BCMA-BBZ-OX40 CAR-T Therapy Using an Instant Manufacturing Platform or Traditional Production Process in Relapsed/Refractory Multiple Myeloma

Supplementary material

Principle investigator: Hui Liu

Department of Hematology, Beijing Hospital,

National Center of Gerontology, Institute of Geriatric Medicine,

Chinese Academy of Medical Sciences, Beijing, China

Table of Contents

Study protocol

1 Title of study

BCMA-BBZ-OX40 CAR-T Therapy Using an Instant Manufacturing Platform or Traditional Production Process in Relapsed/Refractory Multiple Myeloma

2 Background & Rationale

Multiple myeloma (MM) is a hematologic malignancy originating from bone marrow hematopoietic cells and the second most common hematologic malignancy after non-Hodgkin's lymphoma. MM currently remains incurable with high relapse rates.

Chimeric antigen receptor engineered T cell (CAR-T) therapy is a revolutionary new approach to treat cancer malignancies and is considered one of the most significant recent breakthroughs in cancer treatment. This approach combines an antibody that recognizes tumor-associated antigens with T cell activating domains to take advantage of both the high affinity of antibodies for tumor antigens and the tumor killing capability of T lymphocytes.

BCMA is a receptor protein specifically expressed on MM cells and belongs to the TNF receptor superfamily. With the exception of plasma cells, BCMA protein expression has not been detected in normal tissues or CD34+ hematopoietic cells. Binding of B cell activating factor and proliferation inducing ligand promotes MM cell growth and adhesion of bone marrow stromal cells. BCMA antibodies have shown cytotoxic effects against MM cell lines and primary MM cells. Mice deficient in BCMA have normal B cell numbers but impaired B cell function. The above research results indicate that BCMA is well suited as a therapeutic target for MM without significantly affecting normal B cell function.

Current clinical studies have shown that anti-BCMA CAR-T cell therapy can achieve significant efficacy in treating relapsed/refractory MM patients. A study on LCAR-B38M, a dual epitope-binding CAR-T cell therapy directed against two distinct BCMA epitopes, showed that 50 out of 57 (88%) patients with relapsed/refractory MM achieved clinical remission.(1) The objective response rate was 85% in patients with advanced relapsed/refractory MM in the phase 1 clinical trial of anti-BCMA CAR-T cell therapy bb2121.(2) But there are several limitations, including long vein-to-vein time and limited persistence. In some cases, patients lost their eligibility for CAR-T-cell treatment due to rapid disease progression during the long vein-to-vein interval. Thus, it is critical to reduce the

waiting period.

In terms of CAR design, CAR-T cell technology has undergone three generations of evolution. First generation CARs: In vivo studies showed that first generation CARs can provide T cells with the first signal for activation, activating T cells and initial cytotoxic responses and eliciting anti-tumor effects such as inducing interleukin-2 secretion from effector T cells. However, clinical trials have shown that first generation CAR engineered T cells have very limited in vivo proliferation capability, mainly due to incomplete T cell activation caused by lack of the second co-stimulatory signal necessary for T cell activation and maintenance. Second and third generation CARs: Both in vitro and in vivo tests have confirmed that compared to first generation CARs, providing T cells with a second co-stimulatory signal in second and third generation CARs results in stronger proliferation capability, improved persistence, and tumor tissue chemotaxis. Costimulation signaling contributes to the effector function and persistence of T cells.(3, 4) In this regard, OX40 (CD134) activated by the ligand OX40L has broad effects on T-cell activation, proliferation, differentiation, and survival.(5-8) At the molecular level, OX40 ligation recruits TNF receptor-associated factor 2 (TRAF2), TRAF3, or TRAF5 to activate NF-κB and induce antiapoptotic genes, including *Bcl-2*, and promotes the PI3K/AKT pathway to enhance cell survival and cell cycle progression.(9-12) The performance of OX40 expression in CAR-T cells has never been reported in clinical trials for MM therapy.

The BCMA directed CAR-T cells with 4-1BB as the costimulatory domain involved in this study were developed integrating an independently expressed OX40 as an armored domain (named BCMA-BBZ-OX40 CAR-T). An Instant Manufacturing Platform (named InstanCART) was established, and CAR-T cells were produced within 3 days. CAR-T cells were manufactured using the InstanCART process or traditional manufacturing process (TraditionCART).

