Clinical Cancer Research

Elacestrant (RAD1901), a Selective Estrogen Receptor Degrader (SERD), Has Antitumor Activity in Multiple ER$^+$ Breast Cancer Patient-derived Xenograft Models

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Abstract

Purpose: Estrogen receptor–positive (ER$^+$) breast cancers are typically treated with endocrine agents, and dependence on the ER pathway is often retained even after multiple rounds of antiestrogen therapy. Selective estrogen receptor degraders (SERD) are being developed as a strategy to more effectively target ER and exploit ER dependence in these cancers, which includes inhibiting both wild-type and mutant forms of ER. The purpose of this study was to evaluate the efficacy of a novel orally bioavailable SERD, elacestrant (RAD1901), in preclinical models of ER$^+$ breast cancer.

Experimental Design: Elacestrant was evaluated as a single agent and in combination with palbociclib or everolimus in multiple ER$^+$ breast cancer models, including several patient-derived xenograft models.

Results: Elacestrant induces the degradation of ER, inhibits ER-mediated signaling and growth of ER$^+$ breast cancer cell lines *in vitro* and *in vivo*, and significantly inhibits tumor growth of multiple PDX models. Furthermore, we demonstrate that elacestrant in combination with palbociclib or everolimus can lead to greater efficacy in certain contexts. Finally, elacestrant exhibits significant antitumor activity both as a single agent and in combination with palbociclib in two patient-derived breast cancer xenograft models harboring *ESR1* mutations.

Conclusions: These data underscore the potential clinical utility of elacestrant as a single agent and as a combination therapy, for both early- and late-stage ER$^+$ disease. *Clin Cancer Res; 23(16); 4793–804. ©2017 AACR.*

Introduction

Breast cancer is subdivided into categories based on the tumor receptor status; specifically, whether the tumor expresses estrogen receptor (ER), progesterone receptor (PR) or Her2, with ER$^+$ disease making up the majority of patients in the breast cancer patient population (1). Given the dependence on ER in this disease segment, most treatment modalities focus on inhibiting various aspects of this pathway. Indeed, inhibition of estrogen synthesis (e.g., with aromatase inhibitors (AI)) or modulation of ER pathway activity (e.g., with tamoxifen), continue to be mainstays in the standard of care for ER$^+$ breast cancer patients both in the adjuvant and metastatic setting (ref. 2; https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf). While many patients with advanced and metastatic breast cancer initially respond to these agents, a majority of patients will have progressive disease (3, 4). Interestingly, even in these patients who have seen multiple prior endocrine therapies, dependence on ER for tumor growth and sensitivity to other ER-targeting agents is often retained (5–7). On the basis of this continued dependence on ER, novel selective estrogen receptor degraders (SERD) have gained widespread attention as a means of delivering more durable responses and increasing progression-free survival in this setting (8–11).

Currently, fulvestrant (Faslodex, AstraZeneca) is the only approved SERD for the treatment of postmenopausal women with advanced ER$^+$ breast cancer who have progressed on endocrine therapies (http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021344s015lbl.pdf). In the metastatic setting, fulvestrant has been shown to be effective as a single agent and a recent study demonstrated that the increased dose of 500 mg fulvestrant is superior to the historical 250 mg dose in terms of overall survival (8, 12, 13). However, there is evidence to suggest that even at the 500 mg dose, suboptimal occupancy of the estrogen receptor can occur in some patients, which may correlate with an earlier progression of disease (14). These data, combined with fulvestrant's intramuscular route of administration underscore the need for a novel orally bioavailable SERD for the treatment of ER$^+$ breast cancer.

Several reports have shown that the PI3K/mTOR and Rb/E2F signaling pathways can compensate for and crosstalk with the ER signaling pathway, contributing to endocrine treatment resistance (15–18). Given the activation of these growth- and survival-promoting pathways after endocrine therapy, current clinical treatment strategies have begun combining endocrine-based agents with small-molecule inhibitors of these pathways (19, 20). The increase in progression-free survival demonstrated in clinical studies such as BOLERO-2 and PALOMA-1 where an

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Advanced ER$^+$ breast cancers often retain a dependence on ER and maintain sensitivity to multiple rounds of endocrine therapy. Nevertheless, treatment resistance and progressive disease remain a clinical challenge and have been attributed to *ESR1* mutations and/or the activation of compensatory pathways. Here, we demonstrate the preclinical efficacy of elacestrant, a novel SERD that is under clinical investigation for the treatment of advanced ER$^+$ breast cancer. Using multiple patient-derived xenograft (PDX) models, including some derived from heavily pretreated patients, we demonstrate the efficacy of elacestrant as a single agent and in combination with palbociclib or everolimus. Importantly, elacestrant was also effective at inhibiting the growth of PDX models harboring *ESR1* mutations. This study provides rationale for the ongoing clinical investigation of elacestrant in ER$^+$ breast cancer patients, both as a single agent and in combination with approved agents, such as palbociclib and everolimus.

aromatase inhibitor was combined with everolimus (mTOR inhibitor) or palbociclib (CDK4/6 inhibitor), respectively, highlights the effectiveness of this strategy (21–23). Furthermore, recent data from the PALOMA-3 trial validate the use of a SERD (fulvestrant) in combination with CDK4/6 inhibitors as a potential new treatment strategy for metastatic disease (24).

While combinations have demonstrated superior efficacy compared with the use of aromatase inhibitors alone, recent studies have also demonstrated that mutations in the *ESR1* gene itself can also drive estrogen-independent activity leading to clinical treatment resistance (25–31). It has been reported that patients treated with AIs are more likely to develop tumors harboring *ESR1* mutations, resulting in ligand-independent transcriptional activity of ER (27, 32). In addition, some *ESR1* mutations can lead to conformational changes of ER, leading to decreased binding of tamoxifen, thereby potentially reducing its activity (29, 31). These findings highlight the need for a novel SERD that can bind and inhibit both wild-type and mutant forms of ER in order to more effectively treat this patient population. Recent clinical data have demonstrated that while the presence of *ESR1* mutations can be a prognostic indicator of AI resistance, the extent of fulvestrant efficacy is not influenced by *ESR1* status (32, 33), suggesting SERDs have therapeutic value in this clinical context.

The changing breast cancer treatment landscape together with emerging molecular mechanisms underlying ER$^+$ disease provide strong rationale for the further clinical development of an orally bioavailable SERD in patients with ER$^+$ breast cancer. Elacestrant (RAD1901), an orally bioavailable, nonsteroidal small-molecule SERD, is currently under clinical investigation for the treatment of ER$^+$ breast cancers in postmenopausal women (NCT02650817, NCT02338349). In a previous report, we demonstrated that elacestrant selectively binds and induces the degradation of ER (34). Furthermore, we demonstrated that elacestrant antagonized estrogen-mediated uterotrophic effects and had bone protective effects in a rat osteopenia model, suggesting a favorable profile for use in patients. Here, we evaluate elacestrant activity in multiple ER$^+$ breast cancer cell lines and ER$^+$ patient-derived tumor xenograft (PDX) models as a single agent, and in combination with palbociclib or everolimus. In addition, we also demonstrate elacestrant has potent antitumor effects in two PDX models that harbor *ESR1* mutations. Taken together, these results provide strong preclinical data and mechanistic rationale for further clinical investigation of elacestrant.

Methods

Reagents and cell lines

Elacestrant (6R)-6-(2-(N-(4-(2-(ethylamino)ethyl)benzyl)-N-ethylamino)-4-methoxyphenyl)-5,6,7,8-tetrahydronaphthalen-2-ol dihydrochloride) was manufactured by Patheon. Elacestrant lots used in this study were periodically checked to ensure purity, stability and chirality.

