

**Efficacy of Rapamycin (Sirolimus) in
the Treatment of Peutz-Jeghers
Syndrome**

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I. Background

Peutz-Jeghers syndrome (PJS) is an autosomal dominant hereditary syndrome, characterized by multiple hamartomatous polyps in gastrointestinal tract, pigmentation in skin mucosa and increased risk of cancer^[1]. PJS is a rare disease, with 1 patient in every 8000 to 200,000 births^[2]. Large intestinal polyps of PJS patients may lead to local necrosis, hemorrhage, intussusception and intestinal obstruction^[3, 4]. The risk of lifelong occurrence of cancer among PJS patients is as high as 64%, with colorectal cancer being the most common one. Average age of cancer diagnosis in PJS patients is 41^[5]. At present, there is only invasive treatment for PJS, such as endoscopic polypectomy and surgery, without any drug treatment for choice. Endoscopic polypectomy applies to polyps over 0.5 cm in diameter. However, intestinal segmental resection is required when the polyps are too numerous, or progress into cancer, in which case endoscopic polyps control is infeasible^[6]. Invasive treatment exacerbates patients' suffering and increases risk of complications with high medical cost. It may also affect food digestion and absorption, even causing intestinal adhesions and short-bowel syndrome. Therefore, pharmaceutical intervention of PJS' underline pathogenesis is warranted,

The disease is most frequently caused by mutations in LKB1 which is detectable in 50-80% of the PJS family^[7, 8]. LKB1 is a tumor suppressor gene. It encodes a serine/threonine kinase which activates the tumor suppressor genes TSC1 and TSC2 via stimulating adenosine 5'-monophosphate-activated protein kinase (AMPK). Activated TSC1-TSC2 complex inhibits the mammalian target of rapamycin (mTOR)^[9]. Lkb1 deficiency causes PJS through activation of mTOR signaling^[10]. Therefore, mTOR inhibitor sirolimus (rapamycin) is a potential therapy for PJS.

Sirolimus is a macrolide antibiotics. It has been used to treat tuberous sclerosis complex, a benign tumor syndrome. The safety of rapamycin is widely recognized. The possible side effects are usually mild and may cause oral ulcers, hyperlipidemia, rash, acne and some other symptoms. While the efficacy of rapamycin on PJS has been demonstrated in mouse model, no clinical trial has been reported.

Cancer susceptibility is a major life-threatening factor of PJS patients. PJS-associated carcinogenesis is related to LKB1-AMPK-mTOR pathway, thus endowing rapamycin with great clinical significance in the treatment or prevention of PJS-related cancer. In 2011, Klümper et al reported that everolimus, a derivative of sirolimus, was partially effective in the treatment of a PJS patient suffering from advanced pancreatic cancer. No intestinal polyps larger than 1 cm could be found after 8 months' treatment. There have been no evident adverse reactions except for muscle and joint pain of grade 1^[11].

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II. Objectives

1. Evaluation of efficacy of sirolimus on Peutz-Jeghers Syndrome.
2. Evaluation of safety of sirolimus in treatment of Peutz-Jeghers Syndrome.

III. Research design

1. This study is a prospective, nonrandomized, open-label, single-arm clinical trial. The sirolimus capsule (Rapamycin, Yixinke®), 0.5 mg/grain*20/box, is produced by North China Pharmaceutical Co., Ltd.
2. All patients who meet inclusion criteria and sign informed consents receive sirolimus treatment for at least 6 months. Patients are followed up remotely in a regular schedule.
3. No less than 10 patients should be enrolled.

IV. Selection of subjects

A. Inclusion criteria

- (1) Patients are diagnosed with PJS.
- (2) Patients have gastrointestinal polyps related syndromes, including abdominal pain, abdominal distension, gastrointestinal bleeding, etc, with imageological

examination suggesting intestinal obstruction or intussusception; or whose symptoms recur after previous digestive system endoscopic treatment and surgery; or who are inappropriate or unwilling to accept the above treatment again and wish to receive pharmacotherapy.

