Clinical Trial Protocol and Statistical Analysis Plan

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Original Clinical Trial Protocol

Efficacy of Clarithromycin Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment: a Multicenter Randomized Controlled Trial

Protocol version: 1.0

Approved Date: Jul 8, 2016

Funding Source: Study On Helicobacter pylori Eradication Treatment and Infection Related Risk Factor project funded by Clinical Research Center, Shanghai Jiao Tong University School of Medicine, DLY201608.
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Principal Investigator

Hong Lu, Division of Gastroenterology and Hepatology, Shanghai Institute of Digestive Disease; Key Laboratory of Gastroenterology & Hepatology, Ministry of Health; Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China.

Co-investigator

1. Yunwei Sun, Department of Gastroenterology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

2. Hong Gao, Department of Gastroenterology, Zhongshan Hospital, Fudan University, Shanghai, China.

3. Yan Zhan, Department of Gastroenterology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China.

4. Gang Xu, Department of Gastroenterology and Hepatology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Statistician

Yanyan Song, Department of Biostatistics, Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, China
## Protocol Abstract

<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>Efficacy of Clarithromycin Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment: a Multicenter Randomized Controlled Trial</th>
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<tbody>
<tr>
<td><strong>Trial registration number</strong></td>
<td>ClinicalTrials.gov, NCT02935010</td>
</tr>
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<td><strong>Objective</strong></td>
<td>Comparing the efficacy, safety and compliance of PCR molecular method and genotypic clarithromycin susceptibility-based tailored therapy with empiric modified bismuth quadruple therapy in treating naïve patients with H. pylori infection</td>
</tr>
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<td><strong>Condition</strong></td>
<td>H. pylori infection naïve to treatment</td>
</tr>
<tr>
<td><strong>Study Design</strong></td>
<td>This study is a prospective, superiority-designed, multi-center, open-label, randomized controlled trial. Eligible patients will be randomly allocated to receive either clarithromycin susceptibility-based tailored therapy (tailored therapy) or empirical bismuth quadruple therapy (empirical therapy) in a 3:1 ratio. Six weeks after completion of therapy, H. pylori eradication will be assessed by $^{13}$C-urea breath testing.</td>
</tr>
<tr>
<td><strong>Sample size</strong></td>
<td>500</td>
</tr>
</tbody>
</table>
| **Inclusion Criteria** | 1. Participants with non-ulcer functional dyspepsia or scarred peptic ulcer disease;  
2. Ability and willingness to participate in the study and to sign and give informed consent;  
3. confirmed H. pylori infection with positive results on both rapid urease testing and culture |
| Exclusion Criteria | 1. Previous H. pylori eradication therapy  
|                   | 2. Less than 18 years old  
|                   | 3. With history of H. pylori infection treatment  
|                   | 4. With previous gastric surgery  
|                   | 5. Major systemic diseases  
|                   | 6. Pregnancy or lactation  
|                   | 7. Allergy to any of the study drugs  
|                   | 8. Administration of antibiotics, bismuth, antisecretory drugs in 8 weeks prior to inclusion |

| Arms and Interventions | 1. **Empirical therapy**  
|                        | After review of clarithromycin-taking medical history, if have used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if have not used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid.  
|                        | 2. **Tailored therapy**  
|                        | After molecular genetic assays for identification of resistance to clarithromycin of Helicobacter pylori from biopsy samples, if resistance to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if susceptible to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid. |
### Schedule

**1. Enrollment**

- Obtain written informed form
- Check eligibility criteria
- Collect baseline characteristics
- Undergo upper endoscopy with gastric biopsy and rapid urease testing
- Conduct bacterial culture and antibiotic susceptibility testing

**2. Allocation**

After the written informed consents would be obtained from eligible patients, the independent research assistant will open envelopes by randomization sequence and telephone study staff to give them each patient’s treatment allocation. Eligible patients will be randomly allocated to receive either tailored therapy or empirical therapy in a 3:1 ratio.

**3. Treatment**

Study treatment regimens were all given 14 days. During the treatment period, the subjects kept a diary to monitor compliance and symptoms.

**4. Follow-up**

After the end of treatment, adverse events and compliance was surveyed. Six weeks after completion of therapy, H. pylori eradication was assessed by $^{13}$C-urea breath testing.

### Outcome Measures

**Primary Outcome Measure:**

1. Helicobacter pylori eradication rate

Six weeks after completion of therapy, H. pylori eradication success is
defined as negative result from urea breath test (<4‰)

**Secondary Outcome Measure**

1. Rate of adverse events
2. Compliance rate

**Other Pre-specified Outcome Measures:**

1. H. pylori eradication rate of patients with clarithromycin resistance strains
2. H. pylori eradication rate of patients with clarithromycin sensitive strains
3. Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification
4. Medical cost per patient of tailored or empiric therapy
5. Ratio of medical cost to H. pylori eradication rate of each therapy
6. Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy
# Protocol Schedule

<table>
<thead>
<tr>
<th>Study Period</th>
<th>Enrollment</th>
<th>Allocation</th>
<th>Treatment</th>
<th>Follow-up</th>
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<td>Baseline characteristics</td>
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<td><em>H. pylori</em> culture and susceptibility testing</td>
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<tr>
<td>Adverse events</td>
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<td>Compliance assessment</td>
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</tr>
<tr>
<td>$^{13}$C-urea breath test</td>
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<tr>
<td>Medical cost</td>
<td>√</td>
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</table>
1. Background

*Helicobacter pylori (H. pylori)* resistance to antibiotics was main challenge to previous efficacy antibiotic regimens such as standard triple therapy (1). The prevalence of antibiotic resistance have been markedly increasing with time in most parts of the world. Concordantly, the antimicrobial eradication rate of *H. pylori* has been declining globally (2). In China, clarithromycin, metronidazole and levofloxacin resistance show similar trends, and resistance rates have now reached 21~52% for clarithromycin, 61~95% for metronidazole, 20~54% for levofloxacin, and 21~37% for both clarithromycin and metronidazole (3–5). Standard triple therapy had not been suitable for first-line therapy in China because the pooled eradication rate had declined to 74.5% as a meta-analysis showed (6).

As a general rule, therapy for an infectious disease starts with identification of potentially useful antimicrobials and is largely based on the results of susceptibility testing. Systematic reviews or meta-analysis had proved susceptibility-based therapy was superior to 7–10 day triple therapies as first-line treatment (7–9). Therefore, there is a critical need to antimicrobial susceptibility testing (AST) for individualized analysis of antibiotic resistance prior to definitive treatment (2).

Now culture-based susceptibility testing for *H pylori* is rarely performed in standard practice. This may be due to several major limitations: they are time-consuming, costly, and invasive procedures, culture difficulty, and et al. In the age of molecular diagnostics, assays based on genetic material identification have become common as they enable acquiring rapid and accurate results at relatively low costs. The mechanism of resistance to clarithromycin in *H. pylori* is attributed to a decreased potency in binding of macrolides to the ribosome, caused by point mutations within the *rrl* gene in the peptidyl transferase-encoding region of the 23S rRNA gene. Three major point mutations in two positions, A2142C, A2142G, and A2143G, is the main cause of resistance to clarithromycin (10).
So in this study, we will evaluate the efficacy, safety and compliance of PCR molecular method and genotypic clarithromycin susceptibility-based tailored therapy to treat naïve patients with *H. pylori* infection. The comparator is novel bismuth quadruple therapy which has been proved highly effective.(11)

## 2. Objective

### 2.1 The primary objective

To Comparing the efficacy of tailored therapy with empiric modified bismuth quadruple therapy in treating naïve patients with *H. pylori* infection.

### 2.2 The secondary objective

(1) To compare the safety and compliance of tailored therapy with empiric modified bismuth quadruple therapy;

(2) To evaluate the impact of clarithromycin resistance on eradication rates;

(3) To evaluate the diagnosis accuracy of molecular genetic assays in clarithromycin resistance identification

(4) To evaluate the cost-effectiveness of tailored therapy in treating naïve patients with *H. pylori* infection.

