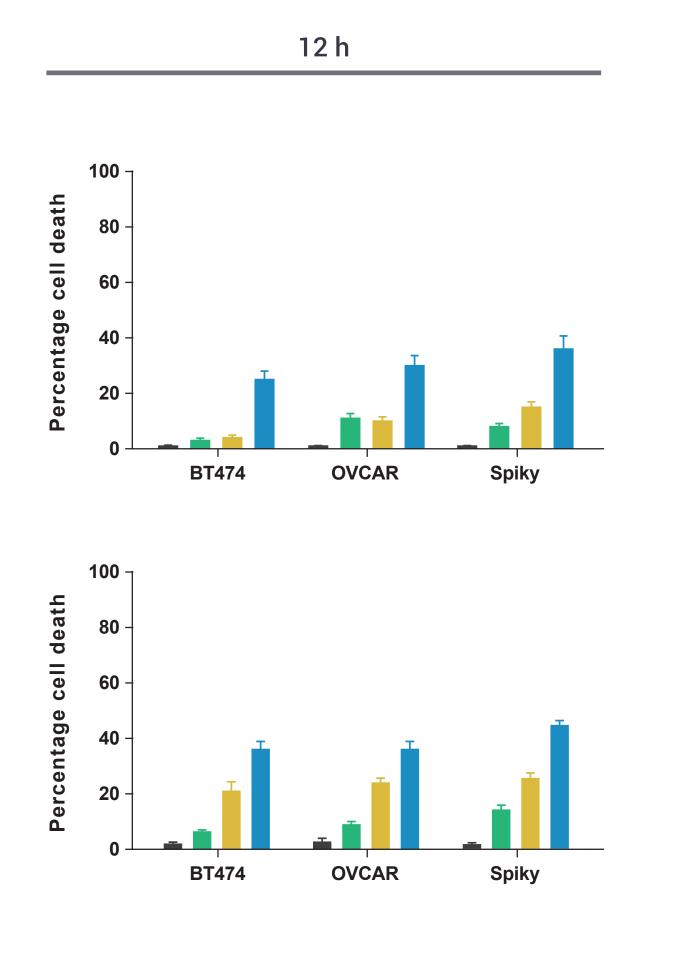
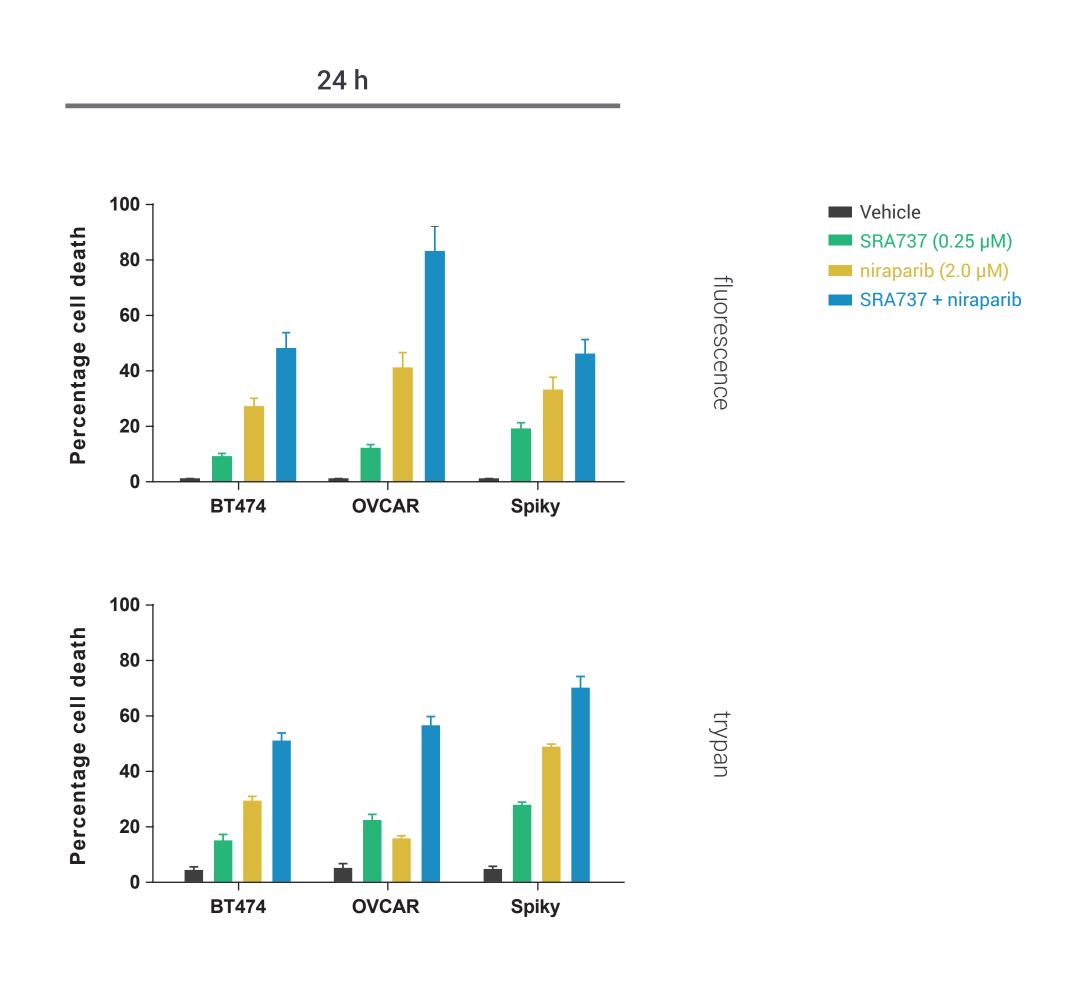


