Study Protocol

PD-1 Inhibitor Combined With Azacytidine and Homoharringtonine，Cytarabine, G-CSF for Refractory or Relapsed AML

Vision 1.0

Dec. 20, 2020

ClinicalTrials.gov Identifier:

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Applicable Section
Study center: The First Affiliated Hospital of Soochow University, Suzhou 215006, China;

Protocol Summary

Study center • The First Affiliated Hospital of Soochow University, Suzhou 215006, China;
Principal Investigator Yue Han, MD, PhD; Depei Wu, MD, PhD.
Methodology A prospective, single-arm, single-center, randomized clinical trial.
Primary objective To assess the efficacy and safety of PD-1 inhibitor combined with azacytidine and homoharringtonine，cytarabine, G-CSF for refractory or relapsed AML .
Inclusion criteria • Chinese guidelines for the diagnosis and treatment of relapsed and refractory acute myeloid leukemia (2017 edition)，excludes acute promyelocytic leukemia (M3、APL); • Hematopoietic stem cell transplantation ≥3 months, Discontinue immunosuppressant ≥3 weeks, Patients without graft-versus-host disease; • Be at least 18 years of age on day of signing informed consent; • Have a performance status of less than or equal to 2 on the Eastern Cooperative Oncology Group（ECOG） Performance Scale; • Demonstrate adequate organ function as defined below, all screening labs should be performed before treatment initiation： 1. ALT(SGPT) less than or equal to 2.5 × Upper Limit of Norma（ULN）； 2. AST (SGOT) less than or equal to 2.5 × ULN；

3. Serum total bilirubin Less than or equal to 2.0 × ULN;Note: If total bilirubin >2.0×ULN, subjects with Gilbert syndrome records are allowed to join the group;

4. Serum Creatinine ≥ 30 mL/min

5. Total white blood cell (WBC) count ≤10,000/μL； Note: hydroxyurea therapy is allowed to reduce white blood cells to meet this inclusion criteria.white blood cells should be determined ≥24 hours after the last hydroxyurea administration. Final hydroxyurea administration should not ≤3 days prior to the first azacytidine administration.

Exclusion criteria

- Patients with chronic myeloid leukemia，AML of other myeloproliferative disorders Malignant neoplasms with other progression ;
- Those who can not control severe infections and other underlying diseases can not tolerate chemotherapy ;
- Patients with cardiac insufficiency： ejection fraction (EF)<30%，New York Heart Association（NYHA） standards，Cardiac insufficiency II or above;
- Patients with liver and kidney dysfunction：Serum bilirubin (SB)≥2mg/dl，AST is 2.5 times higher than normal upper limit, serum creatinine (SCr) is more than 2.5 mg/dl Serious mental illness uncooperative Refusal to join the study;

Intervention

- Azacytidine 75mg/(m2.d) by IV on days 1-7 of every cycle.
- Anti-PD-1 mAb 200mg by IV on day 8 of every cycle.
- Homoharringtonine(HHT) 2mg/(m2.d) by IV on days 1-6 of every cycle Cytarabine 10mg/(m2.d) by SC on days 1-7 of every cycle Granulocyte colony-stimulating factor(G-CSF) 300ug/d by SC on days 1-7 of every cycle，until absolute neutrophil count(ANC) ＞ 5X109 ／L or white blood cell（WBC）＞ 20X109 ／L.

Total number of patients: 30

Total duration of the study: 3 years

Background

Acute myeloid leukemia (AML) is one of the most common hematological malignancies, a malignant, clonal disease with poor prognosis.The rate of complete remission (CR) was 60%-90% in patients with first induction chemotherapy,10%~40% became refractory. 70% of AML patients who received the first CR maybe relapsed in the first year after the CR[1].The 5 years total survival (OS) rate of refractory or relapsed AML (RR-AML) was 5%~10%, and the CR rate was not more than 30% in general[2].there is still no unified treatment regimen for RR-AML , according to Chinese guidelines for diagnosis and treatment (2017 Edition)[3] and United States National Integrated Cancer Network (NCCN)2019 edition AML clinical practice guidelines standard[4], they still emphasize individualized treatment and salvage chemotherapy.Therefore, how to treat refractory or relapsed acute myeloid leukemia is still an urgent clinical problem.

