Protocol- Study 2 (CG1006)

This trial protocol has been provided by the authors to give readers additional information about their work.

**A Clinical Study of Autologous Chimeric Antigen Receptor T Cells Targeting Glypican-3 in the Treatment of Refractory Hepatocellular Carcinoma**

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| **Study Site:** | **Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine**  **NO.145, Middle Shandong Road, Shanghai, China**  **200001** |
| **Principal Investigator:** | **Bo Zhai, MD, PhD**  **Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine** |
| **Protocol Number:** | **CG1006** |
| **Study Drug:** | **CAR-GPC3 T cells** |
| **Study Phase:** | **Exploratory study** |
| **Version Number and Date:** | **V2.0, Nov 03, 2017** |

*This study will follow the protocol, Good Clinical Practice (GCP), the Declaration of Helsinki Declaration, and other relevant regulations.*

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**Declaration of Principal Investigator**

**Title**: A Clinical Study of Autologous Chimeric Antigen Receptor T Cells Targeting Glypican-3 in the Treatment of Refractory Hepatocellular Carcinoma

This protocol has been vigorously reviewed. The information of this protocol is in accordance with the known risk and benefits of the study drug, and the moral, ethical and scientific principles of the conduction of clinical study in the Helsinki Declaration and Good Clinical Practice (GCP).

**Principal Investigator**

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Bo Zhai, MD, PhD Date

Chief Physician   
Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine

# Synopsis

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| **Title** | A Clinical Study of Autologous Chimeric Antigen Receptor T Cells Targeting Glypican-3 in the Treatment of Refractory Hepatocellular Carcinoma |
| **Protocol Number:** | CG1006 |
| **Study Phase** | Exploratory study |
| **Principal Investigator** | Bo Zhai, MD, PhD |
| **Study Site:** | One site (Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine) |
| **Objectives** | Primary objective:  To observe and identify the safety, tolerability and pharmacokinetics of lentiviral vector-transduced CAR-GPC3 T cells in patients with hepatocellular carcinoma (HCC).  Secondary objective:  To observe the efficacy of CAR-GPC3 T cells in the treatment of HCC by the following parameters:  Progression-free survival (PFS)  Disease control rate (DCR) and objective response rate (ORR)  Overall survival (OS) |
| **Study Design** | Open-label, single-arm prospective study  Refractory or relapsed HCC patients without effective treatment methods will receive single or multiple doses of intravenous CAR-GPC3 T cells for the observation of safety and efficacy of CAR-GPC3 T cells. |

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| **Dosage and Usage** | | Study Drug:  CAR-GPC3 T cells  Administration route:  Intravenous  Dosage:  Intravenous administration for hepatocellular carcinoma,  ① Intra-patient dose-escalation trial: the first 3-6 enrolled subjects will receive self-dose escalation. The first subject will receive ≤1.4 × 106 cells/kg as an initial split dose, followed by observation for at least 24 hours; if no dose-limiting toxicity (DLT) occurs and the investigator considers it necessary, the increased dose will be given at the interval of 1-2 days for 1-2 times, with the dose and dosing schedule details determined by the investigator. The above described is considered 1 treatment course.  After the last infusion of the single course, if there is no DLT during the observation period of at least 4 weeks and the investigator considers it necessary, more treatment courses can be given, with the dose and dosing schedule details determined by the investigator. The dose for the second and third subjects could be escalated based on the safety and efficacy results for the first subject.  ②“3+3” dose escalation trial:  Based on the results of the above intra-patient escalation trial, the subsequent subjects will start at one dose level that has been validated as safe; 4-6 dose levels could be tested according to the infusion amount per body weight during every treatment course, with 3-6 subjects per level.  For each subject, after the last infusion of a single course, if there is no DLT during the observation period of at least 4 weeks and the investigator considers it necessary, more treatment courses can be given, with the dose and dosing schedule details determined by the investigator. The observation period will last from the first dose until 2 years after the first infusion.  The study dose will start from one dose level, if no DLT occurs, investigators will proceed to the next dose level. If 2 or more subjects experience DLT at one level, the previous level will be defined maximum tolerated dose (MTD). If 1 case of DLT occurs at one level, 3 more subjects will be enrolled, and if there is no DLT for these 3 subjects, the study will proceed to next dose level. If more than one subject experience DLT at this dose level in these 3 subjects, the previous dose level will be defined MTD. Finally, the highest dose level for which the percentage of subjects with DLT is ≤1/6 will be defined as MTD.  The addition of treatment courses:  If the subject is considered to have possibly benefited from cell therapy according to the investigator's judgment, one or more treatment courses can be added.  For additional courses, the investigator will decide if lymphodepletion is needed according to the physiological or pathological condition of the subject.  If more courses are added, baseline and follow-up schedule will not be influenced.  Lymphodepletion regimen for CAR-GPC3 T cells:  The lymphodepletion regimen will be given at Day-5 to Day-2 before the infusion of CAR-GPC3 T cells, which can improve the proliferation and survival of T cells in vivo; each subject will receive the lymphodepletion regimen before infusion according to the following schedule, which will be adjusted by the investigator according to the patient’s details.  Fludarabine, 20 to 25 mg/m2/day × 4 days  Cyclophosphamide, 500 mg/m2/day ×2 days | |
| **Number of Subjects** | | 20 patients with refractory hepatocellular carcinoma. | |
| **Study Population** | **Inclusion Criteria:**   1. Patients between 18- and 70-years-old who have refractory HCC that has relapsed at least twice within two years, and without effective treatment methods 2. A liver cancer tissue sample tests positive for GPC3 by immunohistochemistry (IHC) 3. Estimated life expectancy > 12 weeks 4. At least one measurable tumor lesion (≥ 10 mm) 5. Liver cirrhosis condition: Child-Pugh A, or B with a score of 7 6. The longest diameter of single tumor < 5 cm; the number of multiple tumors < 10; accumulated tumor volume < 1/4 liver volume 7. Controllable lung metastasis (number of metastatic lesions < 6, longest diameter of lesions < 5 cm) 8. Eastern Cooperative Oncology Group (ECOG) score is 0 to 1, or Karnofsky Performance Status (KPS) score > 70 9. Have venous accesses for leukapheresis or blood sampling, and have no other contraindications for blood collection 10. Hematology: WBC ≥ 2.5×109/L, PLT ≥ 60×109/L, Hb ≥ 9.0 g/dL, MID ≥ 1.0×109/L, LY ≥ 0.4×109/L 11. Biochemistry: serum Alb ≥ 30 g/L, serum lipase and amylase ≤ 1.5 ULN, serum creatinine ≤ 1.5 ULN, ALT ≤ 5 ULN, AST ≤ 5 ULN, total bilirubin ≤ 2.5 ULN 12. Coagulation: prolonged prothrombin time ≤ 4 s 13. Be able to understand and sign the informed consent   All laboratory results should be stably within the ranges above without continuous supportive treatments.  **Exclusion Criteria:**  Patients are excluded from the study if any of the following criteria apply:   1. Diffuse hepatocellular carcinoma 2. Positivity of T cells after transduction < 10%, or expansion of T cells in response to αCD3/CD2 stimulation < 5-fold, which is considered as product manufacture failure 3. Pregnant or breastfeeding women 4. HIV positive 5. Any uncontrollable active infection 6. Current systemic use of steroids. Those who recently used or currently use inhaled steroids are not excluded 7. Allergic to immunotherapy and related drugs 8. Previous or present hepatic encephalopathy 9. The patient has active ascites that requires treatment 10. Active heart disease requiring treatment or poorly controlled high blood pressure determined by the investigator 11. Unstable or active ulcers and gastrointestinal hemorrhage 12. Patient with a history of organ transplantation or waiting for organ transplantation 13. Hyponatremia, with serum sodium < 125 mmol/L 14. Baseline serum potassium < 3.5 mmol/L (supplement of potassium is allowed, and patients who recover to above this limit are not excluded) 15. Patient who requires anti-coagulation therapy (e.g. warfarin or heparin) 16. Patient who needs long-term anti-platelet therapy (aspirin with dose > 300 mg/day; clopidogrel with does > 75 mg/day) 17. Patient who received chemotherapy, radiotherapy, targeted antitumor drugs or interventional therapy for the study disease within 4 weeks prior to leukapheresis 18. Other concurrent antitumor therapies 19. According to the investigators’ evaluation, patients who are unable or unwilling to comply with the requirements of the study protocol.   **Withdrawal criteria:**  At any time, a patient can voluntarily withdraw from the study. For safety, behavioral or managerial reasons, the investigator and sponsor can require a subject to withdraw from the study at any time according to their judgment. If a subject fails to complete the visits as scheduled, the investigator must make every possible contact with the subject. If possible, under any situation, the investigator should make every effort to record the subject’s treatment results. The investigator should inquire about the subject’s reason for withdrawal, request that the subject return to the study site for the last visit if possible and follow any unresolved adverse events as far as possible.   1. If confirmed progressive disease (PD) in accordance with RECIST1.1 criteria is observed, all study treatment should be discontinued; 2. If there are intolerable adverse events or deterioration of disease or health condition that make the subject unsuitable for trial continuously according to the investigator’s judgment; 3. The subject uses other antitumor treatments prohibited in this study, including but not limited to radiotherapy, chemotherapy, and surgical treatment; 4. The subject can voluntarily withdraw the consent from the study; 5. Pregnancy; 6. CAR-T manufacture failure   For subjects who withdraw from the study, the relevant data should be collected continuously unless if they refuse subsequent data collection from the patient consent. The data are no longer to be collected for the below situations:   * Lost follow-ups * Death * Termination of clinical trial proposed by the investigator, ethics committee or regulatory authority. | |
| **Study endpoints** | **Primary endpoint:**  **Safety and tolerability endpoints:**  Treatment-related adverse events, defined as laboratory toxicities and clinical events possibly related or related to treatment which occur within 24 weeks after cell infusion, including infusion-related toxicities and any CAR-GPC3 T‑cell–related toxicities. Including but not limited to:   * Fever * Chill * Nausea, vomiting and various symptoms related to stomach discomfort * Fatigue * Hypotension * Respiratory distress * Tumor lysis syndrome * Cytokine release syndrome * Neutrophil decrease, platelet decrease * Liver and kidney dysfunction * Other toxicities   The severity of adverse events will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03.  **Implantation endpoint:**  The persistent survival of CAR-GPC3 T cells in vivo, defined as the period of persistent survival of “implanted” CAR-GPC3 T cells in vivo. “Implantation endpoint” of the cells in the copy number of CAR-GPC3 DNA in peripheral blood which is detected by q-PCR at each visit point from 4 weeks after the first implantation until any two consecutive test results are negative.  **Secondary endpoints:**  The antitumor response of CAR-GPC3 T-cell infusion:   * Objective response rate (ORR) * Progression-free survival (PFS) * Disease control rate (DCR) * Overall survival (OS) | |
| **Tumor evaluation** | The baseline examination for this study is conducted after lymphodepletion and before CAR-GPC3 T cell infusion, which includes imaging of abdomen (including magnetic resonance imaging, CT, etc.) or other locations (in case of metastasis) and tumor markers examinations. Imaging and tumor marker examinations are performed every 8 weeks between Weeks 4 and 24, and every 3 months from Week 28 to the end of first year; and every 6 months from the end of first year to the end of second year. Imaging evaluation is performed according to RECIST1.1. | |
| **Statistical method** | Refer to regular statistical methods.  All AEs or serious adverse events (SAEs) will be listed by subject, and the number and percentage of subjects with treatment emergent adverse events (TEAEs) will be calculated by system organ class (SOC), the preferred term (PT) and group, to conduct descriptive statistics or inter-group comparison. Descriptive analysis or inter-group comparison of laboratory examinations, vital signs, and changes in the electrocardiogram and other safety parameters from relative baseline (if any) will be performed by groups. See “Statistical Analysis Plan” for details. | |

