Clinical trial protocol

Name: Clinical Study to Evaluate the Safety and Clinical Efficacy of Anti-human CD19/CD22 Bispecific CAR-T Cells in Subjects with Refractory/Relapsed Central Nervous System B-cell Tumors

Scheme Number: HRAIN02-CNSL 01

Research type: Interventional research

Funding Source: Partial funding (Shanghai Hrain Biotechnology Co., Ltd.)

二、Abstract

test drug	Anti-human CD19/CD22 bispecific CAR-T cells
Indications	B-cell tumors involving the central nervous system (mainly including: relapsed B-cell acute lymphoblastic leukemia with central nervous system involvement, refractory/relapsed central nervous system B-cell lymphoma)
Dosage and administration	Intravenous /intrathecal administration : 1 to 3 sachets per administration (depending on individual differences), ivgtt D0 (X × 10^7 live cells/ml, infusion volume = patient weight × required CAR $^+$ T cell amount per kg of body weight ÷ [cell concentration X × 10^7 ^{live} cells/ml × CD3 $^+$ T cell ratio × infection efficiency]). Investigators may administer multiple doses to subjects based on their clinical benefit-risk assessment. For intrathecal administration, one sachet per administration, starting with an initial dose of 1 × 10^6 ^{live} cells/dose, can be administered to no more than 3 subjects intrathecally. The dosage and number of subjects to be enrolled can be increased based on the subject's condition .
Research Topic	Clinical studies evaluating the safety and clinical efficacy of anti-human CD19/CD22 bispecific CAR-T cells in patients with refractory/relapsed central nervous system B-cell tumors.
Research Objective	**Main research objective:** the safety and clinical efficacy of anti-human CD19/CD22 bispecific CAR-T cells in treating patients with refractory/relapsed B-cell tumors involving the central nervous system .
	Secondary research objective: **1)** To evaluate the distribution, expansion, and survival of anti-human CD19/CD22 CAR-T cells in vivo after administration of anti-human CD19/CD22 bispecific CAR-T cells; 2) of the overall response rate (ORR) 3 months after a single dose of anti-human CD19/CD22 bispecific CAR-T cell therapy in subjects with refractory/relapsed central nervous system B-cell tumors. 3) the overall time to response (DOR) in subjects with refractory /relapsed central nervous system B-cell tumors treated with anti-human CD19/CD22 bispecific CAR-T cell therapy . 4) overall survival (OS) in subjects treated with anti-human CD19/CD22 bispecific CAR-T cell therapy for refractory/ relapsed B-cell tumors of the central nervous system.
Experimental Design	This is a single-center, single-arm, open-label , exploratory early-stage clinical study to investigate the safety, tolerability, and pharmacokinetic characteristics of the drug. It also aims to preliminarily observe the efficacy of the drug in inhibiting human CD19/CD22 CAR-T cells in subjects with refractory/relapsed central nervous system B-cell tumors, and to explore the appropriate dosage and dosing regimen for subsequent clinical trials. In this study, we will determine the recommended dosage for subsequent clinical trials based on efficacy and pharmacokinetic endpoints (ORR, CAR-T cell expansion and duration of action AUC $_{0\text{-}28\ days}$) and previous clinical trial experience.
Sample size	In the early clinical study, the plan was to enroll 10-20 subjects who completed

	cell infusion. However, considering the possibility of cell preparation failure or the inability to perform cell infusion due to rapid disease progression during cell preparation, the number of subjects participating in cell preparation in this study may be greater than the planned number.
Research period	Expected 3 years
Selection criteria	refractory/relapsed B-cell tumors affecting the central nervous system , male or female. (1) CD19 or CD22 positive or double positive B-cell tumors, including patients with relapsed B-cell acute lymphoblastic leukemia with central nervous system involvement, and refractory/relapsed central nervous system B-cell lymphoma ; (2) Age 18-70 (inclusive), gender not limited; (3) Expected survival time exceeds 12 weeks; (4) ECOG score 0-2 points; (5) Subjects diagnosed with B-cell tumors involving the central nervous system by cerebrospinal fluid examination and/or imaging examinations such as magnetic resonance imaging and PET/CT . (6) A venous access can be established for the collection, and the researcher determines that mononuclear cell collection is possible; (7) Liver and kidney function, and cardiopulmonary function meet the following requirements: a) Creatinine levels are within the normal range; b) Left ventricular ejection fraction >50%; c) Baseline oxygen saturation >92%; d) Total bilirubin ≤ 2 × ULN; e) ALT and AST ≤ 2.5 times the upper limit of normal; (8) Can understand this experiment and has signed an informed consent form.
Exclusion criteria	Individuals with any of the following conditions are ineligible to be selected as participants: （1） B-cell tumors within the 5 years prior to screening , in addition to fully treated uterine lesions Cervical carcinoma in situ, basal cell or squamous cell skin cancer, localized prostate cancer after radical mastectomy, ductal carcinoma in situ after radical mastectomy, and thyroid cancer after radical mastectomy. （2） for hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HbcAb) and whose peripheral blood HBV DNA titer is ≥ the lower limit of detection; subjects who are positive for hepatitis C virus (HCV) antibody and whose peripheral blood HCV RNA is positive; subjects who are positive for human immunodeficiency virus (HIV) antibody; and subjects who are positive for syphilis. （3） Any unstable systemic disease, including but not limited to unstable angina, cerebrovascular accident or transient ischemic attack (within 6 months prior to screening), myocardial infarction (within 6 months prior to screening), congestive heart failure (NYHA class III or higher), serious arrhythmia requiring medical treatment, liver, kidney or metabolic disease;

