Discovery and characterization of PVTX-321, an estrogen receptor heterobifunctional degrader

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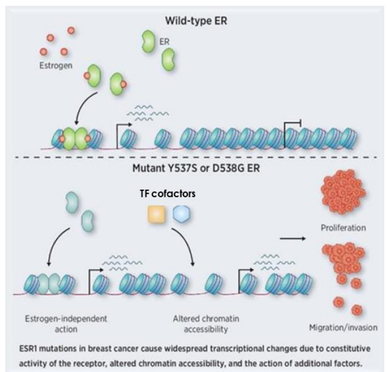
Background

- The Estrogen Receptor (ER) is a nuclear hormone receptor that in the presence of its ligand, estradiol, drives proliferative gene transcription programs in luminal breast tissue
- 70% of breast cancer is ER+
- Endocrine therapies targeting the ER pathway (anti-estrogens and aromatase inhibitors) are used to treat hormone positive, HER2 negative breast cancer
- ER mutations are rare in primary BC, but enriched in metastatic and endocrine therapy resistant BC
- ER mutations often result in constitutive activation of ER in the absence of its ligand, estradiol
- Patients with mutant ER have a worse outcome on current endocrine therapies than patients with WT ER
- Degradation of both WT and mutant ER should have a better outcome than current standard of care agents targeting the upstream ER pathway

Key Findings

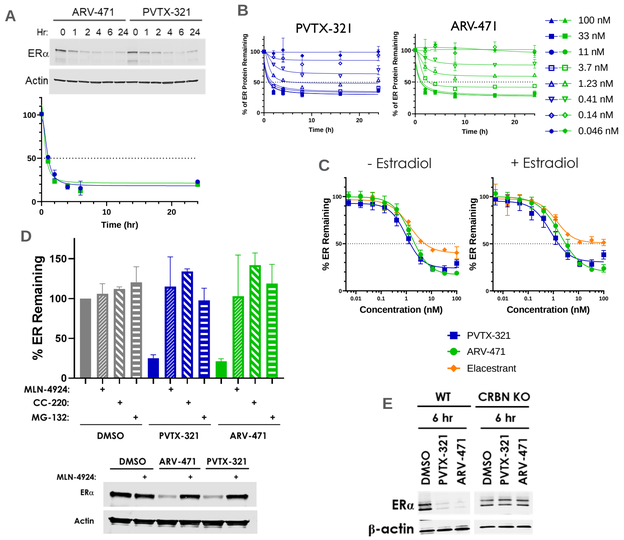
- **PVTX-321 is a potent degrader of wild-type estrogen receptor (ER) & clinically relevant ER mutants**
- **Five-fold more potent ER antagonist than ARV-471**
- **Superior antitumor efficacy in xenograft tumor model**
- **Favorable safety profile in non-GLP rat & dog studies**
- **Fit-for purpose API manufacturing process developed for IND enabling studies**

Introduction



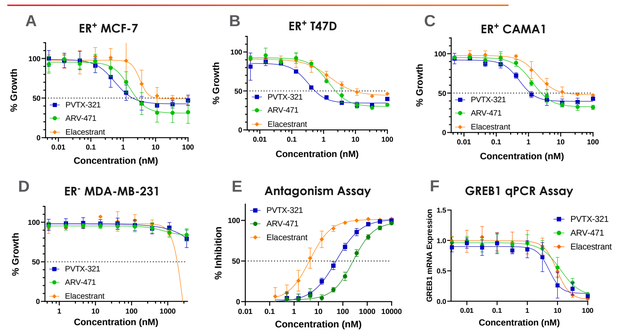
(**A**) Cartoon depicting the functional differences between WT ER transcriptional regulation and what happens in breast cancer with ER mutations, such as Y537S or D538G. Modified from Arnesen et al., Cancer Res, 2021

Figure 1. ER degraders show superb selectivity and potency in vitro



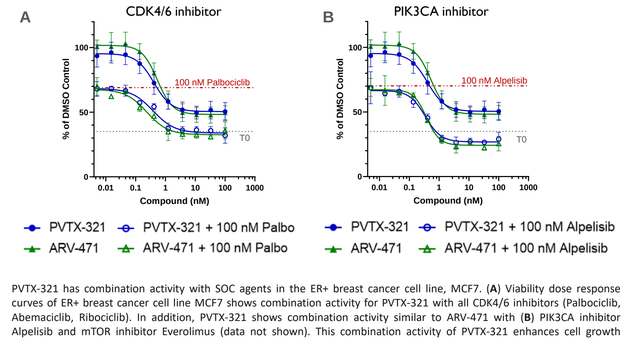
(**A**) 50% of ER is degraded by 1 hr, while maximal degradation of ER is achieved by 4-6 hr, as measured by Western blot and quantified below **(A)** or in-cell Western **(B)** using MCF7 cells. (**C**) Dose response decrease of ER measured using an ER HiBiT-knock-in MCF7 cell line in the presence or absence of 10 nM estradiol. At 4 hr. (**D**) PVTX-321 mediated destruction of ER is dependent on the UPS system as it is inhibited by either the proteasome inhibitor MG-132, the Neddylation inhibitor MLN-4924 and the potent CRBN-binding compound CC-220. (**D**) CRBN is required for degradation of ER, as CRISPR knock out of CRBN abrogates PVTX-321 mediated ER destruction.

