This supplement contains the following items:

1. Protocol Ver 1.0 (the original protocol), 2.0 (updated before patients recruitment), 3.0 (the final protocol), and summary of changes.

2. Original statistical analysis plan, final statistical analysis plan, and summary of changes.

Thymosin alpha1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis

Protocol and Statistical Analysis Plan Amendment History

Contents

Thymosin Alpha 1 in the Prevention of Pancreatic Infection Following Acute Necrotizing Pancreatitis

Protocol Ver: 1.0 dated May. 11. 2015

Principal Investigator

Professor. Weiqin Li

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Nantong people's Hospital

The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School

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1.Abrreviations

AP	acute pancreatitis
IPN	infected pancreatic necrosis
ANP	acute necrotizing pancreatitis
CRP	C-reaction protein
HLA	human leukocyte antigen
WBC	white blood cell
PMBC	peripheral blood mononuclear cell
TLR	toll-like receptor
DC	dendritic cell
CT	computered tomography
MRI	magnetic resonance imaging
APACHE II	Acute Physiology and Chronic Health Evaluation II
RRT	Renal replacement therapy
MV	Mechanical ventilation
CDMC	Coordinating and Data Management Center
DSMB	Data and Safety Monitoring Board
SAE	Serious adverse events
ICU	Intensive care unit
SOFA	Sequential Organ Failure Assessment
NOK	Next Of Kin
ITT	Intention-to-treat
FAS	Full-Analysis Set
PPS	Per-protocol set
SS	Safety set
AEs	Adverse events

2. Study Administrative information

2.1 Steering and management committee

The steering and management committee is responsible for the approval of the full protocol, database, and related methods. The members of the committee will also oversee the implementation of the study and play an advisory role.

Members of the steering committee are listed below

Prof. Weiqin Li (Principal investigator)

Prof. Zhihui Tong

Dr. Lu Ke

Dr. Jing Zhou

Prof. Wenhua He

Prof. Weili Gu

Prof. Jianfeng Tu

Prof. Mingdong Liu

2.2 Coordinating and data management center

Coordinating and data management centers (CDMC) will be organized before the implementation of the current study. They are responsible for the day to day management of the trial, assistance for ethic application in each center, protocol and case report form design, online database design and maintenance, protocol training for the participating centers, randomization, data entry and quality control, severe adverse event monitor and notification and data analysis. The CDMC plans to meet before enrollment, three months after initial enrollment, and six months after initial enrollment to ensure qualified data entry.

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Members of DSMB are listed below

Prof. Qiang Li

Dr. Mengjie Lu

Prof. Wenkui Yu

3. Background and rationale

3.1 The role of immunosuppression and infected pancreatic necrosis in critical care

Infected pancreatic necrosis (IPN) and its related septic complications are the major causes of death in patients with acute necrotizing pancreatitis(ANP). When compared with patients with sterile necrosis, patients with IPN suffered a substantial increase in mortality ranging from 14% to 69% due to sepsis and its related multiple organ failure, despite advances in critical care, surgical interventions, and antibiotics. Therefore, the prevention of pancreatic infection is of great clinical value in the treatment of ANP. In past years, numerous attempts had been made to prevent or delay the development of IPN, including antibiotic prophylaxis, selective gut decontamination, and probiotics, but none of them has been clinically proved with high-quality evidence [3-5]. Immunosuppression and disorders characterized by decreased HLA-DR expression and unbalanced CD3/CD4+/CD8+ T cells of peripheral blood mononuclear cell (PBMC) are reported to be associated with the development of IPN [6, 7]. Our previous study also found that early enteral could moderate the excessive immune response during the early stage of severe acute pancreatitis without leading to subsequent immunosuppression and ultimately improve the outcome of the patients. Thus immunomodulatory treatment could potentially intervene in the evolution of secondary infection of pancreatic necrosis, resulting in better outcomes. Unfortunately, study regarding the immune status in patients with any type of acute pancreatitis (AP) is rare, let alone appropriate treatment aiming to balance patients" immune function.

3.2 The rationale for conducting this study

Thymosin alpha 1 has been shown to have immunomodulatory properties and is reported to be beneficial in patients with sepsis [9, 10], majorly through the involvement of distinct Toll-like receptors (TLRs) acting on different dendritic cells (DC) subsets

and involving the MyD88-dependent signaling pathway. However, the effect of thymosin alpha 1 in patients with AP is rarely reported in the literature. The only randomized clinical study conducted by our group years before proved that the use of Thymosin alpha 1 is associated with improved cellular immunity and reduced infection rate in a group of ANP patients. However, no further study was published yet, and the clinical significance of this study is limited due to the small sample size and single-center nature. Therefore, a multi-center, randomized study with a large sample size and proper design is warranted to evaluate the role of Thymosin Alpha 1 in treating ANP.

4. Study Design

4.1 Aims and hypotheses

To evaluate the effects of Tα1 used in the early phase on preventing pancreatic infection, immunomodulation, and clinical outcomes in patients with ANP.

4.2 General study setting

The present clinical interventional study will be performed in 10-15 different sites across China. It is an investigator-initiated, randomized, multi-centered, double-blinded, placebo-controlled study.

5. Study population

5.1 Patient recruitment

We are going to recruit approximately 510 patients presenting to the participating sites across China into the study. Based on the volume of all the participating centers, the aim should be able to be achieved within the study period.

5.2 Eligibility Criteria

5.2.1 Inclusion Criteria

1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of acute necrotizing pancreatitis (ANP), serum amylase at least three times the upper limit of normal, and/or characteristic findings of ANP on computed tomography;

2. Within a week from the onset of the disease;

3. Age between 18 to 70 years old;

4.APACHEII≥8 and/or Ranson score ≥3

5.2.2 Exclusion Criteria

1. Pregnant pancreatitis;

2. Receive percutaneous drainage or surgery before randomization or need of early surgery due to abdominal compartment syndrome, etc.

3. Patients with a known history of severe cardiovascular, respiratory, renal, hepatic, hematologic, or immunologic disease defined as (1) greater than New York Heart Association class II heart failure, (2) active myocardial ischemia, or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with the requirement for home oxygen;

4. Patients with preexisting immune disorders such as AIDS.

6. Main methods and materials

6.1 Patients screening procedures

All patients presenting to each participating hospital will be assessed by the treating physician and received medical care immediately according to the current best clinical practice. The treating clinician team will be responsible for identifying potential patients and contact the TRACE coordinator or a member of the TRACE study team who will assess the patients for inclusion and exclusion criteria and eligibility into the study. Screening tools will be provided, and a screening log will be kept.

Each participating center will be led by a TRACE project leader (Local Principal Investigator) and a site coordinator. The former will be responsible for the prescription of the Thymosin alpha 1 or matching placebo and monitor the TRACE trial, and the latter will be responsible for daily screening and data management.

6.2 Recruitment

All patients admitted to the participating sites will be considered for enrollment in the study and assessed for inclusion and exclusion criteria. Where possible, informed consent will be obtained from the patient or next of kin (NOK) before enrollment into the study.

6.3 Randomization procedures

6.3.1 Sequence generation

The sequence generation will be performed by the Randomization Allocation Tool v. 1.1.4 (RAT, Ratjin, Nanjing). After the initial enrollment, participants will be randomly assigned in a 1:1 ratio to either the treatment group or the control group in a double-

blinded manner according to internet-based computer-generated random numbers in block sizes of 4. The random assignment will be conducted by a third party independent of the study, and the assignment records will not be disclosed until the end of the study.

6.3.2 Blinding method

After the acquisition of written informed consent and the completion of baseline measurements, the allocation will be sequentially delivered to the clinical investigator who is not involved in outcome assessment and is responsible for patient care after entering basic information into the RAT. Participants, data collectors, and investigators assessing outcome data will be blinded to the treatment allocation to minimize potential sources of bias.

6.4. Study procedures

6.4.1 Interventional arms

After confirming that a participant meets the inclusion criteria and does not meet any of the exclusion criteria, the study protocol flow of participants is outlined in Figure1.

Arm#1 Thymosin Group

In addition to the standard treatment, thymosin alpha 1 therapy will be started after randomization: 1.6mg I.H q12h for the first 7 days and 1.6mg I.H, qd for the following 7 days or until discharge.

Arm#2 Placebo group

Placebo will be given at the same dose as Thymosin alpha 1in addition to the standard treatment.

The drug regimen will last for 14 consecutive days, as shown in Figure 1. If the patient dies or is discharged before completing the designed regimen, the rest of the investigational drug will be waived and recycled by the sponsor. The enrolled patients will be followed up until hospital death or discharge.

6.4.2 General management

All patients should receive initial standard treatment, including fluid resuscitation, early enteral nutrition, routine medical treatment, mechanical ventilation if needed, and continuous renal replacement therapy (CRRT) if needed in the light of recently published guidelines. Prophylactic antibiotics are not recommended. All participating centers are able to offer appropriate intensive care in case the patients require organ support or a continuous monitor. The necrotic collection will be intervened when infection was suspected or confirmed, but the intervention should be optimally delayed for 4 weeks where possible.

When pancreatic infection occurs, either surgical or endoscopic step-up approach based on the location of the necrotic collection and technical availability in each participating center will be applied. Initial drainage is optimally performed after the necrotic collection is well walled-off. Principally, either percutaneous catheter drainage or endoscopic transluminal placement of double pig-tail stents are the primary choices of treatment.

7. Outcome measures

7.1 Primary outcome measures

All patients will be followed until hospital death or discharge. The occurrence of IPN at day 28 will serve as the primary outcome measure of the trial.

7.2 Secondary outcome measures

1. Occurrence of new-onset organ failure and persistent organ failure;
2. C-reactive protein(CRP), HLA-DR expression on monocytes, and other laboratory results like white blood cell(WBC) count and lymphocyte count on day0, day 7, and day14 of the observation;
3. Occurrence of bleeding requiring intervention;

4. Occurrence of gastrointestinal fistula;

5. In-hospital mortality;

6. Pancreatic fistula;

7. Need of percutaneous or endoscopic transluminal drainage;

8. Need of minimally-invasive necrosectomy;

9. Need of open surgery;

10. Length of intensive care unit(ICU) stay;

11. Length of hospital stay;

12. In-hospital cost.

7.3 Definition of outcomes

Pancreatic infection: The diagnosis of pancreatic infection/infected pancreatic necrosis (IPN) will be based on the international guidelines when one or more of the following were present: gas bubbles within (peri) pancreatic necrosis on computed tomography; a positive culture of (peri) pancreatic necrosis obtained by image-guided fine-needle aspiration; a positive culture of (peri) pancreatic necrosis obtained during the first drainage and/or necrosectomy.

New-onset: Events occurring after randomization and not present 24 hours before randomization.

Organ failure is defined as an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 points or more for each organ system (Respiration, Cardiovascular, Renal).

New receipt of organ support therapy: Requirement of organ support therapy (mechanical ventilation, renal replacement therapy, and vasoactive agents) not applied at randomization.

8. Ethics considerations

8.1 Ethical issues of this study

The major ethical considerations include:

1. Some potential participants are unable to give consent for themselves;

2. The thymosin alpha 1 therapy may have some side effects.

This study has been approved by the ethics committee of the Jinling Hospital, which is the sponsor of the trial. Ethics approval of each participating center is required before initiation of enrollment.

8.2 Potential risks and benefits

The thymosin α1, which is used in the validation of the patient trial is presented free by the manufacturer (SciClone Pharmaceutical (China) Co., Ltd.). No specific monetary compensation is available for each participant in this study.

8.3 Consent and confidentiality

Informed consent is required for each participant of this study, either signed by the patient himself or next of kin. All the data stored in the electronic database are de-identified to guarantee patients" privacy.

8.4 Dissemination policy

All the principal investigators of the participating sites and the sponsor will have full access to the data after the conclusion of the study. Anyone who wants to do a post-hoc analysis needs to submit a formal writing proposal to the *writing and publication committee*. The only approved author can have access to the database.

9. Data management, analysis, and statistics

9.1 Data collection

All the data that is necessary to define baseline patient characteristics, the implementation of the study intervention, potential confounding co-interventions, and outcomes will be collected.

The principal investigator of each center will be responsible for patient enrollment and data input. A group of statisticians will be accountable for the pre-definition of statistical analysis and subgroup analysis.

A web-based electrical database will be used for data collection and storage. All data will be input by the principal investigator or nominated investigator (less than two for each participating center) approved by the principal investigator. Training for data entry will be performed by the supplier of the electrical database and the sponsor of the trial

9.2 Sample size

The prevalence of pancreatic infection was reported to be around 25% in ANP episodes. To demonstrate a 40% reduction in the prevalence of pancreatic infection considering the results of our pilot study [11] with 80% power at a two-sided alpha level of 0.05, we projected an estimated sample size of 500 participants using the PASS software (PASS, NCSS software, Kaysville, USA). Considering possible 2% withdrawal, we planned to randomize 510 patients in total.

9.3 Basic principle of analysis

The statistical analysis for outcome measures will be based on the intention-to-treat (ITT) population. Missing data will be handled by multiple imputation to evaluate the robustness of the primary endpoint analyses. The normality of continuous variables was

examined using skewness and kurtosis. Continuous variables were compared using the Mann-Whitney method or Student's T-test depending on the distribution of the data. Categorical data were expressed as numbers and percentages. The between-group difference will be compared using the chi-squared test. Statistical tests will be two-sided, and p values < 0.05 will be accepted as significant for the endpoints.

10. Safety issues

10.1 Data and safety monitoring board

The data and safety monitoring board (DSMB) is an independent group of experts that offers advice during the implementation of the study. The DSMB can recommend that a trial be stopped early because of concerns about participant safety or because the main research question has been answered.

10.2 Adverse events

Adverse events (AEs) are defined in accordance with the National Cancer Institute-Common Terminology Criteria for Adverse Events (National Cancer Institute-Common Terminology Criteria for Adverse Events) as any untoward medical occurrence in a patient or clinical investigation subject administered an investigational intervention and which does not necessarily have to have a causal relationship with this treatment.

It is recognized that some patients admitted to ICUs will experience a number of common aberrations in laboratory values, signs and symptoms due to the severity of the underlying disease and the impact of standard therapies. These will not necessarily constitute an adverse event unless they require significant intervention or are considered to be of concern in the investigator's clinical judgment.

In all cases, the condition or disease underlying the symptom, sign or laboratory

value should be reported, e.g., renal failure rather than hyperkalemia, and agitation rather than self-extubation.

10.3 Serious adverse events

SAEs are defined when any untoward medical occurrence that:

1. Results in death
2. Is life-threatening
3. Requires inpatient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability/incapacity
5. Is a congenital anomaly/birth defect
6. Is an important medical event that may require intervention to prevent one of the previously listed outcomes.

In this study, all SAEs will be reported regardless of suspected causality.