Figure 2. SRA737 and niraparib demonstrate additive to synergistic cell killing of mammary and ovarian cancer cells. Mammary (BT474; BRCA1 wt, BRCA2 [+/S3094*]) or ovarian (OVCAR; wt for both BRCA1 and BRCA2, and Spiky; ((+/-) for both BRCA1 and BRCA2) cancer cells were incubated with vehicle and clinically-achievable concentrations of SRA737 (250 nM), niraparib (2.0 µM) or the combination of both agents for 12 or 24 h. Concentrations of each compound were below their respective IC₅₀ values to facilitate the determination of combinational additivity and synergy. The percentage of dead cells was determined by fluorescence using calcein-AM and di-ethidium bromide or manually counted for trypan blue exclusion. The combination of SRA737 and niraparib elicited greater tumor cell death than either agent alone, as early as 12 h after exposure to drug.





References:

Figure 3. SRA737 and niraparib synergize to kill HRR proficient mammary and ovarian **cancer cells.** To determine whether the interaction between SRA737 and niraparib was synergistic, colony formation assays were performed using median dose effect analyses based on the method of Chou and Tallallay¹. For the indicated dose combinations, colony formation was inhibited between 25% and 82% relative to untreated controls. In mammary (SUM149 [*BRCA1*-∆11] and BT474 [*BRCA2* (wt/S3094*)]) or ovarian (Spiky [(+/-) for both BRCA1 and BRCA2] and SKOV3 [wt for both BRCA1 and BRCA2]) lines, SRA737 synergized with niraparib to kill tumor cells as indicated by combination index scores of less than 0.7. Combination Index ranges in four cell lines Tumor Type

Figure 5. SRA737 and niraparib combine to enhance activation of autophagy regulatory proteins and increase the levels of autophagosomes, resulting in subsequent increase in autolysosomes. The drug combination reduced expression of p62 and LAMP2, increased the expression of ATG5, ATG13 and Beclin1, and enhanced the phosphorylation of ATG13 S318, which is also suggestive of autophagy activation (A). Spiky and BT474 cells were transfected with a plasmid that expresses LC3-GFP-RFP (B). Acid-sensitive GFP and acid-insensitive RFP allows visualization of authophagic flux that involves the transition from autophagosome (neutral pH, GFP punctae visible) to autolysosome (acidic pH, only RFP punctae visible). SRA737, niraparib and, to a greater extent, the drug combination, enhanced autophagosome levels as demonstrated by increased cellular GFP+ punctae. At the earliest time point (4 h), there was minimal drug-stimulated increase in autolysosome levels; however, by 8 h after drug exposure the levels of autophagosomes had declined and the levels of RFP+ punctae in the cells, indicative of autolysosome formation, had increased suggesting that autophagic flux was occurring.

