

The PLUM Study:
Pilot of Letrozole for Uterine Myomas

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1.0 BACKGROUND

Women with symptomatic uterine fibroids have limited treatment options. Uterine leiomyoma, or fibroids, are benign smooth muscle tumors that occur in 25% of premenopausal women and cause heavy bleeding, pelvic discomfort or pressure, urinary and bowel dysfunction, and adverse pregnancy outcomes. Major surgery (hysterectomy and myomectomy) is the most common treatment; uterine artery embolization is reserved for women who have completed childbearing, and magnetic resonance guided focused ultrasound ablation is in limited use under research protocols. Many women seek medical management, but there are no FDA approved drugs that decrease fibroid volume and symptoms without causing harm during long-term use.

Letrozole is an aromatase inhibitor (AI) that decreases the synthesis of estrogen by the aromatase enzyme in the ovary and peripheral tissues, including fibroid cells. Fibroids are hormonally responsive tumors that grow with exposure to systemic and local estrogen levels. Letrozole is FDA approved as adjuvant therapy for postmenopausal women with estrogen receptor positive breast cancer. However, letrozole has been used for treatment of a variety of gynecologic conditions including chronic pelvic pain among women with endometriosis and for ovulation induction among women with infertility. Six small studies of AIs have reported a 35-55% decrease in fibroid volume over 8-12 weeks of oral AI treatment.¹⁻⁶ In premenopausal women, low dose letrozole at 2.5mg/day may preferentially inhibit estrogen synthesis in fibroids more than the ovaries, thus avoiding undesirable side effects of systemic hypoestrogenism such as hot flashes and decreased bone density. Letrozole may therefore improve fibroid symptoms without impacting long-term health risks.

Fibroids change volume rapidly in response to fluctuating estrogen levels. Fibroids shrink up to 60% in 3 months among women treated with gonadotropin-releasing hormone agonists (GnRH) that markedly decrease overall serum estrogen by shutting down ovarian hormone production.⁷ But the hypoestrogenism with GnRH use causes hot flashes and rapid loss of bone density. In addition, fibroids regrow to their original volume within 2-3 months after discontinuing GnRH.^{8,9} Therefore, GnRH use is generally limited to short-term use to reduce fibroid volume prior to surgical intervention and cannot serve as long-term medical management.

Fibroids produce estrogen that autoregulates fibroid growth. In premenopausal women, the ovary is the primary source of estrogen production. However, estrogen is also produced locally in fibroid cells through the conversion of androgen by aromatase cytochrome P450 enzyme activity.¹⁰⁻¹² Estrogen has autocrine and paracrine action which results in fibroids demonstrating local control over their own growth.^{13,14}

Aromatase inhibitors rapidly decrease fibroid volume but this effect plateaus after 3 months. Letrozole is a type II, reversible, competitive aromatase inhibitor that rapidly blocks estrogen production in peripheral tissues.^{15,16} In the two trial of letrozole alone for treatment of fibroids, fibroid volume decreased by 45-46% after 3 months of treatment. However, 90% of that volume reduction occurred within 1 month after treatment. This growth pattern indicates that intermittent letrozole dosing may be a viable option for long-term treatment.

Low dose daily letrozole in premenopausal women has few adverse effects. Long-term safety data on letrozole is derived from large trials and meta-analysis of postmenopausal women with estrogen-receptor-positive breast cancer on letrozole for 5 years.^{17,18} In these studies, letrozole has been associated with slightly higher risks of hypercholesterolemia and fractures compared with women taking tamoxifen.