10.4 Monitoring of potential adverse events

The DSMB will be responsible for overseeing all subjects" safety and monitoring total mortality and serious adverse events. All serious adverse events occurring during the trial will be reported to the Coordinating and data management center (CDMC) within 48 hours. Minimum information to report will include:

1. Initials of the patient and study number
2. Course and nature of the event
3. An investigator's opinion of the relationship between study involvement and the event (unrelated, possibly, probably, or definitely related)
4. Whether treatment is required and what treatment was applied

The contact information for CDMC

Telephone number

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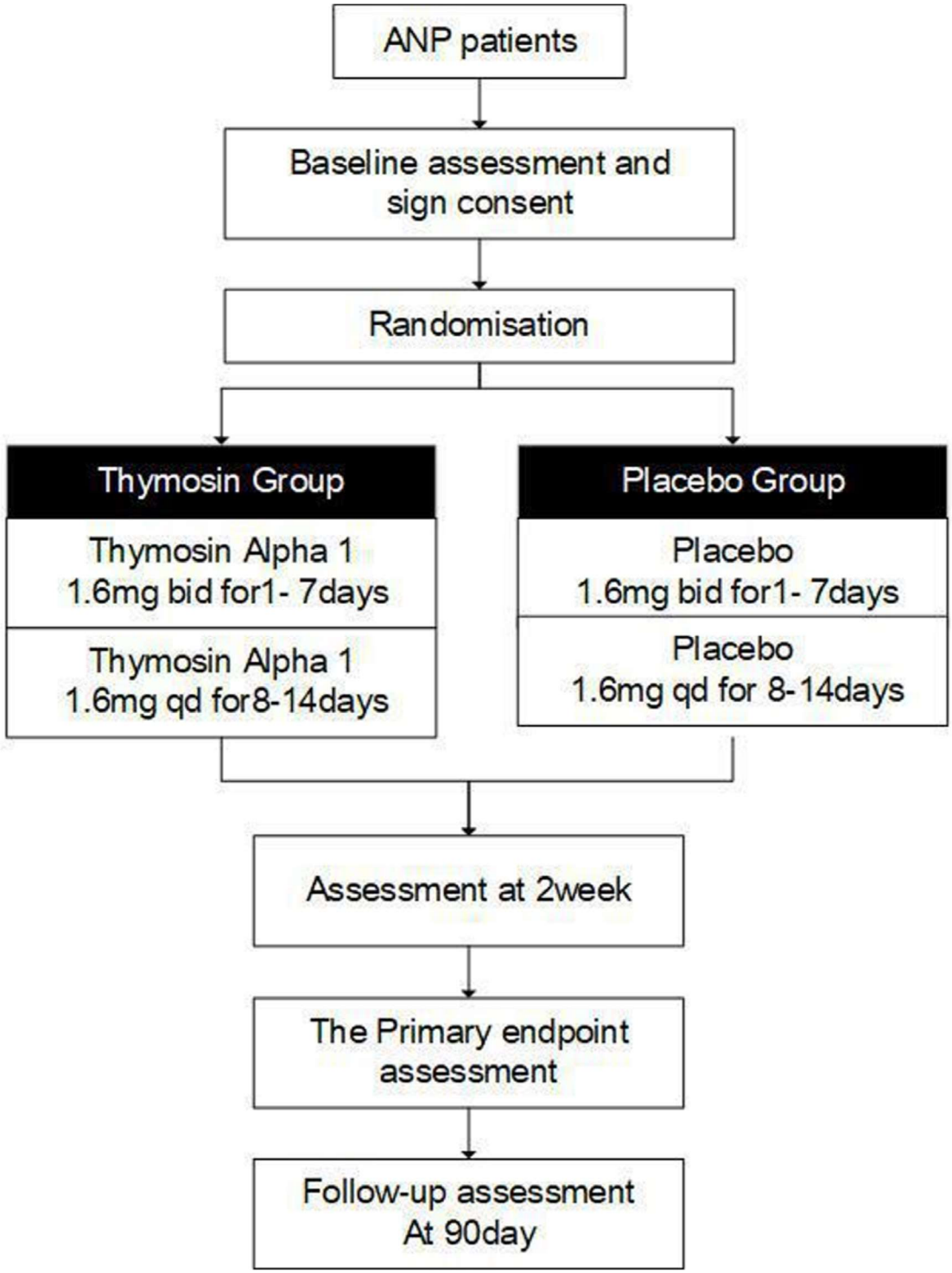
Mobile number of the principal investigator:

+86 13951839654

11 Figure 1: Flow chart



T.R.A.C.E

Thymosin alpha 1 in the pRevention of infected pAncreatic neCrosis following acute nEcrotizing pancreatitis

Protocol Ver: 2.0 dated Dec. 26. 2016

Principal Investigator

Professor. Weiqin Li

Center of severe acute pancreatitis (CSAP), Department of Critical Care Medicine, Jinling Hospital, Nanjing University, Nanjing, Jiangsu province, China

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Participating Centers

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The Affiliated Nanhua Hospital, University of South China
The Affiliated Hospital 2 of Nantong University
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The 94th Hospital of PLA
Zhejiang Provincial People's Hospital
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Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School

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SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Acronym (Short Title)	T.R.A.C.E
Long Title	Thymosin alpha 1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis
Version	2.0
Date	26th Dec 2016
ClinicalTrials.gov Identifier	NCT02473406
Study Design	A double-blind, multicenter, randomized controlled trial
Type of Participants to be studied	Patients aged between18 to 70 years old who were diagnosed with acute necrotizing pancreatitis
Interventions to be Compared	1. Intervention Group: Thymosin alpha 1therapy will be started after randomization: 1.6mg I.H q12h for the first 7 days and 1.6mg I.H, qd for the following 7 days or until discharge. 2. Control Group: Normal saline (placebo) will be given at the same dose as Thymosin alpha 1 in addition to the standard treatment.
Study Hypothesis	To evaluate the effects of Thymosin alpha 1 used in the early phase on the incidence of infected pancreatic necrosis and other clinical outcomes in patients with acute necrotizing pancreatitis.
Primary Outcomes Measure(s)	The incidence of infected pancreatic necrosis during the index admission.
Secondary Outcome Measure(s)	1. Occurrence of new-onset organ failure and persistent organ failure; 2. C-reactive protein(CRP), HLA-DR expression on monocytes, and other laboratory results like white blood cell(WBC) count and lymphocyte count on day0, day7, and day14 of the observation; 3. Occurrence of bleeding requiring intervention; 4. Occurrence of gastrointestinal fistula; 5. In-hospital mortality; 6. Pancreatic fistula; 7. Need of percutaneous or endoscopic transluminal drainage; 8. Need of minimally-invasive necrosectomy;

	9. Need of open surgery; 10. Length of intensive care unit(ICU) stay; 11. Length of hospital stay; 12. In-hospital cost; 13. Incidence of infection within 90 days after enrollment; 14. Mortality within 90 days after enrollment.
Number of Clusters and Participates to be studied	520 patients were planned
Duration	3-4 years
Sponsor	Jinling Hospital of Nanjing University
Founder	Science and technology project, Jiangsu Province of China (No. SBE2016750187), and partly by SciClone Pharmaceuticals Holding Limited.
Chief Investigators	Professor. Weiqin Li

1.Abbreviations

AP	acute pancreatitis
IPN	infected pancreatic necrosis
ANP	acute necrotizing pancreatitis
CRP	C-reaction protein
HLA	human leukocyte antigen
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PPS	per-protocol set
SS	safety set
AEs	adverse events

2. Study Administrative information

2.1 Study timelines

January 2014	Protocol manuscript
April 2014	The first National Expert Symposium
May 2014	Protocol finalized
June 2014	Initial expressions of interest sought from sites
November 2014	Commence study organization
April 2015	Hospital Human Research Ethics Committee (HREC) submissions
May 2015	Study material V.1.0 finalized HREC approvals obtained
August 2015	Commencer phase and protocol assessment in test sites
April 2016	Commence study organization and protocol revision

2.2 Steering and management committee

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Members of the steering committee are listed below

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Prof. Qiang Li

Dr. Mengjie Lu

Prof. Wenkui Yu

2.6 Registration

The TRACE trial was registered on the ClinicalTrials.gov registry (NCT02473406).

2.7 Funding

The study was funded by the Science and Technology project, Jiangsu Province of China (no. SBE2016750187), and partly by SciClone Pharmaceuticals Holding Limited.

3. Background and rationale

3.1 The role of immunosuppression and infected pancreatic necrosis in acute pancreatitis

Infected pancreatic necrosis (IPN) and its related septic complications are the major causes of death in patients with acute necrotizing pancreatitis(ANP). When compared with patients with sterile necrosis, patients with IPN suffered a substantial increase in mortality ranging from 14% to 69% due to sepsis and its related multiple organ failure, despite advances in critical care, surgical interventions, and antibiotics. Therefore, the prevention of IPN is of great clinical value in the treatment of ANP. In past years, numerous attempts had been made to prevent or delay the development of IPN, including antibiotic prophylaxis, selective gut decontamination, and probiotics, but none of them has been clinically proved with high-quality evidence.

Immunosuppression and disorders characterized by decreased HLA-DR expression and unbalanced CD3/CD4+/CD8+ T cells of peripheral blood mononuclear cell (PBMC) are reported to be associated with the development of IPN. Our previous study also found that early enteral nutrition could moderate the excessive immune response during the early stage of severe acute pancreatitis without leading to subsequent immunosuppression and ultimately improve the outcome. Thus immunomodulatory treatment could potentially intervene in the evolution of secondary infection of pancreatic necrosis, resulting in better outcomes. Unfortunately, study regarding the immune status in patients with any type of acute pancreatitis (AP) is rare, let alone

appropriate treatment aiming to modulate patients' immune function.

3.2 The rationale for conducting this study

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4. Study Design

4.1 Aims and hypotheses

To evaluate the effects of Thymosin alpha 1 used in the early phase on preventing infected pancreatic necrosis and other clinical outcomes in patients with acute necrotizing pancreatitis.

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The present clinical interventional study will be conducted in 10-15 different hospitals across China. It is an investigator-initiated, multicenter, double-blind, randomized, placebo-controlled study.

5. Study population

5.1 Patient recruitment

We are going to recruit approximately 520 patients presenting to the participating sites across China into the study. Based on the volume of all the participating centers, the aim should be able to be achieved within 3-4 years.

5.2 Eligibility Criteria

5.2.1 Inclusion Criteria

1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of acute necrotizing pancreatitis (ANP), serum amylase at least three times the upper limit of normal, and/or characteristic findings of ANP on computed tomography;
2. Within a week from the onset of the disease;
3. Age between 18 to 70 years old;
4. APACHEII≥8;
5. Written informed consent obtained.

5.2.2 Exclusion Criteria

1. Pregnant pancreatitis;
2. History of chronic pancreatitis;
3. Underlying malignancy;
4. Receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before randomization;
5. Patients with a known history of severe cardiovascular, respiratory, renal, or hepatic diseases defined as (1) greater than New York Heart Association Class II heart failure(Class II not included), (2) active myocardial ischemia, or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney

disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with the requirement for home oxygen;

6. Patients with preexisting immune disorders such as AIDS.

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All patients presenting to the participating hospitals will be assessed by the treating physician and receive medical care immediately according to the current best clinical practice. The treating clinician team will be responsible for identifying potential patients and contact the TRACE coordinator or a member of the TRACE study team who will assess the patients for inclusion and exclusion criteria and eligibility into the study. Screening tools will be provided, and a screening log will be kept.

Each participating center will be led by a TRACE project leader (local principal investigator) and a site coordinator. The former will be responsible for the prescription of the Thymosin alpha 1 or matching placebo and monitor the TRACE trial, and the latter will be responsible for daily screening and data management.

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All patients admitted to the participating sites will be considered for enrollment in the study and assessed for inclusion and exclusion criteria. Where possible, informed consent will be obtained from the patient or next of kin (NOK) prior to enrollment into the study.

6.3 Randomization procedures

6.3.1 Sequence generation

The sequence generation will be performed by the Randomization Allocation Tool

v. 1.1.4 (RAT, Ratjin, Nanjing). After being screened for eligibility, participants will be randomly assigned in a 1:1 ratio to either the intervention group or the control group according to web-based computer-generated random numbers in block sizes of 4. The random assignment will be conducted by a third party independent of the study, and the assignment records will not be disclosed until the end of the study. Randomization will be stratified by sites.

6.3.2 Blinding method

After the acquisition of written informed consent and the completion of baseline measurements, the allocation will be sequentially delivered to the clinical investigator who is not involved in outcome assessment and is responsible for patient care. Participants, data collectors, and investigators assessing outcome data will be blinded to the treatment allocation to minimize potential sources of bias. All investigators at each site are unable to distinguish the treatment group unless emergency unblinding is required. Adherence to the treatment protocol will be carefully monitored, and protocol deviations will be identified through the data collected by study coordinators. The blinding process will involve labeling the study kits with randomization numbers that will be used as package identification.

6.4. Study procedures

6.4.1 Interventional arms

After confirming that a participant meets the inclusion criteria and does not meet any of the exclusion criteria, the study protocol flow of participants is outlined in Figure1.

Arm#1 Thymosin Group

In addition to the standard treatment, Tα1 therapy will be started after randomization: 1.6 mg, every 12 hours for the first seven days and 1.6 mg once a day for the following seven days or until discharge.

Arm#2 Control group

Placebo will be given at the same dose as Thymosin in addition to the standard treatment.

The drug regimen will last for 14 consecutive days, as shown in Figure 2. If the patient withdraws consent, dies, or is discharged before completing the designed regimen, the rest of the study drugs will be waived and recycled by the sponsor. The eligible patients will be followed up until hospital death or discharge.

6.4.2 General management

All patients should receive initial standard treatment, including fluid resuscitation, early enteral nutrition, routine medical treatment, mechanical ventilation, and continuous renal replacement therapy (CRRT) if needed in the light of recently published guidelines. Prophylactic antibiotics are not recommended. All participating centers are able to offer appropriate intensive care in case the patients require organ support therapy or a continuous monitor. The necrotic collection will be intervened when infection was suspected or confirmed, but the intervention should be optimally delayed for four weeks if the patient could tolerate the symptoms.

When infected pancreatic necrosis occurs, either surgical or endoscopic step-up approach on the basis of the location of the necrotic collection and technical availability in each participating center will be applied. Initial drainage is optimally performed after the necrotic collection is well walled-off. Principally, either percutaneous catheter drainage or endoscopic transluminal placement of double pig-tail stents are the primary choices of treatment.

7. Outcome measures

7.1 Primary outcome measures

The incidence of IPN during the index admission is served as the primary outcome measure of the TRACE trial.

7.2 Secondary outcome measures（censored at death or discharge）

1. Occurrence of new-onset organ failure and persistent organ failure;
2. C-reactive protein(CRP), HLA-DR expression on monocytes and other laboratory results like white blood cell(WBC) count and lymphocyte count on day0, day 7 and day14 of the observation;
3. Occurrence of bleeding requiring intervention;
4. Occurrence of gastrointestinal fistula;
5. In-hospital mortality;
6. Pancreatic fistula;
7. Need of percutaneous or endoscopic transluminal drainage;
8. Need of minimally-invasive necrosectomy;
9. Need of open surgery;
10. Length of intensive care unit (ICU) stay;
11. Length of hospital stay;
12. In-hospital cost;
13. Incidence of infection within 90 days after enrollment;
14. Mortality within 90 days after enrollment.

7.3 Definition of outcomes

IPN： The diagnosis of IPN will be based on the international guidelines when one or more of the following were present: gas bubbles within (peri) pancreatic necrosis on computed tomography; a positive culture of (peri) pancreatic necrosis obtained by image-guided fine-needle aspiration; a positive culture of (peri) pancreatic necrosis obtained during the first drainage and/or necrosectomy[12].

