

**CD19 CAR-T Expressing IL7 and CCL19 Combined With PD1 mAb
for Relapsed or Refractory Diffuse Large B Cell Lymphoma**

Protocol Number: NHL-7×19-2020-01

Study Center: 2nd Affiliated Hospital, School of Medicine,
Zhejiang University

Version: 1.0

Date: March 20, 2020

Overview

Name	Phase Ib clinical study of CD19 CAR-T expressing IL7 and CCL19 combined with PD1 mAb in the treatment of relapsed/refractory diffuse large B cell lymphoma
Purpose	To evaluate the safety and efficacy of CD19 CAR-T (hereinafter referred to as CD19-7×19 CAR-T) expressing IL7 and CCL19 combined with PD1 mAb in the treatment of relapsed / refractory diffuse large B-cell lymphoma, and to provide a new method for the treatment of relapsed / refractory diffuse large B-cell lymphoma.
Sponsor	2nd Affiliated Hospital, School of Medicine, Zhejiang University
Indication	relapsed / refractory diffuse large B-cell lymphoma
Study Design	To evaluate the efficacy and safety of CD19-7×19 CAR-T combined with PD1 mAb in the treatment of relapsed / refractory large B cell lymphoma.
Subjects	Twenty four patients aged 18-75 years were recruited, including 9 patients in the dose increasing stage and 15 patients in the dose expanding stage. If the number of patients in Ib phase increased, the number of patients in the expanding stage decreased accordingly. Patients

	<p>met the inclusion / exclusion criteria. Considering the failure of cell preparation, or the inability of cell transfusion due to disease progression during cell preparation, the number of participants in cell preparation in this study may be more than the number of planned cases.</p>
Study Period	24 months
Selection Criteria	<p>Inclusion Criteria:</p> <p>(1) Age \geq 18, upper limit 75, unlimited for men and women;</p> <p>(2) ECOG score 0-3;</p> <p>(3) Histologically confirmed diffuse large B-cell lymphoma (DLBCL) [according to who 2008];</p> <p>(4) CD19 was positive (immunohistochemistry or flow cytometry).</p> <p>(5) The definition of refractory or relapse of DLBCL is: no complete remission after 2-line treatment; disease progression in any treatment process, or disease stabilization time equal to or less than 6 months; or disease progression or relapse within 12 months after hematopoietic stem cell transplantation;</p> <p>(6) The previous treatment of diffuse large B cell</p>

lymphoma must include rituximab (CD20 mAb) and anthracycline;

(7) There should be at least one measurable focus. It is required that any length of lymph node focus should be greater than 1.5cm or any length of extranodal focus should be greater than 1.0cm. PET-CT scan focuses should have uptake (SUV is greater than liver blood pool);

(8) The absolute value of neutrophils in peripheral blood $\geq 1000 / \mu L$, platelet $\geq 45000 / \mu L$;

(9) Heart, liver and kidney function: creatinine $< 1.5\text{mg/dl}$; ALT (alanine aminotransferase) / AST (aspartate aminotransferase) 2.5 times lower than the normal upper limit; total bilirubin $< 1.5\text{mg/dl}$; heart ejection fraction (EF) $\geq 50\%$;

(10) Sufficient understanding ability and voluntary signing of informed consent;

(11) Those with fertility must be willing to use contraceptive methods;

(12) According to the judgment of the researchers, the expected survival time is more than 4 months;

(13) Willing to follow visit schedule, administration

	<p>plan, laboratory inspection and other test steps.</p> <p>Exclusion Criteria:</p> <ul style="list-style-type: none">(1) History of other tumors;(2) Hematopoietic stem cell transplantation was performed within 6 weeks;(3) Any target car-t treatment was performed within 3 months before the car-t treatment;(4) Previous use of any commercially available PD-1 mAb;(5) Cytotoxic drugs, glucocorticoids and other targeted drugs were received within 2 weeks before cell collection;(6) Active autoimmune diseases;(7) Uncontrollable infection of active bacteria and fungi;(8) HIV infection, syphilis infection; active hepatitis B or C: hepatitis B: HBV-DNA \geq 1000IU / ml; hepatitis C: HCV RNA positive and liver function abnormal.(9) Known central nervous system lymphoma.
	<p>This study is a single arm, single center, open phase I B clinical study. The study will be divided into two phases:</p>