# List of Study Personnel

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# List of Abbreviations

|  |  |
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| ADCC | Antibody-dependent cell-mediated cytotoxicity |
| ADL | Activities of daily living |
| AE | Adverse event |
| ATC | Anatomical Therapeutic Chemical classification system for WHO |
| CAIX | Carbonic anhydrase IX |
| CAR-GPC3 T cells | Chimeric antigen receptor T cells targeting glypican-3 |
| CAR | Chimeric antigen receptor |
| CD3ζ | CD3-ζ chain (also known as zeta chain) |
| CDC | Complement-dependent cytotoxicity |
| CFDA | China Food and Drug Administration |
| CRF | Case report form |
| CRS | Cytokine release syndrome |
| CTCAE | Common Toxicity Criteria for Adverse Events |
| CTL | Cytotoxic T cell |
| DCR | Disease control rate |
| DFS | Disease-free survival |
| DLT | Dose-limiting toxicity |
| DSMB | Data safety and monitoring committee |
| EBV | Epstein-Barr virus |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| EDC | Electronic data capture |
| GCP | Good Clinical Practice |
| GMP | Good Manufacturing Practice |
| HBsAg | Hepatitis B surface antigen |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| hs‑CRP | High-sensitivity C-reactive protein test |
| IEC / IRB | Independent Ethics Committee / Institutional Review Board |
| IRR | Infusion-related reactions |
| ITAM | Immunoreceptor tyrosine-based activation motif |
| KPS | Karnofsky Performance Status |
| LVEF | Left ventricular ejection fraction |
| MedDRA | Medical Dictionary for Drug Regulatory Activities |
| MHC | Major histocompatibility complex |
| MTD | Maximum tolerated dose |
| PBMC | Peripheral blood mononuclear cells |
| PD | Progressive disease |
| PT | Preferred term |
| RCL | Replication-competent lentivirus |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| scFv | Single-chain variable fragment |
| SOC | System organ class |
| SOP | Standard operating procedure |
| TAA | Tumor-associated antigen |
| TCR | T cell receptor |
| Tcm | Central Memory T lymphocytes |
| TEAE | Treatment emergent adverse event |
| Tem | Effective Memory T lymphocytes |
| TNF | Tumor necrosis factor |
| Treg | Regulatory T lymphocyte |
| WOCBP | Women of childbearing potential |

# Introduction

## Background

Glypican-3 (GPC3), alias as DGSX, GTR2-2, MXR7, OCI-5, SDYS, SGB, SGBS or SGBS1, is a therapeutic target for hepatocellular carcinoma (HCC). The GPC3 gene encodes a 70-kDa precursor protein, which can be cleaved by furin to produce a soluble N-terminal peptide (40-kDa) that can enter blood circulation and a membrane-bound C-terminal peptide (30-kDa) that contains two heparan sulfate carbohydrate chains. The GPC3 protein is a member of the heparan sulfate protein polysaccharide family, anchored on the cell membrane through glycosylphosphatidylinositol (GPI) [1]. This cell surface antigen is highly expressed in fetal tissues and is gradually decreased during development. Recently, GPC3 was found in various tumors including squamous cell lung carcinoma, liver cancer, cervical cancer and melanoma [2]. Notably, GPC3 is known as a carcinoembryonic antigen and not expressed in normal adult tissues, which makes GPC3 an ideal candidate target for immunotherapy.

It has been reported that an anti-GPC3 antibody can be used for liver cancer detection and applied as a study treatment by the antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity mechanisms [2]. The C-terminal of GPC3 protein is recognized by the antibody for therapeutic use. However, antibody therapy is restricted by its half-life in circulating blood, and the half-life of most antibodies is within 23 days. Therefore, continuous dosing and/or an increased dose is required for antibody therapy, which increases the therapeutic cost and may lead to the termination of therapy in some situations. Also, as a heterologous protein, the therapeutic antibody can result in allergic reactions and the possible generation of anti-drug antibodies.

Based on the important function of T lymphocytes in tumor immune response and the advantages in overcoming drawbacks of antibody therapy, immunotherapy using adoptive T cell therapy has emerged as a novel treatment for some tumors. However, the efficacy of adoptively transferred T cells is still not able to meet the need for solid tumors. T cell receptors (TCRs) expressed on cytotoxic T lymphocytes (CTL) can recognize a specific antigen on the target cells. Therefore, researchers have engineered chimeric antigen receptors (CARs), which use a single-chain fragment variable (scFv) from the tumor-associated antigen (TAA) antibody fused with intracellular activation signals such as CD3ζ or FcεRIγ of the TCR. The CARs are incorporated into a lentiviral vector and expressed on the surface of T lymphocytes. Such CAR-T lymphocytes are not major histocompatibility complex (MHC) restricted and can selectively target and kill tumor cells. CAR T lymphocytes represent a new strategy for tumor immunotherapy.

Chimeric antigen receptors include an extracellular domain, a transmembrane domain and an intracellular signal domain. Usually, the extracellular domain contains an scFv that specifically recognizes the TAA. The transmembrane domain is derived from CD8, CD28, and other molecules. The intracellular signal domain contains the immunoreceptor tyrosine-based activation motif (ITAM) CD3ζ or FcεRIγ, and costimulatory signals, such as CD28, CD137 or CD134. The intracellular signal domain of first-generation CAR T cells only contained ITAM, and the components of this chimeric antigen receptor were linked as scFv-TM-ITAM. The first-generation CAR-T cells were enabled with antitumor cytotoxic capability, but could only produce limited cytokines, and the antitumor effect did not last for a long time.

In the second generation of CAR-T cells, CD28 or CD137 (also named 4-1BB) was subsequently added to the intracellular signal domain, and components of their chimeric antigen receptor were linked as scFv-TM-CD28 -ITAM or scFv-TM-/CD137-ITAM. The costimulatory effect of B7/CD28 or CD137 from the intracellular signal domain led to the persistent proliferation of T cells, promoted T cells to secrete cytokines like IL-2 and IFN-γ, and simultaneously prolonged the survival period of CAR T in vivo and enhanced their antitumor effects [3].

The third generation of CAR-T cells was developed in recent years. The components of the CAR are linked as scFv-TM-CD28-CD137-ITAM or scFv-TM-CD28-CD134-ITAM, which further prolongs the persistence of CAR-T cells in vivo and enhances their antitumor effects [4]. The CD28 molecule plays an important role in the regulation of lymphocyte proliferation and survival and the establishment of effector and memory T cells. The generation of such effects is due to the recruitment of PI3K, Grb2 and Lck, which regulate the activity of key transcription factors as NFκB and enhance the expression of Bcl-xL and the secretion of IL-2. The receptor CD137 belongs to the tumor necrosis factor (TNF) family and provides the costimulatory signal for T cell response, playing a key role in T cell survival and establishing memory T cells. These receptors recruit adaptors of TNF receptor-associated factor 1 (TRAF1) and TRAF2, and activate downstream signal pathways of JNK, p38, MAPK and NFκB.

Currently, more CARs targeting various tumor surface biomarkers are being developed [5]. The first exciting result of CARs for cancer treatment came from Brenner’s team, using diasialoganglioside-2 (GD2)-targeted CAR-T cells to treat childhood neuroblastoma [6]. In this trial, Epstein-Barr virus (EBV)-specific T cells were transduced with GD2-CAR. Under physiological conditions, endogenous CD3+ T cells were able to recognize EBV-positive cells and become activated, which increased their in vivo survival time and enhanced antitumor response mediated by anti-GD2-CAR T cells. Clinically, EBV-specific CAR T cells were still detected in patient peripheral blood at 6 weeks after infusion; meanwhile, the non-EBV-specific T cells only lasted 1 week. Six out of 11 treated patients showed tumor shrinkage and necrosis at 6 weeks after treatment. Recently the Rosenberg team from NCI reported one case of a lymphoma patient treated with anti-CD19 CAR which contained CD28 and ζ chain as the costimulatory domain [7]. The patient showed remission of CD19+ lymphoma. Two phase I clinical trials for the second generation of autologous CAR-T cells with CD19-specific CARs have been initiated to treat the patients with refractory chronic lymphocytic leukemia (CLL) and relapsed acute lymphocytic leukemia (ALL). Up to now, 8 patients have received the treatment; among 3 patients with relapsed CLL, both of 2 extremely refractory patients who failed multiple prior lines of therapy achieved complete response. Two pediatric patients with relapsed CLL achieved complete response [8,9].

