

1 Thymosin alpha 1 in the prevention of infected pancreatic necrosis
2 following acute necrotizing pancreatitis (TRACE trial): protocol of a
3 multicenter, randomized, double-blind, placebo-controlled, parallel-group
4 trial

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52

53 **Abstract**

54 **Introduction:** Infected pancreatic necrosis (IPN) and its related septic complications
55 are the major causes of death in patients with acute necrotizing pancreatitis (ANP).
56 Therefore, the prevention of IPN is of great clinical value, and immunomodulatory
57 therapy with Thymosin Alpha 1 may be beneficial. This study was designed to test the
58 hypothesis that the administration of Thymosin Alpha 1 during the acute phase of ANP
59 will result in a reduced incidence of IPN.

60 **Methods and analysis:** This is a randomized, multicenter, double-blind, placebo-
61 controlled study. 520 eligible ANP patients will be randomized in a 1:1 ratio to receive
62 either the Thymosin alpha 1 or the placebo using the same mode of administration. The
63 primary endpoint is the incidence of IPN during the index admission. Most of the
64 secondary endpoints will be registered within the index admission including in-hospital
65 mortality, the incidence of new-onset organ failure and new-onset persistent organ
66 failure (respiration, cardiovascular and renal), receipt of new organ support therapy,
67 requirement for drainage or necrosectomy, bleeding requiring intervention, HLA-DR
68 on day0, day7, and day14, etc., and adverse events. Considering the possibility of
69 readmission, an additional follow-up will be arranged 90 days after enrollment, and
70 IPN and death at Day90 will also be served as secondary outcomes.

71 **Ethics and dissemination:** This study was approved by the ethics committee of Jinling
72 Hospital, Nanjing University (No. 2015NZKY-004-02). The TRACE trial was designed
73 to test the effect of a new therapy focusing on the immune system in preventing
74 secondary infection following ANP. The results of this trial will be disseminated in peer-
75 reviewed journals and at scientific conferences.

76 **Trial registration:** The trial has been registered at the ClinicalTrials.gov registry
77 (NCT02473406)

78 **Strengths and limitations of this study**

79 Strength 1: This is a randomized, multicenter, double-blind, placebo-controlled trial
80 providing top-class evidence concerning the efficacy and safety of thymosin alpha 1 for
81 patients with acute necrotizing pancreatitis.

82 Strength 2: The data will be handled by an independent data safety monitoring board

83 (DSMB) to ensure the safety of the participants.

84 Strength 3: Thymosin alpha 1 is a well-studied drug with a favorable safety profile in
85 previous trials.

86 Limitation 1: A sample size of 520 is required to detect the efficacy of Thymosin Alpha
87 1 in preventing infected pancreatic necrosis, which will take years before the conclusion
88 could be drawn.

89 Limitation 2: Continuous immune function assessment is not applied in this study.

90

91 **Background**

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

103 Immunosuppression and disorders characterized by decreased HLA-DR expression
104 and unbalanced CD3/CD4+/CD8+ T cells of peripheral blood mononuclear cell are
105 reported to be associated with IPN[7, 8], especially in those with a more severe type of
106 disease, whose suppressed immune function occurs early and strongly[8, 9]. Our
107 previous observational study found that early enteral nutrition could moderate the
108 excessive immune response during the acute phase of severe acute pancreatitis without
109 leading to subsequent immunosuppression, and ultimately reduce the incidence of
110 infection and ICU stay [10]. Thus, immunomodulatory treatment could potentially
111 intervene in the development of IPN, resulting in better outcomes. Efforts had been
112 made in this field using drugs like lexipafant and octreotide. Still, the hitherto existing
113 evidence failed to show robust clinical benefits of immunomodulation with regard to
114 key clinical outcomes [11].