It is not appropriate to presume that these risks would be associated with letrozole use in premenopausal women. Letrozole use in postmenopausal women significantly reduces overall serum estrogen levels because postmenopausal women primarily derive estrogen from conversion in peripheral tissue via aromatase. However, estrogen in premenopausal women is primarily produced in the ovary with secondary production in peripheral tissue. In one study of 16 women premenopausal women taking letrozole 5.0 mg/day for fibroid treatment, estrogen levels did drop significantly after 1 month, though remained in the normal range for premenopausal women¹⁹⁻²¹ women¹, but in a subsequent study of 33 women taking letrozole at a reduced dose of 2.5mg/day, there was no change in any hormone levels including estradiol, FSH, LH, or testosterone.³ Two studies of the AI anastrozol similarly showed no change in hormone levels among premenopausal women undergoing fibroid treatment.^{2,4}

There is some concern that daily letrozole will induce ovarian cysts because it successfully induces ovulation for women with the polycystic ovarian syndrome.¹⁹ This has led to the use of an oral progestin and/or combined oral contraceptive in addition to letrozole to prevent cysts in the treatment of endometriosis.^{22,23} However, fibroids are hormonally responsive tumors so use of exogenous hormones may counteract the beneficial effect of letrozole. In addition, in the study of 33 women on letrozole 2.5mg monotherapy, there was no significant change in ovarian volume or development of ovarian cysts.³

2.0 STUDY RATIONALE

Although there is preliminary evidence to demonstrate a positive effect of AIs on reducing fibroid volume, current studies are insufficient to support widespread clinical use. There are only 2 trials of letrozole monotherapy for fibroids with a total of 56 women; both are open-label, unblinded studies. A randomized, blinded, placebo-controlled trial is needed for an unbiased assessment of letrozole efficacy. In addition, most women with symptomatic fibroids are 35-50 years old and may require many years of treatment until menopause when fibroids regress spontaneously with falling serum estrogen levels. All current AI studies are short-term with only 2-3 months of drug delivery due to lingering concerns that AIs might induce hypoestrogenism among premenopausal women. There are also no studies that evaluate the rate of fibroid regrowth after stopping AIs when fibroid estrogen levels may rapidly rise. If fibroids regrow slowly over several months after stopping AIs, there may be a role for intermittent AI dosing to maximize benefit and minimize harm. Studies are needed to assess the safety profile of letrozole for >3 months of use and to evaluate changes in fibroid volume after letrozole is discontinued.

Our ultimate goal is to conduct a multi-centered, randomized, trial of letrozole versus placebo among women with symptomatic fibroids. This trial will provide gold standard evidence to determine if letrozole results in symptom improvement but offers the advantages of less morbidity and lower rates of recurrence of fibroid symptoms.

To prepare for a future multi-centered randomized trial of letrozole, we will first conduct a controlled pilot study (the PLUM study). The pilot will allow us to assess the feasibility of recruiting and retaining study participants to this drug intervention trial. We will also complete essential preparatory work for a future randomized trial; we will create and test study forms, a manual of operations, and a study database that can be easily adapted for a multi-site trial. The PLUM pilot study will lay the foundation for a successful future study of letrozole as a treatment for uterine fibroids.

The PLUM study will also provide preliminary data on the efficacy of letrozole in treating uterine fibroids. We will use PLUM outcome data to estimate effect sizes and standard deviations to calculate an appropriate sample size for a future randomized trial.

3.0 STUDY OVERVIEW

This is a randomized, blinded, placebo-controlled trial of oral letrozole among 20 premenopausal women with symptomatic uterine fibroids. Participants will be randomly assigned in a 1:1 ratio to either oral letrozole 2.5mg/day for 6 months (Group A) or intermittent dosing with letrozole 2.5mg/day and an identical placebo capsule (Group B). Group B will receive medication dosing as follows: 2 months of placebo, then 2 months daily letrozole 2.5mg/day, then 2 months of placebo (Table 1). This dosing regimen in the two groups will allow for: 1) between group comparisons in month 1 and 2 to assess the efficacy of letrozole compared with a placebo, 2) within-group comparisons in month 3-6 in group B to evaluate the rate of fibroid regrowth after discontinuing letrozole to evaluate the possibility of intermediate dosing and 3) within-group comparisons in group A in month 1-6 to evaluate the safety of long-term letrozole use. This pilot data will be critical for determining the appropriate long-term dosing regimen in a future, larger, full-scale trial of letrozole.

