A randomized, placebo-controlled, multicenter, prospective clinical study of Berberine hydrochloride in preventing recurrence and carcinogenesis after endoscopic removal of colorectal adenomas

ClinicalTrials.gov number, NCT02226185

STUDY PROTOCOL

Final Version (Dec. 22, 2018)

Leading center:

Renji Hospital, Shanghai Jiao-Tong University School of Medicine

Participating centers:

The Shanghai Tenth People's Hospital, Tongji University

The Seventh Medical Center of PLA General Hospital

General Hospital, Tianjin Medical University

Zhongshan Hospital, Xiamen University,

Nanfang Hospital, Southern Medical Univerisity

The Affiliated Drum Tower of Nanjing University Medical School

SPONSOR:

Renji Hospital, Shanghai Jiao-Tong University School of Medicine

1. Background

Colorectal cancer (CRC) is a leading cause of cancer deaths worldwide and in recent years its incidence is increasing rapidly in China, which constitutes a major public health burden [1]. Almost 90% of CRC cases develop from precursor adenomatous polyps, through a series of genetic changes known as adenoma-carcinoma sequence during at least 10 years [2]. Detection and removal of colorectal adenoma (CRA) could reduce CRC mortality risk by colonoscopy [3], but the recurrence rate is high [4]. Chemoprevention of adenoma and cancer remains its importance in both aspects of public health and cost effectiveness.

Our previous clinical trial has shown that supplementation of 1mg folic acid per day decreases significantly incidence of sporadic CRA in elder healthy people [5]. However, the role of folic acid in the prevention for CRA recurrence has kept controversial to date [6]. Favorable effects of several kinds of medicine on the secondary prevention of CRA have been reported in observational studies and randomized controlled trials (RCTs), including aspirin, vitamin D, etc. A latest meta-analysis summarized that regular intake of aspirin or COX-2 inhibitors seemed to be effective in preventing relapse of adenomas, but meanwhile a dose-related increase of gastrointestinal complications could be observed [7]. To evaluate the protective potential of vitamin D3 and/or calcium, a large-scale randomized trial conducted in 2259 participants with a recent history of CRA showed unfortunately no significant effect of any group [8]. Thus looking for suitable prophylactic drugs for CRA recurrence is urgent needed.

Berberine (BBR) hydrochloride, a natural isoquinoline alkaloid extracted from the Chinese herb Coptis chinensis, has been widely used in China to cure diarrhea and enteritis for centuries with minimal side effects [9], and accumulating evidences have showed its anticancer activity, but its molecular mechanism has not been quite clear. Our previous study presented that BBR could block the colorectal adenoma-carcinoma sequence in mice by changing microbiota structures [10]. In order to further evaluate its clinical potential for CRA recurrence, we have initiated this clinical trial of pharmacological intervention in persons after a recent polypectomy.

2. Objectives

We initiate this placebo-controlled study to investigate the clinical potential of BBR hydrochloride in preventing recurrence and carcinogenesis after endoscopic removal of CRAs.

3. Design

3.1 Overall design

Double-blind, randomized, placebo-controlled trial

3.2 Randomization

Computer-generated randomization is used to allocate participants into BBR hydrochloride group and placebo group in a 1:1 ratio. The person who generates the allocation sequence is an expert on biostatistics, who is not involved in this study. Investigators who enroll participants receive the serial numbers. Participant enrollment must be carried out according strictly to the serial numbers. Those who enroll participants and assign interventions are unaware of the details of grouping.

3.3 Blinding

The participants, the investigators in charge of follow-up, the endoscopists and the pathologists are all blinded of which treatment the participant is receiving.

Unblinding will be advanced for an appropriate clinical management only in case of emergency.

4. Selection of Trial Population

4.1 Inclusion criteria

1. Individuals aged 18-75 years
2. Individuals who had at least one and no more than 6 histologically confirmed CRAs removed within 6 months before recruitment

3. Individuals who are able to swallow pills
4. Individuals who voluntarily sign the consent form after being fully informed and understanding the purpose and procedure of this study, characters of the disease, effect of medication, methods of related examinations, and potential risk/benefits of the study

