Study Protocol

Outcomes of patients after allogenic hematopoietic cell transplantation with decitabine-containing conditioning regimen and acetylcysteine treatment

Vision 1.0

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Protocol Summary

Study centers The First Affiliated Hospital of Soochow University, Suzhou 215006, China;
Principal Investigator Yue Han, MD, PhD; Depei Wu, MD, PhD.
Methodology A prospective, randomized clinical trial.
Primary objective To assess hematopoietic reconstitution, GVHD and relapse rate after allo-HSCT with decitabine containing conditioning regimen and acetylcysteine treatment
Secondary objectives To evaluate the survival of patients after allo-HSCT with decitabine based conditioning regimen and acetylcysteine treatment
Inclusion criteria 1) Diagnosed as hematopoietic malignancy; 2) Achieved complete remission since the last chemotherapy; 3) Age 10-70 years； 4) Be willing to receive allo-HSCT, with HLA matched related or HLA matched unrelated, or HLA mismatched related donor.
Exclusion criteria 1) Active infections, severe organ damage (cardiac, renal and/or hepatic dysfunction greater than grade 2 according to the Common Terminology Criteria for Adverse Events V5.0), or any other conditions that make patients ineligible for allo-HSCT; 2) Allergic to acetylcysteine or decitabine；
Intervention 1) Arm A: Acetylcysteine (1.2g twice a day, oral, from day -10 of conditional regimen to day 180 after HSCT). Conditional regimen: decitabine (20mg/m2 intravenously from day -10 to day -8 of conditional regimen); semustine 250 mg/m²/day on day -9; cytarabine 2 g/m² every 12 hours on day -8; busulfan 3.2mg/kg/day on day -7 to -5; cyclophosphamide 1.8g/m²/day on day -4 to -3; anti-thymocyte globulin (2mg/kg/d on day -5 to day -2) and mycophenolate (500mg, oral, twice a day from day -8) were usually added for transplants with unrelated donor or HLA mismatched donor. 2) Arm B: Conditional regimen: semustine 250 mg/m²/day on day -9; cytarabine 2 g/m² every 12 hours on day -8; busulfan 3.2mg/kg/day on day -

7 to -5; cyclophosphamide 1.8g/m²/day on day -4 to -3; anti-thymocyte globulin (2mg/kg/d on day -5 to day -2) and mycophenolate (500mg, oral, twice a day from day -8) were usually added for transplants with unrelated donor or HLA mismatched donor.
Total number of patients: 100
Total duration of the study: 4 years

Background

Allogeneic hematopoietic stem cell transplantation(allo-HSCT) is the curative treatment for hematological malignancy. Relapse, graft versus host disease (GVHD) and graft failure remain the main causes of treatment failure.

Engraftment of HSCs

Engraftment is the process by which hematopoietic stem cells (HSCs) make their way (homing) to free bone marrow niches where they can find optimal conditions to survive and proliferate. Once they have reached the BM microenvironment, HSC have to proliferate to generate all hematopoietic cell subsets [1]. A fundamental goal for successful engraftment is that the transplanted HSC are capable of sustaining long-term effective hematopoiesis; production of red blood cells, white blood cells, and platelets; and their release to peripheral blood [2].

Various definitions of engraftment exist in the literature. Engraftment is most commonly defined as the first of three consecutive days of achieving a sustained peripheral blood neutrophil count of $>0.5 \times 10^9$/L [3]. Platelet engraftment is usually defined as independence from platelet transfusion for at least 7 days with a platelet count of more than $>20 \times 10^9$/L [4]. The two major factors affecting engraftment are the graft source and the hematopoietic stem cell transplant conditioning regimen.

Emerging evidence from mouse studies has suggested that effective hematopoiesis depends on a particular bone marrow microenvironment in which HSCs reside. The bone marrow microenvironment is a cellular system within the marrow that serves as a vital and dynamic support system which supports hematopoiesis. It is composed of several different cell types (endothelial cells, stromal cells, etc) as well as cytokines and matrix proteins that are essential for the proliferation and differentiation of hematopoietic stem

and progenitor cells and the maturation of lineage specific precursor cells. In patients undergoing HCT, the homeostasis of bone marrow microenvironment is damaged by the intensive conditioning treatments and alloreactivities associated with allogeneic transplantation.