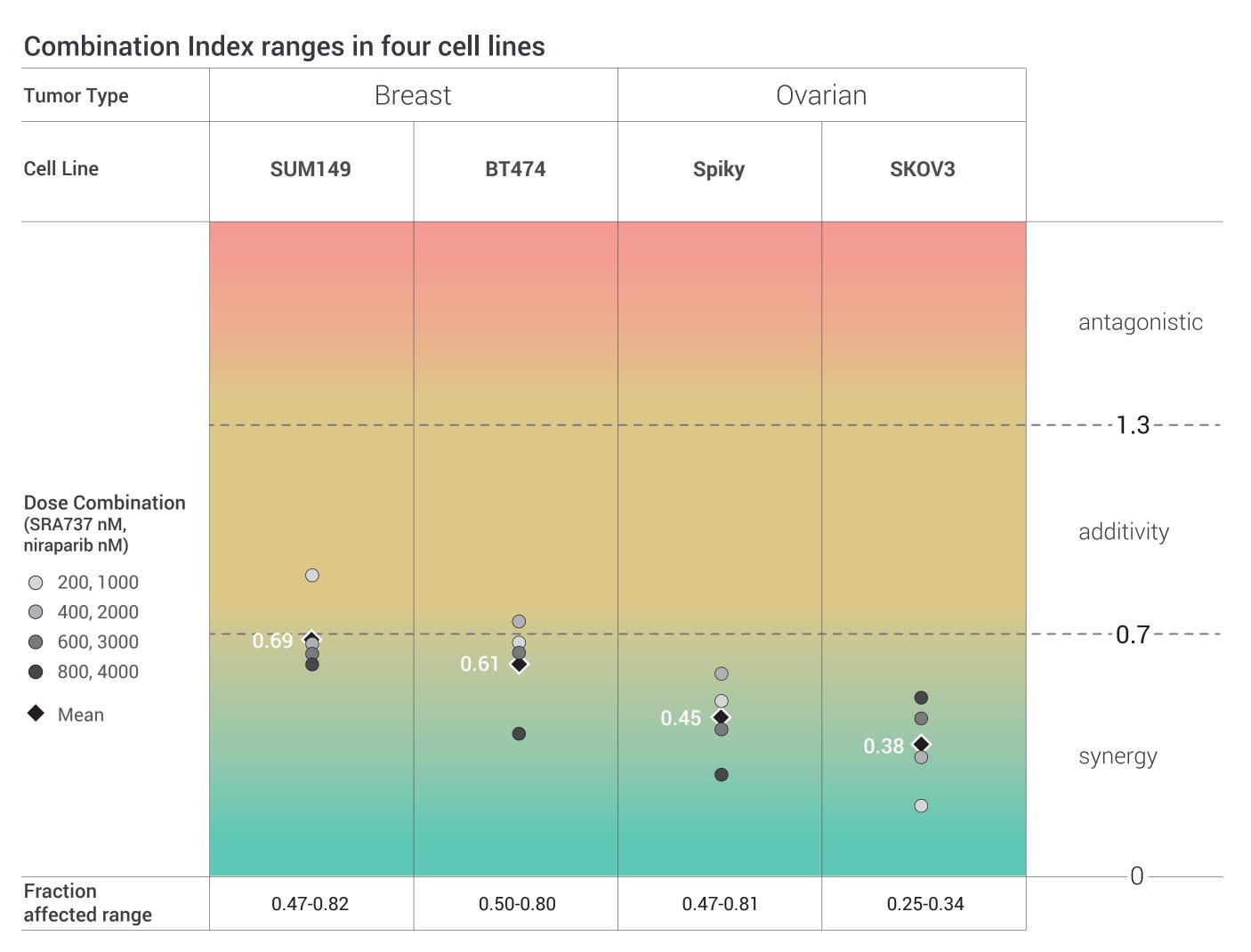
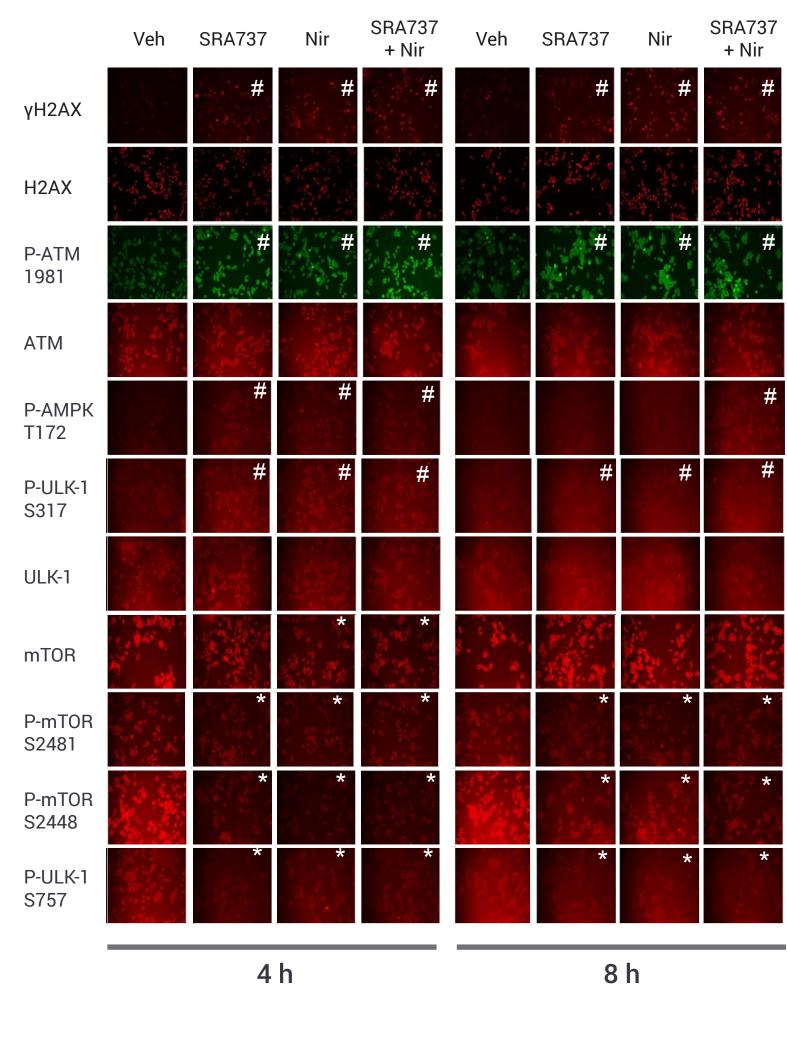


