Efficacy of SERD/SERM Hybrid-CDK4/6 Inhibitor Combinations in Models of Endocrine Therapy–Resistant Breast Cancer

Suzanne E. Wardell1, Matthew J. Ellis2, Holly M. Alley1, Koleen Eisele3, Todd VanArsdale3, Stephen G. Dann3, Kim T. Arndt4, Tina Primeau5, Elizabeth Griffin5, Jieya Shao5, Robert Crowder5, Jin-Ping Lai6, John D. Norris1, Donald P. McDonnell1, and Shunqiang Li5,7

Abstract

Purpose: Endocrine therapy, using tamoxifen or an aromatase inhibitor, remains first-line therapy for the management of estrogen receptor (ESR1)–positive breast cancer. However, ESR1 mutations or other ligand-independent ESR1 activation mechanisms limit the duration of response. The clinical efficacy of fulvestrant, a selective estrogen receptor downregulator (SERD) that competitively inhibits agonist binding to ESR1 and triggers receptor downregulation, has confirmed that ESR1 frequently remains engaged in endocrine therapy–resistant cancers. We evaluated the activity of a new class of selective estrogen receptor modulators (SERM)/SERD hybrids (SSH) that downregulate ESR1 in relevant models of endocrine-resistant breast cancer. Building on the observation that concurrent inhibition of ESR1 and the cyclin-dependent kinases 4 and 6 (CDK4/6) significantly increased progression-free survival in advanced patients, we explored the activity of different SERD–or SSH–CDK4/6 inhibitor combinations in models of endocrine therapy–resistant ESR1+ breast cancer.

Experimental Design: SERDs, SSHs, and the CDK4/6 inhibitor palbociclib were evaluated as single agents or in combination in established cellular and animal models of endocrine therapy–resistant ESR1+ breast cancer.

Results: The combination of palbociclib with a SERD or an SSH was shown to effectively inhibit the growth of MCF7 cell or ESR1-mutant patient-derived tumor xenografts. In tamoxifen-resistant MCF7 xenografts, the palbociclib/SERD or SSH combination resulted in an increased duration of response as compared with either drug alone.

Conclusions: A SERD–or SSH–palbociclib combination has therapeutic potential in breast tumors resistant to endocrine therapies or those expressing ESR1 mutations. Clin Cancer Res; 21(22); 5121–30. ©2015 AACR.

See related commentary by DeMichele and Chodosh, p. 4999

Introduction

Breast cancer remains the most commonly diagnosed cancer among women and a leading cause of cancer mortality in women (1). Although targeted therapies such as the selective estrogen

1Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, North Carolina. 2Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, Texas. 3Pfizer Oncology Research Unit, La Jolla, California. 4Pfizer Oncology Research Unit, Pearl River, New York. 5Division of Oncology, Department of Internal Medicine, Washington University in St Louis, Saint Louis, Missouri. 6Department of Pathology, Saint Louis University, Saint Louis, Missouri. 7Siteman Cancer Center Breast Cancer Program, Washington University in St. Louis, Saint Louis, Missouri.

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

S.E. Wardell and M.J. Ellis contributed equally to this article.

Corresponding Authors: Donald P. McDonnell, Duke University School of Medicine, Pharmacology and Cancer Biology, LSRC Building Room C238, Box 3813, Durham, NC 27710. Phone: 919-684-6035; Fax: 919-681-7139; E-mail: donald.mcdonnell@duke.edu; and Shunqiang Li, Section of Breast Oncology, Division of Oncology, Department of Medicine, 600 South Euclid Avenue, St Louis, MO 63110. Phone: 314-747-9311; Fax: 314-362-7086; E-mail: SQLi@DOM.wustl.edu


©2015 American Association for Cancer Research.

www.aacjr.org
Translational Relevance

Resistance to endocrine therapies is a significant clinical issue in patients with estrogen receptor (ESR1)–positive breast tumors. In refractory disease, however, ESR1 remains active and engaged, providing the impetus to evaluate third-generation selective estrogen receptor modulators (SERM) and selective estrogen receptor downregulators (SERD) as therapeutic interventions. In this study, we demonstrate in both cell-line–derived and patient-derived xenograft models of endocrine therapy–resistant breast cancer that the efficacy of the SERM/SERD hybrid (SSH) molecules bazedoxifen and pipexifen, or the SERD fulvestrant, was increased when coadministered with palbociclib, an inhibitor of the cyclin-dependent kinases 4 and 6. Importantly, these drug combinations were also effective in tumors expressing ESR1 ligand binding domain mutations associated with resistance to endocrine therapy. Bazedoxifen and fulvestrant are approved for the treatment of osteoporosis or breast cancer, respectively; thus, an evaluation of their activity in combination with palbociclib is a priority for clinical investigation.