115 Thymosin alpha 1 had been shown to have immunomodulatory properties and was
116 reported to be clinically beneficial in patients with sepsis[12, 13], majorly through the
117 involvement of distinct Toll-like receptors acting on different dendritic cells subsets and
118 involving the MyD88-dependent signaling pathway. However, for acute pancreatitis
119 (AP), the only randomized controlled study was the pilot one conducted by our group
120 years ago, suggesting that the use of Thymosin alpha 1 was associated with improved

121 cellular immunity and reduced infection rate in a group of 24 patients[14]. Due to the
122 single-center set and small sample size, the clinical implication and generalizability of
123 this study are thought to be limited. Therefore, we designed this multicenter trial, the
124 Thymosin Alpha 1 in the Prevention of Infected Pancreatic Necrosis Following Acute
125 Necrotizing Pancreatitis (TRACE), with sufficient power to test the hypothesis that the
126 administration of Thymosin Alpha 1 during the acute phase of ANP will result in a
127 reduced incidence of IPN.

128

129 ***Study objectives***

130 The primary objective of the TRACE trial is to determine whether Thymosin Alpha
131 1 is superior to placebo in reducing the incidence of IPN in patients with ANP.
132 Secondary objectives are to determine the safety and the impact on the immune function
133 of Thymosin Alpha 1 among patients with ANP.

134

135 ***Study Design***

136 The present study is an investigator-initiated, multicenter, individually-randomized,
137 double-blind, placebo-controlled, parallel-group study. This trial was registered on June
138 16th, 2015, in the CT.gov registry (NCT02473406, <https://www.clinicaltrials.gov/>) and
139 was approved by the ethics committee of Jinling Hospital, Nanjing University (No.
140 2015NZKY-004-02). Local ethics approval was also obtained before enrollment in each
141 participating center. The TRACE trial was designed and coordinated by the Center of
142 Severe Acute Pancreatitis at Nanjing University and the coordinating and data
143 management center of the Chinese Acute Pancreatitis Clinical Trials Group (CAPCTG).
144 The trial steering committee (TSC) was formed to oversee the implementation of the
145 study, and a data safety monitoring board (DSMB) will regularly (every six months)
146 review the safety report prepared by the trial statistician from the accumulating data of
147 this trial.

148

149 ***Study population***

150 This trial is performed in 16 hospitals from China. All adult patients with AP admitted
151 to the participating centers will be assessed for eligibility after admission. The inclusion

152 and exclusion criteria are as follows:

153 *Inclusion criteria*

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

164 *Exclusion criteria*

- 165 1. Pregnant pancreatitis;
- 166 2. History of chronic pancreatitis;
- 167 3. Malignancy related acute pancreatitis
- 168 4. Receiving early intervention or surgery due to abdominal compartment syndrome
169 or other reasons before admission;
- 170 5. Patients with a known history of severe cardiovascular, respiratory, renal or hepatic
171 diseases defined as (1) greater than New York Heart Association Class II heart
172 failure(Class II not included), (2) active myocardial ischemia or (3) cardiovascular
173 intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney
174 disease with creatinine clearance $<$ 40 mL/min, or (6) chronic obstructive pulmonary
175 disease with the requirement for home oxygen;
- 176 6. Patients with preexisting immune disorders such as AIDS.

177 A patient will be considered eligible if he/she meets the inclusion criteria and does
178 not meet any of the exclusion criteria. Allocation will be performed after signed consent
179 is obtained. The study protocol flow of participants is outlined in Figure 1.

180

181 ***Randomization and blinding methods***

182 After the completion of screening measurements and the acquisition of written
183 informed consent, eligible participants will be randomized in a 1:1 ratio to either the
184 treatment group or the placebo group. The randomization code was computer-generated
185 with a block size of 4, and the randomization was stratified by sites.

186 Participants, clinical investigators, and investigators assessing outcome data will be
187 blinded to the treatment allocation to minimize potential sources of bias. The trial
188 statistician will also be blinded regarding the treatment code when developing the
189 statistical programs, which will be validated and completed using dummy
190 randomization codes. The actual allocation will only be provided to the study team after
191 locking of the database and approval of the statistical analysis plan.

