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Prospective, Multi-center Randomized Intermediate Biomarker Study of Oral Contraceptive vs. Depo-Provera for Prevention of Endometrial Cancer in Women with Lynch Syndrome

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Abstract

Women with Lynch syndrome have a 40–60% lifetime risk for developing endometrial cancer, a cancer associated with estrogen imbalance. The molecular basis for endometrial-specific tumorigenesis is unclear. Progestins inhibit estrogen-driven proliferation, and epidemiologic studies have demonstrated that progestin-containing oral contraceptives (OCP) reduce the risk of endometrial cancer by 50% in women at general population risk. It is unknown if they are effective in women with Lynch syndrome. Asymptomatic women age 25–50 with Lynch syndrome were randomized to receive the progestin compounds depo-Provera (depoMPA) or OCP for three months. An endometrial biopsy and transvaginal ultrasound were performed before and after treatment. Endometrial proliferation was evaluated as the primary endpoint. Histology and a panel of surrogate endpoint biomarkers were evaluated for each endometrial biopsy as secondary endpoints. A total of 51 women were enrolled, and 46 completed treatment. Two of the 51 women had complex hyperplasia with atypia at the baseline endometrial biopsy and were excluded from the study. Overall, both depoMPA and OCP induced a dramatic decrease in endometrial epithelial proliferation and microscopic changes in the endometrium characteristic of progestin action. Transvaginal ultrasound measurement of endometrial stripe was not a useful measure of endometrial response or baseline hyperplasia. These results demonstrate that women with Lynch

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syndrome do show an endometrial response to short term exogenous progestins, suggesting that OCP and depoMPA may be reasonable chemopreventive agents in this high-risk patient population.

Keywords

endometrial cancer; chemoprevention; Lynch syndrome; progestin

Introduction

Lynch syndrome, or hereditary non-polyposis colorectal cancer syndrome (HNPCC), is an autosomal-dominant inherited condition characterized by early onset colon cancer and endometrial cancer. In women with Lynch syndrome, risk of endometrial cancer (EC) equals or exceeds risk of colon cancer. Women with Lynch syndrome gene mutations have a 40–60% lifetime risk of developing EC, compared with 3% for the general population (1, 2). Endometrial cancer tends to occur at an earlier age in women with Lynch syndrome (mean age of diagnosis – 48 years) (3, 4). In the general risk patient population, endometrial cancer, especially endometrioid-type, is known to be an estrogen-driven malignancy. Progesterone is well-known to antagonize the effects of estrogen. Accordingly, the Cancer and Steroid Hormone Study (CASH) demonstrated that use of progestin-containing oral contraceptive pills (OCP) reduces the risk of EC by 50% in women at general population risk (5). It is unclear whether OCP are effective in high risk women, such as women with Lynch syndrome.

The goal of this short term 3 month Phase II biomarker endpoint study was to examine the effects of progestin-containing OCP or depo-medroxyprogesterone acetate (depoMPA) on the endometrium of women with Lynch syndrome. Given the substantial risk of endometrial cancer in these women, it is essential to determine if progestins are able to induce the characteristic microscopic changes in the endometrium of Lynch syndrome women, similar to those observed in average-risk women. As a step towards determining the chemopreventive potential of OCP and depoMPA in this population, we examined the short term effect of depoMPA and OCP on the endometrium of women with Lynch syndrome using markers of progestin responsiveness, including pre- and post-treatment endometrial histology, endometrial epithelial proliferation index, and a panel of endometrial gene expression biomarkers of estrogen action. Gene expression biomarkers were selected based on previous microarray studies and targeted gene expression analysis identifying estrogen responsive genes in the endometrium, including several that are differentially expressed in estrogen-related endometrial cancer (6–11).

Methods

Patients

The Institutional Review Boards (IRB) at MD Anderson Cancer Center, University of California, San Francisco and Creighton University approved the study (trial registration ID: NCT00033358). Women between the ages of 25–50 with a known mutation in *MLH1*, *MSH2*, or *MSH6* were eligible. In addition, women who had a personal history of a Lynch syndrome associated cancer and who fulfilled Amsterdam criteria based on family history were eligible. Additional inclusion criteria included no prior hysterectomy, no history of prior pelvic radiation, no chemotherapy for two years, and no use of OCP or hormones for 4 months prior to initiation of study. Women also had to have no medical contraindication to use of OCP or depoMPA, including known or suspected pregnancy, undiagnosed vaginal bleeding, active thrombophlebitis or past history of thromboembolic disorders or cerebral

vascular disease, gallbladder disease, history of diabetes, coronary artery disease, or a current tobacco smoker age ≥ 35 .

