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Selective Progesterone Receptor Modulators in Early-Stage Breast Cancer: A Randomized, Placebo-Controlled Phase II Window-of-Opportunity Trial Using Telapristone Acetate

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ABSTRACT

Purpose: Selective progesterone receptor modulators (SPRMs) show preclinical activity against hormone-sensitive breast cancer, but have not been tested in patients with early, treatment-naïve tumors.

Patients and Methods: In a double-blind presurgical window trial of oral telapristone acetate (TPA) 12 mg daily versus placebo, 70 patients with early-stage breast cancer were randomized 1:1 (stratified by menopause) and treated for 2 to 10 weeks. The primary endpoint was change in Ki67 between diagnostic biopsy and surgical specimens. Gene expression pre- and posttherapy was assessed using RNA-sequencing and gene set enrichment analysis was performed to determine pathways enriched in response to TPA and placebo treatments.

Results: Among 61 evaluable women (29 placebo and 32 telapristone acetate), 91% of tumors were ER/PR positive. The mean Ki67 declined by 5.5% in all women treated with telapristone acetate ($P = 0.003$), and by 4.2% in all women treated with placebo ($P = 0.04$). After menopausal stratification, the Ki67 decline remained significant in 22 telapristone acetate–treated premenopausal women ($P = 0.03$). Differential gene expression analysis showed no significant modulation overall. However, in a subset of tumors that demonstrated $\geq$30% relative reduction in Ki67 in the telapristone acetate group, genes related to cell-cycle progression, and those in the HER2 amplicon were significantly downregulated. In contrast, no significantly enriched pathways were identified in the placebo group.

Conclusions: Patients treated with telapristone acetate whose Ki67 decreased by $\geq$30% demonstrated a selective antiproliferative signal, with a potentially important effect on HER2 amplicon genes. Evaluation of SPRMs in a neoadjuvant trial is merited, with attention to predictors of response to SPRM therapy, and inclusion of pre- and postmenopausal women.

Introduction

Despite significant improvements in hormonal therapy for patients with estrogen and progesterone receptor (ER and PR) positive breast cancer, the risk of long-term relapse remains significant (1). In particular, therapeutic interventions for premenopausal women remain limited to ER-targeting drugs, with the recent addition of inhibitors of cyclin-dependent kinases in the metastatic setting (2). Limited progress has been made in exploiting PR for the treatment and prevention of this disease. Activated PR is a context-dependent mitogen in these cancers and it is known that PR independently governs estrogen action and breast cancer biology (3–5). We and others have previously reported that selective progesterone receptor modulators (SPRMs) inhibit proliferation of PR-positive breast cancer cell lines and suppress rodent mammary tumor formation in a progestin-rich environment (6–10). In addition, mifepristone (RU486) reduces proliferation in normal breast tissue (11), and progestin exposure contributes to higher breast cancer risk in postmenopausal women (12). This evidence motivates investigation of antiprogestin therapies for breast cancer treatment and prevention (3). Preclinical data suggest that second-generation SPRMs such as ulipristal acetate and telapristone acetate are superior to mifepristone, with more selective anti-PR activity, greater inhibition of cell growth in T47D cells, and growth suppression of established ER-positive mammary tumors in rats (8, 10). These drugs display significantly less glucocorticoid receptor-antagonist activity than mifepristone (13). Ulipristal acetate is approved for treatment of uterine fibroids in the European Union, although liver enzyme abnormalities have generated some caution (14–16). Telapristone acetate is a 21-substituted analogue of 19-norprogesterone, which has been investigated for therapy of uterine fibroids and endometriosis in the United States.

Although clinical trials evaluating PR antagonists in advanced cancer settings have reported limited success (17–19), significant differences exist in PR signaling in early-stage versus late-stage breast cancers (20). No clinical studies have tested PR antagonism in early stages of breast tumorigenesis, especially in treatment-naïve patient populations. These gaps in knowledge contribute to suboptimal

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

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Clin Cancer Res 2020;26:25–34

doi: 10.1158/1078-0432.CCR-19-0443

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Translational Relevance

Progesterone receptor (PR) antagonists have not been tested in patients with early-stage breast cancer; recent data support the rationale for such testing. We show, in a randomized placebo-controlled presurgical window trial with 61 evaluable women, that telapristone acetate produces a clear antiproliferative signal, particularly in premenopausal patients. Gene expression analyses show selective suppression of proliferation-related gene pathways, as well as genes in the HER2 amplicon, in the telapristone acetate arm. These findings support the formal testing of selective PR modulators in early-stage breast cancer, and for breast cancer prevention. They offer the important possibility of a new mode of endocrine therapy in premenopausal women; and point to the need to identify biomarkers predictive of therapeutic success.

exploitation of PR-targeting agents for prevention and treatment of early-stage breast cancers. For these reasons, we conducted a randomized, placebo-controlled window-of-opportunity clinical trial to investigate effects of 12 mg/day telapristone acetate treatment in women newly diagnosed with stage I–II breast cancer.

Patients and Methods

Study design and procedures

Pre- and postmenopausal women presenting to the Lynn Sage Breast Center of Northwestern Medicine with a stage I–II primary breast cancer diagnosis were recruited under a protocol approved by the Northwestern University Institutional Review Board, and the FDA (NU12B09, NCT01800422). Randomization was stratified by menopausal status (two-thirds premenopausal), anticipating that the drug may be more effective in a progesterone-rich environment. Initially, we allowed inclusion of women with hormone receptor (HR)-negative tumors, but restricted this to patients with HR-positive disease early in the course of the study. Eligibility criteria included a diagnostic core needle biopsy (DCNB) containing an aggregate tumor deposit of $\geq$5 mm; ECOG performance status of <2, and liver and kidney function tests that were within 1.5-fold of normal values (with exceptions for Gilbert syndrome). A negative pregnancy test was required for women with child-bearing capacity, $\leq$5 days prior to study drug initiation. Participants were asked to refrain from supplements containing natural estrogens. Telapristone acetate (12 mg) and placebo capsules were supplied by Repros Therapeutics, Inc. Consented women were randomized 1:1 to telapristone acetate or placebo, with a treatment duration of 2 to 10 weeks, culminating in surgery. All participants completed the Breast Cancer Prevention Trial Eight Symptom Scale (BESS) questionnaire (21) at study entry and at the end of therapy. Compliance was assessed through participant diaries and counts of returned pills. Participants who took at least 80% of the prescribed dose were considered compliant. Participants were also questioned about adverse events, which were coded using the NCI Common Terminology Criteria for Adverse Events version 3.0.