Acetylcysteinee (NAC), a ROS scavenger, has been widely used as an exogenous antioxidant [5-7]. Emerging evidence from in vitro and murine studies showed that NAC could enhance defective HSCs by repairing dysfunctional bone marrow endothelial cells of thrombocytopenia patients after HSCT. NAC has been also proved to overcome the exhaustion of HSCs and enhance the engraftment of HSCs [6-8].

Decitabine, a hypomethylating agent, can incorporate into deoxyribonucleic acid (DNA) and subsequently bind to DNA methyltransferase (DNMT) covalently, and is widely used for patients with AML and MDS. Our recent study showed decitabine could restore bone marrow microenvironment by repairing endothelial cells and endothelial progenitor cells, as well as cytokines and chemokines which are crucial to HSCs proliferation and differentiation, thereby promote platelet recovery after HSCT [9].

GVHD and Relapse

Decitabine therapy was shown to be associated with reduced incidence of GVHD, lower relapse rate, and increased overall survival of patients after HSCT in several studies [10-12]. It was found to be involved in various immune modulatory effects. Decitabine could induce immunosuppressive effects by increasing the number of regulatory T cells with upregulation of the expressions of Foxp3 and HLA-G [13-16]. Besides, it was shown to exert a protective role after HSCT by modulating the proliferation and differentiation of T cells in a TET2-dependent way. Furthermore, decitabine could alleviate tissue damage by inhibiting the production of proinflammatory cytokines, such as interferon γ and tumor necrosis factor β [17].

Decitabine was also used as salvage therapy for relapse after allo-HSCT in many studies [18-23]. It may upregulate the expressions of antioncogenes that are silenced because of hypermethylation [24], and further remodel specific immune responses by inducing the expression of tumor-associated antigen, restoring the expression of HLA class I antigen, upregulating the expressions of costimulatory molecules expression, inducing heterogeneous expression of killer immunoglobulin-like receptor (KIR) genes, and promoting enrichment or differentiation of tumor-derived APCs [25-28]. In summary, decitabine exerts its antitumor effects via direct cytotoxicity and strengthened tumor immunity, without sacrificing its GVHD protective effects.

In summary, we will conduct this prospective and randomized clinical trial, to evaluate the hematopoietic reconstitution, GVHD and relapse rate of patients after allo-HSCT with decitabine containing conditional regimen and NAC treatment.

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Rationale of this study

- Relapse, graft versus host disease (GVHD) and graft failure are the main causes of treatment failure of allo-HSCT.
- The bone marrow microenvironment plays an important role in supporting hematopoietic reconstitution. NAC and decitabine could potentially promote engraftment by restoring bone marrow microenvironment.
- Decitabine was found to be involved in various immune modulatory effects, and be associated with reduced incidence of GVHD and lower relapse rate.

Study objectives

Primary objective and primary endpoint

Primary objective:

This study aims to validate the outcome of patients after allo-HSCT with decitabine containing conditional regimen and NAC treatment.

Primary endpoint:

The primary endpoint is the hematological engraftment, GVHD and relapse rate after HSCT.

Secondary objectives and secondary endpoints:

To evaluate the survival of patients after HSCT.

Research design

Type of study

This is a prospective, open-label, randomized clinical trial.

Experimental diagram

The overall design of the study is shown in Figure 1.