Materials and Methods

Reagents
ESR1 ligands included 17β-estradiol (Sigma), ICI 182,780 (Toceis), and 4-hydroxytamoxifen (Sigma). Palbociclib, pipexifen (PIP), and BZA were provided by Pfizer. (S)-3-(3-hydroxyphenyl)-4-methyl-2-(4-{(S)-2-[(R)-3-methylpyrrolidin-1-yl]propoxy}phenyl)-2H-chromen-6-ol (BPN1) and (E)-3-{4-[(E)-2-(2-chloro-4-fluorophenyl)-1-(1H-indazol-5-yl)but-1-en-1-yl]phenyl}acrylic acid (BPL2) were synthesized as described previously (14, 15). Ligands were dissolved in ethanol or DMSO.

Cell culture
MCF7 cells were provided by Dr. Kenneth Korach (National Institute of Environmental Health Sciences) in 2004. TamR cells were derived from a tamoxifen-resistant MCF7 xenograft tumor (20) in 2001. Both cell lines were maintained in DMEM/F12 media (Invitrogen) supplemented with 8% fetal bovine serum (FBS; Gemini), non-essential amino acids (Invitrogen), and sodium pyruvate (Invitrogen), with 100 nmol/L 4OHT added for TamR cells. Unless otherwise indicated, cells were plated for experiments in phenol red-free (PRF) media supplemented with 8% charcoal stripped FBS (CS; Gemini). LTED MCF7 cells, derived in vitro as previously described (21), were maintained and plated for experiments in PRF DMEM/F12 media supplemented with 8% CS. Forty-eight hours after plating, cells were treated as indicated, and were harvested for immunoblot or real time quantitative PCR analysis 24 hours later. MCF7, LTED, and TamR cell lines were authenticated by STR analysis in 2013. T47D cells were received from the ATCC in 2007 (authenticated by STR analysis) and were maintained in DMEM media + 10% FBS.

Immunoblot analysis
Protein expression was analyzed as described (22) using antibodies from Cell Signaling Technology (pRB ab6075) and Santa Cruz Biotechnology (cytokerin 18 sc-6259), (lamin A sc-20680), (α-tubulin sc-5546), and (ESR1 sc-8005, sc-543).
In-cell Western blot analysis

MCF7 cells (2.5 × 10^6 well) were plated in clear-bottom 96-well black plates for 24 hours prior to addition of ligand for 18 hours. Fixation, detection of ESR1 (sc-543), and analysis were performed per LI-COR manufacturer’s protocol using the LI-COR ODYSSEY infra-red imaging system. Data were normalized to DNA content (DRAQ5).

RNA Isolation and Real-Time Quantitative PCR

RNA isolation and analysis was performed as described (23). mRNA abundance was calculated using the ΔΔCt method (22). Primer sequences are available upon request.

Proliferation assays

Proliferation assays were performed essentially as previously described (17) and analyzed using FluoroReporter (Gibco) or Cell Titer Glo (Promega) kits per manufacturer’s instructions.

In vivo studies

All procedures were approved by the Institutional Animal Care and Use Committee at Duke University or Washington University in St. Louis. The human tissues for the experiments were processed in compliance with NIH regulations and institutional guidelines, and approved by the institutional review board at Washington University in St. Louis.

TamR xenograft tumor study. Tumors were initiated in the mammary gland of tamoxifen-treated (5-mg s.c. pellet; Innovative Research of America) ovariectomized 6-week-old female NU/NU mice by serial transfer as previously described (17). Tumors were measured 3 × weekly by caliper (volume = (A^2 × B)/2, where A is the shorter axis). At ~0.1-cm^3 volume, mice were randomized to treatment (28 days) with vehicle, BZA (5 or 10 mg/kg/day), PIP (5 or 10 mg/kg/day), or ICI (200 mg/kg weekly). Treatment groups were further subdivided into vehicle or palbociclib (100 mg/kg/day p.o.) treatment. SERDs were dissolved in corn oil and injected s.c. or i.m. as indicated. Palbociclib was dissolved in 10 mmol/L sodium lactate, pH 4. Following euthanasia, tissues were cryopreserved for analysis.