Grossly normal tissue and tumor, if available, were collected from the surgical sample, snap frozen, and stored at −80°C for measurement of drug concentration. The paraffin block of the DCNB and surgical excision samples were sectioned in batches (with pre- and posttreatment samples in the same batch) at the NU Pathology Core Facility. Ten sections from each specimen were submitted to the Research Histology and Tissue Imaging Core at the University of Illinois at Chicago (Chicago, IL). The sections were shipped cold and processed for IHC within 4 weeks.

Study endpoints

The primary efficacy endpoint of this study was to demonstrate that daily oral administration of telapristone acetate results in a 50% reduction in the Ki67 labeling index (LI) of tumor cells, comparing the DCNB to the matching lesion in the surgical excision. Secondary endpoints were (i) to compare concentrations of telapristone acetate and its active monodemethylated metabolite (CDB-4453) in normal tissue, tumor, and plasma obtained on the day of surgery; (ii) to assess changes in circulating ovarian hormone concentrations (17β-estradiol and progesterone) and follicle-stimulating hormone (FSH) induced by drug intervention; and (iii) to evaluate quality-of-life endpoints using the BESS questionnaire (21).

Laboratory methods

IHC assessment of Ki67 was performed on formalin-fixed paraffin-embedded (FFPE) sections of the DCNB and excision specimens, using a dual IHC staining method (pancytokeratin and Ki67) and Definiens Tissue Studio software for digital scoring as described in the Supplementary Materials and Methods. Samples were processed in a blinded batch fashion, with pre- and postintervention samples in the same batch, along with positive and negative controls. Manual, blinded scoring of 10 randomly selected sections performed by the study pathologist (M.E. Sullivan) yielded a correlation of 0.78 ($P = 0.01$). An independent reanalysis of Ki67 using the HALO software (with resetting all optimization parameters in the algorithms) produced a correlation coefficient of 0.95 with the Tissue Studio results. RNA was isolated from FFPE sections and sequenced as described in the Supplementary Materials and Methods. Sequencing was performed at the Center for Medical Genomics at Indiana University. Analysis of differential gene expression was performed with service from Artificial Intelligence Inc. A set of 47 differentially expressed target genes and three housekeeping genes was selected to confirm RNA-sequencing (RNA-seq) results by nCounter gene expression assay (NanoString, Inc.). Assay input comprised 100 ng of RNA; data normalization and analysis was performed using nSolver Analysis Software according to the company's user manual. Plasma and breast tissue concentration measurement of telapristone acetate (CDB-4124) and its active monodemethylated metabolite (CDB-4453) were determined using LC/MS-MS as described in the Supplementary Materials and Methods. Serum concentrations of estradiol, progesterone, and FSH were measured using a validated protocol at Ligand Assay and Analysis Core Laboratory, University of Virginia (Charlottesville, VA). Estradiol assay were done with ELISA Kit (catalog No. ES180S; Calbiotech Inc.). Progesterone and FSH assays were performed with Immulite Technology (catalog Nos. L2KPW2 and L2KFS2; Siemens Corp.). Each hormone assay was performed in duplicate for each sample.

Statistical design and analysis

The study was powered to detect a relative 50% decline in Ki67 index from baseline to posttherapy in the telapristone acetate arm, with no significant change in the placebo arm. We assumed that Ki67 at baseline would be 20%, and the correlation between the baseline and postintervention values in the placebo group would be 0.5, with a SD of 10%. The significance of changes from baseline to posttreatment within groups was evaluated with the paired signed-rank test. Categorized demographic variables were compared between arms via the Fisher exact test. For RNA-seq differential gene expression analysis,

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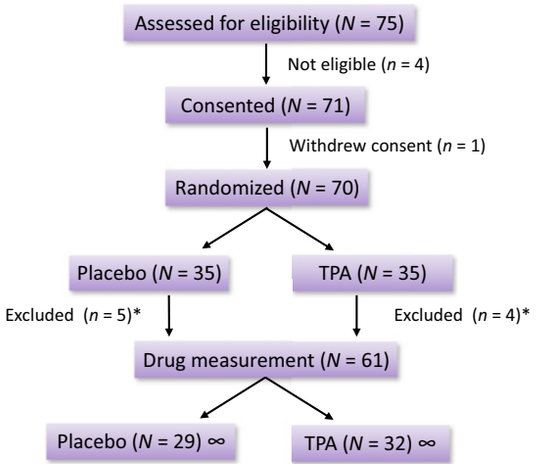

Figure 1.

CONSORT diagram showing participant flow. Of 75 women assessed for eligibility, 71 were consented and 70 were randomized 1:1 to placebo or telapristone acetate (TPA) treatment. Women who did not meet 80% compliance based on the returned-pill counts and patient diary or women whose surgery rescheduled for logistical reasons were excluded from the analysis (*). Because of drug dispensing error during the study, we confirmed treatment group for each woman by detecting drug in plasma samples. The reassignment was based on plasma drug concentration measurement. One subject was reassigned from the placebo to the telapristone acetate group due to a pharmacy dispensing error (∞). The final study sample consisted of 29 placebo and 32 telapristone acetate–treated women.

P values were calculated by Wald test and adjusted for multiple comparisons by Benjamini–Hochberg method (twofold gene expression cutoff and $P_{adj} < 0.05$). For differential gene expression analysis by nSolver software, the significance of changes between baseline and posttreatment within groups were evaluated with paired *t* test and adjusted for multiple comparisons by Benjamini–Yekutieli method (22). The 33 symptoms in the BESS questionnaire were divided into eight clusters as described by Cella and colleagues (21). The mean score within each cluster was used to evaluate significance of changes from baseline to posttreatment within groups as well as the differences between treatment groups using the Wilcoxon signed-rank test.