Many studies have shown that epigenetic changes such as DNA methylation are common in AML and play an important role in the transformation of myelodysplastic syndrome (MDS) to AML. Demethylation drugs have become the first-line treatment of AML drugs.The azacytidine is a 5- azide analogues, which has dual mechanism of demethylation and integrates into DNA and RNA to produce antitumor activity, approved by Europe in 2008 and China in 2018 for treatment of AML with 20%~30% bone marrow blast cells[5-6].Chinese guidelines for the diagnosis and treatment of relapsed and refractory acute myeloid leukemia (version 2017)[3] recommended demethylation drugs for patients with poor tolerance and unsuitable for strong chemotherapy regimens.

Demethylation drugs have been reported to increase the sensitivity of CAG、HAG and other chemotherapy regimens, thereby improving clinical efficacy[7-8].Homoharringtonine is a cell cycle specific alkaloid. There are clinical studies showing that the CR rate of the homoharringtonine combined

with CAG regimen is higher than the CAG regimen for RR-AML[9].Based on this study, azacytidine combined with HAG regimen was used to treat refractory and relapsed patients.

Ørskov AD [10] found that MDS patients treated with demethylated drugs (HMAs) lead to the demethylation of programmed death receptor 1(PD-1) promoter in T cells.And then the expression of PD-1 increased in T cell surface, and the combination of tumor cell surface PD-L1 hindered the anti-tumor immune response.Therefore, this immune checkpoint upregulation also provides strong evidence for the use of PD-1 or PD-L1 pathway inhibitors to overcome tumor immune resistance. In a phase II clinical trial, PD-1 inhibitor nivolumab combined with azacytidine treated 70 patients with recurrent AML[11], regimen：Azacytidine 75 mg/m2,d1-7;nivolumab3mg/kg,d1,d14;4-5w treatment. The total response rate was 33%, patients treated with HMAs for the first time was 58%, other patients was 22%. 11% incidence of immune-related side effects at level 3-4.The median overall survival of all patients was 6.3 months, According to the institutional experience of MD Anderson Cancer Center, it has advantages over azacytidine-based chemotherapy.

To improve the prognosis and survival of RR-AML patients, we propose to conduct a prospective study to explore the efficacy and safety of PD-1 inhibitor combined with azacytidine and HAG regimen in the treatment of RR-AML, and to study its related mechanisms.

Reference

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Rationale of this study

- Acute myeloid leukemia (AML) is one of the most common hematological malignancies, a malignant, clonal disease with poor prognosis.there is still no unified treatment regimen for RR-AML.
- Demethylation drugs have been reported to increase the sensitivity of CAG、HAG and other chemotherapy regimens, thereby improving clinical efficacy.
- HMAs lead to the demethylation of programmed death receptor 1(PD-1) promoter in T cells.And then the expression of PD-1 increased in T cell surface, Therefore, this provides strong evidence for the use of PD-1 or PD-L1 pathway inhibitors to overcome tumor immune resistance.

Study objectives

Primary objective and primary endpoint

Primary objective:

This study aims to validate the efficacy and safety of PD-1 inhibitor combined with azacytidine and homoharringtonine，cytarabine, G-CSF for refractory or relapsed AML

Primary endpoint:

Number of Participants (Responders) achieving CR+CRi+PR after the eighth cycle treatments.

Secondary objectives and secondary endpoints:

Number of Participants (Responders) Achieving Overall Response Rate(ORR) After the Eighth Cycle Treatments;time from randomization to death from any cause;The time between the beginning of the group and the occurrence of any event, including death, progression of the disease, chemotherapy regimen, conversion to chemotherapy, addition of other treatment, occurrence of fatal or intolerable side effects, etc;Time between the beginning of randomization and the progression (in any way) of tumorigenesis or (for any reason) death

Research design

Type of study

This is a prospective, single-arm, single-center randomized clinical trial.