PDX tumor studies. Six- to 8-week-old female SCID Beige (Charles River Laboratories) mice were used in therapeutic efficacy studies as previously described (13). Tumor cells (2–3 × 10^6) were s.c. injected into each mouse. Tumor size was measured every 3 days. Tumor-bearing mice were randomized at 50- to 150-mm^3 tumor volume. BZA (10 mg/kg/day) and palbociclib (125 mg/kg/day p.o.) were administered as above. At euthanasia, tumors were fixed in 10% buffered formalin.

Analysis of animal tissues

Frozen tissues were pulverized prior to protein extraction essentially as above. Total RNA was extracted using the Direct-zol RNA isolation kit (Génesee Scientific) per manufacturer’s instructions. mRNA expression was detected as above. IHC analysis was conducted as described previously (13). Using antibodies purchased from Thermo Scientific (ESR1, RM-9101-S), (Ki67, RM-9106-S1) and Cell Signaling (pRb S807/811, 8516).

Statistical analyses

Tumor growth was analyzed (GraphPad Prism 6) by exponential growth curve analysis and by two-way ANOVA of matched values followed by Bonferroni multiple comparisons to establish significance (P < 0.05) between groups at each day of treatment.

Results

As an initial step in these studies, we performed a comparative analysis of the in vitro pharmacology of (i) the SERD fulvestrant (ICI 182,780; ICI) and (ii) SSHs that have either been evaluated in humans or are currently being evaluated for clinical development: GW5638/DPC974 (active metabolite GW7604), BZA, PIP (ERα-923), and the benzopyran BPN1 (described in U.S. patent 20140107095A1), and benzopyrazole BPL2 (described in U.S. patent 20130231333A1; Fig. 1A; refs. 20, 24, 25). In this study, the ability of each compound to (i) induce ESR1 degradation, (ii) reverse 17β-estradiol (E2)–dependent regulation of target gene transcription, and (iii) inhibit the growth of ESR1+ MCF7 breast cancer cells in response to E2 or growth factors was assessed. When analyzed using an in-cell Western or by standard Western immunoblotting (not shown), all of the compounds tested were found to quantitatively downregulate ESR1, albeit with differences in both efficacy and potency (Fig. 1B, quantitated on the right). However, these drugs exhibited similar efficacy, despite differences in potency, when assessed for their ability to (i) inhibit E2-dependent induction of the mRNAs encoding the progesterone receptor (PGR) and trefoil factor 1 (TFF1; Fig. 1C) and (ii) inhibit E2-dependent proliferation of MCF7 cells (Fig. 1D). Interestingly, we noted discrepancies in the efficacy and potency with which SERDs/SSHs inhibit ESR1 activity when compared with their ability to downregulate ESR1 expression. Specifically, although BZA exhibited the least efficacy in assays of receptor degradation, it inhibited ESR1 activity (gene transcription and cell proliferation) with efficacy and potency similar to ICI, the most efficacious antagonist. Conversely, BPL2 potently induced ESR1 degradation, but was the least potent of the compounds tested when evaluated in ESR1 activity assays. Previously, we have shown that although receptor degradation is a desirable trait of ESR1 antagonists, the primary inhibitory activity of SERDs and SSHs with regard to ESR1 action relates to their ability to function as high-affinity competitive antagonists that drive the receptor into a conformation that precludes interaction with coactivators (17, 22). Growth factor–mediated activation of ESR1 has been implicated as a mechanism by which breast cancer cells may overcome the inhibitory effects of tamoxifen (26). Therefore, we next analyzed the ability of these compounds to attenuate insulin-stimulated proliferation of MCF7 cells, an estrogen-independent activity that requires ESR1 (not shown). This assay enabled the SERDs/SSHs tested to be differentiated, as we observed that while all of the drugs tested inhibited insulin-stimulated proliferation, the most effective downregulators of ESR1 (IC1, BPN1, and BPL2) exhibited inverse-agonist activity (Supplementary Fig. S1). 4-Hydroxytamoxifen did not attenuate the activity of insulin when analyzed under these conditions. Thus, it is likely that the SERD activity reinforces ESR1 inhibition, although the relative importance of receptor degradation versus inhibition on the overall pharmacological activity of these drugs remains an unresolved issue.