## 3. Follow Diagram
4. Study Design

This study is a prospective, superiority-designed, multi-center, open-label, randomized controlled trial conducted in five hospitals in Shanghai, China including Renji Hospital, Ruijin Hospital, Zhongshan Hospital, Shanghai general Hospital, and Shanghai Tenth People's Hospital.

5. Study Population

5.1 Inclusion criteria

(1) Participants with non-ulcer functional dyspepsia or scarred peptic ulcer disease;

(2) Ability and willingness to participate in the study and to sign and give informed consent;

(3) confirmed H. pylori infection with positive results on both rapid urease testing and culture
5.2 Exclusion criteria

(1) Previous H. pylori eradication therapy
(2) Less than 18 years old
(3) With history of H. pylori infection treatment
(4) With previous gastric surgery
(5) Major systemic diseases
(6) Pregnancy or lactation
(7) Allergy to any of the study drugs
(8) Administration of antibiotics, bismuth, antisecretory drugs in 8 weeks prior to inclusion

5.3 Drop out standard

Subjects who sign the informed consent and do not complete the prescribed observation period regardless of when and where to exit, are called drop out.

(1) Patients found in the study course who do not meet the inclusion criteria or do follow the exclusion standards.
(2) Patients who get other irrelevant diseases that could affect the assessment on the efficiency and safety of the drug during the test.
(3) Patients who receive any drug that influences the assessment on the drug.
(4) Patients who do not strictly take standard medication because of any reason (e.g. severe side effects).

5.4 Termination standard

Test termination refers to that clinical trials stop before the end, for the purpose to protect the rights and interests of subjects and to ensure the test quality.
(1) Sever safety issues occur during the trials;

(2) Intervention drug was proved poor or no efficacy;

(3) Serious medical errors in protocol that makes estimation impossible;

(4) Administration reasons;

(5) Withdraw by State food and drug administration.

6. Allocation and Treatment

6.1 Randomization and blinding

Eligible patients will be randomly allocated to receive either susceptibility-based tailored therapy (tailored therapy) or empirical bismuth quadruple therapy (empirical therapy) in a 3:1 ratio. An independent statistician at Shanghai Jiao Tong University College of Basic Medical Sciences generates the computerized random number sequence and used a permuted block randomization with a block size of eight. All investigators is masked to the randomization sequence. Allocation is concealed in an opaque envelope until the intervention was assigned. Envelopes will be kept at Renji Hospital. After the written informed consents would be obtained from eligible patients, the independent research assistant will open envelopes by randomization sequence and telephone study staff to give them each patient’s treatment allocation. This study is an open-labeled trial and patients are not blinded. The technicians, who perform the H. pylori tests (rapid urease test, culture, antimicrobial susceptibility testing, and urea breath test) or fill in the questionnaires, will be blinded to treatment allocation.

6.2 Intervention

(1) Empirical therapy

After review of clarithromycin-taking medical history, if have used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if have not used clarithromycin, give
esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid for 14 days.

(2) Tailored therapy

After molecular genetic assays for identification of resistance to clarithromycin of Helicobacter pylori from biopsy samples, if resistance to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if susceptible to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid for 14 days.

6.3 Drugs

Esomeprazole, 20mg/tablet, AstraZeneca AB, Sodertalje, Sweden.

Bismuth potassium citrate, 300mg/capsule (110mg elemental bismuth), Dawnrays Pharmaceutical Limited, Suzhou, Jiangsu, China.

Amoxicillin, 250mg/capsule, Ruiyang Pharmaceutical Co. Ltd., Shandong, China.

Clarithromycin, 250mg/tablet, Shanghai Abbott Laboratories Co. Ltd., Shanghai, China.

Metronidazole, 200mg/tablet, Shanghai Xinyi Wanxiang Pharmaceutical Industry Company Limited, Shanghai, China.

7. H. pylori real-time PCR and Culture Based Antibiotic Susceptibility Testing

Tissue samples will be obtained from gastroscopic biopsy.

DNAs will be isolated from biopsy specimens and mutation of clarithromycin resistance will be identified by standard molecular (real-time PCR) instrument systems and The Helicobacter pylori Analyte Specific Reagents (BIOLINE USA Inc., MA,
USA) according to the manufacturer’s guidelines.

The biopsy specimens will be cultured and maintained on brain heart infusion agar medium (OXOID, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (85% N2, 10% CO2, 5% O2) at 37°C. All isolates will be stored in brain heart infusion broth (Difco Laboratory, Detroit, MI, USA) supplemented with 30% glycerol at -80°C. Clinical isolates will be also identified as H. pylori using positive tests for urease, oxidase, catalase and Gram staining.

Minimal inhibitory concentrations (MIC) of clarithromycin and metronidazole will be determined by the two-fold agar dilution method. H. pylori is suspended in saline and measured using a spectrophotometer. The bacterial suspensions (10^8 colony forming units per milliliter) are then plated with an inoculator (Sakuma Seisaku, Tokyo, Japan) onto agar plates containing various concentrations of above antibiotics. After three days of microaerophilic incubation, MIC is defined as the lowest drug concentration that prevented visible growth of bacteria. ATCC43504 is used as the quality control. Clarithromycin >2 µg/mL, and metronidazole >8 µg/mL is defined as resistance breakpoints.

8. Safety evaluation

From the beginning of that patients signed the informed consent and are selected for trial to one month after the end of treatment, any adverse medical events, regardless of whether a causal relationship with the study medication, will be judged to be Adverse Event (AE).

8.1 AE’s degree

Mild: patients are easier to accept without induced questions, or patients have only mild discomfort which does not affect their daily lives and there is no need for clinical treatment.

Moderate: patients actively describe the symptoms that affect the life, but they can
tolerate, which doesn’t need clinical treatment.

Severe: patients have objective manifestations, which significantly affect the life, but patients can or can’t bear, which needs clinical treatment.

**8.2 Records and follow-up of AE**

During the study, AE will be accurately recorded, including the time of occurrence, severity, duration, the measures taken and the outcome. Researchers will follow all the AE until the symptoms of patients disappeared or condition become stable. SAE should be tracked until a proper solution is found even though the study is over.

**8.3 Serious adverse event (SAE)**

(1) The judgments of SAE:

- Death, Life-threatening,
- Leading to hospitalization or prolong hospitalization time,
- Permanent or severe disability,
- Congenital Anomaly/Birth Defect.

(2) SAE report system:

Principal investigator and the hospital ethics committee should be reported and SAE report form should be filled in within 24 hours by phone no matter whether any kinds of SAE are related with the drug in 30 days after the treatment. And the form should be reported to national drug administration in time by principal investigator. SAE should be promptly handled, closely tracked until it is properly solved.

Contact method:

1) Contact persons: Hong Lu 86-13611958022

2) SFDA Safety Supervision

**9. Outcome Measures**
9.1 Primary Outcome Measure:

1. Helicobacter pylori eradication rate

Six weeks after completion of therapy, H. pylori eradication success is defined as negative result from urea breath test (<4‰). If patients would be lost to follow-up urea breath testing, they will be scored as treatment failures in the primary ITT analysis. The eradication rate is defined as the number of patients who successful eradicate *H. pylori* divided by the total number of population for analysis.

9.2 Secondary Outcome Measure

(1) Rate of adverse events

During the 14-day treatment period, the subjects will keep a diary to score any possible side effects or discomforts. The subjects will be asked to grade the severity of adverse events according to their influence on daily activities, experienced as “mild” (transient and well tolerated), “moderate” (causing discomfort and partially interfering with daily activities), or “severe” (causing considerable interference with daily activities). The side effect score recorded is based on the most severe event. Rate of adverse events is defined as the number of patients with any adverse event divided by the total number of population for analysis.

(2) Compliance rate

Compliance was defined as either poor when they had taken less than 80% of the total medication or good when they had taken more than 80% medication. Compliance rate is defined as the number of patients with good compliance divided by the total number of population for analysis.