On day 5–10 of the menstrual cycle, corresponding to the endometrial proliferative phase, a transvaginal ultrasound (TVS) and then endometrial biopsy using a 3–4 mm pipelle were performed for each participant. Half the biopsy was placed into formalin for routine histological processing, pathologic assessment, and Ki67 immunohistochemistry. The remaining half was frozen under liquid nitrogen and used for subsequent qRT-PCR assays. The TVS measured the endometrial stripe and the size, morphology, and any abnormalities of the uterus and ovaries. Women were randomized to receive OCP (LoOvral, 0.3 mg ethinyl estradiol and 0.3 mg norgestrel daily for 21 days followed by 7 days of placebo) or a single dose of 150 mg depoMPA. For women randomized to the OCP arm, the second TVS and endometrial biopsy were performed on day 4–10 after initiation of the 4th pack of study medication. For women randomized to the depoMPA arm, the second TVS and endometrial biopsy were performed on day 90 ± 5 days. Women were considered inevaluable if they missed more than 4 consecutive non-placebo pills and/or missed a total of more than 10 non-placebo pills during a 4 month period.

Assays

All endometrial biopsies were microscopically examined. The baseline biopsies were examined to confirm proliferative phase histology and to rule out presence of endometrial hyperplasia or carcinoma. Post-treatment biopsies were evaluated for response to progestin treatment. The response was considered good if the glands were inactive or secretory and there was lack of epithelial cell mitotic figures, and if there was evidence of stromal pre-decidualization, characterized by stromal cells with increased eosinophilic cytoplasm and acquisition of epithelioid shape (Figure 2A). A poor response was characterized by the absence of stromal cellpseudo-decidualization, the absence of inactive endometrial glands, or the presence of proliferative type endometrial epithelial glands with mitotic figures. Presence of endometrial hyperplasia or carcinoma in the post-treatment biopsy was characterized as a pathologic response. Ki67 immunohistochemistry (MIB-1, Dako, Carpinteria, CA) was performed per manufacturer's instructions; its expression was determined by microscopically quantifying the percentage of endometrial epithelial cells with positively-stained nuclei.

Estrogen is well-known to be a stimulator of endometrial proliferation and uterine growth. We have previously characterized a panel of genes that are expressed in the endometrium and modulated by estrogen (Supplementary Table S1) (6–14). Importantly, these genes are differentially expressed in endometrial carcinoma, with several showing significantly higher expression in endometrioid-type carcinomas (estrogen-related) compared to non-endometrioid carcinomas (not related to estrogen excess). This panel includes *IGF-1*, *IGF-1R*, *IGF-2*, *EIG121* (a.k.a. *KIAA1324*), *RALDH2* (a.k.a. *ALDH1A2*), *sFRP1*, *sFRP4*, and *survivin* (a.k.a. *BIRC5*). *IGFBP1* was also analyzed as a positive control for tissue action of progesterone. All quantitative real-time PCR assays for genes in this panel were performed from the frozen endometrial biopsy using an ABI7700 instrument (Life Technologies, Grand Island, NY). All assays were validated using standards comprised of a synthetic oligonucleotide that corresponds exactly to the amplicon that was measured in sample RNA to ensure linearity of signal and to determine the lower limit of detection of each assay. Assays must have been able to detect a minimum of 1000 molecules of target and were linear ($r^2 > 0.98$) over a concentration range of at least 5 logs. Each sample was assayed in quadruplicate including a negative control that was minus the reverse transcriptase. This negative control served to measure any contaminating DNA in the RNA samples. All transcripts were normalized using the transcripts 18S mRNA, β -Actin (*ACTB*),

and 36B4 (a.k.a. *RPLPO*). Assays for these 3 transcripts were performed on each sample and “gNORM” was calculated as the geometric mean of the normalizer transcripts.

Statistical Analysis

The primary outcome was the change in Ki-67 expression prior to and after treatment. We analyzed these changes within, as well as between, treatment groups. Secondary endpoints included the change from pre- to post-treatment in histology and endometrial thickness (as measured by transvaginal ultrasound), as well as the estimation of the frequency of endometrial abnormalities in this patient population on presentation. Another secondary endpoint included the examination of the change in expression of estrogen-induced genes measured by qRT-PCR.

Measurements of each potential surrogate endpoint biomarker (SEB) were taken before and after hormone treatment. Although the actual data values observed were used in the analysis of the trial, for simplicity in the computation of sample size we assumed only that the value for a given biomarker increased or decreased. If the treatment had no effect, then the probability of a marker increasing (or decreasing) was 0.50. This study was designed to have 80% power to detect a change in this probability to 0.82 within each treatment arm. Using an exact test (2-sided) with a significance level of 0.05 we needed 22 patients in a treatment arm to detect a change in the proportion of patients in that arm with markers increasing (or decreasing) from 0.50 to 0.82 with 80% power. To allow for an approximate 15% dropout rate, we enrolled 51 patients total on this multi-center trial.

This was an exploratory study, and any biomarkers found to be elevated or depressed was of interest. However, since there were 9 potential biomarkers of special interest (Ki67 immunohistochemistry and qRT-PCR of 8 estrogen-modulated genes), to achieve an overall type I error rate of 5% we tested each biomarker at the nominal level of $0.05/9 = 0.0056$. The proportion of patients with a marker increasing (or decreasing) had to have been 0.86 for us to be able to detect a statistically significant change in the proportion of patients with markers increasing (or decreasing) from 0.50 and maintain an overall significance level of 0.05.