These studies demonstrate that, when corrected for minor differences in potency, the antagonist efficacy of the SERDs/SSHs tested were very similar. Thus, we selected fulvestrant (ICI), BZA,
and PIP for further study as single agents and in combination with palbociclib as (i) ICI is currently available for clinical use, (ii) BZA is approved for clinical use in Europe and Japan, and (iii) PIP was evaluated in a previously completed phase II clinical trial in patients with advanced breast cancer. Further studies will be undertaken with BPN1 and BPL2 (third-generation SERDs currently in clinical development), as information on their clinical activities emerges. No further studies were performed with GW7604/DPC974 as its development has been halted.

Fulvestrant, bazedoxifene, and pipendoxifene inhibit ESR1 activity in relevant models of endocrine therapy–resistant breast cancer

SERDs/SSHs are likely to be utilized and to have specific advantages in the setting of relapsed/resistant tumors in patients who have already progressed during tamoxifen and/or AI therapy. Thus, it was important to evaluate the efficacy of ICI, BZA, and PIP in cellular models of endocrine therapy–resistant breast cancer. Therefore, in vitro experiments similar to those in Fig. 1 were conducted in MCF7 sublines that were adapted to grow under conditions of estrogen deprivation (LTED—a validated model of aromatase resistance; refs. 27–29) or which were derived from tamoxifen-resistant xenografts (TamR; ref. 20). As was observed for the endocrine therapy–sensitive cells, ICI, BZA, and PIP inhibited proliferation of the LTED (Fig. 2A) and TamR (Fig. 2B) cells with similar potency, and their ability to downregulate ESR1 in these resistant models mirrored that observed in the parental MCF7 cells (Fig. 2C; Supplementary Fig. S2A and not shown). Further, several mutations of ESR1 have recently been identified that are likely to contribute to disease progression (3–5). In agreement with prior studies, we observed that several of these mutants exhibit ligand-independent activation of ESR1 target gene transcription (data not shown). While these mutations reduce the potency of ICI, BZA, and PIP, all three compounds inhibited the proliferation of MCF7 cells expressing these mutations with similar efficacy (Fig. 2D). In addition, in MCF7 cells expressing an ESR1-Y537N/D538G double mutation (recently identified in a fulvestrant-resistant patient tumor (5), we observed...
Figure 2.
Full-length, BZA, and PIP inhibit ESR1 activity in relevant models of endocrine therapy–resistant breast cancer. A, LTED MCF7 cells were plated in PRF media supplemented with FBS that was stripped of growth factors twice using charcoal. Cells were treated with ICI, BZA, and PIP (10^{-12}–10^{-6} mol/L) on days 1, 4, and 6 of an 8-day proliferation assay and analyzed as in Fig. 1. B, TamR cells were plated in media supplemented with CFS 24 hours prior to treatment, and were treated with 1 nmol/L E2 as well as with ICI, BZA, and PIP (10^{-12}–10^{-6} mol/L) on days 1, 4, and 6 of an 8-day proliferation assay. Cell proliferation was quantitated as in Fig. 1. C, TamR cells were plated in PRF media supplemented with charcoal-stripped FBS 48 hours prior to treatment with ICI, BZA, or PIP (10^{-12}–10^{-6} mol/L) for 24 hours. Expression of ESR1 (C) and loading control cytokeratin 18 (CK18; Supplementary Fig. S2A) in whole-cell extracts were detected by immunoblot (left). ESR1 levels relative to CK18 were quantitated by densitometry using Adobe Photoshop (right). D, MCF7 cells engineered to express WT ESR1 or Y537S, Y537N, or D538G ESR1 mutations in T47D breast cancer cell models are particularly sensitive to CDK4/6 inhibitors (30). Importantly, we observed that the expression of the ESR1 Y537S, Y537N, or D538G mutations of ESR1 were treated for 7 days with increasing (10^{-12}–10^{-6} mol/L) concentrations of ICI, BZA, or PIP. DNA content, as assessed by luminescence (CellTiter-Glo assay), serves as an indicator of cell number in each condition. Data represent average detection (mean ± SD of triplicate wells). Data are representative of at least 3 independent experiments. IC_{50} values listed in the figure legend were calculated by nonlinear curve regression using GraphPad Prism 6.