Statement of ethics

All research was conducted in accordance with the Declaration of Helsinki and with full approval from Northwestern University's Institutional Review Board Office. Informed written consent was obtained from each subject included in this research.

Results

Of 75 women assessed for eligibility, 70 were randomized 1:1 to placebo or telapristone acetate treatment and 64 women completed the study with 80% compliance (**Fig. 1**). However, the NU research pharmacy reported drug dispensing errors during the study so that one subject assigned to the placebo arm was given active drug. The study statistician used plasma drug concentration values to reassign the participant to the telapristone acetate group. This reassignment was performed well before the unblinding of endpoint data. The final study sample consisted of 29 placebo and 32 telapristone acetate–treated participants (**Fig. 1**). Baseline participant and tumor characteristics were balanced (**Table 1**); 97% of tumors were ER or PR positive and 91% were ER and PR positive.

Table 1. Participant characteristics at baseline and the duration of treatments according to treatment groups.

	Placebo (*N* = 29)	TPA (*N* = 32)	*P*
Age at diagnosis, y[a]	50.2 ± 8.9	49.9 ± 11.2	0.86
Menopausal status			
Pre	19 (66%)	22 (69%)	0.99
Post	10 (34%)	10 (31%)	
Race			
Caucasian	20 (69%)	26 (81%)	0.37
Non-Caucasian	9 (31%)	6 (19%)	
Tumor grade at surgery			
1	8 (28%)	11 (34%)	0.80
2	14 (48%)	13 (41%)	
3	7 (24%)	8 (25%)	
Tumor size at surgery (cm)[a]	2.04 ± 1.12	2.72 ± 1.76	0.23
ER status			
Negative (<1%)	1 (3%)	1 (3%)	0.99
Positive	28 (97%)	31 (97%)	
PR status			
Negative (<1%)	3 (10%)	3 (9%)	0.99
Positive	26 (90%)	29 (91%)	
HER-2/neu status			
Negative/equivocal	25 (86%)	27 (84%)	0.99
Positive	2 (7%)	2 (7%)	
unknown	2 (7%)	3 (9%)	
%Ki67 LI[a]			
Low (≤10%)	7 (24%)	9 (28%)	0.40
Intermediate (11%–20%)	6 (21%)	2 (6%)	
High (>20%)	9 (31%)	9 (28%)	
Unknown	7 (24%)	12 (38%)	
Surgery type			
Breast conservation	19 (66%)	17 (53%)	0.44
Mastectomy	10 (34%)	15 (47%)	
Dosing d[a]	25.4 ± 10.2	25.7 ± 9.9	
12–21 d	11 (38%)	13 (41%)	0.99
>21 d	18 (62%)	19 (59%)	

[a]Values are reported as mean with SD. These data are extracted from the clinical pathology reports, and do not represent the research Ki67 values, which were batch-measured at the end of the study and were used for the primary endpoint analysis, shown in **Table 2**. The unknown values are largely on patients diagnosed at outside institutions, where Ki67 analysis was not routinely done on core needle biopsies.

Primary endpoint, Ki67 changes from baseline to posttreatment

We compared lesion-matched DCNB and excision samples from all 61 compliant participants. Our prespecified endpoint of a relative 50% reduction in the Ki67 index in the telapristone acetate arm was achieved in eight women treated with telapristone acetate, but was also seen in five women treated with placebo. The mean postintervention Ki67 LI decreased significantly from baseline in both groups (mean reduction of 5.5% in telapristone acetate group, $P = 0.003$ and mean reduction of 4.2% in the placebo group, $P = 0.04$; see **Table 2** and **Fig. 2A**). We explored the staining intensity for Ki67 (H-score), because this avoids selecting a single threshold for cell positivity and can reduce the contribution of weakly stained cells (potential false positives). There was a larger mean H-score reduction in the telapristone acetate group (−14, $P = 0.003$) than in the placebo group (−10, $P = 0.06$). Our randomization scheme was stratified 2:1 for pre- and

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Table 2. Measurement of Ki67 changes according to the treatment groups.

	Placebo ($N = 29$)		TPA ($N = 32$)	
	Mean ± SD	P^a	Mean ± SD	P^a
%Ki67 LI				
Baseline	22 ± 18		19 ± 11	
Posttreatment	18 ± 14		14 ± 8	
Changes from baseline	−4.2 ± 10	0.04	−5.5 ± 9.6	0.003
H-score				
Baseline	56 ± 47		49 ± 28	
Posttreatment	46 ± 38		34 ± 21	
Changes from baseline	−10 ± 25	0.06	−14 ± 24	0.003
Premenopausal women	$N = 19$		$N = 22$	
Baseline	21 ± 17		19 ± 12	
Posttreatment	17 ± 12		14 ± 7.3	
Changes from baseline	−3.6 ± 9.4	0.17	−5.2 ± 9.9	0.03
Postmenopausal women	$N = 10$		$N = 10$	
Baseline	24 ± 10		19 ± 9.7	
Posttreatment	19 ± 18		13 ± 9.8	
Changes from baseline	−5.4 ± 11	0.16	−6.1 ± 9.4	0.08

[a]Paired signed-rank test for changes from baseline within a treatment group.

postmenopausal women, therefore we performed an analysis stratified by menopause. A significant Ki67 decrease was found only in premenopausal women treated with telapristone acetate (mean reduction of 5.2 in the telapristone acetate group, $P = 0.03$ and mean reduction of 3.6 in the placebo group, $P = 0.17$). The change in Ki67 was unrelated to the abundance of PR expression in the tumors (Supplementary Fig. S1). There was also no correlation between the duration of therapy and change in Ki67 ($P = 0.25$ for both telapristone acetate and placebo arms; Supplementary Fig. S2).