New-onset: Events occurring after randomization and not present 24 hours before randomization.

Organ failure is defined as an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 points or more for each organ system(Respiration, Cardiovascular, Renal).

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1. Some potential participants are unable to give consent for themselves;
2. The Tα1 therapy may have some side effects.

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The Tα1, which is used in the validation of the current trial, is presented free by the manufacturer (SciClone Pharmaceutical (China) Co., Ltd.). No specific monetary compensation is available for each participant in this study.

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All the principal investigators of the participating hospitals and the sponsor will have full access to the data after the conclusion of the study. Anyone who wants to do a post-hoc analysis needs to submit a formal writing proposal to the *writing and publication*

committee. Only approved authors can have access to the database.

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9.1 Data collection

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The principal investigator of each center will be responsible for patient enrollment and data input. A group of statisticians will be accountable for the pre-definition of statistical analysis and subgroup analysis.

A web-based electrical database will be used for data collection and storage. All data will be input by the principal investigator or nominated investigator (less than two for each participating center) approved by the principal investigator. Training for data entry will be performed by the supplier of the electrical database and the sponsor of the TRACE trial.

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The statistical analysis for outcome measures will be based on the intention-to-treat

(ITT) population. Missing data will be handled by multiple imputation to evaluate the robustness of the primary endpoint analyses. The normality of continuous variables will be examined using skewness and kurtosis. Continuous variables will be compared using the Mann-Whitney method or Student's T-test depending on the distribution of the data. Categorical data will be expressed as numbers and percentages. The between-group difference will be compared using the chi-squared test. Statistical tests will be two-sided, and p values < 0.05 will be accepted as significant for the endpoints. No interim unblinding and analysis is planned for the TRACE trial.

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In all cases, the condition or disease underlying the symptom, sign or laboratory value should be reported, e.g. renal failure rather than hyperkalemia, and agitation rather than self-extubation.

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6. Is an important medical event that may require intervention to prevent one of the previously listed outcomes.

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The DSMB will be responsible for overseeing all subjects' safety and monitoring total mortality and serious adverse events. All serious adverse events occurring during the trial will be reported to the Coordinating and data management center (CDMC) within 48 hours. Minimum information to report will include:

1. Initials of the patient and study number
2. Course and nature of the event
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The contact information for CDMC

Telephone number

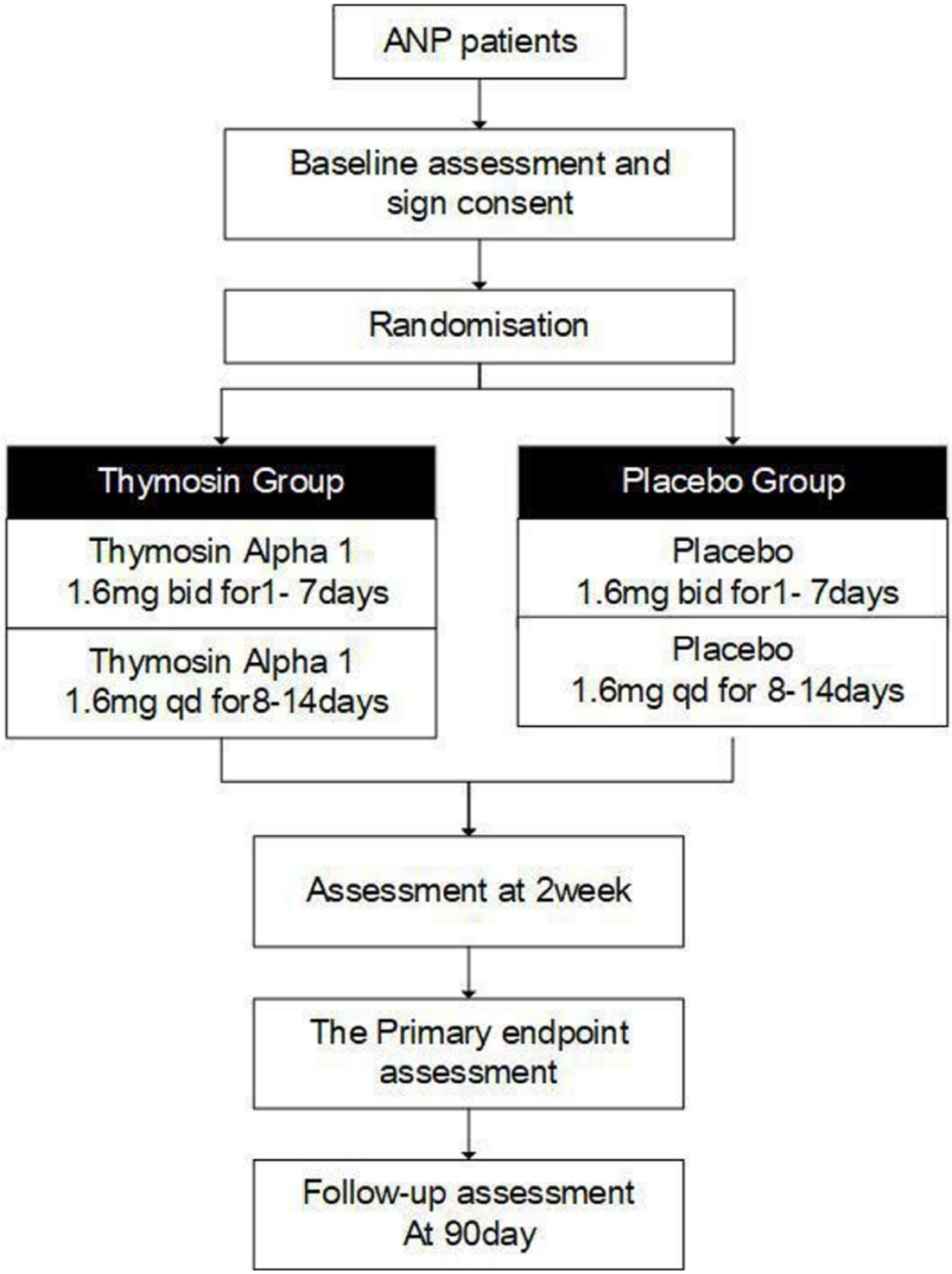
+86 80860007

+86 80863073

Mobile number of the principal investigator:

+86 13951839654

11. Figure 1: study protocol flow of participants.



12. Figure 2: Schedule for participants enrolment, drug administration and data collection.

	Study period							
	Enrollment	Allocation	Index admission					Follow-up
TIMEPOINT	< 24h	0	day1-6	day7	day8-13	day14	discharge/death	90 day
ENROLLMENT								
Eligibility screening	X							
Informed consent	X							
Allocation		X						
INTERVENTIONS:								
Drug injection 1.6mg bid			X	X				
Drug injection 1.6mg qd					X	X		
ASSESSMENTS:								
Incidence of IPN			←	—	—	→		
Major complications			←	—	—	→		
Laboratory test	X			X		X		
Organ failure assessment	X			X		X		
Status of vitality and infection								X

T.R.A.C.E

Thymosin alpha 1 in the pRevention of infected pAncreatic neCrosis following acute nEcrotizing pancreatitis

Protocol Ver: 3.0 dated July. 01. 2019

Principal Investigator

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Participating Centers

The First Affiliated Hospital of Nanchang University
The Affiliated Hospital of Qingdao University Medical School
The Affiliated Hospital of Zunyi Medical University
The Affiliated Nanhua Hospital, University of South China
The Affiliated Hospital 2 of Nantong University
The First Affiliated Hospital of Wannan Medical College
Shangqiu First People's Hospital
The 94th Hospital of PLA
Jiangsu Provincial Hospital of Integrated Chinese and Western Medicine
Zhejiang Provincial People's Hospital
Luoyang Central Hospital
The First Affiliated Hospital and College of Clinical Medicine of Henan University of Science and Technology
Clinical Medical College of Yangzhou University
Qilu Hospital of Shandong University

The First Affiliated Hospital of Anhui Medical University For the

Chinese Acute Pancreatitis Clinical Trials Group (CAPCTG)

Table of contents

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Acronym (Short Title)	T.R.A.C.E
Long Title	Thymosin alpha 1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis
Version	3.0
Date	1st July 2019
ClinicalTrials.gov Identifier	NCT02473406
Study Design	A double-blind, multicenter, randomized controlled trial
Type of Participants to be studied	Patients aged between18 to 70 years old who were diagnosed with acute necrotizing pancreatitis
Setting	16 hospitals
Interventions to be Compared	1. Intervention Group: Thymosin alpha 1therapy will be started after randomization: 1.6mg I.H q12h for the first 7 days and 1.6mg I.H, qd for the following 7 days or until discharge. 2. Control Group: Normal saline (placebo) will be given at the same dose as Thymosin alpha 1 in addition to the standard treatment.
Study Hypothesis	Thymosin alpha 1 could reduce the incidence of infected pancreatic necrosis in patients with predicted severe acute necrotizing pancreatitis.
Primary Outcomes Measure(s)	The incidence of infected pancreatic necrosis during the index admission.
Secondary Outcome Measure(s)	Part I: Clinical outcomes during the index admission 1. The incidence of new-onset organ failure and new-onset persistent organ failure (SOFA score for respiration, cardiovascular, or renal system ≥2). 2. In-hospital mortality 3. Bleeding requiring intervention 4. Gastrointestinal fistula requiring intervention 5. Positive blood culture 6. Incidence of pancreatic fistula 7. New receipt of mechanical ventilation/renal replacement therapy /New receipt of vasoactive agents not applied 24 hours before randomization 8. The requirement for catheter drainage/Number of drainage procedures

	required 9. The requirement for minimally-invasive debridement/Number of minimally invasive necrosectomy required 10. The requirement for open surgery/Number of open surgery required 11. Length of intensive care unit (ICU) stay/Length of hospital stay 12. SOFA score/ CRP level/ HLA-DR level/ Lymphocyte count on day0, day7, and day14 13. In-hospital cost. Part II: Clinical outcomes within 90 days after enrollment 1. Incidence of infection 2. 90-d mortality
Number of Clusters and Participates to be studied	520 patients from 16 hospitals were planned
Duration	4 years
Sponsor	Jinling Hospital of Nanjing University
Founder	Science and technology project, Jiangsu Province of China (No. SBE2016750187), and partly by SciClone Pharmaceuticals Holding Limited.
Chief Investigators	Professor. Weiqin Li

1.Abrreviations

AP	acute pancreatitis
IPN	infected pancreatic necrosis
ANP	acute necrotizing pancreatitis
CRP	C-reaction protein
HLA	human leukocyte antigen
WBC	white blood cell
PMBC	peripheral blood mononuclear cell
TLR	toll-like receptor
DC	dendritic cell
CT	computered tomography
MRI	magnetic resonance imaging
APACHE II	acute physiology and chronic health evaluation II
RRT	renal replacement therapy
MV	mechanical ventilation
CDMC	coordinating and data management center
DSMB	data and safety monitoring board
SAE	serious adverse events
ICU	intensive care unit
SOFA	sequential organ failure assessment
NOK	next of kin
ITT	intention-to-treat
FAS	full analysis Set
PPS	per-protocol set
SS	safety set
AEs	adverse events

2. Study Administrative information

2.1 Study timelines

January 2014	Protocol manuscript
April 2014	The first National Expert Symposium
May 2014	Protocol finalized
June 2014	Initial expressions of interest sought from sites
November 2014	Commence study organization
April 2015	Hospital Human Research Ethics Committee (HREC) submissions
May 2015	Study material V.1.0 finalized HREC approvals obtained
August 2015	Commencer phase and protocol assessment in test sites
April 2016	Commence study organization
March 2017	Patient recruitment commences (all sites)

2.2 Steering and management committee

The steering and management committee is responsible for the approval of the full protocol, database, and its related methods. The members of the committee will also oversee the implementation of the study and play an advisory role.

Members of the steering committee are listed below

Prof. Weiqin Li (Principal investigator)

Prof. Zhihui Tong

Prof. Lu Ke

Dr. Jing Zhou

Prof. Wenhua He

Prof. Xinting Pan

Prof. Miao Chen

Prof. Weili Gu

Prof. Chengjian He

Prof. Jingyi Wu

Prof. Xiangyang Zhao

Prof. Jianfeng Tu

Prof. Junli Sun

Prof. Guoxiu Zhang

Prof. Jingchun Song

Prof. Hong Zhang

Prof. Weiwei Chen

Prof. Haibin Ni

Prof. Shuming Tu

Dr. Yu Zhou

Dr. Youdong Wan

Dr. Kang Li

Dr. Feng Zhou

Dr. Hongguo Yang

Dr. Keke Xin

Dr. Dahuan Li

Dr. Qinbo Zeng

Dr. Dongsheng Zhao

Dr. Qincheng Xu

Dr. Xiaofei Huang

Dr. Bing Xue

2.3 Coordinating and data management center

Coordinating and data management centers (CDMC) will be organized before the implementation of the current study. They are responsible for the day to day management of the trial, assistance for ethic application in each center, protocol and case report form design, online database design and maintenance, protocol training for the participating centers, randomization, data entry and quality control, severe adverse event monitor and notification and data analysis. The CDMC plans to meet before enrollment, three months after initial enrollment and six months after initial enrollment to ensure qualified data entry.

Members of CDMC are listed below

Dr. Lu Ke

Dr. Yuxiu Liu

Dr. Baiqiang Li

Dr. Jing Zhou

Dr. Wenjian Mao and all the research nurses and coordinators from the participating centers. Meetings will be organized as required and no routine meeting is planned.

2.4 Writing and publication committee

The writing and publication committee is responsible for drafting the manuscript and submission of the manuscript to adequate journals. The Writing and publication committee will also decide on the authorship of this study. After the conclusion of this study, every participating centers are welcome to submit proposals for post-hoc analysis to the writing and publication committee is responsible for reviewing and rating all the proposals for further analysis.

Members are listed below

Prof. Weiqin Li

Dr. Lu Ke

Dr. Jing Zhou

Prof. John Windsor

2.5 Data and safety monitoring board

The data and safety monitoring board (DSMB) is an independent group of experts that offers advice during the implementation of the study. The DSMB can recommend that a trial be stopped early because of concerns about participant safety or because the main research question has been answered.

Members of DSMB are listed below

Prof. Qiang Li

Dr. Mengjie Lu

Prof. Wenkui Yu

2.6 Registration

The TRACE trial was registered on the ClinicalTrials.gov registry (NCT02473406).

2.7 Funding

The study was funded by the Science and technology project, Jiangsu Province of China (no. SBE2016750187), and partly by SciClone Pharmaceuticals Holding Limited.

3. Background and rationale

3.1 The role of Immunosuppression and infected pancreatic necrosis in acute pancreatitis

Infected pancreatic necrosis (IPN) and its related septic complications are the major

causes of death in patients with acute necrotizing pancreatitis(ANP). When compared with patients with sterile necrosis, patients with IPN suffered a substantial increase in mortality ranging from 14% to 69% due to sepsis and its related multiple organ failure, despite advances in critical care, surgical interventions and antibiotics. Therefore, the prevention of IPN is of great clinical value in the treatment of ANP. In past years, numerous attempts had been made to prevent or delay the development of IPN including antibiotic prophylaxis, selective gut decontamination and probiotics, but none of them have been clinically proved with high-quality evidence.