Month	1	2	3	4	5	6
Group A	L	L	L	L	L	L
Group B	P	P	L	L	P	P

We will assess changes in fibroid symptoms, quality of life, and sexual functioning monthly using standardized, validated questionnaires. In addition, at baseline, month 2, 4 and 6, we will assess changes in the following measures:

- 1) fibroid and uterine volume assessed by pelvic ultrasound
- 2) serum follicle stimulating hormone (FSH) and estradiol levels to determine ovarian function

Study participants will be recruited at UCSF. All study data will be stored securely in a HIPAA compliant, secure database monitored by the UC San Francisco Coordinating Center. A data safety and monitoring board will oversee participant safety and protection.

4.0 STUDY OBJECTIVES

The PLUM study will collect essential preliminary data to guide and inform a future multi-centered, randomized trial of letrozole versus placebo. The 3 primary study goals are:

1. To assess the feasibility of conducting a randomized, placebo-controlled trial of letrozole for premenopausal women with fibroids.
2. To collect pilot data that examines differences in fibroid symptoms, fibroid volume, and hormone levels among premenopausal women who take letrozole compared with a placebo.

These will be between-group analyses in Months 1 and 2 (Group A vs. Group B) that serve as preliminary data on the efficacy of letrozole in a placebo-controlled trial.

3. To collect pilot data that examines changes in fibroid symptoms, fibroid volume, and hormone levels among premenopausal women taking continuous and intermittent letrozole dosing.

These will be within-group analyses as follows: Months 1-6 for Group A, Months 3-6 for Group B. These data will provide preliminary information on the safety of letrozole use beyond 3 months (Group A) and the rate of fibroid regrowth after letrozole is discontinued (Group B).

5.0 STUDY POPULATION

We will recruit women with symptomatic fibroids who meet the following criteria:

INCLUSION CRITERIA

1. ≥ 21 years old
2. Premenopausal (at least one menses in last 3 months)
3. Symptomatic fibroids (fibroids visualized on ultrasound or MRI and heavy uterine bleeding, pelvic pressure or discomfort, urinary or bowel abnormalities, dyspareunia)
4. Fibroids that are ≤ 4 in total number or Fibroids that are ≤ 7 in total number if all fibroids are less than 4cm (40 mm) each.
5. Fibroids that are ≤ 7 cm (70mm) in maximum diameter, on screening imaging, if ≤ 4 fibroids in total number. (fibroid is defined as any mass with radiographic characteristics of fibroid > 2 cm or 20mm)
6. Up to date in Pap smear screening and surveillance
7. Endometrial biopsy (required if age > 45 years with irregular bleeding) does not indicate premalignant or malignant cells
8. Agree to use non-hormonal barrier method of contraception during study period if at risk for pregnancy
9. Has primary care provider or gynecologist
10. Agrees not to start new medications/treatments for fibroids during the study
11. Able to give informed consent

EXCLUSION CRITERIA

1. Fibroids treated by surgery, radiologic procedure, or GnRH agonist or antagonist in the last 3 months
2. Any submucosal fibroid ≥ 2 cm (20mm) that is $> 50\%$ in uterine cavity (FIGO Type 0 or Type 1 fibroids) amenable to hysteroscopic resection
3. Use of exogenous estrogen and/or progestin in the last month. (for 3 month long-acting depoprovera injection, no use in last 3 months)
4. Pregnant, lactating, or planning to become pregnant in the next 6 months
5. Hematocrit $< 27\%$ or visit to emergency room or hospitalization for fibroid symptoms in the last 3 months (cannot be safely randomized to a placebo)
6. History of osteopenia or osteoporosis
7. History of hyperlipidemia
8. Current liver or kidney disease
9. History of breast cancer or current breast cancer
10. Unable or unwilling to attend 4 study visits
11. Pelvic imaging concerning for gynecologic cancer or cancer of the genitourinary or gastrointestinal system
12. Does not have primary care provider or gynecologist

5.1 Participant Recruitment

We will identify potentially eligible participants with symptomatic fibroids who present to the UCSF Women's Health Center and the UCSF Comprehensive Fibroid Center (CFC) where Dr. Jacoby sees patients. The CFC is a high volume specialty clinic that offers the full range of available surgical and nonsurgical treatments. Many women come to the CFC seeking minimally invasive interventions because of our ongoing trials of new and emerging fibroid treatments as described in Preliminary Data. There are 20-25 new fibroid consultations per week among the CFC physicians; many of these patients inquire about the availability of medical management of

their symptoms. The patient population is racially and ethnically diverse with a general enthusiasm for supporting fibroid studies. Therefore, we believe it is feasible to recruit 20 women to this pilot trial over 6 months in this pilot study.

