A study on curative effects of rapamycin (sirolimus) on symptomatic uterine fibroids and myomatosis and its safety

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I. Background and objectives of research: foreign and domestic research status, preliminary research foundation and design basis of research etc.

Uterine fibroids are benign tumors from smooth muscle cells of the uterus. The pathophysiological principle of fibroids is not clear yet. Genetic predisposition, exposure to steroid hormones, and growth factors can promote formation and growth of fibroids. Uterine fibroids are common among women aged between 35 and 50. Nearly half of reproductive-age women suffer from uterine fibroids. The morbidity of uterine fibroids has been stubbornly high for years and increases year by year. The most common symptoms are menorrhagia and pressure symptoms, such as hypogastralgia, frequent urination and difficult defecation. Hysterectomy is the one and only radical treatment that can completely avoid recurrence of uterine fibroids. However, more and more women expect to avoid hypogastralgia, for they would like to choose an operation that can reserve their uterus in spite of their will of pregnancy. Though there are many surgical options for uterine fibroids, each option has certain risks and a possibility of reoperation caused by recurrence (which increases over time with symptoms). In addition, some rare types of uterine fibroids, including intravenous leiomyomatosis, leiomyomatosis peritonealis disseminate and benign metastasising leiomyomas, are intractable, for the scope of relevant surgery is large and sometimes a single operation cannot affect a radical cure. Hence, it is urgent to explore a treatment that can preserve fertility.

It's no accident that many women are not willing to undergo surgery because of surgical risks, a possibility of recurrence and postoperative residual fibroids. Therefore, there's a large demand of effective drugs for long-term treatment of uterine fibroids that can replace surgery in medical treatment. Traditionally, it is believed that uterine fibroids are ovarian-hormone-dependent benign tumors, and main drugs for uterine fibroids include GnRH-a, progestin receptor antagonist and combined oral contraceptive etc. These drugs are mainly used to shrink fibroids, inhibit growth of fibroids and prevent deterioration of the disease. However, though there are many medicines for uterine fibroids, their curative effects are of great difference. After drug withdrawal, the disease is easy to recur and many of these medicines can cause numerous adverse effects, leading to short-term administration only. Thus, it is urgent to find a new well-tolerated drug with a lasting effect for uterine fibroids.

mTOR is a type of conservative serine-threonine kinases regulating the process of protein synthesis through multiple target molecules in terms of transcription and translation, promoting and coordinating cell growth and proliferation. Research showed that 24-hour treatment of primary smooth muscle cells with rapamycin can cause irreversible growth arrest. An in vivo study showed that to inhibit mTOR of female Eker rats with WAY-129327, analogue of rapamycin, could significantly reduce incidence, multiplicity and sizes of tumors. Fritsch et al. designed a nude mouse model of patient-derived tumor xenografts (PDX) (which retains histologic and functional characteristics) and first proved that rapamycin could inhibit growth of PDX effectively with an in-vivo model. As the mTOR pathway is a type of tumor target molecules of high specificity and specificity in tissue of uterine fibroids, it can be used as a target molecule of intervention treatment, providing a new idea for non-surgical treatment of uterine fibroids. Hence, the application of mTOR inhibitors can offer a new therapy for women with related symptoms of uterine fibroids.

Evidence has shown that LAM does not originate from lungs but migrates to lungs through lymph vessels or blood. Lot of research showed that LAM might be variants outside the uterus rooting in

uterine smooth muscle cells. Food and Drug Administration (FDA) approved the application of rapamycin to treatment of moderate-severe LAM. Results of its clinical application showed that mTOR inhibitors brought landmark change to LAM patients. Before mTOR inhibitors were applied for treatment of LAM, lung transplantation was almost the only therapy for LAM. At present, application of mTOR inhibitors is the main clinical therapy for LAM with significant effects and high safety. Hence, the prospect of application of mTOR inhibitors to treatment of uterine fibroids is promising, especially for patients with symptomatic uterine fibroids or myomatosis who refuse or are not suitable for operative treatment. Thus, treatment with mTOR inhibitors may become the best long-term therapeutic schedule that can replace operative treatment for patients with uterine fibroids.