Immunosuppression and disorders characterized by decreased HLA-DR expression and unbalanced CD3/CD4+/CD8+ T cells of peripheral blood mononuclear cell (PBMC) are reported to be associated with the development of IPN. Our previous study also found that early enteral nutrition could moderate the excessive immune response during the early stage of severe acute pancreatitis without leading to subsequent immunosuppression, and ultimately improve the outcome. Thus immunomodulatory treatment could potentially intervene in the evolution of secondary infection of pancreatic necrosis, resulting in a better outcome. Unfortunately, study regarding the immune status in patients with any type of acute pancreatitis (AP) is rare, let alone appropriate treatment aiming to modulate patients' immune function.

3.2 The rationale for conducting this study

Thymosin alpha 1 (Tα1) has been shown to have immunomodulatory properties and is reported to be beneficial in patients with sepsis, majorly through the involvement of distinct Toll-like receptors (TLRs) acting on different dendritic cells (DC) subsets and involving the MyD88-dependent signaling pathway. However, the effect of Tα1 in patients with AP is rarely reported in the literature. The only randomized clinical study conducted by our group years before proved that the use of Tα1 is associated with improved cellular immunity and reduced infection rate in a group of ANP patients. However, no further study has been published yet and the clinical significance of this

study is limited due to the small sample size and single-center nature. Therefore, a multi-center, randomized controlled study with a large sample size and proper design is warranted to evaluate the role of Tα1 in treating ANP.

4. Study Design

4.1 Aims and hypotheses

We aimed to evaluate the efficacy of Thymosin Alpha 1 in the prevention of IPN and its impact on immune function and other clinical outcomes in patients with ANP.

4.2 General study setting

The present clinical interventional study will be conducted in 16 different hospitals across China. It is an investigator-initiated, multi-center, double-blind, randomized, placebo-controlled study.

5. Study population

5.1 Patient recruitment

We are going to recruit approximately 520 patients presenting to the participating sites across China into the study. Based on the volume of all the participating centers, the aim should be able to be achieved within 4-5 years.

5.2 Eligibility Criteria

5.2.1 Inclusion Criteria

1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of AP, serum amylase at least three times the upper limit of normal, and/or characteristic

findings of AP on computed tomography or less commonly magnetic resonance imaging (MRI) or transabdominal ultrasonography according to the Revised Atlanta Criteria;

2. Less than one week from the onset of abdominal pain;

3. Age between 18 to 70 years old;

4. Acute Physiology and Chronic Health Evaluation(APACHE II) score ≥8 during the last 24 hours before randomization

5. Balthazar CT score ≥5 (presence of pancreatic necrosis).

6. Written informed consent obtained

5.2.2 Exclusion Criteria

1. Pregnant pancreatitis;

2. History of chronic pancreatitis;

3. Underlying malignancy;

4. Receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before randomization;

5. Patients with a known history of severe cardiovascular, respiratory, renal, or hepatic diseases defined as (1) greater than New York Heart Association Class II heart failure(Class II not included), (2) active myocardial ischemia, or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with a requirement for home oxygen;

6. Patients with preexisting immune disorders such as AIDS.

6. Main methods and materials

6.1 Patients screening procedures

All patients presenting to the participating hospitals will be assessed by the treating physician and receive medical care immediately according to the current best clinical

practice. The treating clinician team will be responsible for identifying potential patients and contact the TRACE coordinator or a member of the TRACE study team who will assess the patients for inclusion and exclusion criteria and eligibility into the study. Screening tools will be provided, and a screening log will be kept.

Each participating center will be led by a TRACE project leader (center primary investigator) and a site coordinator. The former will be responsible for the prescription of the Thymosin alpha 1 or matching placebo and monitor the TRACE trial, and the latter will be responsible for daily screening and data management.

6.2 Recruitment

All patients admitted to the participating sites will be considered for enrollment in the study and assessed for inclusion and exclusion criteria. Where possible, informed consent will be obtained from the patient or next of kin (NOK) prior to enrollment into the study.

6.3 Randomization procedures

6.3.1 Sequence generation

The sequence generation was performed by the Randomization Allocation Tool v. 1.1.4 (RAT, Ratjin, Nanjing). After being screened for eligibility, participants will be randomly assigned in a 1:1 ratio to either the intervention group or the control group according to internet-based computer-generated random numbers in block sizes of 4. The random assignment will be conducted by a third party independent of the study, and the assignment records will not be disclosed until the end of the study. Randomization will be stratified by sites.

6.3.2 Blinding method

After the acquisition of written informed consent and the completion of baseline measurements, the allocation will be sequentially delivered to the clinical investigator

who is not involved in outcome assessment and is responsible for patient care. Participants, data collectors, and investigators assessing outcome data will be blinded to the treatment allocation to minimize potential sources of bias. All investigators at each site are unable to distinguish the treatment group unless emergency unblinding is required. Adherence to the treatment protocol will be carefully monitored and protocol deviations will be identified through the data collected by study coordinators. The blinding process will involve labeling the study kits with randomization numbers that will be used as package identification.

6.4. Study procedures

6.4.1 Interventional arms

After confirming that a participant meets the inclusion criteria and does not meet any of the exclusion criteria, the study protocol flow of participants is outlined in Figure1.

Arm#1 Thymosin Group

In addition to the standard treatment, Tα1 therapy will be started after randomization: 1.6 mg, every 12 hours for the first seven days and 1.6 mg once a day for the following seven days or until discharge.

Arm#2 Control group

Placebo (normal saline, Chengdu Tongde Pharmaceuticals) will be given at the same dose as Thymosin in addition to the standard treatment.

The drug regimen will last for 14 consecutive days as shown in Figure 2. If the patient dies or is discharged before completing the designed regimen, the rest of the study drugs will be waived and recycled by the sponsor. The eligible patients will be followed up until hospital death or discharge.

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All patients should receive initial standard treatment, including fluid resuscitation, early enteral nutrition, routine medical treatment, mechanical ventilation, and

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10. The requirement for open surgery/Number of open surgery required during the index admission during the index admission

11. Length of intensive care unit(ICU) stay/Length of hospital stay during the index admission

12. SOFA score/ CRP level/ HLA-DR level/ Lymphocyte count

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The contact information for CDMC

Telephone number

+86 80860007

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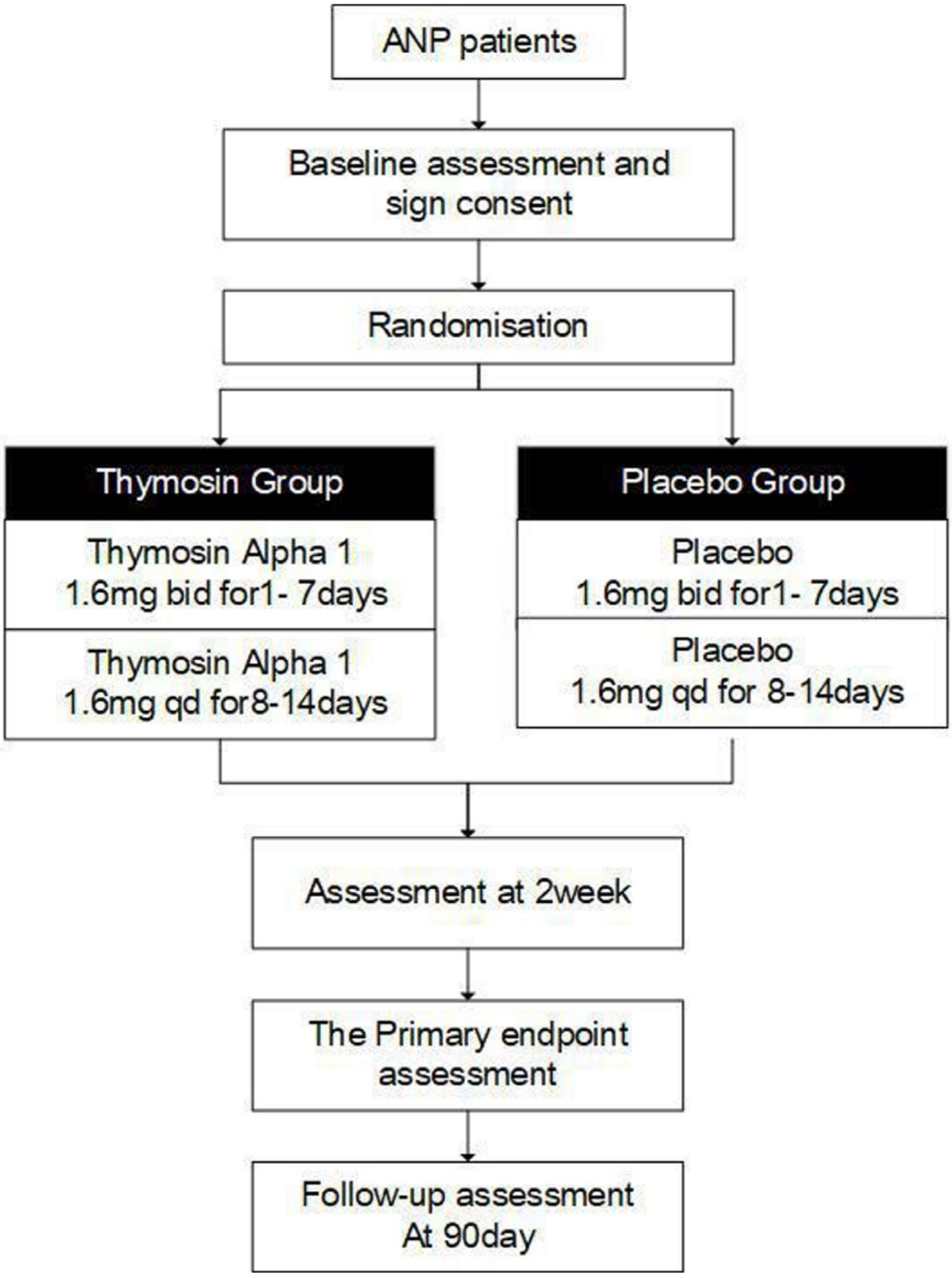
Mobile number of the principal investigator:

+86 13951839654

11. Figure 1: study protocol flow of participants



12. Figure 2: Schedule for participants enrolment, drug administration and data collection.

	Study period							
	Enrollment	Allocation	Index admission					Follow-up
TIMEPOINT	< 24h	0	day1-6	day7	day8-13	day14	discharge/death	90 day
ENROLLMENT								
Eligibility screening	X							
Informed consent	X							
Allocation		X						
INTERVENTIONS:								
Drug injection 1.6mg bid			X	X				
Drug injection 1.6mg qd					X	X		
ASSESSMENTS:								
Incidence of IPN			←	—	—	→		
Major complications			←	—	—	→		
Laboratory test	X			X		X		
Organ failure assessment	X			X		X		
Status of vitality and infection								X

Summary of protocol changes

List of Changes

Protocol Amendment (version 1 to version 2)

Page/ Line No.	Original Text	New Text	Reason
Page 1, Participating Center	Participating Center The First Affiliated Hospital of Nanchang University Zhejiang Provincial People's Hospital Nantong people's Hospital The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School	Participating Centers The First Affiliated Hospital of Nanchang University The Affiliated Hospital of Qingdao University Medical School The Affiliated Hospital of Zunyi Medical University The Affiliated Nanhua Hospital, University of South China The Affiliated Hospital 2 of Nantong University The First Affiliated Hospital of Wannan Medical College The 94th Hospital of PLA Zhejiang Provincial People's Hospital Qilu Hospital of Shandong University Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School	New sites enrolled
Page 5, Section 2. Study Administrative information Line 1	Blank	2.1 Study timelines January 2014 Protocol manuscript April 2014 The first National Expert Symposium May 2014 Protocol finalized June 2014 Initial expressions of interest sought from sites Nov 2014Commence study organization	Add Study timelines

		April 2015 Hospital Human Research Ethics Committee (HREC) submissions May 2015 Study material V.1.0 finalized HREC approvals obtained August 2015 Commence vanguard phase and protocol assessment in test sites April 2016 Commence study organization and protocol revision	
Page 5, Section 2. Study Administrative information Line 6	The steering and management committee is responsible for the approval of the full protocol, database, and its related methods. The members of the committee will also oversee the implementation of the study and play an advisory role. Members of the steering committee are listed below Prof. Weiqin Li (Primary investigator) Prof. Zhihui Tong Dr. Lu Ke Dr. Jing Zhou Prof. Wenhua He Prof. Weili Gu Prof. Jianfeng Tu Prof. Mingdong Liu	2.2 Steering and management committee The steering and management committee is responsible for the approval of the full protocol, database, and its related methods. The members of the committee will also oversee the implementation of the study and play an advisory role. Members of the steering committee are listed below Prof. Weiqin Li (Principal investigator) Prof. Zhihui Tong Prof. Lu Ke Dr. Jing Zhou Prof. Wenhua He Prof. Xinting Pan Prof. Miao Chen Prof. Weili Gu Prof. Chengjian He Prof. Jingyi Wu Prof. Xiangyang Zhao Prof. Jianfeng Tu Prof. Jingchun Song	Add members of steering and management committee

		Prof. Mingdong Liu	
Page 5, Section 2.2 Coordinating and data management center Line 10	Members of CDMC are listed below Dr. Lu Ke Dr. Yuxiu Liu Dr. Wenjian Mao and all the research nurses and coordinators from the participating centers. Meetings will be organized as required, and no routine meeting is planned.	Members of CDMC are listed below Dr. Lu Ke Dr. Yuxiu Liu Dr. Baiqiang Li Dr. Jing Zhou Dr. Wenjian Mao and all the research nurses and coordinators from the participating centers. Meetings will be organized as required and no routine meeting is planned.	Add members of coordinating and data management centers
Page 9, Section 5.2 Eligibility Criteria Line 1	5.2.1 Inclusion Criteria 1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of acute necrotizing pancreatitis(ANP), serum amylase at least three times the upper limit of normal, and/or characteristic findings of ANP on computed tomography; 2. Within a week from the onset of the disease; 3. Age between 18 to 70 years old;	5.2.1 Inclusion Criteria 1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of acute necrotizing pancreatitis (ANP), serum amylase at least three times the upper limit of normal, and/or characteristic findings of ANP on computed tomography; 2. Within a week from the onset of the disease; 3. Age between 18 to 70 years old; 4. APACHEII≥8; 5. Written informed consent obtained. 5.2.2 Exclusion Criteria 1. Pregnant pancreatitis; 2. History of chronic pancreatitis;	The original eligibility criteria is not detailed enough, and we deleted the Ranson score, which is no longer frequently used.