Previous in vitro studies showed that in cytotoxicity experiments using BCMA positive H929 cancer cells as target cells, BCMA-BBZ-OX40 CAR-T cells showed higher cytotoxicity against BCMA positive H929 cancer cells within 6 hours under the same culture conditions compared to ordinary T cells. In receptor binding assays using BCMA positive H929 cancer cells as target cells, under the same culture conditions, after 2 hours incubation,

BCMA-BBZ-OX40 CAR-T cells showed significantly enhanced binding capability for BCMA positive H929 cancer cells compared to ordinary T cells. Additionally, the killing activity of BCMA-BBZ-OX40 CAR-T cells is dependent on the culture duration after viral infection. Experiments compared the tumor cell killing efficacy of CAR-T cells cultured for different durations after viral infection. Results found that efficacy peaked at post-infection days 10-14. Preclinical studies in a BCMA positive multiple myeloma mouse model showed that intravenous injection of BCMA-BBZ-OX40 CAR-T cells (10^7 cells/mouse) could significantly inhibit paralysis and prolong survival and survival rates.

The CAR-T cell doses in an earlier clinical study on BCMA targeted CAR-T cell therapy with 4-1BB as the costimulatory domain (BCMA-BBZ CAR-T) in 10 relapsed/refractory MM patients were 3×10^5-3×10^6/kg. The objective response rate was 70% with no related serious adverse events. Based on preclinical and earlier clinical trial results and referenced results from similar therapies, a dose range of 1×10^5/kg-1×10^7/kg was selected for this trial.

The safety and efficacy of BCMA-BBZ-OX40 CAR-T cells therapy using an instant manufacturing platform or traditional production process in relapsed/refractory MM will be evaluated in this study.

References

1. Zhao WH, Liu J, Wang BY, Chen YX, Cao XM, Yang Y, et al. A phase 1, open-label study of LCAR-B38M, a chimeric antigen receptor T cell therapy directed against B cell maturation antigen, in patients with relapsed or refractory multiple myeloma. J Hematol Oncol. 2018;11(1):141.

2. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-Cell Therapy bb2121 in Relapsed or Refractory Multiple Myeloma. N Engl J Med. 2019;380(18):1726-37.

3. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013;13(4):227-42.

4. Zhang Q, Vignali DA. Co-stimulatory and Co-inhibitory Pathways in Autoimmunity. Immunity. 2016;44(5):1034-51.

5. Rogers PR, Song J, Gramaglia I, Killeen N, Croft M. OX40 promotes Bcl-xL and Bcl-2 expression and is essential for long-term survival of CD4 T cells. Immunity. 2001;15(3):445-55.

6. Salek-Ardakani S, Song J, Halteman BS, Jember AG, Akiba H, Yagita H, et al. OX40 (CD134)

controls memory T helper 2 cells that drive lung inflammation. J Exp Med. 2003;198(2):315-24.

7. So T, Song J, Sugie K, Altman A, Croft M. Signals from OX40 regulate nuclear factor of activated T cells c1 and T cell helper 2 lineage commitment. Proc Natl Acad Sci U S A. 2006;103(10):3740-5.

8. Song J, So T, Cheng M, Tang X, Croft M. Sustained survivin expression from OX40 costimulatory signals drives T cell clonal expansion. Immunity. 2005;22(5):621-31.

9. Arch RH, Thompson CB. 4-1BB and Ox40 are members of a tumor necrosis factor (TNF)-nerve growth factor receptor subfamily that bind TNF receptor-associated factors and activate nuclear factor kappaB. Mol Cell Biol. 1998;18(1):558-65.

10. Kawamata S, Hori T, Imura A, Takaori-Kondo A, Uchiyama T. Activation of OX40 signal transduction pathways leads to tumor necrosis factor receptor-associated factor (TRAF) 2- and TRAF5-mediated NF-kappaB activation. J Biol Chem. 1998;273(10):5808-14.

11. So T, Choi H, Croft M. OX40 complexes with phosphoinositide 3-kinase and protein kinase B (PKB) to augment TCR-dependent PKB signaling. J Immunol. 2011;186(6):3547-55.

12. So T, Croft M. Regulation of PI-3-Kinase and Akt Signaling in T Lymphocytes and Other Cells by TNFR Family Molecules. Front Immunol. 2013;4:139.

3 Trial Objectives

To evaluate the safety and efficacy of BCMA-BBZ-OX40 CAR-T cells therapy using an instant manufacturing platform or traditional production process in patients with relapsed or refractory MM.

The primary objectives were the incidence and severity of adverse events within 1 month after CAR-T-cell infusion. The secondary objective was the best overall response rate (i.e., the percentage of patients who had a complete or partial response using IMWG criteria). Exploratory analysis included time to best response, progression-free survival, overall survival, pharmacokinetic profile, and cytokine kinetics.

