Clinical study on the safety and efficacy of CD7 CAR-T cell in patients with relapsed/refractory acute leukemia

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Objective	To evaluate the clinical safety and tolerability of CD7 CAR-T in the treatment of relapsed refractory acute leukemia
Investigational drug	CD7 CAR-T
Planned number of cases	200
indication	relapsed/refractory acute leukemia
Research purpose	Main purpose: ➢ To evaluate the clinical safety and tolerability of CD7 CAR-T therapy. Secondary purpose: ➢ Preliminary evaluation of the clinical efficacy of CD7 CAR-T therapy (according to clinical evaluation criteria) Exploratory purpose: ➢ To explore the expansion and persistence of CD7 CAR-T in vivo and its correlation with clinical efficacy ➢ To explore the correlation between pharmacodynamic (PD) biomarkers of CD7 CAR-T and clinical efficacy ➢ To explore in vivo expansion and persistence of CD7 CAR-T and the association of pharmacodynamic (PD) biomarkers with cytokine release syndrome (CRS) and neurological events
Research design	This is an open, single-arm, single-center, prospective clinical study. Main contents of clinical research: To evaluate the clinical safety and tolerability of CD7 CAR-T in the treatment of relapsed refractory acute leukemia Dosage plan: It is planned to enroll 200 subjects for dose-limited toxicity (DLT) assessment, and the presence of non-evaluable subjects will increase the number of subjects, which will lead to an increase in the actual sample size.

	CD7 expression was determined by flow cytometry in 200 subjects. After the target was determined, the subjects received the target dose of CD7 CAR-T from 1×10^6 to 1×10^8 /kg. Each subject will start at a low dose of 1 x 10^6/kg and increase to the next dose if there are no significant side effects until the maximum tolerated dose is reached. If the incidence of DLT during the study is greater than 1/3, the SRC will discuss the conduct of the trial based on the safety profile of the subjects and the in vivo pharmacokinetics and pharmacodynamics data of CD7 CAR-T. CRS will be graded according to the revised grading System (Lee 2014). Crs-related adverse events will be evaluated to determine whether they are DLT based on CRS overall grading. Consistent with non-CRS toxicity, all grade 3 CRS lasted >7 days and all grade 4 CRS were judged to be DLT, except as described above. Dlt-evaluable subjects: For accurate assessment of DLT, DLT-evaluable subjects were defined as receiving CD7 CAR-T infusion therapy. DLT non-evaluable subjects will be replaced and will result in an increase in the actual sample size. Drug Safety Monitoring Committee (SRC) During the trial, a Drug Safety Monitoring Committee (SRC) composed of principal investigators and laboratory members will be established to evaluate the safety data generated during the trial and jointly make recommendations for further trials. Evaluation of curative effect: • Remission criteria for acute leukemia will be applied to the NCCN guideline evaluation criteria until 24 months after CAR T cell infusion or disease progression, whichever occurs first, as specified in the Efficacy Evaluation Criteria. Security assessment: The study will collect adverse events (including neurological events, hematological events, infections, and secondary tumors) from the day after the CAR T cell infusion to 24 months after the infusion.
Research treatment procedures	Screening period: ➢ flow cytometry: 5ml of bone marrow was collected by deep purple tube and sent for flow cytometry. The expression of CD7 was determined by flow cytometry.