mTOR inhibitor rapamycin (sirolimus), is a new type of macrolide immunosuppressors. Initially, sirolimus was used as low-toxicity antifungal antibiotics. In 1978, it was found that it could realize immunosuppression of autoimmune diseases. In 1989, it was on trial as a new type of immunosuppressors against rejection of organ transplantation. In September, 1999, FDA formally approved its appearance on the market. So for, more than twenty countries have approved or registered it for prevention of acute rejection of patients receiving organ transplantation. Currently, there are 2082 clinical trials relating to rapamycin and 924 of them have been finished. In China, as a new drug of the second category, sirolimus was permitted to be used clinically in 2002. With 15-year clinical application, its safety data also show its favorable safety features without related security problems.

However, there's no application of mTOR inhibitor sirolimus to treatment of uterine fibroids yet and its curative effects and safety still need to be verified through rigorous clinical trials. Therefore, this study used sirolimus to treat patients with symptomatic uterine fibroids or myomatosis who refused or were not suitable for operative treatment, discussed its therapeutic value for uterine fibroids or myomatosis and provided a clinical basis for formulation of the best therapeutic schedule of non-surgical treatment for uterine fibroids or myomatosis.

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II. Research objectives

1. Evaluation of curative effects of sirolimus on symptomatic and recurrent uterine fibroids and/or various rare types of myomatosis
2. 1.Evaluation of safety of sirolimus in treatment of symptomatic and recurrent uterine fibroids and/or various rare types of myomatosis

III. Research content

1. Symptom control rates after medication for 3 and 6 months
2. Recurrence rates of symptoms after 12 months of drug withdrawal
3. Evaluation of safety and side effects of the drug

IV. Research design

1. This study is a non-random open-label clinic trial carried out by the department of gynaecology and obstetrics, Peking Union Medical College Hospital.
2. All patients who accorded with inclusion criteria and signed informed consents received sirolimus treatment for at least three months. After drug withdrawal, there patients were followed up for at least one year.
3. There should be at least 25 patients included in this study without an upper limit of included patients.

V. Selection of subjects

A. Inclusion criteria
1. Patients who suffer from menorrhagia and pressure symptoms and whose pelvic ultrasound or pelvic MRI indicates uterine fibroids; or who has underwent myomectomy but suffer from menorrhagia and pressure symptoms again with pelvic ultrasound or pelvic MRI indicating uterine fibroids; or whose other imageological examinations or established surgical diagnosis indicate various rare types of myomatosis and who expect a drug therapy.
2. Symptoms of uterine fibroids can be confirmed if one or multiple symptoms below exist:
1) . MP shows an excessive amount of bleeding during menstruation (＞80.0 mL)
2) . A subject report shows three excessive amounts of bleeding during menstruation in latest six months

3) .A subject report indicates pelvic pressure symptoms/pain that are likely related to uterine fibroids.

3. According to results of medical history, physical examinations, gynecological examinations and laboratory examinations, patients are in a good overall condition (except uterine fibroids).

4. Cervical smears show a normal result or have no clinical significance, where further follow-up is unnecessary. If there's a normal result in the latest six month in the medical record of a subject, the subject can pass the inspection of cervical smears. A HPV test can be applied to subjects with equivocal ASCUS as an auxiliary test. ASCUS subjects who get a negative result of a HPV test can be included into this study.