4.2 Exclusion criteria

1. Individuals whose adenoma was not completely removed during previous colonoscopy
2. Individuals with a history of familial adenomatous polyposis (FAP) or hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome)
3. Individuals who are taking regularly aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), cyclo-oxygenase 2 (COX2) inhibitors, calcium or vitamin D
4. Individuals with a history of subtotal/total gastrectomy or partial bowel resection
5. Individuals who are intolerant to another colonoscopy examination
6. Individuals who are hypersensitive or intolerant to the drugs
7. Individuals with severe heart, liver or kidney disease, or any cancer history
8. Individuals presenting severe constipation
9. Pregnant women, women during breast-feeding period, or women with expect pregnancy
10. Individuals with mental diseases who are not able to cooperate
11. Individuals who are involved in designing, planning or performing this trial

4.3 Elimination criteria

1. Individuals who do not meet the inclusion/exclusion criteria.
2. Serious violation of the study protocol which has impact on the drug efficacy.
3. Presence of lost data or data unable to get.

5. Numbers of Cases & Allocation

According to the statistical sample size, we are planning to enroll 1000 cases in our study, 500 cases in each group.

6. Interventions

6.1 Medication

6.1.1 Drug 1

- Ingredient: BBR hydrochloride
- Dosage form: Tablet

6.1.2 Drug 2

- Ingredient: Placebo
- Dosage form: Tablet

All the study pills are provided by Shanghai SINE Pharmaceutical Co., Ltd.

6.2 Dose & usage

BBR hydrochloride 300mg Bid

Placebo 3# Bid

6.3 Duration

Until the last enrolled patient reached the 2-years follow-up.

6.4 Concomitant treatment

Conventional symptomatic treatment can be used in both group during the study period. When severe constipation occurs in participants after taking study pills, they are allowed to take one prokinetic drug within 2 weeks.

6.5 Participant timeline

The time schedule of enrollment, interventions and visits for participants is shown in a schematic diagram (Table 1) following the instructions in the SPIRIT 2013 (Standard Protocol Items: Recommendations for Interventional Trials) statement [11].

7. Observation Items

7.1 Demographic characteristic & medical history

Gender, age, height, weight, medical history, recent medication, smoking status, history of CRA, family history of CRC, clinical manifestations before enrollment, recent colonoscopy and biopsy, etc.

Table1. Participant timeline

	Study Period										
	Alloca -tion	Run-in	Post-allocation								Closeout
Time points	0	-2wks	M3	M6	M9	12	15	18	21	24	M24-M36 Final visit
Enrollment:											
Eligibility screen	X										
Informed consent	X										
Interventions:											
BBR hydrochloride or placebo		◆	—	—	—	—	—	—	—	◆	Return unused pills
Assessments:											
Demographics	X										
Smoking status	X										
Height	X					X				X	
Weight	X					X				X	
Medical history	X										
Family history	X										
Manifestations	X	◆	—	—	—	—	—	—	—	◆	
Laboratory tests	X					X				X	
Colonoscopy	X					X				X	
Histology	X					X				X	
Stool samples	X					X				X	
Drug distribution		◆	—	—	—	—	—	—	—	◆	
Adverse event		X	◆	—	—	—	—	—	—	—	◆
Medication		◆	—	—	—	—	—	—	—	—	◆

7.2 Observation index

7.2.1 Clinical manifestations

We record the clinical manifestations before recruitment and during the follow-up period, including symptoms (fatigue, lack of appetite, nausea, emesis, abdominal distension, diarrhea, change of stool character and abdominal pain) and signs (abdominal tenderness, mass, rebound tenderness, etc.).

7.2.2 Laboratory examination

We record the results of laboratory tests, including routine blood tests, hepatic and renal function at the baseline, and during the follow-up period.

7.2.3 Colonoscopic examination

Participants at each center are informed to undergo at least one or two follow-up colonoscopies until the last enrolled patient reached the 2-years follow-up, by a training endoscopist to investigate whether new adenoma will be taken place, using a standard colonoscope.

Bowel preparation includes 1000ml polyethylene glycol electrolyte solution administered the previous evening and 2000ml in the early morning before an intraday examination. If the colonoscopy does not reach the caecum, it cannot be included for further analysis and the patient will be required to undergo colonoscopy by another skilled endoscopist on next occasion. The mean colonoscopy withdrawal time are required to be 6 minutes or more.

According to a multiple-center study based of Chinese population [12], the peak of recurrence of adenomas is almost entirely concentrated in the first year, and the first three years follow-up after polypectomy is of critical importance in China. The similar opinions are recommended in *2011 Consensus on the Prevention of Colorectal Tumors in China [13]*.