Because the mean reduction of Ki67% in the treated arm was more modest than expected, and changes were seen in the placebo arm, we postulated that the Ki67 changes seen in each group would be clarified using gene expression data. For this purpose, we set a *post hoc* threshold of 30% reduction to stratify participants into "responders" and "nonresponders" and explore the association of gene expression changes with Ki67 response. Using this response parameter, we identified 12 of 31 (39%) "responders" in the telapristone acetate group, and eight of 29 (28%) "responders" in the placebo group (**Fig. 2A**).

RNA sequencing and quantitative technical confirmation

Transcriptome analysis was performed on samples from 60 women (31 telapristone acetate and 29 placebo) with high-quality RNA (mean %DV200-800 was 62 ± 12%). Overall, differential gene expression analysis comparing baseline with posttreatment values for each tumor showed no significant modulation of genes in either group. A nominal $P < 0.05$ was seen in 15 of 17,109 genes in the telapristone acetate group, and 11 of 17,205 genes in the placebo group, but none survived adjustment for multiple comparisons. We then focused on the subgroup of participants whose samples showed a relative Ki67 reduction of ≥30%. In "telapristone acetate nonresponders," there were no genes that were significantly altered posttherapy. Among "telapristone acetate responders," we found 103 of 6,623 genes to be significantly modulated (i.e., $\log_2$ fold change > ±1, $P_{adj} < 0.05$) by telapristone acetate treatment (64 upregulated and 39 downregulated; **Fig. 2B** and **D**; Supplementary Table S1A). The pathway enrichment analysis revealed that these downregulated transcripts were associated with the progression of cell cycle, mitosis, and chromatin organization, among others; the upregulated transcripts were enriched for vesicle-mediated transport pathway (*HBA1/HBA2/HBB/GJA1*; $P < 0.05$, FDR $q < 0.25$; **Fig. 2E**; Supplementary Table S1B). Of note, the IFN signaling pathway was suppressed (*STAT1/HLA-G/KPNA2/IFI6*; $P = 0.054$, FDR q = 0.174); and the activation of the complement cascade pathway of the immune system was activated close to the significance cutoffs (*CFD/CFB/C4A/C4B*; $P = 0.052$, FDR q = 0.174; Supplementary Table S1B). At an individual gene level, there was significant suppression of progestin target genes (*PTTG1, FADS2, PLK1, SLC1A1*) and *ERBB2* expression and an increase of *PGR* expression (Supplementary Table S1A). Notably, 11 downregulated genes (*FBXL20, PSMD3, MED1, STARD3, ORMDL3, PGAP3, PPP1R1B, GSDMB, MIEN1, GRB7,* and *ERBB2*) are located together on the HER2 amplicon locus at chromosome 17 cytoband q12 (**Fig. 3**; Supplementary Table S1A). In contrast, the placebo "responder" group showed significant modulation of 124 of 7,921 genes: 115 upregulated and nine downregulated (**Fig. 2B**; Supplementary Table S2A). However, there were no significantly enriched pathways except the upregulation of Fc epsilon receptor (FCERI) signaling (*FOS/NR4A1/JUN/EGFR/FGF7/CDKN1A*), which is part of immune response signaling pathway ($P = 0.029$, FDR $q = 0.218$; Supplementary Table S2B).

There were four genes (*ALDH1A1, CFD, PFKFB3, PLIN1*) that were common between placebo and telapristone acetate group and upregulated in both groups (**Fig. 2C**). These genes are associated with metabolism and innate immune response signaling pathways. Importantly, none of the pathways significantly modulated in telapristone acetate responders were modulated in placebo responders.

We also examined a variety of published gene signatures related to progesterone effects on cancer stem cells (23, 24), tumor angiogenesis (25), metastasis (26), and the PR isoform–specific signature (9), and did not observe any distinct pattern of modulation compared with placebo responders.

To confirm the findings of the RNA-seq transcriptome analysis, we evaluated a total of 47 target genes with three housekeeping genes (*GUSB, HPRT1, PGK1*) using quantitative gene expression assay (nCounter assay; NanoString Inc.) in 50 subjects with sufficient residual RNA amounts (>100 ng; Supplementary Table S3A). The selection was based on significant modulation in the telapristone acetate responder group; 12 downregulated genes were associated with the top-ranked enriched pathways (cell-cycle progression, mitosis, cell division, and cytokine IFN signaling). Five upregulated genes were also selected (*CYBRD1, SLC1A1, TFAP2B, PGR, ERBB4*), but these were not significantly enriched in pathway analysis. An additional 26 genes were selected from the published literature (5, 7, 9, 27); they included progestin target response genes (*ASPM, MGP, CDC20, CDK1, TOP2A, ACAT2, ADCY2, ISG15, L1CAM*). We also included three cell proliferation markers (*CDC20, RRM2*, and *UBE2C*) from the PAM50 gene set (28, 29). Finally, we included the *ESR1*. The threshold of statistical significance was $P < 0.05$ adjusted by Benjamini–Yekutieli method ($-\text{Log}_{10}P_{adj} > 1.3$). The results are summarized in Supplementary Fig. S3 and Supplementary Table S3B. Of note, we found that the 13 of 26 (50%) genes that demonstrated close to significant modulation by sequencing (*HIST2H3A, HIST2H3C, ASPM, TOP2A, RAD54L, KIFC1, MYBL2, ISG15, ATAD3C, AURKA, CDK1, ECT2*, and *RBP4*) were significantly modulated in the nCounter assay. Finally, we found significant suppression in four of five cell proliferation markers (*CEP55, MKI67, RRM2*, and *UBE2C*) with a borderline significant suppression of *CDC20* expression ($P_{adj} = 0.051$). None of these significant changes in telapristone acetate–treated responders were observed in placebo-treated Ki67 "responders" (Supplementary Fig. S3, left). Within the telapristone acetate–treated group, we found that *RBP4* gene was significantly downregulated in both Ki67