5. Endometrial biopsy should be conducted to eliminate non-endometrial lesions if necessary.

6. Subjects didn't receive any drug therapy for uterine fibroids three months before the clinical test.

7. Female adults have menstruation (>18 years old) and are not during pregnancy and lactation.

8. Subjects have good organ function and results of their biochemical examinations meet the following conditions: AST≤2.5×the upper limit of normal (ULN), ALT≤2.5×the upper limit of normal (ULN), Serum total bilirubin≤1.5×the upper limit of normal (ULN), Creatinine≤1.5×the upper limit of normal (ULN)

9. Patients have signed the informed consent.

B. Exclusion criteria

1. Patients are in a period of pregnancy and lactation (patients delivered, miscarried or breast-feed in three months before the treatment)

2. Patients are allergic to any ingredient of the medicine

3. Patients suffer from a disease requiring immediate blood transfusion

4. Patients suffer from a disease that may impact implementation of the study or explanation of results. This type of diseases includes:

1) Known severe blood coagulation disorders

2) Known anemia that is not caused by HMB

3) Known hemoglobinopathy

4) Patients suffered or suffer from cancer of the uterus, cervical carcinoma, ovarian cancer or breast cancer

5) An ultrasonic examination shows one or multiple ovarian cysts with a diameter ＞30 mm

6) Ovarian tumors or pelvic mass of unknown origin

7) Known or suspected endometrial polyp ＞15 mm

5. Alcohol or drug (such as aperient) abuse

6. Undiagnosed abnormal bleeding of the reproductive system.

7. Patients also participate in another clinical medicine study

8. Patients took part in another clinical trial that may influence this study before this study

C. Exit criteria

1. A subject suffers from an anaphylactic reaction

2. A patient asks to quit the study because of one's own will or the request of her legal representative. A subject can refuse to follow a further step of this study at any time during the study without a reason. Consequently, the subject won't be affected negatively.

3. A subject is asked to quit this study in some condition (such as obvious poor compliance and security problems)

4. Other conditions leading to termination of this study. For example, researchers think the

continuation of this study may impair health of subjects.

