Selective Androgen Receptor Modulator RAD140 Inhibits the Growth of Androgen/Estrogen Receptor Positive Breast Cancer Models with a Distinct Mechanism of Action

Ziyang Yu¹, Suqin He¹, Dannie Wang¹, Hitisha K. Patel¹, Chris P. Miller¹, Jeffrey L. Brown¹, Gary Hattersley¹ and Jamal C. Saeh¹

¹Radius Health, Inc. Waltham, MA, USA

Running Title: SARM inhibits AR/ER+ breast cancer

Keywords: Breast cancer, estrogen receptor, androgen receptor, selective androgen receptor modulator, RAD140

Correspondence: Ziyang Yu, Radius Health, Inc., 950 Winter Street, Waltham, MA 02451; phone 617-551-4065; email: zyu@radiuspharm.com; or Jamal C. Saeh, Radius Health, Inc., 950 Winter Street, Waltham, MA 02451; phone 617-551-4033; email: jsaeh@radiuspharm.com

Abbreviations: E2, 17β-estradiol; AR, androgen receptor; ARE, androgen responsive element; CSS, charcoal dextran-stripped serum; CDK, cyclin dependent kinase; CK, cytokeratin; DHT, dihydrotestosterone; DMSO, dimethyl sulfoxide; ER, estrogen receptor α; GR, glucocorticoid receptor; HER2, human epidermal growth factor receptor 2; IC₅₀, half maximal inhibitory concentration; IHC, immunohistochemistry; IACUC, institutional animal care and use committee; LTED, long term estrogen depleted; PDX, patient-derived xenograft; PR, progesterone receptor; qPCR, real-time quantitative PCR; SARM, selective androgen receptor modulator; SERM, selective estrogen receptor modulator; SERD, selective estrogen receptor downregulator; SD, standard deviation; SEM, standard error of the mean; TGI, tumor growth inhibition; WB, western blot
Abstract

**Purpose:** Steroidal androgens suppress androgen receptor and estrogen receptor positive (AR/ER+) breast cancer cells and were used to treat breast cancer eliciting favorable response. The present study evaluates the activity and efficacy of the oral selective AR modulator (SARM) RAD140 in *in vivo* and *in vitro* models of AR/ER+ breast cancer.

**Experimental Design:** A series of *in vitro* assays were used to determine the affinity of RAD140 to 4 nuclear receptors and evaluate its tissue-selective AR activity. The efficacy and pharmacodynamics of RAD140 as monotherapy or in combination with palbociclib were evaluated in AR/ER+ breast cancer xenograft models.

**Results:** RAD140 bound AR with high affinity and specificity and activated AR in breast cancer but not prostate cancer cells. Oral administration of RAD140 substantially inhibited the growth of AR/ER+ breast cancer patient-derived xenografts (PDXs). Activation of AR and suppression of ER pathway, including the *ESR1* gene, were seen with RAD140-treatment. Co-administration of RAD140 and palbociclib showed improved efficacy in the AR/ER+ PDX models. In line with efficacy, a subset of AR-repressed genes associated with DNA replication were suppressed with RAD140-treatment, an effect apparently enhanced by concurrent administration of palbociclib.

**Conclusions:** RAD140 is a potent AR agonist in breast cancer cells with a distinct mechanism of action including the AR-mediated repression of *ESR1*. It inhibits the growth of multiple AR/ER+ breast cancer PDX models as a single agent, and in combination with palbociclib. The preclinical data presented here supports further clinical investigation of RAD140 in AR/ER+ breast cancer patients.
Statement of Translational Relevance

The expression of the androgen receptor (AR) in estrogen receptor (ER) positive breast cancer has been well described. Androgens inhibit the proliferation of AR and ER positive (AR/ER+) breast cancer cells, and androgen-based treatment showed clinical benefit in breast cancer patients. However, the clinical utility of the classic steroidal androgen-based therapies has been limited. We report here that RAD140, an oral, tissue selective androgen receptor modulator (SARM), exhibited potent activity in inhibiting the growth of multiple AR/ER+ breast cancer xenograft models. The co-administration of RAD140 with CDK4/6 inhibitor elicited enhanced efficacy in these AR/ER+ models. Mechanistically, RAD140 potently regulates AR target genes, while suppressing the ER targets and the $ESR1$ mRNA. These suggest a distinct AR-mediated mechanism of action compared to the traditional ER-targeted agents. RAD140 may present a novel therapeutic approach for AR/ER+ breast cancer. The first-in-human study of RAD140 in recurrent breast cancer has been initiated.
Introduction

Breast cancer is the second leading cause of cancer death in women, with an estimated 246,660 newly diagnosed cases and 40,450 deaths in the United States alone in 2016 (1). Breast cancer is a heterogeneous disease categorized into several histopathological subtypes based on the status of estrogen receptor α (ER), progesterone receptor (PR), and human epidermal growth factor 2-neu (HER2) receptor. While ER-positive (ER+) breast cancers are now treated with standard-of-care agents targeting the ER-axis, including tamoxifen, fulvestrant and aromatase inhibitors (AI), and HER2-positive tumors are treated with HER2 inhibitors such as trastuzumab, novel therapeutic approaches are still needed to address resistance emerging from these established regimens (2, 3). More recently, combined administration of ER-targeted therapies with the inhibitors of cyclin-dependent kinase (CDK) 4/6 (4) or mammalian target of rapamycin (mTOR) (5) have yielded improved therapeutic efficacy in ER+ breast cancer, and these combination therapies now represent a new generation of standard-of-care for this indication.

Recent histopathological studies revealed that the androgen receptor (AR) is the most commonly expressed hormone receptor in breast cancer, with 75-90% of ER-positive and approximately 30% of ER-negative breast cancers expressing AR (6, 7). While AR is widely present in breast cancer tissue, accumulating evidence have demonstrated that the role of AR in these tumors are subtype-dependent (8-11). AR antagonists including bicalutamide and enzalutamide have been shown to reduce the growth of AR-positive but ER-negative, including triple negative, breast cancer in preclinical models (12-14) and in patients (11). In contrast, in ER-positive breast cancers, AR has been considered anti-proliferative and been associated with a favorable prognosis. Clinically, until the 1970s, breast cancers were often treated with non-selective steroidal androgens including testosterone derivatives and danazol with response rates of 20-25% (9, 15-18). In line with these clinical experiences, preclinical studies have also...
shown treatment with classic androgens including dihydrotestosterone (DHT) reduced the growth of AR and ER-positive (AR/ER+) breast cancer cells in vitro (19-21) and in vivo (22). Classic androgen-based therapy for breast cancer declined due to its virilizing effects, potential risk of further aromatization to estrogens, and the emergence of ER-targeted agents including tamoxifen. However, it is worth noting that more recent clinical evidence showed androgen treatment also led to remission in patients who were progressing after ER-targeted therapy with objective response rate around 17-39% (23, 24). Fulvestrant at second line or later in large phase III trials elicited objective response rates of 2.1-11% (25-28).