192

193 ***Trial drugs***

194 After randomization, the participant will receive:

- 195 1. Thymosin Alpha 1 1.6mg I.H q12h for the first seven days and 1.6mg I.H, qd
196 for the following seven days. The administration will be terminated any day
197 during the treatment when the patient is deemed as qualified for hospital
198 discharge, or dead.
- 199 2. Matching placebo(normal saline) using the same mode of administration as
200 the above mentioned.

201 As shown in Figure 1, the recruited patients will start to receive randomized drugs
202 subcutaneously from the day after the allocation day. Thymosin Alpha will be provided
203 by SciClone Pharmaceuticals and the matching placebo by Chengdu Tongde
204 Pharmaceuticals. All study drugs will be stored in a secure area with access limited to
205 the investigators and authorized study site personnel, and under appropriate storage
206 conditions.

207

208 ***General treatment regimen***

209 All patients will receive standard treatment including fluid therapy, early enteral
210 nutrition, routine medical treatment like proton pump inhibitor as indicated, mechanical

211 ventilation if needed, and continuous renal replacement therapy (CRRT) if needed in
212 the light of recently published guidelines[17]. All participating centers are able to offer
213 appropriate intensive care in case the patients require organ support or continuous
214 monitoring. The necrotic collection will be intervened when infection is suspected or
215 confirmed, preferably after four weeks from the onset of the disease when the patient
216 could tolerate the symptoms as suggested by the guidelines[17].

217 When pancreatic infection occurs, either a surgical or endoscopic step-up approach
218 considering the location of the necrotic collection and the technical availability in each
219 participating center will be applied. Principally, either percutaneous catheter drainage
220 or endoscopic transluminal placement of double pig-tail stents, rather than debridement,
221 are the primary choices of treatment.

222

223 ***Endpoints***

224 *Primary outcome measure*

225 The incidence of IPN during the index admission will be served as the primary
226 outcome measure of the TRACE trial. The diagnosis of IPN will be based on the
227 international guidelines when one or more of the following were present: gas bubbles
228 within (peri) pancreatic necrosis on computed tomography; a positive culture of (peri)
229 pancreatic necrosis obtained by image-guided fine-needle aspiration; a positive culture
230 of (peri) pancreatic necrosis obtained during the first drainage and/or necrosectomy[15].

231 *Secondary outcome measures*

232 *Part I: Secondary outcomes during the index admission*

- 233 1. The occurrence of new-onset organ failure and new-onset persistent organ failure
234 (SOFA score for respiration, cardiovascular, or renal system ≥ 2). New-onset is
235 defined as events that occur after randomization and not present 24 hours before
236 randomization;
- 237 2. In-hospital mortality;
- 238 3. Bleeding requiring intervention;
- 239 4. Gastrointestinal perforation or fistula requiring intervention;
- 240 5. Incidence of pancreatic fistula

- 241 6. New receipt of mechanical ventilation (not applied 24 hours before
242 randomization);
- 243 7. New receipt of renal replacement therapy (not applied 24 hours before
244 randomization);
- 245 8. New receipt of vasoactive agents (not applied 24 hours before randomization);
- 246 9. The requirement for catheter drainage (either percutaneous or endoscopic)
- 247 10. Number of drainage procedures required;
- 248 11. The requirement for minimally-invasive debridement;
- 249 12. Number of minimally invasive necrosectomy required;
- 250 13. The requirement for open surgery;
- 251 14. Number of open operations required;
- 252 15. Length of intensive care unit(ICU) stay;
- 253 16. Length of hospital stay;
- 254 17. SOFA score on day0, day7, and day14;
- 255 18. CRP level on day0, day7, and day14;
- 256 19. HLA-DR level on day0, day7, and day14;
- 257 20. Lymphocyte count on day0, day7 and day 14;
- 258 21. In-hospital cost.