	4.APACHEII≥8 and/or Ranson score ≥3 5.2.2 Exclusion Criteria 1. Pregnant pancreatitis; 2. Receive percutaneous drainage or surgery before randomization or need of early surgery due to abdominal compartment syndrome, etc. 3. Patients with a known history of severe cardiovascular, respiratory, renal, hepatic, hematologic, or immunologic disease defined as (1) greater than New York Heart Association class II heart failure, (2) active myocardial ischemia, or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease	3. Underlying malignancy; 4. Receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before randomization; 5. Patients with a known history of severe cardiovascular, respiratory, renal, or hepatic diseases defined as (1) greater than New York Heart Association Class II heart failure(Class II not included), (2) active myocardial ischemia, or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with the requirement for home oxygen; 6. Patients with preexisting immune disorders such as AIDS.	

	with the requirement for home oxygen; 4. Patients with preexisting immune disorders such as AIDS.		
Page 12, Section 7. Outcome measures Line 2	7.1 Primary outcome measures All patients will be followed until hospital death or discharge. The occurrence of IPN at day 28 will serve as the primary outcome measure of the trial. 7.2 Secondary outcome measures 1. Occurrence of new-onset organ failure and persistent organ failure; 2. C-reactive protein(CRP), HLA-DR expression on monocytes, and other laboratory results like white blood cell(WBC) count and lymphocyte count on day0, day 7 and day14 of the observation;	7.1 Primary outcome measures The incidence of IPN during the index admission is served as the primary outcome measure of the TRACE trial. 7.2 Secondary outcome measures（censored at death or discharge） 1. Occurrence of new-onset organ failure and persistent organ failure; 2. C-reactive protein(CRP), HLA-DR expression on monocytes and other laboratory results like white blood cell(WBC) count and lymphocyte count on day0, day 7 and day14 of the observation; 3. Occurrence of bleeding requiring intervention; 4. Occurrence of gastrointestinal fistula; 5. In-hospital mortality; 6. Pancreatic fistula; 7. Need of percutaneous or endoscopic transluminal drainage; 8. Need of minimally-invasive necrosectomy; 9. Need of open surgery; 10. Length of intensive care unit(ICU) stay; 11. Length of hospital stay; 12. In-hospital cost; 13. Incidence of infection within 90 days after enrollment; 14. Mortality within 90 days after enrollment.	Describe in more detail. The time interval of the primary endpoint was changed for a practical reason.

	3. Occurrence of bleeding requiring intervention; 4. Occurrence of gastrointestinal fistula; 5. In-hospital mortality; 6. Pancreatic fistula; 7. Need of percutaneous or endoscopic transluminal drainage; 8. Need of minimally-invasive necrosectomy; 9. Need of open surgery; 10. Length of intensive care unit(ICU) stay; 11. Length of hospital stay; 12. In-hospital cost.		
Page 15, Section 9.2. Sample size Line 5-6	9.2 Sample size The prevalence of pancreatic infection was reported to be around 25% in ANP episodes. To demonstrate a 40% reduction in the prevalence of pancreatic infection considering the results of our pilot study [11] with 80% power at a two-	The prevalence of infected pancreatic necrosis was reported to be around 25% in ANP episodes. To demonstrate a 40% reduction in the prevalence of infected pancreatic necrosis considering the results of our pilot study with 80% power at a two-sided alpha level of 0.05, we projected an estimated sample size of 500 participants using the PASS software (PASS, NCSS software, Kaysville, USA). Considering possible 4% withdrawal, we planned to randomize 520 patients in total.	The possible withdrawal was changed from 2% to 4% for a practical reason.

	sided alpha level of 0.05, we projected an estimated sample size of 500 participants using the PASS software (PASS, NCSS software, Kaysville, USA). Considering possible 2% withdrawal, we planned to randomize 510 patients in total.		

Protocol Amendment (version 2 to version 3)

Page/ Line No.	Original Text	New Text	Reason
Page 19, Participating Center	Participating Centers The First Affiliated Hospital of Nanchang University The Affiliated Hospital of Qingdao University Medical School The Affiliated Hospital of Zunyi Medical University The Affiliated Nanhua Hospital, University of South China The Affiliated Hospital 2 of Nantong University The First Affiliated Hospital of Wannan Medical College The 94th Hospital of PLA Zhejiang Provincial People's Hospital Qilu Hospital of Shandong University Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School	Participating Centers The First Affiliated Hospital of Nanchang University The Affiliated Hospital of Qingdao University Medical School The Affiliated Hospital of Zunyi Medical University The Affiliated Nanhua Hospital, University of South China The Affiliated Hospital 2 of Nantong University The First Affiliated Hospital of Wannan Medical College Shangqiu First People's Hospital The 94th Hospital of PLA Jiangsu Provincial Hospital of Integrated Chinese and Western Medicine Zhejiang Provincial People's Hospital Luoyang Central Hospital The First Affiliated Hospital and College of Clinical Medicine of Henan University of Science and Technology Clinical Medical College of Yangzhou University Qilu Hospital of Shandong University The First Affiliated Hospital of Anhui Medical University For the Chinese Acute Pancreatitis Clinical Trials Group (CAPCTG)	New sites enrolled

Page 25, Section 2.1 Study timelines Line 10	Blank	March 2017 Patient recruitment commences (all sites)	Add more information of timelines
Page 25, Section 2.2 Steering and management committee Line 5	Members of the steering committee are listed below Prof. Weiqin Li (Principal investigator) Prof. Zhihui Tong Prof. Lu Ke Dr. Jing Zhou Prof. Wenhua He Prof. Xinting Pan Prof. Miao Chen Prof. Weili Gu Prof. Chengjian He Prof. Jingyi Wu Prof. Xiangyang Zhao Prof. Jianfeng Tu Prof. Jingchun Song Prof. Mingdong Liu	Members of the steering committee are listed below Prof. Weiqin Li (Principal investigator) Prof. Zhihui Tong Prof. Lu Ke Dr. Jing Zhou Prof. Wenhua He Prof. Xinting Pan Prof. Miao Chen Prof. Weili Gu Prof. Chengjian He Prof. Jingyi Wu Prof. Xiangyang Zhao Prof. Jianfeng Tu Prof. Junli Sun Prof. Guoxiu Zhang Prof. Jingchun Song Prof. Hong Zhang Prof. Weiwei Chen Prof. Haibin Ni Prof. Shuming Tu Dr. Yu Zhou Dr. Youdong Wan	Add members of steering and management committee

		Dr. Kang Li Dr. Feng Zhou Dr. Hongguo Yang Dr. Keke Xin Dr. Dahuan Li Dr. Qinbo Zeng Dr. Dongsheng Zhao Dr. Qincheng Xu Dr. Xiaofei Huang Dr. Bing Xue	
Page 30, Section 5.2 Eligibility Criteria Line 1	5.2.1 Inclusion Criteria 1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of acute necrotizing pancreatitis (ANP), serum amylase at least three times the upper limit of normal, and/or characteristic findings of ANP on computed tomography; 2. Within a week from the onset of the disease; 3. Age between 18 to 70 years old; 4. APACHEII≥8; 5. Written informed consent obtained. 5.2.2 Exclusion Criteria 1. Pregnant pancreatitis;	5.2.1 Inclusion Criteria 1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of AP, serum amylase at least three times the upper limit of normal, and/or characteristic findings of AP on computed tomography or less commonly magnetic resonance imaging (MRI) or transabdominal ultrasonography according to the Revised Atlanta Criteria; 2. Less than one week from the onset of abdominal pain; 3. Age between 18 to 70 years old; 4. Acute Physiology and Chronic Health Evaluation(APACHE II) score ≥8 during the last 24 hours before randomization 5. Balthazar CT score ≥5 (presence of pancreatic necrosis). 6. Written informed consent obtained 5.2.2 Exclusion Criteria	Balthazar CT score was added to specify the definition of ANP.

	2. History of chronic pancreatitis; 3. Underlying malignancy; 4. Receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before randomization; 5. Patients with a known history of severe cardiovascular, respiratory, renal, or hepatic diseases defined as (1) greater than New York Heart Association Class II heart failure(Class II not included), (2) active myocardial ischemia, or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with the requirement for home oxygen; 6. Patients with preexisting immune disorders such as AIDS.	1. Pregnant pancreatitis; 2. History of chronic pancreatitis; 3. Underlying malignancy; 4. Receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before randomization; 5. Patients with a known history of severe cardiovascular, respiratory, renal or hepatic diseases defined as (1) greater than New York Heart Association Class II heart failure(Class II not included), (2) active myocardial ischemia or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with a requirement for home oxygen; 6. Patients with preexisting immune disorders such as AIDS.	
Page 33, Section 7. Outcome measures Line 1	7.1 Primary outcome measures The incidence of IPN during the index admission is served as the primary outcome measure of the TRACE trial. 7.2 Secondary outcome measures（censored at death or discharge）	7.1 Primary outcome measures The incidence of IPN during the index admission is served as the primary outcome measure of the TRACE trial. 7.2 Secondary outcome measures	Describe in more detail.

	1. Occurrence of new-onset organ failure and persistent organ failure; 2. C-reactive protein(CRP), HLA-DR expression on monocytes and other laboratory results like white blood cell(WBC) count and lymphocyte count on day0, day7 and day14 of the observation; 3. Occurrence of bleeding requiring intervention; 4. Occurrence of gastrointestinal fistula; 5. In-hospital mortality; 6. Pancreatic fistula; 7. Need of percutaneous or endoscopic transluminal drainage; 8. Need of minimally-invasive necrosectomy; 9. Need of open surgery; 10. Length of intensive care unit(ICU) stay; 11. Length of hospital stay; 12. In-hospital cost; 13. Incidence of infection within 90 days after enrollment; 14. Mortality within 90 days after enrollment.	1. The incidence of new-onset organ failure and new-onset persistent organ failure during the index admission 2. In-hospital mortality during the index admission 3. Bleeding requiring intervention during the index admission 4. Gastrointestinal fistula requiring intervention during the index admission 5. Positive blood culture 6. Incidence of pancreatic fistula during the index admission 7. New receipt of mechanical ventilation/renal replacement therapy during the index admission 8. The requirement for catheter drainage/Number of drainage procedures required during the index admission 9. The requirement for minimally-invasive debridement/Number of minimally invasive necrosectomy required during the index admission 10. The requirement for open surgery/Number of open surgery required during the index admission during the index admission 11. Length of intensive care unit(ICU) stay/Length of hospital stay during the index admission 12.SOFA score/ CRP level/ HLA-DR level/ Lymphocyte count 13. In-hospital cost during the index admission 14. Incidence of infection within 90 days after enrollment 15. Mortality within 90 days after enrollment	

Thymosin alpha1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis: a multicenter, randomized, double-blind, placebo-controlled, parallel-group trial

Statistical Analysis Plan

Version 1.0-22 Aug 2020

ClinicalTrials.gov: NCT02473406

Protocol version and date: Version3.0- 01/July/2019

Chief Investigator: *Prof. Weiqin Li*

Trial statisticians: *Dr. Tao Chen*

SAP authors: *Dr. Tao Chen, Dr Lu Ke*

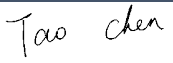
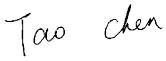
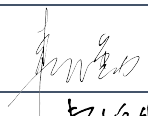
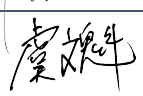
Signatures		
	Signature	Date
(Statistician)		2020.08.22
(Trial Statistician)		2020.08.22
(Chief Investigator)		2020.08.22
(IDMC Chair)		2020.08.22

Table of Contents

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the outcome variables, statistical methods, and analysis strategies to address the study objectives in Thymosin alpha1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis: a multicenter, randomized, double-blind, placebo-controlled, parallel-group trial (Protocol version: Version3.0- 01/July/2019)

2. BACKGROUND AND RATIONALE:

Infected pancreatic necrosis (IPN) and its related septic complications contribute substantially to morbidity and mortality in patients with acute necrotizing pancreatitis(ANP) 1. Compared with patients with sterile necrosis, those with IPN suffered a significant increase in mortality ranging from 14% to 69%, despite advances in critical care, surgical and endoscopic interventions, and antibiotics [2]. Therefore, the prevention of pancreatic necrosis infection is of great clinical value. Over the past years, numerous attempts had been made to prevent or delay the development of IPN, including antibiotic prophylaxis, early enteral nutritional, selective gut decontamination, and probiotics. Still, none of them had been proved to improve patient-centered outcomes with high-quality evidence [3-6]. More efficient treatment aiming at reducing infectious complications of ANP is in need.

Immunosuppression and disorders characterized by decreased HLA-DR expression and unbalanced CD3/CD4+/CD8+ T cells of peripheral blood mononuclear cell (PBMC) are reported to be associated with IPN [7, 8], especially in those with a more severe type of disease, whose suppressed immune function occurs early and persistently [8, 9]. Our previous observational study found that early enteral nutrition could moderate the excessive immune response during the early stage of severe acute pancreatitis without leading to subsequent immunosuppression, and ultimately reduce the incidence of infection and ICU stay [10]. Thus, immunomodulatory treatment could potentially intervene in the development of secondary infection of pancreatic necrosis, resulting in

better outcomes. Efforts had been made in this field using drugs like lexipafant and octreotide, but the hitherto existing evidence failed to show robust clinical benefits of immunomodulation with regard to patient-centered clinical outcomes [11].

Thymosin alpha 1 had been shown to have immunomodulatory properties and was reported to be clinically beneficial in patients with sepsis [12, 13], majorly through the involvement of distinct Toll-like receptors (TLRs) acting on different dendritic cells (DC) subsets and involving the MyD88-dependent signaling pathway. However, for acute pancreatitis, the only randomized controlled study was the pilot one conducted by our group years ago, suggesting that the use of thymosin alpha 1 was associated with improved cellular immunity and reduced infection rate in a group of 24 patients [14]. Due to the single-center nature of and limited sample size, the clinical implication and generalizability of this study are in doubt. Therefore, we conducted a multicenter, randomized, controlled trial, the thymosin alpha 1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis (TRACE trial) with sufficient power to confirm the findings from the pilot study. In the TRACE trial, we hypothesized that administration of Thymosin Alpha 1 during the acute phase of ANP would result in a reduced incidence of IPN

3. STUDY OBJECTIVES

3.1 Primary objective

To determine whether thymosin alpha 1 is superior to placebo in reducing the incidence of infected pancreatic necrosis in patients with ANP.

3.2 Secondary objectives

To determine the safety of thymosin alpha 1, and its impact on immune function and other patient-centered clinical outcomes among patients with ANP.

4. ELIGIBILITY CRITERIA

4.1 Inclusion Criteria

1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of AP, serum amylase at least three times the upper limit of normal, and/or characteristic findings of AP on computed tomography or less commonly magnetic resonance imaging (MRI) or transabdominal ultrasonography according to the Revised Atlanta Criteria [15];
2. Less than one week from the onset of abdominal pain;
3. Age between 18 to 70 years old;
4. Acute Physiology and Chronic Health Evaluation (APACHE II) score ≥8 during the last 24 hours before enrollment;
5. Balthazar CT score ≥5 (presence of pancreatic necrosis) [16];
6. Written informed consent obtained;

4.2 Exclusion Criteria

1. Pregnant pancreatitis;
2. History of chronic pancreatitis;
3. Underlying malignancy;
4. Receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before randomization;
5. Patients with a known history of severe cardiovascular, respiratory, renal or hepatic diseases defined as (1) greater than New York Heart Association Class II heart failure(Class II not included), (2) active myocardial ischemia or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with the requirement for home oxygen;
6. Patients with preexisting immune disorders such as AIDS;

A patient will be considered eligible if he/she meets the inclusion criteria and does not meet any of the exclusion criteria. Allocation will be performed after signed consent is obtained.