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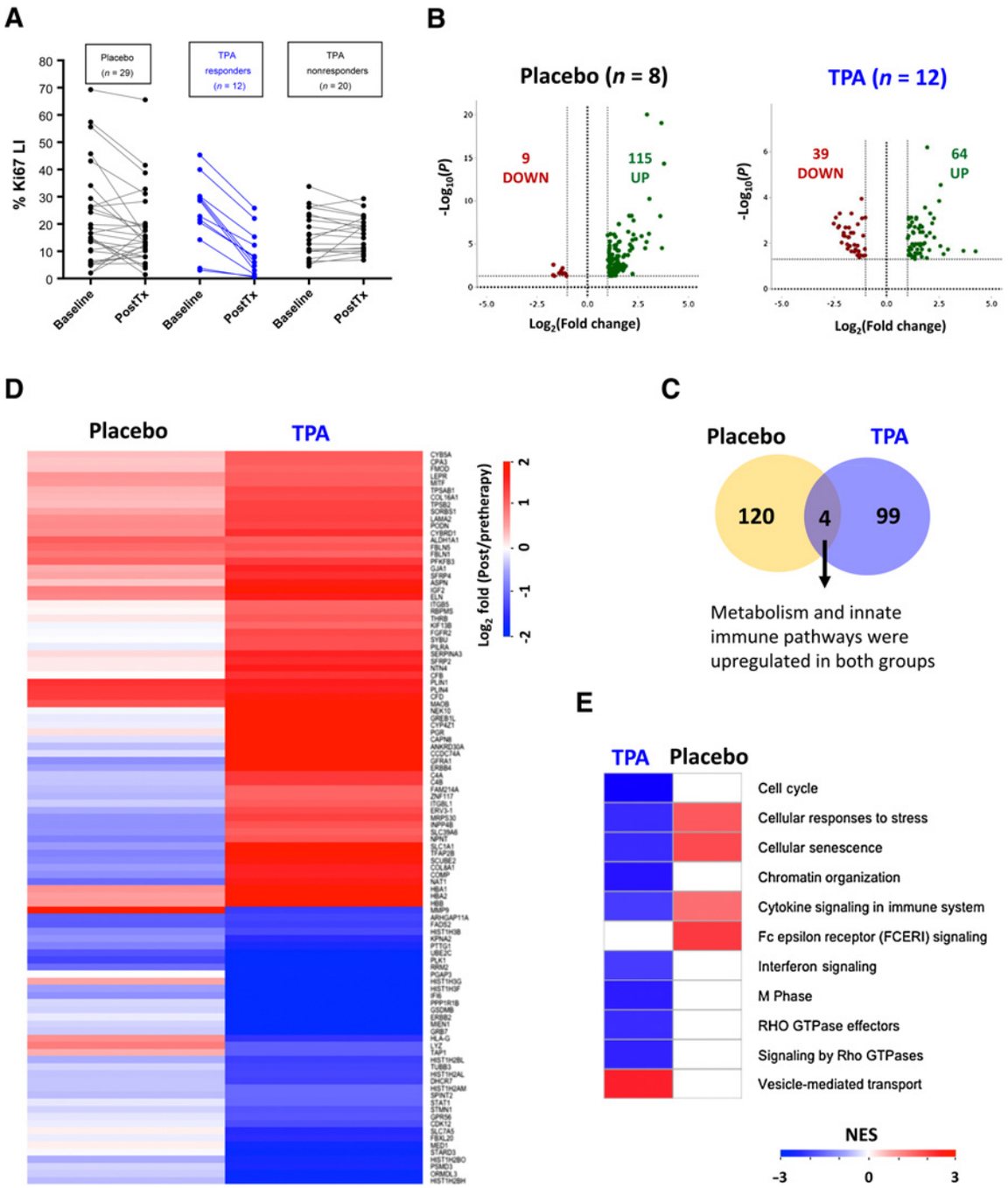

Figure 2.

Gene expression and functional analysis of Ki67 responders in patients treated with telapristone acetate (TPA) or placebo. **A,** Ki67 Change in placebo arm, and in telapristone acetate arm stratified by Ki67 response. Threshold of the Ki67 response was ≥ relative 30% reduction. **B,** Volcano plots display gene expression changes in response to telapristone acetate or placebo treatments. Threshold of significance was $\log_2$ (fold change) > ±1 (*x*-axis) and the adjusted $P < 0.05$ (*y*-axis). Left, there were the nine genes downregulated including MKI67 and the 115 genes upregulated in placebo responders ($n = 8$). Right, there were the 39 genes downregulated and the 64 genes upregulated in telapristone acetate responders ($n = 12$). **C,** Overlap between the genes significantly regulated in response to the telapristone acetate– and placebo therapy. There was minimal overlap between two groups except 4 genes (*ALDH1A1, CFD, PFKFB3, PLIN1*), which were upregulated in both groups. These genes map to metabolism and innate immune system pathways. **D,** Heatmap displays $\log_2$ fold change of the 103 genes that are significantly regulated in response to telapristone acetate therapy. Fold change in both telapristone acetate– and placebo arms is plotted. The induction (red) or repression (blue) of gene expression is shown for each gene. **E,** Heatmap plots the normalized enrichment scores (NES) of the pathways significantly enriched in the gene set enrichment analysis (GSEA) on the expression changes observed in response to telapristone acetate therapy. Each color represents negative enrichment (blue), no enrichment (white) and positive enrichment (red). The cutoff for GSEA was the gene set size of at least three and FDR $q < 0.25$. IFN signaling pathway was suppressed and complement cascade pathway was increased close to the statistical significance.

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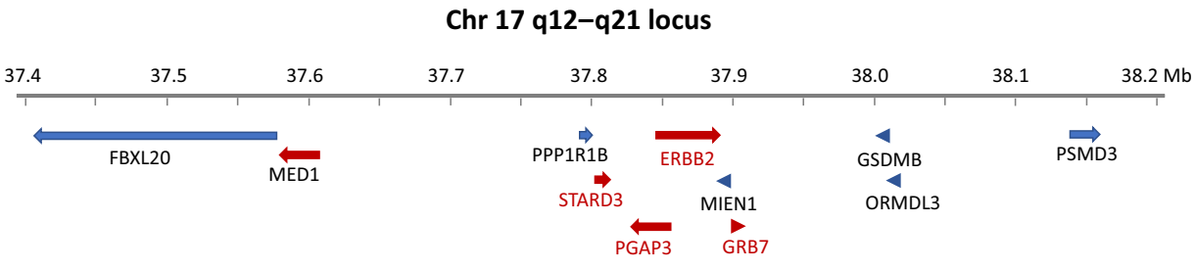

Figure 3.

