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Evaluation of the Combination of Vepdegestrant, a PROTAC Estrogen Receptor Degrader, Plus Palbociclib in CDK4/6 Inhibitor–Resistant Wild-Type and Estrogen Receptor Y537S–Mutant Patient-Derived Xenograft Models

Jessica Teh[1*], Nanni Huser[2*], Alissa Wynne[1], Christopher Kuhlberg[1], Bernadette Pascual[2], Ian Taylor[1]

[1]Arvinas Operations, Inc., New Haven, CT, USA; [2]Pfizer, Inc., La Jolla, CA, USA

*Co-first authors

Objective

- To evaluate the mechanism underlying the synergy of vepdegestrant (ARV-471) and palbociclib in vitro, and to evaluate the in vivo combination of vepdegestrant and cyclin-dependent kinase 4/6 (CDK4/6) inhibitors (palbociclib, abemaciclib, or ribociclib) in CDK4/6 inhibitor–resistant patient-derived xenograft (PDX) models ST1799-PBR (palbociclib resistant; wild-type [WT] estrogen receptor 1 gene [*ESR1*]) and ST941-PBR (*ESR1* Y537S)

Key Findings

- Consistent with the previously described synergistic interaction between vepdegestrant and palbociclib (**Figure 1**), enhanced apoptosis was observed in estrogen receptor–positive (ER+) breast cancer cells treated with this combination (**Figure 2**)
- Vepdegestrant displayed greater antitumor activity as a single agent in both CDK4/6 inhibitor–resistant models (ST1799-PBR: 76% tumor growth inhibition [TGI], ST941-PBR: 101% TGI) than fulvestrant alone (ST1799-PBR: 11% TGI, ST941-PBR: 2% TGI; **Figure 3A and 3B**)
- In combination with palbociclib, vepdegestrant displayed greater antitumor activity (ST1799-PBR: 84% TGI, ST941-PBR: 107% TGI) than that observed with fulvestrant plus palbociclib (ST1799-PBR: 12% TGI, ST941-PBR: 23% TGI) or vepdegestrant alone in both CDK4/6 inhibitor–resistant models tested (**Figure 3A and 3B**)
- In combination with abemaciclib and ribociclib, vepdegestrant displayed greater antitumor activity (abemaciclib combination: 106% TGI, ribociclib combination: 105% TGI) than that observed with vepdegestrant alone (92% TGI) in the ST941-PBR model (**Figure 3C**)

Conclusions

- These data suggest that the combination of vepdegestrant and palbociclib leads to enhanced apoptosis in ER+ breast cancer cells in vitro and that vepdegestrant alone or vepdegestrant plus palbociclib results in greater antitumor activity than fulvestrant alone or fulvestrant plus palbociclib, respectively, in an in vivo CDK4/6 inhibitor–resistant setting
- Additionally, vepdegestrant plus abemaciclib or ribociclib demonstrates enhanced TGI and tumor regressions compared with vepdegestrant alone in the ST941-PBR model

References

1. Gough SM, et al. Clin Cancer Res. 2024;30(16):3549-63.
2. Hamilton EP, et al. Presented at ESMO; October 20–24, 2023; Madrid, Spain. Poster 390P.
3. Burstein HJ, et al. J Clin Oncol. 2021;39:3959-77.
4. Teh J, et al. Presented at AACR; April 14–19, 2023; Orlando, FL, USA. Poster 3075.

Acknowledgments

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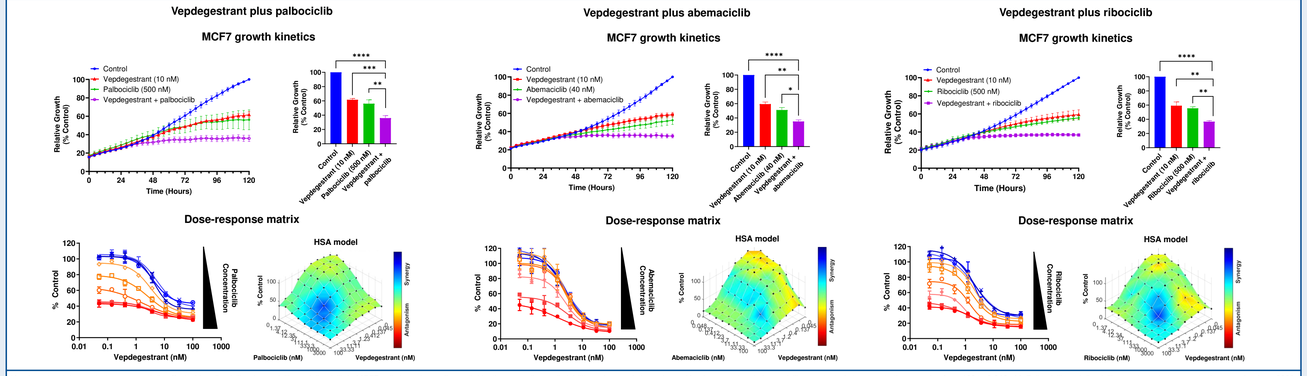
Background