4 Trial Design

Single-arm, open-label clinical trial.

5 Target Population

This study plans to enroll about 20 patients with relapsed or refractory MM for single

infusion treatment with BCMA-BBZ-OX40 CAR-T cells therapy using an instant manufacturing platform or traditional production process.

Inclusion criteria:

1) Patients with R/R MM who had disease progression after their last line of therapy according to International Myeloma Working Group (IMWG) criteria.

2) Previous history of at least two lines of standard of care therapy, including an immunomodulatory agent and a proteasome inhibitor.

3) 18 years of age or older.

4) Measurable disease.

5) Expected survival time >three months.

6) Left ventricular ejection fraction >45%.

7) Volunteer to participate in the study and sign the informed consent.

Exclusion criteria:

1) Patients with high-risk organ involvement: tumor invasion of gastrointestinal tract, lung, pericardium or one of the great vessels.

2) Graft versus host disease requiring immunosuppressive agents.

3) Chemotherapy or radiotherapy within three days before blood collection period.

4) Use of systemic steroids within five days before blood collection period (except for recent or current use of inhaled steroids).

5) Use of bone marrow hematopoiesis stimulators within five days before blood collection period.

6) Patients had received CAR-T or BCMA-targeted treatment.

7) Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) >three times the upper limit of normal; or bilirubin >twice the upper limit of normal.

8) Active hepatitis B, hepatitis C virus, HIV or other active infection.

9) Any condition that may increase the risk to the subject or interfere with the results of the trial, in the opinion of the investigators.

Withdrawal Criteria:

1) The patient requests to withdraw.

2) Serious protocol deviations.

3) Insufficient T cells collected to manufacture CAR-T cell products meeting specifications.

4) Treatment side effects that can't be tolerated.

5) The investigator considered it inappropriate to continue participating in the study.

Study Termination Criteria:

1) Major errors discovered in protocol during study causing inability to evaluate therapies.

2) The investigator requests termination while ensuring participant rights and safety.

3) Terminated by regulatory authority or ethics committee.

6 Study Workflow and Follow-up Schedule

This study consists of five stages: Screening Period, Apheresis, Lympho-depleting Chemotherapy, Infusion and Follow-up.

Visit 1: Screening

Before screening, participants must voluntarily sign the informed consent in writing and be assigned a unique screening code. Participant screening codes consist of the screening identifier "BJ", followed by the participant number "01, 02, 03, 04..." For example, if this participant is the third screening participant, the screening code would be "BJ03".

Only participants meeting all inclusion and not meeting any exclusion criteria can enter the trial. Screening examinations include:

1) Demographics.

2) Medical history, treatment history.

3) Concomitant diseases and medications.

4) Height , weight, and body mass index (BMI).

5) Eastern Cooperative Oncology Group (ECOG) score.

6) Vital signs (temperature, respiratory rate, blood pressure and pulse rate).

7) Physical examinations: skin and mucosa, lymph nodes, head and neck, chest, abdomen, musculoskeletal system, neurological system, and others.

8) Virological testing (HBV antibody, HCV antibody, HIV antibody detection).

9) BCMA expression detection.

10) Routine lab tests: blood cell analysis, urinalysis, blood biochemistry (liver and kidney function, electrolytes), coagulation function.

11) M protein.

12) Serum free light chains and urinary total light chain.

13) Serum cytokines including IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, IL-17A.

14) Other tests: ECG, echocardiogram, CT scans, MRI scans or PET-CT, bone marrow examinations, and CSF if necessary.

Visit 2: Apheresis

The purpose of apheresis is mainly to isolate mononuclear cells from other blood components using a blood cell separator, which is then transfused back to the patient. The entire process takes approximately two-four hours.

1) Apheresis eligibility criteria: Meet all inclusion criteria with no exclusion criteria and completed all screening procedure; no fever or signs of infection before apheresis.

2) Peripheral blood mononuclear cell collection volume requirements:

<50kg body weight: 60-100ml, harvest no less than 4×10^8 cells;

≥50kg body weight: 80-120ml, harvest no less than 8×10^8 cells.

Harvested blood cells are stored short term and transported in blood bags under transport conditions of 2~25°C to manufacturing center within 24 hours.