The chromosomal location of HER2 amplicon or genes close proximity to it. Eleven genes located in chromosome 17 (q12–q21) locus were significantly downregulated in the telapristone acetate–treated responder group. Among these, five genes (ERBB2, MED1, STARD3, PGAP3, GRB7) were the known HER2 amplicon genes (red arrows and font; refs. 36, 51) and six other genes (FBXL20, PPP1R1B, MIEN1, GSDMB, ORMDL3, PSMD3) were adjacent to this locus.

responders and nonresponders (Supplementary Fig. S3, right; Supplementary Tables S3B and S3D). Unlike RNA-seq analysis, the *PGR* expression was unchanged in TPA-treated responders. Of note, the *ESR1* expression was not modulated by TPA treatment in both RNA-seq and the nCounter assay.

Serum hormones changes and missed periods in premenopausal women

Given the known effect of telapristone acetate on the suppression of ovulation in premenopausal women (30), we measured serum concentrations of estradiol, progesterone, and FSH prior to and following intervention. The mean concentration of each hormone is summarized by menopausal status (unadjusted for menstrual cycle phase) in Supplementary Table S4. telapristone acetate–treated premenopausal women showed a significant decline of the mean concentrations of estradiol and progesterone (-39.7 ± 66.8 pg/mL, $P = 0.01$ for estradiol; -2.95 ± 4.69 ng/mL, $P = 0.003$ for progesterone), but changes in the placebo group were not significant. Similarly, the concentration of FSH, decreased significantly in telapristone acetate–treated participants (-6.37 ± 18.6 mIU, $P = 0.01$), without a similar change in the placebo group. We used the preintervention last menstrual period (LMP) date and the usual menstrual cycle length to assess the fraction of premenopausal women who were expected to have a period during therapy but did not. In the telapristone acetate arm, 12 of 22 premenopausal women (54.5%) missed an expected period, whereas only three of 19 women (15.8%) of placebo group did. To evaluate whether changes in serum hormones explained the telapristone acetate response, we examined serum hormone changes among telapristone acetate responders, nonresponders, and the placebo group (Supplementary Fig. S4). Serum estradiol was significantly decreased in telapristone acetate responders, but there were no other significant decreases in either hormone by treatment group or response.

Plasma and tissue concentrations of TPA and its metabolite

Of 61 participants, 32 women presented detectable plasma concentrations of telapristone acetate (CDB-4124) and its active metabolite (CDB-4453). Of these, benign tissue samples were available for drug quantitation from 31 women, and tumor samples were available for quantitation from 22 women. The mean plasma and tissue concentrations of each analyte are summarized in **Table 3**. Overall, we observed that the ratio of parent drug to metabolite was approximately 3:1 in plasma and 4:1 in tissue. The concentrations of both parent drug and its metabolite were twofold higher in benign tissue than in tumor samples (326 ng/g vs. 157 ng/g for CDB-4124 and 78 ng/g vs. 41 ng/g for CDB-4453). There were strong positive correlations between benign and tumor tissue concentration (coefficient $r = 0.71$, $P = 0.0002$ for CDB-4124 and $r = 0.56$, $P = 0.007$ for CDB-4453) and with plasma concentration ($r = 0.62$, $P = 0.0003$ for CDB-4124). These strong correlations were similar in pre- and postmenopausal women for parent drug, but not for the metabolite.

Quality-of-life assessment and adverse events

Quality-of-life (QoL) parameters assessed by the BESS questionnaire at study entry and on the day prior to surgery are summarized in Supplementary Table S5. At baseline, the mean scores for all clusters were similar across arms. There were no between-group differences in the change of symptom severity from baseline to posttreatment. Following treatment, the mean score for body image symptoms (unhappy with the appearance of body, weight gain or loss, and decreased appetite) decreased significantly in both groups ($P = 0.01$

Table 3. Concentrations of telapristone acetate and its metabolite (CDB-4453) in plasma and breast tissue samples.

	Concentrations (mean ± SD)			Spearman correlations			
		Tissue (ng/g)		(Benign with tumor)		(Benign with plasma)	
Analytes	Plasma (ng/mL)	Benign	Tumor	*r*	*P*	*r*	*P*
Total	($N = 32$)	($N = 31$)	($N = 22$)				
TPA	147 ± 111	326 ± 287	157 ± 130	0.71	0.0002	0.62	0.0003
CDB-4453	51 ± 25	78 ± 64	41 ± 27	0.56	0.007	0.30	0.11
Premenopausal	($N = 22$)	($N = 21$)	($N = 17$)				
TPA	152 ± 120	282 ± 243	149 ± 124	0.56	0.02	0.54	0.01
CDB-4453	48 ± 26	64 ± 50	40 ± 28	0.47	0.06	0.32	0.15
Postmenopausal	($N = 10$)	($N = 10$)	($N = 5$)				
TPA	138 ± 92	420 ± 359	185 ± 162	0.90	0.04	0.77	0.009
CDB-4453	58 ± 25	107 ± 82	44 ± 27	0.70	0.19	0.07	0.85

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for placebo and $P = 0.009$ for telapristone acetate). In addition, adverse events (AEs) reports are summarized in Supplementary Table S6. A total of 70 randomized participants were eligible for evaluation of AEs. All of these were grade 1 events, with the exception of a single case of atrial fibrillation in the placebo group (the subject was under treatment for hyperthyroidism). Since grade 1 events consisted of commonly experienced minor symptoms, we evaluated the events that occurred at least three times during therapy. Among telapristone acetate–treated women, there were three who met this criterion: one with gastrointestinal symptoms (nausea, abdominal pain, constipation/abdominal distension). One subject reported musculoskeletal symptoms (back pain, neck pain, and myalgia), and another experienced nervous system disorders (dizziness, paresthesia, and headache). In the placebo group, one subject experienced musculoskeletal symptoms (back pain, neck pain, and myalgia). Overall, there was no between-group difference ($P = 0.99$).