Figure 4. SRA737, niraparib, and the combination, activate a DNA damage response that correlates with the activation of AMPKa and the inactivation of mTOR. Treatment of BT474 cells with 250 nM SRA737, 2.0 µM niraparib, or the combination, enhanced the phosphorylation of H2AX (yH2AX) and of ATM within 4 h of treatment, indicating induction

of DNA double strand breaks and activation of DDR signaling. Concurrent changes in phosphorylation of mTOR (reduced S2448 and S2481) and AMPK (increased T172) and their downstream target ULK1 (reduced S757, increased S317) suggest an induction of autophagy.



p < 0.05 greater than vehicle control; * p < 0.05 less than vehicle control

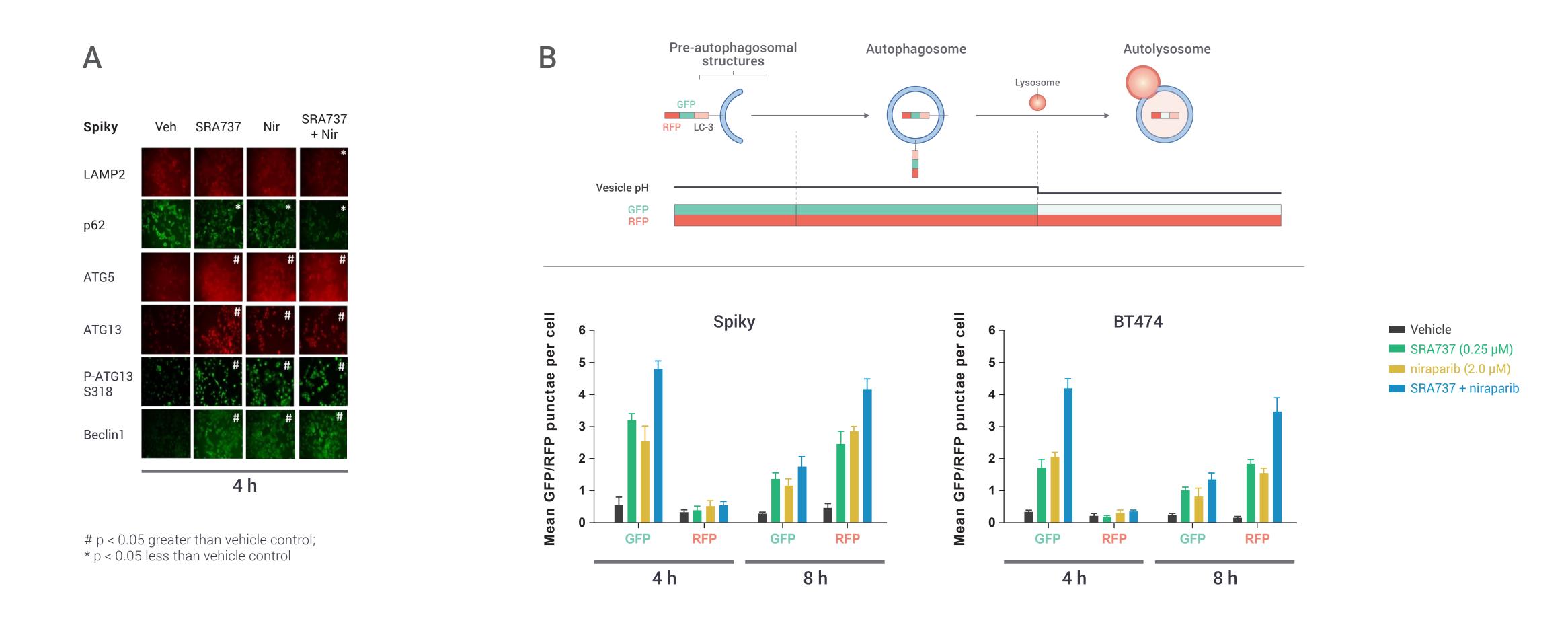


Figure 6. Cell death induced by the SRA737 and niraparib combination involves DNA damage signaling and a functional autophagy pathway. Tumor cells were transfected with a scrambled control siRNA (siSCR) or with siRNA molecules to knock down the expression of the proteins indicated. At 24 h after transfection, cells were treated with vehicle control or the two drugs in combination (250 nM SRA737 and 2.0 µM niraparib). After 24 h, cells were isolated and cell viability determined by a live/dead assay. Knock down of ATM, AMPKa, ULK1, ATG5 and Beclin1 resulted in reduced killing by SRA737 combined with niraparib, suggesting autophagy-dependent cell death may represent a mechanism of cytotoxicity for this DDR combination.