In one recent clinical trial of 17 patients who were treated with HER2-targeted T cells, none of the patients showed serious toxic side effects, and some patients showed the elimination of tumor lesions. In a recent HER2-targeted treatment for glioblastoma multiforme (GBM), 5 of 15 patients had objective response; among them 1 patient’s tumor shrunk by 62%, 1 patient gained stable disease of 4 months, and the other 3 patients gained stable disease who still survived after follow-ups of 18 to over 24 months [10,11]. In the CAR T clinical trial targeting mesothelin through mRNA transduction technology, 6 pancreatic patients did not experience serious toxic side effects; among them 2 patients achieved stable disease and 1 patient’s hepatic metastatic lesion disappeared [12].

Although CAR-T cells have demonstrated promising perspectives in tumor immunotherapy, some potential risks should also be considered. For example, due to low expression of some specific antigens in some normal tissues that can be recognized by a CAR, CAR-T cells may cause damage to such normal tissues. The first case of off-target effects was reported in a CAR-T cell therapy that targeted the antigen carbonic anhydrase IX (CAIX) on a renal cell carcinoma tumor. The patient experienced grade 2-4 hepatic toxicity after multiple infusions of CAR-T cells. The reason was attributed to the low expression of CAIX on epithelial cells of the hepatic duct, and the clinical trial was forced to be discontinued, excluding any efficacy evaluation [13]. Among the above-mentioned ALL and CLL patients treated with CAR-T cells, all of them had grade 3-4 toxicities; 1 of 2 pediatric ALL patients had severe cytokine release syndrome (CRS) and B cell dysplasia, and the other had immune escape leading to relapse. On the other side, the threshold required for effector cell activation can be decreased by too many costimulatory signals in genetically modified T cells; they are activated even with low level of antigens or without antigen stimulation, which results in the release of a large amount of cytokines called “cytokine storm.” Such signal leakage will lead to off-target toxicity and nonspecific tissue injury. For example, third-generation CAR-T cells targeting HER2 were used for the treatment of a patient with advanced colon cancer with liver and lung metastases; cytokine storm induced by the low expression of HER2 in normal lung tissues caused the sudden death of the patient [14].

For the first case in a HER2-targeted CAR-T-cell clinical trial, the patient experienced fatal side effects However, during a separate clinical trial of second-generation HER2-targeted CAR-T cells that enrolled 17 subjects, none of the subjects experienced serious toxicity, and targeted lesions were eliminated in some subjects. The death in the first case was probably due to the application of lymphodepletion with high dose of third-generation CAR-T cells, which resulted in the damage to the HER2-expressing pulmonary tissue and led to respiratory failure. In contrast, less than 1×108 cells of the second-generation CAR-T cells were given to the other trial’s 17 subjects, and no lymphodepletion regimen was given.

The depletion of lymphocytes including regulatory T cells by chemotherapy such as cyclophosphamide can make some cytokines (e.g. IL-7, IL-15) work better in antitumor activity [15]. This hypothesis has been proved in the regular therapy of patients with refractory metastatic melanoma. It was reported that the application of cyclophosphamide (60 mg/kg × 2 days) and fludarabine (25 mg/m2 × 2 days) to eliminate lymphocytes in myeloma patients before adoptive T-cell infusion improved the antitumor efficacy of adoptive T cells [16,17].

Although many subjects experienced CRS, these side effects resolved after the administration of corticosteroids or the anti-IL-6R antibody, tocilizumab. According to most recent studies, CAR-T cells targeting CD19 can bring very good clinical effects to patients. As shown in Maude et al.’s clinical trial, 30 patients with relapsed ALL were treated with CAR-T cells targeting CD19; 90% of patients achieved complete response, with 67% of patients attaining event-free survival in 6 months [18]. In the present prospective phase 1 study, we explored the safety and potential efficacy of CAR-GPC3 T-cell therapy in adult Chinese patients with advanced GPC3+HCC.

## Study drug

The study drug in this trial is the CAR T cells targeting GPC3, named CAR-GPC3 T cells. The clinical grade viral vector is developed and manufactured at CARsgen Therapeutics. The detailed manufacturing process is shown in Figure 1.2‑1. The cells were cryopreserved and stored in a liquid nitrogen tank after production completed.



Figure ‑ The manufacturing process for CAR-GPC3 T cells

Notes: PBMC, peripheral blood mononuclear cell; FCM, flow cytometry.

## Benefits and risks

Subjects in this study will be administered with genetically engineered autologous T cells. Although there was one fatal report, most recent clinical trials showed manageable safety profiles for CAR-T cells. Many subjects experienced CRS, and these side effects resolved after the administration of corticosteroids or the anti-IL-6R antibody, tocilizumab. According to most recent studies, CAR-T cells targeting CD19 showed significant clinical benefits to the patients in most of the studies. As shown in Maude et al.'s clinical trial, 30 patients with relapsed acute lymphocytic leukemia were treated with CAR-T cells targeting CD19; 90% of patients achieved complete response, with 67% of patients attaining event-free survival in 6 months [18].

# Objectives

## Primary objective

To observe and identify the safety, tolerability and pharmacokinetics of CAR-GPC3 T cells in the patients with hepatocellular carcinoma.

## Secondary objective

To observe the efficacy of CAR-GPC3 T cells in the treatment of hepatocellular carcinoma by the following parameters:

* Progression-free survival (PFS)
* Disease control rate (DCR) and objective response rate (ORR)
* Overall survival (OS)

# Study design and plan

## Overall design

This study is a single-arm, open-label trial. It is aimed to confirm the safety, tolerability and implantation potential of CAR-GPC3 T cells in the treatment of patients with GPC3-positive hepatocellular carcinoma. The detailed study procedure is shown in [Table 3.1‑1](#Table_3_1_1).

The subjects who enrolled in this study must have positive GPC3 expression in tumor tissues. Large numbers of autologous peripheral blood mononuclear cells (PBMCs) need to be obtained from the patients. The PBMCs will be transduced with the CAR-GPC3 lentivirus vectors and expanded in vitro to produce a large number of CAR-GPC3 T cells and cryopreserve them for future use. Tumor burden should be re-evaluated after lymphodepletion, and CAR-GPC3 T cells will be thawed at bedside for infusion.

Patients will undergo lymphodepletion prior to the administration of the study drug. For additional treatment courses, the investigator will decide if lymphodepletion is needed according to the clinical condition of the subject.

Intravenous administration for hepatocellular carcinoma,

① Intra-patient dose-escalation trial: the first 3-6 subjects will receive self-dose escalation. The first subject will receive ≤ 1.4 × 106 cells/kg as an initial dose and will be observed for at least 24 hours. If no DLT occurs and the investigator considers it necessary, the increased dose will be given at the interval of 1-2 days for 1-2 times, with the dose the dosing schedule details determined by the investigator. The above described is considered 1 treatment course.

After the last infusion of a single course, if there is no DLT during the observation period of at least 4 weeks, and the investigator considers it necessary, more treatment courses can be given, with the dose the dosing schedule details determined by the investigator. The dose for the second and third subjects will be escalated according to the safety and efficacy results for the first subject.

② “3+3” dose-escalation trial:

Based on the results of the intra-patient dose-escalation trial, the subsequent subjects will start from one dose level that has been validated as safe. At least 4-6 dose levels will be tested according to the infusion amount per body weight during every treatment course, with 3-6 subjects per level (Figure 3.1‑1).

For each subject, after the last infusion of a single course, if there is no DLT during the observation period of at least 4 weeks, and the investigator considers it necessary, more treatment courses can be given, with the dose the dosing schedule details determined by the investigator. The observation period will last from the first dose until 2 years after the first infusion.

The study dose will start from one dose level (cell numbers per body weight) that has been validated as safe, then escalate with 3 subjects per dose level; if no DLT occurs, investigators will proceed to the next dose level. If 2 or more subjects experience DLT at one level, the previous level will be defined maximum tolerated dose (MTD). If 1 case of DLT occurs at one level, 3 more subjects will be enrolled, and if there is no DLT for these 3 subjects, the study will proceed to next dose level. If 1 or more of these 3 subjects experience DLT at this dose level, the previous dose level will be defined MTD. Finally, the highest dose level for which the percentage of subjects with DLT is ≤1/6 will be defined as MTD.

The addition of treatment courses:

If the subject is considered to have possibly benefited from cell therapy according to the investigator’s judgment, one or more treatment courses can be added.

For additional courses, the investigator will decide if lymphodepletion is needed according to the clinical condition of the subject.

If more courses are added, baseline and follow-up schedule will not be influenced.

Lymphodepletion regimen for CAR-GPC3 T cells:

The lymphodepletion regimen will be given at Day-5 to Day-2 before the infusion of CAR-GPC3 T cells, which can improve the proliferation and survival of T cells in vivo; each subject will receive the lymphodepletion regimen before infusion according to the following schedule, which will be adjusted by the investigator according to the patient’s details.

Lymphodepletion regimen, comprising fludarabine combined with cyclophosphamide:

* Fludarabine, 20 to 25 mg/m2/day × 4 days
* Cyclophosphamide, 500 mg/m2/day × 2 days

Within 4 weeks after infusion, the safety, implantation and survival of CAR-GPC3 T cells will be evaluated by q-PCR test (See [Table 7.1-1](#Table_7_1_1) for details). At various time points after infusion, T cell subsets including CAR-GPC3 T cells will be examined and compared with the baseline sample.