5. RANDOMIZATION AND BLINDING

After the completion of screening measurements and the acquisition of written informed consent, eligible participants will be randomized in a 1:1 ratio to either the treatment group or the placebo group. The randomization code was computer-generated with a block size of 4, and the randomization was stratified by sites. Trial drugs were then prepared according to the sequence to ensure blindness. The eligible patients were allocated to receive medication in individually numbered packs, according to the sequential order of the randomization center. Sealed envelopes were prepared for emergency unmasking. Participants, clinical investigators, and investigators assessing outcome data will be blinded to the treatment allocation to minimize potential sources of bias. The trial statistician will also be blinded regarding the treatment code when developing the statistical programs, which will be validated and completed using dummy randomization codes. The allocation will only be provided to the study team after locking the database and approval of the statistical analysis plan.

6. INTERVENTION ARM

After randomization, the participant will receive:

1. Thymosin Alpha 1 1.6mg *IH* every 12 hours for the first seven days and 1.6mg *IH* daily for the following seven days. The administration will be terminated any day during the treatment when the patient is deemed as qualified for discharge or dies.
2. Matching placebo (normal saline) using the same mode of administration as the above mentioned.

As shown in Figure 1, the recruited patients will start randomized drugs

subcutaneously from the day after the enrollment day. Thymosin Alpha will be provided by SciClone Pharmaceuticals and the matching placebo by Chengdu Tongde Pharmaceuticals. All study drugs will be stored in a secure area with access limited to the investigators and authorized study site personnel, and under appropriate storage conditions.

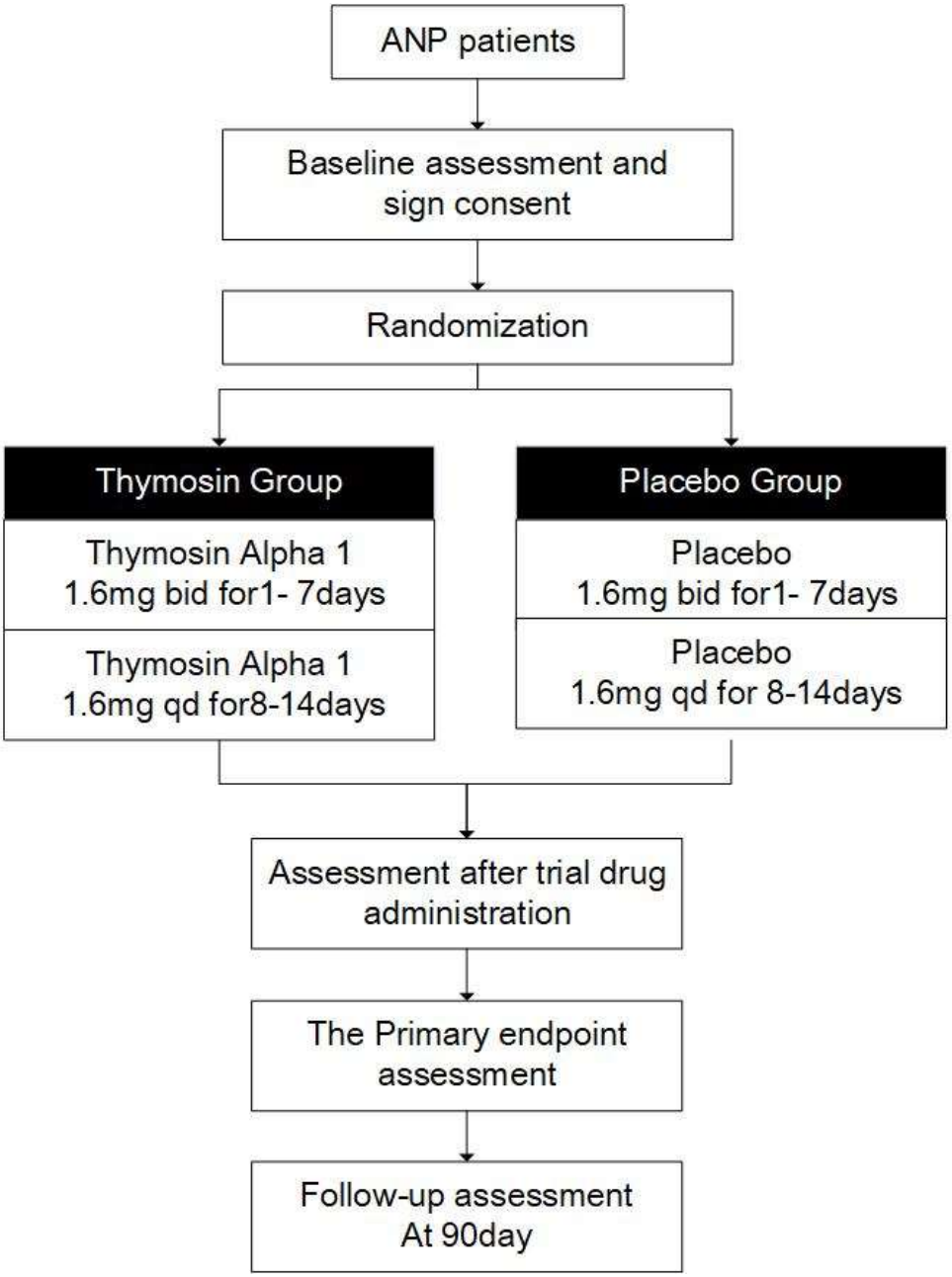


Figure 1: Trial flow chart. ANP denotes acute necrotizing pancreatitis

All patients will receive standard treatment for ANP according to the guidelines[17],

including fluid therapy, early enteral nutrition, routine medical treatment like proton pump inhibitor as indicated, mechanical ventilation if needed, and continuous renal replacement therapy (CRRT) if needed. Prophylactic antibiotics are not recommended. All participating centers are capable of offering appropriate intensive care in case the patients require organ support or continuous monitoring. The necrotic collection will be intervened when an infection is suspected or confirmed. Still, the intervention should be optimally delayed for four weeks when the patient could tolerate the symptoms, as suggested by the guidelines[17].

When infected pancreatic necrosis occurs, either a surgical or endoscopic step-up approach considering the location of the necrotic collection and the technical availability in each participating center will be applied. Initial drainage is optimally performed after the necrotic collection is well walled-off based on guideline recommendations[17]. Principally, either percutaneous catheter drainage or endoscopic transluminal placement of double pig-tail stents， rather than debridement, are the primary choices of treatment.

7. OUTCOME MEASURES

7.1 Primary outcome measures

The incidence of IPN during the index admission will be served as the primary outcome measure of the TRACE trial.

The diagnosis of IPN will be based on the international guidelines when one or more of the following were present: gas bubbles within (peri) pancreatic necrosis on computed tomography; a positive culture of (peri) pancreatic necrosis obtained by image-guided fine-needle aspiration; a positive culture of (peri) pancreatic necrosis obtained during the first drainage and/or necrosectomy [15].

7.2 Secondary outcome measures

Part I: Secondary outcomes during the index admission

1. The occurrence of new-onset organ failure and new-onset persistent organ failure (SOFA score for respiration, cardiovascular, or renal system ≥ 2). New-onset is defined as events that occur after randomization and not present 24 hours before randomization;
2. In-hospital mortality;
3. Bleeding requiring intervention;
4. Gastrointestinal fistula requiring intervention;
5. Positive blood culture;
6. Incidence of pancreatic fistula;
7. New receipt of mechanical ventilation (not applied 24 hours before randomization);
8. New receipt of renal replacement therapy (not applied 24 hours before randomization);
9. New receipt of vasoactive agents (not applied 24 hours before randomization);
10. The requirement for catheter drainage (either percutaneous or endoscopic);
11. Number of drainage procedures required;
12. The requirement for minimally-invasive debridement;
13. Number of minimally invasive necrosectomy required;
14. The requirement for open surgery;
15. Number of open surgery required;
16. Length of intensive care unit (ICU) stay;
17. Length of hospital stay;
18. SOFA score on day0, day7, and day14;
19. CRP level on day0, day7, and day14;
20. HLA-DR level on day0, day7, and day14;
21. Lymphocyte count on day0, day7 and day 14;

22. In-hospital cost.

Part II: *Secondary outcomes within 90 days after enrollment*

23. Incidence of infected pancreatic necrosis within 90 days after enrollment;

24. Mortality within 90 days after enrollment;

7.3 Adverse events

Adverse events (AEs) are defined in accordance with the National Cancer Institute-Common Terminology Criteria for Adverse Events as any untoward medical occurrence in a patient or clinical investigation subject administered an investigational intervention and which does not necessarily have to have a causal relationship with this treatment.

It is recognized that the patient population (ANP with a relatively high APACHE II score) will experience a number of common aberrations in laboratory values, signs, and symptoms due to the severity of the underlying disease and the impact of standard therapies. These will not necessarily constitute an adverse event unless they require significant intervention or are considered to be of concern in the investigator's clinical judgment. The DSMB will review the safety report every six months.

8. SAMPLE SIZE AND POWER

The incidence of pancreatic infection during the index admission was reported to be around 25% in ANP episodes combined with an APACHE II score≥8 in our previous studies [18, 19]. To demonstrate at least a 40% reduction in the incidence of pancreatic infection on the basis of our pilot study [14], we projected a sample size of 500 participants with 80% power at a two-sided alpha level of 0.05 using the PASS software (PASS 11, NCSS software, Kaysville, USA). In our study, we planned to randomize 520 patients after considering 4% of lost follow-up.

9. DATA COLLECTION AND FOLLOW UP

A web-based electrical database (access through the website of the CAPCTG, https://capctg.medbit.cn/) will be used for data collection and storage. All data will be input by the principal investigator or nominated investigator (less than two for each participating center) approved by the principal investigator, and a double check will be done by the research coordinator. Training for data entry will be performed by the provider of the electrical database and the coordinating and data management center of the CAPCTG. According to the data collection schedule, the investigator will collect data during the index admission and on day 90 after enrollment.

10. WITHDRAWALS

In the study period, patients or their next of kin could choose to opt-out from participating in the collection of outcome measures, while remaining under the intervention if appropriate. Patients who discontinued completing the data collection prior to the end of the trial period will still be included in the full analysis population unless they requested otherwise. Reasons for withdrawal should be documented wherever possible and communication between the principal investigator and the site investigators should be arranged.

11. ANALYSIS POPULATION

The principle of intention-to-treat (ITT) will be the main strategy of the analysis adopted for the primary outcome and all the secondary outcomes. The membership of each analysis set will be determined and documented and the reasons for exclusion will be given prior to database lock. A summary table will list the individual subjects sorted by treatment group and describe their protocol deviation/violation.

11.1 Intention-to-treat (ITT) population

This population consists of all randomized subjects, regardless of whether they are ineligible, prematurely discontinue treatment, or are otherwise protocol violators/deviators.

11.2 Per-protocol (PP) population

This population is a subset of the ITT population. Subjects with major protocol violations will be excluded from the PP population.

A major violation is defined as：

- An event that leads to unplanned discontinuation of the experimental drug for more than seven doses of the total 21 planned doses. Subjects who are deemed as cured and discharged before administrating all the study doses will not be counted.
  - An AE;
  - Withdrawal of consent from the study;
  - Unblinding of the study drug;
  - Medical objection to further use of the study drug proposed by the treating physician
- More than seven doses of the total 21 planned doses are missed.
- Violation of the inclusion/exclusion criteria found after enrollment.

11.3 Safety population

This population consists of all randomized subjects who receive at least one dose of the study drug.

12. GENERAL CONSIDERATIONS FOR DATA ANALYSES

12.1 Reporting guidelines

We will follow the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement: updated guidelines for reporting parallel group randomized trials (http://www.consort-statement.org/).

12.2 Participant disposition and Flow chart

A flow chart will be drawn up showing the number of patients screened, enrolled, and followed-up in each study arm, and the number contributing to the ITT, per-protocol and safety analysis.

The number screened and not enrolled and the reasons for non-enrollment will be reported, as well as the number and reasons of patients who were lost for follow up, or who were withdrawn from the study for safety reasons, or who crossed over between study arms, or because of other reasons, et al.

A list of major protocol deviations will be presented after being unblindly confirmed by TSC.

12.3 Data Summaries

Continuous variables will be summarised according to the number of subjects with non-missing data (n), mean, standard deviation (SD), median, minimum, and maximum.

Categorical variables will be summarised according to the absolute frequency and percentage of subjects (%) in each category level. The denominator for the percentages is the number of subjects in the treatment arm with data available unless noted otherwise.

12.4 Planned Covariates

Covariate analyses will be performed, in particular the primary outcome on the ITT population. The prespecified covariates in this study will be:

- Site
- Severity of AP (severe and non-severe),
- Age (>60 and ≤60)
- Etiologies (biliary and non-biliary)
- The extent of pancreatic necrosis(>50% and ≤50%)
- Organ failure (Yes and No)

12.5 Subgroup Analysis

Prespecified subgroup analyses will be performed for the primary outcomes on the ITT population. The subgroup variables are defined as below:

- Severity of AP (severe and non-severe),
- Age (>60 and ≤60)
- Etiologies (biliary and non-biliary)
- The extent of pancreatic necrosis(>50% and ≤50%)

The treatment effect within each category of the above-selected variables will be estimated and the interaction effect between treatment and each variable assessed to explore effect modification.

12.6 Missing Data

12.6.1 Baseline covariates

The following are baseline prognostic variables ascertained at the time of study enrollment: Age, Gender, BMI (as a continuous variable), etiologies, CTSI score for acute pancreatitis, urine output for the day before enrollment, use of antibiotics, APACHE II score, CRP, absolute lymphocyte count and status of organ failure (SOFA score).

Missing baseline prognostic variables will be replaced with mean values calculated from the observed non-missing instances of that baseline prognostic variable. The imputed means will be calculated using pooled data from both arms. Imputed means will not be calculated within the treatment arm using treatment arm-specific data, nor will any post-randomization information be incorporated into the calculation.

Furthermore, replacement values for missing calculated constructs such as BMI and APACHE II score will be estimated using non-missing component-level information. For example, if one of the components of BMI is missing, such as height, overall mean height will be imputed, and BMI will be calculated with the known weight and imputed mean height.

If a baseline prognostic variable requires an imputation of missing values, the percent of cases that were originally missing will be reported.

12.6.2 Efficacy Outcomes

Missing values for efficacy outcomes will be imputed by means of multiple imputation (10 multiple datasets will be created) using the SAS procedure MI or similar procedures if deemed necessary.

Missing data will be assumed missing at random (MAR), (probability that an observation is missing can depend on the observed values of the individual, but not on the missing variable values of the individual). Imputations will be done on continuous as well as categorical variables. If categorical variables are created from continuous variables the imputations will be conducted on the continuous variable. We will first investigate the Missing Completely at Random (MCAR) assumption by modeling the probability of missing data on treatment assignment and other independent variables. If any of the independent variables are significant then missing data depends on covariates, a violation of MCAR. Then missing data will be assumed MAR. Results derived from multiple under MAR imputation and complete-cases analysis without multiple imputation will be compared in a sensitivity analysis. Models under Missing Not At Random assumption (selection and pattern mixture) will not be done. The focus will be on MAR assumption and how its violation can be investigated in a sensitivity analysis.

12.7 Interim Analyses and early stopping guidelines

No interim unblinding and analysis is planned.

The data and safety monitoring board (DSMB) was composed of an independent group of experts, including clinicians and statisticians that offered advice during the implementation of the study. The DSMB will review the safety report every six months，can recommend that a trial should be stopped early because of concerns about participant safety.