MCF7, T47D, and HCC1428 cells were purchased from ATCC and were routinely maintained in phenol red-free minimal essential medium (MEM) or RPMI medium supplemented with 0.01 mg/mL bovine insulin and 10% FBS (HyClone, GE Healthcare Life Sciences) at 5% CO_2. Cells were authenticated using STR analyses and confirmed to be mycoplasma-free using the MycoAlert kit (Lonza). Charcoal-dextran–stripped FBS was obtained from Gemini Bio and HyClone (GE Healthcare Life Sciences).

In vitro cancer cell proliferation

The 50% growth inhibition concentration (IC_{50}) of elacestrant was determined in MCF7 and T47D using the CellTiter-Glo luminescent cell viability assay (Promega). Briefly, cells were incubated in phenol-red free media with charcoal-stripped FBS for 72 hours before seeding into 96-well plates. Twenty-four hours postplating, cells were treated with increasing doses of elacestrant in the presence of 0, 0.01 nmol/L, and 1 nmol/L 17β-estradiol. Cells were incubated in the indicated treatment for 7 days and the CellTiter-Glo assay (Promega) was performed as per manufacturer's instructions.

Western blots

For analysis of ER protein expression in response to treatment, MCF7, T47D, or HCC1428 cells were seeded in 6-well plates containing phenol-red free media supplemented with 10% charcoal-stripped FBS. Forty-eight hours later, cells were treated with elacestrant or fulvestrant at the indicated concentrations. Cells were harvested and lysed in CellLytic M lysis buffer (Sigma-Aldrich) after 24 or 48 hours of treatment, and total protein was separated by SDS-PAGE and transferred to membranes and immunoblotted using antibodies specific to the indicated proteins. For *in vivo* pharmacodynamic studies, end of study flash-frozen tumors were fractured using a cryoPREP instrument (Covaris) and pulverized tissue was lysed in Cell Lysis Buffer (Cell Signaling Technology). Total protein was analyzed by Western blot analysis as described above. Antibodies specific to ERα (Cell Signaling Technology, #13258; Santa Cruz Biotechnology, sc-543), PR (Cell Signaling Technology, sc-3153), and anti-vinculin (Sigma-Aldrich, V9131) as an internal control were used.

Quantitative reverse transcriptase PCR analyses

For cell lines, quantitative reverse transcriptase PCR analysis (qRT-PCR) was performed using the Cells to C_T kit (Life Technologies) using TaqMan probes. For *in vivo* pharmacodynamic studies, end of study flash-frozen tumors were fractured using a cryoPREP instrument (Covaris). From pulverized tissue, total RNA was extracted using the RNeasy Mini Kit (Qiagen) and qRT-PCR was performed using the 1-step RNA to C_T (Carlsbad)

using TaqMan probes. The C_T values were analyzed to assess relative changes in expression of the *TFF1* (trefoil factor 1/breast cancer estrogen-inducible protein), *GREB1* (gene regulated by estrogen in breast cancer 1) and *PGR* (progesterone receptor) genes, with *GAPDH* as an internal control, using the $2^{-\Delta\Delta CT}$ method (35).

In vivo xenograft experiments

All study protocols were reviewed by Radius, approved by Institutional Animal Care and Use Committees (IACUC), and conducted in accordance with US and International regulations for protection of laboratory animals. Female athymic nude mice (NU(NCr)-*Foxn1nu or* BALB/cAnNCrl-*Foxn1nu*) were obtained from Envigo RMS, Inc., Harlan Laboratories, or Charles River Laboratories and acclimated for 3 to 7 days prior to implantation. Mice were given water (reverse osmosis, 1 ppm Cl) and fed a daily complete diet *ad libitum*, and were housed on irradiated bedding on a 12- to 14-hour light cycle under controlled temperature and humidity. Preformulated, clinical-grade fulvestrant (Faslodex, manufactured by AstraZeneca) was obtained through third party vendors and administered by subcutaneous injection once weekly. Elacestrant, palbociclib and everolimus were administered daily by oral gavage. In the ST941 study, groups receiving palbociclib were initially administered 100 mg/kg and dose reduced to 75 mg/kg on day 14 of treatment. At the end of this study, average body weight loss for all treatment groups did not exceed 15%.

MCF-7 xenografts. Twenty-four hours prior to implantation of MCF-7 cells, estrogen pellets (0. 18 mg/pellet 17β−estradiol, 90-day release, Innovative Research of America) were implanted subcutaneously between the scapulae of female athymic nude mice using a sterilized trochar. MCF7 cells (5×10^6 per mouse) in 50:50 Matrigel (Corning Inc.):MEM were implanted in the rear flank. When mean tumor volumes reached approximately 150 to 200 mm^3, mice were randomized to treatment groups based on tumor size. For pharmacodynamic analyses, MCF7 xenograft-bearing mice were treated daily for seven days, animals were euthanized, and tumors collected 4 and 24 hours post-last dose.

Patient-derived xenograft models. HBCx-21, HBCx-3 and HBCx-19 patient-derived tumor xenografts (PDX) were derived at and studies run at XenTech. The ST986, ST941, and ST2177 PDX models were derived at and studies run at South Texas Accelerated Research Therapeutics (San Antonio, TX). MAXF-713 was derived at and studies run at Charles River Discovery (OncoTest). All animals were subcutaneously implanted with PDX models and began receiving estrogen supplementation in the drinking water from the date of tumor implant to the end of the study. The HBCx-19, HBCx-3, and HBCx-21 (XenTech) models were supplemented with 8.5 milligrams of 17β-estradiol to each liter of drinking water. The MAXF-713 model (Charles River Discovery, Oncotest) was supplemented with 10 milligrams of 17β-estradiol to each liter of drinking water. When tumors grew to 150–200 mm^3, mice were randomized on the basis of tumor volume and administered the indicated treatments. At the end of study, tumors were harvested 4 hours post-last dose unless otherwise indicated.

In vivo pharmacokinetic analyses

Terminal plasma was collected via heart puncture and nonterminal plasma was collected via orbital bleeding. For all mice, blood samples were collected in potassium-EDTA–containing tubes and processed for pharmacokinetic analysis. Analysis of fulvestrant in mouse plasma samples was carried out using high-performance liquid chromatography on a Pursuit XRs 3 Diphenyl 100 × 2.0 mm column (Agilent).

IHC

IHC was performed using the Leica Bond III system (Leica Biosystems) according to the manufacturer's instructions. Primary rabbit mAbs were obtained from Cell Marque and were directed against HER2/Neu (SP3), Ki67 (D2H10), estrogen receptor (SP1), and progesterone receptor (SP42).

In vitro binding assays

In vitro binding affinity of elacestrant was determined using purified ligand-binding domain of wild-type and mutant ERα in the PolarScreen ERα Competitor Assay (Thermo Scientific, #A15883) as per manufacturer's instructions.

Statistical and data analysis

Statistical analysis and graphical presentations were performed using GraphPad Prism 6 or 7 and Microsoft Excel. Descriptive data were generally expressed as mean ± SD or SEM. Statistical evaluations of the differences between groups were assessed using Student *t* test analyses, with a prespecified alpha of 0.05. For cell proliferation assays, the IC_{50} was calculated by fitting a dose–response curve using a nonlinear regression model with a sigmoidal dose response. The formula for calculating surviving rate is shown below; Relative IC_{50} is calculated where *y*-axis was set at 50% using GraphPad Prism 5.0 curve fitting software. For all tumor xenograft models, body weights and tumor volumes were evaluated twice weekly. Tumor volume was calculated using the formula: tumor volume (mm^3) $= 0.5 \times L \times W^2$, where W = width and L = length in mm of the tumor. Relative tumor volumes were defined as tumor volume$_{\text{treatment day X}}$ − tumor volume$_{\text{treatment day 0}}$. Tumor growth inhibition (%TGI) was calculated as $(1-(\text{average relative tumor volume}_{\text{treatment group}}/\text{average relative tumor volume}_{\text{vehicle group}}))\times 100$). Tumor volume data were analyzed using a linear mixed-effects model with $\log_{10}$ transformation of postbaseline value over baseline as response variable, treatment, day, and treatment-by-day interaction as fixed effects, and animal as random effect assuming a first-order autoregression correlation between days for a specific animal. Treatment group comparisons were performed using least square means of the $\log_{10}$ transformed postbaseline value over baseline at the end of study, with *P* values adjusted using the Bonferroni method, a conservative *P* value adjustment method (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; Supplementary Table).