Table ‑ Study flow chart

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Screening Period | | Therapy period | | | | | | | | | | | | | Follow-up within first 6 months | | | | | | | | Follow-up within 6-12 months | | | Follow-up within 2 years | |
|  | |  | W0 | | | | | | | W1 | | | | W2 | First 6 months | | | | | | | | Between 6 to 12 months | | | 1 -2 years | |
|  | D0  base line | D1 | | D0 | | D1 | | D3 | | D7 | | D13 | W3 | W4 | W6 | W8 | W12 | W16 | W20 | W24 | W28 | W40 | W52 | W78 | W104 |
| D-22 to  Pre-LD | | LD |  | ±1 day | |  | | ±1 day | | ±1 day | | ±2 days | | ±2 days | ±2 days | ±2 days | ±7 days | ±7 days | ±7 days | ±7 days | ±7 days | ±7 days | ±7 days | ±14 days | ±14 days | ±14 days | ±14 days |
| ICF  GPC3 testing\*  Screening⁑  Blood collection and culture | | 1-4 days | First CAR T infusion |  | | Second and/or third CAR T infusion | |  | |  | |  | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Blood testing | X | X | X | | X |  | X | | X | | X | | X | | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Imaging | X |  | X | |  |  |  | |  | |  | |  | |  | X |  |  | X |  | X |  | X | X | X | X | X |

D = day; ICF = informed consent form signed; LD = lymphodepletion; W = week.

\*Screening fails if the pathological examination does not find GPC3 positivity

⁑Screening fails if the eligibility criteria are not met

1. For GPC3 IHC screening, both archived tumor tissues or fresh samples collected within the previous 6 months are accepted.
2. For subjects who are HbsAg positive, HBV titer should be monitored in the follow-up visits; for subjects with HbsAg negative, the test of full panel for hepatitis B and HBV copy numbers are not required in the follow-ups.
3. Before lymphodepletion, during lymphodepletion, and within 1 week after lymphodepletion, T lymphocyte subsets and absolute counting numbers will be monitored.
4. During the screening period, the imaging results within 1 month of inform consent is acceptable, but imaging examination must be performed at baseline.
5. When more treatment courses are required, peripheral blood should be re-collected when the remaining CAR-T cells are not enough for the dosage.
6. When more treatment courses are added, the investigator will judge if lymphodepletion should be repeated or not.
7. Collect blood samples until any 2 successive test results are CAR-T cell negative.
8. During each treatment course, T-cell infusion can be divided into 3 times at most, with an interval of 1 day, and the subject should be followed on D1 after each infusion; the subject should be followed on D7 and D13 after the last infusion of each course.

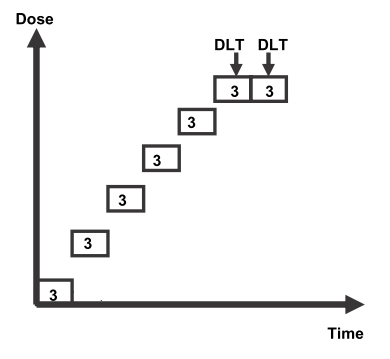


Figure ‑ Dose escalation flow chart

## Study evaluation indicators

### Primary endpoint

**Safety and tolerability endpoints**

Treatment-related adverse events, defined as laboratory toxicities and clinical events possibly related or related to treatment that occur within 24 weeks after cell infusion, including infusion-related toxicities and any CAR-GPC3 T-cell–related toxicities. Including but not limited to:

* Fever
* Chill
* Nausea, vomiting and various symptoms related to stomach discomfort
* Fatigue
* Hypotension
* Respiratory distress
* Tumor lysis syndrome
* Cytokine release syndrome
* Neutrophil decrease, platelet decrease
* Liver and kidney dysfunction
* Other toxicities

The severity of adverse events will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03.

**Persistence endpoint:**

The persistence of CAR-GPC3 T cells in vivo, defined as the period of persistent survival of infused CAR-GPC3 T cells in vivo. The “persistence endpoint” of the cells is the copy number of CAR-GPC3 DNA in peripheral blood that is detected by q-PCR at each visit point from 4 weeks after the first implantation until any two consecutive test results are negative.

### Secondary endpoint

The antitumor response of CAR-GPC3 T cells infusion:

* Objective response rate (ORR)
* Progression-free survival (PFS)
* Time to progression (TTP)
* Overall survival (OS)

# Study population

Before any study-specific activities/procedures, the appropriate written informed consent form (ICF) must be signed. All subjects shall meet all inclusion criteria and not meet any exclusion criteria.

## Inclusion Criteria

1. Patients between 18- and 70-years-old who have refractory HCC relapsed at least twice within two years, and without effective treatment methods
2. A liver cancer tissue sample tests positive for GPC3 expression by immunohistochemistry (IHC)
3. Estimated life expectancy > 12 weeks
4. At least one measurable tumor lesion (≥ 10 mm)
5. Liver cirrhosis condition: Child-Pugh A, or B with a score of 7
6. The longest diameter of single tumor < 5cm; the number of multiple tumors < 10; accumulated tumor volume < ¼ liver volume
7. Controllable lung metastasis (number of metastatic lesions < 6, longest diameter of lesions < 5 cm)
8. Eastern Cooperative Oncology Group (ECOG) score is 0 to 1, or KPS score >70
9. Have venous accesses for leukapheresis or blood sampling, and have no other contraindications for blood collection;
10. Hematology: WBC ≥ 2.5×109/L, PLT ≥ 60×109/L, Hb ≥ 9.0 g/dL, MID ≥ 1.0×109/L, LY ≥ 0.4×109/L
11. Biochemistry: serum Alb ≥ 30 g/L, serum lipase and amylase ≤ 1.5 ULN, serum creatinine ≤ 1.5 ULN, ALT ≤ 5 ULN, AST ≤5 ULN, total bilirubin ≤ 2.5 ULN;
12. Coagulation: prolonged prothrombin time ≤ 4 s
13. Be able to understand and sign the informed consent

All laboratory results should be stably within the ranges above without continuous supportive treatments.

## Exclusion Criteria

Patients are excluded from the study if any of the following criteria apply:

1. Diffuse hepatocellular carcinoma
2. Positivity of T cells after transduction < 10%, or expansion of T cells in response to αCD3/CD2 stimulation < 5-fold, which is considered as product manufacture failure.
3. Pregnant or breastfeeding women
4. HIV positive
5. Any uncontrollable active infection
6. Current systemic use of steroids. Those who recently used or currently use inhaled steroids are not excluded
7. Allergic to immunotherapy and related drugs
8. Previous or present hepatic encephalopathy
9. The patient has active ascites that requires treatment
10. Active heart disease requiring treatment or poorly controlled high blood pressure determined by the investigator
11. Unstable or active ulcers and gastrointestinal hemorrhage
12. Patient with a history of organ transplantation or waiting for organ transplantation
13. Hyponatremia, with serum sodium < 125 mmol/L
14. Baseline serum potassium < 3.5 mmol/L (supplement of potassium is allowed, and patients who recover to above this limit are not excluded)
15. Patient who requires anti-coagulation therapy (e.g. warfarin or heparin)
16. Patient who requires long-term anti-platelet therapy (aspirin with dose > 300 mg/day; clopidogrel with does > 75 mg/day)
17. Patient who received chemotherapy, radiotherapy, targeted antitumor drugs or interventional therapy for the study disease within 4 weeks prior to leukapheresis
18. Other concurrent antitumor therapies
19. According to the investigators’ evaluation, patients who are unable or unwilling to comply with the requirements of the study protocol.

## Subject withdrawal and replacement criteria

At any time, a patient can voluntarily withdraw from the study. For safety, behavioral or managerial reasons, the investigator and sponsor can require a subject to withdraw from the study at any time according to their judgment. If a subject fails to complete the visits as scheduled, the investigator must make every possible contact with the subject. If possible, under any situation, the investigator should make every effort to record the subject’s treatment results. The investigator should inquire about the subject’s the reason for withdrawal, request that the subject to return to study site for the last visit if possible and follow any unresolved adverse events as far as possible.

1. If confirmed progressive disease (PD) in accordance with RECIST1.1 criteria is observed, all study treatment should be discontinued;
2. If there are intolerable adverse events or deterioration of disease or health condition that make the subject unsuitable for trial continuously according to the investigator’s judgment;
3. The subject uses other antitumor treatments prohibited in this study, including but not limited to radiotherapy, chemotherapy, and surgical treatment;
4. The subject can voluntarily withdraw the consent from the study;
5. Pregnancy;
6. CAR-T manufacture failure

For subjects who withdraw from the study, the relevant data should be collected continuously except if they refuse subsequent data collection. The data are no longer to be collected for the below situations:

* Lost follow-ups
* Death
* Termination of clinical trial proposed by the investigator, sponsor, ethics committee or regulatory authority.

Withdrawn subjects will not be replaced.

## Sample size and number of study sites

This study plans to enroll 20 subjects in a single study site.

## Subjects identification

In this study, each subject will receive a unique screening number. Once the screening number is assigned to the subject, it cannot be assigned to another subject for reuse even if the subject withdraws from the study in advance. Each eligible subject will receive a unique enrollment number before infusion.

# Study Drug

## Properties of study drug

CAR-GPC3 T cells are genetically engineered autologous T lymphocytes transduced with the lentiviral vectors expressing CAR-GPC3 transgene. CAR-GPC3 T cells will be suspended in the cryopreservation medium and cryopreserved in separate bags. Each bag will contain 10-50 mL of 1×108 ~ 2×109 CAR-GPC3 T cells. The cryopreservation medium consists of the following solution (%/v/v): 31.25% multiple electrolytes injection, 31.25% of 5% glucose solution, 0.45% sodium chloride, no more than 7.5% DMSO, 1% dextran 40, 5% human serum albumin.