13. STATISTICAL ANALYSES

The principle of intention-to-treat (ITT) will be the main strategy of the analysis adopted for the primary outcome and the secondary outcomes. Analysis among the PP population will be deemed as supportive evidence. The safety analysis set will be used to analyze safety endpoints.

SAS® (version 9.4) will be used to perform all data analyses and generate the majority of data displays. STATA or R may also be used for some data analyses.

13.1 Primary Outcome Analysis

13.1.1 ITT analysis of the primary outcome - the primary analysis

A generalized linear model (GLM) will be used to test our hypothesis. In the GLM model, the incidence of IPN during the index admission will be treated as the response variable following a binomial distribution and the treatment as fixed effect and site as a covariate, and the identity link function will be used. From this model, a point estimate in the incidence of IPN risk difference and its two-sided 95% CI for the group comparison will be estimated. The GLM model will be estimated using SAS GENMOD.

Meanwhile, the relative risk and its two-sided 95% CI will be provided by the GLM model with a binomial distribution and log link function. If the above log-binomial regression model does not converge, the Mantel-Haenszel method will be used to

calculate the RR and its 95% CI stratifying by the site.

13.1.2 Per-protocol analysis of the primary outcome

A supportive analysis of the primary outcome will also be performed on the PP populations. Statistical methods will be the same as above.

13.1.3 Missing primary outcomes

Missing primary outcomes will be assumed to be missing at random (MAR) and thus will be 'ignored' in the primary analysis. However, if greater than 5% of all primary outcomes that should be available for analysis are missing, a sensitivity analysis will be undertaken in addition to the primary analysis.

13.1.4 Covariate adjusted analysis of the primary outcome

Adjusted analyses will also be carried out on the analysis of the primary endpoint to determine whether the treatment effect estimate or standard error is affected by the inclusion of covariables. The covariables that will be included in the adjusted analyses are listed above. Baseline imbalances detected will be included in the adjusted analysis as well but were deemed as sensitivity analysis.

The above log-binomial GLM model may not converge when all covariates are introduced into the model simultaneously. To avoid the non-convergence issue, we will first calculate a propensity score with treatment as the dependent variable (1 for intervention treatment and 0 for the control group) and all covariates listed above as independent variables through a logistic regression model, and then include the calculated propensity score (continuous variable) as a covariate in the log-binomial GLM model.

Imputation for baseline missing covariates will be made for covariate-adjusted analysis.

13.1.5 Subgroup analysis of the primary outcome

If treatment differences can not estimable due to the small numbers within each category, the nearest category will be combined.

Assessment of the homogeneity of treatment effect by a subgroup variable will be conducted by a GLM with the treatment, subgroup variable, and their interaction term as predictors, and the P-value presented for the interaction term.

13.2 Secondary Outcome Analysis

We consider all secondary analyses to be exploratory and hypothesis-generating and therefore, do not adjust for multiple comparisons.

13.2.1 Analysis of binary outcomes

For binary secondary outcomes with single measurement will be analyzed similarly as the primary endpoint analysis using a GLM of the binomial distribution and identity link functions will be used.

For binary secondary outcomes with repeated measurements, they will be summarised using the number (%) of events at each time point and analyzed using a generalized estimating equation (GEE) model, in which treatment, time, the interaction between treatment and time as fixed effects, baseline measurement as the covariate, and subject as cluster effect. An exchangeable covariance structure will be used. The RR together with their 95% CI at each time point will be derived.

13.2.2 Analysis of continuous outcomes

The continuous variables with single measurement will be summarised using the number of subjects (n), mean, standard deviation (SD), median (IQR), minimum, and maximum by treatment group, and will be analyzed by a GLM model with treatment as the fixed effect and with normal distribution and identity link function. The difference in mean outcome and mean differences with their two-sided 95% confidence intervals between two groups will be derived from the GLM model. Log transformed method will be applied if skewed data were identified using the Shapiro-Wilk test.

The secondary continuous outcomes with repeated measurements will be summarized using descriptive statistics at each time point and analyzed using a GEE model, in which treatment, time, the interaction between treatment and time as fixed effects, baseline measurement as the covariate, and subject as cluster effect. An exchangeable covariance structure will be used. For GEE model analysis with skewed data, log-transformed data will be used as the response variable. The geometric mean ratio together with their 95% CI at each time point will be derived.

13.3 SAFETY Analyses

Safety variables will be mainly analyzed in a descriptive way using the number of AEs, the number (%) of participants with AEs by treatment arms.

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Thymosin alpha1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis: a multicenter, randomized, double-blind, placebo-controlled, parallel-group trial

Statistical Analysis Plan

Version 2.0-20 Jan 2021

ClinicalTrials.gov: NCT02473406

Protocol version and date: Version3.0- 01/July/2019

Chief Investigator: *Prof. Weiqin Li*

Trial statisticians: *Dr. Tao Chen*

SAP authors: *Dr. Tao Chen, Dr Lu Ke*

Signatures		
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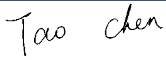
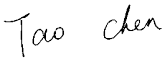
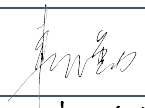
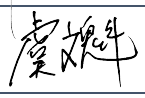
(Statistician)		2021.01.20
(Trial Statistician)		2021.01.20
(Chief Investigator)		2021.01.20
(IDMC Chair)		2021.01.20

Table of Contents

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the outcome variables, statistical methods, and analysis strategies to address the study objectives in Thymosin alpha1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis: a multicenter, randomized, double-blind, placebo-controlled, parallel-group trial (Protocol version: Version3.0- 01/July/2019)

2. BACKGROUND AND RATIONALE:

Infected pancreatic necrosis (IPN) and its related septic complications contribute substantially to morbidity and mortality in patients with acute necrotizing pancreatitis(ANP) 1. Compared with patients with sterile necrosis, those with IPN suffered a significant increase in mortality ranging from 14% to 69%, despite advances in critical care, surgical and endoscopic interventions, and antibiotics [2]. Therefore, the prevention of pancreatic necrosis infection is of great clinical value. Over the past years, numerous attempts had been made to prevent or delay the development of IPN, including antibiotic prophylaxis, early enteral nutritional, selective gut decontamination, and probiotics. Still, none of them had been proved to improve patient-centered outcomes with high-quality evidence [3-6]. More efficient treatment aiming at reducing infectious complications of ANP is in need.

Immunosuppression and disorders characterized by decreased HLA-DR expression and unbalanced CD3/CD4+/CD8+ T cells of peripheral blood mononuclear cell (PBMC) are reported to be associated with IPN [7, 8], especially in those with a more severe type of disease, whose suppressed immune function occurs early and persistently [8, 9]. Our previous observational study found that early enteral nutrition could moderate the excessive immune response during the early stage of severe acute pancreatitis without leading to subsequent immunosuppression, and ultimately reduce the incidence of infection and ICU stay [10]. Thus, immunomodulatory treatment could potentially intervene in the development of secondary infection of pancreatic necrosis, resulting in

better outcomes. Efforts had been made in this field using drugs like lexipafant and octreotide, but the hitherto existing evidence failed to show robust clinical benefits of immunomodulation with regard to patient-centered clinical outcomes [11].

Thymosin alpha 1 had been shown to have immunomodulatory properties and was reported to be clinically beneficial in patients with sepsis [12, 13], majorly through the involvement of distinct Toll-like receptors (TLRs) acting on different dendritic cells (DC) subsets and involving the MyD88-dependent signaling pathway. However, for acute pancreatitis, the only randomized controlled study was the pilot one conducted by our group years ago, suggesting that the use of thymosin alpha 1 was associated with improved cellular immunity and reduced infection rate in a group of 24 patients [14]. Due to the single-center nature of and limited sample size, the clinical implication and generalizability of this study are in doubt. Therefore, we conducted a multicenter, randomized, controlled trial, the thymosin alpha 1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis (TRACE trial) with sufficient power to confirm the findings from the pilot study. In the TRACE trial, we hypothesized that administration of Thymosin Alpha 1 during the acute phase of ANP would result in a reduced incidence of IPN

3. STUDY OBJECTIVES

3.1 Primary objective

To determine whether thymosin alpha 1 is superior to placebo in reducing the incidence of infected pancreatic necrosis in patients with ANP.

3.2 Secondary objectives

To determine the safety of thymosin alpha 1, and its impact on immune function and other patient-centered clinical outcomes among patients with ANP.

4. ELIGIBILITY CRITERIA

4.1 Inclusion Criteria

1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of AP, serum amylase at least three times the upper limit of normal, and/or characteristic findings of AP on computed tomography or less commonly magnetic resonance imaging (MRI) or transabdominal ultrasonography according to the Revised Atlanta Criteria [15];
2. Less than one week from the onset of abdominal pain;
3. Age between 18 to 70 years old;
4. Acute Physiology and Chronic Health Evaluation (APACHE II) score ≥8 during the last 24 hours before enrollment;
5. Balthazar CT score ≥5 (presence of pancreatic necrosis) [16];
6. Written informed consent obtained;

4.2 Exclusion Criteria

1. Pregnant pancreatitis;
2. History of chronic pancreatitis;
3. Underlying malignancy;
4. Receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before randomization;
5. Patients with a known history of severe cardiovascular, respiratory, renal or hepatic diseases defined as (1) greater than New York Heart Association Class II heart failure(Class II not included), (2) active myocardial ischemia or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with the requirement for home oxygen;
6. Patients with preexisting immune disorders such as AIDS;

A patient will be considered eligible if he/she meets the inclusion criteria and does not meet any of the exclusion criteria. Allocation will be performed after signed consent is obtained.

5. RANDOMIZATION AND BLINDING

After the completion of screening measurements and the acquisition of written informed consent, eligible participants will be randomized in a 1:1 ratio to either the treatment group or the placebo group. The randomization code was computer-generated with a block size of 4, and the randomization was stratified by sites. Trial drugs were then prepared according to the sequence to ensure blindness. The eligible patients were allocated to receive medication in individually numbered packs, according to the sequential order of the randomization center. Sealed envelopes were prepared for emergency unmasking. Participants, clinical investigators, and investigators assessing outcome data will be blinded to the treatment allocation to minimize potential sources of bias. The trial statistician will also be blinded regarding the treatment code when developing the statistical programs, which will be validated and completed using dummy randomization codes. The allocation will only be provided to the study team after locking the database and approval of the statistical analysis plan.

6. INTERVENTION ARM

After randomization, the participant will receive:

1. Thymosin Alpha 1 1.6mg *IH* every 12 hours for the first seven days and 1.6mg *IH* daily for the following seven days. The administration will be terminated any day during the treatment when the patient is deemed as qualified for discharge or dies.
2. Matching placebo (normal saline) using the same mode of administration as the above mentioned.

As shown in Figure 1, the recruited patients will start randomized drugs

subcutaneously from the day after the enrollment day. Thymosin Alpha will be provided by SciClone Pharmaceuticals and the matching placebo by Chengdu Tongde Pharmaceuticals. All study drugs will be stored in a secure area with access limited to the investigators and authorized study site personnel, and under appropriate storage conditions.

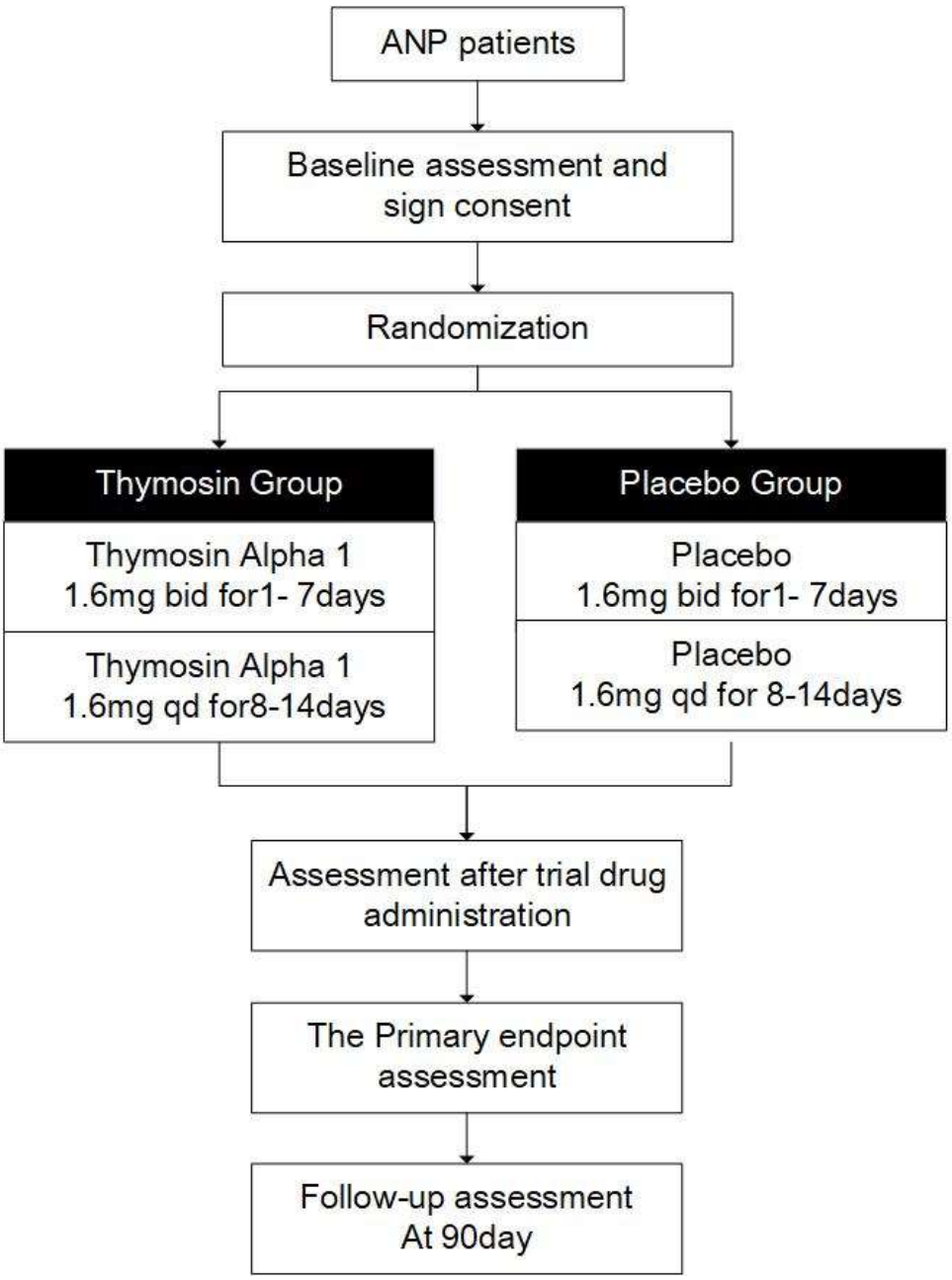


Figure 1: Trial flow chart. ANP denotes acute necrotizing pancreatitis

All patients will receive standard treatment for ANP according to the guidelines[17],

including fluid therapy, early enteral nutrition, routine medical treatment like proton pump inhibitor as indicated, mechanical ventilation if needed, and continuous renal replacement therapy (CRRT) if needed. Prophylactic antibiotics are not recommended. All participating centers are capable of offering appropriate intensive care in case the patients require organ support or continuous monitoring. The necrotic collection will be intervened when an infection is suspected or confirmed. Still, the intervention should be optimally delayed for four weeks when the patient could tolerate the symptoms, as suggested by the guidelines[17].