9.3 Other Pre-specified Outcome Measures:

(1) H. pylori eradication rate of patients with clarithromycin resistance strains

(2) H. pylori eradication rate of patients with clarithromycin sensitive strains
(3) Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification

(4) Medical cost per patient of tailored or empiric therapy

(5) Ratio of medical cost to H. pylori eradication rate of each therapy

(6) Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy

10. Statistical Methods

10.1 Sample size

Assuming 95.7% eradication rate of tailored therapy in our trial, 88.8% eradication rate of empirical therapy, a superiority margin of >0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 340 subjects in susceptibility-based therapy and 114 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration of 10% lost to follow-up, at least 500 participants (374 for tailored therapy and 126 for empiric therapy) is expected to be recruited for the study.

10.2 Statistical Analysis Data

Intention–to-treat (ITT) Analysis population: According to the Intention-to-treat principle: All randomized patients will be included in the ITT analysis. Patients who did not return for a follow-up 13C-UBT will be recorded as treatment failures.

Per-protocol (PP) analysis population: All individuals who violated the study protocol, such as patients not taking at least 80% of treatment drugs, or with unknown post-treatment H. pylori status will be excluded from the PP analysis.

Safety analysis population: The patients with missing safety data in ITT population will excluded from the safety analysis.
10.3 Statistical methods

Comparative superiority of the two groups was assessed through hypothesis testing (one-sided Z test) and derivation of a two-sided 95% confidence interval (CI) of difference based on H. pylori eradication rate. If p-value of the testing less than 0.025 and the lower bound of the 95% CI greater than zero, superiority of susceptibility-based therapy over empirical therapy could be concluded.

Between-group differences were evaluated using Student’s t-test for continuous variables and Pearson’s \( \chi^2 \)/corrected \( \chi^2 \) or Fisher’s exact test for categorical variables, as appropriate. All p-values were two-sided except the testing of superiority, and were considered statistically significant if p-value less than 0.05.

Subgroup analyses of eradication efficacy were performed based on the results of antibiotic susceptibility test and compliance assessment.

11 Ethical principles

The study is registered in ClinicalTrials.gov. The study protocol is approved by the Ethics Committee at all institutions, and the study will be performed according to good clinical practice and the Declaration of Helsinki.

Before each patient is selected into the study, the physician of the study have the responsibility to tell patients or their designated agents the purpose, process, completion and comprehensively introduce possible adverse reactions, risks to bear, possible benefits and other information all-round and detailed, making the patients know their rights. We are ordered to inform the patients of having the right to decide whether to participate in the study, as well as the right to withdraw the study at any time without any discrimination. The patients or their legal representatives should sign the informed consent after carefully reading and fully understanding, and retain the signature page of the copy.
12 References


Sep;70(9):2447–55.


Signature Page

Protocol Title:

Efficacy of Antibiotic Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment: a Multicenter Randomized Controlled Trial

After reviewing the protocol, each investigator and statistician signed this page as an attachment.

Investigator:

I will earnestly fulfill my duties as an investigator in accordance with the Chinese Good Clinical Practice (GCP).

This study will be conducted in accordance with the principles of morality, ethics and science laid down in the Helsinki Declaration and the Chinese GCP. I agree to carry out this clinical trial in accordance with this protocol.

I will be responsible for making timely and appropriate medical decisions for adverse events that occur in subjects during the study period. I know the requirements for correctly reporting serious adverse events and I will record and report these events.

I guarantee that the data will be accurately, completely, timely, and legally filled in the Case Report Form. I will accept the inspections to ensure the quality of the study.

I agree that the findings of the study are published.

Principal Investigator: Hong Lu

Institution: Renji Hospital, School of Medicine, Shanghai Jiao Tong University,
Shanghai, China.

**Signature:** ________________________

**Co-investigator: Yunwei Sun**

**Institution:** Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

**Signature:** ________________________

**Co-investigator: Hong Gao**

**Institution:** Zhongshan Hospital, Fudan University, Shanghai, China

**Signature:** ________________________

**Co-investigator: Yan Zhan**

**Institution:** Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China.

**Signature:** ________________________

**Co-investigator: Gang Xu**

**Institution:** Department of Gastroenterology and Hepatology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

**Signature:** ________________________
Statistical unit

We will conduct statistical analysis based on the Chinese "guideline of Biostatistics for Clinical Trials of Chemical Drugs and Biological Products" and related regulations.

Statistician: Yanyan Song

Institution: Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Signature: ______________________________
Final Clinical Trial Protocol

Efficacy of Antibiotic Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment: a Multicenter Randomized Controlled Trial

Protocol version: 1.1

Revised Date: Jan 10, 2017

Funding Source: Study On Helicobacter Pylori Eradication Treatment and Related Risk Factors of Infection project funded by Clinical Research Center, Shanghai Jiao Tong University School of Medicine, DLY201608.
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Principal Investigator

Hong Lu, Division of Gastroenterology and Hepatology, Shanghai Institute of Digestive Disease; Key Laboratory of Gastroenterology & Hepatology, Ministry of Health; Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China.

Co-investigator

1. Yunwei Sun, Department of Gastroenterology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

2. Hong Gao, Department of Gastroenterology, Zhongshan Hospital, Fudan University, Shanghai, China.

3. Yan Zhan, Department of Gastroenterology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China.

4. Gang Xu, Department of Gastroenterology and Hepatology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Statistician

Yanyan Song, Department of Biostatistics, Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, China
## Protocol Abstract

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<td><strong>Trial registration number</strong></td>
<td>ClinicalTrials.gov, NCT02935010</td>
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<td><strong>Objective</strong></td>
<td>Comparing the efficacy, safety and compliance of pretreatment culture and susceptibility-based tailored therapy with empiric modified bismuth quadruple therapy in treating naïve patients with H. pylori infection</td>
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<tr>
<td><strong>Condition</strong></td>
<td>H. pylori infection naïve to treatment</td>
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<td>This study is a prospective, superiority-designed, multi-center, open-label, randomized controlled trial. Eligible patients will be randomly allocated to receive either susceptibility-based tailored therapy (tailored therapy) or empirical bismuth quadruple therapy (empirical therapy) in a 3:1 ratio. Six weeks after completion of therapy, H. pylori eradication will be assessed by $^{13}$C-urea breath testing.</td>
</tr>
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<td><strong>Sample size</strong></td>
<td>382</td>
</tr>
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| **Inclusion Criteria** | 1. Participants with non-ulcer functional dyspepsia or scarred peptic ulcer disease;  
2. Ability and willingness to participate in the study and to sign and give informed consent;  
3. confirmed H. pylori infection with positive results on both rapid urease testing and culture |
### Exclusion Criteria

1. Previous H. pylori eradication therapy
2. Less than 18 years old
3. With history of H. pylori infection treatment
4. With previous gastric surgery
5. Major systemic diseases
6. Pregnancy or lactation
7. Allergy to any of the study drugs
8. Administration of antibiotics, bismuth, antisecretory drugs in 8 weeks prior to inclusion

### Arms and Interventions

1. **Empirical therapy**
   
   Esomeprazole 20 mg, bismuth potassium citrate 600 mg taken twice a day, amoxicillin 1 g, and metronidazole 500 mg taken three times a day for 14 days.

2. **Tailored therapy**
   
   Tailored therapy is based on antimicrobial susceptibility of clarithromycin, metronidazole and levofloxacin. According to antibiotic resistance pattern included esomeprazole 20mg bid, amoxicillin 1g bid, with a third drug (clarithromycin 500mg bid, metronidazole 400mg bid, or levofloxacin 500mg qd) for susceptible strains, or with bismuth 220mg bid plus metronidazole 400mg qid for triple-resistant strains. Study treatment regimens were all given 14 days.

### Schedule

1. **Enrollment**

   Obtain written informed form
Check eligibility criteria

Collect baseline characteristics

Undergo upper endoscopy with gastric biopsy and rapid urease testing

Conduct bacterial culture and antibiotic susceptibility testing

2. Allocation

After the written informed consents would be obtained from eligible patients, the independent research assistant will open envelopes by randomization sequence and telephone study staff to give them each patient’s treatment allocation. Eligible patients will be randomly allocated to receive either tailored therapy or empirical therapy in a 3:1 ratio.

3. treatment

Study treatment regimens were all given 14 days. During the treatment period, the subjects kept a diary to monitor compliance and symptoms.