Results

Elacestrant is a SERD that inhibits breast cancer cell proliferation *in vitro* and *in vivo*

To determine the ER degrading activity of elacestrant in the breast cancer setting, ER protein expression was examined in multiple ER$^+$ breast cancer cell lines (MCF7, T47D, and HCC1428) following treatment with increasing doses of elacestrant or fulvestrant. Elacestrant treatment resulted in a dose-dependent and marked decrease in ER protein expression, inducing degradation of ER to a similar extent as fulvestrant in all three cell lines tested (Fig. 1A). In addition, elacestrant treatment

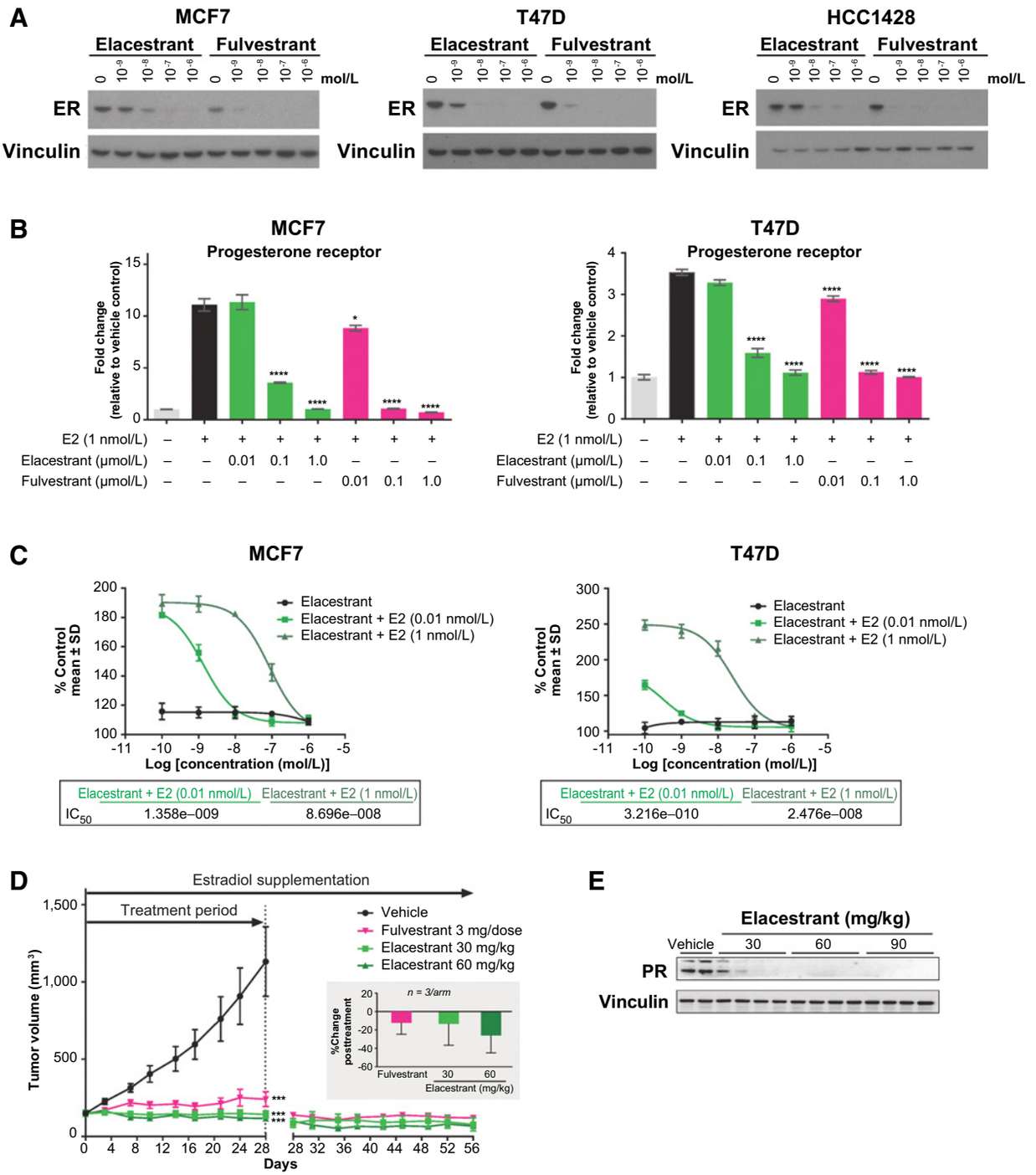

Figure 1.

Elacestrant induces ER degradation, and inhibits estrogen-mediated growth of ER$^+$ breast cancer cells *in vitro* and *in vivo*. Western blot analyses of ER (**A**) or qRT-PCR of *PGR* in indicated cells treated with elacestrant or fulvestrant at the indicated doses (**B**). Histogram depicts the mean fold change ($n = 3$) relative to the mean of vehicle control ± SD. One-way ANOVA was used to detect statistically significant differences between treatment + E2 groups versus E2 alone. *, $P < 0.05$; ****, $P < 0.0001$. **C,** CellTiter-Glo assay of cells treated with increasing doses of elacestrant for seven days in the presence of 10 pmol/L or 1 nmol/L E2. Control refers to cells grown in the absence of estradiol and in the presence of DMSO solvent. **D,** Mean tumor volumes ($n = 8$/arm from day 0 to day 28, $n = 3$/arm from day 28 to day 56) ± SEM of MCF7 xenografts. Asterisks represent significant differences of the indicated treatment groups relative to vehicle control calculated on day 28. **E,** Western blot analyses of individual MCF7 tumors 24 hours post-seventh dose, duplicated in Supplementary Fig. S3D.

resulted in decreased expression of progesterone receptor (*PGR*, PR), an ER target gene, in both MCF7 and T47D cell lines (Fig. 1B; Supplementary Fig. S1A). Regardless of the 17β-estradiol (E2) concentration used (10 pmol/L or 1 nmol/L representative of a postmenopausal and premenopausal setting, respectively; ref. 36), elacestrant was able to decrease *PGR* to a basal level similar to control cells grown in the absence of E2 (Fig. 1B; Supplementary Fig. S1A). Importantly, elacestrant did not increase ER target genes in the absence of estradiol, suggesting elacestrant has an antagonistic profile in the breast cancer setting (Supplementary Fig. S1). Analysis of additional ER target genes, *TFF1* and *GREB1*, revealed similar effects following elacestrant treatment (Supplementary Fig. S1B). Consistent with the ability of elacestrant to induce ER degradation and block ER signaling, elacestrant treatment also inhibited E2-mediated proliferation in MCF7 and T47D cells in a dose-dependent manner (Fig. 1C). These findings confirm the ability of elacestrant to effectively induce ER degradation and inhibit estrogen-mediated signaling and growth of multiple ER$^+$ cell lines.