Visit 3: Lympho-depleting Chemotherapy (D-5 to D-3)

All enrolled participants require effective lympho-depleting chemotherapy to facilitate expansion of CAR-T cells: intravenous fludarabine 25mg/m^2/day, combined with intravenous cyclophosphamide 250mg/m^2/day on days D-5, D-4 and D-3 prior to cell infusion. Physicians may adjust chemo- therapy regimen according to the patient's condition, but the lympho-depleting chemotherapy should be completed no later than 3 days prior to infusion.

Required examinations after lympho-depleting chemotherapy and before cell infusion include:

1) Vital signs, physical examinations.

2) Routine lab tests: blood cell analysis, urinalysis, blood biochemistry (liver/kidney function, electrolytes), coagulation function, immunoglobulins, ferritin.

3) M protein.

4) Serum free light chains and urinary total light chain.

5) Serum cytokines.

6) Other tests: ECG if necessary.

Visit 4: Cell Infusion (D0)

Cell products meeting quality control requirements can be transfused back. The dose of CAR-T cells in the infused product was the dose assigned by the subject. Products are configured by laboratory technicians in the cell processing lab according to patient weight, cryopreserved in liquid nitrogen, transported to study site, thawed at 37°C, and infused back within 60 minutes. Cell product components mainly consist of CAR-T cells, saline, and human serum albumin. Specifications: 20-100ml.

CAR-T cells are infused on D0. Antihistamines are started within two hours prior to CAR-T cell delivery to study site and infusion. Anti-allergic drugs such as calcium gluconate (1.0g, iv) and promethazine hydrochloride (12.5mg, im) are recommended.

Required infusion visit examinations:

1) Vital signs (within 1 hour prior and 4 hours after infusion).
2) Physical examinations (within 1 hour prior and 4 hours after infusion).
3) Other tests: ECG (within 1 hour prior and 4 hours after infusion).

Infusion should be stopped or delayed if any of the following are observed within 48 hours prior to infusion:

1) Fever ≥38.0°C or uncontrollable infections.
2) New onset cardiac arrhythmias.
3) Hypotension requiring vasopressor treatment.
4) Participant requires to use concomitant medications listed in the exclusion criteria.
5) For the patients who are delayed exceeding 2 weeks, if peripheral WBC $<1\times10^9$/L, no repeated lympho-depleting chemotherapy is necessary; if WBC $\geq1\times10^9$/L, repeated lympho-depleting chemotherapy is required.

Follow-up (D1 to Year 2):

Visit 5 (D1 post-infusion)

Required examinations: Vital signs, physical examinations.

Visit 6 (D4±1 post-infusion), Visit 7 (D7±1 post-infusion), Visit 8 (D10±1 post-infusion), Visit 9 (D14±1 post-infusion), Visit 10 (D21±2 post-infusion)

Required examinations:

1) Vital signs, physical examinations.

2) Routine lab tests: blood cell analysis, urinalysis, blood biochemistry (liver/kidney function, electrolytes), coagulation function, immunoglobulins, ferritin, CRP.

3) CAR-T cells count, serum cytokines.

Visit 11 (D28±2 post-infusion)

Required examinations:

1) Vital signs, physical examinations.

2) Routine lab tests: blood cell analysis, urinalysis, blood biochemistry (liver/kidney function, electrolytes), coagulation function, immunoglobulins.

3) M protein.

4) Serum free light chains and urinary total light chain.

5) CAR-T cells count, serum cytokines.

6) CT scans, MRI scans or PET-CT, bone marrow examinations, MRD if necessary.

Visit 12-22 (follow-up monthly ±4 days in months 2-6 post-infusion; then every 3 months ±7 days in months 6-24)

Required examinations:

1) Vital signs, physical examinations.

2) Routine lab tests: blood cell analysis, urinalysis, blood biochemistry (liver/kidney function, electrolytes), coagulation function, immunoglobulins.

3) M protein.

4) Serum free light chains and urinary total light chain.

5) CAR-T cells count, serum cytokines (if already normal by month 2, no further testing needed).

6) CT scans, MRI scans or PET-CT, bone marrow examinations, MRD if necessary.

7) Replicating lentivirus (RCL).

Unscheduled Visits

For safety considerations, investigators may request participants to conduct additional unscheduled visits or examinations. Results of unscheduled visits or exams should also be recorded in the Case Report Form (CRF).

Lab Test Details

1) Blood cell analysis: red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), white

blood cells (WBC), platelets (PLT), white blood cell classification.

2) Urinalysis: specific gravity, pH, bilirubin, protein, glucose, ketones, urobilinogen, nitrites, RBC count, WBC count.