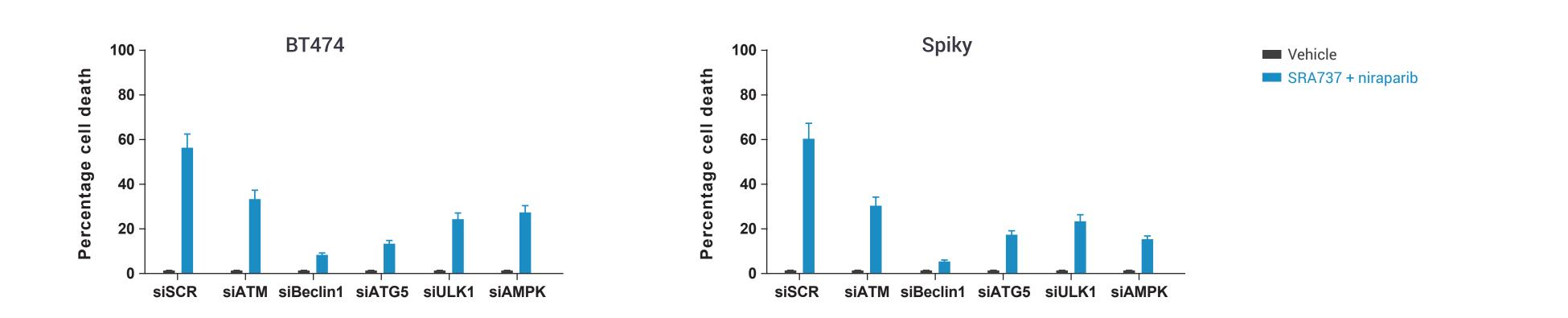
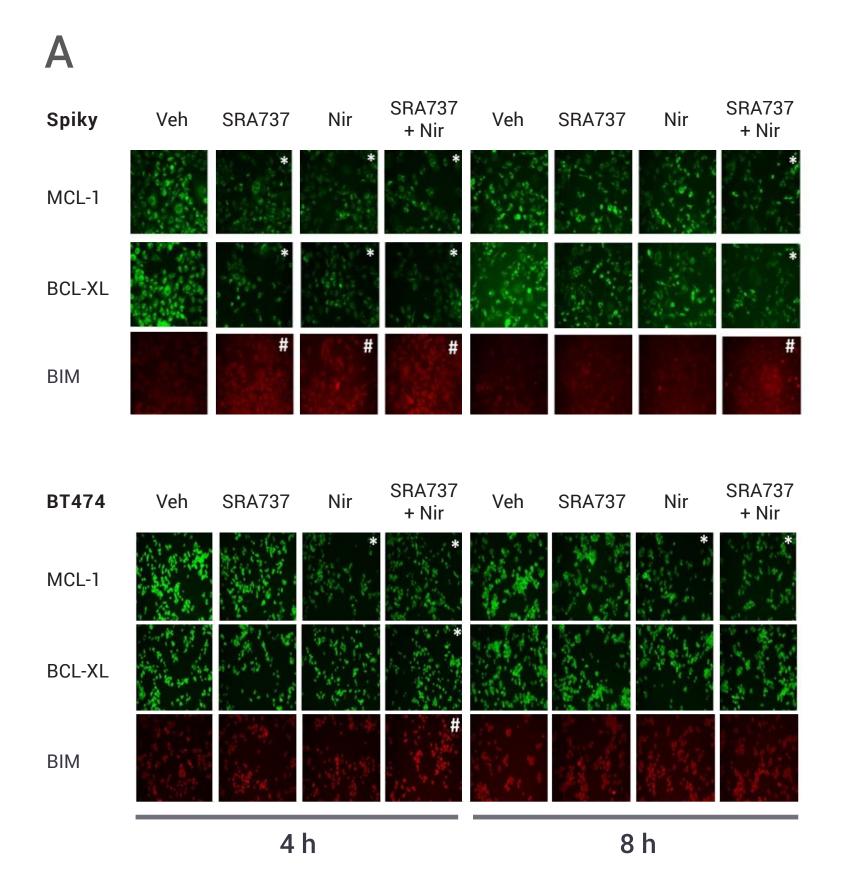