Participants will also be recruited from the greater San Francisco Bay Area community. The investigative team will use a multi-component IRB-approved recruitment approach, including contacting a database of women with fibroids who have given permission to be contacted about future research opportunities, direct community-based media efforts (newspaper notices, radio advertising, brochures in local clinics, talks to local community groups, notices in churches), social media/networking sites). Recruitment efforts will be based at UCSF. To facilitate recruitment.

5.2 Informed Consent

Before entering the study, all study procedures, time requirements, risks and potential benefits will be explained to each potential study participant using the information in the UCSF IRB-approved informed consent form. The potential study participant will be given adequate time to read the informed consent document and ask questions. Eligible participants who choose to enter the study will sign the informed consent form and Health Insurance Portability and Accountability Act (HIPAA) form prior to beginning the study intervention. Each participant will be given a copy of the signed documents and the original will be a part of the research record. All study-specific data will be kept confidential and stored in locked files at the clinical center.

6.0 MEASURED VARIABLES

6.1. Outcome Variables

Outcomes will be measured at baseline prior to randomization and at multiple time points for 6 months. Questionnaires will be administered to evaluate patient-reported fibroid symptoms, study medication compliance, adverse events, and the rate of recurrence for fibroid symptoms. In the following section, outcome measurements are described in detail.

6.1.A Questionnaires to Assess Symptoms

These are validated questionnaires to assess the change in symptoms over time.

1. Uterine Fibroid Symptom-Quality of Life (UFS-QOL): The Uterine Fibroid Symptom and Quality of Life Questionnaire (UFS-QOL) will be the primary outcome to assess fibroid symptoms. The UFS-QOL is a validated fibroid-specific questionnaire that assesses fibroid symptoms and their impact on quality-of-life.^{20,21}
2. EuroQoL 5-D: The EuroQoL 5D is a widely used, validated questionnaire to assess overall quality of quality of life across a broad spectrum of diseases.
3. Menstrual Impact Questionnaire (MIQ): The MIQ is a 12-question, validated questionnaire to determine the impact of heavy menstrual bleeding on a woman's quality of life.²⁴ The MIQ has been used in trials of heavy bleeding that includes women with uterine fibroids.

6.1.B Pelvic Ultrasound

Pelvic ultrasound including trans-abdominal and trans-vaginal images will be used to assess changes in uterine volume as well as fibroid number, size, and volume from baseline to the end of month 2, 4 and 6. All pelvic ultrasound will be performed by either Dr. Heather Huddleston or Dr. Evelyn Mok-Lin, faculty members in the Division of Reproductive Endocrinology and Infertility (REI) in the Department of Obstetrics, Gynecology, and Reproductive Sciences. Dr. Huddleston and Mok-Lin have specialty training in performing pelvic ultrasound to assess uterine fibroids as well as ovarian cysts. They perform up to 100 pelvic ultrasounds every week in their clinical practice and their expertise in this area will be invaluable. All pelvic ultrasound will be performed in a private room within the Ob/Gyn Division of Reproductive Medicine clinical space located adjacent to Mission Bay Hospital at 499 Illinois St or Mt Zion Women's Health.

6.1.C Serum hormone levels

Estradiol and follicle-stimulating hormone (FSH) levels will be measured at baseline and the end of month 2, 4 and 6. These hormones will detect the development of hypoestrogenism and assess changes in the hypothalamic-pituitary-ovarian axis that regulate normal menstrual function. All blood draws will be performed by certified phlebotomists in the REI practice.