Experimental diagram

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

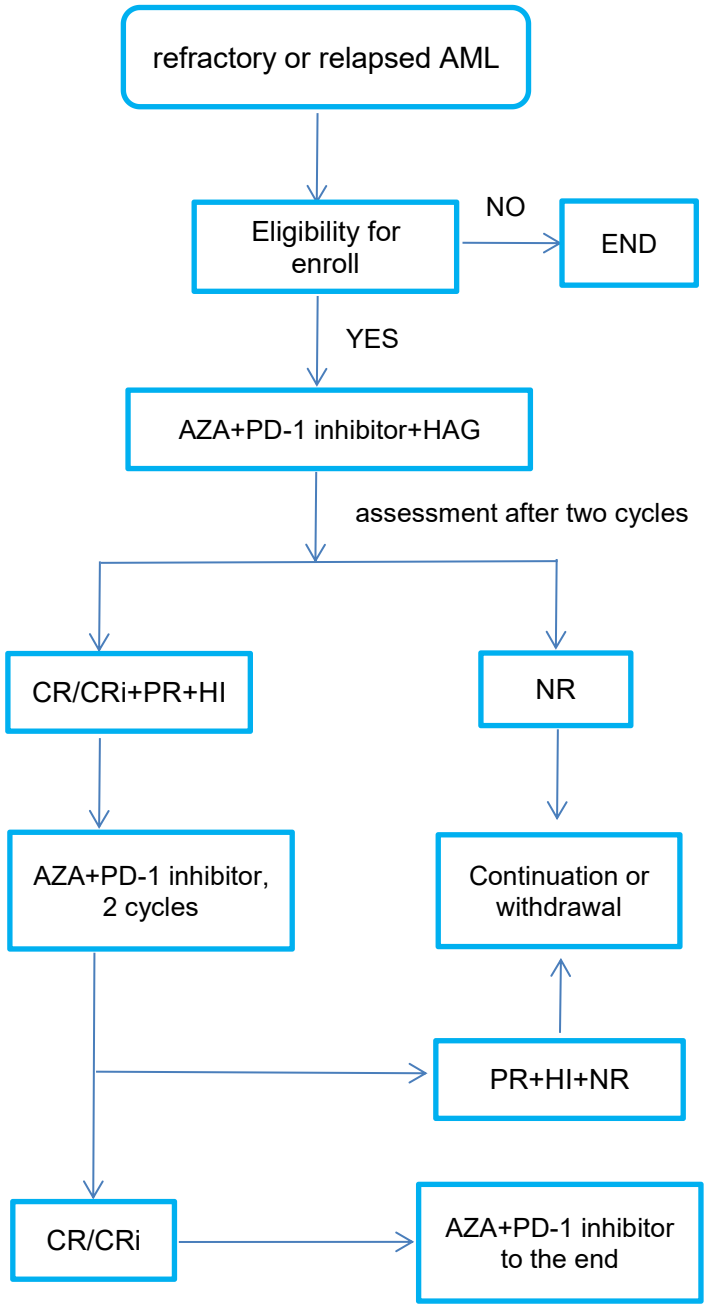

Figure 1. The flow chart of the overall study design

Dosage and administration

Treatment:

Azacytidine 75mg/(m2.d) by IV on days 1-7 of every cycle;

PD-1 inhibitor 200mg by IV on day 8 of every cycle;

Homoharringtonine(HHT) 2mg/(m2.d) by IV on days 1-6 of every cycle Cytarabine 10mg/(m2.d) by SC on days 1-7 of every cycle Granulocyte colony-stimulating factor(G-CSF) 300ug/d by SC on days 1-7 of every cycle，until absolute neutrophil count(ANC) ＞ 5X109 / L or white blood cell（WBC）＞ 20X109 / L.

Study process

Screening phase (3 days)

- Inclusion /Exclusion Criteria
- Demographic data: age, sex, nationality, address, spouse's characteristics, contact information.
- 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
  - BM examination: marrow morphology examination, Flow cytology,etc.

Treatment period (28 days)

- Drugs will be given based on the protocol;
- Blood routine test and blood biochemistry are performed once a day and once a week, respectively;
- T Cellular Subjects and PD-1 are performed before AZA;
- Important vital signs are recorded;
- Adverse events will be recorded and managed according to this protocol.

Termination phase

- Demographic data: age, sex, nationality, address, spouse's characteristics, contact information.
- General information: past history, allergic history, previous disease and status.

- 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,
- T Cellular Subjects and PD-1 are performed ;
- Bone marrow examination: marrow morphology examination, flow cytology.