Paired plots showing pre- and post-treatment values (so called “box plots”) were examined for each biomarker. Paired t-tests (or signed rank test) were used to compare the biomarkers. In addition, to determine whether patterns of changes in the biomarkers were associated with treatment, we performed a multivariate analysis of the data. We examined the correlation in the changes following treatment among the biomarkers using either Pearson’s or Spearman’s correlations as appropriate.

Results

A total of 51 women were enrolled, and their demographics are summarized in Table 1. There were no significant differences between the treatment arms for age, race, mutation status, BMI, parity, or gravity. Twenty-four women had an *MLH1* mutation, 22 women had an *MSH2* mutation, and 2 women had an *MSH6* mutation. In addition, two women fulfilled Amsterdam criteria and had a history of colon cancer. One patient was Amsterdam positive, had a history of a benign ovarian tumor and had previously undergone a unilateral salpingo-oophorectomy.

As shown in Figure 1, both depoMPA and OCP caused a dramatic decrease in endometrial epithelial proliferation as measured by Ki-67 positive cells (depoMPA mean pre- 51.8%, mean post- 13.1% ($p < 0.001$) and OCP mean pre- 48.3%, mean post- 3.1% ($p < 0.001$)). When histology was examined, 20 of 23 patients in the depoMPA group and 22 of 23

patients in the OCP group demonstrated inactive and/or secretory-type glands (Table 2). Interestingly, the three patients who had a poor histologic response to treatment were all in the depoMPA group, the arm in which treatment compliance was not an issue (Figure 2B and 2C). An additional patient, who had a normal endometrial biopsy at baseline and was randomized to receive OCP, was found to have a focus of complex hyperplasia without atypia in a background of inactive glands on her 3 month biopsy (pathologic response; Figure 2C). Two of 51 patients had baseline endometrial abnormalities (3.9%, 95% CI 0.5% to 13.5%). Both abnormalities were complex atypical hyperplasia (Supplementary Figure S1), and both were found to have Grade 1 endometrioid endometrial carcinoma in the subsequent hysterectomy.

Transvaginal ultrasound measurement of baseline endometrial thickness revealed a mean of 5.5 mm (range 2.6–10.1) for the depoMPA arm and a mean of 6.5 mm (range 2.0–19.0) ($p = \text{NS}$) for the OCP arm. Despite the changes in endometrial histology post-treatment, the mean follow-up endometrial thickness was not significantly decreased, with the mean in the depoMPA arm of 4.5 mm (range 1.0–9.3) and 4.5 mm (range 2.0–10.0) in the OCP arm (Table 2).

In addition to Ki-67, transcripts for 8 different estrogen-modulated genes were quantified (Figure 3). We also analyzed the endometrial expression of *IGFBP1*, which is well-known to be induced by progesterone (15, 16), to verify tissue action of OCP and depoMPA. *IGFBP1* was significantly induced in the post-treatment endometrial biopsies for both treatment arms (Figure 3). For both treatment groups, the post-treatment endometrial biopsies had significantly altered expression of *IGF-1*, *IGF-2*, *sFRP1*, *sFRP4*, and *survivin* transcripts. For the depoMPA group, *EIG121* was also decreased in the post-treatment biopsy. These results are consistent with the fact that progesterone typically antagonizes the biological effects of estrogen. For 2 of the 3 poor histologic responders (all 3 in the depoMPA group), endometrial tissue was available for molecular analyses. In contrast to patients with a good histologic response, the two poor responders showed elevated *sFRP1*, *sFRP4*, and *survivin* in the post-treatment endometrial biopsies (Figure 4).

Discussion

While women with Lynch syndrome have a substantial lifetime risk for the development of endometrial cancer, studies regarding the efficacy of preventive strategies are few. We previously reported that surgical prevention with hysterectomy and bilateral salpingo-oophorectomy is highly effective for the prevention of endometrial and ovarian cancer in this high risk population (17). Whether OCP or depoMPA are effective chemopreventive agents for women with Lynch syndrome, as they are for women in the general population, is unknown. As a step towards determining the chemopreventive potential of OCP and depoMPA in this population, we examined the short term effect of depoMPA and OCP on the endometrium of women with Lynch syndrome using several endometrial tissue markers of progestin-responsiveness. With each woman as her own control, after 3 months of treatment we observed a significant decrease in endometrial epithelial proliferation (Ki-67) in both the depoMPA and OCP arms. Histologically, 20 of the 23 women in the depoMPA arm and 21 of 22 women in the OCP arm demonstrated the presence of inactive and/or secretory-type glands. While the endpoint of this study was not efficacy, the significant response both with Ki-67 and histology suggests that both depoMPA and OCP may be reasonable chemopreventive agents in this high risk cohort. It was interesting that 3 women in the depoMPA arm had a poor histologic response, although all 3 demonstrated a significant decrease in Ki-67. Given the small numbers of non-responders overall, it would be difficult to conclude that OCPs were more effective than depoMPA. In addition, longitudinal studies will be necessary to determine if poor histologic response to progestins

such as depoMPA can be a marker for increased risk of endometrial cancer in women with Lynch syndrome. The 1 woman in the OCP arm who had a focus of endometrial hyperplasia without atypia in a background of inactive glands had a pre-treatment biopsy that demonstrated normal proliferative endometrium. It is possible that this small focus of hyperplasia had been present prior to treatment and was missed with pipelle sampling. After completion of the study the patient chose to remain on OCP and had a dilation and curettage 4 months later demonstrating benign endometrium.