## Preparation and administration

Preparation

CAR-GPC3 T cells will be prepared in a clinical grade manufacture facility and will not be released until they meet all release criteria for infusion (including cell viability, CAR T cell positivity, CAR T cell activity in vitro, required tests, and magnetic bead residue, etc.). The cell infusion bag for CAR-GPC3 T cells is considered as the first packaging bag that is labeled and the outer layer with a vacuum packaging bag. After packaging, the products are released by the quality control and quality assurance department of CARsgen Therapeutics and preserved in a liquid nitrogen tank; before infusion the cells will be transported to the study site on dry ice or in liquid nitrogen, and thawed at bedside immediately before infusion.

Thawing of cells at the clinical study site

When the study site receives CAR-GPC3 T cells, the preservation temperature and transportation temperature (including deviation) of CAR-GPC3 T cells, the appearance of the package bag and its integrity should be recorded. Cryopreserved cells will be delivered to the patient’s bedside on dry ice or in liquid nitrogen. The cells will be thawed in a water bath at 37°C to 38°C. Thawing procedure is as below: completely immerse the second vacuum packaging bag into the warm water bath, once per bag, and gently rub the bag until the cells are just thawed and no frozen clumps were left; The cells will be ready for infusion. If the second packaging bag (protective bag) of CAR-GPC3 T cells is damaged or leaky, but the first packaging bag is carefully checked and confirmed to be intact, the product can still be infused. If the infusion bag is broken, the cells should not be infused, and the bag should be destroyed.

## Administration method:

The CAR T cells will be intravenously infused at a rate of 5 mL/min through a blood transfusion apparatus at the study center. One or two bags of CAR-GPC3 T cells will be sent to the bedside after thawing. Each bag will be labeled with “only for autologous T cell infusion” and two unique identifiers such as the subject’s initials and study number. Before infusion, two persons should independently verify the all the subject’s information to confirm the product is correctly matched with the subject.

If any infusion-related reactions (IRR) occur, the infusion can be suspended, or the infusion speed can be reduced. The specific treatment is decided by the investigators. Any changes to the infusion start and end time will be recorded in the source document.

## Storage, distribution and return

### Storage

Long-term cryopreserved CAR-GPC3 T cells will be kept in a liquid nitrogen tank at CARsgen Therapeutics. Part of CAR-GPC3 T cells can be transported to the study site under the intended temperature before infusion, and the temperature should be recorded.

### Preparation of study drug

Before infusion, cryopreserved CAR-GPC3 T cells will be transported in liquid nitrogen to the patient’s bedside at the study site. The cells will be thawed in the water bath at 37oC – 38oC, one bag at a time; the bag should be rubbed gently until the cells are just thawed and there are no frozen clumps were left in the bag. If the packaging bag of CAR-GPC3 T cells is found broken or leaking, the infusion should be stopped, and the product will be returned to CARsgen Therapeutics.

### Return and destruction of study drug

There are multiple reasons for CAR-GPC3 T cells to return to CARsgen Therapeutics, including but not limited to 1) A product with a wrong label; 2) The patient is under conditions unsuitable for the infusion; 3) The patient refuses the infusion. Any unused CAR-GPC3 T cells will be returned to CARsgen Therapeutics and destroyed by autoclave. In addition, after infusion, the packaging bag for CAR-GPC3 T cells will be destroyed at the study site in accordance with the product destruction standard operating procedure (SOP) developed by CARsgen Therapeutics.

## Drug Compliance

The subjects will be given the study drug at the site by the investigator to ensure compliance.

## Concomitant medications and prohibited medications

This study allows the use of drugs solely for supportive treatment (such as antipyretics, analgesics, drugs for first-aid treatment, AE treatment, etc.) and the use of any appropriate treatment regimens in addition to treatment according to the study protocol if the patient has progressive disease, withdraws from the study, etc.

The patient is not allowed to receive any other medications, other local or systemic chemotherapy and radiotherapy, and other drugs with antitumor indications (including herbals). Immune enhancers should not be used during treatment with GPC3 T cells because they can enhance the phagocytosis and killing of CAR-T cells by immune system.

Any concomitant drugs should be documented with the following information: generic name, route of administration, start date, end date, and indications. Dosage adjustments or any changes in treatment regimens for concomitant medication should be recorded. At each visit, the investigator will ask the subjects about their use of concomitant drugs.

# Assessment parameters and methods

## Tumor response evaluation

The baseline examination for this study is conducted after lymphodepletion and before CAR-GPC3 T cell infusion, which includes imaging of the abdomen (including magnetic resonance imaging, CT, etc.) or other locations (in case of metastasis) and tumor marker examinations should be performed. Imaging and tumor marker examinations are performed every 8 weeks between Weeks 4 and 24, and every 3 months from Week 28 to the end of the first year; and every 6 months from the end of the first year to the end of the second year.

Imaging evaluation is performed according to RECIST1.1; images of intrahepatic lesions should be enhanced in the arterial phase after angiography and can be measured repeatedly and steadily until confirmed progressive disease (PD) is determined.

## Implantation endpoint of CAR-GPC3 T cells

The “implantation endpoint” of the cells is the CAR-GPC3 DNA copy number in peripheral blood that is detected by q-PCR at each visit point from 4 weeks after first implantation until any two consecutive test results are negative, recorded as the persistent survival of CAR-GPC3 T cells.

## Safety parameters

### Adverse events

#### Collection of adverse events

The investigator is responsible for collecting all AEs that occur during the study, including those reported by the subjects themselves, observed or learned of by the investigator by asking questions such as “How have you felt since your last clinic visit?”.

#### Definition of AE

AE refers to any adverse medical event that occurs after a patient or clinical subject receives a drug and does not necessarily have a causal relationship with the treatment. Therefore, AE can be any adverse or unforeseen sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of the study drug, regardless of whether it is related to the study drug.

All AEs occurring during the study, regardless of their source (e.g. physical examination, laboratory examination, electrocardiogram (ECG), voluntarily reported), should be recorded in electronic data capture (EDC).

Adverse reaction refers to harmful reaction occurring when drug is normally used which is not related to medication purpose or incidental. It does not refer to the reaction induced by unintentional or intentional overdose or induced by misuse.

Main adverse events include, but are not limited to:

* Abnormal examination results
* Clinically significant symptoms and signs
* Abnormal findings in physical examination
* Allergy
* Drug abuse
* Drug dependence
* Drug overdose
* Drug discontinuation
* Drug interactions
* Pregnancy exposure
* Breastfeeding exposure
* Medication errors

Symptoms and signs associated with the baseline phase of the tumor should be recorded as adverse events if the severity or frequency of the disease increases during the study. However, progressive disease assessed by radiological measurements of cancer lesions should not be reported as adverse events.

The type, incidence, severity, time, seriousness and causality of the adverse events are categorized per CTCAE 4.03.

##### Cytokine release syndrome

Cytokine release syndrome (CRS) is common cytokine-related toxicity in CAR T therapy, which is non-antibody-specific toxicity caused by a high level of immune activation, and often occurs within one month after infusion. All expected adverse events can be also CRS-relevant symptoms.

The investigator will manage the CRS according to the guidelines of medical practice.

#### The evaluation of adverse events

The investigator will evaluate AEs based on the following criteria.

##### Serious adverse event

Serious adverse events refer to any adverse events under any dose:

* Resulting in death;
* Life-threatening: meaning the subject is at risk of death during an adverse event, rather than assuming that a more serious event may lead to death;
* Requires or prolongs hospitalization;
* Results in permanent or significant disability/incapacity;
* Congenital anomalies/birth defects;
* Significant medical events that may not immediately endanger life, lead to death, or require hospitalization, but may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above. Examples of such incidents are intensive treatment of anaphylactic tracheal spasm in the emergency room or at home; hematologic disease or convulsions not requiring hospitalization; continuously progressive drug dependence, or drug abuse.

Clinical and scientific judgment is needed when judging the severity of the event and whether an expedited report is needed.

##### Severity

The investigator should judge the severity of AE according to the CTCAE. The severity grading per CTCAE v4.03:

Grade 1: Mild; no or mild symptoms; clinical or diagnostic detection; no need for treatment.

Grade 2: Moderate; minimal, local or noninvasive treatment indications; limited age-appropriate activities of daily living (ADL); instrumental activities of daily living refer to preparing meals, grocery shopping, using telephone, money management, and so on.

Grade 3: Severe or important medical significance, but will not immediately endanger life; indications of hospitalization or extended hospital stay; disability; limited self-care activities of daily living; self-care activities of daily living refer to bathing, dressing and undressing, eating, going to the restroom, taking medications, and not being bedridden.

Grade 4: Life-threatening event and requiring emergency treatment.

Grade 5: AE-related deaths.

##### Causality

The investigator should assess the possible relationships between AEs and the study drugs against the following criteria and record them in the EDC system.

Definitely related: There is a plausible temporal relationship between the onset of the adverse event and administration of study treatment; the adverse event conforms to the known reactions of the study drug; it is relieved after the reduction and discontinuation of the drug and occurs once again after the drug is used again;

Possibly related: There is a plausible temporal relationship between the onset of the adverse event and administration of study treatment; the adverse event conforms to the known reactions of the study drug; the subject’s clinical status or other treatments may also produce the adverse event;

Possibly unrelated: There is no plausible temporal relationship between the onset of the adverse event and administration of study treatment; the adverse event does not conform to the known reactions of the study drug; the subject’s clinical status or other treatments may also produce the adverse event;

Unrelated: There is no plausible temporal relationship between the onset of the adverse event and administration of study treatment; the adverse event conforms to the known reactions of non-study drugs; the subject’s clinical status or other treatments may also produce the adverse event; the adverse event abates or resolves upon discontinuation of study treatment and, reappears upon reuse;

Unable to determine: There is no definite temporal relationship between the onset of the adverse event and administration of study treatment; the adverse event is similar to the known reactions of the study drug; other drugs may also produce the adverse event.