Follow-up phase (6 months)

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

Response evaluation

- Response: According to the 2020 NCCN guidelines**.**

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 myelosuppression**

  Neutropenia related infections, fever;

  Bleeding: ecchymosis, petechial, haematemesis, hemoptysis, melena, hematuresis, etc;

  Weary, somnolence, pale, etc (due to anemia);

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

  Liver dysfunction;

  Renal dysfunction;

- **Related to the haematological disease**

  Death

  Relapse of any kind

- **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 haemovigilance/pharmacovigilance of the study and acts as an additional warning sign against haemovigilance/pharmacovigilance of the study.

Contact

Yue Han (Email: hanyue@suda.edu.cn), Depei Wu (wudepei@suda.edu.cn), The First Affiliated Hospital of Soochow University, Suzhou 215006, China.

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 two groups.

AZA CR/CRi	PD-1+AZA+HAG CR/CRi	Testing effectiveness
18%	38.5 %	80 %

α=0.05, bilateral test

Statistical analysis

Kaplan- Meier method was performed to estimate the 1-year survival rate

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.

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. ECOG score

Appendix 2. NCCN Guidelines Version 2.2020 Acute Myeloid Leukemia

Appendix 3. Patient consent form for study participation

Appendix 1. 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 2.NCCN Guidelines Version 2.2020 Acute Myeloid Leukemia

RISK STRATIFICATION BY GENETICS IN NON-APL AML[1,2]

Risk Category*	Genetic Abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Biallelic mutated *CEBPA* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}†
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}† Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡ Cytogenetic abnormalities not classified as favorable or adverse
Poor/Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)* -5 or del(5q); -7; -17/abn(17p) Complex karyotype,§ monosomal karyotype‖ Wild-type *NPM1* and *FLT3*-ITD^{high}† Mutated *RUNX1*¶ Mutated *ASXL1*¶ Mutated *TP53*#

[1] Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424-447.
[2] Frequencies, response rates, and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated.
* Prognostic impact of a marker is treatment-dependent and may change with new therapies.
† Low, low allelic ratio (<0.5); high, high allelic ratio (≥0.5); semiquantitative assessment of *FLT3*-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve "*FLT3*-ITD" divided by area under the curve "*FLT3*-wild type"; regardless of *FLT3* allelic fractions, patients should be considered for hematopoietic stem cell transplant, though recent studies indicate that AML with *NPM1* mutation and *FLT3*-ITD low allelic ratio may also have a more favorable prognosis and patients should not routinely be assigned to allogeneic HCT. *FLT3* allelic ratio is not yet pervasively used, and IF not available, the presence of an *FLT3* mutation should be considered high risk unless it occurs concurrently with an *NPM1* mutation, in which case it is intermediate risk. As data emerge, this measure will evolve.
‡ The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.
§ Three or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11) (v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*.
‖ Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).
¶ These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.
# *TP53* mutations are significantly associated with AML with complex and monosomal karyotype.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

RESPONSE CRITERIA DEFINITIONS FOR ACUTE MYELOID LEUKEMIA[1]

- **Morphologic leukemia-free state**
  - **Bone marrow <5% blasts in an aspirate with spicules**
  - **No blasts with Auer rods or persistence of extramedullary disease**
- **If there is a question of residual leukemia, a bone marrow aspirate/biopsy should be repeated in one week.**
- **A bone marrow biopsy should be performed if spicules are absent from the aspirate sample.**
- **Complete response (CR)**
  - **Morphologic CR - patient independent of transfusions**
    - ◊ **Absolute neutrophil count >1000/mcL (blasts <5%)**
    - ◊ **Platelets ≥100,000/mcL (blasts <5%)**
    - ◊ **No residual evidence of extramedullary disease**
  - **Cytogenetic CR - cytogenetics normal (in those with previously abnormal cytogenetics)**
  - **Molecular CR - molecular studies negative[2]**
  - **CRi - There are some clinical trials that include a variant of CR referred to as CRi. This has been defined as <5% marrow blasts, either ANC <1000/mcL or platelets <100,000/mcL, and transfusion independence but with persistence of cytopenia (usually thrombocytopenia).**
  - **Responses less than CR may still be meaningful depending on the therapy.**
- **Partial remission[3]**
  - **Decrease of at least 50% in the percentage of blasts to 5% to 25% in the bone marrow aspirate and the normalization of blood counts, as noted above.**
- **Relapse following CR is defined as reappearance of leukemic blasts in the peripheral blood or the finding of more than 5% blasts in the bone marrow, not attributable to another cause (eg, bone marrow regeneration after consolidation therapy) or extramedullary relapse.**
- **Induction failure - Failure to attain CR following exposure to at least 2 courses of intensive induction therapy (2 cycles of 7+3 or one cycle of 7+3 and one cycle of HiDAC).[4]**