Trial group and treatment plan	<p>Stage I is a dose increasing study. Nine effective subjects are planned to be recruited to complete cell reinfusion. The efficacy and safety of cd19-7 × 19 car-t combined with PD1 antibody in the treatment of relapsed / refractory B cell tumors are preliminarily evaluated.</p> <p>Stage II is the extended study stage. According to the data of safety tolerance, proliferation and survival of cd19-7×19 CAR-T in vivo, clinical efficacy and other data obtained in stage I, the recommended dose for entering this stage is determined after comprehensive consideration, and 15 effective subjects are recruited to further evaluate its effectiveness and safety.</p> <p>In the dose increasing stage, the last subject in each dose group can enter the next dose group after completing the dose limiting toxicity (DLI) evaluation window 30 days after reinfusion and 14 days after the application of PD-1 monoclonal antibody, and after the safety supervision committee combined with the clinical safety and pharmacokinetic data evaluation consent.</p>
---------------------------------------	--

	<p>In the extended study phase, the dose is selected by the PI according to the first phase and reported to the Ethics Committee (Safety Supervision Committee) for approval.</p>
<p>Treatment process</p>	<p>Preparation of test preparation: preparation of CD19-7×19 CAR-T cells</p> <p>In the experimental group, blood cells were separated and PBMCs were separated by Ficoll lymphocyte separation technique. The cell density of 2×10^6 PBMC / ml was inoculated into the cell culture flask. According to the ratio of beads: cell = 3:1, the pre washed human t-activator CD3 / CD28 Dynabeads (cat#11161D, life technologies) were added to activate T cells. After 24 hours, the cell density was concentrated to $3-5 \times 10^6$ cells / ml, and the lentivirus vector carrying CD19-7×19 CAR was infected in a volume ratio of 1:10. At the same time, the transfection reagents Polybrene (4ug/ml) and IL-2 (300IU/ml) were added. After 4 hours, the fresh medium was supplemented and the cell density was adjusted to 1×10^6 cell/ml. On the second day, the cells were centrifuged to remove the uninfected virus solution and</p>

replaced with fresh culture medium. After that, the cells were replaced with fresh culture medium every 2-3 days according to the growth of cells. The transfection efficiency of CD19-7×19 CAR was measured by 0.5-1 × 10⁶ cells on the 4th-5th day. After 12-14 days of culture, cells were harvested to prepare 300ml cells for reinfusion.

CD19-7 × 19 car-t cell detection index:

① Cell number (number of CD19 positive car cells):

Target dose: 1×10⁶/kg, 2×10⁶/kg, 3×10⁶/kg (allowable fluctuation range: ± 20%).

② Cell morphology: the surface of microscope cell is smooth;

③ Cell viability: more than 90%;

④ Cell surface markers: CD3 +, CD8 +, CD107a +, Car19 +, CD45RA, CCR7;

⑤ CD19-7×19 CAR transfusion method: 300ml of cell suspension product (CD19-7×19 CAR-T+normal saline + 2% human albumin) was infused back to the patient by intravenous infusion.

Dosage of the test preparation: CD19-7×19 CAR-T treatment is divided into two stages, stage I is divided

	<p>into three dose ramps: $1 \times 10^6/\text{kg}$, $2 \times 10^6/\text{kg}$, $3 \times 10^6/\text{kg}$ car to transduce auto T cells; stage II is determined by the researcher based on safety tolerance, expansion and duration of CD19-7\times19 CAR-T in vivo, clinical efficacy and previous clinical research experience. On day 0, an intravenous transfusion was performed.</p> <p>FC pretreatment scheme: $30\text{mg}/\text{m}^2/\text{day}$, 3 days (D-5, -4, -3); CTX $500\text{ mg}/\text{m}^2$, 3 days (D-5, -4, -3). See section 3.9 for chemotherapy plan.</p> <p>Dosage and usage of PD1 mAb: the dosage is 200mg / time, and it is used for three weeks for six consecutive courses (PD-1 can be stopped for those who are assessed as disease progression for three months).</p>
<p>Program</p>	<p>At the specific time points specified in the evaluation plan, subjects will be subject to the following procedures:</p> <p>(1) Obtain informed consent, general medical history (including previous treatment of lymphoma), physical examination (including vital signs and physical condition), neurological evaluation, blood routine test, biochemical test,</p>

cytokine, C-reactive protein, lymphocyte subtype and other tests.

(2) Women with fertility will be tested for pregnancy in urine or serum.

(3) Subjects will also receive baseline electrocardiogram (ECG), echocardiography (echo), brain magnetic resonance imaging (MRI), B ultrasound, positron emission tomography - computed tomography (PET-CT).