4. Follow-up

After the end of treatment, adverse events and compliance was surveyed. Six weeks after completion of therapy, H. pylori eradication was assessed by ¹³C-urea breath testing.

<table>
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<th>Outcome Measures</th>
<th>Primary Outcome Measure:</th>
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<tr>
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<td>1. Helicobacter pylori eradication rate</td>
</tr>
<tr>
<td></td>
<td>Six weeks after completion of therapy, H. pylori eradication success is defined as negative result from urea breath test (&lt;4‰)</td>
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Secondary Outcome Measure
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<td><strong>2. Ratio of medical cost to H. pylori eradication rate of each therapy</strong></td>
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<td><strong>3. Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy</strong></td>
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# Protocol Schedule

<table>
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<tr>
<th>Study Period</th>
<th>Enrollment</th>
<th>Allocation</th>
<th>Treatment</th>
<th>Follow-up</th>
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<tbody>
<tr>
<td>Visit</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
<td>4th</td>
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<tr>
<td>Time point (day)</td>
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<td>0-14</td>
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<tr>
<td>Eligibility screen</td>
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<tr>
<td>Baseline characteristics</td>
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<td>Endoscopy with biopsy and RUT</td>
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<tr>
<td><em>H. pylori</em> culture and susceptibility testing</td>
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<tr>
<td>Written informed content</td>
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<tr>
<td>Therapy Allocation</td>
<td>√</td>
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<tr>
<td>Treatment</td>
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<tr>
<td>Adverse events</td>
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<td>Compliance assessment</td>
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<td>$^{13}$C-urea breath test</td>
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<td>Medical cost</td>
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</table>
1. Background

*Helicobacter pylori* (*H. pylori*) resistance to antibiotics was main challenge to previous efficacy antibiotic regimens such as standard triple therapy(1). The prevalence of antibiotic resistance have been markedly increasing with time in most parts of the world. Concordantly, the antimicrobial eradication rate of *H. pylori* has been declining globally.(2) In China, clarithromycin, metronidazole and levofloxacin resistance show similar trends, and resistance rates have now reached 21%–52% for clarithromycin, 61%–95% for metronidazole, 20%–54% for levofloxacin, and 21%–37% for both clarithromycin and metronidazole.(3–5) Standard triple therapy had not been suitable for first-line therapy in China because the pooled eradication rate had declined to 74.5% as a meta-analysis showed.(6)

As a general rule, therapy for an infectious disease starts with identification of potentially useful antimicrobials and is largely based on the results of susceptibility testing. Systematic reviews or meta-analysis had proved susceptibility-based therapy was superior to 7–10 day triple therapies as first-line treatment.(7–9) Therefore, there is a critical need to antimicrobial susceptibility testing (AST) for individualized analysis of antibiotic resistance prior to definitive treatment.(2)

Now culture-based susceptibility testing for *H pylori* is rarely performed in standard practice. This may be due to several major limitations: they are time-consuming, costly, and invasive procedures, culture difficulty, and et al. So empirical therapy would be needed for most patients over the world. In the settings with high antibiotic resistance, only bismuth quadruple therapy (BQT) is the recommended first-line treatment, and any non-BQT therapy (triple, concomitant, hybrid, or sequential) would not be more effective option.(1) Our previous study showed that substitution of amoxicillin for tetracycline in a 14-day BQT containing 1,600mg/d of metronidazole eradicated 96.4%(54/56) of metronidazole susceptible strains and 93.3%(42/45) of metronidazole resistant strains when used as first-line *H. pylori* therapy.(10). Fewer studies had
compared the effect of susceptibility-based therapy versus BQT for first-line H. pylori eradication, except one.(11)

In this study, we will evaluate the efficacy, safety and compliance of pretreatment culture and susceptibility-based tailored therapy to treat naïve patients with \textit{H. pylori} infection. Susceptibility-based therapy will use Pref. Graham DY et al’s recommendation, in which proton pump inhibitors, amoxicillin, and third susceptible drug triple therapies will be given after testing the susceptibility to clarithromycin, metronidazole and levofloxacin, or bismuth quadruple therapy was given if no susceptible antibiotics.(12) The comparator is novel bismuth quadruple therapy in which tetracycline was replaced by amoxicillin, without reference to antimicrobial susceptibility.

2. Objective

2.1 The primary objective

To Comparing the efficacy of tailored therapy with empiric modified bismuth quadruple therapy in treating naïve patients with H. pylori infection.

2.2 The secondary objective

(1) To compare the safety and compliance of tailored therapy with empiric modified bismuth quadruple therapy;

(2) To evaluate the impact of antibiotic resistance on eradication rates;

(3) To evaluate the cost-effectiveness of susceptibility-based tailored therapy in treating naïve patients with H. pylori infection.

3. Follow Diagram
4. Study Design

This study is a prospective, superiority-designed, multi-center, open-label, randomized controlled trial conducted in five hospitals in Shanghai, China including Renji Hospital, Ruijin Hospital, Zhongshan Hospital, Shanghai general Hospital, and Shanghai Tenth People's Hospital.

5. Study population

5.1 Inclusion criteria

(1) Participants with non-ulcer functional dyspepsia or scarred peptic ulcer disease;

(2) Ability and willingness to participate in the study and to sign and give informed consent;

(3) confirmed H. pylori infection with positive results on both rapid urease testing and culture
5.2 Exclusion criteria

(1) Previous H. pylori eradication therapy

(2) Less than 18 years old

(3) With history of H. pylori infection treatment

(4) With previous gastric surgery

(5) Major systemic diseases

(6) Pregnancy or lactation

(7) Allergy to any of the study drugs

(8) Administration of antibiotics, bismuth, antisecretory drugs in 8 weeks prior to inclusion

5.3 Drop out standard

Subjects who sign the informed consent and do not complete the prescribed observation period regardless of when and where to exit, are called drop out.

(1) Patients found in the study course who do not meet the inclusion criteria or do follow the exclusion standards.

(2) Patients who get other irrelevant diseases that could affect the assessment on the efficiency and safety of the drug during the test.

(3) Patients who receive any drug that influences the assessment on the drug.

(4) Patients who do not strictly take standard medication because of any reason (e.g. severe side effects).

5.4 Termination standard

Test termination refers to that clinical trials stop before the end, for the purpose to protect the rights and interests of subjects and to ensure the test quality.
(1) Sever safety issues occur during the trials;
(2) Intervention drug was proved poor or no efficacy;
(3) Serious medical errors in protocol that makes estimation impossible;
(4) Administration reasons;
(5) Withdraw by State food and drug administration.

6. Allocation and Treatment

6.1 Randomization and blinding

Eligible patients will be randomly allocated to receive either susceptibility-based tailored therapy (tailored therapy) or empirical bismuth quadruple therapy (empirical therapy) in a 3:1 ratio. An independent statistician at Shanghai Jiao Tong University College of Basic Medical Sciences generates the computerized random number sequence and used a permuted block randomization with a block size of eight. All investigators is masked to the randomization sequence. Allocation is concealed in an opaque envelope until the intervention was assigned. Envelopes will be kept at Renji Hospital. After the written informed consents would be obtained from eligible patients, the independent research assistant will open envelopes by randomization sequence and telephone study staff to give them each patient’s treatment allocation. This study is an open-labeled trial and patients are not blinded. The technicians, who perform the H. pylori tests (rapid urease test, culture, antimicrobial susceptibility testing, and urea breath test) or fill in the questionnaires, will be blinded to treatment allocation.

6.2 Intervention

(1) Empirical therapy

Esomeprazole 20 mg, bismuth potassium citrate 600 mg taken twice a day, amoxicillin 1 g, and metronidazole 500 mg taken three times a day for 14 days.
(2) Tailored therapy

Tailored therapy is based on antimicrobial susceptibility of clarithromycin, metronidazole and levofloxacin. a) If the H. pylori strain of one patient was sensitive to clarithromycin, clarithromycin triple therapy (EAC) will be given: esomeprazole 20 mg, amoxicillin 1 g, and clarithromycin 500 mg taken twice a day; b) If the strain was resistant to clarithromycin but sensitive to metronidazole, metronidazole triple therapy (EAM) will be given: esomeprazole 20 mg, amoxicillin 1 g, and metronidazole 500 mg taken twice a day. c) If the strain was resistant to both clarithromycin and metronidazole but sensitive to levofloxacin, levofloxacin triple therapy (EAL) will be given: esomeprazole 20 mg, amoxicillin 1 g taken twice a day, and levofloxacin 500 mg taken once a day. d) If the strain was resistant to all three antibiotics, bismuth quadruple therapy (EBA₂M₄) will be given: esomeprazole 20 mg, bismuth potassium citrate 600 mg, amoxicillin 1 g taken twice a day, and metronidazole 500 mg taken four times a day. Study treatment regimens will be all given 14 days.