Discussion

We report the first study of the effects of telapristone acetate, a second-generation SPRM, in women with early-stage, predominantly ER-/PR-positive breast cancer. We chose a window-of-opportunity design because the safety of this agent at the dose we used has been established in several trials in women with uterine fibroids or endometriosis. However, given lack of efficacy data in patients with breast cancer, a formal neoadjuvant study was not justified. We found that cell proliferation (Ki67 labeling) decreased significantly in the telapristone acetate–treated group, from a mean of 19% to 14% ($P = 0.003$); there was a smaller, more varied, but still significant decrease in the placebo group ($P = 0.04$). The results in the telapristone acetate arm represent a 74% reduction rather than the prespecified 50% and therefore do not formally meet the criterion for success. However, our goal was to perform a preliminary evaluation of this class of agents in a treatment-naïve early breast cancer population, and the findings discussed below provide sufficient encouragement to investigate SPRMs further for breast cancer therapy. Our trial was stratified by menopausal status, and we observed a significant within-group decrease in Ki67 LI in telapristone acetate–treated premenopausal women, but not in the premenopausal placebo group. Among postmenopausal women, there was a nonsignificant decline in both placebo and telapristone acetate–treated groups. We weighted our recruitment 2:1 toward premenopausal women, expecting a larger effect in a population that is currently exposed to progesterone. Thus, the postmenopausal data are arguably inadequate for evaluation of this class of agents; further studies should not therefore exclude postmenopausal women.

Our ability to demonstrate a significant between-group decrease in Ki67 index was limited by a decline in Ki67 labeling, with greater variation (SD 10% expected vs. 18% observed), within the placebo arm. Similar decreases in Ki67 index in the placebo group have been seen in other studies (31–33), likely explained by intratumoral heterogeneity (34), and preanalytic processing differences between DCNB samples and surgical excisions. A recent report comparing *MKI67* gene expression in core biopsies with the surgical specimen showed that *MKI67* expression was not significantly correlated in the two samples ($r = 0.35$, $P = 0.10$; ref. 35), although other genes and pathways showed good correlations. Our DCNB samples came from a variety of regional hospitals, and therefore preanalytic variation is likely. However, we took great care with quality control for the staining and scoring protocols for Ki67; pre- and posttherapy samples were batch-sectioned, kept cold, and stained within 4 weeks; two different image analysis systems produced highly concordant results, and correlated well with manual reads by our study pathologist. Our quality checks for scoring showed results that were similar to an international comparison of Ki67 IHC evaluation, where intralaboratory reproducibility was high (36).

Another source of Ki67 variation may be an imbalanced distribution of placebo and treated participants by menstrual phase, which has been shown to affect Ki67 positivity and expression of proliferation-related genes in ER-positive breast tumors (37, 38). Horimoto and colleagues evaluated the Ki67 index in paired biopsy and surgical specimens from 146 patients, and found that Ki67 expression was similar in menstrual phase-concordant diagnostic and surgical specimens, but declined when the diagnostic sample was luteal and the surgical sample was nonluteal. Although we collected menstrual cycle data and measured serum hormones at study enrollment, the DCNB was performed days or weeks prior to enrollment, and menstrual phase at the time of CNB could not be reliably ascertained. A random excess of luteal phase DCNB samples in the premenopausal placebo participants may therefore explain the decline in Ki67 index between DCNB and surgery. However, because KI67 declines were also seen in postmenopausal placebo patients, it is likely that the causes are multifactorial. In the telapristone acetate group, these influences may have diluted the drug effect. Future trials should include strategies to control these sources of variation.

Despite the modest antiproliferative effect suggested by the Ki67 changes in the telapristone acetate arm, and the unexpected declines in the placebo arm, our RNA-seq and nCounter results add considerable weight to the conclusion that telapristone acetate treatment did in fact have significant antiproliferative effects on breast cancers. Gene expression changes and enriched pathways in the TPA-responsive group, but not the placebo group, consisted largely of cell-cycle genes relating to mitosis, chromatin modification, and cell-cycle regulation. Furthermore, the observed gene expression changes were almost completely nonoverlapping with those in the placebo arm, and no significant changes were seen on pathway analysis in the placebo group. These clinical trial findings are consistent with our previous report on multiple ER-/PR-positive breast cancer cell lines, where telapristone acetate inhibited cell-cycle progression (G_2–M; ref. 7); and in rodent mammary carcinogenesis models, where telapristone acetate treatment greatly decreased cell proliferation, angiogenesis, and tumor incidence (10).

Eleven downregulated genes in the telapristone acetate arm are members of the HER2 amplicon on chromosome 17 or immediately adjacent to it: *FBXL20, PSMD3, MED1, STARD3, ORMDL3, PGAP3, PPP1R1B, GSDMB, MIEN1, GRB7*, and *ERBB2*. Although the association of HER2 overexpression or amplification with breast cancer is well known, much less is known about the other genes within this region of chromosome 17. Shiu and colleagues used siRNA technology to knockdown the expression of a number of genes within the amplicon to determine if any of these genes are critical for survival of breast tumor cells. Silencing of *PMSD3* expression was correlated with a significant loss of viability in almost all cell lines tested; the effect of *STARD3* was much more modest (39). The coincident downregulation of these genes in the telapristone acetate responder group suggest coordinate regulation of transcription. Alcala-Corona and colleagues used gene expression data to construct a model of the hierarchical modular structure of the HER2-enriched transcriptional network (40). One of the 162 components in this network contains 4 of the 11 telapristone acetate–genes: *ERBB2, GRB7, PGAP3*, and *STARD3*. Their fully connected pattern in the model indicates "close coexpression," which may be due either to *cis* and/or *trans* regulatory elements, or to coregulator expression, possibilities we will pursue in future studies. Overall, our data suggest that telapristone acetate administration downregulates the expression of *ERBB2* as well as a number of other

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genes colocated on chromosome 17q12, which is predicted to result in apoptosis of the tumor cells. Examination of intrinsic cluster 5 of METABRIC, which contains the *ERBB2*-amplified cancers (PAM50 HER2), shows that luminal B and A tumors also segregate within this cluster (41). These may be the luminal tumors that respond to antiprogestin therapy.