- (3) Conventional treatment doesn't work well in patients with PJS-related malignant tumors.
- (4) Physical condition (ECGO): 0~3
- (5) Organ function is good and biochemical indices meet the following conditions:
 - a) $AST \leq 2.5 \times$ upper limit of normal value (ULN),
 - b) $ALT \leq 2.5 \times$ upper limit of normal value (ULN),
 - c) Serum total bilirubin (TSB) $\leq 1.5 \times$ upper limit of normal value (ULN),
 - d) Creatinine $\leq 1.5 \times$ upper limit of normal value (ULN).
- (6) No other medications have been received for intestinal polyps within 3 months prior to the clinical trial.
- (7) Patients volunteer to participate in the trial. Participant or his/her legal guardian signs the informed consent form.

B. Exclusion criteria

- (1) Patients underwent a surgery within 2 weeks.
- (2) Patients may need emergency surgery in the near future.
- (3) Patients are allergic to any ingredient of rapamycin.
- (4) Patients suffer from a disease requiring immediate blood transfusion.
- (5) Patients suffer from any disease or condition that may impact implementation of the study or interpretation of the results. This type of diseases includes:
 - a) Known severe blood coagulation disorders
 - b) Known anemia that is not caused by intestinal polyps
 - c) Known hemoglobinopathy
 - d) Other gastrointestinal infectious diseases
 - e) Serious heart, liver, kidney and other concomitant diseases that may endanger lives
- (6) Patients are in pregnancy and lactation.
- (7) Alcohol or drug (such as aperient) abuse
- (8) Patients took part in another clinical trial that may influence this study.
- (9) The researchers believe that there are other unfavorable reasons for the patient to become a subject.

C. Exit criteria

- (1) An allergic reaction occurs.
- (2) The patient requests withdrawal: at his or her own discretion or at the request of his or her legal representative. Subjects may refuse to participate in further studies at any time without providing reasons. Subjects will not be affected because of such decision.
- (3) Subjects are required to withdraw from the study in certain special circumstances. (eg, there is significant issues of compliance, safety, or surgery for venous malformations)
- (4) Other situations in which the study must be terminated. For example, the

investigators believe that continuing the study may be harmful to the health of subjects.

D. Rejection criteria

- (1) Patients who violate the requirements of the test protocol
- (2) Patients with poor-recording, incomplete, or inaccurate data

V. Therapeutic schedule

A. Enrollment

Before starting the study, the investigator clearly and colloquially explains the study and its potential risks and benefits to the patient or his/her authorizer. Patient or his/her authorizer and the investigator sign and date the informed consent form. Only after signing the informed consent form can patient or his/her authorizer enter the screening section and then participate in the study. Patients who sign a written informed consent need to be assessed to determine if the patient meets the study criteria:

- 1) Demographic data and medical history of the patient;
- 2) vital signs;

B. Children's dosage regimen

Oral administration

The initial dose of oral rapamycin intake is 1 mg/m² of body surface area. Drug trough concentration in patient's blood is maintained at 5-10 ng/ml.

C. Adults' dosage regimen

- 1) Initial dose and dosing schedule: sirolimus 2mg, once a day. Maintain drug trough concentration of 5-10ng / mL in blood. If adverse events are too severe to tolerate, daily dosage can be reduced by 0.5mg every week. The minimum dosage is 1 mg/day. If patient still cannot tolerate minimum dosage, withdraw from the study. If patient could tolerate the possible adverse events, continue to take the original dose for 3 months, and evaluate improvement of disease condition.
- 2) Subsequent dose and dosing schedule: After taking sirolimus 2 mg once a day for 3 months, those with improved condition continue to take the original dose. If improvement of disease condition is not obvious, change dosage to 2.5mg-3mg once a day. If this dosage cannot be tolerated, patients may withdraw from the study. If it can be tolerated, continue to take sirolimus for another 3 months and reassess the improvement of condition.
- 3) Blood drug concentration test: Blood samples are taken at 1st month, 3rd months, and 6th months of dosage schedules.
- 4) Iron supplementation: For subjects with hemoglobin \leq 10.9 g / dl, iron supplementation should be provided according to the standard dosing regimen, but iron supplementation is not considered as study treatment and will be recorded as concomitant medication.