We further evaluated the *in vivo* activity of elacestrant as a single agent in the MCF7 cell line xenograft model. Following once daily oral administration of either 30 mg/kg or 60 mg/kg, elacestrant induced complete tumor growth inhibition after four weeks of treatment (Fig. 1D). Of note, the plasma concentration of these elacestrant doses are clinically achievable (manuscript in preparation). Fulvestrant treatment also resulted in tumor growth inhibition in this model following a 3 mg/week dose (Fig. 1D). We determined the 3 mg/week dose of fulvestrant in mice achieved a plasma concentration approximately 1.8-fold higher than that of the approved 500 mg clinical dose (Supplementary Fig. S2). Importantly, at the end of the study, body weight loss did not exceed 10% in any of the treatment groups suggesting both elacestrant and fulvestrant were well-tolerated (Supplementary Fig. S3A). To further understand treatment effects, tumor volumes were evaluated after treatment had been withdrawn. Interestingly, tumor growth inhibition was maintained for four weeks after elacestrant or fulvestrant withdrawal (Fig. 1D, inset), suggesting these treatments resulted in sustained tumor growth inhibition.

To better understand the pharmacodynamic effects on the ER pathway, downstream ER signaling was examined in MCF7 tumors treated with elacestrant for seven days. Notably, PR expression was significantly decreased in all elacestrant-treated groups and this decrease was maintained up to 24 hours following the seventh dose (Fig. 1E; Supplementary Fig. S3). These results demonstrate that elacestrant can produce a stable and continued pharmacodynamic effect on ER signaling and sustained tumor growth inhibition across a range of doses.

Elacestrant treatment results in tumor regressions in MCF7 tumor xenografts as a single agent and in combination with palbociclib or everolimus

To support our hypothesis that elacestrant would be an effective endocrine backbone for combination strategies, MCF7 tumor xenografts were treated with elacestrant (60 mg/kg) as a single agent or in combination with either everolimus or palbociclib. The combination of elacestrant with either everolimus or palbociclib resulted in significantly greater tumor growth inhibition as compared with elacestrant single-agent effects (Fig. 2A and B). Evaluation of the changes in tumor volume pre- and posttreatment revealed that elacestrant treatment produced tumor regressions in the majority of animals when used as a single agent or in combination with either palbociclib or everolimus (Fig. 2C and D). Of note, a lower dose of elacestrant (30 mg/kg) elicited similar effects on tumor volume as a single agent and in combination with palbociclib or everolimus (Supplementary Fig. S4). Similarly, the %TGI observed with fulvestrant in combination with palbociclib or everolimus was greater than fulvestrant alone; however, the tumor volume change was not significantly different between these treatment groups (Supplementary Table).

ER and PR protein expression was assessed in individual tumors at the end of study and both proteins were decreased in all elacestrant-treated groups, including the combination groups (Fig. 2E and F). Interestingly, everolimus treatment appeared to increase ER expression, and despite this increase, elacestrant was able to induce ER degradation, suggesting elacestrant effectively counteracts feedback pathways that can induce ER expression (Fig. 2F).

Elacestrant demonstrates significant antitumor efficacy in multiple PDX models

The molecular classification of ER$^+$ disease in the clinical setting is routinely determined using IHC staining, where a tumor can be designated ER-positive with as few as 1% of cells within the tumor expressing ER (37). Patients who have ER$^+$ tumors, regardless of the extent of ER positivity, are typically treated with endocrine agents (37, 38). To more closely mimic the tumor heterogeneity of ER in the clinical setting, we evaluated effects of elacestrant and fulvestrant on five ER$^+$ PDX models with varied ER/PR expression levels (Table 1).

Three of the models chosen, ST986 (*ESR1*:WT, *PIK3CA*:E542K), HBCx-21 (*ESR1*:WT, *PIK3CA*:WT), and MAXF-713 (*ESR1*:WT, *PIK3CA*:WT), were selected on the basis of relatively high expression levels of wild-type ER and PR (Table 1; Supplementary Fig. S5A). Importantly, each model displayed differential sensitivity to fulvestrant (1 mg/week), a dose that we determined achieved an equivalent plasma exposure as the 500 mg clinical dose (Supplementary Fig. S2). This dose of fulvestrant was sufficient to induce complete tumor growth inhibition of the HBCx-21 model, whereas it resulted in partial growth inhibition of the MAXF-713 model and did not result in significant tumor growth inhibition in the ST986 model (Fig. 3A–C). Interestingly, increasing the fulvestrant dose to 5 mg/week, equivalent to approximately 2.9 times the clinical exposure (Supplementary Fig. S2), resulted in growth inhibition of ST986 tumors (Fig. 3A). Elacestrant at all doses tested (30 mg/kg, 60 mg/kg and 120 mg/kg), consistently resulted in significant tumor growth inhibition in all three PDX models (Fig. 3A–C).

Tumors collected at the end of treatment were assessed for changes in ER and PR expression. Consistent with the results seen in MCF7 tumors (Figs. 1 and 2), elacestrant treatment decreased ER protein expression in all three models and this was accompanied by a decrease in PR (Fig. 3D; Supplementary Fig. S5B and S5C).

HBCx-21–treated tumors were also examined following treatment withdrawal in animals treated with the lowest 30 mg/kg dose of elacestrant, or 1 mg/week of fulvestrant. Similar to the result in MCF7 xenografts, tumor growth inhibition in the elacestrant treatment group and the fulvestrant treatment group was maintained for almost two months posttreatment (Supplementary Fig. S5D). Furthermore, analysis of the limited tumor material that remained displayed sustained decreases in ER expression in the elacestrant and fulvestrant-treated tumors, despite lack of