6.3 Drugs

Esomeprazole, 20mg/tablet, AstraZeneca AB, Sodertalje, Sweden.

Bismuth potassium citrate, 300mg/capsule (110mg elemental bismuth), Dawnrays Pharmaceutical Limited, Suzhou, Jiangsu, China.

Amoxicillin, 250mg/capsule, Ruiyang Pharmaceutical Co. Ltd., Shandong, China.

Clarithromycin, 250mg/tablet, Shanghai Abbott Laboratories Co. Ltd., Shanghai, China.

Metronidazole, 200mg/tablet, Shanghai Xinyi Wanxiang Pharmaceutical Industry Company Limited, Shanghai, China.

Levofloxacin, 500mg/tablet, Daiichi Sankyo Pharmaceutical Beijjing Co., Ltd., Beijing, China.
7. *H. pylori* Culture and Antibiotic Susceptibility Testing

Tissue samples will be obtained from gastroscopic biopsy. The biopsy specimens will be cultured and maintained on brain heart infusion agar medium (OXOID, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (85% N2, 10% CO2, 5% O2) at 37°C. All isolates will be stored in brain heart infusion broth (Difco Laboratory, Detroit, MI, USA) supplemented with 30% glycerol at -80°C. Clinical isolates will be also identified as *H. pylori* using positive tests for urease, oxidase, catalase and Gram staining.

Minimal inhibitory concentrations (MIC) of clarithromycin, metronidazole, and levofloxacin will be determined by the two-fold agar dilution method. *H. pylori* is suspended in saline and measured using a spectrophotometer. The bacterial suspensions (108 colony forming units per milliliter) are then plated with an inoculator (Sakuma Seisaku, Tokyo, Japan) onto agar plates containing various concentrations of above antibiotics. After three days of microaerophilic incubation, MIC is defined as the lowest drug concentration that prevented visible growth of bacteria. ATCC43504 is used as the quality control. Clarithromycin >2 µg/mL, metronidazole >8 µg/mL, levofloxacin >2 µg/mL is defined as resistance breakpoints.

8. Safety evaluation

From the beginning of that patients signed the informed consent and are selected for trial to one month after the end of treatment, any adverse medical events, regardless of whether a causal relationship with the study medication, will be judged to be Adverse Event (AE).

8.1 AE’s degree

Mild: patients are easier to accept without induced questions, or patients have only mild discomfort which does not affect their daily lives and there is no need for clinical treatment.
Moderate: patients actively describe the symptoms that affect the life, but they can tolerate, which doesn’t need clinical treatment.

Severe: patients have objective manifestations, which significantly affect the life, but patients can or can’t bear, which needs clinical treatment.

**8.2 Records and follow-up of AE**

During the study, AE will be accurately recorded, including the time of occurrence, severity, duration, the measures taken and the outcome. Researchers will follow all the AE until the symptoms of patients disappeared or condition become stable. SAE should be tracked until a proper solution is found even though the study is over.

**8.3 Serious adverse event (SAE)**

(1) The judgments of SAE:

- Death, Life-threatening,
- Leading to hospitalization or prolong hospitalization time,
- Permanent or severe disability,
- Congenital Anomaly/Birth Defect.

(2) SAE report system:

Principal investigator and the hospital ethics committee should be reported and SAE report form should be filled in within 24 hours by phone no matter whether any kinds of SAE are related with the drug in 30 days after the treatment. And the form should be reported to national drug administration in time by principal investigator. SAE should be promptly handled, closely tracked until it is properly solved.

Contact method:

1) Contact persons: Hong Lu 86-13611958022

2) SFDA Safety Supervision
9. Outcome Measures

9.1 Primary Outcome Measure:

1. Helicobacter pylori eradication rate

Six weeks after completion of therapy, H. pylori eradication success is defined as negative result from urea breath test (<4‰). If patients would be lost to follow-up urea breath testing, they will be scored as treatment failures in the ITT analysis. The eradication rate is defined as the number of patients who successfully eradicate *H. pylori* divided by the total number of population for analysis.

9.2 Secondary Outcome Measure

(1) Rate of adverse events

During the 14-day treatment period, the subjects will keep a diary to score any possible side effects or discomforts. The subjects will be asked to grade the severity of adverse events according to their influence on daily activities, experienced as “mild” (transient and well tolerated), “moderate” (causing discomfort and partially interfering with daily activities), or “severe” (causing considerable interference with daily activities). The side effect score recorded is based on the most severe event. Rate of adverse events is defined as the number of patients with any adverse event divided by the total number of population for analysis.

(2) Compliance rate

Compliance was defined as either poor when they had taken less than 80% of the total medication or good when they had taken more than 80% medication. Compliance rate is defined as the number of patients with good compliance divided by the total number of population for analysis.

9.3 Other Pre-specified Outcome Measures:

(1) Medical cost per patient of tailored or empiric therapy
Medical cost of one patient is overall cost including visit, endoscopy, \( H. \) pylori culture, susceptibility testing, eradication treatment, and adverse event treatment.

(2) Ratio of medical cost to \( H. \) pylori eradication rate of each therapy

(3) Ratio of incremental medical cost to incremental \( H. \) pylori eradication rate of tailored compared with empiric therapy

10. Statistical Methods

10.1 Sample size

Assuming 96.5\% eradication rate of tailored therapy in our trial, 88.9\% eradication rate of empirical therapy, a superiority margin of >0, a power of 80\%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 260 subjects in susceptibility-based therapy and 87 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of \( H. \) pylori eradication rate. Taking into consideration of 10\% lost to follow-up, at least 382 participants (286 for tailored therapy and 96 for empiric therapy) is expected to be recruited for the study.

10.2 Statistical Analysis Data

**Intention--to-treat (ITT) Analysis population:** According to the Intention-to-treat principle: All randomized patients will be included in the ITT analysis. Patients who did not return for a follow-up 13C-UBT will be recorded as treatment failures.

**Per-protocol (PP) analysis population:** All individuals who violated the study protocol, such as patients not taking at least 80\% of treatment drugs, or with unknown post-treatment \( H. \) pylori status will be excluded from the PP analysis.

**Safety analysis population:** The patients with missing safety data in ITT population will excluded from the safety analysis.

10.3 Statistical methods
Comparative superiority of the two groups was assessed through hypothesis testing (one-sided Z test) and derivation of a two-sided 95% confidence interval (CI) of difference based on H. pylori eradication rate. If p-value of the testing less than 0.025 and the lower bound of the 95% CI greater than zero, superiority of susceptibility-based therapy over empirical therapy could be concluded.

Between-group differences were evaluated using Student’s t-test for continuous variables and Pearson’s $\chi^2$/corrected $\chi^2$ or Fisher’s exact test for categorical variables, as appropriate. All p-values were two-sided except the testing of superiority, and were considered statistically significant if p-value less than 0.05.

Subgroup analyses of eradication efficacy were performed based on the results of antibiotic susceptibility test and compliance assessment.

### 11 Ethical principles

The study is registered in ClinicalTrials.gov. The study protocol is approved by the Ethics Committee at all institutions, and the study will be performed according to good clinical practice and the Declaration of Helsinki.