3) Blood biochemistry: sodium, potassium, chloride, creatinine, urea nitrogen, glucose, total bilirubin, direct bilirubin, indirect bilirubin, ALT, AST, GGT, ALP, total protein, albumin.

4) Coagulation function: prothrombin time, INR, activated partial thromboplastin time, fibrinogen, d-dimer.

5) Serum cytokines including IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, IL-17A.

7 Study Assessments

Assessment of Safety

The primary objectives were the incidence and severity of adverse events within 1 month after CAR-T cell infusion. Adverse events were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0. Cytokine release syndrome and neurological toxic effects were graded using the American Society for Transplantation and Cellular Therapy (ASTCT) consensus criteria.

Investigators should collect and record any serious adverse events (SAEs) occurring from start of participant blood collection until 24 months after infusion of study medication or until disease progression, whichever occurs first. The initial SAEs report should include the following information if possible: reporting source, participant demographics, details of the SAEs (name, duration, severity, relatedness to study treatment, treatment and outcome of event) and contact information.

For enrolled but untreated participants, investigators should collect and record any serious adverse events occurring within two weeks after last medical procedure (such as blood collection, chemotherapy conditioning).

Any deaths occurring during the serious adverse event reporting period should be reported regardless of any interventions.

Pre-determined or planned hospitalizations such as for routine health examinations, participant education, protocol-specified procedures, social reasons should not be reported as SAEs.

Assessment of Efficacy

The secondary objective was the best overall response rate (i.e., the percentage of patients who had a complete or partial response using IMWG criteria).

MRD-negative: absence of clonal plasma cells by flow cytometry in bone marrow.

Exploratory analysis included time to best response, progression-free survival, overall survival, pharmacokinetic profile (CAR-T cell persistence and duration in participants within 2 years after CAR-T cell infusion), and cytokine kinetics.

8 Statistical Analysis

Statistical analyses will be performed using Stata 17.0 (Stata Corp., College Station, TX, USA) and SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics are presented as the mean ± SD or medians with minimum and maximum for continuous variables and counts and percentages for categorical variables. The duration of response, progression-free survival, and overall survival are estimated using the Kaplan–Meier method. P values less than 0.05 are considered statistically significant.

Concomitant medications and prohibited drugs The secondary objective was the best overall response rate (i.e., the percentage of patients who had a complete or partial response using IMWG criteria).

MRD-negative: absence of clonal plasma cells by flow cytometry in bone marrow.

Exploratory analysis included time to best response, progression-free survival, overall survival, pharmacokinetic profile (CAR-T cell persistence and duration in participants within 2 years after CAR-T cell infusion), and cytokine kinetics.

9 Concomitant medications and prohibited drugs

Concomitant medications: All drugs or treatments used during the study should be recorded in detail in the CRF, including name, dosage, dates taken and indications used; treatment methods, courses, dates and indications etc.

Prohibited drugs: Systemic corticosteroids (excluding CRS or ICANS treatment), other anti-tumor drugs.

10 Data Management Plan

1) Investigators should collect participant data according to GCP and protocol requirements, and accurately, promptly, completely, and standardly complete CRF.

2) Investigators must retain study records and data, including electronic source data and

electronic documents. All data points should be verifiably documented in study site source documents.

3) Clinical trial data should be entered into databases as soon as possible after each visit following project requirements.

4) Databases will be built according to trial protocols and undergo verification testing prior to officially entering data.

5) Data managers will use the Electronic Data Capture System (EDC) to enter data into databases. Data managers will manage data entry/check workflows to ensure data quality.

6) Medical dictionary MedDRA (22.0) will be used to code adverse events, drug coding will use WHODrug (Mar 2019) pharmacopoeia codes and above.

7) After data entry completion, query resolution and review/sign-off by relevant personnel databases will be locked.

8) Information retention: investigators agree to retain all study information for 5 years after study completion, including source documents on participant admissions, informed consent forms, CRFs and detailed medication management records etc.

11 Ethical Considerations

This study will strictly comply with the Declaration of Helsinki and relevant Chinese laws and regulations on ethical review, rigorously adhering to protocol implementation. Related documentation will be submitted to the hospital ethics committee for approval prior to study initiation. Participants should provide signed informed consent prior to enrollment. During the study, protocol and informed consent form modifications should undergo ethics committee re-review and approval before implementation. Investigators are responsible for submitting periodic progress reports to the ethics committee per requirements, and notifying the committee upon study completion. Participant identities will not be disclosed in publications or scientific conferences on study information and data obtained.