6.1.D Total Cholesterol

Since hyperlipidemia is a rare side effect of letrozole, total cholesterol will be measured at baseline and month 6. All blood draws will be performed by certified phlebotomists in the REI practice. Approximately 5 ml (1 tsp) will be drawn at Baseline and Month 6 for this test.

6.1.E Other Outcomes

Other outcomes related to study intervention and fibroid symptoms will include measures related to:

Treatment Failure: We will ask participants to report any additional fibroid treatments including medical management for fibroid-related symptoms, myomectomy, hysterectomy, uterine artery embolization, MR Guided Focused Ultrasound, endometrial ablation, dilation and curettage, or any new and emerging fibroid treatments.

Adverse Events

This is the first trial of letrozole monotherapy for >12 weeks among premenopausal women. Therefore, a full range of adverse events will be assessed every 2 months for all body systems including known potential side effects such as hot flashes, myalgias, and fatigue as well as unknown side effects that will be identified by using the open ended question "Have you had any changes to your health that impact your ability to perform your normal activities?". In addition to assessing adverse events monthly during the trial, we will query participants about adverse events 30 days after completing the last dose of medication.

Pregnancy and pregnancy outcomes: In this study pregnancy is considered a Serious Adverse Event. Participants will report all known pregnancies while receiving study medication. For each pregnancy, we will query participants about whether the pregnancy resulted in a miscarriage, abortion, or continued pregnancy. For continued pregnancies, we will ask participants to report obstetric outcomes including gestational age at birth, mode of delivery (vaginal or cesarean), and complications (e.g. placenta previa, placental abruption, preeclampsia).

6.1.F Long-term storage of blood

During blood draws at baseline and month 2, 4, and 6, we will ask participants if they are willing to allow long-term storage of their blood (serum) for future fibroid studies. Participants may decline this long term storage and still participate in the study. Specimens will be stored long-term in the Specimen Bank within the UCSF Reproductive Medicine Lab.

6.1.G Schedule of Outcomes Assessment

Outcomes will be assessed at varying timepoints from Baseline to 6 months (Table 2). In-person study visits will occur prior to randomization at the Baseline Visit (BV), Month 2, Month 4, and Month 6. All other visit data will be collected through mailed or on-line questionnaires, and/or phone interviews.

	BV	Mo 1	2	3	4	5	6	7
Symptom questionnaires	X	X	X	X	X	X	X	
EQ5D questionnaire	X		X		X		X	
Pelvic ultrasound	X		X		X		X	
Hormone levels	X		X		X		X	
Total Cholesterol	X						X	
Adverse event assessment		X	X	X	X	X	X	X
Specimen Storage*	X		X		X		X	
Compensation	\$25		\$25		\$25		\$25	

*Optional

6.2 ADDITIONAL MEASUREMENTS

We will collect additional subjective and objective measurements that will be used to: 1) describe the study population and 2) serve as confounding factors in multivariable analysis of the change in fibroid-related symptoms over time.

6.2.A Pre-Enrollment Assessment, Study Participants

At baseline, we will query women about their general and reproductive health and obtain baseline data on fibroid size, number, and location as outlined in Table 3.

Participant-Reported Baseline Measures	<ul style="list-style-type: none"> • Demographic characteristics (age, race/ethnicity, education) • Medical and surgical history • Reproductive history (including pregnancy and pregnancy outcomes) • Medication use • Complementary and alternative therapy use • Desire for future fertility
Objective Baseline Measures	<ul style="list-style-type: none"> • Weight • Height • Vital signs • Uterine size by trans-vaginal ultrasound

	<ul style="list-style-type: none"> • Fibroid characteristics on ultrasound <ul style="list-style-type: none"> *Total number *Location of each fibroid * dimensions in mm of each fibroid * dimensions in mm of each ovary *Presence of ovarian cysts and sizes
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7.0 ADVERSE EVENTS

This is the first trial of letrozole monotherapy for >12 weeks among premenopausal women. Therefore, a full range of adverse events will be assessed by using the open ended question “Have you had any changes to your health that impact your ability to perform your normal activities?”. In addition to assessing adverse events monthly during the trial, we will query participants about adverse events 30 days after completing the last dose of medication.