The high prevalence of AR observed in ER-positive breast cancers, along with the prior clinical efficacy demonstrated with classic androgens, provide a strong rationale for the development of a new generation of oral, selective AR agonists and to further exploit their therapeutic benefits in AR/ER+ breast cancer. Here we describe the tissue-selective AR agonist activity of RAD140, an oral nonsteroidal selective AR modulator (SARM), and its distinct AR-mediated mechanism of action including the suppression of ESR1. RAD140 exhibited potent anti-tumor activity in the in vivo models of AR/ER+ breast cancer both as a single agent and in combination with the CDK4/6 inhibitor palbociclib. RAD140 represents a new generation of tissue selective AR agonists and potentially a novel therapeutic option for AR/ER+ breast cancer.

**Material and Methods**

**Nuclear receptor binding assay**

The PolarScreen nuclear receptor competitor assays (Thermo Fisher, Grand Island, NY) for AR, ER, PR, and glucocorticoid receptor (GR) were used to determine the binding affinity and specificity of RAD140. Briefly, RAD140 and fluorescence-labelled ligands (Fluormones) for AR, ER, PR, or GR, respectively, were added to each reaction well and the plate was incubated for 4 hours at room temperature in the dark. Polarization values for each well were measured and plotted against the concentration of RAD140. As a reference, the binding affinity of the standard
ligands DHT, 17β-estradiol, progesterone, and dexamethasone (for AR, ER, PR and GR, respectively) was also measured. The relative binding affinity of RAD140 vs the 4 standard ligands is reflected by the ratios of IC$_{50}$(standard ligand)/ IC$_{50}$(RAD140). A commercially available spectrum screen was performed for RAD140 (MDS Pharma Services, Taipei, Taiwan). The binding of RAD140 (1 µM) to a panel of cellular targets was assessed (Supplementary Table S1). Appreciable binding was defined as a larger than 50% inhibition of the reference radio-ligand.

**Cell culture and treatment**

The ZR-75-1, HCC1428 and T47D breast cancer cells and LNCaP prostate cancer cells were purchased from American Tissue Type Culture Collection (ATCC, Manassas, VA) and maintained in medium and condition as recommended by the vendor. All cell lines involved in the study were tested negative for mycoplasma contamination (MycoAlert Detection Kit, Lonza, Switzerland) and subjected to annual authentication using STR profiling (Thermo Fisher). All the cells were under passage 15 at the time of experiments. HCC1428 Long Term Estrogen Depleted (LTED) cells were established by culturing these cells in medium with 10% charcoal dextran-stripped serum (CSS) for 16 weeks and the expression of AR, ER, PR were confirmed by western blotting (Supplementary Figure S1A). For proliferation assay, cells were seeded in medium with 10% CSS at 30,000 cells/well in 24-well plates and subjected to treatment with RAD140, DHT or DMSO for 14 days with treatments being renewed every 3 days. At the end of the treatment period, trypsinized cells were stained with trypan blue before subjected to live cell counting in a Nexcelom Cellometer (Nexcelom Bioscience, Lawrence, MA). The PC3-AR cell line, described previously (29), was a generous gift from Dr. Steve Balk of Beth Israel Deaconess Medical Center, Boston, MA and Dr. Changemeng Cai of University of Massachusetts Boston, Boston, MA. For ZR-75-1 cells-based assays, cells were seeded and incubated in medium with 5% CSS for 48 h. Cells were then treated with 17β-estradiol at 1 nM.
final concentration or vehicle (ethanol) before being incubated with dimethyl sulfoxide (DMSO), RAD140 (Radius Health, Inc., as described previously (30)), or DHT (Sigma Aldrich, Natick, MA) in the presence or absence of enzalutamide or ARN-509 (Selleckchem, Houston, TX) for 24 h. The experiments were performed in duplicate. The nuclear and cytoplasmic fractions of the cells were prepared using the NE-PER nuclear and cytoplasmic extraction kit (Thermo Fisher) following manufacturer’s instructions.

AR reporter gene assays

AR reporter gene assay was carried out using a Cignal Androgen Receptor Reporter (luc) Kit (Qiagen, Valencia, CA). ZR-75-1 breast cancer cells, LNCaP and PC3-AR prostate cancer cells were transfected with a tandem androgen responsive elements (ARE)-driven firefly luciferase construct and a Renilla luciferase construct in RMPI-1640 media containing 5% CSS. Forty-eight hours later, transfected cells were treated with DMSO, RAD140, or DHT. The luciferase activity was determined using the Dual-Luciferase Reporter Assay (Promega, Madison, WI) as a representation of AR transcription activity and normalized to the activity of Renilla luciferase. Each set was carried out in triplicate. The values were presented as average fold-changes ± standard deviation (SD) over the vehicle control.