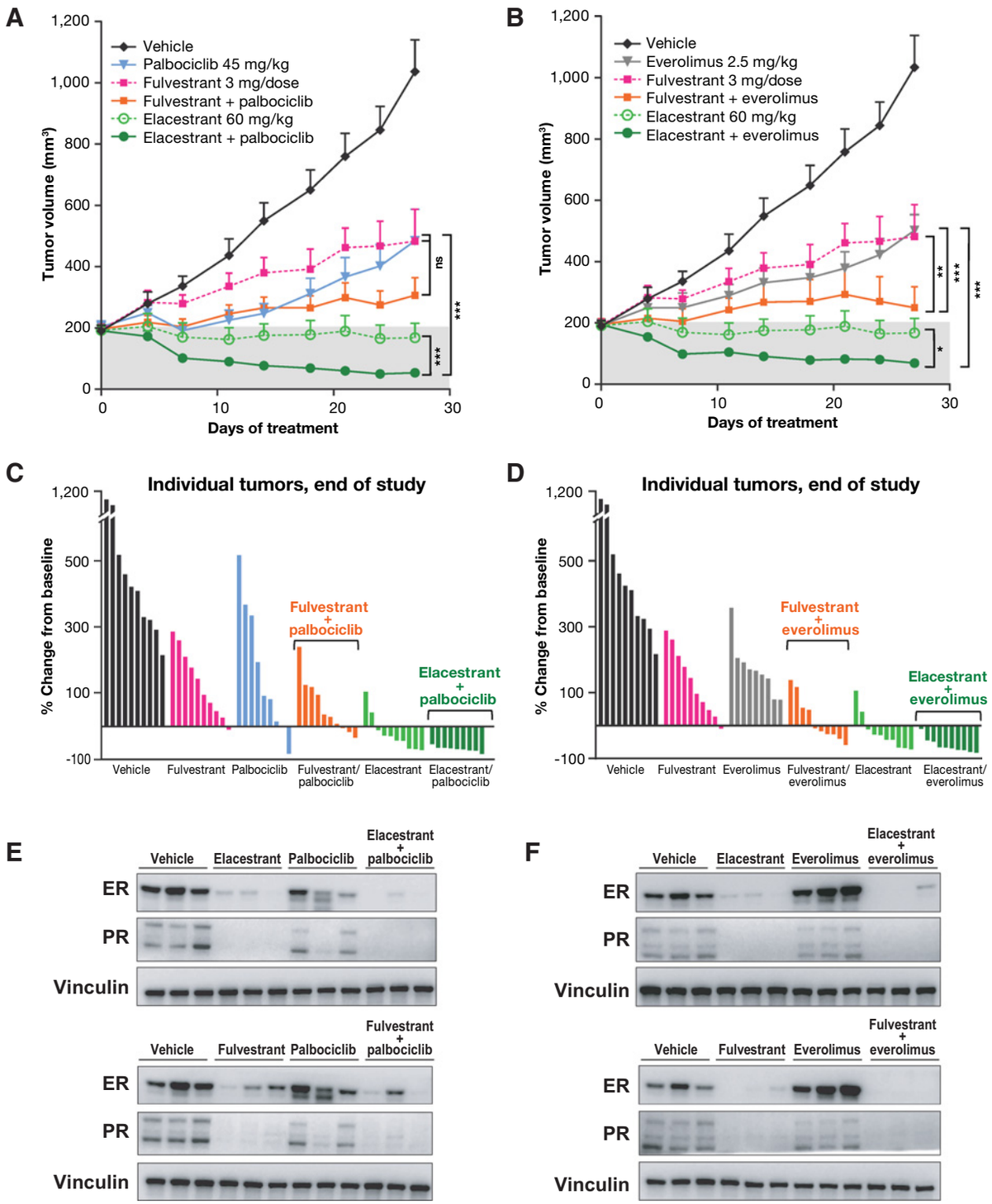

Figure 2.

Elacestrant drives tumor regressions alone and in combination with approved clinical agents in MCF7 xenografts. Animals bearing MCF7 tumors were treated for 27 days with elacestrant alone, fulvestrant alone, or either SERD in combination with palbociclib or everolimus. **A** and **B,** Mean tumor volumes ($n = 9$–10/arm) ± SEM. Asterisks represent significant differences between the indicated treatment groups on day 27. **C** and **D,** Percent change in tumor volumes from individual animals from start of treatment to end of treatment. **E** and **F,** Representative Western blots from individual tumors from **A–D** collected 2 hours posttreatment on day 27. Data representing single agent vehicle-, elacestrant-, and fulvestrant-treated animals are duplicated in **A** and **B**, **C** and **D,** and **E** and **F**.

Table 1. Characterization of PDX models

Model	Patent Rx-history	ER (Intensity)	ER (% positive cells)	PR (Intensity)	PR (% positive cells)	Her2
ST986	Rx-naïve	2	84	2	77	+
MAXF-713	Rx-naïve	3	98	3	85	–
HBCx-21	Rx-naïve	3	99	3	98	–
HBCx-3	Rx-naïve	2	61	3	51	+
HBCx-19	Rx-naïve	2	34	3	8	+
ST2177 (ESR1:Y537S^{hom})	tam, AI	2	45	3	83	+
ST941 (ESR1:Y537S^{het})	AI	2	52	3	73	+

NOTE: Patient Rx-history refers to therapies administered to patients prior to development of PDX. IHC analyses on vehicle-treated tumors using antibodies specific for human ER, PR and Her2. Mean of %positive cells shown (n = 3–6 tumors/model analyzed).
Abbreviations: AI, aromatase inhibitor; Rx, treatment; Tam, tamoxifen.

SERD treatment for an extended period (Supplementary Fig. S5E). These results confirm the potent and stable antitumor efficacy of elacestrant in clinically relevant patient-derived tumor models expressing high levels of ER signaling and further validate that the elacestrant-mediated biological effects extend beyond cell line–derived tumor xenograft models.

We also evaluated the effect of elacestrant and fulvestrant in PDX models expressing intermediate (HBCx-3) and low (HBCx-19) expression levels of ER and PR (Table 1; Supplementary Fig. S5A). On the basis of IHC analyses, approximately 60% of cells expressed ER and 50% of cells expressed PR in the HBCx-3 model (Table 1). In HBCx-3, both fulvestrant and elacestrant resulted in

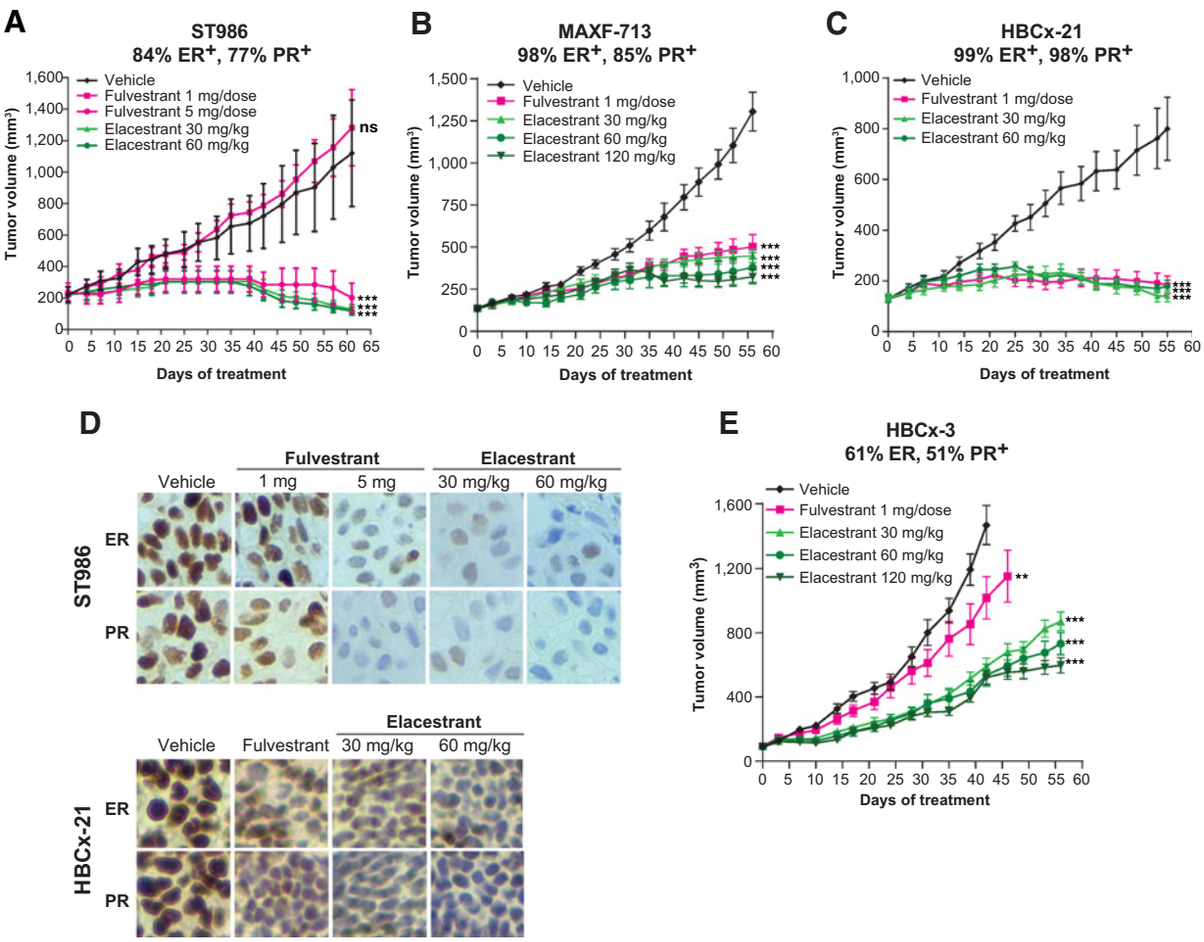

Figure 3.
Elacestrant displays potent anti-tumor activity in multiple PDX models. **A–C,** Mean tumor volumes ± SEM of indicated PDX models treated with elacestrant or fulvestrant at the indicated doses. ST986, n = 6/arm. MAXF-713 and HBCx-21, n = 9–10/arm. Asterisks represent significant differences of the indicated treatment groups relative to vehicle control at end of treatment. **D,** IHC analyses of tumors harvested 4 hours posttreatment at the end of study from ST986 (top) and HBCx-21 (bottom) using antibodies specific for ER or PR. Representative photomicrographs shown (40× magnification). **E,** Mean tumor volumes (n = 10/arm) ± SEM of HBCx-3 model. Asterisks represent significant differences of the indicated treatment groups relative to vehicle control on day 42.