	（4） Within 14 days prior to enrollment, there was an active or uncontrollable infection requiring systemic treatment; （5） Women who are pregnant or breastfeeding, and female subjects who plan to become pregnant within 1 year after cell infusion or male subjects whose partners plan to become pregnant within 1 year after cell infusion; （6） Individuals who received CAR-T therapy or other gene-modified cell therapy before screening; （7） Subjects who were receiving systemic steroid therapy prior to screening and were determined by the investigator to require long-term systemic steroid therapy during the treatment period (excluding inhaled or topical use); and subjects who received systemic steroid therapy within 72 hours prior to cell infusion (excluding inhaled or topical use). （8） Other situations where researchers deem the study unsuitable for inclusion.
Definition of dose-limiting toxicity (DLT)	The severity of adverse events is classified according to the NCI-CTCAE version 5.0, and the investigator determines whether the subject has DLT. Given the specific nature of central nervous system involvement in B-cell tumors , DLT is defined as: grade 3 or higher (CTCAE version 5.0) possible or confirmed symptoms, laboratory toxicities, and clinical events that are likely or definitively related to the study treatment and occur within 28 days after a subject receives anti-human CD19/CD22 bispecific CAR-T cell infusion. This also includes any of the following investigational drug-related conditions that occur despite treatment intervention: 1) Grade 4 or 5 cytokine release syndrome induced by anti-human CD19/CD22 bispecific CAR-T cell therapy; 2) Grade 3 cytokine release syndrome induced by anti-human CD19/CD22 bispecific CAR-T cell therapy and which did not improve to grade 2 or below within 7 days; 3) Grade 3 or higher organ toxicity reactions occurred (nervous system, heart, skin, gastrointestinal tract, liver, lung, and kidney, except for the AE that resolved to Grade 2 or lower within 3 days). 4) Any unintended toxicity that necessitates termination of treatment based on the investigator's or sponsor's judgment.
Definition of Maximum Tolerable Dose (MTD)	The maximum tolerated dose is defined as the highest dose at which no more than 2 out of 5 patients develop DLT.
Study endpoints	**Primary study endpoint:** The proportion of DLT and adverse events exceeding CTCAE grade ≥ 3 . **Secondary study endpoints:** 1) Cmax) of anti-human CD19/CD22 bispecific CAR-T cells expanded in peripheral blood after drug administration , the time to reach the highest concentration (T_{max}), and the area under the curve (AUC) $_{0\text{-}28d}$ at 28 days ; The proportion

	and duration of CAR-T in cerebrospinal fluid ; 2) PD parameters: Expression of CAR-T activation-related cytokines such as IL-6, INF-γ, and IL-15; 3) CRi) at 3 months after administration ; 4) Duration of response after administration (DOR): refers to the time from the first assessment of CR or CRi to the first assessment of disease progression or death from any cause. 5) Overall survival (OS) after drug administration: the time from cell reinfusion to death from any cause.
Statistical methods	General principles: Statistical analysis will be performed using SAS statistical analysis software version 9.2 or later. For continuous variables, descriptive statistics will include the mean, median, standard deviation, maximum, and minimum values. For categorical variables, descriptive statistics will include the number of subjects and the percentage. The main statistical analysis set is as follows: All Subjects Enrolled Set: All subjects who signed informed consent, regardless of whether they were successfully screened or received anti-human CD19/CD22 bispecific CAR-T cells ; Full Analysis Set (FAS): This dataset contains the infusion of anti-human CD19/CD22 bispecific CAR-T cells, performed according to the trial protocol, within the selected subject dataset. The FAS set is the primary dataset for PK, PD, and immunogenicity analyses. Safety Set: In the subject screening dataset, this includes all subjects who received the investigational drug and had subsequent safety follow-ups. The safety set is an analytical dataset that summarizes safety data. Efficacy Set: This is the dataset used to select participants who received the investigational drug and underwent subsequent efficacy evaluations. The efficacy set is an analytical dataset that summarizes efficacy data.

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