Figure 3.
A, Estrogen deprivation and cotreatment with CDK4/6 inhibitors can significantly left-shift the IC_{50} of tamoxifen-resistant breast cancer xenografts. B, Growth factor depletion decreases ESR1 expression in breast cancer cells. C, Treatment of MCF7 cells with increasing doses of palbociclib reduced Rb phosphorylation, but did not significantly change endogenous ESR1 expression (Fig. 3A and Supplementary Fig. S3A and S3B). Similarly, analysis of ESR1-dependent target gene expression revealed that neither the efficacy nor the potency of 17β-estradiol was influenced by treatment with increasing doses of palbociclib (Fig. 3B). Together, these data suggest that CDK4/6 inhibition itself is unlikely to directly impact ESR1 activity. Not surprisingly, using pRB as a readout in MCF7 cells, we observed that when compared with the IC_{50} of 43 nmol/L observed in Fig. 3A using growth factor depleted media (i) alone (2 nmol/L) or (ii) supplemented with E2 (13 nmol/L; Supplementary Fig. S3A and S3B, respectively). These findings underscore the importance of administering palbociclib concurrently with other agents that inhibit key cell growth pathways. A comparison of the ability of palbociclib to inhibit the proliferation of MCF7, LTED, and TamR cells revealed that all exhibited a similar sensitivity to CDK4/6 inhibition (Supplementary Fig. S3C). As expected, E2-dependent MCF7 cell proliferation was attenuated by the addition of palbociclib (Fig. 3C). Importantly, palbociclib did not negatively impact the antiproliferative activity of ICI, BZA, or PIP, nor did it change the IC_{50} of these drugs in this assay (Fig. 3C). A similar response to SERD/SSH-palbociclib combinations was observed in the LTED and TamR cells (not shown). Considering these data, and that which has already been published on these drugs, it is likely that their antiproliferative activities, while converging on common growth-stimulatory pathways, occur by distinct mechanisms. Importantly, we observed that the expression of the Y537S, Y537N, or D538G ESR1 mutations in T47D breast cancer cells did not negatively impact the efficacy of the BZA–palbociclib combination (Supplementary Fig. S3D).

Palbociclib increases the efficacy with which SERDs inhibit the growth of tamoxifen-resistant breast cancer xenografts

Previously, we have reported the development and characterization of an in vivo–derived xenograft model of tamoxifen resistance. When engrafted in mice, this xenograft exhibits tamoxifen-dependent growth, an activity that can be attributed, at least in part, to increased FOXA1 activity, as has been reported in endocrine therapy–resistant breast tumors (refs. 20, 31; unpublished data). The ability of palbociclib, BZA, and PIP to inhibit the
tameisoxifen-stimulated growth of TamR xenograft tumors in vivo as monotherapies or as SERD/SHER-palbociclib combination thera-
pies was next examined. For comparative purposes, we also in-
cluded ICI and ICI-palbociclib arms in this study, although the
dose of ICI used, and that which we and others have found to
be required to inhibit tumor growth (~200 mg/kg weekly), far
exceeds that administered to breast cancer patients (12, 32).
Importantly, when corrected for species equivalency by body
surface area (33), BZA and PIP were administered at doses similar
to those previously evaluated in the clinic (18, 24, 34). Due to the
high number of experimental groups, the data are presented in
several panels to facilitate relevant comparisons. In this well-
validated model of tamoxifen-resistant breast cancer (20, 35),
we noted that palbociclib, BZA, PIP, and ICI, when evaluated as
monotherapies, were similarly effective at inhibiting tumor
growth (Fig. 4 and Supplementary Fig. S4A). The inhibition of
tumor growth was similar in animals treated with 5 mg/kg BZA or
PIP as compared with 10 mg/kg (Fig. 4 and Supplementary
Fig. S4A). Of the combinations evaluated, only BZA-palbociclib
significantly extended the duration of response as compared with
either treatment alone, a result observed using either 5 or 10 mg/
kg BZA (Supplementary Fig. S4B and not shown). Interestingly,
tumor regression (an uncommon response in this model) was
initially observed for palbociclib treatment, followed by robust
regression in a subset of animals after 2 to 3 weeks of treatment
(Fig. 4). This variability in response to palbociclib correlated with
the level of pRb apparent in the progressing tumors assessed at
sacrifice (Supplementary Fig. S4C), demonstrating resistance to
palbociclib without loss of Rb expression. The mechanism by
which these tumors develop resistance to palbociclib is currently
under investigation. We noted that pRb levels were reduced in the
ICI-, BZA-, and PIP-treated animals, likely because cyclin D1 is a
direct transcriptional target of ESR1 (Supplementary Fig. S4C).
However, whereas the impact of BZA, PIP, and ICI, on tumor
growth was statistically similar, higher levels of pRb were observed
in the tumors of animals treated with PIP alone (Supplementary
Fig. S4C). Regardless, coadministration of palbociclib resulted in
a sustained inhibition of Rb phosphorylation in all of the SSH/
SERD-palbociclib-treated tumors (Supplementary Fig. S4C). Consistent with our in vitro analyses, it was observed that all of
these compounds reduced the levels of intratumoral ESR1, where-
as palbociclib treatment alone had no significant effect on receptor
expression (Supplementary Fig. S4C). Finally, evaluation of the
intratumoral expression of the tamoxifen-induced genes AGR2
and KRT13 indicated that (i) palbociclib treatment alone had little
effect on ESR1 target gene activation, and (ii) all SSH/SERDs
efficiently inhibited ESR1 transcriptional activity regardless of
palbociclib treatment (Supplementary Fig. S4D). Overall, these
findings highlight the efficacy of SSH/SERD-palbociclib com-
bined treatment in a clinically relevant model of endocrine
therapy-resistant breast cancer.