	➢ The length of the screening period for eligible subjects depends on tumor burden at the time of enrollment, immunosuppressive drug use, and vital signs Treatment period: ➢ Pretreatment chemotherapy: cyclophosphamide 500 mg/m^2 and fludarabine 30 mg/m^2 by intravenous infusion, all administered on days -5, -4, and -3 ➢ CAR T cell input: Subjects were hospitalized to complete CD7 CAR T input and were required to be hospitalized for observation for at least 2 weeks after the input. Follow-up period: To CAR T cell infusion for two years
Inclusion Criteria	1. Patients diagnosed with acute leukemia. 2. Acute leukemia complex/refractory cases with poor response to conventional chemotherapy: 1) patients who did not achieve complete remission after 2 courses of treatment with standard induced remission regimen; 2) Recurrence within 6 months after the first remission; 3) Relapse 6 months after the first remission, but failure to be treated again with the original induced remission regimen; 4) Recurrent patients. 3. At least 2 weeks or 5 half-lives (whichever is shorter) from the start of preconditioning chemotherapy after prior systemic treatment, except for immune checkpoint inhibitors/agonists; Systemic immune checkpoint inhibitor/agonist treatment is at least 3 half-lives away from pre-treatment chemotherapy (e.g., ipilimumab, etc.). 4. Toxic reactions caused by previous antitumor therapy must be stabilized and returned to ≤ grade 1 (except for clinically insignificant toxicity, such as baldness). 5. Over 14 years old, under 65 years old. 6. Physical Strength score 0-3 (ECOG standard) 7. No obvious active infection or graft-versus-host disease 8. Expected survival ≥3 months 9. Adequate kidney, liver, lung and heart function, defined as: Creatinine clearance (estimated by Cockcroft Gault formula) > 60 mL/min; Serum ALT/AST ≤ 2.5 ULN; Total bilirubin ≤1.5 ULN, excluding subjects with Gilbert's syndrome; Cardiac ejection fraction ≥ 50%, echocardiography confirmed centropericardial effusion, and ECG showed no clinically significant abnormal findings.

	There was no clinically significant pleural effusion. Baseline blood oxygen saturation under indoor ventilation was > 92%. 10. The serum pregnancy test results of fertile women must be negative (women who have undergone surgical sterilization or at least 2 years after menopause are considered to be infertile).
Exclusion Criteria	1. The subject has had other malignancies, non-melanoma skin tumors, carcinoma in situ (e.g. Cervix, bladder, breast), unless disease-free survival of at least 3 years 2. Presence or suspicion of uncontrollable fungal, bacterial, viral or other infections. 3. Known human immunodeficiency virus (HIV) infection 4. Known history of hepatitis B (HBsAg positive) or hepatitis C (HCV antibody positive). Subjects with latent or prehepatitis B infection (defined as HBcAb positive and HBsAg negative) can be enrolled only if PCR tests for HBV DNA are negative. In addition, these subjects were required to undergo a monthly PCR test for HBV DNA. Participants who are serologically positive for HCV antibodies can also be enrolled if their PCR test results for HCV RNA are negative. 5. Existing or past CNS disease, such as seizures, cerebrovascular ischemia/bleeding, dementia, cerebellar disease, or any CNS-related autoimmune disease 6. Subjects with severe heart disease, such as uncontrolled or symptomatic arrhythmias, congestive heart failure, or myocardial infarction within 6 months prior to screening, or any grade 3 (moderate) or 4 (severe) heart disease (according to the New York Heart Society Functional Grading Method NYHA) with lymphoma infiltrating the heart's atria or ventricles 7. A history of myocardial infarction, angioplasty or stent placement, unstable angina pectoris, or other clinically significant heart disease in the 12 months prior to enrollment 8. Emergency treatment is expected or likely to occur within 6 weeks due to rapid tumor progression (e.g. tumor mass compression) 9. Primary immune deficiency 10. History of symptomatic deep vein thrombosis or pulmonary embolism within 6 months prior to enrollment 11. Any medical condition that may affect the evaluation of safety or efficacy 12. Have had severe rapid hypersensitivity reactions to any of the drugs to be used in this study 13. Administer live vaccine within ≤6 weeks prior to initiation of the pretreatment regimen 14. Pregnant or lactating female subjects

