Study Protocol

A Safety and Efficacy Study of NAC in Patients With TA-TMA

Vision 1.0 Nov. 01, 2017

ClinicalTrials.gov Identifier: NCT03252925

Principal Investigator Yue Han, MD, PhD,
hanyue@suda.edu.cn;

Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Suzhou 215006, China.

Protocol version	Vision 1.0
Issue date	Nov. 01, 2017
Study center	The First Affiliated Hospital of Soochow University

Protocol Summary

Study center The First Affiliated Hospital of Soochow University
Principal Investigator Yue Han, MD, PhD
Methodology A phase III, open-label, single-center, randomized placebo-controlled trial
Primary objective The primary outcome was the incidence of transplant-associated thrombotic microangiopathy (TA-TMA).
Inclusion criteria Patients with malignancies between the ages of 12 and 70 who are scheduled to undergo allogeneic hematopoietic stem cell transplantation are eligible to enter the study. Eligible patients met the following criteria. (i) planned to receive allo-HSCT; (ii) established stable donor hematopoiesis; (iii) Eastern Cooperative Oncology Group (ECOG) performance status of 0-2.
Exclusion criteria

Exclusion criteria were allergy to any component of NAC, bronchial asthma, peptic ulcer, pregnancy and lactation, absence of uncontrolled infection or severe liver, kidney, lung, or heart disease.
Intervention Patients were randomized 1:1 by interactive response technique to receive either oral NAC (50 mg/kg/day continuously from 9 days before HSCT to 30 days after HSCT) or a placebo. A placebo (identical-looking tablets) was administered similarly to NAC for patients randomized to the placebo group.
Total number of patients: 300
Total duration of the study: 3 years

Background

Transplant-associated thrombotic microangiopathy (TA-TMA) is a severe complication in patients following hematopoietic stem cell transplantation (HSCT). Previous investigations suggest that multiple factors resulting in endothelial cell injury lead to the occurrence of TA-TMA. Specifically, the abnormity of complement system contributes to the development of TA-TMA. TA-TMA shares a similar clinical manifestation with thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic uremic syndrome (aHUS), which is characteristic with microangiopathic hemolytic anemia, platelet consumption, fibrin deposition in the microcirculation, and, ultimately, end-organ injury [1]. However, the diagnostic criteria of TA-TMA are far from accurate. Although several diagnostic criteria

had been proposed, no criterion has been rigorously evaluated clinically due to lack of pathological diagnosis. Moreover, the pathogenesis of TA-TMA is still unclear. Conventional treatment management is less efficient [2]. Plasma infusions or therapeutic plasma exchange is considered as limited efficacy, with relatively low response rates (20–50%) in comparison to idiopathic TTP (75%) generally reported [3].

N-acetylcysteine (NAC) is an antioxidant synthesized from cysteine that increases glutathione biosynthesis and scavenges reactive oxygen species directly or indirectly [4]. NAC was recently suggested as a potential treatment for patients with thrombotic thrombocytopenic purpura (TTP) [5-7]. Li and colleagues provide a case report of a TTP patient relieved with NAC, providing the first clinical evidence for the efficacy of NAC in treating TTP [5]. In our study, we explored whether TA-TMA patients might benefit from NAC via targeting complement.

Reference

1. Jodele S, Licht C, Goebel J, et al. Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. Blood. 2013;122:2003-2007.

2. Gavriilaki E, Sakellari I, Anagnostopoulos A, et al. Transplant-associated thrombotic microangiopathy: opening Pandora's box. Bone marrow transplantation. 2017;52:1355-1360.

3. Kennedy GA, Kearey N, Bleakley S, et al. Transplantation-associated thrombotic microangiopathy: effect of concomitant GVHD on efficacy of therapeutic

plasma exchange. Bone marrow transplantation. 2010;45:699–704.

4. G. F. Rushworth and I. L. Megson. Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits. Pharmacol Ther. 2014;141:150-9.

5. G. W. Li, S. Rambally, J. Kamboj, S. Reilly, J. L. Moake, et al. Treatment of refractory thrombotic thrombocytopenic purpura with N-acetylcysteine: a case report. Transfusion. 2014;54:1221-4.