significant, yet partial growth inhibition (Fig. 3E) with a corresponding decrease in *PGR* mRNA (Supplementary Fig. S5F). In both HBCx-3 (61% ER, 51% PR) and MAXF-713 (98% ER, 85% PR), the addition of fulvestrant to elacestrant did not achieve a significant change in tumor volume compared with elacestrant as a single agent, suggesting elacestrant alone achieves maximal efficacy at the doses tested in these models (Supplementary Fig. S6A and S6B). Furthermore, neither compound was efficacious in the HBCx-19 model (Supplementary Fig. S6C). The IHC results demonstrated that 34% of cells expressed ER and 8% of cells expressed PR in the HBCx-19 tumors (Table 1), suggesting the ER signaling pathway may play a less significant role in driving tumor growth in this model. In addition to the SERDs tested, tamoxifen was also ineffective at inhibiting tumor growth in the HBCx-19 model, confirming the lack of efficacy seen in this model is consistent across different classes of ER-targeting agents (Supplementary Fig. S6C). Collectively, these data suggest that elacestrant acts *in vivo* maximally to inhibit ER signaling and tumor growth in these PDX models. Furthermore, these data also demonstrate that PDX models with lower levels of ER and PR expression were generally less sensitive to antiestrogens.

To explore the correlation between ER/PR and sensitivity to endocrine agents further, we performed an efficacy screen with elacestrant in eight PDX models derived from patients that were clinically diagnosed with luminal A or luminal B breast cancers. The models used for the screen were confirmed for ER, PR, and Her2 expression and in large part retained similar ER and PR levels as described in the clinical diagnoses (Supplementary Fig. S7). These models represented a wide range of ER and PR levels, with the assumption that models with higher ER/PR expression would rely more heavily on the ER pathway for growth, and therefore be more susceptible to ER-antagonism (Supplementary Fig. S7). Indeed, the *in vivo* screen validated our hypothesis demonstrating that models with higher ER and PR expression generally displayed increased sensitivity to elacestrant. While ER signaling alone is unlikely to be the sole determinant for single-agent activity, these results further corroborated our findings from the initial PDX studies (Fig. 3; Supplementary Fig. S7).

Correlation of hormone receptor status and combination efficacy

The aforementioned correlation with ER status led us to further hypothesize that PDX models with intermediate ER and PR expression may represent a setting that would benefit from combinations with approved agents. To this end, we treated HBCx-3 tumors with elacestrant in combination with palbociclib or everolimus. Interestingly, while the combination of elacestrant with fulvestrant did not result in greater efficacy compared with elacestrant alone (Supplementary Fig. S6A and S6B), the combination of elacestrant with either palbociclib or everolimus in the HBCx-3 model resulted in significantly greater efficacy than any single-agent alone (Fig. 4A and B). As a comparison, combinations of elacestrant with palbociclib or everolimus were also examined in the MAXF-713 PDX model that expressed relatively high levels of ER and PR. In this model, elacestrant treatment alone resulted in a significant decrease in tumor volume relative to vehicle treatment (Fig. 3B; Supplementary Fig. S6). The addition of everolimus to elacestrant did not significantly decrease tumor volumes and the addition of palbociclib to elacestrant resulted in a modest, yet significant decrease in tumor volumes compared with elacestrant alone in this model (Supplementary Fig. S6D). These data suggest elacestrant can induce greater %TGI in a model with higher ER and PR expression (Table 1; Supplementary Fig. S5A) and that models with intermediate ER and PR expression might benefit from combination strategies.

Elacestrant effectively inhibits growth of PDX models harboring ESR1 mutations

As mentioned above, patients who have received multiple rounds of endocrine therapies often progress, yet many maintain sensitivity to ER-targeting agents (7). This is further supported by data demonstrating elacestrant displays single-agent efficacy in multiple models that were derived from heavily pretreated patients that received prior endocrine therapies (Supplementary Fig. S7). The emergence of clinical *ESR1* mutations has recently gained attention as an increasingly important mechanism of clinical resistance to endocrine therapies (25–31). To test the potential for elacestrant to inhibit the growth of *ESR1*-mutant tumors, we utilized two PDX models (ST2177 and ST941) that harbor a Y537S mutation in the *ESR1* gene (Table 1). This specific mutation is among the most frequently reported aberrations found in tumors from patients treated with aromatase inhibitors (27), and has also been shown to result in high levels of constitutive ER signaling. Consistent with this, these two *ESR1* mutant PDX models were derived from patients who were treated with aromatase inhibitors and the models were confirmed to have high levels of PR expression (Table 1; Supplementary Fig. S5A).

The *in vivo* efficacy of elacestrant and fulvestrant was examined to evaluate the relative efficacy of these agents in tumors expressing *ESR1* mutations. Fulvestrant at 3 mg/dose did not result in a significant decrease in tumor volumes in the ST941 model and the 1 mg/dose was partially effective in the ST2177 model (Fig. 5A–D). It should be noted, however, higher doses of fulvestrant were not tested in these models; therefore, we cannot determine whether a greater efficacy could have been achieved. Elacestrant induced complete growth inhibition of ST2177 tumors at both doses tested (30 mg/kg and 60 mg/kg), and demonstrated a dose-dependent growth inhibition in ST941 tumors (Fig. 5A–D). The addition of palbociclib to elacestrant (60 mg/kg) resulted in a significant decrease in tumor volumes relative to elacestrant alone in ST941, suggesting an additional benefit may be realized in some patients with *ESR1*-mutant tumors using a combination strategy.

PR expression was also examined at the end of treatment in these two models using both IHC and qRT-PCR (Fig. 5E and F). Interestingly, elacestrant resulted in a dose-dependent decrease of PR in both models. This result was consistent in the ST941 model as a corresponding dose-dependent efficacy was also observed. Interestingly, in ST2177, a dose-dependent decrease in PR was observed despite complete %TGI observed with both doses of elacestrant (Fig. 5E and F).

Together, these results further demonstrate the potent *in vitro* and *in vivo* activity of elacestrant to inhibit ER expression, downstream signaling and ER-mediated growth of multiple ER^+ breast cancer models. These data also suggest elacestrant has consistent and maximum inhibitory effects on the ER pathway, and that the combination of elacestrant with other targeted therapies can provide additional benefit. Finally, we have demonstrated that elacestrant is capable of producing regressions in tumor models derived from heavily pretreated patients including those harboring *ESR1* mutations.

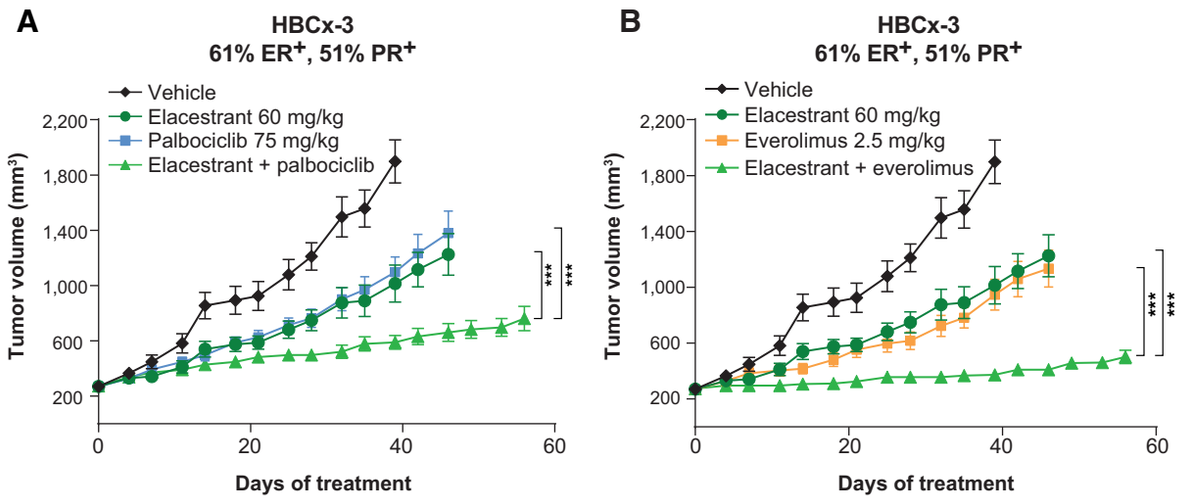

Figure 4.