In vivo efficacy study

All study protocols were reviewed by Institutional Animal Care and Use Committees (IACUC) and Radius Health, Inc. and conducted in compliance with US and European regulations of protection of laboratory animals. The HBCx-22, HBCx-21 and HBCx-3 breast cancer patient-derived xenograft (PDX) models were established and evaluated at XenTech (Ervy, France) by implanting tumor fragments subcutaneously in the flank female athymic nude mice (Foxn1nu, Envigo RMS, Indianapolis, IN). The ST897 breast cancer PDX model was established in female athymic nude mice (Foxn1nu, Charles River Laboratories, Stone Ridge, NY) and studies
conducted at South Texas Accelerated Research Therapeutics (San Antonio, TX). These models were characterized as ER and PR positive and negative for HER2 overexpression based on immunohistochemistry (IHC) and later confirmed using Western Blot analysis and real-time quantitative polymerase chain reaction (PCR) (Supplementary Table S2 and Supplementary Figure S1). The efficacy study in T-47D breast cancer cell line-derived xenograft model, established in NOD/Shi-scid/IL-2Rγnull mice, was conducted at WuXi AppTec (Shanghai, China). These xenograft models were supplemented with exogenous estradiol in drinking water (HBCx-3, HBCx-21, HBCx-22 and ST897), or subcutaneous pellet (0.18 mg/90-day release, Innovative Research America, Sarasota, FL) implanted 3 days prior to tumor cell inoculation (T-47D). The PDX-bearing mice were randomized once their tumor volumes reached 60 to 200 mm³, as previously described (31). The cell line-derived xenografts with volumes between 125-250 mm³ were enrolled in treatment groups. RAD140, DHT or fulvestrant were administered as single agent or in combination with palbociclib. RAD140, palbociclib, and the vehicle (0.5% carboxymethyl cellulose) were given orally (PO). Fulvestrant (clinical formulation, AstraZeneca, London, UK) was given as subcutaneous (SC) injection. DHT was administered as subcutaneously implanted pellets (12.5 mg/60-day release, Innovative Research America) from the day of randomization to the end of treatment (14). Tumor volume and body weight were measured twice a week. Tumor growth inhibition (TGI %) was calculated based on the change of mean tumor volume from treatment Day 1 to the last day of assessment and presented as a percentage of that of the vehicle control group (100 × (1- ΔTVtreatment / ΔTVvehicle)), as an indication of anti-tumor efficacy. Statistical analyses were performed for the changes in individual tumor volume (ΔTV, from treatment Day 1 to the last day of assessment) between the groups compared.

**Immunohistochemistry (IHC)**
Formalin-fixed paraffin-embedded sections of xenograft tumors underwent antigen retrieval and were then blocked using 5% goat serum and avidin blocking solution. IHC was performed in a Leica Bond III auto-stainer (Leica Biosystems, Buffalo Grove, IL) according to the manufacturer's instructions. The primary rabbit monoclonal antibodies against ER (SP1) and PR (SP42) were obtained from Cell Marque (Rocklin, CA). Rabbit monoclonal antibody against Ki67 (D2H10) and phospho-retinoblastoma protein (phospho-Rb) were from Cell Signaling (Danvers, MA). Mouse antibody against AR (441) was obtained from Dako (Carpinteria, CA). The AR IHC staining protocol included the use of ImmunoDNA Background Blocker (Bio SB, Santa Barbara, CA). Sections shown in each figure were stained and photographed under identical conditions. Staining and scoring of the samples were performed by a pathologist blinded to the treatment groups.

**Western blot (WB) analysis**

Tumor lysates from the *in vivo* studies were prepared using Cell Lysis Buffer with protease and phosphatase inhibitors (Cell Signaling) in a FastPrep Sample Preparation System (MP Biomedicals, Santa Ana, CA). Cell lysates were prepared using Cell Lysis Buffer as described above. Total protein was quantitated and subjected to WB analysis using antibodies against AR (PG-21, EMD Millipore, Billerica, MA; Clone 441, Thermo Fisher), ERα, PR, GAPDH, β-tubulin, and HDAC2 (Cell Signaling).

**Gene expression analysis**

Total RNA from xenograft samples and cells was extracted using the RNeasy Mini Kit (Qiagen), with a DNase incubation step included to ensure complete removal of genomic DNA. The expression of genes was evaluated using real-time quantitative PCR (qPCR) and RNA sequencing (RNA-seq). For qPCR, TaqMan primer and probe sets for AR, KLK2, FKBP5, ZBTB16, ESR1, PGR, TFF1, GREB1, BLM, FANCI, LMNB1, MCM2, MCM4, MCM7, 18S, and
$GAPDH$ were purchased from ThermoFisher Scientific (Foster City, CA). The PCR reaction was set up with TaqMan Fast Virus 1-Step Master Mix in a Quant Studio 6 Flex qPCR system (Thermo Fisher Scientific). Samples from a minimum of 3 tumors from each treatment group or those from a minimum of 2 replicated cell-based assays were included for the analysis. The expression of genes of interest were normalized to that of $GAPDH$ or $18S$ and presented as the mean fold-change compared to the vehicle control group. For RNA-seq analysis, total RNA samples were converted into cDNA libraries using the TrueSeq Stranded mRNA sample preparation kit (Illumina, San Diego, CA). Libraries are quantified, normalized, and pooled before being subjected to HiSeq 2×50bp paired end sequencing on an Illumina sequencing platform. De-multiplexed FASTQ files were aligned to HG19 human genome using the STAR aligner version 2.4 and genetic features were quantified using RSEM version 1.2.14. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE104177. The AR and ER target genes with significant changes in expression (false discovery rate, FDR<0.05) were analyzed and Log$_2$ fold changes presented in heat map. Global pathway analysis was performed for genes with significant changes in expression (FDR<0.001) using the functional annotation tools (Gene Ontology) available on the National Health Institute DAVID Bioinformatics Resources platform (https://david.ncifcrf.gov).

**Results**

**RAD140 binds to AR with high affinity and high specificity**

The binding affinity of RAD140 was assessed using fluorescence polarization-based competitive binding assays for AR, ER, PR, and GR and compared against the standard ligands for each nuclear receptor. As positive controls, assays evaluating the binding of the standard ligands including DHT, $17\beta$-estradiol (E2), progesterone, and dexamethasone to AR, ER, PR, and GR, respectively, were performed. In the competitive binding assay against the AR-Fluoromone,
RAD140 exhibited 1.6-fold lower AR affinity than DHT (Figure 1A). This suggests binding affinity of RAD140 to AR is slightly lower but comparable to that of natural androgen. RAD140 exhibited 33-fold lower affinity to PR compared to progesterone. No binding of RAD140 to ER or GR was detected. In addition, a cellular target spectrum screen was performed to determine the binding selectivity of RAD140 against a broad panel of 165 molecular targets that included many pharmacologically relevant molecules including ion channels, G-protein-coupled receptors (GPCRs), enzymes, and nuclear hormone receptors (Supplementary Table S1). No appreciable interaction of RAD140 with any targets screened, other than AR and PR, was detected. These results indicate RAD140 binds to AR with high affinity and specificity.