6. A. Rottenstreich, S. Hochberg-Klein, D. Rund and Y. Kalish. The role of N-acetylcysteine in the treatment of thrombotic thrombocytopenic purpura. J Thromb Thrombolysis. 2016;41:678-83.

7. G. Cabanillas and A. Popescu-Martinez. N-Acetylcysteine for Relapsing Thrombotic Thrombocytopenic Purpura: More Evidence of a Promising Drug. Am J Ther. 2016;23:e1277-9.

Study Outcomes

Primary Outcome:

The primary outcome was the incidence of TA-TMA.

Secondary Outcome Measures:

1. The level of VWF multimers in patients post HSCT.
2. The level of endothelial micro particle in patients post HSCT.
3. The level of TNF-α in patients post HSCT.
4. The level of ROS in patients post HSCT.

Dosage and administration

Patients were randomized 1:1 by interactive response technique to receive either oral NAC (50 mg/kg/day continuously from 9 days before HSCT to 30 days after HSCT) or a placebo. A placebo (identical-looking tablets) was administered similarly to NAC for patients randomized to the placebo group.

- Drug: N-Acetylcysteine

50mg/Kg.d, oral

Other Name: NAC

- Drug: Placebo Oral Tablet

50mg/Kg.d, oral

Other Name: Placebo

Statistical methods

Kaplan-Meier survival analysis was used to estimate cumulative incidence of TA-TMA, the cumulative predicted overall survival (OS) rate and event-free survival (EFS) rate, cumulative recurrence rate of malignant hematologic disease, cumulative mortality associated with transplantation, and log-rank test was performed. The statistical process and graph were all run on SPSS 13.0 software. All tests were bilateral, and $P \leq 0.05$ was considered to be significant. OS and EFS are estimated by Kaplan-Meier method.

Ethical statement Ethical principles

The study should be carried out in accordance with Helsinki declaration proposed by World Medical Association and subsequent amendments and approved by the Independent Ethics Committee (IEC) before implementation.

Information and informed consent

➢All the patients who agree to participate should be asked to sign the informed consent form to prove their approval for participation in this study. The informed consent form must be signed and dated personally by the patients and investigators.

➢Before obtaining the informed consent, the investigators must provide sufficient information to the patients who are potential participants in this study:

- The investigators should orally inform the patients of all the relevant circumstances of this study;

- The information provided to patients must be fully and easily understood by non-professionals, so that they can make a decision according to their own willingness based on their full understanding of this study;

- Additionally, the patients voluntarily participate in the study, and are free to quit from the study at any time, without any reasons. The subsequent treatment of patients is not under the influence of unwilling to participate or quitting from the study.

➢All the patients who agree to participate should be asked to sign the Informed Consent Form (ICF) to prove their approval for participation in this study. The ICF must be

signed and dated personally by the patients and the investigators;

➢The signed ICF will be kept in the Data Center and must be safely kept for future review at any time throughout the study.

Withdrawal from the study

The main withdrawal criteria are as following:

➢Changes in the patient's condition after inclusion, which suggests that the study protocol is unsuitable for the patient;

➢Severe complications affecting the implementation of the study treatment;

➢Patients who are confirmed to require emergency treatment due to other diseases after inclusion;

➢Treatment unmated with the study protocol;

➢Patients who voluntarily quit or discontinue any examination, treatment and monitoring required by the study for personal reasons at any stage after inclusion in this study. The PI also has the right to withdraw patients from the study if he/she feels that the withdrawal is in the best interests of the patients.

Identity and privacy of patients

➢After obtaining an ICF, each enrolled patient is assigned a subject number (allocation number). This number will represent the identity of the patient during the study and for the retrieving of dedicated clinical research database;

➢Throughout the study, several measures will be taken to minimize any breaches of personal information, including:

- Only the PI and co-investigators will be able to link to the research data of the patients to themselves through the identifiable table after authorization;

- Collection, transmission, handling and storage of the study data must comply with the data protection and privacy regulations.