Those definitely related, possibly related, and unable to determine are judged as adverse drug reactions, and their incidence will be calculated.

SAE will also be evaluated and recorded based on the above criteria.

#### Recording of adverse events

All AEs occurring from when the inform consent is signed to discontinuation (including switching from on-site visit to telephone visit) should be reported. The investigator should report the events occurring after study completion to the sponsor if they are believed to be related to the study drug. All AEs should be recorded, regardless of their causality to the study drug. The record of all AEs should include a brief description of medical terms, date and time of onset, date and time of recovery, severity, treatment required or not, causal relationship of AEs to the study drug, action taken and seriousness.

#### Recording of serious adverse events

All SAEs that occur during the study (starting from the initial use of the drug specified in the protocol to the time when a subject withdraws from the study), no matter whether they are related to the study drug, should be reported to the drug safety contact within 24 hours after the knowledge of it. See the list of the study-related personnel for the contact information.

The investigator should report the events occurring after study completion to the sponsor if they are believed to be related to study drug.

An SAE reported for the first time should contain at least the following information:

* The name of the event reporter (such as the name and address of the investigator)
* Subject information (screening / random number, name acronym; no disclosure of the full name of the subjects)
* Protocol number
* Descriptions of SAE
* Causality evaluation, if possible

Complete all information in the SAE report as much as possible in the initial report and send the completed SAE report to the sponsor's drug safety contact. In addition, the SAE must be recorded and reported to the Ethics Committee to be put on record.

#### Follow up of adverse events

All AEs experienced by the subjects, whether or not they were related to the drug, should be followed up until the AE subsides, abnormal laboratory tests return to baseline values or stable levels acceptable to researchers and medical inspectors, until the observed changes could be reasonably explained, or until the subject is lost or dies.

#### Pregnancy

Pregnancy is not considered to be AE. Pregnancies that occur between the administration of the study drug and the completion of the study should be followed up until the end/termination of the pregnancy and recorded. The investigator must report the pregnancy within 24 hours after learning of it.

#### Laboratory parameters

In this study, laboratory testing will be performed at the study site. Sample collection, data analysis and evaluation will follow the requirements of local laboratories.

Any abnormal laboratory test results with clinical significance should be reported as AEs, except for those noted during the screening period. If necessary, blood samples used to assess safety may be collected repeatedly. Clinically significant results conform to one or more of the following situations:

* The results are accompanied by symptoms;
* Lead to changes in study medication (e.g. dose change, interruption or permanent discontinuation);
* Require other diagnostic examinations or medical/surgical interventions;
* Require significant increase in concomitant drugs or other treatments;
* The investigator or the sponsor thinks the examination result should be reported as an adverse event.

An abnormal test result will not be defined as an adverse event if it is simply to repeat the test and does not meet any of the above criteria. Any abnormal test result should not be reported as an adverse event if it is judged to be an error.

### Vital signs

The vital signs will be evaluated according to the [schedule of events](#Table_7_1_1):

* Blood pressure (systolic and diastolic blood pressure; mmHg)
* Heart rate (beats per minute)
* Body temperature (°C)
* Respiratory rate (breaths per minute)

Vital sign examinations should be conducted after the subject rests for 5 minutes in the supine position. The investigator will assess whether the abnormal measurements are clinically significant or not.

### Electrocardiogram

The 12-lead ECG will be conducted according to the [schedule of events](#Table_7_1_1). Subjects should rest in a supine position for 5 minutes and then the ECG should be conducted while they are awake. If the subject is asleep, the ECG should be performed 5 minutes after he/she is awakened and changes position. All ECGs should be evaluated by qualified doctors. Any clinically significant findings (e.g. QTc interval > 500 ms) should be recorded as AE by the investigator.

### Physical examination

The following physical systems will be examined according to the study flow chart: general condition, head (ears, nose, throat, eyes), respiratory system, abdomen, urogenital system, skeletal muscle system, nervous system, lymphatic system and skin.

## Demographic information and baseline characteristics

Demographic information and baseline characteristics only contain information gathered/evaluated during the screening period/at baseline.

### Demographic information of the subjects

Demographic information of the subjects includes:

* Age
* Height
* Weight
* Sex
* Body surface area

### Medical history

The medical history of the subjects, including past medical history and current medical history, should be recorded. The coding of medical history is referred to Section 9.4.

### Past and concomitant medication

Past medication and concomitant medication will be described in Section 5.6.

# Schedule of events

## Schedule of events

The detailed schedule of study visits is shown in Table 7.1‑1.

Table ‑ Schedule of events

|  | **Screening period** | **Treatment period** | | | | | | | | **Follow-up period** | | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Visit Week** |  | **LD period** | | **W1~W2** | | | | | | **W3** | **W4** | **W6** | **W8** | **W12** | **W16** | **W20** | **W24** | **W28** | **W40** | **W52** | **W78** | **W104** |
| **Visit Day** | **D-22~ pre-LD** | **pre- LD8** | **LD** | **D-2~ D0 10** | **D0** | **D111** | **D3** | **D7** | **D13** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Window** |  |  | **1-4 days** | **baseline** |  | **±1** | **±1** | **±2** | **±2** | **±2** | **±2** | **±7** | **±7** | **±7** | **±7** | **±7** | **±7** | **±7** | **±14** | **±14** | **±14** | **±14** |
| ICF | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Eligibility criteria | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Pathological test 1 | X |  |  | \* |  |  | \* | \* |  |  | \* |  | \* |  |  |  |  |  |  |  |  |  |
| Demographic data | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Medical history and treatment history | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Concomitant disease and concomitant medication | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Pregnancy test (WOCBP) | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Five markers of hepatitis B, HBV-DNA2 | X |  |  | \* |  |  |  |  |  |  | \* |  |  | \* |  |  |  | \* | \* | \* | \* | \* |
| HIV | X |  |  |  |  |  |  |  |  |  |  |  |  | X |  |  | X |  | X | X | X | X |
| HCV, syphilis and other infectious disease | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Physical examination | X |  |  | X |  |  |  |  |  | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Temperature | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Heart rate, respiratory, blood pressure | X | X | X9 | X | X12 |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| ECOG | X |  |  |  |  |  |  |  |  |  |  |  | X | X | X | X | X | X | X | X | X | X |
| Blood test | X | \* | X9 | X |  | \* | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Lymphocyte subpopulation3 | X | X | X9 | X |  | \* | \* | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Urinalysis | X | \* |  | X |  |  |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Hepatic and renal function | X | \* |  | X |  |  |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Electrolyte | X | \* |  | X |  |  |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Coagulation | X | \* |  | X |  |  |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| CRP | X | \* | X9 | X |  | \* | X | X | X | X | X |  |  |  |  |  |  |  |  |  |  |  |
| Tumor markers | \* |  |  | X |  |  |  | X | X | X | X | \* | X | X | X | X | X | X | X | X | X | X |
| ECG | X | \* |  | X |  |  |  | X |  |  | X |  | X | X | X | X | X | X | X | X | X | X |
| Imaging4 | X |  |  | X |  |  |  |  |  |  | X |  | \* | X | \* | X | \* | X | X | X | X | X |
| Peripheral blood apheresis5 | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Lymphodepletion6 |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| CAR T cells infusion |  |  |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Cytokines and CAR copies7 |  |  |  | X |  | X | \* | \* | \* | \* | \* | \* | \* | \* | \* | \* | \* | \* | \* | \* | \* | \* |
| AEs and concomitant medication | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |

X: mandatory test

\*: optional test

AEs = adverse events; CAR = chimeric antigen receptor; CRP = C-reactive protein; D = day; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; ICF = informed consent form; LD = lymphodepletion; W = week; WOCBP = women of childbearing potential

1. During the screening, both of the archived tumor tissue samples or fresh samples within 6 months before screening are accepted. The liver tissue biopsy will be performed when the investigator considers it necessary at visit on D3, D7, W4 and W8 after the last CAR-T cells infusion., and GPC3 and CAR-T cell expression will be evaluated.
2. For subjects with HBsAg positive, HBV titer should be monitored in the follow-ups; for subjects with HBsAg negative, the test of full panel for hepatitis B and HBV titer are not required in the follow-ups.
3. Before lymphodepletion, during lymphodepletion, and within 1 week after lymphodepletion, T lymphocyte subsets and absolute counting numbers will be monitored.
4. During screening period, the imaging examination results within 1 month of inform consent is acceptable, but imaging examination must be performed at baseline.
5. When more treatment courses are added, if remaining CAR-T cells are not enough, the peripheral blood should be re-collected when the remaining CAR-T cells are not enough for the dosage.
6. When more treatment courses are added, the investigator will judge if lymphodepletion should be repeated or not.
7. Collect blood samples until any 2 successive test results are CAR-T cell negative; cytokines test should include IL-6, TNF-alpha, IFN-γ, IFN-β, IL-15, IL‑10.
8. The examinations prior to lymphodepletion should be performed within 48 hours before lymphodepletion; if the screening data is obtained within 7 days before the lymphodepletion, repeat examinations will be unnecessary.
9. The examination in the middle of lymphodepletion should be performed after second dosing of chemotherapy, but before third dosing.
10. Baseline examination can be performed either on the same day of infusion or on the day before infusion. The examinations done at baseline are not required to be repeated on the day of infusion.
11. During each treatment course, T-cell infusion can be divided into 3 times at most, with an interval of 1 day, and the subject should be followed on D1 after each infusion. Blood examination and T lymphocyte subset detection should be performed on D1, and C-reactive protein should be monitored when the subject had fever of over 38°C or obvious toxicity; the subject should be followed on D7 and D13 after the last infusion of each course.
12. The vital signs should be monitored within 1 hour before infusion and after infusion, every 15 minutes.

## Visit procedure

All the subjects should complete the following process.

### Screening period (D-22~pre-lymphodepletion)

All screened subjects should sign informed consent form before any study-related procedure begins. The screening will be carried out from Day -22 to Day -6 day before T-cell therapy.