**RAD140 is a potent tissue-selective AR agonist**

SARMs are selective AR ligands that have been shown to act as agonists or antagonists in a tissue-context-dependent manner (32). We previously demonstrated that RAD140 exhibits differential activity in prostate versus in the muscle (30). To further assess the activity of RAD140 on AR transcription in tissue-context, an AR reporter assay was performed in ZR-75-1, a human breast cancer cell line expressing endogenous AR and ER (21), and the AR-positive prostate cancer cell line LNCaP (29). Dose responses by RAD140 were compared to the known AR agonist DHT. Treatment of steroid-depleted ZR-75-1 cells with 1, 10, and 100 nM RAD140 induced AR transcription activity by 1.9 to 2.9-fold, comparable to the induction seen with DHT at the same concentrations (Figure 1B, left). In contrast, in the low passage, androgen-sensitive LNCaP cells treated with RAD140 at 0.1 to 10 nM, no induction of AR activity was observed while DHT at 1 or 10 nM led to more than a 10-fold induction of AR transcription activity (Figure 1B, right). In order to rule out the possibility that the attenuated AR activity of RAD140 is due to the expression of a mutant AR T877A in LNCaP cells, we also performed AR reporter assay in PC3-AR cells which express ectopic wildtype AR (29). Similar to the finding in LNCaP cells, RAD140 exhibited much attenuated activity on ectopic wildtype AR in prostate cancer cells.
compared with DHT (Supplementary Figure S2). These results indicated that RAD140 is a tissue-specific AR agonist, with selective activity in breast cancer cells but not in prostate cancer cells.

Classic AR agonists are known to induce AR conformation change and subsequent localization to the nuclei, while pure AR antagonist such as enzalutamide and ARN-509 act partially via blocking AR nuclear localization (33, 34). We then examined whether RAD140 induces the nuclear expression of AR in breast cancer cells. Treatment of ZR-75-1 cells with RAD140 led to increased AR expression in the nuclear fraction while the estrogen (E2) showed no apparent effect (Figure 1C). The positive control, DHT, substantially increased the nuclear expression of AR. ARN-509 substantially reduced the nuclear expression induced by DHT and RAD140, suggesting a direct antagonism against these two AR agonists. This provided further evidence that RAD140 is an AR agonist in breast cancer cells.

RAD140 monotherapy suppresses the growth of AR/ER+ breast cancer patient-derived xenograft (PDX) models

It has been previously reported that DHT inhibits the proliferation of ER positive breast cancer cells driven by supplemental estradiol (21). We evaluated the activity of RAD140 and DHT in an estrogen-independent AR/ER+ breast cancer line HCC1428 LTED, in which ER signaling appears to be active as judged by PR expression (Supplementary Figure S1). RAD140 exhibited inhibitory effect on the proliferation of these endocrine-resistant breast cancer cells at concentrations as low as 10 nM, with an apparent maximal effect seen at 100 nM (Figure 1D). This was comparable with the inhibitory effect seen with DHT in the 0.1-10 nM range. To further assess how the AR agonist activity of RAD140 translates into antitumor activity in vivo, RAD140 was evaluated as a single agent in AR/ER+ breast cancer PDX models. Oral administration of RAD140 induced significant tumor growth inhibition (TGI) of 76%, 59%, and 58% when compared with the vehicle control groups in HBCx-22, HBCx-3 and HBCx-21, respectively.
(Figure 2A, Supplementary Figures S3A and S3B). In the HBCx-22 model, fulvestrant, a selective estrogen receptor downregulator (SERD) and standard-of-care for ER-positive breast cancer dosed at 1 mg weekly, exhibited a statistically significant antitumor activity, as judged by a TGI of 59%, comparable to a previous report of this model and indicative of its dependency on ER (31). It has been previously determined that the 1 mg weekly administration of fulvestrant achieves exposure equivalent to the 500 mg monthly dose in human (35). The terminal tumor volumes of the RAD140- or fulvestrant-treated HBCx-22 xenografts appeared to be lower compared with those treated with vehicle (Figure 2B). To further verify the concept of inhibiting the growth of AR/ER+ breast cancer tumors with AR agonists, T-47D cell line-derived xenografts were treated with DHT, RAD140 or fulvestrant (Supplementary Figure S3C). Treatment with DHT or RAD140 inhibited the growth of these T-47D tumors to comparable extent. Fulvestrant also elicited potent growth inhibition. In addition, the activity of the combination of fulvestrant with RAD140 was evaluated in HBCx-22 model but no appreciable improvement in tumor growth inhibition was observed, suggesting a potential overlap in the pathway inhibition induced by these two agents in this model (Supplementary Figure S3D). This observation is consistent with previous clinical experience with anastrozole-fulvestrant combination and preclinical data of fulvestrant-RAD1901 combination, both of which showed such intense ER blockade failed to yield additional benefit (35, 36). Together, these results indicated that RAD140 as a single agent is effective in inhibiting the growth of AR/ER+ breast cancer xenografts.

We then examined the changes in common breast cancer biomarkers in the HBCx-22 xenografts treated with RAD140 or fulvestrant using IHC. As shown in Figure 2C, HBCx-22 tumors in the control group were positive for AR, ER, and PR, consistent with prior characterization of this model (Supplementary Figure S1). An apparently increased AR expression, predominantly localized in the nuclei, was seen in RAD140-treated tumors. This
demonstrated that RAD140 possesses AR agonist properties such as stabilizing AR and promoting its nuclear localization which are commonly seen with classic androgens (37, 38). Interestingly, RAD140 treatment led to substantially decreased expression of ER and its downstream target PR. A profound decrease in Ki67 expression was also seen in these RAD140-treated tumors, indicating a suppressive effect on the proliferation of these tumor cells. Treatment with fulvestrant also led to substantial decreases in ER, PR and Ki67 expression, to levels seemingly comparable to those seen with RAD140. Furthermore, the epithelial nature of the terminal tumor mass was confirmed using IHC assays for Cytokeratin (CK) 18 and Vimentin (Supplementary Figure S4). This suggests SARM and SERD both inhibit the ER pathway and breast cancer cell proliferation.

**Pharmacodynamic analysis reveals RAD140-mediated regulation of AR and ER target genes and ESR1**

It has been proposed that androgens suppress ER signaling in AR/ER+ breast cancer cells, which may contribute to the anti-proliferative activity (19, 21). To better understand the mechanism of action of the SARM RAD140 in AR/ER+ breast cancer models, we examined the pharmacodynamic changes with a focus on the AR and ER pathways. In the HBCx-22 model, a profound induction of AR target genes including \( \text{FKBP5}, \text{KLK2}, \text{and ZBTB16} \) was seen in RAD140-treated tumors (Figure 3A, upper) while the expression of the \( \text{AR} \) gene was not affected. Fulvestrant, as expected, did not induce the expression of these AR target genes, although a slight increase in \( \text{AR} \) message was seen. The ER target genes \( \text{PGR}, \text{TFF1}, \text{and GREB1} \) were found to be substantially suppressed in RAD140-treated tumors to levels comparable or even lower than that seen with fulvestrant (Figure 3A, lower). Interestingly, in these RAD140-treated tumors, the mRNA expression of \( \text{ESR1} \), the coding gene of ER, was profoundly suppressed. In contrast, the fulvestrant-treatment did not lead to appreciable change in \( \text{ESR1} \) gene expression. At protein level, both RAD140 and fulvestrant exhibited a profound
effect in decreasing the expression of ER and PR in HBCx-22 xenografts (Figure 3B, left), consistent with the IHC findings described above. Similar decrease in ER and PR expression was seen in HBCx-3 AR/ER+ xenografts treated with RAD140 (Figure 3B, middle). In the T-47D xenografts, treatment with RAD140, DHT or fulvestrant, also led to decreased ER and PR expression (Figure 3B, right).