7.2.4 Electrocardiograph (ECG)

We record the ECG results before recruitment and during the follow-up period.

7.2.5 The tests of gut microbiota in feces specimens

A net pot is delivered to each participant at the initial visit and during the follow-up period for collection of stool. Participants are required to deliver the pot with fresh stool to hospital within 4 hours, and the samples will be collected and marked by our trained study staff and frozen immediately at -80℃. The feces specimens are preserved for further test of gut microbiota in the future.

8. Management of Trial Drugs

8.1 Registration & usage

Patients who met the inclusion/exclusion criteria will be randomized into group A or B, and each participant will receive a number that will remain the same throughout the trial. The serial number will be recorded on the case report form (CRF). Each selected patient can only receive the corresponding medication.

The study drugs should be kept and distributed by a special person and a registration system should be established. Each patient should return the rest of drugs at the end. Patients are required to avoid taking study drugs outside the trial.

8.2 Dispense & storage

To well manage and use the study drugs, each study center should establish a strict personal drug management system. At the end of the trial, the remaining drugs should be uniformly recovered and disposed. All the study drugs must be stored safely under suitable conditions.

9. Compliance

To ensure a better trial completion rate, we should pay attention to the measures of subject compliance. Each participant should be fully informed of the potential benefits and risks of the study drugs and sign voluntarily the consent form. Enrollment is followed by a 2-week run-in period to identify and exclude participants who have been considered unlikely to follow the study procedures.

We should confirm that the test or control drugs are taken effectively and adequately by each participant. Follow-up is performed by telephone calls and clinical visits. A regular monthly phone call will be made by our study staffs in charge to monitor adherence and the curative and side effects of the medication until the end of the trial. A clinical visit every 3 months is planned to return any unused pills and bottle, in exchange for another 3-month dosage of drugs with the same label. Bottles of study pills were mailed to participants who had difficulties in reaching the hospital.

During the study period, if one participant is found to refuse to take the drugs (including the placebo), we should have a positive conversation with the participant and try to

persuade him/her to continue the study, and report the situation to the doctor in charge meanwhile.

10. Discontinuation of Trial

1. The case found to be inconsistent with the inclusion criteria after recruitment.
2. It is difficult to adhere to the treatment for the patient who is in a worse condition after medication. In that case, the investigator can suspend the trial and treat as an invalid case.
3. Serious or intolerable adverse events.
4. Serious violation of the study protocol during the study period.
5. Poor compliance.
6. Lost in contact for various reasons.
7. Withdrawal of the consent form by the participant.
8. Discontinuation of trial asked by the sponsor.
9. Discontinuation of trial suggested by the investigators.

11. Endpoints

The primary endpoint is the recurrence of CRA at any follow-up colonoscopy in both groups during the whole trial. The number, size and location of all adenomas are assessed.

The secondary outcomes are the incidence rates of all polypoid lesions (hyperplastic polyps, inflammatory polyps, serrated polyps, etc.), as well as advanced adenoma or CRC at any follow-up colonoscopy in both groups.

All retrieved polypoid lesions should be sent to local pathology laboratory for histologic evaluation. Two experienced pathologists at each center, who are unaware of the treatment assignments, must review the slides for all removed colorectal lesions. Disagreements in the histologic diagnosis are resolved by re-examination by these two pathologists until agreement is reached. We classify the location of a lesion as right

sided (caecum, ascending colon, hepatic flexure, and transverse colon) or left sided (splenic flexure, descending colon, sigmoid colon, and rectum), and define advanced CRA (A-CRA) as an adenoma with a diameter of 10 mm or more, a villous adenoma (≥25% villous) or an adenoma with high-grade dysplasia.

12. Safety Monitoring

12.1 Observation index

Safety assessments include adverse events, laboratory tests, EKG, vital signs, etc.

12.2 Adverse events (AE)

An adverse event is any unexpected or unfavorable condition related to any medical treatment during the study period, including an inappropriate sign or symptom, an abnormal lab test or a temporary illness related to the use of the drugs.

We record in time the performance, type, time, extent, duration and outcome of the adverse event.

12.3 Serious adverse events (SAE)

Serious adverse events are defined as those events that meet any of the following criteria:

• Requires inpatient hospitalization or prolongation of existing hospitalization;

• Results in persistent or significant disability or incapacity;

• Is immediately life threatening or results in death.