- Vepdegestrant (ARV-471) is an oral, small-molecule, PROteolysis TArgeting Chimera (PROTAC) ER degrader with a unique mechanism of action that directly harnesses the ubiquitin-proteasome system to induce degradation of WT ER and clinically relevant ER mutants[1]
- In endocrine-sensitive and endocrine-resistant xenograft models, vepdegestrant demonstrated greater antitumor activity compared with the selective ER degrader fulvestrant[1]
- Vepdegestrant showed robust ER degradation and promising clinical benefit in heavily pretreated patients with ER+/human epidermal growth factor receptor 2–negative (HER2-) breast cancer[2]
- CDK4/6 inhibitors are now a standard of care treatment in combination with endocrine therapy in patients with ER+/HER2-advanced breast cancer, demonstrating significantly longer progression-free survival (and with some combinations, prolonged overall survival) compared with endocrine therapy alone[3]
- Our previous studies revealed evidence of synergistic interactions between vepdegestrant and CDK4/6 inhibitors (palbociclib, abemaciclib, and ribociclib) in ER+ breast cancer cells (**Figure 1**)[1,4]
- Here, we further explore the mechanism underlying the synergy between vepdegestrant and palbociclib in vitro and evaluate the combination of vepdegestrant and palbociclib, abemaciclib, or ribociclib in CDK4/6 inhibitor–resistant PDX models

Figure 1: Vepdegestrant in combination with palbociclib, abemaciclib, and ribociclib: evidence of synergistic activity in vitro[1]



Vepdegestrant demonstrates a synergistic effect with palbociclib, abemaciclib, and ribociclib in MCF7 cells based on cell growth kinetics, as well as cell viability and synergistic analyses. Vepdegestrant and abemaciclib were tested at concentrations ranging from 100 to 0.046 nM. Palbociclib and ribociclib were tested at concentrations ranging from 3000 to 1.3 nM. Error bars represent ± SEM. Vepdegestrant plus palbociclib: **P=0.005; ***P=0.0007; ****P=0.0001 (one-way ANOVA). Vepdegestrant plus abemaciclib: *P<0.025; **P≤0.008; ****P<0.0001 (one-way ANOVA). Vepdegestrant plus ribociclib: **P<0.01; **** P<0.0001 (one-way ANOVA).
ANOVA=analysis of variance; HSA=highest single agent; SEM=standard error of the mean.
Adapted from Gough SM, et al. Clin Cancer Res. 2024;30(16):3549-63.

Methods

- The live-cell imaging proliferation and dose-response matrix assays shown in **Figure 1** were conducted as previously reported[1]

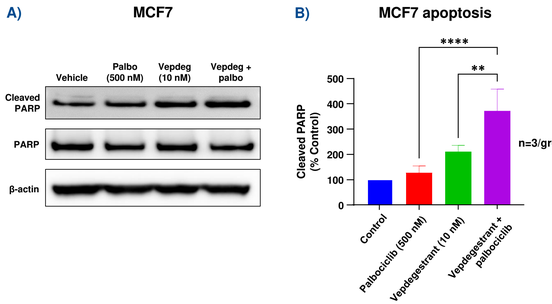
Lysate Generation and Western Blotting

- MCF7 cells were treated as indicated after 24 hours and then lysed after 72 hours
- A bicinchoninic acid assay was performed to determine protein concentrations in the lysates; lysates were then normalized and run on western blots
- The western blots were probed with primary antibodies for poly(ADP-ribose) polymerase (PARP), cleaved PARP, and β-actin overnight and imaged using a ChemiDoc system (Bio-Rad)