After signing the informed consent form, the patients will receive routine assessment of their hepatocellular carcinoma stage, including:

1. Medical history (including previous antineoplastic treatment, and current medications)
2. Physical examination and ECOG score
3. Vital signs, including heart rate, respiration, blood pressure and body temperature, and body weight
4. Examine the medication history taken by the subjects and the adverse reactions that occurred
5. Assessment of patients with hepatocellular carcinoma: Assessment of tumor by CT or MRI. Evaluation is performed according to RECIST1.1 criteria
6. Electrocardiogram

Tumor tissue samples: Tumor tissue samples will be collected to analyze the expression of GPC3, which will be performed at the central laboratory of CARsgen Therapeutics. The pathological tissues that meet the testing requirements include formalin preserved fresh samples, paraffin blocks, blank specimens, etc.

Blood sample collection:

1. Routine blood test and peripheral blood classification examination (complete blood count [CBC])
2. Biochemistry examination: liver function, renal function, amylase, lipase, electrolytes
3. Coagulation examination
4. Detection of tumor marker
5. Detection of HIV infection (AIDS), hepatitis B and hepatitis C, syphilis and other infectious diseases
6. Women of childbearing potential (WOCBP) should receive a pregnancy test, and negative results should be obtained within 2 weeks before cell infusion. If the interval is expected to be 2 weeks, the test should be repeated before lymphodepletion.
7. Urine sample collection: routine urine test, urine pregnancy test (if blood pregnancy test is not accepted by the subject)

If all above inclusion criteria are met and no exclusion criteria are met, the blood sample will be collected; at least 1.0×109 leukocytes should be collected for leukapheresis or at least 200 mL peripheral whole blood should be collected, and transported to the laboratory of CARsgen Therapeutics at the temperature between 2°C and 8°C. The sample will be used for the isolation of at least 1.0 × 108 PBMC by Ficoll technique. Initial PBMCs will also be cryopreserved for retrospective analysis and research required by the China Food and Drug Administration (CFDA).

### Lymphodepletion

In order to improve the survival, stability, and antitumor function of the transduced autologous CAR-GPC3 T cells in the patients, the lymphodepletion regimen is applied before infusion. Lymphodepletion will be conducted 4-9 days before CAR-GPC3 T-cell infusion, and the regimen is initially designed as follows:

Fludarabine + cyclophosphamide regimen (fludarabine 20~25 mg/m2/day ×4 days, cyclophosphamide 500 mg/m2/day ×2 days).

Fludarabine will be used in combination with cyclophosphamide on the first to second day of lymphodepletion, and fludarabine will be given on the third to fourth day.

Calculate the subject’s body surface area according to Stevenson’s formula: body surface area (m2) = 0.0061 × height (cm) + 0.0128 × weight (kg) - 0.1529

The detailed chemotherapy regimen will be chosen and given by the physician according to the subject's underlying disease and previous treatment. Before and after lymphodepletion, the physician is advised to closely monitor the blood through routine examination (closely observe the change of white blood cells and lymphocytes after lymphodepletion), serum biochemistry parameters and urination. In case of oliguria, please implement the annotated hydration and alkalization regimen, and document in the patient’s record. Clinicians are advised to carry out many protective measures for subjects, such as wearing masks and reducing changes of personnel, to minimize the patient’s exposure to opportunistic infections. The infusion of CAR T cells can be conducted 1 to 2 days after the subject completes the lymphodepletion regimen. CBC and C-reactive protein detection should be performed on Day -3 of lymphodepletion.

### Baseline measurement

The following examinations should be completed after lymphodepletion and before infusion, to provide the baseline of tumor burden measurement:

1. Physical examination;
2. Vital signs;
3. Imaging examination and tumor marker;
4. CBC, urinalysis, liver and kidney function, bleeding and coagulation time, electrolytes, hs‑CRP, electrocardiogram, and virus titer for HBsAg positive subjects;
5. Collection of serology sample, for the monitoring of cytokines and survival of CAR-GPC3 T cells. This detection will be conducted in the laboratory of CARsgen Therapeutics.

### Infusion (D0)

The infusion of CAR-GPC3 T cells will be initiated after baseline examination, with the infusion day recorded as Day 0. All subjects will receive lymphodepletion and CAR-GPC3 T cell infusion. CAR-GPC3 T cells are applied.

The first subject will receive ≤1.4 × 106 cells/kg as an initial split dose, followed by observation for at least 24 hours. If no DLT occurs and the investigator considers it necessary, the increased dose will be given at the interval of 1-2 days for 1-2 times, with the dose and dosing schedule details determined by the investigator. The above described is considered 1 treatment course.

After the last infusion of the single course, if there is no DLT during the observation period of at least 4 weeks, and the investigator considers it necessary, more treatment courses can be given, with the dose and dosing schedule details determined by the investigator. The dose for the second and third subjects could be escalated based on the safety and efficacy results for the first subject. Doses will be escalated in this way until the study proceeds into the “3+3” trial.

If the subject is considered to have possibly benefited from the T-cell therapy according to the investigator's judgment, one or more treatment courses can be added.

The vital signs should be monitored within 1 hour before infusion and after infusion, every 15 minutes.

The added courses with lymphodepletion will not change the scheduled visits.

### Observation within 2 weeks after infusion

* Pharmacodynamics samples should be collected on Day 1, Day 3 and Day 7 after last infusion, to examine cytokines and survival of CAR-GPC3 T cells. This detection will be conducted in the laboratory of CARsgen Therapeutics.
* Blood and urine routine examinations, liver and kidney function, electrolytes, bleeding and coagulation, hs-CRP, tumor markers and electrocardiogram should be examined on Day 1, Day 3 and Day 7 after last infusion of every treatment course, and tissue samples for pathological detection will be collected if feasible. Refer to the study procedure table for the specific testing items.
* Blood and urine routine examinations, liver and kidney function, electrolytes, bleeding and coagulation, hs-CRP, tumor markers and electrocardiogram should be examined 2 weeks after first infusion, and tissue samples for pathological detection will be collected if feasible. Refer to the study procedure table for the specific testing items.

### Follow-up period (W3 to W24 after infusion)

* From W3 to W24, the visits will be arranged every 1 week or 2 weeks or 4 weeks, i.e. The following tests should be performed at W3, W4, W8, W10, W12, W16, W20, W24:

Physical examination, vital signs, routine blood and urine examinations, liver and kidney function, bleeding and coagulation time, electrolytes, hs-CRP, imaging, tumor markers, and HIV. Pharmacodynamics samples will be collected for the monitoring of cytokines and survival of CAR-GPC3 T cells as determined in the laboratory of CARsgen Therapeutics. Refer to the [schedule of events table](#Table_7_1_1) for the specific testing items.

### Follow-up period (W28 to 2 years after infusion)

* From W28 to one and half a year after baseline, the visits will be arranged every 12 weeks or 3 months. After that, only one follow-up visit is required with the following examinations:

Physical examination, vital signs, routine blood and urine examinations, liver and kidney function, bleeding and coagulation time, electrolytes, hs-CRP, imaging, tumor markers, and HIV. Pharmacodynamics samples will be collected for the monitoring of cytokines and survival of CAR-GPC3 T cells as determined in the laboratory of CARsgen Therapeutics. Refer to the [schedule of events table](#Table_7_1_1) for the specific testing items.

The above visit contents and frequency can be increased according to clinical needs.

### Early withdrawal from the study and study completion visit

For subjects who withdraw from the study early and who complete the visits in 2 years, they should be contacted every 8 weeks by telephone, outpatient or mail, for the continuous collection of the following data:

The recording of all subsequent antitumor therapy, until death or study completion;

The recording of survival status, until death or study completion.

For subjects who withdraw from the study, the relevant data should be collected continuously except they refuse subsequent data collection. The data are no longer to be collected for the below situations:

* Lost follow-ups
* Death

Termination of clinical trial proposed by the investigator, sponsor, ethics committee or regulatory authority.

## Restrictions

### Medication restrictions

The patient is not allowed to receive any other medications, including other local or systemic chemotherapy and radiotherapy. Immune enhancers should not be used in the treatment with GPC3 T cells (since they can enhance the phagocytosis and killing of CAR-T cells by immune system).

### Other restrictions

Subjects are not allowed to donate sperm during the study and within 3 months after receiving the study drug.

Subjects must agree that they and their spouses/partners will use reliable contraceptives from the start of the study to study completion or provide evidence that the subjects are infertile.

# Statistical method

The statistical considerations summarized in this chapter outline the data analysis plan for this study, and the statistical methods are described in detail in a separate statistical analysis plan (SAP).

## Study population

### Subject enrollment

The number and percentage of subjects enrolled in each dose level and those who complete the study will be calculated. The reason for discontinuation before and after enrollment will be summarized.

### Protocol Deviation

All major deviations from the protocol will be listed by subject.

### Analysis set

Enrollment set: Including all subjects who signed ICF

Safety set (SS): Including all subjects who received study drug

Enrollment set will be used for the description and tabulation of subject enrollment, and Safety set will be used for all safety analysis.

Safety set will be used for demographic and baseline characteristics analysis.

## General consideration

SAS version 9.2 or above will be used for statistical analysis.

A list of all raw data, including all recorded data, and all computed data, will be generated. Continuous variables will be analyzed using descriptive statistics (e.g. number of cases, mean, median, standard deviation, minimum and maximum). Categorical variables will be analyzed by frequency table (frequency and percentage).

**Analysis and data processing:**

Definition of baseline assessment

Baseline assessment refers to the latest assessment before receiving the study drug.

Visit window

Assessments beyond the protocol’s permissible window will be presented in accordance with the investigator’s record on the Case Report Form (CRF).

Unscheduled assessment

Additional assessments (laboratory data or vital signs collected during unscheduled visits or during AE evaluation or processing) will be tabulated, but not summarized. If there are multiple laboratory examinations during a visit, the first valid examination value will be used as a summary and other values will be tabulated. Invalid laboratory data may not be used for analysis.

