Enhanced Efficacy of Vepdegestrant (ARV-471), a Novel PROTAC® Estrogen Receptor Degrader, in Combination with Targeted Agents in ER+ Breast Cancer Models

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Objective

- To assess the effects of vepdegestrant (ARV-471) in combination with CDK4/6 or PIK3CA/mTOR pathway inhibitors in preclinical models of ER+ breast cancer.

Key Findings

- *In vitro* studies revealed evidence of synergistic interactions between ARV-471 and the CDK4/6 inhibitors abemaciclib and ribociclib, the mTOR inhibitor everolimus, or the PIK3CA inhibitors alpelisib and inavolisib in ER+ breast cancer cells.
- Evidence of synergistic effects between ARV-471 and everolimus was also observed in ER+ breast cancer cells expressing ER Y537S or D538G mutations.
- ARV-471 in combination with CDK4/6, PIK3CA or mTOR inhibitors led to enhanced tumor regressions in MCF7 xenografts as compared to single agents alone.
- ARV-471 displayed greater anti-tumor activity in combination with abemaciclib, ribociclib or inavolisib than that observed with fulvestrant in combination with these agents.

Conclusions

- Taken together, these data highlight the potential utility of vepdegestrant (ARV-471) as a combination partner for clinically relevant targeted agents for treatment of early and late-stage ER+ disease.

References

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Background

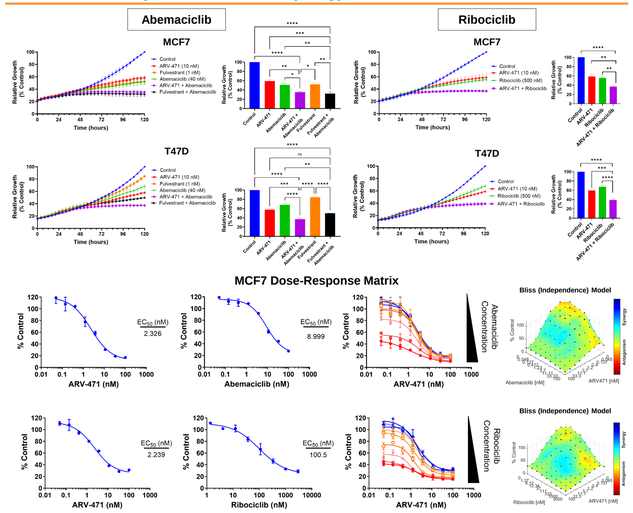
- Vepdegestrant (ARV-471) is a selective, orally bioavailable PROteolysis-TArgeting Chimera (PROTAC®) small molecule that induces wild-type and mutant estrogen receptor alpha (ER) degradation via the ubiquitin-proteasome system. ARV-471 demonstrates superior ER degradation and antitumor activity compared to fulvestrant in endocrine sensitive and resistant xenograft models (1,2) and has shown significant ER degradation and promising clinical benefit in late-line ER+ breast cancer patients (3,4). Dual pathway inhibition combining ER targeting agents with CDK4/6 or PIK3CA/mTOR pathway inhibitors is now a central strategy for treatment of advanced ER+ breast cancer. However, resistance to aromatase inhibitors resulting from ESR1 gene mutations, the suboptimal ER degradation and intramuscular route of administration of fulvestrant underscore a need for superior orally bioavailable endocrine therapy backbones for these combinations.

Methods

- **Live-cell imaging proliferation assay**
  - MCF7 or T47D cells were seeded in 6 well plates and treated with the indicated concentrations of compounds. The plate was then placed in the Incucyte® S3 Live-Cell Analysis System and images were acquired every 4 hours for a total of 120 hours. Data were analyzed using the Incucyte® Software v2020C which quantified cell surface area coverage as confluence values. Relative growth was calculated based on the confluence value observed for the control at 120 hours. Graphing and statistical analyses were performed using GraphPad Prism (GraphPad Software).
- **Dose-response matrix assay**
  - Cells were seeded at $2x10^3$ cells in 200 µl of media per well in 96 well plates and incubated overnight at 37°C. Three-fold serial dilutions were performed for each compound to generate 8-point dose-response curves. ARV-471, abemaciclib and everolimus were tested at concentrations ranging from 100 to 0.046 nM. Inavolisib was tested at concentrations ranging from 1000 to 0.46 nM. Alpelisib and ribociclib were tested at concentrations ranging from 3000 to 1.37 nM. At Day 5, cell viability was measured using Cell-Titer Glo (Promega). Data were analyzed with the Combenefit Software (5) and GraphPad Prism (GraphPad Software).
- **MCF7 xenograft model**
  - MCF7 cells were orthotopically implanted into the mammary fat pads of NOD/SCID female mice.17β-estradiol 0.72 mg 90-day pellet (Innovative Research of America) were implanted 2-3 days prior to MCF7 cell implant. For combination arms, ARV-471 was administered first followed by combination partners 1 hour later. ARV-471- and/or combination partner- treated mice were dosed orally once daily. Fulvestrant-treated mice were dosed subcutaneously twice per week for 2 weeks followed by once weekly for 2 weeks.