When infected pancreatic necrosis occurs, either a surgical or endoscopic step-up approach considering the location of the necrotic collection and the technical availability in each participating center will be applied. Initial drainage is optimally performed after the necrotic collection is well walled-off based on guideline recommendations[17]. Principally, either percutaneous catheter drainage or endoscopic transluminal placement of double pig-tail stents， rather than debridement, are the primary choices of treatment.

7. OUTCOME MEASURES

7.1 Primary outcome measures

The incidence of IPN during the index admission will be served as the primary outcome measure of the TRACE trial.

The diagnosis of IPN will be based on the international guidelines when one or more of the following were present: gas bubbles within (peri) pancreatic necrosis on computed tomography; a positive culture of (peri) pancreatic necrosis obtained by image-guided fine-needle aspiration; a positive culture of (peri) pancreatic necrosis obtained during the first drainage and/or necrosectomy [15].

7.2 Secondary outcome measures

Part I: Secondary outcomes during the index admission

1. The occurrence of new-onset organ failure and new-onset persistent organ failure (SOFA score for respiration, cardiovascular, or renal system ≥ 2). New-onset is defined as events that occur after randomization and not present 24 hours before randomization;
2. In-hospital mortality;
3. Bleeding requiring intervention;
4. Gastrointestinal fistula requiring intervention;
5. Positive blood culture;
6. Incidence of pancreatic fistula;
7. New receipt of mechanical ventilation (not applied 24 hours before randomization);
8. New receipt of renal replacement therapy (not applied 24 hours before randomization);
9. New receipt of vasoactive agents (not applied 24 hours before randomization);
10. The requirement for catheter drainage (either percutaneous or endoscopic);
11. Number of drainage procedures required;
12. The requirement for minimally-invasive debridement;
13. Number of minimally invasive necrosectomy required;
14. The requirement for open surgery;
15. Number of open surgery required;
16. Length of intensive care unit (ICU) stay;
17. Length of hospital stay;
18. SOFA score on day0, day7, and day14;
19. CRP level on day0, day7, and day14;
20. HLA-DR level on day0, day7, and day14;
21. Lymphocyte count on day0, day7 and day 14;
22. In-hospital cost.

Part II: Secondary outcomes within 90 days after enrollment

23. Incidence of infected pancreatic necrosis within 90 days after enrollment;

24. Mortality within 90 days after enrollment;

7.3 Adverse events

Adverse events (AEs) are defined in accordance with the National Cancer Institute-Common Terminology Criteria for Adverse Events as any untoward medical occurrence in a patient or clinical investigation subject administered an investigational intervention and which does not necessarily have to have a causal relationship with this treatment.

It is recognized that the patient population (ANP with a relatively high APACHE II score) will experience a number of common aberrations in laboratory values, signs, and symptoms due to the severity of the underlying disease and the impact of standard therapies. These will not necessarily constitute an adverse event unless they require significant intervention or are considered to be of concern in the investigator's clinical judgment. The DSMB will review the safety report every six months.

8. SAMPLE SIZE AND POWER

The incidence of pancreatic infection during the index admission was reported to be around 25% in ANP episodes combined with an APACHE II score≥8 in our previous studies [18, 19]. To demonstrate at least a 40% reduction in the incidence of pancreatic infection on the basis of our pilot study [14], we projected a sample size of 500 participants with 80% power at a two-sided alpha level of 0.05 using the PASS software (PASS 11, NCSS software, Kaysville, USA). In our study, we planned to randomize 520 patients after considering 4% of lost follow-up.

9. DATA COLLECTION AND FOLLOW UP

A web-based electrical database (access through the website of the CAPCTG, https://capctg.medbit.cn/) will be used for data collection and storage. All data will be input by the principal investigator or nominated investigator (less than two for each participating center) approved by the principal investigator, and a double check will be done by the research coordinator. Training for data entry will be performed by the provider of the electrical database and the coordinating and data management center of the CAPCTG. According to the data collection schedule, the investigator will collect data during the index admission and on day 90 after enrollment.

10. WITHDRAWALS

In the study period, patients or their next of kin could choose to opt-out from participating in the collection of outcome measures, while remaining under the intervention if appropriate. Patients who discontinued completing the data collection prior to the end of the trial period will still be included in the full analysis population unless they requested otherwise. Reasons for withdrawal should be documented wherever possible and communication between the principal investigator and the site investigators should be arranged.

11. ANALYSIS POPULATION

The principle of intention-to-treat (ITT) will be the main strategy of the analysis adopted for the primary outcome and all the secondary outcomes. The membership of each analysis set will be determined and documented and the reasons for exclusion will be given prior to database lock. A summary table will list the individual subjects sorted by treatment group and describe their protocol deviation/violation.

11.1 Intention-to-treat (ITT) population

This population consists of all randomized subjects, regardless of whether they are ineligible, prematurely discontinue treatment, or are otherwise protocol violators/deviators.

11.2 Per-protocol (PP) population

This population is a subset of the ITT population. Subjects with major protocol violations will be excluded from the PP population.

A major violation is defined as：

- An event that leads to unplanned discontinuation of the experimental drug for more than seven doses of the total 21 planned doses. Subjects who are deemed as cured and discharged before administrating all the study doses will not be counted.
  - An AE;
  - Withdrawal of consent from the study;
  - Unblinding of the study drug;
  - Medical objection to further use of the study drug proposed by the treating physician
- More than seven doses of the total 21 planned doses are missed.
- Violation of the inclusion/exclusion criteria found after enrollment.

11.3 Safety population

This population consists of all randomized subjects who receive at least one dose of the study drug.

12. GENERAL CONSIDERATIONS FOR DATA ANALYSES

12.1 Reporting guidelines

We will follow the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement: updated guidelines for reporting parallel group randomized trials (http://www.consort-statement.org/).

12.2 Participant disposition and Flow chart

A flow chart will be drawn up showing the number of patients screened, enrolled, and followed-up in each study arm, and the number contributing to the ITT, per-protocol and safety analysis.

The number screened and not enrolled and the reasons for non-enrollment will be reported, as well as the number and reasons of patients who were lost for follow up, or who were withdrawn from the study for safety reasons, or who crossed over between study arms, or because of other reasons, et al.

A list of major protocol deviations will be presented after being unblindly confirmed by TSC.

12.3 Data Summaries

Continuous variables will be summarised according to the number of subjects with non-missing data (n), mean, standard deviation (SD), median, minimum, and maximum.

Categorical variables will be summarised according to the absolute frequency and percentage of subjects (%) in each category level. The denominator for the percentages is the number of subjects in the treatment arm with data available unless noted otherwise.

12.4 Planned Covariates

Covariate analyses will be performed, in particular the primary outcome on the ITT population. The prespecified covariates in this study will be:

- Site
- Severity of AP (severe and non-severe),
- Age (>60 and ≤60)
- Etiologies (biliary and non-biliary)
- The extent of pancreatic necrosis(>50% and ≤50%)
- Organ failure (Yes and No)

12.5 Subgroup Analysis

Prespecified subgroup analyses will be performed for the primary outcomes on the ITT population. The subgroup variables are defined as below:

- Severity of AP (severe and non-severe),
- Age (>60 and ≤60)
- Etiologies (biliary and non-biliary)
- The extent of pancreatic necrosis(>50% and ≤50%)

The treatment effect within each category of the above-selected variables will be estimated and the interaction effect between treatment and each variable assessed to explore effect modification.

12.6 Missing Data

12.6.1 Baseline covariates

The following are baseline prognostic variables ascertained at the time of study enrollment: Age, Gender, BMI (as a continuous variable), etiologies, CTSI score for acute pancreatitis, urine output for the day before enrollment, use of antibiotics, APACHE II score, CRP, absolute lymphocyte count and status of organ failure (SOFA score).

Missing baseline prognostic variables will be replaced with mean values calculated from the observed non-missing instances of that baseline prognostic variable. The imputed means will be calculated using pooled data from both arms. Imputed means

will not be calculated within the treatment arm using treatment arm-specific data, nor will any post-randomization information be incorporated into the calculation. Furthermore, replacement values for missing calculated constructs such as BMI and APACHE II score will be estimated using non-missing component-level information. For example, if one of the components of BMI is missing, such as height, overall mean height will be imputed, and BMI will be calculated with the known weight and imputed mean height.

If a baseline prognostic variable requires an imputation of missing values, the percent of cases that were originally missing will be reported.

12.6.2 Efficacy Outcomes

Missing values for efficacy outcomes will be imputed by means of multiple imputation (10 multiple datasets will be created) using the SAS procedure MI or similar procedures if deemed necessary.

Missing data will be assumed missing at random (MAR), (probability that an observation is missing can depend on the observed values of the individual, but not on the missing variable values of the individual). Imputations will be done on continuous as well as categorical variables. If categorical variables are created from continuous variables the imputations will be conducted on the continuous variable. We will first investigate the Missing Completely at Random (MCAR) assumption by modeling the probability of missing data on treatment assignment and other independent variables. If any of the independent variables are significant then missing data depends on covariates, a violation of MCAR. Then missing data will be assumed MAR. Results derived from multiple under MAR imputation and complete-cases analysis without multiple imputation will be compared in a sensitivity analysis. Models under Missing Not At Random assumption (selection and pattern mixture) will not be done. The focus will be on MAR assumption and how its violation can be investigated in a sensitivity analysis.

12.7 Interim Analyses and early stopping guidelines

No interim unblinding and analysis is planned.

The data and safety monitoring board (DSMB) was composed of an independent group of experts, including clinicians and statisticians that offered advice during the implementation of the study. The DSMB will review the safety report every six months，can recommend that a trial should be stopped early because of concerns about participant safety.

13.STATISTICAL ANALYSES

The principle of intention-to-treat (ITT) will be the main strategy of the analysis adopted for the primary outcome and the secondary outcomes. Analysis among the PP population will be deemed as supportive evidence. The safety analysis set will be used to analyze safety endpoints.

SAS® (version 9.4) will be used to perform all data analyses and generate the majority of data displays. STATA or R may also be used for some data analyses.

13.1 Primary Outcome Analysis

13.1.1 ITT analysis of the primary outcome - the primary analysis

A generalized linear model (GLM) will be used to test our hypothesis. In the GLM model, the incidence of IPN during the index admission will be treated as the response variable following a binomial distribution and the treatment as fixed effect and site as a covariate, and the identity link function will be used. From this model, a point estimate in the incidence of IPN risk difference and its two-sided 95% CI for the group comparison will be estimated. The GLM model will be estimated using SAS GENMOD.

Meanwhile, the relative risk and its two-sided 95% CI will be provided by the GLM model with a binomial distribution and log link function. If the above log-binomial regression model does not converge, the Mantel-Haenszel method will be used to

calculate the RR and its 95% CI stratifying by the site.

13.1.2 Per-protocol analysis of the primary outcome

A supportive analysis of the primary outcome will also be performed on the PP populations. Statistical methods will be the same as above.

13.1.3 Missing primary outcomes

Missing primary outcomes will be assumed to be missing at random (MAR) and thus will be 'ignored' in the primary analysis. However, if greater than 5% of all primary outcomes that should be available for analysis are missing, a sensitivity analysis will be undertaken in addition to the primary analysis.

13.1.4 Covariate adjusted analysis of the primary outcome

Adjusted analyses will also be carried out on the analysis of the primary endpoint to determine whether the treatment effect estimate or standard error is affected by the inclusion of covariables. The covariables that will be included in the adjusted analyses are listed above. Baseline imbalances detected will be included in the adjusted analysis as well but were deemed as sensitivity analysis.

The above log-binomial GLM model may not converge when all covariates are introduced into the model simultaneously. To avoid the non-convergence issue, we will first calculate a propensity score with treatment as the dependent variable (1 for intervention treatment and 0 for the control group) and all covariates listed above as independent variables through a logistic regression model, and then include the calculated propensity score (continuous variable) as a covariate in the log-binomial GLM model.

Imputation for baseline missing covariates will be made for covariate-adjusted analysis.

13.1.5 Subgroup analysis of the primary outcome

If treatment differences can not estimable due to the small numbers within each category, the nearest category will be combined.

Assessment of the homogeneity of treatment effect by a subgroup variable will be conducted by a GLM with the treatment, subgroup variable, and their interaction term as predictors, and the P-value presented for the interaction term.

13.2 Secondary Outcome Analysis

We consider all secondary analyses to be exploratory and hypothesis-generating and therefore, do not adjust for multiple comparisons.

13.2.1 Analysis of binary outcomes

For binary secondary outcomes with single measurement will be analyzed similarly as the primary endpoint analysis using a GLM of the binomial distribution and identity link functions will be used.

For binary secondary outcomes with repeated measurements, they will be summarised using the number (%) of events at each time point and analyzed using a generalized estimating equation (GEE) model, in which treatment, time, the interaction between treatment and time as fixed effects, baseline measurement as the covariate, and subject as cluster effect. An exchangeable covariance structure will be used. The RR together with their 95% CI at each time point will be derived.

13.2.2 Analysis of continuous outcomes

The continuous variables with single measurement will be summarised using the number of subjects (n), mean, standard deviation (SD), median (IQR), minimum, and maximum by treatment group, and will be analyzed by a GLM model with treatment as the fixed effect and with normal distribution and identity link function. The difference in mean outcome and mean differences with their two-sided 95% confidence intervals between two groups will be derived from the GLM model. Log transformed method will be applied if skewed data were identified using the Shapiro-Wilk test.

The secondary continuous outcomes with repeated measurements will be summarized using descriptive statistics at each time point and analyzed using a GEE model, in which treatment, time, the interaction between treatment and time as fixed effects, baseline measurement as the covariate, and subject as cluster effect. An exchangeable covariance structure will be used. For GEE model analysis with skewed

data, log-transformed data will be used as the response variable. The geometric mean ratio together with their 95% CI at each time point will be derived.

13.2.3 Analysis of time-to-event outcomes

Time-to-event outcomes (e.g. time from randomization to the occurrence of death from any cause or Time to infections during the study) and will be summarised by the number (%) of participants with the event, person-years, and incidence rate by treatment arm.

The trial arms will be compared using the log-rank test, as a two-sided test. The Kaplan-Meier plots will be drawn to describe the process of death by treatment arms. Cox regression model will be used to derive the hazard ratio and its 2-sided 95% confidence interval for comparing two treatment groups.

13.3 SAFETY Analyses

Safety variables will be mainly analyzed in a descriptive way using the number of AEs, the number (%) of participants with AEs by treatment arms.

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Summary of statistical analysis plan changes

List of Changes

Statistical Analysis Plan Amendment (version 1 to version 2)

Page/ Line No.	Original Text	New Text	Reason
Page 97, Section 13.2 Secondary Outcome Analysis, Line 29	NA	13.2.3 Analysis of time-to-event outcomes Time-to-event outcomes (e.g. time from randomization to the occurrence of death from any cause or Time to infections during the study) and will be summarised by the number (%) of participants with the event, person-years, and incidence rate by treatment arm. The trial arms will be compared using the log-rank test, as a two-sided test. The Kaplan-Meier plots will be drawn to describe the process of death by treatment arms. Cox regression model will be used to derive the hazard ratio and its 2-sided 95% confidence interval for comparing two treatment groups.	Description about Time to event analyses was added