(4) The patients were assessed to complete leukocyte separation.

(5) Pre treatment of FC scheme shall be carried out within - 5 to - 3 days after car-t back transmission.

(6) CD19-7×19 CAR-T infusion.

(7) On the 31st day of CD19-7×19 CAR-T infusion, PD1 MAb (baizean mAb) was infused intravenously.

Subjects were regularly asked to report concomitant medication and adverse events and to assess their disease throughout the study.

<p>Observation index</p>	<p>1. Primary research objectives:</p> <p>(1) To evaluate the safety of CD19-7×19 CAR-T cells combined with PD1 mAb in the treatment of relapsed or refractory diffuse large B cell lymphoma;</p> <p>(2) Objective remission rate (CR + PR) of CD19-7×19 CAR-T cells combined with PD1 mAb in the treatment of recurrent or refractory diffuse large B-cell lymphoma was evaluated.</p> <p>2. Secondary research objectives:</p> <p>(1) 2-year PFS, relapse rate, OS and dor;</p> <p>(2) After treatment, biological markers such as car T cells and cytokines were monitored;</p> <p>(3) To study the relationship between the efficacy and side effects of different car-t cell dosage groups and PD1 mAb combination.</p>
<p>Safety analysis</p>	<p>Vital signs, adverse events, changes in clinical laboratory data and early withdrawal, etc.</p>
	<p>1. Selection of statistical analysis data</p> <p>(1) Fas (full analysis set): according to the principle of intent to treatment (ITT), all patients will enter the full analysis set of this trial. For patients who withdraw from the study in advance for various reasons, the</p>

<p>Statistical analysis</p>	<p>results of the corresponding evaluation points will be filled with the last observation carry forward (LOCF).</p> <p>(2) Per protocol set (PPS): all of them met the entry conditions to enter the study and complete the treatment, with good compliance.</p> <p>(3) Safety analysis population: defined as patients receiving car-t cell infusion and patients receiving a course of post-treatment safety evaluation of PD-1 mAb constitute the safety analysis set of this study.</p> <p>(4) Subgroup analysis: the safety and efficacy of car-t cells in different dose groups and in the groups receiving 1-6 times of PD-1 mAb were analyzed.</p> <p>(5) Intermediate analysis sets: DLT evaluation is applicable to patients with IB dose climbing. DLT evaluation time: 30 days after car-t infusion and 15 days after the first treatment with PD-1 mAb. Mid term evaluation patients: all patients in IB stage were enrolled, and the efficacy evaluation was completed for 3 months.</p> <p>2. Analysis plan</p> <p>According to the research plan, biostatisticians and main researchers make statistical analysis plan, and</p>
------------------------------------	--

complete and form documents before data locking.

The statistical analysis software applies SAS statistical analysis software 9.1.1.

(1) Efficacy evaluation

The measurement data were expressed by $X \pm s$, and the comparison of different time points was analyzed by repeated measurement analysis of variance. $P < 0.05$ was statistically significant. Kaplan Meier survival curve analysis was used for survival analysis to calculate the median survival time and progression free survival time.

(2) Safety evaluation

The safety evaluation will be descriptive, including adverse events, laboratory examination indicators, vital signs, etc. The type, severity, frequency and relationship with the study drug of all adverse events occurred during the study will be described in a list. The suspension of the study due to adverse events and cases of serious adverse events will be specially noted. For laboratory examination, all completed examination items are listed in the form of a cross table before and after treatment (according to

	<p>the judgment of the clinician). Items with abnormal values and clinical significance need to be listed.</p>
<p>Dose limited toxicity (DLT)</p>	<p>Dose limiting toxicity (DLT) is defined as:</p> <p>CD19-7×19 CAR-T cell infusion within 30 days is likely or definitely related to any of the following conditions:</p> <p>(1) Grade 3-5 anaphylaxis;</p> <p>(2) In addition to B-cell clearance, the level of CTCAE was 2-5;</p> <p>(3) The non hematologic toxicity of any major organ \geq Level 3 was not relieved (reduced to level 3 toxicity) or lasted for more than 28 days after intravenous administration of dexamethasone 10mg / 12h for 7 days (or the same dose of corticosteroids) or trozumab 8mg/kg for 3 times.</p>
<p>Maximum tolerable dose (MTD)</p>	<p>The incidence of dose limited toxicity (DLT) was lower than the highest dose level of 33% (at least 6 subjects were required to accept this dose level to evaluate the toxicity).</p>