Responsibility of independent ethics committee

The responsibilities of independent ethics committee include:
➢Review of this study;

➢Evaluate this study to determine if risks to which patients are exposed have been duly minimized and whether these risks are reasonable compared to expected benefits;

➢Check the study protocol and relevant documents (patient information sheet, ICF, CRF, etc.) submitted by the Research Committee before beginning of the study;

➢Provide the written proof of ethical review opinions, the written proof of the date of the review meeting, the written proof of the members presenting at the meeting and voting members, the written proof of recording of the reviewed versions of study protocol, ICF and other related documents, and if possible, a copy of the minutes, to the Research Committee. The study can begin only after obtaining the written proof of favorable opinions/approval of the IEC;

➢Supervise the legitimacy of the process of informed consent;

➢Ensure the safety of patients during the research process by examining the reported safety information. The

investigators should report to the IEC on any therapeutic complications which may affect the safety of patients. When complications that affect the patient safety occur, the IEC should inform PI to withdraw patients from the study and use any possible treatment to cure patients. If the PI decides not to withdraw patients from the study, he should provide a written proof of explanation to the IEC; ➢Review the reasonableness of the costs incurred by patients in the course of the study through a selective check for hospitalization expenses. When the patient has indications for off-label use of drugs, the IEC will receive the notice and the description of relevant situation from the investigators. The IEC should record the relevant records;

➢Review the revisions of the study protocol, and any changes must be approved by the IEC before they are adopted. Unless the change is necessary to eliminate an immediate hazard to the patients, in which case the IEC should be informed as soon as possible;

➢Review the progress of the study annually and the close-out report submitted by the Research Committee at the end of the trial.

Responsibility of investigator

The responsibilities of investigators are as follows:

➢Investigators, participated in the clinical trial, should be obtained certificate for assistant practicing doctor, resigned and attended the training of the GCP guidelines and the correlation of laws and regulations, possessed specialty, qualification and ability in clinical trial.

➢Subjects are adequately recruited by the investigator during required time according to the clinical trial， and

operation capacity and possibility to be qualified the item should be evaluated by the previous working experience.

➢Investigators are familiar with clinical trial protocols and execute instruction strictly according to the protocols and case report form.

➢In all adverse events, subjects must be taken appropriate therapies and made a contact with the investigator as soon as possible. Investigators will take responsibilities of relevant medical decision of clinical trial to ensure subjects that is taken adequate medical treatment.

➢Any information on the case report form possesses original data, to ensure the case history and case report form filled in truly, accurately, completely, timely, regularly and legally.

➢Subjects will be illustrated the relevant situation where the clinical trial is fully consent by Ethics Committee. The informed consent will be acquired and, during the process, it must meet the specification.

➢Supervision and inspection will be received by supervisor and inspector assigned by sponsor to ensure the quality of clinical trial.

➢Any advices and suggestions focused on the study protocols might be submit to the principal institution of clinical research, and made a discussion with sponsors to decide whether adopting the above advice or suggestions, during the clinical trial.

Publications

➢All the data collected during this study are the property of the study sponsor and cannot be communicated in any case

to a third party without the written agreement of the investigator.

➢Any publication or communication (oral or written) will be decided from a common agreement between the investigators and will respect the international recommendations: "Uniforms Requirements for Manuscripts Submitted to Biomedical Journals" (http://www.cma.ca/publications/mwc/uniform.htm): notably, an authorship will be proposed to each clinical center participating in the study and to each member of the steering committee according to his/her actual participation.

Organization and responsibility

The Research Committee is responsible for developing study protocol, auditing patient eligibility and guiding interpretation of informed consent. It is also responsible for collection of complication reports, guiding diagnosis and treatment of complications and emergency intervention for serious complications, as well as approving the content and distribution of all publications related to the study.

Patient consent form for study participation

Title：A safety and efficacy study of NAC in patients with TA-TMA	
Patient Name	
Please **tick** each box below ☐ if you have known the corresponding statement.	
☐**What you should know about a research study**	

Someone will explain this research study to you.

- A research study is something you volunteer for.
- Presence or absence of participation in the research study is up to you.
- You can choose not to take part in the research study.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- You can feel free to ask all the questions that you want before you decide.