D. Rejection criteria

1. A subject violates the requirement of the test scheme

2. Data recorded by a patient are of poor quality and incomplete and inaccurate.

VI. Therapeutic schedule

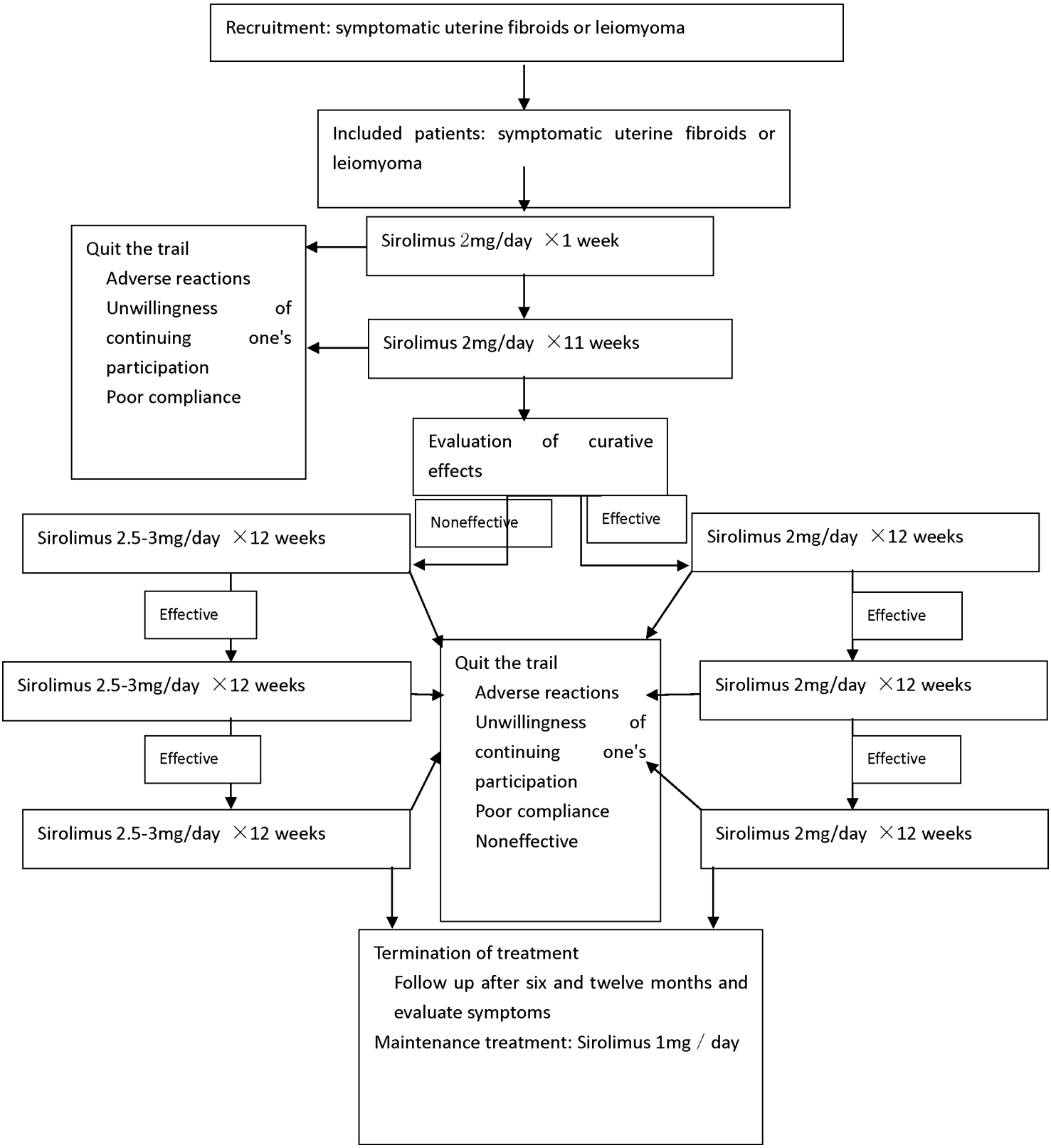
1. Initial dosage of administration and dosage regimen: take 2mg of sirolimus once a day since the fifth to seventh day of menstruation. After one week of medicine taking, subjects are called for an interview. If a subject has severe adverse reactions and fails to tolerate the drug, her weekly dosage can be reduced to 0.5mg, where the minimum daily dosage should be 1mg / day. If the subject still fails to tolerate the drug, she should quit; if she feel unwell but tolerable, the administration should be continued for another three months. After the three months, evaluate the condition of this subject.