Palbociclib and BZA inhibit the growth of xenograft tumors
derived from patients resistant to endocrine interventions

We next sought to evaluate the activity of selected ESR1
modulators and palbociclib as monotherapy or combination in
patient-derived xenograft (PDX) tumor models derived from
biopsies of ESR1+ tumors that had progressed during or after
endocrine therapies. Because palbociclib had not previously been
evaluated in PDX models, we initially assessed the efficacy of
palbociclib treatment alone in tumors expressing wild-type (WT)
ESR1 (WHIM11), having amplified ESR1 expression (WHIM16),
or expressing the ESR1-D538G mutation (WHIM43). Palbociclib
significantly inhibited the growth of the WHIM11 and WHIM16
SERM- or SERD–CDK4/6 Inhibitor Combination in Breast Cancer

Figure 4. Palbociclib increases the efficacy with which SERDs or SERM/SERD hybrids inhibit the growth of tamoxifen-resistant breast tumor xenografts. TamR tumors were implanted into tamoxifen-treated mice. When Tam-stimulated tumors attained ~0.1-cm³ tumor volume, animals were randomized (7–9 mice per group) to receive continued tamoxifen treatment as well as vehicle or SSH/SERD (BZA, 5 or 10 mg/kg/day s.c.; PIP, 5 or 10 mg/kg/day s.c.; or ICI, 5 mg/mouse 1× weekly i.m.), and also vehicle or palbociclib (100 mg/kg/day, p.o.). Tumor growth for each group (separated by SERD treatment for legibility) is presented as average tumor volume ± SEM per study arm at each day of treatment, with the initial day of treatment at randomization considered to be day 0. Tam control and palbociclib only treatments presented on each graph are identical. Tumor growth for animals treated with 5 mg/kg BZA or PIP are shown above, while measurements for animals treated with 10 mg/kg BZA or PIP are depicted in Supplementary Fig. S4C. By day 14, responses to all treatments were significant as compared with the tam treatment-only control (P < 0.01). Significant differences (P < 0.05) between combination treatments and SERD only (●), palbociclib only (●), or both single treatments (●) are indicated at appropriate time points.

tumors, but was without effect on the WHIM43 tumors (Fig. 5A–C). IHC analysis revealed that this lack of response was likely due to loss of Rb expression in this tumor (Fig. 5C).

Having demonstrated the activity of palbociclib as a monotherapy in these clinically relevant models, we next evaluated its activity when administered in combination with BZA. From the ESR1+ PDXs available to us, we selected the WHIM20 tumor for these studies as it expresses ESR1-V537S, an ESR1 mutation that exhibits significant SSH/SERD resistance (Fig. 2D). In this model, it was observed that BZA, palbociclib, or the combination were similarly effective in blocking tumor growth (Fig. 5D). IHC analysis of these tumors revealed that the BZA–palbociclib combination resulted in a more complete suppression of Ki67 expression as compared with either treatment alone (Fig. 5D). Together, these data confirm the likely utility of the BZA–palbociclib combination in achieving maximal suppression of tumor growth and may explain why, in the xenograft studies described in Fig. 4, the duration of the response to the BZA–palbociclib combination is longer than that observed in tumors treated with each drug alone.

