PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Thymosin alpha 1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis (TRACE trial): protocol of a multicenter, randomized, double-blind, placebo-controlled, parallel-group trial
AUTHORS	Zhou, Jing; Mao, Wenjian; Ke, Lu; Chen, T; He, Wenhua; Pan, Xinting; Chen, Miao; He, Chengjian; Gu, Weili; Wu, Jingyi; Song, Jingchun; Ni, Haibin; Tu, Jianfeng; Sun, Junli; Zhang, Guoxiu; Chen, Weiwei; Xue, Bing; Zhao, Xiangyang; Shao, Min; Liu, Yuxiu; Tong, Zhihui; Li, Weiqin

VERSION 1 – REVIEW

REVIEWER	Pascal Probst University of Heidelberg, Germany
REVIEW RETURNED	19-Feb-2020

GENERAL COMMENTS	Zhou et al. present a randomised-controlled trial comparing Thymosin alpha 1 versus placebo in patients with acute necrotizing pancreatitis. The rationale to perform this study is given by a pilot trial. The methods are adequate. I have a few minor concerns to be addressed: Abstract The word prospective can be deleted throughout the manuscript, since this is clear by the randomised design. Please also state how the hypothesis (superiority) for the primary endpoint is and what the estimated effect will be. Endpoints Please state for the primary endpoint in which time interval you assess it after randomisation. Sample Size estimation Can you please say in another words what you mean by a 40% reduction. Please say the infection rate decreases from 25% in the placebo group to (I assume) 15% in the verum group.

REVIEWER	Ambareen Kausar East Lancashire NHS Trust United Kingdom
REVIEW RETURNED	16-Apr-2020

GENERAL COMMENTS	Please expand on the safety data of thymosin alpha1 in humans, and the adequacy of consent process in particular are they consented for possible excess adverse reactions and mortality in the experimental group. Is there stratification by centre-if not is this a limitation?

VERSION 1 – AUTHOR RESPONSE

Reviwer#1 :

Zhou et al. present a randomised-controlled trial comparing Thymosin alpha 1 versus placebo in patients with acute necrotizing pancreatitis. The rationale to perform this study is given by a pilot trial. The methods are adequate.

I have a few minor concerns to be addressed:

Abstract:

Q1:The word prospective can be deleted throughout the manuscript, since this is clear by the randomised design.

Response:

We agreed with that and deleted “prospective” accordingly. Thanks for your suggestion.

Q2: Please also state how the hypothesis (superiority) for the primary endpoint is and what the estimated effect will be.

Response:

Thanks for your suggestion. We added a sentence in the Introduction section of the Abstract to clearly state our hypothesis(L57-59). Also, we added a similar sentence in the main manuscript(L123-127).

Endpoints

Q3:Please state for the primary endpoint in which time interval you assess it after randomization

Response:

The time interval for the primary endpoint is “during the index admission”. We highlighted that both in the Abstract and Methods section(L62-63 and L225-226) as below:

> This study was designed to test the hypothesis that the administration of Thymosin Alpha 1 during the acute phase of ANP will result in a reduced incidence of IPN.
>
> Therefore, we designed this multicenter trial, the Thymosin Alpha 1 in the Prevention of Infected Pancreatic Necrosis Following Acute Necrotizing Pancreatitis (TRACE), with sufficient power to

test the hypothesis that the administration of Thymosin Alpha 1 during the acute phase of ANP will result in a reduced incidence of IPN.

Sample size

Q4. Can you please say in another words what you mean by a 40% reduction. Please say the infection rate decreases from 25% in the placebo group to (I assume) 15% in the verum group.

Response：

We reworded the sentence and highlighted it according to your suggestion. Please check the revised manuscript in L266-268 as below:

To reduce the incidence of IPN from 25% to 15% on the basis of our pilot study [1], we projected 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)

Reviewer#2

Q1：Please expand on the safety data of thymosin alpha1 in humans,

Response:

Thymosin α1 was very well tolerated at high doses in phase I trials and following clinical trials conducted in cancer patients also reported no particular safety concerns[2, 3]. We have added this under the subtitle “Adverse events” with corresponding references(L306-307) as below.

Thymosin alpha 1 is a well-studied drug with a favorable safety profile in previous trials [2].

Q2：The adequacy of consent process in particular are they consented for possible excess adverse reactions and mortality in the experimental group.