Investigators must inform SFDA, sponsor, relevant contract research organization (CRO), ethic committee, and relevant government department by telephone/fax within 24 hours after being aware of the serious adverse event, and file the event in the report form with detailed documents.

The patient should be followed up until clinical recovery or his/her condition has been stabilized.

12.4 Criteria for accessing relationship between adverse events and study drugs

A. The time of the adverse event is consistent with the time of medication.

B. The adverse event is consistent with the known adverse reaction to the drug.

C. The adverse event cannot be explained by concomitant medication or other reasons.

D. The adverse event disappears after drug withdrawal.

E. The same adverse reaction occurs when reusing the drug.

According to the above principles, the causal relationship is divided into five levels: definitely relevant, probably relevant, possibly relevant, possibly irrelevant, and definitely irrelevant.

Definitely relevant: meet the A, B, C, D, E criteria.

Probably relevant: meet the A, B, C, D criteria.

Possibly relevant: meet the A, B criteria.

Possibly irrelevant: meet the A criterion.

Definitely irrelevant: all symptoms can be explained by other reasons, and do not meet all above criteria.

13. Statistical Analysis Plan (SAP)

The statistical analysis plan is made and modified by biostatistician and main investigators according to the study protocol before the data is locked. Statistical analysis is based on the statistical plan.

13.1 Sample size

We estimated 918 individuals for recruitment after calculation on the basis of an expected CRA recurrence rate of 21% (30% reduction) for the intervention group and 30% for the placebo group. This study sample size was decided to provide power of 80% at a statistical significance level of 0.05, allowing a 20% dropout rate.

13.2 Statistical analysis

13.2.1 Balance analysis

Full Analysis Set (FAS) is used in the balance analysis. This analysis set includes all the subjects to the principle of intention to treat (ITT). The subjects must have received at least one follow-up colonoscopy.

A balance analysis is performed for baseline data to evaluate the comparability of the two groups. The baseline data include the number of patients enrolled, the number of clinically evaluable patients, age, weight, symptoms, signs and laboratory tests. In the balance analysis, quantitative variables are described using the mean, standard deviation (SD), normal distribution W test and *P* value, etc. The t test or the Wilcoxon rank sum test (if abnormal distribution) is used for comparison the difference between the test drug group and the control group in the statistical analysis. Categorical variables are described using cases and percentages. The chi-square ($\chi 2$) test, the Mantel-Haenzel $\chi 2$ test or the Fisher's exact test is used for analyzing categorical data.

13.2.2 Efficacy analysis

The trial results are evaluated by FAS and Per Protocol Set (PPS). FAS is mainly used to compare the treatment effect for the primary endpoint, and regarding the statistical results from PPS as supplementary analysis. If the subjects have not completed all the treatment, the last exam result will be treated as last observation carried forward (LOCF). Risk ratios (RR) and 95% confidence intervals (CI) are used as the efficacy indicator, comparing by Mantel-Haenszel $\chi 2$ test methods between the two groups.

Unconditional logistic regression is conducted to compute the odds ratio to estimate the relation between BBR intervention and recurrence of A-CRA and non A-CRA (NA-CRA). Kaplan–Meier method is conducted to estimate progression-free survival of no recurrence of adenoma with log-rank test to compare the survival curve between BBR and placebo group. Survival Curve is used to illustrate time to recurrence of adenoma and RRs are illustrated by Forest plots. We perform predefined subgroup analyses of the primary outcomes to assess BBR effect in terms of age, gender, smoking status, BMI, family history of CRC, number of adenoma and history of CRA.

13.2.3 Safety analysis

Safety Set (SS) for safety analysis includes all the subjects having received at least one dose of treatment.

Adverse events should be listed, and the $\chi 2$ test or the Fisher's exact test is used for analyzing the incidence between two groups.

13.2.4 Statistical significance

A two-sided *P* value of less than 0.05 is considered to indicate statistical significance.

13.2.5 Statistical software

Statistical analyses are conducted with the use of SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

14. Data Management

Establish the procedure for data storage, transfer, and query. The archived documents include: the participant's CRF, subject code form, serious adverse event report form, completed GCP form, follow-up report form and related original medical documents. The transferred data include: the subject random table, CRF, serious adverse event report form, and the documents required for summary. The main investigator at each center has the right to inspect the relevant trial data and original records.