Interestingly, we have observed that IFN signaling pathway was specifically inhibited in the telapristone acetate responder group, which relates to the recent observation that PR attenuates the expression of IFN-stimulated genes in both the presence and absence of ligand (42). The IFN metagene is reported to indicate poor prognosis, in HR-positive/HER2-negative disease (43). Our novel finding that the IFN-stimulated genes (*ISG15* and *IFI-6*) were inhibited by telapristone acetate treatment, along with *STAT1*, is particularly noteworthy in light of recent evidence, suggesting that exosome transfer from the stroma to breast cancer cells initiates antiviral signaling (44, 45). The expression of a specific set of IFN-stimulated genes (the IFN-related DNA damage resistance signature or IRDS) predicts radiotherapy and chemotherapy resistance (46). In breast cancer, the IRDS clinical classifier comprises seven genes that include *STAT1* and *ISG15*. Exosomes transferred from stromal cells to breast cancer cells appear to increase the expression of the IRDS genes, with *STAT1/IRDS* and Notch 3 acting cooperatively to expand tumor-initiating cells and tumor-resistant cells (44). Down-regulation of *STAT1* by telapristone acetate may be responsible for the decreased expression of *ISG15* and *IFI-6* observed in our study. The consequences of this effect of telapristone acetate on IFN-stimulated genes on therapeutic resistance, and stemness (47), deserve further investigation using SPRMs such as ulipristal acetate or onapristone.

Going forward, an important aspect of defining the role of SPRMs in breast cancer therapy will be the identification of parameters that select patients for therapy. Candidates that have been suggested by others revolve around phosphorylated PR (23) and the expression of PR isoforms (5, 29). We were unable to evaluate these in this study due to limited tissue availability from the DCNB, and our prioritization of RNA-seq studies; but our findings do point to potentially important pathways that should be evaluated in the future (HER2- and IFN-related genes, among others). Other important avenues for investigation relate to the combination of anti-progesterone and anti-estrogen therapy, as suggested by others (5, 48–50), as well as combinations with drugs targeting protein kinases such as MEK (51) and CDK4/6 inhibitors such as palbociclib (52).

The dose of telapristone acetate that we used (12 mg) was selected on the basis of safety in trials for benign gynecologic diseases. This may not be optimal for antitumor efficacy, but we chose it with the longer term objective of testing SPRMs for breast cancer prevention, where safety is a primary concern. Higher doses of single-agent mifepristone have been tested in patients with metastatic breast cancer (200–400 mg; refs. 18, 53, 54); but showed insufficient activity in advanced disease. More recently, phase I trial testing of mifepristone in combination with chemotherapy yielded promising results (55). Because telapristone acetate has been removed from the clinical development pipeline (after acquisition by Allergan), the appropriate dose for other SPRMs will need to be determined. Therapeutic indications will allow some tolerance for adverse effects, but preventive indications will not. Reassuring data regarding tolerability and liver safety continue to accumulate from trials of uterine fibroids (16, 56).

Our data also document the suppression of menstruation and of serum progesterone levels in premenopausal women, which points to the possibility that some of the effects of telapristone acetate on tumor cell proliferation may be indirect. It is also consistent with the present interest in the long-term contraceptive potential of these drugs (30) and NCT03296098. The effects of ulipristal acetate on breast epithelial proliferation are currently being assessed in NCT02922127; and those of mifepristone on the breast epithelium of in BRCA 1/2 mutation carriers is under study in NCT01898312. Thus, it is possible that SPRMs will present premenopausal women with an option that provides both breast cancer prevention and contraception.

We measured plasma and breast tissue concentrations of telapristone acetate and its active metabolite, and found that benign breast tissue drug concentrations related well to those in the plasma, and to those in tumor tissue. However, there was no significant correlation between tumor drug levels and plasma concentrations; or between tumor drug concentration and Ki67 response (data not shown).

In summary, our results are sufficiently encouraging to justify additional trials with SPRMs in both the treatment and the prevention setting. Each will need to be evaluated individually, because differences exist between these agents, but ulipristal acetate and onapristone (ZK98299) are excellent candidates for development in this direction. Longer term trials in women with fibroids have now been completed with ulipristal acetate (56), and trials in stage IV breast cancer have been initiated with onapristone, so that it is now possible to consider a true neoadjuvant trial with a clinical response endpoint in early breast cancer. Predictors of benefit clearly need to be pursued further; and if the downregulation of HER2 amplicon genes is corroborated, SPRMs combinations with anti-HER2 agents should be tested in the treatment of HR-positive tumors where HER2 and other genes on this amplicon are overexpressed. Finally, the possibility of a novel intervention for premenopausal women with HR-positive breast cancer will be a very welcome addition, because recent innovations in endocrine therapy have not benefited this important group of patients.

Disclosure of Potential Conflicts of Interest

H. Singhal is an employee/paid consultant for and holds ownership interest (including patents) in Hoffman-La Roche. No potential conflicts of interest were disclosed by the other authors.

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Other (developing clinical study protocol and lab manual for collection, processing, handling, and storage of biospecimen): O. Lee

Acknowledgments

This study was funded by the Breast Cancer Research Foundation (to S.A. Khan) and NIH R01CA192124 (to S.A. Khan and J.J. Kim). Telapristone and placebo were supplied by Repros Therapeutics, who also supported the drug concentration assays. We thank Xiaoling Xuei and Yunlong Liu at the Center for Medical Genomics at Indiana University School of Medicine, for performing the RNA-seq study and Ryan Deaton, Rami Hayajneh, and the Research Histology and Tissue Imaging Core at the University of Illinois at Chicago, for performing the Ki67 study.

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Received February 14, 2019; revised June 19, 2019; accepted September 26, 2019; published first September 30, 2019.

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