Next, the modulation of AR and ER target genes by RAD140 was further examined in vitro. As determined in ARE-luc reporter assay and proliferation assay (Figure 1B and D), RAD140 at 100 nM exhibited maximal effects, comparable with DHT at 10 nM, therefore 100 nM was selected as the RAD140 concentration for further in vitro assays. In ZR-75-1 cells treated with RAD140, substantial induction of FKB5 and ZBTB16 messages was seen (Figure 3C), which was comparable to that seen with DHT. The induction of these AR targets by RAD140 or DHT was completely blocked by the antagonist enzalutamide. The expression of the AR gene did not seem to be affected by RAD140- or DHT-treatment. The ER target genes PGR and TFF1 were induced by 1 nM of E2. Treatment with either RAD140 or DHT was found to repress the E2-induced expression of these genes. Similar to the effects seen in xenograft models, treatment with RAD140 or DHT decreased ESR1 mRNA. The effects of RAD140 and DHT on AR pathway and ER pathway genes appear to be comparable, suggesting an AR-specific effect. Furthermore, competitive blockade of AR activation by enzalutamide effectively reversed the AR and ER pathway modulation by RAD140 or DHT. The incomplete reversal of ESR1 suppression by enzalutamide may be due to the relatively lower affinity of this compound compared to AR agonists RAD140 and DHT. These in vivo and in vitro observations further demonstrated the AR agonist activity of RAD140 in AR/ER+ breast cancer models. More importantly, RAD140 and DHT both inhibited ER downstream targets, as well as the ESR1 gene, suggesting a novel mechanism of action of AR agonists in inhibiting the ER pathway in breast cancer cells.

**Global modulation of signaling pathways by SARM in AR/ER+ breast cancer xenografts**
To further understand the global effect of RAD140 on signaling cascades in AR/ER+ breast cancer cells, RNA-seq analysis of HBCx-22 xenografts was performed. We examined a broader range of AR and ER target genes with significantly altered expression in RAD140-treated tumors (Figure 4A). The results indicated that in addition to the targets examined by qPCR, RAD140-treated tumors had higher levels of AR-activated genes in addition to those shown in qPCR. A subset of the ER target genes was found to be suppressed in RAD140-treated tumors, consistent with the qPCR findings. Using a stringent filtering criteria (FDR<0.001), we found that globally 86 genes were significantly upregulated and 77 were downregulated by at least 2-fold in RAD140-treated HBCx-22 xenografts (Figure 4B, Supplementary Tables S3 and S4). In the Gene Ontology analysis, these upregulated genes were found to be most significantly enriched in the metabolic process. This was consistent with the previous report describing the role of AR as a master regulator of central metabolism and biosynthesis in prostate cancer cells (39). The downregulated genes in RAD140-treated tumors were found to be enriched in categories associated with cell division, DNA replication and cell cycle progression. This negative regulation of gene transcription was in line with previous reports on the transcription suppressor role of AR (40, 41). These data further confirmed the regulation of AR and ER pathways by RAD140 and suggested a unique mechanism of action of RAD140 via the AR-mediated transcription repression. These data offer evidence for further uncovering the underlying mechanism of RAD140 as a single agent in AR/ER+ breast cancer models.

Enhanced anti-tumor activity of combined administrations of RAD140 with CDK4/6 inhibitor

Given the observed effect of RAD140 on cell cycle and DNA replication-related genes, we hypothesized that combined administration of this SARM and a CDK4/6 inhibitor, which inhibits the phosphorylation and subsequent degradation of Rb, thus blocking cell cycle entry (42-44), may produce improved anti-tumor activity in AR/ER+ breast cancer models. Palbociclib, a
CDK4/6 inhibitor recently approved for breast cancer treatment in combination with ER-targeted agents in as early as first line of therapy (45-47), was selected for this study. Two AR/ER+ PDX models, HBCx-3 (Figure 5A) and ST897 (Figure 5B), were treated with RAD140, palbociclib, or a combination of RAD140 with palbociclib. Compared to the vehicle-treated group, RAD140 as monotherapy produced statistically significant inhibition of tumor growth in these models compared with vehicle control. Palbociclib as a single agent also inhibited the growth of these xenografts to a comparable level. Importantly, RAD140 and palbociclib when administered together elicited improved efficacy over palbociclib or RAD140 monotherapies. Similarly, RAD140 and palbociclib combination produced improved efficacy compared with palbociclib alone in an additional AR/ER+ PDX model, HBCx-22 (Supplementary Figure S5A). An additional TGI analysis performed for HBCx-3 xenografts treated with RAD140 and palbociclib with lower starting volume ($\leq 108$ mm$^3$) versus those with higher starting volume (>108 mm$^3$) did not reveal appreciable difference between the two subgroups, suggesting the combination treatment had similar effect in tumors of different starting size. As the combination of RAD140 with palbociclib induced apparent regression in ST897 tumors, we examined the expression of apoptotic marker cleaved Caspase 3 in ST897 and HBCx-3 tumors treated with RAD140, palbociclib or combination but did not observe clear signs of apoptosis (Supplementary Figure S5B), suggesting a cytostatic but not cytotoxic effect. Together, these results indicate that the combination of RAD140 with palbociclib led to improved anti-tumor activity compared with each of these agents given as single agent.