A secure electronic database (Bo Shi, China) that conformed to GCP requirements is used for data management. A timely training of personnel has been conducted before electronic data input, and access to the corresponding system rights has been granted. The investigator of each center can only reach their own data page by using a specific account and pass code, and the principal investigator in Ren-Ji Hospital can view and centralize all the baseline and follow-up information of participants, yet with no permission to modify the data of other centers.

It is necessary to adopt standardized statistical methods and invite familiar biostatistician to summarize and analyze the results.

To ensure the reliability and completeness of the trial data, the principle investigator

has the responsibility to monitor systematically the data of each center at regular intervals, and evaluate whether the execution is consistent with the protocol and whether the reported data are consistent with the records of each center. A report should be prepared for each visit.

15. Quality Control

Standard operating procedure (SOP) should be used at all study centers for quality control.

In order to ensure the quality of the multi-center trial, the main investigators should discuss together and develop a comprehensive clinical research plan before the start of the formal trial. The medical personal participating in the trial should also receive GCP training.

All observed results and abnormal finding in the clinical trial should be cautiously verified and recorded in time to ensure data reliability. All kinds of instruments, equipment, reagents and standards used in various examination items in the clinical trial should have strict quality standards, and we should make sure that they works properly. Data record and transfer must be performed by an experienced physician and checked by a special person to ensure data scientificity and accuracy. The conclusions of the clinical trial must be based on the original data.

The personal in charge should write CRFs in a complete, detailed and accurate manner. The CRFs should be completed as soon as possible after the examinations, and reported or preserved according to the procedure after been signed by the main investigator of each center. All data related to the trial should be centrally managed and analyzed.

16. Ethics

The trial will be carried out in accordance with the Chinese Guideline for Good Clinical Practice (GCP) and the principles laid down in the Declaration of Helsinki. The trial will not be initiated before the protocol has been approved by Institutional Review Board (IRB) of the clinical research center.

The study personal has the responsibility to provide a complete and comprehensive introduction in writing of the study purpose, procedure and potential risk to each participant or his representative before enrollment, and let the participant know that he/she has the right to withdraw from the study at any time. An informed consent form must be given to each patient prior to enrollment, and the study personal should retrieve the signed consent before the patient enters the study.

17. Follow-up & Medical Measures After the Trial

If adverse events persist after discontinuation of the study drugs, the patients should be followed and receive reasonable treatment until clinical recovery or their conditions have been stabilized with corresponding records on the CRFs. If the participant suffers from health damage due to adverse drug reactions, he/she will receive appropriate medical compensation given by sponsor.

18. References

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SUMMARY OF PROTOCOL CHANGES

Protocol Changes Version 1.0 (July 16, 2014) to 1.1 (Nov. 20, 2014):

1) Cover page: Participating centers

Addition of the following:

The Affiliated Drum Tower of Nanjing University Medical School

Deletion of the following:

Zhongshan Hospital, Fudan University

Protocol Changes Version 1.1 (Nov. 20, 2014) to 1.2 (Dec. 20, 2014):

1) Section 4. Selection of Trial Population:

Addition of the following:

4.1 Inclusion criteria

1. Individuals aged 18-75 years
2. Individuals who had at least one and no more than 6 histologically confirmed CRAs removed within 6 months before recruitment
3. Individuals who are able to swallow pills
4. Individuals who voluntarily sign the consent form after being fully informed and understanding the purpose and procedure of this study, characters of the disease, effect of medication, methods of related examinations, and potential risk/benefits of the study

4.2 Exclusion criteria

1. Individuals whose adenoma was not completely removed during previous colonoscopy
2. Individuals with a history of familial adenomatous polyposis (FAP) or hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome)
3. Individuals who are taking regularly aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), cyclo-oxygenase 2 (COX2) inhibitors, calcium or vitamin D
4. Individuals with a history of subtotal/total gastrectomy or partial bowel resection
5. Individuals who are intolerant to another colonoscopy examination
6. Individuals who are hypersensitive or intolerant to the drugs
7. Individuals with severe heart, liver or kidney disease, or any cancer history
8. Individuals presenting severe constipation
9. Pregnant women, women during breast-feeding period, or women with expect pregnancy
10. Individuals with mental diseases who are not able to cooperate
11. Individuals who are involved in designing, planning or performing this trial

Deletion of the following:

4.1 Inclusion criteria

1. Individuals aged 18-70 years, without gender limitation
2. Individuals who had at least one and no more than 6 histologically confirmed CRAs removed by EMR, ESD or APC within 1 month
3. Excluding individuals with familial adenomatous polyposis (FAP) by asking medical and family history
4. Individuals who voluntarily sign the consent form

4.2 Exclusion criteria

1. Individuals suffering from other malignancy at the same time
2. Individuals with other severe diseases and the expected survival is less than 3 years
3. Individuals who are intolerant to another colonoscopy examination
4. Pregnant women, women during breast-feeding period, or women with expect pregnancy
5. Individuals with a history of subtotal/total gastrectomy or partial bowel resection
6. Individuals presenting severe constipation
7. Individuals who are taking regularly aspirin, cyclo-oxygenase 2 (COX2) inhibitors or calcium
8. Individuals with mental diseases who are not able to cooperate
9. Individuals who are involved in designing, planning or performing this trial
10. Individuals who are hypersensitive or intolerant to the drugs
11. The investigators think that the subject is not suitable for inclusion

2) Section 6.2 Dose & usage

Addition of the following:

BBR hydrochloride 300mg Bid×36 months

Placebo 3# Bid×36 months

Deletion of the following:

BBR hydrochloride 300mg Tid×36 months

Placebo 3# Tid×36 months

Protocol Changes

Version 1.2 (Dec. 20, 2014) to Final Version (Dec. 22, 2018):

1) Cover page: Participating centers

Addition of the following:

The Seventh Medical Center of PLA General Hospital

Deletion of the following:

PLA Army General Hospital

- The Renaming Declarations of PLA Army General Hospital have been filed for reference and approved by IRB of the leading center.

2) Section 1

Addition of the following:

1. Background

Colorectal cancer (CRC) is a leading cause of cancer deaths worldwide and in recent years its incidence is increasing rapidly in China, which constitutes a major public health burden [1]. Almost 90% of CRC cases develop from precursor adenomatous polyps, through a series of genetic changes known as adenoma-carcinoma sequence during at least 10 years [2]. Detection and removal of colorectal adenoma (CRA) could reduce CRC mortality risk by colonoscopy [3], but the recurrence rate is high [4]. Chemoprevention of adenoma and cancer remains its importance in both aspects of public health and cost effectiveness.

Our previous clinical trial has shown that supplementation of 1mg folic acid per day decreases significantly incidence of sporadic CRA in elder healthy people [5]. However, the role of folic acid in the prevention for CRA recurrence has kept controversial to date [6]. Favorable effects of several kinds of medicine on the secondary prevention of CRA have been reported in observational studies and randomized controlled trials (RCTs), including aspirin, vitamin D, etc. A latest meta-analysis summarized that regular intake of aspirin or COX-2 inhibitors seemed to be effective in preventing relapse of adenomas, but meanwhile a dose-related increase of gastrointestinal complications could be observed [7]. To evaluate the protective potential of vitamin D3 and/or calcium, a large-scale randomized trial conducted in 2259 participants with a recent history of CRA showed unfortunately no significant effect of any group [8]. Thus looking for suitable prophylactic drugs for CRA recurrence is urgent needed.

Berberine (BBR) hydrochloride, a natural isoquinoline alkaloid extracted from the Chinese herb Coptis chinensis, has been widely used in China to cure diarrhea and enteritis for centuries with minimal side effects [9], and accumulating evidences have showed its anticancer activity, but its molecular mechanism has not been quite clear. Our previous study presented that BBR could block the colorectal adenoma-carcinoma sequence in mice by changing microbiota structures [10]. In order to further evaluate

its clinical potential for CRA recurrence, we have initiated this clinical trial of pharmacological intervention in persons after a recent polypectomy.

Deletion of the following:

1. Title

A randomized, placebo-controlled, multicenter, prospective clinical study of Berberine (BBR) hydrochloride in preventing recurrence and carcinogenesis after endoscopic removal of colorectal adenomas (CRAs)

3) Section 6 Interventions

Addition of the following:

6. Interventions

6.1 Medication

6.1.1 Drug1

- Ingredient: BBR hydrochloride
- Dosage form: Tablet

6.1.2 Drug 2

- Ingredient: Placebo
- Dosage form: Tablet

All the study pills are provided by Shanghai SINE Pharmaceutical Co., Ltd.