As a secondary endpoint, we found that the point estimate of endometrial abnormalities in women with Lynch syndrome under age 50 was 3.9% (95% CI 0.5% to 13.5%). Both women were completely asymptomatic and had complex atypical hyperplasia on their baseline biopsy. Both went on to have a hysterectomy, and both were found to have Grade 1 endometrial cancer. Given that the median age of women was 36.8 in the depoMPA arm and 37 in the OCP arm, the finding of complex endometrial hyperplasia (CAH) and cancer in 2 asymptomatic women highlights that these women are at extremely high risk of developing endometrial cancer. The point estimate of endometrial hyperplasia/cancer in the general population is difficult to ascertain, but there are some data from large studies of hysterectomy specimens derived from uterine prolapse patients. One study of 372 women with hysterectomies for uterine prolapse (84% of patients older than 55 years of age) found 4 cases (1.1%) of simple/complex hyperplasia with atypia or endometrial carcinoma (18). Another study of 644 similar hysterectomies from women with a mean age of 59.7 ± 12 years found 12 patients (1.9%) with incidental endometrial complex hyperplasia or carcinoma (19). In both of these studies, the patient age was significantly older than the median age of 36.8–37 years for this study.

As another secondary endpoint, we examined the ultrasound measurement of the endometrial stripe in women before and after treatment. While 1 of the 2 women with baseline CAH had a thickened endometrial stripe (19 mm), the other woman did not (7 mm). Of note, the patient with the 19 mm stripe had had a transvaginal ultrasound 6 weeks earlier (outside the study) in which the stripe was measured at 7 mm. For the other women in the study, there was no significant difference with respect to the change in endometrial thickness. The three patients with poor response in the depoMPA treatment group had changes in endometrial thickness -3.0 , 0 , and 3.6 mm. The 1 patient with a pathologic response in the follow-up biopsy (in the OCP treatment group) had no change in endometrial thickness from before therapy to after therapy. Therefore, measurement of endometrial stripe as a correlate of response to progestin is not useful. In addition, consistent with other published studies, ultrasound measurement of endometrial stripe is not a sensitive screening method for detecting endometrial abnormalities.

As an additional secondary endpoint, we examined a panel of qRT-PCR biomarkers of estrogen action in the endometrium. First, we demonstrated the feasibility of performing a number of transcript assays using endometrial tissue obtained from a pipelle. Second, we found a statistically significant decrease in the expression of *EIG121*, *IGF-1*, *sFRP-1*, *sFRP4* and *survivin* in the post-treatment biopsies. Therefore, these molecular biomarkers can be modulated by short term exogenous progestins (OCP and depoMPA). Third, we found that the women who received depoMPA who had a poor histologic response (only 2 of 3 had tissue available for qRT-PCR) demonstrated elevated post-treatment *sFRP1*, *sFRP4*, and *survivin* compared to baseline biopsy values. The survivin protein inhibits apoptosis and is increased in multiple tumor types, including endometrial cancers (14, 20–24). While both OCP and depoMPA decreased *survivin* in the endometrium, the 2 poor responders demonstrated an increase in *survivin*. The proteins encoded by *RALDH2*, *sFRP1*, and *sFRP4* are all thought to act as “brakes” to inhibit physiological estrogen-induced endometrial proliferation. In this way, normal endometrial growth induced by estrogen is

controlled. The enzyme encoded by *RALDH2* catalyzes the synthesis of retinoic acid, a known inhibitor of uterine growth (7). Both sFRP1 and sFRP4 act as molecular antagonists to ligands in the Wnt signaling pathway (25), binding to Wnt7a to act as “brakes” to decrease Wnt-associated proliferation in the endometrium. In this study, *sFRP1* and *sFRP4* expression were down-regulated by both OCP and depoMPA. Considering progesterone’s known antagonism of estrogen action, we speculate that under conditions of endometrial quiescence induced by long-term progestin exposure, expression of such estrogen-regulated genes is turned off. The abnormally elevated post-treatment levels of *sFRP1*, *sFRP4*, and *survivin* observed in the 2 non-responders may represent biomarkers predictive of an even greater increased risk of endometrial cancer development in these women, but this will require longitudinal studies to verify. From Figure 4, it can be seen that the baseline endometrial expression of these genes is quite variable, but the vast majority of post-treatment biopsies have a narrower range of gene expression. This, plus our observations of different gene expression in the 2 non-responders, introduces the concept of assessing tissue biomarkers following some type of exogenous stimulus/treatment rather than in untreated tissues. In other words, expression of biomarkers in response to a stimulus may be more informative than a baseline measure of a tissue biomarker. We are particularly intrigued whether molecular analysis of gene transcripts after a progestin challenge may be a clinically useful test to identify which women with Lynch syndrome are at particularly high risk for endometrial cancer.