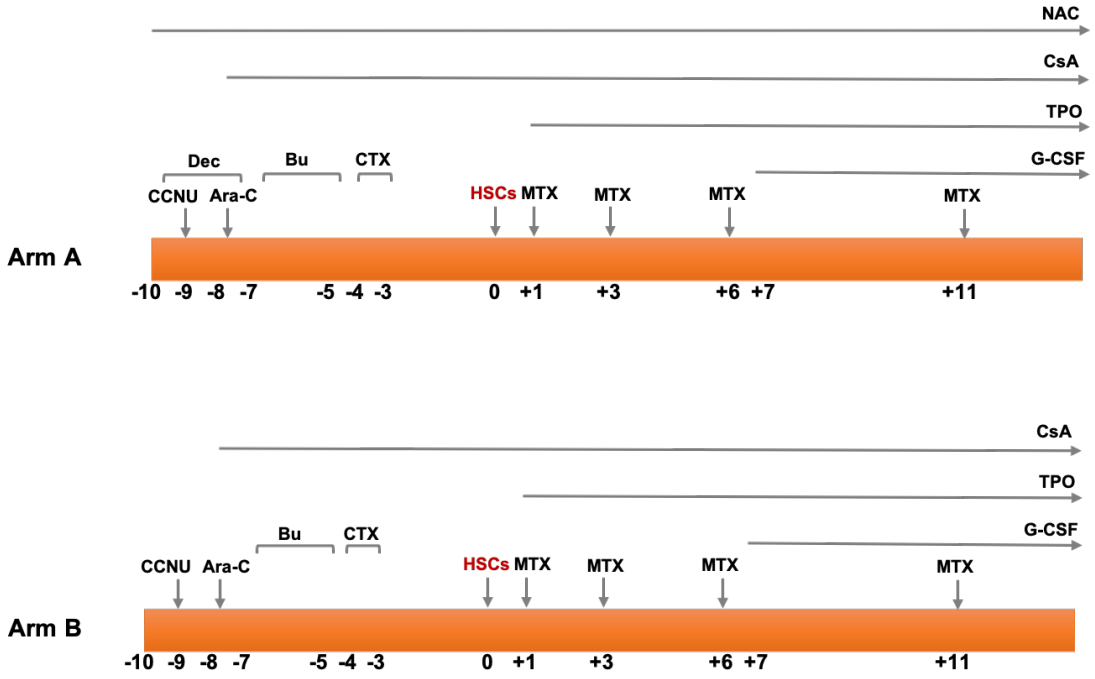

Figure 1. The flow chart of the conditioning regimen.

Notes: CCNU: semustine; Dec: decitabine; Bu: busulfan; CTX: cyclophosphamide; Ara-C: cytarabine; MTX: methotrexate; CsA: cyclophosphamide; TPO, recombinant human thrombopoietin.

Subject selection

Inclusion criteria

- Diagnosed as hematopoietic malignancy;
- Achieved complete remission since the last chemotherapy;
- Age 10-70 years；
- Be willing to receive allo-HSCT, with HLA matched related or HLA matched unrelated, or HLA mismatched related donor.

Exclusion criteria

- Active infections, severe organ damage (cardiac, renal and/or hepatic dysfunction greater than grade 2 according to the Common Terminology Criteria for Adverse Events V5.0), or any other conditions that make patients ineligible for allo-HSCT;
- Allergic to acetylcysteine or decitabine.

Treatment Allocation

Subjects will be randomized after the investigator has verified that all eligibility criteria have been met. Subjects will be randomized in a 1:1 ratio to either Arm A or Arm B.

Procedures for randomization

Completely randomized block design method was performed to assign the patients into the 2 arms. For each study site, we produced the random series and corresponding assigning arm A and B.

Dosage and administration

Treatment:

Arm A: Acetylcysteine (1.2g twice a day, oral administration, from day -10 to day 365 after HSCT). Conditional regimen: decitabine (20mg/m^2 intravenously from day -10 to day -8 of conditional regimen); semustine 250 mg/m^2/day on day -9; cytarabine 2 g/m^2 every 12 hours on day -8; busulfan 3.2mg/kg/day on day -7 to -5; cyclophosphamide 1.8g/m^2/day on day -4 to -3; cyclosporin A:

3mg/kg/d from day -8. Anti-thymocyte globulin (2mg/kg/d on day -5 to day -2) and mycophenolate (500mg, oral, twice a day from day -8) were usually added for transplants with unrelated donor or HLA mismatched donor.

Arm B: Conditional regimen: semustine 250 mg/m^2/day on day -9; cytarabine 2 g/m^2 every 12 hours on day -8; busulfan 3.2mg/kg/day on day -7 to -5; cyclophosphamide 1.8g/m^2/day on day -4 to -3; cyclosporin A: 3mg/kg/d from day -8. Anti-thymocyte globulin (2mg/kg/d on day -5 to day -2) and mycophenolate (500mg, oral, twice a day from day -8) were usually added for transplants with unrelated donor or HLA mismatched donor.