6.2 Dose & usage

BBR hydrochloride 300mg Bid

Placebo 3# Bid

6.3 Duration

Until the last enrolled patient reached the 2-years follow-up.

6.4 Concomitant treatment

Conventional symptomatic treatment can be used in both group during the study period. When severe constipation occurs in participants after taking study pills, they are allowed to take one prokinetic drug within 2 weeks.

6.5 Participant timeline

The time schedule of enrollment, interventions and visits for participants is shown in a schematic diagram (Table 1) following the instructions in the SPIRIT 2013 (Standard Protocol Items: Recommendations for Interventional Trials) statement [11].

Table1: Participant timeline

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Enrollment:											
Eligibility screen	X										
Informed consent	X										
Interventions:											
BBR hydrochloride or placebo		◆	—	—	—	—	—	—	—	◆	Return unused pills
Assessments:											
Demographics	X										
Smoking status	X										
Height	X					X				X	
Weight	X					X				X	
Medical history	X										
Family history	X										
Manifestations	X	◆	—	—	—	—	—	—	—	◆	
Laboratory tests	X					X				X	
Colonoscopy	X					X				X	
Histology	X					X				X	
Stool samples	X					X				X	
Drug distribution		◆	—	—	—	—	—	—	—	◆	
Adverse event		X	◆	—	—	—	—	—	—	—	◆
Medication		◆	—	—	—	—	—	—	—	—	◆

Deletion of the following:

6. Interventions

6.1 Medication

6.1.1 Drug1

- Ingredient: BBR hydrochloride
- Dosage form: Tablet

6.1.2 Drug 2

- Ingredient: Placebo
- Dosage form: Tablet

6.2 Dose & usage

BBR hydrochloride 300mg Bid×36 months

Placebo 3# Bid×36 months

6.3 Duration 36 months.

6.4 Concomitant treatment

Conventional symptomatic treatment can be used in both group during the study period.

4) Section 7.2 Observation index

Addition of the following:

7.2.1 Clinical manifestations

We record the clinical manifestations before recruitment and during the follow-up period, including symptoms (fatigue, lack of appetite, nausea, emesis, abdominal distension, diarrhea, change of stool character and abdominal pain) and signs (abdominal tenderness, mass, rebound tenderness, etc.).

7.2.2 Laboratory examination

We record the results of laboratory tests, including routine blood tests, hepatic and renal function at the baseline, and during the follow-up period.

7.2.3 Colonoscopic examination

Participants at each center are informed to undergo at least one or two follow-up colonoscopies until the last enrolled patient reached the 2-years follow-up, by a training endoscopist to investigate whether new adenoma will be taken place, using a standard colonoscope.

Bowel preparation includes 1000ml polyethylene glycol electrolyte solution administered the previous evening and 2000ml in the early morning before an intraday examination. If the colonoscopy does not reach the caecum, it cannot be included for further analysis and the patient will be required to undergo colonoscopy by another skilled endoscopist on next occasion. The mean colonoscopy withdrawal time are required to be 6 minutes or more.

According to a multiple-center study based of Chinese population [12], the peak of recurrence of adenomas is almost entirely concentrated in the first year, and the first three years follow-up after polypectomy is of critical importance in China. The similar opinions are recommended in *2011 Consensus on the Prevention of Colorectal Tumors in China [13]*.

7.2.4 Electrocardiograph (ECG)

We record the ECG results before recruitment and during the follow-up period.

7.2.5 The tests of gut microbiota in feces specimens

A net pot is delivered to each participant at the initial visit and during the follow-up period for collection of stool. Participants are required to deliver the pot with fresh stool to hospital within 4 hours, and the samples will be collected and marked by our trained study staff and frozen immediately at -80℃. The feces specimens are preserved for further test of gut microbiota in the future.

Deletion of the following:

7.2.1 Clinical manifestations

We record the clinical manifestations before recruitment and after 12 months, 24 months, 36 months of intervention, including symptoms (fatigue, lack of appetite, nausea, emesis, abdominal distension, diarrhea, change of stool character and abdominal pain) and signs (abdominal tenderness, mass, rebound tenderness, etc.). 0, 1, 2, 3 points are used to record none, light, medium and severe, respectively.

7.2.2 Laboratory examination

We record the results of laboratory tests, including blood, urine and stool routines, hepatic and renal function, carcinoembryonic antigen (CEA) and carbohydrate antigen 199 (CA199) level.