Discussion

Tamoxifen and aromatase inhibitors remain the first-line interventions of choice for the treatment of ESR1+ breast cancers (36). Further, the SERMs tamoxifen and raloxifene and the AI exemestane are active chemopreventative agents (37, 38). The enduring presence of tamoxifen and aromatase inhibitors in contemporary breast cancer treatment regimens attests to the central importance of ESR1 as a driver of tumor growth and progression. However, resistance to endocrine therapy is nearly universal in advanced disease, and ESR1+ tumors remain the majority cause of death from breast cancer. Although the mechanisms underlying resistance to endocrine therapy are complex and varied, loss of dependence on ESR1 and its downstream signaling pathways is an infrequent event. Therefore, ESR1 and the estrogen signaling axis remain important therapeutic targets, an observation that has driven the search for agents that target this pathway by new mechanisms. In this study, we demonstrate that ICI, BZA, and PIP, high-affinity competitive antagonists of ESR1 that down-regulate the receptor to varying degrees, are effective in relevant models of endocrine therapy–resistant disease. Importantly, these modulators inhibited the activity of clinically relevant ESR1 mutations with efficacy similar to that observed for the WT ESR1 despite significantly reduced potency. Most importantly, we observed in both endocrine-resistant breast cancer xenograft tumors, and in PDX tumors expressing a relevant mutation of ESR1 (Y537S), that the SSH/SERD BZA in combination with palbociclib resulted in significant tumor growth control.

It is generally accepted that suboptimal pharmacological properties of the only currently approved SERD, fulvestrant, limit the achievable tumor exposure (12, 39–41). Despite these difficulties, higher doses of fulvestrant have improved overall survival in two trials, highlighting the promise of more effective ESR1 targeting. The identification of treatment-associated ESR1 mutations emphasizes the need for improved SERDs able to inhibit these mutations at therapeutically achievable levels. Fortunately, most of the newer, third-generation ESR1 antagonists have improved pharmaceutical properties and their efficacy should not be limited by drug exposure. One SERD, ARN810 (BPL2), is currently being evaluated in a phase I/IIa trial enrolling relapsed/recurrent breast cancer patients (42). However, BZA, a low-toxicity drug that has already been approved for the treatment and prevention of osteoporosis, may have immediate utility in advanced disease (16, 17).

Several new SERMs and SERDs will be available for clinical use in the next 5 years, and, by virtue of their distinct mechanisms of action, it may be possible to sequence their use to increase the duration of treatment response. However, more durable responses will likely be achieved by appropriate combination of SERDs/SERMs with other drugs that inhibit pathways of importance in cancer. Recently, we reported that the Notch signaling pathway is activated in tamoxifen-resistant breast cancers and that the growth of resistant xenografts could be inhibited using γ-secretase inhibitors; thus, the combination of a SERM/SERD with a Notch inhibitor may be beneficial (43). There are also considerable experimental data that support the combination of ESR1 modulators with inhibitors of ERK or PI3K signaling.
When tumors reached patients receiving a palbociclib the duration of progression-free survival was nearly doubled in combination use have come from the PALOMA-1 trial, in which may limit their use (44). The most provocative data thus far on although toxicities observed with the latter two classes of drug may limit their use (44). The most provocative data thus far on letrozole alone (19).

![Figure 5.](image)

Palbociclib and BZA inhibit the growth of xenograft tumors derived from patients resistant to endocrine interventions. A–C, WHIM11 (A), WHIM16 (B), or WHIM43 (C) PDX tumors were implanted into intact NSG mice. Animals bearing tumors of equivalent size (200–500 mm³, depending on the tumor model) were randomized (7–10 mice per group) to treatment with vehicle or palbociclib (125 mg/kg/day, p.o.). Top, tumor growth for each group is presented as average tumor volume ± SEM per study arm at each day of treatment, with the initial day of treatment at randomization considered to be day 0. Bottom, pRb and Ki67 were detected by IHC analysis of representative tumors for each group. A–D, *: significance (P < 0.0001) as compared with vehicle control. D, WHIM20 PDX tumors were implanted as above. When tumors reached ~200 mm³ volume, animals (4 mice per group) were randomized to palbociclib (125 mg/kg/day, p.o) or BZA (10 mg/kg/day sc) alone or in combination. Left, tumor growth for each group is presented as average tumor volume ± SEM per study arm at each day of treatment, with the initial day of treatment at randomization considered to be day 0. Center, Ki67 expression in one representative tumor per treatment group as detected by IHC analysis. Right, 38 sectors per tumor were quantitated by duplicate manual scoring of 3 tumors per group. Mean percentage of positive cells/tumor of individual tumors, as well as the average (±SEM) per treatment group, are depicted on the right. Significant differences between treatments were detected by ANOVA followed by the Holm-Sidak multiple comparison test and are indicated: a, P < 0.0001; b, P < 0.001; c, P < 0.01; d, P < 0.05; n.s., comparison did not detect significant difference.