Missing value

Missing data for safety analysis will not be imputed.

If an abnormal value is found, it will be checked. If necessary, the sponsor will ask the investigator to correct it or identify it as an abnormal value.

## Demographic information, medical history, baseline characteristics and concomitant medications

The safety set will be used to summarize demographic information and baseline characteristics. Demographic information and baseline characteristic data, medical history and concomitant medications will be analyzed using descriptive statistics (number of cases [n], mean, standard deviation, median, minimum and maximum) or frequency tables.

The medical history will be coded according to the Medical Dictionary for Drug Regulatory Activities (MedDRA) for pharmaceutical administration; the concomitant medication will be coded according to WHO-DDE, which is classified by the Anatomical Therapeutic Chemical (ATC) classification system. The medical history and concomitant medications will be summarized in the safety set.

The version of the encoding dictionary will be described in CSR.

## Drug compliance

Treatment compliance will be analyzed using descriptive statistics (number of cases [n], mean, standard deviation, median, minimum and maximum) or frequency tables.

Drug compliance (%) will be calculated by actual dosage/ planned dosage × 100%.

## Implantation endpoint of CAR-GPC3 T cells

A list of all raw data, including all recorded data, and all computed data, will be generated. The implantation endpoint of CAR-GPC3 T cells will be analyzed using descriptive statistics (e.g. number of cases, mean, median, standard deviation, minimum and maximum).

## Safety analysis

All AEs will be collected by subjects in Safety Set. The number and percentage of subjects with treatment emergent adverse events (TEAEs) will be calculated by system organ class (SOC), preferred term (PT) and group. SAEs including death and TEAEs leading to discontinuation will be summarized.

Descriptive analysis of laboratory examinations, vital signs and echocardiography including left ventricular ejection fraction (LVEF) and their changes from baseline (if any) will be performed by group. Other safety parameters will be described in a similar way and tabulated by subjects.

## Interim analysis

One interim analysis will be conducted when 10 subjects have enrolled in this study.

# Ethics, regulations and project management

## Data quality assurance

The sponsor or the personnel designated by the sponsor will conduct on-site visits at the study site to verify the qualifications of the investigator, inspect the facilities of the site and inform the investigator of his or her responsibilities and procedures to be performed to ensure the integrity and accuracy of the records.

The investigator should keep a complete and accurate record of each subject’s study-related observations and other data in the medical records. All information recorded in CRF should be consistent with that in the source files of the subjects (e.g. medical records).

## Data management and quality control

Data obtained by all study sites and their laboratories will be recorded in the CRF. Once clinical data in the CRF is transferred to the data management team and the database is locked, changes in the data will be recorded as audit trails. The reason for the change, the name of the person making the change, the time and date of the change will be recorded as audit trail records.

During routine monitoring, relevant monitors or data managers will ask questions about the CRF. Relevant personnel at the study center will answer questions asked by the investigator. The names of the people answering the questions, time and date will be recorded. Once all the original data are checked and all the questions are answered, the inspector or data manager will freeze the database.

## Case report form and source files

All data obtained in the study should be recorded in the CRF in a timely manner. All source files for data entered into the CRF should be kept in the medical record, which usually includes laboratory examinations, ECG and echocardiography results. The data entered into the CRF directly will be considered as raw data. The clinical monitor checks the original data entered in the CRF with the source files while performing the central inspections. After completing the check, the clinical monitor will discuss the missing data and unexplained data with the investigator.

## Data collection

The investigator (and appropriate authorized personnel) will be given access to the CRF, and only the investigator and authorized personnel can enter and correct data on the CRF.

The investigator (or appropriate authorized personnel) should complete the CRF of each enrolled subject to reflect the findings during the last study observation. Therefore, after completing the visit or assessment, the investigator should fill in the CRF immediately. The investigator should verify the accuracy of data entry in the CRF. The investigator should indicate in the CRF if certain assessments are not feasible, or certain specific information is not available or is not applicable, or unknown.

The investigator should sign his or her name in the CRF after completion.

The dose and dose change of each subject should be recorded on CRF.

## Data processing

Data audit and data processing programs include specific requirements for data consistency and authenticity verification, as well as processing principles for data with obvious errors. The database will be updated according to the signed corrections.

Previous and concomitant medications will be coded according to WHO-DDE classified by ATC, and past/current disease history and AE will be coded according to MedDRA.

## Archiving of study records

The investigator should keep clinical study data for five years after the completion of the clinical study, and the sponsor should keep clinical study data for five years after the drug is approved for marketing.

## Good Clinical Practice

This study protocol will be implemented by following GCP, Declaration of Helsinki (2008) and local regulations.

## Informed consent

According to local laws and regulations, the ICF signed and dated by the subject will be obtained before study process begins. The investigator will keep the original signed ICF as study data. The signing date of ICF will be recorded in CRF.

If the protocol needs to be amended, the ICF may need to be amended to reflect the updated information in the protocol. If the ICF is amended, it must be submitted to the IEC for written approval and the signatures of subsequent enrollees and those currently under study should be obtained.

## Protocol approval and amendment

According to local regulations, the study protocol and/or other relevant documents will be approved by the IEC/competent authority before the study begins. The sponsor must ensure that all ethical and regulatory requirements have been met before the first subject is enrolled.

This study will be carried out strictly in accordance with this protocol. The proposed amendments to the protocol must be approved in writing by the relevant personnel before implementation and submitted to the IEC/IRB/competent authority for examination and approval.

Management changes (which do not affect the benefit-risk ratio of subjects) do not require formal protocol amendments. Each amendment of the protocol will be distributed to all protocol recipients, with corresponding instructions attached.

## Study cycle

The longest participation period for each subject in the study is 2 years.

## Early termination

If the investigator, the sponsor, or medical inspector is aware that certain conditions or events may endanger the subject if he/she continues to carry out the study, the study may be terminated after discussion by the relevant personnel. Even without the above findings, the sponsor can decide to terminate the study ahead of schedule.

Early termination of the study can be done for the following reasons, but not for these reasons only:

* Unexpected, significant or unacceptable risks for enrolled subjects
* Slow enrollment
* The sponsor decides to suspend or stop the development of drugs

## Confidentiality

All findings and documents related to the study will be classified as confidential. The investigator and members of his/her study team should not disclose such information without the written consent of the sponsor.

The subjects of the study should be kept anonymous. In the CRF and submitted documents, the subjects are identified by subject number, name acronym and/or date of birth, instead of the subjects’ names. The investigator should keep confidential documents, such as signed ICFs, that do not require delivery but confirm the identity of the subject.

## Liability and insurance

The sponsor will provide insurance for the subjects of the study and will cover the cost of treatment and the corresponding financial compensation for the subjects who suffer damage related to the drug or study process according to laws and regulations.

## Publishing policy

The signing of the study protocol indicates that the investigator has agreed that the results of this study can be used for domestic or foreign registration, publishing or providing information to medical professionals. If necessary, the regulatory agency will be informed of the name, address, qualifications, and responsibilities of the investigator in the study.

The investigator should not publish any data related to this study (posters, abstracts, papers, etc.) without prior communication with the sponsor. Detailed information will be described in another document.

# Study monitoring and audit

## Clinical monitoring plan

The study will be monitored according to the clinical monitoring plan. The investigator should arrange enough time to cooperate with monitoring activities. The investigator should also ensure that the monitor or other quality assurance reviewer will have access to all relevant study documents and study-related facilities (such as pharmacies, diagnostic laboratories, etc.) and have sufficient space for field monitoring.

## Clinical monitoring

The clinical monitor should routinely monitor the study center and regularly review all CRFs and source documents for each subject during and after the study. During the monitoring of the study center, the monitor should determine that the study-related files are appropriately documented, provide training and GCP guidance for the investigators and other staff in the study, and determine that there are appropriate facilities and adequate professional staff.

During the study, the monitor will conduct central visits to check compliance with the protocol, CRF entry, subjects’ disease history, drug counts, and to confirm that the study is carried out in accordance with the relevant regulatory requirements. The CRF entries will be checked against the original data. The medical history check will be carried out in such a way that protects the subject’s privacy.

Regular monitoring during the study process provides an opportunity for the investigator to assess the progress and understand potential problems in the study. The monitor should ensure that the data submitted are accurate and in accordance with the source documents; review the study products and properly preserve them; obtain the informed consent form signed by the subjects correctly and archive them; confirm that the subjects enrolled in the study meet the criteria for inclusion and exclusion of the study, and determine and preserve all the necessary documents according to GCP requirements.

The integrity and clarity of CRF entry should be checked and compared with the original data to monitor the study process. Furthermore, regulatory authorities, IEC / IRB and/or the sponsor’s clinical quality assurance department may check the original data and/or go to the center for on-site audit or inspection. During the audit or inspection, original data will be directly accessed; parties with direct access should ensure the data and medical confidentiality.

## Audit and inspection

The investigator should allow the study-related ethics committee, sponsor, government regulatory agencies and quality assurance team to monitor, review and examine all study-related documents (such as source documents, regulatory documents, data collection tools, research data, etc.). The investigator should ensure the ability of study-related facilities, such as pharmacies, diagnostic laboratories, etc.

Participants in the study should accept the examinations by government regulatory agencies and relevant quality assurance offices.

# Ethics

This study protocol and any amendments will be submitted to the appropriate independent institutional review board (ethics committee) in compliance with the law for official approval. The ethics committee’s decision on the study will be notified in writing to the investigator and a copy will be provided to the sponsor before the study begins. The investigator should provide the sponsor with a list of the members of the ethics committee and their work units.

# Confidentiality and publicity of the study findings

The trial results will be released according to the requirements of the study site. Without the consent of the sponsor, no complete or partial results of the study or information provided by any sponsor should be published or disclosed to any third party concerning the trial protocol. Any investigator involved in the study is obliged to provide the sponsor with complete trial results and data.

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