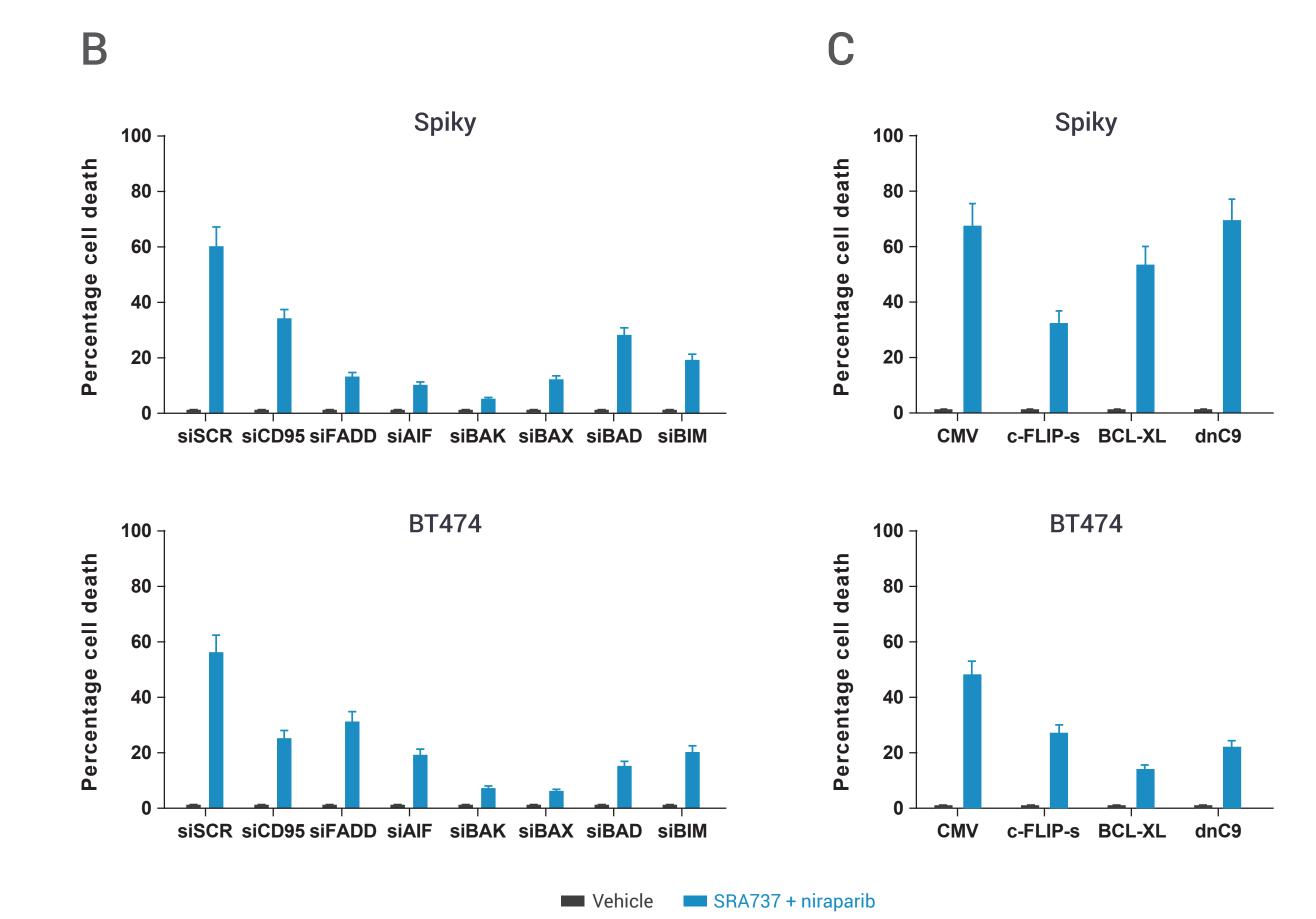