VI. Follow-up plan and content

A. Evaluation before the trial

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) VAS pain score
- 5) (Patients suspected with secondary malignant lesions) tumor markers examination, including CEA, CA199 and CA125
- 6) (Female aged 25 or elder) ultrasonic examination of uterus and its appendages
- 7) Blood and urine routine examination
- 8) Liver and kidney function, blood lipid test
- 9) (Patients with secondary malignant tumor) chest CT and other related examinations
- 10) Abdominal and pelvic cavity contrast-enhanced CT + small intestine reconstruction CT examination
- 11) Digestive system endoscopy
- 12) (Female of childbearing age) blood/urine pregnancy test

B. Evaluation during sirolimus treatment

Adverse reactions are evaluated weekly during the first month of drug treatment. The efficacy and safety are then evaluated at the 3rd and 6th months, and the contents of the examination is the same as the pre-trial evaluation.

- 1) Efficacy assessment:
 - a. Clinical symptom assessment (including ileus, VAS pain score, gastrointestinal bleeding and some other related symptoms);
 - b. Changes in hemoglobin;
 - c. Nutritional status (including albumin, etc.);
 - d. Abdominal and pelvic cavity contrast-enhanced CT + small intestine reconstruction CT examination, digestive system endoscopy to measure the size, quantity and total load of intestinal polyps;
 - e. (For patients with secondary malignant tumor) Tumor response to the drug, such as changes of tumor marker, tumor regression shown by imaging, metastasis and etc.
- 2) Safety assessment: Safety parameters (such as asking adverse events, measuring laboratory values, vital signs, gastrointestinal bleeding, etc.) will be closely monitored on a regular basis throughout the study.
- 3) Treatment compliance: In order to monitor compliance, subjects are required to complete an electronic diary during the entire study period. Dates of drug intake will be tracked by this diary. At every visit, the investigator and the subject will review the integrity of the electronic diary data together.

C. 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. Its content is the same as pre-trial evaluation.

VII. Records and reports of adverse events

A. Adverse events

The term of adverse events encompass any signs, symptoms, syndromes or diseases affecting health condition of subjects during the trial. The term also includes clinically relevant conditions found by laboratory test or other diagnostic procedures. It may call for unplanned

treatment, or lead to withdrawal from the trial. Adverse events may be: new disease; deterioration of symptoms accompanying disease progression during treatment; certain events not related to the trial; combination of one or more events above.

Serious adverse events

At any dose of the test drug or at any time during the observation period, the following adverse events occurs: death; immediate life-threatening events; requirement of hospitalization or prolonged hospital stay; disability; congenital malformations; other events with important medical implications (those do not immediately endanger life or cause death or require hospitalization, but may harm the patient or require interventions to prevent one of the consequences defined above) and requiring medical treatment to prevent permanent injury.

B. Adverse reactions

Harmful and unexpected reactions caused by pharmaceutical applications under normal use of drugs and prescribed doses.

C. Degree of adverse reactions:

- 1) Mild: causing mild discomfort which can be tolerated and does not affect daily life; under this circumstance, the subject does not need to discontinue medication.
- 2) Moderate: causing discomfort which affects patients' daily life.
- 3) Severe: affecting daily life; cannot be endured by the subject or cause organ damage and need to withdraw medication.
- 4) Very serious: risk of disability or death; require emergent rescue and drug withdrawal.