7.1 Adverse Event Grading Scale

Adverse events will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) and Common Toxicity Criteria (CTC), version 4. Using this system, adverse events are classified as follows:

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to Adverse Event

7.2 Reporting of Adverse Events

Grade 4 or 5 adverse events will be reported to the respective institution’s Institutional Review Board (IRB) and the UCSF Coordinating Center within 24 hours. The UCSF Coordinating Center will notify the study sponsor, the UCSF IRB, and the Data and Safety Monitoring Board (DSMB) Chair within 24 hours of the adverse event. The DSMB will receive a report of all adverse events every 4 months, categorized with the appropriate severity grading. The DSMB report will include details of the adverse event as reported in the Adverse Event Case Report Form.

8.0 DATA AND SAFETY AND MONITORING PLAN

The Data and Safety and Monitoring Board will consist of a single Data Safety Monitor (DSM), an obstetrician/gynecologist with expertise in clinical studies of gynecologic interventions and the conduct of clinical trials.

The DSM will be charged with overseeing participant safety and monitoring the scientific validity of the trial. Prior to initiating the study, the DSM will review and approve the study protocol.

Assessment and Reporting of Adverse Events: The DSM will receive updates on adverse events every 3 months during the study. These will be presented to her by study group in a blinded fashion. All serious adverse events will be reported to the DSM within 24 hours of notification to the investigator.

Assessment and Reporting of Study Conduct: Every 3 months, we will report to Dr. Goldman updated data on the number of potential participants screened, excluded, and enrolled in the study. We will also report adherence to study visits and medication.

Interim Analysis and Stopping rules: This is a small pilot study of 20 women with a short duration of 6 months. Therefore, the sample size and length of study is not appropriate for an interim analysis. In addition, we do not believe a stopping rule is indicated in this small pilot.

Full details of the DSMB are described in the PLUM DSMB Charter.

9.0 Participant Remuneration

Study participants will be provided with small values of debit or gift cards for their time and commitment to the study as follows: \$25 at the Baseline, Month 2, Month 4, and Month 6 visits. Participants will also be paid for the cost of parking and transportation for study related activities.

10.0 STATISTICAL CONSIDERATIONS

Data analysis plan: We will use descriptive statistics to characterize the feasibility and process measures listed in Table 5, with 95% confidence intervals. Changes in UFS-QOL scores, the primary outcome, as well as secondary outcomes including hormone levels and fibroid volume, will be compared using linear mixed models for repeated measures, adjusting for baseline. We will estimate 1) between-group differences in change from baseline to months 1 and 2; 2) changes from baseline to months 1-3 and 4-6 in group A; and 3) changes from month 3 to months 5 and 6 within group B.

Sample size calculations: This is a pilot study with the aim of assessing the feasibility of implementing a definitive letrozole trial. Therefore, we chose a sample size that would be large enough to assess feasibility, but small enough to complete the pilot within a the1 year timeframe and with a limited budget. Accordingly, the study will not have power to detect small treatment effects. In the PROMISe pilot study discussed in Preliminary Studies, the standard deviation of the UFS-QOL SSS was 20, and correlation of baseline with follow-up scores declined from 0.5 at 1 month to nearly zero at 3 months. In the analysis for AIM 2 of between-group differences in the change in UFS-QOL from baseline to 2 months , the sample size of 20 will provide 80% power to detect a between-group difference of 13.4 points in average SSS scores at months 1 and 2, after accounting for short term attrition of 10%. In the analysis for AIM 3, assessing longer term changes in SSS scores within group A, the sample of 15 women have 80% power to detect average changes from baseline to months 1-3 and 4-6 of 16.0 points. In additional analysis for AIM 3, assessing changes from month 3 to months 5-6 in group B, the corresponding minimum detectable effect will be 18 points. Effects of this magnitude are

clinically relevant because 15-20 points is the mean difference in SSS between women with symptomatic fibroids and women without fibroids.

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