Note:

- Donor for allogeneic HCT was selected based on the results of human leukocyte antigen (HLA) typing, age, sex, and health status, etc. Generally, HLA-matched related younger male donor was prioritized and alternative donor including HLA haplo-identical donor and HLA-matched unrelated donor were chosen according to the institutional guidelines. Grafts from bone marrow or peripheral blood were regularly primed by granulocyte colony stimulating factor (G-CSF) before collecting.
- Prophylactic platelet transfusion is required for no-bleeding patients with platelet counts lower than 20×10^9/L and those with bleeding and platelet counts lower than 30×10^9/L. For those with severe anemia (hemoglobin ≤ 60 g/L), washed red blood cells should be given. Platelet and red blood cells should be irradiated with Co^{60} before transfusion for those share ABO inconsistency between donor and recipient.
- Decitabine treatment should be suspended when some non-hematologic toxicities appear as follows:

- Serum creatinine ≥ 2.0mg/dL;
- Serum aminotransferase or total bilirubin higher than 2 fold the upper-limit of normal level.

Concomitant therapy

Except the study drug, other therapies (including immunosuppressant, antibiotics, proton-pump inhibitor, diuretic, nutritional therapy, etc) must be recorded in the eCRF for the main study treatment period.

Study process

Screening phase (3 days)

- Inclusion /Exclusion Criteria
- Demographic data: age, sex, nationality, address, contact information.
- General information: past history, allergic history, previous disease and status, complications, previous treatment.
- Physical examination: height, body weight, blood pressure, body temperature, heart rate, body surface area, respiratory and cardiovascular system examination, nervous system examination, Eastern Cooperative Oncology Group (ECOG) score, etc.
- Laboratory examination:
  - Blood analysis: blood routine test, blood biochemistry, virological testing, T-lymphocyte subsets.
  - BM examination: marrow morphology examination, minimal residual disease, chromosome，mutational analyses, gene rearrangement detection and donor chimerism.
- Electrocardiogram test.

Study period (365 days)

- Drugs will be given based on the protocol;
- Blood routine test and blood biochemistry will be performed once a day and once a week from day -10 to day 90, and once a week and once a month from day 90 to day 360, respectively. For patients with poor graft function, prolonged intensive monitoring of blood is recommended.

- Bone marrow examination will be performed once a month, including marrow morphology, minimal residual disease, chromosome, mutational analyses, gene rearrangement detection and donor chimerism.
- Important vital signs will be recorded.
- Adverse events will be recorded and managed according to this protocol.

Termination phase

- Physical examination: height, body weight, blood pressure, body temperature, heart rate, body surface area, respiratory and cardiovascular system examination, nervous system examination, ECOG score, etc.
- Laboratory examination:
- Blood analysis: blood routine test, blood biochemistry, virological testing, T-lymphocyte subsets.
- BM examination: marrow morphology examination, minimal residual disease, chromosome， mutational analyses, gene rearrangement detection and donor chimerism.
- Electrocardiogram test.

Follow-up phase (3 years)

- Routine blood examination will be repeated once every 1 month;
- The changes of other complications;
- Survival will be long-term recorded until death.

Safety evaluations

Safety and tolerability are assessed throughout the study.

Safety endpoints

White blood cell count, hemoglobin, liver function, renal function.

Clinical symptoms:

Newly occurred symptoms during the study period: fever, nausea, cough, somnolence, depression, constipation, diarrhea, petechia, edema, pharyngitis, pulmonary edema, atrial fibrillation, tachycardia, mycobacterium avium complex infectious, hyperglycemia, neutropenia, anemia.

Laboratory examination

Blood routine test, blood biochemistry, virological testing, electrocardiogram, etc.

Adverse event

Definition and record of an adverse event

Adverse events are defined as any harmful medical manifestations occurring in a person who participates in a biomedical research whether this manifestation is related to the research or the product used in the research. An adverse effect may be any unfavourable or unexpected sign (including abnormal laboratory results), any symptom or temporary disorder associated with the use of the product, with or without relation to the product.
The clinically significant abnormality must be reported as an adverse event (AE) in the eCRF, and AE is supposed to record the time of occurrence, the stage of severity, the duration of time, measures and outcomes.