PDX Models

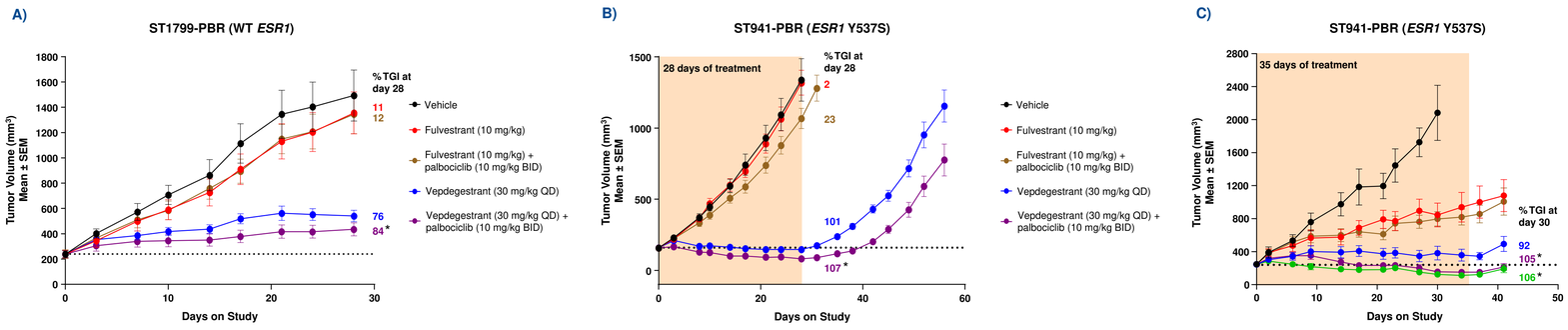
- For the combination of vepdegestrant and palbociclib, PDX mouse models were generated using ST941-PBR and STI799-PBR tumor fragments; the ST941-PBR study was conducted at Pfizer, and the STI799-PBR study was conducted at xenoSTART
  - When mean tumor volume reached approximately 200 mm^3, mice were assigned to treatment groups (n=10 per group) and dosed with vehicle, vepdegestrant 30 mg/kg, fulvestrant 10 mg/kg, vepdegestrant 30 mg/kg plus palbociclib 10 mg/kg, or fulvestrant 10 mg/kg plus palbociclib 10 mg/kg
  - Vepdegestrant was administered orally once daily, palbociclib was administered orally twice daily, and fulvestrant was administered subcutaneously twice in the first week, then weekly thereafter
  - All mice received continuous treatment for 28 days; for the ST941-PBR model, the vepdegestrant and vepdegestrant plus palbociclib groups were then taken off treatment, and tumor growth was monitored up to day 56
- For the combinations of vepdegestrant and abemaciclib or ribociclib, PDX mouse models were generated using ST941-PBR tumor fragments; these studies were conducted at xenoSTART
  - When mean tumor volume reached 250 mm^3, mice were assigned to treatment groups (n=8 per group) and dosed with vehicle, vepdegestrant 30 mg/kg, abemaciclib 50 mg/kg, ribociclib 150 mg/kg, vepdegestrant 30 mg/kg plus abemaciclib 50 mg/kg, or vepdegestrant 30 mg/kg plus ribociclib 150 mg/kg
  - Vepdegestrant, abemaciclib, and ribociclib were administered orally once daily
  - All mice received continuous treatment for 35 days; all arms were then taken off treatment, and tumor growth was monitored up to day 41

Figure 2: Enhanced apoptosis with vepdegestrant in combination with palbociclib in vitro



Western blot of cleaved PARP, PARP, and β-actin in MCF7 cell lysates (panel A). Mean levels of cleaved PARP protein in cells treated with vepdegestrant and/or palbociclib compared with vehicle (panel B). Error bars represent ± SEM. **P<0.01; ****P<0.0001 (one-way ANOVA).
ANOVA=analysis of variance; palbo=palbociclib; PARP=poly(ADP-ribose) polymerase; SEM=standard error of the mean; vepdeg=vepdegestrant.

Figure 3: Antitumor effects of vepdegestrant in combination with palbociclib in CDK4/6 inhibitor–resistant WT *ESR1* and *ESR1* Y537S–mutant PDX models and in combination with abemaciclib or ribociclib in a CDK4/6 inhibitor–resistant *ESR1* Y537S–mutant PDX model



Mean tumor volume of mice with ST1799-PBR (WT *ESR1*, panel A) and ST941-PBR tumor fragments (*ESR1* Y537S, panel B), dosed with single agents (fulvestrant or vepdegestrant) or combinations (fulvestrant with palbociclib or vepdegestrant with palbociclib). *ESR1* Y537S–mutant models receiving vepdegestrant or vepdegestrant with palbociclib were taken off treatment at day 28, and tumor growth was monitored up to day 56. Mean tumor volume of mice with ST941-PBR tumor fragments (*ESR1* Y537S) dosed with single agents (vepdegestrant, ribociclib, or abemaciclib) or combinations (vepdegestrant with ribociclib or vepdegestrant with abemaciclib; panel C). *ESR1* Y537S–mutant models receiving abemaciclib, ribociclib, vepdegestrant, vepdegestrant with ribociclib, or vepdegestrant with abemaciclib were taken off treatment at day 35, and tumor growth was monitored up to day 41 with the study ongoing at the time of data cutoff. Vepdegestrant, palbociclib, abemaciclib, and ribociclib were administered orally. Fulvestrant was administered subcutaneously twice in the first week, then weekly thereafter. No significant loss in mouse body weight was observed for any single agents or combination (data not shown). All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by Pfizer's Institutional Animal Care and Use Committee (or through an ethical review process if the studies were done outside of Pfizer).
Panels A and B: *P<0.05 at day 28 compared with vepdegestrant alone (ANCOVA). Panel C: *P<0.05 at day 30 compared with vepdegestrant alone (t-test).
ANCOVA=analysis of covariance; BID=twice daily; CDK4/6=cyclin-dependent kinase 4/6; *ESR1*=estrogen receptor 1 gene; PBR=palbociclib-resistant; PDX=patient-derived xenograft; QD=once daily; SEM=standard error of the mean; TGI=tumor growth inhibition; WT=wild-type.