The expression of AR, ER, PR, Ki67, and phospho-Rb in the HBCx-3 PDX were assessed using IHC (Supplementary Figure S6). Similar to that seen in HBCx-22 (Figure 2C), RAD140 treatment led to apparent enrichment of AR in the nuclei, suggesting AR activation. ER and PR expression was profoundly decreased in tumors treated with a RAD140-containing regimen. Palbociclib alone also appeared to decrease the expression of ER and PR, a finding similar to
the decrease of ER in breast cancer cells treated with a pan-CDK inhibitor roscovitine (48). The phosphorylation of Rb was found to be substantially suppressed in tumors treated with palbociclib as a single agent and in combination with RAD140, while RAD140 alone did not seem to affect phospho-Rb level. Ki67 expression was suppressed in tumors treated with RAD140 or palbociclib, and this suppression appeared to be further enhanced in tumors that received both agents. These results further confirmed the inhibitory effect of RAD140 on ER signaling cascade. Gene expression analysis of the HBCx-3 xenografts treated with RAD140, palbociclib, or their combination showed that the AR target genes *FKBP5* and *ZBTB16* were robustly induced in RAD140-treated tumors, while no appreciable effect on the *AR* gene was seen (Figure 5C, upper). The ER target genes *PGR* and *TFF1* were substantially suppressed in the tumors of the RAD140-treated groups (Figure 5C, middle). Notably, a profound suppression of the *ESR1* mRNA level was also observed in these RAD140-treated HBCx-3 xenografts. Palbociclib alone, in contrast, did not have an apparent effect on AR target genes but did seem to repress *PGR* and induce *TFF1* and *ESR1* to a relatively modest degree compared to the changes induced by RAD140 treatment.

We next explored the underlying mechanism for the improved efficacy and enhanced inhibition of cell proliferation seen with the RAD140-palbociclib combination. It has been recently reported that in castration-resistant prostate cancer cells, activated AR recruits hypo-phosphorylated Rb to the loci of genes implicated in DNA replication and suppress their transcription (41). We hypothesized that AR activated by RAD140 inhibits DNA replication-related genes in breast cancer cells, and this effect is enhanced by co-administration of palbociclib. Indeed, the HBCx-3 tumors treated with RAD140 alone had decreased expression of the DNA replication-related genes including bloom syndrome recQ like helicase (*BLM*), Fanconi anemia complementation group gene (*FANC1*), laminin B1 (*LMNB1*), and minichromosome maintenance genes 2, 4, and 7 (*MCM2, MCM4, and MCM7*) (Figure 5C, lower). These decreases were comparable with
those seen with palbociclib alone. In tumors treated with both RAD140 and palbociclib, the expression of these genes appeared to be further suppressed. Together, the enhanced effect of RAD140-palbociclib combination on the expression of DNA replication-related genes may have contributed to the enhanced inhibition on tumor growth.

Discussion

This study demonstrates for the first time that RAD140, an orally available SARM, is an AR agonist in breast cancer cells and suppresses the growth and proliferation of multiple AR/ER+ breast cancer cell line and xenograft models. The AR pathway was found to be activated in RAD140-treated breast cancer cells and xenografts, while genes within the ER pathway, including \textit{ESR1}, were suppressed. In addition, RAD140-treatment was found to decrease the expression of DNA-replication related genes in breast cancer cells, consistent with previous report that these genes were suppressed in androgen-treated prostate cancer cells. Combined administration of RAD140 with the CDK4/6 inhibitor palbociclib was more efficacious compared with either of the agents used alone. These findings suggest a distinct mechanism of action of RAD140 which includes the AR-mediated suppression of \textit{ESR1} in inhibiting AR/ER+ breast cancer growth.

Accumulating evidence in the recent years have further defined the role of AR in breast cancer and led to renewed interests in evaluating AR-targeted agents for breast cancer. The high prevalence of AR in the predominant ER positive subtype of breast cancers (6), clinical benefit rates as high as 39% seen with androgen-therapy in breast cancer patients progressing on ER-targeted treatments (23, 24), along with prior experience with steroidal androgens in breast cancer together lend support to the development of a new generation of oral, non-steroidal AR agonists for the treatment of AR/ER+ breast cancer.
SARMs are tissue selective AR agonists by design and may offer a novel approach to inhibit the growth of AR/ER+ breast cancers, with substantially attenuated side effects commonly seen with classic non-tissue selective androgens (32, 49). The activity of RAD140 are AR specific, as evidenced by the similar effects observed with DHT and RAD140 on AR and its downstream targets, along with the reversal of these effects by AR antagonists (Figures 1C and 3C). In addition, due to its non-steroidal structure, the SARM RAD140 is not subject to further conversion to estrogens by CYP19 aromatase, or to DHT by 5α-reductase, thus reducing the potential risk of stimulating ER positive tumor growth or increasing virilization. As a proof-of-concept study, we report here that RAD140 treatment inhibited the growth of the breast cancer xenograft models supplemented by exogenous estrogen. Of note, RAD140, and its classic androgen comparator DHT, also inhibited the proliferation of an endocrine-resistant breast cancer cell line model, HCC1428 LTED, suggesting AR agonists may inhibit the growth of ER positive breast cancer models with or without estrogen-supplementation. Furthermore, in multiple PDX models, RAD140 inhibited tumor growth as a single agent and this effect was enhanced by combining with palbociclib. PDX models have been demonstrated to closely recapitulate human tumors with regards to tumor heterogeneity and response to standard-of-care agents (31, 50). Indeed, heterogeneity was observed in the PDX models used in this study, as indicated by variable levels of AR, ER, PR expression, suggesting a good representation of the patient tumors with a spectrum of expression levels of these nuclear receptors. Also, the growth pattern and response to RAD140 seen with HBCx-3 and HBCx-22 PDX models, each evaluated in two independent studies (Figures 2A, 5A and Supplementary Figures S3A and S5A), seemed to be consistent. This suggests good reproducibility of these studies. Therefore, the efficacy seen with RAD140 in these PDX models may be suggestive of the potential therapeutic benefit in breast cancer patient population. It is worth noting that although RAD140 and fulvestrant led to similar tumor growth inhibition in HBCx-22 tumors, further studies are needed to evaluate the efficacy of RAD140 relative to that of fulvestrant or tamoxifen in
hormone-dependent models to predict its value in first-line setting. It is interesting to note that although HBCx-3 (Supplementary Figure S6) and HBCx-22 (Figure 2) xenografts contain comparable percentage of AR positive cells, RAD140 induced higher degree of inhibition in growth and Ki-67 expression in HBCx-22 than in HBCx-3. This may be due to the presence of higher percentage of ER positive cells in HBCx-22 tumors than in HBCx-3 (Supplementary Table S2). As summarized previously, AR agonists exhibit inhibitory effect on AR/ER+ breast cells but not on AR+/ER- cells (9, 51). It is conceivable that the varying degree of growth inhibition seen with SARM may be attributed to different ER positivity of these models.