Classification of adverse event severity

Every case report is performed by the grade of severity about drug adverse reactions：

- Mild: usually transient symptoms unaffecting the daily life;
- Moderate: symptoms probably affecting the daily life;
- Severe: symptoms seriously affecting the daily life.

Relevance

- Definite relevance: The adverse event is clearly related to the investigational agent. For example, an event that follows a reasonable temporal sequence from administration of the study intervention abides by a known or expected response pattern to the suspected intervention, which improves by stoppage and reappears through repeated exposures, but is unable to be reasonably explained by the known characteristics of the subject's clinical state;
- Possible relevance: An adverse event that follows a reasonable temporal sequence from administration of the study intervention abides by a known or expected response pattern to the suspected intervention, which can be easily induced by various other factors;
- Irrelevance: The adverse event is clearly uncorrelated with the research agent or procedure. For example, there exists another plausible cause, and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the study intervention and/or a causal relationship is considered biologically implausible.

Serious adverse event:

A serious adverse event (SAE) event is defined as the event with one of the following consequences:

- Death;
- Life-threatening condition;
- Hospitalization or extension of hospitalization;
- Temporary or permanent disability;
- Congenital anomaly or malformation;
- Important medical events: although the event caused by the drug which may endanger the subjects may not be life-threatening and result in death or require hospitalization, medical or surgical intervention requires to be

taken to prevent one of the outcomes based on the appropriate medical judgment.

Definition of an unexpected adverse event:

An unexpected adverse event of the product is defined as the event with the nature, severity or course non-corresponding to the product information mentioned in the summary when it is authorized or in the investigator's brochure when it is not authorized.

Expected adverse events:

- **Due to other non-hematological toxicity**

  Liver dysfunction;

  Renal dysfunction;

- **Related to the hematological disease**

  Death

- **Related to drug allergy**

  Erythra, edema, pruritus, swelling, etc.

Procedures of all adverse event

Investigators must immediately take necessarily measures at once to protect subjects' safety, when serious adverse events occur. Furthermore, all adverse events should be followed up and investigated, and detailed records are supposed to be made and signed.

All serious and non-serious AEs and special situation will be reported from the signature to completion of the subject's last study-related procedure (which may include the follow-up contact for safety). SAEs must be reported using the Serious Adverse Event Form. The sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

All AEs, regardless of the severity or presumed relationship with the study treatment, must be recorded in the source document and eCRF using medical terminology. Whenever possible, diagnosis should be made when signs and symptoms are caused by the common etiology. Investigators must record their opinions on the relationship between AEs and the study treatment in the eCRF. All measures required for (serious) AEs must be recorded in the source document and reported according to sponsor instructions.

Monitoring committee

A safety committee comprised of 3 persons independent of the trial will be organized and will meet to check the safety related to the study treatment during the study. In terms of the overall mortality of each arm and serious adverse events, review will be performed by the independent safety committee. The committee is responsible for hemovigilance/pharmacovigilance of the study and acts as an additional warning sign against hemovigilance/pharmacovigilance of the study.

Statistical methods

Statistical analysis will be done by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the efficacy and safety data is outlined below. Baseline for all analyses will be the day of randomization unless otherwise specified.

Sample size estimation

We used the formula of sample size estimate when comparing the independent rates more two groups. The sample size for each group at least was 50, and the total sample size should at least be 100.

Statistical analysis

Completely randomized block design method was performed to assign the patients into the 2 arms. Numerical data was presented as medians with interval quartile range (IQR), and categorical data were shown as proportions. One-way ANOVA was used to compare the difference for the means among 3 treatment arms. Chi-square test or Wilcoxon rank test was used to compare the difference for the proportions between 2 treatment arms. Kaplan-Meier method was performed to estimate the 1-year survival rate for the 2 arms respectively and log-rank test to compare their difference for the survival curves. Cox model and logistic model were used to estimate independent association for the HR (hazard ratio), OR (odds ratio) and 95% CI (confidence interval) among the treatment arms. A significance level was set as a 2-tailed P value of < .05. All the analyses used the SPSS 19.0 (SPSS Inc.).

Management of product quality complaint

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, ie, any dissatisfaction relative to the identity, quality, durability, or reliability of a product, including its labeling or package integrity. PQCs may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from clinical studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide.