Before each patient is selected into the study, the physician of the study have the responsibility to tell patients or their designated agents the purpose, process, completion and comprehensively introduce possible adverse reactions, risks to bear, possible benefits and other information all-round and detailed, making the patients know their rights. We are ordered to inform the patients of having the right to decide whether to participate in the study, as well as the right to withdraw the study at any time without any discrimination. The patients or their legal representatives should sign the informed consent after carefully reading and fully understanding, and retain the signature page of the copy.
12 References


Signature Page

Protocol Title:

Efficacy of Antibiotic Susceptibility-based Tailored Versus Empirc Therapy for Helicobacter pylori First-line Treatment: a Multicenter Randomized Controlled Trial

After reviewing the protocol, each investigator and statistician signed this page as an attachment.

Investigator:

I will earnestly fulfill my duties as an investigator in accordance with the Chinese Good Clinical Practice (GCP).

This study will be conducted in accordance with the principles of morality, ethics and science laid down in the Helsinki Declaration and the Chinese GCP. I agree to carry out this clinical trial in accordance with this protocol.

I will be responsible for making timely and appropriate medical decisions for adverse events that occur in subjects during the study period. I know the requirements for correctly reporting serious adverse events and I will record and report these events.

I guarantee that the data will be accurately, completely, timely, and legally filled in the Case Report Form. I will accept the inspections to ensure the quality of the study.

I agree that the findings of the study are published.

Principal Investigator: Hong Lu

Institution: Renji Hospital, School of Medicine, Shanghai Jiao Tong University,
Shanghai, China.

**Signature:** ____________________________

**Co-investigator: Yunwei Sun**

**Institution:** Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

**Signature:** ____________________________

**Co-investigator: Hong Gao**

**Institution:** Zhongshan Hospital, Fudan University, Shanghai, China

**Signature:** ____________________________

**Co-investigator: Yan Zhan**

**Institution:** Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China.

**Signature:** ____________________________

**Co-investigator: Gang Xu**

**Institution:** Department of Gastroenterology and Hepatology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

**Signature:** ____________________________


Statistical unit

We will conduct statistical analysis based on the Chinese "guideline of Biostatistics for Clinical Trials of Chemical Drugs and Biological Products" and related regulations.

Statistician: Yanyan Song

Institution: Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Signature: ____________________________
## Summary of protocol changes

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<thead>
<tr>
<th>Change</th>
<th>Original</th>
<th>Final</th>
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</thead>
<tbody>
<tr>
<td><strong>Title</strong></td>
<td>Efficacy of Clarithromycin Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment: a Multicenter Randomized Controlled Trial</td>
<td>Efficacy of <strong>Antibiotic</strong> Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment: a Multicenter Randomized Controlled Trial</td>
</tr>
<tr>
<td><strong>2.2 The secondary objective</strong></td>
<td>(1) To compare the safety and compliance of tailored therapy with empiric modified bismuth quadruple therapy; (2) To evaluate the impact of clarithromycin resistance on eradication rates; (3) To evaluate the diagnosis accuracy of molecular genetic assays in clarithromycin resistance identification (4) To evaluate the cost-effectiveness of tailored therapy in treating naïve patients with H. pylori infection.</td>
<td>(1) To compare the safety and compliance of tailored therapy with empiric modified bismuth quadruple therapy; (2) To evaluate the impact of antibiotic resistance on eradication rates; (3) To evaluate the cost-effectiveness of susceptibility-based tailored therapy in treating naïve patients with H. pylori infection.</td>
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</table>
### Summary of protocol changes

<table>
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<th>6.2 Intervention</th>
<th>(1) Empirical therapy</th>
<th>(2) Tailored therapy</th>
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<tbody>
<tr>
<td>naïve patients with H. pylori infection.</td>
<td>After review of clarithromycin-taking medical history, if have used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if have not used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid for 14 days.</td>
<td>Tailored therapy is based on antimicrobial susceptibility of clarithromycin, metronidazole and levofloxacin. a) If the H. pylori strain of one patient was sensitive to clarithromycin, clarithromycin triple therapy (EAC) will be given: esomeprazole 20 mg, amoxicillin 1 g, and clarithromycin 500 mg taken twice a day; b) If the strain was resistant to clarithromycin but sensitive to metronidazole, metronidazole triple therapy (EAM) will be given: esomeprazole 20 mg, amoxicillin 1 g, and metronidazole 500 mg taken twice a day. c) If the strain was resistant to both clarithromycin and metronidazole but sensitive to levofloxacin, levofloxacin triple therapy (EAL) will be given:</td>
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(1) Empirical therapy

Esomeprazole 20 mg, bismuth potassium citrate 600 mg taken twice a day, amoxicillin 1 g, and metronidazole 500 mg taken three times a day for 14 days.

(2) Tailored therapy

Tailored therapy is based on antimicrobial susceptibility of clarithromycin, metronidazole and levofloxacin. a) If the H. pylori strain of one patient was sensitive to clarithromycin, clarithromycin triple therapy (EAC) will be given: esomeprazole 20 mg, amoxicillin 1 g, and clarithromycin 500 mg taken twice a day; b) If the strain was resistant to clarithromycin but sensitive to metronidazole, metronidazole triple therapy (EAM) will be given: esomeprazole 20 mg, amoxicillin 1 g, and metronidazole 500 mg taken twice a day. c) If the strain was resistant to both clarithromycin and metronidazole but sensitive to levofloxacin, levofloxacin triple therapy (EAL) will be given:
<table>
<thead>
<tr>
<th>6.3 Drugs</th>
<th>Esomeprazole, 20mg/tablet, AstraZeneca AB, Sodertalje, Sweden.</th>
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<tr>
<td>Bismuth potassium citrate, 300mg/capsule (110mg elemental bismuth), Dawnrays Pharmaceutical Limited, Suzhou, Jiangsu, China.</td>
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<tr>
<td>Metronidazole, 200mg/tablet, Shanghai Xinyi Wanxiang Pharmaceutical</td>
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|  | esomeprazole 20 mg, amoxicillin 1 g taken twice a day, and levofloxacin 500 mg taken once a day. d) If the strain was resistant to all three antibiotics, bismuth quadruple therapy (EBA$_2$M$_4$) will be given: esomeprazole 20 mg, bismuth potassium citrate 600 mg, amoxicillin 1 g taken twice a day, and metronidazole 500 mg taken four times a day. Study treatment regimens will be all given 14 days. |
| | Esomeprazole, 20mg/tablet, AstraZeneca AB, Sodertalje, Sweden. |
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## Summary of protocol changes

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<tr>
<td>Tissue samples will be obtained from gastroscopic biopsy. DNAs will be isolated from biopsy specimens and mutation of clarithromycin resistance will be identified by standard molecular (real-time PCR) instrument systems and The <em>Helicobacter pylori</em> Analyte Specific Reagents (BIOLINE USA Inc., MA, USA) according to the manufacturer’s guidelines. The biopsy specimens will be cultured and maintained on brain heart infusion agar medium (OXOID, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (85% N2, 10% CO2, 5% O2) at 37°C.</td>
<td>Tissue samples will be obtained from gastroscopic biopsy. The biopsy specimens will be cultured and maintained on brain heart infusion agar medium (OXOID, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (85% N2, 10% CO2, 5% O2) at 37°C.</td>
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<thead>
<tr>
<th>9.3 Other Pre-specified Outcome</th>
<th>(1) H. pylori eradication rate of patients with clarithromycin resistance strains</th>
<th>(1) Medical cost per patient of tailored or empiric therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2) H. pylori eradication rate of patients with clarithromycin sensitive</td>
<td>Medical cost of one patient is overall cost including visit, endoscopy, <em>H. pylori</em> culture, susceptibility testing, eradication treatment, and adverse event</td>
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<thead>
<tr>
<th>Measures:</th>
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<tbody>
<tr>
<td>strains</td>
<td>treatment.</td>
</tr>
<tr>
<td>(3) Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification</td>
<td>(2) Ratio of medical cost to H. pylori eradication rate of each therapy</td>
</tr>
<tr>
<td>(4) Medical cost per patient of tailored or empiric therapy</td>
<td>(3)Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy</td>
</tr>
<tr>
<td>(5) Ratio of medical cost to H. pylori eradication rate of each therapy</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample size</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Assuming 95.7% eradication rate of tailored therapy in our trial, 88.8% eradication rate of empirical therapy, a superiority margin of &gt;0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 340 subjects in susceptibility-based therapy and 114 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration</td>
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</tr>
</tbody>
</table>
### Summary of protocol changes

<table>
<thead>
<tr>
<th>Consideration of 10% lost to follow-up, at least 500 participants (374 for tailored therapy and 126 for empiric therapy) is expected to be recruited for the study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>of 10% lost to follow-up, at least 382 participants (286 for tailored therapy and 96 for empiric therapy) is expected to be recruited for the study.</td>
</tr>
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Original Statistical Analysis Plan

This statistical analysis plan includes the details of study sample size calculation and statistical methods for analysis of outcomes and safety.