Figure 2. PVTX-321 is efficacious by inhibiting growth of multiple ER+ breast cancer cell lines and a potent antagonist



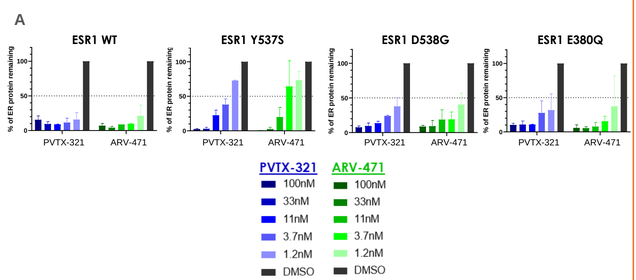
PVTX-321 inhibits cell growth in ER+ breast cancer cell lines (**A**) MCF7, (**B**) T47D and (**C**) CAMA1; however, (**D**) PVTX-321 does not affect proliferation in the ER negative breast cancer cell line MDA-MB-231. (**E**) PVTX-321 is a 5-fold more potent antagonist than ARV-471 in a biochemical assay. (**F**) PVTX-321 inhibits GREB1 transcription in the presence of 100 nM estradiol.

Figure 3. PVTX-321 shows combination activity with standard of care combination agents



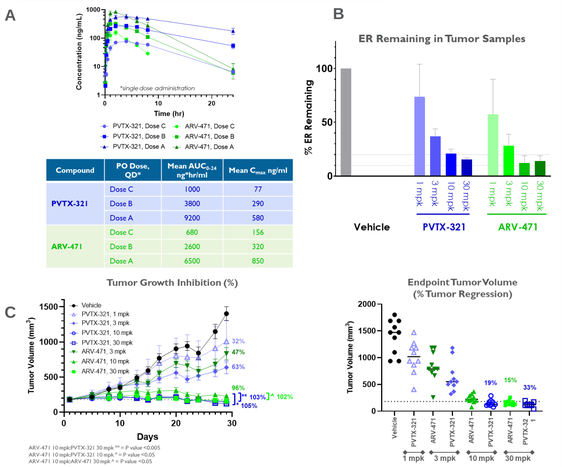
PVTX-321 has combination activity with SOC agents in the ER+ breast cancer cell line, MCF7. (**A**) Viability dose response curves of ER+ breast cancer cell line MCF7 shows combination activity for PVTX-321 with all CDK4/6 inhibitors (Palbociclib, Abemaciclib, Ribociclib). In addition, PVTX-321 shows combination activity similar to ARV-471 with (**B**) PIK3CA inhibitor Alpelisib and mTOR inhibitor Everolimus (data not shown). This combination activity of PVTX-321 enhances cell growth inhibition and cell killing (open circle or triangle), compared to single agent (solid circle or triangle) which is cytostatic.

Figure 4. PVTX-321 is a potent degrader of WT and clinically relevant ER mutations



(**A**) The ER gene was edited with CRISRP in MCF7 breast cancer cells to produce cell lines that express the clinically relevant ER point mutations Y537S, D538G, and Q380E. Cells were treated with varying doses of PVTX-321 or ARV-471 for 24 hr and then Western blots were quantified and results shown in representative graphs. Inhibition of cell growth was measured upon treatment with PVTX-321, ARV-471, or Elacestrant in (**B**) MCF7-Y537S or (**C**) MCF7-D538G cells. Cell lines engineered with ER point mutations Y537S or D538G show better cell growth inhibition and approach the T0 (dashed line) compared to parental cells (compare to figure 3A and B).

Figure 5. PVTX-321 has dose-dependent oral exposure, ER degradation and tumor growth inhibition in vivo



Compound	PO Dose, QD*	Mean $AUC_{0\text{-}24}$ ng*hr/ml	Mean C_{max} ng/ml
PVTX-321	Dose C	1000	77
	Dose B	3800	290
	Dose A	9200	580
ARV-471	Dose C	680	156
	Dose B	2600	320
	Dose A	6500	850

(**A**) PVTX-321 demonstrates dose linear pharmacokinetics after a single oral dosing in mice. PVTX-321 has longer plasma half-life in mice and greater duration of exposure in tumor tissue (data not shown) compared to ARV-471. (**B**) PVTX-321 achieves dose dependent ER destruction in MCF7 tumor model. PVTX-321 and ARV-471 achieve similar levels ER degradation at 30 mpk in tumors (86% and 84%, respectively) at 6 hrs post the 3rd dose, once daily administration. (**C**) Daily oral administration of PVTX-321 in MCF7 mouse Xenografts leads to tumor growth inhibition and tumor shrinkage over multiple doses. 10 mpk and 30 mpk of PVTX-321 show significantly improved efficacy than 10 mpk of ARV-471. PVTX-321 shows tumor regression at 10 and 30 mpk, similar to 30 mpk of ARV-471

Conclusions

- We developed a heterobifunctional Estrogen Receptor (ER) degrader comprised of a novel ERα binder and a proprietary Cereblon ligand connected with a conformationally constrained linker
- PVTX-321 is a potent ER degrader and antagonist with activity against wild-type and clinically relevant ER mutants
- Superior efficacy to the clinical stage ER heterobifunctional degrader candidate ARV-471 in estradiol-dependent MCF-7 mouse xenograft model
- Favorable safety profile in non-GLP rat & dog studies
- Fit-for purpose API manufacturing process developed for IND enabling studies

Hevener et al, Molecular Metabolism, 2018
Clusan et al., Int J Mol Sci, 2021
Arnesen et al., Cancer Res, 2021