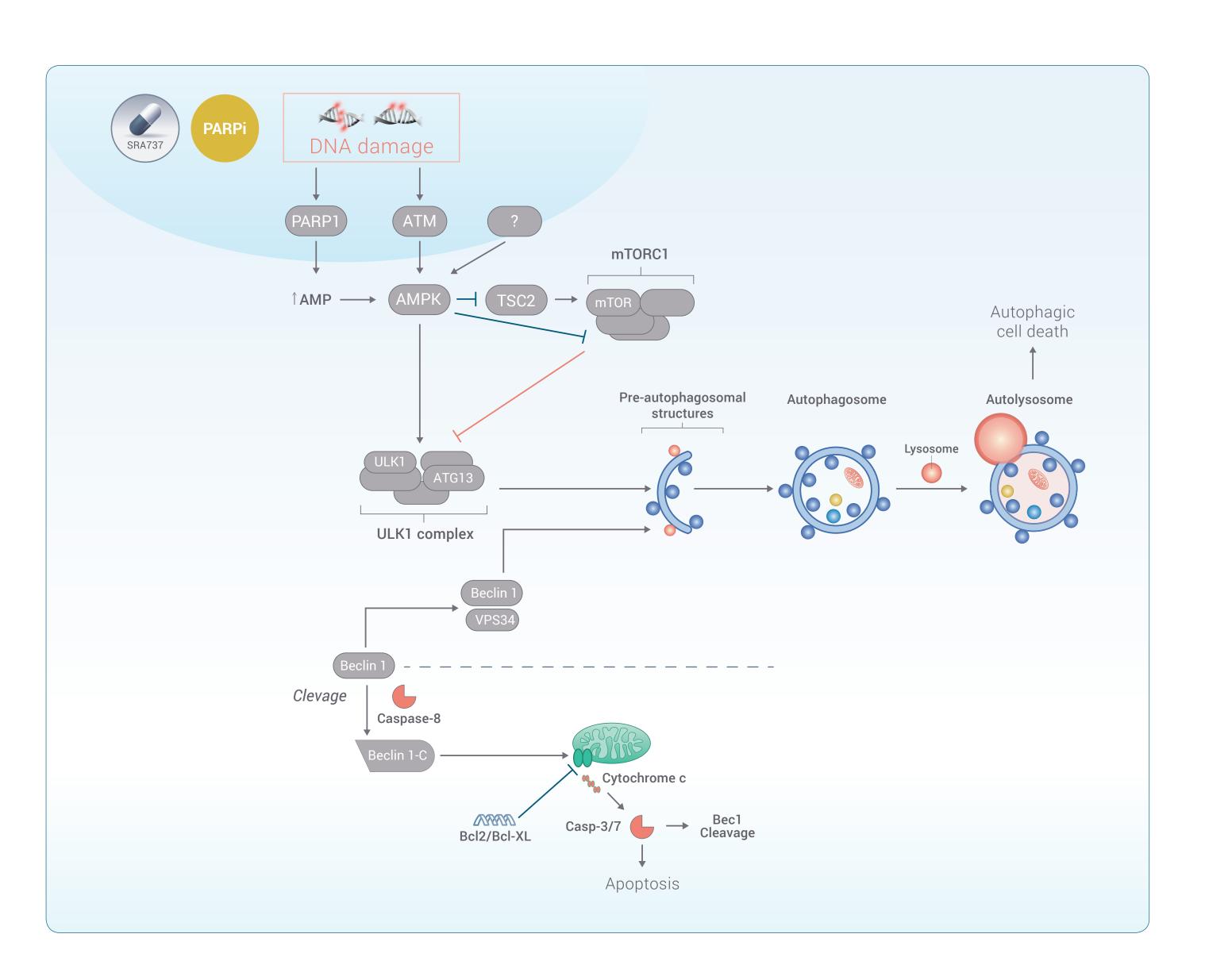
Figure 7. Cell death induced by the SRA737 and niraparib combination involves both intrinsic and extrinsic apoptotic pathways. SRA737 (250 nM), niraparib (2.0 µM), and the drug combination, reduced the expression of anti-apoptotic proteins BCL-XL and MCL-1 in Spiky and BT474 cell lines. The drugs also enhanced expression of the cytotoxic BH3 domain protein BIM (A). Tumor cells were transfected with a scrambled control siRNA (siSCR) or with siRNA directed against the indicated proteins. Knock down of CD95, FADD, AIF, BAX, BAK, BAD and BIM all reduced drug combination lethality (B). Conversely, over-expression of antiapoptotic proteins partially rescued combination-induced cell death (C). These results suggest that both intrinsic and extrinsic apoptotic pathways contribute to SRA737 and niraparib-induced killing.