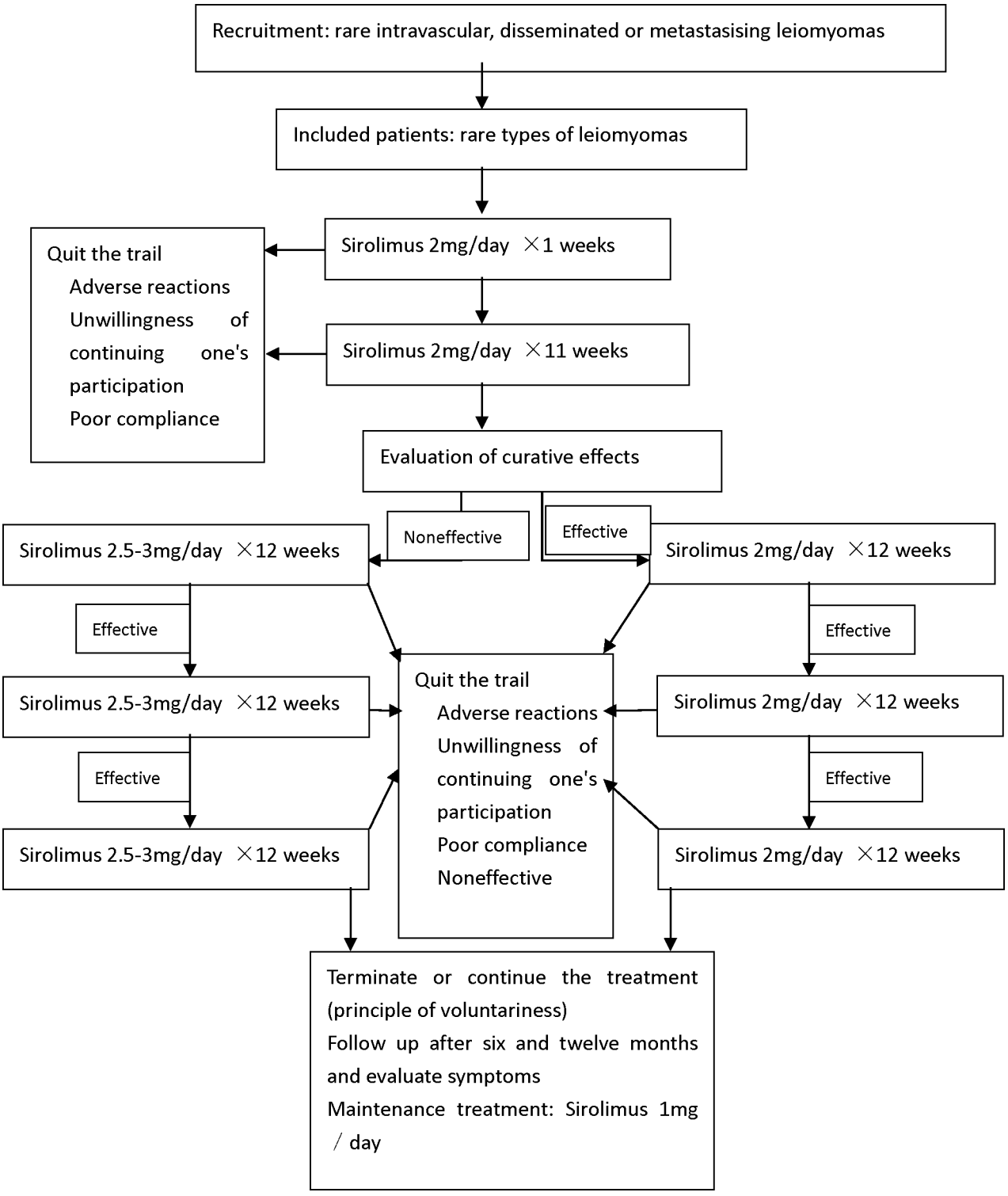
2. Subsequent dosage of administration and dosage regimen: if the condition of a subject is significantly improved after taking 2mg of sirolimus once a day for three months, the initial administration should be continued. If the condition of a subject shows no significant improvement, the dosage should be changed into 2.5mg-3mg once a day. If this subject fails to tolerate the dosage, she should quit this study; if she can tolerate the dosage, this dosage regimen should be executed for three months. After that, her condition should be evaluated again.

3. Iron supplement: if hemoglobin of a subject $\leq$10.9 g/dL, she should receive iron supplement in accordance with the standard dosage regimen. However, the iron supplement is not regarded as a part of treatment of this study and will be recorded as a part of drug combination.

4. If the condition of a patient with a rare type of uterine fibroids (including intravenous leiomyomatosis, leiomyomatosis peritonealis disseminate and benign metastasising leiomyomas) is under control with a tolerable drug, the patient can continue this drug therapy after this six-month study.

VII. Flow charts of research




Recruitment: rare intravascular, disseminated or metastasising leiomyomas
Included patients: rare types of leiomyomas
Sirolimus 2mg/day ×1 weeks
Quit the trail
Adverse reactions
Unwillingness of continuing one's participation
Poor compliance
Sirolimus 2mg/day ×11 weeks
Evaluation of curative effects
Noneffective
Effective
Sirolimus 2.5-3mg/day ×12 weeks
Sirolimus 2mg/day ×12 weeks
Effective
Effective
Sirolimus 2.5-3mg/day ×12 weeks
Quit the trail
Adverse reactions
Unwillingness of continuing one's participation
Poor compliance
Noneffective
Sirolimus 2mg/day ×12 weeks
Effective
Effective
Sirolimus 2.5-3mg/day ×12 weeks
Sirolimus 2mg/day ×12 weeks
Terminate or continue the treatment (principle of voluntariness)
Follow up after six and twelve months and evaluate symptoms
Maintenance treatment: Sirolimus 1mg / day