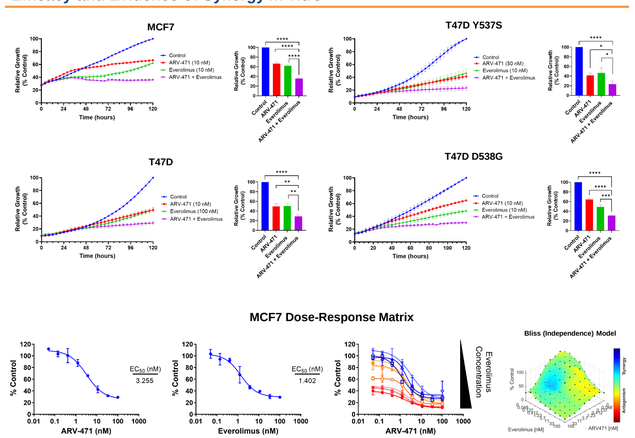
Results

Figure 1: ARV-471 in Combination with CDK4/6 Inhibitors Demonstrates Enhanced Efficacy and Evidence of Synergy *in vitro*



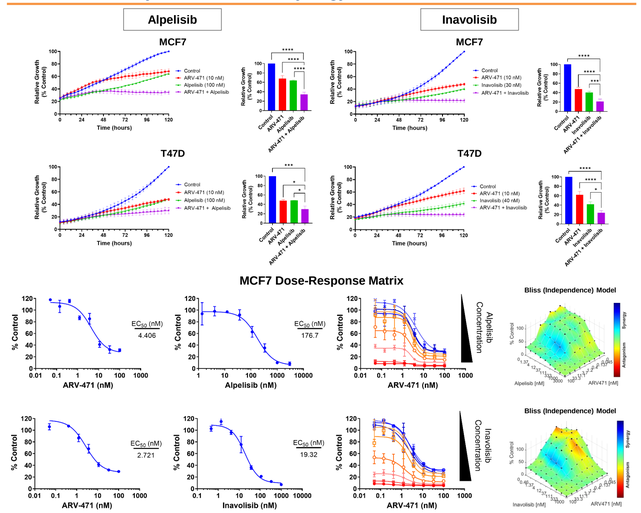
Bar graphs depict relative cell growth after 120 hours of treatment; EC_{50} = Half maximal effective concentration, nM = nanomolar

Figure 2: ARV-471 in Combination with Everolimus Demonstrates Enhanced Efficacy and Evidence of Synergy *in vitro*



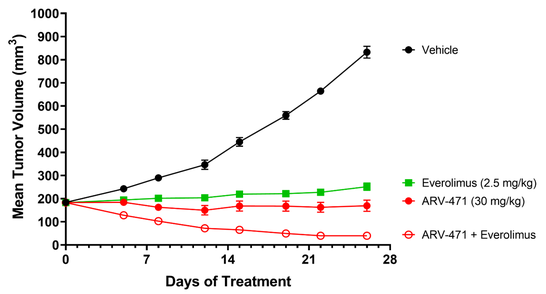
Bar graphs depict relative cell growth after 120 hours of treatment; EC_{50} = Half maximal effective concentration, nM= nanomolar

Figure 3: ARV-471 in Combination with PIK3CA Inhibitors Demonstrates Enhanced Efficacy and Evidence of Synergy *in vitro*



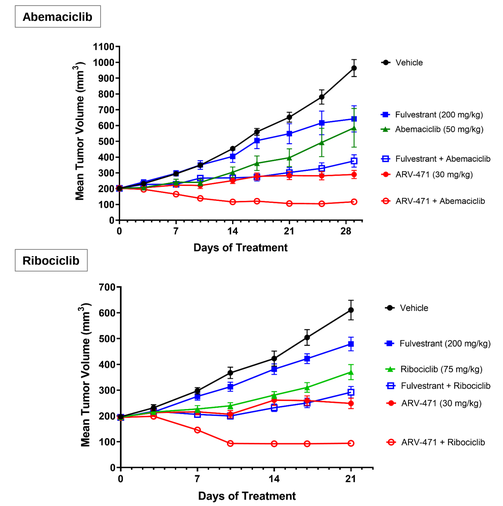
Bar graphs depict relative cell growth after 120 hours of treatment; EC_{50} = Half maximal effective concentration, nM = nanomolar

Figure 4: Antitumor Effects of ARV-471 in Combination with the mTOR Inhibitor Everolimus



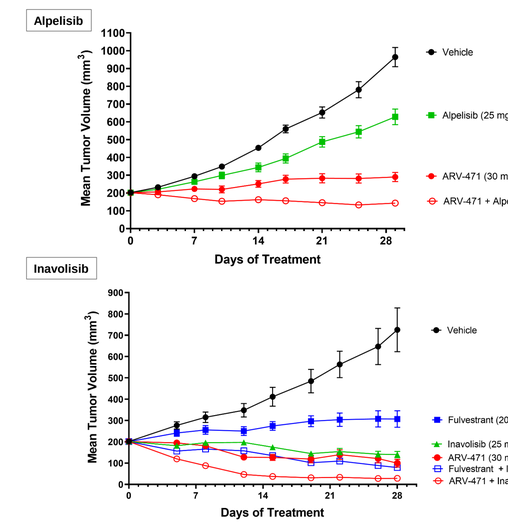
mg/kg = milligrams per kilograms

Figure 5: Antitumor Effects of ARV-471 in Combination with the CDK4/6 Inhibitors Abemaciclib and Ribociclib



mg/kg = milligrams per kilograms

Figure 6: Antitumor Effects of ARV-471 in Combination with the PIK3CA Inhibitors Alpelisib and Inavolisib



mg/kg = milligrams per kilograms