Elacestrant in combination with palbociclib or everolimus in a PDX model. Mean tumor volumes ($n = 8$–10/arm) ± SEM of HBCx-3 xenografts treated with elacestrant (60 mg/kg) and either palbociclib (**A**) or everolimus (**B**). Asterisks represent significant differences between the indicated treatment groups on day 46.

Discussion

In this report, we describe an orally bioavailable SERD, elacestrant, and its potent growth-inhibitory activity in multiple models of ER$^+$ breast cancer. We demonstrate that the *in vitro* effects of elacestrant (ER degradation, ER signaling blockade, and growth inhibition) translate to *in vivo* efficacy in multiple patient-derived tumor xenograft models (Table 1; Supplementary Fig. S8). Specifically, elacestrant as a monotherapy demonstrated efficacy in attenuating the growth of multiple clinically relevant PDX models, many of which were derived from patients who were heavily pretreated (Supplementary Figs. S7 and S8A). Moreover, in models with high ER/PR expression, elacestrant demonstrated maximal efficacy as a single agent, whereas in models expressing lower ER/PR levels a combination with palbociclib or everolimus was required to achieve similar efficacy (Supplementary Fig. S8B). Furthermore, elacestrant treatment resulted in significant tumor growth inhibition in two PDX models that harbor *ESR1* mutations (Table 1; Fig. 5). Together, these data suggest the potential clinical utility of elacestrant in the treatment of ER$^+$ breast cancers, both in the early and late stages of disease.

To date, fulvestrant remains the only SERD approved in the clinic for the treatment of ER$^+$ breast cancer. In the *in vitro* setting, we demonstrate the ability of elacestrant to induce ER degradation to the same extent as fulvestrant and this corresponded to a blockade of ER signaling (Fig. 1). We further demonstrate elacestrant is able to inhibit tumor growth similar to fulvestrant in *in vivo* MCF7 tumor xenografts. In this study, elacestrant demonstrated potent antitumor activity in multiple models at the clinically achievable and well-tolerated dose of 30 mg/kg. Importantly, at this dose, elacestrant achieved mouse plasma exposure similar to the human plasma exposure corresponding to a clinical dose that produces a robust estrogen receptor engagement based on 16α-[^{18}F]Fluoro-17β-estradiol (FES) uptake (manuscript in preparation).

The data presented here suggest a correlation between the efficacy of an ER-targeting agent with the expression of ER (allowing target engagement of a SERM or SERD) and PR (suggesting dependence on the ER pathway signaling for growth and survival). This is an important observation to consider, given that standard clinical practice recommends endocrine treatment for patients with ER$^+$ tumors with as few as 1% of cells with the tumor expressing ER (37). Given the results presented here demonstrating minimal efficacy with ER-targeting agents in PDX models with low/no ER/PR expression, this concept warrants further investigation. Indeed, a recent retrospective study comparing patients with tumors that were 1%–9% ER-positive versus greater than 10% ER positive suggested that the patients with lower ER positivity did not benefit from endocrine therapy (39). In fact, the lack of response to endocrine therapies in these patients was similar to patients with basal (ER-negative) disease; however, it would be important to confirm this with a randomized clinical study.

Furthermore, the data in the HBCx-3 model suggest that a model with intermediate levels of ER and PR is only partially sensitive to a SERD, and the addition of a second SERD did not result in additional efficacy at the doses tested (Supplementary Fig. S6A). These data suggest that these tumors are likely driven by other pathways in addition to ER signaling. Our data demonstrating increased efficacy with combinations of elacestrant and palbociclib or everolimus in HBCx-3 confirm this hypothesis (Fig. 4). Similarly, in the MAXF-713 model, the combination of fulvestrant and elacestrant did not significantly increase the %TGI compared with the %TGI with elacestrant alone. However, the combination of palbociclib and elacestrant resulted in a slight, yet significant increase in %TGI compared with elacestrant alone, suggesting additional pathways can remain active despite high ER/PR levels. Collectively, these data demonstrate that (i) elacestrant can elicit potent antagonism on the ER pathway and (ii) elacestrant may serve as an effective endocrine backbone for combinations with multiple agents in the treatment of ER$^+$ breast cancer. This idea is supported by the recent data from the PALOMA-3 trial, which demonstrated increased progression-free survival when palbociclib was combined with fulvestrant compared with patients treated with fulvestrant alone (24). In