Response:

We rephrased the description of the consent process under the subtitle “Consent to participate”(L352-356) as below ,and we attached an English version of the patient consent form as a supplemental file to offer full information.

The consents for this study is obtained from each patient or his/her next of kin with full information regarding the possible adverse effects of the experimental drug and potential

consequences. The translated patient consent form is attached as a supplemental file(Supplement Materials).

Q3:Is there stratification by centre-if not is this a limitation?

Response:

Thanks for your comments. To account for the heterogeneity among each center, we did use stratified randomization. We have added the details to clarify this under the subtitle “Randomization and blinding methods” in L184-185 as below:

The randomization code was computer-generated with a block size of 4, and the randomization was stratified by sites.

Editorial Requests:

Q1. Please work to improve the quality of the English throughout your manuscript. We recommend asking a native English speaking colleague to assist you or to enlist the help of a professional copyediting service.

Response:

We edited the manuscript scrupulously with aid from a native speaker. We also reworded the study title to keep the terminology consistent. Please check the revised version as below:

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

Q2. Please revise the Strengths and Limitations section of your manuscript (after the Abstract). This section should contain five short bullet points, no longer than one sentence each, that relate specifically to the methods.

Response:

We have rewritten the Strengths and Limitations section, please check the revised version.

Q3. Please reformat the main text so that it follows the structure recommended in the journal’s instructions for authors for study protocols, for example the main text of your manuscript should contain an Ethics and Dissemination section. See: https://bmjopen.bmj.com/pages/authors/#protocol

Response:

We checked the instruction and revised the manuscript accordingly. Please check the revised version in L336-L356 as below:

Ethics approval and dissemination

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

Consent to participate

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

Q4. Please include the name of all the ethics committees that have provided approval in your Ethics and Dissemination sectio of the main text.

Response:

We have added all the site ethics committees under the subtitle "ethics approval and disseminations" in L336-350 as below:

This study was approved by the ethics committee of Jinling Hospital. The ethical approval document ID is 2015NZKY-004-02. Even when central ethical approval has been confirmed, we will not begin recruiting at other participating centers in the trial until the local ethics committee approved the study. Site ethical approvals were obtained from ethics committees of First Affiliated Hospital of Nanchang University, The Affiliated Hospital of Qingdao University, Affiliated Hospital of Zunyi Medical College, Nanhua Hospital, Second Affiliated Hospital of Nantong University, Yijishan Hospital of Wannan Medical College, 908th Hospital of Chinese People's Liberation Army,

Jiangsu Province Hospital on Integration of Chinese and Western Medicine, Zhejiang Provincial People's Hospital, Luoyang Central Hospital, The Affiliated Hospital of Henan University of Science and Technology, Northern Jiangsu People's Hospital, First People's Hospital of Shangqiu, Qilu Hospital of Shandong University, and First Affiliated Hospital of Anhui Medical University. The results of this trial will be reported in peer-reviewed journals and presented at scientific conferences.

Q5. Along with your revised manuscript, please include a copy of the SPIRIT checklist indicating the page/line numbers of your manuscript where the relevant information can be found (http://www.spirit-statement.org/)

Response:

We added the checklist accordingly, please check the updated file named "CHECKLIST".

Q6. Along with your revised manuscript, please provide an English language examples of the patient consent form as a supplementary file as per item #32 of the SPIRIT checklist.

Response: we added the translated consent form as a supplemental file, please check the updated file named "TRACE ICF ENG".

Thank you again for all the editor/reviewers' efforts.

Yours Sincerely

Lu Ke

Jinling Hospital, Nanjing University

Refernces

1. Wang X, Li W, Niu C, Pan L, Li N, Li J: **Thymosin alpha 1 is associated with improved cellular immunity and reduced infection rate in severe acute pancreatitis patients in a double-blind randomized control study**. *Inflammation* 2011, **34**(3):198-202.
2. Schulof RS: **Thymic peptide hormones: basic properties and clinical applications in cancer**. *Critical reviews in oncology/hematology* 1985, **3**(4):309-376.
3. Costantini C, Bellet MM, Pariano M, Renga G, Stincardini C, Goldstein AL, Garaci E, Romani L: **A Reappraisal of Thymosin Alpha1 in Cancer Therapy**. *Frontiers in oncology* 2019, **9**:873.

VERSION 2 – REVIEW

REVIEWER	Pascal Probst University of Heidelberg
REVIEW RETURNED	27-Jul-2020

GENERAL COMMENTS	All queries are resolved.