D. Evaluation of the relationship between adverse reactions and experimental drug

According to the 5-level judgment system, the relationships can be divided into 5 types: definitely related, very likely related, may be related, may be irrelevant, and definitely irrelevant. The first three are counted as adverse reactions, in which case the incidence of adverse reactions and adverse events should be reported.

E. Record and report

The investigator should explain to the patient or his/her legal guardian that it is required to truthfully reflect the changes in the condition after administration. Doctors should avoid suggestive questions. While observing the efficacy, pay close attention to the observation of adverse events or unanticipated side effects (including symptoms, signs, laboratory tests), analyze the causes, make judgments, and report the incidence of adverse reactions and adverse events.

For adverse events occurring during the trial, time, symptoms, extent, duration, treatment measures, outcomes, and some other items should be recorded in the case report form to evaluate the correlation with the test drug. Investigator should record it in detail, and then signs and dates.

F. Treatment of adverse events

- 1) reporting system

Any adverse events, such as subjective discomfort and abnormal laboratory tests, should be taken seriously and carefully analyzed. Immediate measures should be taken to protect safety of the subject.

- 2) treatment procedure

Physicians are pre-trained to prevent certain types of adverse events or reactions that may occur in the study, so as to protect safety of patients. Treatment procedure of adverse event should be recorded in detail. Persistence and disappearance of adverse events should also be recorded.

3) treatment of serious adverse events

On the occurrence of any serious adverse event, research physician should report to primary PI within 24 hours, in addition to emergency treatment.

4) follow-up of unresolved adverse events

All adverse events should be tracked until they are properly resolved. Adverse events with abnormal laboratory values should be tracked until the value returns to baseline.

VIII. Statistical analysis

Statistical analysis will be conducted by SPSS.

A. Methods of statistical analysis

The categorical variables will be described by frequency distribution (number of cases and percentage). Continuous variables will be described with mean, median, minimum, maximum, first and third quartile. The confidence interval of all parameters is set to be 95%. All analyses will be conducted with SPSS.

B. Contents of statistical analysis

- 1) General data analysis: Demographic variables and baseline characteristics will be summarized comprehensively by mean of descriptive statistics and/or appropriate frequency tables.
- 2) Evaluation of efficacy: According to follow-up and reexamination results, the patients' quality of life, size and volume of the polyps, total polyp load control and improvement of symptoms will be analyzed.
- 3) Analysis of adverse events: Side effects and adverse events during medication will be assessed.
- 4) Evaluation of the relationship between adverse events and the tested drug

IX. Measures of quality control

1. Supervision by the project leader
2. Establish a standardized evaluation method, unify various diagnostic criteria and efficacy criteria
3. Formulate plans for monitoring and treatment of adverse reactions
4. All researchers are trained before the study begun
5. Designate quality controllers and develop a plan for quality control, with regular inspections
6. Devise a follow-up plan and system

X. Expected research results

The results will be published in a paper and fed back to the subjects.

XI. Potential risks, benefits or advantages of this study

As a drug that has been on the market for years, it is applied to this study beyond its indication. It is recommended by doctors considering that its benefits outweigh its risks and the subjects have no better choice. For subjects, it may relieve their symptoms and improve quality of life, and complications associated with surgery and damage to intestinal function can be avoided. The testing drug is free during the study and subjects should bear the risks of treatment.

XII. Data preservation and confidentiality

It will be stated in the informed consent that personal information of subjects will be strictly confidential in this study. Results of this study may be published in scientific paper, but personal information of subjects, including their names, will not appear in the paper. All data relevant to this study will be preserved by Peking Union Medical College Hospital and Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, and strict confidentiality measures will be established.

XIII. Measures to protect subjects and minimize risks

In the whole course of this study, safety supervision will be carried out by experts from the digestive surgery department of our hospital to strictly control diagnostic criteria and treatment indicators of Peutz-Jeghers's syndrome, making the collected clinical data meet international diagnostic criteria and providing favorable treatment for these patients.