Procedures

All initial PQCs must be reported to the sponsor by the investigational staff within 24 hours after being made aware of the event.
If the defect is combined with an SAE, the investigational staff must report the PQC to the sponsor according to the SAE reporting timelines. A sample of the

suspected product should be maintained for further investigation if requested by the sponsor.

Study drug information

- Decitabine is a kind of white sterile lyophilized powder, placed in a clear, colorless vial. Each 20 mL vial contains 25 mg decitabine.
- NAC is an effervescent tablet containing 600 mg for each pill.

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 sponsor and coordination institutions to decide whether adopting the above advice or suggestions, during the clinical trial executed by each institutions.

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.

Appendix

Appendix 1. The score of bleeding

Appendix 2. ECOG score

Appendix 3. Criteria for IBMTR severity index for acute GVHD

Appendix 4. NIH global severity of chronic GVHD

Appendix 5. Patient consent form for study participation

Appendix 1. The score of bleeding*

Grade	Manifestation
Score 0	No bleeding.
Score 1	Occult bleeding in secretion, including mild petechia or minimal vaginal bleeding.
Score 2	Minor bleeding without the requirement of RBC transfusion, such as ecchymosis, epistaxis, line oozing, vaginal bleeding, skin bleeding, mild hematemesis, melena, mild hematuria.
Score 3	Bleeding that can cause a rapid fall of hematocrit and needs one or more units of RBC transfusion per day over the expected rate of an individual, or fail to obtain a post-transfusion increment at active bleeding.
Score 4	Life-threatening bleeding, defined as either massive bleeding causing severe hemodynamic compromise or bleeding into a vital organ, such as intracranial hemorrhage, pericardial hemorrhage, diffuse alveolar hemorrhage.

Minor bleeding signified bleeding less than a week of daily score 2;

Moderate bleeding was defined as more than a week of daily score 2, or 1–2 days of score 3 (within 7 days);

Severe bleeding included score 3 for 3 or more days, or any score 4.

Notes: RBC=red blood cell; * Nevo S, Swan V, Enger C, et al. Acute bleeding after bone marrow transplantation (BMT)- incidence and effect on survival. A quantitative analysis in 1,402 patients. Blood. 1998;91(4):1469-77.

Appendix 2. ECOG score*

Grade	Performance status
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Able to move freely and take mild physical activity, such as housework and office work, but restricted in severe physical activity.
2	Able to move freely and take care of himself, but loss of work capability; doing activities for more than half of the waking hours.
3	Capable of taking care of himself partially, confined to the bed or chair for more than half of the waking hours.
4	Completely disabled, unable to take care of himself and totally confined to the bed or chair.
5	Dead

Notes: ECOG=Eastern Cooperative Oncology Group; * Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649-655.

Appendix 3. Criteria for IBMTR severity index for acute GVHD*

Skin involvement				Liver involvement			Gastrointestinal involvement	
Index	Stage (max.)	Rash range		Stage (max.)	Total bilirubin (μmol/L)		Stage (max.)	Volume of diarrhea (ml/d)
A	1	<25%		0	<34		0	<500
B	2	25%- 50%	or	1-2	34-102	or	1-2	550-1 500
C	3	>50%	or	3	103-255	or	3	>1 500
D	4	Bullae	or	4	>255	or	4	Severe pain and ileus

Notes: GVHD=graft-versus-host disease; *Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol. 1997;97(4):855-64.

Appendix 4. Criteria of chronic GVHD*

Severity	Manifestation
Mild	1 or 2 Organs involved with no more than score 1 plus Lung score 0.
Moderate	3 or More organs involved with no more than score 1 OR At least 1 organ (not lung) with a score of 2 OR Lung score 1
Severe	At least 1 organ with a score of 3 OR Lung score of 2 or 3

Notes: GVHD=graft-versus-host disease; In skin: higher of the 2 scores to be used for calculating global severity. In lung: FEV1 is used instead of clinical score for calculating global severity. If the entire abnormality in an organ is noted to be unequivocally explained by a non-GVHD documented cause, that organ is not included for calculation of the global severity. If the abnormality in an organ is attributed to multifactorial causes (GVHD plus other causes) the scored organ will be used for calculation of the global severity regardless of the contributing causes (no downgrading of organ severity score). * Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21(3):389-401.