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2. Definition of populations for analysis ........................................... 57
3. Evaluation of baseline characteristics .......................................... 57
4. Evaluation of primary outcomes .................................................... 59
5. Evaluation of secondary outcomes ............................................... 60
6. Additional analyses ................................................................. 61
7. Table Design ........................................................................... 63
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According to the Intention-to-treat principle: All randomized patients will be included in the ITT analysis. Patients who did not return for a follow-up 13C-UBT will be recorded as treatment failures.

2.2 Per-protocol (PP) analysis population

All individuals who violated the study protocol, such as patients not taking at least 80% of treatment drugs, or with unknown post-treatment H. pylori status will be excluded from the PP analysis.

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The patients with missing safety data in ITT population will excluded from the safety analysis.

3. Evaluation of baseline characteristics

3.1 presentation of baseline characteristics
Variables of baseline characteristics collected are age (years), gender (male/female), diagnosis (Dyspepsia/PUD), clarithromycin-taking history, 23S rRNA mutation, antibiotic resistance pattern, poor adherence, and loss of follow-up. The following table shows the types and presentation of variables.

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</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>(Phenotypic)</td>
<td></td>
<td></td>
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<td>Clarithromycin, n (%)</td>
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<td>Number (percentage)</td>
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<td>Categorical</td>
<td>Number</td>
</tr>
<tr>
<td><strong>Loss of follow-up</strong></td>
<td>Categorical</td>
<td>Number</td>
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</tbody>
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Comparisons of baseline characteristics between the tailored therapy and empiric therapy will be performed in the ITT analysis populations. Comparisons between the both groups will be performed using the Pearson’s χ², corrected χ² or Fisher’s exact test for categorical variables and using the Student t test for continuous variables.
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Six weeks after completion of therapy, H. pylori eradication success is defined as negative result from urea breath test (<4‰). If patients would be lost to follow-up urea breath testing, they will be scored as treatment failures in the ITT analysis.

The eradication rate is defined as the number of patients who successful eradicate *H. pylori* divided by the total number of population for analysis. These data are presented with number, percentage, and 95% confidence interval (CI).

4.2 Testing the differences of eradication rates

Testing the differences of eradication rates between the tailored therapy and empiric therapy will be performed using the Pearson’s $\chi^2$, corrected $\chi^2$ or Fisher’s exact test in the ITT and PP analysis populations.

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Testing the superiority of tailored therapy group to empiric therapy group will be performed in the ITT and PP analysis populations using hypothesis testing (one-sided Z test) and derivation of a two-sided 95% confidence interval (CI) of difference based on H. pylori eradication rate. If p-value of the testing less than 0.025 and the lower bound of the 95% CI greater than zero, superiority of susceptibility-based therapy over empirical therapy could be concluded.

5. Evaluation of secondary outcomes

5.1 Rate of adverse events
During the 14-day treatment period, the subjects will keep a diary to score any possible side effects or discomforts. The subjects will be asked to grade the severity of adverse events according to their influence on daily activities, experienced as “mild” (transient and well tolerated), “moderate” (causing discomfort and partially interfering with daily activities), or “severe” (causing considerable interference with daily activities). The side effect score recorded is based on the most severe event. Rate of adverse events is defined as the number of patients with any adverse event divided by the total number of population for analysis. These data are presented with number, percentage.

Comparisons of presence of each adverse event and any adverse event between the tailored therapy and empiric therapy will be performed in the safety analysis populations using the Pearson’s $\chi^2$, corrected $\chi^2$ or Fisher’s exact test.

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Compliance was defined as either poor when they had taken less than 80% of the total medication or good when they had taken more than 80% medication. Compliance rate is defined as the number of patients with good compliance divided by the total number of population for analysis. These data are presented with number, percentage.

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(1) Medical cost per patient of tailored or empiric therapy

Medical cost of one patient is overall cost including visit, endoscopy, H. pylori culture, susceptibility testing, eradication treatment, and adverse event treatment.
Comparisons of medical cost between the tailored therapy and empiric therapy will be performed in the PP analysis populations using Student t test.

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Ratio of medical cost to H. pylori eradication rate of each therapy is defined as average medical cost of the therapy divided by H. pylori eradication rate of one therapy.

(3) Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy

If one therapy cost less and achieved higher eradication rate than the other one, it will be referred to as dominant strategy. If one therapy yielded higher eradication rate but also cost more, an incremental cost-effectiveness ratio (ICER) will be calculated. Obviously, the tailored therapy will cost more because of additional bacterial culture and antibiotic susceptibility testing. So, if eradication rate of the tailored therapy was higher, the ICER of the tailored therapy will be calculated as the ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy (in terms of RMB per incremental eradication percentage). If eradication rate of the tailored therapy was equal to or lower than the empiric therapy, the tailored therapy will not be more cost-effective option and the economic evaluation will not be conducted.

6. Additional analyses

6.1 Antibiotic resistance rate

Minimal inhibitory concentrations (MIC) of clarithromycin >2 µg/mL, metronidazole >8 µg/mL, levofloxacin >2 µg/mL is defined as resistance breakpoints. Antibiotic resistance rate is defined as the number of patients with antibiotic resistant strains divided by the total number of population for analysis. These data are presented
with number, percentage.

6.2 Effect of antibiotic resistance on eradication rate

Comparisons of eradication rate of one therapy between antibiotic susceptible and resistant strains will be performed in the PP analysis populations using the Pearson’s $\chi^2$, corrected $\chi^2$ or Fisher’s exact test.

Additional comparisons of eradication rate of antibiotic resistant strains between the tailored therapy and the empiric therapy will be performed in the PP analysis populations using the Pearson’s $\chi^2$, corrected $\chi^2$ or Fisher’s exact test.

6.3 Effect of 23S rRNA mutation on eradication rate

Comparisons of eradication rate of clarithromycin-containing therapy between strains with 23S rRNA mutation and those without 23S rRNA mutation will be performed in the PP analysis populations using the Pearson’s $\chi^2$, corrected $\chi^2$ or Fisher’s exact test.

6.4 Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification

Using culture based antibiotic susceptibility testing as the gold standard, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 23S rRNA mutation in identification of *H. pylori* resistance to clarithromycin will be estimated.

\[
Sensitivity = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}
\]

\[
Specificity = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}
\]

\[
PPV = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false positives}}
\]

\[
NPV = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false negatives}}
\]

**True positives:** the strains with 23S rRNA mutation and MIC of clarithromycin $>2$
µg/mL

**False positives:** the strains with 23S rRNA mutation but MIC of clarithromycin ≤2 µg/mL

**True negatives:** the strains without 23S rRNA mutation and MIC of clarithromycin ≤2 µg/mL

**False negatives:** the strains without 23S rRNA mutation but MIC of clarithromycin >2 µg/mL

6.5 Effect of presence of adverse events on compliance.

Comparisons of compliance rate of one therapy between presence and absence of adverse event will be performed in the safety analysis populations using the Pearson’s χ², corrected χ² or Fisher’s exact test.

6.6 Effect of compliance on eradication rate

Comparisons of eradication rate of one therapy between good and poor compliance will be performed in the ITT analysis populations who had UBT results using the Pearson’s χ², corrected χ² or Fisher’s exact test.