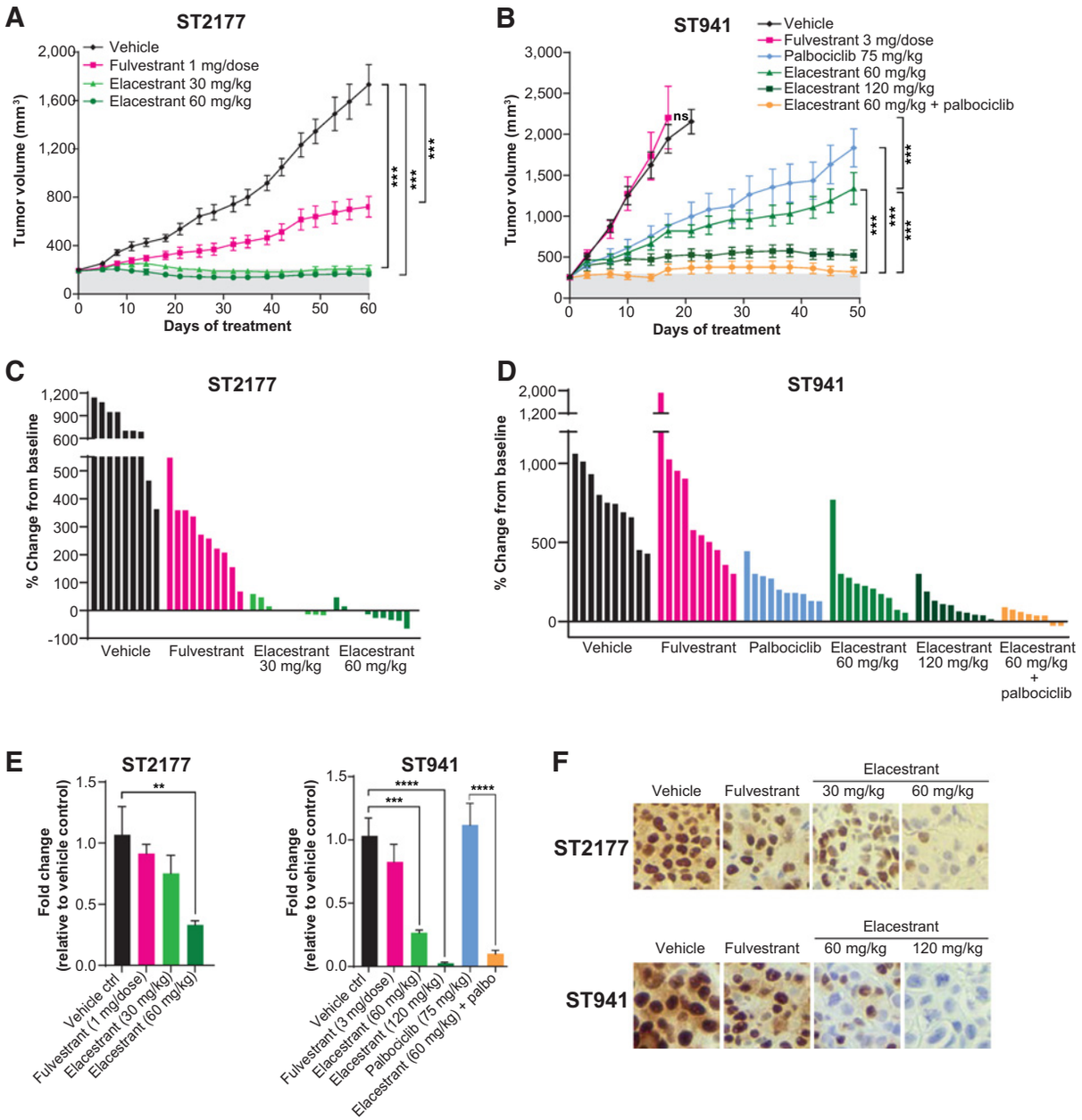

Figure 5.

Elacestrant inhibits growth of two PDX models with Y537S mutations in *ESR1*. **A** and **B,** (top) Mean tumor volumes ($n = 8$–10/arm) ± SEM of ST2177 (**A**) or ST941 treated with the indicated compounds at the indicated doses (**B**). For ST2177, asterisks represent significant differences between the indicated groups at the end of study. For ST941, statistical analyses of elacestrant-treated groups versus vehicle-treated groups were performed on the day that vehicle-treated tumors were harvested (day 21). Statistical analyses of fulvestrant-treated groups were compared with vehicle-treated groups on the day that fulvestrant-treated animals were harvested (day 17). **C** and **D,** Percent change in tumor volumes from individual animals at end of treatment for ST2177 and on the days in which ST941 were analyzed for statistical significance as outlined above. **E,** qRT-PCR analyses of *PGR* in ST2177 or ST941 tumors treated with elacestrant or fulvestrant at the indicated doses. One-way ANOVA was used to detect statistical significance between the indicated groups. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. Histogram depicts the mean fold change ($n = 4$) relative to the mean of vehicle control ($n = 4$) ± SD. **F,** IHC of tumors collected 4 hours posttreatment from **A** and **B** using an antibody specific for PR. Representative photomicrographs shown (40× magnification).

summary, these results suggest ER/PR levels should be considered along with the heterogeneity of ER$^+$ breast cancers, to provide patients with the most appropriate therapeutic strategies and to improve the chance of clinical success.

Consistent with clinical reports (40), the results presented here suggest expression of progesterone receptor in addition to ER, might have predictive value in determining antiestrogen treatment response. While baseline PR levels in each model might be

predictive of response, a complete decrease in PR after SERD treatment might not be required for efficacy. For example, both doses of elacestrant tested (30 mg/kg and 60 mg/kg) produced a consistent and significant tumor growth inhibition in the ST2177 model; however, the lowest dose of elacestrant, 30 mg/kg, appeared to have a limited effect on PR expression (Fig. 5E and F). It is possible that changes in ER target genes beyond *PGR* could be more correlative with SERD response. Indeed, treatment of ST2177 tumors with 30 mg/kg elacestrant did result in a significant decrease in *TFF1* expression (Supplementary Fig. S9A). In a second example, a decrease in PR expression was observed in elacestrant treated MCF7 tumors 4 hours and 24 hours following dosing (Supplementary Fig. S3C). When elacestrant-treated tumors were evaluated four weeks posttreatment, *PGR* expression was no longer decreased, whereas *GREB1* expression was decreased (Supplementary Fig. S9B). It will be important to understand specific pharmacodynamic changes that correlate with sustained tumor growth inhibition in the context of long-term treatment and posttreatment settings. Recently, a study evaluating PR levels and response to fulvestrant demonstrated that while initial PR levels indicated sensitivity to fulvestrant in patients, the reduction in PR levels did not appear to correlate with long-term efficacy (41). These data and reports suggest it will be important to evaluate a broader panel of ER target genes in response to elacestrant, with the notion that global ER antagonism might more accurately correlate with response to ER-targeting agents. Interestingly, a recent report demonstrated that fulvestrant can effectively antagonize ER signaling and E2-dependent proliferation in the absence of ER degradation (42). This further highlights the importance of global ER antagonism and its link to efficacy. It will be important to further understand the mechanisms of ER degradation by elacestrant as it relates to the downstream effects on tumor growth inhibition. Finally, it will be interesting to determine whether the ER target gene profile and the ER coactivator/corepressor recruitment might be different in response to elacestrant versus fulvestrant. Indeed, a recent report suggested a unique ER-targeting profile for each SERM/SERD tested (9). This might explain, in part, the differences in efficacy observed in the models tested here in response to fulvestrant or elacestrant treatment.

The prevalence of *ESR1* mutations is gaining increasing attention as current advances in technology readily allow the detection of these and other mutations from circulating tumor DNA in plasma samples, highlighting their clinical importance (43). Recent studies have demonstrated that patients treated with aromatase inhibitors are more likely to develop treatment-resistant tumors that harbor *ESR1* mutations (26, 27, 32). In fact, approximately one-third of patients previously treated with an AI were shown to have a mutation in *ESR1* (32). Some evidence in the literature suggests a shift in potency of fulvestrant when evaluated in preclinical models that express *ESR1* mutations (30). However, recent data from the PALOMA-3 trial demonstrated that the presence of *ESR1* mutations did not affect the extent of clinical benefit observed in fulvestrant-treated patients (32). Consistent with this, we demonstrate elacestrant was able to potently inhibit the growth of PDX models derived from patients previously treated with multiple endocrine therapies, including two that harbor an *ESR1* Y537S mutation (Fig. 5). Preliminary data suggests that elacestrant is able to bind to wild-type *ESR1* and multiple mutant forms of *ESR1* with similar potency in an *in vitro* binding assay, suggesting elacestrant could have activity against a broader panel of mutations beyond Y537S (Supplementary Fig. S10). Given that multiple clinical *ESR1* mutations have been identified, it will be interesting to continue to evaluate elacestrant efficacy against these other *ESR1* mutations. Finally, it will be important to track *ESR1* allele burden in future clinical studies when evaluating response to elacestrant, as many patients that are heavily pretreated with aromatase inhibitors have tumors with multiple mutations in *ESR1* (32).

The data presented here further describe the novel, orally bioavailable SERD, elacestrant, which potently inhibits ER-mediated signaling and growth of multiple ER$^+$ breast cancer models. Elacestrant significantly inhibits tumor growth of clinically relevant patient-derived wild-type and mutant *ESR1* xenograft models as a single agent, and may serve as an effective endocrine backbone for use in combination therapy with other approved agents.

Collectively, these results provide strong rationale for further clinical investigation of elacestrant in multiple settings, including the potential treatment of endocrine naïve patients, as well as heavily pretreated patients with ER$^+$ breast cancer.

Disclosure of Potential Conflicts of Interest

All authors hold ownership interest in Radius Health, Inc.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Bihani, H. Arlt, N. Tao, J.L. Brown, H.K. Patel

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Bihani, H. Arlt, N. Tao, H. Jiang, G. Hattersley, F. Garner, H.K. Patel

Writing, review, and/or revision of the manuscript: T. Bihani, H. Arlt, N. Tao, H. Jiang, J.L. Brown, D.M. Purandare, G. Hattersley, F. Garner, H.K. Patel

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