	15. Male or female subjects who do not consent to effective contraception from the time they sign informed consent until 6 months after completing AT19 treatment 16. Subjects judged by the investigator had difficulty completing all visits or procedures required by the study protocol (including follow-up visits), or were not compliant enough to participate in the study 17. In the past 2 years, subjects have had other malignancies, non-melanoma skin tumors, carcinoma in situ (e.g. Cervix, bladder, breast), end-organ damage due to autoimmune diseases (e.g. Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus), or need to systematically administer immunosuppressive or other drugs for systemic disease control. Unless disease free survival of at least 3 years 18. Participate in other clinical experimenters during the same period
Evaluation index of curative effect	Remission criteria for acute leukemia • Complete Remission (CR) : ➢ Bone marrow: primitive + naive cell count ⩽ 5%, red blood cell and megakaryocyte line normal. ➢ Hematography: hemoglobin (Hb) ⩾100g /L (male) or ⩾90g /L (female and children), neutrophil absolute value ⩾ 1.5 ×10^9/L, platelet count ⩾100×10^9/L, no leukemia cells in the classification. ➢ Clinical manifestations: no symptoms and signs caused by leukemia infiltration, life is normal or nearly normal. • Partial response (PR) : The number of primitive + naive cells in the bone marrow is > 5%, ⩽ 20%, or one of the clinical or hematologic two criteria does not meet the complete response criteria. • No response (NR) : Those who did not meet the above criteria in bone marrow, blood, or clinical Settings. leukemia recurrence • Relapse occurs in one of the following: • The number of primitive + naive cells in bone marrow was > 5%, ⩽ 20%, and clinical remission could not be achieved after 1 course of anti-leukemia therapy. • Bone marrow primitive + naive cell count > 20%. • Patients with infiltration of extramedullary leukemia cells. Complete response rate (CR) and partial response rate (PR) after 3 months of CD7 CAR-T reinfusion; Recurrence rate, progression-free survival (PFS) and overall survival (OS) after 1 to 5 years of CD7 CAR-T reinfusion; The amount and duration of CD7 CAR-T in vivo.

	Objective response rate (ORR) : defined as investigator-determined complete response rate (CR) and partial response rate (PR). All subjects who did not meet objective response criteria were considered to be in no response at the analysis deadline. Duration of response (DOR) : defined as the time from the subject's first response (CR or PR) to the subject's disease progression or death from any cause. Subjects who did not develop disease progression or death by the analysis deadline were excluded at the time of the last tumor evaluation. Progression-free survival (PFS) : defined as the time from initiation of CAR T cell infusion to disease progression or death from any cause. Subjects who did not develop disease progression or death by the analysis deadline were excluded at the time of the last tumor evaluation. Overall survival (OS) : defined as the time from initiation of immunization infusion until death from any cause. All subjects who did not die by the analysis deadline were deleted based on the last contact time.
Statistical method	Sample size determination: The sample size of this experiment was not determined based on assumptions. Approximately 200 subjects are planned to be recruited based on the occurrence of DLT. Subjects whose DLT is not evaluable will be replaced, which may result in more subjects being enrolled than expected. Statistical analysis: In general, continuous variables such as age will be described statistically using observations, mean, median, standard deviation, minimum, and maximum; Categorical variables are described statistically using the frequency of each category and its percentage. To the event occurrence time variable, Kaplan-Meier method was used to summarize the data. If the data allows, calculate the median and quartile. The number of incidents and deletions will also be presented. The final analysis of the study will be based on data collected by the subjects throughout the study period. The efficacy evaluable analysis included all subjects treated with a dose of at least 1.0×106CAR T cells /kg who had baseline tumor evaluation and at least one post-baseline tumor evaluation. The safety analysis set included all subjects who received CAR T cell transfusions. The statistical methods will be detailed in the statistical analysis plan. Safety evaluation: Safety was evaluated by summary of adverse events, changes in laboratory results, and changes in vital signs. All subjects receiving CD7 CAR-T infusion will

	be included in the safety analysis (i.e., the safety analysis set). Adverse events will be graded according to NCI CTCAE (version 4.03). Adverse events were coded using the International Dictionary of Medical Terms (MedDRA). Summarize the number and frequency of adverse events according to the classification of human organ systems with appropriate terms. All SAEs (including death), DLT, AESI need a separate list summary. Changes in laboratory results will be summarized according to NCI CTCAE (version 4.03) grading. For laboratory indicators, the highest level of toxicity that occurred during the test was summarized in the form of counts and percentages. Changes in vital signs and physical strength scores were compared to baseline levels for descriptive statistics. Some vital signs and laboratory test results will be graphically summarized for each subject's data over time. Curative effect evaluation: Descriptive statistical methods were used in this study, including complete response rate, partial response rate, objective response rate, progression-free survival, overall survival, recurrence rate, and CD7 CAR-T retention and duration in vivo. All adverse events (including serious adverse events) are described and their incidence is calculated with a 95% confidence interval. Survival information was analyzed using Kaplan-Meier curves.