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

- 260 1. Incidence of infection within 90 days after enrollment;
- 261 2. Mortality within 90 days after enrollment.

262

263 ***Sample size estimation***

264 The incidence of IPN during the index admission was reported to be around 25% in
265 ANP episodes combined with an APACHE II score \geq 8 in our previous studies[18, 19].
266 To reduce the incidence of IPN from 25% to 15% on the basis of our pilot study [14],
267 we projected a sample size of 500 participants with 80% power at a two-sided alpha
268 level of 0.05 using the PASS software (PASS 11, NCSS software, Kaysville, USA). In
269 our study, we planned to randomize 520 patients after considering 4% of lost follow up.

270

271 ***Statistical analysis***

272 Primary analyses will be based on the intention-to-treat (ITT) population, and
273 secondary supportive analyses will be done on the PP population. The safety analysis
274 will be performed on the safety population. Missing data will be handled by multiple
275 imputations to evaluate the robustness of the primary endpoint analyses[20]. The
276 populations are defined as follows:

- 277 1. ITT population: This population consists of all randomized subjects, regardless of
278 whether they are ineligible, prematurely discontinue treatment, or are otherwise
279 protocol violators/deviators.
- 280 2. Per-protocol (PP) population: This population is a subset of the ITT population.
281 Subjects with major protocol deviations will be excluded from the PP population.
282 Major protocol deviations will be defined in the statistical analysis plan.
- 283 3. Safety population: This population will be the same as the ITT population, which
284 consists of all randomized subjects, who receive at least one dose of study drug.

285 The normality of continuous variables was examined using skewness and kurtosis.
286 Categorical data were expressed as number and percentage. A generalized linear
287 model (GLM) will be employed to compare group differences in the primary outcome.
288 No interim analysis was planned in our study. The detailed analysis strategies for
289 secondary outcomes and subgroup analyses by the severity of AP(severe and non-
290 severe), age(dichotomized at 60 years old), etiologies of AP (biliary and non-biliary)
291 and extent of pancreatic necrosis(>50% and ≤50%), will be included in the statistical
292 analysis plan. Statistical tests will be two-sided, and p values < 0.05 will be deemed as
293 significant.

294

295 ***Adverse events***

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

301 It is recognized that the study patient population (ANP with relatively high APACHE
302 II score) will experience a number of common aberrations in laboratory values, signs,
303 and symptoms due to the severity of the underlying disease and the impact of standard
304 therapies. These will not necessarily constitute an adverse event unless they require
305 significant intervention or are considered to be of concern in the investigator's clinical
306 judgment. Thymosin alpha 1 is a well-studied drug with a favorable safety profile in
307 previous trials [21]. The DSMB will review the safety report every six months.

308

309 ***Recruiting process***

310 The trial was registered on June 16th, 2015, in the CT.gov registry(NCT02473406
311 <https://www.clinicaltrials.gov>). The first patient was randomized on March 22nd, 2017.
312 So far, 426 patients had been randomized, and the enrollment keeps to the schedule.

313

314 ***Patient and Public Involvement***

315 Patients or the public were not involved in the design, or conduct, or reporting, or
316 dissemination plans of our research.

317

318 ***Data collection and management***

319 A web-based electrical database (access through the website of the CAPCTG,
320 <https://capctg.medbit.cn/>) will be used for data collection and storage. All data will be
321 input by the primary investigator or nominated investigators (less than two for each
322 participating center) approved by the primary investigator, and a double check will be
323 done by the research coordinator. Training for data entry will be performed by the
324 provider of the electrical database (Unimed Scientific Inc., Wuxi, China) and the
325 coordinating and data management center of the CAPCTG. According to the schedule
326 shown in Figure 2, the investigator will collect data during the index admission and on
327 day 90 after enrollment. If a study subject wishes to discontinue the study drug or the
328 treating physician believes a study subject should discontinue the study drug due to
329 medical considerations, the investigator will communicate with the study subject and
330 the treating physician to obtain the reasons. Further evaluation and follow-up will still

331 be performed unless the study subject withdraws consent for disclosure of information.
332 The study blinding will only be broken in a medical emergency when the treating
333 physician believes that the administration of the study drug is associated with the
334 emergency.