This study also highlights the challenges in performing Phase II gynecologic chemoprevention trials in high risk populations. We screened over 700 women in order to enroll 51 over a 6 year period (data not shown). Primary reasons for exclusion included no identified Lynch syndrome mutation, not wanting to come off OCP prior to enrollment, not willing to take depoMPA, unwilling to undergo 2 endometrial biopsies, smoker and over age 35, planned pregnancy, prior or planned hysterectomy, high cholesterol, or not having flexibility to undergo baseline endometrial biopsy on days 5–10 of menstrual cycle. Based on our experience from this trial, three factors are necessary for the completion of Phase II gynecologic chemoprevention trials – 1) steady commitment of the research staff at all sites for recruitment of participants, 2) multi-disciplinary and multi-institutional commitment and cooperation from geneticists, GI and GYN services, and 3) sponsor (NCI) patience in keeping the study open as long as there is steady accrual.

In conclusion, this Phase II biomarker study found that women with Lynch syndrome have a normal response to short term exogenous progestins, based on histology and proliferation indices, compared to previous reports in the general population (26, 27). This suggests that oral contraceptives and depoMPA may be reasonable chemopreventive agents for this high risk cohort, as they are in women at general population risk (5). In addition, young women with Lynch syndrome who are asymptomatic have a high baseline rate of complex atypical hyperplasia and endometrial cancer. Finally, while requiring coordinated, multi-institutional efforts, we demonstrated the capacity to complete a Phase II chemoprevention study in women with Lynch syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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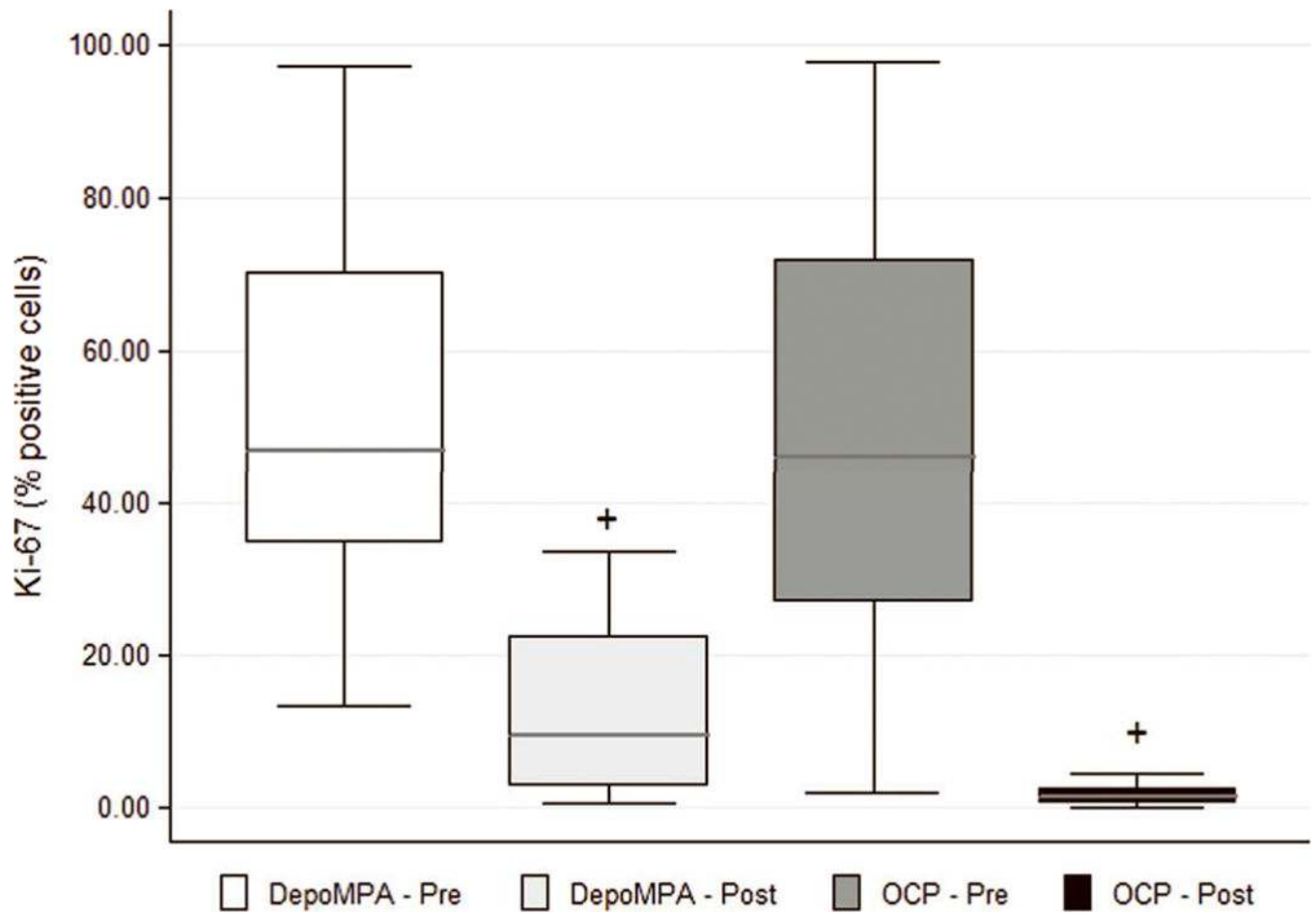