p < 0.05 greater than vehicle control; * p < 0.05 less than vehicle control

Model of SRA737 and niraparib-induced tumor cell killing by autophagic cell death and apoptosis



Conclusions

- Checkpoint kinase 1 (Chk1) is a key regulator of the cellular response to replication stress (RS), stabilizing stalled replication forks, abrogating new 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 and NCT02797977).
- Other 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 their effects, thereby limiting the clinical potential of these agents.
- In short-term cell viability assays, the combination of SRA737 and niraparib in HRR proficient ovarian and breast tumor cell lines elicited enhanced tumor cell death compared to either agent alone, with evidence of cell death as early as 12 h after exposure to drug. Combination indices determined from colony forming assays indicated highly synergistic activity (CI < 0.7), which were observed employing clinically achievable concentrations of each agent.
- Quantitative immunofluorescence studies indicated an induction of DNA double strand breaks, activation of DDR signaling, induction of autophagy, and activation of extrinsic and intrinsic apoptotic signaling.
- Collectively these results argue that autophagic cell death, as well as apoptotic pathways, contribute to SRA737 and niraparib-induced tumor cell killing. The involvement of multiple cell death mechanisms may decrease the potential of tumors to develop resistance to these agents.
- These findings support further clinical investigation of SRA737 in combination with PARPi, such as niraparib, in HRR proficient cancers.
- 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 (mCRPC).