335

336 ***Ethics approval and dissemination***

337 This study was approved by the ethics committee of Jinling Hospital. The ethical
338 approval document ID is 2015NZKY-004-02. Even when central ethical approval has
339 been confirmed, we will not begin recruiting at other participating centers in the trial
340 until the local ethics committee approved the study. Site ethical approvals were obtained
341 from ethics committees of First Affiliated Hospital of Nanchang University, The
342 Affiliated Hospital of Qingdao University, Affiliated Hospital of Zunyi Medical
343 College, Nanhua Hospital, Second Affiliated Hospital of Nantong University, Yijishan
344 Hospital of Wannan Medical College, 908th Hospital of Chinese People's Liberation
345 Army, Jiangsu Province Hospital on Integration of Chinese and Western Medicine,
346 Zhejiang Provincial People's Hospital, Luoyang Central Hospital, The Affiliated
347 Hospital of Henan University of Science and Technology, Northern Jiangsu People's
348 Hospital, First People's Hospital of Shangqiu, Qilu Hospital of Shandong University,
349 and First Affiliated Hospital of Anhui Medical University. The results of this trial will
350 be reported in peer-reviewed journals and presented at scientific conferences.

351

352 ***Consent to participate***

353 The consents for this study is obtained from each patient or his/her next of kin with
354 full information regarding the possible adverse effects of the experimental drug and
355 potential consequences. The translated patient consent form is attached as a
356 supplemental file(Supplement Materials).

357

358 **Discussion**

359 The TRACE trial was designed to test the efficacy of a new therapy targeting the
360 immune system in preventing IPN following ANP, which is a potentially lethal

361 complication causing substantial morbidity and mortality. We also aimed to investigate
362 the efficacy of immunomodulatory treatment with thymosin alpha 1 in patients with
363 different clinical characteristics using predefined subgroup analysis. The results of the
364 TRACE trial would potentially provide a novel therapeutic option in the management
365 of ANP and identify the patient population who may benefit most from the
366 administration of thymosin alpha 1.

367 Immunomodulation is of significant clinical value in critically ill settings and the
368 treatment of sepsis[12]. While, acute pancreatitis, which has a lot in common with
369 sepsis like overwhelmed inflammation and infection related complications, might be
370 another suitable target for immunomodulatory therapy. In general, previously studied
371 drugs such as lexipafant and octreotide were aimed to control cytokines, which are
372 thought to be the pivotal part in the early inflammatory response of AP, rather than
373 preventing the development of IPN[11]. However, like what we learned in sepsis,
374 immunosuppression quickly following the initial inflammatory cascades should be the
375 target of treatment during the course of ANP, as well, especially in those with organ
376 failure[8, 22]. A pilot study published by our group several years ago indicated that the
377 administration of thymosin alpha 1 could improve compromised monocyte HLA-DR
378 expression and reduce infection rate in a small group of patients (n=24) with severe
379 acute pancreatitis defined by the original Atlanta Classification. The result of this study
380 is encouraging which drive us to conduct this large multi-center RCT to obtain more
381 reliable clinical evidences[14].

382 The TRACE trial was sponsored by the CSAP at Jinling Hospital, Nanjing University,
383 which is the national referral center for acute pancreatitis (AP) admitting more than 600
384 cases of AP annually and coordinated by the CAPCTG coordinating and data
385 management center, which could cover the whole country. The trial is performed in 16
386 centers across China and aims to recruit 520 patients. Due to the limitation of the budget
387 and technical availability, we can not conduct a continuous immune assessment with
388 multiple markers and more time points. Alternatively, we choose monocyte HLA-DR,
389 which is a representative parameter of the immune system, majorly reflecting the
390 antigen presentation capacity to assess the immunomodulatory effect of thymosin alpha

391 1. HLA-DR was widely used in previous studies regarding immune function in different
392 diseases like sepsis[12, 23].