Figure 1. Proliferation index (Ki-67) is significantly reduced following short-term treatment with Depo-Provera (depoMPA) or oral contraceptive (OCP). + $p < 0.05$ for comparison of pre- and post-treatment.

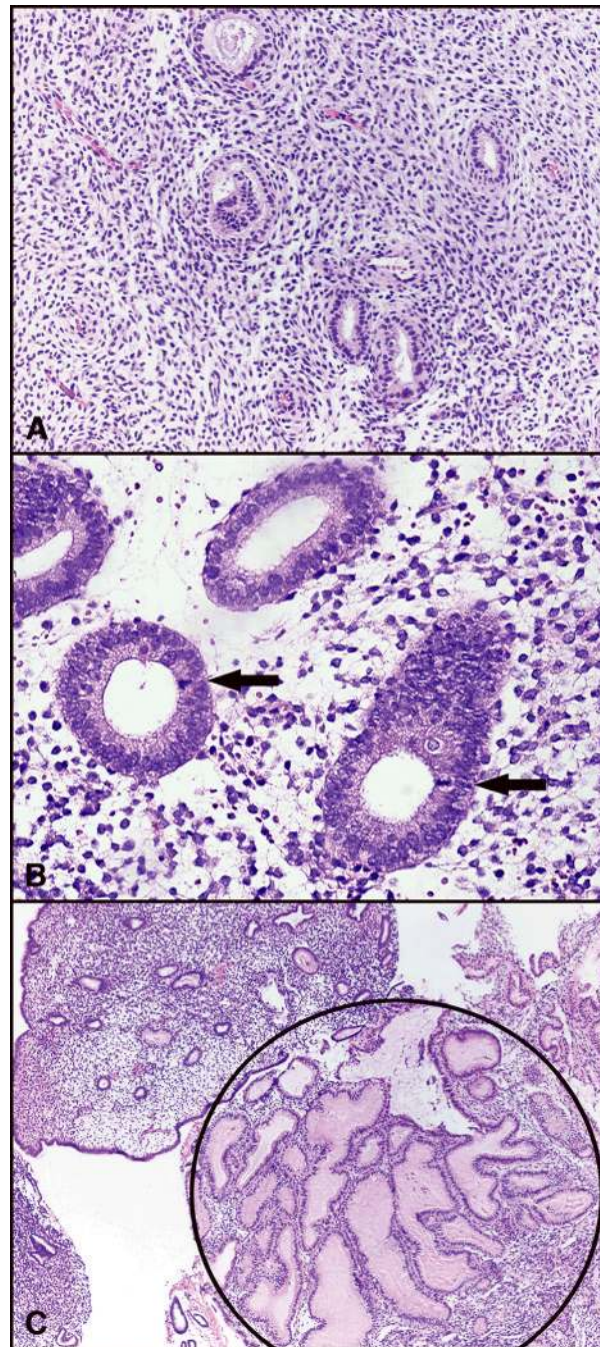


Figure 2. Photomicrographs of post-treatment endometrial biopsies from women enrolled in this chemoprevention trial. A. Endometrial biopsy showing typical good response to progestin treatment. Endometrial glands are small, inactive appearing, and have no mitotic figures. The stromal cells are showing pre-decidualized changes. H&E, 10X. B. Post-treatment endometrial biopsy from a non-responder. Note the presence of mitotic figures (arrows) in the endometrial glands. H&E, 20X. C. Post-treatment endometrial biopsy from a different non-responder. The circled area shows a focus of complex endometrial hyperplasia, in which the endometrial glands are greatly enlarged and hyperplastic compared to adjacent smaller, inactive endometrial glands. H&E, 4X.