Appendix 5: Patient consent form for study participation

Title: Outcomes of patients with hematopoietic malignancy after allogenic hematopoietic cell transplantation with decitabine-containing conditioning regimen and acetylcysteine			
Investigator OR institution Name		**Study Site**	
Patient Name			
Please **tick** each box below ☐ if you have known the corresponding statement. **(*to be completed by the patient)***			

☐ 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?

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the main curative treatment for hematological malignancy. Relapse, graft versus host disease (GVHD) and graft failure are the main causes of treatment failure. NAC was found to be able to enhance defective HSCs by repairing dysfunctional bone marrow endothelial cells, and overcome the exhaustion of HSCs and enhance the engraftment of HSCs. Decitabine could restore bone marrow microenvironment by repairing endothelial cells and endothelial progenitor cells, as well as cytokines and chemokines which are crucial to HSCs proliferation and differentiation, thereby promote platelet recovery after HSCT. Besides, decitabine therapy was shown to be associated with reduced incidence of GVHD, lower relapse rate, and increased overall survival in several studies. Thereby we will conduct this clinical trial to evaluate the hematopoietic reconstitution, GVHD and relapse rate of patients with

hematological malignancy after allo-HSCT with decitabine containing conditional regimen and NAC treatment.

☐ Why have I been chosen?

We are inviting patients who

- Diagnosed as hematopoietic malignancy;
- Achieved complete remission since the last chemotherapy;
- Age 10-70 years；
- Be willing to receive allo-HSCT, with HLA matched related or HLA matched unrelated, or HLA mismatched related donor.

☐ 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.

If you are included in this research, you will be randomly assigned into the one of two treatment groups. Randomization means that neither you nor your doctor will be able to select which treatment you will receive. We will use a computer to allocate you to one of this two groups with equal chances of each treatment approach being the one you will receive. This procedure will ensure that the number of patients in each of the two treatment groups is similar.

- If you are assigned in the group A:

You will be treated with decitabine (20mg/m2 intravenously from day -10 to day -8 of conditional regimen) plus NAC (1.2g twice a day, oral administration, from day -10 to day 180 after HSCT), together with modified Bu/Cy regimen.

➢ If you are assigned in the group B:

You will be treated with modified Bu/Cy regimen without decitabine and NAC. Your doctors will introduce the detailed treatment procedures to you. You are free to ask any question about the treatments you may have.

☐ How long will I be in the study

If you participate in this study, you will receive therapy for 1 year. Follow up will continue indefinitely, for as long as 3 years or longer.

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first.

☐ What are the risks of the study?

➢ **Potential adverse event of decitabine includes:**

- **Other non-hematological toxicity:**

  Liver dysfunction;
  Renal dysfunction;

- **Drug allergy**

  Erythra, edema, pruritus, swelling, etc

➢ **Others:**

There also may be other unexpected complications or discomforts that we cannot predict.

Your doctors will do their best to prevent complications and to treat them if occur. You will receive the best medical care available during and after the study and in the unlikely event of an injury arising from taking part in this study, you will be provided with the necessary care.

☐Are there benefits you to taking part in the study?

You'll get free treatment of decitabine and NAC (Group A) and free test of blood and bone marrow (all participants). Another possible benefit you may experience from this research includes better hematopoietic reconstitution and lower rates of GVHD and relapse, and prolongation of survival time. However, there is no guarantee that each patient will benefit from the treatment in this research.

Furthermore, the results obtained from this study may be useful in guiding the treatment for patients with such disease in the future.

☐ 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 the treatment, 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.

☐Whom do I call if I have questions or problems?

For information about your disease and research-related injury, you may contact:

Name (institution) : _____ Telephone Number:_____

For information about this study, you may contact:

Name (institution) : _____ Telephone Number:_____

For information about your rights as a research subject, you may contact:

Name (institution) : _____ Telephone Number:_____

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		**Name (print)**		**Date signed**	

Investigator (institution) Statement and Signature

to be completed by the person taking consent

I have discussed this clinical research study with the patient and/or his or her authorized representative using a language that is understandable and appropriate. I believe that I have fully informed the participant of the nature of this study and the possible benefits and risks of taking part. I believe the participant has understood this explanation.

Investigator OR institution signature		**Name (print)**		**Date signed**	