VIII. Follow-up plan and content

1. Evaluation before the trail

Each subject should complete the following items in four weeks before the treatment:

1) Complete medical history

2) Detailed personal profile

3) A physical examination

4) A blood routine examination

5) Examinations of hepatic and renal function and blood fat

6) A detection of female sex hormones between the second and fourth days of menstruation

7) A routine urine test + an urine pregnancy test]

8) A pelvic MRI inspection

2. Evaluation during sirolimus treatment

In the first month of treatment, evaluation of adverse reactions is conducted every week. In the third and sixth months, curative effects and safety of sirolimus were assessed.

1) Evaluation of curative effects: sizes of the uterus and fibroids were measured through pelvic MRI. Blood was drawn to monitor the female hormone level and impacts of bleeding and symptoms on daily life of subjects.

2) Evaluation of safety: safety parameters (such as to enquire AE and measure laboratory values, vital signs, thickness of endometrium, abnormal uterine bleeding and sizes of follicular structure including follicle and functional ovarian cysts) are monitored periodically during the study.

3) Treatment compliance: in order to monitor compliance, subjects are asked to keep an E-diary every day. Researchers track each date of studied drug taking in accordance with E-diaries. In each interview, researchers and subjects examine the data integrity of E-diaries together.

3. Evaluation after treatment

Evaluation of curative effects and safety is conducted at the end of the drug therapy, six and twelve months after the treatment, where the evaluation content is the same as that before the trail.

IX. Statistical analysis

1. Methods of statistical analysis

Classified variables are expressed by frequency distribution (case number and percentage). Continuous variables are described through mean, median, minimum, maximum, first and third quartile. The confidence interval of all parameters is the significance level = 0.05. All analyses are conducted with SPSS.

2. Contents of methods of statistical analysis

1) General data analysis: demographic variables and baseline characteristics are summarized with mean of descriptive statistic and/or appropriate frequency tables.

2) Evaluation of curative effects: according to follow-up and reexamination results, quality of life, fibroid control and improvement of symptoms are analyzed.

3) Analysis of adverse reactions: assessment of side effects and adverse events during medication.

4) Judgement of relations between adverse events and the tested drug

X. Measures of quality control

1) Supervision by the project leader
2) Establish a standard evaluation method, unify criteria of diagnosis and effect assessment etc.
3) Formulate plans for monitoring and treatment of adverse reactions
4) All researchers receive training before this study
5) Assign quality controllers and develop a plan for quality control with regular inspections
6) Devise a follow-up plan and system

XI. Expected research results

After this study, its research results will be published in a paper and subjects will be informed of these results.

XII. Potential risks, benefits or advantages of this study

As a drug coming into the market for years, it is applied to this study beyond instruction, where physicians know that its benefits outweigh its risks and have no other choice. For subjects in this study, their symptoms can be relieved, their quality of life can be improved, their uterus can be retained and surgery-related complications and psychological harm caused by loss of fertility can be avoided. During this study, all tested drugs are free and subjects should bear risks of the treatment.

XIII. Expected effects and preservation and confidentiality of data etc

According to the informed consent: personal information of subjects is strictly confidential in this study. Results of this study may be published in a paper, but personal information of subjects, including their names, will not appear in the paper. All data related to this study will be kept by Peking Union Medical College Hospital and strict confidentiality measures will be established.

XIV. Measures to protect subjects and minimize risks

In the whole course of this study, safety supervision of subjects will be carried out by experts and professors from the department of gynaecology and obstetrics of this hospital to strictly control diagnostic criteria and treatment indicators of symptomatic uterine fibroids and myomatosis, leading to collected clinical data according with the international diagnostic standard and favorable guarantee of various patients in treatment.