[1] Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol 2003;21(24):4642-4649.
[2] This is clinically relevant only in APL and Ph+ leukemia at the present time. Molecular remission for APL should be performed after consolidation, not after induction as in non-APL AML.
[3] Partial remissions are useful in assessing potential activity of new investigational agents, usually in phase I trials.
[4] Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424-447.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

Appendix 3: Patient consent form for study participation

Title: Low-dose decitabine for refractory prolonged isolated thrombocytopenia after hematopoietic cell transplantation			
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?**

Acute myeloid leukemia (AML) is one of the most commonhematological malignancies, a malignant, clonal disease with poor prognosis.The rate of complete remission (CR) was 60%-90% in patients with first induction chemotherapy,10%~40% became refractory. 70% of AML patients who received the first CR maybe relapsed in the first year after the CR1.The 5 years total survival (OS) rate of refractory or relapsed AML (RR-AML) was 5%~10%, and the CR rate was not more than 30% in general.there is still no unified treatment regimen for RR-AML.The immune checkpoint inhibitor (ICB) PD-1 can overcome the immune tolerance of tumor cells. It has advantages over azacytidine-based chemotherapy. Demethylation drugs have been reported to increase the sensitivity of CAG、HAG and other chemotherapy regimens, thereby improving clinical efficacy.

To improve the prognosis and survival of RR-AML patients, we propose to conduct a prospective study to explore the efficacy and safety of PD-1 inhibitor combined with azacytidine and HAG regimen in the treatment of RR-AML, and to study its related mechanisms.

☐ **Why have I been chosen?**

We are inviting patients who

- Chinese guidelines for the diagnosis and treatment of relapsed and refractory acute myeloid leukemia (2017 edition)，excludes acute promyelocytic leukemia (M3、APL);
- Hematopoietic stem cell transplantation ≥3 months, Discontinue immunosuppressant ≥3 weeks, Patients without graft-versus-host disease;

☐ **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 treated with Azacytidine 75mg/(m2.d) by IV on days 1-7 of every cycle. Anti-PD-1 mAb 200mg by IV on day 8 of every cycle.Homoharringtonine(HHT) 2mg/(m2.d) by IV on days 1-6 of every cycle Cytarabine 10mg/(m2.d) by SC on days 1-7 of every cycle Granulocyte colony-stimulating factor(G-CSF) 300ug/d by SC on days 1-7 of every cycle，until absolute neutrophil count(ANC) ＞ 5X109 / L or white blood cell（WBC）＞ 20X109 / L.
- 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 1 therapy for 4 weeks. Follow up will continue indefinitely, for as long as 8 months 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 azacytidine includes:

- Myelosuppression:
  Neutropenia related infections, fever;
  Bleeding: ecchymosis, petechial, haematemesis, hemoptysis, melena, hematuresis, etc;
  Weary, somnolence, pale, etc (due to anemia);
- Other non-hematological toxicity:
  Liver dysfunction; Renal dysfunction;
- Drug allergy
  Erythra, edema, pruritus, swelling, etc

Potential adverse event of PD-1 inhibitor includes:

- immune-related adverse events (irAEs). Such as rash, pruritus, diarrhea, pneumonia, liver and kidney function damage, endocrine dysfunction, 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 2 cycles PD-1 inhibitor and related test (e.g., immune function, PD-1/PD-L1 expression, cytokines, etc).Patients will receive 100 RMB travel subsidy for each follow-up.Another possible benefit you may experience from this research includes 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 cancer 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.

☐ **Who is organizing the research?**

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

☐ **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**	