Although AI–palbociclib combinations are in late-stage clinical development, there remains considerable enthusiasm for strategies in which palbociclib is combined with a SERD or SERM. One important advantage of a SERM or SSH/SERD over an AI is that its competitive activity will prevent the activation of ESR1, either independent of ligand or by endogenous steroidal estrogens or other nonsteroidal compounds that exhibit estrogenic activity (e.g., 27-hydroxycholesterol). Indeed, several trials have been opened evaluating palbociclib combined with fulvestrant or tamoxifen in patients who have progressed after endocrine therapies. As reported here, and by others in the past, resistance to endocrine therapy can be attributed in some instances to somatic mutations in ESR1 that reduce the IC50 of the known SERMs and SERDs (3–5, 13). The pharmaceutical properties of fulvestrant are likely to limit its utility in treating tumors having ESR1 mutations. Likewise, preexisting resistance to tamoxifen in advanced breast cancers makes it unsuitable for the combined therapy. Importantly, when appropriately dose corrected, all of the SERDs/SSHs evaluated in this study were able to inhibit the activity of the common receptor mutants. Given the pharmaceutical properties
of BZA, it is reasonable to expect that this compound would have efficacy, as a single agent, in tumors expressing ESR1 mutations. Our studies also highlight the potential utility of palbociclib and BZA-palbociclib combinations in endocrine therapy–resistant tumors. The mechanistic basis for the favorable activity of SERM/SERD–palbociclib combinations is currently under investigation.

In conclusion, there are substantial data highlighting the multiple points of convergence between the ESR1 and cyclin D1/E2F1/Rb signaling pathway. The results of the studies presented herein highlight how knowledge of these interactions has led to the development of useful strategies to inhibit multiple key points in this pathway to yield useful responses in preclinical models of advanced breast cancer. These findings underscore the need for the near-term clinical evaluation of SERD–palbociclib combinations in patients with advanced breast cancer.

Disclosure of Potential Conflicts of Interest

S.E. Wardell and M.J. Ellis are consultants/advisory board members for Pfizer. D.P. McDonnell reports receiving a commercial research grant from and is a consultant/advisory board member for Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: S.E. Wardell, M.J. Ellis, T. VanAndale, K.T. Arndt, D.P. McDonnell, S. Li

Development of methodology: S.E. Wardell, M.J. Ellis, K. Eisele, T. VanAndale, J. Shao, S. Li

References


Acknowledgments

The authors thank Dr. D. Clay Rouse, DVM (Duke University) for surgical expertise.

Grant Support

Supporting funding provided by the NIH (2R01DK048807; to S.E. Wardell, H.M. Alley, and D.P. McDonnell), Susan G Komen Grants KG090422, BCTR0707808, and Promise Grant PG 12220321 (all to M.J. Ellis), Washington University Institute for Clinical and Translational Sciences (NIH/NCRR UL1TR002493, to M.J. Ellis and S. Li) and Siteman Cancer Center (NIH/NCI P30 CA91842) support for the Washington University HamLET core, Brown Shoe, Theresa’s Research Foundation, and research grants from Pfizer Pharmaceuticals (D.P. McDonnell and S. Li).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Wardell et al.


22. Wardell SE, Marks JR, McDonnell DP. The turnover of estrogen receptor alpha by the selective estrogen receptor degrader (SERD) fulvestrant is a saturable process that is not required for antagonism efficacy. Biochem Pharmacol 2011;82:122–30.


Efficacy of SERD/SERM Hybrid-CDK4/6 Inhibitor Combinations in Models of Endocrine Therapy–Resistant Breast Cancer

Suzanne E. Wardell, Matthew J. Ellis, Holly M. Alley, et al.


Updated version
Access the most recent version of this article at:

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2015/06/10/1078-0432.CCR-15-0360.DC1

Cited articles
This article cites 38 articles, 17 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/22/5121.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/21/22/5121.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.