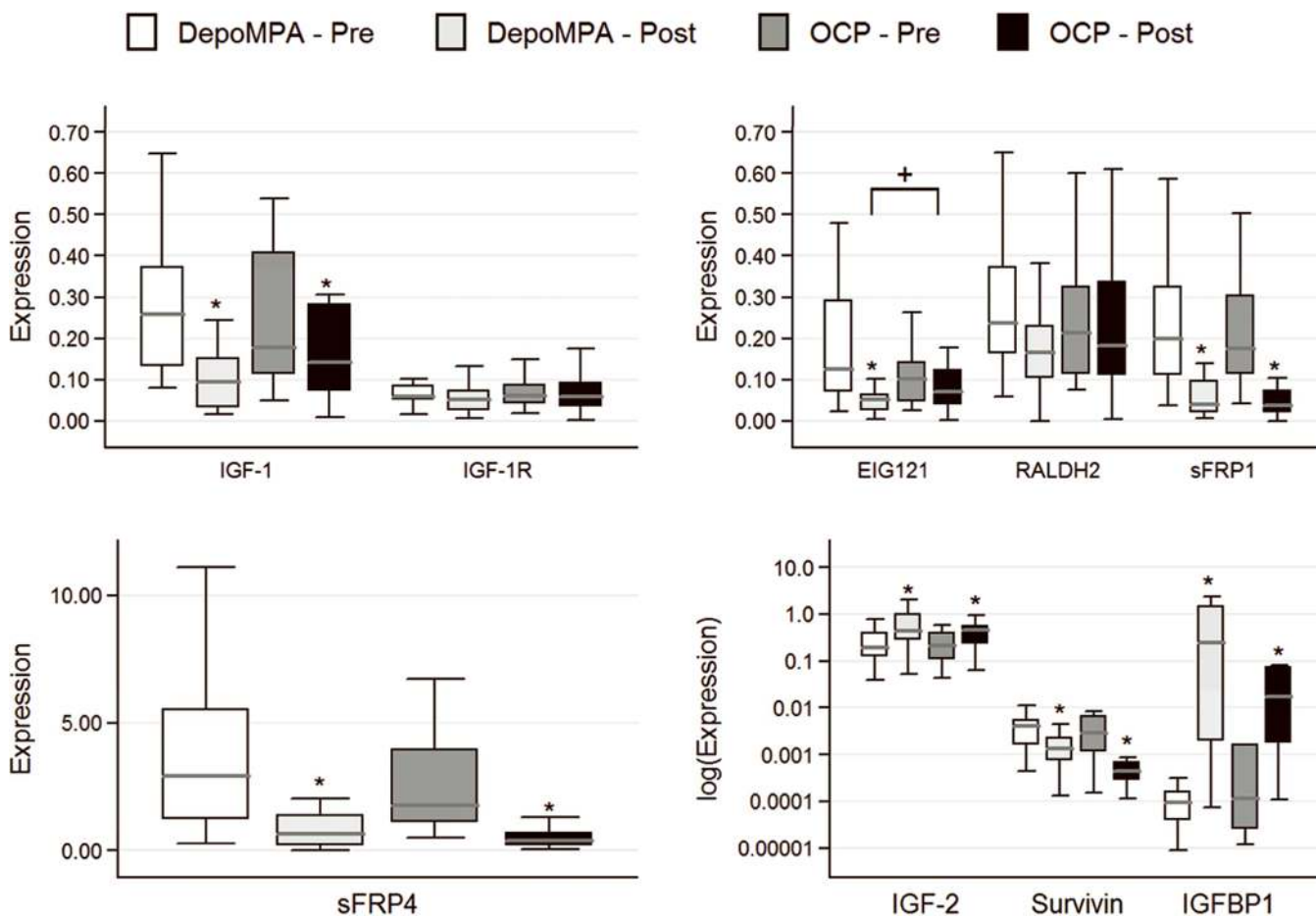


Figure 3. Treatment with Depo-Provera (depoMPA) and oral contraceptives (OCP) alter gene expression of several biomarkers. * $p < 0.05$ for paired pre-and post-treatment groups. + $p < 0.05$ for a difference in gene expression changes between treatment with depoMPA compared to OCP.