7. Table Design

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis (Dyspepsia/PUD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin-taking history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23S rRNA mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic resistance pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Levofloxacin, n (%)  
<table>
<thead>
<tr>
<th>All-susceptible, n (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Triple-resistant, n (%)</td>
<td></td>
</tr>
</tbody>
</table>

Poor adherence*  
Loss of follow-up  

NUD, non-ulcer dyspepsia; PUD, healed peptic ulcer disease.  
* Poor adherence, took less than 80% of drugs

Table 2. Eradication rate of each group in ITT and PP analysis

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
<th>Difference</th>
<th>p value for inequality*</th>
<th>p value for superiority **</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT (%)</td>
<td>n/N</td>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP (%)</td>
<td>n/N</td>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI= confidence interval; ITT = intention-to-treat; PP = per-protocol.  
* The p values were for testing the difference between tailored therapy group and empiric therapy group.  
** The p values were for testing the superiority of tailored therapy group to empiric therapy group.

Table 3. antibiotic resistance pattern of ITT population

<table>
<thead>
<tr>
<th>antibiotic resistance pattern (CLA-MET-LEV)</th>
<th>ITT population</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>All susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-S-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-S-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-R-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-S-R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dual resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-R-S</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CLA, clarithromycin; LEV, levofloxacin; MET, metronidazole; R, resistant; S, susceptible.

Table 4. Eradication rates of tailored and empiric therapy in the presence of antibiotic resistance in the per-protocol population

<table>
<thead>
<tr>
<th>PP population</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>23S rRNA mutation (genotypic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin resistance (phenotypic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole resistance (phenotypic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Adverse events and adherence in the treatment groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, n/N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None/Mild/Moderate/Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE variety</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste alteration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspepsia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>......</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>......</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Skin rash

**Discontinued drugs**

*because of AEs*

**Good Adherence (Took at least 80% of drugs)**

| | 
|---|---|
| **Subjects without AEs** | 
| **Subjects with AEs** | 
| **Total** | 

AE, adverse event.
Final SAP

Final Statistical Analysis Plan

This statistical analysis plan includes the details of study sample size calculation and statistical methods for analysis of outcomes and safety.

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Ratio of medical cost to H. pylori eradication rate of each therapy is defined as average medical cost of the therapy divided by H. pylori eradication rate of one therapy.

(3) Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy

If one therapy cost less and achieved higher eradication rate than the other one, it will be referred to as dominant strategy. If one therapy yielded higher eradication rate but also cost more, an incremental cost-effectiveness ratio (ICER) will be calculated. Obviously, the tailored therapy will cost more because of additional bacterial culture and antibiotic susceptibility testing. So, if eradication rate of the tailored therapy was higher, the ICER of the tailored therapy will be calculated as the ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy (in terms of RMB per incremental eradication percentage). If eradication rate of the tailored therapy was equal to or lower than the empiric therapy, the tailored therapy will not be more cost-effective option and the economic evaluation will not be conducted.

6. Additional analyses

6.1 Antibiotic resistance rate

Minimal inhibitory concentrations (MIC) of clarithromycin >2 µg/mL, metronidazole >8 µg/mL, levofloxacin >2 µg/mL is defined as resistance breakpoints. Antibiotic resistance rate is defined as the number of patients with antibiotic resistant strains divided by the total number of population for analysis. These data are presented with number, percentage.

6.2 Effect of antibiotic resistance on eradication rate

Comparisons of eradication rate of one therapy between antibiotic susceptible and
resistant strains will be performed in the PP analysis populations using the Pearson’s χ2, corrected χ2 or Fisher’s exact test.

Additional comparisons of eradication rate of antibiotic resistant strains between the tailored therapy and the empiric therapy will be performed in the PP analysis populations using the Pearson’s χ2, corrected χ2 or Fisher’s exact test.

6.3 **Effect of presence of adverse events on compliance.**

Comparisons of compliance rate of one therapy between presence and absence of adverse event will be performed in the safety analysis populations using the Pearson’s χ2, corrected χ2 or Fisher’s exact test.

6.4 **Effect of compliance on eradication rate**

Comparisons of eradication rate of one therapy between good and poor compliance will be performed in the ITT analysis populations who had UBT results using the Pearson’s χ2, corrected χ2 or Fisher’s exact test.

### 7. Table Design

**Table 1. Baseline characteristics**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis (Dyspepsia/PUD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic resistance pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levofoxacin, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-susceptible, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triple-resistant, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor adherence*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
NUD, non-ulcer dyspepsia; PUD, healed peptic ulcer disease.

* Poor adherence, took less than 80% of drugs

Table 2. Eradication rate of each group in ITT and PP analysis

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
<th>Difference</th>
<th>p value for inequality*</th>
<th>p value for superiority **</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT (%) n/N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP (%) n/N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI = confidence interval; ITT = intention-to-treat; PP = per-protocol.

* The p values were for testing the difference between tailored therapy group and empiric therapy group.

** The p values were for testing the superiority of tailored therapy group to empiric therapy group.

Table 3. antibiotic resistance pattern of ITT population

<table>
<thead>
<tr>
<th>antibiotic resistance pattern (CLA-MET-LEV)</th>
<th>ITT population</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>All susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-S-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-S-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-R-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-S-R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dual resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-R-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-S-R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-R-R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triple resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-R-R</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CLA, clarithromycin; LEV, levofloxacin; MET, metronidazole; R, resistant; S, susceptible.
Table 4. Eradication rates of tailored and empiric therapy in the presence of antibiotic resistance in the per-protocol population

<table>
<thead>
<tr>
<th>PP population</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levofloxacin resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistance pattern (Clarithromycin-Metronidazole-Levofloxacin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-X-X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-S-X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-R-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-R-R</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R=resistant; S=susceptible; X=resistant or susceptible.

Table 5. Adverse events and adherence in the treatment groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tailored therapy</th>
<th>Empiric therapy</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, n/N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None/Mild/Moderate/Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE variety</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste alteration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspepsia</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>......</td>
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<td></td>
<td></td>
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<td>......</td>
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<td></td>
</tr>
</tbody>
</table>
Skin rash

<table>
<thead>
<tr>
<th>Discontinued drugs because of AEs</th>
</tr>
</thead>
</table>

**Good Adherence (Took at least 80% of drugs)**

<table>
<thead>
<tr>
<th>Subjects without AEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with AEs</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

AE, adverse event.
## Summary of Statistical Analysis Plan Changes

<table>
<thead>
<tr>
<th>Change</th>
<th>Original</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sample size estimation</td>
<td>Assuming 95.7% eradication rate of tailored therapy in our trial, 88.8% eradication rate of empirical therapy, a superiority margin of &gt;0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 340 subjects in susceptibility-based therapy and 114 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration of 10% lost to follow-up, at least 500 participants (374 for tailored therapy and 126 for empiric therapy) is expected to be recruited for the study.</td>
<td>Assuming 96.5% eradication rate of tailored therapy in our trial, 88.9% eradication rate of empirical therapy, a superiority margin of &gt;0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 260 subjects in susceptibility-based therapy and 87 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration of 10% lost to follow-up, at least 382 participants (286 for tailored therapy and 96 for empiric therapy) is expected to be recruited for the study.</td>
</tr>
<tr>
<td>3.1 presentation of</td>
<td>Variables of baseline characteristics collected are age (years), gender (male/female), diagnosis (Dyspepsia/PUD), clarithromycin-taking</td>
<td>Variables of baseline characteristics collected are age (years), gender (male/female), diagnosis (Dyspepsia/PUD), clarithromycin-taking</td>
</tr>
</tbody>
</table>
### Summary of SAP changes

<table>
<thead>
<tr>
<th>baseline characteristics</th>
<th>history, 23S rRNA mutation, antibiotic resistance pattern, poor adherence, and loss of follow-up. The following table shows the types and presentation of variables.</th>
</tr>
</thead>
</table>

#### 6. Additional analyses

<table>
<thead>
<tr>
<th>6.3 Effect of 23S rRNA mutation on eradication rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparisons of eradication rate of clarithromycin-containing therapy between strains with 23S rRNA mutation and those without 23S rRNA mutation will be performed in the PP analysis populations using the Pearson’s $\chi^2$, corrected $\chi^2$ or Fisher’s exact test.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6.4 Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using culture based antibiotic susceptibility testing as the gold standard, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 23S rRNA mutation in identification of <em>H. pylori</em> resistance to clarithromycin will be</td>
</tr>
<tr>
<td>7. Table Design</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>