7.2.3 Colonoscopic examination

We record the colonoscopic results before recruitment and after 12 months, 24 months, 36 months of intervention. Additional exams may be done after 18 months, 30 months of intervention if necessary.

7.2.4 Electrocardiograph (ECG)

We record the ECG results before recruitment and after 12 months, 24 months, 36 months of intervention.

7.2.5 The tests of gut microbiota in the tissue and feces specimens

5) Section 11. Endpoints

Addition of the following:

The primary endpoint is the recurrence of CRA at any follow-up colonoscopy in both groups during the whole trial. The number, size and location of all adenomas are assessed.

The secondary outcomes are the incidence rates of all polypoid lesions (hyperplastic polyps, inflammatory polyps, serrated polyps, etc.), as well as advanced adenoma or CRC at any follow-up colonoscopy in both groups.

All retrieved polypoid lesions should be sent to local pathology laboratory for histologic evaluation. Two experienced pathologists at each center, who are unaware of the treatment assignments, must review the slides for all removed colorectal lesions. Disagreements in the histologic diagnosis are resolved by re-examination by these two pathologists until agreement is reached. We classify the location of a lesion as right sided (caecum, ascending colon, hepatic flexure, and transverse colon) or left sided (splenic flexure, descending colon, sigmoid colon, and rectum), and define advanced CRA (A-CRA) as an adenoma with a diameter of 10 mm or more, a villous adenoma (≥25% villous) or an adenoma with high-grade dysplasia.

Deletion of the following:

The primary endpoint is the recurrence and carcinogenesis of CRA at any follow-up colonoscopy in both groups.

6) Section 13.2.2 Efficacy analysis

Addition of the following:

Unconditional logistic regression is conducted to compute the odds ratio to estimate the relation between BBR intervention and recurrence of A-CRA and non A-CRA (NA-CRA). Kaplan–Meier method is conducted to estimate progression-free survival of no recurrence of adenoma with log-rank test to compare the survival curve between BBR and placebo group. Survival Curve is used to illustrate time to recurrence of adenoma and RRs are illustrated by Forest plots. We perform predefined subgroup analyses of the primary outcomes to assess BBR effect in terms of age, gender, smoking status, BMI, family history of CRC, number of adenoma and history of CRA.

Deletion of the following:

Exploratory subgroup analyses and safety analysis will also be conducted.

7) Section 13.2.5 Statistical software

Addition of the following:

Statistical analyses are conducted with the use of SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Deletion of the following:

Statistical analyses are conducted with the use of SAS software, Version 8.1.

8) Section 14. Data Management

Addition of the following:

Establish the procedure for data storage, transfer, and query. The archived documents include: the participant's CRF, subject code form, serious adverse event report form, completed GCP form, follow-up report form and related original medical documents. The transferred data include: the subject random table, CRF, serious adverse event report form, and the documents required for summary. The main investigator at each center has the right to inspect the relevant trial data and original records.

A secure electronic database (Bo Shi, China) that conformed to GCP requirements is used for data management. A timely training of personnel has been conducted before electronic data input, and access to the corresponding system rights has been granted. The investigator of each center can only reach their own data page by using a specific account and pass code, and the principal investigator in Ren-Ji Hospital can view and centralize all the baseline and follow-up information of participants, yet with no permission to modify the data of other centers.

It is necessary to adopt standardized statistical methods and invite familiar biostatistician to summarize and analyze the results.

To ensure the reliability and completeness of the trial data, the principle investigator has the responsibility to monitor systematically the data of each center at regular intervals, and evaluate whether the execution is consistent with the protocol and whether the reported data are consistent with the records of each center. A report should be prepared for each visit.

Deletion of the following:

Establish the procedure for data storage, transfer, and query. The archived documents include: the participant's CRF, subject code form, serious adverse event report form, completed GCP form, follow-up report form and related original medical documents. The transferred data include: the subject random table, CRF, serious adverse event report form, and the documents required for summary. The main investigator at each

center has the right to inspect the relevant trial data and original records.

It is necessary to adopt standardized statistical methods and invite familiar biostatistician to summarize and analyze the results.

To ensure the reliability and completeness of the trial data, the principle investigator has the responsibility to monitor systematically the data of each center at regular intervals, and evaluate whether the execution is consistent with the protocol and whether the reported data are consistent with the records of each center. A report should be prepared for each visit.

9) Section 18

Addition of the following:

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