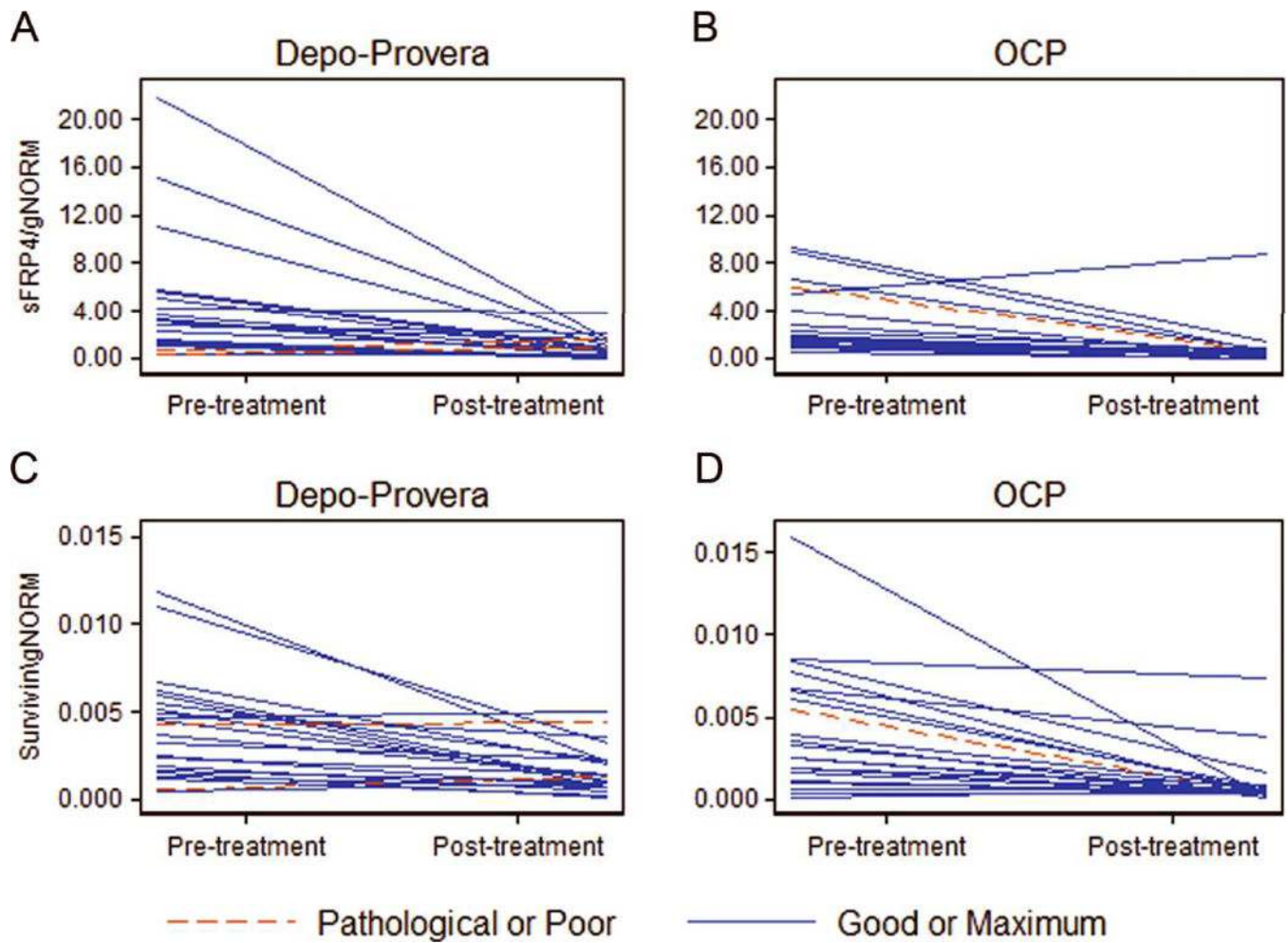


Figure 4.

Anti-proliferative gene expression biomarkers measured in endometrial biopsies pre- and post-treatment. *sFRP4* expression is decreased in the vast majority of patients receiving Depo-Provera (A) and oral contraceptive (B). However, two patients with poor histologic response demonstrate an increase in *sFRP4*. Survivin expression is also decreased in the vast majority of patients following treatment with Depo-Provera (C) or oral contraceptives (D). However, two patients with poor histologic response demonstrate an increase in *survivin*.

Table 1

Demographic and Clinical Characteristics

	depoMPA (N = 25)	OCP (N = 26)	P-value
Age (years)			
Mean	36.8	38.0	0.495
Range	25–48	25–48	
Age at Menarche (years)			
Mean	12.7	12.7	0.841
Range	10–16	11–16	
Race			
White	20	23	0.244
Black	0	1	
Hispanic	4	1	
Asian	1	0	
Native American	0	1	
Mutation			
MLH1	11	13	0.841
MSH2	12	10	
MSH6	1	1	
Amsterdam + Colon Cancer	1	1	
Amsterdam + Benign Ovarian Tumor	0	1	
BMI			
Mean	28.4	26.2	0.267
Range	19.3–48.4	18.2–44.7	
Gravity			
0	4	7	0.331
1	2	0	
2	7	4	
3	6	10	
4	4	2	
5	1	3	
6	1	0	
Parity			
0	5	9	0.209
1	6	1	
2	9	8	
3	4	7	
4	1	1	

Table 2

Histology and Endometrial Thickness

	depoMPA (N = 25)	OCP (N = 26)	P-value
Baseline Biopsy			
Proliferative	23	24	0.999
Not Proliferative	1	1	
Pathologic	1	1	
Follow-Up Biopsy			
Good	20	22	0.233 ^a
Poor	3	0	
Pathologic	0	1	
No Biopsy	2	3	
Baseline Endometrial Thickness (mm)			
Mean	5.5	6.5	0.189
Range	2.6–10.1	2.0–19.0	
Follow-Up Endometrial Thickness (mm)			
Mean	4.5	4.5	0.933 ^b
Range	1.0–9.3	2.0–10.0	
Change in Endometrial Thickness (mm)			
Mean	0.9	1.7	0.225 ^b
Range	-5.0–6.0	-1.0–5.0	

^aExcludes “No Biopsy” group.

^b2 patient on depoMPA and 3 patients on OCP did not have follow-up endometrial thickness.