393 In conclusion, the TRACE trial aims to assess the efficacy of thymosin α 1
394 administered early during the ANP on the incidence of IPN and other major clinical
395 outcomes and thereby potentially offer a novel therapeutic option in the treatment of
396 ANP patients.

397

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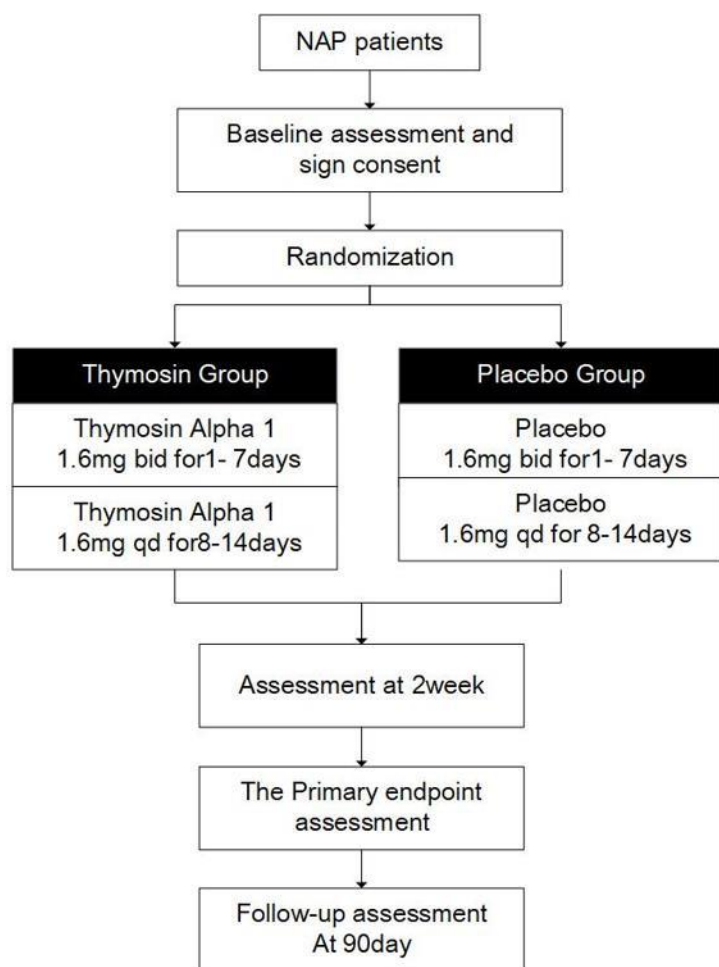
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449

450 Figure 1: Trial flow chart.

451

TIMEPOINT	Study period							
	Enrollment	Allocation	Index admission					Follow-up
	<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

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

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468 *Declarations*

469 **Authors' contributions:**

470 All authors were involved in the study design, and read and approved the final manuscript. During
471 the study, J Z, W M and Y L are responsible for randomizing the patients and ensuring the blinding.
472 J Z, W H, X P, M C, C H, W G, J W, J S, H N, J T, J S, G Z, W C, B X, X Z, M S are responsible
473 for carrying out recruitment, managing the treatment of the patients and collecting data. W L, Z T, L
474 K, J Z, T M and W H, X P, M C, C H, W G, J W, J S, H N, J T, J S, G Z, W C, B X, X Z, M S are
475 members of the TSC. J Z, L K, Z T and T C drafted the manuscript.

476 **Competing interests**

477 This study is supported by SBE2016750187 of Science and technology project, Jiangsu Province,
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485 *Consent for publication*

486 Not Applicable.

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