The tissue selectivity of RAD140 is evidenced by potent androgen-like effects in bone and muscle, with much attenuated effects in other androgen-responsive tissues such as prostate and seminal vesicles (30). Notably, when administered along with testosterone, RAD140 was found to partially antagonize the growth effect of testosterone in prostate and seminal vesicles, suggesting that RAD140 acts as a competitive AR ligand with attenuated activity. Here we demonstrated for the first time that the SARM RAD140 is a potent AR agonist in breast cancer cells. It exhibited much attenuated activity on AR in prostate cancer cells, a finding consistent with the previously described attenuated effect on prostate growth (30). It has been proposed that the tissue-selective AR activity of SARMs is due to the differential allosteric activation of AR compared to classic androgens, which impacts the recruitment of co-activators needed for AR-mediated gene transcription (52, 53). An earlier study on the selective estrogen receptor modulator (SERM) tamoxifen showed that its tissue selectivity may be attributed to the differential expression of ER co-activators such as SRC-1 in breast versus endometrial cancer cells (54). Similarly, it is conceivable that AR upon binding to RAD140 assumes a conformation that only allows the interaction with a subset of co-regulators that are uniquely expressed in breast epithelial cells but not in the prostate.
Despite the preclinical evidence and favorable clinical outcome seen with androgens in ER positive breast cancer (9, 10), improved understanding of the underlying mechanism is needed to further improve the design and development of new AR agonist-based therapy. It has been proposed that activated AR suppresses ER signaling by competing with ER for transcriptional co-activators or directly competing for binding sites, and subsequently leads to inhibited cell proliferation (55, 56), suggesting a functional interference between these two nuclear receptors. Here we show AR agonists including RAD140 and DHT activate AR target genes in breast cancer cells in vitro and in vivo, while suppressing a subset of ER target genes. Importantly, we found that treatment with these AR agonists also led to substantial suppression of ER at both mRNA and protein levels. This suggests that AR may negatively regulate ER signaling by two mechanisms: while AR may compete with ER for co-activators (functional interference), it may directly downregulate ER expression (direct suppression). The reduction of ER expression by AR agonists has not been extensively documented but can be seen in a study by Poulin et al. (57), in which DHT-treatment in ZR-75-1 cells for 8 or 12 days led to decreases of ER message by 50% or 65%, respectively. Another study also showed reduced ER protein level in ZR-75-1 cells treated with DHT, along with decreased expression of classic ER targets PGR and TFF1 (56), although the mechanism for the decrease in ER expression had remained to be further elucidated. Our data show both RAD140- and DHT-treatment led to decreased ESR1 expression at as early as 24 h, which may present a novel mechanism by which RAD140 suppresses ER signaling and differentiates from traditional ER-targeted agents that act via inhibiting ER protein function or leading to its degradation. This may translate into unique therapeutic benefit of SARMs in overcoming resistance to ER-targeted therapy seen in tumors with reactivated ER signaling, either via post-translational modification or enrichment of ESR1 alterations. The inhibitory effect of RAD140 on the proliferation of HCC1428 LTED cells shed light on its efficacy in such E2-independent, albeit ER-active, models but further studies are needed to fully evaluate the activity of SARMs in similar models in vivo. In addition to the
suppression of ER signaling, our RNA-seq analysis suggests that RAD140 may inhibit tumor growth via other AR-mediated mechanisms. Of note, RAD140-treatment suppressed genes implicated in DNA replication and cell cycle progression, which were further suppressed by concurrent CDK4/6 inhibition. Gao et al. (41) described the role of AR as a tumor suppressor in castration-resistant prostate cancer (CRPC) cells by inhibiting DNA replication-related genes via the recruitment of hypo-phosphorylated Rb. In addition, the clinical and preclinical efficacy seen with high dose androgen therapy in CRPC was also attributed to androgen-induced stabilization of AR which prevents the re-licensing of DNA for replication and subsequent cell cycle progression (58, 59). In the present study, the AR-mediated suppression of ESR1 and its downstream pathway, along with the suppression of DNA replication- and cell cycle-related genes seen with RAD140-treatment suggest a distinct mechanism of action of this SARM in AR/ER+ breast cancer cells. The potent anti-tumor activity and tissue-selective AR activity, along with overall tolerability in animal models and oral availability together lend support to further clinical investigation of RAD140 in AR/ER+ breast cancer patients.

Acknowledgments

The authors would like to acknowledge Dr. Steven P. Balk of Beth Israel Deaconess Medical Center and Dr. Changmeng Cai of University of Massachusetts Boston for their comments on the manuscript. The authors thank Drs. Fiona Garner and Teeru Bihani, and Margaret Ward, of Radius Health, Inc. for their comments and suggestions on the project and the manuscript. The authors thank Dr. Jim Humphreys and the histopathology team at Precision Pathology (San Antonio, TX) for the immunohistochemistry work.


Figure Legends

Figure 1. Binding affinity and specificity of RAD140 and its tissue-selective AR agonist activity.

A, The binding affinity of RAD140 to AR, ER, PR, and GR was determined using PolarScreen Competitive Binding Assays for each of these nuclear receptors. Dihydrotestosterone (DHT), 17β-estradiol, progesterone, and dexamethasone were included as positive control for the binding to AR, ER, PR, and GR, respectively. The relative affinity of RAD140, versus the standard ligands, to these receptors was calculated based on the IC₅₀ values using Prism. Data presented are an average of at least three independent experiments. B, ZR-75-1 breast cancer cells and LNCaP prostate cancer cells were transfected with ARE-luc and Renilla luciferase constructs in medium containing 5% CSS. Twenty-four hours after the transfection, cells were treated with RAD140 or DHT at the indicated final concentrations. ARE-driven luciferase activity was measured and normalized to that of Renilla luciferase. Data presented are an average of biological triplicates. C, Steroid-deprived ZR-75-1 cells were treated with ethanol (vehicle) or 1 nM of 17β-estradiol (E2) for 2 h, followed by treatment with RAD140 at 100 nM in the presence or absence of ARN-509 (10 μM) for overnight. Whole cell lysates and nuclear fractions were prepared and subjected to western blot analyses for AR. HDAC2 and β-tubulin were probed as loading controls for nuclear and whole cell extracts, respectively. D, HCC1428 LTED cells were maintained in steroid-depleted condition and treated with RAD140 or DHT at the indicated concentrations for 14 days, with medium and compounds replenished every 3 days. At the end of the treatment, cells were stained with trypan blue before subjected to live-cell counting in a Nexcelom Cellometer. Average cell number ± SD of biological triplicates is presented. *, p<0.05 for comparison with the DMSO-treated group.