☐ What is the purpose of this study?

Transplant-associated thrombotic microangiopathy (TA-TMA) is a severe complication in patients following hematopoietic stem cell transplantation (HSCT). Previous investigations suggest that multiple factors resulting in endothelial cell injury lead to the occurrence of TA-TMA. Specifically, the abnormity of complement system contributes to the development of TA-TMA. TA-TMA shares a similar clinical manifestation with thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic uremic syndrome (aHUS), which is characteristic with microangiopathic hemolytic anemia, platelet consumption, fibrin deposition in the microcirculation, and, ultimately, end-organ injury. However, the diagnostic criteria of TA-TMA are far from accurate. Although several diagnostic criteria had been proposed, no criterion has been rigorously evaluated clinically due to lack of pathological diagnosis. Moreover, the pathogenesis of TA-TMA is still unclear. Conventional treatment management is less efficient. Plasma infusions or therapeutic plasma exchange is considered as limited efficacy, with relatively low

response rates (20–50%) in comparison to idiopathic TTP (75%) generally reported.
N-acetylcysteine (NAC) is an antioxidant synthesized from cysteine that increases glutathione biosynthesis and scavenges reactive oxygen species directly or indirectly. NAC was recently suggested as a potential treatment for patients with thrombotic thrombocytopenic purpura (TTP). Li and colleagues provide a case report of a TTP patient relieved with NAC, providing the first clinical evidence for the efficacy of NAC in treating TTP. In our study, we explored whether TA-TMA patients might benefit from NAC via targeting complement.

☐ Do I have to take part?

- Participation in the study is entirely voluntary.
- You will be given sufficient time to consider whether you want to participate in the study.
- Non-participation in the study will not affect your future care that you will receive from your medical and nursing team in our hospital.
- If you decide to join the study, you will be asked to sign an informed consent form.
- You are free to withdraw from this research at any time and without giving a reason. Any of your current medical care and future treatment in our hospital will not be affected.

☐ What will happen to me if I take part?

- All patients who agree to take part and provide a written informed consent form will be included into this study.

🎬 You will be randomized 1:1 by interactive response technique to receive either oral NAC (50 mg/kg/day continuously from 9 days before HSCT to 30 days after HSCT) or a placebo.

🎬 Your doctors will introduce the detailed treatment procedures to you. You are free to ask any question about the treatments you may have.

☐ **What are the risks of the study?**

Potential adverse event of NAC includes: bronchial asthma, peptic ulcer, pregnancy and lactation, absence of uncontrolled infection or severe liver, kidney, lung, or heart disease.

☐ **What are my responsibilities in this study?** If you choose to take part in this study, you will need to:

🎬Keep your study appointments.

🎬Tell your doctor about: All medications and supplements you are taking; Any discomforts or complications you suspect; Any doctors' visits or hospital stays outside of this study; Whether you have been or are currently in another research study.

☐ **How will my privacy be protected?**

🎬Your health information, such as your response to drug, results of study tests, and medicines you took, will be kept by the data center of this research. This information is strictly confidential. However, your name and contact information will not be put in the database. If information from this study is published or presented at scientific meetings, there will be no personally identifiable information.

☐ **What happens if I change my mind during the study?**

We would certainly recommend that you continue the study, however, participation in the study is voluntary and you may stop the participation at any time that you wish without losing any of your rights as a patient here. Your treatment at our hospital will not be affected in any way. The PI of this study also has the right to withdraw patients from the study if he feels that the withdrawal is in your best interests. Your follow-up information will still be kept by doctor after you have stopped participating in the research.

☐ **Who is organizing the research?**

The study is organized by the The First Affiliated Hospital of Soochow University, Suzhou 215006, China.

Signature page

I have read all the above, asked questions, and received answers concerning areas I did not understand. I have had the opportunity to take this consent form home for review or discussion.

I willingly give my consent to participate in this program. Upon signing this form, I will receive a copy. I may also request a copy of the protocol *(full study plan).*

Patient signature

Date signed

Investigator signature

Date signed