Figure 2. The effect of oral administration of RAD140 on the growth of AR/ER+ breast cancer patient-derived xenografts (PDX). A, Mean tumor volumes ± SEM of HBCx-22 breast cancer
xenografts treated with vehicle, RAD140 (100 mg/kg once daily) or fulvestrant (1 mg once weekly) for the indicated period of time (N=8/group). 

B, Changes in tumor volume of individual HBCx-22 xenografts from the beginning to the end of the treatment. 

C, IHC analysis of HBCx-22 xenografts collected at the end of the study was performed using antibodies against AR, ER, PR, and Ki67. Representative images of 3 individual tumors from each treatment group are shown.

Figure 3. Expression of AR and ER pathway genes and ESR1 in RAD140-treated AR/ER+ breast cancer cells and xenografts. 

A, Tumor samples from the HBCx-22 collected 6 h post last treatment were subjected to qPCR analysis for the expression of AR, ER, and the indicated downstream target genes. 

B, samples from HBCx-22, HBCx-3 and T-47D xenograft models were collected 6 h post last treatment and subjected to western blot analysis for the expression of AR, ER and PR. 

C, Steroid-deprived ZR-75-1 cells were pre-treated with 1 nM of E2 for 2 h and then treated with RAD140 (100 nM) or DHT (10 nM) in the presence or absence of enzalutamide (10 μM) for 24 h. RNA of the treated cells were isolated and subjected to qPCR analysis for the expression of AR, ESR1 and the indicated downstream targets.

Figure 4. Global pathway modulation by RAD140 in the HBCx-22 PDX model. 

A, Heat maps of mRNA expression fold change of the known AR and ER pathway genes in RAD140- versus vehicle-treated tumors (N=3/group), with downregulation represented by shades of blue and upregulation by shades of red. Gene Ontology analyses of the significantly upregulated (B) or downregulated (C) genes in RAD140-treated tumors.

Figure 5. Combined administration of RAD140 with CDK4/6 inhibitor inhibited AR/ER+ PDX growth. 

A, Mean tumor volumes ± SEM of HBCx-3 breast cancer xenografts treated with vehicle,
RAD140 (100 mg/kg twice daily), palbociclib (75 mg/kg once daily) or a combination of RAD140 and palbociclib for the indicated period of time. N=7/group. Inter-group comparisons were carried out using student’s t-test based on changes in tumor volume (ΔTV) from the beginning to the end of the study. For the comparison between RAD140 and vehicle groups, p<0.05. *, the combination group vs palbociclib, p<0.05; The combination group vs RAD140, p<0.01. B, Mean tumor volumes ± SEM of ST897 breast cancer xenografts treated with vehicle, RAD140 (100 mg/kg twice daily), palbociclib (75 mg/kg once daily) or a combination of RAD140 and palbociclib for the indicated period of time. N=8/group. Statistical analysis was performed as in A. For the comparison between RAD140 and vehicle groups, p<0.001. *, combination group vs RAD140 group, p=0.0012; **, combination group vs palbociclib group, p=0.0027; C, Tumor samples from the HBCx-3 model, as described in A, were collected 6 h post last treatment and subjected to qPCR analysis for the expression of AR, ESR1, and their downstream targets, and the genes implicated in DNA replication including BLM, FANCI, LMNB1, MCM2, MCM4, and MCM7. Expression of these genes were normalized to that of ribosomal RNA 18S. Mean normalized expression ± SD from 3 individual tumors of each group is presented.
Figure 1
Figure 2
Figure 4

A

AR Signaling Pathway

ER Signaling Pathway

Log₂ (fold change over vehicle)

B

Upregulated genes in SARM-treated xenografts

Log₁₀ (p-value)

-8 -6 -4 -2 0

- metabolic process
- xenobiotic metabolic process
- steroid biosynthetic process
- oxidation-reduction process
- response to retinoic acid
- mineralocorticoid biosynthetic process
- acetyl-CoA biosynthetic process
- response to organic cyclic compound
- oxygen homeostasis
- gluconolactone biosynthetic process
- response to drug
- cellular response to insulin stimulus
- androgen biosynthetic process
- activation of cysteine-type endopeptidase activity
- fatty acid oxidation

C

Downregulated genes in SARM-treated xenografts

Log₁₀ (p-value)

-30 -20 -10 0

- cell division
- mitotic nuclear division
- sister chromatid cohesion
- anaphase-promoting complex-dependent catabolic process
- mitotic cytokinesis
- G2/M transition of mitotic cell cycle
- protein ubiquitination involved in ubiquitin-dependent catabolic process
- DNA replication
- mitotic metaphase plate congression
- microtubule-based movement
- regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle
- mitotic sister chromatid segregation
- mitotic spindle midzone assembly
- positive regulation of ubiquitin protein ligase activity
- regulation of cell cycle
- retrograde vesicle-mediated transport, Golgi to ER
- antigen processing and presentation of exogenous peptide antigen via MHC class II
- G1/S transition of mitotic cell cycle
- cell proliferation
- mitotic spindle organization
- positive regulation of ubiquitin ligase activity involved in regulation of mitotic cell cycle transition
- mitotic spindle assembly
- DNA repair
- positive regulation of cytokinesis
- microtubule depolymerization
Figure 5

A: Tumor volume (mm$^3$) over treatment days for different treatments:

- **HBCx-3**
  - Vehicle
  - RAD140
  - Palbociclib
  - RAD140 + palbociclib

- **ST897**
  - Vehicle
  - RAD140
  - Palbociclib
  - RAD140 + palbociclib

B: Gene expression analysis:

- **FKBP5**
- **ZBTB16**
- **AR**
- **PGR**
- **TFF1**
- **ESR1**
- **BLM**
- **FANCI**
- **LMNB1**
- **MCM2**
- **MCM4**
- **MCM7**

C: AR, ER, and DNA replication pathways with relative mRNA expression levels for RAD140 and palbociclib treatments.
Selective Androgen Receptor Modulator RAD140 Inhibits the Growth of Androgen/Estrogen Receptor Positive Breast Cancer Models with a Distinct Mechanism of Action

Ziyang Yu, Suqin He, Dannie Wang, et al.

Clin Cancer Res  Published OnlineFirst October 3, 2017.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-17-0670

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2017/10/03/1078-0432.CCR-17-0670.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.